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Syntheses of Lignin-Derived Thioacidolysis Monomers and Their Uses as Quantitation Standards

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ABSTRACT: Analytical thioacidolysis releases diagnostic monomers from lignins by selectively cleaving alkyl aryl ether bonds. High yielding syntheses of the three thioacidolysis monomers were developed, and the compounds were used as standards for gas chromatography−mass spectrometry (GC−MS) and gas chromatography−flame ionization detector (GC−FID) quantitation of monomers released from lignocellulosics. First, syringyl, guaiacyl, and *p*-hydroxyphenyl glycerols were synthesized from the corresponding ethyl cinnamates and used as thioacidolysis substrates to prepare the monomers in high yields. These monomers were then used as standard compounds to measure their GC−MS and GC−FID response factors against two internal standards, 4,4′-ethylenebisphenol and tetracosane. For quantitation, it was shown that 4,4′-ethylenebisphenol is the better internal standard for GC−MS, whereas tetracosane remains the choice for GC−FID. When the obtained response factors were applied, the thioacidolysis monomer yields from white spruce, loblolly pine, poplar, bamboo, and sugar cane bagasse were determined by GC−MS. The obtained results were consistent with those reported in the literature.

KEYWORDS: *Lignin, thioacidolysis, arylglycerol, GC−MS, synthesis, response factor*

INTRODUCTION

Lignin is derived from hydroxycinnamyl alcohols, mainly *p*-coumaryl, coniferyl, and sinapyl alcohols, by enzyme-catalyzed free-radical generation, followed by combinatorial chemical copolymerization. It plays essential roles in the development of plant cell walls and affects their use as renewable biomass materials.[1] As a highly abundant natural polymer, lignin has drawn much research and industrial attention for decades.[2] However, because of its complexity and heterogeneity, it has been a most challenging problem for scientists to understand the composition and structure of lignin for various purposes ranging from plant breeding and genetic alteration to the ultimate use of lignocellulosics.[3]

Historically, chemical degradation methods for lignin analysis have played very important roles for researchers to obtain compositional and structural information about the complex polymer. Those methods included nitrobenzene oxidation, permanganate oxidation, acidolysis, thioacetolysis, hydrogenolysis, and the current widely used thioacidolysis and derivatization followed by reductive cleavage (DFRC) methods.[4−6] As an analytical method for characterization of lignin, thioacidolysis has been successfully applied to a wide array of lignocellulosic materials and isolated lignins in various studies related to the biosynthesis of monolignols, biological processing of plant biomass, and the pulping industry.[7] Following thioacidolysis reactions, which cleave alkyl aryl ether linkages and notably *β*-*O*-4-ether-linked lignin units, diagnostic *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) monomers **6a**−**6c** (Figure 1) are released from lignin polymers.[8,9] The measurement of these thioacidolysis monomers gives syringyl/guaiacyl (S/G, **6b**/**6c**) ratios for lignins, important compositional information correlated to various properties of lignocellulosic biomass; for example, hardwoods with higher S/G require less energy for pulping because of their higher *β*-*O*-4-ether contents that are dictated by the syringyl level.[10] In principle, the thioacidolysis monomer yield (normally expressed as *μ*mol/g of lignin) reflects the proportion of "noncondensed" *β*-*O*-4-ether structural units, an important feature of lignins.

For quantitative analysis by gas chromatography−flame ionization detector (GC−FID) or gas chromatography−mass spectrometry (GC−MS), it is essential to have response factors (RFs) of the target compounds versus a standard (internal or external). To measure such RFs, authentic and pure target compounds are required. In the case of thioacidolysis, monomers **6b** and **6c** derived from guaiacyl and syringyl units have been isolated from scale-up thioacidolysis reactions with isolated milled wood lignins (MWLs), which involved a series of time-consuming purification steps, including high-performance liquid chromatography (HPLC) separation.[8,11] Monomer **6a** does not appear to have been assessed. By applying the purified monomers **6b** and **6c**, RFs versus tetracosane for GC−FID were measured and then used for quantitating compounds **6b** and **6c**. It turned out that the RFs for compounds **6b** and **6c** were the same (or very close). GC−

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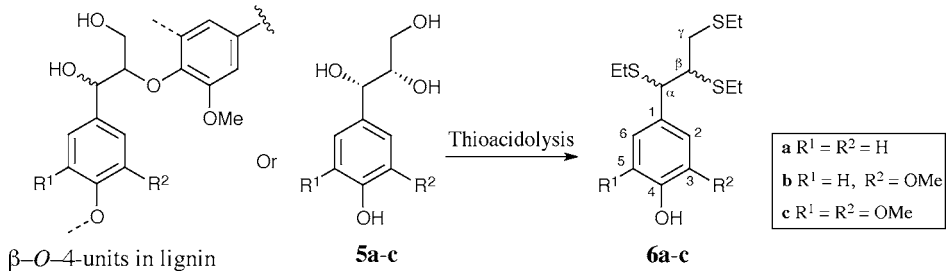

Figure 1. Major lignin-derived monomers **6a−6c** resulting from cleaving *β-O*-4-ether linkages, the most abundant linkage type in lignin, or from arylglycerols **5a−5c**, by thioacidolysis.

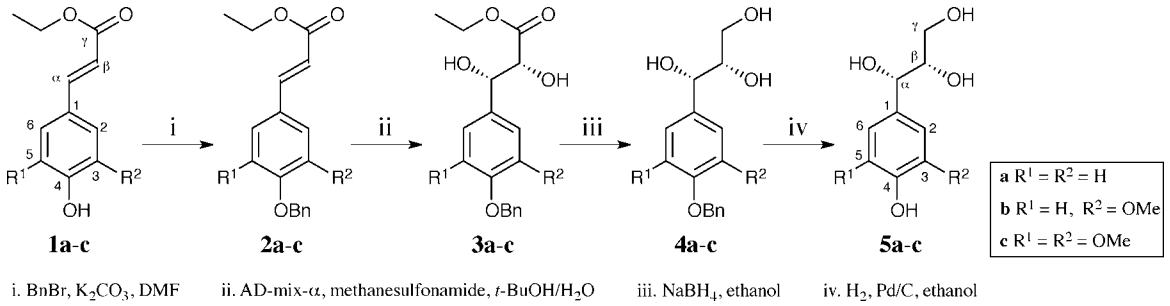

Figure 2. Synthetic route to the preparation of arylglycerols **5a−5c**.

MS is now popular and widely used by various analytical methods because of the ready availability of relatively cheap instrumentation. In addition to its powerful ability to detect/identify compounds of interest, GC−MS is also useful for quantitative analysis when suitable internal standards are used in a similar way to those used in GC−FID. GC−MS may be even better when selected-ion monitoring (SIM) is used to obtain selected-ion chromatograms (SICs) for quantitation because significantly higher sensitivity and better resolution (from potentially interfering compounds) can be obtained in SIM detection. However, although we are aware of many laboratories using GC−MS for thioacidolysis analysis, no GC−MS method has been reported for such applications probably because of the difficulty of obtaining the required pure thioacidolysis monomers **6a−6c**. Here, we report on the syntheses of the three lignin-derived thioacidolysis monomers **6a−6c** produced via standard thioacidolysis of the corresponding arylglycerols that can be synthesized in a convenient and efficient way. To obtain RFs required for quantitative thioacidolysis of lignins, we also report on the RFs of the synthesized thioacidolysis monomers versus 4,4′-ethylenebisphenol, a new standard that we are recommending for GC−MS work, or tetracosane determined by GC−MS and GC−FID. Moreover, to test and verify the feasibility of the obtained RFs, lignocellulosic samples representative of softwoods, hardwoods, and grasses were subjected to thioacidolysis and their lignin-derived monomers were quantitated by GC−MS using the newly established RFs and compared to the values obtained herein and, where possible, in prior literature using GC−FID.

MATERIALS AND METHODS

Materials. All chemicals and solvents used in this study were purchased from Aldrich (Milwaukee, WI) and used as supplied. Lignocellulosic samples were coarse-ground using a Wiley (Thomas Scientific, Swedesboro, NJ) mill with a 0.5 mm screen and extracted with 80% ethanol (3 times, 6 h at 50 °C for each extraction) to remove extractives. The Klason lignin content was measured by the standard method.[12]

Flash chromatography was performed with Biotage snap silica cartridges on an Isolera One (Biotage, Charlottesville, VA). All synthesized compounds were characterized by nuclear magnetic resonance (NMR) and/or GC−MS methods. NMR spectra were acquired on a Bruker Biospin (Billerica, MA) AVANCE 500 (500 MHz) spectrometer fitted with a cryogenically cooled 5 mm TCI gradient probe with inverse geometry (proton coils closest to the sample) and spectral processing used Bruker's Topspin 3.1 (Mac) software. Standard Bruker implementations of one- and two-dimensional [gradient-selected correlation spectroscopy (COSY), heteronuclear single-quantum coherence (HSQC), and heteronuclear multiple-bond correlation (HMBC)] NMR experiments were used for routine structural assignments of newly synthesized compounds. The conditions used for all samples were 0.5−60 mg in 0.5 mL of NMR solvent (acetone-d_6, methanol-d_4, or chloroform-d) with the central solvent peaks as internal references (δ_H/δ_C 2.04/29.80, 3.31/49.15, or 7.24/77.23, respectively). To best compare NMR data of the synthesized compounds to those in the literature, all three NMR solvents were used.

Syntheses of Arylglycerols. Ethyl cinnamates **1a−1c** (Figure 2) were made from the corresponding hydroxycinnamic acids and ethanol catalyzed by dry HCl (prepared by adding 5 mL of acetyl chloride to 100 mL of ethanol). Crystallization of the crude products from ethyl acetate/hexane gave white or light yellow crystals in about 75−85% yields. Melting points: **1a**, 70−71 °C (literature 65−68 °C); **1b**, 61−62 °C (literature 63−65 °C); and **1c**, 76−78 °C.

Benzylation. Benzylation of compounds **1a−1c** with benzyl bromide to protect the phenolic hydroxyl groups was accomplished in a traditional way as exemplified here by the formation of compound **2b**. Ethyl ferulate **1b** (9.15 g, 41.2 mmol) was dissolved in 40 mL of *N*,*N*-dimethylformamide (DMF), to which anhydrous K_2CO_3 powder (8.28 g, 60.0 mmol) and 6.5 mL of benzyl bromide (9.36 g, 54.7 mmol) were added. The resultant mixture was stirred overnight, after which time thin-layer chromatography (TLC) (3:1 EtOAc/cyclohexane, v/v) showed no compound **1b** remaining. The DMF was removed by evaporation at 55 °C under reduced pressure. EtOAc and water (200 mL each) were added to the mixture followed by the addition of 1 M HCl to quench the residual base. After all liquids were transferred to a separatory funnel, the two phases were separated. The

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water phase was re-extracted with EtOAc (100 mL). The combined EtOAc phase was washed with saturated NH_4Cl, dried over anhydrous $MgSO_4$, and evaporated to dryness. The crude product was crystallized from ethanol (200 mL) to give white crystals (11.12 g, 86.5% yield, melting point, 71−72 °C). Compounds **2a** and **2c** were made as white crystals (melting points, **2a**, 68−69 °C; **2c**, 76−77 °C) similarly in 71.0 and 65.4% yields. The NMR data of compounds **2a**−**2c** are consistent with those previously published.[13−15]

Asymmetric Dihydroxylation. Dihydroxylation of compounds **2a**−**2c** (Figure 2) was via sharpless catalytic asymmetric dihydroxylation (AD) reactions using AD-mix-α as the oxidant as described. To a well-stirred *tert*-BuOH/H_2O (55 mL, 1:1, v/v) solution at 0 °C (ice-water bath) was added AD-mix-α (1.4 g/mmol of substrate) followed by the addition of methanesulfonamide (1.0 equiv). The resultant mixture was continuously stirred for 5 min before compound **2** (8 mmol) was added. The resulting solution was well-stirred at 0 °C for 2 h, and then the mixture was warmed to room temperature and stirred for 24 h. The reaction was checked by TLC (1:1 cyclohexane/EtOAc, v/v) to ensure that no starting material remained. Then, the reaction was quenched by adding excess saturated $Na_2S_2O_3$ solution (60 mL), and the resultant solution was stirred for an additional 30 min. The product was extracted twice with EtOAc (200 mL × 2), and the combined organic phase was washed with distilled water and saturated NaCl solution. Then, the EtOAc solution was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (Biotage, 100 g silica column) using hexane/EtOAc (1:1, v/v) to obtain compounds **3** as oils that became solids or crystals after standing overnight. The yields of dihydroxylation products were 97.6% (**3a**), 91.4% (**3b**), and 83.0% (**3c**). The NMR data of compounds **3a**−**3b** are consistent with the previously published data.[16,17] Compound **3c**, NMR (acetone-d_6) δ_H: 1.20 (3H, t, J = 7.20 Hz, CH_3), 3.82 (6H, s, 2/6-OMe), 4.15 (2H, q, J = 7.20 Hz), 4.26 (1H, br-t, β), 4.92 (1H, br, α), 4.93 (2H, s, Bn-CH_2), 6.78 (2H, s, 2/6), 7.28−7.51 (5H, Bn). NMR (acetone-d_6) δ_C: 14.45 (CH_3), 56.34 (OMe), 61.39 (CH_2), 75.02 (Bn, α-CH_2), 75.45 (α), 76.50 (β), 104.74 (C2/6), 128.29 (Bn-C4), 128.71 (Bn-C3/5), 128.82 (Bn-C2/6), 137.11 (C1), 138.32 (C4), 139.53 (Bn-C1), 154.09 (C3/5), 173.07 (γ). HRMS: M + Na^+ m/z calculated 399.1415, found 399.1415.

Sodium Borohydride Reductions. Compounds **4a**−**4c** (Figure 2) were synthesized from **3a**−**3c** by sodium borohydride reduction in ethanol. The detailed procedure is illustrated for the preparation of compound **4a**. To compound **3a** (640 mg, 2.03 mmol) dissolved in 95% ethanol (20 mL) was added $NaBH_4$ (309 mg, 4.0 equiv), and the mixture was stirred for overnight, although the reaction can be completed in about 4 h as indicated by TLC (20:1 CH_2Cl_2/MeOH, v/v). Then, the excess reducing agent was quenched by slowly adding 1 M aqueous HCl solution until the mixture turned clear and H_2 evolution ceased. The mixture was evaporated at 40 °C under reduced pressure to remove ethanol, and the residue was dissolved in EtOAc (50 mL). The resultant product in EtOAc was hydrolyzed by shaking with 1 M aqueous HCl (40 mL). The aqueous phase was extracted with further EtOAc (20 mL). The two EtOAc fractions were combined and mixed with 1 M aqueous HCl (40 mL) in a separatory funnel. The two phases in the funnel were well-mixed by shaking vigorously for 10 min. Such operation was repeated to make sure (by TLC) all borate intermediates were converted to product **4a**. The EtOAc solution was washed with 0.4 M $NaHCO_3$ (10 mL) and with saturated NH_4Cl solution. After drying over anhydrous $MgSO_4$ and filtering, the EtOAc solution was evaporated at 40 °C under reduced pressure to result in product **4a** (525 mg, 94.5% yield). Compounds **4b** and **4c** were obtained similarly from the corresponding compounds **3b** and **3c** in 92.0% (**4b**) and 81.4% (**4c**) yields. The NMR data of compounds **4a**−**4c** are consistent with those previously published in the literature.[15,18,19]

The last step (debenzylation) to accomplish the synthesis of arylglycerols was via standard catalytic hydrogenation with palladium on activated carbon (Pd/C, 10% Pd) in ethanol. Thus, for example, 3.3 g (10.85 mmol) of compound **4b** was dissolved in ethanol (30 mL) with stirring, followed by the addition of 100 mg of Pd/C. The resultant mixture was stirred under a hydrogen balloon for 3 h, after which TLC showed that no starting material **4b** remained. The solid catalyst was filtered off with a polyamide membrane (0.2 μm), and the product was recovered by evaporation of the filtrate resulting in compound **5b** as a oil that became solid (2.22 g, 10.37 mmol, 95.6% yield) when dried under high vacuum. Compounds **5a** and **5c** were obtained similarly in 94.7 and 95.5% yields from compounds **4a** and **4c**. The NMR data for compounds **5a**−**5c** are consistent with those previously published in the literature.[20−22]

Syntheses of Thioacidolysis Monomers 6a−6c. For the preparative-scale synthesis of compounds **6a**−**6c**, a modified thioacidolysis procedure was used as follows: 4.0 mL of EtSH and 1.0 mL of BF_3 etherate were added to a 25 mL screw-cap glass bottle containing 20 mL of freshly distilled dioxane. Using thioacidolysis of compound **5a** as the example, compound **5a** (0.95 g, 5.16 mmol) was added. The cap was screwed on tightly, and the bottle was kept in a heated sand bath at 100 °C for 4 h with stirring. Then, the bottle was cooled to room temperature. The product mixture was transferred into a separatory funnel, and 50 mL of 0.4 M $NaHCO_3$ was added to quench the excess reagents. HCl (1 M) solution was added to the reaction mixture to adjust the pH of the mixture to less than 3. After the normal workup with CH_2Cl_2 extraction, the crude products were loaded onto a Biotage flash chromatography Snap cartridge (100 g of silica gel) and eluted with hexane/ethyl acetate (6:1, v/v) to produce pure **6a** as a clear oil (1.28 g, 78.7% yield). Compounds **6b** and **6c** were made in the same way as described above from the corresponding compounds **5b** and **5c** in 55.8 and 44.8% yields. Although GC−MS analysis of the crude mixtures showed that thioacidolysis reactions performed in such a way were high yielding (around 90%), only modest isolated yields were attained because only fractions with high purity were collected for use as standards. Because of the nature of the reactions involved in the thioacidolysis, the products **6a**−**6c** obtained were mixtures of two diastereomers, *erythro* (*anti*) and *threo* (*syn*), even after the flash chromatographic purification. Compound **6a** (two isomers), NMR (acetone-d_6) δ_H: 1.06 (3H, t, J = 7.40 Hz, CH_3), 1.10 (3H, t, J = 7.40 Hz, CH_3), 1.11 (3H, t, J = 7.40 Hz, CH_3), 1.14 (3H, t, J = 7.40 Hz, CH_3), 1.18 (3H, t, J = 7.40 Hz, CH_3), 1.21 (3H, t, J = 7.40 Hz, CH_3), 2.27 (2H, q, J = 7.40 Hz, CH_2), 2.30 (2H, m, CH_2), 2.44 (2H, m, CH_2), 2.46 (2H, m, CH_2), 2.54 (2H, q, J = 7.40 Hz, CH_2), 2.58 (2H, q, J = 7.40 Hz, CH_2), 2.68 (1H, m, γ_1), 2.86 (1H, m, γ_2), 2.73 (1H, m, γ_1), 3.00 (1H, m, γ_2), 3.00 (1H, m, β), 3.16 (1H, m, β), 4.36 (1H, d, J = 6.00 Hz, α), 4.40 (1H, d, J = 4.35 Hz, α), 6.77 (2H, m, 3/5), 6.80 (2H, m, 3/5), 7.29 (2H, m, 2/6), 7.34 (2H, m, 2/6). NMR (acetone-d_6) δ_C: 14.79 (CH_3), 14.88 (CH_3 × 2), 15.04 (CH_3), 15.22 (CH_3), 15.29 (CH_3), 25.92 (CH_2), 26.04 (CH_2), 26.68 (CH_2), 26.77 (CH_2), 27.00 (CH_2 × 2), 37.02 (γ), 37.19 (γ), 52.38 (α), 52.45 (β), 53.00 (α), 53.65 (β), 115.31 (C3/5), 115.49 (C3/5), 130.60 (C2/6), 130.98 (C1), 131.03 (C2/6), 132.56 (C1), 157.18 (C4), 157.31 (C4). HRMS (TOF−MS) M + Na^+ m/z calculated 339.0882, found 339.0875. The NMR data of compounds **6b** and **6c** are consistent with the previously published data in the literature.[9,11]

Measurement of GC−MS RFs. Each pure compound **6** in a preweighed vial was left under high vacuum (about 100 mT) overnight and weighed (on an analytical balance, accurate to 0.1 mg). Mixing given amounts (about 15 mg, accurately weighed) of each compound **6** and internal standards (tetracosane and 4,4′-ethylenebisphenol) in CH_2Cl_2 produced a stock solution (30 mL). Duplicate or triplicate replicates of such solutions were made for each compound **6**, and then 10 μL aliquots of each stock solution (about 0.2 mg mass) were taken for silylation with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (100 μL) and pyridine (50 μL) at 50 °C for 40 min. The silylated sample was injected into the GC−MS, and RFs were calculated on the basis of the following equation:

$$RF = (W_S/A_S)/(W_{IS}/A_{IS})$$

where W_S is the mass (weight) of the sample (compound **6**), W_{IS} is the mass of the internal standard (tetracosane or 4,4′-ethylenebisphenol), A_S is the peak area of the sample in the chromatogram, and A_{IS} is the peak area of the internal standard.

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GC−MS analysis of thioacidolysis monomers was carried out on a GCMS-QP2010plus instrument (Shimadzu Co., Addison, IL) with a 30 m × 0.25 μm film, SHR5XLB capillary column. Helium was used as the carrier gas. GC conditions were as follows: initial column temperature, 150 °C, held for 1 min, ramped at 10 °C/min to 310 °C, and held for 8 min; injector temperature, 250 °C; splitless mode; and EI mode at 0.2 kV for ionization. When SIM was chosen, the corresponding SICs were obtained and the RFs under SIM were also calculated similarly.

The RFs for GC−FID were also measured on a GC-2010plus workstation (Shimadzu Co., Addison, IL) with a 30 m × 0.25 μm film, SHR5XLB capillary column. GC conditions were the same as those for GC−MS described above.

Thioacidolysis of Lignocellulosic Samples. Thioacidolysis of lignocellulosic samples was performed according to a published standard procedure.[23] The thioacidolysis reagent was prepared freshly by adding 2.5 mL of EtSH and 0.7 mL of BF_3 etherate to a 25 mL volumetric flask containing 20 mL of distilled dioxane and then complemented with dioxane to exactly 25 mL. A solution of 4,4′-ethylidenebisphenol in dioxane or tetracosane in dichloromethane was made and used as an internal standard. Freshly made thioacidolysis reagent (4.0 mL) and internal standard (about 0.2 mg, added by an accurately measured volume) were added to a 5 mL screw-cap reaction vial containing extractive-free lignocellulosic material (5 mg). The vial cap was screwed on tightly, and the vial was kept in a heating block at 100 °C for 4 h with occasional shaking. After the vial was cooled in ice water for 10 min, the product mixture was transferred into a separatory funnel and 7 mL of 0.4 M $NaHCO_3$ was added to adjust the pH to 7. Then, 2 mL of 1 M HCl solution was used to adjust the pH of the solution to below 3. After the normal workup with CH_2Cl_2 extraction (10 mL × 3), the combined organic phase was washed with saturated NH_4Cl, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure at 40 °C. The residues were dissolved in about 1 mL of CH_2Cl_2, and 100 μL of the solution was silylated (BSTFA, 100 μL; pyridine, 20 μL) at 50 °C for 40 min for GC−MS analysis. A total-ion chromatogram was acquired on the GC−MS instrument under the same GC and ionization conditions as used for the determination of RFs. Thioacidolysis monomers from loblolly pine and poplar woods were also measured by GC−FID for comparison purposes.

RESULTS AND DISCUSSION

Syntheses of Lignin-Derived Thioacidolysis Monomers 6a−6c. Lignin-derived thioacidolysis monomers **6a−6c** result from *β-O*-4-ether cleavage accomplished by ethanethiol and BF_3-etherate in dioxane solutions. Therefore, a logical synthetic way to make these monomers could be using the corresponding *β-O*-4-dimeric lignin models via thioacidolysis. However, synthesis of *β-O*-4-dimeric lignin models is not a trivial practice, although there have been various synthetic routes to prepare such models.[24,25] Also, using these dimers would produce at least two kinds of products, from the two different units linked via the *β-O*-4-ether, including the desired thioacidolysis monomers **6**. Possible purification problems would be obviated if monomeric arylglycerols **5** could be converted to thioacidolysis monomers **6** by thioacidolysis. Our preliminary results with compounds **5** indicated that, under the standard thioacidolysis conditions, arylglycerols **5** were converted efficiently (~85% yields) into the corresponding thioacidolysis monomers (Figure 1). Accordingly, a multi-step synthesis strategy was developed, via modifications to published procedures, to prepare compounds **5** from readily available ethyl hydroxycinnamates **1** (Figure 2).

Protection of the phenols in compounds **1** was required to perform the dihydroxylation reaction. Benzylation of compounds **1** with benzyl bromide and potassium carbonate in DMF produced compounds **2** that can be crystallized in high yield. Although substrates for thioacidolysis are not required to be single isomers and certainly do not need to be single enantiomers, the sharpless asymmetric dihydroxylation was chosen to convert compounds **2** to compounds **3** because of the easy operating conditions, high yields of product, and readily available oxidant (AD-mix-*α* or AD-mix-*β*). Moreover, using AD-mix-*α* (or AD-mix-*β*) here is helpful to make crystalline target products **5**, which is desirable when attempting to obtain the purest compounds for performing thioacidolysis to make pure thioacidolysis monomers to measure GC−FID or GC−MS RFs.

Reduction of the *α*,*β*-dihydroxy esters **3** with $NaBH_4$ in 95% ethanol gave the corresponding arylglycerols **4** in almost quantitative yields without requiring purification. Compounds **4** have been made by dihydroxylation with AD-mix-*α* from 4-benzoxycinnamyl alcohols, which were synthesized from compounds **2** by $LiAlH_4$ reduction in tetrahydrofuran (THF). Using $NaBH_4$ as a reducing agent instead of $LiAlH_4$ has advantages because of simple and convenient operations as well as lower cost with $NaBH_4$. However, for the $NaBH_4$ reduction step, TLC should be used to make sure that the acid hydrolysis of the intermediate borates is complete. The last step, debenzylation, performed in ethanol with palladium/carbon under a hydrogen atmosphere, was a traditional and convenient way that produced the desired arylglycerols **5**. Although we did not have any problems carrying out such hydrogenation experiments, it should be cautioned that adding Pd/C to hot ethanol solutions can ignite and any residual Pd/C powder left on the neck of a flask should be cleaned off with wet tissue paper before the hydrogen balloon is put on, via an adaptor or directly, to avoid potential fire hazards.

Determination of GC−MS RFs and Their Applications. Tetracosane has been used as an internal standard for quantitation of thioacidolysis monomers by GC−FID, and the RF 1.5 (for GC−FID) has been applied for all H, G, and S monomers.[9,11] Although being structurally quite different from thioacidolysis monomers, tetracosane is the internal standard used universally to date for thioacidolysis thus far, at least for GC−FID. Tetracosane was also used as an internal standard for the DFRC method when it was first introduced in 1997;[6] 4,4′-ethylenebisphenol was later introduced because it was structurally more similar to the DFRC monomers.[26] Being phenolic in nature, 4,4′-ethylenebisphenol is also closer to thioacidolysis monomers with respect to equilibrium partitioning concentrations between organic and water phases. Therefore, in this study, both tetracosane and 4,4′-ethylenebisphenol were tested as internal standards to measure GC RFs of thioacidolysis monomers using the synthesized and purified compounds **6a−6c**.

From the data listed in the Table 1, it is clear that 4,4′-ethylenebisphenol is a better standard than tetracosane for thioacidolysis monomers when MS detection is used for GC analysis because the RFs of H, G, and S monomers versus 4,4′-ethylenebisphenol were 1.0−1.2, whereas RFs versus tetracosane were 0.4−0.5. These data indicate that tetracosane produces more total fragment ions per weight unit than thioacidolysis monomers do under the ionization conditions used, whereas 4,4′-ethylenebisphenol produces similar amounts. Choosing 4,4′-ethylenebisphenol as the internal standard for thioacidolysis should produce more accurate results when GC−MS is used for quantitation of the lignin-derived monomers **6a−6c** (H, G, and S) because the RFs are closer to 1. When SIM is used to enhance sensitivity for determination of compounds with low abundance or to obtain better resolution

Table 1. GC−MS and GC−FID RFs of Thioacidolysis Monomers 6a−6c versus 4,4′-Ethylenebisphenol or Tetracosane[a]

	GC−MS				
	versus 4,4′-ethylenebisphenol		versus tetracosane	GC−FID	
lignin-derived monomers	TIC	SIC	TIC	versus 4,4′-ethylenebisphenol	versus tetracosane
6a	1.00	1.24	0.42	1.46	1.06
6b	1.21	1.56	0.47	1.95	1.44
6c	1.23	1.91	0.53	2.03	1.51

[a]TIC, total-ion chromatogram; SIC, selected-ion chromatogram. *m/z* 239, 269, 299, and 343 were chosen for compounds **6a**, **6b**, and **6c** and 4,4′-ethylenebisphenol, respectively. No RF was measured for tetracosane under SIC conditions because there is no obvious characteristic fragment in the mass spectrum of tetracosane (Figure 3).

with complex mixtures, the selected characteristic or base mass fragments are commonly used for quantitation. In the case of lignin-derived thioacidolysis monomers, characteristic fragment ions are also the base (highest abundant) peaks; i.e., *m/z* 239 for compound **6a** (H), 269 for compound **6b** (G), and 299 for compound **6c** (S) were used to measure their RFs versus the characteristic or base mass fragment (*m/z* 243) of 4,4′-ethylenebisphenol (Figure 3). The calculated RFs listed in Table 1 indicated that 4,4′-ethylenebisphenol could still be used as an internal standard for quantitation of thioacidolysis monomers when SIM is chosen for MS detection. For tetracosane, no RFs under SIM were measured because no obvious characteristic fragment ions could be identified (Figure 3). With the synthesized thioacidolysis monomers **6a−6c**, the RFs versus 4,4′-ethylenebisphenol or tetracosane were also measured under similar GC conditions when FID is used for detection. Although the RF value for G monomer was slightly lower compared to the reported value, the RF value obtained for S monomer was very close to the previously published data (Table 1). Comparing these RFs suggested that tetracosane is a more suitable internal standard than 4,4′-ethylenebisphenol for GC−FID quantitation of lignin-derived thioacidolysis monomers. It should be noted that compound **6a**, the H monomer (derived from *p*-hydroxyphenyl units in lignin), was synthesized and characterized here for the first time. Note that its GC−FID RF value is considerably lower than those for the G and S monomers, implying that H units would be significantly overestimated if the same RF (1.5) was used. Although the H unit is generally less significant than the other units (G and S) in lignin, its accurate quantitation is still needed and becomes crucial in some cases, notably in softwood compression woods (where its level can reach 30%)[27] and in plants downregulated in coumarate 3-hydroxylase[28] or hydroxycinnamoyl-CoA transferase,[29] where it can reach essentially 100% levels. Therefore, in this work, the obtained RFs of the thioacidolysis H monomer versus 4,4′-ethylenebisphenol or tetracosane for GC quantitation provide an important basis for future studies involving lignin compositional analysis.

To test and verify the obtained RFs, several lignocellulosic materials were analyzed by thioacidolysis using 4,4′-ethylenebisphenol as an internal standard. The released lignin-derived monomers **6a−6c** were quantitated by GC−MS under the same ionization and GC conditions as for the RF

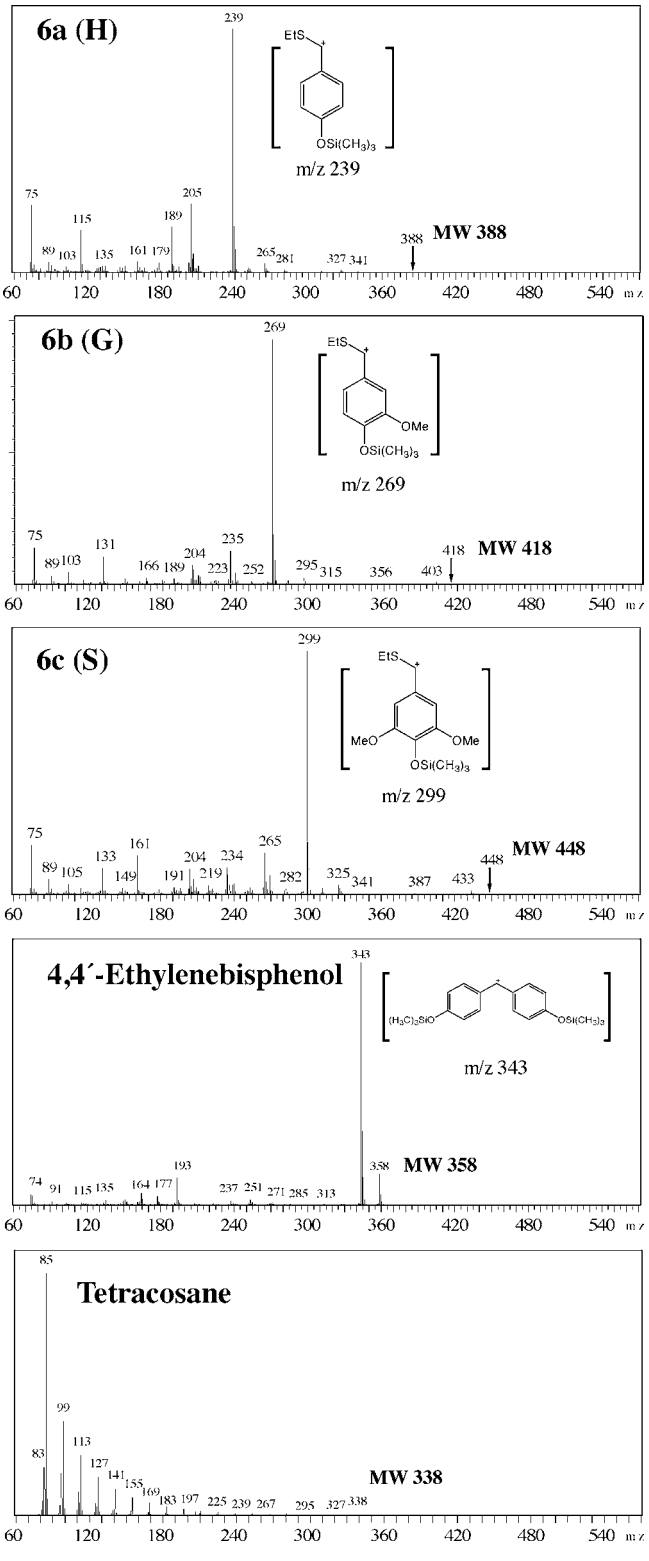

Figure 3. Mass spectra of lignin-derived thioacidolysis monomers **6a−6c** (H, G, and S) and internal standards 4,4′-ethylenebisphenol and tetracosane. The structures of characteristic base peak fragment ions are shown with each individual spectrum (except for that from tetracosane).

measurements. The yields of thioacidolysis monomer (μmol/g of Klason lignin; Table 2), calculated by applying the RFs from 4,4′-ethylenebisphenol or tetracosane, were obtained from several extract-free lignocellulosic samples representative of

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Table 2. Yields[a] (μmol/g of Klason Lignin) of Lignin-Derived Thioacidolysis Monomers from Various Lignocellulosic Materials, Measured by GC−MS and GC−FID[b]

		monomer yields				molar ratios	
lignocellulosic material	detector (IS)	H (**6a**)	G (**6b**)	S (**6c**)	total yields	H/G	S/G
poplar	TIC (EBP)		848	1342	2190		1.58
	FID (EBP)		820	1244	2064		1.52
	FID (C_{24})		797	1194	1991		1.50
loblolly pine	TIC (EBP)	45	1020		1065	0.04	
	FID (EBP)	46	1009		1055	0.05	
	FID (C_{24})	45	999		1044	0.05	
white spruce	TIC (EBP)	21	1109		1130	0.02	
bamboo	TIC (EBP)	73	602	411	1086	0.12	0.68
bagasse	TIC (EBP)	8	303	265	576	0.03	0.87

[a]Using RFs from Table 1. [b]H, *p*-hydroxyphenyl monomer (**6a**); G, guaiacyl monomer (**6b**); S, syringyl monomer (**6c**); EBP, 4,4′-ethylenebisphenol; C_{24}, tetracosane.

softwoods, hardwoods, and grasses under the standard thioacidolysis degradation conditions.[23] The monomer yields for loblolly pine but less so for poplar were quite consistent, regardless of which detection method or internal standard were used for quantitation, and were close to the reported data.[5] Two grass samples, bamboo and sugar cane bagasse, were also analyzed by thioacidolysis with GC−MS quantitation, and their monomer yields were reported here for the first time. It has been found that yields of thioacidolysis monomer for grass samples are underestimated because of the acylation of lignin units by *p*-coumarates that do not cleave efficiently under thioacidolysis conditions.[30] In the case of bagasse, the total monomer yield was 570 μmol/g of lignin, which is close to that of corn or rice straws.[5]

In conclusion, an efficient multi-step approach was designed and used to synthesize substituted arylglycerols as starting materials to prepare the three authentic thioacidolysis monomers **6a**−**6c**. Using these synthesized and purified thioacidolysis monomers, GC−MS and GC−FID RFs of these monomers versus 4,4′-ethylenebisphenol or tetracosane (as internal standards) have been determined. The obtained results suggest that 4,4′-ethylenebisphenol is a better internal standard than tetracosane for thioacidolysis analysis of lignins when MS detection is used for quantitation, whereas tetracosane remains suitable for FID detection. When selected-ion monitoring is used for MS detection, 4,4′-ethylenebisphenol can still be used for quantitation of thioacidolysis monomers, although the obtained results are less accurate than those from integration of total-ion chromatograms. Applying the obtained RFs, yields of thioacidolysis monomers from various lignocellulosic samples were found to be consistent with those reported in the literature.

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