Corroboration of Techniques for Assigning Absolute Configuration: Lacinilene C Methyl Ether as an Exemplary Study

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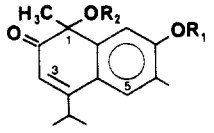
Methods for determination of absolute configuration were independently applied to the phytoalexin lacinilene C methyl ether. The results from circular dichroism, NMR spectroscopy utilizing chiral solvating agents, and chiral stationary phase chromatography allow assignment of the *R* configuration to (+)-lacinilene C methyl ether. This assignment was verified by X-ray diffraction. Chemical conversion permitted assignment of the *R* configuration to (+)-lacinilene C.

Assignment of the absolute configuration is an important aspect of the structure determination process for a great many compounds. Of the various options available for this task, single-crystal X-ray diffraction analysis of a diastereomeric derivative containing a center of known configuration provides an unequivocal answer. Unfortunately, application of this method requires the availability of a suitable crystal, a requirement that places a serious limitation on the method in spite of impressive technological advances in instrumentation and data analysis. Limitations on access to costly equipment and availability of special expertise further restrict the general utility of modern X-ray diffraction analysis.

Thus, it continues to be important to explore the capabilities and limitations of alternative methods for assignment of absolute configuration, such as CD spectroscopy,[1] NMR spectroscopy utilizing chiral solvents,[2] and liquid chromatography employing chiral stationary phases.[3-5] However, these alternatives are only as reliable as our understanding of underlying molecular behavior: conformational preferences in solution in the case of CD spectroscopy; molecular interactions in solution in the case of NMR spectroscopy; and dominant functional group interactions with the chiral stationary phase in the case of liquid chromatography.

Lacinilene C (**1a**) and its methyl ether (LCME, **1b**) have one asymmetric carbon atom and therefore may exist as either of two enantiomers. Lacinilene C and LCME have



1a $R_1, R_2 = H$
1b $R_1 = CH_3, R_2 = H$
1c $R_1 = CH_3, R_2 = CH_3CO$

been isolated from foliar cotton plant tissue.[6,7] The absolute configuration of lacinilene C may have significant biological implications, since it inhibits growth of the cotton bacterial pathogen, *Xanthomonas campestris* pv. *malvacearum*. Studies showed that the (−) enantiomer of lacinilene C is more inhibitory to *Xanthomonas* than is the (+) enantiomer and, that on standing in solution, (+)-lacinilene C slowly racemizes and gains inhibitory activity.[8] LCME is less active against *Xanthomonas*. LCME is a chemotactic agent and is a putative causative agent of byssinosis.[9,10] Determination of the absolute configuration of these compounds and characterization of the interactions of the assigned enantiomers with specific chiral stationary phases may be useful in understanding the molecular basis for observed biological activities.

Our need to determine the absolute configuration of LCME[6,7] was confounded by the absence of a suitable crystal, which led us to consider the applicability of alternative methods. Owing to the presence of a variety of functionality appended to the aromatic ring, this particular structure, together with the related structures **1a** and **1c**, offered an opportunity to explore the application of the spectroscopic and chromatographic methods mentioned above. Thus, we decided to apply these methods simultaneously at different laboratories to determine the absolute configuration of LCME.

The use of chiral stationary phases for liquid chromatographic separations is of special interest, because this technique not only promises to provide a relatively inexpensive and rapid method for configurational assignment but also can be employed to prepare useful quantities of the pure enantiomers for biological and spectroscopic evaluation. However, application of the technique for configurational assignment is tenuous when the enantiomers contain multiple functional groups that can contribute to interaction with the chiral stationary phase.

Assignment of LCME absolute configuration was recognized as such a case. Both **1a** and **1b** may be considered as benzyl alcohols, whose interaction with certain chiral stationary phases is thought to be understood.[11,12] However, the two compounds differ in the presence or absence

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Table I. Circular Dichroism of (+)-Lacinilene C Methyl Ether

solvent[a]			
hexane		ethanol	
λ_{max}, nm	$[\theta] \times 10^{-3}$	λ_{max}, nm	$[\theta] \times 10^{-3}$
371	10.2	376	10.2
329	-12.2	333	-7.5
247	5.2	248	6.0
226	-3.5	227	-1.3
208	15.3	210	13.5

[a] Data was obtained with a 1-mm path length cell; concentration, 0.25 mg/mL. The sample employed was 82% ee and the values were corrected for 100% ee.

of the polar phenolic hydroxyl group, which can potentially give rise to additional strong interactions with these chiral phases. The results that we now report regarding the apparent relative importance of these different functional groups, together with those of other studies, add to the confidence with which this method can be applied in the future.

Results and Discussion

Circular Dichroism. (+)-LCME gives a strong positive Cotton effect from the carbonyl n → π* transition at 371 nm in hexane and 376 nm in ethanol (Table I). The octant rule, based on the sign of the Cotton effect for the n → π* transition,[13,14] has been used to determine the absolute configuration of cyclic ketones of known conformation.

Application of the octant rule for assignment of LCME absolute configuration is complicated by ambiguity regarding the preferred conformation in solution. LCME can exist in either of two stable conformers with either the C-1 methyl group or the C-1 hydroxyl group in a pseudoaxial position. Considering (*R*)-LCME, one conformation places the C-1 methyl group in the pseudoaxial position, where it rests in the upper-left octant and therefore makes a positive contribution to the sign of the Cotton curve, and the C-1 hydroxyl group in pseudoequatorial position, where it lies approximately in one of the orthogonal planes passing through the carbonyl group and therefore makes no significant contribution to the sign of the Cotton curve. In the other conformer, the hydroxyl group makes a negative contribution to the Cotton curve owing to its location in the lower-left octant, and the methyl group makes no significant contribution owing to its position in one of the orthogonal planes.

Fortunately, the ^{1}H NMR spectrum is helpful in determining the preferred conformation of LCME. The Curphy–Morrison additivity constants[15,16] predict a chemical shift for the methyl group at the chiral center in LCME of δ 1.9 on the basis of models in which free rotation around all bonds of the benzylic carbon atom is assumed. The actual chemical shift is found to be δ 1.43 (CCl_4), indicating the methyl group is in a more shielded (e.g., pseudoaxial) region.

In addition, for α,β-unsaturated carbonyl systems, a hypsochromic shift of the n→π* transition is commonly observed in hydroxylic solvents due to the effects of hydrogen bonding.[17] For LCME, a bathochromic shift is noted in ethanol, suggesting that a significant degree of intramolecular hydrogen bonding occurs between the hydroxyl group and the carbonyl oxygen in hexane. Hydrogen bonding is most effective when the methyl group is in a pseudoaxial position.

The preferred conformation can also be assigned on the basis of $A^{(1,3)}$ strain. Thus, the larger methyl substituent encounters less steric interaction (e.g., $A^{1,3}$ strain) when located in the pseudoaxial position.[18,19] In LCME the methyl group is therefore assigned the pseudoaxial orientation, an assignment which leads one to expect a positive Cotton effect for (*R*)-LCME. Since (+)-LCME is the enantiomer that gives a positive Cotton effect, we assign the *R* configuration to (+)-LCME.

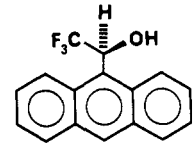
Table II. NMR Spectral Nonequivalence Observed for (*S*)-(-)-Enriched Lacinilene C Methyl Ether with Two Chiral Solvating Agents[a]

		nonequivalence and sense, |δ|	
proton	δ	chiral (*S*)-(+)-**2**	solvating agent (*R*)-(+)-**3**
Ar–OCH_3	3.31 (s)	0.044 (H)	0.057 (L)
Ar–CH_3	2.23 (s)	0.025 (H)	0.075 (L)
C_5–H	7.09 (s)	0.024 (H)	
C_3–H	6.04 (s)		0.072 (H)

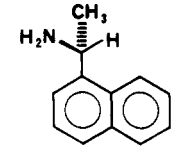
[a] The spectra were obtained in benzene-d_6 with a Varian HR-220 spectrometer. Nonequivalence of the resonances listed was obtained with 4 molar equiv of (*S*)-(+)-**2** or 10 molar equiv of (*R*)-(+)-**3**. The major resonance corresponding to the (*S*)-(-)-lacinilene C methyl ether enantiomer is indicated as the highfield (H) or lowfield (L) signal.

Proton Magnetic Resonance and Chiral Solvating Agents. The addition of a chiral solvating agent can produce nonequivalence of enantiomers in the ^{1}H NMR spectrum. If the solute–chiral solvating agent interactions which beget these spectral differences are understood, the absolute configuration may be determined.

On addition of (*S*)-2,2,2-trifluoro-1-(9-anthryl)ethanol (**2**) or (*R*)-1-(1-naphthyl)ethylamine (**3**) to LCME, nonequivalence is observed for the protons shown in Table II.



2



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To determine the sense of nonequivalence (i.e., which enantiomers affords the upfield and which the downfield signal), a sample of LCME enriched in the (-) isomer (38% ee) was employed.

A proposed dibasic solvation model[2] for **2** predicts a primary interaction between the acidic hydroxyl group of the fluoro alcohol and a hydrogen bond receptor in the enantiomeric solute, as well as a secondary interaction between the carbinyl hydrogen (weakly acidic due to the inductive effect of the trifluoromethyl group) and a secondary basic site of the solute.[20] In acyclic α-hydroxy carbonyl compounds, the hydroxyl group serves as the primary basic site and the carbonyl group as the secondary site for carbinyl hydrogen bonding.[2,21] Using these in-

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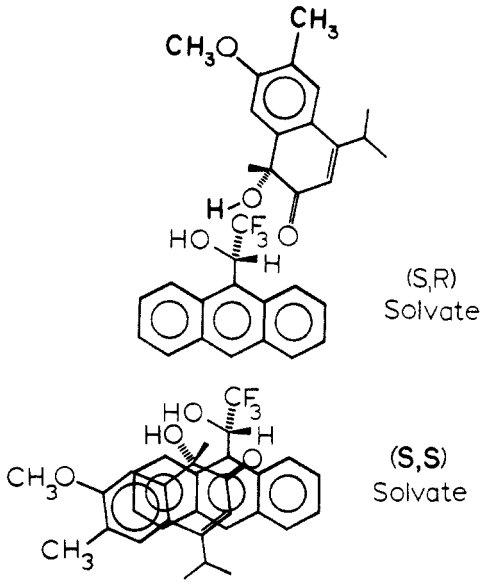


Figure 1. Proposed structures of the diastereomeric solvates derived from (*S*)-**2** and the enantiomers of LCME.

teractions, the diastereomeric adsorbate from (*S*)-**2** and (*S*)-LCME is expected to cause aromatic methoxy and methyl substituents of LCME to be in a region shielded by the anthryl ring, thus producing an upfield shift for these groups as compared to those of the corresponding groups in the *S,R* solvate. This is depicted in Figure 1. The (−) enantiomer of LCME exhibits such upfield shifts for these groups and is thus assigned the *S* configuration.

(*R*)-α-(1-Naphthyl)ethylamine (**3**) has previously been used as a chiral solvating agent for α-hydroxy carbonyl compounds.[2,22] The primary interaction between **3** and alcoholic solutes such as LCME is hydrogen bonding between the hydroxyl group of the solute and the amino group of **3**.[2,22] The aromatic and enone portions of LCME constitute an extended π-system somewhat electron deficient relative to the naphthyl group of **3** and capable of undergoing π−π interaction with the latter. For the solvate derived from (*S*)-LCME and (*R*)-**3**, the π−π interaction and a weak carbinyl hydrogen bonding interaction between the methine hydrogen of **3** and the carbonyl oxygen of LCME are suggested to lead to formation of the solvates shown in Figure 2. The vinyl hydrogen is shielded by the magnetic anisotropy of the naphthyl system. However, the chemical shifts of the more remote methoxyl and aryl methyl groups are relatively unaffected. In the solvate derived from (*R*)-LCME, the carbinyl hydrogen bonding interaction is thought to occur at the methoxyl oxygen rather than carbonyl oxygen. Hence, the relative position of the two ring systems differs, and the methyl and aryl methyl substituents, now positioned more nearly over the naphthyl system, experience shielding. The vinyl hydrogen is more remote from the naphthyl and is relatively unshielded. Therefore, one expects and sees opposite senses of nonequivalence for the vinyl proton compared to the aromatic methyl and methoxyl substituents. Since (−)-LCME exhibits a highfield sense of nonequivalence for the vinyl proton and a lowfield sense for the aromatic methyl and methoxyl groups, it is assigned the *S* configuration.

Chromatographic Separations. The chromatographic resolution of the enantiomers of lacinilene C and its derivatives **1b** and **1c** was investigated on chiral stationary

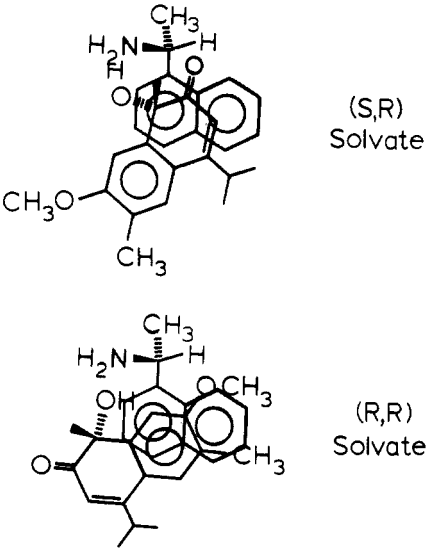
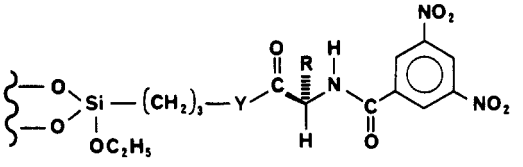


Figure 2. Proposed structures of the diastereomeric solvates derived from (*R*)-**3** and the enantiomers of LCME.

phases representing two types, the covalently linked **4a,b** and the ionically bound **5a,b**. Separation factors are



4) Y = NH
5) Y = $NH_3^{\oplus}O^{\ominus}$

R
a phenyl
b isobutyl

sufficiently large to allow preparative separation of modest quantities of the enantiomers for spectroscopic evaluation or chemical elaboration. For example, we required a highly enriched sample of **1b** for chiroptical measurements and a moderately enriched sample (e.g., 30–50% ee) for NMR studies with a chiral solvating agent. This was accomplished by resolution of 9.0 mg of LCME in a single chromatographic run on a column (250 × 10 mm i.d.) containing chiral stationary phase **4b**. Two fractions were collected, affording (−)- and (+)-LCME in 38% and 82% ee, respectively. Under identical chromatographic conditions, a 4-mg sample can be base-line resolved ($\alpha = 1.11$), whereby each enantiomer is obtained in greater than 95% ee. Chiral stationary phases (**4a,b** and **5a,b**) resolve the enantiomers of lacinilene C and LCME affording separation factors, α, ranging from 1.05 to 1.13.

Owing to the variety of functional groups found in lacinilene C and LCME, numerous chiral recognition mechanisms can be envisioned to account for resolution on chiral stationary phases **4** and **5**. A chiral recognition model has been proposed for the resolution of benzyl alcohols on **5a**.[11,12] Considering LCME as a benzyl alcohol, resolution on **5a** can be rationalized in terms of three interactions: (1) π−π interaction between the electron-rich aromatic ring of LCME and the electron-deficient dinitrobenzoyl group of the chiral stationary phase; (2) hydrogen bonding between the amide hydrogen and the hydroxyl group of LCME; and (3) differential steric interaction between either the carboxylate group or the phenyl group of the chiral stationary phase and the enone in LCME, as depicted in Figure 3. This analysis leads to the conclusion that the *R* enantiomer of LCME will form the more stable disastereomeric adsorbate with (*R*)-**5a**

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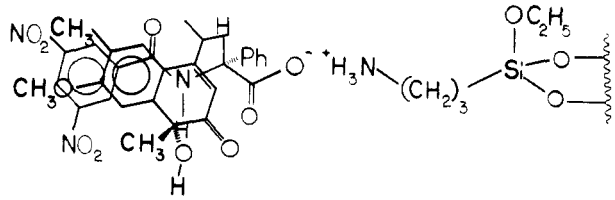

Figure 3. Proposed structure for the diastereomeric adsorbate derived from *R* chiral stationary phase **5a** and the *R* enantiomer of LCME. This is the most stable of the two diastereomeric adsorbates and causes (*R*)-LCME to be more strongly retained than its antipode.

since it encounters the least steric interaction. Experimentally, (+)-LCME is retained more strongly on (*R*)-**5a** (α = 1.13) and is thus assigned the *R* configuration.

The relationship between the sign of rotation and absolute configuration of **1a**, **1b**, and **1c** was established by treating an enriched sample of (+)-lacinilene C (**1a**) with diazomethane to give an enriched sample of (+)-LCME. When LCME was acetylated, (+)-LCME gave the (+) enantiomer of LCME-*O*-acetate, **1c**. Thus the dextrarotatory enantiomers of **1a**, **1b**, and **1c** are of the same absolute configuration.

Interestingly, chromatographic elution order of the enantiomers of **1a** on chiral stationary phase **5a** [α = 1.07, (–) enantiomer more retained] is reversed compared to those of LCME [α = 1.13, (+) enantiomer more retained]. Elution order reversal has been noted in other cases of phenols and their methyl ethers and is ascribed to the preferential operation of different retention mechanisms.[24,25] In addition, an elution order reversal is observed for LCME depending on whether ionically bound **5a** or covalently bound **4a** is employed. This behavior could not have been predicted with our present level of understanding. It strongly suggests that, for these compounds, there are multiple retention mechanisms which give rise to enantioselection. The observed selectivity is a time-weighted average of these retention processes, some of which operate in opposite enantioselective senses. Changes in the solute or support structure can effect the relative contributions of competing retention mechanisms and thus alter the magnitude of the separation or the order of elution of the enantiomers.

Experiments using chiral stationary phase **5a** revealed that the elution order of the enantiomers of LCME acetates also was reversed on chiral stationary phase **5a** [for LCME-*O*-acetate, α = 1.11, (–) enantiomer more retained], compared to LCME enantiomers. Reversal of elution order upon acetylation of chiral alcohols has been reported.[26] Acetylation renders the chiral recognition model shown in Figure 3 inoperative by masking the essential hydroxyl group.

Single-Crystal X-ray Diffraction. Results of single-crystal X-ray diffraction analysis unequivocally verify the prior assignments of absolute configuration. For this work a suitable, crystalline diastereomeric derivative was required. We chose to derivatize LCME with D-glucose to provide the chiral probe for our X-ray study.

LCME was glycosolated by the Hanessian modification[27] of the Koenigs–Knorr reaction. Of the several methods investigated, this proved to be the most effective for derivatizing this hindered, sluggishly reacting tertiary benzyl aclohol. Purification of the reaction products on a silica gel column accomplished partial resolution of the anomers. The initial aomeric ratio of α-, β-glucosides was approximately 2:1, favoring the more mobile α isomer. The β isomer was recrystallized from ether, providing yellow crystals, mp 185–186 °C.

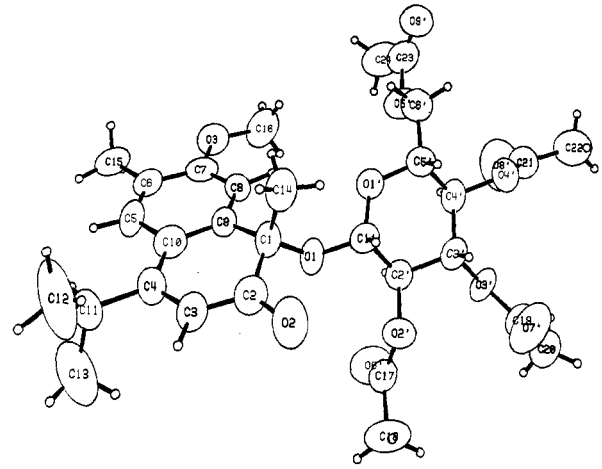

Figure 4. X-ray structure of tetra-*O*-acetyl-α-D-glucopyranoside of (*S*)-LCME.

Figure 4 shows the structure of the tetra-*O*-acetyl-α-D-glucopyranoside of (*S*)-LCME as determined by the single-crystal X-ray defraction study. Bond distances are normal. Except for C-1 and C-2, the atoms of the two fused six-membered rings are nearly coplanar [with the maximum deviation being 0.059 (8) Å for C-3]. C-1 is 0.219 (7) Å above that plane while C-2 is 0.085 (8) Å below it. That plane makes a dihedral angle of 39° with the O-1′, C-1′, C-3′, C-4′ plane (the seat of the chair conformation observed for the sugar ring). There is probably some disorder in the positions of C-12 and C-13, accounting for the rather large thermal motion corresponding to rotation around the C-4–C-11 bond. The positional parameters, thermal parameters, and bond distances and angles are available as supplemental material.

In order to establish the optical rotation of (*S*)-LCME, the α-D-glucopyranoside of (*S*)-LCME was hydrolyzed by refluxing for 1.5 h in a 1:1 solution of MeOH:10% (aq) HCl. The resulting LCME was isolated and chromatographed on the chiral stationary phase **5a**. The chromatogram demonstrated that only partial racemization occurred during hydrolysis and that the first and major enantiomer (75%) eluting from the column has a negative rotation. Thus (*S*)-LCME is the initially eluted levorotatory enantiomer, verifying the conclusions based on the chromatographic, NMR, and CD investigations.

Conclusion. These experiments indicate that our understanding of conformational preferences even in relatively complex molecular systems permits successful application of a variety of techniques for determination of absolute configuration. Application of the less tedious approaches involving CD, NMR chiral solvates, or chiral phase liquid chromatography can lead to correct assignments and will become more widely useful as additional results from studies of diverse compounds become available. With the present state of understanding in this area, however, use of more than one technique is strongly recommended.

Experimental Section

The ^{1}H NMR spectra were acquired on a Nicolet NT-300 spectrometer with observation of ^{13}C at 75.45 MHz and ^{1}H at 300 MHz or on a JEOL FX-90Q spectrometer, observing ^{1}H at 89.55 MHz or a Varian HR-2201 observing ^{1}H at 220 MHz. Optical rotations were taken on a Perkin-Elmer Model 241 polarimeter;

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CD spectra were recorded on a Cary Model 60 recording spectropolarimeter or a JASCO J-40A spectrometer.

Analytical HPLC was performed by using an Altex 100 or 100A pump, a Valco 7000 psi or Altex 210 injector equipped with a 10-μL and 20-μL sample loop, respectively, and an Altex Model 152 dual wavelenth (254 nm and 280 nm) detector. Chromatography columns containing chiral stationary phases **4a,b** or **5a,b** were obtained from Regis Chemical Co. and Baker Chemical Co. Preparative HPLC was accomplished with a Beckman 110A pump, an Altex 210 injector equipped with sample loops from 75 μL to 500 μL, and an ISCO UA-5 absorbance monitor.

The X-ray data were collected on an Enraf Nonius CAD4 diffractometer, and all calculations were done with the Nonius SDP system on a PDP11/34 computer. A crystal of approximate dimensions 0.15 × 0.20 × 0.30 mm was used for the data collection. The cell dimensions were determined from 25 carefully centered reflections with $10° < \theta < 15°$. The crystals are orthorhombic: a = 12.121 (3), b = 12.494 (3), and c = 20.198 (4) Å. There are four molecules of $C_{30}H_{38}O_{12}$ (F.W. = 590.6) in the unit cell (space group $P2_12_12_1$) giving a calculated density of 1.275 (2) g/cm^3. Mo Kα radiation (a = 0.7107 Å) was used to measure 3950 reflections of which 2289 were independent. Only those 1566 reflections with $F_o > 2\sigma(F_o)$ were considered observed and used to solve and refine the structure. Three intensity standards monitored after every 6000 s of X-ray exposure indicated no significant decomposition. Orientation was maintained by periodic (after every 150 reflections) checks of the centering reflections. Data collection was by the θ–2θ technique. The structure was solved from Multan.[28] Refinement was by full-matrix least squares [minimizing $\Sigma w(|F_o| - |F_c|)^2$ where $w^{-1} = \sigma^2_{counting} + (0.05F_o^2)^2/4F_o$]. The final agreement factor ($R = \Sigma||F_o| - |F_c||/\Sigma F_o$) = 0.058. There were 379 variables (x, y, z, 6β's for non-hydrogen atoms). Hydrogen atoms were not refined but placed in positions from difference Fourier syntheses and chemical reasonability. The final difference Fourier synthesis had no features greater than 0.5 e/Å^3.

Lacinilene C Methyl Ether Tetra-*O*-acetyl-α- and -β-D-glucopyranosides. To a mixture of 300 mg (1.15 mmol) of lacinilene C methyl ether,[29] 650 mg (2.53 mmol) of silver trifluoromethanesulfonate,[30] and 0.42 mL (3.45 mmol) of tetramethylurea in 20 mL of CH_2Cl_2 was added dropwise 946 mg (2.30 mmol) of tetra-*O*-acetyl-α-D-glucopyranosyl bromide (prepared from D-glucose)[31] in 15 mL of CH_2Cl_2 at 22 °C. The resulting mixture was refluxed for 2 h and then stirred at 22 °C for 19 h. This mixture was combined with a mixture prepared on $^1/_6$ the scale by using simular proportions of reagents. The resulting mixture was passed through a short column of Celite, eluting with 200 mL of CH_2Cl_2. The eluate was concentrated to give a brown oil which was purified by column chromatography (54 g of Merck, PF_{254} silica gel 60, eluted with toluene/EtOAc, 4/1), providing 72 mg (20.6%) of recovered LCME, 667 mg of fractions containing both α and β anomers, and 154 mg (19.4%) of the less mobile β anomer as a crystalline solid. Recrystallization of the β anomer provided 129 mg of the pure tetra-*O*-acetyl-β-D-glucopyranoside of laciniline C methyl ether, mp 185–186 °C, R_f 0.18 (toluene/EtOAc, 2/1). The crystal used for the X-ray examination was selected from this sample. The ^{1}H NMR of this tetraacetate did not permit clear observation of the homonuclear HC-1′–HC-2′ coupling which is indicative of the anomeric stereochemistry; thus, the ^{1}H NMR data are given for the glucoside itself (see below). Additional chromatography on silica gel as described above and using preparative plates (eluted with toluene/EtOAc, 1/1) ultimately provided 310 mg (39.0%) of the purified more mobile, tetra-*O*-acetyl-α-D-glucopyranoside of laciniline C methyl ether, R_f 0.21 (toluene/EtOAc, 2/1).

Lacinilene C Methyl Ether β-D-Glucopyranoside. Treatment of 75 mg (0.127 mmol) of the tetraacetate, prepared as described above, with 0.3% sodium methoxide in MeOH (7 mL) at 22 °C for 1 h, followed by purification on silica gel (plates, $CHCl_3$/MeOH, 5/1) afforded 37 mg (69.6%) of the desired glucoside: R_f 0.44 ($CHCl_3$/MeOH, 5/1); ^{1}H NMR (MeOH-d_4) δ 7.56 (s, HC-5), 7.53 (s, HC-8), 6.02 (s, HC-3), 4.13 (d, J = 7.09 Hz, HC-1′), 3.96 (s, H_3C-16), 2.22 (s, H_3C-15), 1.51 (s, H_3C-14), 1.28 (d, J = 6.1 Hz, C-12), 1.25 (d, J = 6.1 Hz, C-13); ^{13}C NMR (MeOH-d_4) δ 204.5 (C-2), 167.0 (C-4), 160.7 (C-7), 145.5 (C-9), 128.6 (C-3), 127.5 (C-5), 120.6 (C-10), 118.0 (C-6), 112.0 (C-8), 102.4 (C-1′), 83.5 (C-1), 78.2 (C-3′), 77.7 (C-5′), 76.1 (C-2′), 71.0 (C-4′), 62.3 (C-6′), 56.3 (C-16), 32.4 (C-14), 30.3 (C-11), 22.6 and 22.4 (C-12 and C-13), 16.1 (C-15).

Hydrolysis of β-D-Glucopyranoside of Lacinilene C Methyl Ether. The glycoside (0.4 mg) was dissolved in warm MeOH (2 mL); 10% HCl (2 mL) was added and the solution refluxed for 1.5 h. The solution was poured into an equal volume of a 1:1 solution of H_2O and saturated brine (50% brine) and extracted with Et_2O (3 × 4 mL). The combined Et_2O extracts were washed with 50% brine, dried (Na_2SO_4), and evaporated to dryness. The product was compared (fluorescence and R_f value) to synthetic LCME on TLC as previously described.[32] The product and the product mixed with authentic LCME were dissolved in hexane/isopropyl alcohol (95:5) (running solvent; flow 1 mL/min) and injected onto an ionic Pirkle column (Type 1-A). The (−) enantiomer aluted first and was the major product (75%).

General Procedure for the Preparation of *N*-(3,5-Dinitrobenzoyl)amino Acids. To a slurry of 0.05 mol of enantiomerically pure amino acid and 11.5 g (0.05 mol) of 3,5-dinitrobenzoyl chloride in 150 mL of dry THF was bubbled ethylene oxide. The solution was cooled with an ice bath and addition continued until a total of 4.0 g (0.09 mol) of ethylene oxide had been added. The mixture was allowed to stir for 1 h, filtered by suction, and the solvents were removed in vacuo to afford the *N*-(3,5-dinitrobenzoyl)amino acid in 80–95% yield. Enantiomeric purity, as determined by HPLC analysis, was greater than 98% ee. The material may be recrystallized from acetone–carbon tetrachloride. Physical properties agreed with those previously reported.[33]

Procedure for in Situ Modification of Packed Amino Columns. Preparation of covalently bound amino acid DNB columns, **4a–b**, is outlined below for a semipreparative (250 mm × 10 mm i.d.) column. A solution of 0.02 mol of amino acid DNB and 5.0 g (0.02 mol) of 1-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) in 100 mL of CH_2Cl_2 was prepared by treatment of the freshly mixed components in an ultrasonic bath for 15 min. The filtered solution was immediately pumped through a commercially packed aminopropyl silanized silica gel column (dp, 5 μm; Regis Chemical Co.) at a flow rate of 4 mL/min. The column was subsequently washed with 50 mL of CH_2Cl_2, 50 mL of methanol, and 10% 2-propanol in hexane until a stable base line was achieved. Preparation of ionically bound amino acid DNB columns, **5a–b**, followed the previously reported procedure.[33]

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Supplementary Material Available: X-ray data collection tables include a table of positional parameters and their estimated standard deviations, a table of general temperature factor expressions, a table of bond angles, and a table of bond distances (10 pages). Ordering information is given on any current masthead page.

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