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Regioselectivity of Enzymatic and Photochemical Single Electron Transfer Promoted Carbon−Carbon Bond Fragmentation Reactions of Tetrameric Lignin Model Compounds

Dae Won Cho,*[,†] John A. Latham,[†] Hea Jung Park,[‡] Ung Chan Yoon,[‡] Paul Langan,[§] Debra Dunaway-Mariano,[†] and Patrick S. Mariano*[,†]

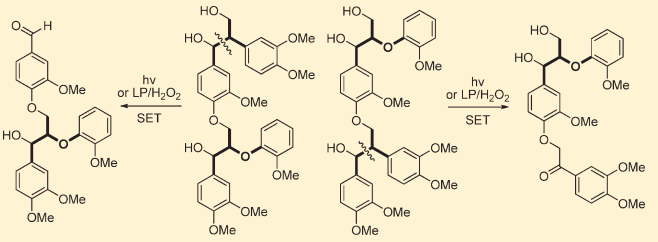
[†]Department of Chemistry and Chemical Biology, University of New Mexico, Albuquerque, New Mexico 87131, United States

[‡]Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Busan 609-735, Korea

[§]Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545, United States

Supporting Information

ABSTRACT: New types of tetrameric lignin model compounds, which contain the common β-O-4 and β-1 structural subunits found in natural lignins, have been prepared and carbon−carbon bond fragmentation reactions of their cation radicals, formed by photochemical (9,10-dicyanoanthracene) and enzymatic (lignin peroxidase) SET-promoted methods, have been explored. The results show that cation radical intermediates generated from the tetrameric model compounds undergo highly regioselective C−C bond cleavage in their β-1 subunits. The outcomes of these processes suggest that, independent of positive charge and odd-electron distributions, cation radicals of lignins formed by SET to excited states of sensitizers or heme-iron centers in enzymes degrade selectively through bond cleavage reactions in β-1 vs β-O-4 moieties. In addition, the findings made in the enzymatic studies demonstrate that the sterically large tetrameric lignin model compounds undergo lignin peroxidase-catalyzed cleavage via a mechanism involving preliminary formation of an enzyme−substrate complex.

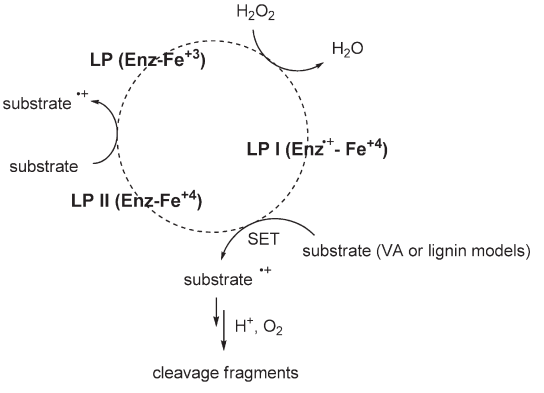


INTRODUCTION

The cellulose found in plant cell walls can be transformed to glucose, the starting materials for fermentation that efficiently yields bioethanol. However, a large barrier exists to accessing and hydrolytically cleaving cellulose in most plant materials, owing to its encasement in networks comprised of lignin. Lignin is a natural, heterogeneous arylpropanoid polymer that is biosynthesized in plants in order to provide structural rigidity and prevent hydrolysis of cellulose and, thereby, to protect plants from external chemical and/or biological attack.[1,2] As a result, a large effort is underway to develop mild, nonenergy-intensive and eco-friendly methods to bring about delignification of plant materials so that cellulose can be easily converted to glucose, the precursor of bioethanol.

One approach to delignification employs fungi (e.g., the white rot fungus *Phanerochaete chrysosporium*) that excrete iron-heme containing enzymes, such as lignin peroxidase (LP) and manganese peroxidase (MnP), which catalyze oxidation reactions that lead to cleavage of C−C bonds in lignin.[3] The cleavage reactions result in a decrease in the structural integrity and an increase in the permeability of the arylpropanoid polymer. The mechanistic pathway for LP induced degradation of lignin involves initial single electron transfer (SET) from the aromatic groups in the polymer to the doubly oxidized form of LP (i.e., LP I) to generate lignin radical cations, which undergo carbon−carbon bond cleavage (Scheme 1).[4−13] An alternative route has been suggested that involves mediation by small molecules (e.g., veratryl alcohol) whose cation radicals formed by SET to LP I are responsible for one-electron oxidation of lignin.

Scheme 1



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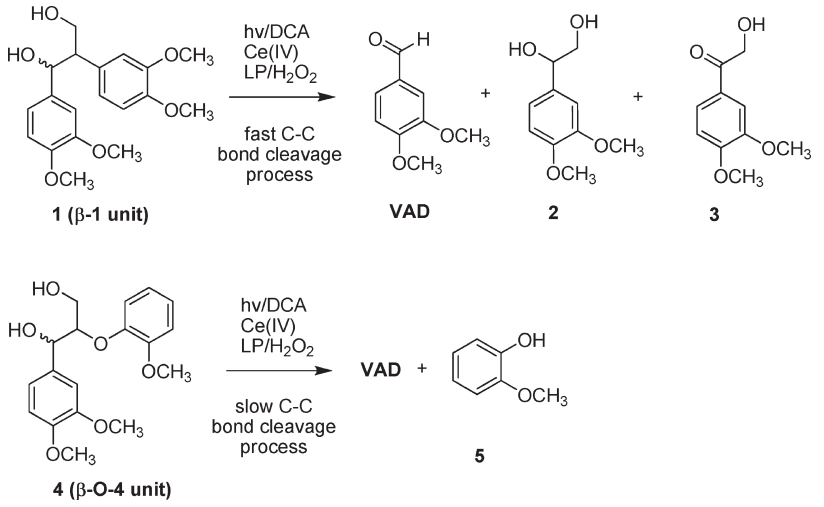
Scheme 2



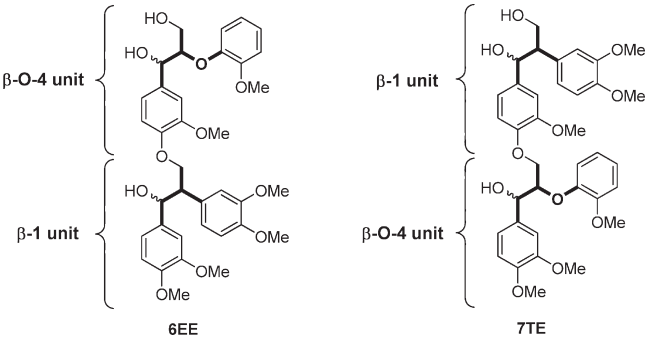
Studies are underway in our laboratory to gain fundamental information about how the composition of lignins might govern the efficiencies of delignification reactions that proceed via SET pathways. We believe that this information is potentially relevant to ethanol production from plant materials[14,15] since it could provide a framework for the genetic design of plants[16−19] that have the type of lignin that are more readily cleaved by enzymatic or other oxidative processes. Prior to our efforts in this area, nothing was known about the C−C bond cleavage reactivity of sites in lignin where radical cation formation can take place. This is an extremely important issue since it is possible that SET from lignin to either LP I directly and/or to hole carrier mediator cation radicals produces a mixture of intermediates that differ in the site where the cation radical center exists and that these intermediates undergo rapid and reversible interconversion by an electron-hopping mechanism. As we have shown in previous investigations,[20] in this event, the site(s) at which cation radical C−C bond cleavage takes place more rapidly will strongly influence the nature and overall efficiency of the lignin cleavage process.

In an earlier study,[21] information was gained about the efficiencies/rates of C−C bond cleavage of arylpropanoid units in lignin. For this purpose, SET photochemical, Ce(IV), and LP-promoted oxidation reactions were carried out on dimeric lignin model compounds **1** and **4** that represent the β-1 (1,2-diarylpropanoid) and β-O-4 (1-aryl-2-aryloxypropanoid) groups that are present in the lignin skeleton (Scheme 2). The observations made in that effort show that, regardless of the method used for their generation, cation radicals derived by SET oxidation of β-1 lignin model undergo C−C bond cleavage more rapidly than do those produced from β-O-4 model compounds.

Another approach to assessing how structure governs the C−C bond cleavage reactivity of lignin cation radicals involves the use of the more complex lignin models. This approach was employed in an earlier study of trimeric lignin models by Baciocchi and his co-workers.[22] The results of that investigation showed that LP generated radical cations of trimeric models composed of two β-O-4 units are degraded by C−C or benzyl C−H bond cleavage processes to give aldehyde and β-hydroxyketone products by pathways that mimic fragmentation patterns seen in dimeric model compounds. To our knowledge, no investigation has been conducted using more complex lignin models that contain different arylpropanoid units to assess the sites at which cation radical C−C bond cleavage takes place more efficiently.

As part of our continuing efforts in this area we have designed a study of SET-photochemical and LP-catalyzed reactions of tetrameric lignin model compounds that are comprised of both β-1 and β-O-4 moieties. We envisaged that analysis of products produced in low conversion reactions of these models would yield information about the relative rates of C−C bond cleavage in interconverting radical cations in which the charge and odd electron is distributed over β-1 and β-O-4 moieties. For example, if the β-1 > β-O-4 reactivity pattern observed in our earlier investigation with dimeric models is general, it is anticipated that cation radicals of mixed β-1 and β-O-4 tetrameric models will undergo predominant cleavage of 1,2-diaryl rather than 1-aryl-2-aryloxy C−C bonds.

Scheme 3



To probe this feature, two tetrameric lignin model compounds, **6EE** and **7TE** (Scheme 3), in which β-1 and β-O-4 groups are connected via an ether linkage in a manner that mimics arylpropanoid arrays in the natural polymer, were prepared. Studies of the time courses of SET-promoted photochemical and LP enzymatic reactions of these substances were carried out. The results of this effort demonstrate that the most rapid reaction pathways followed by **6EE** and **7TE** involve C−C

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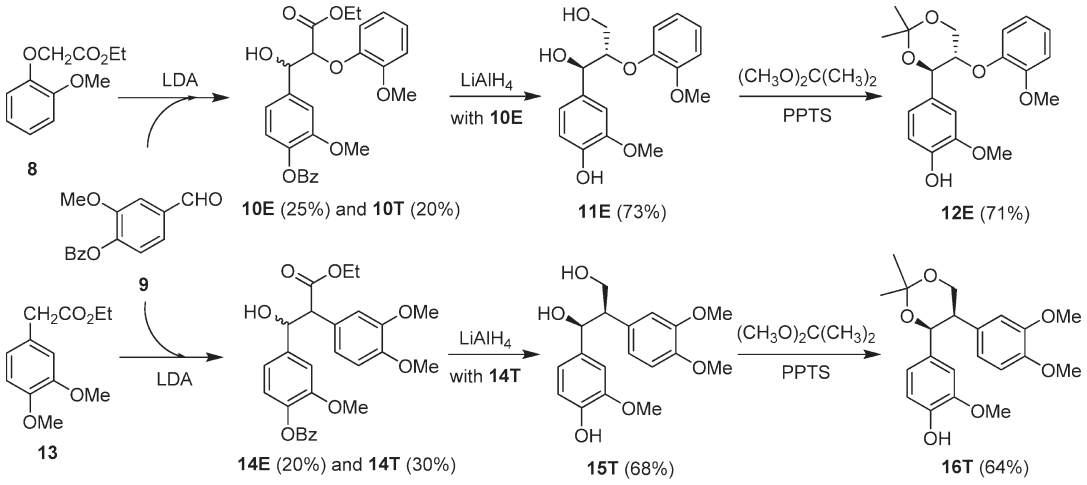
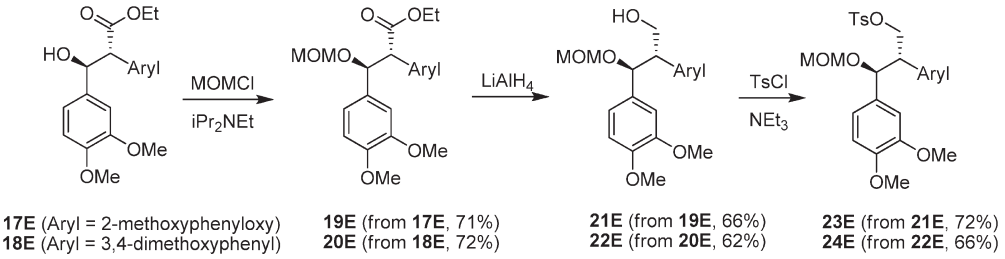
Scheme 4



Scheme 5



bond cleavage of β-1 units within their tetrameric skeletons. These findings along with observations made in probing the kinetics of the LP processes are described and discussed below.

RESULTS

Synthesis of Tetrameric Lignin Models. A convergent strategy was used for the preparation of the tetrameric model compounds **6EE** and **7TE** (Scheme 4). The approach involves the preparation and coupling of the selectively protected, diastereomerically pure phenols **12E** and **16T**[21] with the respective primary tosylates **23E** and **24E**. The synthesis of **12E** was initiated by aldol condensation of the aryloxyacetate **8** with the selectively protected veratraldehyde derivative **9** (Scheme 4). This process afforded a separable mixture of the diastereomeric esters **10T** and **10E**, the latter of which was treated with $LiAlH_4$ followed by acetonide protection of the resulting 1,3-diol to form the erythro phenol **12E**. A similar route beginning with the arylacetate **13** was employed to generate the threo phenolic acetonide **16T**. The stereochemical assignments to these substances were made by comparison of their spectroscopic properties with those previously reported for closely related compounds whose stereochemistry was determined by using X-ray crystallographic analysis.[21,23,24]

The tosylate coupling partners **23E** and **24E** were synthesized by using processes developed in our earlier effort[21] beginning with MOM protection of the erythro β-hydroxyesters **17E** and **18E**. The protected hydroxyesters were then transformed to the respective target tosylates **23E** and **24E** by sequential treatment with $LiAlH_4$ and *p*-toluenesulfonyl chloride (Scheme 5).

Preparation of the tetrameric lignin model compounds **6EE** and **7TE** was accomplished by using respective heterocoupling reactions of the β-O-4 phenol **5E** with the β-1 tosylate **24E**, and of the β-1 phenol **16T** with the β-O-4 tosylate **23E** (Scheme 6). These processes were promoted by treatment of the phenol derivatives with NaH. The products of these reactions, **25EE** and **26TE**, were then treated with 3 N HCl to remove the acetonide and MOM protecting groups and furnish the desired tetrameric models **6EE** and **7TE**. Owing to the fact that enantiomeric mixtures of the phenol and tosylate subunits were used in the coupling reactions, **6EE** and **7TE** are produced as ca. 1:1 mixtures of inseparable diastereomers.

DCA-Promoted Photoreaction of Tetrameric Lignin Models. One method employed for the generation of cation radicals of the tetrameric lignin model compounds **6EE** and **7TE** involves SET-photosensitization with the acceptor 9,10-dicyanoanthracene (DCA). Prior to beginning photochemical studies, the oxidation potentials and rates of DCA fluorescence quenching were determined for **6EE** and **7TE** (see the Supporting Information for fluorescence spectra and Stern−Volmer plots). The results of these experiments show that SET from tetrameric lignin models to the singlet excited state of DCA ($E_{1/2}(-)DCA^{S1}$ = +2.8 V) is both predicted and observed to take place at diffusion controlled rates (Table 1).

Photochemical reactions of **6EE** and **7TE** with DCA as the electron acceptor were performed on oxygenated 5% aqueous

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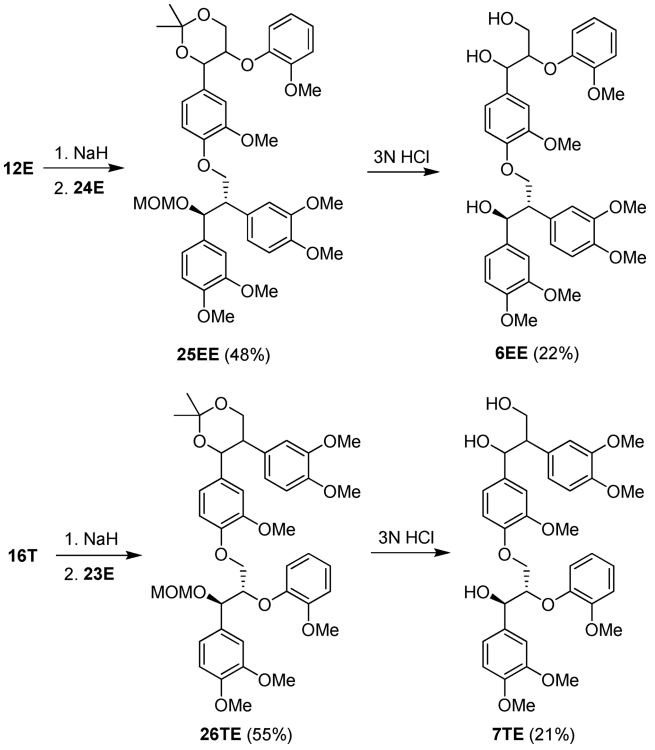
Scheme 6



acetonitrile solutions. Inspection of the results displayed in Scheme 7 and Table 2 shows that low conversion (18%) SET-photochemical reaction of the β-O-4 (top)−β-1 (bottom) tetramer **6EE** takes place cleanly to form nearly equal amounts of veratrylaldehyde (**VAD**) and aryloxyketone **27E**. In a similar manner, the DCA-sensitized, low conversion (32%) photoreaction

Table 1. Oxidation Potentials and DCA Fluorescence Quenching Rate Constants of Tetrameric Models 6EE and 7TE

substrate	oxidation potentials $E_{1/2}(+)$ (V vs Ag/AgCl)	DCA fluoroescence quenching rate constants $k_q \times 10^{-10}$ (M^{-1} s^{-1})[a]
6EE	1.38	1.15
7TE	1.38	1.06

[a] τ_{DCA} = 14.9 ns

Table 2. Products and Yields of DCA-Promoted Photoreactions of Tetrameric Models 6EE and 7TE[a]

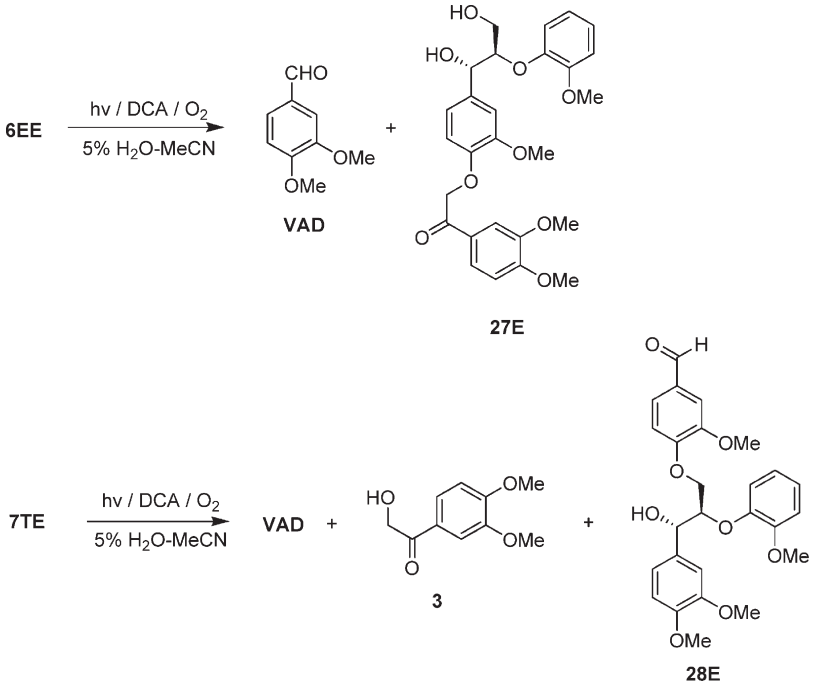
		product (% yield)[c]			
substrate	percent conversion[b]	**VAD**	**27E**	**3**	**28E**
6EE	18	14	15		
7TE	32	5		2	19

[a] Uranium glass filtered light irradiation (0.5 h) of O_2 satd 5% aq MeCN solutions of DCA (0.27 mM) and substrate (0.22 mM of **6EE**, 0.46 mM of **7TE**). [b] Based on recovered substrates determined by HPLC analysis. [c] Determined by HPLC analysis.

of the β-1 (top)−β-O-4 (bottom) tetramer **7TE** in the presence of O_2 produces aldehyde **28E** as the major product along with minor amounts of **VAD** and the ketol **3** (Scheme 7 and Table 2).

Structural assignments to photoproducts **27E** and **28E** were made by comparing their spectroscopic data to those of independently synthesized substances. The route employed to prepare ketone **27E** (Scheme 8) involves condensation of phenol **12E** and 3,4-dimethoxyphenacyl bromide to form the aryloxyketone **30E**. Removal of the acetonide protecting group then

Scheme 7



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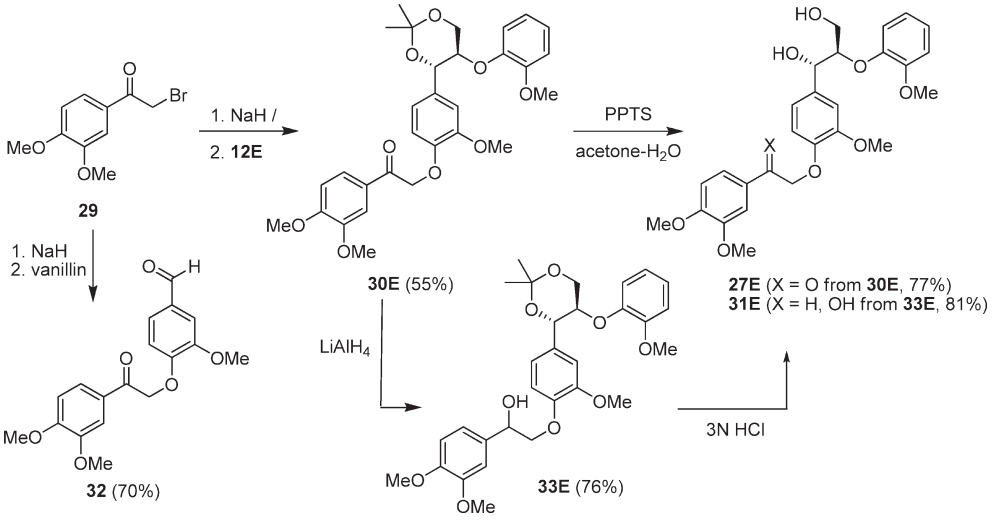
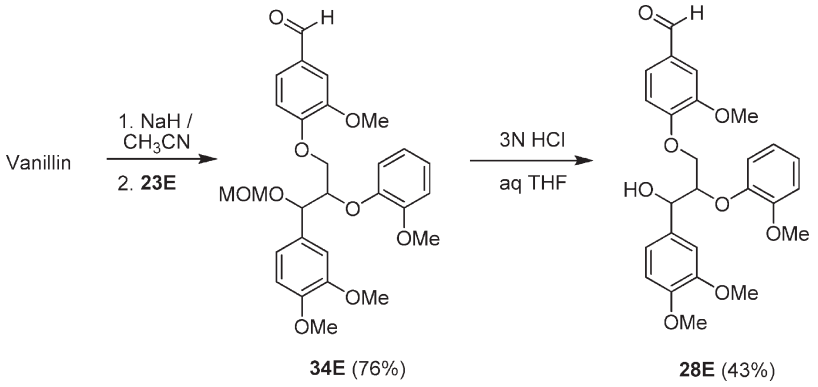
Scheme 8



Scheme 9



furnishes photoproduct **27E**. An authentic sample of the aldehyde photoproduct **28E** was produced by using a sequence starting with condensation of vanillin with tosylate **23E** to yield aldehyde **34E**, followed by MOM-deprotection (Scheme 9). Another possible product that could arise by cleavage of the β-1 C−C bond in tetrameric model **6EE** (see below) is the benzylic alcohol **31E**. This substance was independently prepared (Scheme 8) in order to unambiguously rule out its presence by inspection of the product mixture produced by DCA irradiation of **6EE**. Likewise, the aryloxyketone **32**, a possible product of β-O-4 C−C bond cleavage reaction of the initially formed photoproduct **27E**, produced by irradiation of **6EE**, was prepared (Scheme 8) to demonstrate that it does form in a secondary reaction. Finally, it is important to note that careful ^{1}H NMR and HPLC analysis of the crude mixtures arising by DCA-promoted photoreactions of **6EE** and **7TE** failed to reveal the presence of products other than those displayed in Scheme 7.

Monitoring product distributions generated in the DCA-sensitized photoreactions of **6EE** and **7TE** as a function of irradiation time gives more detailed information about preferences displayed in C−C bond cleavage processes (Figure 1). The time course of the low conversion (<10%) reaction of **6EE** shows that C−C bond cleavage in the β-1 subunit takes place exclusively to form **VAD** and ketone **27E** (Figure 1A). Thereafter, formation of **VAD** increases continuously but production of **27E** reaches a maximum at ca. 50% conversion of tetramer **6EE**. This observation suggests that both reactant **6EE** and fragment **27E** undergo SET-induced photoreactions that generate **VAD** as the only detectable product in the latter case. Importantly, even though the secondary SET reaction of **27E** might have been expected to undergo β-O-4 C−C bond cleavage, ketone **32** that would have formed by such a pathway is not observed (see above).

Study of the time dependence of the product distributions in the photoreaction of the tetrameric model **7TE** (Figure 1B) showed that at low conversion (<40%), **28E**, **VAD**, and ketol **3** were formed in ratios that were invariant with time. At higher conversions, the initially formed β-1 C−C bond cleavage product **28E** slowly disappears while the amount of **VAD** gradually increases.

A brief study was carried out to explore the reactivity of the ketone **27E**, a product of SET-induced cleavage of **6EE**. Irradiation of a DCA and O_2 saturated solution of this substance in 5% aqueous MeCN with uranium glass filtered light leads to clean production of the acetophenone derivative **35** (10%) and 1,4-diketone **36**[25,39] (70%) (Scheme 10). This process appears to be initiated by direct excitation of **27E**, which absorbs light, albeit weakly, in the wavelength region transmitted by the uranium

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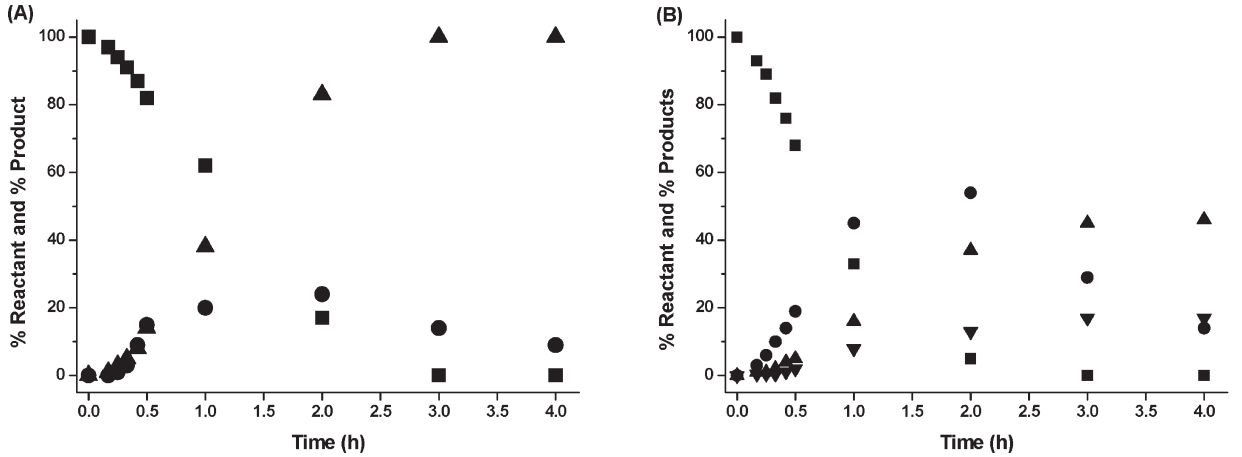
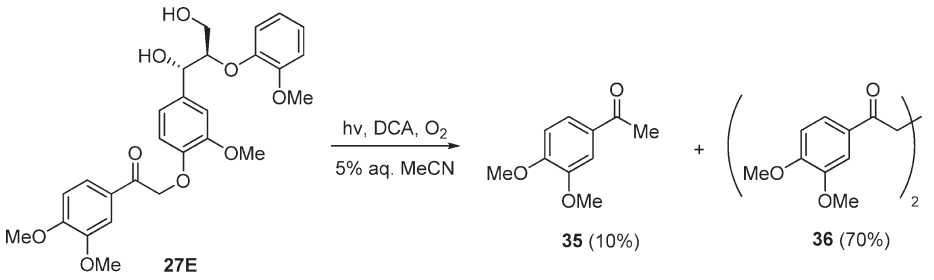


Figure 1. Plots of percentages of reactants remaining (**6EE** and **7TE** (■)) and products formed (**VAD** (▲) and **27E** (●), and **VAD** (▲), **3** (▼), and **28E** (●)) in DCA-promoted photoreactions of **6EE** (A) and **7TE** (B) in O_2 saturated conditions as functions of irradiation times.

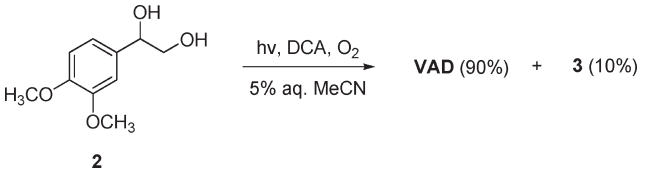
Scheme 10



glass filter, followed by documented[25] α-aryloxyketone homolytic cleavage. The α-acyl radical produced in this manner undergoes H-atom abstraction and coupling to form the respective products **35** and **36**.

The observation that **VAD** and ketol **3** are generated in the C−C bond cleavage reaction of the β-1 unit in **7TE** is interesting since these substances would need to arise via a radical intermediate produced in this process or through secondary reaction of the initially formed diol **2**, which we have observed earlier[21] as a minor product in reactions of related β-1 model compounds. To explore the latter possibility, diol **2** was subjected to the DCA-sensitized photochemical reaction conditions. As shown in Scheme 11, under these conditions **2** is efficiently converted to **VAD** and ketol **3**.

Scheme 11



Enzymatic Reactions of Tetrameric Lignin Model Compounds. Low conversion, lignin peroxidase catalyzed reactions of **6EE** and **7TE** initiated by H_2O_2 were performed in tartrate buffer solutions (pH 3.4) containing 17% acetonitrile. HPLC analysis of the product mixtures provided the results listed in Table 3. As can be seen by viewing the data, the LP-catalyzed reaction generates the same products in the same relative yields as those arising from DCA-promoted photoreactions of **6EE** and **7TE** by way of selective C−C bond cleavage in the β-1 unit.

Table 3. LP-Catalyzed Reaction of Tetramers 6EE and 7TE

		product (% yield)[c]			
substrate	percent conversion[a,b]	VAD	27E	28E	3
6EE	16	16	2		
6EE	31	25	8		
7TE	7	1		4	
7TE	18	4		12	1

[a] LP (8 μM), **6EE** and **7TE** (0.2 mM), and H_2O_2 (1.2 mM for low conversion, 2.4 mM for high conversion) in 17% MeCN−buffer solution (pH 3.4) were used. [b] Percent conversion based on recovered substrate determined by HPLC analysis. [c] Determined by HPLC.

Steady state kinetic constants for the LP-catalyzed bond cleavage reactions of **6EE** and **7TE** were determined (Figure 2 and Table 4). As can be seen by the results displayed in Figure 2A, **6EE** and **7TE** form complexes with LP, in which SET take place to produce cation radical intermediates that undergo β-1 bond breaking. Regardless of the K_M and k_{cat} differences between **6EE** and **7TE**, their catalytic efficiencies (k_{cat}/K_M) are approximately the same. Furthermore, in agreement with previously reported results by other groups,[22,26,27] the catalytic efficiency is influenced by the size of the lignin model compounds; the tetrameric models have lower catalytic efficiencies than dimeric models as compared to our previous studies.[21]

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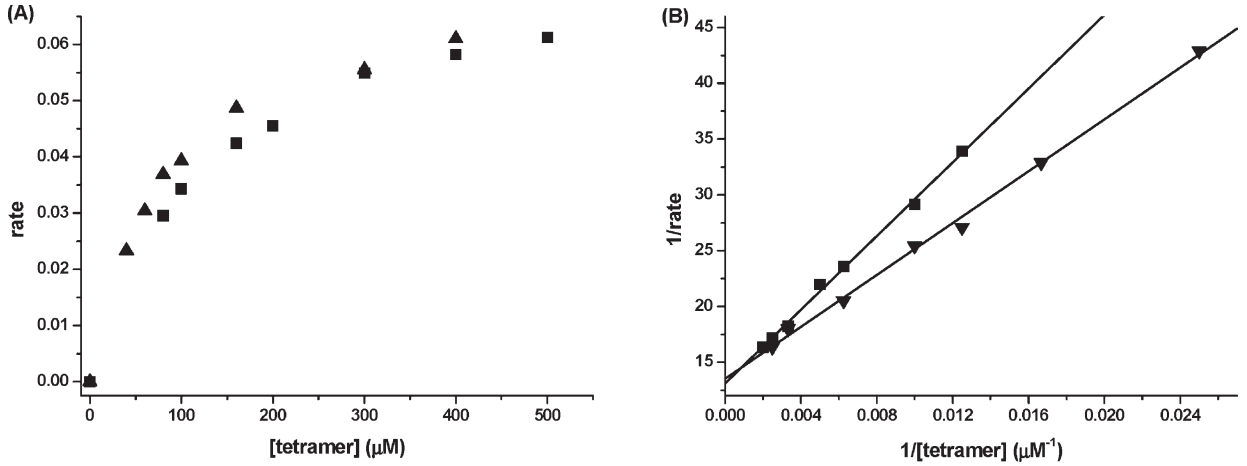


Figure 2. Plots of the (A) rate vs tetrameric model concentrations in LP-catalyzed reactions of **6EE** (■) and **7TE** (▼), and (B) Lineweaver−Burke plots of the reciprocals of rates vs reciprocals of concentration of **6EE** (■) and **7TE** (▼).

Table 4. Steady State Kinetic Constants of the LP-Catalyzed Reaction of Tetramers 6EE and 7TE[a]

substrate	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (s^{-1} M^{-1})
6EE	0.39 ± 0.01	130	3.0×10^3
7TE	0.30 ± 0.01	80	3.75×10^3

[a] 20−300 μM substrates in 25% MeCN−tartrate buffer solution (50 mM, pH 3.4, 25 °C), 0.26 μM LP, 50 μM H_2O_2 were used.

DISCUSSIONS

As stated in the Introduction, one of the major hurdles that needs to be surmounted in the conversion of plant materials to ethanol is related to the development of efficient and low energy requiring pretreatment methods that facilitate access of cellulase enzymes to cellulose that is encased in lignin networks in plant cell walls. An interesting approach to this problem relies on the use of microbial or enzyme based lignin degradation processes. It is known that wood rotting fungi (e.g., the white rot fungus *Phanerochaete chrysosporum*) secrete enzymes like LP that catalyze depolymerization of lignin[3] through SET-promoted C−C bond cleavage pathways. Being a complex heterogeneous polymer, lignin is comprised of several major types of dimeric structural units containing propanoid moieties with 1-aryl-2-aryloxy (β-O-4), 1,2-diaryl (β-1), benzofuran (β-5), and spirodienone structural frameworks (Figure 3). It should be noted that the β-5 unit is a cyclized form of the β-1 structure and that spirodienone units[28] likely convert to β-1 moieties under the highly acidic conditions present in LP containing secretions of wood rotting the fungi.

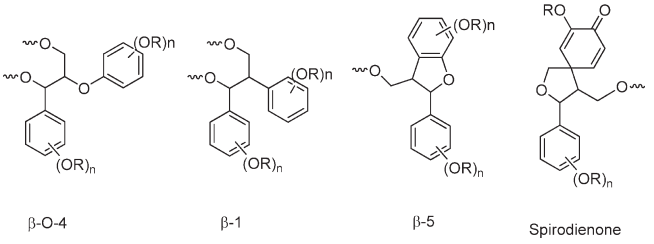
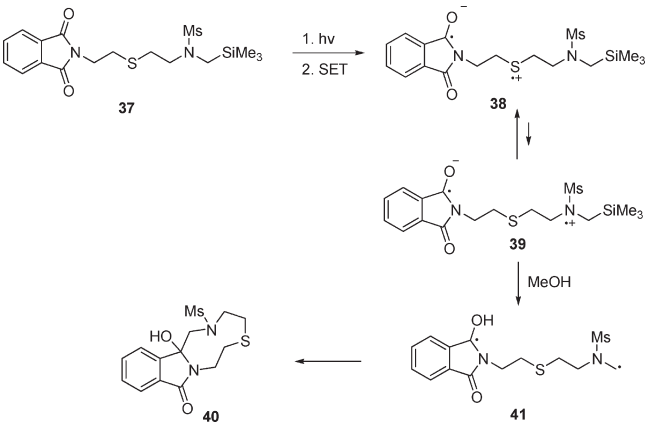


Figure 3

Cation radicals arising by SET from alkoxyaryl sites in lignin to the oxidized form of LP (termed LP I) are believed to undergo C−C bond cleavage to generate cation and radical intermediates, the latter of which are further oxidized under aerobic conditions. It is highly probable that the lignin cation radicals are comprised of a mixture of potentially rapidly interconverting species that differ in the location of the arene ring delocalized positively charged radical centers. As we have demonstrated in earlier studies[20] with more simple polydonor derived cation radicals, the sites at which bond cleavage reactions take place more rapidly in systems of this type are governed by the rates of the processes and not the relative populations of the interconverting species. An example taken from this earlier effort that demonstrates this principle is found in the SET-promoted photochemical reaction of the thioether linked, α-silymethansulfonamide terminated phthalimide **37** (Scheme 12). In this system, SET from the two possible heteroatom donor sites to the phthalimide excited state gives rise to two interconverting zwitterionic biradicals **38** and **39**, whose population is governed by differences in the oxidation potentials of the thioether (ca. +1.4 V)[29] and sulfonamide (ca. +2.0 V).[30] As a result, **38** is heavily favored at equilibrium. However, as product yield determinations show, the exclusive reaction pathway followed involves α-desilylation of **39** to form biradical **41**, which then undergoes cyclization to yield **40**. Thus, the much larger rate of methanol-promoted

Scheme 12



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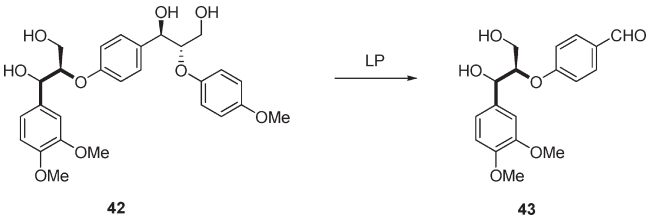
α-desilylation at the methansulfonamide cation radical site vs α-deprotonation at the thioether cation radical site is the major factor controlling the nature of the SET process.[31]

An example in which the rates of bond cleavage are important in governing product profiles of reactions proceeding through the intermediacy of competitively formed cation radicals that arise by SET from sites of differing oxidation potential is found in studies by Baciocchi and his co-workers.[22] Specifically, the major product produced in the LP-promoted reaction of the trimeric lignin model **42** (Scheme 13) is aldehyde **43**, formed by C−C bond cleavage at the monoalkoxy-substituted β-O-4 center, which in contrast to the dialkoxy substituted unit has a higher oxidation potential.

Scheme 13

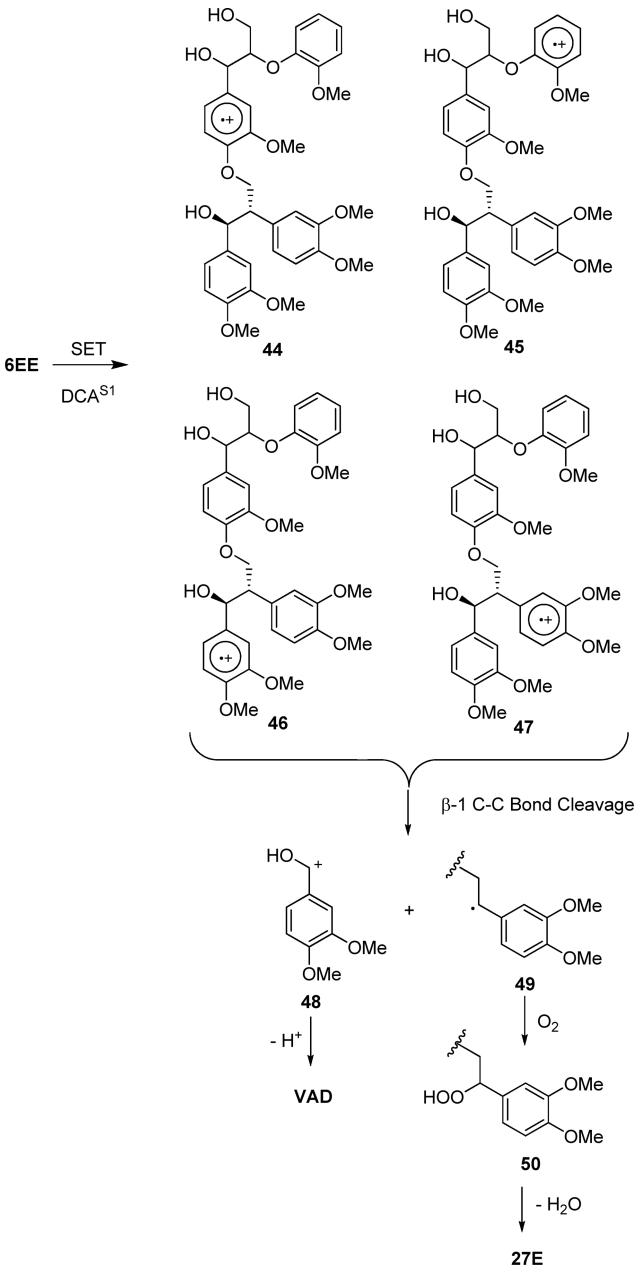


In the same manner, it is expected that preferences for C−C bond cleavage in reactions of lignin cation radicals will be governed by reactivity rather than population factors. In an earlier effort,[21] we probed reactions of cation radicals of simple dimeric lignin model compounds that possess either β-1 or β-O-4 structures that were generated by using DCA-photosensitized, CAN and LP initiated SET processes. The results showed that β-1 cation radicals undergo C−C bond cleavage at rates that far exceed those of their β-O-4 counterparts. We also determined that these findings are consistent with the results of DFT calculations, which indicate that the C−C bond dissociation energies of β-1 cation radicals are significantly lower than those of similarly substituted β-O-4 analogues.

The major aim of the current effort was to determine if the reactivity profiles observed with use of dimeric models can be employed to predict the site(s) of C−C bond cleavage in more complex lignin cation radicals. For this purpose, the tetrameric models **6EE** and **7TE**, which contain both β-1 and β-O-4 units, were prepared and subjected to SET-promoted reactions by using DCA-photosensitization and LP catalysis. It is important to note that **6EE** and **7TE** contain an array of alkoxy-substituted arene rings that should have nearly the same oxidation potentials. As a result, cation radicals of these substances are expected to exist as mixtures of interconverting, near equal energy positively charged radical species (Scheme 14).

Reaction Mechanism. The photosensitized reactions are initiated by SET from **6EE** and **7TE** to the singlet excited state of DCA, processes that take place at diffusion controlled rates as judged by the results of fluorescence quenching studies summarized in Table 1. Using **6EE** as an example, SET forms a mixture of four interconverting radical cations (**44**−**47**) that differ in the arene ring where the odd electron and positive charge are localized. Two of the four cation radicals, **46** and **47**, are capable of undergoing β-1 type C−C bond cleavage to generate the cation radical pair **48** + **49**, while one (**42**) could undergo β-O-4 type bond cleavage. That the former reaction pathway dominates in the SET photoreaction of **6EE** is reflected in the exclusive formation of **VAD** and α-aryloxyketone **27E**. **VAD** arises by

Scheme 14

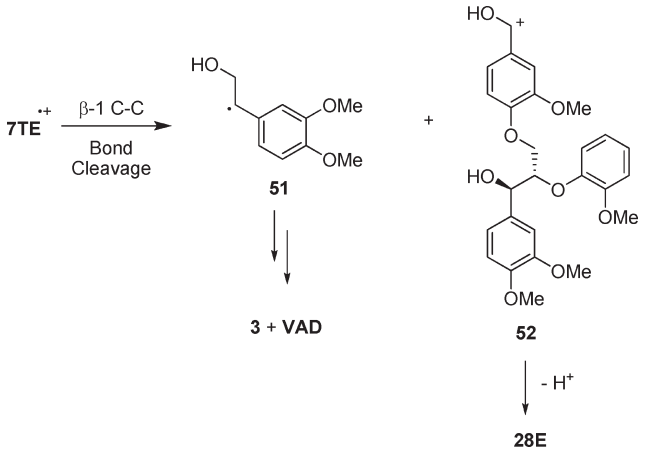


deprotonation of cation **48** while **27E** is produced by reaction of radical **49** with oxygen followed by loss of water from the resulting hydroperoxide **50**. Importantly, the failure to detect substances that would have been formed by β-O-4 type C−C bond cleavage of cation radical **44** further evidence the exclusive operation of β-1 type cleavage in **6EE**.

In a similar fashion, β-1 C−C bond fragmentation in the cation radical arising from **7TE** leads to formation of the radical cation pair **51** + **52** (Scheme 15). The major products formed in this process are aldehyde **28E**, coming from proton loss from cation **52**, and ketol **3**, arising by oxygen capture of radical **51** followed by dehydration of the resulting hydroperoxide. The production of **VAD** in this process is interesting since this substance could arise by β-O-4 type cleavage. However, another reasonable route exists for the formation of **VAD**

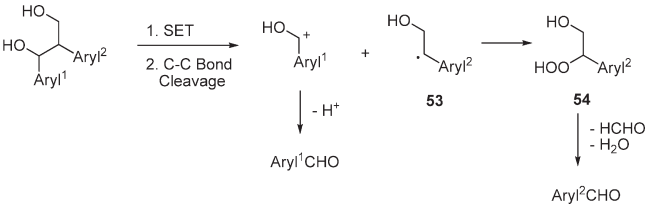
dx.doi.org/10.1021/jo200253v |J. Org. Chem. 2011, 76, 2840–2852

Scheme 15



following β-1 C−C bond cleavage of the **7TE** cation radical. This proposal is based on the results of unpublished studies with unsymmetrically substituted dimeric β-1 lignin model compounds (Scheme 16), which show that cation radical C−C bond cleavage gives aldehydes that originate from both aryl moieties. This observation suggests that the β-hydroxy-hydroperoxides **54** formed by oxygen addition to the C2 radical fragment **53** are capable of undergoing retro-aldol type cleavage in competition with dehydration to yield aldehyde products. Thus, radical **51**, produced by cation radical β-1 bond fragmentation (Scheme 15), is likely one source of **VAD** in the DCA photosenstitized reaction of **7TE**.

Scheme 16



LP-Catalyzed Processes. The outcomes of LP-catalyzed reactions of tetrameric lignin models **6EE** and **7TE** are remarkably similar to those of the DCA-sensitized processes, despite the fact that the reactions are carried out under quite different conditions. For example, LP-promoted reaction of **7TE**, occurring in pH 3.4 aq tartrate buffer containing 15% MeCN, produces a ca. 1:2.4−4 ratio of **VAD**+**3** and aldehyde **28E**. In contrast, a ca. 1:3 ratio of these products is generated in the DCA-sensitized photoreaction in 5% aq MeCN. Furthermore, although differences exist in the relative amounts of **VAD** and α-aryloxyketone **27E** formed in the photochemical (ca. 1:1) and LP (ca. 3−8:1) reactions of **6EE**, the reduced amount of **27E** generated in the former process is a likely consequence of its highly efficient photochemical reactivity (Scheme 10) and its UV-spectroscopic properties (λ_{max} 278 and 310 nm with absorption extending to 330 nm) that enable it to absorb light competitively with DCA under the photochemical reaction conditions. Very closely related direct irradiation reactions of 3,4-dimethoxyphenacyl phenol ethers have been observed to take place to generate the acetophenone derivative **35** and 1,4-diketone **36**. However, it is possible that a DCA-sensitized SET reaction of **27E** is responsible for the formation of **35** and **36**.

The chemical mechanism of the LP-catalyzed C−C bond cleavage reactions of **6EE** and **7TE** are the same as those for the DCA-sensitized processes. However, an interesting difference exists in the way the reactive cation radical intermediates are produced. In the photochemical process, SET occurs from the tertrameric substrates to the singlet excited state of DCA in a diffusion governed manner. However, as the kinetic data displayed in Figure 2 and Table 4 show, SET in the enzymatic reactions occurs in LP−substrate complexes. The K_M values for LP-promoted reactions of **6EE** (130 μM) and **7TE** (80 μM) reflect reasonably tight binding of these substrates that resemble those for C−C bond cleavage of simple dimeric lignin model compounds (50−250 μM)[21] and for oxidation of the monoarene ring containing substrate veratrylalcohol (72 μM).[6] It should be noted that, by using a resonant mirror biosensor system, it has been shown that a synthetic lignin formed by polymerization of coniferyl alcohol binds reversibly to both LP and its oxidized form, LP I, with respective dissociation constants of 330 and 350 μM.[12] The k_{cat} values for LP-catalyzed cleavage of **6EE** and **7TE** fall in the range of 0.3−0.4 s^{-1} and are approximately 1 order of magnitude smaller than those of simple β-1 dimeric model compounds (ca. 4−9 s^{-1}).[21]

On the basis of these observations, a plausible enzymatic mechanism for the β-1 C−C bond cleavage reactions involves SET in complexes of **6EE** and **7TE** with LP I. Since no structures of LP or LP I complexed with substrates or substrate analogues have been determined thus far, the exact manner in which the tetrameric models bind to LP I is not known. However, it is reasonable to expect that only one of the four arene rings of these substances is positioned close to the porphyrin-π-cation, which serves as the electron acceptor in LP I. The results of X-ray crystallographic studies conducted by Poulos and his co-workers[32] suggest that the heme moiety in LP is buried in the protein skeleton but modeling shows that the active site could accommodate the aromatic ring of verytrylalcohol. In addition, kinetic studies[33,34] have led to the conclusion that LP oxidizes natural lignins through a long-range SET pathway much in the same way that cytochrome *c* peroxidase oxidizes its substrates.[35,36] In any event, SET from the tetrameric models to LP I generates cation radicals that are initially localized in one arene ring but that can undergo delocalization by intramolecular SET. Two of the four possible cation radicals derived from either **6EE** or **7TE** (exemplified by **46** and **47** for **6EE** in Scheme 13) can participate in β-1 C−C bond fragmentation, whereas one of the other two (e.g., **44** for **6EE**) can only undergo less efficient β-O-4 cleavage. Since C−C bond cleavage in these cation radicals is expected to compete with back SET to regenerate LP I and nonoxidized substrate, the fact that less reactive or unreactive species comprise the mixture of cation radicals arising from **6EE** and **7TE** could be one of the reasons why the k_{cat} values for LP-promoted reactions of the tetrameric substrates are lower than those for dimeric β-1 models in which all cation radicals are reactive.

Summary. The results of this effort have provided interesting information about the regioselectivities of C−C bond cleavage reactions of cation radical intermediates formed by SET from tetrameric lignin model compounds that contain both β-1 and β-O-4 structural units. The findings could be relevant to LP-catalyzed reactions of natural lignins and, consequently, to the genetic design of plants that contain lignins that more efficiently undergo microbial and/or enzymatic delignification.

EXPERIMENTAL SECTION

General. All reagents were obtained from commercial sources and used without further purification and solvents were dried by using standard procedures. ^{1}H and ^{13}C NMR (500 MHz) spectra were recorded on $CDCl_3$ solutions and the chemical shifts of resonances are reported in parts per million relative to $CHCl_3$ (7.24 ppm in ^{1}H NMR, 77.0 ppm in ^{13}C NMR) serving as an internal standard. HRMS data were obtained by using electrospray ionization or fast atom bombardment. Photochemical reactions were conducted with an apparatus consisting of a 450 W Hanovia medium vapor pressure mercury lamp surrounded by a uranium glass filter in a water-cooled quartz immersion well and quartz glass tubes containing solutions of substrates in a merry-go-round photoreactor. All products were isolated as oils unless otherwise specified and the purity of each was determined to be >90% by ^{1}H and ^{13}C NMR analysis. Column chromatography was performed with 230−400 mesh silica gel. Identification of products from photochemical and enzymatic reactions was identified by comparing their spectroscopic and chromatographic properties with those of independently synthesized or commercially available compounds. Product yields were obtained by using HPLC analysis (a 4.6 mm diameter Restek Ultra Aqueous C-18 reverse phase column with a pore size of 5 μm, and a MeOH/H_2O gradient) based on calibration curves constructed by using known or synthesized substances.

Synthesis of *erythro*-1-(4-Hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-diol Acetonide (12E)

Diastereomeric ethyl 3-(4-(benzoyloxy)-3-methoxyphenyl)-2-(2-methoxyphenoxy)-3-hydroxypropionate (10E and 10T): A solution of diisopropylamine (4.6 mL, 32.8 mmol) in dry THF (40 mL) containing 13 mL (33 mmol) of 2.5 M *n*BuLi at −78 °C was stirred for 30 min. Acetate ester **8** (6.9 g, 32.8 mmol) was added dropwise and the resulting solution was stirred for 1 h followed by addition of aldehyde **9** (7.0 g, 27.3 mmol). After 3 h of additional stirring at the same temperature, the mixture was diluted with H_2O and extracted with EtOAc. The organic layer was dried and evaporated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:3) to yield erythro **10E** (3.2 g, 25%) and threo **10T** (2.9 g, 20%).

10E: ^{1}H NMR ($CDCl_3$) 1.15 (t, 3H, J = 7.5 Hz), 3.80 (s, 3H), 3.84 (s, 3H), 4.13 (q, 2H, J = 7 Hz), 4.75 (d, 1H, J = 5 Hz), 5.21 (d, 1H, J = 5 Hz), 6.83−7.19 (m, 7H), 7.48 (t, 2H, J = 7.5 Hz), 7.61 (t, 1H, J = 7.5 Hz), 8.19 (d, 2H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) 14.0, 55.8, 55.9, 61.3, 73.7, 83.7, 111.3, 112.4, 119.1, 119.2, 121.1, 122.5, 124.0, 128.5, 129.5, 130.3, 133.4, 138.1, 139.7, 147.1, 150.6, 151.1, 164.6, 169.2; HRMS (ES) m/z 489.1515 (M + Na, $C_{26}H_{26}O_8Na$ requires 489.1525).

10T: ^{1}H NMR ($CDCl_3$) 1.11 (t, 3H, J = 7 Hz), 3.80 (s, 3H), 3.85 (s, 3H), 4.08 (q, 2H, J = 7 Hz), 4.51 (d, 1H, J = 6.5 Hz), 5.13 (d, 1H, J = 7 Hz), 6.84−7.13 (m, 7H), 7.48 (t, 2H, J = 8 Hz), 7.61 (t, 1H, J = 7.5 Hz), 8.19 (d, 2H, J = 8 Hz); ^{13}C NMR ($CDCl_3$) 13.8, 55.7, 55.9, 61.3, 74.7, 85.0, 111.2, 112.3, 118.3, 119.3, 121.0, 122.5, 123.9, 128.4, 129.3, 130.2, 133.3, 137.3, 139.9, 147.2, 150.3, 151.3, 164.5, 169.3; HRMS (ES) m/z 489.1522 (M + Na, $C_{26}H_{26}O_8Na$ requires 489.1525).

***erythro*-1-(4-Hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-diol (11E):**[37]**.** To a solution of THF (50 mL) containing 1.0 M $LiAlH_4$ (7.0 mL, 7.0 mmol) was added **10E** (1.6 g, 3.4 mmol) at room temperature. After 3 h of stirring, H_2O (20 mL) and 1 N HCl (20 mL) were added at 0 °C and the solution was extracted with CH_2Cl_2. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:1) to yield **11E** (0.8 g, 73%). ^{1}H NMR ($CDCl_3$) 3.64 and 3.89 (dd, 2H, J = 3.5, 12 Hz), 3.86 (s, 3H), 3.87 (s, 3H), 4.14 (m, 1H), 4.95 (d, 1H, J = 4.5 Hz), 5.58 (s, 1H), 6.80−7.06 (m, 7H).

***erythro*-1-(4-Hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-diol acetonide (12E):** A solution of **11E** (4.7 g, 14.7 mmol) in CH_2Cl_2 (80 mL) containing pyridinium *p*-toluenesulfonate (0.74 g, 3.0 mmol) and 2,2-dimethoxypropane (7.64 g, 73.4 mmol) was stirred at room temperature for 8 h and concentrated in vauco to give a residue that was portioned between CH_2Cl_2 and sat. $NaHCO_3$. The organic layer was dried and concentrated in vacuo to give a residue, which was subjected to column chromatography (EtOAc:hexane 1:3) to yield **12E** (3.76 g, 71%). ^{1}H NMR ($CDCl_3$) 1.49 (s, 3H), 1.62 (s, 3H), 3.74 (s, 3H), 3.81 (s, 3H), 3.98−4.01 (m, 1H), 4.11−4.17 (m, 2H), 4.88 (d, 1H, J = 9 Hz), 5.55 (s, 1H), 6.48 (d, 1H, J = 8 Hz), 6.69 (t, 1H, J = 8 Hz), 6.77 (d, 1H, J = 8 Hz), 6.83−6.88 (m, 2H), 6.98 (s, 1H), 7.02 (d, 1H, J = 8 Hz); ^{13}C NMR 19.6, 28.5, 55.7, 55.8, 62.9, 74.6, 99.4, 109.9, 112.1, 114.0, 117.4, 120.5, 120.8, 122.7, 131.1, 145.4, 146.2, 147.1, 150.4; HRMS (ES) m/z 397.1630 (M + Na, $C_{21}H_{26}O_6Na$ requires 397.1627).

Synthesis of *erythro*-2-(2-Methoxyphenoxy)-3-(3,4-dimethoxyphenyl)-3-methoxymethyloxy-1-tosylpropane (23E)

Ethyl *erythro*-2-(2-methoxyphenoxy)-3-(3,4-dimethoxyphenyl)-3-methoxymethyloxypropionate (19E): A solution of **17E**[21] (0.64 g, 1.7 mmol) in THF (40 mL) containing diisopropylethylamine (2.4 mL, 13.6 mmol) and methoxymethyl ether (2.7 g, 34 mmol) was stirred at room temperature for 15 h and concentrated in vauco to give a residue that was portioned between CH_2Cl_2 and H_2O. The organic layer was dried and concentrated in vacuo to give a residue, which was subjected to column chromatography (EtOAc:hexane 1:3) to yield **19E** (3.76 g, 71%). ^{1}H NMR ($CDCl_3$) 1.24 (t, 3H, J = 7 Hz), 3.31 (s, 3H), 3.71 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 4.22 (q, 2H, J = 7 Hz), 4.57 (s, 2H), 4.66 (d, 1H, J = 7.5 Hz), 5.03 (d, 1H, J = 7.5 Hz), 6.59 (d, 1H, J = 8 Hz), 6.72 (t, 1H, J = 7.5 Hz), 6.78−6.83 (m, 2H), 6.89 (t, 1H, J = 8 Hz), 7.00 (d, 1H, J = 8 Hz), 7.05 (s, 1H); ^{13}C NMR ($CDCl_3$) 14.2, 55.7, 55.8, 61.2, 82.4, 93.9, 110.5, 111.0, 112.5, 117.3, 120.7, 120.8, 123.1, 129.8, 147.3, 148.7, 149.0, 150.4, 170.0; HRMS (ES) m/z 443.1689 (M + Na, $C_{22}H_{28}O_8Na$ requires 443.1682).

Ethyl *erythro*-2-(2-methoxyphenoxy)-3-(3,4-dimethoxyphenyl)-3-methoxymethyloxy-1-propanol (21E): To solution of THF (50 mL) containing 1.0 M $LiAlH_4$ (8.6 mL, 8.6 mmol) was added **19E** (3.6 g, 8.6 mmol) at room temperature. After 3 h of stirring, H_2O (20 mL) and 1 N HCl (20 mL) were added at 0 °C and the solution was extracted with CH_2Cl_2. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:2) to yield **21E** (2.1 g, 66%). ^{1}H NMR ($CDCl_3$) 3.39 (s, 3H), 3.80 (s, 3H), 3.80 (s, 3H), 3.82 (s, 3H), 3.82−3.94 (m, 2H), 4.10−4.13 (m, 1H), 4.59 (s, 2H), 4.88 (d, 1H, J = 7 Hz), 6.54 (d, 1H, J = 8 Hz), 6.75 (t, 1H, J = 7.5 Hz), 6.83 (d, 2H, J = 8 Hz), 6.93−6.96 (m, 3H); ^{13}C NMR ($CDCl_3$) 55.7, 55.8, 55.9, 61.4, 76.3, 87.0, 94.1, 110.5, 110.8, 112.0, 120.4, 120.5, 121.3, 123.6, 131.2, 147.6, 148.7, 148.9, 151.1; HRMS (ES) m/z 401.1578 (M + Na, $C_{20}H_{26}O_7Na$ requires 401.1576).

***erythro*-2-(2-Methoxyphenoxy)-3-(3,4-dimethoxyphenyl)-3-methoxymethyloxy-1-tosylpropane (23E):** To solution of CH_2Cl_2 (70 mL) containing **21E** (3.67 g, 9.7 mmol) was added triethylamine (4.1 mL, 29.0 mmol) at 0 °C. After 1 h of stirring, TsCl (2.8 g, 14.5 mmol) was added and the solution was stirred for 10 h at room temperature. The solution was extracted with CH_2Cl_2 and sat. $NaHCO_3$. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:2) to yield **23E** (3.7 g, 72%). ^{1}H NMR ($CDCl_3$) 2.40 (s, 3H), 3.31 (s, 3H), 3.67 (s, 3H), 3.80 (s, 3H), 3.84 (s, 3H), 4.30−4.33 (m, 1H), 4.37−4.40 (m, 1H), 4.45−4.48 (m, 1H), 4.52−4.56 (m, 2H), 4.84 (d, 1H, J = 6 Hz), 6.61 (d, 1H, J = 7 Hz), 6.70 (t, 1H, J = 7 Hz), 6.76 (d, 2H, J = 8 Hz), 6.84−6.90 (m, 3H), 7.24 (d, 2H, J = 8 Hz), 7.68 (d, 2H, J = 8 Hz); ^{13}C NMR ($CDCl_3$) 21.6, 55.6, 55.8, 55.8, 55.9, 68.5, 76.2, 81.3, 94.4, 110.6, 110.7, 112.3, 118.8, 120.5, 120.8, 123.1, 128.0, 129.7, 129.8, 132.8, 144.6, 147.1, 148.8, 150.7; HRMS (ES) m/z 555.1666 (M + Na, $C_{27}H_{32}O_9NaS$ requires 555.1665).

Synthesis of *erythro*-2,3-(3,4-Dimethoxyphenyl)-3-methoxymethyloxy-1-tosylpropane (24E)

Ethyl *erythro*-2,3-(dimethoxyphenyl)-3-methoxymethyloxypropionate (20E): A solution of the **18E**[21] (2.5 g, 6.4 mmol) in

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THF (40 mL) containing diisopropylethylamine (8.9 mL, 51.2 mmol) and methoxymethyl ether (10.3 g, 128.1 mmol) was stirred at room temperature for 15 h and concentrated in vauco to give a residue that was portioned between CH_2Cl_2 and H_2O. The organic layer was dried and concentrated in vacuo to give a residue, which was subjected to column chromatography (EtOAc:hexane 1:3) to yield **20E** (2.0 g, 72%). ^{1}H NMR ($CDCl_3$) 1.26 (t, 3H, J = 7 Hz), 3.34 (s, 3H), 3.72 (s, 3H), 3.73 (s, 3H), 3.75 (s, 3H), 3.76 (s, 3H), 3.84−3.86 (m, 1H), 4.11−4.16 (m, 1H), 4.21−4.26 (m, 1H), 4.51 (s, 2H), 5.03 (d, 1H, J = 10.5 Hz), 6.54−6.61 (m, 5H), 6.67 (s, 1H); ^{13}C NMR ($CDCl_3$) 14.1, 55.7, 55.7, 55.9, 58.8, 60.9, 79.9, 94.0, 110.0, 110.3, 110.6, 111.5, 120.5, 121.2, 127.2, 130.6, 148.2, 148.4, 148.5, 148.6, 172.6; HRMS (ES) m/z 457.1837 (M + Na, $C_{23}H_{30}O_8Na$ requires 457.1838).

***erythro*-2,3-(Dimethoxyphenyl)-3-methoxymethyloxy-1-propanol (22E):** To solution of THF (50 mL) containing 1.0 M $LiAlH_4$ (4.9 mL, 4.9 mmol) was added **20E** (2.1 g, 4.8 mmol) at room temperature. After 3 h of stirring, H_2O (20 mL) and 1 N HCl (20 mL) were added at 0 °C and the solution was extracted with CH_2Cl_2. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:1) to yield **22E** (1.05 g, 62%). ^{1}H NMR ($CDCl_3$) 3.04−3.07 (m, 1H), 3.43 (s, 3H), 3.71 (s, 3H), 3.74 (s, 3H), 3.78 (s, 3H), 3.79 (s, 3H), 3.91−3.96 (m, 1H), 4.15−4.19 (m, 1H), 4.49−4.53 (m, 2H), 4.78 (d, 1H, J = 10 Hz), 6.43 (s, 1H), 6.52−6.55 (m, 2H), 6.61−6.66 (m, 3H); ^{13}C NMR ($CDCl_3$) 54.5, 55.7, 55.8, 56.0, 66.0, 82.0, 93.8, 110.0, 110.3, 110.9, 111.8, 120.3, 120.5, 131.5, 131.8, 147.7, 148.4, 148.5, 148.6; HRMS (ES) m/z 415.1735 (M + Na, $C_{21}H_{28}O_7Na$ requires 415.1733).

***erythro*-2,3-(3,4-Dimethoxyphenyl)-3-methoxymethyloxy-1-tosylpropane (24E):** To solution of CH_2Cl_2 (60 mL) containing **22E** (1.6 g, 4.1 mmol) was added triethylamine (1.7 mL, 12.2 mmol) at 0 °C. After 1 h of stirring, TsCl (1.2 g, 6.1 mmol) was added and the solution was stirred for 10 h at room temperature. The solution was extracted with CH_2Cl_2 and sat. $NaHCO_3$. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:1) to yield **24E** (1.5 g, 66%). ^{1}H NMR ($CDCl_3$) 2.34 (s, 3H), 3.17−3.21 (m, 1H), 3.29 (s, 3H), 3.64 (s, 3H), 3.70 (s, 3H), 3.78 (s, 6H), 4.41−4.51 (m, 2H), 4.46 (s, 2H), 4.68 (d, 1H, J = 8.5 Hz), 6.33 (s, 1H), 6.39 (d, 1H, J = 7.5 Hz), 6.51−6.62 (m, 4H), 7.21 (d, 2H, J = 8 Hz), 7.57 (d, 2H, J = 8 Hz); ^{13}C NMR ($CDCl_3$) 21.6, 51.2, 55.6, 55.7, 56.0, 70.7, 78.1, 94.0, 110.1, 110.3, 110.5, 111.7, 120.3, 121.2, 127.8, 129.6, 129.8, 131.0, 132.8, 144.5, 147.8, 148.3, 148.4, 148.5; HRMS (ES) m/z 569.1808 (M + Na, $C_{28}H_{34}O_9NaS$ requires 569.1821).

Synthesis of Tetrameric Model *β*-O-4 (top)−*β*-1 (bottom) (6EE)

Tetrameric model *β*-O-4 actonide (top)−*β*-1 MOM (bottom) (25EE): To a solution of CH_3CN (60 mL) containing **12E** (3.9 g, 10.8 mmol) was added NaH (60% in mineral oil) (450 mg, 11.3 mmol) at room temperature. After 1 h of stirring at 80 °C, **24E** (3.94 g, 7.2 mmol) was added and the solution was stirred for 24 h at the same temperature. The solution was concentrated following extraction with CH_2Cl_2 and H_2O. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:1) to yield the diastereomeric mixture **25EE** (2.54 g, 48%). Diastereomeric mixture: ^{1}H NMR ($CDCl_3$) 1.49 (s, 3H), 1.62 (s, 3H), 3.22 (s, 3H), 3.32−3.36 (m, 1H), 3.66 (s, 3H), 3.72 (s, 6H), 3.74 (s, 3H), 3.76 (s, 3H), 3.78 (s, 3H), 3.98−4.01 (m, 1H), 4.08−4.17 (m, 2H), 4.23−4.25 (m, 1H), 4.46−4.50 (m, 1H), 4.50 (s, 2H), 4.88 (d, 1H, J = 8.5 Hz), 4.98−5.01 (m, 1H), 6.45 (d, 1H, J = 8 Hz), 6.51 (d, 1H, J = 8 Hz), 6.55 (s, 1H), 6.59−6.66 (m, 4H), 6.75−6.85 (m, 4H), 7.00 (s, 2H); ^{13}C NMR ($CDCl_3$) 19.6, 28.5, 51.5, 55.6, 55.9, 62.8, 69.3, 74.5, 77.1, 78.1, 94.1, 99.4, 110.2, 110.4, 111.4, 112.0, 112.6, 113.1, 117.3, 117.4, 119.8, 119.9, 120.3, 121.4, 122.6, 131.7, 132.0, 147.0, 147.5, 148.1, 148.3, 149.4, 150.3; HRMS (ES) m/z 757.3195 (M + Na, $C_{41}H_{50}O_{12}Na$ requires 757.3200).

Tetrameric model *β*-1 (top)−*β*-O-4 (bottom) (6EE): To a solution of THF (60 mL) containing **25EE** (1.2 g, 1.63 mmol) was added 3 N HCl (20 mL) at room temperature and the solution was stirred for 12 h at the room temperature. The solution was concentrated following extraction with CH_2Cl_2 and 1 N HCl. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 2:1) to yield the diastereomeric mixture **6EE** (230 mg, 22%). Diastereomeric mixture: ^{1}H NMR ($CDCl_3$) 3.32−3.36 (m, 1H), 3.69 (s, 3H), 3.72 (s, 3H), 3.77 (s, 3H), 3.78 (s, 3H), 3.85 (s, 6H), 3.85−3.91 (m, 1H), 4.11−4.17 (m, 2H), 4.28−4.31 (m, 1H), 4.41−4.43 (m, 1H), 4.94−4.97 (m, 1H), 5.18 (d, 1H, J = 5 Hz), 6.61−7.05 (m, 13H); ^{13}C NMR ($CDCl_3$) 52.3, 55.6, 557, 55.8, 55.9, 60.6, 72.6, 77.5, 87.2, 109.5, 109.7, 110.4, 110.8, 112.1, 112.2, 114.1, 118.4, 119.1, 120.8, 121.6, 124.2, 131.7, 133.9, 135.1, 146.7, 147.8, 148.0, 148.1, 148.4, 148.4, 149.7, 151.5; HRMS (ES) m/z 673.2632 (M + Na, $C_{36}H_{42}O_{11}Na$ requires 673.2625).

Synthesis of Tetrameric Model *β*-1 (top)−*β*-O-4 (bottom) (7TE)

Tetrameric model *β*-1 acetonide (top)−*β*-O-4 MOM (bottom) (26TE): To a solution of CH_3CN (60 mL) containing **16T** (1.34 g, 3.6 mmol) was added NaH (60% in mineral oil) (143 mg, 3.6 mmol) at room temperature. After 1 h of stirring at 80 °C, **23E** (1.6 g, 3.0 mmol) was added and the solution was stirred for 24 h at the same temperature. The solution was concentrated following extraction with CH_2Cl_2 and H_2O. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:1) to yield the diastereomeric mixture **26TE** (1.2 g, 55%). Diastereomeric mixture: ^{1}H NMR ($CDCl_3$) 1.62 (s, 3H), 1.64 (s, 3H), 3.30 (s, 3H), 3.49 and 3.51 (s, 3H), 3.65 and 3.66 (s, 3H), 3.75 (s, 6H), 3.77 and 3.78 (s, 3H), 3.83 (s, 3H), 3.75−3.78 (m, 1H), 4.08 (d, 1H, J = 7 Hz), 4.10−4.15 (m, 1H), 4.18−4.22 (m, 1H), 4.50−4.53 (m, 1H), 4.57−4.64 (m, 2H), 4.71−4.75 (m, 1H), 5.02 (d, 1H, J = 5 Hz), 5.28 (d, 1H, J = 3 Hz), 6.37 (d, 1H, J = 16.5 Hz), 6.50−6.61 (m, 4H), 6.76−6.78 (m, 3H), 6.86−6.98 (m, 4H), 7.10 (s, 1H); ^{13}C NMR ($CDCl_3$) 18.8, 29.9, 45.0, 55.6, 55.8, 65.4, 67.9, 73.6, 82.0, 94.4, 99.3, 110.0, 110.4, 110.9, 112.1, 112.8, 112.9, 113.2, 118.2, 118.2, 120.5, 120.6, 120.7, 122.0, 122.3, 130.2, 130.3, 132.5, 133.5, 147.2, 147.3, 147.9, 147.9, 148.5, 148.6, 149.0, 150.6; HRMS (ES) m/z 757.3190 (M + Na, $C_{41}H_{50}O_{12}Na$ requires 757.3200).

Tetrameric model *β*-1 (top)−*β*-O-4 (bottom) (7TE): To a solution of THF (60 mL) containing **26TE** (0.24 g, 0.33 mmol) was added 3 N HCl (10 mL) at room temperature and the solution was stirred for 12 h at the room temperature. The solution was concentrated following extraction with CH_2Cl_2 and 1 N HCl. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 2:1) to yield the diastereomeric mixture **7TE** (43 mg, 21%). Diastereomeric mixture: ^{1}H NMR ($CDCl_3$) 3.00−3.05 (m, 1H), 3.70 (s, 3H), 3.78, (s, 3H), 3.81 (s, 3H), 3.84 (s, 6H), 3.88 (s, 3H), 3.92−3.94 (m, 1H), 3.98−4.00 (m, 1H), 4.12−4.21 (m, 2H), 4.53−4.57 (m, 1H), 4.87−4.90 (m, 1H), 4.97−5.01 (m, 1H), 6.45−6.56 (m, 2H), 6.64−6.82 (m, 5H), 6.87−6.91 (m, 3H), 6.97−7.05 (m, 2H), 7.15−7.18 (m, 1H); ^{13}C NMR ($CDCl_3$) 54.5, 55.4, 55.7, 55.8, 64.2, 66.3, 68.2, 68.3, 72.9, 75.6, 79.3, 84.8, 109.5, 110.0, 110.8, 111.0, 111.3, 112.1, 112.2, 118.7, 118.9, 120.4, 120.9, 121.1, 121.4, 123.9, 130.8, 132.1, 147.3, 147.6, 148.3, 148.6, 148.8, 149.0, 149.2, 149.5, 151.4; HRMS (ES) m/z 673.2628 (M + Na, $C_{36}H_{42}O_{11}Na$ requires 673.2625).

Synthesis of Potential Degradation Products of Tetrameric Model Compounds 27E, 28E, 31E, and 32

Compound 30E: To a solution of CH_3CN (60 mL) containing **12E** (2.5 g, 6.94 mmol) was added NaH (60% in mineral oil) (280 mg, 6.94 mmol) at room temperature. After 1 h of stirring at 80 °C, **29** (2.7 g, 10.4 mmol) was added and the solution was stirred for 24 h at the same temperature. The solution was concentrated following extraction with CH_2Cl_2 and H_2O. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography

(EtOAc:hexane 1:3) to yield **30E** (2.1 g, 55%). ^{1}H NMR ($CDCl_3$) 1.45 (s, 3H), 1.61 (s, 3H), 3.71 (s, 3H), 3.82 (s, 3H), 3.90 (s, 3H), 3.93 (s, 3H), 3.95−4.01 (m, 1H), 4.10−4.12 (m, 1H), 4.12−4.16 (m, 2H), 4.88 (d, 1H, J = 8.5 Hz), 5.22 (s, 2H), 6.45 (d, 1H, J = 8 Hz), 6.66 (t, 1H, J = 7.5 Hz), 6.75−6.77 (m, 2H), 6.83−6.87 (m, 2H), 6.99 (d, 1H, J = 8.5 Hz), 7.03 (s, 1H), 7.56 (s, 1H), 7.63 (d, 1H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) 19.6, 28.5, 55.7, 55.8, 56.0, 56.1, 62.8, 72.1, 74.4, 99.4, 110.1, 110.4, 111.2, 112.0, 114.2, 117.4, 119.7, 120.7, 122.7, 122.8, 127.8, 133.4, 147.0, 147.2, 149.1, 149.3, 150.3, 153.7, 193.2; HRMS (ES) m/z 561.2098 (M + Na, $C_{30}H_{34}O_9Na$ requires 561.2101).

Compound 27E: A solution of **30E** (0.54 g, 1.0 mmol) in acetone:H_2O (v/v 3:2) (30 mL) containing pyridinium p-toluenesulfonate (0.76 g, 3.0 mmol) was stirred at 80 °C for 10 h and concentrated in vauco to give a residue that was portioned between EtOAc and sat. $NaHCO_3$. The organic layer was dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:1) to yield **27E** (0.38 g, 77%). ^{1}H NMR ($CDCl_3$) 3.62 (d, 1H, J = 12 Hz), 3.86 (s, 3H), 3.86−3.88 (m, 1H), 3.91 (s, 3H), 3.93 (s, 3H), 4.10−4.14 (m, 1H), 4.95 (d, 1H, J = 4 Hz), 5.26 (s, 2H), 6.76−6.81 (m, 2H), 6.87−6.94 (m, 4H), 6.98 (s, 1H), 7.03−7.06 (m, 1H), 7.56 (s, 1H), 7.64 (d, 1H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) 55.9, 56.0, 56.1, 60.7, 72.0, 72.6, 87.4, 109.9, 110.1, 110.3, 112.1, 114.3, 118.3, 121.2, 121.6, 122.7, 124.3, 127.7, 133.9, 146.8, 146.9, 149.2, 149.7, 151.6, 153.8, 193.1; HRMS (ES) m/z 521.1799 (M + Na, $C_{27}H_{30}O_9Na$ requires 521.1788).

Compound 33E: To a solution of 50 mL of THF containing 1.0 M $LiAlH_4$ (4.0 mL, 4.0 mmol) was added **30E** (2.0 g, 3.7 mmol) at room temperature. After the mixture was stirred for 3 h, 20 mL of H_2O and 20 mL of 1 N HCl solution at 0 °C were added and the solutions were extracted with CH_2Cl_2. The extracts were dried and concentrated in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 1:2) to yield **33E** (1.52 g, 76%). ^{1}H NMR ($CDCl_3$) 1.50 (s, 3H), 1.62 (s, 3H), 3.74 (s, 3H), 3.82 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 3.88−3.93 (m, 1H), 4.00−4.02 (m, 1H), 4.08−4.10 (m, 1H), 4.11−4.18 (m, 2H), 4.91 (d, 1H, J = 9 Hz), 4.98 (d, 1H, J = 7.5 Hz), 6.47 (d, 1H, J = 6.5 Hz), 6.69 (t, 1H, J = 7.5 Hz), 6.77 (d, 1H, J = 8.5 Hz), 6.82−6.90 (m, 4H), 6.96 (s, 1H), 7.04−7.06 (m, 2H); ^{13}C NMR ($CDCl_3$) 19.6, 25.6, 28.5, 55.7, 55.8, 55.8, 55.9, 62.8, 68.0, 71.9, 74.4, 76.6, 99.5, 109.3, 110.9, 111.1, 111.2, 112.1, 115.8, 115.9, 117.3, 118.5, 119.9, 120.0, 120.7, 122.7, 132.0, 133.7, 147.0, 147.6, 147.6, 148.7, 149.0, 149.8, 150.3; HRMS (ES) m/z 563.2262 (M + Na, $C_{30}H_{36}O_9Na$ requires 563.2257).

Compound 31E: A solution of **33E** (1.54 g, 2.85 mmol) in acetone:H_2O (v/v 3:2) (80 mL) containing pyridinium p-toluenesulfonate (2.15 g, 8.6 mmol) was stirred at 80 °C for 24 h and concentrated in vauco to give a residue that was portioned between EtOAc and sat. $NaHCO_3$. The organic layer was dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:1) to yield **31E** (1.15 g, 81%). ^{1}H NMR ($CDCl_3$) 3.62 (dd, 1H, J = 3, 12.5 Hz), 3.84 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 3.89−3.91 (m, 1H), 3.94 (dd, 1H, J = 4, 10 Hz), 4.09−4.14 (m, 2H), 4.96 (d, 1H, J = 5 Hz), 5.02 (d, 1H, J = 9.5 Hz), 6.82−7.06 (m, 10H); ^{13}C NMR ($CDCl_3$) 55.8, 55.9, 60.6, 72.0, 72.5, 76.2, 87.3, 109.3, 109.7, 111.0, 112.1, 115.3, 118.6, 121.0, 121.6, 124.3, 132.1, 134.1, 146.7, 147.3, 148.7, 149.0, 149.9, 151.6; HRMS (ES) m/z 523.1942 (M + Na, $C_{27}H_{32}O_9Na$ requires 523.1944).

Compound 34E: To a solution of CH_3CN (50 mL) containing vanilin (0.4 g, 2.6 mmol) was added NaH (60% in mineral oil) (105 mg, 2.6 mmol) at room temperature. After 1 h of stirring at 80 °C, **23E** (0.93 g, 1.75 mmol) was added and the solution was stirred for 24 h at the same temperature. The solution was concentrated following extraction with CH_2Cl_2 and H_2O. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:3) to yield **34E** (680 mg, 76%). ^{1}H NMR ($CDCl_3$) 3.34 (s, 3H), 3.68 (s, 3H), 3.79 (s, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 4.34−4.37 (m, 1H), 4.42−4.45 (m, 1H), 4.65 (dd, 2H, J = 7, 26.3 Hz), 4.75−4.78 (m, 1H), 5.08 (d, 1H, J = 5 Hz), 6.80 (d, 3H, J = 8 Hz), 6.89−6.96 (m, 5H), 7.35−7.37 (m, 2H); ^{13}C NMR ($CDCl_3$) 55.6, 55.7, 55.8, 68.0, 82.4, 94.6, 109.2, 110.6, 111.8, 112.1, 118.8, 120.3, 120.8, 122.8, 126.6, 130.1, 130.2, 147.8, 148.7, 148.8, 150.0, 150.8, 153.9190.9; HRMS (ES) m/z 535.1935 (M + Na, $C_{28}H_{32}O_9Na$ requires 535.1944).

Compound 28E: To a solution of THF (60 mL) containing **34E** (0.64 g, 1.2 mmol) was added 3 N HCl (10 mL) at room temperature and the solution was stirred for 12 h at the room temperature. The solution was concentrated following extraction with CH_2Cl_2 and 1 N HCl. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 2:1) to yield the diastereomeric mixture **28E** (240 mg, 43%). ^{1}H NMR ($CDCl_3$) 3.79 (s, 3H), 3.84 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 4.11−4.14 (m, 1H), 4.30−4.33 (m, 1H), 4.61−4.64 (m, 1H), 5.02 (d, 1H, J = 3.5 Hz), 6.79−6.83 (m, 2H), 6.88−6.94 (m, 4H), 6.98 (s, 1H), 7.03−7.07 (m, 1H), 7.21−7.23 (m, 1H), 7.34−7.38 (m, 1H), 9.82 (s, 1H); ^{13}C NMR ($CDCl_3$) 29.7, 55.7, 55.8, 55.8, 55.9, 67.8, 72.5, 84.9, 109.2, 109.3, 110.9, 111.8, 112.1, 118.5, 121.2, 121.5, 124.2, 126.6, 130.3, 131.7, 147.1, 148.4, 148.9, 149.9, 151.5, 153.6, 190.9; HRMS (ES) m/z 491.1684 (M + Na, $C_{26}H_{28}O_8Na$ requires 491.1682).

Compound 32:[38]. To a solution of CH_3CN (50 mL) containing vanillin (2.2 g, 14.5 mmol) was added NaH (60% in mineral oil) (580 mg, 14.5 mmol) at room temperature. After 1 h of stirring at 80 °C, **29** (2.5 g, 9.6 mmol) was added and the solution was stirred for 24 h at the same temperature. The solution was concentrated following extraction with CH_2Cl_2 and H_2O. The organic extracts were dried and concentrated in vacuo to give a residue that was subjected to column chromatography (EtOAc:hexane 1:3) to yield **32** (2.2 g, 70%). ^{1}H NMR ($CDCl_3$) 3.92 (s, 3H), 3.95 (s, 6H), 5.42 (s, 2H), 6.83 (d, 1H, J = 8.5 Hz), 6.90 (d, 1H, J = 8 Hz), 7.36 (d, 1H, J = 8 Hz), 7.43 (s, 1H), 7.63 (s, 1H), 7.63 (d, 1H, J = 8.5 Hz), 9.82 (s, 1H); ^{13}C NMR ($CDCl_3$) 56.0, 56.0, 56.1, 71.1, 109.7, 110.1, 110.2, 112.4, 122.6, 126.3, 127.3, 130.8, 149.3, 149.9, 152.8, 154.1, 190.8, 191.8; HRMS (ES) m/z 353.0996 (M + Na, $C_{18}H_{18}O_6Na$ requires 353.1001).

DCA Fluorescence Quenching by Tetrameric Lignin Models 6EE and 7TE. Fluorescence spectra were recorded on 2 mL of MeCN solutions of DCA (5.4×10^{-6} M) each containing 0, 0.25, 0.5, 1.0, 2.0 mM of the respective tetrameric lignin model compounds. The excitation wavelength was 400 nm.

DCA-Promoted Photoreactions of Tetrameric Lignin Models 6EE and 7TE. Independent DCA-saturated, O_2-purged solutions containing each of the tetrameric lignin model compounds (0.22 mM of **6EE**, 0.46 mM of **7TE**) in 4 mL of 5% aqueous MeCN in quartz tubes were simultaneously irradiated by using uranium-filtered light in a merry-go-round apparatus for time periods of 0.5, 1, 1.5, 2, 3, 4, and 5 h. Each photolysate was subjected to HPLC analysis, giving the yields reported in Table 2 and Figure 1.

Photoreaction of 27E. A DCA-saturated, O_2-purged solution of **27E** (150 mg, 0.3 mmol) in 150 mL of 5% aqueous MeCN was irradiated by using uranium glass filtered light for 6 h (50% conversion). Concentration of the photolysate gave a residue that was subjected to silica gel chromatography (1:3 EtOAc−hexane) to yield **35** (3 mg, 10%) and **36**[25,39] (38 mg, 70%).

DCA-Promoted Photoreaction of 2. A 7 mL sample of a DCA-saturated, 5% aq MeCN solution containing **2** (6.6 mg, 3.3×10^{-5} mol, 4.75 mM) was added to a quartz tube and the tube was sealed. After bubbling with O_2 gas for 5 min, uranium-filtered UV light was irradiated for 7 h, which gave ca. 96% conversion of **2**. The photolysate was subjected to HPLC to yield **VAD** (90%) and **3** (10%).

Lignin Peroxidase (LP)-Catalyzed Reactions of Tetrameric Lignin Models 6EE and 7TE. To 200 μL of 50 mM tartrate buffer (pH 3.4) were added 200 μL of tetrameric lignin models (0.5 mM dissolved in 17% MeCN−tartrate buffer, final concentration 0.2 mM) and 40 μL of lignin peroxidase (100.5 μM, final concentration 8 μM, 17.2 units per mL). After 60 μL of H_2O_2 (10 mM, final concentration

1.2 mM) was added, the solutions were agitated for 30 min and then subjected to HPLC analysis to yield the following products: from **6EE** (16% conversion), **VAD** (16%) and **27E** (2%); and from **7TE** (7% conversion), **VAD** (1%) and **28E** (4%). For high conversion, an additional 60 μL of H_2O_2 was added and the solutions were agitated for 30 min again and then subjected to HPLC analysis to yield the following products: from **6EE** (31% conversion), **VAD** (25%) and **27E** (8%); and from **7TE** (18% conversion), **VAD** (4%), **28E** (12%), and **3** (1%).

Determination of Steady State Kinetic Constants of LP-Catalyzed Reactions of Tetrameric Models 6EE and 7TE. LP reactions were carried out by monitoring the formation of bond cleavage products at 310 nm. Reactions were performed in 50 mM tartate buffer (pH 3.4 at 25 °C) with fixed concentrations of LP (0.26 μM for **6EE** and **7TE**), varying concentrations of substrate dissolved in 25% MeCN−buffer solution, and initiated by the addition of a fixed concentration of H_2O_2 (50 μM). For all measurements, the initial velocity data, measured as a function of substrate concentration, were analyzed by using the following equations: $V = V_{max}[S]/([S] + K_M)$, where V is initial velocity, V_{max} is maximum velocity, [S] is substrate concentration, and K_M is the Michaelis constant. The k_{cat} was calculated from $V_{max}/[E]$, where [E] is the total enzyme concentration.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H and ^{13}C NMR spectra of all previously unidentified compounds, and DCA fluorescence quenching plots. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: cdw@pusan.ac.kr and mariano@unm.edu.

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■ REFERENCES

(1) Adler, E. *Wood Sci. Technol.* **1977**, *11*, 169–218.
(2) Sarkanen, K. V. *Lignins: Occurrence, Formation, Structure and Reactions*: Sarkanen, K. V., Ludwig, C. H., Eds.; Wiley-Interscience: New York, 1971; pp 95−495.
(3) Hammel, K. E.; Jensen, K. A.; Mozuch, M. D.; Landucci, L. L.; Tien, M.; Pease, E. A. *J. Biol. Chem.* **1993**, *268*, 12274.
(4) Tien, M.; Kirk, T. K. *Science* **1983**, *221*, 661.
(5) Harvey, P. J.; Schoemaker, H. E.; Bowen, R. M.; Palmer, J. M. *FEBS Lett.* **1985**, *183*, 13.
(6) Tien, M.; Kirk, T. K.; Bull, C.; Fee, J. A. *J. Biol. Chem.* **1986**, *261*, 1687.
(7) Schmidt, H. W.; Haemmerli, S. D.; Schoemaker, H. E.; Leisolar, M. S. *Biochemistry* **1989**, *28*, 1776.
(8) Hammel, K. E.; Kalyanaraman, B.; Kirk, T. K. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3708.
(9) Glenn, J. K.; Morgan, M. A.; Mayfield, M. B.; Kuwahara, M.; Gold, M. H. *Biochem. Biophys. Res. Commun.* **1983**, *114*, 1077.
(10) Umezawa, T.; Higuchi, T. *FEBS Lett.* **1987**, *218*, 255.
(11) Kawai, S.; Shoji, S. I.; Nabeta, K.; Okuyama, H.; Higuchi, T. *Mokuzai Gakkaishi* **1990**, *36*, 126.
(12) Johjima, T.; Itoh, N.; Kabuto, M.; Tokimura, F.; Nakagawa, T.; Wariishi, H.; Tanaka, H. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1989.
(13) Have, R. T.; Teunissen, P. J. M. *Chem. Rev.* **2001**, *101*, 3397.
(14) de Wild, P.; Van der Laan, R.; Kloekhorst, A.; Heeres, E. *Environ. Prog. Sustainable Energy* **2009**, *28*, 461.
(15) Dabo, P.; Cyr, A.; Lessard, J.; Brossard, L.; Menard, H. *Can. J. Chem.* **1999**, *77*, 1225.
(16) Hu, W. J.; Kawaoka, A.; Tsai, C. J.; Lung, J.; Osakabe, K.; Ebinuma, H.; Chiang, V. L. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 5407.
(17) Ralph, J.; MacKay, J. J.; Hatfield, R. D.; O'Malley, D. M.; Whetten, R. W. *Science.* **1997**, *277*, 235.
(18) Ralph, J.; Kim, H.; Peng, J.; Lu, F. *Org. Lett.* **1999**, *1*, 323.
(19) Tsai, C. J.; Podila, G. K.; Chiang, V. L. *Plant Cell Rep.* **1994**, *14*, 94.
(20) Yoon, U. C.; Kwon, H. C.; Hyung, T. G.; Choi, K. H.; Oh, S. W.; Yang, S.; Zhao, Z.; Mariano, P. S. *J. Am. Chem. Soc.* **2004**, *126*, 1110.
(21) Cho, D. W.; Parthasarathi, R.; Pimentel, A. S.; Maestas, G. D.; Park, H. J.; Yoon, U. C.; Dunaway-Mariano, D.; Gnanakaran, S.; Langan, P.; Mariano, P. S. *J. Org. Chem.* **2010**, *75*, 6549.
(22) Baciocchi, E.; Fabbri, C.; Lanzalunga, O. *J. Org. Chem.* **2003**, *68*, 9061.
(23) Langer, V.; Lundquist, K. *Acta Crystallogr., Sect. E: Struct. Rep. Online* **2002**, *E58* (4), 433.
(24) Stomberg, R.; Lundquist, K. *Nord. Pulp Paper Res. J.* **1994**, *9* (1), 37.
(25) Castellan, A.; Zhu, J. H.; Colombo, N.; Nourmamode, A.; Davidson, R. S.; Dunn, L. *J. Photochem. Photobiol., A* **1991**, *58*, 263.
(26) Ciofi-Baffoni, S.; Banci, L.; Brandi, A. *J. Chem. Soc., Perkin Trans. 1* **1998**, *1*, 3207.
(27) Banci, L.; Ciofi-Baffoni, S.; Tien, M. *Biochemistry* **1999**, *38*, 3205.
(28) Setala, H.; Pajunen, A.; Rummakko, P.; Brunow, G. *J. Chem. Soc., Perkin Trans. 1* **1999**, *461*.
(29) Cottrell, P. T.; Mann, C. K. *J. Electrochem. Soc.* **1969**, *116*, 1499.
(30) Yoon, U. C.; Kim, J. W.; Ryu, J. Y.; Cho, S. J.; Oh, S. W. *J. Photochem. Photobiol., A* **1997**, *106*, 145.
(31) Su, Z.; Mariano, P. S.; Falvey, D. E.; Yoon, U. C.; Oh, S. W. *J. Am. Chem. Soc.* **1998**, *120*, 10676.
(32) Poulos, T. L.; Edwards, S. L.; Warishi, H.; Gold, M. H. *J. Biol. Chem.* **1993**, *268*, 4429.
(33) Shoemaker, H. E.; Lundell, T. K.; Hataka, A. I.; Piontek, K. *FEMS Microbiol. Rev.* **1994**, *13*, 321.
(34) Wariishi, H.; Sheng, D.; Gold, M. H. *Biochemistry* **1994**, *33*, 5545.
(35) Beratan, D. N.; Onuchic, J. N.; Winkler, J. R.; Gray, H. B. *Science* **1992**, *258*, 1740.
(36) Moser, C. C.; Keske, J. M.; Waruche, K.; Farid, R. S.; Dutton, L. *Nature (London)* **1992**, *355*, 796.
(37) Pardini, V. L.; Smith, C. Z.; Utley, J. H. P.; Vargas, R. R.; Viertler, H. *J. Org. Chem.* **1991**, *56*, 7305.
(38) Criss, D. L.; Fisher, T. H.; Schultz, T. P. *Holzforschung* **1998**, *52*, 57.
(39) Nishiyama, Y.; Kobayashi, A. *Tetrahedron Lett.* **2006**, *47*, 5565.