Supporting Information

Enzyme Cascade Reactions: Synthesis of Furandicarboxylic Acid (FDCA) and Unactivated Carboxylic Acids

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1. General experimental information and materials

The *E. coli* TP1000 mutant strain used for PaoABC expression is a derivative of MC4100 with a kanamycin cassette inserted in the mobAB gene region. *E. coli* xanthine dehydrogenase and catalase were sourced from Sigma-Aldrich. Starting materials were purchased from Alfa Aesar and Sigma-Aldrich and used as received. HPLC analysis was performed on an Agilent 1200 system equipped with a G1379A degasser, G1312A binary pump, a G1329 autosampler unit, a G1315B diode array detector and a G1316A temperature controlled column compartment. The columns used were Thermofisher Hypurity C18 (5 µm particle size, 4.6 mm diameter x 250 mm), Thermofisher ODS Hypersil C18 (5 µm particle size, 4.6 mm diameter x 250 mm) and Bio-Rad Aminex HPX-87H, 300 mm × 7.8 mm pre-packed column. GC analysis was performed on an Agilent 7890A chromatograph using a Alltech SE-30, 30.0 m x 320 µm x 0.25 µm column. Conditions are indicated separately for each compound. ^{1}H spectra were recorded on a BrukerAvance 400 or 500 without additional internal standard.

2. Preparation of biocatalysts

Galactose oxidase variant $M_{3\text{-}5}$ (GOase $M_{3\text{-}5}$)

GOase mutant $M_{3\text{-}5}$[1] was transformed into *E. coli* BL21 Star™ (DE3) cells (Invitrogen) according to manufacturer's specifications. A single colony was picked from an overnight LB plate containing 1 µL of kanamycin of a 30 mg/mL stock solution per mL of agar and used to inoculate 5 mL LB medium supplemented with 5 µL kanamycin and grown overnight at 37 °C and 250 rpm. 500 µL of the overnight culture was used to inoculate 250 mL of an autoinduction medium (8ZY-4LAC) as described by Deacon and McPherson[2] and supplemented with 250 µL of kanamycin in a 2-L-baffled Erlenmeyer flask. The cells were grown at 26 °C and 250 rpm for 60 h. Cells were harvested by centrifugation at 6000 rpm and 4 °C for 20 min and subsequently prepared for protein purification.

Purification of GOase $M_{3\text{-}5}$

The cell pellet from a 250-mL-culture was resuspended in 30 mL lysis buffer containing 50 mMpiperazine-*N*,*N*'-bis(2-ethanesulfonic acid) (PIPES), 25 % sucrose (w/v), 1 mg mL^{-1} lysozyme, 5 mM $MnCl_2$ and 1 % Triton X-100 (v/v). The suspension was gently shaken at 4 °C for 20 min. Afterwards, cells were mechanically disrupted *via* ultrasonication (30 s on, 30 s off; 20 cycles) followed by ultracentrifugation (20000 x *g*, 30 min, 4 °C). The cleared crude extract was transferred into a flexible tubing (30 kDa cut-off), dialysed into buffer C (50 mMNaPi buffer, 300 mMNaCl, pH 8.0) for 12 h at 4 °C and subsequently passed through a syringe filter with a 0.22 µm pore size. Protein purification was accomplished with a peristaltic tubing pump (Thermo Scientific) equipped with a 5-mL-Strep-Tag®-II column (GE Healthcare) pre-equilibrated with buffer C. After loading with crude extract, the column was washed with 5 column volumes of buffer C followed by protein elution with 70 mL of buffer D (50 mMNaPi buffer, 300mM NaCl, 5 mM desthiobiotin, pH 8.0).

For copper-loading, GOase $M_{3\text{-}5}$-containing fractions were pooled and subsequently transferred into flexible dialysis tubing (30 kDa cut-off) and dialysed twice for 12 h into buffer

E (50 mM NaPi buffer saturated with $CuSO_4$, pH 7.4) at 4 °C. Removal of excess $CuSO_4$ was attained by two cycles of dialysis into buffer E (without $CuSO_4$) for 12 h at 4 °C and protein samples concentrated to approximately 3 mg/mL using a *Sartorius* Vivaspin 6 spin column (30 kDa mass cut-off). The protein samples were aliquoted and aliquots were frozen in liquid nitrogen prior to storage at -80 °C.

E. coli perisplasmic aldehyde oxidase (PaoABC)

For PaoABC expression,[3] the plasmid pMN100 derived from pTrcHisA (Invitrogen), containing the PaoABC genes with a His6 tag fused to the N-terminus of PaoA, was used. For heterologous expression in *E. coli*, pMN100 was transformed into *E. coli* TP1000 cells, containing a deletion in the mobAB genes responsible for Moco dinucleotide formation. One liter of LB supplemented with 1 mM sodium molybdate and 10 µM isopropyl thio-β-D-galactoside was inoculated with 2 mL of an overnight culture and incubated for 24 h at 22 °C and 100 rpm. The cells were harvested by centrifugation at 4000 x *g* for 15 min.

Purification of PaoABC

The cell pellet was resuspended in 8 volumes of 50 mM sodium phosphate, 300 mM NaCl, pH 8.0, 10 mM imidazole and cell lysis was achieved by sonication (MSE Soniprep) with cooling on ice (20 bursts of 20s on/off at 14u). After addition of DNase I, the lysate was incubated for 30 min. After centrifugation at 17000 x *g* for 25 min the supernatant was filtered through 0.45 and 0.2 µM membranes before loading onto Ni_2-nitrilotriacetic agarose (HiTrap 1mL column (GE Healthcare). The column was washed with 2 column volumes of 10 mM imidazole, 50 mM sodium phosphate, 300 mM NaCl, pH 8.0, followed by a wash with 10 column volumes of the same buffer with 20 mM imidazole. His-tagged PaoABC was eluted with 20 mL of 100 mM imidazole in 50 mM sodium phosphate, 300 mM NaCl, pH 8.0. Fractions containing PaoABC were buffer exchanged into 50 mM Tris, 1 mM EDTA, pH 7.5. The yield of protein was about 13 mg/L of *E. coli* culture.

Xanthine dehydrogenase variants E232V and E232VR310 (XDH E232V, XDH E232VR310)[4,5]

For expression of XDH mutants, the plasmid pSL207 derived from pTrcHisA (Invitrogen), containing the xdh genes with a His6 tag fused to the N-terminus of XDHA, was used. For heterologous expression in *E. coli*, pSL207 was transformed into *E. coli* TP1000 cells, containing a deletion in the mobAB genes responsible for Moco dinucleotide formation. The enzyme was expressed in 500-mL-cultures of TP1000 cells carrying plasmid pSL207 grown at 30 °C in LB medium supplemented with 150 µg/mL ampicillin, 1 mM molybdate, and 0.02 mM isopropyl-D-thiogalactopyranoside until the OD_{600nm} = 1. This culture was then transferred to a bottle containing 8 liters of supplemented LB medium and subsequently grown at 30 °C for 18 - 20 h. Cells were harvested by centrifugation at 5000 x *g* at 4 °C and subsequently prepared for protein purification.

Purification of XDH E232V and XDH E232VR310

The cell pellet was resuspended in 8 volumes of 50 mM sodium phosphate, 300 mM NaCl, pH 8.0, and cell lysis was achieved by several passages through a French press. After addition of DNase I, the lysate was incubated for 30 min. After centrifugation at 17000 x *g* for 25 min, imidazole was added to the supernatant to a final concentration of 10 mM. The

supernatant was mixed with 2 mL of Ni_2-nitrilotriacetic agarose (Qiagen) per liter of cell growth, and the slurry was equilibrated with gentle stirring at 4 °C for 30 min. The slurry was poured into a column, and the resin was washed with 2 column volumes of 10 mM imidazole, 50 mM sodium phosphate, 300 mM NaCl, pH 8.0, followed by a wash with 10 column volumes of the same buffer with 20 mM imidazole. His-tagged XDH was eluted with 100 mM imidazole in 50 mM sodium phosphate, 300 mM NaCl, pH 8.0. Fractions containing XDH were combined and dialyzed against 50 mM Tris, 1 mM EDTA, 2.5 mM dithiothreitol, pH 7.5. The dialyzed sample was applied to a Q-Sepharose fast protein liquid chromatography column and eluted with a linear gradient of 0-250 mM NaCl. To the pool of fractions containing XDH, 15 % ammonium sulfate was added, and the protein was then applied to a phenyl-Sepharose column equilibrated with 50 mM Tris, 1 mM EDTA, 2.5 mM dithiothreitol, 15 % ammonium sulfate, pH 7.5. XDH E232V was eluted from the column with a linear gradient of 15 to 0 % ammonium sulfate. During purification, fractions were monitored using SDS-PAGE, whereas enzyme activity was measured spectrophotometrically as described earlier.[4]

3. Screening of xanthine oxidoreductases for oxidation of 1, 4 and 5

Table S1. Screening of XORs.

Entry[a]	Enzyme	HMF **1**	DFF **4**	FFCA **5**	Oxidant
1	*E.coli* XDH[b]	Active	Not active	Not active	O_2
2	XDH E232V[c]	Active	Active	Active	DCPIP
3	XDH E232V R310[d]	Active	Active	Active	DCPIP
4	PaoABC[e]	Active	Active	Active	O_2

[a] Reaction conditions: Potassium phosphate buffer (50 mM, pH 7.6), 3 µL 0.1 M 1 (in MeCN), 30 µL 0.01 M DCPIP (aq.) final volume 300 µL, 36°C. Activity was determined by the colour change from blue to colourless; [b] *E. coli* XDH (1.1 mg/mL); [c] XDH E232V (25.4 mg/mL); [d] XDH E232V/R310M (23 mg/mL); [e] PaoABC (13.3 mg/mL); [b and e] Activity detected using NBT assay[6]

4. Optimisation of the HMF 2-step oxidation cascade

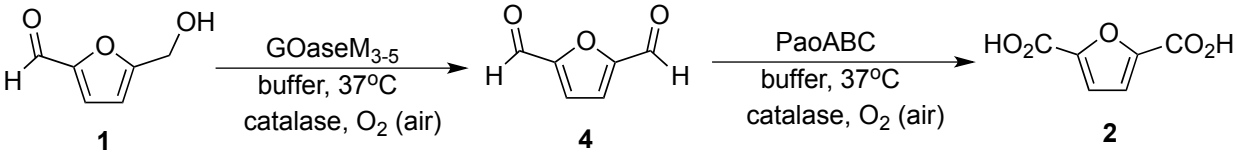


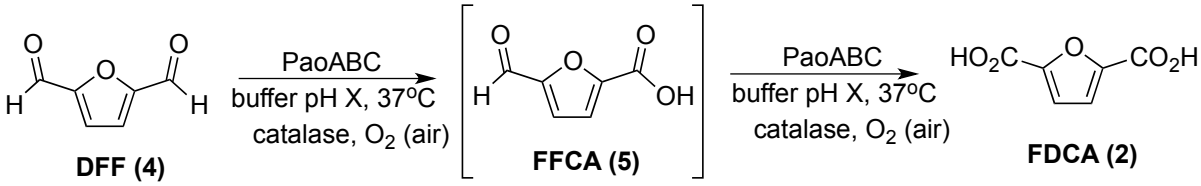
Table S2 . Optimisation of the HMF 2-step oxidation cascade.

Entry[a]	**HMF 1 (mM)**	**pH**	**Buffer (mM)**	**DFF 4[b] (%)**	**FDCA 2[c] (%)**
1	10	7.5	50	-	97
2	20	7.5	50	-	55
3	20	7.5	50	>99	>99
4	30	"	"	>99	>99
5	50	"	"	>99	0

6	"	"	100	>99	>99
7	70	7	300	>99	>99 (80)[e]
8	100	"	400	>99	0
9[d]	"	"	"	>99	>99
10[d]	"	"	"	0	>99 (74)[e]

Reaction Conditions: Entries 1&2 are one-pot with all enzymes present; entries 3-10 are sequential with PaoABC added after complete conversion to **4**. [a] GOase M_{3-5} (3.3 mg/mL) 103 µL, catalase (3.3 mg/mL) 33 µL, 3 µL of xM HMF (in MeCN), potassium phosphate buffer (x mM, pH 7.6) final volume 300 µL, then after full conversion to **4**, PaoABC (13.3 mg/mL) 5 µL. [b] Formation of **4** was monitored via RP-HPLC using a Thermofisher Hypurity C18 column with flow rate 0.6mL/min using 85% water + 0.1% Acetic acid and 15% MeCN. [c] Formation of **2** was monitored via RP-HPLC using a Thermofisher HypurityC18 column with flow rate 1 mL/min using a 98% 10 mM phosphate buffer (pH 6.5) and 2% MeCN mobile phase. [d] Additional quantity of catalase was added with PaoABC. [e] Reactions on preparative scale.

5. Optimisation of DFF (4) oxidation using PaoABC



DFF (4) **FFCA (5)** **FDCA (2)**

To a solution of potassium phosphate buffer was added **4** (2 M in MeCN), 33 µl catalase (3.3 mg/mL) and PaoABC (13.3 mg/mL), final volume 300 µL. The reaction was vigorously shaken and placed in a shaking incubator at 37˚C. Aliquots of the reaction mixture were taken out, acidified with 2 M HCl and analysed by RP HPLC.

HPLC Conditions: Thermo Fisher Hypurity C18, 98% 10 mM phosphate buffer pH 6.5, 2% MeCN with a flow rate of 1 mL/min.

Table S3. PaoABC catalysed oxidation of DFF (**4**).

Entry	DFF (4) (mM)	pH[c]	Buffer (mM)	PaoABC (µL)	Time (h)	FFCA (5) (%)	FDCA (2)[b] (%)
1	50	6	200	5	2	100	0
2	"	7	200	"	"	0	>99
3	"	8	200	"	"	0	>99
4	"	9	200	"	"	18	0
5	80	7	200	"	4	0	>99
6	"	8	200	"	"	0	66
7[a]	100	7	200	"	16	40	60
8	**"**	**7**	**400**	**"**	**6**	**0**	**>99**

[a] pH after 16 h = 4.5. [b] Conversion adjusted by analysing a 1:1 standard of the aldehyde:acid by NMR and comparing the HPLC trace of the same sample and adjusting the absorbance accordingly. [c] Initial pH.

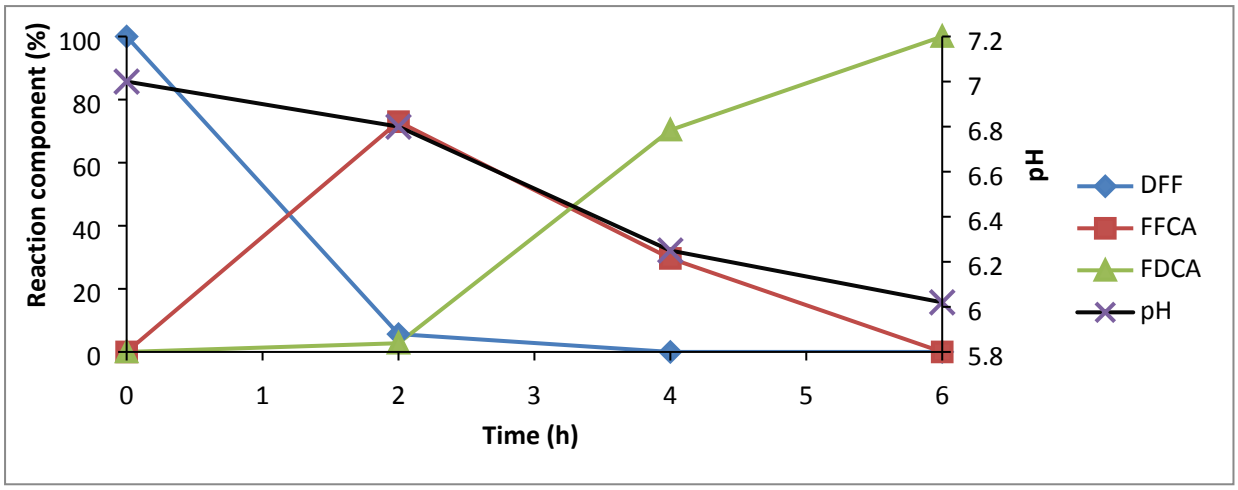
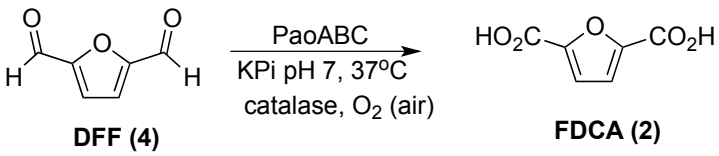


Figure S1. Time course of FDCA **5** production showing change in pH over time for entry 8.

5.1 Preparative scale oxidation of DFF (4) with PaoABC



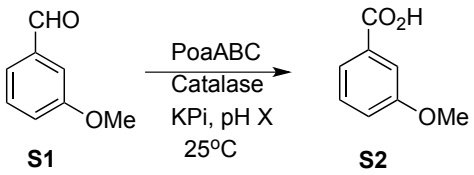
DFF (4) **FDCA (2)**

To a solution of 400 mM pH 7 phosphate buffer was added DFF (**4**) (37 mg, 0.29 mmol), 330 µl catalase (3.3 mg/mL), 150 µL MeCN and 50 uL PaoABC (13.3 mg/mL), final volume 3 mL. The reaction was vigorously shaken and placed in a shaking incubator at 37°C. The pH was maintained at pH 7 by the careful addition of 1 M NaOH. After this time the reaction was heated to 80°C for 5 minutes and left cool. The solution containing denatured protein was centrifuged and the supernatant removed. The supernatant was then cooled to 0°C and concentrated HCl was added until a precipitate formed. The solution was then centrifuged and the supernatant removed and the pellet washed with 1 M HCl. The pellet was dissolved in acetone and then concentrated *in vacuo* three times yielding FDCA (**2**) as a slight yellow solid (41 mg, 0.26 mmol, 90%).

^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 13.63 (bs, 2H), 7.29 (s, 2H)

^{13}C (500 MHz, DMSO-d_6) δ ppm: 159.4, 147.5, 118.86

6. pH-Profile for oxidation of *m*-anisaldehyde S1 with PaoABC



S1 **S2**

3 µL of a 1 M solution of *m*-anisaldehyde **S1** was added to 33 µL catalase (3.3 mg/ml) in 262 µL of various pH phosphate buffer (50 mM). 1 µL of PaoABC was then added and the reaction was shaken vigorously. Aliquots of the reaction mixture were removed, acidified with 2 M HCl, centrifuged and analysed by RP HPLC.

RP-HPLC Conditions: Thermofisher Hypurity C18 (5 µm particle size, 4.6 mm diameter x 250 mm) with eluent system 25% MeCN, 75% water + 0.1%TFA at 1mL/min.

Table S4. PaoABC catalysed oxidation of **S1**.

		Conversion (%)[a]				
Entry	**pH**	**20min**	**40min**	**60min**	**90min**	**120min**
1	5	5.68	11.27	14.03	31.48	46.2
2	5.5	9.32	14.2	23.15	38.9	54.6
3	6	12.3	30.6	39.2	63.5	82.2
4	6.5	11.8	20.2	41.2	62.2	89.2
5	7	16.2	34.7	50.2	72.2	90.2
6	7.5	11.2	28.2	34.2	65.2	83.2
7	8	12.2	26.2	-	72.2	91.2

[a] Conversion adjusted by analysing a 1:1 standard of the aldehyde:acid by NMR and comparing the HPLC trace of the same sample and adjusting the absorbance accordingly.

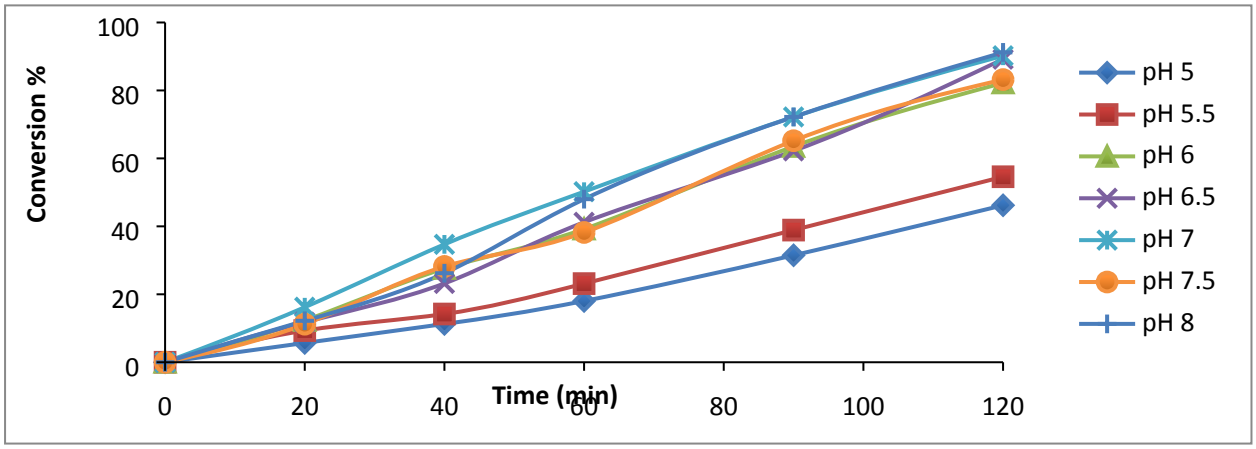


Figure S2. Rate of oxidation of **S1** by PaoABC at different pHs.

7. NMR-analysis of DFF (4) and FFCA (5) at pH 5-8 in deuterated buffers (KPi buffer, 400mM)

7.1 DFF (4) analysis

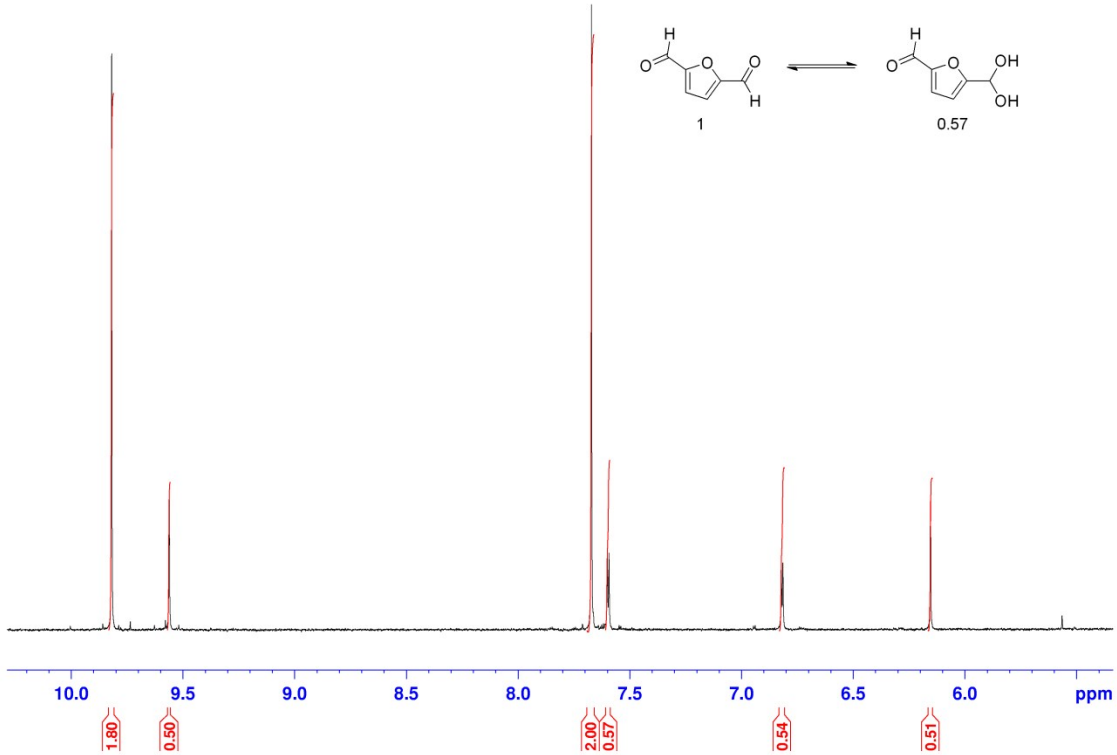


Figure S3. NMR analysis of DFF (**4**) clearly showing hydrate formation, peaks at 9.58, 7.59, 6.82 and 6.15ppm. Ratio of hydrate: aldehyde was identical for each pH.

7.2 FFCA (5) analysis

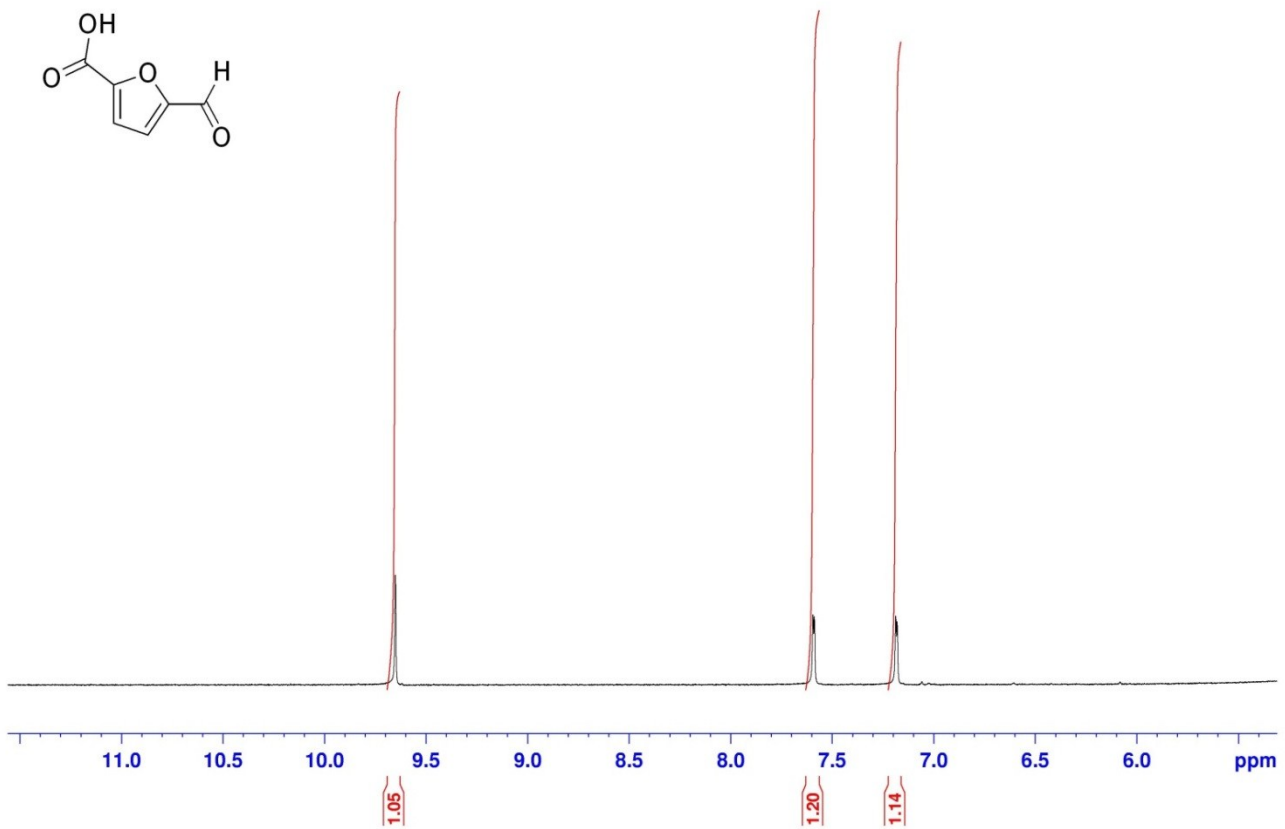
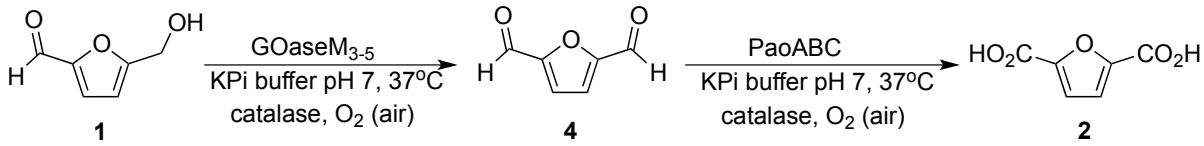


Figure S4. NMR analysis of FFCA (**5**) showing no hydrate formation.

8. Preparative scale synthesis of FDCA (2)

0.1 M HMF (1) 3 mL oxidation procedure



To a solution of 400 mM pH 7 potassium phosphate buffer (1.09 mL), MeCN (0.03mL) and catalase (0.33 mL of a 3.3 mg/mL solution) was added **1** (38 mg, 0.3 mmol) (final concentration = 100mM). GOase M_{3-5} (1.5 mL of a 3.3 mg/mL solution) was then added and the reaction shaken at 37°C in an incubated shaker for 10 h. After this time, another portion of catalase (0.33 mL of a 3.3 mg/mL solution) was added along with PaoABC (0.05 mL of a 13.2 mg/mL solution) and left for another 5 h in the shaking incubator. The pH was carefully monitored and adjusted to pH 7 with 1 M NaOH. After this time the reaction was heated to 80°C for 5 minutes and left to cool. The solution containing denatured protein was centrifuged and the supernatant removed. The supernatant was then cooled to 0°C and concentrated HCl was added until a precipitate formed. The solution was then centrifuged and the supernatant removed and the pellet washed with 1M HCl. The pellet was dissolved in acetone and then concentrated in vacuo three times yielding **2** as a slight yellow solid (35 mg, 0.22 mmol, 74% yield).

^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 13.63 (bs, 2H), 7.29 (s, 2H)

^{13}C (500 MHz, DMSO-d_6) δ ppm: 159.4, 147.5, 118.86

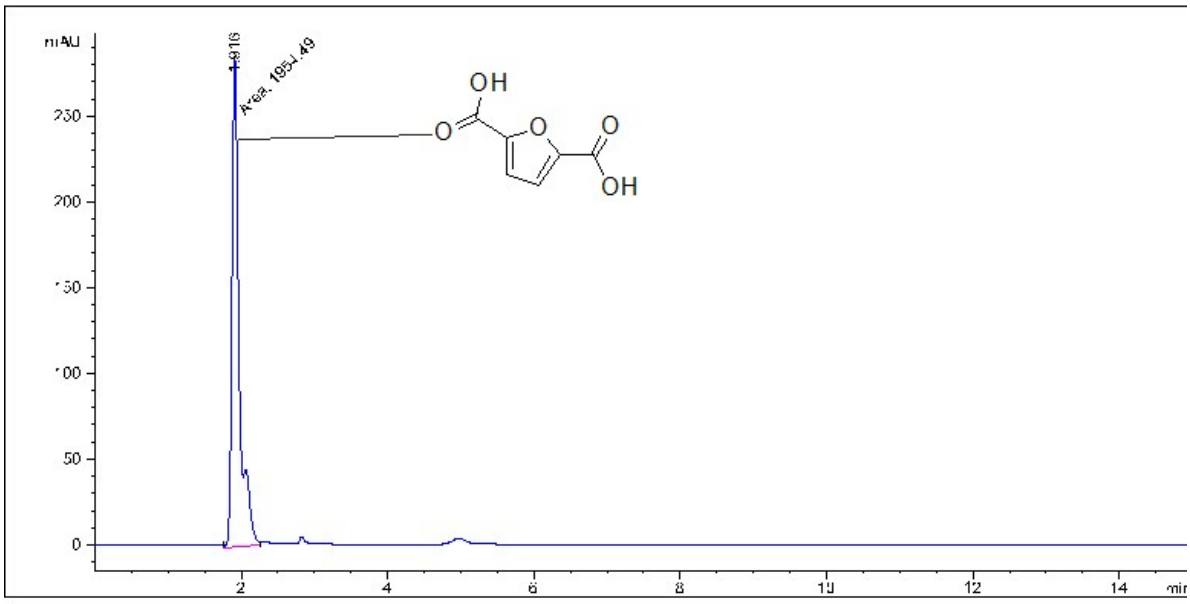


Figure S5. HPLC trace of FDCA (**2**). HPLC conditions: Thermo Fisher Hypurity C18, 98% 10 mM phosphate buffer pH 6.5, 2% MeCN with a flow rate of 1mL/min.

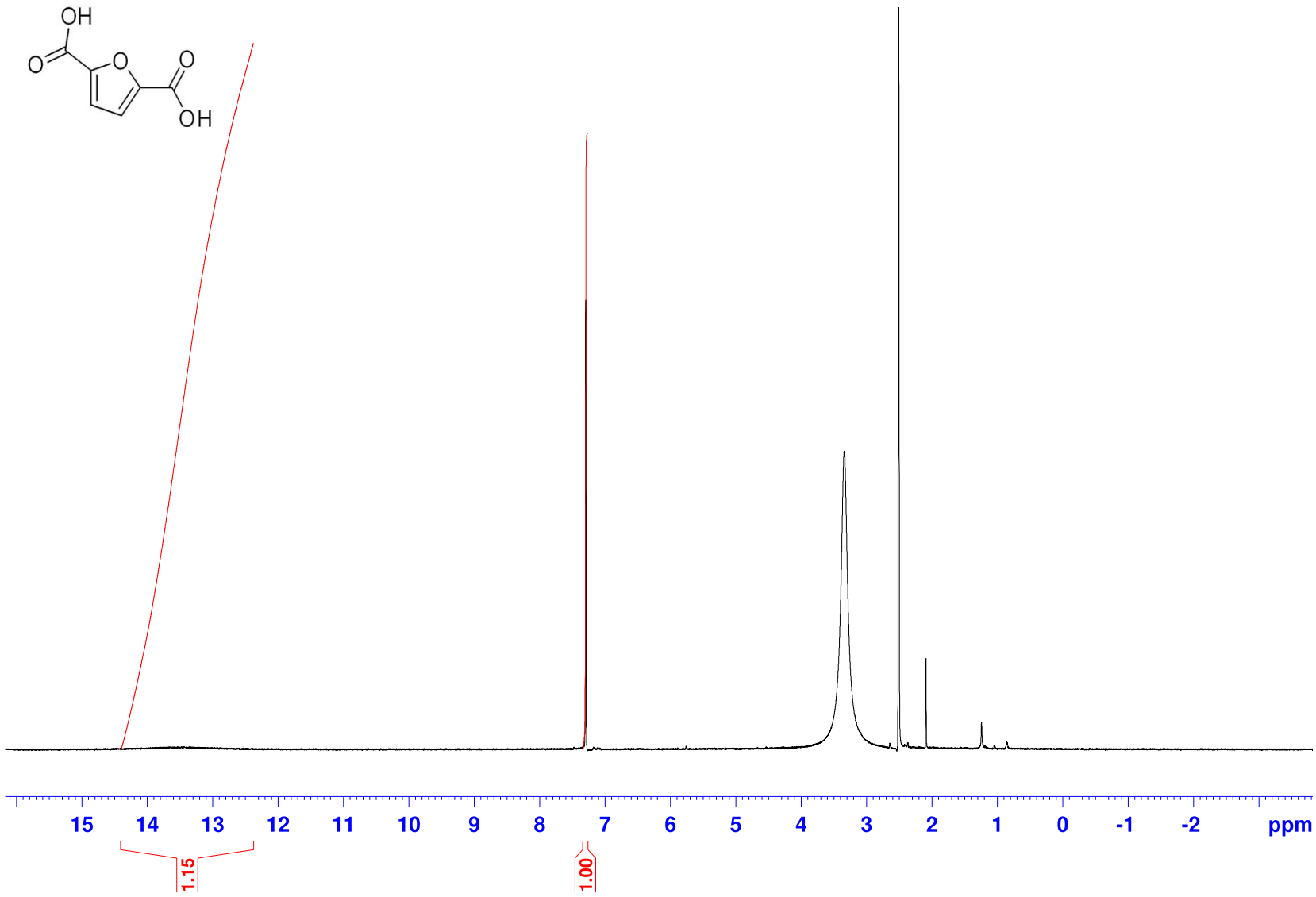
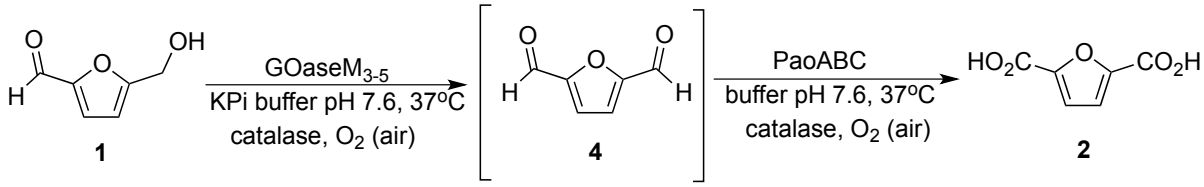


Figure S6. ^{1}H- NMR analysis of recrystallised FDCA (**2)** from section 6.

9. 10 mM and 20 mM combined dual enzyme (GOase M_{3-5} +PaoABC) time course oxidation of HMF (1) to FDCA(2)



To a solution of 200 mM potassium phosphate buffer (pH 7) (156 µL) and catalase 33 µL (3.3 mg/mL) was added HMF **1** (xM in MeCN). 103 µL GOase M_{3-5} (3.3 mg/mL solution) and 5µL PaoABC (13.3 mg/mL) was then added and the reaction incubated at 37°C in a shaking incubator. Aliquots (10 µL) of the reaction were then diluted with 50 µL distilled water and 10 µL 1 M HCl. The aliquot was then centrifuged and analysed by RP-HPLC.

HPLC conditions: Bio-Rad Aminex HPX-87H, 300 mm × 7.8 mm pre-packed column with 100% 0.005 M H_2SO_4 at 60°C with flow rate 0.6mL/min.

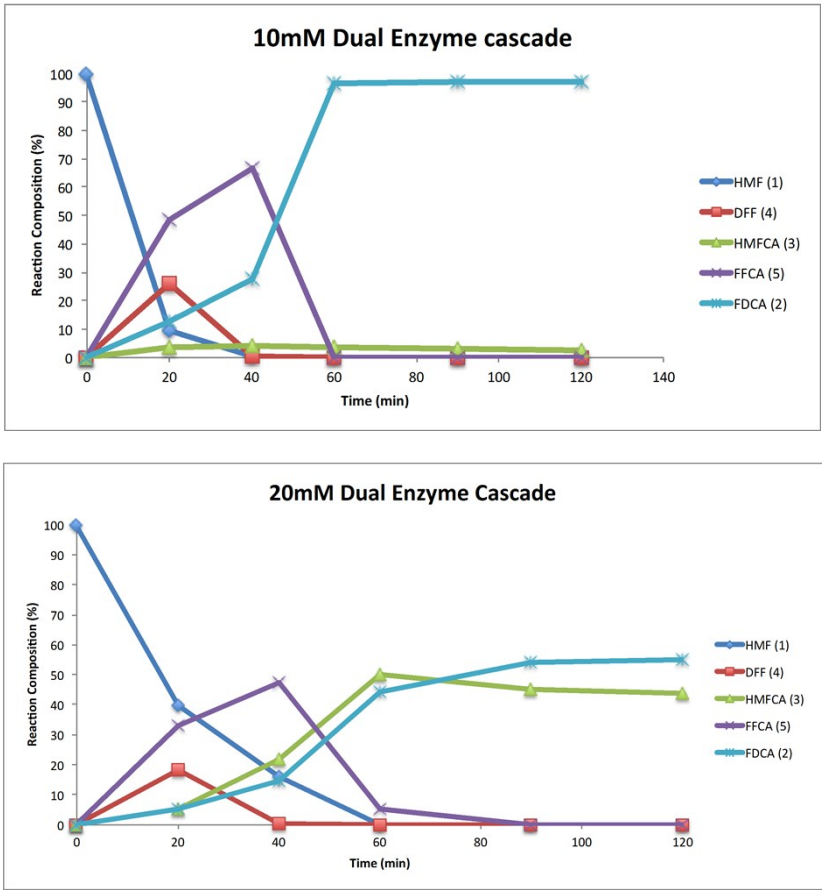


Figure S7. Enzyme cascades for conversion of HMF (**1**) with combined enzymes (GOase M_{3-5} + PaoABC). HMF (**1**):DFF (**4**):HMFCA (**3**):FFCA (**5**):FDCA (**2**).

10. 50 mM 2 step time course oxidation of HMF (1) using GOase M_{3-5} and PaoABC

To a solution of 400 mM potassium phosphate buffer (pH 7) (156 µL) and catalase 33 µL (3.3 mg/mL) was added 7.5 µL **1** (2 M in MeCN). 103 µL GOase M_{3-5} (3.3 mg/mL solution) was then added and incubated at 37°C in a shaking incubator. Aliquots (10 uL) of the reaction were then diluted with 90 µL distilled water and 10 µL 1 M HCl. The aliquot was then centrifuged and analysed by RP-HPLC. After all **1** was oxidised, as determined by HPLC, 5 µL PaoABC (13.3 mg/mL) was added and the reaction placed back into the shaking incubator and aliquots taken as previously.

HPLC conditions: Bio-Rad Aminex HPX-87H, 300 mm × 7.8 mm pre-packed column with 100% 0.005 M H_2SO_4 at 60°C with flow rate 0.6 mL/min.

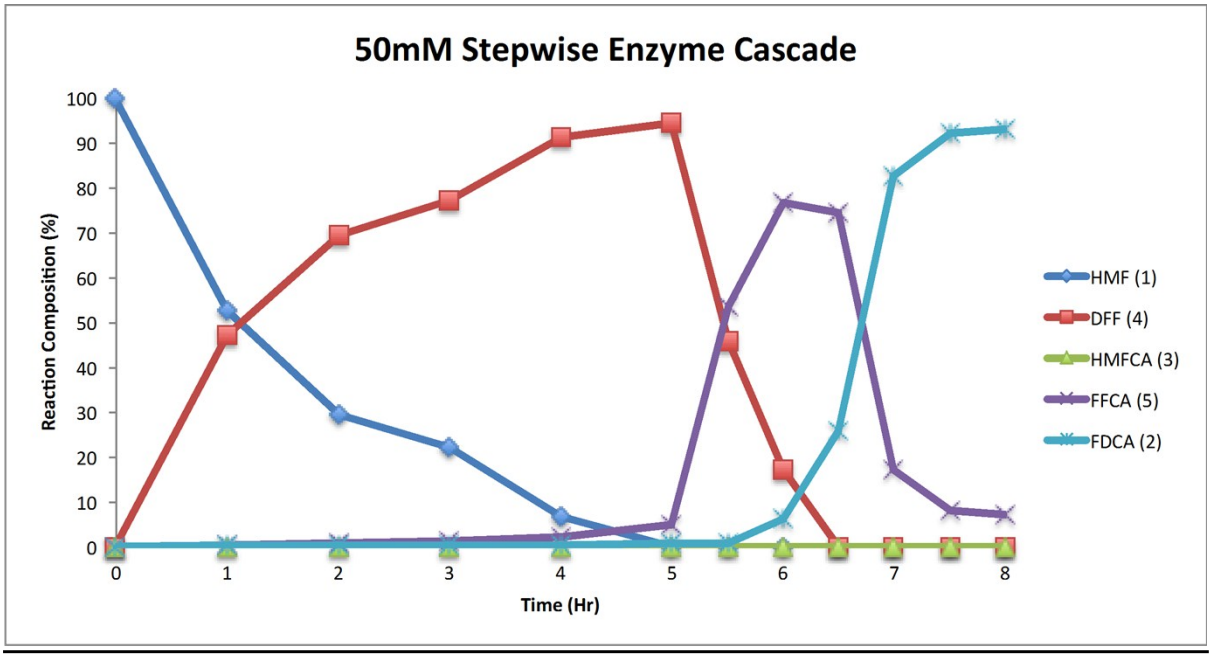


Figure S8. 2-Step oxidation showing high conversion to DFF (**4**) after 5 h and subsequent rapid oxidation of DFF (**4**) to FFCA (**5**) and FDCA (**2**). (NMR analysis was conducted on a 1:1:1:1:1 standard of HMF (**1**):DFF (**4**):HMFCA (**3**):FFCA (**5**):FDCA (**2**) and compared to a HPLC trace of the same sample (relative absorptions were adjusted accordingly).

11.0 Combined dual enzyme (GOase M_{3-5} +PaoABC) time course oxidation of HMF (1) (50mM)

To a solution of 400 mM potassium phosphate buffer (pH 7) (156 µL) and 33 µL of catalase (3.3 mg/mL) was added 7.5 µL HMF (**1**) (2 M in MeCN). 103 µL GOaseM_{3-5} (3.3 mg/mL solution) and 5 µL PaoABC was then added and incubated at 37°C in a shaking incubator. Aliquots (10 µL) of the reaction was then diluted with 90 µL distilled water and 10 µL 1 M HCl. The aliquot was then centrifuged and analysed by RP-HPLC.

HPLC conditions: Bio-Rad Aminex HPX-87H, 300 mm × 7.8 mm pre-packed column with 100% 0.005 M H_2SO_4 at 60°C with flow rate 0.6mL/min.

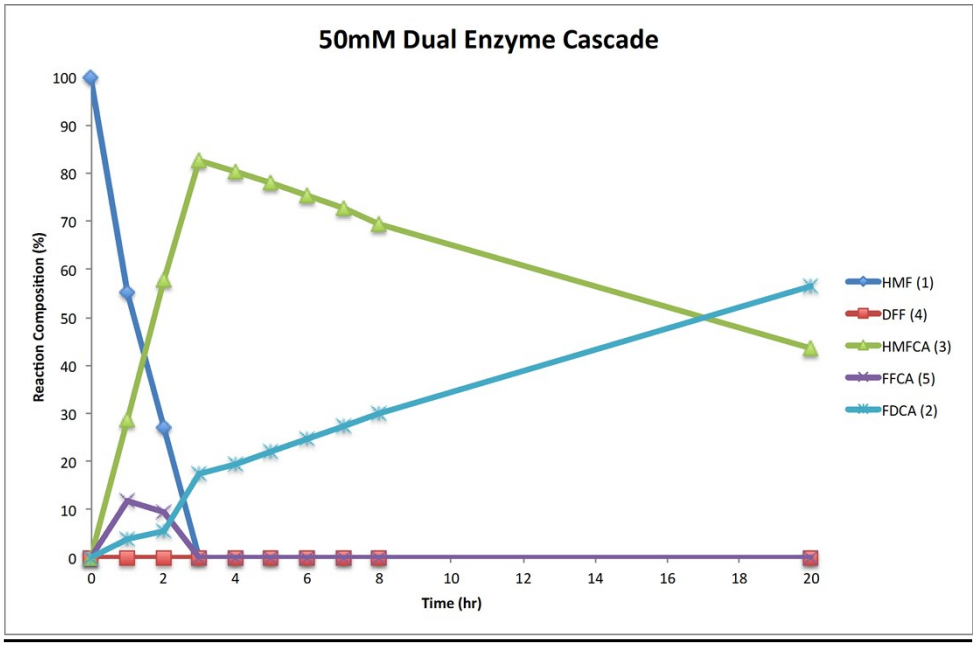
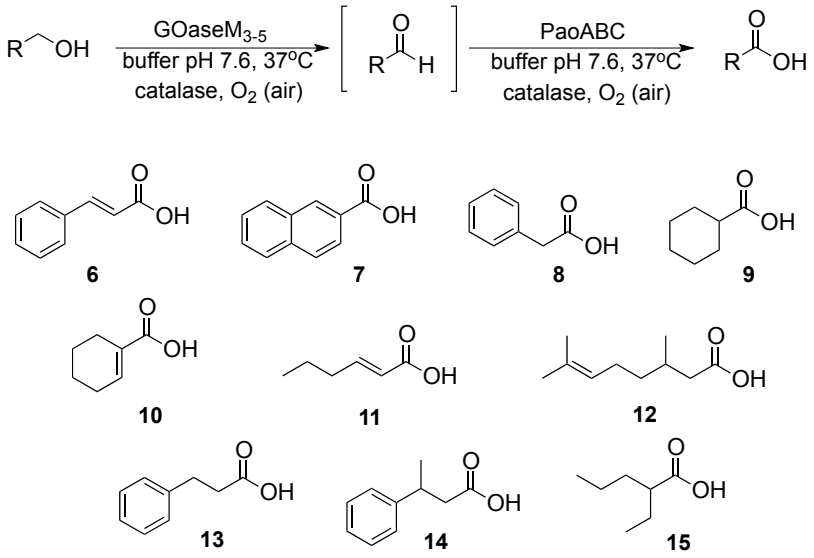


Figure S9. Combined dual enzyme oxidation showing high conversion of HMF (**1**) to HMFCA (**3**) after 2.5 h and slow oxidation to FDCA (**2**) over 20 h. NMR analysis was conducted on a 1:1:1:1:1 standard of HMF (**1**):DFF(**4**):HMFCA(**3**):FFCA(**5**):FDCA(**2**) was compared to a HPLC trace of the same sample (relative absorptions were adjusted accordingly).

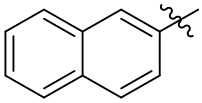
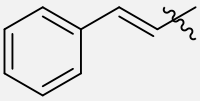
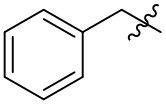
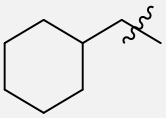
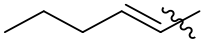
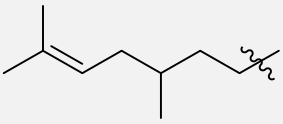
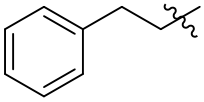
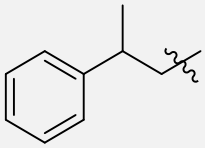
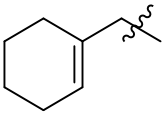
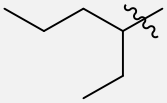
12. GOaseM_{3-5} - PaoABC- oxidation of alcohols 6-15



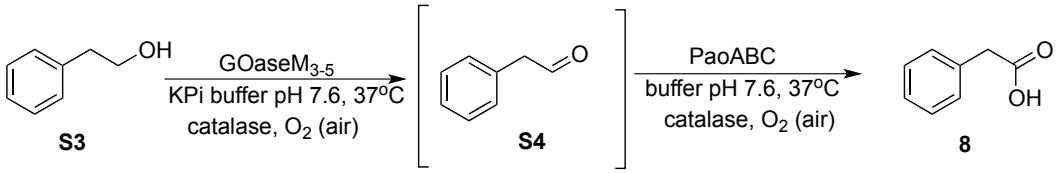
To 50 mM pH 7.6 potassium phosphate buffer (159 µL) and 33 µL catalase (3.3 mg/mL) was added 3 µL of substrate alcohol (1 M in MeCN, 10 mM final concentration). 103 µL of GOase M_{3-5} (1.3 mg/mL) and 5 µL PaoABC (13.2 mg/mL) was then added. The reaction was incubated at 37°C in a shaking incubator and left shake overnight. The reaction was quenched by the addition of 50 µL 1 M HCl and extracted into 200 µL DCM. The DCM was then analysed by GC.

GC Conditions: All samples were analysed using an Alltech SE-30, 30.0 m x 320 µm x 0.25 µm GC capillary column (40°C for 5 min, 50°C/min to 140°C and held for 5 min, 10°C/min to 300°C).

Table S5. GC Retention times of substrates **6**-**15**.

R=	-CH_2OH	-CHO	-CO_2H	Conversion
	14.91	14.52	16.94	100
	11.87	11.25	14.0	100
	8.9	7.57	11.48	100
	7.18	Not available	9.58	100
	4.71	Not available	8.449	100
	10.9	9.68	12.78	100
	10.92	9.6	13	100
	11.49	10.4	13.6	100
	7.6	Not available	11.8	81
	7.8	6.2	10.4	50

13. 10 mM Cascade for oxidation of phenylethanol S3



To a solution of 200 mM pH 7.6 potassium phosphate buffer (159 µL) and 33 µL catalase (3.3 mg/mL) was added 3 µL of substrate (1 M in MeCN, 10mM final concentration). 103 µL of GOase M_{3-5} (3 mg/mL) and 5 µL PaoABC (13.2 mg/mL) was then added and the reaction incubated at 36°C in a shaking incubator and left to shake. 30 µL aliquots of reaction mixture were taken out, acidified by addition of 20 µL of 1M HCl, centrifuged and analysed by RP-HPLC

HPLC conditions: ODS-Hypersil C18 column, 1 mL/min, 82% water (0.1% TFA) 18% MeCN.

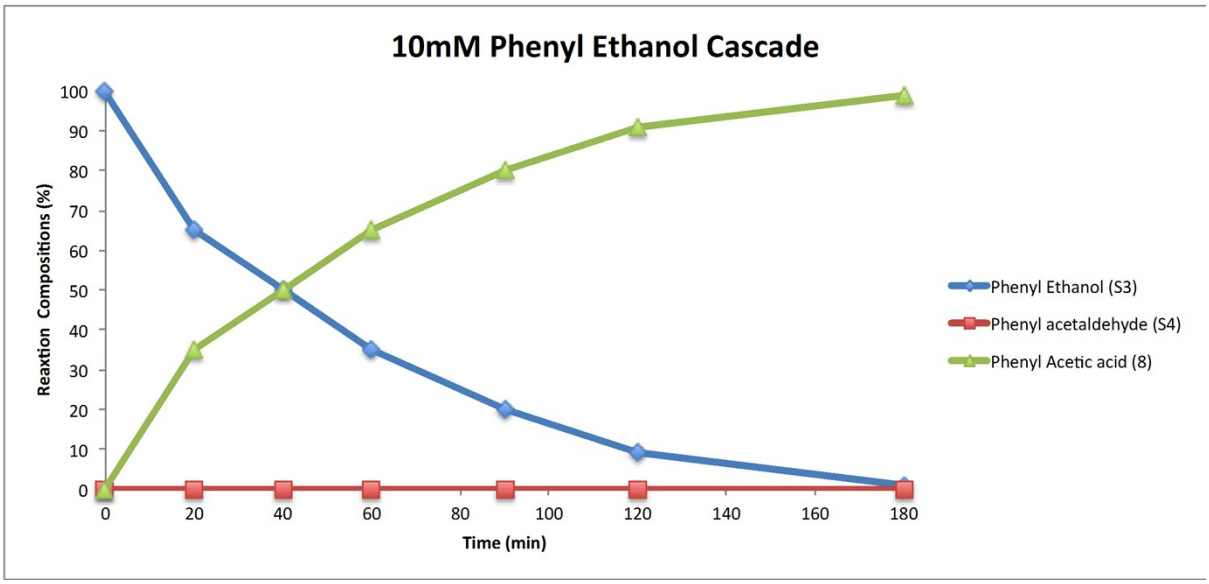
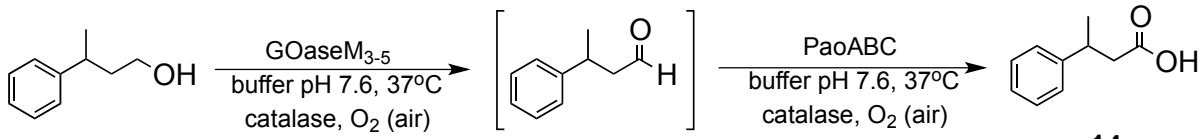


Figure S10. Oxidation of phenyl ethanol (**S3**) showing high conversion to phenyl acetic acid (**8**) after 3 h. NMR analysis was conducted on a 1:1:1 standard of phenylethanol (**S3**): phenylacetaldehyde (**S4**): phenylacetic acid (**8**): and compared to a HPLC trace of the same sample (relative absorptions were adjusted accordingly).

14. GOaseM_{3-5} PaoABC 30mM scale conversion of 3-phenylbutanol S5



S5 **S6** **14**

To a solution of pH 7.6 50 mM phosphate buffer was added 15 µL of 3-phenylbutanol **S5** (2 M in MeCN). GOase M_{3-5} (1.3 mg/mL) and PaoABC (13.3 mg/mL) was then added to final volume 1 mL. The reaction was then placed in a shaking incubator for 16 h. The reaction was then acidified to pH 1 using 1 M HCl and extracted into $CDCl_3$ and analysed by H^1 NMR.

Table S6. 30 mM scale conversion of 3-phenylbutanol **S5**.

Entry	mM	Temp	Catalase (3.3 mg/mL)	GOaseM_{3-5} (1.3 mg/mL)	PaoABC (µL) (13.3 mg/mL)	Conversion (S5:S6:14)[a]
1	30	25	109	268	16.6	62:0:38
2	“	37	“	400	“	30:0:70
3	“	“	180	“	“	12:0:88
4	“	“	“	500	“	15:0:85

[a] Conversion calculated by ^{1}H NMR of crude isolated product.

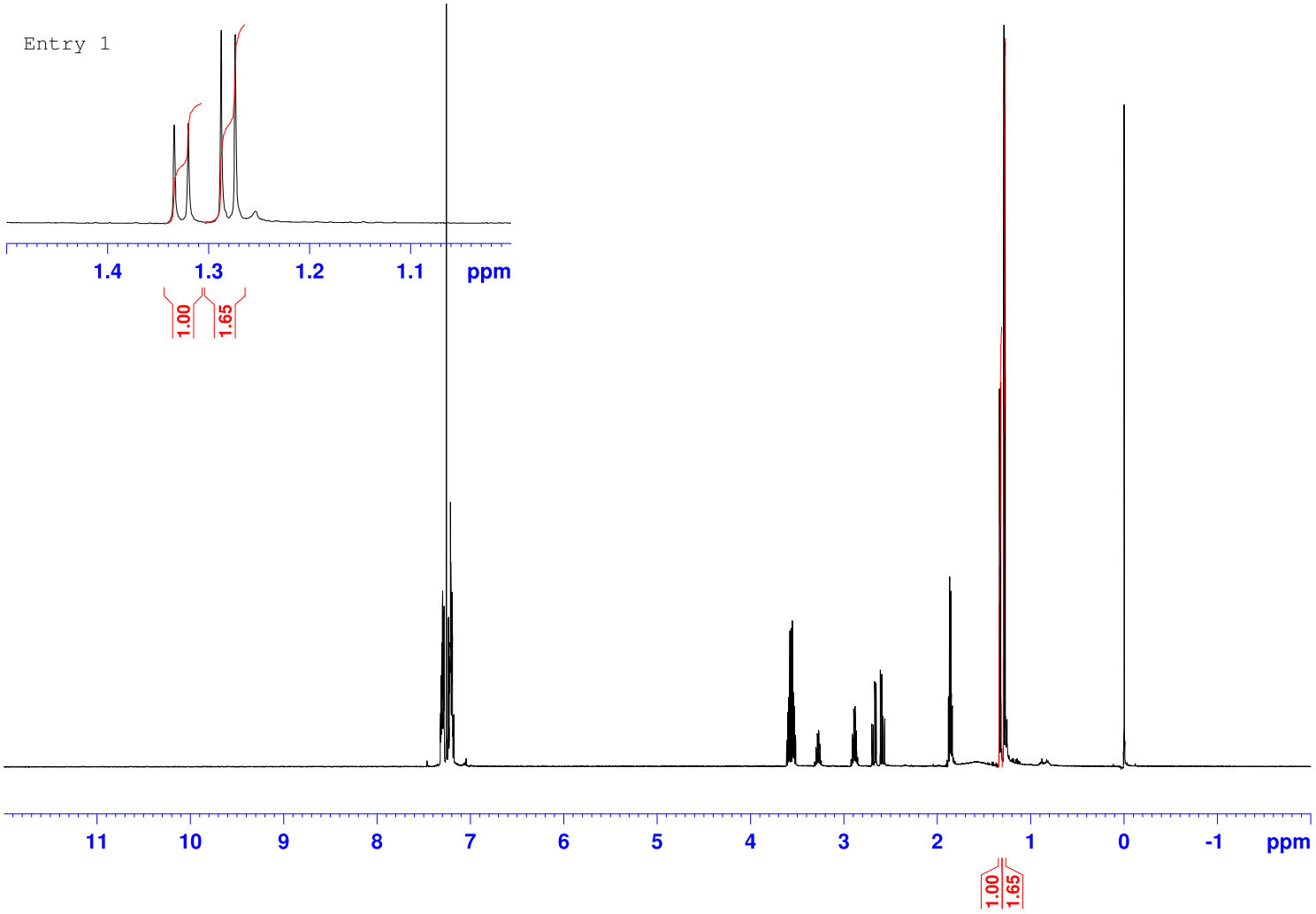


Figure S11. [1] H NMR of crude reaction (entry 1).

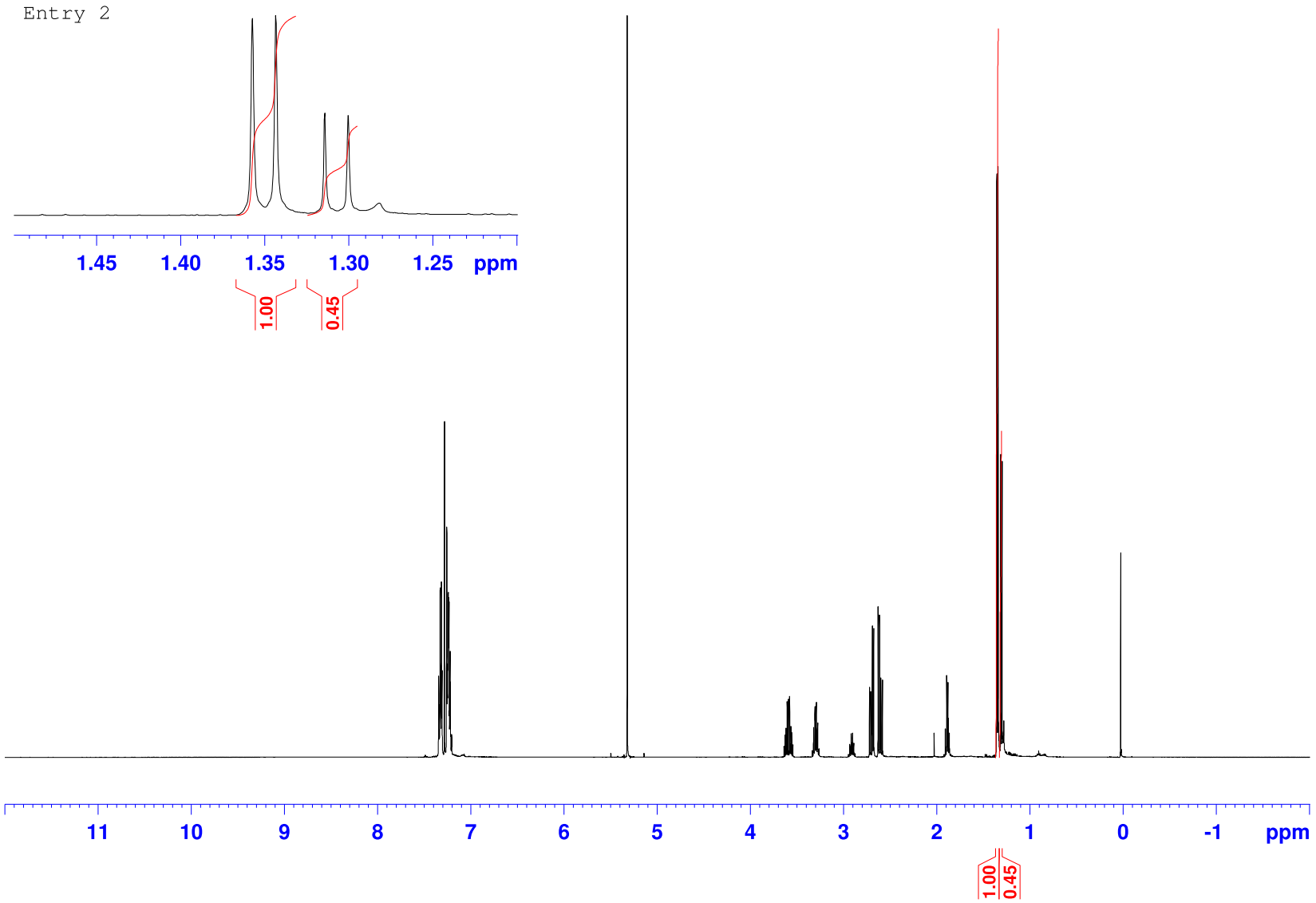


Figure S12. [1] H NMR of crude reaction (entry 2).

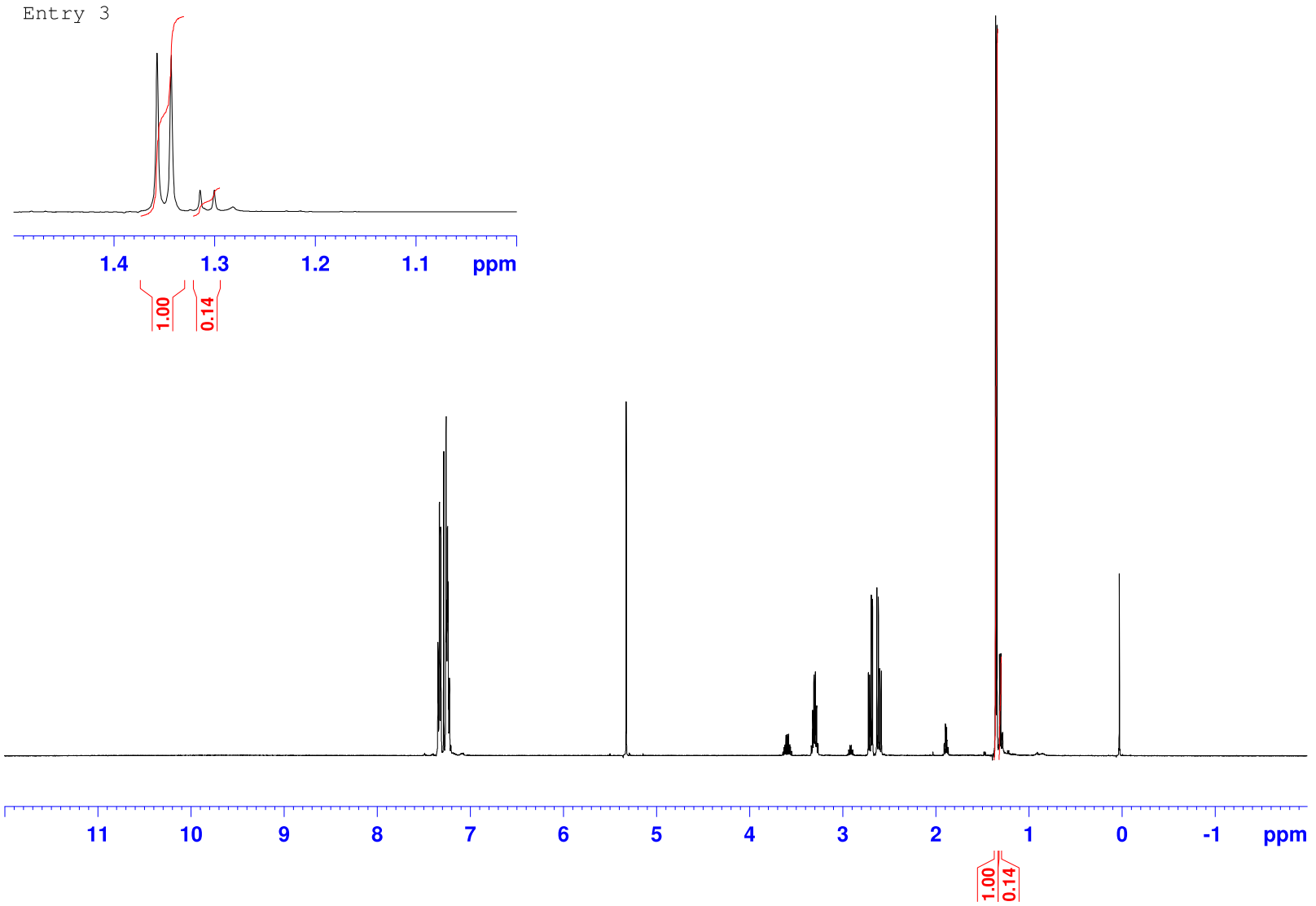


Figure S13. [1] H NMR of crude reaction (entry 3).

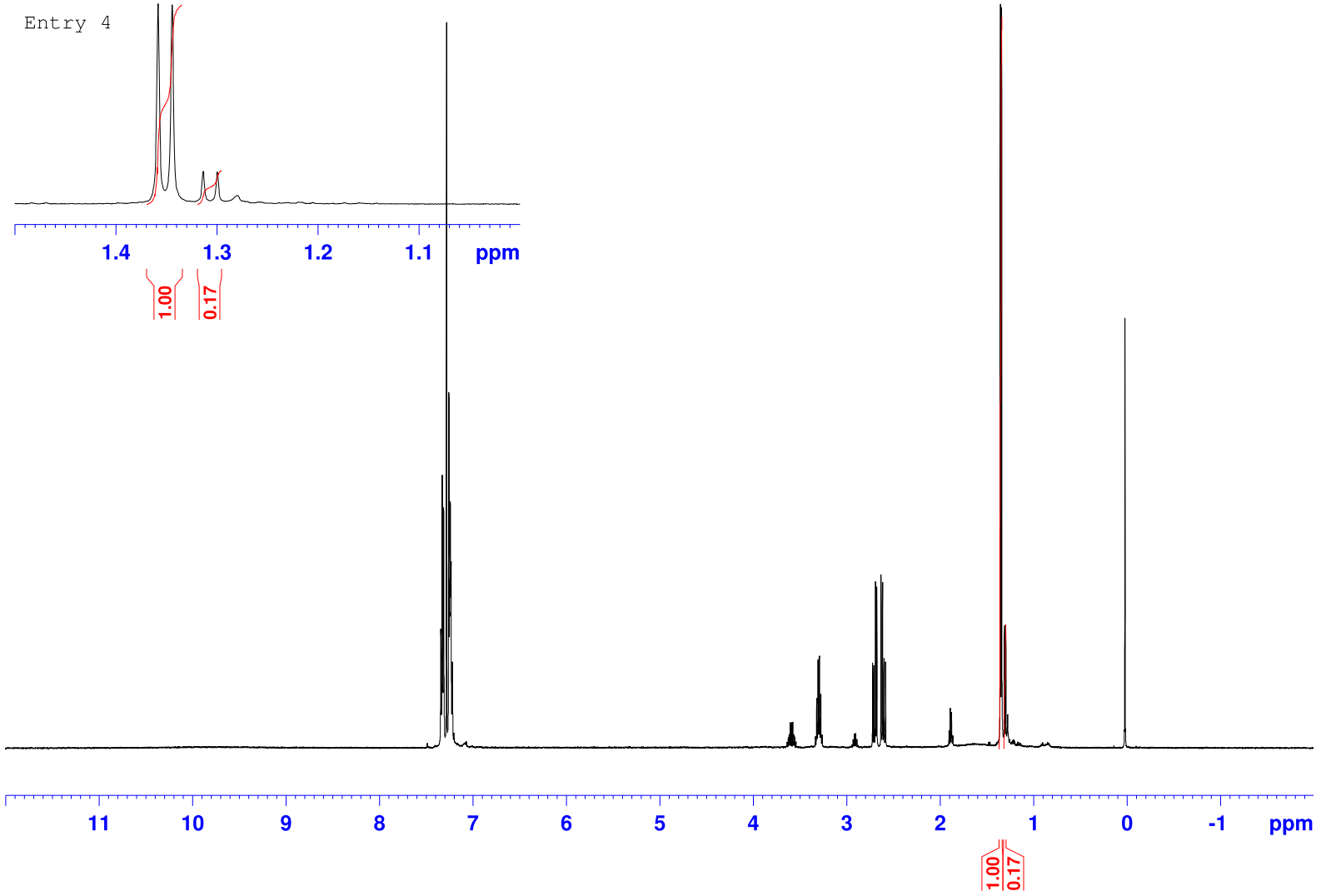
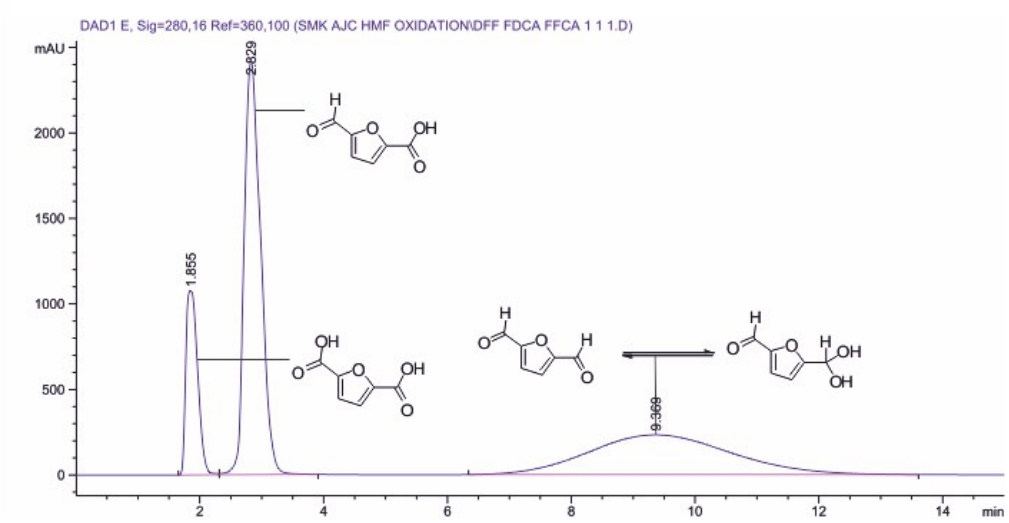


Figure S14. [1] H NMR of crude reaction (entry 4).

15. Standards for absorbance calibration



Signal 1: DAD1 E, Sig=280,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.855	BV	0.2087	1.44457e4	1075.79065	14.9186
2	2.829	VB	0.2709	4.49283e4	2416.34424	46.3992
3	9.369	BB	2.4234	3.74560e4	233.00818	38.6823
Totals :				9.68300e4	3725.14307	

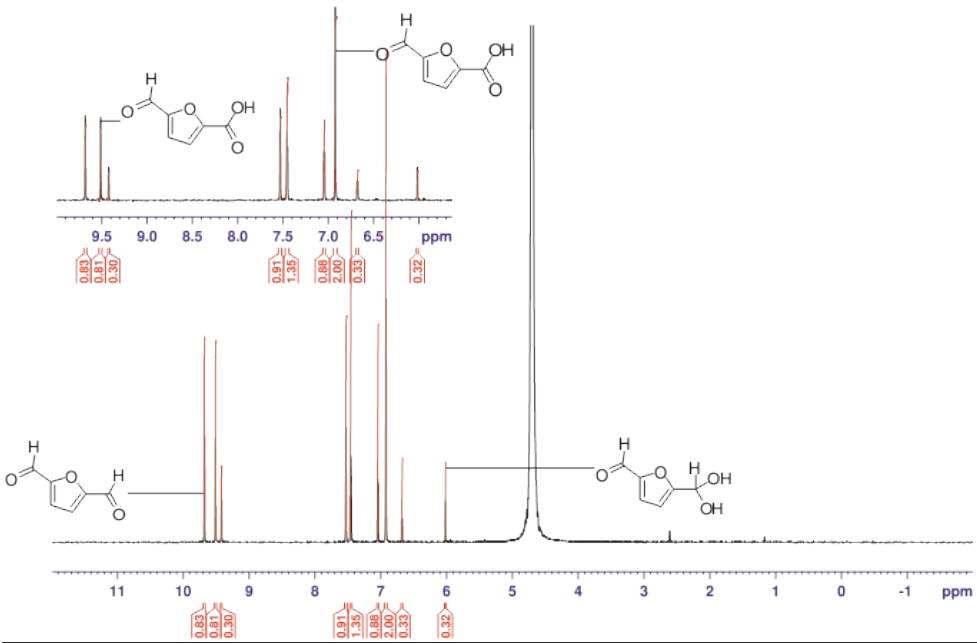
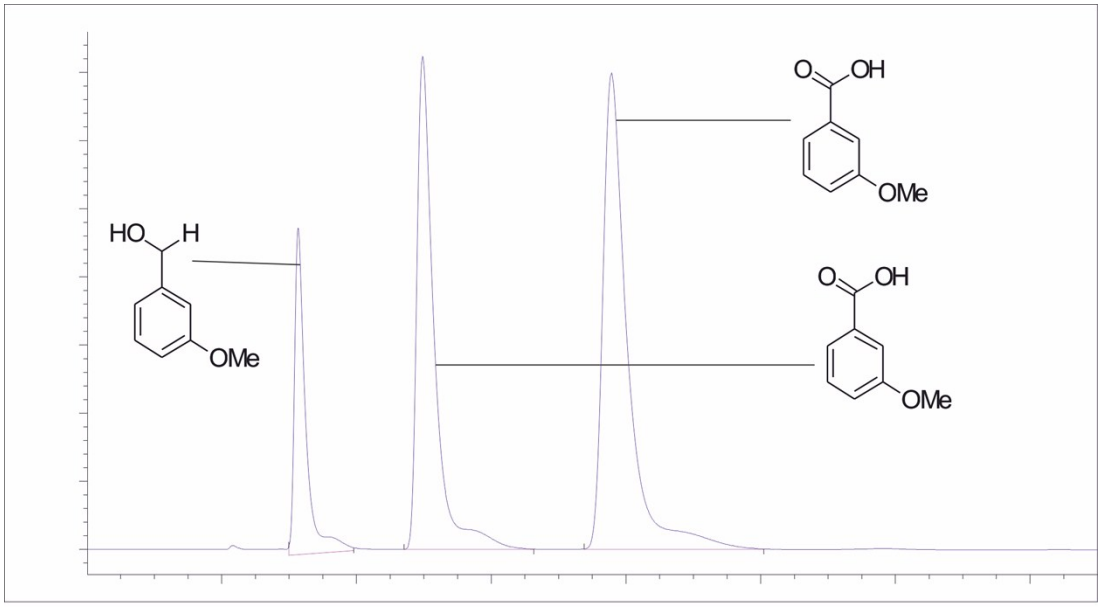


Figure S15. 1:1:1 Standard of HMF (**1**), FDCA (**2**), FFCA (**5**) used to adjust absorbance in section 3. HPLC Conditions: Thermo Fisher Hypurity C18, 98% 10 mM phosphate buffer pH 6.5, 2% MeCN with a flow rate of 1mL/min.



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.135	MM	0.1922	5539.78662	480.34412	15.2654
2	4.981	BB	0.2612	1.29815e4	723.98010	35.7718
3	7.783	BB	0.3704	1.77685e4	699.28815	48.9628

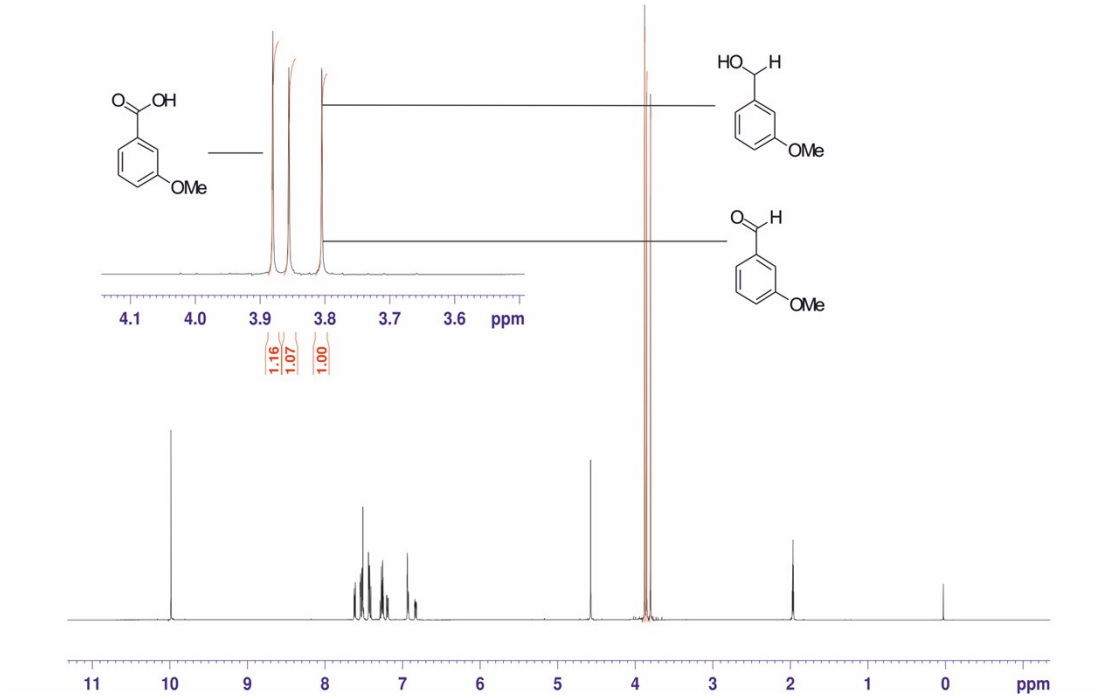


Figure S16. 1:1:1 standard of 3-methoxybenzyl alcohol, aldehyde (**S1)** and acid (**S2**) used to adjust absorbance in section 4.0. HPLC Conditions: Thermofisher Hypurity C18 (5 µm particle size, 4.6 mm diameter x 250 mm) with eluent system 25% MeCN, 75% water + 0.1%TFA at 1 mL/min.

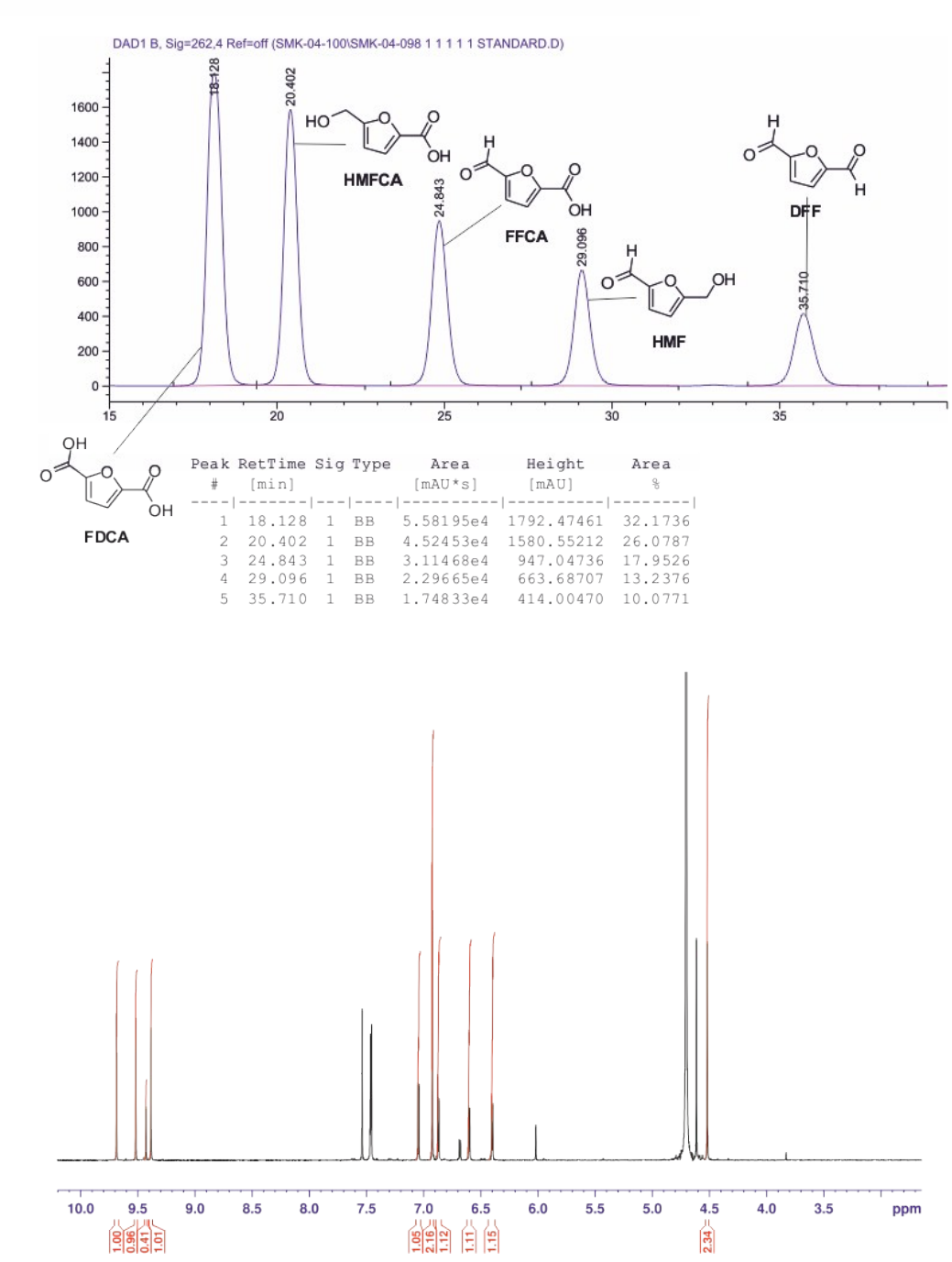


Figure S17. 1:1:1:1 standard of HMF (**1**), DFF (**4**), HMFCA (**3**), FFCA (**4**) and FDCA (**2**) used to adjust absorbance in section 7.0. Peaks used in NMR are FFCA = 9.67, DFF = 9.51, DFF Hydrate = 9.421, HMF = 9.38, FDCA = 6.92 and HMFCA = 4.4. HPLC Conditions: Bio-Rad Aminex HPX-87H, 300 mm × 7.8 mm pre-packed column with 100% 0.005 M H_2SO_4 at 60°C with flow rate 0.6 mL/min.

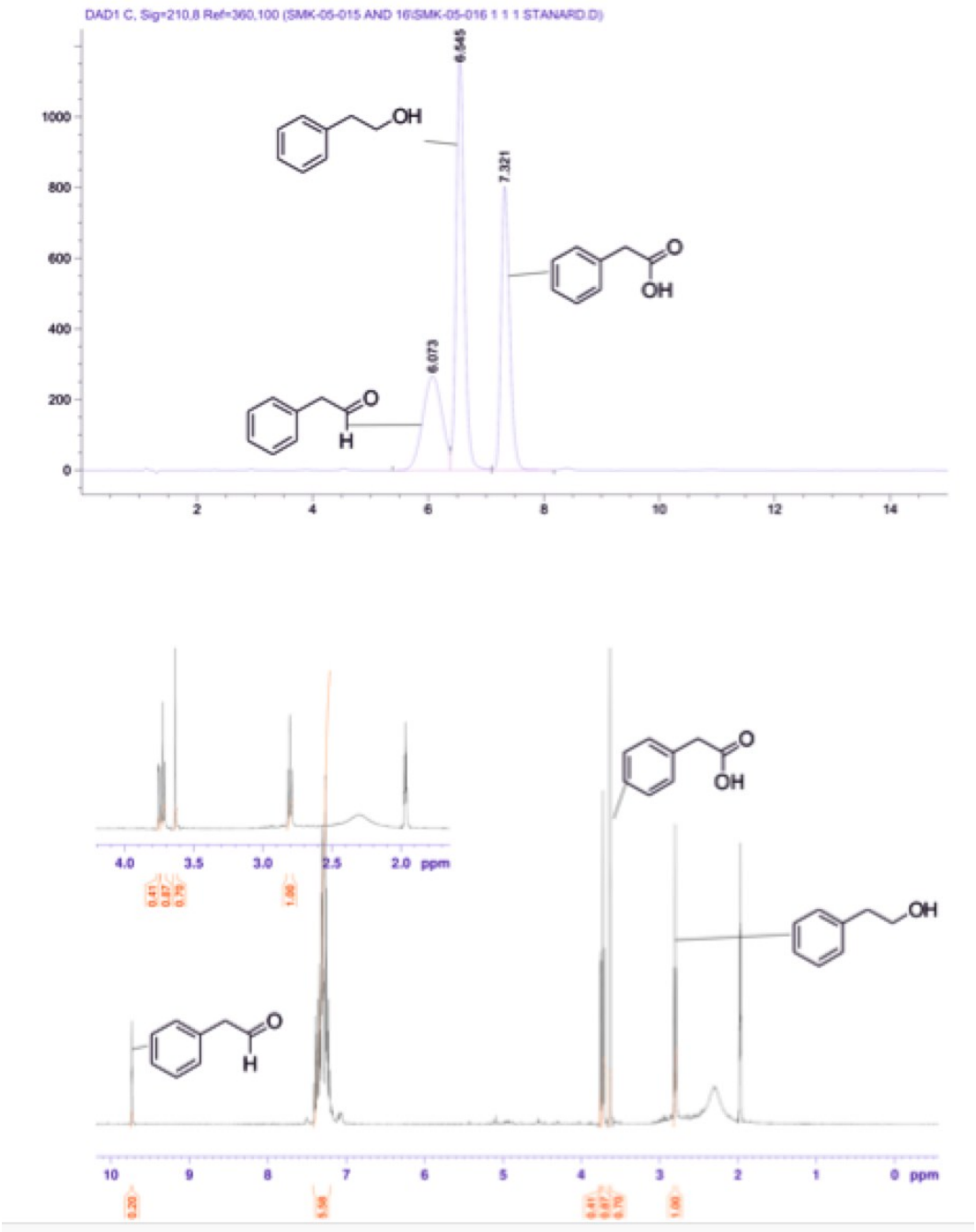


Figure S18. 1:1:1 Standard phenyl acetaldehyde (**S4**): phenyl acetic acid (**8)** used to adjust absorbance in section 11. Peaks used in NMR are phenyl ethanol = 2.81, phenyl acetaldehyde = 9.7, phenyl acetic acid = 3. HPLC Conditions: ODS-Hypersil C18 column, 1 mL/min, 82% Water (0.1% TFA) 18% MeCN.

16. References

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2. Deacon, S.E. and McPherson, J. *ChemBioChem.*, **2011**, *12*, 593-601.

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5. Schumann, S., Terao, M., Garattini, E., Saggu, M., Lendzian, F., Hildebrandt, P., and Leimkühler, S. *PLoS ONE*, **2009**, *4*, e5348.

6. A. Agarwal and U. C. Banerjee, *Open Biotech J.*, 2009, ***3***, 46–49.