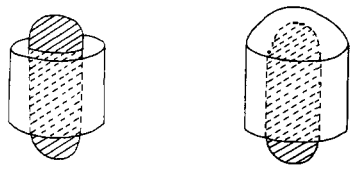


a. binding by a common cylindrical host

b. binding by a capped cylindrical host

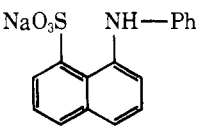
Figure 1. Schematic representation of hydrophobic binding. Obliquely lined areas are exposed in water, not contributing to binding. Obliquely dotted lined areas are contributing to binding.

sodium 1-anilino-8-naphthalenesulfonate 11–24 times stronger than the parent cyclodextrin.

Treatment of β-CD (0.010 mol) with diphenylmethane-*p,p'*-disulfonyl chloride (**1**) (0.012 mol) in pyridine gave crude **2** in ca. 70% yield based on TLC analysis. Careful workup (decantation, condensation, precipitation with $CHCl_3$, recrystallization from water) gave pure **2** in 20% yield. IR (KBr), 1595, 1495, 1360, 1180, and 880 cm^{-1}; UV (H_2O) 236 and 257 nm. NMR spectrum, multiplet centered at 7.69 (aromatic 8 H), multiplet centered at 4.87 (C_1H, 7 H), and multiplet centered at 3.50 (others, 42 H)

The reaction of β-CD (0.0020 mol) and terephthaloyl chloride (0.0026 mol) in pyridine afforded practically pure **3** in 15% yield after repeated recrystallizations from water. IR (KBr), 1710, 1280, and 730 cm^{-1}; UV (H_2O) 246 nm. NMR spectrum, multiplet centered at 7.97 (aromatic 4 H), multiplet centered at 4.80 (C_1H, 7 H), and multiplet centered at 3.50 (others, 42 H)

Binding of 1,8-ANS, **4**, by capped β-CD's was investigated by fluorescence measurements.[6] A strong increase in the fluorescence intensity was observed, and the Benesi-Hildebrand plot[7] gave a straight line in the concentration range of a capped β-CD from 4.2×10^{-5} to 2.5×10^{-4} M at a concentration of 5×10^{-6} M of **4**, which gave the binding constant for the complexation of **4** by **2** to be 1.3×10^3 M^{-1}. The constant for the binding of **4** by **3** was 6.4×10^2 M^{-1}, interestingly in a good accord with that by **2**. Thus, binding by a capped β-CD becomes 11–24 times stronger than that by the parent β-CD and is relatively insensitive to the capping groups.



4

A cyclodextrin with pendant hydrophobic substituents[5] was reported to bind aromatic guests more poorly ($K_{diss} = 2.6 \times 10^{-2}$ for *m*-nitrophenyl acetate) than the parent CD ($K_{diss} = 5.3 \times 10^{-3}$) does but it binds 1-adamantanecarboxylic acid more strongly ($K_{diss} = 6.7 \times 10^{-5}$) than the parent CD ($K_{diss} = 1.6 \times 10^{-3}$). The capped CD **2** in the present work also binds adamantanecarboxylic acid strongly ($K_{diss} = 2.0 \times 10^{-5}$ at pH 6.86).[8] That capped CD's in this present work bind even an aromatic **4** much more strongly than the parent CD is, therefore, very interesting, and is in good agreement with the Nèmethy-Sheraga's concept of the hydrophobic interaction.

Thus, we may safely say that one can design a structure of an appropriate hydrophobic host primarily based on this concept.

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(8) Although pH for the present measurement and the Emert–Breslow measurement were different, being 6.86 and 9.00, respectively, the K_{diss} for the binding of 1-adamantanecarboxylic acid was only slightly dependent on pH of this region, since its pK_a is ca. 5.
(9) The binding of *m*-nitrophenyl acetate, a Emert–Breslow substrate, by the capped cyclodextrin **2** has not been examined and, therefore, a direct comparison of **2** with the Emert–Breslow's cyclodextrin is not possible. One of the referees pointed out the reasonable possibility that the polar nitro group may destabilize the inclusion complex to show large K_{diss} value. However, the solubility of ANS, the present guest, in water (more than 2.2 M) is considerably higher than that of *m*-nitrophenyl acetate (ca. 0.1 M), suggesting that polar destabilization is not the sole determining factor.

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Stereoselective Formation of Diepoxides by an Enzyme System of *Pseudomonas oleovorans*

Sir:

In previous work, we have established that an enzyme system from *Pseudomonas oleovorans* catalyzes the conversion of terminal olefins to the corresponding 1,2-oxides.[1-12] Among the highly unusual characteristics of this enzymatic epoxidation reaction are a specificity far different from that observed in peracid epoxidations, a lack of retention of the original olefinic geometry during the course of the reaction, and the fact that the conversion of 1,7-octadiene to 7,8-epoxy-1-octene proceeds with a high degree of stereoselectivity with more than 90% of the product being the *R*(+) isomer. Although each of these characteristics imparts potential synthetic utility to this enzymatic system, the latter is of particular interest since optically active epoxides with a high degree of optical purity cannot be produced from simple olefins via any known chemical epoxidizing agent.[13] We now wish to report that highly stereoselective syntheses of diepoxides from simple diolefins can also be achieved using this enzymatic system. Furthermore, we have found that the configuration of a preformed asymmetric center at one end of a substrate profoundly affects the stereochemical consequences of enzymatic oxygen insertion into a double bond at the other end of the molecule. These findings not only establish the unique suitability of this system for the facile production of both mono and diepoxides of high optical purity, but also provide information about the mode of substrate binding at the active site.

Enzymatic epoxidation of 1,7-octadiene to produce 7,8-epoxy-1-octene or 1,2;7,8-diepoxyoctane on a preparative scale was accomplished using growing cells of *P. oleovorans* in the presence of octane according to the procedures that we have previously described.[5,7] Enzymatic epoxidation of racemic 7,8-epoxy-1-octene was accomplished as follows. Shake flasks (100 300-ml) each containing 100 ml of P_1 minimal salts medium,[5] 1 ml of octane, 100 μl (87 mg) racemic 7,8-epoxy-1-octene and a 10-ml inoculum of a resting cell suspension of *P. oleovorans* (~10^9 cells/ml) were incubated for 20 h at 30 °C on a gyrotory shaker. The broths were then extracted with toluene and concentrated, and both the diepoxide product and unreacted 7,8-epoxy-1-octene isolated by vacuum distillation. Typical recoveries (before distillation) from a single shake flask

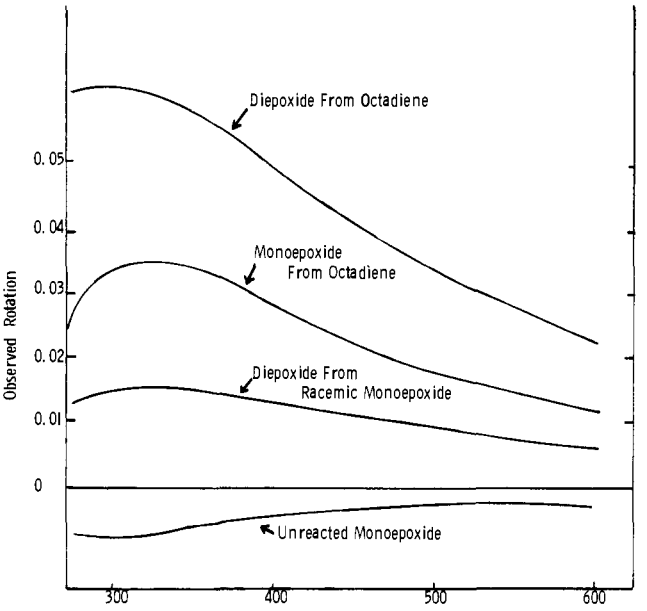

Figure 1. ORD curves for various enzymatically produced mono- and diepoxide products. The spectra were obtained using neat samples in a 0.1-mm path length cell.

were 30–40 mg of unreacted monoepoxide and 15–20 mg of diepoxide. No attempt was made to systematically optimize the conversion of monoepoxide to diepoxide. In all cases, purities of the various products were established according to our previously described criteria.[2,7]

Figure 1 shows the ORD curves obtained with neat samples of the various enzymatic and chemical products. As reported previously, neat 7,8-epoxy-1-octene produced enzymatically from octadiene exhibits a positive rotation, while that produced from the peracid epoxidation of octadiene is racemic. The data in the figure now establish for the first time that the same is true of enzymatically produced 1,2;7,8-diepoxyoctane. However, it is apparent that the magnitude of the positive rotation exhibited by neat samples of enzymatically produced 1,2;7,8-diepoxyoctane is strikingly dependent on whether the starting substrate is octadiene or racemic 7,8-epoxy-1-octene. In the latter case, the recovered unreacted monoepoxide substrate exhibits only a slight negative rotation, which confirms our contention that, once formed, the epoxide functionality does not isomerize to any significant extent under the reaction conditions. Optical purities of the various epoxide products were determined by NMR using the chiral shift reagent tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato]europium(III) according to the procedure that we have previously described.[7] Table I summarizes the results of these determinations.

Conceptually, several limiting possibilities can be envisioned for the stereochemical course of the enzymatic epoxidation of (*R,S*)-7,8-epoxy-1-octene. On the one hand, in view of the preferential formation of *R* monoepoxide from octadiene, it might be expected that enzymatic oxygen insertion always proceeds virtually exclusively from the *si-si* face of the reactive double bond, irrespective of the configuration of a preformed asymmetric center at the other end. Thus, racemic monoepoxide would give rise to a mixture of (*R,R*) and (*S,R*) diepoxide molecules, and the enantiomeric excess of *R* sites should approach 50%. A second limiting possibility is that the configuration of the preformed asymmetric center somehow alters the course of the enzymatic oxygen insertion process so that, for example, *R*-monoepoxide might give rise to only (*R,R*)-diepoxide and *S*-monoepoxide to only (*S,S*), resulting in racemic diepoxide from racemic monoepoxide. Obviously, any number of intermediate possibilities with *R*- or *S*-monoepoxide functionalities exerting various degrees of influence on the stereochemical course of the enzymatic diepoxidation process may be envisioned. In addition, the monoepoxide enantiomers may be enzymatically oxygenated at different rates, with the limiting cases being that either one or the other is essentially unreactive in this process, a common phenomenon in enzyme-catalyzed reactions.

Table I. Enantiomeric Composition of Epoxides

Compd	NMR determination[a] % *R* sites[b]	% *S* sites[b]
Peracid epoxidation of 1,7-octadiene		
7,8-Epoxy-1-octene	50	50
1,2;7,8-Diepoxyoctane	50	50
Enzymatic epoxidation of 1,7-octadiene		
7,8-Epoxy-1-octene	92	8
1,2;7,8-Diepoxyoctane	83	17
Enzymatic epoxidation of racemic 7,8-epoxy-1-octene		
1,2;7,8-Diepoxyoctane	59	41
Unreacted 7,8-epoxy-1-octene	47	53

[a] Optical purity determinations were carried out using the europium shift reagent as described previously (ref 7), except that the concentration ratios of epoxide to shift reagent were 0.08 for the monoepoxide products and 0.302 for the diepoxide products. The downfield multiplet arises from the isomer with the positive rotation which has the *R* configuration (ref 7, 13, and 14). [b] All values are averages of at least three determinations. Average deviations never exceeded about 2%.

The results presented here establish that the diepoxide product produced from racemic monoepoxide exhibits an enantiomeric excess of *R* sites of only 20%, while diepoxide produced from octadiene, through the obligate intermediacy of monoepoxide which is generated almost exclusively with the *R* configuration,[2,4,7,8,10] exhibits an enantiomeric excess of *R* sites of nearly 70%. These findings indicate that the configuration of the preformed epoxide group indeed influences the stereochemical course of oxygen insertion in the diepoxidation reaction and thus it is clear that *the opposite ends of a straight chain substrate do not function independently in this enzymatic reaction.* We have carried out a mathematical analysis of the data and find that they are consistent with a scheme which postulates that the two monoepoxide enantiomers react at somewhat different rates and that diepoxidation of *S*-monoepoxide produces predominantly *S,S*-diepoxide with the same degree of stereoselectivity observed in the product of *R,R*-diepoxide from *R*-monoepoxide. However, other more complex schemes cannot be ruled out by the data in hand[15] and a final determination on this point must await the development of new methods capable of generating the *S*-monoepoxide in high optical yield. In any case, irrespective of the detailed mechanism, the synthetic potential of this system for the stereoselective production of diepoxides from olefins is clear, and such compounds may find application in the synthesis of stereoregular polymers or as specific cross-linking agents for proteins or other substances. We have previously noted the unusually critical role played by the mode of substrate binding in moderating the reactivity of this enzymatic system,[8-10,12] and our results with the racemic monoepoxide substrate undoubtedly reflect such factors. In view of these findings, it will be interesting to evaluate the effects of other types of asymmetric centers and of variations in substrate geometry on the stereoselectivity of this enzymatic reaction.

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Benzoylamination of Uridine Derivatives

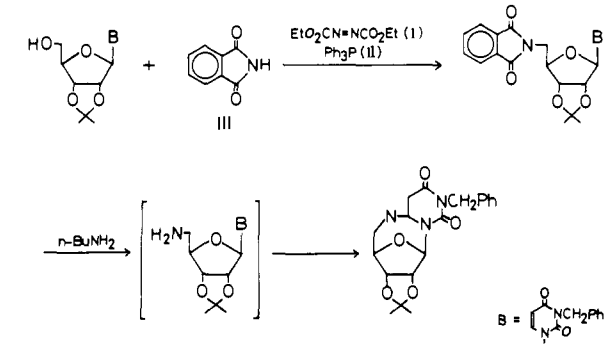
Sir:

Conversion of hydroxyl groups of sugars and nucleosides into acylamino functions is an important reaction for the synthesis of biologically active compounds such as antibiotics.[1] The conversion generally requires many reaction steps, e.g., replacement of the hydroxyl group by an azide group via halides or sulfonates followed by reduction and acylation.[2] In this communication we wish to report an alternative method for the preparation of acylaminonucleosides. N-Protected uridines were utilized in the present study.

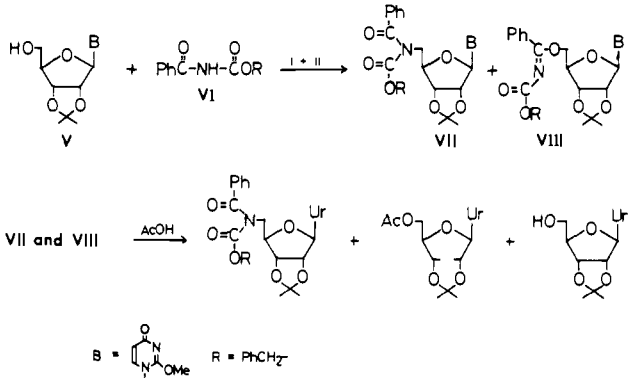
When N^3-benzyl-2′,3′-*O*-isopropylideneuridine (5 mmol) was allowed to react with diethyl azodicarboxylate (I; 6 mmol), triphenylphosphine (II; 6 mmol), and phthalimide (III; 5 mmol) in tetrahydrofuran (THF; 25 ml) at room temperature overnight, 5′-deoxy-5′-phthaloylamino-N^3-benzyl-2′,3′-*O*-isopropylideneuridine (IV) was obtained in a 47% yield (mp 160–161 °C, from ethanol).[3] On treatment with methanol (15 ml)-*n*-butylamine (3 ml) under reflux for 11 h, IV afforded 5′-deoxy-5′,6-epimino-5,6-dihydro-3-benzyl-2′,3′-*O*-isopropylideneuridine in a 28% yield (mp 129–131 °C, from CCl_4) (Scheme I).[4]

Since the N^3-benzyl group of uridine derivatives is difficult to remove,[5] O^2-methyl-2′,3′-*O*-isopropylideneuridine (V)[6] was used as a protected uridine. O^2-Methyl-2′,3′-*O*-isopropylideneuridine reacted smoothly with III in the presence of 1.5 molar equiv each of I and II under the same conditions as above giving 5′-deoxy-5′-phthaloylamino-O^2-methyl-2′,3′-*O*-isopropylideneuridine (77% yield, amorphous solid) which, on treatment with 20% acetic acid under reflux for 16 h, afforded 5′-deoxy-5′-phthaloylaminouridine[7] in a yield of 78% (mp 228–229 °C, from 3% aqueous acetic acid).

Scheme I



Scheme II



The conversion of the 5′-hydroxyl group of uridine into the benzoylamino group could be accomplished in a similar way (Scheme II). When V (2 mmol) was allowed to react with 1.5 molar equiv each of I, II, and *N*-benzyloxycarbonylbenzamide (VI) in THF (5 ml) at room temperature overnight, a 50% yield of a condensation product was isolated by preparative layer chromatography (methanol–ethyl acetate = 1:40). The complexity of its ^{1}H NMR spectrum suggested that it was a mixture of 5′-deoxy-5′-(*N*-benzyloxycarbonyl-*N*-benzoyl)amino-O^2-methyl-2′,3′-*O*-isopropylideneuridine (VII) and O^2-methyl-2′,3′-*O*-isopropylideneuridinyl 5′-(*N*-benzyloxycarbonyl)benzenecarboxyimidate (VIII). The mixture was again applied on silica gel plate and developed by the same system. This procedure was repeated three times giving the desired VII in a 20% yield. The VIII was presumably hydrolyzed on the plate during manipulation.[8] Alternatively, on treatment with acetic acid under reflux for 5 h, the mixture of VII and VIII afforded 5′-*O*-acetyl-2′,3′-*O*-isopropylideneuridine, 2′,3′-*O*-isopropylideneuridine, and 5′-deoxy-5′-(*N*-benzyloxycarbonyl-*N*-benzoyl)amino-2′,3′-*O*-isopropylideneuridine (amorphous solid) in 21, 8, and 20% yields, respectively. These products could be easily separated by preparative layer chromatography (the yields are based on V used).

As described in the previous paper,[8] the alkylation of VI by means of I, II, and alcohol gave rise to both *N*- and *O*-alkylated products. On the other hand, the reaction of the sodium salt of VI with primary alkyl halide resulted in the predominant formation of the corresponding *N*-alkylimide. Therefore the reaction of 5′-deoxy-5′-bromo-O^2-methyl-2′,3′-*O*-isopropylideneuridine (IX)[9] was next tried. When IX (1 mmol) was allowed to react with tetrabutylammonium salt of VI (1.5 mmol) in DMF (5 ml) at room temperature for 8 days, VII was, as expected, isolated in a 74% yield without accompanying the formation of VIII (Scheme III).

The deprotection of VII was carried out by usual manners. Thus VII was hydrogenated on Pd in ethanol giving 5′-