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Effects of Alkoxy Groups on Arene Rings of Lignin β-O-4 Model Compounds on the Efficiencies of Single Electron Transfer-Promoted Photochemical and Enzymatic C−C Bond Cleavage Reactions

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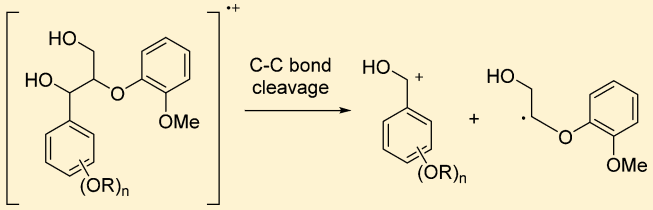
Supporting Information

ABSTRACT: To gain information about how alkoxy substitution in arene rings of β-O-4 structural units within lignin governs the efficiencies/rates of radical cation C1−C2 bond cleavage reactions, single electron transfer (SET) photochemical and lignin peroxidase-catalyzed oxidation reactions of dimeric/tetrameric model compounds have been explored. The results show that the radical cations derived from less alkoxy-substituted dimeric β-O-4 models undergo more rapid C1−C2 bond cleavage than those of more alkoxy-substituted analogues. These findings gained support from the results of DFT calculations, which demonstrate that C1−C2 bond dissociation energies of β-O-4 radical cations decrease as the degree of alkoxy substitution decreases. In SET reactions of tetrameric compounds consisting of two β-O-4 units, containing different degrees of alkoxy substitution, regioselective radical cation C−C bond cleavage was observed to occur in one case at the C1−C2 bond in the less alkoxy-substituted β-O-4 moiety. However, regioselective C1−C2 cleavage in the more alkoxy-substituted β-O-4 moiety was observed in another case, suggesting that other factors might participate in controlling this process. These observations show that lignins containing greater proportions of less rather than more alkoxylated rings as part of β-O-4 units would be more efficiently cleaved by SET mechanisms.



INTRODUCTION

Lignin, a complex, heterogeneous biopolymer with a backbone structure that contains mainly 1-aryl-2-aryloxypropan-1,3-diol and 1,2-diarylpropan-1,3-diol units, is found in plant cell walls and, along with cellulose and hemicellulose, is a major component of lignocellulose.[1−3] Because of its structural rigidity and intimate association with cellulose, lignin protects plant cell walls from chemical/biological degradation. However, the structural complexity and unreactive nature of lignin also make cellulose-based ethanol production utilizing natural plant materials difficult. As a result, a preliminary delignification step is required for cellulase enzymes to transform entrapped cellulose to monomeric glucose, which serves as the starting material for cellulosic ethanol production. Thus, finding effective and cheap ways to liberate cellulose from rigid lignin networks (delignification) is a goal of numerous studies aimed at enhancing cellulosic ethanol production.[4−10]

Lignin is biosynthesized by pathways initiated by radical polymerizations of three precursors, including *p*-hydroxycinnamyl- (coumayl), 4-hydroxy-3-methoxycinnamyl- (coniferyl), and 4-hydroxy-3,5-dimethoxycinnamyl- (sinapyl) alcohol. Oxidative polymerization of these components generates 1,2-diarylpropanoid (β-1), 1-aryl-2-aryloxypropanoid (β-O-4), phenylcoumaran propanoid (β-5), spirodienone,[11] and other substructures of the heterogeneous polymer (Scheme 1). In addition, the 1-arene rings of these structural units typically contain mono- (coumaryl), di- (guaiacyl), and tri- (syringyl) hydroxyl, alkoxyl, and/or aryloxyl (oxy) substituents depending upon the substrates that participate in the radical polymerization processes (Scheme 1). Although the number and disposition of the oxy substituents in these subunits vary from plant to plant, generally the β-O-4 structural unit is the most abundant.[8c,12]

A variety of physical (e.g., milling, comminuting, steam), chemical (e.g., acid or base hydrolysis), and biological (e.g., enzyme) approaches have been explored for efficient delignification methods. Since the discovery that lignin peroxidase (LP) and related oxidase enzymes found in fungi (e.g., *Phanerochaete chrysosporium*) degrade lignin,[4] a number of studies have focused on the development of enzymatic methods for delignification.[13−17] The results of mechanistic investigations of LP-catalyzed reactions of lignin and lignin model compounds (Scheme 2) show that the processes are initiated by single electron transfer (SET) from lignin **1** to either LP or a hole carrier mediator (e.g., veratrylalcohol, VA) to form radical

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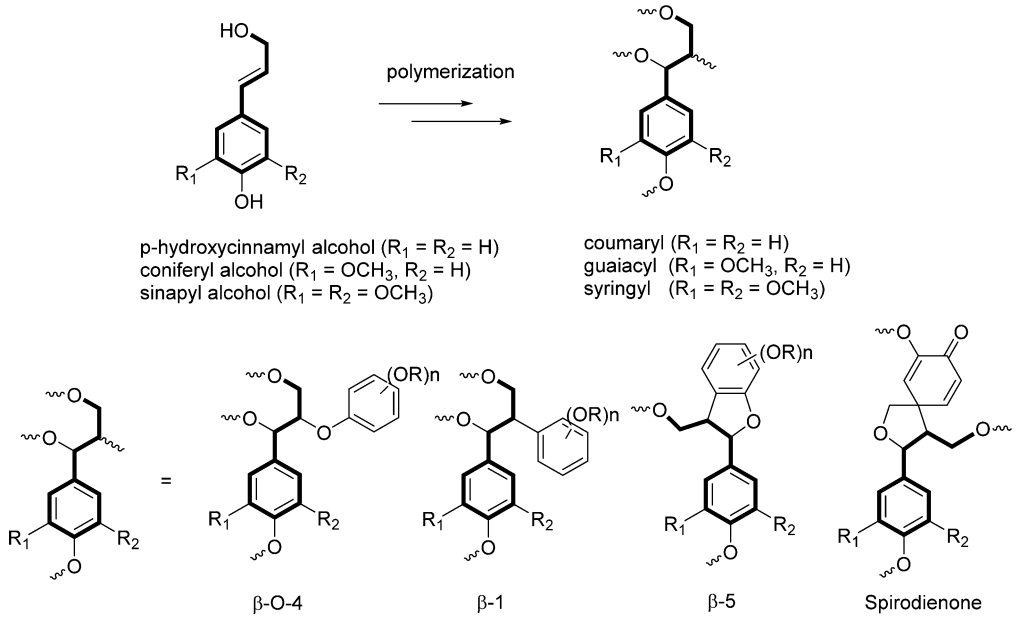
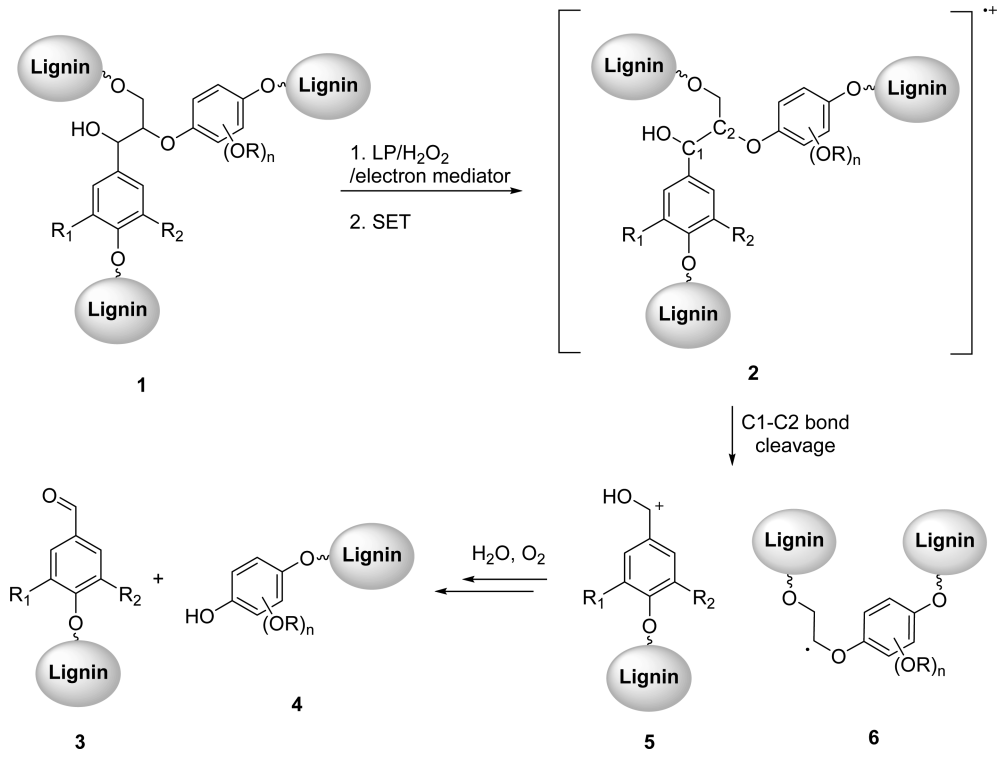
Scheme 1



Scheme 2



cation **2**.[6a,18,19] C1−C2 bond cleavage in this intermediate results in formation of a cation **5** and a radical **6**, which undergo respective deprotonation and further oxidation to generate aldehyde **3** and phenol **4** products.

Some time ago, we embarked on a program designed to obtain information about the dependence of the efficiencies of SET-promoted C−C bond cleavage reactions of lignins on the substructural (e.g., *β*-1 and *β*-O-4 units) features and arene ring substitution patterns.[20,21] We believed that information gained from this effort is important because initial SET from lignin **7** to LP or a hole carrier could produce a mixture of radical cation intermediates (e.g., **8a**−**8c** in Scheme 3) that differ in the sites where the charged odd electron centers exist. These intermediates are likely to undergo rapid and reversible interconversion by way of an intrasite-SET process (electron hopping) (Scheme 3).[22,23] In this event, the overall rate of C−C bond cleavage reactions of lignin radical cations will be governed by the rates of C−C bond cleavage of the radical cation of the most reactive substructural (*β*-1, *β*-O-4, etc.) or substituted arene ring containing unit. Importantly, this reasoning suggests that efficiencies of SET-promoted delignification processes, like those promoted by LP, will be governed

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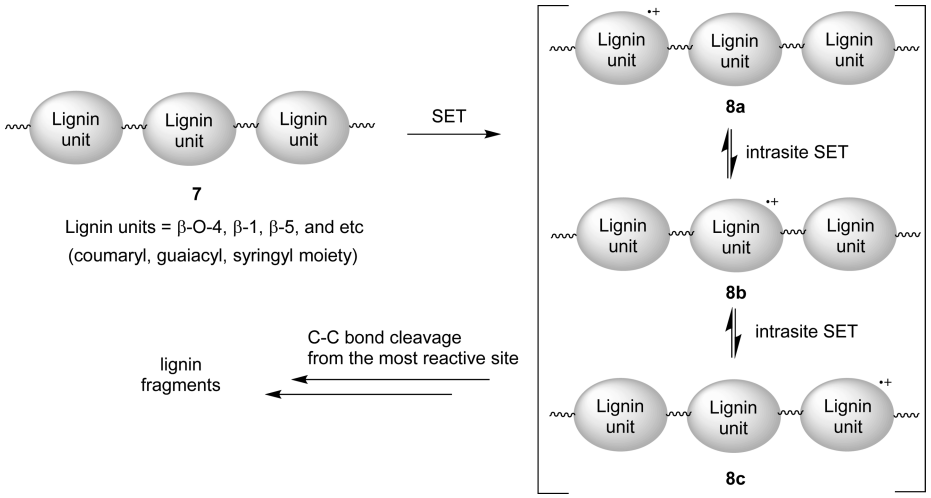
Scheme 3



by the composition of the lignin (i.e., substructural units and arene ring substituents).

In earlier studies,[20,21] we investigated photochemical, Ce(IV), and LP-promoted SET oxidation reactions of dimeric and tetrameric lignin model compounds that contain *β*-1 and *β*-O-4 type structure. The results of these efforts showed that radical cations derived from *β*-1 models (i.e., 1,2-diarylpropanoid) undergo C1−C2 bond cleavage more rapidly than do those of *β*-O-4 models (i.e., 1-aryl-2-oxyarylpropanoid) (Figure 1). In addition, theoretically determined C1−C2 bond dissociation energies of these radical cations were found to agree well with experimental observation.

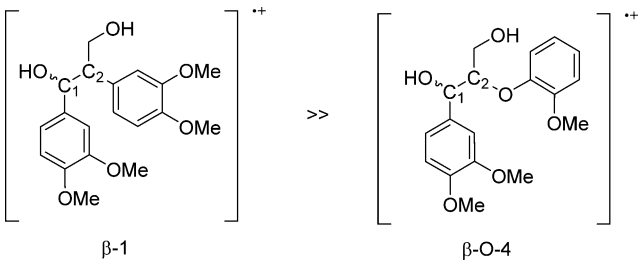


Figure 1. Reactivities of radical cations of *β*-1 and *β*-O-4 lignin model compounds.

The investigation, described below, was designed to evaluate if and how the presence of alkoxy, aryloxy, and hydroxy substituents on the C1-aryl rings of *β*-O-4 units in lignins determine the efficiencies of radical cation C1−C2 bond cleavage reactions. To our knowledge, a systematic investigation seeking an answer to this question has not been carried out previously.[24] However, this is an important question because it is known that both the structural nature and the degree of alkoxy and hydroxy substitution in lignins vary among plant species.[25,26] In addition, it is possible to genetically engineer plants by regulating enzymes in the lignin biosynthetic pathway to control the substituent composition of structural units in the lignin. For example, Chiang and his co-workers[27,28] showed that the coumayl content of lignin of a transgenic aspen, in which 4-coumaric acid Co-A ligase is suppressed, is decreased by 40%. In another study, Meyer and Chapple[29] found that a ferulate 5-hydroxylase (F5H) deficient mutant of aspen produces a plant that contains lignin devoid of syringyl units. Moreover, upregulation of the gene encoding F5H leads to a plant that has nearly 100% syringyl containing lignin. Thus, it appears that it is possible to engineer plants so that their lignins contain aromatic moieties that have between one and three hydroxyl, aryloxy, or alkoxy groups.[30]

In the current effort, we prepared three lignin *β*-O-4 dimeric model compounds (**9E**, **10E**, and **11E**), which differ in the number of methoxy groups on the C1-arene ring, and determined the efficiencies/rates of their photoinduced (9,10-dicyanoanthracene (DCA) sensitized) and enzyme-catalyzed (LP) SET oxidation reactions (Figure 2). In addition, we

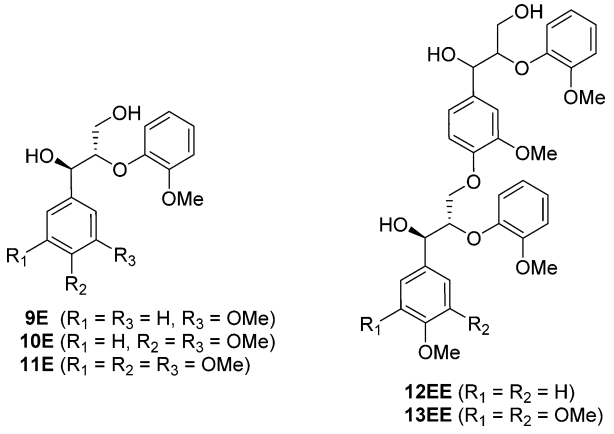


Figure 2. *β*-O-4 dimeric and tetrameric model compounds explored in this investigation.

investigated the regioselectivity of the C−C bond cleavage reaction of the radical cation derived by SET oxidation of the tetrameric model compounds, **12EE** and **13EE**. Analysis of the experimentally determined product distributions, relative quantum efficiencies (Φ_{rel}), and catalytic rate constants (k_{cat}) of the respective photochemical and enzymatic processes suggest that lignins substructures containing less rather than more oxy-substituted arene rings are more efficiently cleaved in reactions that operate by electron transfer mechanisms.

RESULTS

Synthesis of the Dimeric *β*-O-4 Lignin Model Compounds 9E, 10E, and 11E. The *erythro* diastereomers

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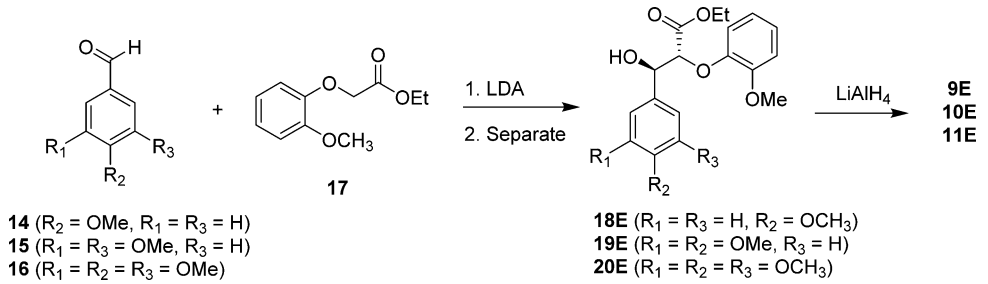
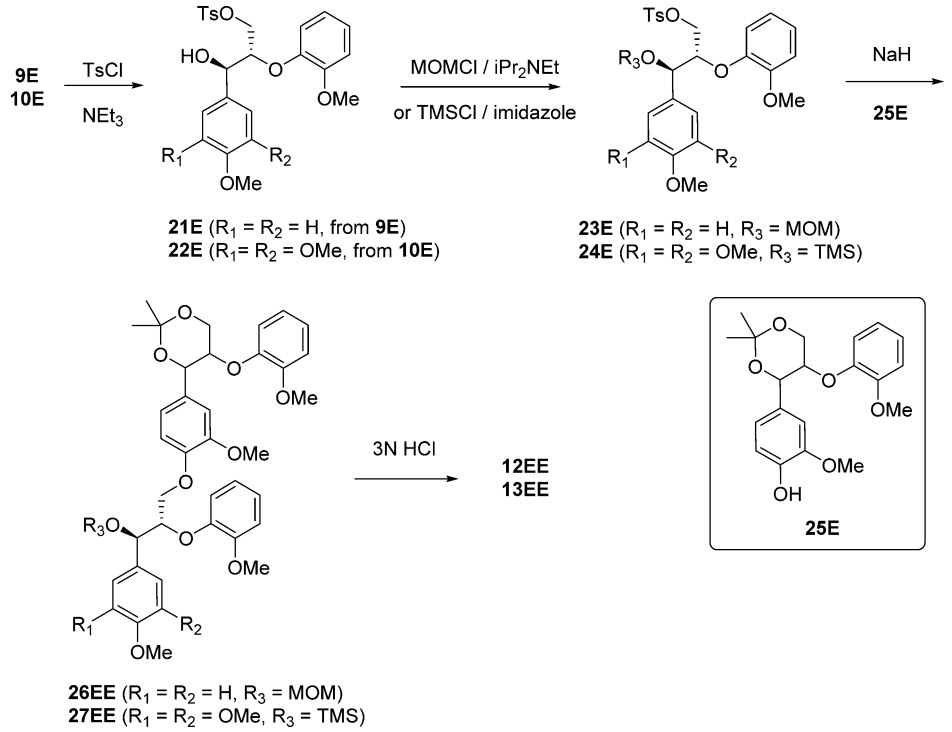
Scheme 4



Scheme 5



of the β-O-4 model compounds **9E**, **10E**, and **11E** were prepared using previously reported methods.[20,21,31−35] The synthetic routes begin with aldol condensation reactions of the substituted benzaldehydes **14**−**16** with 2-aryloxy acetate **17**, which, followed by column chromatographic separation, yield the respective, diastereomerically pure β-hydroxyesters **18E**−**20E** (Scheme 4). Reduction of these esters using $LiAlH_4$ generates the target *erythro* β-O-4 compounds **9E**−**11E**.

Synthesis of the Tetrameric Lignin Model Compounds 12EE and 13EE. On the basis of a previously developed synthetic protocol,[21] the tetrameric compounds **12EE** and **13EE** containing two β-O-4 subunits were prepared through coupling reactions of the lignin dimers **23E**, **24E**, and **25E**.[21] In the pathways, selective tosylation of the primary alcohol and MOM or TMS protection of the secondary alcohol groups of the respective *erythro*-diols **9E** and **11E** (Scheme 4) are employed to generate the corresponding substrates **23E** and **24E** in reactions with the phenol **25E**. These processes, initiated by treatment of NaH, produce **26EE** and **27EE**, respectively, which upon treatment with 3 N HCl form the respective tetrameric products **12EE** and **13EE** (Scheme 5).

SET-Photochemical Reactions of Dimeric β-O-4 Lignin Model Compounds. To assess the C−C bond cleavage reactivity of cation radical intermediates derived from dimeric lignin model compounds **9E**−**11E**, SET-photochemical reactions using DCA as the excited state electron acceptor sensitizer were carried out. To ensure that SET from the lignin models to the singlet excited state of DCA (DCA^{S1}, E^{S1}_{red} = +2.76 V vs Ag/AgCl) is thermodynamically/kinetically favorable, fluorescence quenching rate constants (k_q) and oxidation potentials (E_{ox}) of dimeric lignin model compounds were determined (Table 1). As can be seen by viewing the results displayed in Table 1, the diastereomerically pure dimeric models have similar E_{ox} values that are independent of number of methoxy substituent on C1- or C2-aryl ring moiety and that fall below the E^{S1}_{red} of DCA. The slight variations observed in the E_{ox} values are likely a result of competitive contributions to the

Table 1. Oxidation Potentials and Rate Constants of DCA-Fluorescence Quenching of the Lignin Model Compounds 9E−11E

substrate	E_{ox} (+) (V vs Ag/AgCl)	$k_q \times 10^{-10}$ (M^{-1} s^{-1})
9E	1.19	0.98
10E	1.22	1.16
11E	1.15	0.84

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Scheme 6

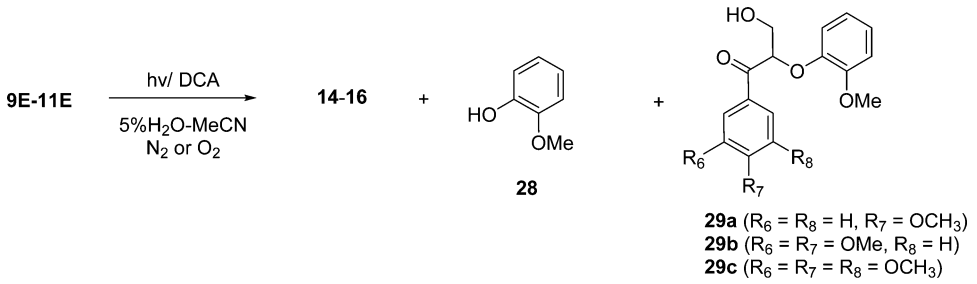

Table 2. Products and Yields of DCA-Photochemical Reactions of β-O-4 Lignin Model Compounds 9E−11E

			% yield[b]				
			aldehyde				
substrate	condition[a]	% conversion	**14**	**15**	**16**	**28**	**29**
9E	N_2	16	15(94)			trace	
9E	O_2	55	36(65)			trace	
10E	N_2	16		15(94)		trace	trace(**29b**)
10E	O_2	50		26(52)		trace	trace(**29b**)
11E	N_2	14			7(50)	trace	
11E	O_2	51			10(20)	trace	

[a]Solutions containing substrate (2.1 mM) and saturated DCA (0.27 mM) were irradiated for the same time periods (28 h in N_2 and 7 h in O_2 environment). [b]Yields in parentheses are based on recovered substrate **9E−11E**.

oxidation potentials by the two aryl rings in these substances. Thus, the lowest energy oxidation of **9E** likely takes place in the C2-aryloxy ring, while those of **10E** and **11E** occur in the C1-arene rings. Because of these SET donor propensities, **9E−11E** quench the fluorescence of DCA with nearly equal, diffusion controlled (1×10^9−10^{10} M^{-1} s^{-1}) rates.

To determine photoproduct distributions, 5% aqueous MeCN solutions (7 mL) containing DCA and β-O-4 model compounds **9E−11E** were irradiated ($\lambda > 330$ nm) under N_2 (28 h) and O_2 (7 h) atmospheres. The results of DCA-promoted photoreactions are displayed in Scheme 6 and Table 2. The major products generated in these processes are the respective benzaldehydes **14−16**, formed via radical cation C1−C2 bond cleavage. Phenol **28** is produced in trace amounts in all processes, and ketone **29b**, generated through cation radical C1−H bond cleavage, is formed in trace quantities in the reaction of **11E** only. The low quantity of **28** isolated from the photoreaction mixtures can be ascribed to the fact that this phenol is likely labile under photochemical oxidative conditions.[16a,36] Finally, the structure of **29b** was unambiguously assigned by comparison of its spectroscopic properties to those of the known substance.[20]

Table 3. Products and Yields of LP-Catalyzed Reactions of β-O-4 Lignin Model Compounds 9E−11E[a]

		% yield[b]			
		aldehyde			
substrate	% conversion[a]	**14**	**15**	**16**	**28**
9E	24	17(71)			trace
10E	27		24(89)		trace
11E	22			6.5(30)	trace

[a]Solution containing substrate (0.4 mM) and LP (8 μM) and 60 μL of H_2O_2 were used. [b]Yields in parentheses are based on recovered substrate **9E−11E**.

Enzymatic Reactions of Dimeric β-O-4 Lignin Model Compounds. Enzymatic oxidation reactions of the dimeric compounds **9E−11E**, catalyzed by *Phanerochaete chrysosporium* LP in the presence of H_2O_2, were performed. Analysis of the product distributions and yields of these reactions, each carried out for a fixed time period of 30 min (Table 3), show that C−C bond cleavage pathways take place exclusively to generate the respective benzaldehydes **14−16** along with a trace amount of phenol **28**. Interestingly, reaction of the trimethoxy-substituted C1-arene containing substrate **11E** promoted by LP/H_2O_2 leads to **16** with an efficiency that is 2.6 times lower than the other β-O-4 dimeric compounds.

Relative Quantum Yields (Φ_{rel}) and Steady-State Kinetic Constants (k_{cat} and K_M) of DCA- and LP-Promoted SET Oxidation Reactions of Lignin Model Compounds 9E−11E. To determine the C−C bond cleavage reactivities of dimeric β-O-4 compounds bearing varying numbers of C1 arene ring methoxy substituents, relative quantum efficiencies (Φ_{rel}) of DCA-promoted photoreactions and steady-state kinetic constants (k_{cat} and K_M) of enzymatic reactions of **9E−11E** were determined (Table 4). Φ_{rel} values were determined by measuring product yields of photochemical processes conducted using a standard simultaneous irradiation technique. Specifically, deoxygenated 5% aqueous MeCN solutions, containing concentrations of the substrate (ca. 2.1 mM) that bring about equal absorbances at their wavelength maximum and saturated DCA, were simultaneously irradiated

Table 4. Relative Quantum Yield (Φ_{rel}) and Steady-State Kinetic Constant (k_{cat}/K_M) of DCA-Promoted and LP-Catalyzed Reactions of Dimeric Lignin Models 9E−11E

substrate	Φ_{rel}	k_{cat} (s^{-1})	K_M (μM)	k_{cat}/K_M (10^3 s^{-1} μM^{-1})
9E	4	3.9 ± 0.2	440 ± 40	15
10E	3.3	1.07 ± 0.04	200 ± 20	9.9
11E	1	0.87 ± 0.01	270 ± 10	8

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Table 5. Product Distributions of DCA-Promoted Photoreactions of Tetrameric Lignin Models 12EE and 13EE[a]

			% yield[b]				
substrate	condition	% conversion	**30E**	**31E**	**14**	**16**	**28**
12EE	O_2/DCA 20 min	12	5(42)		trace		trace
12EE	O_2/DCA 1 h	39	18(46)		4(10)		trace
13EE	O_2/DCA 20 min	6		3(50)		trace	trace
13EE	O_2/DCA 1 h	21		12(57)		3(14)	trace

[a]Solutions containing substrate (0.525 mM) and saturated DCA (0.27 mM) were irradiated for the same time period. [b]Yields in parentheses are based on recovered substrate **12EE** and **13EE**.

Scheme 7

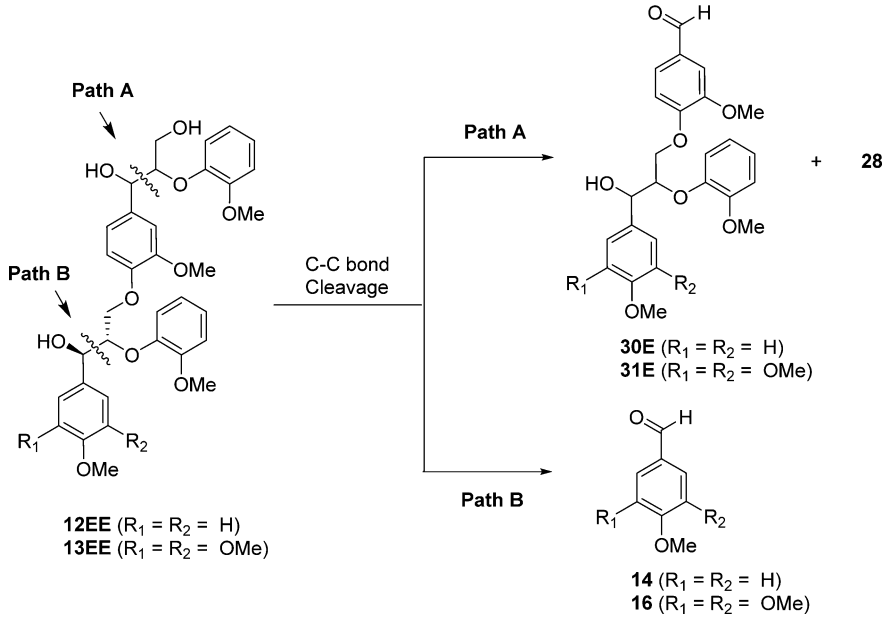

Table 6. Product Distributions of LP-Catalyzed Reactions of Tetrameric Lignin Models 12EE and 13EE[a]

			% yield[b]				
substrate	condition	% conversion	**30E**	**31E**	**14**	**16**	**28**
12EE	0.6 μM LP, 0.2 mM H_2O_2	12	5.4(45)		trace		trace
12EE	3 μM LP, 0.8 mM H_2O_2	35	15(43)		2(6)		trace
13EE	0.6 μM LP, 0.2 mM H_2O_2	16		3(19)		trace	trace
13EE	3 μM LP, 0.8 mM H_2O_2	40		12(30)		2(5)	trace

[a]Solution containing substrate (0.2 mM) and LP (0.6 μM) and 10 μL of H_2O_2 were used. [b]Yields in parentheses are based on recovered substrate **12EE** and **13EE**.

for a time period that promotes low substrate conversions (ca. 10%). Product yields were then determined by utilizing HPLC analysis of the crude photolyzates.

Steady-state kinetic data (k_{cat} and K_M) for the LP-catalyzed reactions were determined from initial rates of reactions of varying concentrations of **9E**−**11E** in the range of 0.05−2.5 mM using 10 mM of H_2O_2. The rises of absorbances at 310 nm, associated with formation of the benzaldehyde products, versus time were linear in all cases and used to define initial rates. The steady-state kinetic constants (Table 5) for each substrate were calculated by plotting the reciprocal of the initial rates versus the reciprocal of the concentrations of each dimeric compound (Lineweaver−Burk plots, see the Supporting Information).[20,21,37]

The observations show that the C1−C2 bond cleavage reactivities of radical cations derived from the dimeric β-O-4 lignin model compounds are highly influenced by the number of alkoxy substituents on the C1-arene ring. Specifically, a comparison of the Φ_{rel} and k_{cat} values arising from the respective photochemical (in 5% aqueous MeCN) and enzyme (in 0.1 M tartrate buffer, pH 3.4) experiments show that the radical cation arising from the monomethoxy substrate **9E** undergoes C1−C2 bond cleavage with a rate that is 4 times greater than that of the trimethoxy-substituted analogue **11E**.

Photochemical and Enzymatic SET Oxidation Reactions of the Tetrameric Lignin Model Compounds. SET-promoted C−C bond cleavage reactions of the tetrameric model compounds **12EE** and **13EE** were performed by using the photochemical and enzymatic methods described above for the dimeric model compounds. Photoirradiation (λ > 330 nm) of an oxygenated 5% aqueous MeCN solution containing DCA and **12EE** gives rise to the formation of aldehyde **30E** as the major product and trace amounts of benzaldehyde **14** and guaiacol **28** (Table 5). In this process, **30E** is formed by

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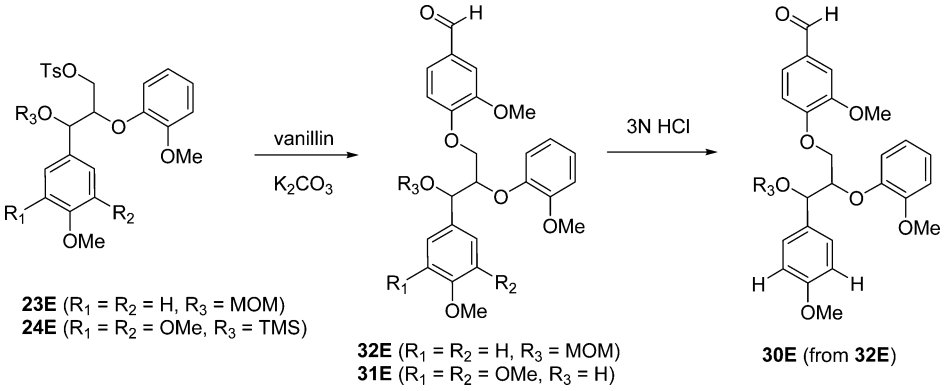
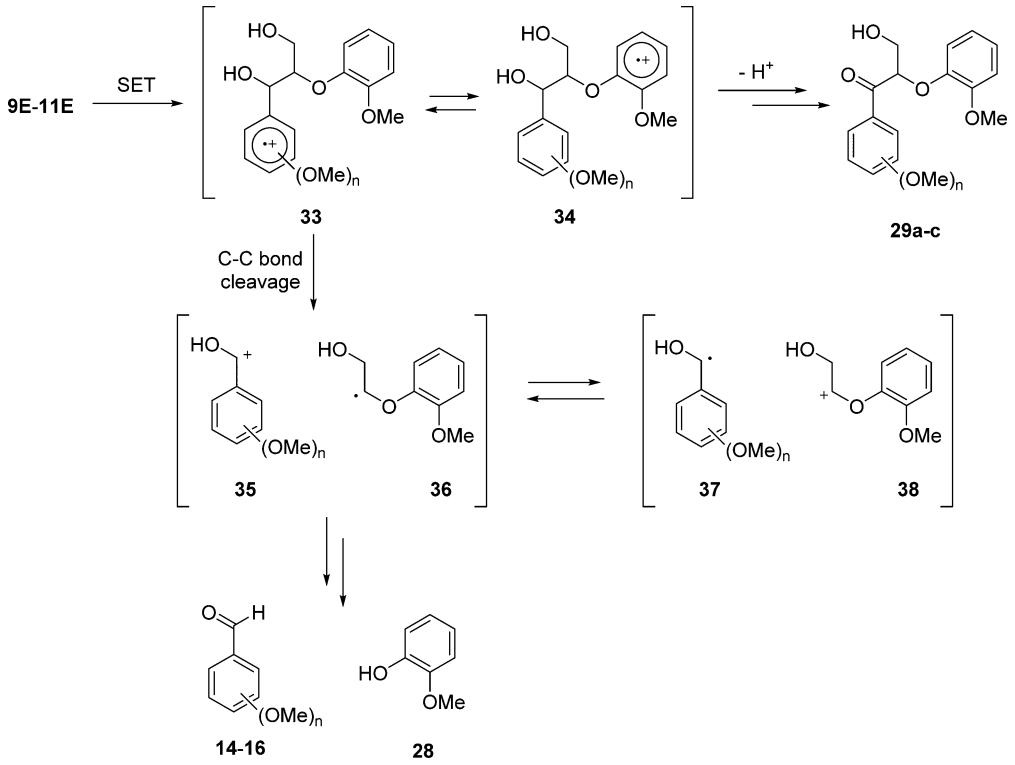
Scheme 8



Scheme 9



cleavage of the upper C1−C2 bond (path A in Scheme 7) in the cation radical of **12EE**, while **14** is formed by cleavage of the lower C1−C2 bond (path B). The LP-catalyzed reaction of **12EE** displays the same C−C bond cleavage selectivity as that seen in the photoreaction (Scheme 7, Table 6).

Product distributions and yields of DCA- and LP-promoted reactions of **13EE** are given in Tables 5 and 6. In both of these processes, **13EE** reacts more favorably through path A (Scheme 7), as reflected by the fact that aldehyde **31E** is the major product generated along with a trace amount of guaiacol **28**.

Structural assignments to aldehydes **30E** and **31E** were made unambiguously by comparisons of their spectroscopic properties with those of authentic substances, independently synthesized using the routes shown in Scheme 8.

■ DISCUSSION

Reaction Mechanism. The mechanistic pathway for the oxidative C1−C2 bond cleavage reactions described above begins with formation of the key radical cation intermediate, arising by SET from the β-O-4 model compounds to either the singlet excited state of DCA or LP. The positive charge and odd electron density should be distributed over both arene rings in the β-O-4 radical cation (represented by **33** and **34** in Scheme 9) in a manner that depends on the electron-donating methoxy group substitution. C1−C2 bond cleavage of the radical cation, taking place in a formal sense from **33** due to interactions between the arene π-system and the C1−C2 σ-bond, generates a benzylic cation **35** and oxy-substituted radical **36**. Assignment of preferred location of the charge and odd electron centers in **35** and **36**, versus the alternative **37** and **38**, is made on the basis of the relative thermodynamic stabilities determined by using the results of unpublished DFT calculations, which match those that come from redox potential data determined by Griller and Wayner[38,39] and a consideration of substituent effects on the energies of aryl- and hydroxyl-substituted carbocations. Independent deprotonation of **35** and oxidation followed by hydrolysis of **36** then produces benzaldehydes **14**–

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16 and guaicol **28**, respectively. β-Hydroxyketone **29b**, formed in trace quantities in the photoreaction of **10E**, arises by benzylic C1−H deprotonation of radical cation **33**. These mechanistic considerations are consistent with the results of proposals in studies conducted earlier by Baciocchi,[24,40] Arnold,[41−43] and Maslak[44,45] using lignin models and 1-aryl-2-ethyl ethers.

Oxy-Substituent Effects on Reactivity of β-O-4 Cation Radical. In our previous studies of DCA photosensitized and LP-catalyzed reactions of lignin model compounds, we observed that C1−C2 bond cleavage of radical cations derived from substances bearing β-1 lignin model structures are more rapid than those from β-O-4 model compounds.[20,21] This difference was attributed to the fact that β-1 radical cations have lower C1−C2 bond dissociation energies than those of β-O-4 analogues, a proposal that gained support from the results of DFT calculations.

Observations made in the current study demonstrate that the C−C bond cleavage reactivities of β-O-4 radical cations depend on the numbers of alkoxy substituents on the C1-arene ring and, specifically, that the cleavage rates decrease as the numbers of alkoxy substituents increase (Table 3, **9E** > **10E** > **11E**). Information that relates to this issue is found in the results of earlier investigations, which demonstrate arene ring substituents control the rates of C−C bond cleavage of related radical cations. For example, observations made in studies by Maslak and his co-workers[44,45] show that the efficiencies of SET induced photoreactions of substituted 1,2-diarylethanes are governed by substituent effects, which correlate with C−C BDEs.

To determine if the experimental observations made in the studies described above can be explained by using BDE-based considerations, we have carried out DFT calculations on the β-O-4 model compounds **9E**−**11E**. For this purpose, geometry optimizations were performed using DFT with a B3LYP/6-31G+G(d,p) basis set. BDEs of the neutral states of the lignin models in the gas phase were calculated by using differences in energies between the neutral compounds and those of corresponding radicals derived by homolytic C1−C2 bond cleavage, and BDEs of the radical cation states of the compounds were obtained by using differences between the energies of the optimized radical cations and the lower energy set of radical cation pairs ($C1^+/C2^\bullet$ and $C1^\bullet/C2^+$) (Table 7).

Table 7. DFT Calculated C1−C2 Bond Dissociation Energies (BDE) of Dimeric β-O-4 Model Compounds 9E−11E

		BDE of radical cations	
models	BDE of neutral models (kcal/mol)	C1 cation + C2 radical (kcal/mol)	C1 radical + C2 cation (kcal/mol)
9E	62.7	28.9	42.6
10E	62.3	30.4	47.3
11E	63.6	36.5	49.7

As can be seen by inspecting the results in Table 7, the BDEs of the neutral states of **9E**−**11E** do not vary greatly. However, the radical cation BDEs, corresponding to formation of C1 benzylic cations and C2 radicals, are both significantly different and follow a trend that well correlates with the rates of these processes. Specifically, the calculated BDEs of the radical cations, which fall in the order **9E** < **10E** < **11E**, are fully consistent with the rates of C1−C2 bond cleavage of these radical cations, which follows the order **9E** > **10E** > **11E**. It should be noted that the trends seen in the calculated BDEs in the gas phase of these cation radicals are matched by those calculated for the radical cations in the modestly polar MeCN solvent (e.g., 19.4, 20.1, and 24.8 kcal/mol for **9E**, **10E**, and **11E**).

Regioselectivity of C−C Bond Cleavage of Tetrameric Lignin Model Compounds. Another approach to assessing how arene ring oxy-substitution governs the C1−C2 bond cleavage reactivity of lignin radical cations relies on a determination of the regioselectivity of SET-promoted photochemical and enzymatic reactions of model compounds containing differently substituted β-O-4 units. In each of the tetrameric model compounds, **12EE** and **13EE**, which we have prepared for this purpose, the initially generated radical cation should have positive charge and odd electron densities that are distributed over four arene rings as a consequence of intrasite SET (or electron hopping) processes.[22,23] However, C1−C2 bond cleavage reactions of each radical cation in a formal sense can only occur when the charged radical centers exist on the two C1 arene ring moieties A and C in **39** and **40**, respectively (Scheme 10). If interconversions between the potentially reactive species take place rapidly relative to bond cleavage, the relative rates of C1−C2 bond cleavage in **39** (path B) and **40** (path A) will govern the regioselectivity of the SET photochemical and enzyme-catalyzed reactions of the tetrameric model compounds.[21] In accord with this assumption and in a manner that is consistent with both the Φ_{rel} and k_{cat} data and the calculated large BDE differences between C1−C2 bond cleavage of radical cation derived from di- and trimethoxy-substituted C1-arene containing models, the DCA-photosensitized and LP-catalyzed reactions of tetramer **13EE** take place by highly selective cleavage of the more reactive β-O-4 moiety containing the dialkoxy rather than trialkoxy-substituted C1 arene ring (path A in Scheme 10).

In contrast, the radical cation derived from **12EE**, which contains monoalkoxy (A)- and dialkoxy (C)-substituted C1 arene rings, undergoes selective cleavage (path A) of the C1−C2 bond that contains the more highly methoxy-substituted arene ring. The cause of this unusual disparity might be a consequence of the incorrect assumption that the oxidation potential controlled location of highest positive charge and odd electron densities in radical cations **39** do not govern C−C bond cleavage selectivities. Thus, if this factor contributes to governing regioselectivity, the preference for reaction by path A in the radical cation derived from **12EE** would be consistent with the expected greater localization of the positive charge and odd electron density on the more alkoxy-substituted C1 arene ring. However, considering the fact that the calculated BDE differences between the two types of C1−C2 bonds in the radical cation arising from **9E** and **10E** are likely small, a range of other factors including ring geometry controlled, orbital overlap might be operating to govern the bond cleavage selectivity in reaction of this tetrameric cation radical. No doubt, further studies probing this issue are required.

■ CONCLUSIONS

Observations made in the study described above have potential relevance to the major problem confronting the use of plant materials for ethanol. The biggest challenge facing this process is associated with the difficulty in accessing cellulose that is encased in lignin structures within plant cell walls. As mentioned above, the nature of lignins, and in particular their

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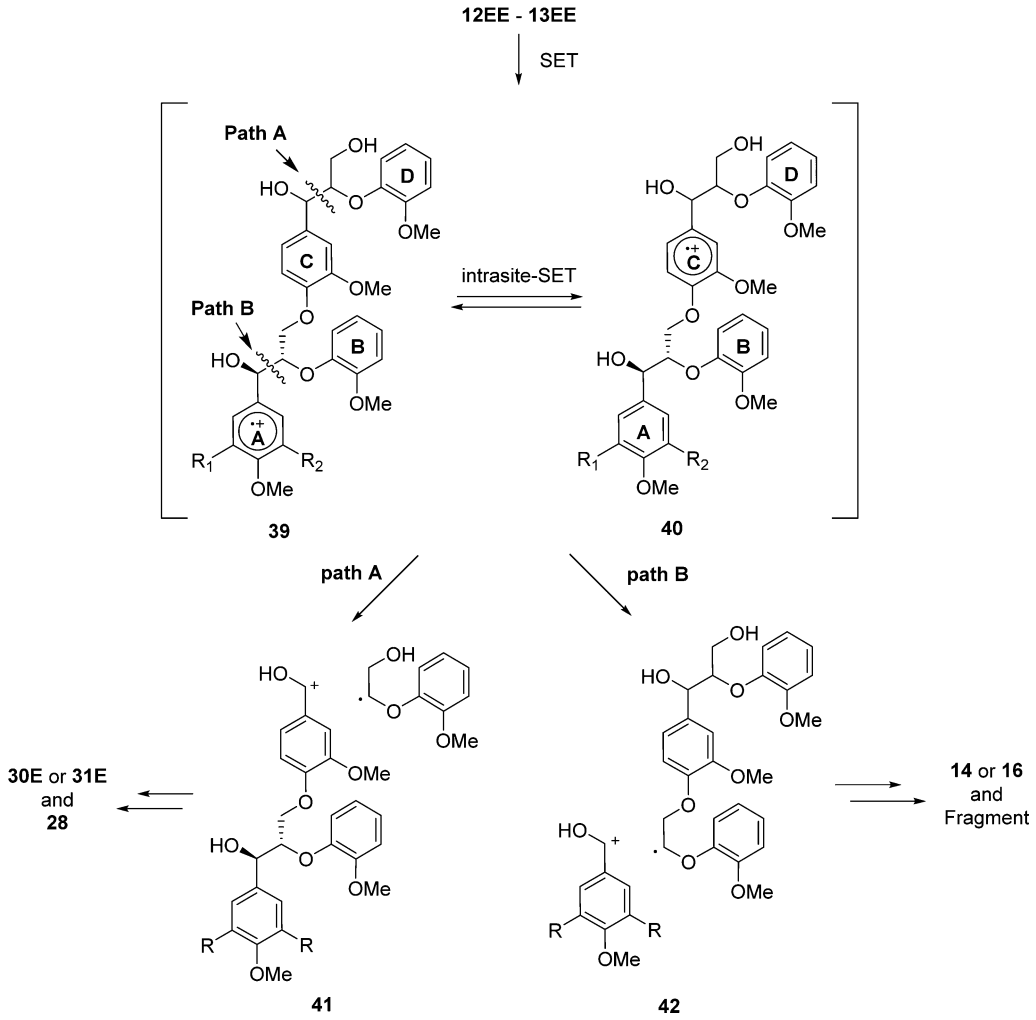
Scheme 10



structural unit compositions as well as their coumaryl (monooxy), guaiacyl (dioxy), and syringyl (trioxy) contents, vary from plant to plant. In addition, the compositions of lignins can be genetically controlled. Although it remains to be determined if the reactivity profile uncovered in this effort applies to the oxidative delignification reactivities of lignins found in plant environments, the results suggest that plants containing lignins with lower syringyl contents will be more susceptible to oxidative delignification and therefore more useful for ethanol production.

EXPERIMENTAL SECTION

General Procedures. 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 (300 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. The purity of each was determined to be >90% by ^{1}H and ^{13}C NMR analysis. High resolution (HRMS) mass spectra were obtained by use of a quadrupole mass analyzer, electron spray ionization unless otherwise noted. 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 the Dimeric Model Compounds 9E and 11E. Ethyl 3-Hydroxy-3-(4-methoxyphenyl)-2-(methoxyphenyloxy) Propionate 18E. A solution of diisopropylamine (10.0 mL, 70.5 mmol) in dry THF (70 mL) containing 28.2 mL (28.2 mmol) of 2.5 M *n*BuLi at −78 °C was stirred for 30 min. Acetate ester **17** (14.8 g, 70.5 mmol) was added dropwise, and the resulting solution was stirred for 1 h followed by addition of *p*-anisaldehyde **14** (8.0 g, 58.8 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* **18E** (14.3 g, 70%) exclusively. ^{1}H NMR ($CDCl_3$) δ 1.12 (t, 3H, J = 7 Hz), 3.77 (s, 3H), 3.84 (s, 3H), 3.87 (d, 1H, J = 6.5 Hz), 4.11 (dd, 2H, J = 7 Hz, J = 14 Hz), 4.70 (d, 1H, J = 4.5 Hz), 5.13 (t, 1H, J = 5.5 Hz), 6.80−6.92 (m, 5H), 7.00 (t, 1H, J = 8 Hz), 7.36 (d, 2H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 55.2, 55.8, 61.1, 73.6, 84.1, 112.2, 113.5, 119.2, 121.0, 124.0, 128.1, 131.2, 147.2, 150.6, 159.3, 169.2; HRMS (ES) m/z 369.1309 (M + Na, $C_{19}H_{22}O_6Na$ requires 369.1314).

Ethyl 3-Hydroxy-3-(3,4,5-trimethoxyphenyl)-2-(methoxyphenyloxy) Propionate 20E. A solution of diisopropylamine (10.0

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mL, 70.5 mmol) in dry THF (70 mL) containing 28.2 mL (28.2 mmol) of 2.5 M *n*BuLi at −78 °C was stirred for 30 min. Acetate ester **17** (14.8 g, 70.5 mmol) was added dropwise, and the resulting solution was stirred for 1 h followed by addition of aldehyde **16** (11.5 g, 58.8 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* **20E** (18.2 g, 76%) exclusively. ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, J = 7 Hz), 3.66 (d, 1H, J = 6 Hz), 3.81 (s, 3H), 3.83 (s, 6H), 3.84 (s, 3H), 4.14 (dd, 2H, J = 7 Hz, J = 14 Hz), 4.71 (d, 1H, J = 5.5 Hz), 5.12 (t, 1H, J = 5.5 Hz), 6.70 (s, 2H), 6.82−6.85 (m, 1H), 6.88−6.90 (m, 2H), 6.99−7.02 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.0, 55.8, 56.0, 60.8, 61.3, 74.0, 83.6, 103.9, 112.2, 118.6, 121.0, 123.9, 134.7, 137.6, 147.1, 150.5, 153.0, 169.3; HRMS (ES) m/z 429.1520 (M + Na, $C_{21}H_{26}O_8Na$ requires 429.1525).

1-(4-Methoxyphenyl)-2-(methoxyphenyloxy)-1,3-propandiol 9E. To a solution of THF containing 1.0 M $LiAlH_4$ (12.8 mL, 12.8 mmol) was added **18E** (4.4 g, 12.8 mmol) at room temperature. After being stirred for 3 h, 20 mL of H_2O and 20 mL of 1 N HCl solution at 0 °C were added, and the solution was 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 1:1) to yield **9E** (2.1 g, 55%). ^{1}H NMR ($CDCl_3$) δ 2.84−2.86 (m, 1H), 3.54 (d, 1H, J = 3.5 Hz), 3.60−3.65 (m, 1H), 3.78 (s, 3H), 3.85 (s, 3H), 3.85−3.91 (m, 1H), 4.11−4.14 (m, 1H), 4.96−4.98 (m, 1H), 6.85−6.94 (m, 5H), 7.04 (t, 1H, J = 8 Hz), 7.29 (d, 2H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ 55.2, 55.8, 60.6, 72.5, 87.4, 112.1, 113.8, 121.0, 121.6, 124.2, 127.2, 131.9, 146.8, 151.6, 159.0; HRMS (ES) m/z 327.1211 (M + Na, $C_{17}H_{20}O_5Na$ requires 327.1208).

1-(3,4,5-Trimethoxyphenyl)-2-(methoxyphenyloxy)-1,3-propandiol 11E. To a solution of THF containing 1.0 M $LiAlH_4$ (7.4 mL, 7.4 mmol) was added **20E** (3.0 g, 7.4 mmol) at room temperature. After being 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 1:1) to yield **11E** (1.6 g, 59%). ^{1}H NMR ($CDCl_3$) δ 2.69−2.72 (m, 1H), 3.56 (d, 1H, J = 3 Hz), 3.61−3.65 (m, 1H), 3.81 (s, 3H), 3.82 (s, 6H), 3.87 (s, 6H), 3.87−3.93 (m, 1H), 4.13−4.15 (m, 1H), 4.93−4.95 (m, 1H), 6.59 (s, 2H), 6.90−6.98 (m, 3H), 7.05−7.08 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 55.9, 56.1, 60.7, 60.8, 72.8, 87.4, 102.9, 112.1, 121.2, 121.6, 124.4, 135.4, 137.3, 146.7, 151.6, 153.3; HRMS (ES) m/z 387.1417 (M + Na, $C_{19}H_{24}O_7Na$ requires 387.1420).

Synthesis of Tetrameric Model Compound 12EE. Tosylate 21E. To a solution of CH_2Cl_2 containing **9E** (1.9 g, 6.2 mmol) was added NEt_3 (1.6 mL, 11.4 mmol) at 0 °C. After being stirred for 1 h, TsCl (1.3 g, 6.8 mmol) was added at 0 °C, and the solution was stirred for 12 h at room temperature. The solution was extracted with CH_2Cl_2, followed by drying and concentration in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 1:2) to yield **21E** (2.0 g, 70%). ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H), 3.77 (s, 3H), 3.81 (s, 3H), 4.07 (d, 1H, J = 10.5 Hz), 4.28−4.32 (m, 1H), 4.32−4.36 (m, 1H), 4.85 (d, 1H, J = 3 Hz), 6.81 (d, 2H, J = 8.5 Hz), 6.88 (d, 2H, J = 8.0 Hz), 6.95 (d, 1H, J = 7.5 Hz), 7.03 (t, 1H, J = 7.5 Hz), 7.18 (d, 2H, J = 8.5 Hz), 7.23 (d, 2H, J = 8.5 Hz), 7.65 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 21.5, 55.2, 55.8, 68.4, 71.6, 84.4, 112.3, 113.8, 114.0, 121.1, 121.5, 124.3, 127.2, 127.9, 129.7, 130.5, 132.8, 144.6, 145.6, 151.5, 159.1; HRMS (ES) m/z 481.1300 (M + Na, $C_{24}H_{26}O_7SNa$ requires 481.1297).

MOM Protected Tosylate 23E. To a solution of THF containing **21E** (1.0 g, 2.2 mmol) was added iPr$_2$NEt (1.5 mL, 8.7 mmol) at 0 °C. After being stirred for 30 min, MOMCl (1.0 mL, 13.1 mmol) was added at 0 °C, and the solution was stirred for 12 h at room temperature. The solution was extracted with CH_2Cl_2 followed by drying and concentration in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 1:3) to yield **23E** (0.69 g, 62%). ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H), 3.30 (s, 3H), 3.68 (s, 3H), 3.77 (s, 3H), 4.32 (d, 1H, J = 7.5 Hz), 4.40−4.46 (m, 1H), 4.51 (d, 1H, J = 6.5 Hz), 4.56 (d, 1H, J = 6.5 Hz), 4.87 (d, 1H, J = 1.5 Hz), 6.63 (d, 1H, J = 8.0 Hz), 6.71 (t, 1H, J = 7.0 Hz), 6.76−6.81 (m, 4H), 7.22−7.27 (m, 3H), 7.68 (d, 2H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ 21.5, 55.2, 55.7, 55.8, 68.5, 76.1, 81.6, 94.4, 112.5, 113.6, 119.2, 120.8, 123.1, 127.9, 128.9, 129.6, 133.0, 144.5, 147.1, 150.9, 159.4; HRMS (ES) m/z 525.1561 (M + Na, $C_{26}H_{30}O_8SNa$ requires 525.1559).

Acetonide and MOM Protected Tetramer 26EE. To a solution of MeCN (60 mL) containing **25E** (1.15 g, 3.2 mmol) was added NaH (60% mineral oil) (190 mg, 4.8 mmol) at room temperature. After 1 h of stirring at 80 °C, **23E** (1.6 g, 3.2 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, drying, and concentration in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 1:1) to yield diastereomeric **26EE** (1.1 g, 48%). ^{1}H NMR ($CDCl_3$) δ 1.49 (s, 3H), 1.62 (s, 3H), 3.31 (s, 3H), 3.68 (s, 3H), 3.72 (s, 6H), 3.77 (s, 3H), 3.97−4.01 (m, 1H), 4.11−4.13 (m, 2H), 4.20−4.23 (m, 1H), 4.29−4.32 (m, 1H), 4.59 (d, 1H, J = 6.5 Hz), 4.66 (d, 1H, J = 6.0 Hz), 4.70−4.73 (m, 1H), 4.86−4.88 (m, 1H), 5.07 (d, 1H, J = 4 Hz), 6.42−6.45 (m, 1H), 6.63−6.67 (m, 1H), 6.72−6.83 (m, 8H), 6.87−6.90 (m, 1H), 6.96−6.99 (m, 3H), 7.34 (d, 1H, J = 8 Hz); ^{13}C NMR ($CDCl_3$) δ 19.7, 28.5, 55.2, 55.6, 55.7, 55.9, 62.9, 68.0, 74.6, 82.5, 94.6, 99.4, 111.6, 112.2, 112.4, 113.5, 117.6, 118.0, 118.8, 119.8, 120.8, 122.4, 122.7, 129.1, 130.1, 132.3, 147.2, 148.1, 148.3, 148.6, 150.5, 150.9, 159.3; HRMS (ES) m/z 713.2943 (M + Na, $C_{39}H_{46}O_{11}Na$ requires 713.2938).

Tetramer 12EE. To solution of THF (60 mL) containing **26EE** (1.8 g, 2.5 mmol) was added 3 N HCl (3 mL) at room temperature, and the solution was stirred for 12 h at 50 °C. The solution was concentrated following extraction with CH_2Cl_2, drying, and concentration in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 2:1) to yield diastereomeric **12EE** (0.81 g, 51%). ^{1}H NMR ($CDCl_3$) δ 3.60−3.63 (m, 1H), 3.77 (s, 3H), 3.80 (s, 3H), 3.85 (s, 6H), 4.00−4.02 (m, 1H), 4.10−4.13 (m, 2H), 4.35−4.40 (m, 1H), 4.55−4.58 (m, 1H), 4.93 (d, 1H, J = 3.5 Hz), 5.01 (d, 1H, J = 3.5 Hz), 6.71 (d, 1H, J = 8 Hz), 6.78 (t, 2H, J = 8 Hz), 6.83−6.93 (m, 8H), 7.00−7.05 (m, 2H), 7.32 (d, 2H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 55.2, 55.9, 60.8, 68.4, 72.7, 73.8, 84.9, 87.4, 109.9, 112.0, 113.7, 113.9, 118.4, 121.1, 121.3, 121.4, 121.6, 123.9, 124.2, 127.6, 128.4, 131.8, 133.4, 146.9, 147.5, 147.6, 148.4, 149.8, 150.9, 151.6, 151.7, 159.0; HRMS (ES) m/z 629.2351 (M + Na, $C_{34}H_{38}O_{10}Na$ requires 629.2363).

Synthesis of Tetrameric Model Compound 13EE. Tosylate 22E. To a solution of 50 mL of CH_2Cl_2 containing **11E** (1.0 g, 2.7 mmol) was added NEt_3 (1.2 mL, 8.2 mmol) at 0 °C. After being stirred for 1 h, TsCl (1.1 g, 5.8 mmol) was added at 0 °C, and the solutions was stirred for 12 h at room temperature. The solution was extracted with CH_2Cl_2 followed by drying and concentration in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 1:2) to yield **22E** (0.94 g, 66%). ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H), 3.79 (s, 6H), 3.81 (s, 6H), 4.07 (d, 1H, J = 11 Hz), 4.28−4.32 (m, 1H), 4.35−4.38 (m, 1H), 4.83 (d, 1H, J = 3.5 Hz), 6.51 (s, 2H), 6.89 (d, 2H, J = 8.0 Hz), 6.97 (d, 1H, J = 7 Hz), 7.04 (t, 1H, J = 7.5 Hz), 7.23 (d, 2H, J = 8.5 Hz), 7.64 (d, 2H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 21.6, 55.8, 56.1, 60.8, 68.5, 71.9, 84.4, 103.1, 112.3, 121.1, 121.6, 124.4, 127.9, 129.7, 132.8, 134.0, 137.5, 144.8, 146.5, 151.6, 153.3; HRMS (ES) m/z 541.1510 (M + Na, $C_{26}H_{30}O_9SNa$ requires 541.1508).

TMS Protected Tosylate 24E. To a solution of THF containing **22E** (1.4 g, 2.7 mmol) was added imidazole (0.6 g, 8.1 mmol) at room temperature. After being stirred for 30 min, TMSCl (0.9 g, 8.1 mmol) was added at the same temperature, and the solution was stirred for 10 h. The solution was extracted with CH_2Cl_2 followed by drying and concentration in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 1:3) to yield **24E** (0.77 g, 48%). ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 9H), 2.39 (s, 3H), 3.68 (s, 3H), 3.78 (s, 6H), 3.80 (s, 3H), 4.30−4.33 (m, 2H), 4.37−4.40 (m, 1H), 6.53 (d, 1H, J = 8.5 Hz), 6.55 (s, 2H), 6.68 (t, 1H, J = 6.5 Hz), 6.76 (d, 1H, J = 7.0 Hz), 6.87 (t, 1H, J = 7.0 Hz), 7.22 (d, 2H, J = 8.0 Hz), 7.66 (d, 1H, J = 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ 0.0, 21.6, 55.6, 56.1, 60.8,

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68.9, 73.9, 82.5, 104.0, 112.4, 118.5, 120.8, 122.8, 127.9, 129.6, 133.0, 136.5, 137.5, 144.5, 147.3, 150.7, 152.9; HRMS (ES) m/z 613.1909 (M + Na, $C_{29}H_{38}O_9SSiNa$ requires 613.1904).

Acetonide and TMS Protected Tetramer 27EE. To a solution of MeCN (60 mL) containing **25E** (0.8 g, 2.2 mmol) was added NaH (60% mineral oil) (100 mg, 2.5 mmol) at room temperature. After 1 h of stirring at 80 °C, **24E** (1.2 g, 2.0 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, drying, and concentration in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 2:1) to yield **27EE** (0.64 g, 45%). ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 3H), 1.61 (s, 3H), 3.73 (s, 6H), 3.74 (s, 3H), 3.78 (s, 3H), 3.79 (s, 3H), 3.84 (s, 3H), 3.94−3.99 (m, 1H), 4.06−4.13 (m, 3H), 4.21−4.23 (m, 1H), 4.53−4.57 (m, 1H), 4.88 (d, 1H, J = 8.5 Hz), 4.99 (d, 1H, J = 2 Hz), 6.46 (d, 1H, J = 8 Hz), 6.63−6.68 (m, 3H), 6.73−6.77 (m, 2H), 6.83−6.91 (m, 3H), 6.98−7.02 (m, 3H), 7.10 (d, 1H, J = 7.5 Hz); ^{13}C NMR 19.7, 28.5, 55.7, 55.9, 56.0, 60.8, 62.9, 68.6, 73.5, 74.4, 84.3, 99.4, 103.6, 111.2, 112.2, 114.0, 117.4, 119.8, 120.8, 121.0, 121.4, 122.7, 123.9, 133.1, 135.4, 137.3, 147.1, 147.3, 147.7, 149.4, 150.4, 151.5, 153.1; HRMS (ES) m/z 729.2891 (M + Na, $C_{39}H_{46}O_{12}Na$ requires 729.2887).

Tetramer 13EE. To a solution of THF (60 mL) containing **27EE** (1.0 g, 1.4 mmol) was added 3 N HCl (3 mL) at room temperature, and the solution was stirred for 12 h at room temperature. The solution was concentrated following extraction with CH_2Cl_2, drying, and concentration in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 2:1) to yield **13EE** (0.33 g, 38%). ^{1}H NMR ($CDCl_3$) δ 3.61 (d, 1H, J = 11.5 Hz), 3.76 (s, 6H), 3.79 (s, 3H), 3.82 (s, 3H), 3.86 (s, 3H), 3.89 (s, 3H), 4.08−4.11 (m, 3H), 4.21−4.26 (m, 1H), 4.56−4.59 (m, 1H), 4.94 (d, 1H, J = 4 Hz), 5.00 (d, 1H, J = 2 Hz), 6.63 (s, 2H), 6.74−6.76 (m, 1H), 6.80−6.82 (m, 1H), 6.88−6.95 (m, 6H), 7.01−7.04 (m, 2H), 7.12−7.14 (m, 1H); ^{13}C NMR δ 55.9, 56.0, 60.7, 60.8, 68.4, 72.7, 73.4, 84.4, 87.4, 103.6, 109.9, 112.2, 114.0, 114.1, 118.5, 121.0, 121.1, 121.4, 121.7, 123.9, 124.3, 133.6, 135.3, 137.3, 146.9, 147.3, 147.4, 149.4, 149.7, 151.5, 151.7, 153.1; HRMS (ES) m/z 689.2570 (M + Na, $C_{36}H_{42}O_{12}Na$ requires 689.2574).

Synthesis of Photochemical and Enzymatic Reaction Products 30E and 31E. 32E. To a solution of MeCN (60 mL) containing vanillin (0.3 g, 1.9 mmol) was added K_2CO_3 (0.54 g, 4.0 mmol) at room temperature. After 1 h of stirring at 80 °C, **23E** (0.8 g, 1.6 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, drying, and concentration in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 1:2) to yield **32E** (0.32 g, 41%). ^{1}H NMR ($CDCl_3$) δ 3.34 (s, 3H), 3.69 (s, 3H), 3.77 (s, 3H), 3.82 (s, 3H), 4.37−4.39 (m, 1H), 4.46−4.49 (m, 1H), 4.61 (d, 1H, J = 5 Hz), 4.69 (d, 1H, J = 5 Hz), 5.11 (d, 1H, J = 5 Hz), 6.78−6.98 (m, 6H), 7.32−7.36 (m, 4H), 9.80 (s, 1H); ^{13}C NMR δ 55.2, 55.6, 55.7, 55.8, 67.9, 76.6, 82.8, 94.6, 109.3, 111.8, 112.2, 113.6, 119.2, 120.8, 122.9, 126.5, 128.8, 129.9, 130.1, 147.7, 150.0, 150.9, 154.0, 159.3; HRMS (ES) m/z 505.1844 (M + Na, $C_{27}H_{30}O_8Na$ requires 505.1838).

Synthesis of 30E. To a solution of THF (40 mL) containing **32E** (0.3 g, 0.6 mmol) was added 3 N HCl (3 mL) at room temperature, and the solution was stirred for 12 h at 50 °C. The solution was concentrated following extraction with CH_2Cl_2, drying, and concentration in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 1:1) to yield **30E** (0.15 g, 55%). ^{1}H NMR ($CDCl_3$) δ 3.77 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 4.10−4.4.13 (m, 1H), 4.31−4.35 (m, 1H), 4.60−4.62 (m, 1H), 5.02 (d, 1H, J = 3.5 Hz), 6.81 (d, 1H, J = 8 Hz), 6.85 (d, 2H, J = 8.5 Hz), 6.92 (d, 2H, J = 7.5 Hz), 7.05 (t, 1H, J = 7.5 Hz), 7.22 (d, 1H, J = 7.5 Hz), 7.31−7.35 (m, 4H); ^{13}C NMR δ 55.2, 55.8, 55.9, 67.8, 72.4, 85.2, 109.2, 111.8, 112.1, 113.8, 114.0, 121.5, 124.2, 126.6, 127.3, 130.3, 131.2, 147.1, 149.9, 151.6, 153.7, 159.1, 190.9; HRMS (ES) m/z 461.1579 (M + Na, $C_{25}H_{26}O_7Na$ requires 461.1576).

Synthesis of 31E. To a solution of MeCN (30 mL) containing vanillin (0.19 g, 1.2 mmol) was added K_2CO_3 (0.32 g, 2.4 mmol) at room temperature. After 1 h of stirring at 80 °C, erythro rich **24E** (0.6 g, 1.0 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, drying, and concentration in vacuo to afford a residue that was subjected to column chromatography (EtOAc:hexane 1:1) to yield diastereomeric **31E** (0.1 g, 21%). ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 6H), 3.79 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 4.13−4.19 (m, 1H), 4.33−4.37 (m, 1H), 4.61−4.63 (m, 1H), 5.00 (s, 1H), 6.61 (s, 1H), 6.85 (d, 1H, J = 8.5 Hz), 6.92 (d, 2H, J = 8 Hz), 7.05 (t, 1H, J = 7.5 Hz), 7.19 (d, 1H, J = 8 Hz), 7.35−7.38 (m, 2H), 9.80 (s, 1H); ^{13}C NMR δ 55.8, 55.9, 56.0, 60.8, 67.8, 72.8, 84.6, 103.1, 109.2, 111.9, 112.1, 121.3, 121.5, 124.2, 126.5, 130.4, 134.8, 137.3, 147.0, 149.9, 151.5, 153.2, 153.5, 190.8; HRMS (ES) m/z 521.1787 (M + Na, $C_{27}H_{30}O_9Na$ requires 521.1788).

DCA-Promoted Photoreactions of Dimeric Lignin Model Compounds 9E−11E. Independent DCA saturated, N_2 or O_2 purged solutions containing each dimeric lignin model compound (1.5×10^{-5} mol, 2.1 mM) in 7 mL of 5% aqueous MeCN in quartz tubes were simultaneously irradiated by using uranium filtered light in a merry-go-round apparatus for 28 h (for N_2 purged solution) and 7 h (for O_2 purged solution). Each photolysate was subjected to HPLC analysis.

Relative Quantum Yields of DCA-Promoted Photoreactions of Dimeric Lignin Model Compounds 9E−11E. Independent DCA saturated, N_2 purged solutions containing each dimeric lignin model compound (1.5×10^{-5} mol, 2.1 mM) in 7 mL of 5% aqueous MeCN in quartz tubes were simultaneously irradiated by using uranium filtered light in a merry-go-round apparatus for 14 h. Each photolysate was subjected to HPLC analysis.

DCA Fluorescence Quenching by Dimeric Lignin Model Compounds 9E−11E. 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, 2.5 mM of the respective dimeric lignin model compounds. The excitation wavelength was 400 nm.

Lignin Peroxidase-Catalyzed Reactions of Dimeric Lignin Model Compounds 9E−11E. To 200 μL of 0.1 M tartrate buffer (pH 3.4) were added 200 μL of dimeric lignin model compounds (1 mM dissolved in 17% MeCN−tartrate buffer, final concentration 0.4 mM) and 40 μL of lignin peroxidase (100.5 μM, final concentration 8 μM). 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.

DCA-Promoted Photoreactions of Tetrameric Lignin Models 12EE and 13EE. Independent DCA saturated, O_2 purged solutions containing each tetrameric compound **12EE** and **13EE** (3.68×10^{-6} mol, 0.525 mM) in 7 mL of 5% aqueous MeCN in quartz tubes were simultaneously irradiated by using uranium filtered light in a merry-go-round apparatus for 20 min (for low conversion)/1 h (for high conversion). Each photolysate was subjected to HPLC analysis.

Lignin Peroxidase-Catalyzed Reactions of Tetrameric Lignin Models 12EE and 13EE. To 388 μL of 0.1 M tartrate buffer (pH 3.4) were added 100 μL of **12EE** and **13EE** (1 mM dissolved in 17% MeCN−tartrate buffer, final concentration 0.2 mM) and 2 μL of lignin peroxidase (150 μM, final concentration 0.6 μM). After 10 μL of H_2O_2 (10 mM, final concentration 0.2 mM) was added, the solutions were agitated for 30 min and then subjected to HPLC analysis.

Determination of Steady-State Kinetic Constants of LP-Catalyzed Reactions of Dimeric 9E−11E. The extent of product formation in the LP-catalyzed reactions of **9E−11E** was monitored by measuring absorbance increases at 310 nm corresponding to the respective formation of anisaldehyde (**14**, $\Delta\varepsilon_{310}$ = 9920), veratrylaldehyde (**15**, $\Delta\varepsilon_{310}$ = 10 237), and trimethoxybenzaldehyde (**16**, $\Delta\varepsilon_{310}$ = 6206). Reactions were performed in 0.1 M tartrate buffer (pH 3.4 at 25 °C) with a fixed concentration of H_2O_2 (1.2 mM), concentrations of substrate dissolved in acetonitrile varying from 0.05 to 2.5 mM, and initiated by the addition of fixed concentrations LP. For all measurements, the initial velocity data measured as a function of substrate concentration were subjected to Lineweaver−Burk analysis using Enzyme Kinetics version 1.3 (SigmaPlot) and the equation $V = V_{max}[S]/([S] + K_M)$, where V is initial velocity, V_{max} is maximum velocity, [S] is substrate concentration, and K_M is the Michaelis

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constant. k_{cat} values were 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, fluorescence quenching spectra, and Stern−Volmer plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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