Supporting Information

Catecholase catalyzed synthesis of wedelolactone, a natural coumestan and its analogs

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MATERIALS AND METHODS:

Section I: General information for synthesis

All reactions were carried out in a two-way dry round bottom flask or a Schlenk tube. All reagents were purchased from commercial suppliers and used without further purification. ^{1}H NMR spectra were recorded on a Bruker DPX 300 MHz, JEOL JNM-ECZ 400 MHz, Bruker AV-400 MHz and Bruker DRX 600 MHz NMR instrument at an ambient temperature either in $CDCl_3$, acetone-d_6 or DMSO-d_6. ^{13}C NMR spectra were recorded at 75 MHz, 100 MHz and 150 MHz at ambient temperature. The chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. ^{1}H NMR is reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, dd=doublet of doublet, brs=broad singlet, t=triplet, q=quartet, m=multiplet), coupling constant and integration. Coupling constant (*J*) values are given in Hertz (Hz). All ^{13}C NMR spectra were recorded with complete proton decoupling. Mass spectral data correspond to ESI-MS and are given in *m/z* unit. ESI-HRMS were done on LC-MS Xevo G2 XS Q-Tof Mass Spectrometer, Agilent(R) 6538 UHD HRMS/Q-TOF high-resolution spectrophotometer and Waters(R) Micromass(R) Q-TOF Micro™ Mass Spectrometer. The X-ray diffraction measurements were carried out at 298 K on a Bruker APEX2 CCD diffractometer. Analytical thin-layer chromatography (TLC) was carried out on Merck 20 × 20 cm silica gel 60-F_{254} plates. Column chromatography was done with Biotage flash, silica gel 100-200 mesh.

Purification of catecholase enzyme from sweet potato:[1]

600 g of potato tubers were taken in 250 mL isolation buffer (50 mM NaOAc, 0.1 M NaCl, 0.5% sodium ascorbate of pH 6.0) and homogenized in a mixer grinder. To precipitate out phenolic compounds of low molecular mass, 24 g polyvinylpolypyrrolidone was added to it. This was kept for an hour at 4 °C for the precipitate to settle. The mixture was filtered through a cheesecloth. Successive salting out of the extract was done at 4 °C. 62.5 g solid $(NH_4)_2SO_4$ (in the proportion 25 g/100 mL solution) was added to it to 35% saturation and stirred for 30 min. The precipitate was removed by centrifugation (10000 × g, 4 °C, 1.5 h). To the supernatant part, $(NH_4)_2SO_4$ was added to make 85% saturation. The solution was stirred for an hour. The solution was again kept for centrifugation: 10000 × g, 4 °C for 1.5 h. The precipitate obtained hence dissolved in 250 mL of the previously prepared buffer. This

resultant solution was saturated further to 35% by addition of $(NH_4)_2SO_4$. The pH of this solution is adjusted to 4.0 with 2 N HCl and stirred for 15 min before subjecting it to centrifuge again at 14000 × g, 4 °C for 20 min. The supernatant was taken and its pH was adjusted to 6.0 with 0.2 M NaOH. To it, $(NH_4)_2SO_4$ was added and the solution was saturated to 85%. It was centrifuged at 14000 × g, 4 °C for 1 h. A brown pellet was formed and this was lyophilized. The resultant dried pellet was dissolved in 50 mM NaOAc buffer (which contains 0.5 M NaCl of pH 6.0).

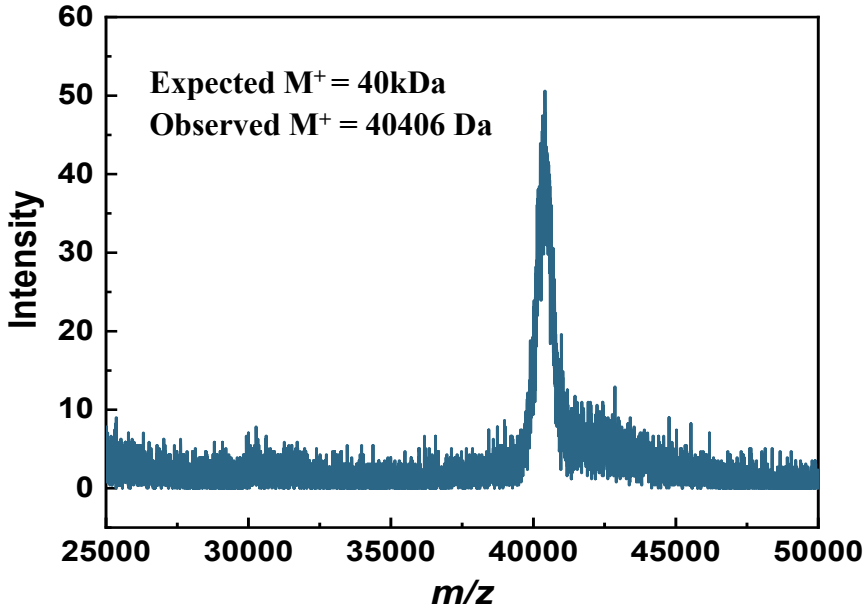
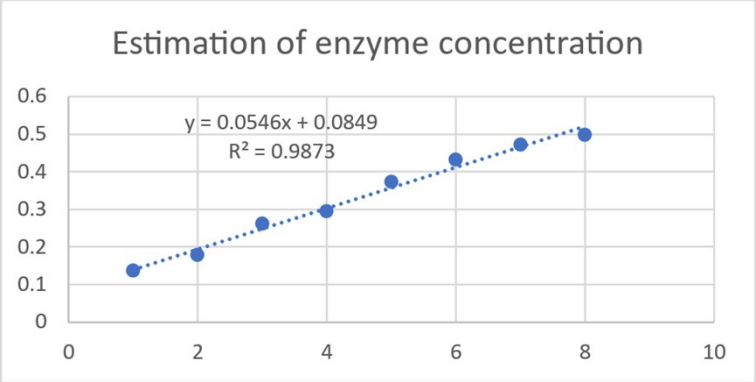


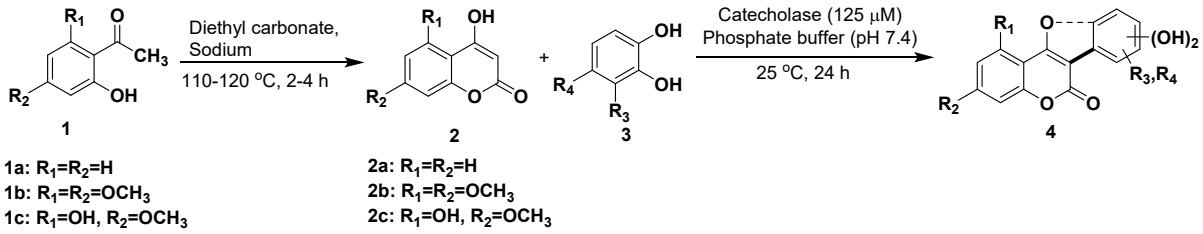
Figure S1: MALDI-MS of Catechol oxidase (*Ipomoea batatas*) in 2,5-dihydroxybenzoic acid as matrix

Estimation of catecholase concentration

BSA (5 µg/µL) was used as a standard protein to estimate and normalize the catecholase concentration. Catecholase was centrifuged at 16000 × g for 10 min at 4 °C. The supernatant was collected and measured the protein concentration using the Lowry assay by CLARIOstar (BMG Labtech). The catecholase concentration was further normalized to 5 µg/µL (125 µM) by adding a required volume of cold PBS. This normalized protein was used for the reaction to catalyze.



Typical experimental procedure for the synthesis of 4a-4h, 4k-4n

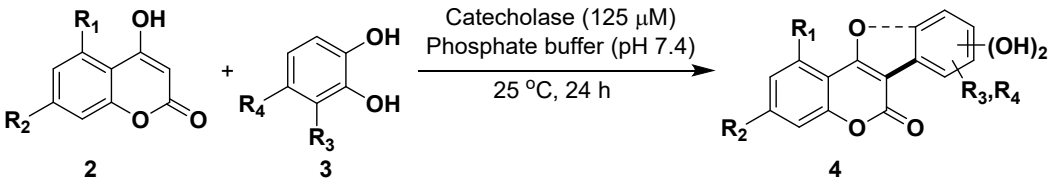


1a: $R_1=R_2=H$
1b: $R_1=R_2=OCH_3$
1c: $R_1=OH$, $R_2=OCH_3$

2a: $R_1=R_2=H$
2b: $R_1=R_2=OCH_3$
2c: $R_1=OH$, $R_2=OCH_3$

The selective monomethylation of 2,4,6-trihydroxyacetophenone by dimethyl sulfate yielded the corresponding 2,6-dihydroxy-4-methoxyacetophenone **(1c)**, which was cyclized on being heated with diethylcarbonate in the presence of sodium to afford 4,5-dihydroxy-7-methoxycoumarin **(2c)**. Oxidative cyclization of **2** with catechol (**3**) using catecholase enzyme from sweet potato was performed next to afford the desired product in phosphate buffer medium (pH 7.4) at ambient temperature.

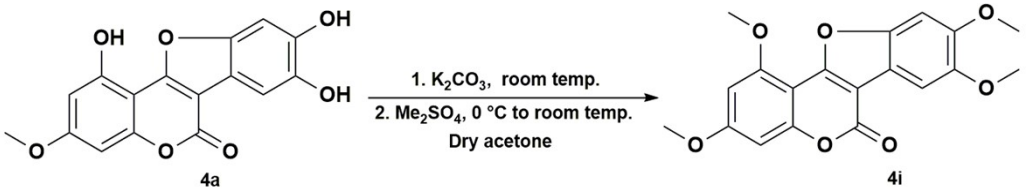
General experimental procedure for 4a-4h, 4k-4n



2,4,6-trihydroxyacetophenone (168.1 mg, 1 mmol) was dissolved in dry acetone (3 mL) at room temperature, and K_2CO_3 (165.8 mg, 1.2 mmol) was added and stirred until the solution turned turbid. To it, dimethyl sulfate (142 μL, 1.5 mmol) was added slowly at 0 °C and the reaction mixture was stirred at room temperature for 5-6 h to yield 2,6-dihydroxy-4-methoxyacetophenone (**1c**). This formed 2,6-dihydroxy-4-methoxyacetophenone (**1c**) was further reacted with diethylcarbonate for concomitant cyclization to yield 4,5-dihydroxy-7-

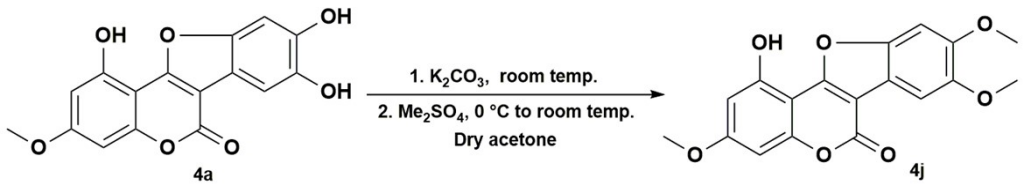
methoxycoumarin (**2b**). In a 25 mL round bottom flask, 4,5-dihydroxy-7-methoxycoumarin (**2b**; 104.1 mg, 0.5 mmol) and catechol (**3**; 60.5 mg, 0.55 mmol) were taken with 2.0 mL phosphate buffer (pH 7.4). Then semi-purified catecholase (30 µL, 125 µM) was added to it. The resulting mixture was stirred at room temperature for 24 h. The reaction mixture was extracted with chloroform (2 × 15 mL) and washed with water (10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure. The desired product was isolated by flash chromatography (silica gel 100- 200) and eluted with ethyl acetate/ hexane (60:40) to give **4a** as pale brown solid.

Experimental procedure of 4i



To a solution of 1,8,9-trihydroxy-3-methoxy-6*H*-benzofuro[3,2-*c*]chromen-6-one (**4a**, 314 mg, 1 mmol) in dry acetone (8 mL) at room temperature, K_2CO_3 (483 mg, 3.5 mmol) was added and stirred until the solution turned turbid. To it, dimethyl sulfate (332 µL, 3.5 mmol) was added slowly at 0 °C and the reaction mixture was stirred at room temperature for 5-6 h. After completion of the reaction, acetone was evaporated, and the reaction mixture was worked up with ethyl acetate (3 × 30 mL) and water. The organic layer was then dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford 1,3,8,9-tetramethoxy-6*H*-benzofuro[3,2-*c*]chromen-6-one (**4i**, 185 mg, 52%) as yellow solid.

Experimental procedure for 4j

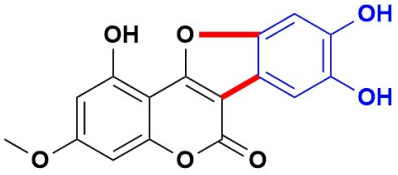


To a solution of 1,8,9-trihydroxy-3-methoxy-6*H*-benzofuro[3,2-*c*]chromen-6-one (**4a**, 314 mg, 1 mmol) in dry acetone (8 mL) at room temperature, K_2CO_3 (345 mg, 2.5 mmol) was added and stirred until the solution turned turbid. To it, dimethyl sulfate (237 µL, 2.5 mmol) was added slowly at 0 °C and the reaction mixture was stirred at room temperature for 5-6h.

After completion of the reaction, acetone was evaporated, and the reaction mixture was worked up with ethyl acetate (3×30 mL) and water. The organic layer was then dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford 1-hydroxy-3,8,9-trimethoxy-6*H*-benzofuro[3,2-*c*]chromen-6-one (**4j**, 188 mg, 55%) as yellow solid. A minor amount of **4i** was also formed in the course of the reaction which was recovered by flash chromatography.

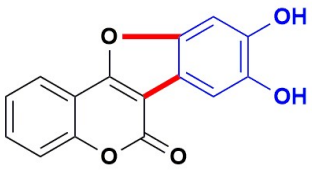
Spectral characterization

1, 8, 9-Trihydroxy-3-methoxy-6*H*-benzofuro[3,2-*c*] chromen-6-one (4a)



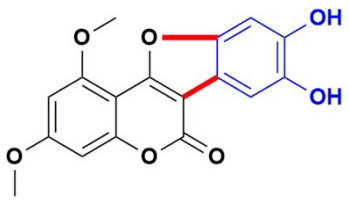
Pale cream solid. 207.2 mg (66% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.77 (s, 3H), 6.41 (d, J = 2.0 Hz, 1H), 6.57 (s, 1H), 7.12 (s, 1H), 7.19 (s, 1H), 9.35 (d, 2 -OH), 10.91 (s, 1 -OH). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 56.2 (CH_3), 93.7 (CH), 97.2 (C), 98.6 (CH), 99.4 (CH), 102.2 (C), 105.1 (CH), 114.2 (C), 144.8 (C), 145.9 (C), 149.4 (C), 155.3 (C), 155.8 (C), 158.3 (C), 159.4 (C), 162.7 (C). HRMS (ESI) m/z: calc. for $C_{16}H_{11}O_7$ $[M+H]^+$ is 315.0505; found 315.0497.

8, 9-Dihydroxy-6*H*-benzofuro[3,2-*c*] chromen-6-one (4b)



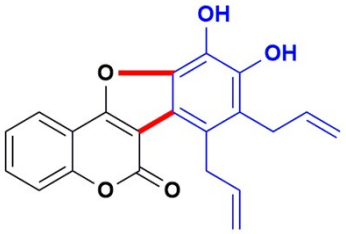
Off-white solid. 187.6 mg (70% yield). ^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.26 (s, 1H), 7.32 (s, 1H), 7.50 (t, J = 7.8 Hz, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.68 (m, 1H), 8.02 (dd, J = 7.8 Hz, 1.2 Hz, 1H), 9.60 (s, 1H), 9.72 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 99.4 (CH), 105.3 (CH), 105.9 (C), 112.9 (C), 114.3 (C), 117.6 (CH), 121.6 (CH), 125.4 (CH), 131.8 (CH), 145.2 (C), 147.0 (C), 149.9 (C), 152.8 (C), 157.9 (C), 158.3 (C). HRMS (ESI) m/z: calc. for $C_{15}H_9O_5$ $[M + H]^+$ 269.0450; found 269.0440.

8, 9-Dihydroxy-1,3-dimethoxy-6*H*-benzofuro[3,2-*c*] chromen-6-one (4c)



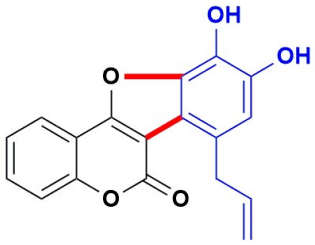
Brownish solid. 180.4 mg (55% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.89 (s, 3H), 4.01 (s, 3H), 6.66 (brs, 1H), 6.79 (brs, 1H), 7.16 (s, 1H), 7.25 (s, 1H), 9.43 (s, 1H), 9.49 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 56.1 (CH_3), 56.6 (CH_3), 94.1 (CH), 95.7 (CH), 97.2 (C), 98.8 (CH), 102.2 (C), 104.5 (CH), 113.6 (C), 144.4 (C), 145.6 (C), 148.9 (C), 155.1 (C), 156.2 (C), 157.6 (C), 158.3 (C), 162.6 (C). HRMS (ESI) m/z: calc. for $C_{17}H_{13}O_7$ $[M + H]^+$ 329.0661; found 329.0654.

7, 8-Diallyl-9,10-dihydroxy-6*H*-benzofuro[3,2-*c*]chromen-6-one (4d)



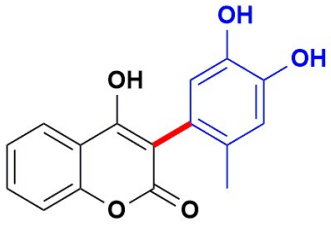
Pale cream solid. 174 mg (50% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.69 (d, J = 6.2 Hz, 2H), 4.12 (d, J = 6.1 Hz, 2H), 4.97 – 4.86 (m, 2H), 5.03 (d, J = 10.0 Hz, 1H), 5.12 (d, J = 17.1 Hz, 1H), 6.01 (m, 2H), 7.49 – 7.44 (m, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.66 (t, J = 7.8 Hz, 1H), 8.06 – 8.01 (m, 1H), 8.48 (s, 1H), 9.10 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 28.42 (CH_2), 31.43 (CH_2), 106.81 (C), 109.25 (C), 112.61 (C), 114.12 (CH_2), 114.85 (CH_2), 116.09 (C), 117.15 (CH), 118.61 (C), 121.85 (CH), 125.34 (CH), 132.04 (CH), 135.91 (CH), 138.19 (CH), 142.81 (C), 144.82 (C), 149.09 (C), 152.74 (C), 157.68 (C), 158.81 (C). HRMS (ESI) m/z: calc. for $C_{21}H_{17}O_5$ $[M + H]^+$ 349.1076; found 349.1068.

7-Allyl-9,10-dihydroxy-6*H*-benzofuro[3,2-*c*]chromen-6-one (4e)



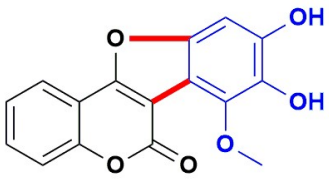
Brown solid. 163.2 mg (53% yield). ^{1}H NMR (300 MHz, DMSO -d_6): δ = 3.67 (d, J = 6.0 Hz, 2H), 5.02 – 5.15 (m, 2H), 5.98 – 6.12 (m, 1H), 7.24 (s, 1H), 7.49 (t, J = 7.5 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.67 (t like, 1H), 8.04 (d, J = 7.8 Hz, 1H), 9.03 (s, 1H), 9.95 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 27.9 (CH_2), 102.5 (CH), 105.7 (C), 110.1 (C), 112.5 (C), 113.2 (C), 115.6 (CH_2), 117.1 (CH), 121.2 (CH), 124.9 (CH), 131.3 (CH), 135.3 (CH), 144.0 (C), 144.4 (C), 148.8 (C), 152.3 (C), 157.6 (C), 157.7 (C). HRMS (ESI) m/z: calc. for $C_{18}H_{13}O_5$ $[M + H]^+$ 309.0763; found 309.0753.

3-(4,5-Dihydroxy-2-methylphenyl)-4-hydroxy-2*H*-chromen-2-one (4f)



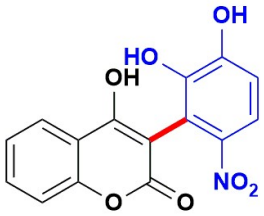
Yellowish-white solid. 127.8 mg (45% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.92 (s, 3H), 6.53 (s, 1H), 6.65 (s, 1H), 7.38 (m, 2H), 7.64 (t, J = 7.2 Hz, 1H), 7.91 (d, J = 7.5 Hz, 1H), 8.74 (s, 1H), 8.85 (s, 1H), 10.94 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 19.08 (CH_3), 105.87 (C), 116.64 (CH), 116.83 (C), 117.76 (CH), 119.07 (C), 121.64 (CH), 124.14 (CH), 124.40 (CH), 128.94 (C), 132.53 (CH), 143.41 (C), 145.71 (C), 152.96 (C), 160.66 (C), 162.24 (C). HRMS (ESI) m/z: calc. for $C_{16}H_{13}O_5$ $[M + H]^+$ 285.0763; found 285.0756.

8,9-Dihydroxy-7-methoxy-6*H*-benzofuro[3,2-*c*] chromen-6-one (4g)



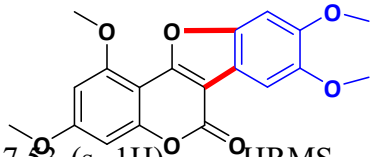
Dark yellow solid. 155 mg (52% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.09 (s, 3H), 7.07 (s, 1H), 7.49 (t, J = 7.5 Hz, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.68 (t, J = 7.5 Hz, 1H), 8.08 (d, J = 7.8 Hz, 1H), 8.31 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 60.7 (CH_3), 99.4 (CH), 105.6 (C), 112.3 (C), 114.2 (C), 117.1 (CH), 121.4 (CH), 125.0 (CH), 131.5 (CH), 133.7 (C), 138.2 (C), 141.8 (C), 145.9 (C), 152.4 (C), 157.5 (C), 158.0 (C). HRMS (ESI) m/z: calc. for $C_{16}H_{11}O_6$ $[M + H]^+$ 299.0556; found 299. 0543.

3-(2, 3-dihydroxy-6-nitrophenyl)-4-hydroxy-2*H*-chromen-2-one (4h)



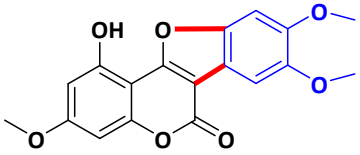
Pale brown solid. 183 mg (58% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 6.94 (d, J = 8.3 Hz, 1H), 7.39 - 7.42 (m, 3H), 7.62-7.66 (m, 1H), 7.94 (d, J = 7.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 99.2 (C), 112.6 (C), 116.3 (CH), 117.5 (CH), 123.9 (CH), 124.6 (CH), 132.0 (CH), 142.5 (C), 146.0 (C), 152.0 (C), 153.1 (C), 162.6 (C). Three peaks probably merged. HRMS (ESI) m/z: calc. for $C_{15}H_{10}NO_7$ $[M + H]^+$ 316.0457; found 316.0448.

1, 3, 8, 9-tetramethoxy-6*H*-benzofuro[3, 2-*c*]chromen-6-one (4i)



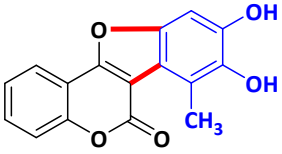
Yellow solid. 185 mg (52% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.89 (s, 3H), 3.98 (s, 3H), 4.01 (s, 3H), 4.05 (s, 3H), 6.44 (d, J =2.0 Hz, 1H), 6.64 (d, J = 2.4 Hz, 1H), 7.26 (s, 1H merged), 7.53 (s, 1H). HRMS (ESI) m/z: calc. for $C_{19}H_{17}O_7$ $[M + H]^+$ 357.0974; found 357.1041.

1-hydroxy-3,8,9-trimethoxy-6*H*-benzofuro[3,2-*c*] chromen-6-one (4j)



Yellow solid. 188 mg (55% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.89 (s, 3H), 3.99 (s, 3H), 4.04 (s, 3H), 5.66 (s, 1H), 6.43 (d, J = 2.0 Hz, 1H), 6.63 (d, J = 2.0 Hz, 1H), 7.23 (s, 1H), 7.59 (s, 1H). HRMS (ESI) m/z: calc. for $C_{18}H_{15}O_7$ $[M + H]^+$ 343.0818; found 343.0919.

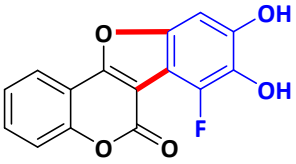
8,9-dihydroxy-7-methyl-6H-benzofuro[3,2-c]chromen-6-one (4k)



Yellowish-white solid. 138.1 mg, (49%) .^{1}H NMR (400 MHz, DMSO -d_6) : δ = 2.41 (s, 3H), 7.20 (s, 1H), 7.20 (s, 1H), 7.48 (t, J = 7.5 Hz, 1H), 7.58 (d, J = 8.3 Hz, 1H), 7.69 – 7.64 (m, 1H), 8.05 (dd, J = 7.8,

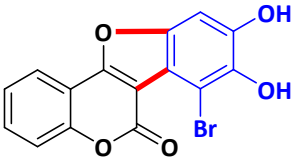
1.4 Hz, 1H), 8.95 (s, 1H), 9.81 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) : δ = 9.55 (CH_3), 102.42 (CH), 106.259 (C), 109.12 (C), 113.04 (C), 113.52 (C), 117.61 (CH), 121.76 (CH), 125.45 (CH), 131.78 (CH), 144.75 (C), 144.78 (C), 149.60 (C), 152.86 (C), 158.14 (C), 158.22 (C). HRMS (ESI) m/z: calc. for $C_{16}H_{11}O_5$ $[M + H]^+$ 283.0606; found 283.0603.

7-fluoro-8,9-dihydroxy-6H-benzofuro[3,2-c]chromen-6-one (4l)



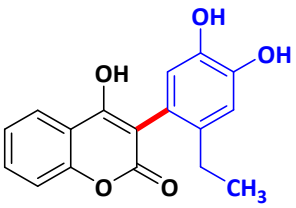
Off-white solid. 100.2 mg, (35%) .^{1}H NMR (400 MHz, DMSO-d_6) : δ = 7.08 (s, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.66 (t, J = 7.2 Hz, 1H), 7.97 (d, J = 7.1 Hz, 1H), 9.34 (s, 1H), 10.25 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) : δ = 95.42 (CH), 104.51 (C), 105.11 (C), 112.39 (C), 117.43 (CH), 121.93 (CH), 125.40 (CH), 132.25 (CH), 132.78 (C), 143.25 (C), 145.71 (C), 149.19 (C), 153.10 (C), 156.51 (C), 158.57 (C). HRMS (ESI) m/z: calc. for $C_{15}H_8O_5F$ $[M + H]^+$ 287.0356; found 287.0346.

7-bromo-8,9-dihydroxy-6H-benzofuro[3,2-c]chromen-6-one (4m)



Brown solid. 176.5 mg (51%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.25 (s, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.54 (d, J = 8.3 Hz, 1H), 7.67 (t, J = 7.8 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H), 9.30 (s, 1H), 10.61 (s, 1H). ^{13}C NMR(100 MHz, DMSO-d_6) : δ = 98.26 (CH), 100.90 (C), 105.77 (C), 112.35 (C), 115.88 (C), 117.14 (CH), 121.92(CH), 125.26(CH), 132.36(CH), 143.29(C), 146.99(C), 149.61 (C), 152.98(C), 155.95(C), 159.19(C). HRMS (ESI) m/z: calc. for $C_{15}H_8O_5Br$ $[M + H]^+$ 346.9555; found 346.9539.

7-ethyl-8,9-dihydroxy-6H-benzofuro[3,2-c]chromen-6-one (4n)



Yellowish sticky solid. 164.8 mg, (55%) .^{1}H NMR (400 MHz, DMSO -d_6) : δ = 0.98 (t, J = 7.5 Hz, 3H), 2.23 (q, J = 7.5 Hz, 2H), 6.50 (s, 1H), 6.69 (s, 1H), 7.48 – 7.29 (m, 2H), 7.63 (t, J = 7.7 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 8.76 (s, 1H), 8.86 (s, 1H), 10.98 (s, 1H). ^{13}C NMR (100 MHz, DMSO- d_6) : δ = 15.68 (CH_3), 25.78 (CH_2), 105.81 (CH), 116.12 (C), 116.66(C), 116.71 (C), 119.06 (C), 120.97 (CH), 124.11 (CH), 124.45 (CH), 132.58 (CH), 135.12 (C), 143.52 (C), 145.93 (C), 152.94 (C), 160.81 (C), 162.53 (C). HRMS (ESI) m/z: calc. for $C_{17}H_{15}O_5$ $[M + H]^+$ 299.0919; found 299.0918.

Section II: ^{1}H and ^{13}C NMR spectra of 4a – 4n

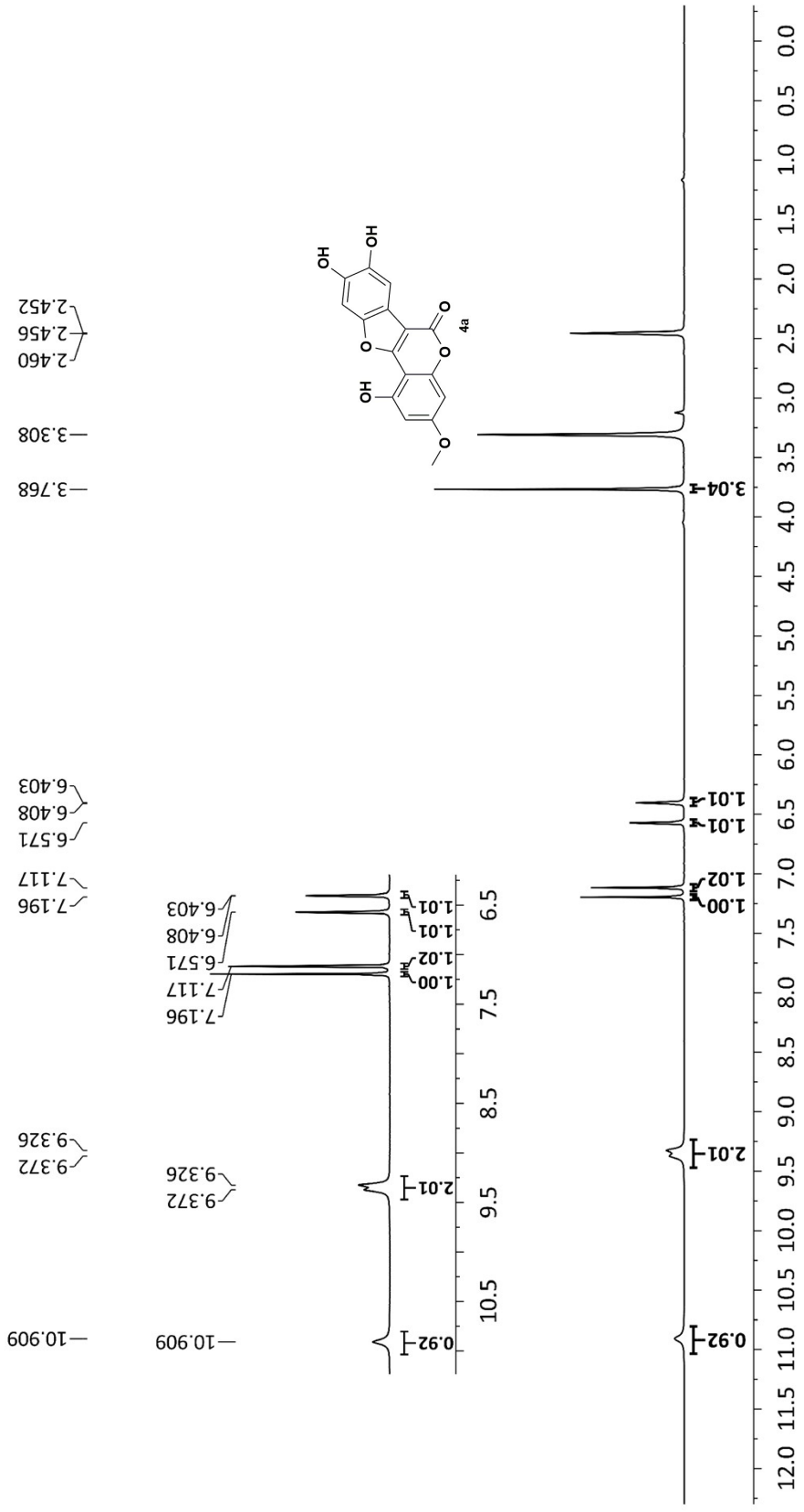


Figure S2: ^{1}H NMR spectrum of **4a** in DMSO-d_6 at 400 MHz

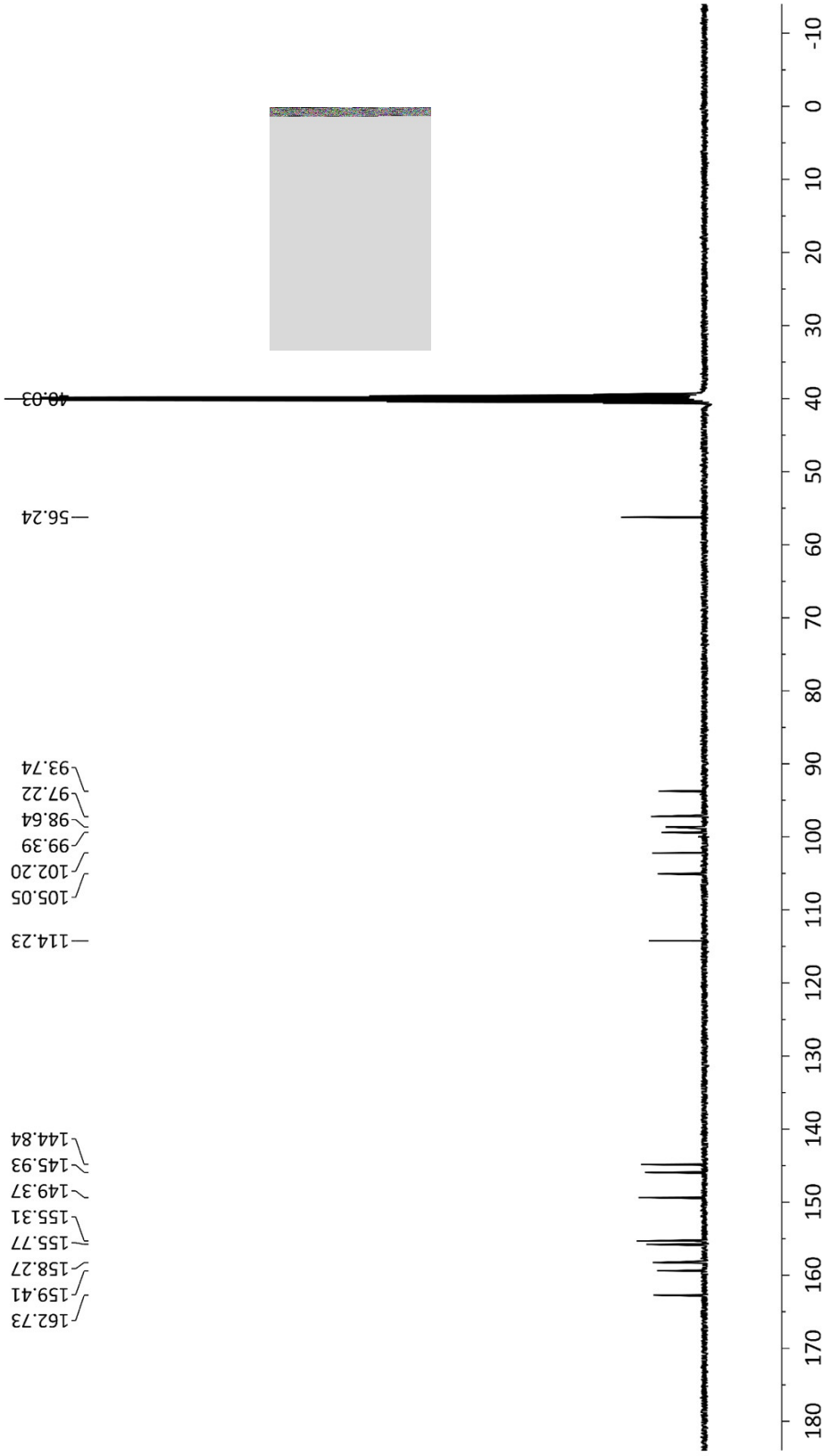


Figure S3: ^{13}C NMR spectrum of **4a** in DMSO-d_6 at 100 MHz

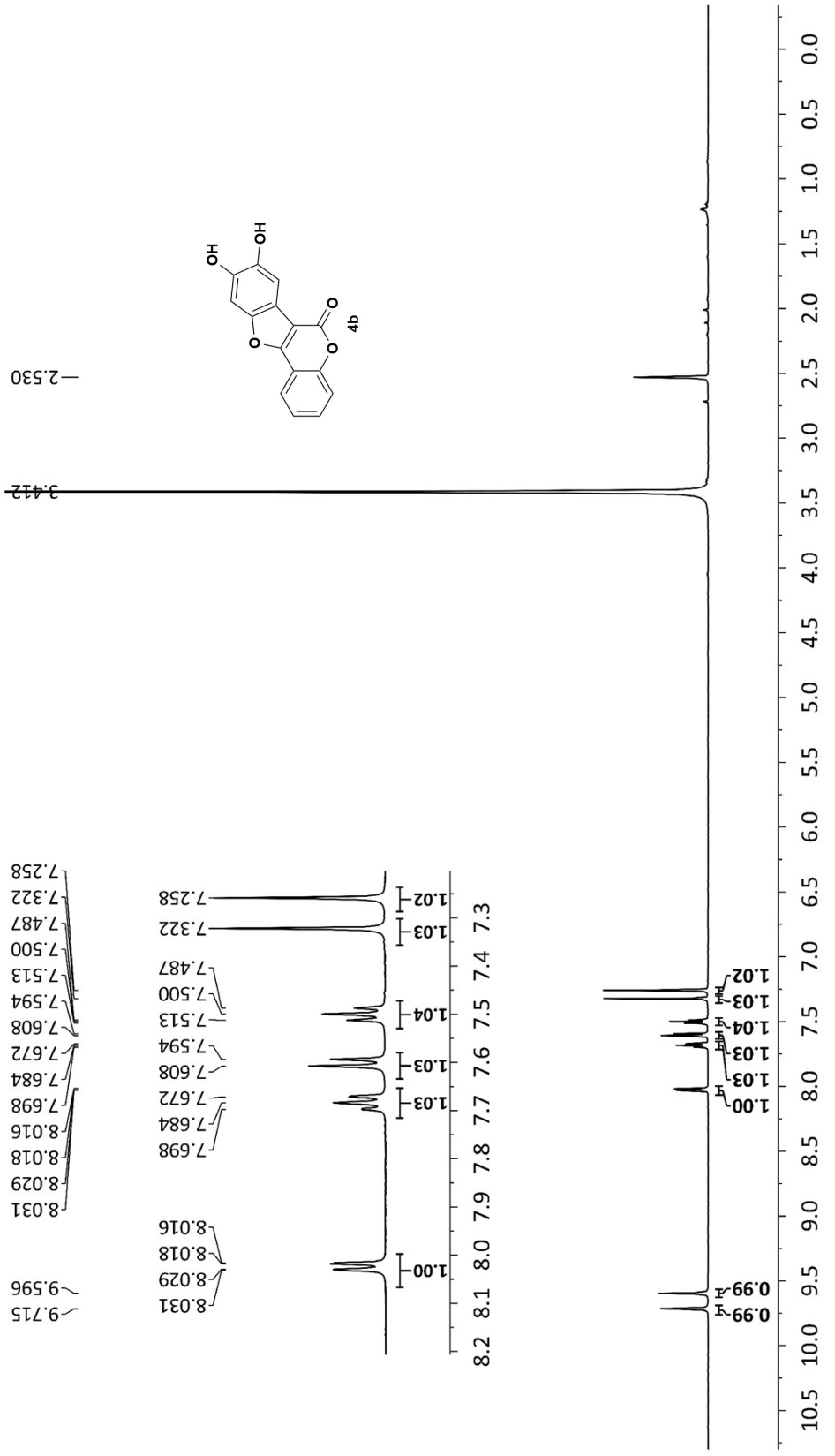


Figure S4: ^{1}H NMR spectrum of **4b** in DMSO-d_6 at 600 MHz

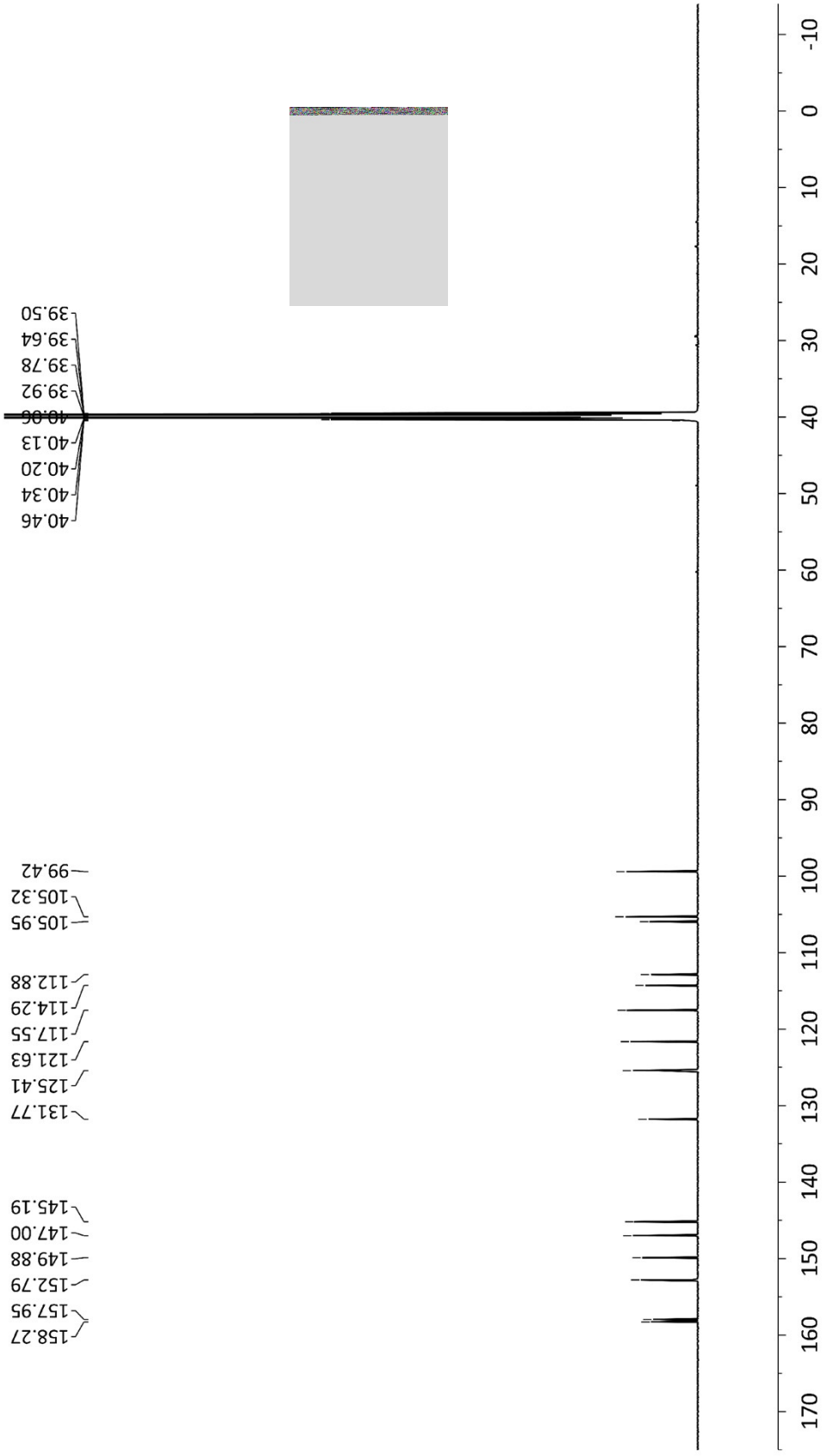


Figure S5: ^{13}C NMR spectrum of **4b** in DMSO-d_6 at 150 MHz

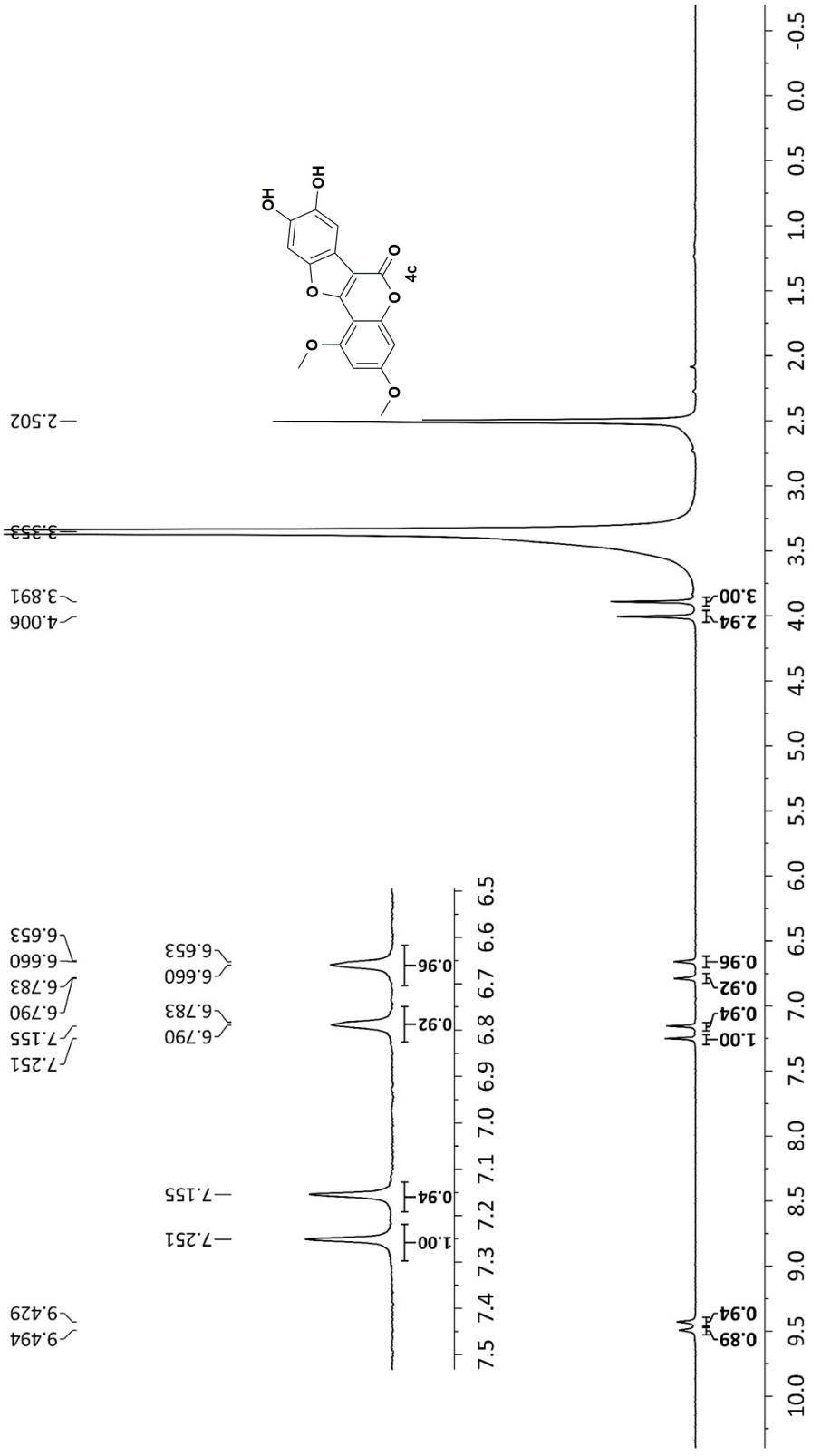


Figure S6: ^{1}H NMR spectrum of **4c** in DMSO-d_6 at 300 MHz

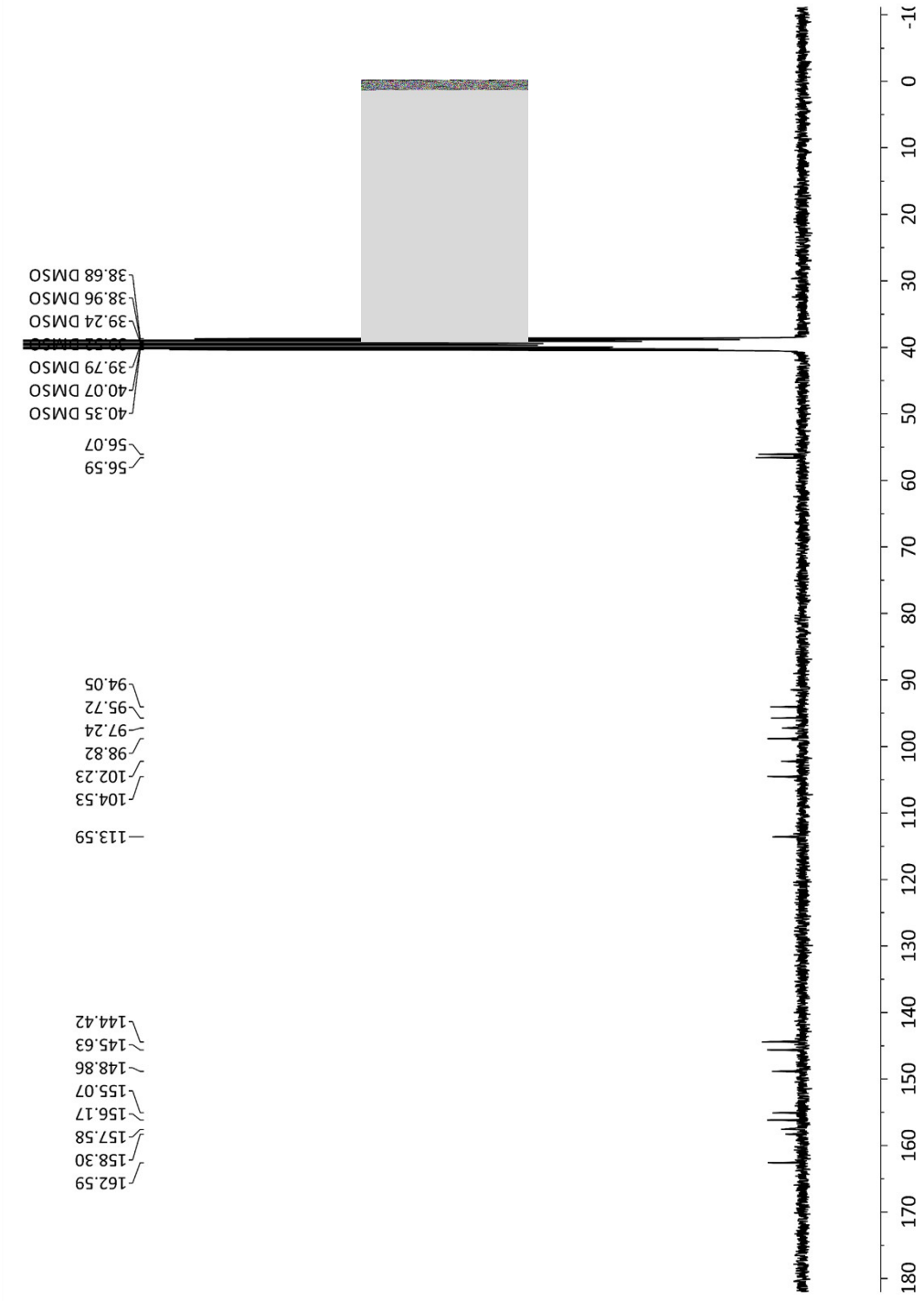


Figure S7: ^{13}C NMR spectrum of **4c** in DMSO-d_6 at 75 MHz

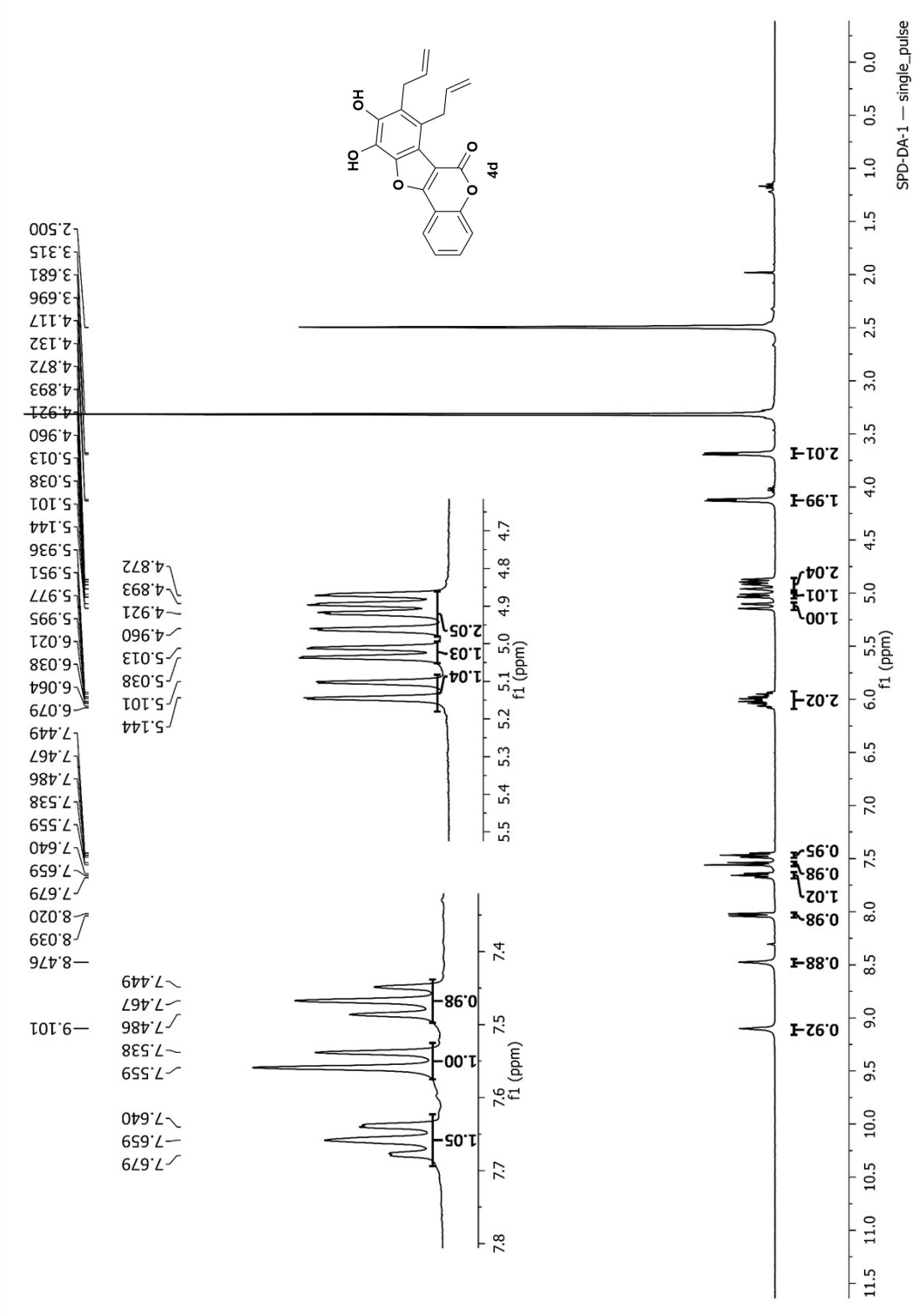


Figure S8: ^{1}H NMR spectrum of **4d** in DMSO-d_6 at 400 MHz

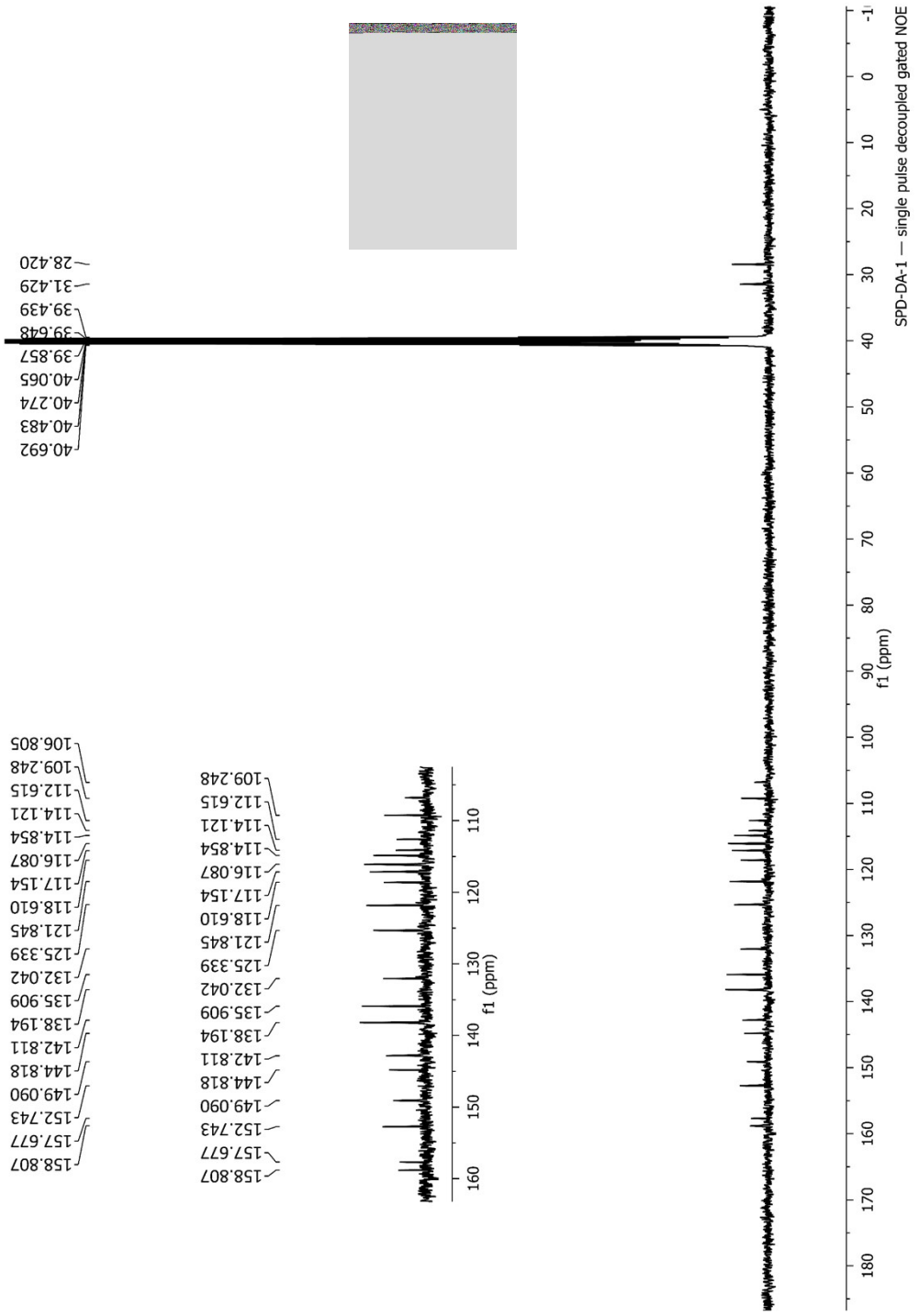


Figure S9: ^{13}C NMR spectrum of **4d** in DMSO-d_6 at 100 MHz

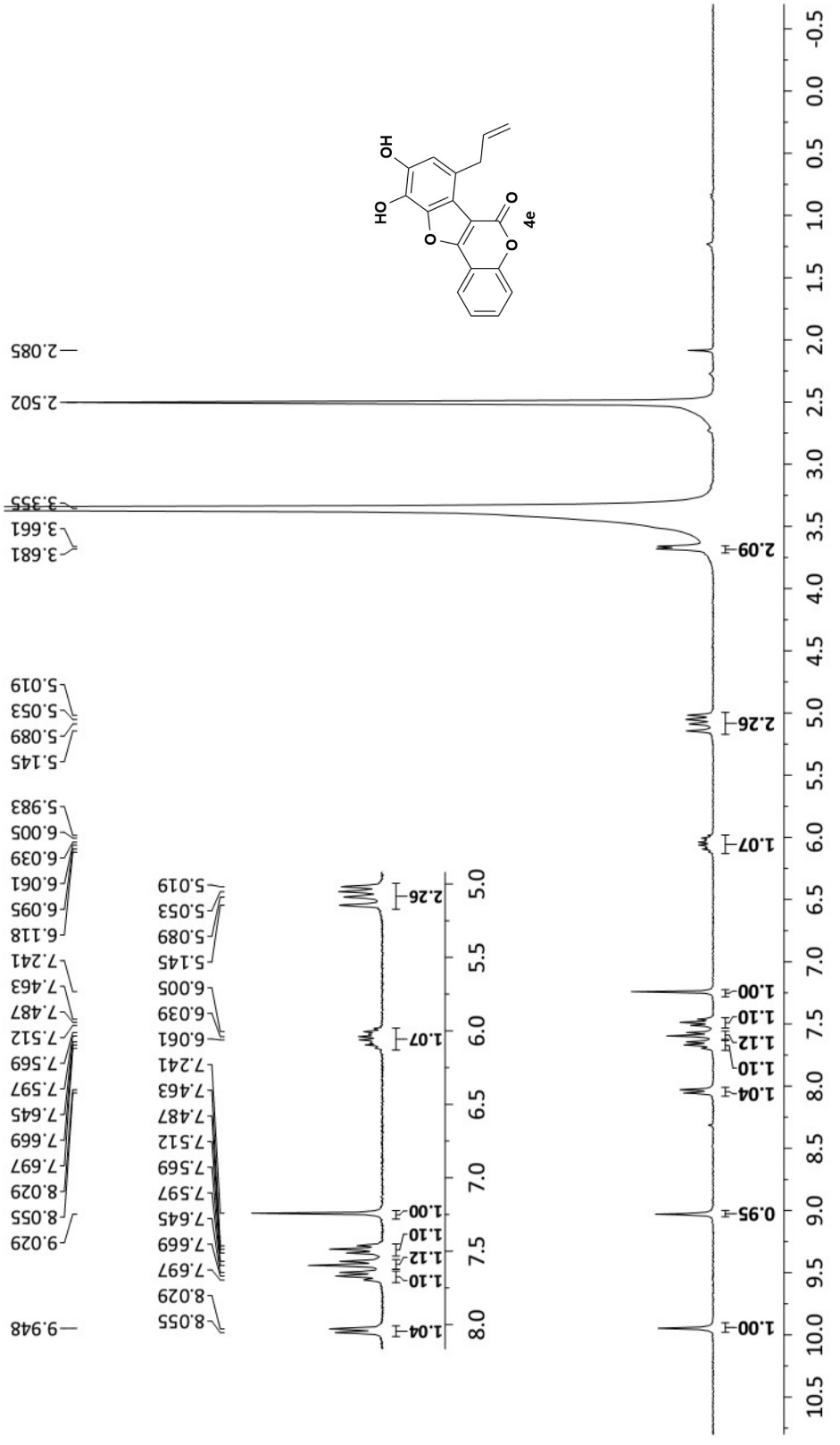


Figure S10: ^{1}H NMR spectrum of **4e** in DMSO-d_6 at 300 MHz

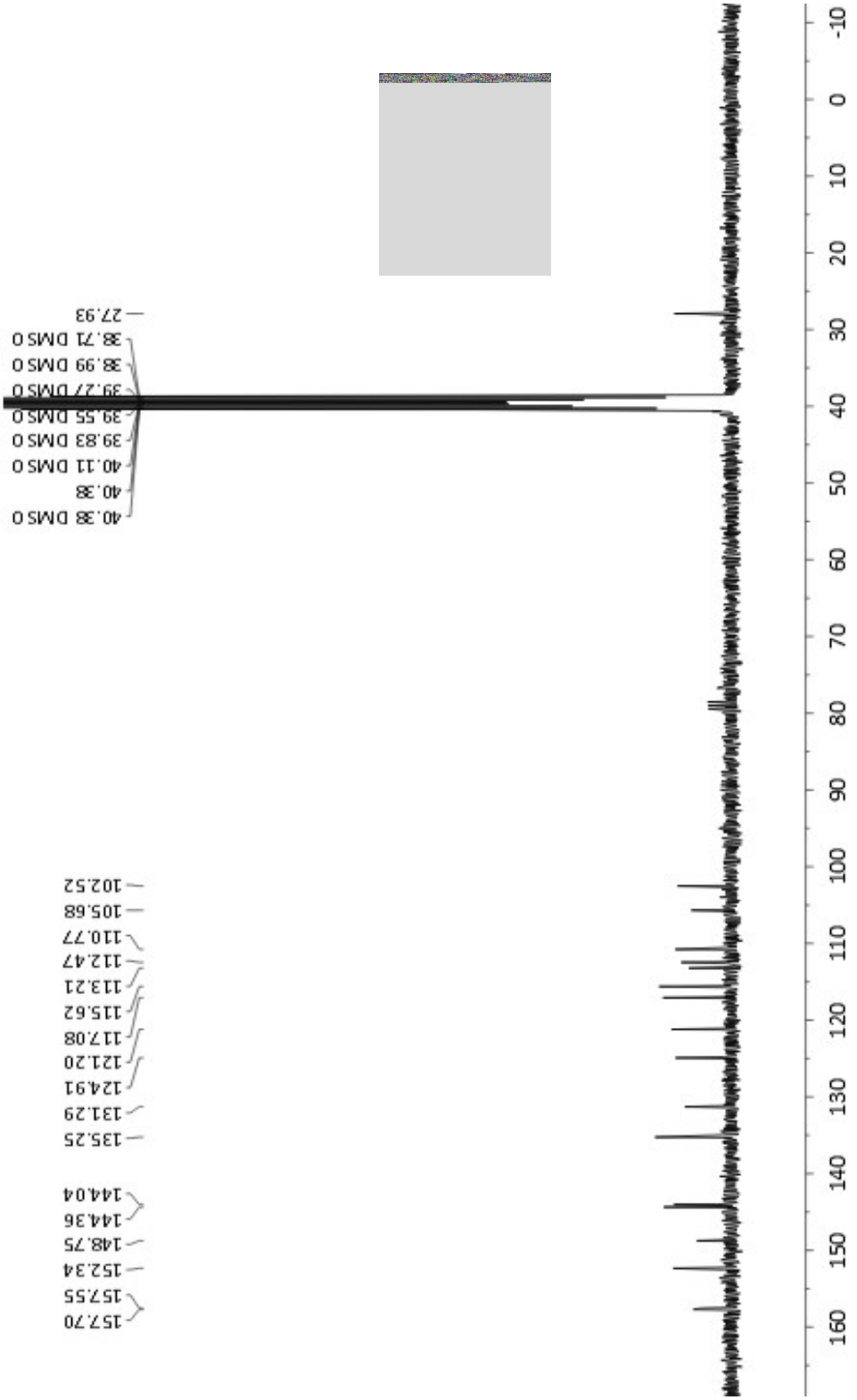


Figure S11: ^{13}C NMR spectrum of **4e** in DMSO-d_6 at 75 MHz

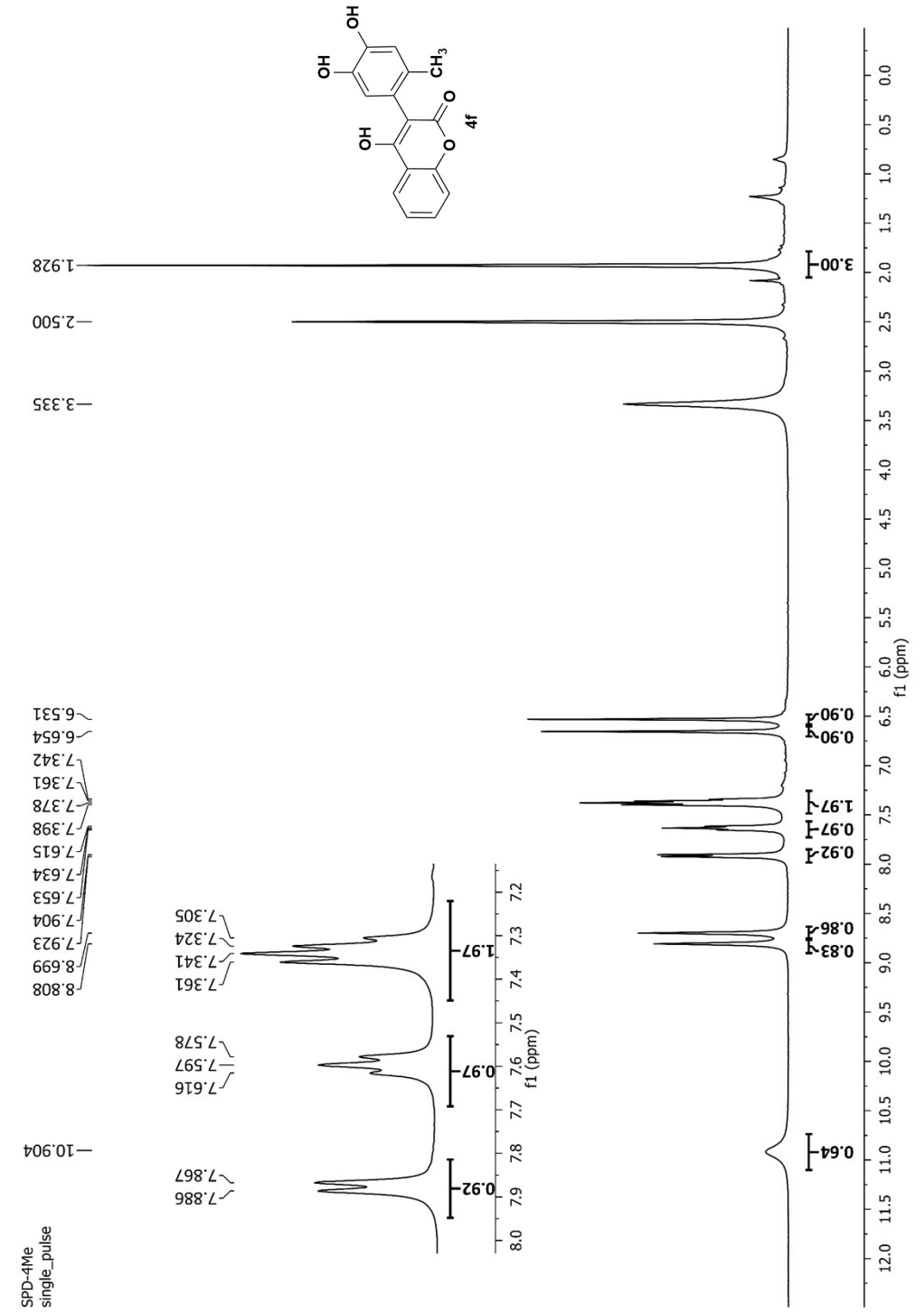


Figure S12: ^{1}H NMR spectrum of **4f** in DMSO-d_6 at 400 MHz

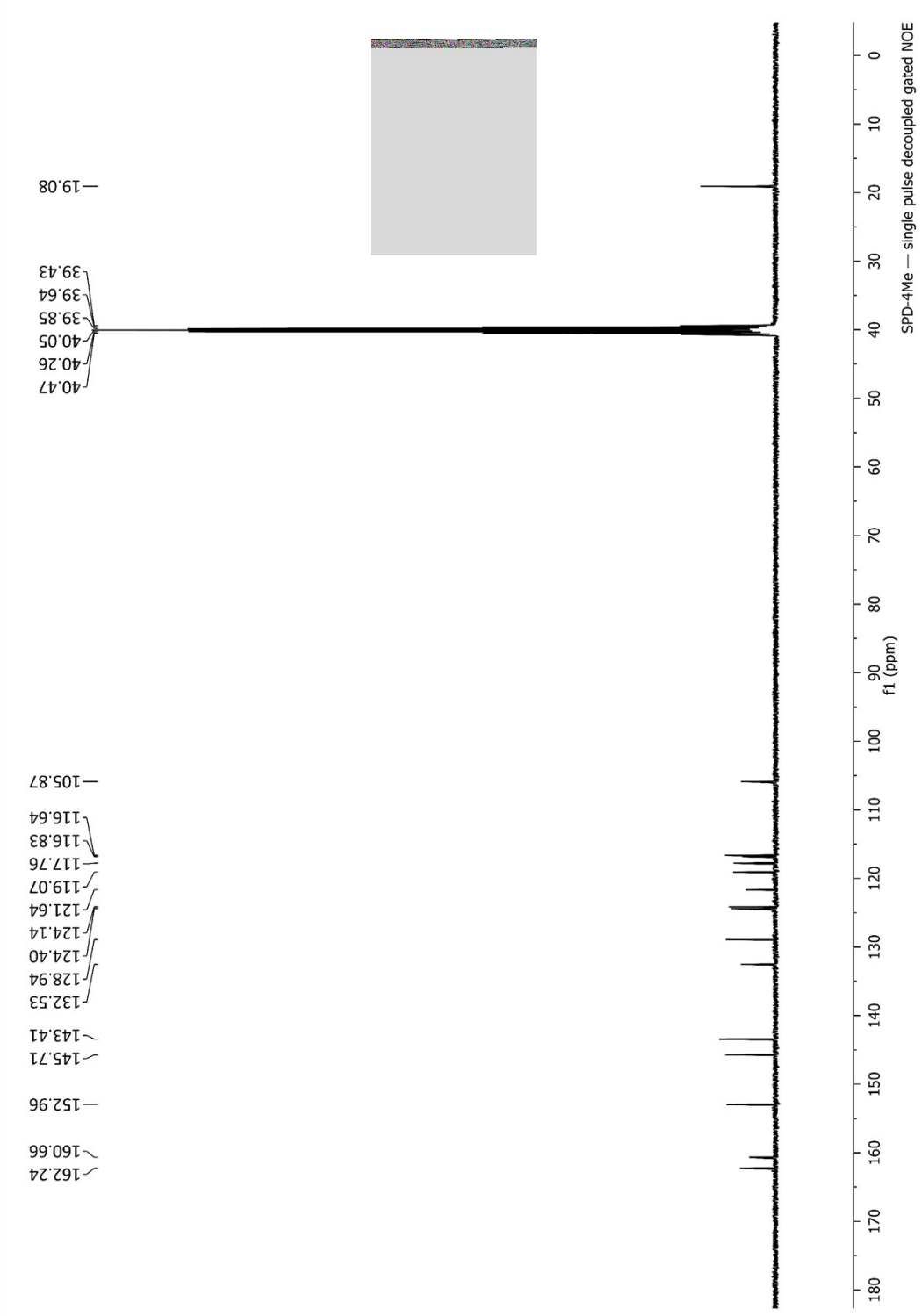


Figure S13: ^{13}C NMR spectrum of **4f** in DMSO-d_6 at 100 MHz

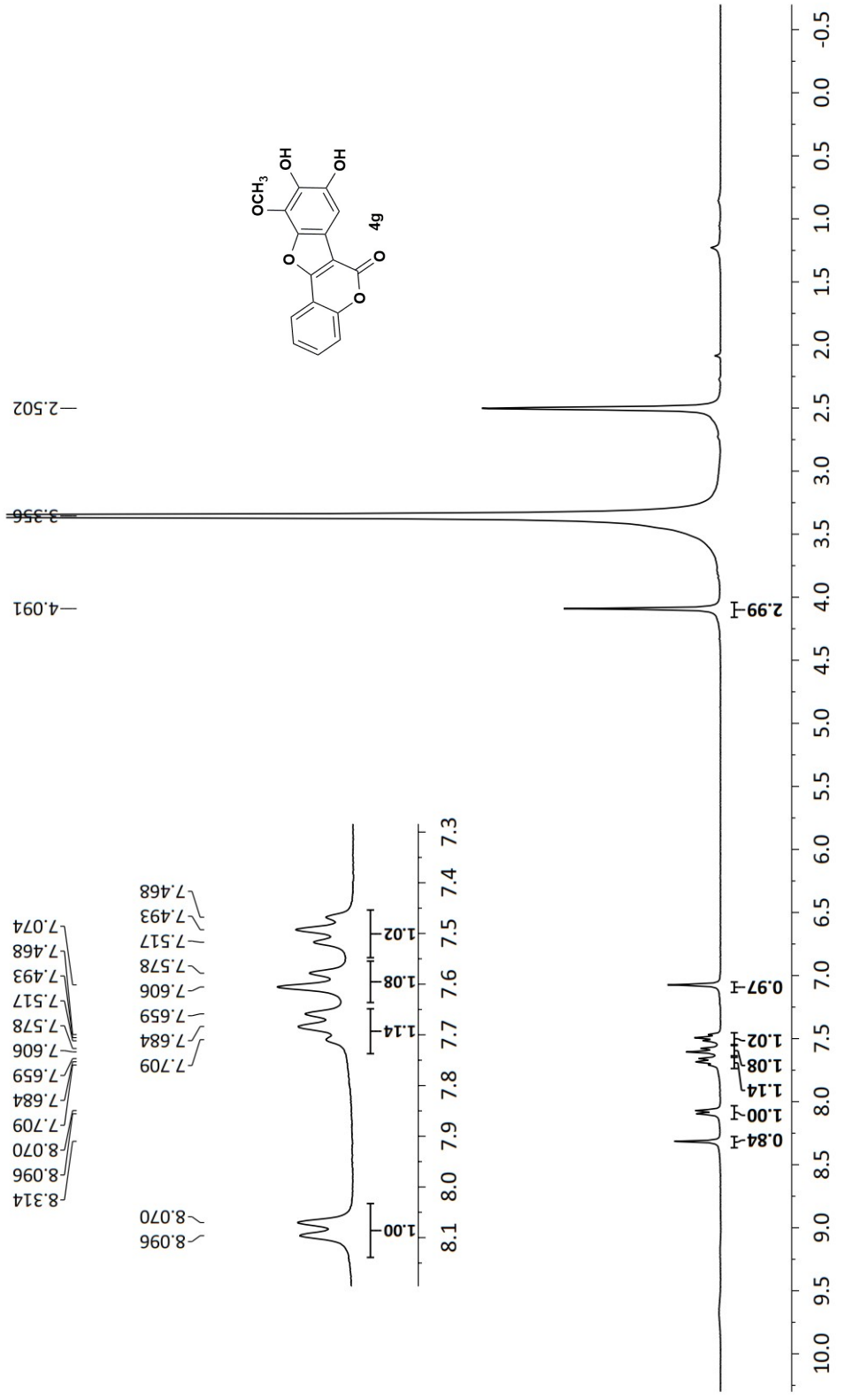


Figure S14: ^{1}H NMR spectrum of **4g** in DMSO-d_6 at 300 MHz

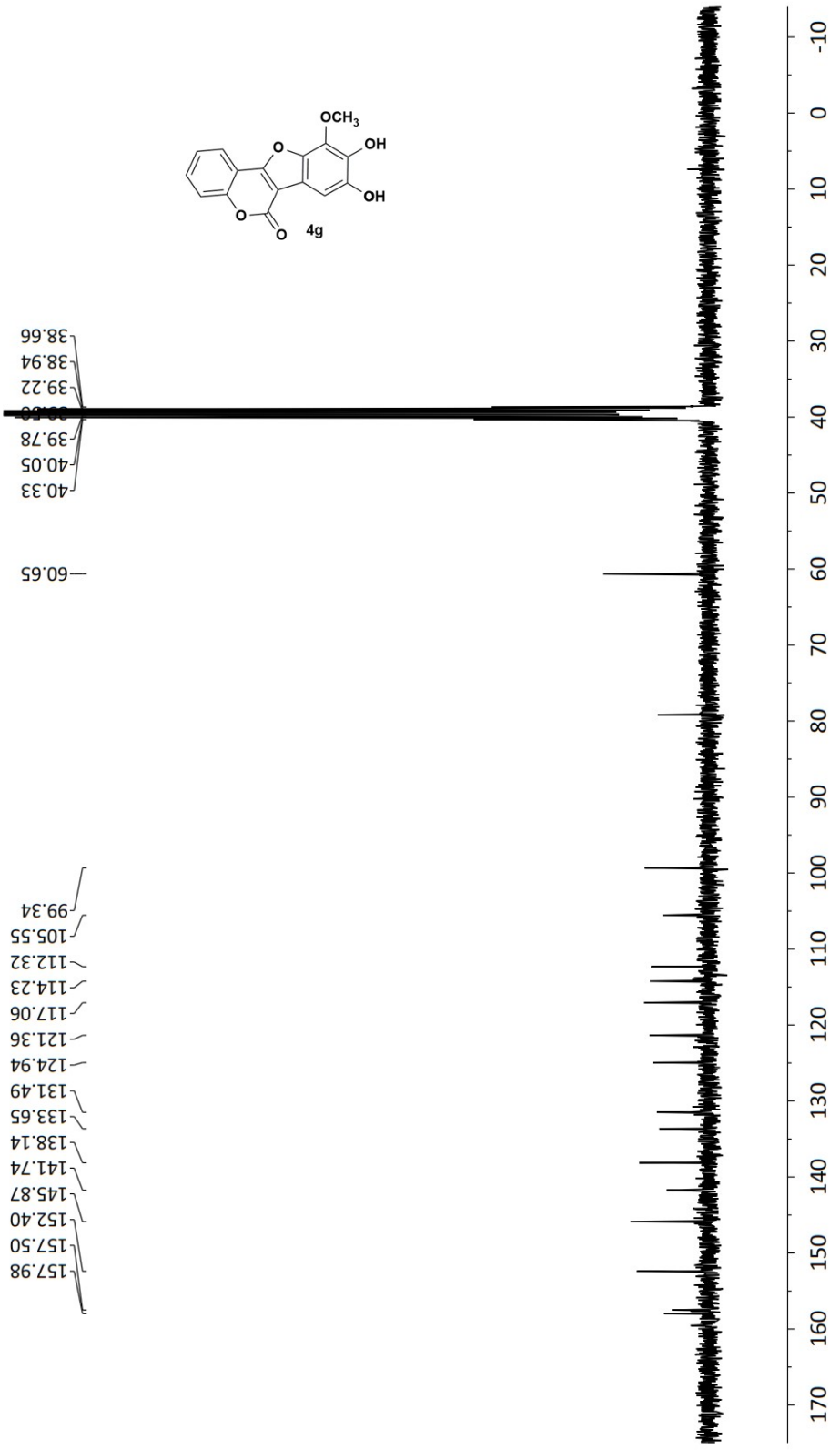


Figure S15: ^{13}C NMR spectrum of **4g** in DMSO-d_6 at 75 MHz

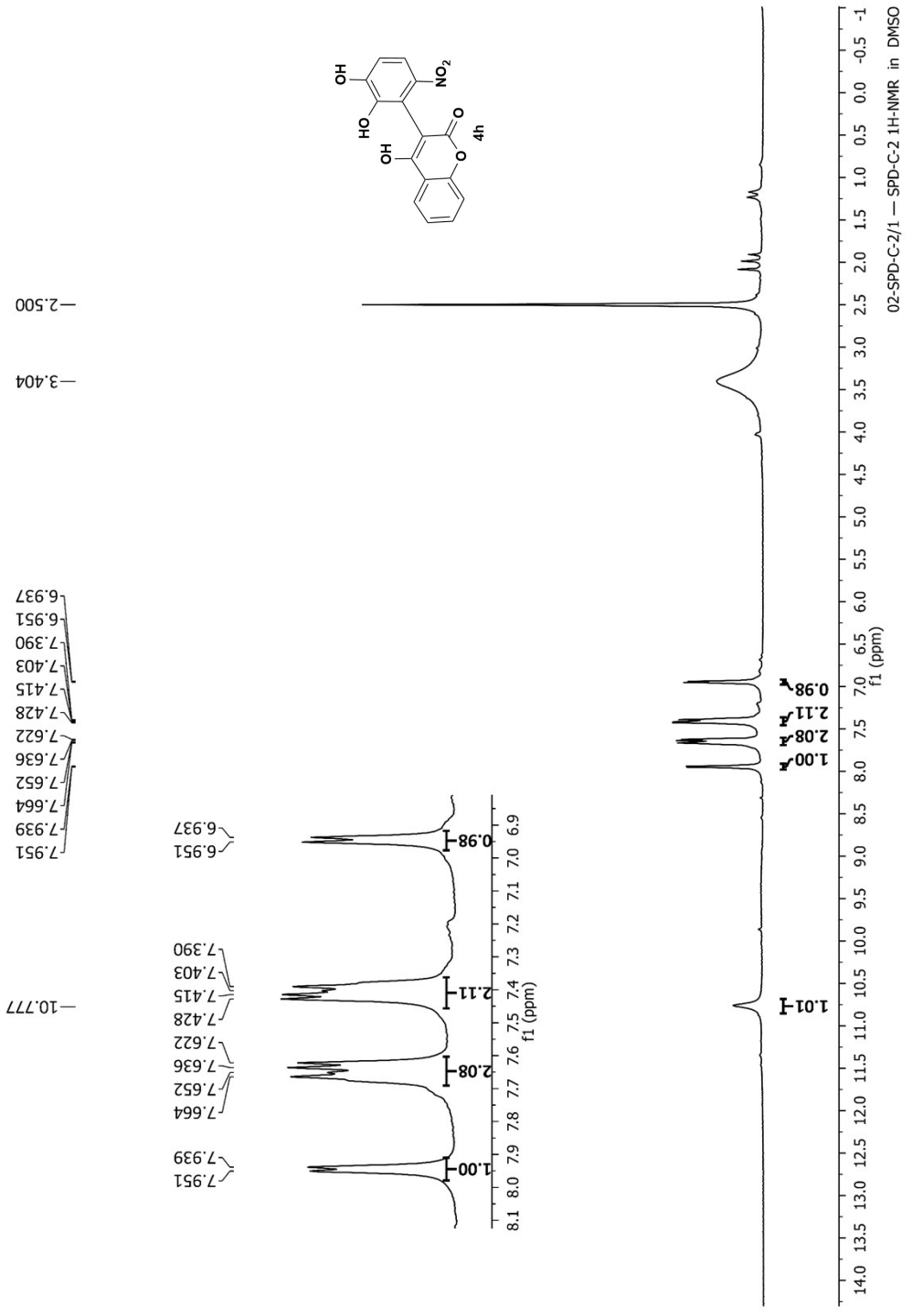


Figure S16: ^{1}H NMR spectrum of **4h** in DMSO-d_6 at 300 MHz

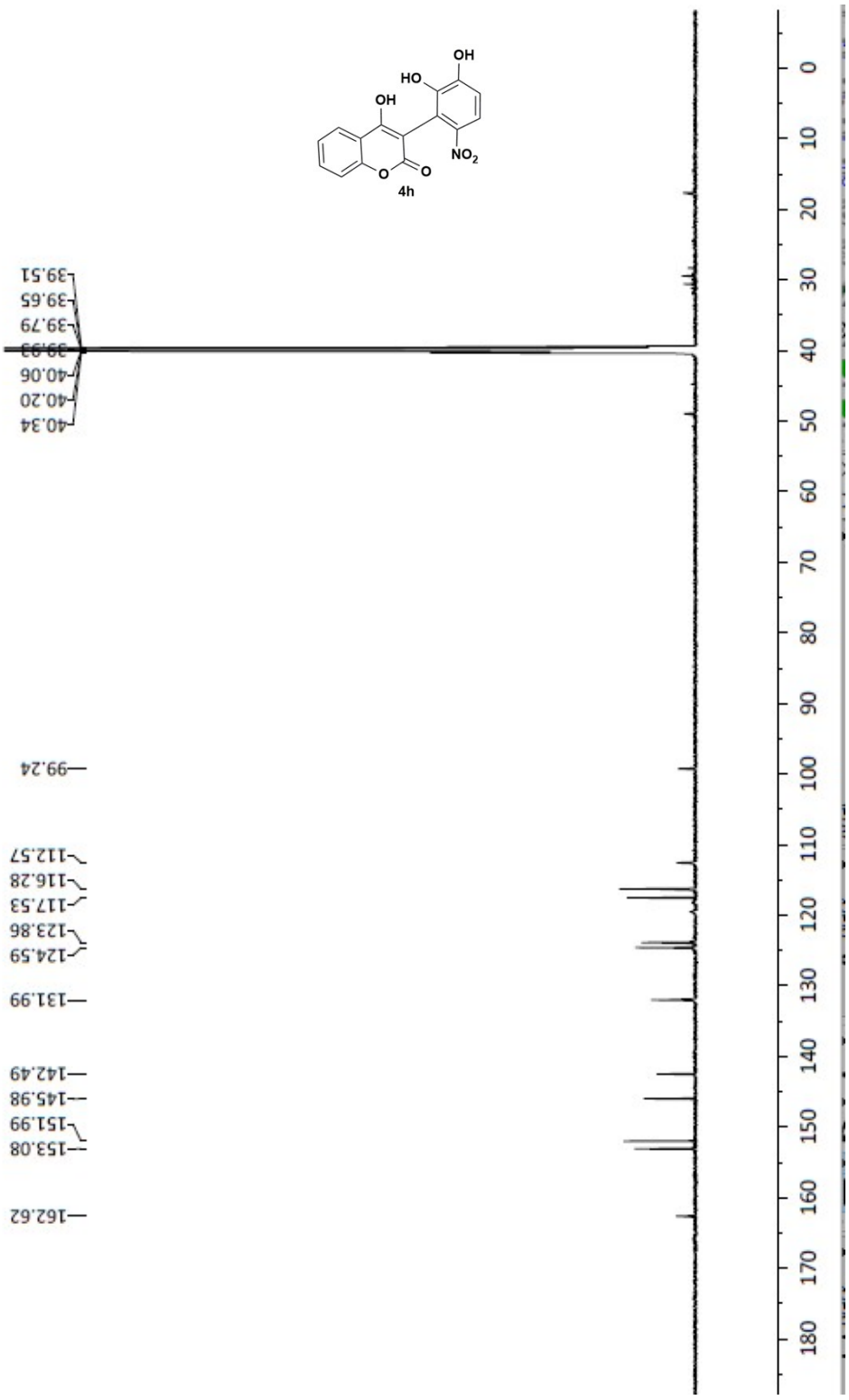


Figure S17: ^{13}C NMR spectrum of **4h** in DMSO-d_6 at 75 MHz

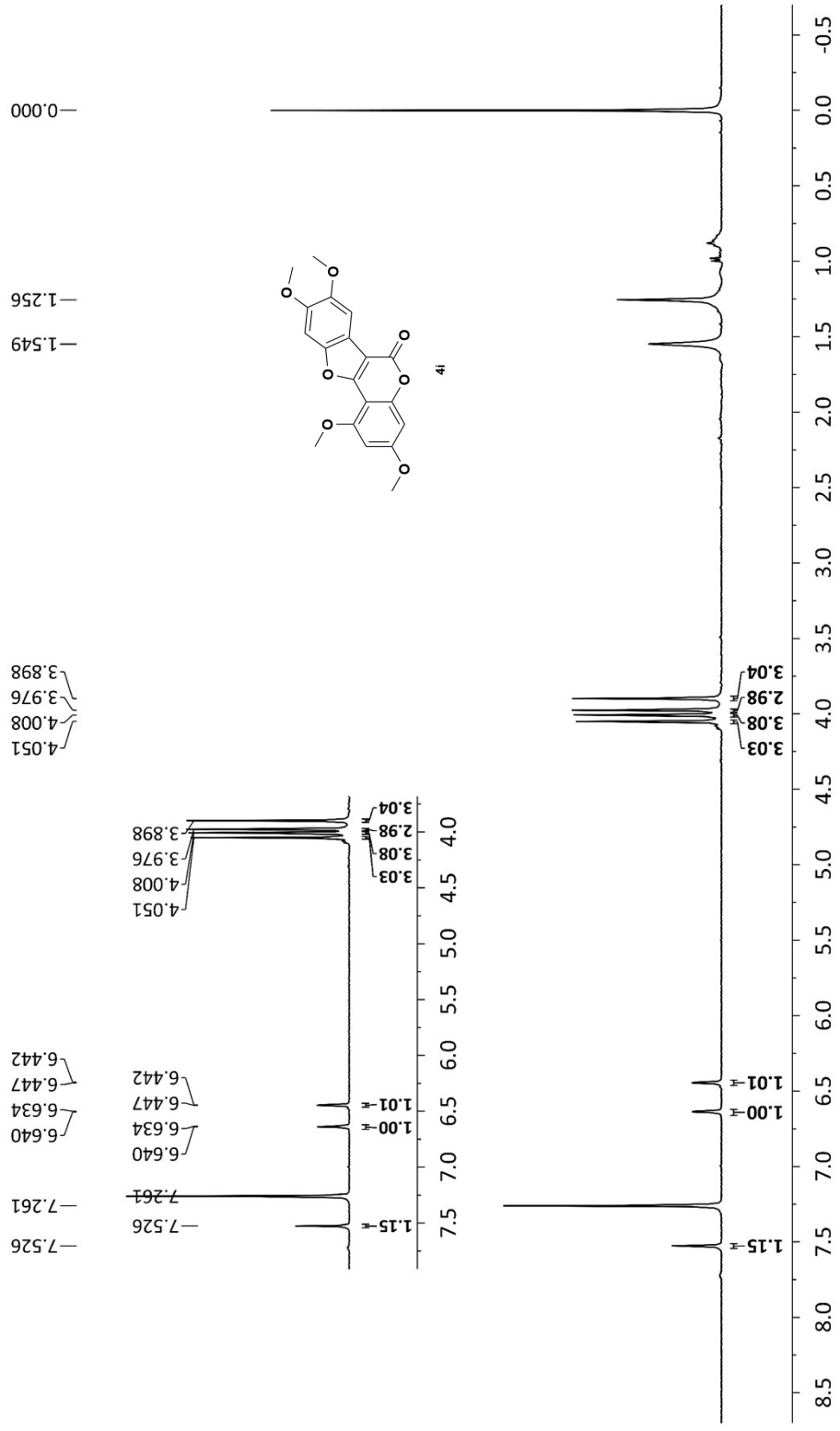


Figure S18: ^{1}H NMR spectrum of **4i** in $CDCl_3$ at 400 MHz

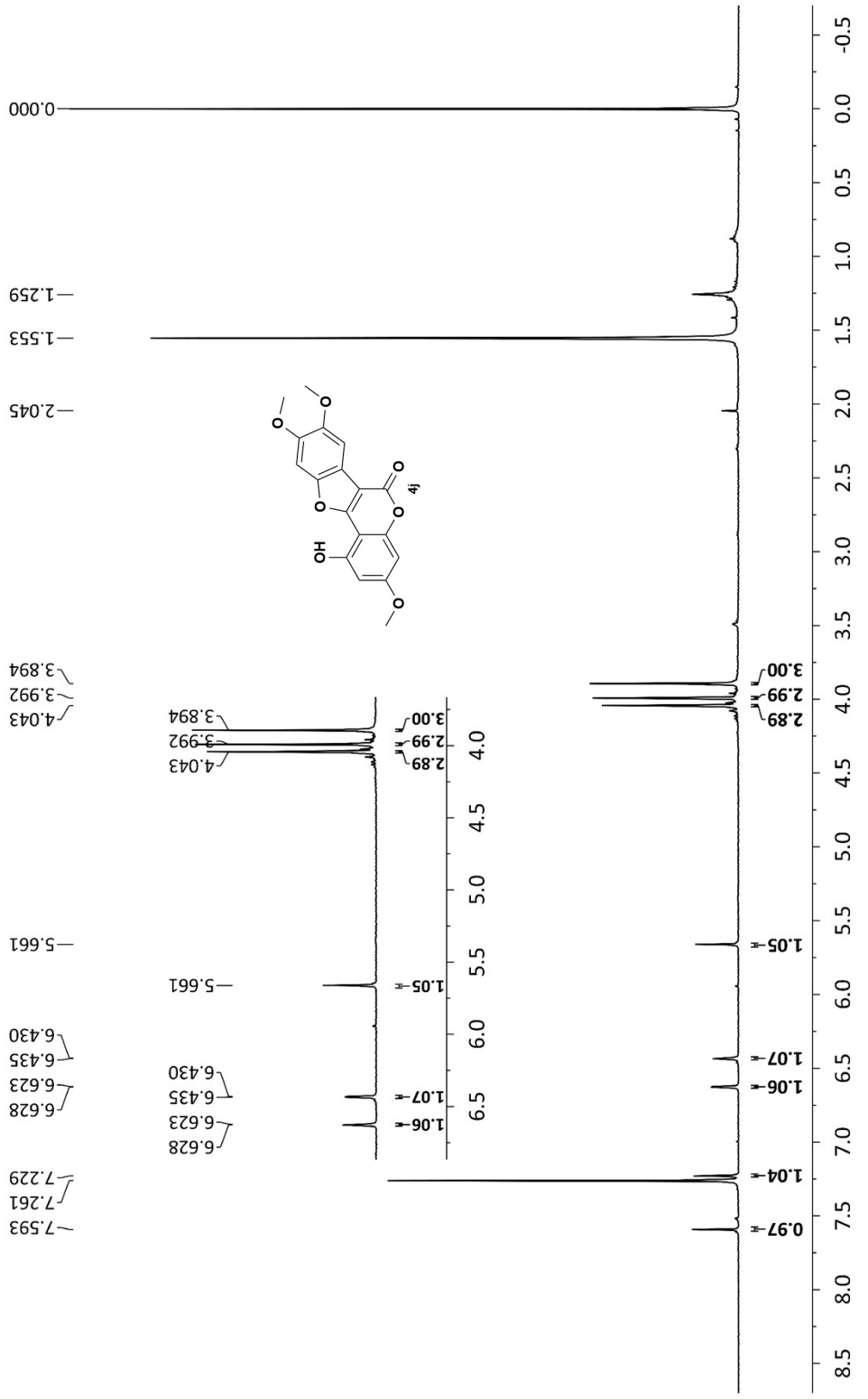


Figure S19: ^{1}H NMR spectrum of **4j** in $CDCl_3$ at 400 MHz

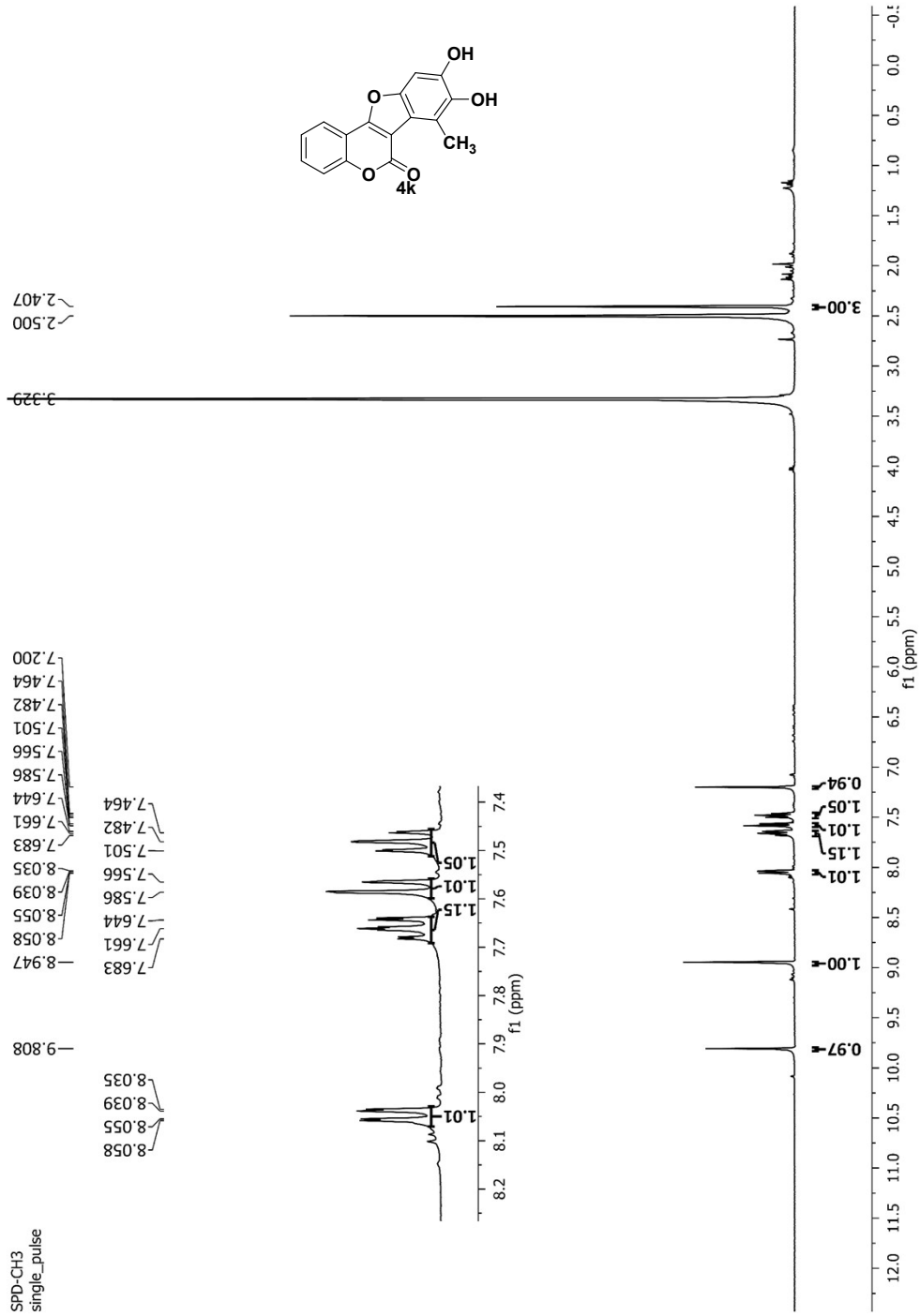


Figure S20: ^{1}H NMR spectrum of **4k** in DMSO-d_6 at 400 MHz

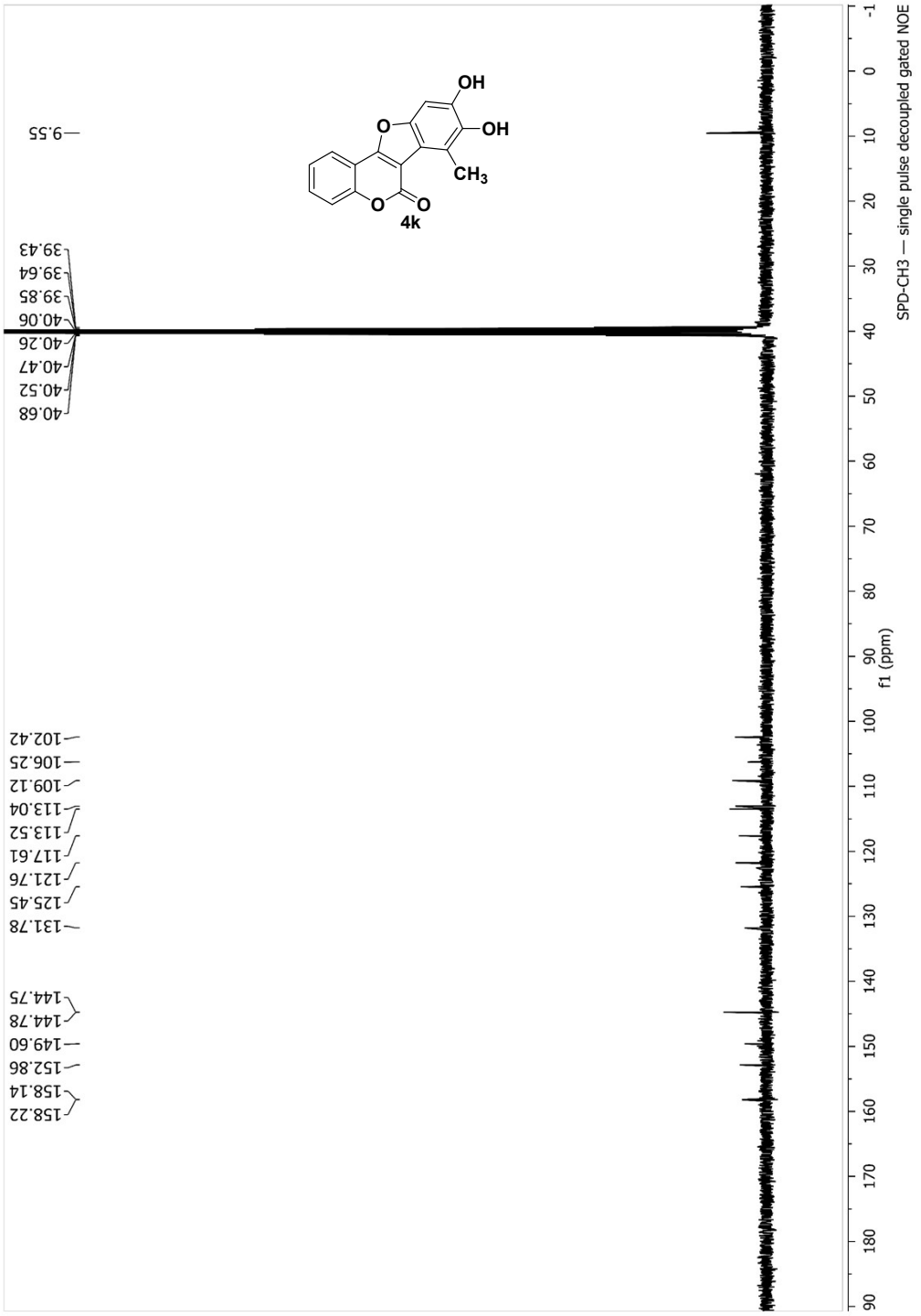


Figure S21: ^{13}C NMR spectrum of **4k** in DMSO-d_6 at 100 MHz

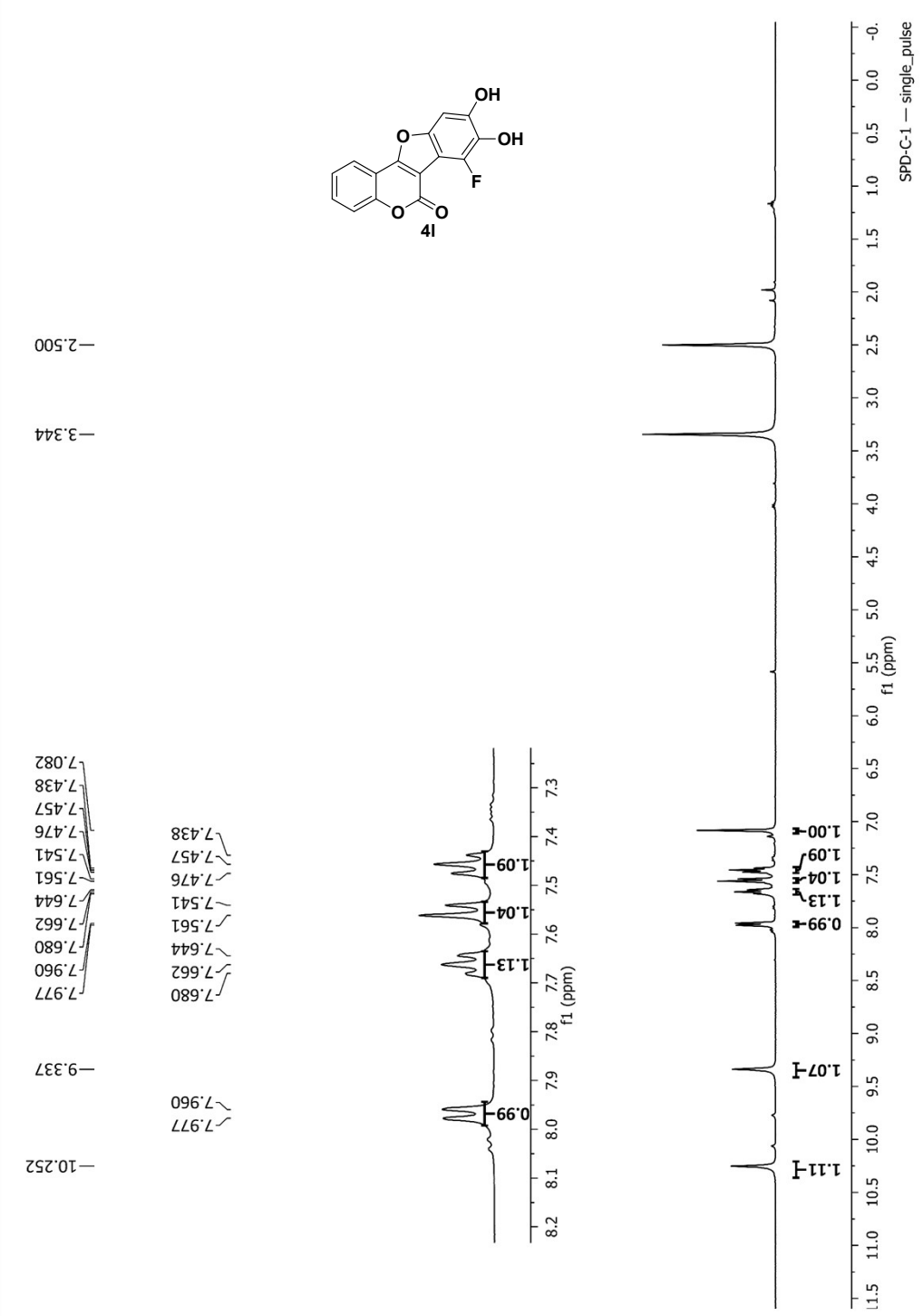


Figure S22: ^{1}H NMR spectrum of **4l** in DMSO-d_6 at 400 MHz

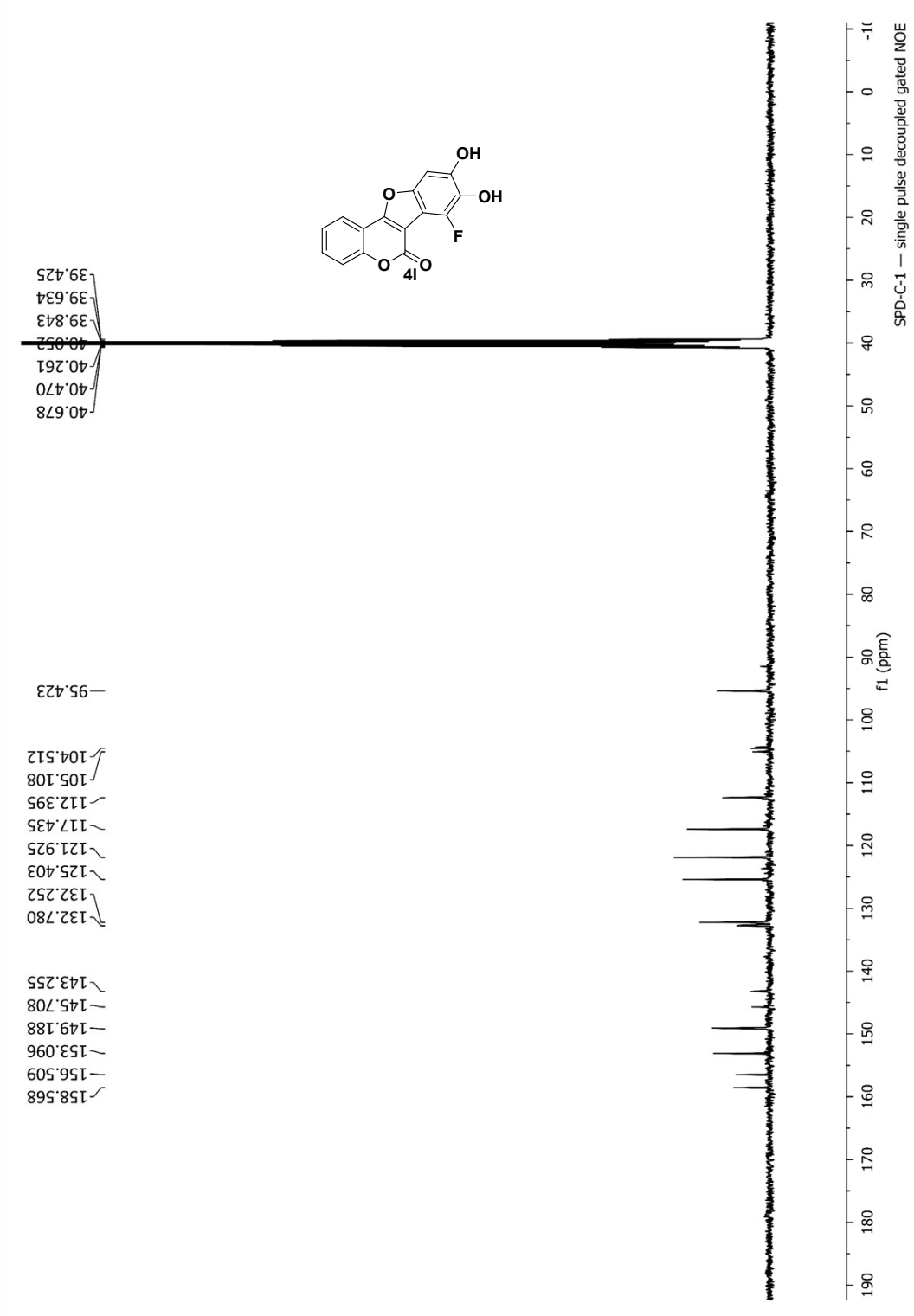


Figure S23: ^{13}C NMR spectrum of **4l** in DMSO-d_6 at 100 MHz

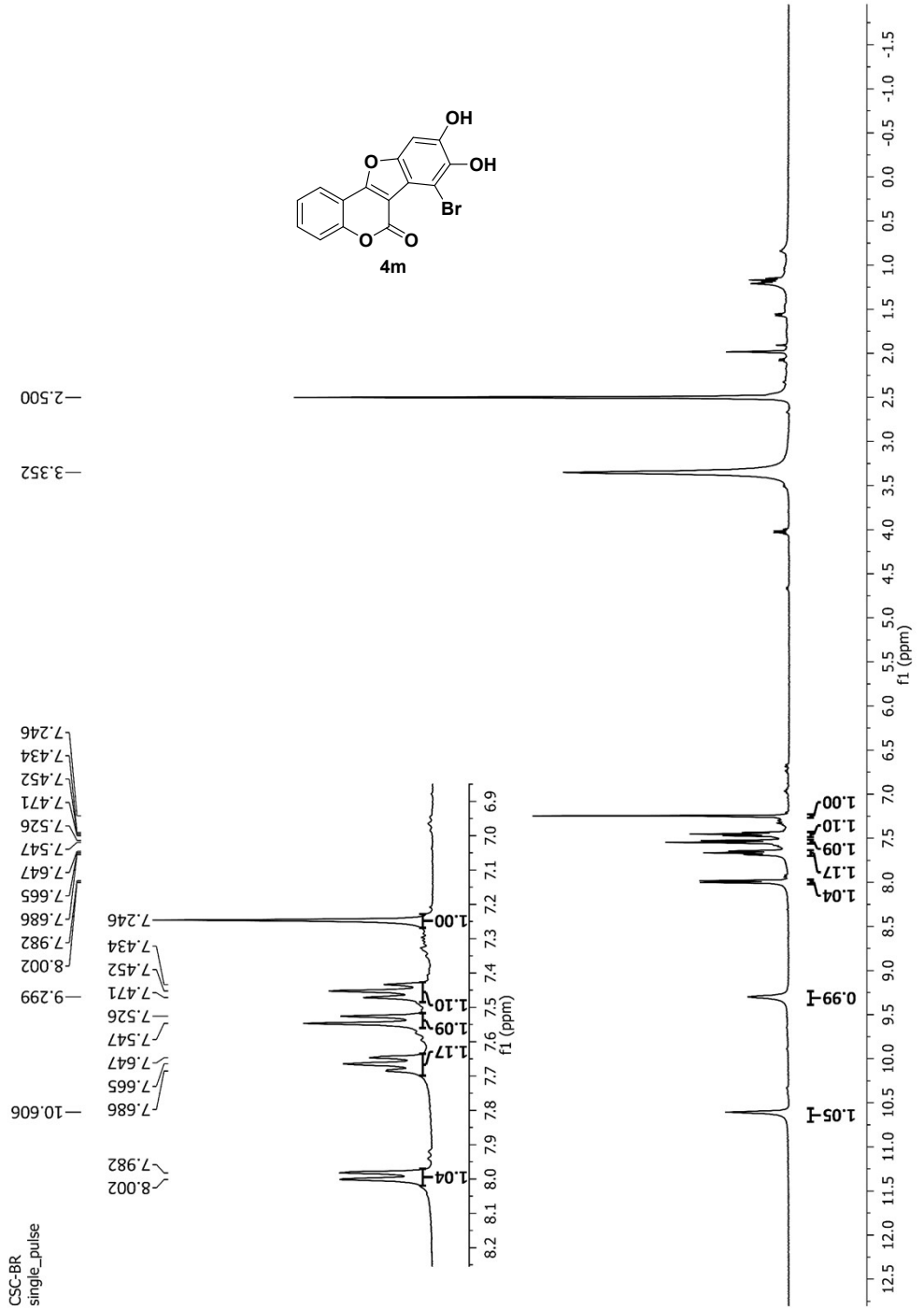


Figure S24: ^{1}H NMR spectrum of **4m** in DMSO-d_6 at 400 MHz

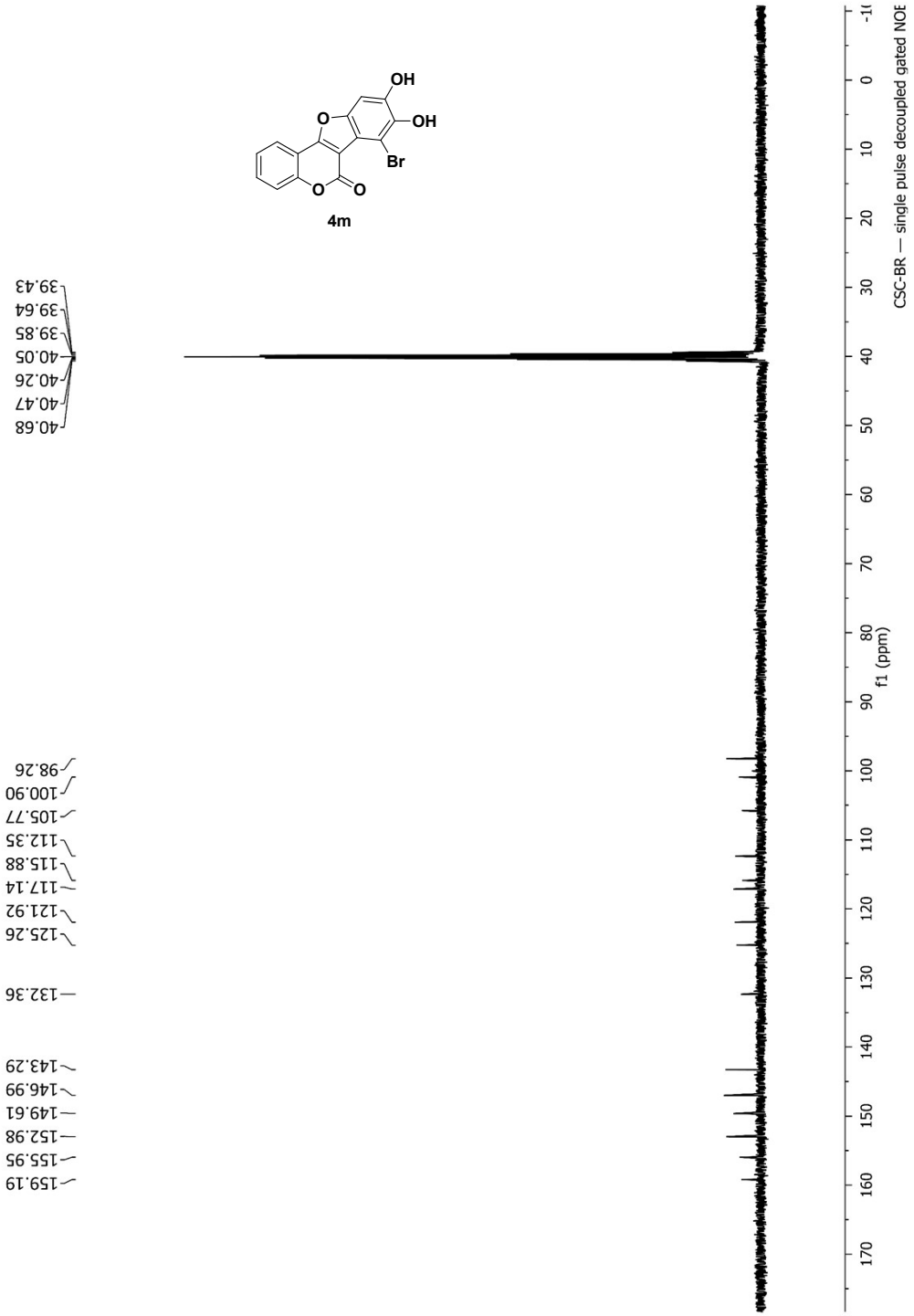


Figure S25: ^{13}C NMR spectrum of **4m** in DMSO-d_6 at 100 MHz

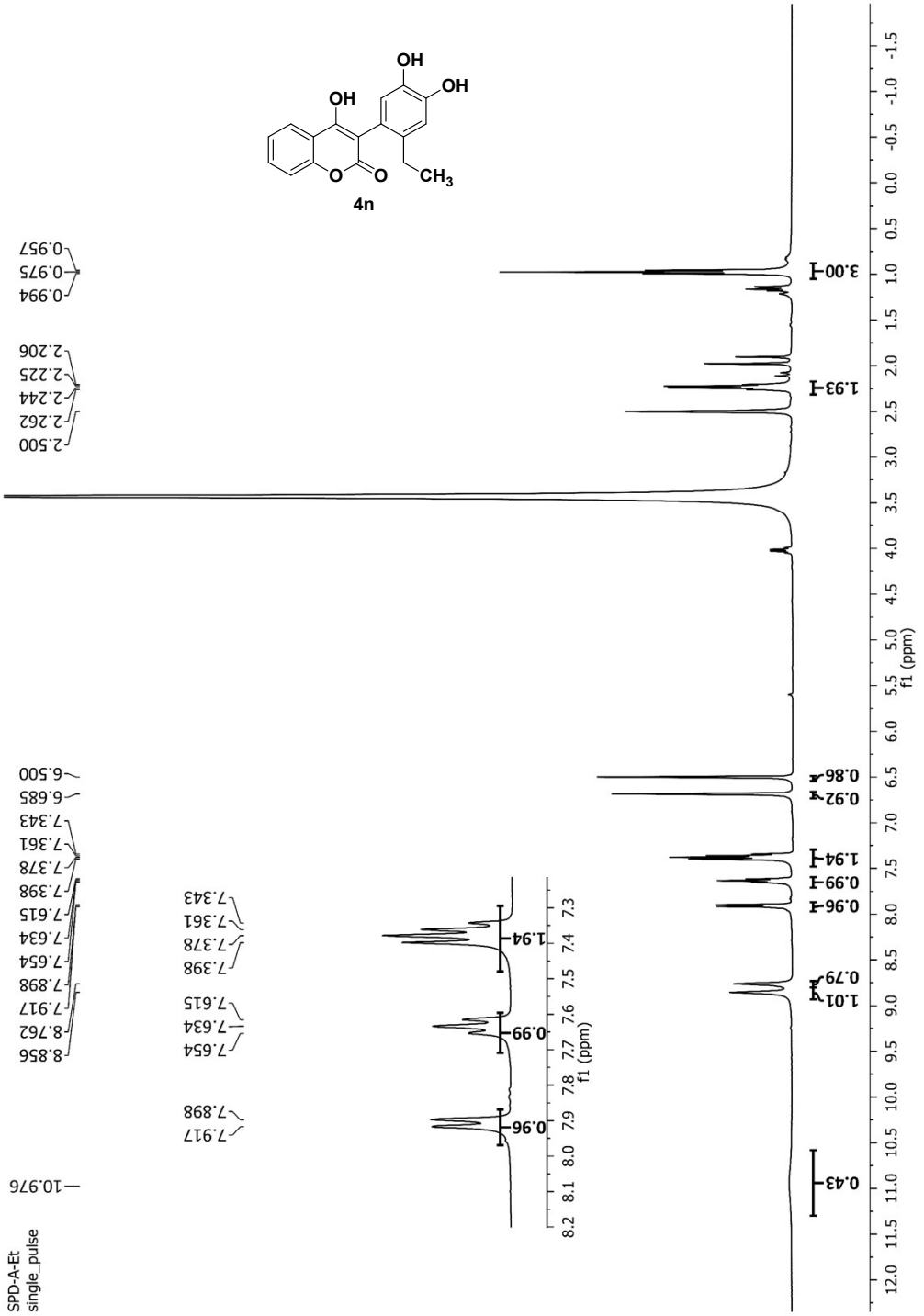


Figure S26: ^{1}H NMR spectrum of **4n** in DMSO-d_6 at 400 MHz

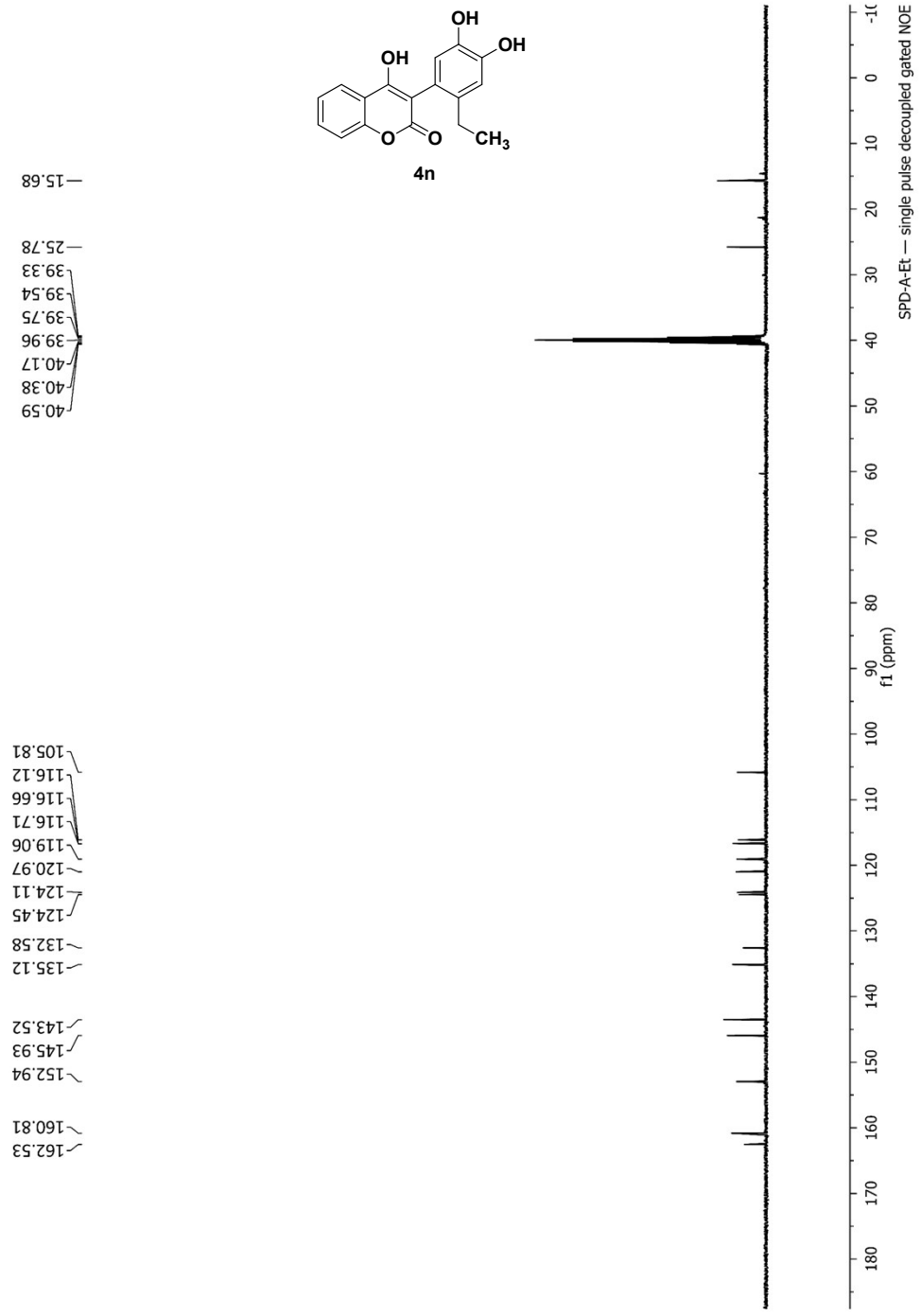
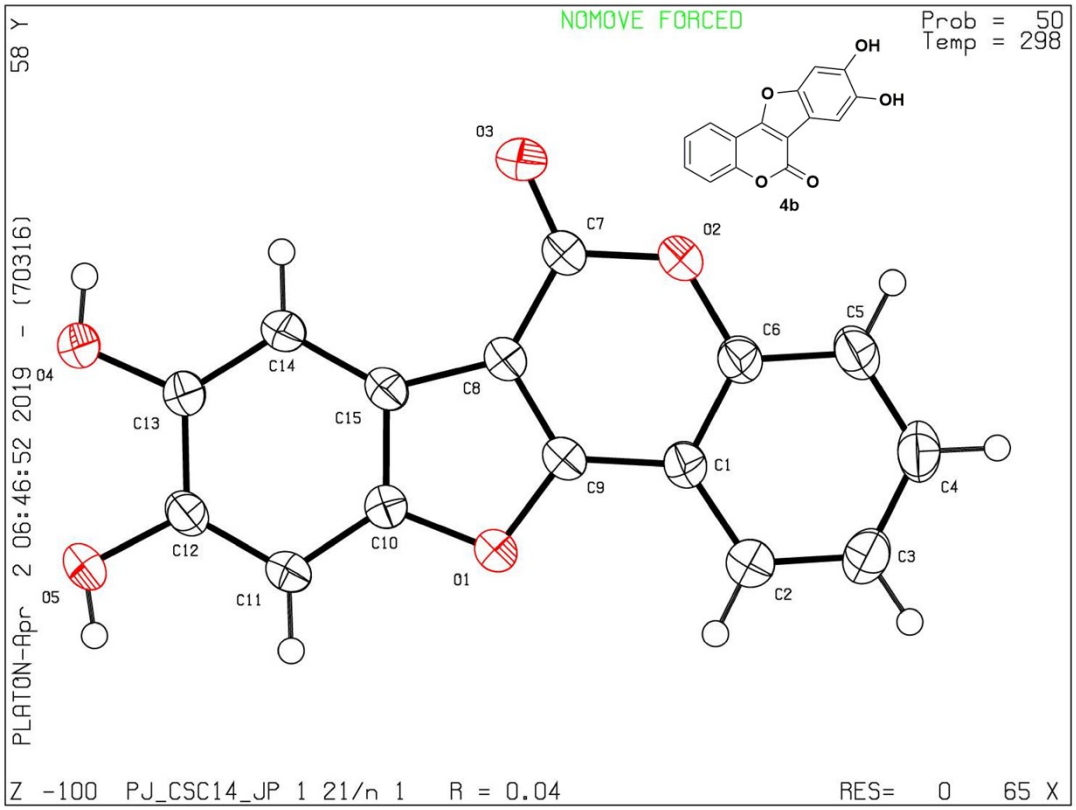


Figure S27: ^{13}C NMR spectrum of **4n** in DMSO-d_6 at 100 MH

Section III: X-ray crystal structure determination for 4b (CCDC 1907579)



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Bond precision:  C-C = 0.0019 A          Wavelength=0.71073

Cell:            a=8.1252(9)     b=6.7892(8)         c=20.442(2)
                 alpha=90        beta=97.880(5)      gamma=90
Temperature:     298 K

                Calculated                Reported
Volume          1117.0(2)                 1117.0(2)
Space group     P 21/n                    P 1 21/n 1
Hall group      -P 2yn                    -P 2ybc (x-
Moiety formula  C15 H8 O5                 C15 H8 O5
Sum formula     C15 H8 O5                 C15 H8 O5
Mr              268.21                    268.23
Dx,g cm-3       1.595                     1.595
Z               4                         4
Mu (mm-1)       0.122                     0.122
F000            552.0                     552.4
F000'           552.35
h,k,lmax        10,8,26                   10,8,26
Nref            2567                      2535
Tmin,Tmax       0.941,0.952               0.874,1.000
Tmin'           0.941

Correction method= # Reported T Limits: Tmin=0.874 Tmax=1.000
AbsCorr = MULTI-SCAN

Data completeness= 0.988          Theta(max)= 27.500

R(reflections)= 0.0417( 2231)     wR2(reflections)= 0.1225( 2535)

S = 1.091                 Npar= 183
```

References:

1. Eicken C., Zippel F., Karentzopoulos K. B., Krebs B. (1998) Biochemical and spectroscopic characterization of catechol oxidase from sweet potatoes (Ipomoea batatas) containing a type-3 dicopper center. *FEBS Letters*. 436, 293-299.