Supporting Information

Fluorescence-based chemical tools for monitoring ultrasound-induced hydroxyl radical production in aqueous solution and in cells

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1. General Information and Methods

1.1 Materials and Chemicals

All chemicals and reagents were purchased commercially and were of analytical grade. Absorption spectra were obtained on a Jasco V-770 spectrophotometer and fluorescence spectra were obtained on a PerkinElmer LS55 Luminscence spectrometer using quartz cuvettes of 1 cm path length. Column chromatography was carried out using Merck® silica gel 60 under a positive pressure of nitrogen. NMR spectra were recorded on a Bruker AVIII 400, Bruker NEO 600, Bruker AVII 500 (with cryoprobe) and Bruker AVIII 500 spectrometers. Chemical shifts are reported as δ values in ppm. Mass spectra were performed using Waters Micromass LCT and Bruker microTOF spectrometers. Ultrasound-based experiments were performed using a custom-built sonoreactor and a commercially available WED-100 instrument (Honda Hi-Tech, Shenzhen, China). The method for •OH radical was adapted from a previous report,[1] in which •OH radical was generated by the Fenton reaction. The concentration of ·OH was equal to the Fe(II) concentration: $FeCl_2 \cdot 4H_2O$ (10 mM) and Na_2EDTA (10 mM) in PBS (10 mL). The Fe(II)-EDTA solution should be made fresh for each experiment. The Fe(II)-EDTA solutions were diluted accordingly into PBS containing the probe and H_2O_2 (10 mM). Other species were made by following previous methods.[2]

1.2 Cell Experiments

HeLa cervical cancer cell line (ATCC-CCL2) was maintained in a Dulbecco's Modified Eagle's Medium supplemented with 10 % FBS in a humidified atmosphere of 5 % CO_2 and 95 % air at 37 °C and split when the cells reached 90 % confluency.

1.2.1 Cytotoxicity assay

Standard Cell Counting Kit-8 (CCK-8) assay was used to evaluate the cytotoxicity of **Res-DHB** with HeLa cells. Cells were incubated in 96-well microplates in DMEM media containing 10% FBS overnight. The cells were cultured with different concentrations of **Res-DHB** (0, 2.5, 5 and 10 μM) for 12 h, respectively. Subsequently, CCK-8 solution (10 μL) was added to per well and sequentially incubated for 30 min. The absorbance at 450 nm was recorded on a microplate reader.

1.2.2 Fluorescence Imaging

Cells were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight at 37 °C in a humidified atmosphere of 5 % CO_2 and 95 % air. Cells were incubated with **Res-DHB** (10 μM), followed by sonication operations at different acoustic intensities (0.5, 1, 1.5, 2 W/cm^2) and times (1, 3, 5, 10 min). All experiments were conducted at a 50% duty cycle with a pulse time of 6 ms. Here, the acoustic intensity was the listed intensity on the device. A 2 cm of pork tissue was placed between the ultrasound source and the cells, followed by ultrasound treatment (1MHz, 1.5 W/cm^2, 5 min). Subsequently, cells were incubated

for a further 120 minutes after sonication. The cells were then washed three times with PBS. The fluorescence images were recorded using the high content imaging system (Operetta CLS, PerkinElmer, USA).

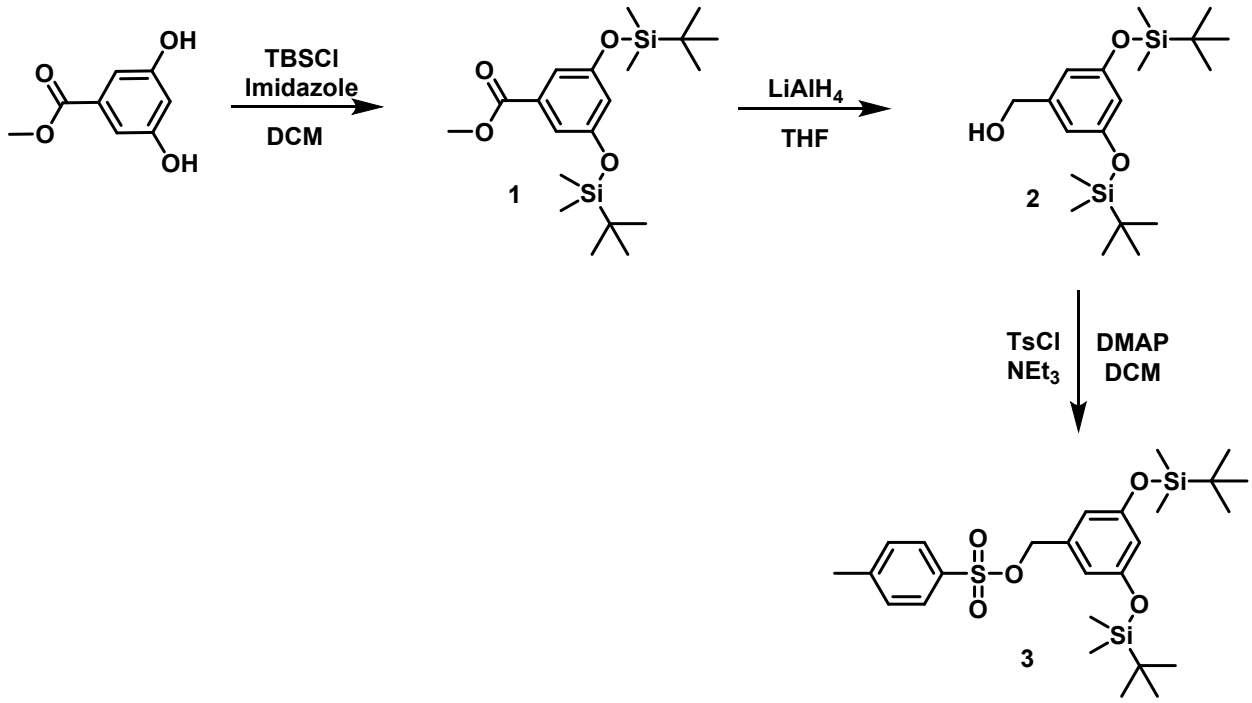
Live subject statement: The animal tissue used in this study was purchased from a supermarket in Yantai and these refrigerated products were used without further treatment. The samples were stored in a refrigerator at −18 °C to prevent sample deformation. Since researchers have no control over antemortem procedures or euthanasia, the occupational health risk is nil; therefore, the Institutional Animal Care and Use Committee at the Shandong Laboratory of Yantai Drug Discovery does not require a protocol. The structure of slaughtered pork generally consists of skin, subcutaneous fat, and muscle layers. In addition, the intermuscular and intramuscular fat layers are also contained within the muscle layer.

1.2.3. Ultrasound Exposure Experiments

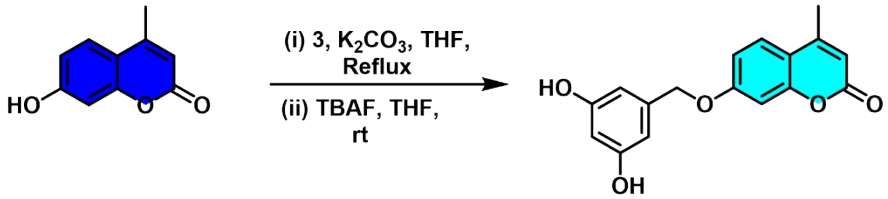
The ultrasound exposure experiments were performed with a bespoke sonoreactor (University of Oxford). The sonoreactor created a converging acoustic pressure wave to form an intense localized region for acoustic cavitation.

The operating acoustic parameters used were 1.083 MHz drive frequency, 100 cycles, and 1 ms burst period resulting in a 9.23% duty cycle. The power per unit area of the surface of the transducer (29.83 cm^2) was 12.7 W/cm^2 per pulse with a time-averaged power per unit area of 1.2 W/cm^2. The samples were exposed to ultrasound for different exposure times (1 min, 2 min, 3 min, 4 min, 5 min, and 6 min). Before each reaction, the solution was saturated with air by vigorously shaking the reaction vessel. All reactions were performed at ambient pressure and room temperature.

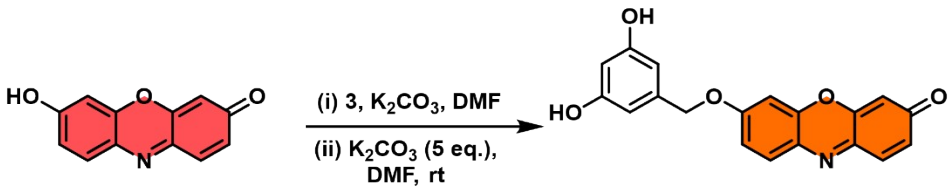
2. Synthetic Schemes



Scheme S1. Synthesis of Tosyl-based silyl-protected dihydroxylbenzyl (**3**)



Scheme S2. Synthesis of **Umb-DHB** from the fluorophore, 4-methylumbeliferone



Scheme S3. Synthesis of **Res-DHB** from the fluorophore, resorufin

3. Additional Analyses

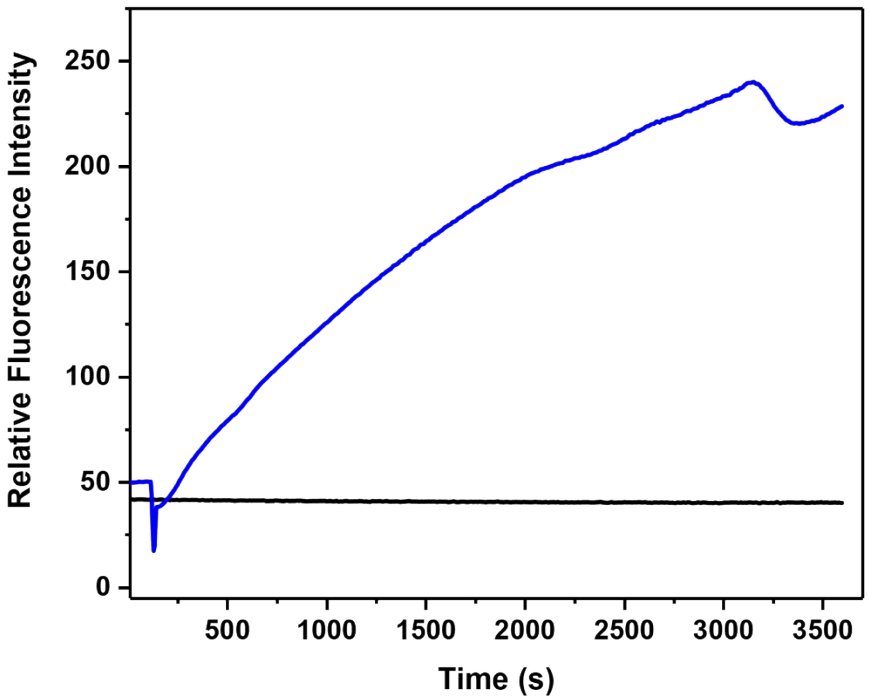


Figure S1. Change in relative fluorescence emission intensity of **Umb-DHB** (5 μM) at 450 nm in PBS buffer and H_2O_2 (10 mM) (pH =7.40). λ_{ex} = 350 nm, slit widths: ex = 10 nm and em = 2.5 nm. **Blue** – Fe(II)-EDTA (100 μM) addition. **Black** – control.

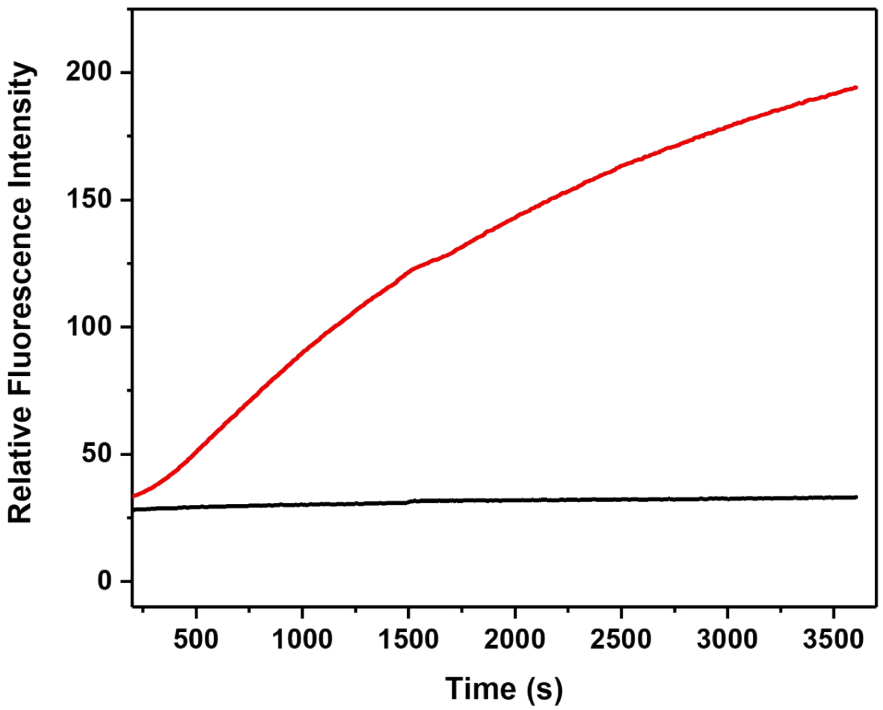


Figure S2. Change in relative fluorescence emission intensity of **Res-DHB** (5 μM) at 585 nm in PBS buffer and H_2O_2 (10 mM) (pH =7.40). λ_{ex} = 350 nm, slit widths: ex = 10 nm and em = 3.5 nm. **Red** – Fe(II)-EDTA (100 μM) addition. **Black** – control.

Figure S3. Photograph of quartz cuvettes containing **Res-DHB** in PBS buffer and H2O2 (10 mM, pH = 7.40) before (left) and after the addition of Fe(II)-EDTA (100 μM)(right)

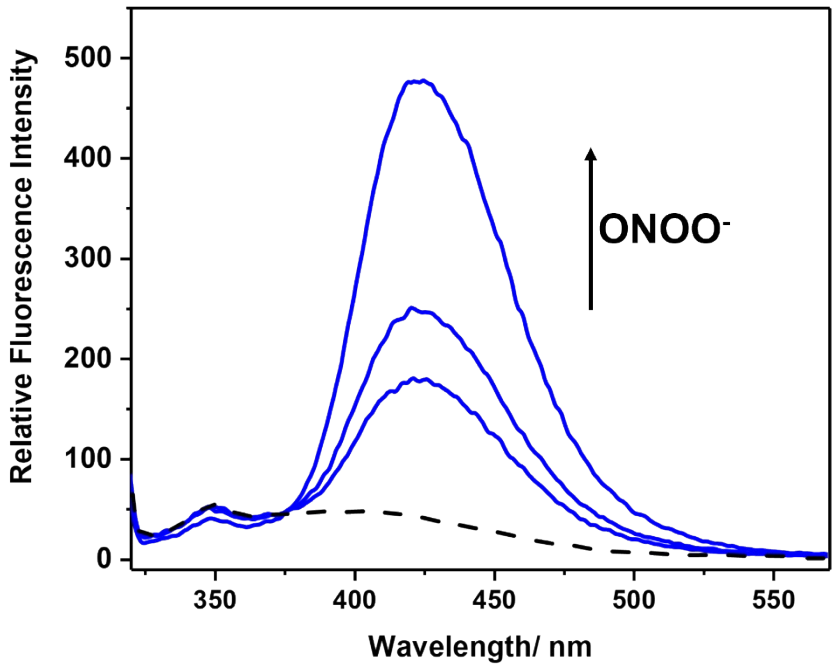


Figure S4. Fluorescence spectra of terephthalic acid (2 mM) in PBS buffer (pH = 7.20) with increasing concentrations of $ONOO^-$ (0 – 300 μM). λ_{ex} = 310 nm, Slit widths: 10 and 4 nm.

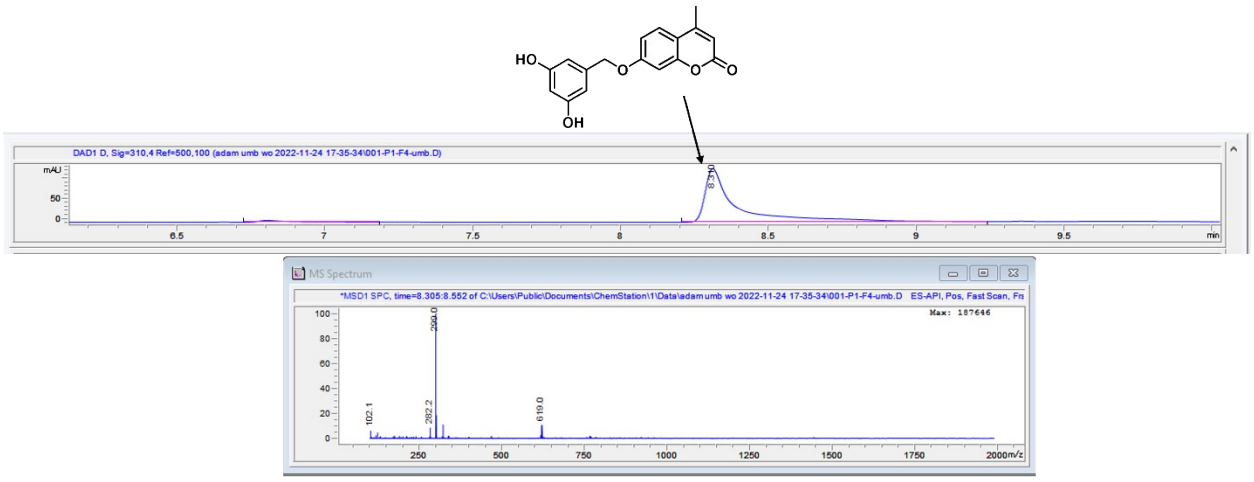


Figure S5. LC-MS spectra of **Umb-DHB** (5 μM) in PBS buffer, pH =7.4 solution before ultrasound exposure.

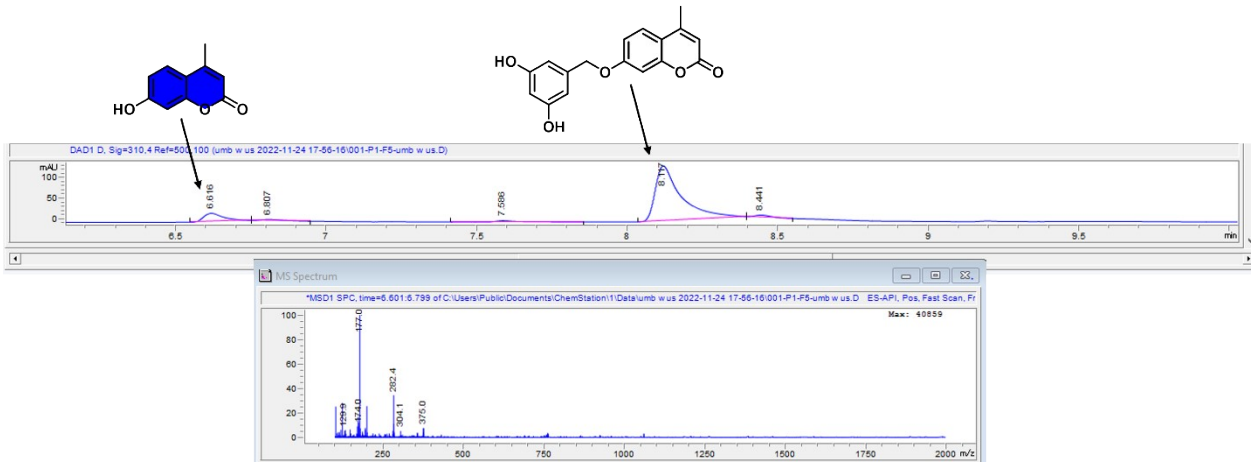


Figure S6. LC-MS spectra of **Umb-DHB** (5 µM) in PBS buffer, pH =7.4 solution after ultrasound exposure (2 min).

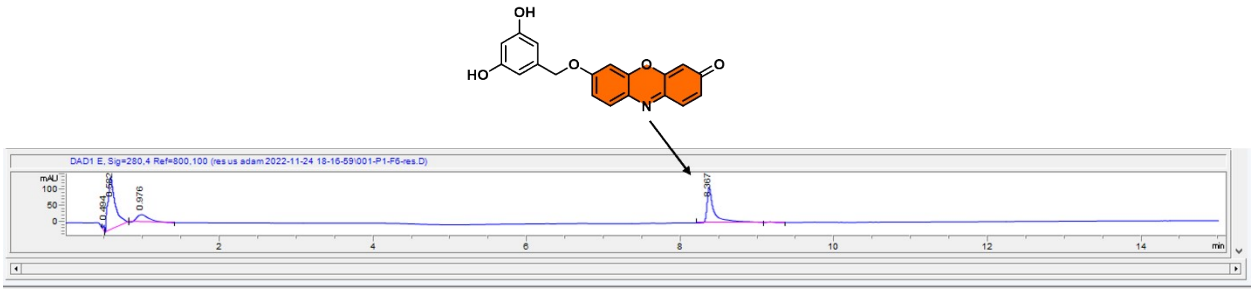


Figure S7. LC spectra of **Res-DHB** (5 µM) in PBS buffer, pH =7.4 solution before ultrasound exposure.

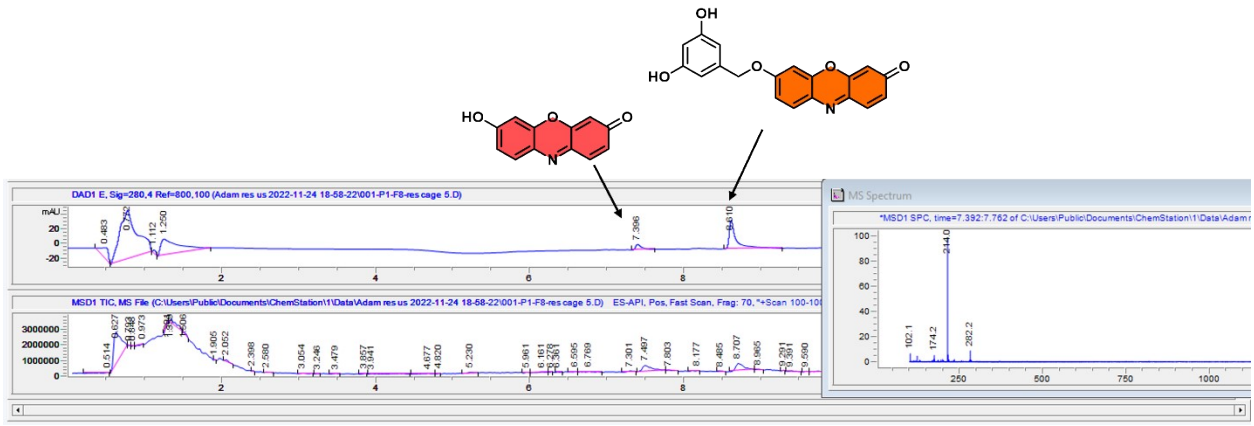


Figure S8. LC-MS spectra of **Res-DHB** (5 µM) in PBS buffer, pH =7.4 solution after ultrasound exposure (2 min).

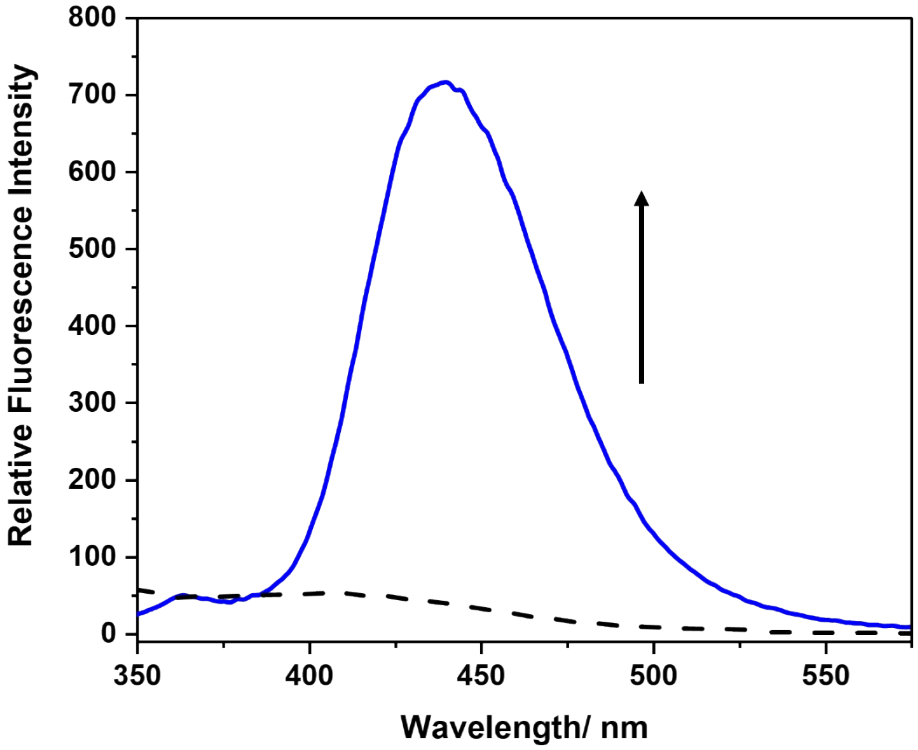


Figure S9. Fluorescence spectra of terephthalic acid (2 mM) in PBS buffer, pH = 7.2 before and after ultrasound exposure (6 min). λex = 310 nm, slit widths: ex = 10 nm and em = 4 nm.

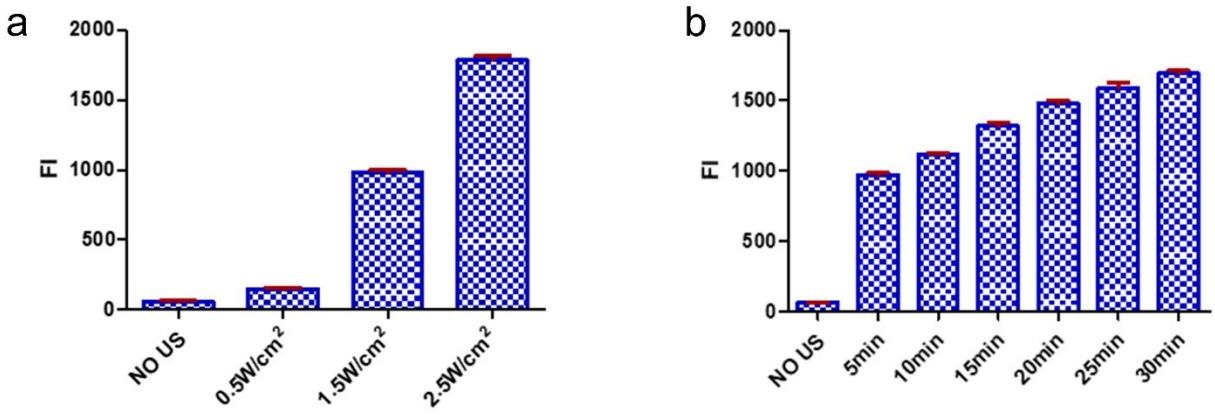


Figure S10. Fluorescence changes of **Res-DHB** upon sonication under (a) different power intensities and (b) ultrasound time. Ultrasound: 1 MHz, 1.5 W/cm^2, 50% duty cycle. Mean values ± standard deviation, N = 3.

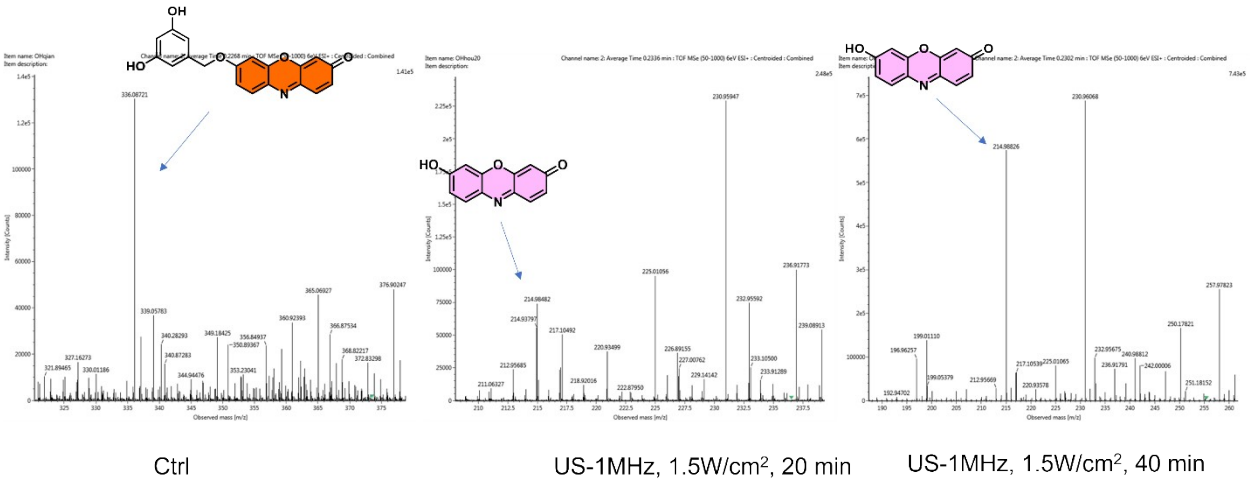


Figure S11. Mass spectra of **Res-DHB** before and after ultrasound exposure in aqueous solution

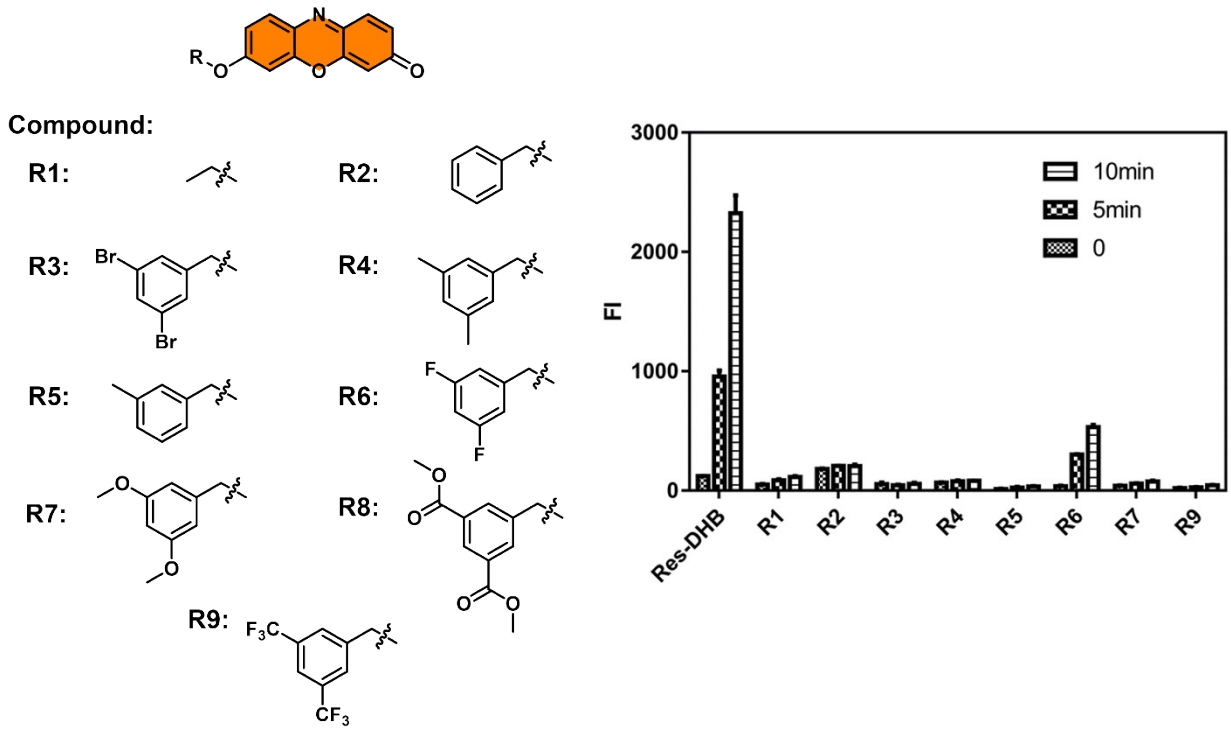


Figure S12. Fluorescence changes of **Res-DHB** and analogues (10 μM) upon sonication over time (0, 5, 10 min). Ultrasound: 1 MHz, 1.5 W/cm^2, 50% duty cycle. Mean values ± standard deviation, N = 3. Note - **R8** was found to be too insoluble.

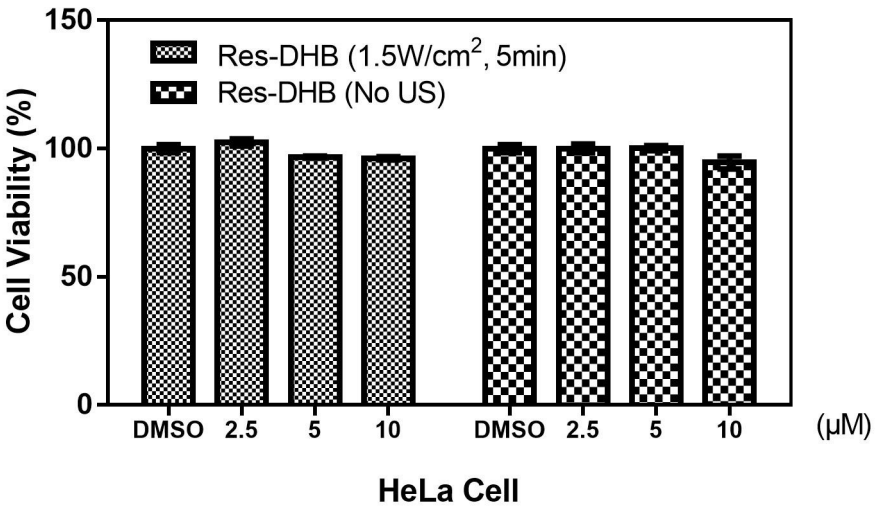


Figure S13. Cytotoxicity assessments of **Res-DHB** (0, 2.5, 5, 10 μM) with and without US treatment towards HeLa cells for 12 h by the method of CCK-8.

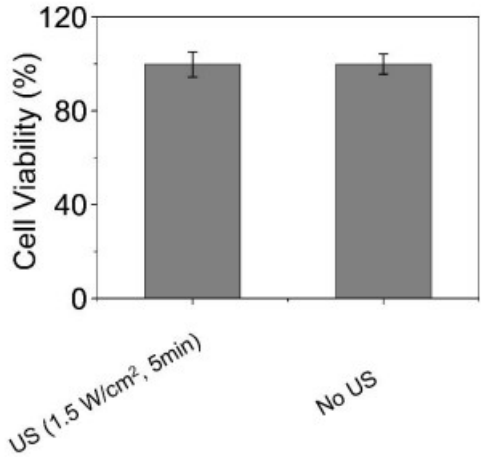


Figure S14. Cytotoxicity assessments of HeLa cells with and without US treatment by the method of CCK-8

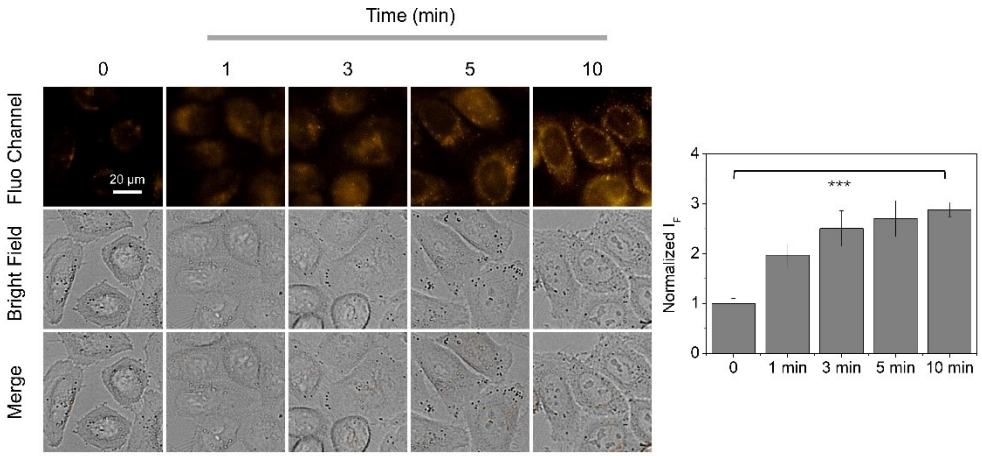


Figure S15. Time-dependent fluorescence imaging of HeLa cells incubated with probe **Res-DHB** (10 μM) upon sonication. Ultrasound: 1 MHz, 1.5 W/cm^2, 50% duty cycle. Mean values ± standard deviation, N = 3. ***P < 0.001.

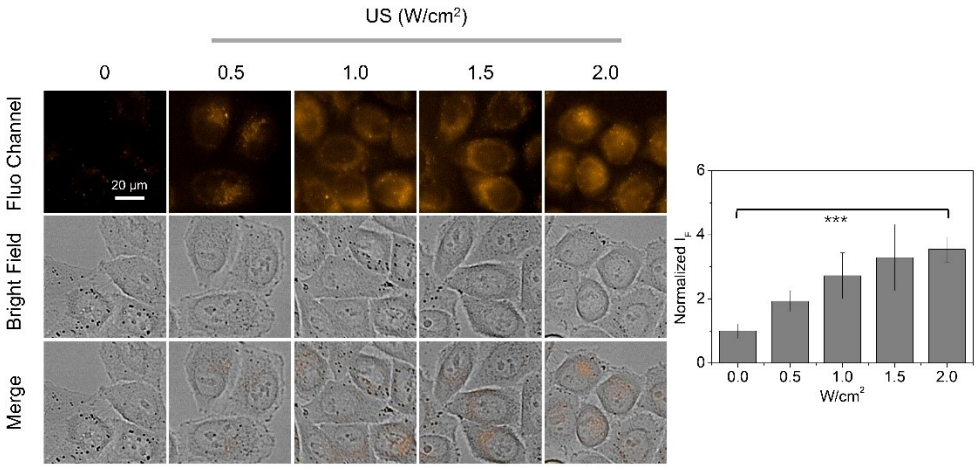


Figure S16. Power-dependent fluorescence imaging of HeLa cells incubated with probe **Res-DHB** (10 μM) upon sonication. Ultrasound: 1 MHz, 1.5 W/cm^2, 50% duty cycle. Time: 5min. Mean values ± standard deviation, N = 3. ***P < 0.001.

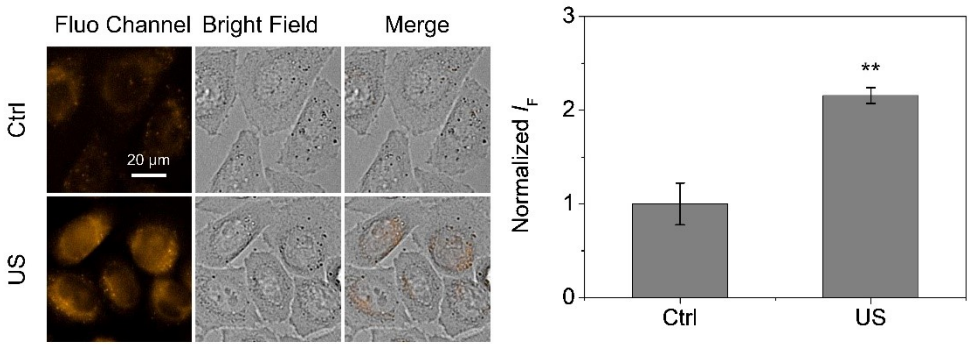
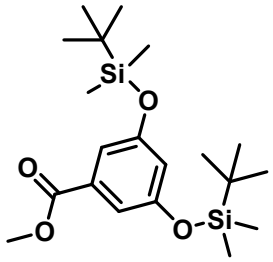


Figure S17. Fluorescence imaging of HeLa cells covered by 2 cm pork tissue incubated with **Res-DHB** (10 μM) upon sonication. Ultrasound: 1 MHz, 1.5 W/cm^2, 50% duty cycle. Time: 5min. Mean values ± standard deviation, N = 3. $^{**}P < 0.01$.

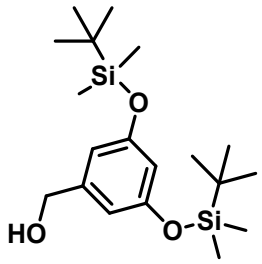
4. Synthetic Procedures

Methyl 3,5-bis((tert-butyldimethylsilyl)oxy)benzoate (1)



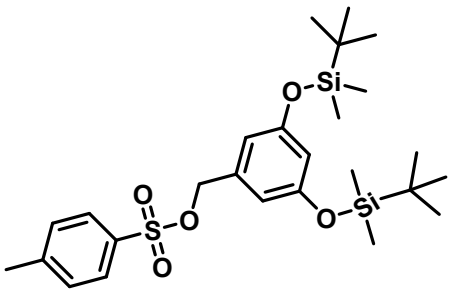
tert-Butyldimethylsilyl chloride (9.90 g, 65.68 mmol) was added to methyl 3,5-dihydroxybenzoate (5.00 g, 29.76 mmol) and imidazole (5.10 g, 74.40 mmol) in DCM (200 mL) at 0 ^{o}C. The reaction mixture was stirred at room temperature for approximately 16 hours (reaction progress followed by TLC). The reaction mixture was partitioned with H_2O (200 mL) and the organic layer was washed with brine (3 x 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude mixture was deemed suitable for the subsequent reaction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, *J* = 2.3 Hz, 2H), 6.52 (t, *J* = 2.3 Hz, 1H), 3.88 (s, 3H), 0.98 (s, 18 H), 0.20 (s, 12 H). Data was consisted with reported literature data.[1]

(3,5-Bis((tert-butyldimethylsilyl)oxy)phenyl)methanol (2)



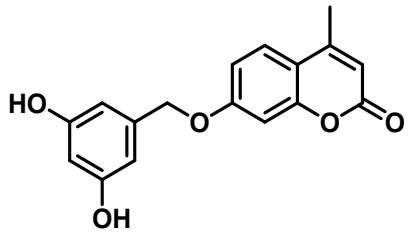
$LiAlH_4$ (1.00 g, 12.60 mmol) was added portion wise to a solution of methyl 3,5-bis((tert-butyldimethylsilyl)oxy)benzoate (**1**) in anhydrous THF (100 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stir overnight (~ 16 hours). Upon completion, the reaction was quenched with H_2O (50 mL) and partitioned with EtOAc (50 mL). The aqueous layer was extracted with EtOAc (3 x 50 mL) and the combined organics were washed with brine (3 x 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure to afford the crude material. Purification was performed via silica chromatography (10:90 EtOAc:Hexane) to afford the title compound as a clear oil (3.85 g, 10.44 mmol, 83 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (d, J = 2.2 Hz, 2H), 6.25 (t, J = 2.2 Hz, 1H), 4.57 (s, 2H), 0.98 (s, 18H), 0.19 (s, 12H). Data was consisted with reported literature data.[1]

3,5-Bis((tert-butyldimethylsilyl)oxy)benzyl 4-methylbenzenesulfonate (3)



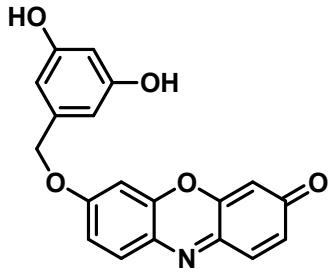
4-Toluenesulphonyl chloride (1.16 g, 6.08 mmol) was dissolved in DCM (15 mL) and added dropwise to a solution of 3,5-bis((tert-butyldimethylsilyl)oxy)phenyl)methanol (2) (1.50 g, 4.07 mmol), NEt_3 (0.82 mL, 6.08 mmol), and DMAP (0.10 g, 0.81 mmol) in DCM (15 mL) at 0 ^oC. The reaction was monitored by TLC and upon completion, the reaction mixture was partitioned between H_2O (15 mL) and DCM (15 mL). The organic layer was washed with H_2O (15 mL), brine (15 mL), dried ($MgSO_4$) and concentrated under reduced pressure to afford the crude material. The crude mixture was purified via silica chromatography (10:90 DCM:Hexane to 30:70 DCM:Hexane) to afford the title compound as a clear pale waxy solid (0.75 g, 1.43 mmol, 35 %). ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 8.1 Hz, 2H), 6.31 (d, J = 2.3 Hz, 2H), 6.26 (t, J = 2.3 Hz, 1H), 4.93 (s, 2H), 2.44 (s, 3H), 0.96 (s, 18H), 0.16 (s, 12H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.74, 144.73, 135.12, 133.39, 129.81, 127.96, 113.32, 112.49, 71.63, 25.64, 21.64, 18.17, -4.44. HRMS: m/z calculated for $C_{26}H_{42}O_5SSi_2$: requires 523.2364 for $[M+H]^+$, found 523.2383.

7-((3,5-Dihydroxybenzyl)oxy)-4-methyl-2H-chromen-2-one (Umb-DHB)

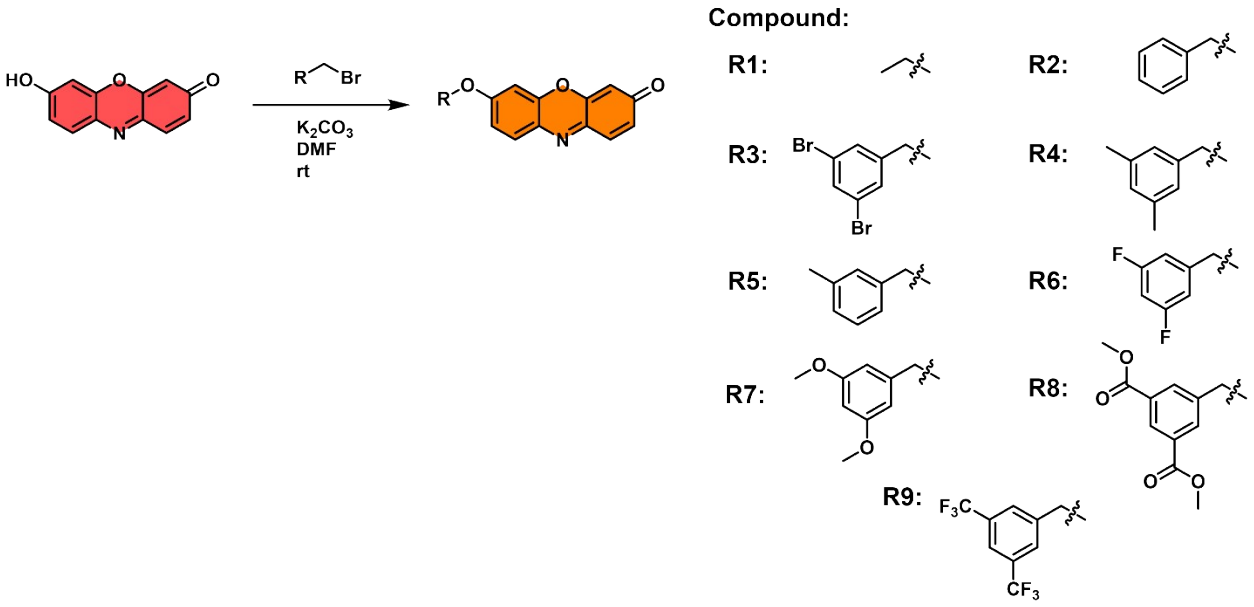


A mixture of 4-methylumbeliferone (0.20 g, 1.14 mmol), **3** (0.59 g, 1.14 mmol), and K_2CO_3 (0.31 g, 2.27 mmol) in THF (5 mL) was heated to reflux and monitored by thin layer chromatography (TLC). Once the reaction was deemed complete, the mixture was cool to room temperature and partitioned between EtOAc (50 mL) and H_2O (50 mL). The organic layer was washed with H_2O (50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude material was dissolved in THF (2 mL) and cooled to 0 ^oC. TBAF - 1 M in THF (0.76 mL) was added dropwise to the mixture and the reaction mixture was stirred for 30 mins at room temperature. EtOAc (50 mL) was then added, and the organic layer was washed with H_2O (50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude material was purified via silica chromatography to afford the title compound as a white solid (0.055 g, 0.18 mmol, 16 %). ^1H NMR (600 MHz, DMSO-d6) δ 9.27 (s, 2H), 7.68 (d, *J* = 9.2 Hz, 1H), 7.00 (m, 2H), 6.28 (d, *J* = 2.3 Hz, 2H), 6.22 – 6.14 (m, 2H), 5.06 (s, 2H), 2.39 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d6) δ 161.85, 160.58, 159.00, 155.13, 153.87, 138.78, 126.95, 113.69, 113.18, 111.66, 105.83, 102.47, 102.16, 70.26, 18.59. HRMS: m/z calculated for $C_{17}H_{14}O_5$: requires 299.0914 for $[M+H]^+$, found 299.0906.

7-((3,5-Dihydroxybenzyl)oxy)-3H-phenoxazin-3-one (Res-DHB)



3 (0.49 g, 0.94 mmol) was added to a pink colored mixture of resorufin (0.20 g, 0.94 mmol) and K_2CO_3 (0.13 g, 0.94 mmol) in DMF (5 mL). The reaction mixture was stirred at room temperature for 14 hours and monitoring via TLC showed partial silyl ether deprotection. Once the reaction was deemed complete, the mixture was partitioned between EtOAc (50 mL) and H_2O (50 mL). The organic layer was washed with H_2O (3 x 50 mL), brine (3 x 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The excessive aqueous washes removed unreacted resorufin. The crude material was dissolved in DMF (2 mL) and K_2CO_3 (0.65 g, 4.70 mmol) was added and the deprotection was followed by TLC. Upon completion, EtOAc (50 mL) was added, and the organic layer was washed with H_2O (50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The crude material was purified via silica chromatography with EtOAc followed by MeOH to afford the title compound as a red solid (0.085 g, 0.25 mmol, 27 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 9.42 (s, 2H), 7.77 (d, *J* = 8.7 Hz, 1H), 7.52 (d, *J* = 9.9 Hz, 1H), 7.17 – 7.04 (m, 2H), 6.78 (d, *J* = 9.4 Hz, 1H), 6.28 (m, 3H), 6.16 (s, 1H), 5.11 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d6) δ 185.79, 162.84, 159.10, 150.19, 145.68, 138.44, 135.38, 134.19, 131.78, 128.42, 114.87, 106.13, 105.85, 102.62, 101.64, 70.77. HRMS: m/z calculated for $C_{19}H_{13}NO_5$: requires 336.0867 for $[M+H]^+$, found 336.0860.



Scheme S4. General synthesis of resorufin analogues **R1-R9**

General synthesis of R1-R9. To a solution of resorufin, K_2CO_3 (1.5 eq.) in DMF, the appropriate alkyl/ benzyl halide (1.5 eq.) was added. The reaction was monitored by TLC. After stirring at room temperature for ~ 14 hours, the reaction was partitioned between EtOAc (50 mL) and H_2O (50 mL). The organic layer was washed with H_2O (3 x 50 mL), brine (3 x 50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The excessive aqueous washes removed unreacted resorufin. The crude material was triturated (Et_2O) or purified via silica chromatography to afford the title compounds **R1**-**R9** as red/orange solids.

R1: 73 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.9 Hz, 1H), 7.42 (d, *J* = 9.7 Hz, 1H), 6.93 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.83 (dd, *J* = 9.8, 2.0 Hz, 1H), 6.80 (d, *J* = 2.6 Hz, 1H), 6.33 (d, *J* = 2.0 Hz, 1H), 4.15 (q, *J* = 7.0 Hz, 2H), 1.48 (t, *J* = 7.0 Hz, 3H). 13C NMR (151 MHz, $CDCl_3$) δ 186.29, 163.11, 149.90, 145.74, 145.44, 134.70, 134.16, 131.58, 128.31, 114.08, 106.70, 100.45, 64.67, 14.56. HRMS: m/z calculated for $C_{14}H_{11}NO_3$: requires 242.0812 for $[M+H]^+$, found 242.0803.

R2: 56 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.9 Hz, 1H), 7.46 – 7.40 (m, 5H), 7.40 – 7.35 (m, 1H), 7.02 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.89 (d, *J* = 2.6 Hz, 1H), 6.83 (dd, *J* = 9.8, 2.0 Hz, 1H), 6.32 (d, *J* = 2.1 Hz, 1H), 5.18 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 186.30, 162.67, 149.82, 145.74, 145.63, 135.43, 134.71, 134.28, 131.63, 128.86, 128.57, 128.52, 127.52, 114.27, 106.78, 101.10, 70.91. HRMS: m/z calculated for $C_{19}H_{13}NO_3$: requires 304.0968 for $[M+H]^+$, found 304.0971.

R3: 46 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.9 Hz, 1H), 7.67 (t, *J* = 1.8 Hz, 1H), 7.53 (d, *J* = 1.7 Hz, 2H), 7.43 (d, *J* = 9.8 Hz, 1H), 7.00 (dd, *J* = 8.9, 2.7 Hz, 1H), 6.88 – 6.81 (m, 2H), 6.33 (d, *J* = 2.0 Hz, 1H), 5.11 (s, 2H). The product was too insoluble to obtain a ^{13}C NMR spectrum. HRMS: m/z calculated for $C_{19}H_{11}Br_2NO_3$: requires 459.9178 for $[M+H]^+$, found 459.9178.

R4: 83 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.9 Hz, 1H), 7.42 (d, *J* = 9.8 Hz, 1H), 7.05 (br. s, 2H), 7.01 (m, 2H), 6.88 (d, *J* = 2.6 Hz, 1H), 6.83 (dd, *J* = 9.8, 2.0 Hz, 1H), 5.10 (s, 2H), 2.35 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 186.31, 162.84, 149.85, 145.64, 138.54, 135.25, 134.70, 134.24, 131.60, 130.23, 128.49, 125.39, 114.31, 106.75, 101.05, 71.06, 21.30. HRMS: m/z calculated for $C_{21}H_{17}NO_3$: requires 332.1281 for $[M+H]^+$, found 332.1280.

R5: 72 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.9 Hz, 1H), 7.42 (d, *J* = 9.7 Hz, 1H), 7.31 (t, *J* = 7.5 Hz, 1H), 7.25 (m 2H), 7.19 (m, 1H), 7.01 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.88 (d, *J* = 2.6 Hz, 1H), 6.85 – 6.82 (m, 1H), 6.32 (d, *J* = 2.0 Hz, 1H), 5.14 (s, 2H), 2.39 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 186.30, 162.75, 149.83, 145.70, 145.64, 138.65, 135.33, 134.70, 134.26, 131.61, 129.34, 128.76, 128.50, 128.25, 124.63, 114.28, 106.77, 101.08, 70.99, 21.43. HRMS: m/z calculated for: $C_{20}H_{15}NO_3$ requires 318.1125 for $[M+H]^+$, found 318.1131.

R6: 63 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.9 Hz, 1H), 7.42 (d, *J* = 9.8 Hz, 1H), 7.03 – 6.94 (m, 3H), 6.87 – 6.76 (m, 3H), 6.32 (d, *J* = 2.1 Hz, 1H), 5.16 (s, 2H). The product was too insoluble to obtain a 13C NMR spectrum. HRMS: m/z calculated for $C_{19}H_{11}F_2NO_3$: requires 340.0780 for $[M+H]^+$, found 340.0771.

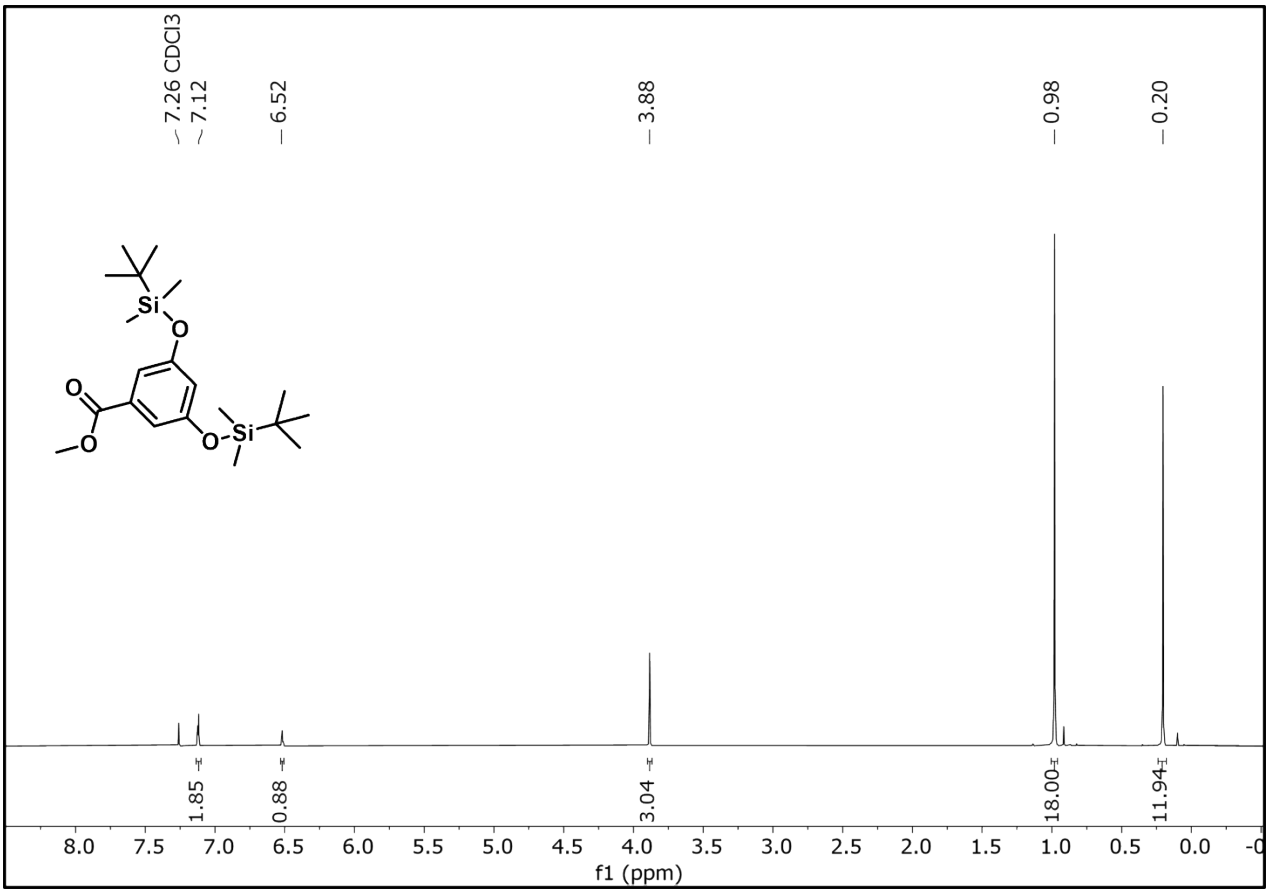
R7: 56 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.9 Hz, 1H), 7.42 (d, *J* = 9.8 Hz, 1H), 7.01 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.86 (d, *J* = 2.6 Hz, 1H), 6.83 (dd, *J* = 9.8, 2.0 Hz, 1H), 6.57 (dd, *J* = 2.3, 0.7 Hz, 2H), 6.44 (t, *J* = 2.3 Hz, 1H), 6.32 (d, *J* = 2.0 Hz, 1H), 5.12 (s, 2H), 3.81 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 186.29, 162.58, 161.23, 149.81, 145.76, 145.61, 137.79, 134.70, 134.28, 131.62, 128.55, 114.26, 106.78, 105.25, 101.16, 100.10, 70.79, 55.43. HRMS: m/z calculated for $C_{21}H_{17}NO_5$: requires 364.1180 for $[M+H]^+$, found 364.1179.

R8: 37 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.35 (s, 2H), 7.76 (d, *J* = 8.8 Hz, 1H), 7.45 (d, *J* = 9.8 Hz, 1H), 7.05 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.91 (d, *J* = 2.6 Hz, 1H), 6.88 – 6.85 (m, 1H), 6.35 (d, *J* = 2.0 Hz, 1H), 5.27 (s, 2H), 4.00 (s, 6H). ^{13}C NMR (151 MHz, CDC_{l3}) δ 186.29, 165.84, 162.00, 149.72, 146.09, 145.59, 136.63, 134.73, 134.41, 132.60, 131.76, 131.34, 130.72, 128.73, 114.01, 106.90, 101.19, 69.71, 52.58. HRMS: m/z calculated for $C_{23}H_{17}NO_7$: requires 420.1078 for $[M+H]^+$, found 420.1090.

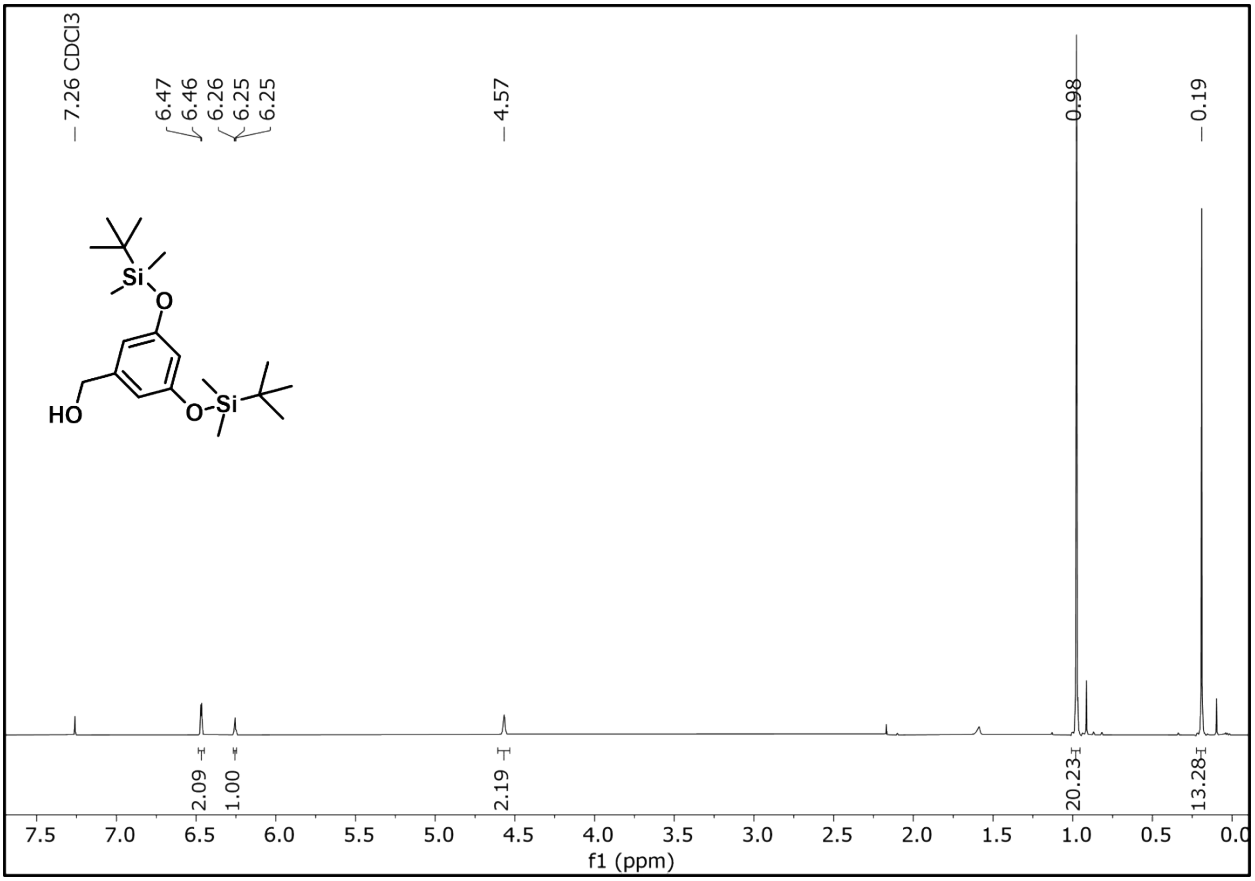
R9: 54 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (d, *J* = 10.5 Hz, 3H), 7.77 (d, *J* = 8.9 Hz, 1H), 7.43 (d, *J* = 9.8 Hz, 1H), 7.04 (dd, *J* = 8.9, 2.7 Hz, 1H), 6.91 (d, *J* = 2.7 Hz, 1H), 6.85 (dd, *J* = 9.8, 2.1 Hz, 1H), 6.34 (d, *J* = 2.0 Hz, 1H), 5.27 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 186.27, 161.55, 149.65, 146.39, 145.59, 138.09, 134.76, 134.53, 132.63, 132.41, 132.18, 131.96, 131.88, 128.90, 127.36, 127.34, 124.01, 122.54, 122.52, 122.49, 122.47, 122.44, 122.20, 113.72, 107.00, 101.19, 69.15. HRMS: m/z calculated for $C_{21}H_{11}F_6NO_3$: requires 440.0716 for $[M+H]^+$, found 440.0714.

5. NMR and HRMS Spectra

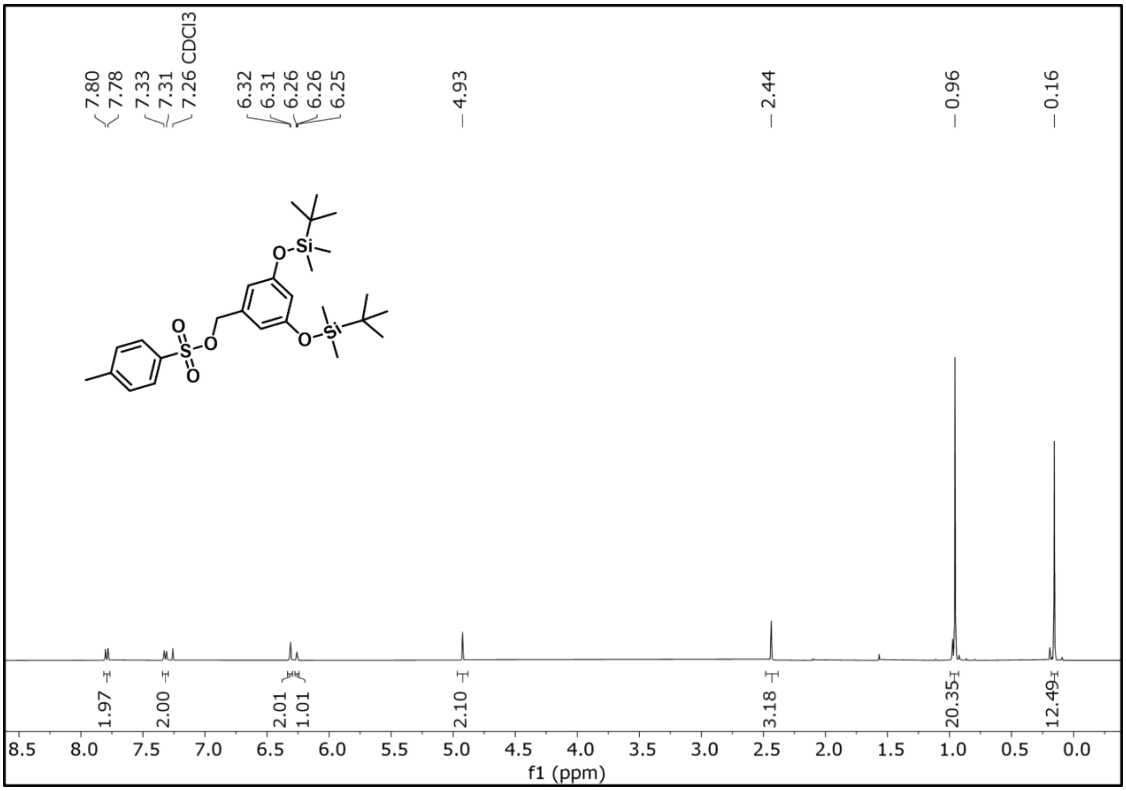
Methyl 3,5-bis((tert-butyldimethylsilyl)oxy)benzoate (1) (^{1}H NMR, 400 MHz, $CDCl_3$)



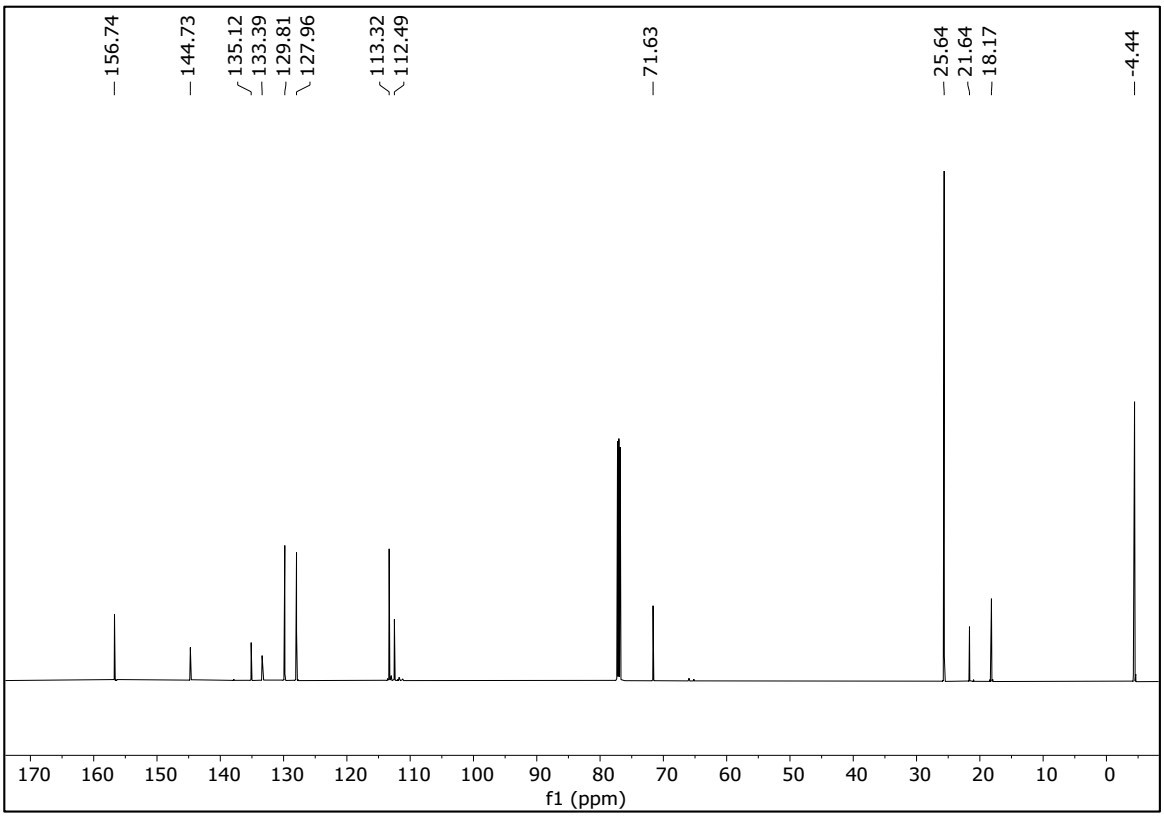
3,5-Bis((*tert*-butyldimethylsilyl)oxy)phenylmethanol (2) (^{1}H NMR, 400 MHz, $CDCl_3$)



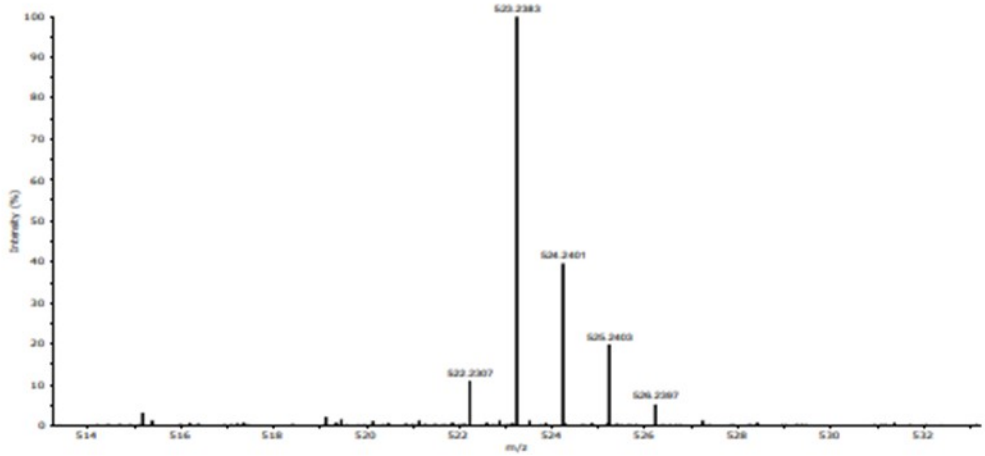
3,5-Bis((tert-butyldimethylsilyl)oxy)benzyl 4-methylbenzenesulfonate (3) (^{1}H NMR, 400 MHz, $CDCl_3$)



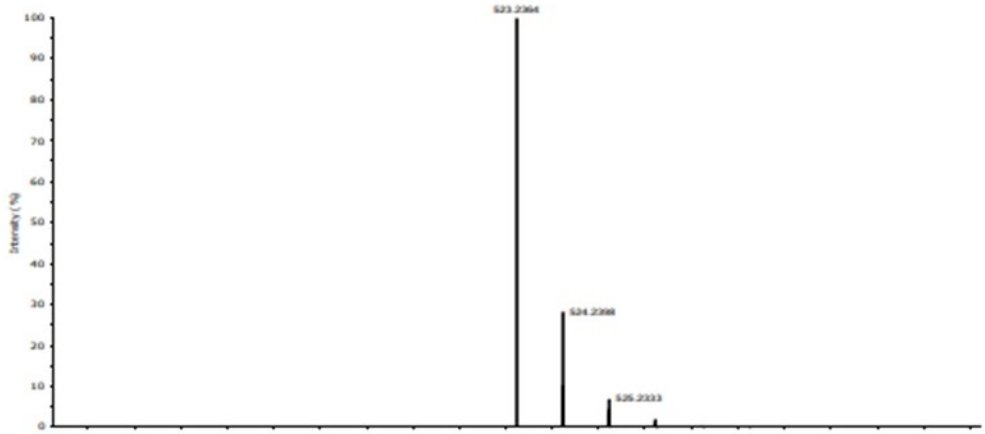
3,5-Bis((tert-butyldimethylsilyl)oxy)benzyl 4-methylbenzenesulfonate (3) (^{13}C NMR, 151 MHz, $CDCl_3$)



Expanded Spectrum RT 0.11, NL 3747743, Peak [1], Target Mass 523.2364

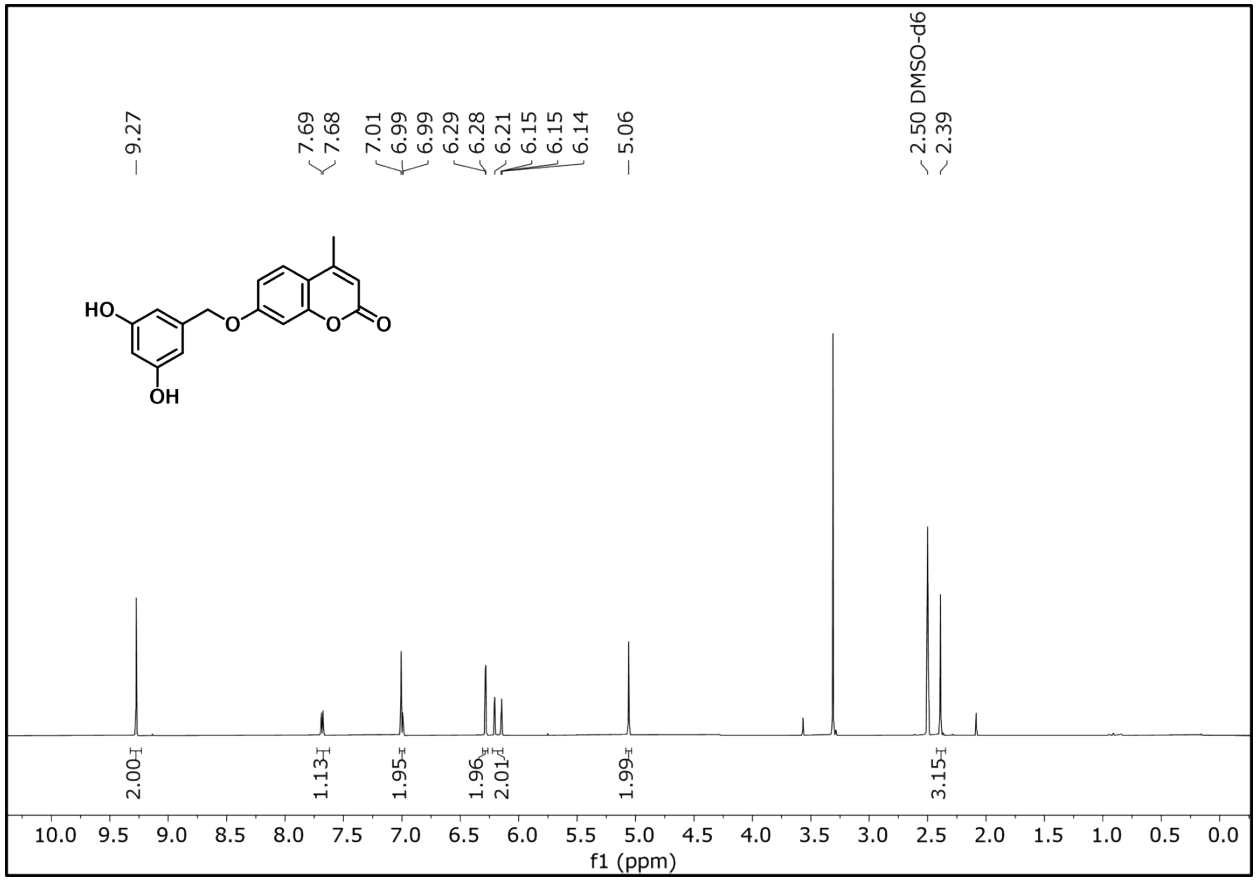


Theoretical Spectrum for C26H43O5SSi2, Minimum Abundance 0.01%

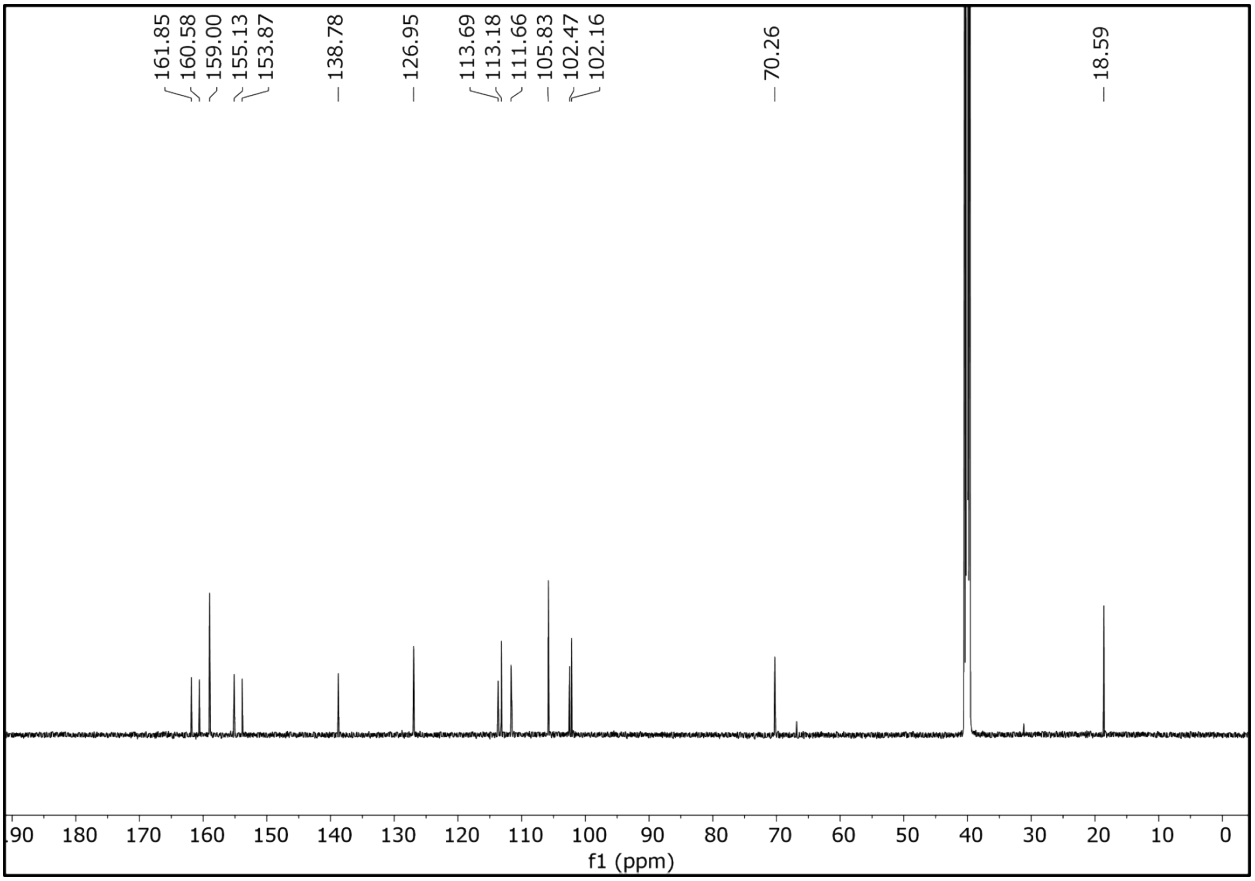


Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
523.2383	523.2364	1.87	3.57	$C_{26}H_{43}O_5SSi_2$	2147568

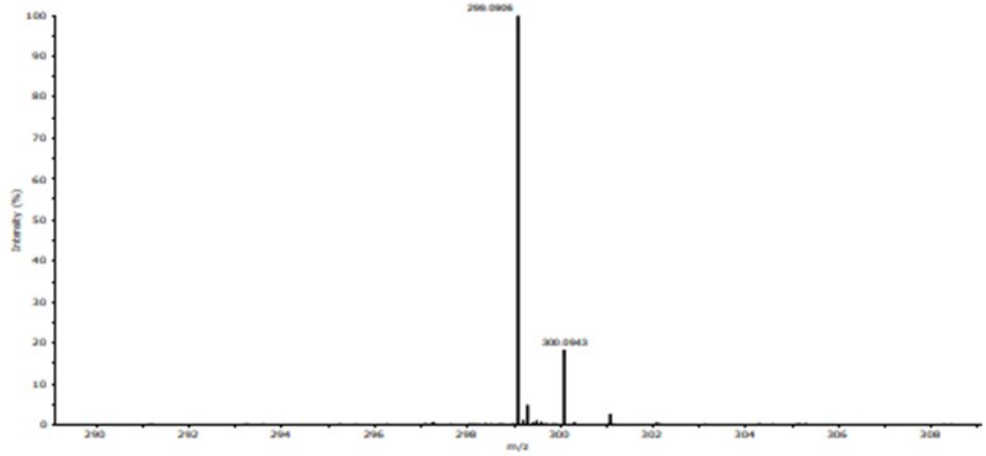
7-((3,5-Dihydroxybenzyl)oxy)-4-methyl-2H-chromen-2-one (4) (^{1}H NMR, 600 MHz, DMSO-d6)



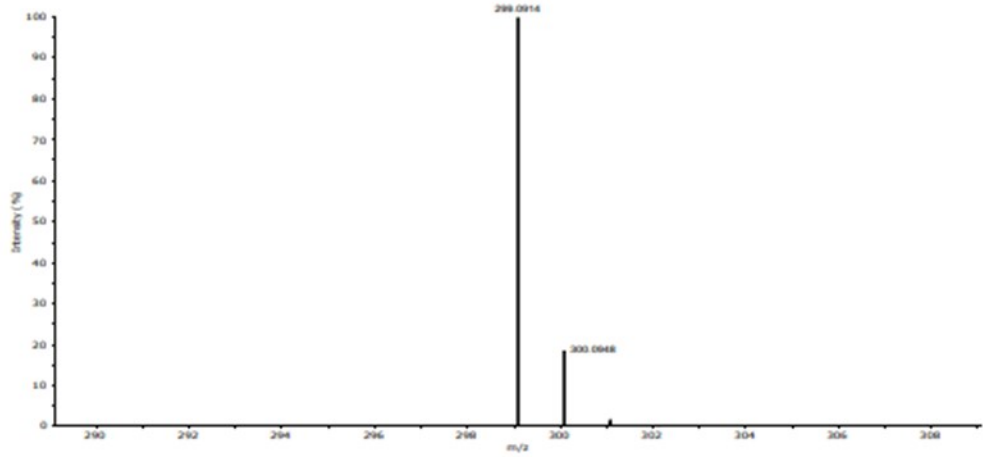
7-((3,5-Dihydroxybenzyl)oxy)-4-methyl-2H-chromen-2-one (4) (^{13}C NMR, 151 MHz, $CDCl_3$)



Expanded Spectrum RT 0.11, NL 6971573, Peak [1], Target Mass 299.0914

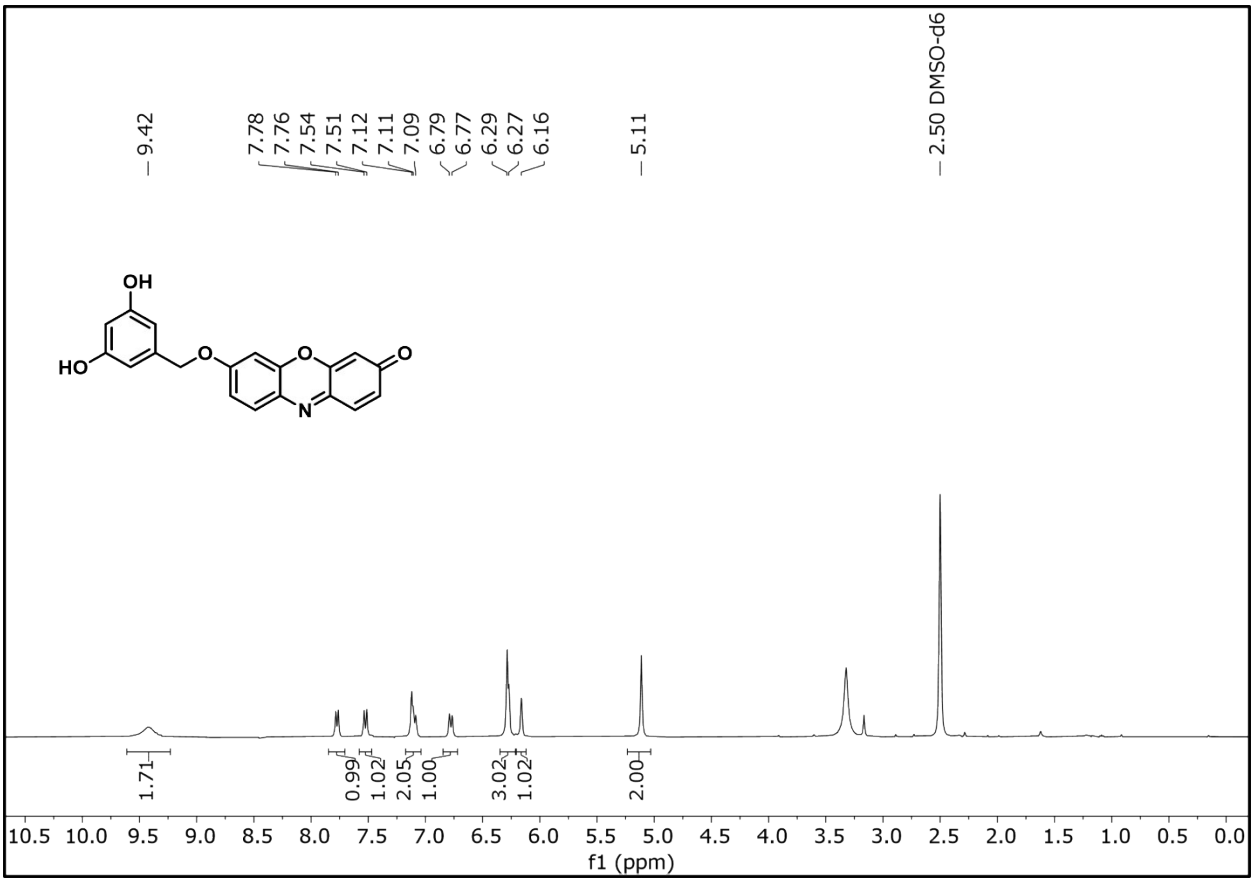


Theoretical Spectrum for C17H15O5, Minimum Abundance 0.01%

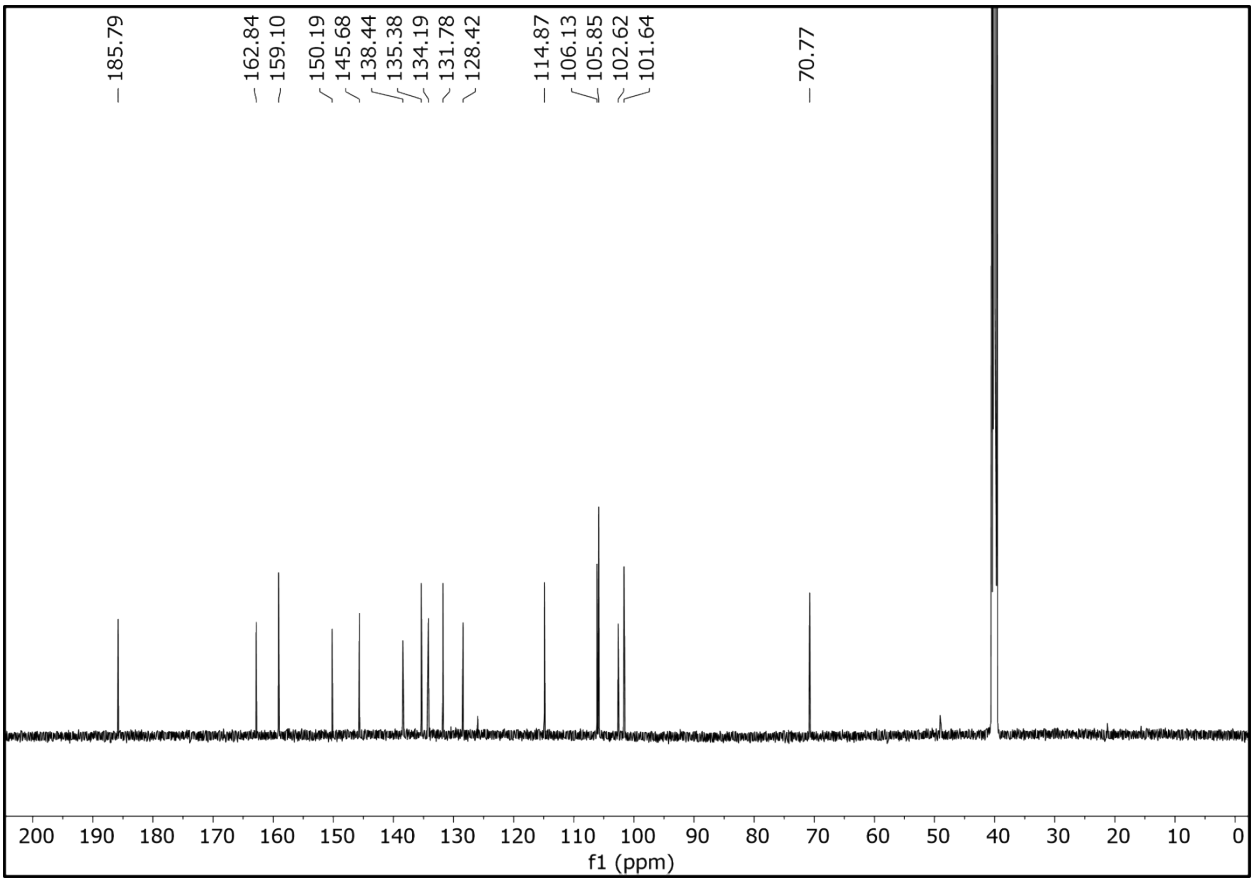


Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
299.0906	299.0914	-0.81	-2.69	$C_{17}H_{15}O_5$	6684555

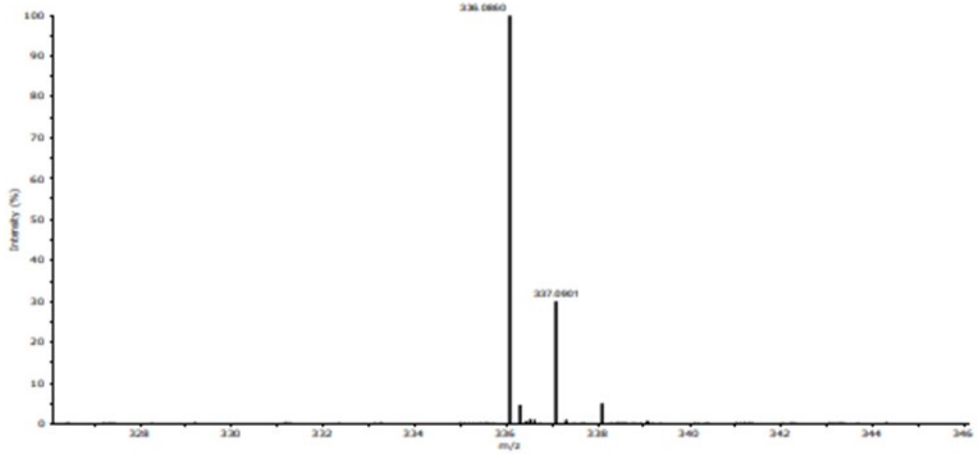
7-((3,5-Dihydroxybenzyl)oxy)-3H-phenoxazin-3-one (Res-DHB) (^{1}H NMR, 400 MHz, DMSO-d6)



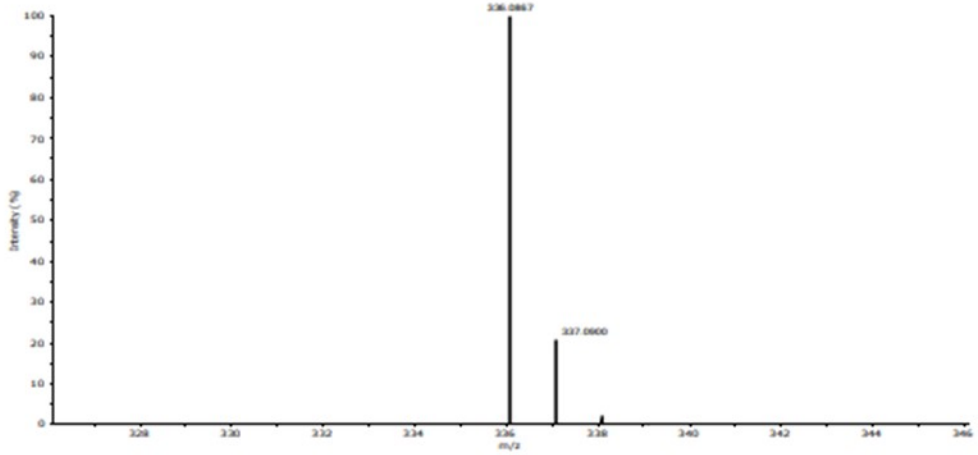
7-((3,5-Dihydroxybenzyl)oxy)-3H-phenoxazin-3-one (Res-DHB) (^{13}C NMR, 151 MHz, DMSO-d6)



Expanded Spectrum RT 0.11, NL 6953070, Peak [1], Target Mass 336.0867

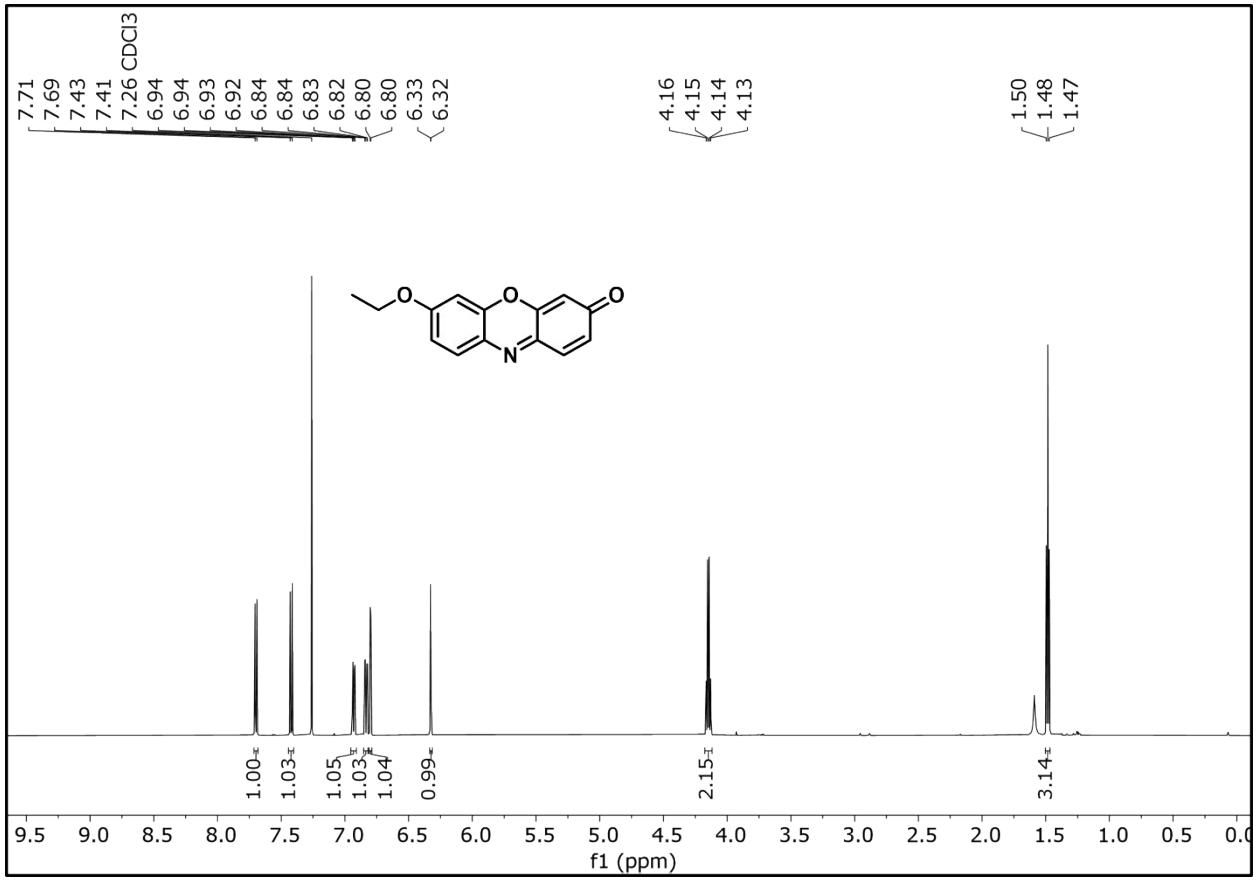


Theoretical Spectrum for C19H14NO5, Minimum Abundance 0.01%

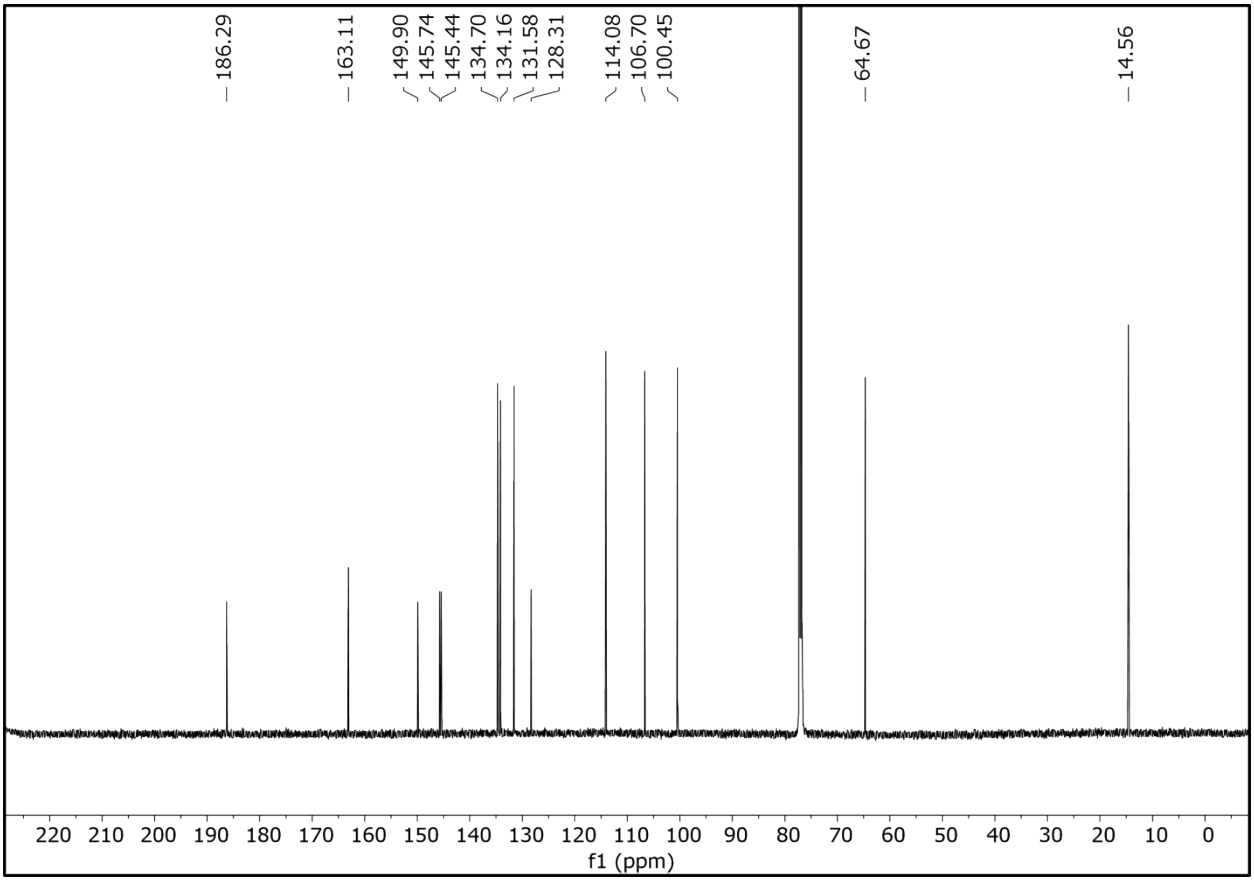


Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
336.0860	336.0867	-0.65	-1.95	$C_{19}H_{14}NO_5$	29549876

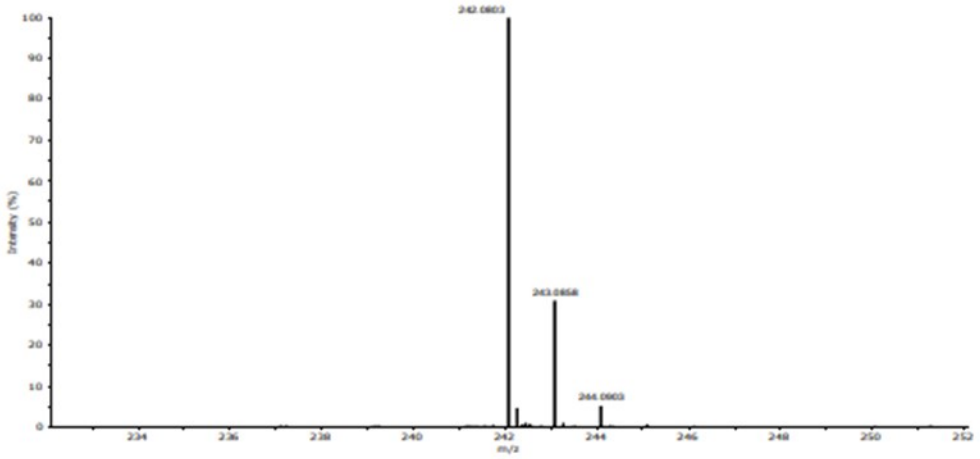
7-Ethoxy-3H-phenoxazin-3-one (R1) (^{1}H NMR, 600 MHz, $CDCl_3$)



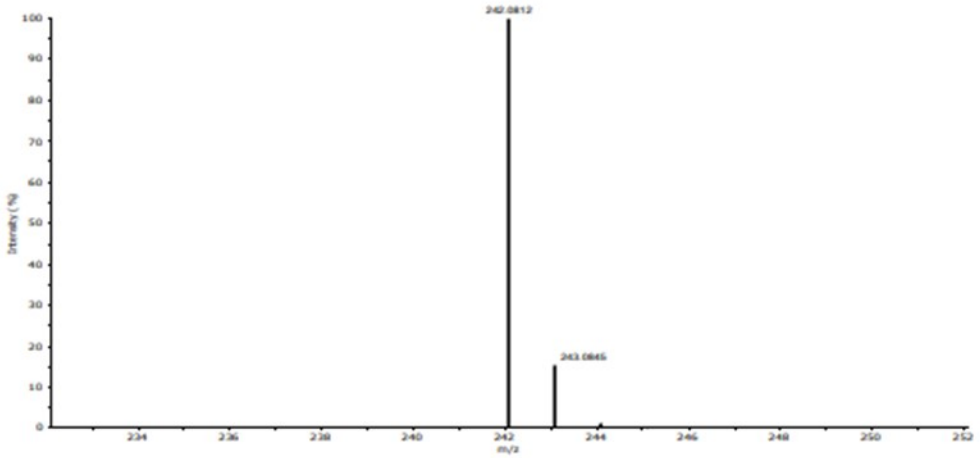
7-Ethoxy-3H-phenoxazin-3-one (R1) (^{13}C NMR, 151 MHz, $CDCl_3$)



Expanded Spectrum RT 0.13, NL 1623560, Peak [1], Target Mass 242.0812

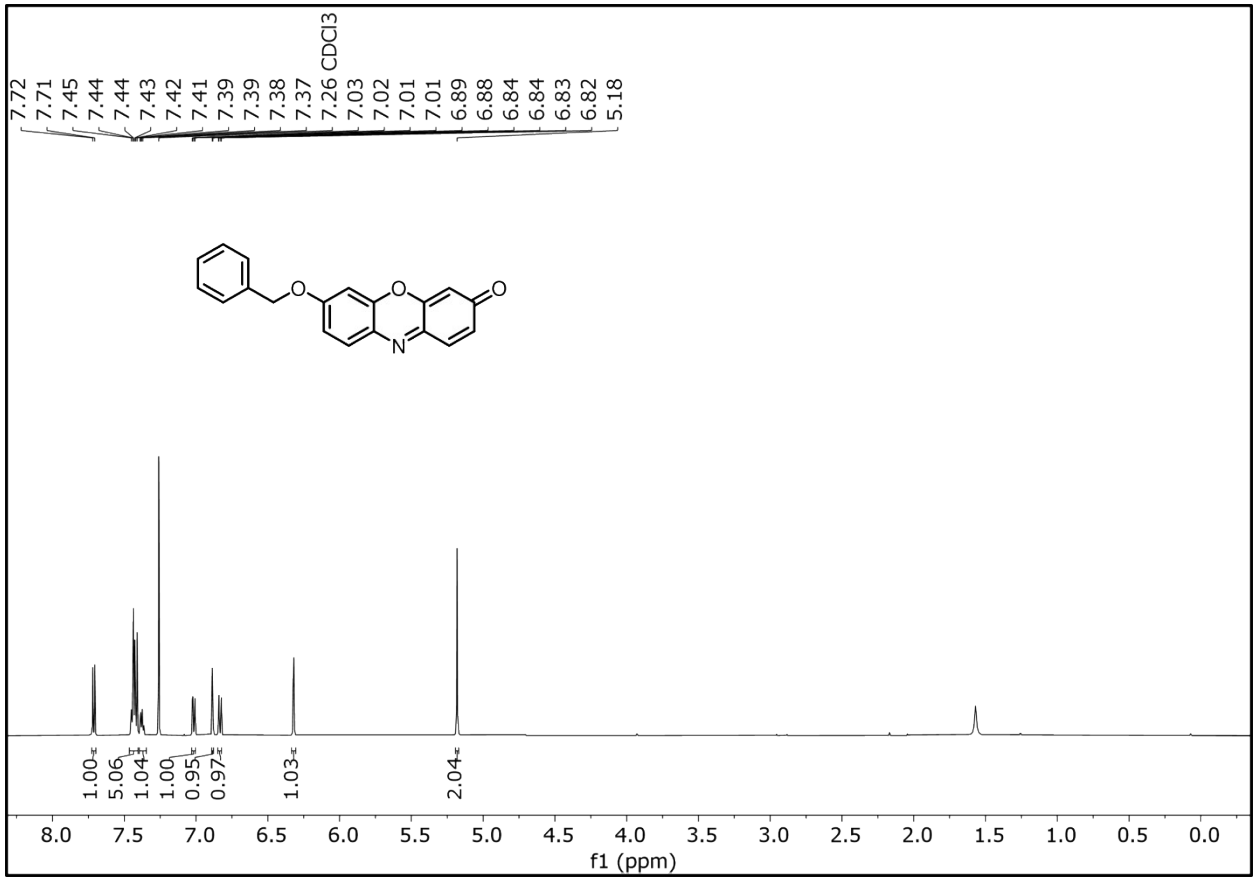


Theoretical Spectrum for C14H12NO3, Minimum Abundance 0.01%

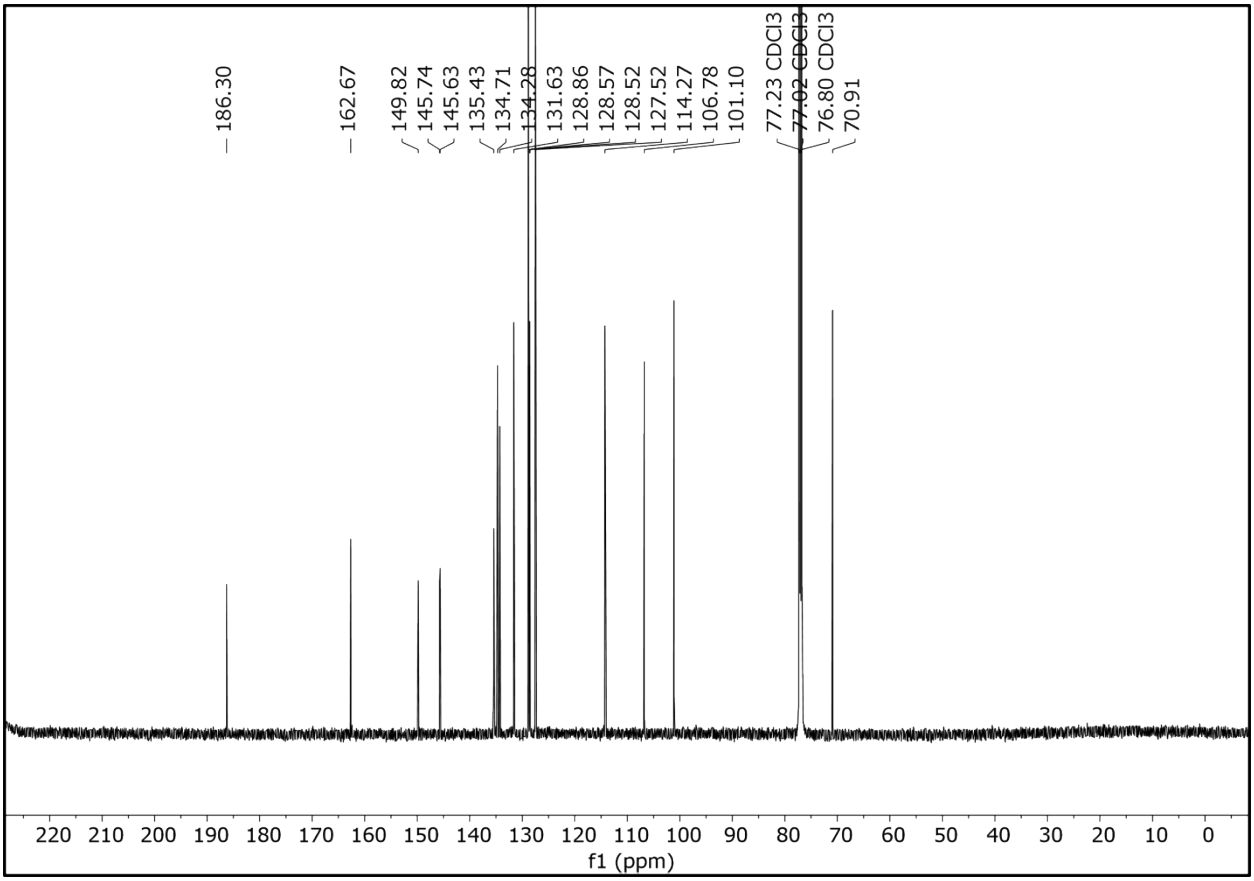


Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
242.0803	242.0812	-0.87	-3.61	$C_{14}H_{12}NO_3$	4969378

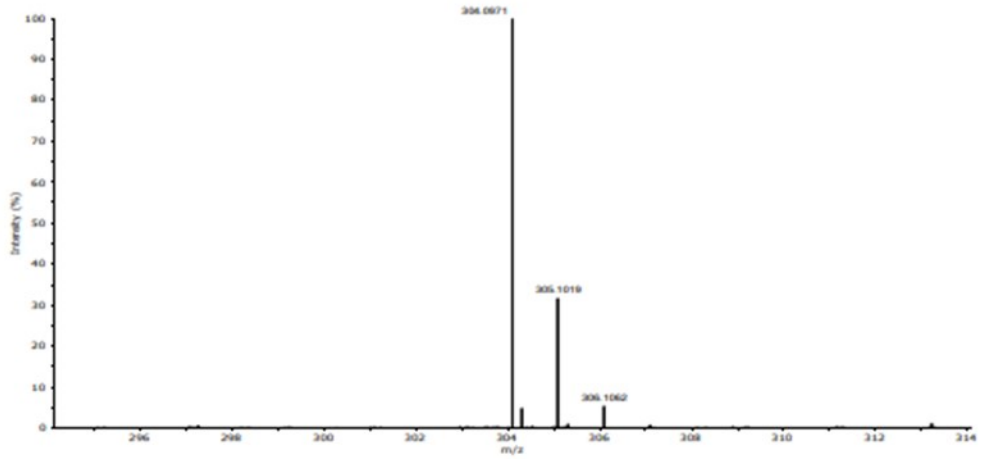
7-(Benzyloxy)-3H-phenoxazin-3-one (R2) (^{1}H NMR, 600 MHz, $CDCl_3$)



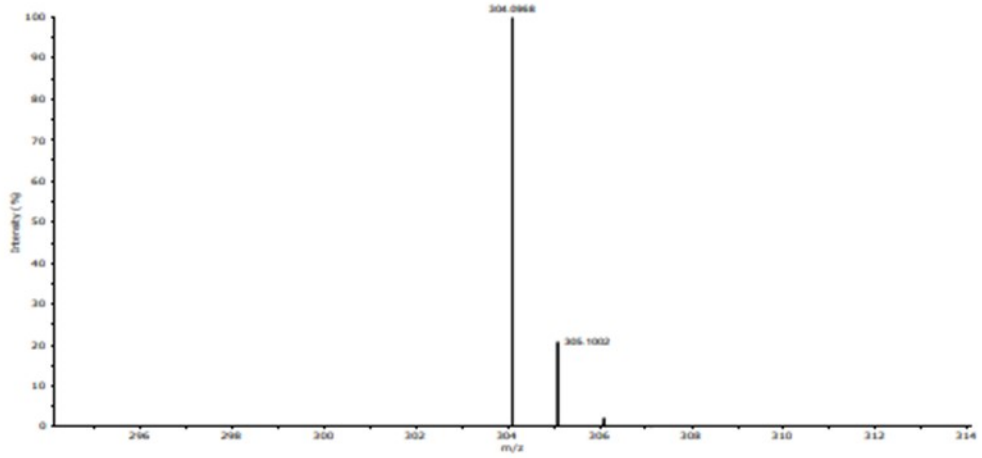
7-(Benzyloxy)-3H-phenoxazin-3-one (R2) (^{13}C NMR, 151 MHz, $CDCl_3$)



Expanded Spectrum RT 0.13, NL 1147489, Peak [1], Target Mass 304.0968

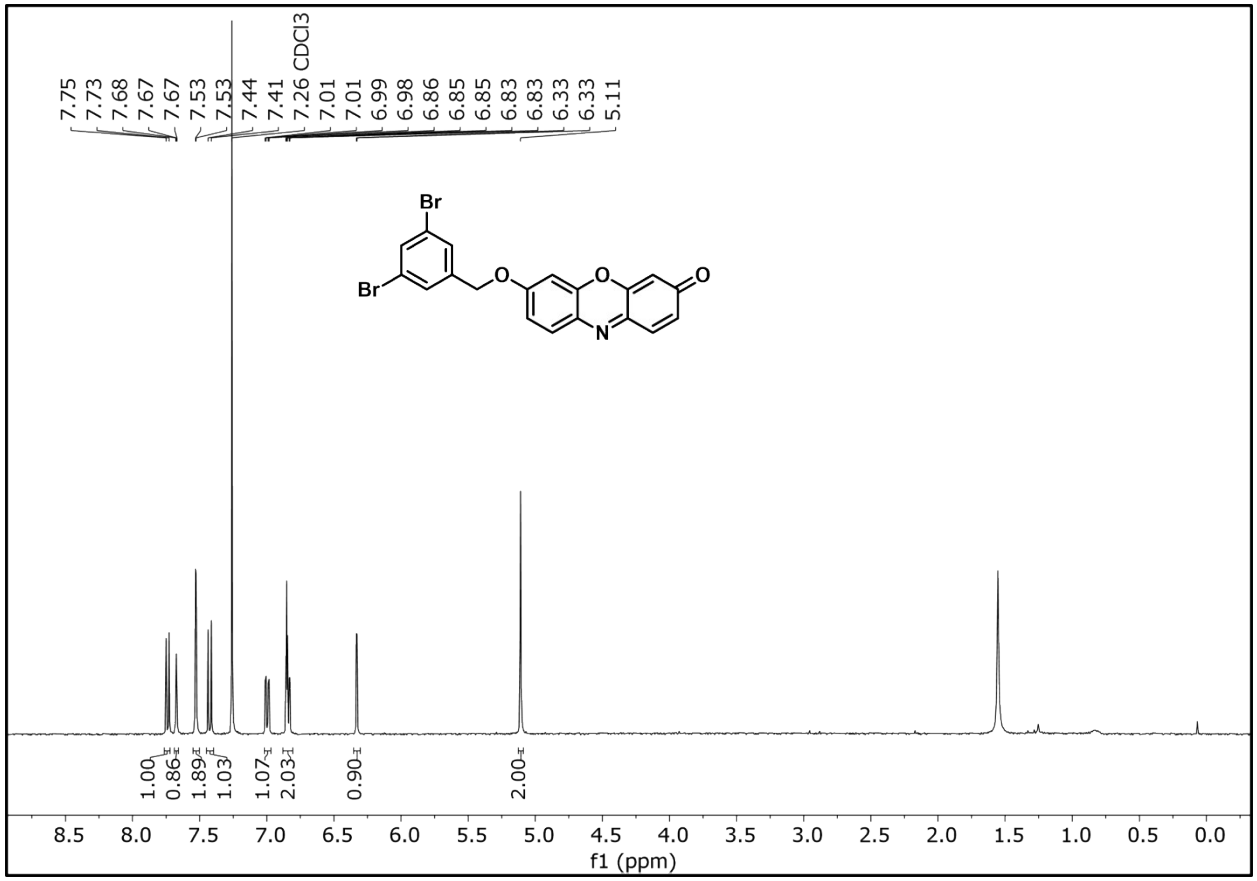


Theoretical Spectrum for C19H14NO3, Minimum Abundance 0.01%

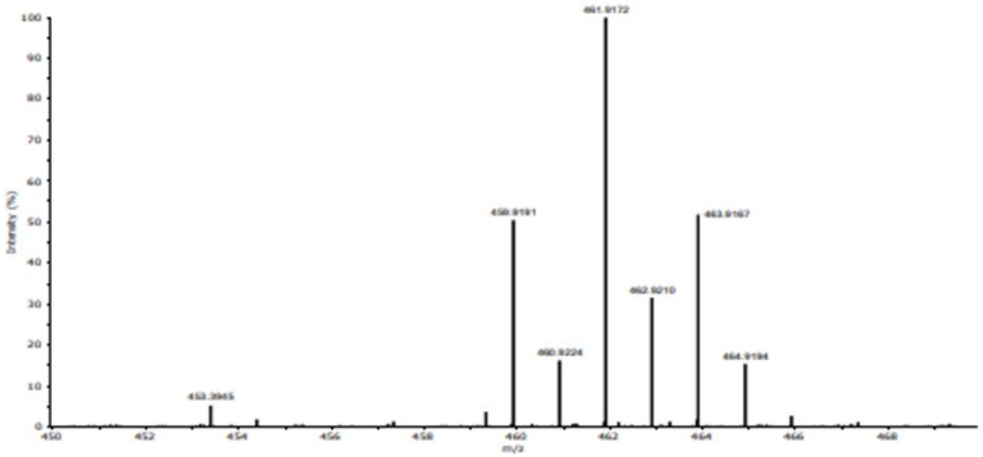


Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
304.0971	304.0968	0.28	0.91	$C_{19}H_{14}NO_3$	4128866

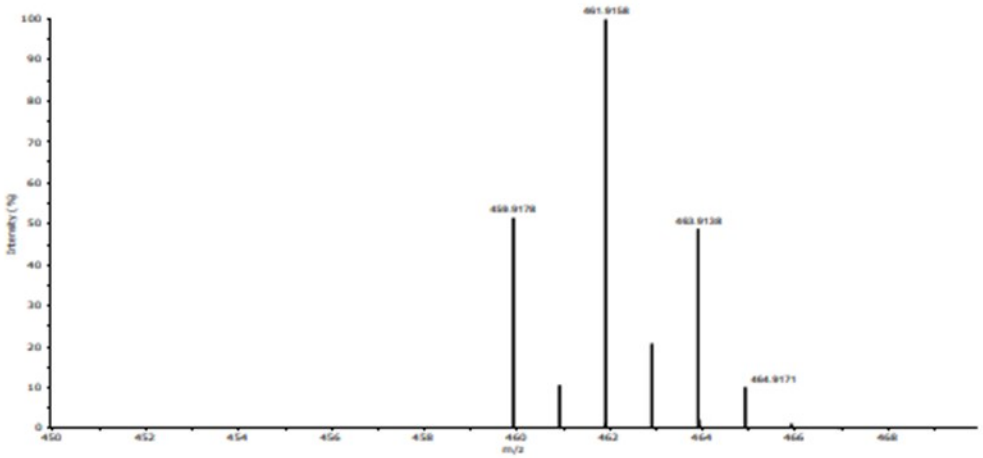
7-((3,5-Dibromobenzyl)oxy)-3H-phenoxazin-3-one (R3) (^{1}H NMR, 400 MHz, $CDCl_3$)



Expanded Spectrum RT 0.14, NL 421636, Peak [1], Target Mass 459.9178

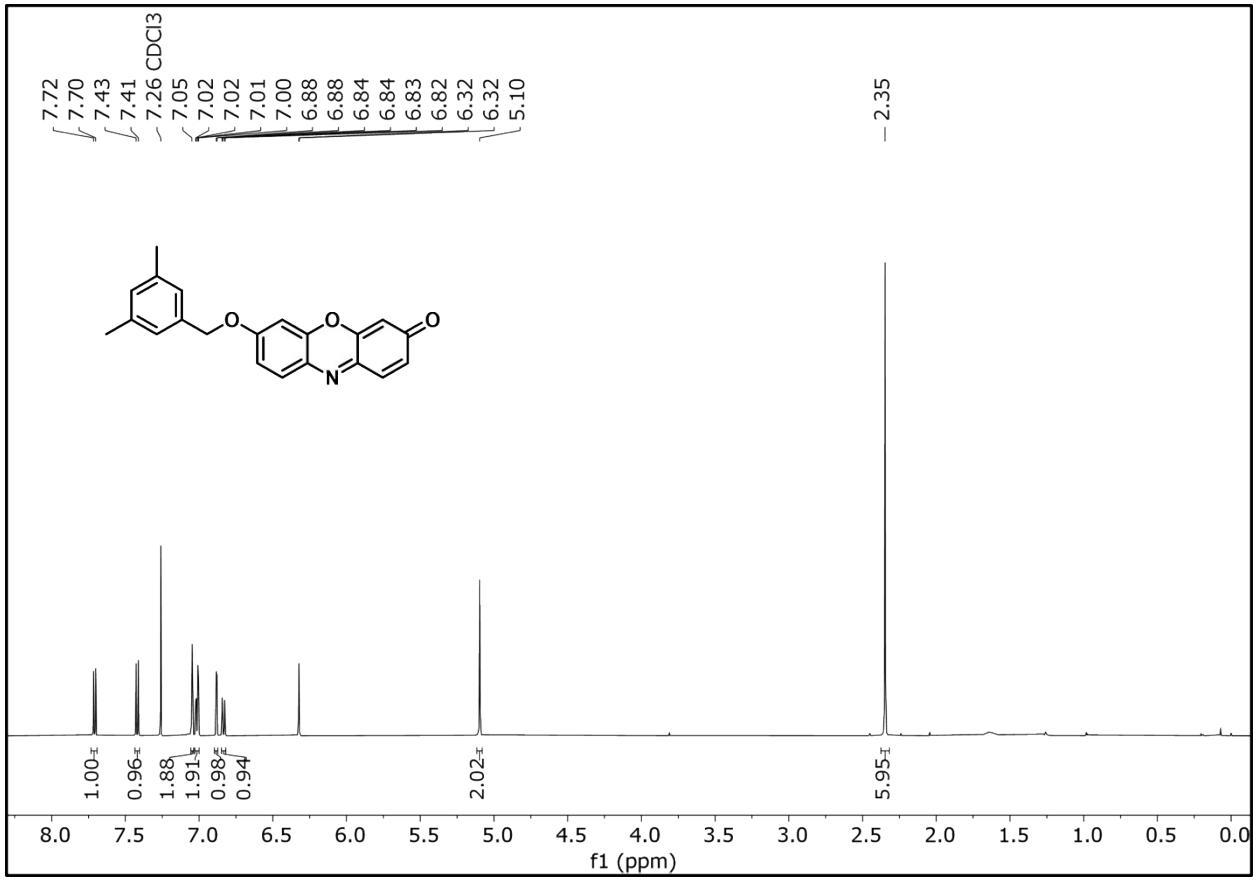


Theoretical Spectrum for C19H12Br2NO3, Minimum Abundance 0.01%

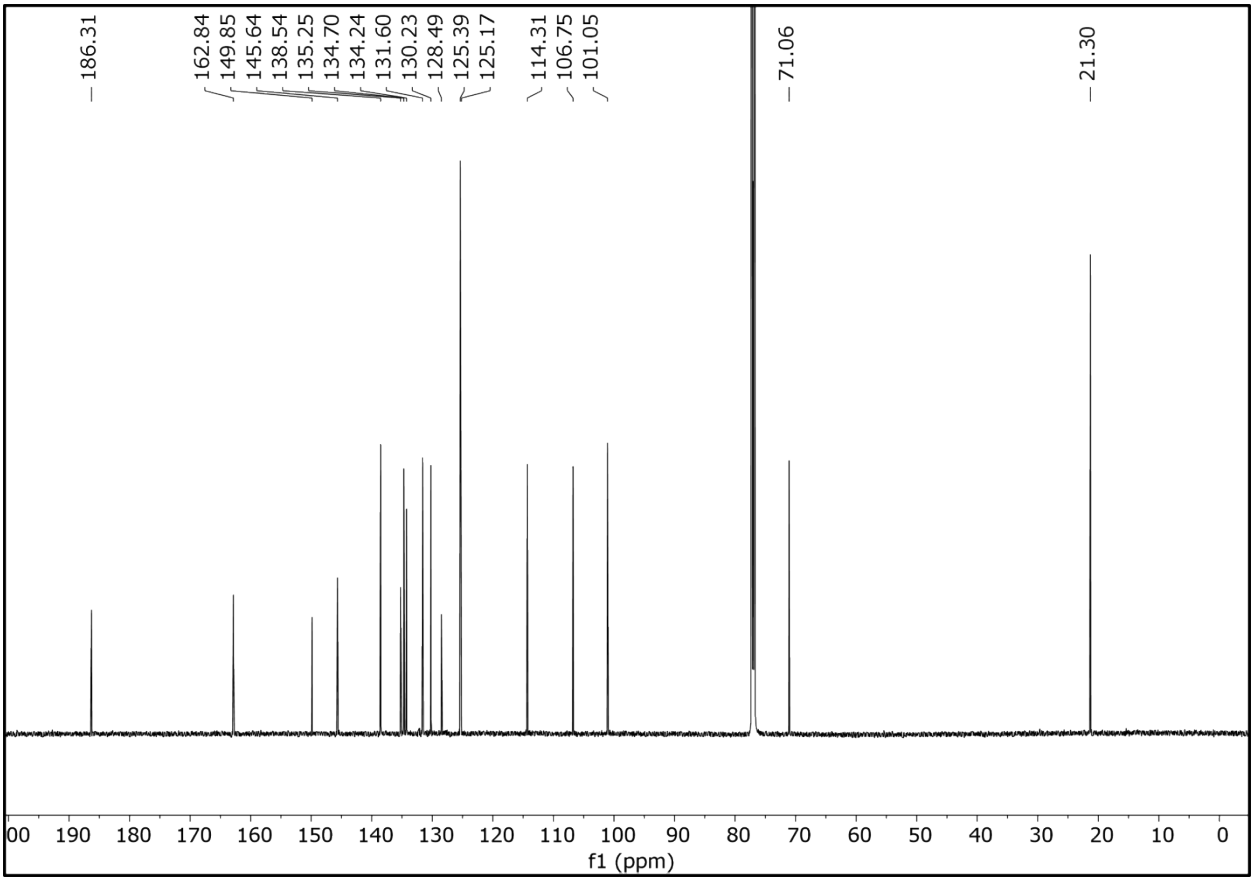


Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
459.9191	459.9178	1.25	2.73	$C_{19}H_{12}Br_2NO_3$	1669901

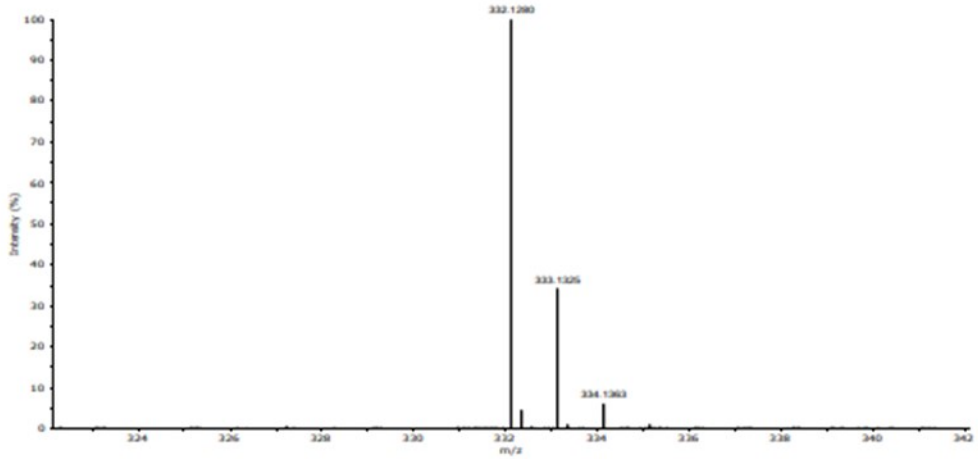
7-((3,5-Dimethylbenzyl)oxy)-3H-phenoxazin-3-one (R4) (^{1}H NMR, 600 MHz, $CDCl_3$)



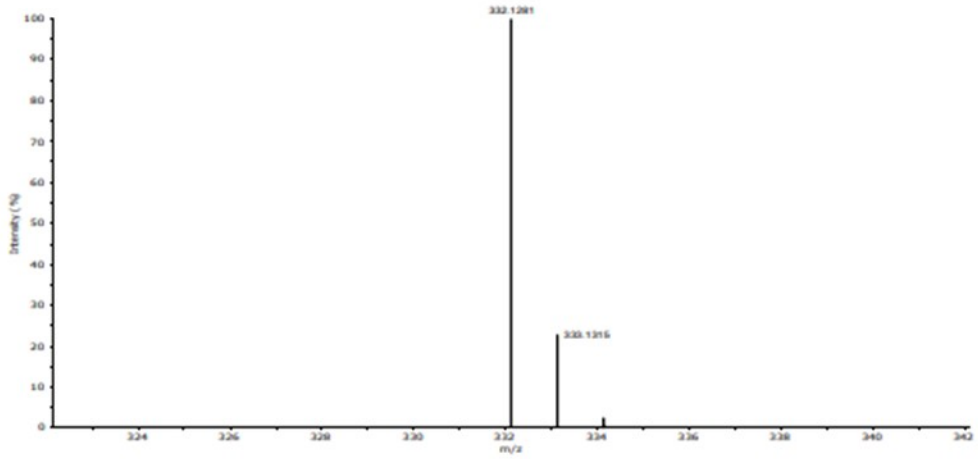
7-((3,5-Dimethylbenzyl)oxy)-3H-phenoxazin-3-one (R4) (^{13}C NMR, 151 MHz, $CDCl_3$)



Expanded Spectrum RT 0.13, NL 4015208, Peak [1], Target Mass 332.1281

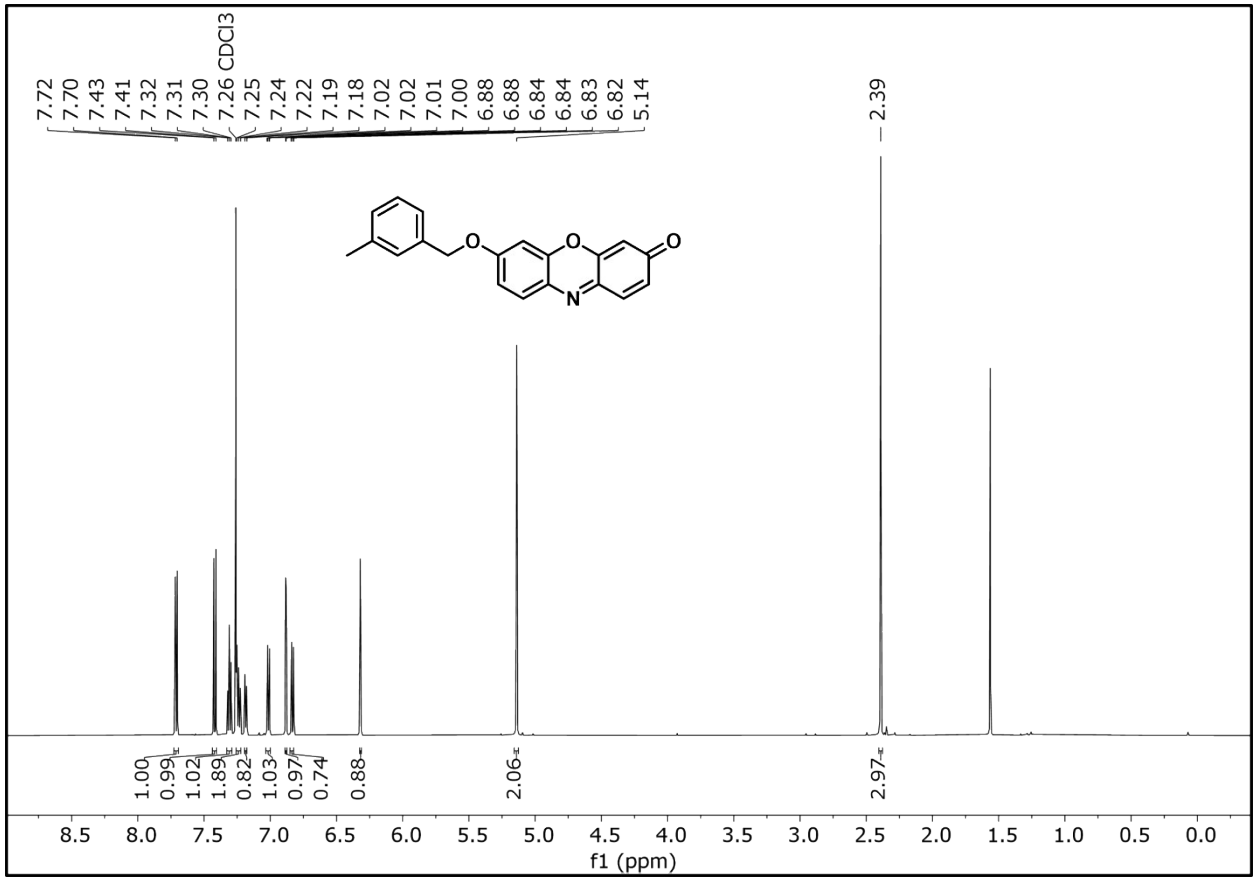


Theoretical Spectrum for C21H18NO3, Minimum Abundance 0.01%

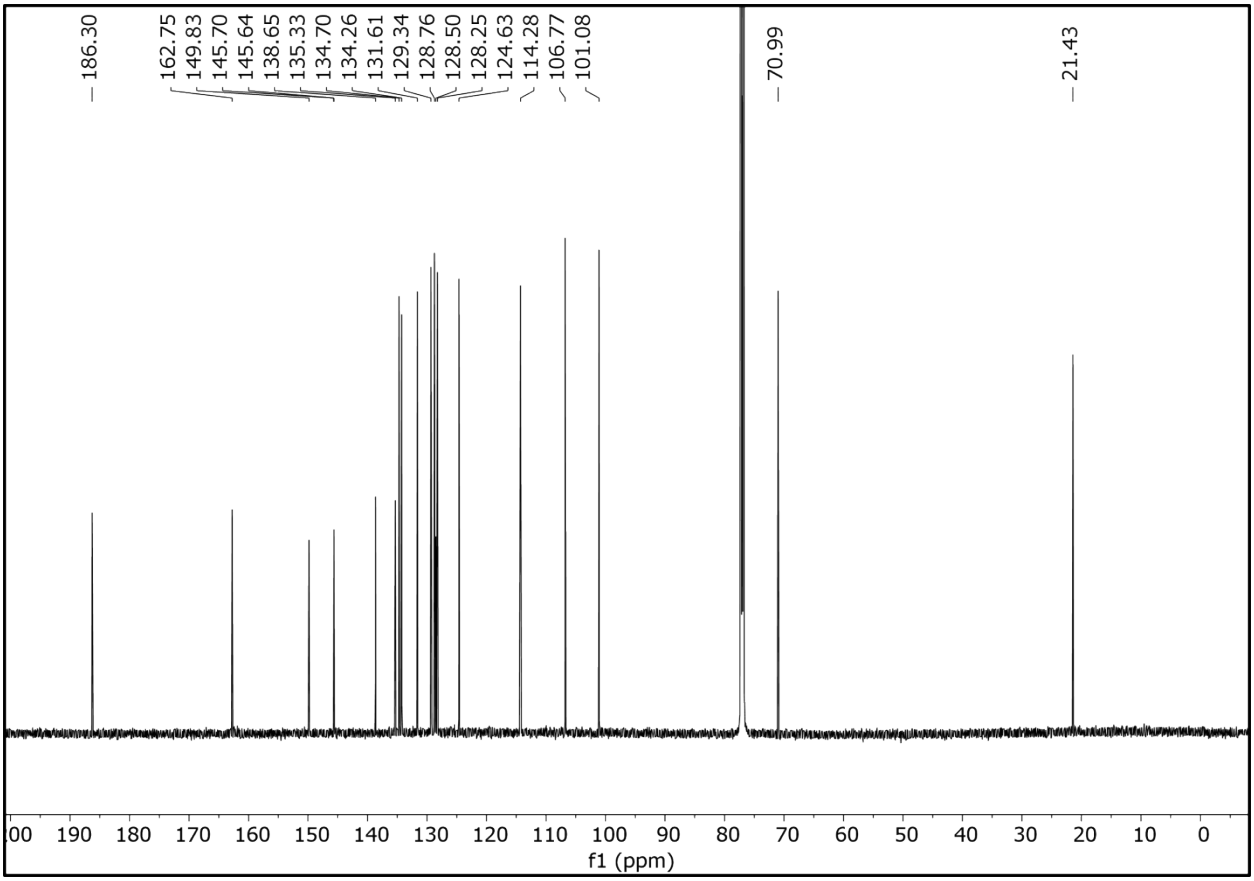


Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
332.1280	332.1281	-0.12	-0.37	$C_{21}H_{18}NO_3$	17123631

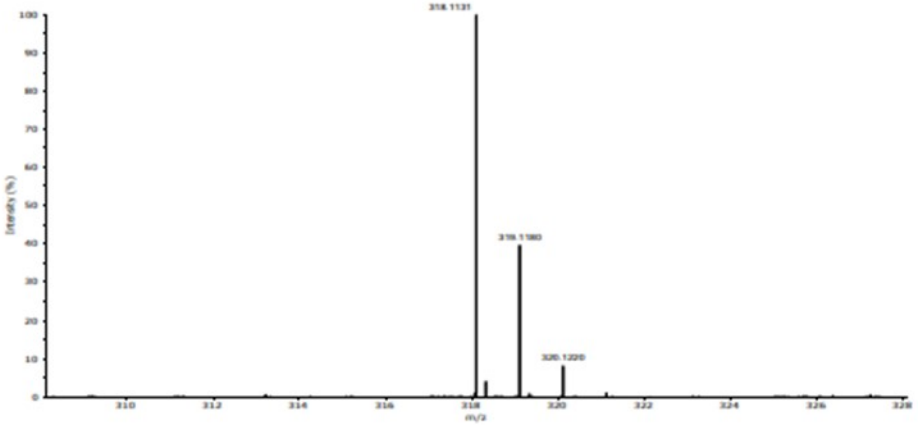
7-((3-Methylbenzyl)oxy)-3H-phenoxazin-3-one (R5) (^{1}H NMR, 600 MHz, $CDCl_3$)



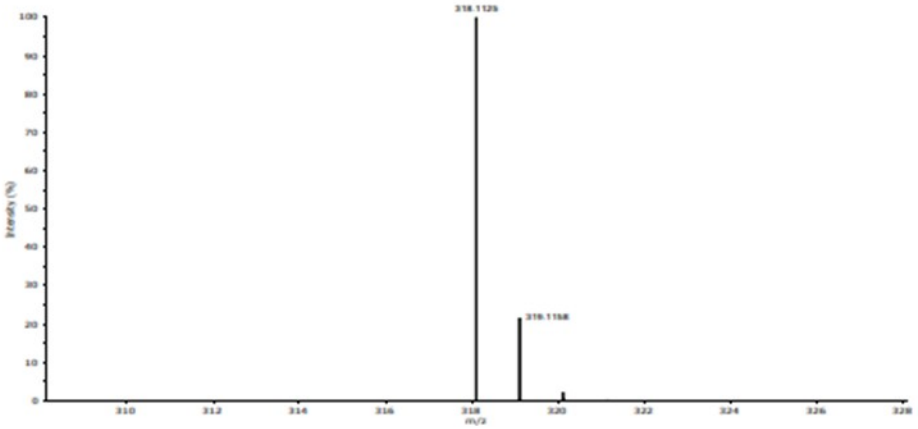
7-((3-Methylbenzyl)oxy)-3H-phenoxazin-3-one (R5) (^{13}C NMR, 151 MHz, $CDCl_3$)



Expanded Spectrum RT 0.13, NL 1509664, Peak [1], Target Mass 318.1125

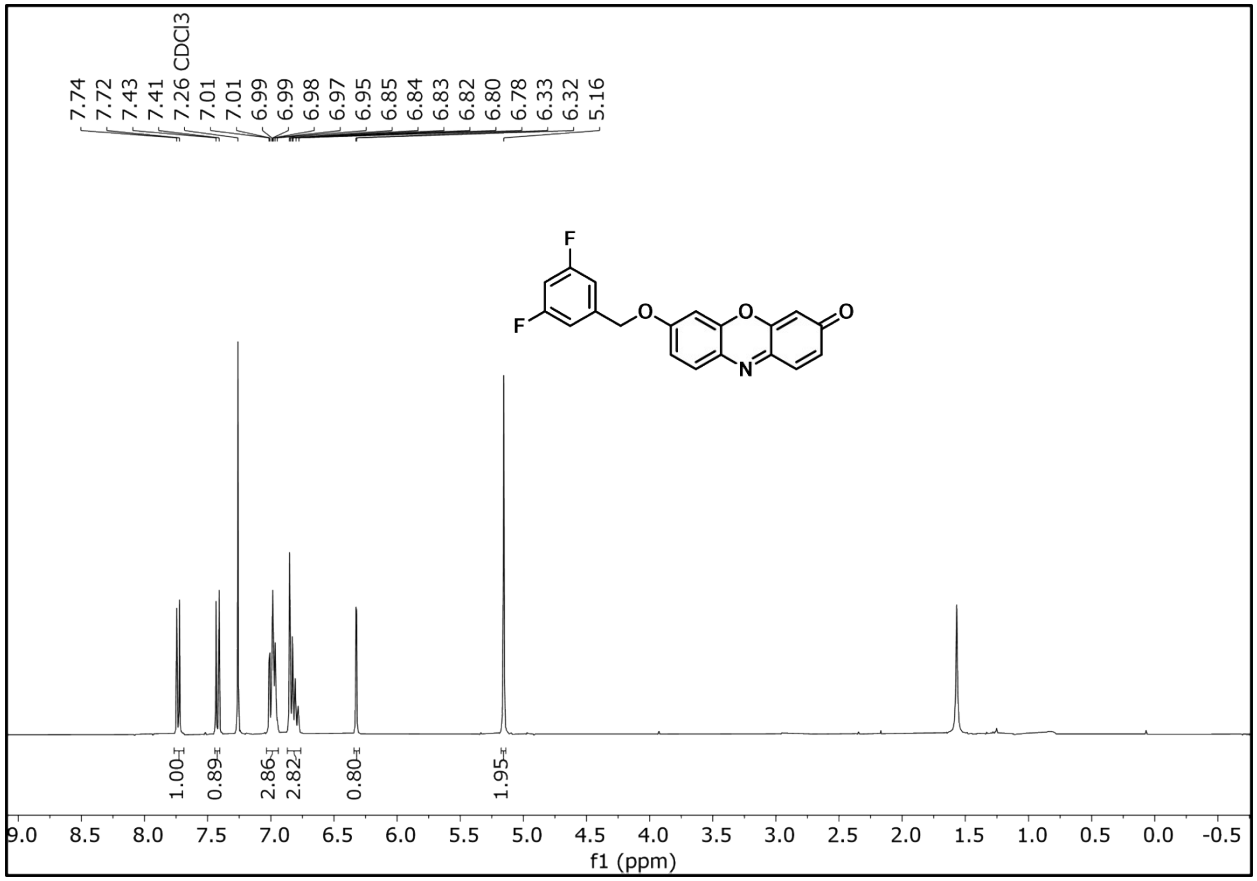


Theoretical Spectrum for C20H16NO3, Minimum Abundance 0.01%

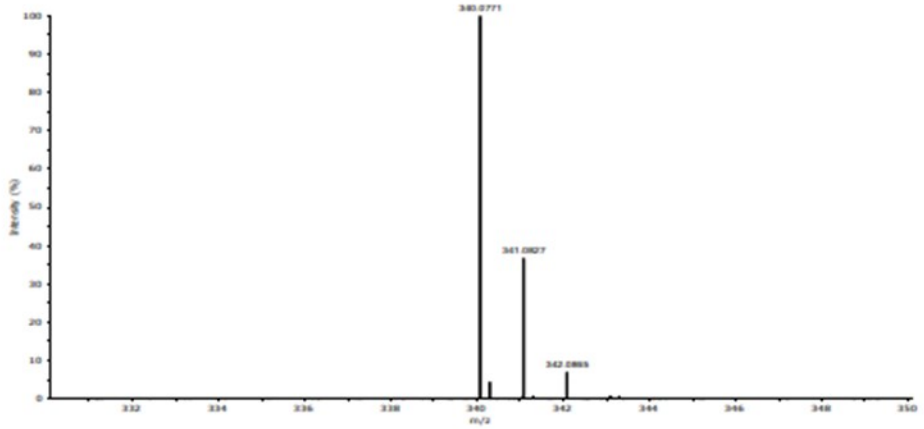


Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
318.1131	318.1125	0.63	1.97	$C_{20}H_{16}NO_3$	5528037

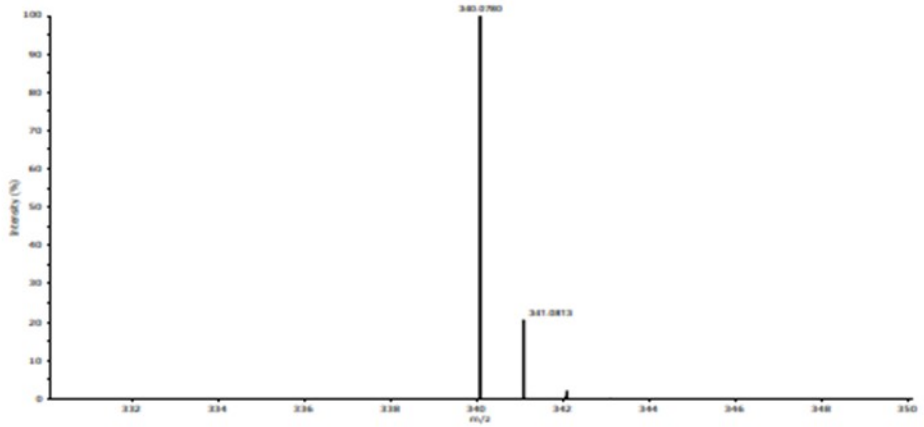
7-((3,5-Difluorobenzyl)oxy)-3H-phenoxazin-3-one (R6) (^{1}H NMR, 400 MHz, $CDCl_3$)



Expanded Spectrum RT 0.13, NL 3076289, Peak [1], Target Mass 340.0780

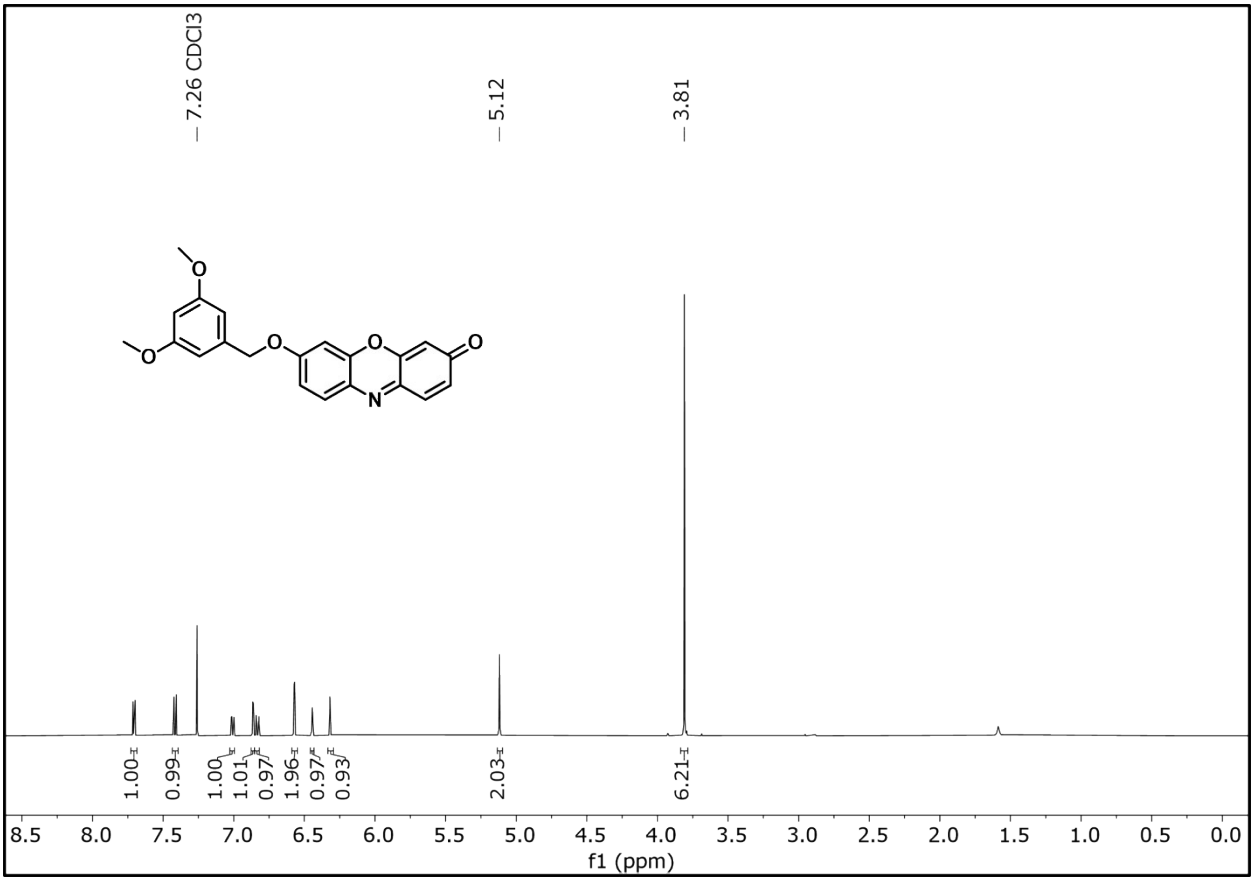


Theoretical Spectrum for C19H12F2NO3, Minimum Abundance 0.01%

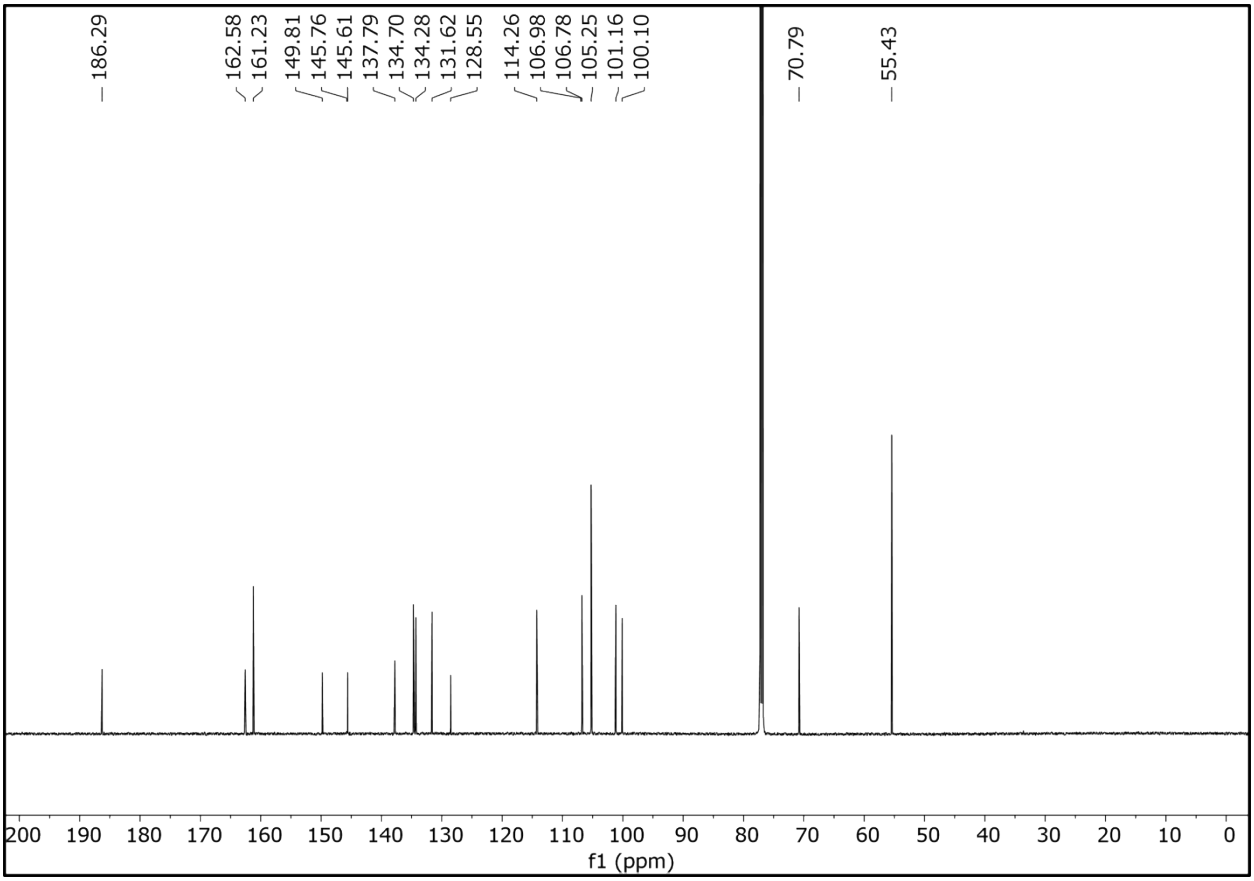


Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
340.0771	340.0780	-0.88	-2.59	$C_{19}H_{12}F_2NO_3$	12001504

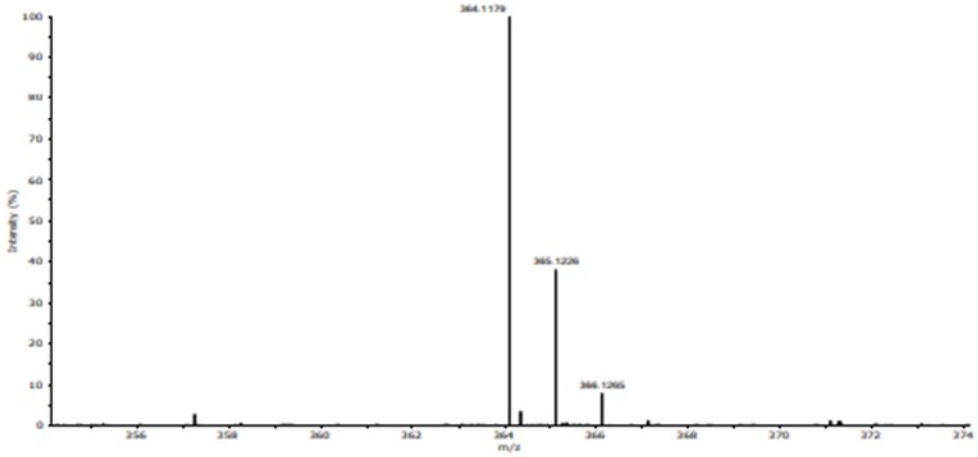
7-((3,5-Dimethoxybenzyl)oxy)-3H-phenoxazin-3-one (R7) (^{1}H NMR, 600 MHz, $CDCl_3$)



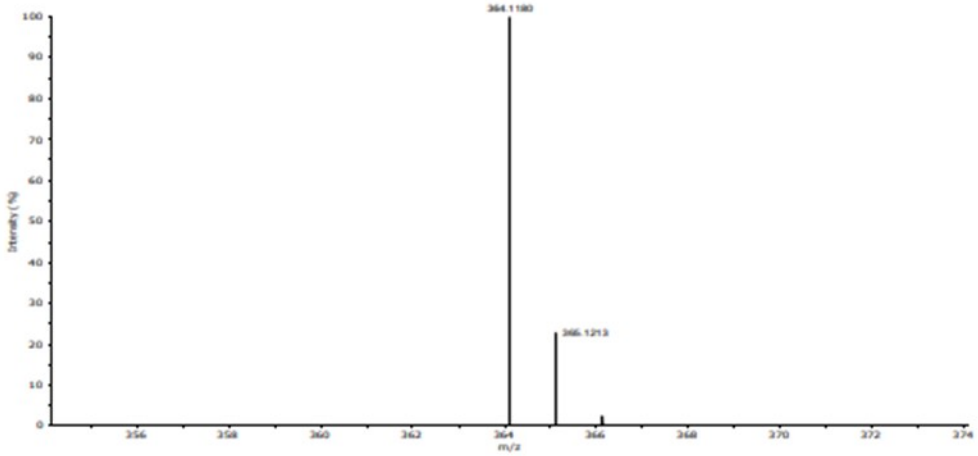
7-((3,5-Dimethoxybenzyl)oxy)-3H-phenoxazin-3-one (R7) (^{13}C NMR, 151 MHz, $CDCl_3$)



Expanded Spectrum RT 0.13, NL 1525322, Peak [1], Target Mass 364.1180

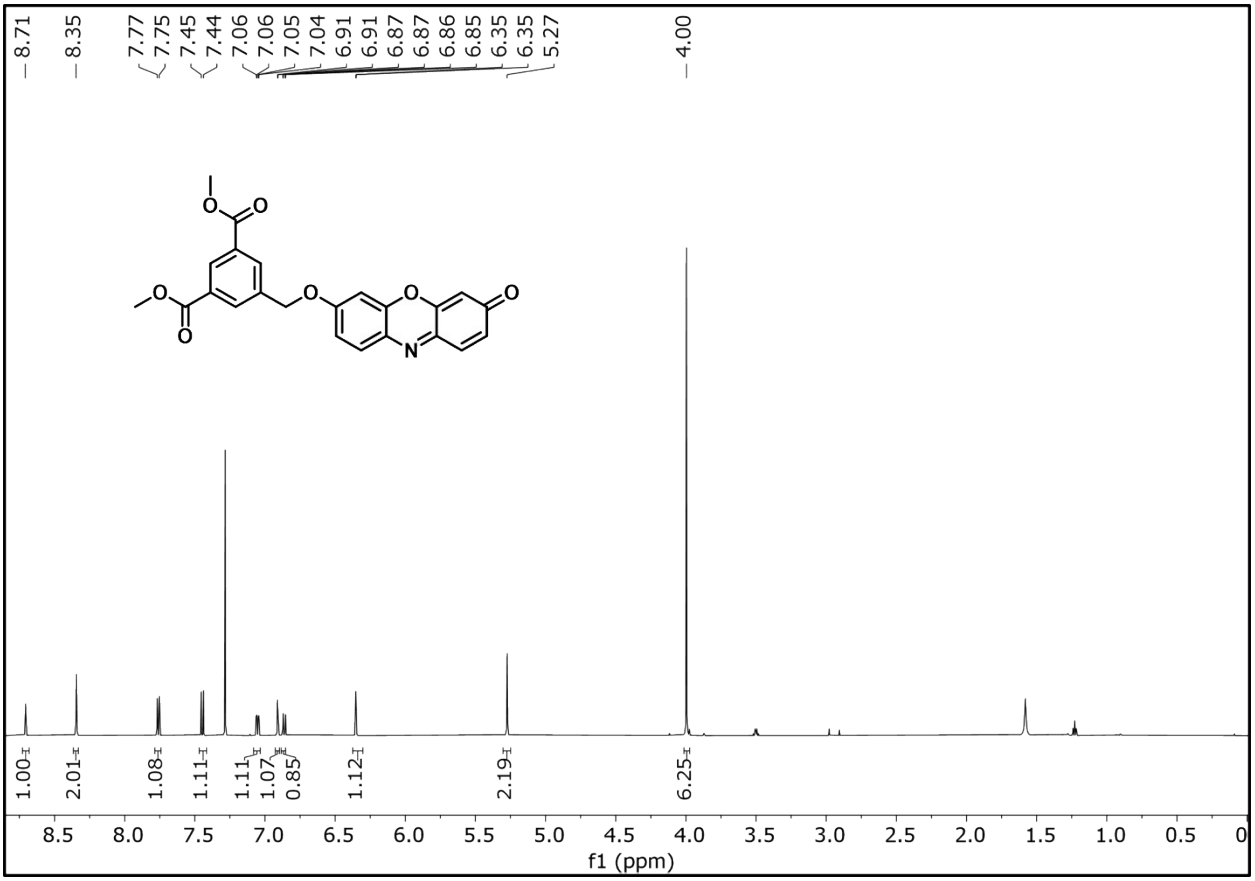


Theoretical Spectrum for C21H18NO5, Minimum Abundance 0.01%

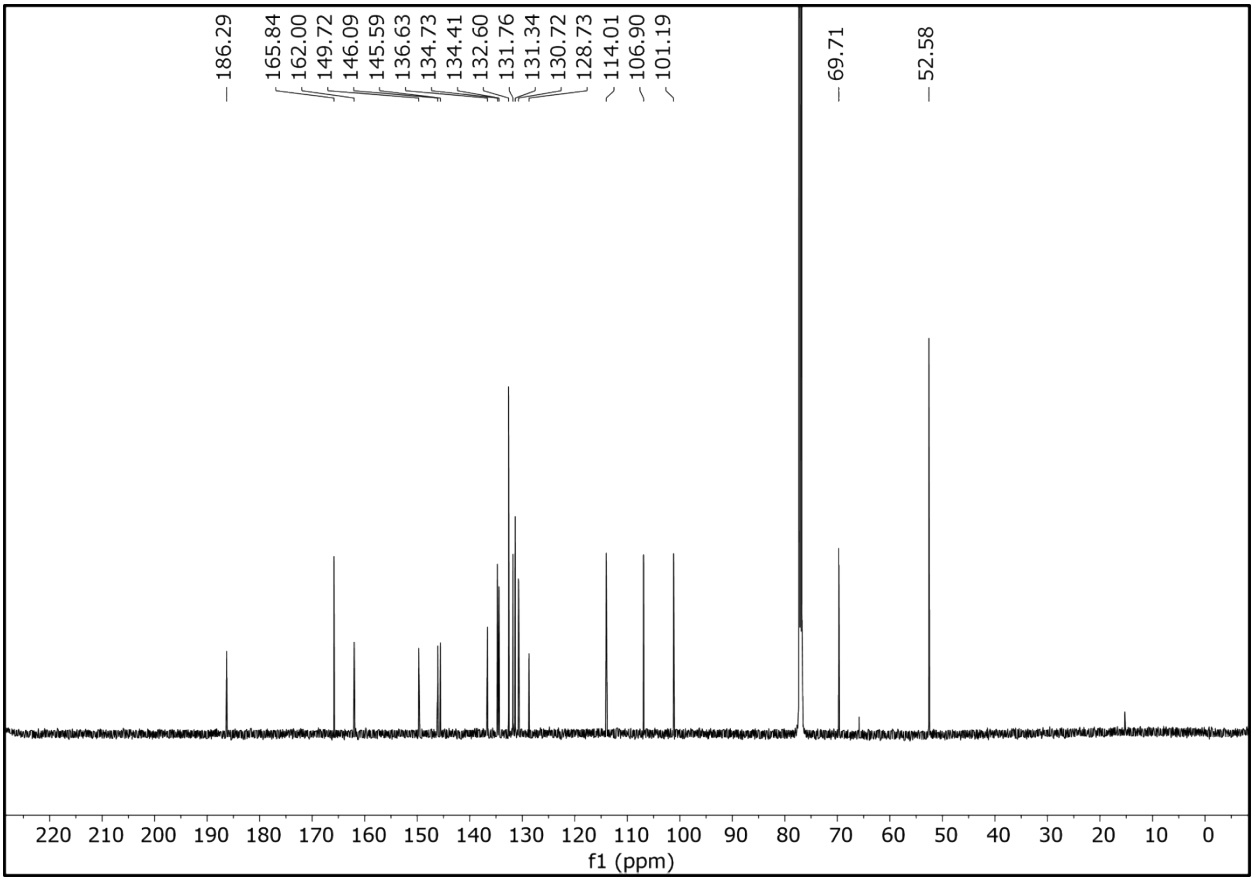


Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
364.1179	364.1180	-0.05	-0.15	$C_{21}H_{18}NO_5$	5823945

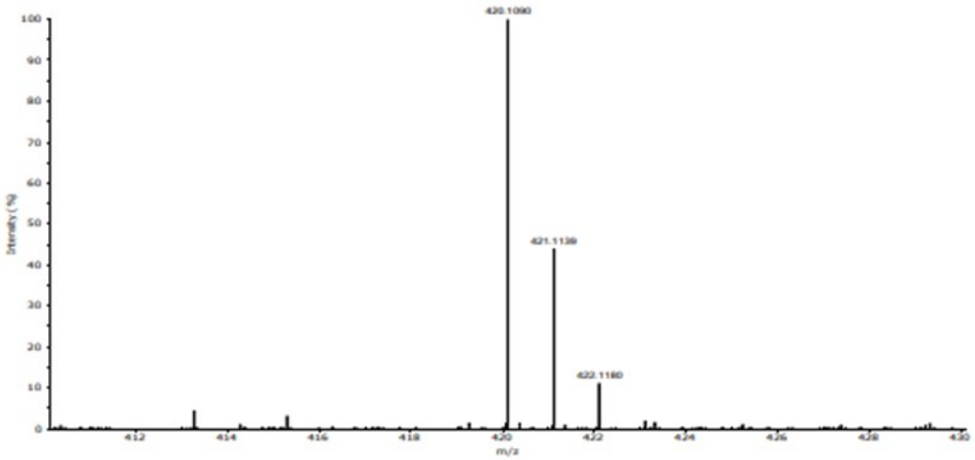
Dimethyl 5-(((3-oxo-3H-phenoxazin-7-yl)oxy)methyl)isophthalate (**R8**) (^{1}H NMR, 600 MHz, $CDCl_3$)



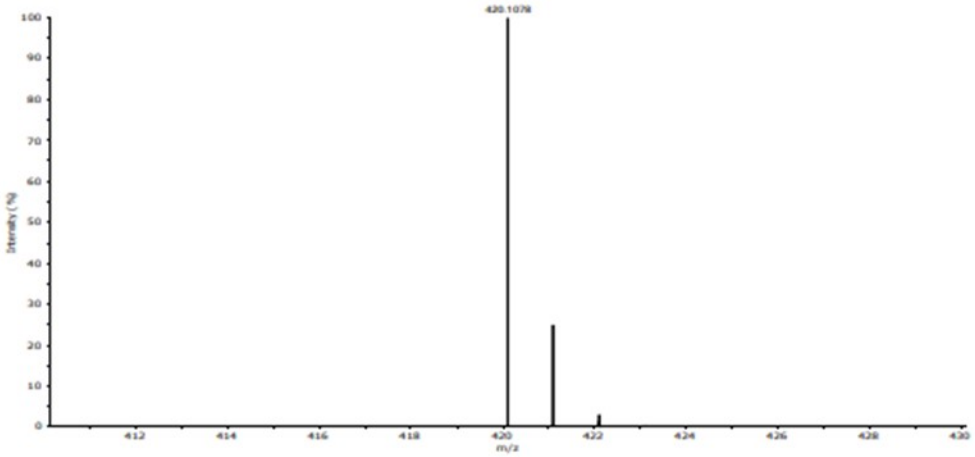
Dimethyl 5-(((3-oxo-3H-phenoxazin-7-yl)oxy)methyl)isophthalate (**R8**) (^{13}C NMR, 151 MHz, $CDCl_3$)



Expanded Spectrum RT 0.13, NL 500812, Peak [1], Target Mass 420.1078

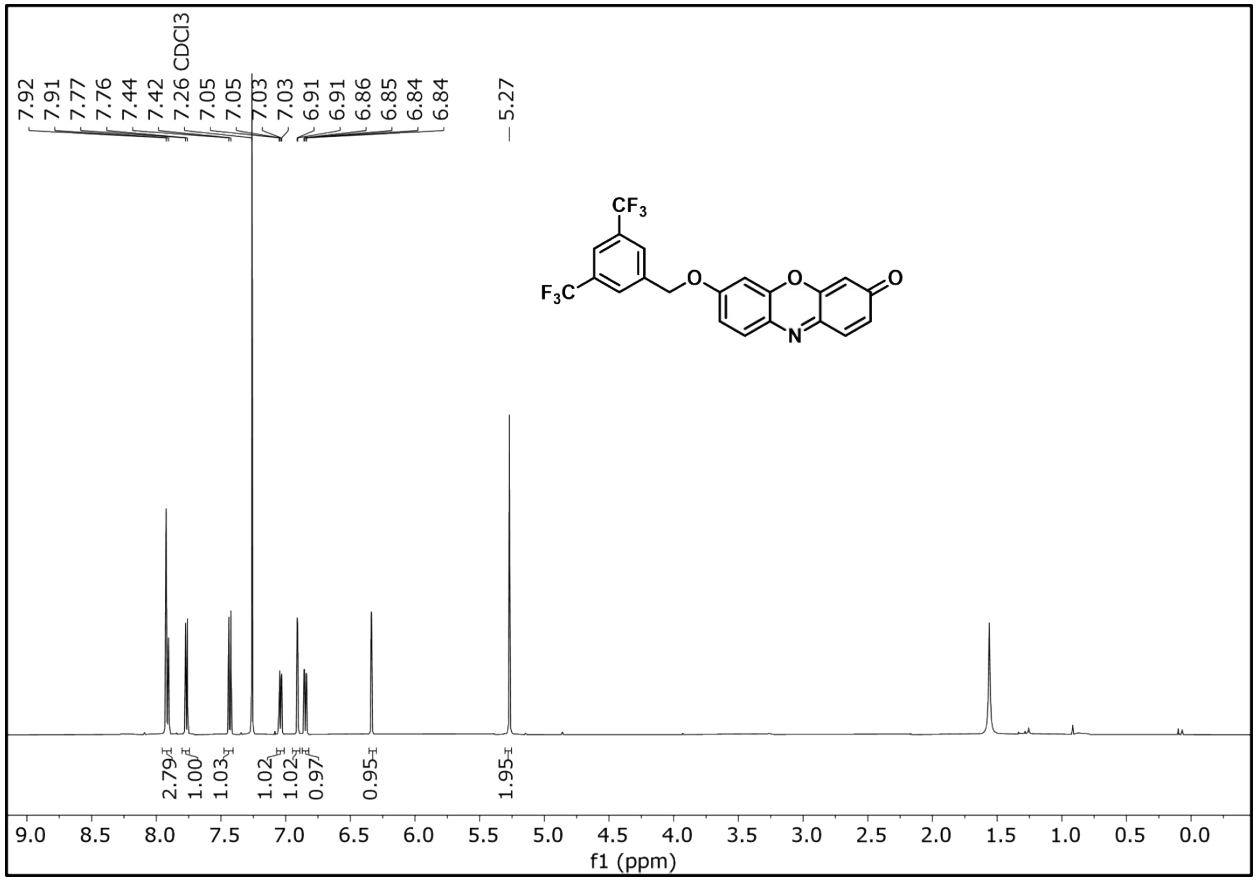


Theoretical Spectrum for C23H18NO7, Minimum Abundance 0.01%

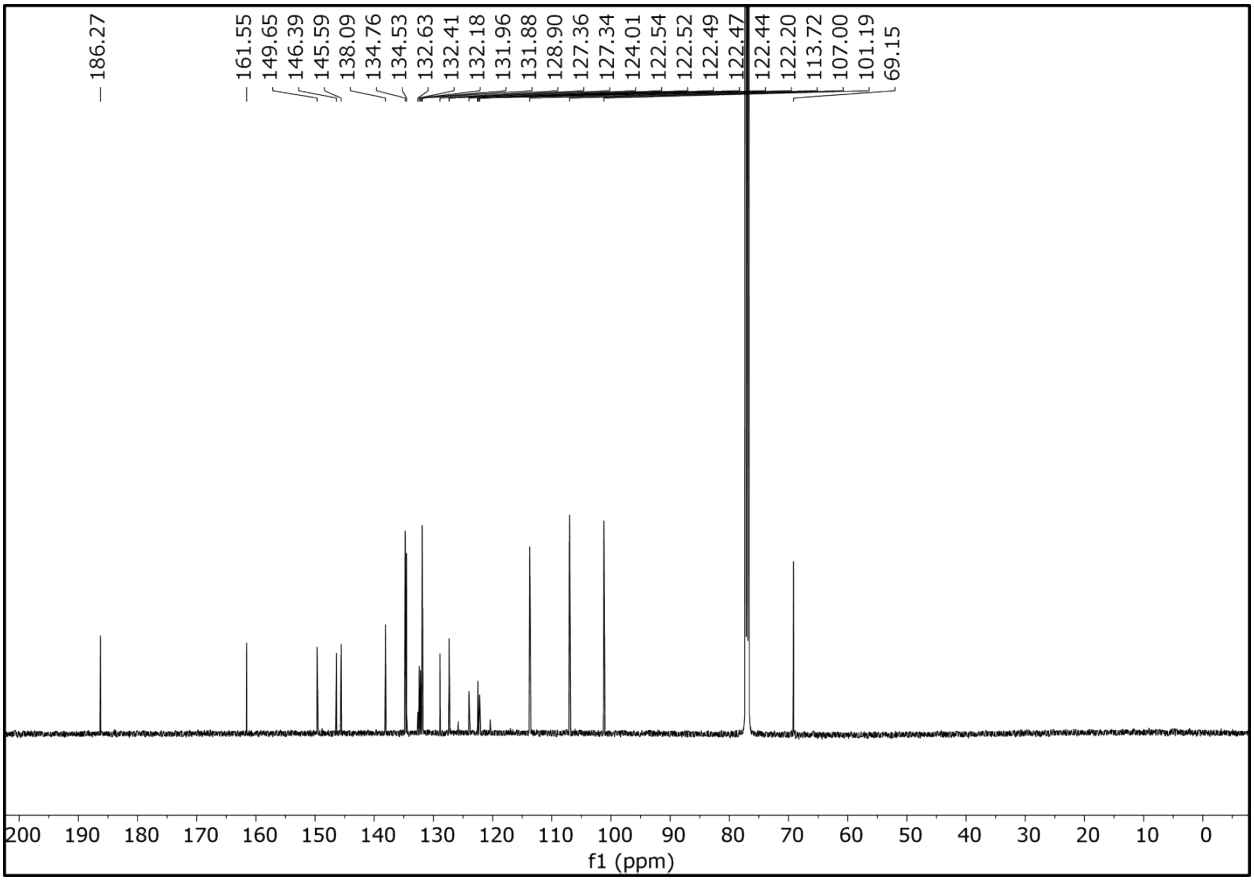


Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
420.1090	420.1078	1.22	2.89	$C_{23}H_{18}NO_7$	1922147

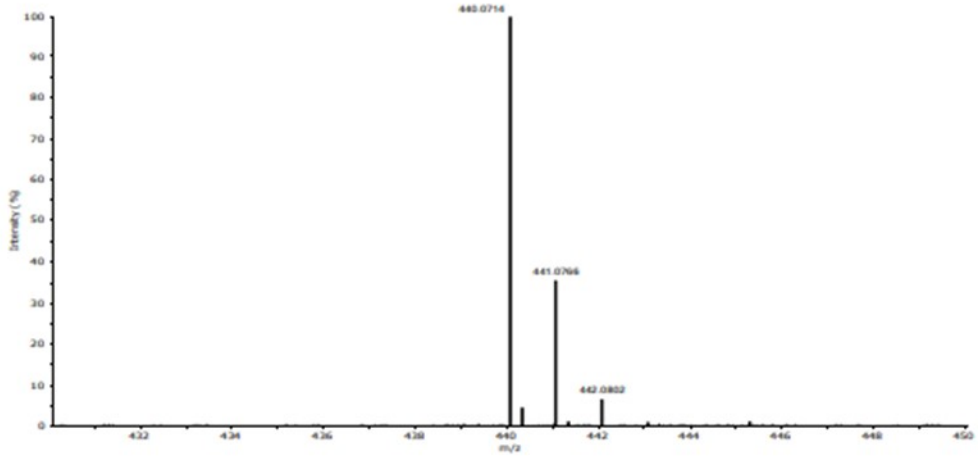
7-((3,5-Bis(trifluoromethyl)benzyl)oxy)-3H-phenoxazin-3-one (**R9**) (^{1}H NMR, 600 MHz, $CDCl_3$)



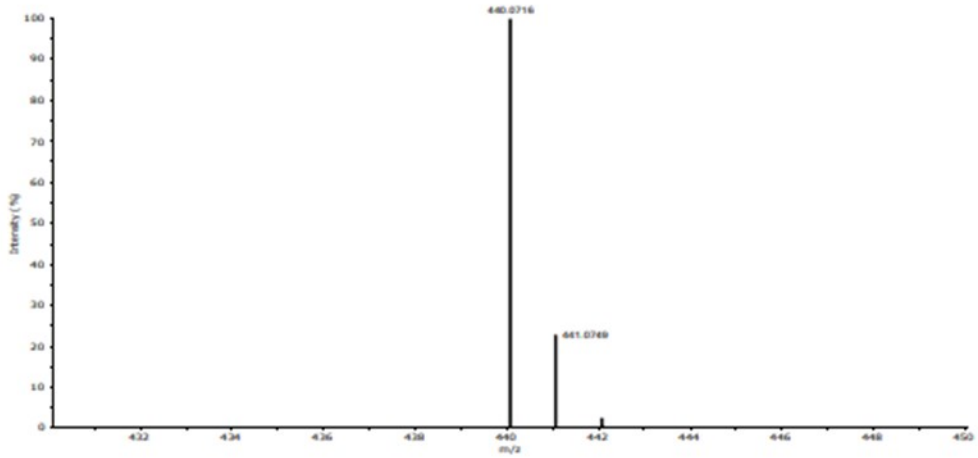
7-((3,5-Bis(trifluoromethyl)benzyl)oxy)-3H-phenoxazin-3-one (**R9**) (^{13}C NMR, 151 MHz, $CDCl_3$)



Expanded Spectrum RT 0.13, NL 2785460, Peak [1], Target Mass 440.0716



Theoretical Spectrum for C21H12F6NO3, Minimum Abundance 0.01%



Measured Mass	Calculated Mass	Error (mDa)	Error (ppm)	Formula $[M+H]^+$	Response
440.0714	440.0716	-0.19	-0.44	$C_{21}H_{12}F_6NO_3$	14946275

6. References

1. Q. F. Fu, H. Y. Li, D. B. Duan, C. L. Wang, S. Y. Shen, H. M. Ma and Z. B. Liu, *Angew. Chem. Intl. Ed.*, 2020, **59**, 21546-21552.
2. L. L. Wu, Q. Y. Yang, L. Y. Liu, A. C. Sedgwick, A. J. Cresswell, S. D. Bull, C. S. Huang and T. D. James, *Chem. Commun.*, 2018, **54**, 8522-8525.