January 2017

Quantification Of Lignin Hydroxyl Containing Functional Groups Via 31p{1h} Nmr Spectroscopy And Synthesis Of Lignin Degradation Standards

Joshua E. Schumaker

QUANTIFICATION OF LIGNIN HYDROXYL CONTAINING FUNCTIONAL GROUPS VIA $^{31}P\{^{1}H\}$ NMR SPECTROSCOPY AND SYNTHESIS OF LIGNIN DEGRADATION STANDARDS

by

Joshua E. Schumaker
Bachelor of Science, University of North Dakota, 2015

A Thesis

Submitted to the Graduate Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Master of Science

Grand Forks, North Dakota
December
2017

This thesis, submitted by Joshua E. Schumaker in partial fulfillment of the requirements for the Degree of Master of Arts (insert appropriate degree) from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

_____
Dr. Alena Kubátová

_____
Dr. Irina Smoliakova

_____
Dr. David Pierce

This Thesis is being submitted by the appointed advisory committee as having met all of the requirements of the School of Graduate Studies at the University of North Dakota and is hereby approved.

_____
Dr. Grant McGimpsey
Dean of the School of Graduate Studies

_____
Date

PERMISSION

Title QUANTIFICATION OF LIGNIN HYDROXYL CONTAINING FUNCTIONAL GROUPS VIA $^{31}P\{^{1}H\}$ NMR SPECTROSCOPY AND SYNTHESIS OF DEGRADATION STANDARDS

Department Chemistry

Degree Master of Science

In presenting this thesis in partial fulfillment of the requirements for a graduate degree from the University of North Dakota, I agree that the library of this University shall make it freely available for inspection. I further agree that permission for extensive copying for scholarly purposes may be granted by the professor who supervised my thesis work, or in her absence, by the Chairperson of the department or the dean of the School of Graduate Studies. It is understood that any copying or publication or other use of this thesis or part thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and the University of North Dakota in any scholarly use which may be made of any material in my thesis.

Joshua Eugene Schumaker
11/28/2017

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SCHEMES

LIST OF TABLES

ABBREVIATIONS

Abbreviations	Full Meaning
TMDP	2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane
GPC	gel permeation chromatography
FT-IR	Fourier transform infrared
TGA	thermal gravimetric analysis
TCA	thermal carbon analysis
NMR	nuclear magnetic resonance
SEC	size exclusion chromatography
GC-MS	gas chromatography mass spectrometry
LC-MS	liquid chromatography mass spectrometry
s	seconds
m/z	mass to charge ratio
py	pyridine
$CDCl_3$	deuterated chloroform
rt	room temperature
h	hour

ACKNOWLEDGEMENTS

I would like to thank my advisors Dr. Alena Kubátová and Dr. Irina Smoliakova for both taking me in to the group after the departure of my first advisor, as well as their guidance along my way to graduation. I would also like to thank Dr. David Pierce for serving on my committee and his support as well as Dr. Julia Zhao and Dr. Yun Ji for serving as past members on my committee. I would also like to thank past and present research group members, especially Anastasia Andrianova, Clayton Geib, and Thomas DiProspero for their assistance on the project.

I would also like to recognize the financial support of the National Science Foundation North Dakota EPSCoR Programs I, Center for Sustainable Materials Science, and IIA-1330840 DakotaBioCon and the Department of Chemistry at the University of North Dakota.

I would finally like to thank my wife Megan for always encouraging me, and my parents, Bruce and Lynette Schumaker, who fostered my love of learning.

ABSTRACT

The focus of this thesis is on the structural characterization of lignin, a complex phenol-based heteropolymer. In the first part of the study, functionalization of lignin and its known degradation products by 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) and analysis by ^{31}P NMR spectroscopy was investigated. Chemical shifts of derivatized alcohols, phenols, and carboxylic acids were determined that allowed for qualitative characterization of lignin. This approach was further applied for the quantitative analysis of OH-containing groups present in lignin employing the internal standard. Lignin samples, fractionated by gel permeation chromatography (GPC) based on molecular weights, were derivatized by TMDP and characterized by ^{31}P NMR spectroscopy. It was determined that the number of hydroxyl-containing functional groups quantified in lignin samples increased by 366% after GPC.

In the second part of the study, six possible lignin degradation products were synthesized. It was confirmed that one of them, (*E*)-4,4'-(ethane-1,2-diyl)bis(2-methoxyphenol), was present in lignin degradation samples.

CHAPTER I

LIGNIN AND METHODS OF CHARACTERIZATION

I.1 Introduction

In the last decade, lignin research has grown in significance because of a variety of new industrial applications using this material. Lignin (Figure 1) is a large organic and primarily aromatic biopolymer found in virtually all terrestrial plants, and as such, is the third most abundant polymer found on earth after cellulose and hemicellulose.[1] It is one of the primary structural materials that comprise the support tissues of most vascular plants, i.e. flowering plants, ferns, and trees.[2] Approximately 15–40% of all dry plant weight is comprised of lignin.[1] Due to the abundance of lignin, the United States currently has the capability to produce up to 1.3 billion tons of lignin per year, which far exceed the current global market for lignin products.[3] Lignin is primarily obtained from the paper industry as a byproduct, used most frequently as a fuel for the recovery of energy.[4] Multiple kinds of lignin are formed depending on the process of its isolation, purification, and modification.[5] It is projected that in the future, lignin will have an estimated market more than $130 billion, as a result of the potential conversion to hydrocarbons, thus serving as a crude oil substitute.[6]

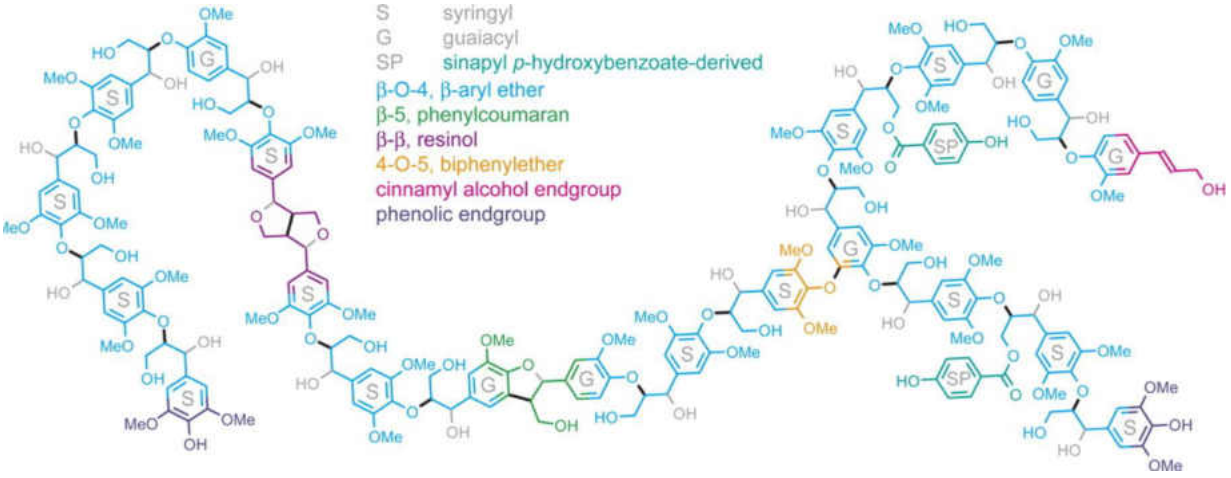


Figure 1. Proposed chemical structure of lignin.[7]

Lignin has been previously used as an additive in items such as concrete and cement, animal feed stocks, adhesives, resins, and fertilizers. However, starting in the early 2000s several research groups found various high value chemicals and polymers that has potential to be produced from lignin, such as polyurethane and carbon fiber.[2] Carbon fiber has typically been formed from traditional fossil fuels and, if derived from lignin, could have industrial application in creating high strength composites for aircrafts, cars, and blades found on wind turbines used to generate wind energy.[3] However, only fractions of the total raw lignin would be able to be utilized for these endeavors, as lignin has the most complex and recalcitrant nature among the biopolymers, e.g., cellulose or hemicellulose.[8] This complexity arises from the heterogeneous nature (see Figure 1) of the constituent monomers, as well as the potential for repolymerization to occur.[9-10] The complexity causes difficulty in understanding the degradation processes that occur and the reactivity of individual products. As a result, individual chemicals can only be retrieved from the depolymerization process in low yield, ranging from 2.5–10% of total lignin weight depending on the technique used.[9] The most common depolymerization/degradation techniques include pyrolysis, hydro-solvothermolysis, oxidation, and hydrogenolysis.[11] To use

lignin in these applications, understanding the chemical composition of both initial lignin feedstock and its products is essential.

I.2 Methods for the Characterization of Lignin and Lignin Degradation Products

Characterization of lignin and lignin depolymerization samples is usually provided by a variety of methods that can be separated into two categories: bulk techniques, which cannot distinguish individual compounds but provides characterization of the sample as a whole, and separation techniques, which target individual compounds in the sample. The former category includes Fourier transform infrared (FTIR) spectroscopy, thermal gravimetric analysis (TGA), thermal carbon analysis (TCA), nuclear magnetic resonance (NMR) spectroscopy, and size exclusion chromatography (SEC).[10] The latter category includes separation methods such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS).[10]

For the mass balance evaluation TGA or a TCA method, recently developed by the Dr. Kubatova group, may be employed allowing for quantitative characterization of lignin and its products. The TCA is based on measurements of the total carbon present in the sample by heating it in increasing temperature steps with and without the presence of oxygen.[12] Organic compounds are vaporized or pyrolyzed in temperature steps between 200 and 890 °C, oxidized to CO_2, which is then converted to methane. The methane is detected via a flame ionization detector (FID). TCA may be used to determine the volatile fraction corresponding to the total monomeric products produced from lignin depolymerization as well as larger oligomeric and coked fractions.[12] The temperature step in which the carbon is evolved indicates what type of carbon is present in samples. Thus the TCA may provide quantification of the total sample,

including the non-volatile fractions, as well as the "coked" fraction, or solid residue. While providing quantitative information, TCA is best used in conjunction with other separation techniques to provide a more comprehensive look at structure of the samples. GC-MS is widely used for lignin depolymerization samples, as many derived monomeric species are volatile. GC-MS provides qualitative information on compounds within the samples; however, volatility limits the scope of the technique, leaving significant fractions unanalyzed. LC-MS too may be used in conjunction with TCA, but is instead limited to compounds for which standards are available.

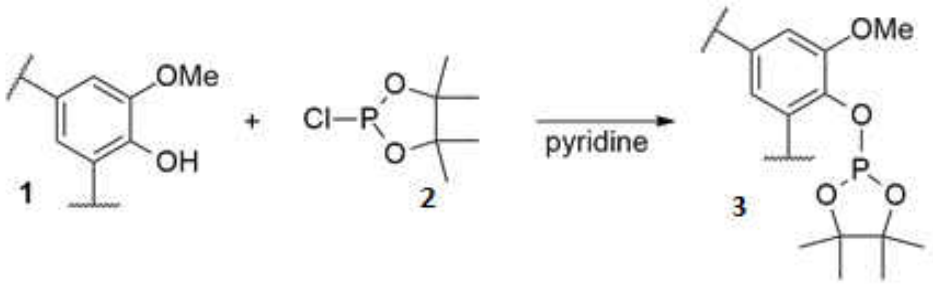
GC-MS is useful for the detailed identification of individual compounds, especially for the monomeric compounds. However, the quantification of the total degradation fraction is problematic, especially since a large percentage of lignin (and often products) is non-volatile. In addition, specific standards are required for quantification. Gel Permeation Chromatography (GPC), a form of size exclusion chromatography, estimates the molecular weight (MW) of the polymer before and after the degradation process occurs to measure the degradation efficiency. GPC uses a separation principle based on the molecular sieve effect; however, it may not account for potential steric effects or the effects of functional groups.[13] Representative calibration standards are necessary for the validation of the method.

One of the reported approaches for the structural characterization of lignin is derivatization followed by ^{31}P $\{^{1}H\}$ NMR spectroscopic analysis.[2, 14-19] This method may be used to identify and quantify hydroxyl-containing moieties found in lignin. In general, ^{31}P NMR spectroscopy is well suited for quantitative studies, as phosphorus, ^{31}P, is a monoisotopic element.[20] This is in contrast to magnetically active ^{13}C isotopes used in ^{13}C NMR spectroscopy, which constitute only 1% of all carbon atoms and thus limiting the sensitivity. In ^{1}H NMR

spectroscopy, the main isotope of hydrogen, ^{1}H, constitutes 99.9% of all atoms of this element; however, the resulting spectra of complex molecules and especially mixtures are often too convoluted to give pertinent information. One advantage of ^{31}P NMR spectra, particularly proton-decoupled ($^{31}P\{^{1}H\}$ NMR), is their simplicity, as the number of P atoms in common analytes is low. The chemical shift values in ^{31}P NMR vary significantly and depend on the valence of the P atom as well as on electronic and steric properties of the substituents bonded to this atom. As a result, signal overlapping in ^{31}P NMR spectra is not common, which simplifies the assignment of the resulting phosphorus signals.

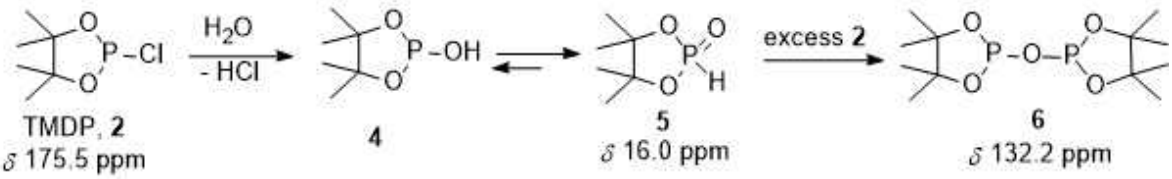
Since lignin molecules have no P atoms, this polymer and its degradation products have to be derivatized to introduce a P-containing moiety. Lignin and compounds produced during its degradation have a variety of OH-containing groups, such as the hydroxyl group in alcohols and carbohydrates, the phenolic OH moiety, the carboxyl fragment in carboxylic acids, and the hemiacetal functionality in carbohydrates (Figure 1). Naturally, the most convenient way to introduce P atoms into a lignin degradation product is derivatization of all hydroxyl-containing moieties. One known method for derivatization of these OH groups is to introduce a P atom through the process of phosphitylation. The qualitative and quantitative analysis of the mixtures containing alcohols, phenols, and carboxylic acids using phosphitylation followed by ^{31}P NMR analysis was first described by Verkade et al. in 1986.[21] Six reagents have been utilized for the derivitization.[22] Two of them, 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP, **2**)[2, 15-16, 22-25] and 2-chloro-1,3,2-dioxaphospholane, are commercially available and, therefore, have been used more frequently.[15] Phosphitylation requires the presence of a base, such as pyridine, to capture the by-product hydrogen chloride. The organic products of phosphitylation reactions

using these two reagents are called cyclic phosphites (Scheme 1). Since a saturated five-membered P-containing ring is named a phospholane, compounds phosphitylated using 2-chloro-1,3,2-dioxophospholane and its derivatives are sometimes called phospholanes as well. They are relatively stable compounds and provide single peaks in ^{31}P NMR spectra in the range of 130–150 ppm.



Scheme 1. Phosphitylation reaction of a possible lignin fragment using TMDP.

It has to be noted that water present in the analyte also reacts with TMDP providing additional products and, therefore, ^{31}P NMR signals. The structures of these products and their ^{31}P NMR chemical shifts are given in Scheme 2.[22]



Scheme 2. Reaction of TMDP with water.

Applying a special set of parameters, ^{31}P NMR spectra can be used for quantitative studies by measuring signal integrations relative to that of the known amount of an internal standard. In typical ^{31}P NMR spectra, signal intensities are not always proportional to the number of P

atoms giving those peaks. The major reason for this phenomenon is that different P atoms undergo relaxation between pulses at a different rate. In NMR spectroscopy, relaxation is the process by which an excited magnetic state returns to its equilibrium distribution. Two major types of relaxation occur during resonance; the first is spin-spin relaxation, and the second is spin-lattice relaxation. Spin-spin relaxation is also referred to as transverse relaxation, as it describes the decay of the excited magnetization perpendicular to the applied magnetic field, while spin-lattice relaxation, or longitudinal relaxation, describes the return to equilibrium parallel to the magnetic field.[26] To eliminate the effect of relaxation on signal intensity, the time between pulses (the so-called delay time, d1) is increased from the commonly used 2 seconds (s) to a higher value. A delay time of 25 s was used in closely related studies.[22, 24-25, 27-29] By adding a known amount of a standard, it is possible to obtain spectra for quantification with a proper relaxation time. The most common standards are cyclohexanol, triphenylphosphine oxide and *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide. For *in situ* analyses, all three compounds can be used; however, the phosphitylated norbornene derivative slowly decomposes.

It is well known that chemical shift values in NMR spectra are affected by a number of factors, the most significant of them is the electronic properties of the substituents attached to the atom whose nucleus is undergoing resonance.[15] In the case of phosphitylated phenol derivatives, the ^{31}P NMR chemical shifts are expected to be in a range of 138–143 ppm. Phosphitylated alcohols provide signals shifted downfield 143–150 ppm due to less pronounced electron-withdrawing properties of alkoxy groups compared to aryloxy moieties. Phosphitylated carboxylic acids, which have strongly electron-withdrawing carboxylate groups, are expectedly give ^{31}P NMR signals upfield, 130–138 ppm, compared to derivatized phenols and alcohols.

The introduction and position of a functional group on the aromatic rings of phosphitylated phenol derivatives lead to differences in the NMR chemical shifts of the corresponding compounds. Taking phosphitylated phenol as a reference compound with the signal at 138.68 ppm, a methoxy group in the ortho, meta, and para position alters the position of the resonance peak.[21, 25] The signal of the phosphitylated phenol with the ortho-methoxy group is shifted downfield, though insignificantly (138.78 ppm) compared to that of the reference.[30] These downfield shifts can be explained by the electron-donating effect of the methoxy group through resonance (Figure 2A). In resonance structures of the ortho- and para-substituted derivatives, a partial negative charge is created at the carbon nearest to the phospholane moiety. As a result, the P atom is more shielded, and its signal has a higher chemical shift value. It is noteworthy that the effect of the ortho-methoxy group is much more pronounced than that of the para substituent because the ortho position is closer to the P-containing group. The meta isomer provided the ^{31}P NMR signal at 137.72 ppm.[30] The upfield shift can be explained by the electron-withdrawing inductive effect of the methoxy substituent as the resonance-governed electron-donating properties of this group do not operate in the meta position (Figure 2B).

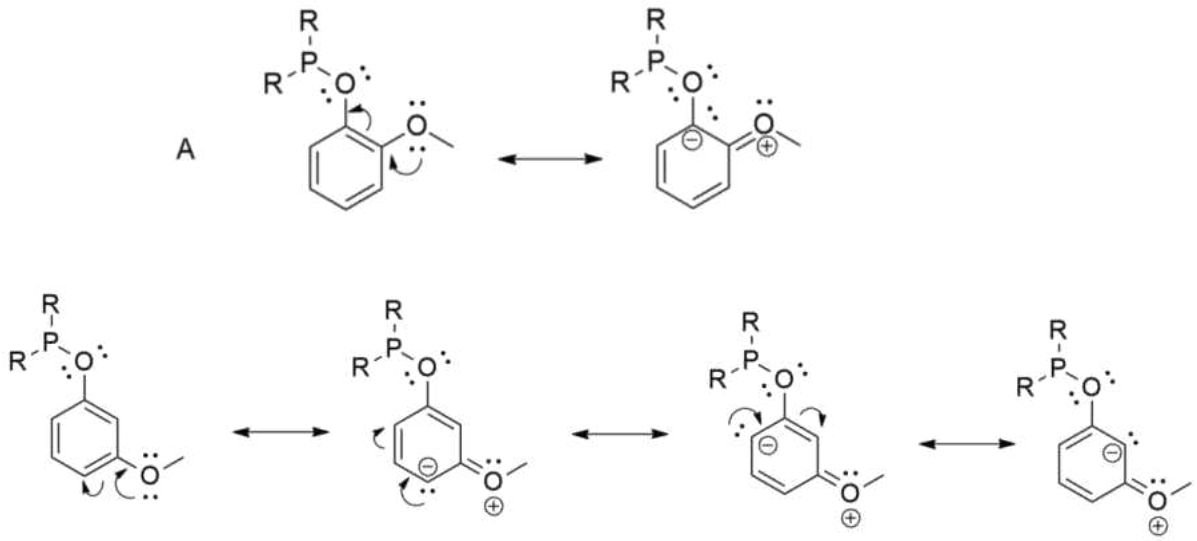


Figure 2. Resonance structures for ortho-methoxy phenol derivatives (**A**) and the corresponding meta analogs (**B**)

Due to specific resonance around the substituted phenol compounds which make up lignin and the specificity of the subsequent phospholanes on the spectrum, the $^{31}P\{^{1}H\}$ NMR spectroscopic method is the ideal candidate for quantitative studies of compounds with a limited number of hydroxyl moieties per product. Previously, most quantitative studies have focused on overall percentages of the connective bonds found in lignin, as well as quantifying the type of hydroxyl group (i.e., aliphatic, phenolic, or guaiacyl phenolic OH groups).[31-34] However, with a sufficient number of scans, resolution will be sufficient to distinguish and quantify individual compounds through the ^{31}P NMR spectra, as well as the overall types of lignin degradation products and bonds present.

CHAPTER II

STATEMENT OF PURPOSE

The present study had two main goals. The first goal was to develop a $^{31}P\{^{1}H\}$ NMR method for quantitative and qualitative characterization of lignin and lignin degradation products utilizing the phosphitylation of hydroxyl-containing functional groups. Specifically, we developed a method suitable to quantify lignin and lignin degradation samples by both (i) the type of hydroxyl-containing moieties (i.e., alcoholic (sp^3)C-OH, phenolic (sp^2)C-OH, and carboxylic C(O)OH), and (ii) the presence and amount of a particular compound (e.g., guaiacol and syringol) in an analyte. $^{31}P\{^{1}H\}$ NMR spectroscopy is advantageous compared to other NMR methods as phosphorus exists as only one isotope and, in the absence of other P atoms in close proximity in the structure, each signal appears as a singlet. Also, with proper delay and relaxation times, integration intensities of all signals can be measured and thus provide relative quantities. Using known amounts of an internal standard allows one to determine not only relative but also absolute amounts of the compounds or specific moieties in the given sample. Another advantage of NMR spectroscopy is that researchers can study samples, which lack volatility, e.g., higher MW compounds. The method is complementary to other techniques used for analysis of lignin and lignin degradation products such as GC-MS and LC-MS.

The second goal of the study was the synthesis of lignin degradation standards for accurate identification and quantification of lignin and lignin degradation products. GC-MS

electron ionization mass spectra of lignin degradation products commonly contain signals with *m/z* = 272, 274, and 302, which have not been assigned using commercially available standards.[12,13] In order to identify the compounds giving these ions, several structures relevant to lignin with the masses of 272, 274, and 302 were identified. As a part of this thesis, we proposed synthetic routes to these substances and then prepare them. All these compounds were tested by ESI-TOF and GC-MS to determine if they are components of lignin degradation mixtures prepared in the research lab of Dr. Kubatova.

CHAPTER III
^{31}P AND ^{1}H NMR CHARACTERIZATION OF LIGNIN AND LIGNIN DEGRADATION PRODUCTS

III.1 Materials and Methods

2-Chloro-4,4,5,5-tetramethyldioxaphospholane (TMDP) and alkali lignin were obtained from Sigma Aldrich. Both pyridine and $CDCl_3$ were purified by distillation, and kept over NaOH and 4 Å molecular sieves, respectively.

The ^{1}H, ^{13}C, and ^{31}P $\{^{1}H\}$ NMR spectra were recorded using a Bruker Avance 500 NMR spectrometer. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded in $CDCl_3$ unless specified otherwise. ^{31}P NMR spectra were taken in a mixture of pyridine (py) and $CDCl_3$ in a ratio of 1.6:1. For quantitative studies using $^{31}P\{^{1}H\}$ NMR spectroscopy, the pulse width was optimized to give the 90˚ flip angle at approximately 10 μs. The optimized pulse delay was 20 s. The $^{31}P\{^{1}H\}$ NMR spectra of TMDP and its hydrolysis product were obtained at 256 scans. $^{31}P\{^{1}H\}$ NMR spectra of phosphitylated lignin, lignin degradation products and other analytes were obtained using 1024 scans.

To determine the signals of the hydroxylated TMDP, two drops of water were added to the solution containing 250 μL of py, 150 μL of $CDCl_3$, and 15 μL of TMDP. The mixture was stirred for 5 min to allow the organophosphorus compound to react. The signal of resulting hydroxylated TMDP, 2-hydroxy-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane **2** (Scheme 2), was

observed at 132.2 ppm. A signal at 0.6 ppm of an unidentified compound was also observed. The signal at 16.0 ppm was observed in the spectrum.

A general procedure for the phosphitylation reaction: 400 μL of 1.6:1 (v/v) mixture of pyridine and $CDCl_3$ were added to a 4.0 mL vial with a magnetic stir bar. Then a compound to be phosphitylated was introduced to the vial. During quantification studies, chromium acetylacetonate (1 mg) and cyclohexanol (10 μL) were added to the vial before introducing the phosphitylation reagent. Two molar equivalents of TMDP were added dropwise to the solution. After stirring at rt for approximately 5 min, the phosphitylated sample was transferred to a NMR tube. NMR spectra were recorded within 1 hour after preparation of the sample. Since most samples tended to be dissolved in DCM/water solution, which is not conducive to the phosphitylation reaction due to the solvent containing hydroxyl groups, some sample preparation was before the phosphitylation reaction could be performed. Samples that were dissolved in solvent were dried using a rotary evaporator at 20 torr for 60 seconds to remove most solvents from the system.

III.2 Results and Discussion

III.2.1 Determination of Spectral Parameters Necessary for Qualitative and Quantitative Analysis of Lignin Degradation Products Using $^{31}P\{^{1}H\}$ NMR Spectroscopy

The first parameter that required optimization was the pulse angle, which is controlled by the pulse width, or length of time during which excitation occurs. Typically, a pulse angle of 45° or 90° is used for recording spectra. A 90° pulse angle would provide the largest peaks by

area, which would make discerning low concentration compounds easier, especially when in a mixture. To determine the 90° angle, the 180° angle is found experimentally by altering the pulse width parameter (p1) where the peak is functionally nonexistent. The 90° and 45° angles are then half and a fourth of the value at 180°, respectively. Starting at a p1 value of 2 μs, 2 μs intervals were measured to determine the 180° pulse. In our study, we used a p1 value equal to 10 μs.

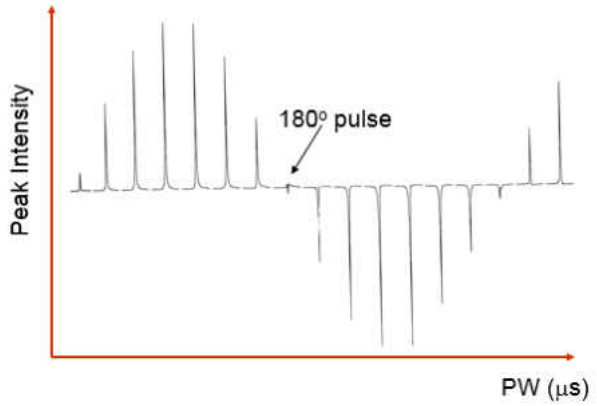


Figure 3. Peak intensity *vs*. pulse width (PW), which correlates to the pulse angle.[35]

To ensure that the resolution of the spectra was adequate, the number of scans was optimized. The number of scans used in NMR spectroscopy directly correlates to the resolution of the resulting spectrum; however, too many scans would significantly increase the time necessary for collecting the data. Resolution increase by square root of the number of scans, meaning a four-fold increase will double the resolution. Spectra of the samples were compared at 16, 64, 256, and 1024 scans. As expected, the spectrum obtained using 1024 scans was sufficient for identifying signals in the ^{31}P NMR spectra, as seen in Figure 6. It is noteworthy that recording a ^{31}P NMR spectrum after scans with 256 scans with the delay time of 20 s took approximately 1.5 h and with 1024 scans with the same time took 6 hours.

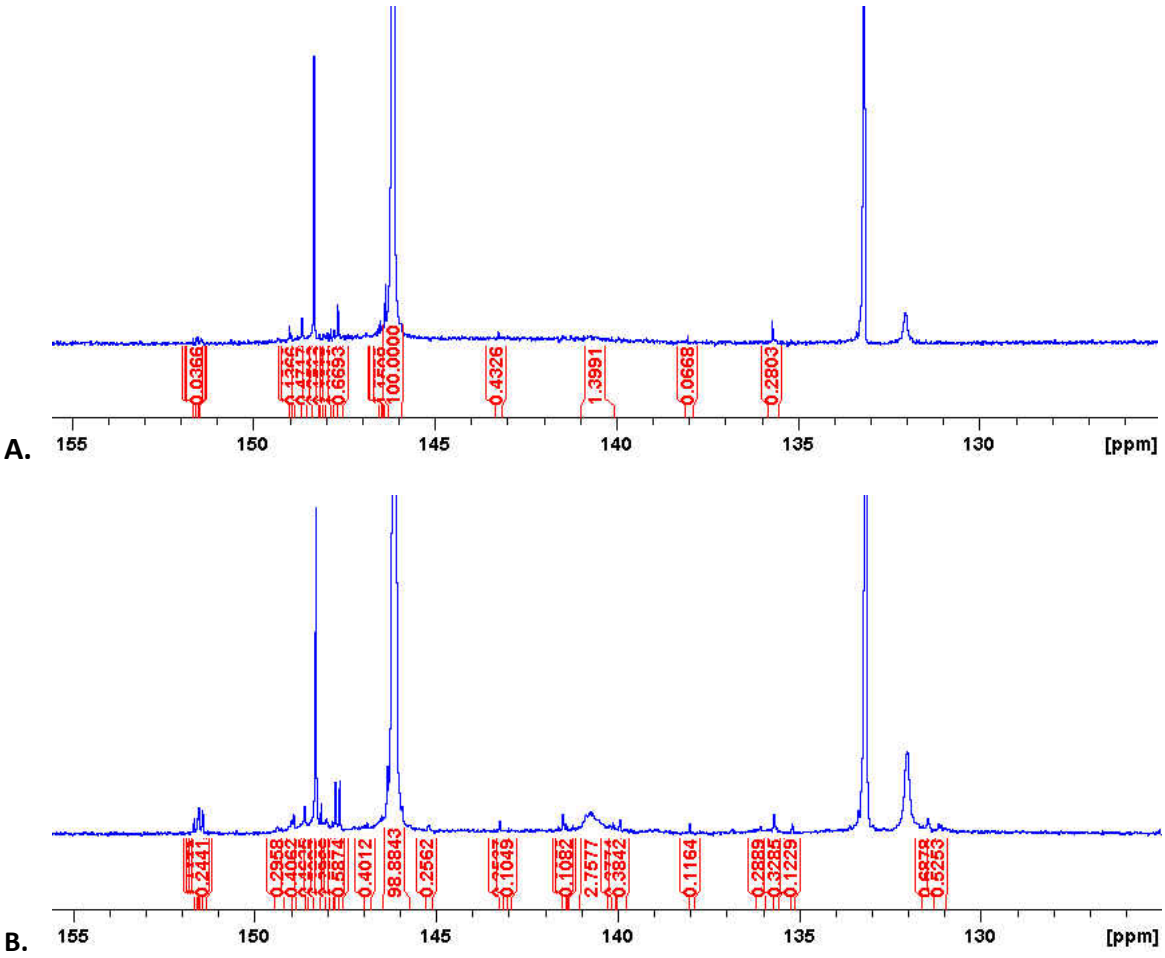


Figure 4. $^{31}P\{^{1}H\}$ NMR spectra of the weight fractionated sample with 256 scans (A) and 1024 scans (B), showing an increase in resolution in the latter.

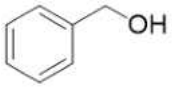
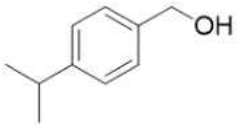
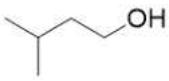
III.2.2 Determination of ^{31}P NMR Chemical Shifts of OH-Containing Compounds

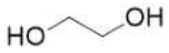
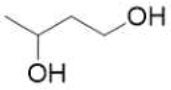
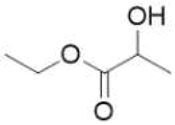
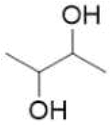
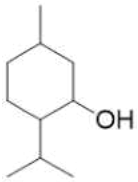
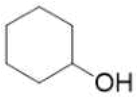
The lignin degradation products may include a broad array of phenolic derivatives, including but not limited to guaiacol, and vanillin, and their derivatives.[36-37] Our first task was to determine chemical shift values of these compounds after phosphitylation with TMDP. A test mixture containing 2.5% of each of the following compounds was prepared: phenol, guaiacol, methyl guaiacol, propyl guaiacol, ethyl guaiacol, vanillin, acetovanillin, syringaldehyde, vanillic acid, homovanillic acid, and bicreosol. This test mixture was phosphitylated with TMDP in the

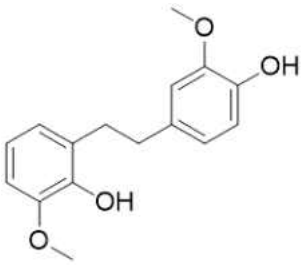
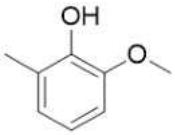
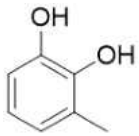
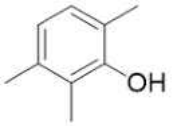
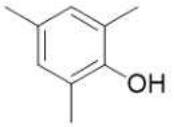
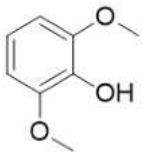
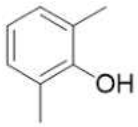
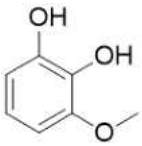
presence of pyridine, and then its $^{31}P\{^{1}H\}$ NMR spectrum was recorded. The number of $^{31}P\{^{1}H\}$ NMR signals in the 130–150 ppm region corresponded to the number of compounds in the test mixture confirming that each of the phosphitylated lignin degradation products provided a unique signal with a specific chemical shift. One by one, standards of degraded compounds were then added to the test mixture in order to assign the signals found in the test mix. As expected, an increase in concentration for a particular compound did not change the compound's chemical shift in the mixture. A number of other OH-containing compounds were analyzed by ^{31}P NMR spectroscopy after phosphitylation in order to determine structural factors affecting chemical shift values of derivatized alcohols, phenols, carbohydrates, and carboxylic acids.

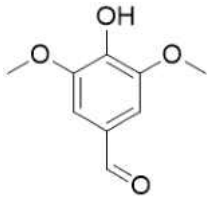
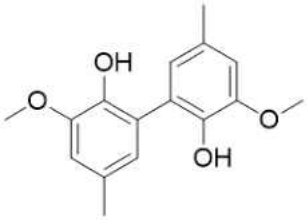
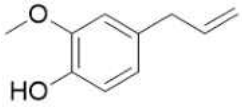
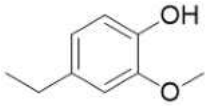
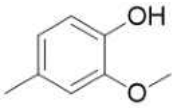
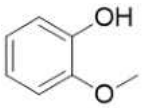
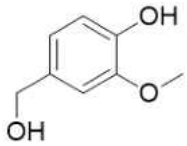
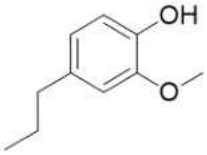
Our data on the ^{31}P NMR chemical shift values of a number of phosphitylated OH-containing standards are given in Table 1. For comparison, previously reported ^{31}P NMR chemical shifts of some phosphitylated compounds are also included into Table 1.[30, 35]

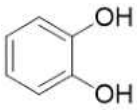
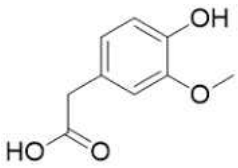
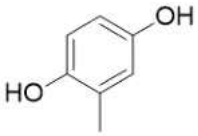
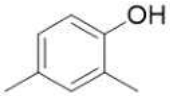
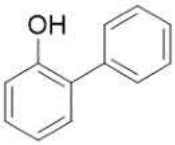
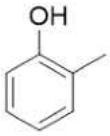
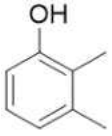
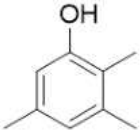
Table 1. ^{31}P NMR chemical shift determined for phosphitylated hydroxyl-containing compounds.

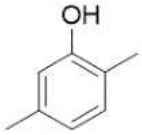
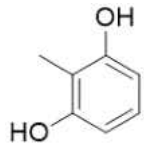
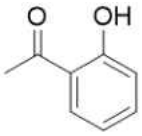
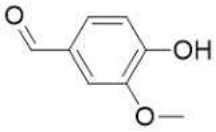
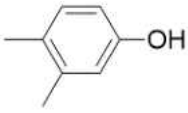
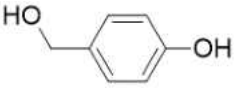
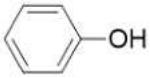
Name	Structure	Formula	Measure Chemical Shifts (ppm)	Reported Chemical Shifts (ppm)
Alcohols				
Methanol		CH_3OH	no data	148.93[33]
Benzyl alcohol (phenylmethanol)		C_7H_8O	no data	148.74[33]
4-Isopropylbenzyl alcohol		$C_{10}H_{14}O$	no data	148.22[33]
2,2-Dimethylpropane-1,3-diol		$C_5H_{12}O_2$	no data	148.03[33]
Ethanol		C_2H_5OH	no data	147.99[28]
Isoamyl alcohol (3-methyl-1-butanol)		$C_6H_{12}O$	no data	147.76[33]

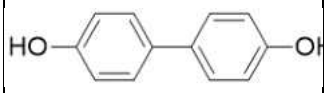
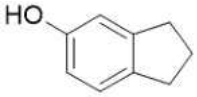
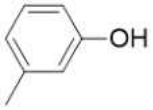
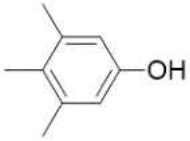
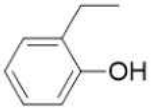
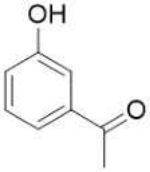
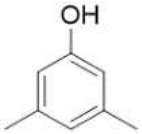
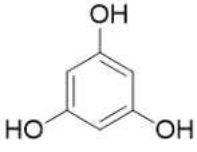
Ethylene glycol (ethane-1,2-diol)		$C_2H_6O_2$	no data	147.75[33]
Butane-1,3-diol		$C_4H_{10}O_2$	no data	147.58;[33] 146.39[33]
Ethyl lactate (ethyl-2-hydroxypropanoate)		$C_5H1_0O_3$	no data	146.99[33]
Butane-2,3-diol		$C_4H_{10}O_2$	no data	146.70;[33] 146.89[33]
L-Menthol (5-methylcyclohexanol)		$C_{10}H_{20}O$	no data	146.53;[33] 146.25[28]
Cyclohexanol		$C_6H_{12}O$	145.10	NR
tert-Butyl alcohol		$C_4H_{10}O$	no data	142.83[33]
<u>Phenols</u>				

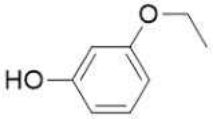
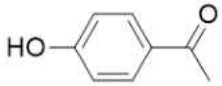
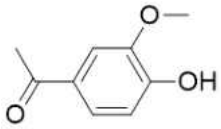
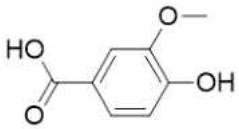
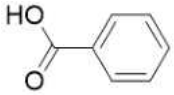
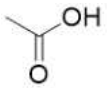
4-[2-(2-Hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol		$C_{16}H_{18}O_4$	144.71; 141.12	NR
2-Methoxy-6-methylphenol		$C_8H_{10}O_2$	no data	143.73[33]
3-Methylbenzene-1,2-diol		$C_8H_{10}O3$	142.86	142.87[33]
2,3,6-Trimethylphenol		$C_9H_{12}O$	no data	143.58[33]
2,4,6-Trimethylphenol		$C_9H_{12}O$	no data	143.35[33]
Syringol (1,3-dimethoxy-2-hydroxybenzene)		$C_7H_8O_2$	143.14	143.06[28]
2,6-Xylenol (2,6-dimethylphenol)		$C_8H_{10}O$	no data	143.04[33]
3-Methyoxybenzene-1,2-diol		$C_7H_8O_3$	no data	142.72;[28] 138.60[28]

Syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde)		$C_9H_{10}O_4$	142.31	NR
Bicreosol (4-formyl-2-methoxyphenyl benzoate)		$C_{15}H_{12}O_4$	141.84	141.87[28]
Eugenol (4-allyl-2-methoxyphenol)		$C_{10}H_{12}O_2$	139.92	NR
Ethyl guaiacol (4-ethyl-2-methoxyphenol)		$C_9H_{12}O_2$	139.90	NR
Methyl guaiacol (2-methoxy-4-methylphenol)		$C_8H_{10}O_2$	139.89	NR
Guaiacol (2-methoxyphenol)		$C_7H_8O_2$	139.76	139.78[28]
Vanillyl alcohol (4-hydroxylmethyl-2-methoxyphenol)		$C_8H_{10}O_3$	no data	139.75;[28] 148.40[28]
Propyl guaiacol (2-methoxy-4-propylphenol)		$C_{10}H_{14}O_2$	139.75	NR

Catechol (benzene-1,2-diol)		$C_6H_6O_2$	no data	139.71;[33] 138.92[33]
Homovanillic acid (2-(4-hydroxy-3-methoxyphenyl)acetic acid)		$C_9H_{10}O_4$	139.71	NR
2-Methyl-1,4-benzenediol		$C_7H_8O_2$	no data	139.69;[33] 138.87[33]
2,4-Xylenol (2,4-dimethylphenol)		$C_8H_{10}O$	no data	139.52[33]
2-Phenylphenol		$C_{12}H_{10}O$	no data	139.32[33]
o-Cresol (2-methylphenol)		C_7H_8O	no data	139.29[33]
2,3-Xylenol (2,3-dimethylphenol)		$C_8H_{10}O$	no data	139.29[33]
2,3,5-Trimethylphenol		$C_9H_{12}O$	no data	139.21[33]

2,5-Xylenol (2,5-dimethylphenol)		$C_8H_{10}O$	no data	139.21[33]
2-Methylresorcinol		$C_7H_8O_2$	no data	139.14[33]
2-Hydroxyacetophenone		$C_8H_8O_2$	no data	138.97[33]
Vanillin (4-hydroxy-3-methoxybenzaldehyde)		$C_8H_8O_3$	138.87	NR
p-Cresol (4-methylphenol)		C_7H_8O	no data	138.83;[33] 138.15[28]
3,4-Xylenol (3,4-dimethylphenol)		$C_8H_{10}O$	no data	138.72[33]
4-Hydroxybenzyl alcohol		$C_7H_8O_2$	no data	138.69;[33] 148.74[33]
Phenol		C_6H_6O	137.69	138.68[33]

Biphenyl-4,4'-diol		$C_{12}H_{10}O_2$	no data	138.68;[33] 138.00[28]
5-Indanol		$C_9H_{10}O$	no data	138.67[33]
m-Cresol (3-methylphenol)		C_7H_8O	no data	138.60;[33] 137.98[28]
3,4,5-Trimethylphenol		$C_9H_{12}O$	no data	138.58[33]
2-Ethylphenol		$C_8H_{10}O$	no data	138.56[33]
3-Hydroxyacetophenone		$C_8H_8O_2$	no data	138.53[28]
3,5-Xylenol (3,5-dimethylphenol)		$C_8H_{10}O$	no data	138.46[33]
Benzene-1,3,5-triol		$C_6H_6O_3$	no data	138.28[33]

3-Ethoxyphenol		$C_8H_{10}O_2$	no data	137.83[28]
4-Hydroxyacetophenone		$C_8H_8O_2$	no data	137.65[28]
Acetovanillone (1-(4-Hydroxy-3-methoxyphenyl)ethanone)		$C_9H_{10}O_3$	TBD	NR
Acids				
Vanillic acid (4-hydroxy-3-methoxybenzoic acid)		$C_8H_8O_4$	139.78; 135.57	NR
Benzoic acid		CH_3OOH	134.63	134.63[28]
Acetic acid		CH_3OOH	134.68	134.63[28]

According to Table 1, the relevant chemical shifts of phospholane derivatives in ^{31}P NMR spectra can be loosely divided into three regions: 143–150 ppm of phosphitylated alcohols, 138–143 ppm for phenols, and 135–138 ppm for carboxylic acids. Validation of the quantitative method can be seen in appendices A–D.

III.2.3 Quantification of OH-Containing Groups in Lignin

The next part of the present study was to estimate the number of hydroxyl groups in alkali lignin using $^{31}P\{^{1}H\}$ NMR spectroscopy and compare the results with the known data. In general, quantitative information on the number of OH groups in lignin samples is scarce. For example, *Meadwest Vaco*, a company that produced Indulin lignin for commercial purposes, reported that there are 6.52 mmoles of hydroxyl groups present per g of lignin. Moreover, the company provided the data for three types of hydroxyl groups: phenolic, benzylic, and aliphatic (Table 2). It is noteworthy that the *Meadwest Vaco* lignin specifications contain no information on either the amount of hydroxyl groups from the carboxyl functionality nor the methods used to obtain their data. (*Meadwest Vaco* was purchased by another company and now defunct.)

In the present study, a sample of the alkali lignin purchased by Sigma Aldrich was used. It was shown previously that this alkali lignin has the same structural characteristics as Indulin lignin, for which quantitative information on hydroxyl groups is available. The lignin sample was derivatized by TMDP using the same procedure described above for qualitative determination of OH-containing compounds. To apply the method for qualitative studies, a known amount of cyclohexanol was added to the lignin sample before functionalization. The data obtained in the experiment, which was repeated three times, are presented in Table 2. It is important to note that the phosphitylated benzylic and aliphatic hydroxyl groups are indistinguishable using ^{31}P NMR spectroscopy, and the number of aliphatic moieties given in Table 2 includes hydroxyl groups at the benzylic position. While the number of aliphatic and especially phenolic hydroxyl groups determined by ^{31}P NMR spectroscopy is somewhat lower, the difference between the

Meadwest Vaco and our data for the total amount of OH-containing groups was negligible when the acids were added.

To conclude, using the quantitative ^{31}P NMR method, we were able to determine the number of mmoles per g of lignin using ^{31}P NMR spectroscopy, and the number is similar to that reported previously.

Table 2. Comparison of the number of mmoles of hydroxyl groups present per g of alkali lignin found by Meadwest Vaco *vs* the results obtained in the present study using ^{31}P NMR spectroscopy, also seen in appendix A.

		^{31}P NMR data	
	Meadwest Vaco data	alkali	Indulin
Phenolic OH	3.6	2.85 ± 0.04	2.4 ± 0.3
Benzylic OH	0.06	2.7 ± 0.2	2.4 ± 0.3
Aliphatic OH	2.9		
Carboxylic Acid OH	Not Reported	0.78 ± 0.01	0.7 ± 0.1
Total	6.5	6.3 ± 0.2	5.5 ± 0.6

III.2.4 Quantification of OH-Containing Groups in GPC Weight Fractionated Samples of Lignin

After checking the ^{31}P NMR spectroscopy-based method for quantitative determination of OH-containing groups in lignin, we applied this approach to weight fractionated samples of lignin. With the help of Dr. Anastasia Andrianova, a member of Dr. Kubatova's group, six weight fractionated samples of lignin were obtained using GPC. These samples, designated as 3NA-81-x (x = 1-6), contained a high volume of THF and water. The later solvent can critically interfere with phosphitylation reaction and the subsequent ^{31}P NMR measurements. Therefore, the phosphitylation procedure of the weight fractionated samples required modifications, and samples of the weight fractionation were added to the Schlenk flask by syringe, and the vacuum

was applied for 20 s, until the solvent was removed. Concentration of cyclohexanol, utilized as the internal standard was 4982 μg/mL. The quantity was then used to find the concentration of the other phospholane signals. The ^{31}P NMR spectra can be seen in Figure 5 below.

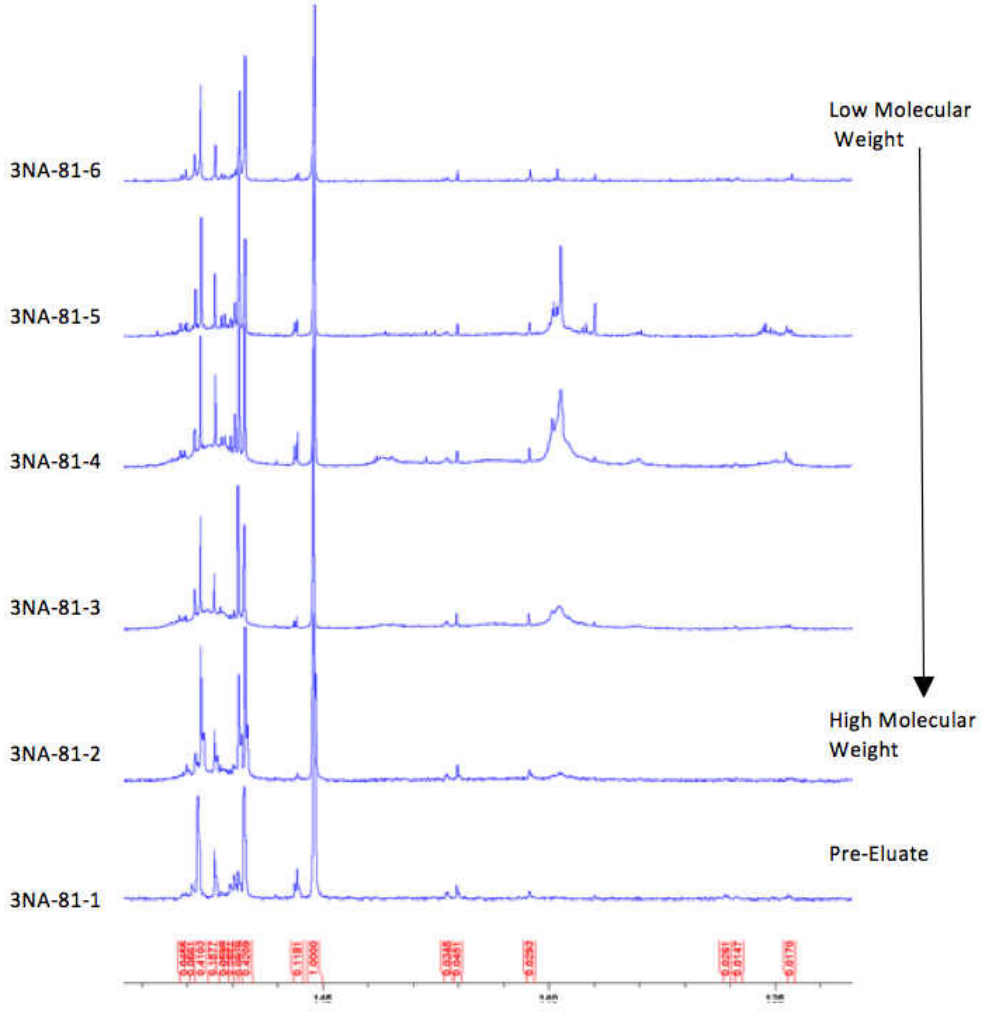


Figure 5. ^{31}P NMR spectra of GPC weight fractionated lignin samples. The top spectrum spectrum (3NA-81-6) is the lowest molecular weight fraction, while 3-NA-2 is the highest molecular weight fraction. The bottom spectrum is a pre-eluate fraction comprised mostly of impurities of carbohydrate origins.

The number of hydroxyl groups present in each sample was calculated through integration relative to the cyclohexanol peak found at 145.2 ppm with a known concentration. The same loosely divided regions were used for the classification of hydroxyl groups found in the weight fractionated samples: 143–150 ppm for alcohols, 138–143 ppm for phenols, and 135–138 ppm for carboxylic acids. The number of mmoles found in each sample are summarized in Table 3. It is noteworthy that the spectra of all fractions contained a cluster of unidentified signals centered around 150.6 ppm. These signals have not been reported in other studies of phosphitylated lignin-based samples, nor were they observed in the spectra of other lignin and lignin-derived samples investigated by our group. It is possible that TMDP is reacting with some other highly electronegative species such as sulfur, which is present in alkali lignin.

Table 3. Number of mmoles found in NMR samples in the GPC weight fractions per g of alkali lignin.

	mmoles				
Sample	Unidentified	Alcohols	Phenols	Acids	Total
3NA-81-1	0.48	0.99	0.05	0.04	1.55
3NA-81-2	0.49	1.61	0.40	0.00	2.50
3NA-81-3	0.71	3.10	1.78	0.15	5.74
3NA-81-4	0.57	3.79	2.08	1.82	6.68
3NA-81-5	0.78	2.48	1.82	0.37	5.45
3NA-81-6	0.46	1.30	0.13	0.08	1.96
Total	3.5	13.3	6.3	0.9	23.9

Overall, there is a general upward trend in the number of μmoles found as the molecular weight of the fraction decreases. This is similar to the findings of Dr. Andrianova, as most of the lignin was concentrated in the lower molecular weight fractions especially 3-5.[38] The first fraction was mostly impurities from lignin, which is most likely comprised largely of carbohydrate derivatives, and the sixth fraction contained only a small amount of lignin, which can easily be

seen when looking at the diode array detector (DAD) chromatogram of the high pressure (HP) size exclusionary chromatography (SEC) elution profiles of the fractions in Figure 6.[38]

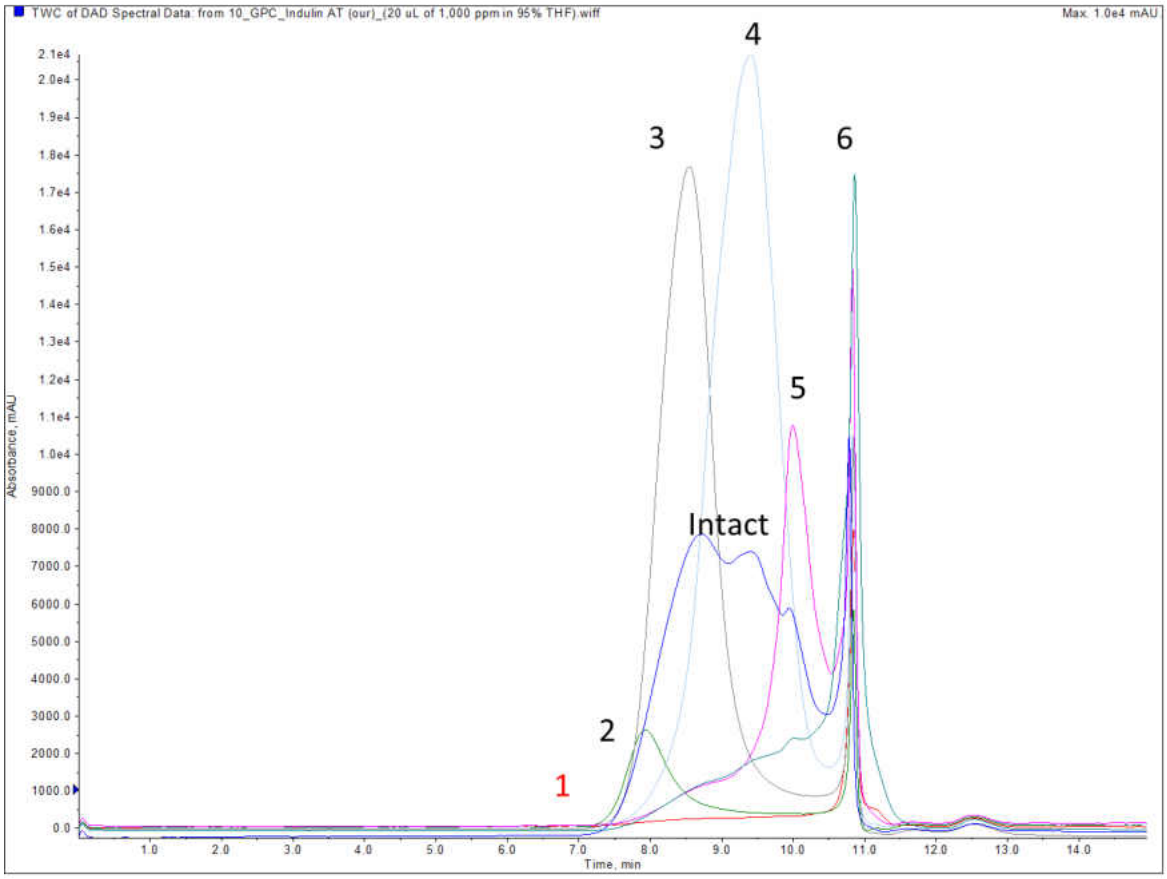


Figure 6. Overlayed HPLC-DAD chromatograms of the GPC elution profiles of the lignin fractions.[36]

As seen in the GPC chromatograms, fractions 3–5 contained the highest concentration of lignin, with a relatively small concentration this polymer in the remaining three samples. This is mirrored in the ^{31}P NMR spectra and summarized in Table 3. The number of moles of hydroxyl groups post GPC is significantly larger than the number determined in alkali lignin before the weight fractionation occurs. When extrapolating the number of theoretical moles from the number of actual mmoles per g of lignin, we should expect approximately 619 mmoles of

hydroxyl groups in total, instead we determined there were about 2269 mmoles, which is shown in Table 4.

Overall, we observed a positive upward trend for the number of hydroxyl groups for most types of OH-containing compounds present in lignin fractions after GPC. The phenolic signals have significantly higher relative intensities as the molecular weight of lignin decreases. There is also growth for the aliphatic hydroxyl groups as the molecular weight decreases, but by percentage, phenols see significantly higher growth, with the highest number of phenols present in almost equal amounts in fractions 4 and 5. Carboxylic acids are present in low concentration regardless of the fraction, but peak in the low molecular weight fraction 5.

The number of moles of hydroxyl groups post GPC was significantly larger than the number determined in alkali lignin before the weight fractionation. When estimating the number of theoretical mmoles from the number of actual mmoles per g of lignin, we should expect approximately 6.52 mmoles of hydroxyl groups in total. Instead, the number of hydroxyl groups determined using ^{31}P NMR spectroscopy was 23.9 mmoles, which constitutes 370% relative to the expected amount (Table 4).

Table 4**.** Calculation of the yield of hydroxyl group mmoles found in lignin after GPC weight fractionation per gram of lignin. 95 mg were fractionated in total.

	mmoles
Actual number of μmoles of hydroxyl groups	23.9
Theoretical number of μmoles of hydroxyl groups	6.52
Percent increase of hydroxyl groups after GPC	370

The obtained quantitative ^{31}P NMR data suggest that after weight fractionation, there is a 366% increase in the number of hydroxyl groups undergoing the phosphitylation reaction.

There are several possible explanations for the large discrepancy found before and after GPC. The first possible explanation is that the hydroxyl groups in lignin before GPC are unavailable for phosphitylation due to steric hindrance to undergo phosphitylation. The weight fractionation process is possibly causing modifications of the complex three-dimensional structure of lignin is such a way as to allow more hydroxyl groups to undergo phosphitylation.

THF used in the GPC method could also be a source of some additional OH groups determined in our experiments. For example, THF could react with lignin under the conditions used during the GPC resulting in the cleavage of the THF ring to allow for the formation of a new hydroxyl group. THF peroxide formation is also a possibility, and the compound has a new OH group and, therefore, will readily react with TMDP. Finally, the ether bond cleavage in THF could be occurring either before or during the GPC method. The ^{31}P NMR spectrum of concentrated THF does contain two phospholane signals. The first is a signal in the aliphatic hydroxyl group region at 147.4 ppm, while the other is in the phenolic region at 142.0 ppm. While this does add to the μmolar total, both have relatively small integrations and an insignificant impact on the final total, and certainly does not account for the total 366% increase in hydroxyl groups.

Another segment of the increase is due to the presence of the unidentified portion between 150 and 152 ppm in the spectra of the GPC fractions, which accounts for approximately 16% of the number of moles. These signals could not belong to phosphitylated alcohols, phenols, carboxylic acids, and carbohydrate derivatives. We can hypothesize that the signals in the region between 150 and 152 ppm belong to phosphitylated sulfur-containing compounds, such as thiols, since sulfur is known to be present in lignin. However, this suggestion still needs to be verified.

III.3 Conclusions

We further developed the previously described method of lignin analysis based on phosphitylation of the polymer followed by ^{31}P NMR spectroscopic measurements. This method was applied for qualitative and quantitative analysis of weight fractionated lignin samples obtained by GPC. We found that after GPC weight fractionation, lignin samples exhibit an increase in the number of hydroxyl groups present. This could be either due to a result of modification in the complex lignin 3-dimensional structure. THF used in the method could also cause additional signals, either through reactions with lignin or THF peroxide formation. In total after GPC, the hydroxyl groups quantified increased by 366% over alkali lignin prior to weight fractionation possibly due to steric hinderance and thus incomplete derivatization of intact lignin.

CHAPTER IV

SYNTHESIS OF LIGNIN DEGRADATION STANDARDS

IV.1 Experimental

IV.1.1 Materials and Methods

Column chromatography was performed using Natland Silica gel 60 (230–400 mesh). Preparative thin-layer chromatography was performed on a 200 × 250 mm glass plate with a loose layer of Natland silica gel 60 (230–400 mesh) mixed with approximately 10% of Sigma-Aldrich TLC standard grade silica gel with a fluorescent indicator. Analytical TLC was performed using Merck silica gel 60 (F_{254}) 250 μm pre-coated aluminum plates. To visualize compounds on TLC plates, either UV light (254 nm) or iodine was used.

^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker AVANCE 500 NMR spectrometer. Chemical shifts are reported with $SiMe_4$ as an internal standard. Spin-spin coupling constants are given in Hz. NMR spectra are recorded in $CDCl_3$ unless otherwise specified. $CDCl_3$ was distilled over CaH_2. Acetone was distilled over $KMnO_4$ before use, or HPLC grade acetone was used. Benzoyl chloride, benzyl chloride, guaiacol, creosol, vanillin, 3,4-dimethoxybenzyl alcohol, 2-methoxyphenol, sodium thiosulfate, and chromium acetylacetonate were purchased from Sigma Aldrich. Triethylamine and thionyl chloride were acquired from Acros Organics. Triphenylphosphine was received from Eastman Chemical Co, $FeSO_4 \cdot 7H_2O$ was purchased from MCB Reagents, and $K_3[Fe(CN)_6]$ was acquired from Mallinckrodt. Benzoyl chloride, benzyl

chloride, trimethylamine, and thionyl chloride were distilled prior to their use. Other chemicals were used as purchased.

IV.1.2 Synthesis of Standards

IV.1.2.1 Synthesis of 1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)-1-propanone (**10)**

4-Propionylphenyl benzoate (6). The compound was prepared using the reported procedure for the O-benzoylation of the propiophenone in 48% yield.[39] The ^{1}H NMR data of the synthesized product were identical to the data reported previously.[39]

4-(2-Bromopropionyl)phenyl benzoate (7). Compound **7** was prepared by using a known procedure for bromination of related ketones in 59% yield.[40] Column chromatography was performed for additional purification. The eluent used was a mixture of hexane and ethyl acetate (50:1, 36:1, 10:1, and pure ethyl acetate).[40] ^{1}H NMR spectrum (δ, ppm): 1.93 (d, J = 7 Hz, 3H), 5.28 (q, J = 7 Hz, 1H), 7.37 (m, 2H), 7.55 (m, 2H), 7.67 (m, 1H), 8.14 (m, 2H), 8.22 (m, 2H).

1-(4-Hydroxyphenyl)-2-(2-methoxyphenoxy)-1-propanone (10). The compound was synthesized in 10% yield using a modified published procedure.[41] A mixture of benzoate **7** (0.25 g, 0.75 mmol), guaiacol (0.14 g, 1.13 mmol), and anhydrous K_2CO_3 (0.25 g, 1.88 mmol) in acetone (20 mL) was refluxed for 1 h. After cooling, the mixture was filtered. The filtrate was diluted with H2O (60 mL) and extracted with chloroform. After extraction, an aqueous solution of 2M NaOH was used to wash the organic layer to remove excess guaiacol. Preparative TLC (on silica gel) was performed using a 2:1 mixture of hexane and ethyl acetate to collect a mixture of two compounds, 2-(2-methoxyphenoxy)propanoylphenyl benzoate (**9**) and the target compound **10**. After TLC, the mixture was recrystallized using ethyl acetate and hexane. Compound **10**

precipitated as a yellow powder. ^{1}H NMR spectrum (δ, ppm): 1.72 (d, J = 7 Hz, 3H), 3.83 (s, 3H), 5.42 (q, J = 7 Hz, 1H), 6.81 (s, 1H), 6.75−6.95 7.37 (m, 6H), 8.96 (m, 2H).

IV.1.2.2 Synthesis of 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol (**17**)

2-Benzyloxy-3-methoxybenzaldehyde (12). The compound was prepared using a known procedure in 86% yield as pale yellow crystals.[42] The ^{1}H NMR data matched those reported in the literature.[42]

2-Benzyloxy-3-methoxybenzyl alcohol (13). Compound **13** was obtained in 77% yield as colorless powder by reduction of compound 12 using a known procedure.[42] The spectral data matched those reported in the literature.[42]

2-Benzyloxy-1-chloromethyl-3-methoxybenzene (14). Compound **14** was synthesized from alcohol **13** in 89% yield as a pale yellow oil using a known procedure.[43] ^{1}H NMR spectrum (δ, ppm): 3.90 (s, 3H), 4.60 (s, 2H), 5.12 (t, J = 8 Hz, 2H), 6.94 (m, 1H), 6.99 (m, 1H), 7.08 (t, J = 8 Hz, 1H), 7.33–7.42 (m, 3H), 7.48-7.53 (m, 2H).

(2-Benzyloxy-3-methoxybenzyl)triphenylphosphonium chloride (15). Compound **15** was obtained from chloride **14** in 89% yield as a white powder using a previously reported procedure.[43] ^{1}H NMR spectrum (δ, ppm): 3.90 (s, 3H), 4.59 (s, 2H), 4.83 (s, 1H), 5.12 (s, 2H), 6.86 (dd, J_1 = 8, J_2 = 1 Hz, 1H), 6.92 (dd, J_1 = 8, J_2 = 1 Hz, 1H), 7.12 (t, J = 8 Hz, 1H), 7.33-7.42 (m, 3H), 7.48–7.53 (m, 20H).

2,4-Bisbenzyloxy-3,3'-dimethoxystilbene (16). Compound **16** was synthesized from the phosphonium salt **15** and O-benzylvanillin in 18% yield as a white powder using a previously

reported procedure.[43] ^{1}H NMR spectrum (δ, ppm): 3.89 (s, 3H), 3.90 (s, 3H), 5.00 (s, 2H), 5.17 (s, 2H), 6.84 (m, 3H), 6.99 (m, 2H), 7.06 (m, 1H), 7.21−7.51 (m, 10H).

4-[2-(2-Hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol (also known as **2',6-dimethoxy-2,4'-ethylenebisphenol**) **(17).** Compound **17** was synthesized from alkene **16** in 63% yield as colorless needles using a previously reported procedure. ^{1}H NMR spectrum (δ, ppm): 2.89 (m, 4H), 3.86 (s, 3H), 3.87 (s, 3H), 5.60 (br. s, 2H), 6.60−7.10 (m, 6H).

IV.1.2.3 Synthesis of 6,6'-dihydroxy-5,5'-dimethoxy-[1,1'-biphenyl]-3,3'-dicarboxaldehyde (**19**)

6,6'-Dihydroxy-5,5'-dimethoxy-(1,1'-biphenyl)-3,3'-dicarboxaldehyde (19). Compound **19** was synthesized in one step from 4-hydroxy-3-methoxybenzaldehyde (**18**) by using the reported procedure.[44] Yield was 32%. ^{1}H NMR spectra (d_6-DMSO, δ, ppm): 3.93 (s, 3H), 7.43 (s, 2H), 9.81 (s, 1H).

IV.1.2.4 Synthesis of 3,3'-dimethoxy-5,5'-dimethyl-[1,1'-biphenyl]-2,2'-diol (**21**)

3,3'-Dimethoxy-5,5'-dimethyl-(1,1'-biphenyl)-2,2'-diol (also known as **bicreosol**) **(21).** Compound **23** was obtained following a known procedure with minor modifications.[45] Creosol (5.0 g, 36 mmol) was added to a flask with anhydrous sodium acetate (7.7 g, 94 mmol) and water (366 mL). A second solution of $K_3[Fe(CN)_6]$ (30 g, 90 mmol) was prepared in water (208 mL). This solution was slowly added to the creosol containing solution. The mixture was vigorously stirred for 4 h at rt. The solution was cooled and extracted with DCM (2 × 50 mL). The organic layer was collected and washed with water and dried with sodium sulfate. After drying, the solvent was removed under reduced pressure. The solid residue was recrystallized using ethyl ether. The

precipitate that formed was filtered. The filtrate was cooled to 0 °C and filtered again. The solvent from the filtrate was removed under reduced pressure. The solid residue was dissolved in toluene. After addition of pentane, a solid precipitate was formed. The crude product in the precipitate was purified using column chromatography. M.p. 119–121 °C. Yield was 0.63 g (13%). ^{1}H NMR spectrum (δ, ppm): 2.33 (s, 6H), 3.91 (s, 1H), 5.98 (s, 2H), 6.72 (two br. s, 4H).

IV.1.2.5 Synthesis of 1,2-dimethoxy-4-[(2-methoxyphenoxy)methyl]benzene (**24**)

3,4-Dimethoxybenzyl bromide (23). Compound **23** was obtained following a known procedure with minor modifications.[45] 3,4-Dimethoxybenzyl alcohol (5.0 g, 30 mmol), toluene (41 mL), hexane (156 mL), and 48% aqueous solution of HBr (9.1 mL, 81 mmol) were stirred and refluxed for 1 h. The mixture was dried over Na_2SO_4 and concentrated on a rotary evaporator. After cooling in a freezer for 12 h, a white solid formed. The crude product was collected using vacuum filtration and then recrystrallized using diethyl ether. After storing the mixture in a freezer for 12 hrs, white crystals appeared. These crystals were filtered, rinsed with pentane, and dissolved in warm diethyl ether. Hexane was added to get the product as a white solid. Yield of compound **23** was 0.47 g or 18%. ^{1}H NMR spectrum (δ, ppm): 3.89 and 3.91 (two s, 6H) 4.51 (s, 2H), 6.82 (d, J = 8 Hz, 1H), 6.92 (d, J = 2 Hz, 1H), 6.96 (dd, J_1 = 8, J_2 = 2 Hz, 1H).

1,2-Dimethoxy-4-[(2-methoxyphenoxy)methyl]benzene (24). The compound was obtained using a known procedure described for a closely related analog.[41] A solution of compound **23** (0.70 g, 3.0 mmol) in toluene (5.1 mL) was placed in a three-neck round-bottom flask with a stirring bar, a dropping funnel and a condenser. A solution of sodium 2-methoxyphenolate (0.79 g, 5.3 mmol) in toluene (4 mL) was slowly added to the flask. (The latter

compound was obtained by slow mixing equimolar amounts of guaiacol and NaOH in EtOH followed by solvent removal in vacuum.) The mixture was refluxed for 2 h. After cooling, the mixture was filtered to remove NaBr. The filtrate was washed with a dilute aqueous solution of NaOH followed by washing with distilled water. The organic layer was dried over sodium sulfate. After drying, the solution was filtered, and the solvent was removed under reduced pressure. The solid was recrystallized using diethyl ether and hexane. The crude product was further purified by preparative TLC using a 2:1 mixture of pentane and diethyl ether as the eluent. The compound was isolated in 14% yield as white crystals. M.p. 64−65 °C. ^{1}H NMR spectrum (δ, ppm): 3.88 and 3.89 (two s, 3H and 6H, respectively), 5.09 (s, 2H), 6.82−7.02 (m, 7H).

IV.1.2.6 Synthesis of (E)-4,4'-(ethene-1,2-diyl)bis(2-methoxyphenol) (**26**)

(*E*)-4,4'-(ethene-1,2-diyl)bis(2-methoxyphenol) (**26**). The compound was obtained using the McMurry coupling following the procedure described previously.[46] The yield was 3% (due to high instability of the compound and multiple attempts to purify it). ^{1}H NMR spectrum (DMSO-d_6, δ, ppm): 3.83 (s, 3H), 6.75 (d, J = 8 Hz, 1H), 6.93 (dd, J_1 = 8, J_2 = 2 Hz, 1H), 6.95 (s, 1H), 7.14 (d, J = 2 Hz, 1H), 9.08 (s, 1H).

IV.2 Synthesis of Diarene Lignin Degradation Product Standards

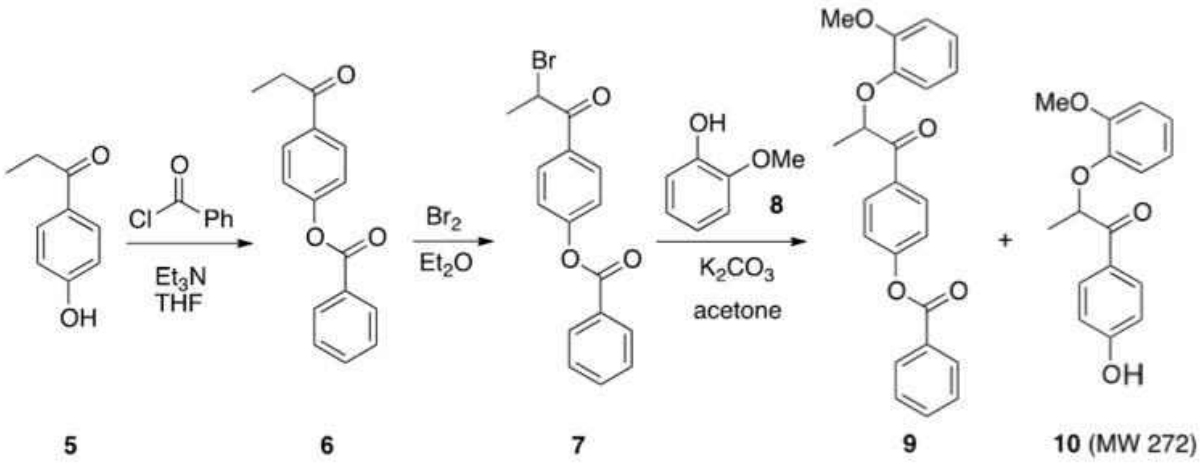
In order to fully quantify lignin degradation samples fully using various methods, the final products must be known. Members of our group found that mass spectra of alkali lignin samples after hydrodegradation contain signals corresponding to unknown products with molecular

masses of 272, 274, and 302. Several compounds with these molecular masses have been hypothesized as possible degradation products based on reported lignin structures and after eliminating a number of compounds with known mass spectra and LC retention times that did not match experimental data. In the present work, we selected six compounds with molecular masses 272, 274, and 302 for synthesis in order to match their mass spectra, GC, and LC retention times with those obtained for unknown degradation products with the same molecular mass. Those five compounds are 1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)-1-propanone (**10**), 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol (**17**), 6,6'-dihydroxy-5,5'-dimethoxy-(1,1'-biphenyl)-3,3'-dicarboxaldehyde (**19**), 3,3'-dimethoxy-5,5'-dimethyl-(1,1'-biphenyl)-2,2'-diol (**21**), 1,2-dimethoxy-4-[(2-methoxyphenoxy)methyl]benzene (**24**), and 4,4'-(ethene-1,2-diyl)bis(2-methoxyphenol) (**26**).

IV.2.1 Preparation of 1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)-1-propanone, **10**

Synthesis of the previously reported 1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)-1-propanone, **10**, was accomplished in three steps (Scheme 3).[39-41] The starting organic compound was 1-(4-hydroxyphenyl)-1-propanone (**5**). It was treated with benzoyl chloride to produce 4-propionylphenyl benzoate (**6**). This compound was brominated using Br_2 to give 4-(2-bromopropionyl)phenyl benzoate (**7**).[40] The bromide reacted with guaiacol (**8**) in the presence of potassium carbonate to furnish a mixture of 4-[2-(2-methoxyphenoxy)propanoyl]phenyl benzoate (**9**) and the desired final product, 1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)-1-propanone, **10**.[41] Ester **9** was partially converted to phenol **10** during washing the reaction mixture with a 2M solution of NaOH. The latter compound was isolated from the mixture of **9**

and **10** using preparative TLC, followed by recrystallization. The structure of this target product and its precursors **6**, **7**, and **9** were confirmed by ^{1}H NMR spectroscopy. The molecular masses of compounds **9** and **10** were confirmed using TOF-MS in positive mode.



Scheme 3. Synthesis of 1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)-1-propanone, **10**.

After purification, compound **10** was analyzed using GC-MS in comparison with thermally degraded lignin, sample KF-15-5. While the extracted ion 272 was not present in the chromatogram, upon derivatization using *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) a molecular ion peak was found with the appropriate mass-to-charge ratio, at 344. The signal after extracting ion 344 had a retention time of 11.04 minutes. The mass spectrum of the signal also verifies that the signal in question belongs to compound **10**, as the fragmentation pattern shows a signal at *m/z* 222 that is caused by the cleavage of the sp^3 C-O bond. A peak at *m/z* 193 is likely due to the cleavage of a C-C bond in the aliphatic chain adjacent to the carbonyl group. The chromatogram and mass spectrum can be seen in Figure 6.

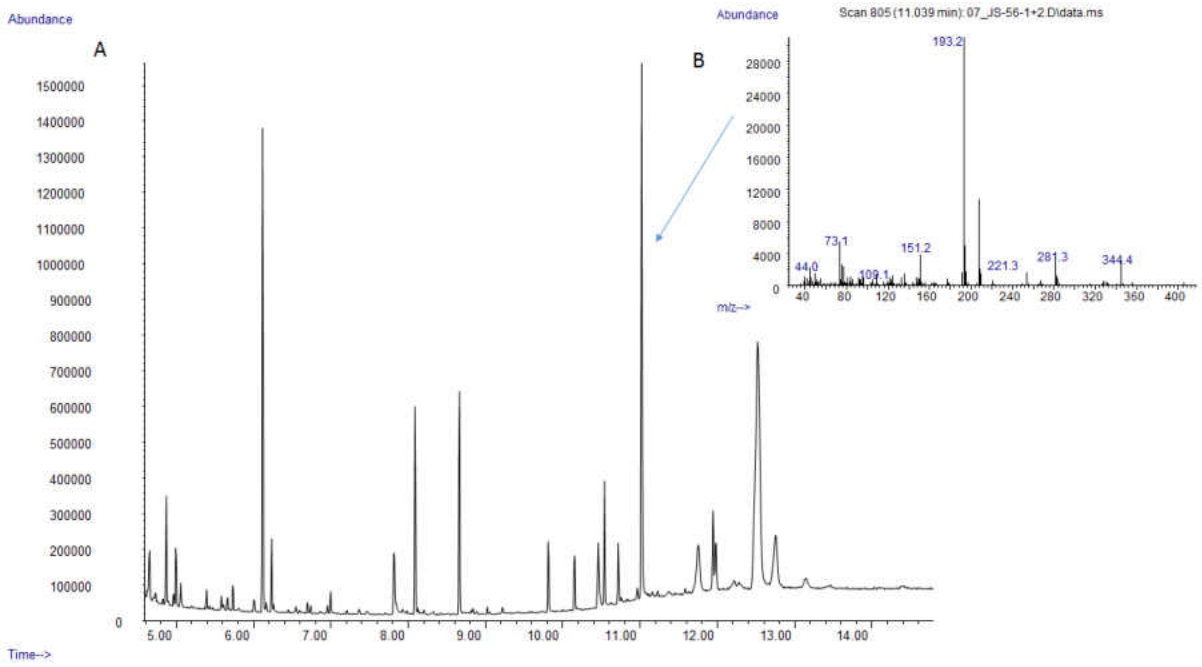
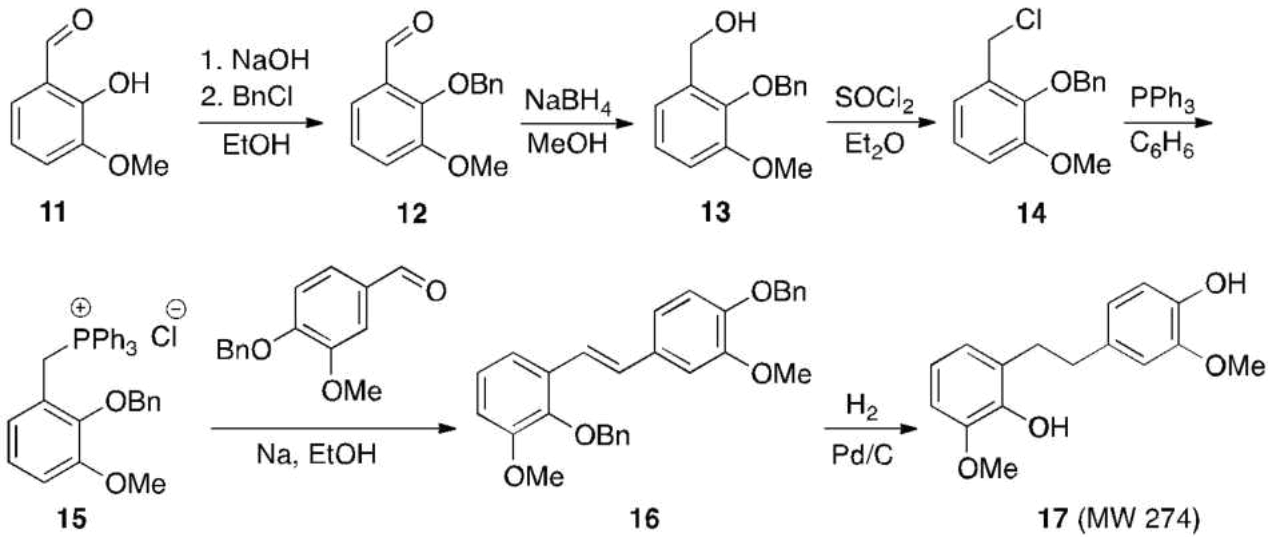


Figure 7. (A) GC-MS chromatogram of the derivatized 1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)-1-propanone, **10**, and (B) electron ionization mass spectrum of the compound eluting at retention time 11.039 minutes.

The lack of signals in non-derivatized samples suggests that compound **10** is not present in the chromatograms of the thermally degraded lignin. Thermally degraded lignin was derivatized using N, O-bistrifluoroacetamide and run in the same sequence, with the same method as derivatized compound **10**. When ion 344 was extracted, the chromatogram contained multiple signals with a variety of retention times. Several signals were near but not at a retention time of 11.04; however, the molecular fragments were different from the derivatized compound **10**. The different retention time and different molecular fragments both suggest that compound **10** is not present in thermally degraded lignin derived from alkali lignin. Indulin lignin was not thermally degraded to test.

IV.2.2 Preparation of 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol, **17**

Synthesis of the previously reported 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol,[43, 47] **17**, was accomplished in six steps (Scheme 4). The starting organic compound was 2-hydroxy-3-methoxybenzaldehyde (**11**). It reacted with sodium hydroxide followed by treatment with benzyl chloride to produce the ether 2-benzyloxy-3-methoxybenzaldehyde (**12**).[47] This compound was then reduced using $NaBH_4$ to afford alcohol **13**.[47] The compound was reacted with thionyl chloride to give the corresponding benzyl chloride, **14**.[43] This was refluxed with triphenylphosphine to produce the phosphonium salt (**15**).[43] The Wittig reaction of this salt with O-benzylvanillin in the presence of Na in ethanol afforded the rather unstable alkene **16**.[43] This compound was reduced over Pd/C with H_2 to give the final product 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol, **17**.[43] The compound was obtained in a total yield of 23% in 5 steps. The structure of the substituted phenol **17** and its precursors **11**–**16** were confirmed by ^1H NMR spectroscopy. The molecular mass of the final product was confirmed by TOF-MS in positive mode.



Scheme 4. Synthesis of 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol, **17**.

Compound **17** was analyzed GC-MS and the retention time of the extracted ion 274 was compared to thermally degraded lignin. The chromatogram can be seen in Figure 7. The extracted ion 274 can be seen at 11.2 minutes, which does not match signals from thermally degraded lignin. However, since only alkali lignin was tested, and others such as indolin or organosolv lignins remain untested, we cannot conclude that 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol is not present in lignin.

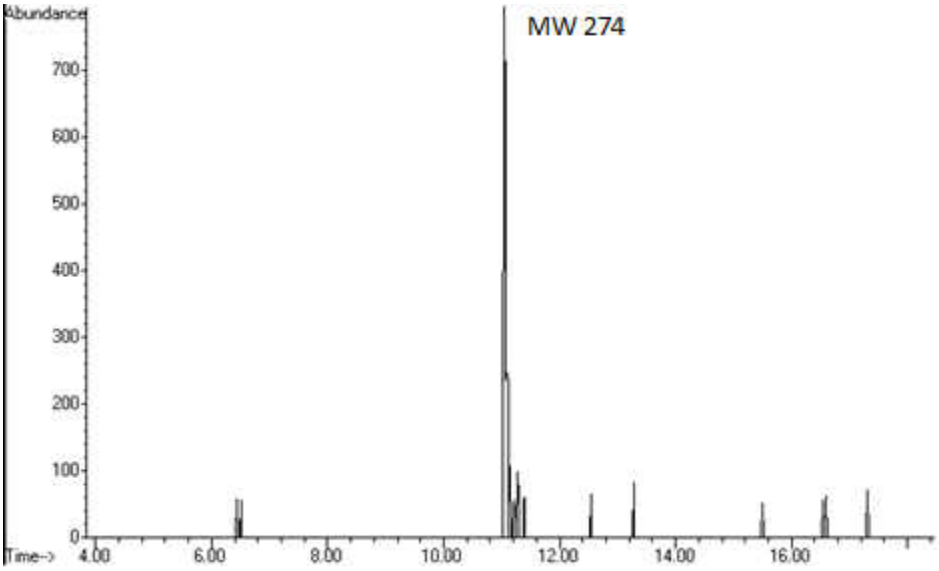
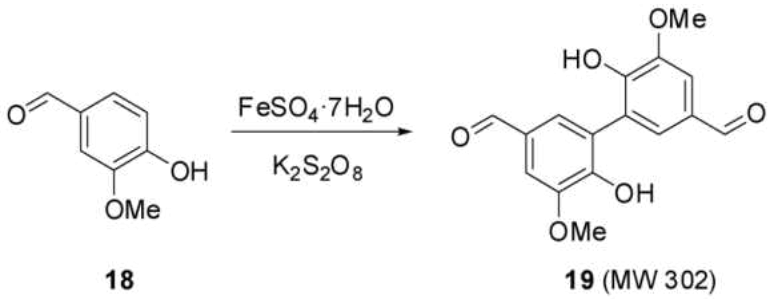


Figure 8. GC-MS extracted ion chromatogram for *m/z* of 274 of a 100 ppm 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol sample, run in splitless mode with 1 μL injection and an injection port at 250 °C.

IV.2.3 Preparation of 6,6'-dihydroxy-5,5'-dimethoxy-(1,1'-biphenyl)-3,3'-dicarboxaldehyde, **19**

Synthesis of the previously reported 6,6'-dihydroxy-5,5'-dimethoxy-(1,1'-biphenyl)-3,3'-dicarboxaldehyde, **19**, was accomplished in a single step (Scheme 5).[41] The starting organic compound was 4-hydroxy-3-methoxy-benzaldehyde (**18**). Treatment of compound **18** with iron(II) sulfate hydrate and potassium persulfate provided the desired product **19** in 32% yield. The structure of this compound was supported by ^{1}H NMR spectroscopy. The molecular mass of compound **19** was confirmed using TOF-MS in negative mode with a mass accuracy of 10 ppm error.

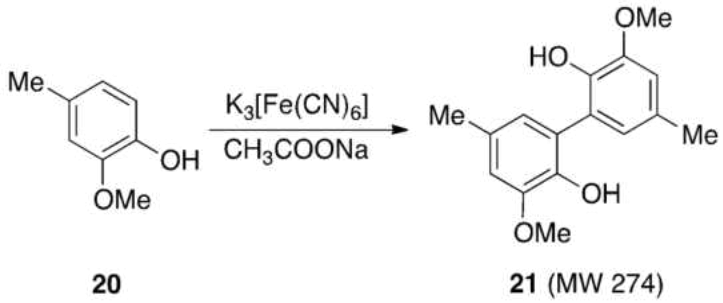


18 **19** (MW 302)

Scheme 5. Synthesis of 6,6'-dihydroxy-5,5'-dimethoxy-(1,1'-biphenyl)-3,3'-dicarboxaldehyde **19**.

IV.2.4 Preparation of 3,3'-dimethoxy-5,5'-dimethyl-(1,1'-biphenyl])-2,2'-diol, **21**

Synthesis of the previously reported 3,3'-dimethoxy-5,5'-dimethyl-[1,1'-biphenyl]-2,2'-diol, **21**, was accomplished in a single step (Scheme 6).[41] The starting organic compound was 2-methoxy-4-methylphenol (**20**). Treatment of phenol **20** $K_2[Fe(CN)_6]$ in the presence of NaOAc in water provided compound **21** in 5% yield. The structure of this phenol was confirmed by ^{1}H NMR spectroscopy. The molecular mass and the dimeric structure of compound **21** were confirmed using TOF-MS in negative mode with a mass accuracy of 18 ppm error.

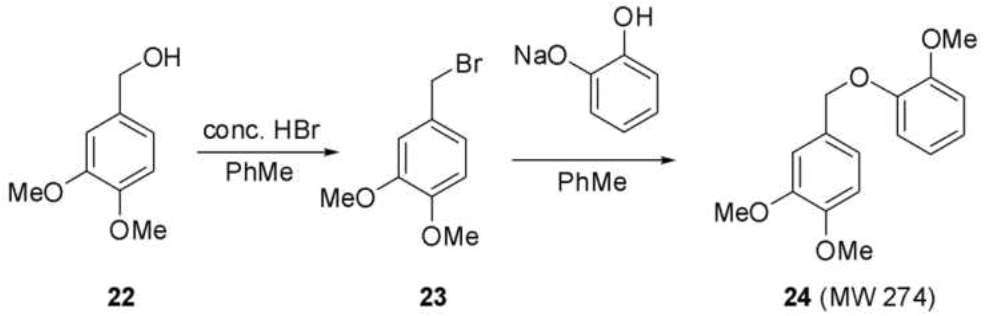


20 **21** (MW 274)

Scheme 6. Synthesis of 3,3'-dimethoxy-5,5'-dimethyl-[1,1'-biphenyl]-2,2'-diol, **21**.

IV.2.5 Preparation of 1,2-dimethoxy-4-[(2-methoxyphenoxy)methyl]benzene, **24**

Synthesis of the previously reported 1,2-dimethoxy-4-[(2-methoxyphenoxy) methyl]benzene, **24**, was accomplished in two steps (Scheme 7).[41] The starting organic compound was 3,4-dimethoxybenzyl alcohol (**22**). Treatment of the alcohol with concentrated hydrobromic acid produced bromide **23** in 4% yield. Compound **23** reacted with sodium 2-methoxyphenolate in toluene affording the desired product **24** in 14% yield. The structure of **24** was confirmed by ^{1}H NMR spectroscopy. The molecular mass of compound **24** was confirmed by TOF-MS in positive mode with a mass accuracy of 5 ppm error.

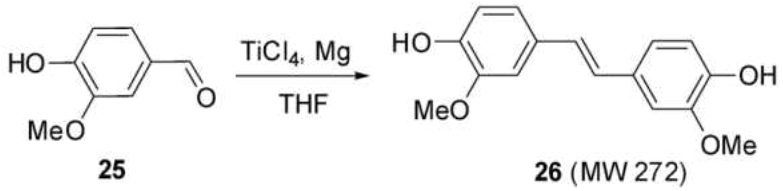


22 **23** **24** (MW 274)

Scheme 7. Synthesis of 1,2-dimethoxy-4-[(2-methoxyphenoxy)methyl]benzene, **24**.

IV.2.6 Preparation of (E)-4,4’-(ethene-1,2-diyl)bis(2-methoxyphenol), **26**

Synthesis of (E)-4,4’-(ethene-1,2-diyl)bis(2-methoxyphenol), **26**, was accomplished in a single step using a previously reported procedure for the preparation of this alkene via a McMurry coupling (Scheme 8).[46] The starting compound was 4-hydroxy-3-methoxybenzaldehyde (**25**). Compound **25** was treated with Mg and $TiCl_4$ in toluene under an atmosphere of N_2 to produce compound **26** in 3% yield. The structure of phenol **26** was supported by ^1H NMR data. The molecular mass of compound **26** was confirmed by GC-MS and TOF-MS in negative mode. It has to be noted that the compound is quite unstable and rapidly changes its structure and color.



Scheme 8. Synthesis of 4,4’-(ethene-1,2-diyl)bis(2-methoxyphenol), **26**.

Compound **26** was analyzed by GC-MS by contrasting the retention times of the compound with the retention time of the degraded lignin with the extracted ion of 272 *m/z*, which is seen in Figure 8. The peak at retention time 12.6 minutes has the same retention time in both cases, as well as matching LC-MS signals, and mass spectrum, suggesting that 4,4’-(ethene-1,2-diyl)bis(2-methoxyphenol) is present in the thermally degraded alkali lignin.

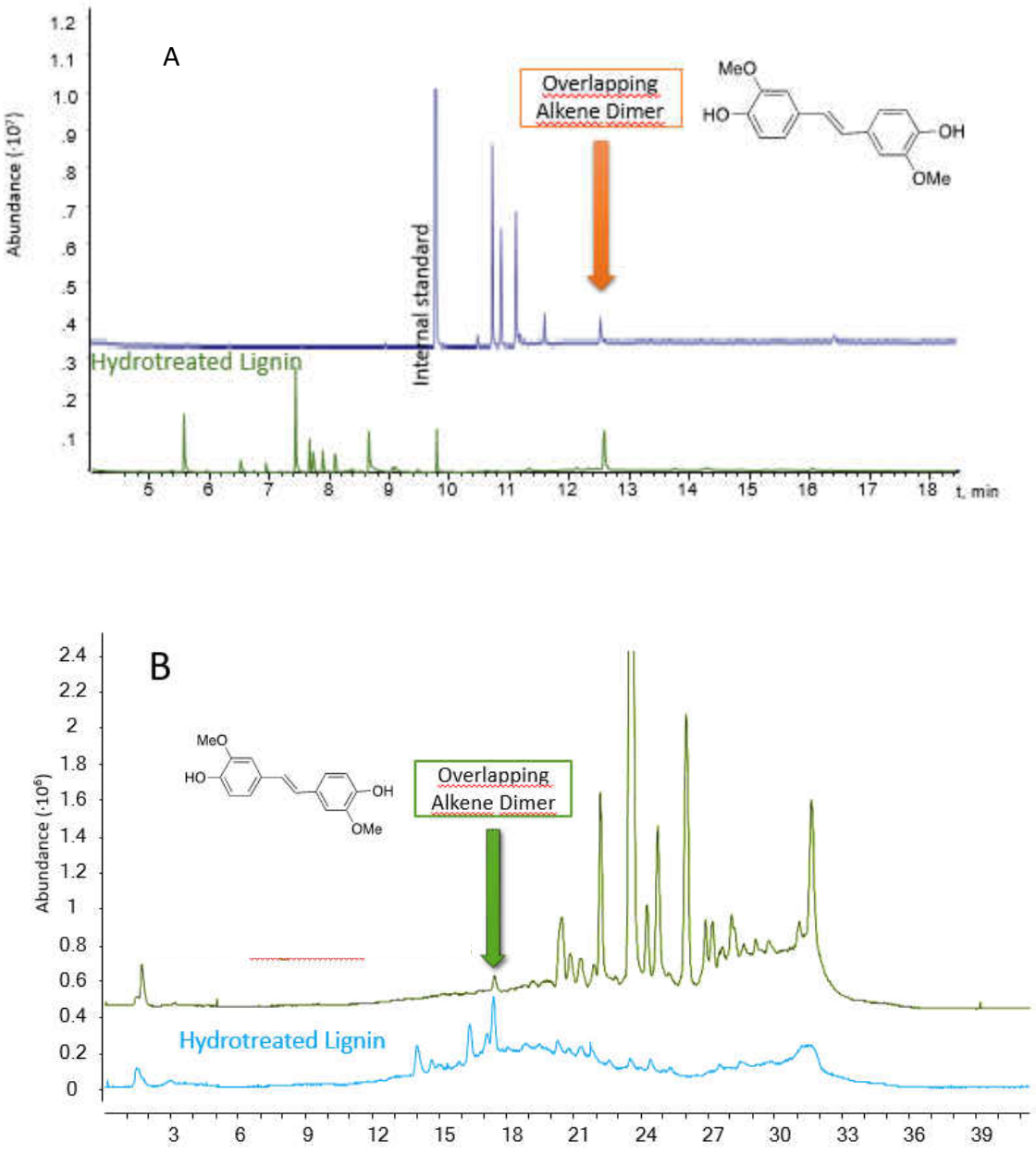


Figure 9. (A) GC-MS chromatogram of (*E*)-4,4'-(ethene-1,2-diyl)bis(2-methoxyphenol) (blue) and hydrotreated lignin (green) and (B) LC-MS chromatogram of **26** (green) and hydrotreated lignin (light blue).

IV.3 Conclusions

We have synthesized and characterized six previously reported but not commercially available six compounds with the *m/z* of 272, 274, and 302 to determine if they are possible products of lignin degradation. After characterization, five of the synthesized compounds (1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)-1-propanone (**10**), 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol (**17**), 6,6'-dihydroxy-5,5'-dimethoxy-(1,1'-biphenyl)-3, 3'-dicarboxaldehyde (**19**), 3,3'-dimethoxy-5,5'-dimethyl-(1,1'-biphenyl)-2,2'-diol (**21**), and 1,2-dimethoxy-4-[(2-methoxyphenoxy)methyl]benzene) (**24**) were found to not be products of alkali thermal degradation. The sixth product, 4,4'-(ethene-1,2-diyl)bis(2-methoxyphenol), had a matching retention time and mass spectrum. These results strongly suggest that 4,4'-(ethene-1,2-diyl)bis(2-methoxyphenol) is one of the degradation products found in alkali lignin after its thermal decomposition. While the other five compounds synthesized in the study may not be present in the thermal degradation of alkali lignin, they still may be present in other forms of degraded lignin such as Indulin, Kraft, or organosolv lignin, each of which has variations in the amount of bio-oil, GC-elutable fractions, solid residue, and gas phases.

APPENDICES

Appendix A

$^{31}P\{^{1}H\}$ NMR spectrum showing validation of the spectroscopic method with alkali and Indulin lignin (5 mg).

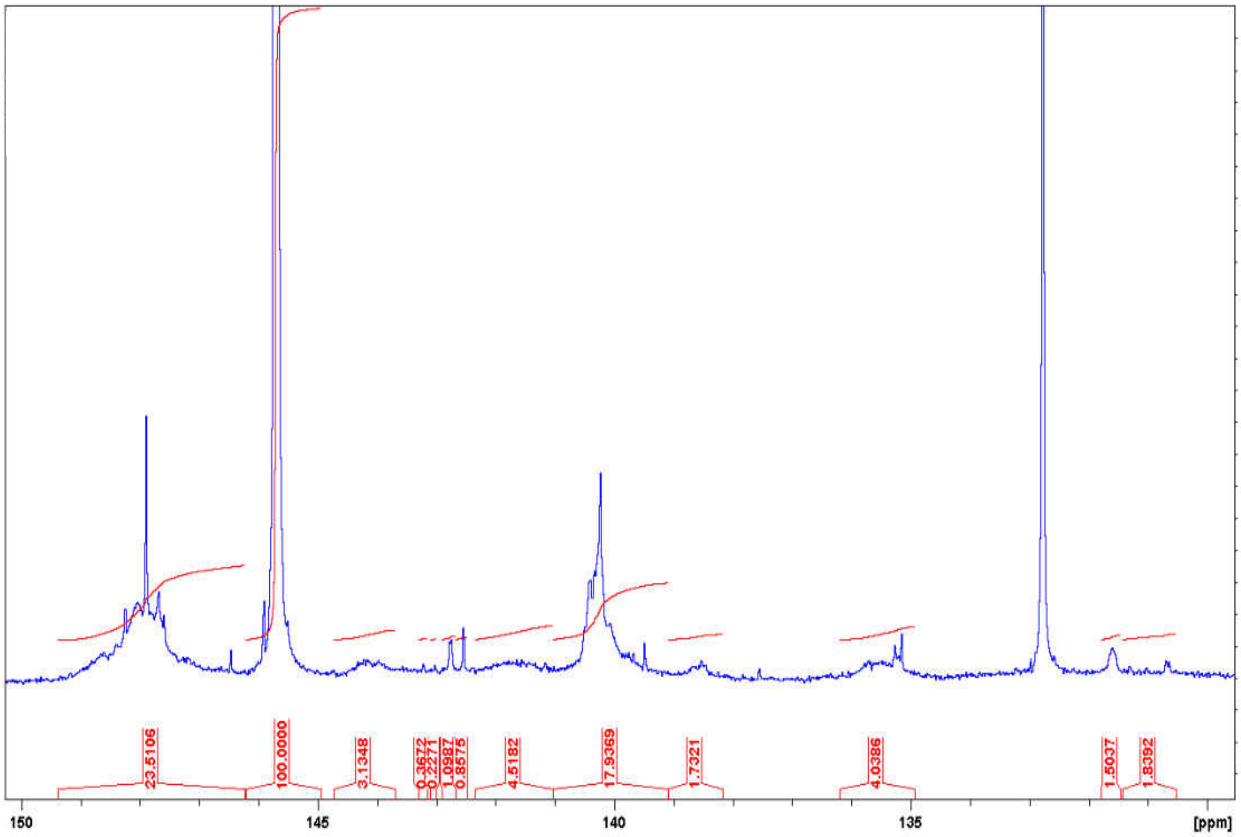


Figure 10. ^{31}P NMR spectrum of 5 mg of alkali lignin. Integration is relative to cyclohexanol.

Table 5. Repeatability of alkali lignin characterization using ^{31}P NMR spectroscopy. The table shows the number of mmoles of lignin hydroxyl groups per gram of lignin found by the ^{31}P NMR spectra in relation to cyclohexanol. Expected mmoles is the number of extrapolated mmoles reported by Meadwest Vaco.

	mmoles			
	Alcohols	Phenols	Acids	Total
alkali lignin 1	2.4	2.9	0.8	6.1
alkali lignin 2	2.7	2.8	0.8	6.3
alkali lignin 3	2.8	2.8	0.8	6.4
mean	**2.7**	**2.85**	**0.78**	**6.3**
SD	0.2	0.04	0.01	0.2
RSD	8	1	2	3
expected mmoles	2.9	3.6	0	6.5

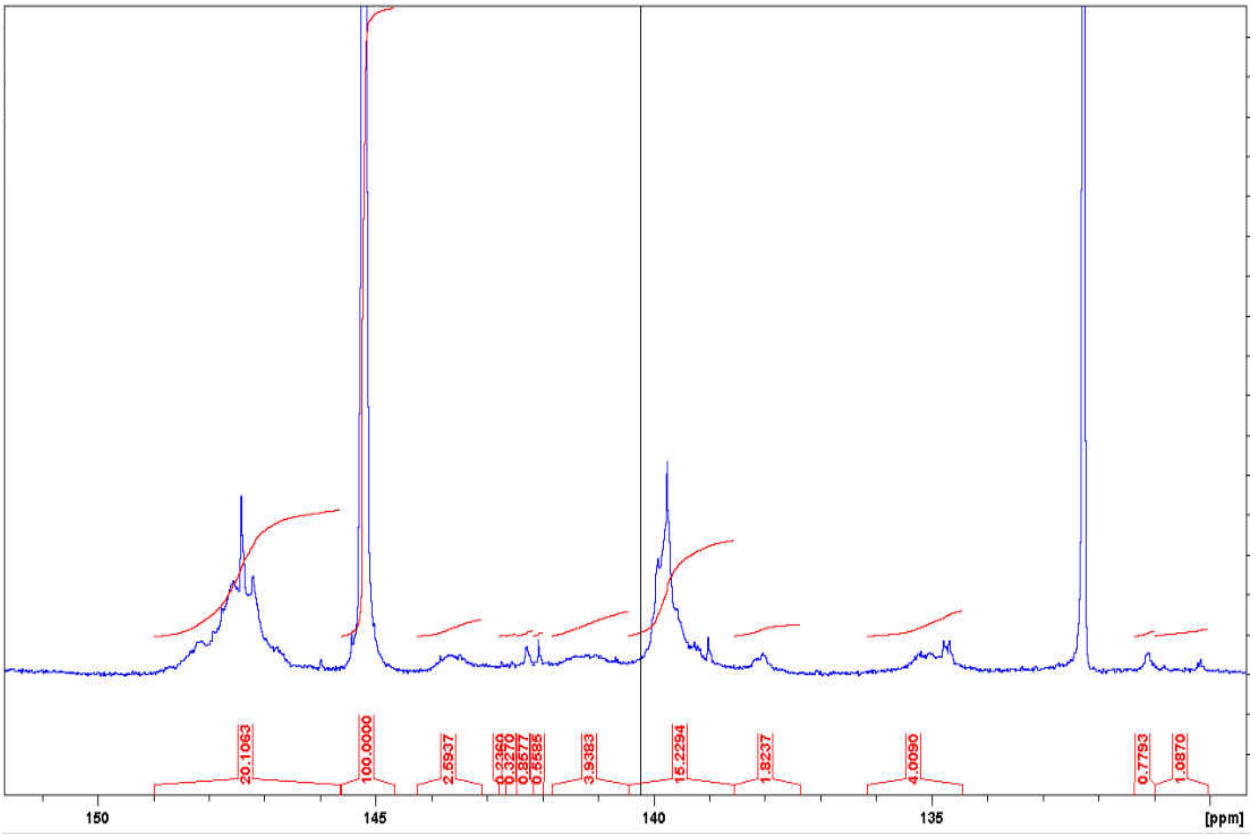


Figure 11. ^{31}P NMR spectrum of 5 mg of Indulin lignin. Integration is relative to cyclohexanol.

Table 6. Repeatability of Indulin lignin characterization using ^{31}P NMR spectroscopy. The table shows the number of mmoles of lignin hydroxyl groups per gram of lignin found by the ^{31}P NMR spectra in relation to cyclohexanol. Expected mmoles is the number of extrapolated mmoles reported by Meadwest Vaco.

	mmoles			
	Alcohols	Phenols	Acids	Total
Indulin lignin 1	2.6	2.6	0.8	5.9
Indulin lignin 2	2.2	2.2	0.6	5.0
mean	**2.38**	**2.40**	**0.69**	**5.47**
SD	0.25	0.25	0.10	0.61
RSD	11	11	14	11
expected mmoles	2.9	3.6	0	6.5

Appendix B

$^{31}P\{^{1}H\}$ NMR spectrum showing validation of the spectroscopic method with carboxylic acids.

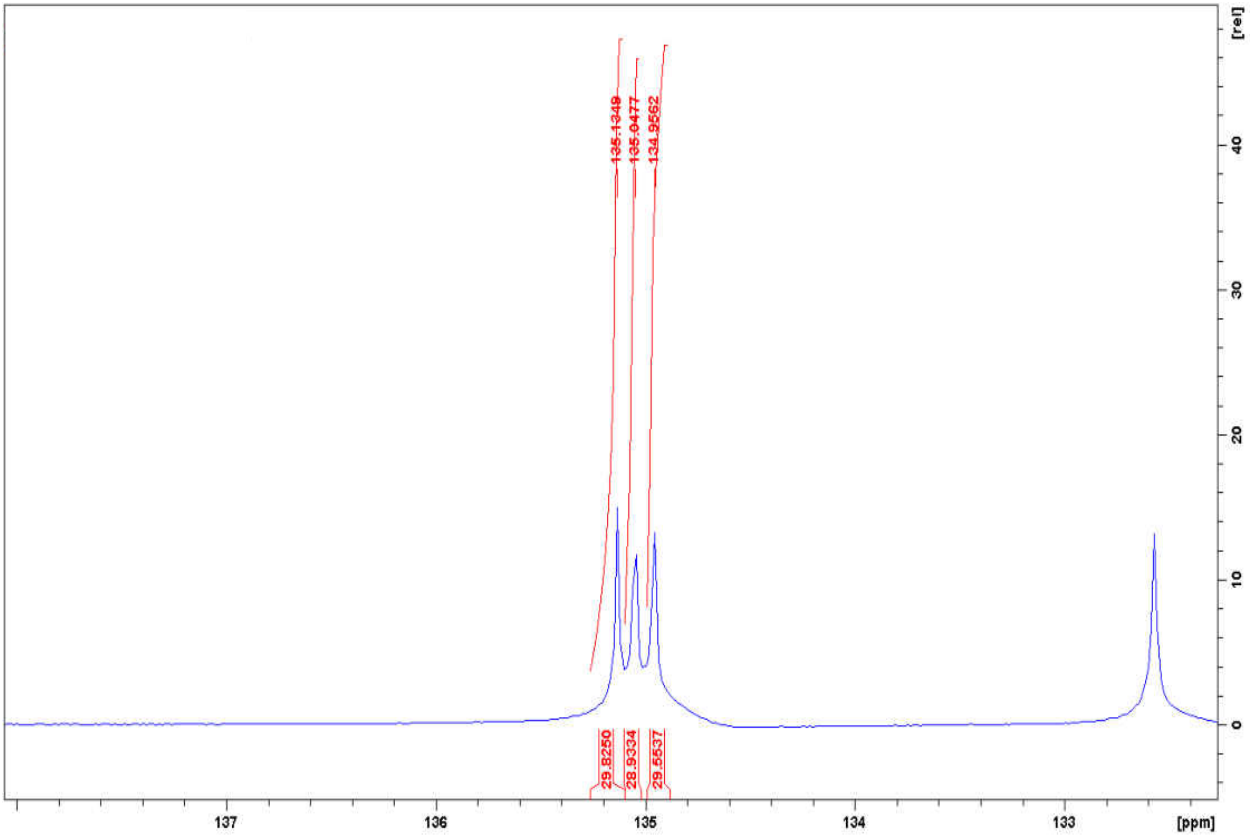


Figure 12. ^{31}P NMR spectrum of 0.34 mmoles of acetic acid, isobutyric acid, and heptanoic acid. Integration is expressed as a ratio to cyclohexanol.

Table 7. Confirmation of quantification of carboxylic acids using ^{31}P NMR spectroscopy with comparison of moles submitted to the analysis and those measure. The table shows the number of moles of hydroxyl groups found by the ^{31}P NMR spectra in relation to cyclohexanol.

	acetic acid	isobutyric acid	heptanoic acid
Run #1	3.7E-04	3.6E-04	3.7E-04
Run #2	3.6E-04	3.5E-04	3.8E-04
Run #3	3.6E-04	3.6E-04	3.7E-04
Actual number of moles	3.5E-04	3.4E-04	3.5E-04
mean	3.6E-04	3.6E-04	3.8E-04
SD	5.7E-06	3.3E-06	8.1E-06
RSD	2	1	2

Appendix C

$^{31}P\{^{1}H\}$ NMR spectrum showing validation of the spectroscopic method with alcohols.

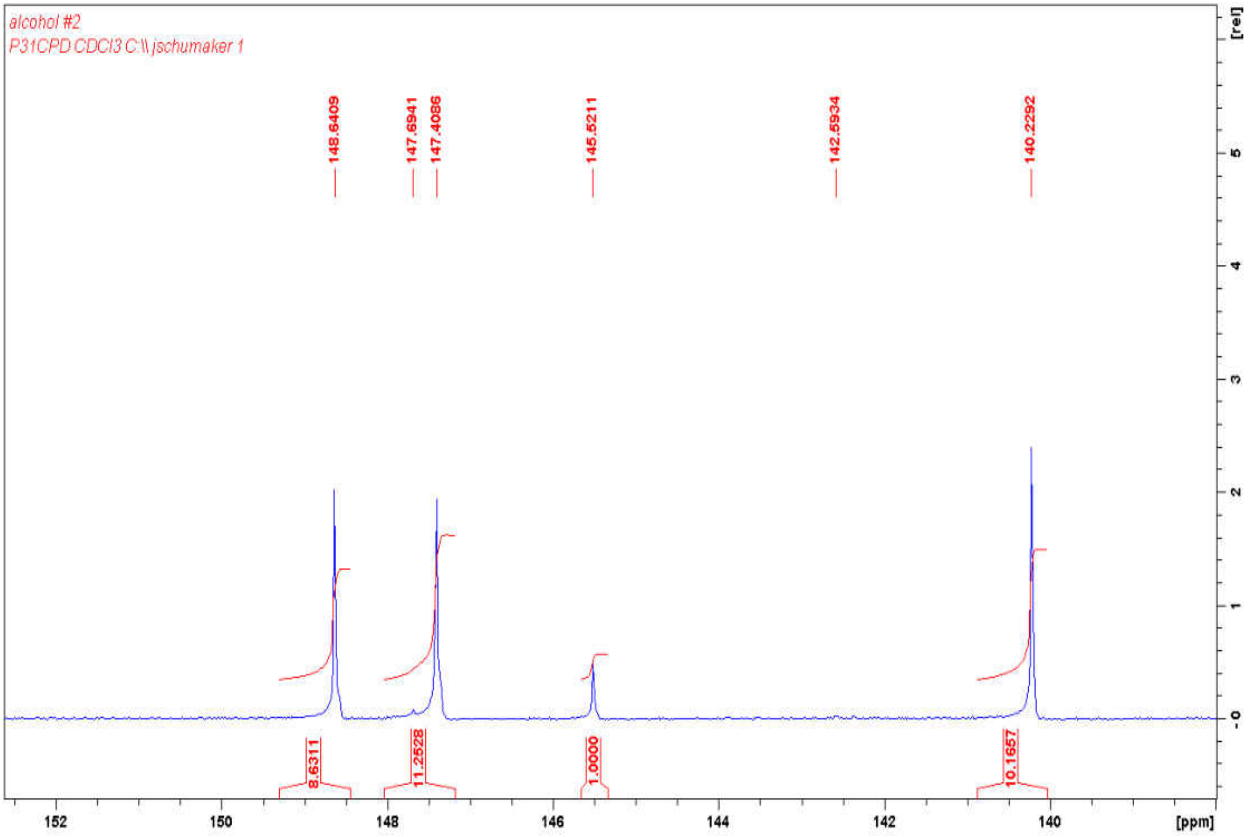


Figure 13. ^{31}P NMR spectrum of 0.13 mmoles of allyl alcohol and homovanillyl alcohol. Integration is expressed as a ratio to cyclohexanol.

Table 8. Confirmation of quantification of alcohols using ^{31}P NMR spectroscopy with comparison of moles submitted to the analysis and those measure. The table shows the number of moles of hydroxyl groups found by the ^{31}P NMR spectra in relation to cyclohexanol.

	allyl alcohol	homovanillyl alcohol	
Run #1	9.3E-05	1.2E-04	1.1E-04
Run #2	1.1E-04	1.2E-04	1.2E-04
Run #3	1.1E-04	1.3E-04	1.4E-04
actual	1.3E-04	1.2E-05	
mean	1.0E-04	1.2E-04	1.3E-04
SD	1.1E-05	6.3E-06	1.1E-05
RSD	10	5	9

Appendix D

$^{31}P\{^{1}H\}$ NMR spectrum showing validation of the spectroscopic method with phenols.

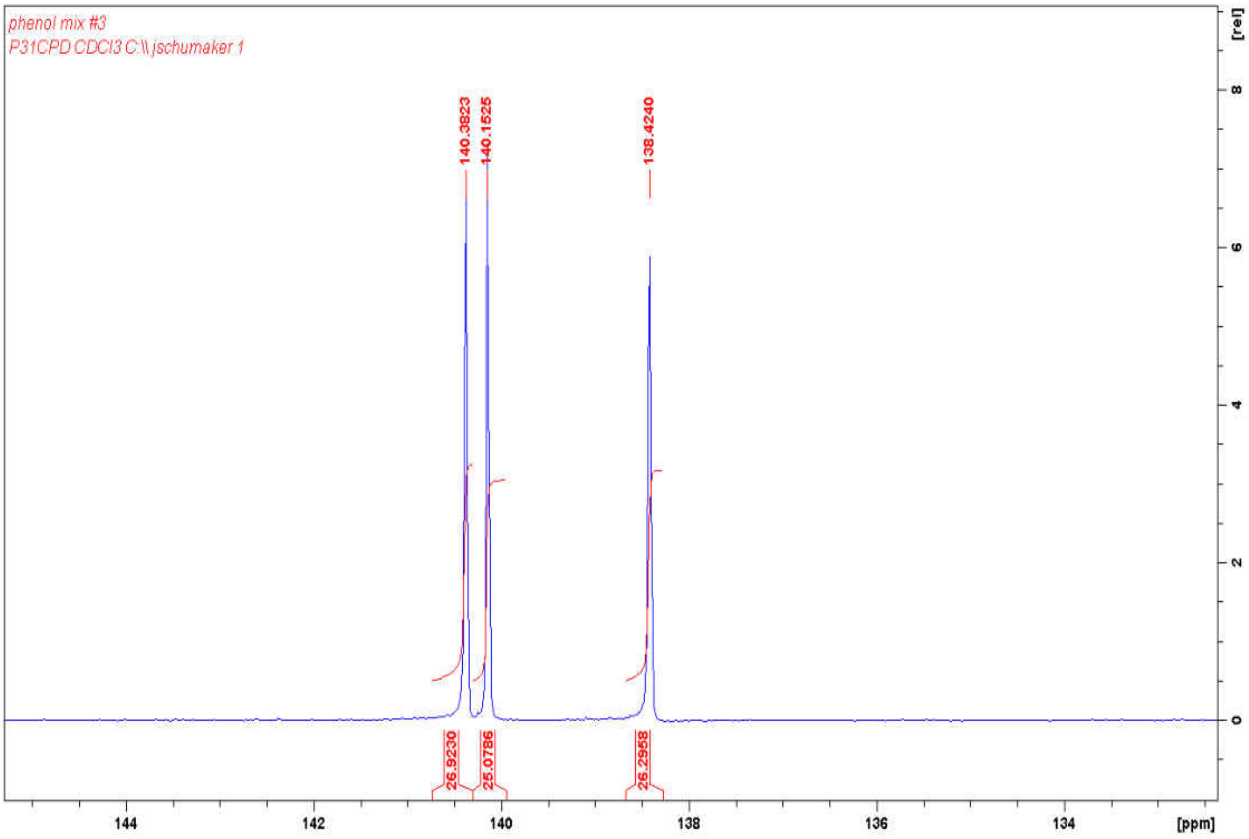


Figure 14. ^{31}P NMR spectrum of 0.32 mmoles of phenol, guaiacol, and methyl guaiacol. Integration is expressed as a ratio to cyclohexanol.

Table 9. Confirmation of quantification of phenols using ^{31}P NMR spectroscopy with comparison of moles submitted to the analysis and those measure. The table shows the number of moles of hydroxyl groups found by the ^{31}P NMR spectra in relation to cyclohexanol.

	phenol	guaiacol	methyl guaiacol
Run #1	3.1E-04	3.3E-04	3.3E-04
Run #2	3.3E-04	3.1E-04	3.3E-04
actual	3.3E-04	3.1E-04	3.2E-04
mean	3.2E-04	3.2E-04	3.3E-04
SD	9.9E-06	1.1E-05	3.4E-06
RSD	3	4	1

Appendix E

Expanded ^{31}P NMR spectra between 130—152 ppm.

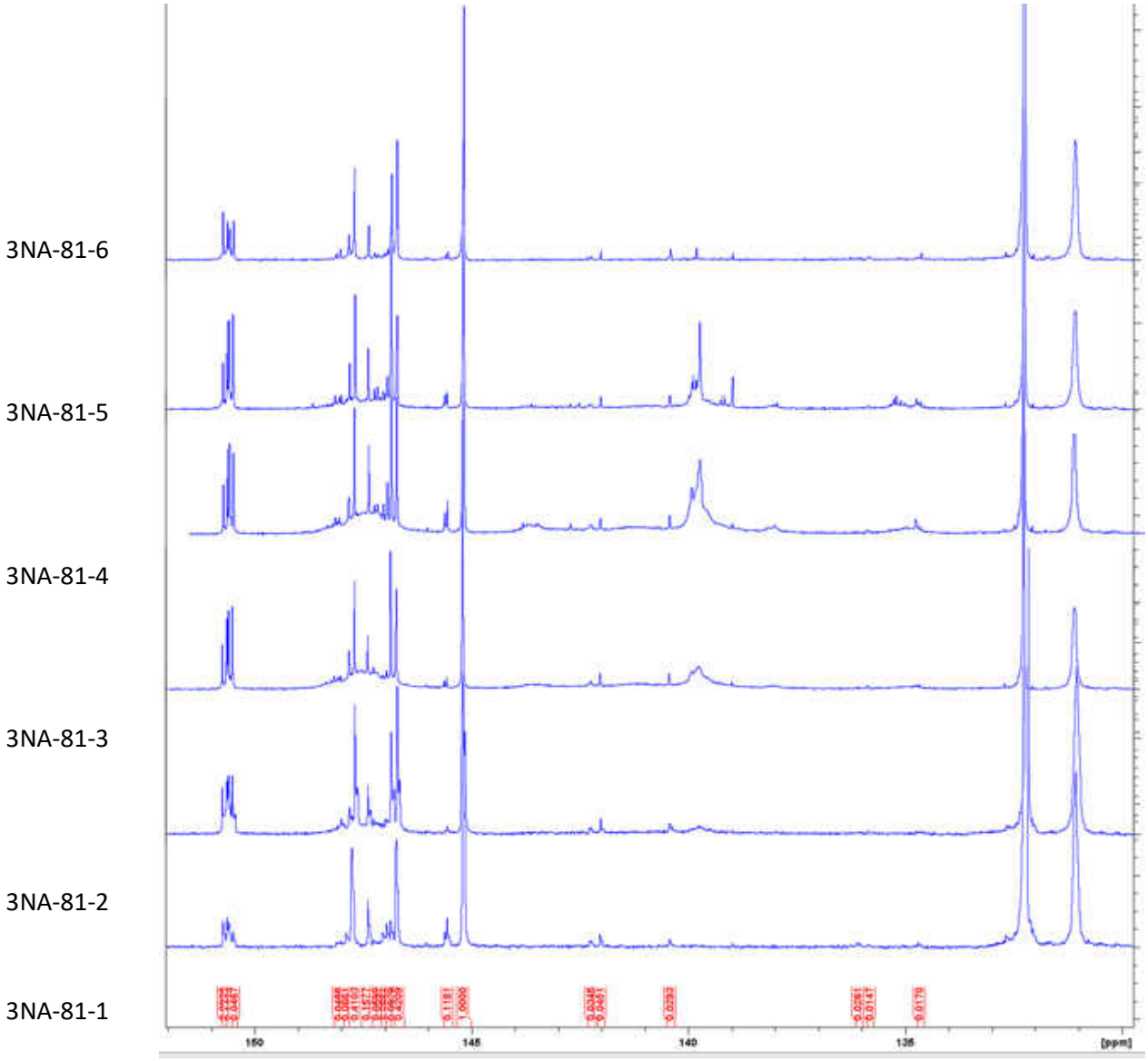


Figure 15. Expanded overlayed ^{31}P NMR spectra of the GPC weight fractionated lignin samples with the top spectrum as the lowest molecular weight sample, and 3NA-81-2 as the heaviest molecular weight fraction. The bottom spectrum is not lignin, but rather a carbohydrate derivative pre-eluate.

Appendix F

Bar graph showing mmoles of hydroxyl groups per analyzed sample.

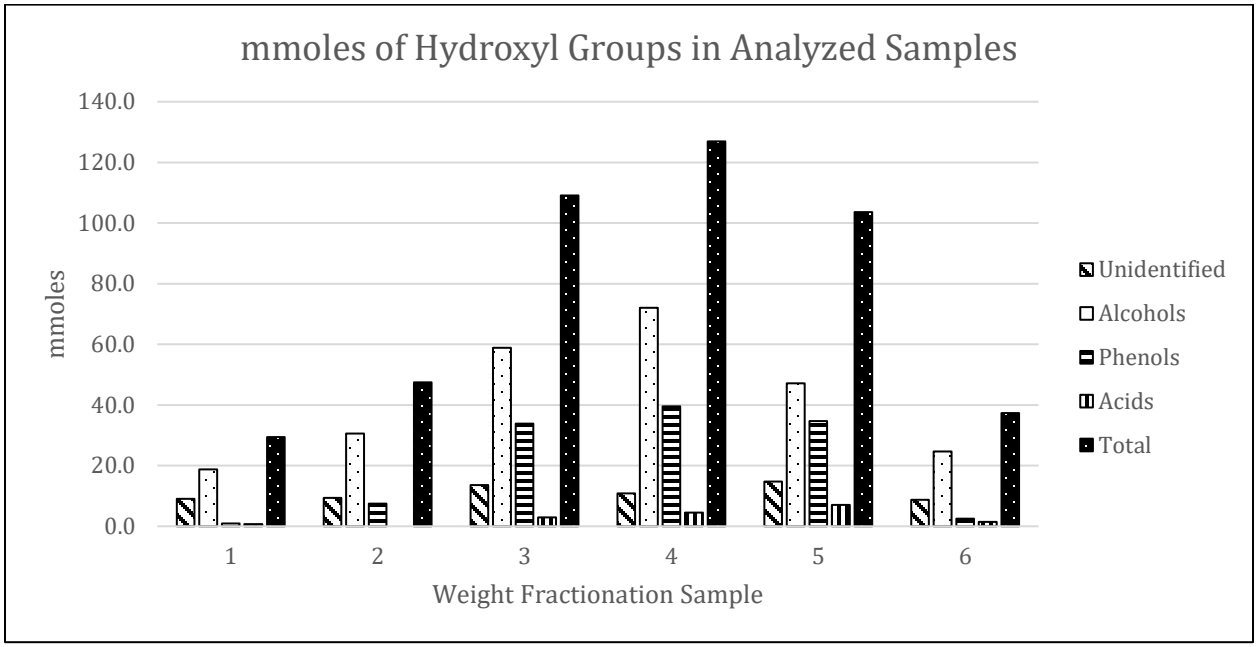


Figure 16. Bar graph showing mmoles of hydroxyl groups separated by weight fractionation sample and type of hydroxyl group.

Appendix G

$^{31}P\{^{1}H\}$ NMR spectroscopy spectra identifying delay times for 90° pulse angle.

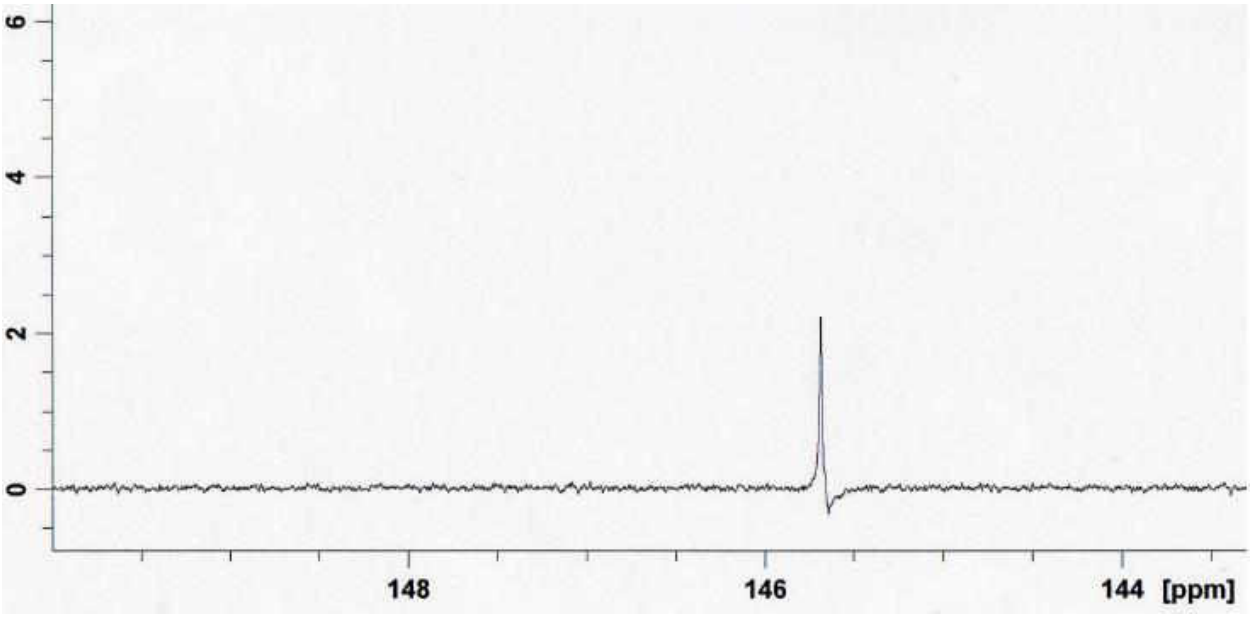


Figure 17. $^{31}P\{^{1}H\}$ NMR spectrum with a P1 of 21 seconds.

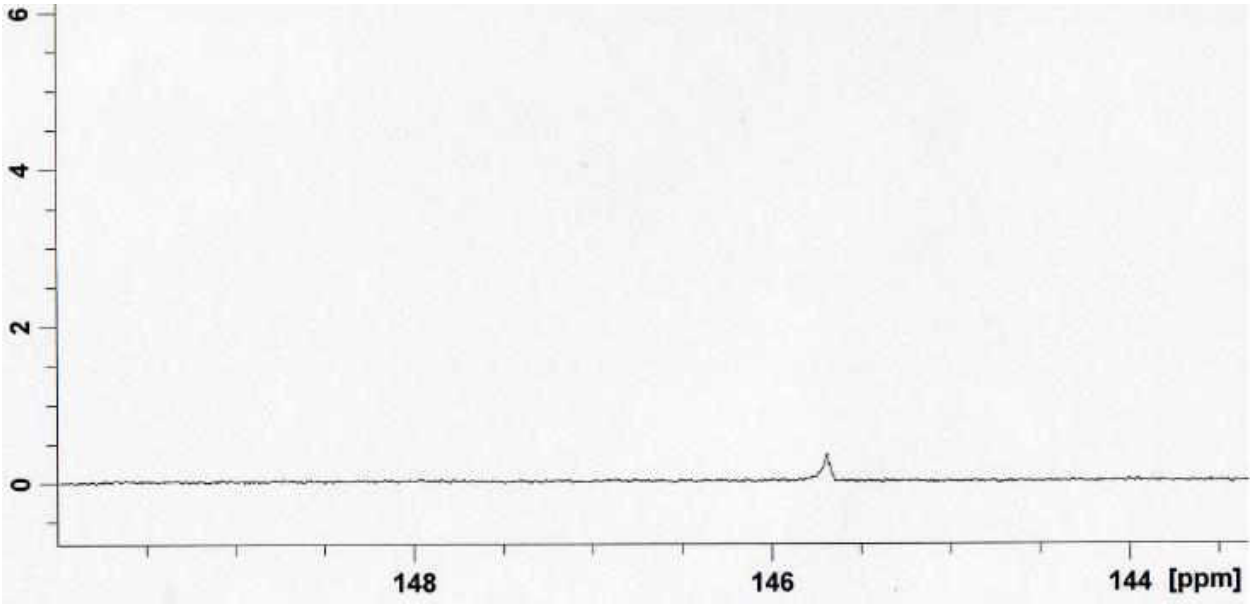


Figure 18. $^{31}P\{^{1}H\}$ NMR spectrum with a P1 of 20 seconds.

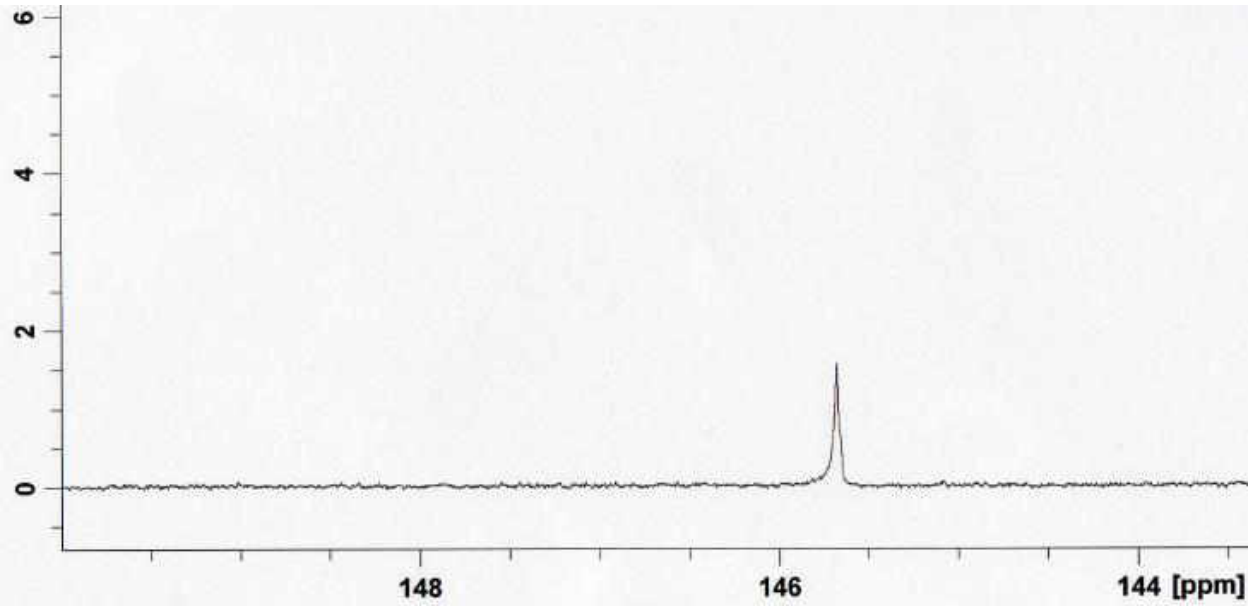


Figure 19. $^{31}P\{^{1}H\}$ NMR spectrum with a P1 of 19 seconds.

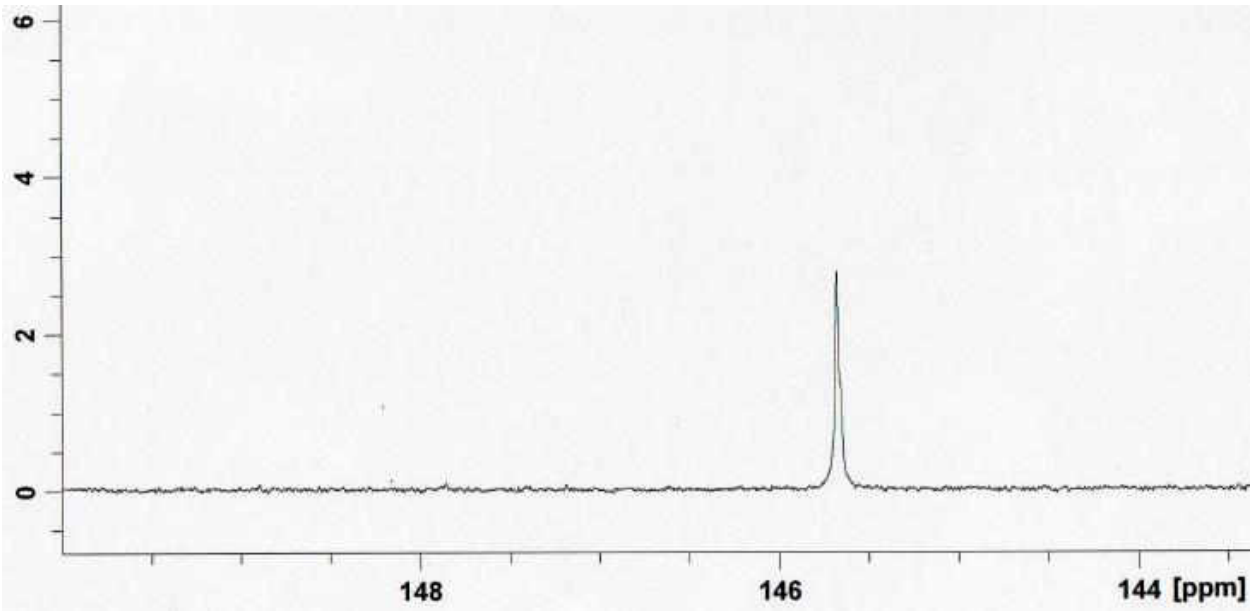


Figure 20. $^{31}P\{^{1}H\}$ NMR spectrum with a P1 of 18 seconds.

Appendix H

^{1}H, $^{13}C\{^{1}H\}$ NMR, and ESI-TOF spectra of 1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)- 1-propanone, **10.**

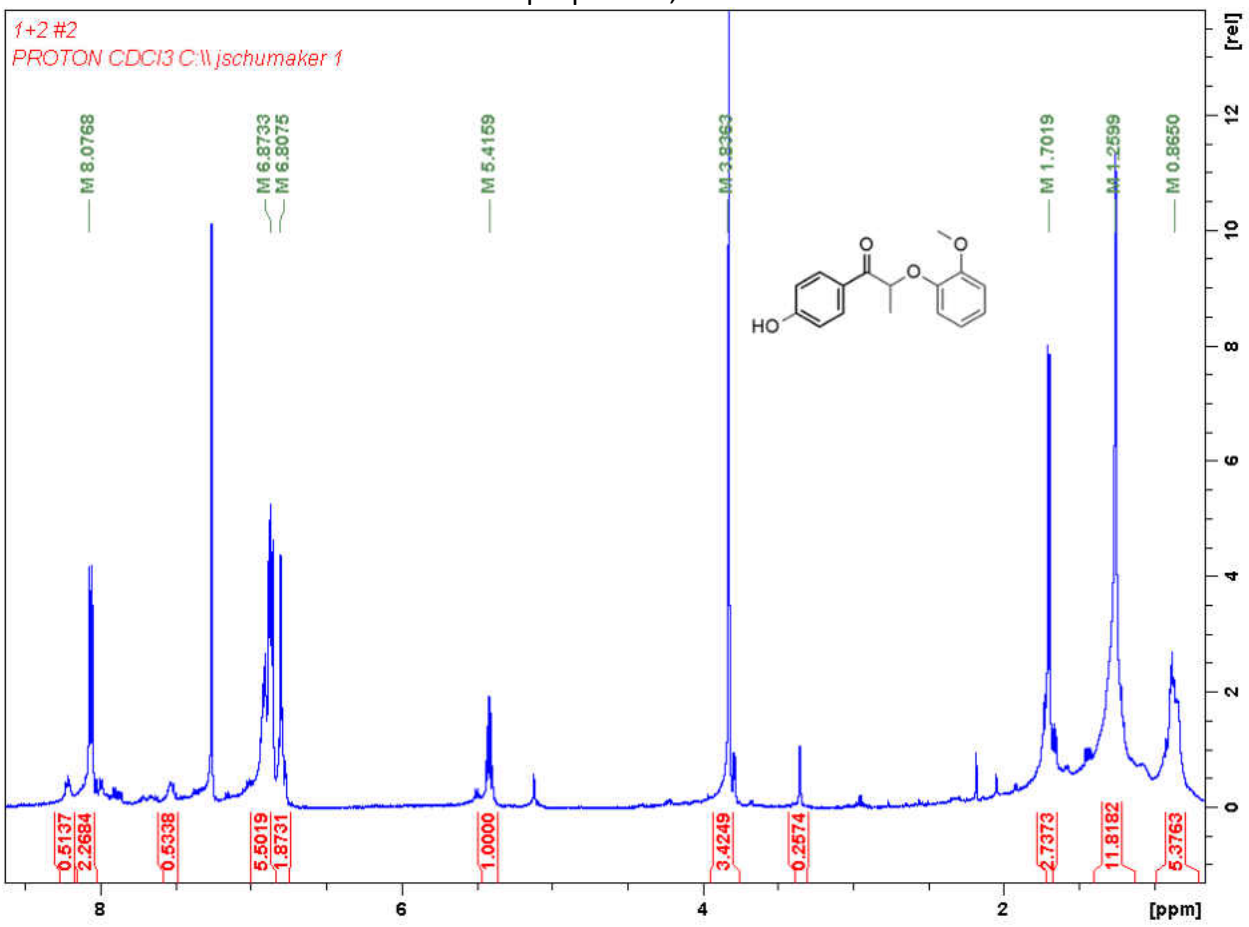


Figure 21. ^{1}H NMR spectrum of 1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)- 1-propanone, **10.**

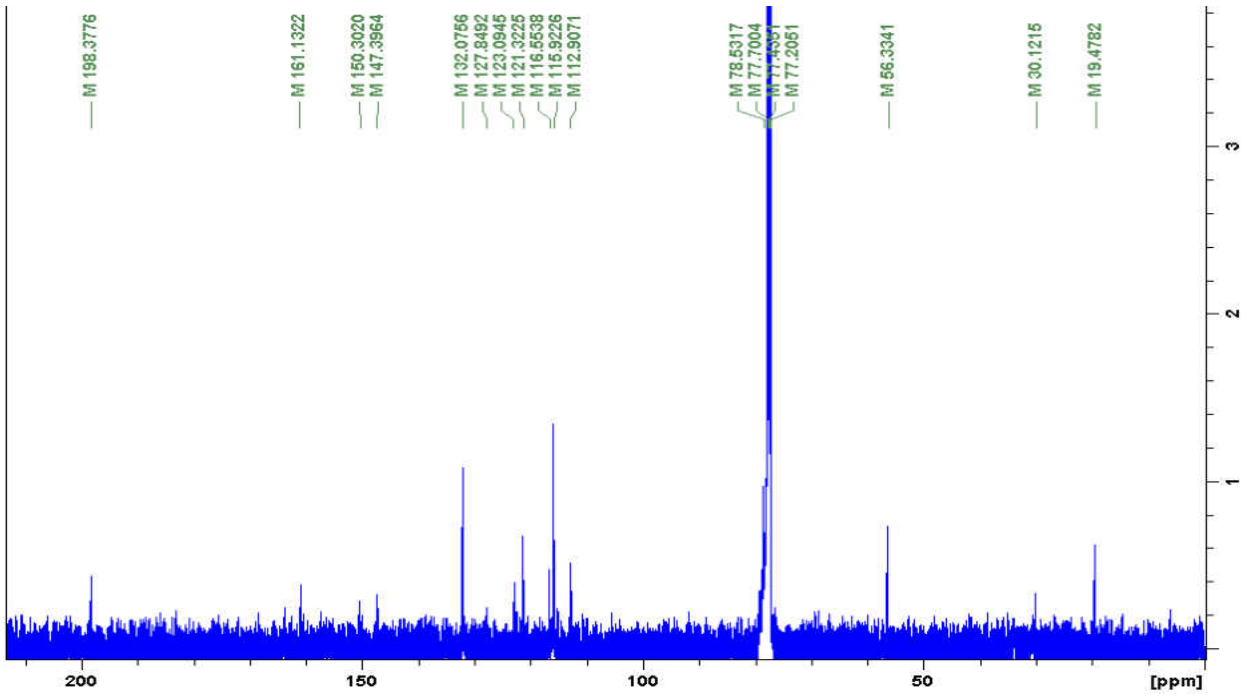


Figure 22. $^{13}C\{^{1}H\}$ NMR spectrum of 1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)- 1-propanone, **10.**

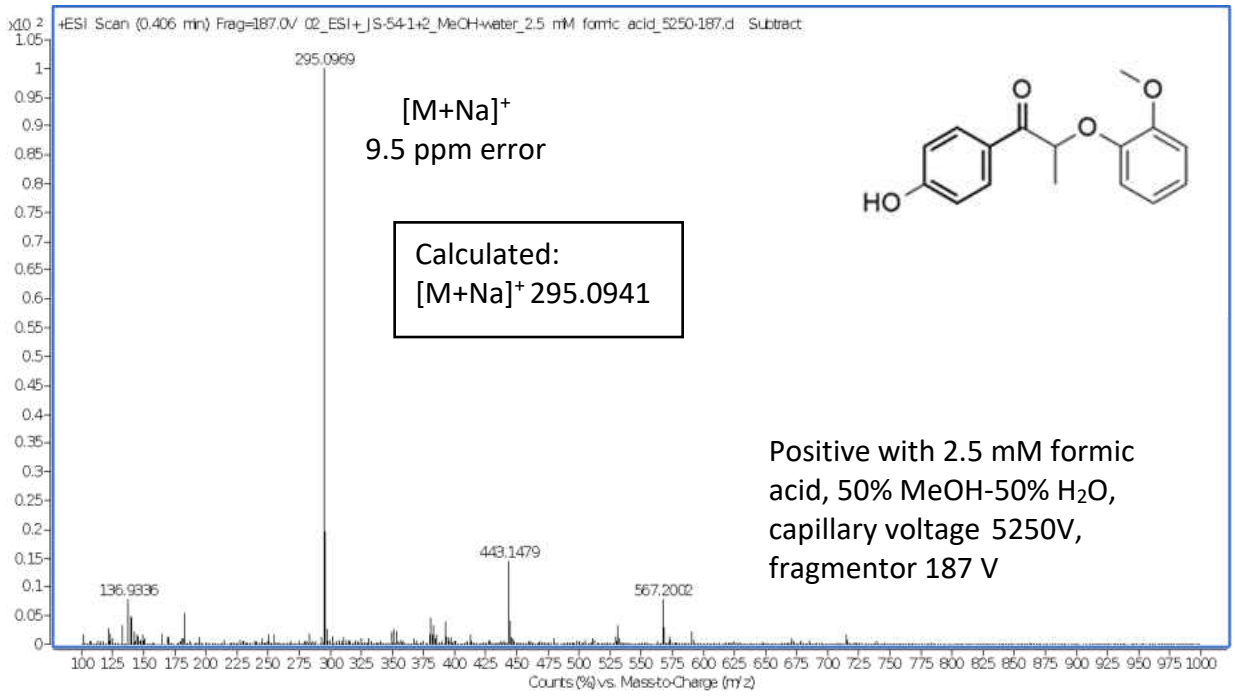


Figure 23. ESI-TOF spectrum run in the negative mode with 2.5 mM formic acid, 50% MeOH–50% H_2O, a capillary voltage of 3000 V, and a fragmentor voltage of 125 V. 1-(4-Hydroxyphenyl) -2-(2-methoxyphenoxy)- 1-propanone, **10**, had a concentration of 5 ppm.

Appendix I

^{1}H, $^{13}C\{^{1}H\}$ NMR, and ESI-TOF spectra of 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol, **17.**

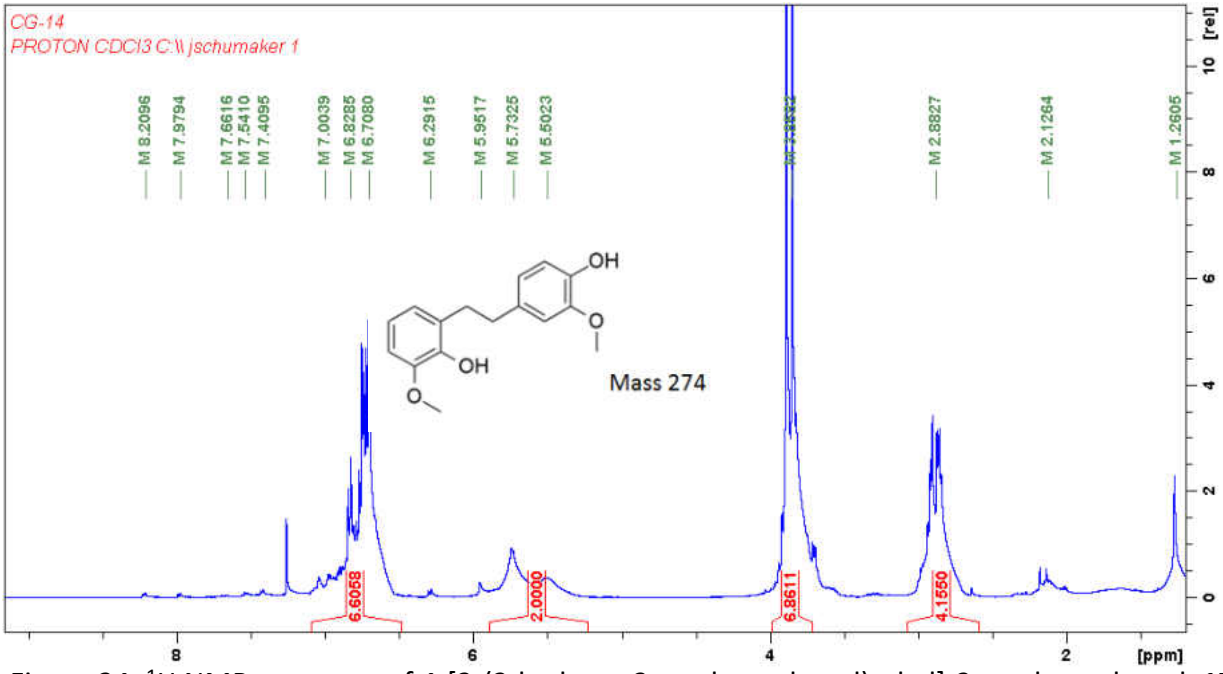


Figure 24. ^{1}H NMR spectrum of 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol, **17**.

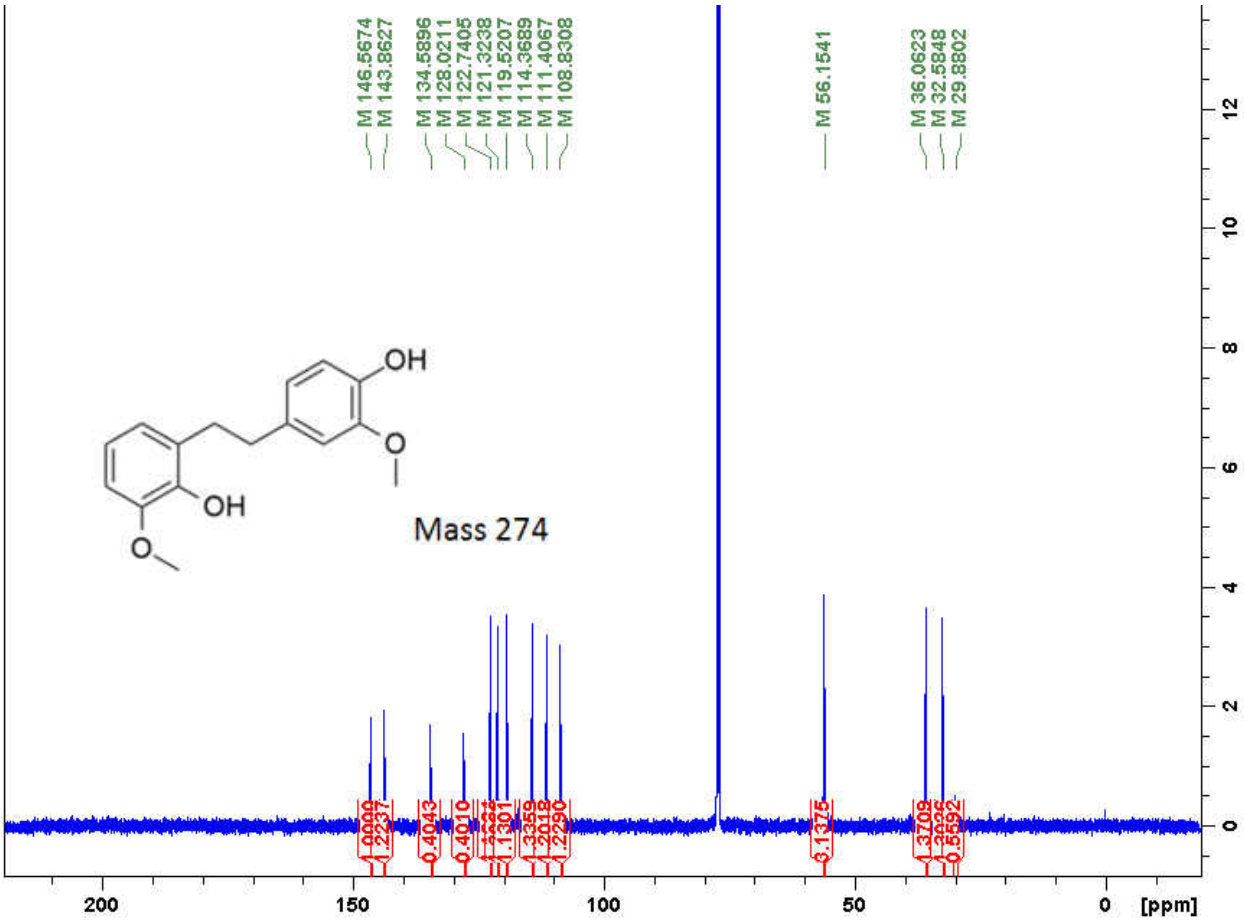


Figure 25. $^{13}C\{^{1}H\}$ NMR spectrum of 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol, **17**.

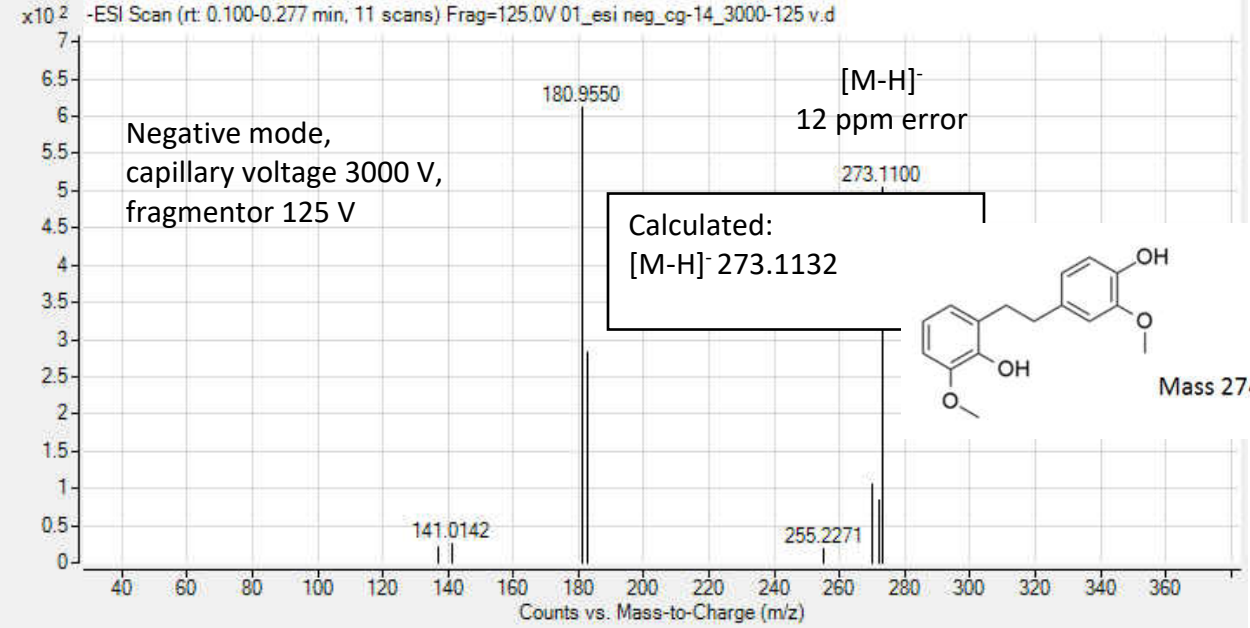


Figure 26. ESI-TOF spectrum run in the negative mode with 2.5 mM formic acid, 50% MeOH–50% H_2O, a capillary voltage of 3000 V, and a fragmentor voltage of 125 V. 4-[2-(2-hydroxy-3-methoxyphenyl)ethyl]-2-methoxyphenol, **17**, had a concentration of 5 ppm.

Appendix J

^{1}H NMR and ESI-TOF spectra of 6,6'-dihydroxy-5,5'-dimethoxy-(1,1'-biphenyl)-3,3'-dicarboxaldehyde, **19**.

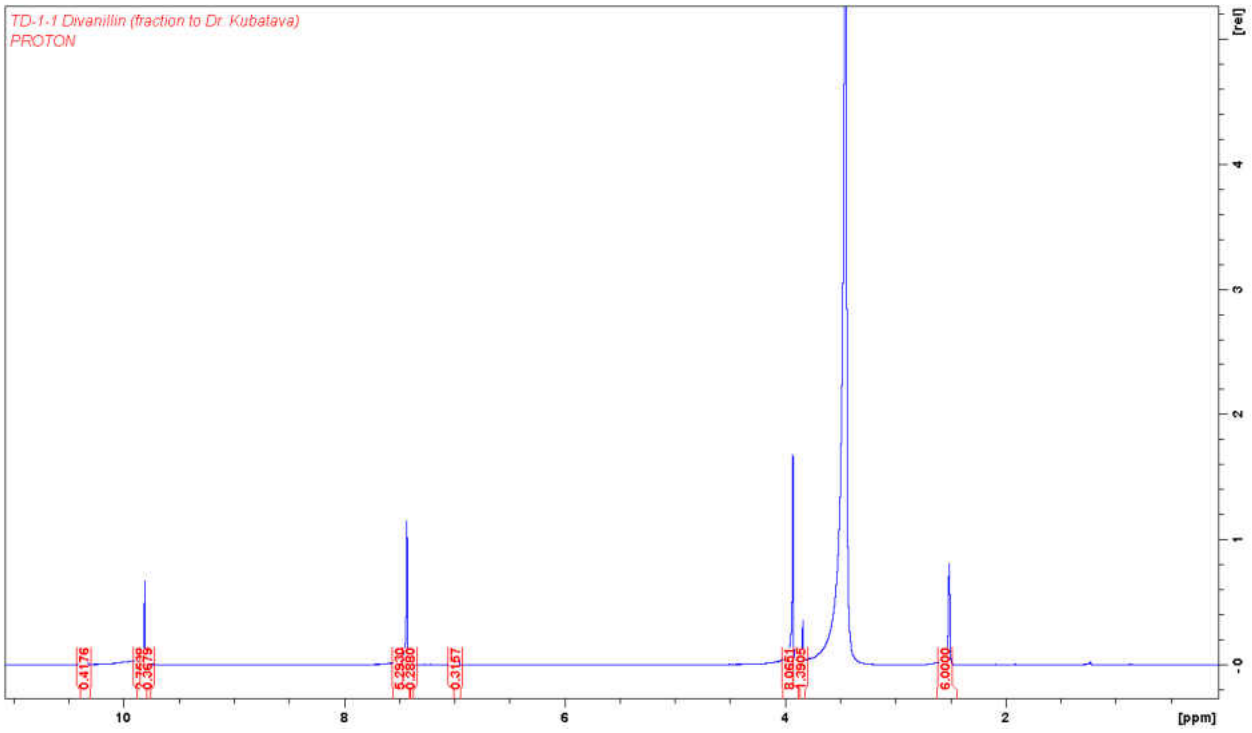


Figure 27. Spectrum of 6,6'-dihydroxy-5,5'-dimethoxy-(1,1'-biphenyl)-3,3'-dicarboxaldehyde, **19**.

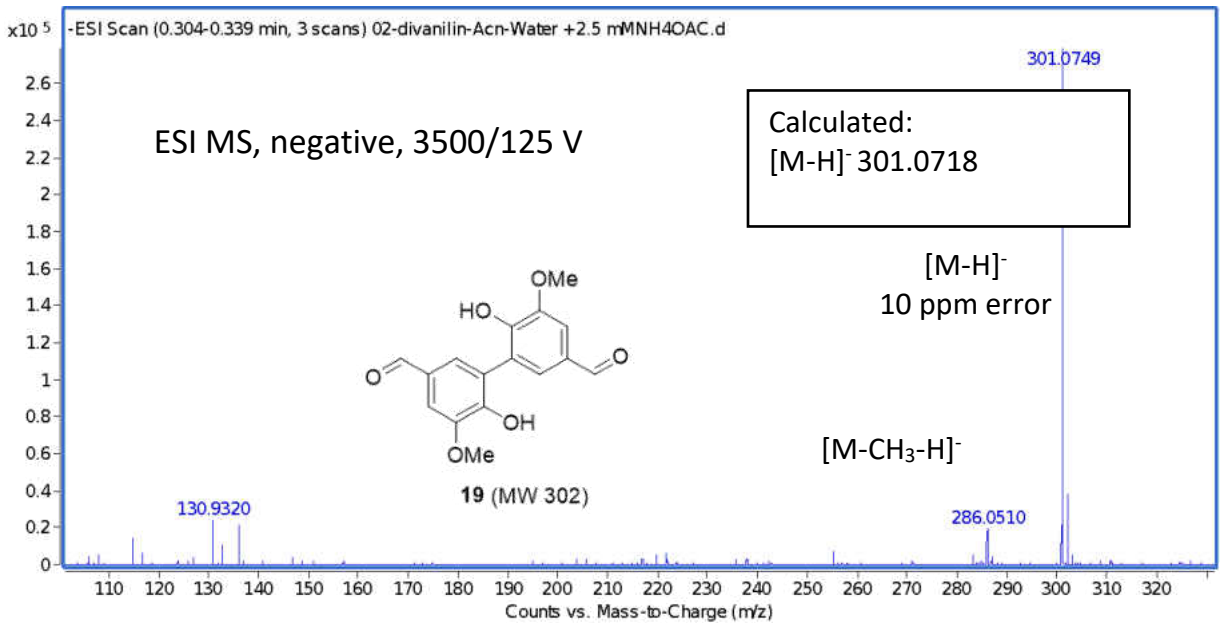


Figure 28. ESI-TOF spectrum run in the negative mode with 2.5 mM ammonium acetate acid, 50% ACN–50% H_2O, a capillary voltage of 3000 V, and a fragmentor voltage of 125 V. 6,6'-dihydroxy-5,5'-dimethoxy-(1,1'-biphenyl)-3,3'-dicarboxaldehyde, **19**, had a concentration of 5 ppm.

Appendix K

^{1}H NMR and ESI-TOF spectra of 3,3'-dimethoxy-5,5'-dimethyl-(1,1'-biphenyl])-2,2'-diol, **21**.

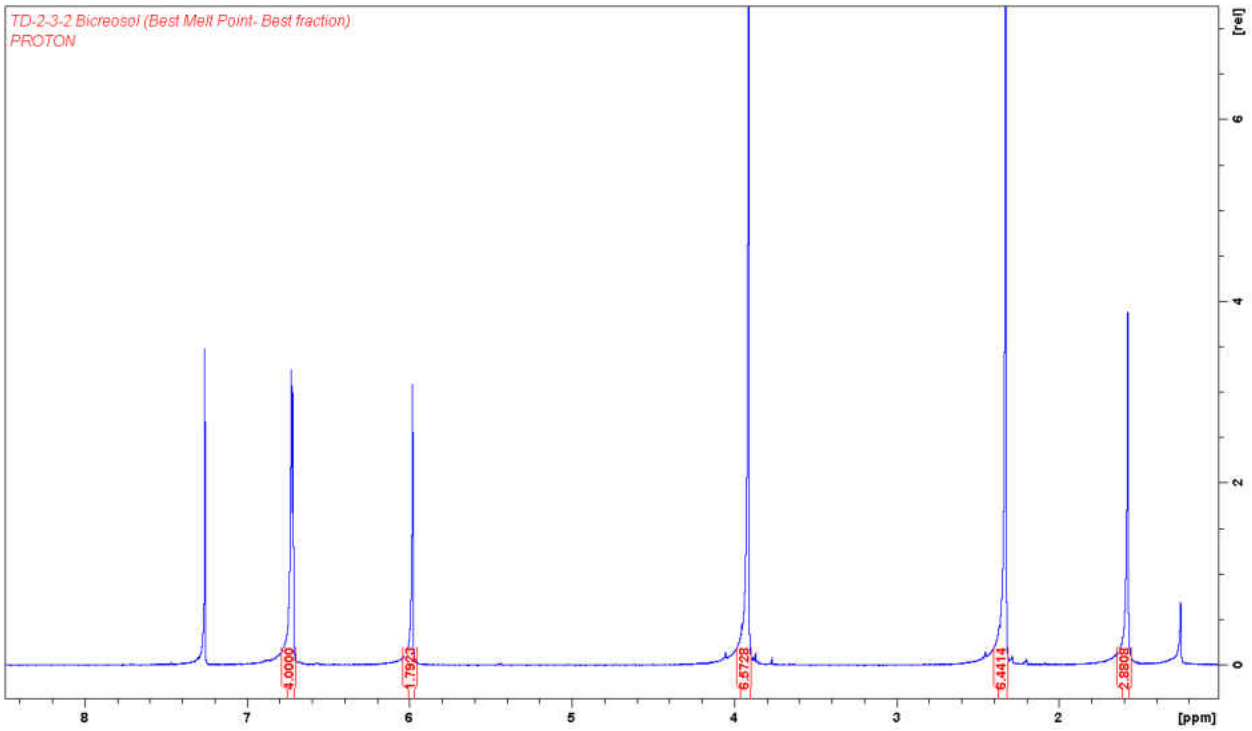


Figure 29. ^{1}H NMR spectrum of 3,3'-dimethoxy-5,5'-dimethyl-(1,1'-biphenyl])-2,2'-diol, **21**.

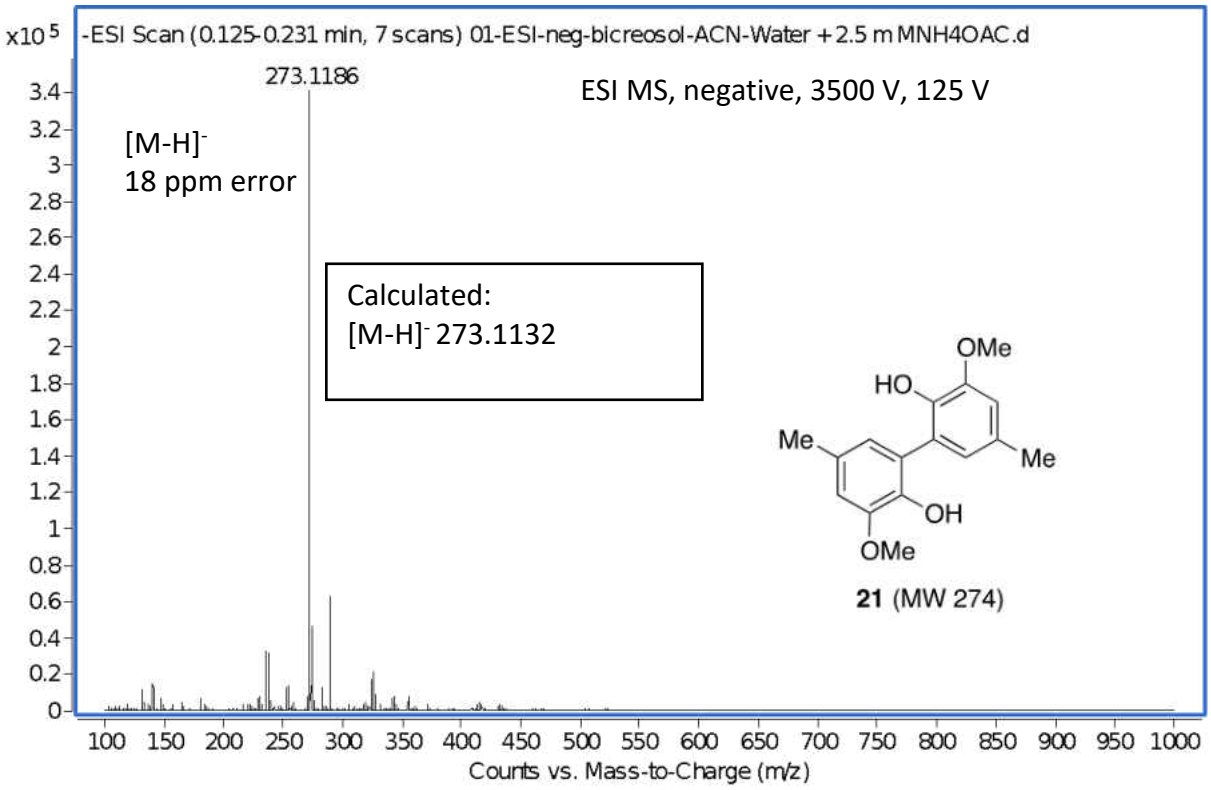


Figure 30. ESI-TOF spectrum run in the negative mode with 2.5 mM ammonium acetate acid, 50% ACN–50% H_2O, a capillary voltage of 3500 V, and a fragmentor voltage of 125 V. 3,3'-dimethoxy-5,5'-dimethyl-(1,1'-biphenyl])-2,2'-diol, **21**, had a concentration of 5 ppm.

Appendix L

^{1}H NMR and ESI-TOF spectra of 1,2-dimethoxy-4-[(2-methoxyphenoxy)methyl]benzene, **24**.

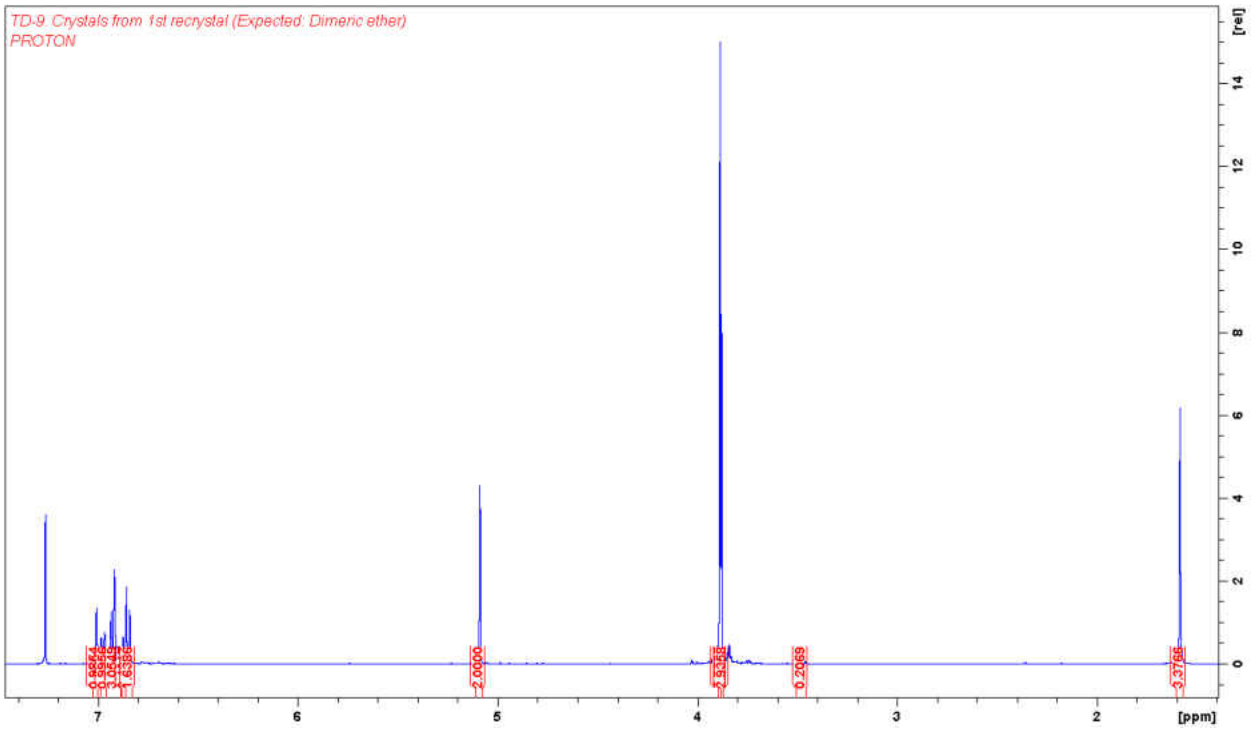


Figure 31. ^{1}H NMR spectrum of 1,2-dimethoxy-4-[(2-methoxyphenoxy)methyl]benzene, **24**.

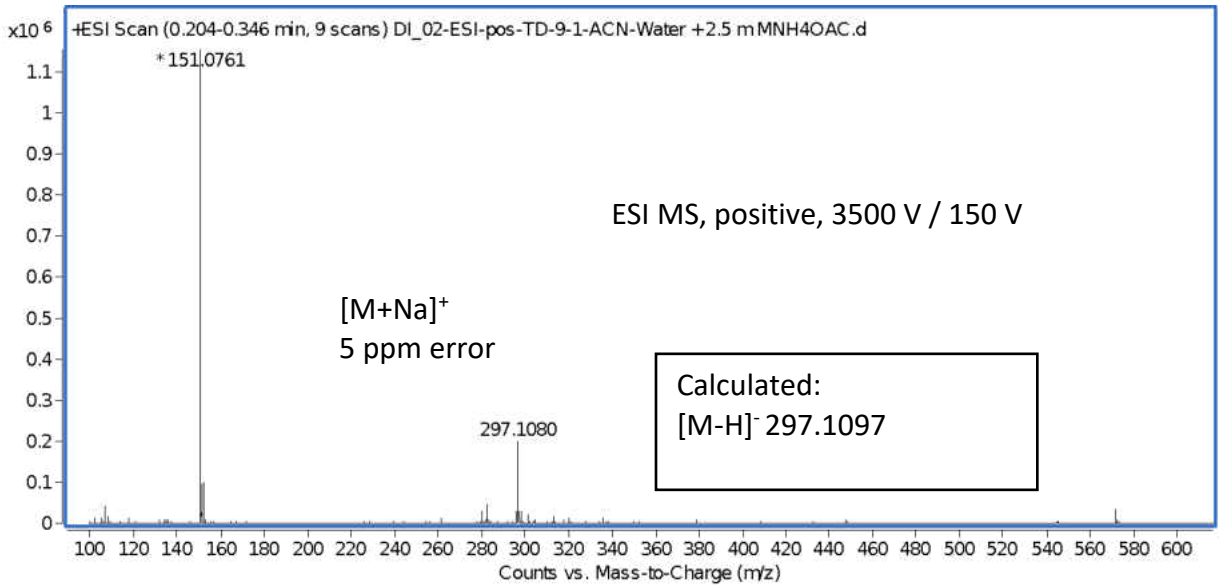


Figure 32. ESI-TOF spectrum run in the positive mode with 2.5 mM ammonium acetate acid, 50% ACN–50% H_2O, a capillary voltage of 3500 V, and a fragmentor voltage of 150 V. 1,2-dimethoxy-4-[(2-methoxyphenoxy)methyl]benzene, **24**, had a concentration of 5 ppm.

Appendix M

^{1}H NMR spectrum of (*E*)-4,4’-(ethene-1,2-diyl)bis(2-methoxyphenol), **26**.

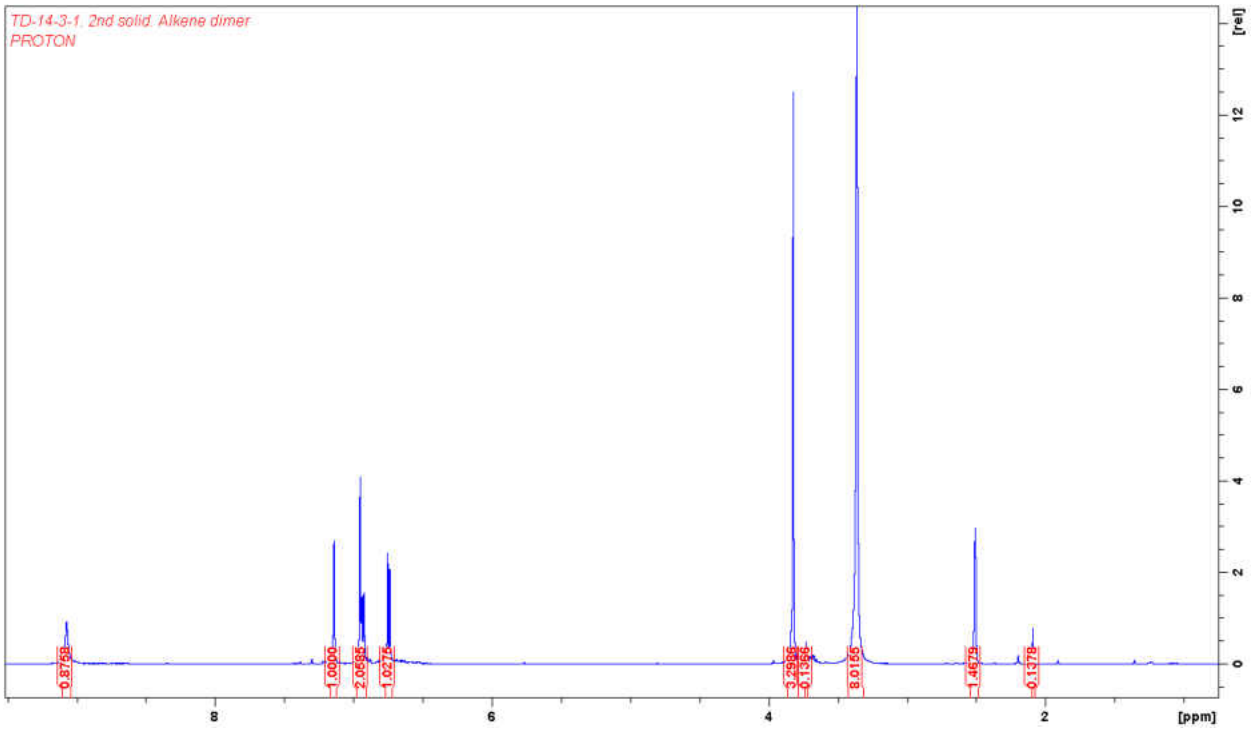


Figure 33. ^{1}H NMR spectrum of (*E*)-4,4’-(ethene-1,2-diyl)bis(2-methoxyphenol), **26**.

CHAPTER V

REFERENCES

1. Gabov, K.; Gosselink, R. J.; Smeds, A. I.; Fardim, P., Characterization of Lignin Extracted from Birch Wood by a Modified Hydrotropic Process. *J. Agric. Food. Chem.* **2014,** *62*, 10759-10767.

2. Argyropoulos, D. S., Quantitative Phosphorus-31 NMR Analysis of Lignins, a New Tool for the Lignin Chemist. *J. Wood Chem. Tech.* **1994,** *14* (1), 45-63.

3. Ragauskas, A. J.; Beckham, G. T.; Biddy, M. J.; Chandra, R.; Chen, F.; Davis, M. F.; Davison, B. H.; Dixon, R. A.; Gilna, P.; Keller, M.; Langan, P.; Naskar, A. K.; Saddler, J. N.; Tschaplinski, T. J.; Tuskan, G. A.; Wyman, C. E., Lignin Valorization: Improving Lignin Processing in the Biorefinery. *Science* **2014,** *344*, 709-721.

4. Brebu, M.; Vasile, C., Thermal Degradation of Lignin - A Review. *Cellul. Chem. Technol.* **2009,** *44* (9), 353-363.

5. Yuan, T.; Xu, F.; Sun, R., Role of Lignin in a Biorefinery: Separation, Characterization, and Valorization. *J. Chem. Tech. Biotech.* **2013,** *88* (3), 346-352.

6. Smolarski, N., High-value Opportunities for Lignin: Unlocking its Potential Market Insights. **2012**.

7. Vanholme, R.; Demedts, B.; Morreel, K.; Ralph, J.; Boerjan, W., Lignin Biosynthesis and Structure. *Plant Physiol.* **2010,** *153* (3), 895-905.

8. Lee, H. S.; Jae, J.; Ha, J. M.; Suh, D. J., Hydro- and Solvothermolysis of Kraft Lignin for Maximizing Production of Monomeric Aromatic Chemicals. *Bioresour. Technol.* **2016,** *203*, 142-149.

9. Wang, H. T., M. Ji, Y., Recent Development in Chemical Depolymerization of Lignin: A Review. *J. Appl. Chem.* **2013,** *2013*, 1-9.

10. Kozliak, E. I.; Kubátová, A.; Artemyeva, A. A.; Nagel, E.; Zhang, C.; Rajappagowda, R. B.; Smirnova, A. L., Thermal Liquefaction of Lignin to Aromatics: Efficiency, Selectivity, and Product Analysis. *ACS Sus. Chem. Eng.* **2016,** *4* (10), 5106-5122.

11. Kang, S.; Li, X.; Fan, J.; Chang, J., Hydrothermal Conversion of Lignin: A Review. *Ren. Sus. Energy Rev.* **2013,** *27*, 546-558.

12. Voeller, K., M., Characterization of Kraft Alkali Lignin and Products of its Thermal Degradation by Fractional Pyrolysis Method. *Thesis.* **2015**.

13. Bilek, J. Determination of Lignin Degradation Products by GC-MS and Thermal Carbon Analysis (TCA). *Thesis.* **2017.**

14. Andrianova, A. A.; Yeudakimenka, A. A.; Lilak, S. L.; Kozliak, I. I.; Ugrinov, A.; Sibi, M. P.; Kubátová, A., Size Exclusion Chromatography of Lignin: The Mechanistic Aspects and Elimination of Undesired Secondary Interactions. *J. Chromatogr., A.* **2017, submitted**.

15. Anderson, R. C.; Shapiro, M. J., 2-Chloro-(R),5(R)-dimethyl-2-oxo- 1,3,2-dioxaphospholane, a New Chiral Derivatizing Agent. *J. Org. Chem.* **1984,** *49*, 1304-1305.

16. Archipov, Y.; Argyropoulos, D. S.; Bolker, H.; Heitner, C., ^{31}P NMR Spectroscopy in Wood Chemistry. Phosphite Derivatives of Carbohydrates. *Carbohydr. Res.* **1991,** (220), 49-61.

17. Archipov, Y.; Argyropolous, D. S.; Bolker, H. I.; Heitner, C., ^{31}P NMR Spectroscopy in Wood Chemistry Part 1 - Model Compounds. *J. Wood. Chem. Techn.* **1991,** *11*, 137-157.

18. Archipov, Y.; Argyropoulos, D. S.; Bolker, H. I.; Heitner, C., 31P NMR Spectroscopy in Wood Chemistry. I. Model Compounds. *J. Wood Chem. Tech.* **1991,** *11* (2), 137-157.

19. Argyropoulos, D. S., Quantitative Phosphorus-31 NMR Analysis of Six Soluble Lignins. *J. Wood Chem. Tech.* **1994,** *14* (1), 65-82.

20. Argyropoulos, D. S., ^{31}P NMR in Wood Chemistry: A Review of Recent Progress. *Res. Chem. Intermed.* **1995,** *21*, 373-395.

21. Meija, J.; Coplen, T. B.; Berglund, M.; Brand, W. A.; De Bièvre, P.; Gröning, M.; Holden, N. E.; Irrgeher, J.; Loss, R. D.; Walczyk, T.; Prohaska, T., Atomic Weights of the Elements 2013 (IUPAC Technical Report). *Pure Appl. Chem.* **2016,** *88* (3), 265-291.

22. Wroblewski, A. E.; Lensink, C.; Markuszewski, R.; Verkade, J. G., ^{31}P NMR Spectroscopic Analysis of Coal Pyrolysis Condensates and Extracts for Heteroatom Functionalities Possessing Labile Hydrogen. *Energy Fuels* **1988,** *2*, 765-774.

23. Ben, H.; Ferrell III, J. R. In-depth Investigation on Quantitative Characterization of Pyrolysis Oil by ^{31}P NMR. *RSC Adv.* **2016,** *6* (21), 17567-17573.

24. Argyropoulos, D. S., Quantitative Phosphorus-31 NMR Analysis of Six Soluble Lignins. *J. Wood. Chem. Techn.* **1994,** *14*, 65-82.

25. Granata, A.; Argryopolous, D. S. 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, a Reagent for the Accurate Determination of Uncondensed and Condensed Phenol Moieties in Lignins. *J. Agric. Food Chem* **1995,** (43), 1538-1544.

26. Pu, Y.; Cao, S.; Ragauskas, A. J., Application of Quantitative ^{31}P NMR in Biomass Lignin and Biofuel Precursors Characterization. *Energy Environ. Sci.* **2011,** *4*, 3154-3166.

27. Reich, J., H. Relaxation in NMR Spectroscopy. (accessed 12/19/2016).

28. Constant, S.; Basset, C.; Dumas, C.; Di Renzo, F.; Robitzer, M.; Barakat, A.; Quignard, F., Reactive Organosolv Lignin Extraction from Wheat Straw: Influence of Lewis Acid Catalysts on Structural and Chemical Properties of Lignins. *Ind. Crops Prod.* **2015,** *65*, 180-189.

29. Jasiukaitytė, E.; Kunaver, M.; Crestini, C., Lignin Behaviour during Wood Liquefaction—Characterization by Quantitative ^{31}P, ^{13}C NMR and Size-Exclusion Chromatography. *Catal. Today* **2010,** *156* (1-2), 23-30.

30. Xin, J.; Zhang, P.; Wolcott, M. P.; Zhang, X.; Zhang, J., Partial Depolymerization of Enzymolysis Lignin via Mild Hydrogenolysis over Raney Nickel. *Bioresour. Technol.* **2014,** *155*, 422-426.

31. Jiang, Z.; Argyropolous, D. S.; Granata, A., Correlation Analysis of ^{31}P NMR Chemical Shifts with Substituent Effects of Phenols. *Mag. Res. Chem.* **1995,** *33*, 375-382.

32. Ben, H.; Ragauskas, A. J., NMR Characterization of Pyrolysis Oils from Kraft Lignin. *Energy Fuels* **2011,** *25* (5), 2322-2332.

33. Ben, H.; Ragauskas, A. J., Pyrolysis of Kraft Lignin with Additives. *Energy Fuels* **2011,** *25* (10), 4662-4668.

34. Kosa, M.; Ben, H.; Theliander, H.; Ragauskas, A. J., Pyrolysis Oils from CO_2 Precipitated Kraft Lignin. *Green Chem.* **2011,** *13* (11), 3196-3202.

35. Ben, H.; Ragauskas, A. J., One Step Thermal Conversion of Lignin to the Gasoline Range Liquid Products by Using Zeolites as Additives. *RSC Adv.* **2012,** *2* (33), 12892-12898.

36. Wroblewski, A. E. L., C.; Markuszewski, R.; Verkade, J. G., ^{31}P NMR Spectroscopic Analysis of Coal Pyrolysis Condensates and Extracts for Heteroatom Functionalities Possessing Labile Hydrogen. *Energy Fuels* **1988,** *2*, 765-774.

37. Yoshikawa, T.; Shinohara, S.; Yagi, T.; Ryumon, N.; Nakasaka, Y.; Tago, T.; Masuda, T., Production of Phenols from Lignin-Derived Slurry Liquid using Iron Oxide Catalyst. *Appl. Catal., B* **2014,** *146*, 289-297.

38. Yoshikawa, T.; Yagi, T.; Shinohara, S.; Fukunaga, T.; Nakasaka, Y.; Tago, T.; Masuda, T., Production of Phenols from Lignin via Depolymerization and Catalytic Cracking. *Fuel Process. Technol.* **2013,** *108*, 69-75.

39. Andrianova, A. A. Novel Approaches in Lignomics Employing Liquid Chromatography and Mass Spectrometry. Dissertation, University of North Dakota, 2017.

40. Siddaraju, Y.; Prabhu, K. R., Iodine Promoted α-hydroxylation of Ketones. *Org. Biomol. Chem.* **2015,** (13), 6749-6753.

41. Wen, G.; Su, Y.; Zhang, G.; Lin, Q.; Zhu, Y.; Zhang, Q.; Fang, X., Stereodivergent Synthesis of Chromanones and Flavanones via Intramolecular Benzoin Reaction. *Org. Lett.* **2016,** *18* (16), 3980-3983.

42. Zakis, G. F., *Synthesis of Model Lignin Compounds*. Zinatne: Riga, **1980**.

43. Nawrat, C. C.; Palmer, L. I.; Blake, A. J.; Moody, C. J., Two Approaches to the Aromatic Core of the Aminonaphthoquinone Antibiotics. *J. Org. Chem.* **2013,** *78* (11), 5587-5603.

44. Choundhury, A. M., Acid-catalysed Rearrangement of a Spirocyclohexadienone Involving an External Nucleophile. *J.C.S. Perkin I* **1974**, 132-134.

45. Elbs, K.; Lerch, H., Über Dehydrodivanillin. *J. Prak. Chem.* **1916,** *93* (1), 1-9.

46. TenBrink, R. E.; McCall, J. M., The Synthesis of l-Haloalkyl-l,3,4,5-tetrahydro-2-benzoxepins. *J. Heterocycl. Chem* **1981,** *18* (4), 821-824.

47. Harvey, B. G.; Guenthner, A. J.; Meylemans, H. A.; Haines, S. R. L.; Lamison, K. R.; Groshens, T. J.; Cambrea, L. R.; Davis, M. C.; Lai, W. W., Renewable Thermosetting Resins and Thermoplastics from Vanillin. *Green Chem.* **2015,** *17* (2), 1249-1258.

48. Nawrat, C. C. P., L. I.; Blake, A. J.; Moody, C. J., Two Approaches to the Aromatic Core of the Aminonaphthoquinone Antibiotics. *J. Org. Chem.* **2013,** *78*, 5587-5603.