Electronic Supplementary Material (ESI) for Green Chemistry.

Laccase/TEMPO-mediated system for the thermodynamically disfavored oxidation of 2,2-dihalo-1-phenylethanol derivatives

Kinga Kędziora, Alba Díaz-Rodríguez, Iván Lavandera, Vicente Gotor-Fernández and Vicente Gotor

Electronic Supplementary Information (page S1 of S46)

Table of Contents

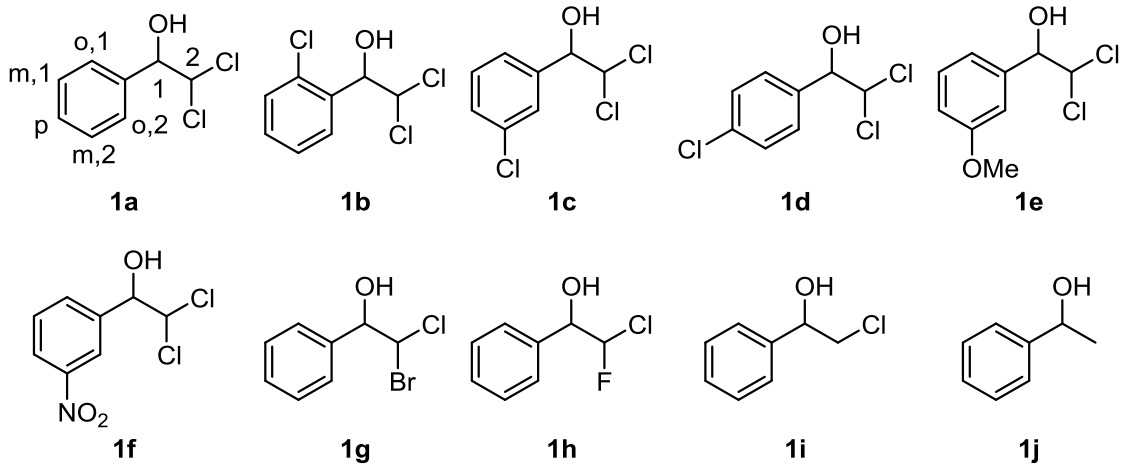
1. General

Acetophenone derivatives, 1-phenylethanol **1j**, α-chloroacetophenone **2i**, α,α-dichloroacetophenone **2a**, benzoic acid **3a**, benzaldehyde **4a**, and mandelic acid **5a** were purchased from Aldrich or Alfa Aesar. TEMPO was acquired from Aldrich (catalogue number: 214000). Other chemical reagents were purchased from different commercial sources and used as received. Solvents for chemical reactions were of high purity grade. Laccase from *Trametes versicolor* was obtained from Aldrich (13.6 U/mg) and overexpressed ADH from *Rhodococcus ruber* DSM 44541 (*E. coli*/ADH-A) was used as lyophilized cells as described elsewhere.[1]

Thin-layer chromatography (TLC) was conducted with Merck Silica Gel 60 F254 precoated plates and visualized with UV and potassium permanganate stain. Flash chromatography was performed using silica gel 60 (230-400 mesh). Melting points were obtained on a Gallenkamp apparatus and are reported uncorrected. IR spectra were recorded on a Perkin-Elmer 1720-X infrared Fourier transform spectrophotometer on NaCl pellets. ^{1}H-, ^{13}C-NMR, and DEPT were obtained using a Bruker DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) spectrometer for routine experiments. The chemical shifts (δ) are given in ppm and the coupling constants (J) in Hertz (Hz). ESI^{+} mode was used to record mass spectra (MS) and ESI-TOF for HRMS. The mass spectra acquisition was not feasible with the techniques described above for compounds **1c**, **1g**, and **2f**. Gas chromatography (GC) analyses were performed on a Hewlett Packard 6890 Series II chromatograph. HPLC analyses were performed with Hewlett Packard 1100 LC liquid chromatograph. Optical rotations were measured using a Perkin-Elmer 241 polarimeter and are quoted in units of 10^{-1} deg cm^2 g^{-1}.

2. Experimental procedures

2.1. Chemical structure of the alcohols used throughout the study



*2.2. General procedure for the synthesis of ketones **2b-f***[2]

To a solution of the corresponding acetophenone (3.7 mmol) and *p*-toluenesulfonic acid (708.8 mg, 3.7 mmol) in acetonitrile, *N*-chlorosuccinimide was added (1 g, 7.6 mmol). The reaction mixture was heated at 50 °C and stirred till disappearance of the starting material (16 h or 48 h for **2b**). After completion, the solvent was evaporated under reduced pressure and the crude was purified using flash chromatography (50-80% CH_2Cl_2/hexane) affording ketones in high yields (75-90%).

Compound 2b: 2,2-dichloro-1-(2-chlorophenyl)ethanone; yellowish pale oil. IR (neat): ν 3071, 3005, 1728, 1589 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 6.77 (*s*, 1H, H_2), 7.31-7.47 (*m*, 3H, H_m+H_p), 7.55 (*d*, $^3J_{HH}$ 8.3 Hz, 1H, H_o). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 69.1 (CH, C_2), 126.9 (CH, C_{ar}), 130.1 (CH, C_{ar}), 130.3 (CH, C_{ar}), 131.1 (C, C_i), 132.9 (CH, C_{ar}), 133.8 (C, C_o), 188.2 (C, C_1); HRMS (ESI^+, m/z): calcd for $C_8H_5Cl_3ONa$: 244.9298, found: 244.9289.

Compound 2c: 2,2-dichloro-1-(3-chlorophenyl)ethanone; yellowish pale oil. IR (neat): ν 3070, 3007, 2360, 1707, 1573 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 6.60 (s, 1H, H_2), 7.47 (*ap t*, $^3J_{HH}$ 8.0 Hz, 1H, H_m), 7.63 (*ddd*, $^3J_{HH}$ 8.0 $|^4J_{HH}|$ 2.1 $|^4J_{HH}|$ 1.1 Hz, 1H, H_p), 7.99 (*ddd*,

$^3J_{HH}$ 8.0 $|^4J_{HH}|$ 2.1 $|^4J_{HH}|$ 1.1 Hz, 1H, $H_{o,1}$), 8.07 (*ap t*, $|^4J_{HH}|$ 1.8 Hz, 1H, $H_{o,2}$). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 67.8 (CH, C_2), 127.8 (CH, $C_{o,1}$), 129.6 (CH, $C_{o,2}$), 130.3 (CH, C_m), 132.8 (C, C_m), 134.6 (CH, C_p), 135.3 (C, C_i), 184.9 (C, C_1). Spectral data for this compound are in agreement with the previously published ones.[3]

Compound 2d: 2,2-dichloro-1-(4-chlorophenyl)ethanone; white solid. m.p.: 60-61 °C. IR (KBr): ν 3383, 3027, 2583, 1707, 1590, 1402 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 6.59 (*s*, 1H, H_2), 7.49 (*dd*, $^3J_{HH}$ 6.8 $|^4J_{HH}|$ 2.1 Hz, 2H, H_m), 8.04 (*dd*, $^3J_{HH}$ 6.8 $|^4J_{HH}|$ 2.1 Hz, 2H, H_o). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 67.7 (CH, C_2), 129.2 (CH, $2C_{ar}$), 129.2 (C, C_p), 131.1 (CH, $2C_{ar}$), 141.1 (C, C_i), 184.8 (C, C_1). Spectral data for this compound are in agreement with the previously published ones.[4]

Compound 2e: 2,2-dichloro-1-(3-methoxyphenyl)ethanone; yellowish pale solid. m.p.: 65-66 °C. IR (KBr): ν 3078, 3008, 2965, 2837, 1707, 1597, 1487 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 3.87 (*s*, 3H, Me), 6.69 (*s*, 1H, H_2), 7.19 (*dd*, $^3J_{HH}$ 8.3 $|^4J_{HH}|$ 2.6 Hz, 1H, H_p), 7.42 (*ap t*, $^3J_{HH}$ 8.1 Hz, 1H, H_m), 7.62 (*m*, 2H, H_o). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 55.4 (CH_3), 67.6 (CH, C_2), 113.9 (CH, C_m), 121.1 (CH, C_{ar}), 121.9 (CH, C_{ar}), 129.7 (CH, C_{ar}), 132.5 (C, C_i), 159.8 (C, C_m), 185.7 (C, C_1). HRMS (ESI^+, m/z): calcd for $C_9H_8Cl_2O_2Na$: 240.9794, found: 240.9788.

Compound 2f: 2,2-dichloro-1-(3-nitrophenyl)ethanone; yellowish pale solid. m.p.: 56-57 °C. IR (KBr): ν 3086, 2991, 1709, 1526 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 6.63 (*s*, 1H, H_2), 7.76 (*ap t*, $^3J_{HH}$ 8.0 Hz, 1H, H_m), 8.48 (*m*, 2H, $H_{o,1}$+H_p), 8.90 (*s*, 1H, $H_{o,2}$). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 67.6 (CH, C_2), 124.6 (CH, C_{ar}), 128.5 (CH, C_{ar}), 130.1 (CH, C_{ar}), 132.3 (C, C_i), 135.2 (CH, C_{ar}), 148.4 (C, C_m),184.1 (C, C_1).

*2.3. Synthesis of α-bromo-α-chloro ketone **2g***

To a solution of α-chloroacetophenone **2i** (500 mg, 3.2 mmol) and *p*-toluenesulfonic acid (615 mg, 3.2 mmol) in acetonitrile (4 mL), *N*-bromosuccinimide was added (863 mg, 4.85 mmol). The reaction mixture was heated at 50 °C and stirred till disappearance of the starting material (16 h). After completion of the reaction, the solvent was evaporated under

reduced pressure and the crude was purified using flash chromatography (20% CH_2Cl_2/hexane) yielding **2g** as a white solid (0.64 g, 85%).

Compound 2g: 2-bromo-2-chloro-1-phenylethanone; m.p.: 40-41 °C. IR (KBr): ν 3066, 3029, 1701, 1594, 1448, 1219 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 6.77 (*s*, 1H, H_2), 7.53 (*ap t*, $^3J_{HH}$ 7.7 Hz, 2H, H_m), 7.63 (*dt*, $^3J_{HH}$ 7.5 $|^4J_{HH}|$ 1.3 Hz, 1H, H_p), 8.07 (*dd*, $^3J_{HH}$ 8.5 $|^4J_{HH}|$ 1.3 Hz, 2H, H_o). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 54.1 (CH, C_2), 128.8 (CH, $2C_{ar}$), 129.5 (CH, $2C_{ar}$), 131.1 (C, C_i), 134.4 (CH, C_p), 185.9 (C, C_1). HRMS (ESI^+, m/z): calcd for $C_8H_6BrClONa$: 254.9183, found: 254.9174. Spectral data are in agreement with the previously published data.[5]

*2.4. Synthesis of α-chloro-α-fluoro ketone **2h***

Ketone **2h** was synthesized following the protocol described by Yamazaki and coworkers.[6] To a solution of ethyl chlorofluoroacetate (4.35 mmol, 0.5 mL) in dry toluene (5 mL) at -80 °C under nitrogen atmosphere, 1.1 equiv. of phenyl magnesium bromide (1.6 mL of a 3 M solution in Et_2O) was added dropwise and the reaction was stirred for one hour. After that time, the reaction mixture was warmed up to 0 °C and then left for 10 min. Then the reaction was quenched with a saturated solution of ammonium chloride. The crude was extracted with Et_2O (3 x 10 mL), dried over anhydrous Na_2SO_4 and was slowly evaporated under reduced pressure in an ice bath to prevent the loss of the volatile product. The crude mixture was purified using flash chromatography (100% pentane to 70% pentane/CH_2Cl_2) affording **2h** as white crystals (0.42 g, 56% yield).

Compound 2h: 2-chloro-2-fluoro-1-phenylethanone; m.p.: 45-46 °C. IR (KBr): ν 3054, 2927, 2341, 1711, 1265 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 6.92-6.75 (*d*, $^2J_{HF}$ 51 Hz, 1H, H_2), 7.52 (*ap t*, $^3J_{HH}$ 7.4 $|^4J_{HH}|$ 1.5 Hz 2H, H_m), 7.66 (*dt*, $^3J_{HH}$ 7.4 $|^4J_{HH}|$ 1.3 Hz, 1H, H_p), 8.1 (*dd*, $^3J_{HH}$ 7.4 $|^4J_{HH}|$ 1 Hz, 2H, H_o). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 93.3-96.6 (*d*, $^1J_{CF}$ 249 Hz, CH, C_2), 128.8 (CH, $2C_m$), 129.5-129.6 (*d*, $|^4J_{CF}|$ 5.55 Hz, CH, C_o), 131.1 (C, C_i), 134.9 (CH, C_p), 187.7 (C, C_1). ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -146.5.

*2.5. Synthesis of racemic alcohols **1a-i***

To a solution of the corresponding ketone **2a-i** (0.2 mmol) in methanol (1 mL) at 0 ºC, $NaBH_4$ was added (4.6 mg, 0.1 mmol) and the temperature was maintained at 0 °C. The reaction mixture was stirred till disappearance of the starting material at room temperature (16 h). After completion of the reaction, few drops of diluted HCl were added to neutralize the excess of $NaBH_4$ followed by evaporation of the solvent under reduced pressure. Water was added and then extracted with CH_2Cl_2 (3 x 5 mL). The combined organic fractions were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude reaction was purified using flash chromatography (50-80% CH_2Cl_2/hexanes) yielding the alcohols with high yields (70-95%).

Compound 1a: 2,2-dichloro-1-phenylethanol; white solid. m.p.: 50-51 °C. IR (KBr): ν 3414, 3065, 3033, 2916, 1495, 1454 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 3.40 (*s*, 1H, OH), 4.95 (*br s*, 1H, H_1), 5.83 (*d*, $^3J_{HH}$ 5.3 Hz, 1H, H_2), 7.38-7.43 (*m*, 5H, H_{ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 76.3 (CH, C_2), 78.7 (CH, C_1), 127.2 (CH, $2C_{ar}$), 128.5 (CH, $2C_{ar}$), 129.0 (CH, C_p), 137.4 (C, C_i). Spectral data were in agreement with the previously published data.[7]

Compound 1b: 2,2-dichloro-1-(2-chlorophenyl)ethanol; yellowish pale oil. IR (neat): ν 3419, 3071, 2999, 2920, 1473, 1440 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 3.00 (*s*, 1H, OH), 5.48 (*d*, $^3J_{HH}$ 2.9 Hz, 1H, H_1), 6.10 (*d*, $^3J_{HH}$ 3.1 Hz, 1H, H_2), 7.25-7.40 (*m*, 3H, H_{ar}), 7.63 (*dd*, $^3J_{HH}$ 7.5 $|^4J_{HH}|$ 2.2 Hz, 1H, H_m). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 74.8 (CH, C_2), 74.9 (CH, C_1), 127.0 (CH, C_{ar}), 128.7 (CH, C_{ar}), 129.4 (CH, C_{ar}), 129.8 (CH, C_{ar}), 132.1 (C, C_i), 135.0 (C, C_o). Spectral data were in agreement with the previously published data.[8]

Compound 1c: 2,2-dichloro-1-(3-chlorophenyl)ethanol; yellowish pale oil. IR (neat): ν 3400, 3069, 2994, 2916, 2360, 1598, 1576, 1478, 1433 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 3.29 (*br s*, 1H, OH), 4.82 (*d*, $^3J_{HH}$ 5.2 Hz, 1H, H_1), 5.68 (*d*, $^3J_{HH}$ 4.9 Hz, 1H, H_2), 7.15-7.26 (*m*, 3H, $H_{o,1}$+H_m+H_p), 7.32 (*s*, 1H, $H_{o,2}$). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 75.9 (CH, C_2), 78.1 (CH, C_1), 125.5 (CH, C_{ar}), 127.4 (CH, C_{ar}), 129.2 (CH, C_{ar}), 129.8 (CH, C_{ar}), 134.4 (C, C_i), 139.3 (C, C_m).

Compound 1d: 2,2-dichloro-1-(4-chlorophenyl)ethanol; yellowish pale oil. IR (neat): ν 3428, 2994, 2900, 1598, 1493 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 3.15 (*br s*, 1H, OH), 4.93 (*d*, $^{3}J_{HH}$ 5.3 Hz, 1H, H_1), 5.76 (*d*, $^{3}J_{HH}$ 4.3 Hz, 1H, H_2), 7.35 (*s*, 4H, H_{ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 75.9 (CH, C_2), 77.9 (CH, C_1), 128.4 (CH, $2C_{ar}$), 128.5 (CH, $2C_{ar}$), 134.7 (C, C_{ar}), 135.5 (C, C_{ar}). Spectral data were in agreement with the previously published data.[9]

Compound 1e: 2,2-dichloro-1-(3-methoxyphenyl)ethanol; yellowish pale oil. IR (neat): ν 3445, 3003, 2940, 2837, 1602, 1491 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 2.87 (*s*, 1H, OH), 3.74 (*s*, 3H, Me), 4.87 (*d*, $^{3}J_{HH}$ 5.3 Hz, 1H, H_1), 5.74 (*d*, $^{3}J_{HH}$ 5.5 Hz, 1H, H_2), 6.81 (*ddd*, $^{3}J_{HH}$ 8.1 $|^{4}J_{HH}|$ 2.4 $|^{4}J_{HH}|$ 1 Hz, 1H, $H_{o,2}$), 6.91 (*m*, 2H, $H_{o,1}$+H_p), 7.22 (*m*, 1H, H_m). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 55.2 (CH_3), 76.2 (CH, C_2), 78.6 (CH, C_1), 112.6 (CH, C_{ar}), 114.3 (CH, C_{ar}), 119.2 (CH, C_{ar}), 129.5 (CH, C_{ar}), 138.8 (C, C_i), 159.6 (C, C_m). HRMS (ESI^+, m/z): calcd for $C_9H_{10}Cl_2O_2Na$: 242.9950, found: 242.9936.

Compound 1f: 2,2-dichloro-1-(3-nitrophenyl)ethanol; yellowish pale oil. IR (neat): ν 3494, 3092, 3000, 2918, 1531, 1350 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 3.95 (*s*, 1H, OH), 5.13 (*d*, $^{3}J_{HH}$ 4.9 Hz, 1H, H_1), 5.84 (*d*, $^{3}J_{HH}$ 5.3 Hz, 1H, H_2), 7.56 (*ap t*, $^{3}J_{HH}$ 7.9 Hz, 1H, H_m), 7.79 (*d*, $^{3}J_{HH}$ 7.7 Hz, 1H, $H_{o,1}$), 8.19 (*ddd*, $^{3}J_{HH}$ 8.2 $|^{4}J_{HH}|$ 2.2 $|^{4}J_{HH}|$ 1.1 Hz, 1H, H_p), 8.31 (*s*, 1H, $H_{o,2}$). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ 75.4 (CH, C_2), 77.4 (CH, C_1), 122.3 (CH, C_{ar}), 123.7 (CH, C_{ar}), 129.3 (CH, C_{ar}), 133.4 (CH, C_{ar}), 139.3 (C, C_i), 147.9 (C, C_m). HRMS (ESI^+, m/z): calcd for $C_8H_8Cl_2NO_3$: 235.9876, found: 235.9880.

Compound 1g: 2-bromo-2-chloro-1-phenylethanol; white solid. IR (KBr): ν 3407, 3063, 1451, 1189 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): major diastereoisomer: δ 2.96 (*dd*, $^{3}J_{HH}$ 4.1 $|^{4}J_{HH}|$ 1.4 Hz, 1H, OH), 4.95 (*ap t*, $^{3}J_{HH}$ 4.1 Hz, 1H, H_1), 5.85 (*dd*, $^{3}J_{HH}$ 5.4 $|^{4}J_{HH}|$ 1.4 Hz, 1H, H_2), 7.40-7.55 (*m*, 5H, H_{ar}); minor diastereoisomer: δ 3.02 (*dd*, $^{3}J_{HH}$ 4.1 $|^{4}J_{HH}|$ 1.4 Hz, 1H, OH), 5.07 (*ap t*, $^{3}J_{HH}$ 4.1 Hz, 1H, H_1), 5.87 (*dd*, $^{3}J_{HH}$ 5.2 $|^{4}J_{HH}|$ 1.6 Hz, 1H, H_2), 7.41 (*m*, 5H, H_{ar}). ^{13}C-NMR ($CDCl_3$, 75.5 MHz): major diastereoisomer: δ 65.6 (CH, C_2), 78.8 (CH, C_1), 126.9 (CH, $2C_{ar}$), 128.4 (CH, $2C_{ar}$), 128.9 (CH, C_p), 137.5 (C, C_i); minor diastereoisomer: δ 64.4 (CH, C_2), 78.9 (CH, C_1), 126.9 (CH, $2C_{ar}$), 128.4 (CH, $2C_{ar}$), 128.9 (CH, C_p), 137.5 (C, C_i).

Compound 1h: 2-chloro-2-fluoro-1-phenylethanol; colourless oil. IR (neat): ν 3400, 3034, 2922, 1454.9 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 3.03 (*s*, 1H, OH), 4.92 (*m*, 1H, H_2), 6.18 (*dd*, $^{2}J_{HF}$ 49.6 $^{3}J_{HH}$ 5.0 Hz, 1H, H_1), 7.42 (*m*, 5H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): major diastereoisomer: δ 75.8-76.1 (*d*, $^{2}J_{CF}$ 23.4 Hz, 1C, C_1), 102-105.2 (*d*, $^{1}J_{CF}$ 247.4 Hz, 1C, C_2),

126.9 (CH, $2C_{ar}$), 128.5 (CH, $2C_{ar}$), 128.9 (CH, C_p), 136.4 (*d*, $^3J_{CF}$ 1.9 Hz C, C_i), minor diastereoisomer: δ 76.7-76.9 (*d*, $^2J_{CF}$ 20.8 Hz, 1C, C_1), 104.1 (*d*, $^1J_{CF}$ 247.8 Hz, 1C, C_2), 127.2 (CH, $2C_{ar}$), 128.8 (CH, $2C_{ar}$), 128.9 (CH, C_p), 136.3 (C, C_i); ^{19}F-NMR ($CDCl_3$, 282 MHz): δ -143.7 and -140.5. Spectral data were in agreement with the previously published data.[6]

Compound 1i: 2-chloro-1-phenylethanol; pale yellowish oil. IR (neat): ν 3400, 3063, 3031, 2955, 2895, 2360, 1494, 1454 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300.13 MHz): δ 2.48 (*br s*, 1H, OH), 3.65 (*dd*, $^2J_{HH}$ 11.2, $^3J_{HH}$ 8.8 Hz, 1H, H_2), 3.75 (*dd*, $^2J_{HH}$ 11.2, $^3J_{HH}$ 3.5 Hz, 1H, H_2), 4.91 (*dd*, $^2J_{HH}$ 8.7, $^3J_{HH}$ 3.5 Hz, 1H, H_1), 7.38 (*m*, 5H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): 50.8 (C_2), 74.0 (C_1), 126.0 (CH, 2C), 128.4 (CH, C_p), 128.6 (CH, 2C), 139.8 (C_i). Spectral data were in agreement with the previously published data.[10]

2.6. Enzymatic protocols

*2.6.1. Optimization of the conditions for the laccase/TEMPO-mediated oxidation of **1a** in the monophasic aqueous/organic solvent system*

2.6.1.1. Effect of oxygen supply (open air)

To a test tube containing 1.8 mL of acetate buffer (50 mM, pH 4.5) saturated with O_2 for an hour prior to the experiment, were added **1a** (19.1 mg, 0.1 mmol) dissolved in 200 µL of acetonitrile (final concentration: 10% v/v), TEMPO (3.1 mg, 0.02 mmol) and laccase from *Trametes versicolor* (2.3 mg, 31.3 U). The solution was vigorously shaken to dissolve all reactants. The resulting solution was left to open air and stirred at 30 ºC for 24 h. Then, the reaction was stopped by addition of EtOAc (2 x 2 mL). The organic layers were combined, dried over Na_2SO_4 and an aliquot was filtered before spiking to GC for conversion of **2a** (33±1.4%).

2.6.1.2. Effect of the temperature

To a microwave tube containing 1.8 mL of acetate buffer (50 mM, pH 4.5), were added **1a** (19.1 mg, 0.1 mmol) dissolved in 200 µL of acetonitrile (final concentration: 10% v/v), TEMPO (3.1 mg, 0.02 mmol) and laccase from *Trametes versicolor* (2.3 mg, 31.3 U).

The tube was sealed and the reaction mixture was vigorously shaken prior to the attachment of a balloon that was filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet into the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture was left stirring at 20, 30 or 40 ºC for 24 h and the balloon was refilled with oxygen for the first 8 h of the reaction (0.4±0.1 mL/s). Then, the reaction was stopped by addition of EtOAc (2 x 2 mL). Organic layers were combined, dried over Na_2SO_4 and an aliquot was filtered before spiking to GC for conversion of **2a**: 20 ºC (56%), 30 ºC (53%), and 40 ºC (8%)

2.6.1.3. Effect of the TEMPO equivalents

To a microwave tube containing 1.8 mL of acetate buffer (50 mM, pH 4.5), were added **1a** (19.1 mg, 0.1 mmol) dissolved in 200 µL of acetonitrile (final concentration: 10% v/v), TEMPO (3.1-6.3 mg, 0.02-0.04 mmol) and laccase from *Trametes versicolor* (2.3 mg, 31.3 U). The tube was sealed and the mixture was vigorously shaken prior to the attachment of a balloon that was filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture was left stirring at 30 ºC for 24 h and the balloon was refilled with oxygen for the first 8 h of reaction (0.4±0.1 mL/s). Then, the reaction was stopped by addition of EtOAc (2 x 2 mL). Organic layers were combined, dried over Na_2SO_4 and an aliquot was filtered before spiking to GC for conversion of **2a**: 20% mol (53±4.2%), 30% mol (40±9.9%), and 40% mol (63±5.7%).

2.6.1.4. Effect of the NaOAc buffer pH

To a microwave tube containing 1.8 mL of acetate buffer (50 mM, pH 3.5-6.0), were added **1a** (19.1 mg, 0.1 mmol) dissolved in 200 µL of acetonitrile (final concentration: 10% v/v), TEMPO (3.1 mg, 0.02 mmol) and laccase from *Trametes versicolor* (2.3 mg, 31.3 U). The tube was sealed and the mixture was vigorously shaken prior to the attachment of a

balloon that was filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture was left stirring at 30 ºC for 24 h and the balloon was refilled with oxygen for the first 8 h of reaction (0.4±0.1 mL/s). Then, the reaction was stopped by addition of EtOAc (2 x 2 mL). Organic layers were combined, dried over Na_2SO_4 and an aliquot was filtered before spiking to GC for conversion of **2a**: pH 3.5 (13%), pH 4.0 (26%), pH 4.5 (53%), pH 5.5 (37%), and pH 6.0 (23%).

2.6.1.5. Effect of the buffer type

To a microwave tube containing 1.8 mL of acetate or citrate buffer (50 mM, pH 4.5), were added **1a** (19.1 mg, 0.1 mmol) dissolved in 200 µL of acetonitrile (final concentration: 10% v/v), TEMPO (3.1 mg, 0.02 mmol) and laccase from *Trametes versicolor* (2.3 mg, 31.3 U). The tube was sealed and the reaction mixture was vigorously shaken prior to the attachment of a balloon that was filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture was left stirring at 30 ºC for 24 h and the balloon was refilled with oxygen for the first 8 h of reaction (0.4±0.1 mL/s). Then, the reaction was stopped by addition of EtOAc (2 x 2 mL). Organic layers were combined, dried over Na_2SO_4 and an aliquot was filtered before spiking to GC for conversion of **2a**: NaOAc buffer (53±4.2%) and citrate buffer (35±2.8%).

2.6.1.6. Effect of the organic solvent (Figure S1)

To a microwave tube containing **1a** (19.1 mg, 0.1 mmol) dissolved in 40 µL of DMSO or 200 µL of acetonitrile, CH_2Cl_2, toluene or MTBE (final concentration: 2% for DMSO; 10% v/v for the others), 1.96 mL for DMSO and 1.80 mL of acetate buffer (50 mM, pH 4.5) for the other solvents were added followed by TEMPO (3.1 mg, 0.02 mmol) and laccase from

Trametes versicolor (2.3 mg, 31.3 U). The tube was sealed and the reaction mixture was vigorously shaken prior to the attachment of a balloon that was filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture was left stirring at 20 ºC for 24 h and the balloon was refilled with oxygen for the first 8 h of reaction (0.4±0.1 mL/s). Then, the reaction was stopped by addition of EtOAc (2 x 2 mL). Organic layers were combined, dried over Na_2SO_4 and an aliquot was filtered before spiking to GC for conversion of **2a**: DMSO (43±8.5%), MeCN (56±4.9%), CH_2Cl_2 (49±4.9%), toluene (70±0.7%), and MTBE (62±8.5%). In the reaction with MTBE, the evaporation of the organic solvent was noticed. As a control experiment, the same reaction was achieved only in NaOAc buffer pH 4.5 50 mM (2 mL), obtaining a conversion of 47±2.1%.

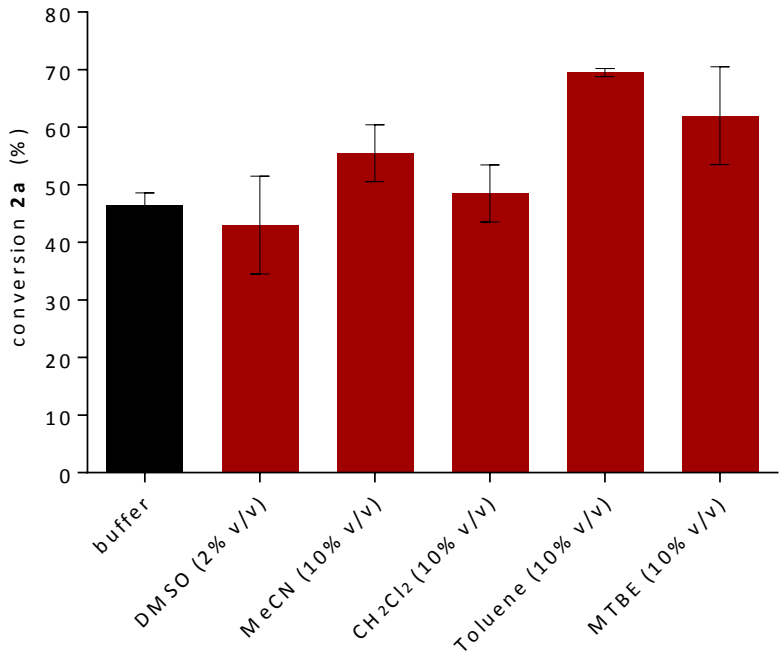


Figure S1. Effect of the organic solvent in the laccase/TEMPO-catalyzed oxidation of **1a**.

2.6.1.7. Effect of the organic solvent concentration (Figure 1 in the article)

To a microwave tube containing **1a** (19.1 mg, 0.1 mmol) dissolved in 40-1330 µL of MeCN, toluene or MTBE (final concentration: 2-66% v/v, respectively), were added 1.96-0.66 mL of acetate buffer (50 mM, pH 4.5), TEMPO (3.1 mg, 0.02 mmol) and laccase from *Trametes versicolor* (2.3 mg, 31.3 U). The tube was sealed and the mixture was vigorously shaken prior to the attachment of a balloon that was filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture was left stirring at 20 ºC for 24 h and the balloon was refilled with oxygen for the first 8 h of reaction (0.4±0.1 mL/s). Then, the reaction was stopped by addition of EtOAc (2 x 2 mL). Organic layers were combined, dried over Na_2SO_4 and an aliquot was filtered before spiking to GC for conversion of **2a** (Table S1). In the reaction with MTBE, the evaporation of the organic solvent was noticed.

Table S1. Effect of the organic solvent concentration in the laccase/TEMPO-catalyzed oxidation of **1a**.

organic solvent content (% v/v)	**2a (%)**[a]		
	MeCN	**toluene**	**MTBE**
2	42±7.1	73±2.1	39±6.4
10	56±4.9	70±0.7	62±8.5
20	52±7.8	74±2.8	82±12.7
50	9±2.1	89±0.7	80±3.5
66	n.d.	65±4.9	95±2.8

[a] Conversion values measured by GC. n.d. not determined.

*2.6.2. Oxidation of **1a** in a biphasic aqueous/MTBE system*

2.6.2.1. Use of a lower amount of TEMPO equivalents

Laccase from *Trametes versicolor* (4.6 mg, 62.6 U) was dissolved in 2 mL of acetate buffer (50 mM, pH 4.5) in a microwave tube, followed by addition of 4 mL of MTBE (final concentration: 66% v/v), alcohol **1a** (38.2 mg, 0.2 mmol) and TEMPO (1.55-6.2 mg, 0.01-0.04 mmol). The tube was sealed and the mixture was vigorously shaken prior to the attachment of a balloon that was filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture was left stirring at 20 ºC for 24 h and the balloon was refilled with oxygen for the first 8 h of reaction (0.4±0.1 mL/s). Then, the reaction was stopped by addition of EtOAc (2 x 4 mL). Organic layers were combined, dried over Na_2SO_4 and an aliquot was filtered before spiking to GC for conversion of **2a**: 5% mol (51±4.2%), 10% mol (85±7.8%), and 20% mol (95±4.2%).

2.6.2.2. Time course study of the laccase/TEMPO-mediated oxidation of **1a** in a biphasic aqueous/MTBE system or in plain buffer (Figure 2 in the article)

Laccase from *Trametes versicolor* (2.3 mg, 31.3 U) was dissolved in 1 mL of acetate buffer (50 mM, pH 4.5) in a microwave tube, followed by addition of 2 mL of MTBE (final concentration: 66% v/v), alcohol **1a** (28.5 mg, 0.15 mmol), and TEMPO (4.6 mg, 0.03 mmol). The tube was sealed and the reaction mixture was vigorously shaken prior to the attachment of a balloon filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture was left stirring at 20^{o}C, the balloon being refilled with oxygen during the reaction course (0.4±0.1 mL/s). Then, the reaction was stopped at different times (see Table S2) by addition of EtOAc (2 x 3 mL). Organic layers were combined, dried over Na_2SO_4 and

a filtered aliquot was spiked to GC instrument to measure conversion of **2a**. In the oxidations with a reaction time higher than 2-3 h, the evaporation of the organic solvent was noticed.

For a comparative study, into 3 mL of NaOAc buffer pH 4.5 (50 mM) were added: substrate **1a** (50 mM, 28.5 mg, 0.15 mmol), TEMPO (4.6 mg, 20% mol, 0.03 mmol) and laccase from *Trametes versicolor* (2.3 mg, 31.3 U). The reaction was left at 20 ºC with oxygen bubbling (1 atm, 0.4±0.1 mL/s) for the first 8 h of reaction. Then, the reaction was stopped at certain time by adding EtOAc (2 x 3 mL) and worked-up in an analogous way as for the biphasic system.

Table S2. Oxidation of **1a** at different reaction times using the laccase/TEMPO system in a biphasic medium aqueous/MTBE system or just in plain buffer.

***t* (h)**	**2a (%)**[a,b]	**2a (%)**[a,c]
1	10.7±2.5	1.1±0.2
2	27.5±4.6	1.9±0.2
3	51.7±4.5	2.4±0.2
4	58.4±2.2	4.2±1.9
6	77.3±4.6	10.7±1.7
8	82.5±4.9	13±2.5
24	95±4.2	42±4.2

[a] Conversion values measured by GC. [b] In a buffer/MTBE biphasic system. [c] In solely buffer.

2.6.2.3. Effect of the substrate concentration

Laccase from *Trametes versicolor* (2.3 mg, 31.3 U) was dissolved in 1 mL of acetate buffer (50 mM, pH 4.5) in a microwave tube, followed by addition of 1 mL of MTBE (final concentration: 50% v/v), alcohol **1a** (9.5-76.4 mg, 0.05-0.4 mmol) and 20% mol of TEMPO

(1.5-12.4 mg, 0.01-0.08 mmol). The tube was sealed and the mixture was vigorously shaken prior to the attachment of a balloon filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture was left stirring at 20 ºC for 24 h and the balloon was refilled with oxygen for the first 8 h of reaction (0.4±0.1 mL/s). Then, the reaction was stopped by addition of EtOAc (2 x 4 mL). Organic layers were combined, dried over Na_2SO_4 and a filtered aliquot was spiked to GC instrument to measure conversion of **2a**: 25 mM (65±1.4), 100 mM (93±6.8), and 200 mM (93±2.1). After 2-3 h, the evaporation of the organic solvent was noticed.

*2.6.3. General method for the oxidation of alcohols **1a-j** using laccase and TEMPO in a biphasic aqueous/organic solvent system*

Laccase from *Trametes versicolor* (2.3 mg, 31.3 U) was dissolved in 1 mL of acetate buffer (50 mM, pH 4.5) in a microwave tube, followed by addition of 2 mL of MTBE (final concentration: 66% v/v), the corresponding alcohol **1a-j** (0.1 mmol) and TEMPO (3.1 mg, 0.02 mmol). The tube was sealed and the mixture was vigorously shaken prior to the attachment of a balloon filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture (Figure S2) was left stirring at 20 ºC for 24 h and the balloon was refilled with oxygen for the first 8 h of reaction (0.4±0.1 mL/s). Then, the reaction was stopped by addition of EtOAc (2 x 2 mL). Organic layers were combined, dried over Na_2SO_4 and a filtered aliquot was spiked to GC instrument to measure conversions of alcohols **2a-j** (see Table 1 in the article). After 2-3 h, the evaporation of the organic solvent was noticed.

Figure S2. Reaction set-up without thermostatic control.

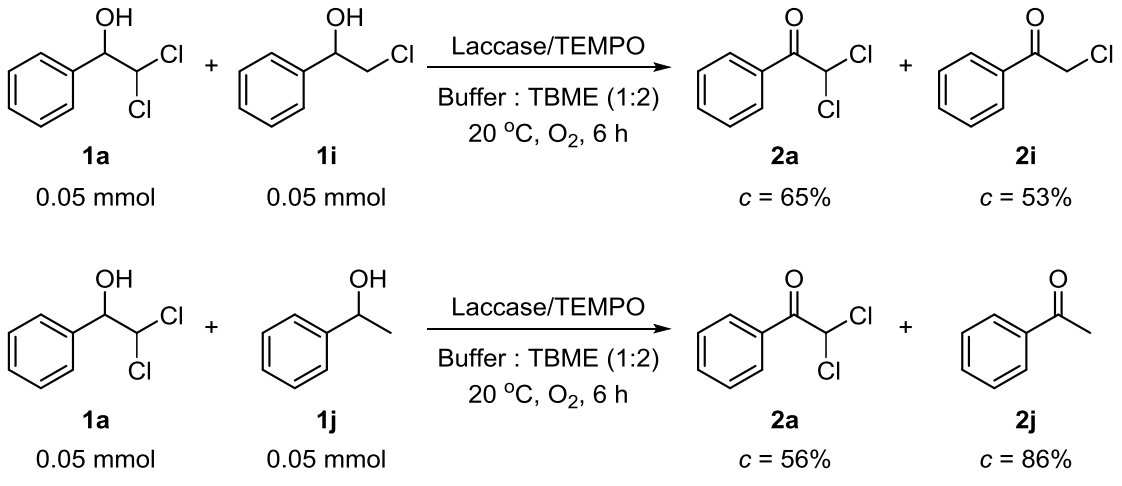
*2.6.4. Scale-up of the laccase/TEMPO-mediated synthesis of dihalogenated ketones **2a**,**c**,**d** in a biphasic system*

To 5.2 mL of acetate buffer (50 mM, pH 4.5) in a 25 mL round bottom flask 12 mg of laccase from *Trametes versicolor* (163 U) and 100 mg of the corresponding alcohols **1a**, **1c**, or **1d** were added. Later, 10.4 mL of MTBE were transferred to the reaction vessel and 16 mg of TEMPO (20% mol) were added to the reaction mixture. The flask was sealed with a rubber and the mixture was vigorously shaken prior to the attachment of a balloon filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction was stirred at 20 °C for 24 h and the balloon was refilled with oxygen for the first 8 h of experiment (0.4±0.1 mL/s). The flask was closed with a stopper and the pressure equilibrated by a needle outlet. The reaction was ended by addition of EtOAc (3 x 5 mL). The organic fractions were combined and dried over Na_2SO_4. A small aliquot was used for GC analysis (>95%

conversion). After filtration and solvent removal under reduced pressure, the crude was loaded on a silica column. Flash chromatography was performed using 90% hexane/dichloromethane as mobile phase and pure ketones **2a** (76% isolated yield), **2c** (81% isolated yield), and **2d** (80% isolated yield).

2.6.5. Selectivity in the laccase/TEMPO system to oxidize simultaneously two sec*-alcohols*

Laccase from *Trametes versicolor* (2.3 mg, 31.3 U) was dissolved in 1 mL of acetate buffer (50 mM, pH 4.5) in a microwave tube, followed by addition of 2 mL of MTBE (final concentration: 66% v/v), substrate **1a** (9.5 mg, 0.05 mmol), substrate **1i** or **1j** (0.05 mmol), and TEMPO (3.1 mg, 0.02 mmol). The tube was sealed and the reaction mixture was vigorously shaken prior to the attachment of a balloon filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture was left stirring at 20 ºC for 6 h and the balloon was refilled with oxygen during the reaction course (0.4±0.1 mL/s). After that time, the reactions were stopped by addition of EtOAc (2 x 2 mL). Organic layers were combined, dried over Na_2SO_4 and a filtered aliquot was spiked to GC instrument to measure conversions of **2a** and **2i** or **2j** (Scheme S1). After 2-3 h, the evaporation of the organic solvent was noticed.



Scheme S1. Oxidation of *sec*-alcohols **1a** and **1i** or **1j** mediated by the laccase/TEMPO system.

2.6.6. One-pot two-step sequential reaction with the laccase/TEMPO system and E. coli*/ADH-A for the deracemisation of racemic alcohol **1a** (Scheme 2 in the article)*

To 2 mL of acetate buffer (50 mM, pH 4.5) in a microwave tube, laccase from *Trametes versicolor* (4.6 mg, 62.6 U) and 40 mg of alcohol **1a** (0.2 mmol) were added. Later, 4 mL of MTBE (final concentration: 66% v/v) were transferred to the reaction vessel and 6.2 mg of TEMPO were added to the reaction mixture (0.04 mmol). The tube was sealed and the reaction mixture was vigorously shaken prior to the attachment of a balloon filled with oxygen (1 atm). The flask was closed with a stopper and the pressure was equilibrated by a needle outlet. The reaction was stirred at 20 °C for 24 h and the balloon was refilled with oxygen for the first 8 h of the experiment (0.4±0.1 mL/s). Then, an aliquot of 50 µL was taken for routine conversion analysis by GC (97%) and the pH was readjusted to 7.5 with 2 mL of Tris base solution in water (300 mM) and a few drops of HCl (2 M). Subsequently, 10% v/v of isopropanol (440 µL), 100 mg of lyophilized *E. coli*/ADH-A cells as well as NAD^+ (final concentration: 1 mM), were added to the reaction mixture and left at 30°C for 48 h shaking at 250 rpm. After that time, the reaction mixture was centrifuged at 5000 rpm for 5 min and the supernatant was collected. The pellet was washed with EtOAc (3 x 5 mL) and the reaction products present in the supernatant were also extracted with EtOAc (4 x 5 mL) and added to the previous organic fraction. Na_2SO_4 was added, filtered and an aliquot was spiked to GC analysis to assess conversion (94.5%) and *ee* (97%). After filtration and solvent removal under reduced pressure, the crude was loaded on a silica column. Flash chromatography was performed using 40% dichloromethane/hexanes as mobile phase and enantiopure alcohol (*R*)-**1a** was obtained as a white solid (isolated yield: 20 mg, 53%, *ee* 97%).

*2.7. Other chemical systems used to oxidize alcohol **1a** (Figure 3 in the article)*

2.7.1. Laccase from Trametes versicolor*/4-OH-TEMPO*

To a microwave tube containing 1.8 mL of acetate buffer (50 mM, pH 4.5), were added **1a** (19.1 mg, 0.1 mmol) dissolved in 200 µL of acetonitrile (final concentration: 10% v/v), 4-OH-TEMPO (3.4 mg, 0.02 mmol) and laccase from *Trametes versicolor* (2.3 mg, 31.3 U). The tube was sealed and the mixture was vigorously shaken prior to the attachment of a balloon that was filled with oxygen (1 atm). Pressure was equilibrated placing an additional needle outlet in the rubber lid and the oxygen flow was set to gently purge the reaction mixture. The reaction mixture was left stirring at 30 ºC for 24 h and the balloon was refilled with oxygen for the first 8 h of reaction (0.4±0.1 mL/s). Then, the reaction was stopped by addition of EtOAc (2 x 2 mL). Organic layers were combined, dried over Na_2SO_4 and an aliquot was filtered before spiking to GC for conversion (see Table S3).

2.7.2. NaOCl/TEMPO system

This procedure was adapted from Anelli *et al.*[11] In a round bottom flask, **1a** (40 mg, 0.2 mmol) was resuspended in water (5 mL), followed by addition of TEMPO (6.2 mg, 0.04 mmol) and 9 equiv. of NaOCl (1.16 mL of 10% solution). The reaction mixture was stirred at 0 ºC for 7 h. After that time, the crude was acidified with HCl solution (1 M) and extracted with EtOAc (3 x 5 mL). Organic fractions were combined and dried over Na_2SO_4. Later the solvent was evaporated and the crude was dried under high vacuum for ^{1}H-NMR analysis (see Table S3).

2.7.3. NaOCl/KBr/TEMPO system

This procedure was adapted from Anelli *et al.*[11] In a round bottom flask, **1a** (40 mg, 0.2 mmol) was dissolved in 0.42 mL of acetonitrile and 3.76 mL of phosphate buffer (50 mM, pH 7.5), followed by addition of TEMPO (6.2 mg, 0.04 mmol), 1 equiv. of NaOCl (0.123 mL of 10% solution), and KBr (4.8 mg, 0.04 mmol). The reaction mixture was stirred at room

temperature for 24 h. After that time, the crude was acidified with HCl solution (1 M) and extracted with EtOAc (3 x 5 mL). Organic fractions were combined and dried over Na_2SO_4. Later the solvent was evaporated and the crude was dried under high vacuum for ^{1}H-NMR analysis (see Table S3).

2.7.4. $NaClO_2$/NaOCl/TEMPO system

This procedure was adapted from Zhao *et al*.[12] In a round bottom flask, **1a** (40 mg, 0.2 mmol) was dissolved in 0.4 mL of acetonitrile and 3.6 mL of phosphate buffer (100 mM, pH 7.5), followed by addition of TEMPO (6.2 mg, 0.04 mmol), 1.5 equiv. of $NaClO_2$ (34 mg, 0.3 mmol) and NaOCl (25 µL of 10% solution, 0.04 mmol). The reaction mixture was stirred at room temperature for 24 h. After that time, the crude was acidified with HCl solution (1 M) and extracted with EtOAc (3 x 5 mL). Organic fractions were combined and dried over Na_2SO_4. Later the solvent was evaporated and the crude was dried under high vacuum for ^{1}H-NMR analysis (see Table S3).

2.7.5. CuCl/TEMPO system

This procedure was adapted from Semmelhack *et al*.[13] In a round bottom flask, **1a** (40 mg, 0.2 mmol) was dissolved in 1 mL of acetonitrile and 4 mL of water, followed by addition of TEMPO (6.2 mg, 0.04 mmol) and CuCl (4 mg, 0.04 mmol). The reaction mixture was stirred at room temperature for 24 h. After that time, the crude was acidified with HCl solution (1 M) and extracted with EtOAc (3 x 5 mL). Organic fractions were combined and dried over Na_2SO_4. Later the solvent was evaporated and the crude was dried under high vacuum for ^{1}H-NMR analysis (see Table S3).

2.7.6. $CuBr_2$/TEMPO system

This procedure was adapted from Miyazawa and Endo.[14] In a round bottom flask, **1a** (40 mg, 0.2 mmol) was dissolved in 1 mL of acetonitrile and 4 mL of water, followed by addition of TEMPO (6.2 mg, 0.04 mmol) and $CuBr_2$ (9 mg, 0.04 mmol). The reaction mixture

was stirred at room temperature for 24 h. After that time, the crude was acidified with HCl solution (1 M) and extracted with EtOAc (3 x 5 mL). Organic fractions were combined and dried over Na_2SO_4. Later the solvent was evaporated and the crude was dried under high vacuum for ^{1}H-NMR analysis (see Table S3).

2.7.7. I_2/TEMPO system

This procedure was adapted from Miller and Hoerrner.[15] In a round bottom flask, **1a** (19 mg, 0.1 mmol) was resuspended in 2.5 mL of Tris-HCl buffer (50 mM, pH 7.5), followed by addition of TEMPO (3.1 mg, 0.02 mmol) and I_2 (38 mg, 0.15 mmol). The reaction mixture was stirred at 30 ºC for 24 h. After that time, the excess of iodine was quenched with a $Na_2S_2O_3$ solution (10% w/v) and the crude was extracted with EtOAc (3 x 5 mL). Organic fractions were combined and dried over Na_2SO_4. Later the solvent was evaporated and the crude was dried under high vacuum for ^{1}H-NMR analysis (see Table S3).

2.7.8. NaOCl

In a round bottom flask, **1a** (40 mg, 0.2 mmol) was resuspended in water (5 mL), followed by addition of 9 equiv. of NaOCl (1.16 mL of 10% solution). The reaction mixture was stirred at 30 ºC for 24 h. After that time, the crude was acidified with HCl solution (1 M) and extracted with EtOAc (3 x 5 mL). Organic fractions were combined and dried over Na_2SO_4. Later the solvent was evaporated and the crude was dried under high vacuum for ^{1}H-NMR analysis (see Table S3).

2.7.9. NaOCl/KBr system

In a round bottom flask, **1a** (40 mg, 0.2 mmol) was resuspended in 5 mL of water, followed by addition of 9 equiv. of NaOCl (1.16 mL of 10% solution) and KBr (4.8 mg, 0.04 mmol). The reaction mixture was stirred at 0 ºC for 7 h. After that time, the crude was acidified with HCl solution (1 M) and extracted with EtOAc (3 x 5 mL). Organic fractions

were combined and dried over Na_2SO_4. Later the solvent was evaporated and the crude was dried under high vacuum for ^{1}H-NMR analysis (see Table S3).

2.7.10. $NaClO_2$

In a round bottom flask, **1a** (40 mg, 0.2 mmol) was resuspended in water (5 mL), followed by addition of 9 equiv. of $NaClO_2$ (213 mg, 1.88 mmol). The reaction mixture was stirred at 0 ºC for 7 h. After that time, the crude was acidified with HCl solution (1 M) and extracted with EtOAc (3 x 5 mL). Organic fractions were combined and dried over Na_2SO_4. Later the solvent was evaporated and the crude was dried under high vacuum for ^{1}H-NMR analysis (see Table S3).

2.7.11. Dess-Martin periodinane in aqueous media

In a round bottom flask, **1a** (50 mg, 0.26 mmol) was resuspended in 5 mL of H_2O, followed by addition of the Dess-Martin periodinane reagent (133 mg, 0.314 mmol). The reaction mixture was stirred at 30 ºC for 24 h. After that time, the excess of the reagent was quenched with $Na_2S_2O_3$ (10% w/v solution), and then 10 mL of $NaHCO_3$ (saturated solution) was added and finally was extracted with CH_2Cl_2 (3 x 5 mL). Organic phases were combined, dried over Na_2SO_4 and the solvent was evaporated. Finally, the crude was dried under high vacuum prior ^{1}H-NMR analysis (see Table S3).

2.7.12. Dess-Martin periodinane in CH_2Cl_2

This procedure was adapted from Pace *et al.*[16] In a round bottom flask, **1a** (50 mg, 0.26 mmol) was dissolved in 5 mL of CH_2Cl_2, followed by addition of the Dess-Martin periodinane reagent (133 mg, 0.314 mmol). The reaction mixture was stirred at room temperature for 1.5 h. After that time, 10 mL of Et_2O, 2.5 mL of a 1% w/v solution of $Na_2S_2O_3$ and 10 mL of $NaHCO_3$ (saturated solution) were added and left stirring for 15 min. The organic phase was separated and dried over Na_2SO_4. Later the solvent was evaporated and the crude was dried under high vacuum prior ^{1}H-NMR analysis (see Table S3).

Table S3. Other chemical methods employed to oxidize alcohol **1a**.

system	areas (%)[a]			
	1a	**2a**	**3a+4a**	**5a**
Laccase *T. versicolor*/4-OH-TEMPO	95	5	<3	<3
NaOCl/TEMPO	6	3	88	3
NaOCl/KBr/TEMPO	39	39	22	<3
$NaClO_2$/NaOCl/TEMPO	90	<3	5	4
CuCl/TEMPO	>97	<3	<3	<3
$CuBr_2$/TEMPO	>97	<3	<3	<3
I_2/TEMPO	>97	<3	<3	<3
NaOCl	<3	<3	97	3
NaOCl/KBr	10	<3	45	45
$NaClO_2$	>97	<3	<3	<3
Dess-Martin periodinane/H_2O	>97	<3	<3	<3
Dess-Martin periodinane/CH_2Cl_2	46	54	<3	<3

[a] Measured by NMR.

3. *Ab initio* calculations

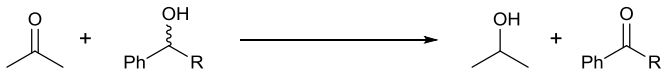
To emphasize the ability of the laccase/TEMPO system to oxidize secondary alcohol substrates with varying redox strength, we evaluated the relative thermodynamic stability of the halogenated alcohol/ketone pairs **1a**,**i**,**j**/**2a**,**i**,**j** with respect to the acetone/2-propanol pair by computing the Gibbs free energy change in aqueous solution ($\Delta_r G$) for the isodesmic reactions shown in Table S4.

The computed values, which could be correlated with the standard reduction potential of the corresponding alcohol/ketone pairs, showed clearly that the dichlorinated alcohol **1a** was a weaker reducing agent compared to the mono- (**1i**, by 2.5 kcal/mol) or unsubstituted (**1j**, by 6.7 kcal/mol) derivatives. Since **1a** showed a larger conversion value (95%) than those of **1i**,**j** (62-78%), it turned out that the differences in the intrinsic redox strength of **1a**,**i**,**j** did not affect their oxidation, highlighting thus the flexibility of the laccase/TEMPO system. Moreover, the lack of thermodynamic effects due to the alcohol substituents in the experimentally observed conversions, pointed out that these values for substrates **1i** or **1j** could be further improved by tuning the reaction conditions.[17]

We applied the same computational protocol that has been used in our previous work[10] to calculate the Gibbs free energies of reactants and products in aqueous solution. Thus, preliminary conformational search calculations were carried out by means of gas-phase Molecular Dynamics simulations using the generalized AMBER force field (GAFF) and the *Sander* program.[18] The resulting conformers were then optimized at the HF/cc-pVDZ level and characterized by analytical frequency calculations using the *Gaussian03* program.[19] Subsequently, all the structures were reoptimized at the MP2/cc-pVTZ level of theory. MP2 energies were extrapolated to the complete basis set limit (CBS) by means of single-point MP2/cc-pVXZ (X=Q,5) calculations and using the Schwartz extrapolation scheme. Solvation free energies were estimated from single-point COSMO-MP2/cc-pVTZ calculations in the

framework of the conductor-like screening model representing continuum solvent effects.[20] All the MP2 and COSMO calculations were performed with the TURBOMOLE program[21] package. Further details of the computational protocol can be found elsewhere.[10]

Table S4. Total number of conformers (N_{conf}) of the alcohol/ketone pairs examined computationally and Boltzmann-averaged free energies (kcal/mol) both in solution ($\Delta_r G$) and in the gas-phase ($\Delta_r G_{gas}$) for the redox equilibrium with respect to 2-propanol/acetone. The corresponding changes in the free energy over the most stable conformers in solution ($\Delta_r G_{min}$) are also indicated.



1a,**i**,**j** **2a**,**i**,**j**

alcohol/ketone	**R**	N_{conf}	$\Delta_r G$	$\Delta_r G_{gas}$	$\Delta_r G_{min}$	**Reference**
a	$CHCl_2$	9/1	+7.35	+7.61	+6.92	this work
i	CH_2Cl	6/2	+4.86	+4.98	+4.29	10
j	CH_3	2/1	+0.61	+0.20	+0.36	10

4. Environmental assessment using EATOS

E-factor calculations (Figure S3) were performed using the EATOS (v. 1.1) software tool,[22] while solvent demand was calculated using Microsoft Excel (determining the solvent use of each protocol to obtain 1 g of **2a**). All reactions were treated as proceeding to the corresponding conversion, hence all losses in yield are accounted for as 'unknown by-products'. The EATOS and Excel files used for the calculations are also available as ESI material.

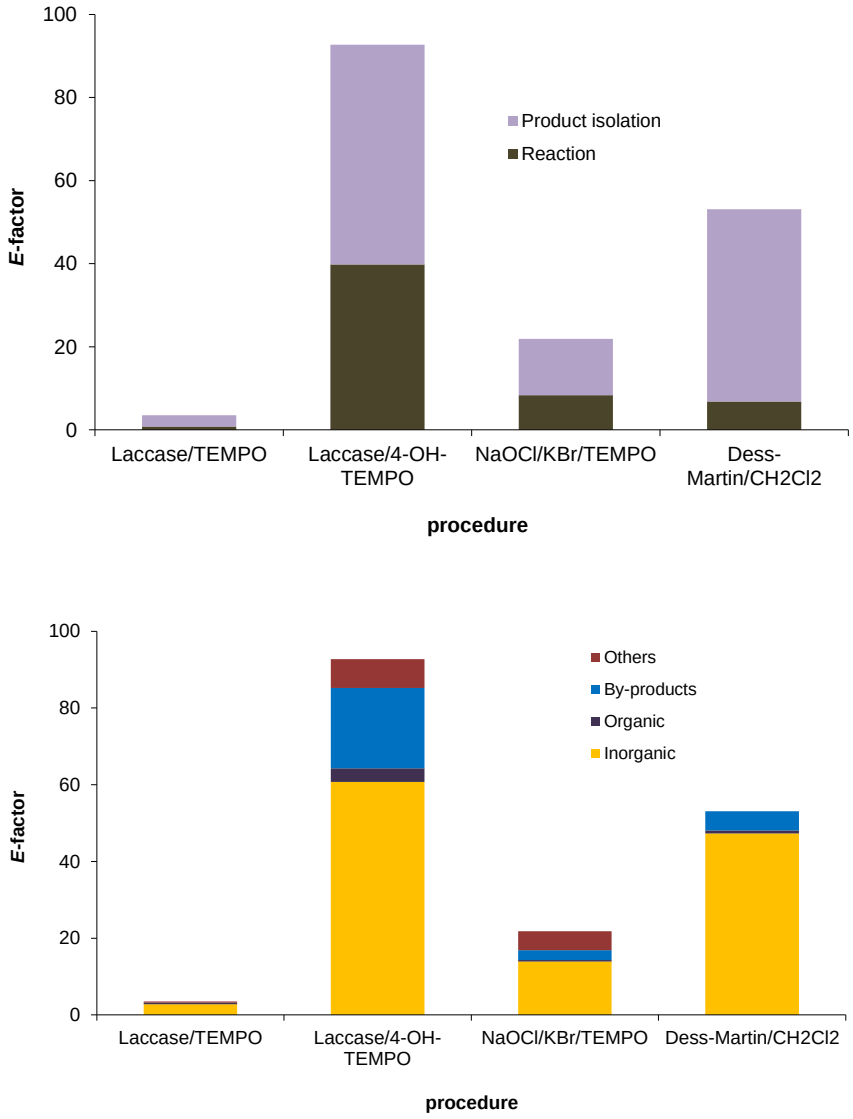


Figure S3. Contribution to *E*-factor (excluding solvents) for each procedure to synthesize ketone **2a**.

5. Analytical data

GC analyses for determination of conversions

The following chiral and achiral GC columns were used:

- Column **A**: Restek RT-BetaDEXse (30 m x 0.25 mm x 0.25 μm, 12.2 psi N_2).
- Column **B**: Varian Chirasil Dex CB (25 m x 0.25 mm x 0.25 μm, 12.2 psi N_2).
- Column **C**: Hewlett Packard HP1 (30 m x 0.32 mm x 0.25 μm, 12.2 psi N_2).

Table S5. Determination of conversions by GC.

compound	column	program[b]	retention time (min)	
			alcohol	ketone
			1	**2**
a[a]	A	90/5/3/180/10	32.8 (*S*), 33.5 (*R*)	26.9
b[a]	A	110/0/5/160/20/20/180/10	28.3, 31.4	18.2
c[a]	A	110/0/5/160/20/20/180/10	36.9, 38.3	21.9
d[a]	A	110/0/5/160/20/20/180/10	35.8, 37.1	21.1
e[a]	B	110/0/3/180/7	26.8, 27.4	19.1
f[a]	C	110/0/10/210/5	8.1	6.5
g[a]	A	90/5/3/180/10	35.9, 36.5	31.2
h[a]	A	90/5/3/180/10	29.3, 29.7, 30.3	19.5, 19.9
i[a]	A	90/0/5/130/15/5/200/2	27.1, 28.2	22.9
j	A	90/0/5/130/15/5/200/2	12.6	9.8

[a] Change in Cahn-Ingold-Prelog (CIP) priority. [b] Program: initial temp. (°C)/ time (min)/ slope (°C/min)/ temp. (°C)/ time (min)/ slope (°C/min)/ final temp. (°C)/ time (min).

6. Supporting references

1. K. Edegger, C. C. Gruber, T. M. Poessl, S. R. Wallner, I. Lavandera, K. Faber, F. Niehaus, J. Eck, R. Oehrlein, A. Hafner and W. Kroutil, *Chem. Commun.*, 2006, 2402-2404.

2. J. C. Lee, Y. H. Bae and S. K. Chang, *Bull. Korean Chem. Soc.*, 2003, **24**, 407-408.

3. J. Liu, W. Li, C. Wang, Y. Li and Z. Li, *Tetrahedron Lett.*, 2011, **52**, 4320-4323.

4. A. O. Terent'ev, S. V. Khodykin, N. A. Troitskii, Y. N. Ogibin and G. I. Nikishin, *Synthesis*, 2004, 2845-2848.

5. J. Barluenga, L. Llavona, J. M. Concellón and M. Yus, *J. Chem. Soc., Perkin Trans. 1*, 1991, 297-300.

6. T. Yamazaki, T. Terajima and T. Kawasaki-Takasuka, *Tetrahedron*, 2008, **64**, 2419-2424.

7. M. Fujita, M. Obayashi and T. Hiyama, *Tetrahedron*, 1988, **44**, 4135-4145.

8. R. K. Freidlina, V. I. Dostovalova, N. A. Kuz'mina and E. C. Chukovskaya, *Org. Magn. Resonance*, 1981, **15**, 133-138.

9. J. Blum, S. Shtelzer, P. Albin and Y. Sasson, *J. Mol. Catal.*, 1982, **16**, 167-174.

10. F. R. Bisogno, E. Garcia-Urdiales, H. Valdes, I. Lavandera, W. Kroutil, D. Suarez and V. Gotor, *Chem. Eur. J.*, 2010, **16**, 11012-11019.

11. P. L. Anelli, S. Banfi, F. Montanari and S. Quici, *J. Org. Chem.*, 1989, **54**, 2970-2972.

12. M. Zhao, J. Li, E. Mano, Z. Song, D. M. Tschaen, E. J. J. Grabowski and P. J. Reider, *J. Org. Chem.*, 1999, **64**, 2564-2566.

13. M. F. Semmelhack, C. R. Schmid, D. A. Cortés and C. S. Chou, *J. Am. Chem. Soc.*, 1984, **106**, 3374-3376.

14. T. Miyazawa and T. Endo, *J. Mol. Catal.*, 1985, **32**, 357-360.

15. R. A. Miller and R. S. Hoerrner, *Org. Lett.*, 2003, **5**, 285-287.

16. V. Pace, A. Cortez Cabrera, M. Fernández, J. V. Sinisterra and A. R. Alcántara, *Synthesis*, 2010, **20**, 3545-3555.

17. In fact, this result improves a previous study which showed that ketone **2j** could be attained in 35% conversion using laccase from *Trametes versicolor* and TEMPO (10% mol)

in plain 100 mM phosphate buffer pH 4.5. For more information, see: I. Matijosite, Ph D Thesis, Technical University of Delft, 2008.

18. D. A. Case, T. A. Darden, T. E. Cheatham, III, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, M. Crowley, R. C. Walker, W. Zhang, K. M. Merz, B. Wang, S. Hayik, A. Roitberg, G. Seabra, I. Kolossváry, K. F. Wong, F. Paesani, J. Vanicek, X. Wu, S. R. Brozell, T. Steinbrecher, H. Gohlke, L. Yang, C. Tan, J. Mongan, V. Hornak, G. Cui, D. H. Mathews, M. G. Seetin, C. Sagui, V. Babin and P. A. Kollman, AMBER 10, University of California, San Francisco, 2008.

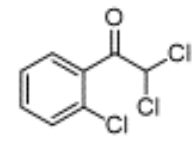
19. Gaussian 03, Revision C.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, Gaussian, Inc., Wallingford CT, 2004.

20. A. Klamt and G. Schüürmann, *J. Chem. Soc., Perkin Trans. 2*, 1993, **5**, 799-805.

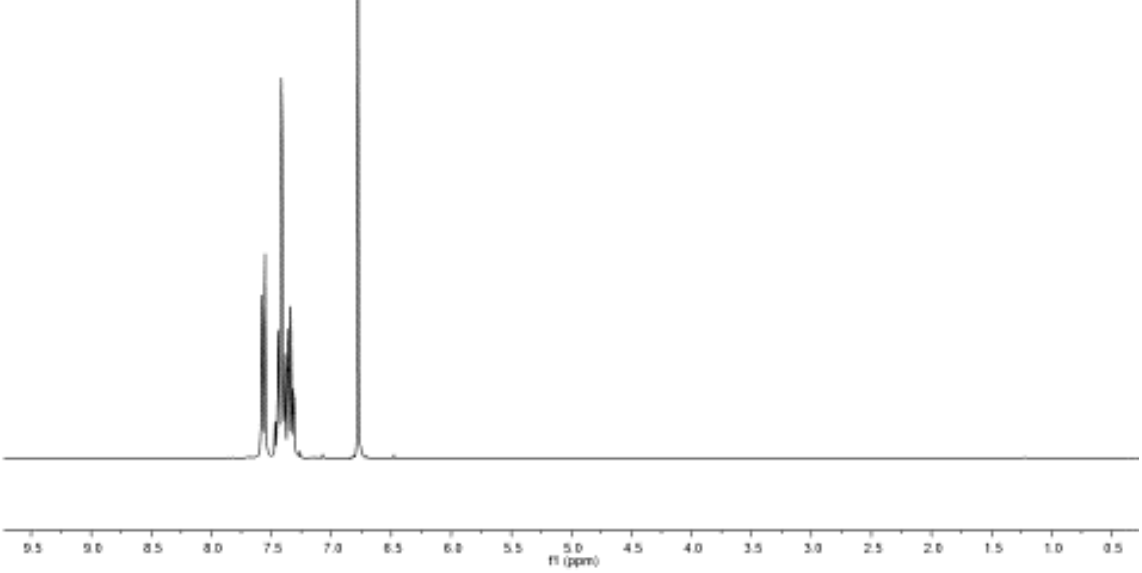
21. R. Ahlrichs, M. Bär, M. Häser, H. Horn and C. Kölmel, *Chem. Phys. Lett.*, 1989, **162**, 165-169.

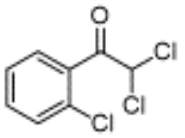
22. (*a*) M. Eissen and J. O. Metzger, *Chem.–Eur. J.*, 2002, **8**, 3580-3585; (*b*) EATOS: Environmental Assessment Tool for Organic Syntheses, http://www.metzger.chemie.uni-oldenburg.de/eatos/english.htm.

7. Compound NMR spectra

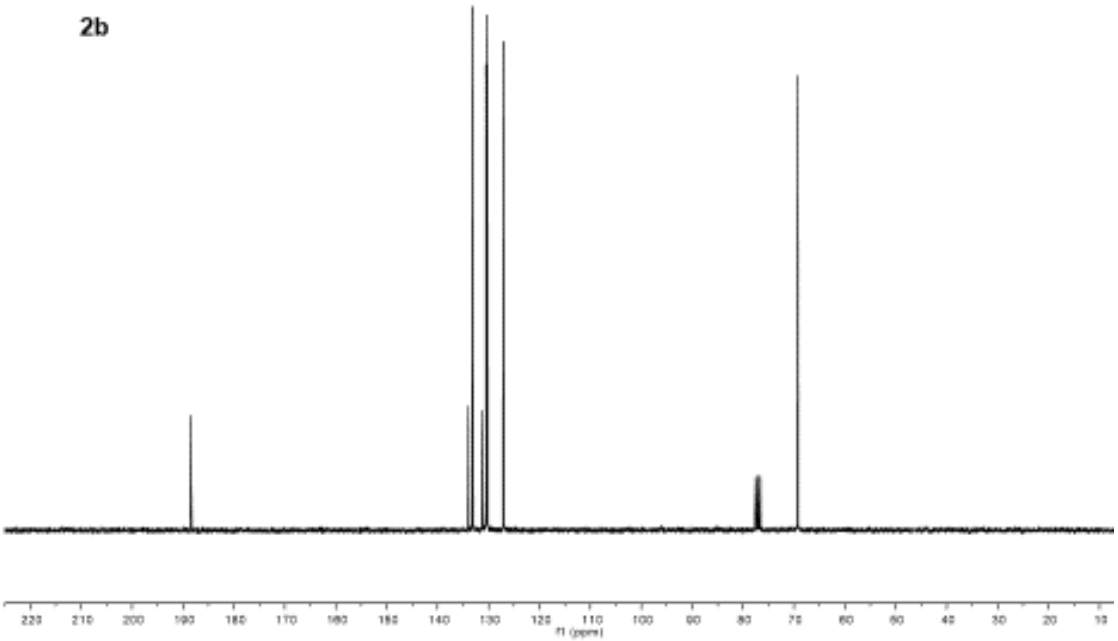


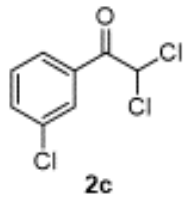
2b





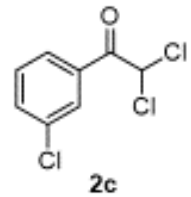
2b



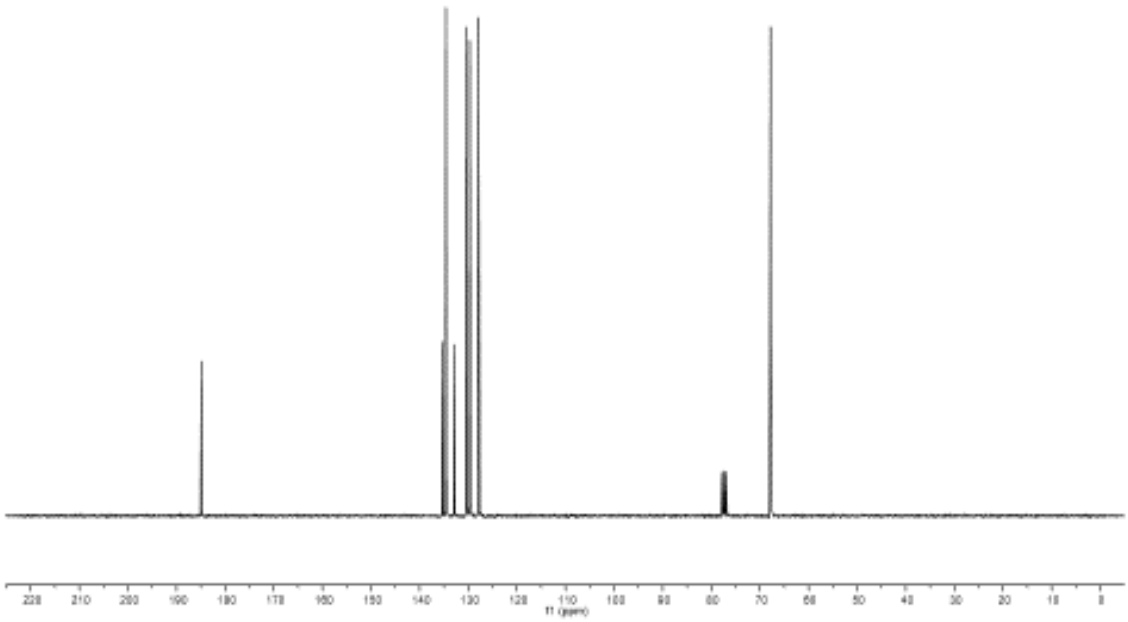

O
Cl
Cl
Cl

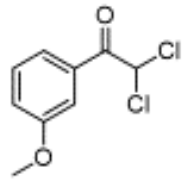
2c

10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
f1 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5


O
Cl
Cl
Cl

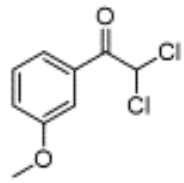
2c


220
210
200
190
180
170
160
150
140
130
120
110
f1 (ppm)
100
90
80
70
60
50
40
30
20
10
0

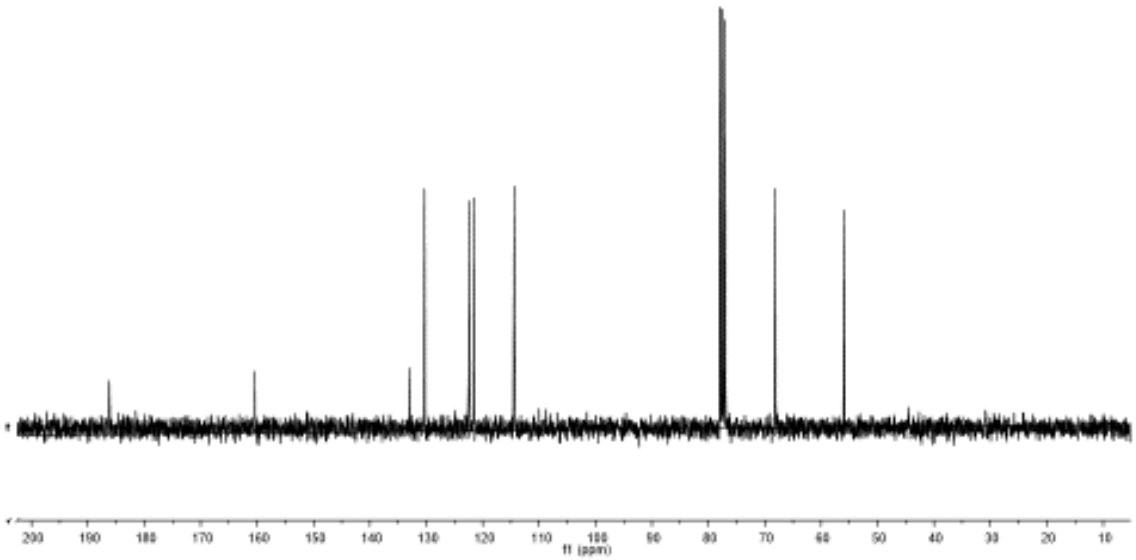

O
Cl
Cl
O

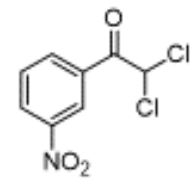
2d

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
f1 (ppm)


O
Cl
Cl
O

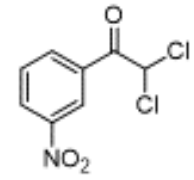
2d


200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

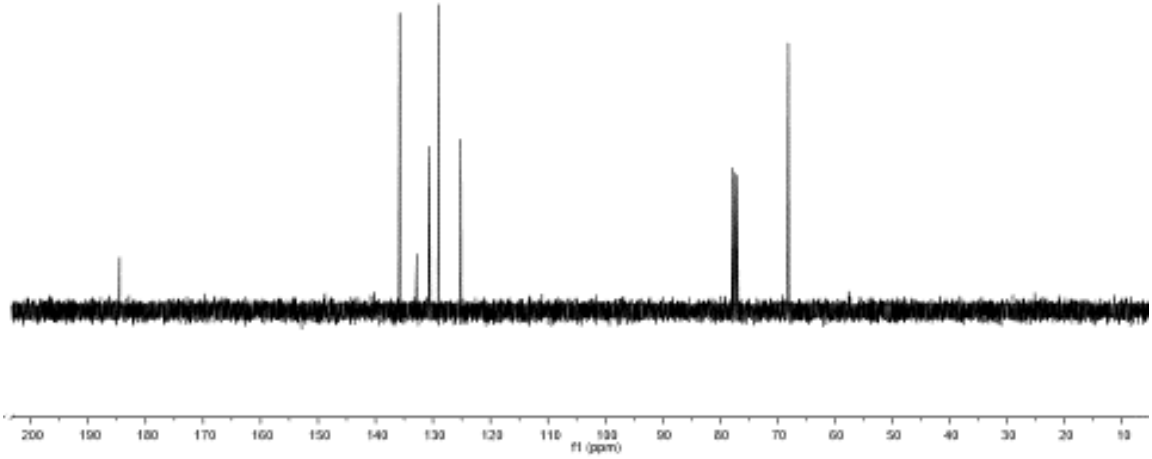

O
Cl
Cl
NO2

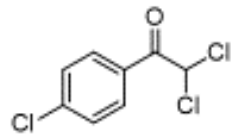
2e

11.0 10.5 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
f1 (ppm)


O
Cl
Cl
NO2

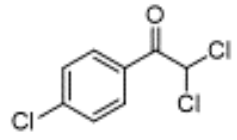
2e


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

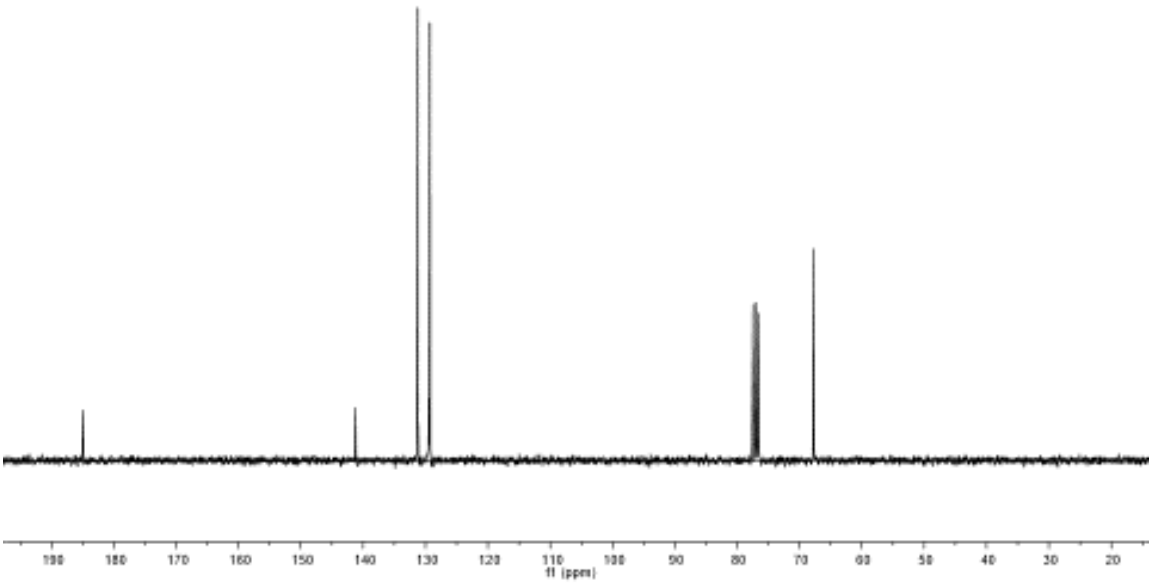

O
Cl
Cl
Cl

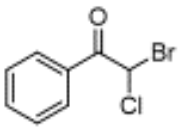
2f

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
f1 (ppm)

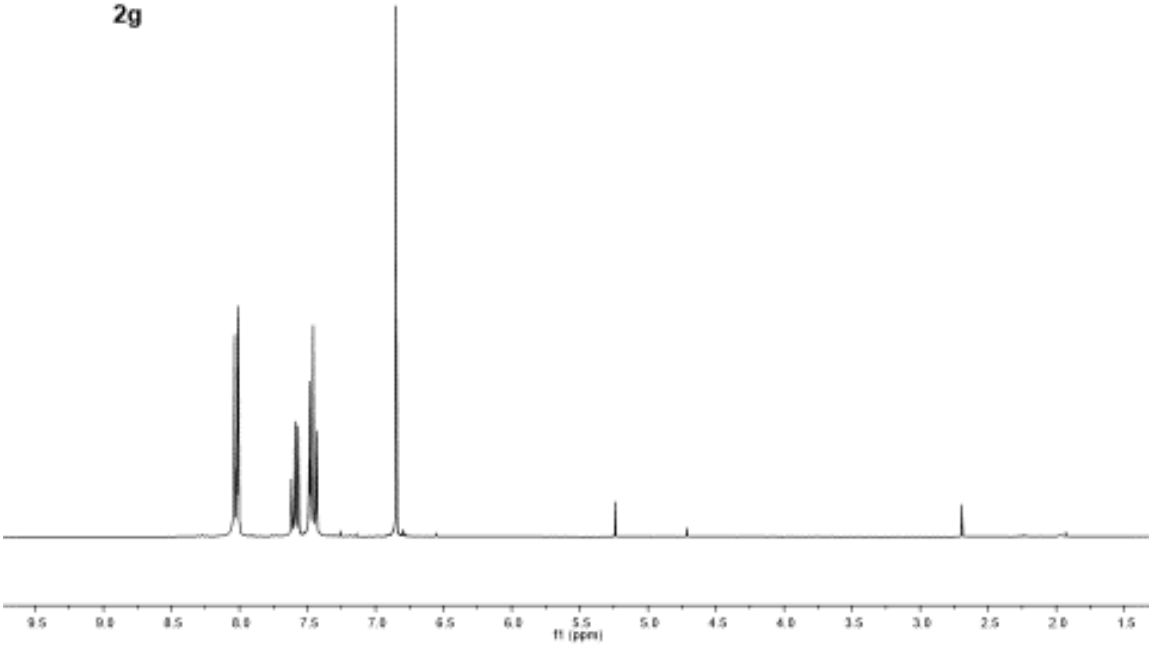

O
Cl
Cl
Cl

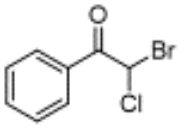
2f


190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)


O
Br
Cl

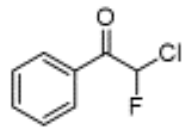
2g


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
f1 (ppm)

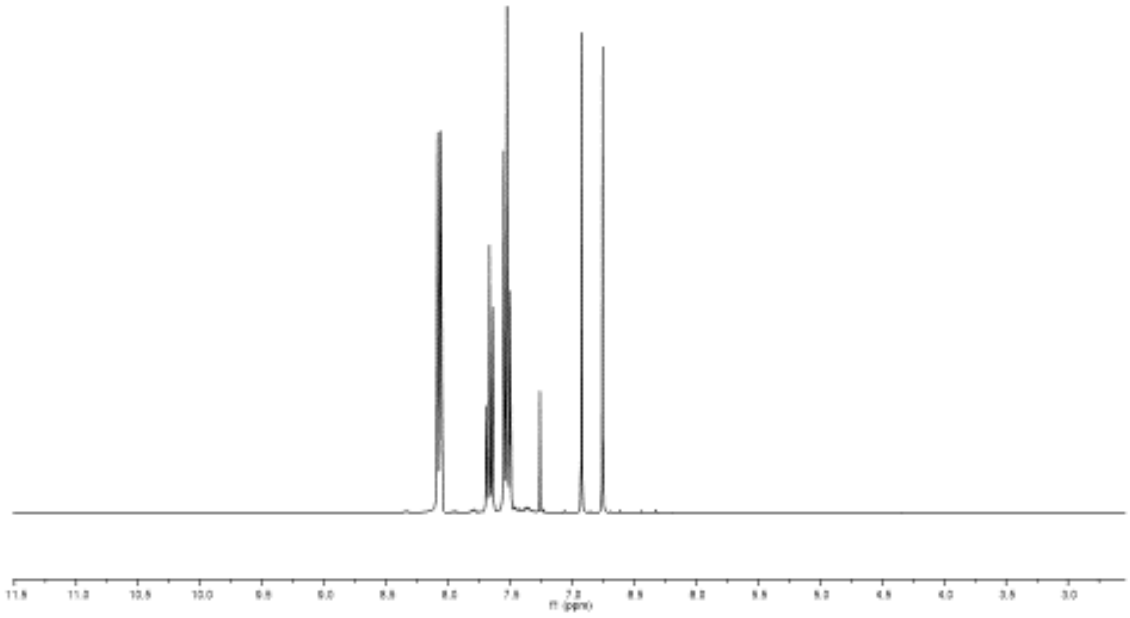

O
Br
Cl

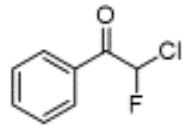
2g

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

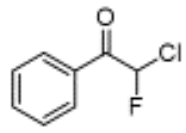


2h

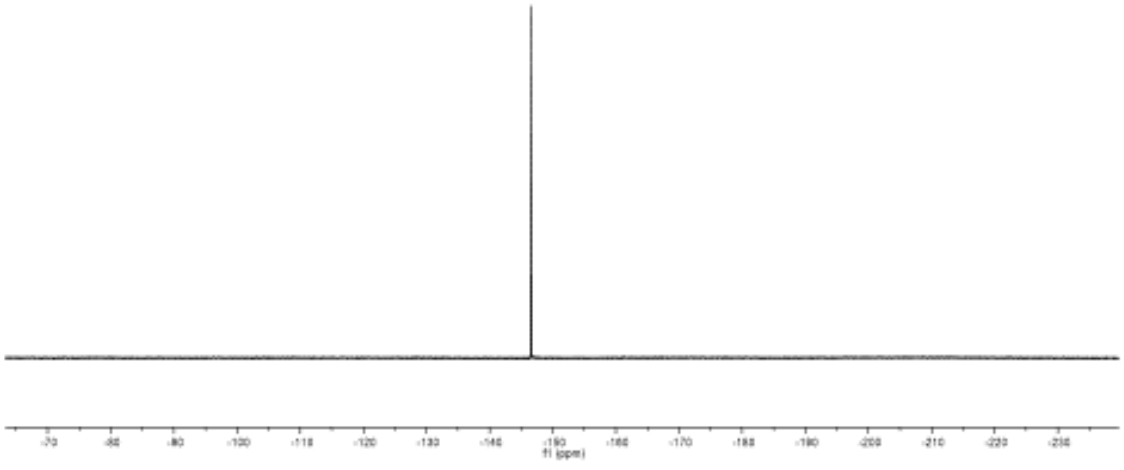


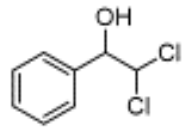


2h

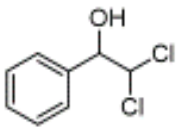


2h

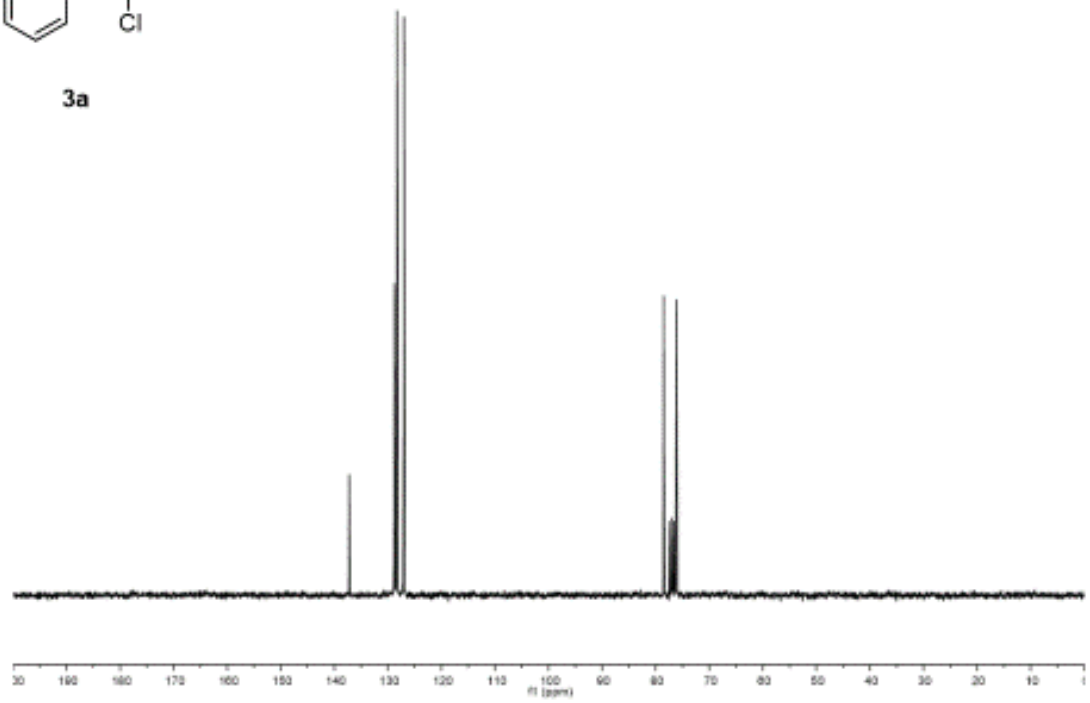


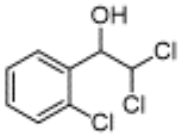


3a

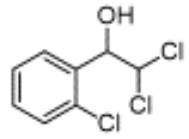


3a

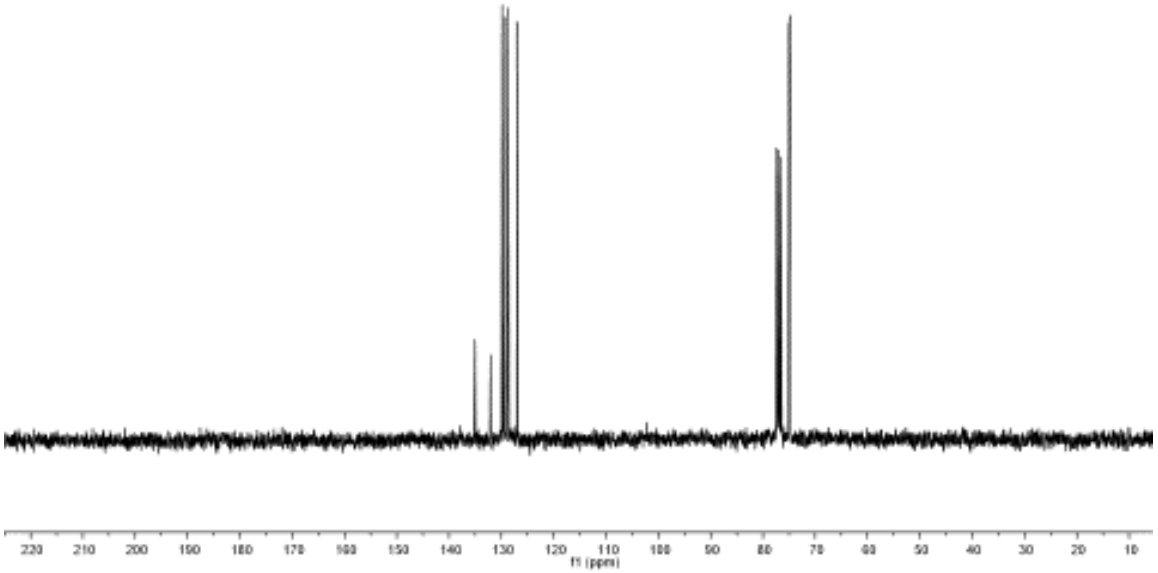


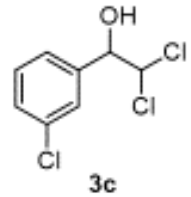


3b

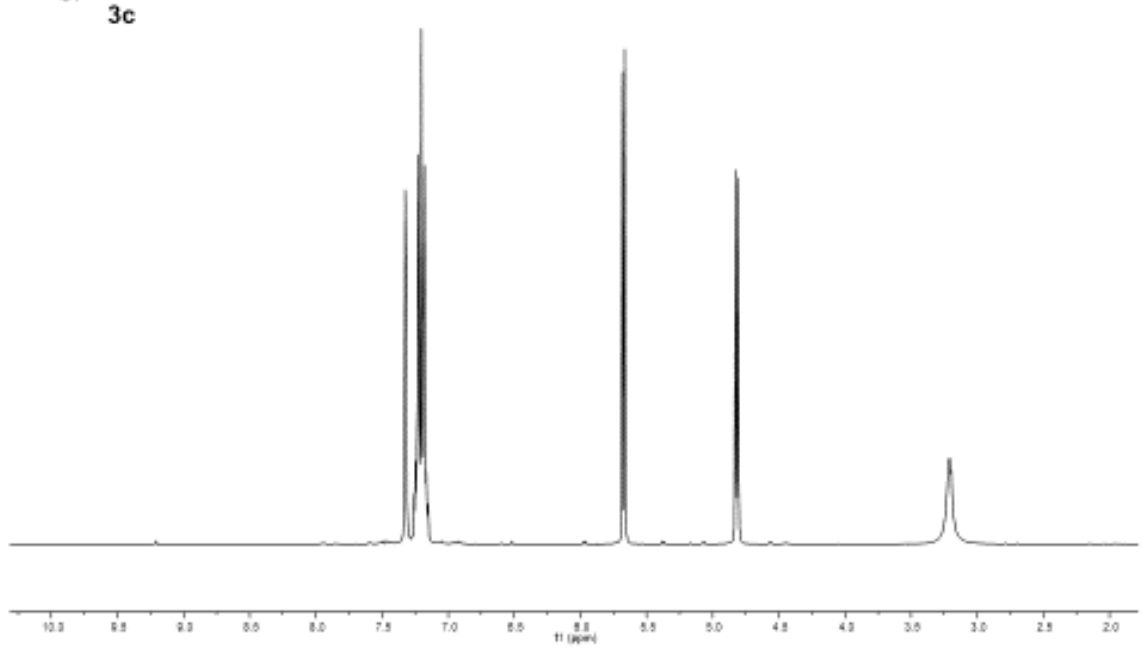

OH
Cl
Cl
Cl

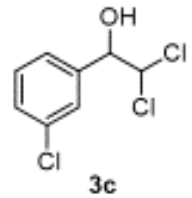
3b


220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

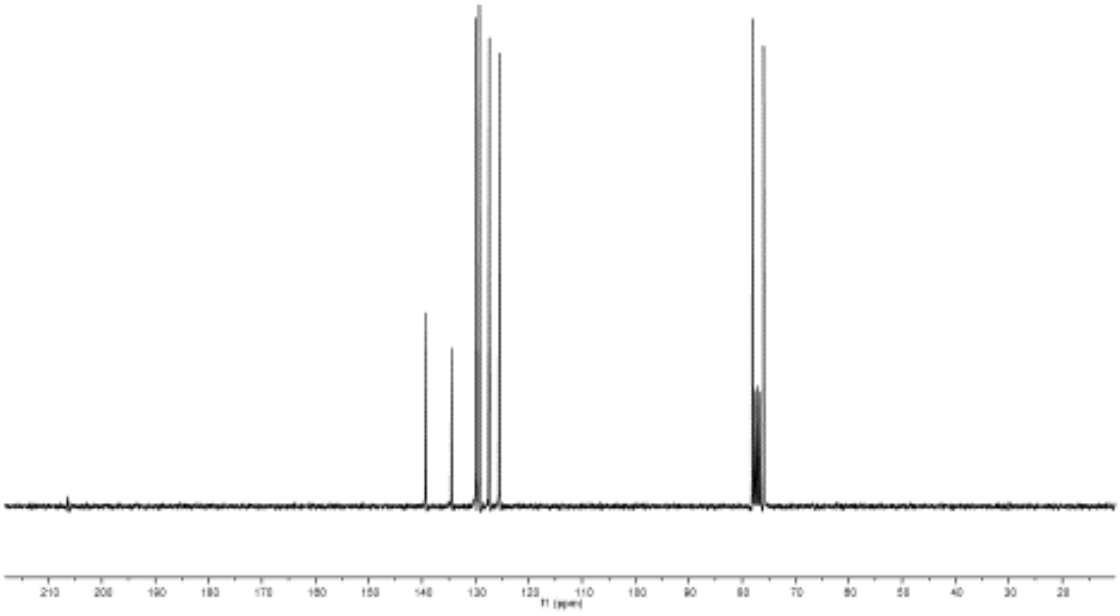

OH
Cl
Cl
Cl

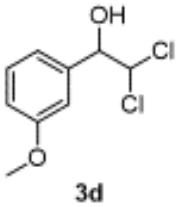
3c


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
f1 (ppm)

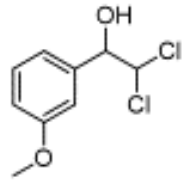


3c

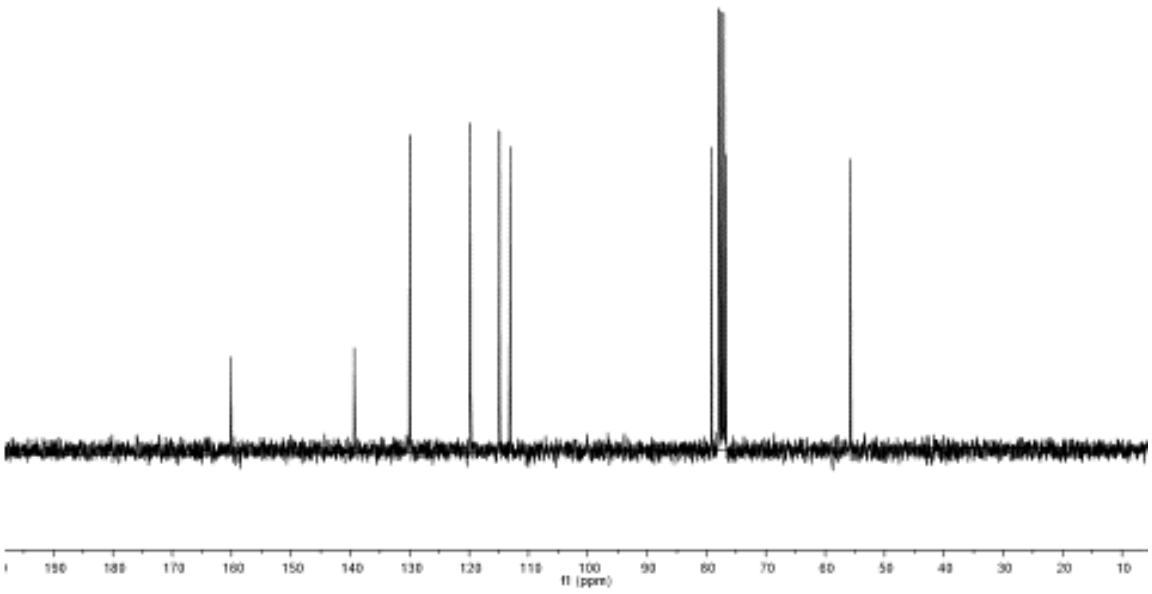


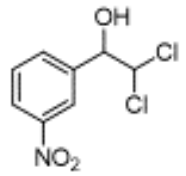


3d

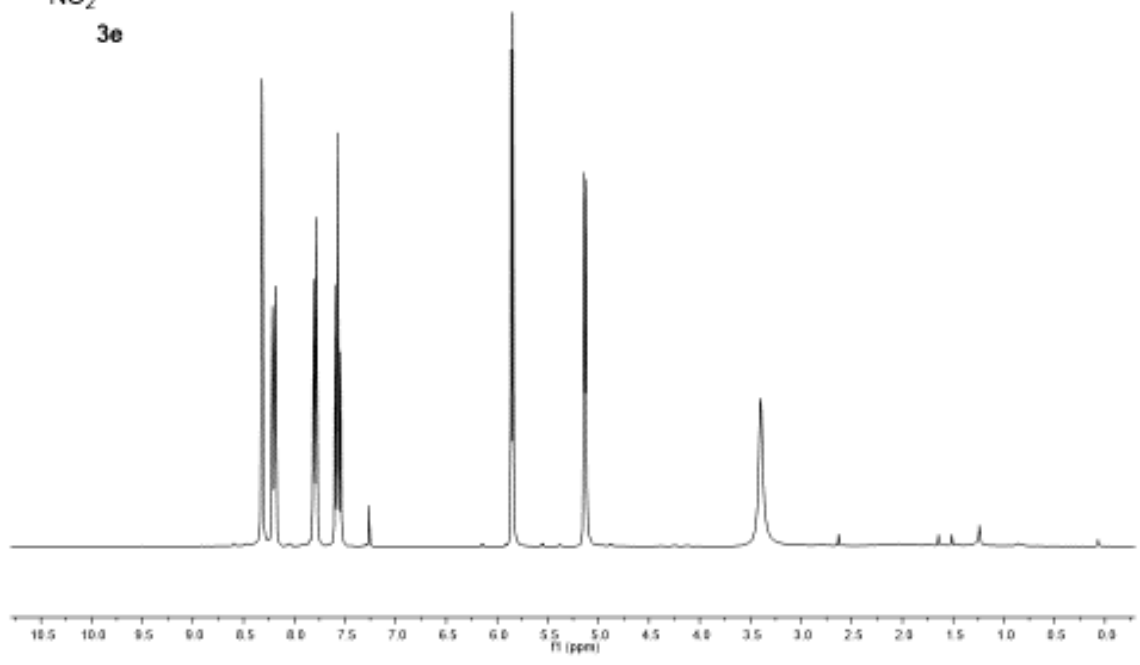


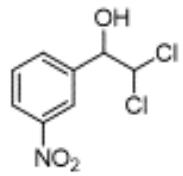
3d



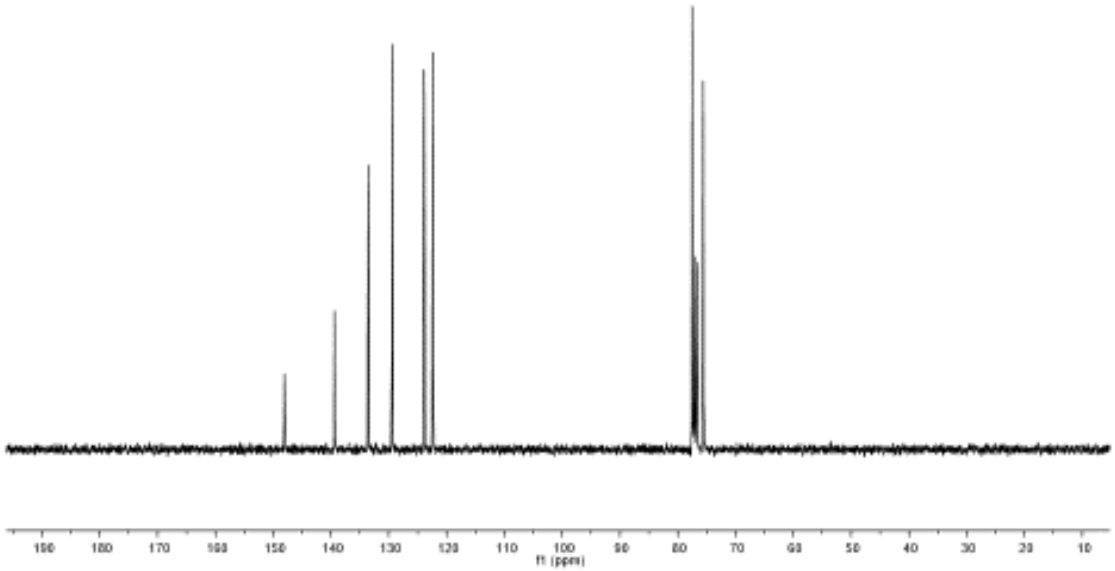


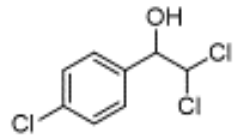
3e



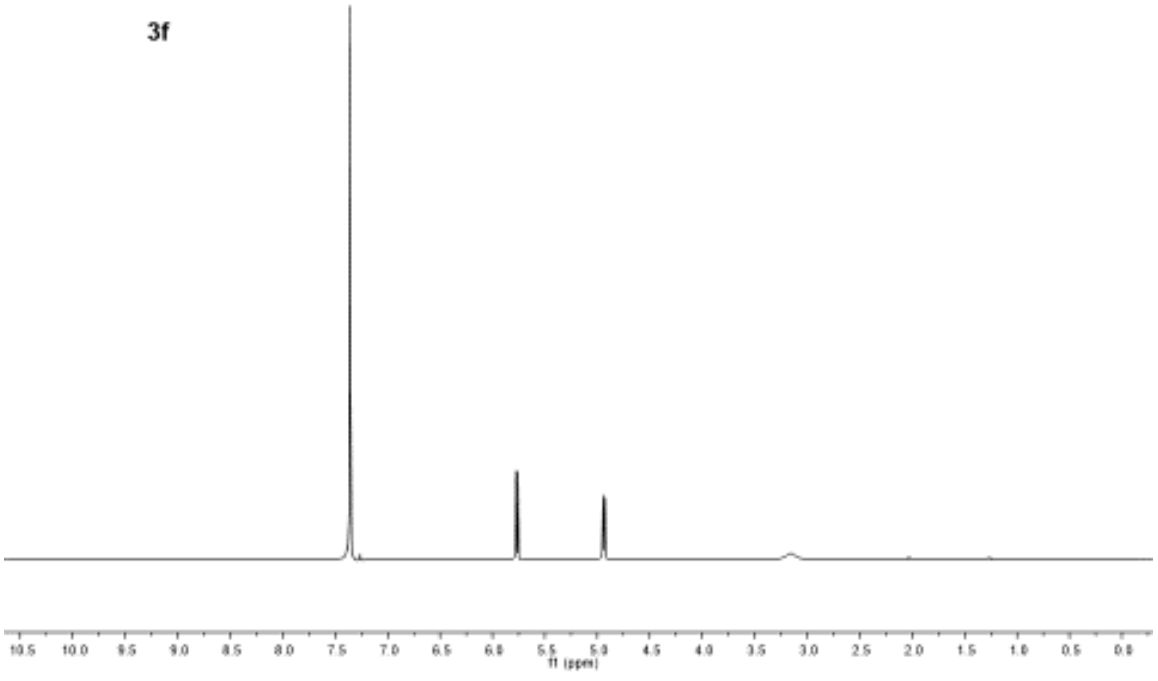


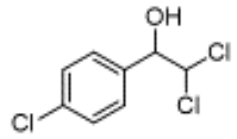
3e





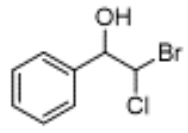
3f



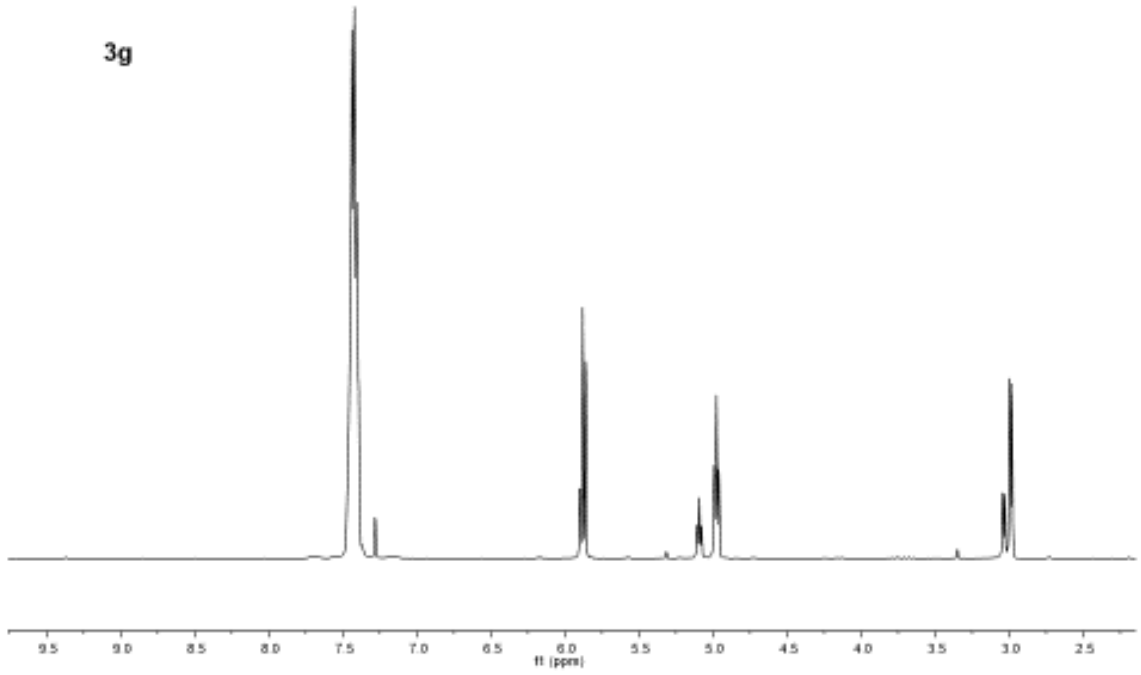

OH
Cl
Cl
Cl

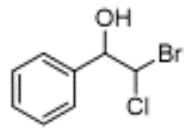
3f

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

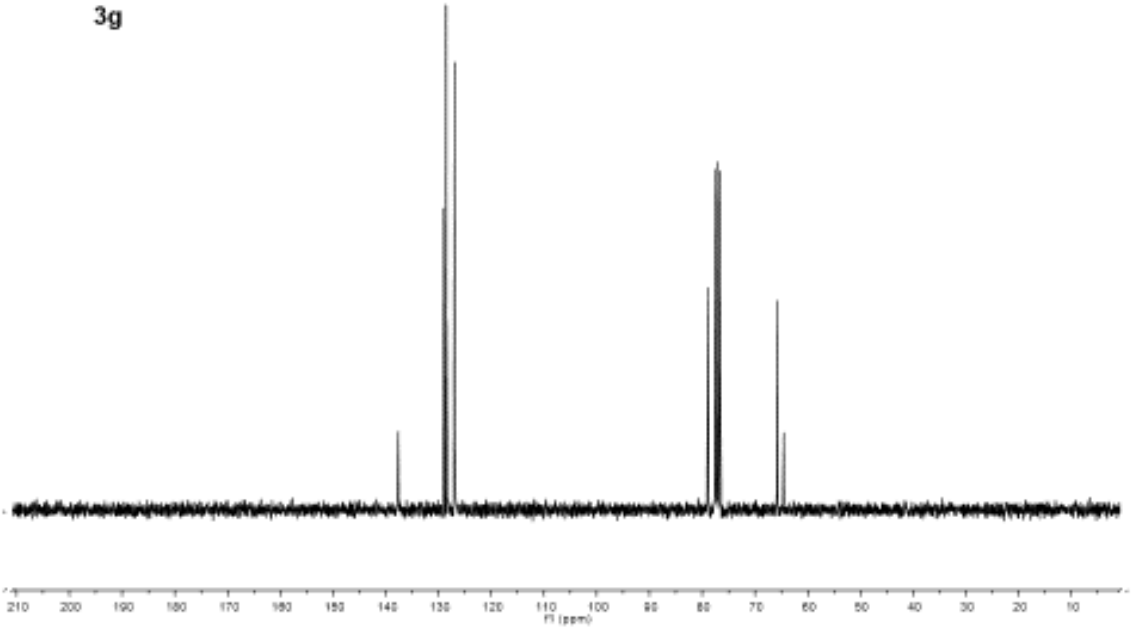

OH
Br
Cl

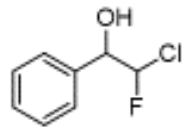
3g


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
f1 (ppm)

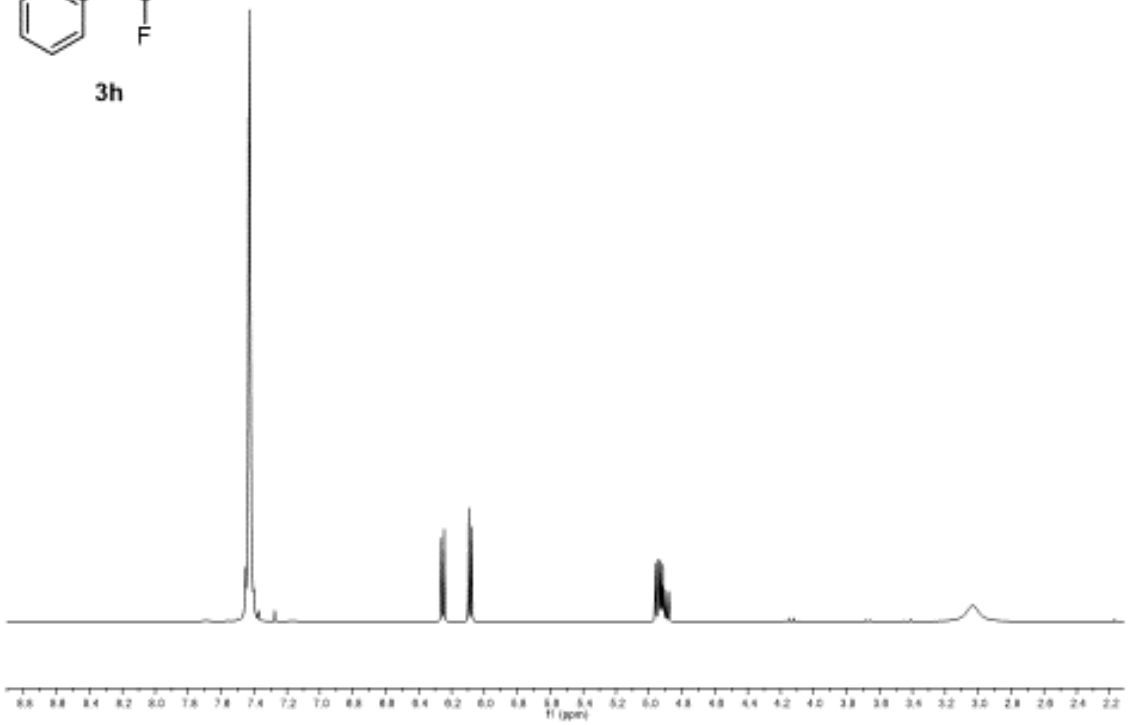


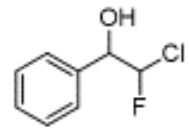
3g



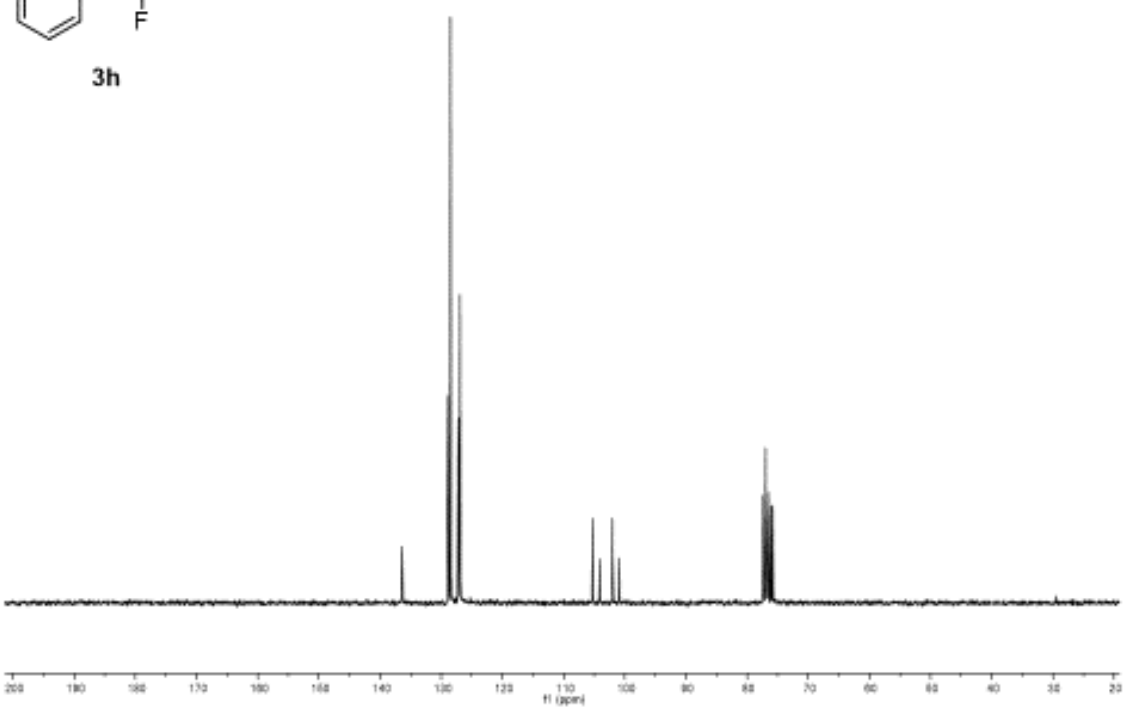


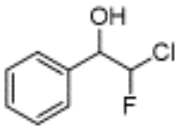
3h



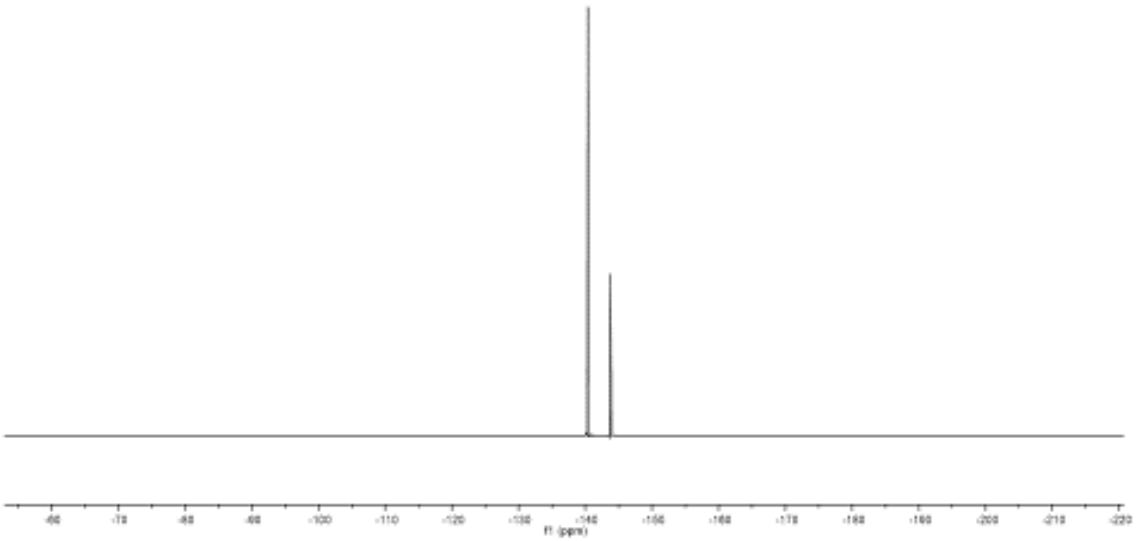


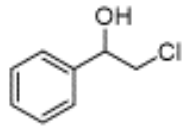
3h



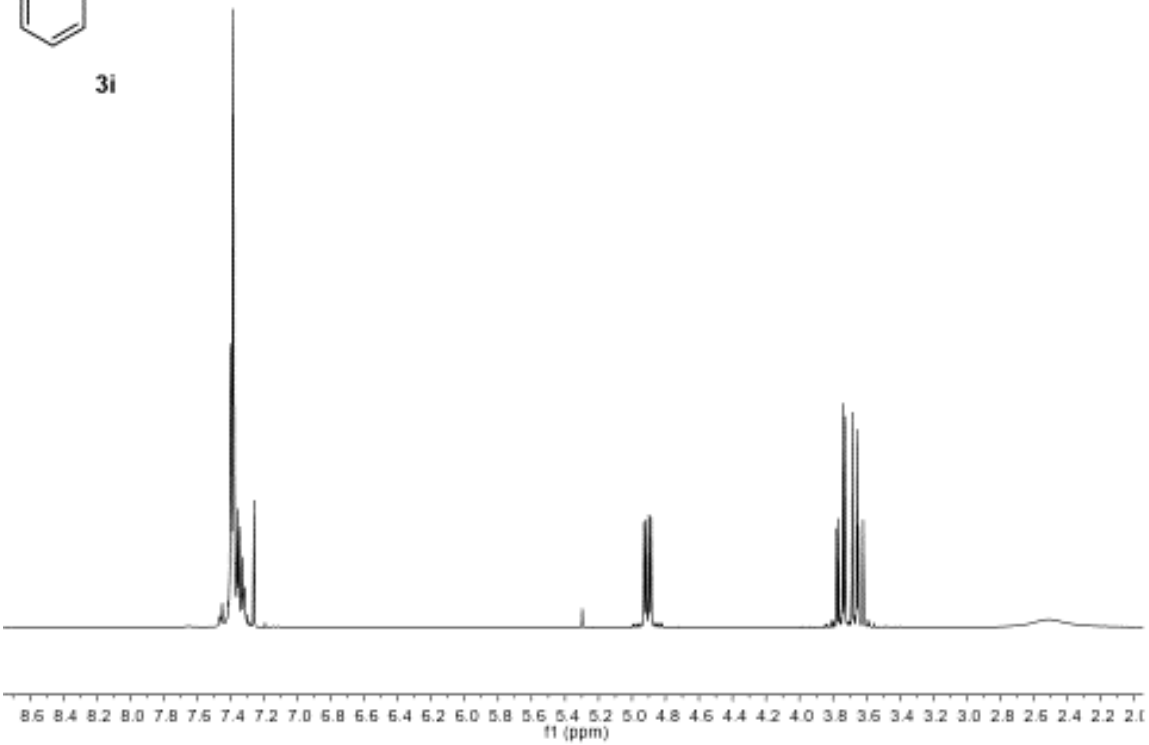


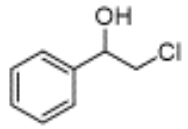
3h



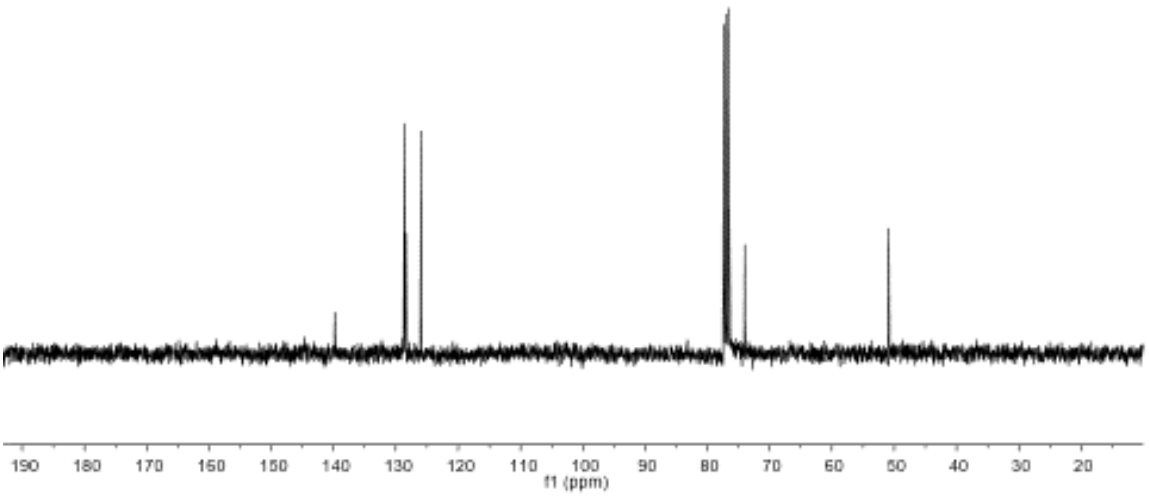

OH
Cl

3i


8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2
f1 (ppm)


OH
Cl

3i


190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)