Tunable Boc Modification of Lignin and Its Impact on Microbial Degradation Rate

Liuqun Gu,* † §, Meifeng Wang, ♀‡ Hui Li, ♀† Kai Ni Teh,§ Yiqun Li*‡ and Ye Liu*§

§ Institute of Materials Research and Engineering (IMRE), Agency for Science, Technology and Research (A-STAR); 4 Fusionopolis Way, Innovis Building, 138635, Singapore E-mail: ye-liu@imre.a-star.edu.sg† Department of Biomedical Engineering, Jinan University; #601, Huangpudadaoxi, Guangzhou, China E-mail: guliuqun@jnu.edu.cn or guliuqun@yahoo.com

‡ Department of Chemistry, Jinan University; #601, Huangpudadaoxi, Guangzhou, China E-mail: tlyq@jnu.edu.cn

KEYWORDS: Lignin, Modification, Reactive filler, $(Boc)_2O$, Microbial degradation.

ABSTRACT: A new type of modified lignin, lignin-p-Boc, was obtained through reaction with di-*tert*-butyl dicarbonate (Boc_2O) in aqueous media which was catalyzed using 4-dimethylaminopyridine (DMAP) . Boc modification occurred regardless of types of lignin, was tunable, and proceeded well in recovering lignin at high purity from sodium lignosulfonate (a common byproduct from pulping industry see ref 15; lignin content: 60%). Lignin-p-BOC was demonstrated as a potential reactive filler in green plastic. Furthermore, the effects of the modification on the breakdown rate of alkali lignin by microbial (white rot fungus *P.*

1 *chrysosporium*) was investigated, and the results showed that the modification obviously

2 decreased the breakdown rate.


3 **INTRODUCTION**

4   Lignin is one of most abundant biopolymers and the most with rich aromatic components in

5 nature. However, for utilization in industry it is much lagging behind than other biopolymers

6 such as cellulose.[1,2] One of main reasons is due to its random designed structure by nature; and

7 lignin structure can be different after separation as a waste from the same bio-resource via

8 different processes in pulping plants,[3] which adds more difficulty for quality control in its

9 utilization.[3,4] Not surprisingly, the employment of lignin as macromolecule filler attracted a lot

10 of attention because the negative effect of its less defined structure could be minimized.

11 Especially the use of lignin as reinforcement in biodegradable thermoplastic was extensively

12 explored due to the recent public concern to environmental impact of conventional plastic.[5]

13   But chemically lignin is very polar composing of significant amount of phenolic and aliphatic

14 hydroxyl group as well as some acidic functional groups, which would lead to incompatible or

15 immiscible composite when mixing with non-polar thermoplastic.[6,7] A common approach to

16 increase the hydrophobicity is to modify lignin by masking the free hydroxyls. The most widely

17 used method is to protect hydroxyl groups of lignin with anhydrides[8,9,10] or fatty acids[11-14]

18 forming new ester bond which is less polar. The advantage is simple and cost can be very cheap;

19 another method is to modify lignin with "graft to" or "graft from" strategies forming star

20 polymers.[6,7] For example: lignin-g-PLA,[15] rosin-lignin copolymer[16,17] and hydroxypropyl lignin

21 (hydropropyl group with certain length)[18,19]. The advantage is longer hydrophobic chain;

22 however it can be a difficulty to remove short-chain polymers after grafting and the resulted cost

1   might hamper the potential application. General methods for modification of lignin in low cost

2   for versatile utilization are still desirable.

3   Recently, tosylated lignin was synthesized in an aqueous medium in the absence of any

4   organic solvent; it is more reactive than unmodified lignin and shows potential for several

5   applications.[20] Inspired by this work, here we develop a new practical method to modify lignin

6   with di-*tert*-butyl dicarbonate (Boc$_2$O) without using any organic solvents. The degree of the

7   modification can be tuned by adjusting the loading of catalyst and the amount of Boc$_2$O used.

8   The Boc modified lignin shows more reactive than unmodified lignin, which is useful for new

9   green composite via reactive blending with thermopolyesters because of the possible newly

10   formed O-Boc/cyclic carbonates group on modified lignin. In addition, under optimized

11   conditions various aromatic hydroxyl groups could all be converted into single syringyl type

12   ones in basic aqueous solution which increases the regularity of lignin in structure. This method

13   was also demonstrated to be very efficient in recovering lignin in high purity from industrial

14   lignosulfonate lignin (lignin content: 60%). Further evaluation of the microbial (white rot fungus

15   P. chrysosporium) breakdown rate with both alkali lignin and alkali lignin-p-BOC and results

16   indicated that the presence of Boc groups in lignin obviously decreased the rate of degradation.

17   **Scheme 1.** Structure of Lignin and a Postulated Modified Structure of Lignin

18
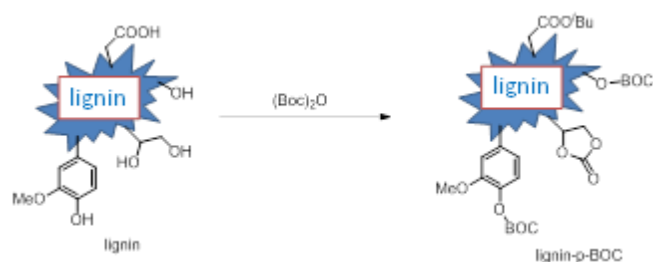
19   Di-tert-butyl dicarbonate (Boc$_2$O) is well known as a reagent for protection of amines and

20   alcohols in organic synthesis in the presence of base catalyst.[21-23] In addition, it is also used as a

1   dehydrating agent in reaction with carboxylic acids.[24] A recent revisited reaction of alcohols with

2   Boc$_2$O in the presence of 4-dimethylaminopyridine (DMAP) indicated that the pKa of the

3   alcohols strongly affect formation of products (symmetrical carbonates or O-Boc) and the

4   reaction rate; More acidic alcohol reacts faster with Boc$_2$O.[21] Inspired by this finding, we

5   envisage that the acidic hydroxyl (phenolic) and carboxylic acid groups which are of much

6   importance to the polar property of lignin could be easily protected with Boc$_2$O (Scheme 1).

7   Furthermore, 1,2-diols or 1,3-diols might possibly be converted into cyclic carbonates according

8   to the literature (Scheme 1) [22, 25]. In a result, the modified lignin would be more hydrophobic.

9   **EXPERIMENTAL SECTION**

10   **Materials.** Alkaline lignin and sufite lignin were all purchased from Tokyo Chemical Industry

11   Co., Ltd. (TCI), Singapore and Shanghai. The raw sulfite lignin (purity: around 60%) was

12   purchased from Shaanxi New Leader Enterprise; Di-*tert*-butyl dicarbonate (Boc$_2$O) was

13   purchased from Sigma-Aldrich and Scientific Resource Pte Ltd (Alfa-Aesar) as well as Aladdin,

14   Shanghai. All other common chemicals were purchased either from Sigma-Aldrich, Singapore,

15   Aladdin or Macklin, China. Double beam TU-1901 UV–visible spectrophotometer was from

16   Beijing Persee General Instrument Ltd. The white rot fungus *P. chrysosporium* (ATCC 24725)

17   was brought from Guangdong Culture Collection Center.

18   **Characterization of Lignin.** All lignin samples were freeze-dried under reduced pressure

19   before use for FTIR, NMR analysis. For reactions with Boc$_2$O, lignin was used without any

20   pretreatment from bottle.

21   **^{31}P NMR Spectroscopy.** Quantitive ^{31}P NMR Spectra of all lignin were obtained according to

22   published method. Alkaline lignin, sulfite lignin and all lignin-p-BOCs were freeze-dried under

23   reduced pressure for 2 days before measurement. ***General method:*** To a dry vial (1 mL) with 30

mg lignin sample was added a solvent mixture of anhydrous pyridine and deuterated chloroform (0.5 mL in 1.6/1 V/V), then phosphitylating reagent (2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP), 50 µL), cylcohexanol (100 µL, 10.85 mg in a solvent mixture of anhydrous pyridine and deuterated chloroform in 1.6/1 V/V) as internal standard, and chromium (III) acetylacetonate solution (100 µL, 5.0 mg in a solvent mixture of anhydrous pyridine and deuterated chloroform in 1.6/1 V/V) as a relaxation reagent. Finally the solution was made up to 1 mL with solvent mixture of anhydrous pyridine and deuterated chloroform (in 1.6/1 V/V), then the vial was sealed tightly and the solution was shaken for 2 hours to ensure thorough mixing. It was transferred into a dry NMR tube for subsequent analysis. All experiments were carried out on a JEOL 500 MHz or a Bruker 400 MHz spectrometer. ^{31}P NMR chemical shifts were referenced with respect to water signal at 132.2 or cyclohexanol signal at 145.2. Peak of phosphitylating reagent TMDP was at 176.0. The content of hydroxyl groups for phosphitylating reagent was obtained by integration of the following spectral regions: aliphatic hydroxyls (150.3 - 145.3 ppm), condensed phenolic units (144.6 – 141.2 ppm), syringyl phenolic units (143.3 - 142.0 ppm), guaiacyl phenolic hydroxyls (140.5 -138.6 ppm), *p*-hydroxyphenolic units (138.5 – 137.3 ppm) and carboxylic acids (135.9 – 134.0 ppm).

^{1}H NMR Spectroscopy. Quantitive ^{1}H NMR Spectra of all lignin were obtained with pentafluorobenzaldehyde as internal standard. All experiments were carried out on a JEOL 500 MHz or Bruker 300 MHz for 1024 scans. 40 mg pentafluorobenzaldehyde (0.2 mmol) was added into 10 mL d_6-DMSO in a vial. The solution was shaken for a thorough mixing and then kept for use (Standard A). To a vial (1 mL) with 10 mg lignin sample was added Standard A (0.5 mL), then the solution was shaken for 2 hours to ensure thorough mixing. It was transferred into a NMR tube for subsequent analysis.

1   **Boc Protection of Lignin.** *General procedure A*: A mixture of 2N NaOH (5 mL), alkali lignin

2   (1 g) and Boc_2O (2.5 mL or other amount) with a stirring bar in a 20 mL vial was placed into an

3   ice-water bath, then DMAP (339 mg or 100 mg) was added and the mixture was warmed to room

4   temperature after 20 mins. The reaction continued to be stirred at room temperature for 3 hours

5   or 1 day. After then, 2 N HCl was added drop by drop to adjust the pH value to about 2. The

6   modified lignin was isolated by centrifugation and subsequent washing (3 × 20 mL) deionized

7   water. The isolated product was freeze-dried under reduced pressure to give dry sample for

8   characterization. *General procedure B*: A mixture of MeCN (5 mL), alkali lignin (1 g) and

9   Boc_2O (2.5 mL) with a stirring bar in a 20 mL vial was placed into an ice-water bath, then

10   DMAP (339 mg) was added and the mixture was warmed to room temperature after 20 mins.

11   The reaction continued to be stirred at room temperature for 1 day. The solvent was removed by

12   air flowing and the latter workup procedure was same to that of the above *General procedure A*.

13   **Demonstration as potential reactive filler by simple mixing of lignin-p-BOC and**

14   **polyethylene glycol.** A mixture of lignin-p-BOC (0.25 g) and polyethylene glycol (Mw = 2000

15   or 400) (1 mL) was stirred at 60 ℃ for 1 hour; the mixture became homogeneous after reaction.

16   Then the mixture was cooled to room temperature and was precipitated in hexane/ethyl acetate

17   solution. Pale yellow powder was obtained as a product lignin-p-PEG in 0.197 g (PEG 400:

18   0.185 g) and further dried *in vaccum* for hours (molar ratio 1/1 to total hydroxyls) before NMR

19   characterization. ^{1}HNMR data of both products indicated PEG reacted with lignin-p-BOC and

20   considerate amount of PEG chains were attached onto lignin core successfully.

21   **Preparation of hydropropyl alkaline lignin**. The preparation procedure is according to

22   reported procedure[8b]. ^{31}P NMR result indicated that almost all the aromatic hydroxyls were

23   masked with hydropropyl groups (total alphatic OH: 3.80 mmol/g).

1   **Demonstration as potential reactive filler with BOC protection of hydropropyl alkaline**

2   **lignin.** Boc Modification reactions in acetonitrile and in NaOH solution were similar to *General*

3   *procedure A* and *General procedure B* with Boc_2O (molar ratio 1/1 to total hydroxyls) in the

4   presence of 10 wt% DMAP for 1d.

5   **FT-IR Analysis.** Fourier transform infrared spectra were obtained with powdered solid lignin

6   on KBr disks. Spectra were collected from 4000 to 400 (or 600) cm^{-1} with 16 cans. A typical

7   *BOC modified lignin*: -OH, 3000 - 3700 cm^{-1}; C-H stretch, 2976 and 2940 cm^{-1}; C=C stretch,

8   2050 – 2070 cm^{-1}; C=O (bicarbonate) stretch, 1755, 1650 (conjugated) cm^{-1}; C=C (aromatic)

9   bending and ring puckering, 1566 and 1511 cm^{-1}; C-O vibration, 1278, 1257, 1215, 1145, 1038

10   cm^{-1}.

11   **General methods for microbial degradation of alkali lignin and lignin-p-BOC 2.** A new

12   batch of BOC modified lignin was synthesized for microbial degradation testing according to the

13   same procedure in preparation of lignin-p-BOC **2**. Many microorganisms are capable of

14   degrading lignin and one of the best characterized species is white-rot fungi. This strain we

15   selected is *Phanerochaete chrysosporium* (*P. chrysosporium*). The culture was maintained on

16   malt agar slants at 4 ℃, and then transferred to malt agar plates and cultured at 39 ℃ for 7 days.

17   The spores on the agar were scraped and suspended in the sterile water. The spore concentration

18   was determined by measuring absorbance at 650 nm and adjusted to 5.0×10^6 spores/ml. Lignin

19   degradation was studied in 50 mL Kirk's nitrogen limitation cultures without veratryl alcohol

20   adding in 200-ml Erlenmeyer flasks. Lignin was added as a solution in 1 ml of 10% DMSO to

21   give a concentration of 1 g/L. 5 ml of spore suspension was added to each flask, and the pH was

22   adjusted to 4.5 with H_3PO_4. Lignin degradation were carried out at 39 ℃ for 18 days and flushed

23   with oxygen every day. Control groups contained no spores were incubated like spore-containing

1   cultures. All experiments were carried out in triplicates. Absorption of solution of lignin was

2   measured with double beam UV–visible spectrophotometer. 0.5 ml samples were removed from

3   the cultures every day and then sonicated for 30s. 4.5 ml of 0.55% (W/V) NaOH was added to

4   the culture in order to precipitate mycelia and protein. Then the mixture was centrifuged. The

5   supernatant was diluted to proper concentration and measured at 280 nm.

6   **RESULTS AND DISCUSSION**

7   **The Effect of Solvent and Loading of DMAP on Total Hydroxyls of Alkali Lignin.**

8   Initially a reaction of alkali lignin and Boc_2O in acetonitrile with catalytic amount of DMAP was

9   performed at room temperature for 1 day (**Table 1**). Boc modification of lignin occurred as

10 expected smoothly by comparison of ^{1}H NMR/FT-IR spectra of commercial lignin and lignin-p-

11 BOC after modification.[26] Quantitive ^{31}P NMR of purified lignin-p-BOC **1** indicated after

12 modification the content of the total free hydroxyls and carboxylic acids dropped to 41% (2.53

13 mmol/g, entry 1, **Table 1**) of that of commercial alkali lignin (6.12 mmol/g, **Figure 1**). Since

14 poor solubility of lignin in acetonitrile was observed, a reaction in NaOH solution was tested.

15 Remarkable increase on reaction efficiency was achieved and the units of total free OH of lignin-

16 p-BOC **2** were reduced to 10% (0.63 mmol/g, entry 2, **Table 1**) of the original one. Reaction rate

17 for Boc protection of lignin was very fast at room temperature and in 3 hours the content of free

18 OH for lignin-p-BOC **3** was comparable with lignin-p-BOC **2** that achieved in 1 day (11% (0.67

19 mmol/g) VS 10% (0.63 mmol/g)) of original one, entries 2 and 3, **Table 1**).

20 **Table 1.** Boc modification of alkali lignin with Boc_2O[a]

Entries	Solvent	(Boc)$_2$O (mL)	DMAP (mg)	Lignin-p-BOC (g) (Number)	Aliphatic OH (mmol/g) (%)[c]	Aromatic OH (mmol/g) (%)[c]	Carboxylic acid (mmol/g) (%)[c]	Total free OH and acid (mmol/g) (%)[c]

1	MeCN	2.5	339	0.79 (**1**)	0.75（64）	1.50（54）	0.28（64）	2.53（59）
2	NaOH (2N)	2.5	339	1.08 (**2**)	0.33（84）	0.30（91）	0.32（59）	0.95（84）
3[b]	NaOH (2N)	2.5	339	1.13 (**3**)	0.46（78）	0.21（93）	0.35（55）	1.02（83）
4	NaOH (2N)	2.5	0	1.08 (**4**)	1.39（34）	0.38（88）	0.18（83）	1.95（68）
5	NaOH (2N)	1.4	339	0.88 (**5**)	0.37（82）	0.51（84）	0.24（77）	1.12（82）
6	NaOH (2N)	2.5	100	1.21 (**6**)	0.53（75）	0.51（84）	0.31（60）	1.35（78）
7	NaOH (2N)	0.5	50	0.97 (**13**)	0.64（70）	1.27（61）	0.36（54）	2.27（63）
8	-	-	-	Alkali lignin	2.11	3.23	0.78	6.12

a Reaction conditions: DMAP (339 mg) was added into a mixture of 2 N NaOH (5 mL), alkali lignin (1g) and Boc$_2$O (2.5 mL) with a stirring bar in a 20 mL vial. The solution was stirred at rt for 1d; *b*: The reaction was performed at rt for 3 hrs; c: Conversion percent of hydroxyls or acid.

A control reaction of lignin and Boc$_2$O in the absence of DMAP as a catalyst was also examined, and the result indicated NaOH solution could also slightly promote Boc protection of lignin, but in a much lower efficiency on masking of aliphatic hydroxyls (units of free aliphatic hydroxyls: 66% (1.39 mmol/g) VS 16% (0.33 mmol/g) of original one, entries 2 and 4, **Table 1**). In concern of cost, reactions with low amount of Boc$_2$O and DMAP were also examined, comparable concentration of residual hydroxyls (18% VS 16%, entries 2 and 5, **Table 1**) for lignin-p-BOCs was observed, however a lower yield was achieved with equivalent amount of Boc$_2$O. The reaction yield could be maintained well in the presence of 10 wt% DMAP meanwhile the concentration of free hydroxyls on new product slightly increased (17% VS 10%, entries 2 and 6, **Table 1**).
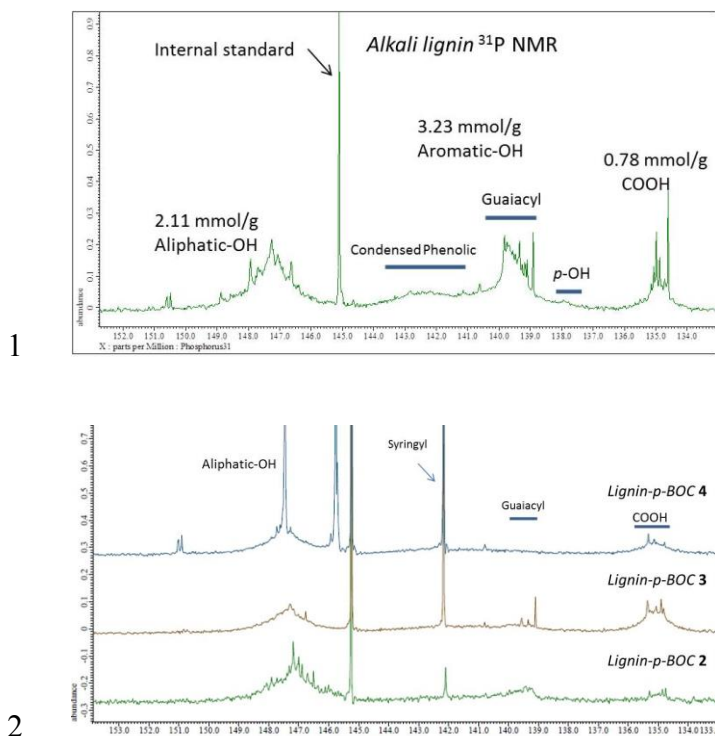
1

2

Figure 1. Quantitive ^{31}P NMR Spectra and signal assignment of commercial alkali lignin and alkali lignin-p-BOC **2 - 4** in CDCl$_3$/pyridine.

The Effect of Solvent and Loading of DMAP on Total Hydroxyls of Sulfite Lignin. In current market, lignin byproducts (lignosulfonate) from sulfite process for chemical pulping of wood are dominant although the kraft process is the operation pre-eminently employed.[3, 27] hence to develop an economic method to utilize sodium lignosulfonate is in great need. Subsequently Boc modification of sodium lignosulfonate was also optimized similarly and generally similar trend was observed (**Table 2**). However, lower overall yields were achieved probably because of their better solubility in water. Interestingly, with this method, lignin-p-BOC can be recovered easily on water from industrial sodium lignosulfonate lignin (lignin purity: 60%)[28] without additional NaOH base because the inside base promoted the reaction (Entry 7, **Table 2**). This

1 process not only combines the modification and pre-purification in one pot, but also reuses the
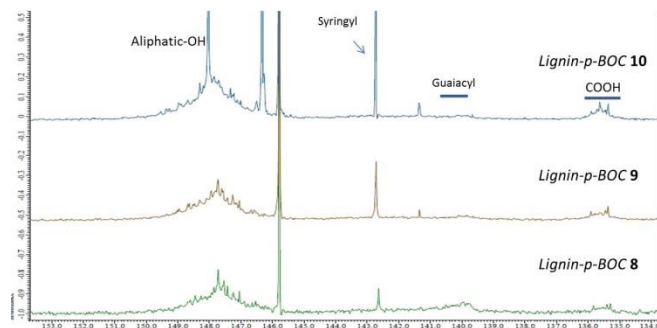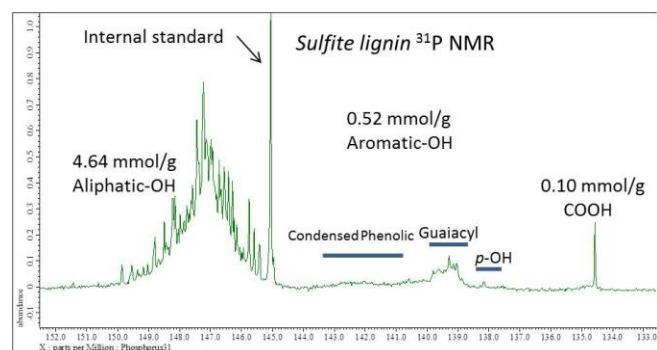
2 waste impurity[29] as a base.

5 **Figure 2.** Quantitative ^{31}P NMR spectra and signal assignment of commercial sulfite lignin and

6 sulfite lignin-p-BOC **8 - 10** in CDCl$_3$/pyridine.

7   **The Effect of Reaction Conditions on Selective Masking of Lignin.** The presence of

8 catalytic DMAP accelerated BOC modification of aliphatic OH groups (units of free aliphatic

9 hydroxyls: 16% (0.33 mmol/g) VS 66% (1.39 mmol/g) of original one, entries 2 and 4, **Table 1**),

10 meanwhile NaOH showed its preference in modification of aromatic OH groups (units of free

11 aliphatic hydroxyls: 66% (1.39 mmol/g) VS aromatic hydroxyls 12% (0.38 mmol/g) of original

12 one, entry 4, **Table 1** and **Figure 1**). The aromatic hydroxyls were masked in faster rate probably

13 because they are more acidic and the deprotonation process is fast. Interestingly, all the aromatic

14 hydroxyls could selectively either be protected by Boc or be converted into syringyl OH when

11

1 the Boc modification reaction was performed in NaOH solution for 1 day in the absence of

2 DMAP as a catalyst (lignin-p-BOCs **4** and **10**, **Figure 2**), which is not observed in other methods

3 for modification of lignin probably generated by Friedel-Crafts alkylation reaction of guaiacyl

4 part (2-substituted phenolic moiety) with Boc$_2$O (a known chemistry under similar conditions).

5 The existence of DMAP had negative effect on this selective transformation especially when

6 reaction time was prolonged, althou gh it benefited Boc protection of aliphatic OH.

7 **Table 2.** BOC modification of sulfite lignin with BOC$_2$O. [a]

Entries	Solvent	(Boc)$_2$O (mL)	DMAP (mg)	Lignin-p-BOC (g) (Number)	Aliphatic OH (mmol/g) (%)[d]	Aromatic OH (mmol/g) (%)[d]	Carboxylic acid (mmol/g) (%)[d]	Total free OH and acid (mmol/g) (%)[d]
1	MeCN	2.5	339	0.23 (**7**)	0.79 （83）	0.43 （17）	0.12	1.33 （75）
2	NaOH (2N)	2.5	339	0.48 (**8**)	0.55 （88）	0.19 （63）	0.03 （70）	0.77 （85）
3[b]	NaOH (2N)	2.5	339	0.57 (**9**)	0.88 （81）	0.17 （67）	0.12	1.17 （78）
4	NaOH (2N)	2.5	0	0.19 (**10**)	1.98 （57）	0.26 （50）	0.13	2.37 （55）
5	NaOH (2N)	1.2	339	0.57 (**11**)	0.69 （85）	0.27 （48）	0.10	1.06 （80）
6	NaOH (2N)	2.5	100	0.47 (**12**)	0.95 （80）	0.31 （40）	0.09	1.35 （74）
7[c]	NaOH (2N)	1.5	100	0.62 (**14**)	1.06 （77）	0.44 （15）	0.45	1.95 （63）
8	-	-	-	Sulfite lignin	4.64	0.52	0.10	5.26

8   a Reaction conditions: DMAP (339 mg) was added into a mixture of 2 N NaOH (5 mL), sulfite
9 lignin (1g) and Boc$_2$O (2.5 mL) with a stirring bar in a 20 mL vial. The solution was stirred at rt
10 temperature for 1d; b: The reaction was performed at rt for 3 hrs; c: Reaction conditions: DMAP

1 (100 mg) was added into a mixture of DI water (5 mL), industrial sulfite lignin (purity 60%, 2g)

2 and Boc$_2$O (1.5 mL) with a stirring bar in a 20 mL vial. The solution was stirred at rt temperature

3 for 3 hrs; d: Conversion percent of hydroxyls or acid.

4 **Reactivity after Boc Modification.** The units of Boc groups calculated based on quantitative

5 ^{1}H NMR of lignin-p-BOC were not always consistent with the units of decreased free hydroxyls

6 calculated based on ^{31}P NMR, which indicated other reactions including O-Boc further reactions

7 with free OH into cyclic carbonates or Boc$_2$O promoted lactones formation might occur as

8 expected[24, 25]. The fact that less amount in units of O-Boc in lignin-p-BOC **2** (in 1d) compared to

9 those in lignin-p-BOC **3** (in 3h) from quantitative ^{1}H NMR suggested that longer reaction

10 benefited further reactions of O-Boc with other free hydroxyls resulting to less total free

11 hydroxyls. To achieve a structurally compatible polymer blends based on lignin usually required

12 a pretreatment of lignin by grafting short chains on isolated lignin[30-31]. In addition, a simple

13 reactive lignin can potentially increase the highest content of lignin for compatible polymer

14 blends. To be reactive filler in new thermoplastic composites, O-Boc groups on lignin-p-BOC

15 are expected to be still reactive with other free nucleophilic groups (such as aliphatic OHs, NHs)

16 from thermoplastic during extrusion. In order to demonstrate the possible reactivity, hydropropyl

17 alkaline lignin[32] was selected as a model substrate because it has excellent solubility in common

18 deuterium solvents (CDCl$_3$/pyridine or d_6-DMSO) and newly formed long chain hydropropyl

19 groups can also act as free nucleophilic OHs similar to those from thermoplastics.

20 In order to prove that reactive extrusion with lignin-p-BOC is possible, polyethylene glycol

21 (PEG) was chosen for simple mixing with lignin-p-BOC at 60 ℃ for 1 hour in the absence of

22 other reagents. The new PEG peaks on ^{1}HNMR spectra of products with PEG$_{2000}$ and PEG$_{400}$

23 indicated that further reactions occurred as expected and PEG chains were introduced onto lignin
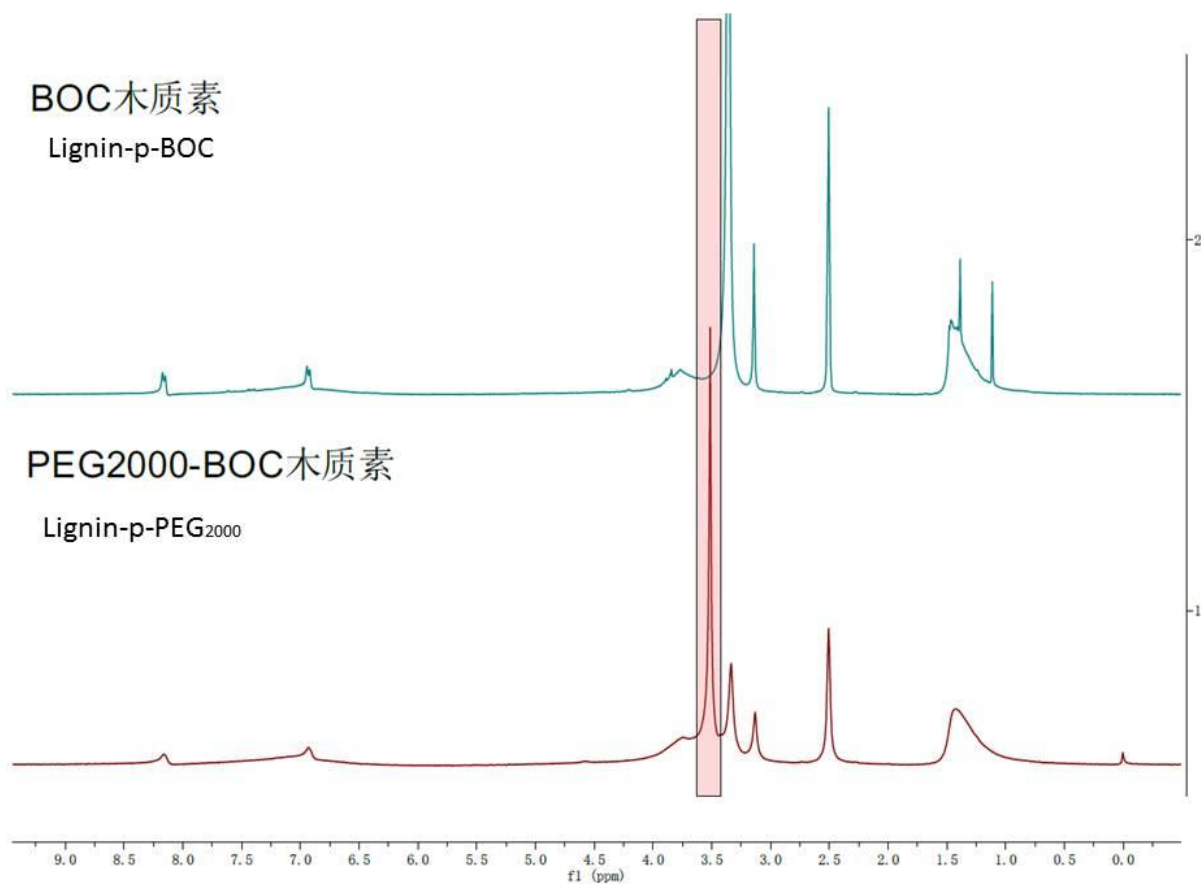
24 core successfully (**Figure 3**).

Figure 3. Comparison of lignin-p-BOC with further reacted product lignin-p-PEG$_{2000}$.

Reactions in acetonitrile and in NaOH solution with Boc$_2$O (molar ratio 1/1 to total hydroxyls) in the presence of 10 wt% DMAP were parallel performed for 1d. The isolated Boc modified product in acetonitrile showed no free hydroxyl and carboxylic acids signal on ^{31}P NMR spectra surprisingly. Significant amount of samples became insoluble in CDCl$_3$/pyridine or d_6-DMSO after reaction, which indicated that intermolecular carbonation involved with O-Boc and other free OH groups occurred as expected, forming larger polymer with more cross-linking structure. Interestingly, the degree of intermolecular reaction are remarkably decreased in aqueous solution (NaOH) and the Boc modified hydropropyl alkaline lignin (aliphatic hydroxyls: 1.33 mmol/g)

14

1   was fully soluble in CDCl$_3$/pyridine or d_6-DMSO, which shows that the future reactive extrusion

2   might also be tunable by controlling the moisture content (details see SI).

3   **Investigation on Microbial Degradation.** Concerning the Boc modification might have effect

4   on degradation of lignin and with which fully biodegradable single-used plastics[33], we evaluated

5   the microbial (white rot fungus P. chrysosporium) breakdown rate of both alkali lignin and alkali

6   lignin-p-BOC in parallel according to literature[34]. The presence of Boc groups in lignin

7   obviously decreased the rate of degradation by white rot fungus *P. chrysosporium* within 18 days

8   (**Figure 4**), which is consistent with proposed enzyme breakdown mechanism initiated from

9   hydroxyl parts[35]. This finding is of much importance to future design of "green plastics" with

10   modified lignin as a major component in balancing the stability in storage and biodegradability
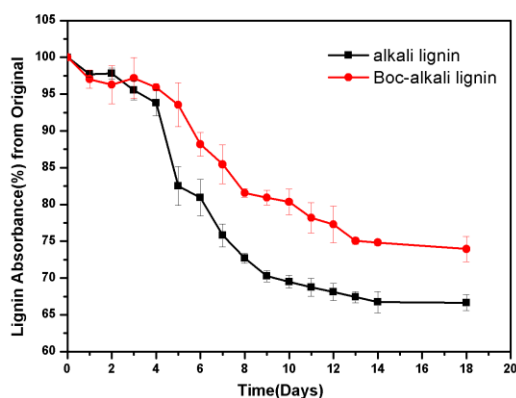
11   after filled in field.

12

13   **Figure 4.** Absorption change of alkali lignin and Boc-alkali lignin at 280nm.

14   **CONCLUSION**

15   In summary, a new type Boc modified lignin was synthesized under mild conditions with

16   Boc$_2$O and the modification was generally tunable on the degree of Boc protection and

17   preference on the different hydroxyls by slight change of reaction conditions. Simple

1 optimization indicated that Boc modification occurred more efficiently in aqueous medium in the

2 absence of any organic solvent, and both types of commercial lignin (alkaline and lignosulfonate)

3 were applicable. This method demonstrated to be very efficient in recovering lignin in high

4 purity from industrial lignosulfonate lignin (lignin content: 60%). This process not only

5 combines the modification and pre-purification in one pot, but also reuses the waste impurity as a

6 base. The Boc modified lignin-p-BOC also shows potential as reactive filler for new composite.

7 The reactive potentiality of O-Boc group with aliphatic OH was demonstrated in Boc

8 modification of hydropropyl alkaline lignin. Evaluation of the microbial (white rot fungus P.

9 chrysosporium) breakdown rate of both alkali lignin and alkali lignin-p-BOC in parallel

10 indicated that the presence of Boc groups in lignin obviously decreased the rate of degradation.

11

12 ASSOCIATED CONTENT

13 **Supporting Information**. Data and spectrum of ^{1}H NMR、FT-IR and ^{31}P NMR of lignin-p-

14 BOC prepared under different conditions as well as experimental details in microbial degradation

15 of alkali lignin and alkali lignin-p-BOC see are available free of charge in *Supporting*

16 *Information*.

17 AUTHOR INFORMATION

18 ORCID
19 Liuqun Gu: 0000-0002-4254-6824
20
21 Notes
22 The authors declare no competing financial interest.

23 **Author Contributions**

24 ♀：Both authors contributed equally

6 REFERENCES

7 (1) Mülhaupt, R. Green Polymer Chemistry and Biobased Plastics: Dreams and Reality.

8 *Macromol. Chem. Phys.* **2013**, *214 (2)*, 159-174.

9 (2) Bruijnincx, P. C. A.; Rinaldi, R.; Weckhuysen, B. M. Unlocking the potential of a sleeping

10 giant: lignins as sustainable raw materials for renewable fuels, chemicals and materials**.** *Green.*

11 *Chem.* **2015**, *17 (11)*, 4860-4861.

12 (3) Culebras, M.; Sanchis, M. J.; Beaucamp, A.; Carsí, M.; Kandola, Baljinder K.; Horrocks,

13 A. R.; Panzetti, G.; Birkinshawf, C. and Collins, M. N. Understanding the thermal and

14 dielectric response of organosolv and modified kraft lignin as a carbon fibre precursor *Green.*

15 *Chem.* **2018**, *in press*, DOI: 10.1039/C8GC01577E;

16 (4) Laurichesse, S.; Avérous, L. Chemical modification of lignins:Towards biobased

17 polymers. *Prog. Polym. Sci.* **2014**, *39 (7),* 1266-1290.

18 (5) Saito, T.; Brown, R. H.; Hunt, M. A.; Pickel,D. L.; Pickel, J. M.; Messman, J. M.; Baker, F.

19 S.; Keller, M.; Naskar, A. K. Turning renewable resources into value-added polymer:

20 development of lignin-based thermoplastic**.** *Green. Chem.* **2012**, *14 (12)*, 3295-3303;

(6) Duval, A.; Lawoko, M. A review on lignin-based polymeric, micro- and nano-structured materials. *React. Funct. Polym.* **2014**, *85*, 78-96;

(7) Sen, S.; Patil, S.; Argyropoulos, D. S. Thermal properties of lignin in copolymers, blends, and composites: a review. *Green. Chem.* **2015**, *17 (11)*, 4862-4887.

(8) Li, Y.; Sarkanen, S. Miscible Blends of Kraft Lignin Derivatives with Low-Tg Polymers. *Macromolecules.* **2005**, *38 (6)*, 2296-2306;

(9) Guo, Z.-X.; Gandini, A.; Pla, F. Polyesters from lignin. 1. The reaction of kraft lignin with dicarboxylic acid chlorides. *Polym. Int.* **1992**, *27 (1)*, 17-22;

(10) Sailaja, R. R. N.; Deepthi, M. V. Mechanical and thermal properties of compatibilized composites of polyethylene and esterified lignin**.** *Mater. Des.* **2010**, *31 (9)*, 4369-4379.

(11) Firdaus, M.; Meier, M. A. R. Renewable co-polymers derived from vanillin and fatty acid derivatives. *Eur. Polym. J.* **2013**, *49 (1)*, 156-166;

(12) Thiebaud, S.; Borredon, M. E. Solvent-free wood esterification with fatty acid chlorides. *Bioresour. Technol.* **1995**, *52 (2)*, 169-173;

(13) Thiebaud, S.; Borredon, M. E.; Baziard, G.; Senocq, F. Properties of wood esterified by fatty acid chlorides. *Bioresour. Technol.* **1997**, *59 (2 & 3)*, 103-107;

(14) Tamminen, T.; Ropponen, T.; Hult, E.; K. Poppius-Levlin, US patent: *20140243511.*

(15) Chung, Y. L.; Olsson, J. V.; Li, R. J.; Frank, C. W.; Waymouth, R. M.; Billington, S. L.; Sattely, E. S. A Renewable Lignin-Lactide Copolymer and Application in Biobased Composites. *ACS Sustainable Chem. Eng.* **2013**, *1 (10)*, 1231-1238.

(16) a) Wang, J.-F.; Yao, K.-J.; Korich, A. L.; Li, S.-G.; Ma, S.-G.; Ploehn, H. J.;Lovine, P. M.; Wang, C.-P.; Chu, F.-X.; Tang, C.-B. Combining renewable gum rosin and lignin: Towards hydrophobic polymer composites by controlled polymerization**. *J. Polym. Sci. Part A: Polym. Chem.* **2011**, *49 (17)*, 3728-3738;

(17) Thielemans, W.; Can, E.; Morye, S. S.; Wool, R. P. Novel applications of lignin in composite materials**. *J. Appl. Polym. Sci.* **2002**, *83 (2)*, 323-331.

(18) a) Pavier, C.; Gandini, A. Oxypropylation of sugar beet pulp. 2. Separation of the grafted pulp from the propylene oxide homopolymer. *Carbohydr. Polym.* **2000**, *42 (1)*, 13-17;

(19) Sadeghifar, H.; Cui, C.; Argyropoulos, D. S. Toward Thermoplastic Lignin Polymers. Part 1. Selective Masking of Phenolic Hydroxyl Groups in Kraft Lignins via Methylation and Oxypropylation Chemistries. *Ind. Eng. Chem. Res.* **2012**, *51 (51)*, 16713-16720.

(20) Diop, A.; Awada, H.; Zerrouki, R.; Daneault, C.; Montplaisir, D. Tosylation and Characterization of Lignin in Water. *Ind. Eng. Chem. Res.* **2014**, *53 (43)*, 16771-16776.

(21) Basel, Y.; Hassner, A. Di-tert-butyl Dicarbonate and 4-(Dimethylamino)pyridine Revisited. Their Reactions with Amines and Alcohols. *J. Org. Chem.* **2000**, *65 (20)*, 6368-6380;

(22) Chankeshwara, S. V.; Chebolu, R.; Chakraborti, A. K. Organocatalytic methods for chemoselective O-tert-butoxycarbonylation of phenols and their regeneration from the O-t-Boc derivatives. *J. Org. Chem.* **2008**, *73 (21)*, 8615-8618;

(23) Cheraiet, Z.; Hessainia, S.; Ouarna, S.; Berredjem, M.; Aouf, N.-E. A simple and eco-sustainable method for the O-Boc protection/deprotection of various phenolic structures under water-mediated/catalyst-free conditions. *Green Chem. Lett. Rev.* **2013**, *6 (3)*, 211-216.

(24) Pozdnev, V. F. Activation of carboxylic acids by pyrocarbonates. Application of di-tert-butyl pyrocarbonate as condensing reagent in the synthesis of amides of protected amino acids and peptides. *Tetrahedron Lett.* **1995**, *36 (39)*, 7115-7118.

(25) Nagarajan, M.; Kumar, V. S.; Rao, B. V. Di-tert-butyl pyrocarbonate mediated synthesis of macrocyclic lactones from ω-hydroxy acids. *Tetrahedron Lett.* **1997**, *38 (33)*, 5835-5838.

(26) The details in identifying different functional groups of lignin-p-BOC see *Supporting Information*.

(27) Wang, Y.-Y.; Chen, Y.; Sarkanen, S. Path to plastics composed of ligninsulphonates (lignosulfonates). *Green. Chem.* **2015**, *17 (11)*, 5069-5078;

(28) Lignin product data was from Alibaba website.

(29) The raw sodium lignosulfonate (purity: around 60%) was purchased from Shaanxi New Leader Enterprise Ltd, China; the major impurities are sodium oxide (Na_2O, 16%) and volatile acid (8%) according to a product report from the supplier.

(30) Nge, T. T.; Tobimatsu, Y.; Takahashi, S.; Takata, E.; Yamamura, M.; Miyagawa, Y.; Ikeda, T.; Umezawa, T.; and Yamada, T. Isolation and Characterization of Polyethylene Glycol (PEG)-Modified Glycol Lignin via PEG Solvolysis of Softwood Biomass in a Large-Scale Batch Reactor. *ACS Sustainable Chem. Eng.* **2018**, *6*, 7841-7848.

(31) Culebras, M.; Beaucamp, A.; Wang, Y.; Clauss, M. M.; Frank, E.; and Collins, M. N. Biobased Structturally Compatible Polymer Blends Based on Lignin and Thermoplastic Elastomer Polyurethane as Carbon Fiber Precursor. *ACS Sustainable Chem. Eng.* **2018**, *6*, 8816-8825;

1 (32) Hydropropyl alkaline lignin was prepared according to reported literature (reference [8b]).

2 ^{31}P NMR result indicated that almost all the aromatic hydroxyls were masked with hydropropyl

3 groups (total alphatic OH: 3.80 mmol/g).

4 (33) A recent report on marine pollution by plastic waste inputs from land sees: Jambeck, J. R.;

5 Geyer, R.; Wilcox, C.; Siegler, T. R.; Perryman, M.; Andrady, A.; Narayan, R,; Law, K. L.

6 Plastic waste inputs from land into the ocean. *Science* **2015**, *347 (6223)*, 768-771.
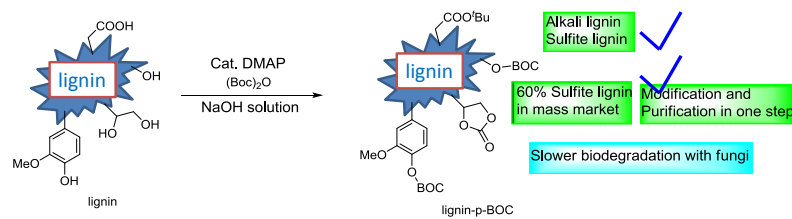
7 (34) Ulmer, D. C.; Leisola, M. S. A.; Schmidt, B. H.; Fiechter, A. Rapid degradation of isolated

8 lignins by Phanerochaete chrysosporium. *Appl. Environ. Microbiol.* **1983**, *45 (6)*, 1795-1801.

9 (35) Bugg, T. D. H.; Ahmad, M.; Hardiman, E. M.; Rahmanpour, R. Pathways for degradation of

10 lignin in bacteria and fungi**.** *Nat. Prod. Rep.* **2011**, *28 (12)*, 1883-1896.

11

12

13

14

15

16

17 Synopsis

18 A method for lignin-p-Bocs was developed with various sources of lignin; lignin-p-Bocs showed

19 potential as reactive fillers for composite plastics.

1   TOC

2

3