One-pot conversion of engineered poplar into biochemicals and biofuels using biocompatible deep eutectic solvents

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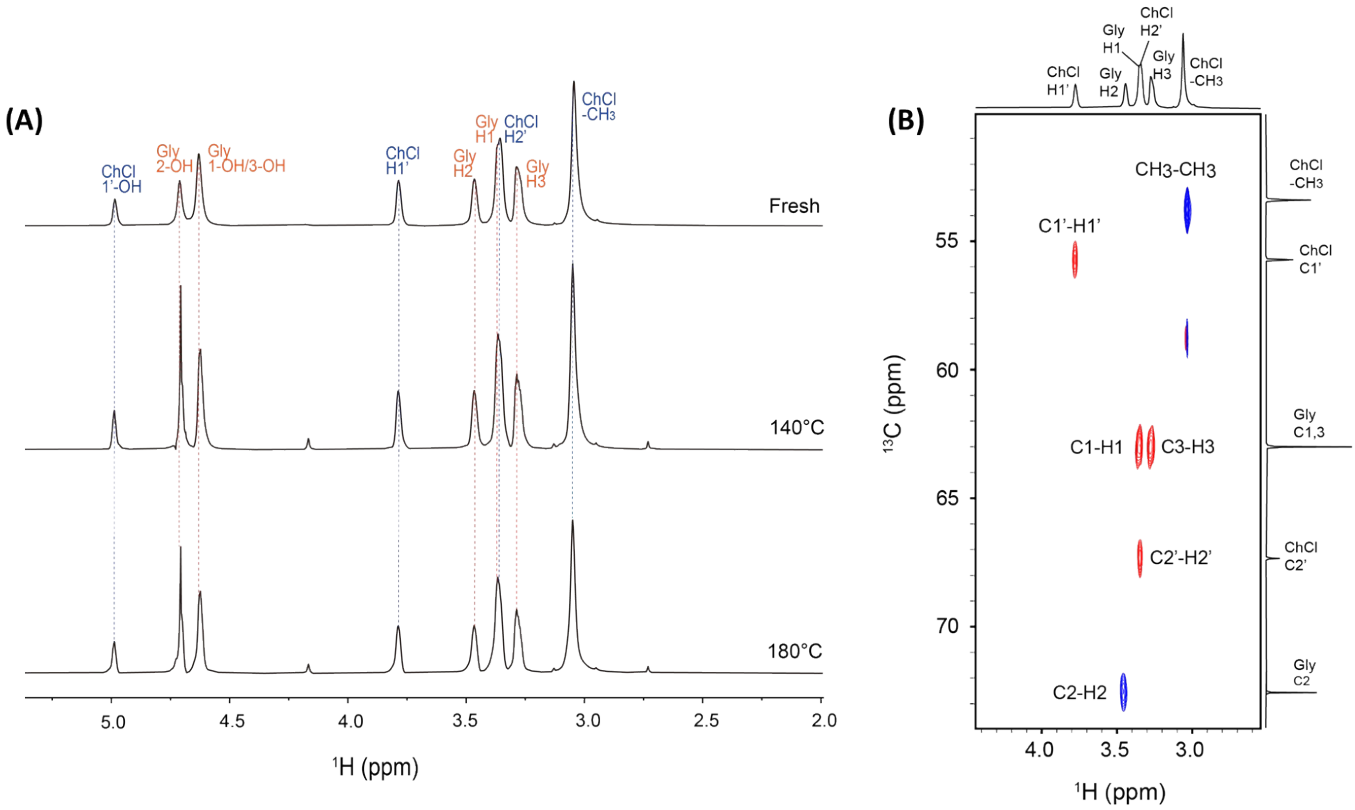


Fig. S1. (A) 1D ^{1}H NMR and (B) 2D ^{1}H–^{13}C HSQC spectra of fresh ChCl–Gly. Positively (CH, CH_3) and negatively (CH_2) phased peaks are shown in blue and red, respectively.

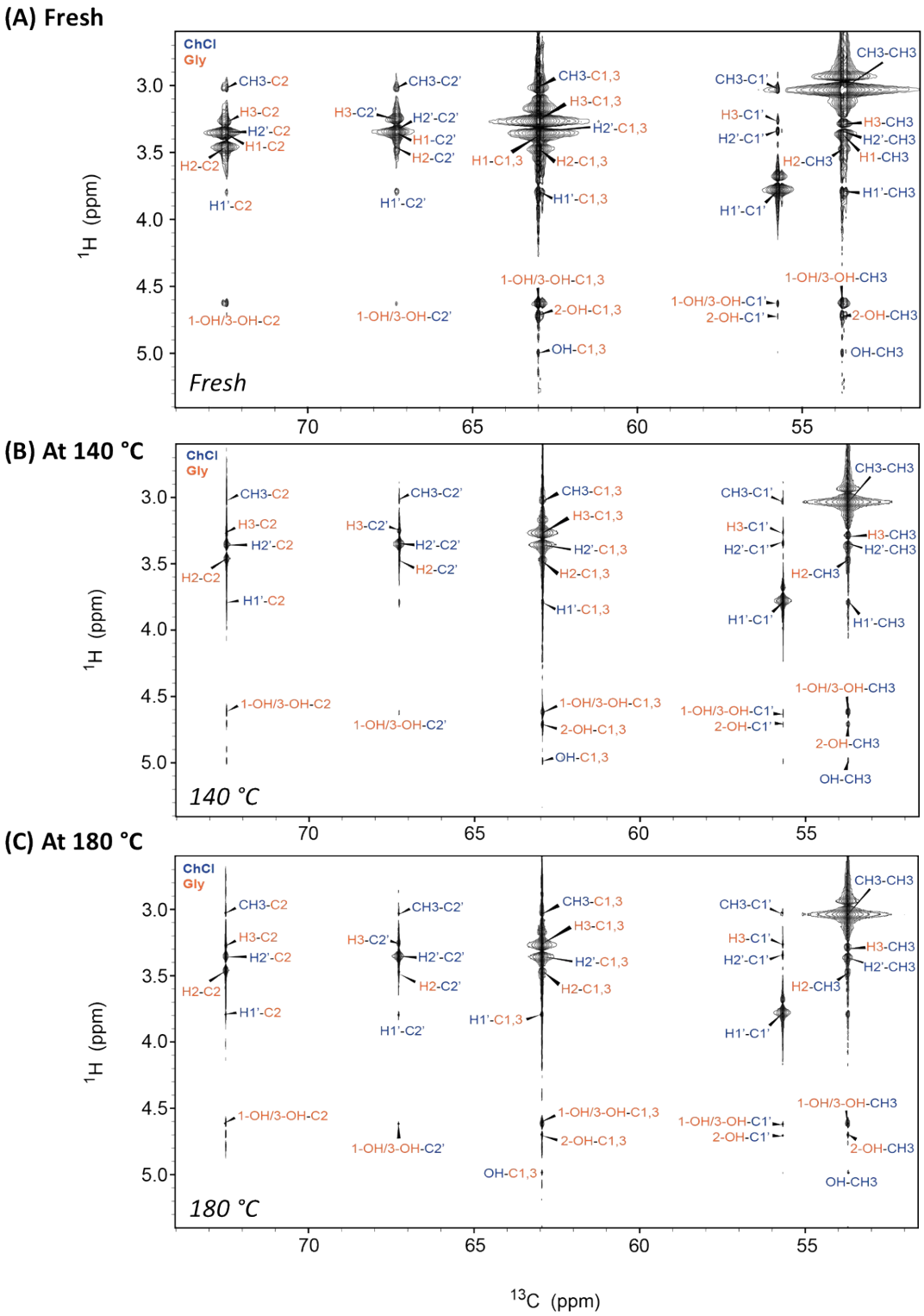


Fig. S2. 2D ^{1}H–^{13}C HOESY spectra of (A) fresh ChCl–Gly, and after treatment at (B) 140 °C and (C) 180 °C. Resonances from choline chloride and from glycerol are colored in blue and orange, respectively.

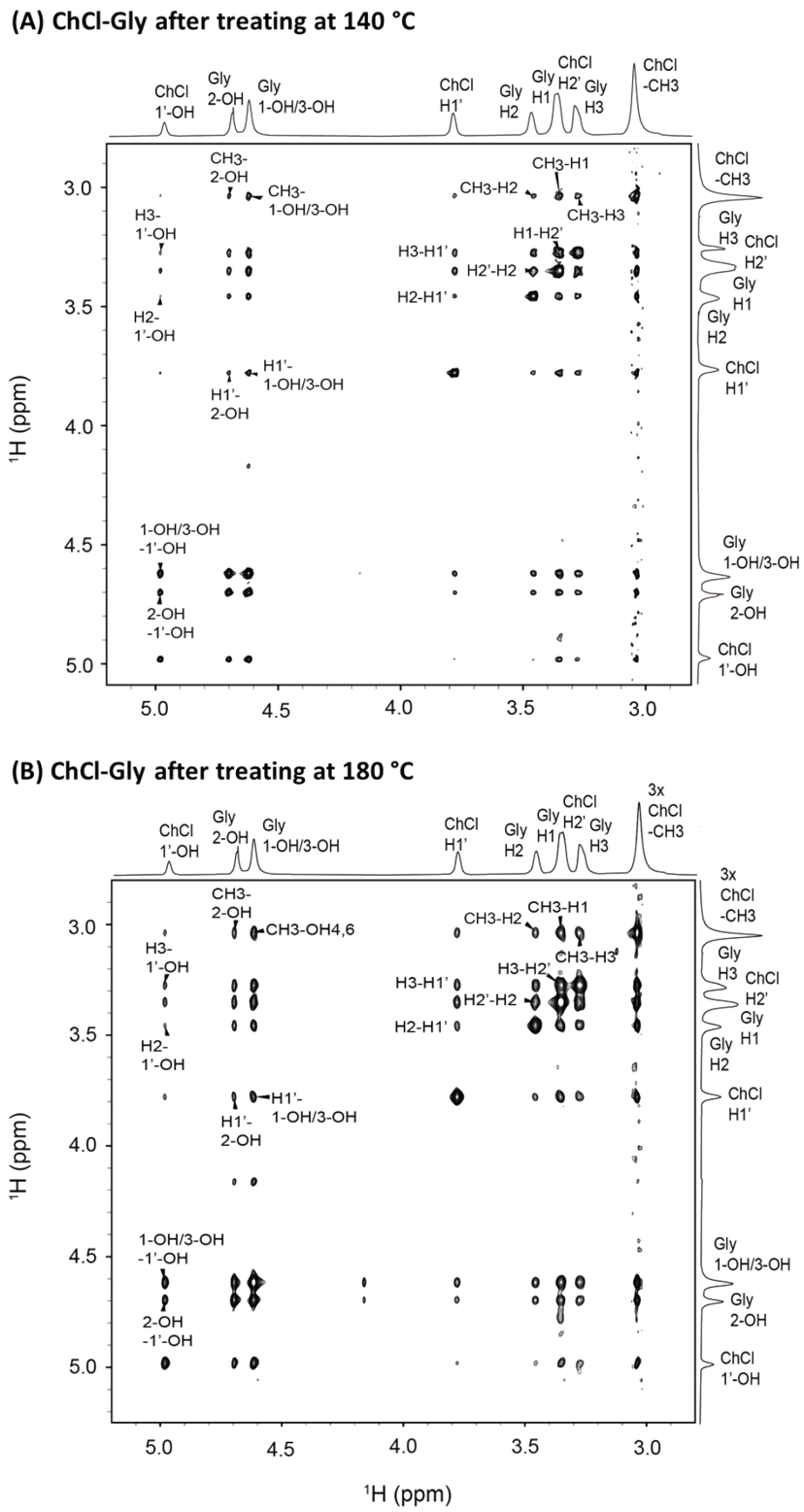


Fig. S3. 2D NOESY spectra of ChCl–Gly after treatment at (A) 140 °C and (B) 180 °C.

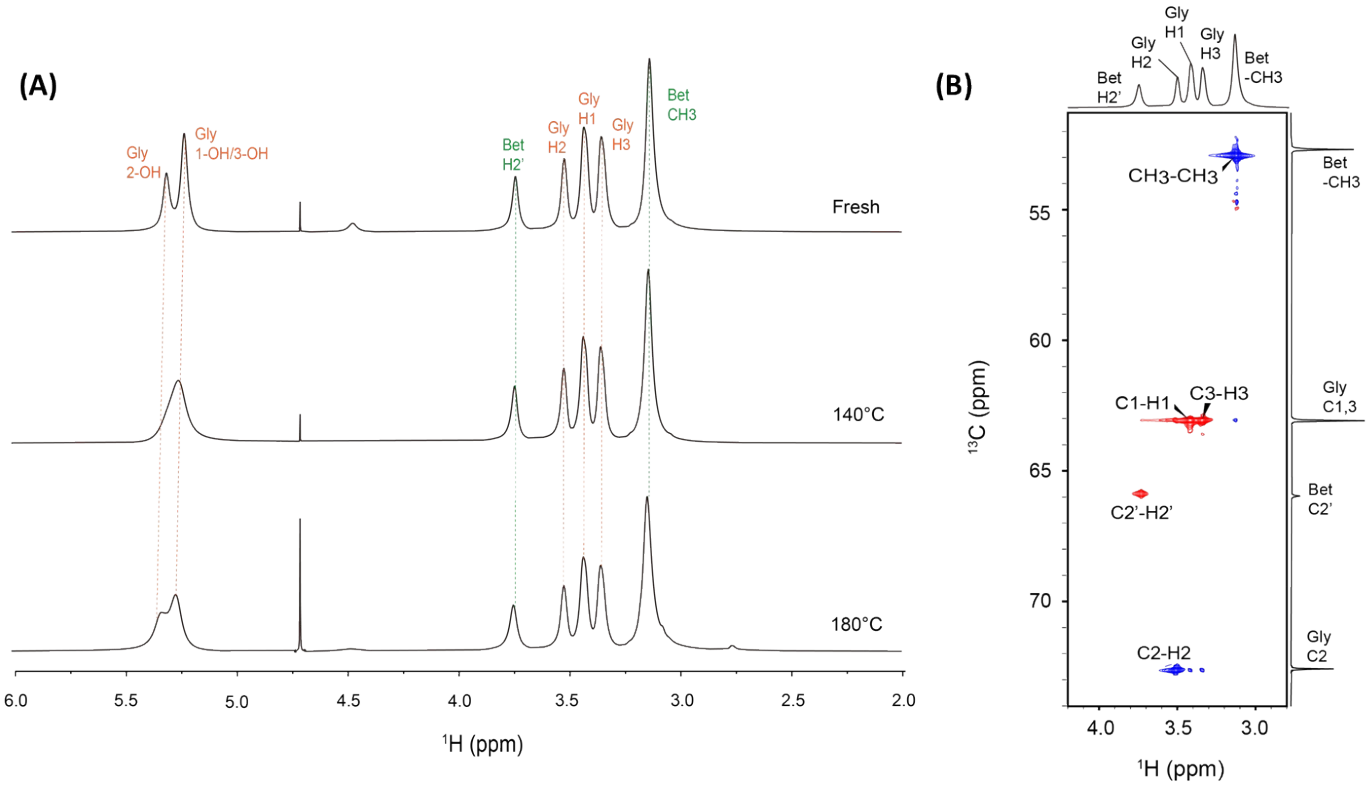


Fig. S4. (A) ^{1}H NMR and (B) HSQC spectra of fresh Bet–Gly. Positively (CH, CH_3) and negatively (CH_2) phased peaks are shown in blue and red, respectively.

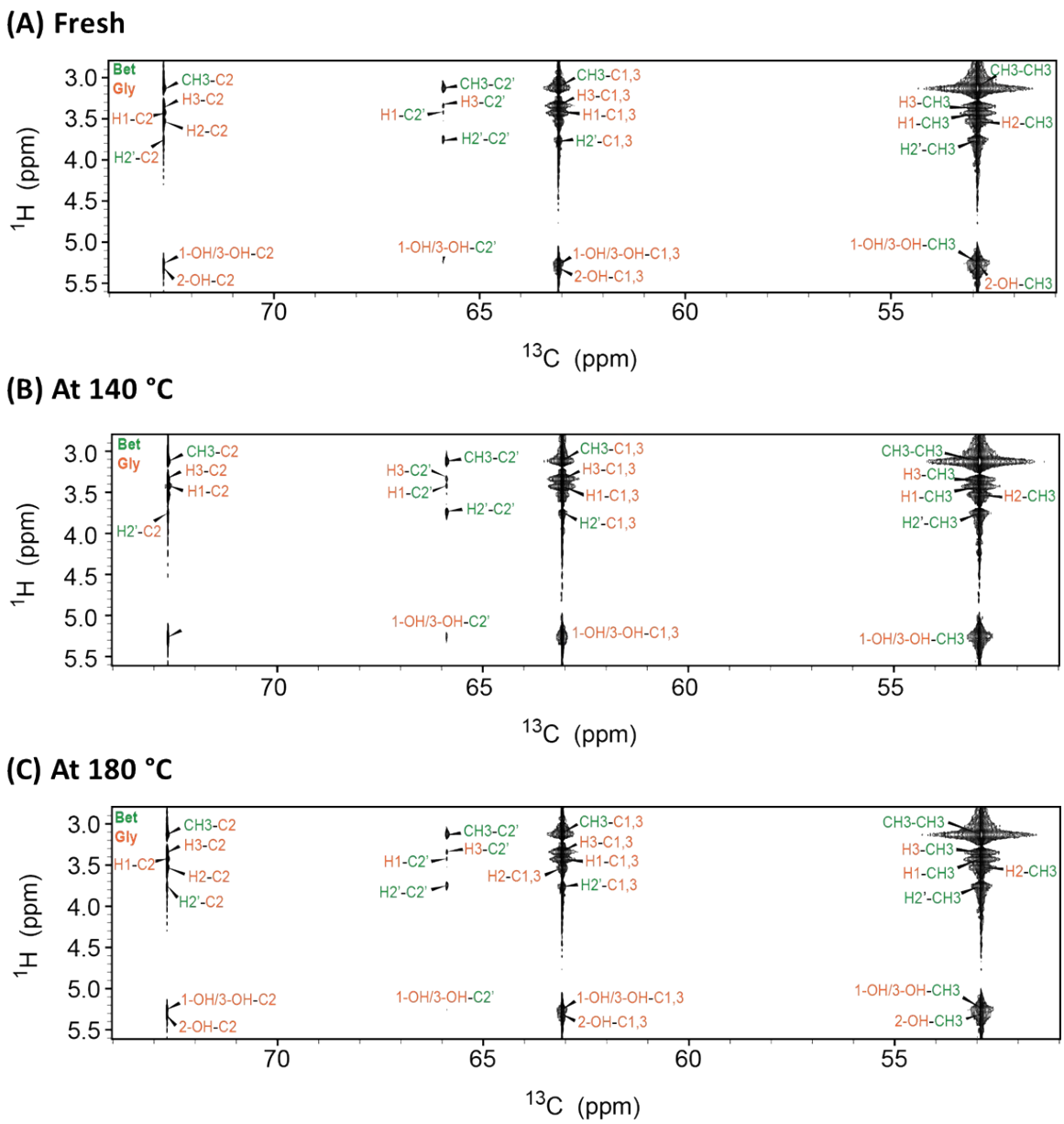


Fig. S5. 2D ^{1}H–^{13}C HOESY spectra of (A) fresh Bet–Gly, and after treatment at (B) 140 °C and (C) 180 °C. Resonances from betaine and from glycerol are colored in green and orange, respectively.

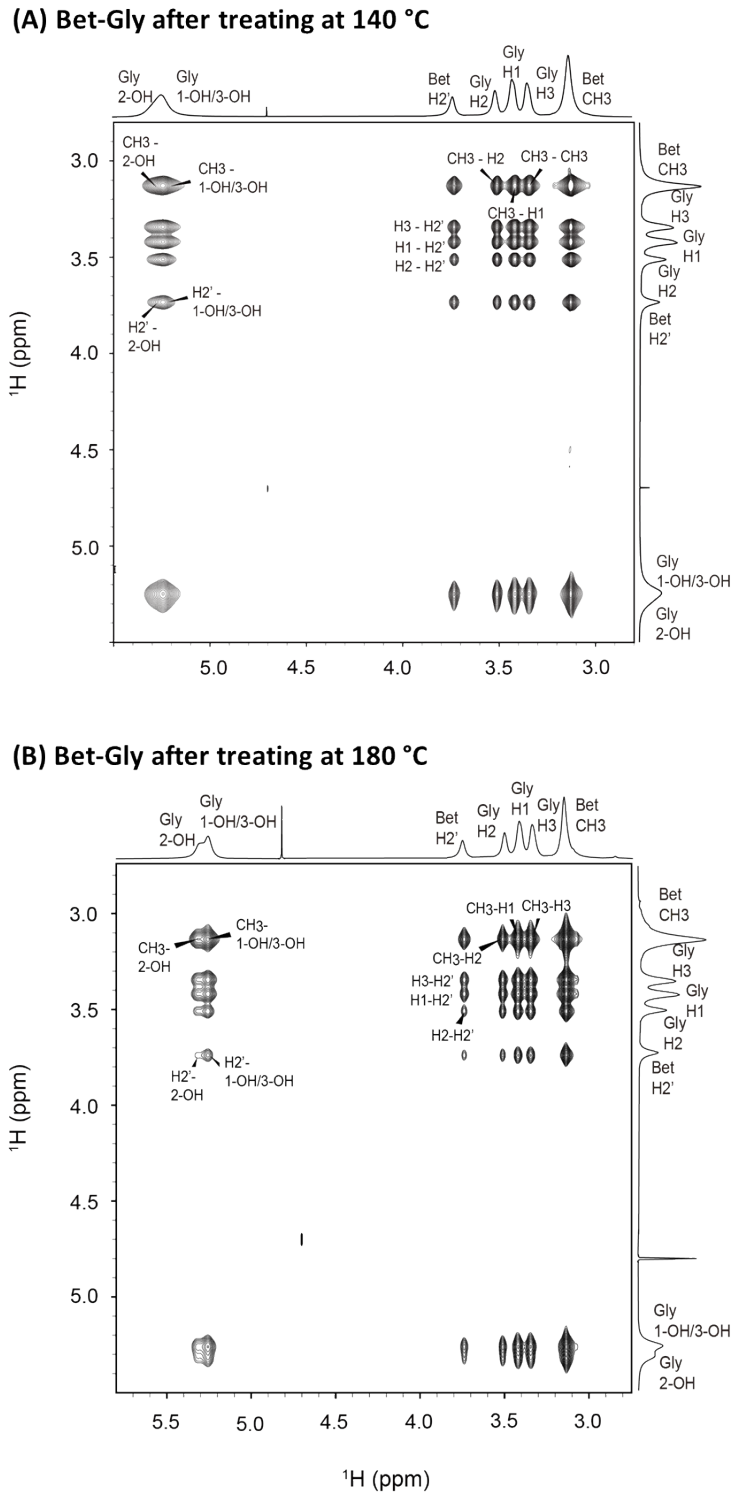


Fig. S6. 2D NOESY spectra of Bet–Gly after treatment at (A) 140 °C and (B) 180 °C.

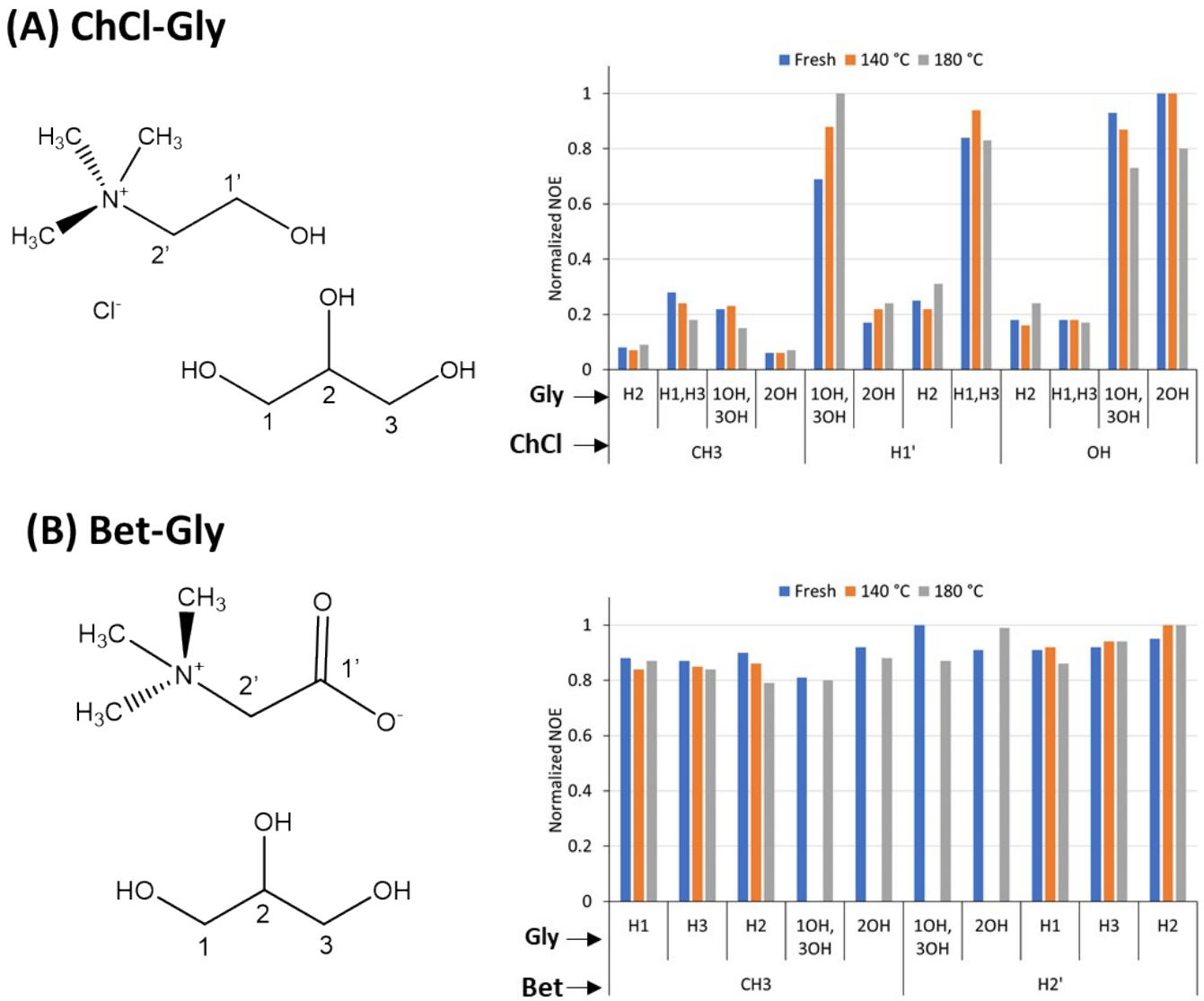
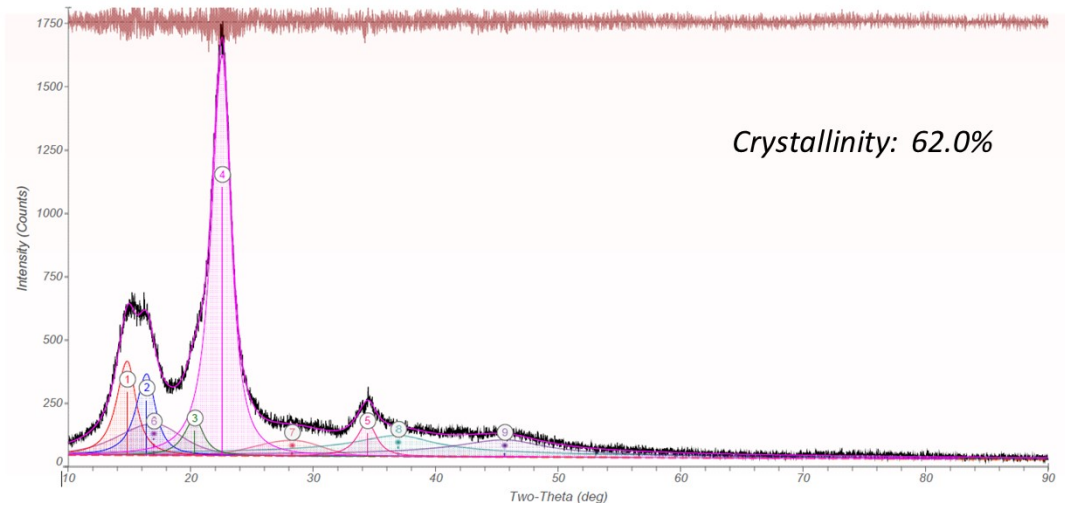


Fig. S7. Chemical structure and the normalized nuclear Overhauser effect (NOE) values of (A) ChCl–Gly and (B) Bet–Gly before and after treatment at 140 and 180 °C. (Data for Bet–Gly after treating at 140 °C not shown due to the presence of unresolved peaks)

(A) Cellulose after treated with ChCl-Gly at 140 °C

#	Angle(°)	d(Å)	Centroid	Centroid	BG	Height	Area(α1)	Area(α1)%	Skew¹	Shape¹	FWHM(°)¹	XS(Å)	AP
1	14.799 (0.016)	5.9813 (0.0132)	14.706°	6.0188Å	45.9	248.1 (6.1)	623.4 (11.7)	22.8 (0.4)%	0.196	1.255p	1.879 (0.007)	32	
2	16.387 (0.021)	5.4051 (0.0138)	16.294°	5.4356Å	45.6	214.8 (8.5)	546.6 (?)	20.0 (?)%	0.196	1.255p	1.879 (Tied)	32	
3	20.308 (0.049)	4.3693 (0.0208)	20.215°	4.3892Å	44.7	96.5 (6.6)	248.5 (?)	9.1 (?)%	0.196	1.255p	1.879 (Tied)	32	
4	22.546 (0.010)	3.9404 (0.0033)	22.453°	3.9565Å	44.2	1058.7 (8.9)	2738.8 (?)	100.0 (?)%	0.196	1.255p	1.879 (Tied)	32	
5	34.416 (0.025)	2.6037 (0.0037)	34.323°	2.6106Å	41.5	88.6 (3.2)	230.1 (?)	8.4 (?)%	0.196	1.255p	1.879 (Tied)	32	
6	16.975 (0.221)	5.2189 (0.1350)	16.604°	5.3348Å	45.4	83.3 (8.7)	563.7 (86.1)	20.6 (3.1)%	0.240	3.119p	6.100 (0.413)	12	•
7	28.259 (0.189)	3.1554 (0.0414)	27.876°	3.1979Å	42.9	41.3 (3.5)	277.8 (34.0)	10.1 (1.2)%	0.240	50.000p	6.290 (0.320)	12	•
8	36.934 (0.250)	2.4318 (0.0318)	36.311°	2.4721Å	40.9	55.6 (2.8)	1049.7 (73.2)	38.3 (2.7)%	0.240	0.500p	10.226 (0.610)	4	•
9	45.617 (0.127)	1.9871 (0.0105)	44.994°	2.0131Å	39.0	45.4 (2.5)	801.9 (36.2)	29.3 (1.3)%	0.240	0.616p	10.226 (0.285)	5	•



(B) Cellulose after treated with ChCl-Gly at 180 °C

#	Angle(°)	d(Å)	Centroid	Centroid	BG	Height	Area(α1)	Area(α1)%	Skew¹	Shape¹	FWHM(°)¹	XS(Å)	AP
1	14.896 (0.016)	5.9426 (0.0126)	14.807°	5.9780Å	46.9	288.5 (12.2)	734.5 (23.4)	24.9 (0.8)%	0.184	1.284p	1.916 (0.006)	32	
2	16.390 (0.035)	5.4040 (0.0230)	16.301°	5.4333Å	46.7	228.9 (28.7)	589.5 (?)	20.0 (?)%	0.184	1.284p	1.916 (Tied)	31	
3	20.382 (0.043)	4.3537 (0.0181)	20.293°	4.3725Å	46.2	109.3 (5.4)	284.5 (?)	9.7 (?)%	0.184	1.284p	1.916 (Tied)	31	
4	22.684 (0.009)	3.9169 (0.0031)	22.595°	3.9320Å	45.9	1126.1 (7.7)	2944.8 (?)	100.0 (?)%	0.184	1.284p	1.916 (Tied)	31	
5	34.579 (0.023)	2.5918 (0.0033)	34.490°	2.5983Å	44.4	102.8 (3.6)	269.8 (?)	9.2 (?)%	0.184	1.284p	1.916 (Tied)	32	
6	17.127 (0.342)	5.1731 (0.2049)	17.146°	5.1673Å	46.6	112.8 (19.2)	600.2 (102.5)	20.4 (3.5)%	-0.020	1.124p	3.886 (0.312)	15	•
7	28.848 (0.171)	3.0924 (0.0358)	28.892°	3.0877Å	45.2	78.8 (4.2)	1172.0 (100.6)	39.8 (3.4)%	-0.020	0.663p	8.949 (0.591)	6	•
8	37.816 (0.181)	2.3771 (0.0219)	37.849°	2.3751Å	44.0	38.4 (4.4)	268.6 (41.8)	9.1 (1.4)%	-0.020	50.000p	6.553 (0.394)	12	•
9	45.298 (0.283)	2.0003 (0.0237)	45.348°	1.9982Å	43.1	71.2 (3.6)	1185.0 (47.3)	40.2 (1.6)%	-0.020	0.692p	10.002 (0.212)	5	•

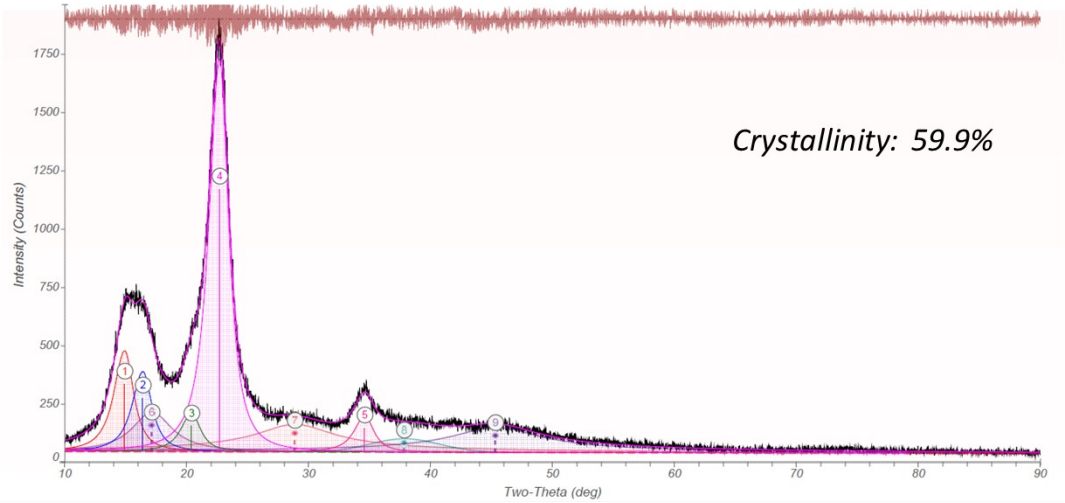


Fig. S8. X-ray diffraction spectra and crystallinity index of cellulose after treating with ChCl–Gly at (A) 140 °C and (B) 180 °C.

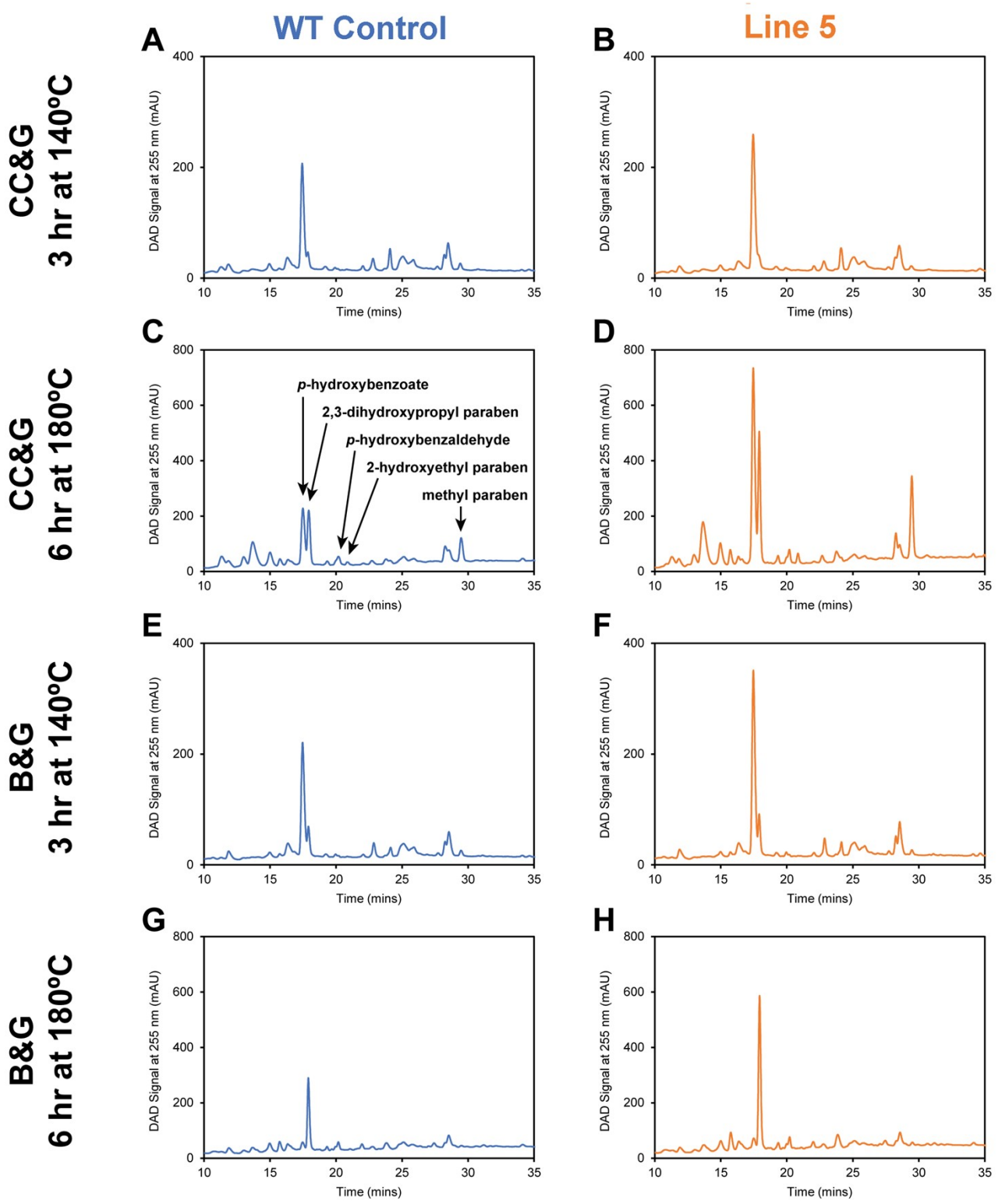


Fig. S9. HPLC-DAD chromatograms at 255 nm for DES hydrolysates of the WT (shown in blue) and line 5 CPL poplar (shown in orange) for treatments with ChCl–Gly (A-D) and Bet–Gly (E-H).

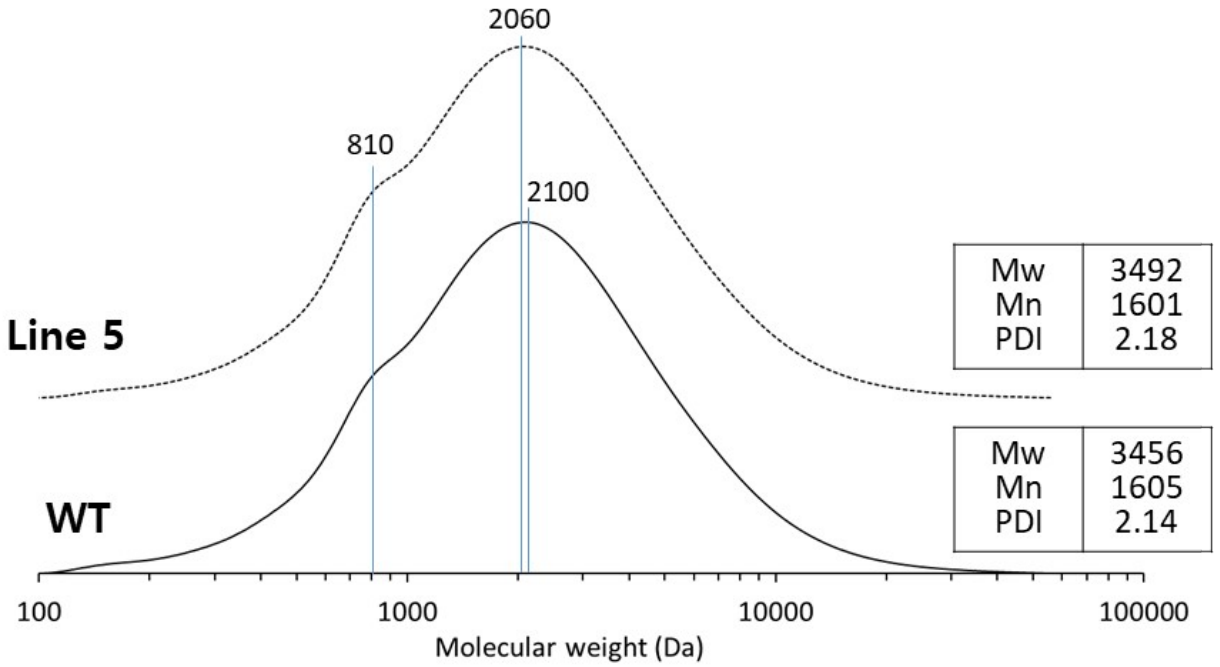


Fig. S10. The average molecular weight and molecular weight distribution of residual lignin recovered after ChCl–Gly one-pot treatment (pretreatment conditions: 180 °C for 6 h, enzymatic saccharification: 50 °C for 72 h).

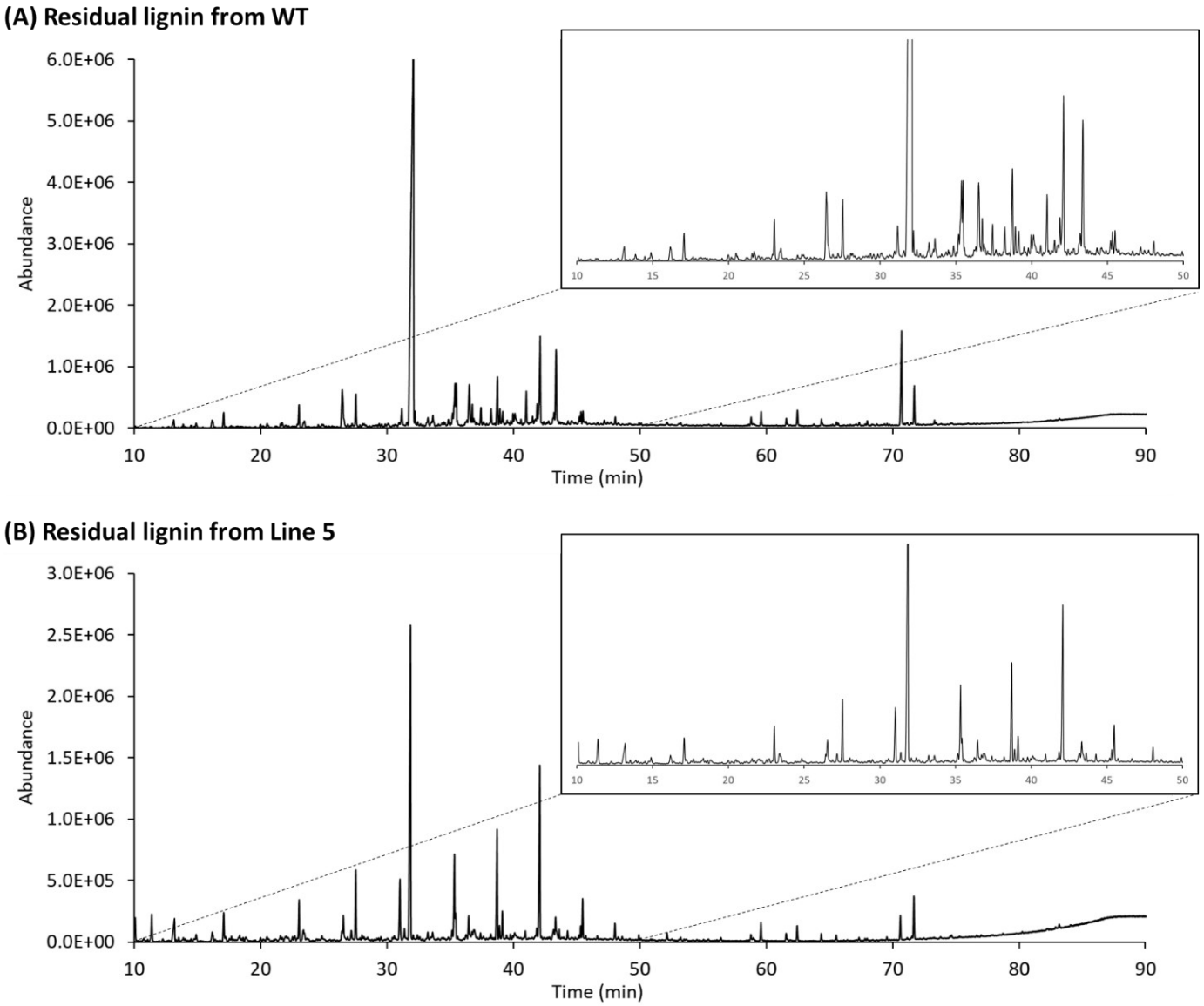


Fig. S11. Gas chromatogram of products in the liquid phase produced from the hydrogenolysis of (A) WT poplar and (B) transgenic poplar line 5-derived residual lignin.

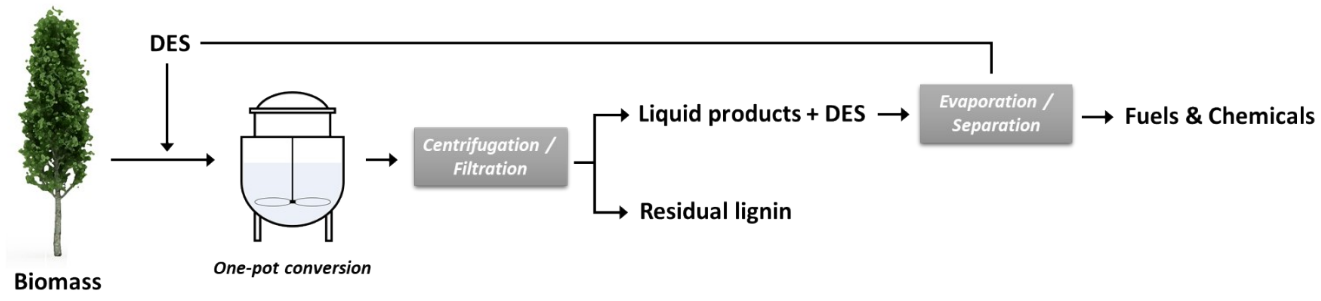


Fig. S12. Simplified process flow diagram for the biocompatible DES-based biorefinery with DES recycling.

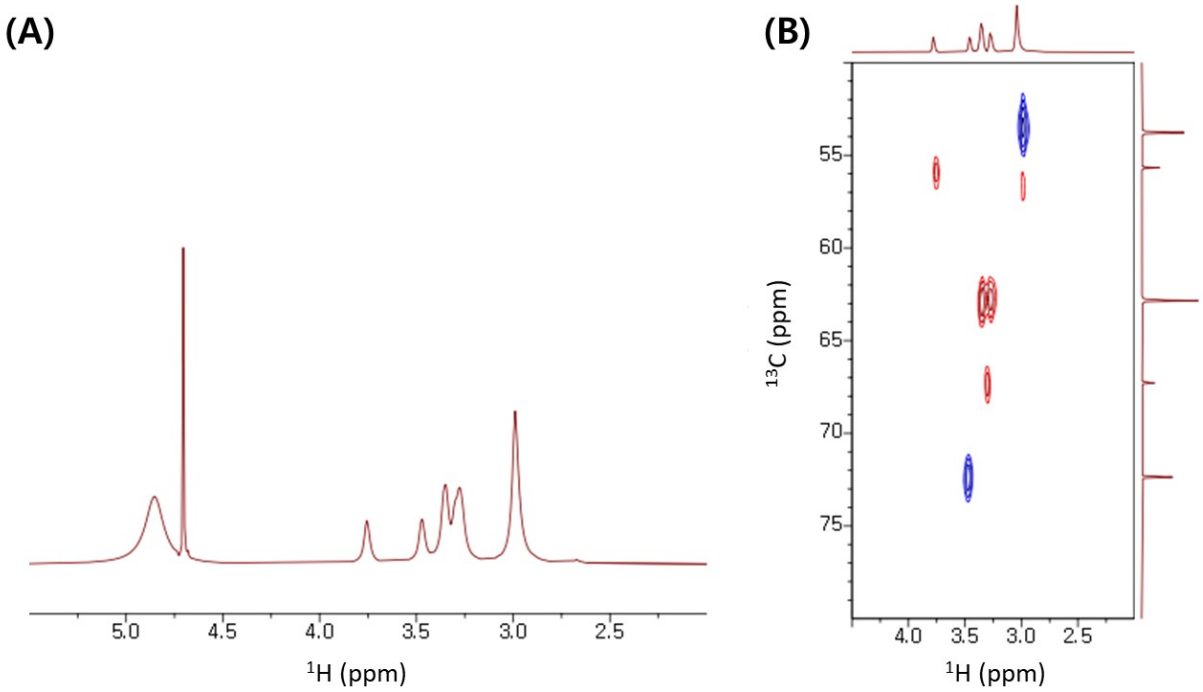


Fig. S13. (A) 1D ^{1}H NMR and (B) 2D ^{1}H–^{13}C HSQC spectra of recovered ChCl–Gly. Positively (CH, CH_3) and negatively (CH_2) phased peaks are shown in blue and red, respectively.

Table S1. The amount of free *p*HB and *p*HB derivatives in the hydrolysates after one-pot DES pretreatment and enzymatic saccharification.

Conditions	Samples	*p*HB	*p*HB aldehyde	2,3-dihydroxypropyl paraben	Methyl paraben	2-hydroxyethyl paraben
ChCl–Gly	WT	1,044 ± 157	21 ± 0	220 ± 15	43 ± 13	6 ± 2
140 °C	Line 1	1,397 ± 38	15 ± 2	190 ± 3	36 ± 12	5 ± 2
3 h	Line 2	1,535 ± 54	16 ± 0	185 ± 3	31 ± 5	4 ± 2
	Line 3	1,157 ± 3	15 ± 1	190 ± 18	33 ± 6	5 ± 2
	Line 4	1,263 ± 4	20 ± 5	159 ± 15	21 ± 1	5 ± 2
	Line 5	1,714 ± 419	19 ± 2	217 ± 18	38 ± 5	4 ± 1
ChCl–Gly	WT	1,263 ± 231	689 ± 20	1,113 ± 47	57 ± 0	57 ± 0
180 °C	Line 1	2,697 ± 457	744 ± 22	1,684 ± 119	114 ± 7	114 ± 7
6 h	Line 2	2,296 ± 96	761 ± 39	1,716 ± 187	109 ± 12	109 ± 12
	Line 3	2,397 ± 134	713 ± 75	1,590 ± 221	98 ± 15	98 ± 15
	Line 4	2,472 ± 180	731 ± 33	1,285 ± 36	87 ± 10	87 ± 10
	Line 5	3,857 ± 488	760 ± 86	1,892 ± 352	137 ± 24	137 ± 24
Bet–Gly	WT	1,115 ± 172	17 ± 0	303 ± 18	42 ± 8	5 ± 1
140 °C	Line 1	1,363 ± 23	15 ± 1	309 ± 15	30 ± 4	4 ± 1
3 h	Line 2	1,624 ± 64	14 ± 0	345 ± 19	30 ± 3	4 ± 1
	Line 3	1,158 ± 38	16 ± 1	298 ± 3	35 ± 2	5 ± 1
	Line 4	1,369 ± 106	17 ± 1	270 ± 44	19 ± 6	3 ± 1
	Line 5	1,958 ± 424	17 ± 1	436 ± 27	39 ± 0	4 ± 0
Bet–Gly	WT	155 ± 96	299 ± 76	1,415 ± 148	47 ± 17	2 ± 0
180 °C	Line 1	209 ± 58	318 ± 11	2,169 ± 150	57 ± 6	3 ± 0
6 h	Line 2	116 ± 9	314 ± 36	1,984 ± 167	60 ± 18	13 ± 11
	Line 3	197 ± 91	337 ± 15	2,018 ± 136	57 ± 13	3 ± 0
	Line 4	272 ± 36	341 ± 2	1,764 ± 18	58 ± 11	15 ± 14
	Line 5	267 ± 144	375 ± 39	2,678 ± 155	77 ± 26	3 ± 0

Table S2. Amounts of interunit linkages in recovered lignin samples. .

Linkages	Amount (% of total aromatics)	
	WT	**Line 5**
β–O–4 aryl ether, **A**	8.3	9.0
β–5 phenylcoumaran, **B**	4.9	2.8
β–β resinol, **C**	5.6	3.6

Reaction conditions: Treated with ChCl–Gly at 180 °C for 6 h. The amounts of each interunit linkage were determined by integration of the peak volumes (A_α, B_α, and $0.5 \times C_\alpha$, respectively) on a basis of total aromatics. Total aromatics = $(0.5\times H_{2/6} + G_2 + 0.5\times S_{2/6})$.

Table S3. The amount of alkylphenols from the hydrogenolysis of residual lignin.

Compounds	Amount (mg/g feedstock)	
	Residual lignin from WT	**Residual lignin from Line 5**
p-cresol (H, C1C6)	1.72 ± 0.33	1.33 ± 0.01
4-propylphenol (H, C3C6)	8.36 ± 0.02	3.35 ± 0.97
guaiacol (G, C6)	1.93 ± 0.35	1.87 ± 0.41
4-methylguaiacol (G, C1C6)	2.84 ± 0.32	2.41 ± 0.55
4-ethylguaiacol (G C2C6)	3.20 ± 0.15	3.22 ± 0.18
4-propylguaiacol (G, C3C6)	90.58 ± 6.21	16.78 ± 0.96
syringol (S, C6)	3.64 ± 0.03	4.03 ± 0.64
4-methylsyringol (S, C1C6)	7.04 ± 0.89	5.47 ± 1.09
4-ethylsyringol (S, C2C6)	7.74 ± 0.06	8.27 ± 0.88
4-propylsyringol (S, C3C6)	17.75 ± 1.05	14.69 ± 0.88
Sum	**144.80 ± 5.16**	**61.41 ± 2.71**