Advanced Synthesis & Catalysis

Supporting Information

Regioselective Enzymatic β-Carboxylation of *p*-Hydroxystyrene Derivatives Catalyzed by Phenolic Acid Decarboxylases

Christiane Wuensch,[a,b,†] Tea Pavkov-Keller,[a,c,†] Georg Steinkellner,[a,c] Johannes Gross,[a,b]Michael Fuchs,[a]Altijana Hromic,[a,c] Andrzej Lyskowski,[a,c] Kerstin Fauland,[a,c] Karl Gruber,[c]Silvia M. Glueck[a,b]* and Kurt Faber[b]*

† These authors contributed equally to this work.

[a] Austrian Centre of Industrial Biotechnology, c/o

[b] Department of Chemistry, Organic & Bioorganic Chemistry, Heinrichstrasse 28, University of Graz, 8010 Graz, Austria
Fax: (+43)-316-380-9840; phone: (+43)-316-380-5332; Email: Si.Glueck@Uni-Graz.at, Kurt.Faber@Uni-Graz.at

[c] Institute of Molecular Biosciences, Humboldtstrasse 50, University of Graz, 8010 Graz, Austria

Supporting Information

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Protein mutagenesis

The amino acid residues which were mutated are highlighted in green. The following mutants have been prepared: FDC_Es Y19F, FDC_Es Y21F, FDC_Es Y39F, FDC_Es E72A.

Enzyme sequence of ferulic acid decarboxylase from *Enterobacter* sp. Px6-4 (FDC_Es):
MNTFDKHDLSGFVGKHLVYTYDNGWEYEIYVKNENTLDYRIHSGLVGNRWVKDQQ
AYIVRVGESIYKISWTEPTGTDVSLIVNLGDSLFHGTIFFPRWVMNNPEKTVCFQNDHI
PLMNSYRDAGPAYPTEVIDEFATITFVRDCGANNESVIACAASELPKNFPDNLK

CDS:
ATGAACACCTTCGATAAACATGATCTGAGCGGTTTTGTTGGTAAACATCTGGTGT
ATACCTATGATAATGGCTGGGAGTATGAGATCTATGTGAAAAATGAAAACACCCT
GGATTATCGCATTCATAGCGGTCTGGTTGGTAATCGTTGGGTTAAAGATCAGCAG
GCATATATTGTTCGTGTGGGTGAAAGCATCTATAAAATCAGCTGGACCGAACCGA
CCGGCACCGATGTTAGCCTGATTGTTAATCTGGGTGATAGCCTGTTTCATGGCACC
ATCTTTTTTCCGCGTTGGGTGATGAATAATCCGGAAAAAACCGTTTGCTTTCAGAA
CGATCATATTCCGCTGATGAATAGCTATCGTGATGCAGGTCCGGCATATCCGACC
GAAGTTATTGATGAATTTGCCACCATTACCTTTGTTCGTGATTGTGGTGCAAATAA
CGAAAGCGTTATTGCATGTGCAGCAAGCGAACTGCCGAAAAACTTTCCGGATAAT
CTGAAATAA

Tyrosine (Y): **TAT**/TAC
Phenylalanine (F): **TTT**/TTC
Glutamic acid (E): GAG/**GAA**
Alanine (A): **GCG**/GCT/GCC/GC

SDS Pages to verify overexpression of enzyme variants

Successful overexpression[1,2] of soluble enzyme was obtained for all mutants of ferulic acid decarboxylase from *Enterobacter* sp. (FDC_Es). The mutated amino acid residues are the following (see also above): Y19F, Y21F, Y39F, E72A. Enzyme preparations with and without induction using IPTG are shown.

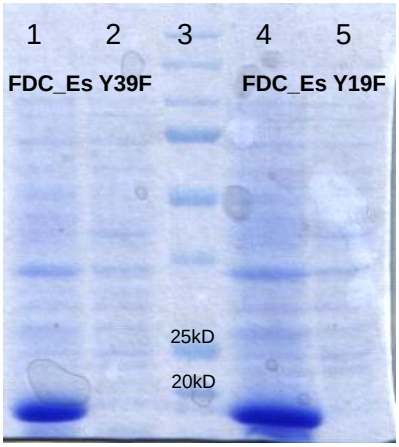


Figure S1: SDS-PAGE analysis of enzyme variants FDC_Es Y19F and FDC_Es Y39F.
Lane 1: FDC_Es Y39F mutant with IPTG induction; lane 2: FDC_Es Y39F mutant without induction; lane 3: Precision Plus Protein Standard All Blue Standard (5 µL, BIORAD); lane 4: FDC_Es Y19F mutant with IPTG induction; lane 5: FDC_Es Y19F mutant without induction.

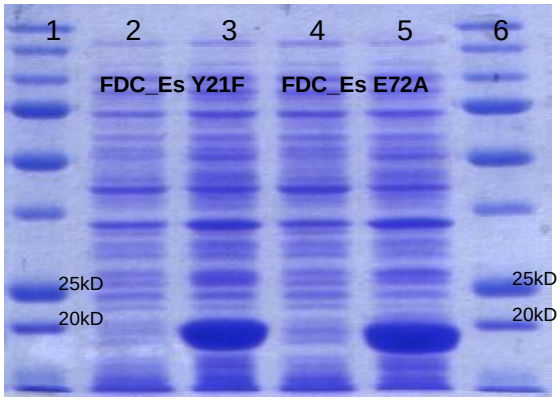


Figure S2: SDS-PAGE analysis of enzyme variants FDC_Es Y21F and FDC_Es E72A.
Lane 1/6: Precision Plus Protein Standard All Blue Standard (5 µL, BIORAD); lane 2: FDC_Es Y21F mutant without IPTG induction; lane 3: FDC_Es Y21F mutant with IPTG induction; lane 4: FDC_Es E72A mutant without IPTG induction; lane 5: FDC_Es E72A mutant with IPTG induction.

Crystal structure determination

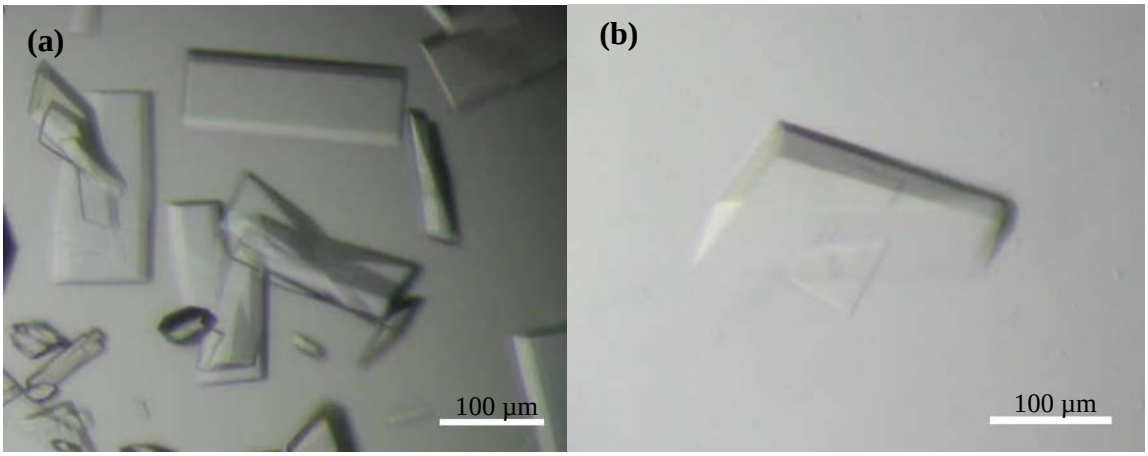


Figure S3. Typical crystals of ferulic acid decarboxylases from *Enterobacter* sp. (FDC_Es); (a) FDC_Es WT (wild type) and (b) FDC_Es E72A variant.

Table S1. Data collection and refinement statistics.

	FDC_Es native	**FDC_Es E72A**
Data collection		
Space group	P 2 2_12_1	P 2 2_12_1
a, b, c (Å)	39.4, 83.8, 133.3	39.21, 83.9, 132.0
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	50-1.13 (1.20-1.13)	41.9 – 1.49 (1.59 -1.49)
R_{merge}(%)	7.4 (70.3)	10.4 (71.4)
I/δ	14.2 (2.2)	11.8 (2.4)
Completeness (%)	99.7 (98.4)	98.7 (93.2)
Multiplicity	9.1 (7.2)	8.4 (7.8)
Refinement		
Resolution (Å)	50-1.15	41.9-1.49
No. reflections	157334	71216
R_{work}/R_{free}	0.12/0.13	0.16/0.18
No. atoms		
Protein	5943	3302
Water	533	478
Mean B factor	13.54	20.99
Rmsd		
Bond lengths (Å)	0.007	0.006
Bond angles (°)	0.998	1.030
Ramachandran plot		
Most favored residues (%)	97.1	96.8
Outliers (%)	0	0
PDB	4UU3	4UU2

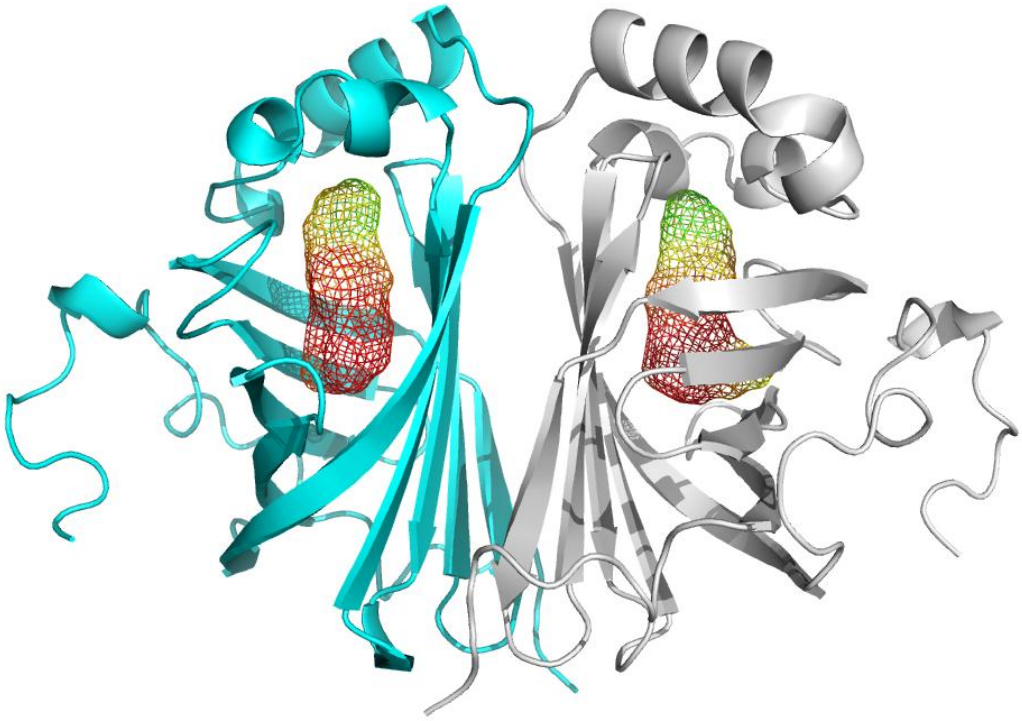


Figure S4. Overall dimeric structure of FDC_Es. The structure shows a lipocalin-like fold, which is common for this enzyme family. The cavity in the active site, as calculated by Casox,[3] is show in mash presentation and colored according to hydrophobicity (red – more hydrophobic, green – more hydrophilic). Individual monomers are shown in ribbon representation and are colored in cyan and grey. The figure was prepared using the program PyMOL (Schrodinger Inc.).

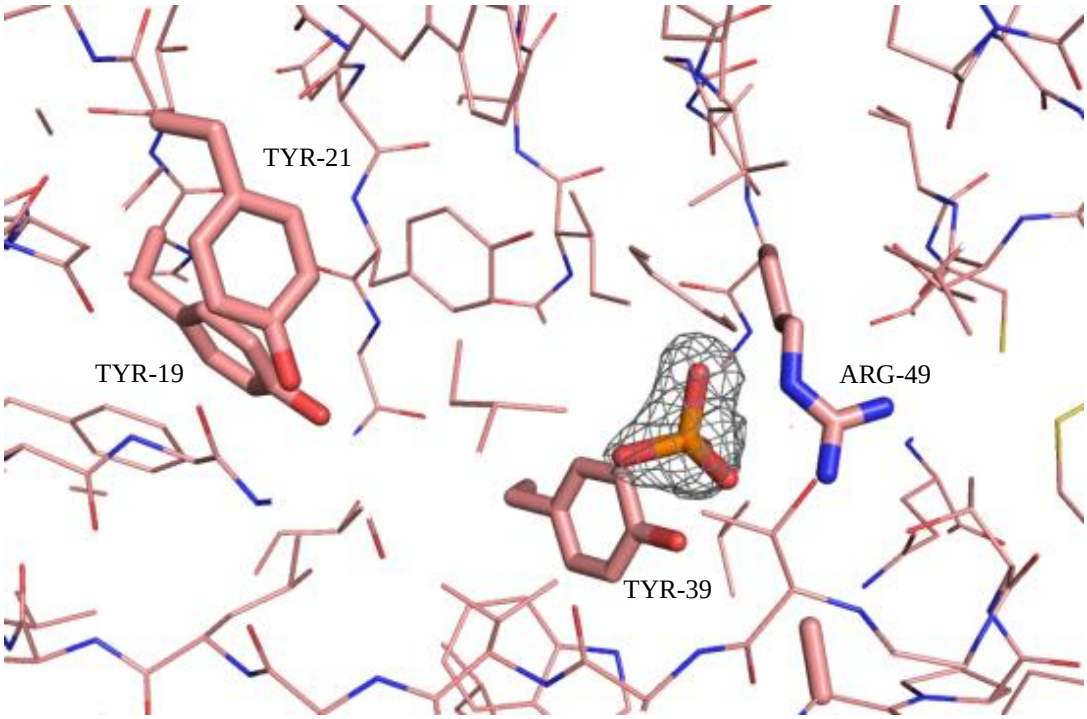


Figure S5. Electron density map. Fo-Fc omit density (grey, contoured at 3σ) in the active site of FDC_Es E72A variant. The orientation of the active site corresponds to that shown in Figure 2. The bound $[PO_4]^{3-}$ (or $[SO_4]^{2-}$) is shown is orange. The catalytically important amino acid residues are shown in stick representation and are labeled. The figure was prepared using the program PyMOL (Schrodinger Inc.).

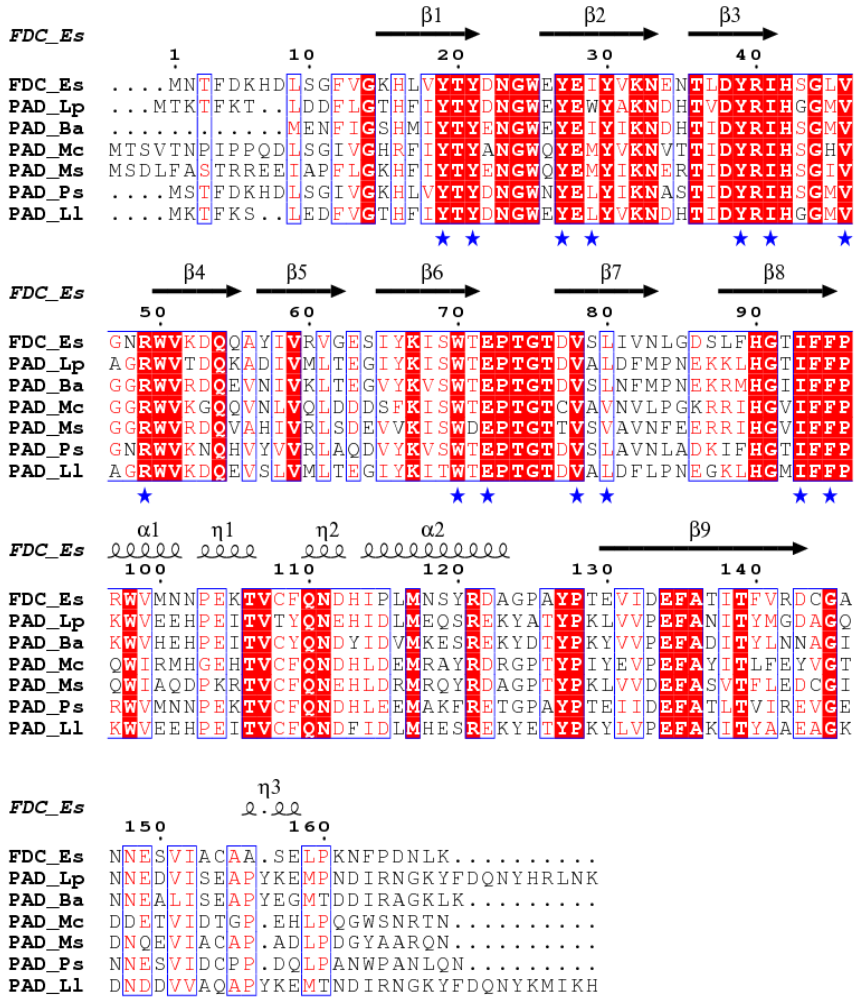
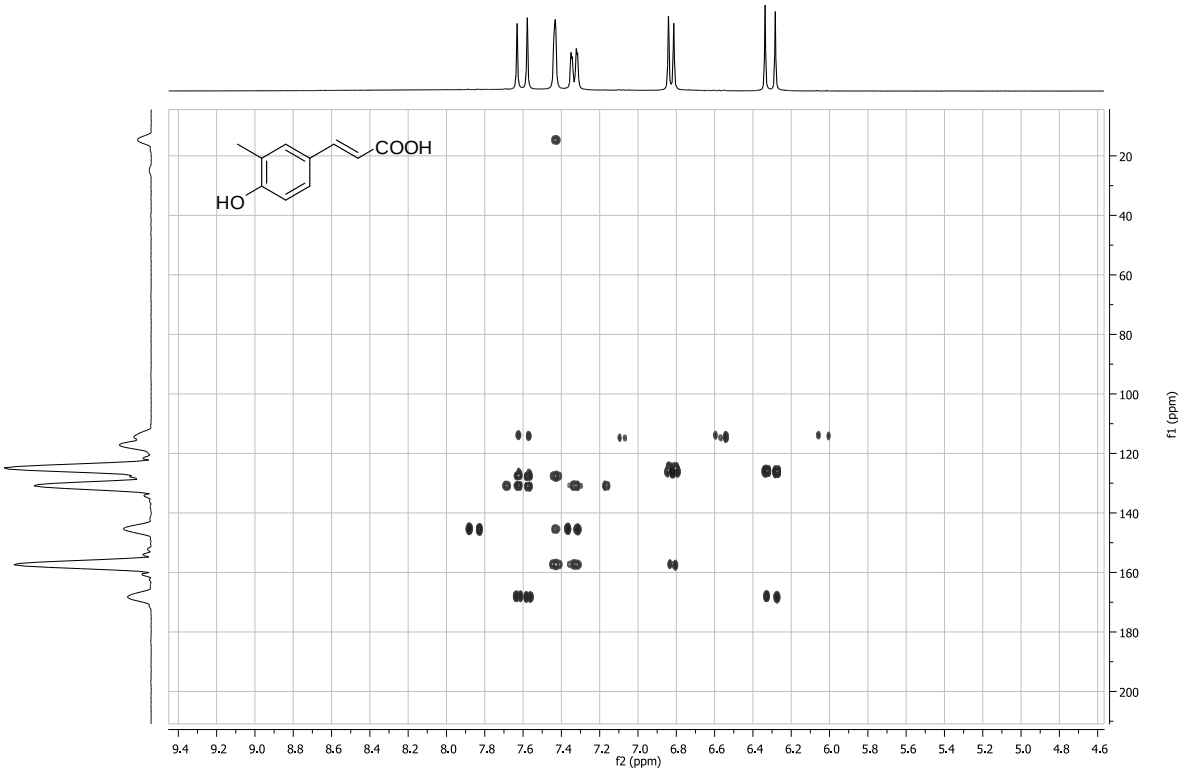


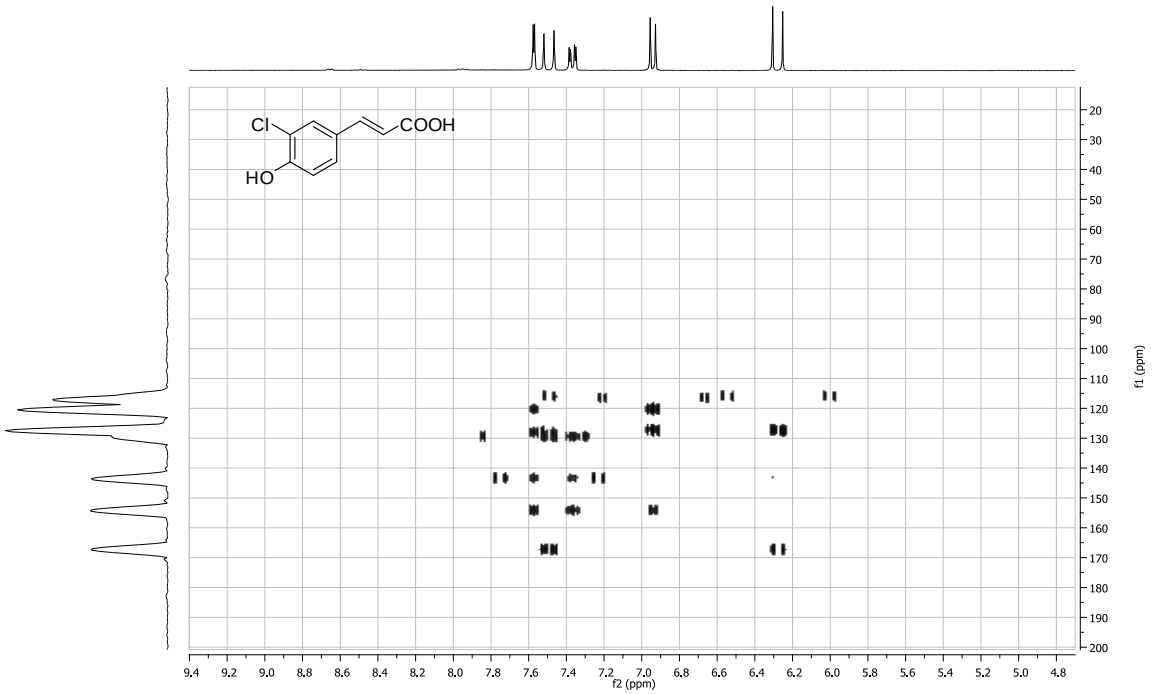
Figure S6. Sequence alignment of investigated PADs and FDC_Es (see Table 2). Residues situated in the active site are indicated with a blue star. The only variations can be observed for the residues in position 29 and 80 (referring to FDC_Es). Position 80 accommodates a hydrophobic residue: Leu or Val, respectively, whereas position 29 shows the most variation accommodating Ile, Trp, Met or Leu. The secondary structure elements are derived from the FDC_Es structure.

HMBC-NMR spectra

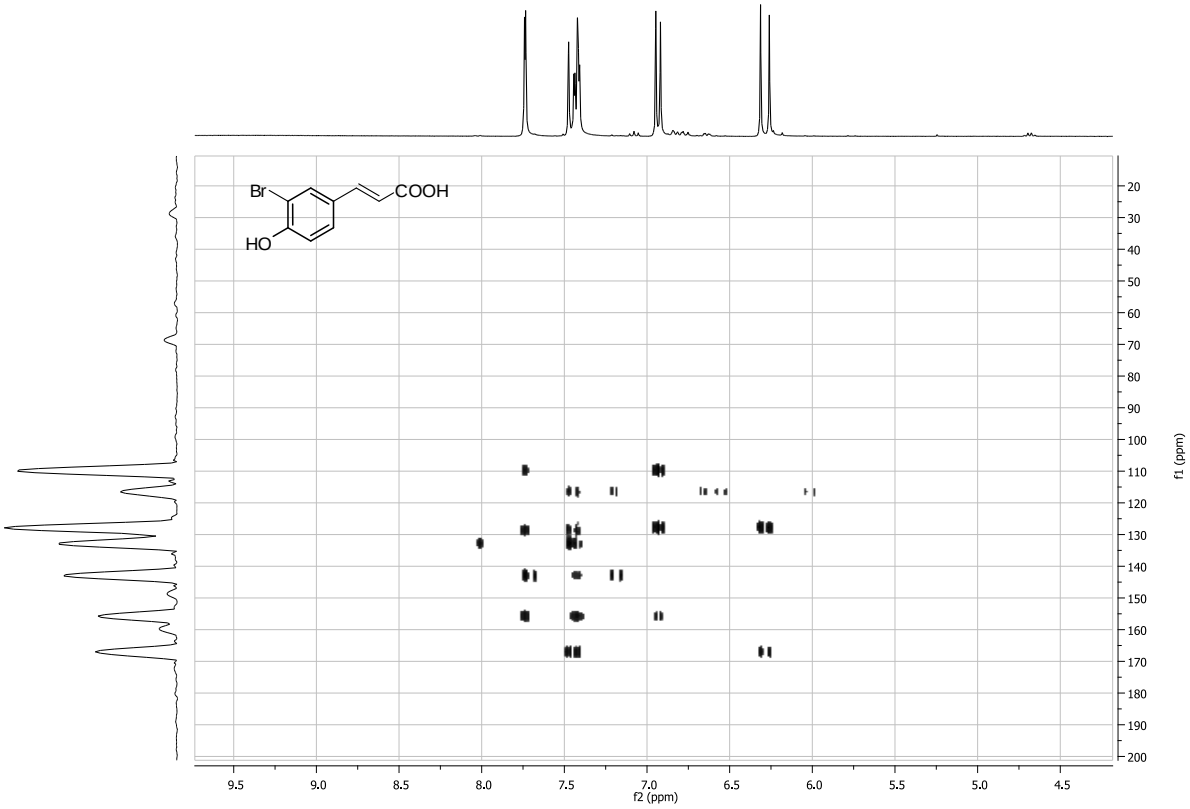
(*E*)-3-(4-Hydroxy-3-methylphenyl)acrylic acid (4b)



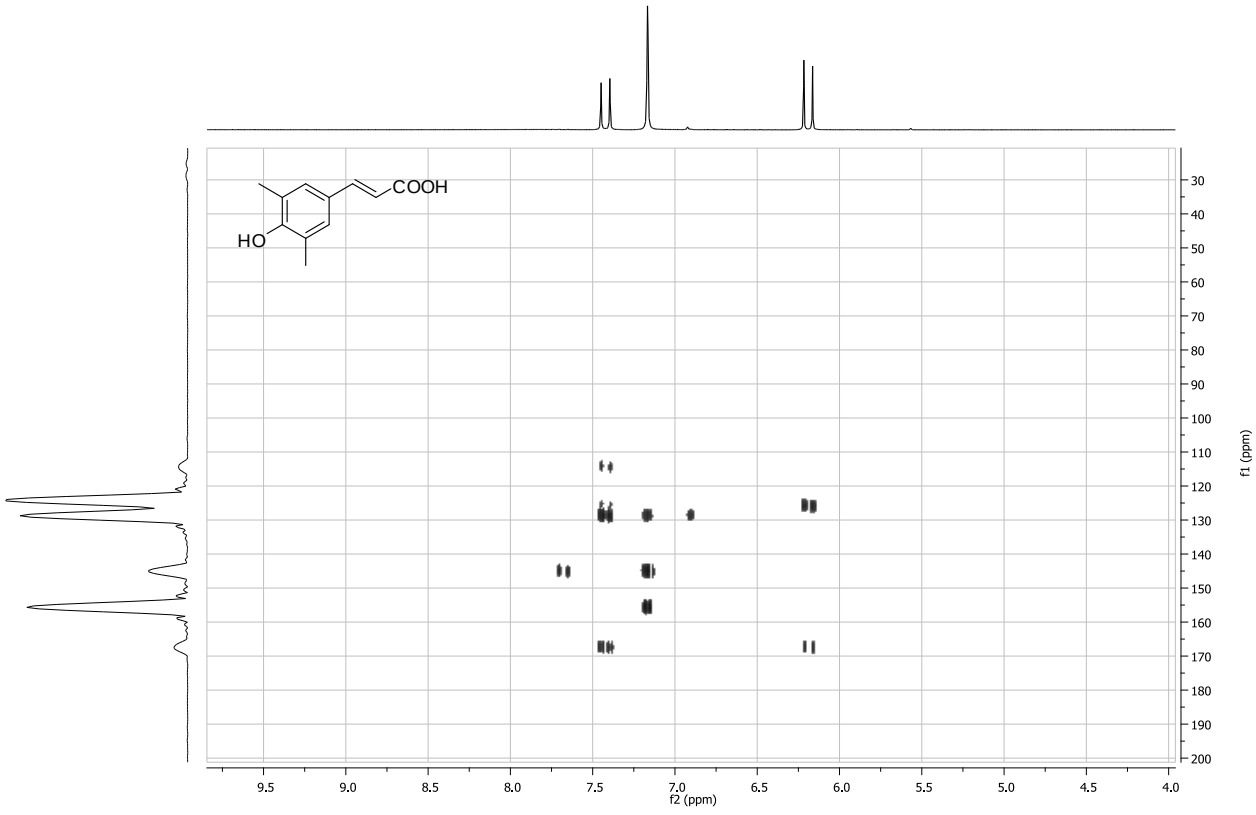
(*E*)-3-(3-Chloro-4-hydroxyphenyl)acrylic acid(5b)



(*E*)-3-(3-Bromo-4-hydroxyphenyl)acrylic acid(6b):

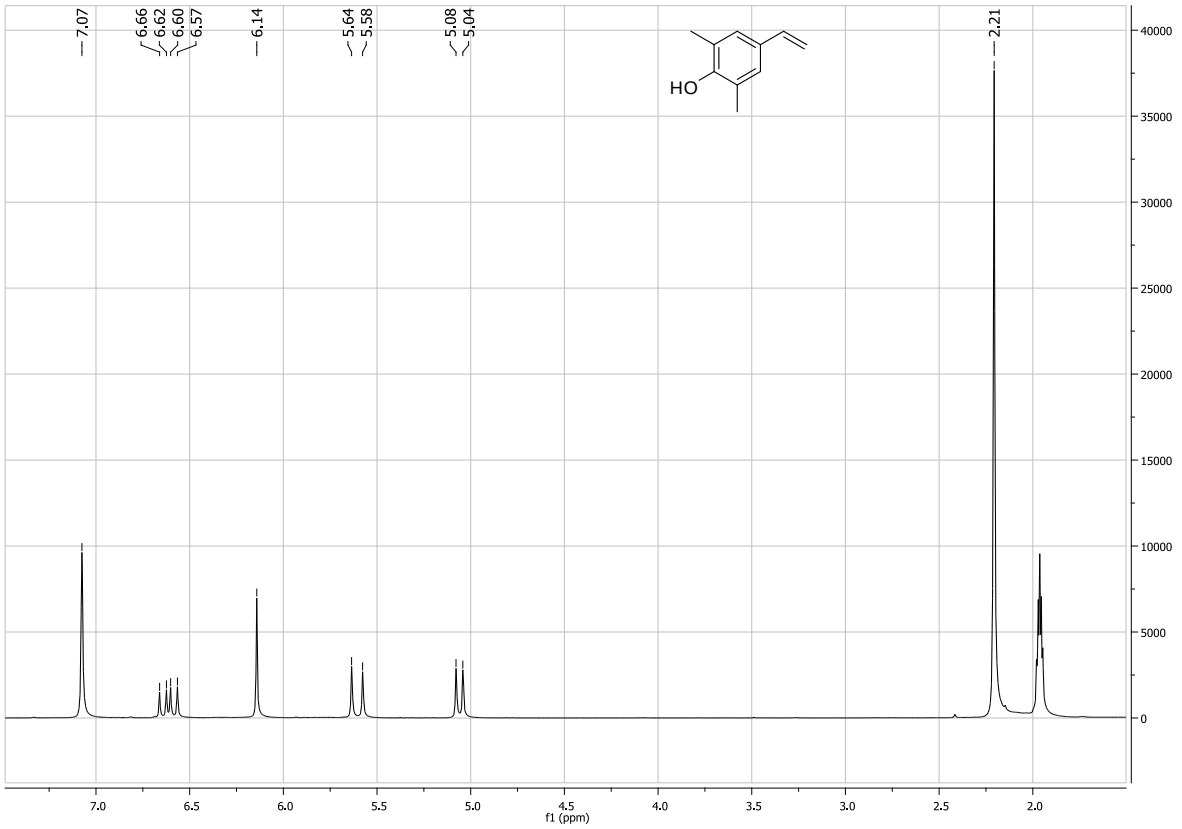


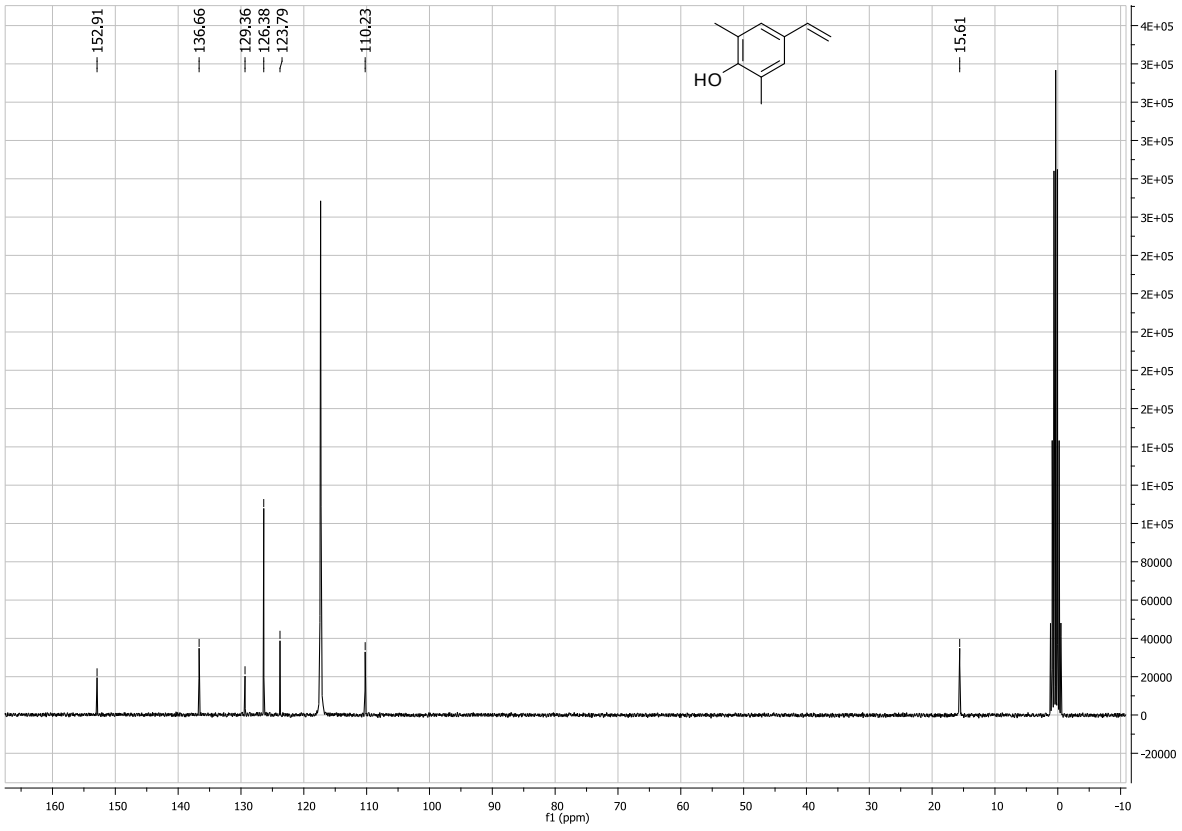
(*E*)-3-(4-Hydroxy-3,5-dimethylphenyl)acrylic acid(7b)



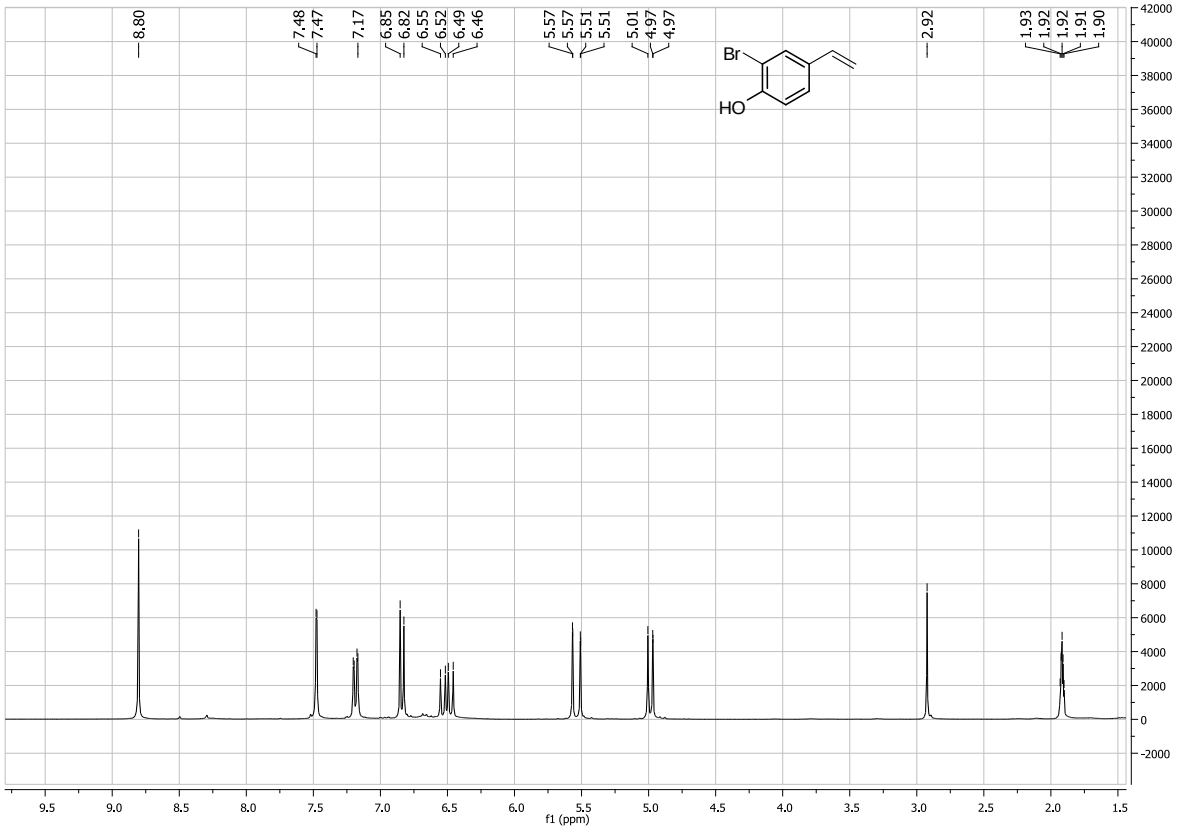
^{1}H and ^{13}C-NMR spectra

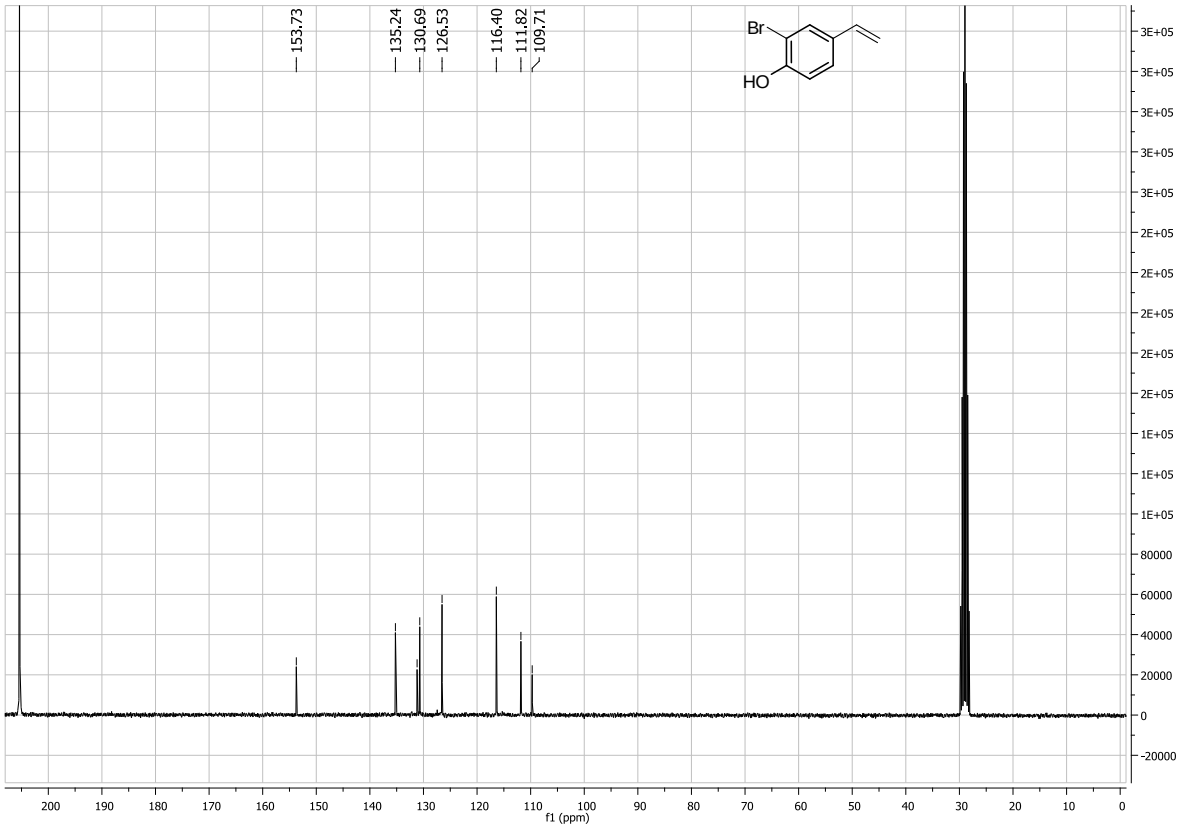
2,6-Dimethyl-4-vinylphenol (7a)



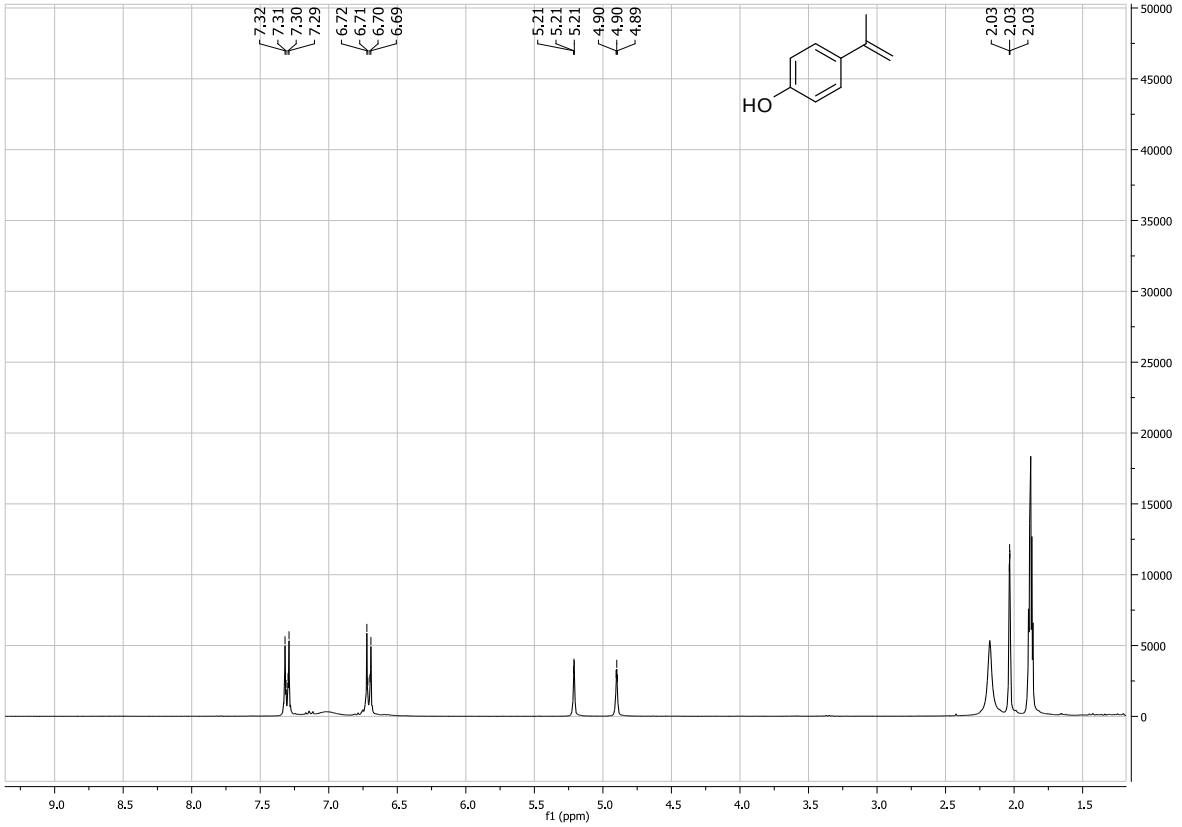


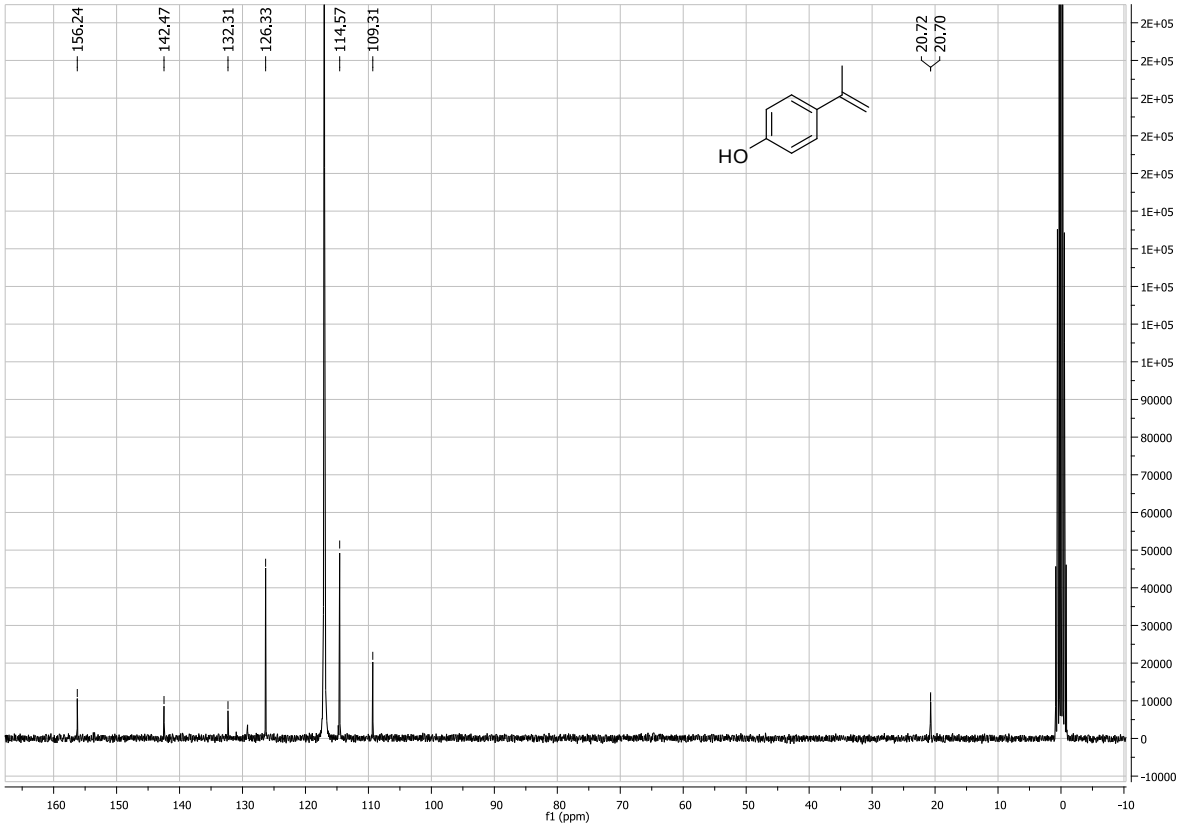
2-Bromo-4-vinylphenol (6a)



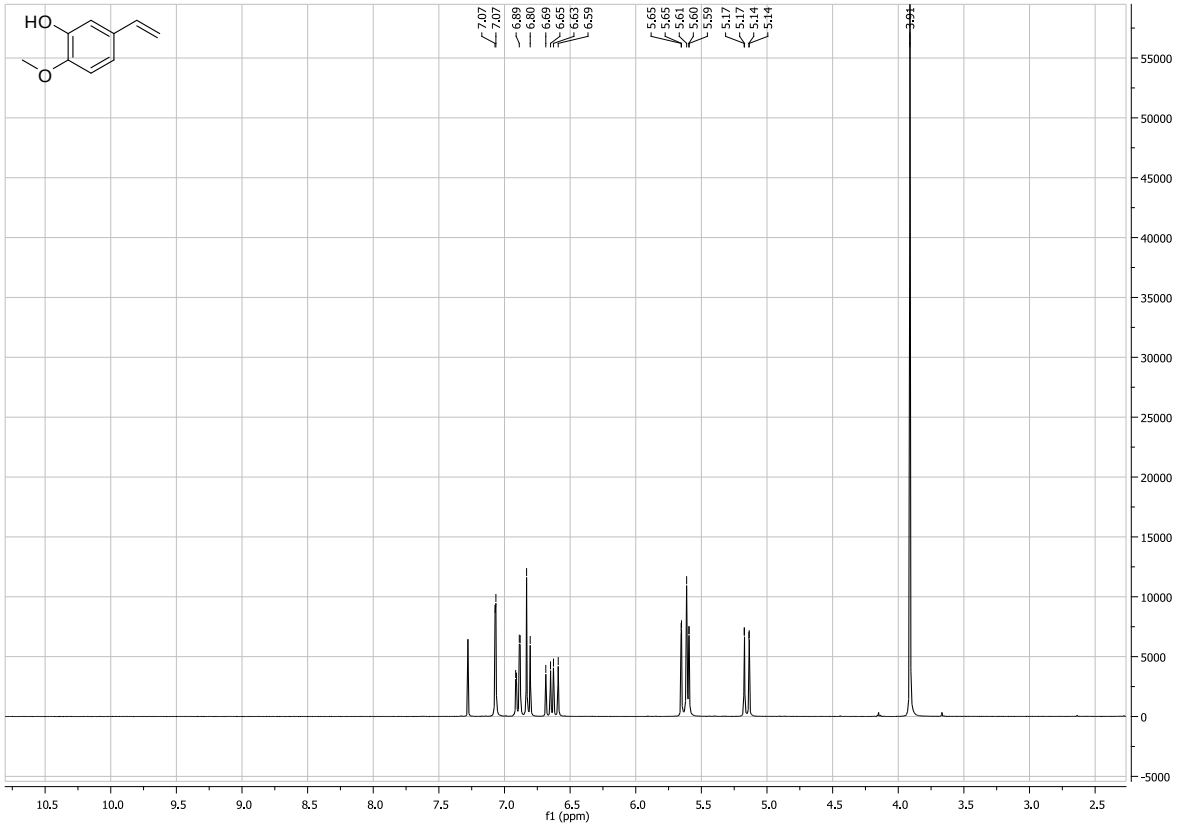


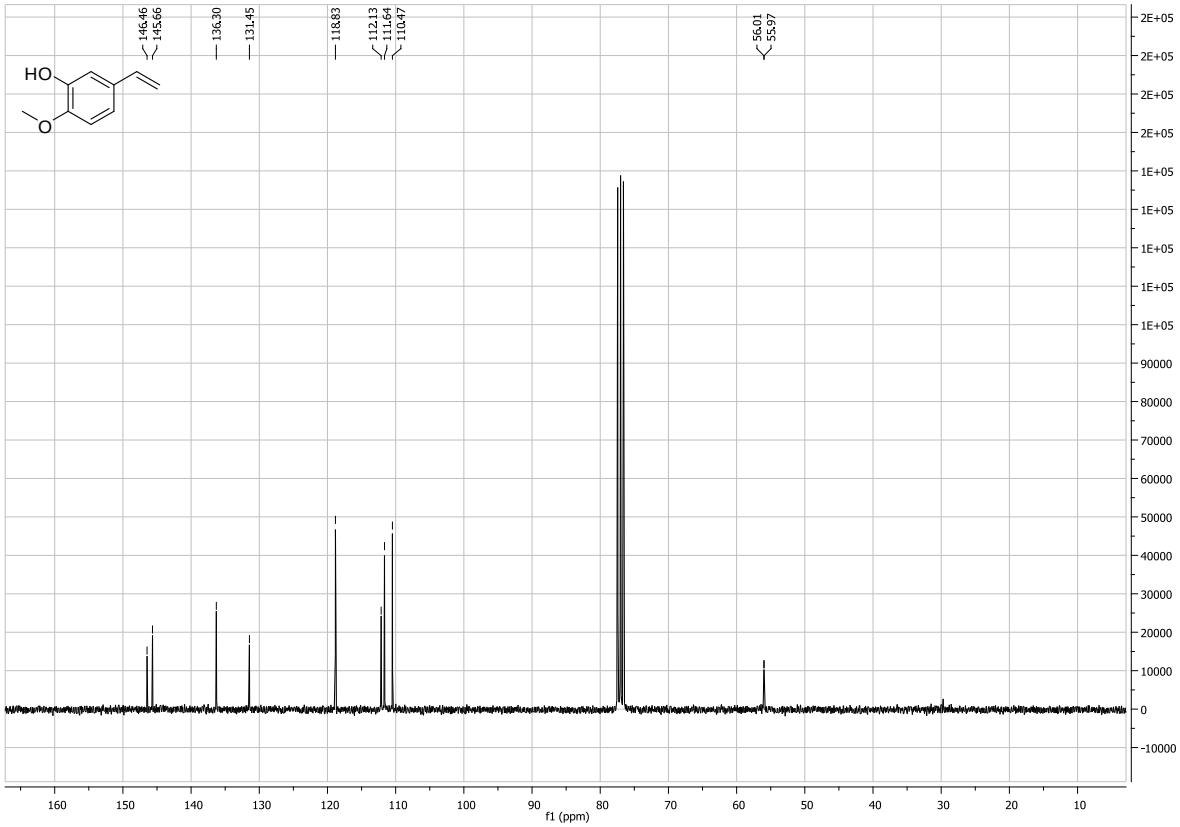
4-(Prop-1-en-2-yl)phenol(10a)





2-Methoxy-5-vinylphenol(11a)





References

[1] C. Wuensch, S. M. Glueck, J. Gross, D. Koszelewski, M. Schober, K. Faber, *Org. Lett.* **2012**, *14*, 1974-1977.

[2] C. Wuensch, J. Gross, G. Steinkellner, K. Gruber, S. M. Glueck, K. Faber, *Angew. Chem. Int. Ed.* **2013**, *52*, 2293-2297.

[3] G. Steinkellner, R. Rader, G. G. Thallinger, C. Kratky, K. Gruber, *BMC Bioinform.* **2009**, *10*, 32.