Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supporting Information for

Halofunctionalization of alkenes by vanadium chloroperoxidase from *Curvularia inaequalis*

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Content:

(1) General procedures

All chemicals were purchased from Sigma-Aldrich and were used without further purification unless other-wise specified.

^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 (400 MHz) spectrometer in $CDCl_3$ or D_2O. Chemical shifts (ppm), multiplicity (s = singlet, d = doublet,t = triplet, q = quartet, m = multiplet), integration, coupling constant (J-values, Hz) and assignment are given. Data for ^{13}C NMR are re-ported in terms of chemical shift (ppm).

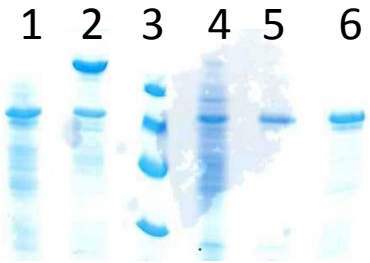
Flash Column chromatography was carried out with Acros silica gel (35-70 µm) and with mixtures of petroleumether (PE) and ethyl acetate (EtOAc) as eluent. Thin-layer chroma-tography (TLC) was performed on 0.20 mm silica gel 60-F plates. Organic solutions were concentrated under reduced pressure with a rotary evaporator.

HPLC measurements were performed on a Shimadzu LC-20 system with a Shimadzu mSPD-20A Photo Diode Array detector using Chiralpak OD column (46 × 25 mm). At intervals, aliquots from the organic phase were extracted 1:1 in heptane/isopropanol (70 : 30) and aliquots from the aqueous phase were extracted 1:1 in heptane/isopropanol (70 : 30) and analysed by HPLC.

(2) Preparation of vanadium chloroperoxidase from *Curvularia inaequalis* (CiVCPO)

For heterologous expression and purification of CiVCPO a slightly modified literature procedure[1]: A 2 L culture of *Escherichia coli* transformant [*E. coli* TOP10 (Invitrogen) with the construct pBAD- *Ci*VCPO] was grown at 37 °C in Lysogeny broth medium supplemented with 100 µg/mL ampicillin to an OD 600 nm of 0.6-0.8. Protein expression was induced after cooling the fermentation broth to 20 °C and addition of 0.02 % L arabinose, followed by another 72 hours of incubation. The expression of CiVCPO in E. coli yielded an enzyme content of 15 $mg \cdot L^{-1}$ culture. Cells were harvested by centrifugation at 8000 rpm for 10 min at 4 °C. The cells were re-suspended to 1 g mL^{-1} in 50 mM Tris/H_2SO_4, pH 8.1 fortified with protease inhibitors, lysozyme (2 mg mL^{-1}) and DNaseI. Cells were lysed using a Cell disruptor and debris was removed by centrifugation at 15000 rpm for 1 h at 4 °C. After centrifugation an equal volume of isopropyl alcohol was added to the supernatant to precipitate nucleic acids and unstable proteins. After centrifugation (30 min at 15000 rpm), the clear supernatant was applied to a DEAE Sephacel column (Amersham Pharmacia Biotech) (5mL min^{-1}) equilibrated with 50 mM Tris/H_2SO_4 pH 8.1. After washing of the column with 2 volumes of 50 mM Tris/H_2SO_4, pH 8.1, and 2 volumes of 0.1 M NaCl in 50 mM Tris/H_2SO_4, pH 8.1, the enzyme was eluted with 1 M NaCl in 50 mM Tris/HCl, pH 8.1. Finally the pure apoenzyme was dialyzed against 100 µM orthovanadate in 50 mM Tris H_2SO_4, pH 8.1 to obtain the reconstituted holoenzyme. As illustrated below, SDS-PAGE monitoring of the purification process showed that the whole soluble fraction from the *E. coli* cultures (lane 4) was considerably enriched in the *Ci*VCPO (67.5 kDa) band, incubation and centrifugation with isopropanol (lane 3) partially removed undesired proteins and finally after DEAE chromatography (lanes 5 and 6) protein was $\geqslant$ 90 % pure. Protein concentration was estimated by the BSA assay and *Ci*VCPO activity was determined to be 120 U mg^{-1}. One unit of the enzyme activity was defined as the amount of the enzyme that catalyzes the

bromination of 1 µmol Monochlorodimedon per min at pH 5 and 30 °C (using a saturating concentration of bromide (5 mM) in 0.1 M citrate (pH 5) after the addition of 10 mM of H_2O_2) . Purification steps of *Ci*VCPO (lane 1 crude extract with isopropanol; lane 2 crude extract with isopropanol after centrifugation; lane 3 standard (99 kDa, 66 kDa, 45 kDa and 30 kDa); lane 4 crude extract, lanes 5-6 purified enzyme).



(3) Procedure for oxidation of alkenes

In 0.1 M of pH 5 citrate buffer with 160 mM of KBr, 40 mM alkene (0.1 mmol) was added, followed by 100 nM of vanadium-dependent chloroperoxidase from the fungus *Curvularia inaequalis* (*Ci*VCPO) and 170 mM of H_2O_2 was added in one goal or 22 mM of H_2O_2 per hour were added for 5 hours while the solution is stirring at room temperature. The reaction mixtures were extracted by dichloromethane, dried over anhydrous Na_2SO_4. The combined organic layers were reduced in vacuo. The bromohydrin was isolated by flash column chromatography on silica gel.

(4) pH effect on selectivity of products

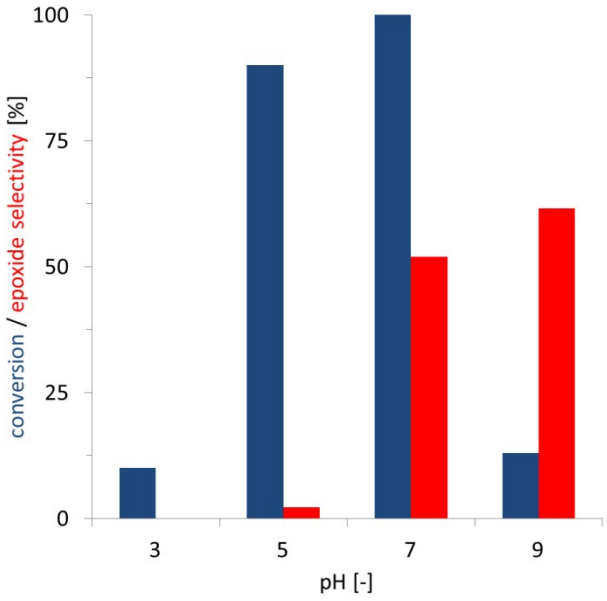
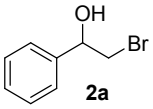


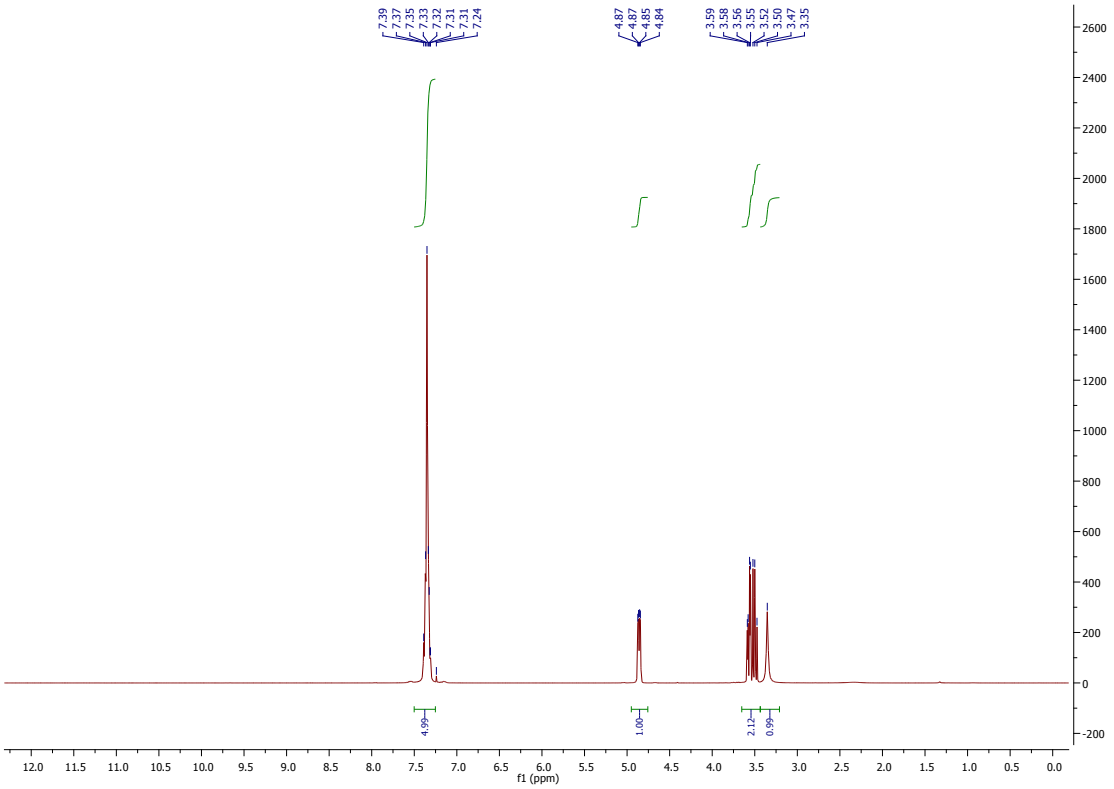
Figure S1. Activity and selectivity of the chemoenzymatic halohydroxylation of styrene at different pH values. General conditions: c(*Ci*VCPO)= 100 nM, c(styrene) = 40 mM, c(KBr) = 160 mM, c(H_2O_2)= 170 mM, T= 25 °C, t = 20 h.

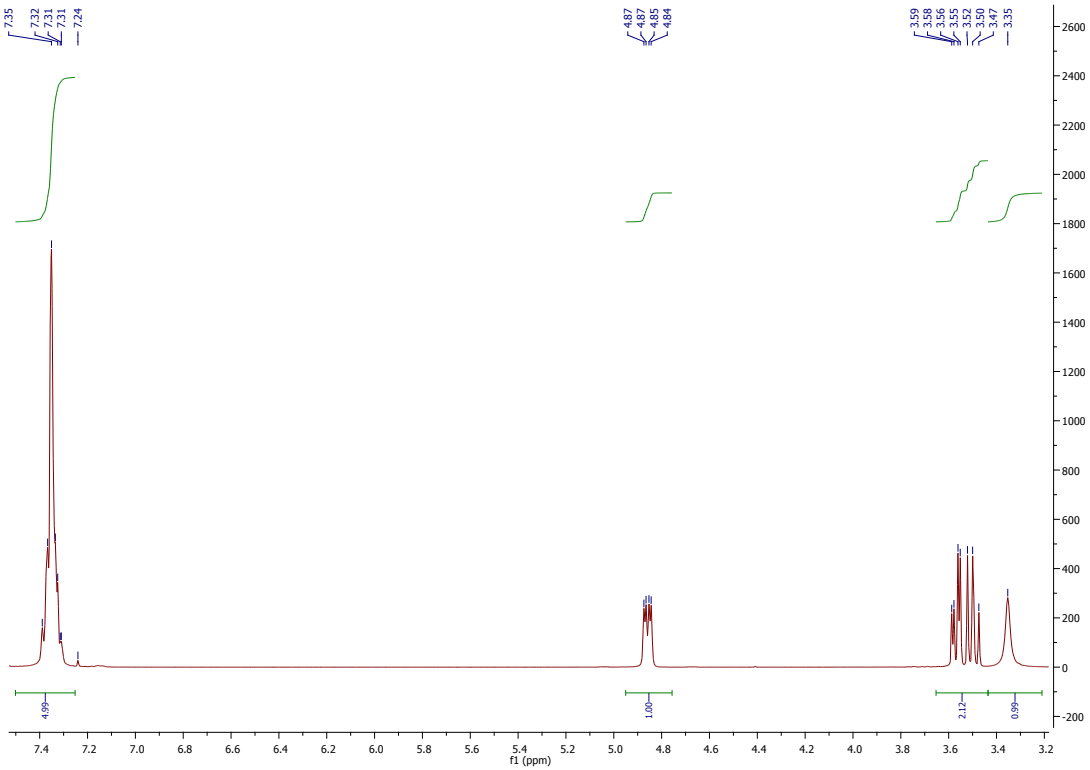
(5) NMR spectra of Scheme 3



2a

2-bromo-1-phenylethanol was purified by flash column chromatography to give 81% isolated yield as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.35 (1H, brs), 3.41-3.70 (2H, m), 4.82-4.90 (1H, dd, *J* = 8.10 Hz, 4.00 Hz), δ 7.25-7.50 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.8, 73.7, 126.1, 128.4, 128.6, 140.5. (in accordance with literature)[2]

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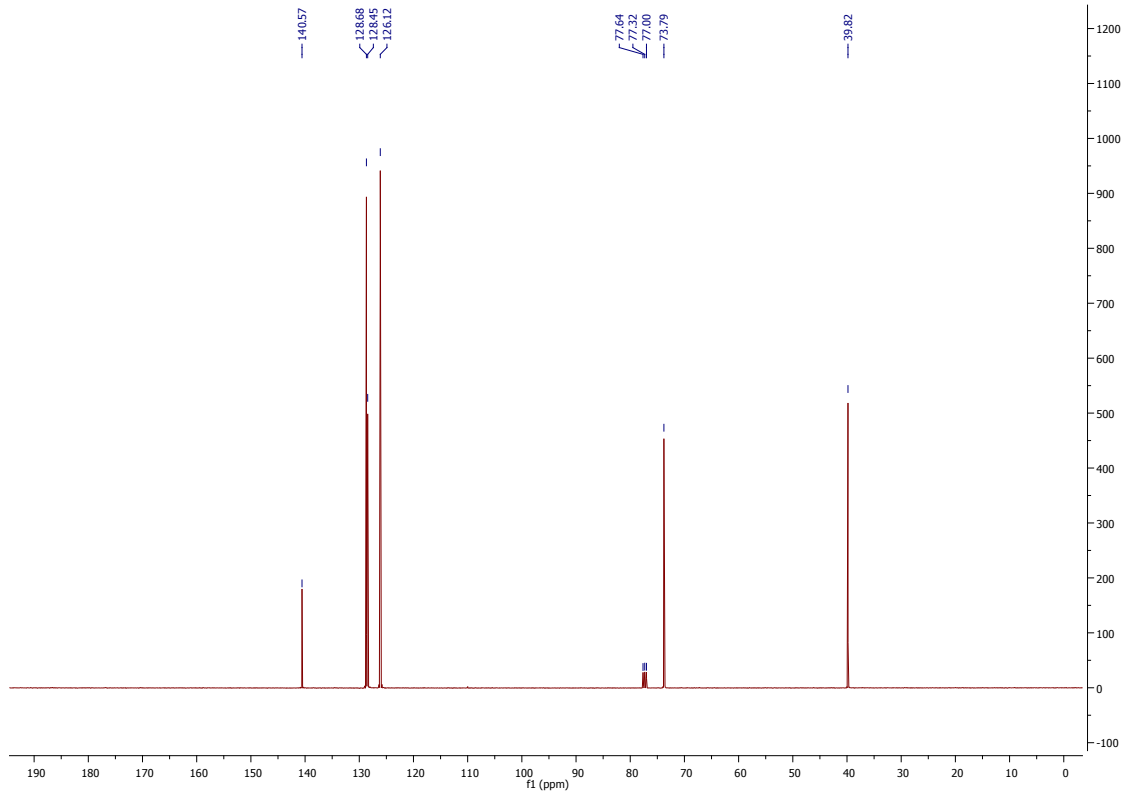
7.32
7.31
7.31
7.24
4.87
4.87
4.85
4.84
3.59
3.58
3.56
3.55
3.52
3.50
3.47
3.35
4.99
1.00
2.12
0.99
f1 (ppm)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73.79
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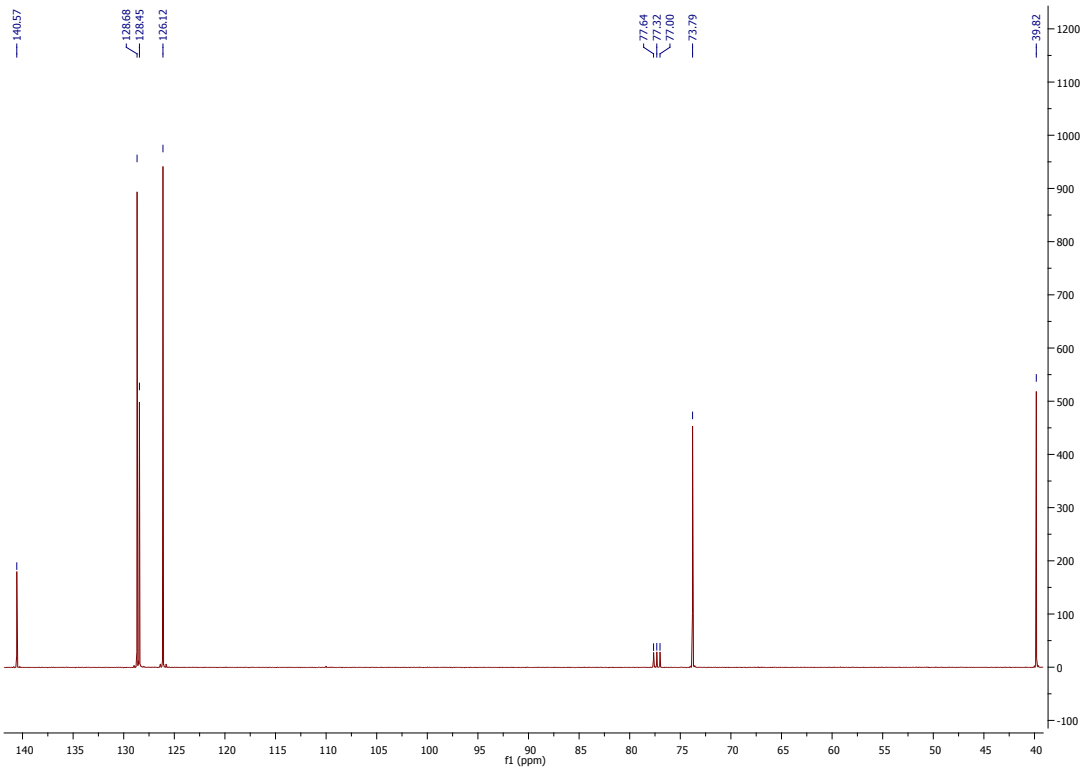
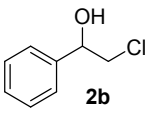
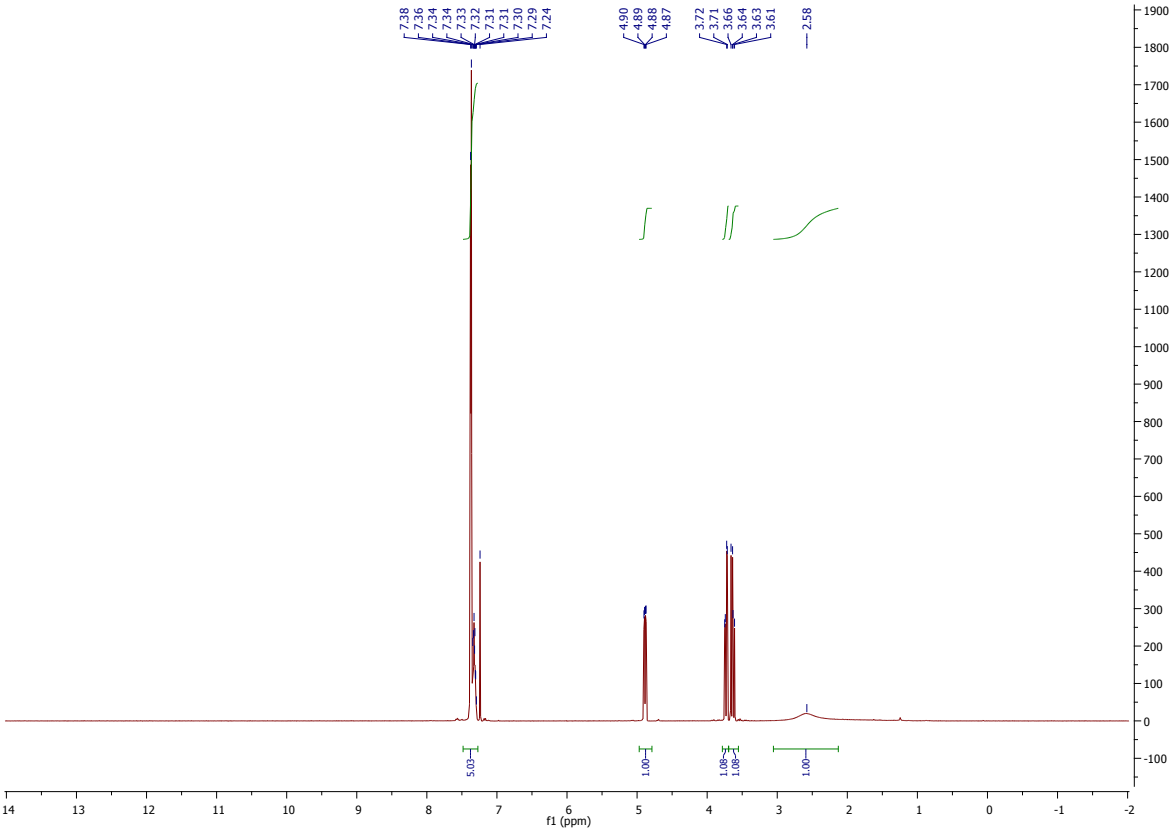
f1 (ppm)





2b 2-chloro-1-phenylethanol was purified by flash column chromatography to give 77% isolated yield as a colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.58 (1H, brs), 3.60-3.67 (1H, dd, *J* = 11.2, 8.8 Hz), 3.70-3.76 (1H, dd, *J* = 11.2, 3.4 Hz), 4.82-4.90 (1H, dd, *J* = 8.8, 3.2 Hz,), δ 7.23-7.40 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 50.8, 74.0, 126.0, 128.4, 128.6, 139.8. (in accordance with literature)[3]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7.34
7.34
7.33
7.32
7.31
7.31
7.30
7.29
7.24
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4.89
4.88
4.87
3.72
3.71
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3.63
3.61
2.58
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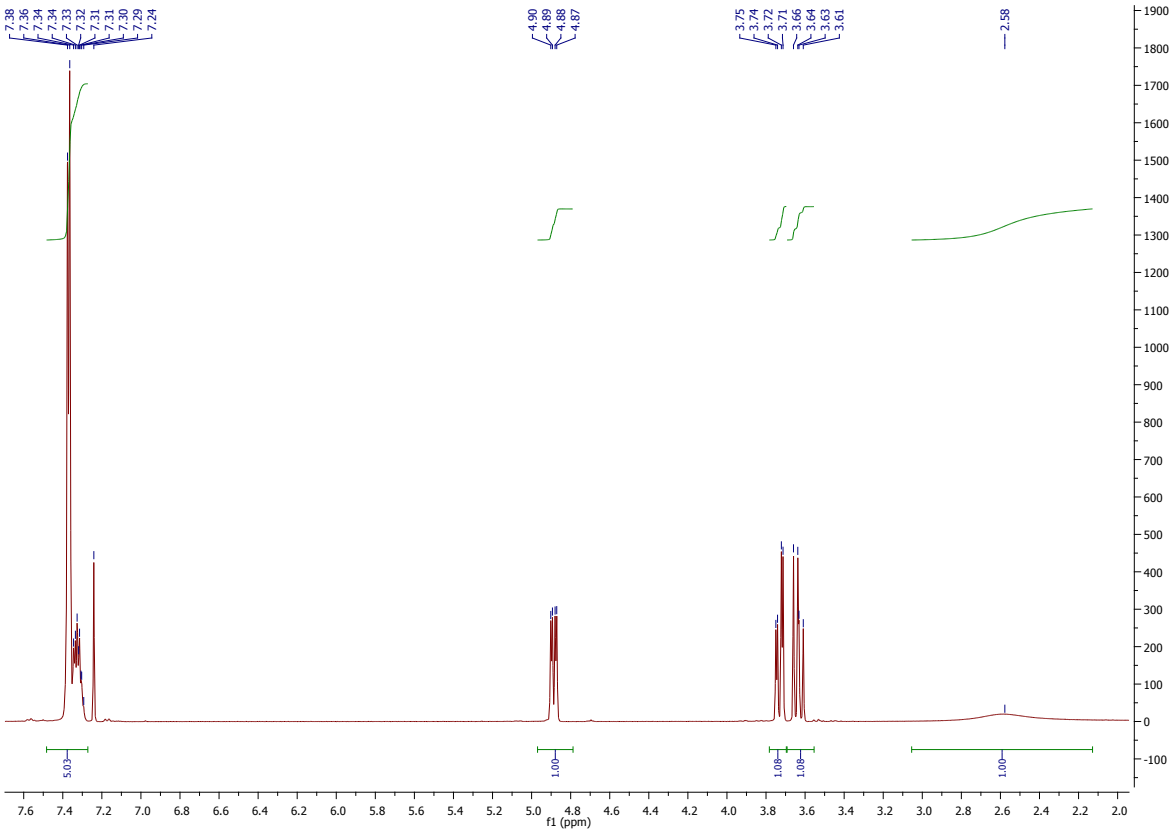
1.08
1.00
f1 (ppm)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7.32
7.31
7.31
7.30
7.29
7.24
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4.89
4.88
4.87
3.75
3.74
3.72
3.71
3.66
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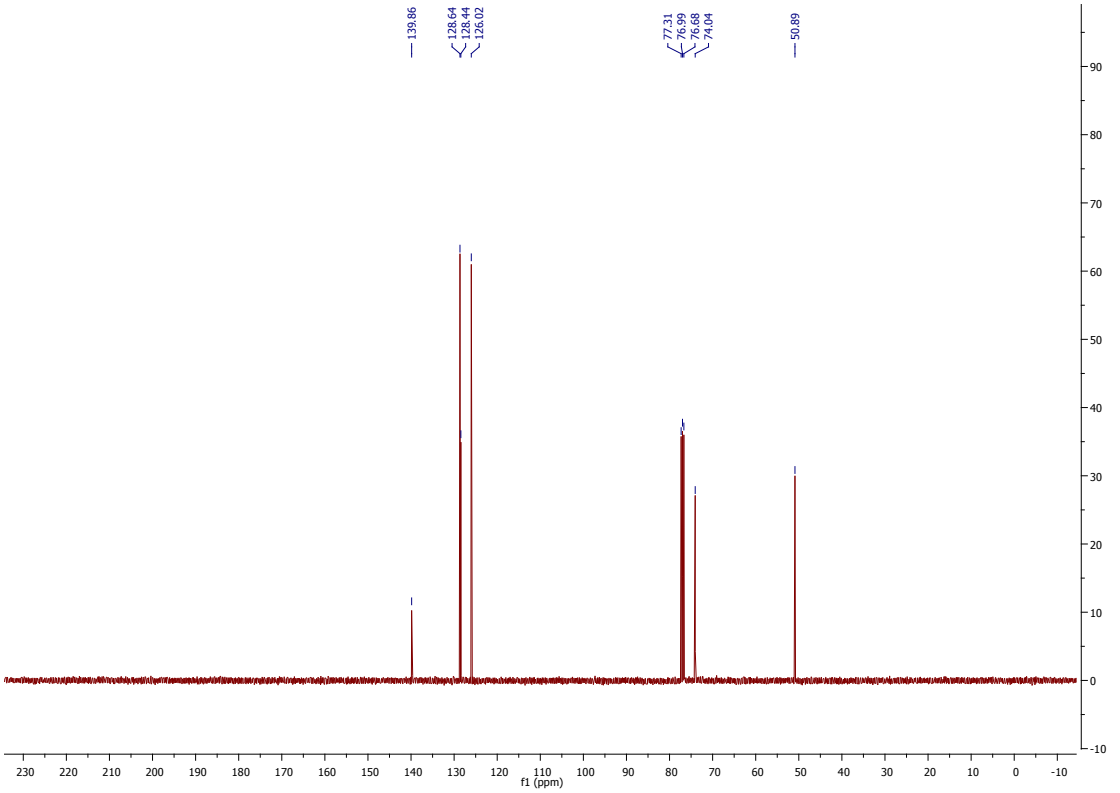
3.63
3.61
2.58
5.03
1.00
1.08
1.08
1.00
f1 (ppm)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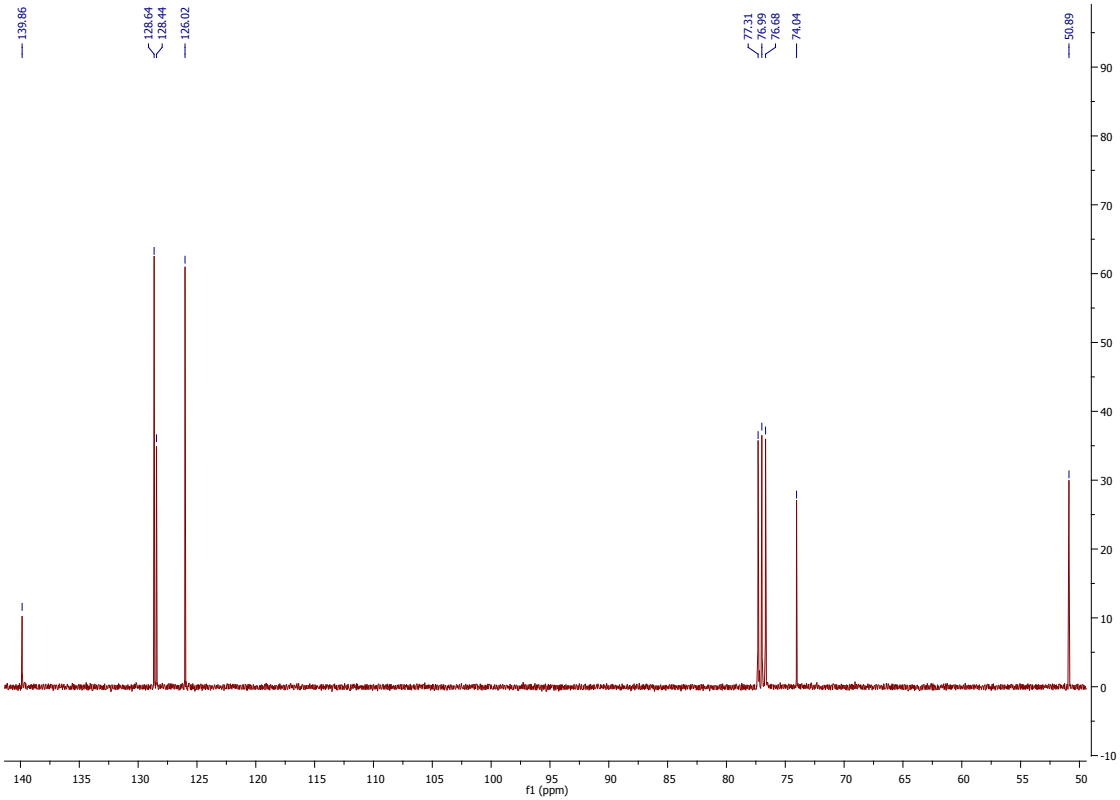
74.04
50.89
f1 (ppm)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128.64
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126.02
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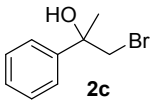
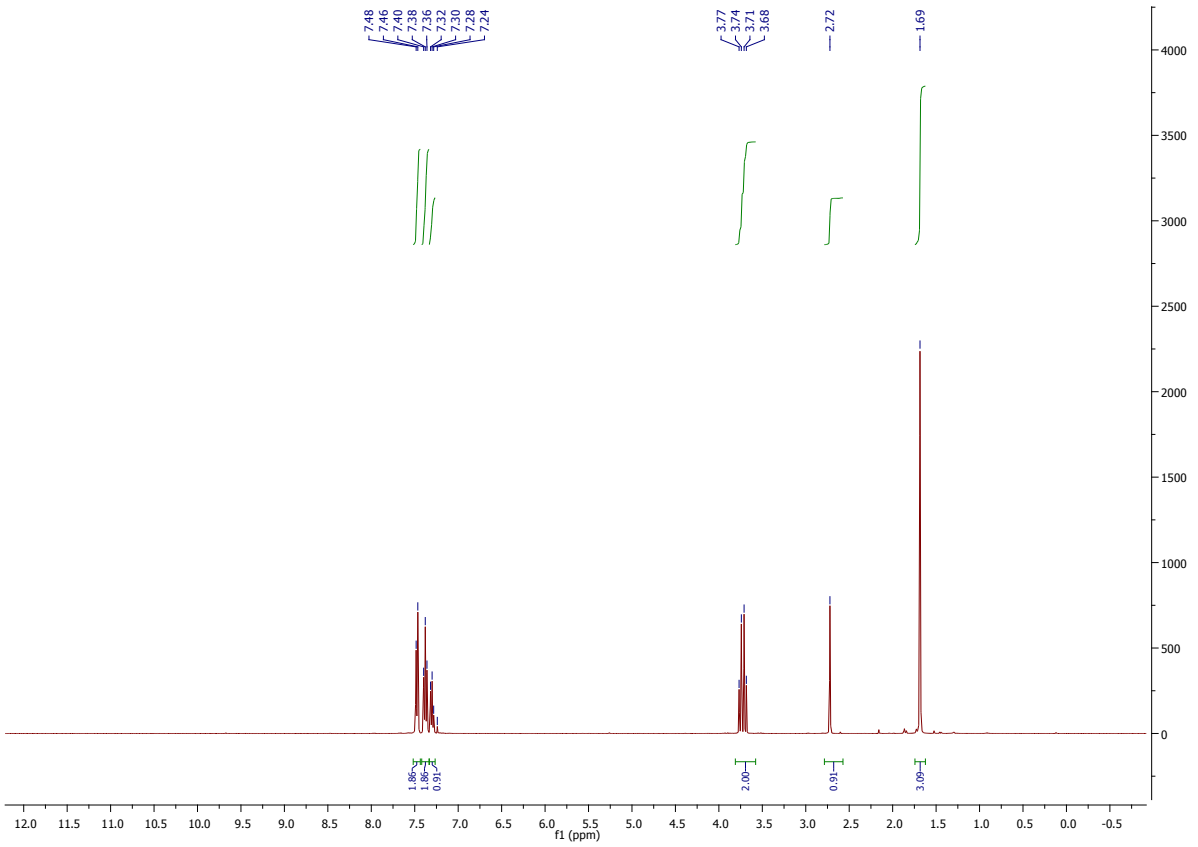
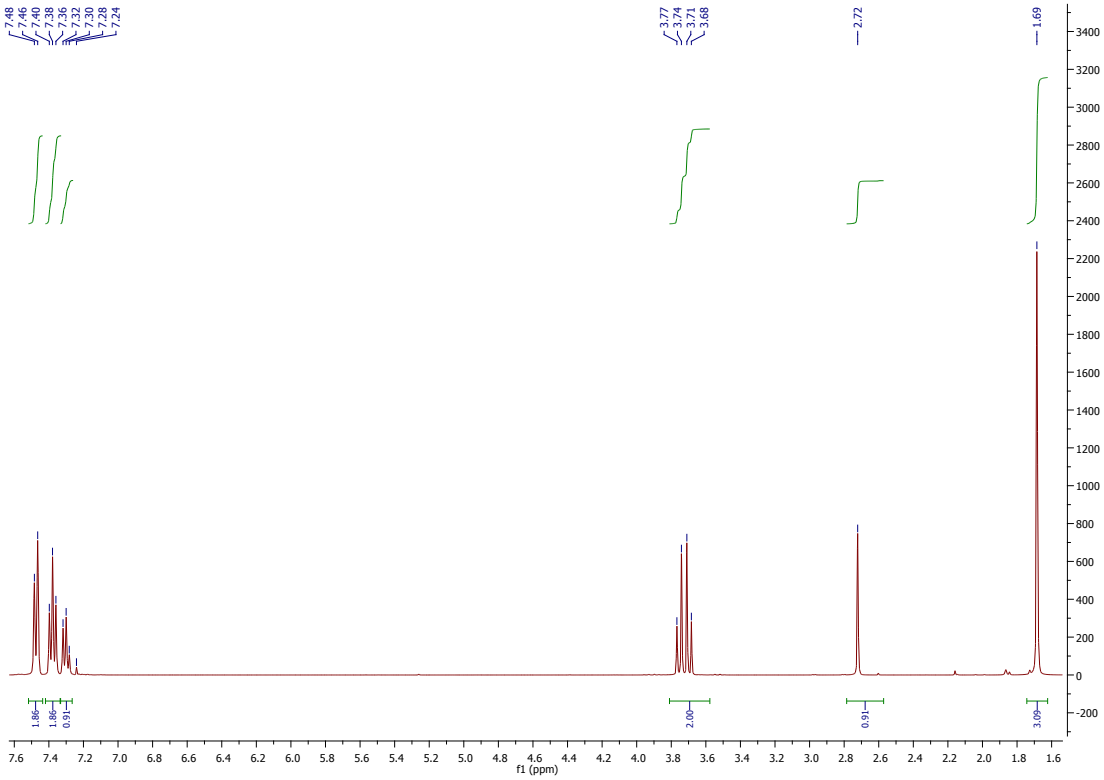
50.89
f1 (ppm)



2c 1-bromo-2-phenylpropan-2-ol was purified by flash column chromatography to give 83% isolated yield as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.69 (s, 3H), 2.72 (brs, 1H), 3.65-3.73 (d, 1H, *J* = 10.4 Hz), 3.74-3.79 (d, 1H, *J* = 10.4 Hz); 7.35-7.64 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 28.0, 46.2, 73.2, 124.9, 127.5, 128.4, 144.2. (in accordance with literature)[4]

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7.40
7.38
7.36
7.32
7.30
7.28
7.24
3.77
3.74
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2.72
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1.86
1.86
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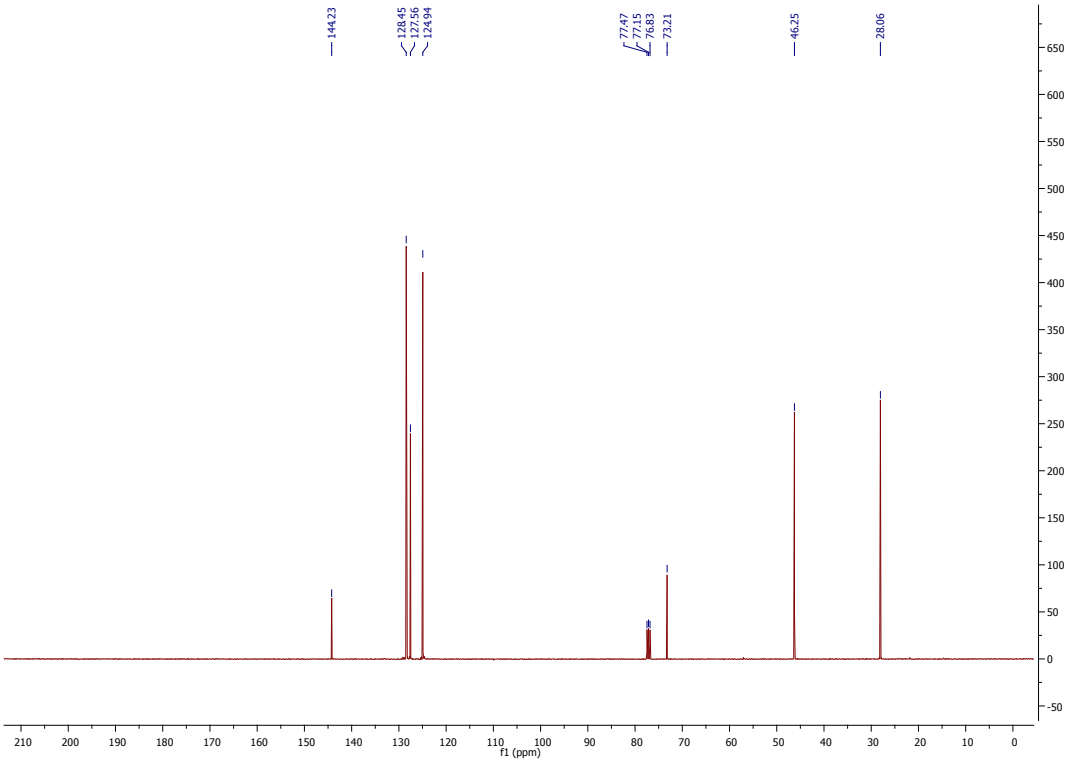
2.00
0.91
3.09
f1 (ppm)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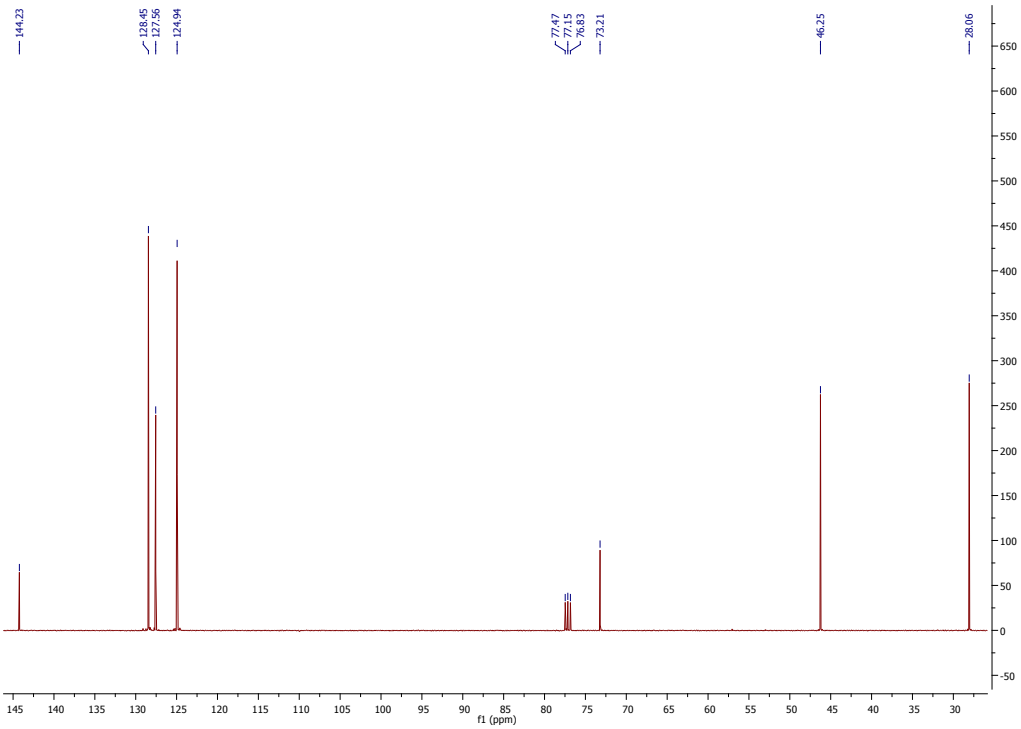
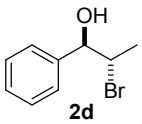
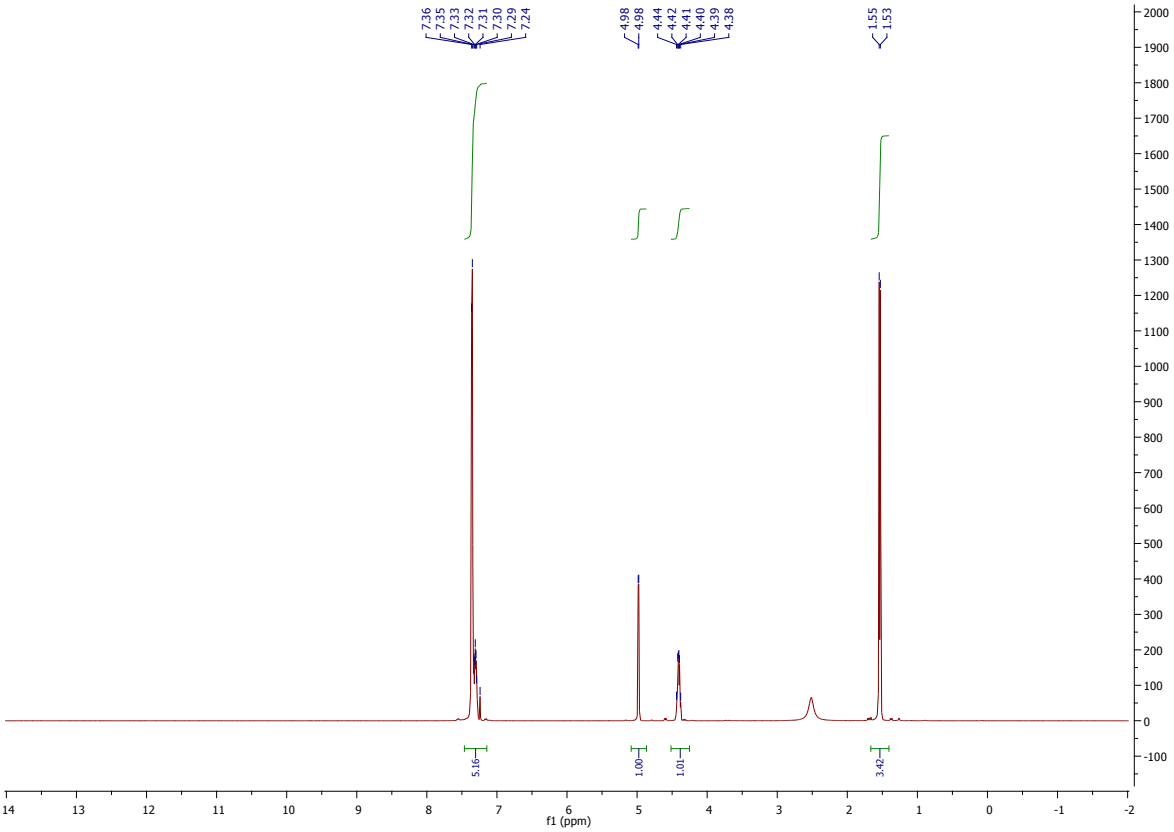
77.15
76.83
73.21
46.25
28.06
f1 (ppm)





2d *anti*-2-bromo-1-phenylpropan-1-ol was purified by flash column chromatography to give 68% isolated yield as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.53-1.55 (d, 3H, *J* = 6.8 Hz), 2.50 (brs, 1H), 4.38-4.44 (dq, 1H, *J* = 6.8, 3.6 Hz), 4.98 (d, 1H, *J* = 3.6 Hz), 7.30-7.38 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ:18.8, 56.0, 77.3, 126.3, 128.0, 128.3, 139.6. (in accordance with literature)[5]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7.33
7.32
7.31
7.30
7.29
7.24
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4.98
4.44
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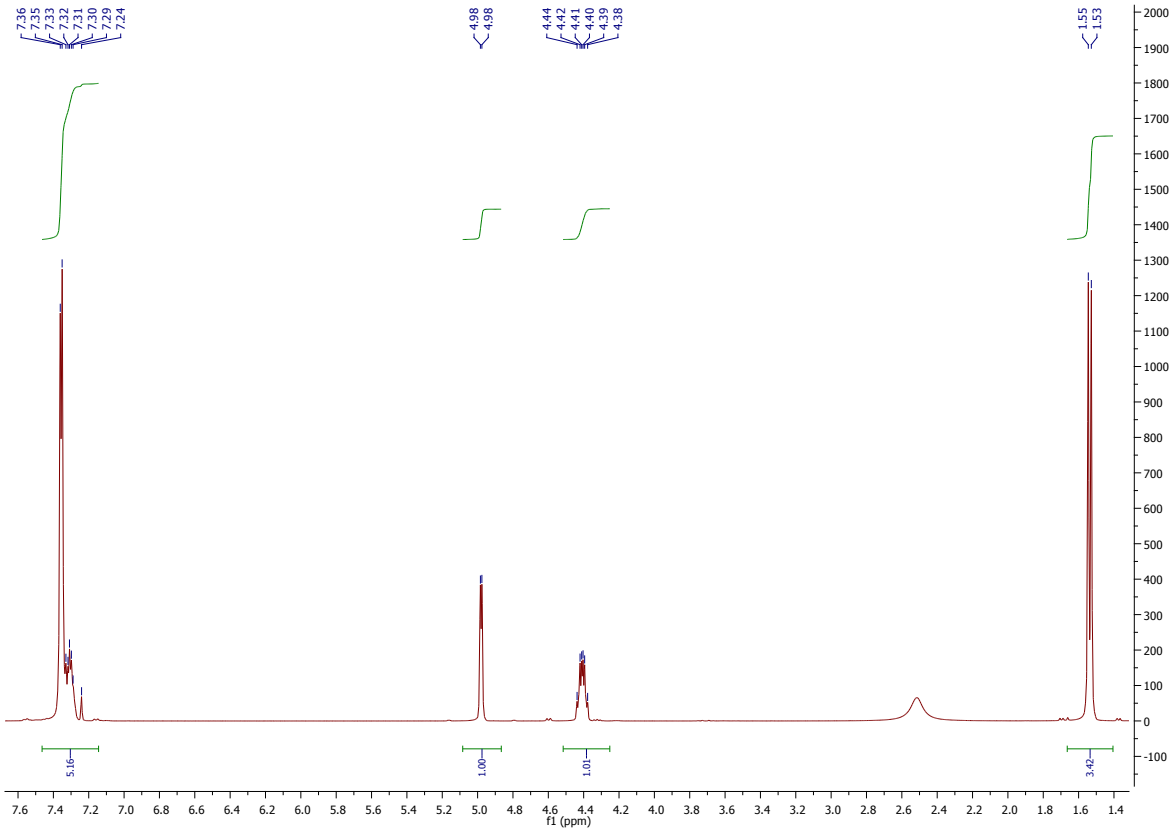
3.42
f1 (ppm)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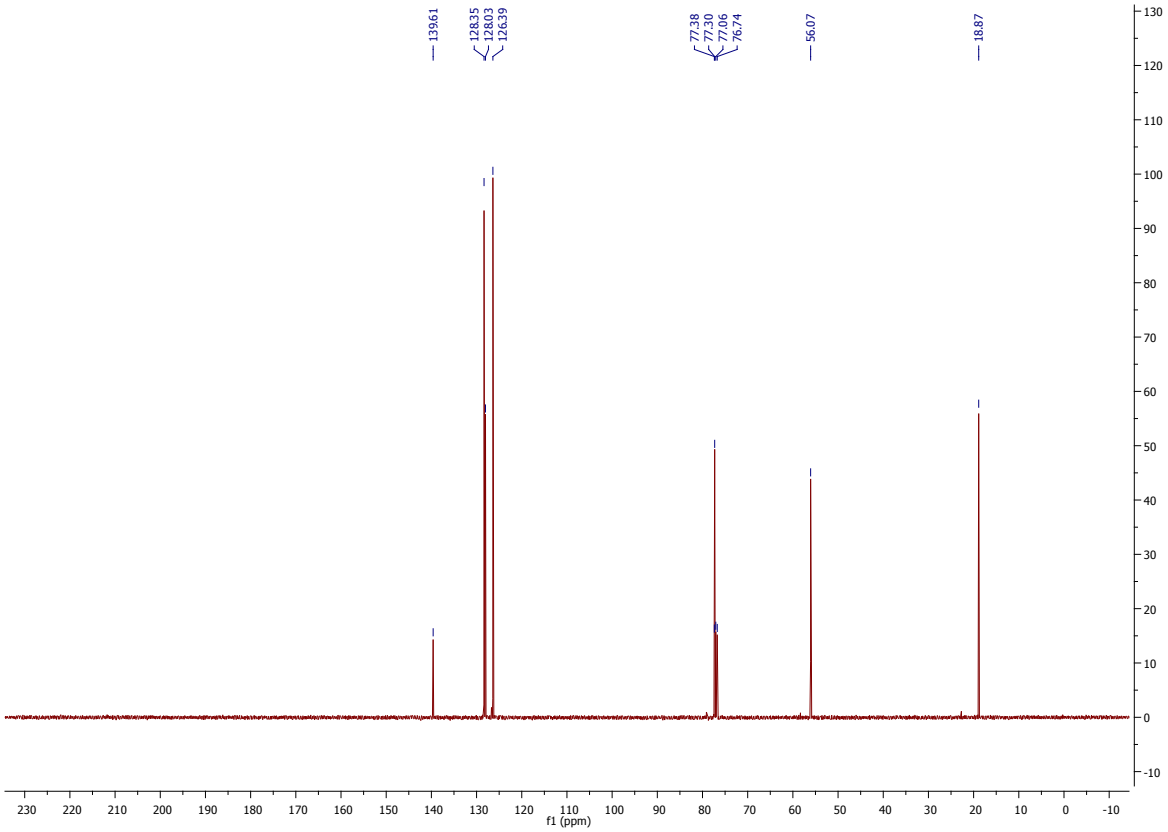
1.00
1.01
3.42
f1 (ppm)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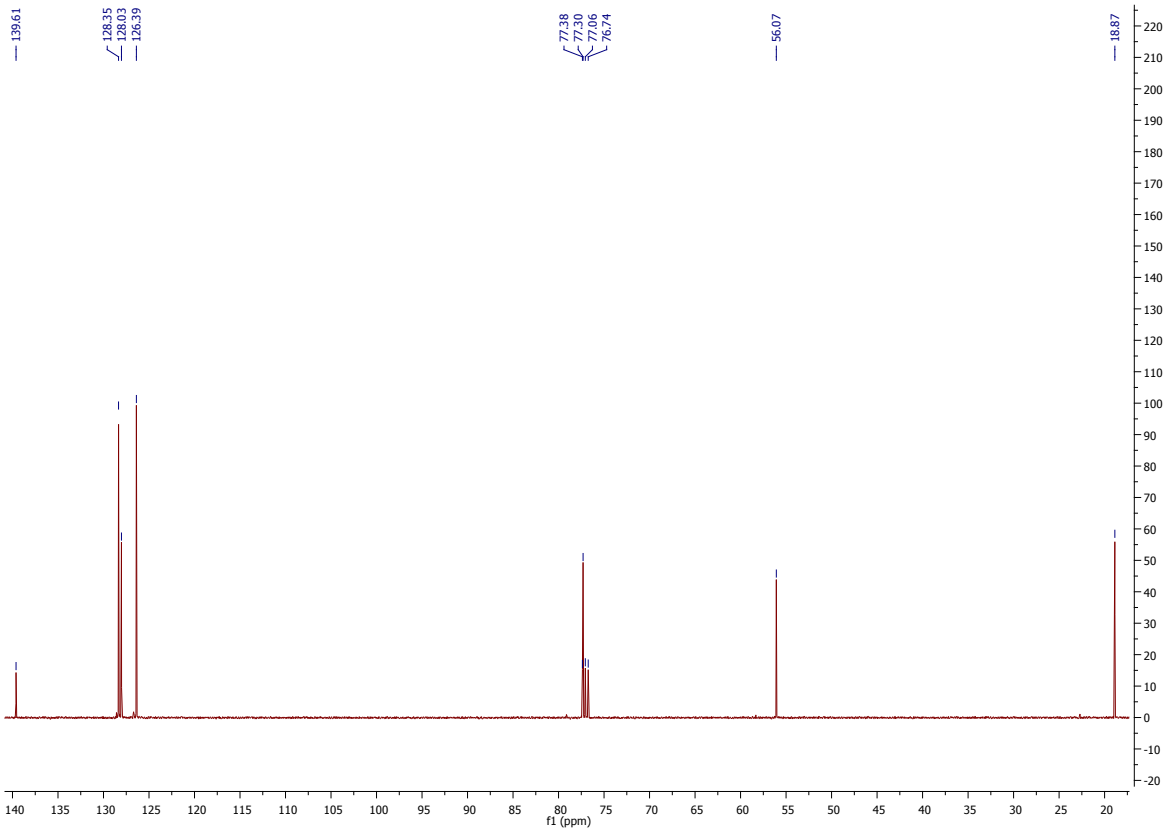
f1 (ppm)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76.74
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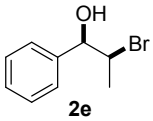
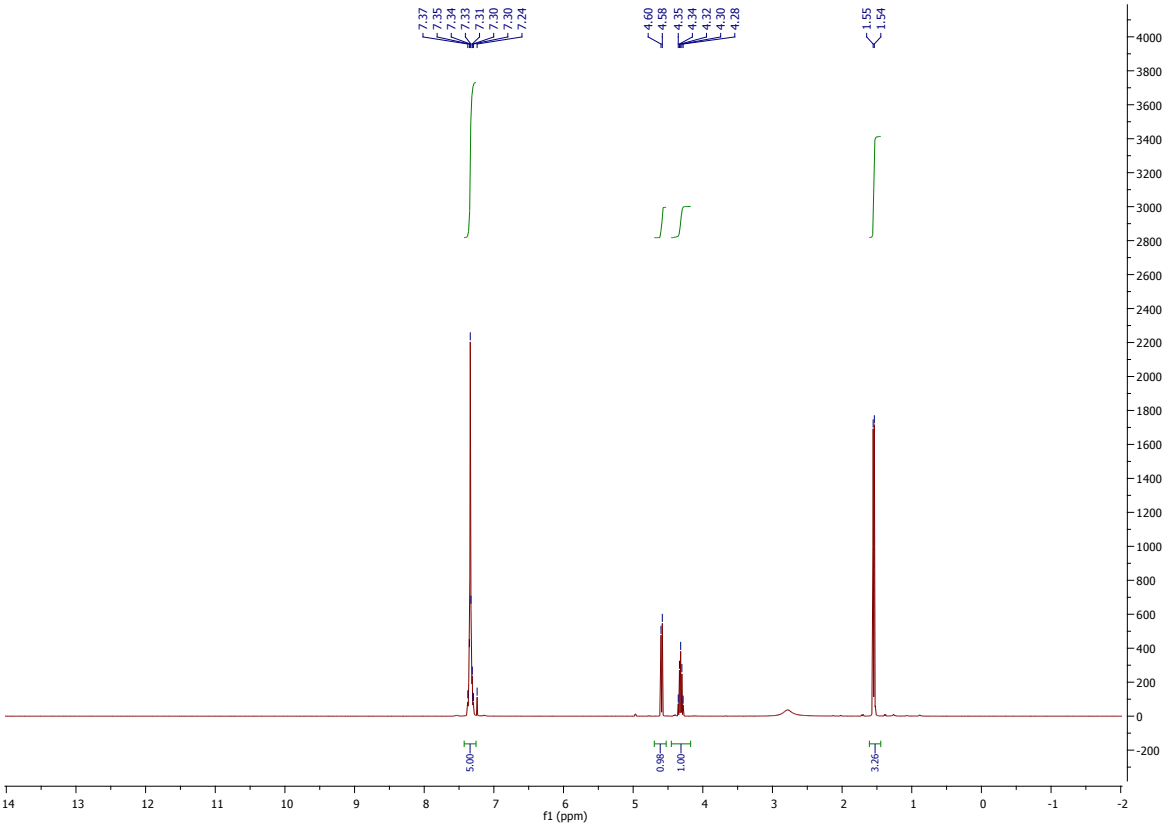
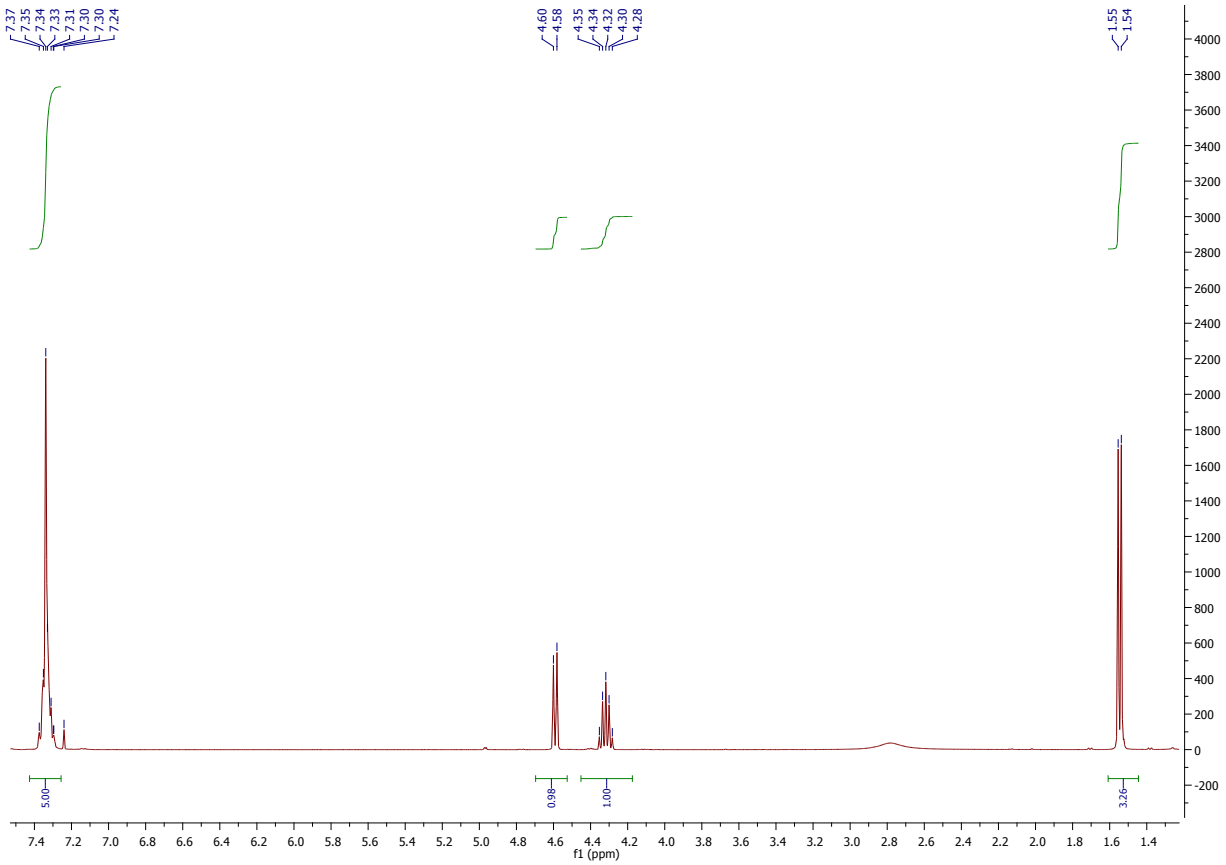
18.87
f1 (ppm)



2e

Syn-2-bromo-1-phenylpropan-1-ol was purified by flash column chromatography to give 78% isolated yield as a colorless liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.54-1.55 (d, 3H, *J* = 6.8 Hz), 2.75 (brs, 1H), 4.28-4.35 (m, 1H), 4.58-4.60 (d, 1H, *J* = 7.5 Hz), 7.30-7.38 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ:22.7, 58.3, 79.0, 126.7, 128,4, 128.5, 139.6. (in accordance with literature)[6]

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7.35
7.34
7.33
7.31
7.30
7.30
7.24
4.60
4.58
4.35
4.34
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4.30
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1.54
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1.00
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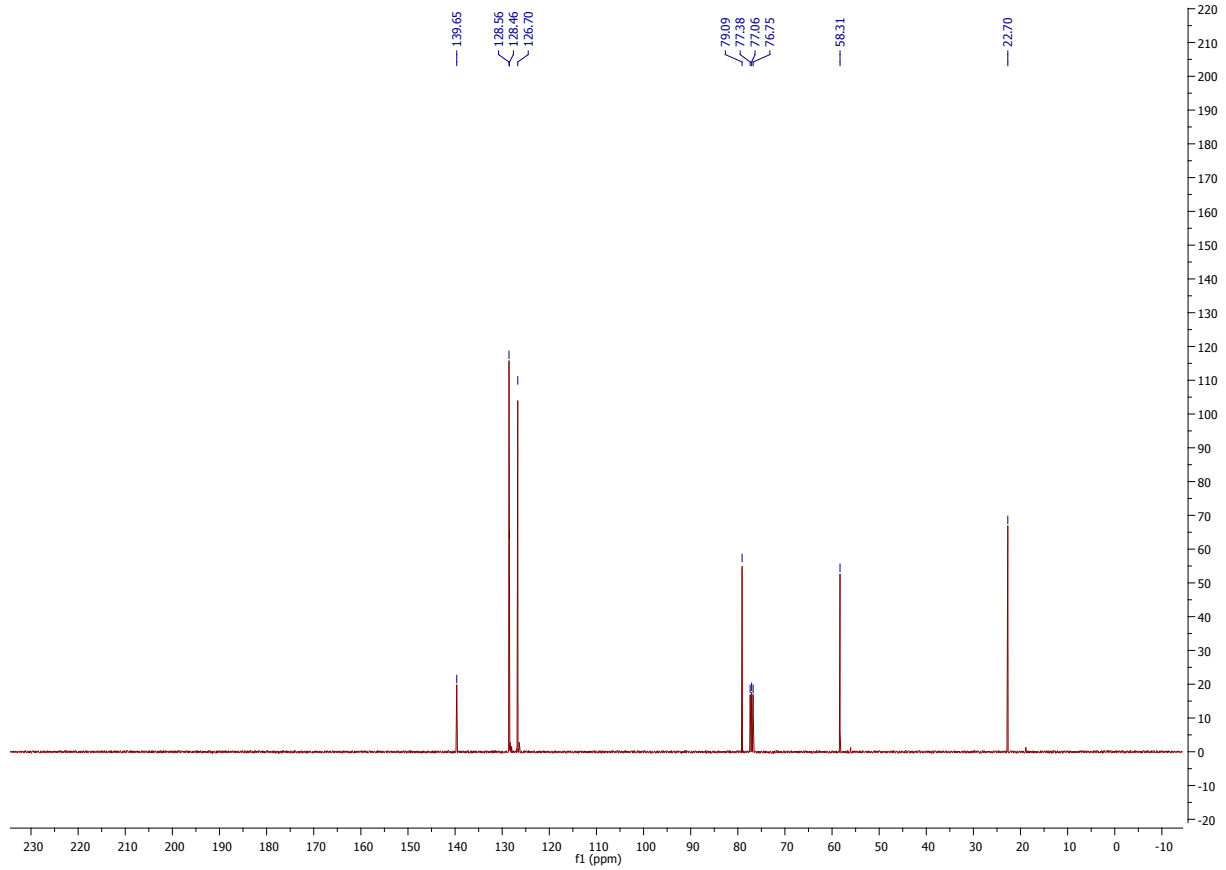
f1 (ppm)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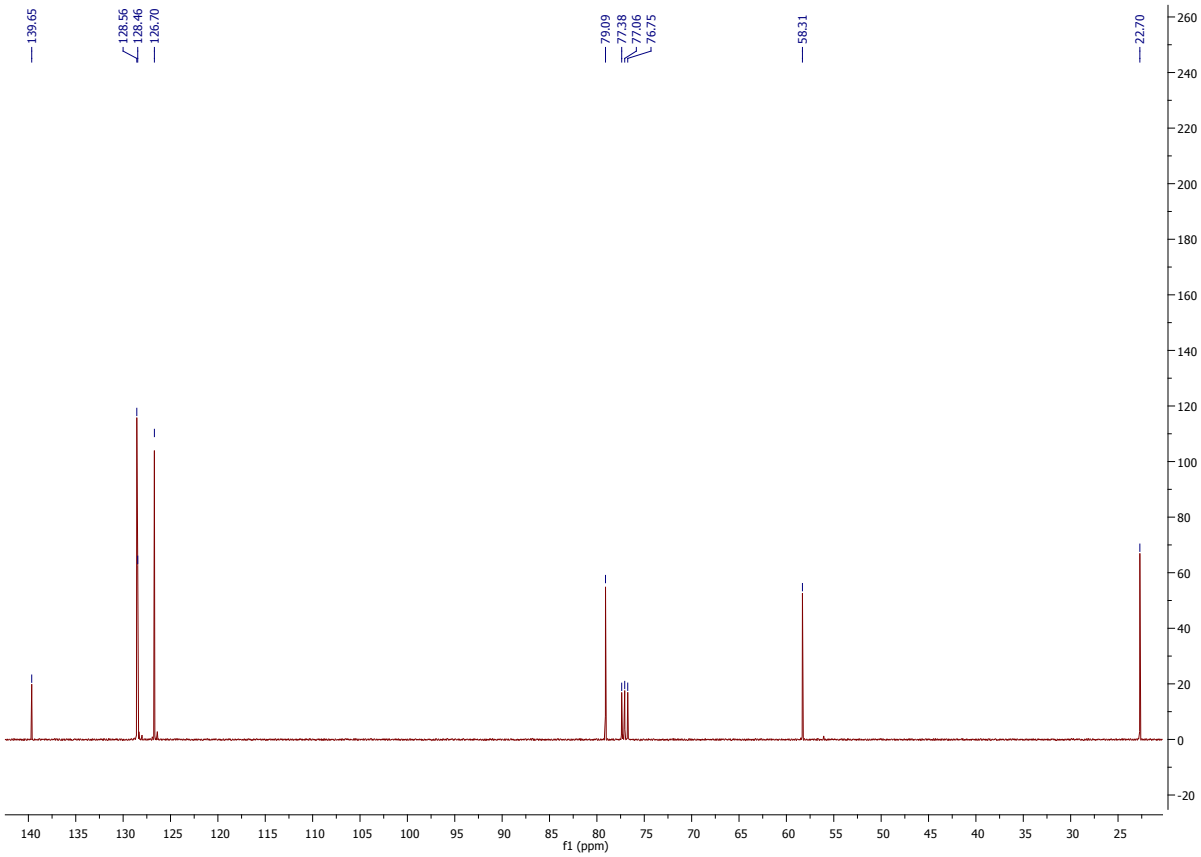
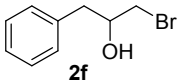
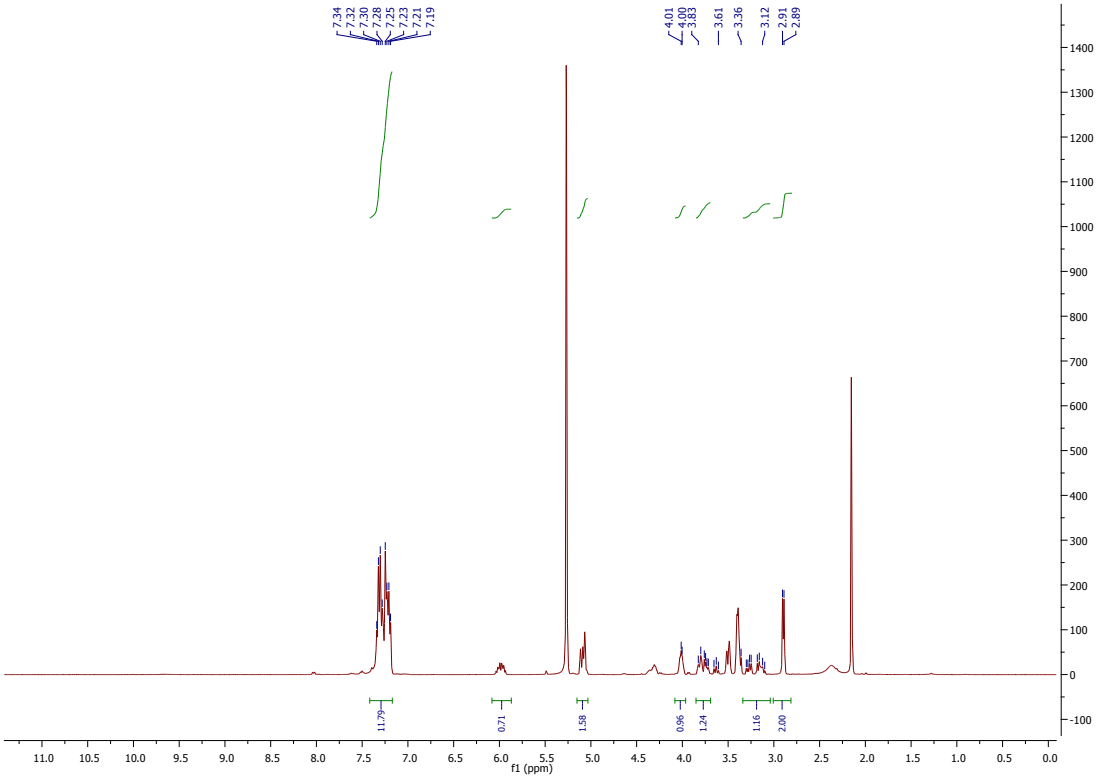
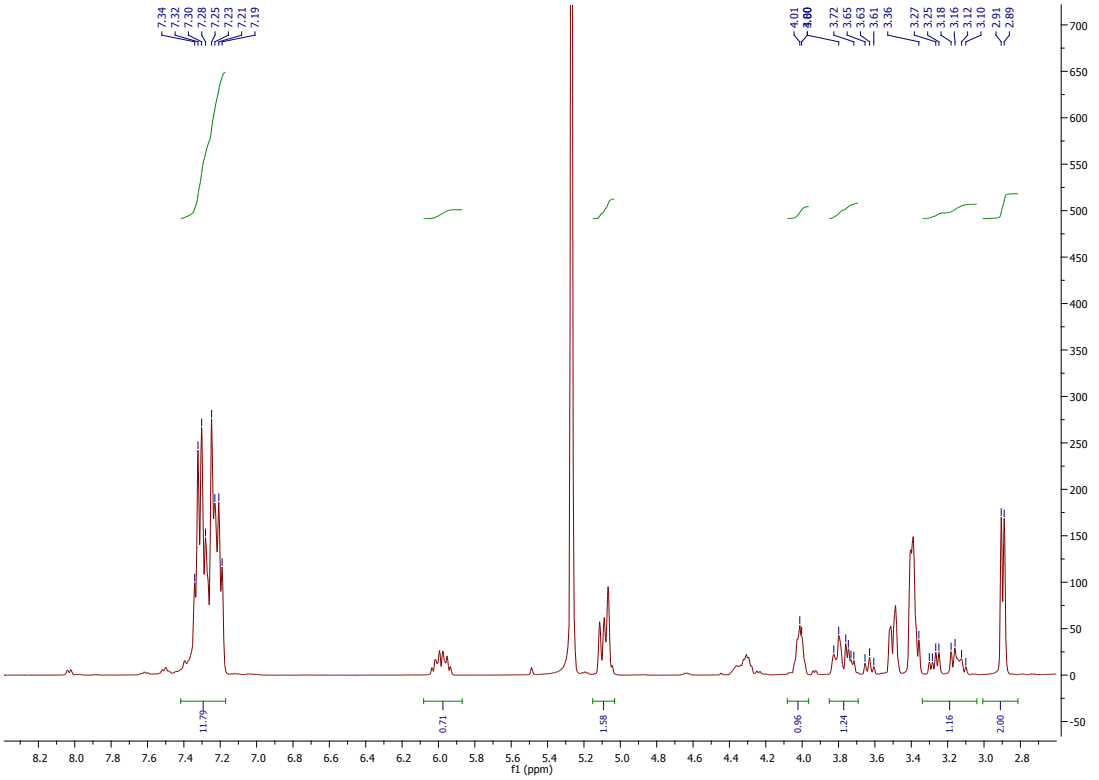
f1 (ppm)

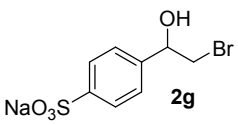




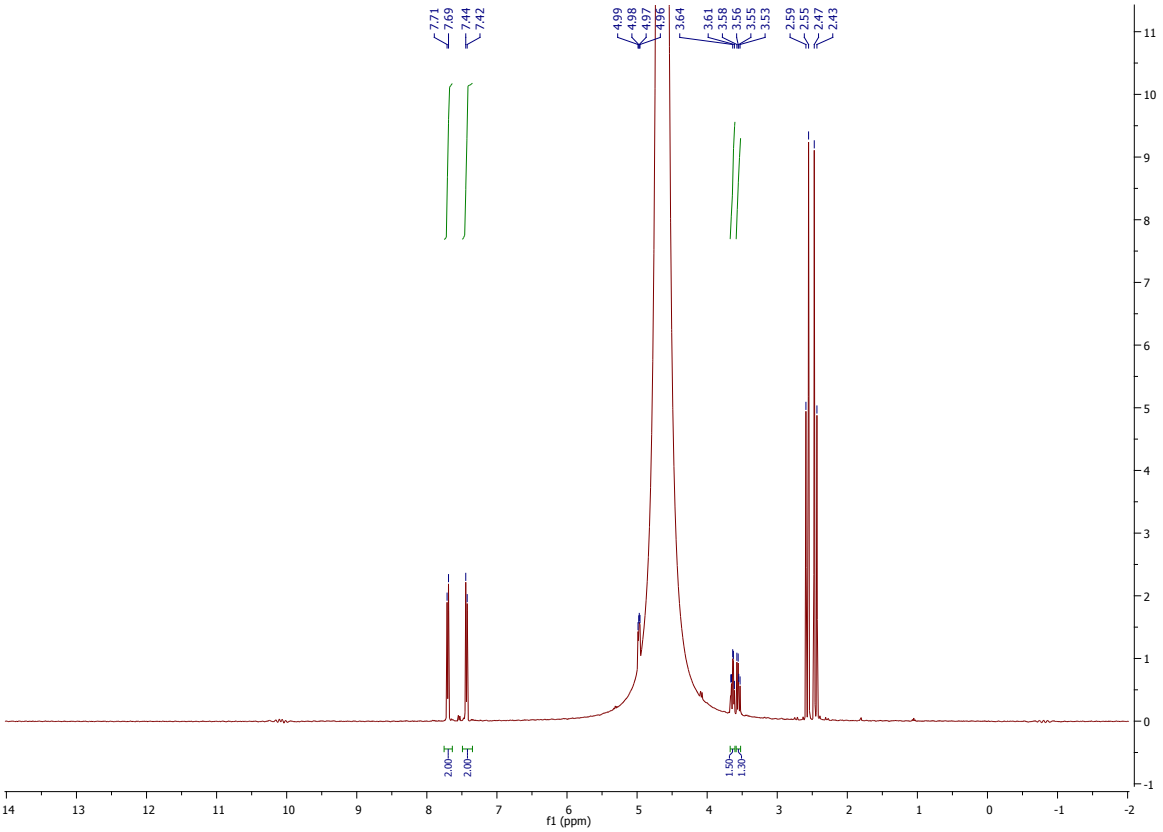
2f 1-bromo-3-phenylpropan-2-ol was identified by crude ^{1}H NMR sepctra. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.89-2.91 (d, 2H, *J*=6.4 Hz), 3.10-3.83 (m, 2H), 4.00 (m, 1H), 7.19–7.35 (m, 5H). (in accordance with literature)[7]







2g sodium 4-(2-bromo-1-hydroxyethyl)benzenesulfonate was identified by crude ^{1}H NMR spectra. ^{1}H NMR (400 MHz, D_2O) δ: 3.53-3.66 (2H, m), 4.96-4.99 (1H, dd, J= 4.2, 7.0 Hz), 7.42-7.44(2H, d, *J*= 8.2 Hz), 7.69-7.71 (2H, d, *J*= 8.2 Hz).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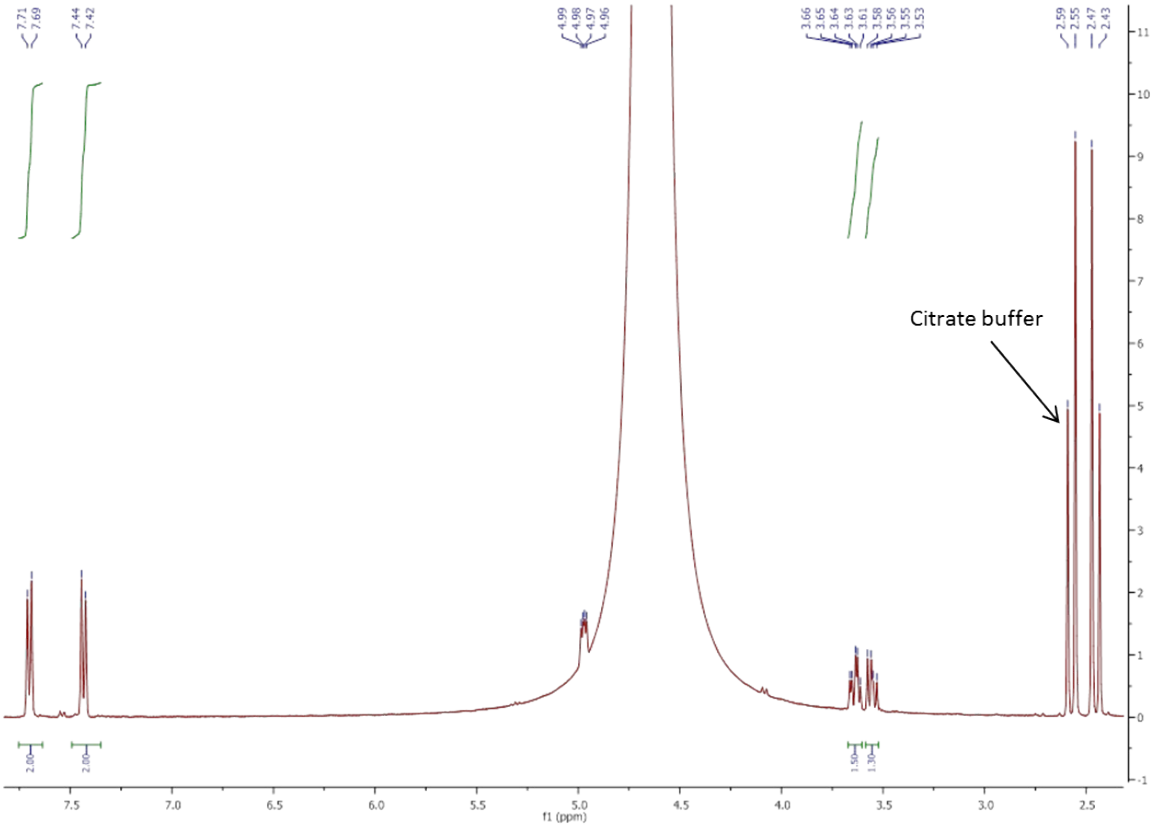
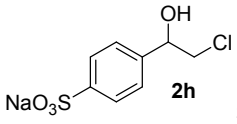
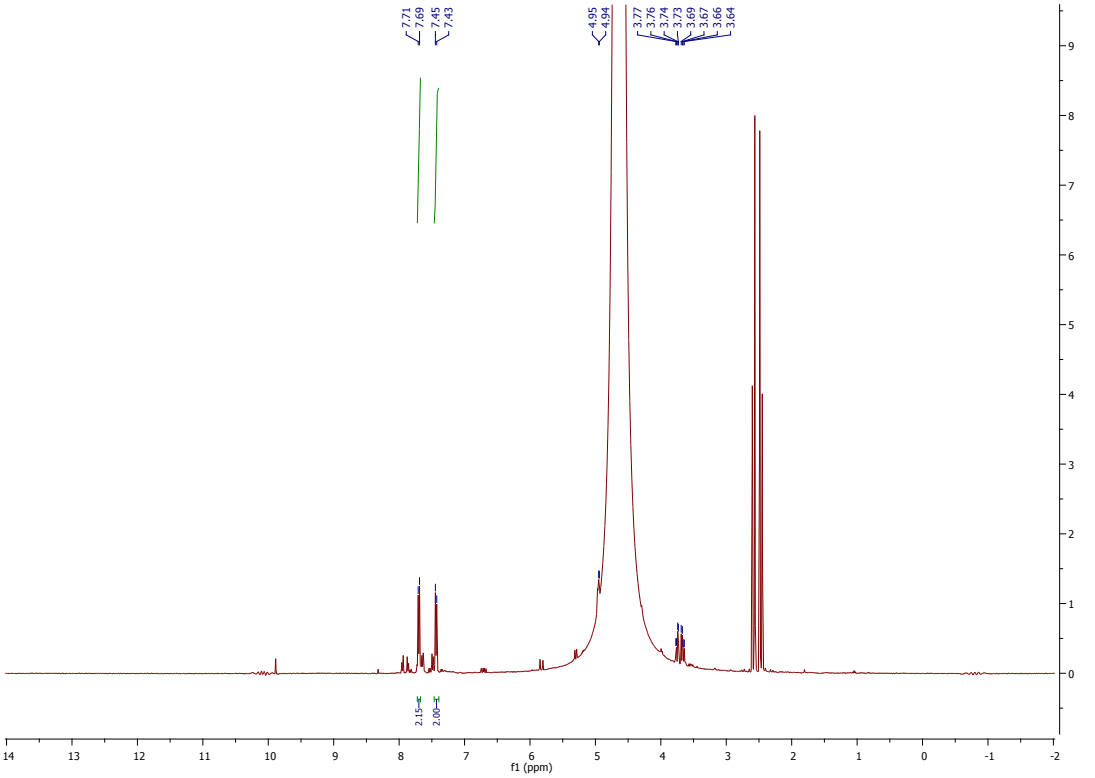
7.42
4.99
4.98
4.97
4.96
3.64
3.61
3.58
3.56
3.55
3.53
2.59
2.55
2.47
2.43
2.00
2.00
1.50
1.30
f1 (ppm)

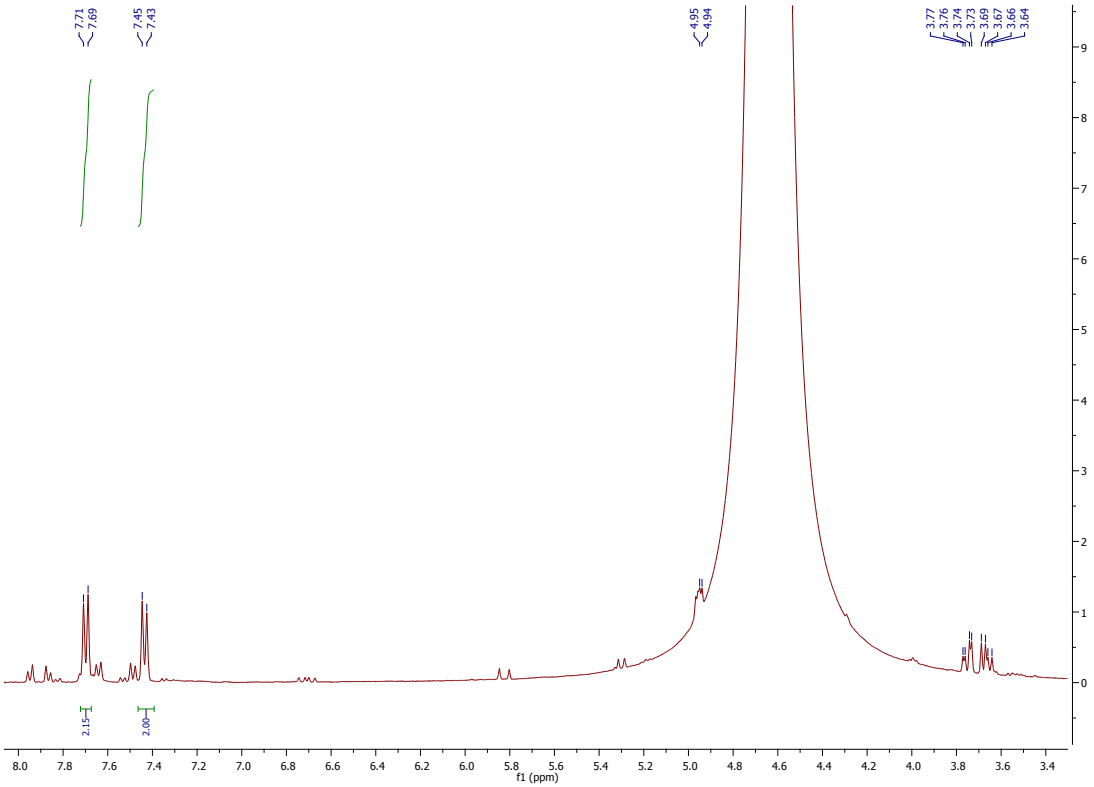

7.71
7.69
7.44
7.42
4.99
4.98
4.97
4.96
3.66
3.65
3.64
3.63
3.61
3.58
3.56
3.55
3.53
2.59
2.55
2.47
2.43
Citrate buffer
2.00
2.00
1.50
1.30
f1 (ppm)



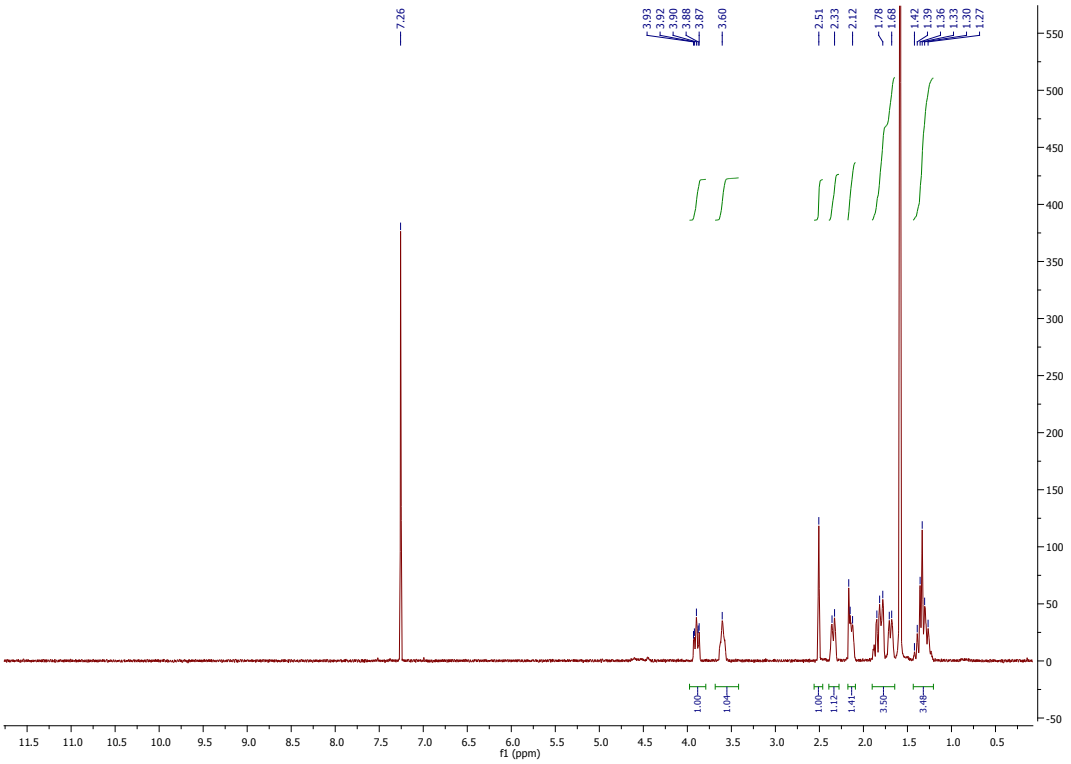
2h

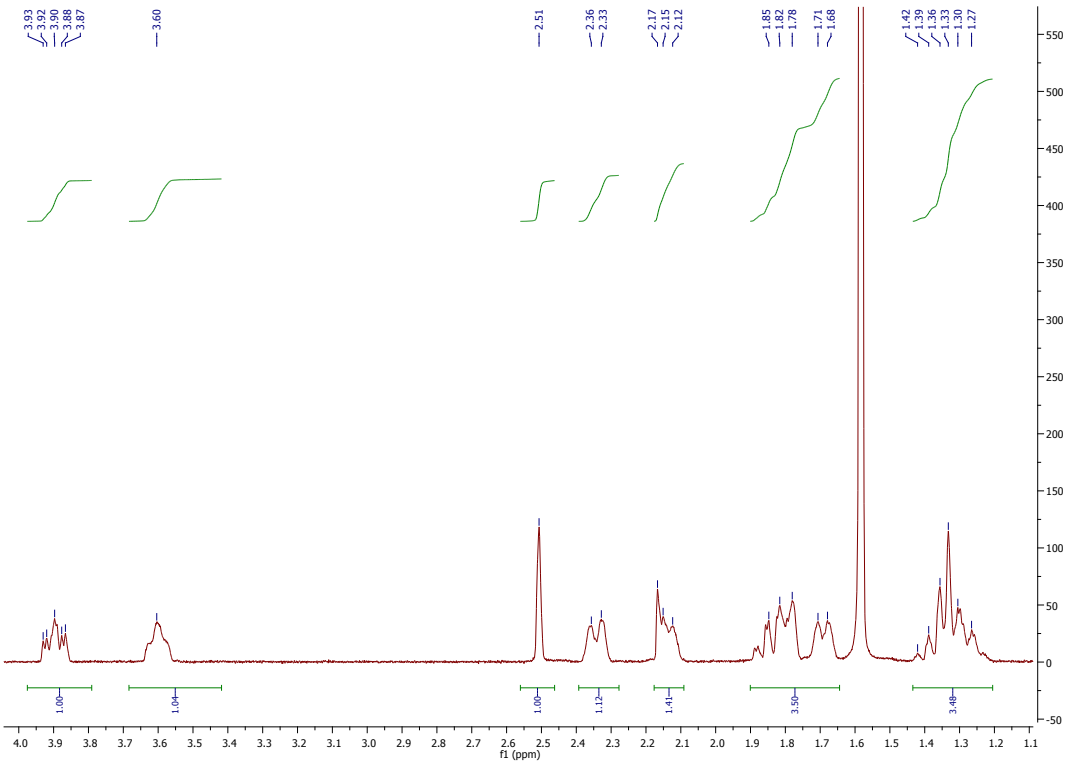
sodium 4-(2-chloro-1-hydroxyethyl)benzenesulfonate was identified by crude ^{1}H NMR spectra. ^{1}H NMR (400 MHz, D_2O) δ: 3.64-3.77 (2H, m), 4.94-4.95 (1H, dd, J= 4.2, 6.8Hz), 7.43-7.45 (2H, d, *J*= 8.2 Hz), 7.69-7.71 (2H, d, *J*= 8.2 Hz).

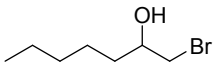




2i anti-2-bromocyclohexan-1-ol was identified by crude ^{1}H NMR spectra. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.27-1.42 (3H, m), 1.68-1.85 (3H, m), 2.12-2.17 (2H, m), 2.33-2.36 (2H, m), 2.51 (s, 1H), 3.60 (1H, m), 3.87-3.93 (1H, m). in accordance with literature)[8]

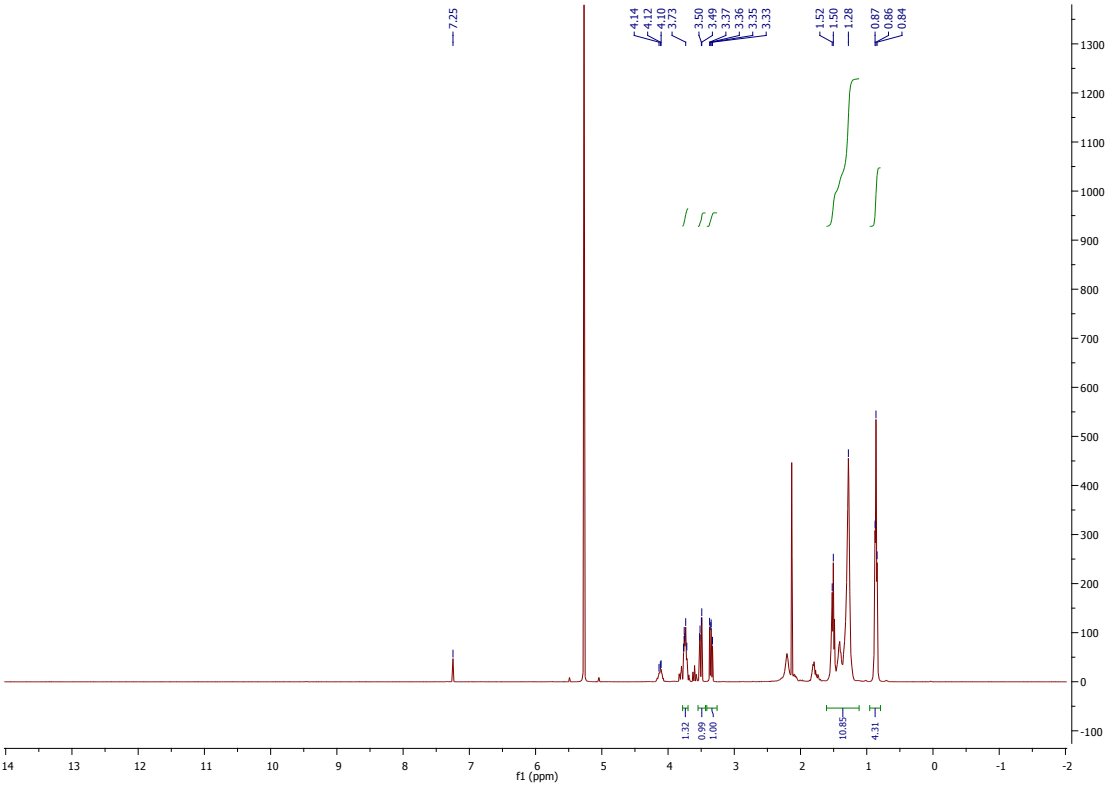


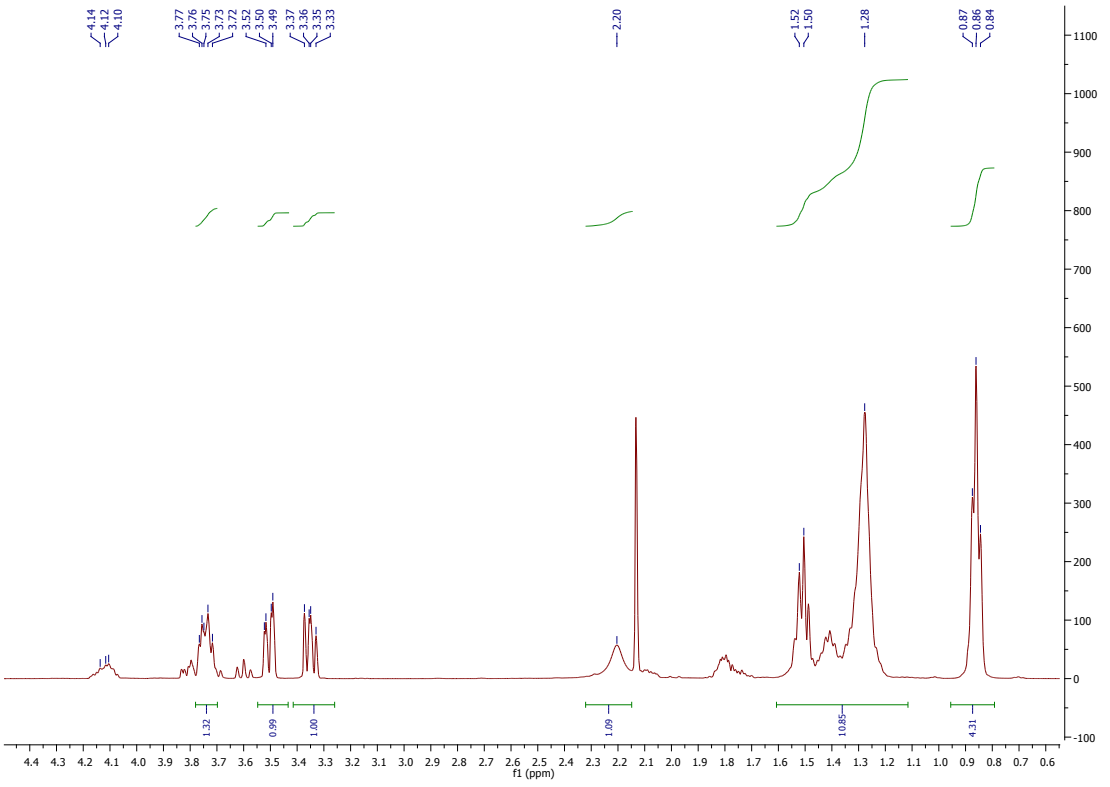


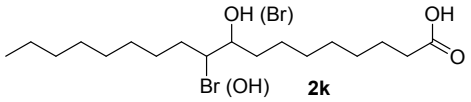


2j

1-bromoheptan-2-ol was identified by crude ^{1}H NMR spectra. ^{1}H NMR (400 MHz, CDCl3) δ: 0.84-0.87 (3H, m), 1.28-1.52 (m, 8H), 2.20 (brs, 1H), 3.33-3.37 (1H, m), 3.49-3.52 (1H, m), 3.72-3.77 (1H, m).

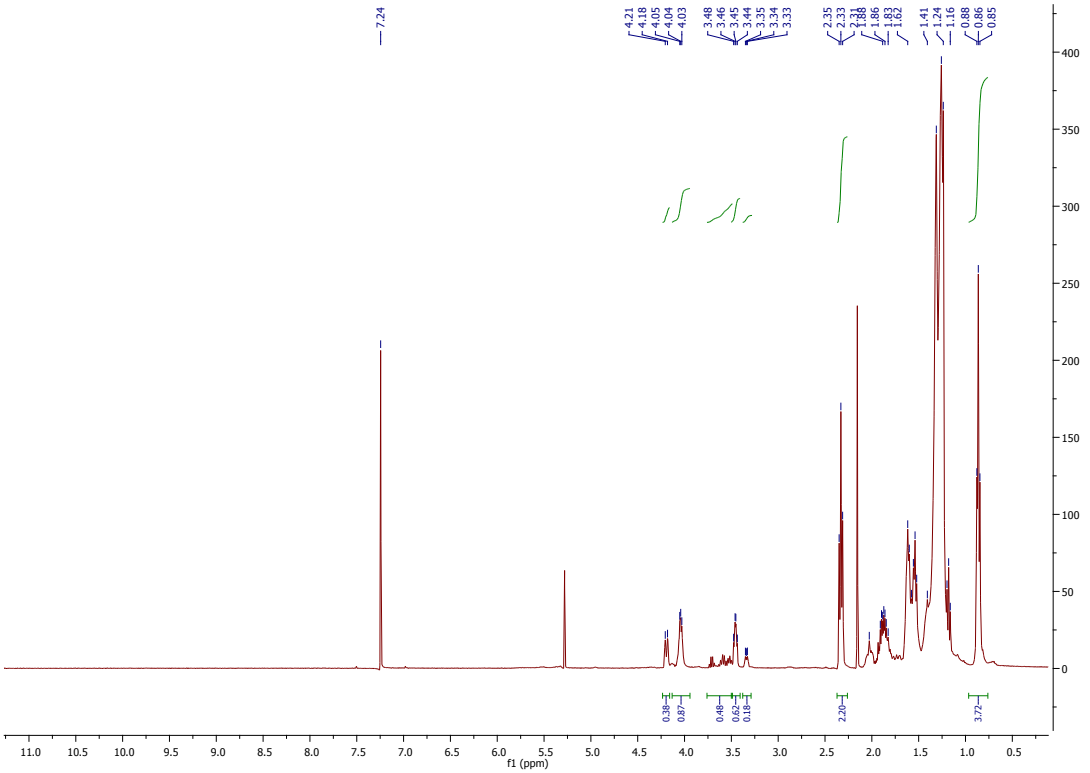


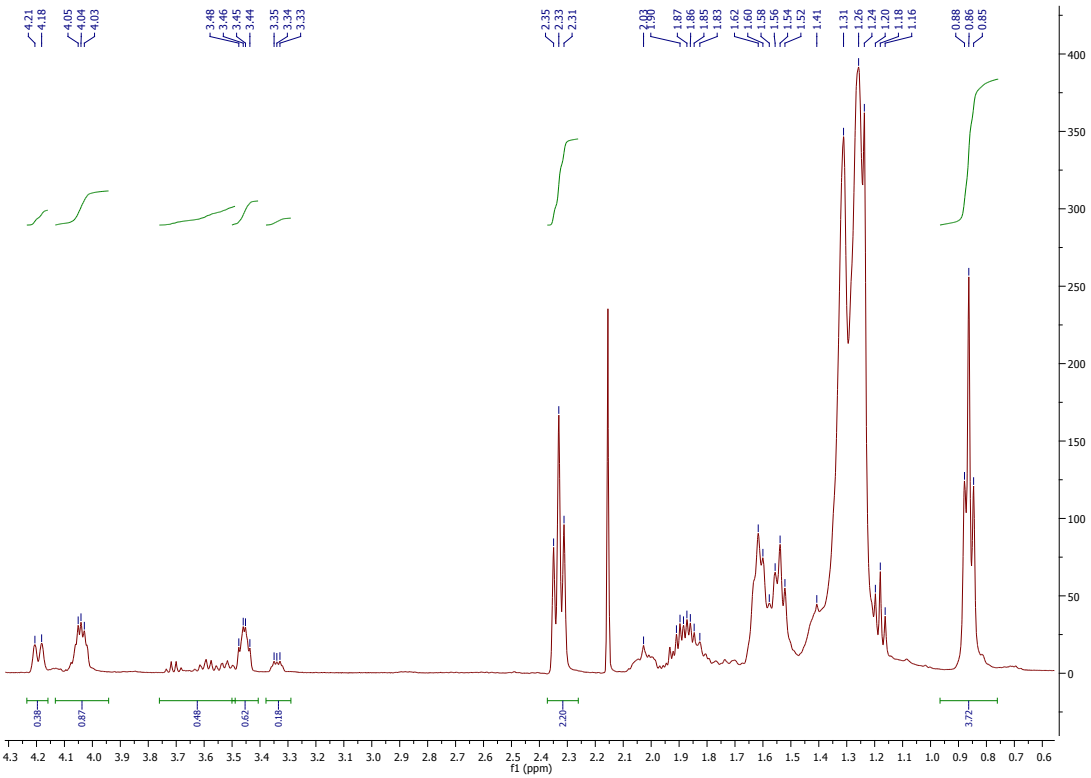




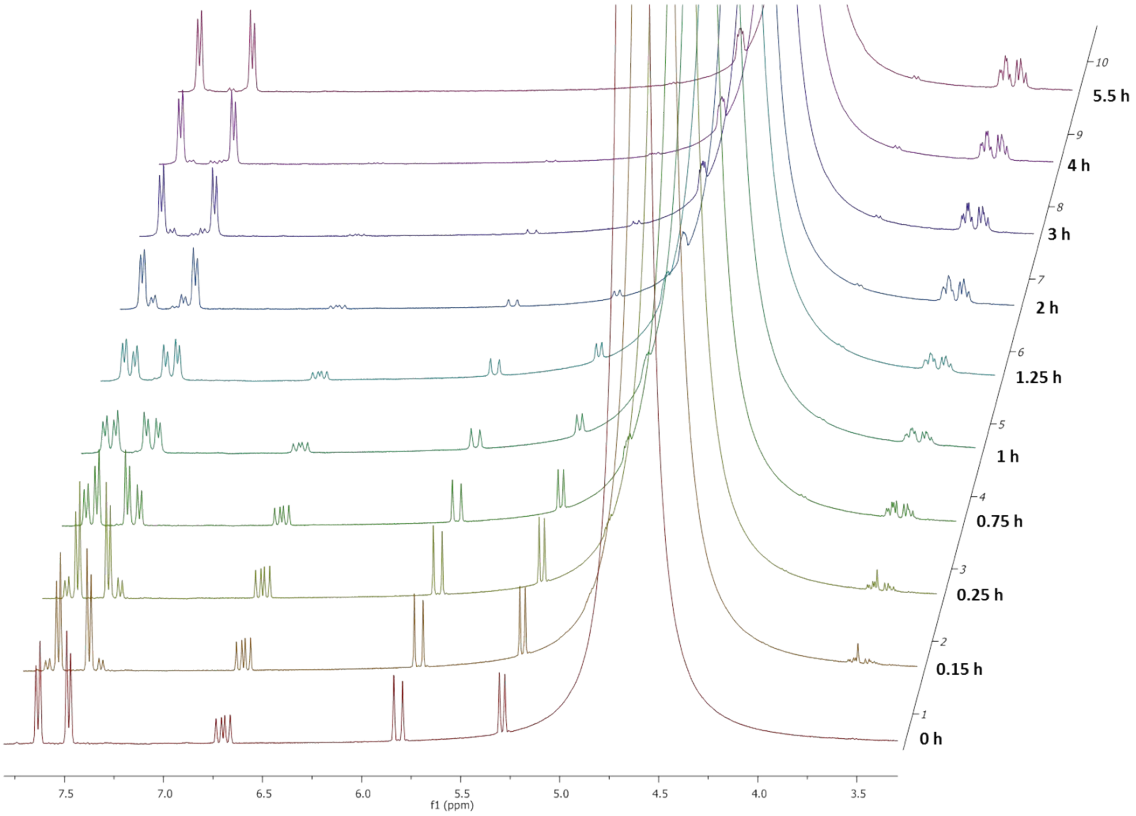
2k

10-bromo-9-hydroxyoctadecanoic acid and 9-bromo-10-hydroxyoctadecanoic acid was identified by crude ^{1}H NMR spectra. ^{1}H NMR (400 MHz, CDCl3) δ: 0.85 (3H, m), 1.16-1.87 (26H, m), 2.31-2.36 (2H, m), 3.33-3.35 (1H, m), 4.03-4.05 (1H, m).

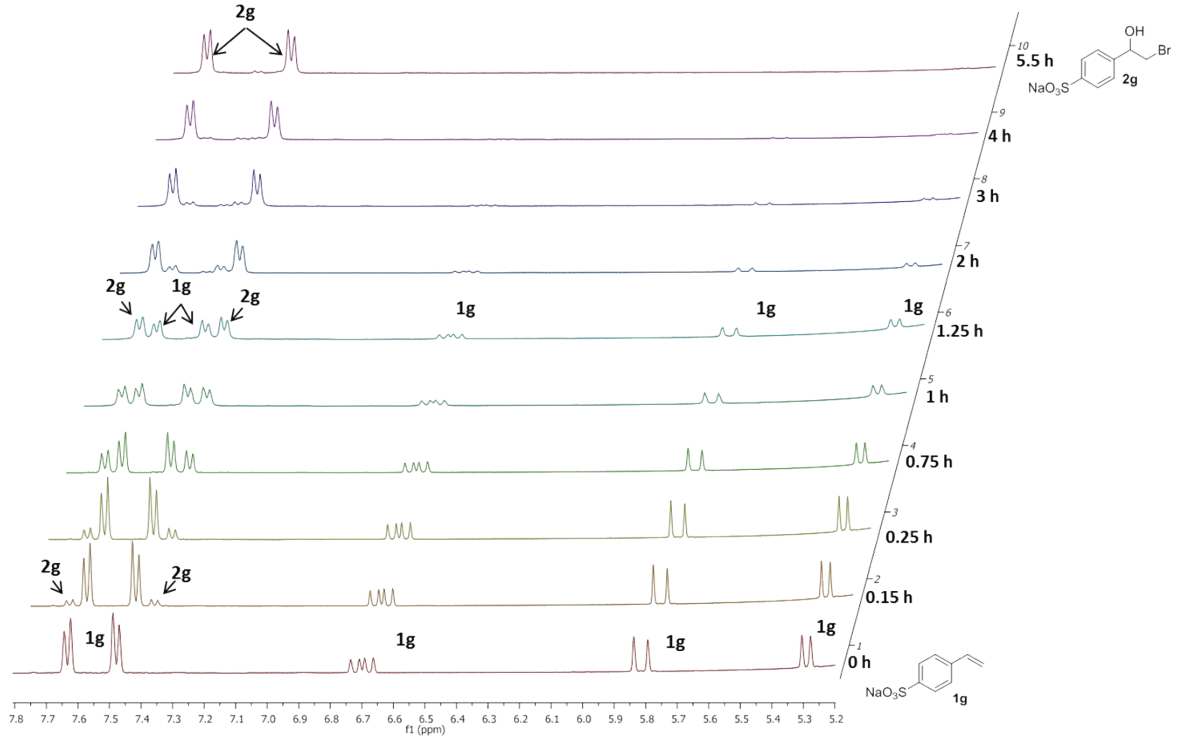




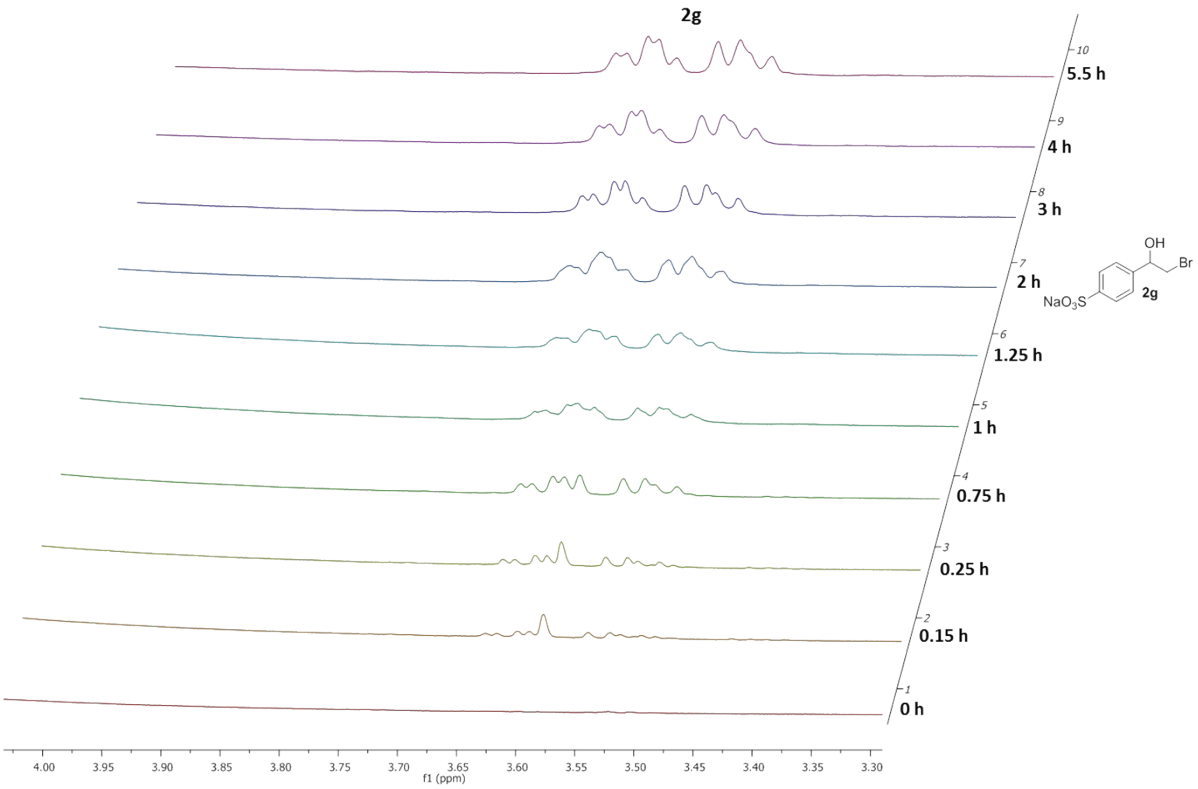
(6) ^{1}H NMR spectra for time course in Figure 2.



In aqueous medium (D_2O) 40 mM substrate 1g, 0.1 M citrate (pH5), 160 mM KBr, 170 mM H_2O_2, 100 nM CiVCPO was mixed at room temperature. The ^{1}H NMR spectra were measured in 7 hours followed by time.



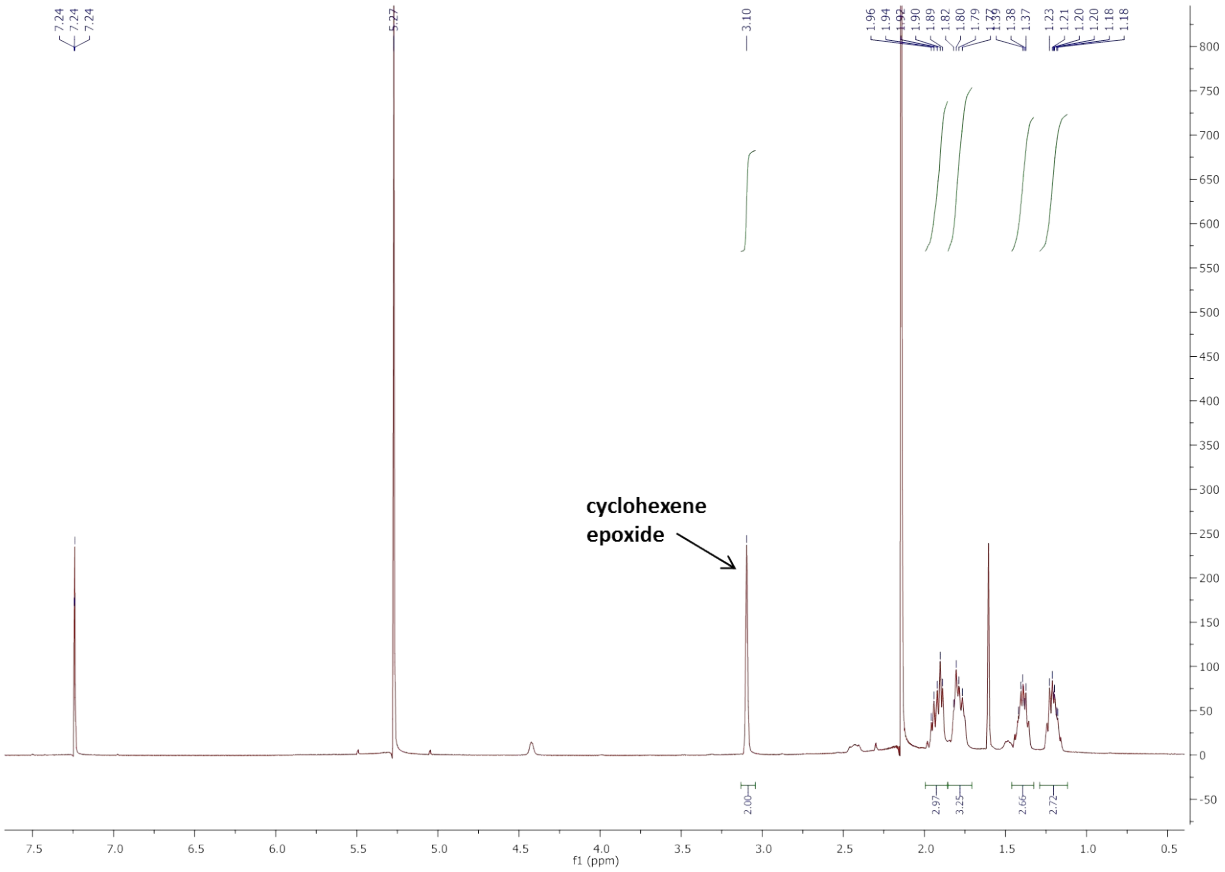
Zoom in ^{1}H NMR spectra from 5.2 ppm to 7.8 ppm.

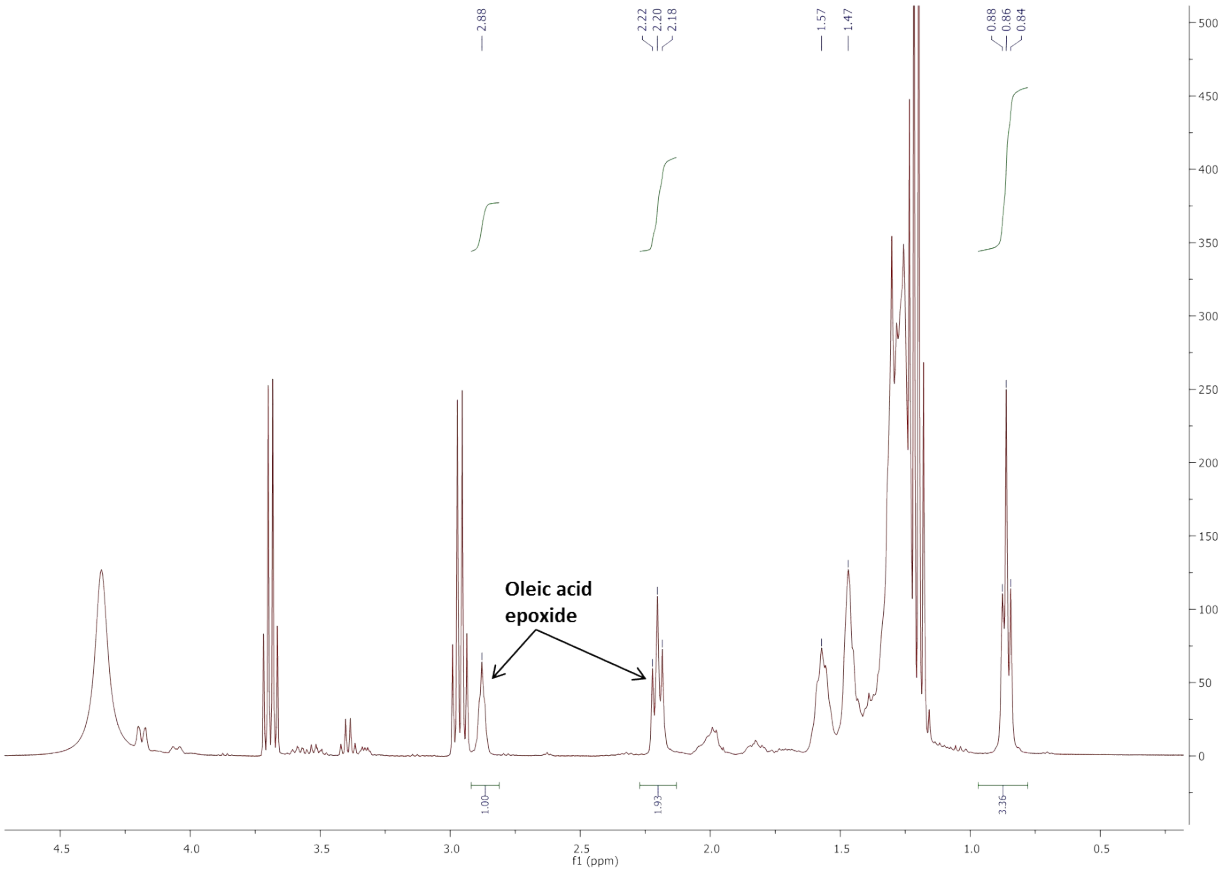


Zoom in ^{1}H NMR spectra from 3.3 ppm to 4.0 ppm.

(7) procedure of epoxide formation by *Ci*VCPO and base

In 0.1 M of citrate buffer pH 5 with 160 mM of KBr, 40 mM styrene was added, followed by 100 nM of vanadium-dependent chloroperoxidase from the fungus *Curvularia inaequalis* (*Ci*VCPO) and 170 mM of H_2O_2 were added while the solution is stirring at room temperature. After the reaction completed, base (NaOH or Et_3N) was added in situ to adjust pH to 10. The reaction solution was continuously stirring at room temperature for 16 hours. The reaction mixtures were extracted by dichloromethane, dried over anhydrous Na_2SO_4. The combined organic layers were concentrated in *vacuo*. Epoxides was determined by ^{1}H NMR spectra.


cyclohexene
epoxide
f1 (ppm)


Oleic acid
epoxide
f1 (ppm)

(8) preparation of *Ar*HHE

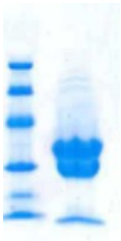
E. coli expression

The *Ar*HHE coding sequence was cloned into the expression vector PET15 and the resulting plasmids (pET15-HHDL) was used for expression. *Ar*HHE was produced in *E. coli* BL21(DE3). Cells were grown for 24 h in autoinduction media (2L of ZYM) at 25^{o}C.

Purification

Cells were harvested by centrifugation at 8000 rpm for 10 minutes at 4^{o}C. Bacteria were resuspended in 100 ml TEMG buffer (10 mM Tris-SO4, 1 mM EDTA, 1 mM b-mercaptoethanol, and 10% glycerol, pH 7.5), supplemented with lysozyme (2mg/ml) and DNAseI. After 0.5 hour of incubation, cells were sonicated and debris was removed by centrifugation at 10000 rpm for 0.5 hours.

*Ar*HHE was purified using an HPLC system, in one single step. The separation was performed on a Ni column 5-mL cartridge at a flow rate of 5 mL min^{-1}. After 20 mL, the retained proteins were eluted with a 0–50% imidazol gradient in 150 mL and 100% imidazol in 20 mL. The appropriate fractions were pooled, concentrated and dialyzed against TEMG buffer . The purification of the *Ar*HHE was confirmed by sodium dodecyl sulfate (SDS)–PAGE in 12% gels stained with Coomassie brilliant blue R-250 (Sigma).



Purification of *Ar*HHE: lane 1 standard (99 kDa, 66 kDa, 45 kDa, 30 kDa and 22 KDa); lane 2 purified *Ar*HHE.

(9) procedure of epoxide formation by *Ci*VCPO and *Ar*HHE

In 0.1 M of Britton-Robinson buffer pH 7 with 160 mM of KBr, 20 mM styrene, cyclohexene or oleic acid was added, followed by adding 100 nM of CiVCPO together with *Ar*HHE and 22 mM of H_2O_2 per hour were added for 5 hours while the solution is stirring at room temperature. The reaction mixtures were extracted by dichloromethane, dried over anhydrous Na_2SO_4. The combined organic layers were reduced in vacuo. Styrene oxide was obtained as major product.

(10) pH effect on the cascade process of *Ci*VCPO and *Ar*HHE

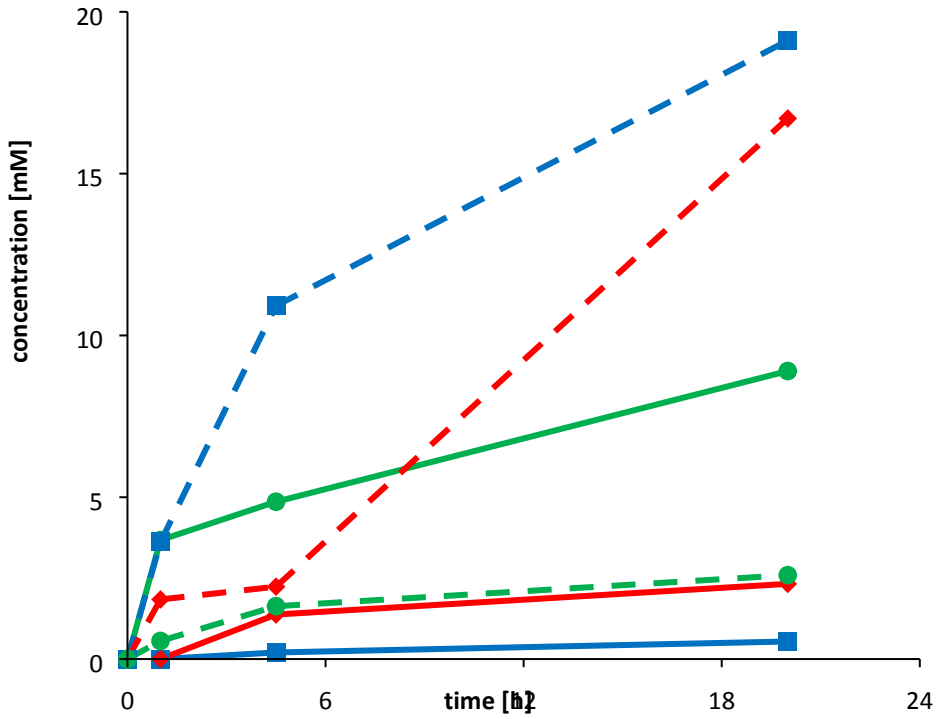


Figure S2. Time courses of the bienzymatic cascade reaction at pH 5 (○), pH 6 (◆) and pH 7 (ஜ). Halohydrin: dottet lines, epoxide: solid line. Reaction conditions: 80 mM buffer B&R, 20 mM styrene, 40 mM H_2O_2 , 40 mM KBr, 100 nM *Ci*VCPO and 5 µM *Ar*HHE in 2mL reaction at 30°C and 800 rpm.

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