Supporting information

Disconnecting the Estrogen Receptor Binding Properties and Antimicrobial Properties of Parabens Through 3,5-Substitution.

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General Fisher Experimental Procedure

All reactions were performed in flame dried flasks equipped with a reflux condenser. Reactions were then heated using a mineral oil bath with stirring. All reagents were used as purchased from Acros and Sigma-Aldrich. Reaction progress was monitored by analytical TLC using TLC Gel 60W $F_{254}S$ plates made my Millipore. TLC plates were visualized by UV light (254 nm) and/or potassium permanganate ($KMnO_4$) stain. Product purification was performed using flash chromatography with Zeochem silica gel 60 (0.063-0.200mm, 70-230 mesh) at medium pressure. All melting points were determined using a Barnstead Electrothermal 9100 and are uncorrected. Infrared spectra were obtained using a Nicolet iS10 FT-IR spectrometer (Thermo) and reported in cm^{-1} (%T). Proton ^{1}H and ^{13}C NMR were taken on an Anasazi EFT-60 (15 MHz carbon) or a 400 MHz (100 MHz carbon) JOEL FT-NMR with multi-nuclear probe. Chemical shifts are reported in δ (ppm) units using a TMS reference peak as a standard (0 ppm). Purity determination by HPLC was performed on a Shimadzu Prominence UFLC System with the eluents listed. High resolution mass spectroscopy samples were obtained from the NCSU Mass Spectrometry Facility located in the Department of Chemistry. These samples were carried out on a high resolution mass spectrometer – the *Thermo Fisher Scientific Exactive Plus MS*, a benchtop full-scan Orbitrap™ mass spectrometer – using Heated Electrospray Ionization (HESI). Samples were dissolved in methanol and analyzed via flow injection into the mass spectrometer at a flow rate of 200μL/min. The mobile phase was 90% methanol with 0.1% formic acid and 10% water with 0.1% formic acid. The mass spectrometer was operated in positive and negative ion modes using the following HESI parameters:

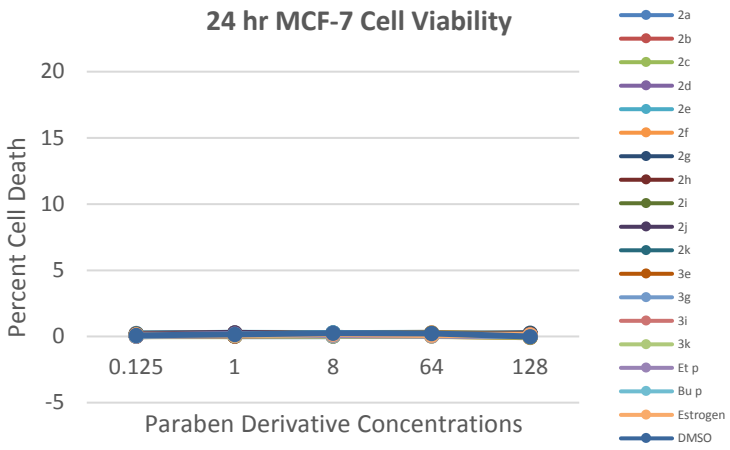
HESI Source Parameters	
Spray voltage	3.5 kV
Capillary temperature	350 ºC
Heater temp	100 ºC
S Lens RF level	70 V
Sheath gas flow rate	50
Resolution	70,000
Scan Range	180-1000 m/z

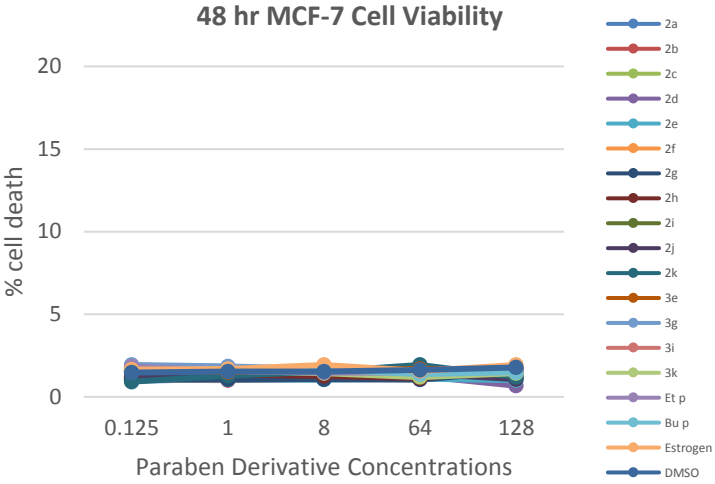
General Synthesis of Esters. Fisher esterification was performed within a 25 mL round bottom flask containing about 500 mg of the substituted benzoic acid derivatives **1** dissolved in 5 mL of the desired alcohol. Then 8 drops of 18 M sulfuric acid was added to the solution. The solution was then heated at reflux overnight (~16 hrs). The reactions were monitored by thin layer chromatography. The reaction was diluted with 50 mL of EtOAc and rinsed with deionized water (20 mL) in triplicate. The organic layer was dried with $MgSO_4$ then filtered. The reaction crude material was concentrated under vacuo to afford a solution of product in trace alcohol. Flash chromatography was used to purify crude products and in many cases involved a second column in which the first eliminated most of the alcohol. Yields were then recorded for the spectroscopically pure solids obtained after complete purification.

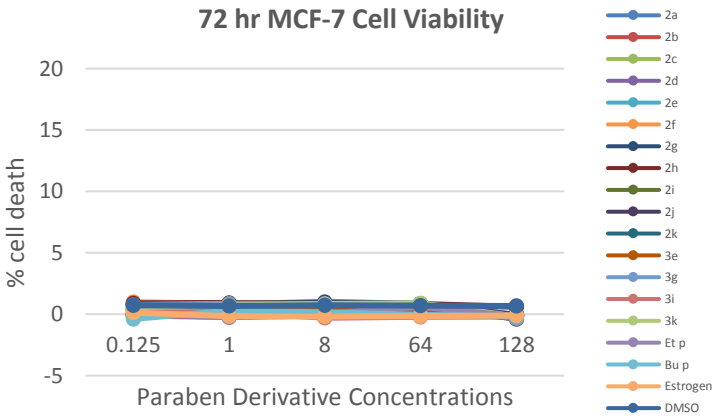
Correlation of Bacterial Optical Density to Colony Forming Units (CFUs). Day cultures were incubated by adding two *S. aureus* (Carolina Biologicals 155554A) colonies grown on sterilized Mueller-Hinton Broth Agar (MHB agar) to 2 mL of sterilized MHB. These cultures were incubated for 6-8 hrs at 37 ^{o}C. After 6-8 hrs the cultures were diluted ten-fold then half-fold serially diluted to provide four dilute solutions of the cultures (1/10, 1/20, 1/40, and 1/80 dilutions). The optical density at 600 nm (OD_{600}) was then recorded for each of these solutions. The original day cultures were then diluted to perform colony counts through serial ten-fold dilutions which provided 9 dilutions (10^{-2} through 10^{-12}). Aliquots (100 µL, notice additional ten-fold reduction) of each culture were then incubated on MHB agar for 18 hrs and plates that contained 10-200 colonies were count to obtain a CFU for the original day culture. This was repeated three days with each day consisting of a pair of trials starting from day cultures that originate from differing colonies, which corresponds to 6 biological replicates. The linear relationship between the average CFUs and OD_{600} was found and used to determine the average OD_{600} that corresponds to 5×10^5 CFU mL^{-1}.

Microdilution Assay for Minimum Inhibitory Concentrations (MIC). Sterilized MHB was inoculated to 5×10^5 CFU mL^{-1} (OD_{600} = 0.00983) with *S. aureus* (Carolina Biologicals 155554A). The inoculum was aliquoted (1 mL) into separate sterilized test tubes and the resulting paraben derivative (from 16 mg/mL DMSO stocks) was added to give the highest concentration to be tested (512 µg/mL or 256 µg/mL). These sample solutions were then aliquoted (200 µL) into the top row of a 96-well plate while retaining the final well (column) for untreated bacterial cells to act as the control. Rows 2-12 of the plate were then aliquoted (100 µL) with the original inoculant. Row 1 wells were mixed 6 to 8 times, and then 100 µL was transferred to row 2. Row 2 wells were mixed 6 to 8 times, followed by a 100 µL transfer from row 2 to row 3. This procedure was repeated to serially dilute the rest of the rows of the microtiter plate. The plate was then sealed with GLAD Press n' Seal® and incubated under stationary conditions at 37 °C. After 16 hrs, MIC values were recorded as the lowest concentration of test compound at which no visible growth of bacteria was observed. This was repeated for a minimum of three days with each experiment consisting of a pair of trials originating from differing day cultures for a minimum of 6 biological replicates.

Cell Line and Cytotoxicity Assay. MCF7 cells (human breast cancer cell line) were used in this study. Cells were grown in complete RPMI media (Gibco Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, 10 mM Hepes, and 0.1 mM non-essential amino acids in a 5% CO_2 atmosphere at 37 °C. To determine the cytotoxicity of the compounds, 2×10^5 cells were plated into flat-bottom 96-well plates (Thermo Scientific Nunc, Waltham, MA) in 0.2 mL of complete medium and treated with different concentrations of each compound (from 16 mg/mL DMSO stocks). Cytotoxicity was determined after 24, 48, and 72 hours of culture with the compounds using an LDH cytotoxicity assay according to manufacturer's protocol (Thermo Scientific Pierce). These protocols were then performed in triplicate with two biological replicates. The percent cell death was determined by using the formula: ((Experimental sample release-spontaneous re-lease) / (Maximum release-spontaneous re-lease)) x 100. The figures to the right illustrate the results from this assay at each time point. Note that no compound demonstrates cytotoxicity greater than 2% cell death while the assay's accuracy was 2-3% (average 2.7%) thus indicating that all compounds were statistically non-toxic at all time points.

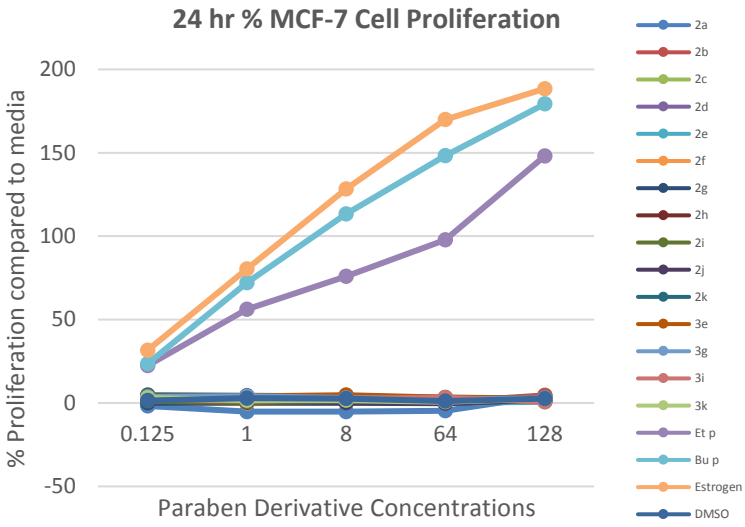


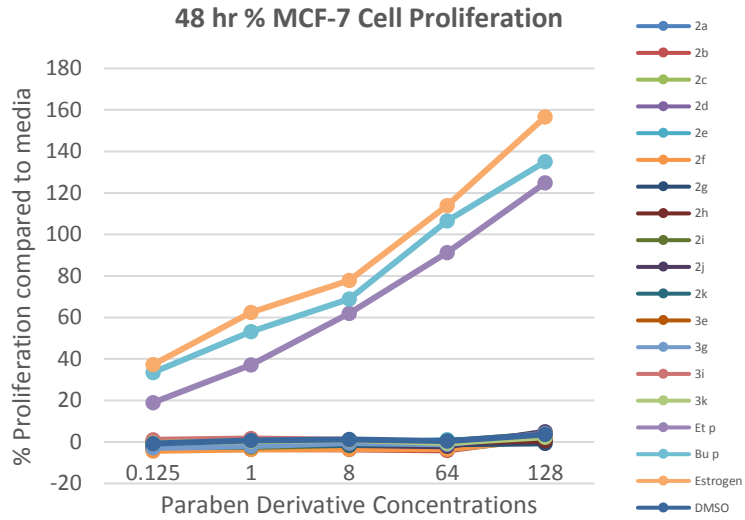


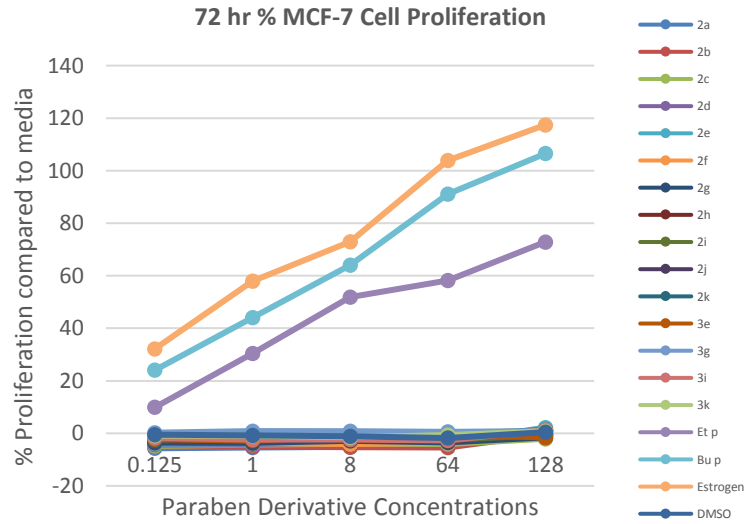


Cell Proliferation Assay. To determine the effect of the compounds on cell proliferation, 2×10^5 MCF7 cells were plated into flat-bottom 96-well plates in 0.2 mL of complete medium and treated with different concentrations of each compound. For comparison, ethyl paraben, butyl paraben, and estrogen were used as standards. Proliferation was determined after 24, 48, and 72 hours of culture with the compounds using a CellTiter 96 Nonradioactive Cell Proliferation Assay (MTT) according to manufacturer's protocol (Promega Madison, WI). These protocols were then performed in triplicate with two biological replicates.

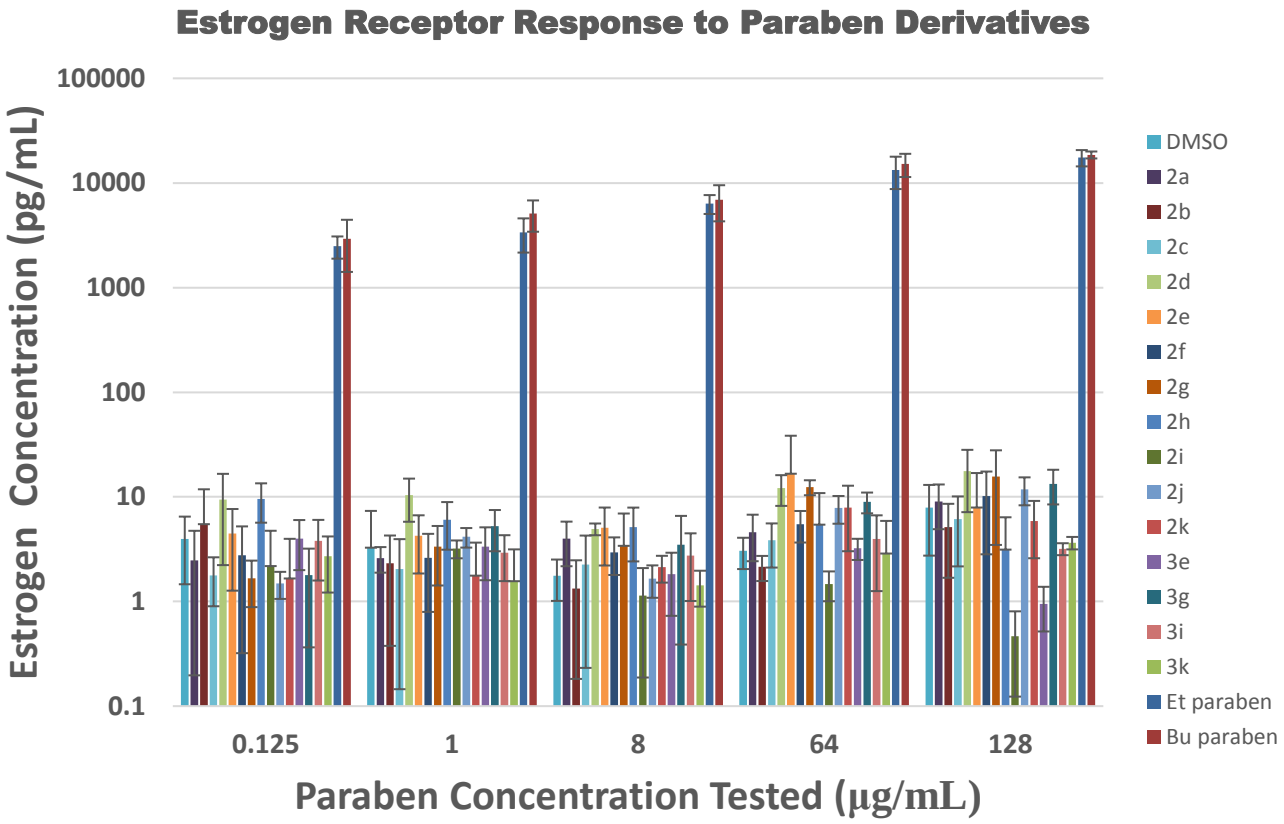
The figures to the right illustrate the snapshot (24 hrs, 48 hrs, and 72 hrs) of cellular proliferation relative to untreated cells or cells in media. It is important to note that when reporting these data in percent to the control as opposed to cellular count that it is typical to see percent proliferation degrade (estrogen percent proliferation is 190% at 24 hrs but 120% at 72 hrs). This is a result of the compound degrading or being metabolized and the inevitability that the MCF-7 cellular count will ultimately grow lessening the cell to compound ratio. Inside of this 72 hr time we saw cellular proliferation from commercial parabens and estrogen but no substituted paraben derivative stimulated cells relative to the control.







Estradiol ELISA Assay. To determine if the compounds stimulated estradiol secretion from MCF7 cells, 2×10^5 cells were plated into flat-bottom 96-well plates in 0.2 mL of complete medium and treated with different concentrations of each compound. Estradiol secretion was determined after 24 hours of culture with the compounds using a 17β Estradiol ELISA kit (Sigma-Aldrich St. Louis, MO or Enzo Life Sciences Farmingdale, NY). These protocols were then performed in triplicate. Data for all compounds tested shown below:



LanthaScreen TR-FRET Er Alpha Competitive Binding Assay Procedure. This assay was run by Select-Screen Services which is a subsidiary group of Thermo Fisher Scientific. They provide assay conditions as listed below, but these conditions are available online at:

http://assets.thermofisher.com/TFS-Assets/LSG/brochures/lanthascreen-tr-fret-competitive-binding-assay-protocol.pdf

Which is the SelectScreen service of the Thermo Fisher product A15887 (LanthaScreen TR-FRET ER alpha competitive binding kit) and the manual for this kit can be found at:

https://assets.thermofisher.com/TFS-Assets/LSG/manuals/lanthascreen_tr_fret_er_alpha_comp_binding_assay_man.pdf

LanthaScreen TR-FRET Competitive Binding Assay Conditions

Target	Target Conc (nM)	Antibody	Antibody Conc (nM)	Tracer	Tracer Conc (nM)	Tracer Kd (nM)	Buffer	Known Inhibitor	IC50 (nM)
ER-alpha	2.1	Tb-anti-GST	2	Fluor. ES2 Green	3	3.5	NRK	17-beta-Estradiol	0.600

Test Compounds
The Test Compounds are screened in 1% DMSO (final) in the well. For 10 point titrations, 3 fold serial dilutions are conducted from the starting concentration of the customer's choosing.

Target/Antibody Mixtures
All Target/Antibody Mixtures are diluted to a 2X working concentration in the appropriate Assay Buffer.

Tracer
The 4X Fluorescein labeled Tracer is prepared in Assay Buffer.

Assay Protocol
Bar-coded Corning, low volume, black 384-well plate (Corning Cat. #4511 or #4514)
1. 4.0 µL –160 nL 100X Test Compound in 100% DMSO plus 3.84 µL Assay Buffer
2. 8.0 µL – 2X Target/Antibody Mixture
3. 4.0 µL – 4X Tracer
4. 60-minute incubation at room temperature
5. Read on fluorescence plate reader and analyze the data

LanthaScreen TR-FRET Competitive Binding Assay Controls

The following controls are made for each individual target and are located on the same plate as the target:

0% Displacement Control
The maximum Emission Ratio is established by the 0% Displacement Control wells, which do not contain known inhibitor in the reaction and therefore exhibits no displacement of the tracer.

100% Displacement Control
The minimum Emission Ratio is established by the 100% Displacement Control wells, which contain the highest concentration of the known inhibitor used in that assay.

Known Inhibitor
A known inhibitor control standard curve, 10 point titration, is run for each individual target on the same plate as the target to ensure the inhibitor is displaced within an expected IC_{50} range previously determined.

LanthaScreen TR-FRET Competitive Binding Assay Data Analysis

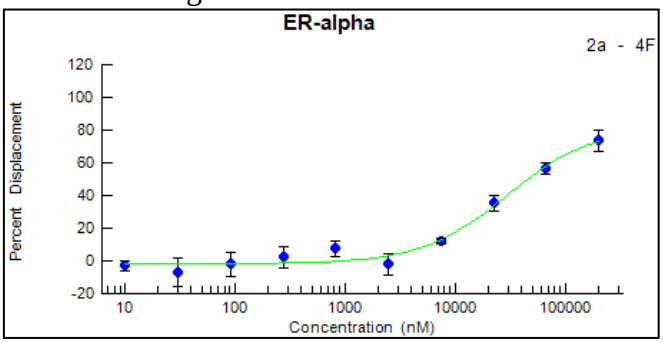
The following equations are used for each set of data points:

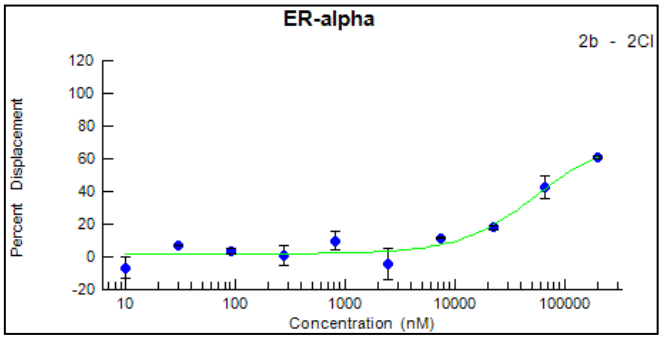
	Equation
Emission Ratio (ER)	$\frac{\text{Fluorescein Emission (520 nm)}}{\text{Terbium Emission (495 nm)}}$
% Displacement	$\left\{ \frac{ER_{0\%\ Disp\ Ctrl} - ER_{Sample}}{ER_{0\%\ Disp\ Ctrl} - ER_{100\%\ Disp\ Ctrl}} \right\} * 100$
Difference Between Data Points (single point only)	$\lvert \%\ Displacement_{Point\ 1} - \%\ Displacement_{Point\ 2} \rvert$
Test Compound Interference	For each emission wavelength, fluorescence interference is flagged for a compound well that is more than 20% outside the range of the controls.
Z´ (using Emission Ratio values)	$1 - \frac{3 * Stdev_{0\%\ Disp\ Ctrl} + 3 * Stdev_{100\%\ Disp\ Ctrl}}{\lvert Mean_{0\%Disp\ Ctrl} - Mean_{100\%\ Disp\ Ctrl} \rvert}$

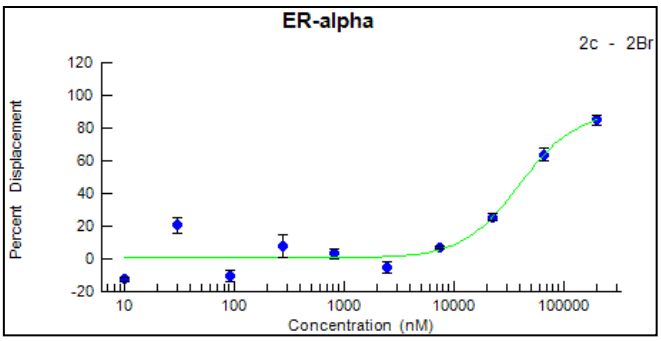
Graphing Software

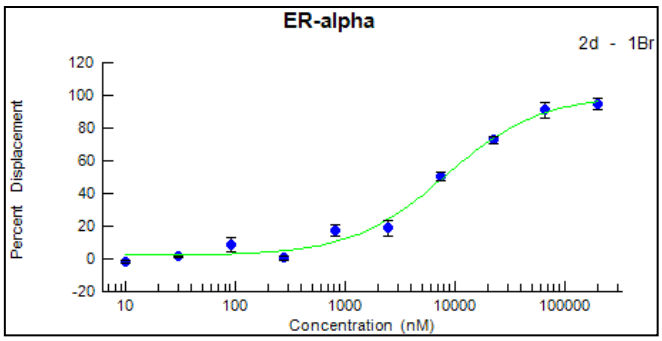
SelectScreen Profiling Service uses XL*fit* from IDBS. The dose response curve is curve fit to model number 205. If the bottom of the curve does not fit between -20% & 20% inhibition, it is set to 0% inhibition. If the top of the curve does not fit between 70% and 130% inhibition, it is set to 100% inhibition.

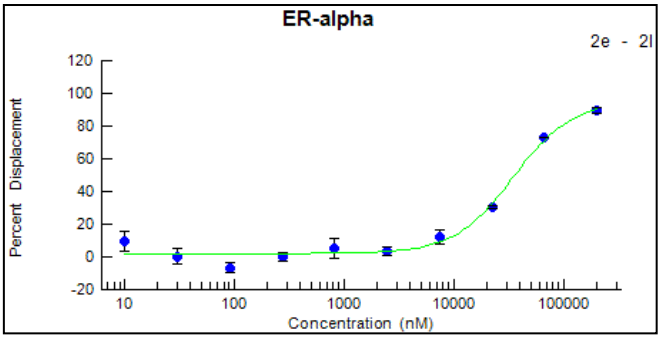
The results are provided in table and figure format. The figures below illustrate the % labelled estrogen displaced by the compounds tested. Select Screen does not provide an IC50 curve for the standard (7-β-estradiol) however the standard is run on each plate to confirm accuracy to past averages (0.600 nM). 7-β-estradiol correlated to IC_{50}s of 0.452 and 0.407 nM on the plates used in these assays. The compound names are given in the upper right-hand corner of the images. Although, 3a, 3b, 3c, and 3d corresponds to samples **3e**, **3g**, **3i**, and **3k** respectively. It is important to note that for the highest con-centrations tested of the nitro substituted compounds **2k** and **3k** exhibited fluorescence interference at the wavelengths associated with the lanthanide donor.

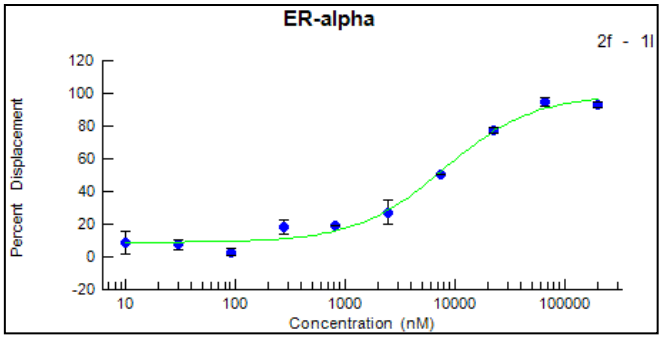


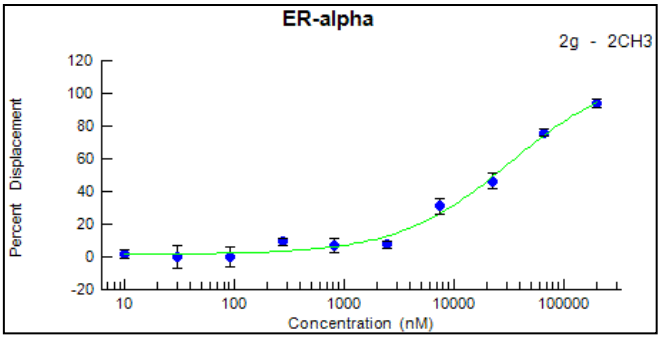


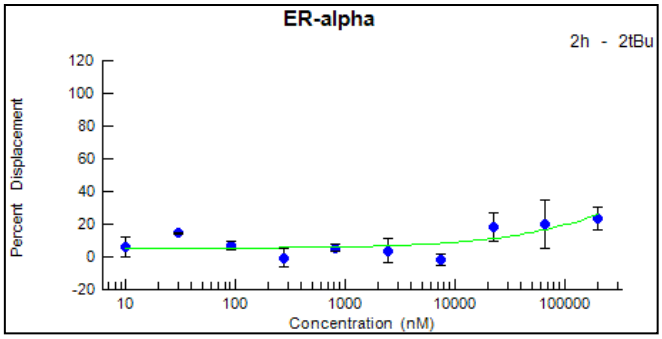

ER-alpha
2c - 2Br
Percent Displacement
Concentration (nM)

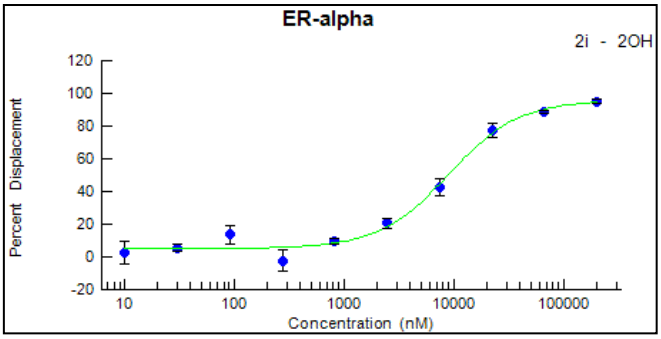

ER-alpha
2d - 1Br
Percent Displacement
Concentration (nM)

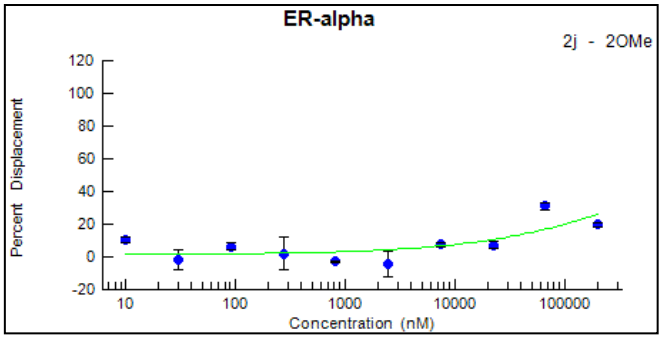

ER-alpha
2e - 2I
Percent Displacement
Concentration (nM)

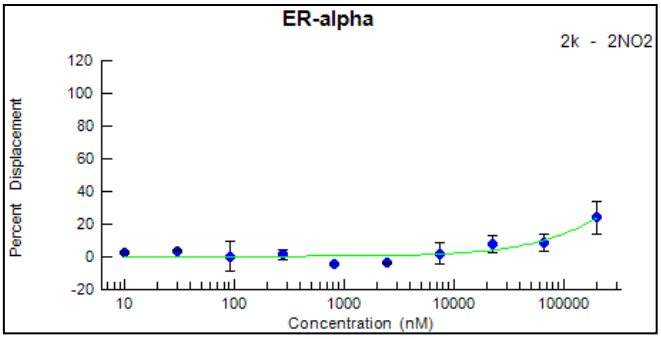

ER-alpha
2f - 1I
Percent Displacement
Concentration (nM)

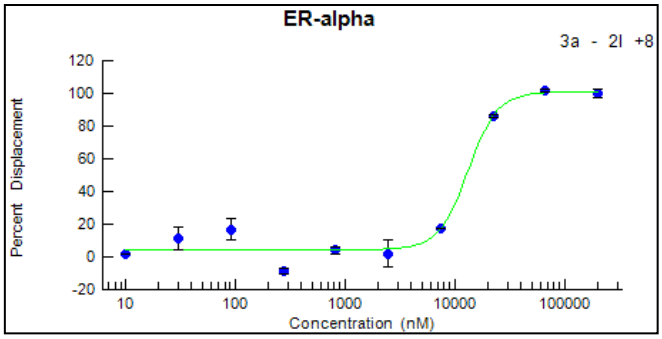

ER-alpha
2g - 2CH3
Percent Displacement
Concentration (nM)

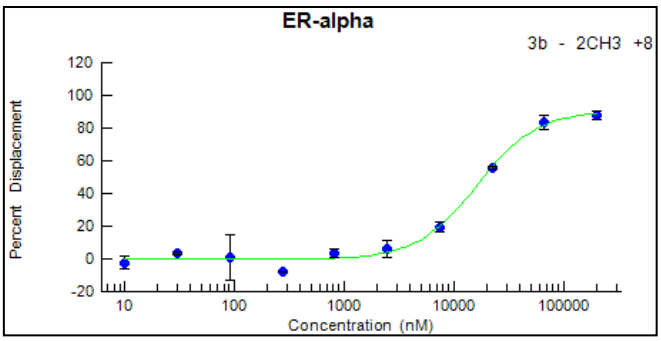

ER-alpha
2h - 2tBu
Percent Displacement
Concentration (nM)

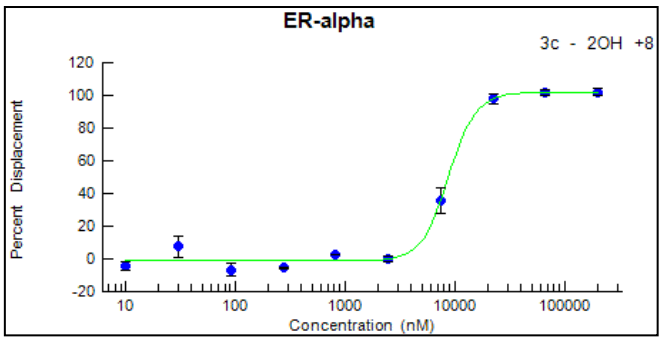

ER-alpha
2i - 2OH
Percent Displacement
Concentration (nM)

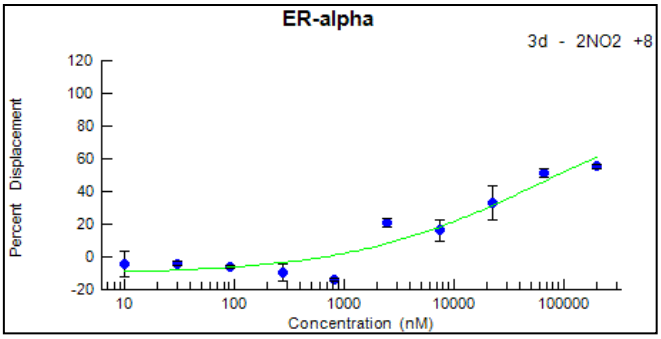

ER-alpha
2j - 2OMe
Percent Displacement
Concentration (nM)

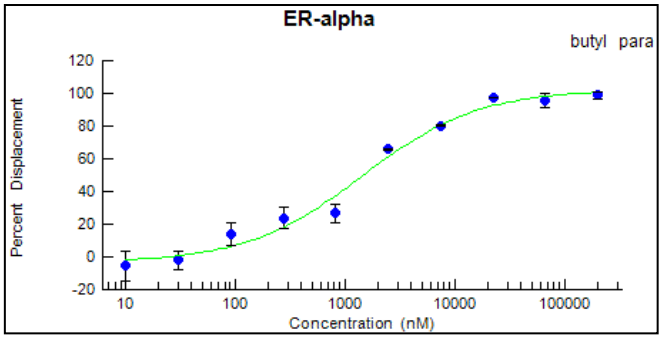

ER-alpha
2k - 2NO2
Percent Displacement
Concentration (nM)

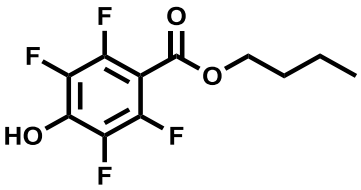

ER-alpha
3a - 2I +8
Percent Displacement
Concentration (nM)


ER-alpha
3b - 2CH3 +8
Percent Displacement
Concentration (nM)

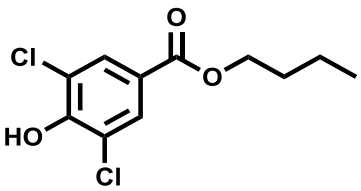

ER-alpha
3c - 2OH +8
Percent Displacement
Concentration (nM)


ER-alpha
3d - 2NO2 +8
Percent Displacement
Concentration (nM)

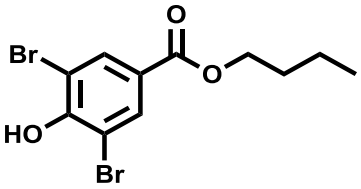

ER-alpha
butyl para
Percent Displacement
Concentration (nM)



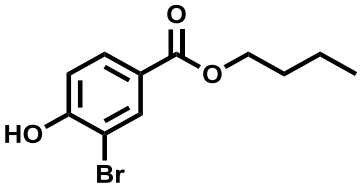
Butyl 2,3,5,6-tetra-fluoro-4-hydroxybenzoate. **2a.** The general procedure was used to prepare **2a** and it was purified by flash chromatography using 15% ethyl acetate in hexanes to provide a white powder (224 mg, 35% yield). Mp = 35-37ºC. R_f (20% ethyl acetate/hexanes)= 0.35. ^{1}H NMR:δ6.67(s, 1H), 4.50(t, J=6.1 Hz, 2H), 2-1.3 (m, 4H), 0.97 (t, J=6.8 Hz, 3H). ^{13}C NMR: 161.9, 146.3 (dddd, J=260 Hz, 50 Hz, 8 Hz, 4 Hz), 139.5 (tt, J=13 Hz, 5 Hz), 138.0 (dddd, J=244 Hz, 15 Hz, 4 Hz, 4 Hz), 102.5 (t, J=14 Hz), 66.9, 30.6, 19.2, 13.7. IR(CH_2Cl_2 film): br. 3267, s. 2964, s. 2939, s. 2876, s. 1699, w. 1649, w. 1526, s. 1497, s. 1324.HRMS (ESI-) m/*z* 265.04913 [$(M-H)^-$; calculated mass for $C_{11}H_9F_4O_3^-$: 265.04933 amu].



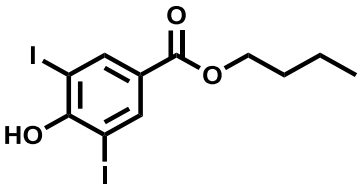
Butyl 3,5-di-chloro-4-hydroxybenzoate. **2b.** The general procedure was used to prepare **2b** and it was purified by flash chromatography using 10% ethyl acetate in hexanes to provide a white powder (460 mg, 73% yield). Mp = 80-82ºC. R_f (20% ethyl acetate/hexanes)= 0.54. ^{1}H NMR:δ7.96(s, 2H), 6.38(brs, 1H), 4.31(t, J=6.3 Hz, 2H), 2.0-1.1(m, 4H), 0.98(t, J=5.7 Hz, 3H). ^{13}C NMR: 164.6, 152, 129.8, 123.6, 121.4, 66.6, 30.7, 19.2, 13.7. IR(CH_2Cl_2 film): br. 3229, s. 2962, s. 2928, s. 2872, s. 1696, w. 1591, w. 1569, w. 1489, w. 1416.HRMS (ESI-) m/*z* 261.00886 [$(M-H)^-$; calculated mass for $C_{11}H_{11}Cl_2O_3^-$: 261.00907 amu].



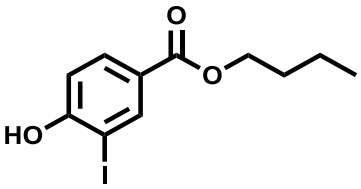
Butyl 3,5-di-chloro-4-hydroxybenzoate. **2c.** The general procedure was used to prepare **2c** and it was purified by flash chromatography using 10% ethyl acetate in hexanes to provide a white powder (401 mg, 53% yield). Mp = 77-79ºC. R_f (25% ethyl acetate/hexanes)= 0.71.^{1}H NMR:δ8.13(s, 2H), 6.15(brs, 1H), 4.32(t, J=6.3 Hz, 2H), 2.0-1.1(m, 4H), 0.94(t, J=5.9 Hz, 3H). ^{13}C NMR: 164.2, 153.4, 133.7, 125.1, 109.8, 65.6, 30.8, 19.4, 13.9. IR(CH_2Cl_2 film): br. 3298, s. 2959, s. 2925, s. 2871, s. 1699, w. 1588, w. 1555, w. 1480.HRMS (ESI+) m/*z* 350.92165 [$(M+H)^+$; calculated mass for $C_{11}H_{13}Br_2O_3^+$: 350.92260 amu].



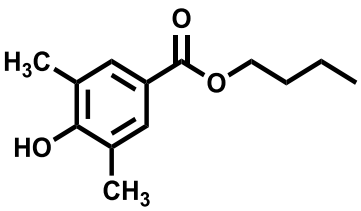
Butyl 3-bromo-4-hydroxybenzoate. **2d.** The general procedure was used to prepare **2d** and it was purified by flash chromatography using 20% ethyl acetate in hexanes to provide a white powder (580mg, 87% yield). Mp = 83-85ºC. R_f (25% ethyl acetate/hexanes)= 0.326. ^{1}H NMR: δ8.18(d, J=2.1 Hz, 1H), 7.92 (dd, J=2.0 Hz, 8.5 Hz, 1H), 7.04 (d, J=8.6 Hz, 1H), 6.647(brs, 1H), 4.315 (t, J=6.4 Hz, 2H), 2.1-1.2 (m, 4H), 0.977 (t, J=6.0 Hz, 3H). ^{13}C NMR: 165.9, 157, 134.3, 130.8, 123.8, 115.9, 110.0, 65.3, 30.7, 19.2, 13.9. IR(CH_2Cl_2 film): 3321, 2959, 2933, 3056, 1685, s. 1600, s. 1577, s. 1507, 1476.HRMS (ESI-) m/*z* 270.99773 [$(M-H)^-$; calculated mass for $C_{11}H_{12}BrO_3^-$: 270.99753 amu].



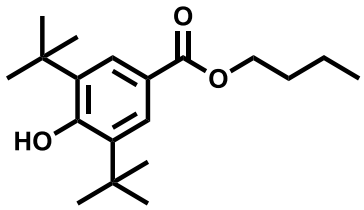
Butyl 3,5-di-iodo-4-hydroxybenzoate. **2e.** The general procedure was used to prepare **2e** and it was purified by flash chromatography using 10% ethyl acetate in hexanes to provide a white powder (514 mg, 90% yield). Mp = 83-85ºC. R_f (20% ethyl acetate/hexanes)= 0.50. ^{1}H NMR:δ8.35(s, 2H), 6.12(brs, 1H), 4.30(t, J= 6.3 Hz, 2H), 1.9-1.2(m, 4H), 0.98 (t, J= 5.8 Hz, 3H). ^{13}C NMR: 163.5, 157.1, 140.8, 126.3, 81.7, 65.3, 30.7, 19.1, 13.7. IR(CH_2Cl_2 film): br. 3368, s. 3070, s. 2957, s. 2935, s. 2871, s. 1699, w. 1579, w. 1546, w. 1458, w. 1391.HRMS (ESI+) m/*z*446.89419 [$(M+H)^+$; calculated mass for $C_{11}H_{13}I_2O_3^+$: 446.89486 amu].



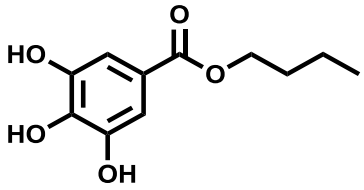
Butyl 3-iodo-4-hydroxybenzoate. **2f.** The general procedure was used to prepare **2f** and it was purified by flash chromatography using 10% ethyl acetate in hexanes to provide a white powder (175 mg, 42% yield). Mp = 89-91ºC. R_f (20% ethyl acetate/hexanes)= 0.46. ^{1}H NMR:δ 8.38(d, J=2.0 Hz, 1H), 7.93(dd, J=3.06 Hz, 2.1 Hz, 1H), 7.00 (d, J=8.5 Hz, 1H), 6.67(brs, 1H), 4.32(t, J=6.3 Hz, 2H), 2.0-1.2(m, 4H), 0.97(t, J=6.7 Hz, 3H). ^{13}C NMR: 165.6, 159.2, 140.5, 131.7, 124.2, 114.6, 84.5, 65.2, 30.6, 19.1, 13.6. IR(CH_2Cl_2 film): s. 3316, s. 2955, s. 2931, s. 2871, s. 1694, w. 1597, w. 1570, w. 1502, w. 1420.HRMS (ESI+) m/*z* 320.99751 [$(M+H)^+$; calculated mass for $C_{11}H_{14}IO_3^+$: 320.99821 amu].



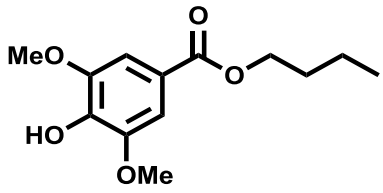
Butyl 3,5-di-methyl-4-hydroxybenzoate. **2g.** The general procedure was used to prepare **2g** and it was purified by flash chromatography using 15% then 30% ethyl acetate in hexanes to provide a white powder (580 mg, 87% yield). Mp = 72-73ºC. R_f (25% ethyl acetate/hexanes)= 0.45. ^{1}H NMR: δ7.7(s, 2H), 5.4(brs, 1H), 4.30(t, J=4.8 Hz, 2H), 2.28(s, 6H), 2-0.8(m, 4H), 0.98(t, J=3.0 Hz, 3H). ^{13}C NMR: 167.5, 157.3, 130.4, 123.6, 121.4, 64.5, 30.8, 19.2, 15.9, 13.5.IR(CH_2Cl_2 film): br. 3379, s. 2982, s. 2960, w. 2935, s. 1685, s. 1598, s. 1490, s. 1480, w. 1464. HRMS (ESI+) m/*z* 245.11450 [$(M+Na)^+$; calculated mass for $C_{13}H_{18}O_3Na^+$: 245.11482 amu].



Butyl 3,5-di-tert-butyl-4-hydroxybenzoate. **2h.** The general procedure was used to prepare **2h** and it was purified by flash chromatography using 3% ethyl acetate in hexanes to provide a white powder (568 mg, 90% yield). Mp = 84-85ºC. R_f (25% ethyl acetate/hexanes)= 0.73. ^{1}H NMR: δ7.93(s, 2H), 5.70(brs,1H), 4.30(t, J=5.9 Hz, 2H), 2.0-1.1 (m, 4H), 0.98(t, J=5.0 Hz, 3H). ^{13}C NMR: 167.3, 158.2, 135.9, 127.1, 121.7, 64.4, 34.4, 31.0, 30.2, 19.4, 13.8. IR(CH_2Cl_2 film): br. 3416, s. 2959, s. 1701, s. 1603, s. 1516, s. 1459, s. 1424. HRMS (ESI+) m/*z* 307.22653 [$(M+H)^+$; calculated mass for $C_{19}H_{32}O_3^+$: 307.22677 amu].

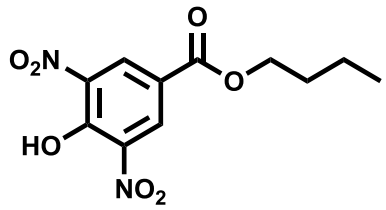


Butyl 3,4,5-trihydroxybenzoate. **2i.** The general procedure was used to prepare **2i** and it was purified by flash chromatography using 40% ethyl acetate in hexanes to provide a white powder (472 mg, 71% yield). Mp = 129-132ºC. R_f (20% ethyl acetate/hexanes)= 0.26. ^{1}H NMR:δ9.14 (s, 3H), 6.98(brs, 2H), 4.16 (t, J=5.9 Hz, 2H), 1.7-1.1(m, 4H), 0.90(t, J=5.8 Hz, 3H). ^{13}C NMR: 166, 145.6, 138.5, 119.7, 108.6, 63.7, 30.4, 18.9, 13.7. IR(MeOHfilm): s. 3493, s. 3461, s. 3223, s. 2962, s. 2930, s. 2876, s. 1688, w. 1613, w. 1538, w. 1465, w. 1400.HRMS (ESI+) m/*z* 249.07309 [$(M+Na)^+$; calculated mass for $C_{11}H_{14}O_5Na^+$: 249.07334 amu].

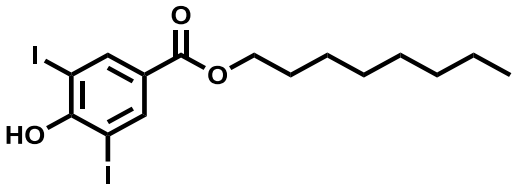


Butyl 3,5-di-methoxy-4-hydroxybenzoate. **2j.** The general procedure was used to prepare **2j** and it was purified by flash chromatography using 25% ethyl acetate in hexanes to provide a white powder (607 mg, 95% yield). Mp = 67-71ºC. R_f (25% ethyl acetate/hexanes)= 0.35. ^{1}H NMR: δ7.33(s, 2H), 5.93(brs, 1H), 4.31(t, J=5.9, 2H), 3.94(s, 6H), 2.1-1.2 (m, 4H), 0.99 (t, J=6.1 Hz, 3H). ^{13}C NMR: 166.0, 146.4, 139.3, 120.5, 106.4, 64.3, 55.8, 30.3, 18.7, 13.2.IR(CH_2Cl_2 film): br. 3381, w. 3116, s. 2895, s. 2874, s. 2844, s.

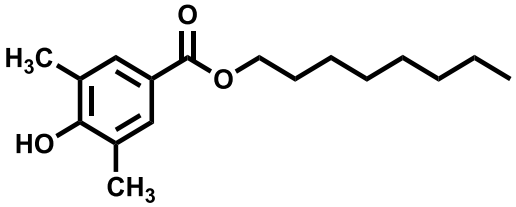
1685, s. 1610, 1598, 1516, 1465, 1426. HRMS (ESI+) m/*z* 277.10442 [$(M+Na)^+$; calculated mass for $C_{13}H_{18}O_5Na^+$: 277.10464 amu].



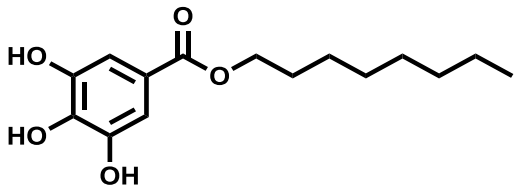
Butyl 3,5-di-nitro-4-hydroxybenzoate. **2k.** The general procedure was used to prepare **2k** and it was purified by flash chromatography using a gradient of 10% to 30% to 80% ethyl acetate in hexanes to provide a yellow powder (568 mg, 90% yield). Mp = 225-230ºC. R_f (80% ethyl acetate/hexanes)= 0.54. ^{1}H NMR: δ8.3(s, 2H), 4.2(t, J=5.1 Hz, 2H), 2-1.2(m, 4H), 0.94(t, J=4.0 Hz, 3H), phenolic O-H/D not observed. ^{13}C NMR: 164.5, 143.0, 130.8, 104.9, 79.3, 64.1, 30.5, 18.9, 13.7. IR(CH_2Cl_2 film): 3388, w. 2985, s. 2958, w. 2946, s. 1708, w. 1552, 1609, w. 1520, w. 1488, w. 1457. HRMS (ESI-) m/*z* 283.05704 [$(M-H)^-$; calculated mass for $C_{11}H_{11}N_2O_7^-$: 283.05717 amu].



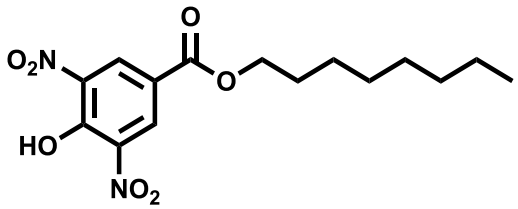
Octyl 3,5-di-iodo-4-hydroxybenzoate. **3e.** The general procedure was used to prepare **3e** and it was purified by flash chromatography using 15% ethyl acetate in hexanes to provide a white powder (639 mg, 99% yield). Mp = 55-59ºC. R_f (20% ethyl acetate/hexanes)= 0.60. ^{1}H NMR:δ8.34(s, 2H), 5.13(brs, 1H), 4.29(t, J=6.3 Hz, 2H), 2.0-1.1(m, 12H), 0.89(t, J=4.4 Hz, 3H). ^{13}C NMR: 163.6, 157.3, 140.8, 126.4, 81.8, 65.8, 31.8, 29.2, 28.7, 26.0, 22.7, 14.2. IR(CH_2Cl_2 film): br. 3389, s. 3079, s. 2950, s. 2915, s. 2853, s. 1701, w. 1580, w. 1543, w. 1465, w. 1400. HRMS (ESI+) m/*z* 502.95713 [$(M+H)^+$; calculated mass for $C_{15}H_{21}I_2O_3^+$: 502.95746 amu].



Octyl 3,5-di-methyl-4-hydroxybenzoate. **3g.** The general procedure was used to prepare **3g** and it was purified by flash chromatography using 10% ethyl acetate in hexanes to provide a white powder (685 mg, 82% yield). Mp = 43-45ºC.R_f (25% ethyl acetate/hexanes)= 0.37. ^{1}H NMR: δ7.70(s, 2H), 5.18(brs, 1H), 4.27(t, J=5.6 Hz, 2H), 2.27(s, 6H), 2-1.2(m, 12H), 0.88(t, 4.3 Hz, 3H). ^{13}C NMR: 167.2, 157.5, 130.2, 123.9, 121.6, 64.7, 31.6, 29.0, 28.5, 25.8, 22.3, 15.6, 13.7, 13.5. IR(CH_2Cl_2 film): br. 3429, 2953, 2924, w. 2895, s.1685, 1601, w. 1558, w. 1538, 1488. HRMS (ESI+) m/*z* 301.17742 [$(M+Na)^+$; calculated mass for $C_{17}H_{26}O_3Na^+$: 301.17743 amu].

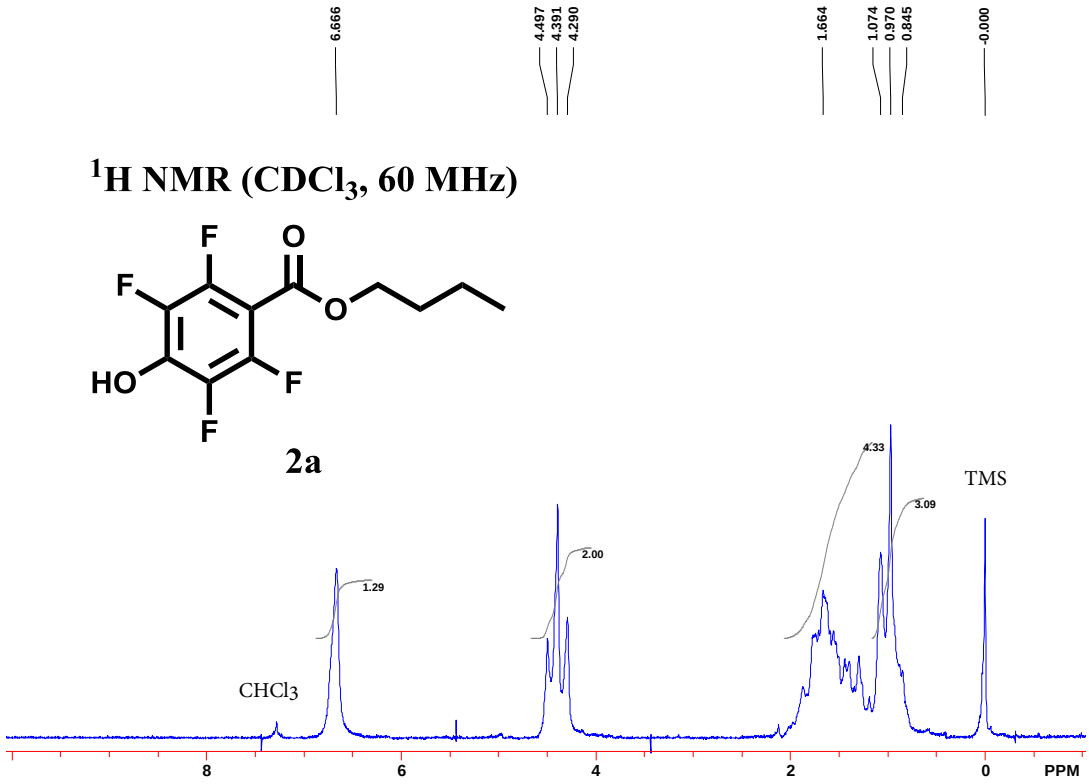


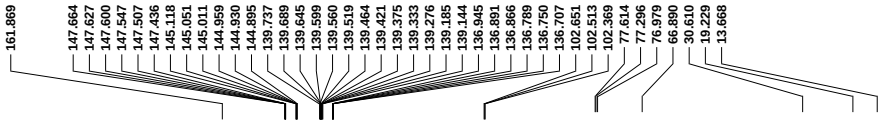
Octyl 3,4,5-trihydroxybenzoate. **3i.** The general procedure was used to prepare **3i** and it was purified by flash chromatography using 45% ethyl acetate in hexanes with 1% acetic acid to provide a white powder (329 mg, 40% yield). Mp = 86-91ºC. R_f (40% ethyl acetate/hexanes)= 0.31. ^{1}H NMR:δ9.08(brs, 3H), 6.96(s, 2H), 4.16(t, J=5.6 Hz, 2H), 1.9-1.1 (m, 12H), 0.86(t, J=4.1, 3H). ^{13}C NMR: 166.8, 144.9, 137.7, 121.5, 109.5, 64.5, 32.0, 29.5, 29.0, 26.3, 22.7, 13.9. IR(MeOHfilm): br. 3318, s. 2939, s. 2833, s. 1700, w. 1609, w. 1534, w. 1448, w. 1341.HRMS (ESI+) m/*z* 305.13575 [$(M+Na)^+$; calculated mass for $C_{15}H_{22}O_5Na^+$: 305.13594 amu].



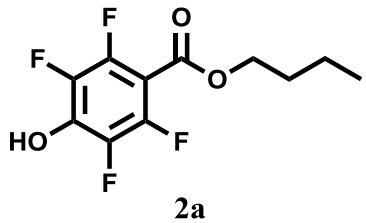
Octyl 3,5-di-nitro-4-hydroxybenzoate. **3k.** The general procedure was used to prepare **3k** and it was purified by flash chromatography using a gradient of 10% to 30% to 80% ethyl acetate in hexanes to provide a yellow powder (607 mg, 82% yield). Mp = 225-230 ºC.R_f (80% ethyl acetate/hexanes)= 0.54. ^{1}H NMR:δ8.34(s, 2H), 4.21(t, J=5.9 Hz, 2H), 1.8-1.1(m, 12H), 0.86(t, J=4.4 Hz, 3H), phenolic O-H/D not observed. ^{13}C NMR: 164.4, 160.5, 142.9, 130.6, 104.9, 64.3, 31.3, 28.7,28.7, 28.3, 25.5, 22.1, 13.9. IR(MeOH film): br. 3330, s. 2958, s. 2930, s. 2867, s. 1702, w. 1639, w. 1614, s. 1545, w. 1467. HRMS (ESI-) m/*z* 339.12000 [$(M-H)^-$; calculated mass for $C_{15}H_{19}N_2O_7^-$: 339.11977 amu].

Characterization images for **2a**

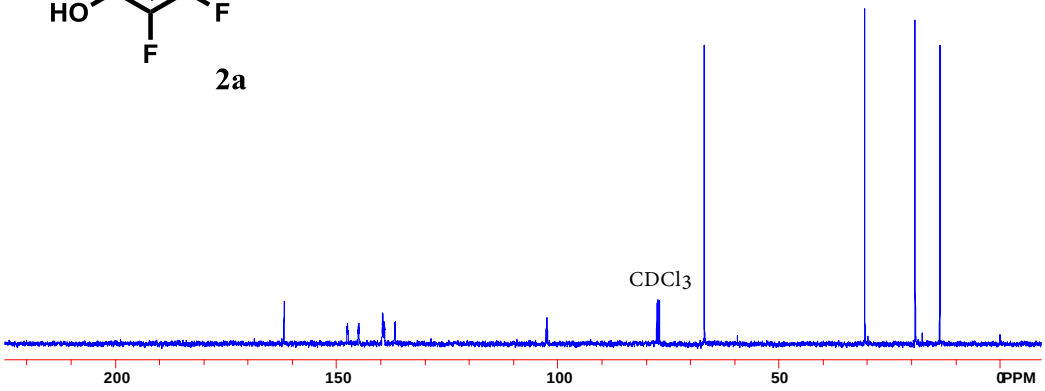




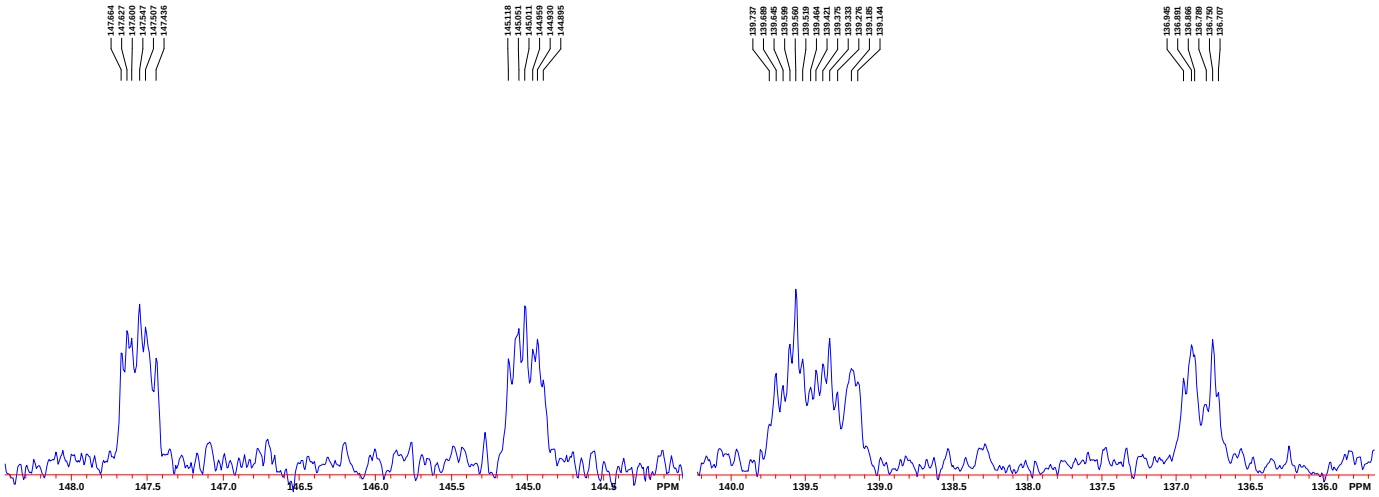
^{13}C NMR ($CDCl_3$, 100 MHz)



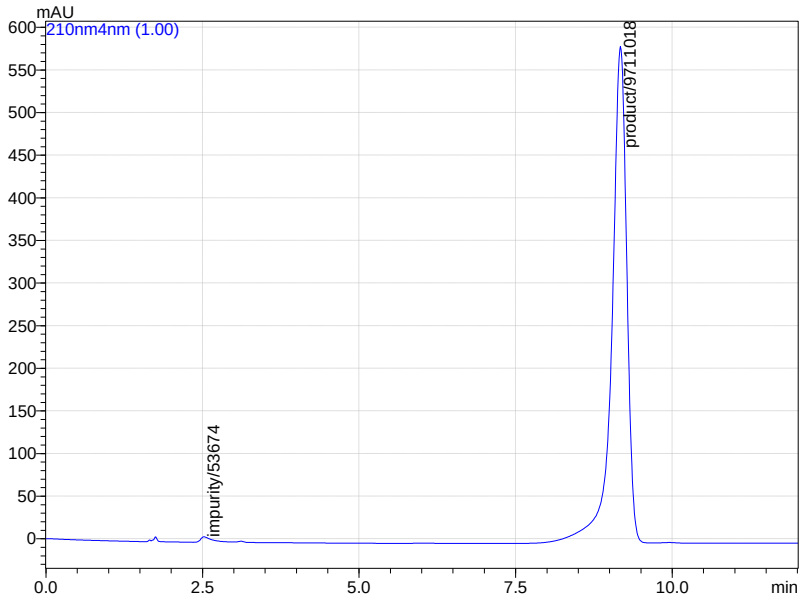
2a



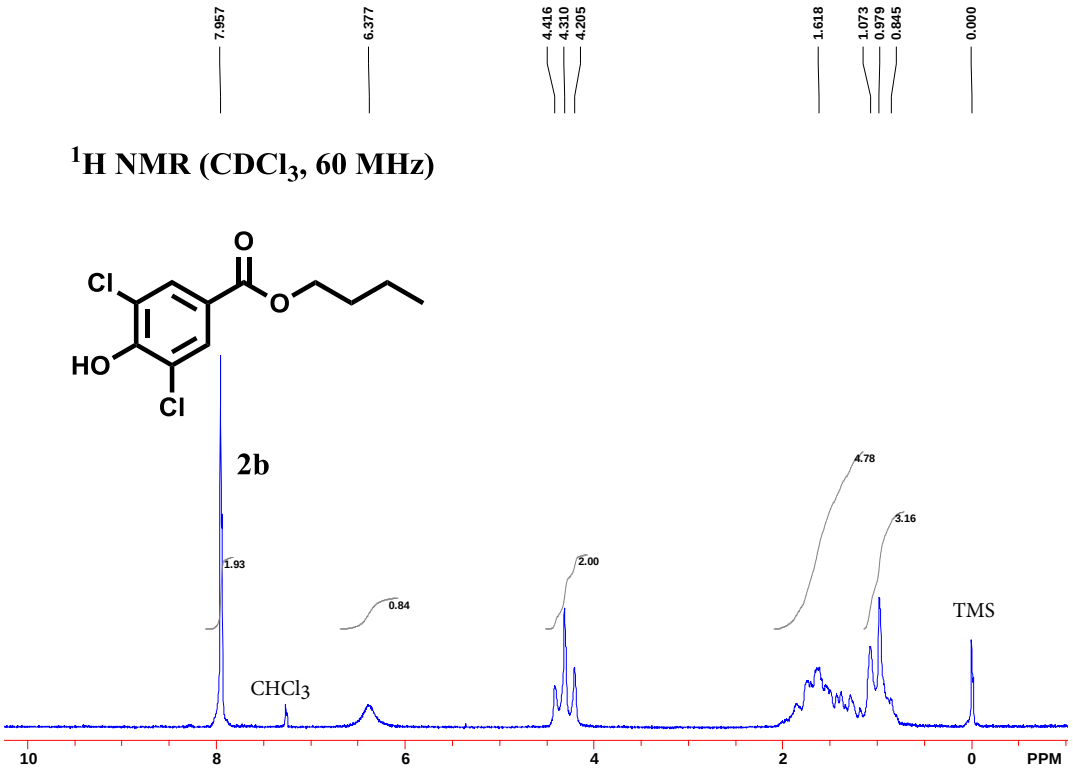
Enlarged C-F coupling from carbon of **2a**

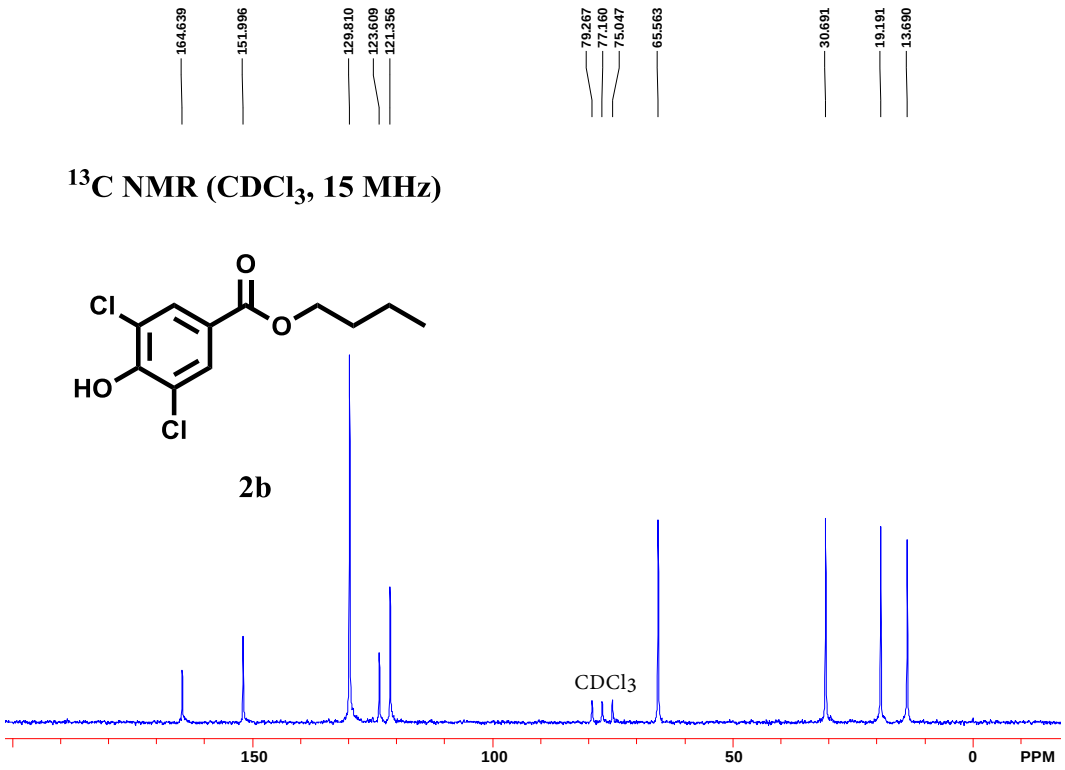


HPLC Analysis of **2a** (eluent 70% MeOH w/ 30% 50 nM phosphoric acid)

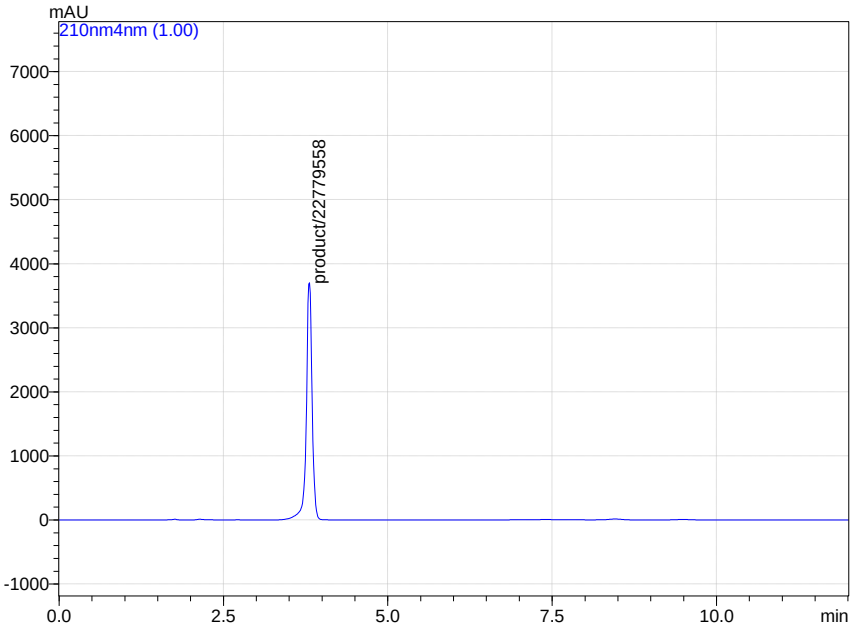


Characterization images for **2b**

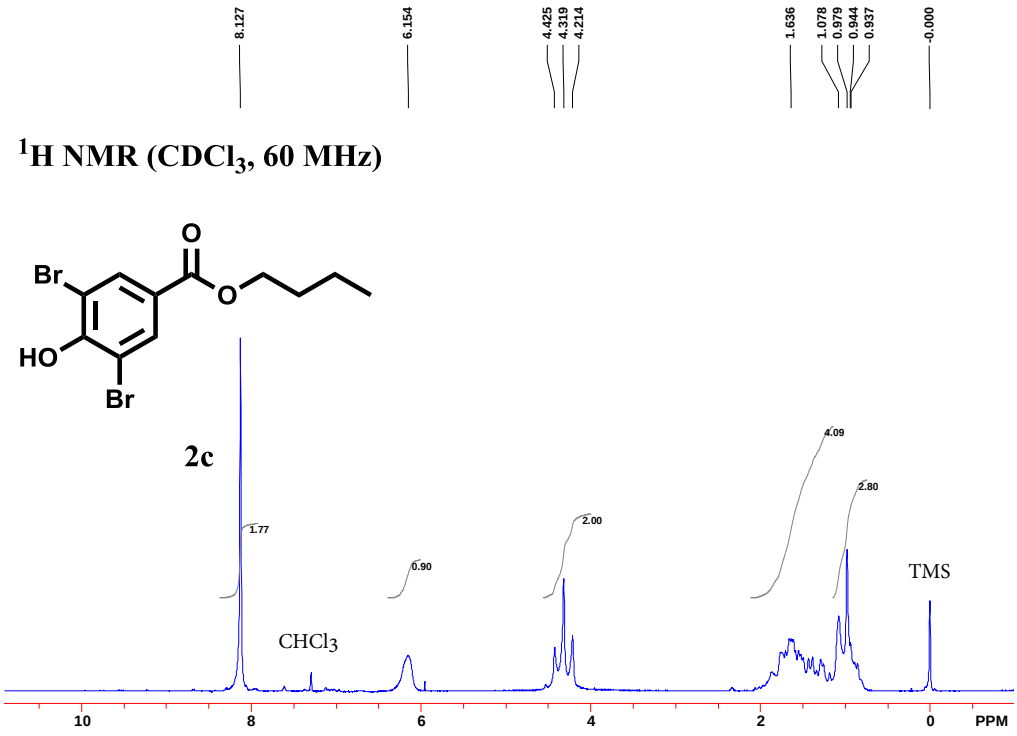


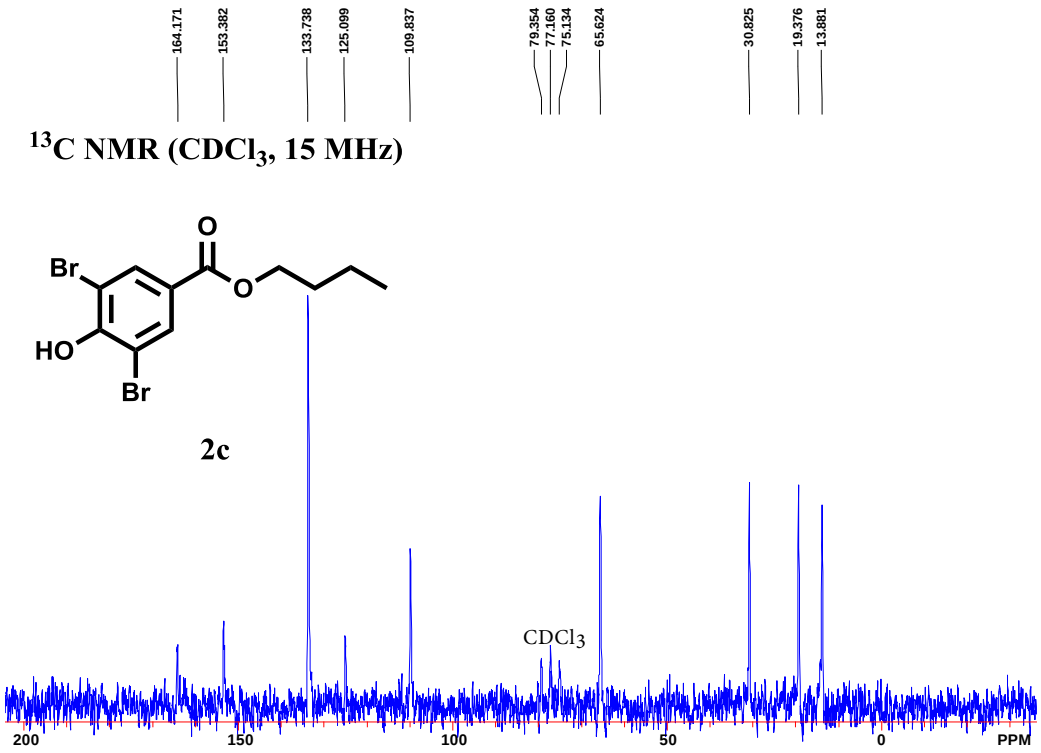


HPLC Analysis of **2b**(eluent 80% MeOH w/ 20% 50 nM phosphoric acid)

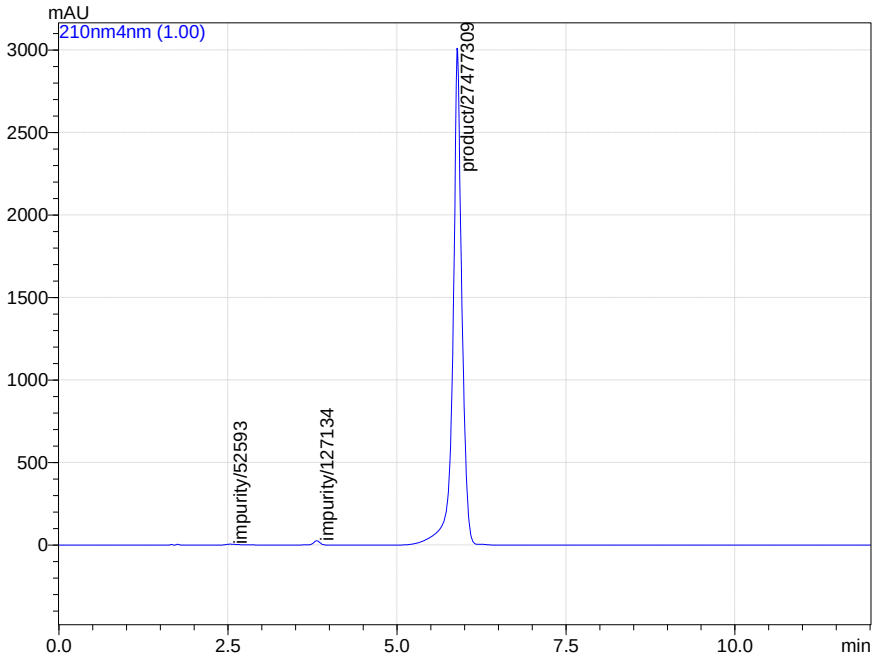


Characterization images for **2c**

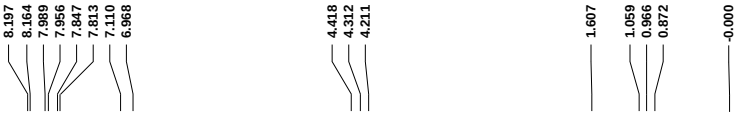




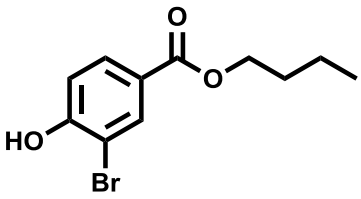
HPLC Analysis of **2c** (eluent 80% MeOH w/ 20% 50 nM phosphoric acid)



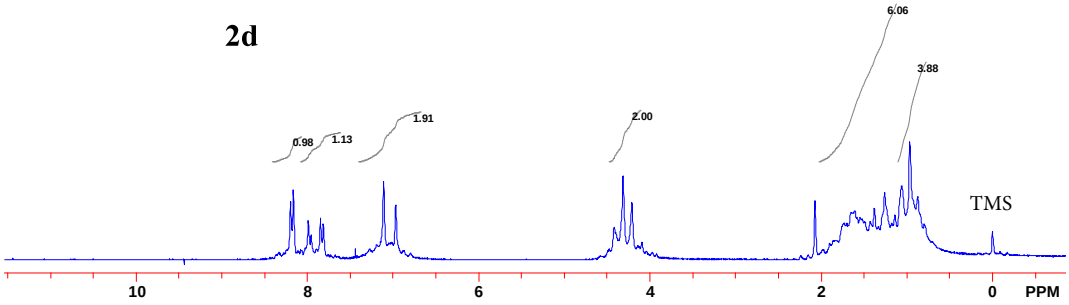
Characterization images for **2d**



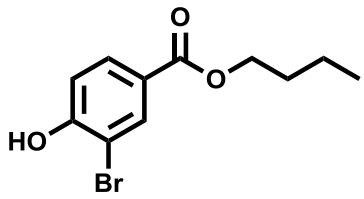
^{1}H NMR ($CDCl_3$, 60 MHz)



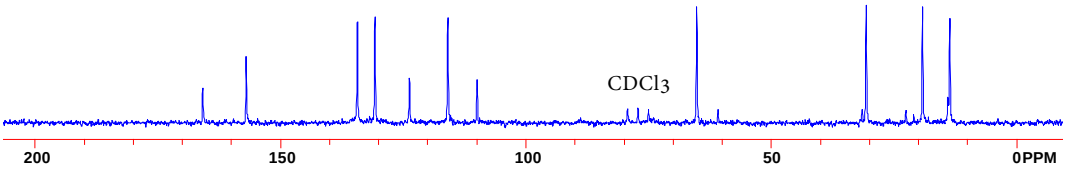
2d



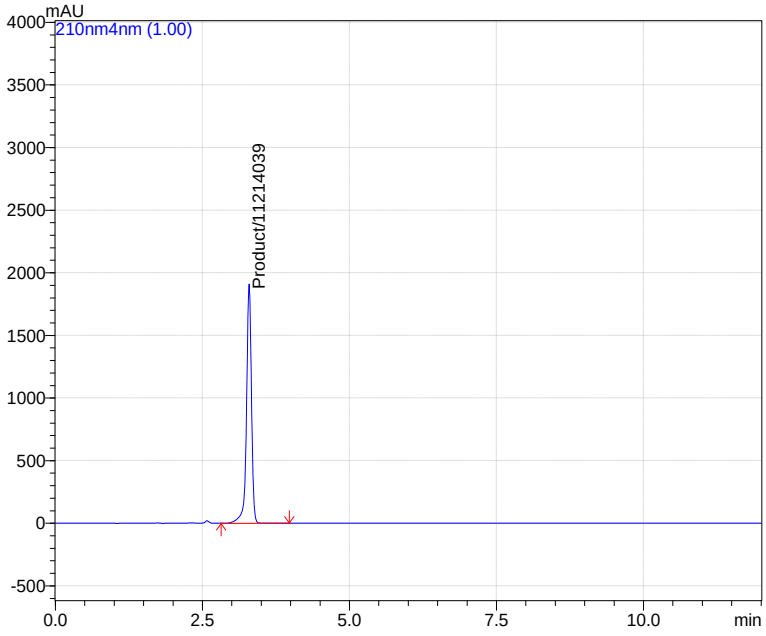
^{13}C NMR ($CDCl_3$, 15 MHz)



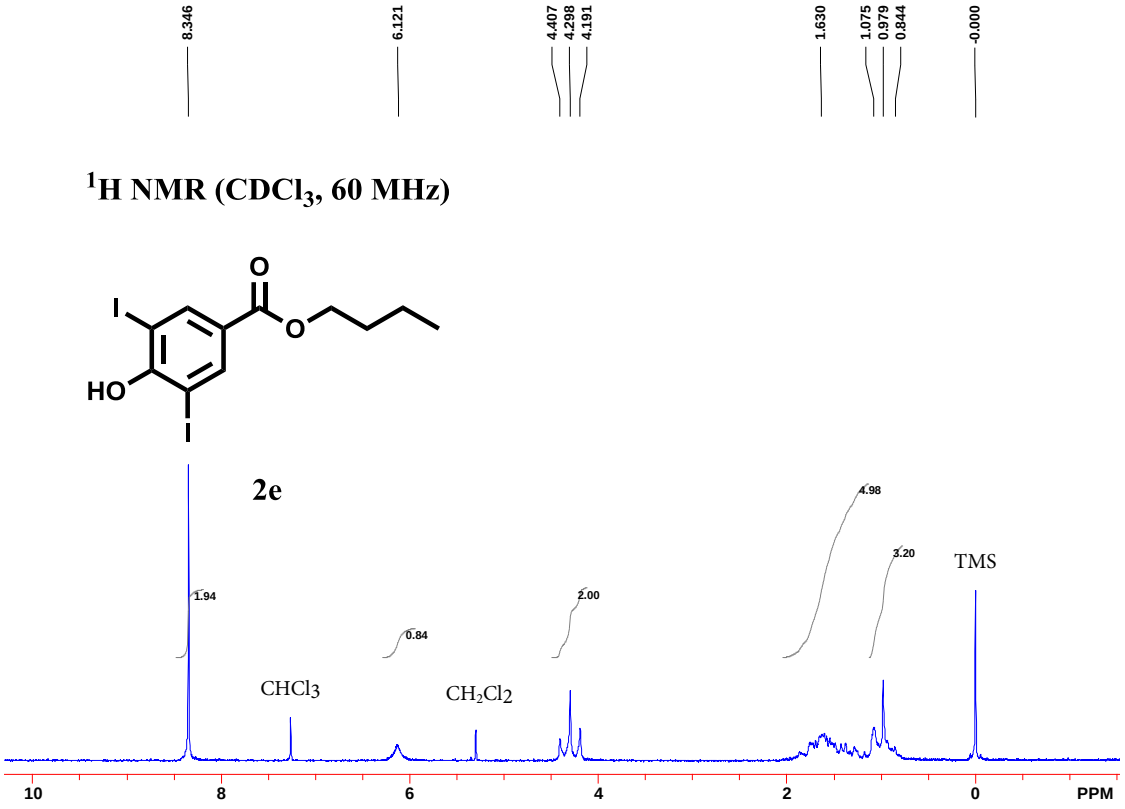
2d

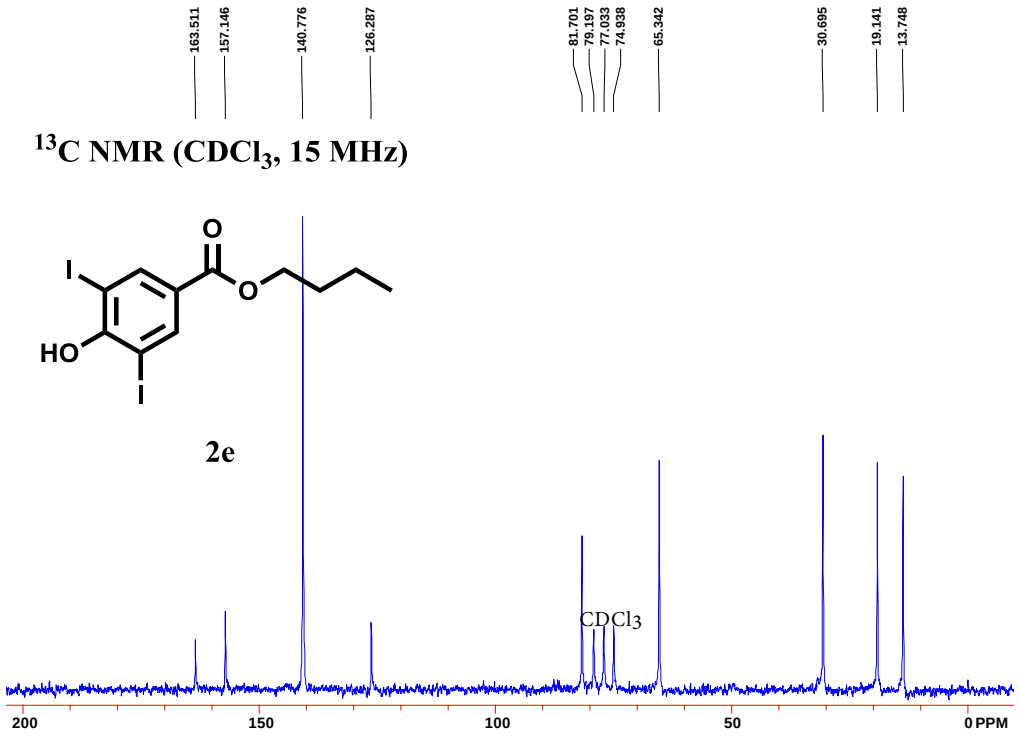


HPLC Analysis of **2d** (eluent 90% MeOH w/ 10% DI water)

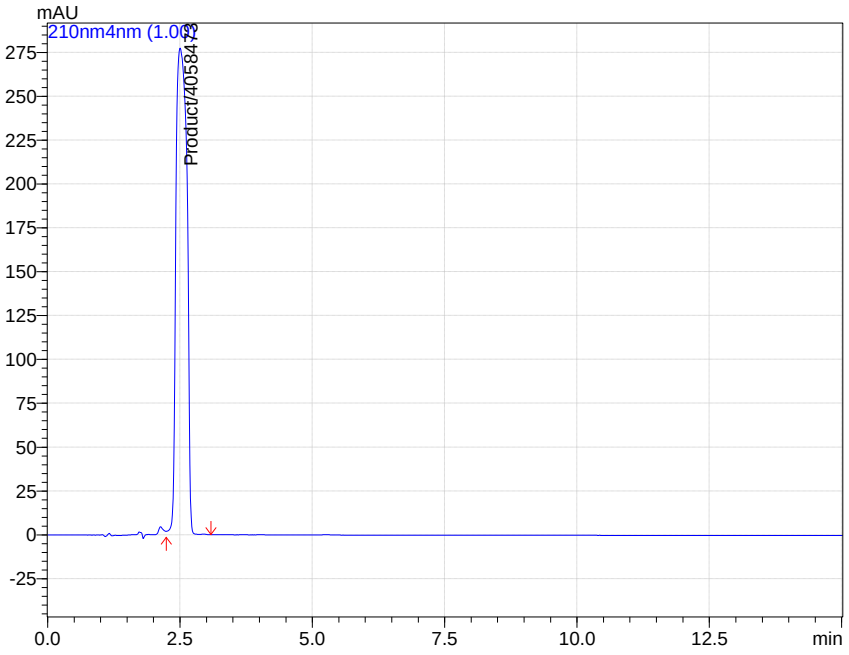


Characterization images for **2e**

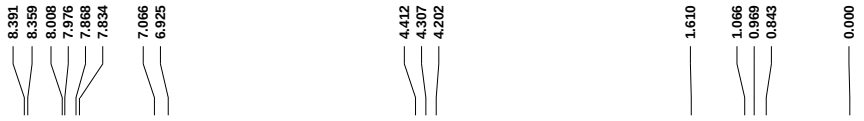




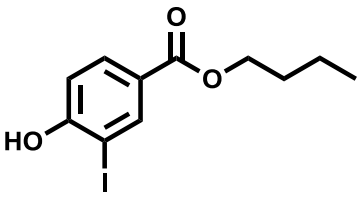
HPLC Analysis of **2e** (eluent 90% MeOH w/ 10% DI water)



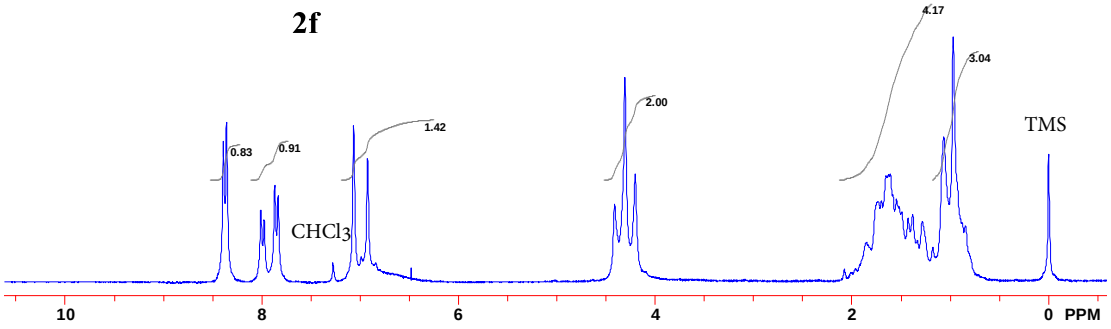
Characterization images for **2f**

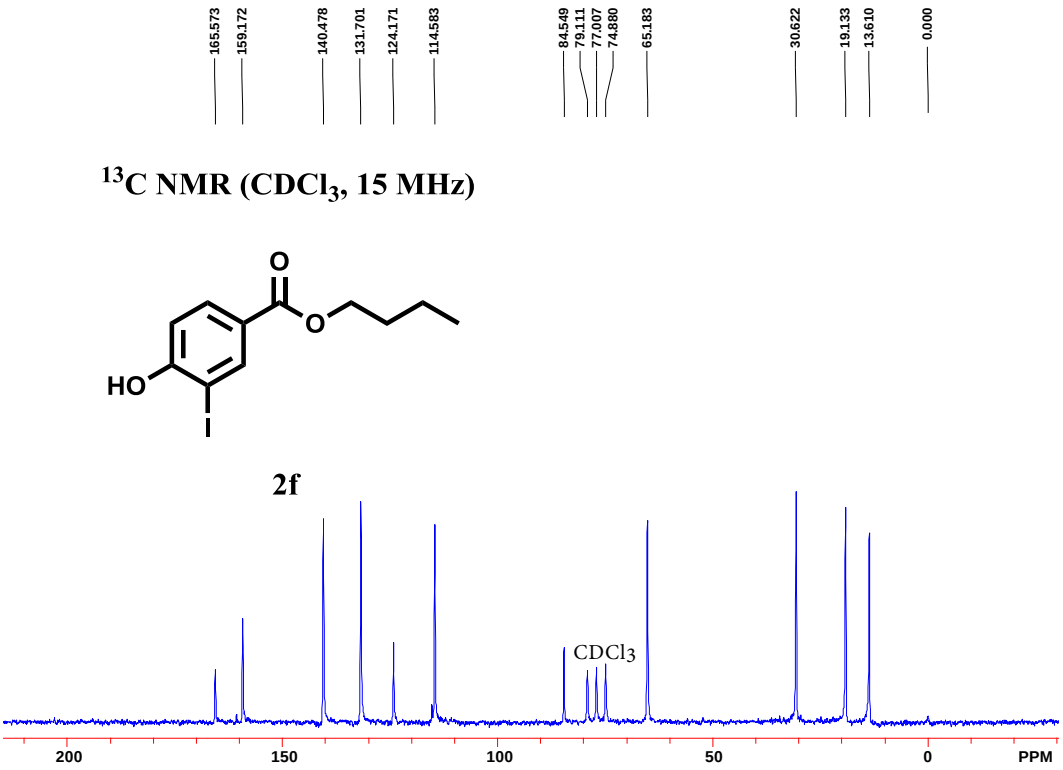


^{1}H NMR ($CDCl_3$, 60 MHz)

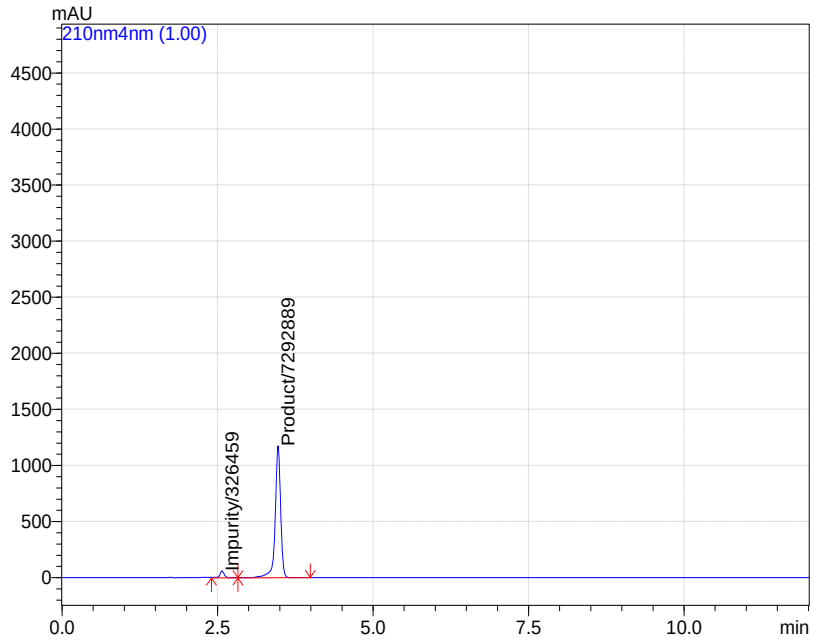


2f

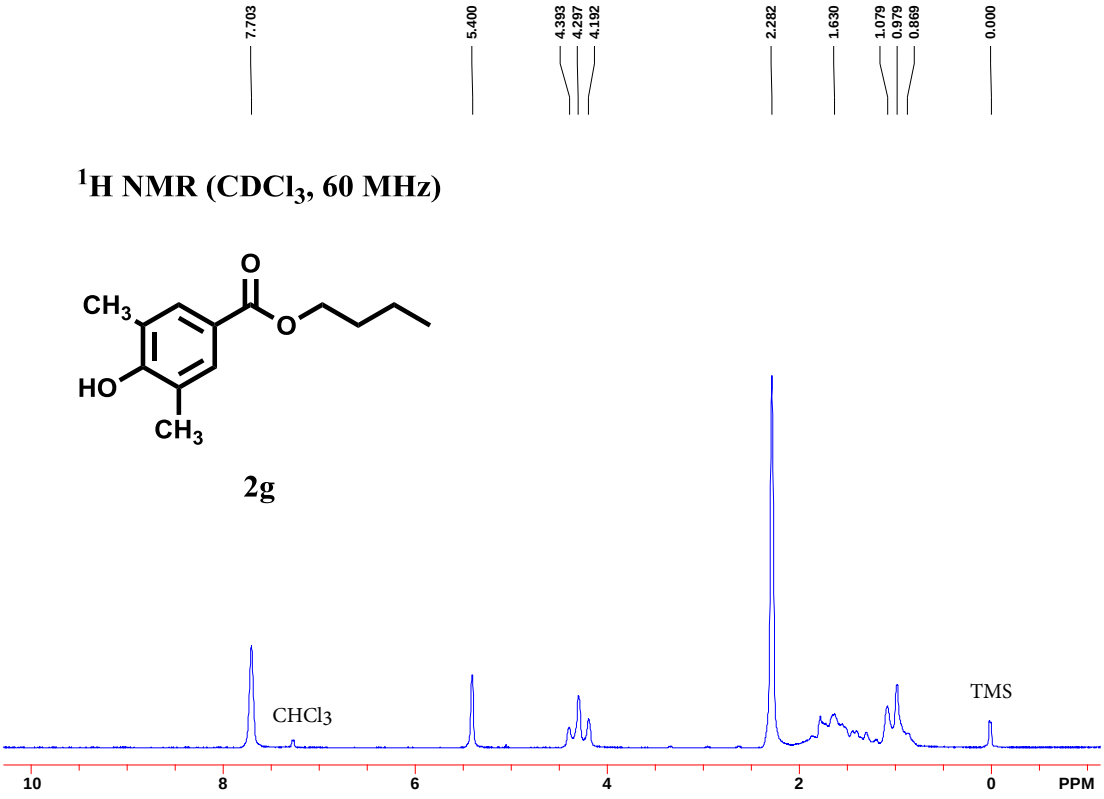


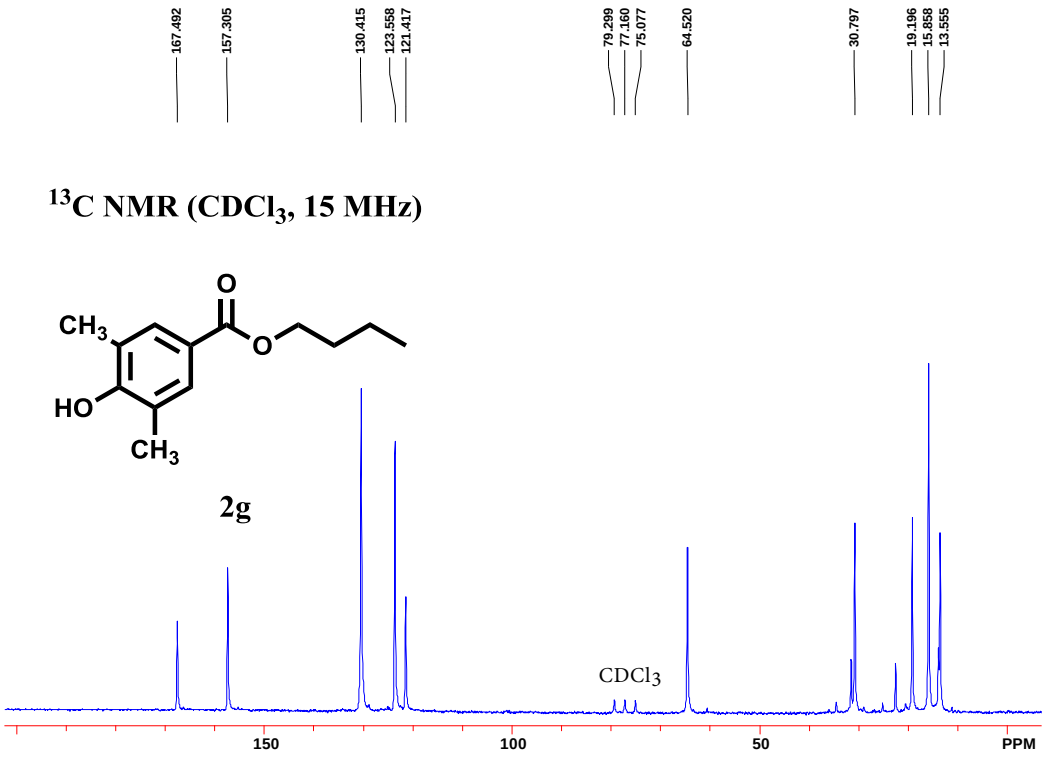


HPLC Analysis of **2f** (eluent 90% MeOH w/ 10% DI water)

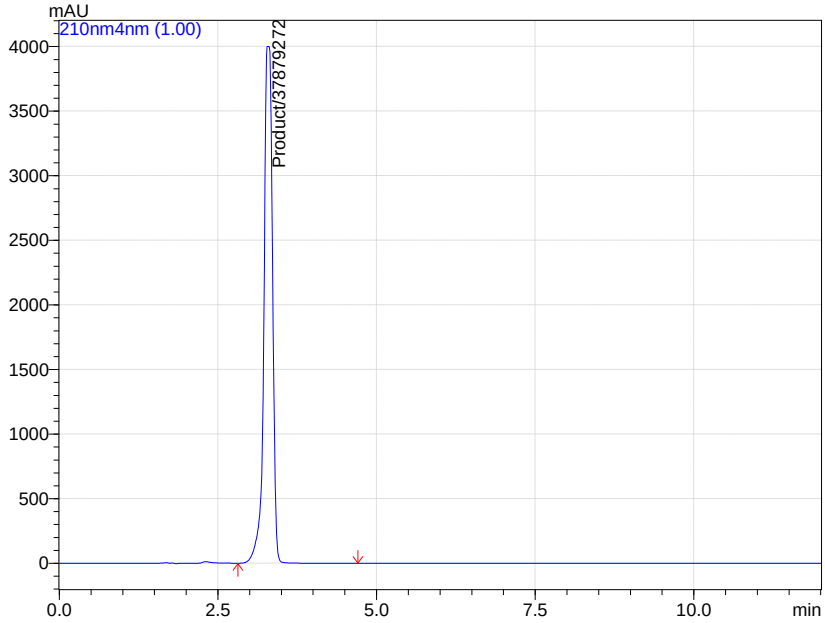


Characterization images for **2g**

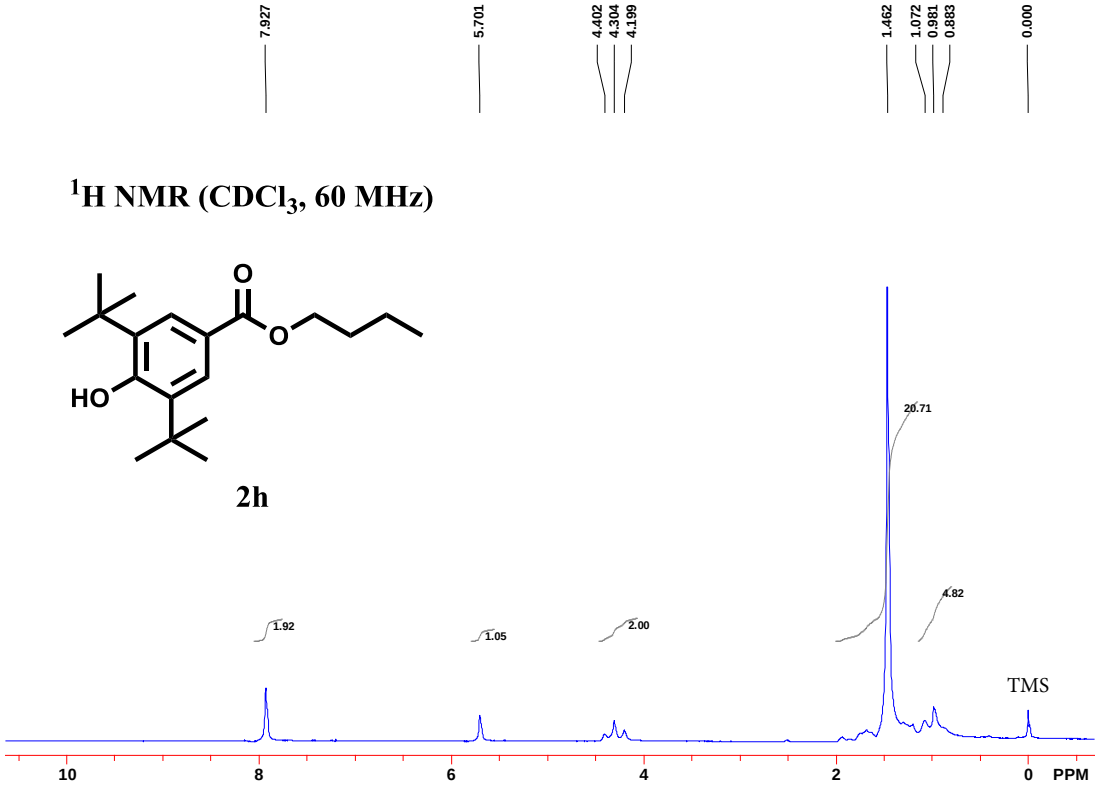


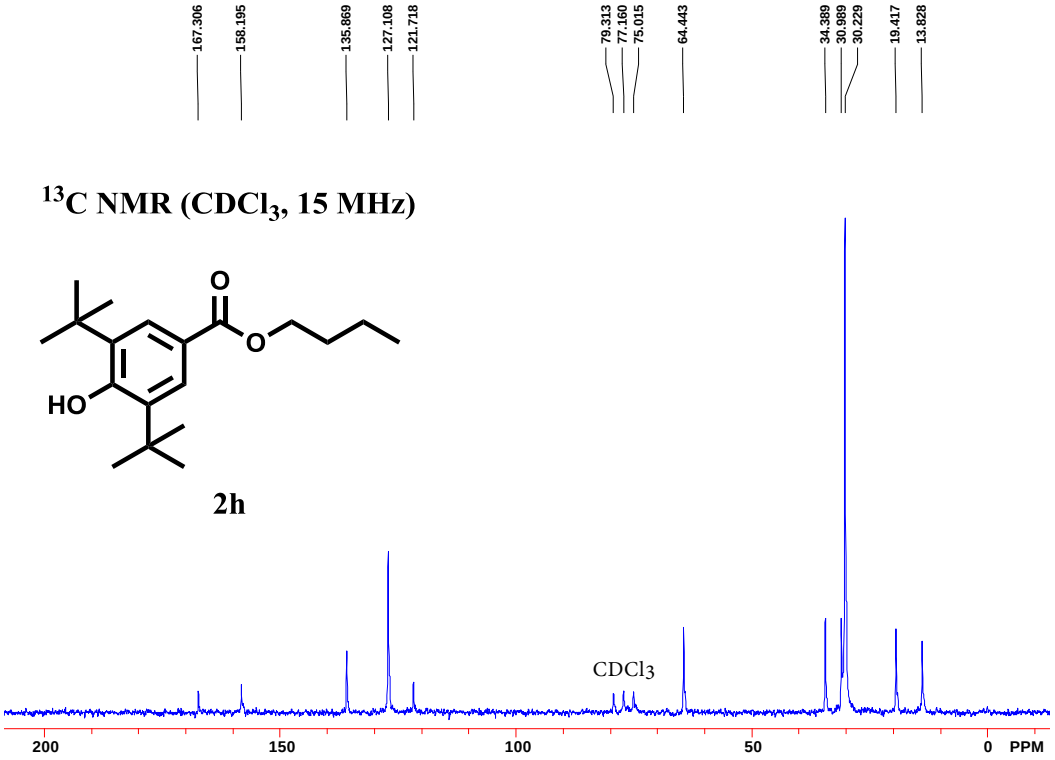


HPLC Analysis of **2g** (eluent 90% MeOH w/ 10% DI water)

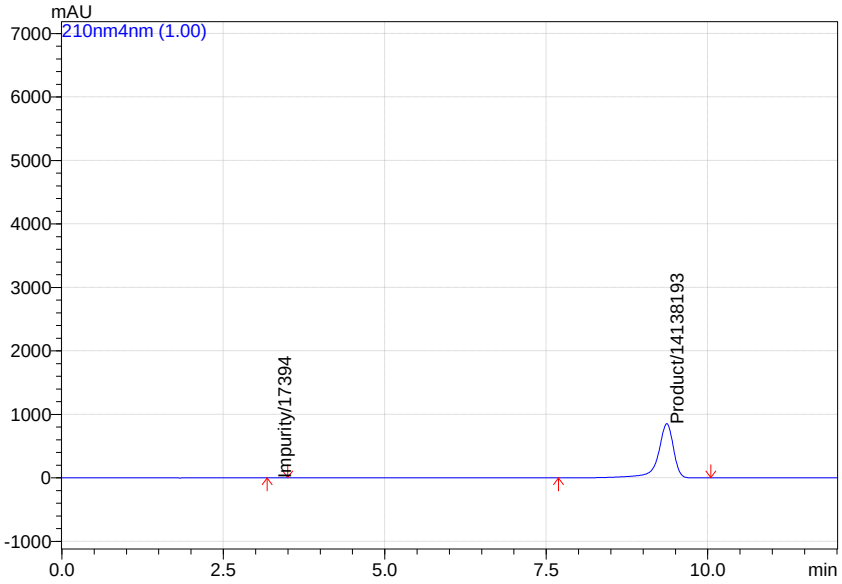


Characterization images for **2h**

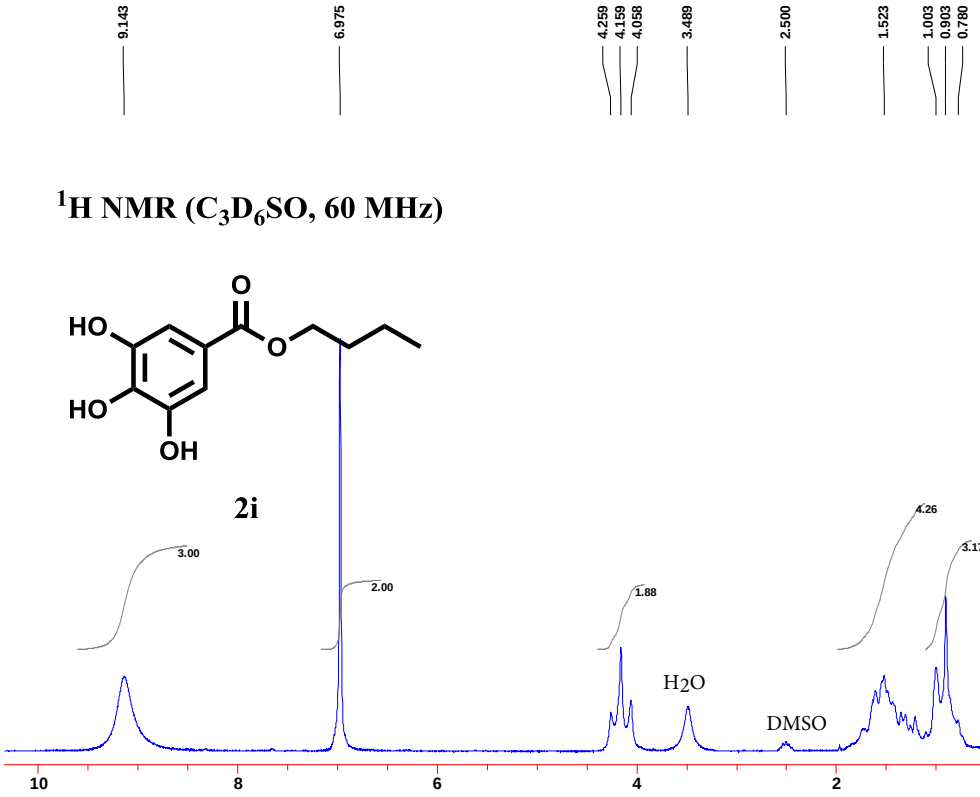




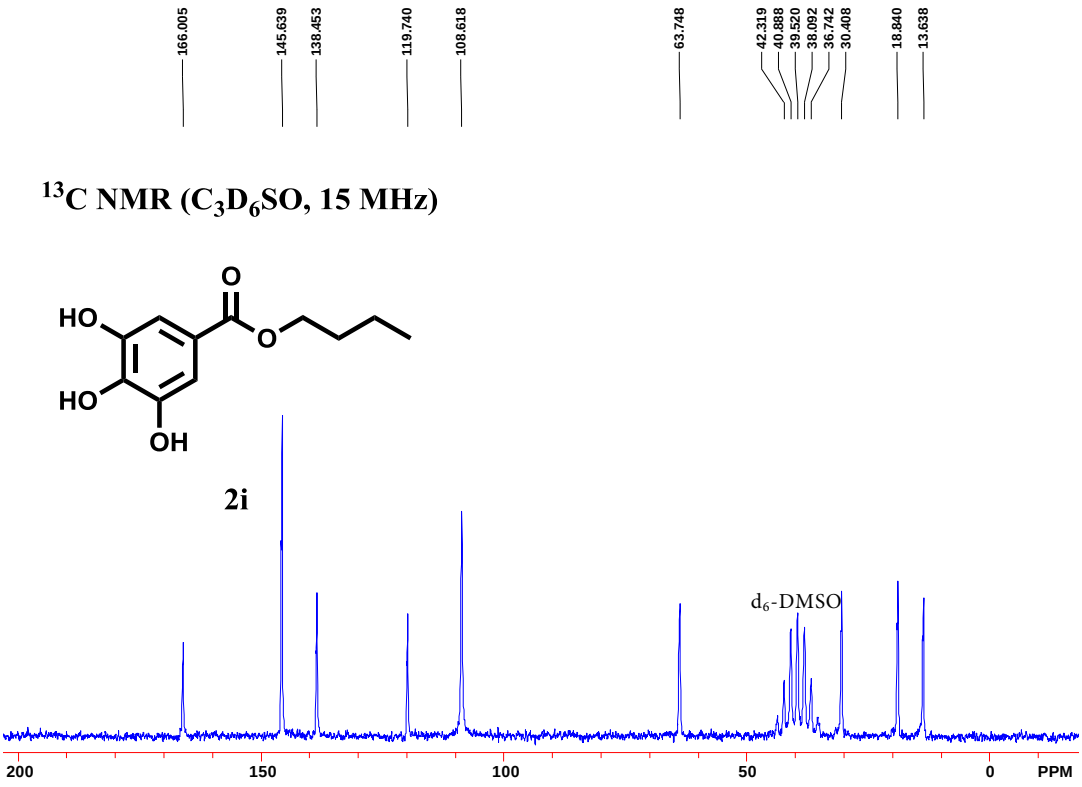
HPLC Analysis of **2h** (eluent 90% MeOH w/ 10% DI water)



Characterization images for **2i**

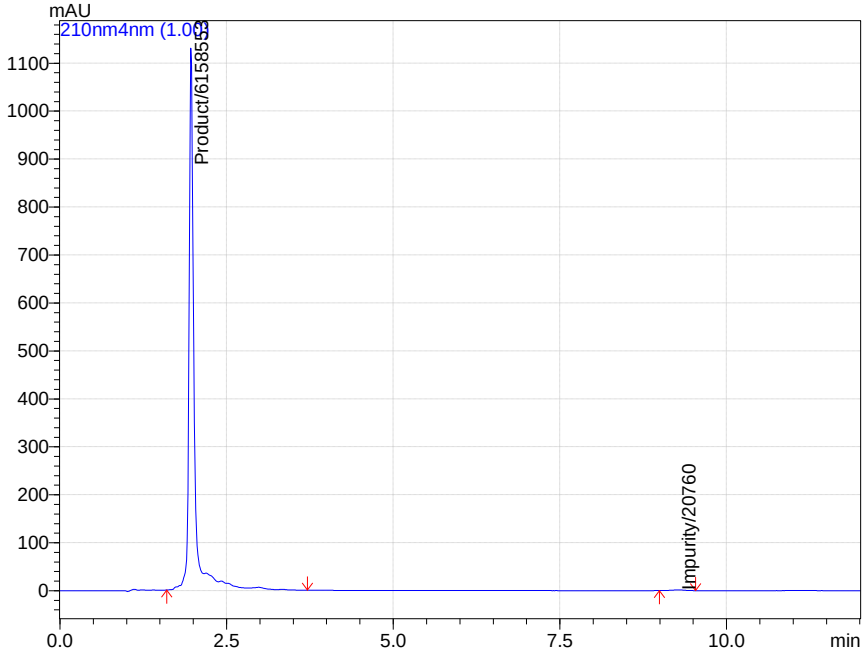


^{1}H NMR (C_3D_6SO, 60 MHz)

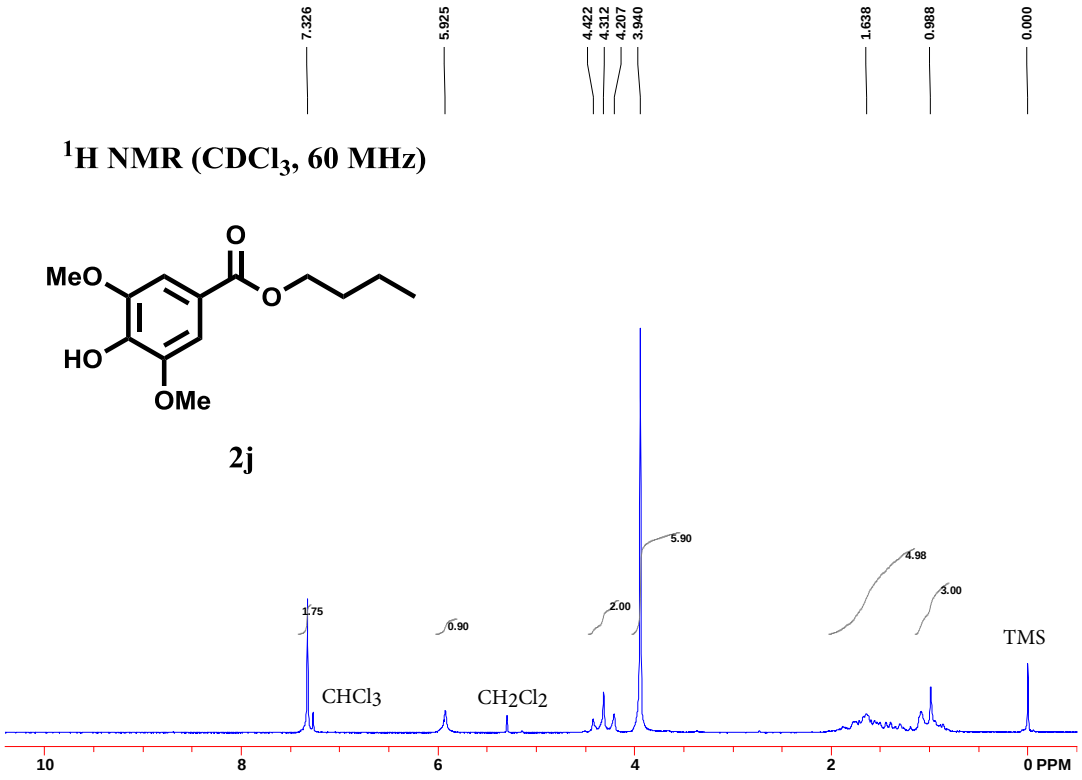


^{13}C NMR (C_3D_6SO, 15 MHz)

HPLC Analysis of **2i** (eluent 90% MeOH w/ 10% DI water)

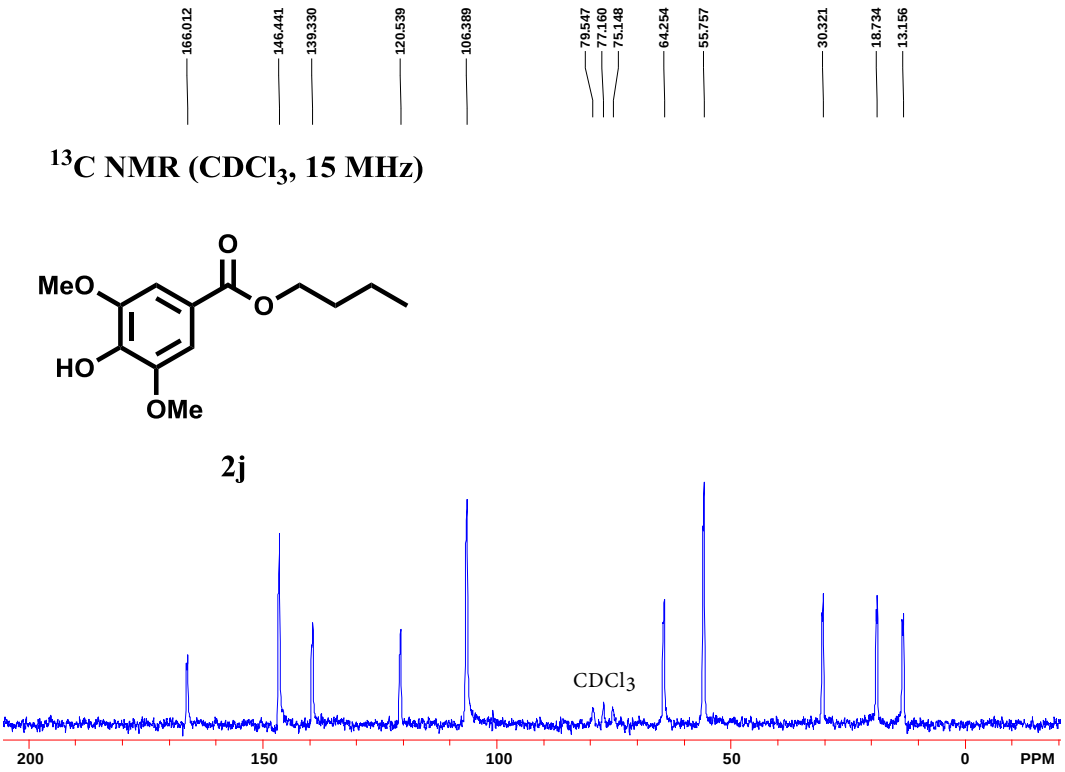


Characterization images for **2j**



^{1}H NMR ($CDCl_3$, 60 MHz)

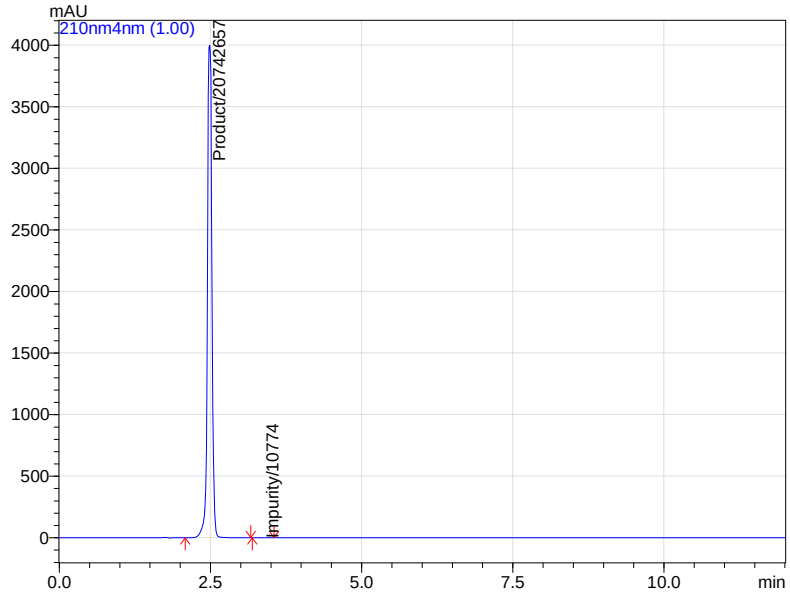
2j



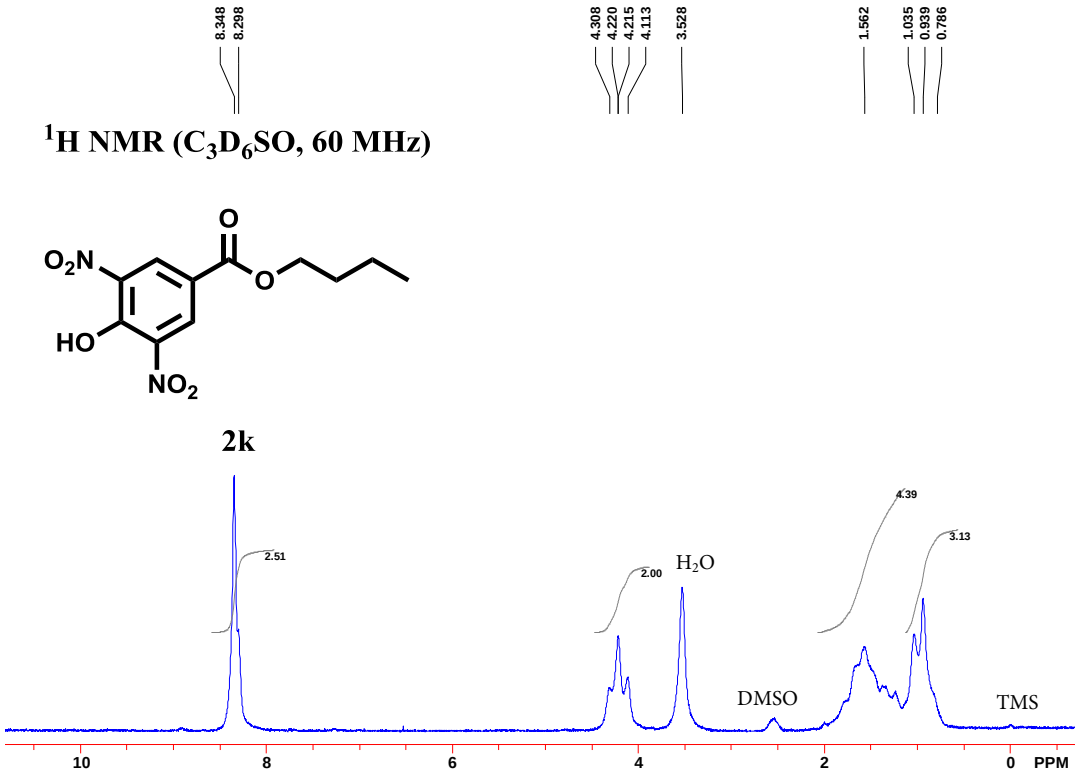
^{13}C NMR ($CDCl_3$, 15 MHz)

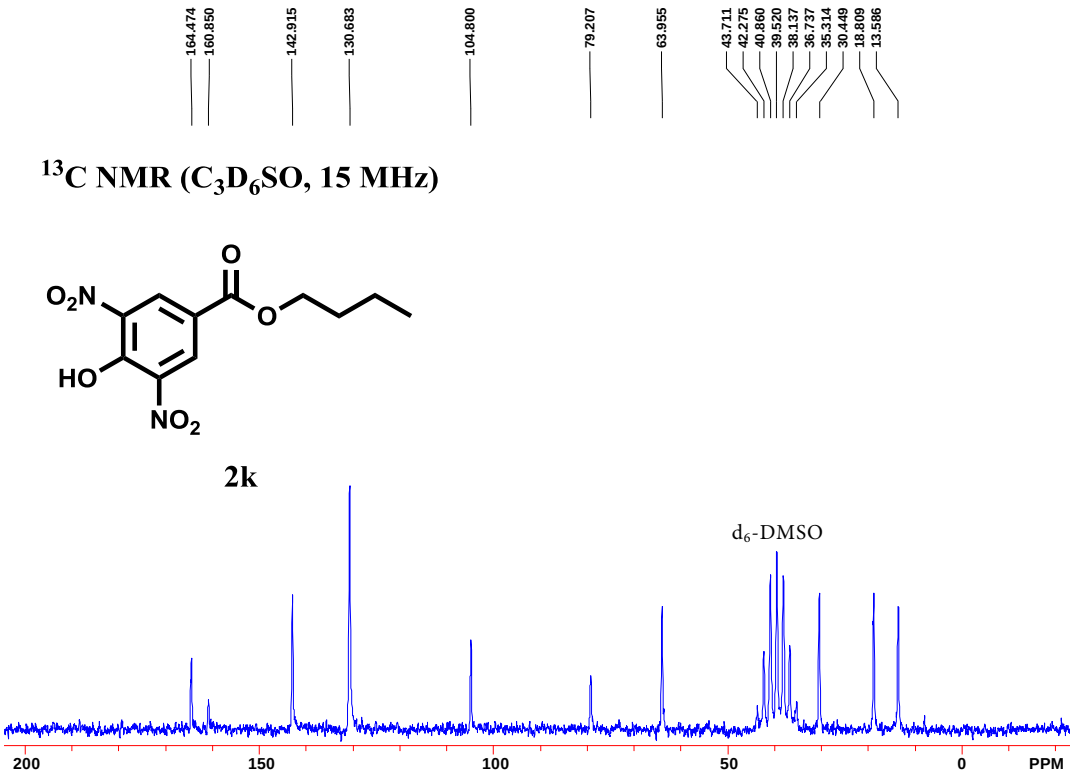
2j

HPLC Analysis of **2j** (eluent 90% MeOH w/ 10% DI water)

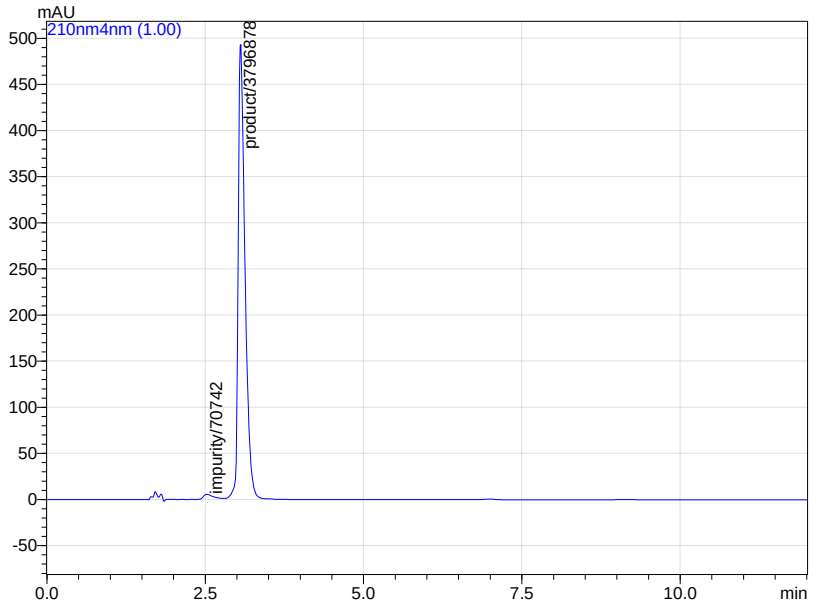


Characterization images for **2k**

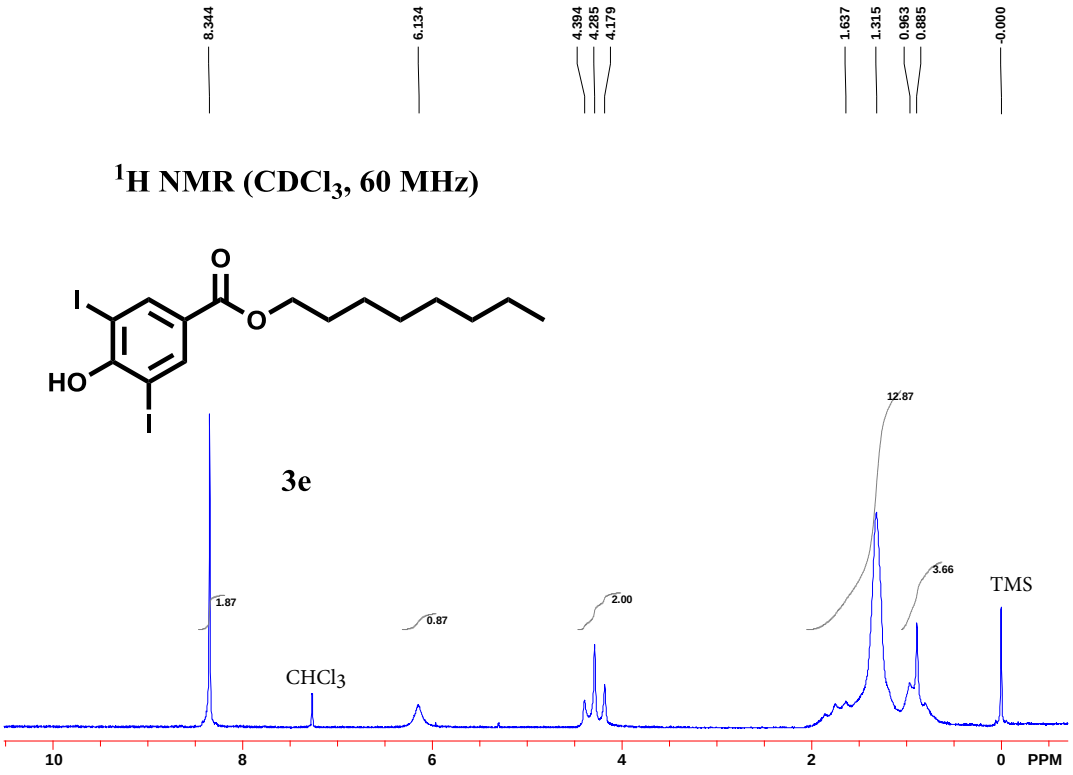


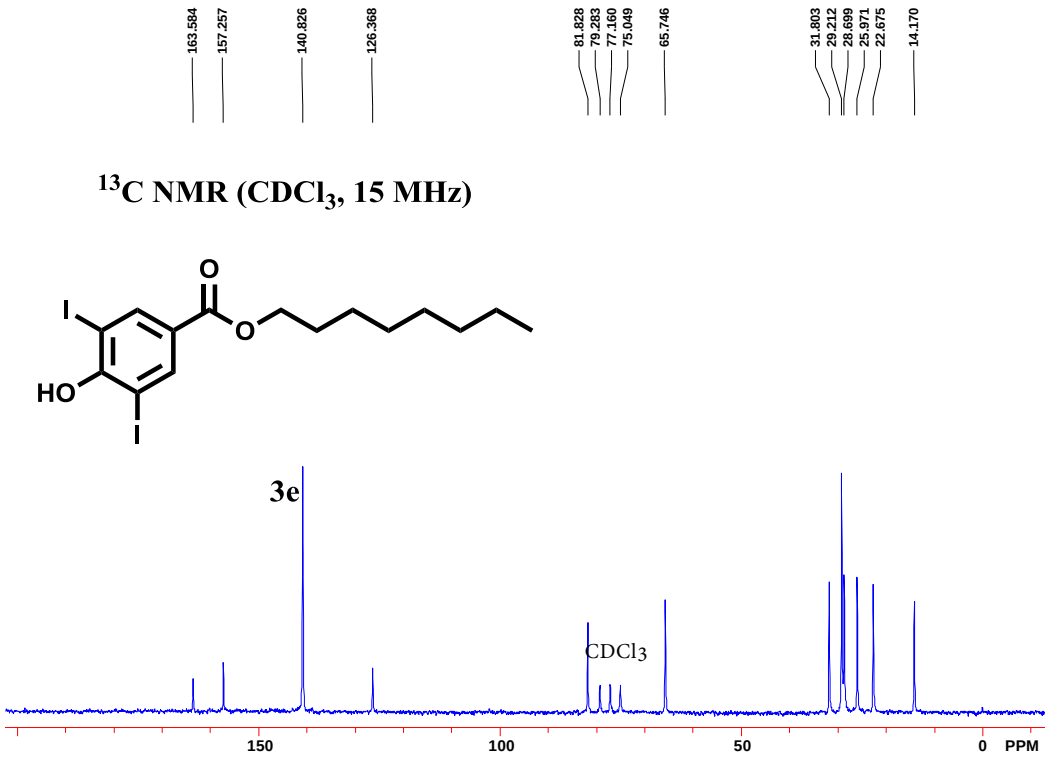


HPLC Analysis of **2k** (eluent 70% MeOH w/ 30% 50 nM phosphoric acid)

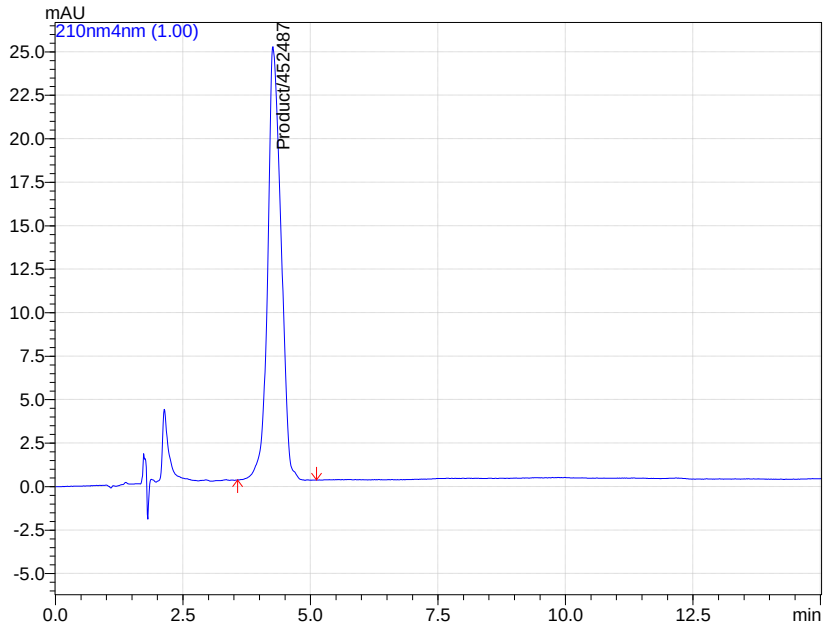


Characterization images for **3e**

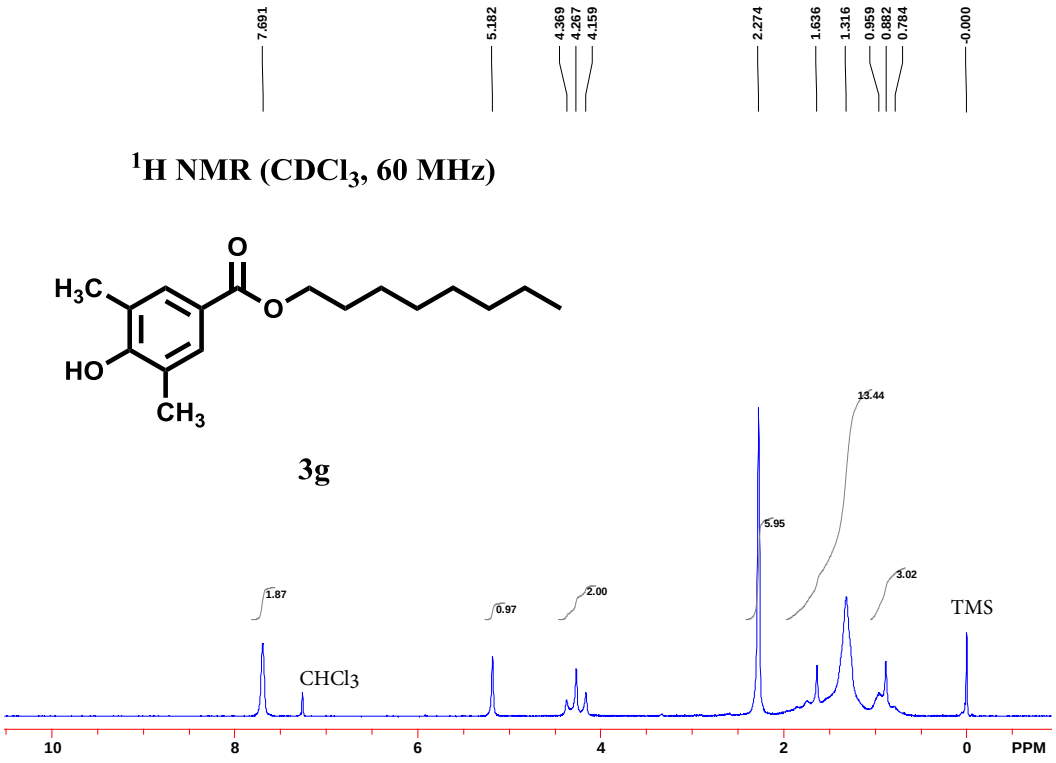


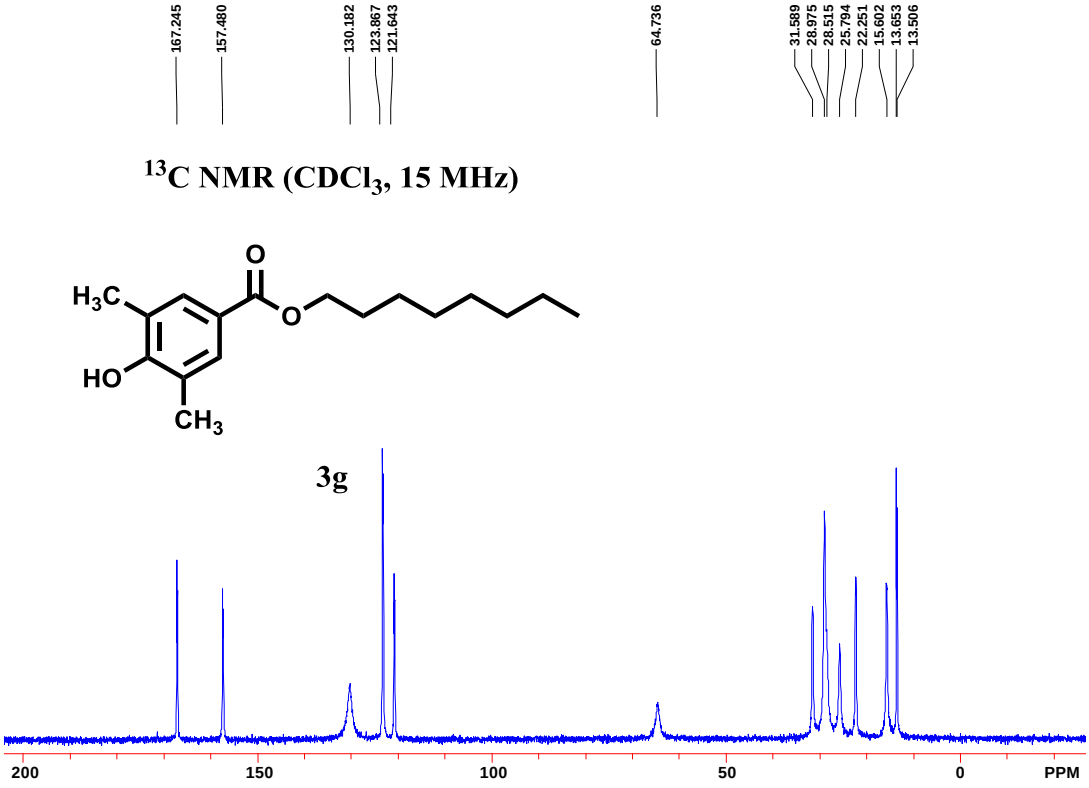


HPLC Analysis of **3e** (eluent 90% MeOH w/ 10% DI water)

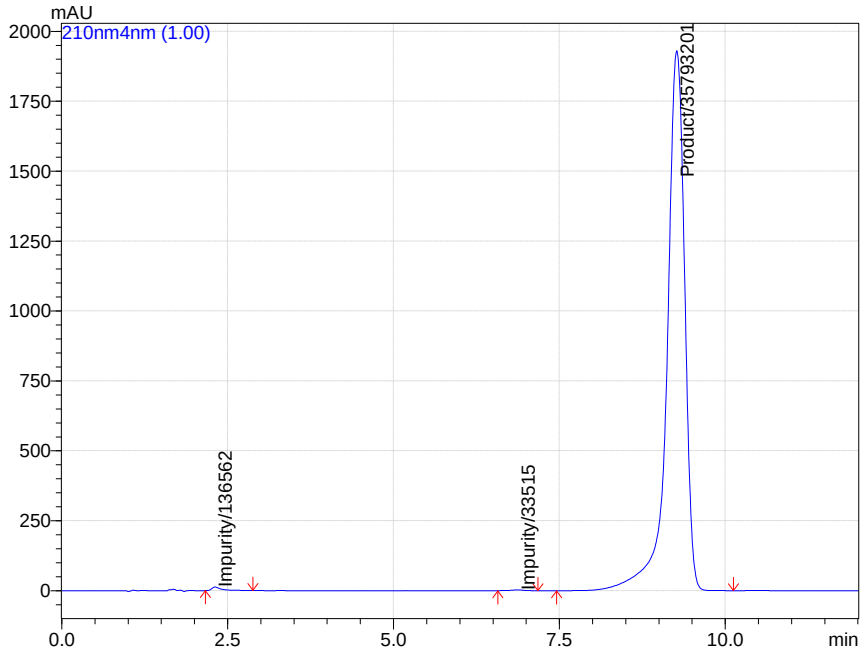


Characterization images for **3g**

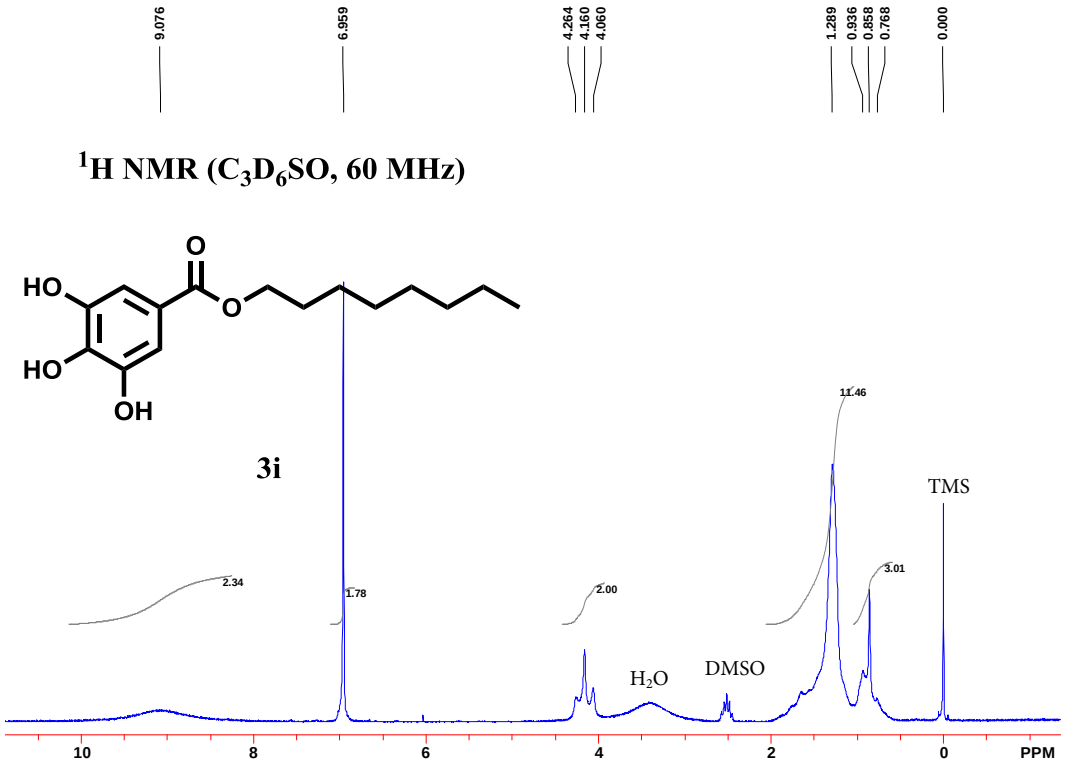


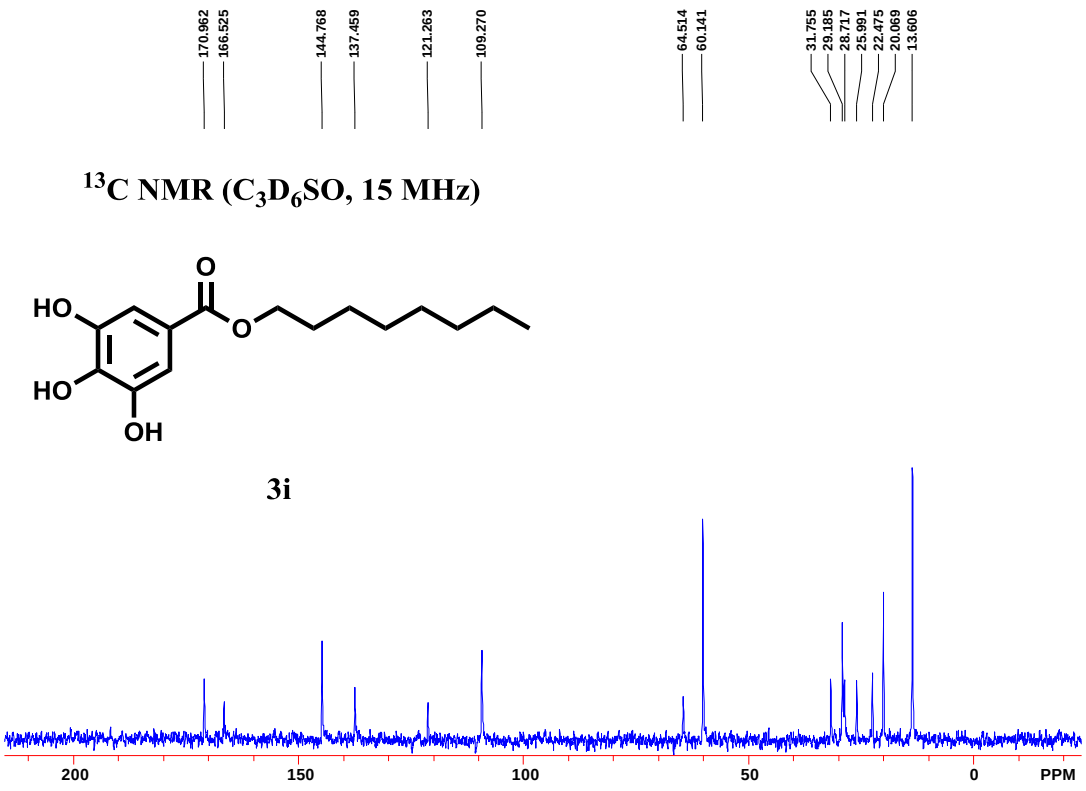


HPLC Analysis of **3g** (eluent 90% MeOH w/ 10% DI water)



Characterization images for **3i**

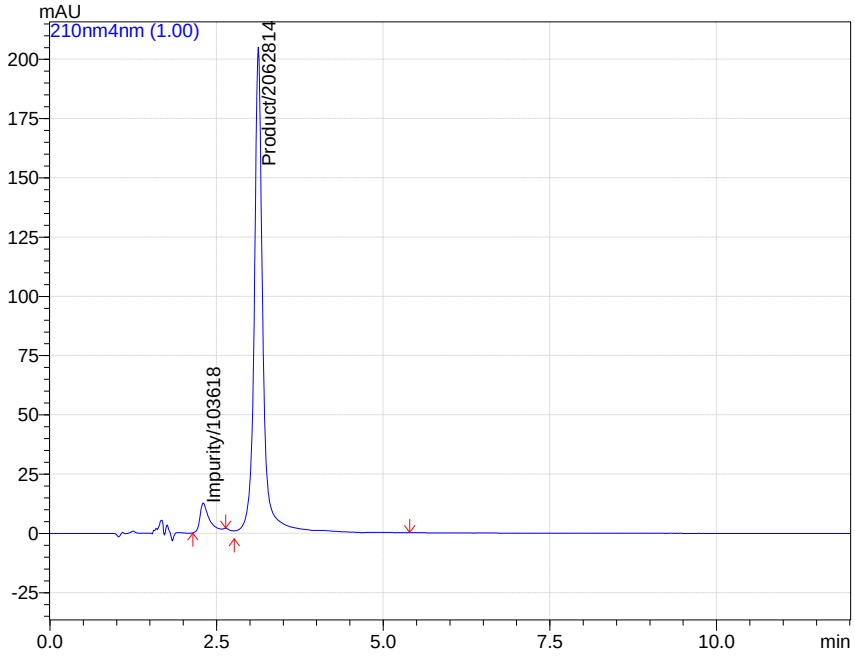




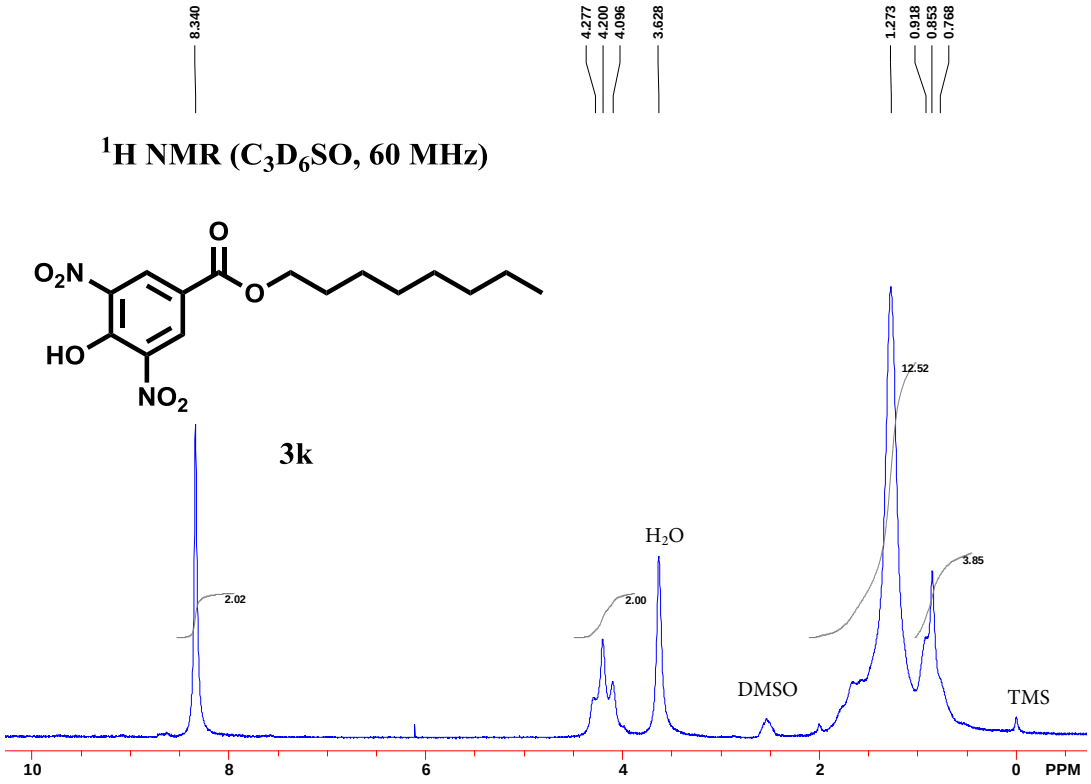
^{13}C NMR (C_3D_6SO, 15 MHz)

3i

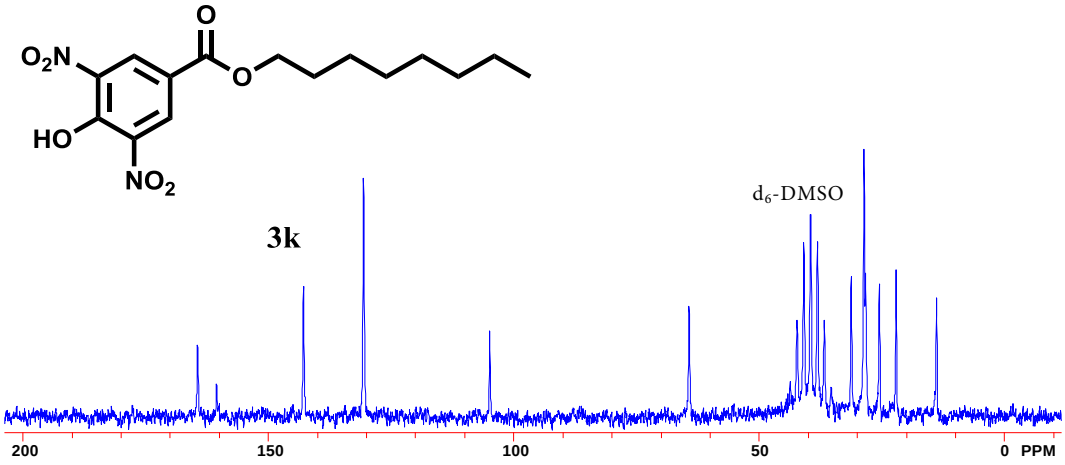
HPLC Analysis of **3i** (eluent 90% MeOH w/ 10% DI water)



Characterization images for **3k**



^{13}C NMR (C_3D_6SO, 15 MHz)



HPLC Analysis of **3k** (eluent 90% MeOH w/ 10% DI water)