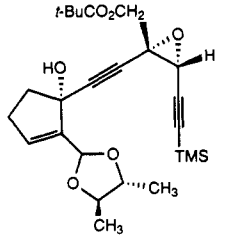
C_6H_5SeCl (1.05 equiv) and pyridine (1.1 equiv) in CH_2Cl_2 affords the α-(phenyl selenide) in 69% yield. Acetal formation with (2*R*,3*R*)-2,3-butanediol (1.2 equiv, 98%, Aldrich Chemical Co.) and camphorsulfonic acid (CSA, azeotropic removal of water) followed by selenide oxidation and elimination (*m*-chloroperbenzoic acid (MCPBA); *i*-Pr_2NH, CH_2Cl_2, 0–23 °C)[12] provides enone **6** in 85% overall yield. 1,4-Addition of 2-naphthalenethiol (1.2 equiv) to **6** (Et_3N (4 equiv), THF, 23 °C) proceeds in high yield to form a 1:1 mixture of the two trans diastereomers. Pure (2*R*,3*R*)-**7** is obtained by crystallization from hexanes (50% of theory after recrystallization, mp 100 °C, stereochemistry determined by X-ray analysis of the corresponding anti oxime).[13] Concentration of the mother liquors and treatment of the residue with triethylamine (5 equiv) and 2-naphthalenethiol (0.2 equiv, 0.1 M) in THF at 23 °C reestablishes a 1:1 mixture of trans diastereomers and allows for the recycling of (2*S*,3*S*)-**7**.



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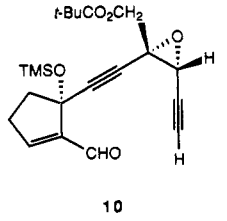

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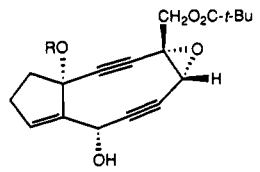
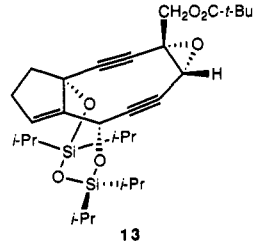


11 R = TMS
12 R = H



13

Metalation of epoxy acetylene **5** with $NaN(TMS)_2$ (1.05 equiv, 1.0 M in THF) in toluene at −78 °C followed by addition of ketone **7** (1.15 equiv), also at −78 °C, produces an 18:1 mixture of coupling product **8** and the β-hydroxy epimer, respectively, which are separated by flash column chromatography to provide **8** in 40% yield.[14] Sulfoxide formation (MCPBA, CH_2Cl_2, −78 °C; 1:1 mixture of diastereomers) and elimination (*i*-Pr_2NEt, toluene reflux, 4 h) proceed smoothly with exclusive formation of the trisubstituted cyclopentene **9** (84% overall). Deprotection of the silylacetylene is accomplished in quantitative yield upon exposure of **9** to $KF{\cdot}2H_2O$ in methanol at 23 °C for 3 h. Acetal hydrolysis (1:1 CH_3CN/water, 0.05 M CSA, 0 °C, 20 h) and silylation of the tertiary hydroxyl group (2,6-lutidine (20 equiv), $(CH_3)_3SiO$-SO_2CF_3 (8 equiv), CH_2Cl_2, −78 °C) then afford aldehyde **10** in 80% combined yield. Cyclization of **10** is achieved by treating a slurry of **10** and anhydrous $CeCl_3$ (3 equiv) in THF at −78 °C with excess $LiN(TMS)_2$ (25 equiv) for 1 h. After quenching with pH 7 phosphate buffer solution, aqueous workup, and flash column chromatography, the cyclic epoxy diyne **11** is obtained as a single diastereomer in 87% yield. Cyclizations conducted in the absence of $CeCl_3$ are less clean and do not proceed to completion. Spectroscopic data for **11** are in full accord with the assigned structure; in particular, ^{13}C NMR data are consistent with strained acetylenic bonds.[15] Though neat samples of **11** readily decompose, solutions of **11** can be stored at −20 °C without serious deterioration. The cyclization reaction which converts **10** to **11** involves an intramolecular acetylide addition similar to that employed in syntheses of molecules related to the calichemicin–esperamicin antibiotics.[16] It is noteworthy that this type of reaction is effective in forming the more strained cyclononadiyne ring of **11** and proves to be compatible with the epoxy diyne functional group. Desilylation of **11** ($Et_3N{\cdot}3HF$, CH_3CN, 23 °C, 2 h) affords diol **12** in high yield which, upon treatment with 1,3-dichlorotetraisopropyldisiloxane and imidazole in *N*,*N*-dimethylformamide at 23 °C for 4 h, efficiently produces disiloxane **13**, thereby establishing the cis-stereochemical relationship of the hydroxyl groups of **12**. This stereochemistry results from acetylide attack on the s-trans aldehyde rotamer of **10**, a stereochemical outcome observed in the earlier studies of Danishefsky and co-workers.[16a]

The synthetic route to **11** outlined above is convergent and enantioselective and demonstrates a viable strategy for construction of the strained and reactive core functionality of **1**, potentially applicable to a synthesis of **1** itself. It is further anticipated that **11** will be of value as a direct precursor to molecules of importance in elucidation of the mechanism of DNA cleavage by **1**.

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Supplementary Material Available: High-resolution ^1H NMR spectra of compounds **2**–**11**, a ^{13}C NMR spectrum of **1**, and an ORTEP representation of the anti oxime of (2*R*,3*R*)-**7** (14 pages). Ordering information is given on any current masthead page.

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(14) Approximately 50% of epoxide **5** can be recovered from the coupling reaction. Stereochemical assignments are based on NOE studies of the diimide reduction products (saturation of the silylacetylene, cis reduction of the internal acetylene) of **8** and the β-hydroxy diastereomer. Acetylide addition to form **8** is apparently directed by the acetal appendage.

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Effect of the Solvent on Enzyme Regioselectivity

Eduardo Rubio,† Alfonso Fernandez-Mayorales,‡ and Alexander M. Klibanov*

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
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The realization that enzymes can act as catalysts in neat organic solvents[1] has led to the introduction of a new fundamental variable, the reaction medium, into studies of enzyme–substrate (and also antibody–antigen[2]) interactions. It has been found that the nature of the solvent has a profound effect on substrate specificity[3] and enantioselectivity[4] of enzymes. In the present investigation, we have addressed the question of whether it is possible to regulate

† On leave from the Department of Organometallic Chemistry, University of Oviedo, Oviedo, Spain.

‡ Permanent address: Institute of Organic Chemistry, Madrid, Spain.

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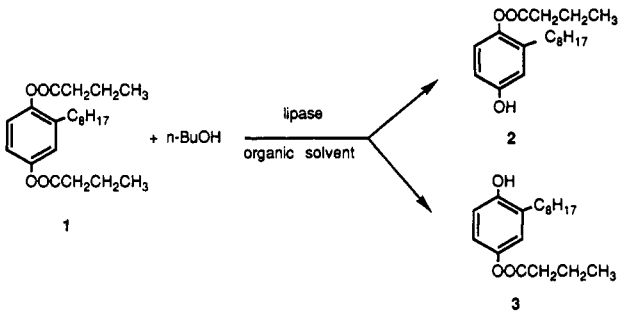
0002-7863/91/1513-695$02.50/0

Scheme I



the regioselectivity of enzymes by changing the solvent.

We selected, as a target molecule, octylhydroquinone (an analogue of some important natural compounds[5]) butyrylated at both phenolic groups. This diester (**1**) can undergo enzymatic transesterification with an alkanol via two alternative pathways, depicted in Scheme I, to form either 4-(butyryloxy)-3-octylphenol (**2**) or 4-(butyryloxy)-2-octylphenol (**3**). We examined the initial rates (v_2 and v_3, respectively) of the formation of **2** and **3** catalyzed by different lipases[6] in two disparate anhydrous organic solvents, toluene and acetonitrile. One would expect that for steric reasons the formation of **2** should be preferred over that of **3**. Indeed, for lipases from *Chromobacterium viscosum*, *Candida cylindracea*, and *Aspergillus niger*, **2** was formed at least 10 times faster than **3** in both solvents. For porcine pancreatic and *Penicillium roqueforti* lipases, as well as for nonenzymatic transesterifications (catalyzed by *p*-toluenesulfonic acid in toluene and by KCN in acetonitrile), the preference was the same, albeit more modest: v_2/v_3 in both solvents ranged from 1 to 4. However, surprising results were obtained with *Pseudomonas cepacia* lipase and *Pseudomonas* lipoprotein lipase: while in toluene v_2/v_3 was in agreement with the aforementioned data, 2.0 and 2.4, respectively, in acetonitrile with rate ratios were 0.5 and 0.8, respectively. Thus the regioselectivity of these two enzymes reverses upon a transition from toluene to acetonitrile as the reaction medium.

Lipase from *P. cepacia* was used in further work to investigate this phenomenon. We hypothesized that the enzyme has a hydrophobic cleft in the vicinity of the catalytic site[7] and that **1** can bind to the enzyme in two distinct modes. In the first one, the octyl moiety does not occupy the putative hydrophobic cleft, thus placing the distal butyryl group in the catalytic site, leading to formation of **2**. In the second mode, the octyl moiety fills the cleft and places the proximal butyryl moiety in the catalytic site, thus leading to formation of **3**. In hydrophobic toluene, transfer of the octyl moiety from the solvent to the cleft offers no thermodynamic advantage and therefore, the first binding mode, yielding **2** (the upper route in Scheme I), prevails. Conversely, in hydrophilic acetonitrile the free energy of partitionining of the octyl moiety from the solvent into the hydrophobic cleft is favorable; consequently, the second binding mode, yielding **3**, is preferred.

We tested this hypothesis experimentally. First, one would expect that replacement of **1**'s octyl moiety with a smaller alkyl group should abolish the effect of partitioning and, in turn, the change in regioselectivity. Indeed, we found that v_2/v_3 for the dibutyl ester of methylhydroquinone, while similar to that for **1** in toluene, in acetonitrile is 1.1 instead of 0.5 (i.e., no more reversal of regioselectivity upon solvent change). Second, in agreement

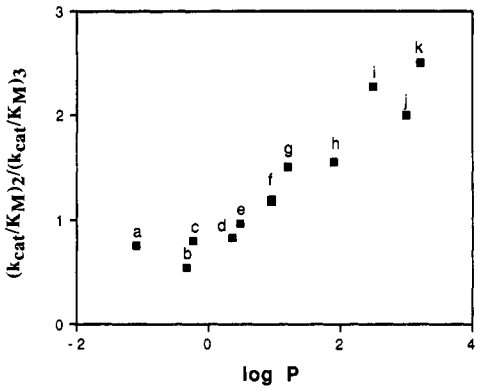


Figure 1. The dependence of regioselectivity of *P. cepacia* lipase in the transesterification of **1** (Scheme I) on the hydrophobicity of the solvent used as the reaction medium: a, dioxane; b, acetonitrile; c, acetone; d, *tert*-butyl alcohol; e, tetrahydrofuran; f, cyclohexanone; g, isopropyl acetate; h, methyl *tert*-butyl ether; i, toluene; j, carbon tetrachloride; and k, cyclohexane. All organic solvents were dried prior to use by shaking with 3-Å molecular sieves to bring the water content below some 0.01% (Zaks, A.; Klibanov, A. M. *J. Biol. Chem.* **1988**, *263*, 3194). The values of k_{cat}/K_M were determined by varying the concentration of **1** from 10 to 30 mM at 100 mM butanol and 5 mg/mL enzyme (suspensions were shaken at 300 rpm and 30 °C and assayed by gas chromatography under the conditions allowing to distinction between **2** and **3** following a precolumn derivatization (Stalling, D. L.; Gehrke, C. W.; Zumwalt, R. W. *Biochem. Biophys. Res. Commun.* **1968**, *31*, 616)).

with the postulated model, replacement of the butyryl moiety in **1** with crotonyl, caproyl, or γ-chlorobutyryl had little effect on enzyme regioselectivity (v_2/v_3 values were 1.9, 1.5, and 1.4 in toluene and 0.5 for all in acetonitrile, respectively), whereas the replacement with the bulky isobutyryl moiety resulted in v_2/v_3 greater than 14 in both solvents. Third, 100 mM octyl pivalate, a designed inhibitor[8] competing for the enzyme's hydrophobic cleft, expectedly increased the v_2/v_3 ratio in both toluene and acetonitrile (by 35% and 53%, respectively). Finally, our hypothesis predicts that there should be a correlation between regioselectivity of *P. cepacia* lipase in the transesterification of **1** and the hydrophobicity of the reaction medium. As seen in Figure 1, $(k_{cat}/K_M)_2/(k_{cat}/K_M)_3$ values in fact increase when log *P* of the solvent[9] (where *P* is its partition coefficient between octanol and water) is raised.[10]

It would be of both mechanistic and preparative significance to extend these studies of enzyme regioselectivity as a function of the solvent to recently reported acylations/deacylations of carbohydrates,[11] related compounds,[12] and steroids[13] catalyzed by lipases and proteases in organic solvents.

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(6) Lipases were obtained from Sigma Chemical Co., Amano International Enzyme Co., Biocatalysts Ltd., and FinnSugar Biochemicals. *P. cepacia* lipase (lipase PS, formerly lipase P) was previously incorrectly referred to as *Pseudomonas fluorescens* lipase by the manufacturer (Dr. S. Mihara of Amano, private communication). Enzyme powders were added to substrate solutions (20 mM **1** and 100 mM butanol) in anhydrous solvents, and the resultant suspensions were shaken at 300 rpm and 30 °C over the time period of 3–12 h. Periodically, aliquots were withdrawn and analyzed by gas chromatography.

(7) The existence of a hydrophobic cleft in the active center of this lipase has been recently postulated: Kalaritis, P.; Regenye, R. W.; Partridge, J. J.; Coffen, D. L. *J. Org. Chem.* **1990**, *55*, 812.

(8) It roughly resembles the relevant portion of **1** and, yet, is unable to undergo the enzymatic transesterification because of the bulkiness of the $(CH_3)_3$ acyl moiety.

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(10) Analogous dependence was observed for *Pseudomonas* sp. lipoprotein lipase. Conversely, for *C. cylindracea* lipase (i) there was no correlation between regioselectivity and log *P* of the solvent and (ii) in all the solvents listed in Figure 1 regioselectivity was greater than 8.

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(14) This work was financially supported by NIH Grant GM39794. E.R. and A.F.-M. were recipients of Spanish MEC/Fulbright and CSIC fellowships, respectively. We are grateful to Paul A. Burke for helpful discussions.