drogen bonds to the side-chain functions of Arg-244, Ser-130, and Ser/Thr-235 upon active site anchoring of the substrate.[10] This mode of active site binding brings one N_η of the Arg-244 side chain from the β-face of an active site-bound carbapenem to approximately 2.5–3 Å from the C_2 of the substrate (Chart I). It appeared to us that the Arg-244 guanidinium moiety could serve—in a truly adventitious manner—as the source of proton for the tautomerization of **2** to yield **3**.[11] To test this concept, we have studied the turnover of imipenem (*N*-formimidoylthienamycin) by the wild-type TEM-1 β-lactamase and the Arg-244-Ser mutant enzyme.[12] The wild-type enzyme hydrolyzes imipenem in the characteristic biphasic manner reported for other carbapenems (Figure 1).[4,5] The more rapid first phase of hydrolysis leads to a slower steady-state phase, which does not end until the substrate is exhausted. Extrapolation of the rate of the linear second phase back to time zero indicated a burst size of 2.5 imipenem molecules hydrolyzed by each enzyme molecule prior to the onset of the slower second phase, as reported for other carbapenems.[4,5] However, the Ser-244 mutant enzyme showed a monophasic hydrolysis of imipenem with a rate close to that of the first phase of hydrolysis by the wild-type enzyme;[13] the transition to the slower second phase was entirely eliminated. This observation suggests that the Ser-244 enzyme catalyzes a steady-state hydrolysis of imipenem without tautomerization of **2** to **3** (i.e., $\mathbf{1} \rightarrow \mathbf{2} \rightarrow \mathbf{4}$). The first-order rate for deacylation of **3** was calculated for the slow phase of imipenem turnover by the wild-type enzyme at 3.3×10^{-3} s^{-1}, according to the method of Glick et al.[14] The corresponding deacylation rates for both the Ser-244 enzyme and the first phase of the wild-type protein were fast and could not be measured accurately. Steady-state kinetic parameters for the turnover of imipenem by the Ser-244 enzyme were evaluated at $K_m = 27$ μM and $k_{cat} = 0.04$ s^{-1}. The value of k_{cat} indicates the lower limit on the rate of deacylation of **2** ($k_{H_2O} \geq k_{cat} = 0.04$ s^{-1}). These findings reveal that the rate of hydrolysis of **2** is at the minimum 12-fold faster than that of **3**. Inhibition of the wild-type β-lactamase as monitored by turnover of benzylpenicillin, which is due to transient active site acylation by imipenem, is rapid and is reversed considerably after all the substrate is consumed (Figure 1). On the other hand, the Ser-244 mutant enzyme was inhibited only marginally (<15%), in part because of rapid hydrolysis of the acyl-enzyme intermediate.

In conclusion, we have shown that Arg-244 of the TEM-1 β-lactamase is responsible for the biphasic turnover profile of imipenem, a representative carbapenem antibiotic, and we were able to estimate the hydrolysis rates of the pyrroline tautomers **2** and **3** from the active site. Furthermore, we have shown that the Ser-244 mutant enzyme is not effectively inhibited by imipenem. The recent report of the occurrence of the plasmid-borne Ser-244 mutant TEM-1 β-lactamase in a clinical isolate might herald an anticipated compromise of the continued effectiveness of imipenem as a backup drug against multiresistant clinical isolates.[15]

Acknowledgment. We thank Dr. James Knox for kindly providing us with the crystal coordinates for the *Bacillus licheniformis* 749/C β-lactamase, and Dr. Elias Manavathu for carrying out the mutagenesis experiments.

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(10) Zafaralla, G.; Manavathu, E. K.; Lerner, S. A.; Mobashery, S., unpublished results. It was suggested previously that Lys-234 interacts with substrate carboxylate.[7,8] However, recent modeling by Knox and Moews indicated that the Lys-234 side chain does not have any surface accessibility to the active site.[9]

(11) An additional possibility is that a coordinated water molecule to the electrophilic guanidinium moiety of Arg-244, whose pK_a would be lowered by this interaction, is the source of proton. Calculations at ab initio level are being carried out to provide a more quantitative estimate of the actual decrease in pK_a of a water molecule that is hydrogen bonded to a positive ammonium ion center—collaboration with R. Bach of this Department. Preliminary data support the basic idea set forth in this footnote.

(12) Mutagenesis was carried out according to the method of Kunkel: Kunkel, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 488. The wild-type and Ser-244 mutant β-lactamases were purified to homogeneity by a slight modification of a literature method: Fisher, J.; Belasco, J. G.; Khosla, S.; Knowles, J. R. *Biochemistry* **1980**, *19*, 2895. The far-UV circular dichroic (CD) spectra of the Ser-244 β-lactamase were identical to the wild-type enzyme in the pH range of 4–9. Furthermore, the two enzymes behave similarly in turnover of typical penicillins and cephalosporins.

(13) Our measurements of the binding energies of the Arg-244 guanidinium group to the substrate carboxylate (see Chart I) attributed 1.3–2.3 and 0.3–1.0 kcal/mol to the stabilization of both the transition and ground states of penicillins and cephalosporins, respectively (unpublished results). Therefore, the slightly slower rate for hydrolysis of imipenem by the Ser-244 mutant β-lactamase, compared to the early parts of the fast phase of hydrolysis by the wild-type enzyme, is due to the loss of a weak hydrogen bond between imipenem and the mutant protein in the enzyme–substrate complex.

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Switchable Liquid Crystalline Photopolymer Media for Holography

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Received October 8, 1991

Liquid crystalline materials for optical information storage have been sought by several groups in the last few years.[1] The generally high birefringence of liquid crystals offers an attractive means to achieve large refractive index modulations (Δn) through photochemical manipulation of order and/or orientation. In this report, results from a simple approach to orientational control are described, with the introduction of a new type of highly efficient and switchable holographic recording medium. Very few holographic media are known that allow for modulation of the diffracted light intensity (i.e., efficiency).[2]

Photopolymerization of liquid crystalline monomers has been shown in some cases to "lock in" or even increase the mesomorphic order.[3] If the photopolymerization is induced by interfering coherent beams, then a grating will be produced (Figure 1) with the regions of constructive interference becoming locked by polymerization and the regions of destructive interference remaining as fluid monomer. Upon application of an electric field, the monomeric regions should align with the field, increasing Δn to approximately the birefringence of the monomer.[4] The large increase in Δn should produce a corresponding increase in diffraction efficiency.

We have tested this approach with two monomers from the literature: the aromatic diacrylate **1**[5] and the cholesteryl methacrylate **2**.[6] The holographic results were obtained by using either a phase insensitive holography (PIH) or a phase modulated holography (PMH) apparatus similar to those described by Bräuchle and co-workers.[7] These methods use crossed laser beams to create

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(4) In order for Δn to approach the birefringence of the monomer, the liquid crystal would have to be prealigned, for example, by surface treatments, in a direction orthogonal to the subsequent electric field. Our samples were not prealigned, so further improvement in efficiency might be obtained with surface treatments.

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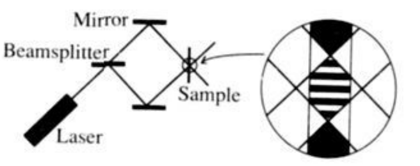


Figure 1. Crossed-beam irradiation generates interference fringes that are recorded in the sample through photochemistry. The resulting grating can then diffract one of the beams into the other.

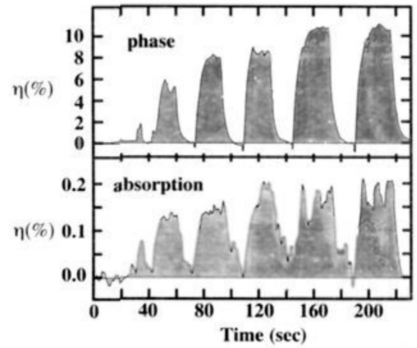
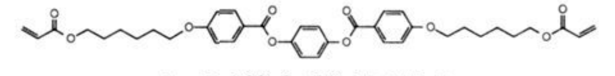
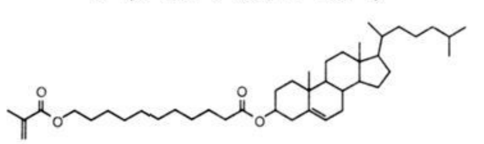


Figure 2. Diffraction efficiency due to phase (refractive index) and absorption for **1** in its nematic phase (with photoinitiator) at 130 °C. Crossed-beam irradiation was begun at $t = 0$ s, and an electric field was applied intermittently, corresponding to the sharp increases in efficiency.

interference fringes that are recorded in the sample through its photochemistry (Figure 1). Simultaneous reading is accomplished in PIH by chopping one beam and measuring the diffracted intensity with a photodiode. In PMH, one beam is phase-modulated and the resulting beat signal is detected in both beams. In our experiments, the 647-nm line of a Spectra Physics 2020 Kr ion laser was used. The monomer, with a small amount of dissolved photoinitiator,[8] was drawn by capillary action into a 6-μm thermostated cell made from indium-tin oxide coated glass (from Applied Films Laboratory). No aligning surface treatments were used for these experiments.[4]



1 (C 108 S (88) N 155 I)



2 (C 53.2 S 54.8 Ch 64.4 I)

Results from a representative PMH experiment with **1** in its nematic liquid crystalline phase at 130 °C are shown in Figure 2. The PMH experiment allows separate detection of diffraction due to phase and absorption effects, and plots for both are shown. A relatively weak phase diffraction was observed during the initial photopolymerization, and a dramatic 25-fold increase was observed upon application of an alternating electric field (100 V, 1 kHz) to the sample. The diffraction efficiency rose from 0.4% without the field to a peak of 11% with the field on.[9] The reversibility of this modulation was also demonstrated.[10] The sign of the modulation indicates that the regions of destructive interference have the larger refractive index, which is consistent with the model stated above that depicts the liquid crystalline monomer in these regions to be aligned with the field. The diffracted intensity due to absorption was about 50 times weaker than the phase signal.

Similar results were obtained when the photopolymerization was carried out under an applied electric field followed by *removal* of the field. With this procedure, diffraction efficiencies up to 18% were observed, although the modulation was somewhat reduced, due to more efficient diffraction during the initial polymerization.

Modulation was also observed with **2** in its cholesteric phase at 58 °C; however, the increases were very small (10-30%). That **2** gives rise to lower modulation is not surprising, since the relative lack of unsaturation should lead to smaller birefringence for this liquid crystal. In addition, the side-chain polymer of **2** may possess significant fluidity, since the mesogens are only anchored at one end. The cross-linked network polymer of **1** has been shown to remain oriented in a smectic phase up to 300 °C.[5]

In conclusion, we have demonstrated that a liquid crystalline monomer, **1**, performs well as a highly efficient, switchable optical recording medium.[11] Even at this preliminary stage, this medium has the properties necessary for application in holographic displays, spatial light modulators, and holographic associative memories.[12] Work is continuing toward the optimization of these media.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, and to the Camille and Henry Dreyfus Foundation New Faculty Award Program, for partial support of this work.

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(9) The switching occurs on a time scale of about 2 s.

(10) The durability of the switching medium can presumably be improved by reading the hologram with a wavelength of light that is not absorbed by the photoinitiator. We have not yet carried out these experiments.

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$RLi{\cdot}LiCuR_2$ Dimers: An Explicit Structural Alternative to "Higher Order" Homocuprates

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Received April 19, 1991

Appearance of the now-classic Gilman reagents[1] in 1952 spawned an era of organocopper chemistry that dominates transition-metal-directed carbon-carbon bond formation today.[2-4] Based on the ratio of alkyllithium to methylcopper, a range of stoichiometries[5-7] and reactivities[8] can be observed for the lithium-copper reagents. Although a list of informative X-ray structures is growing,[9-16] a clear correspondence between chem-

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