A colorimetric assay adapted to fragment screening revealing aurones and chalcones as new arginase inhibitors.

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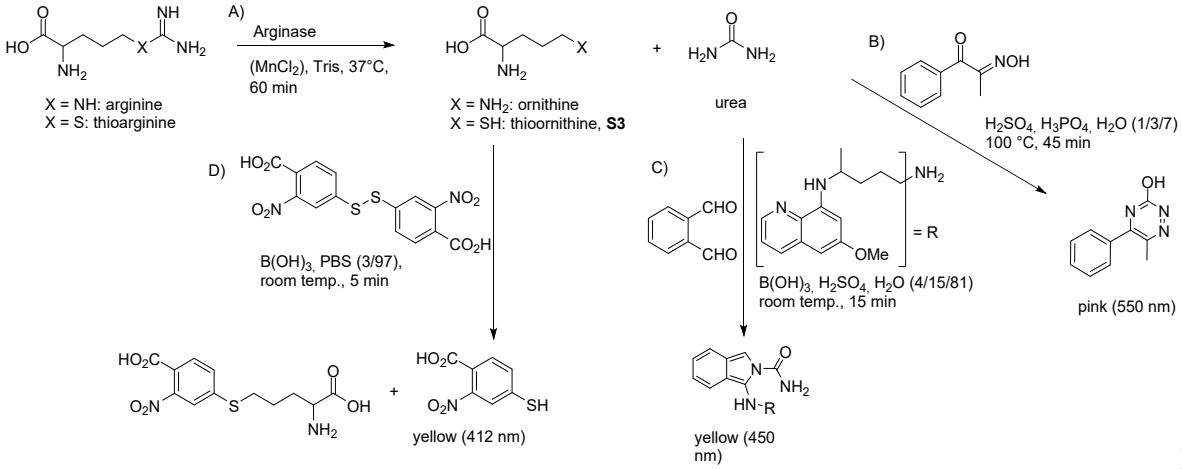


Fig. S1: Arginase assays: A) enzymatic reaction, B) urea revelation by α-isonitrosopropiophenone (INPP) [1,2], C) urea revelation by ortho-phtalaldehyde and primaquine (OPA) [3,4], D) thioornithine revelation by 5,5-dithiobis(2-nitrobenzoic acid) (TOGA) [5]

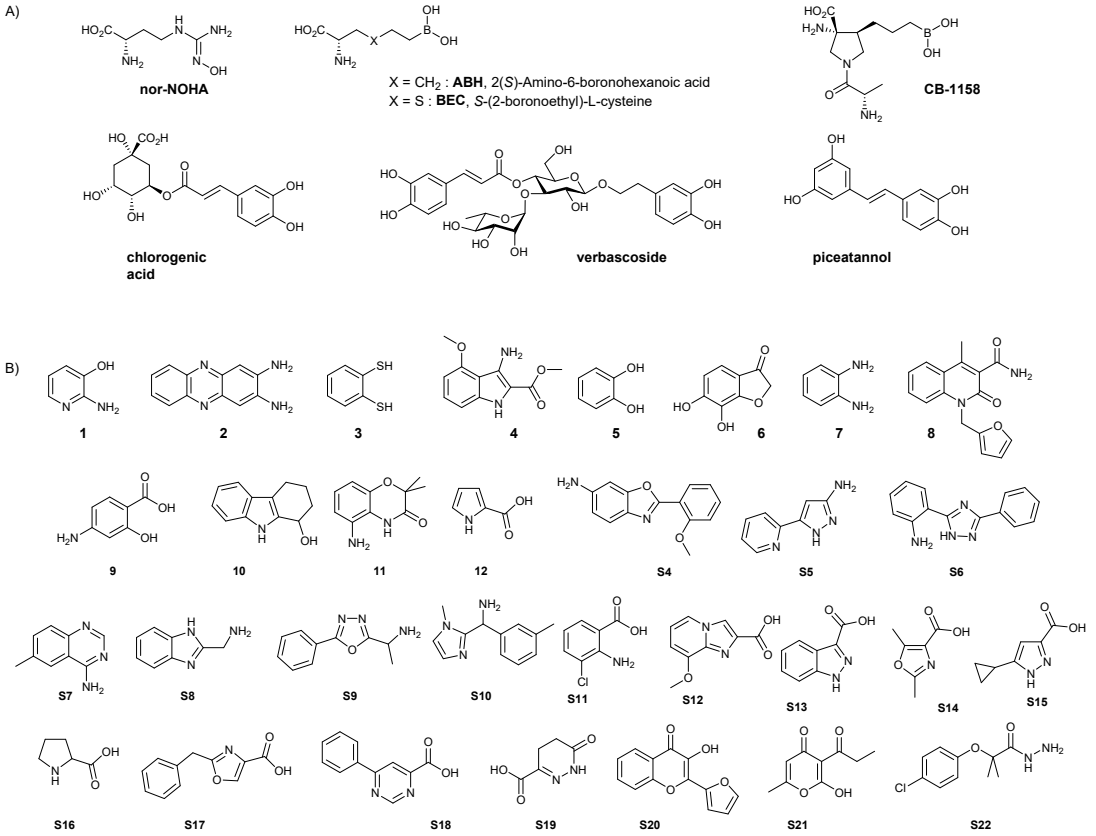


Fig. S2: A) arginase reference inhibitors B) Representative set of compounds from OTAVA® chelator fragment library

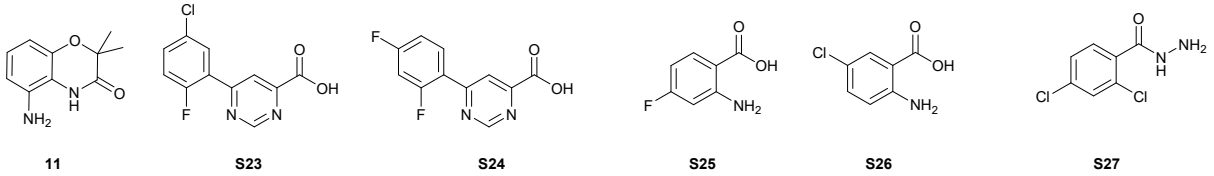


Fig S3: False positive compounds from full screening, **S23-S28**.

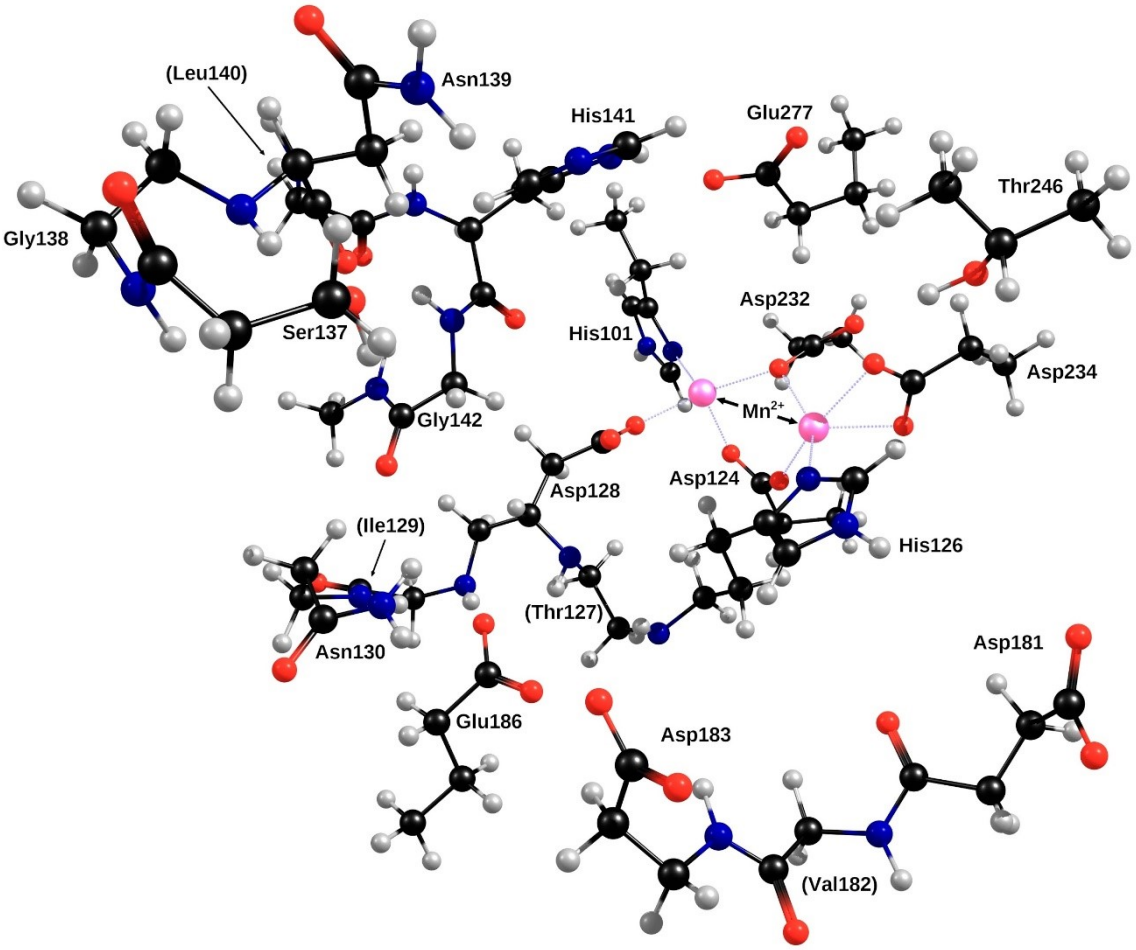


Fig. S4: Representation of the human arginase model used in this study. For clarity reasons, some residues had to be slightly shifted. The side chains of the amino acids in parentheses were not included in the model**.**

Table S1 : Percentage of inhibition (Inhib) and of interference (Inter) of the representative set of compounds from the OTAVA® fragment library.

substrate		*L-arginine*								Thioarginine			
Colorimetric reagent		α-isopropionitosophenone				*Ortho*-phtalaldehyde - primaquine				Ellman reagent			
Supplementation Mn^{2+}		4 mM		0 mM		4 mM		0 mM		4 mM		0 mM	
results		inhib	inter	inhib	inter	inhib	inter	inhib	inter	inhib	inter	inhib	inter
Fragments	1	16	7	14	7	29	0	21	0	67	68	51	52
	2	93	82	92	88	24	36	24	0	100	104	100	63
	3	12	4	52	21	7	0	3	0	100	100	100	100
	4	90	88	90	87	18	7	13	7	38	15	20	0
	5	84	69	20	17	51	8	11	0	100	0	0	3
	6	71	64	80	78	44	0	20	0	100	48	54	13
	7	73	76	75	78	23	13	23	17	47	39	13	14
	8	76	75	86	84	8	0	11	0	29	14	13	7
	9	51	55	57	56	21	18	23	5	30	0	24	0
	10	63	57	69	68	20	3	23	4	19	4	30	0
	11	63	51	65	64	44	13	25	11	7	32	22	2
	12	56	64	64	71	14	7	17	7	31	4	28	8
	S4	62	13	61	38	30	10	28	19	43	2	6	0
	S5	9	0	16	0	12	0	6	0	22	0	9	0
	S6	41	43	39	35	17	0	22	10	33	17	41	10
	S7	2	0	0	0	0	5	4	0	26	6	15	10
	S8	22	0	24	0	14	2	14	1	31	20	29	0
	S9	13	0	10	0	0	0	9	0	29	7	25	2
	S10	23	0	9	0	23	0	20	0	36	0	25	0
	S11	14	4	24	21	25	15	35	30	27	0	22	0
	S12	14	26	23	28	8	0	7	0	37	42	34	22
	S13	7	0	13	0	0	0	16	0	22	0	20	5
	S14	13	5	20	0	0	0	3	1	34	3	25	5
	S15	6	3	8	2	10	0	22	0	28	14	34	15
	S16	13	0	9	0	4	0	9	0	37	6	29	4
	S17	28	0	24	0	14	17	19	0	55	9	37	15
	S18	0	12	0	3	5	0	13	0	16	4	12	5
	S19	9	4	3	2	11	0	18	0	32	7	27	8
	S20	37	48	49	50	17	27	7	0	53	18	35	0
	S21	12	0	10	0	2	0	9	0	23	0	15	0
	S22	1	0	8	0	39	0	38	19	21	0	28	2
polyphenolic arginase inhibitors	Verb	98	76	60	50	85	22	11	3	16	16	19	0
	Pic	98	60	82	89	92	23	22	15	100	73	9	24
	CGA	86	81	37	46	48	4	9	0	77	0	17	0
aminoacid based arginase inhibitors	Nor	100	4	100	5	100	0	99	0	100	2	93	0
	BEC	100	0	98	2	96	0	96	0	100	11	97	0
	ABH	100	2	100	0	97	0	100	4	99	13	100	0

An heatmap from red (100%) to white (0%) has been applied.

Arginase assays

Reagents and solutions

Bovine liver arginase I was purchased from MP Biomedicals (1 international unit convert 1.0 µmole of L-arginine to ornithine and urea per minute at pH 9.5 and 37 °C). Stock solutions at a final concentration of 12.5 mg/mL (b-ARGI) were prepared in 50 mM Tris-HCl buffer at pH 7.5, containing 100 mM of NaCl, 0.1% of bovine serum albumin and 20% v/v of glycerol. Reagents were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France) apart 2-amino-5-mercaptopentanoic acid (thioornithine) which is was obtained as previously described.[6] Solvent used were of analytical grade and Ultrapurified water (resistivity 18 MΩ/cm) was obtained using an ELGA water purification system (ELGA LabWater). Spectrophotometric measurements were made on a Synergy HT BioTeck apparatus.

Chemical library

Chelator Fragment Library from OTAVA® chemicals, Vaughan, Ontario, Canada. compounds containing metal chelator functions with molecular weight under 300 g/mol, calculated partition coefficient under 3, calculated water solubility over -5, polar surface area under 80 Å, number of rotatable bonds under 3, number of hydrogen bond donor under 3, number of hydrogen bond acceptor under 4, number of rings over 0 and number of halogens under 4. The representative set is shown in **Fig. S2**.

L-arginine or thioarginine hydrolysis

Arginase 1 stock solution (1 U.I. per µL) was diluted at 1/250 in a Tris-HCl buffer (50 mM, pH 7.5) containing 0.1% of bovine serum albumin (TBSA). Solutions of enzymatic substrates were prepared in ultra-pure water at pH 9.7 and contain, respectively, 50 mM of L-arginine (L-ARG) and 27 mM of thioarginine (THIO). Manganese supplementation containing 10 mM $MnCl_2$ (THM) was prepared in Tris-HCl buffer (50 mM, pH 7.5). Solutions of urea and thioarginine were prepared in TBSA and contain respectively, 15 mM of urea (urea sol) and 7.1 mM of thioornithine (thioOrn sol). Solutions of the compounds to be tested were prepared in DMSO at an initial concentration of 3.5 mM. To each well of a 96-well microtiter plate was added: 10 µL of TBSA with arginase or 10 µL of TBSA (blank), or 10 µL of urea sol / thioOrn sol (measurement of enzymatic product degradation); 30 µL of THM (manganese supplementation) or 30 µL of Tris-HCl buffer (without manganese supplementation); 10 µL of a solution of the inhibitor or DMSO (as 100% activity of arginase); 20 µL of the substrate solution. The microplate was covered with a plastic sealing film and incubated for 60 min at 37 °C in a dry bath.

Revelation by α-isonitrosopropiophenone, adapted from [7]

120 µL of $H_2SO_4/H_3PO_4/H_2O$ (1:3:7) were added to quench the enzymatic reaction. Then, 10 µL of α-isonitrosopropiophenone (5% in absolute EtOH) was added, the microplate was covered with an aluminum sealing film and heated at 100°C for 45 min in an oven. After return to room temperature and centrifugation, the microplate was shaken for 2 min and the absorbance was read at 550 nm.

Revelation by o-phtalaldehyde and primaquine, adapted from [3]

The colorimetric reagent was prepared extemporaneously by a 1:1 mixture of a solution of *o*-phtalaldehyde 10 mM in 15% sulfuric acid with solution of primaquine diphosphate 4 mM

with boric acid 130 mM in 15% sulfuric acid. 60 µL of this mixture was added to the microplate. After 15 min at room temperature, the microplate was shaken, and the absorbance was read at 450 nm.

Revelation by 5,5-dithiobis(2-nitrobenzoic acid), adapted from [8]

30 µL of an aqueous solution of boric acid 100 mM were added to quench the enzymatic reaction. Immediately after, 30 µL of a solution of 5,5-dithiobis(2-nitrobenzoic acid) 3 mM in PBS at pH 7.8 was added. The microplate was shaken for 1 min and the absorbance was read at 412 nm.

All experiments were repeated independently three times. The absorbances were normalized with the blank and the percent inhibition is relative to the 100% activity of arginase. For IC_{50} determination, compounds were tested at final concentrations of 1.0, 3.0, 10, 30, 100, 300, 1000 and 3000 µM. The normalized absorbances were used to generate a concentration-response curve and the IC_{50} values were estimated by nonlinear sigmoidal curve-fitting by using Prism (GraphPad Software, version 7).

Organic synthesis

Generality

Starting material reagents and analytical grade solvents were purchased from Sigma Aldrich and TCI Chemicals. All reactions were routinely checked by TLC using Merck Kieselgel 60 F254 aluminium plates and visualised by UV light. IR spectra were performed on a Spectrum 65 PerkinElmer with UATR and principal absorption values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d6 using a Bruker AC 400 MHz (^{1}H) or 75 MHz (^{13}C) instruments. Chemical shifts are given in parts per million (ppm) and referenced to residual solvent pics and coupling constants J is given in Hertz (Hz). ESI – MS analyses were carried out at the Service Commun d'Analyse, ICMR – UMR CNRS 6229 – 51 100 Reims.

Thioornithine synthesis, as previously described and in accordance with [9]

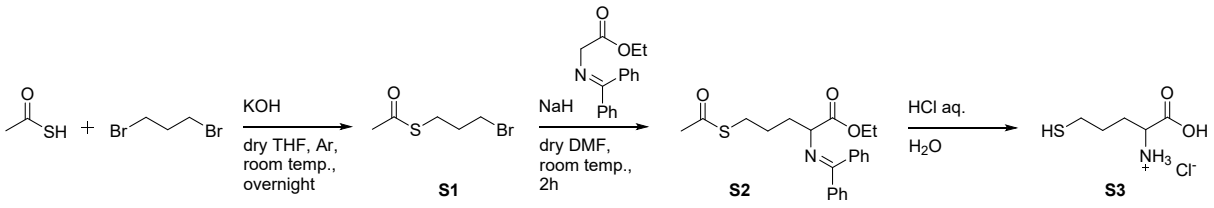


Fig. S5: synthesis of 1-carboxy-4-mercaptobutan-1-aminium chloride, thioornithine hydrochloride [9]

S-(3-bromopropyl)ethanethioate, **S1**

To a suspension of 1,3-dibromopropane (1.1 equiv., 5.9 mL, 5.79 10^{-2} mol) and potassium hydroxide (1 equiv., 2.95 g, 5.26 10^{-2} mol) in dry THF was added thioacetic acid (1 equiv., 3.73 mL, 5.26 10^{-2} mol) and the mixture was stirred at room temp. under argon overnight. The mixture was then concentrated under vacuum and the title product was purified by two successive fraction distillation (120-130 °C, 30 mmHg) as a yellow liquid (2.7 g, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.05 (t, J = 6.7 Hz, 2H), 2.27 (s, 3H), 2.93 (t, J = 6.7 Hz, 2H), 3.38 (t, J = 6.7 Hz, 2H), in accordance with [9].

ethyl 5-(acetylthio)-2-((diphenylmethylene)amino)pentanoate, **S2**

To a suspension of NaH (1.1 equiv., 237 mg, 4.94 mmol) in dry DMF, was added a solution of the ethyl 2-((diphenylmethylene)amino)acetate (1 equiv., 1.17 g, 4.48 mmol) and the mixture was heated at 30 – 40 °C under argon until a red solution is obtained (few minutes). A solution of S-(3-bromopropyl)ethanethioate **S1** (1.2 equiv., 1.06 g, 5.38 mmol) in a minimum amount of dry DMF was slowly added. The mixture is then stirred under argon until the starting material disappears (TLC monitoring, CH_2Cl_2-MeOH 1%). The reaction was quenched with a small amount of absolute ethanol and the solvents were removed under reduce pressure. The title product was purified by flash chromatography (hexane to hexane-ethylacetate 10% gradient) as a yellow oil (115 mg, 67%). IR (UATR): 3059, 2933, 2858, 1738, 1691, 1624, 1445 cm^{-1};^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.26–1.31 (m, 5H), 1.99 (q, J = 7.3 Hz, 2H), 2.32 (s, 3H), 2.83 (t, J = 7.3 Hz, 2H), 4.06 (t, J = 6.5 Hz, 1H), 4.17–4.22 (m, 2H), 7.18–7.69 (m, 10H); HRMS (ES^-) : $[M+H]^-$ calculated for $C_{22}H_{24}NO_3S$: 382.1555; found: 382.1553; in accordance with [9].

1-carboxy-4-mercaptobutan-1-aminium chloride, thioornithine hydrochloride **S3**

The ethyl 5-(acetylthio)-2-((diphenylmethylene)amino)pentanoate (215 mg, 5.61 10^{-4} mol) was suspended in a 6H hydrochloric acid solution and refluxed under argon overnight. The aqueous phase was washed three times by diethylether and concentrated under reduced pressure to give the title compound as a white solid (77 mg, 92%). IR (UATR): 3182, 2933, 2551, 1739, 1634, 1504, 1451. ^{1}H NMR (400 MHz, D_2O) δ ppm: 1.59–1.72 (m, 2H), 1.92–2.01 (m, 2H), 2.13 (m, 2H), 2.51 (m, 3H), 3.98 (t, J = 6.3 Hz, 1H); HRMS (ESI) calcd. For $C_5H_{11}NO_2S$ 150.0583, found 150.0601; in accordance with [9].

Synthesis of **14**

(*E*)-3-(5-chloro-2-hydroxyphenyl)-1-(furan-2-yl)prop-2-en-1-one **13** (CAS: 1688703-85-6)

To a solution of 5-chloro-2-hydroxybenzaldehyde (1.1 equiv., 1.50 g, 9.56 mmol) in EtOH (22 mL) was added at room temperature 1-(furan-2-yl)ethan-1-one (1 equiv., 957 mg, 8.69 mmol) followed by tBuOK (2 equiv., 1.15 g, 17.4 mmol). The resulting mixture was stirred at room temperature overnight. The reaction mixture was quenched with water (50 mL) and acidified to pH 4-5. The resulting aqueous phase was extracted with EtOAc (3 x 30 mL) and the organic phases were combined, washed with brine (20 mL), dried over $MgSO_4$, filtrated and concentrated under vacuum in order to get a crude compound (1.2 g) as brown oil. This crude compound was then purified by flash column chromatography, solid deposit (Celite), 15 g SiO_2, DCM/MeOH 100/0 for 5 CV, 100/0 to 95/5 for 15 CV, to give pure compound (1.98 g, 92% yield) as a yellow solid. Rf = 0.48 (DCM/MeOH, 95/5). Mp = 163°C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 6.79 (dd, J=3.5, 1.7 Hz, 1 H), 6.94 (d, J=8.8 Hz, 1 H), 7.30 (dd, J=8.8, 2.6 Hz, 1 H), 7.72 (d, J=15.9 Hz, 1 H), 7.82 (dd, J=3.5, 0.6 Hz, 1 H), 7.94 (d, J=15.9 Hz, 1 H), 7.95 (d, J=2.6 Hz, 1 H), 8.06 (dd, J=1.6, 0.6 Hz, 1 H), 10.56 (s, 1 H). ^{13}C NMR (400 MHz, DMSO-d6) δ ppm: 112.70, 112.70, 117.90, 119.53, 122.02, 122.84, 123.20, 127.36, 131.40, 136.34, 153.00, 155.92, 176.71.

4-chloro-2-(3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenol **14** (CAS: 2061968-74-7)

To a solution of (E)-3-(5-chloro-2-hydroxyphenyl)-1-(furan-2-yl)prop-2-en-1-one **13** (1 equiv., 134 mg, 0.539 mmol) in EtOH (12 mL) was added at room temperature hydrazine monohydrate (2 equiv., 54 mg, 53 µL, 1.78 mmol). The resulting mixture was refluxed for 5 min. The reaction mixture was then concentrated under vacuum to get the desired compound as a yellow solid (145 mg, quant). Rf = 0.78 (DCM/MeOH, 95/5). Mp = 192°C. ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 2.65 (dd, J=16.3, 9.7 Hz, 1 H), 3.36 (dd, J=16.3, 10.8 Hz, 2 H), 4.92 (dd, J=10.8, 9.7 Hz, 1 H), 6.54 (dd, J=3.4, 1.7 Hz, 1 H), 6.62 (dd, J=3.4, 0.7 Hz, 1 H), 6.83 (d, J=8.6 Hz, 1 H), 7.11 (dd, J=8.6, 2.7 Hz, 1 H), 7.22 (d, J=2.7 Hz, 1 H), 7.49 (br. s., 1 H), 7.71 (dd, J=1.7, 0.7 Hz, 1 H), 9.92 (s, 1 H).^{13}C NMR (400 MHz, DMSO-d6) δ ppm : 39.48, 57.56, 109.19, 111.60, 116.60, 122.37, 126.15, 127.48, 130.70, 141.14, 143.41, 148.35, 153.53. HRMS (ES^-) : $[M+H]^-$ calculated for $C_{13}H_{12}ClN_2O_2$: 263,7010; found: 263,7020.

Synthesis of aurones **15a-h**.

6,7-dihydroxybenzofuran-3(*2H*)-one **6**

A solution of chloroacetic acid (1.1 equiv., 1.24 g, 13.1 mmol) in $POCl_3$ (2 equiv., 3.64 g, 2.2 mL, 23.8 mmol) was added under stirring at 0 °C pyrogallol (1 equiv., 1.5 g, 11.9 mmol). The mixture is then heated to reflux for 2h. Water (25 mL) is added, and the aqueous layer is extracted with Ethyl Acetate (4 x 25 mL). Organic layers are gathered and washed with brine

(50 mL). The resulting layer was dried over Na_2SO_4, and the solvent are evaporated under reduced pressure. The resulting crude is filtrated with silica gel chromatography column using DCM/MeOH:97/3 as solvent. The result is evaporated under reduced pressure and water is added (5 mL) with one drop of methanol. A precipitate is then filtrated and collected while the filtrate is concentrated and putted at 0 °C for a night. A second precipitate is filtrated, and a second fraction is collected. The 2-chloro-1-(2,3,4-trihydroxyphenyl)ethan-1-one is obtained (1.2 g, 51%) as a brown powder. Mp 170 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 5.04 (s, 2H), 6.43 (d, J=8.8 Hz, 1H), 7.30 (d, J=8.8 Hz, 1H), 8.73 (s, 1H), 10.26 (s, 1H), 11.60 (s, 1H); in accordance with [10].

To a solution of 2-chloro-1-(2,3,4-trihydroxyphenyl)ethan-1-one (1 equiv., 1.55 g, 7.7 mmol) in ethanol (30 mL) vigorously stirred is added sodium acetate (3 equiv., 1.90 g, 23.0 mmol). The mixture is heated to reflux for 4 h. The solvent is then evaporated under reduced pressure and water (60 mL) are added. The aqueous layer is extracted with EtOAc (4 x 80 mL). Organic layers are gathered and washed with brine (160 mL) and dried over Na_2SO_4. Solvent is removed under reduced pressure and the pure product is obtained as a brown powder (907 mg, 71%). Mp 230 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 4.71 (s, 2H), 6.60 (d, J=8.4 Hz, 1H), 6.97 (d, J=8.4 Hz, 1H), 9.14 (s, 1H), 10.34 (s, 1H) in accordance with [10].

General procedure for the Claisen-Schmidt condensation

To a solution of the aldehyde (1.5 equiv.) in MeOH (15 mL for 1 mmol per equiv.) is added the ketone (1 equiv.) and an aqueous solution of potassium hydroxide 40% (30 equiv.). The mixture is then vigorously stirred at room temperature under argon overnight. Water (15 mL for 1 mmol per equiv.) is added, and pH is adjusted to 4-5 with HCl 3%. Aqueous layer is extracted with EtOAc (3 x 10 mL for 1 mmol per equiv.). The organic phases are combined and washed with brine (15 mL for 1 mmol per equiv.) and dried over Na_2SO_4. Crude product is then purified by flash column chromatography.

(Z)-2-(4-fluorobenzylidene)-6,7-dihydroxybenzofuran-3(*2H*)-one **15a**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 6,7-dihydroxybenzofuran-3(*2H*)-one **6** (1 equiv., 200 mg, 1.2 mmol), 4-fluorobenzaldehyde (1.5 equiv., 224 mg, 190 µL, 1.8 mmol), KOH_{aq} 40% (30 equiv., 2 g, 5 mL, 36 mmol) and MeOH (10 mL) as an orange solid (180 mg, 55%). Mp > 266°C; IR (UATR, cm^{-1}): 3526, 1585; ^{1}H NMR (400 MHz, MeOD) δ ppm: 6.73 (d, J=8.4 Hz, 1H), 6.79 (s, 1H), 7.20-7.22 (m, 3H), 8.00-8.15 (m, 2H); ^{13}C NMR (100 MHz, DMSO) δ ppm: 109.14, 112.88, 114.01, 115.59, 116.04 (d, J=21 Hz), 128.88 (d, J=2.9 Hz), 130.09, 133.57 (d, J=8.1 Hz), 147.25, 154.84, 155.19, 162.52 (d, J=248 Hz), 182.21; HRMS (ES$^-$) : $[M+H]^-$ calculated for $C_{15}H_8FO_4$: 271.0407; found: 271.0406; in accordance with [11].

(*Z*)-2-(3,4-difluorobenzylidene)-6,7-dihydroxybenzofuran-3(2H)-one **15b**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 6,7-dihydroxybenzofuran-3(*2H*)-one **6** (1 equiv., 200 mg, 1.2 mmol), 3,4-difluorobenzaldehyde (1.5 equiv., 257 mg, 200 µL, 1.8 mmol), KOH_{aq} 40% (30 equiv., 2 g, 5 mL, 36 mmol) and MeOH (10 mL) as an orange solid (225 mg, yield = 65%). Mp: 262°C; IR (UATR, cm^{-1}): 3526, 3068, 1610, 1128; ^{1}H NMR (400 MHz, MeOD) δ ppm: 6.73 (d, J=8.4 Hz, 1H), 6.74 (s, 1H), 7.20 (d, J= 8.4 Hz, 1H), 7.36 (m, 1H), 7.75 (m, 1H), 8.09 (m, 1H); ^{13}C NMR (100 MHz, DMSO) δ ppm: 108.01, 113.05, 113.79, 115.71, 118.06 (d, J=16.7 Hz),

119.50 (d, J=18.2 Hz), 128.71 (dd, J=6.5, 2.9 Hz), 129.98 (dd, J=6.6, 3.7 Hz), 130.07, 147.73, 148.45 (dd, J=248.6, 30 Hz), 150.95 (dd, J=249.6, 28 Hz), 155.08, 155.11, 182.10; HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_7F_2O_4$: 289.0312; found: 289.0311.

(*Z***)-6,7-dihydroxy-2-((perfluorophenyl)methylene)benzofuran-3(2H)-one 15c**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 6,7-dihydroxybenzofuran-3(*2H*)-one, **6** (1 equiv., 200 mg, 1.2 mmol), 2,3,4,5,6-pentafluorobenzaldehyde (1.5 equiv., 353 mg, 220 µL, 1.8 mmol), KOH_{aq} 40% (30 equiv., 2g, 5 mL, 36 mmol) and MeOH (10 mL) as an orange solid (264 mg, 64%). Mp = 158°C; IR (UATR, cm^{-1}): 3028, 1599, 1308; ^1H NMR (400 MHz, MeOD) δ ppm: 6.64 (s, 1H), 6.72 (d, J = 8.6 Hz, 1H), 7.21 (d, J= 8.6 Hz, 1H); HRMS (ES^-) : $[M+H]^-$ calculated for $C_{16}H_8F_5O_3$: 343,2290; found: 343,2294.

(*Z*)-2-(3,4-dihydroxybenzylidene)-6,7-dihydroxybenzofuran-3(2H)-one **15d**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 6,7-dihydroxybenzofuran-3(*2H*)-one **6** (1 equiv., 200 mg, 1.2 mmol), 3,4-dihydroxybenzaldehyde (1.5 equiv., 268 mg, 1.8 mmol), KOH_{aq} 40% (30 equiv., 2 g, 5 mL, 36 mmol) and MeOH (10 mL) as a yellow solid (165 mg, 48%). Mp: 266°C; IR (UATR, cm^{-1}): 3180, 1593, 1282, 1038; ^1H NMR (400 MHz, MeOD) δ ppm: 6.71 (s, 1H), 6.72 (d, J=8.4 Hz, 1H), 6.86-6.89 (m, 1H), 7.19 (d, J=8.4 Hz, 1H), 7.39 (dd, J=8.4, 2.1 Hz, 1H), 7.53 (d, J=2.1 Hz, 1H); ^{13}C NMR (100 MHz, MeOD) δ ppm: 111.81, 112.54, 114.56, 115.21, 115.95, 118.37, 123.52, 124.53, 130.15, 145.46, 145.85, 147.95, 154.19, 154.99, 182.02; HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_9O_6$: 285.0399; found: 285.0395; in accordance with [11].

(*Z*)-6,7-dihydroxy-2-(3,4,5-trihydroxybenzylidene)benzofuran-3(2H)-one **15e**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 6,7-dihydroxybenzofuran-3(*2H*)-one **6** (1 equiv., 200 mg, 1.2 mmol), 3,4,5-trihydroxybenzaldehyde (1.5 equiv., 280 mg, 1.8 mmol), KOH_{aq} 40% (30 equiv., 2 g, 5 mL, 36 mmol) and MeOH (10 mL) as a yellow solid (163 mg, 45%). Mp: 222°C; IR (UATR, cm^{-1}): 3203, 1596, 1127 ; ^1H NMR (400 MHz, DMSO) δ ppm: 6.50 (s, 1H), 6.72 (d, J = 8.2 Hz, 1H), 6.98 (s, 2H), 7.10 (d, J=8.2 Hz, 1H); ^{13}C NMR (100 MHz, MeOD) δ ppm: 111.08, 112.12, 113.80, 114.96, 115.55, 123.11, 130.06, 136.31, 145.63, 146.69, 154.51, 155.57, 183.92; HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{10}O_7$: 301.0348; found: 301.0344; in accordance with [11].

(*Z*)-6,7-dihydroxy-2-(2,3,4-trihydroxybenzylidene)benzofuran-3(2H)-one **15f**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 6,7-dihydroxybenzofuran-3(*2H*)-one **6** (1 equiv., 200 mg, 1.2 mmol), $C_7H_6O_4$ (1.5 equiv., 280 mg, 1.8 mmol), KOH_{aq} 40% (30 equiv., 2 g, 5 mL, 36 mmol) and MeOH (10 mL) as a yellow solid (120 mg, yield = 33%). Mp>266°C; IR (UATR, cm^{-1}): 3159, 1602, 1041; ^1H NMR (400 MHz, DMSO) δ ppm: 5.99 (d, 1H), 6.48 (d, 1H), 6.64 (s, 1H), 7.13 (d, 1H), 7.14 (d, 1H), 8.64 (s, 1H), 8.65 (s, 1H), 9.17 (s, 1H), 9.21 (s, 1H), 9.43 (s, 1H); HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{10}O_7$: 301.0348; found: 301.0349; in accordance with [12].

(*Z*)-6,7-dihydroxy-2-(4-hydroxybenzylidene)benzofuran-3(*2H*)-one **15g**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 6,7-dihydroxybenzofuran-3(*2H*)-one **6** (1 equiv., 230 mg, 1.4 mmol), 4-hydroxybenzaldehyde (1.5 equiv., 253 mg, 2.1 mmol), KOH_{aq} 40% (30 equiv., 2.3 g, 5.8 mL, 42 mmol) and MeOH (10 mL) as a yellow solid (215 mg, 57%). Mp >266°C; IR (UATR, cm^{-1}): 3226, 1578, 1131; ^{1}H NMR (400 MHz, MeOD) δ ppm: 6.73 (d, J=8.4 Hz, 1H), 6.79 (s, 1H), 6.91 (d, J=8.8 Hz, 2H), 7.20 (d, J=8.4 HZ, 1H), 7.93 (d, J=8.8 Hz, 2H); ^{13}C NMR (100 MHz, MeOD) δ ppm: 113.81, 114.40, 116.11, 116.40, 117.10, 125.30, 132.72, 135.07, 148.12, 156.22, 156.38, 161.07, 185.44; HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_9O_5$: 269.0450; found: 269.0449; in accordance with [11].

(Z)-6,7-dihydroxy-2-(3-hydroxybenzylidene)benzofuran-3(2H)-one, **15h**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 6,7-dihydroxybenzofuran-3(*2H*)-one **6** (1 equiv., 230 mg, 1.4 mmol), 3-hydroxybenzaldehyde (1.5 equiv., 253 mg, 2.1 mmol), KOH_{aq} 40% (30 equiv., 2.3 g, 5.8 mL, 42 mmol) and MeOH (10 mL) as a yellow solid (204 mg, 54%). Mp: 266°C; IR (UATR, cm^{-1}): 3145 (νOH), 1578 (νC=O), 1131; ^{1}H NMR (400 MHz, MeOD) δ ppm: 6.71 (s, 1H, H_{15}), 6.73 (d, J=8.4 Hz, 1H), 6.85 (ddd, J=8, 2.4, 0.8 Hz, 1H), 7.20 (d, J=6.4 Hz, 1H), 7.29 (t, J=8 Hz, 1H), 7.45 (t, J=2 Hz, 1H), 7.48 (m, 1H); ^{13}C NMR (100 MHz, DMSO) δ ppm: 110.50, 112.76, 114.05, 115.58, 117.54, 117.60, 122.41, 129.84, 130.17, 130.26, 147.46, 154.92, 155.302, 157.56, 182.29; HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_9O_5$: 269.0450; found: 269.0445; in accordance with Hou *et al.*[12]

Synthesis of chalcones **21a-j**

General procedure for the MOM protection

To a solution of the hydroxybenzaldehyde or the hydroxyacetophenone (1 equiv.) in DCM or ACN (0.75 mL per mmol of the starting material) vigorously stirred and cooled at 0°C was added MOMBr (2.1 equiv. per hydroxyl) followed 30 minutes later by the addition of DIPEA (2.1 equiv. per hydroxyl +1 equiv.). The ice bath is removed, and the mixture is stirred for 3 hours at room temperature. The mixture is then treated with HCl 1N (0.75 mL per mmol of the starting material) and the aqueous phase is extracted with DCM (0.75 mL per mmol of the starting material). Organic phases are gathered and washed with NaOH 1N (0.75 mL per mmol of the starting material), HCl 1N (0.75 mL per mmol of the starting material) and, brine. The organic phase is finally dried over Na_2SO_4, and solvent is removed by distillation under reduced pressure. The crude is purified by flash column chromatography.

1-(3,4-bis(methoxymethoxy)phenyl)ethan-1-one **18a**

The title product is obtained following the general procedure for the MOM protection with 3,4-dihydroxyacetophenone (1 equiv., 500mg, 3.3 mmol); MOMBr (4.2 equiv., 1.73 g, 1.15 mL, 13.8 mmol); DIPEA (5.2 equiv., 2.21 g, 2.9 mL, 17.1 mmol); DCM (25 mL). The crude is purified by flash column chromatography (gradient of MeOH in DCM from 0 to 10%) to obtain the title product as a white solid (550 mg, 69%). Mp: 70°C; ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 2.54 (s, 3H), 3.50 (d, J=5.6 Hz, 6H), 5.27 (d, J=9.6 Hz, 4H), 7.18 (d, J=8.4 Hz, 1H), 7.59 (dd, J=8.4, 2.0 Hz, 1H), 7.75 (d, J=2.0 Hz, 1H).

1-(4-(methoxymethoxy)phenyl)ethan-1-one **18b**

The title product is obtained following the general procedure for the MOM protection with 4-hydroxyacetophenone (1 equiv., 500 mg, 3.70 mmol); MOMBr (1.5 equiv., 689 mg, 0.50 mL, 5.51 mmol); DIPEA (3 equiv., 1.43 g, 2.0 mL, 11.1 mmol); DCM (25 mL). The crude is purified by flash column chromatography (gradient of MeOH in DCM from 0 to 10%) to obtain the title product as a yellowish oil (651 mg, 98%). IR (UATR, cm^{-1}): 1673, 1149, 981; ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 2.54 (s, 3H), 3.47 (s, 3H), 5.22 (s, 2H), 7.14 (d, J=8.9 Hz, , 2H), 7.91 (d, J=8.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 26.33, 56.33, 94.15, 115.79, 130.09, 131.14, 161.49, 197.12.

1-(3-(methoxymethoxy)phenyl)ethan-1-one **18c**

The title product is obtained following the general procedure for the MOM protection with 3-hydroxyacetophenone (1 equiv., 500 mg, 3.70 mmol); MOMBr (1.5 equiv., 689 mg, 0.50 mL, 5.51 mmol); DIPEA (3 equiv., 1.43 g, 2.0 mL, 11.1 mmol); DCM (25 mL). The crude is purified by flash column chromatography (gradient of MeOH in DCM from 0 to 10%) to obtain the title product as a yellow oil (567 mg, 85%). IR (UATR, cm^{-1}): 1682, 1265, 1005; ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 2.58 (s, 3H), 3.48 (s, 3H), 5.21 (s, 2H), 7.23 (ddd, J=8.5, 2.1, 1 Hz, 1H), 7.37 (dd, J= 9.7, 6.1, 1H), 7.58 (m, 1H), 7.60 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 26.84, 56.24, 94.55, 115.74, 121.26, 122.11, 129.76, 138.70, 157.54, 197.88.

1-(3,5-bis(methoxymethoxy)phenyl)ethan-1-one **18d**

The title product is obtained following the general procedure for the MOM protection with 3,5-dihydroxyacetophenone (1 equiv., 522 mg, 3.43 mmol); MOMBr (4 equiv., 1.75 g, 1.14 mL, 14 mmol); DIPEA (4 equiv., 1.81 g, 2.4 mL, 14 mmol); dry ACN (4 mL). The crude is purified by flash column chromatography (gradient of EtOAc in hexane from 0 to 40%) to obtain the title product as a colourless oil (391 mg, 47%). IR (UATR, cm^{-1}): 1694, 1141, 1011. (; ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 2.49 (s, 3H), 3.41 (s, 6H), 5.12 (s, 4H), 6.87 (s, 1H), 7.20 (d, J=2.3 Hz, 2H).

3,4-bis(methoxymethoxy)benzaldehyde **19a**

The title product is obtained following the general procedure for the MOM protection with 3,4-dihydroxybenzaldehyde (1 equiv., 500mg, 3.6 mmol), MOMBr (4.2 equiv., 1.73 g, 1.15 mL, 13.8 mmol), DIPEA (5.2 equiv., 2.21 g, 2.9 mL, 17.1 mmol) and DCM (25 mL) and purified by flash chromatography (gradient of MeOH in DCM from 0 to 10%) as a white solid (574 mg, yield = 71%). IR (UATR, cm-1): 2935, 2833, 1673, 1584, 1505; ^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm: 3.41 (s, 3 H), 3.42 (s, 3 H), 5.27 (s, 2 H), 5.33 (s, 2 H), 7.30 (d, *J*=6.9 Hz, 1 H), 7.58 (dd, *J*=6.9, 2.0 Hz, 1 H), 7.59 (d, *J*=2.0 Hz, 1 H), 9.85 (s, 1 H); ^{13}C NMR (101 MHz, DMSO-*d*6) δ ppm: 55.80, 55.90, 94.30, 94.70, 115.40, 115.59, 126.25, 130.54, 146.96, 152.19, 191.42.

4-(methoxymethoxy)benzaldehyde **19b**

The title product is obtained following the general procedure for the MOM protection with 4-hydroxybenzaldehyde (1 equiv., 500mg, 4.1 mmol); MOMBr (1.5 equiv., 767.3 mg, 0.501 mL, 6.14 mmol); DIPEA (3 equiv., 1.60 g, 2.9 mL, 12.3 mmol); DCM (25 mL). The crude is purified by flash column chromatography (gradient of MeOH in DCM from 0 to 10%) to obtain the title product as a yellowish oil (592 mg, 87%). IR (UATR, cm^{-1}): 2829, 1597, 1147 ; ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 3.49 (s, 3H), 5.25 (s, 2H), 7.14 (d, J=8.7 Hz, 2H), 7.83 (d, J=8.8 Hz, 2H),

9.90 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 56.48, 94.23, 116.40, 130.87, 132.00, 162.33, 190.00.

3-(methoxymethoxy)benzaldehyde **19c**

The title product is obtained following the general procedure for the MOM protection with 3-hydroxybenzaldehyde (1 equiv., 500mg, 4.1 mmol); MOMBr (1.5 equiv., 767.3 mg, 0.501 mL, 6.14 mmol); DIPEA (3 equiv., 1.6 g, 2.9 mL, 12.3 mmol); DCM (25 mL). The crude is purified by flash column chromatography (gradient of MeOH in DCM from 0 to 10%) to obtain the title product as a colourless oil (592 mg, 80%). IR (UATR, cm^{-1}): 2827, 1698, 1007; ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 3.48 (s, 3H), 5.22 (s, 2H),7.29 (ddd, J=8.1, 2.6, 1.2 Hz, 1H), 7.45 (dd, J=9.6, 6.0 Hz, 1H), 7.51 (t, J=1.3 Hz, 1H) 7.53 (m, 1H), 9.97 (s, 1H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 56.28 , 94.53 , 116.07, 122.9, 123.94, 130.24, 137.96, 157.90, 192.09.

3,5-bis(methoxymethoxy)benzaldehyde **19d**

The title product is obtained following the general procedure for the MOM protection with 3,5-dihydroxybenzaldehyde (1 equiv., 200mg, 1.45 mmol), MOMBr (4 equiv., 0.72 g, 0.5 mL, 5.6 mmol), DIPEA (4 equiv., 0.75 g, 1 mL, 5.6 mmol) and dry ACN (4 mL) and purified by flash chromatography (gradient of EtOAc in hexane from 10% to 30%) as a colourless oil (232 mg, yield = 71%). IR (UATR, cm-1): 2904, 1698, 1140, 1018, (^{1}H NMR (400 MHz, DMSO-*d*6) δ ppm: 3.44 (s, 3 H), 3.42 (s, 6 H), 5.17 (s, 4 H), 6.94 (t, *J*= 2.3 Hz, 1 H), 7.17 (d, *J*=2.3 Hz, 2 H), 9.85 (s, 1 H).

3,4,5-tris(methoxymethoxy)benzaldehyde **19e**

The title product is obtained following the general procedure for the MOM protection with 3,4,5 trihydroxybenzaldehyde (1 equiv., 491 mg, 3.20 mmol); MOMBr (6.3 equiv., 2.5 g, 1.63 mL, 20 mmol); DIPEA (10 equiv., 4.14 g, 5.44 ml, 32 mmol); DCM (25 mL). The crude is purified by flash column chromatography (gradient of EtOAc in hexane from 0 to 40%) to obtain the title product as a yellowish oil (800 mg, 87%). IR (UATR, cm^{-1}): 2921, 1693, 1037, 918. ^{1}H NMR (400 MHz $CDCl_3$) δ ppm: 3.51 (s, 6H), 3.61 (s, 3H), 7.38 (s, 2H), 9.84 (s, 2H).

(*E*)-1,3-bis(3,4-bis(methoxymethoxy)phenyl)prop-2-en-1-one, **20a**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 1-(3,4-bis(methoxymethoxy)phenyl)ethan-1-one **18a** (1 equiv., 400 mg, 1.6 mmol), 3,4-bis(methoxymethoxy)benzaldehyde **19a** (1.5 equiv., 565 mg, 2.5 mmol), KOH_{aq} 40% (30 equiv., 2.8g, 6.9 mL, 48 mmol) and MeOH (25 mL) and purified by flash chromatography (gradient of MeOH in DCM from 0 to 10%) as a white solid (520 mg, 71%). Mp: 82°C; IR (UATR, cm^{-1}): 1500 (νC=O), 1248, 916; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.45 (m, 12H), 5.23 (m, 8H), 7.17 (d, J=8.4 Hz, 1H), 7.23 (d, J=8.4 Hz, 1H), 7.25 (dd, J=7.6, 2 Hz, 1H), 7.35 (d, J= 15.6 Hz, 1H), 7.44 (d, J=2 Hz, 1H), 7.66 (dd, J=8.4, 2 Hz, 1H), 7.69 (d, J=15.6 Hz, 1H), 7.82 (d, J= 2 Hz, 1H); HRMS (ES^+) : $[M+H]^+$ calculated for $C_{23}H_{28}O_9$: 449.1812; found: 449.1816.

(*E*)-1-(3,4-bis(methoxymethoxy)phenyl)-3-phenylprop-2-en-1-one **20b**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 1-(3,4-bis(methoxyethoxy)phenyl)ethan-1-one **18a** (1 equiv., 400 mg, 1.6 mmol), benzaldehyde (1.5 equiv., 254 mg, 2.4 mmol), KOH_{aq} 40% (30 equiv., 2.7 g, 6.7 mL,

48 mmol) and MeOH (25 mL) and purified by flash chromatography (gradient of MeOH in DCM from 0 to 10%) as a white solid (365 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.52 (m, 6H), 5.3 (m, 4H), 7.39 (m, 3H), 7.48 (d, J=16 Hz, 1H), 7.62 (m, 3H), 7.68 (dd, J=8.4, 2 Hz, 1H), 7.79 (d, J=16 Hz, 1H), 7.84 (d, J=2 Hz, 1H).

(*E*)-1-(3,4-bis(methoxymethoxy)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one **20c**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 1-(3,4-bis(methoxymethoxy)phenyl)ethan-1-one **18a** (1 equiv., 200 mg, 0.83 mmol), 4-(methoxymethoxy)benzaldehyde **19b** (1.5 equiv., 208 mg, 1.25 mmol), KOH_{aq} 40% (30 equiv., 1.4g, 3.6 mL, 25 mmol) and MeOH (10 mL) and purified by flash chromatography (gradient of EtOAc in DCM from 0 to 20%) as a white- yellow solid (231 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.47 (s, 3H), 3.52 (s, 3H), 3.53 (s, 3H), 5.20 (s, 2H), 5.30 (s, 2H), 5.31 (s, 2H), 7.06 (d, J=8.7 Hz, 2H), 7.22 (d, J=8.3, 1H), 7.39 (d, J=15.6 Hz, 1H), 7.58 (d, J=8.7 Hz 2H), 7.68 (dd, J=8.5, 2.1 Hz, 1H), 7.76 (d, J=15.6, 1H), 7.84 (d, J=2.1, 1H), ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 56.24, 56.47, 94.27, 95.05, 95.58, 115.17, 116.58, 116.77, 123.94, 128.86, 130.14, 132.84, 144.06, 147.05, 151.39, 159.20, 188.81.

(*E*)-1-(3,4-bis(methoxymethoxy)phenyl)-3-(3-(methoxymethoxy)phenyl)prop-2-en-1-one **20d**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with 1-(3,4-bis(methoxymethoxy)phenyl)ethan-1-one **18a** (1 equiv., 375 mg, 1.60 mmol), 3-(methoxymethoxy)benzaldehyde **19c** (1.2 equiv., 316 mg, 1.90 mmol), KOH_{aq} 40% (30 equiv., 2.69 g, mL, 48 mmol) and MeOH (7 mL) and purified by flash chromatography (gradient of EtOAc in Hexane from 0 to 40%) as an off white solid (462 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.53 (s, 3H), 3.56 (s, 3H), 3.57 (s, 3H), 5.24 (s, 2H), 5.34 (s, 2H), 5.35 (s, 2H), 7.13 (ddd, J=7.9, 2.4, 1.2, 1H), 7.32 (m, 4H) 7.50 (d, J=15.7 Hz, 1H), 7.72 (dd, J=8.5, 2.1 Hz, 1H), 7.78 (d, J=15.6, 1H), 7.88 (d, J=2.1, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 56.20, 56.72, 94.60, 95.08, 95.62, 115.19, 115.94, 116.83, 118.41, 122.31, 122.35, 124.13, 130.07, 132.62, 136.61, 144.14, 147.13, 151.59, 157.78, 188.84.

(*E*)-1-(3,4-bis(methoxymethoxy)phenyl)-3-(3,5-bis(methoxymethoxy)phenyl)prop-2-en-1-one **20e**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with , 3,5-bis(methoxymethoxy)benzaldehyde **19e** (1.5 equiv., 235 mg, 1.03 mmol), 1-(3,4-bis(methoxymethoxy)phenyl)ethan-1-one **18a** (1 equiv., 166 mg, 0.70 mmol), KOH_{aq} 40% (30 equiv., 1.12 g, 3 mL, 21 mmol) and MeOH (10 mL) and purified by flash chromatography (EtOAc in Hexane, gradient from 10% of 40%) as a yellow solid (240 mg, 77%). Mp: 70°C; IR (UATR, cm^{-1}): 1590; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.43 (s, 6H), 3.46 (s, 3H), 3.48 (s, 3H), 5.13 (s, 4H), 5.24 (s, 2H), 5.25 (s, 2H), 6.73 (t, *J*= 2.2 Hz, 1H), 6.91 (d, *J*= 2.2 Hz, 2H), 7.17 (d, *J*=8.5 Hz, 1H), 7.37 (d, *J*= 15.6 Hz, 1H), 7.62 (m, 2H), 7.77 (d, *J*=2.2 Hz, 1H).

(*E*)-1-(3,4-bis(methoxymethoxy)phenyl)-3-(3,4,5-tris(methoxymethoxy)phenyl)prop-2-en-1-one **20f**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with , 3,4,5-tris(methoxymethoxy)benzaldehyde **19e** (1.5 equiv., 447 mg, 1.60 mmol), 1-(3,4-bis(methoxymethoxy)phenyl)ethan-1-one **18a** (1 equiv., 264 mg, 1.10 mmol),

KOH_{aq} 40% (30 equiv., 1.85 g, 4.7 mL, 33 mmol) and MeOH (10 mL) and purified by flash chromatography (EtOAc in Hexane 20% isocratic) as a yellow oil (527 mg, 94%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 3.54 (s, 9H), 3.56 (s, 3H), 3.63 (s, 3H), 5.20 (s, 2H), 5.26 (s, 4H), 5.32 (s, 2H), 5.34 (s, 2H), 7.15 (s, 2H), 7.26 (d, *J*= 8.7 Hz, 1H), 7.36 (d, *J*= 15.6 Hz, 1H), 7.67 (m, 2H), 7.84 (d, *J*= 1.7 Hz, 1H).

(*E*)-3-(3,4-bis(methoxymethoxy)phenyl)-1-phenylprop-2-en-1-one **20g**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with acetophenone (1 equiv., 142 mg, 145 µL, 1.2 mmol), 3,4-bis(methoxymethoxy)benzaldehyde **19a** (1.5 equiv., 400 mg, 1.8 mmol), KOH_{aq} 40% (30 equiv., 2 g, 5 mL, 36 mmol) and MeOH (25 mL) and purified by flash chromatography (gradient of MeOH in DCM from 0 to 10%) as a white solid (330 mg, 81%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 3.51 (s, 3H), 3.53 (s, 3H), 5.26 (s, 2H), 5.27 (s, 2H), 7.17 (d, J=8.4 Hz, 1H), 7.25 (dd, J=8.4, 2.2 Hz, 1H), 7.37 (d, J=15.6 Hz, 1H), 7.45 (d, J= 2.2 Hz, 1H), 7.48 (t, J= 7.2 Hz, 2H), 7.55 (tt, J= 7.2, 1.2 Hz, 1H), 7.71 (d, J=15.6 Hz, 1H), 7.98 (dt, J= 7.2, 1.2 Hz, 2H).

(*E*)-3-(3,4-bis(methoxymethoxy)phenyl)-1-(4-(methoxymethoxy)phenyl)prop-2-en-1-one **20h**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with , 3,4-bis(methoxymethoxy)benzaldehyde **19a** (1.5 equiv., 697 mg, 3.10 mmol), 1-(4-(methoxymethoxy)phenyl)ethan-1-one **18b** (1 equiv., 370 mg, 2.10 mmol), KOH_{aq} 40% (30 equiv., 3.53g, 9 mL, 63 mmol) and MeOH (15 mL) and purified by flash chromatography (gradient of EtOAc in Hexane from 0 to 40%) as a yellow solid (699 mg, 58%). Mp: 81°C; IR (UATR, cm^{-1}): 1505, 1252, 954; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 3.42 (s, 3H), 3.45 (s, 3H) , 3.48 (s, 3H), 5.18 (s, 2H) , 5.21 (s, 2H) , 5.21(s, 2H), 7.04 (d, J=8.9, 2H) , 7.11(d, J=8.4, 1H) ,7.19 (dd, J=8.5, 2 Hz), 7.32 (d, J=15.6, 1H), 7.39 (d, J=2 Hz, 1H), 7.65 (d, J=15.6, 1H), 7.94 (d, J=8.9, 2H),^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 55.24, 55.30, 55.32, 93.09, 94.11, 94.53, 114.80, 114.94, 115.19, 119.58, 122.83, 128.55, 129.63, 131.15, 142.97, 146.39, 148.31, 148.36, 159.86, 187.96.

(*E*)-3-(3,4-bis(methoxymethoxy)phenyl)-1-(3-(methoxymethoxy)phenyl)prop-2-en-1-one **20i**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with , 3,4-bis(methoxymethoxy)benzaldehyde **19a** (1.5 equiv., 700 mg, 3.10 mmol), 1-(3-(methoxymethoxy)phenyl)ethan-1-one **18c** (1 equiv., 372 mg, 2.10 mmol), KOH_{aq} 40% (30 equiv., 3.53g, 9 mL, 63 mmol) and MeOH (15 mL) and purified by flash chromatography (gradient of EtOAc in Hexane from 0 to 40%) as a yellow solid (643 mg, 79%). Mp: 66°C; IR (UATR, cm^{-1}): 1506, 1253; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 3.43 (s, 3H), 3.45 (s, 3H), 3.47 (s, 3H) , 5.17 (s, 2H), 5.21(s, 4H), 7.11 (d, J=8.4, 1H), 7.18 (m, 2H), 7.28 (d, J=15.7, 1H), 7.34 (t, J= 7.9, 1H), 7.39 (d, J=2, 1H), 7.57 (m, 2H) , 7.65 (d, J=15.7, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 55.12, 55.30, 55.33, 93.46, 94.09, 94.53, 115.00, 115.07, 115.17, 119.57, 119.84, 121.01, 122.92, 128.32, 128.61, 138.85, 143.71, 146.39, 148.48, 156.43.

(*E*)-3-(3,4-bis(methoxymethoxy)phenyl)-1-(3,5-bis(methoxymethoxy)phenyl)prop-2-en-1-one **20j**

The title product is obtained following the general procedure for the Claisen-Schmidt condensation with , 3,4-bis(methoxymethoxy)benzaldehyde **19a** (1.5 equiv., 509 mg, 2.25

mmol), 1-(3,5-bis(methoxymethoxy)phenyl)ethan-1-one **18a** (1 equiv., 360 mg, 1.50 mmol), KOH_{aq} 40% (30 equiv., 2.5 g, 6.42 mL, 45 mmol) and MeOH (10 mL) and purified by flash chromatography (EtOAc in Hexane, gradient from 0% of 40%) as a yellow solid (406 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.43 (s, 6H), 3.45 (s, 3H), 3.48 (s, 3H), 5.15 (s, 4H), 5.21 (s, 2H), 5.22 (s, 3H), 6.88 (t, *J*= 2.2 Hz, 1H), 7.12 (d, *J*= 8.4 Hz, 1H), 7.21 (m, 4H), 7.65 (d, *J*= 15.7, 1H).

General procedure for the MOM deprotection

To a solution of the starting material (1 equiv.) in MeOH (0.6 mL per mmol of starting material) was added drop wise HCl_{aq} 3N (20 equiv.). The mixture is heated at reflux for 10 min. MeOH is evaporated under reduced pressure and the aqueous solution is lyophilised or diluted with water (Vol tot x5) extracted by AcOEt (Vol tot x2), dried over Na_2SO_4 and finally the solvent was removed under reduced pressure without heating.

(*E*)-1,3-bis(3,4-dihydroxyphenyl)prop-2-en-1-one, **21a**

To a solution of (*E*)-1,3-bis(3,4-bis(methoxymethoxy)phenyl)prop-2-en-1-one, **20a** (1 equiv., 130 mg, 0.29 mmol) in MeOH (5 mL) was added drop wise HCl_{aq} 3N (20 equiv., 2 mL, 6 mmol). The mixture is heated at reflux for 10 min. MeOH is evaporated under reduced pressure and the aqueous solution is lyophilised to give the title compound as a dark red solid (74 mg, 94%). Mp: 142°C; IR (UATR, cm^{-1}): 3362 (νOH), 1500 (νC=O), 1248; ^{1}H NMR (400 MHz, MeOD) δ ppm: 6.83 (d, J=8.4 Hz, 1H), 6.90 (d, J=8.4 Hz, 1H), 7.10 (dd, J=8.4, 2 Hz, 1H), 7.18 (d, J=2 Hz, 1H), 7.48 (d, J=15.2 Hz, 1H), 7.52 (d, J=2 Hz, 1H), 7.56 (dd, J=8.4, 2 Hz, 1H, 7.64 (d, J=15.2 Hz, 1H); ^{13}C NMR (100 MHz, MeOD) δ ppm: 124.78, 124.95, 124.97, 125.46, 128.11, 131.34, 131.38, 136.12, 139.61, 152.96,155.05, 155.23, 158.00, 160.21, 196.73; HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{11}O_5$: 271.2480; found: 271.2483; in accordance with Cai *et al.*[13] and Hofmann *et al.*[14]

(*E*)-1-(3,4-dihydroxyphenyl)-3-phenylprop-2-en-1-one **21b**

The title product is obtained following the synthesis procedure of the MOM deprotection with (*E*)-1-(3,4-bis(methoxymethoxy)phenyl)-3-phenylprop-2-en-1-one **20b** (1 equiv., 120 mg, 0.4 mmol), HCl 3N (20 equiv., 2.7 mL, 8 mmol) and MeOH (7.5 mL) and purified by lyophilisation as a yellow solid (83 mg, 86%). Mp: 184°C; IR (UATR, cm^{-1}): 3486, 3229, 1569; ^{1}H NMR (400 MHz, DMSO) δ ppm: 6.86 (d, J=8.4 Hz, 1H), 7.20 - 7.70 (m, 7H), 7.80-7.90 (m, 2H), 9.38 (s, 1H), 9.95 (s, 1H); ^{1}H NMR (400 MHz, MeOD) δ ppm: 6.89 (d, J=8.4Hz, 1H), 7.40-7.50 (m, 2H), 7.53 (d, J=2 Hz, 1H), 7.59 (dd, J=8.4/2 Hz, 1H), 7.70-7.75 (m, 3H); ^{13}C NMR (100 MHz, DMSO) δ ppm: 115.06, 115.25, 115.34, 122.16, 128.61, 128.88, 129.56, 130.24, 134.90, 142.39, 145.50, 150.94, 187.18; HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{11}O_3$: 239.0708; found: 239.0711.

(*E*)-1-(3,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one **21c**

The title product is obtained following the synthesis procedure of the MOM deprotection with (*E*)-1-(3,4-bis(methoxymethoxy)phenyl)-3-(4-(methoxymethoxy)phenyl)prop-2-en-1-one **20c** (1 equiv., 231 mg, 0.60 mmol); MeOH (7 mL); HCl_{aq} 3N (20 equiv., 4 mL, 12 mmol). The mixture is then extracted to afford the crude product as an orange solid. Hence, the crude was triturated with a mixture of Et_2O and hexane 1:1 to give the title compound as a yellow solid (100 mg, 67%). Mp: 188°C; IR (UATR, cm^{-1}): 3372, 1511, 1163; ^{1}H NMR (400 MHz, DMSO-

d) δ ppm: 6.85 (d, J=8.6, 2H), 6.89 (d, J=8.3, 1H), 7.53 (d, J=2.1, 1H), 7.62 (m, 3H), 7.71 (m, 2H), ^{13}C NMR (100 MHz, DMSO-d) δ ppm: 115.09, 115.35, 115.83, 118.64, 121.86, 126.06, 129.97, 130.69, 142.92, 145.46, 150.66, 159.80, 187.18. HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{13}O_4$: 257.0815; found: 257.0714; in accordance with [13].

(*E*)-1-(3,4-dihydroxyphenyl)-3-(3-hydroxyphenyl)prop-2-en-1-one **21d**

The title product is obtained following the synthesis procedure of the MOM deprotection with (*E*)-1-(3,4-bis(methoxymethoxy)phenyl)-3-(3-(methoxymethoxy)phenyl)prop-2-en-1-one **20d** (1 equiv., 442 mg, 1.14 mmol); MeOH (7 mL); HCl_{aq} 3N (20 equiv., 7.6 mL, 23 mmol). The mixture is then extracted to afford the crude product as an orange solid. Hence, the crude was triturated with Et_2O to give the title compound as a yellow solid (280 mg, 96%). Mp: 214°C; IR (UATR, cm^{-1}): 3506, 3196, 2973, 1580; ^{1}H NMR (400 MHz, DMSO-d) δ ppm: 6.89 (s, 1H), 6.90 (d, J=2.9, 1H), 7.26 (m, 3H), 7.60 (m, 3H), 7.76 (d, J=15.5, 1H), 9.66 (broad s, 3H). ^{13}C NMR (100 MHz, DMSO-d) δ ppm: 115.02, 115.12, 115.34, 117.45, 119.58, 121.99, 122.12, 129.60, 129.87, 136.31, 142.65, 145.51, 150.94, 157.72, 187.20. HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{13}O_4$: 257.0814; found: 257.0815.

(*E*)-1-(3,4-dihydroxyphenyl)-3-(3,5-dihydroxyphenyl)prop-2-en-1-one **21e**

The title product is obtained following the synthesis procedure of the MOM deprotection with (*E*)-1-(3,4-bis(methoxymethoxy)phenyl)-3-(3,5-bis(methoxymethoxy)phenyl)prop-2-en-1-one, **20e** (1 equiv., 240 mg, 0.53 mmol), HCl 3N (20 equiv., 3.6 mL, 10.7 mmol) and MeOH (10 mL). The mixture is then extracted and the crude product was triturated with a mixture of EtOH 0.1% in Et_2O to afford the desired product as a brown solid (120 mg, 83%). IR (UATR, cm^{-1}): 3282, 1571. ^{1}H NMR (400 MHz, DMSO-d) δ ppm: 6.36 (s, 1H), 6.67 (s, 2H), 6.89 (d, *J*=8.3 Hz, 1H), 7.49 (d, *J*=15.7 Hz, 1H), 7.58 (s, 1H), 7.61 (d, *J*=8.3 Hz, 1H), 7.64 (d, *J*=15.5 Hz, 1H), 9.52 (broad s, 4 OH) ^{13}C NMR (100 MHz, DMSO-d) δ ppm: 104.81, 106.62, 115.16, 115.32, 121.73, 122.04, 129.61, 136.58, 143.01, 145.51, 150.94, 158.69, 187.23. HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{13}O_5$: 273.0763; found: 273.0764.

(*E*)-1-(3,4-dihydroxyphenyl)-3-(3,4,5-trihydroxyphenyl)prop-2-en-1-one **21f**

The title product is obtained following the synthesis procedure of the MOM deprotection with (*E*)-1-(3,4-bis(methoxymethoxy)phenyl)-3-(3,4,5-tris(methoxymethoxy)phenyl)prop-2-en-1-one **20f** (1 equiv., 475 mg, 0.93 mmol); HCl 3N (30 equiv., 9.34 mL, 28 mmol) and MeOH (10 mL). The mixture is then extracted, and the crude product was triturated with Et_2O to afford the desired product as a brown solid (200 mg, 75%). Mp>266°C. IR (UATR, cm^{-1}): 3245, 1571. ^{1}H NMR (400 MHz, DMSO-d) δ ppm: 6.80 (s, 2H), 6.93 (d, *J*= 8.2 Hz, 1H), 7.45 (s, 2H), 7.54 (m, 2H), 9.03 (broad s, 1H), 9.17 (broad s, 2H), 9.46 (broad s, 1H), 10.03 (broad s, 1H). ^{13}C NMR (100 MHz, DMSO-d) δ ppm: 108.21, 115.28, 115.44, 118.51, 121.56, 125.42, 129.96, 136.44, 143.78, 145.44, 146.25, 150.62, 186.96. HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{13}O_6$: 289.0713; found: 289.0712.

(*E*)-3-(3,4-dihydroxyphenyl)-1-phenylprop-2-en-1-one **21g**

The title product is obtained following the synthesis procedure of the MOM deprotection with (*E*)-3-(3,4-bis(methoxymethoxy)phenyl)-1-phenylprop-2-en-1-one, **20g** (1 equiv., 200 mg, 0.6 mmol); HCl 3N (20 equiv., 4 mL, 12 mmol) and MeOH (10 mL). The mixture is then lyophilised, and the crude product was as a yellow solid (136 mg, yield = 95%). Mp: 201°C;

IR (UATR, cm^{-1}): 3481, 3304, 1561; ^{1}H NMR (400 MHz, MeOD) δ ppm: 6.82 (d, J=8 Hz, 1H), 7.12 (dd, J=8.4, 2.2 Hz, 1H), 7.19 (d, J=2.2 Hz, 1H), 7.49 (d, J=15.6 Hz, 1H), 7.54 (t, J=7.6 Hz, 2H), 7.62 (tt, J=7.4, 1.2 Hz, 1H), 7.68 (d, J=15.6 Hz, 1H), 8.03 (dt, J=7.4, 1.6 Hz, 2H); ^{13}C NMR (100 MHz, MeOD) δ ppm: 114.33, 115.23, 118.28, 122.37, 126.84, 128.08, 128.38, 132.52, 138.36, 145.51, 146.06, 148.75, 191.33; HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{11}O_3$: 239.0708; found: 239.0710.

(*E*)-3-(3,4-dihydroxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one **21h**

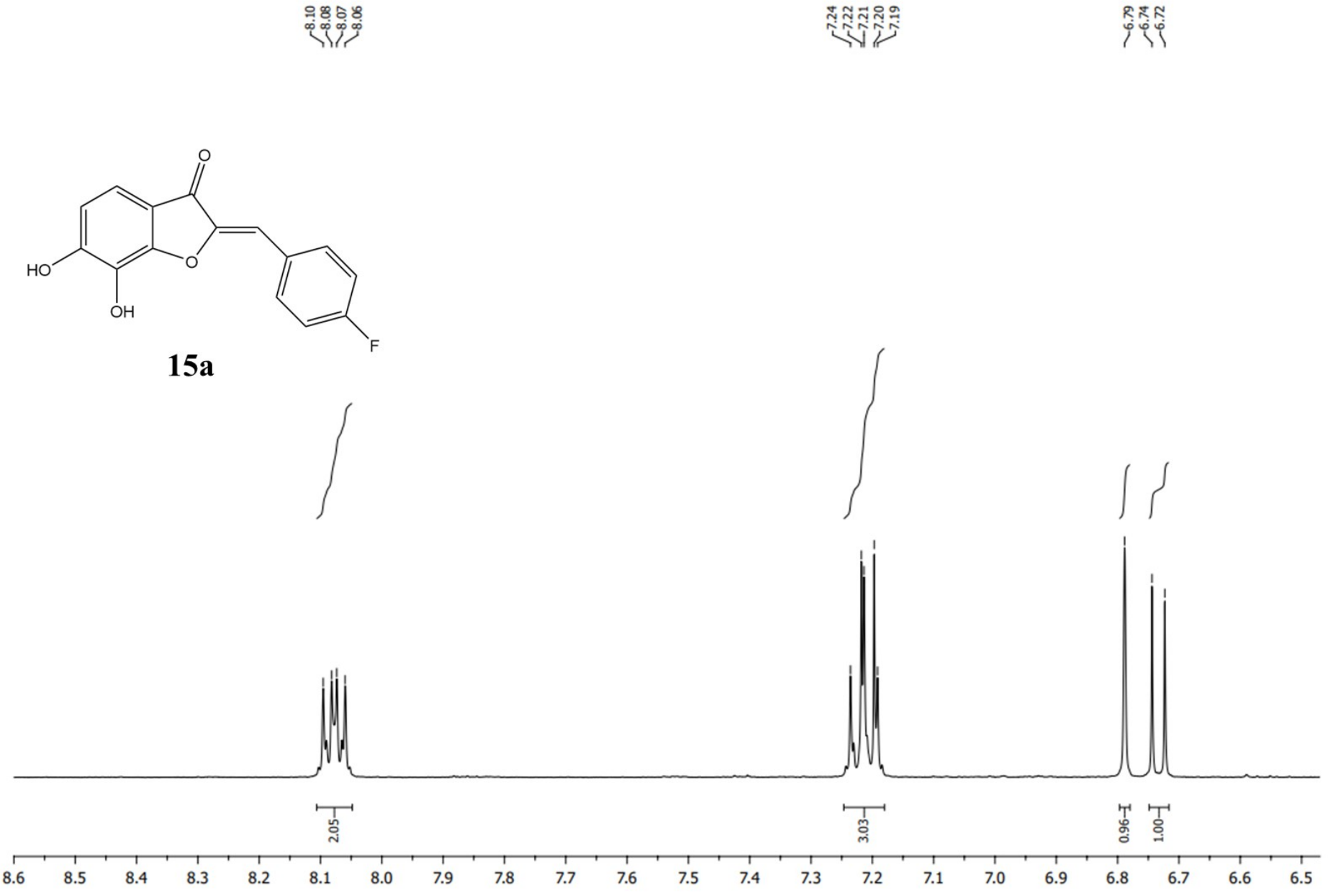
The title product is obtained following the synthesis procedure of the MOM deprotection with (*E*)-3-(3,4-bis(methoxymethoxy)phenyl)-1-(4-(methoxymethoxy)phenyl)prop-2-en-1-one, **20h** (1 equiv., 634 mg, 1.63 mmol); HCl 3N (20 equiv., 11 mL, 33 mmol) and MeOH (20 mL). The mixture was then extracted, and the product is obtained as a yellow solid (300 mg, yield = 72%). Mp 210°C; IR (UATR, cm^{-1}): 3390, 3117, 1566; ^{1}H NMR (400 MHz, DMSO-d) δ ppm: 6.84 (d, J=8.1, 1H), 6.92 (d, J=8.7 Hz, 2H), 7.19 (dd, J=8.2, 1.9 Hz, 1H), 7.27 (d, J=1.9 Hz, 1H), 7.56 (d, J=15.4 Hz, 1H), 7.62 (d, J=15.4 Hz, 1H), 8.05 (d, J=8.7 Hz, 2H) 9.76 (broad s, 3H). ^{13}C NMR (100 MHz, DMSO-d) δ ppm: 115.30, 115.42, 115.73, 118.45, 121.82, 126.48, 129.50, 130.89, 143.62, 145.57, 148.40, 161.87, 187.07. HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{13}O_4$: 257.0814; found: 257.0814; in accordance with [14].

(*E*)-3-(3,4-dihydroxyphenyl)-1-(3-hydroxyphenyl)prop-2-en-1-one **21i**

The title product is obtained following the synthesis procedure of the MOM deprotection with (*E*)-3-(3,4-bis(methoxymethoxy)phenyl)-1-(3-(methoxymethoxy)phenyl)prop-2-en-1-one **20i** (1 equiv., 385 mg, 1 mmol); HCl 3N (20 equiv., 6.6 mL, 19.84 mmol) and MeOH (10 mL). The mixture was then extracted, and the product is obtained as a yellow solid (252 mg, yield quant.). Mp: 198°C; IR (UATR, cm^{-1}): 3325, 1557; ^{1}H NMR (400 MHz, DMSO-d) δ ppm: 6.84 (d, J=8.2 Hz, 1H), 7.07(ddd, J=8.1, 2.5, 0.8 Hz, 1H), 7.20 (dd, J=8.2, 2 Hz, 1H), 7.27(d, J=2 Hz, 1H), 7.39 (t, J=7.9, 1H), 7.45 (m, 1H), 7.54 (d, J=15.5, 1H), 7.59 (dd, J=6.5, 1.2 Hz, 1H), 7.61(d, J=15.5, 1H), 9.15 (broad s, 1H), 9.73 (broad s, 1H), 9.78 (broad s, 1H). ^{13}C NMR (100 MHz, DMSO-d) δ ppm: 114.94, 115.90, 116.24, 119.01, 119.75, 120.35, 122.60, 126.71, 130.23, 139.95, 142.52, 146.08, 149.19, 158.13, 189.36. HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{13}O_4$: 257.0814; found: 257.0815; in accordance with [14].

(*E*)-3-(3,4-dihydroxyphenyl)-1-(3,5-dihydroxyphenyl)prop-2-en-1-one **21j**

The title product is obtained following the synthesis procedure of the MOM deprotection with (*E*)-3-(3,4-bis(methoxymethoxy)phenyl)-1-(3,5-bis(methoxymethoxy)phenyl)prop-2-en-1-one, **20j** (1 equiv., 400 mg, 0.90 mmol), HCl 3N (20 equiv., 6 mL, 18 mmol) and MeOH (10 mL) The mixture was then extracted, and the crude product was triturated with a mixture of EtOH 0.1% in Et_2O to afford the desired product as a brownish solid (190 mg, 76%). Mp: 228°C. IR (UATR, cm^{-1}): 3751, 1563. ^{1}H NMR (400 MHz, DMSO-d) δ ppm: 6.51 (t, *J*= 2.2 Hz, 1H), 6.84 (d, *J*= 8.1 Hz, 1H), 6.93 (d, *J*= 2.2 Hz, 2H), 7.17 (dd, *J*= 8.3, 2 Hz, 1H), 7.24 (d, *J*= 2 Hz, 1H), 7.41 (d, *J*= 15.5 Hz, 1H), 7.57 (d, *J*= 15.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d) δ ppm: 106.31, 106.80, 115.24, 115.82, 118.67, 122.09, 126.24, 140.15, 144.56, 145.65, 140.70, 158.67, 188.98. HRMS (ES^-) : $[M+H]^-$ calculated for $C_{15}H_{13}O_5$: 273.0763; found: 273.0761; in accordance with [14]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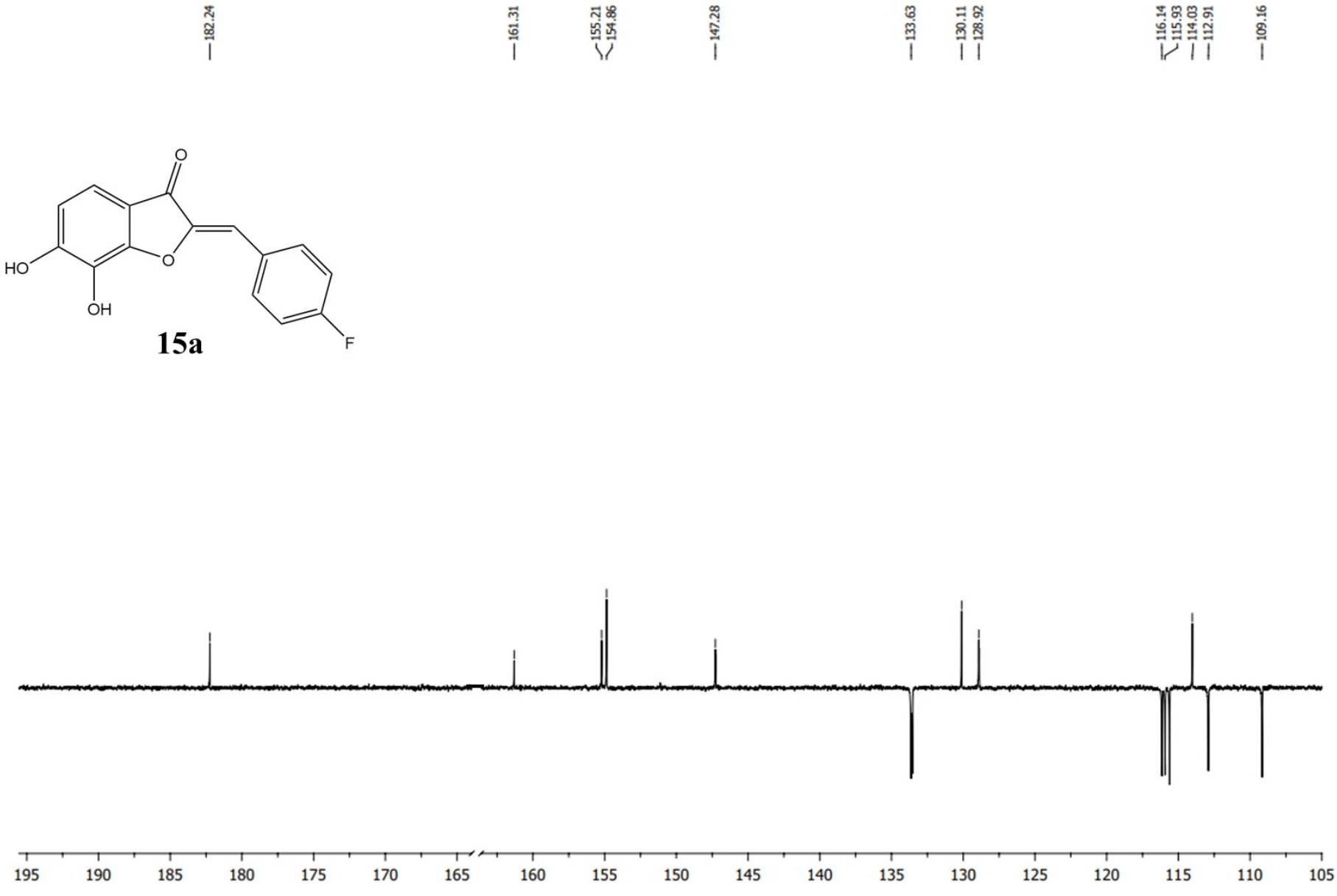
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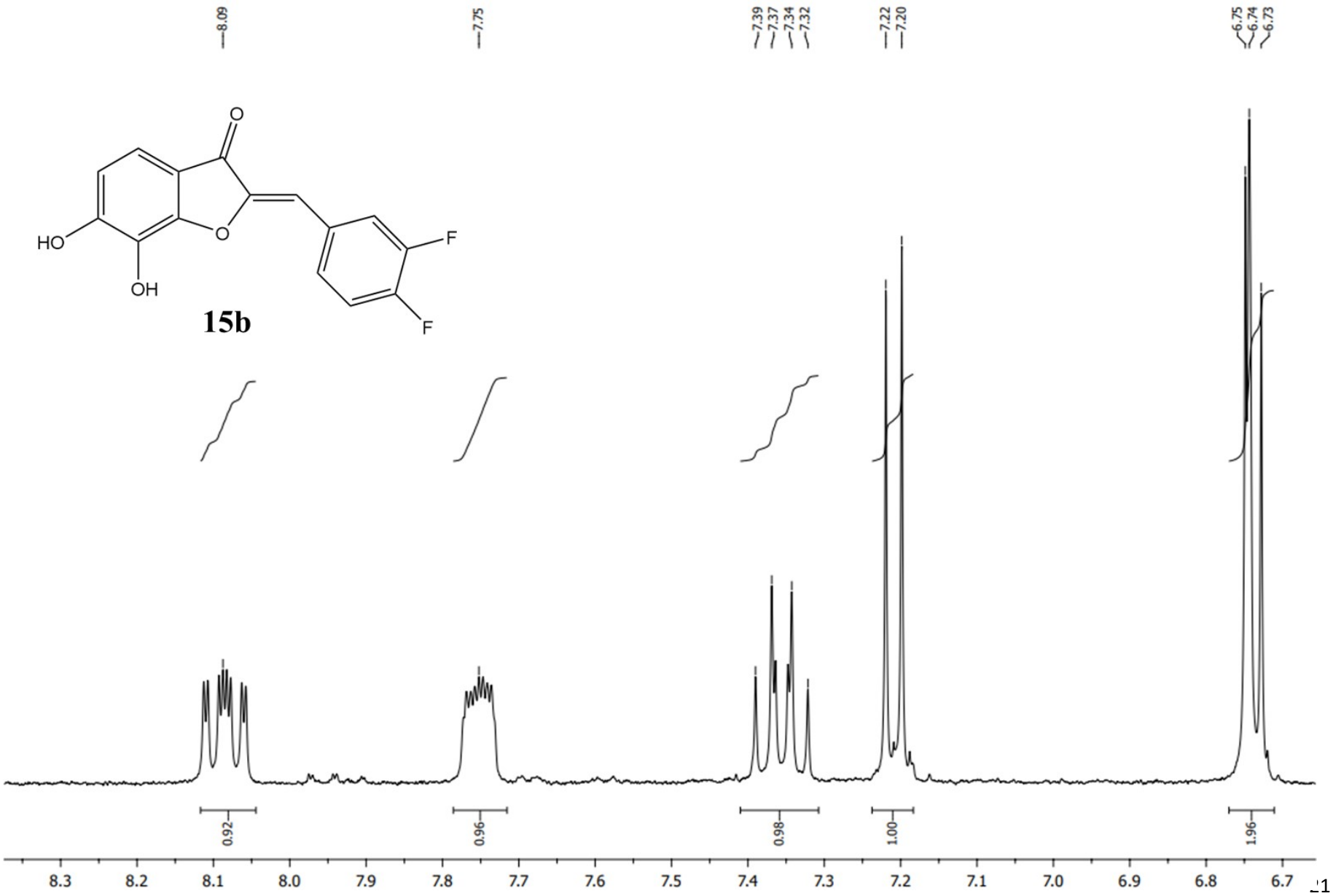
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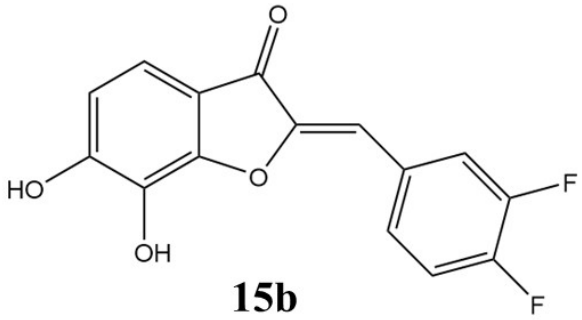
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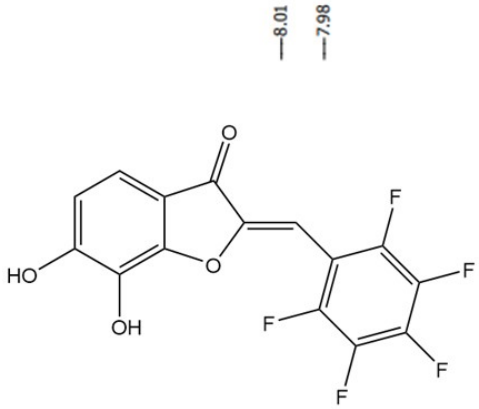
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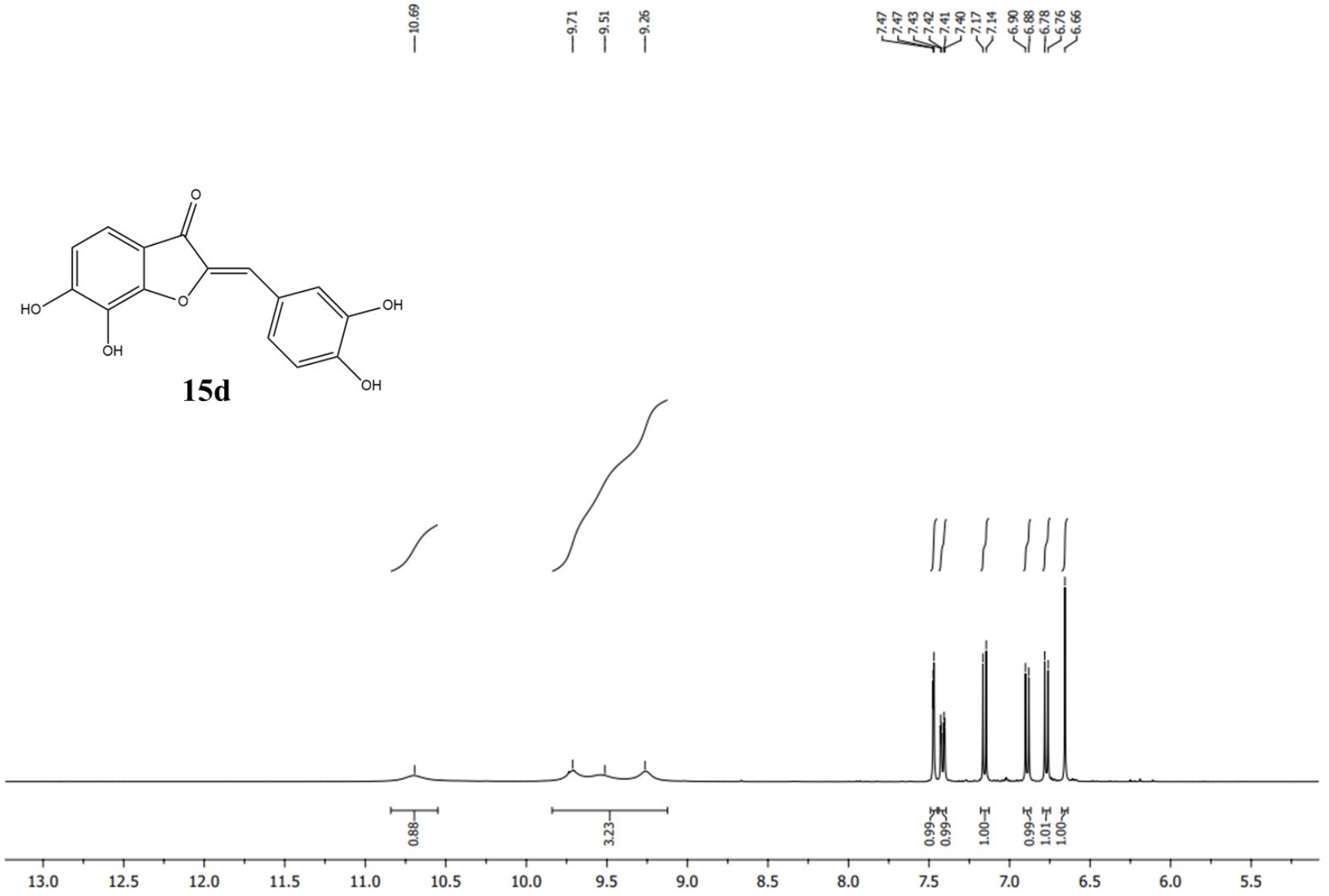
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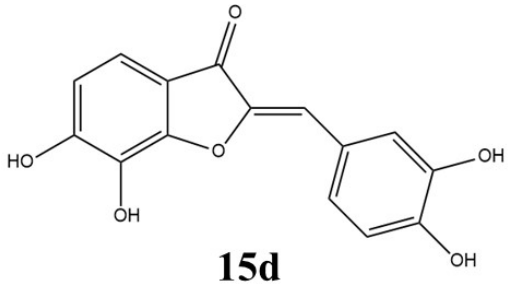
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115
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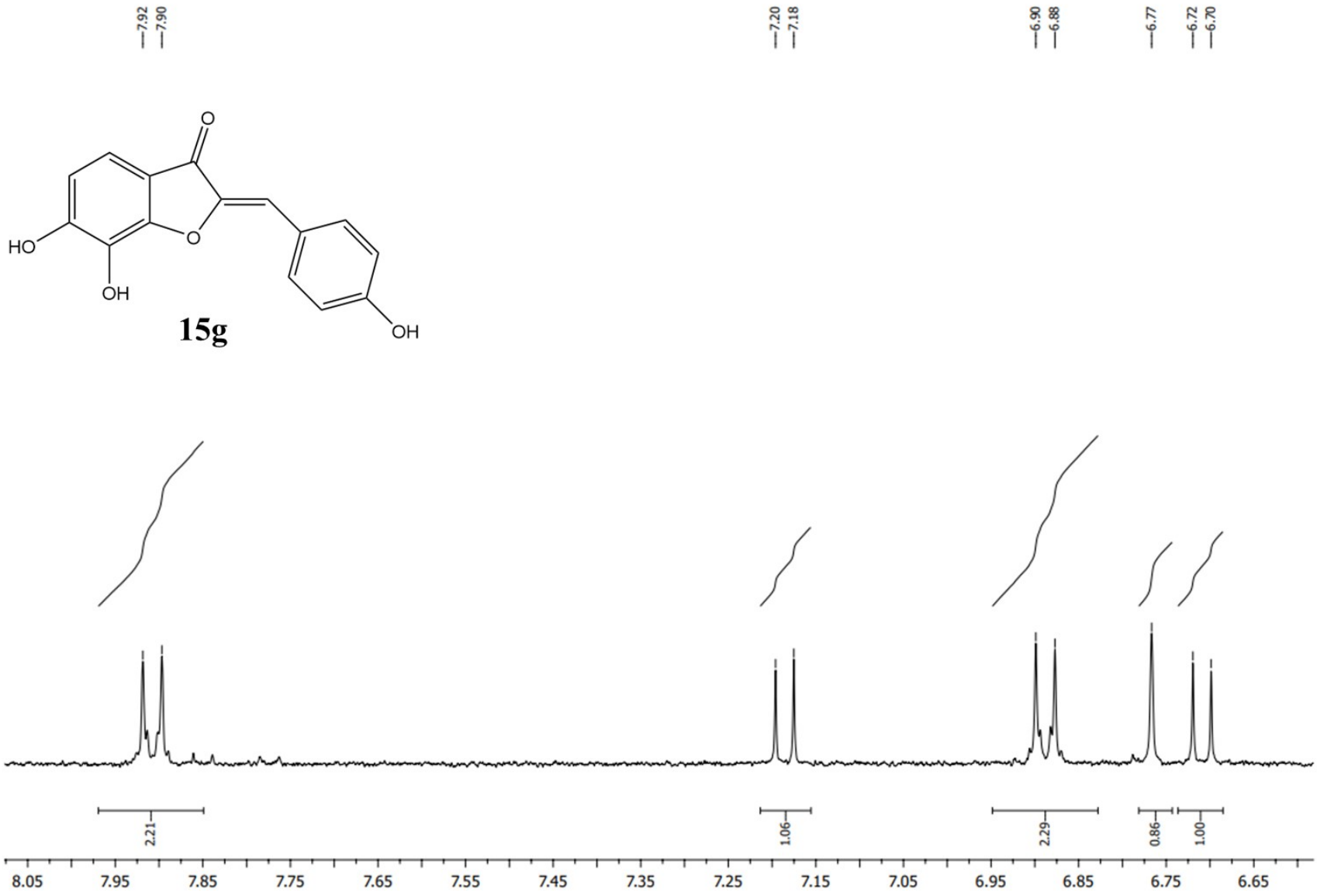
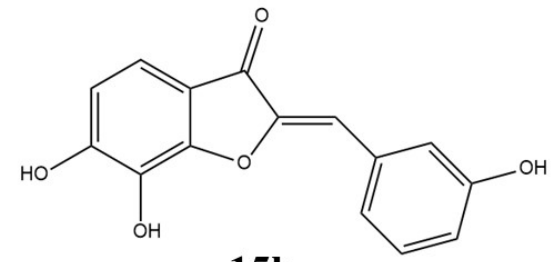
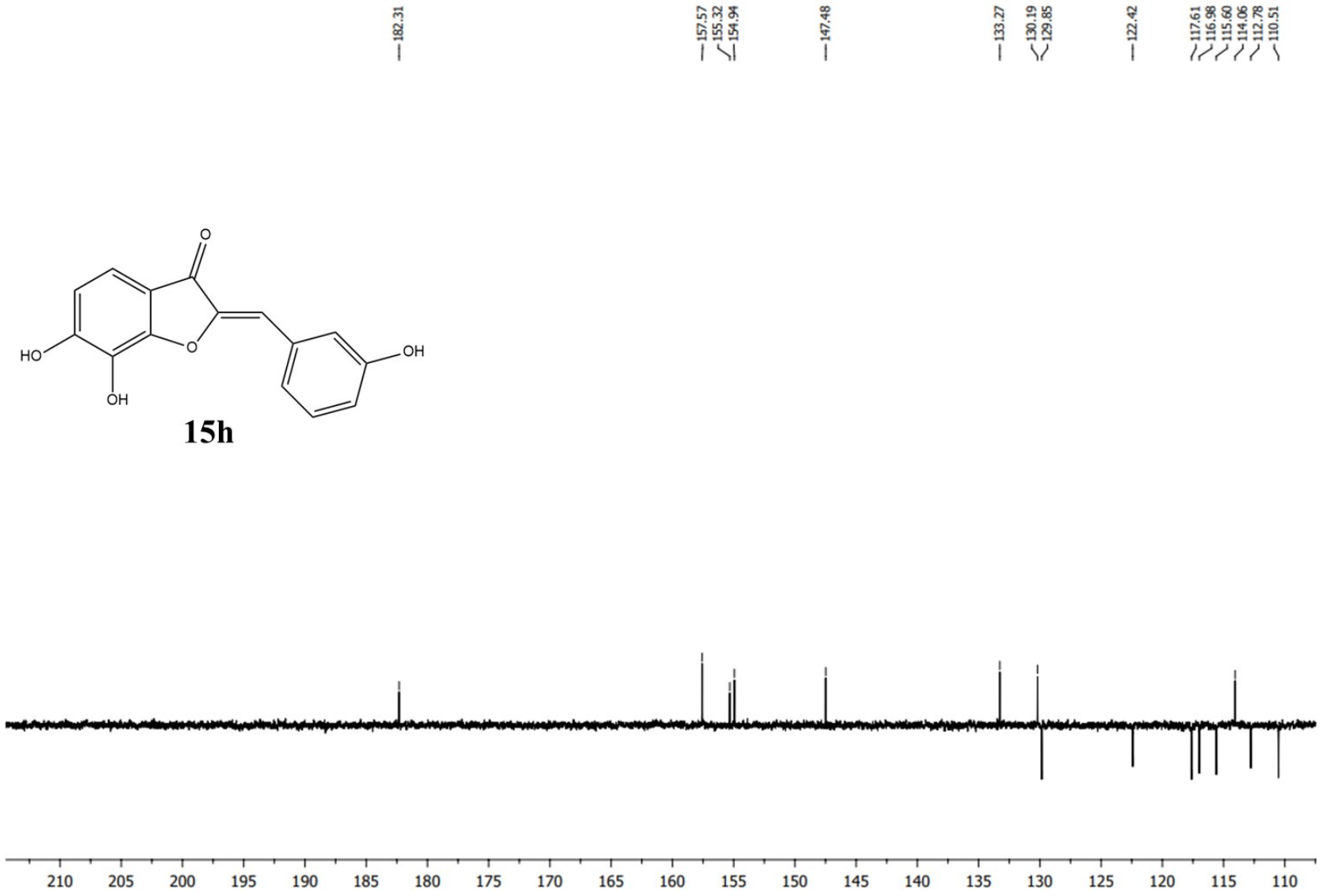

7.92
7.90
7.20
7.18
6.90
6.88
6.77
6.72
6.70
HO
OH
O
O
OH
15g
2.21
1.06
2.29
0.86
1.00
8.05
7.95
7.85
7.75
7.65
7.55
7.45
7.35
7.25
7.15
7.05
6.95
6.85
6.75
6.65

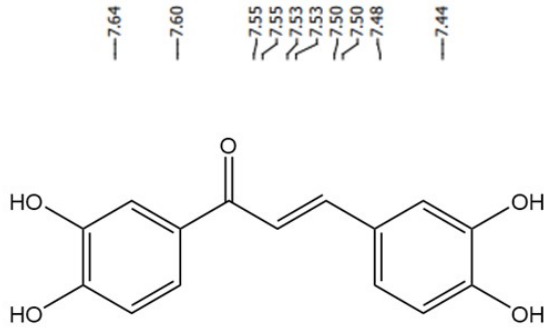

O
HO
O
OH
OH

15h

7.50
7.48
7.45
7.45
7.44
7.31
7.29
7.27
7.21
7.19
6.87
6.86
6.85
6.84
6.74
6.72
6.71
1.11
1.02
1.18
1.11
1.13
1.16
1.00
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.6

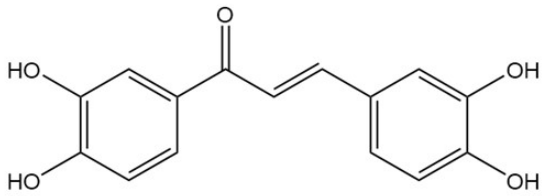

182.31
157.57
155.32
154.94
147.48
133.27
130.19
129.85
122.42
117.61
116.98
115.60
114.06
112.78
110.51
O
HO
O
OH
OH
210
205
200
195
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110

15h


HO
HO
O
OH
OH

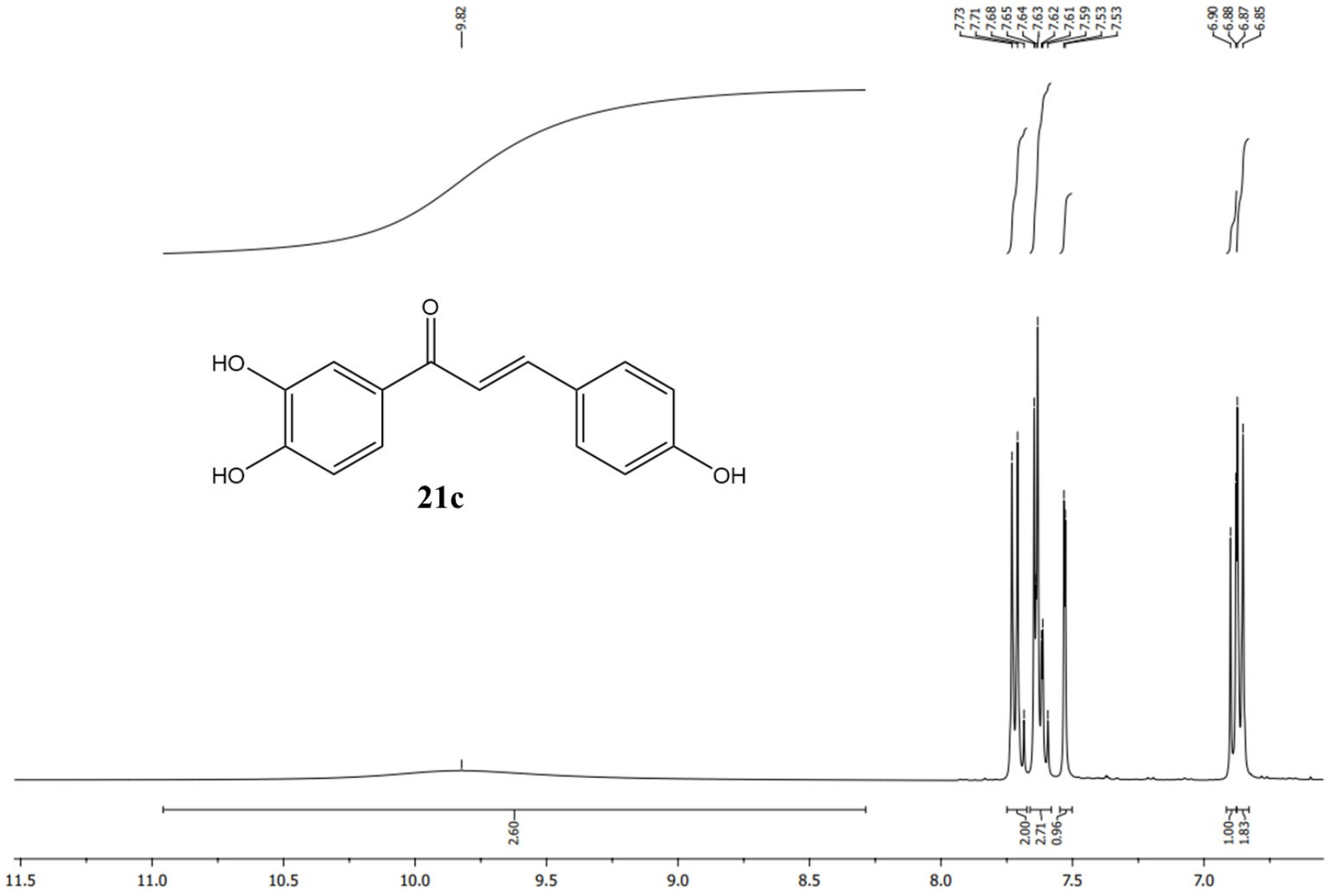
21a

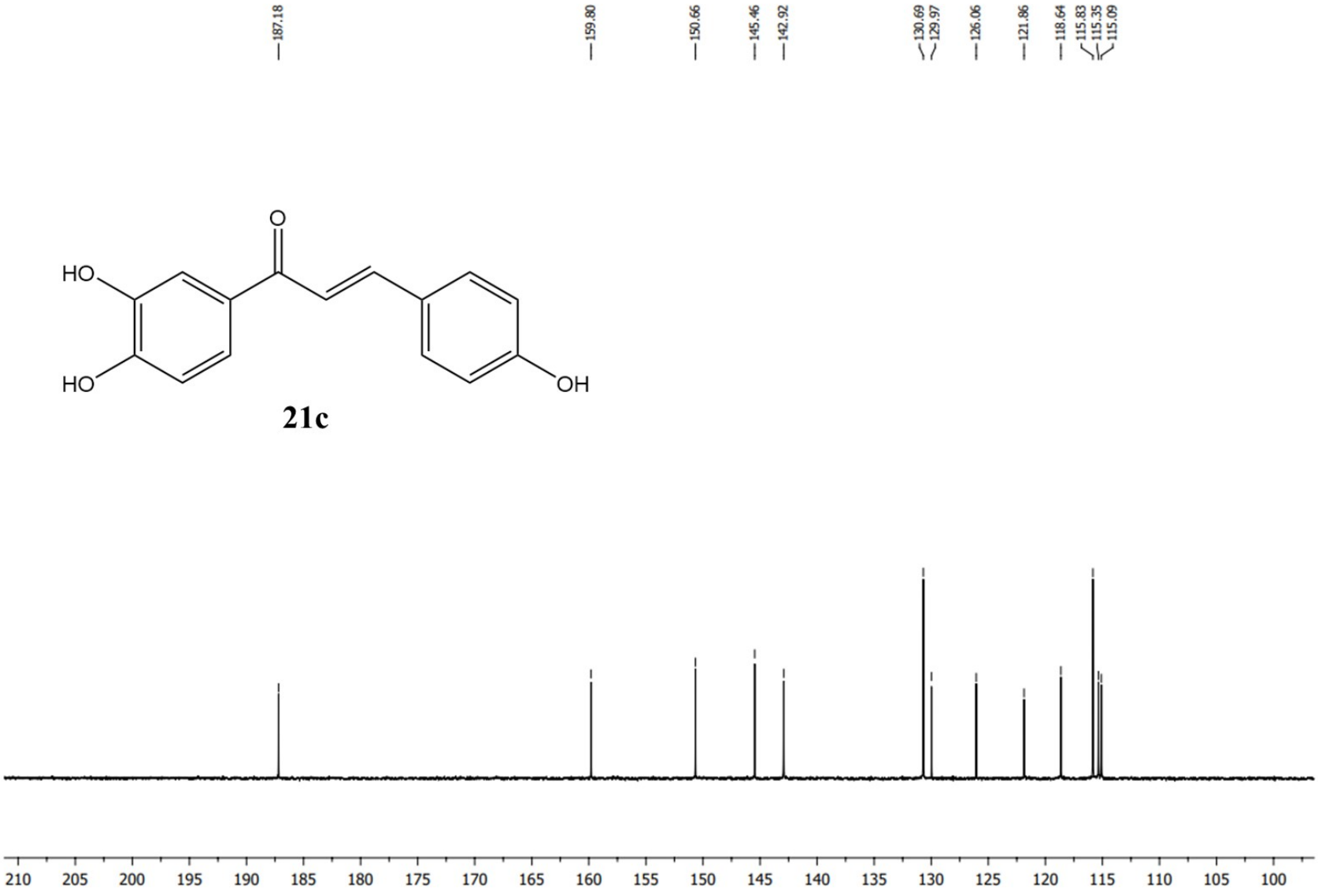
7.64
7.60
7.55
7.55
7.53
7.53
7.50
7.50
7.48
7.44
7.17
7.16
7.09
7.09
7.07
7.07
6.89
6.87
6.82
6.80
1.02
1.01
0.96
1.03
1.00
1.03
1.00
0.98
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
7.30
7.25
7.20
7.15
7.10
7.05
7.00
6.95
6.90
6.85
6.80
6.75
6.70


O
HO
HO
OH
OH

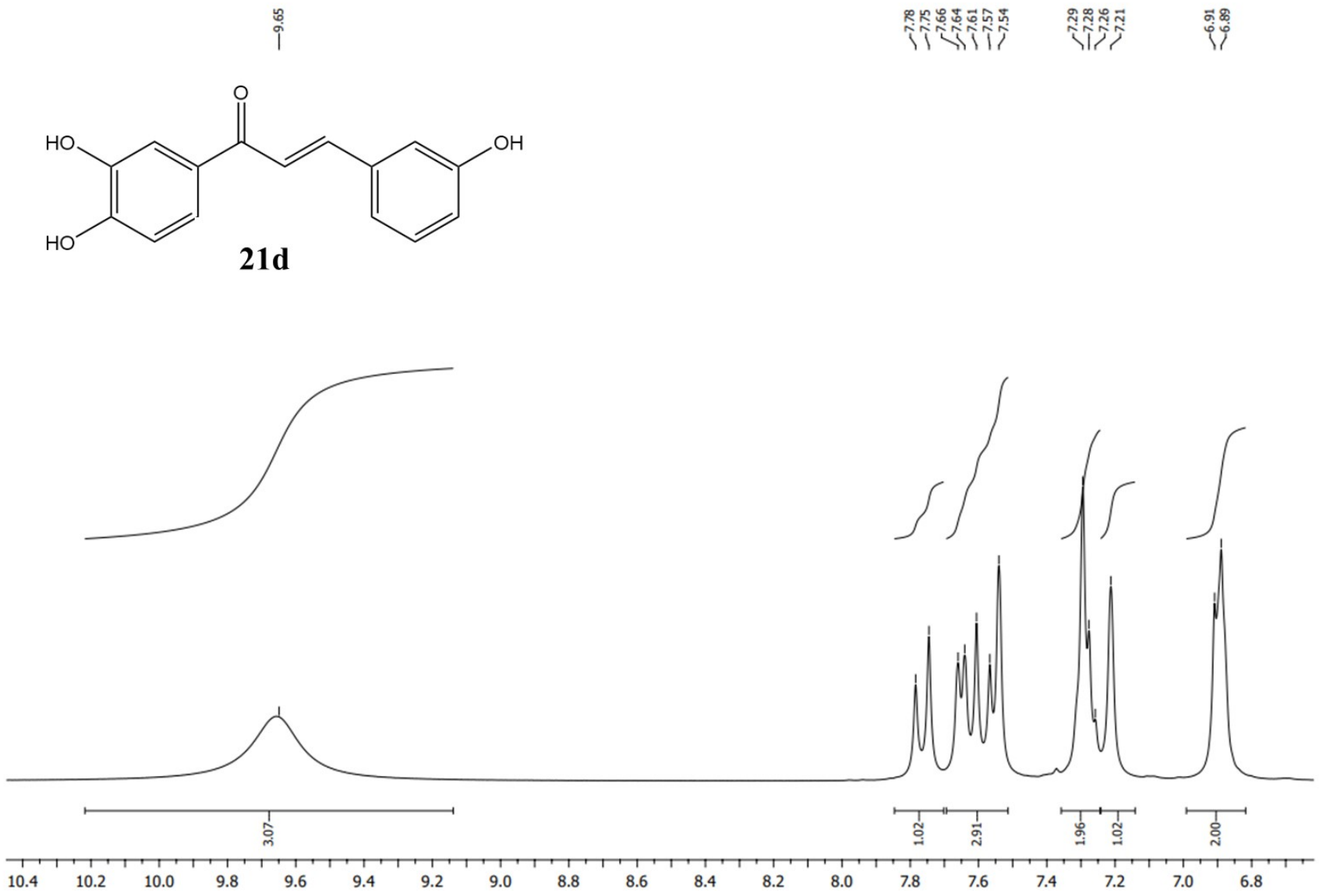
21a

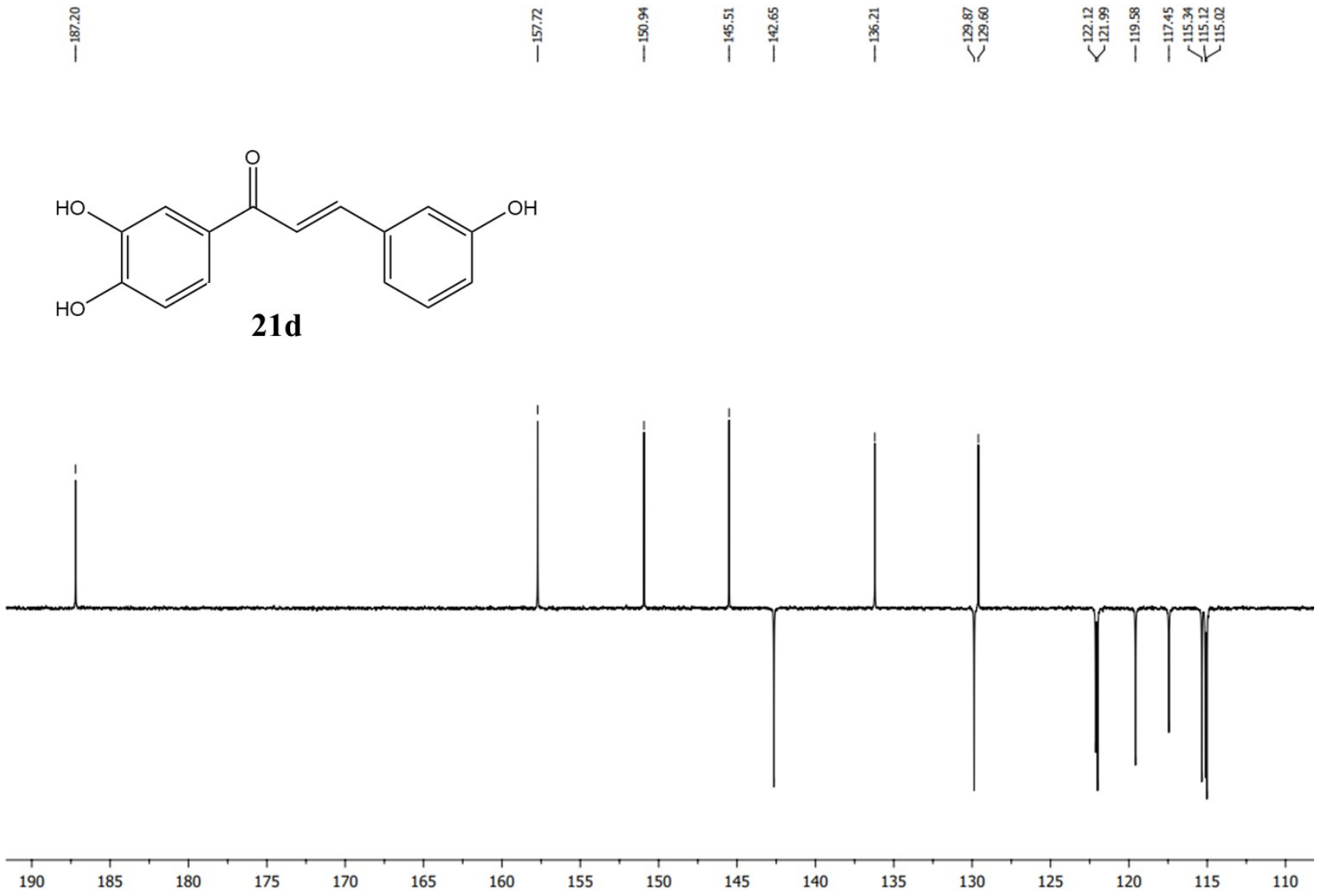
191.19
152.14
149.76
146.83
146.62
146.00
131.79
128.48
123.41
123.39
119.61
116.59
116.34
115.91
115.59
195
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115

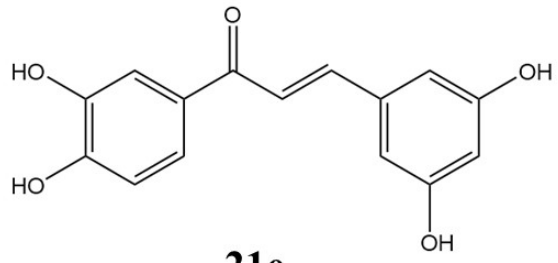

9.82
7.73
7.71
7.68
7.65
7.64
7.63
7.62
7.61
7.59
7.53
7.53
6.90
6.88
6.87
6.85
HO
HO
O
OH
21c
2.60
2.00
2.71
0.96
1.00
1.83
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0


187.18
159.80
150.66
145.46
142.92
130.69
129.97
126.06
121.86
118.64
115.83
115.35
115.09
HO
HO
O
OH
210
205
200
195
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100

21c

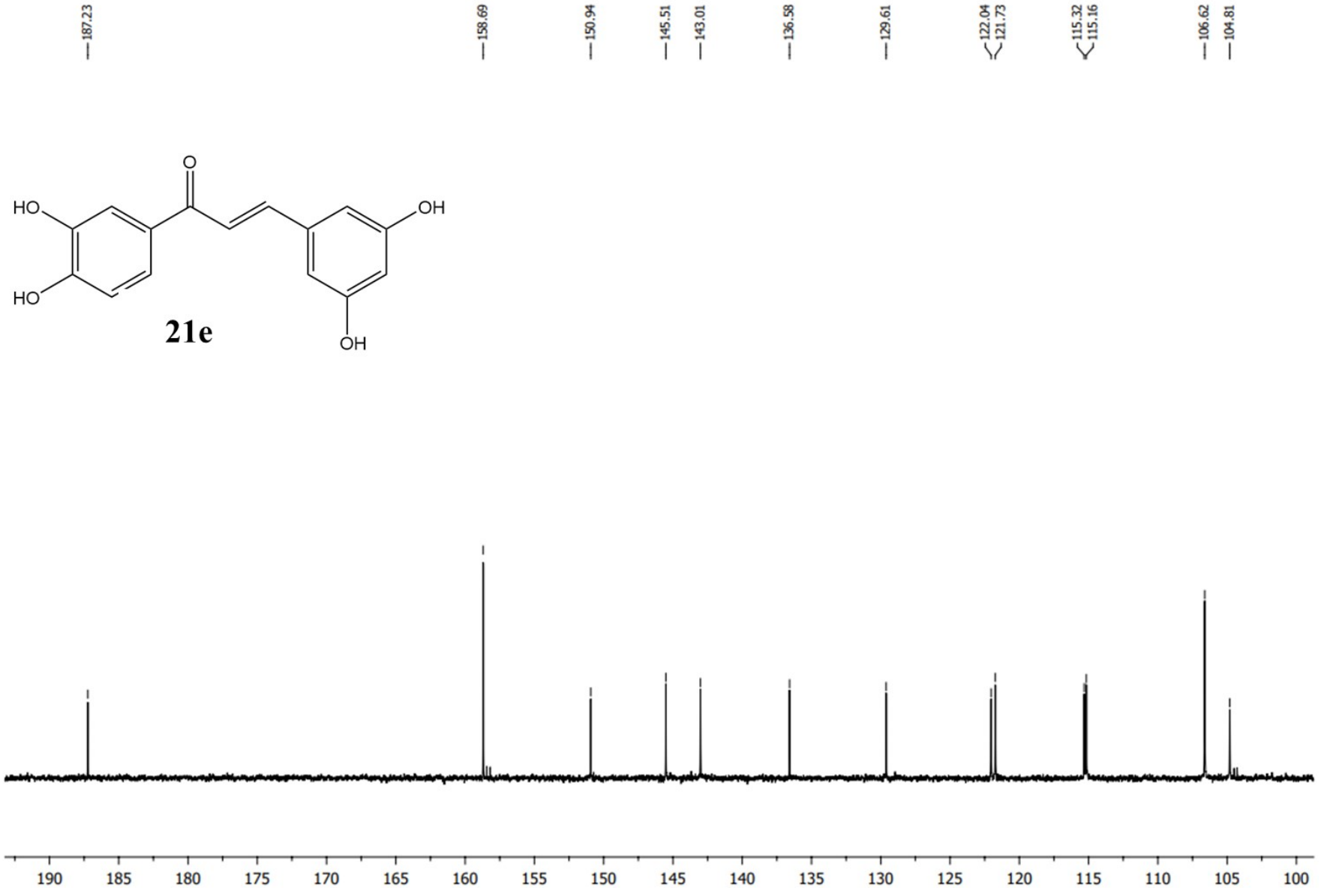

9.65
7.78
7.75
7.66
7.64
7.61
7.57
7.54
7.29
7.28
7.26
7.21
6.91
6.89
HO
HO
O
OH
21d
3.07
1.02
2.91
1.96
1.02
2.00
10.4
10.2
10.0
9.8
9.6
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8

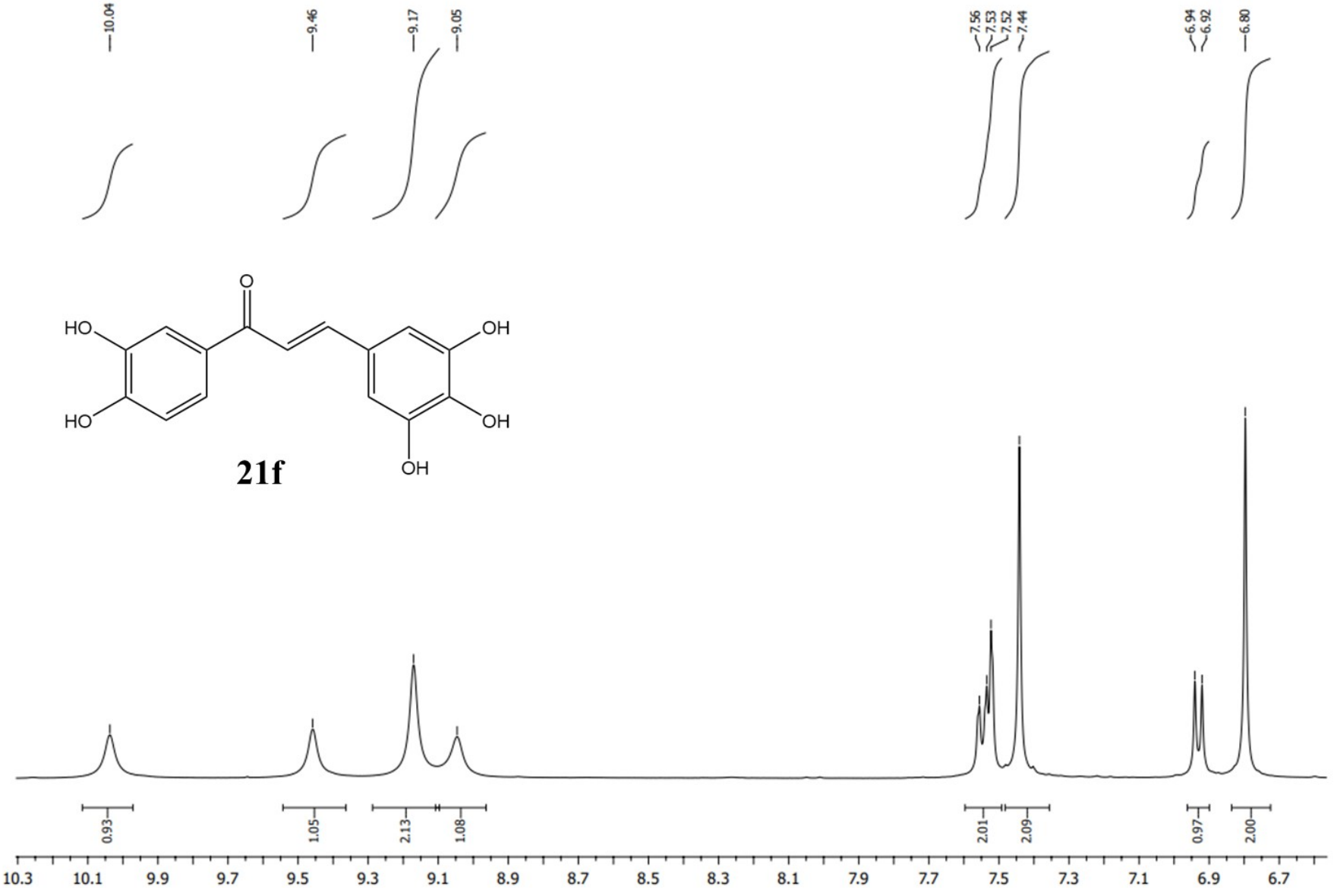

187.20
157.72
150.94
145.51
142.65
136.21
129.87
129.60
122.12
121.99
119.58
117.45
115.34
115.12
115.02
HO
HO
O
OH
21d
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110


O
HO
HO
OH
OH

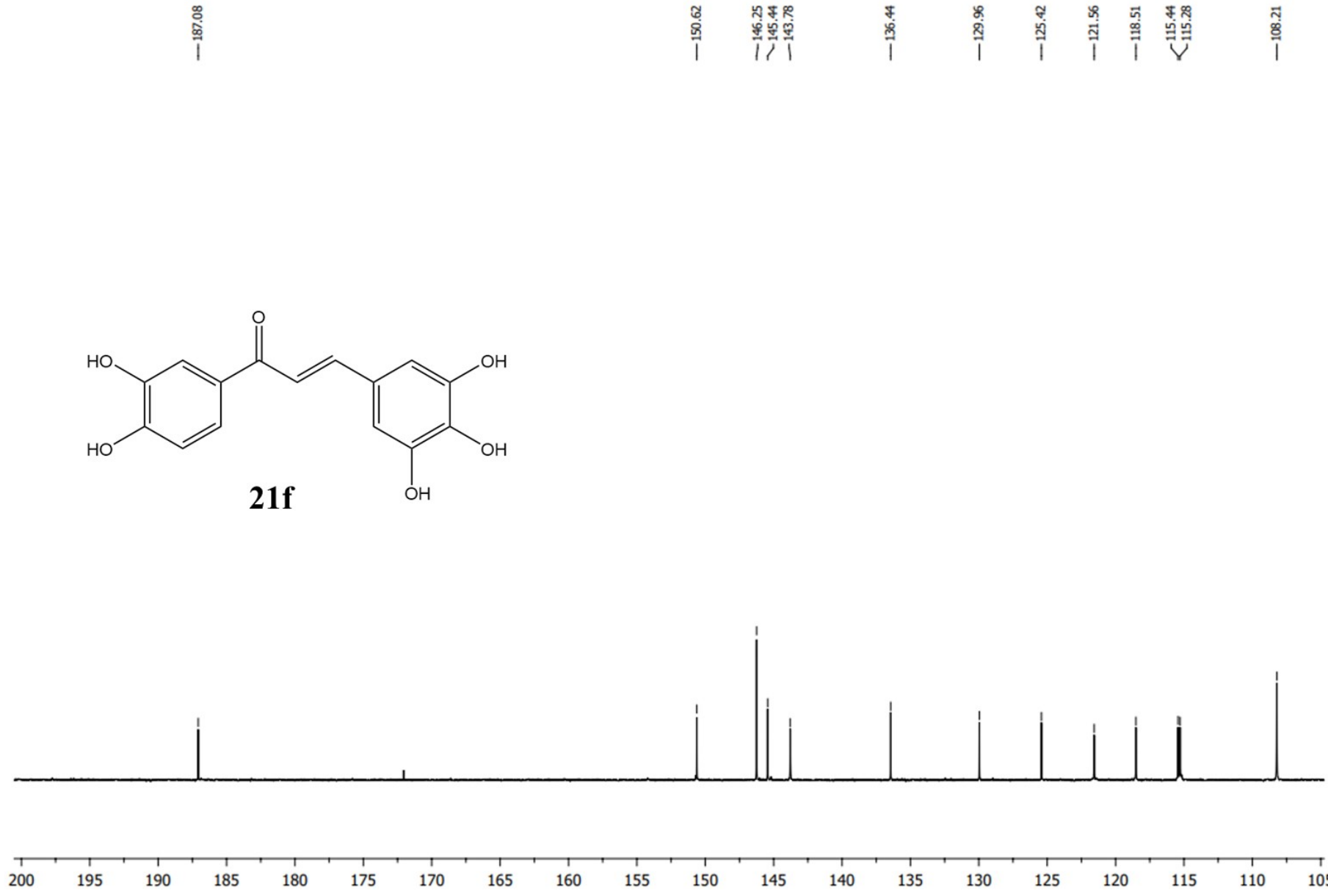
21e

9.56
7.66
7.62
7.60
7.60
7.51
7.49
7.45
6.90
6.88
6.67
6.36
4.02
1.92
0.88
1.04
1.01
1.91
1.00
10.6
10.4
10.2
10.0
9.8
9.6
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4

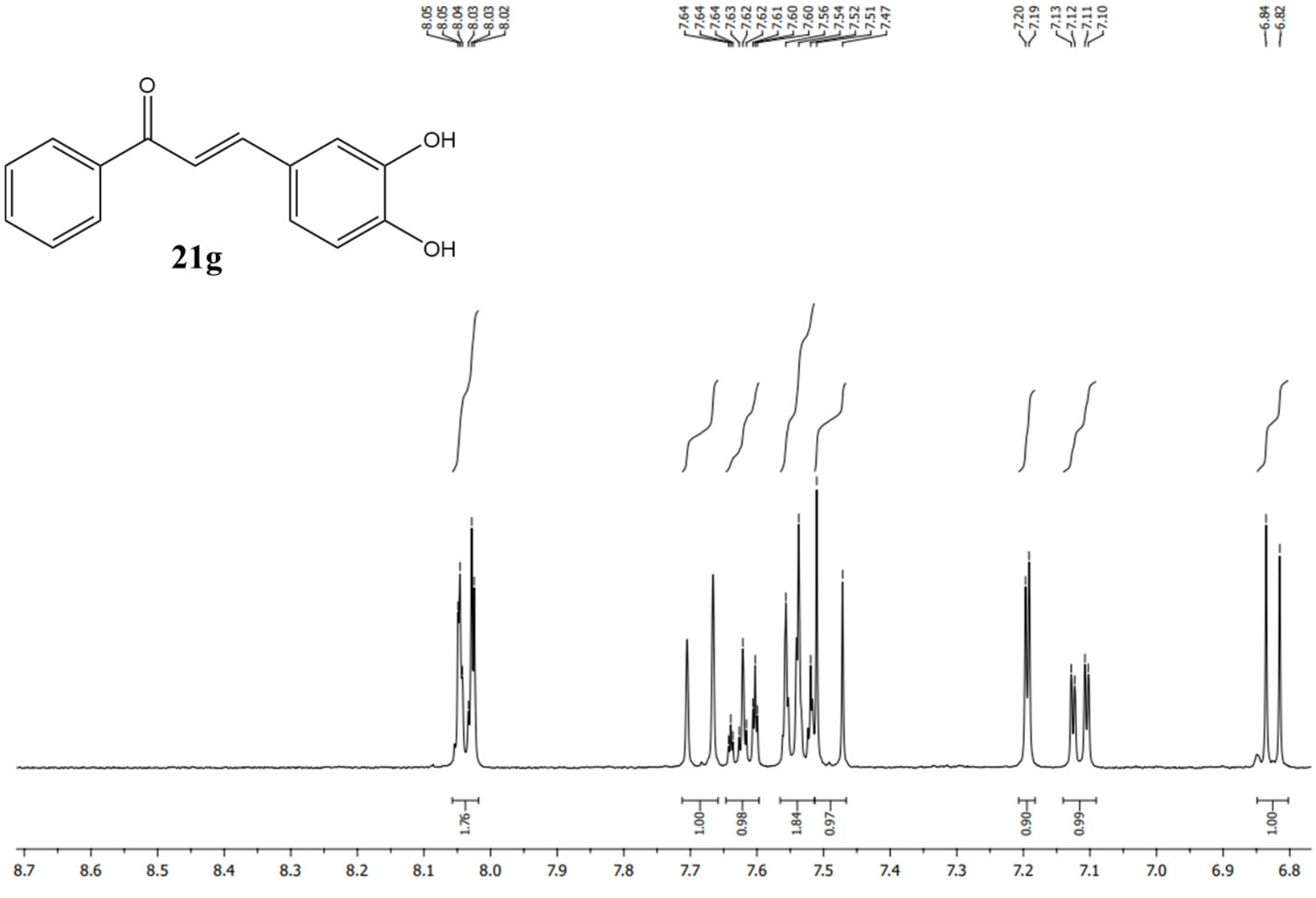

187.23
158.69
150.94
145.51
143.01
136.58
129.61
122.04
121.73
115.32
115.16
106.62
104.81
HO
HO
OH
OH
O
21e
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100


10.04
9.46
9.17
9.05
7.56
7.53
7.52
7.44
6.94
6.92
6.80
HO
HO
O
OH
OH
OH
0.93
1.05
2.13
1.08
2.01
2.09
0.97
2.00
10.3
10.1
9.9
9.7
9.5
9.3
9.1
8.9
8.7
8.5
8.3
8.1
7.9
7.7
7.5
7.3
7.1
6.9
6.7

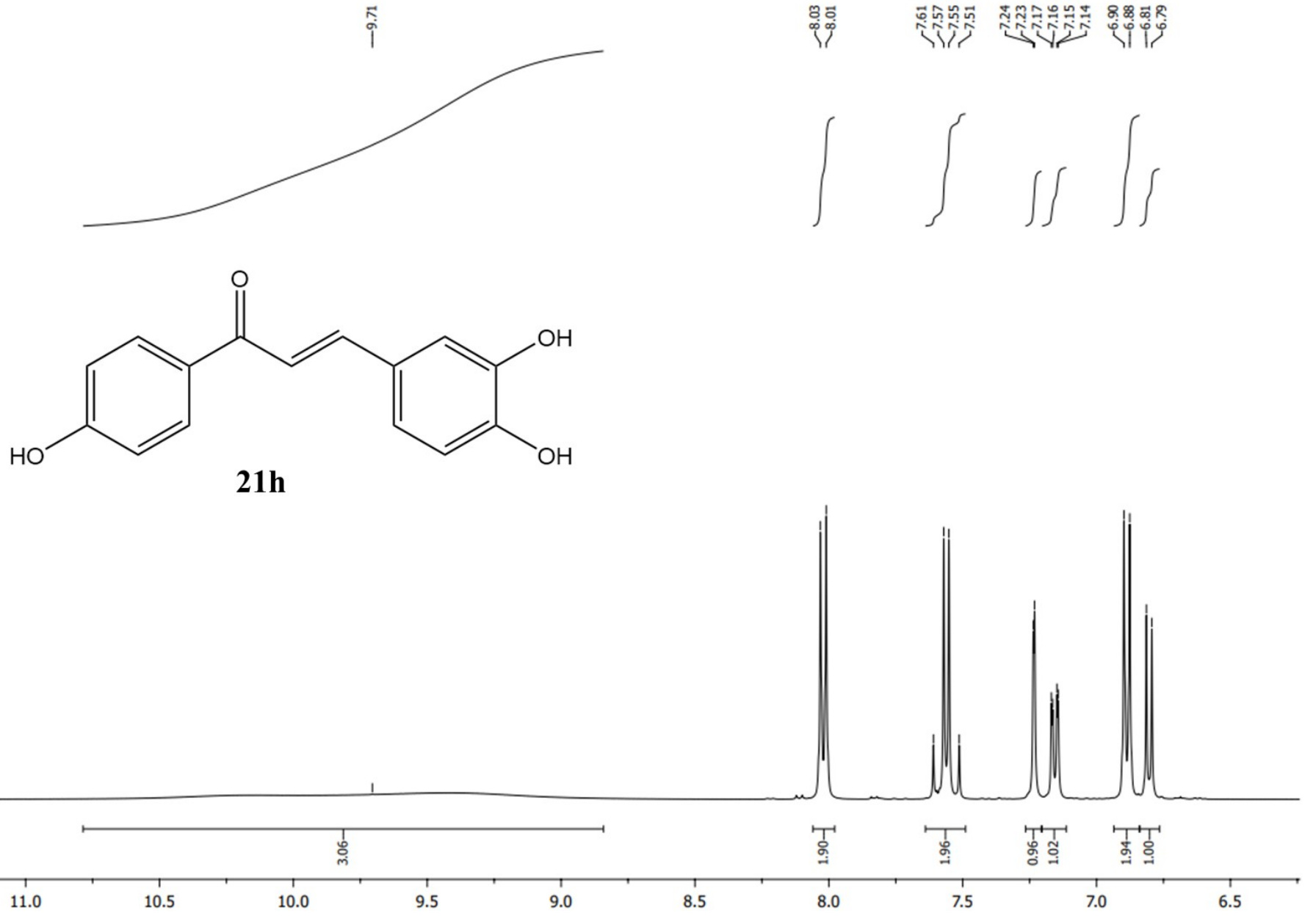
21f


187.08
150.62
146.25
145.44
143.78
136.44
129.96
125.42
121.56
118.51
115.44
115.28
108.21
HO
HO
O
OH
OH
OH
200
195
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105

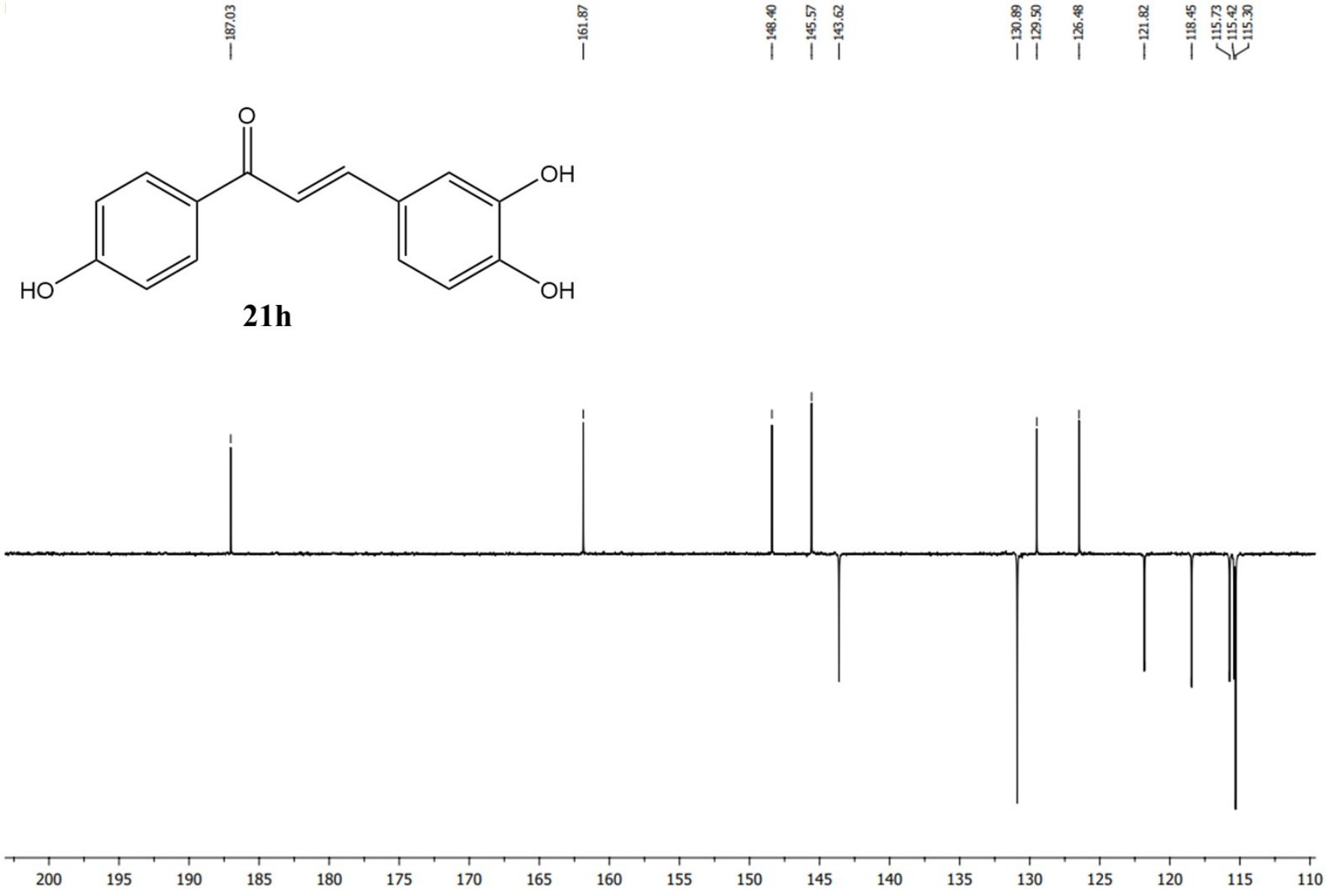
21f


8.05
8.05
8.04
8.03
8.03
8.02
7.64
7.64
7.64
7.63
7.62
7.62
7.61
7.60
7.60
7.56
7.54
7.52
7.51
7.47
7.20
7.19
7.13
7.12
7.11
7.10
6.84
6.82
O
OH
OH
21g
1.76
1.00
0.98
1.84
0.97
0.90
0.99
1.00
8.7
8.6
8.5
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8

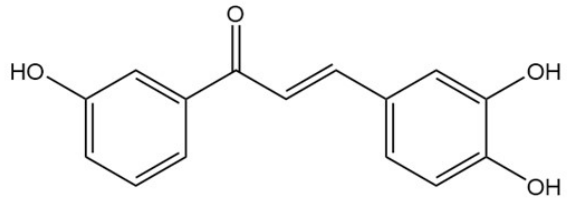

192.73
150.16
147.46
146.91
139.75
133.92
129.77
129.48
128.24
123.76
119.68
116.63
115.73
O
OH
OH
21g
195
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115


9.71
8.03
8.01
7.61
7.57
7.55
7.51
7.24
7.23
7.17
7.16
7.15
7.14
6.90
6.88
6.81
6.79
O
OH
HO
OH
3.06
1.90
1.96
0.96
1.02
1.94
1.00
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5

21h

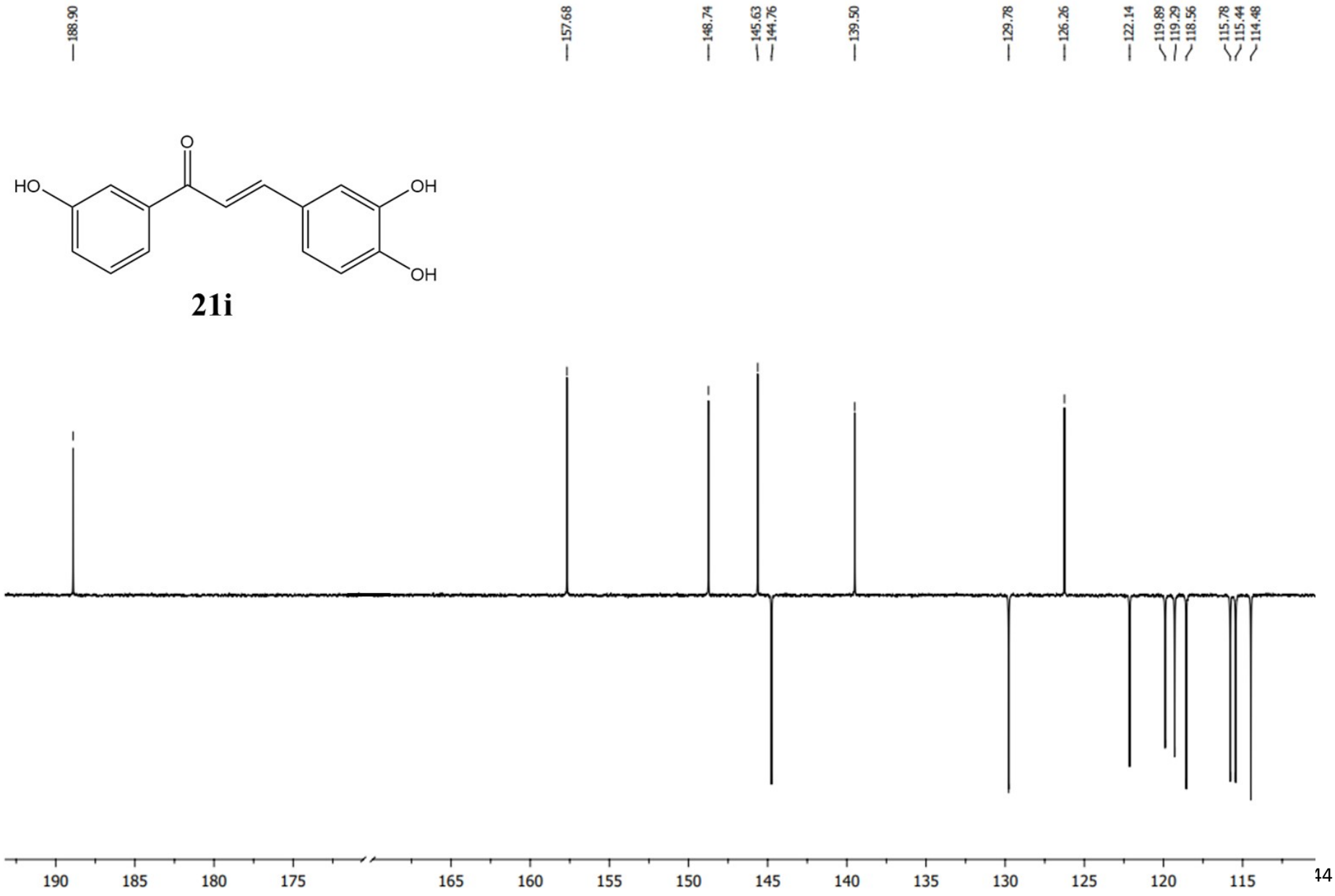

187.03
161.87
148.40
145.57
143.62
130.89
129.50
126.48
121.82
118.45
115.73
115.42
115.30
O
OH
HO
OH
200
195
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110

21h

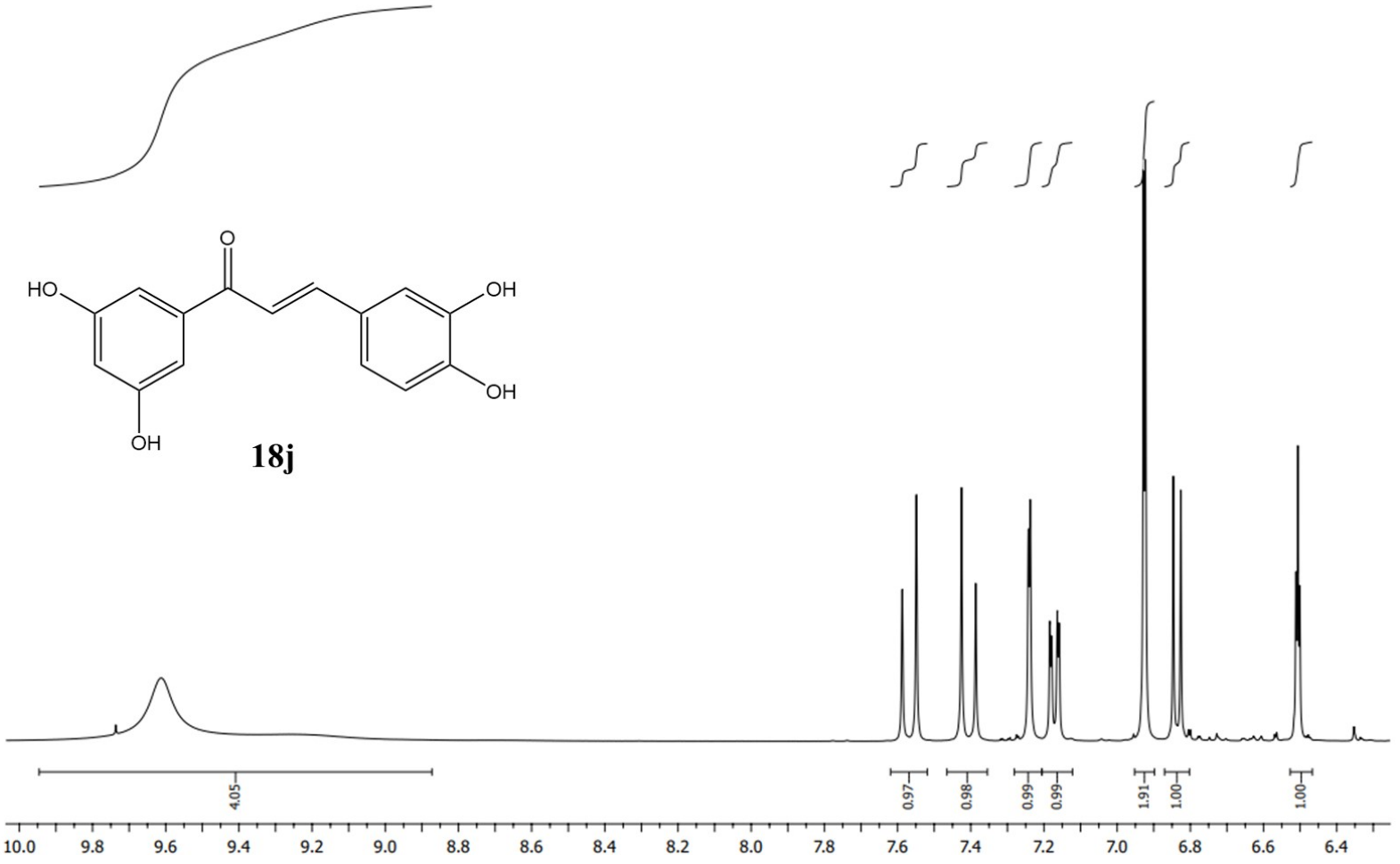

HO
O
OH
OH

21i

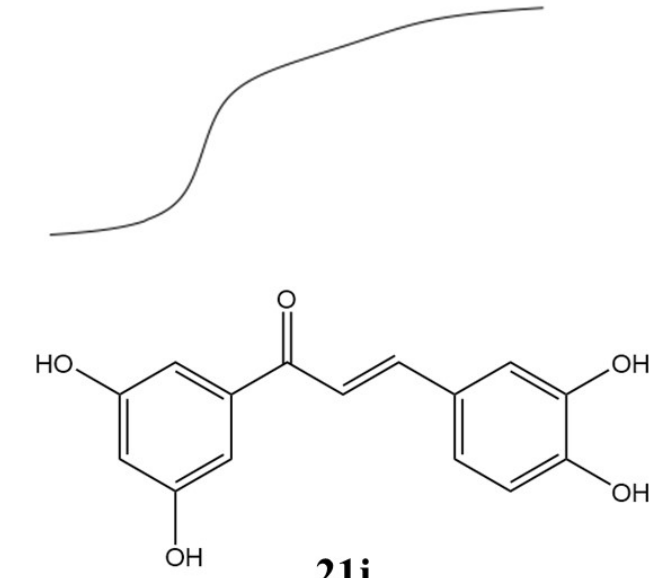
9.78
9.73
9.15
7.63
7.60
7.59
7.58
7.56
7.52
7.45
7.45
7.44
7.41
7.39
7.37
7.28
7.27
7.21
7.21
7.19
7.19
7.08
7.08
7.08
7.07
6.83
1.04
1.02
0.97
3.09
1.00
1.04
0.99
1.00
1.00
1.00
10.1
9.9
9.7
9.5
9.3
9.1
8.9
8.7
8.5
8.3
8.1
7.9
7.7
7.5
7.3
7.1
6.9
6.7
6.5


188.90
157.68
148.74
145.63
144.76
139.50
129.78
126.26
122.14
119.89
119.29
118.56
115.78
115.44
114.48
HO
O
OH
OH
190
185
180
175
165
160
155
150
145
140
135
130
125
120
115

21i

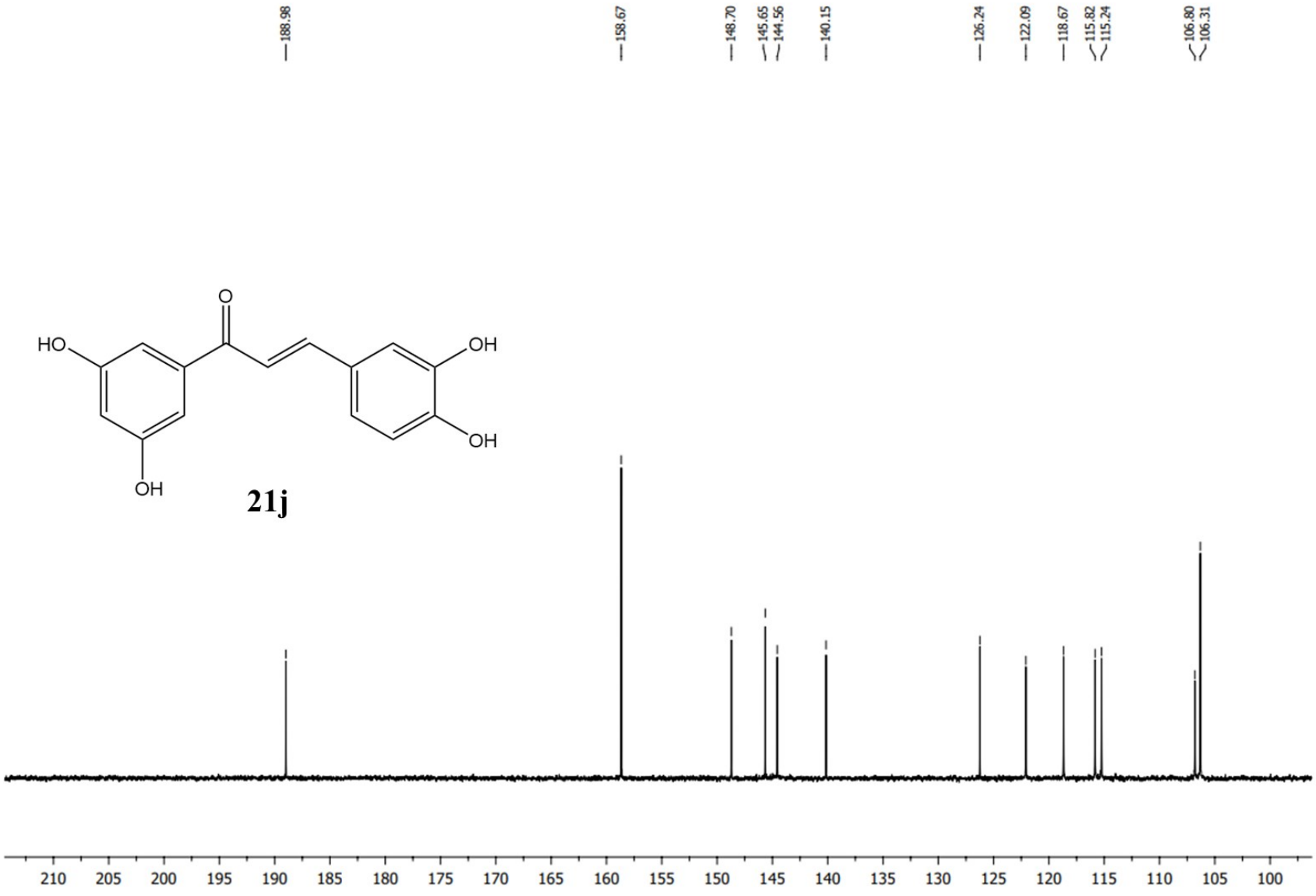

HO
O
OH
OH
OH
4.05
0.97
0.98
0.99
0.99
1.91
1.00
1.00
10.0
9.8
9.6
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4

18j

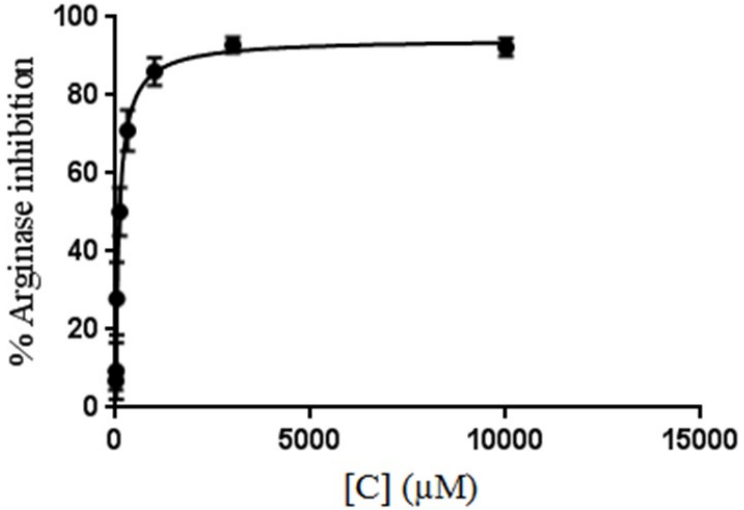

O
HO
OH
OH
OH

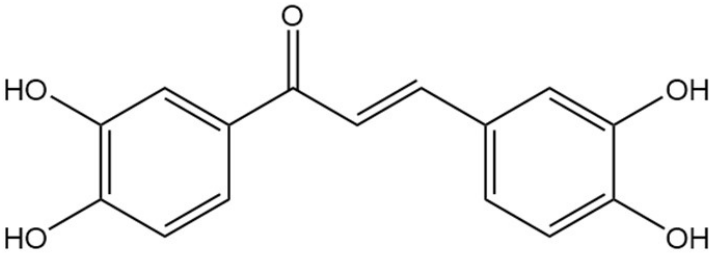
21j

4.05
0.97
0.98
0.99
0.99
1.91
1.00
1.00
10.0
9.8
9.6
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4

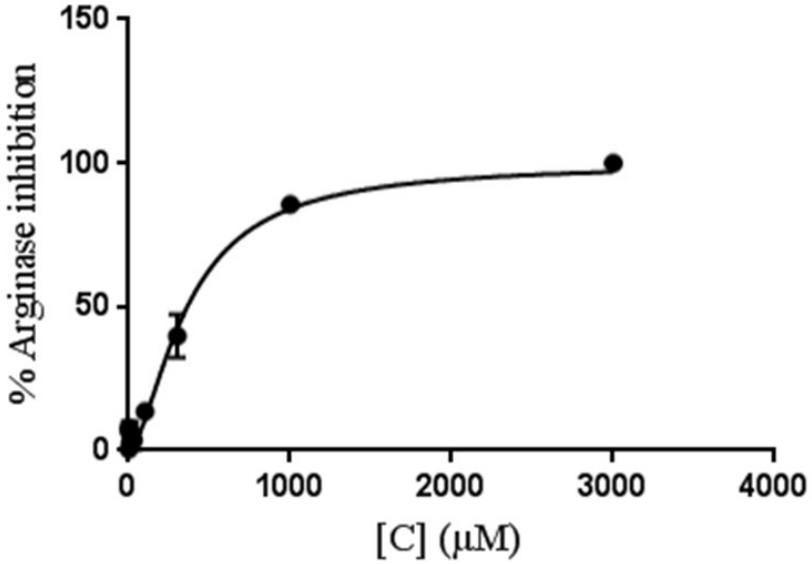

188.98
158.67
148.70
145.65
144.56
140.15
126.24
122.09
118.67
115.82
115.24
106.80
106.31
HO
OH
OH
OH
O
21j
210 205 200 195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100

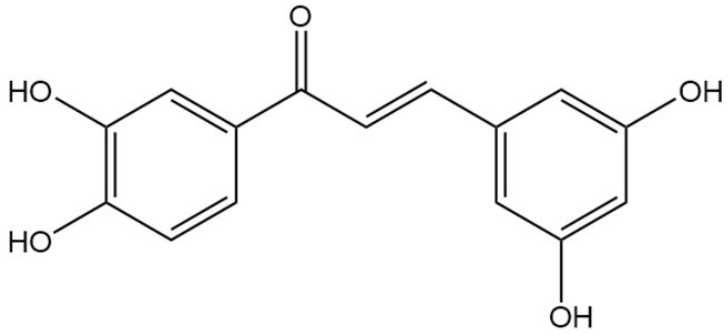
21a


% Arginase inhibition
100
80
60
40
20
0
0
5000
10000
15000
[C] (µM)

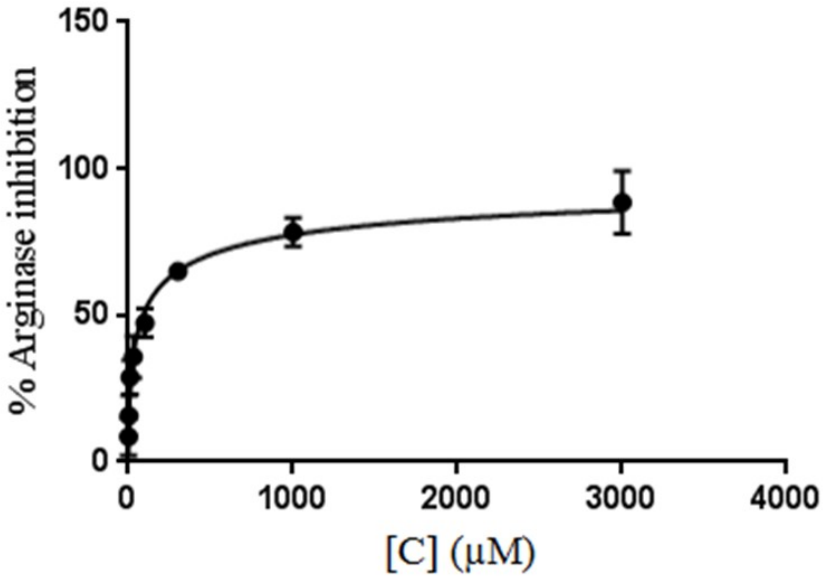

O
HO
HO
OH
OH

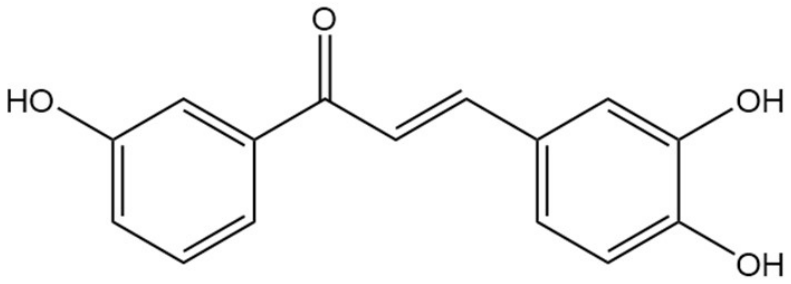
21e


% Arginase inhibition
150
100
50
0
0
1000
2000
3000
4000
[C] (μM)

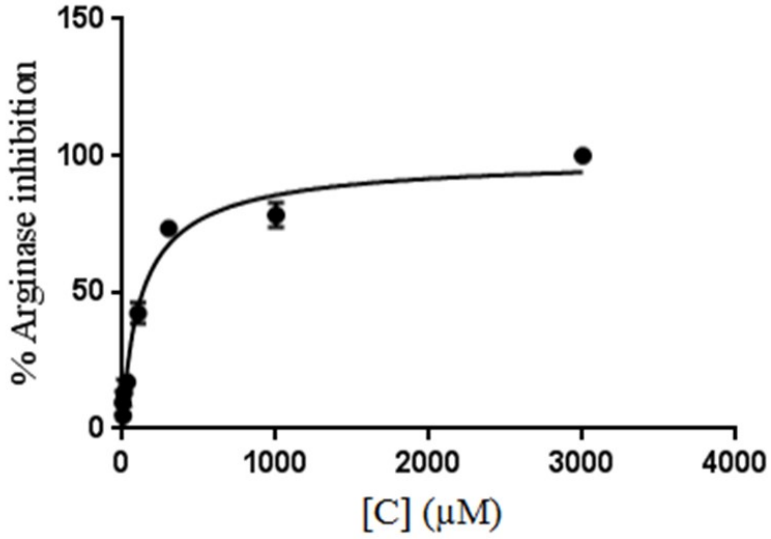

O
HO
HO
OH
OH

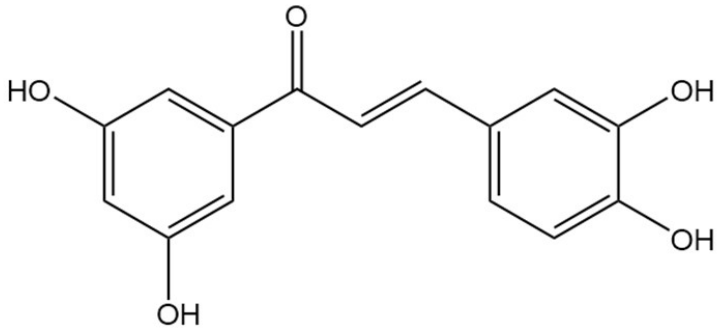
21i


% Arginase inhibition
150
100
50
0
0
1000
2000
3000
4000
[C] (μM)


O
HO
OH
OH

21j


% Arginase inhibition
150
100
50
0
0
1000
2000
3000
4000
[C] (µM)


O
HO
OH
OH
OH

Quantum chemistry modelling

The quantum chemistry model used in this study is taken from the X-ray diffraction structure 3KV2 obtained by Di Constanzo and coworkers.[15] It has been applied successfully to a previous work[16] and technical details can be found in the Supplementary Information section of this article.

The model features 21 residues, for a total of 241 atoms. The Cartesian coordinates of 25 of them, mostly alpha-carbon atoms, were frozen to take into account the rigidity of the enzymatic structure around the manganese cluster. Softer constraints were applied at the entrance of the cavity. The model is represented in **figure S4**.

All optimisations were carried out at the DFT(B3LYP)/6-31G(d) level of theory. The IEFPCM solvent model was applied to the system for all calculations, with a dielectric constant of $\varepsilon = 78$.

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