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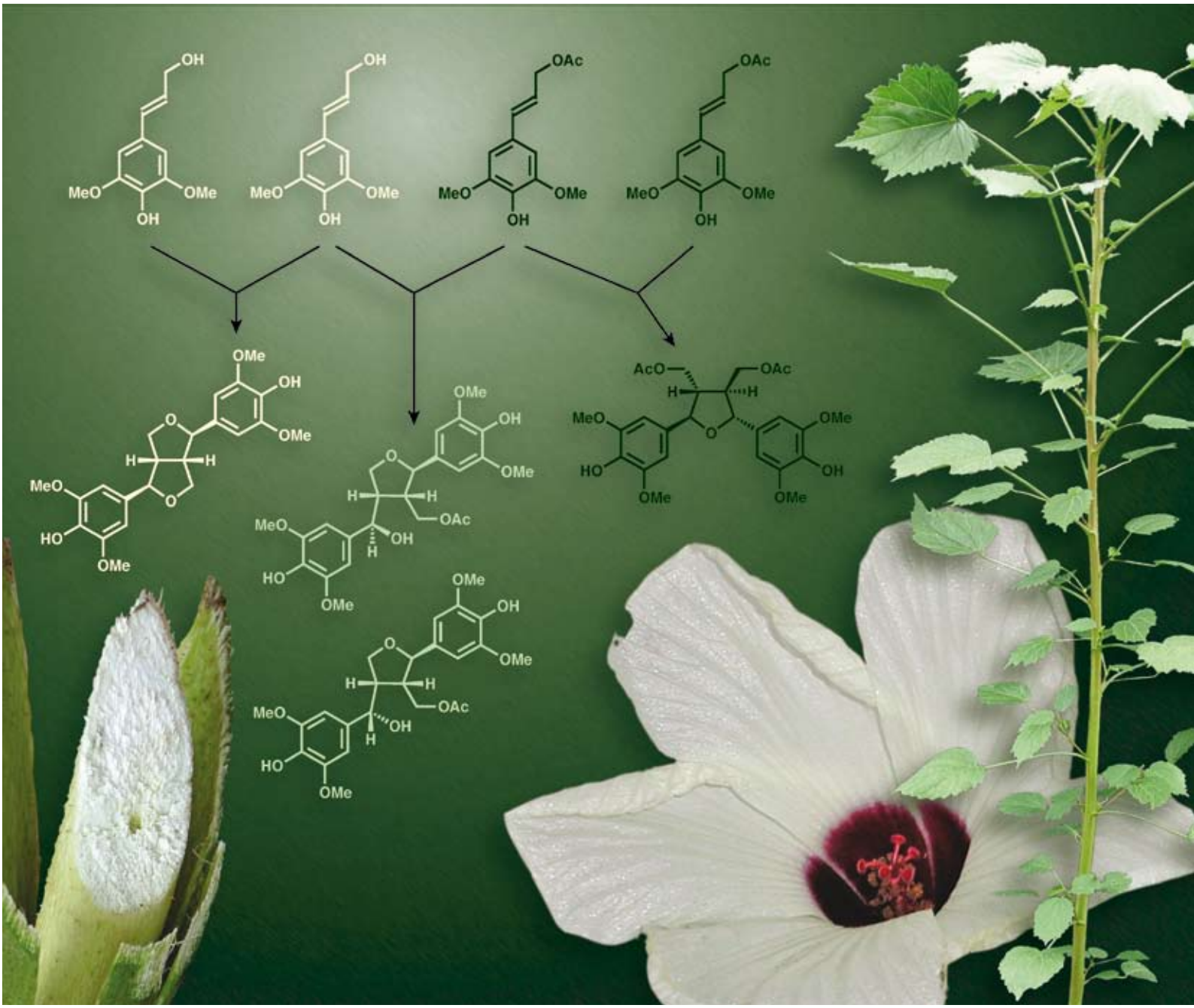

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ARTICLE

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Novel tetrahydrofuran structures derived from β–β-coupling reactions involving sinapyl acetate in Kenaf lignins

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Free radical coupling of sinapyl γ-acetate or cross-coupling between sinapyl acetate and sinapyl alcohol yields novel tetrahydrofuran β–β-(cross-)coupled dehydrodimers. Such substructures are therefore anticipated in naturally acetylated lignins, *e.g.* in Kenaf, if sinapyl acetate is a component of the lignin monomer pool. The DFRC (derivatization followed by reductive cleavage) method, modified by replacing all acetyl reagents and solvents with their propionyl analogs (DFRC′), allows the analysis of naturally acetylated lignins. DFRC′ treatment of the sinapyl acetate-derived dimers or crossed dimers gave diagnostic products that retain at least one acetate group on a sidechain γ-position; the products have been authenticated by comparison of their mass spectra and GC retention times with those of synthesized compounds. DFRC′ of Kenaf lignins produces the same diagnostic products as from the dimers, implicating the presence of the various tetrahydrofuran units in Kenaf lignins. With data from the model compounds in hand, NMR analysis of Kenaf lignins elegantly confirms the presence of such substructures in the polymer, establishing that acetates on Kenaf lignins arise through incorporation of sinapyl acetate, as a lignin precursor, *via* enzyme-mediated radical coupling mechanisms.

Introduction

Lignins are complex phenylpropanoid polymers accounting for a substantial component of terrestrial plant biomass. They are formed through enzyme-initiated radical coupling of three hydroxycinnamyl alcohols (monolignols), although other phenolic monomers may also be significantly involved in lignification in some plants.[1–3] Selected lignins have long been known to be naturally acylated by various acids.[4] The biochemistry associated with such acylation remains unresolved, however, and the presumably required transferases are unknown.

Sites of acylation (regiochemistry) are important to establish as they suggest biochemical incorporation pathways.[5,6] Three pathways have been considered responsible for acylation of lignins, resulting in different regiochemistries:[6] (a) free acids attack lignin quinone methide intermediates, forming lignins with esters only at the α-positions of lignin sidechains; (b) preformed γ-acylated hydroxycinnamyl alcohols are incorporated by radical coupling into lignins during wall lignification so that esters are exclusively on the γ-positions of lignin sidechains; and c) acylation occurs post-lignification, resulting in esters at γ- and/or α-positions, depending on the selectivity of the (presumably required) transferase and the chemical structures of individual lignin units or sub-units. Although early studies suggested mixed regiochemistry for *p*-coumarates on grass lignins,[7–10] more diagnostic investigations into maize lignins established a strict γ-regiochemistry that was subsequently verified in other grasses.[11,12] The observation of *p*-coumarates on the γ-position of several types of units in lignin (β-ethers, both isomers; phenylcoumarans; cinnamyl alcohol endgroups)[11] suggested that pathway (b) (pre-acylation of lignin monomers) is the most likely pathway, as initially suggested by Nakamura and Higuchi.[10] However, unambiguous evidence has not been forthcoming.

The primary aim of this study was to seek evidence for the participation of the presumed pre-acylated monolignol sinapyl acetate in Kenaf lignification. As outlined in a preliminary communication,[13] there is one lignification pathway that is significantly altered by pre-acetylation of the monolignols. That is the pathway in which the γ-OH on the monolignol becomes involved in post-coupling reactions, *i.e.* the pathway normally leading to β–β-coupled (resinol) units **3** (Fig. 1). The key concept is that, with the γ-position acetylated, β–β-coupling or cross-coupling can still presumably occur but the re-aromatization reactions following the radical coupling step can no longer be driven by the internal attack of the γ-OH on the quinone methide intermediates **QM1/2** or **QM2** (Fig. 1, top pathway) – the γ-acetylation prevents such a reaction. Other pathways must therefore be in effect producing other products. The important point is that the acetyl group can remain attached in non-resinol β–β-coupling products, products that could not have arisen from post-coupling acetylation reactions.

Therefore finding the unique non-resinol syringyl structures in Kenaf lignins will establish beyond reasonable doubt that acetates on Kenaf lignins arise through incorporation of pre-acetylated sinapyl alcohol, as a lignin precursor, *via* radical coupling mechanisms. In the previous communication,[13] we reported that β–β-coupled dimeric DFRC′ products containing γ-acetate groups were tentatively identified by GC–MS. In this work, all expected diagnostic β–β-coupled DFRC′ degradation products from Kenaf

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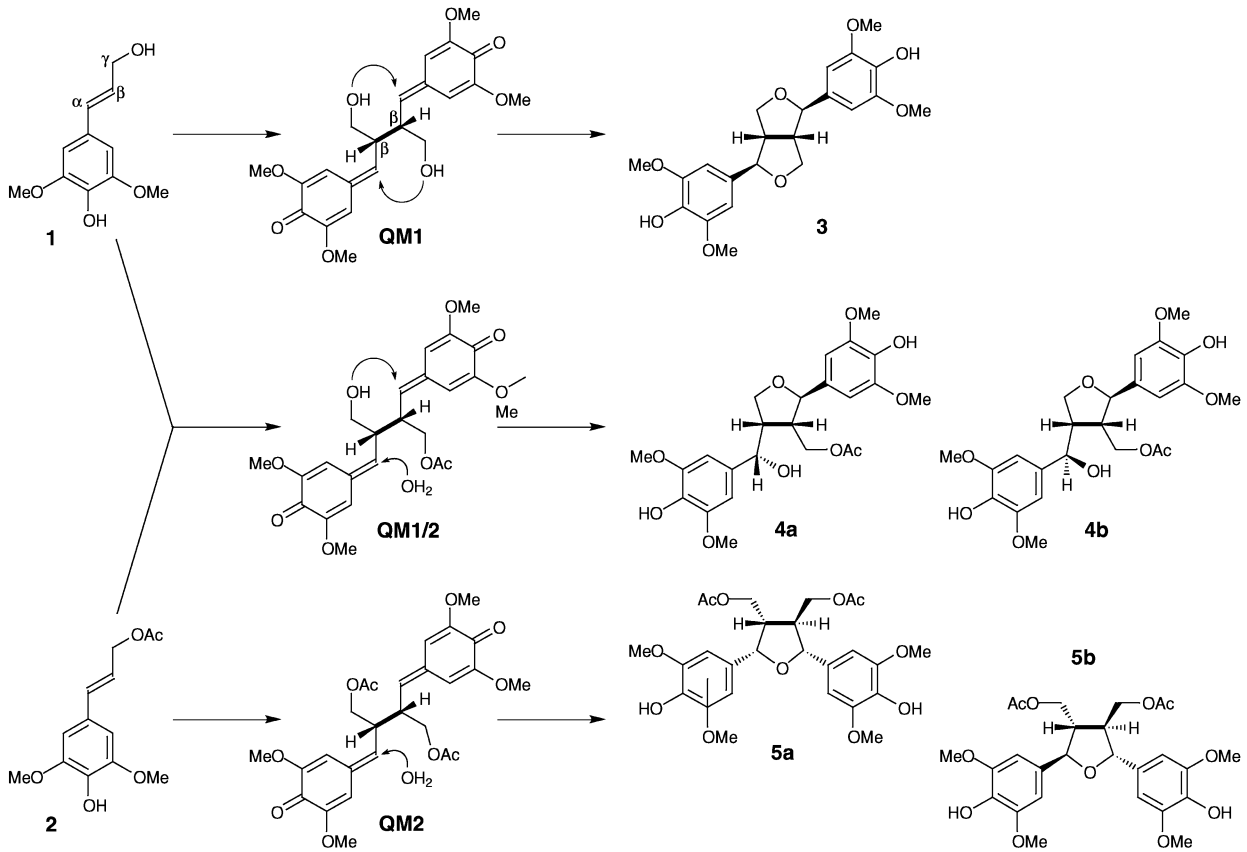

Fig. 1 Dehydrodimerization of sinapyl alcohol **1**, and sinapyl acetate **2**, and cross-coupling of **1** and **2**. The traditional monolignol **1** will dehydrodimerize initially, forming the β–β-coupled bis(quinone methide) intermediate **QM1**, which re-aromatizes by internal γ-OH attack on each quinone methide electrophilic α-carbon to produce syringaresinol **3** as the overwhelmingly major product. When sinapyl acetate **2** dimerizes, it forms an analogous bis(quinone methide) intermediate **QM2**. However, **QM2** can not be re-aromatized by internal trapping; structures **5** arise from water attack on one quinone methide moiety with the resulting α-OH attacking the other quinone methide to form a tetrahydrofuran. When **1** and **2** radicals cross-couple, the intermediate bis(quinone methide) **QM1/2** now has one quinone methide moiety which can be internally trapped by the single γ-OH to form a single tetrahydrofuran ring, but the other quinone methide can only be re-aromatized by attack of an external nucleophile. The β–β-bond formed *via* radical coupling is shown bolded.

lignins were observed and identified by comparison of their mass spectra and GC retention times with those of synthesized authentic compounds. Phenol-methylated tetrahydrofuran model compounds **4M–5M** (see later in Fig. 6), corresponding to non-resinol β–β-structures presumably existing in Kenaf lignins, were also synthesized. With these model compound data, 2D NMR analysis of the Kenaf lignins allows the identification of such tetrahydrofuran structures in the polymer. The findings described here provide strong evidence that acetate groups in Kenaf lignins arise from pre-acetylated sinapyl alcohol, *i.e.* sinapyl γ-acetate, which participates in the formation of lignin macromolecules by conventional radical coupling processes. Logically, other acylated lignins also result from lignification using acylated monolignols as monomers.

Results and discussion

Oxidative coupling products from sinapyl alcohol and sinapyl acetate

Enzyme-catalyzed oxidation of 4-hydroxycinnamyl alcohols (*p*-coumaryl, coniferyl and sinapyl alcohols) has been conducted extensively in studies related to lignin biosynthesis and lignin structures.[14–18] Oxidation of coniferyl alcohol with H_2O_2/peroxidase or metal oxidants leads to complex mixtures of dimeric and oligomeric products.[19,20] However, the oxidation of sinapyl alcohol with H_2O_2/peroxidase gave the β–β-coupled dehydrodimer, syringaresinol, as the predominant product, along with a small amount of the arylglycerol-β-aryl ether (β-O-4-dimer) being reported.[21] In this study, sinapyl alcohol was oxidized with H_2O_2/peroxidase in a 20 mM phosphate buffer solution containing 17% (v/v) acetone to solubilize the substrates. Syringaresinol was found to be the only β–β-coupled dimeric product, consistent with previous results. However, no β-O-4-dimer could be detected here in NMR spectra of the crude product. Oxidation of sinapyl acetate **2** (Fig. 1, lower pathway) under similar conditions yielded several β–β-coupled products from which tetrahydrofurans **5a** (major) and **5b** (minor) were isolated and identified. Compounds structurally similar to **5**, solely *threo*-β–β-coupled products, have been obtained from H_2O_2/peroxidase oxidation of isoeugenol and (*E*)-2,6-dimethoxy-4-propenylphenol.[22] Other 4-propenylphenol derivatives such as (*E*)-coniferyl alcohol, (*E*)-ferulic acid, (*E*)-sinapyl alcohol as well as (*E*)-sinapic acid have also been found to yield *threo*-β–β-coupled tetrahydrofuran products. This stereoselectivity was considered to result from an intermediate

charge-transfer complex in which the two aromatic rings are parallel and aligned in a 'tail to tail' manner.[22] In the case of sinapyl acetate **2**, the β–β-coupling reaction should have a selectivity similar to that of (*E*)-sinapyl alcohol and yield *threo*-products with compound **5a** as the predominant isomer (Fig. 1). Some of the acetyl groups of **2** hydrolyzed during the course of oxidation, so syringaresinol **3** and cross-coupled products **4a** and **4b** were also produced. When equimolar amounts of sinapyl alcohol **1** and sinapyl acetate **2** were oxidized together with H_2O_2/peroxidase, the cross-coupled compounds **4** were formed as the major products, the minor products being **3** and **5a**. This result is particularly interesting because cross-coupled dimers are not generally found to be major products from free radical coupling of two different phenolic monomers. In fact the ratio of **3**:**4**:**5** was essentially the statistical 1 : 2 : 1, suggesting little selectivity in the reaction. Therefore radicals from sinapyl acetate **2** can equally homo-couple or hetero-couple with sinapyl alcohol **1** radicals during the enzyme-catalyzed oxidation process.

It would be reasonable to expect that substructures like compounds **4–5** would exist in Kenaf lignins if sinapyl acetate **2**, as a lignin precursor, participates in lignification. β–β-Coupled syringyl structures are relatively minor in lignins since they are formed only in the initial coupling reactions of the sinapyl alcohol monolignol – lignins derive principally from coupling reactions between a monolignol (primarily at its β-position) and the growing lignin oligomer/polymer (where only the 4-O- and, in guaiacyl units, the 5-position are available; β–β-coupling as a chain-extension reaction is not possible). So a sensitive analytical method, DFRC′ combined with GC–MS, was conveniently used to seek proof for the existence of substructures **4–5** in Kenaf lignins as discussed below.

DFRC′ products from model compounds 3–5 and Kenaf lignins

The DFRC (derivatization followed by reductive cleavage) method is a degradation procedure that produces analyzable monomers and dimers by cleaving α- and β-ethers in lignins.[23] Although the mechanism is completely different, it is conceptually similar to acidolytic methods such as acidolysis[24] and thioacidolysis.[25] An advantage of the DFRC method is that γ-ester groups on β-O-4-linked lignin units remain intact.[26] The method therefore allowed us to confirm that *p*-coumarate groups are at the γ-positions of lignins in grasses such as maize, and that they are predominantly on syringyl units of the lignins examined.[6] A modified version that replaces all acetyl reagents with propionyl analogs (DFRC′, see experimental section) allows natural acetates to be similarly characterized.[26] Analysis of DFRC′ monomers from Kenaf bast fiber established that acetates acylate the γ-position of lignin sidechains. They are also almost entirely on syringyl units (~60% of the released syringyl monomers were acetylated *vs.* <1% of guaiacyl monomers). Acetyl groups, like uronosyl groups but unlike *p*-coumarates, are known to migrate on lignin sidechains[27] so a mixed regiochemistry was anticipated; a small percentage of α-acetylation was observed by NMR.[28] An unambiguous approach to determine whether lignin acetates arise from pre-acetylated monolignols is therefore required.

Model reactions. To test if the DFRC′ method is valid for establishing the presence of structures **4–5** that have been incorporated into Kenaf lignins, it is imperative to determine whether unique and diagnostic products could result from DFRC′ degradation. DFRC′ treatment of syringaresinol **3** produced three major products as indicated by GC (not shown). TLC separation of the crude DFRC′ products gave two major fractions. One fraction was a mixture of two pairs of isomers with MW 570, as indicated by GC–MS. They were characterized and identified by NMR to be tetrahydronaphthalene (aryltetralin-type) dimers **10** and **11** as shown in Fig. 2. The major isomers of **10** and **11** have a *trans*-configuration between substituents on carbons Aα and Aβ, as suggested by the dibenzylic proton NMR signals (Aα) at around δ 4.44–4.46, a broad singlet for **11** or a narrow doublet (J = 1.45 Hz) for **10**, similarly to that in methyl thomasidic acid ester **15** (Fig. 3). The other fraction was a pair of isomers with a MW of 644. The proton NMR and proton–proton COSY NMR of this compound suggested that it also had an aryltetralin-type skeleton and its major isomer was tentatively assigned to be compound **6**. Similar aryltetralin-type compounds are also produced following thioacidolysis of β–β-linked lignin units (pinoresinol and syringaresinol).[25] The GC retention time, mass spectrum and NMR data of **6** (isolated from DFRC′ of **3**) were identical with those from synthesized authentic compound **6** (Fig. 3), confirming its structure.

When compound **5** was subjected to DFRC′ treatment, three compounds were also produced. Two of them were identified by GC–MS and NMR after TLC purification as compounds **12** and **13**, acetate analogs of **10** and **11**. The third product was the di-γ-acetate analog of **6** as revealed by proton NMR. Comparison of the GC retention time, mass spectrum and NMR data of this product isolated from DFRC′ of **5** with synthesized authentic compound **9** (Fig. 3) confirmed its identity to be **9** (Fig. 2). In summary, DFRC′ of the sinapyl acetate dimer **5** produced analogs of the products from syringaresinol in which the latter (**6**, **10–11**) have propionate groups on their Aγ positions and the former (**9**, **12–13**) retain their initial acetate groups.

Following the results above from compounds **3** and **5**, it is not difficult to understand the formation of compounds **7** and **8** as well as compounds **10–13** when compound **4**, the product of cross-coupling between sinapyl acetate **2** and sinapyl alcohol **1** (Fig. 1), was subjected to the DFRC′ degradation conditions (Fig. 2). However, it was not straightforward to confirm that both **7** and **8** exist in DFRC′ products from compound **4** because they have the same MW (630) and similar/identical mass spectra, and are so similar in structure that they were not resolved by TLC or even GC under the conditions used in this study. Moreover, compounds **7** and **8** have almost identical proton NMR data (see experimental section); the ^{13}C NMR spectra are different. Assigning their ^{13}C spectra from mixtures was not possible, so compounds **7** and **8** were each synthesized independently (Fig. 3). Comparing NMR (both ^{13}C and ^{1}H) spectra of synthesized **7** and **8** with those of isolated fractions (mixtures) containing compounds **7** and **8** confirmed the co-existence of compounds **7** and **8** in the DFRC′ products from compound **4**.

It is worthy to note that compound **6** retains all of the γ-oxygen atoms originally in **3** whereas **10** and **11** retain only half of them following DFRC′ treatment. In a similar way, all functional groups originally in compounds **4–5** were retained in DFRC′ products **7–9**. Products **12–13** have only a single acetyl group on each of their sidechains. Since no acetyl-containing reagents or solvents are used in DFRC′, acetyl groups on the products

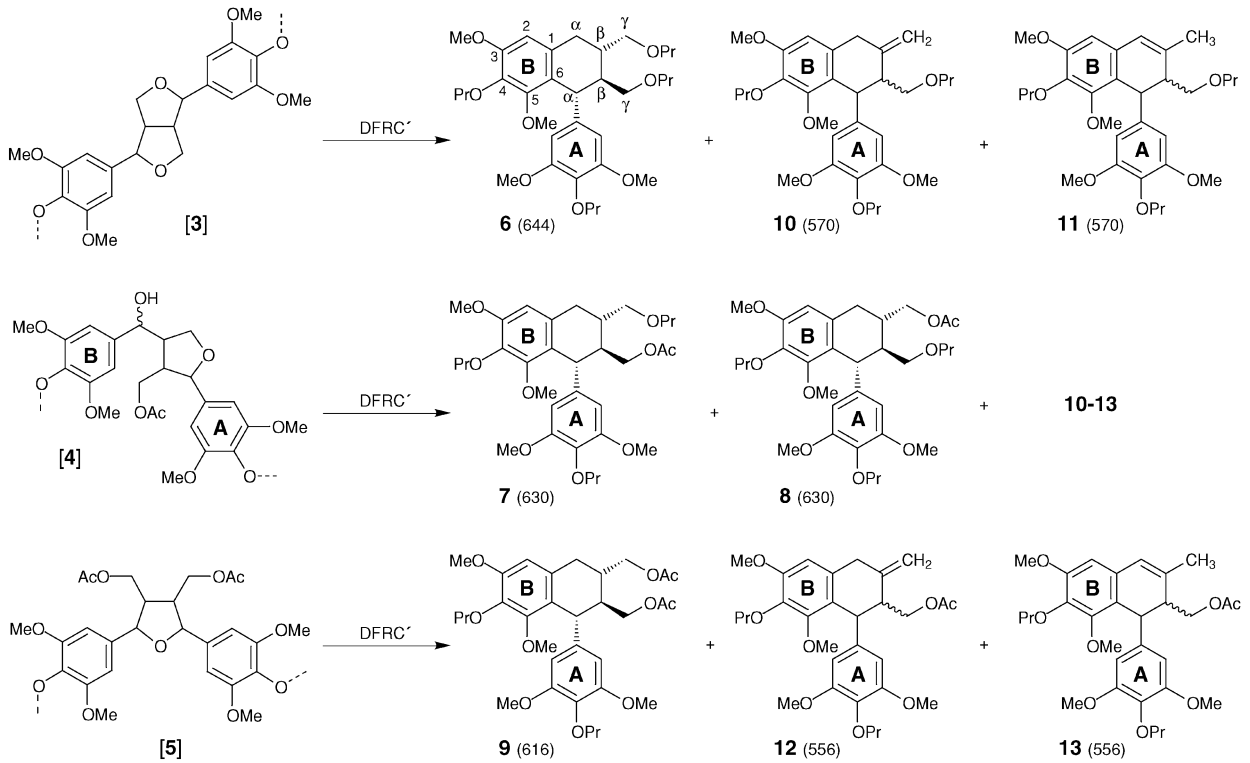

Fig. 2 Major DFRC′ products from model compounds **3–5** that have been incorporated into lignin (and therefore represented as [**3**], [**4**], and [**5**]). Numbers in parentheses are nominal molecular masses for MS.

must derive from the acetates in the original compounds **4** and **5**. Therefore the DFRC′ degradation products **7–9** and **12–13** are diagnostic because they are all β–β-coupled dimers having at least one acetyl group on their sidechains (γ-position) and those acetyl groups are obviously retained from structures **4–5** following DFRC′ degradation. Finding compounds **7–9** and **12–13** from the DFRC′ degradation products of Kenaf lignins will establish the existence of substructures **4–5** in the lignins.

Kenaf. In a preliminary trial communicated previously,[13] isolated Kenaf lignin was subjected to DFRC′ treatment. Crude DFRC′ degradation products were analyzed by GC–MS. Three small peaks in the total-ion chromatograms had characteristics that looked promising. Mass spectra showed that those products had molecular weights (MW) of 644, 630, 616 and had 0, 1, 2 aliphatic acetate and 2, 1, 0 aliphatic propionate groups respectively, like compounds **6–9**. Here, comparison of GC retention times, Fig. 4, and mass spectra of DFRC′ products with those of authentic (synthesized, Fig. 3) compounds confirmed that compounds **6–9** are present in the DFRC′ degradation products from Kenaf lignin.

Compounds **10–13** were not able to be resolved in the complex GC chromatograms of crude DFRC′ products, although comparison of the data obtained from DFRC′ analysis of compounds **3–5** and the Kenaf lignin suggested their presence in the lignin product. Kenaf bast fiber lignin used in this work has a very high syringyl content and a consequently high content of β-O-4-linkages. Therefore it was necessary that the predominant monomers in DFRC′ degradation products be removed and that the dimer fractions be further fractionated in order to resolve and identify the expected products **10–13** by GC–MS analysis. Fig. 4 shows the dimer region of the total-ion chromatograms (TIC) of the TLC-fractionated DFRC′ products from Kenaf lignin. Fraction 2 contains compounds **10–13** and Fraction 3 has compounds **12–13** and **6**. Compounds **7** and **8** fall primarily into Fraction 4 whereas compound **9** clearly showed up in Fraction 5. When the most abundant ion in the mass spectrum of a product was selected, the selected-ion chromatograms (Fig. 4) more clearly showed that all anticipated DFRC′ products from Kenaf lignin, compounds **6–13**, were indeed present. The logical conclusion is that mono- and di-acetylated β–β-coupling products, such as compounds **4–5**, exist as subunits in Kenaf lignins.

Mechanism and implications. As for how DFRC′ products **6–13** are formed, proposed pathways and intermediates are shown in Fig. 5. Under strongly acidic conditions (propionyl bromide in propionic acid), α-ethers (benzyl ethers) are cleaved forming α-bromides **30**; any free γ-hydroxyls are propionylated.[26] For β–β-linked units, the electron-rich syringyl aromatic ring nucleophilically attacks one benzylic position, displacing bromide and forming a 6-membered ring intermediate **31** which rearomatizes to the tetrahydronaphthalene **32**. Compound **32** can also, in part, eliminate HBr forming an α,β-double bond, resulting in formation of dihydronaphthalene **33** from which γ-bromide **34** is formed. In the following DFRC′ step, Zn reductive elimination of bromo-ethers results in cleavage of β–O-4-linkages to produce the normally analyzed monomers and dimers. Elimination of the bromide in **32** produces compounds **6–9** whereas **34** produces compounds **10–13**.

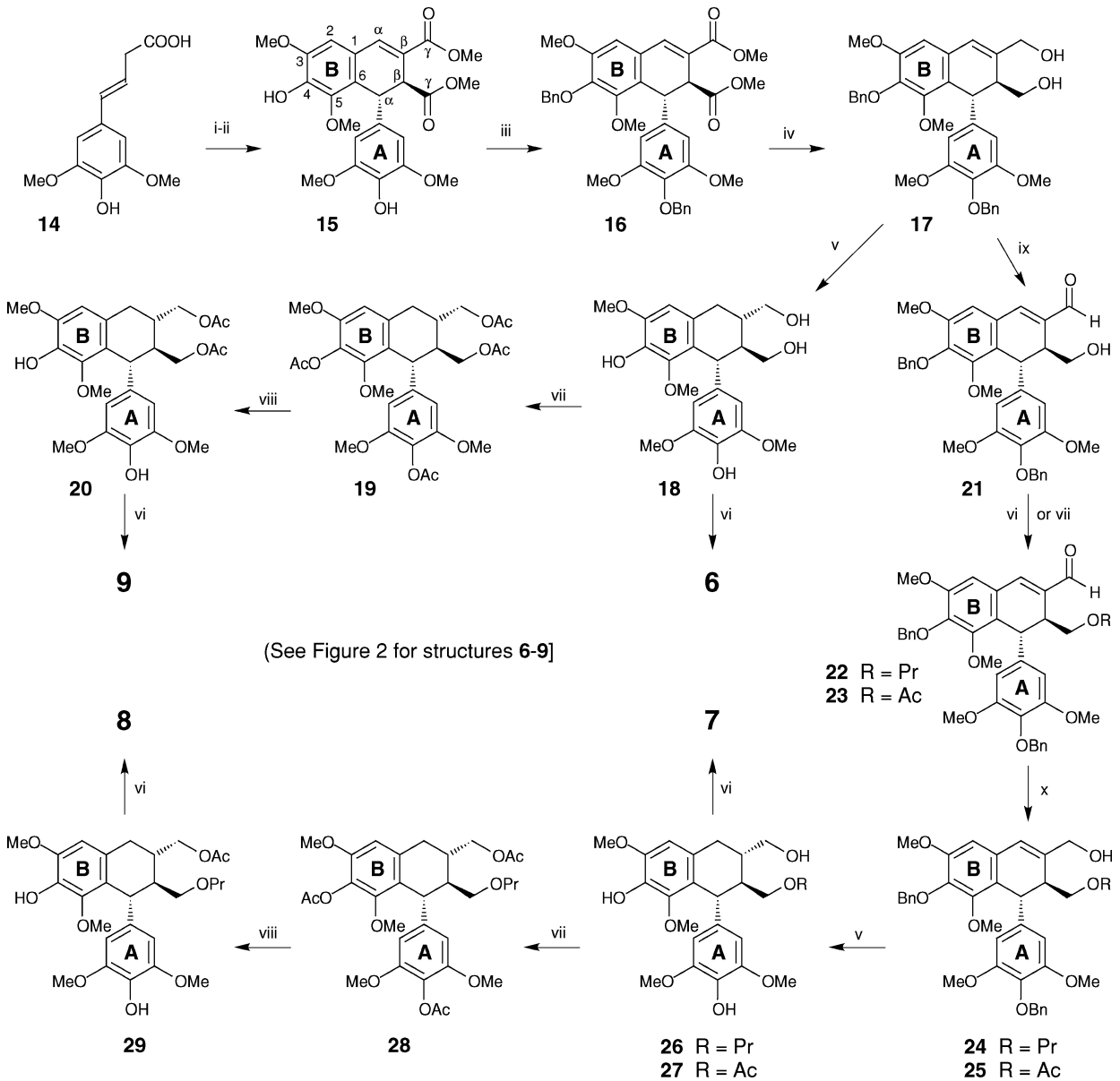

Fig. 3 Synthetic routes to compounds **6–9**. i. MeOH–AcCl, 16 h; ii. $FeCl_3 \cdot 6H_2O$, 80% aq. MeOH–HCl, 48 h; iii. BnCl, cat. KI, K_2CO_3, acetone, reflux, 48 h; iv. LAH, THF, 1 h; v. Pd(en)/C, H_2, MeOH, 10 h; vi. propionic anhydride + pyridine, 16 h; vii. acetic anhydride + pyridine, 16 h; viii. pyrrolidine, 10 min; ix. PDC, DMF, 4 h; x. $NaBH_4$, EtOAc, 2 h.

In general, detection of γ-acetates in syringyl β–β-coupled DFRC′ products **7–9** and **12–13** from Kenaf lignin suggests the existence of the novel non-resinol substructures **4–5** which can be formed only from dehydrogenative coupling of sinapyl acetate itself or cross-coupling with sinapyl alcohol. Direct proof for the presence of these novel β–β-structures in Kenaf lignin by 2D NMR analysis of this Kenaf lignin follows.

2D NMR analysis of Kenaf lignin

Natural acetates on lignins are often neglected, in part due to the common practice of acetylating lignins to improve their solubility and NMR characteristics. A prior study by NMR revealed high levels of acetate on underivatized Kenaf bast fiber lignins and that the acetates are primarily at the γ-sidechain positions.[29] Experiments based on the DFRC′ method confirmed the regiochemistry and further revealed that acetates are almost entirely on syringyl units.[26] Such studies have recently been extended to plants with even higher levels of acylation.[30] As discussed above, the novel β–β-coupled substructures **4–5** (Fig. 1) are expected to exist in Kenaf lignins. Those structures have not been identified by NMR analysis, possibly because of their lower contents in lignin and the prior unavailability of the corresponding model compounds. We initially considered it rather ambitious to attempt to detect such low-level structures directly by NMR. As shown below, however, they are readily found.

In this study, model compounds **4M–5M**, representing etherified structures **4–5** that model the substructures potentially existing in Kenaf lignins, have been synthesized. The characteristic sidechain regions of HSQC NMR spectra were compared with

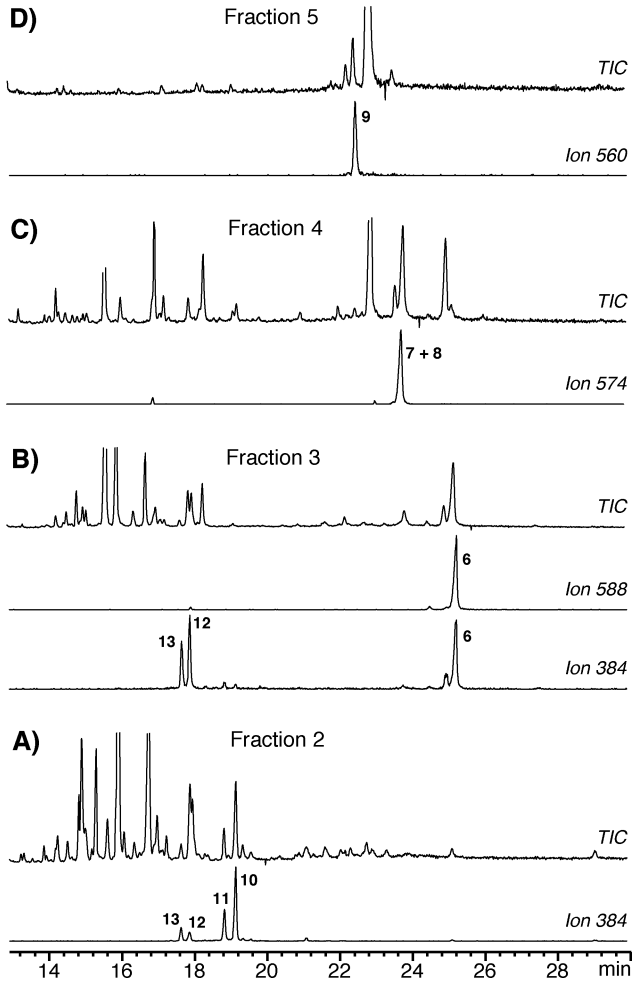

Fig. 4 Total ion and selected-ion chromatograms from TLC-fractioned DFRC′ products from Kenaf lignin.

those of the corresponding spectra from Kenaf lignins in order to identify these β–β-coupled substructures (Fig. 6). Although it is of interest to delineate the presence of naturally acetylated compounds **4–5**, peracetylation was used here to improve NMR characteristics; the tetrahydrofuran structures observed only form when sinapyl acetate is the monomer, so the presence of acetates from the acetylation of the lignin is not a complication here.

In order to find the β–β-structures implicated by DFRC′ results, β–β-coupled model compounds **4–5** were made from peroxidase–H_2O_2 oxidation of sinapyl alcohol **1** and γ-acetylated sinapyl alcohol **2**. Diaryltetrahydrofuran **5b** was also synthesized independently from sinapic acid following a previously described method with slight modification[31] (Fig. 7). The structures of **4–5** were identified by the normal array of 1D and 2D (HSQC, HMBC, COSY) NMR experiments. Methylation of the phenolic-OH groups in compounds **4–5** and acetylation of the α-OH of products **4a**, **4b** provide ideal models **4aM** and **4bM** for identifying these substructures in acetylated Kenaf lignins (in which they are typically 4-O-etherified). Therefore HSQC NMR spectra of these methylated and acetylated model compounds **4aM**, **4bM** and **5bM** were recorded at high resolution. The diagnostic sidechain regions of the HSQC spectra were compared with the corresponding regions of the Kenaf lignin (Fig. 6).

From the HSQC NMR spectra of the lignin in Fig. 6 (left column), it is obvious that all diagnostic sidechain ($^{13}C_\alpha$–H_α, $^{13}C_\beta$–H_β, $^{13}C_\gamma$–H_γ) correlations corresponding to the various β–β-structures are observed and identified by comparison with the synthesized model compounds **4aM**, **4bM** and **5bM** (right column). However, one curious aspect remains. Although peroxidase–H_2O_2 oxidative coupling of sinapyl alcohol **1** and γ-acetylated sinapyl alcohol **2** produces compound **4a** as the major isomer, Kenaf lignins contain both **4a** and **4b**, although **4b** is at a higher level. Only structure **5b**, which is a minor isomer produced by the peroxidase–H_2O_2 oxidative coupling reaction of γ-acetylated sinapyl alcohol **2**, can be found in Kenaf lignins. Both isomers of **5** have the same *trans*-configuration about the β–β-bond, so it is not the coupling reaction that differs, it is the post-coupling water addition to one quinone methide moiety of the intermediate **QM2** (Fig. 1) and the subsequent attack of the resulting α-OH group on the other quinone methide moiety. Observations to date indicate that the stereochemistry of products resulting from post-coupling reactions in lignin biosynthesis is under kinetic control; the isomer ratios of β-O-4- and β–β-units in lignin are consistent with *in vitro* results, as reviewed.[2] To date there has been no evidence that proteins have any role in lignification,[32] particularly since no evidence of any optical activity can be detected in lignins.[33] Whether there is additional control in the post-β–β-coupling water

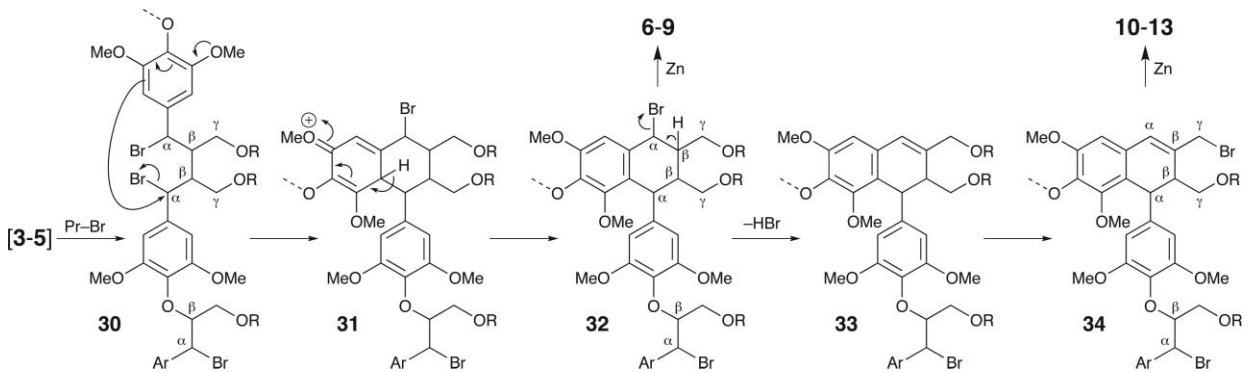

Fig. 5 Proposed pathway to the formation of DFRC′ products **6–13** from units **3–5** that have been incorporated into Kenaf lignin (one β-4-O-linkage is shown explicitly). R = Ac or Pr.

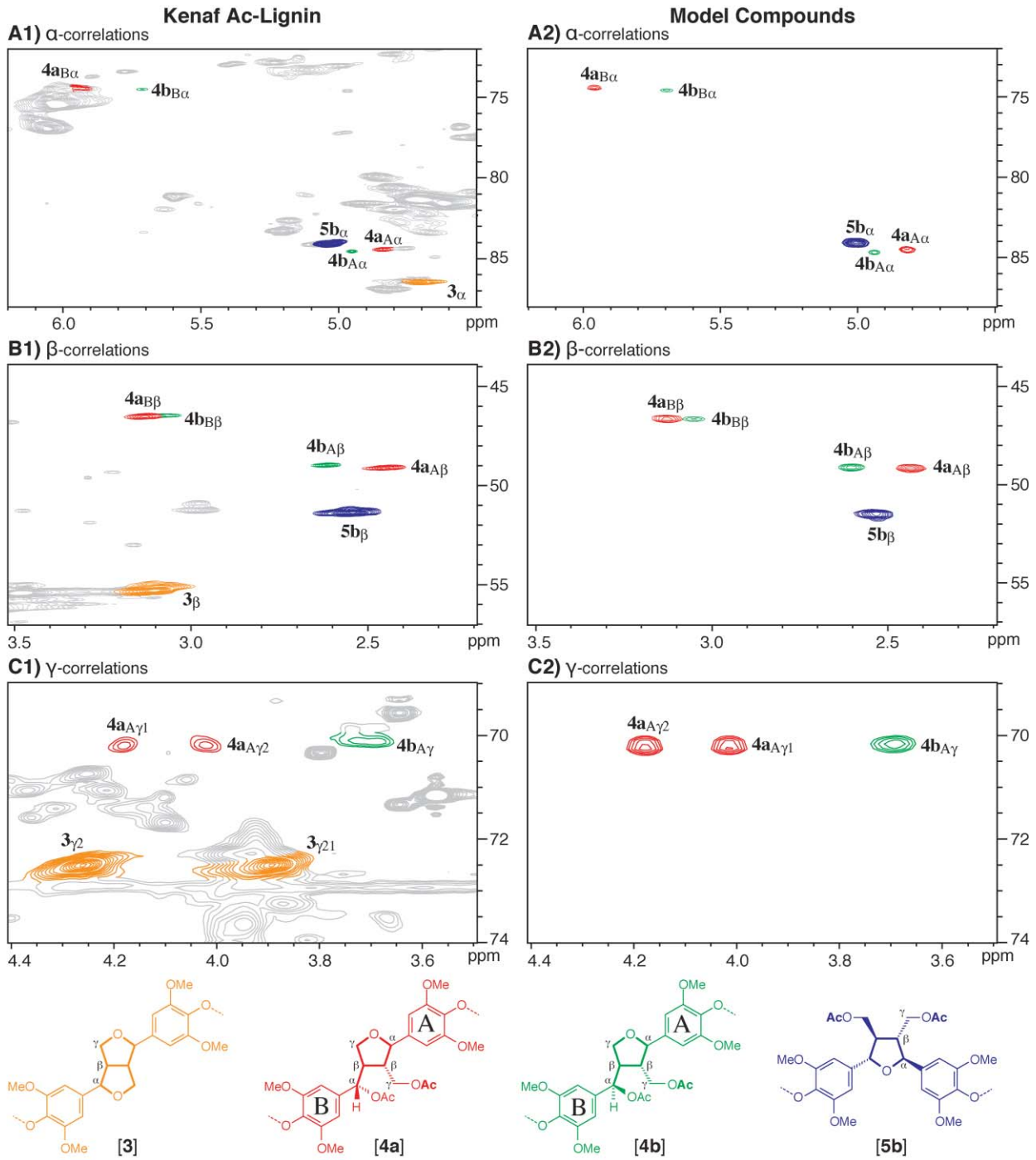

Fig. 6 Partial HSQC ^{13}C–^{1}H correlation NMR spectra showing correlations in the acetylated Kenaf lignin spectrum (A1–C1) matching those in model compound spectra (A2–C2). Model spectra are from the 4-*O*-methylated (and acetylated) compounds **4aM**, **4bM**, and **5bM**. The structures represent the units in Fig. 2 that have been incorporated into lignin or are 4-*O*-methylated models, so are denoted in square brackets under the structures; the assignments on the spectra are not bracketed, for simplicity. Note that individual spectra have been combined so the various structures are in approximately the same proportions as in the Kenaf lignins. Note also that, although the models and the lignins are acetylated, the tetrahydrofuran structures **4–5** evidenced arise only from reactions incorporating sinapyl acetate **2**; the originally present acetates are shown bolded (**Ac**). A) α-^{13}C–^{1}H correlations. B) β-^{13}C–^{1}H correlations. C) γ-^{13}C–^{1}H correlations; correlations from **5b** (not shown) are severely overlapped with γ-^{13}C–^{1}H correlations from other lignin units.

addition to favor **5b** in Kenaf, or whether the specific conditions that differ between the *in vitro* and *in vivo* system can explain the differing stereochemical course of sinapyl acetate dimerization towards isomers **5a** and **5b**, awaits further investigation.

Compounds for structural authentication by NMR

Mass spectra and NMR of TLC-separated DFRC′ products from compound **3** and **5** suggested that compounds with MW 616 and

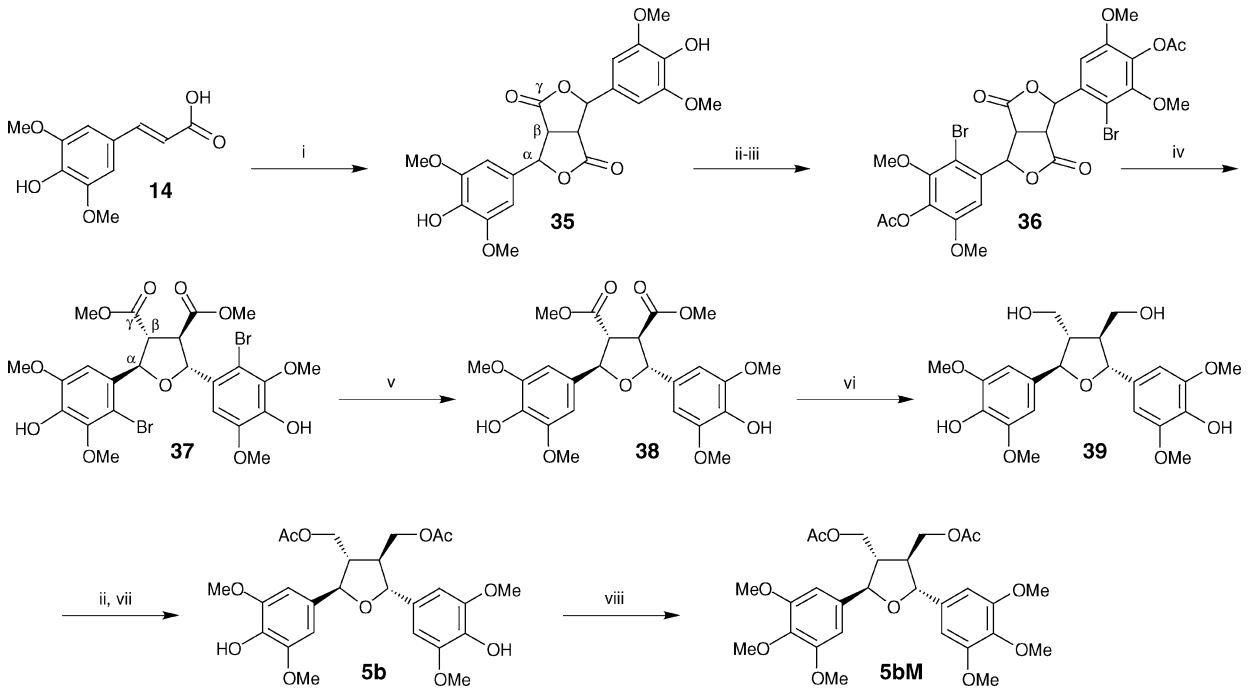

Fig. 7 Synthetic route to model compound **5bM**. i. horseradish peroxidase–H_2O_2, aqueous acetone; ii. acetic anhydride/pyridine; iii. bromine, NH_4OAc, AcOH, 50 °C, 6 h; iv. AcCl–MeOH, 16 h; v. Pd/C–Et_3N, H_2, MeOH, 2 days, 80%; vi. DIBAL-H, toluene, 2 h; vii. pyrrolidine, 3 min;. viii. MeI, K_2CO_3, acetone, 16 h.

644 are tetrahydronaphthalene (aryltetralin) structures (*cf.* **6** and **9**) of which 4 isomers each are possible. Only one pair of peaks corresponding to each MW was detected by GC–MS. As they are derived from β–β-linked units, and the β–β-bonds remained intact during the DFRC′ process, the two methylene substituents at the β-positions should have a *trans*-configuration in accordance with the *threo*-coupling mode of (*E*)-sinapyl alcohol or (*E*)-sinapyl acetate.[22] Thus major isomers of these two DFRC′ products should be compounds **6** and **9**, which have a *trans–trans*-configuration of their three substituents on the 6-membered rings. Therefore synthetic routes in Fig. 3 were designed to make the required isomers of these target compounds.

Methyl thomasidioic acid ester **15**, Fig. 3, was made from $FeCl_3$ oxidation of methyl sinapate in the presence of HCl in 80% aqueous methanol (the esterification and the oxidation were done in a single flask without work-up – see experimental section). Benzyl ether protection of **15** gave compound **16** that was reduced by lithium aluminium hydride in dry THF to produce diol **17**. Hydrogenation and debenzylation of compound **17** in methanol yielded compound **18**. Heterogeneous *cis*-hydrogenation of methyl thomasidioic acid ester **15** or compound **16** followed by LAH reduction gave ~1 : 1 mixtures of the two isomers of **18** rather than the predominantly *trans–trans*-isomer **18** isolated above (results not shown here). Propionylation of **18** finally accomplished synthesis of **6**, while acetylation of **18** produced **19** from which selective deacetylation with neat pyrrolidine led to **20**; propionylation of **20** gave **9**.

As discussed above, it is expected that **7** and **8** would have the same configuration as **6** and **9**. Selective oxidation of diol **17** with pyridinium dichromate, differentiating the two γ-OHs, was the key step to making **7** and **8**. Benzyl ether protection of phenolic hydroxyls was necessary for a clean oxidation (of **17** to yield aldehyde **21**). Propionylation or acetylation of **21** gave **22** or **23**. Sodium borohydride reduction of aldehydes **22** or **23** in ethyl acetate gave the corresponding alcohols **24** or **25**. The following steps have the same strategies as for making **6** and **9** to finish the syntheses of the desired **7** and **8**.

Compounds **10–11** and **12–13** were isolated by TLC from DFRC′ products of syringaresinol **3** and tetrahydrofuran **5** respectively (see experimental section), and their structures were confirmed by NMR.

Compounds **4aM** and **4bM** were synthesized by methylation and acetylation of **4a** and **4b**, which were isolated from cross-coupling of sinapyl alcohol **1** and sinapyl acetate **2**. Compound **5bM** was made by methylation of **5b** synthesized from sinapic acid following modifications (Fig. 7) to the routes based on Stevenson's method for synthesizing Grandisin.[31] Thus dilactone **35** was made in 77% yield from sinapic acid by peroxidase–hydrogen peroxide oxidation in aqueous acetone. Acetylation of dilactone **35** followed by bromination with bromine/ammonium acetate in acetic acid gave rise to brominated dilactone **36**. The total crude **36** was treated with dry HCl in methanol (acetyl chloride in methanol[34]) producing bromo-containing diaryltetrahydrofuran **37**. Compound **38** was obtained from hydrodebromination of **37** in methanol with Pd/C–triethylamine under a hydrogen balloon. Compound **39** was obtained by diisobutylaluminium hydride (DIBAL-H) reduction of **38** in toluene. Acetylation of **39** followed by selective phenolic acetate deacetylation with pyrrolidine resulted in compound **5b**.

Biochemical implications

Sinapyl acetate **2** (Fig. 1) is able to participate in enzyme-catalyzed radical coupling (dehydrogenation) reactions, a mechanism involved in lignin biosynthesis, producing β–β-linked dimer **5** as

a major product. Oxidative coupling of **2** by $CuCl_2$ in aqueous ethanol produced the β-O-4-dimer and even a small amount of the β-O-4-trimer (F. Lu, unpublished), in addition to mixtures of β–β-coupled products **3–5**. When sinapyl alcohol **1** and sinapyl acetate **2** are used in admixture in the H_2O_2/peroxidase system, cross-coupled dimer **4** is preferentially formed. These results demonstrate the potential of **2** to be a precursor of lignin. The detection of compounds **7–9** and **12–13** in DFRC′ products from both Kenaf lignin and models **4–5** suggests the existence of substructures **4** and **5** in Kenaf lignins. 2D NMR analysis of the Kenaf lignins coupled with analysis of synthesized model compounds **4aM**, **4bM** and **5bM** further established that substructures **4** and **5** in Kenaf lignins are present and can be formed from enzyme-catalyzed free radical coupling of sinapyl alcohol **1** and sinapyl acetate **2** during cell wall lignification. It is unlikely that such structures are formed post-lignification. Previous studies indicated that Kenaf bast fiber lignins are naturally highly acetylated overwhelmingly on syringyl units and that over 50% of β-O-4-linked syringyl units are acetylated at sidechain γ-positions. Nor is it likely that the compounds arise from enzymatically-assisted ring-opening reactions on syringaresinol **3**; enzymatic processes also produce chiral products whereas β–β-coupled products released from lignins have always been found to be optically inactive.[2,32,33] All of the above findings can be readily rationalized if the pre-acetylated monolignol, sinapyl acetate **2**, is incorporated into lignin macromolecules by radical coupling mechanisms.

Conclusions

Oxidation of sinapyl acetate **2** by H_2O_2/peroxidase produces compound **5**, whereas β–β-cross-coupled dimer **4** is the major product formed when sinapyl alcohol **1** and sinapyl acetate **2** are co-oxidized. DFRC′ degradation of syringaresinol **3** and compounds **4–5** produce tetrahydronaphthalene (aryltetralin) structures in which the γ-substituents diagnostically establish the existence of acetate groups originally acylating γ-positions of lignin sidechains. Detection of γ-acetates in syringyl β–β-coupled DFRC′ products **7–9** and **12–13** from Kenaf lignins implies the existence of substructures **4–5** that can be formed only from dehydrogenative coupling of sinapyl acetate itself or its cross-coupling with sinapyl alcohol. With ideal model compounds, 2D HSQC NMR analysis of Kenaf lignins shows that all the expected structures **4–5** suggested by DFRC′ are present in the Kenaf lignins. The stereochemical difference regarding the formation of substructure **5** between *in vitro* results and structures in lignin remains to be explored. The results from DFRC′–GC–MS and HSQC analysis of Kenaf lignins in this work provide the strongest evidence to date that acetates on Kenaf lignins are formed through incorporation of sinapyl acetate, as a lignin precursor, into lignin macromolecules by radical coupling. It is likely that the other acylated lignins, *p*-coumaroylated lignins in grasses and *p*-hydroxybenzoylated lignins in palms, poplars, willows, and other species, are similarly derived from the corresponding pre-acylated monolignols.

Confirmation that lignin acylation is *via* acylated monolignols implicates transferase enzymes (and, therefore, responsible genes) in the acylation. Since all lignins analyzed heavily favor sinapyl alcohol acylation, it is anticipated that sinapyl alcohol is the preferred substrate of the presumably involved acyl transferases, which now need to be isolated and characterized. Access to these genes, and up- and down-regulating them in various plants to alter lignin acylation, might finally give better insight into the value of lignin acylation to the plant. It will also open up new ways to alter lignification for altered cell wall properties that may be beneficial to existing agricultural and forestry crops or to their utilization in various natural and industrial processes.

Experimental section

General

All reagents were from Aldrich (Milwaukee, WI) and used as supplied. Solvents (AR grades) were from Mallinckrodt (Paris, KY). Evaporations were conducted under reduced pressure at temperatures <50 °C. Further removal of organic solvents, as well as drying of residues, was accomplished under higher vacuum (100–200 mTorr) at room temperature. Flash chromatography was performed on FLASH 40M cartridges (Biotage, Dyax Corp., Charlottesville, VA) using an UA-6 UV-vis detector (ISCO, Lincoln, NE). Preparative TLC plates (1 mm or 2 mm thickness, normal-phase) were from Alltech (Deerfield, IL). Acetylation and propionylation were performed with acetic or propionic anhydride and pyridine at room temperature overnight (~16 h). Selective removal of phenolic acetates was accomplished using neat pyrrolidine according to a published method[35] based on a prior method.[36] Hydrogenation/debenzylation was conducted in methanol using a hydrogen-filled balloon at room temperature using 10% Pd(en)/C (10% Pd/C treated with ethylenediamine in methanol over 30 h) according to a literature method.[37] Kenaf lignin isolation was described in a previous paper.[29]

^{1}H, ^{13}C and 2D NMR (gradient COSY, HSQC and HMBC) spectra were taken on a Bruker DRX-360 instrument fitted with a 5 mm ^{1}H/broadband gradient probe with inverse geometry (proton coils closest to the sample). The conditions used for all samples were 0.5–60 mg of material in 0.4 mL of acetone-d_6, with the central solvent peak as internal reference (δ_H 2.04, δ_C 29.80). Carbon designations are based on conventional lignin numbering.[28] NMR data for all synthesized compounds are given in Tables 1 (proton) and 2 (carbon).

Oxidative coupling of sinapyl alcohol and sinapyl acetate

Sinapyl alcohol **1** (500 mg) or sinapyl acetate **2** (500 mg) dissolved in acetone (20 mL) was dispersed into a stirred 20 mM phosphate buffer solution (80 mL, pH 4.5), followed by addition of horseradish peroxidase (11 mg; 250 U mg^{-1}). H_2O_2 (3%, 1.1 eq.) was added slowly over 15 min at room temperature, and then the mixture was stirred for 2.5 h. The mixture was saturated with NH_4Cl and the products extracted with dichloromethane (3 × 150 mL). The combined organic solvent fraction was dried over $MgSO_4$ and evaporated under reduced pressure. Products were separated and purified using flash chromatography and/or preparative TLC and analyzed by NMR and MS.

DFRC′ (modified DFRC) procedure

Kenaf lignin (200 mg) was stirred overnight at room temperature with propionyl bromide in propionic acid (1 : 3, 10 mL). Alternatively, compounds **3–5** (30–120 mg) dissolved in 2–6 mL

Table 1 ^{1}H NMR data

Cmpd	A/B	α	β	γ	2(/6)	OMe	Ac
4a	A	4.81 (5.9)	2.39	4.20, 4.45	6.64	3.79	1.93
	B	4.86 (4.46, 1.20)	2.92	4.07, 4.15	6.71	3.80	
4b	A	4.92 (3.16)	2.64	4.35, 4.64	6.60	3.80	2.05
	B	4.6 (10.13, 4.08)	2.78	3.58	6.70	3.80	
4aM	A	4.82 (5.80)	2.43	4.14, 4.26	6.66	3.67 (4-O), 3.80	1.96
	B	5.96 (7.25)	3.12	4.01, 4.17	6.71	3.70 (4-O), 3.81	2.07
4bM	A	4.94 (3.55)	2.60	4.22, 4.36	6.63	3.70, 3.80	2.0
	B	5.76 (10.92)	3.04	3.70	6.73	3.70, 3.81	2.04
5a	A	4.65(7.9)	2.39	4.27 (6.2)	6.86	3.86	1.84*
	B	5.15 (7.1)	2.74	3.80 (6.6)	6.79	3.84	1.98*
5b	A,B	4.96 (7.8)	2.51	3.84	6.76	3.83	1.94
5bM	A,B	5.00 (7.6)	2.52	4.26	6.77	3.84	1.94
6	A	4.21 (6.6)	2.27	4.00, 4.30	6.45	3.69	
	B	2.82	2.03	4.13, 4.21	6.71	3.25(5), 3.80(3)	2.06
7	A	4.19 (7.5)	2.24	4.05, 4.24	6.46	3.69	
	B	2.82	2.03	4.11, 4.20	6.71	3.22(5), 3.79(3)	2.06
8	A	4.18 (7.5)	2.24	4.06, 4.23	6.45	3.69	
	B	2.81	2.03	4.08, 4.19	6.71	3.22(5), 3.80(3)	1.97
9	A	4.20 (6.6)	2.24	4.05, 4.23	6.46	3.69	1.98
	B	2.86	2.03	4.10, 4.20	6.71	3.25(5), 3.80(3)	2.06
10	A	4.47 (1.45)	3.00	4.06, 4.18	6.37	3.66	
	B	3.57, 3.72	—	4.70, 5.00	6.72	3.42(5), 3.80(3)	
11	A	4.44	2.81	3.85, 4.20	6.45	3.66	
	B	6.39 (1.45)	—	1.89(1.45)	6.70	3.59(5), 3.80(3)	
12	A	4.47 (1.45)	2.96	4.06, 4.18	6.37	3.66	1.96
	B	3.57, 3.70	—	4.70, 5.00	6.72	3.42(5), 3.80(3)	
13	A	4.44	2.82	3.85, 4.20	6.45	3.65	1.99
	B	6.39 (1.45)	—	1.89 (1.45)	6.70	3.42(5), 3.82(3)	
15	A	5.00	4.02	—	6.36	3.68	
trans	B	7.66	—	—	6.96	3.62(3), 3.88(5)	
15	A	4.75 (8.8)	4.10 (8.8, 2.63)	—	6.36	3.68	
cis	B	7.42 (2.63)	—	—	6.90	3.47(3), 3.86(5)	
16	A	5.10	4.16	—	6.42	3.68	
	B	7.73	—	—	7.03	3.67(5), 3.89(3)	
17	A	4.67	2.70	3.32, 3.67	6.50	3.69	
	B	6.50	—	4.11	6.70	3.63(5), 3.84(3)	
18	A	4.26 (6.5)	1.96	3.56	6.42	3.70	
	B	2.62	1.64	3.60	6.55	3.35(5), 3.80(3)	
19	A	4.20 (6.7)	2.05	4.00, 4.25	6.46	3.70	1.98
	B	2.82	2.02	4.08, 4.18	6.71	3.26(5),3.80(3)	2.06
20	A	4.20 (6.6)	2.22	3.98, 4.13	6.39	3.72	1.98
	B	2.66	1.92	4.02, 4.21	6.59	3.35(5), 3.83(3)	2.06
21	A	4.92	3.28	3.12, 3.54	6.35	3.67	
	B	7.50	—	9.57	7.06	3.61(5), 3.91(3)	
22	A	4.61	3.44	3.96, 4.03	6.35	3.67	
	B	7.60	—	9.61	7.10	3.62(5), 3.92(3)	
23	A	4.61	3.45	3.92, 4.01	6.35	3.67	
	B	7.60	—	9.61	7.10	3.62(5), 3.92(3)	
24	A	4.48	2.93	3.85, 4.22	6.53	3.71	
	B	6.60 (1.0)		4.18, 4.22	6.71	3.66(5), 3.85(3)	
25	A	4.48	2.88	3.82, 4.18	6.49	3.69	2.02
	B	6.59 (1.0)		4.15,4.18	6.72	3.65(5), 3.86(3)	
26	A	4.21 (6.1)	2.21	3.97, 4.13	6.39	3.71	
	B	2.70	1.66	3.54, 3.62	6.58	3.35(3), 3.82(5)	
27	A	4.20 (6.2)	2.21	3.97, 4.13	6.40	3.71	2.03
	B	2.70	1.67	3.54, 3.64	6.57	3.33(5), 3.82(3)	
28	A	4.18 (7.5)	2.24	4.06, 4.23	6.45	3.69	2.18
	B	2.81	2.02	4.07, 4.19	6.71	3.24(5), 3.82(3)	1.97,2.22
29	A	4.18 (6.5)	2.21	3.99, 4.14	6.38	3.71	
	B	2.69	1.92	4.00, 4.12	6.59	3.32(5), 3.83(3)	1.96
35	A	5.75	4.11	—	6.73	3.83	
36	A	6.15	4.09	—	6.83	3.81, 3.82	2.30
37	A	5.25 (5.25)	3.62	—	7.14	3.82, 3.90	
38	A	5.32 (m)	3.59	—	6.77	3.82	
39	A	4.88 (8.6)	2.28	3.62, 3.72	6.74	3.81	

Table 2 ^{13}C NMR data

Cmpd	A/B	α	β	γ	1	2	3	4	5	6	OMe	Ac	Pr
4a	A	84.78	49.46	63.55	134.32	104.22	148.50	135.87	148.50	104.22	56.58	170.93	
	B	72.51	48.02	68.98	135.85	104.57	148.50	135.87	148.50	104.57	56.58		
4b	A	84.90	49.00	64.02	135.20	103.82	148.55	135.80	148.55	103.82		171.16	
	B	72.94	49.24	70.64	136.27	104.34	148.58	136.22	148.58	104.34			
4aM	A	84.52	49.12	63.04	139.33	104.04	154.32	138.47	154.32	104.04	56.41	170.86	
	B	74.48	46.54	70.25	136.28	104.17	154.41	139.00	154.41	104.17	56.47	170.09	
4bM	A	84.66	49.06	63.40	139.84	103.59	154.32	138.31	154.32	103.59	56.40	171.03	
	B	74.62	46.53	70.22	136.77	104.77	154.41	139.02	154.41	104.77	56.47	170.09	
5a	A	83.74	51.51	64.96	132.61	104.91	148.66	136.44	148.66	104.91	56.58	171.09	
	B	81.67	46.32	65.03	129.72	104.68	148.52	136.02	148.52	104.68	56.63	170.76	
5b	A,B	84.10	51.32	64.19	133.57	104.70	148.62	136.30	148.62	104.70	56.64	20.66, 170.94	
5bM	A,B	84.08	51.42	64.15	138.80	104.48	154.42	138.69	154.42	104.48	56.46	20.67, 170.92	
6	A	43.56	45.23	64.20	145.85	105.88	152.97	128.01	152.97	105.88	56.41		174.34
	B	33.76	36.72	66.80	136.39	107.72	152.06	132.75	152.29	124.92	53.61, 60.35		174.52
7	A	43.58	45.13	64.14	145.88	105.80	152.98	127.88	152.98	105.80	56.36		174.33
	B	33.76	36.57	66.71	136.31	107.62	152.22	132.67	151.98	124.91	56.25, 60.29	171.19	
8	A	43.59	45.19	64.01	145.85	105.80	152.91	127.89	152.91	105.80	56.36		
	B	33.74	36.53	66.90	136.32	107.66	152.25	132.69	152.00	124.91	56.26, 60.30	20.71, 171.00	174.51
9	A	43.54	45.14	64.13	145.83	107.76	152.99	128.19	152.99	107.76	56.36	171.01	
	B	33.73	36.64	66.96	136.31	107.76	152.09	132.79	152.37	124.96	56.26, 60.30	171.20	
10	A	43.22	50.35	65.89	144.62	105.90	152.69	128.13	152.69	105.90	56.34		174.17
	B	34.66	142.13	113.70	134.63	107.65	152.49	132.65	152.22	123.06	56.28, 60.99		
11	A	39.83	47.07	64.55	143.44	105.11	162.88	128.33	162.88	105.11	56.34		174.29
	B	125.19	136.35	22.91	133.05	106.44	152.59	133.27	152.14	120.30	56.46, 61.37		
12	A	43.13	50.20	65.96	144.55	105.74	152.59	127.94	152.59	105.74	56.26	170.78	
	B	34.56	142.03	113.71	134.58	107.57	152.40	132.54	152.14	122.97	56.20, 60.96		
13	A	39.66	46.80	64.55	143.30	104.96	162.77	128.40	162.77	104.96	56.26	170.95	
	B	125.14	136.38	22.92	133.00	106.31	152.50	133.28	152.08	120.20	56.37, 61.35		
15 (*trans*)	A	40.25	47.46	172.79	134.20	106.10	148.49	135.79	148.49	106.10	56.64		
	B	138.43	123.94	167.46	123.30	108.95	146.36	142.83	148.59	124.59	56.58, 60.43		
15 (*cis*)	A	41.09	48.70	172.21	132.11	107.71	148.07	136.03	148.07	107.71	56.71		
	B	125.62	136.88	168.28	125.11	108.59	148.56	142.70	145.83	122.81	56.61, 60.03		
16	A	40.29	47.02	172.50	139.29	105.78	154.29	136.68	154.29	105.78	56.39		
	B	137.82	128.31	167.23	125.32	109.78	154.11	144.08	152.46	123.83	56.43, 61.32		
17	A	38.93	47.40	63.61	141.91	106.06	153.95	136.25	153.95	106.06	56.33		
	B	122.84	140.74	65.48	130.56	107.23	153.51	141.47	153.11	122.31	56.31, 61.20		
18	A	42.52	49.22	63.70	139.20	107.04	148.21	134.70	148.21	107.04	56.63		
	B	33.75	41.11	66.45	129.69	106.97	147.59	138.44	146.98	126.35	56.24, 59.36		
19	A	43.48	45.20	64.09	145.86	105.87	152.93	132.68	152.93	105.87	56.36	170.94	
	B	33.67	36.60	66.93	136.42	107.71	152.03	127.97	152.23	124.88	56.24, 60.23	171.23	
20	A	42.73	45.44	65.10	137.90	106.87	148.40	135.09	148.40	106.87	56.67	170.98	
	B	33.18	37.04	67.44	128.40	107.00	147.96	138.68	146.89	125.30	56.28, 59.35	171.15	
21	A	38.18	43.21	62.49	141.41	105.94	154.22	136.55	154.22	105.94	56.39		
	B	146.48	138.23	192.92	128.43	109.85	153.96	144.89	153.41	125.81	56.50, 61.30		
22	A	39.08	39.59	65.01	140.39	105.85	154.31	136.80	154.31	105.85	56.41		
	B	147.09	137.14	192.64	128.20	110.04	154.14	144.95	153.07	125.46	56.57, 61.31		174.10
23	A	38.92	39.46	64.98	140.26	105.89	154.30	136.80	154.30	105.89	56.42	20.64, 170.80	
	B	147.06	137.18	192.63	128.18	110.03	154.14	144.93	153.12	125.39	56.55, 61.31		
24	A	39.54	42.90	64.86	141.19	106.07	154.04	136.47	154.04	106.07	56.45		174.33
	B	123.69	139.48	65.14	130.41	107.45	153.68	141.54	153.01	121.88	56.40, 61.21		
25	A	39.50	42.94	65.18	140.94	106.09	154.05	136.53	154.05	106.09	56.37	20.75, 170.93	
	B	123.74	139.28	65.19	130.25	107.41	153.75	141.64	153.08	121.80	56.34, 61.18		
26	A	42.61	45.06	65.34	138.53	106.90	148.38	135.01	148.38	106.90	56.69		174.54
	B	33.38	40.38	65.54	129.59	107.11	147.85	138.36	146.94	125.66	56.33, 59.46		
27	A	42.63	44.96	65.31	138.48	106.79	148.32	134.90	148.32	106.79	56.63	20.85, 171.24	
	B	33.39	40.23	65.41	129.51	107.01	147.79	138.37	146.86	125.64	56.27, 59.38		
28	A	43.52	45.09	64.10	145.86	105.77	152.87	127.87	152.87	105.77	56.34		174.51
	B	33.68	36.55	66.90	136.41	107.66	152.22	132.64	151.99	124.86	56.25, 60.26	20.21, 171.00	
29	A	42.73	45.53	65.04	137.90	106.90	148.42	135.13	148.42	106.90	56.69		174.46
	B	33.19	37.13	67.49	128.44	107.04	147.98	138.71	146.92	125.31	56.30, 59.39	20.70, 170.97	
35	A,B	83.39	49.06	176.05	129.87	104.31	149.04	137.51	149.04	104.31	56.77		
36	A,B	81.29	47.10	175.24	136.35	106.29	153.25	135.48	151.65	107.71	56.82, 61.51	20.20, 175.24	
37	A,B	83.22	56.75	171.67	130.61	107.16	145.52	141.48	149.20	109.88	56.77, 60.45		
38	A,B	84.13	57.52	172.22	131.94	104.46	148.67	136.61	148.67	104.46	56.62		
39	A,B	83.90	56.88	62.60	134.47	104.67	148.55	136.10	148.55	104.67	56.60		

of the same reagent were stirred overnight at room temperature. The excess bromide was quenched with a volume of *t*-butyl alcohol equal to that of the propionyl bromide used above, and stirred for 10 min. Then dioxane (the same volume as propionic acid above) and water (10% of the total volume in the flask) were added. To this well-stirred solution, Zn dust (500 mg for lignin, 200 mg for compounds **3–5**) was added and stirring was continued for 30 min. Products were recovered by dichloromethane extraction as usual.[38] Propionylation with propionic anhydride in pyridine completed the procedure. DFRC′ products from compounds **3–5** were analyzed by GC–MS and subjected to preparative TLC separation ($CHCl_3$–EtOAc, 20 : 1) to isolate the major products, compounds **6–13**. For the Kenaf lignin sample, monomers in lignin DFRC′-degraded products were separated by flash chromatography ($CHCl_3$–EtOAc, 10 : 1). The dimer fractions collected were further fractionated by TLC ($CHCl_3$–EtOAc, 20 : 1) into 5 fractions from which fractions 2–5 contained the major β–β-coupled dimers as indicated in GC–MS chromatograms (Fig. 4). DFRC′ products from model compounds **3–5** and Kenaf lignin were analyzed by GC (Hewlett Packard 5980) with a 0.20 mm X 25 m DB-1 (J & W Scientific, Folsom, CA) column, and EI-MS data were collected on a Hewlett Packard 5970 mass-selective detector. GC conditions (He as carrier gas, 10 mL min^{-1}): initial column temperature 150 °C, held for 1 min, ramped at 20 °C min^{-1} to 280 °C, then ramped at 2 °C min^{-1} to 310 °C, held for 15 min; injector 220 °C, detector 300 °C.

Syntheses of DFRC′-degraded products 6–13

Compound **15**, 1,2-dihydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-6,8-dimethoxynaphthalene-2,3-dicarboxylic acid dimethyl ester. The following one-pot method was considerably simpler than methods previously described.[39] To sinapic acid (5 g) in methanol (150 mL) was slowly added acetyl chloride (15 mL) over 15 min and stirred for 16 h. Water (30 mL) and $FeCl_3 \cdot 6H_2O$ (45 g) were added and the mixture was stirred continuously for another 48 h by which time the starting material (methyl sinapate) had been consumed, as indicated by TLC. After methanol was removed by evaporation under reduced pressure, the mixture was poured into EtOAc (350 mL), and then washed with 3% HCl (4 × 200 mL), and saturated NH_4Cl (100 mL). The EtOAc fraction was dried over $MgSO_4$ and evaporated. Crude products were applied to flash chromatography ($CHCl_3$–EtOAc, 5:1). Compound **15** was obtained in 52% yield, along with about 5% of the minor isomer that has a *cis*-configuration between carbons Aα and Aβ.

Compound **16**, 7-benzyloxy-1-(4-benzyloxy-3,5-dimethoxyphenyl)-1,2-dihydro-6,8-dimethoxynaphthalene-2,3-dicarboxylic acid dimethyl ester. Compound **15** (1.8 g, 3.8 mmol) was dissolved in acetone (150 mL) and benzyl chloride (1.01 g 8.0 mmol, 2.1 eq.) and powdered K_2CO_3 (1.1 g, 8.0 mmol, 2.1 eq.) added. The mixture was refluxed for 48 h and the solid was filtered off. The acetone was evaporated and the residue was dissolved in EtOAc (200 mL) and washed with aq. HCl (3%, 50 mL), and saturated aq. NH_4Cl (50 mL). After drying over $MgSO_4$, the EtOAc was evaporated. Flash chromatography of the crude product gave pure compound **16** as a pale yellow foamy solid (2.2 g, 90% yield).

Compound **17**, 7-benzyloxy-1,2-dihydro-1-(4-hydroxy-3,5-dimethoxyphenyl)-2,3-bis(hydroxymethyl)-6,8-dimethoxynaphthalene. To compound **16** (800 mg, 1.22 mmol) dissolved in anhydrous THF (50 mL) was added LAH (200 mg, 5.34 mmol, 4.3 eq.). The mixture was stirred for 1.5 h, then the excess reducing agent was quenched with aq. THF. After removal of THF by evaporation, EtOAc (200 mL) was added to the residue and the organic layer washed with aqueous HCl (3%, 2 × 50 mL), saturated NH_4Cl (50 mL), and dried over $MgSO_4$. Evaporation of the EtOAc gave essentially pure compound **17** as a colorless oil (660 mg, 90% yield).

Compound **18**, 1,2,3,4-tetrahydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-2,3-bis(hydroxymethyl)-6,8-dimethoxynaphthalene. Compound **17** (300 mg, 0.5 mmol) was dissolved in methanol (6 mL) in a 25 mL flask containing Pd(en)/C (30 mg), prepared from 10% Pd/C according to Sajiki's procedure.[37] The flask was flushed with H_2, a H_2-balloon attached, and the mixture was stirred for 10 h at room temperature (20 °C). The reaction mixture was filtered using a 0.2 μm nylon membrane filter (Schleicher & Schuell, Keene, NH). The filtrate was evaporated to give crude product in which **18** was the dominant isomer. After preparative TLC ($CHCl_3$–EtOAc, 1 : 3), pure **18** was obtained (170 mg, 80% yield).

Compound **6**, 1,2,3,4-tetrahydro-6,8-dimethoxy-1-(3,5-dimethoxy-4-propionyloxyphenyl)-7-propionyloxy-2,3-bis-(propionyloxymethyl)naphthalene. Compound **18** was propionylated by propionic anhydride in pyridine to quantitatively give compound **6** as an oil. MS (*m*/*z*) 644 (M^+, 13), 588 (100), 532 (41), 514 (21), 458 (38), 427 (46), 384 (51), 217 (55), 167 (61).

Compound **9**, 2,3-bis(acetoxymethyl)-1,2,3,4-tetrahydro-6,8-dimethoxy-1-(3,5-dimethoxy-4-propionyloxyphenyl)-7-propionyloxy-naphthalene. Acetylation of compound **18** with acetic anhydride/pyridine produced **19** in quantitative yield. Selective deacetylation of compound **19** with pyrrolidine gave compound **20** in 90% yield. Propionylation of **20** produced **9** as an oil in quantitative yield. MS (*m*/*z*) 616 (M^+, 3), 560 (32), 504 (16), 413 (15), 348 (13), 217 (50), 167 (100).

Compound **21**, 7-benzyloxy-1-(4-benzyloxy-3,5-dimethoxyphenyl)-1,2-dihydro-2-hydroxymethyl-6,8-dimethoxynaphthalene-2-carbaldehyde. To compound **17** (200 mg, 0.33 mmol) dissolved in dry DMF (5 mL) in a 25 mL flask was added pyridinium dichromate (188 mg, 0.5 mmol, 1.5 eq.). The mixture was stirred at room temperature for 5 h at which point TLC showed the starting material to have been consumed. The mixture was poured into EtOAc (30 mL) and the precipitated oxidant was filtered off through a Celite pad. The EtOAc fraction was washed with water (3 × 10 mL), saturated NH_4Cl (10 mL), and dried over $MgSO_4$. Evaporation of under reduced pressure gave a crude product from which **21** (160 mg, 80% yield) was purified by TLC ($CHCl_3$–EtOAc, 5 : 1).

Compound **22**, 7-benzyloxy-1-(4-benzyloxy-3,5-dimethoxyphenyl)-1,2-dihydro-6,8-dimethoxy-2-(propionyloxymethyl)-naphthalene-2-carbaldehyde, was from propionylation of compound **21**.

Compound **23**, 2-acetoxymethyl-7-benzyloxy-1-(4-benzyloxy-3,5-dimethoxyphenyl)-1,2-dihydro-6,8-dimethoxynaphthalene-2-carbaldehyde, was from acetylation of compound **21**.

Compound **24**, 7-benzyloxy-1-(4-benzyloxy-3,5-dimethoxyphenyl)-1,2-dihydro-3-hydroxymethyl-6,8-dimethoxy-2-(propionyloxymethyl)naphthalene, was from reduction of compound **22** using sodium borohydride in ethyl acetate.

Compound **25**, 2-acetoxymethyl-7-benzyloxy-1-(4-benzyloxy-3,5-dimethoxyphenyl)-1,2-dihydro-3-hydroxymethyl-6,8-dimethoxynaphthalene, was from reduction of compound **23** using sodium borohydride in ethyl acetate.

Compounds **26**, 1,2,3,4-tetrahydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-6,8-dimethoxy-2-(propionyloxymethyl)naphthalene, was obtained from hydrogenation/debenzylation of compound **24** in 81% yield after TLC purification ($CHCl_3$–EtOAc, 2 : 1 as development solvent).

Compound **27**, 2-acetoxymethyl-1,2,3,4-tetrahydro-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-3-hydroxymethyl-6,8-dimethoxynaphthalene, was obtained from hydrogenation/debenzylation of compound **25** in 85% yield after TLC purification ($CHCl_3$:EtOAc, 2:1 as development solvent).

Compound **7**, 2-acetoxymethyl-1,2,3,4-tetrahydro-6,8-dimethoxy-1-(3,5-dimethoxy-4-propionyloxyphenyl)-7-propionyloxy-3-propionyloxymethyl-naphthalene. Propionylation of compound **27** gave compound **7**. MS (*m*/*z*) 630 (M^+, 11), 574 (100), 518 (41), 483 (11), 458 (11), 427 (25), 384 (37), 353 (19), 217 (47), 167 (17).

Compound **8**, 3-acetoxymethyl-1,2,3,4-tetrahydro-6,8-dimethoxy-1-(3,5-dimethoxy-4-propionyloxyphenyl)-7-propionyloxy-2-(propionyloxymethyl)naphthalene. Acetylation of compound **26** produced compound **28**. Selective deacetylation of compound **28** gave compound **29**, which was propionylated to produce compound **8**. MS (*m*/*z*) 630 (M^+, 11), 574 (100), 518 (41), 483 (11), 458 (11), 427 (25), 384 (37), 353 (19), 217 (47), 167 (17).

Compounds **10–13** (**10**, 1,2,3,4-tetrahydro-6,8-dimethoxy-1-(3,5-dimethoxy-4-propionyloxyphenyl)-3-methylene-7-propionyloxy-2-(propionyloxymethyl)naphthalene; **11**, 1,2,-dihydro-6,8-dimethoxy-1-(3,5-dimethoxy-4-propionyloxyphenyl)-3-methyl-7-propionyloxy-2-(propionyloxymethyl)naphthalene; **12**, 2-acetoxymethyl-1,2,3,4-tetrahydro-6,8-dimethoxy-1-(3,5-dimethoxy-4-propionyloxyphenyl)-3-methylene-7-propionyloxynaphthalene; **13**, 2-acetoxymethyl-1,2,-dihydro-6,8-dimethoxy-1-(3,5-dimethoxy-4-propionyloxyphenyl)-3-methyl-7-propionyloxynaphthalene). Compounds **10–11** were identified by NMR in a mixture (**10**:**11** = 2 : 1) isolated by TLC ($CHCl_3$–EtOAc, 20 : 1) from DFRC′ of syringaresinol **3** whereas compounds **12–13** were isolated by TLC ($CHCl_3$–EtOAc, 20 : 1) and identified by NMR in a mixture (**12**:**13** = 3 : 1) from DFRC′ products of diaryltetrahydrofuran **5**. Compound **10**: MS (*m*/*z*) 570 (M^+, 4), 514 (6), 440 (19), 384 (100), 286 (12), 230 (30), 167 (19). Compound **11**: MS (*m*/*z*) 570 (M^+, 3), 514 (3), 496 (5), 440 (42), 384 (100), 167 (21). Compound **12**: MS (*m*/*z*) 556 (M^+, 3), 500 (19), 440 (16), 384 (100), 286 (17), 230 (42), 167 (16). Compound **13**: MS (*m*/*z*) 556 (M^+, 3), 500 (6), 496 (6), 440 (43), 384 (100), 167 (12).

Syntheses of phenol-methylated model compounds 4aM, 4bM

Compound **4**, 3-acetoxymethyl-2-(4-hydroxy-3,5-dimethoxyphenyl)-4-[hydroxy-(4-hydroxy-3,5-dimethoxyphenyl)-methyl]-tetrahydrofuran, was made from peroxidase-catalyzed hydrogen peroxide oxidative free radical coupling reactions of sinapyl alcohol **1** with sinapyl acetate **2** in 40% yield after chromatographic separation ($CHCl_3$–EtOAc, 9 : 1 to 1 : 1) of the crude products. The isomeric ratio of **4a** to **4b** was 5 : 1.

Model compounds **4aM** and **4bM**, 3-acetoxymethyl-4-[hydroxy-(3,4,5-trimethoxyphenyl)-methyl]-2-(3,4,5-trimethoxyphenyl)tetrahydrofuran, were made by methylation and acetylation of **4a** and **4b** respectively.

Syntheses of compounds 5a and 5b

Compound **5**, 3,4-bis(acetoxymethyl)-2,5-bis(4-hydroxy-3,5-dimethoxyphenyl)tetrahydrofuran, was made from peroxidase-catalyzed hydrogen peroxide oxidative free radical coupling reactions of sinapyl acetate **2**. Compound **5a**, the major isomer, was isolated in 35% yield after chromatographic separation ($CHCl_3$–EtOAc, 9 : 1 to 1 : 1) of the crude products.

Compound **5b**, the minor isomer formed in this reaction, was detected in a mixture with **5a** and was isolated in small amount. To confirm its structure an independent synthetic approach from sinapic acid to **5b** was used (see text and Fig. 7), as follows.

Compound **35**, 3,6-bis(4-hydroxy-3,5-dimethoxyphenyl)tetrahydrofuro[3,4-*c*]furan-1,4-dione. Sinapic acid (5.0 g, 22.32 mmol) was dissolved in 15% (v/v) aqueous acetone. Horseradish peroxidase (15 mg, 181 units mg^{-1}) dissolved in 10 mL water was added. Then hydrogen peroxide urea complex (13.40 mmol, 1.2 eq.) in 10 mL water was introduced to this mixture, which was stirred at room temperature for 2.5 h. After standing overnight, the light yellow precipitate was filtered off using nylon membrane (20 μm pore size), and dried to give dilactone **35** (3.8 g, 8.6 mmol, 77% yield) as a yellow powder.

Compound **36**, 3,6-bis(4-acetoxy-2-bromo-3,5-dimethoxyphenyl)tetrahydrofuro[3,4-*c*]furan-1,4-dione. To a solution of acetylated **35** (4.0 g, 7.5 mmol) and NH_4OAc in acetic acid (100 mL) at room temperature was added a solution of Br_2 (2.6 g, 16.2 mmol) in the same solvent (5 mL). The mixture was stirred and kept at 50 °C for 6 h after which the solvent was removed under reduced pressure. The residue then was diluted with ethyl acetate (200 mL) and washed with water and saturated aq. NH_4Cl. The ethyl acetate solution was dried over anhydrous $MgSO_4$ and evaporated to produce crude **36**, which was purified *via* flash chromatography. Pure **36** (4.0 g, 5.85 mmol, 78% yield) was obtained as a pale yellow oil.

Compound **37**, 2,5-bis(2-bromo-4-hydroxy-3,5-dimethoxyphenyl)tetrahydrofuran-3,4-dicarboxylic acid dimethyl ester. Acetyl chloride (10 mL) was added slowly over 30 min to a solution of **36** (3.6 g, 5.23 mmol) in methanol–dioxane (3 : 1, v/v, 200 mL). This solution was allowed to stand at room temperature for 16 h before all solvents were evaporated under reduced pressure. The crude product were subjected to flash chromatography (hexane–EtOAc, 2 : 1) resulting in pure **37** (2.1 g, 3.23 mmol, 62% yield) as a pale oil.

Compound **38**, 2,5-bis(4-hydroxy-3,5-dimethoxyphenyl)tetrahydrofuran-3,4-dicarboxylic acid dimethyl ester. To a mixture of compound **37** (0.24 g, 0.45 mmol) and 10% Pd/C (50 mg) in methanol (50 mL) was added triethylamine (0.23 g, 2.26 mmol, 5.0 eq). This mixture was stirred for 2 days under a hydrogen-filled balloon (refilled every 6 h) before filtering through a Celite pad in a sintered glass filter, and washing with methanol (10 ml). The filtrate was concentrated to about 5 mL and diluted with ethyl acetate (100 mL). The ethyl acetate solution was washed with aqueous 3% HCl (100 mL), saturated NH_4Cl (30 mL), dried over anhydrous $MgSO_4$, and evaporated under reduced pressure, producing **38** (0.120 g, 0.243 mmol) in 75.5% yield after TLC purification (cyclohexane–EtOAc, 3 : 1).

Compound **39**, 2,5-bis(4-hydroxy-3,5-dimethoxyphenyl)-3,4-(dihydroxymethyl)tetrahydrofuran. To an ice-cold solution of **38** (0.12 g, 0.243 mmol) in toluene (5 mL) was added DIBAL-H (2.5 mL, 1.5 M in toluene, 11.0 eq.) over 3 min while stirring. The solution was kept stirring over an ice–water bath for another 1 h before ethanol was slowly added to quench the excess reagent. The resultant mixture was transferred to a separatory funnel with ethyl acetate (100 mL) and washed with 3% aqueous HCl (100 mL) and saturated NH_4Cl solution (50 mL). The ethyl acetate solution was dried over anhydrous $MgSO_4$. Evaporation under reduced pressure gave crude **39** (0.096 g, 0.22 mmol, 90%), which was used directly for the preparation of **5b**.

Compound **5b**, 3,4-bis(acetoxymethyl)-2,5-bis(4-hydroxy-3,5-dimethoxyphenyl)tetrahydrofuran. Acetylation of **39** with acetic anhydride–pyridine (1 : 1) followed by selective deacetylation in pyrrolidine gave the target compound **5b** in 85% overall yield.

Compound **5bM**, 3,4-bis(acetoxymethyl)-2,5-bis(3,4,5-trimethoxyphenyl)tetrahydrofuran. Methylation of **5b** with methyl iodide and potassium carbonate in acetone at room temperature overnight produced the final diaryltetrahydrofuran model compound **5bM** in 95% yield.

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