Supporting Information

Using deep eutectic solvents to overcome limited substrate solubility in the enzymatic decarboxylation of bio-based phenolic acids

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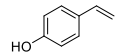
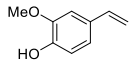
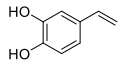
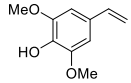
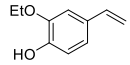
1 Specific activities of PAD WT, I85A, I85V and I85L towards substrates 1a-1e

Table S1. Specific activity of *Bs*PAD WT and mutants (I85L, I85V, I85A) towards substrates 1a-1e. n.c. = not converted.

Entry	Substrate	Product	Specific acitivty [U mg^{-1}]			
			PAD I85L	PAD WT	PAD I85V	PAD I85A
1	**1a**		1.0 ± 0.3	40 ± 4	5 ± 1	1.0 ± 0.2
2	**1b**		1.3 ± 0.4	80 ± 13	8 ± 2	6 ± 1
3	**1c**		0.6 ± 0.5	18 ± 1	1.1 ± 0.2	1.6 ± 0.5
4	**1d**		n.c.	0.36 ± 0.03 [a]	3.0 ± 0.5	10 ± 2
5	**1e**		n.c.	1.1 ± 0.3	7 ± 2	29 ± 1

Reaction conditions: 10 mM substrate, 5 % (v/v) DMSO, 50 mM potassium phosphate buffer (pH 6), 5 µg purified PAD WT or 50 µg PAD mutant (unless otherwise noted), 30 °C, 600 rpm. [a] 100-fold amount of enzyme was used (500 µg).

2 Preparation of *Bs*PAD variants

Table S2. Mutagenesis primer used for QuikChange™ reaction (underlined characters indicate deviations from original plasmid sequence).

bsPAD_I85L_fwd	5'-CATGGCATTCTGTTCTTCCCGAAATGGGTG-3'
bsPAD_I85L_rev	5'-GAAGAACAGAATGCCATGCATGCGTTTTTCATTTG-3'
bsPAD_I85V_fwd	5'-CATGGCATTGTGTTCTTCCCGAAATGGGTG-3'
bsPAD_I85V_rev	5'-GAAGAACACAATGCCATGCATGCGTTTTTCATTTG-3'

3 Structure of *DES* components

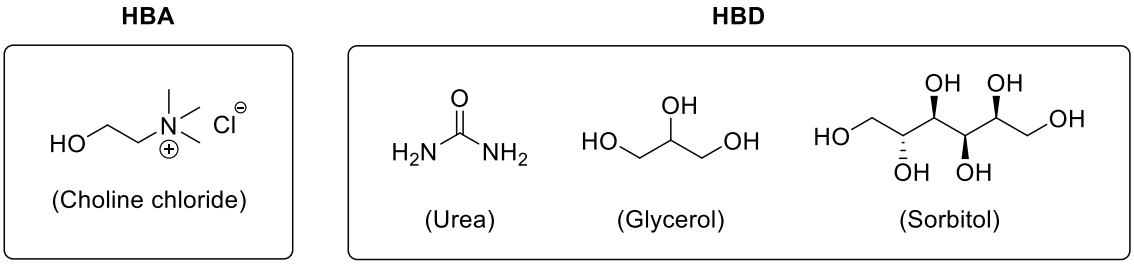


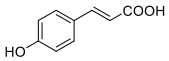
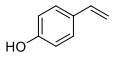
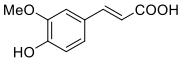
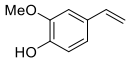
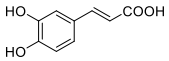
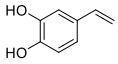
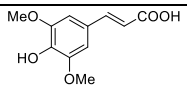
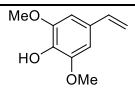
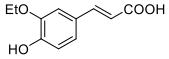
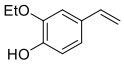
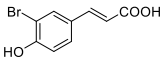
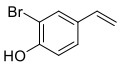
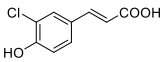
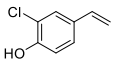
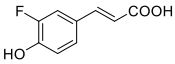
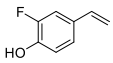
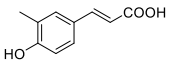
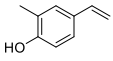
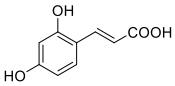
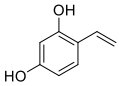
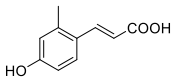
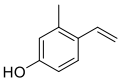
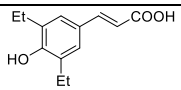
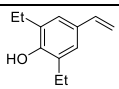
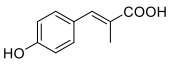
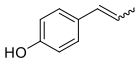
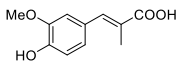
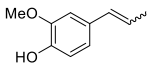
Figure S 1. Hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) components of the employed DES mixtures.

4 Analytics

4.1 HPLC

All calibration samples were prepared in triplicates and the resulting standard curves are shown in Figure S 2-Figure S 12. Table S3 summarizes retention times of all substrates and products and the used detection wavelength.

Table S3: Conditions for HPLC-analysis of substrate consumption with different phenolic acids. n.d. = not determined.

Substrate	t_R (Substrate) [min]	Detection wavelength [nm]	Product	t_R (Product) [min]
 **1a**	3.0	280	 **2a**	5.3
 **1b**	3.0	280	 **2b**	5.5
 **1c**	2.6	280	 **2c**	3.8
 **1d**	2.9	320	 **2d**	5.0
 **1e**	3.4	320	 **2e**	7.3
 **1f**	3.5	320	 **2f**	7.8
 **1g**	3.4	320	 **2g**	7.2
 **1h**	3.1	320	 **2h**	5.9
 **1i**	3.3	320	 **2i**	7.0
 **1j**	2.7	320	 **2j**	4.1
 **1k**	3.1	320	 **2k**	n.d.
 **1l**	5.5	320	 **2l**	n.d.
 **1m**	3.2	280	 **2m**	6.6
 **1n**	3.4	280	 **2n**	n.d.

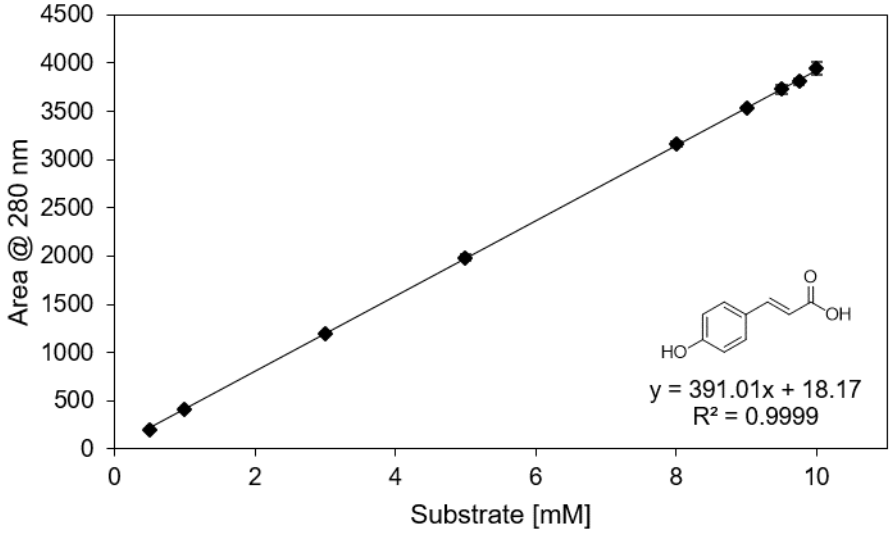


Figure S 2. Calibration curve for *p*-coumaric acid (**1a**) (2 µL injection volume, $\lambda_{detection}$ = 280 nm).

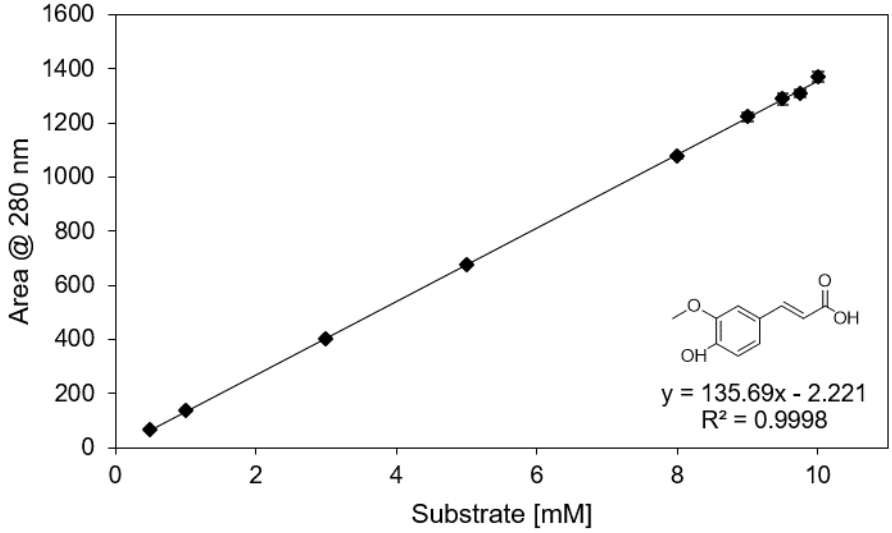


Figure S 3. Calibration curve for ferulic acid (**1b**) (2 µL injection volume, $\lambda_{detection}$ = 280 nm).

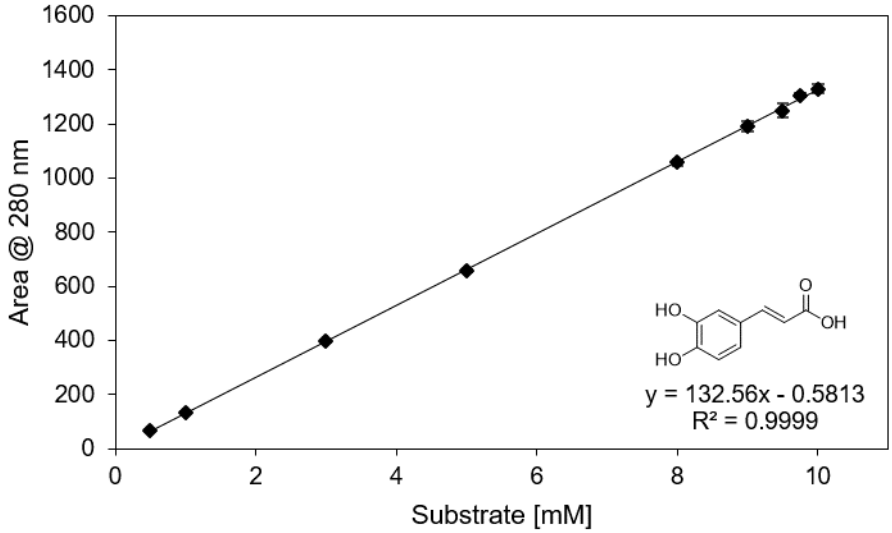


Figure S 4. Calibration curve for caffeic acid (**1c**) (2 µL injection volume, $\lambda_{detection}$ = 280 nm).

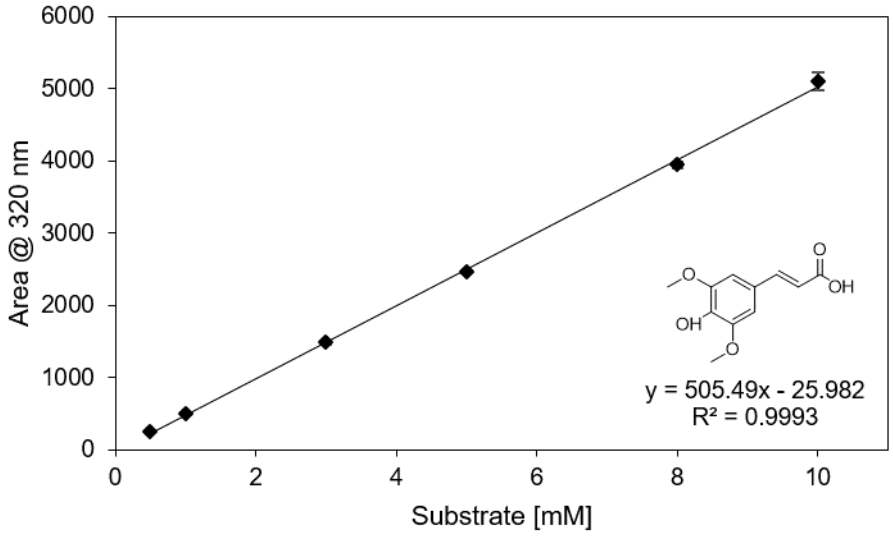


Figure S 5. Calibration curve for sinapinic acid (**1d**) (2 µL injection volume, $\lambda_{detection}$ = 320 nm).

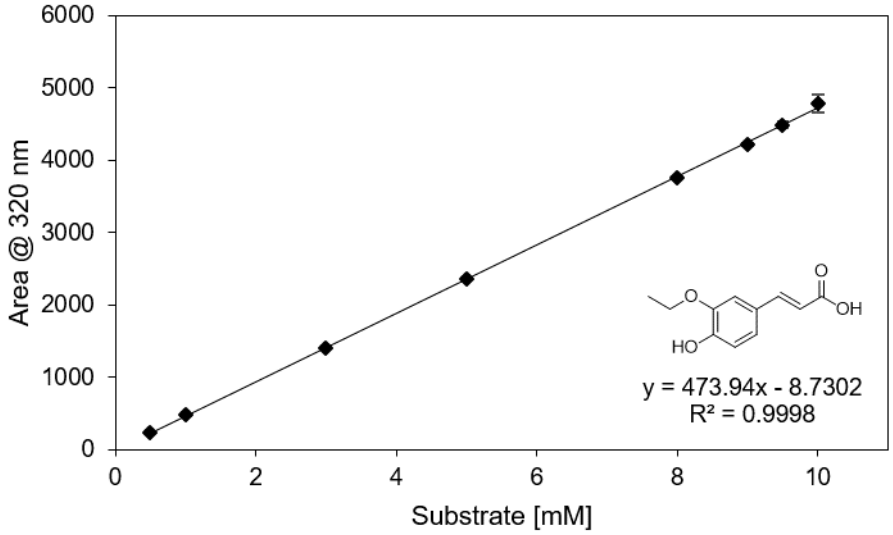


Figure S 6. Calibration curve for (*E*)-3-(3-ethoxy-4-hydroxyphenyl)acrylic acid (**1e**) (2 µL injection volume, $\lambda_{detection}$ = 320 nm).

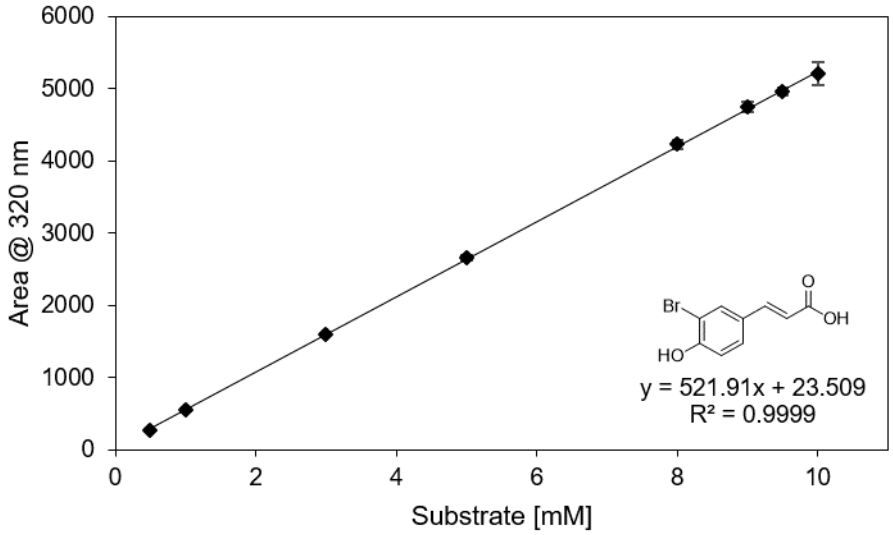


Figure S 7. Calibration curve for (*E*)-3-(3-bromo-4-hydroxyphenyl)acrylic acid (**1f**) (2 µL injection volume, $\lambda_{detection}$ = 320 nm).

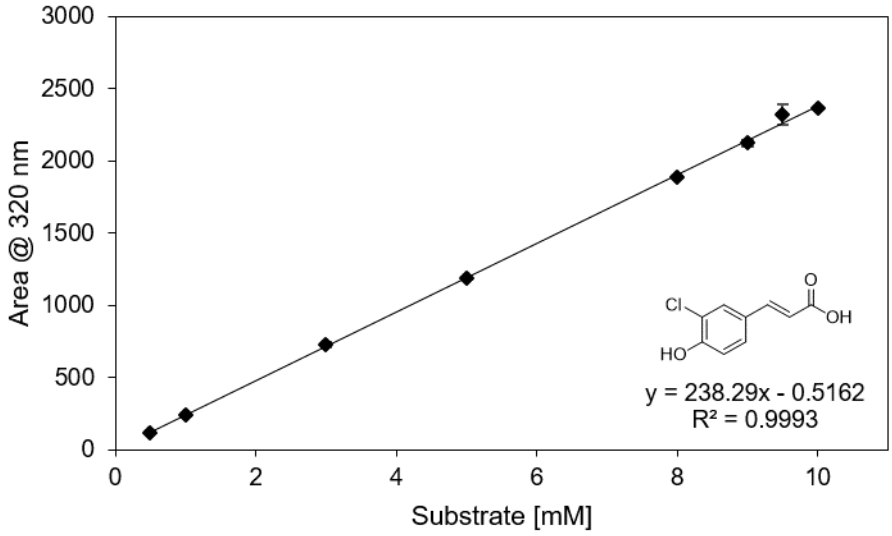


Figure S 8. Calibration curve for (*E*)-3-(3-chloro-4-hydroxyphenyl)acrylic acid (**1g**) (1 µL injection volume, $\lambda_{detection}$ = 320 nm).

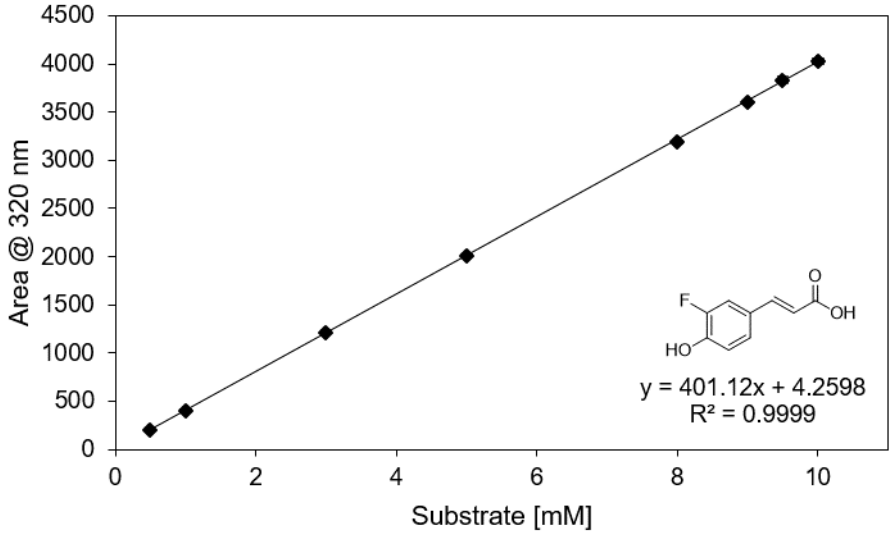


Figure S 9. Calibration curve for (*E*)-3-(3-fluoro-4-hydroxyphenyl)acrylic acid (**1h**) (2 µL injection volume, $\lambda_{detection}$ = 320 nm).

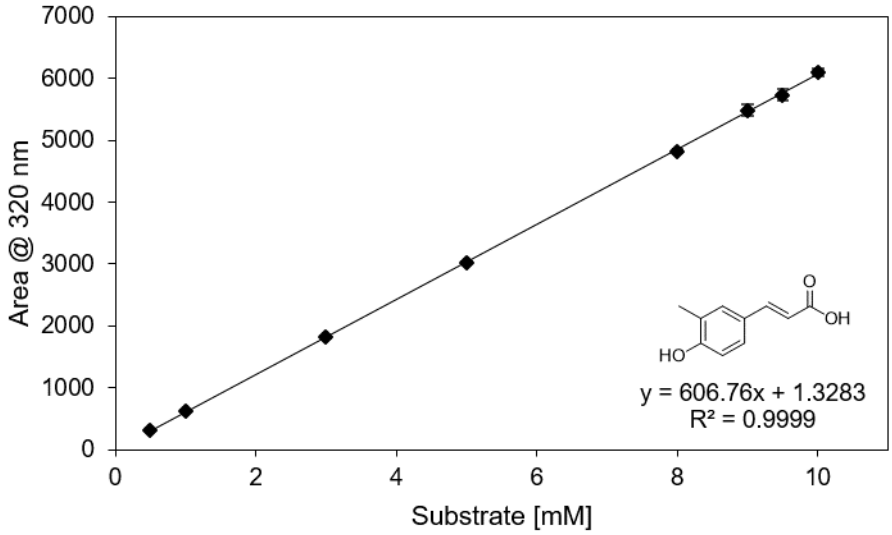


Figure S 10. Calibration curve for (*E*)-3-(4-hydroxy-3-methylphenyl)acrylic acid (**1i**) (2 µL injection volume, $\lambda_{detection}$ = 320 nm).

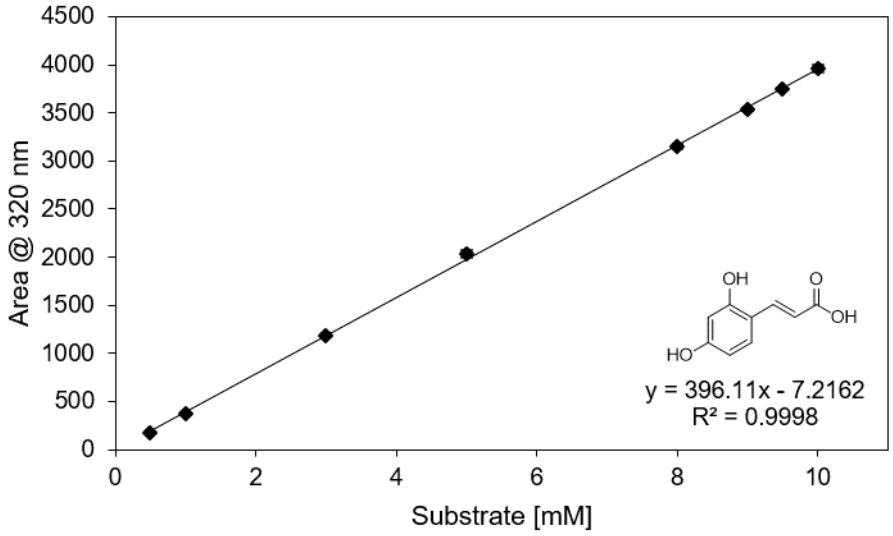


Figure S 11. Calibration curve for (*E*)-3-(2,4-dihydroxyphenyl)acrylic acid (**1j**) (2 µL injection volume, $\lambda_{detection}$ = 320 nm).

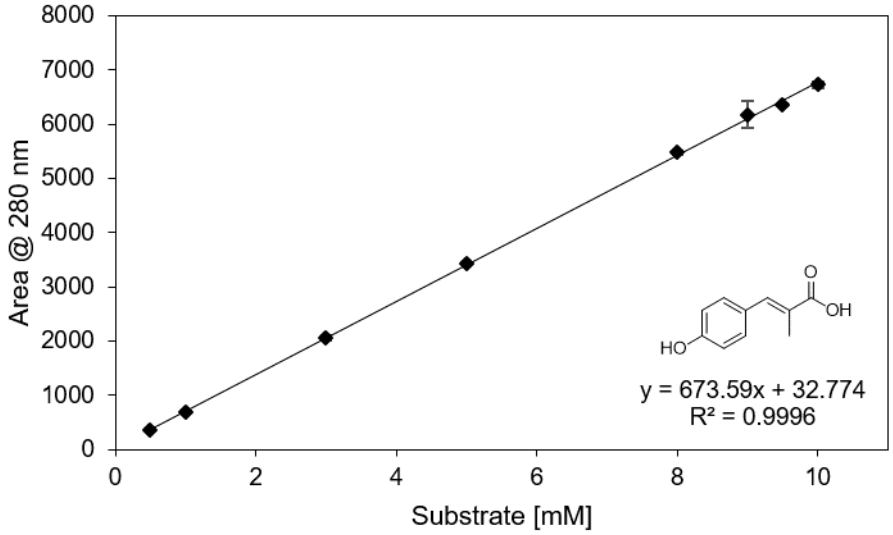


Figure S 12. Calibration curve for (*E*)-3-(4-hydroxyphenyl)-2-methylacrylic acid (**1m**) (2 µL injection volume, $\lambda_{detection}$ = 280 nm).

4.2 GC-MS

Table S4. Method parameters for GC-MS analyses.

Flow control mode	Linear velocity (39.5 cm sec^{-1}) (carrier gas: He)
Total flow	11.5 mL min^{-1}
Column flow	1.13 mL min^{-1}
Injection temperature	250 °C
Injection volume	1 µL
Split ratio	6.5
Temperature program	100 °C for 4 min, 20 °C min^{-1} to 300 °C, 300 °C for 5 min. Total program time: 19 min

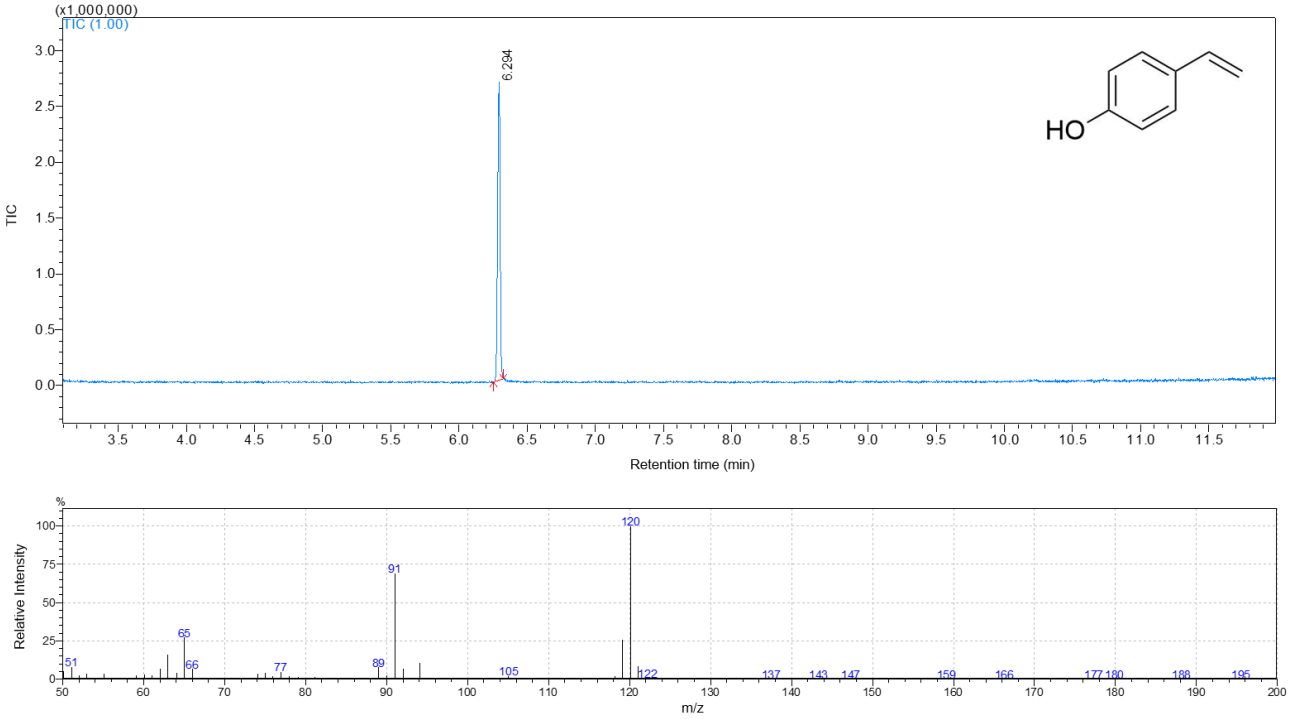


Figure S 13. GC-MS analysis of **2a**. Top: TIC (t_R=6.3 min), Bottom: corresponding mass spectrum; *m/z*=120 (100%) [M^+].

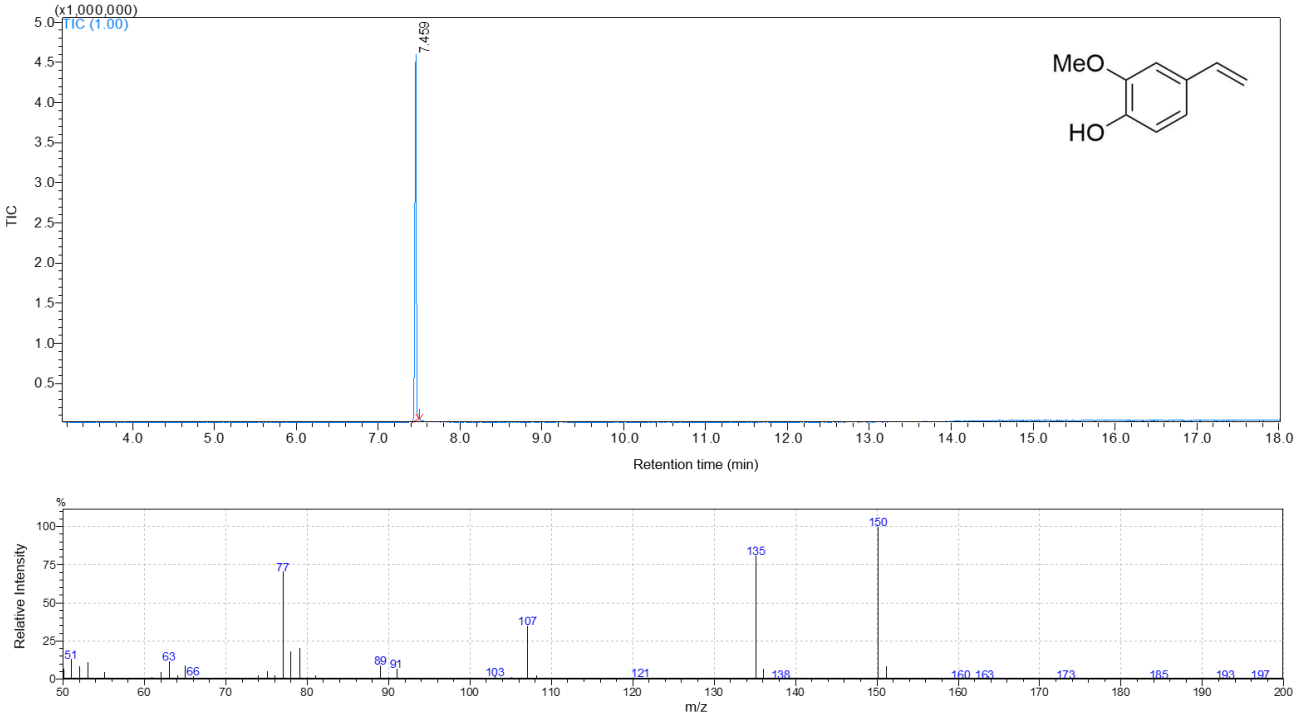


Figure S 14. GC-MS analysis of **2b**. Top: TIC (t_R=7.5 min), Bottom: corresponding mass spectrum; *m/z*=150 (100%) [M^+].

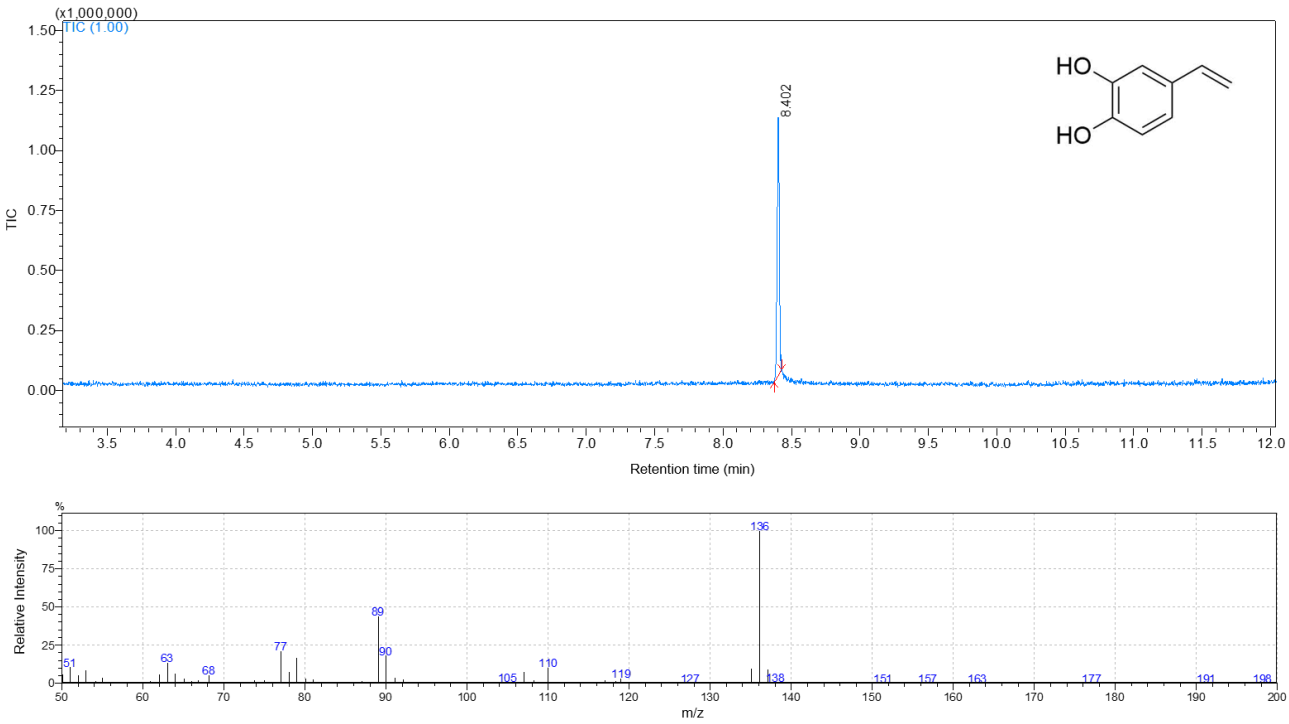


Figure S 15. GC-MS analysis of **2c**. Top: TIC (t_R=8.4 min), Bottom: corresponding mass spectrum; *m/z*=136 (100%) [M^+].

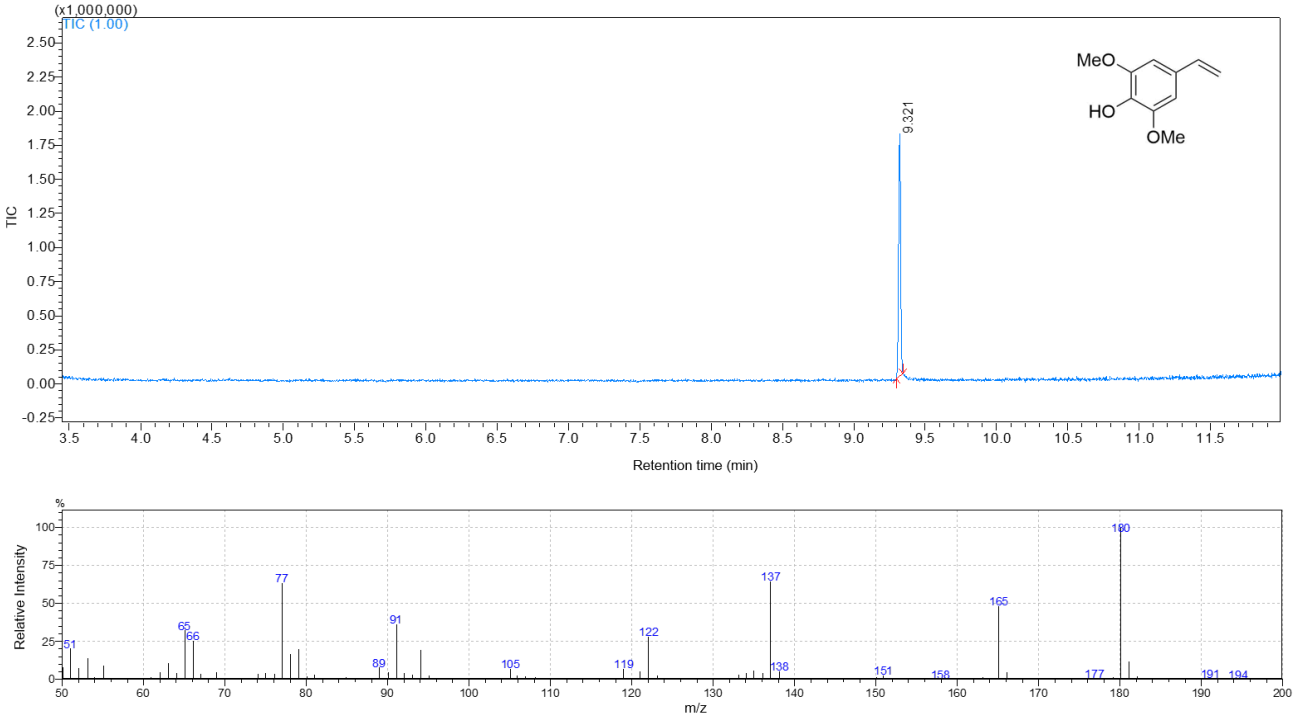


Figure S 16. GC-MS analysis of **2d**. Top: TIC (t_R=9.3 min), Bottom: corresponding mass spectrum; *m/z*=180 (100%) [M^+].

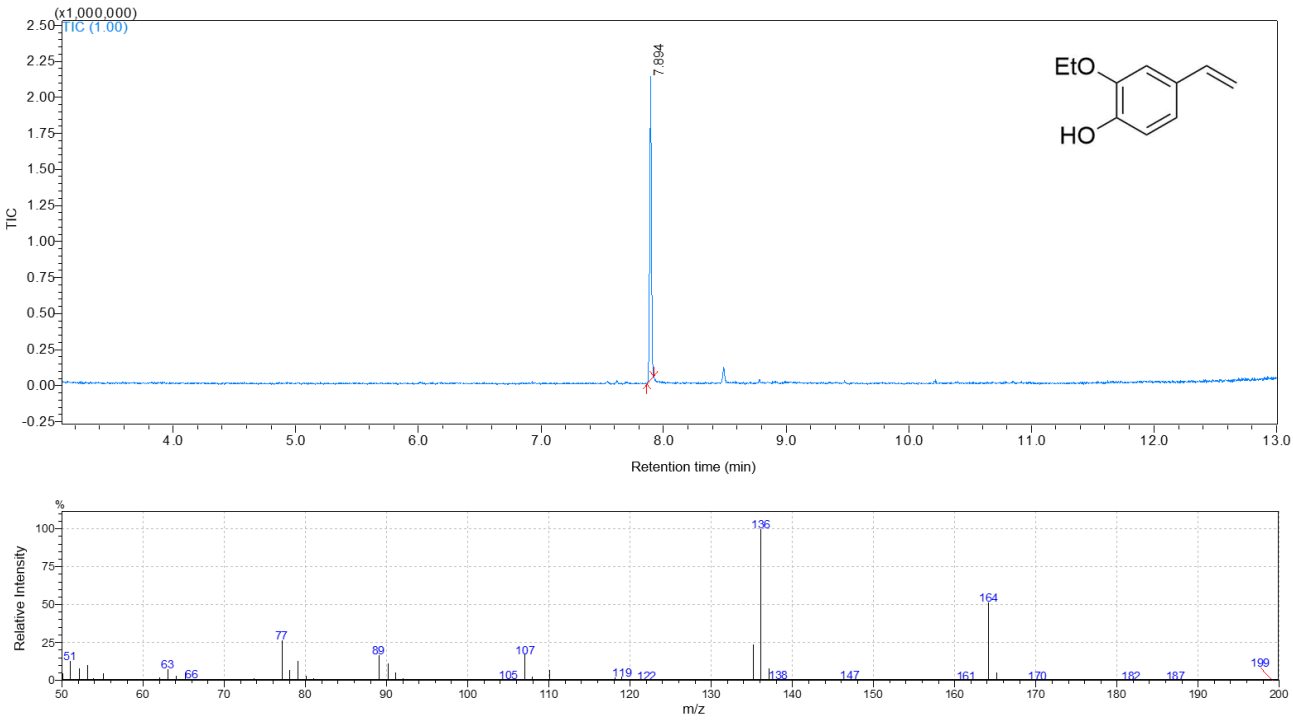


Figure S 17. GC-MS analysis of **2e**. Top: TIC (t_R=7.9 min), Bottom: corresponding mass spectrum; *m/z*=164 (50%) [M^+].

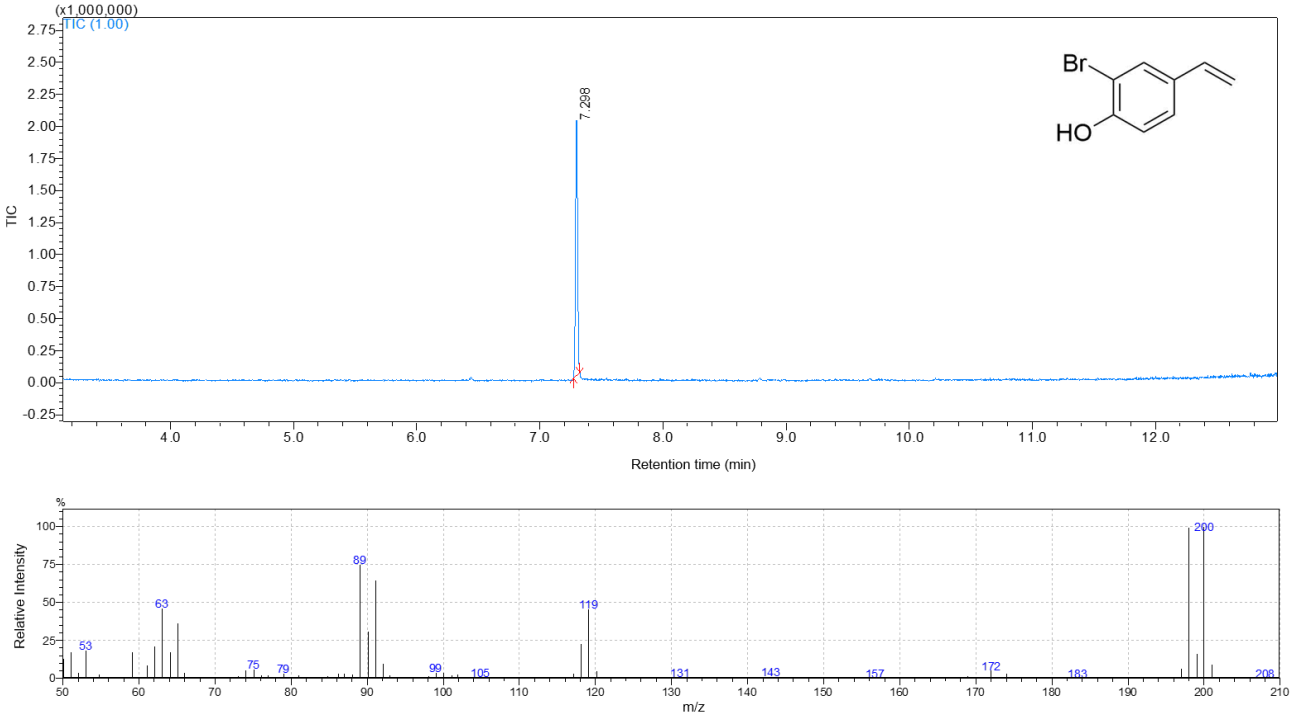


Figure S 18. GC-MS analysis of **2f**. Top: TIC (t_R=7.3 min), Bottom: corresponding mass spectrum; *m/z*=198 (100%) [M^+].

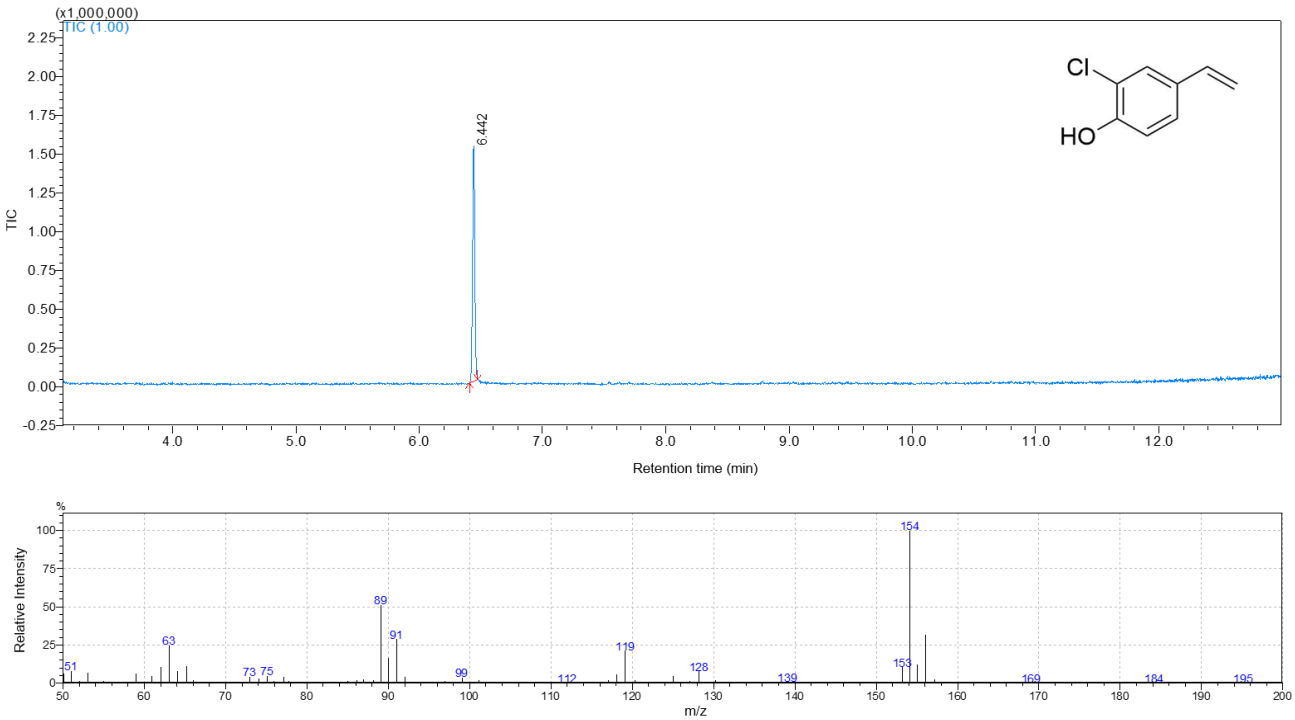


Figure S 19. GC-MS analysis of **2g**. Top: TIC (t_R=6.4 min), Bottom: corresponding mass spectrum; *m/z*=154 (100%) [M^+].

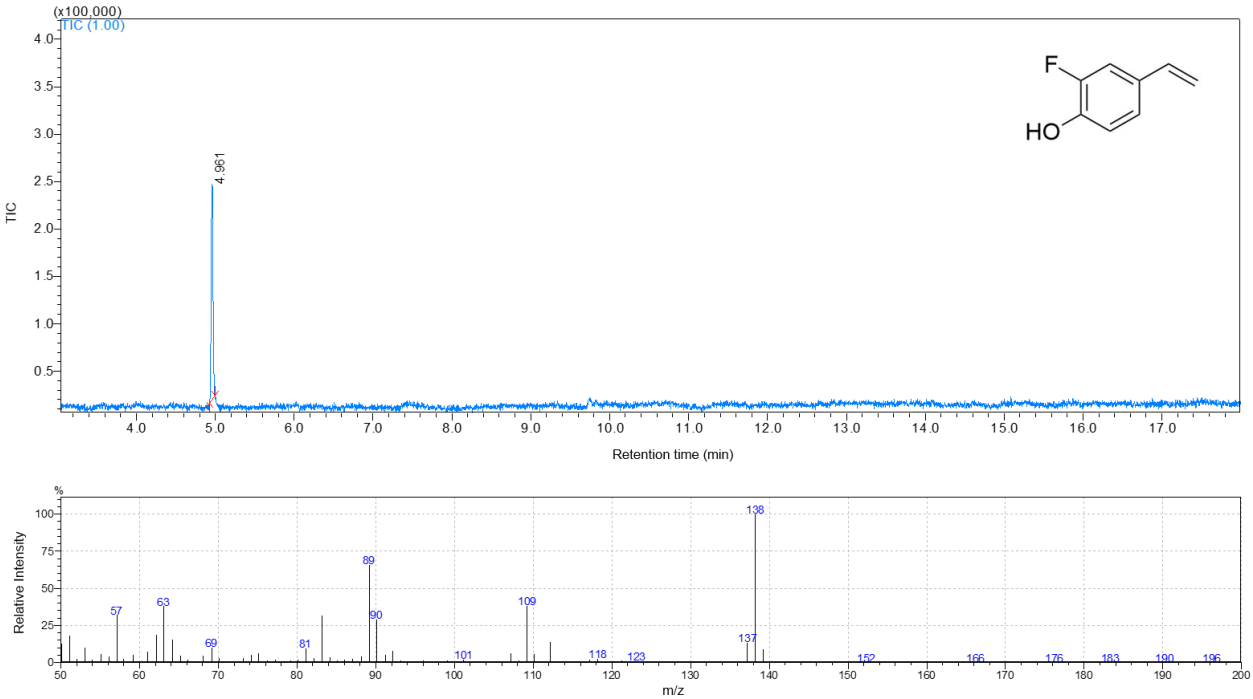


Figure S 20. GC-MS analysis of **2h**. Top: TIC (t_R=5.0 min), Bottom: corresponding mass spectrum; *m/z*=138 (100%) [M^+].

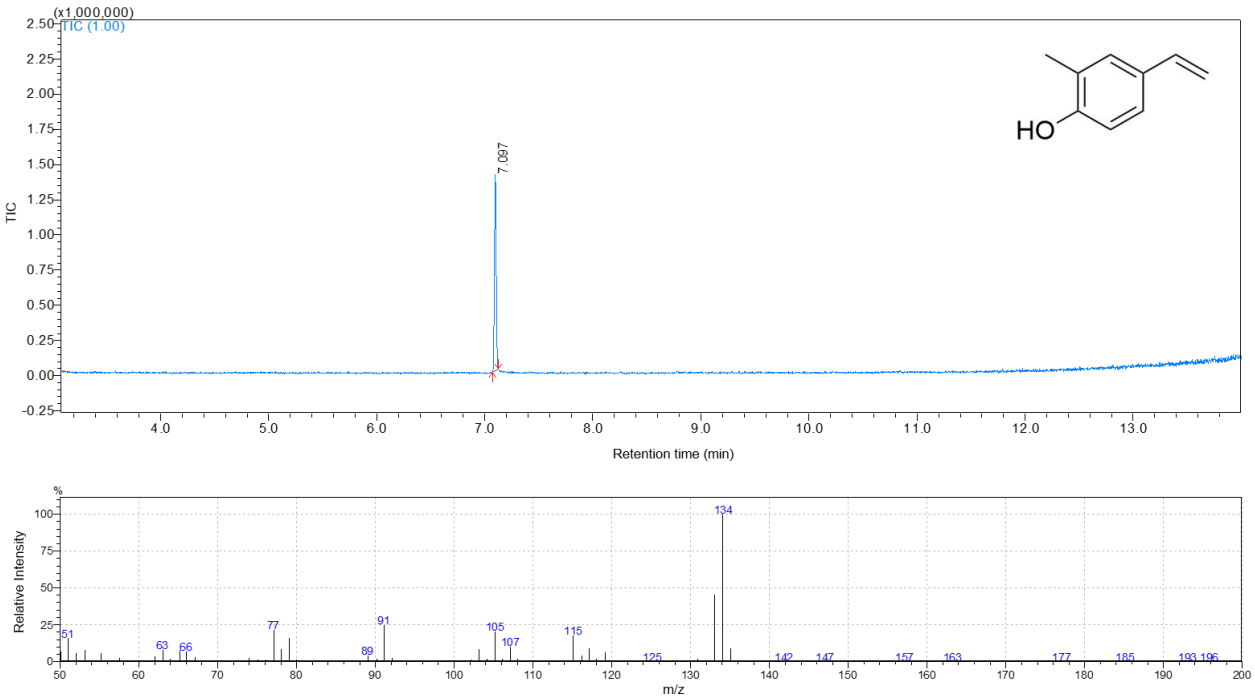


Figure S 21. GC-MS analysis of **2i**. Top: TIC (t_R=7.1 min), Bottom: corresponding mass spectrum; *m/z*=134 (100%) [M^+].

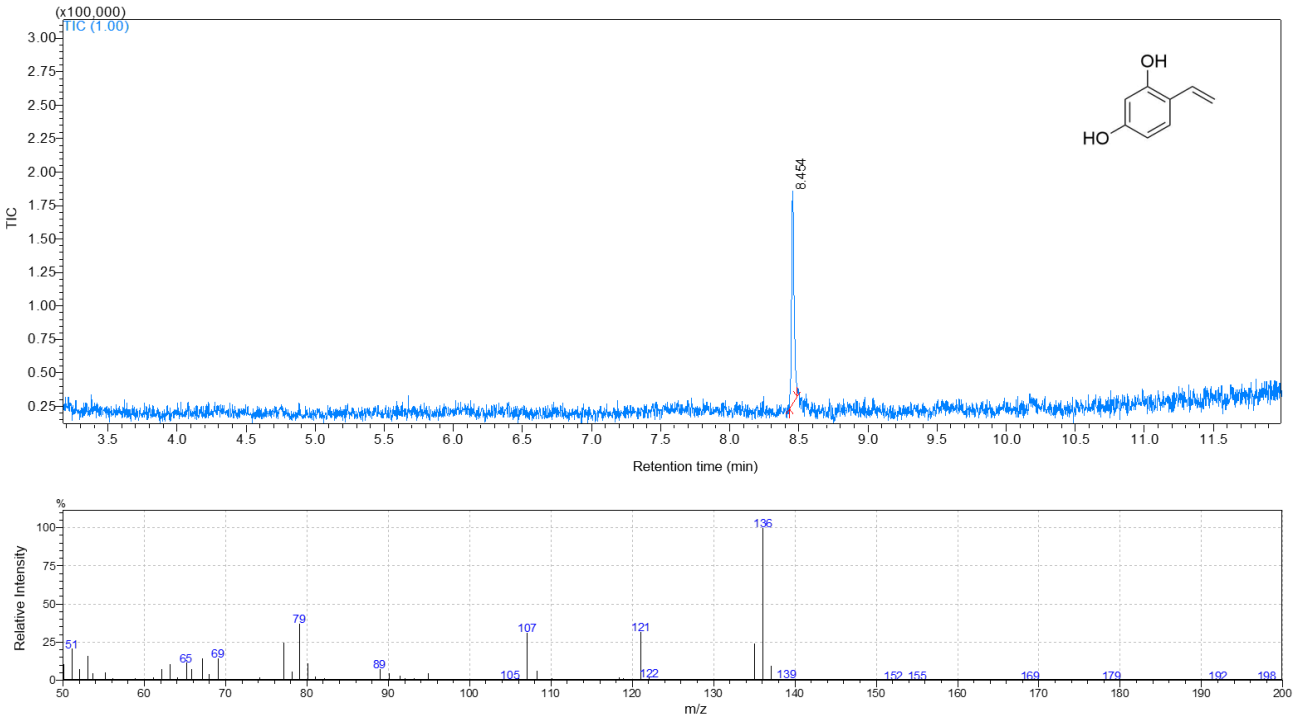


Figure S 22. GC-MS analysis of **2j**. Top: TIC (t_R=8.5 min), Bottom: corresponding mass spectrum; *m/z*=136 (100%) [M^+].

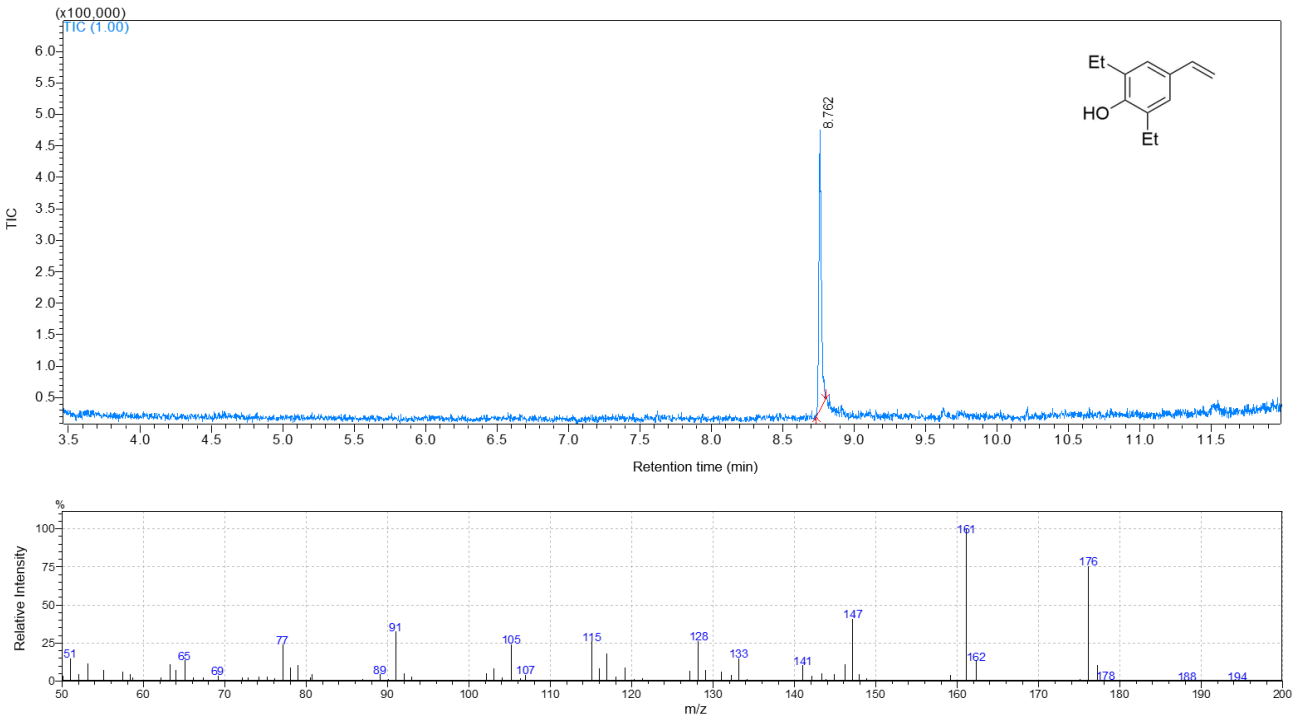


Figure S 23. GC-MS analysis of **2l**. Top: TIC (t_R=8.8 min), Bottom: corresponding mass spectrum; *m/z*=176 (75%) [M^+].

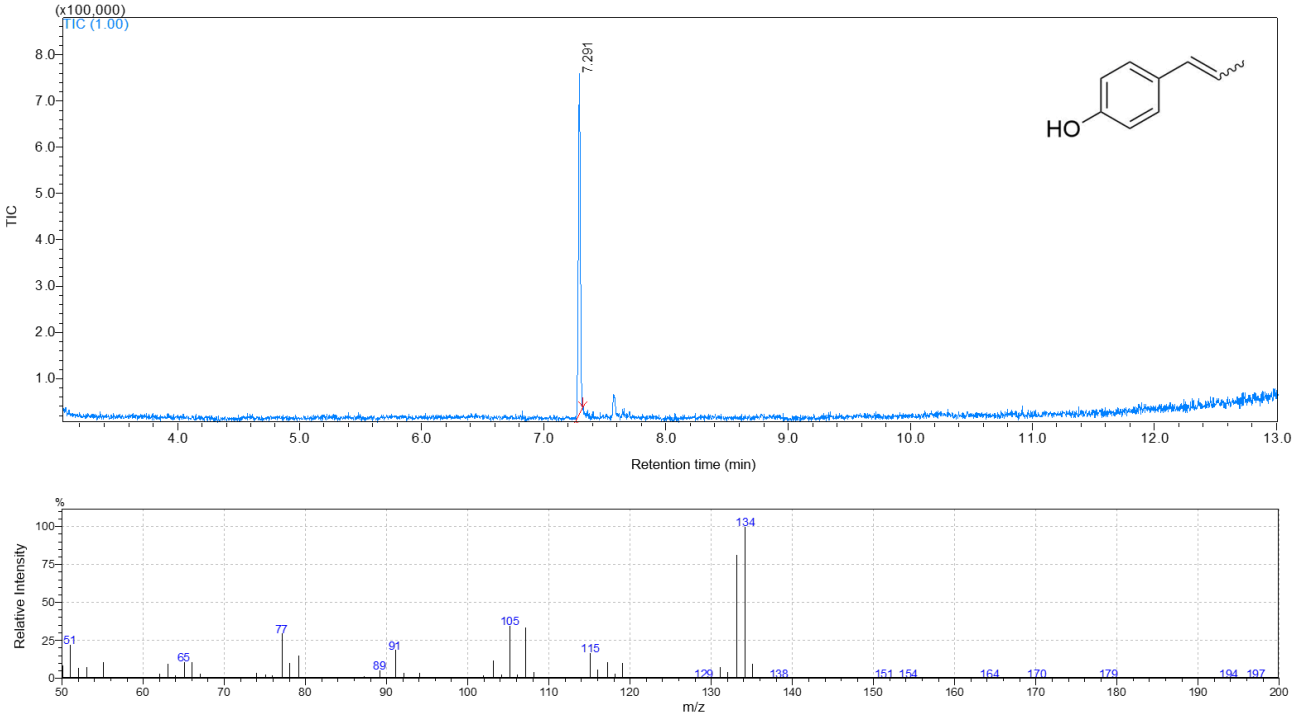


Figure S 24. GC-MS analysis of **2m**. Top: TIC (t_R=7.3 min), Bottom: corresponding mass spectrum; *m/z*=134 (100%) [M^+].

5 Syntheses

5.1 General procedure for Knoevenagel Condensations

The appropriate aldehyde (1 equivalent) was stirred together with malonic acid or methylmalonic acid (2.2 equivalents), in pyridine (6 equivalents) in a round bottom flask at room temperature until all components were dissolved. Then piperidine (0.15 equivalents) was added and the mixture heated to reflux overnight (> 12h). After cooling the mixture to room temperature, cold HCl (0°C, 2M, aqueous) was added until no further precipitate formed. The precipitate was filtered under vacuum, washed with HCl (0°C, 2M, aqueous) and water and dried over

$CaCl_2$ in an evacuated desiccator. When necessary, the product was further purified by fractioned recrystallization from ethanol and HCl (2M, aqueous).

5.2 Synthesis of (*E*)-3-(4-hydroxy-3-methylphenyl)acrylic acid (1i)

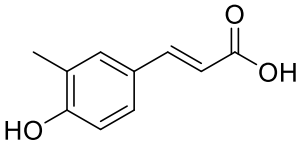


Figure S 25. Structure of (*E*)-3-(4-hydroxy-3-methylphenyl)acrylic acid (**1i**).

1i was synthesized from the corresponding aldehyde (465 mg) according to the general procedure for Knoevennagel Condensations and pure after washing the precipitate. Yield: 468 mg (77%); TLC: eluent: hexanes/EtOAc/AcOH (1:1 + 2,5% AcOH), aldehyde: 0.73, product: 0.25. Spectroscopic data fits to literature values.[1]

^{1}H NMR (300 MHz, MeOD) δ 7.57 (d, *J* = 15.9 Hz, 1H), 7.33 (s, 1H), 7.26 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.76 (d, *J* = 8.3 Hz, 1H), 6.26 (d, *J* = 15.9 Hz, 1H), 2.19 (s, 3H) ppm.

^{13}C NMR (75 MHz, MeOD) δ 169.71, 157.95, 145.62, 130.55, 127.26, 125.65, 124.95, 114.42, 113.73, 14.71 ppm.

5.3 Synthesis of (*E*)-3-(4-hydroxy-2-methylphenyl)acrylic acid (1k)

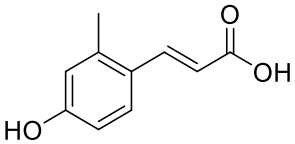


Figure S 26. Structure of (*E*)-3-(4-hydroxy-2-methylphenyl)acrylic acid (**1k**).

1k was synthesized from the corresponding aldehyde (100 mg) according to the general procedure for Knoevennagel Condensations and pure after washing the precipitate. Yield: 42 mg (32%); TLC: eluent: hexanes/EtOAc/AcOH (1:1 + 2,5% AcOH), product: 0.50. Spectroscopic data fits to literature values.[2]

^{1}H NMR (300 MHz, MeOD) δ 7.91 (d, *J* = 15.8 Hz, 1H), 7.50 (d, *J* = 9.3 Hz, 1H), 6.66 (s, 1H), 6.64 (s, 1H), 6.23 (d, *J* = 15.8 Hz, 1H), 2.35 (s, 3H) ppm.

^{13}C NMR (75 MHz, MeOD) δ 169.65, 159.37, 142.39, 139.68, 127.41, 124.33, 116.85, 114.94, 113.40, 18.45 ppm.

5.4 Synthesis of (*E*)-3-(3,5-diethyl-4-hydroxyphenyl)acrylic acid (1l)

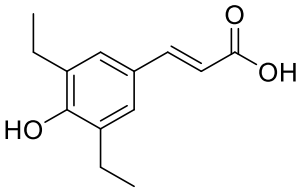


Figure S 27. Structure of (*E*)-3-(3,5-diethyl-4-hydroxyphenyl)acrylic acid (**1l**).

1l was synthesized from the corresponding aldehyde (390 mg) according to the general procedure for Knoevennagel Condensations and pure after washing the precipitate. Yield: 293 mg (60%); TLC: eluent: hexanes/EtOAc/AcOH (1:1 + 2,5% AcOH), aldehyde: 0.66, product: 0.22.

^{1}H NMR (300 MHz, MeOD) δ 7.54 (d, *J* = 15.9 Hz, 1H), 7.16 (s, 2H), 6.23 (d, *J* = 15.9 Hz, 1H), 2.61 (q, *J* = 7.5 Hz, 4H), 1.17 (t, *J* = 7.5 Hz, 6H) ppm.

^{13}C NMR (75 MHz, MeOD) δ 171.16, 156.22, 147.34, 132.37, 128.32, 127.4, 115.13, 24.37, 14.71 ppm.

5.5 Synthesis of (*E*)-3-(3-fluoro-4-hydroxyphenyl)acrylic acid (1h)

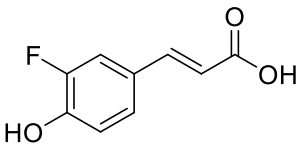


Figure S 28. Structure of (*E*)-3-(3-fluoro-4-hydroxyphenyl)acrylic acid (**1h**).

1h was synthesized from the corresponding aldehyde (250 mg) according to the general procedure for Knoevennagel Condensations and pure after washing the precipitate. Yield: 168 mg (52%); TLC: eluent: hexanes/EtOAc/AcOH (1:1 + 2,5% AcOH), aldehyde: 0.5, product: 0.22. Spectroscopic data fits to literature values.[3]

^{1}H NMR (300 MHz, MeOD) δ 7.56 (d, *J* = 15.9 Hz, 1H), 7.35 (dd, *J* = 12.1, 2.0 Hz, 1H), 7.24 (dd, *J* = 8.4, 1.7 Hz, 1H), 6.93 (t, *J* = 8.6 Hz, 1H), 6.31 (d, *J* = 15.9 Hz, 1H).

^{13}C NMR (75 MHz, MeOD) δ 169.15, 153.15, 149.95, 147.32, 144.07, 126.64, 125.15, 114.85, 114.60.

5.6 Synthesis of (*E*)-3-(3-chloro-4-hydroxyphenyl)acrylic acid (1g)

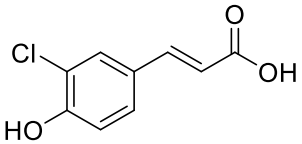


Figure S 29. Structure of (*E*)-3-(3-chloro-4-hydroxyphenyl)acrylic acid (**1g**).

1g was synthesized from the corresponding aldehyde (1063 mg) according to the general procedure for Knoevennagel Condensations and pure after washing the precipitate. Yield: 683 mg (47%); TLC: eluent: hexanes/EtOAc/AcOH (1:1 + 2,5% AcOH), aldehyde: 0.66, product: 0.22. Spectroscopic data fits to literature values.[1]

^{1}H NMR (300 MHz, MeOD) δ 7.61 – 7.49 (m, 2H), 7.39 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.93 (d, *J* = 8.45 Hz, 1), 6.32 (d, *J* = 16.0 Hz, 1H) ppm.

^{13}C NMR (75 MHz, MeOD) δ 169.11, 155.08, 143.69, 129.53, 127.77, 127.07, 120.92, 116.42, 115.91 ppm.

5.7 Synthesis of (*E*)-3-(3-bromo-4-hydroxyphenyl)acrylic acid (1f)

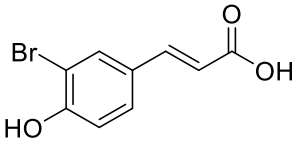


Figure S 30. Structure of (*E*)-3-(3-bromo-4-hydroxyphenyl)acrylic acid (**1f**).

1f was synthesized from the corresponding aldehyde (1056 mg) according to the general procedure for Knoevennagel Condensations and pure after washing the precipitate. Yield: 598 mg (60%); TLC: eluent: hexanes/EtOAc/AcOH (1:1 + 2,5% AcOH), aldehyde: 0.66, product: 0.21. Spectroscopic data fits to literature values.[1]

^{1}H NMR (300 MHz, MeOD) δ 7.73 (d, *J* = 2.1 Hz, 1H), 7.54 (d, *J* = 15.9 Hz, 1H), 7.43 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 6.31 (d, *J* = 15.9 Hz, 1H) ppm.

^{13}C NMR (75 MHz, MeOD) δ 169.10, 156.16, 143.57, 132.78, 128.36, 127.44, 116.00, 115.86, 109.97 ppm.

5.8 Synthesis of (*E*)-3-(4-hydroxyphenyl)-2-methylacrylic acid (1m)

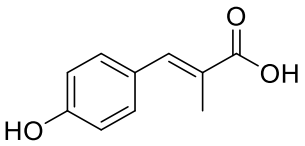


Figure S 31. Structure of (*E*)-3-(4-hydroxyphenyl)-2-methylacrylic acid (**1m**).

1m was synthesized from the corresponding aldehyde (1000 mg) and methylmalonic acid (1 equivalent) according to the general procedure for Knoevennagel Condensations and pure after washing the precipitate. Yield: 324 mg (22%); TLC: eluent: hexanes/EtOAc/AcOH (1:1 + 2,5% AcOH), aldehyde: 0.68, product: 0.22. Spectroscopic data fits to literature values.[4]

^{1}H NMR (300 MHz, MeOD) δ 7.63 (s, 1H), 7.33 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 2.09 (d, *J* = 1.4 Hz, 3H) ppm.

^{13}C NMR (75 MHz, MeOD) δ 171.10, 157.87, 139.06, 131.35, 127.12, 124.86, 114.92, 12.83 ppm.

5.9 Synthesis of (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-methylacrylic acid (1n)

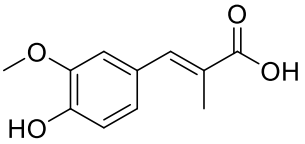


Figure S 32. Structure of (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-methylacrylic acid (**1n**).

1n was synthesized from the corresponding aldehyde (936 mg) and methylmalonic acid (1 equivalent) according to the general procedure for Knoevennagel Condensations and pure after washing the precipitate. Yield: 202 mg (16%); TLC: eluent: hexanes/EtOAc/AcOH (1:1 + 2,5% AcOH), aldehyde: 0.68, product: 0.22. Spectroscopic data fits to literature values.[1]

^{1}H NMR (300 MHz, MeOD) δ 7.64 (s, 1H), 7.05 – 6.91 (m, 2H), 6.83 (d, *J* = 8.2 Hz, 1H), 3.86 (s, 3H), 2.11 (d, *J* = 1.4 Hz, 3H) ppm.

^{13}C NMR (75 MHz, MeOD) δ 171.06, 147.37, 147.11, 139.28, 127.64, 125.14, 123.42, 114.81, 113.15, 54.99, 12.91 ppm.

6 NMR Spectra

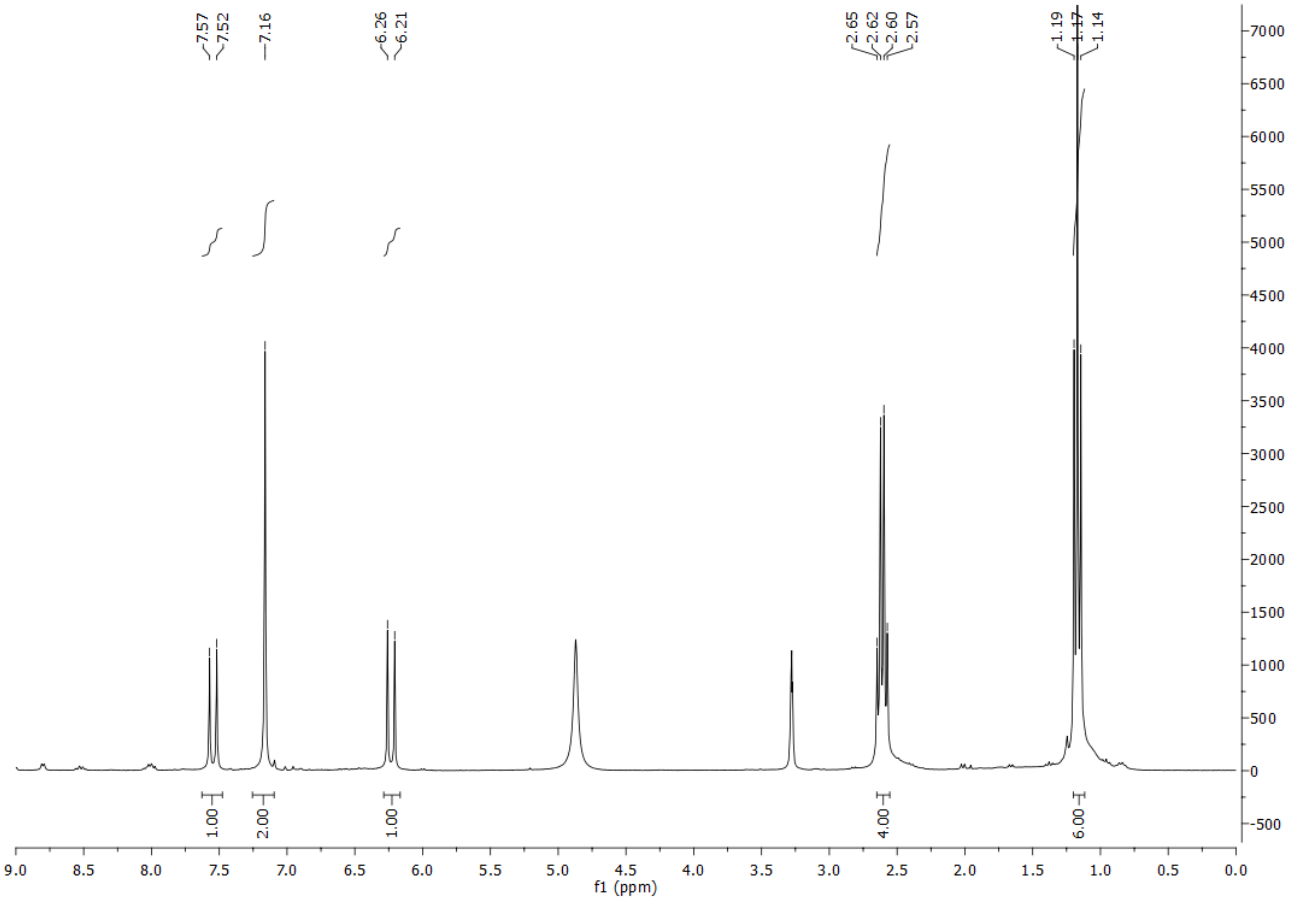


Figure S 33. ^{1}H NMR of (*E*)-3-(3,5-diethyl-4-hydroxyphenyl)acrylic acid (**1l**) (MeOD)

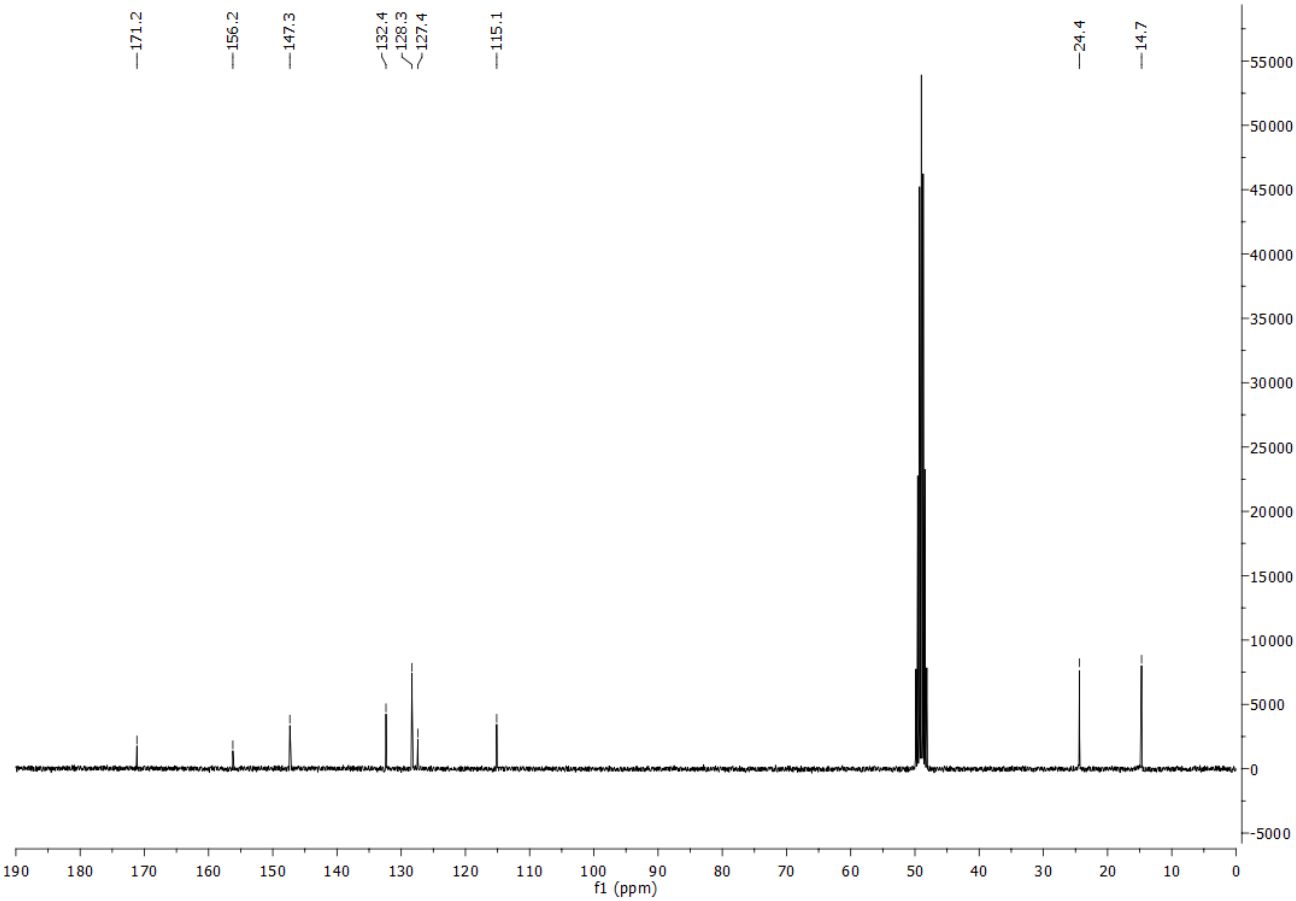


Figure S 34. ^{13}C NMR of (*E*)-3-(3,5-diethyl-4-hydroxyphenyl)acrylic acid (**1l**) (MeOD)

7 References

[1] C. Wuensch, T. Pavkov-Keller, G. Steinkellner, J. Gross, M. Fuchs, A. Hromic, A. Lyskowski, K. Fauland, K. Gruber, S. M. Glueck, et al., *Adv. Synth. Catal.* **2015**, *357*, 1909–1918.

[2] H. Chen, H. Jiang, J. A. Morgan, *Phytochemistry* **2007**, *68*, 306–311.

[3] E. Busto, R. C. Simon, W. Kroutil, *Angew. Chemie Int. Ed.* **2015**, *54*, 10899–10902.

[4] D. Petras, D. Kerwat, A. Pesic, B.-F. Hempel, L. von Eckardstein, S. Semsary, J. Arasté, M. Marguerettaz, M. Royer, S. Cociancich, et al., *ACS Chem. Biol.* **2016**, *11*, 1198–1204.