Supplementary Materials for

# Direct Arene Trifluoromethylation Enabled by Promiscuous Activity of Fungal Laccase

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## I. Materials and Methods

### 1. General Methods

Unless otherwise noted, all chemicals, salts, and solvents were reagent grade and used as received from commercial suppliers without further purification. In particular, laccase from *Trametes versicolor* was purchased from Sigma Aldrich with a specific activity of 0.89 U/mg. The pH of buffers used was adjusted using Mettler Toledo SevenExcellence pH meter with InLab Expert Pro-ISM probe. $^{1}$H NMR, $^{13}$C NMR, and 2D NMR (nuclear magnetic resonance) spectra were acquired on Bruker AVANCE III 400 MHz with DCH CryoProbe. $^{19}$F NMR spectra were recorded on Bruker AVANCE NEO 400 MHz with RT iProbe. Chemical shifts are reported in parts per million (ppm) on the delta (δ) scale. Chemical shifts for $^{1}$H NMR were referenced to the residual protium in the NMR solvent ($CDCl_3$: 7.26 ppm; $CD_2Cl_2$: 5.32; $CD_3OD$: 3.31 ppm). Chemical shifts for $^{13}$C NMR were referenced to the carbon resonance of the NMR solvent ($CDCl_3$: 77.16 ppm; $CD_3OD$: 49.0 ppm). NMR spectra were processed using MestReNova 12.0.2. Data is presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, hept = heptet, m = multiplet), integration and coupling constants (*J*) in Hertz (Hz). Analytical thin layer chromatography (TLC) was performed using Merck 60 F254, 0.25 µm silica gel plates and spots were visualized by UV and/or potassium permanganate, or ceric ammonium molybdate stain. Flash column chromatography was carried out using Merck 60 F254, 0.040-0.063 µm silica gel. Preparative TLC chromatography was carried out using Merck 60 F254, 0.25 µm silica gel plates. High resolution electrospray ionization (HRMS-ESI) mass spectra were recorded using Agilent 6545B Time-of-Flight (TOF) mass spectrometer. GC-MS (gas chromatography–mass spectrometry) analyses were obtained on an Agilent 7890A GC equipped with Agilent 5975C Inert XL MSD with Triple-Axis Detector and HP-5ms Ultra Inert column (30 m x 0.25 mm ID x 0.25 µm). GC-FID (gas chromatography-flame ionization detector) analyses were obtained on an Agilent 8890 GC equipped with an FID detector and CycloSil-B column (30 m x 0.25 mm ID x 0.25 µm). ICP-MS (inductively coupled plasma–mass spectrometry) analyses were performed with PerkinElmer's NexION 2000C ICP Mass Spectrometer. Names of structures were generated using ChemBioDraw Ultra 21.0.0.28.

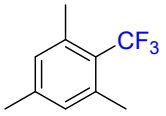
### 2. General procedure for laccase-catalyzed trifluoromethylation in analytical scale

The laccase (4 U) from commercial supplier was dissolved in sodium phosphate buffer (400 µL, 100 mM, pH 7) prior to addition of a stock solution of substrate in DMSO (100 µL, 0.2 M). A stock solution of $NaSO_2CF_3$ (100 µL, 1 M in $H_2O$, 5 equiv.) was then added, followed by aqueous *t*BuOOH solution (29 µL, 70 wt.% in $H_2O$, 10 equiv.). The reactions were shaken on an orbital shaker at room temperature, 600 rpm. After 15 h, a saturated solution of sodium thiosulfate or sodium bicarbonate in water was added (100 µL) and the mixture was extracted with a solution of $CDCl_3$ (1 mL, containing 0.02 mmol 2,2,2-trifluoroacetophenone as internal standard). The organic fraction was washed with water and brine, and dried over $Na_2SO_4$. The solution was filtered and analyzed by GC-MS, $^{19}$F NMR and/or $^{1}$H NMR.

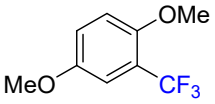
**3. General procedure for laccase-catalyzed trifluoromethylation in preparative scale (for characterization of new compounds)**

Laccase from *Trametes versicolor* (200 U) was dissolved in 10 mL reaction buffer (100 mM NaPi at pH 7 or 250 mM NaOAc at pH 5.4). A solution of arene **1** (2.5 mL, 0.4 M in DMSO, 1 mmol) was added to the catalyst solution, followed by addition of stock solutions of $NaSO_2CF_3$ (2.5 equiv., 2 M in respective buffer) and *t*BuOOH (5 equiv., 70 wt.% in $H_2O$). The mixture was shaken on an orbital shaker at 600 rpm at room temperature. After 4 h, a second batch of $NaSO_2CF_3$ (2.5 equiv., 2 M in respective buffer) and *t*BuOOH (5 equiv., 70 wt.% in $H_2O$) was added and the reaction shaken overnight for another 12 h. The reaction mixture was extracted thrice with diethyl ether (except for **1m** and **1n** - extracted with ethyl acetate). The combined organic fraction was washed twice with brine, dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure (25 °C, 400 mbar). The crude residue was purified by silica gel flash chromatography and, if required, a second purification by silica gel flash chromatography or preparative TLC chromatography was performed to afford the desired trifluoromethylated product **3**. *(Note: Volatile products should not be purified using preparative TLC chromatography.)*

**Product Characterization**




**3a**

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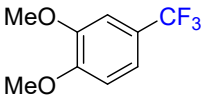
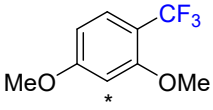
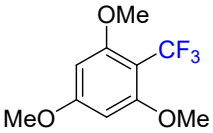


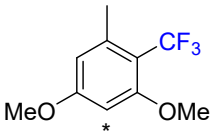

**3c**



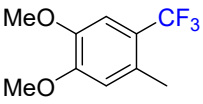

**3d : 3d***



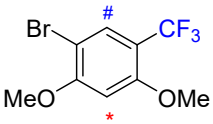

**3e**



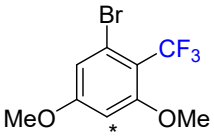

**3f : 3f***




**3g**




**3h : 3h* : 3h#**



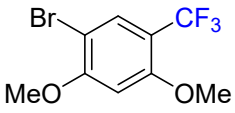

**3i : 3i***

The NMR data for the products above matched those reported in the literature:

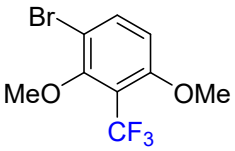
1,3,5-trimethyl-2-(trifluoromethyl)benzene (**3a**),[1] 1,4-dimethoxy-2-(trifluoromethyl)benzene (**3b**),[2] 1,2-dimethoxy-4-(trifluoromethyl)benzene (**3c**),[2] 2,4-dimethoxy-1-(trifluoromethyl)benzene (**3d**),[2] 1,3-dimethoxy-2-(trifluoromethyl)benzene (**3d***),[2] 1,3,5-trimethoxy-2-(trifluoromethyl)benzene (**3e**),[2] 1,5-dimethoxy-3-methyl-2-(trifluoromethyl)benzene (**3f**),[3] 1,3-dimethoxy-5-methyl-2-(trifluoromethyl)benzene (**3f***),[3] 1,2-dimethoxy-4-methyl-5-(trifluoromethyl)benzene (**3g**),[4] 1-bromo-2,4-dimethoxy-5-(trifluoromethyl)benzene (**3h**),[2] 2-bromo-1,5-dimethoxy-3-(trifluoromethyl)benzene (**3h#**),[5] 1-bromo-3,5-dimethoxy-2-(trifluoromethyl)benzene (**3i**),[3] 5-bromo-1,3-dimethoxy-2-(trifluoromethyl)benzene (**3i***).[3]

**Products from reaction of 1h**

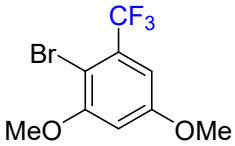
**1-bromo-2,4-dimethoxy-5-(trifluoromethyl)benzene : 1-bromo-2,4-dimethoxy-3-(trifluoromethyl)benzene : 2-bromo-1,5-dimethoxy-3-(trifluoromethyl)benzene (3h : 3h* : 3h#)**



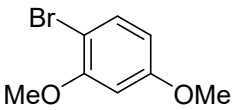

**3h**




**3h***




**3h# (minute)**




**1h (SM)**

**3h* : 3h# (minute) : 1h (SM) isolated as a mixture in ratio of 1 : 0.37 : 9**

**1-bromo-2,4-dimethoxy-5-(trifluoromethyl)benzene (3h)**

$^{1}$H NMR (400 MHz, $CDCl_3$) δ 6.52 (s, 1H), 6.52 (s, 1H), 3.95 (s, 3H), 3.92 (s, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 159.60, 158.52 (q, $J_{C\text{-}F}$ = 3.3, 1.5 Hz), 131.49 (q, $J_{C\text{-}F}$ = 5.4 Hz), 123.20 (q, $J_{C\text{-}F}$ = 271.6 Hz), 112.52 (q, $J_{C\text{-}F}$ = 31.9 Hz), 101.38, 96.97, 56.60, 56.40.

$^{19}$F NMR (376 MHz, $CDCl_3$) δ -61.5 (s, $CF_3$).

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_9H_9BrF_3O_2^+$ 284.9733; Found 284.9730.

Characterization data were in agreement with literature.[2]

**1-bromo-2,4-dimethoxy-3-(trifluoromethyl)benzene (3h*) : 2-bromo-1,5-dimethoxy-3-(trifluoromethyl)benzene (3h#)**

**3h*** and **3h#** were formed in small amount at a ratio of 3.4 : 1 : 0.5 (**3h** : **3h*** : **3h#**) in crude $^{19}$F NMR and isolated as a mixture with starting material **1h** at a ratio of 1 : 0.37 : 9 (**3h*** : **3h#** : **1h**) in $^{1}$H NMR. Hence, no full characterization was obtained.

**3h***: $^{1}$H NMR deduced after matching against NMR data of **3h, 3h#**[5] and **1h**.

$^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 0.6 Hz, 1H), 6.69 (d, *J* = 9.3 Hz, 1H), 3.87 (s, 2 x 3H).

$^{19}$F NMR (376 MHz, $CDCl_3$) δ -56.4 (s, $CF_3$).

**3h#**: Characterization data were in agreement with patent.[5]

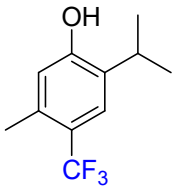
$^{1}$H NMR (400 MHz, $CDCl_3$) δ 6.84 (d, *J* = 2.8 Hz, 1H), 6.63 (d, *J* = 2.8 Hz, 1H), 3.91 (s, 3H), 3.84 (s, 3H).

$^{19}$F NMR (376 MHz, $CDCl_3$) δ -62.3 (s, $CF_3$).

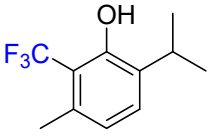
HRMS of **3h* : 3h# : 1h** mixture: (ESI TOF) *m/z*: $[M+H]^+$ Calcd for $C_9H_9BrF_3O_2^+$ *m/z* 284.9733; Found 284.9722.

**<u>Products from reaction of 1j</u>**

**2-isopropyl-5-methyl-4-(trifluoromethyl)phenol : 6-isopropyl-3-methyl-2-(trifluoromethyl)phenol (3j : 3j*)**




**3j**




**3j***

**2-isopropyl-5-methyl-4-(trifluoromethyl)phenol (3j)**

$^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.40 (s, 1H), 6.63 (s, 1H), 5.08 (s, 1H), 3.17 (sept, *J* = 6.9 Hz, 1H), 2.38 (d, $J_{H\text{-}F}$ = 1.6 Hz, 3H), 1.26 (s, 3H), 1.24 (s, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 155.1 (d, $J_{C-F}$ = 1.0 Hz), 135.8 (q, $J_{C-F}$ = 1.8 Hz), 131.7, 125.1 (q, $J_{C-F}$ = 272.5 Hz), 124.7 (q, $J_{C-F}$ = 5.6 Hz), 121.6 (q, $J_{C-F}$ = 29.8 Hz), 118.6, 26.9, 22.5, 19.0 (q, $J_{C-F}$ = 2.1 Hz).

$^{19}$F NMR (376 MHz, $CDCl_3$) δ -60.0 (s, $CF_3$).

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{14}F_3O^+$ 219.0991; Found 219.0983.

HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{11}H_{12}F_3O^-$ 217.0846; Found 217.0841.

**6-isopropyl-3-methyl-2-(trifluoromethyl)phenol (3j*)**

$^1$H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, *J* = 7.8 Hz, 1H), 6.78 (d, *J* = 7.8 Hz, 1H), 6.09 (d, *J* = 7.1 Hz, 1H), 3.28 (sept, *J* = 6.8 Hz, 1H), 2.41 (q, $J_{H-F}$ = 2.8 Hz, 3H), 1.23 (s, 3H), 1.21 (s, 3H).

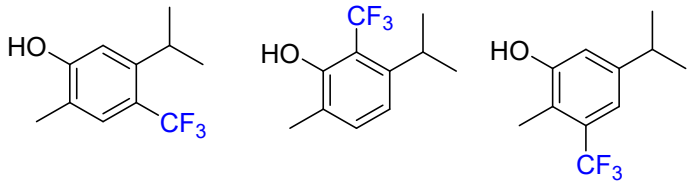
$^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.8 (q, *JC-F* = 2.0 Hz), 135.4 (q, $J_{C-F}$ = 1.8 Hz), 135.1, 129.5, 126.5 (q, *JC-F* = 274.9 Hz), 124.1, 113.9 (q, *JC-F* = 26.4 Hz), 26.8, 22.6, 20.7 (q, *JC-F* = 3.6 Hz).

$^{19}$F NMR (376 MHz, $CDCl_3$) δ -53.2 (s, $CF_3$).

HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{11}H_{12}F_3O^-$ 217.0846; Found 217.0842.

**<u>Products from reaction of 1k</u>**

**5-isopropyl-2-methyl-4-(trifluoromethyl)phenol : 3-isopropyl-6-methyl-2-(trifluoromethyl)phenol : 5-isopropyl-2-methyl-3-(trifluoromethyl)phenol (3k : 3k* : 3k#)**




**3k** **3k*** **3k#**

**5-isopropyl-2-methyl-4-(trifluoromethyl)phenol (3k) : 5-isopropyl-2-methyl-3-(trifluoromethyl)phenol (3k#)**

**3k#** was formed in small amount at a ratio of 1 : 0.74 : 0.33 (**3k : 3k* : 3k#**) in crude $^{19}$F NMR. **3k** and **3k#** were isolated as a mixture at a ratio of 1 : 0.3 (**3k : 3k#**) in $^{19}$F and $^1$H NMR.

**3k**: $^1$H NMR (400 MHz, $CDCl_3$) δ 7.35 (s, 1H), 6.82 (s, 1H), 3.34 – 2.90 ($m_{H-F}$, 1H), 2.23 (s, 3H), 1.23 (s, 3H), 1.22 (s, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 156.64, 148.04, 128.68 (q, $J_{C-F}$ = 5.9 Hz), 125.03 (q, $J_{C-F}$ = 272.7 Hz), 121.04, 120.15 (q, $J_{C-F}$ = 29.5 Hz), 113.62, 29.13 (q, $J_{C-F}$ = 2.1 Hz), 24.39, 23.90, 15.36.

$^{19}$F NMR (376 MHz, $CDCl_3$) δ -57.7 (s, $CF_3$).

**3k#**: $^1$H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, $J_{H-F}$ = 1.8 Hz, 1H), 6.82 (s, 1H), 2.86 (sept, *J* = 6.9 Hz, 1H), 2.30 (q, $J_{H-F}$ = 1.6 Hz, 3H), 1.24 (s, 3H), 1.22 (s, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 154.58, 147.94, 130.25 (q, $J_{C-F}$ = 29.2 Hz), 124.63 (q, $J_{C-F}$ = 274.8 Hz), 120.15 (q, $J_{C-F}$ = 29.5 Hz), 116.61 (q, $J_{C-F}$ = 5.56 Hz), 116.56 , 33.85, 24.39, 23.90, 11.25 (q, $J_{C-F}$ = 2.1 Hz).

$^{19}$F NMR (376 MHz, $CDCl_3$) δ -60.5 (s, $CF_3$).

HRMS of **3k : 3k#** mixture:

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{14}F_3O^+$ 219.0991; Found 219.0984.

HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{11}H_{12}F_3O^-$ 217.0846; Found 217.0847.

**3-isopropyl-6-methyl-2-(trifluoromethyl)phenol (3k*)**

$^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.2 Hz, 1H), 6.92 (d, *J* = 7.9 Hz, 1H), 6.21 (q, *J* = 7.1 Hz, 1H), 3.36 – 3.14 ($m_{H-F}$, 1H), 2.22 (s, 3H), 1.23 (s, 3H), 1.22 (s, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 152.02 (q, $J_{C-F}$ = 2.0 Hz), 147.72 (q, $J_{C-F}$ = 1.6 Hz), 134.16, 126.59 (q, $J_{C-F}$ = 275.3 Hz), 124.52, 118.79, 112.44 (q, $J_{C-F}$ = 26.0 Hz), 29.60 (q, $J_{C-F}$ = 3.3 Hz), 24.67, 16.21.

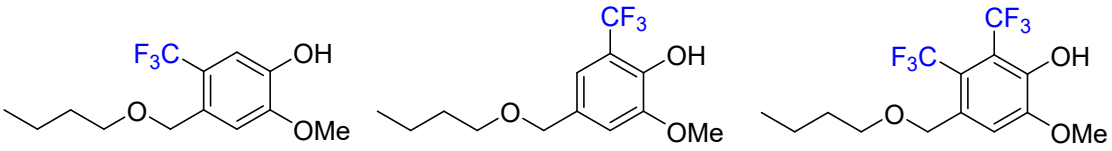
$^{19}$F NMR (376 MHz, $CDCl_3$) δ -50.7 (s, $CF_3$).

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{14}F_3O^+$ 219.0991; Found 219.0996.

HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{11}H_{12}F_3O^-$ 217.0846; Found 217.0843.

**Products from reaction of 1l**

**4-(butoxymethyl)-2-methoxy-5-(trifluoromethyl)phenol : 4-(butoxymethyl)-2-methoxy-6-(trifluoromethyl)phenol : 4-(butoxymethyl)-6-methoxy-2,3-bis(trifluoromethyl)phenol (3l : 3l* : 3l^)**




**3l** **3l*** **3l^**

**4-(butoxymethyl)-2-methoxy-5-(trifluoromethyl)phenol (3l)**

**3l**: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.18 (s, 1H), 7.16 (s, 1H), 4.59 (s, 2H), 3.95 (s, 3H), 3.51 (t, *J* = 6.5 Hz, 2H), 1.67 – 1.60 ($m_{H-F}$, 2H), 1.47 – 1.36 ($m_{H-F}$, 2H), 0.94 (t, *J* = 7.4 Hz, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 148.89, 144.32, 130.30 (q, $J_{C-F}$ = 1.5 Hz), 124.42 (q, $J_{C-F}$ = 273.0 Hz), 120.60 (q, $J_{C-F}$ = 31.4 Hz), 112.43 (q, $J_{C-F}$ = 5.8 Hz), 111.11, 70.72, 68.52 (q, $J_{C-F}$ = 2.7 Hz), 56.19, 31.95, 19.57, 14.08.

$^{19}$F NMR (376 MHz, $CDCl_3$) δ -58.7 (s, $CF_3$).

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{13}H_{18}F_3O_3^+$ 279.1203; Found 279.1179.

HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{13}H_{16}F_3O_3^-$ 277.1057; Found 277.1052.

**4-(butoxymethyl)-2-methoxy-6-(trifluoromethyl)phenol (3l*): 4-(butoxymethyl)-6-methoxy-2,3-bis(trifluoromethyl)phenol (3l^)**

**3l^** was formed in small amount at a ratio of 1 : 1.1 : 0.05 (**3l : 3l* : 3l^**) in crude $^{19}$F NMR. **3l*** and **3l^** were isolated as a mixture at a ratio of 1 : 0.2 (**3l* : 3l^**) in $^{19}$F and $^{1}$H NMR. $^{13}$C NMR data is not available for **3l^** because the signals are weak and many of the peaks are buried under those of **3l***.

**3l***: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.08 (s, 1H), 7.04 (s, 1H), 6.09 (s, 1H), 4.43 (s, 2H), 3.94 (s, 3H), 3.46 (t, *J* = 6.6 Hz, 2H), 1.60 – 1.52 (m, 2H), 1.46 – 1.34 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 147.23, 143.72 (q, $J_{C-F}$ = 3.9, 1.7 Hz), 130.35, 123.65 (q, $J_{C-F}$ = 272.6 Hz), 117.64 (q, $J_{C-F}$ = 4.9 Hz), 115.87 (q, $J_{C-F}$ = 31.6 Hz), 113.23, 72.48, 70.42, 56.54, 31.92, 19.52, 14.06.

$^{19}$F NMR (376 MHz, $CDCl_3$) δ -62.0 (s, $CF_3$).

HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{13}H_{16}F_3O_3^-$ 277.1057; Found 277.1051.

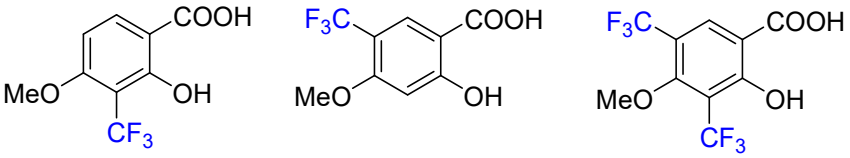
**3l^**: $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.36 (s, 1H), 6. (s, 1H), 4.61 (s, 2H), 4.0 (s, 3H), 3.50 (t, *J* = 6.0 Hz, 2H), 1.60 – 1.52 (m, 2H), 1.46 – 1.33 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H).

$^{19}$F NMR (376 MHz, $CDCl_3$) δ -52.04 (q, *J* = 16.1 Hz), -55.18 (q, *J* = 16.1 Hz).

HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{14}H_{15}F_6O_3^-$ 345.0931; Found 345.0923.

**Products from reaction of 1m**

**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid : 2-hydroxy-4-methoxy-5-(trifluoromethyl)benzoic acid : 2-hydroxy-4-methoxy-3,5-bis(trifluoromethyl)benzoic acid (3m : 3m* : 3m^)**




**3m** **3m*** **3m^**

**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid (3m)**

$^1$H NMR (400 MHz, $CD_3OD$) δ 8.07 (d, *J* = 9.0 Hz, 1H), 6.71 (d, *J* = 9.0 Hz, 1H), 3.93 (s, 3H).

$^{13}$C NMR (101 MHz, $CD_3OD$) δ 173.37, 165.20, 163.43, 136.72, 125.15 (q, $J_{C-F}$ = 273.9 Hz), 107.78, 106.53 (q, $J_{C-F}$ = 30.2 Hz), 103.95, 56.97.

$^{19}$F NMR (376 MHz, $CD_3OD$) δ -56.9 (s, $CF_3$).

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_9H_8F_3O_4^+$ 237.0369; Found 237.0362.

HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_9H_6F_3O_4^-$ 235.0224; Found 235.0230.

**2-hydroxy-4-methoxy-5-(trifluoromethyl)benzoic acid (3m*)**

$^1$H NMR (400 MHz, $CD_3OD$) δ 8.03 (s, 1H), 6.66 (s, 1H), 3.93 (s, 3H).

$^{13}$C NMR (101 MHz, $CD_3OD$) δ 172.62, 168.20, 164.35, 130.83 (q, $J_{C-F}$ = 5.5 Hz), 124.81 (q, $J_{C-F}$ = 270.2 Hz), 111.67 (q, $J_{C-F}$ = 32.0 Hz), 106.08, 101.37, 56.88.

$^{19}$F NMR (376 MHz, $CD_3OD$) δ -63.2 (s, $CF_3$).

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_9H_8F_3O_4^+$ 237.0369; Found 237.0362.

HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_9H_6F_3O_4^-$ 235.0224; Found 235.0225.

**2-hydroxy-4-methoxy-3,5-bis(trifluoromethyl)benzoic acid (3m^)**

**3m^** was formed in small amount at a ratio of 1.2 : 1 : 0.24 (**3m : 3m* : 3m^**) in crude $^{19}$F NMR. **3m^** was isolated as a mixture at a ratio of 1 : 0.13 (**3m : 3m^**) in $^{19}$F and $^1$H NMR. $^{13}$C NMR data is not available for **3m^** because the signals are weak and many of the peaks are buried under those of **3m**.

$^1$H NMR (400 MHz, $CD_3OD$) δ 8.32 (s, 1H), 3.88 (s, 3H).

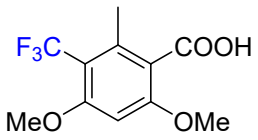
$^{19}$F NMR (376 MHz, $CD_3OD$) δ -58.9 (s, $CF_3$), -61.7 (s, $CF_3$).

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{10}H_7F_6O_4^+$ 305.0243; Found 305.0259.

HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{10}H_5F_3O_4^-$ 303.0098; Found 303.0089.

**Products from reaction of 1n**

**4,6-dimethoxy-2-methyl-3-(trifluoromethyl)benzoic acid (3n)**




**3n**

$^{1}$H NMR (400 MHz, $CD_3OD$) δ 6.67 (s, 1H), 3.92 (s, 3H), 3.91 (s, 3H), 2.35 (q, $J_{H-F}$ = 3.2 Hz, 3H).

$^{13}$C NMR (101 MHz, $CD_3OD$) δ 171.57, 162.01 (q, $J_{C-F}$ = 1.6 Hz), 160.54, 136.91 (q, $J_{C-F}$ = 1.3 Hz), 126.53 (q, $J_{C-F}$ = 273.6 Hz), 121.24, 110.85 (q, $J_{C-F}$ = 29.5 Hz), 95.45, 56.87, 56.52, 17.89 (q, $J_{C-F}$ = 4.6 Hz).

$^{19}$F NMR (376 MHz, $CD_3OD$) δ -54.4 (s, $CF_3$).

HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{11}H_{12}F_3O_4^+$ 265.0682; Found 265.0676.

HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{11}H_{10}F_3O_4^-$ 263.0537; Found 263.058.

**4. Preparation of laccase sample for ICP-MS analysis**

A solution of laccase from *Trametes versicolor* (0.89 U/mg, Sigma Aldrich) was prepared in 100 mM NaPi buffer (pH 7) with a concentration of 10 U/mL. 1 mL of laccase solution was digested with 0.5 mL conc. nitric acid (70%, ≥99.999% trace metals basis, Sigma Aldrich). The digested solution was diluted to 25 mL with ultrapure water and analyzed by ICP-MS. The calibration curve of Cu is shown in Fig. S6 and the data from triplicate ICP-MS measurements are listed in Table S1.

**5. Kinetic isotope experiment**

In kinetic isotope experiment, deuterated analog **1e-D** was prepared following the procedure reported in the literature.[6] This method gave **1e-D** in 99% deuteration(Fig. S7). The crude NMR of KIE experiment using equal amounts of **1e** and **1e-D** shows the formation of isotopic products at a ratio of approximately 1:1 (Fig. S8).

**II. Supplementary figures and tables**

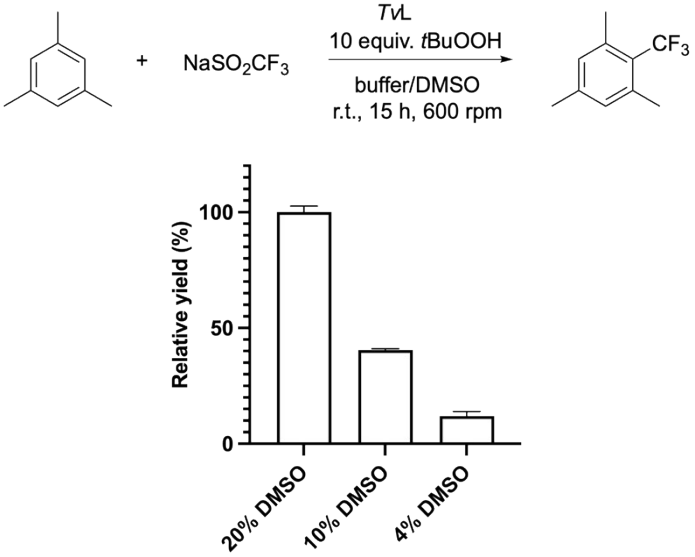



**Figure S1.** Effect of the percentage of DMSO co-solvent on the yield of *Tv*L-catalyzed trifluoromethylation of mesitylene. Reaction conditions: mesitylene (0.02 mmol), $NaSO_2CF_3$ (5 equiv.), *t*BuOOH (10 equiv.), laccase (4 U), x vol.-% DMSO, NaPi buffer (100 mM, pH 7), 25 °C, 600 rpm, 15 h. The relative yields were determined by GC-FID analysis of the crude reaction mixture with 2,2,2-trifluoroacetophenone as internal standard and the yield of the reaction under standard conditions (20 vol.-% DMSO) was normalized as 100%. Data shown are the average from three replicates, with error bars indicating 1 standard deviation.

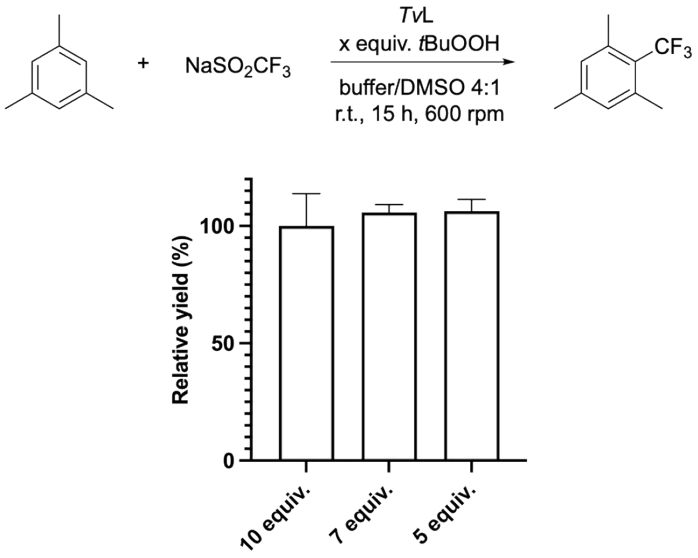



**Figure S2.** Effect of the equivalents of *t*BuOOH on the yield of *Tv*L-catalyzed trifluoromethylation of mesitylene. Reaction conditions: mesitylene (0.02 mmol), $NaSO_2CF_3$ (5 equiv.), *t*BuOOH (x equiv.), laccase (4 U), 20 vol.-% DMSO, NaPi buffer (100 mM, pH 7), 25 °C, 600 rpm, 15 h. The relative yields were determined by GC-FID analysis of the crude reaction mixture with 2,2,2-trifluoroacetophenone as internal standard and the yield of the reaction under standard conditions (10 equiv. *t*BuOOH) was

normalized as 100%. Data shown are the average from three replicates, with error bars indicating 1 standard deviation.

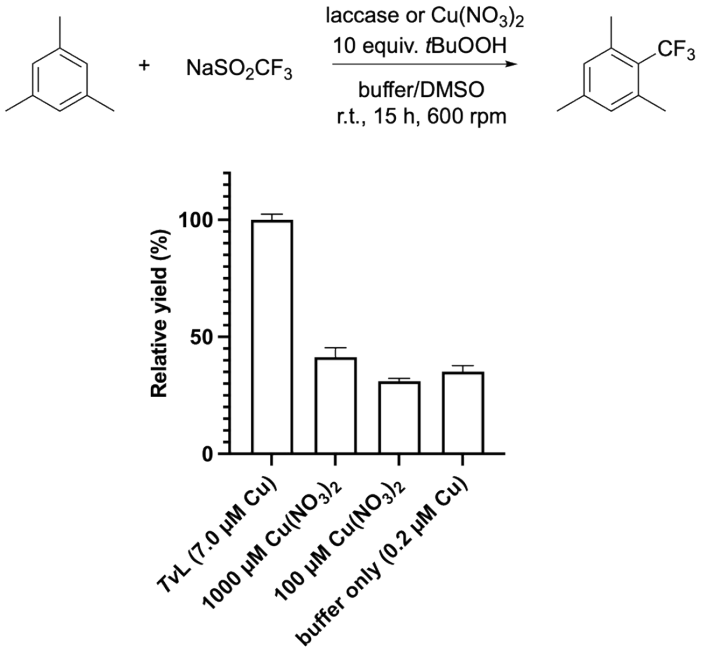



**Figure S3.** Trifluoromethylation of mesitylene catalyzed by *Tv*L and different concentrations of $Cu(NO_3)_2$. Reaction conditions: mesitylene (0.02 mmol), $NaSO_2CF_3$ (5 equiv.), *t*BuOOH (10 equiv.), laccase (4 U), 20 vol.-% DMSO, NaPi buffer (100 mM, pH 7), 25 °C, 600 rpm, 15 h. For reactions catalyzed by $Cu(NO_3)_2$, ultrapure water was used as the solvent as copper phosphate is marginally soluble in water. The relative yields were determined by GC-FID analysis of the crude reaction mixture with 2,2,2-trifluoroacetophenone as internal standard and the yield of the reaction catalyzed by laccase was normalized as 100%. Data shown are the average from three replicates, with error bars indicating 1 standard deviation.

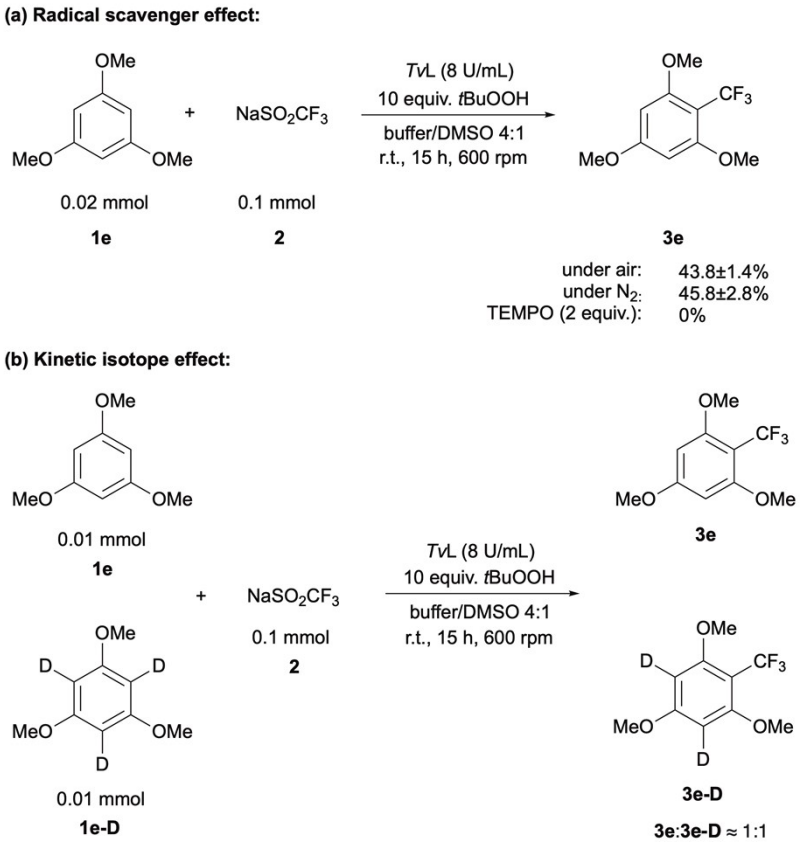



**Figure S4.** Mechanistic experiments.

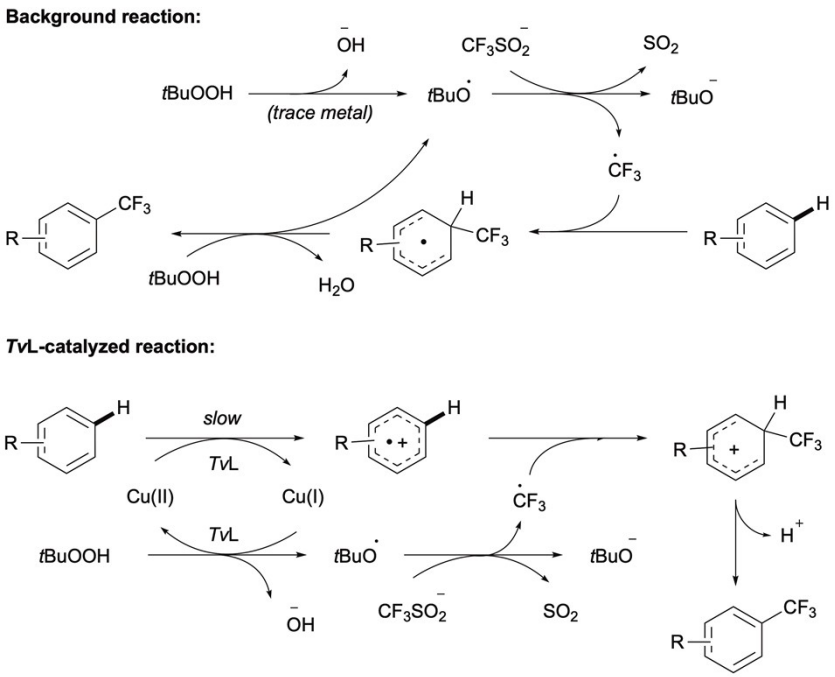



**Figure S5.** Proposed mechanism of laccase-catalyzed trifluoromethylation of arenes.

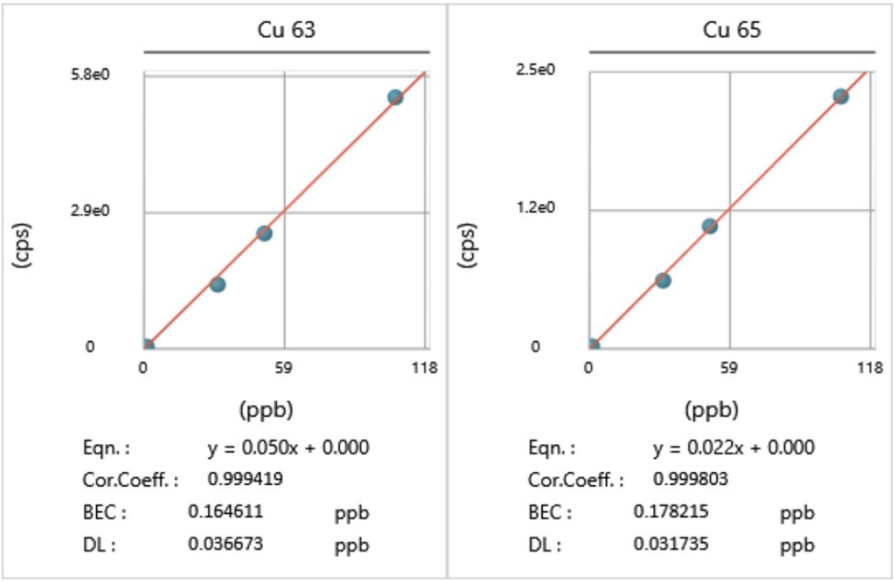



**Figure S6.** Calibration curve for ICP-MS quantification of copper.

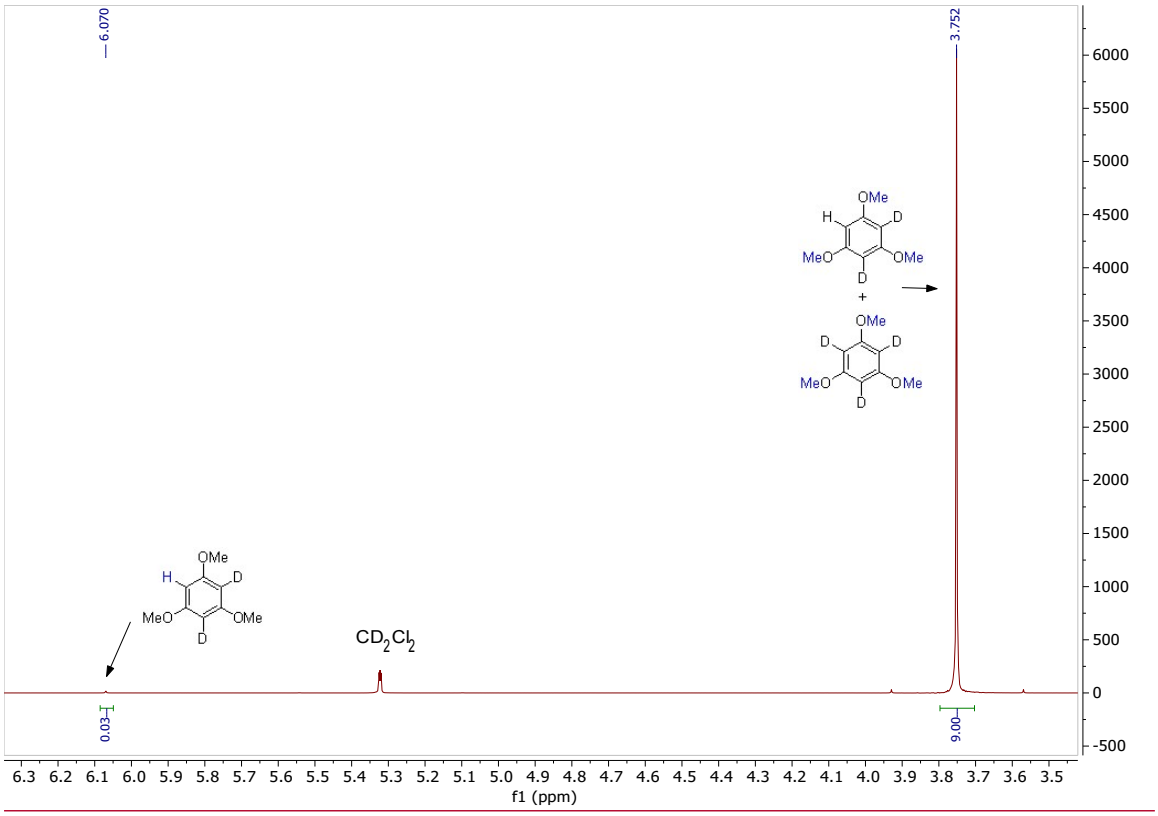



**Figure S7.** $^{1}$H NMR of isolated **1e-D**.

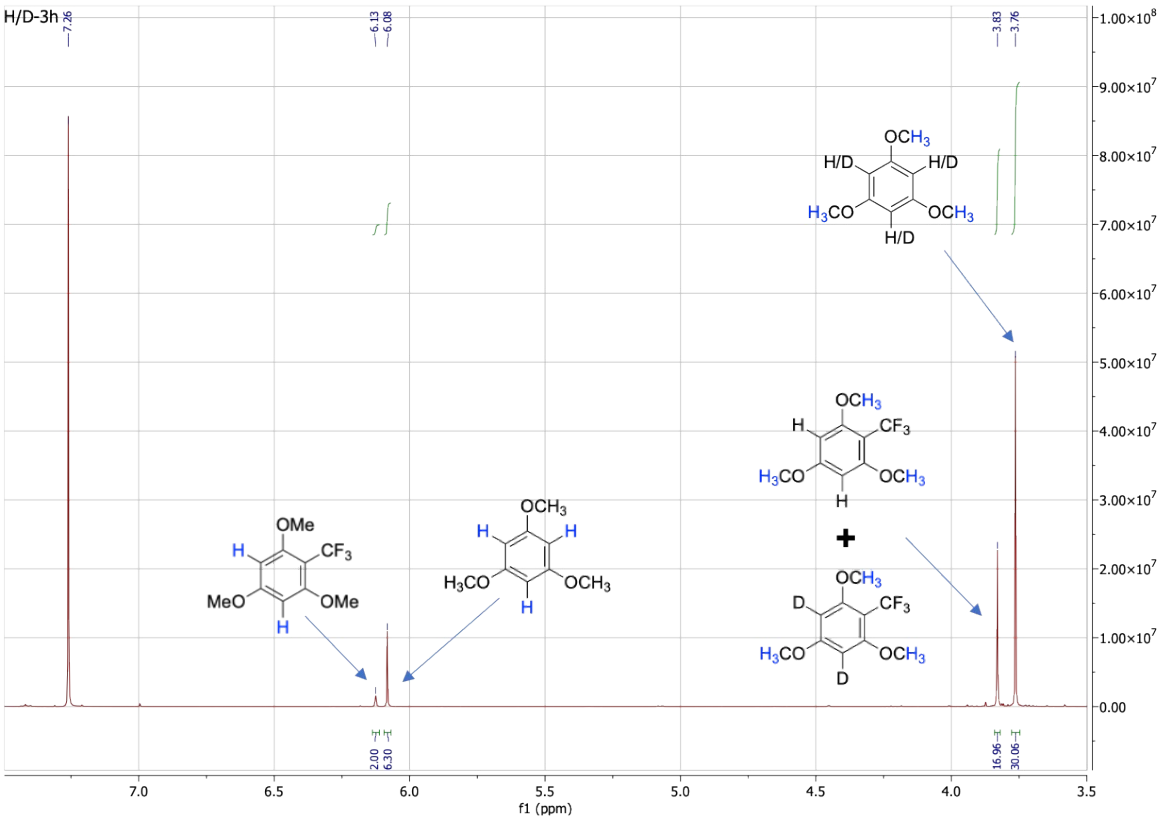


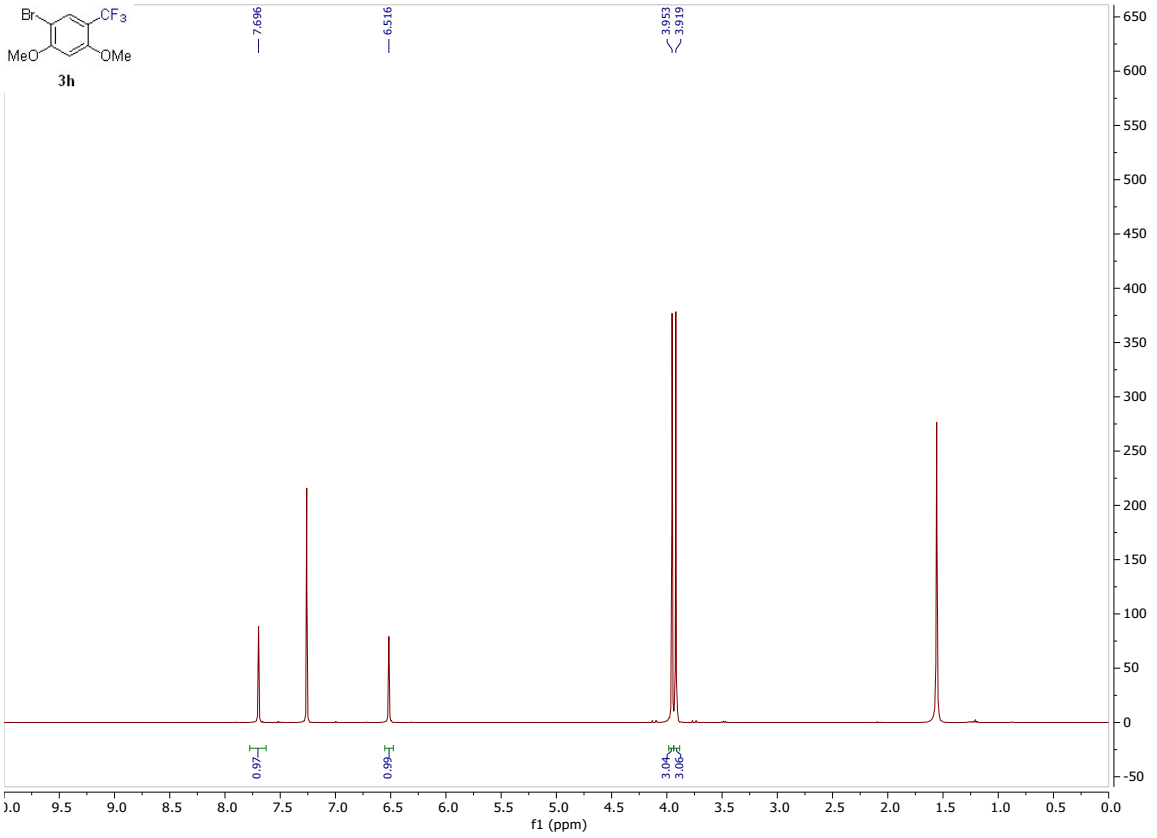

**Figure S8.** $^{1}$H NMR of crude reaction mixture of **1e** and **1e-D**.

**Table S1.** The copper concentration in ultrapure water, NaPi buffer and laccase sample solutions measured by ICP-MS.

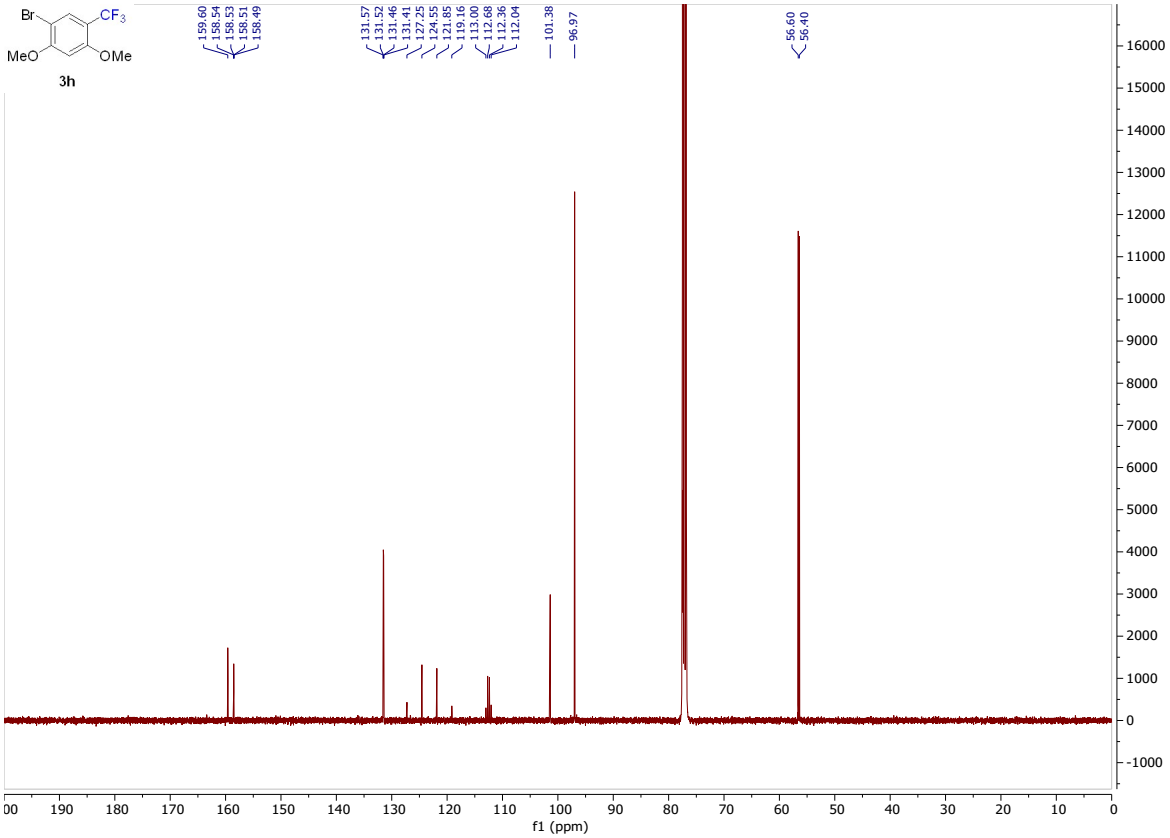
| | Cu 63 (ppb)<br>Ammonia DRC |
|---|---|
| Control ($H_2O$) | 1.305 |
| 100 mM NaPi buffer | 1.806 |
| Sample 1 (laccase) | 19.967 |
| Sample 2 (laccase) | 20.473 |
| Sample 3 (laccase) | 16.882 |
| Average [Cu] in laccase sample | 17.30±1.94 |

**$^{1}$H NMR, $^{13}$C NMR, HSQC, HMBC and $^{19}$F NMR Spectra**

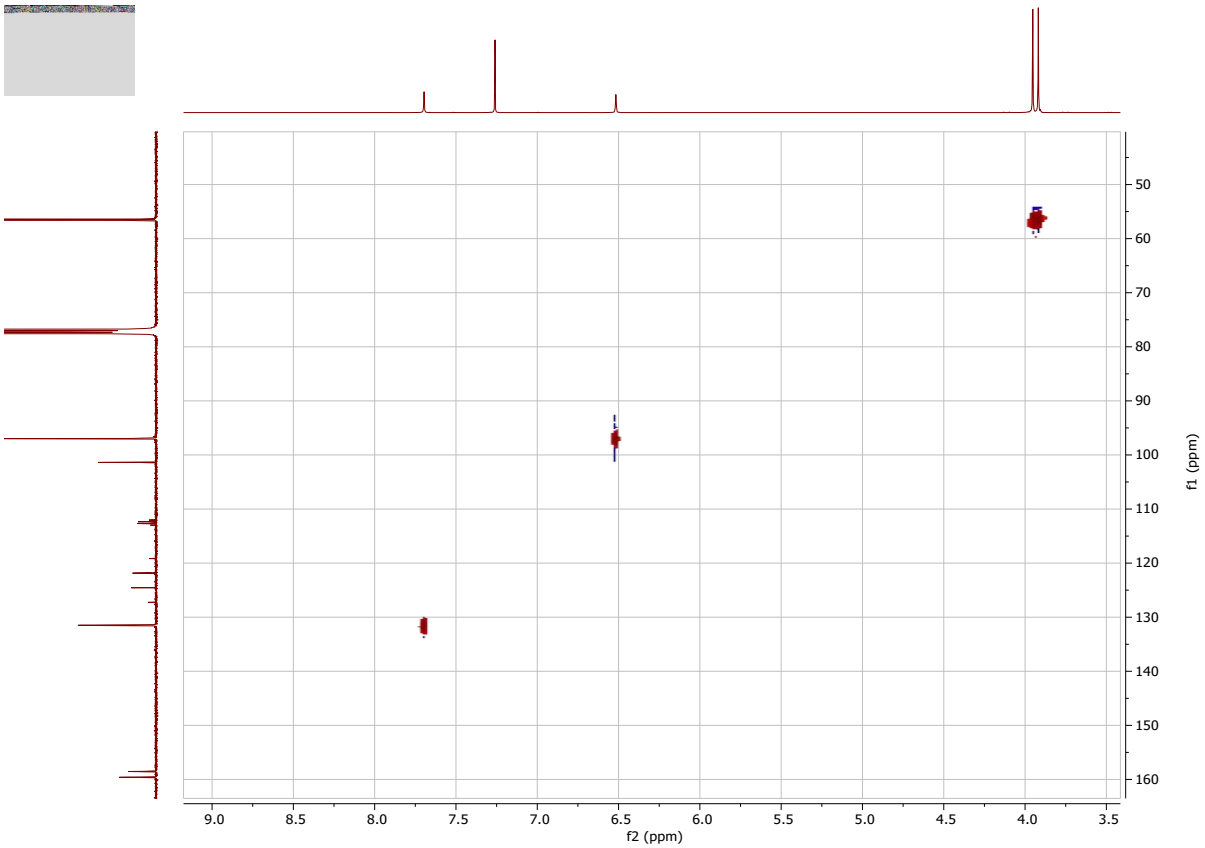
**1-bromo-2,4-dimethoxy-5-(trifluoromethyl)benzene (3h), $^{1}$H NMR (400 MHz, $CDCl_3$)**



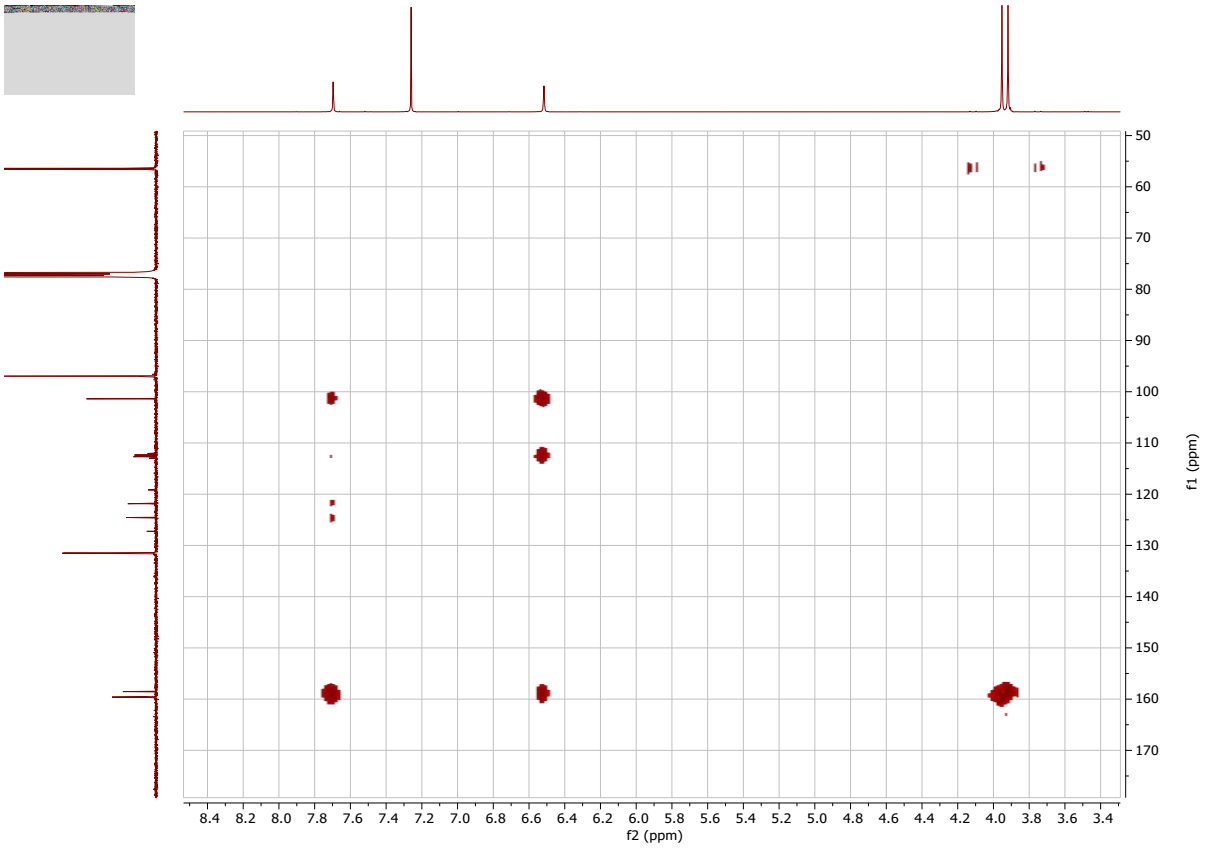

**1-bromo-2,4-dimethoxy-5-(trifluoromethyl)benzene (3h), $^{13}$C NMR (101 MHz, $CDCl_3$)**



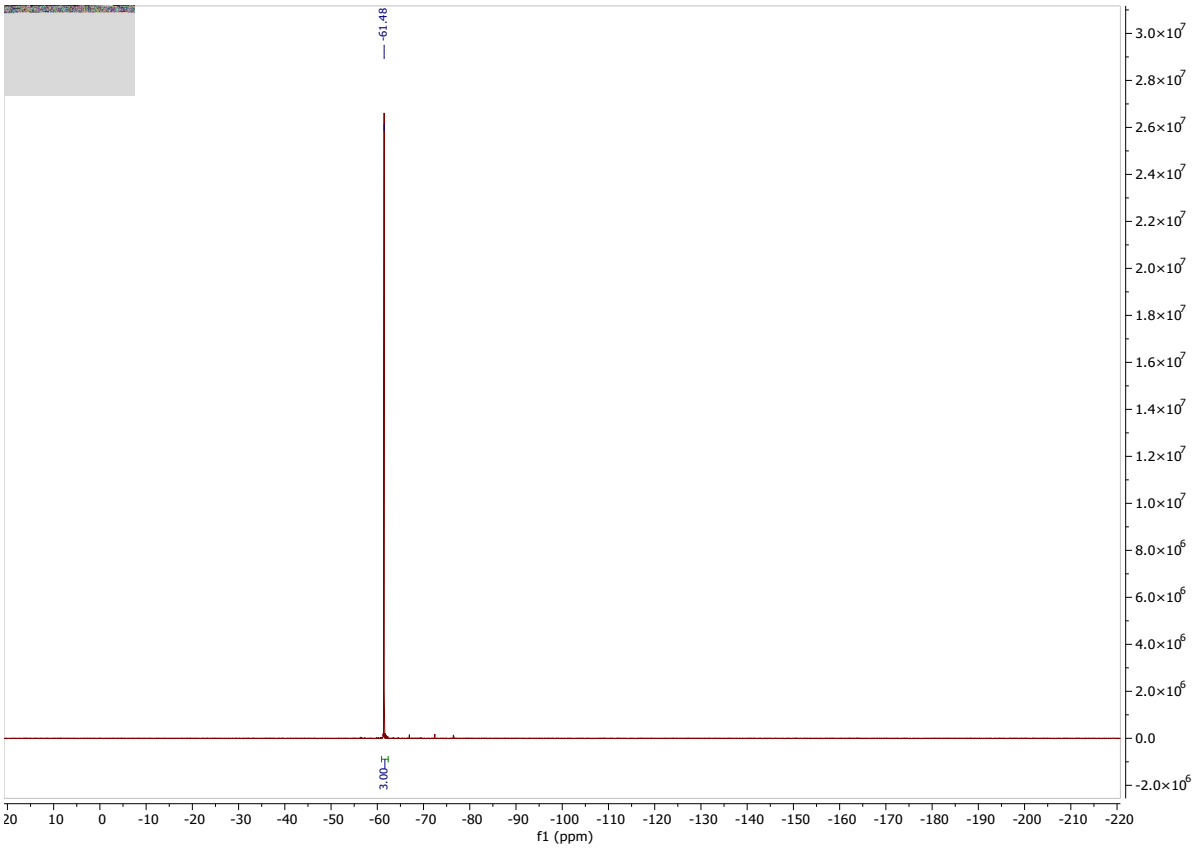
**1-bromo-2,4-dimethoxy-5-(trifluoromethyl)benzene (3h), HSQC ($CDCl_3$)**



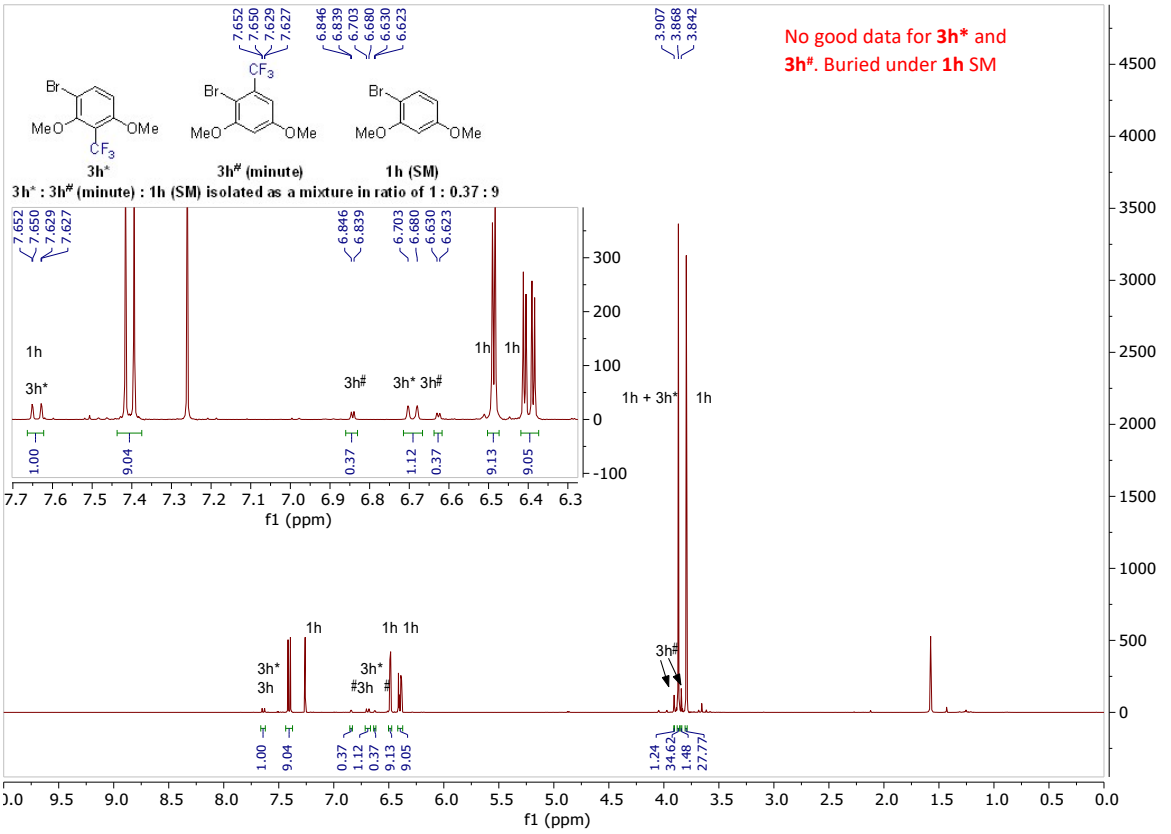

**1-bromo-2,4-dimethoxy-5-(trifluoromethyl)benzene (3h), HMBC ($CDCl_3$)**



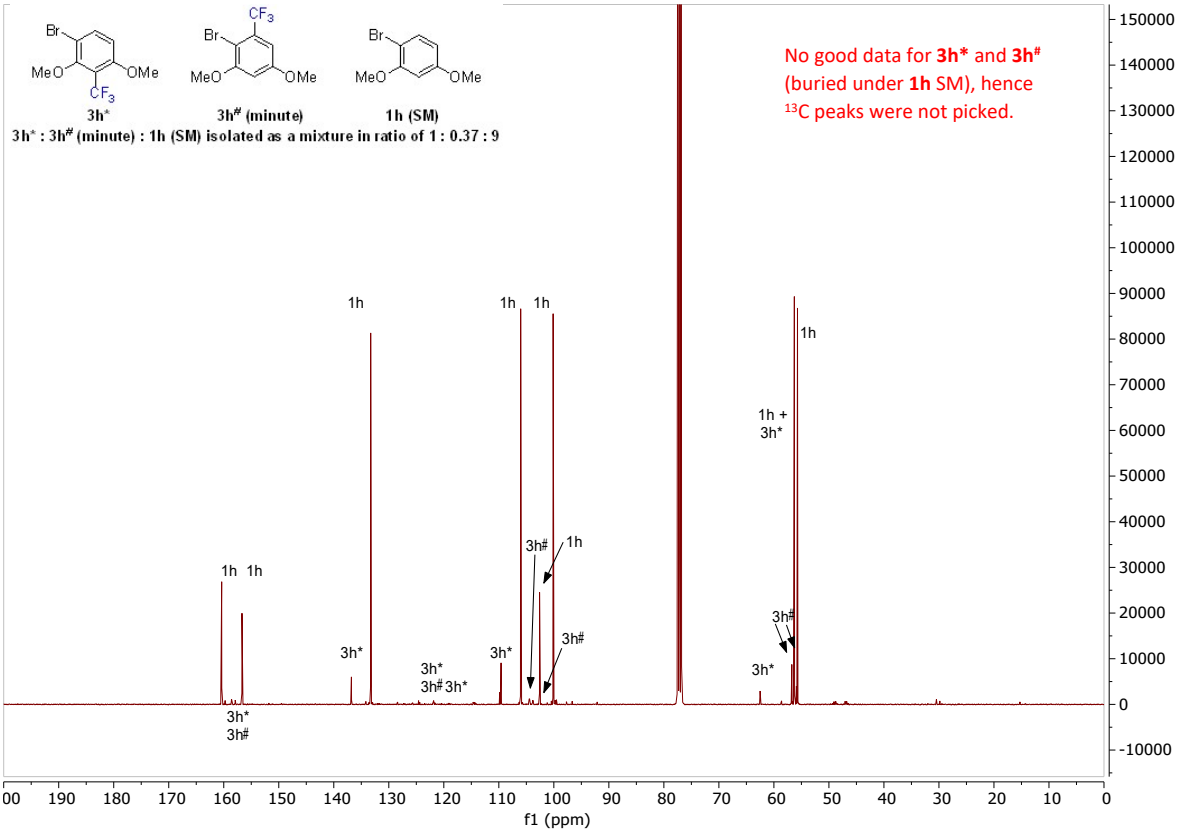
**1-bromo-2,4-dimethoxy-5-(trifluoromethyl)benzene (3h), $^{19}F$ NMR (376 MHz, $CDCl_3$)**



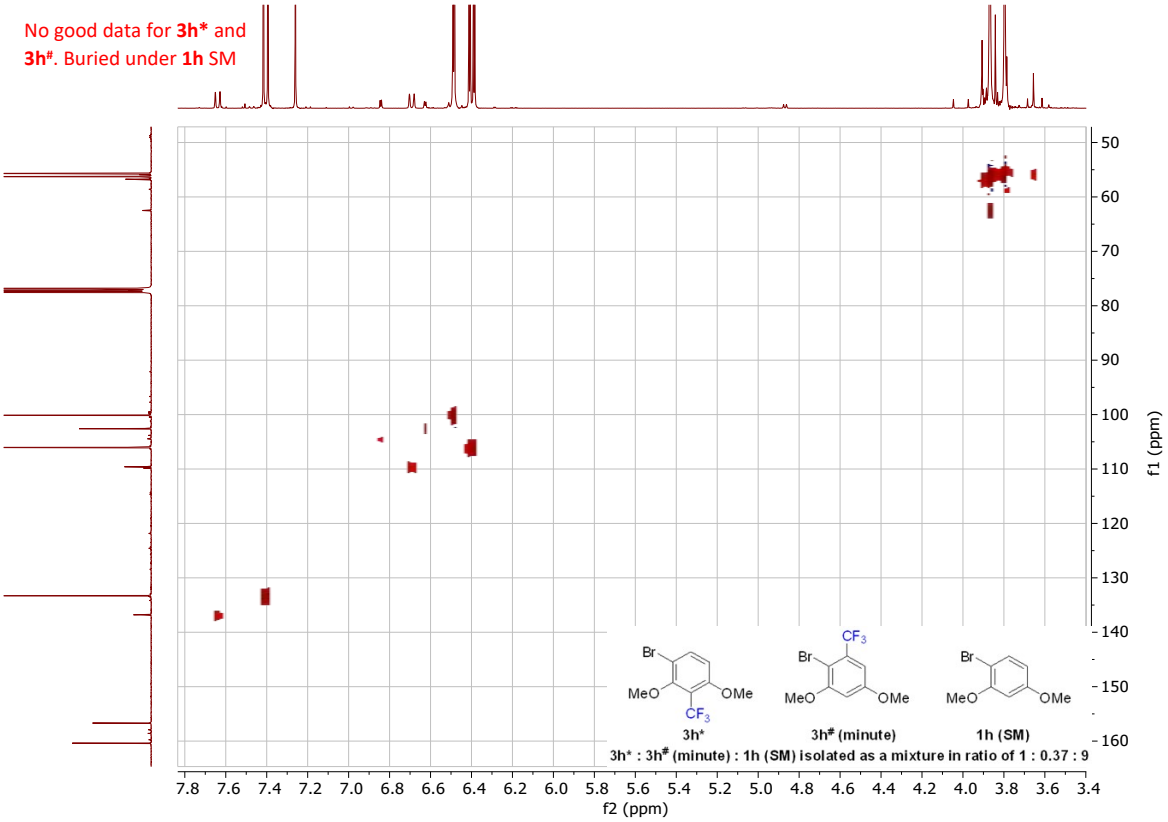
**1-bromo-2,4-dimethoxy-3-(trifluoromethyl)benzene : 2-bromo-1,5-dimethoxy-3-(trifluoromethyl)benzene (3h* : 3h# isolated as a mixture with 1h (SM) in the ratio of 1 : 0.37 : 9), $^{1}$H NMR (400 MHz, $CDCl_3$)**



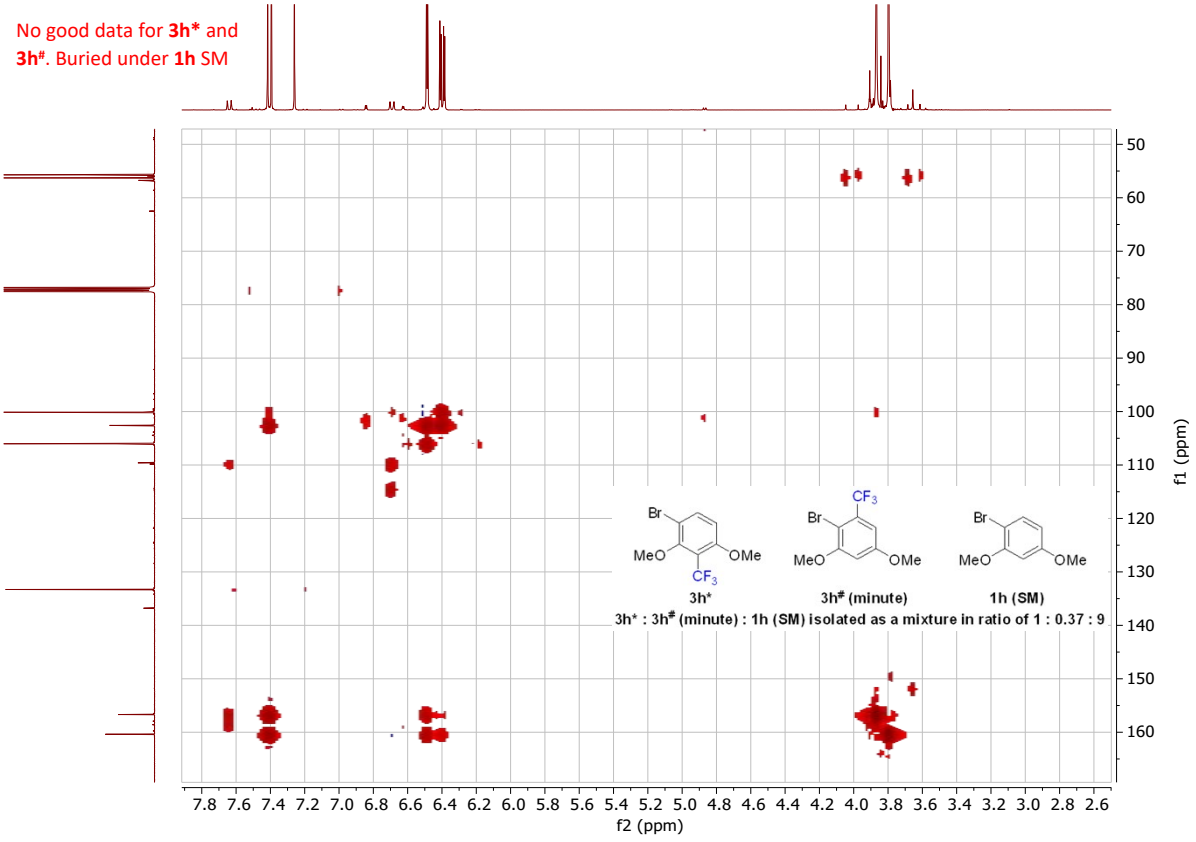

**1- bromo-2,4-dimethoxy-3-(trifluoromethyl)benzene : 2-bromo-1,5-dimethoxy-3-(trifluoromethyl)benzene (3h* : 3h# isolated as a mixture with 1h (SM) in the ratio of 1 : 0.37 : 9), $^{13}$C NMR (101 MHz, $CDCl_3$)**



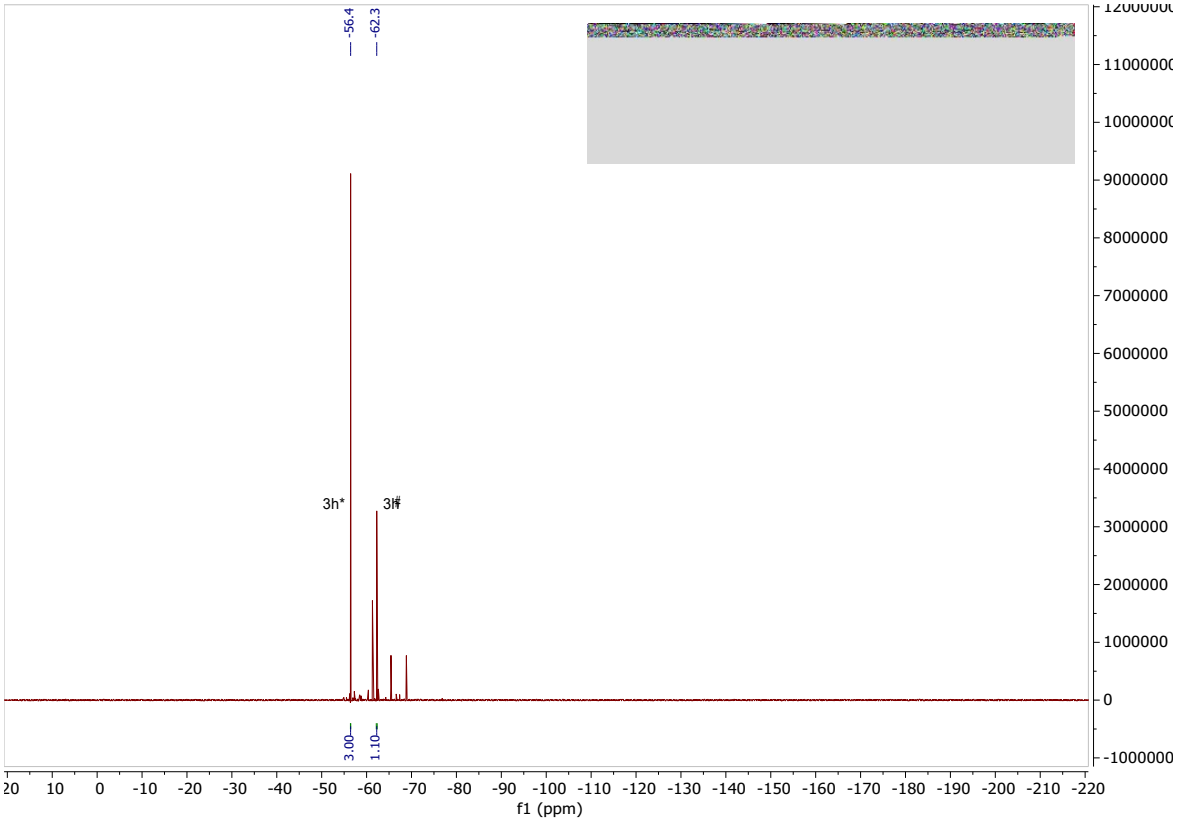
**1-bromo-2,4-dimethoxy-3-(trifluoromethyl)benzene : 2-bromo-1,5-dimethoxy-3-(trifluoromethyl)benzene (3h* : 3h# isolated as a mixture with 1h (SM) in the ratio of 1 : 0.37 : 9), HSQC ($CDCl_3$)**



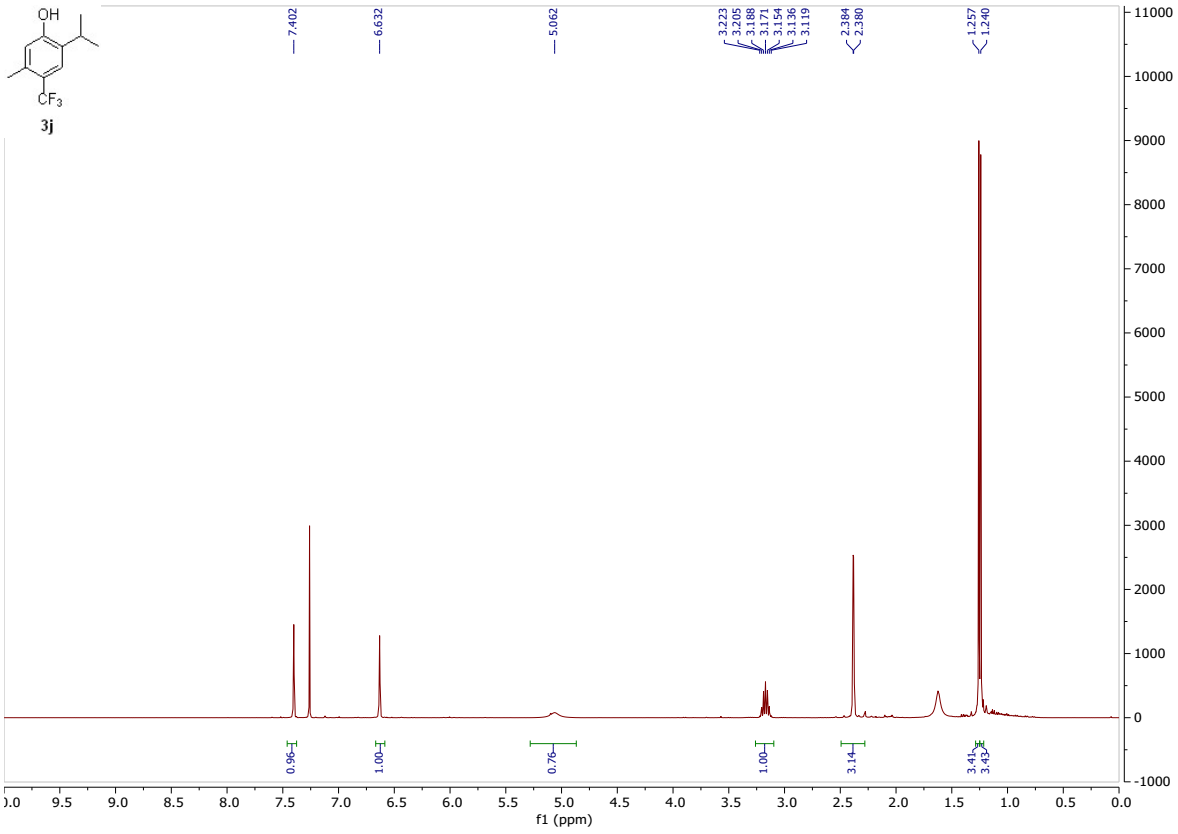

**1-bromo-2,4-dimethoxy-3-(trifluoromethyl)benzene : 2-bromo-1,5-dimethoxy-3-(trifluoromethyl)benzene (3h* : 3h# isolated as a mixture with 1h (SM) in the ratio of 1 : 0.37 : 9), HMBC ($CDCl_3$)**



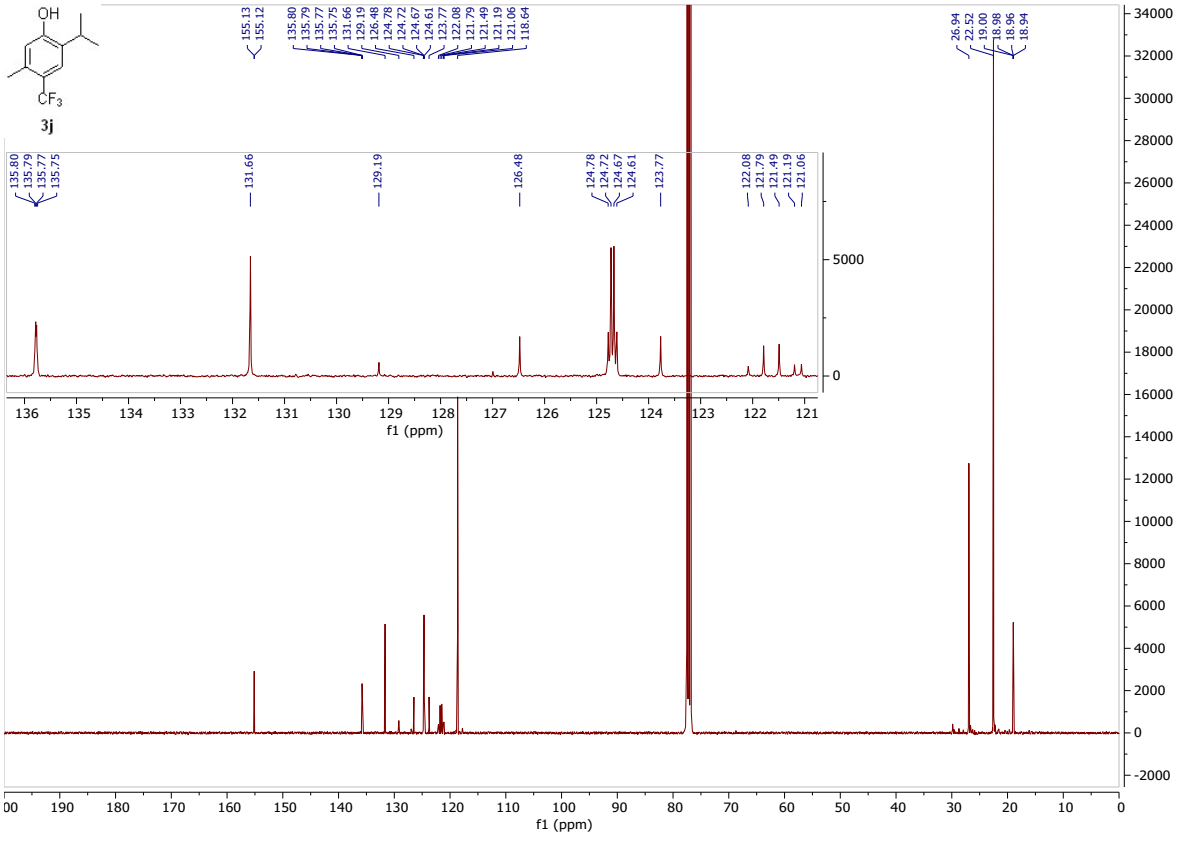
**1-bromo-2,4-dimethoxy-3-(trifluoromethyl)benzene : 2-bromo-1,5-dimethoxy-3-(trifluoromethyl)benzene (3h* : 3h# isolated as a mixture with 1h (SM) in the ratio of 1 : 0.37 : 9), $^{19}F$ NMR (376 MHz, $CDCl_3$)**



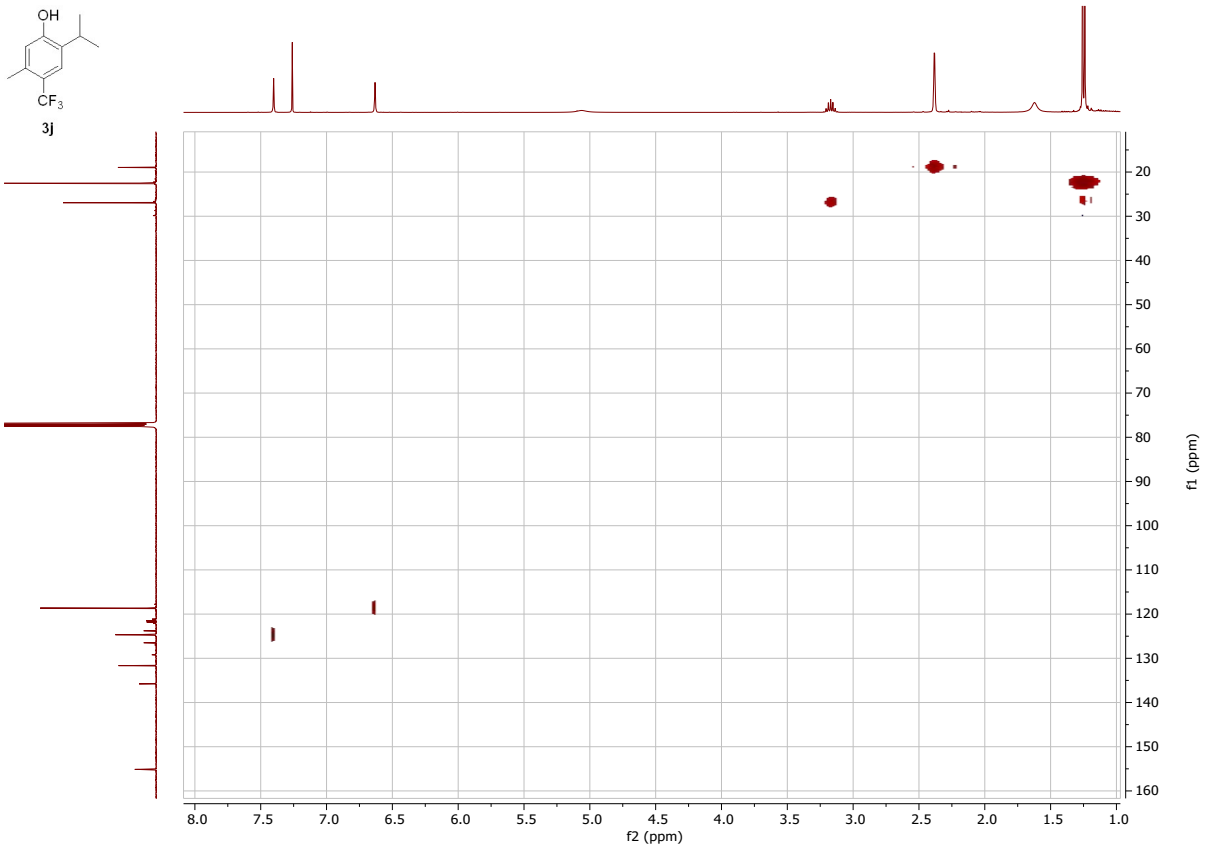
**2-isopropyl-5-methyl-4-(trifluoromethyl)phenol (3j), $^{1}H$ NMR (400 MHz, $CDCl_3$)**



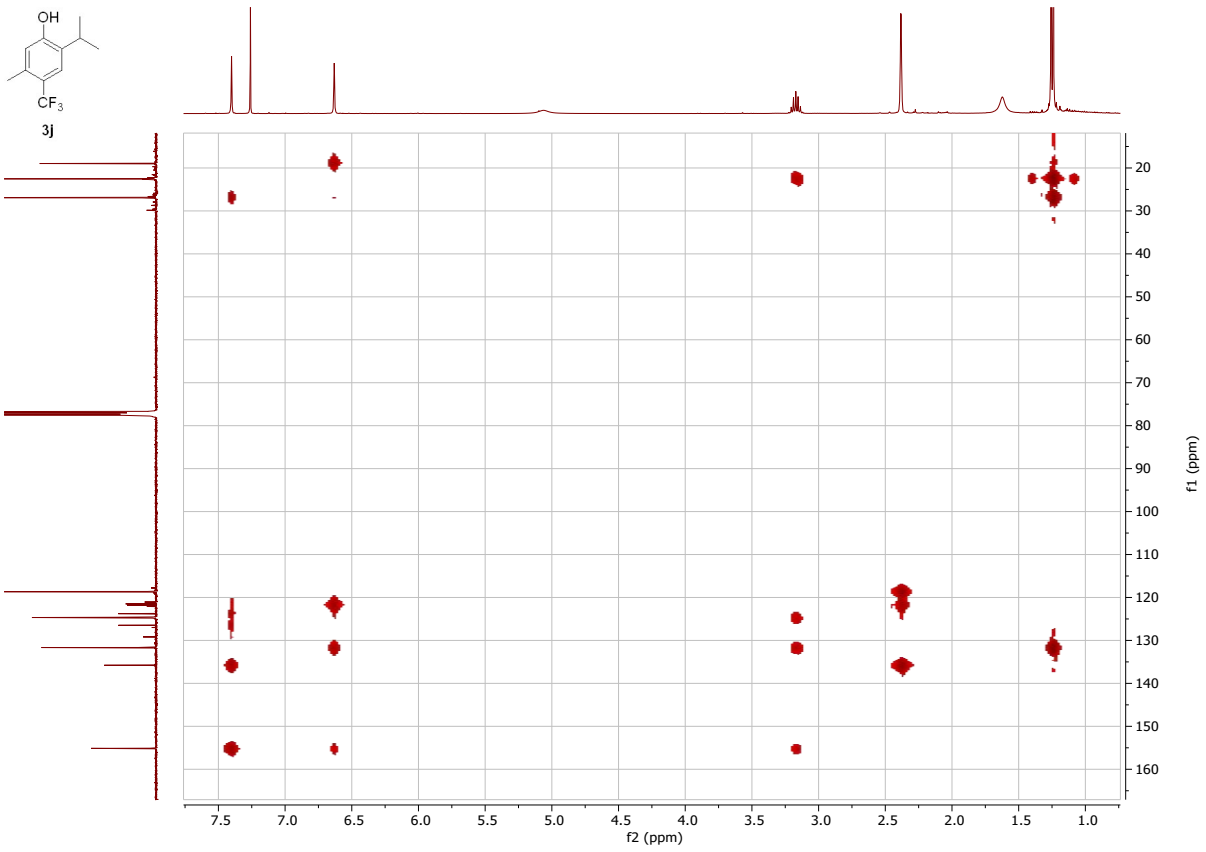

**2-isopropyl-5-methyl-4-(trifluoromethyl)phenol (3j), $^{13}C$ NMR (101 MHz, $CDCl_3$)**



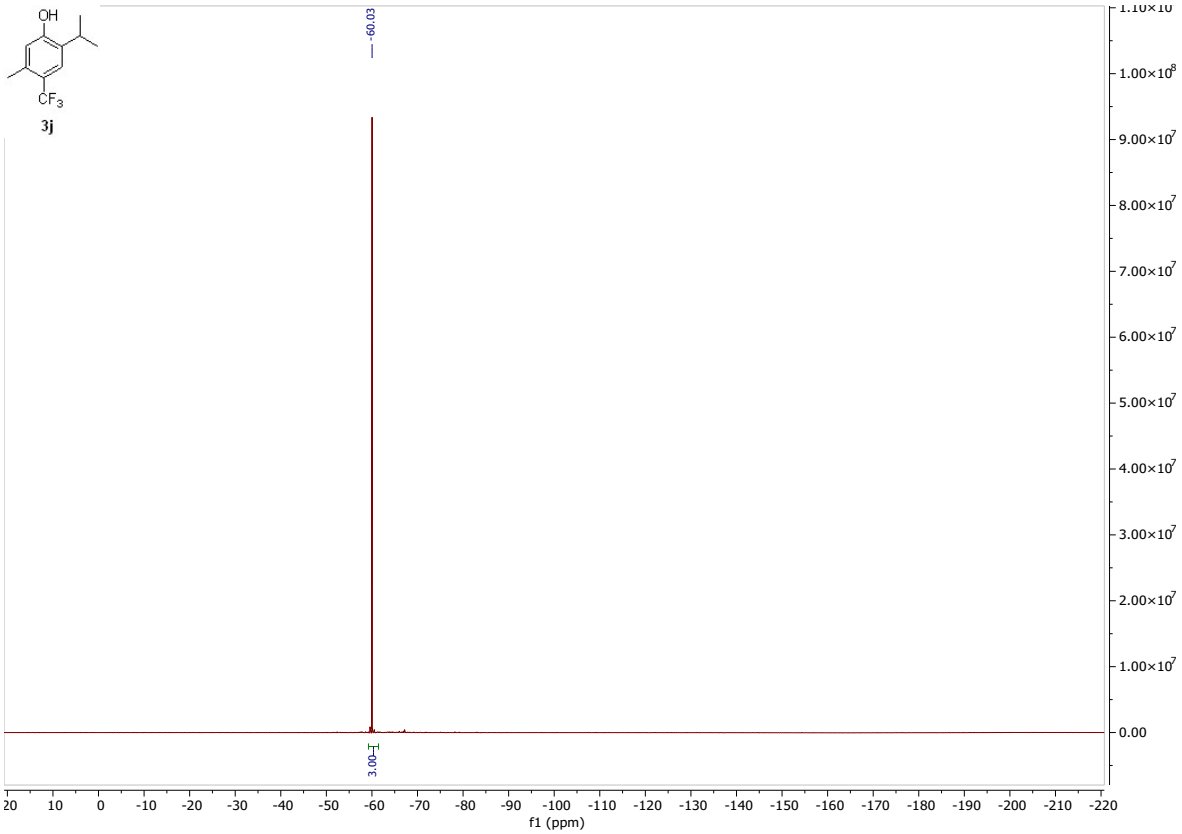
**2-isopropyl-5-methyl-4-(trifluoromethyl)phenol (3j), HSQC ($CDCl_3$)**



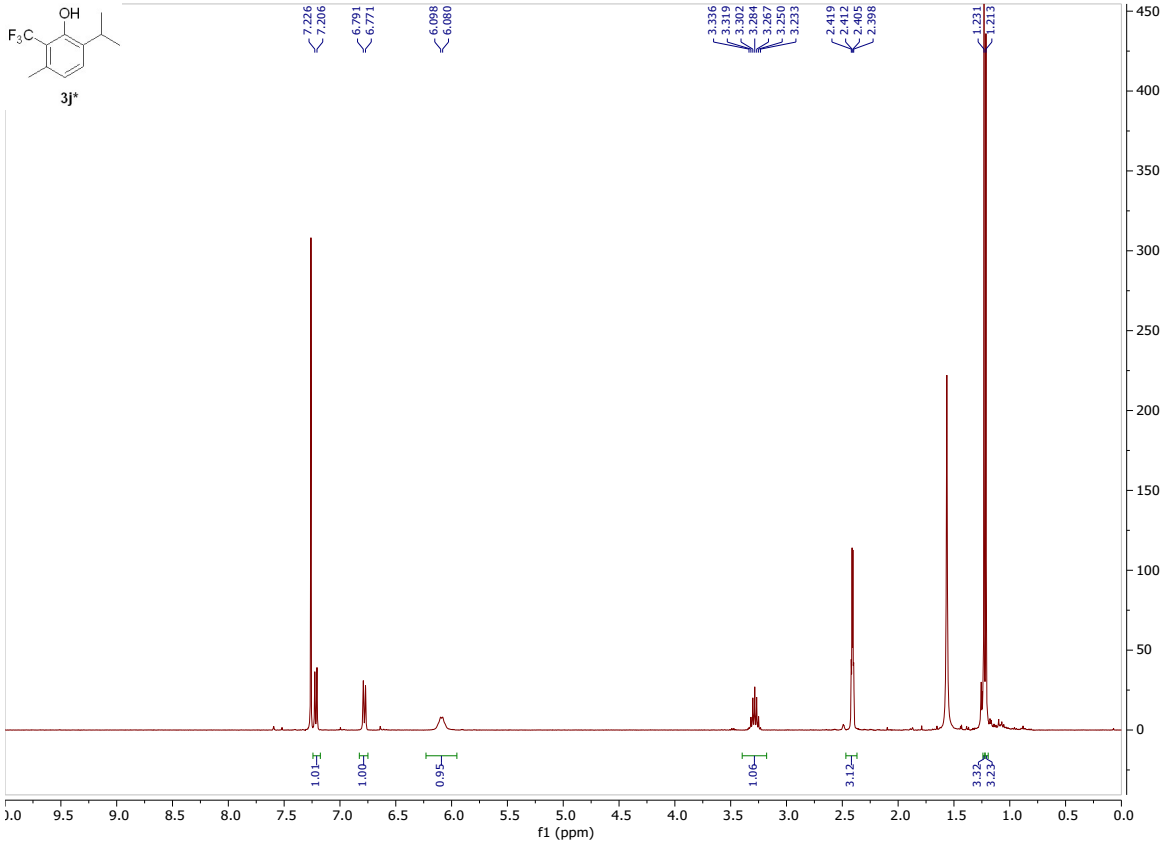

**2-isopropyl-5-methyl-4-(trifluoromethyl)phenol (3j), HMBC ($CDCl_3$)**



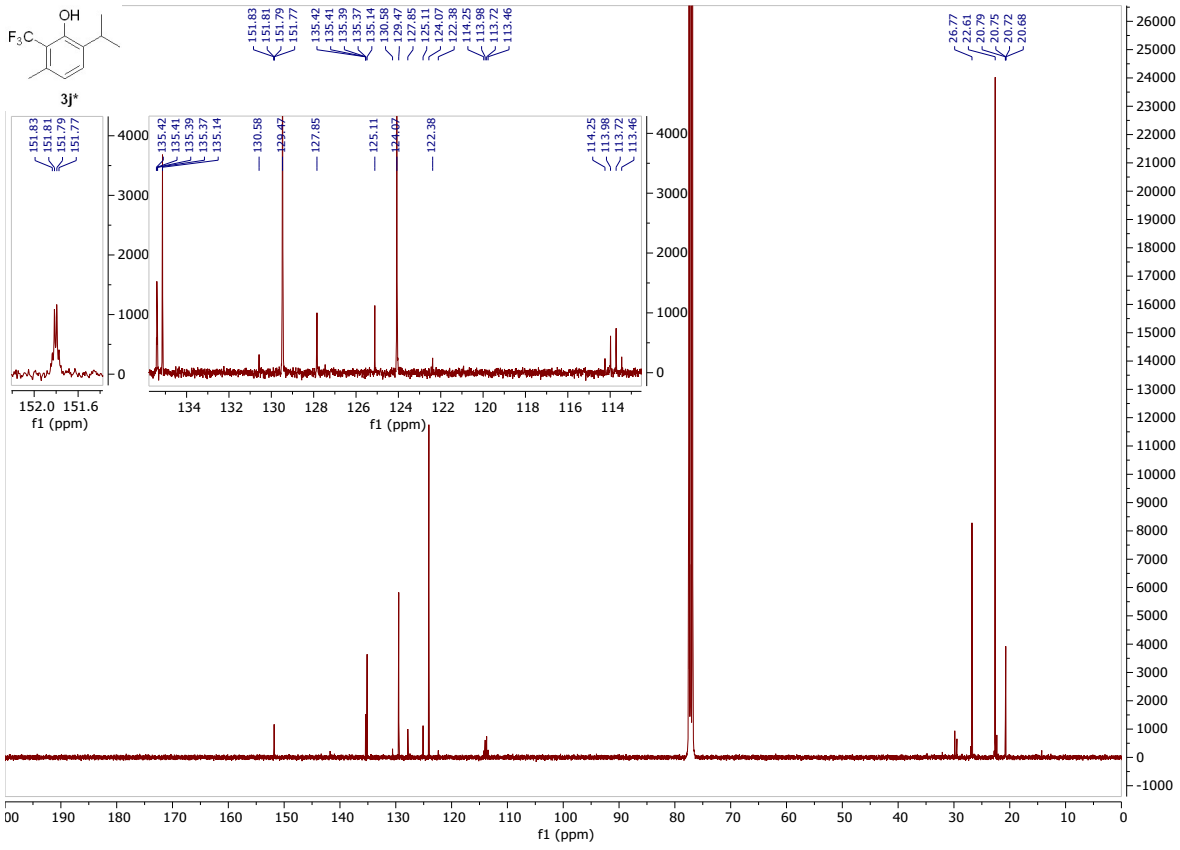
**2-isopropyl-5-methyl-4-(trifluoromethyl)phenol (3j), $^{19}F$ NMR (376 MHz, $CDCl_3$)**



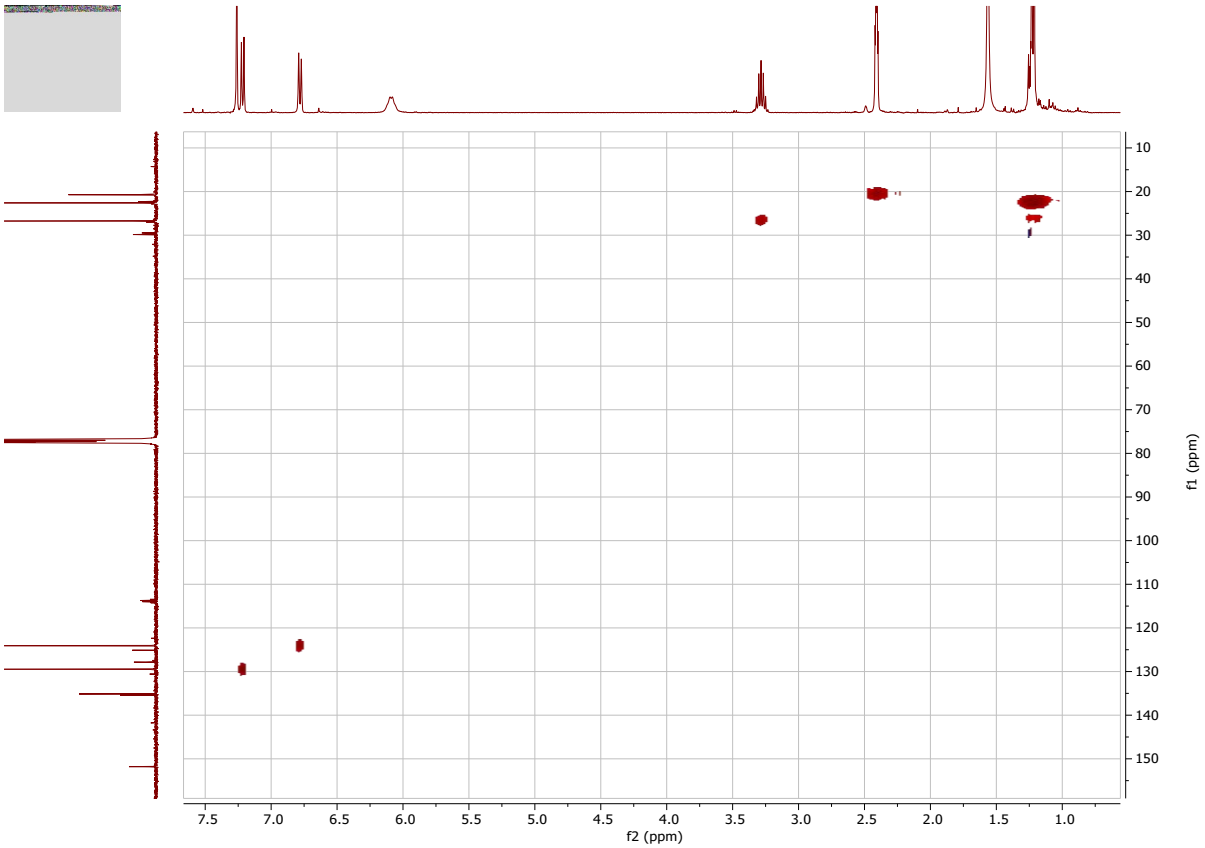
**6-isopropyl-3-methyl-2-(trifluoromethyl)phenol (3j*), $^{1}H$ NMR (400 MHz, $CDCl_3$)**



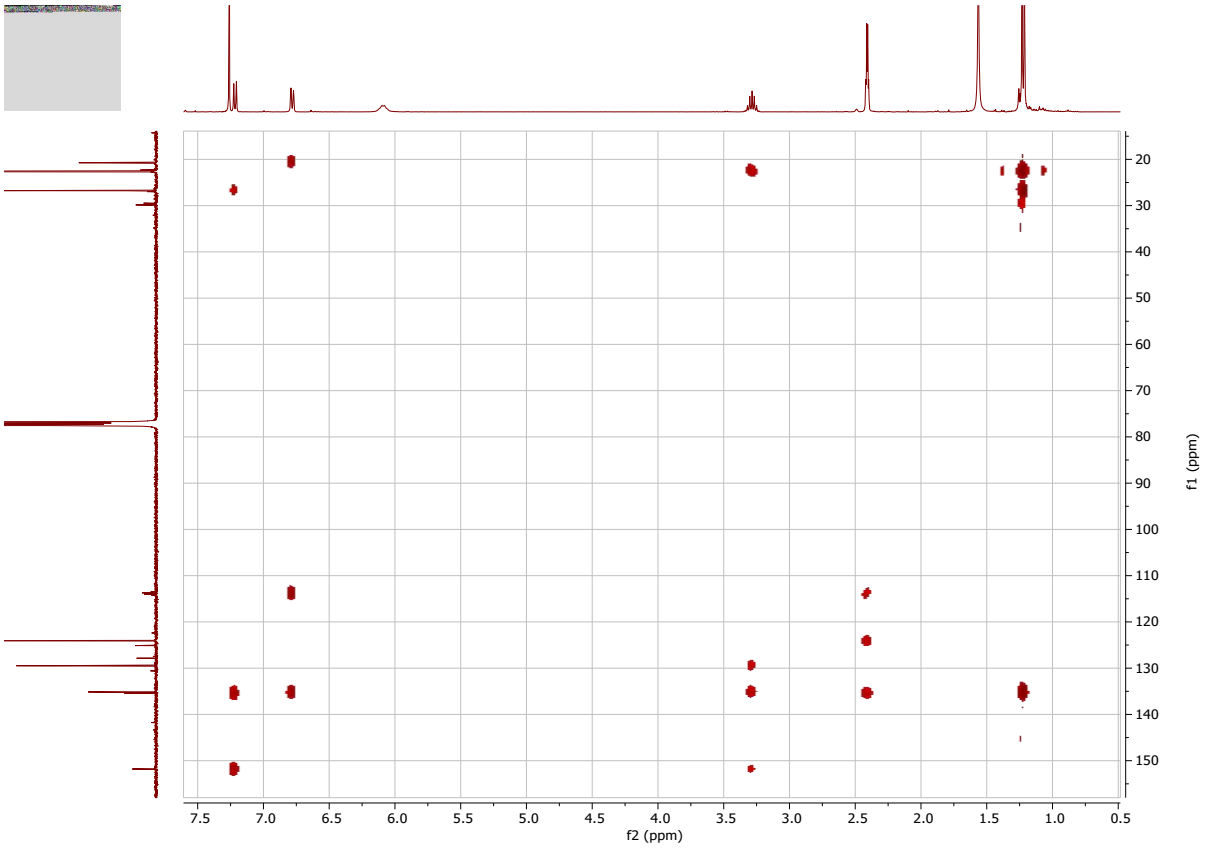

**6-isopropyl-3-methyl-2-(trifluoromethyl)phenol (3j*), $^{13}C$ NMR (101 MHz, $CDCl_3$)**



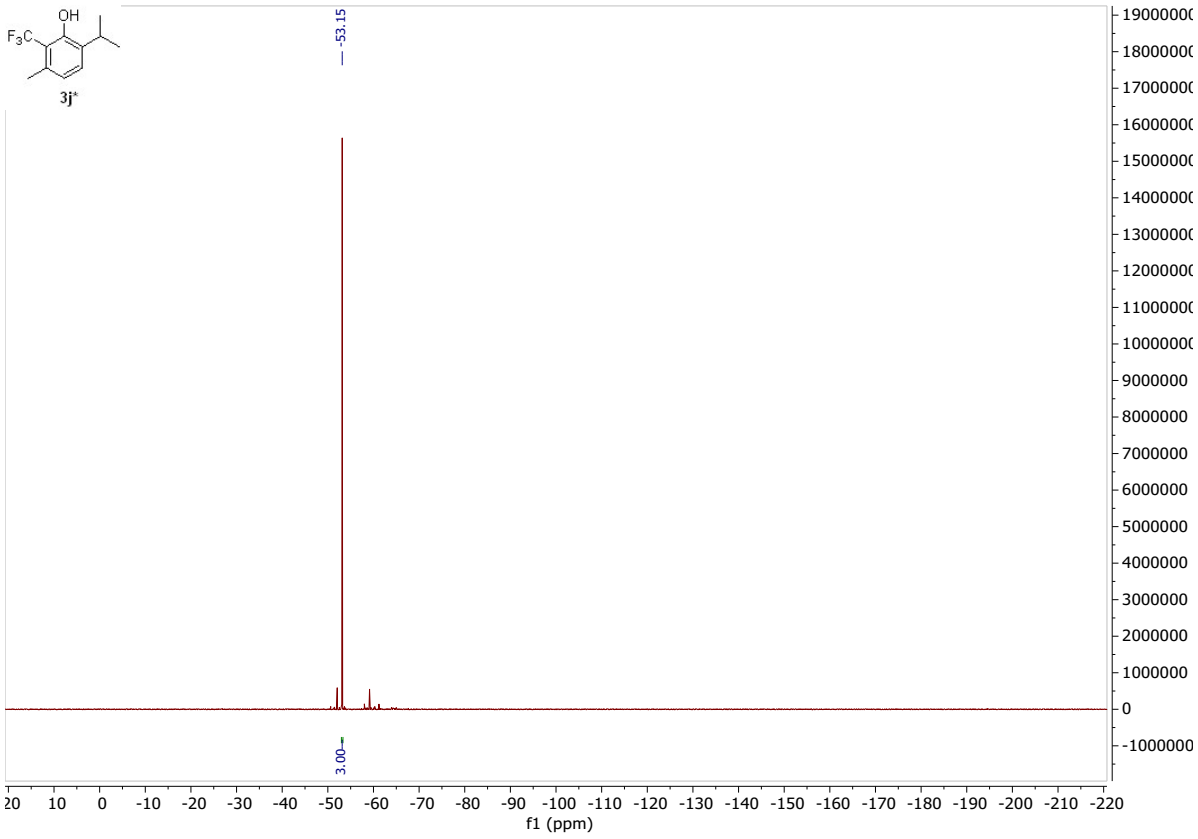
**6-isopropyl-3-methyl-2-(trifluoromethyl)phenol (3j*), HSQC ($CDCl_3$)**



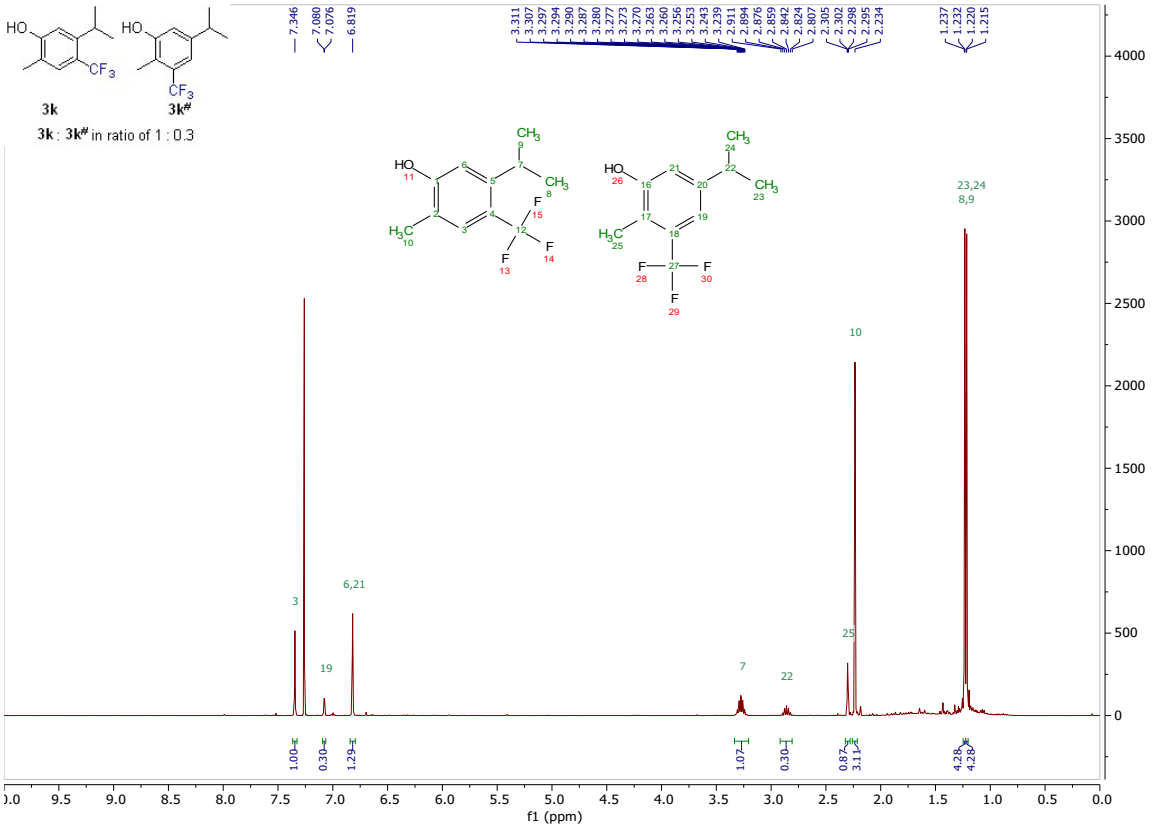

**6-isopropyl-3-methyl-2-(trifluoromethyl)phenol (3j*), HMBC ($CDCl_3$)**



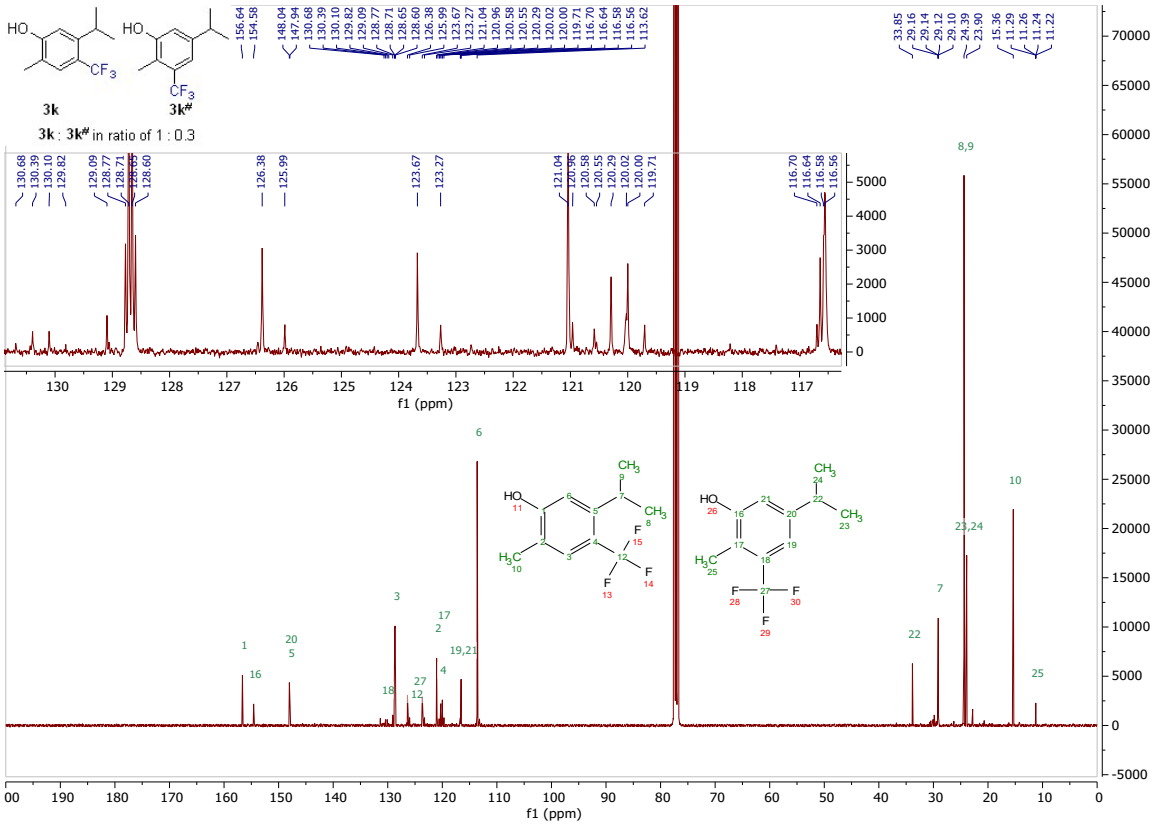
**6-isopropyl-3-methyl-2-(trifluoromethyl)phenol (3j*), $^{19}F$ NMR (376 MHz, $CDCl_3$)**



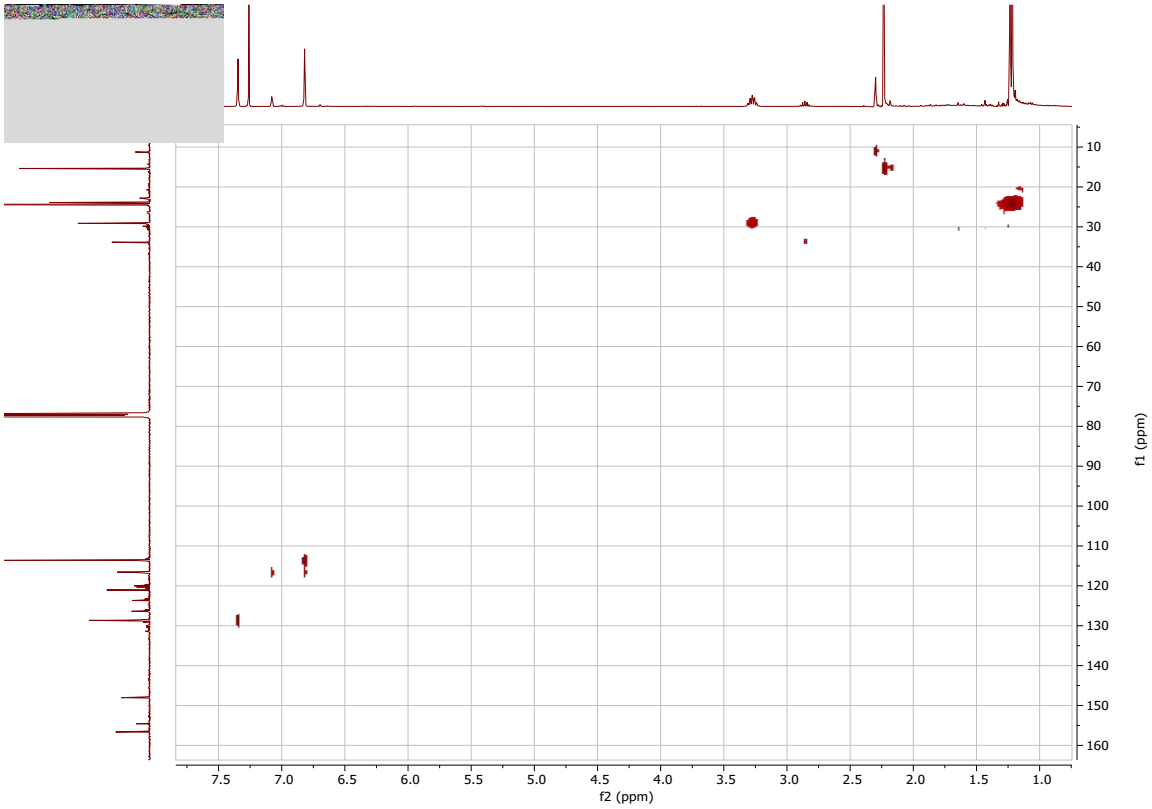
**5-isopropyl-2-methyl-4-(trifluoromethyl)phenol : 5-isopropyl-2-methyl-3-(trifluoromethyl)phenol (3k : 3k$^{\#}$ in ratio of 1 : 0.3), $^{1}$H NMR (400 MHz, $CDCl_3$)**



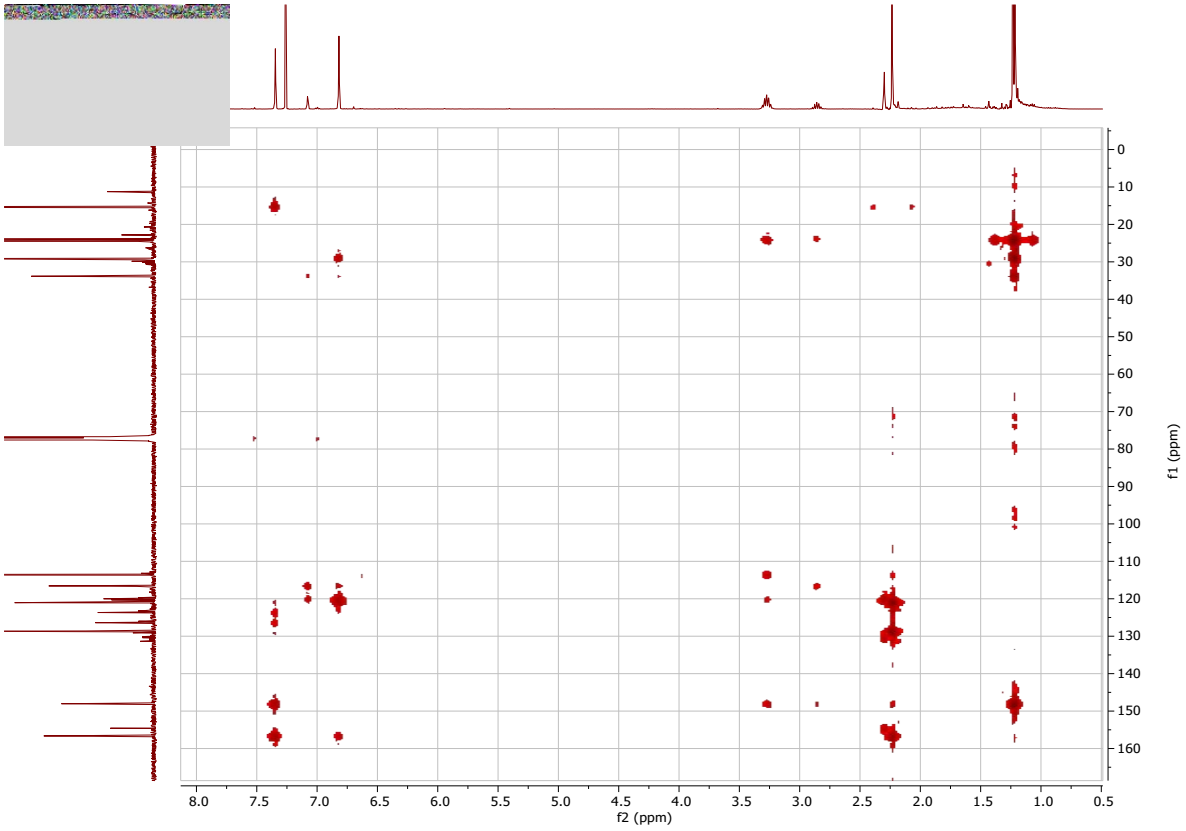

**5-isopropyl-2-methyl-4-(trifluoromethyl)phenol : 5-isopropyl-2-methyl-3-(trifluoromethyl)phenol (3k : 3k$^{\#}$ in ratio of 1 : 0.3), $^{13}$C NMR (101 MHz, $CDCl_3$)**



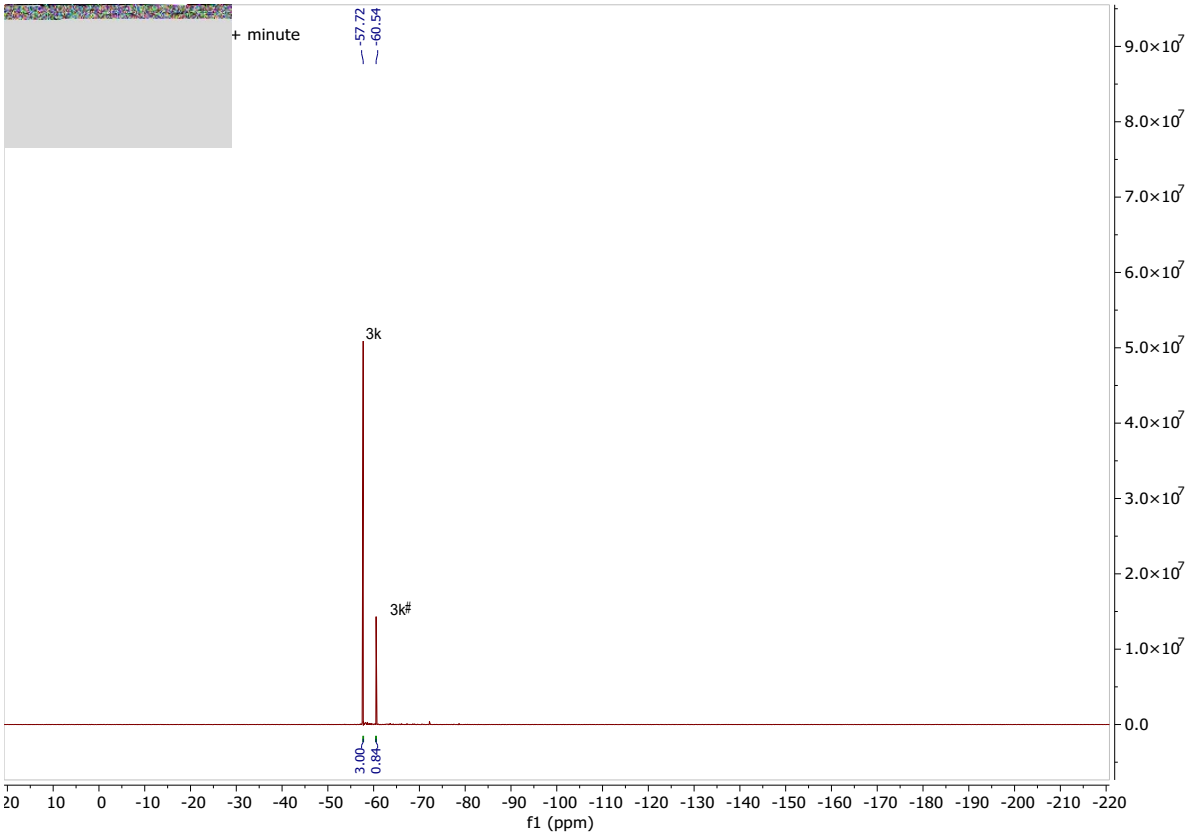
**5-isopropyl-2-methyl-4-(trifluoromethyl)phenol : 5-isopropyl-2-methyl-3-(trifluoromethyl)phenol (3k : 3k# in ratio of 1 : 0.3), HSQC ($CDCl_3$)**



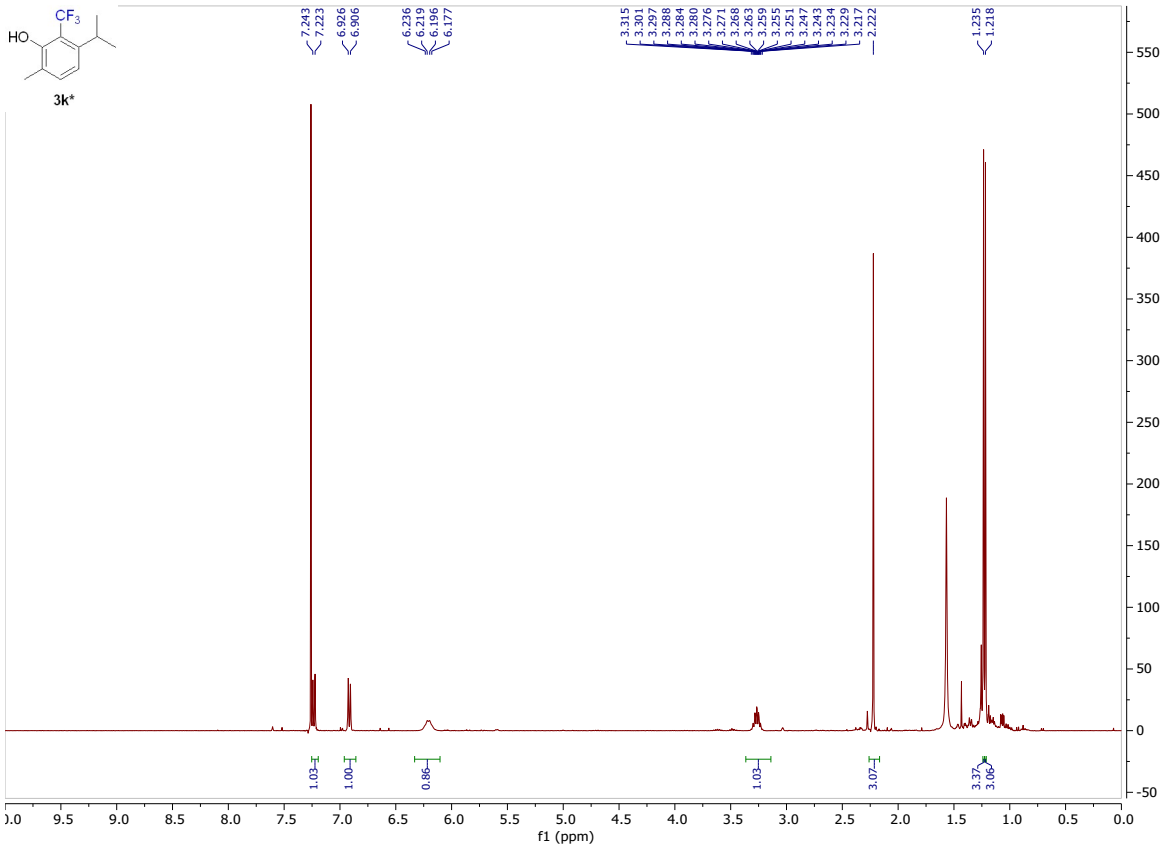

**5-isopropyl-2-methyl-4-(trifluoromethyl)phenol : 5-isopropyl-2-methyl-3-(trifluoromethyl)phenol (3k : 3k# in ratio of 1 : 0.3), HMBC ($CDCl_3$)**



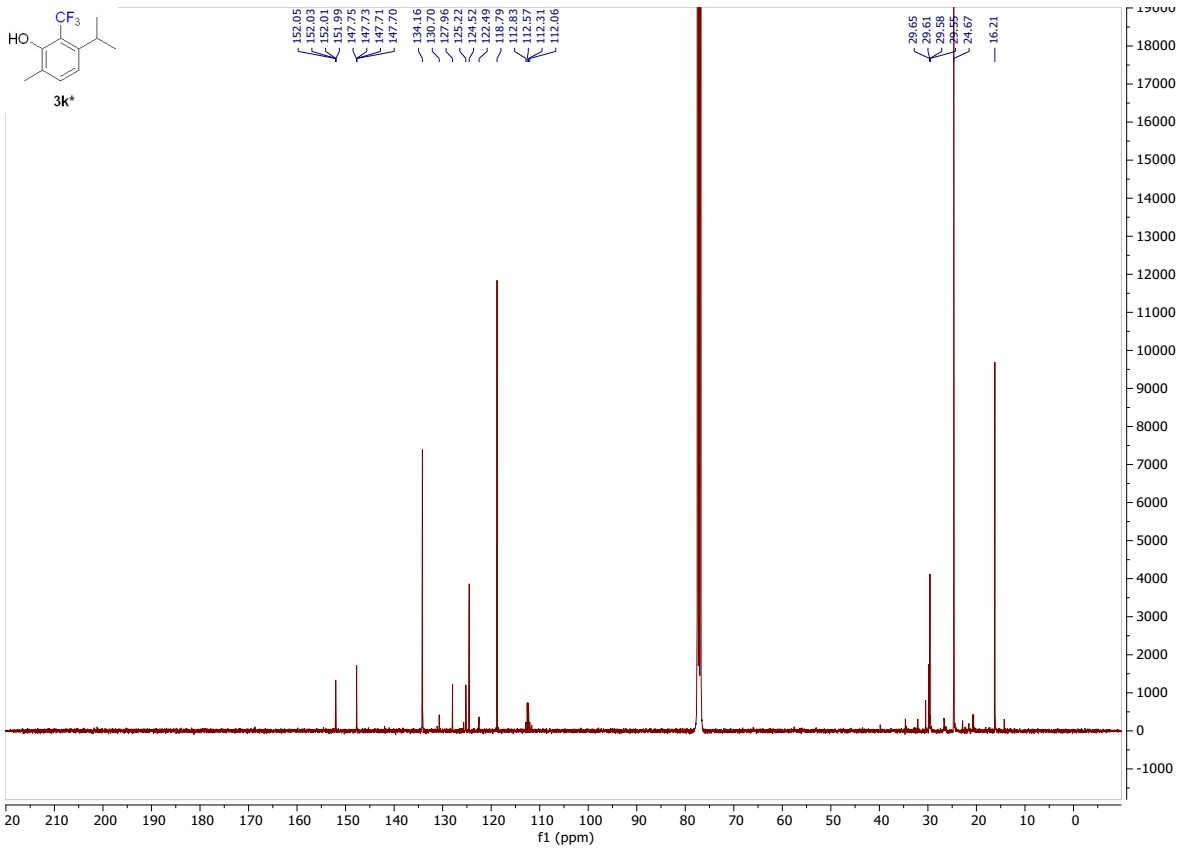
**5-isopropyl-2-methyl-4-(trifluoromethyl)phenol : 5-isopropyl-2-methyl-3-(trifluoromethyl)phenol (3k : 3k# in ratio of 1 : 0.3), $^{19}F$ NMR (376 MHz, $CDCl_3$)**



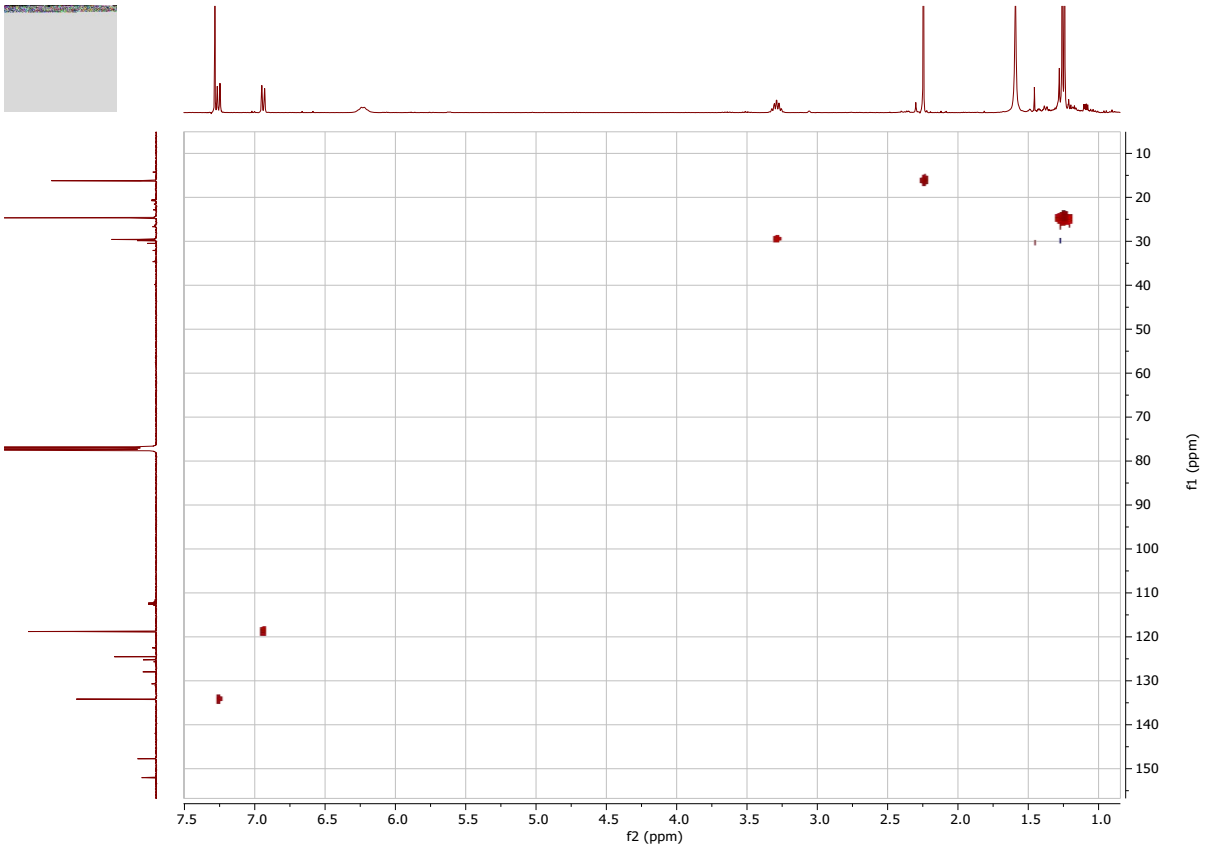
**3-isopropyl-6-methyl-2-(trifluoromethyl)phenol(3k*), $^{1}H$ NMR (400 MHz, $CDCl_3$)**



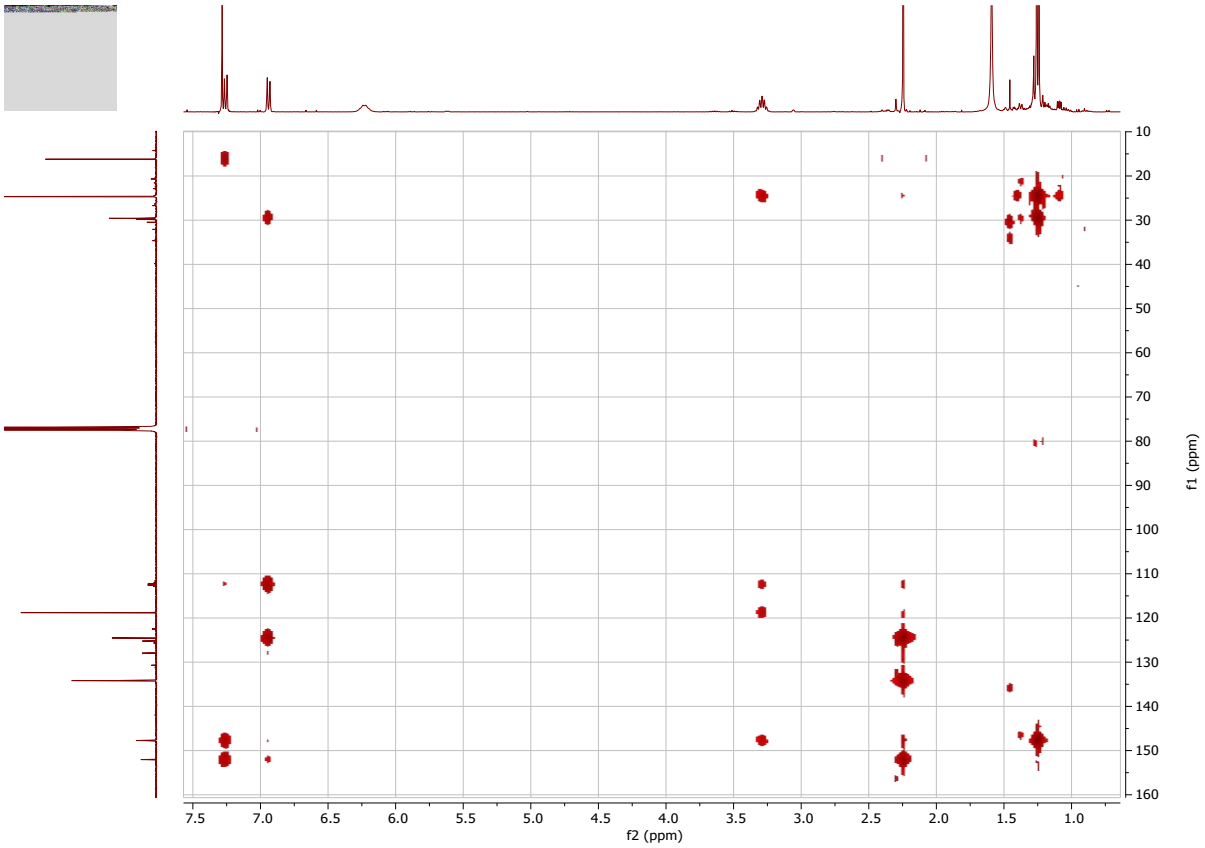

**3-isopropyl-6-methyl-2-(trifluoromethyl)phenol(3k*), $^{13}C$ NMR (101 MHz, $CDCl_3$)**



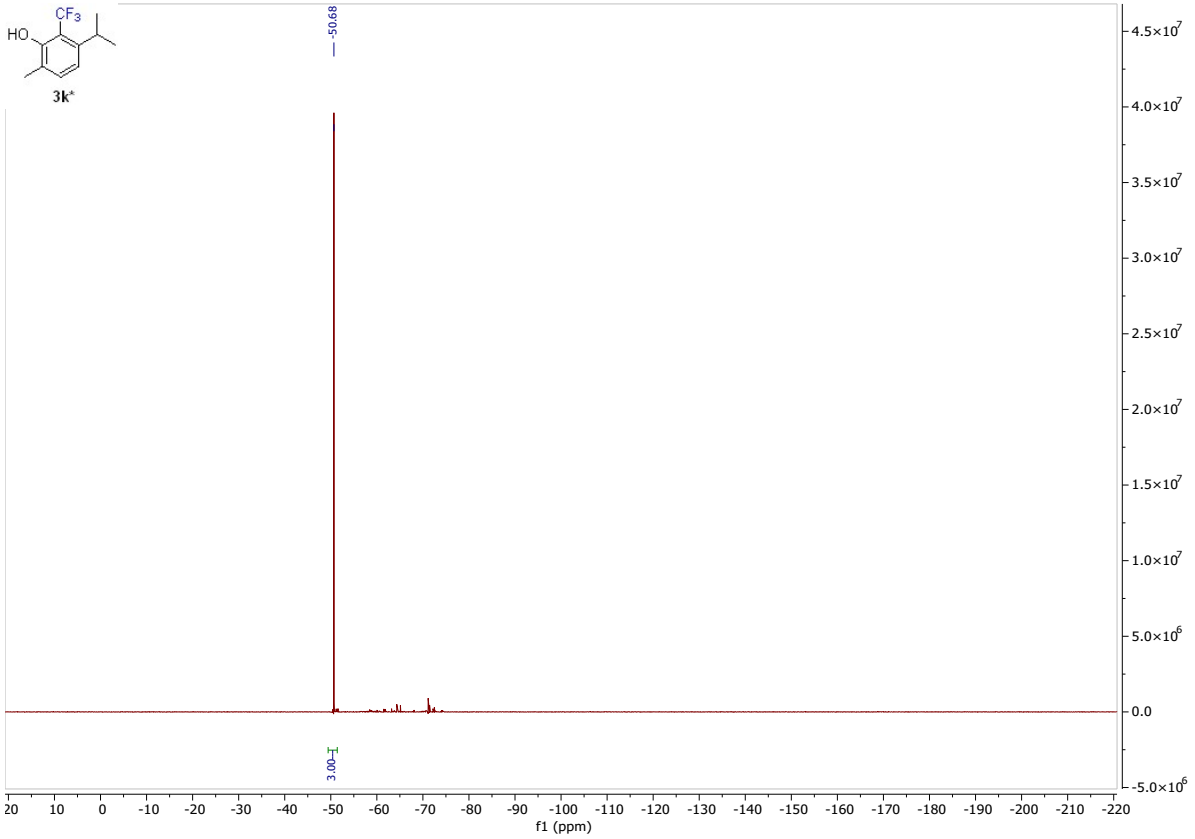
**3-isopropyl-6-methyl-2-(trifluoromethyl)phenol(3k*), HSQC ($CDCl_3$)**



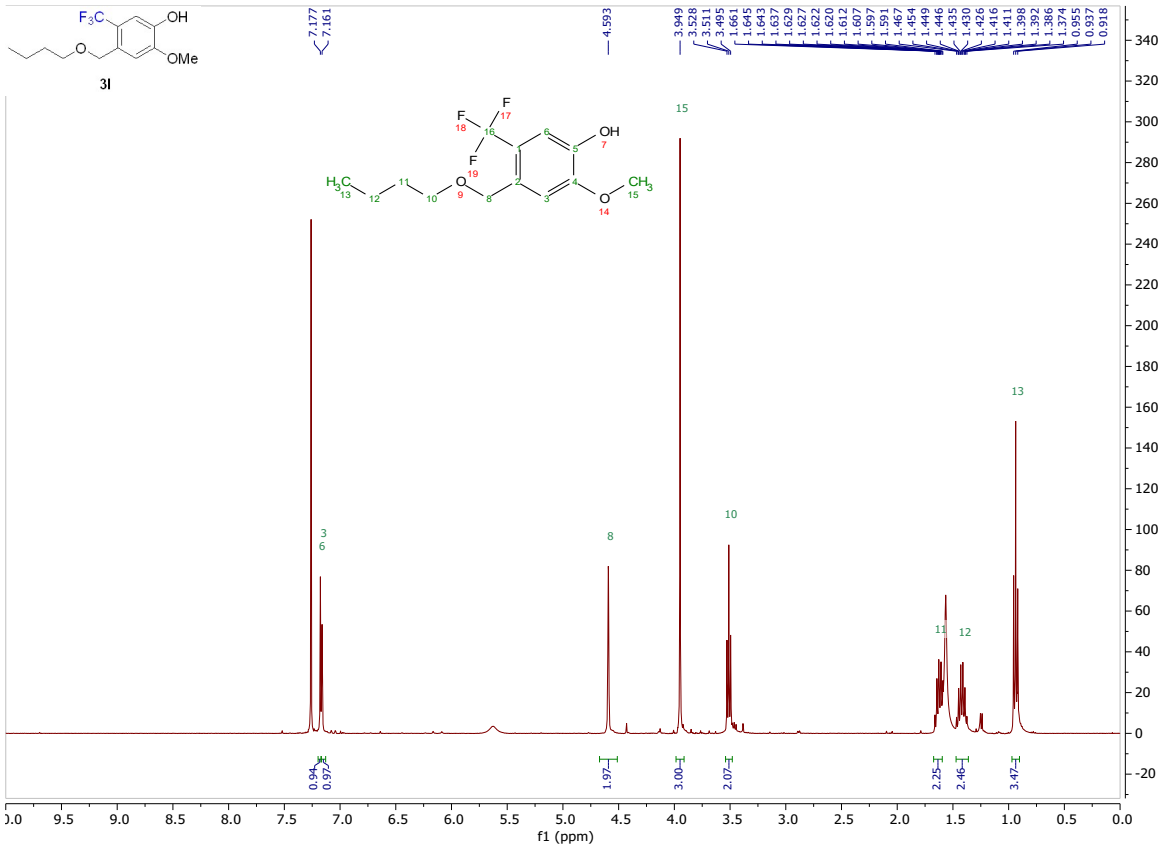

**3-isopropyl-6-methyl-2-(trifluoromethyl)phenol(3k*), HMBC ($CDCl_3$)**



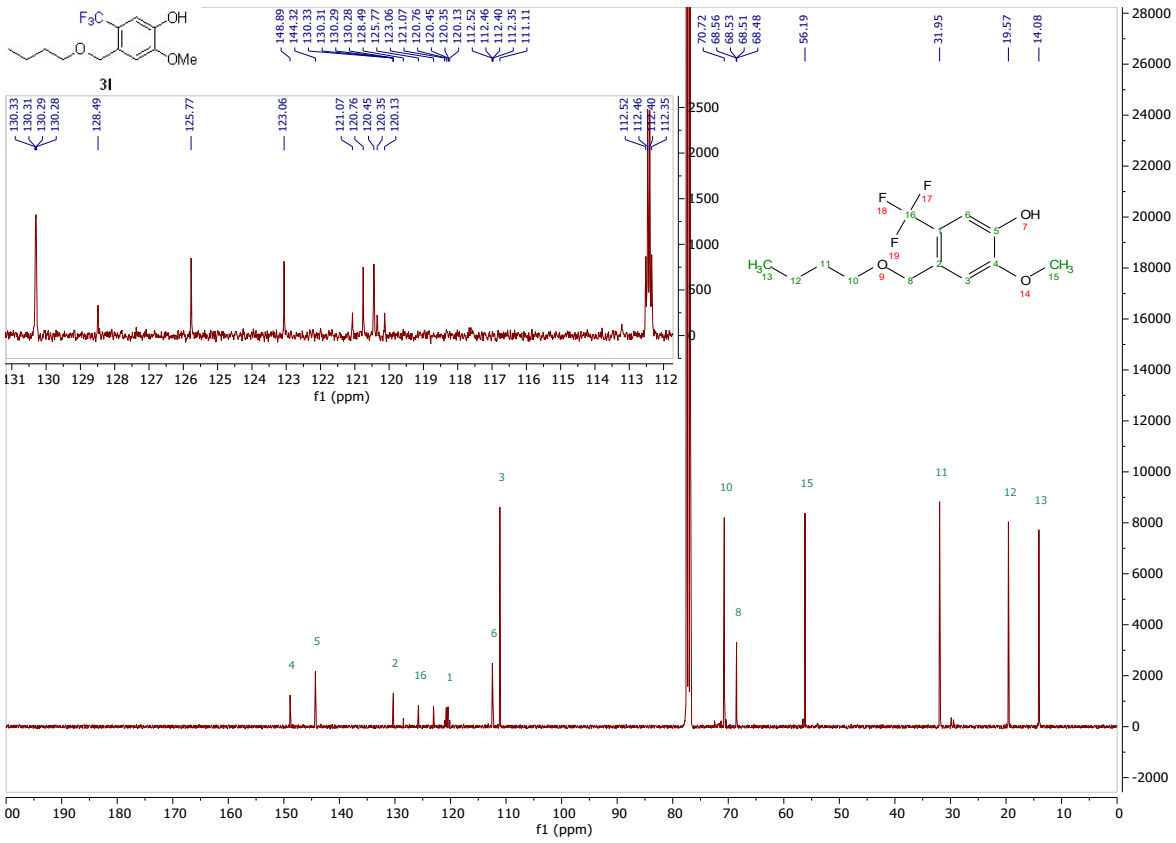
**3-isopropyl-6-methyl-2-(trifluoromethyl)phenol(3k*), $^{19}F$ NMR (376 MHz, $CDCl_3$)**



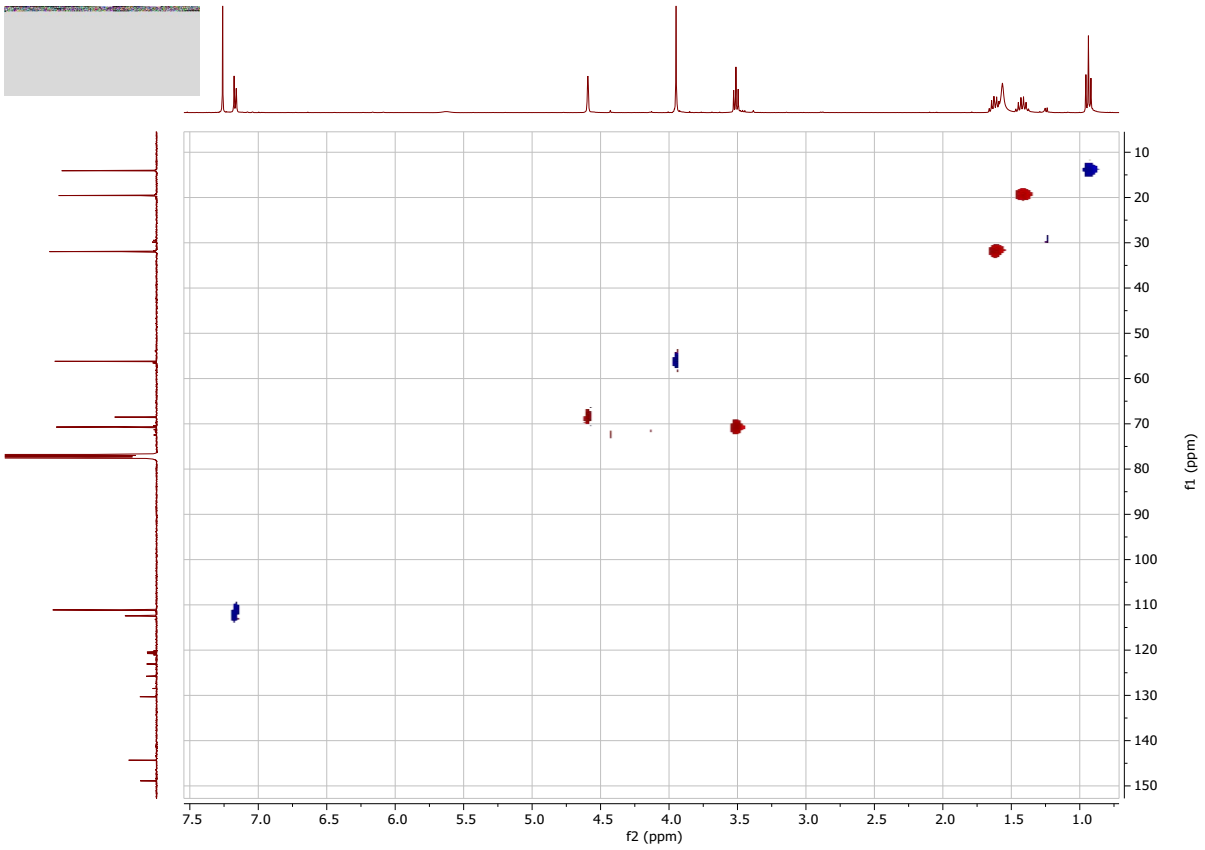
**4-(butoxymethyl)-2-methoxy-5-(trifluoromethyl)phenol (3l), $^{1}$H NMR (400 MHz, $CDCl_3$)**



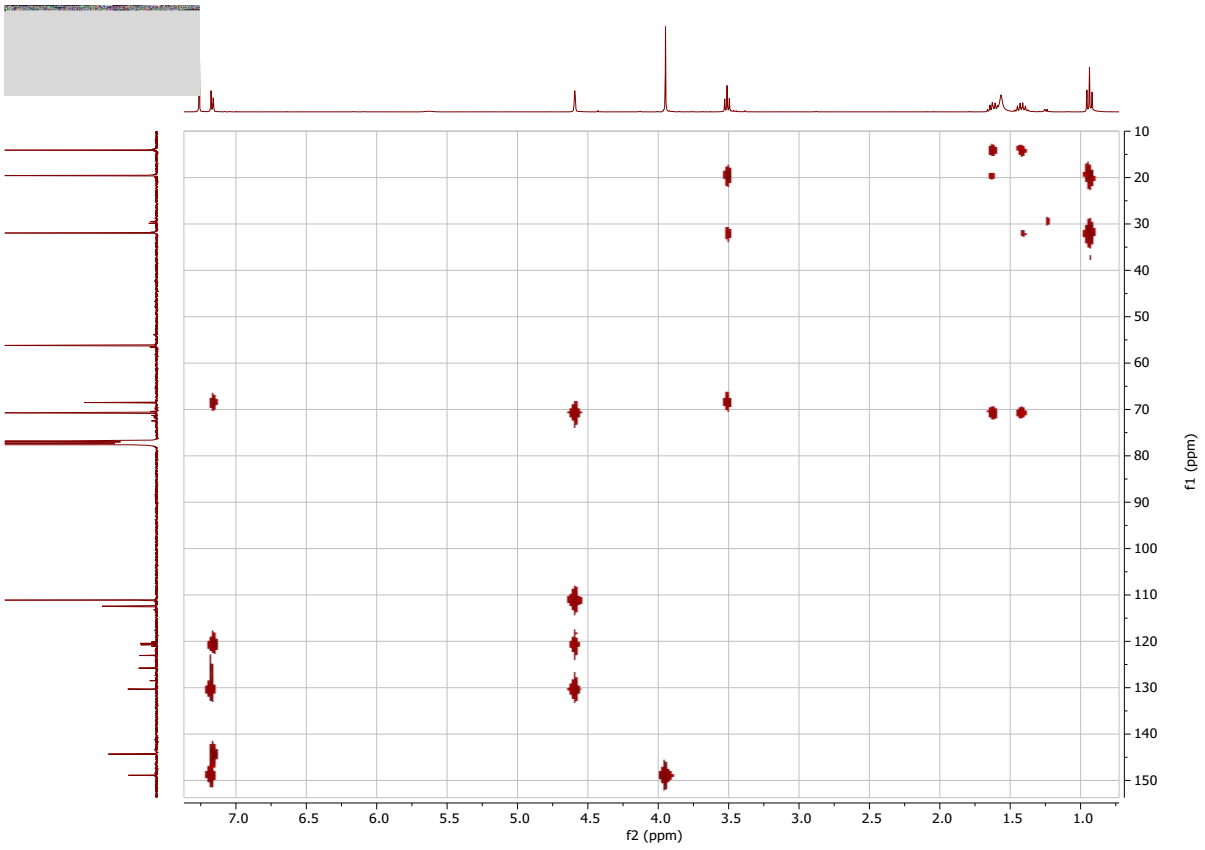

**4-(butoxymethyl)-2-methoxy-5-(trifluoromethyl)phenol (3l), $^{13}$C NMR (101 MHz, $CDCl_3$)**



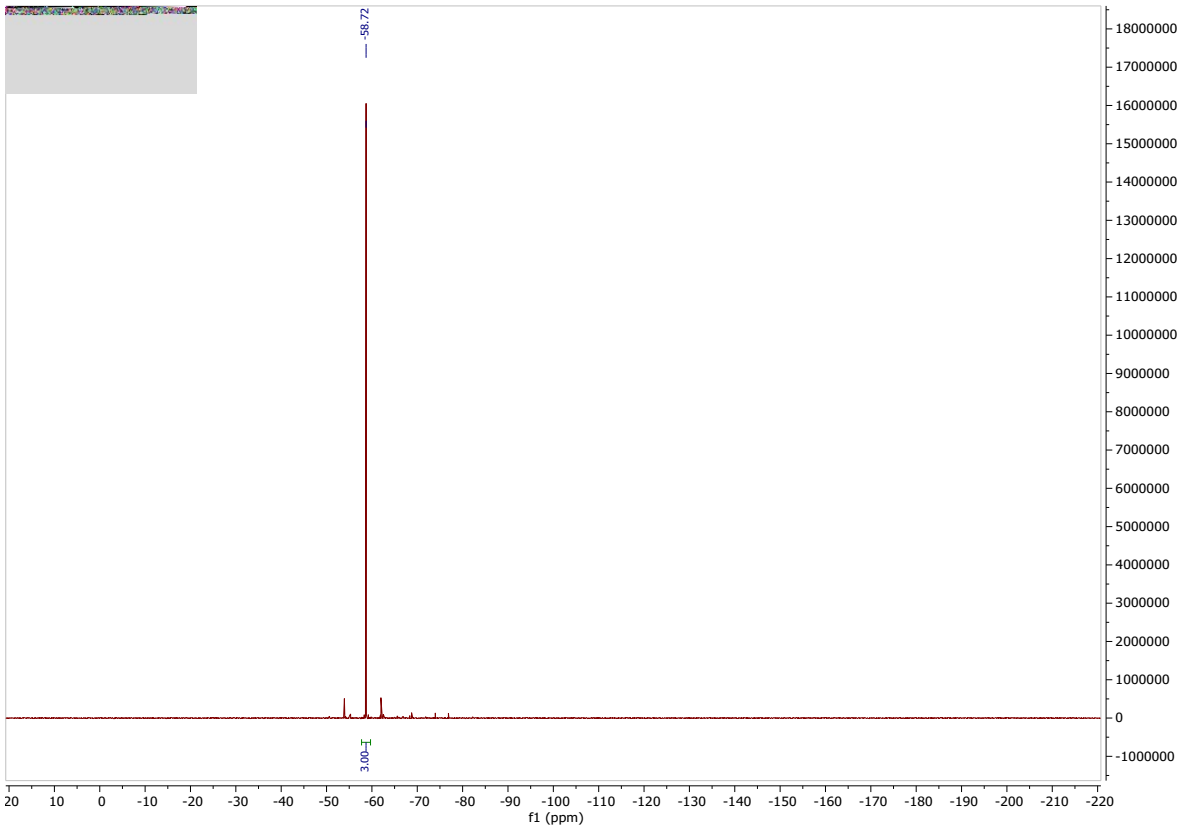
**4-(butoxymethyl)-2-methoxy-5-(trifluoromethyl)phenol (3l), HSQC ($CDCl_3$)**



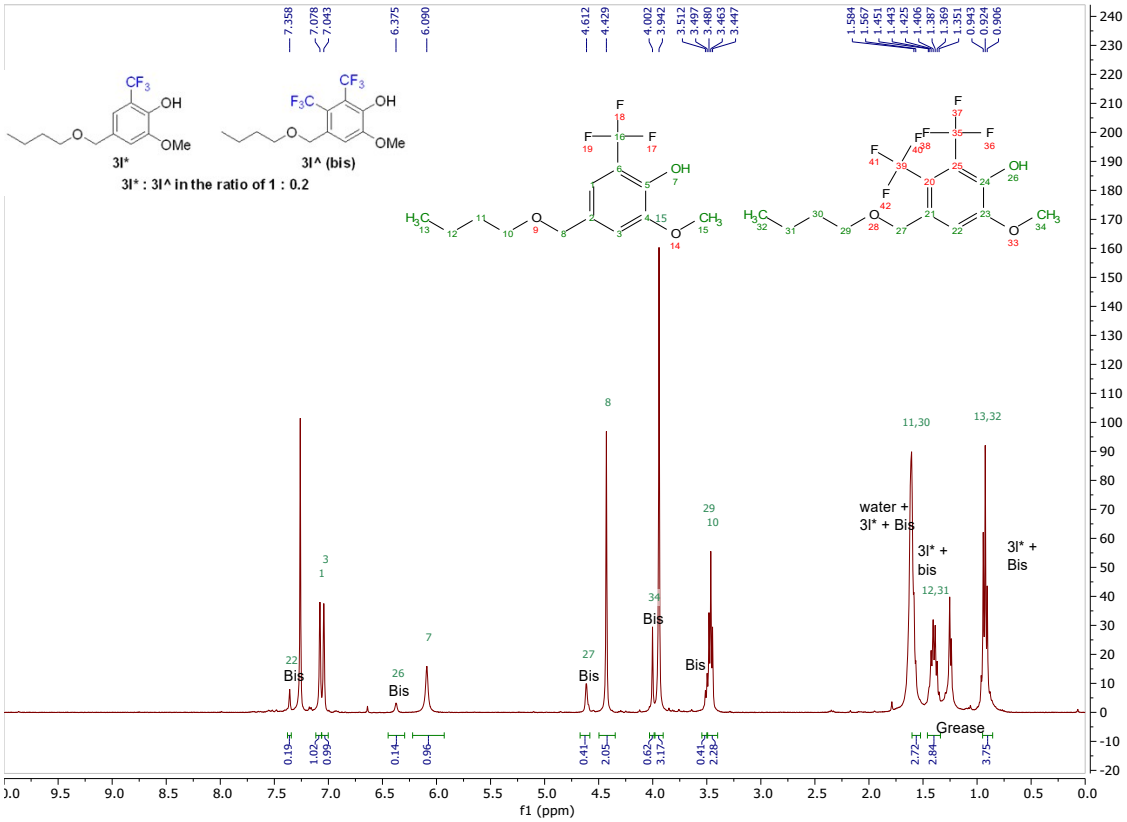

**4-(butoxymethyl)-2-methoxy-5-(trifluoromethyl)phenol (3l), HMBC ($CDCl_3$)**



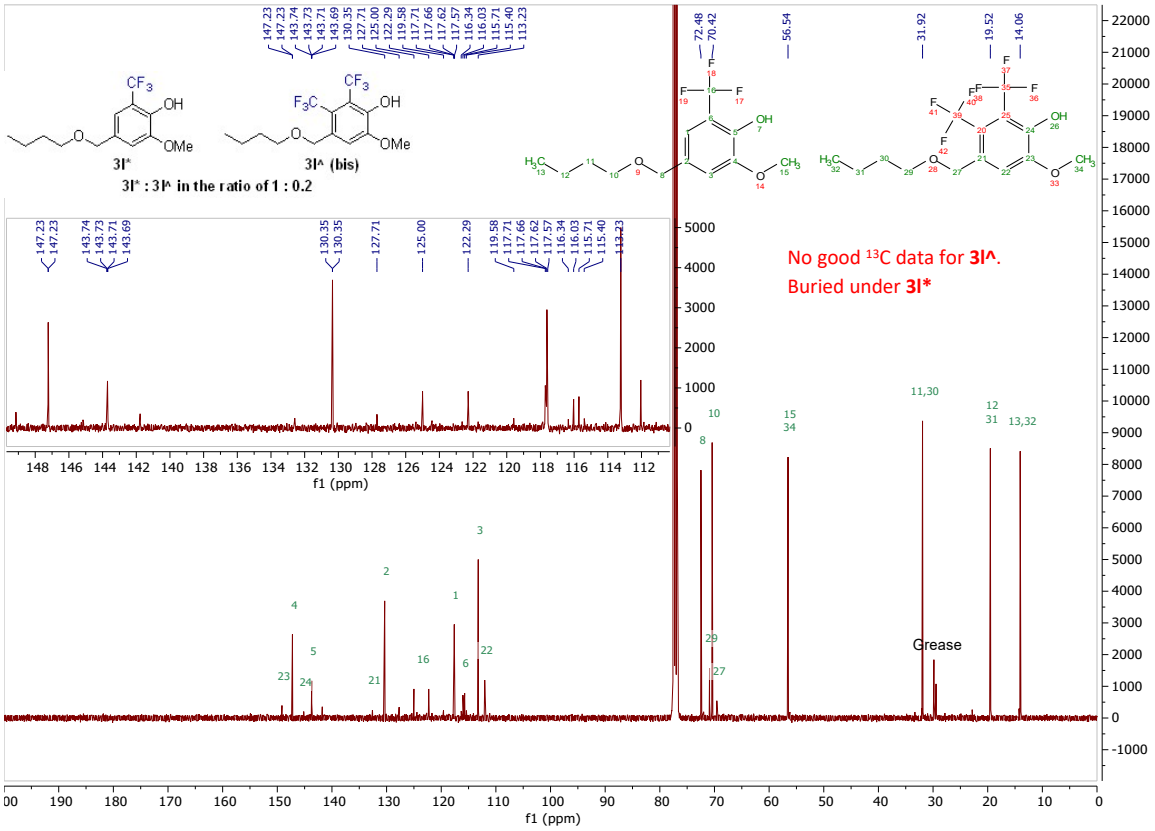
**4-(butoxymethyl)-2-methoxy-5-(trifluoromethyl)phenol (3l), $^{19}F$ NMR (376 MHz, $CDCl_3$)**



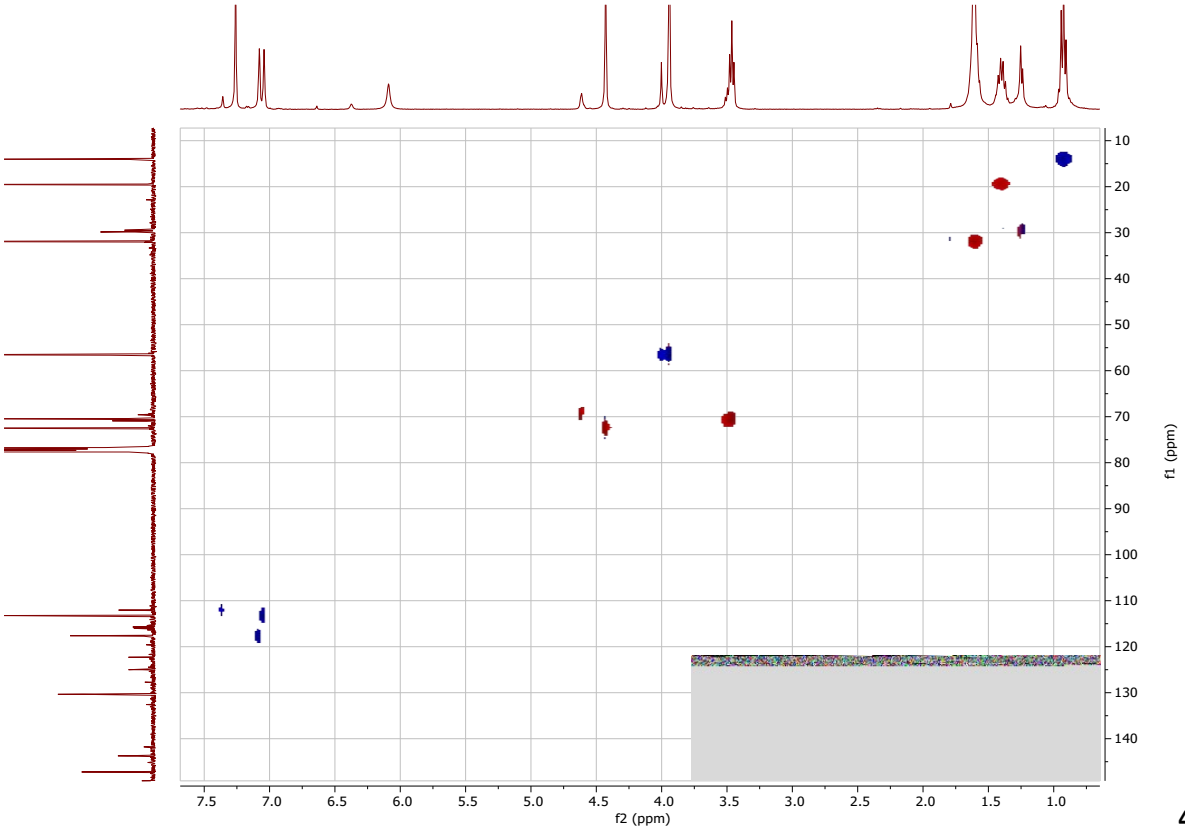
**4-(butoxymethyl)-2-methoxy-6-(trifluoromethyl)phenol : 4-(butoxymethyl)-6-methoxy-2,3-bis(trifluoromethyl)phenol (3l*: 3l^ in the ratio of 1 : 0.2), $^{1}$H NMR (400 MHz, $CDCl_3$)**



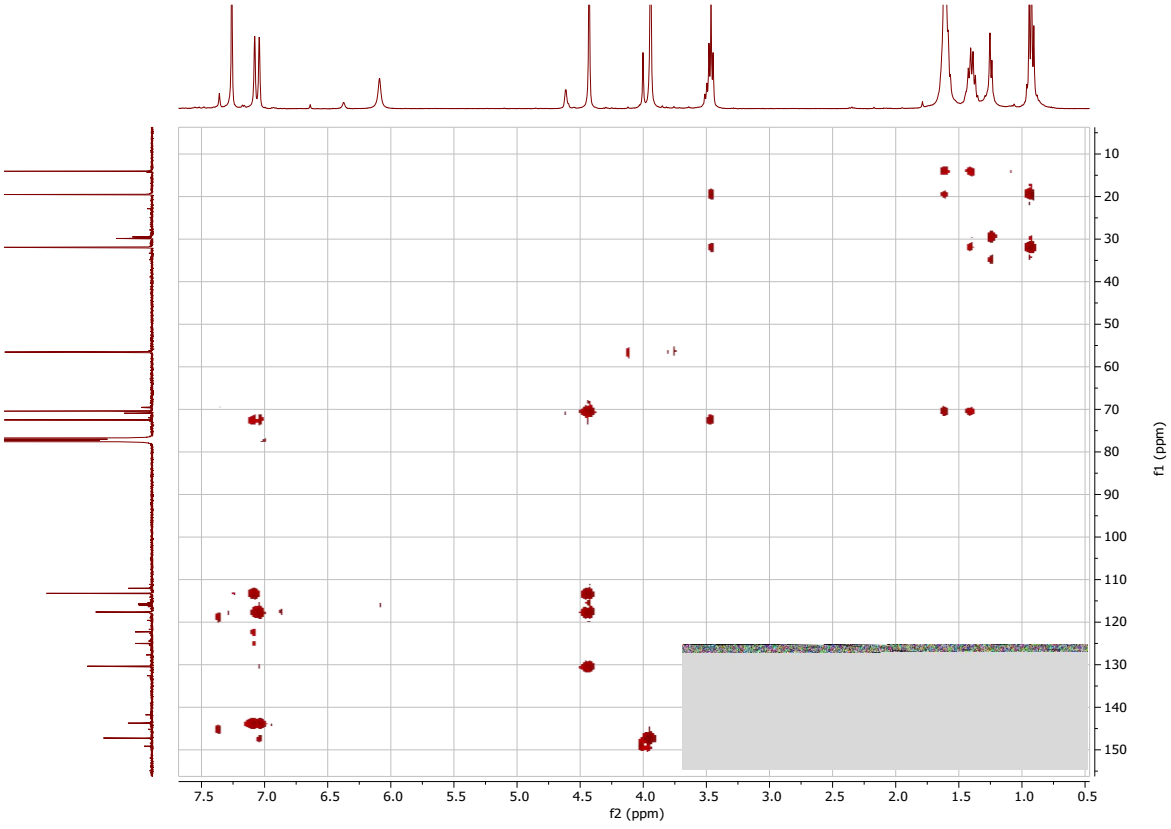

**4(butoxymethyl)-2-methoxy-6-(trifluoromethyl)phenol : 4-(butoxymethyl)-6-methoxy-2,3-bis(trifluoromethyl)phenol (3l*: 3l^ in the ratio of 1 : 0.2), $^{13}$C NMR (101 MHz, $CDCl_3$)**



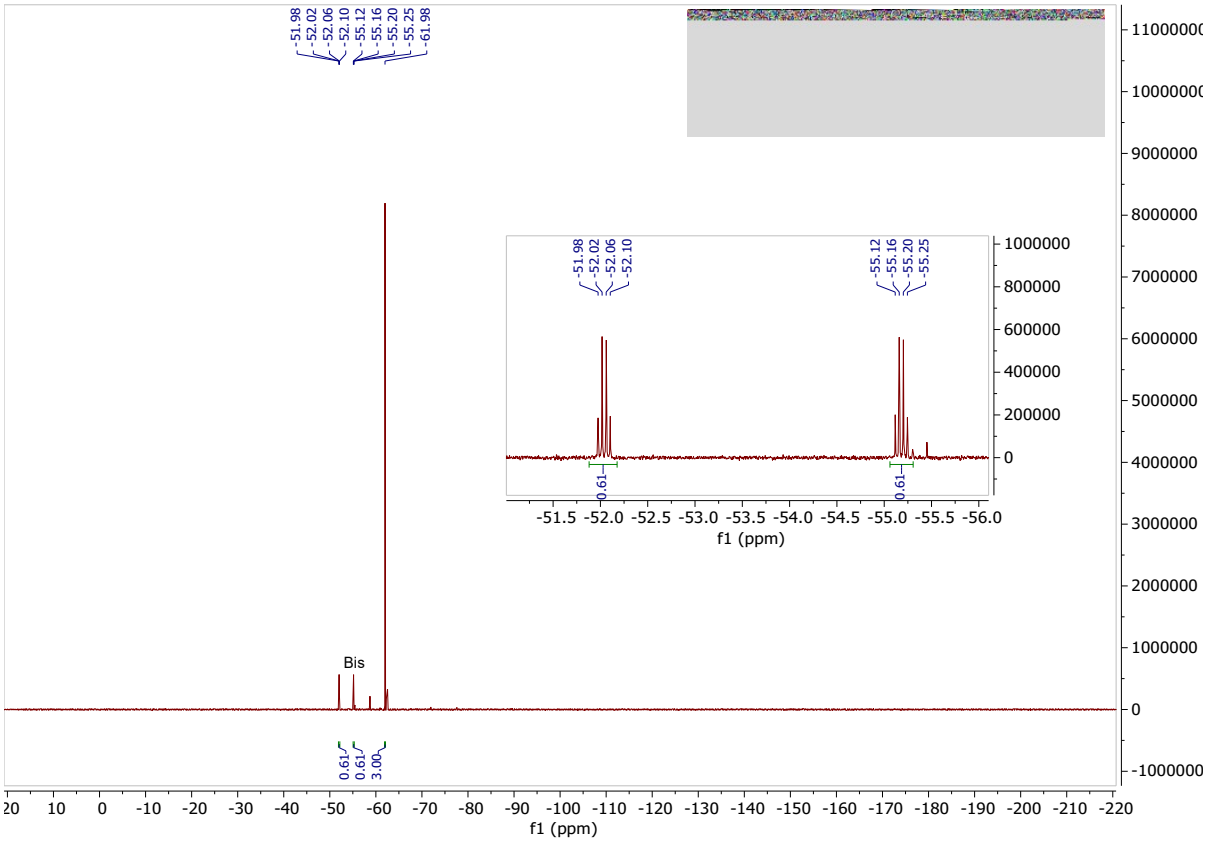
**44-(butoxymethyl)-2-methoxy-6-(trifluoromethyl)phenol : 4-(butoxymethyl)-6-methoxy-2,3-bis(trifluoromethyl)phenol (3l*: 3l^ in the ratio of 1 : 0.2), HSQC ($CDCl_3$)**



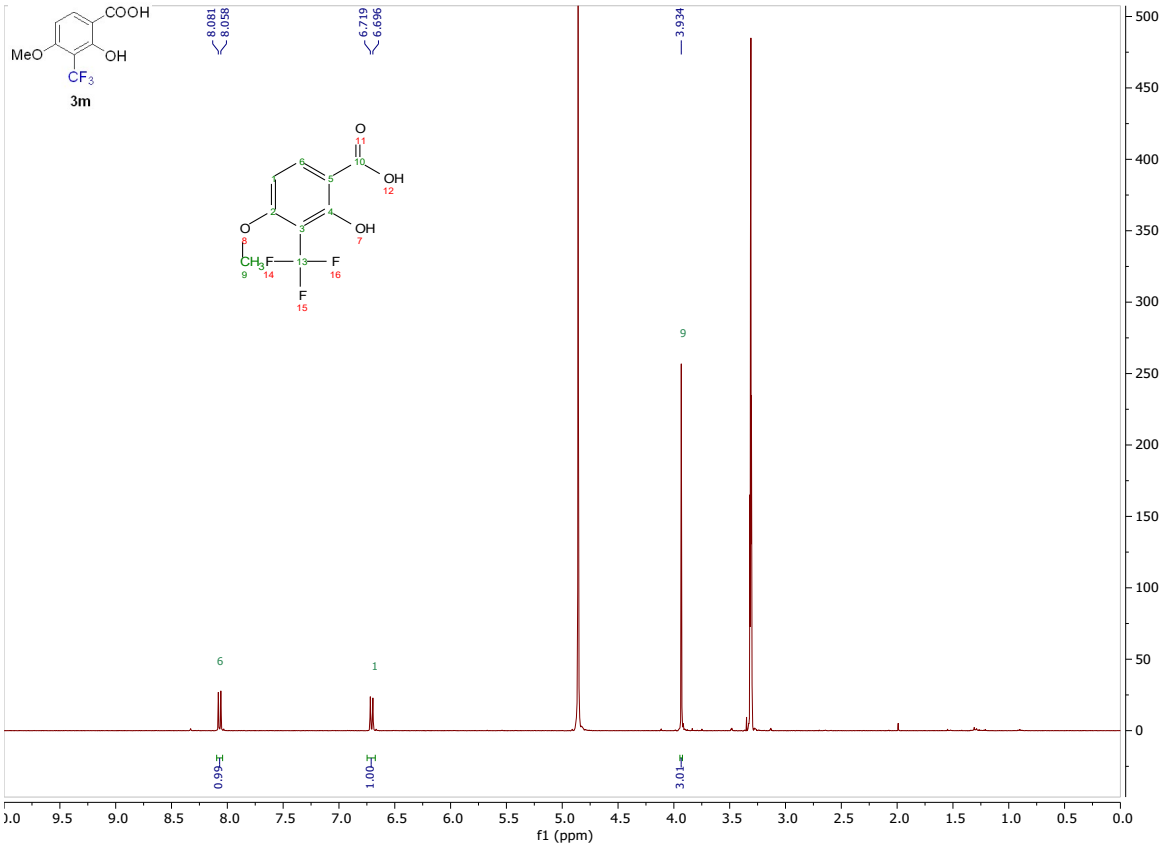

**4-(butoxymethyl)-2-methoxy-6-(trifluoromethyl)phenol : 4-(butoxymethyl)-6-methoxy-2,3-bis(trifluoromethyl)phenol (3l*: 3l^ in the ratio of 1 : 0.2), HMBC ($CDCl_3$)**



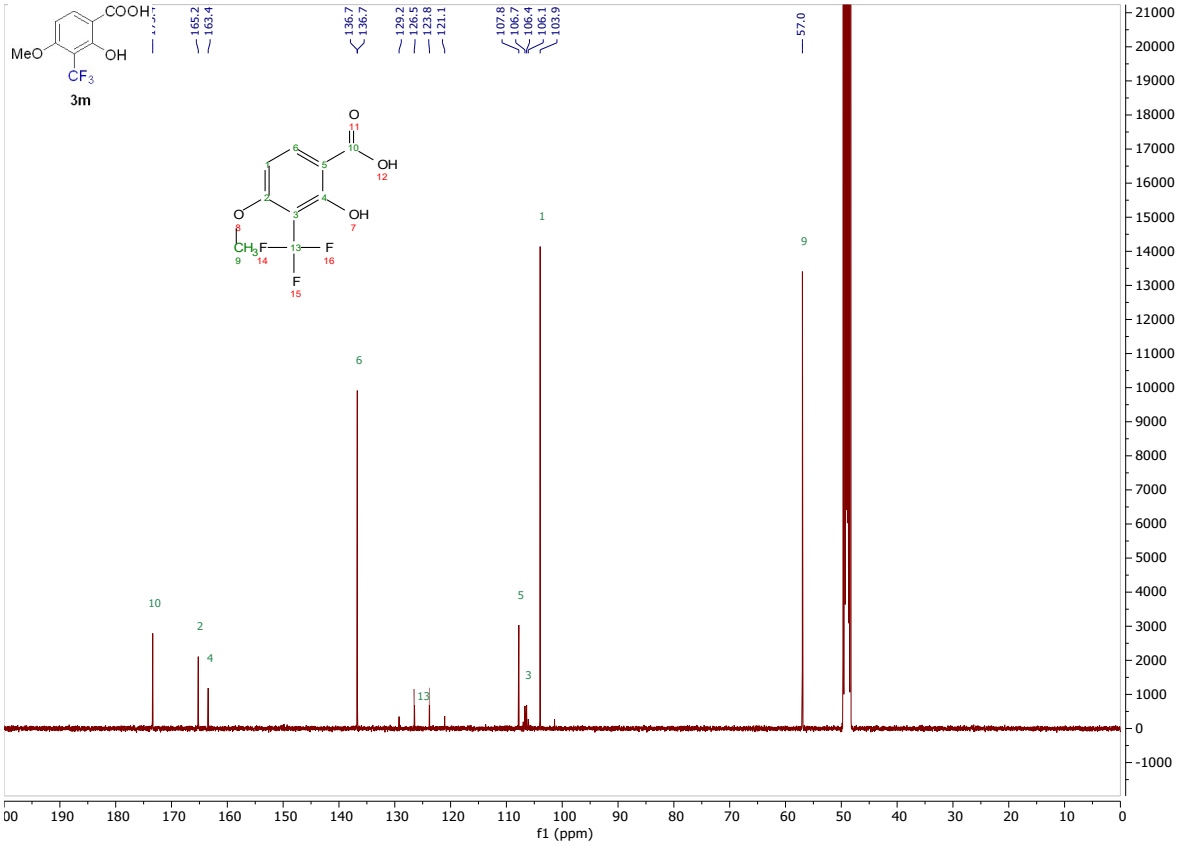
**4-(butoxymethyl)-2-methoxy-6-(trifluoromethyl)phenol : 4-(butoxymethyl)-6-methoxy-2,3-bis(trifluoromethyl)phenol (3l*: 3l^ in the ratio of 1 : 0.2), $^{19}F$ NMR (376 MHz, $CDCl_3$)**



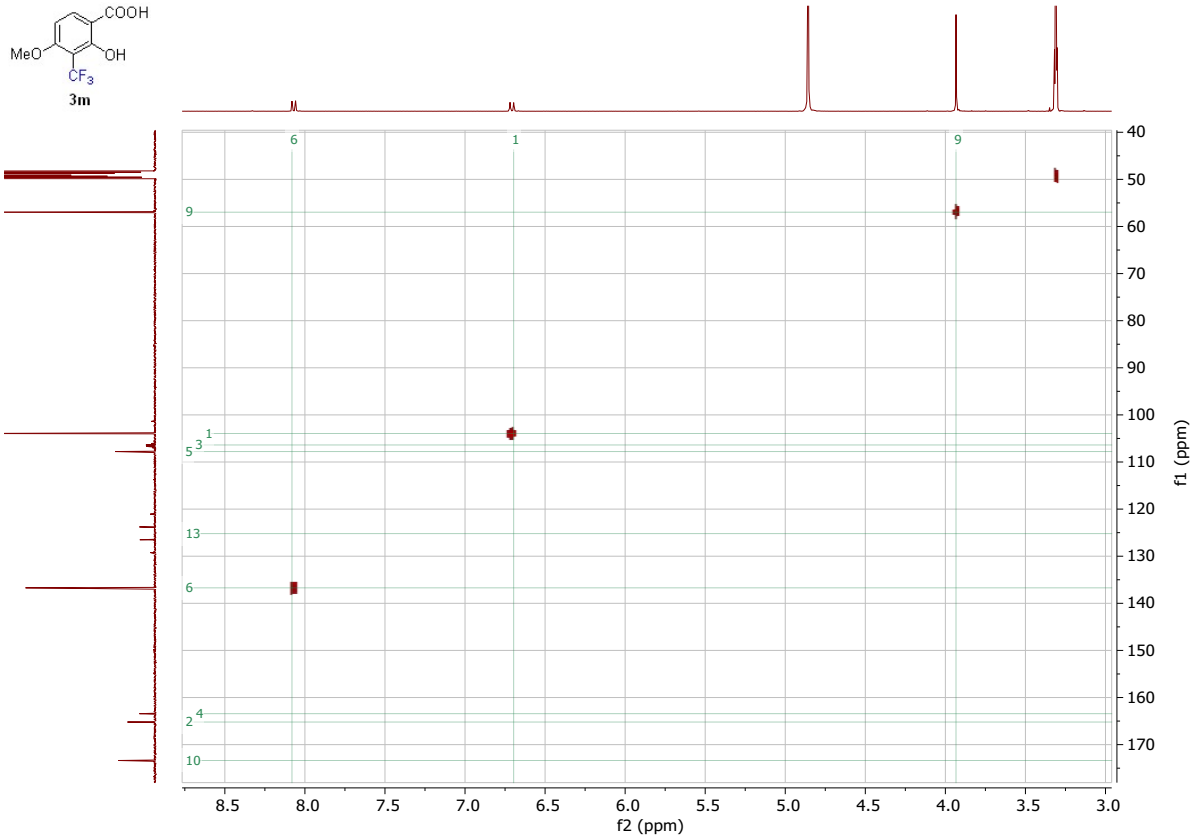
**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid (3m), $^{1}$H NMR (400 MHz, $CD_3OD$)**



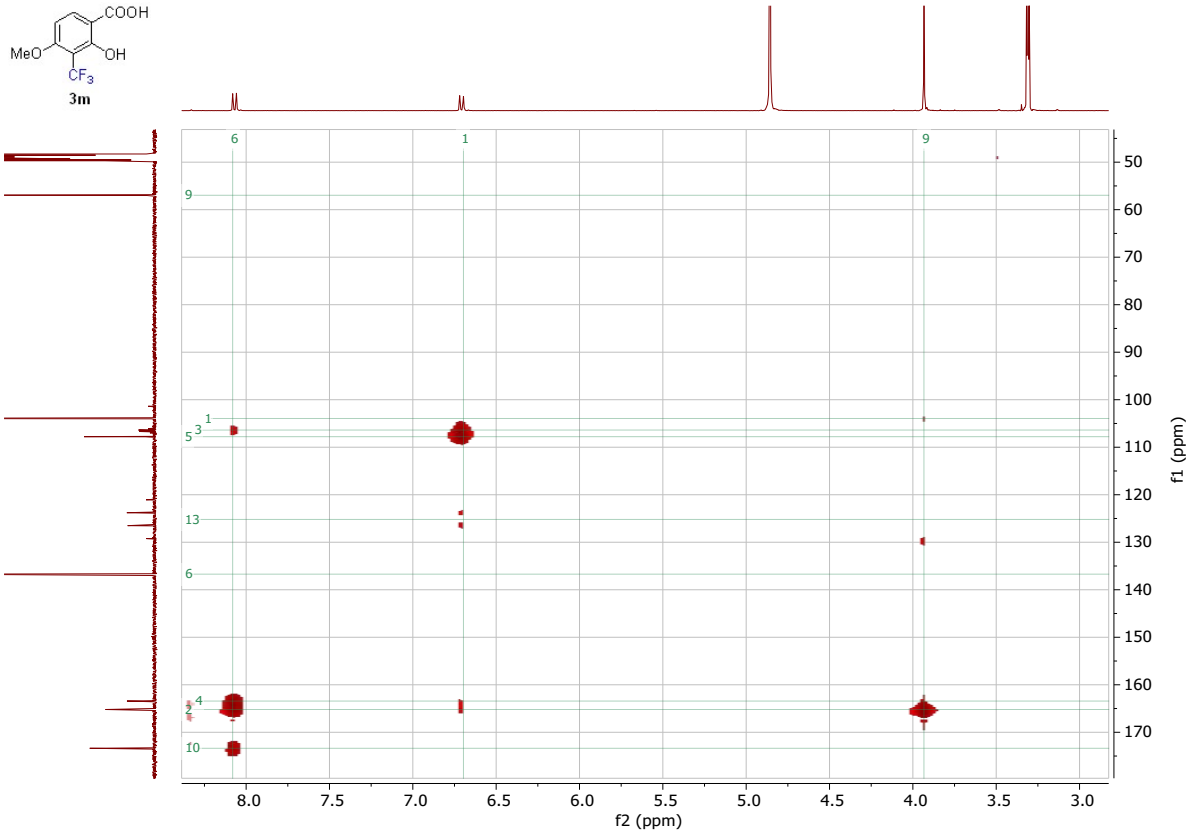

**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid (3m),$^{13}$C NMR (101 MHz, $CD_3OD$)**



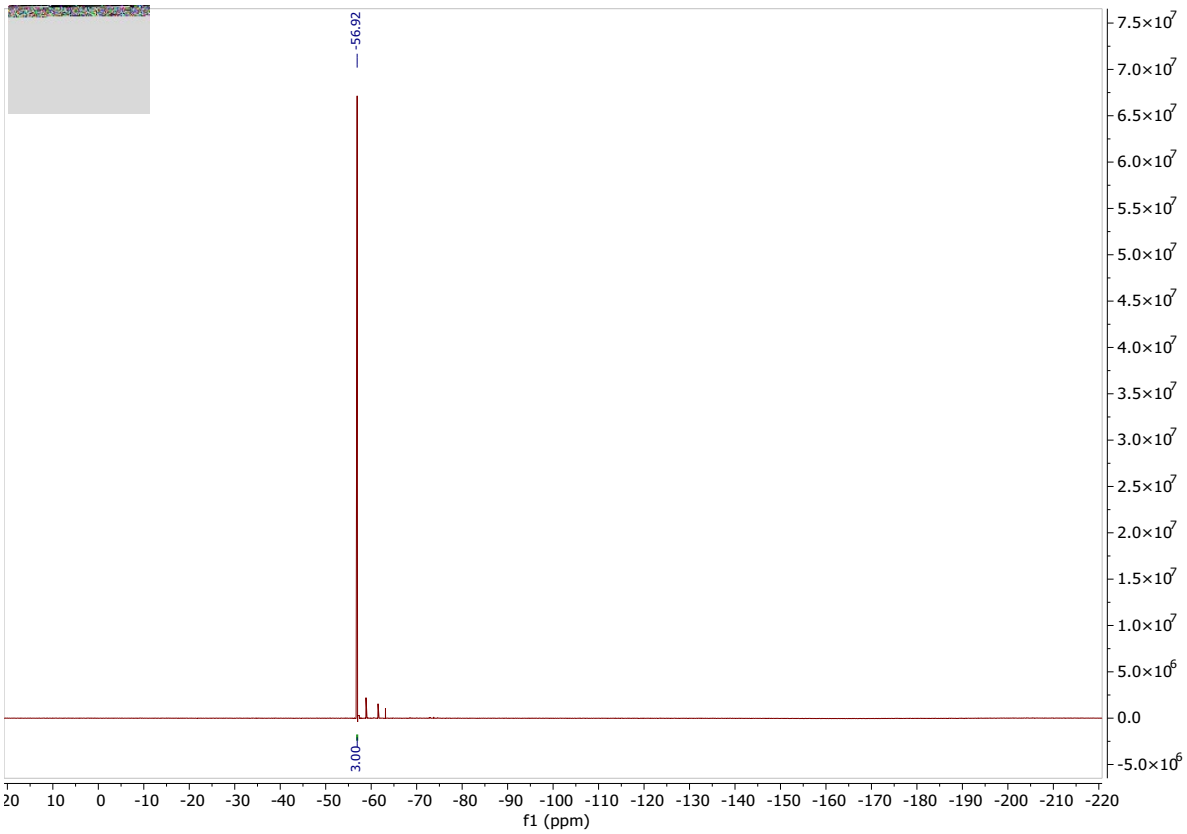
**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid (3m), HSQC ($CD_3OD$)**



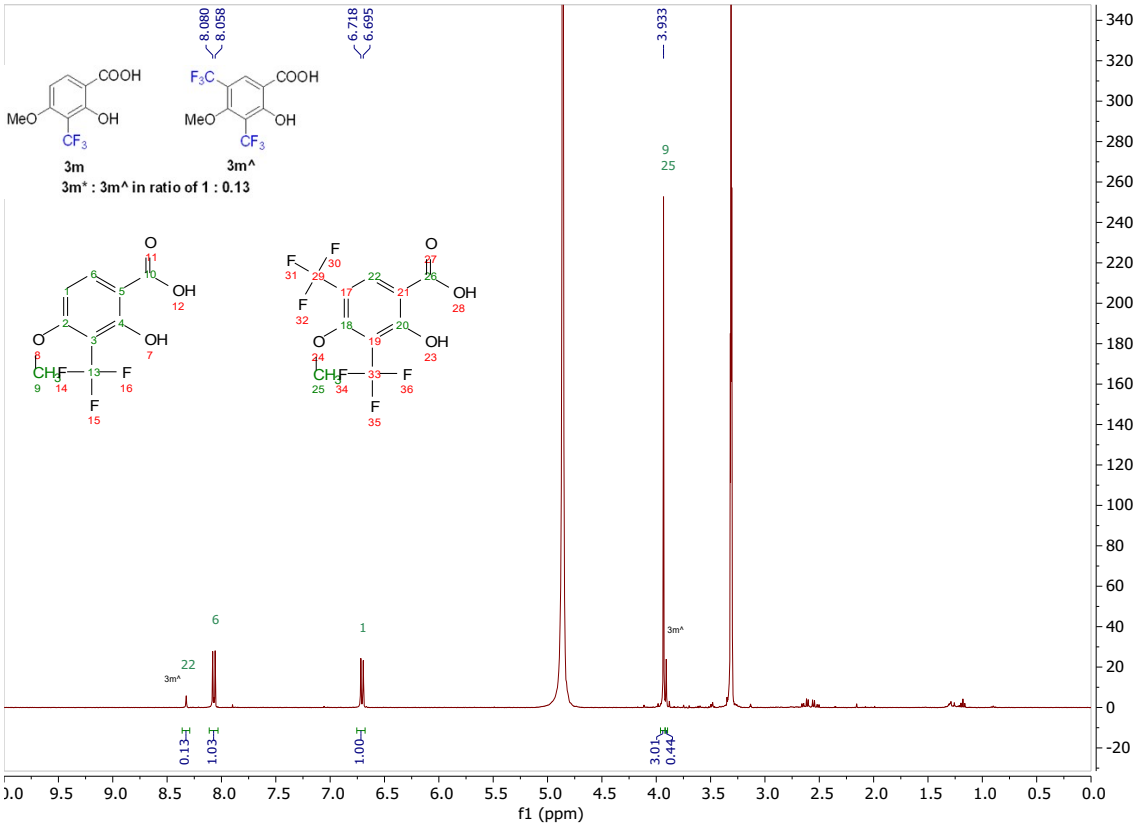

**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid (3m), HMBC ($CD_3OD$)**



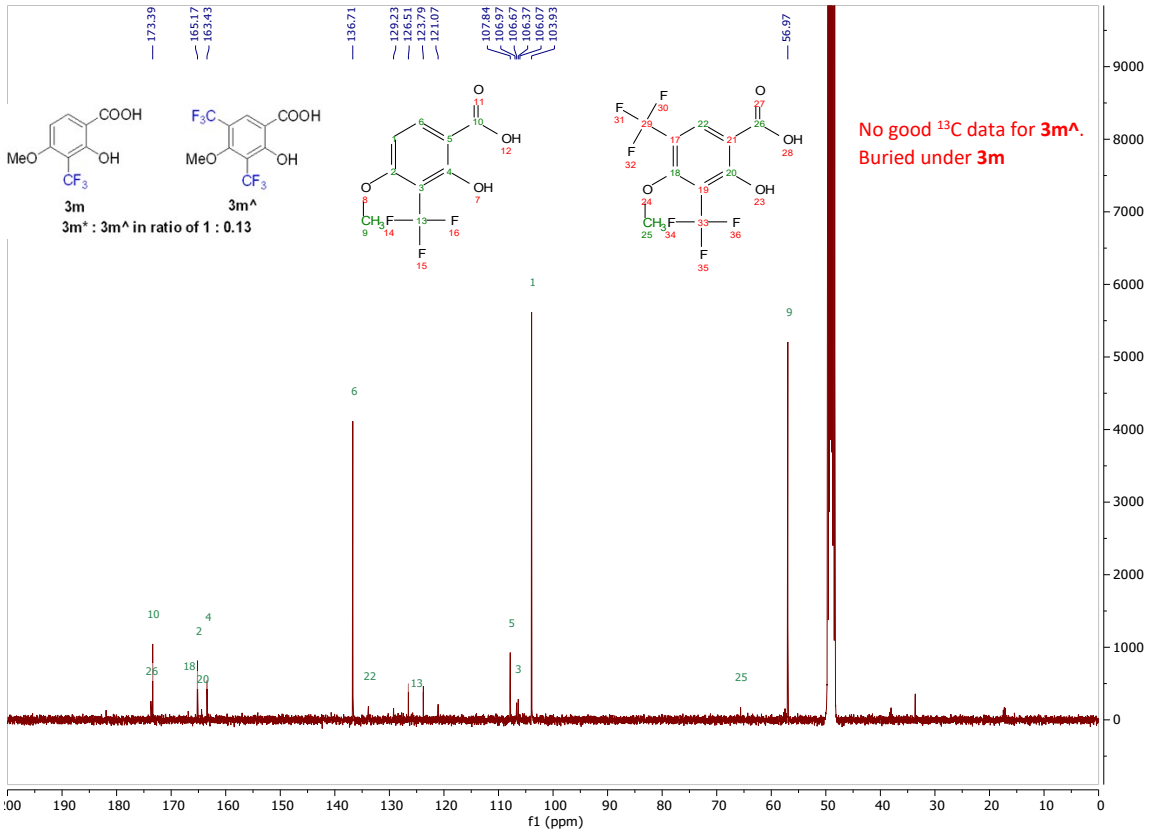
**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid (3m), $^{19}F$ NMR (376 MHz, $CD_3OD$)**



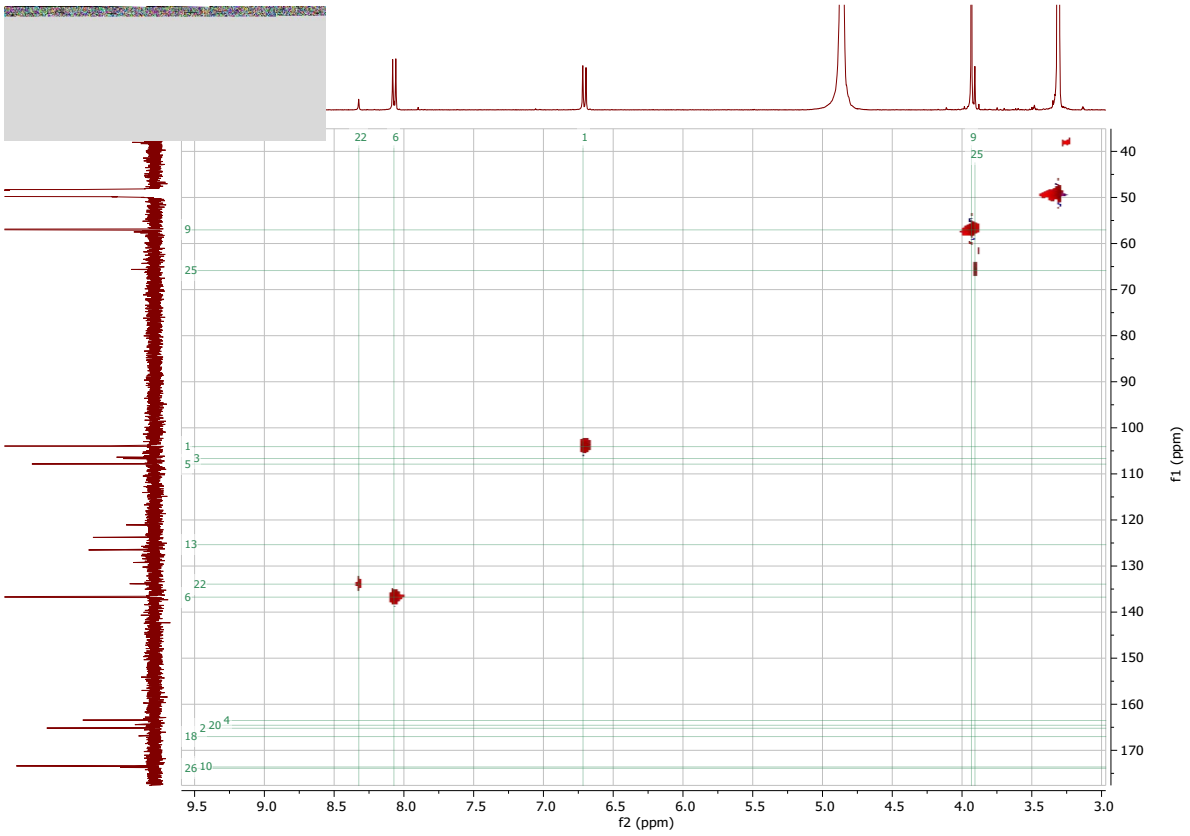
**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid : 2-hydroxy-4-methoxy-3,5-bis(trifluoromethyl)benzoic acid (3m: 3m^ in ratio of 1 : 0.13), $^{1}$H NMR (400 MHz, $CD_3OD$)**



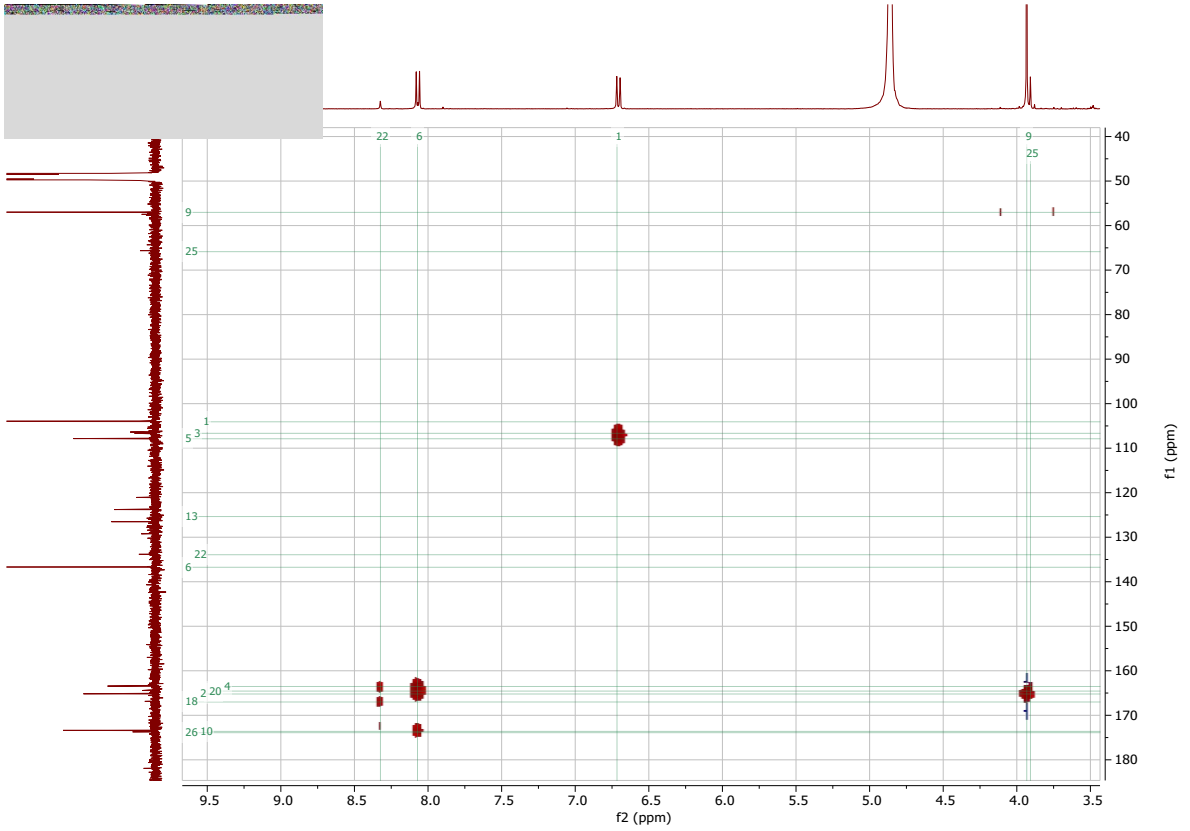

**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid : 2-hydroxy-4-methoxy-3,5-bis(trifluoromethyl)benzoic acid (3m: 3m^ in ratio of 1 : 0.13), $^{13}$C NMR (101 MHz, $CD_3OD$)**



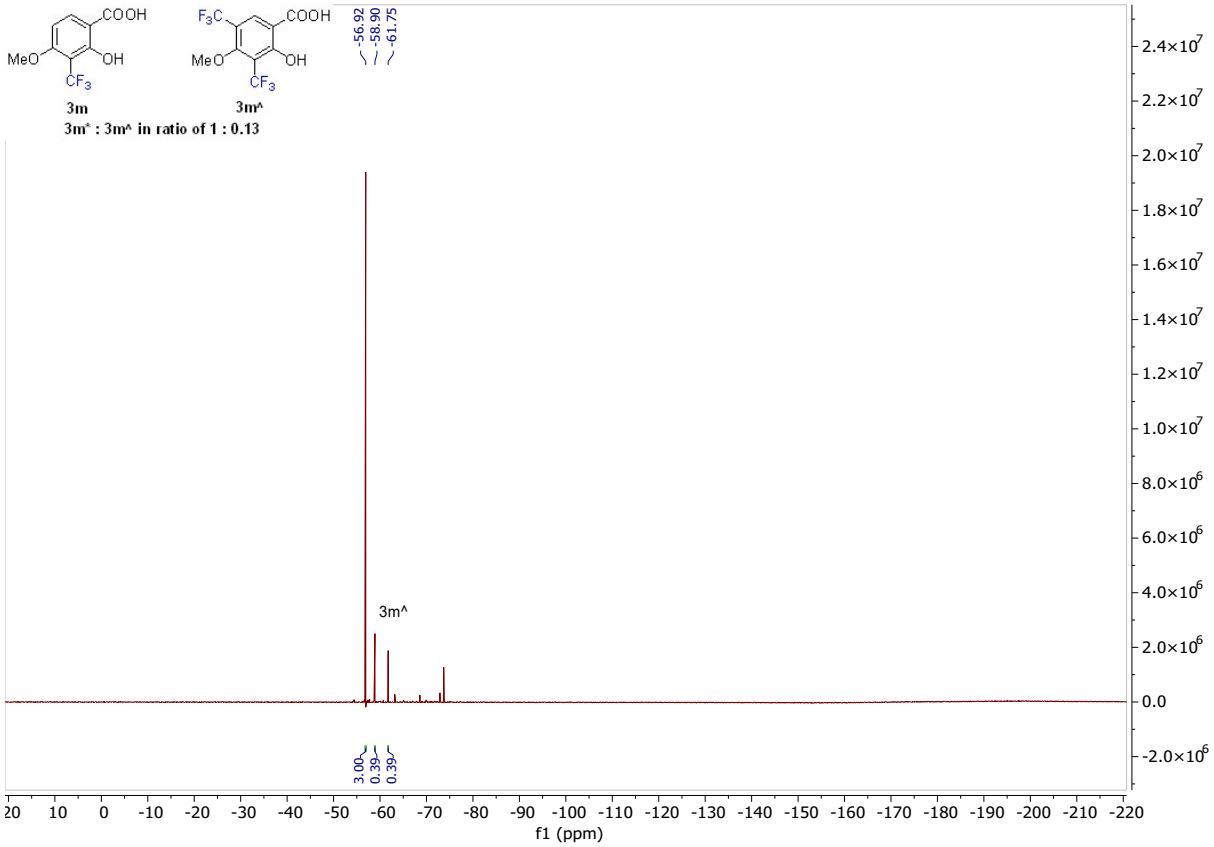
**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid : 2-hydroxy-4-methoxy-3,5-bis(trifluoromethyl)benzoic acid (3m: 3m^ in ratio of 1 : 0.13), HSQC ($CD_3OD$)**



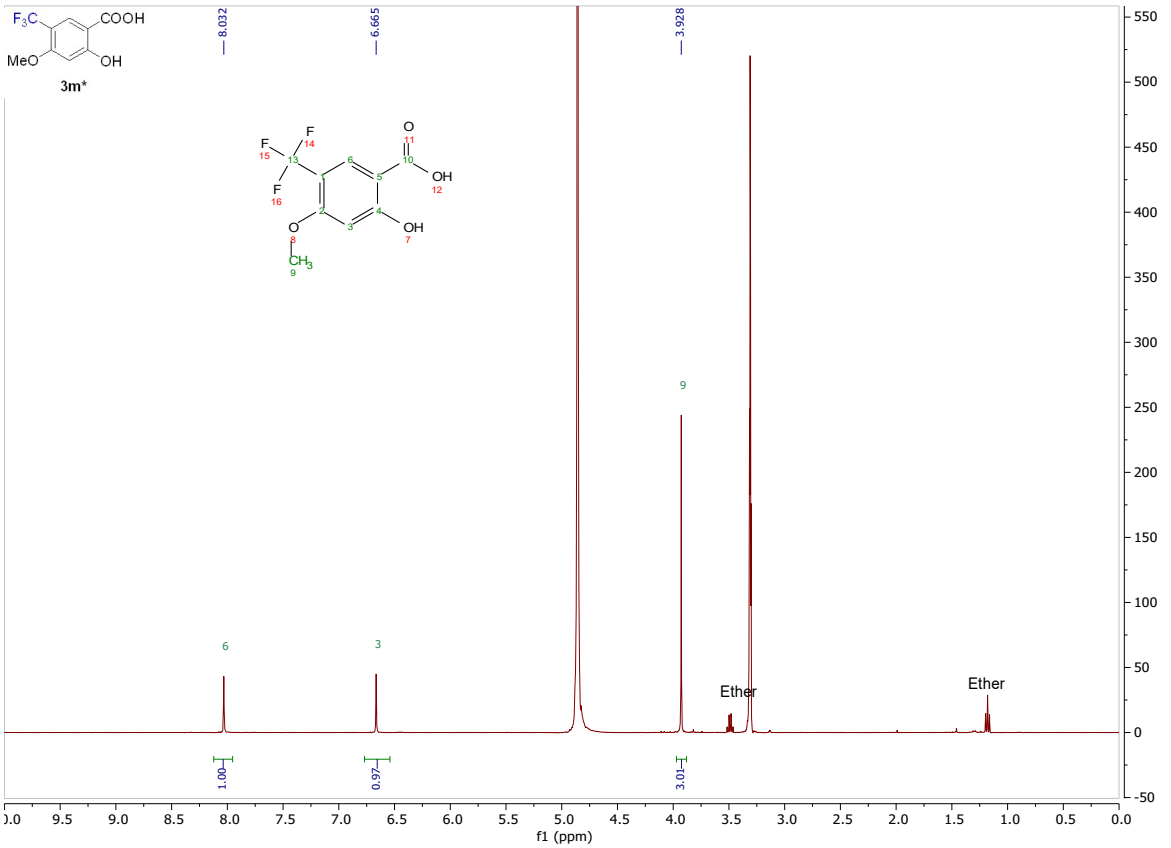

**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid : 2-hydroxy-4-methoxy-3,5-bis(trifluoromethyl)benzoic acid (3m: 3m^ in ratio of 1 : 0.13), HMBC ($CD_3OD$)**



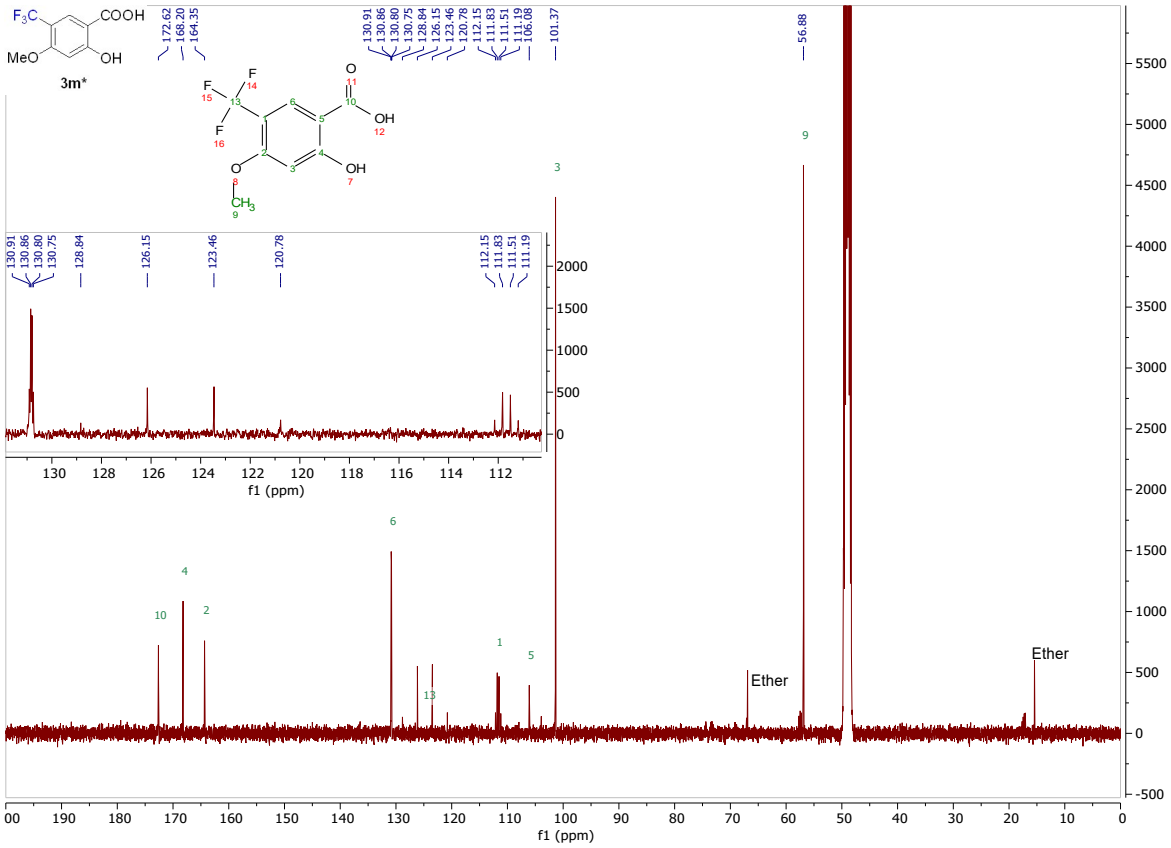
**2-hydroxy-4-methoxy-3-(trifluoromethyl)benzoic acid : 2-hydroxy-4-methoxy-3,5-bis(trifluoromethyl)benzoic acid (3m: 3m^ in ratio of 1 : 0.13), $^{19}$F NMR (376 MHz, $CD_3OD$)**



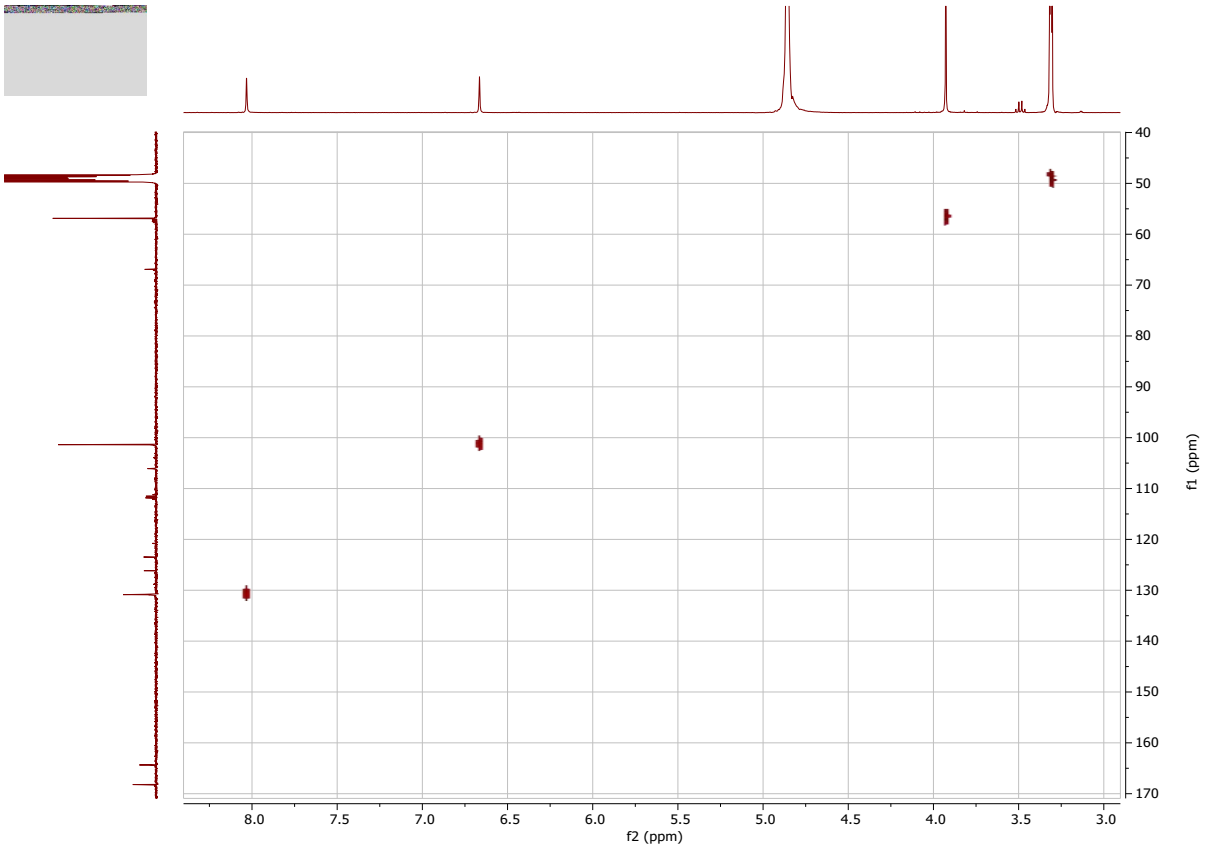
**2-hydroxy-4-methoxy-5-(trifluoromethyl)benzoic acid (3m*), $^{1}$H NMR (400 MHz, $CD_3OD$)**



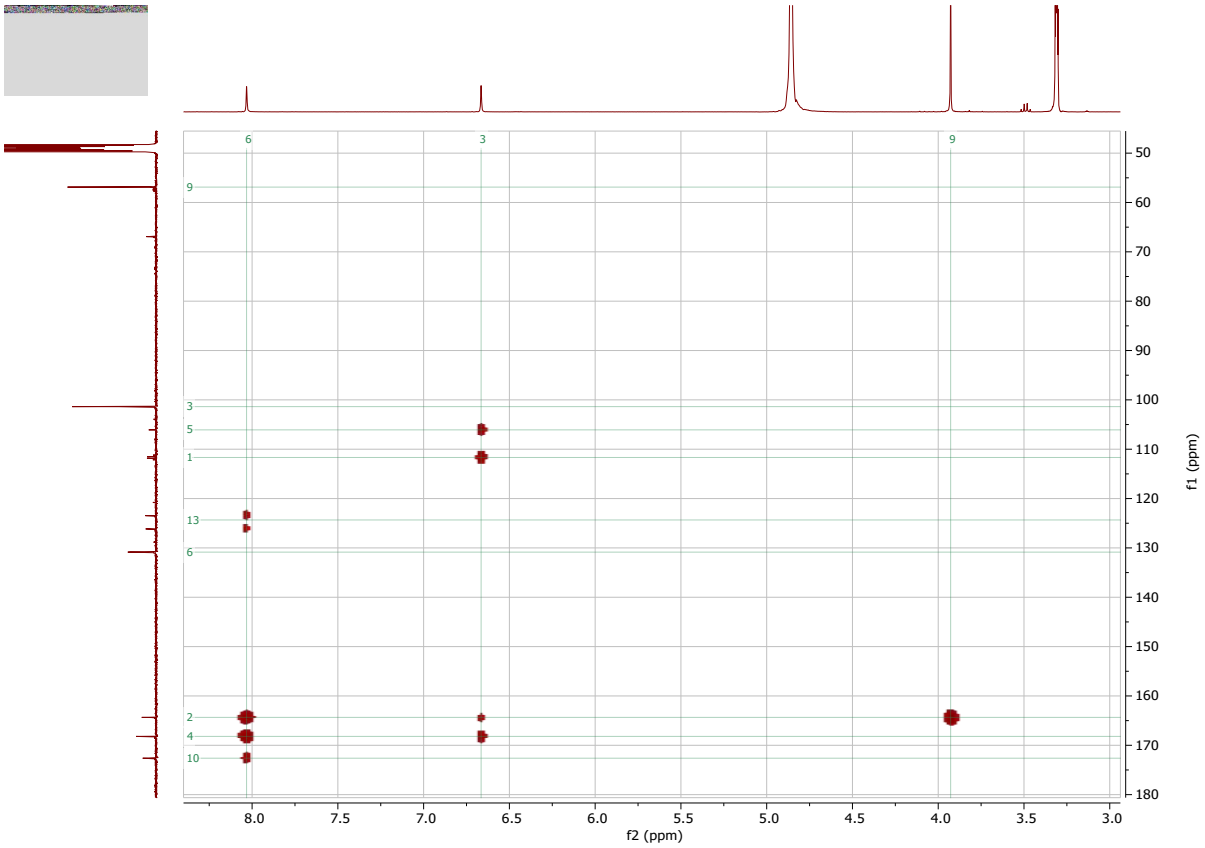

**2-hydroxy-4-methoxy-5-(trifluoromethyl)benzoic acid (3m*), $^{13}$C NMR (101 MHz, $CD_3OD$)**



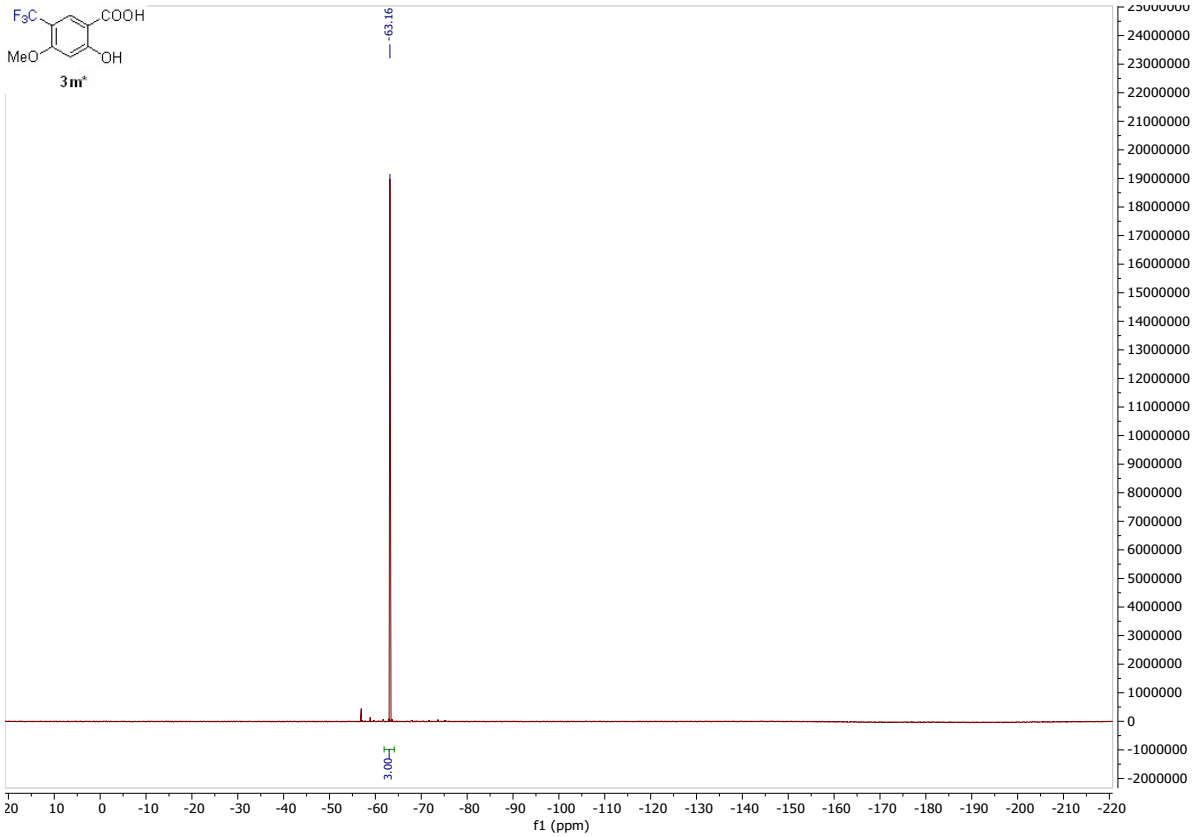
**2-hydroxy-4-methoxy-5-(trifluoromethyl)benzoic acid (3m*), HSQC ($CD_3OD$)**



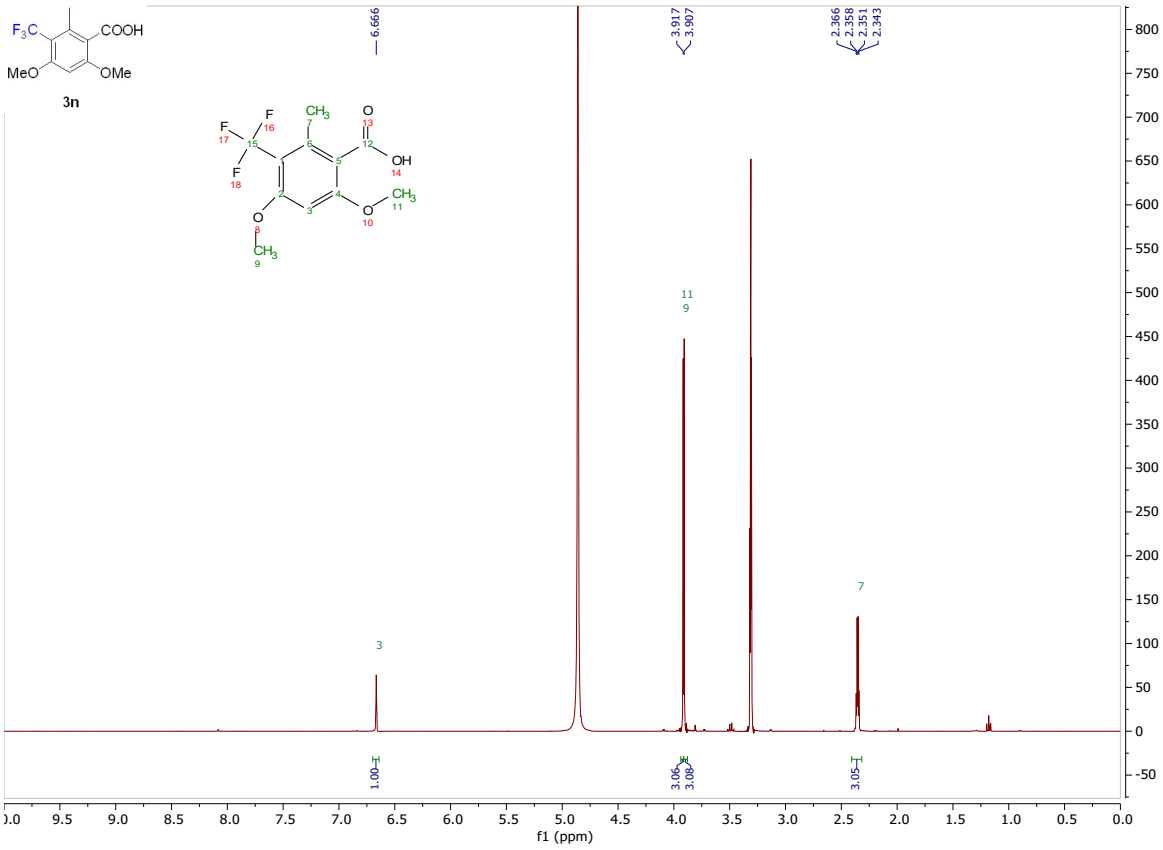

**22-hydroxy-4-methoxy-5-(trifluoromethyl)benzoic acid (3m*), HMBC ($CD_3OD$)**



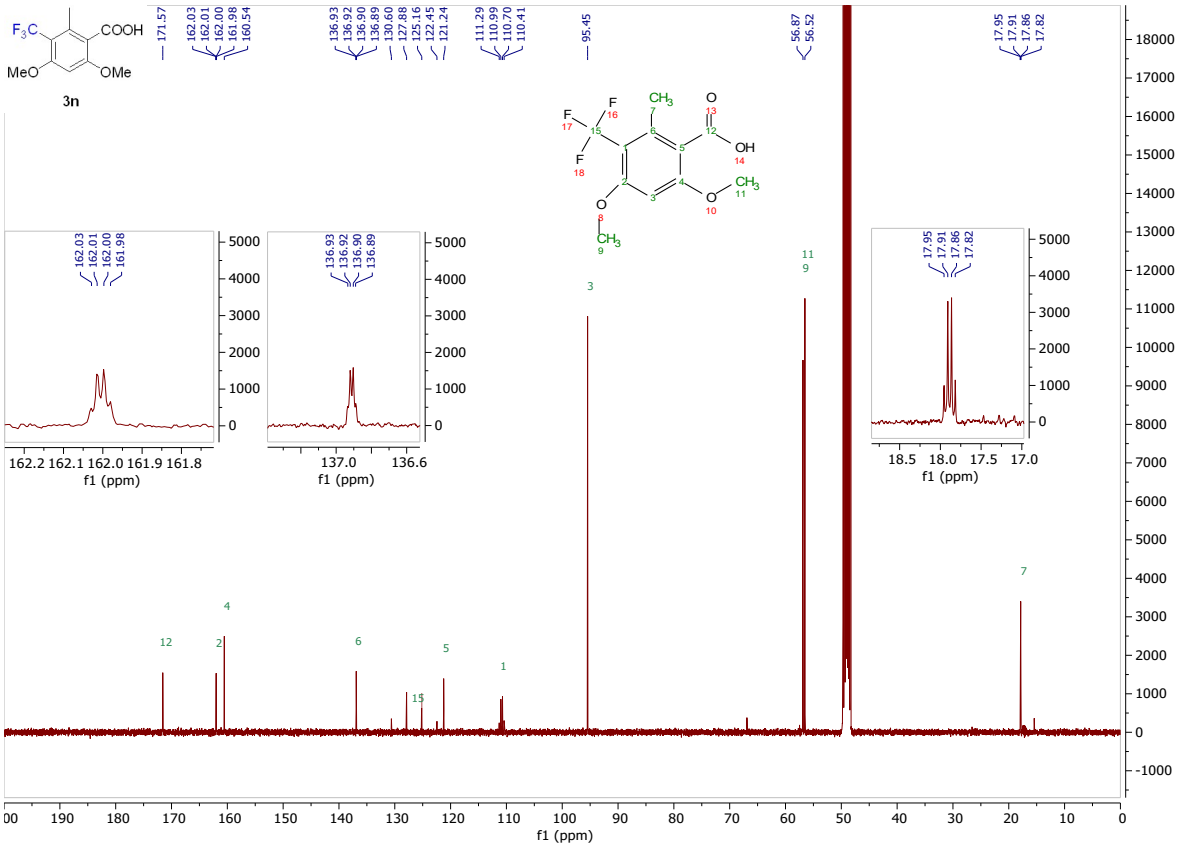
**2-hydroxy-4-methoxy-5-(trifluoromethyl)benzoic acid (3m*), $^{19}$F NMR (376 MHz, $CD_3OD$)**



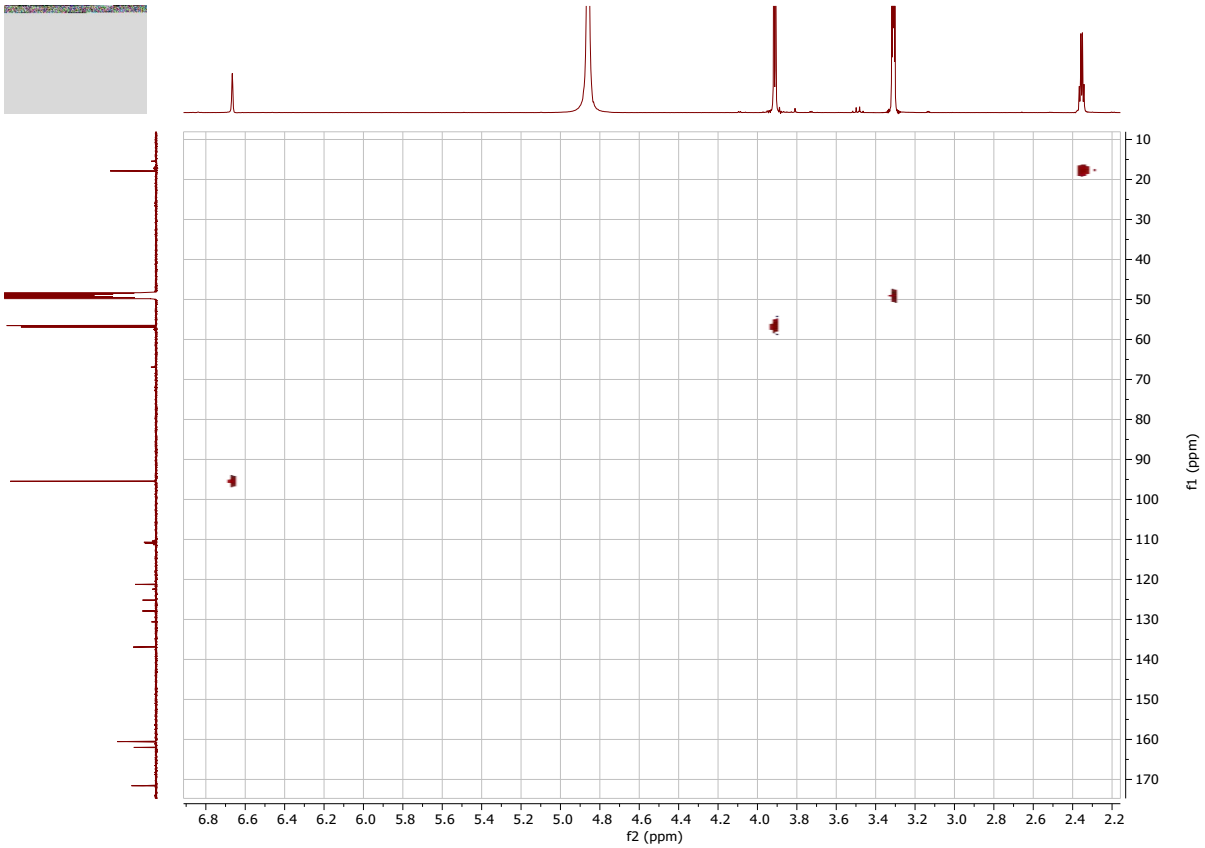
**4,6-dimethoxy-2-methyl-3-(trifluoromethyl)benzoic acid (3n), $^{1}$H NMR (400 MHz, $CD_3OD$)**



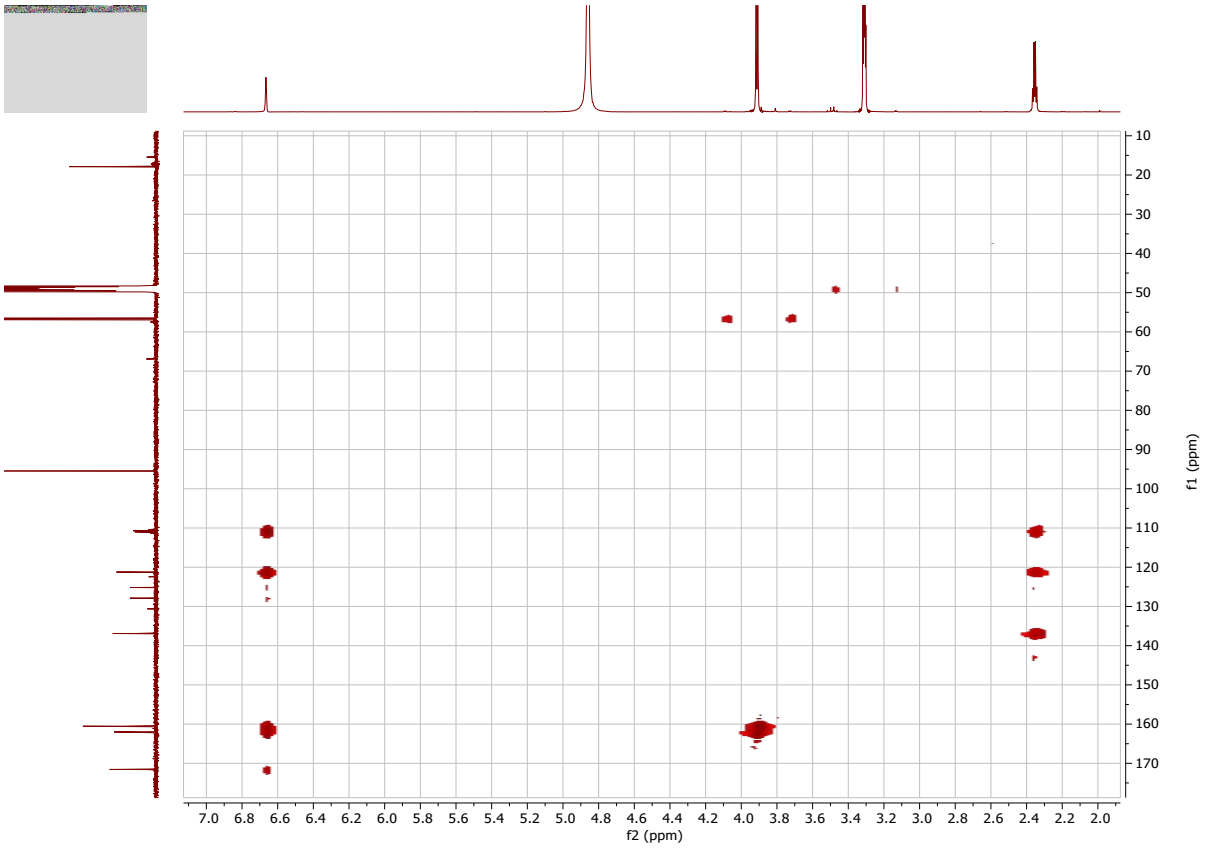

**4,6-dimethoxy-2-methyl-3-(trifluoromethyl)benzoic acid (3n), $^{13}$C NMR (101 MHz, $CD_3OD$)**



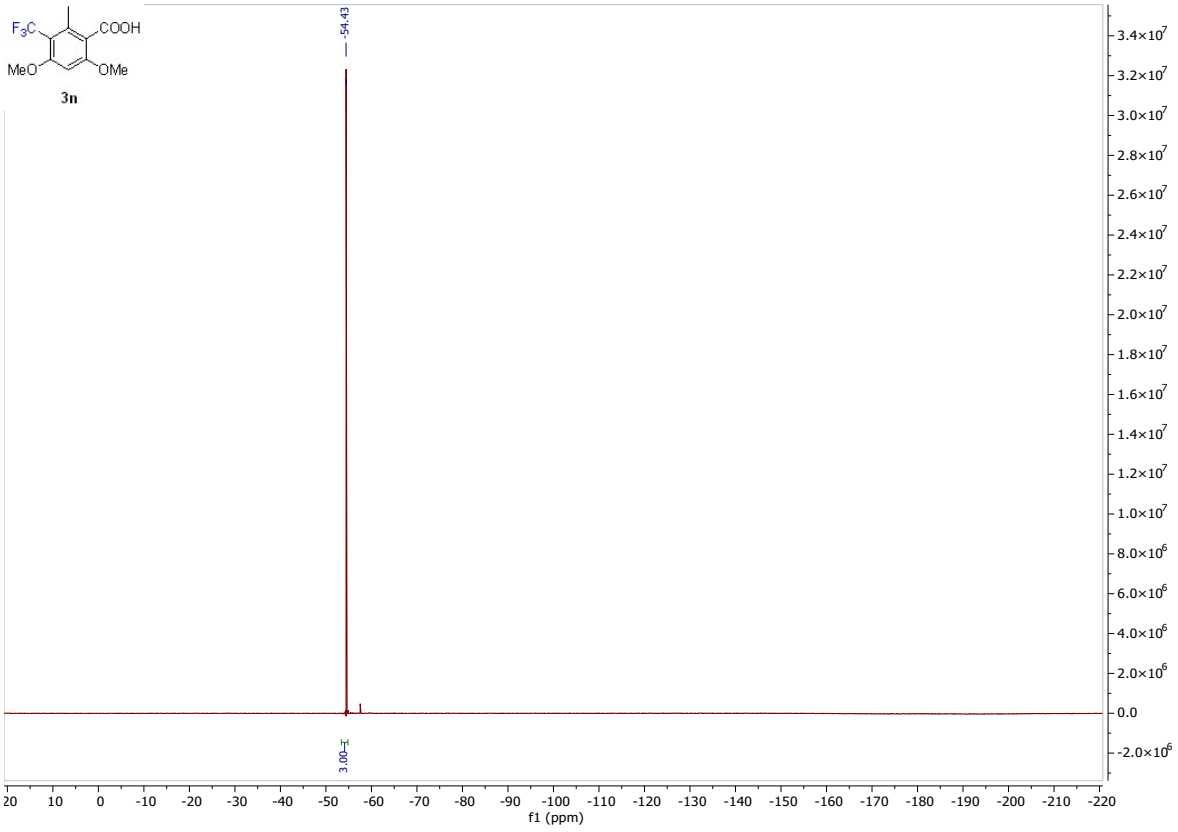
**4,6-dimethoxy-2-methyl-3-(trifluoromethyl)benzoic acid (3n), HSQC ($CD_3OD$)**




**4,6-dimethoxy-2-methyl-3-(trifluoromethyl)benzoic acid (3n), HMBC ($CD_3OD$)**



**4,6-dimethoxy-2-methyl-3-(trifluoromethyl)benzoic acid (3n), $^{19}$F NMR (376 MHz, $CD_3OD$)**

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