reactions, we did not detect any α- or γ-methylated product by TLC, GLC, and ^{1}H NMR analyses. In contrast, as shown in Table I, the mesylate **3c** and the tosylate **4b** gave the regio-, (*E*)-stereo-, and diastereoselective α-alkylation products **7** and **10**, respectively, as the major products by treatment with $Me_2CuLi{\cdot}BF_3$ (entries 1 and 2). Comparable results were obtained by reaction of the mesylate **4c** with $Me_2CuLi{\cdot}BF_3$ or the higher order heterocuprate $Me_2Cu(CN)Li_2$ (entries 3 and 4). In these reactions, the diastereoselection of the α-alkylation products **7** and **10** is over 99% judging from GLC and ^{1}H and ^{13}C NMR analyses. Thus it is shown that the stereochemistry of the α-position of the chirality transfer products **7** and **10** was governed by the stereochemistry at the γ-position of the substrates (anti SN_2' reaction).[16] The stereochemistries of **7** and **10** were determined by converting them into known (2*R*,5*R*)-*trans*-2-methyl-5-hexanolide **8**[17] and its (2*S**,5*R**)-cis isomer **11**,[2b,18] a sex pheromone of the carpenter bee *Xylocopa hirufissima*,[19] respectively, by a sequence of reactions [(i) H_2/10% Pd-C in MeOH; (ii) MeCN-$BF_3{\cdot}Et_2O$-HF (98:1:1), 20 °C, 2.5 h].

Treatment of the mesylate **5b** with *n*-Bu_2CuLi or *n*-$Bu_2CuLi{\cdot}2BF_3$ yielded the α-butylated product **14b** along with the γ-isomer **13** as a minor product. Here also, the α-alkylation proceeded with very high diastereoselectivity (98%). The stereochemistries of **13** and **14b** were determined as depicted in Scheme I by ^{1}H NMR spectral analyses of the lactones **15** and **16b** derived from **13** and **14b**, respectively, by the same sequence of reactions described above.

Although very high diastereoselectivity was realized with the cuprate-BF_3 reagent, the reduction or γ-alkylated product was always accompanied as a byproduct. Fortunately, this difficulty was overcome by use of the higher order heterocuprate-BF_3 reagent,[20] and the desired α-alkylated product was obtained with nearly 100% de in an essentially quantitative yield under a very mild reaction condition (-78 °C, 30 min) (entries 9-14).

It should be noted that the chemical yield of the present chirality transfer varies considerably depending on the solvent used. Although the reason for solvent effects is still not clear, THF-Et_2O (10:2) or THF-hexane (10:2) is the solvent of choice for the chirality transfers since reaction in Et_2O alone is too sluggish to be practicable.[21]

It is apparent from these preliminary data that by judicious selection of organocopper-Lewis acid reagents, the present method offers unusually facile entry to synthetically useful and stereochemically pure α-alkyl-δ-oxygenated (*E*)-β,γ-enoates. Application of this method for a synthesis of natural products is under investigation.

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Supplementary Material Available: Experimental details and data (IR, ^{1}H NMR, ^{13}C NMR) for **7**, **8**, **10**, **11**, **13**, **14**, **15**, and **16** (3 pages). Ordering information is given on any current masthead page.

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Resonance Raman Spectroscopic Evidence for Alternative Structures in the *Native* Ternary Complex Formed with Thymidylate Synthase

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We present evidence from resonance Raman (RR) spectroscopy for coexisting alternative structures in the native ternary inhibitor complex formed with thymidylate synthase (EC 2.1.1.45, TSase) isolated from the bacterium *Lactobacillus casei*. Inhibition of TSase is well established to be an important aspect of the mechanism of action of the widely used cancer chemotherapeutic agent, 5-fluorouracil (5-FU). A metabolite of 5-FU, 5-fluoro-2′-deoxyuridylate (FdUMP), together with the cofactor (+)-5,10-methylenetetrahydrofolate (5,10-CH_2FH_4), forms a potent (K_i = 10^{-11} M^{-1}) ternary inhibitor complex,[1] with distinctive near-UV absorption bands at 322 and 375 nm (with ϵ 59 800, and 7000 M^{-1} cm^{-1}),[2,3] which undergoes slow ($t_{1/2}$ = 10 h) dissociation.[4] The current structural model for this complex,[5] shown as II in Figure 1, is based on extensive characterization[6-9] of a proteolyzed fragment to which FdUMP and CH_2FH_4 are covalently attached.[3]

Figure 2 shows RR spectra of the native ternary complex, obtained with 337- and 356-nm Kr^+ laser excitation near resonance with the two near-UV absorption bands. Also shown at the top is the spectrum of (*p*-aminobenzoyl)glutamate (PABA-Glu) obtained with 266-nm excitation, in resonance with its strong absorption band (λ_{max} = 272 nm). Suggested assignments to the benzenoid ring modes[10] are indicated. Similar spectra are seen

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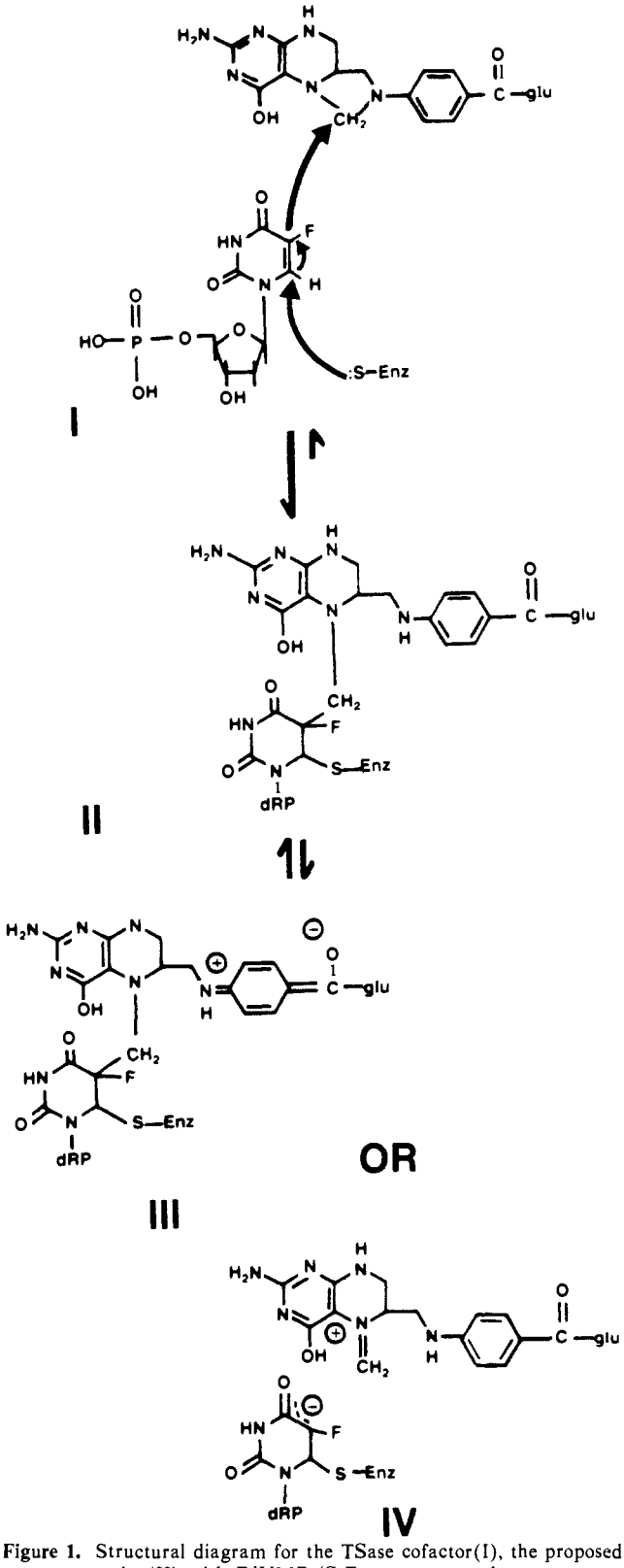

Figure 1. Structural diagram for the TSase cofactor(I), the proposed ternary complex(II) with FdUMP (S-Enz represents the enzyme nucleophile, Cys-198) and the two alternative structures (III, IV) suggested for the species whose RR spectrum is observed with 356-nm excitation.

for methotrexate, which also contains a PABA-Glu moiety,[11] and for *p*-(dimethylamino)benzaldehyde (DABA).[12] The bands in the PABA-Glu spectrum show a one-to-one correspondence with those of the 337-nm ternary complex spectrum, but there are

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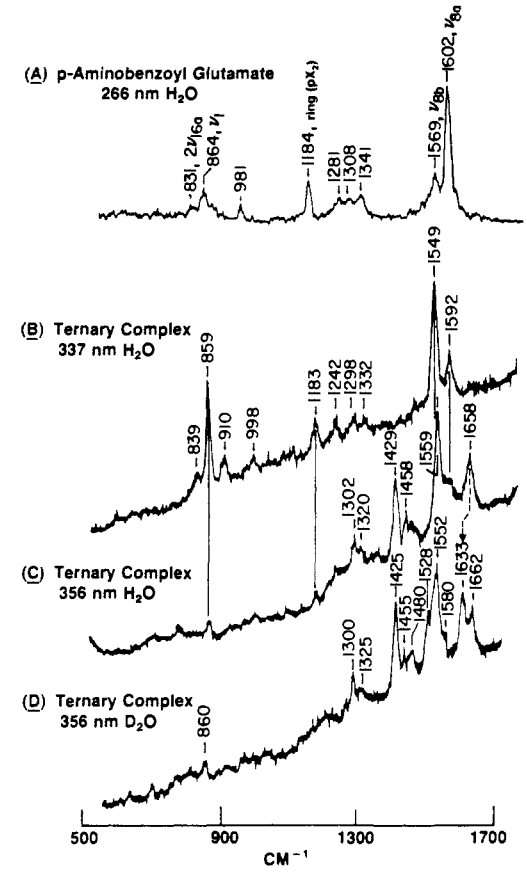

Figure 2. Resonance Raman spectra for (A) aqueous (2.5 mM) (*p*-aminobenzoyl)glutamate (PABA-Glu) obtained with 266-nm excitation (YAG laser fourth harmonic, incident on a flowing free stream of sample; Raman signal collected for 2 s per point with a 1.26 Spex 1269 scanning monochromator with a solar blind phototube and integrator electronics[18]) and for the TSase–FdUMP–CH_2FH_4 ternary complex (2:2:1 complex (prepared as described in ref 6) 1.4 mM in chromophore, in 0.025 M potassium phosphate buffer, pH 6.5, containing 25 mM ethanethiol and 700 mM potassium chloride) held at 4 °C in a spinning Raman cell, with (B) 337-nm excitation (CW Kr^+ laser, 15 mW) and with 356-nm excitation (CW Kr^+ laser, 45 mW) with the sample in (C) H_2O and (D) D_2O. The CW spectra were obtained with a Spex 1401 scanning double monochromator equipped with a cooled photomultiplier and photon counting electronics. Conditions: 8-cm^{-1} spectral band-pass, 1-cm^{-1} increments, 2.5 s/point. The absorption spectrum was monitored before and after Raman spectral acquisition to ensure the integrity of the chromophore.

significant frequency shifts and intensity alterations, especially the 5-cm^{-1} downshift and intensification of ν_1 and the 10- and 20-cm^{-1} downshifts and intensity reversal of ν_{8a} and ν_{8b}. These changes imply a significant structural perturbation of the PABA-Glu portion of the ternary complex, which also produces the 272 → 322 nm absorption red shift. The likeliest sources of these spectral effects are protein interactions involving a positive charge (or H-bond donor) near the PABA carbonyl group and/or a negative charge (or H-bond acceptor) near the NH group. Both the electronic transition energy and the vibrational frequencies are expected to be lowered by these interactions.

The ternary complex spectrum obtained with 356-nm excitation is strikingly different from that obtained at 337 nm. Remnants of the strong PABA-Glu bands at 859, 1183, 1549, and 1592 cm^{-1} can be seen, but the spectrum is dominated by a new set of bands. We therefore conclude that a chemical entity other than PABA-Glu is responsible for the main features of the 356-nm spectrum. When the ternary complex is formed in D_2O, several changes are seen. Of particular interest is the downshift of one component of the broad 1658-cm^{-1} band to 1633 cm^{-1}. A 25-cm^{-1} D_2O shift of a band in this region is strong evidence that it arises from an exocyclic double bond, presumably C=N, whose atoms contain exchangeable protons.

We consider two attractive possibilities for the chromophore excited at 356 nm, III and IV in Figure 1. If structure III is correct, the 1658-cm^{-1} band would be assigned to the quininoid C=N stretch, shifting to 1633 cm^{-1} on replacement of the NH proton in D_2O. A similar assignment (1624 cm^{-1}) has been made for a DABA-Zn^{II} complex,[12] also though to exist in a quininoid form. The remaining bands of the 356-nm ternary complex RR spectrum would also be expected to arise from vibrational modes in the quininoid fragment. Structure III would be favored by the same kind of protein interactions which are suggested to account for the altered PABA-Glu vibrational spectrum. These interactions would be central to the enzyme mechanism because they would favor breaking of the N-10 methylene-PABA bond over the N-5 methylene-tetrahydropterin bond in the cofactor (I, Figure 1) and disfavor reformation of this bond, which is believed to be the process by which the ternary complex undergoes dissociation. The coexistence of structures II and III would imply conformational heterogeneity in the protein, since without different protein interactions II and III are simply resonance forms. Different dispositions of the proposed charges or H-bonds in alternative protein conformations could, however, favor different electronic structures and different positions of the nuclei. Another possibility is that in a fraction of the molecules proton transfer occurs to or from protein acceptor or donor groups so that a tautomer of III is produced, with the proton on the CO rather than the N end of the quininoid fragment.

Alternatively, structure IV (or its N-10 analogue) has been suggested by Santi et al.[13] and others[14,15] to be an intermediate in the TSase catalytic mechanism. It has a $^{+}N{=}CH_2$ bond attached to the tetrahydropterin ring, which would be capable of exchange with D_2O.[16] This bond too could account for the observed 1658-cm^{-1} band, which shifts to 1633 cm^{-1} in D_2O. The remaining bands in the 356-nm spectrum could be attributed to vibrational modes of the tetrahdropterin moiety, with which the $^{+}N{=}CH_2$ bond is in conjugation. Accumulation of IV would also be in keeping with the proposed PABA-Glu perturbing protein interactions, since these would destabilize the methylene-PABA bond, and stabilize IV, which is otherwise a high-energy intermediate.[17]

At this point III and IV are both viable candidates for the species giving rise to the 356-nm spectrum. We hope to resolve the ambiguity via RR and NMR isotope labeling experiments. In either case it is important to recognize that the 322- and 375-nm absorption bands are absent unless the cofactor and FdUMP are both bound to the enzyme. It is only in the ternary complex that the protein interactions which are responsible for the perturbed PABA-Glu absorption and vibrational spectra, and also for the stabilization of either structure III or IV, are developed. During enzyme turnover these same activating interactions can be induced by binding of the natural substrate, 2'-deoxyuridylate, with the cofactor. They may well be involved in other enzymatic reactions utilizing N-10 linked tetrahydrofolates.

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Design and Synthesis of New Ferroelectric Liquid Crystals. 2. Liquid Crystals Containing a Nonracemic 2,3-Epoxy Alcohol Unit

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Tilted smectic liquid crystal (LC) phases, when composed of nonracemic molecules, possess a spontaneous ferroelectric polarization, or macroscopic dipole moment, deriving from anisotropic orientation of molecular dipoles in the LC phase.[2] We have suggested that this polarization (P, in units of nC/cm^2, or D/molecule) may be considered in terms of a novel type of molecular recognition occurring in the LC phase and have developed a model in principle allowing prediction of the sign (handedness) and magnitude of the polarization for specific compounds.[3] In this paper we describe a prototypical example of a new class of liquid crystal materials possessing the *trans*-2,3-epoxy alcohol chiral unit. This compound represents an ideal test of our model for the polarization, and indeed exhibits for the first time *predictable sign of the ferroelectric polarization* in a material with high polarization density.

The model states that individual molecules of the phase are ordered with respect to *conformation* and *rotational orientation* by a time-average surface of constant molecular mean field, or binding site, resulting from interactions with neighboring molecules. When applied to the well-known *p*-alkoxyphenyl *p*-alkoxybenzoates, the binding site takes the shape of a bent cylinder,[3] and the preferred conformation has the alkoxy tail units staggered with the carbons of the tails aligned in a plane, as shown in Figure 1. This plane is congruent with the smectic C tilt plane, and the preferred rotational orientation of the molecules is such that the tails are less tilted than the phenyl benzoate core.

Ferroelectric polarization results when the material is made nonracemic by incorporation of a stereogenic center in one of the tails. Typical ferroelectric LC molecules reported to date possess a 2-methylbutyl or 2-alkoxypropyl chiral tail.[3] Unfortunately, lack of data on the conformations present in the C* phase and the average rotational orientation of the molecules with respect to the tilt plane precludes interpretation of the observed polarization on a molecular level in these cases.

For display device applications, it is desirable to obtain ferroelectric LCs with high polarization density.[4] Thus, compounds with a large dipole moment oriented *normal* to the tilt plane in Figure 1 are desired. Given this goal, incorporation of the *trans*-2,3-epoxy alcohol unit into the tail of alkoxyphenyl alkoxybenzoate LCs, as shown in Figure 2 for an *S,S* enantiomer, seems particularly attractive. Our model suggests that the molecular dipole due to the epoxide unit in such a material should be oriented normal to the tilt plane, exactly as required, and the rigidity inherent in the epoxide ring should reduce conformational averaging of this dipole. This suggests that the polarization should be significantly enhanced relative to the 2-methylbutyl- or 2-

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