Supporting Information

Oxyprenyl-Chalcones as Antibacterial Hits: Design of Experiments-Optimized Synthesis, Antibacterial Evaluation, Early Drug-Like Profiling and Biodegradability Prediction

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Content

1. Experimental Methods - Chemistry

1-(2-Hydroxy-4-((3-methylbut-2-en-1-yl)oxy)phenyl)ethan-1-one (8a). In a round-bottom flask, a mixture of 1-(2,4-dihydroxyphenyl)ethan-1-one **7a** (100 mg, 0.66 mmol), 1-bromo-3-methylbut-2-ene (0.14 mL, 1.0 mmol) and K_2CO_3 (100 mg, 0.72 mmol) were stirred in acetone (7 mL) at 80 °C for 16 h. Upon completion of the reaction, monitored by TLC, the mixture was filtered at the same temperature and the solvent was evaporated under reduced pressure. The crude product was purified through silica gel column chromatography (PE/EtOAc 10:1), affording compound **8a** as colorless oil (143 mg, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.73 (s, 1H, OH), 7.62 (d, *J* = 8.7 Hz, 1H, ArH), 6.49 – 6.40 (m, 2H, ArH), 5.53 – 5.35 (m, 1H, C=CH-), 4.51 (d, *J* = 6.8 Hz, 2H, CH_2), 2.52 (s, 3H, COMe), 1.80 (s, 3H, =CMe_2), 1.75 (m, 3H, =CMe_2). ESI-MS *m/z* $[M+H]^+$ 221; $[M+Na]^+$ 243.

1-{4-[(3-Methylbut-2-en-1-yl)oxy]phenyl}ethan-1-one (8b). Starting from **7b**, the title compound was obtained in 79% yield, following the synthetic procedure described for the synthesis of **8a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.9 Hz, 2H, ArH), 6.96 (d, *J* = 8.9 Hz, 2H, ArH), 5.49 (t, *J* = 7.6 Hz, 1H, C=CH-), 4.60 (d, *J* = 6.8 Hz, 2H, CH_2), 2.55 (s, 3H, COMe), 1.81 (s, 3H, =CMe_2), 1.76 (s, 3H, =CMe_2).

4-(Methoxymethoxy)benzaldehyde (10). To a solution of 4-hydroxybenzaldehyde **9** (500 mg, 4.1 mmol) in anhydrous DCM (38 mL) cooled at 0 °C, *N*,*N*-diisopropylethylamine (DIPEA) (2.16 mL, 12.3 mmol) and chloro(methoxy)methane (0.94 mL, 13.3 mmol) were slowly added under N_2 atmosphere. The reaction mixture was stirred for 1 h, after this time a saturated solution of $NaHCO_3$ (5 mL) was added to the reaction mixture, and the aqueous phase was extracted using DCM (3 x 10 mL). The combined organic extracts were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was used without any further purification (888 mg, 98% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.88 (s, 1H, CHO), 7.82 (d, *J* = 8.7 Hz, 2H, ArH), 7.13 (d, *J* = 8.6 Hz, 2H, ArH), 5.24 (s, 2H, OCH_2), 3.47 (s, 3H, OMe). ESI-MS *m/z* $[M+H]^+$ 167; $[M+Na]^+$ 189.

(2*E*)-1-{2-Hydroxy-4-[(3-methylbut-2-en-1-yl)oxy]phenyl}-3-(4-hydroxyphenyl)prop-2-en-1-one (1a). In a round-bottom flask **8a** (108.0 mg, 0.49 mmol) and **9** (59.7 mg, 0.49 eq) were solubilized in EtOH (0.5 mL), and the mixture was heated at 78 °C, then an aqueous solution of NaOH (50% w/w) (0.2 mL) was slowly added to the reaction mixture. The mixture was stirred at the same temperature for 30 min, then allowed to reach room temperature and further stirred for 24 h. After this time the reaction mixture was diluted with ice-cold H_2O (1 mL) and the pH was adjusted to pH=2 using 2 N HCl, the aqueous phase was extracted with DCM (3x3 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (Hex/EtOAc 2:1), to afford the title compound **1a** (19 mg, 12%) as a colorless oil. ^{1}H NMR (300 MHz, Acetone-d_6) δ 13.67 (s, 1H, -OH), 8.14 (d, *J* = 9.0 Hz, 1H, ArH), 7.91 – 7.65 (m, 4H, ArH, -CH=), 6.92 (d, *J* = 8.7 Hz, 2H, ArH), 6.50 (dd, *J* = 8.9, 2.5 Hz, 1H, ArH), 6.45 (d, *J* = 2.5 Hz, 1H, ArH), 5.46 (t, *J* = 6.7 Hz, 1H, C=CH-), 4.64 (d, *J* = 6.7 Hz, 2H, -CH_2-), 1.77 (s, 3H, =CMe_2), 1.76 (s, 3H, =CMe_2). ^{13}C NMR (101 MHz, Acetone-d_6) δ 193.2, 167.7, 166.6, 161.4, 145.6, 139.0, 132.9, 132.1, 127.6, 120.4, 118.3, 117.0, 115.0, 108.8, 102.5, 66.1, 26.0, 18.4. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{20}H_{21}O_4$ 325.1434, found 325.1437, $[M+Na]^+$ calcd for $C_{20}H_{20}O_4Na$ 347.1254, found 347.1255.

(2*E*)-3-(4-Hydroxyphenyl)-1-{4-[(3-methylbut-2-en-1-yl)oxy]phenyl}prop-2-en-1-one (1b). Starting from **8b** and **9**, the title compound was obtained yield as a yellow solid in 48% yield, following the synthetic procedure described for the synthesis of **1a**. mp 88-90 °C (Hex/EtOAc). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.07 (d, *J* = 8.7 Hz, 2H, ArH), 7.73 (d, *J* = 15.5 Hz, 1H, -CH=), 7.64 – 7.55 (m, 3H, ArH, -CH=), 7.03 (d, *J* = 8.7 Hz, 2H, ArH), 6.85 (d, *J* = 8.4 Hz, 2H, ArH), 5.48 (t, *J* = 6.7 Hz, 1H, C=CH-), 4.64 (d, *J* = 6.7 Hz, 2H, -CH_2-), 1.80 (s, 3H, =CMe_2), 1.78 (s, 3H, =CMe_2). ^{13}C NMR (101 MHz, Methanol-d_4) δ 190.4, 163.9, 161.0, 145.4, 138.7, 131.6, 131.3, 131.1, 127.2, 120.0, 118.9, 116.3, 115.0, 65.5, 25.2, 17.6. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{20}H_{21}O_3$ 309.1485, found 309.1489, $[M+Na]^+$ calcd for $C_{20}H_{20}O_3Na$ 331.1305, found 331.1308.

(2*E*)-1-{2-Hydroxy-4-[(3-methylbut-2-en-1-yl)oxy]phenyl}-3-[4-(methoxymethoxy)phenyl]prop-2-en-1-one (11). Starting from **10** and **8a**, the title compound was obtained in 42% yield, following the synthetic procedure described for the synthesis of **1a**. ^{1}H NMR (300 MHz, Acetone-d_6) δ 13.64 (s, 1H, OH), 8.18 (d, *J* = 9.0 Hz, 1H, ArH), 7.92 – 7.81 (m, 4H, ArH, -CH=), 7.12 (d, *J* = 8.8 Hz, 2H, ArH), 6.55 – 6.45 (m, 2H, ArH), 5.48 (t, *J* = 6.7 Hz, 1H, C=CH-), 5.28 (s, 2H, OCH_2O), 4.66 (d, *J* = 6.7 Hz, 2H, OCH_2CH=), 3.45 (s, 3H, OMe), 1.77 (s, 3H, =CMe_2), 1.79 (s, 3H, =CMe_2). ESI-MS *m/z* $[M+H]^+$, 369; $[M+Na]^+$ 391.

(2*E*)-1-(4-Hydroxyphenyl)-3-{4-[(3-methylbut-2-en-1-yl)oxy]phenyl}prop-2-en-1-one (2b). Starting from **7b** and **12**, the title compound was obtained obtained as a yellow solid in 13% yield, following the synthetic procedure described for the synthesis of **1a**. mp 128-130 °C (Hex/EtOAc). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.01 (d, *J* = 8.7 Hz, 2H, ArH), 7.76 – 7.59 (m, 4H, ArH, -CH=), 6.98 (d, *J* = 8.6 Hz, 2H, ArH), 6.90 (d, *J* = 8.6 Hz, 2H, ArH), 5.48 (t, 1H, C=CH-), 4.60 (d, *J* = 6.7 Hz, 2H, -CH_2-), 1.80 (s, 3H, =CMe_2), 1.77 (s, 3H, =CMe_2). ^{13}C NMR (101 MHz, Methanol-d_4) δ 190.7, 163.8, 162.3, 145.1, 138.8, 132.1, 131.2, 130.8, 128.8, 120.6, 120.1, 116.2, 116.0, 65.8, 25.6, 18.0. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{20}H_{21}O_3$ 309.1485, found 309.1488, $[M+Na]^+$ calcd for $C_{20}H_{20}O_3Na$ 331.1305, found 331.1307.

(2*E*)-1-{2-Hydroxy-4-[(3-methylbut-2-en-1-yl)oxy]phenyl}-3-(4-{[tris(propan-2-yl)silyl]oxy}phenyl)prop-2-en-1-one (14) and (2*E*)-1-{2-hydroxy-4-[(3-methylbut-2-en-1-yl)oxy]phenyl}-3-(4-hydroxyphenyl)prop-2-en-1-one (1a). In a two-necked round-bottom flask, under N_2 atmosphere, aldehyde **13** (20 mg, 0.071 mmol) was solubilized in 2-MeTHF (0.3 mL), the solution was then cooled in an ice-bath at 0 °C, and NaH (60% dispersion in mineral oil) (11 mg, 0.45 mmol) was added in small portions. The mixture was stirred at the same temperature for 10 min, then a solution of **8a** (31 mg, 0.142) in 2-MeTHF (0.2 mL) was added to the reaction mixture. The reaction was stirred for additional 2 h, then the whole mixture was poured in ice-water (3 mL) and further stirred for 30 min. After this time the aqueous phase was extracted with EtOAc (3x3 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was purified through silica gel column chromatography (PE/EtOAc/DCM 20:1:0.5) affording a mixture of **14** (yellow oil, 9.93 mg, 23%) and **1a** (1.8 mg, 8%). **14:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.63 (s, 1H, OH), 7.89 – 7.79 (m, 2H, ArH), 7.57 – 7.49 (m, 2H, ArH), 7.45 (d, *J* = 15.4 Hz, 1H, -CH=), 6.96 – 6.85 (m, 2H, ArH), 6.48 (d, *J* = 8.3 Hz, 2H, ArH), 5.47 (t, *J* = 6.7 Hz, 1H, C=CH-), 4.53 (d, *J* = 6.8 Hz, 2H, -CH_2-), 1.79 (s, 3H, =CMe_2), 1.74 (s, 3H, =CMe_2), 1.36 – 1.21 (m, 3H, CHSi), 1.13-1.12 (m, 18H, *i*Pr-Me).

Methyl (E)-2-(4-(3-(2-(2-methoxy-2-oxoethoxy)-4-((3-methylbut-2-en-1- yl)oxy)phenyl)-3-oxoprop-1-en-1-yl)phenoxy)acetate (15a). To a suspension of **1a** (1.39 g, 4.3 mmol) and K_2CO_3 (1.31 g, 9.50 mmol) in dry CH_3CN (8.0 mL), methyl 2-bromoacetate (0.89 mL, 9.50 mmol) was slowly added. The reaction mixture was stirred under reflux for 12 h. After the completion, the mixture was cooled at room temperature and an aqueous solution of NaCl s.s. (3 mL) was added. The aqueous phase was extracted with EtOAc (3 x 3 mL), the organic extracts were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified through silica gel column chromatography (PE/EtOAc 2:1) affording the title compound **15a** as a white solid (884 mg, 44%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.86 (d, *J* = 15.7 Hz, 1H, -CH=), 7.75 – 7.68 (m, 3H, ArH), 7.61 (d, *J* = 15.7 Hz, 1H, -CH=), 6.97 (d, *J* = 8.8 Hz, 2H, ArH), 6.65 (dd, *J* = 8.7, 2.2 Hz, 1H, ArH), 6.59 (d, *J* = 2.2 Hz, 1H, ArH), 5.45 (t, *J* = 6.6 Hz, 1H, C=CH-), 4.91 (s, 2H, -OCH_2-), 4.78 (s, 2H, -$COCH_2$-), 4.62 (d, *J* = 6.7 Hz, 2H, -$COCH_2$-), 3.75 (s, 3H, OMe), 3.72 (s, 3H, OMe), 1.75 (s, 3H, =CMe_2), 1.73 (s, 3H, =CMe_2).

Methyl 2-{4-[(1*E*)-3-{4-[(3-methylbut-2-en-1-yl)oxy]phenyl}-3-oxoprop-1-en-1-yl]phenoxy}acetate (15b). Starting from **1b**, the title compound was obtained in 53% yield, following the synthetic procedure described for the synthesis of **15a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 8.8 Hz, 2H, ArH), 7.74 (d, *J* = 15.6 Hz, 1H, -CH=), 7.58 (d, *J* = 8.8 Hz, 2H, ArH), 7.42 (d, *J* = 15.6 Hz, 1H, -CH=), 6.90 (d, *J* = 8.8 Hz, 2H, ArH), 6.85 (d, *J* = 8.7 Hz, 2H, ArH), 5.47 (t, *J* = 6.8 Hz, 1H, C=CH-), 4.66 (s, 2H, -$COCH_2$-), 4.57 (d, *J* = 6.8 Hz, 2H, -OCH_2-), 3.79 (s, 3H, Me), 1.79 (s, 3H, =CMe_2), 1.75 (s, 3H, =CMe_2).

(E)-2-(4-(3-(2-(Carboxymethoxy)-4-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-oxoprop-1-en-1-yl)phenoxy)acetic acid (16a). Lithium hydroxide (180 mg, 7.51 mmol) was added to a solution of ester **15a** (0.88 g, 1.87 mmol) in THF/H_2O (7:1, 24 mL). The mixture was stirred at room temperature for 5 h. After completion of the reaction, the organic phase was removed under reduced pressure, the residue was diluted with water, the pH was adjusted to 2 with 1 N HCl and the resulting precipitate was filtered and washed with water. The obtained crude white solid **16a** (684 mg, 76% yield) was used in the next step without further purification. mp 172-175 °C (PE/EtOAc) ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.92 (d, *J* = 15.7 Hz, 1H, -CH=), 7.84 – 7.70 (m, 3H, ArH), 7.65 (d, *J* = 15.7 Hz, 1H, -CH=), 6.98 (d, *J* = 8.8 Hz, 2H, ArH), 6.75 – 6.58 (m, 2H, ArH), 5.47 (t, *J* = 6.7 Hz, 1H, C=CH-), 4.93 (s, 2H, -$COCH_2$-), 4.79 (s, 2H, -$COCH_2$-), 4.66 (d, *J* = 6.7 Hz, 2H, -OCH_2-), 1.77 (s, 3H, =CMe_2), 1.76 (s, 3H, =CMe_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ 189.0, 170.6, 163.5, 159.91, 159.0, 141.8, 138.3, 132.8, 130.6, 128.6, 125.6, 121.6, 119.8 115.3, 107.7, 100.1, 65.7, 65.2, 65.1, 25.9, 18.51. ESI-MS *m/z* $[M+H]^+$ 469. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{24}H_{25}O_8$, 441.1544, found 441.1547, $[M+Na]^+$ calcd for for $C_{24}H_{24}O_8Na$ 463.1363, found 463.1367.

2-{4-[(1E)-3-{2-hydroxy-4-[(3-methylbut-2-en-1-yl)oxy]phenyl}-3-oxoprop-1-en-1-yl]phenoxy}acetic acid (16b). Starting from **15b**, the title compound was obtained in 38% yield, following the synthetic procedure described for the synthesis of **16a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.8 Hz, 2H, ArH), 7.80 (d, *J* = 15.6 Hz, 1H, -CH=), 7.64 (d, 2H, *J* = 8.6 Hz, ArH), 7.48 (d, *J* = 15.6 Hz, 1H, -CH=), 7.04 – 6.92 (m, 4H, ArH), 5.53 (t, *J* = 6.9 Hz, 1H, C=CH-), 4.76 (s, 2H, -$COCH_2$-), 4.63 (d, *J* = 6.8 Hz, 2H, -OCH_2-), 1.84 (s, 3H, =CMe_2), 1.80 (s, 3H, =CMe_2).

(E)-N-(2-(Diethylamino)ethyl)-2-(4-(3-(2-(2-((2-(diethylamino)ethyl)amino)-2- oxoethoxy)-4-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-oxoprop-1-en-1-yl)phenoxy)acetamide (3). In a round-bottom flask intermediate **16a** (100 mg, 0.23 mmol) was solubilized in anhydrous dimethylformamide (DMF, 2 mL), then 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (105 mg, 2.4 eq) and hexafluorophosphate azabenzotriazole tetramethyluranium (HATU) (0.86 mg, 0.02 mmol) were added. The reaction mixture was stirred 10 min at the same temperature, and after this time TEA (46 mg, 0.063 mL, 0.46 mmol) and N^1,N^1-diethylethane-1,2-diamine (0.076 mL, 0.55 mmol) were added. The mixture was stirred at the same temperature for 12 h, after this time NH_4Cl *s.s.* (2 mL) was added, and the aqueous phase was extracted with EtOAc (3 x 3 mL), the organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified through silica gel (PE/EtOAc 2:1) affording the title compound **3** as a pale yellow amorphous solid (51.3 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H, NH), 8.50 (s, 1H, NH), 7.79 – 7.46 (m, 3H, ArH, -CH=), 7.28-7.26 (m, 1H, ArH), 7.05 – 6.81 (m, 3H, ArH, -CH=), 6.62 (dd, *J* = 8.7, 2.2 Hz, 1H, ArH), 5.47 (t, *J* = 6.8 Hz, 1H, C=CH-), 4.66 – 4.48 (m, 6H, -OCH_2-),

3.82 – 3.69 (m, 2H, -NH$\underline{CH_2}$-), 3.66 (q, *J* = 6.4 Hz, 2H,-NH$\underline{CH_2}$-), 3.23-3.14 (m, 12H, -NCH_2-), 1.82 (s, 3H, =CMe_2), 1.77 (s, 3H, =CMe_2), 1.47 – 1.18 (m, 12H, -$CH_2\underline{Me}$). HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{36}H_{53}N_4O_6$, 637.3960, found 637.3960, $[M+Na]^+$ calcd for $C_{36}H_{52}N_4O_6Na$ 659.3779, found 659.3779. ESI-MS *m/z* $[M+H]^+$ 624. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{36}H_{53}N_4O_6$, 637,3960, found 637,3960, $[M+Na]^+$ calcd for $C_{36}H_{52}N_4O_6Na$ 659.3779, found 659.3779.

***N*-[2-(diethylamino)ethyl]-2-{4-[(1E)-3-{4-[(3-methylbut-2-en-1-yl)oxy]phenyl}-3-oxoprop-1-en-1-yl]phenoxy}acetamide (4).** Starting from **16b**, the title compound was obtained as a yellow wax in 38% yield, following the synthetic procedure described for the synthesis of **3**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.6 Hz, 2H, ArH), 7.76 (d, *J* = 15.6 Hz, 1H, -CH=), 7.61 (d, *J* = 8.4 Hz, 2H, ArH), 7.44 (d, *J* = 15.6 Hz, 1H, -CH=), 7.15 – 6.91 (m, 4H, ArH), 5.50 (t, *J* = 7.0 Hz, 1H, $\underline{C=CH}$-), 4.60 (d, *J* = 6.8 Hz, 2H, -OCH_2-), 4.56 (s, 2H, -$COCH_2$-), 3.59 – 3.52 (bs, 2H,-NH$\underline{CH_2}$-), 2.73 – 2.72 (bs, 6H, -NCH_2-), 1.81 (s, 3H, =CMe_2), 1.77 (s, 3H, =CMe_2), 1.21 – 1.10 (bs, 6H, -$CH_2\underline{Me}$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.8, 168.7, 162.84, 159.2, 143.4, 139.1, 131.2, 130.8, 130.2, 129.1, 120.4, 119.1, 115.4, 114.6, 67.2, 65.2, 52.6, 47.9, 35.2, 26.0, 18.4, 9.7. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{28}H_{37}N_2O_4$, 465.2748, found 465.2750.

(2*E*)-1-(2,4-dihydroxyphenyl)-3-{4-[(3-methylbut-2-en-1-yl)oxy]phenyl}prop-2-en-1-one (2a).

Procedure a from Scheme 1: Starting from **12** and **7a**, the title compound was obtained as a brownish solid in 30% yield, following the synthetic procedure described for the synthesis of **1a**.

Procedure b from Scheme 4: In a two-necked round-bottom flask, under N_2 atmosphere, intermediate **17** (40 mg, 0.13 mmol) was solubilized in 2-MeTHF (0.6 mL), the solution was then cooled in an ice-bath at 0 °C, and NaH (60% dispersion in mineral oil) (26 mg, 0.65 mmol) was added in small portions. The mixture was stirred at the same temperature for 10 min, then a solution of intermediate **12** (49.5 mg, 0.26 mmol) in 0.4 mL of 2-MeTHF was added slowly to the reaction mixture. The reaction was stirred for additional 2 h, then the whole mixture was poured in a beaker with ice- water (3 mL) and further stirred for 30 min. After this time the aqueous phase was extracted with EtOAc (3 x 3 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was purified through silica gel column chromatography (PE/EtOAc/DCM 20:1:0.5) affording intermediate (*E*)-1-(2-hydroxy-4-((3-methylbut-2-en-1-yl)oxy)phenyl)-3-(4-((triisopropylsilyl)oxy)phenyl)prop-2-en-1-one as a yellow oil (56.3mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (m, 1H), 7.82 – 7.77 (m, 1H), 7.64 – 7.56 (m, 2H), 7.46 (m, 1H), 7.01 – 6.90 (m, 2H), 6.50 – 6.40 (m, 2H), 5.55 – 5.44 (m, 1H), 4.57 (d, *J* = 6.8 Hz, 2H), 1.79 (m, 6H), 1.37 – 1.22 (m, 4H), 1.12 (d, *J* = 7.4 Hz, 18H). ESI-MS *m/z* $[M+H]^+$ 481. tetra-*n*-Butylammonium fluoride (59.8 mg, 0.228 mmol) was added to a solution of the above compound in THF (3.6 mL) at -30 °C. After stirring at rt for 30 min, the reaction mixture was allowed to warm to rt over a period of 4 h. Then it was poured into saturated aqueous $NaHCO_3$ and extracted with Et_2O. The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. mp 109-111 °C (PE/EtOAc). ^{1}H NMR (300 MHz, Acetone-d_6) δ 13.61 (s, 1H, OH), 9.5 (bs, 1H, OH), 8.14 (d, *J* = 8.9 Hz, 2H, ArH), 7.91 – 7.73 (m, 4H, ArH, -CH=), 7.02 (d, *J* = 8.8 Hz, 2H, ArH), 6.48 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 6.38 (d, *J* = 2.4 Hz, 1H, ArH), 5.47 (t, *J* = 6.6 Hz, 1H, $\underline{C=CH}$-), 4.64 (d, *J* = 6.6 Hz, 2H, -CH_2-), 1.79 (s, 3H, =CMe_2), 1.77 (s, 3H, =CMe_2). ^{13}C NMR (100 MHz, Acetone-d_6) δ 193.0, 167.5, 166.4, 161.1, 145.4, 138.80, 132.7, 131.9, 127.5, 120.2, 118.1, 116.8, 114.8, 108.6, 102.3, 65.9, 25.8, 18.2. ESI-MS *m/z* $[M+H]^+$ 325. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{20}H_{21}O_4$ 325.1434, found 325.1438.

Dimethyl 2,2'-((4-(3-(4-((3-methylbut-2-en-1-yl)oxy)phenyl)acryloyl)-1,3- phenylene)bis(oxy))(E)-diacetate (18a). Starting from **2a**, the title compound was obtained in 76% yield, following the synthetic procedure described for the synthesis of **15a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.6 Hz, 1H, ArH), 7.69 (d, *J* = 15.7 Hz, 1H, -CH=), 7.63 – 7.56 (m, 3H, ArH, -CH=), 6.92 (d, *J* = 8.7 Hz, 2H, ArH), 6.54 (dd, *J* = 8.6, 2.2 Hz, 1H, ArH), 6.47 (d, *J* = 2.2 Hz, 1H, ArH), 5.50 (t, *J* = 6.7 Hz, 1H, $\underline{C=CH}$-), 4.70 (s, 2H, -$COCH_2$-), 4.67 (s, 2H, -$COCH_2$-), 4.54 (d, *J* = 6.8 Hz, 2H, -OCH_2-), 3.82 (s, 3H, OMe), 3.78 (s, 3H, OMe), 1.80 (s, 3H, =CMe_2), 1.75 (s, 3H, =CMe_2). ESI-MS *m/z* $[M+H]^+$ 469.

Methyl 2-{4-[(2E)-3-{4-[(3-methylbut-2-en-1-yl)oxy]phenyl}prop-2-enoyl]phenoxy}acetate (18b). Starting from **2b**, the title compound was obtained in 44% yield, following the synthetic procedure described for the synthesis of **15a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, *J* = 8.9 Hz, 2H, ArH), 7.77 (d, *J* = 15.6 Hz, 1H, -CH=), 7.58 (d, *J* = 8.8 Hz, 2H, ArH), 7.40 (d, *J* = 15.6 Hz, 1H, -CH=), 6.98 (d, *J* = 8.8 Hz, 1H, ArH), 6.94 (d, *J* = 8.7 Hz, 1H, ArH), 5.42 (t, *J* = 6.7 Hz, 1H, $\underline{C=CH}$-), 4.65 (s, 2H, -$COCH_2$-), 4.49 (d, *J* = 6.8 Hz, 2H, -OCH_2-), 3.75 (s, 3H, OMe), 1.74 (s, 3H, =CMe_2), 1.69 (s, 3H, =CMe_2).

(E)-2,2'-((4-(3-(4-((3-Methylbut-2-en-1-yl)oxy)phenyl)acryloyl)-1,3- phenylene)bis(oxy))diacetic acid (19a). Starting from **18a**, the title compound was obtained as white solid in 70% yield, following the synthetic procedure described for the synthesis of **16a**. mp 177-180 °C (PE/EtOAc). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86 (d, *J* = 16.0 Hz, 1H, -CH=), 7.72 (d, *J* = 8.8 Hz, 2H, ArH), 7.65 (d, *J* = 8.7 Hz, 1H, ArH), 7.58 (d, *J* = 15.7 Hz, 1H, -CH=), 6.96 (d, *J* = 8.7 Hz, 2H, ArH), 6.67 (d, *J* = 2.2 Hz, 1H, ArH), 6.62 (dd, *J* = 8.7, 2.3 Hz, 1H, ArH), 5.40 (t, *J* = 6.6 Hz, 1H, $\underline{C=CH}$-), 4.87 (s, 2H, -$COCH_2$-), 4.77 (s, 2H, -$COCH_2$-), 4.58 (d, *J* = 6.7 Hz, 2H, -OCH_2-), 1.75 (s, 2H, =CMe_2), 1.72 (s, 2H, =CMe_2).^{13}C NMR (101 MHz, DMSO-d_6) δ 188.2, 169.5, 169.3, 161.6, 159.6, 157.8, 141.1, 136.9, 131.7, 129.7, 127.0, 124.1, 121.3, 119.0, 114.4, 106.2, 99.4, 64.7, 64.2, 63.9, 24.9, 17.5. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{24}H_{25}O_8$, 441.1544, found 441.1544, $[M+Na]^+$ calcd for for $C_{24}H_{24}O_8Na$ 463.1363, found 463.1362.

2-{4-[(2*E*)-3-{4-[(3-methylbut-2-en-1-yl)oxy]phenyl}prop-2-enoyl]phenoxy}acetic acid (19b). Starting from **18b**, the title compound was obtained in 76% yield, following the synthetic procedure described for the synthesis of **16a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.6 Hz, 2H, ArH), 7.72 (d, *J* = 15.5 Hz, 1H, -CH=), 7.52 (d, *J* = 8.5 Hz, 2H, ArH), 7.33 (d, *J* = 15.5 Hz, 1H, -CH=), 6.95 (d, *J* = 8.6 Hz, 2H, ArH), 6.88 (d, *J* = 8.4 Hz, 2H, ArH), 5.44 (t, *J* = 6.8 Hz, 1H, C=CH-), 4.71 (s, 2H, -$COCH_2$-), 4.49 (d, *J* = 6.8 Hz, 2H, -OCH_2-), 1.74 (s, 3H, =CMe_2), 1.69 (s, 3H, =CMe_2).

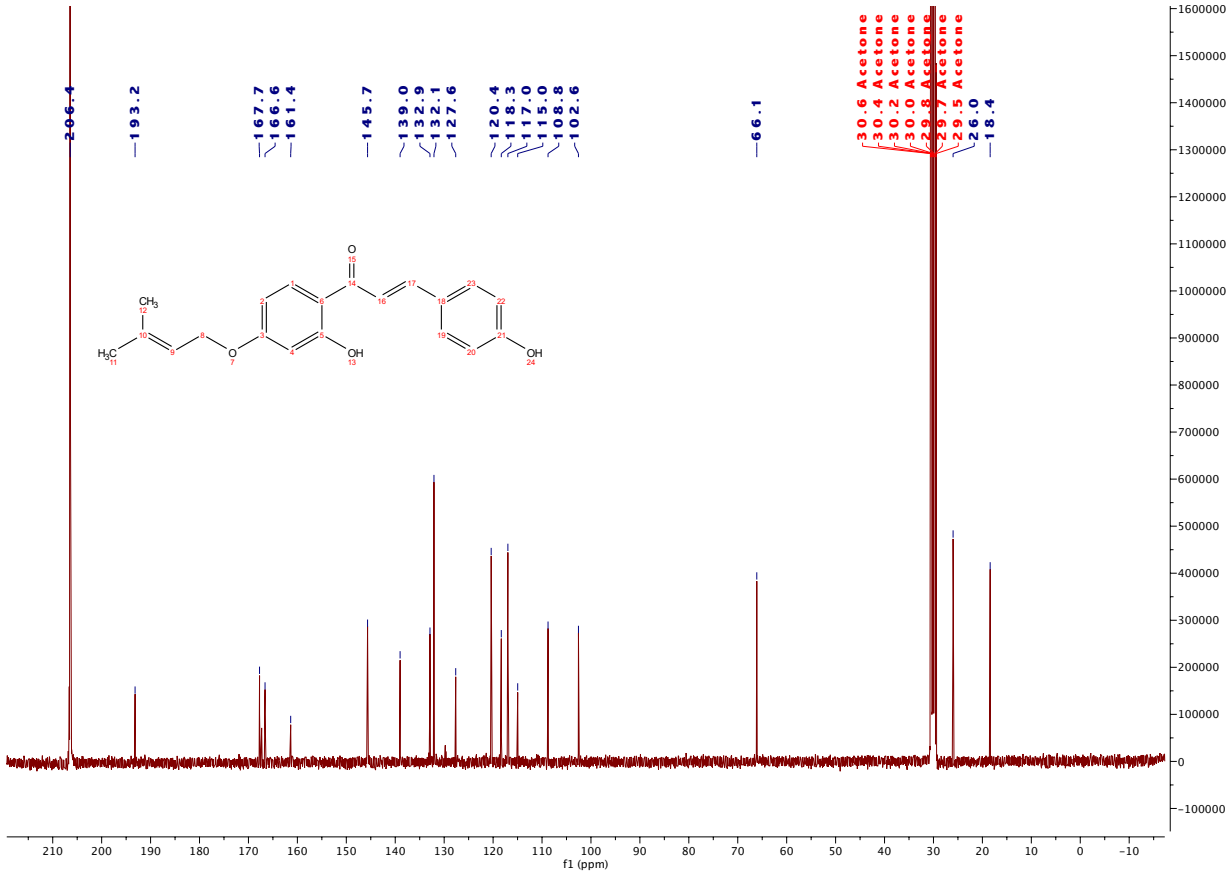
(E)-2,2'-((4-(3-(4-((3-Methylbut-2-en-1-yl)oxy)phenyl)acryloyl)-1,3-phenylene)bis(oxy))bis(N-(2-(diethylamino)ethyl)acetamide) (5). Starting from **19a**, the title compound was obtained as a yellow wax in 12% yield, following the synthetic procedure described for the synthesis of **3**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 1H, NH), 8.63 (s, 1H, NH), 7.75 – 7.47 (m, 4H, ArH, -CH=), 7.21 (d, *J* = 15.8 Hz, 1H, -CH=), 6.98 – 6.88 (m, 2H, ArH), 6.74 – 6.52 (m, 2H, ArH), 5.49 (t, *J* = 7.0 Hz, 1H, C=CH-), 4.69 – 4.43 (m, 6H, -OCH_2-), 3.82 – 3.58 (m, 4H, -NHCH_2-), 3.21-3.18 (m, 12H, -NCH_2-), 1.81 (s, 3H, =CMe_2), 1.76 (s, 3H, =CMe_2), 1.38 – 1.27 (m, 12H, -CH_2Me). ESI-MS *m/z* [M+H]+ 637. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{36}H_{53}N_4O_6$, 637,3960, found 637,3967, $[M+Na]^+$ calcd for $C_{36}H_{52}N_4O_6Na$ 659.3779, found 659.3783.

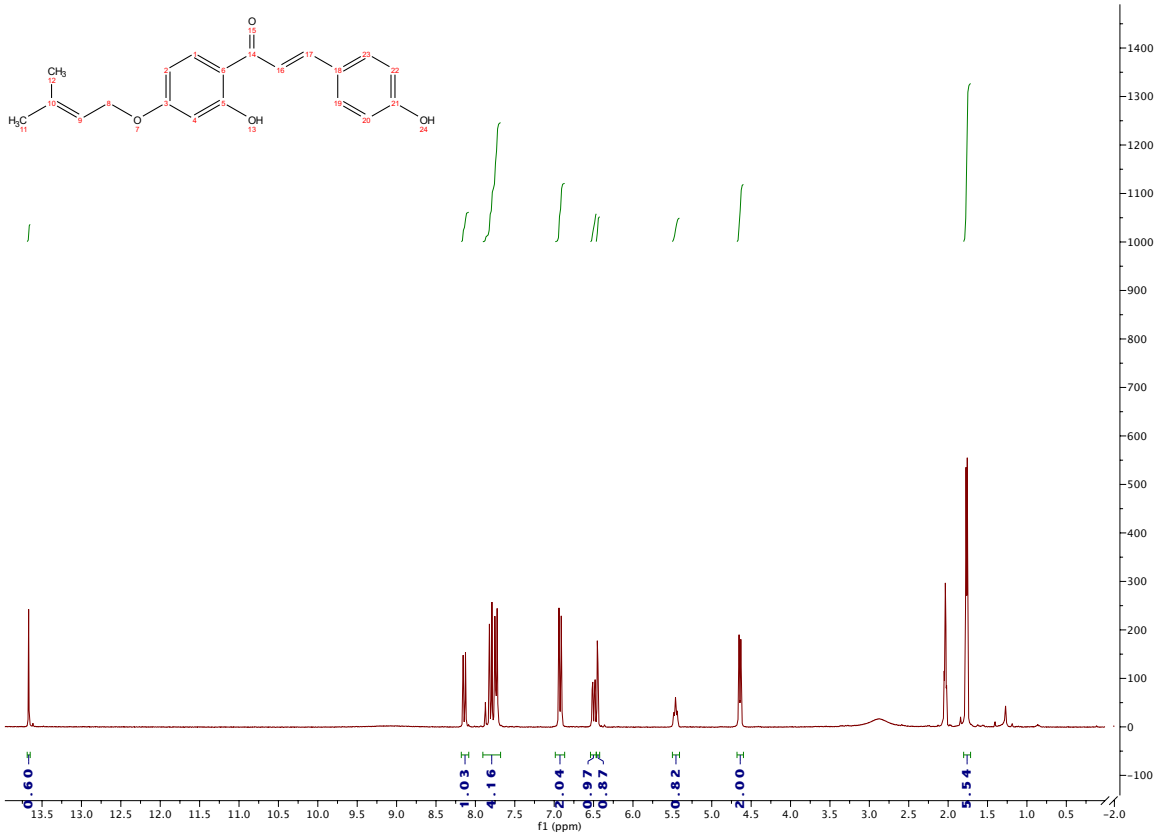
***N*-[2-(Diethylamino)ethyl]-2-{4-[(2*E*)-3-{4-[(3-methylbut-2-en-1-yl)oxy]phenyl}prop-2-enoyl]phenoxy}acetamide (6).** Starting from **19b**, the title compound was obtained as a white solid in 32% yield, following the synthetic procedure described for the synthesis of **3**. mp 100-102 °C (PE/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.4 Hz, 2H, ArH), 7.78 (d, *J* = 15.5 Hz, 1H, -CH=), 7.59 (d, *J* = 8.5 Hz, 2H, ArH), 7.40 (d, *J* = 15.5 Hz, 1H, -CH=), 7.23 (bs, 1H, NH), 7.00 (d, *J* = 8.5 Hz, 2H, ArH), 6.94 (d, *J* = 8.4 Hz, 2H, ArH), 5.49 (t, *J* = 7.2 Hz, 1H, C=CH-), 4.62 – 4.50 (m, 4H, -OCH_2-), 3.37 (q, *J* = 5.7 Hz, 2H, NH-CH_2), 2.57 (t, *J* = 6.0 Hz, 2H, -NCH_2-), 2.50 (q, *J* = 7.1 Hz, 4H-NCH_2-), 1.81 (s, 3H, =CMe_2), 1.76 (s, 3H, =CMe_2), 0.97 (t, *J* = 7.1 Hz, 6H, -CH_2Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.5, 167.4, 160.8, 160.6, 144.1, 138.6, 132.4, 130.6, 130.0, 127.4, 119.1, 119.0, 114.9, 114.3, 67.1, 64.8, 51.4, 46.9, 36.0, 25.6, 18.1, 11.1. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{28}H_{37}N_2O_4$, 465.2748, found 465.2750.

***N*-[2-(Diethylamino)ethyl]-2-({3-[(1E)-2-{4-[(3-methylbut-2-en-1-yl)oxy]phenyl}ethenyl]-1-benzofuran-6-yl}oxy)acetamide (20).** A solution of **19a** (379.0 mg, 0.86 mmol) and 1,1-carbonyldiimidazole (139.5 mg, 0.86 mmol) in anhydrous THF (5 mL) was stirred at room temperature for 1 h. The mixture was then cooled at 0 °C and *N*,*N*-diethylethylendiamine (266 mL, 1.89 mmol) was added. The mixture was stirred at 0 °C for 4 h and then at 25 °C overnight. After this time a further amount of CDI (139.5 mg, 0.86 mmol) and amine (266 mL, 1.89 mmol) were added. The mixture was stirred at 25 °C for 24 h. Then the mixture was concentrated under reduced pressure, and the obtained residue was dissolved in DCM (20 mL) and 1 N HCl (15 mL) was added to adjust pH at 7-8. The organic layer was dried and concentrated under reduced pressure. The resultant crude product was purified by silica gel column chromatography (gradient elution from DCM/MeOH 80:1 to 10:1) to afford the title compound as a brownish solid in 22% yield. mp 78-81 °C (PE/EtOAc).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.6 Hz, 1H, ArH), 7.56 (s, 1H, -OCH=), 7.32 (d, *J* = 8.5 Hz, 2H, ArH), 7.01-6.78 (m, 6H, ArH, -CH=CH-), 5.43 (t, 1H, *J* = 6.7 Hz, C=CH-), 4.50 – 4.33 (m, 4H, -OCH_2-), 3.32 (q, *J* = 5.7 Hz, 2H, NH-CH_2), 2.53 (t, *J* = 6.0 Hz, 2H, -NCH_2-), 2.47 (q, *J* = 7.1 Hz, 4H, -NCH_2-), 1.69 (s, 3H, =CMe_2), 1.64 (s, 3H, =CMe_2), 0.91 (t, *J* = 7.1 Hz, 6H, -CH_2Me). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2, 158.7, 156.8, 155.8, 143.0, 138.4, 130.2, 129.18, 127.443, 121.5, 120.5, 119.7, 116.1, 115.0, 112.0, 97.8, 68.0, 65.0, 51.5, 47.0, 36.4, 25.9, 18.3, 11.6. HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{29}H_{37}N_2O_4$, 477.2749, found 477.2752.

2. NMR spectra

1a





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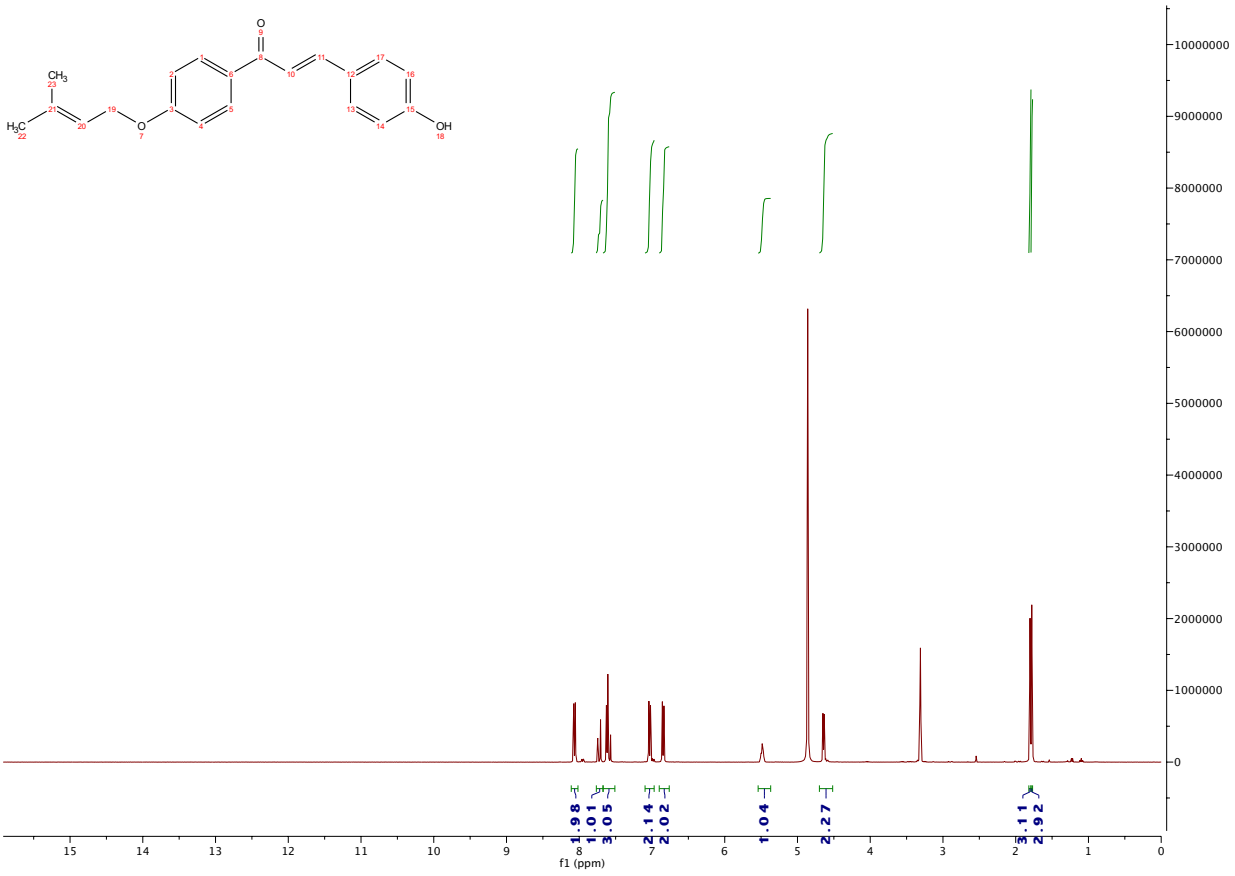
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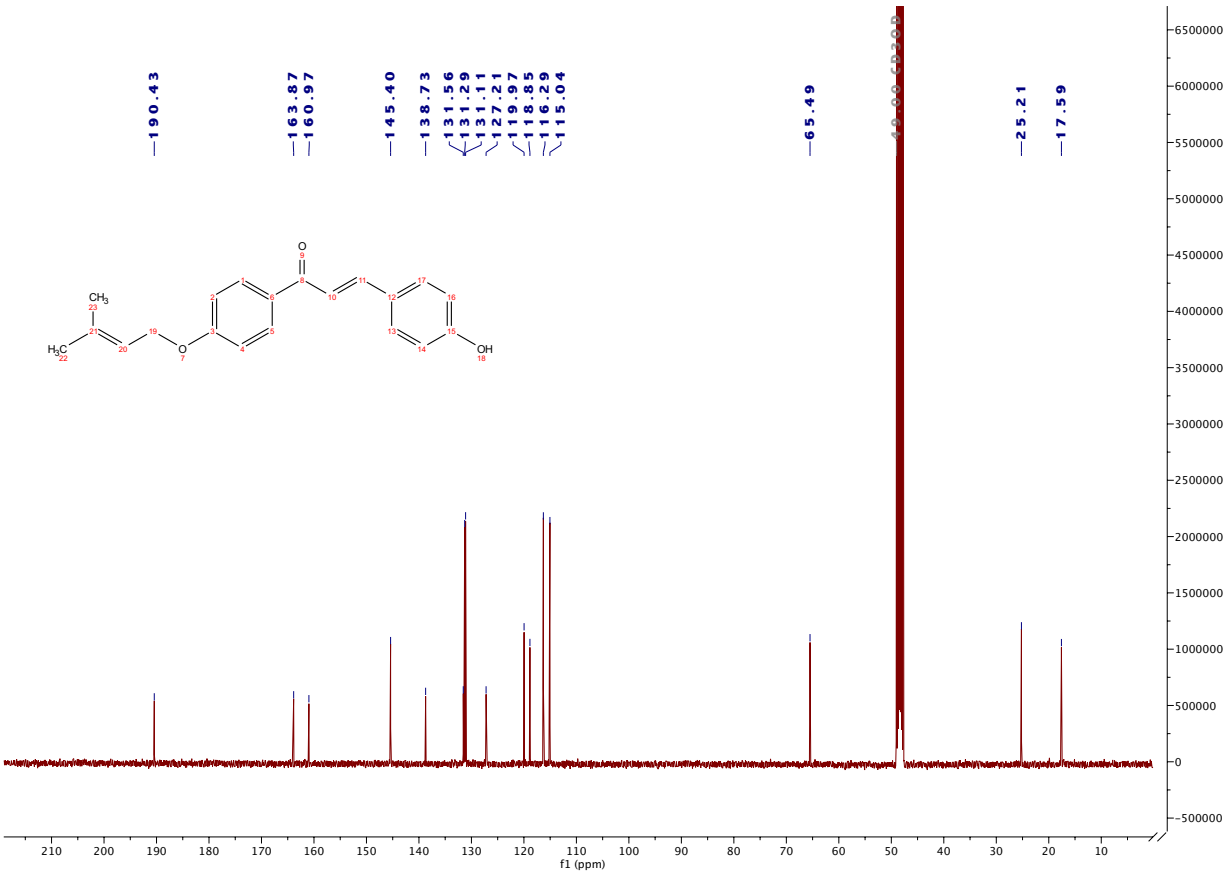
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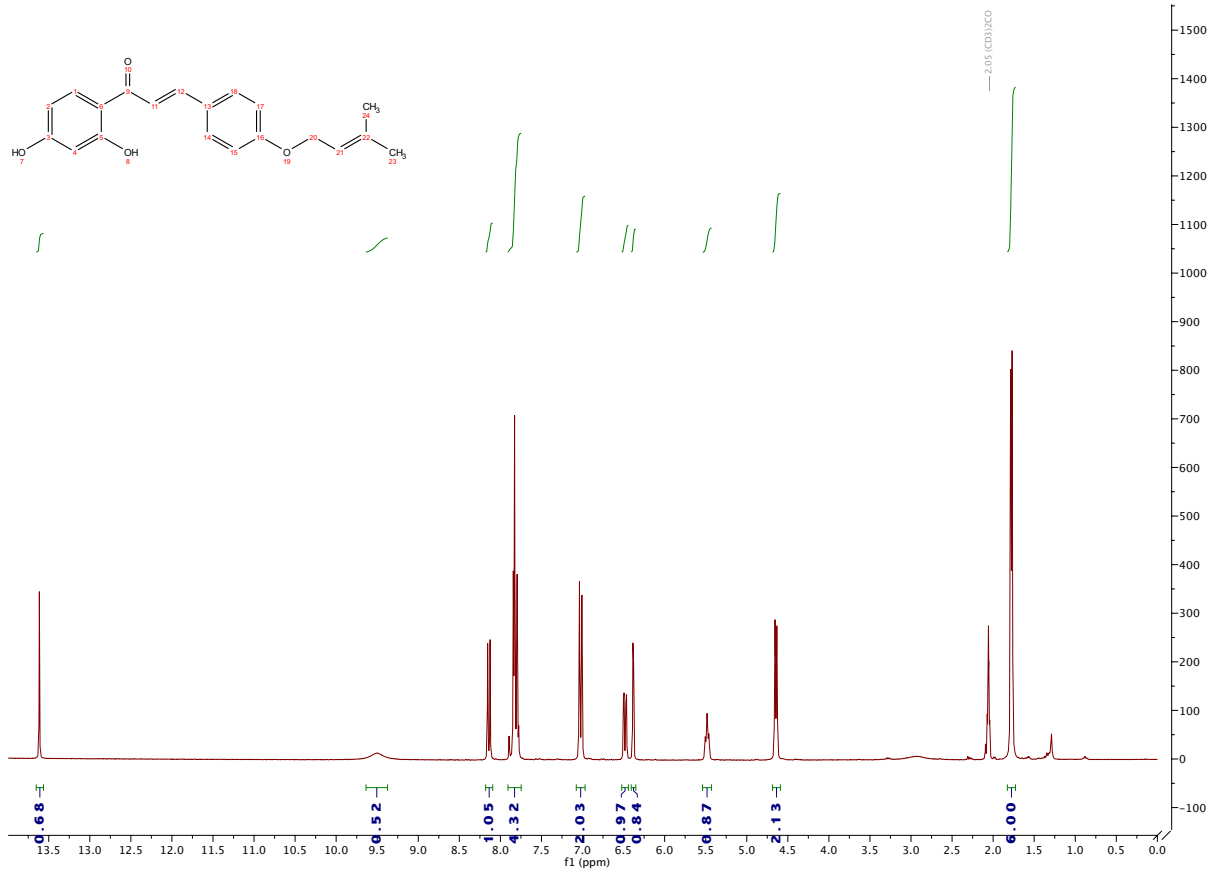
2a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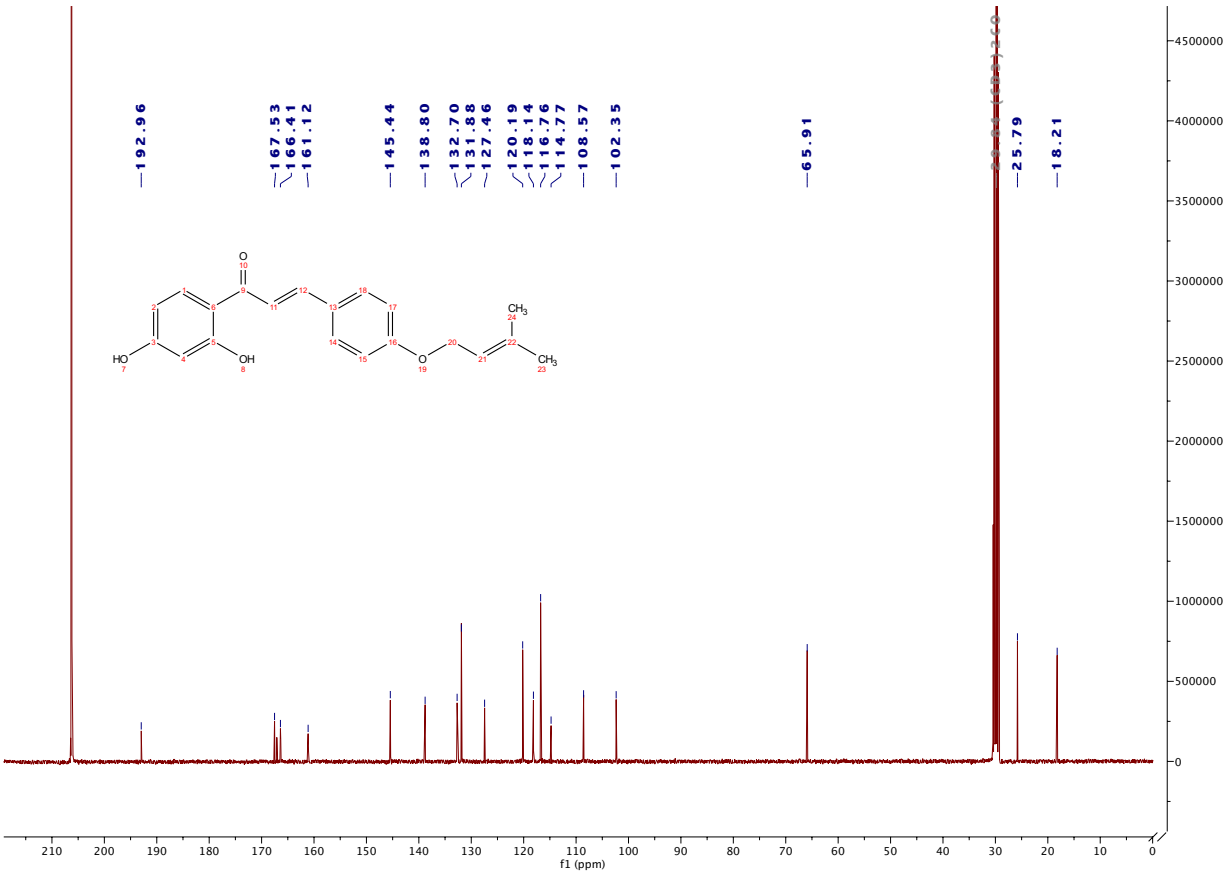
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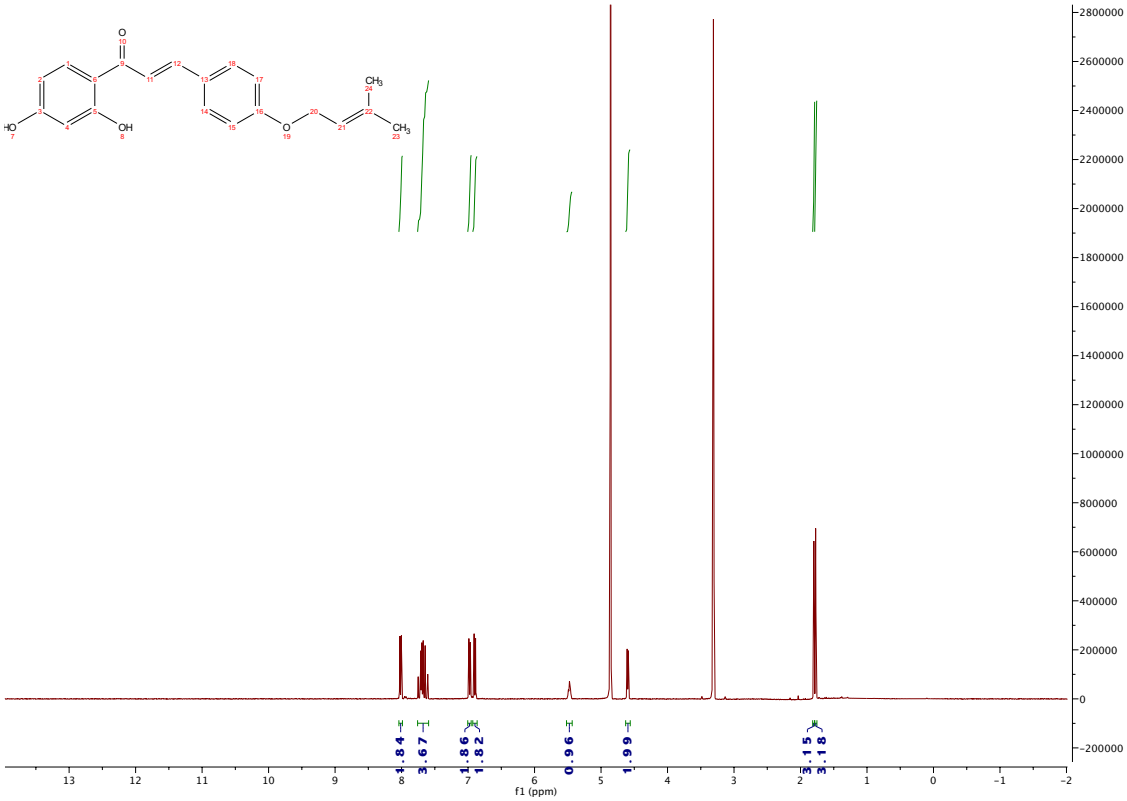
2b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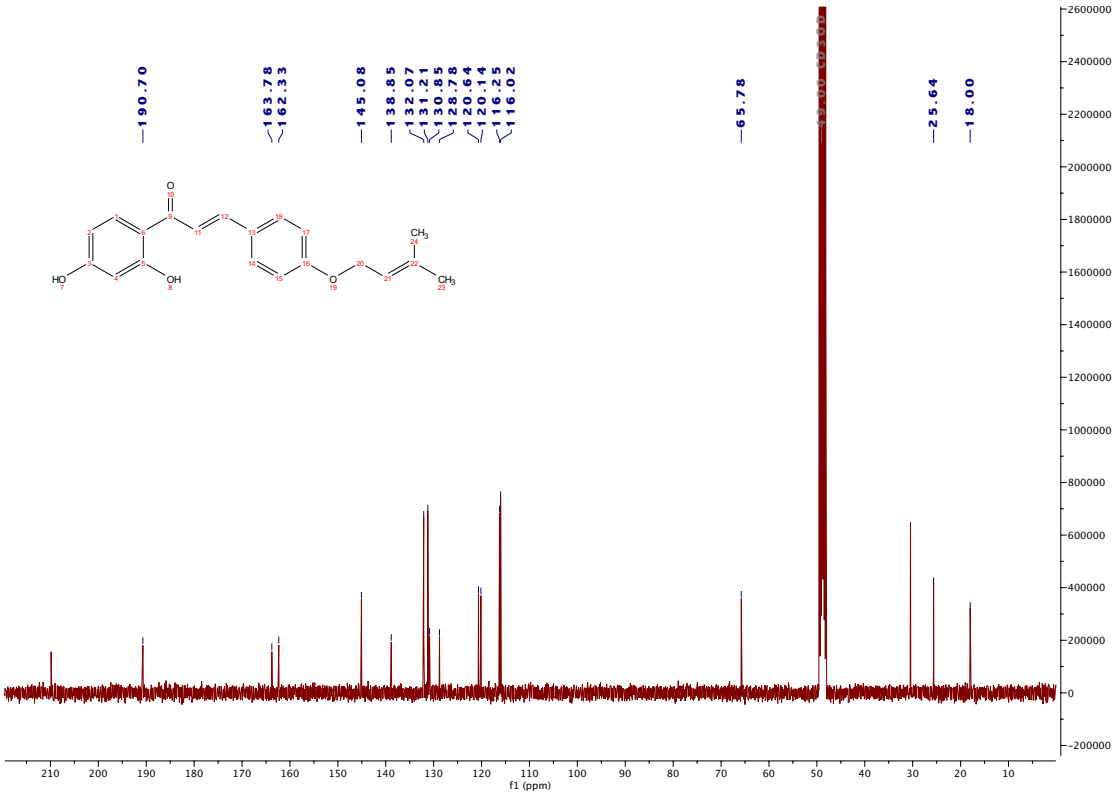
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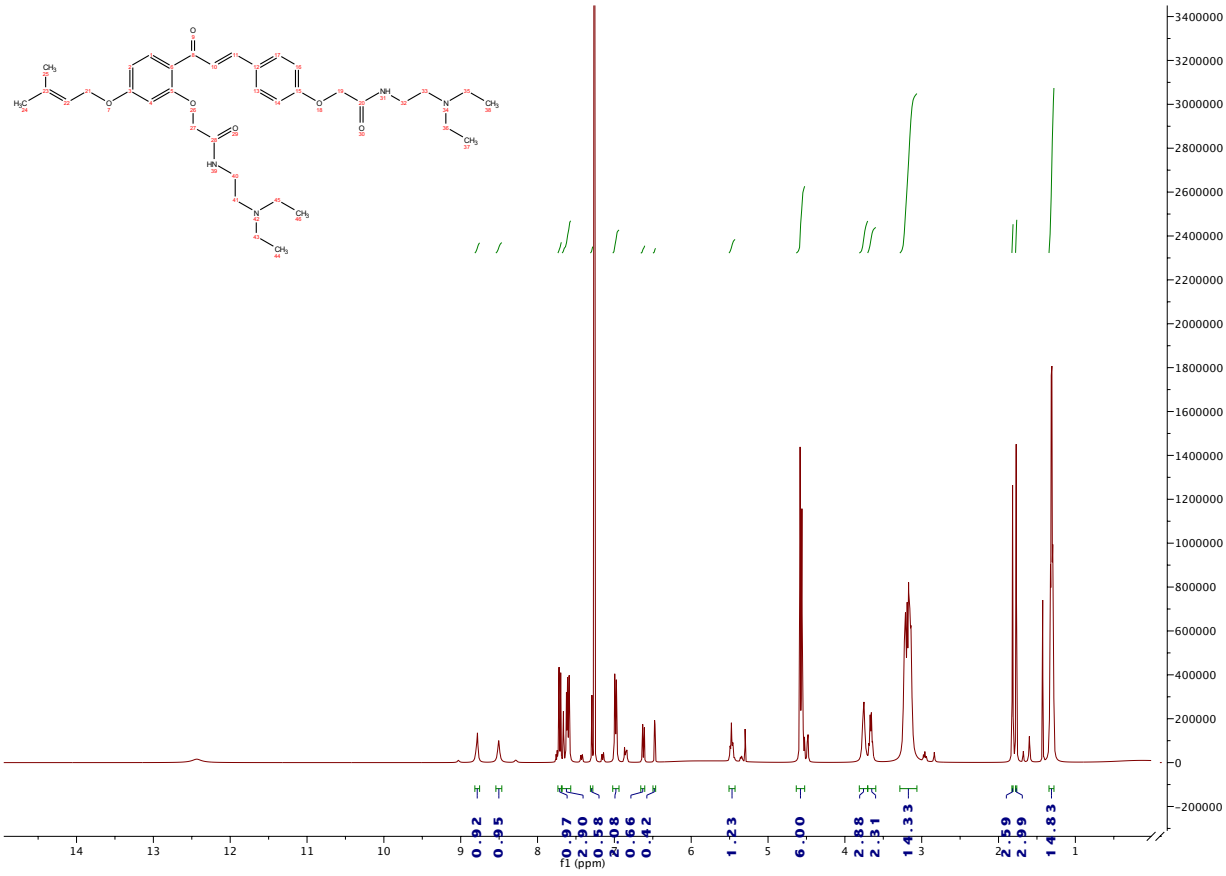
3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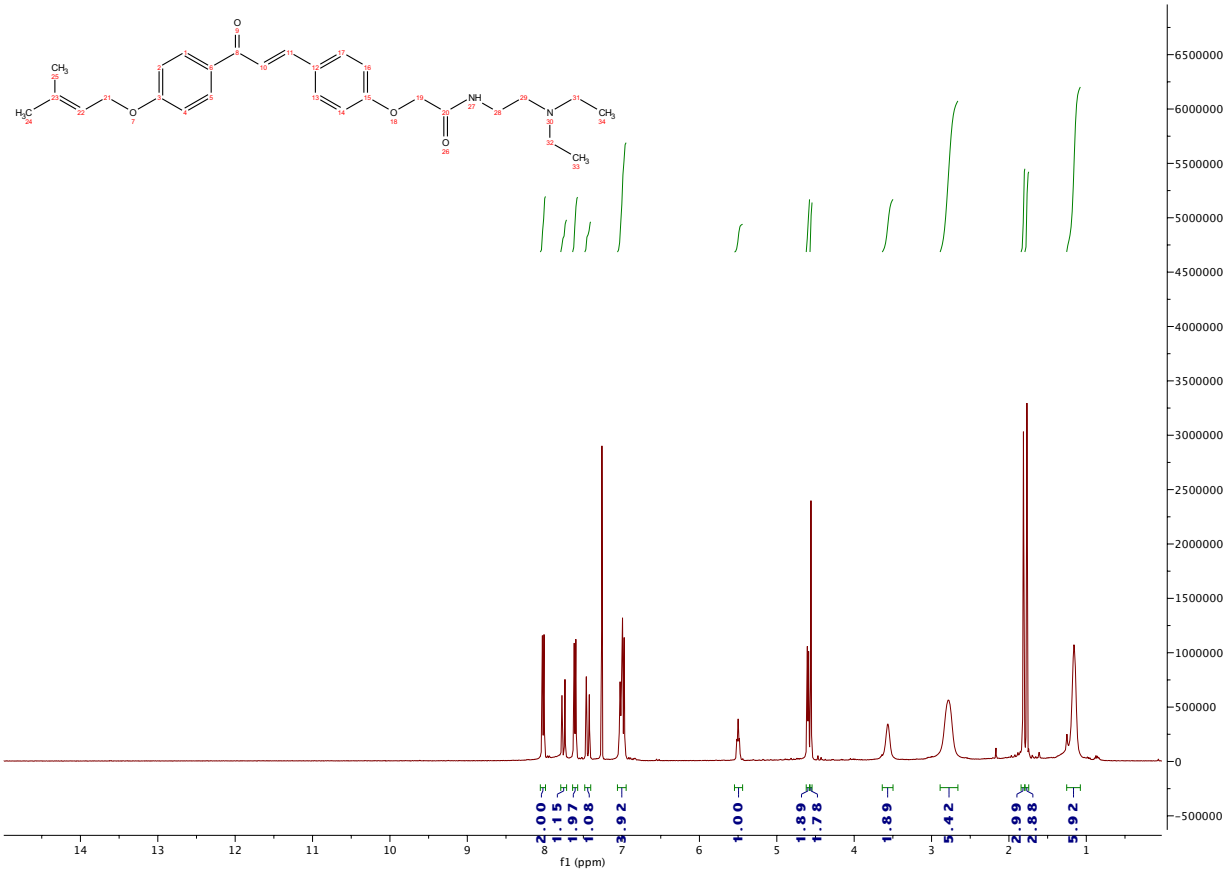
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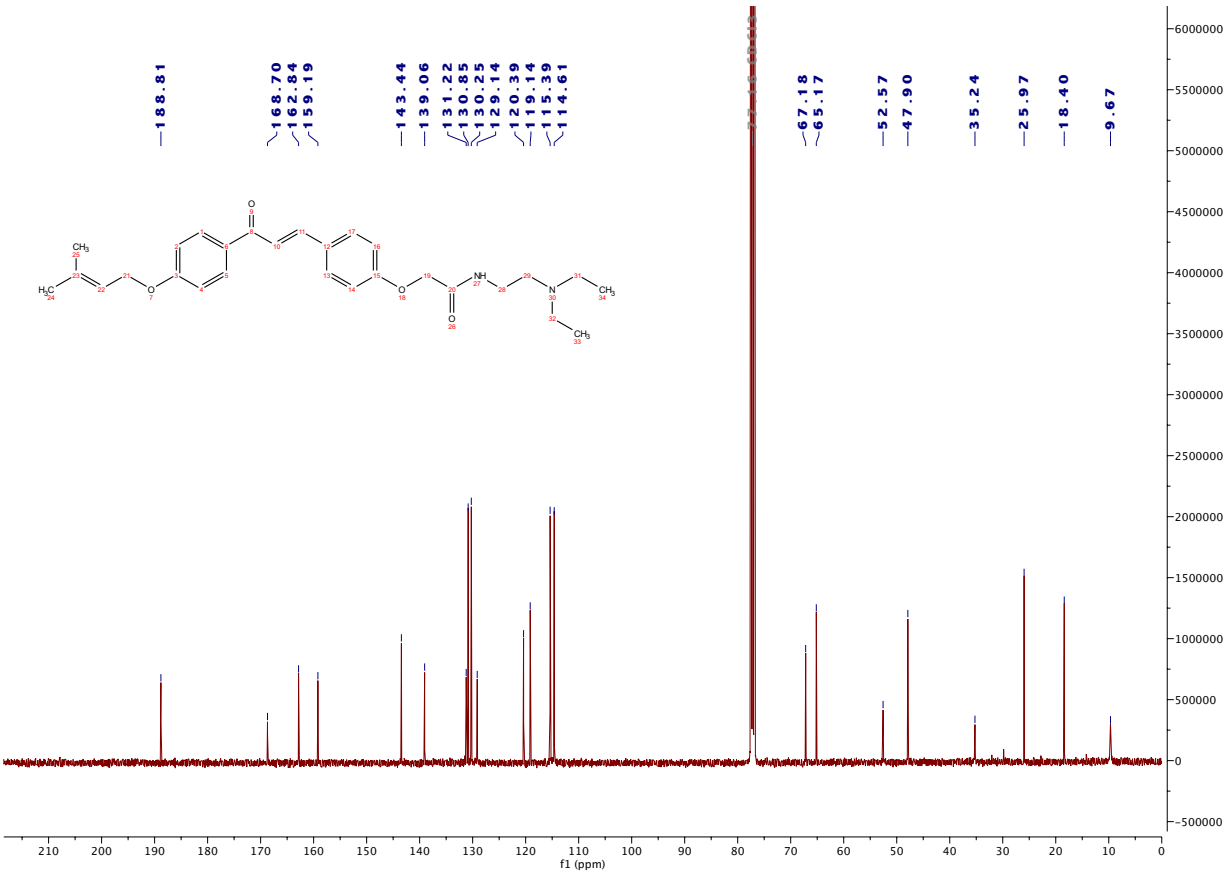
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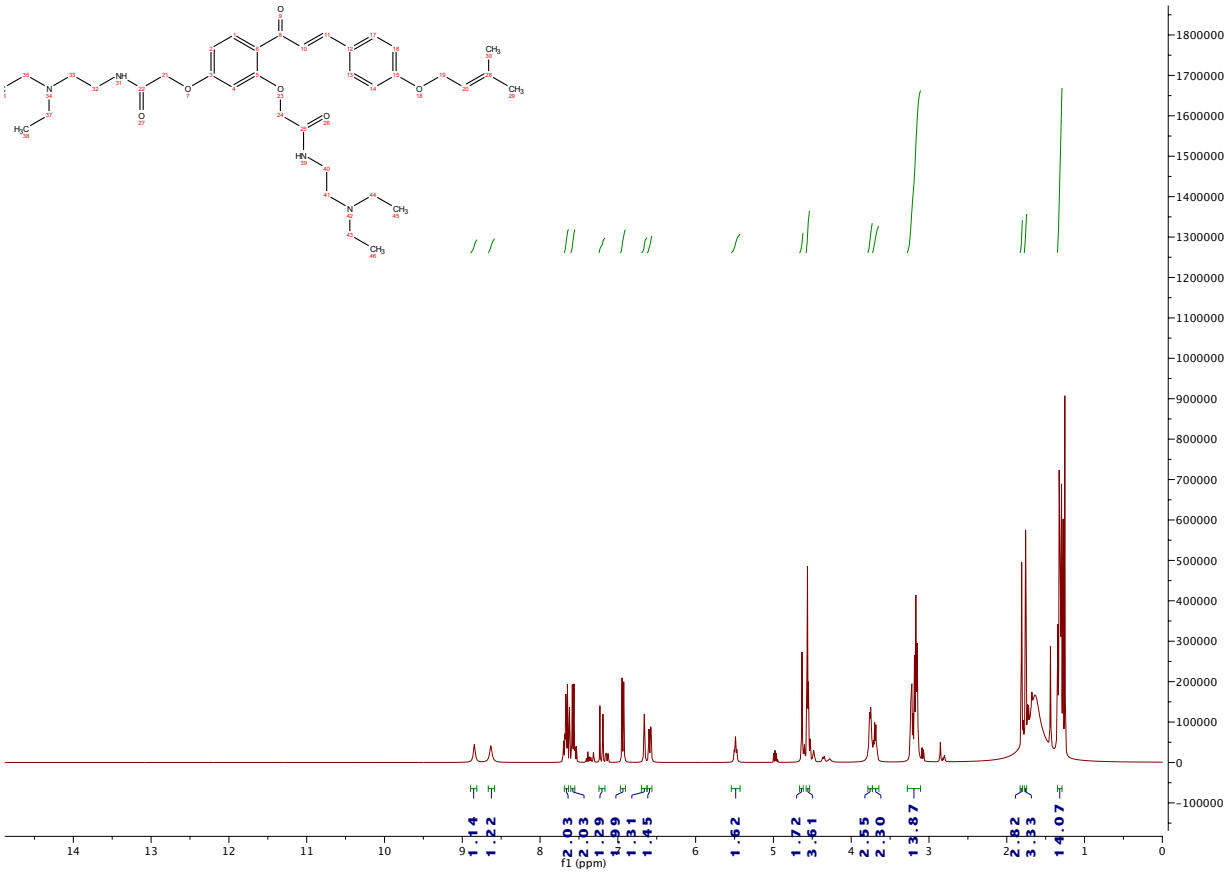
5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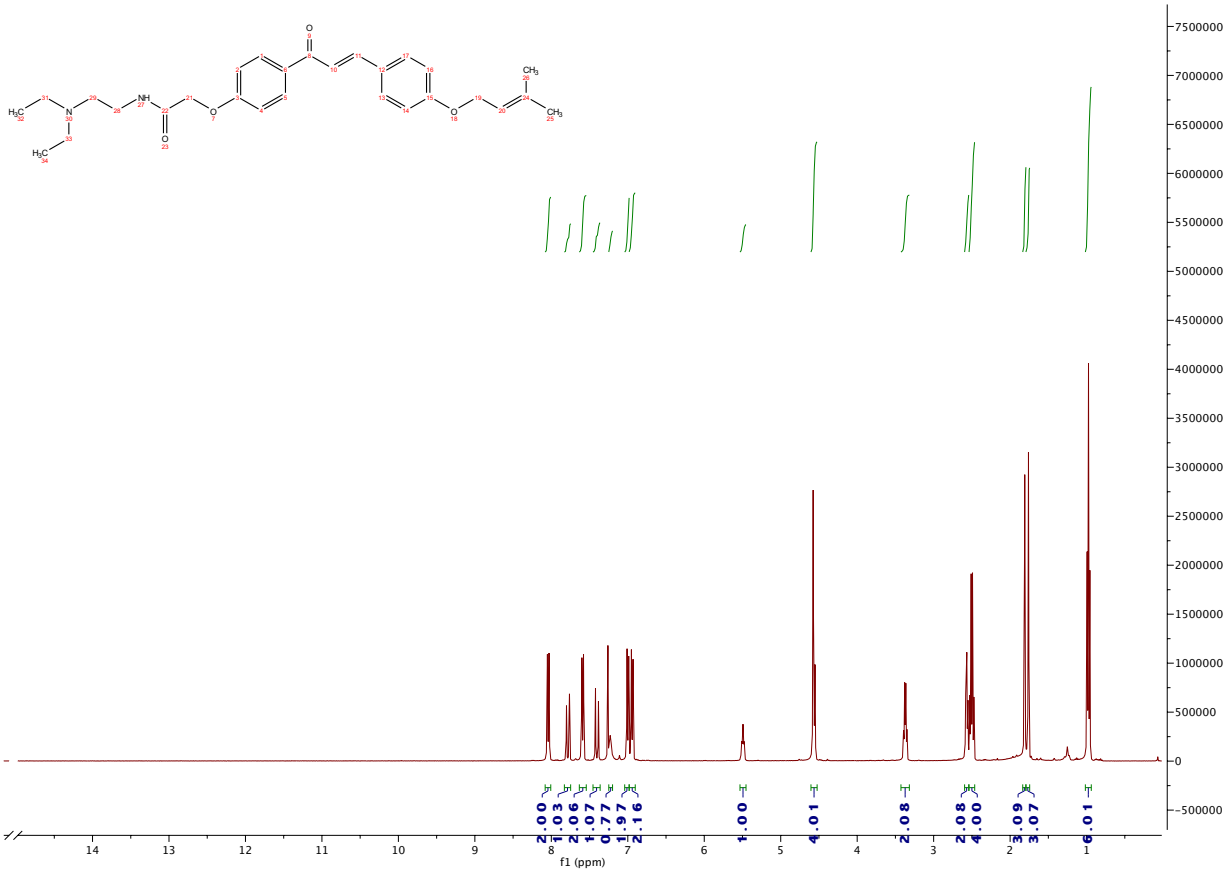
6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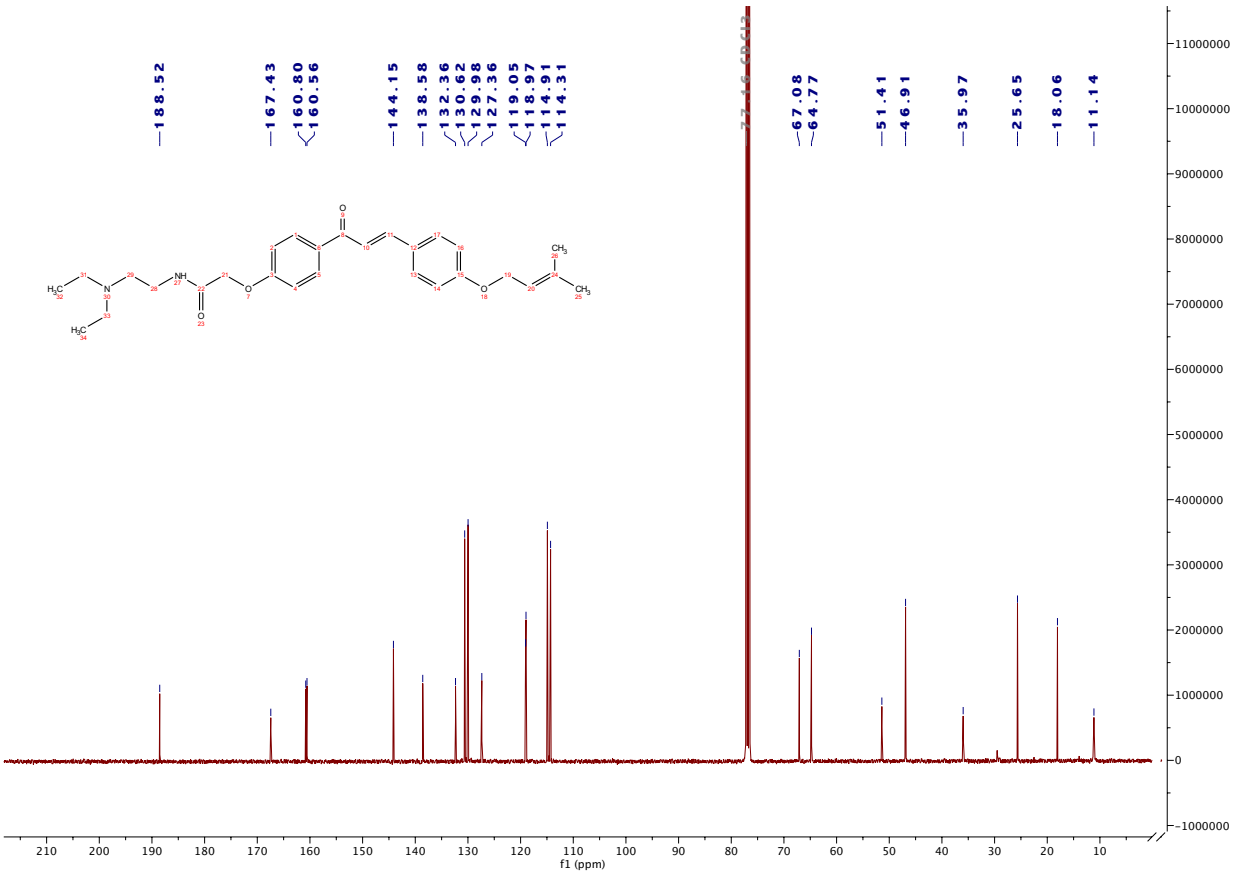
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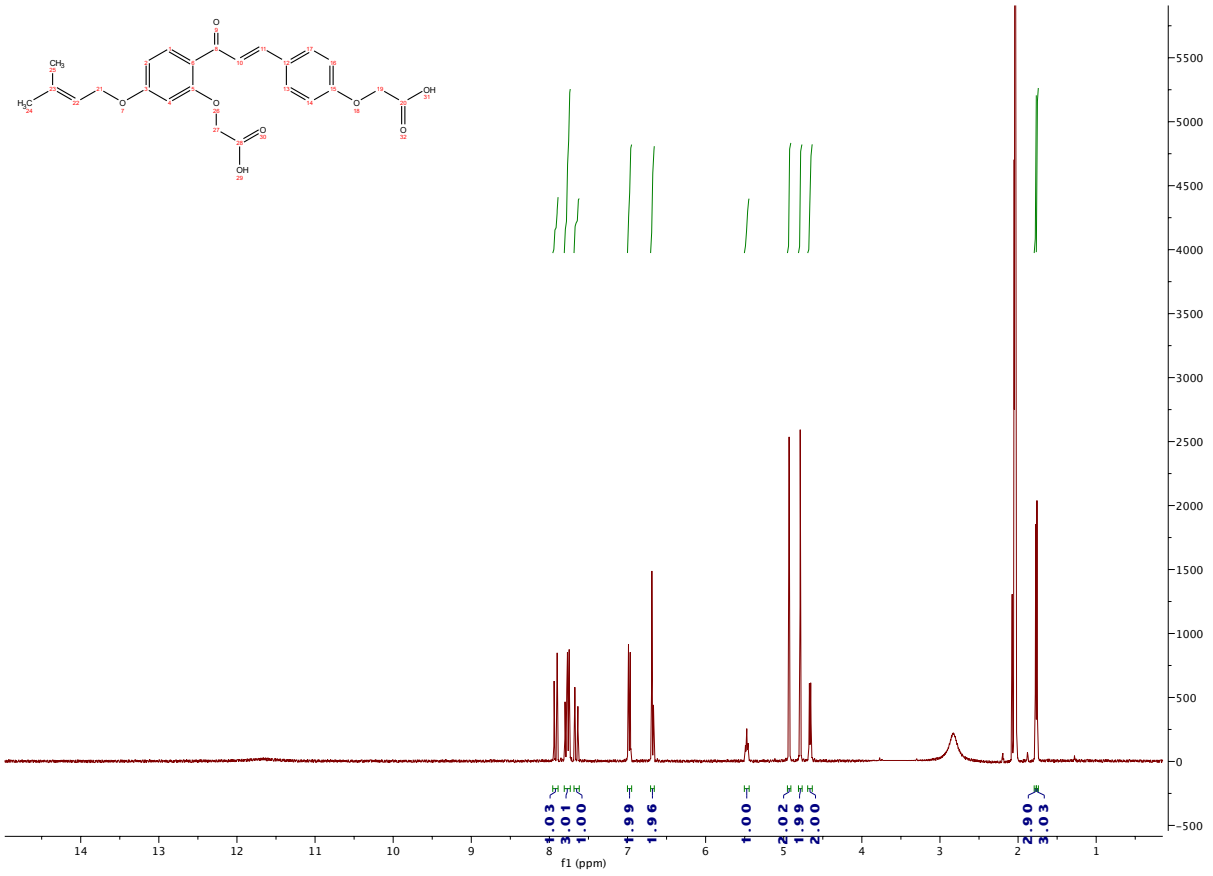
16a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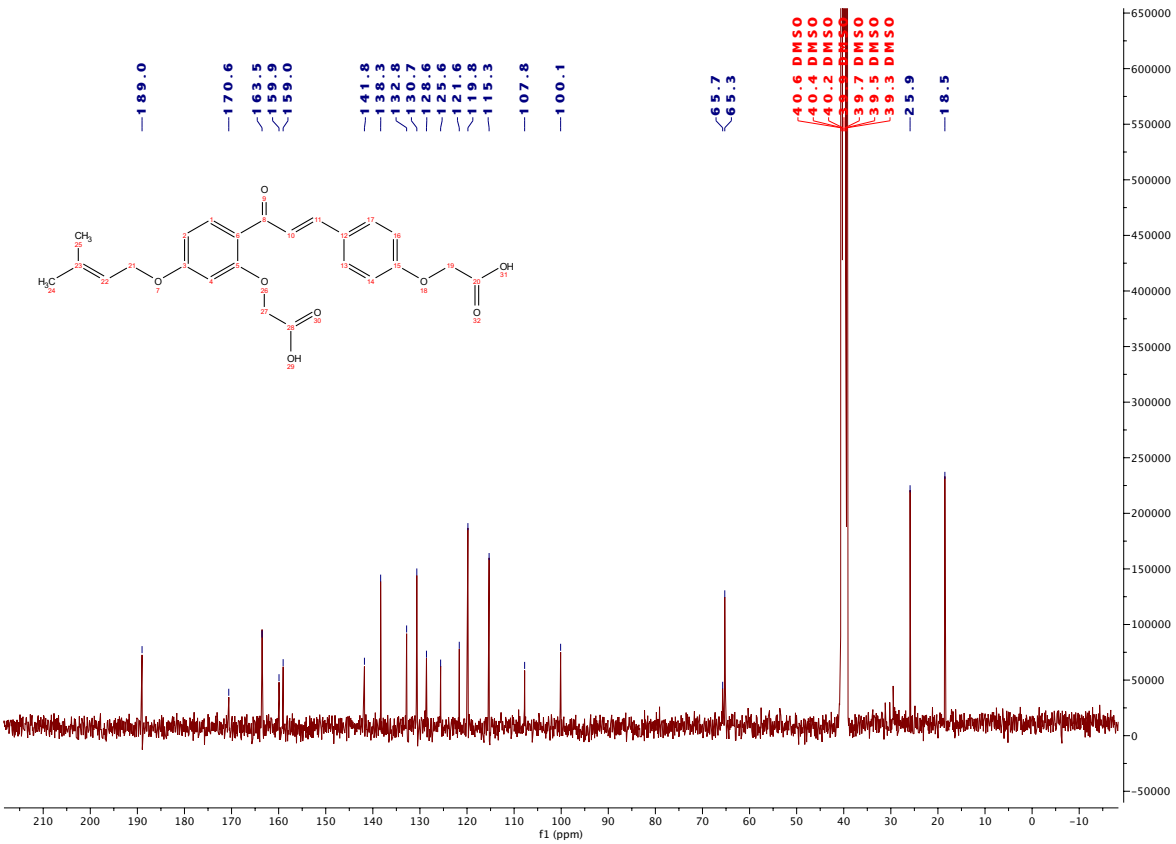
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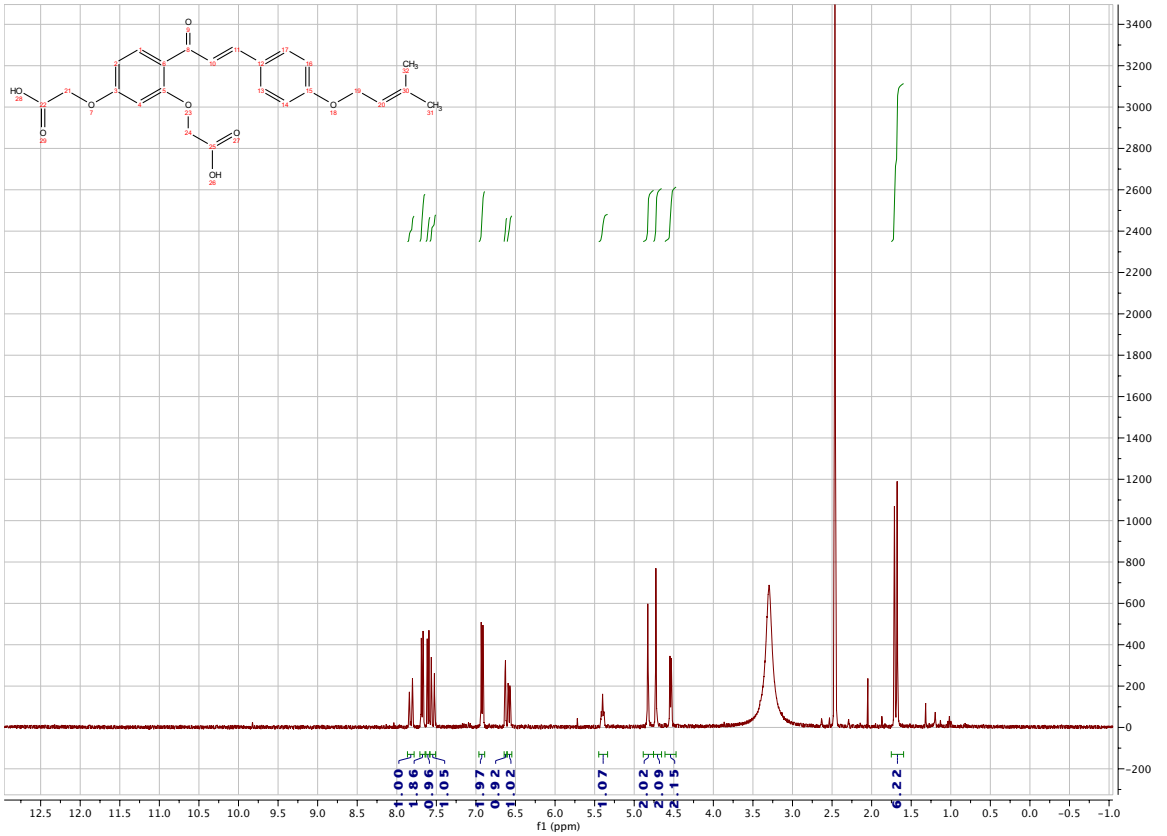
19a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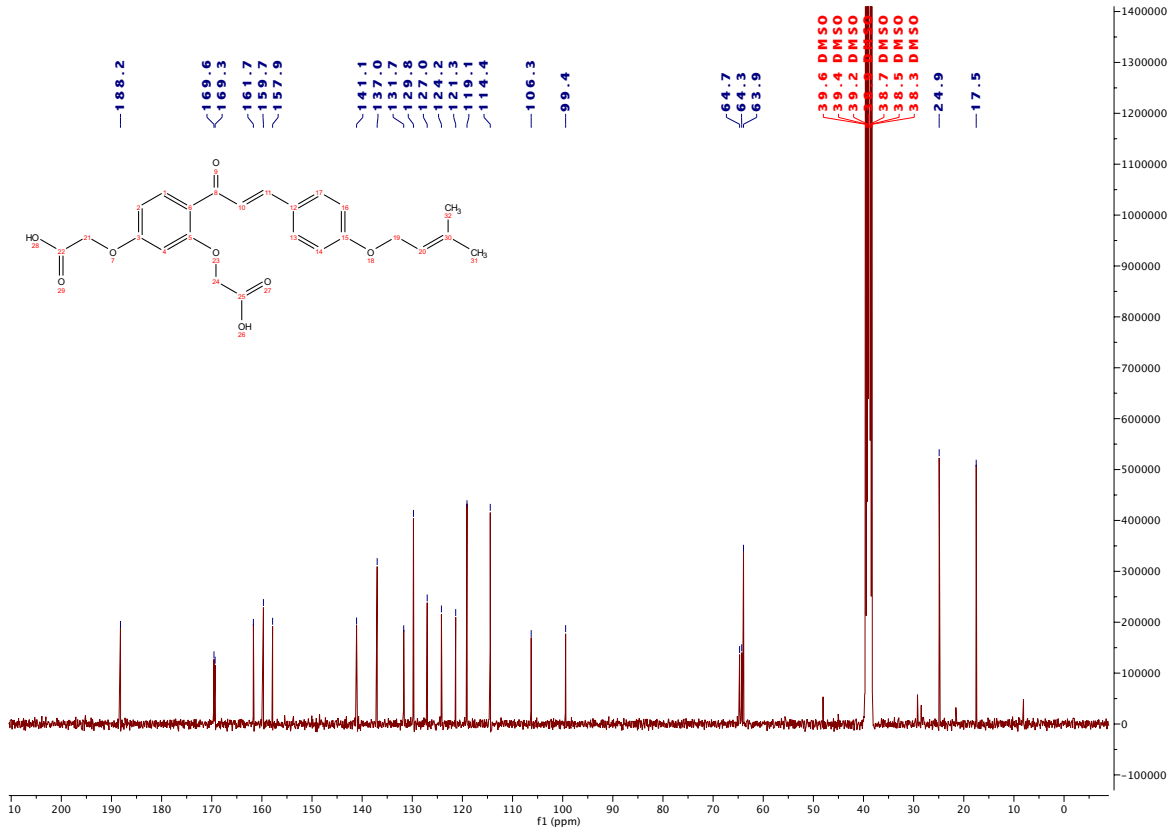
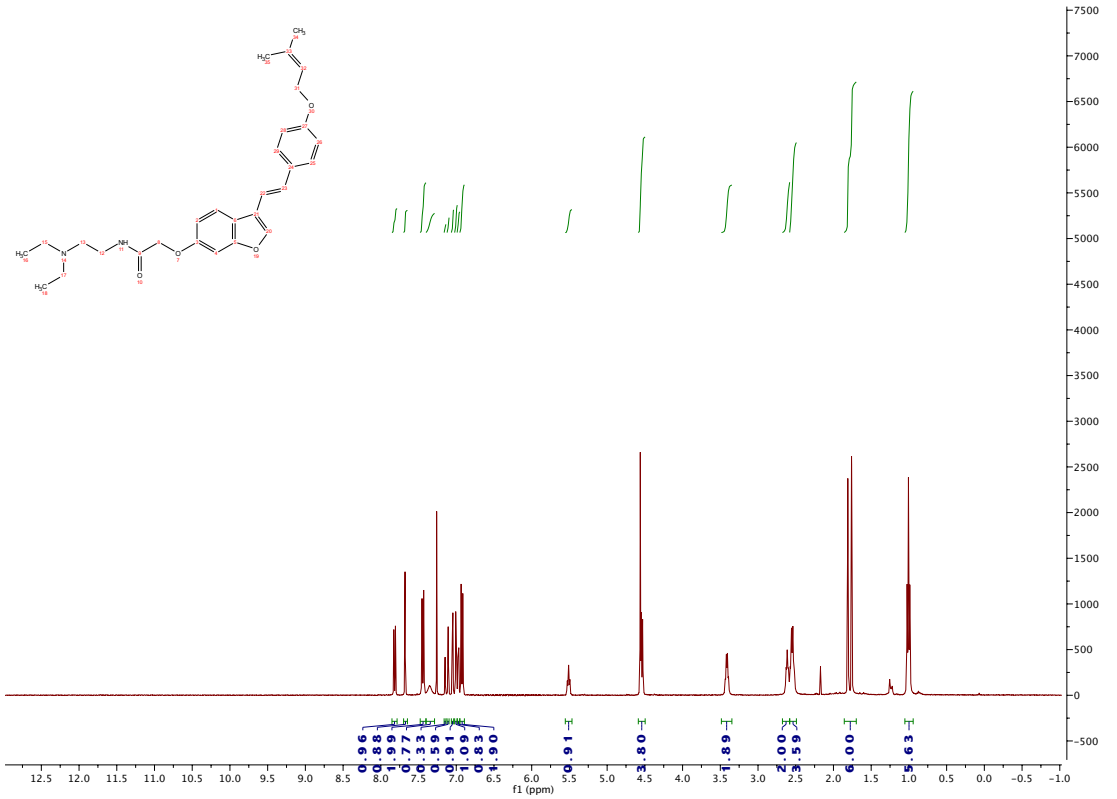
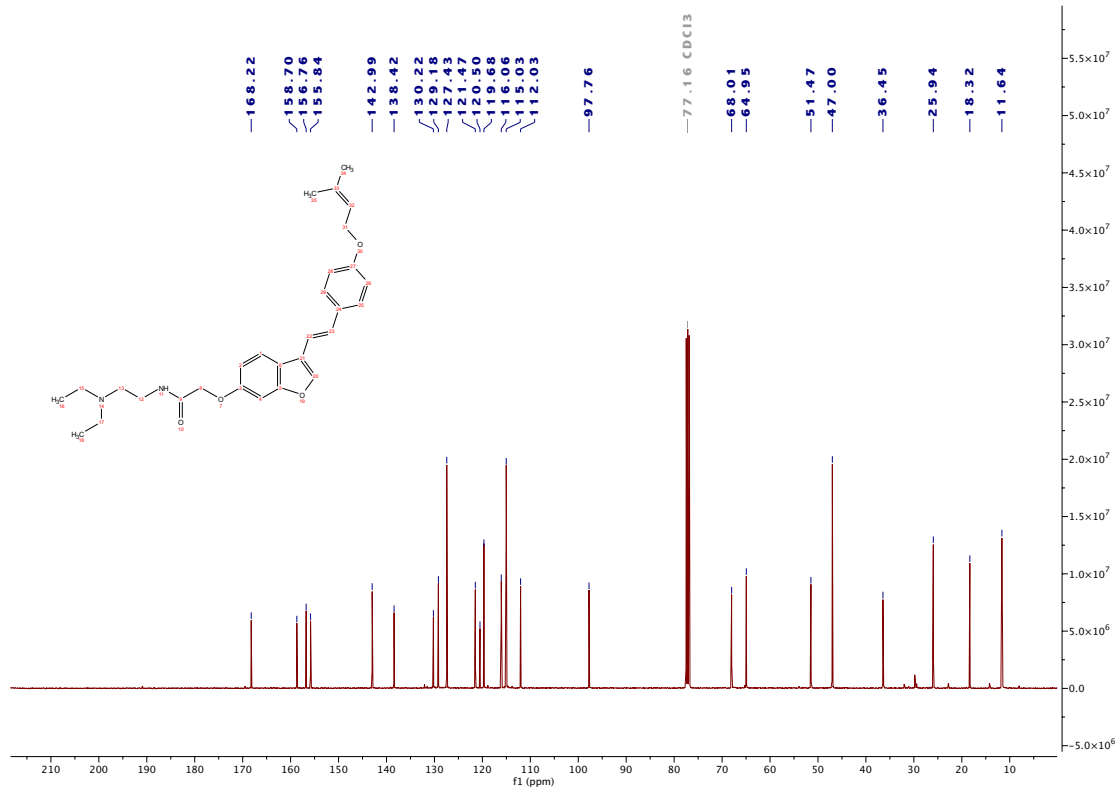
f1 (ppm)


188.2
169.6
169.3
161.7
159.7
157.9
141.1
137.0
131.7
129.8
127.0
124.2
121.3
119.1
114.4
106.3
99.4
64.7
64.3
63.9
39.6 DMSO
39.4 DMSO
39.2 DMSO
38.7 DMSO
38.5 DMSO
38.3 DMSO
24.9
17.5
f1 (ppm)

20


0.96
0.88
1.99
0.77
0.33
0.59
0.91
1.09
0.83
4.90
0.91
3.80
1.89
2.00
3.59
6.00
5.63
f1 (ppm)


168.22
158.70
156.76
155.84
142.99
138.42
130.22
129.18
127.43
121.47
120.50
119.68
116.06
115.03
112.03
97.76
77.16 CDCl3
68.01
64.95
51.47
47.00
36.45
25.94
18.32
11.64
f1 (ppm)

3. Analytical data and purity

Table S1

Compound	RT (min)	% Purity
1a	6.72	96.34
1b	11.35	96.39
2a	6.28	98.44
2b	11.73	95.10
3	19.13	98.93
4	3.32	96.83
5	19.15	98.22
6	3.38	98.97
20	12.91	98.20
16a	16.12	99.0
19a	16.97	95.7

HPLC analysis were performed with a Shimadzu Prominence apparatus equipped with a scanning absorbance UV-VIS detector (Diode Array SPD-M20A) also equipped with a thermostatic chamber using Purospher®STAR, RP-18ε (5 µM) or Poroshell 120 EC-18 (2.7 µm) HPLC columns

4. DoE

The DoE optimisation was designed and analysed using MODDE® Pro 13.1 software (Sartorius Stedim Data Analytics AB).

Date were analysed by multiple linear regression, confidence interval 95%.

General Procedure for the Claisen-Schmidt reaction for the DoE

Ketone **8a** (100 mg) was dissolved in 1.5 mL of 2-methyltetrahydrofuran (2-MeTHF) under a nitrogen atmosphere using a calibrated pipette. Once complete solubilization was achieved, NaH was added under nitrogen, previously weighed on an analytical balance. The amount of base varied depending on the experimental conditions (e.g., 2 eq = 45 mg; 9.0 eq = 98 mg), as reported in the corresponding worksheet. When 9 equivalents of NaH were used, the base was added portionwise over 5 minutes.

After 30 minutes of stirring, aldehyde was added to the reaction mixture (e.g., 0.8 eq = 101 mg; 1.2 eq = 151 mg), and the mixture was stirred under the specified conditions (2 or 16 h) either at room temperature or at 0 °C, according to the experimental design.

At the end of the reaction, 10 µL of the reaction mixture was withdrawn using a calibrated pipette and diluted in 1 mL of a mixture consisting of acetonitrile (850 µL), water (150 µL), and DMSO (50 µL). After complete dissolution, it was filtered and 10 µL were withdrawn and injected to HPLC. The concentration of the product was determined by HPLC analysis.