SUPPORTING INFORMATION

Cellulose Triacetate Synthesis via One-Pot Organocatalytic Transesterification and Delignification of Pretreated Bagasse

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Experimental

Preparation of lignin

Lignin was isolated from bagasse according to previously reported procedures.[1, 2] However, DMSO was newly employed as a co-solvent to assist in the dissolution of bagasse.[3, 4] In order to clarify the difference from the original reports,[1, 2] the detailed experimental protocol is given below.

First, choline acetate (ChOAc) was prepared by the one-pot neutralization method[5] with minor modifications. For isolation of lignin, 10 g each of bagasse (particle size: 250–500 μm), ChOAc, and DMSO were mixed and heated in an incubator (UNI ACE UA-1100, Tokyo Rikakikai Co.) at 110 °C for 16 h. The resulting mixture was transferred to a centrifuge tube with distilled water (200 mL) and the Cellic® CTec2 enzyme (50 filter paper units (FPU)·g^{-1} to bagasse loading). The enzymatic saccharification was then conducted at 50 °C for 72 h. After centrifugation (12,500×g, 4 °C, 30 min), the residual solid was washed three times with distilled water (100 mL). The obtained brown powder (i.e., enzymatic lignin) was dried at 90 °C for longer than 4 h and crushed by a ball-mill (Premium line P-7, Fritsch Japan Co.). Isolated yield; 2.85 g (65%, for the content of lignin in 10 g raw bagasse).

Acetylation of cellulose, xylan, and lignin

Cellulose acetate and xylan acetate. Avicel and xylan were separately acetylated following the procedures described in our previous reports.[6-8]. Avicel and xylan (each 600 mg) in EmimOAc (10 g) were dried under vacuum at 80 °C for 2 h to remove the moisture. DMSO (15 mL) was added to the solution under an Ar atmosphere, then heated to 110 °C and stirred for 16 h to obtain a clear viscous solution with a pale yellow colour. The reaction solution was cooled to 80 °C and further stirred for 30 min with an excess amount of isopropenyl acetate (IPA, 20 mL). The reaction mixture was then poured into 600 mL of MeOH, and repeatedly filtered and washed with MeOH. The pale yellowish gel-like solid was dissolved in chloroform (200 mL). After filtration to remove the partial chloroform-insoluble fraction, the filtrate was concentrated using a rotary evaporator at 40 °C. The concentrated filtrate was reprecipitated in MeOH (300 mL). The residue was then washed with MeOH and distilled water. After freeze drying for two days, cellulose acetate and xylan acetate were obtained. The average yield (n = 3) was 619 mg (59%, cellulose acetate) and 570 mg (59%, xylan acetate).

Lignin acetate. Lignin (600 mg) in EmimOAc (10 g) was dried under vacuum at 80 °C for 2 h to remove the moisture. After DMSO (15 mL) was added to the solution under an Ar atmosphere, the obtained solution was heated to 110 °C and stirred for 16 h to obtain a clear viscous black-brown solution. The reaction solution was then cooled to 80 °C, and stirred for another 30 min with an excess amount of IPA (20 mL). Subsequently, the reaction mixture was poured into 600 mL of MeOH. A small amount of the precipitated polymer was removed by filtration, which might have been derived from the contamination of the polysaccharides. The average yield (n = 3) was 248 mg.

The methanol (MeOH) filtrate was then concentrated via evaporation at 40 °C to afford a black viscous liquor containing EmimOAc, DMSO, and lignin derivatives. Next, acetone (100 mL) was added slowly to the obtained

mixture to yield a clear solution. This homogeneous solution was stirred at room temperature for 2 h along with strong-acidic cation exchange resin (Amberlite® IRN-77 H^+ (50 g)) to remove $Emim^+$. The resins were removed by filtration and the filtrate was evaporated at 40 °C. The concentrated solution was then added dropwise to distilled water (600 mL) to precipitate the product as light brown particles. The precipitate was washed repeatedly with distilled water after centrifugation (12,500×g, 4 °C, 60 min) and freeze dried for two days to afford lignin acetate. The average isolated yield (n = 3) was 411 mg (81%).

Mass balance

Table S1 Mass balance diagram of the products from different starting materials.

	Polysaccharide acetate (MeOH-insoluble) / mg		Lignin acetate / mg
Starting material [a]	CH_3Cl-soluble	Insoluble	MeOH-soluble
Bagasse [b]	326±26	192±1	144±4
Pretreated bagasse [b]	423±47	—	197±2
Avicel [c]	619±22	—	—
Xylan [c]	570±6	—	—
Lignin [c]	—	248±5	411±3

[a] 600 mg was used in each of these reactions. [b] Average results (n = 5). [c] Average results (n = 3).

Characterization

^{13}C NMR

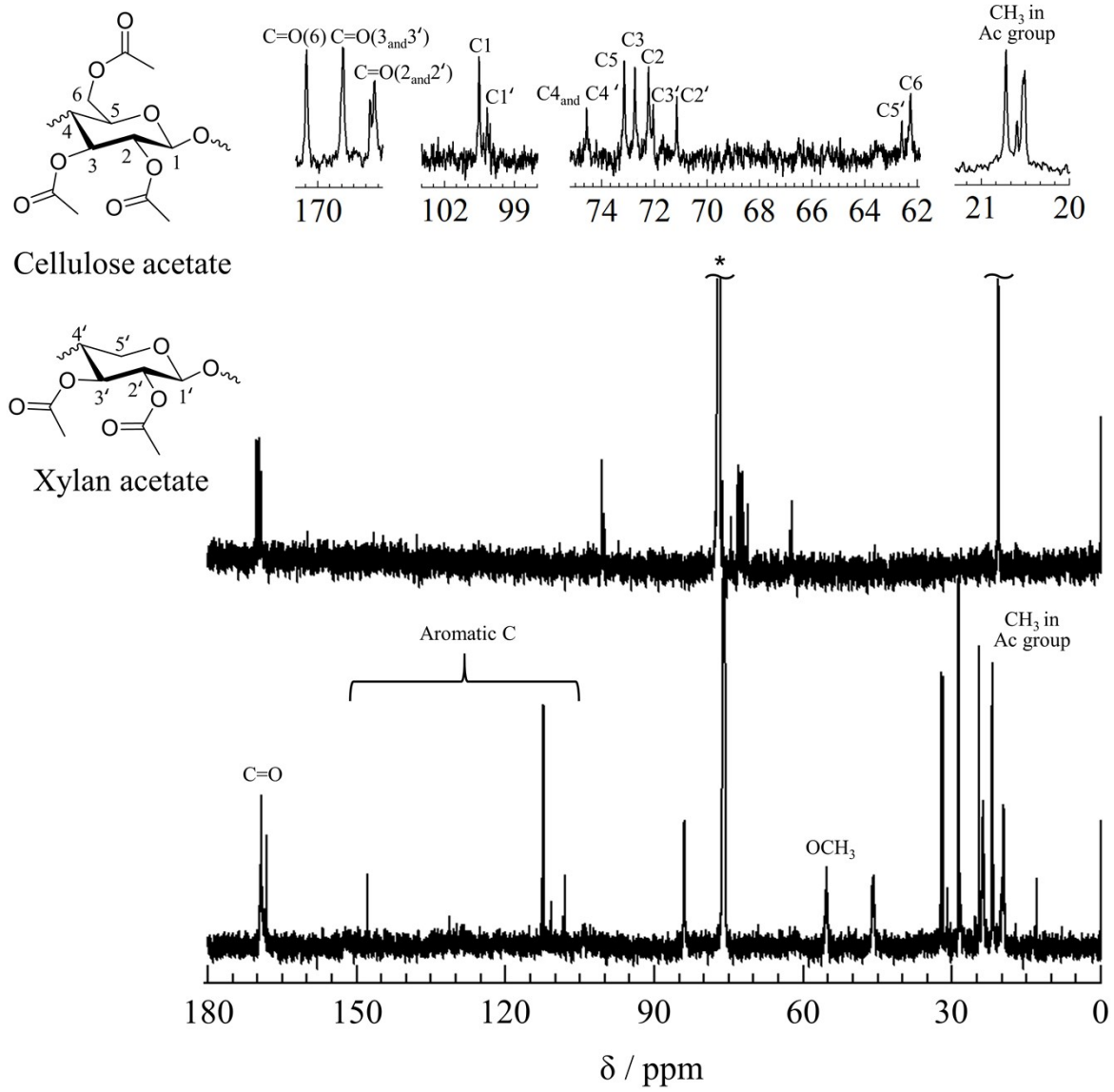


Figure S1. ^{13}C NMR spectra of polysaccharide acetate (upper) and lignin acetate (lower) from bagasse measured in $CDCl_3$ at 55 °C. Peaks corresponding to xylan acetate were assigned according to the literature.[9]

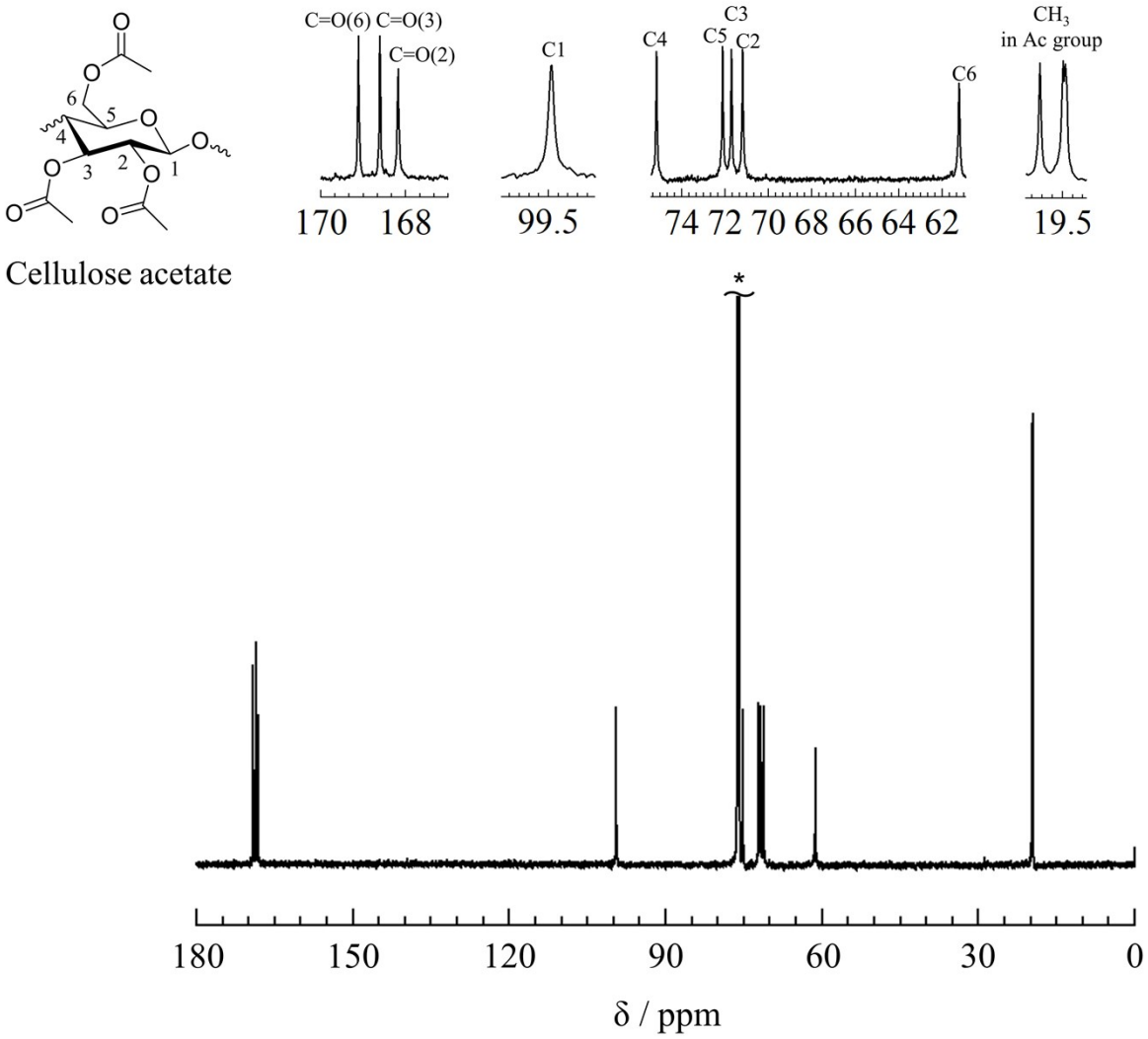


Figure S2. ^{13}C NMR spectrum of cellulose triacetate obtained from pretreated bagasse measured in $CDCl_3$ at 55 °C.

Size exclusion chromatography (SEC)

All samples (10 mg) were dried under vacuum at 70 °C for 24 h prior to dissolution, and the dried samples were weighed in a vial. The eluent (2.0 mL, DMF containing 0.01 mol·L^{-1} LiBr) was added to the vial and the obtained solution was left at room temperature for 24 h prior to the SEC measurement. The resultant solution was filtered using a PTFE-syringe filter (pore size: 0.22 μm) equipped with a glass-prefilter and transferred to another vial for the autosampler. All the SEC measurements were conducted under the conditions listed below.

- Column: TSKgel α-M
- Eluent: 0.01 mol·L^{-1} LiBr in DMF
- Flow rate: 1.0 mL·min^{-1}
- Temperature 40 °C
- Injection 100 μL
- Detector: Refractive index (RI)
- Standard: Polystyrene (PStQuick A and PStQuick C, Tosoh Co.)
- Sample conc. 5.0 g·L^{-1}

The SEC traces of polysaccharide acetate and lignin acetate from the different starting materials are depicted in Figures S5 and S6, respectively. The estimated weight average molecular weight (M_w) and the dispersity ($Đ$) of the acetylated samples are given in Table S2.

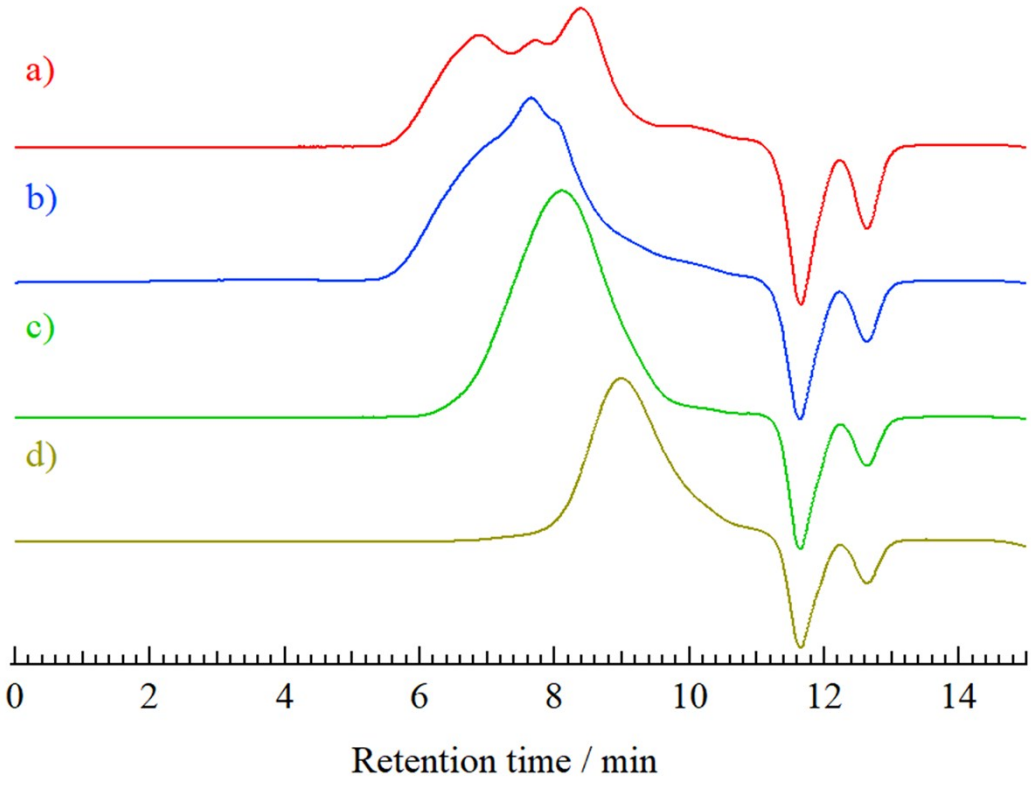


Figure S3. SEC traces of the acetylated samples of (a) polysaccharide acetate from bagasse, (b) cellulose triacetate from pretreated bagasse, (c) cellulose acetate from Avicel, and (d) xylan acetate from xylan, as recorded by an RI detector.

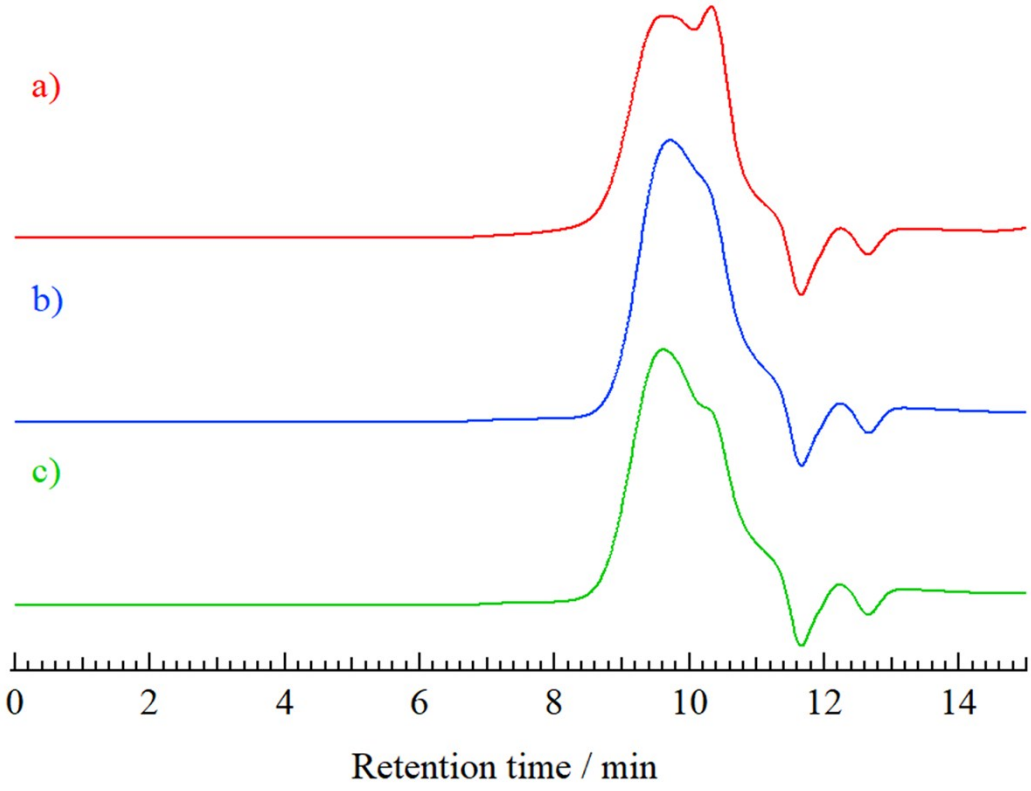


Figure S4. SEC traces of the acetylated samples of lignin acetate from (a) bagasse, (b) pretreated bagasse, and (c) lignin, as recorded by an RI detector.

Table S2 Molecular weights (M_w) and dispersity ($Đ$) of the acetylated products determined by SEC using an RI detector.

Material	M_w / kg·mol^{-1}	$Đ$
Polysaccharide acetate from bagasse	1316	15
Cellulose triacetate from pretreated bagasse	1306	14
Cellulose acetate from Avicel	237	5.3
Xylan acetate from xylan	21.3	7.4
Lignin acetate from bagasse	3.7	15
Lignin acetate from pretreated bagasse	2.9	14
Lignin acetate from lignin	3.8	17

Composition analysis

The cellulose, hemicellulose, and lignin contents of the acetylated products and the corresponding starting materials were determined by a composition analysis according to the standard biomass analytical method provided by the National Renewable Energy Laboratory (NREL).[10] For composition analysis, the acetylated products containing polysaccharide acetate from bagasse, cellulose triacetate from pretreated bagasse, and cellulose acetate from Avicel were deacetylated prior to the measurements as described in the experimental section of the main text.

The amounts of the monosaccharides derived from cellulose and hemicellulose in the hydrolysates were determined by HPLC equipped with an RI detector using the calibration curves of standard materials containing glucose, xylose, galactose, arabinose, and mannose. These standard solutions were prepared as aqueous solutions with concentrations ranging from 0.5–50 mmol·L^{-1}. The calibration curves are shown in Figure S3.

HPLC analysis for monomeric sugars

- Column: CARBOSep CHO-682
- Eluent: Ultra-pure water
- Flow rate: 0.4 mL·min^{-1}
- Temperature: 85 °C
- Detector: RI
- Injection: 20 μL

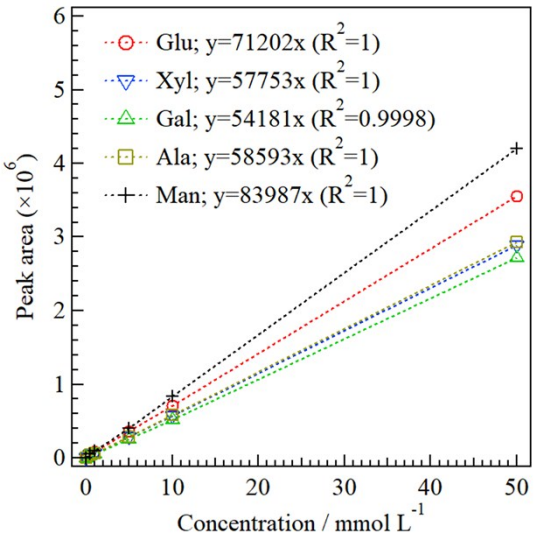


Figure S5. Calibration curves of standard materials in the concentration range from 0.5 to 50 mmol·L^{-1}.

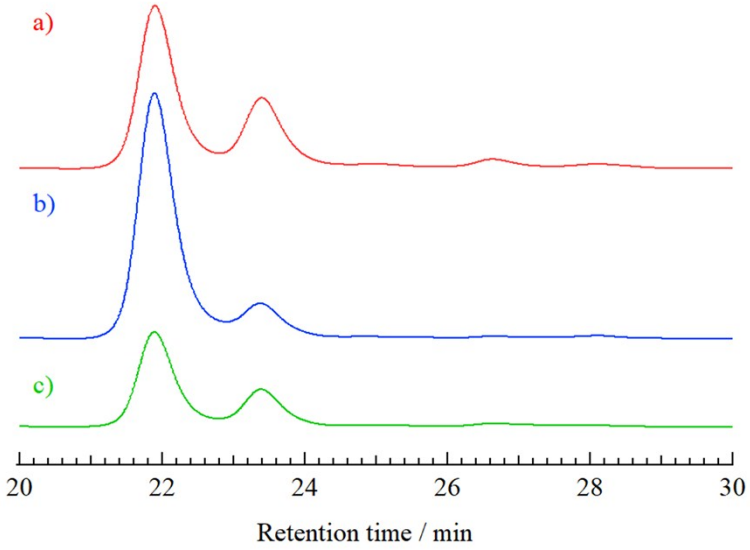


Figure S6. HPLC chromatograms of acid hydrolysates used for the composition analysis of monomeric sugars derived from the starting materials of (a) bagasse and (b) pretreated bagasse, and (c) deacylated polysaccharide acetate from bagasse measured in ultra-pure water at 85 ºC.

Table S3 Concentration of the monomeric sugars in the acid-hydrolyzed mixtures.

	Concentration [a] (mmol· L^{-1})				
Material	Glucose	Xylose	Galactose	Arabinose	Mannose
Bagasse [b]	8.2±0.0	4.9±0.0	0.8±0.1	0.8±0.1	0.5±0.1
Pretreated bagasse [b]	12.4±0.1	2.6±0.1	0.0±0.0	0.1±0.0	0.1±0.0
Polysaccharide acetate from bagasse [c]	4.6±0.1	2.3±0.1	0.1±0.0	0.1±0.0	0.0±0.0
Cellulose triacetate from pretreated bagasse [c]	7.6±0.2	0.1±0.0	0.2±0.0	0.0±0.0	0.0±0.0
Cellulose acetate from Avicel [c]	8.9±0.1	0.0±0.0	0.2±0.0	0.1±0.0	0.0±0.0

[a] Calculated from HPLC data using calibration curves in Figure S1. [b] Average results (n = 5). [c] Average results (n = 3).

Table S4 Compositions of the starting materials.

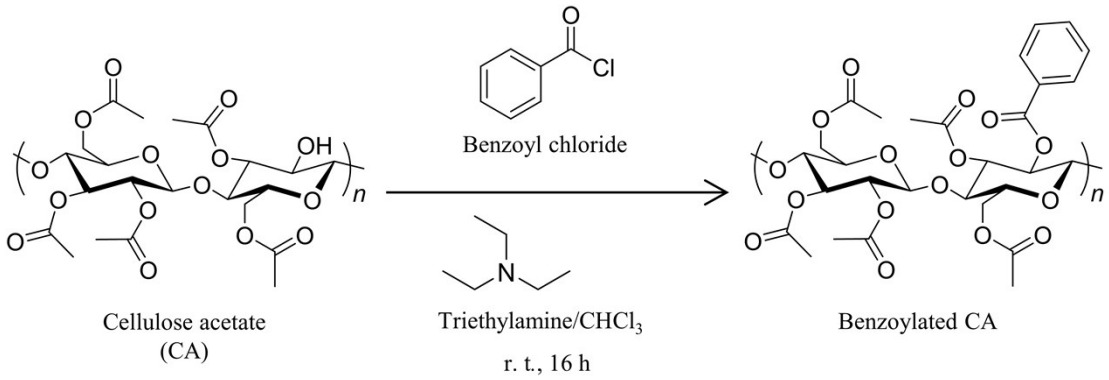
	Composition (wt.%)		
Material	Cellulose	Hemicellulose [a]	Lignin
Bagasse [b, c]	38.6±0.2	27.8±0.9	27.9±0.2
Pretreated bagasse [b]	56.0±0.2	10.4±0.3	33.6±0.1

[a] Composed of xylan as the main chain branching with galactose, arabinose, and mannose as the minor monomeric sugars. [b] Average results (n = 5). [c] Since extractives, protein, ash, etc., were not considered, the sum of the cellulose, hemicellulose, and lignin contents might not total 100%.

Determination of degrees of substitution

^{1}H NMR measurements after benzoylation

The degrees of substitution (DS) of the acetylated polysaccharides were determined by ^{1}H NMR measurements. These polysaccharide derivatives were obtained by the benzoylation of the residual OH groups in the acetylated polysaccharides with an excess amount of benzoyl chloride as shown in Scheme S1, following previously reported procedures.[7-9]



Scheme S1. Benzoylation of the residual hydroxyl groups in cellulose acetate.

In a typical procedure, solution of the acetylated polysaccharide (100 mg), benzoyl chloride (440 mg, 3.1 mmol), and triethylamine (318 mg, 3.1 mmol) in chloroform (4–10 mL) was stirred at room temperature for 24 h. The reaction mixture was then poured into MeOH (300 mL). The precipitate was collected by filtration and washed repeatedly with MeOH. Subsequently, the gel-like solid was dissolved in chloroform (20–30 mL) with stirring at room temperature for more than 1 h. The resulting solution was concentrated using a rotary evaporator at 40 °C and reprecipitated into MeOH (300 mL). The residue was washed repeatedly with MeOH and dried under vacuum at 70 °C for 24 h. ^{1}H NMR spectrum of the benzoylated polysaccharide derivative was measured in DMSO-d_6.

The conversion rate (%) was calculated using the following equation:

$$Conv.\,(\%) = \frac{\int Ac_{1.7-2.3\,ppm} \Big/ 3}{\int Ac_{1.7-2.3\,ppm} \Big/ 3 + \int Bz_{7.2-8.2\,ppm} \Big/ 5} \times 100$$

where

Ac: acetyl group and Bz: benzoyl group

Each anhydrous glucose unit in cellulose has three OH groups, while each anhydrous xylose unit in xylan has two OH groups. Therefore, the conversions (%) of cellulose acetate and xylan acetate can be assessed in terms of DS for the corresponding monomeric sugar units using the following equation:

$$DS\ (–) = Conv.\ (\%) \div 100 \times conversion\ factor$$

where

Conversion factor = 3.0 for cellulose acetate, and 2.0 for xylan acetate

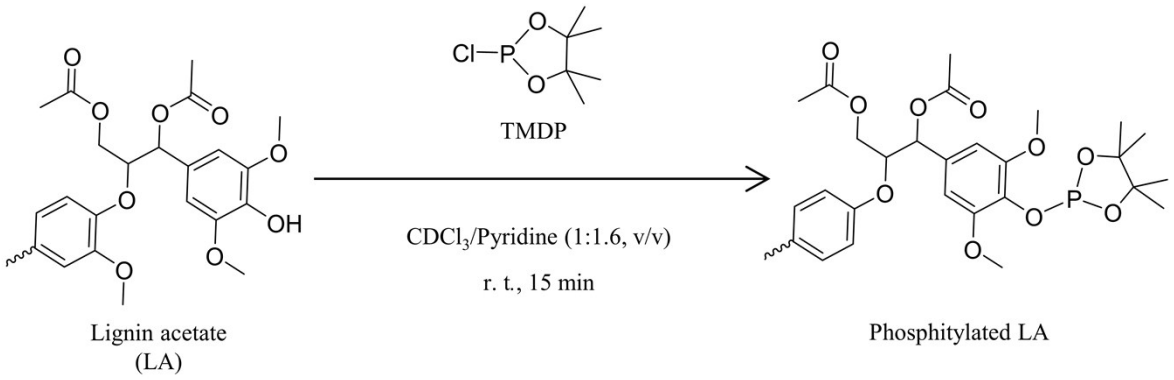
Table S5 Calculated DS of the acetylated products.

Products	Conv. (%) (DS [a])
Polysaccharide acetate from bagasse	99.4±0.1 (n.d. [b])
Cellulose triacetate from pretreated bagasse	99.4±0.1 (2.98±0.00)
Cellulose acetate from Avicel	98.4±0.0 (2.95±0.00)
Xylan acetate from xylan	97.2±0.2 (1.94±0.00)

[a] No units; DS (–) indicates the degree of substitution of the OH groups in cellulose and xylan with acetyl groups for the corresponding anhydrous monomeric sugar units. [b] Not determined as the product was a mixture of cellulose and hemicellulose.

Quantitative ^{31}P NMR measurements after phosphitylation

To determine the conversion rate of the total OH groups to acetyl groups in lignin, the amount of residual OH groups of the acetylated products were estimated by quantitative ^{31}P NMR analysis of the phosphitylation of lignin acetate, in accordance with previous studies.[11,12] The NMR spectrometer was the one described in the experimental section. The inverse gated ^{1}H decoupling sequence was employed with a recycle delay of 25 s. In total, 128 free induction decays were collected and averaged to obtain each ^{31}P spectrum. The phosphitylation reaction was conducted using 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholate (TMDP) which is a common phosphitylation reagent. The schematic representation of the reaction is shown below.



Scheme S2. Phosphitylation of the residual OH groups in lignin acetate.

1) Prepare a mixed solvent with two different internal standard (IS) solutions, and a relaxation reagent solution.
   Solvent: $CDCl_3$/pyridine (1.6:1.0, *v/v*).
   IS-1 solution: 15 g·L^{-1} *N*-hydroxy-5-norbornene-2,3-dicarboximide in $CDCl_3$/pyridine.
   IS-2 solution: 10 g·L^{-1} cyclohexanol in $CDCl_3$/pyridine.
   Relaxation reagent solution: 5 g·L^{-1} tris(2,4-pentanedionato)chromium(III) in $CDCl_3$/pyridine.
2) After vacuum drying at 70 °C for > 4 h, lignin sample (20–30 mg) was weighed into a 1 mL volumetric flask and dissolved in 500 μL of $CDCl_3$/pyridine mixed solvent.
   *NOTE: If the sample (e.g., unmodified lignin) was difficult to dissolve in $CDCl_3$/pyridine, the addition of DMF (100–300 μL) instead of the mixed solvent assisted in the dissolution.
3) 100 μL of both IS-1 and IS-2 solutions were added to the sample solution and mixed using a vortex mixer.
4) 150 μL of TMDP solution was added and reacted at room temperature for 15 min by vortex mixing every 5 min.
5) The relaxation reagent solution (100 μL) was added and vortex mixed again.
6) A small amount of $CDCl_3$/pyridine mixed solvent was dropped into the resulting solution to adjust the total volume to 1.0 mL.
7) The sample was transferred to an NMR tube and ^{31}P NMR spectrum was obtained.

8) The residual OH content (mmol·g^{-1}) in the lignin sample was calculated by the integral values of the ^{31}P NMR peaks corresponding to the aliphatic, aromatic, and carboxylic acidic OH groups. The integral range for each type of OH group is defined in Table S4.

$$C_{Sample} = C_{IS\_1} \times \left(I_{Sample} \div I_{IS\_1}\right),$$

where I_{Sample} and $I_{IS\text{-}1}$ = integral value of the corresponding peak of ^{31}P NMR.

Table S6 Typical chemical shifts and integration regions for lignin OH groups in ^{31}P NMR spectra.

Chemicals or Structure of OH groups in lignin	δ / ppm
TMDP hydrolysate	132.2
N-hydroxy-5-norbornene-2,3-dicarboximide (IS–1)	150.7–153.6
Cyclohexanol (IS–2)	144.9
Aliphatic OH	145.4–150.0
Aromatic OH	137.6–144.0
Carboxylic acidic OH (COOH)	133.6–136.0

9) The conversion rate of hydroxyl groups in the lignin was calculated as follows.

$$Conv.\,\% = \frac{C_{Ref} - C_{Sample}}{C_{Ref}} \times 100,$$

where

C_{Ref} = Total OH content (mmol·g^{-1}) of lignin = 4.53

C_{Sample} = Residual OH content (mmol·g^{-1}) of each lignin acetate determined by quantitative ^{31}P NMR analysis.

Table S7 Amount of OH groups in the lignin samples.

Products or lignin samples	Aliphatic OH	Aromatic OH	COOH	Total OH
	mmol·g^{-1} (Conv.%)			
Lignin acetate from bagasse [a]	0.28 (92±0.3)	0.43 (48±0.8)	0.29 (25±2.4)	1.00 (77±0.3)
Lignin acetate from pretreated bagasse [a]	0.22 (93±0.3)	0.42 (49±1.3)	0.38 (3.5±3.2)	1.02 (78±0.0)
Lignin acetate from lignin [a]	0.09 (97±0.1)	0.49 (41±0.7)	0.47 (—)	1.06 (78±0.6)
Lignin	3.32	0.83	0.39	4.53

[a] Described as average OH amount (n = 3).

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