Electrical Supporting information

Chemo-enzymatic cascades producing 2,5-furandicarboxylic acid precursors via D-gluconate "barbell oxidation" and dehydration

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Table. S1 Ga5DH amino acids sequence

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MSTSLFDLTG RRALITGSSQ GIGFSLAQGL QAAGADIVLN GRDPEKLSIA AEKLGGVKTL
60
PFDVTDHAGV KAAIDRFEAE TGPIDILVNN AGMQHRAPLE DFPPEMFQKV LQTNLTSIFN
120
VGQAVAKHML GRKSGKIINI ASVQTALARP NISPYTATKG AVGNLTKGMA TEWARHGLQC
180
NAIAPGYFDT PLNAALVKDA DFSQWLEKRT PAGRWGQLDE LTGACIFLAS QASSFVNGHV
240
LYVDGGITVS L
253
```

Fig. S1 Multiple sequence alignment of Ga5DH to known.

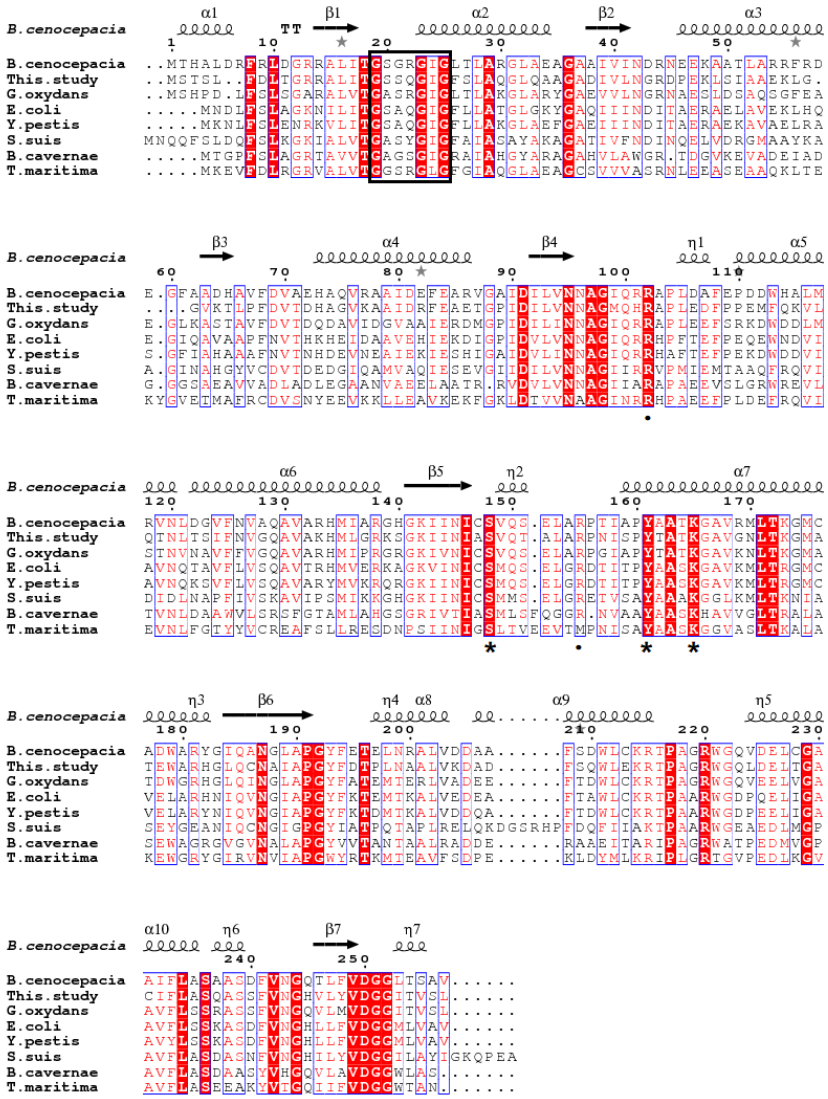

Fig. S1 Strictly conserved residues are highlights. Sequence comparison of Ga5DH from *G. oxydans* H24 with those from *T. maritima*, *E. coli*, *G. oxydans* 621H, *Y. pestis, B. cavernae, B. cenocepacia* and *S. suis* reveals amino acid sequence identities of 38.91%, 51.37%, 58.59%, 48.63%, 37.94%, 59.92% and 41.70%, respectively.

Fig. S2 Results of clone, and purification of Ga5DH.

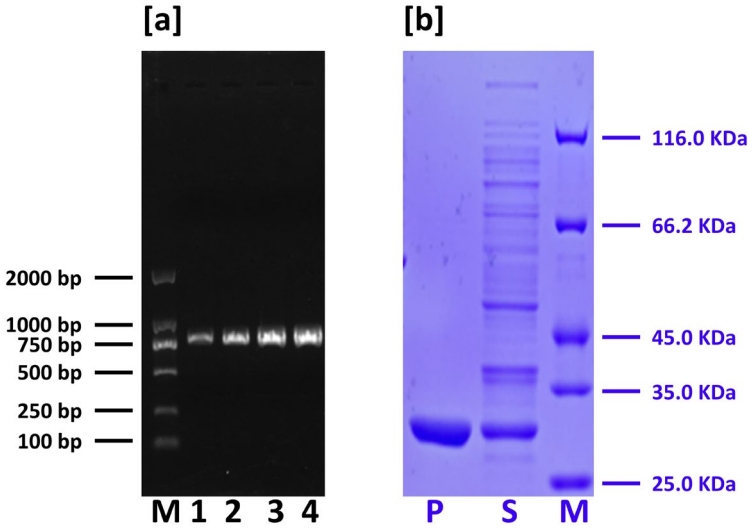

Fig. S2 [a]: Colony PCR of recombinant *E.coli* BL21(DE3) (pET28a-ga5dh). M: DL2000 DNA Marker, 2000, 1000, 750, 500, 250, and 100 bp (from top to bottom). **[b]:** SDS-PAGE analysis of Ga5DH. Lanes P and S: Supernatant and purified protein of Ga5DH (26.6 kDa), M: Protein marker, 116.0, 66.2, 45.0, 35.0, 25.0, 18.4 and 14.4 kDa (from top to bottom).

Table. S2 Kinetic parameters of Ga5DH with cofactor cofactors $NADP^+$ and NAD^+.

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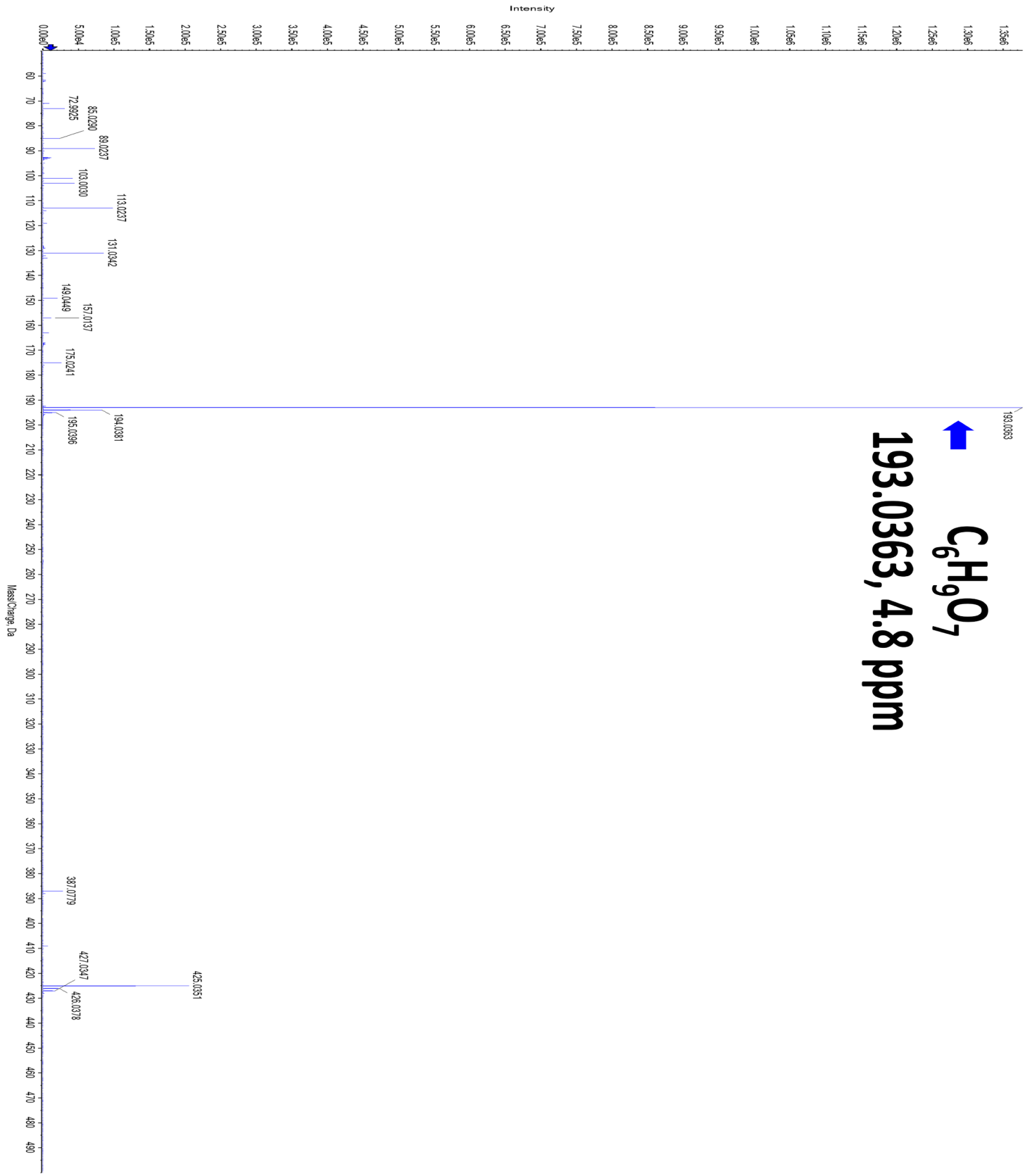
Entry	Cofactor	K_m [mM]	k_{cat}[s^{-1}]	k_{cat}/K_m[$s^{-1}mM^{-1}$]
1	$NADP^+$	1.1	18.25	8.4
2	NAD^+	N.D[b]	N.D[b]	N.D[b]

[a] Reaction conditions: cofactors (0.1-1.0mmol), GA (50 mmol), pure Ga5DH, Tris-HCl buffer (100 mM, pH 8.0, 1 mL), and 30°C. [b] not detected.

Fig. S3 HRMS results of bio-oxidation product.



*Negative TOF MS, range 50-500

Fig. S4 Influences of temperature, pH and metal ions on the Ga5DH, and Michaelis-Menton curve fit of Ga5DH with GA and $NADP^+$.

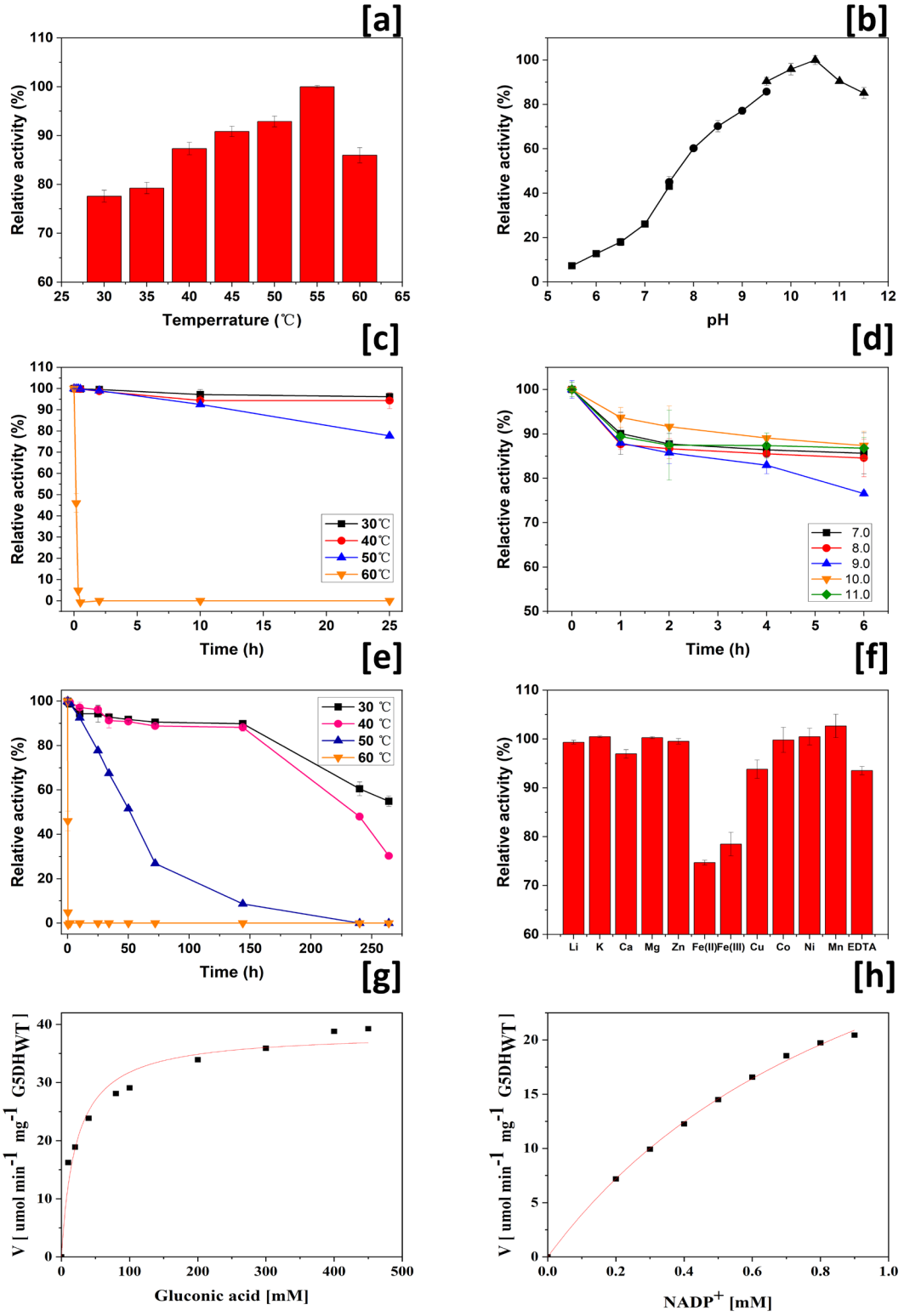


Fig. S4 Influences of temperature (a, c, e), pH (b, d) and metal ions (f) on the Ga5DH, and Michaelis-Menton curve fit of Ga5DH with GA and $NADP^+$ (g, h).

Table. S3 Distribution coefficients of COBE and (S)-CHBE and the tolerances of Ga5DH/PsCR/BmGDH in different solvents

Table. S3 Distribution coefficients of COBE and (S)-CHBE and the tolerances of Ga5DH/PsCR/BmGDH in different solvents

Organic solvents	Distribution coefficients		Relative activity [d] (%)		
	COBE	(*S*)-CHBE	PsCR	BmGDH	Ga5DH
Water	-	-	85.5±0.7	48.5±0.4	92.4±0.7
Ethyl acetate	16.2[a]	23.5[a]	33.2	0	54.4±0.3
n-Butyl acetate	10.6[a]	15.8[a]	72.1±0.4	38.3±0.5	93.6±0.4
Ethyl octanoate	6.7[b]	9.3[b]	65.7±0.9	39.8±0.4	67.3±0.9
1-Octanol	5.3[c]	3.8[c]	72.7±0.4	43.5±0.3	57.8±0.4
Trichloromethane	17.7[a]	7.7[a]	5.5±0.4	0	57.8±0.4
Octane	0.9[c]	0.2[c]	77.5±0.6	20.7±0.7	97.1±0.6
n-Hexane	1.5[c]	0.3[c]	62.6±0.5	13.4±0.5	75.0±0.5
Dibutyl phthalate	6.2[c]	4.2[c]	60.2±0.8	41.3±0.6	63.6±0.8
Heptane	1.0[a]	0.3[a]	75.4±0.6	15.5±0.8	80.6±0.6
Isopropyl ether	2.2[a]	3.9[a]	24.4±0.6	7.2±0.5	67.8±0.6

[a] Data from reference 1. [b] 10 mL 100 mM Tris-HCl (8.0) buffer which contains 10 umol COBE and 10 umol (S)-CHBE, mixed with 10 mL ethyl octanoate and shook under 30℃. the concentrations of COBE and (S)-CHBE in water and ethyl octanoate were detected by GC. [c] Data from reference 2. [d] 1 mL 100 mM Tris-HCl (8.0) buffer which contains PsCR (10 U/mL), BmGDH (10 U/mL), and Ga5DH (10 U/mL), respectively, mixed with 1 mL organic solvent under 30℃ for 2.5 hours, and detected the activity.

a. **Shimizu S, Kataoka M, Katoh M, Morikawa T, Miyoshi T, Yamada H. Stereoselective reduction of ethyl 4-chloro-3-oxobutanoate by a microbial aldehyde reductase in an organic solvent-water diphasic system [J]. Appl Environ Microbiol, 1990, 56: 2374~2377.**

c. **Matsuda T, Yamanaka R, Nakamura K. Recent progress in biocatalysis for asymmetric oxidation and reduction [J]. Tetrahedron: Asymmetry, 2009, 20: 513~557.**

Fig. S5 Docking results between GA and Ga5DH.

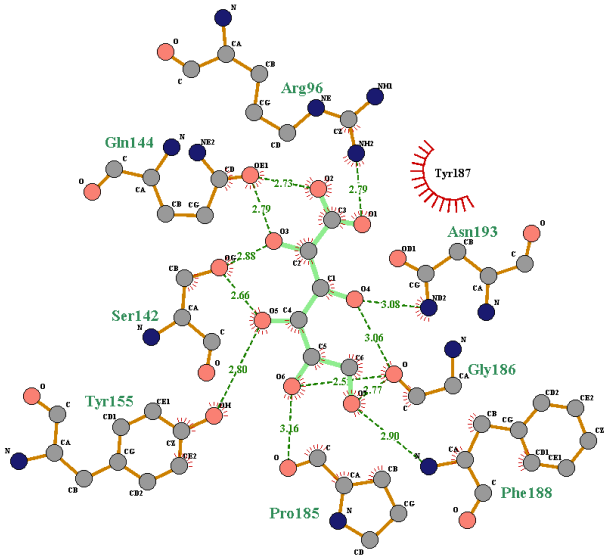


Fig. S5 2D representation of hydrogen bond and hydrophobic interactions by LIGPLOT program analyses. Dashed lines represent hydrogen bonds, and spiked residues form hydrophobic interactions between G5DH and gluconic acid.

Fig. S6 Comparison of three enzymatic cascade reactions.

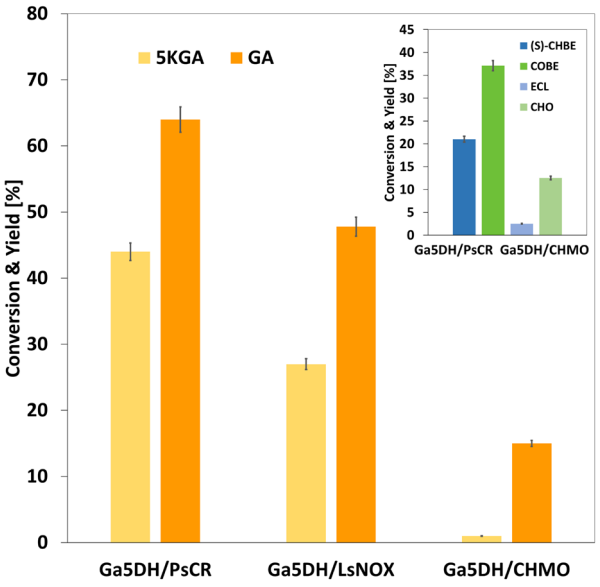


Fig. S6 Comparison of three enzymatic cascade reactions. Conditions: GA (100 mM), co-substrate (150 mM), $NADP^+$ (0.2 mM), Ga5DH (50 U), PsCR (100 U), or LsNOX (100 U), or CHMO (100 U),and buffer (100 mM Tris-HCl, pH 8.0, 10 mL), stirring in a shake flask (50 mL) at 150 rpm and 30 °C for 1 hours.

Fig. S7 Ga5DH/ PsCR enzymatic reaction optimization.

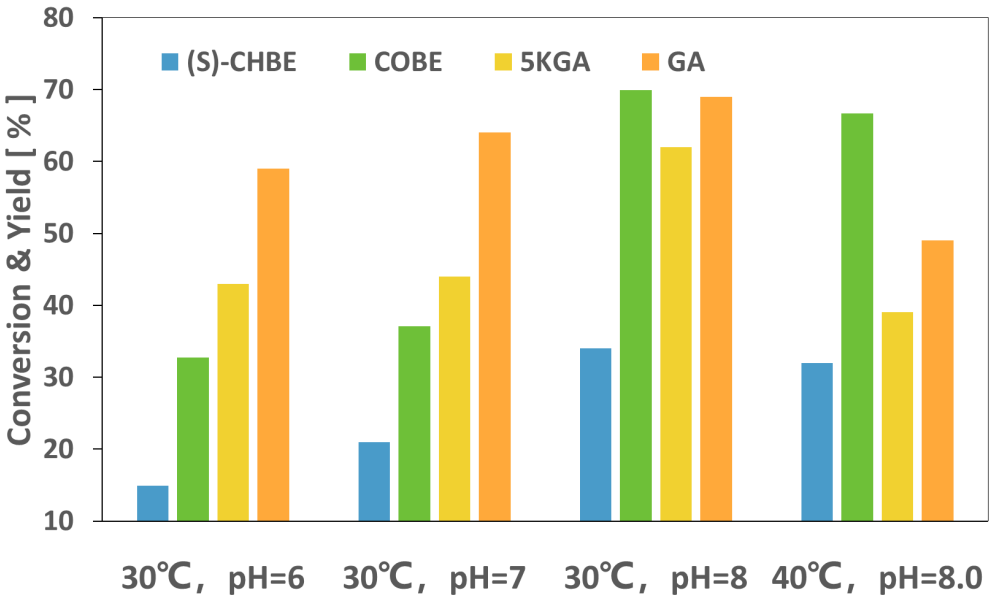


Fig. S7 Ga5DH/ PsCR enzymatic reaction optimization. Conditions: GA (100 mM), COBE (100 mM), $NADP^+$ (0.2 mM), Ga5DH (50 U), PsCR (100 U), and buffer (100 mM Tris-HCl, pH 8.0, 10 mL), stirring in a shake flask (50 mL) at 150 rpm and 30 °C for 1 hours.

Fig. S8 Influences of COBE and (S)-CHBE on Ga5DH.

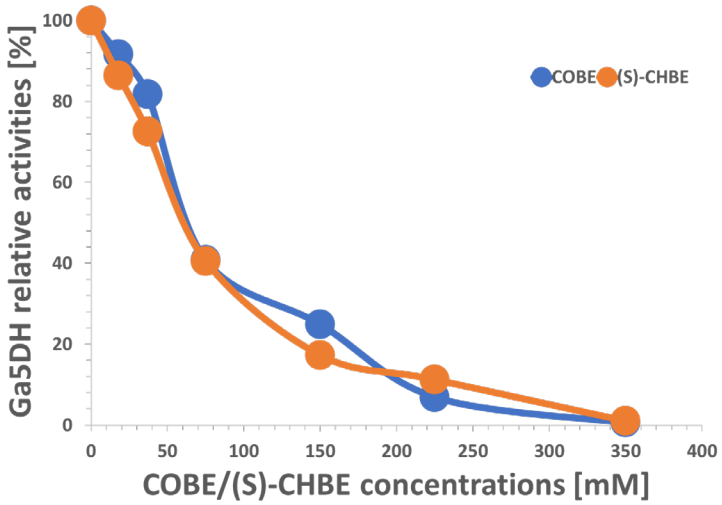


Fig. S8 Influences of COBE and (S)-CHBE on Ga5DH.

Fig. S9 SDS-PAGE results and enzymatic activities of co-express strains.

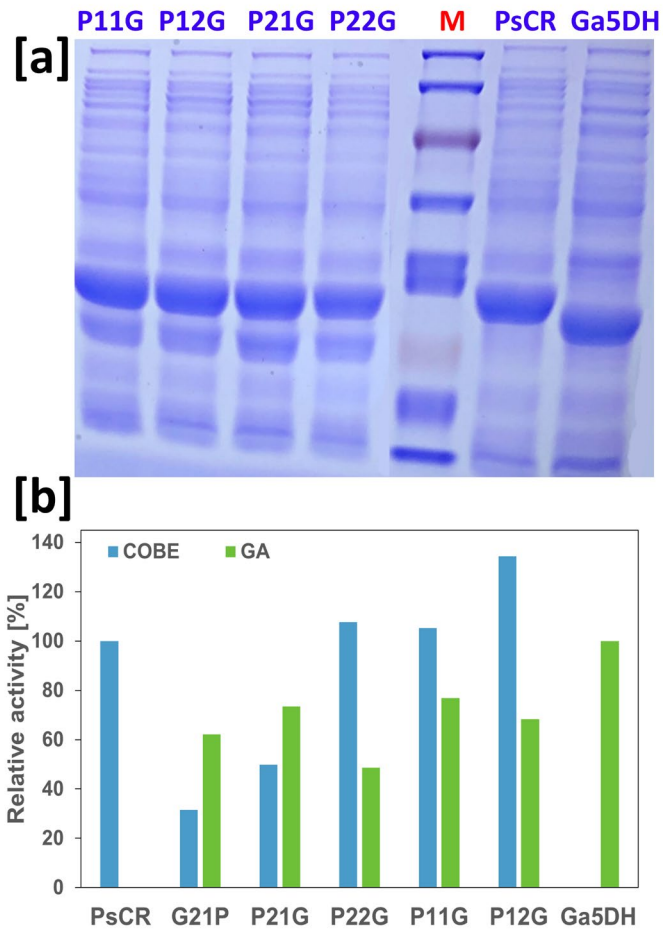


Fig. S9 [a]: SDS-PAGE analysis of co-expression strains. M: Protein marker, 150, 100, 70, 50, 40, 35, 25, 20, and 15 kDa (from top to bottom). SD1: GAAGGAGA SD2: TAAGGAGG AS1: ATATACAT AS2: TACAT. **[b]:** Effects of different gene order, SD regions and AS on PsCR and Ga5DH activity.

Fig. S10 Post-treatment of bio-oxidation mixtures.

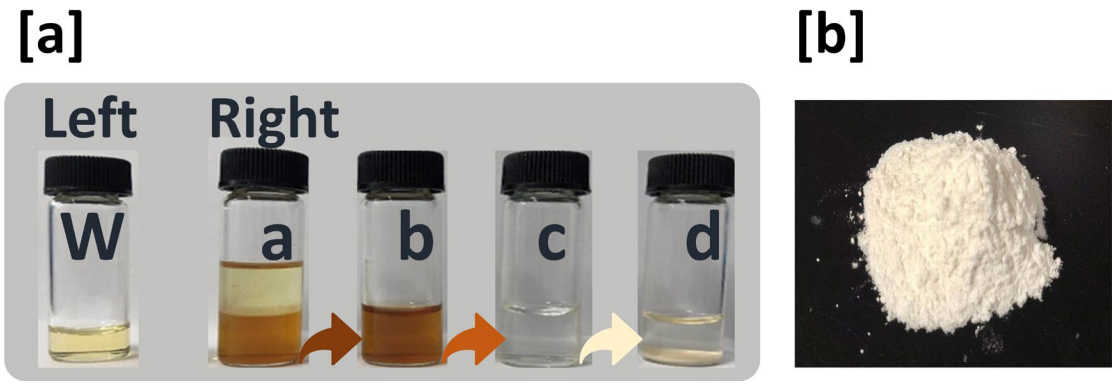


Fig. S10 [a]: Appearance of reaction mixtures during post-processing. **Left: W:** reaction liquid of aqueous catalytic system after boiling and centrifugal. **Right:** reaction liquid via a series of post-treatment from the aqueous-organic biphasic catalytic system. **a:** after boiling and centrifugal; **b:** aqueous phase though biphasic separation of a; **c:** decolorized from b by activated carbon; **d:** CaCl2 precipitation based on c. **[b]:** Calcium 5-keto-D-gluconate from bio-oxidation.

Table. S4 Precipitation of 5KGA based on different metal salts

Table. S4 Precipitation of 5KGA based on different metal salts. [a]

	Cation Concentration [mM]				
Metal salts	**20**	**40**	**60**	**80**	**100**
$MgSO_4$	-	-	-	-	-
$MnSO_4$	-	-	-	-	-
$CuSO_4$	-	-	-	-	-
$FeCl_3$	-	-	-	-	-
$ZnCl_3$	-	-	-	-	-
$FeSO_4•7H_2O$	-	-	-	-	-
$NiSO_4•6H_2O$	-	-	-	-	-
$SrCl_2•H_2O$	-	+	+	+	+
$CrCl_3•6H_2O$	-	-	-	-	-
$CoCl2_6•H_2O$	-	-	-	-	-
$CaCl_2$	+	+	+	+	+
$BaCl_2$	-	-	+	+	+

[a] condition: 10 mL 100 mM Tris-HCl (8.0) buffer (contains 0.1 M 5KGA), added metal salt (contains 20 mM metal cation) per time and shook 10mins under 30 ℃ for dissolving. – not precipitated. + precipitated.

Fig. S11 Bio-oxidation magnification.

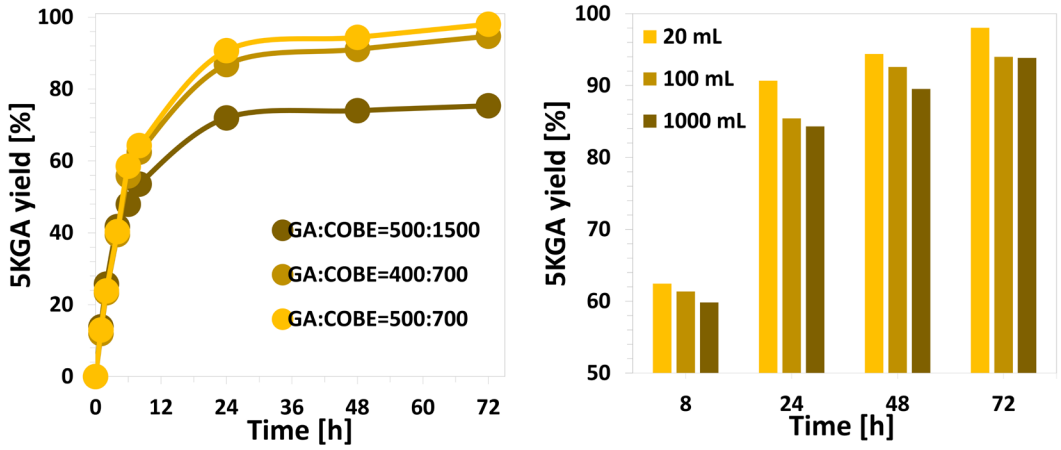


Fig. S12 Reaction curves of bio-oxidation of glucose to GA and COBE to (S)-CHBE via B21P.

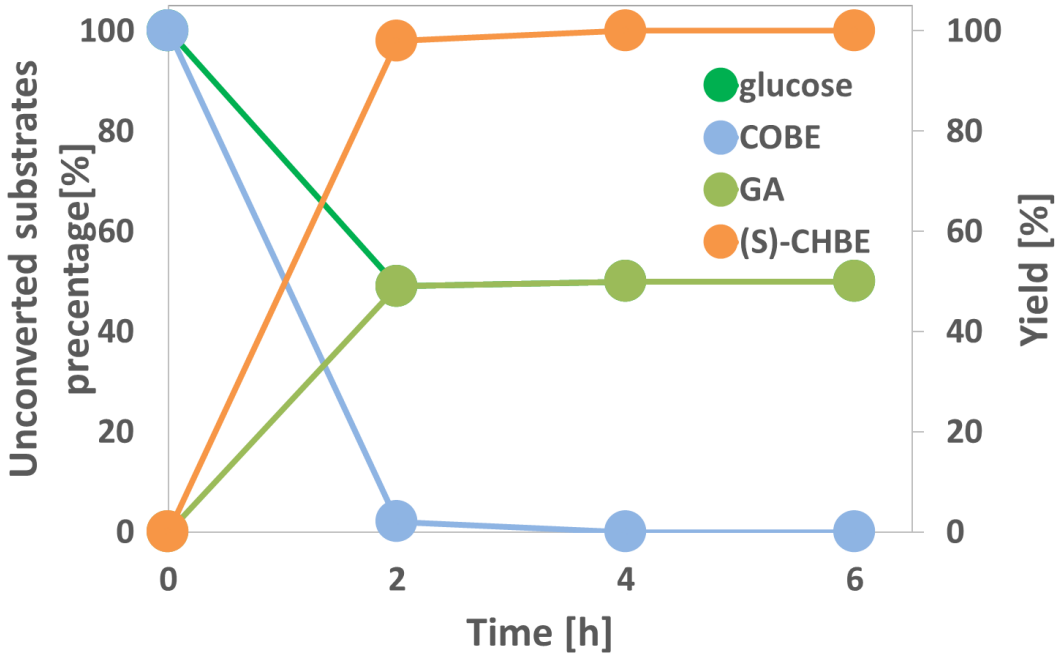
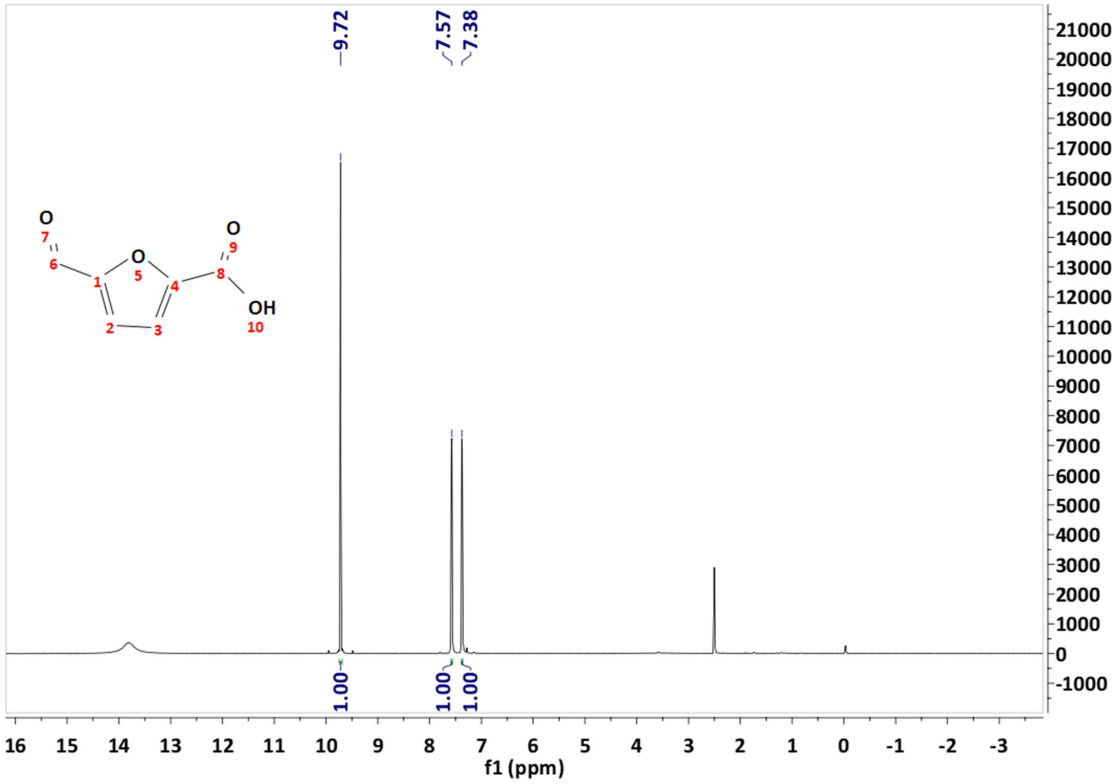
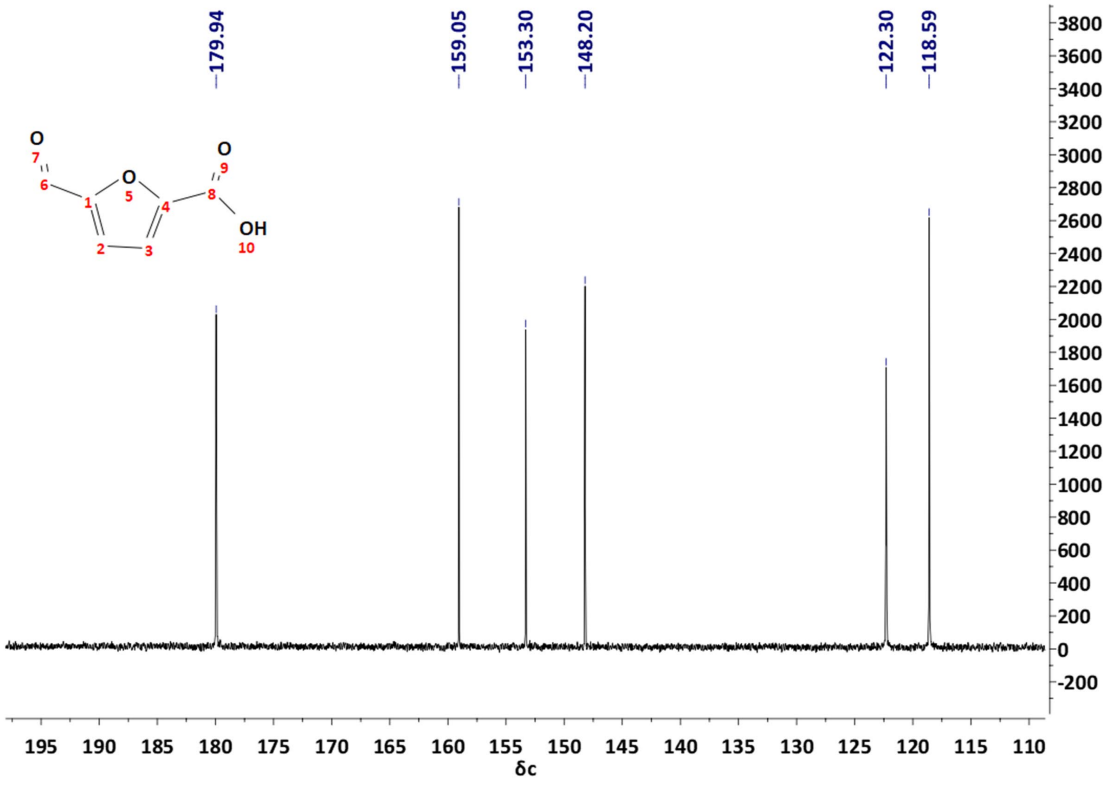


Fig. S12 Reaction conditions: 50 OD whole cells of *E. coli* BL21 harbouring BmGDH-SD2AS1-PsCR. Aqueous-organic biphasic catalytic system: including organic system, 10 mL n-butyl acetate, and aqueous catalytic system, stirring in a shake flask (50 mL) at 250 rpm and 30 °C for 6 hours.

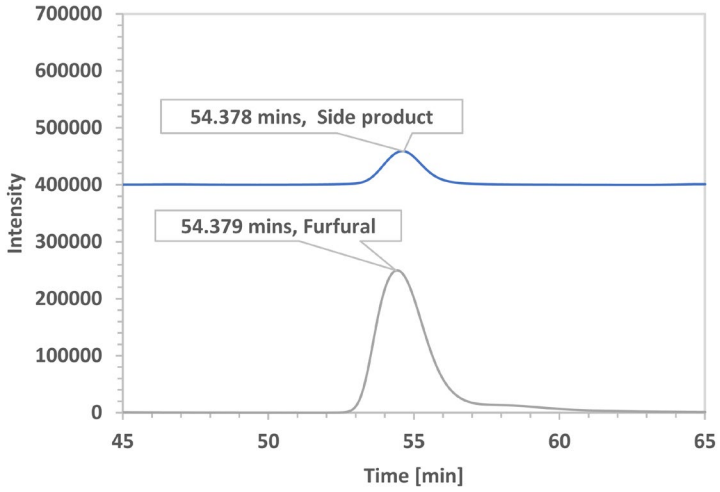
Fig. S13 Characterizations of FFCA and FF.

NMR results of FFCA.

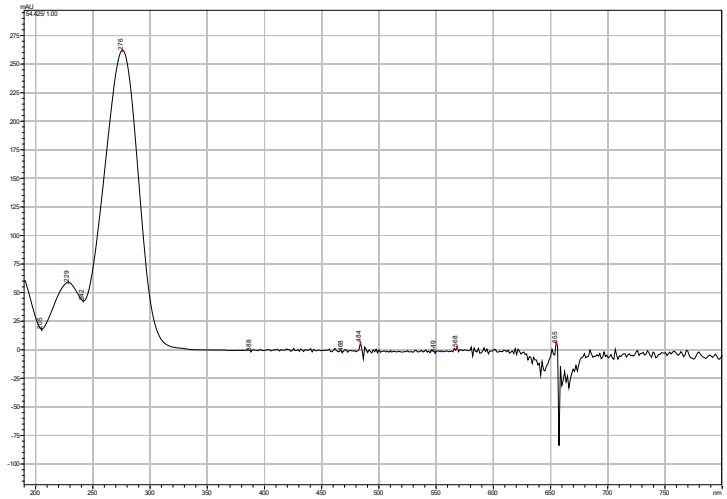




Retention time of side product and furfural at 254 nm.



UV absorption spectrum of side product



UV absorption spectrum of furfural

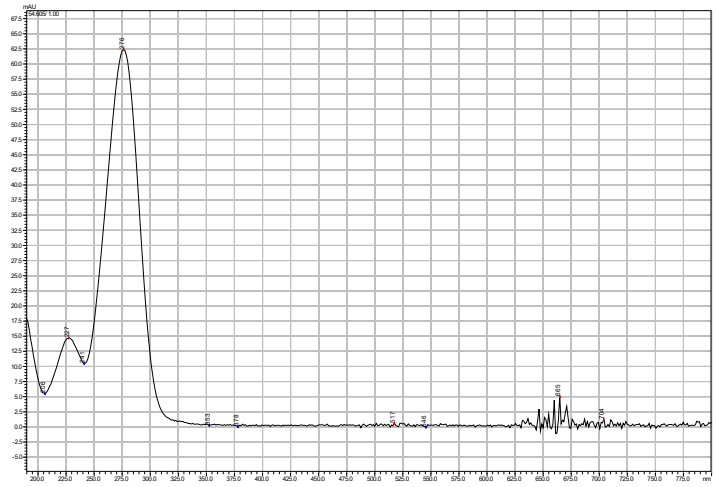
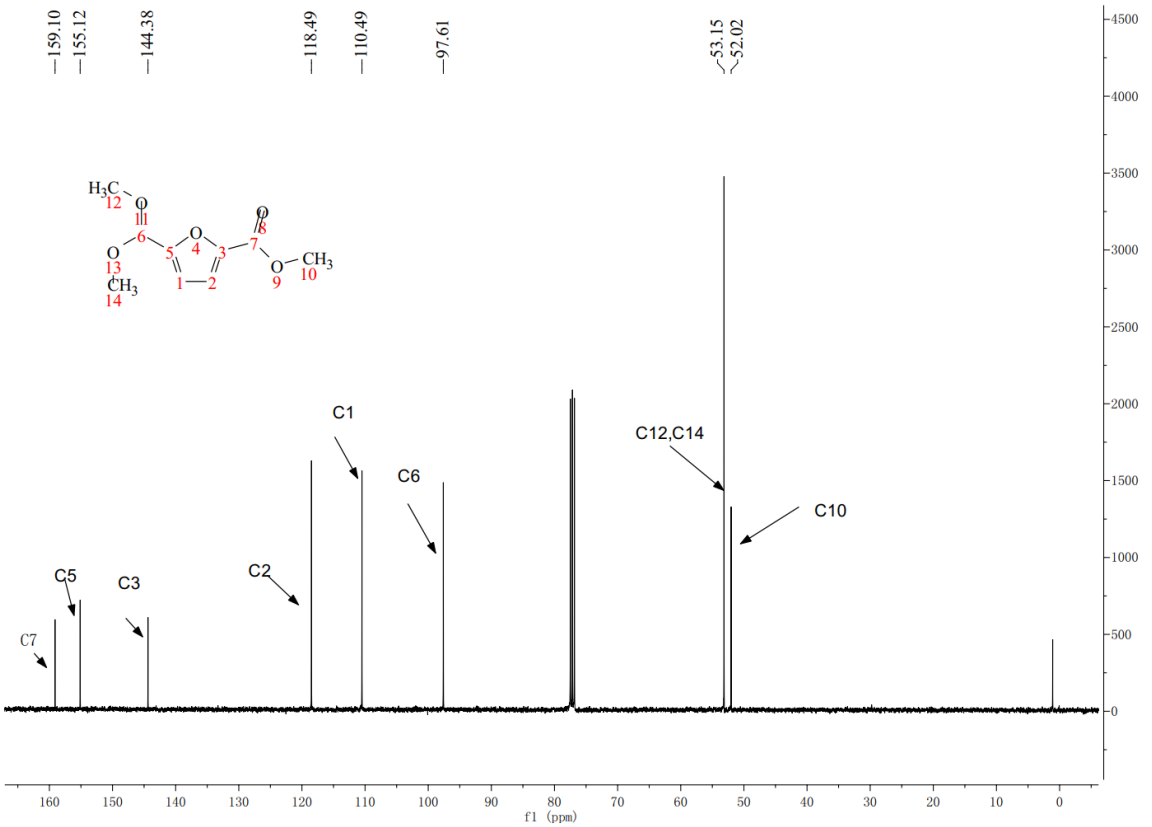
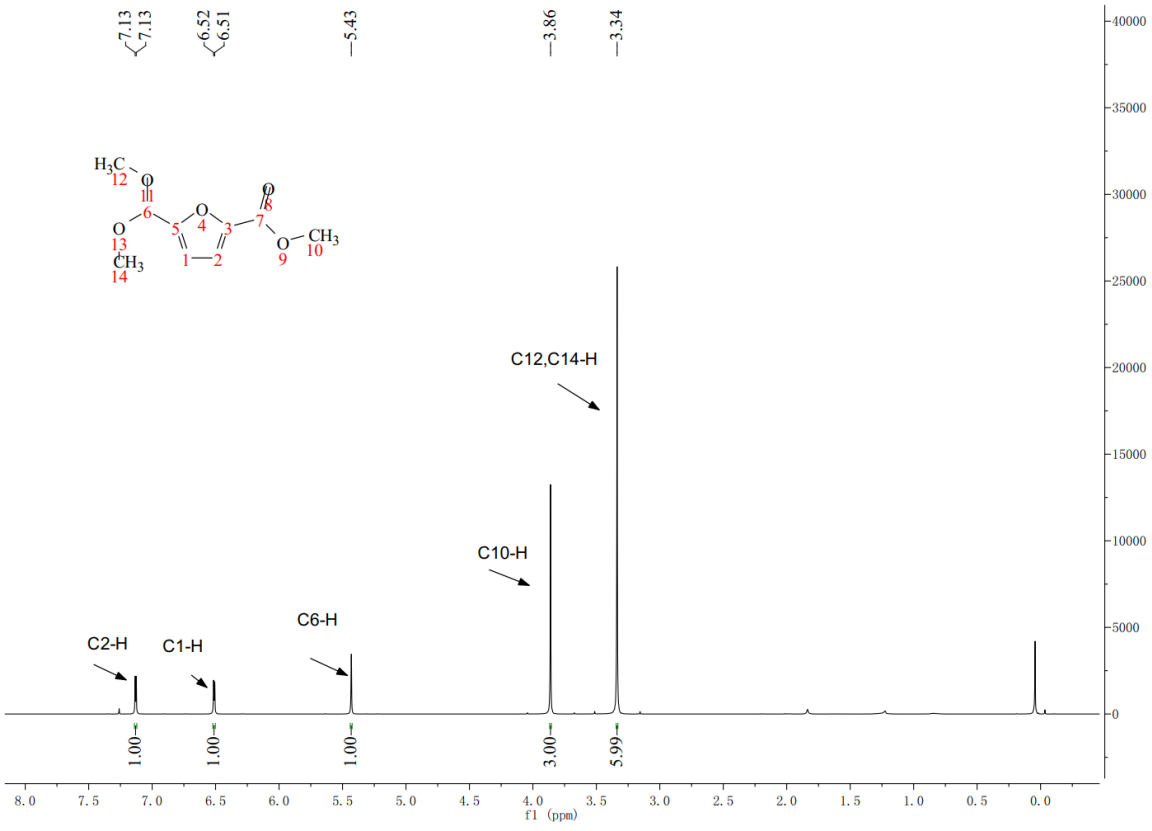


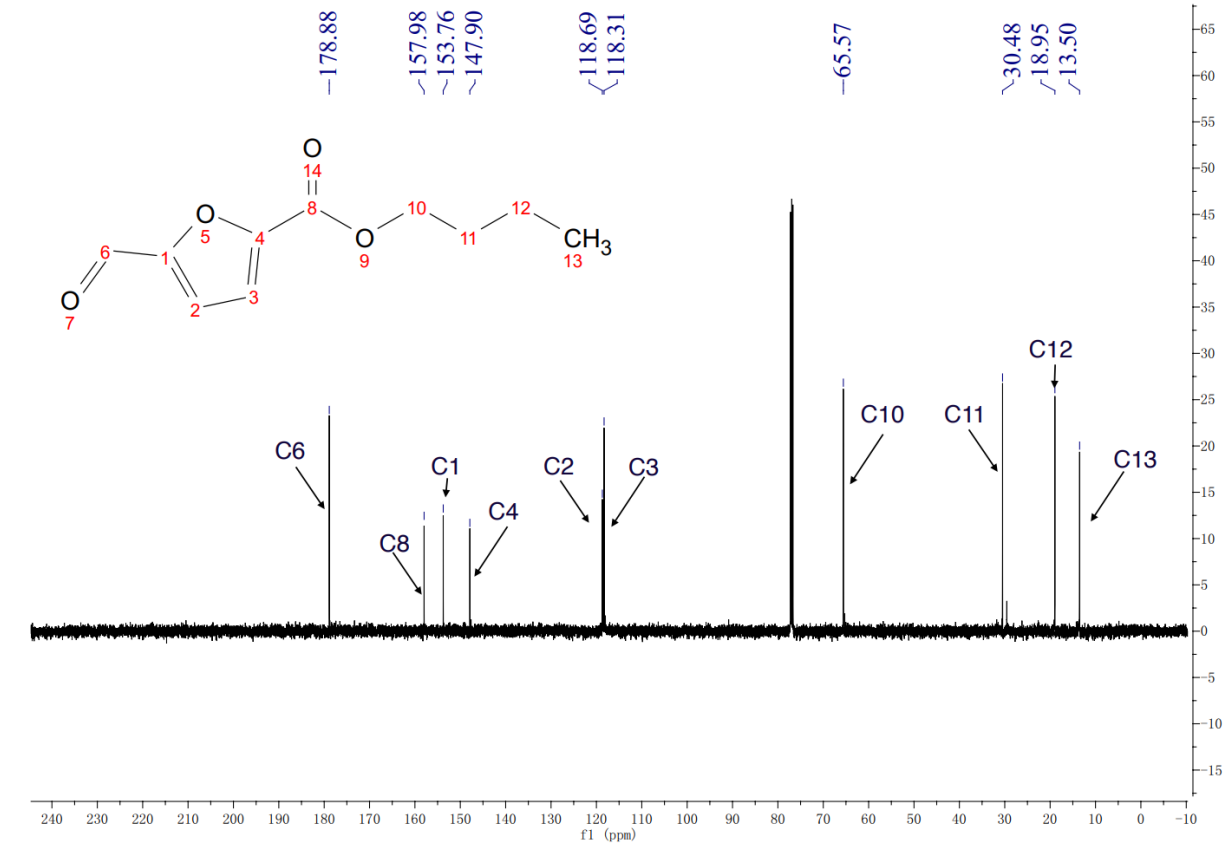
Fig. S14 Characterizations of Me-MMFCA and nBu-FFCA.

NMR analysis of Me-MMFCA





NMR analysis of nBu-FFCA



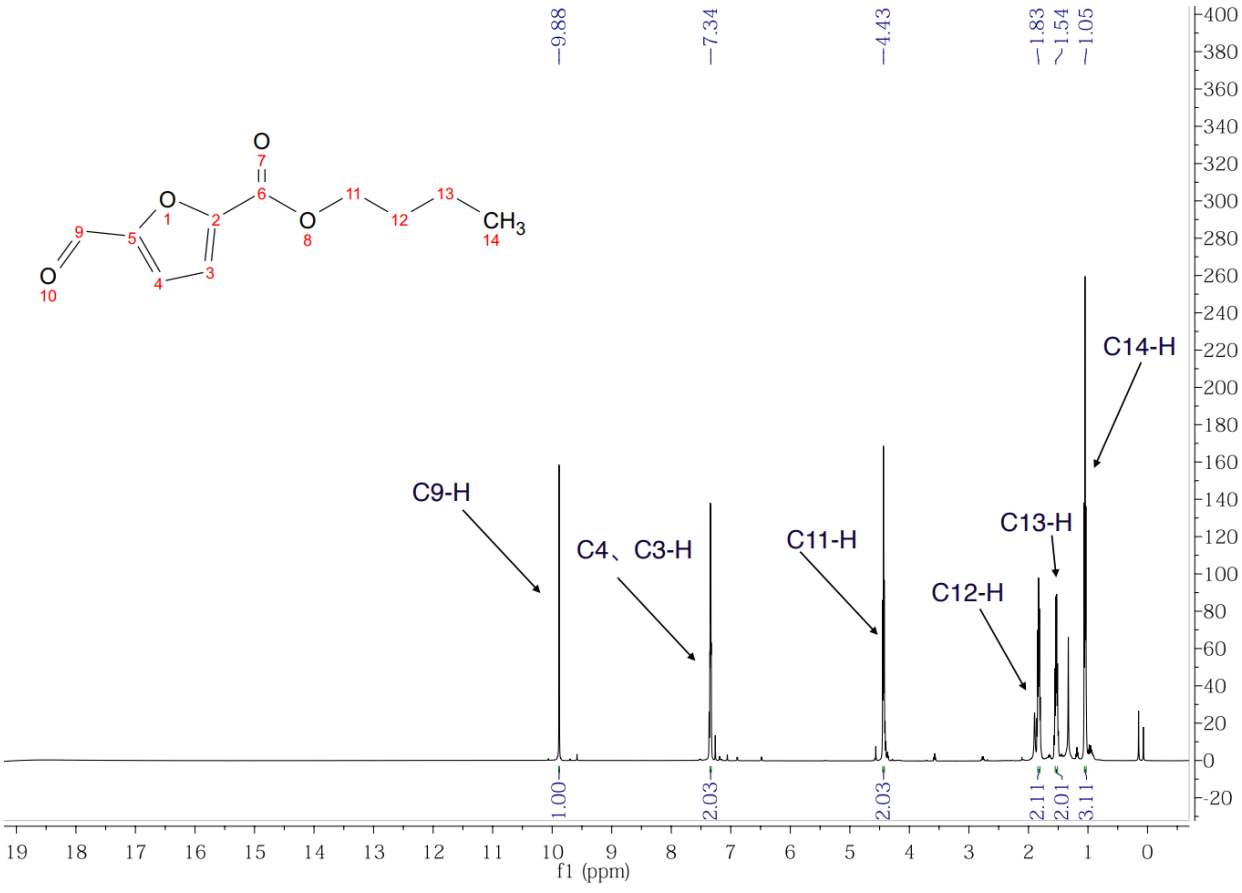


Fig. S15 Appearance of HMF.

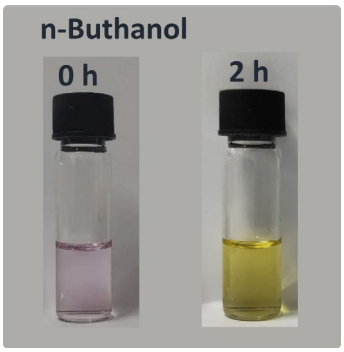


Fig. S15 Appearance of HMF. Reaction condition: HMF 50 mg, n-butanol 5 mL, sulfuric acid 50 mM, under 110℃ for 2 hours.

Fig. S16 Appearance of five commercial solid catalysts.

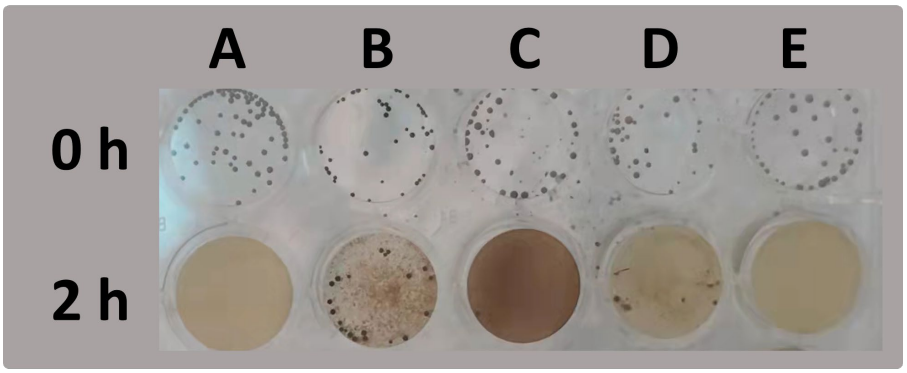


Fig. S16 Appearance of five commercial solid acid catalysts. Reaction condition: 5KGA 50mg, n-butanol 5mL, catalyst 10 mg, under 110 ℃ for 2 hours. **A**: amberlyst-15; **B**: HND-590; **C**: HND-580; **D**: HND-12; **E**: HND-8.

Fig. S17 The addition volumes of H_2SO_4 optimization during 5KGA dehydration.

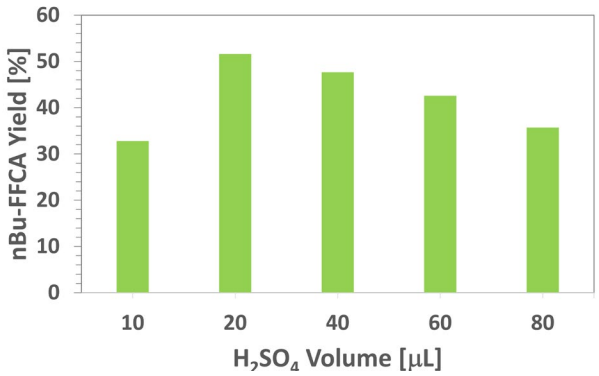
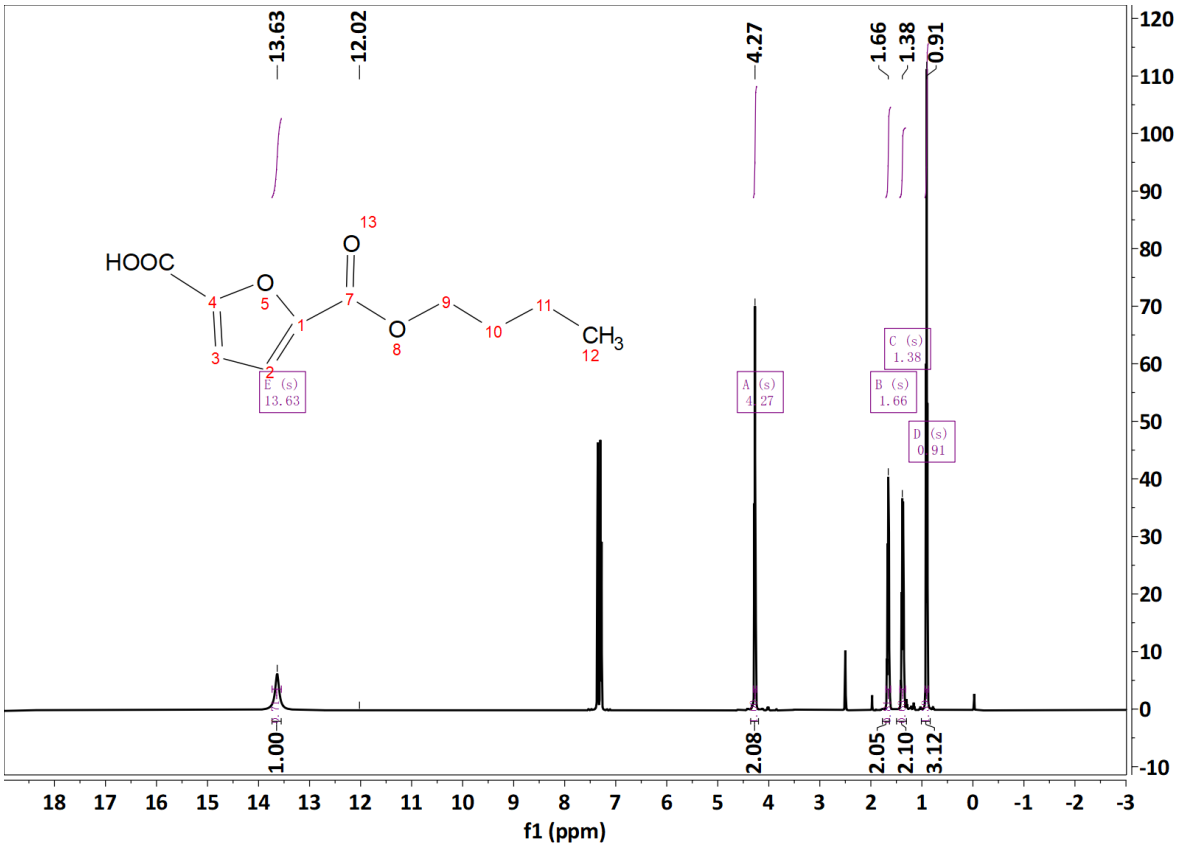


Fig. S17 Reaction condition: 5KGA 50 mg, n-butanol 5 mL, sulfuric acid, under 110℃ for 2 hours. The volumes of sulfuric acid from 10 to 80 μL, the concentration of sulfuric acid (additional volumes= 20 μL) in the system is 50 mM.

Fig. S18 NMR analysis of monobutyl furandicarboxylate.



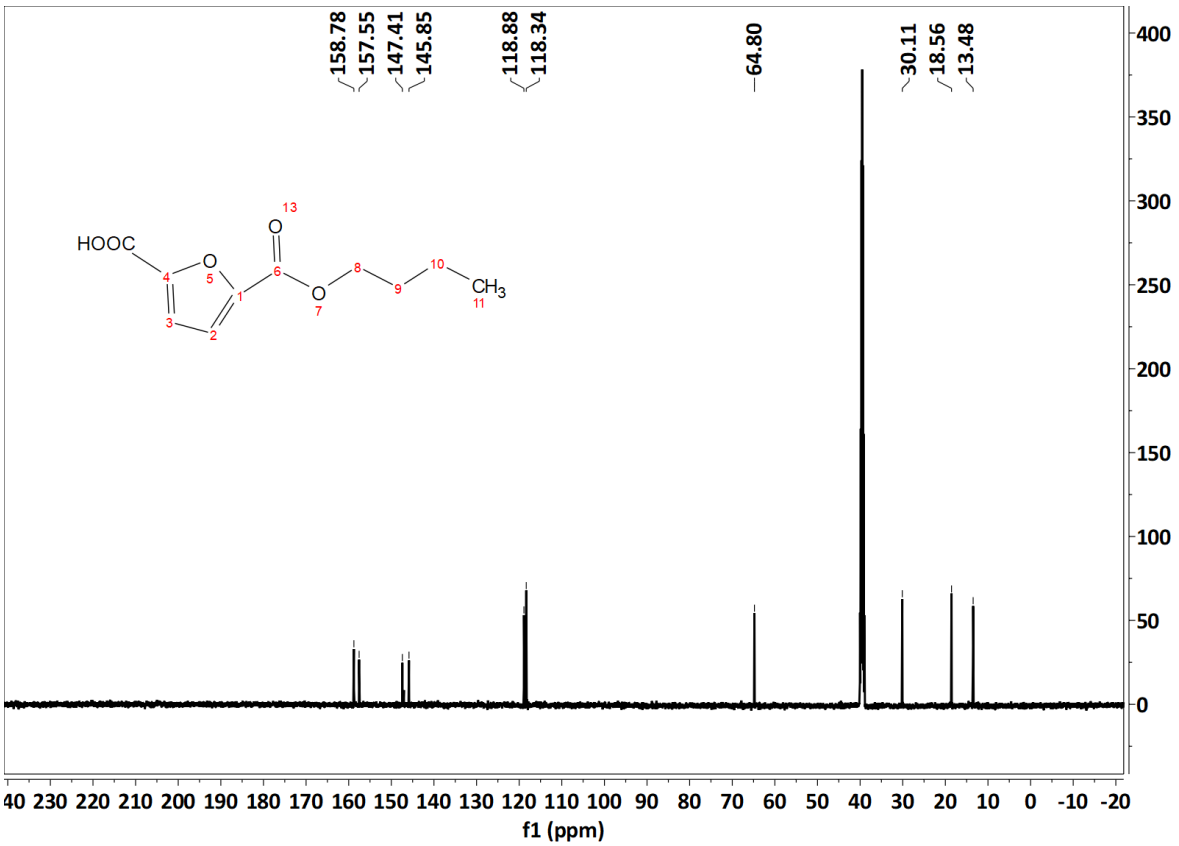


Table. S5 Sustainability evaluation of this study

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Gluconic acid to 5KGA	
Gluconic acid molar weight (g/mol)	196
5KGA molar weight (g/mol)	194
5KGA yield (% 5KGA molar yield)	99.9
Weight of 5KGA produced from 1 mol gluconic acid (g/mol)	193.8
5KGA to nBu-FFCA	
5KGA molar weight (g/mol)	194
n-Butanol molar weight (g/mol)	74
nBu-FFCA molar weight (g/mol)	196
nBu-FFCA yield (% nBu-FFCA molar yield)	77.9
Weight of nBu-FFCA produced from 1 mol 5KGA (g/mol)	152.7
nBu-FFCA to FDCA ester	
nBu-FFCA molar weight (g/mol)	196
O_2 molar weight (g/mol)	32
FDCA ester molar weight (g/mol)	212
FDCA ester yield (% nBu-FFCA molar yield)	91
Weight of FDCA ester produced from 1 mol nBu-FFCA (g/mol)	192.9
Gluconic acid to FDCA ester	
Atom economy [%]	**70.2**
Carbon efficiency [%]	**77.7**
Reaction mass efficiency [%]	**56.6**

Table. S6 Comparison of sustainability of various FDCA preparation routes.

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Substrates	Steps	Yield [%]	CE [a] [%]	RME [b] [%]	SP [c] [¥/kg]	Cost[d] [¥]	Source
GA	GA→5KGA	99.9					
	5KGA→nBu-FFCA	77.9	77.7	56.6	279 311[e]	393,967 443,723[f]	*This study*
	nBu-FFCA →monobutyl furandicarboxylate	91					
Cellulose	Cellulose→HMF	42					
			39.3	34.3	299	760,656	[61]
	HMF→FDCA	93.6					
Glucose	Glucose→HMF	39					
			31.2	25.0	311	997,396	[57]
	HMF→FDCA	80					
Fructose	Fructose→HMF	70.3					
			66.8	49.1	437	665,087	[58]
	HMF→FDCA	95					

[a] Carbon efficiency. [b] Reaction mass efficiency. [c] Substrates price, which were obtained from Aladdin (https://www.aladdin-e.com/zh_cn). [d] The cost of raw materials required to produce one ton of FDCA (calculated based on the starting raw materials). [e] Price of glucose. [f] Calculated from glucose, the yield of GA was 98.97 % based on the reference (10.1016/j.ijbiomac.2017.10.074)

Fig. S19 ^{13}C NMR results of 5KGA in three deuterated reagents within different addition of D_2SO_4.

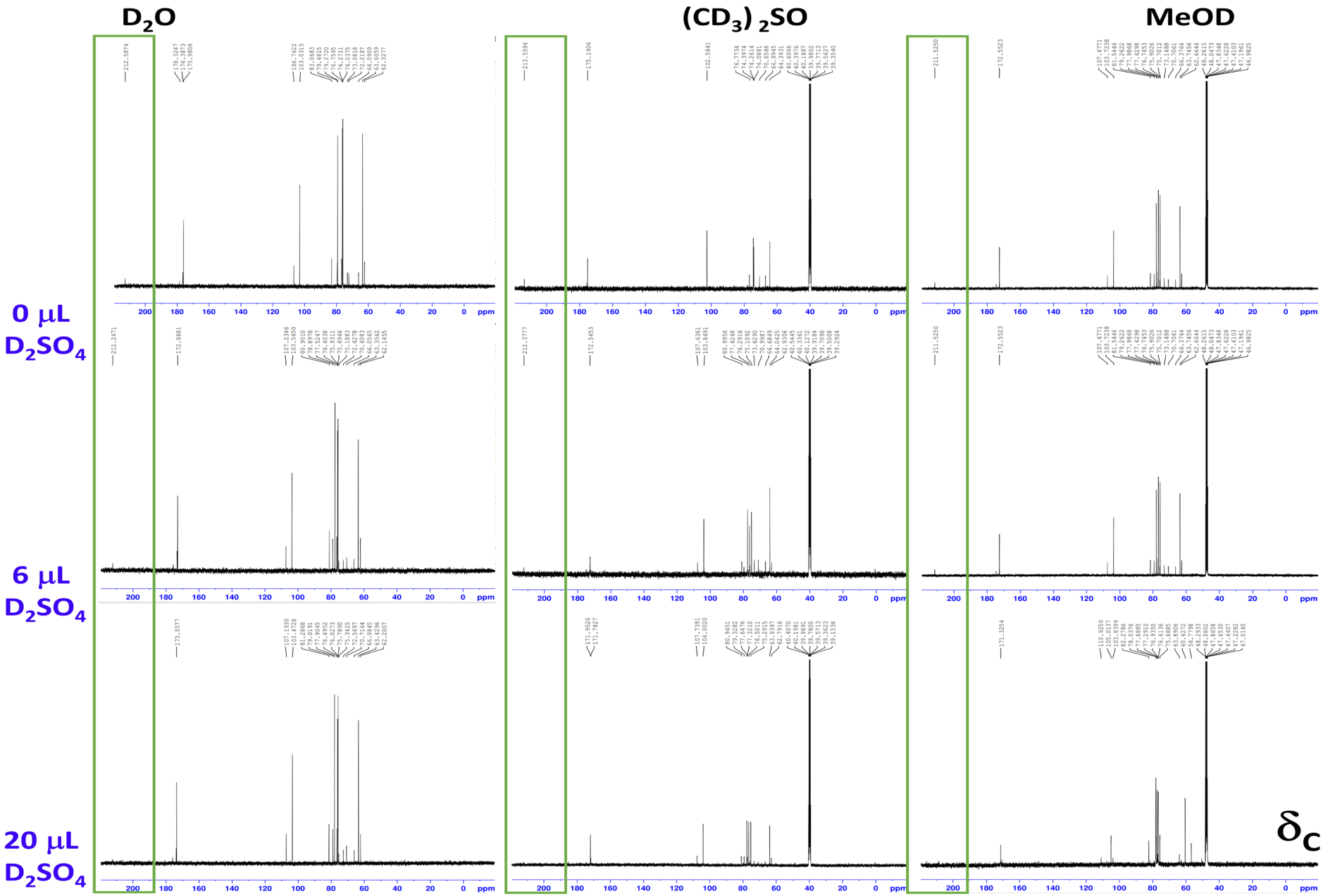
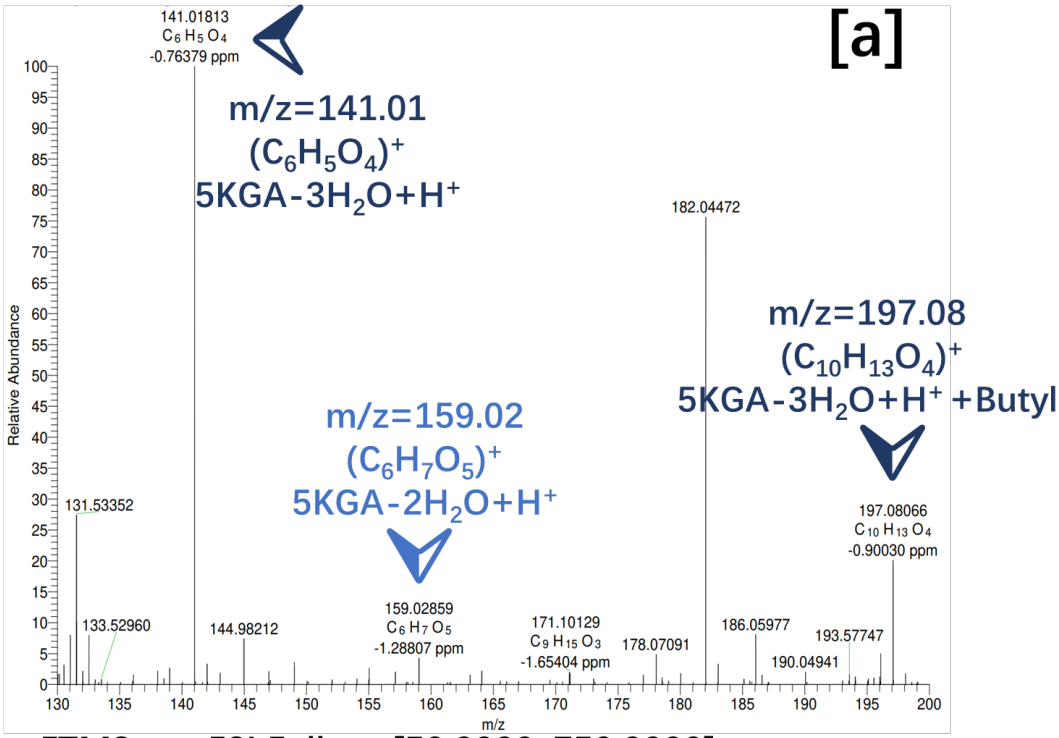


Fig. S20 HRMS result of 5KGA dehydration at 5 mins.



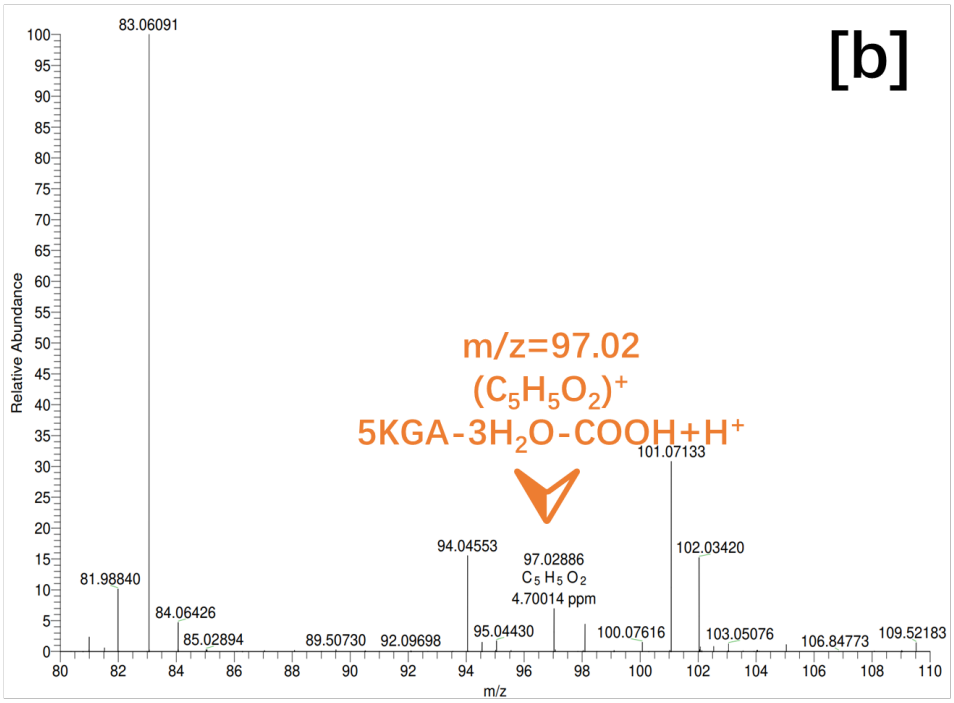


Table. S7 Comparison of FFCA and 5KGA in DMSO and n-butanol.

Table. S7 Comparison of FFCA and 5KGA in DMSO and n-butanol.[a]

Entry	Substrate	Solvents	Product	Conversion [mol %]	FF Yield [mol %]	First product Yield [b]
1	5KGA	DMSO	FFCA, FF	94.3	16.6	42.7
4	5KGA	n-Butanol	nBu-FFCA,	100	5.5	51.6
3	FFCA	DMSO	FFCA	2.4	N.D[c]	98.0
2	FFCA	n-Butanol	nBu-FFCA	91.3	N.D[c]	52.8

[a]: Reaction conditons: 50 mg substrates and 50 mM H_2SO_4 in 5ml solvents, under 110℃ for 1 hour. [b]: calculated with the main product: [c]: Not detected.

Fig. S21 appearances of FFCA and 5KGA in DMSO and n-butanol.

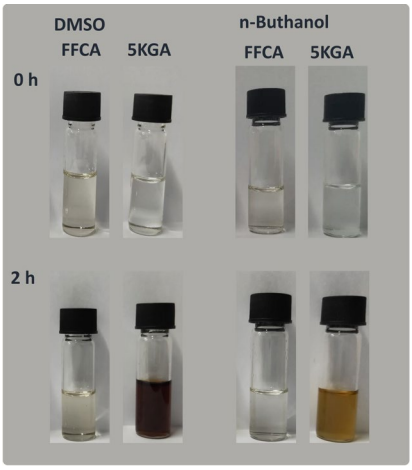


Fig. S22 The key bond distances in angstroms Å .

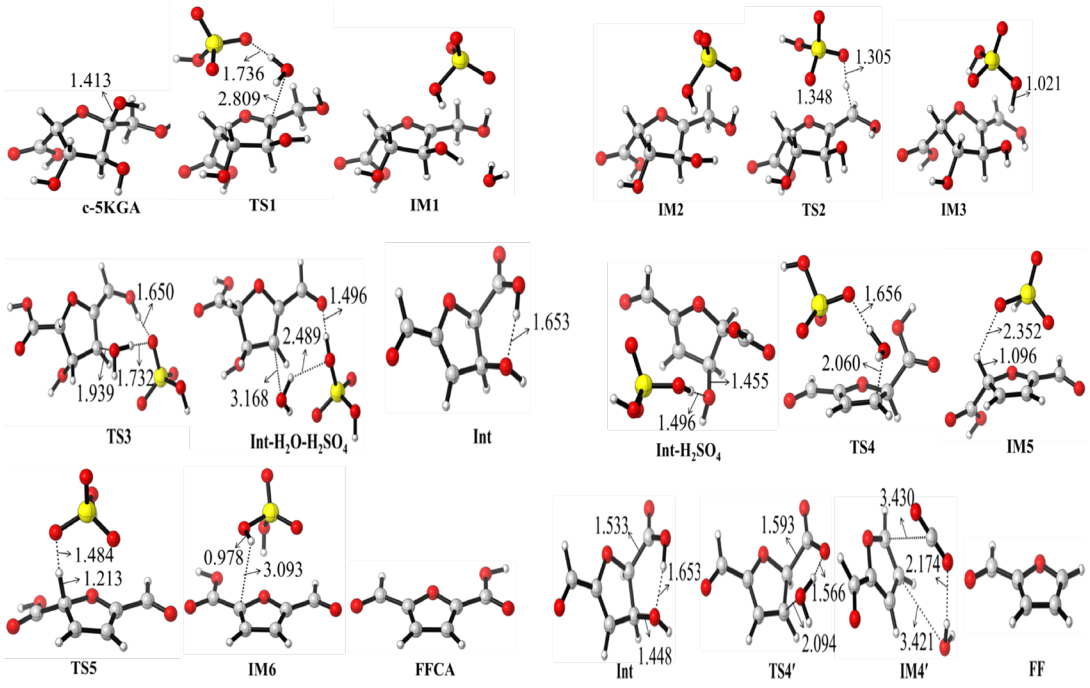


Fig. S22 The key bond distances in angstroms Å . color code, C: gray, H: white, O: red, S: yellow.

Table. S8 Coordinates and energies (in hartree) of the calculated structures

c-5KGA

Energy=-761.5434073

C	1.166391	-0.149730	0.884686
O	-0.015677	-0.951270	0.955610
C	-1.136086	-0.283672	0.396451
C	-0.534615	0.843917	-0.461870
C	0.708589	1.229898	0.335333
H	1.609540	-0.068366	1.880121
H	-0.209760	0.431065	-1.426108
H	0.411067	1.866696	1.176054
O	-1.896852	0.245533	1.463033
H	-2.793184	0.373166	1.107739
C	-1.953488	-1.325663	-0.358189
H	-2.136439	-2.165702	0.322317
H	-1.380129	-1.689468	-1.220009
O	-3.172724	-0.701224	-0.755071
H	-3.841591	-1.391679	-0.860046
C	2.222677	-0.757685	-0.021821
O	3.407872	-0.511969	0.102537
O	1.719558	-1.548185	-0.978015
H	2.459093	-1.860950	-1.531547
O	-1.463316	1.893736	-0.639316
H	-1.067286	2.522298	-1.260572
O	1.637525	1.879566	-0.507665
H	2.376802	2.191059	0.035620

TS1

Energy=-1461.9535623

C	0.939456	-1.159738	-0.021587
O	0.559084	-0.109687	-1.001196
C	0.951259	1.044709	-0.632790
C	1.743177	1.009550	0.646957
C	1.368440	-0.354598	1.238891
H	0.047355	-1.763283	0.113575
H	2.790470	0.954708	0.289742
H	0.494790	-0.238773	1.885951
O	-1.123384	2.501954	0.575760
H	-0.797877	2.121429	1.403536
C	0.782994	2.189226	-1.562871
H	-0.138101	2.042147	-2.141522
H	1.625856	2.102697	-2.267561
O	0.860200	3.426396	-0.905541
H	0.057740	3.426739	-0.330384
C	2.068396	-2.008681	-0.572468
O	2.248922	-3.138614	-0.175483
O	2.822720	-1.363841	-1.461758
H	3.547749	-1.952110	-1.747377
O	1.551486	2.047453	1.561685
H	1.743478	2.885260	1.106369
O	2.473656	-0.897812	1.910337
H	2.164019	-1.631597	2.462851
S	-2.742981	-0.596810	0.090192
O	-1.843269	-0.609977	1.268414
O	-4.129017	-1.015668	0.374397
O	-2.136804	-1.727243	-0.961327
H	-2.403696	-2.609239	-0.644753
O	-2.613602	0.643617	-0.725625
H	-1.726352	1.835535	0.172399

IM1

Energy=-1461.969471

C	2.103117	-1.129132	0.108354
O	1.094129	-0.869093	-0.941309
C	0.391350	0.169936	-0.690659
C	0.878275	0.917170	0.535952
C	1.743498	-0.135098	1.247620
H	1.992657	-2.178256	0.373367
H	1.540293	1.699632	0.125900
H	1.131075	-0.651587	1.992333
O	-0.680858	3.714885	0.209136
H	-1.505621	4.072879	0.571159
C	-0.500608	0.633423	-1.796143
H	-1.002186	-0.243452	-2.222339
H	0.202269	1.003933	-2.558554
O	-1.388351	1.663567	-1.460816
H	-2.174169	1.255346	-1.014211
C	3.499694	-0.885048	-0.433633
O	4.466309	-1.429377	0.052800
O	3.521555	0.000605	-1.428651
H	4.447528	0.140823	-1.704329
O	-0.092381	1.448253	1.384984
H	-0.346041	2.360881	1.051724
O	2.858628	0.496637	1.822380
H	3.267217	-0.114622	2.454133
S	-2.854618	-1.032098	0.166229
O	-3.339988	0.333175	-0.205788
O	-3.566678	-1.612824	1.315425
O	-1.292215	-0.752372	0.698587
H	-1.253671	0.078934	1.236599
O	-2.624210	-1.932397	-0.976055
H	-0.964944	3.173092	-0.559929

IM2

Energy=-1461.969471

C	1.958002	-0.919837	-0.276128
O	0.975338	-0.207428	-1.126380
C	0.345761	0.684875	-0.464727
C	0.896005	0.883476	0.921531
C	1.660838	-0.421056	1.173374
H	1.771646	-1.979889	-0.432683
H	1.630082	1.701225	0.801937
H	1.002782	-1.139086	1.670822
C	-0.563188	1.619495	-1.179738
H	-1.176412	1.060912	-1.896079
H	0.114934	2.261432	-1.764994
O	-1.304807	2.414810	-0.287492
H	-2.146405	1.926337	-0.116923
C	3.372413	-0.578855	-0.708386
O	4.292621	-1.338996	-0.502653
O	3.465534	0.627588	-1.265454
H	4.400960	0.803668	-1.482703
O	-0.039585	1.166747	1.927812
H	-0.542510	1.949323	1.630703
O	2.817130	-0.152020	1.922015
H	3.167386	-0.991446	2.257595
S	-2.906924	-0.699240	-0.125887
O	-3.369759	0.706605	0.070121
O	-3.641314	-1.679992	0.688696
O	-1.343274	-0.685784	0.497211
H	-1.334586	-0.228150	1.366467
O	-2.651395	-1.074664	-1.525815

TS2

Energy=-1385.4546846

C	-1.473528	-0.904152	0.110045
O	-0.699809	-0.150431	1.090942
C	-0.376202	1.043391	0.628832
C	-1.027057	1.332132	-0.689789
C	-1.443990	-0.055621	-1.200545
H	-0.987255	-1.872546	0.004259
H	-1.942458	1.902720	-0.467873
H	-0.668283	-0.443999	-1.861496
C	0.574575	1.830156	1.267938
H	1.614011	1.058228	0.893503
H	0.712433	1.685966	2.340416
O	0.780863	3.147728	0.866855
H	0.675467	3.171077	-0.103150
C	-2.896930	-1.116955	0.588656
O	-3.568305	-2.049985	0.198745
O	-3.316762	-0.162042	1.420264
H	-4.247168	-0.339670	1.654932
O	-0.157171	2.059872	-1.531830
H	-0.696219	2.587141	-2.140300
O	-2.693093	0.042515	-1.846309
H	-2.850982	-0.783356	-2.327970
S	2.602891	-0.807984	-0.171932
O	1.556273	-0.607037	-1.186141
O	3.908483	-1.268460	-0.650376
O	2.038364	-1.915682	0.895042
H	1.961051	-2.778475	0.445766
O	2.731401	0.399391	0.753060

IM3

Energy=-1385.4839444

C	1.445563	-0.947667	-0.322194
O	0.644965	-0.045794	-1.105307
C	0.501047	1.160257	-0.391976
C	0.782973	0.947994	1.060731
C	1.217831	-0.525213	1.163854
H	1.093074	-1.958909	-0.521593
H	1.586079	1.593147	1.431754
H	0.389891	-1.113912	1.566746
C	0.062177	2.260575	-1.004112
H	-1.751964	0.787815	1.097741
H	-0.112456	2.291103	-2.074366
O	-0.224567	3.435447	-0.378859
H	-0.240109	3.305173	0.583940
C	2.921265	-0.883144	-0.679847
O	3.673271	-1.817421	-0.484848
O	3.291674	0.297641	-1.181723
H	4.251354	0.270725	-1.355512
O	-0.424173	1.254137	1.793696
H	-0.291807	1.075211	2.739259
O	2.369916	-0.615326	1.973660
H	2.563608	-1.552887	2.124834
S	-2.580141	-0.828392	-0.127794
O	-1.720544	-1.723867	0.648429
O	-3.961647	-1.184848	-0.399134
O	-1.909522	-0.579051	-1.555269
H	-0.912670	-0.497596	-1.467386
O	-2.599109	0.601280	0.559424

TS3

Energy=-1385.4645459

S	3.330258	0.313235	0.226363
O	2.542913	1.431291	0.784443
O	2.501375	-0.572715	-0.678662
O	4.134529	-0.447180	1.193097

C	-0.904109	-1.146841	-0.165311
O	-2.238743	-1.171462	-0.571278
C	-2.694979	0.185597	-0.658558
C	-1.669925	1.045141	0.128856
C	-0.441254	0.160015	0.063111
H	-1.524037	2.023752	-0.330450
H	0.422799	0.387806	0.673082
O	-1.996166	1.152944	1.506525
H	-2.480489	1.982303	1.632420
O	0.368902	0.774224	-1.587674
H	0.589110	1.714353	-1.453684
C	-0.264992	-2.343370	0.045100
H	1.232907	0.291129	-1.481609
H	-0.851672	-3.259556	0.034816
O	1.014202	-2.531707	0.274111
H	1.587868	-1.743637	0.040804
O	4.349854	0.951131	-0.887762
H	4.945293	1.584494	-0.444697
H	-2.712028	0.494401	-1.709530
C	-4.087125	0.368686	-0.093010
O	-4.648519	1.447355	-0.141833
O	-4.606366	-0.732396	0.447778
H	-5.495423	-0.514049	0.785950

Int-H_2O-H_2SO_4

Energy=-1385.4793752

S	3.326423	-0.081022	-0.412936
O	2.678823	-1.304546	-0.897344
O	2.575358	0.481926	0.850044
O	3.582860	0.985068	-1.373633
C	-1.006178	1.064568	0.393806
O	-2.314838	1.195530	0.790332
C	-2.912242	-0.117880	0.719481
C	-1.842614	-1.076815	0.081948
C	-0.650373	-0.169953	0.006086
H	-1.651152	-1.930485	0.738701
H	0.309358	-0.480055	-0.373910
O	-2.182963	-1.521465	-1.224977
H	-2.696218	-2.338056	-1.133341
O	1.155795	-2.302929	1.497292
H	1.592794	-2.271392	0.631692
C	-0.241142	2.307045	0.382384
H	1.246153	-1.391251	1.809060
H	-0.837647	3.229804	0.419208
O	0.989002	2.382241	0.322603
H	1.919347	1.242557	0.591216
O	4.680486	-0.492112	0.354116
H	5.084662	-1.263470	-0.090877
H	-3.165796	-0.431066	1.734217
C	-4.180067	-0.115506	-0.109716
O	-4.987113	-1.022883	-0.044474
O	-4.291502	0.941299	-0.915775
H	-5.115857	0.843975	-1.428397

Int-H_2SO_4

Energy=-1309.009008

C	-2.275407	0.564523	0.003230
O	-1.326257	1.184780	-0.889196
C	-0.374702	1.763480	-0.086568
C	-0.474862	1.460344	1.216513
C	-1.572298	0.448725	1.376641
H	-3.132245	1.237342	0.104619
H	0.191055	1.790312	2.000042
H	-2.264409	0.629077	2.199151
C	0.627462	2.604911	-0.768508
H	0.567703	2.604389	-1.870940
O	1.456167	3.258498	-0.158372
H	0.014737	-1.282492	0.541113
C	-2.789292	-0.763765	-0.515363
O	-3.752866	-1.306952	-0.013003
O	-2.080696	-1.253018	-1.532084
H	-2.467041	-2.112473	-1.786209
O	-1.035268	-0.894767	1.534159
H	-0.701802	-0.998093	2.440826
S	2.070639	-0.825695	-0.007709
O	2.275345	0.183115	-1.045473
O	2.250186	-0.439227	1.389359
O	3.019034	-2.089121	-0.348416
H	3.806897	-1.773722	-0.832435
O	0.695540	-1.562375	-0.190533

Int

Energy=-608.5789117

C	0.744200	-0.236940	0.718062
O	-0.320305	-1.033596	0.121013
C	-1.430692	-0.227632	0.094641
C	-1.216152	1.046129	0.453421
C	0.253659	1.240017	0.686111
H	0.847929	-0.559845	1.756229
H	0.496763	1.739503	1.625362
C	-2.684791	-0.874128	-0.330218
H	-2.591922	-1.938182	-0.613067
O	-3.750731	-0.281422	-0.369992
C	2.021429	-0.633245	-0.032324
O	2.666771	-1.606168	0.306728

O	2.347032	0.085161	-1.102659
H	1.766449	0.896401	-1.127450
O	0.792964	2.021420	-0.407388
H	1.424544	2.660232	-0.041300
H	-1.955521	1.833527	0.480449

TS4

Energy=-1308.9950469

C	2.261236	0.732982	0.145911
O	1.179701	1.252037	0.955006
C	0.251840	1.704391	0.118174
C	0.545421	1.513939	-1.215605
C	1.747988	0.796699	-1.262836
H	3.100833	1.430900	0.249273
H	-0.071836	1.821404	-2.045019
H	2.422727	0.721084	-2.103105
C	-0.930949	2.396803	0.714537
H	-0.966717	2.411227	1.813187
O	-1.743901	2.945380	-0.000764
H	0.441319	-1.410205	-1.152031
C	2.731607	-0.646137	0.600736
O	3.802106	-1.085569	0.247722
O	1.841593	-1.255365	1.372706
H	2.178444	-2.142502	1.602508
O	1.288056	-1.177348	-1.630232
H	1.056605	-1.170850	-2.573521
S	-1.918075	-0.872041	0.002870
O	-1.585656	-0.061367	1.203808
O	-2.343127	-0.068988	-1.159931
O	-3.191153	-1.820138	0.432160
H	-3.935600	-1.242748	0.683262
O	-0.895050	-1.910857	-0.311224

TS4′

Energy=-608.5497853

C	0.684551	-0.325769	0.744628
O	-0.449471	-1.050985	0.199996
C	-1.459052	-0.189708	0.120583
C	-1.150944	1.086534	0.529244
C	0.220744	1.078075	0.845731
H	0.842650	-0.702955	1.764596
H	0.726036	1.793065	1.478155
C	-2.754177	-0.723735	-0.376393
H	-2.743633	-1.783528	-0.680208
O	-3.744895	-0.022091	-0.432049
C	1.995770	-0.752988	-0.053359
O	2.085585	-1.964556	-0.264821
O	2.797218	0.182790	-0.325340
H	1.929586	1.431513	-0.698880
O	1.120028	2.032026	-0.787108
H	1.388397	2.886573	-0.412492
H	-1.832117	1.923403	0.560796

IM4

Energy=-1308.999635

C	-2.283696	0.846558	-0.098520
O	-1.213919	1.453418	-0.871189
C	-0.245908	1.748403	-0.045439
C	-0.539667	1.441697	1.300839
C	-1.765934	0.848693	1.294441
H	-3.161661	1.492795	-0.212373
H	0.120650	1.622703	2.133849
H	-2.345802	0.489597	2.132050
C	0.965492	2.450729	-0.582546
H	1.015380	2.542903	-1.675977
O	1.768868	2.930559	0.188832
H	-0.443986	-1.988129	0.915243
C	-2.656924	-0.552079	-0.611589
O	-3.681092	-1.078756	-0.247653
O	-1.754972	-1.044098	-1.446417
H	-2.022313	-1.946877	-1.706768
O	-1.183511	-1.755308	1.513199
H	-0.735116	-1.565092	2.349476
S	1.830359	-0.904563	-0.044989
O	1.325130	0.065057	-1.066429
O	2.015593	-0.297459	1.287297
O	3.316507	-1.365644	-0.574358
H	3.907035	-0.589872	-0.563744
O	1.099883	-2.193897	-0.057662

IM4′

Energy=-608.608871

C	-0.410746	-0.860869	1.857505
O	-1.510804	-0.844972	1.069084
C	-1.397820	0.254934	0.244234
C	-0.227925	0.921336	0.523823
C	0.409619	0.198106	1.568655
H	-0.346803	-1.673163	2.566552
H	1.353793	0.426333	2.039974
C	-2.457148	0.470346	-0.712210
H	-3.268566	-0.281120	-0.680887
O	-2.473121	1.407031	-1.507058
C	1.405875	-1.430388	-0.995133
O	0.450927	-2.097953	-1.061216
O	2.374275	-0.776258	-0.942532
H	2.679502	1.289475	-0.337742

O	2.713264	2.191733	0.011531	O	-2.302767	-0.749248	-1.468353
H	3.076545	2.071205	0.900255	H	-1.478044	-0.441089	-1.887944
H	0.138075	1.809834	0.032124	O	-3.447562	-1.085554	0.653554

IM5

Energy=-1232.5191675

C	-1.640543	-0.595269	-0.283952
O	-0.814709	0.214975	0.600186
C	-0.304725	1.190306	-0.099290
C	-0.754120	1.193848	-1.453357
C	-1.562430	0.118204	-1.586589
H	-1.171660	-1.585093	-0.324426
H	-0.465022	1.925513	-2.192599
H	-2.093485	-0.217618	-2.467836
C	0.475934	2.241135	0.633789
H	0.819343	1.956047	1.638637
O	0.642831	3.329133	0.128368
C	-3.068841	-0.746820	0.244229
O	-3.877625	-1.399777	-0.373826
O	-3.269522	-0.108377	1.388771
H	-4.195704	-0.248348	1.666923
S	2.208249	-0.798442	-0.061083
O	1.928427	0.635417	-0.441418
O	1.164721	-1.720869	-0.554592
O	2.089349	-0.829175	1.582134
H	1.168528	-1.042313	1.818254
O	3.603324	-1.174020	-0.315948

IM6

Energy=-1232.5522236

C	-1.848164	-0.293686	-0.055626
O	-0.935009	0.430988	0.658069
C	-0.966741	1.712293	0.162572
C	-1.896084	1.797004	-0.845405
C	-2.463712	0.504061	-0.985931
H	0.688372	-0.506774	-1.812134
H	-2.132945	2.687213	-1.409624
H	-3.227954	0.187918	-1.680479
C	-0.050760	2.674390	0.757620
H	0.573563	2.283480	1.579713
O	0.017763	3.834739	0.377030
C	-1.995545	-1.722339	0.232493
O	-2.764306	-2.441824	-0.379997
O	-1.193221	-2.127529	1.227160
H	-1.315465	-3.086364	1.355337
S	2.145187	-0.496339	-0.233550
O	2.112717	0.945108	-0.453668
O	1.025377	-1.173465	-1.180909
O	1.572039	-0.873269	1.208030
H	0.671363	-0.495953	1.329157
O	3.382904	-1.239551	-0.358152

TS5

Energy=-1232.5127886

C	1.349568	-0.448162	0.304001
O	0.963498	0.523072	-0.674085
C	0.864771	1.675682	-0.052015
C	1.287798	1.617061	1.281443
C	1.640821	0.304969	1.501682
H	0.304498	-1.030828	0.501142
H	1.306119	2.447647	1.971133
H	2.013946	-0.141260	2.414139
C	0.279252	2.816026	-0.811418
H	-0.057595	2.584520	-1.834129
O	0.191700	3.911270	-0.298594
C	2.290965	-1.518685	-0.193048
O	2.861810	-2.260269	0.574654
O	2.346746	-1.557952	-1.520381
H	2.933891	-2.292137	-1.784742
S	-2.134734	-0.660334	0.162289
O	-1.716390	0.722567	0.469177
O	-1.040300	-1.659243	0.494019

FFCA

Energy=-532.1331542

C	0.779982	0.275646	-0.000105
O	-0.199899	-0.667381	-0.000395
C	-1.391305	0.006347	-0.000049
C	-1.167852	1.365645	-0.000128
C	0.236618	1.539259	-0.000086
H	-1.930398	2.130989	0.000026
H	0.791719	2.465653	-0.000004
C	-2.601091	-0.796610	0.000105
H	-2.434524	-1.889177	0.000052
O	-3.723866	-0.306843	0.000249
C	2.182153	-0.148108	0.000074
O	3.108179	0.645963	0.000262
O	2.323661	-1.481488	0.000004
H	3.277578	-1.682549	0.000102

FF

Energy=-343.4815855

C	1.865692	-0.542427	-0.000026

O	0.639068	-1.114995	-0.000010
C	-0.281570	-0.087721	0.000141
C	0.377516	1.119834	-0.000050
C	1.766444	0.823969	0.000103
H	2.706478	-1.220572	-0.000374
H	2.589184	1.524342	-0.000055
C	-1.676980	-0.454207	0.000004
H	-1.868898	-1.543882	0.000032
O	-2.594167	0.363814	-0.000041
H	-0.092582	2.092865	-0.000224

At the B3LYP+D3(BJ)/6-311++G(2d,p) (smd, solvent = 1-butanol)//B3LYP +D3(BJ)/6-31G(d,p) (smd, solvent = 1-butanol) level.