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Acceleration of Oxidations Promoted by Laccase with Red Light

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Electronic Supporting Information

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Experimental Procedures

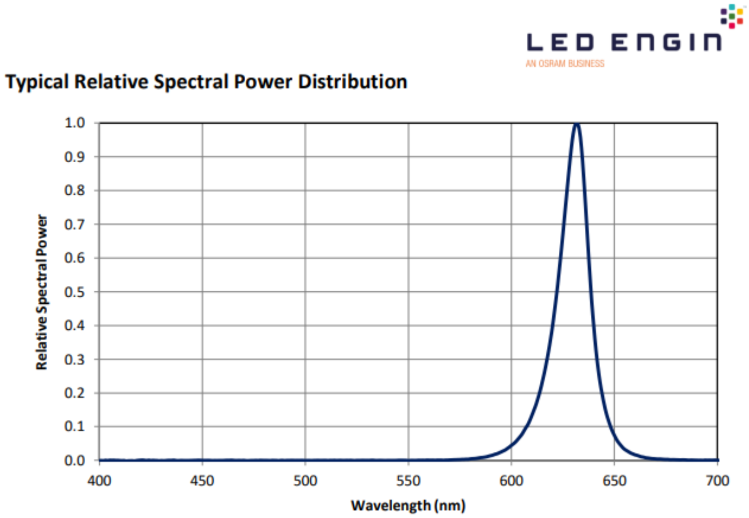
General information

Laccase from *Trametes Versicolor* was purchased from Sigma Aldrich (51639, 38429). Commercially available reagents and ACS grade solvents were used without further purification. Ultrapure water was obtained by MilliQ Millipore system. Profenols **2b-f** were obtained by reduction of the corresponding profens with BH_3•DMS in Et_2O,[1] while 2-pyridin methanol **2n** and *p*-Cl benzylalcohol **2j** by reduction of 2-pyridinecarboxaldehyde and *p*-Cl benzaldehyde with $NaBH_4$, respectively.[2] Merck 60 F254 TLC plates were used in monitoring of the reactions. ^{1}H NMR spectra were recorded with an INOVA 400 instrument with a 5 mm probe. HPLC-MS analysis were obtained with an Agilent Technologies HP1100 instrument, equipped with a ZORBAX-Eclipse XDB-C8 Agilent Technologies column (mobile phase: H_2O/CH_3CN, 0.4 mL/min, gradient from 30 to 80% of CH_3CN in 8 min, 80% of CH_3CN until 25 min) coupled with an Agilent Technologies MSD1100 single-quadrupole mass spectrometer, full-scan mode from m/z 50 to 2600, scan time of 0.1 s in positive ion mode, ESI spray voltage of 4500 V, nitrogen gas of 35 psi (1 psi=6894.7 Pa), drying gas flow of 11.5 mL/min, fragmentor voltage of 20 V.

Reaction mixtures were irradiated with OSRAM LED Engin (see Figure S1 for emission profile) and Eco Star CFL Floraison 5U-125W-2700°K lamp (see Figure S2 for emission profile). The irradiance of the light sources was measured on the reaction solution, using a Delta Ohm Photo-Radiometer HD2302.0, coupled with LP 471 RAD probe (spectral range 400-1050 nm). The OSRAM LED Engin is characterized by an average irradiance on the sample of 0.50 ± 0.02 mW cm^{-2}, while the average irradiance of the Eco Star CFL lamp is 10.5 ± 0.3 mW cm^{-2}.

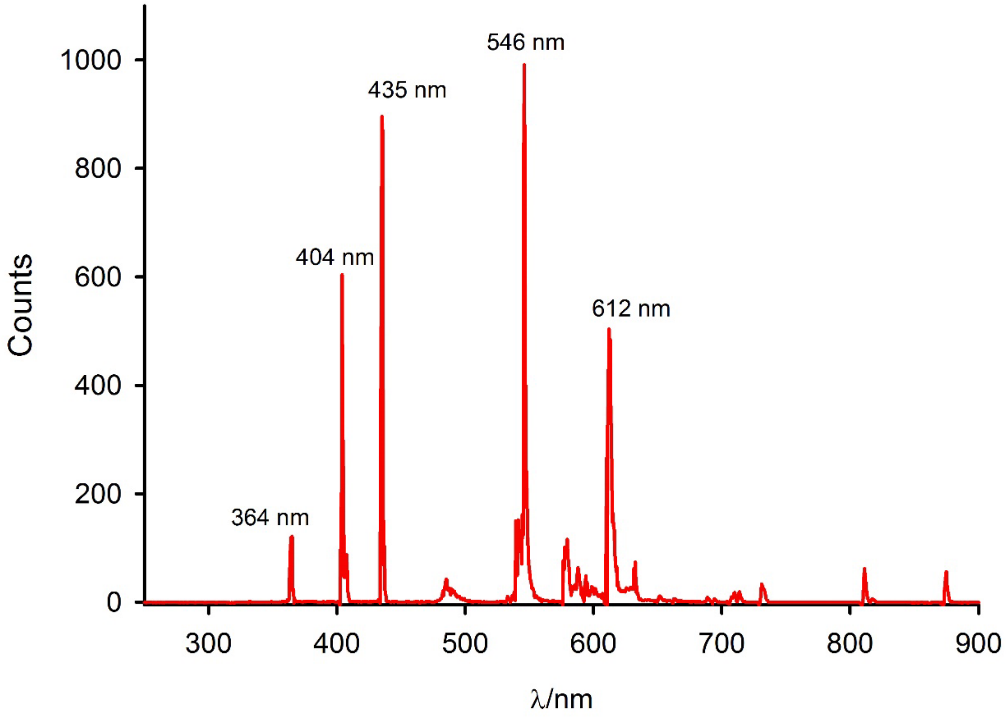
Emission profile of red-LED

Figure S1. Relative spectral power as function of wavelength of LED @623 nm (OSRAM LED Engin) used for irradiation of reaction mixture at 25°C.

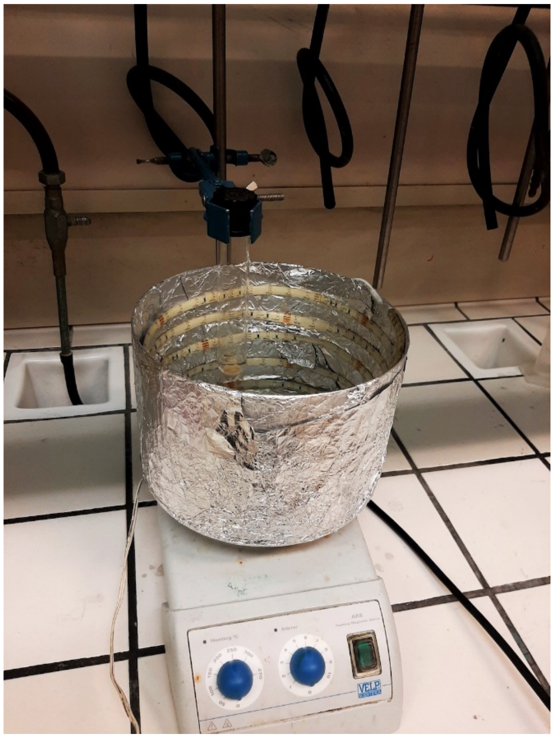


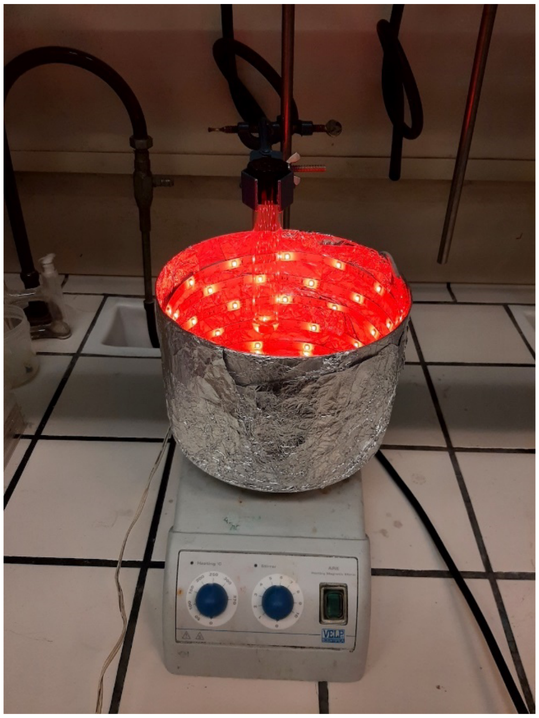
Emission profile of CFL lamp

Figure S2. Relative spectral power as function of wavelength of and Eco Star CFL Floraison 5U-125W-2700°K lamp.



Reaction Set-up





General procedures for oxidation reactions

The Laccase *Tv* Sigma-Aldrich number 51639 with a nominal activity of 10 U/mg, was submitted to catechol assay and thus it was determined the actual activity as 2.4 U/mg.

*A) Procedure for the oxidation of the model substrate 2-phenylpropanol **2a** (Table 1)*

To a stirred solution of the substrate **2a** (0.25 mmol, 34 mg, 1 equiv) in MilliQ water (3 mL) into a screw cap vial (50mL), the mediator **1c,g,h,i** (see % mol in Table 1) and then Laccase *Tv* (2.5 mg, 6 U) were added.
Oxygen was bubbled for 30 seconds, and the closed vial was kept under magnetic stirring and light irradiation (630 nm red LED or CFL lamp). Reaction course was monitored by TLC (cyclohexane/EtOAc 1:1), bubbling O_2 every time the reaction was exposed to air.
At completion, the reaction mixture was adjusted to pH = 2 by addiction of an aqueous HCl 1 M solution. After extraction with EtOAc (3 x 5 mL), the collected organic layers were dried on Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude mixture was analyzed by HPLC-MS and ^1H NMR.
The carboxylic acid product **3a** was isolated by washing the crude mixture dissolved in DCM with a basic solution of $NaHCO_3$. The aqueous layer was then adjusted to pH = 2 and extracted with DCM (3 x 5 mL). The collected organic layers were dried on Na_2SO_4, filtered and, after solvent removal under reduced pressure, the desired acid product **3a** was obtained.

B) General procedure for alcohols oxidation: profenols (Scheme 2), benzylic alcohols and octanol (Table 2)

Into a screw cap vial (50 mL), the alcoholic substrate **2b,e,f,g,h,k,l** (0.25 mmol, 1 equiv) was dissolved in MilliQ water (3 mL). Substrates **2c,d,i,j** were instead firstly dissolved in acetone as a co-solvent (0.3 mL) followed by MilliQ water (2.7 mL) addition. TEMPO (0.05 mmol, 7.8 mg, 0.2 equiv), and Laccase *Tv* (2.5 mg, 6 U) were finally added. Oxygen was bubbled for 30 seconds, and the closed vial was kept under magnetic stirring and 630 nm red LED light irradiation. O_2 was bubbled every time the reaction was exposed to air. At completion (TLC monitoring), the reaction mixture was then adjusted to pH = 2 by addiction of HCl 1 M and the aqueous phase was extracted with EtOAc (3 x 5 mL). Collected organic layers were dried on Na_2SO_4, filtered and the solvent removed under reduced pressure. In the case of octanol **2l,** the aqueous layer was extracted with DCM, then removed by distillation at ambient pressure. The crude mixture was analyzed by HPLC-MS and ^1H NMR.
In the case of Profens, the carboxylic acids **3b-f** were then isolated by washing the crude mixture dissolved in DCM with a basic solution of $NaHCO_3$. The aqueous layer was subsequently adjusted to pH = 2, saturated with brine and extracted with EtOAc (3 x 5 mL). The collected organic layers were dried on Na_2SO_4, filtered and the solvent removed under reduced pressure to yield the desired products **3b-f**.

C) General procedure for aldehydes oxidation (Table 3)

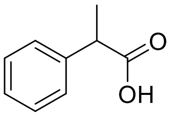
MilliQ water (3 mL), aldehyde **4g,h,m,n** (0.25 mmol, 1 equiv), TEMPO (0.02 equiv, 7.8 mg, 0.05 mmol) and Laccase *Tv* (2.5 mg, 6 U) were added into a screw cap vial (50 mL). Oxygen was bubbled for 30 seconds, and the closed vial was kept under magnetic stirring and 630 nm LED light irradiation. The reaction was monitored by TLC, bubbling oxygen every time the reaction was exposed to air.
For compound **4n**, at reaction completion (TLC monitoring) after 4.5 hours, the reaction mixture was directly lyophilized to obtain the crude.
For substrates **4g,h,m**, the reaction was stopped after 72 h by adding an aqueous solution of HCl 1 M to pH = 2. With substrates **4g,h,** the aqueous phase was extracted with DCM (3 x 5 mL). The collected organic layers were dried on Na_2SO_4 and filtered, and the solvent removed under reduced pressure. For substrate **4m** the acid aqueous layer was extracted with Et_2O (3x5 mL); the collected organic layers were dried on Na_2SO_4 and filtered; the solvent was then removed by distillation at ambient pressure.
The crudes were analyzed through HPLC-MS and ^1H NMR.

D) General procedure for amines oxidation (Table 4)

In a screw cap vial (50 mL), amine **6g,h,m** (0.25 mmol, 1 equiv) was dissolved in 3 mL of sodium acetate buffer 0.5 M at pH = 4.5. Then TEMPO (0.05 mmol, 7.8 mg, 0.2 equiv) and Laccase *Tv* (2.5 mg, 6 U) were added. Oxygen was bubbled for 30 seconds, and the closed vial was kept under magnetic stirring and light irradiation (630 nm led or CFL lamp). The course of the reaction was monitored by TLC.
At completion, the aqueous reaction mixture was extracted with EtOAc or DCM (3 x 5 mL). The collected organic layers were dried on Na_2SO_4, filtered ad the solvent was removed under reduced pressure. For *p*-F benzylamine **6m**, the aqueous layer was extracted with Et_2O, then removed by distillation. The reaction crude was analyzed through HPLC-MS and ^1H NMR.
The unreacted amine was eventually recovered from the aqueous phase after treatment with NaOH 1 M, followed by extraction with EtOAc (3 x 5 mL), drying on Na_2SO_4, filtration and solvent evaporation.

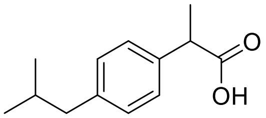
Characterization of the isolated products

*2-phenylpropanoic acid (**3a**)*



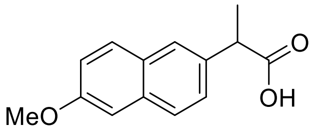
Following procedure A with the conditions of Table 1 (Entry 2), **3a** was obtained as a colorless oil in 90% yield (67 mg)· **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.35 (m, 4H), 7.31 – 7.24 (m, 1H), 3.76 (q, *J* = 7.2 Hz, 1H), 1.54 (d, *J* = 7.2 Hz, 3H)**.** **^{13}C NMR** (100 MHz, $CDCl_3$) δ 181.0, 139.9, 128.8, 127.7, 127.5, 45.5, 18.2. The spectroscopic data are consistent with those previously reported in the literature.[3]

*2-(4-isobutylphenyl)propanoic acid (**3b**)*



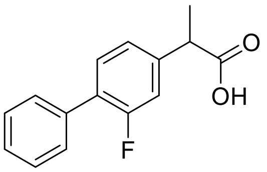
Following procedure B, **3b** was obtained as a colorless oil in 75% yield (38 mg). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.73 (bs, 1H), 7.25 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 7.9 Hz, 2H), 3.73 (q, *J* = 7.1 Hz, 1H), 2.48 (d, *J* = 7.2 Hz, 2H), 1.87 (dp, *J* = 13.5, 6.7 Hz, 1H), 1.52 (d, *J* = 7.2 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 6H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 181.1, 141.0, 137.1, 129.5, 127.4, 45.2, 45.1, 30.3, 22.5, 18.2. **HPLC-MS (ESI^+):** t_R= 8.607 min; 207 m/z $[M+H]^+$; 161 m/z $[M-COOH]^+$. The spectroscopic data are consistent with those previously reported in the literature.[4]

*2-(6-methoxynaphthalen-2-yl)propanoic acid (**3c**)*



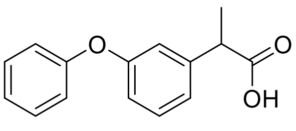
Following procedure B, **3c** was obtained as a white solid in 68% yield (39 mg). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.69 (m, 3H), 7.41 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.16 – 7.07 (m, 2H), 3.90 (s, 3H), 3.87 (q, *J* = 7.2 Hz, 1H), 1.58 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 180.8, 157.8, 135.0, 134.0, 129.4, 129.0, 127.4, 126.3, 126.3, 119.2, 105.7, 55.4, 45.4, 18.3. **HPLC-MS (ESI^+):** t_R= 7.000 min; 231 m/z $[M+H]^+$. The spectroscopic data are consistent with those previously reported in the literature.[4]

*2-(2-fluoro-[1,1'-biphenyl]-4-yl)propanoic acid (**3d**)*



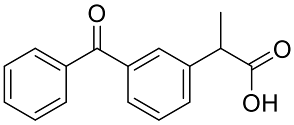
Following procedure B, **3d** was obtained as a white solid in 43% yield (26 mg). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.57 – 7.50 (m, 2H), 7.48 – 7.33 (m, 4H), 7.21 – 7.11 (m, 2H), 3.79 (q, *J* = 7.1 Hz, 1H), 1.57 (d, *J* = 7.2 Hz, 3H).**^{13}C NMR** (100 MHz, $CDCl_3$) δ 180.0, 159.8 (d, *J* = 248.6 Hz), 141.1 (d, *J* = 7.7 Hz), 135.6, 131.0 (d, *J* = 4.1 Hz), 129.1 (d, *J* = 2.9 Hz), 128.6, 128.3 (d, *J* = 13.4 Hz), 127.9, 123.8 (d, *J* = 3.4 Hz), 115.5 (d, *J* = 23.7 Hz), 45.0, 18.2. **^{19}F NMR** (376 MHz, $CDCl_3$) δ -117.45 (dd, *J* = 11.1, 8.3 Hz). **HPLC-MS (ESI^+):** t_R= 7.932 min; 245 m/z $[M+H]^+$; 262 m/z $[M+18]^+$. The spectroscopic data are consistent with those previously reported in the literature.[5]

*2-(3-phenoxyphenyl)propanoic acid (**3e**)*



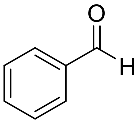
Following procedure B, **3e** was obtained as a yellow oil in 66% yield (40 mg). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.38 – 7.24 (m, 2H), 7.15 – 7.05 (m, 4H), 7.05 – 7.00 (m, 2H), 3.73 (q, *J* = 7.2 Hz, 1H), 1.51 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 180.5, 157.6, 157.1, 141.9, 130.0, 129.9, 123.5, 122.5, 119.1, 118.4, 117.6, 45.4, 18.2. **HPLC-MS (ESI$^+$):** t_R= 7.675 min; 243 m/z $[M+H]^+$; 260 m/z $[M+18]^+$. The spectroscopic data are consistent with those previously reported in the literature.[6]

*2-(3-benzoylphenyl)propanoic acid (**3f**)*



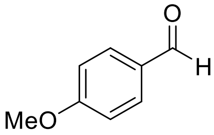
Following procedure B, **3f** was obtained as a yellow oil in 65% yield (41 mg). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.79 (m, 3H), 7.72 – 7.65 (m, 1H), 7.62 – 7.52 (m, 2H), 7.46 (m, 3H), 3.83 (q, *J* = 7.2 Hz, 1H), 1.56 (d, *J* = 7.2 Hz, 3H). **^{13}C NMR** (100 MHz, $CDCl_3$) δ 196.6, 179.8, 140.3, 138.1, 137.6, 132.7, 131.8, 130.2, 129.5, 129.4, 128.7, 128.5, 45.3, 18.3. **HPLC-MS (ESI$^+$):** t_R= 7.241 min; 255 m/z $[M+H]^+$; 272 m/z $[M+18]^+$. The spectroscopic data are consistent with those previously reported in the literature.[6]

*Benzaldehyde (**4g**)*



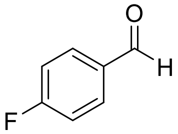
Following procedure D, **4g** was obtained as a colourless liquid in 70% yield (18.5 mg). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 7.93 (d, *J* = 7.2 Hz, 2H), 7.68 (t, *J* = 7.3 Hz, 1H), 7.58 (t, *J* = 7.5 Hz, 2H). The spectroscopic data are consistent with those found in literature.[7]

*4-Methoxybenzaldehyde (**4h**)*



Following procedure D, **4h** was obtained as a colourless liquid in 90% yield (31 mg). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.88 (s, 1H), 7.83 (d, *J* = 8.6 Hz, 2H), 7.00 (d, *J* = 8.5 Hz, 2H), 3.88 (s, 3H). The spectroscopic data are consistent with those found in literature.[7]

*4-Fluorobenzaldehyde (**4m**)*



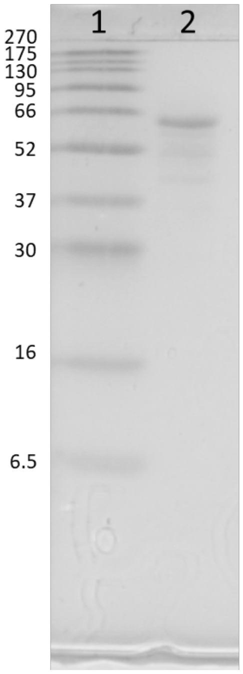
Following procedure D, **4m** was obtained as a colourless liquid in 99% yield (31 mg). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.97 (s, 1H), 8.00-7.81 (m, 2H), 7.21 (t, *J* = 8.5 Hz, 2H). The spectroscopic data are consistent with those found in literature.[7]

Concentration of Laccase Tv

Commercial laccase from *Trametes versicolor* (Sigma-Aldrich, 38429) is a light brown powder. The powder is highly soluble in aqueous media and appears as a transparent brown solution. Due to the high concentration of the soluble byproducts coming from the extraction process, the absorption spectrum does not show the characteristic “blue band” of the laccase.

In order to detect the typical absorption band, centered at 610 nm, further concentration steps of the protein are necessary. A convenient procedure to remove soluble small weight byproducts is centrifugal filtration. Amicon® Ultra-4 Centrifugal Filters - 10 kDa cutoff were used. Potassium phosphate buffer 0.1 M (pH 5.8) was used in all the step of centrifugation and characterization. 800 mg of laccase powder were dissolved in 4 mL of buffer in a vial under gentle stirring. The brown transparent solution was transferred in the centrifugal filter and then centrifuged (Eppendorf 5810R, fixed-angle rotor), setting the centrifuge at 7500 x g for 30 minutes per cycle at 15 °C. Eight successive cycles of centrifugation were performed, restoring every time the initial volume of the solution, using 4 mL of buffer. In the last step of the concentration process, the laccase solution was collected without dilution.

SDS-PAGE



Concetrated laccase was dissolved in protein buffer (62.5 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 0.04 M, DTT and 0.01% bromophenol blue), then boiled for 5 minutes at 100 °C (ThermoMixer HC, S8012-0000; STARLAB). 10 µL of the sample was loaded in a 15% polyacrylamide gel, then a constant voltage of 130 V was applied for 60 minutes, using a Mini-PROTEAN Tetra electrophoresis system (Bio-Rad). In order to visualize the protein bands, the gel was stained with Coomassie Blue G-250 solution and then scanned with ImageQuant LAS 500, under colorimetric acquisition mode. Laccase isoforms from *Trametes versicolor* are extracellular and monomeric glycoproteins with ~462–527 amino acids and a typical weight of ~50–56 kDa.[8] The most common form is characterized by 519-520 aminoacids and a MW of 56 kDa (the apparent MW, due to glycosylation, detected in SDS is close to 66 kDa[9]), as observed in figure S3.

Figure S3. SDS-PAGE analysis of laccase. 1) mPAGE® Color Protein Standard (Millipore, MPSTD4); 2) concentrated laccase (100 µg).

Instrumentation for photophysical studies

Absorption spectra in the 190 -1100 nm range were recorded at room temperature in solutions contained in quartz cuvettes (optical pathlength 1 cm, Hellma®) by using a Perkin Elmer λ40 spectrophotometer. For absorption kinetic measurements a Varian Cary 50 Bio was used. Fluorescence emission spectra in the 250-900 nm range were recorded with a Perkin Elmer LS 55 spectrofluorimeter, equipped with Hamamatsu R928 photomultiplier. Lifetimes were measured by an Edinburgh FLS920 spectrofluorimeter equipped with a TCC900 card for data acquisition in time-correlated single-photon counting experiments (0.5 ns time resolution) with a pulsed diode laser. The estimated experimental errors are: ± 2 nm of the band maximum, 5% of the molar absorption coefficient, 10% of the lifetime measurements.

Results and Discussion

Time course of the reaction

Procedure:

Four identical reactions were conducted in the dark and four under red LED irradiation, following the general procedure A) for the oxidation of 2-phenylpropanol. The reactions were stopped after 0.5, 2, 3.75, and 6.25 hours by addition of HCl 1 M till pH = 2, extraction with EtOAc (3 x 5 mL), and the collected organic layers were dried on Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude mixtures were analyzed by ^1H NMR. The signals of the benzylic proton in the alcohol, aldehyde, and acid, respectively, was chosen for quantization of the three species and reported in Figure S4 as relative amounts % respect the reaction time.

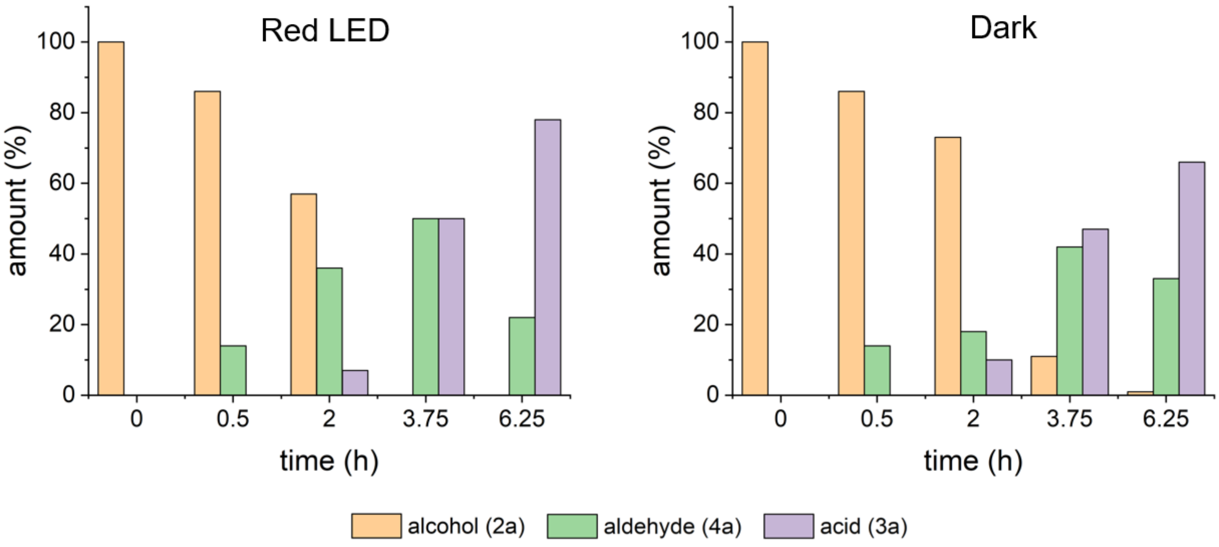


Figure S4. Time course of the oxidation of alcohol **2a** to aldehyde (**4a**) and acid (**3a**) with Laccase Tv and TEMPO (20%) at 28 °C in MilliQ H_2O, under red LED irradiation (left plot) or in the dark (right plot)

Determination of Laccase activity and stability under irradiation.

The stability of Laccase Tv under 630 nm LED-light irradiation in comparison to a normal day-light exposure was examined by measuring the enzyme activity at set time intervals. The activity was determined by catechol assay.[10,11] Four conditions were tested over 6 days. Thus, four samples of Laccase Tv (13 U/mg) L1, L2, L3, and NL1 were prepared and submitted to different conditions, as summarized in Table 1.

Table S1. Laccase Tv solutions and test conditions.

Solution [a]	Solvent	630 nm LED light irradiation	Oxygen bubbled	Laccase Tv concentration
L1	Acetate buffer 0.5 M pH = 4.5	✓	✓	1,01 mg/mL
L2	Acetate buffer 0.5 M pH = 4.5	✓	✗	1,03 mg/mL
L3	MilliQ water	✓	✗	0,97 mg/mL
NL1	Acetate buffer 0.5 M pH = 4.5	✗	✓	1 mg/mL

Sample preparation

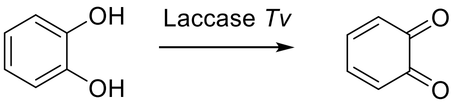
For each solution, 1 mg of Laccase Tv was weighted on a precision balance and then dissolved in 1 mL of the appropriate solvent into a screw cap vial. The actual concentrations obtained can be seen in table 1.

Solutions **L1** and **NL1** differ only for a LED-light or a daylight exposure.
In samples **L1** and **NL1** oxygen was bubbled for 30 seconds after each sampling.
At set time intervals (t = 0 and 24, 48, 72 and 144 hours) aliquotes from the **L1**, **L2**, **L3**, **NL1** solutions were taken to be subjected to catechol assay for laccase activity determination, as described below.

Catechol assay for laccase activity

Catechol is a natural substrate for Laccase Tv, producing benzoquinone. Laccase Tv activity was determined by spectrophotometric assay at 405 nm on monitoring the absorbance increase due to the formation of benzoquinone.[10, 11]

Scheme S1. Catechol oxidation by Laccase Tv.



Tests were conducted in a 96 polystyrene well plate, in duplicate. In each well, 100 µL of a 20 mM catechol solution in 0.5 M acetate buffer (pH=4.5), 90 µL of acetate buffer and 10 µL of laccase solutions (L1, L2, L3 or NL1) were added, giving a total volume of 200 µL. Blank solutions were made of 100 µL of catechol solution and 100 µL of acetate buffer. Immediately after laccase solution addiction, mixtures were shaken for 1 min and 30 s, using the “shake” function of the plate reader (Thermo Scientific Multiskan EX). Absorbance at ʎ=405 nm was then measured in 20 seconds intervals for 2 hours, using the stepping and kinetic mode of the instrument. Data were collected by Ascent software and analyzed by Excel.
Thus, quantitative determination of the enzyme activity can be obtained by the formula:[12]

$$Activity\ (\mu mol \cdot min^{-1} \cdot mg^{-1}) = \frac{{}^{dA}\!/_{dt}\ (min^{-1})}{\varepsilon\ (mL \cdot \mu mol^{-1} \cdot cm^{-1}) \cdot l\ (cm) \cdot [E](mg \cdot mL^{-1})}$$

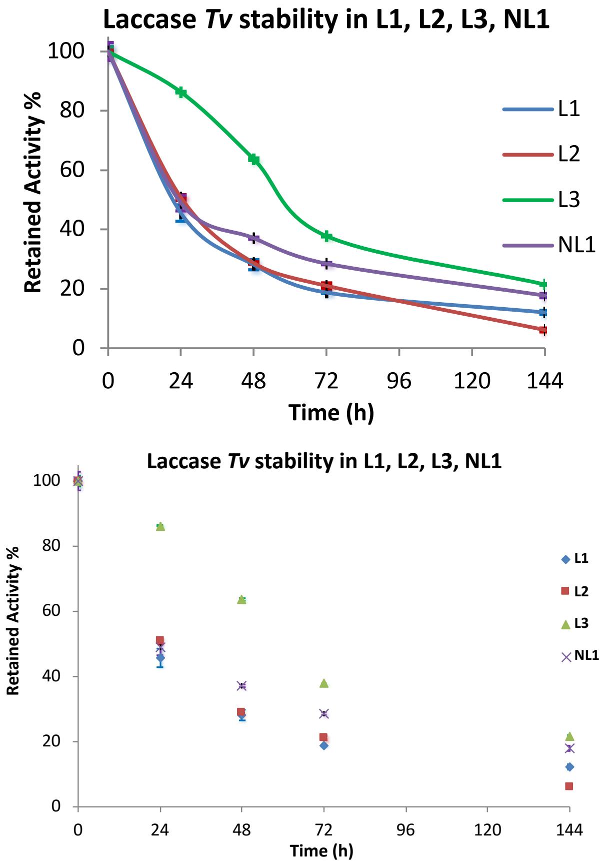
where dA/dt is the slope of Abs-Time curve in the initial phase of the reaction (approximately 0-10 min), where the trend is linear. ε is the molar extinction coefficient of benzoquinone at λ = 405 nm (1,26 mL·µmol^{-1}·cm^{-1}), [E] is the enzyme concentration inside the plate well and *l* is the optical path length of the radiation through the sample. In this apparatus, since the radiation is perpendicular to the sample, optical path length is proportional to the volume of the well solution, in our case *l* = 0.5 cm.

Laccase activity was determined in all four Laccase *TV* samples L1, L2, L3, and NL1; results are in enzyme units as U/mg ± SD in Table S2.

Table S2. Results of activity monitoring in time for each solution.

Time (h)	L1		L2		L3		NL1	
	Activity (U/mg)	% Retained Activity	Activity (U/mg)	% Retained Activity	Activity (U/mg)	% Retained Activity	Activity (U/mg)	% Retained Activity
0	2,24 ± 0,040	100 ± 1,8	1,95 ± 0,0065	100 ± 0,34	2,70 ±0,042	100 ± 1,5	1,86 ± 0,056	100 ± 3,0
24	1,02 ± 0,062	45,5 ± 2,8	0,99 ± 0,013	50,8 ± 0,67	2,33 ± 0,0069	86,3 ± 0,26	0,91 ± 0,049	48,9 ± 2,6
48	0,63 ± 0,038	28,1 ± 1,7	0,56 ± 0,0022	28,7 ± 0,11	1,72 ± 0,012	63,7 ± 0,43	0,69 ± 0,0067	37,1 ± 0,36
72	0,42 ± 0,0067	18,8 ± 0,30	0,41 ± 0,013	21,0 ± 0,67	1,02 ± 0,0069	37,8 ± 0,26	0,53 ± 0,0067	28,5 ± 0,37
6 days	0,27 ± 0,016	12,1 ± 0,71	0,012 ± 0,0065	6,2 ± 0,33	0,58 ± 0,0069	21,5 ± 0,26	0,33 ± 0,013	17,7 ± 0,72

Figure S5. Plots of Laccase Tv activity vs. time in samples **L1**, **L2**, **L3**, and **NL1**.



Photophysical studies

Figure S6. Absorption spectra of a Laccase Tv aqueous solution (5 mg in 6 mL of phosphate buffer 0.1 M, pH 5.8) before (red solid line) and after (black solid line) concentration. The inset shows the difference between the black and red spectra after normalization at 500 nm.

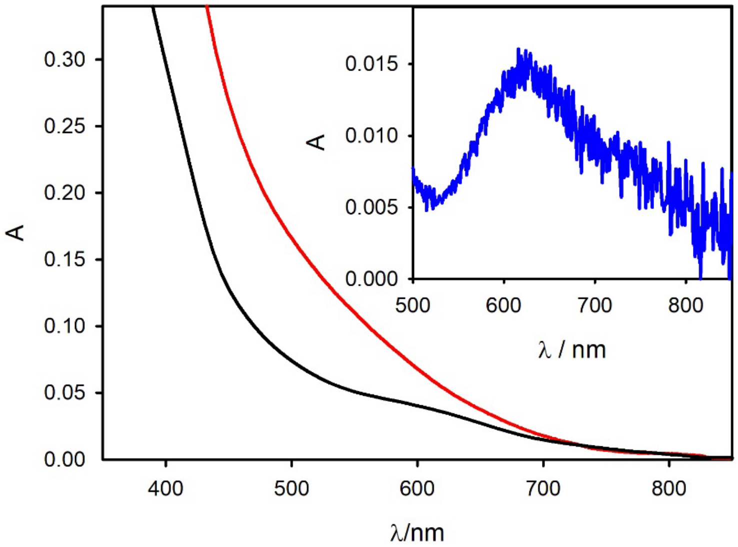


Figure S7. Emission spectrum (black solid line) and emission intensity decay (inset, green dots) of a Laccase Tv aqueous solution (black solid line) upon excitation at 340 nm. τ1 = 0.8 ns (42.3%) and τ2 = 5.2 ns (57.7%) were estimated by double fit exponential function.

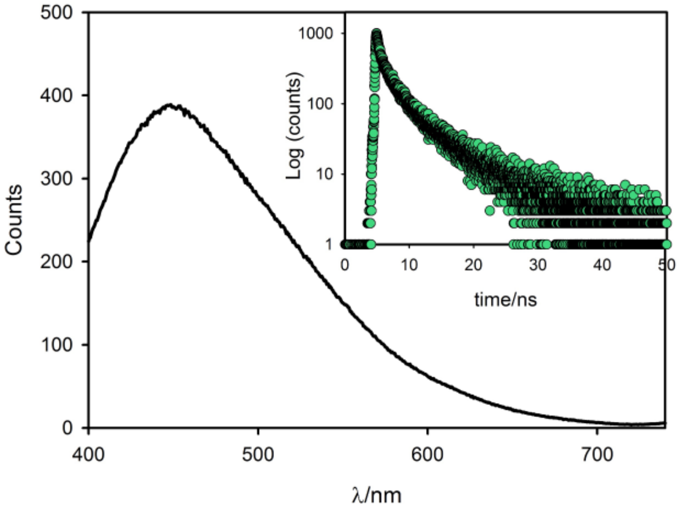
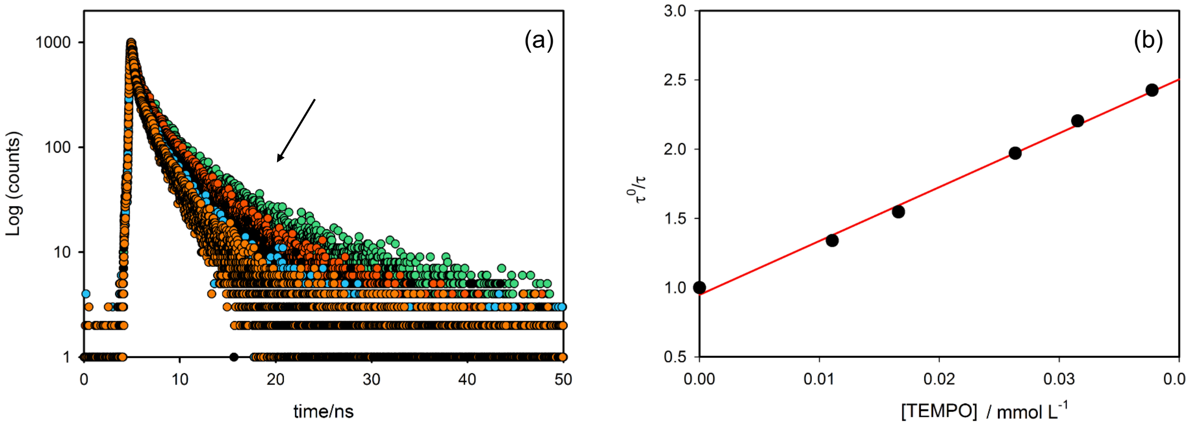


Figure S8. (a) Emission intensity decays of Laccase Tv in air-equilibrated aqueous solution (green dots) and upon addition of increasing amount of TEMPO (up to 0.038 M, orange dots); excitation at 405 nm. (b) Emission intensity decays of Laccase Tv in air-equilibrated aqueous solution in the absence (τ^0) and in the presence (τ) of increasing amount of TEMPO (black dots). The slopes represent the Stern-Volmer constant (KSV), i.e. the product of the quenching constant (k_q) and τ^0.



The Stern-Volmer plots show a linear correlation between the ratio τ^0/τ and the quencher concentration, as expected for a dynamic quenching process according to the Stern-Volmer equation:

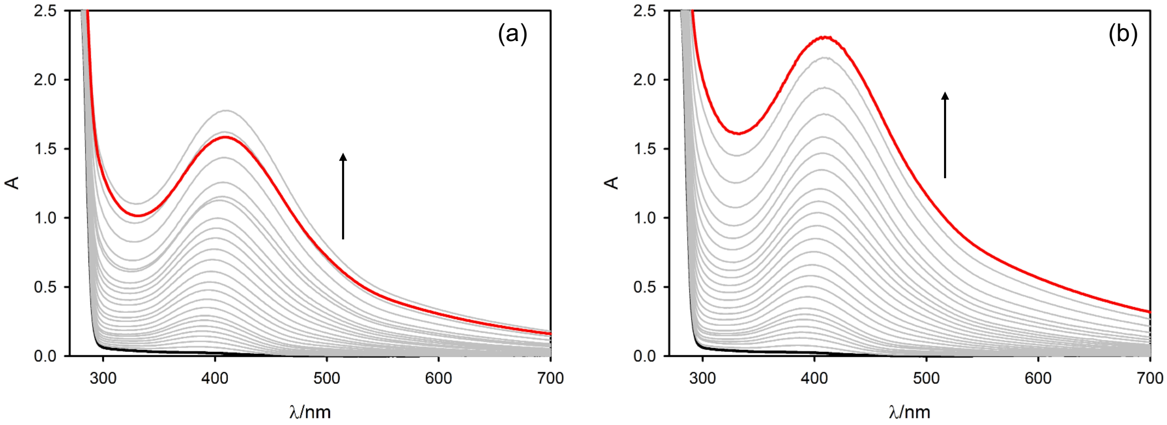
$$(1)\ \tau^0/\tau = 1 + K_{SV}[Q] = 1 + k_q\,\tau^0[Q]$$

where τ^0 and τ are the lifetimes in the absence and in the presence of the quencher Q, respectively, K_{SV} is the Stern-Volmer constant and k_q is the quenching constant.

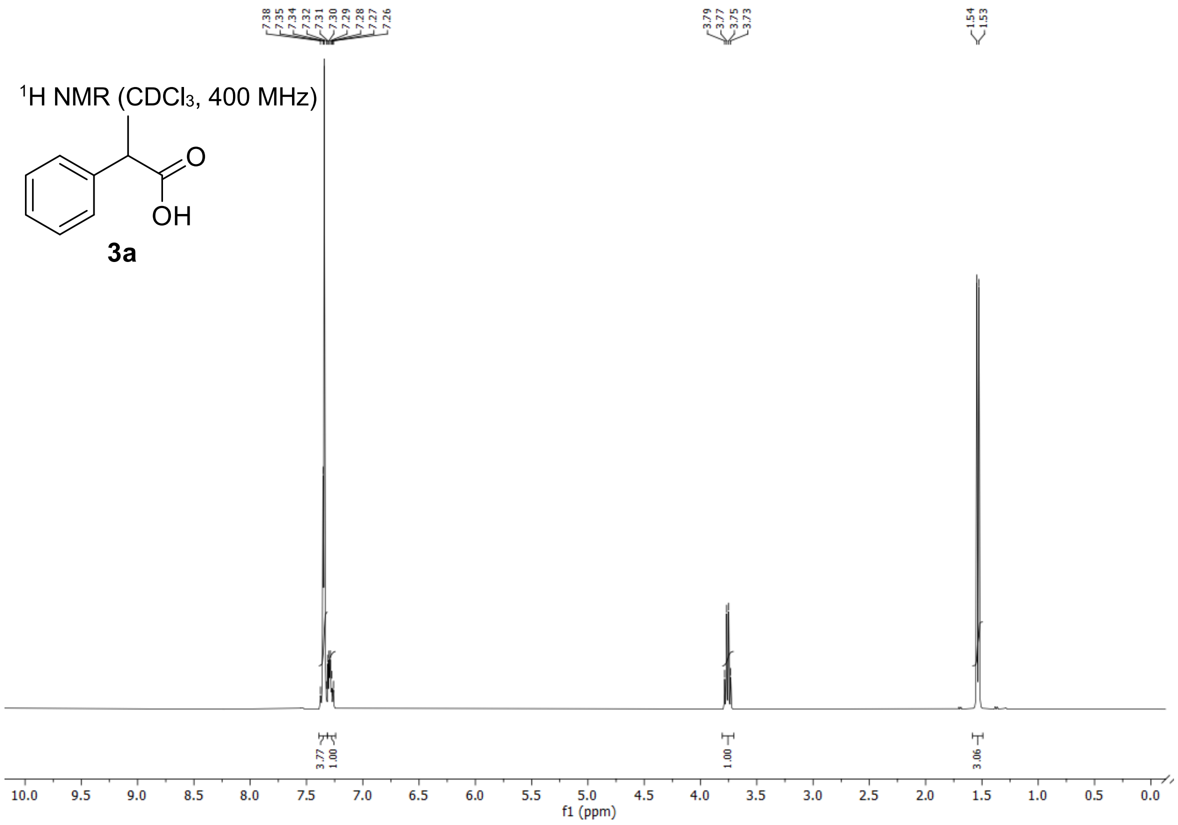
The analysis of the plots reported above yields the following quenching constants:

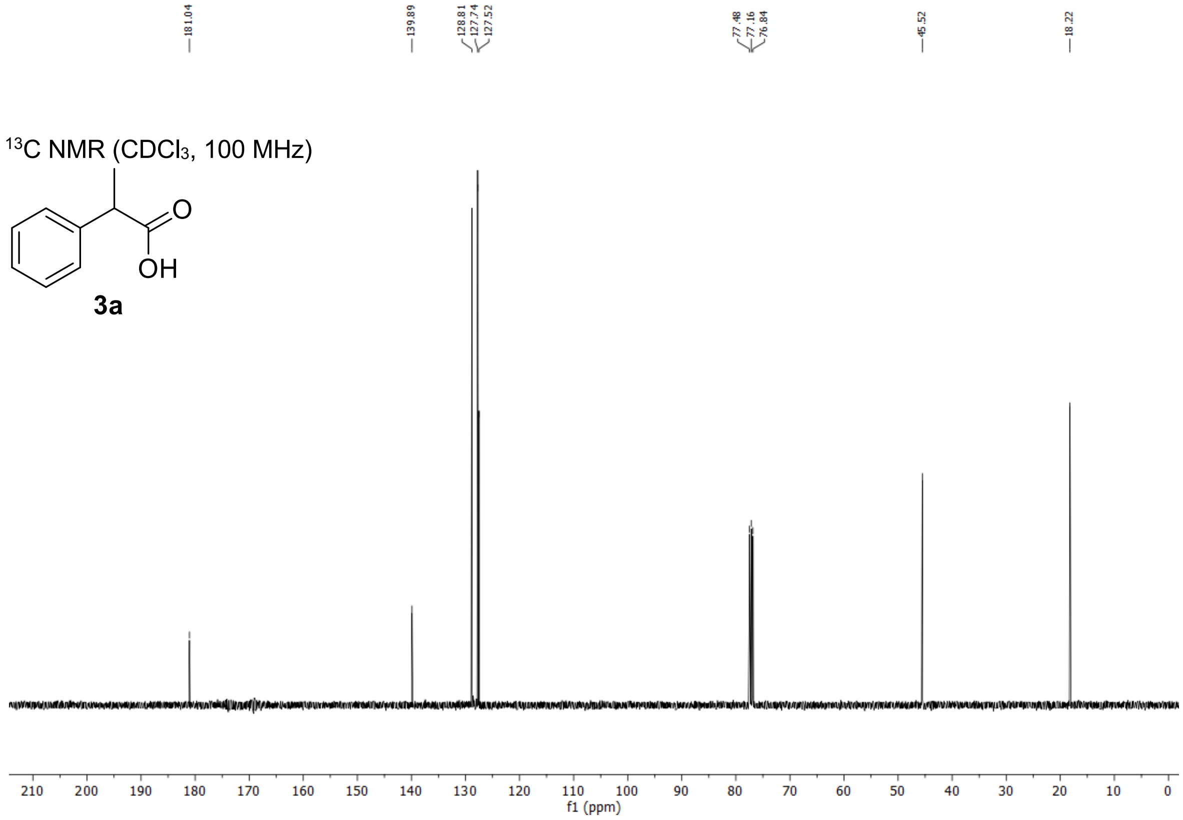
$$(2)\ k_q = 9.5 \times 10^9\ M^{-1}s^{-1} \text{ for TEMPO}$$

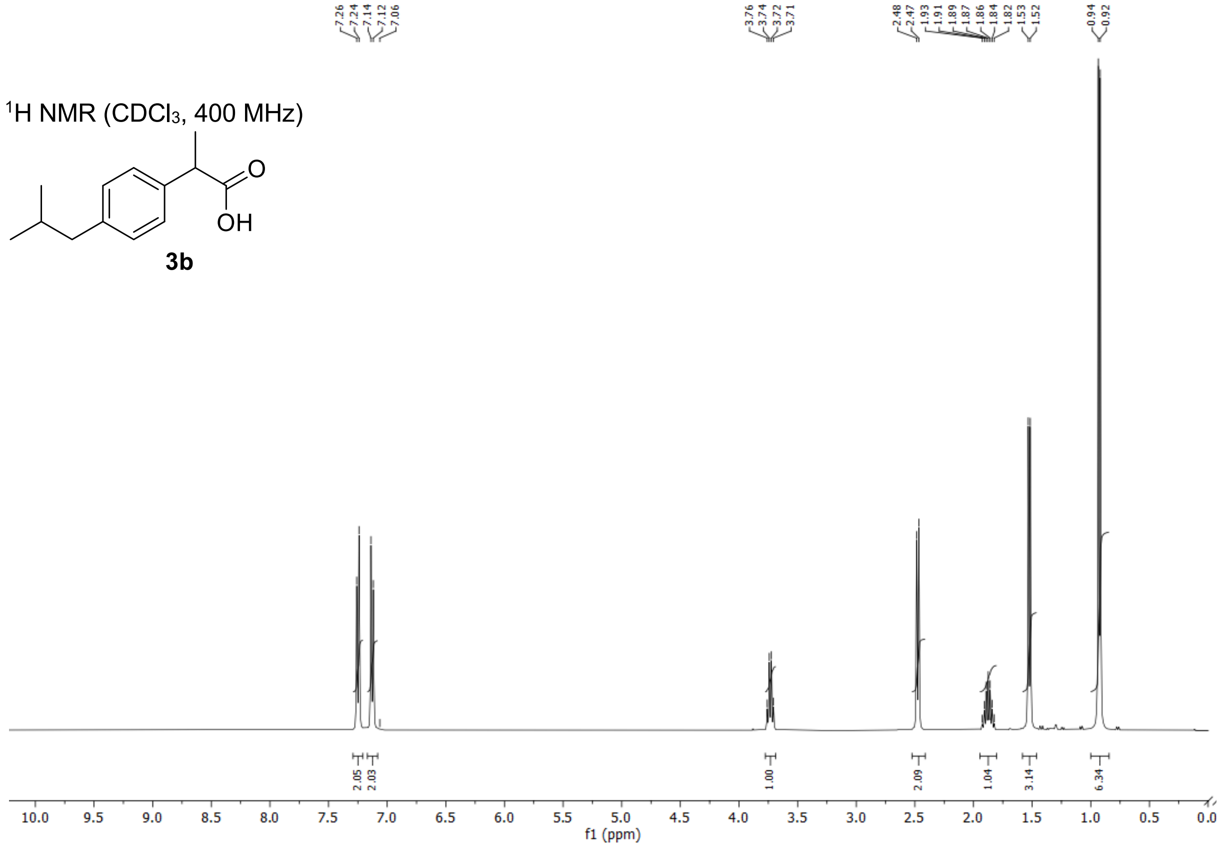
Figure S9. Absorption spectrum of an aqueous solution (acetate buffer 0.5 M, pH 4.5) of catechol 18 mM in presence of Laccase Tv (6 mg in 5 mL) at t = 0 (black solid lines), after 380 min (red solid line in figure (a)) and upon 380 min of red led irradiation (red solid line in figure (b)). Temperature 6°C.



Copies of NMR spectra of isolated compounds

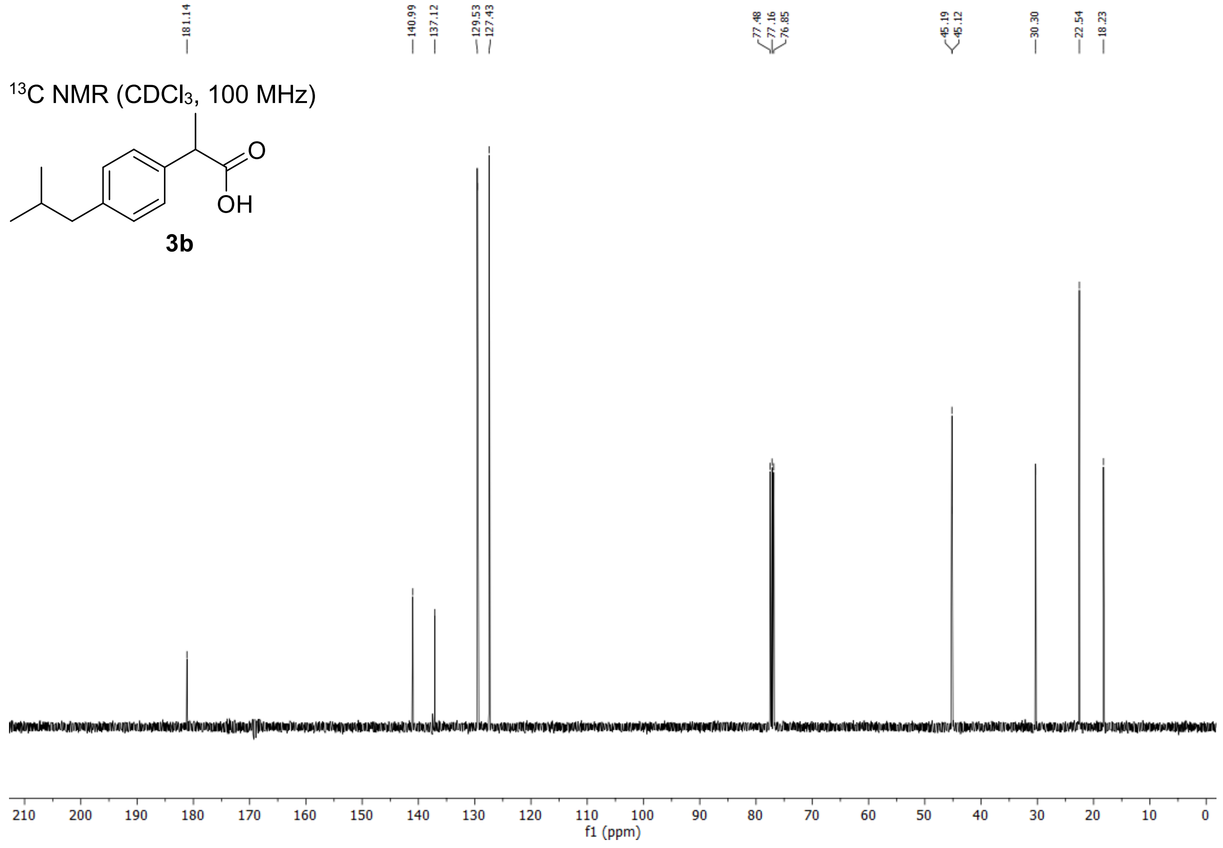






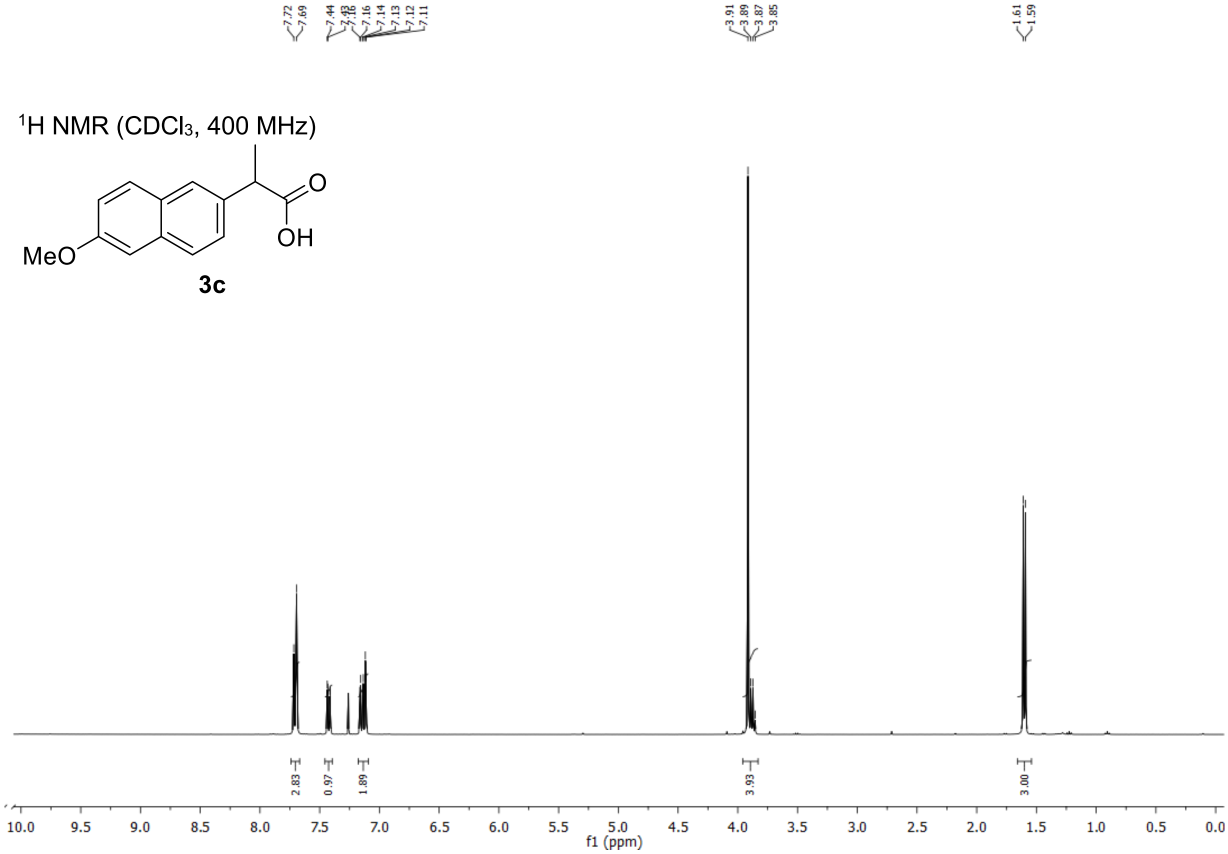
^{1}H NMR ($CDCl_3$, 400 MHz)

3b



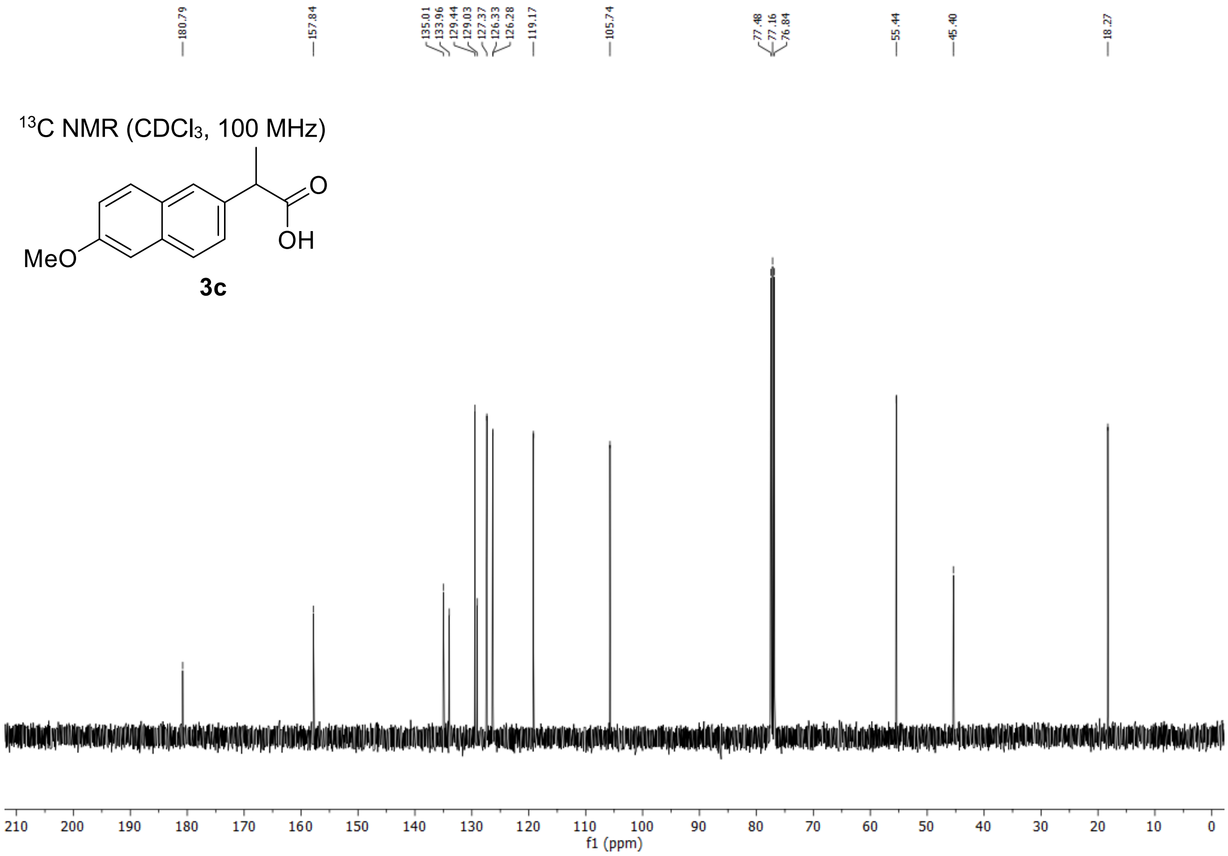
^{13}C NMR ($CDCl_3$, 100 MHz)

3b



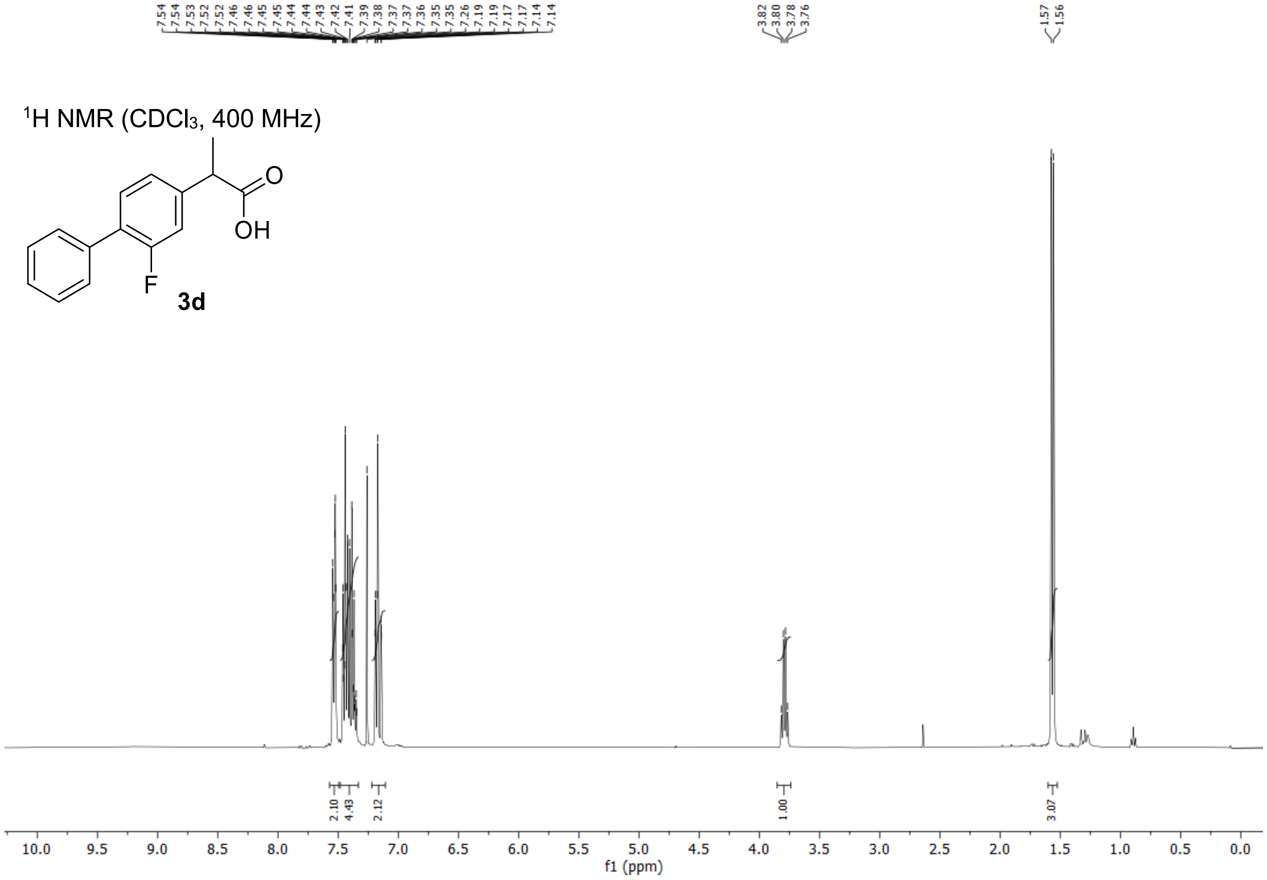
^{1}H NMR (CDCl$_3$, 400 MHz)

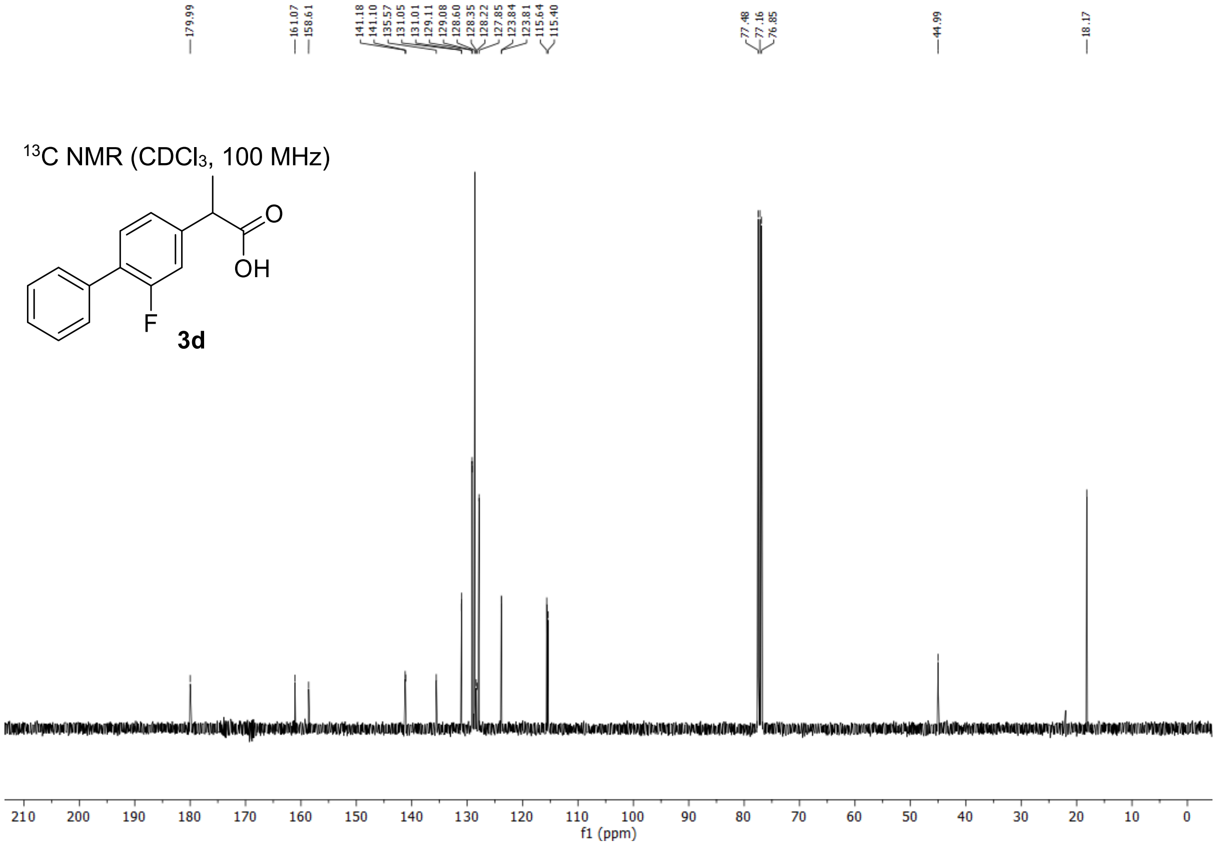
3c



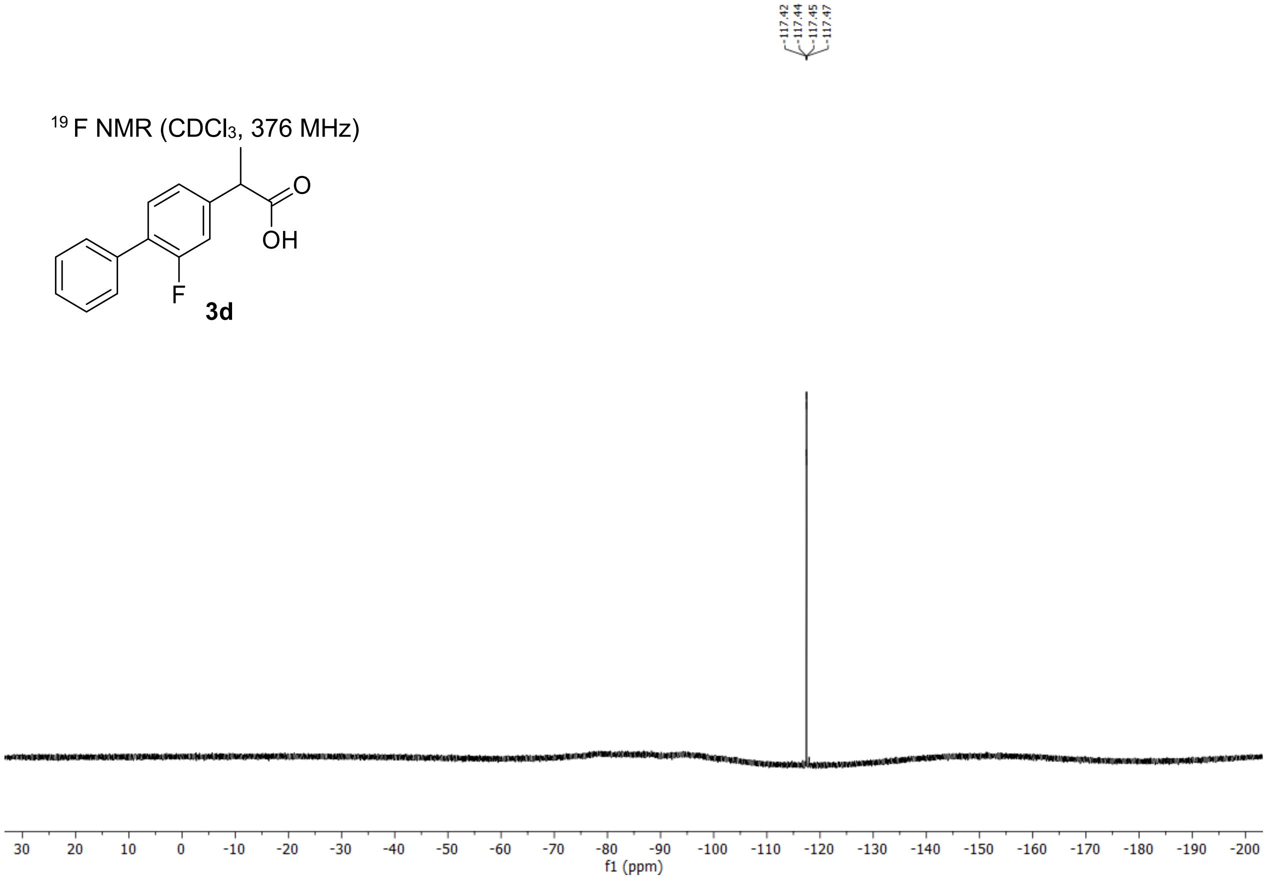
^{13}C NMR (CDCl$_3$, 100 MHz)

3c


7.54
7.54
7.53
7.52
7.52
7.46
7.45
7.45
7.44
7.44
7.43
7.42
7.41
7.39
7.38
7.37
7.37
7.36
7.35
7.35
7.26
7.19
7.19
7.17
7.17
7.14
7.14
3.82
3.80
3.78
3.76
1.57
1.56
^{1}H NMR (CDCl$_3$, 400 MHz)
O
OH
F
3d
2.10
4.43
2.12
1.00
3.07
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

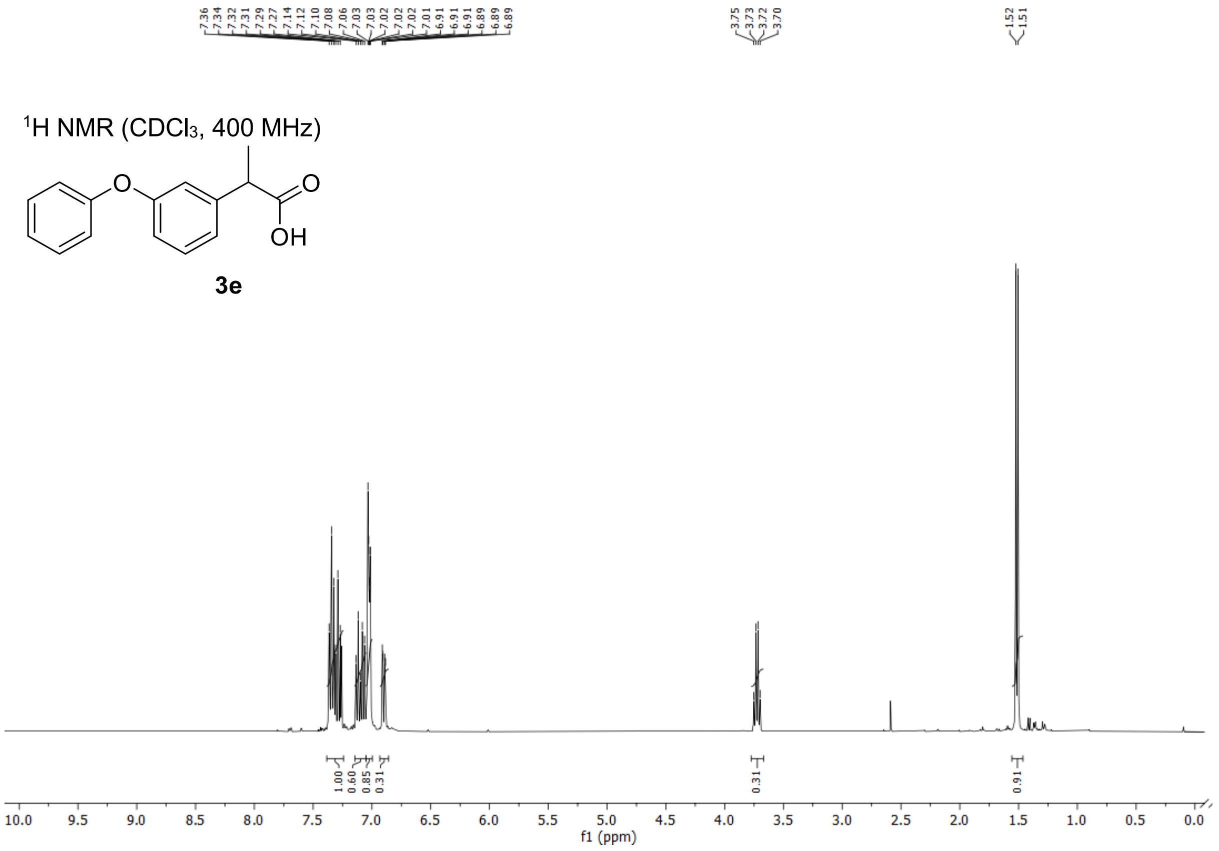

179.99
161.07
158.61
141.18
141.10
135.57
131.05
131.01
129.11
129.08
128.60
128.35
128.22
127.85
123.84
123.81
115.64
115.40
77.48
77.16
76.85
44.99
18.17
^{13}C NMR (CDCl$_3$, 100 MHz)
O
OH
F
3d
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{19}F NMR ($CDCl_3$, 376 MHz)

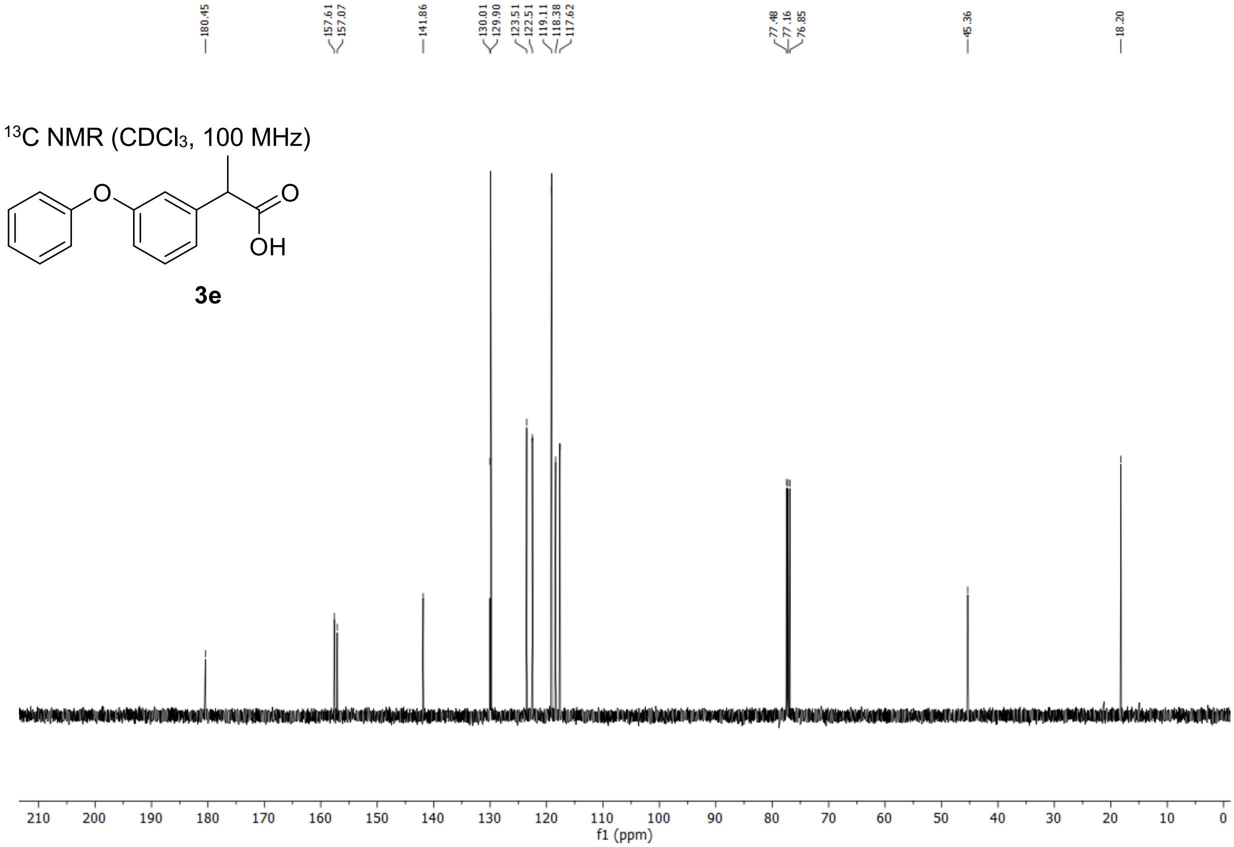


3d

^{1}H NMR ($CDCl_3$, 400 MHz)

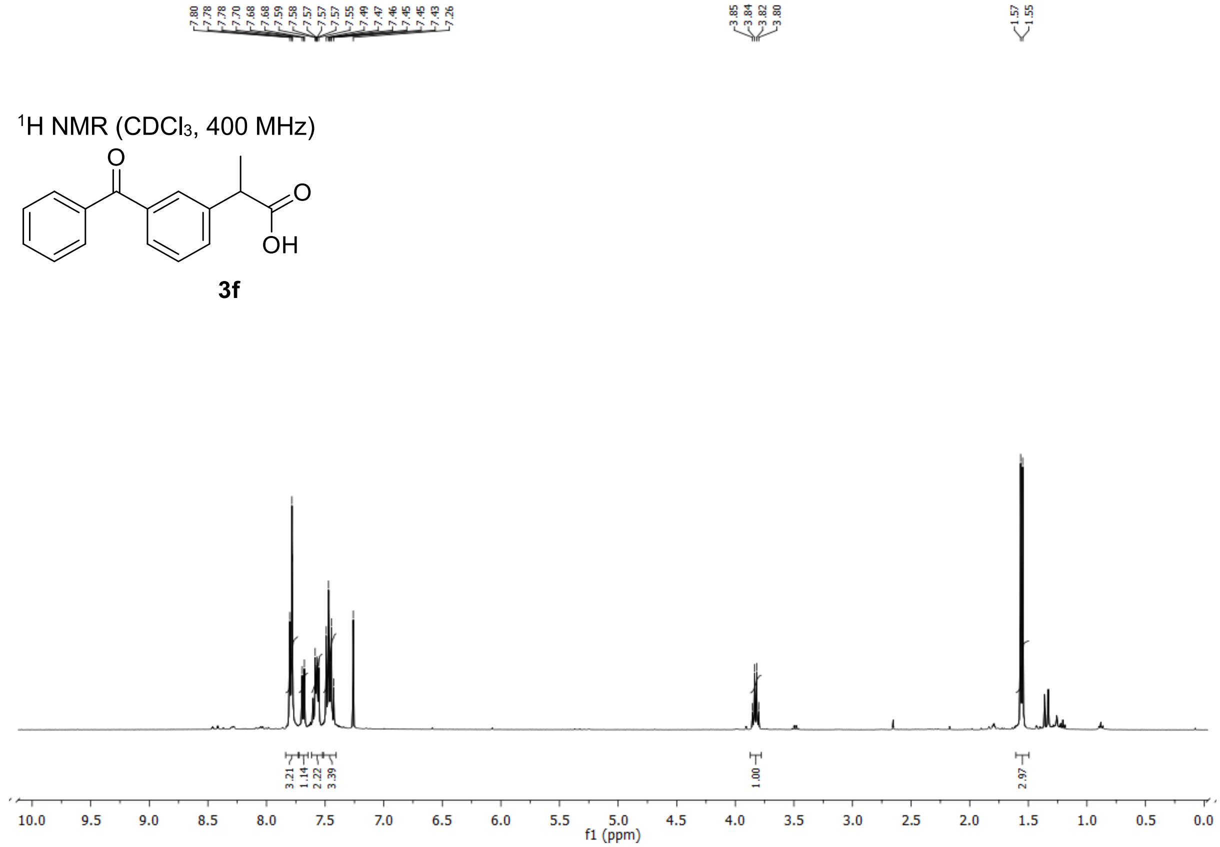


3e



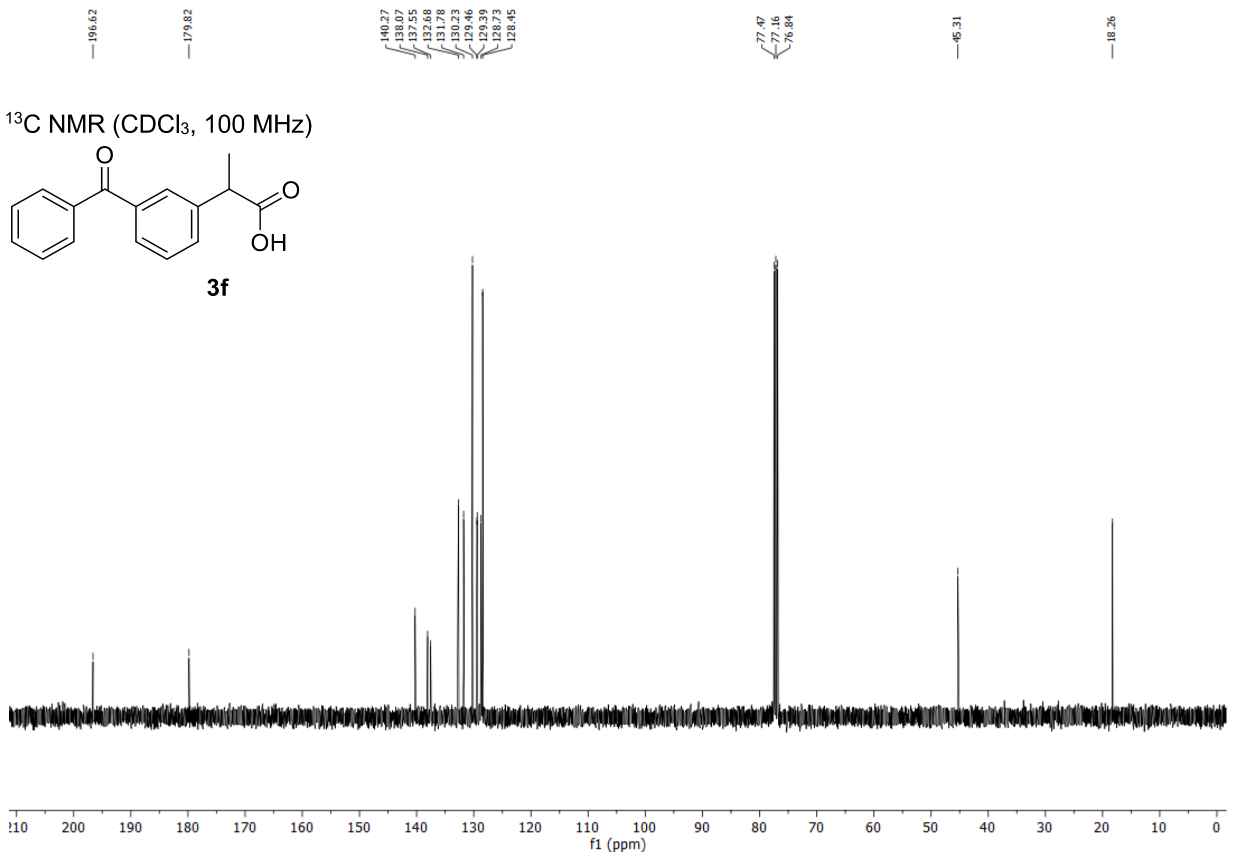
^{13}C NMR ($CDCl_3$, 100 MHz)

3e



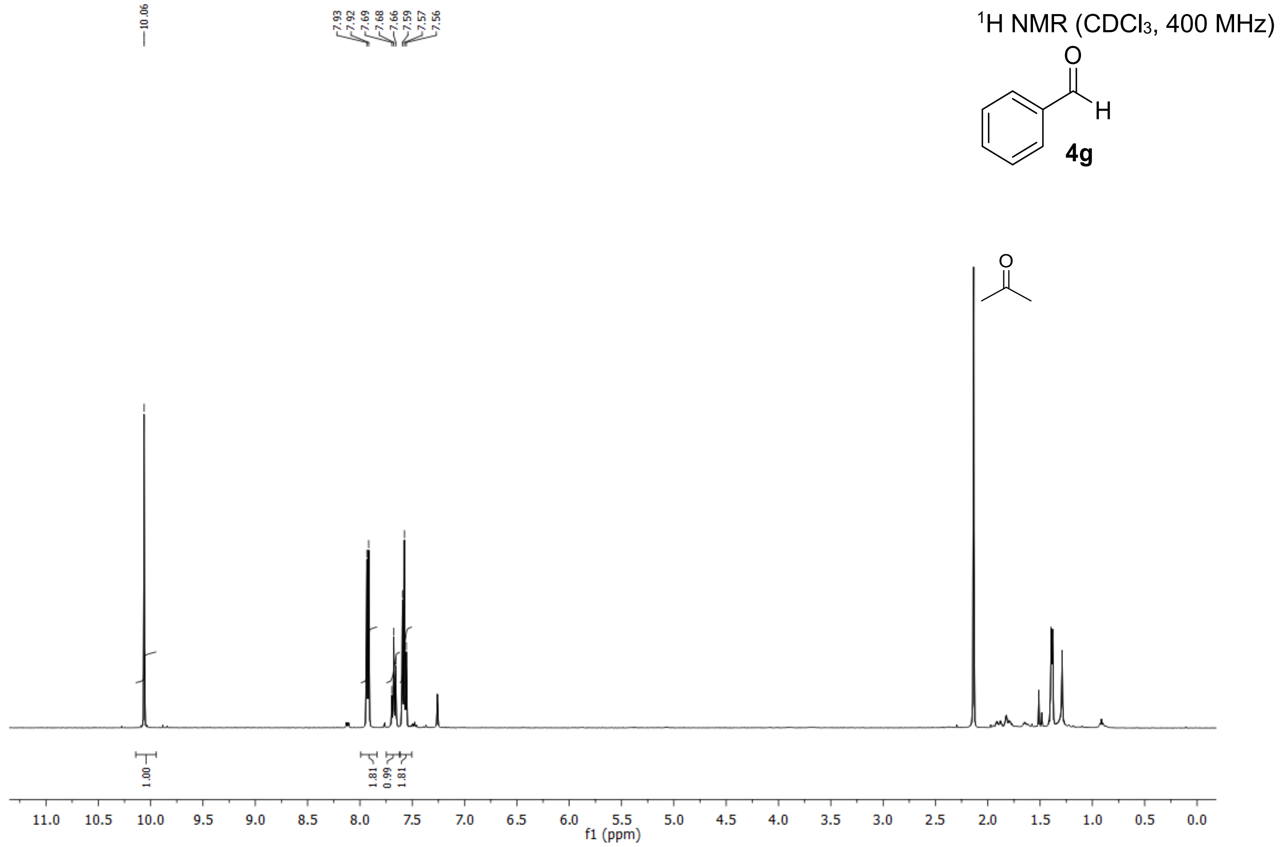
^{1}H NMR ($CDCl_3$, 400 MHz)

3f



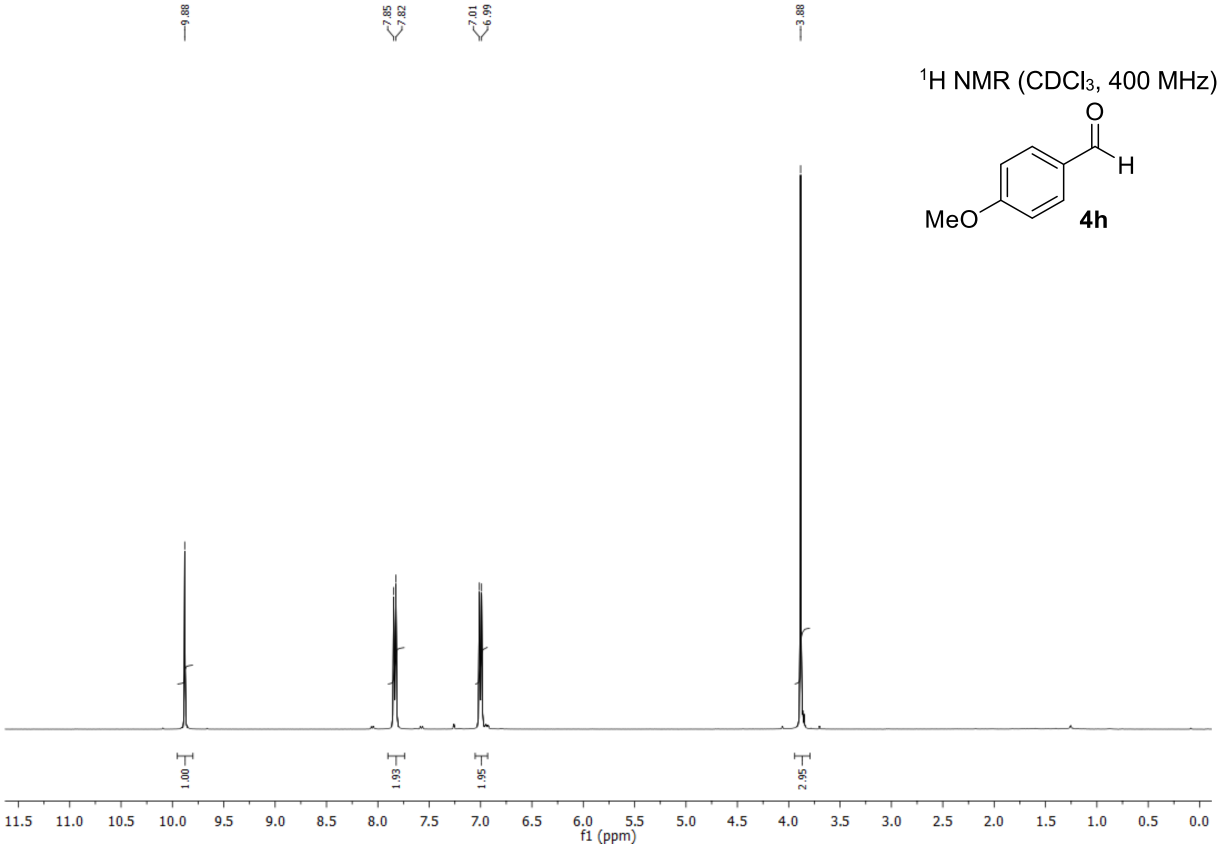
^{13}C NMR (CDCl$_3$, 100 MHz)

3f



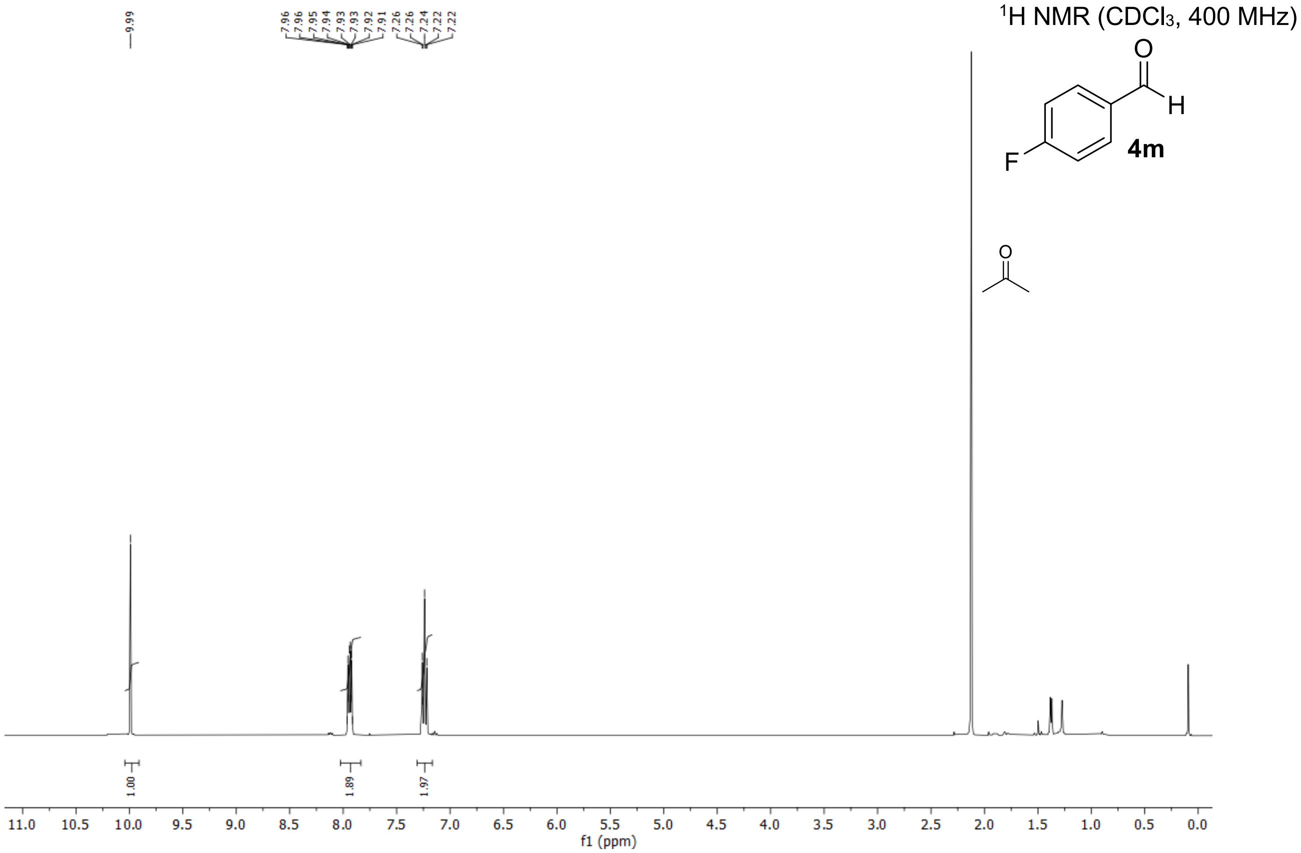
^{1}H NMR (CDCl$_3$, 400 MHz)

4g



^{1}H NMR (CDCl$_3$, 400 MHz)

4h



^{1}H NMR (CDCl$_3$, 400 MHz)

4m

Copies of ^{1}H NMR spectra of crude mixtures (Table 2 and 3)

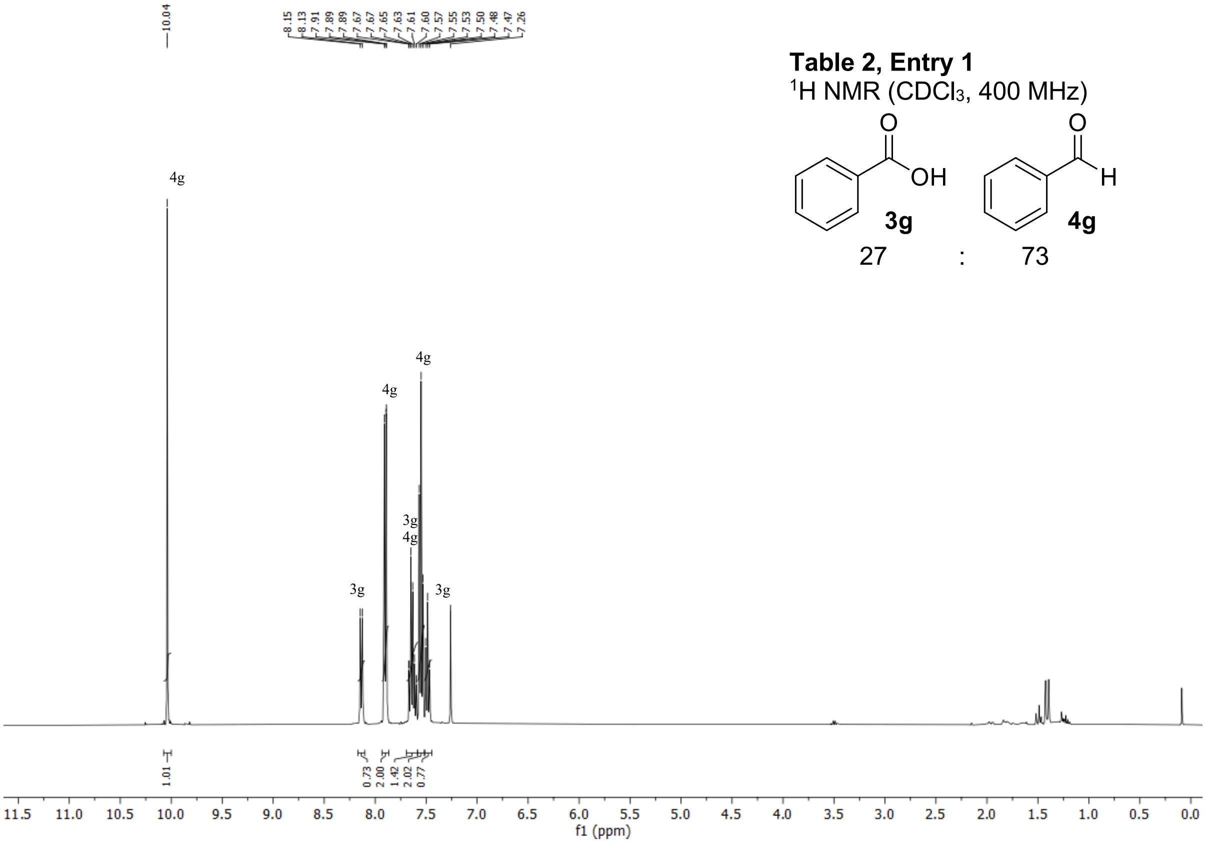


Table 2, Entry 1
^{1}H NMR ($CDCl_3$, 400 MHz)

3g : 4g
27 : 73

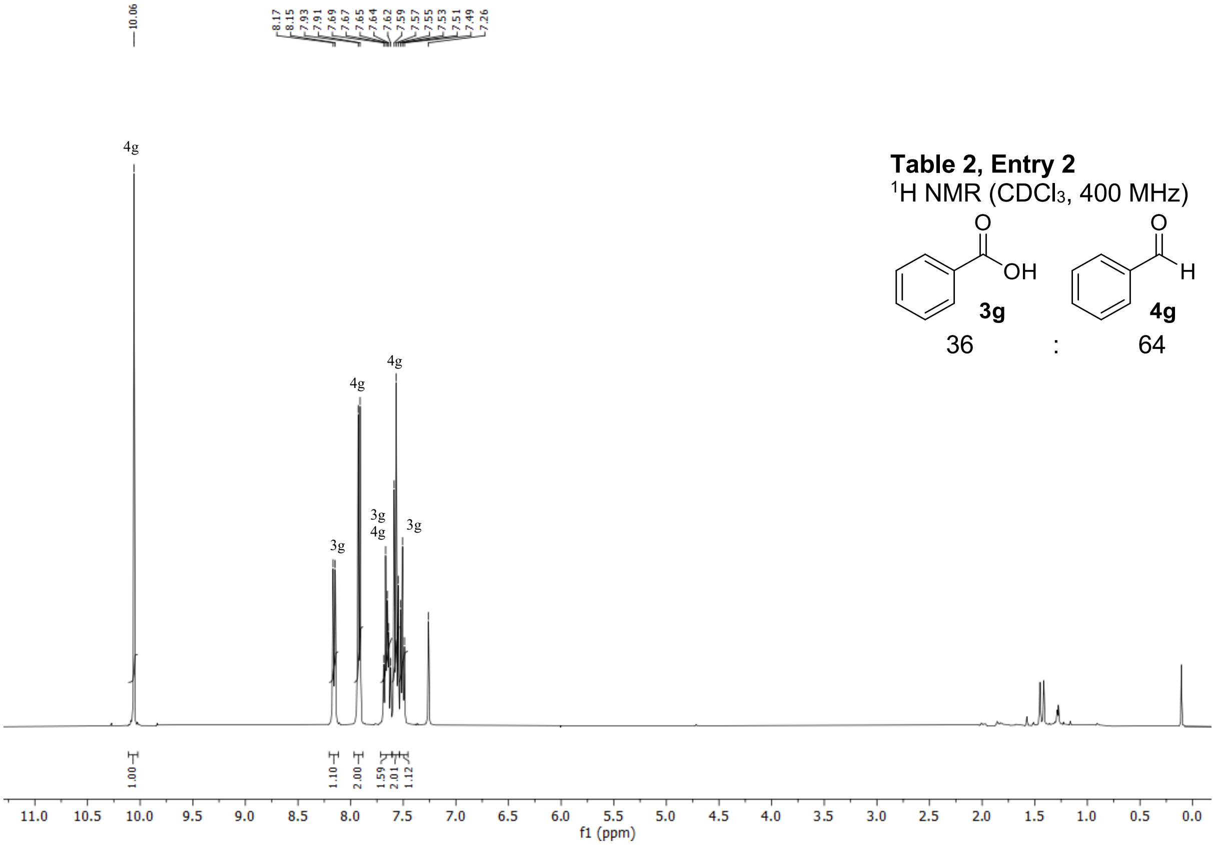


Table 2, Entry 2
^{1}H NMR ($CDCl_3$, 400 MHz)

3g : 4g
36 : 64

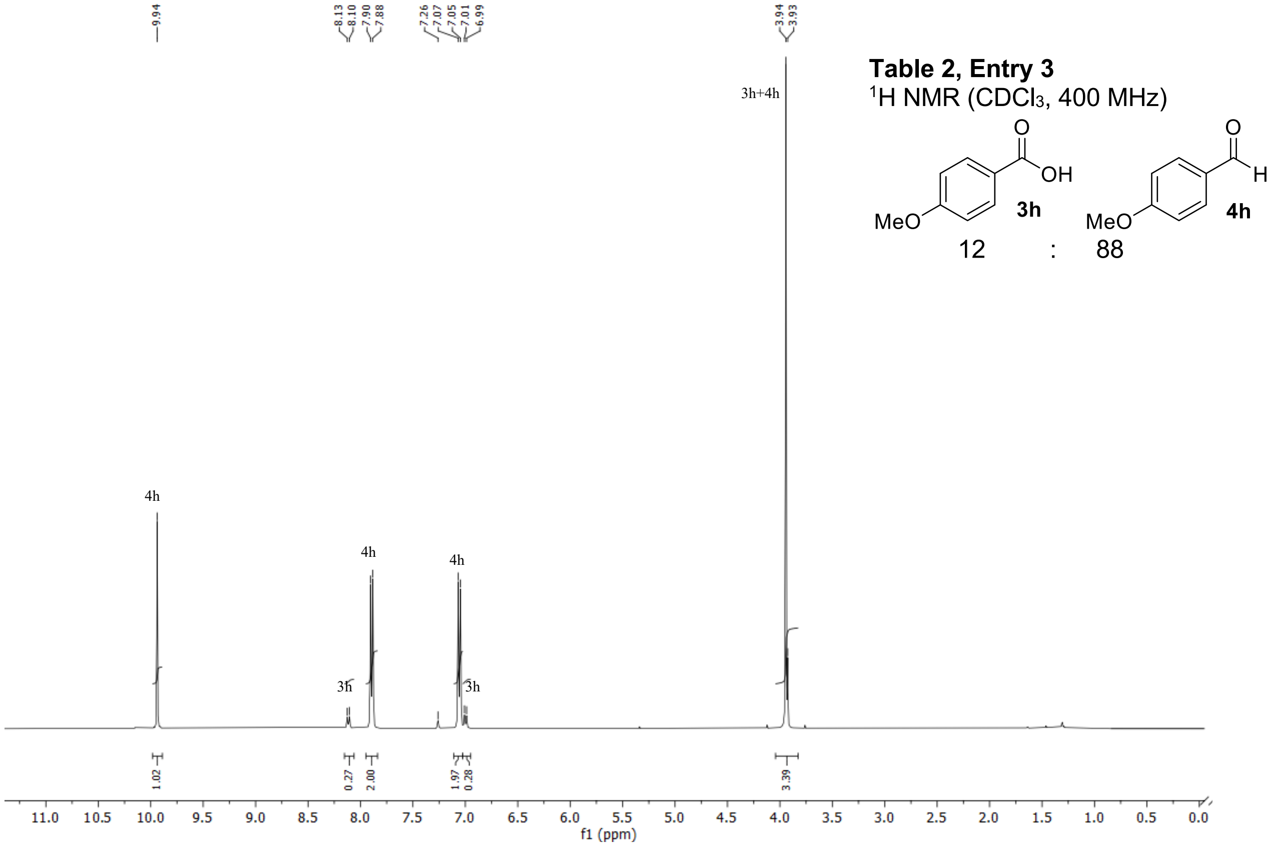


Table 2, Entry 3

^{1}H NMR ($CDCl_3$, 400 MHz)

3h 12 : 88 **4h**

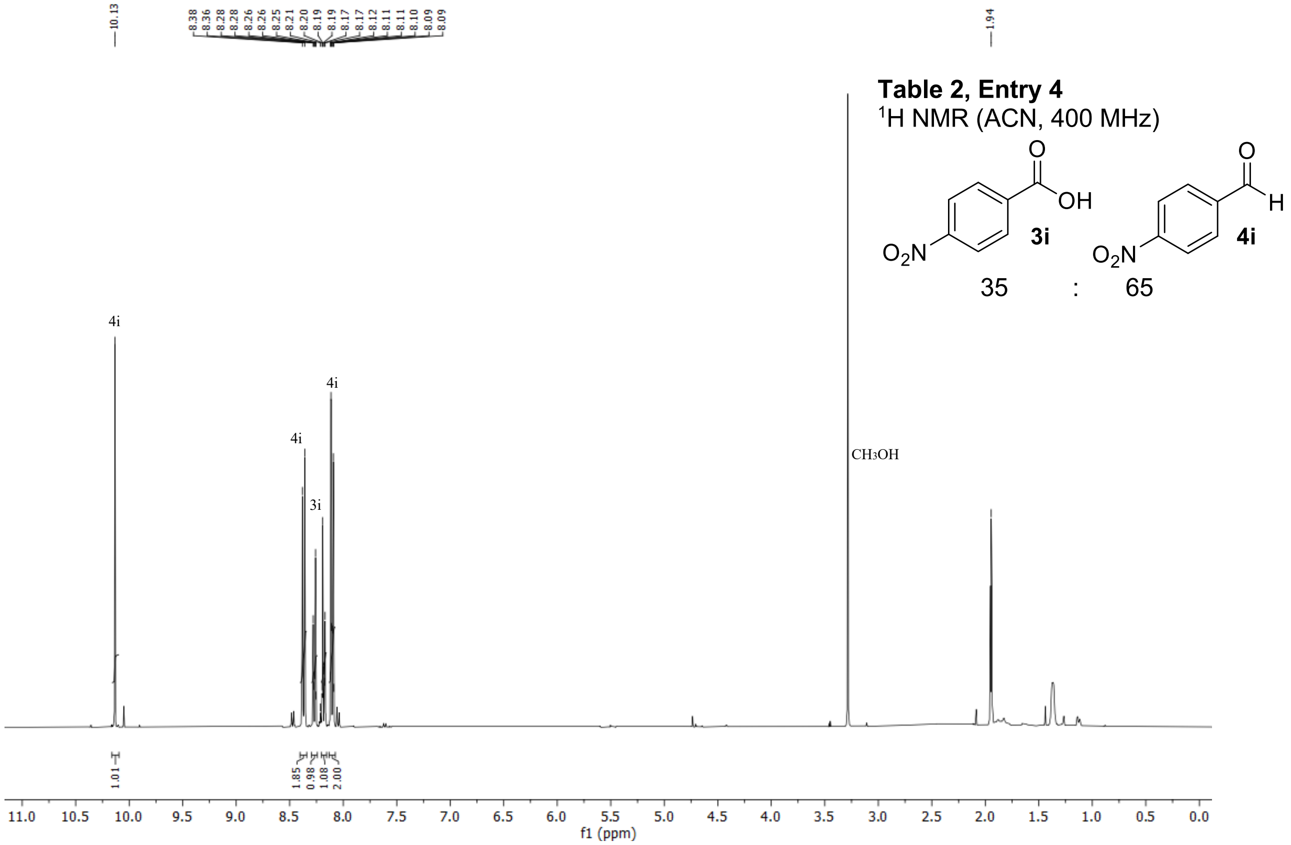
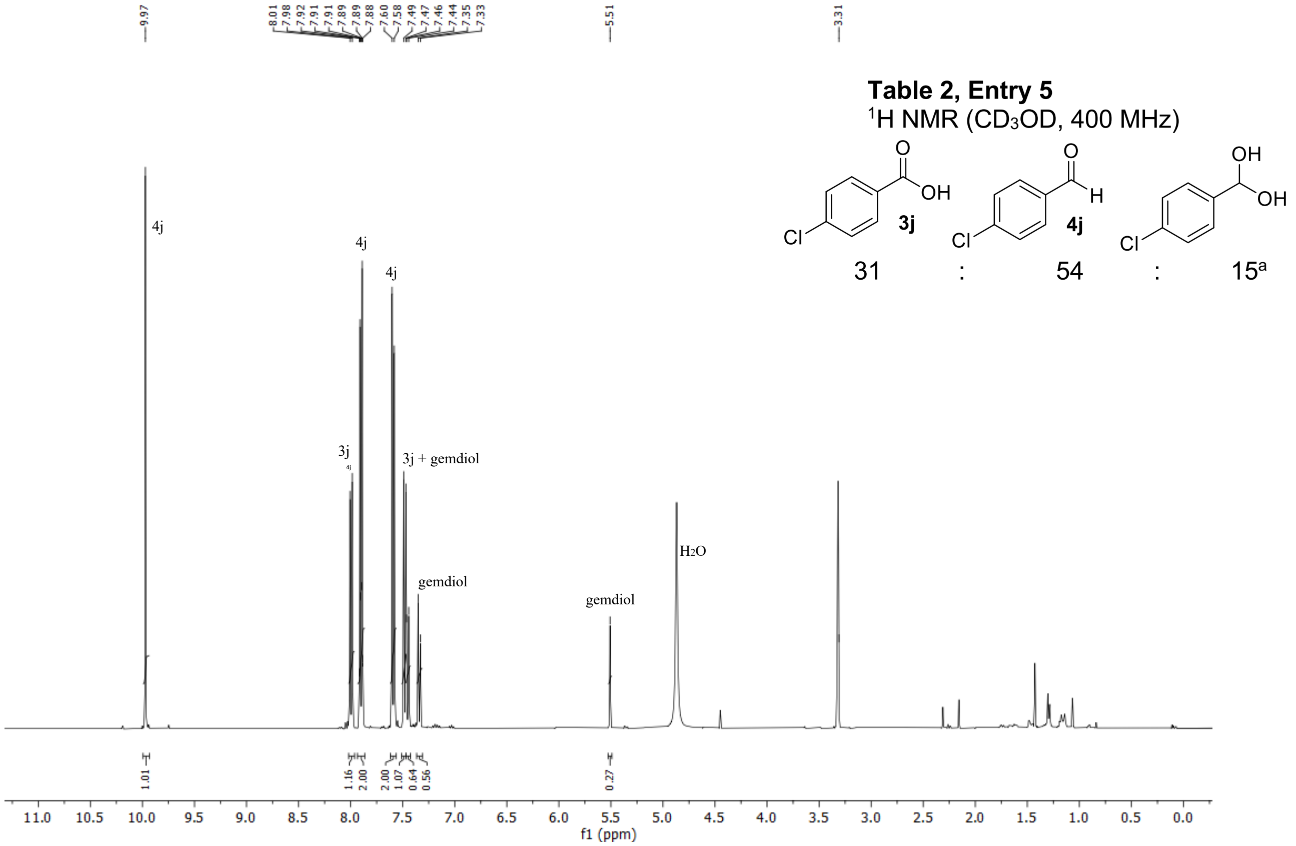
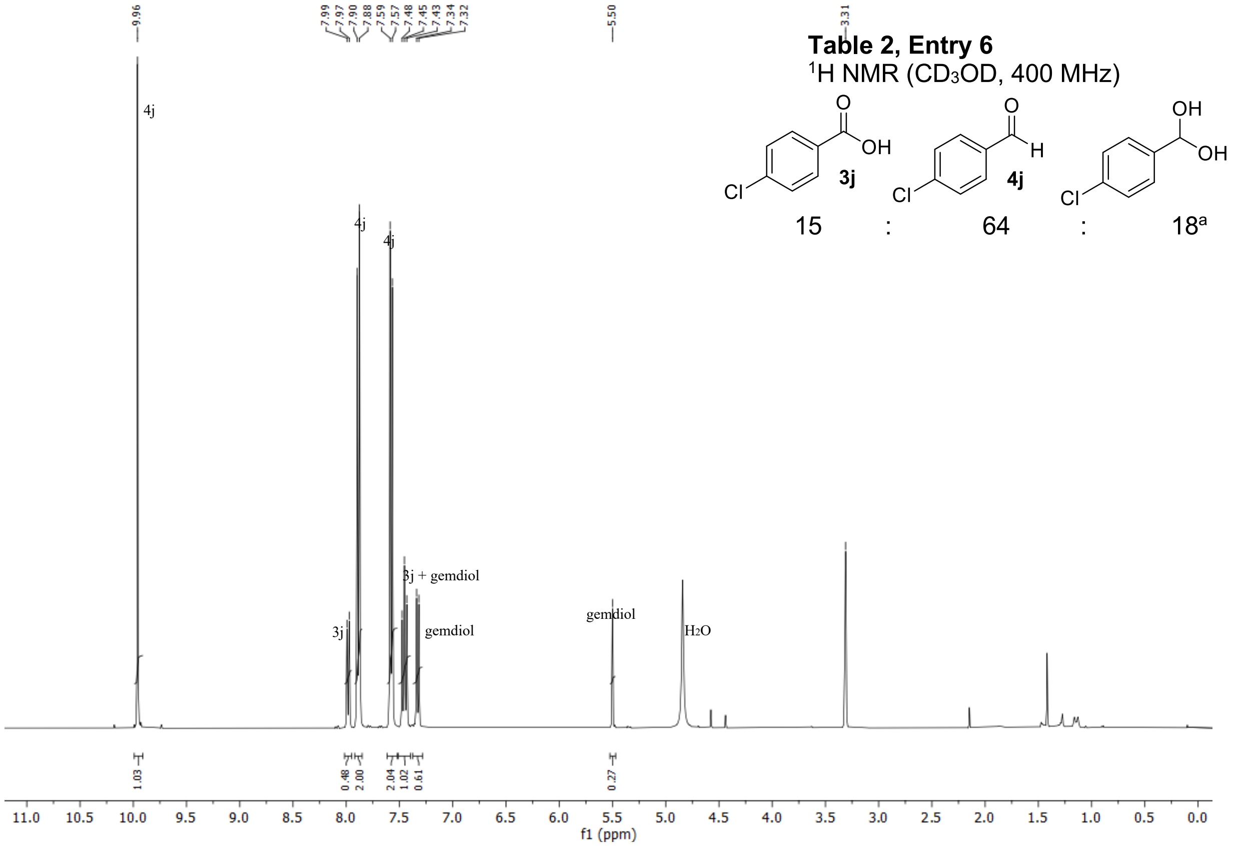


Table 2, Entry 4

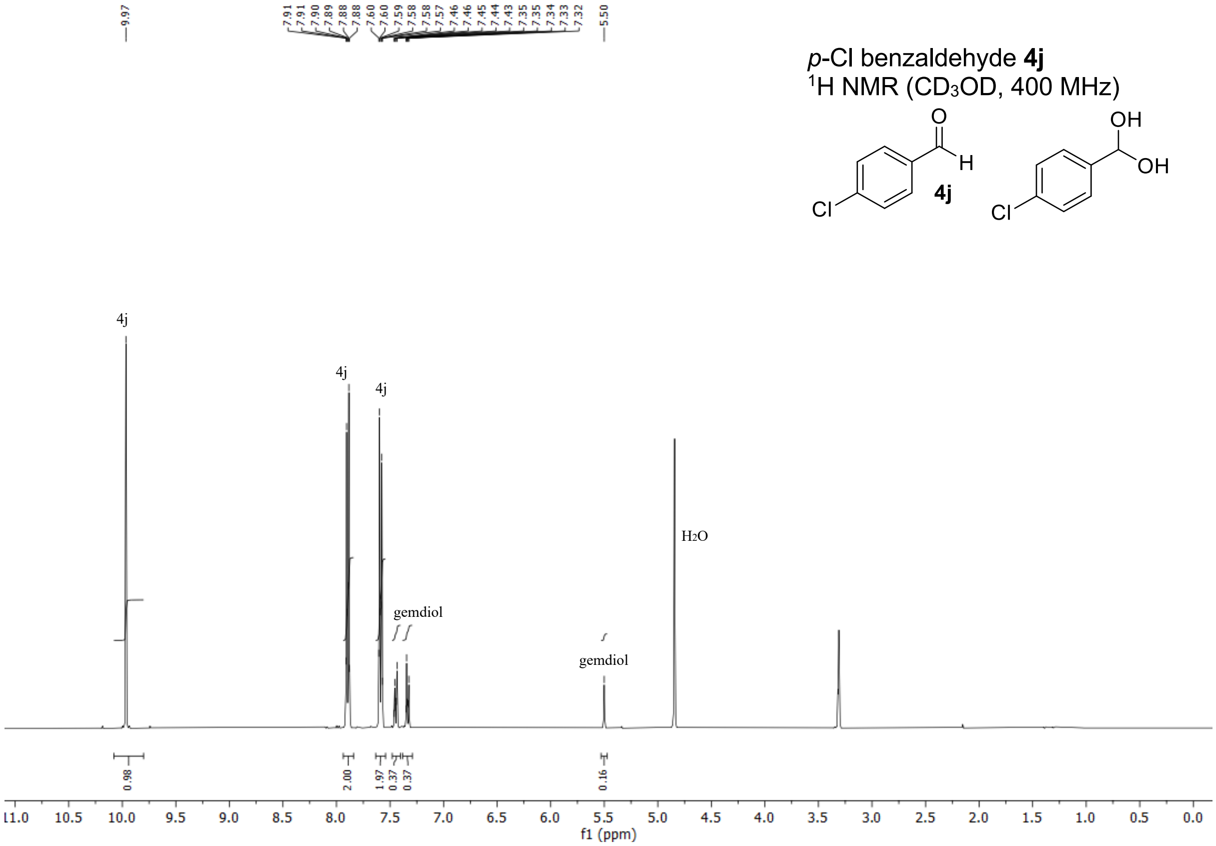
^{1}H NMR (ACN, 400 MHz)

3i 35 : 65 **4i**





a) Presence of gemdiol is observed due to the moisture present in CD$_3$OD (see below for the spectra of commercial *p*-Cl benzaldehyde in the same solvent)



p-Cl benzaldehyde **4j**

^{1}H NMR (CD$_3$OD, 400 MHz)

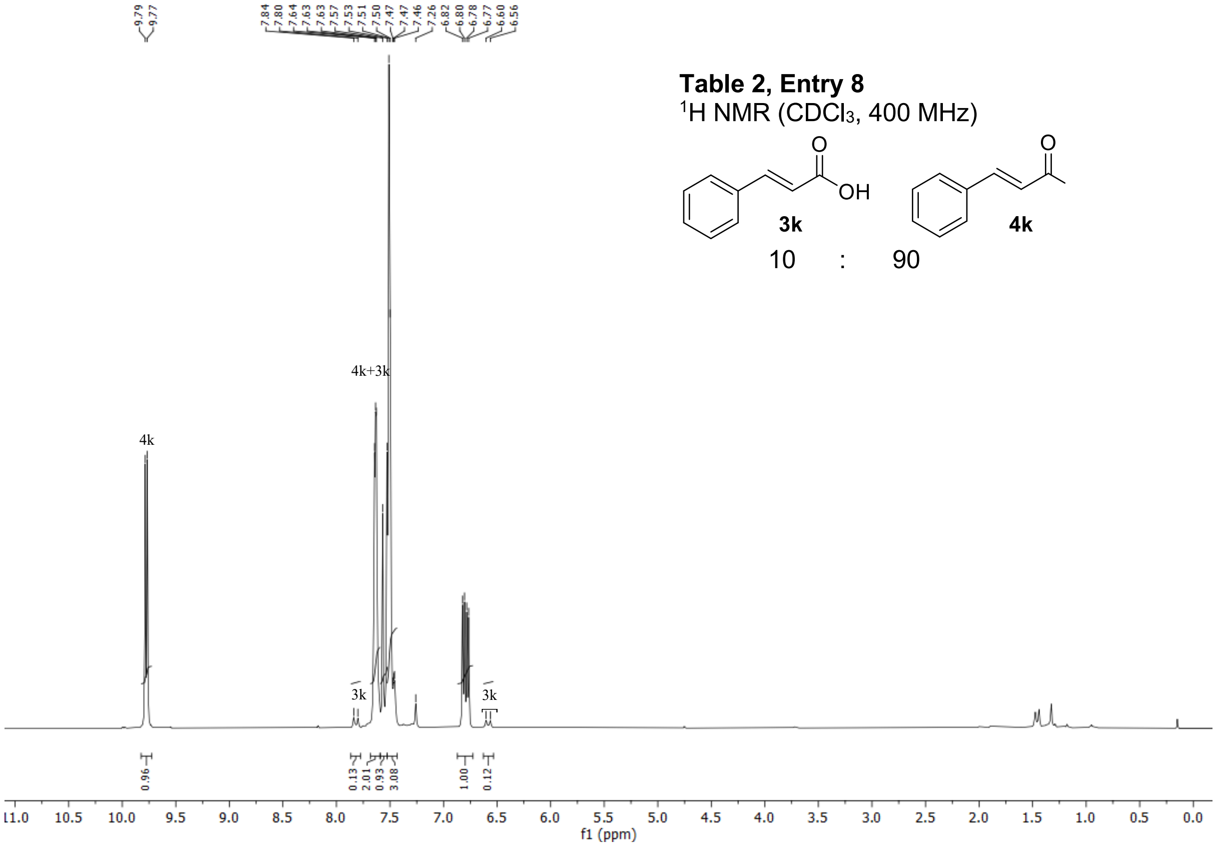
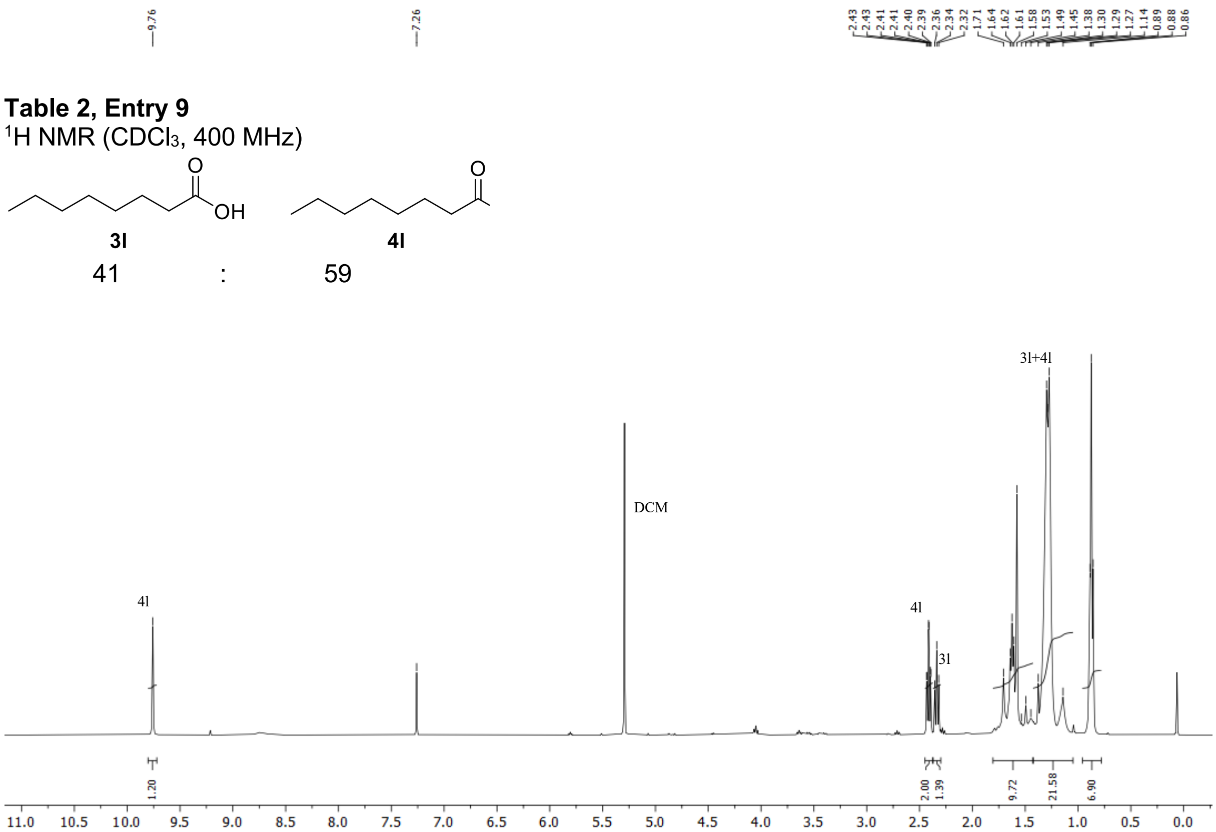


Table 2, Entry 8

^{1}H NMR (CDCl$_3$, 400 MHz)

10 : 90

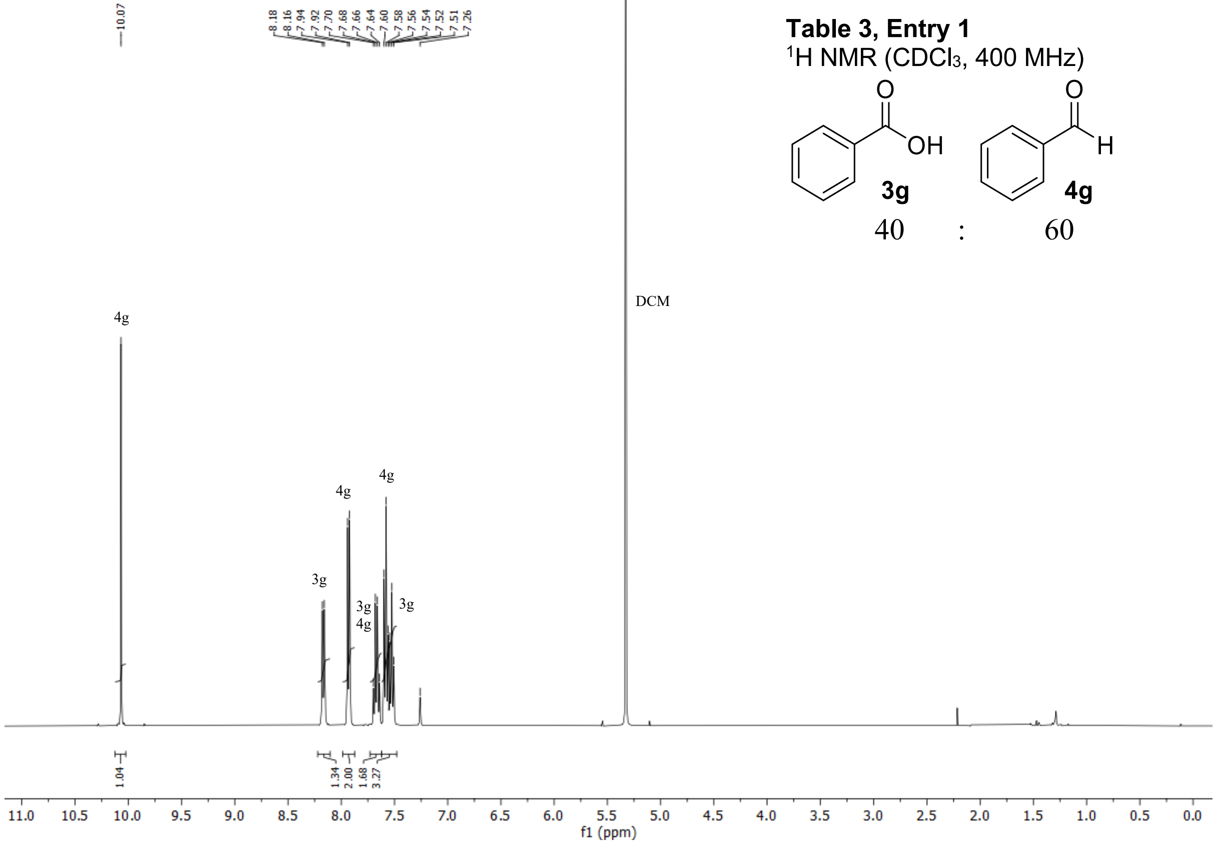
Table 2, Entry 9
^{1}H NMR (CDCl$_3$, 400 MHz)



3l : **4l**

41 : 59

Table 3, Entry 1
^{1}H NMR (CDCl$_3$, 400 MHz)



3g : **4g**

40 : 60

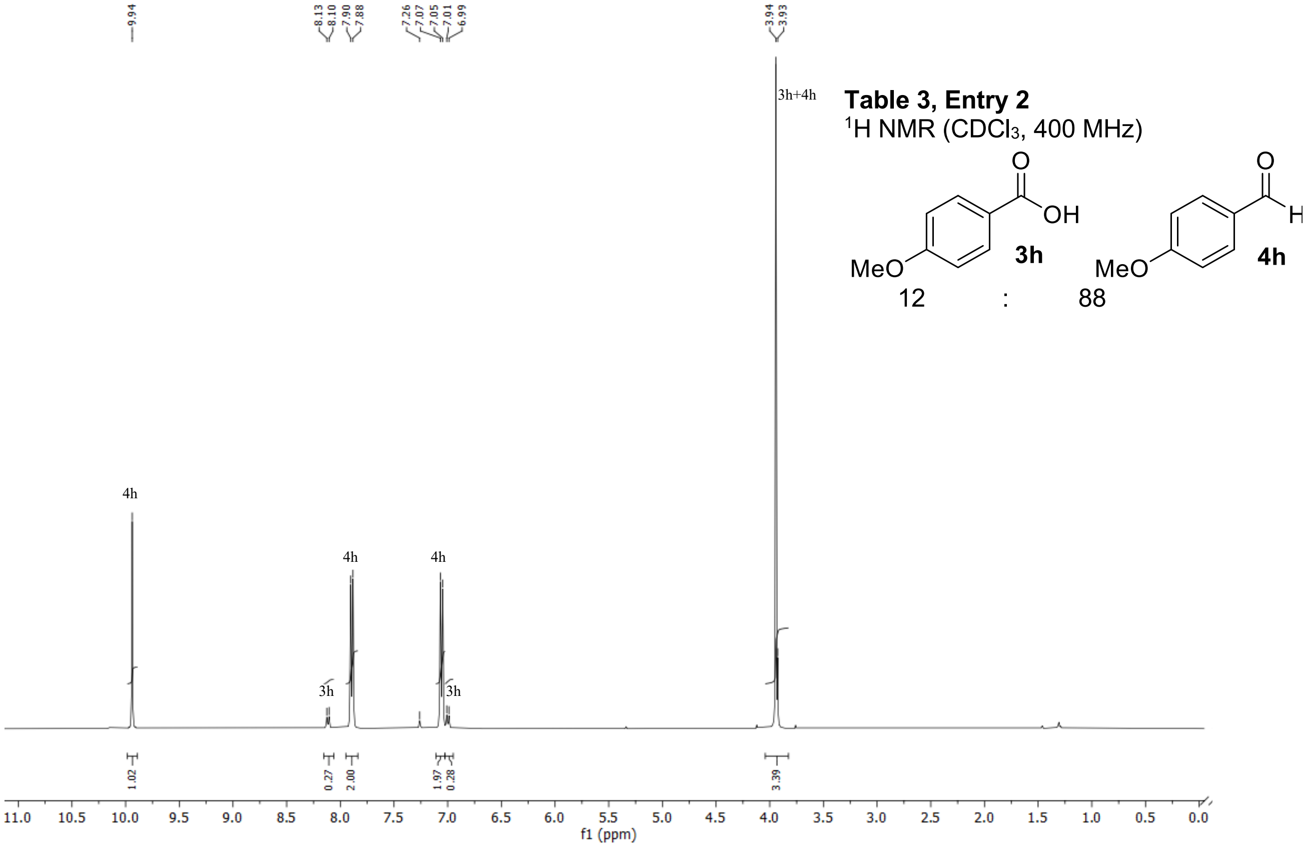


Table 3, Entry 2

^1H NMR ($CDCl_3$, 400 MHz)

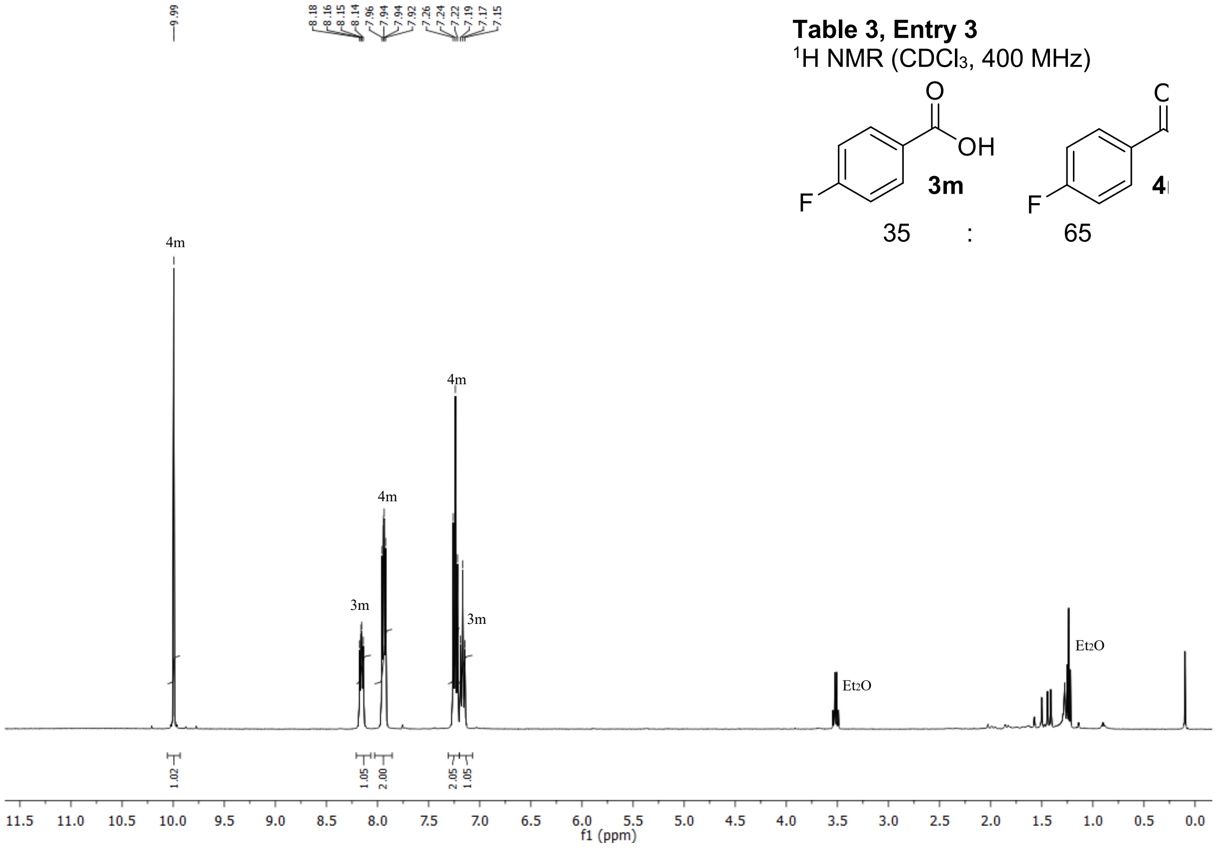


Table 3, Entry 3

^1H NMR ($CDCl_3$, 400 MHz)

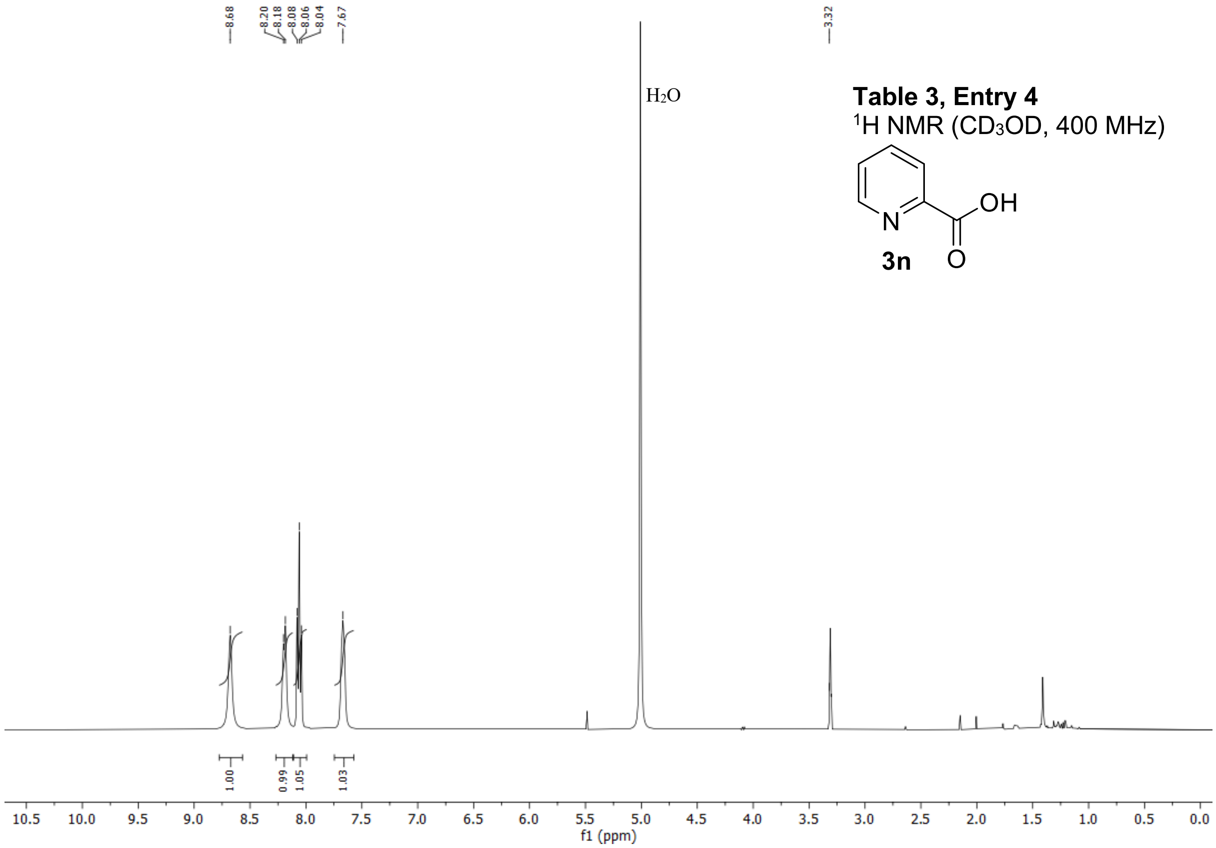


Table 3, Entry 4

^{1}H NMR (CD_3OD, 400 MHz)

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