carrying out the X-ray crystallographic study of 8. We also express our appreciation to Dr. K. Inokuchi and the Fujisawa Pharmaceutical Co., Ltd., Japan, for providing us with a gift of bicyclomycin.

Supplementary Material Available: Experimental procedure for the X-ray analysis of 8, ORTEP drawing of 8 with atom labeling scheme (Figure 2), Table 4 listing the final cell constants, as well as other information pertinent to data collection and refinement, and Tables 5-9 giving a complete listing of atomic coordinates and equivalent isotropic displacement parameters, bond lengths, bond angles, and hydrogen-bonding parameters, select long-range proton-carbon connectivities observed in the proton-detected long-range heteronuclear multiple quantum chemical shift correlation (HMBC) experiments for 8 (Figure 1) and 3 (Figure 3) and ^1H and/or ^{13}C NMR spectra for all new compounds (42 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Separation of Remote Diol and Triol Stereoisomers by Enzyme-Catalyzed Esterification in Organic Media or Hydrolysis in Aqueous Media

J. Shield Wallace, Bruce W. Baldwin, and Cary J. Morrow*

Department of Chemistry, University of New Mexico, Albuquerque, New Mexico 87131

Received April 30, 1992

The separation of symmetric, remote, secondary diol stereoisomers by stereoselective enzyme-catalyzed acetylation with acetic anhydride in anhydrous, low polarity organic solvents or by stereoselective enzyme-catalyzed hydrolysis of the corresponding peracetate in aqueous media is described. Whether or not an alcohol is acetylated or an acetate is hydrolyzed is determined solely by its own stereochemical arrangement and not by the stereochemistry at any other stereogenic center. Since the enzyme used, Amano P lipoprotein lipase from *Pseudomonas* species, acetylates secondary alcohol stereogenic centers of the (*R*)-configuration, an (*R*,*R*)-diol is converted to its diacetate, a meso-diol is converted to the monoacetate at its (*R*)-stereogenic center, and an (*S*,*S*)-diol is left unchanged. Similarly, when hydrolysis is used, (*R*)-stereogenic centers are hydrolyzed so that the (*R*,*R*)-stereoisomer is converted to the corresponding diol while the (*S*,*S*)-stereoisomer remains a diacetate. The resulting mixture is separated, and the remaining acetates are removed by hydrolysis to give diols and triols of high stereochemical purity. Diols successively separated by esterification include α,α'-dimethyl-1,4-benzenedimethanol, **1**, α,α'-dimethyl-1,3-benzenedimethanol, **4**, α,α'-dimethyl-2,6-pyridinedimethanol, **5**, and α,α'-dimethyl-4,4'-biphenylenedimethanol, **6**. For two cases, α,α'-dimethyl-2,6-pyridinedimethanol, **5**, and α,α',α''-trimethyl-1,3,5-benzenetrimethanol, **7**, the separation was achieved using the hydrolysis procedure. The stereochemical purity of each of the separated diol stereoisomers was determined by evaluating the NMR spectrum of its bis-MTPA ester. In most cases, it was possible to establish both the stereochemical purity of the fraction and the amount of each contaminating stereoisomer that was present. The diol products are expected to be of value for preparing optically active polymers and optically active crown ethers.

Diols are valuable intermediates in the preparation of polymers, acetals, and crown ethers, and optically active diols have been widely used for stereochemical control in homochiral syntheses. Unfortunately, the number of optically active diols, other than those associated with carbohydrates, is quite small. Thus, a general source could provide valuable new building blocks for many structures. Most techniques for the preparation of optically active diols focus on the stereospecific synthesis of a single enantiomer.[1,2] The chemicals for preparing both enantiomers via such a procedure are not always available. In many cases, the stereochemistry at the second stereogenic center is determined by that at the first, limiting the allowable distance between the two. Finally, a completely different approach is generally required for preparing the meso stereoisomer.

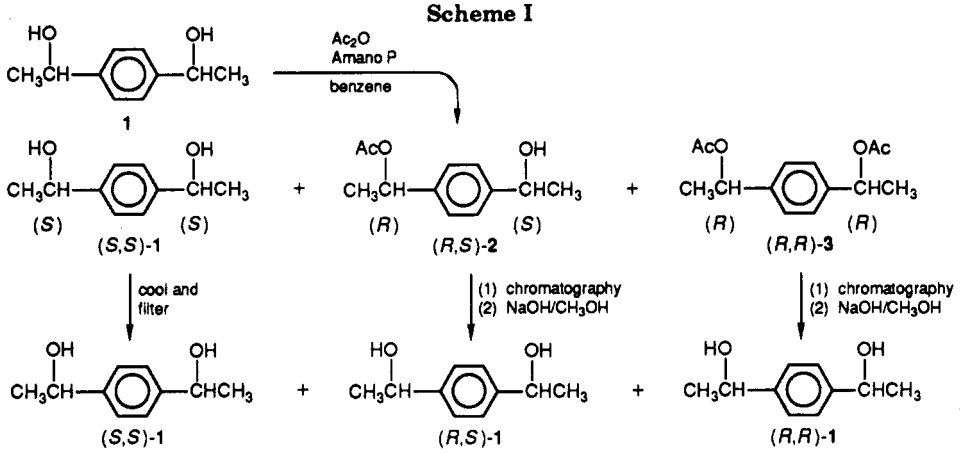
As a result of our recent activity in the synthesis of optically active $[AA-BB]_x$ polyesters,[3] the importance of finding an efficient approach to the preparation of all possible stereoisomers of symmetric, secondary diol monomers in a highly purified form became apparent. Having all three isomers allows, for example, the synthesis of an all (*R*), an all (*S*), or the "pseudo-syndiotactic" (*R*,*S*)-polymer as well as a polymer containing any combination of the above stereochemistries. Moreover, since our interest lay in the use of enzymes to effect polycondensations, preparing diols free of any meso material became particularly important. While such separations can be achieved by VPC,[4] it seems unlikely they will be useful on a preparative scale. Upon consideration of possible alternative methods for reaching this goal, we concluded that a combination of enzymatic and chemical methods should allow a synthetic mixture of symmetric diol stereoisomers to be separated most easily. The most important feature of such a separation is that it would depend only on the ability of an enzyme to distinguish the chemistry at each stereogenic center in the diol, and would be independent of any interaction between the stereogenic centers.

The specificity of hydrolase enzymes for diol stereochemistry has been exploited for some time. However, until recently, their use has been limited to modification of one stereogenic center in a meso diol (or diacylated meso diol)[5] or modification of a specific hydroxyl (or esterified hydroxyl) in a diol bearing a prochiral center.[6] Early

(1) Ito, K.; Harada, T.; Tai, A.; Izumi, Y. *Chem. Lett.* **1979**, 1049.
(2) Kitamura, M.; Ohkuma, T.; Inoue, S; Sayo, N.; Kumobayashi, H.; Akutagawa, S.; Ohta, T.; Takaya, H.; Noyori, R. *J. Am. Chem. Soc.* **1988**, *110*, 629.
(3) Wallace, J. S.; Morrow, C. J. *J. Polym. Sci. Part A: Polym. Chem.* **1989**, *27*, 2553.
(4) Koppenhofer, B.; Walser, M.; Bayer, E.; Abdalla, S. *J. Chromatog.* **1986**, *358*, 159.
(5) See, for example: Hemmerle, H.; Gais, H.-J. *Tetrahedron Lett.* **1987**, *28*, 3471.
(6) See, for example: Ramos Tombo, G. M.; Schar, H.-P.; Fernandez i Busquets, X.; Ghisalba, O. *Tetrahedron Lett.* **1986**, *27*, 5707.

0022-3263/92/1957-5231$03.00/0 © 1992 American Chemical Society

Scheme I



examples of such processes involved stereoselective hydrolysis of one of a pair of esters in aqueous media.[7-9] The recent discovery[10-14] that such enzymes also are effective catalysts in low to moderate polarity organic solvents has allowed development of esterification[15-20] and transesterification[7,15,16,18,21] as viable, though little exploited, alternatives for modifying one of a pair of stereochemically opposite hydroxyls in a diol.

Recently, Sih's group reported a kinetic analysis for the separation of a racemic mixture of (*R*,*R*)- and (*S*,*S*)-diols that is free of the (*R*,*S*)-diol, using enzyme-catalyzed acylation.[22] Application of the method to the resolution of racemic 2,4-pentanediol was also described. The analysis allows one to determine the composition of the mixture of diol substrates, monoacylated intermediates, and diacylated products present at different extents of conversion of the diol racemate to products. The relative rates of the four forward processes underway are defined in terms of ratios of the experimentally determine rate constants for each of those processes. Unfortunately, the analysis is not readily adapted to the case where the meso compound is also present, for, in the latter case, the number of diol substrates and diester products is increased from two of each to three, the number of monoacylated intermediates is increased from two to four, the number of rate constants required to define the forward reactions in the system is increased from four to eight, and the relative rates of the four possible first steps can no longer be reduced to a single ratio.

In a typical organic-phase resolution, the enzyme catalyzes reaction of an activated ester with one enantiomer of a racemic alcohol. When the reaction has reached approximately 50% completion, it is stopped by filtering out the enzyme catalyst. The products of interest are an unchanged, optically active alcohol and an optically active ester. Following separation of the unchanged alcohol enantiomer from the ester, the latter can be hydrolyzed (chemically or enzymatically) to obtain the second enantiomer of the optically active alcohol. In a valuable modification of this procedure, Bianchi, Cesti, and Battistel have shown that acid anhydrides can be used with lipases in place of the activated ester to esterify chiral alcohols stereoselectively.[20] Because this method exhibits both high reaction rates and selectivities, we decided to adapt it for the separation of diol stereoisomers.

Results and Discussion

The substrate chosen to test the separation plan was α,α'-dimethyl-1,4-benzenedimethanol [1,1'-(1,4-benzene)-diethanol], 1. This diol was interesting because its stereogenic centers are well removed from each other so the possibility of one center influencing the stereochemical environment of the other is minimized. The stereochemical mixture of these diols has been used to prepare thermally depolymerizable benzylic polycarbonates.[23] It can be easily synthesized in high yield by the sodium borohydride reduction of 1,4-diacetylbenzene.[24] Acetic anhydride was selected as the esterification agent because of its low cost and high reactivity. Crude Amano P, a lipoprotein lipase from *Pseudomonas* sp. which had been supported on Celite 577, as described by Bianchi and coworkers,[20] was used as the catalyst and anhydrous benzene was chosen as the solvent. These conditions have been shown to acetylate the (*R*) configuration of 1-phenylethanol selectively.[20]

As is summarized in Scheme I, using 1 as an example, the following general procedure has been developed. The diol mixture is dissolved or suspended in anhydrous benzene under a dry nitrogen atmosphere. The catalyst is then added, followed immediately by 2 equiv of acetic anhydride. Reaction progress is followed by VPC, and when the reaction is complete, as indicated by a dramatic reduction in the rate, it is stopped by filtering out the enzyme. (We subsequently showed that a useful modification of the Bianchi et al. method is to quench by adding

(7) Huang, F. C.; Lee, L. F. H.; Mittal, R. S. D.; Ravikumar, P. R.; Chan, J. A.; Sih, C. J.; Caspi, E.; Eck, E. R. *J. Am. Chem. Soc.* **1975**, *97*, 4144.
(8) Ohno, M.; Kobayashi, S.; Limori, T.; Wang, Y.-F.; Izawa, T. *J. Am. Chem. Soc.* **1981**, *103*, 2405.
(9) Chen, C.-S.; Fujimoto, Y.; Sih, C. J. *J. Am. Chem. Soc.* **1981**, *103*, 3580.
(10) Cambou, B.; Klibanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 2687.
(11) Zaks, A.; Klibanov, A. M. *Science* **1984**, *224*, 1249.
(12) Gatfield, I. L. *Ann. N.Y. Acad. Sci.* **1984**, 568.
(13) Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192.
(14) Klibanov, A. M. *CHEMTECH* **1986**, 354.
(15) Cambou, B.; Klibanov, A. M. *Biotechnol. Bioeng.* **1984**, *26*, 1449.
(16) Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 7072.
(17) Langrand, G.; Secchi, M.; Buono, G.; Baratti, J.; Triantaphylides, C. *Tetrahedron Lett.* **1985**, *26*, 1857.
(18) Langrand, G.; Baratti, J.; Buono, G.; Triantaphylides, C. *Tetrahedron Lett.* **1986**, *27*, 29.
(19) Gil, G.; Ferre, F.; Meou, A.; Le Petit, J.; Triantaphylides, C. *Tetrahedron Lett.* **1987**, *28*, 1647.
(20) Bianchi, D.; Cesti, P.; Battistel, E. *J. Org. Chem.* **1988**, *53*, 5531.
(21) Francalanci, F.; Cesti, P.; Cabri, W.; Bianchi, D.; Martinengo, T.; Foà, M. *J. Org. Chem.* **1987**, *52*, 5079.
(22) Guo, Z.-W.; Wu, S.-H.; Chen, C.-S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 4942.
(23) Eichler, E.; Kryczka, B.; Willson, C. G. *Makromol. Chem. Rapid Commun.* **1986**, *7*, 121.
(24) Smejkal, J.; Kopecky, J. Z. *Chem.* **1986**, *26*, 397.

Table I. Specific Rotations of Remote Diol Stereoisomers Separated by Amano P-Catalyzed Stereoselective Acetylation

	R,R		*R,S*		*S,S*	
diol	yield[a] (%)	specific rotation[b] (deg)	yield[a] (%)	specific rotation[b] (deg)	yield[a] (%)	specific rotation[b] (deg)
1	22.4	+80.5	35.3	−0.35	24.1	−79.9
4	16.4	+65.9	41.5[c]	+0.77	16.3	−63.6
5	14.9	+44.01	46.5	+10.21[d]	14.1	−42.30
6	25.0	+76.95	32.5	+1.45	22.5	−76.05[e]

[a] The yield of each component is calculated relative to the total amount of the diol mixture used in the reaction. A statistical mixture would contain 25% (*R,R*), 25% (*S,S*), and 50% (*R,S*) isomer. [b] $[\alpha]^{amb}_{D}$ (c = 2, acetone). [c] After recycling through the esterification to remove an 11.6% impurity of (*R,R*)-diol. [d] Not recycled to remove the (*R,R*)-diol impurity. [e] After recycling to remove an impurity of the (*R,S*)-stereoisomer.

excess methanol to the reaction mixture and stirring for ~0.5 h before filtering off the enzyme. This destroys the excess acetic anhydride which markedly simplifies the remainder of the work up.) Based on the known selectivity of Amano P lipase for catalyzing esterification of secondary alcohol stereogenic centers having the (*R*)-configuration, it can be concluded that the product mixture includes three components: unreacted diol **1**, having the (*S,S*)-configuration, monoacylated diol **2**, having the (*R,S*)-configuration, and diacylated diol **3**, having the (*R,R*)-configuration. The mixture is separated by standard techniques such as chromatography. In the case of α,α'-dimethyl-1,4-benzenedimethanol, and several of the other diols described here, the (*S,S*)-diol **1** crystallizes from the mixture upon cooling, and only the (*R,S*)-monoacetate **2**, and (*R,R*)-diacetate **3** need be separated by chromatography. Following separation, the acylated compounds are hydrolyzed to regenerate the (*R,S*)- and (*R,R*)-diols. The results from separating stereoisomers mixtures of **1**, **4**, **5**, and **6**, using variations of this general method are summarized in Table I.

No valid direct measure of the optical purities of the (*R,R*)- and (*S,S*)-**1** has been reported. The compounds prepared by the method described here have significantly higher values for $[\alpha^{25}]_D$ of −79.9° and +80.5° (c = 2, acetone) or +74.8 (c = 2, chloroform) than the value of +60° (c not reported, chloroform) previously reported for the (*R,R*)-diol formed by enzymatic oxidation of *p*-diethylbenzene.[25] An enantiomeric excess of >97% was claimed for that product on the basis of its behavior in the presence of a chiral NMR shift reagent. Our results, therefore, suggest that this method is of questionable validity for determining the stereochemical purity of these diols.

From the rotation data alone, it seemed reasonable that the two enantiomers prepared in the present study were optically pure, for the two rotations differed by only 0.75% in magnitude (the (*S,S*)-**1** was known to contain minor amounts of impurities) while the rotation of the meso compound (*R,S*)-**1** was essentially zero. Since the separation scheme requires that the enzyme select the (*R,R*)-stereoisomer twice and the (*R,S*)-stereoisomer once but that it always rejects the (*S,S*)-stereoisomer, it seemed unlikely that partially separated stereoisomers having this set of specific rotations would occur fortuitously. That this assumption is not always valid will be demonstrated below.

To verify the stereochemical purities of the diols, we examined the use of α-methoxy-α-trifluoromethylphenylacetyl (MTPA or "Mosher's") esters prepared by reaction of the diols with (+)-MTPA chloride.[26] In contrast with the usual applications of these products, in which only the relative amounts of two enantiomers is sought,[27] this work required distinguishing the relative amounts of three stereoisomers, each of which has two stereogenic centers. Thus, when stereochemically enriched (*R,R*)-**1** is examined, it is not enough to know the concentration of (*S*)-stereogenic centers present. It is also important to determine whether those centers reside in an (*R,S*)- or an (*S,S*)-stereoisomer impurity. To our knowledge, NMR analysis of Mosher's esters has not been used in this way prior to this work.

Table II. Stereochemically Sensitive NMR Regions of the Bis-MTPA Esters from the Stereoisomers of 1

		intensities at chemical shifts (δ)					
		methoxy[a]		*C*-methyl[a]			
		S	*R*	*Sr*[b]	*Ss*[b]	*Rr*[b]	*Rs*[b]
diol	δ	3.55	3.46	1.63	1.62	1.58	1.57
(*R,R*)-**1**		<3	>97	<3	<3	>97	<3
(*R,S*)-**1**		50	50	48	<3	<3	52
(*S,S*)-**1**		>97	<3	<3	>97	<3	<3

[a] The entries show the relative intensities of the absorption at each chemical shift indicated. It is assumed that an absorption that is at least 3% of the intensity of the strongest absorption in the group will be observable. [b] The upper case letter shows the stereochemistry at the stereogenic center bearing the methyl group that gives rise to the observed absorption. The lower case letter shows the stereochemistry at the second stereogenic center in the molecule from which the bis MTPA ester was formed.

It was found that complete conversion of all stereogenic centers to the MTPA ester could only be achieved using the modified procedure described subsequently to the first report of these compounds.[28] In a set of preliminary experiments, the bis-MTPA esters were prepared from the separated stereoisomers of diols **1**, from mixtures of pairs of these same diol stereoisomers, and from the (*R,S*)-diol and racemic MTPA chloride. Through these studies, it was shown that the absorptions in the proton NMR spectra arising from the methoxy group in the acid moieties of the bis-MTPA ester provide an estimate of the total number of (*R*)- and (*S*)-stereogenic centers present. For each diol, the methoxy absorption of an MTPA group on an (*S*)-stereogenic center was found to be from 0.04 to 0.1 ppm downfield from the methoxy absorption of an MTPA group on an (*R*)-center. For example, as seen in Table II, the methoxy absorption from the MTPA ester formed at an (*R*)-stereogenic center in a stereoisomer of **1** appears at 3.46 ppm while the absorption from the MTPA ester formed at an (*S*)-center appears at 3.55 ppm. However, the methoxy absorption does not distinguish between an (*R*)-center in the (*R,S*) (i.e., meso) compound and an (*R*)-center in the (*R,R*)-enantiomer. Neither does it distinguish an (*S*)-center in the (*R,S*)-isomer from an (*S*)-center in the (*S,S*)-stereoisomer. Thus, although a single methoxy absorption shows a diol to be a single, pure enantiomer, two methoxy absorptions of the same size could

(25) Holland, H. L.; Bergen, E. J.; Chenchaiah, C.; Khan, S. H.; Munoz, B.; Ninniss, R. W.; Richards, D. *Can. J. Chem.* **1987**, *65*, 502.
(26) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
(27) Sutowardoyo, K. I.; Sinou, D. *Tetrahedron: Asymmetry* **1991**, *2*, 437.
(28) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.

Table III. Stereochemically Sensitive NMR Regions of the Bis-MTPA Esters from the Stereoisomers of 4

		intensities at chemical shifts (δ)							
		methoxy[a]		C-methyl[a]				trifluoromethyl[b]	
		S	R	Sr[c]	Ss[c]	Rr[c]	Rs[c]	R	S
diol	δ	3.54	3.45	1.62	1.56	1.55	1.52	−72.05	−71.91
(R,R)-4		8	92	6.5	3	84	6.5	8	92
(R,S)-4		48	52	45	3	7	45	53	47
(S,S)-4		88	12	9	73	9	9	86	14

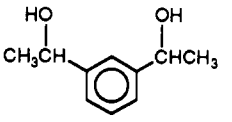
[a] The entries show the relative intensities of the absorption at each chemical shift indicated in the proton NMR spectrum of each bis-MTPA ester. [b] The entries show the relative intensities of the absorption at each chemical shift (relative to $CFCl_3$) indicated in the ^{19}F NMR spectrum of each bis-MTPA ester. [c] The upper case letter shows the stereochemistry at the stereogenic center bearing the methyl group that gives rise to the observed absorption. The lower case letter shows the stereochemistry at the second stereogenic center in the molecule from which the bis-MTPA ester was formed.

be interpreted as a racemic mixture of the two enantiomers, pure meso compound, or any mixture of the racemate and the meso diastereomer. Similarly, two unequal methoxy absorptions may be interpreted as a partially racemic mixture of the two enantiomers, a pure enantiomer contaminated with the meso diastereomer, or a combination of these two. As seen in Tables II–V and VII, this behavior of the methoxy group absorptions was consistant throughout the group of diols and the triol studied.

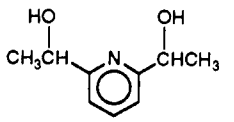
In contrast, for most of the diol stereoisomers studied, the absorption assigned to the C-methyl hydrogens of one stereogenic center in the *bis*-MTPA ester appeared at a unique chemical shift that was determined by whether the second stereogenic center in the diol moiety was of the (R)- or the (S)-configuration. Thus, the methyl doublet absorption for an (R)-center in the (R,R)-enantiomer appears at a different chemical shift than does the methyl doublet for an (R)-center in its (R,S)-diastereomer. Separate sets of doublets also appear that are due to methyl hydrogens at an (S)-stereogenic center when the second center is (R) or (S). Using the methoxy and the methyl hydrogen absorptions, it was shown that the (R,R), (R,S), and (S,S)-1 separated from the stereoisomer mixture were stereochemically pure to the detection limit of the method (≤3%). These results are summarized in Table II.

When the stereoisomers of the meta diol α,α′-dimethyl-1,3-benzenedimethanol, 4, were separated using the procedure described here, the acylation was significantly slower, apparently because of steric interference with the diol's approach to the enzyme or because the enzyme is inhibited by the unreactive stereoisomers. When the reaction was stopped after 22.5 h, not all (R)-stereogenic centers had reacted. Rather, it appeared that acetylation of the hydroxyl at the (R)-center in the (R,S)-stereoisomer and at one of the hydroxyls in the (R,R)-stereoisomer occured rapidly, but the second center of the (R,R)-stereoisomer reacted more slowly. As a result, the (R,S)-diol monoacetate fraction was contaminated with (R,R)-diol monoacetate, and hydrolysis of the monoacetate fraction, therefore, led to diol having $[\alpha]^{25}_D$ + 7.68° rather than the 0.00° expected for a meso compound. On the basis of the specific rotations shown for diols 4 in Table I, both the unchanged (S,S)-diol and the (R,R)-diol, from hydrolysis of the corresponding diacetate, appeared to be stereochemically pure. However, when these diols were converted to the corresponding bis-MTPA esters, it was found from the methoxy absorptions that each stereoisomer sample included both (R)- and (S)-stereogenic centers. As shown in Table III, this fact could be confirmed using the ^{19}F NMR absorptions provided by the CF_3 group in the MTPA moieties. However, neither the methoxy nor the trifluoromethyl absorptions provided information about the second stereogenic center in each stereoisomer. Once again, the C-methyl region of the proton spectra allowed the actual content of each fraction of the separated mixture to be determined. The (R,R)-fraction was found to have an enantiomeric excess of 93% but to be contaminated with about 13% of the meso diastereomer. Following a single recycle through the separation procedure, the meso compound was shown to be contaminated with 7% of the (R,R)-enantiomer and 3% of the (S,S)-enantiomer, accounting for its slight positive optical rotation. The (S,S)-fraction displayed an enatiomeric excess of 78% and was contaminated with 18% of the meso diastereomer as well. Interestingly, it was found during the recycle process for the (R,S)-fraction that, the reaction proceeded more rapidly when the substrate no longer incorporated a significant component of the (S,S)-diol.

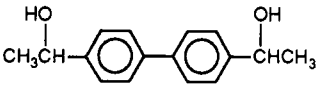
It is noteworthy that, following the methanolic sodium hydroxide hydrolysis of the mono- or diacetate of a diol such as 4 (or 5 or 7 below), in which the hydroxylated side



4



5



6

chains are arranged meta to each other, simply evaporating the methanol led to a mixture of the expected diol and its mono and diacetates. The reason for this unusual reesterification under alkaline conditions in these cases is not clear. Careful experiments ruled out incomplete hydrolysis as the source of the acetates. Other experiments indicated that acetate ion, not methyl acetate, is the source of the acetyl groups. The esterification was suppressed by neutralizing the alkali with methanolic HCl before beginning the evaporation.

Complete acylation of the (R) stereogenic centers was even slower when α,α′-dimethyl-2,6-pyridinedimethanol 5, was used as the substrate than when 4 was used, possibly because of the added effect of hydrogen bonding between unesterified alcohols and the pyridine nitrogen. To develop evidence in support of this hypothesis, the ability of 5 to catalyze its own acylation was explored by preparing a reaction mixture identical with that treated with the enzyme, but with the enzyme omitted. Under these conditions the anhydride was consumed at a rate of 0.4% per hour and gave only the monoacylated product. In the presence of the lipase catalyst, it appears that formation of monoacetate from the (R,R)- and (R,S)-enantiomers is rapid, but the second (R)-center in the (R,R)-stereoisomer reacts much more slowly and formation of the (R,R)-diacetate is incomplete even after 2 days. The monoacetylated (R,R)-diol is found in the fraction containing the monoacetylated meso diastereomer and is the source of the $[\alpha]^{25}_D$ +10° observed for that diol following its

Table IV. Stereochemically Sensitive NMR Regions of the Bis-MTPA Esters from the Stereoisomers of 5

		intensities at chemical shifts (δ)					
		methoxy[a]		C-methyl[a]			
		S	R	Sr[b]	Ss[b]	Rr[b]	Rs[b]
diol	δ	3.58	3.54	1.66	1.64	1.62	1.58
(R,R)-5		3	97	4	<3	92	4
(R,S)-5		50	50	48	<3	4	48
(S,S)-5		>97	<3	<3	>97	<3	<3
(R,R)-5[c]		25	75	18	12	52	18

[a] The entries show the relative intensities of the absorption at each chemical shift indicated. It is assumed that an absorption that is at least 3% of the intensity of the strongest absorption in the group will be observable. [b] The upper case letter shows the stereochemistry at the stereogenic center bearing the methyl group that gives rise to the observed absorption. The lower case letter shows the stereochemistry at the second stereogenic center in the molecule from which the bis-MTPA ester was formed. [c] This entry is for (R,R)-5 prepared using the acetylation method. The resulting stereoisomers gave the rotations provided in Table I.

Table V. Stereochemically Sensitive NMR Regions of the Bis-MTPA Esters from the Stereoisomers of 6

		intensities at chemical shifts (δ)			
		methoxy[a]		C-methyl[a]	
		S	R	S	R
diol	δ	3.57	3.49	1.67	1.61
(R,R)-6		4	96	6	94
(R,S)-6		49	51	48	52
(S,S)-6		94	6	92	8

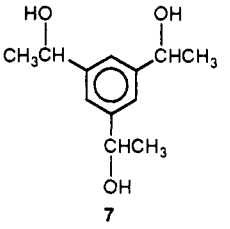
[a] The entries show the relative intensities of the absorption at each chemical shift indicated.

isolation and hydrolysis. Removing this impurity by recycling the fraction through the acylation has not been attempted.

The rotations of +44.0° and −42.3° observed for the (R,R)- and (S,S)-stereoisomers, respectively, shown in Table I, suggest they approach being optically pure. As shown in Table IV, however, upon preparation of the bis-MTPA ester from (R,R)-5, it was found to contain about 18% of the meso diol and about 12% of the (S,S)-enantiomer. Because of the difficulty in acylating the diol enzymatically, the possibility of effecting the separation by hydrolysis was explored. Using a standard procedure, the stereoisomer mixture of 5 was converted to a mixture of the corresponding diacetates, and the diacetate mixture was then subjected to Amano P catalyzed hydrolysis. Because the (R)-stereogenic centers were preferentially hydrolyzed, the resulting mixture contained (R,R)-5 and the diacetate of (S,S)-5 as well as the (S) monoacetate of meso-5. Separation of this mixture as before and hydrolysis of the esters provided the expected diols. Formation of the bis-MTPA esters of the (R,R)-5 and the (S,S)-5 showed each to be free of its enantiomer. However, the (R,R)-5 fraction contained about 4% of *meso*-5 while the *meso*-5 fraction contained about 4% of (R,R)-5.

A final diol α,α′-dimethyl-4,4′-biphenylenedimethanol, 6, was chosen to separate further the two stereogenic centers and ensure that the enzyme acylates each center based only on its own configuration and not the configuration or acylation state of the other stereogenic center. Unfortunately, this goal was impeded by the low solubility of the substrate. After 8 h, the reaction stopped despite the fact that the acetylation would be expected to maintain an equilibrium concentration of the (R,R)- and (R,S)-diols to be acetylated. Apparently, one (or both) of the acetylated products is an effective inhibitor of the lipase. Filtering off the catalyst and undissolved diol followed by evaporation of the benzene provided the expected mixture of 6 and its mono and diacetates. Separation of the mixture as described before gave diol monoacetate and diacetate fractions. However, the (S,S)-diol fraction was contaminated with (R,R)- and (R,S)-diol, and the total weight of material was about one-third of that expected. The solid, consisting of enzyme and undissolved diol, was subjected to the reaction conditions twice more, after which time all diol containing (R)-centers has been consumed. Filtering off the enzyme and cooling the benzene provided (S,S)-6 having $[\alpha]^{25}_D$ −76.1° which compares favorably with $[\alpha]^{25}_D$ +77.0° found for the (R,R)-stereoisomer after hydrolysis of the diacetate. As shown in Table V, when the bis-MTPA esters of these stereoisomer fractions were prepared, it was found that the C-methyl region no longer provided information allowing different diastereomers to be distinguished. However, the (R,R)-6 fraction was shown to incorporate no more than 4% of (S)-stereogenic centers from either (R,S)-6 or (S,S)-6. Similarly, the (S,S)-6 fraction was shown to contain no more than 6% of (R)-centers from (R,S)-6 or (R,R)-6. The (R,S)-6 fraction was found to contain slightly more (R)-centers than (S)-centers.

Separation of the stereoisomers of a triol, α,α′,α″-trimethyl-1,3,5-benzenetrimethanol, 7, was also explored. However, because of the low solubility of this triol, it was concluded that nonenzymatic esterification would become a significant, detrimental side reaction over the long reaction time the process was expected to require. Consequently, it was decided to convert the triol to its triacetate by chemical means and then to attempt a stereoselective hydrolysis with the enzyme.



7

The triacetate was prepared by standard methods then suspended in a phosphate buffer at pH 7.8 to which was added the unsupported Amano P. The pH of the mixture was monitored and restored to 7.8 ± 0.5 with daily additions of alkali. After 28 days, the theoretical amount of base had been consumed and the reaction ceased. Separation of the four products and hydrolysis of the acetates as detailed in the Experimental Section provided the four triols. The optical rotations of the acetylated materials

Table VI. Specific Rotations of the Enzymatic Hydrolysis Products and Fully Hydrolyzed Triol Products in the Separation of the Stereoisomers of α,α′,α″-Trimethyl-1,3,5-benzenetrimethanol, 7

stereochem desig	no. of acetate grps in enzymatic hydrolysis product	$[\alpha]^{25}_D$ (deg) of enzymatic hydrolysis product	$[\alpha]^{21}_D$ (deg) of the fully hydrolyzed triol
R,R,R	0	+95.50	+95.50
S,S,S	3	−173.25	−94.10
R,S,S	2	−114.75	−32.10
R,R,S	1	+14.50	+32.31

Table VII. Stereochemically Sensitive NMR Regions of the Bis-MTPA Esters from the Stereoisomers of 7

		intensities at chemical shifts (δ)							
		methoxy[a]		C-methyl[a,b]					
		S	R						
triol	δ	3.53	3.43	1.60	1.53	1.52	1.49	1.48	1.45
(S,R,R)-7		67	33	<3	66	<3	<3	<3	34
(S,S,R)-7		29	71	27	3	16	<3	54	<3
(S,S,S)-7		>97	<3	<3	<3	<3	>97	<3	<3

[a] The entries show the relative intensities of the absorption at each chemical shift indicated. [b] It has not yet been possible to assign absorptions at specific chemical shifts in the C-methyl region of the spectra to specific stereochemical arrangements.

as well as the triols are shown in Table VI. As summarized in Table VII, conversion of the (S,S,S)-fraction to the corresponding tris MTPA esters showed it to contain no detectable (R)-stereogenic centers as evidenced by single absorptions for the methoxy and the C-methyl protons. It can be concluded that the (R,R,R)-stereoisomer is stereochemically pure as well, on the basis of its specific rotation, compared with that of the (S,S,S)-stereoisomer, as shown in Table VI. Determining the stereochemical purity of the (R,R,S)- and (S,S,R)-enantiomer pair using MTPA esters is more difficult for the two identical groups; i.e., the two S groups in the (S,S,R)-stereoisomer are in diastereotopic environments so need not give a single set of absorptions when the (R)-MTPA group is added to each. That the (R,R,S)-stereoisomer is stereochemically pure is supported by both the methoxy protons and the C-methyl protons appearing as pairs of absorptions in the NMR spectrum of the corresponding MTPA ester, each in a 2:1 ratio of intensities. Although comparison of the specific rotation for the (S,S,R)-enantiomer with that of the (R,R,S)-enantiomer supports it too long being stereochemically pure, this cannot be confirmed by the multiple absorptions in the NMR spectrum of its MTPA ester.

From the results described here, it is apparent that separation of diol and triol stereoisomer mixtures by enzymatic modification of all stereogenic centers in the mixture having the same configuration will provide a valuable source of these compounds, particularly when two or more of the stereoisomers are required. The apparently high selectivity of the enzyme for a single configuration suggests that another, less obvious, application for the methodology is in determining the effectiveness of homochiral syntheses of diols and triols. Such an analysis would require treating the product mixture from the reaction under study with acetic anhydride and the enzyme and then determining the relative amounts of unacetylated, monoacetylated, and diacetylated products. Initially, separating the products and actually determining their stereochemical purity would be desirable, but subsequent analyses of other mixtures of the same diol could be carried out directly on the mixture of products from the acetylation using VPC or HPLC. We have also demonstrated the usefulness of bis-MTPA esters for determining the stereochemical purity of the separated diol stereoisomers.

Experimental Section

General. Reaction mixtures were stirred magnetically under a dry nitrogen atmosphere unless otherwise indicated. Chemicals used were the best available commercial grade and were used without purification unless otherwise indicated. The "anhydrous" benzene used had a water content of <0.05%. Organic extracts were dried with Na_2SO_4 unless otherwise indicated. Melting points are uncorrected. NMR spectra were recorded at 250 MHz. VPC analyses were carried out on a 5-m fused silica capillary column having a bonded liquid phase of cross-linked methyl silicone. Helium was used as the carrier gas at a flow rate of 30 mL/min. The oven temperature was programmed to remain at 50 °C for 2 min and then rise to 250 °C at 16 °C/min and remain there for 10 min. TLC analyses were carried out on silica gel-coated glass plates and were visualized by fluorescence under UV excitation. The elution solvent was a 4:1–1:1 mixture of hexane and ethyl acetate for acetylated materials and pure ethyl acetate for the diols. Microanalysis were carried out by Mrs. Ruby Ju of the University of New Mexico Microanalytical Laboratory.

Preparation of the Stereoisomer Mixture of α,α′-Dimethyl-1,4-benzenedimethanol, (S,S)-, (R,S)-, and (R,R)-1.[24] A 250-mL flask was charged with 10.0 g (61.7 mmol) of 1,4-diacetylbenzene (Aldrich) and 120 mL of anhydrous isopropyl alcohol. To the stirred solution was added 2.3 g (60.8 mmol) of sodium borohydride in three equal portions. After 2 h, TLC analysis showed the starting material had been consumed, and a single new spot remained. The reaction was quenched by addition of dilute HCl with stirring until the pH dropped to 6.5. The salts that precipitated were filtered off then the solvent was evaporated and the residue extracted with 3 × 100 mL of ethyl acetate. The combined organic extracts were washed with 2 × 100 mL of water and dried and the solvent evaporated to yield 9.1 g (89%) of the diol mixture: mp 82–83 °C (lit. mp 80–81 °C);[29] IR (KBr) 3350, 2950, 1515, 1440, 1360, 1295, 1210, 1080, 1005, 900, 830 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (s, 4 H), 4.87 (q, 2 H), 2.27 (s, 2 H), 1.51 (d, 6 H). Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.40; H, 8.47.

Separation of the Stereoisomers of α,α′-Dimethyl-1,4-benzenedimethanol. A 500-mL flask was charged with 6.98 g (42.0 mmol) of the stereoisomer mixture of 1 and 250 mL of anhydrous benzene. The mixture was heated to obtain a clear solution and then cooled to ambient temperature, and 4.4 g of supported Amano P lipoprotein lipase (AMANO Int'l Enzyme Co., Troy, VA), which had been prepared as described elsewhere,[20] was added with stirring followed by addition of 8.57 g (84.0 mmol) of acetic anhydride. After 6 h a marked change in the rate of disappearance of 1 and appearance of products was observed by VPC, and after 7 h the reaction was quenched by filtering off the catalyst.

Isolation of (S,S)-α,α′-Dimethyl-1,4-benzenedimethanol, (S,S)-1. Approximately half of the solvent was evaporated and the concentrated mixture stored at 0 °C overnight. The frozen benzene was allowed to thaw, and the colorless crystals of (S,S)-1 which remained were filtered off (1.68 g, 24.1% of the starting material); mp 128–130 °C after recrystallization from benzene. The ^1H and ^{13}C NMR spectra were identical with those described above for the stereoisomer mixture: $[\alpha]^{25}_D$ −79.9 °C (c = 2, acetone). Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.12; H, 8.54.

Isolation of (R,S)-α,α′-Dimethyl-1,4-benzenedimethanol Monoacetate, (R,S)-2. The filtrate from isolation of (S,S)-1 was washed with 2 × 50 mL of aqueous K_2CO_3 and with 50 mL of water then dried and the solvent evaporated at ambient temperature. The residual yellow oil was purified by flash chromatography on a 15-cm bed of Merck 60/60 Angstrom silica gel eluting with 1:1 hexane/ethyl acetate to give two fractions. The second fraction, having a lower R_f on TLC, and comprising 3.54 g of a colorless oil, was shown to be (R,S)-2 and accounted for 40.1% of the starting diol mixture: $[\alpha]^{25}_D$ ±59.3° (c = 4, acetone); ^1H NMR ($CDCl_3$) δ 7.35 (s, 4 H), 5.84 (q, J = 6.6 Hz, 1 H), 4.83 (q, J = 6.5 Hz, 1 H), 2.68 (br s, 1 H), 2.03 (s, 3 H), 1.51 (d, J = 6.6 Hz, 3 H), 1.45 (d, J = 6.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 170.3, 145.4, 140.5, 126.0, 125.4, 72.0, 69.7, 24.9, 21.9, 21.1. Anal. Calcd for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 69.42; H, 7.51.

Isolation of (R,R)-α,α′-Dimethyl-1,4-benzenedimethanol Diacetate (R,R)-3. The first fraction, comprising 2.56 g of a colorless oil, was shown to be (R,R)-3 and accounted for 24.4% of the starting diol mixture: $[\alpha]^{25}_D$ +152.9° (c = 2, acetone); ^1H NMR ($CDCl_3$) δ 7.33 (s, 4 H), 5.87 (q, J = 6.6 Hz, 2 H), 2.06 (s, 6 H), 1.52 (d, J = 6.6 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ_c 170.1, 141.1, 126.1, 71.8, 21.9, 21.1. Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.48; H, 7.32.

(29) Mowry, D. T.; Renoll, M.; Huber, W. F. *J. Am. Chem. Soc.* **1946**, *68*, 1105.

(*R*,*S*)-α,α′-Dimethyl-1,4-benzenedimethanol, (*R*,*S*)-1. To a solution of 0.396 g (9.90 mmol) of NaOH and 50 mL of commercial absolute methanol was added 2.00 g of the monoacetate (*R*,*S*)-**2**, in 25 mL of methanol. The mixture was stirred for 2.5 h after which time VPC analysis showed the hydrolysis to be complete. The solvent was evaporated, 100 mL of water added to the residue, and the mixture stirred rapidly. The crystalline precipitate which formed was filtered off and dried in vacuo at ambient temperature for 12 h. It was then recrystallized from hot benzene to give 1.41 g (88%) of (*R*,*S*)-**1** having mp 116–118 °C (lit.[29] mp 90–91 or 114–115 °C); $[\alpha]^{25}_D$ −0.35° (*c* = 2, acetone). The ^1H and ^{13}C NMR spectra were identical with those described above for the stereoisomer mixture of **1**. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.55; H, 8.66.

(*R*,*R*)-α,α′-Dimethyl-1,4-benzenedimethanol, (*R*,*R*)-1. In a manner analogous to that described for the monoacetate, 1.50 g (6.0 mmol) of the diacetate, (*R*,*R*)-**3**, was treated with 0.48 g (12.0 mmol) of NaOH. To provide 0.91 g (92%) of (*R*,*R*)-**1** as colorless needles, mp 130–131.5 °C (lit.[25] mp 92–94 °C, lit.[29] mp 90–91 °C, lit.[26] mp 114–115 °C); $[\alpha]^{25}_D$ +80.5° (*c* = 2, acetone), +74.8° (*c* = 2, ethanol) (lit.[25] $[\alpha]^{25}_D$ +60° (*c* not given, ethanol or chloroform)). The ^1H and ^{13}C NMR were identical with those described above for the stereoisomer mixture of **1**. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.13; H, 8.72.

Preparation of the Stereoisomer Mixture of α,α′-Dimethyl-1,3-benzenedimethanol, (*S*,*S*)-, (*R*,*S*)-, and (*R*,*R*)-4. This mixture of stereoisomers was prepared from 1,3-diacetylbenzene by reduction with sodium borohydride as described above for the para stereoisomers. Cooling of the reaction mixture with an ice bath was required to moderate the exothermic reduction. The mixture of stereoisomers was isolated as a semisolid. It appeared as a single spot on TLC and as a single peak on VPC: ^1H NMR ($CDCl_3$) δ 7.2–7.4 (m, 4 H), 4.85 (q, 2 H), 2.95 (br s, 2 H), 1.46 (d, 6 H); ^{13}C NMR ($CDCl_3$) δ_c 146.0, 128.4, 124.8, 123.0, 69.9, 25.0. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: *C*, 72.19; H, 8.70.

Separation of the Stereoisomers of α,α′-Dimethyl-1,3-benzenedimethanol. A benzene solution containing 10.1 g of the mixture of stereoisomeric diols **4** was treated with Amano P and acetic anhydride as described above for the para diols, **1**. The reaction was allowed to continue for 22.5 h, and then the excess acetic anhydride was destroyed by addition of 3 mL of methanol. TLC analysis showed the acetic anhydride to have been consumed after 0.25 h, and, after stirring for 0.5 h, the enzyme was filtered off and the products were separated as before.

Isolation of (*S*,*S*)-α,α′-Dimethyl-1,3-benzenedimethanol, (*S*,*S*)-4. After being cooled overnight, the insoluble (*S*,*S*)-diol, 1.65 g, corresponding to 16.3% of the starting mixture of diols, was filtered off. It displayed $[\alpha]^{25}_D$ −63.6° (*c* = 2, acetone). The ^1H and ^{13}C NMR spectra were identical with those described above for the stereoisomer mixture. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.20; H, 8.61.

Isolation of (*R*,*S*)-α,α′-Dimethyl-1,3-benzenedimethanol Monoacetate. The monoacetate of (*R*,*S*)-**4**, 6.65 g, corresponding to 52.5% of the starting mixture of diol stereoisomers, was isolated by chromatography as described above for the para isomer. It displayed: $[\alpha]^{25}_D$ +53.1° (*c* = 2.2, acetone); ^1H NMR ($CDCl_3$) 7.2–7.4 (m, 4 H), 5.85 (q, *J* = 6.6 Hz, 1 H), 4.85 (q, *J* = 6.5 Hz, 1 H), 2.55 (br s, 1 H), 2.04 (s, 3 H), 1.52 (d, *J* = 6.6 Hz, 3 H), 1.47 (d, *J* = 6.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ_c 170.3, 146.0, 141.6, 128.4, 124.9, 124.8, 123.0, 72.2, 69.9, 25.0, 22.0, 21.1. Anal. Calcd for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 69.23; H, 7.74.

Isolation of (*R*,*R*)-α,α′-Dimethyl-1,3-benzenedimethanol Diacetate. The diacetate of (*R*,*R*)-**4**, 2.77 g, corresponding to 18.2% of the starting mixture of diol stereoisomers, was isolated by chromatography as described above for the para isomer. It displayed: $[\alpha]^{25}_D$ +111.3° (*c* = 2, acetone); ^1H NMR ($CDCl_3$) δ 7.2–7.4 (m 4 H), 5.85 (q, *J* = 6.6 Hz, 2 H), 2.04 (s, 6 H), 1.52 (d, *J* = 6.6 Hz, 6 H); ^{13}C NMR ($CDCl_3$) δ_c 170.3, 141.6, 128.4, 124.9, 123.0, 72.2, 22.0, 21.1. Anal. Calcd for $C_{14}H_{18}O_4$: C, 67.18; H, 7.25. Found: C, 67.48; H, 7.32.

(*R*,*S*)-α,α′-Dimethyl-1,3-benzenedimethanol, (*R*,*S*)-4. The monoacetate of (*R*,*S*)-**4** was hydrolyzed by treatment with sodium hydroxide in absolute methanol as described for the corresponding para isomer above. To prevent reacetylation of the alcohols, it was necessary to neutralize the reaction mixture to pH 6.5 with methanolic HCl before evaporating the methanol. The diol displayed $[\alpha]^{25}_D$ +7.68° (*c* = 2, acetone). The ^1H NMR spectrum was identical with that of the mixture of diol stereoisomers.

Purification of the (*R*,*S*)-α,α′-Dimethyl-1,3-benzenedimethanol by Recycling. A 4.32-g sample of the impure (*R*,*S*)-α,α′-dimethyl-1,3-benzenedimethanol isolated above was again submitted to the Amano P lipase catalyzed acetylation by acetic anhydride as described previously. In the absence of any (*S*,*S*)-diol, the reaction was complete after 11 h. VPC analysis showed the presence of 11.6% of diacetate along with the monoacetate. Separation of the mixture by chromatography gave 2.61 g of the monoacetate of (R,S)-**2** corresponding to a recovery of 2.08 g of the diol (*R*,*S*)-**4**. The monoacetate displayed $[\alpha]^{20}_D$ +57.6° (*c* = 2, acetone). A second fraction contained 0.81 g of the diacetate of (*R*,*R*)-**4** (corresponding to 0.54 g of the (*R*,*R*)-diol) which displayed $[\alpha]^{20}_D$ +110.8° (*c* = 2; acetone). Hydrolysis of the monoacetate as described previously gave meso diol, (*R*,*S*)-**2**, having $[\alpha]^{20}_D$ +0.77° (*c* = 2, acetone), indicating an impurity of 1.1% of (*R*,*R*)-**4**. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 71.97; H, 8.56.

(*R*,*R*)-α,α′-Dimethyl-1,3-benzenedimethanol, (*R*,*R*)-4. The diacetate of (*R*,*R*)-**4**, 0.42 g (1.68 mmol), was hydrolyzed with sodium hydroxide, 0.132 g (3.3 mmol), in anhydrous methanol (50 mL) as described for (*R*,*R*)-**3** above. To prevent reacetylation of the hydrolyzed diol it was necessary to adjust the mixture to pH 6.5 with methanolic HCl before evaporating the methanol. Following extraction of the diol into methylene chloride and evaporation of the solvent, 0.25 g (90%) of (*R*,*R*)-**4** was recovered. It displayed $[\alpha]^{25}_D$ +65.9° (*c* = 2, acetone). The ^1H and ^{13}C NMR spectra were identical with those of the mixture of diol stereoisomers. Anal. Calcd for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 71.96%; H, 8.52.

Preparation of the Stereoisomer Mixture of α,α′-Dimethyl-2,6-pyridinedimethanol, (*S*,*S*)-, (*R*,*S*)-, and (*R*,*R*)-5. A 250-mL flask was charged with 5.0 g (30.6 mmol) of 2,6-diacetylpyridine and 60 mL of anhydrous isopropyl alcohol. To the stirred solution was added 1.16 g (3.06 mmol) of sodium borohydride in three equal portions. The initially colorless solution became yellow and warmed and then slowly returned to pale yellow. After 1 h, VPC analysis showed all of the starting material to have been consumed, and after an additional hour, the excess borohydride was destroyed by adjusting the pH to 6.5 with 5% aqueous HCl, about 50 mL being required. After the solution was stirred for a short time, the pH was readjusted to 8 with dilute aqueous NaOH solution. The precipitate which formed was filtered off and the isopropyl alcohol and some of the water evaporated until a volume of about 40 mL was reached. The concentrated aqueous mixture was extracted with 3 × 100 mL of ethyl acetate. The combined organic phases were dried, and the solvent was evaporated to give 4.20 g (82%) of a nearly colorless oil which crystallized upon standing at 0 °C: mp 49–58 °C; ^1H NMR ($CDCl_3$) δ 7.71 (t, 1 H), 7.21 (d, 2 H), 4.84–4.98 (m, 2 H), 3.91 (s, 2 H), 1.52 (d, 6 H). Anal. Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.84. Found: C, 64.95; H, 7.60.

Separation of the Stereoisomers of α,α′-Dimethyl-2,6-pyridinedimethanol. A 500-mL flask was charged with 3.93 g (23.5 mmol) of the stereoisomer mixture of **5** and 150 mL of dry benzene. To the clear, stirred solution was added 2.5 g of Amano P lipase supported on Celite 577 followed by 4.79 g (47.0 mmol) of acetic anhydride. The reaction was stopped after 45 h by addition of 2 mL of methanol, despite the fact that VPC analysis showed that less than the expected amount of diacetate had been formed. The catalyst was removed by filtration and the filtrate concentrated to yield 4.53 g of a light yellow oil which was separated by flash chromatography on a 15-cm bed of Merck 60/60 Angstrom silica gel eluted with 1:1 hexane/ethyl acetate.

Isolation of (*R*,*R*)-α,α′-Dimethyl-1,3-pyridinedimethanol Diacetate. The first fraction comprising 1.034 g of a pale yellow oil and accounting for 17.5% of the starting diol stereoisomer mixture was shown to be the diacetate of (*R*,*R*)-**5**: $[\alpha]^{amb}_D$ +73.33° (*c* = 2, acetone); ^1H NMR ($CDCl_3$) δ 7.66 (t, 1 H), 7.23 (d, 2 H), 5.93 (q, 2 H), 2.41 (s, 6 H), 1.57 (d, 6 H). Anal. Calcd for $C_{15}H_{21}NO_4$: C, 64.50; H, 7.58; N, 5.01. Found: C, 64.58; H, 7.65; N 5.08.

Isolation of (*R*,*S*)-α,α′-Dimethyl-1,3-pyridinedimethanol Monoacetate. The second fraction, comprising 2.57 g of a pale

yellow oil and accounting for 52.5% of the starting mixture of diol stereoisomers, was shown to be the monoacetate of (*R,S*)-**5**: $[\alpha]^{amb}_D$ +29.43° (*c* = 2.2, acetone); ^{1}H NMR ($CDCl_3$) δ 7.66 (t, 1 H), 7.22 (d, 2 H), 5.92 (q, 1 H), 4.91 (q, 1 H), 2.57 (br s, 1 H), 2.41 (s, 3 H), 1.57 (d, 3 H), 1.51 (d, 3 H). Anal. Calcd for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.70. Found: C, 63.01; H, 7.30; N, 6.74.

Isolation of (*S,S*)-α,α′-Dimethyl-1,3-pyridinedimethanol (*S,S*)-5. The third fraction comprising 0.56 g of a white microcrystalline solid and accounting for 14.2% of the starting mixture of diol stereoisomers was shown to be (*S,S*)-**5**: mp 61–63 °C; $[\alpha]^{amb}_D$ −63.6° (*c* = 2, acetone). The ^{1}H spectrum was identical with that described above for the stereoisomer mixture. Anal. Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.89; H, 7.90; N, 8.45.

(*R,S*)-α,α′-Dimethyl-1,3-pyridinedimethanol, (*R,S*)-5. The monoacetate of (*R,S*)-**5** was hydrolyzed by treatment with sodium hydroxide in absolute methanol as described for the monoacetate of (*R,S*)-**4** above. (*R,S*)-**5** was isolated as a white semisolid, 1.42 g (89%): $[\alpha]^{amb}_D$ +10.21° (*c* = 2, acetone). The ^{1}H NMR spectrum was identical with that of the mixture of diol stereoisomers. Anal. Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.45; H, 7.99; N, 8.44.

(*R,R*)-α,α′-Dimethyl-1,3-pyridinedimethanol, (*R,R*)-5. The diacetate of (*R,R*)-**5** was hydrolyzed with sodium hydroxide in anhydrous methanol as described for the diacetate of (*R,R*)-**4** above. The (*R,R*)-**5**, 0.450 g (85%), was isolated as a white microcrystalline solid: mp 62–64 °C; $[\alpha]^{amb}_D$ +44.01° (*c* = 2, acetone). The ^{1}H NMR spectrum was identical with that of the mixture of diol stereoisomers. Anal. Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.66; H, 7.98; N, 8.38.

Preparation of the Stereoisomer Mixture of α,α′-Dimethyl-2,6-pyridinedimethanol Diacetates. This mixture was prepared from the mixture of diols **5** as described below for the preparation of the stereoisomer mixture of α,α′,α″-trimethyl-1,3,5-benzenetrimethanol triacetates from the mixture of triols **7**.

Separation of α,α′-Dimethyl-2,6-pyridinedimethanol, Stereoisomers by Hydrolysis of the Diacetate Mixture. To a solution of 2.5 g (9.95 mmol) of a stereoisomer mixture of the diacetates of **5** in ether was added 7.5 silica gel. The ether was then evaporated, leaving a granular, free-flowing powder. To this powder was added 1.25 g of Amano P Lipoprotein Lipase and 25 mL of 0.1 N phosphate buffer. Aqueous NaOH was added as required to maintain the pH at 7.8. When the pH ceased changing significantly (about 3 days), the mixture was filtered through a glass fiber filter circle (coarse frits clog easily), and the yellowish filtrate was extracted with ether. The filtrate was dried and evaporated to give a residue that was separated by flash chromatography on silica gel using 1:1 ethyl acetate/hexane followed by neat ethyl acetate as eluants. Three fractions comprising the diacetate of (*S,S*)-**5**, the monoacetate of (*R,S*)-**5**, and (*R,R*)-**5** were isolated. The diacetate and monoacetate were hydrolyzed as described above to provide (*S,S*)-**5** and (*R,S*)-**5**, respectively.

Preparation of the Stereoisomer Mixture of α,α′-Dimethyl-4,4′-biphenylenedimethanol, (*S,S*)-, (*R,S*)-, and (*R,R*)-6. This mixture of stereoisomers was prepared from 4,4′-diacetylbiphenyl (K & K Laboratories) by reduction with sodium borohydride as described above for the stereoisomers of **1**. The mixture of stereoisomers was isolated as an off-white solid, mp 160–163 °C. It appeared as a single spot on TLC: ^{1}H NMR ($CDCl_3$) δ 7.35–7.65 (AA′BB′, q, 8 H), 4.95 (broadened q, 2 H), 1.86 (d, *J* = 2.5 Hz, 2 H), 1.54 (d, *J* = 6.4 Hz, 6 H). Anal. Calcd for $C_{16}H_{18}O_2$: C, 79.24; H, 7.49. Found: C, 79.11; H, 7.43.

Separation of the Stereoisomers of α,α′-Dimethyl-4,4′-biphenylenedimethanol. A mixture containing 4.0 g (16.5 mmol) of the mixture of stereoisomeric diols **6**, 3.37 g (33.05 mmol) of acetic anhydride, and 2.52 g of Amano P lipase supported on Celite 577 in 150 mL of dry benzene was allowed to stir for 10 h. The reaction was stopped by filtering off the catalyst. Evaporation of the solvent provided only 1.62 g of product, so the solid which had been filtered off was resuspended in 150 mL of dry benzene and treated with a further 3.0 g (29.4 mmol) of acetic anhydride, this time for a period of 30 h. Workup as before provided an additional 1.54 g of product. A third repetition of the acetylation provided a final 1.38 g of product. The combined products (4.54 g, 97% of theory) were separated by flash chromatography on a 15-cm bed of Merck 60/60 A silica gel eluting with 1:1.5 hexane/ethyl acetate to give, after evaporation of the solvent, three fractions, all of which crystallized upon standing.

Isolation of (*R,R*)-α,α′-Dimethyl-4,4′-biphenylenedimethanol Diacetate. The first fraction was shown to be the diacetate of (*R,R*)-**6**, 1.57 g, corresponding to 29.1% of the starting mixture of diol stereoisomers: mp 77–80 °C; $[\alpha]^{25}_D$ +170.19° (*c* = 2, acetone); ^{1}H NMR ($CDCl_3$) δ 7.50 (AA′BB′, q, 8 H) 5.92 (q, *J* = 6.6 Hz, 2 H), 2.08 (s, 6 H), 1.57 (d, *J* = 6.6 Hz, 6 H). Anal. Calcd for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.88; H, 6.82.

Isolation of (*R,S*)-α,α′-Dimethyl-4,4′-biphenylenedimethanol Monoacetate. The second fraction was shown to be the monoacetate of (*R,S*)-**6**, 1.69 g, corresponding to 36.0% of the starting mixture of diol stereoisomers: mp 70–72 °C; $[\alpha]^{25}_D$ +62.51° (*c* = 2.2, acetone); ^{1}H NMR ($CDCl_3$) 7.5 (AA′BB′ m, 8 H), 5.92 (q, *J* = 6.6 Hz, 1 H), 4.94 (q, *J* = 6.4 Hz, 1 H), 2.08 (s, 3 H), 1.98 (br s, 1 H), 1.57 (d, *J* = 6.6 Hz, 3 H), 1.53 (d, *J* = 6.4 Hz, 3 H). Anal. Calcd for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09. Found: C, 76.02; H, 7.09.

Isolation of (*S,S*)-α,α′-Dimethyl-4,4′-biphenylenedimethanol, (*S,S*)-6. The third fraction was shown to be (*S,S*)-**6**, 1.10 g, corresponding to 27.5% of the starting mixture of diol stereoisomers: mp 158–160 °C; $[\alpha]^{25}_D$ −55.00° (*c* = 2). Comparison with the mp and rotation found for the (*R,R*)-isomer suggested contamination by (*R,S*)- and/or (*R,R*)-isomer.

Purification of (*S,S*)-α,α′-Dimethyl-4,4′-biphenylenedimethanol, (*S,S*)-6, by Recycling. The impure (*S,S*)-**6** was treated with 0.93 g (9.1 mmol) of acetic anhydride and 0.66 g of the supported Amano P lipase in 100 mL of dry benzene. After 30 h, the catalyst was removed, and upon cooling the benzene solution to 0 °C, the (*S,S*)-diol (0.91 g, 83%) crystallized as a white solid: mp 167–169 °C; $[\alpha]^{25}_D$ −76.05° (*c* = 2, acetone). The ^{1}H and ^{13}C NMR spectra were identical with those described above for the stereoisomer mixture. Anal. Calcd for $C_{16}H_{18}O_2$: C, 79.24; H, 7.49. Found: C, 79.28; H, 7.46.

(*R,S*)-α,α′-Dimethyl-4,4′-biphenylenedimethanol, (*R,S*)-6. The monoacetate of (*R,S*)-**6** was hydrolyzed by treatment with NaOH in absolute methanol, as described for (*R,S*)-**2** above, to give 1.31 g (91%) of (*R,S*)-**6**: mp 168–171 °C; $[\alpha]^{25}_D$ +1.45° (*c* = 2, acetone). The ^{1}H NMR spectrum was identical with that of the mixture of diol stereoisomers. The rotation of +1.45° indicates this meso compound is contaminated with 1.9% of (*R,R*)-(+)-**6** having rotation +76.95°. Anal. Calcd for $C_{16}H_{18}O_2$: C, 79.24; H, 7.49. Found: C, 79.07; H, 7.36.

(*R,R*)-α,α′-Dimethyl-4,4′-biphenylenedimethanol, (*R,R*)-6. The diacetate of (*R,R*)-**6**, 0.42 g (1.68 mmol), was hydrolyzed with NaOH, 0.132 g (3.3 mmol) in anhydrous methanol (50 mL), as described for (*R,R*)-**3** above, to give 1.02 g (88%) of (*R,R*)-**6**: mp 168–170 °C; $[\alpha]^{25}_D$ +76.95° (*c* = 2, acetone). The ^{1}H NMR spectrum was identical with that for the mixture of diol stereoisomers. Anal. Calcd for $C_{16}H_{18}O_2$: C, 79.24; H, 7.49. Found: C, 78.97; H, 7.34.

Preparation of the Stereoisomer Mixture of α,α′,α″-Trimethyl-1,3,5-benzenetrimethanol, (*S,S,S*)-, (*R,S,S*)-, (*S,R,R*)-, and (*R,R,R*)-7. This mixture of stereoisomers was prepared from the poorly soluble 1,3,5-diacetylbenzene by reduction with sodium borohydride as described above for the α,α-dimethyl-1,4-benzenedimethanol stereoisomers. The product was isolated as a very pale yellow solid that appeared as a single spot on TLC: mp 122–126° C; ^{1}H NMR (CD_3COCD_3) δ 7.28 (s, 3 H), 4.87 (q, *J* = 6.5, 3 H), 3.67 (br s, 1.5 H), 2.73 (br s, 1.5 H), 1.46 (d, *J* = 6.5, 9 H). Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63. Found: C, 68.75; H, 8.85.

Preparation of the Stereoisomer Mixture of α,α′,α″-Trimethyl-1,3,5-benzenetrimethanol Triacetates. To a 250-mL flask was added 4.0 g (19.0 mmol) of the stereoisomer mixture of triols **7** mixed with 50 mL of CH_2Cl_2. The solution was stirred, and to it were added 8.74 g (85.6 mmol) of acetic anhydride, 8.67 g (85.7 mmol) of triethylamine, and 0.25 g (2.0 mmol) of 4-(*N,N*-dimethylamino)pyridine (DMAP). The partially soluble triol was completely dissolved after about 0.5 h, and after 2 h, TLC analysis showed only one spot at an R_f much higher than that of the triol. The reaction was quenched by cooling the flask in an ice–water bath and then slowly adding 50 mL of water. The phases were separated, and the organic phase was extracted with

3 × 50 mL of aqueous citric acid solution then 3 × 50 mL of saturated aqueous $NaHCO_3$ solution, and finally, with 2 × 100 mL of water. After drying, the solvent was evaporated to yield a dark yellow oil which was passed through a 4-in. bed of silica gel eluted with 1:1 hexane/ethyl acetate to provide 5.88 g (92%) of a light yellow oil: ^1H NMR ($CDCl_3$) δ 7.26 (s, 3 H), 5.89 (q, J = 6.6, 3 H), 2.09 (s, 9 H), 1.53 (d, J = 6.6, 9 H). The oil was used in the next step without additional characterization.

Separation of the Stereoisomers of α,α',α''-Trimethyl-1,3,5-benzenetrimethanol, 7. To a 500-mL flask fitted with a stirrer and a pH electrode was added 5.86 g (17.42 mmol) of the stereoisomer mixture of α,α',α''-trimethyl-1,3,5-benzenetrimethanol triacetate dissolved in 20 mL of benzene. To this was added 100 mL of a 0.1 M potassium phosphate buffer that had been adjusted to pH 7.8 followed by 0.600 g of *unsupported* Amano P lipase catalyst. The yellow reaction mixture was stirred at room temperature for 672 h (28 days) while maintaining the pH between 7.5 and 8.2 by daily additions of 1.0 M aqueous NaOH solution. After 672 h, 25.7 mL of the theoretical 26.1 mL of base had been added and the pH ceased to change over the next 24 h. The mixture was diluted with 300 mL of CH_2Cl_2 and then stirred rapidly. The resulting emulsion was broken by gravity filtration through a glass fiber filter. The phases were separated, and the organic phase was dried and then the solvent removed and the resulting light yellow oil subjected to flash chromatography on a 15-cm bed of 60/60 Angstrom silica gel eluted with 1:2 hexane/ethyl acetate to give three fractions.

Isolation of (S,S,S)-α,α',α''-Trimethyl-1,3,5-benzenetrimethanol Triacetate. The first fraction (highest R_f) comprised 0.91 g of a colorless oil accounting for 15.5% of the starting triacetate. It was shown to be the triacetate of (S,S,S)-7: $[\alpha]^{amb}_D$ −173.25° (c = 2, acetone). The ^1H NMR spectrum was identical with that described above for the stereoisomer mixture of triacetates. Anal. Calcd for $C_{18}H_{24}O_6$: C, 64.28; H, 7.19. Found: C, 64.64; H, 7.38.

Isolation of (R,S,S)-α,α',α''-Trimethyl-1,3,5-benzenetrimethanol Diacetate. The second fraction comprised 1.33 g of a colorless oil accounting for 25.9% of the starting triacetate. It was shown to be the diacetate of (R,S,S)-7: $[\alpha]^{amb}_D$ −114.75° (c = 2, acetone); ^1H NMR (CD_3COCD_3) δ 7.29 (d, J = 1.5 Hz, 2 H), 7.22 (d, J = 1.5 Hz, 1 H), 5.87 (q, J = 6.6 Hz, 2 H), 4.87 (q, J = 6.4 Hz, 1 H), 2.55–2.70 (br m, 1 H), 2.06 (s, 6 H), 1.52 (d, J = 6.6 Hz, 6 H), 1.48 (d, J = 6.4 Hz, 3 H). Anal. Calcd for $C_{16}H_{22}O_5$: C, 65.28; H, 7.53. Found: C, 65.27; H, 7.64.

Isolation of (R,R,S)-α,α',α''-Trimethyl-1,3,5-benzenetrimethanol Monoacetate. The third fraction comprised 1.41 g of a very pale yellow oil accounting for 32.0% of the starting triacetate. It was shown to be the monoacetate of (R,R,S)-7: $[\alpha]^{amb}_D$ +14.50° (c = 2.2, acetone); ^1H NMR (CD_3COCD_3) 7.28 (d, J = 1.4 Hz, 1 H), 7.23 (d, J = 1.4 Hz, 2 H), 5.84 (q, J = 6.6 Hz, 1 H), 4.84 (q, J = 6.4 Hz, 2 H), 2.55–2.70 (br m, 3 H), 2.04 (s, 3 H), 1.52 (d, J = 6.6 Hz, 3 H), 1.46 (d, J = 6.4 Hz, 6 H). Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.64; H, 7.99. Found: C, 66.57; H, 8.10.

Isolation of (R,R,R)-α,α',α''-Trimethyl-1,3,5-benzenetrimethanol, (R,R,R)-7. The aqueous phase was reduced to near dryness by evaporation of the water and then was extracted with 3 × 50 mL of acetone. The acetone was evaporated to provide 0.605 g of an off-white solid that accounted for 21.4% of the starting triacetate. Recrystallization from 3:1 hexane/ethyl acetate yielded 0.51 g of rectangular plate crystals which were shown to be (R,R,R)-7: $[\alpha]^{25}_D$ +95.50° (c = 2, acetone). The ^1H NMR spectrum was identical with that described above for the triol stereoisomer mixture. Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63. Found: C, 68.75; H, 8.85.

(R,R,S)-α,α',α''-Trimethyl-1,3,5-benzenetrimethanol, (R,R,S)-7. The monoacetate of (R,R,S)-7 was hydrolyzed by treatment with NaOH in absolute methanol as described for the mono- and diacetates of α,α'-dimethyl-1,3-benzenedimethanol, 4, above. The (R,R,S)-triol was isolated as a white microcrystalline powder, 0.45 g (90%): mp 123–125 °C; $[\alpha]^{amb}_D$ +32.31° (c = 2, acetone). The ^1H NMR spectrum was identical with that of the reaction of triol stereoisomers. Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63. Found: C, 68.69; H, 8.78.

(R,S,S)-α,α',α''-Trimethyl-1,3,5-benzenetrimethanol, (R,S,S)-7. The diacetate of (R,S,S)-7 was hydrolyzed with NaOH in anhydrous methanol as described for the mono- and diacetates of 4 above. The (R,S,S)-triol was isolated as a white, microcrystalline powder, 0.47 g (87%): mp 123–124 °C; $[\alpha]^{25}_D$ −32.10° (c = 2, acetone). The ^1H NMR spectrum was identical with that of the mixture of triol stereoisomers. Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63. Found: C, 68.39; H, 8.71.

(S,S,S)-α,α',α''-Trimethyl-1,3,5-benzenetrimethanol, (S,S,S)-7. The triacetate of (S,S,S)-7, was hydrolyzed with NaOH in anhydrous methanol as described for the mono- and diacetates of 4 above. The (S,S,S)-triol was isolated as rectangular plates, 0.384 g (87%): mp 128–129 °C; $[\alpha]^{amb}_D$ −94.10° (c = 2, acetone). The ^1H NMR spectrum was identical with that of the mixture of triol stereoisomers. Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63. Found: C, 68.57; H, 8.45.

Synthesis of the Bis-(R)-MTPA Esters of the Diol and Triol Stereoisomers.[28] To an oven-dried 12 × 75-mm test tube were added a few crystals of (N,N-dimethylamino)pyridine (DMAP) and the vial sealed with a rubber septum. The tube was then purged with dry nitrogen, and 300 μL of pyridine and 50 μL (0.27 mmol) of (R)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride were added by syringe. A solution of 15 mg (0.18 mmol) of a diol stereoisomer in 300 μL of pyridine was added to the tube and it was then shaken over night at ~35 °C. Complete conversion of the starting diol to the bis ester was confirmed by TLC, eluting with 1:1 ethyl acetate/hexane. To the reaction mixture was added 66 μL (0.54 mmol) of 3-(dimethylamino)propylamine, and after 5 min, the solution was diluted with 20 mL of ether and washed succesively with 5% HCl and saturated aqueous Na_2CO_3. (In the case of diols 5, the ether solution was washed only with saturated $NaHCO_3$ solution that had been adjusted to pH 8 with HCl.) The organic phase was dried, the solvent evaporated, and the residue purified by flash chromatography using 30% ethyl acetate/hexane as eluent. The solvent was evaporated from the ester-containing fractions with the last traces being removed under high vacuum over night. The required NMR samples were prepared by dissolving the resulting residues in $CDCl_3$.

Acknowledgment. This work was supported in part by the National Science Foundation under Grant No. DMR-8912065 and by the University of New Mexico Research Allocations Committee.