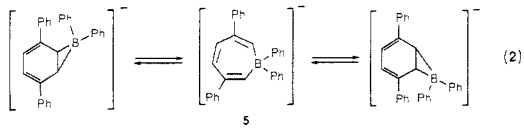
(-20 °C, 500 MHz) reveal that **5** must be less than ca. 10 kcal/mol higher energy than **1**.



(2)

5

The electronic structure of **1** is unusual. In particular, its strong absorption in the visible region is unexpected by analogy with related norcaradienes[15] and by comparison with *trans*-1,1,2,3-tetraphenylboratirane (λ_{max} = 280 nm).[12] The 510-nm absorption of **1** therefore must be due to interaction of the strained three-membered borate ring with the diene chromophore. Some insight into this interaction comes from molecular orbital calculation (Gaussian 86, 3-21G basis set)[16] on parent boratanorcaradiene ($C_6H_8B^-$). Its HOMO is formed from combination of the diene Ψ_2 with the appropriate Walsh-like[17] orbital of the borate ring. For comparison, the HOMO of norcaradiene, calculated at the same level of approximation, has the same nodal properties but is much lower in energy. Clearly, the "excess" electronic charge that results from replacement of a neutral carbon by a negative borate group alters properties profoundly. We are continuing to explore the extent of this perturbation.

Acknowledgment. This work was supported by a grant from the National Science Foundation. David Hartsough and Professor Scott Kahn of this department helped with the molecular orbital calculations.

Supplementary Material Available: Drawings of **1** and **2** containing atom numbering and tables of atomic coordinates, calculated positions, and thermal parameters for $[(CH_3)_4N][C_{30}H_{24}B]$ (7 pages); observed and calculated structure factors for $[(CH_3)_4N][C_{30}H_{24}B]$ (4 pages). Ordering information is given on any current masthead page.

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Free Standing Polydiacetylene Films Cast from Bilayer Membranes

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The formation of supramolecular assemblies of monolayers, bilayers, and/or multilayers is of considerable interest. Monolayers and multilayers are frequently prepared by Langmuir-Blodgett techniques. Recently Kunitake and co-workers introduced an alternative approach to ordered multilayers.[1-5] The molecular ordering present in bilayers was transformed to macroscopic ordering in multilayer films by the casting of aqueous dispersions of bilayers onto solid supports.

It is well known that the topotactic photopolymerization of diacetylenes is acutely sensitive to the molecular order of crystals and supramolecular assemblies. Monolayers of diacetylenic fatty acids are polymerizable only in close packed solid-like monolayers.[6,7] Bilayer membranes of lipid diacetylenes are neither photopolymerizable above the lipid phase transition temperature (T_c) of the membrane[8] nor in small sonicated vesicles where the lipid chain packing is disordered by the sharp radius of curvature of the membrane.[9] The stringent requirements for efficient photopolymerization of diacetylenes provide an excellent test of the ordering in cast multilayer films.

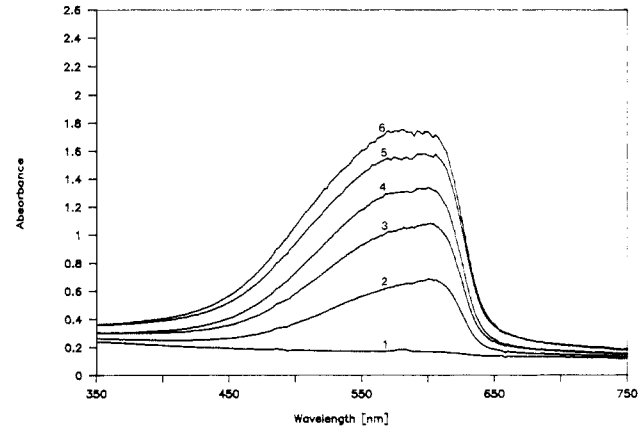
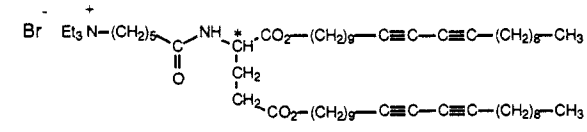


Figure 1. Absorption spectra of a cast multibilayer film of **1**. The spectra were recorded after exposure to 254 nm light for the following times: curve 1, 0 s; 2, 10 s; 3, 30 s; 4, 60 s; 5, 120 s; and 6, 180 s.

We describe here a new diacetylenic lipid (**1**) based on a glutamate backbone, the formation of cast multilayers from bilayers of pure **1**, and the successful photopolymerization of the multilayers to yield free standing polydiacetylene (PDA) films. The rigid, all-conjugated polymer structure renders most PDAs insoluble. Therefore the preparation of films of PDA heretofore has been accomplished only from a few chloroform soluble PDA (i.e., poly-3BCMU, poly-4BCMU)[10] or from amphiphilic diacetylenes via L-B techniques.[11,12]



1

Lipid **1** was synthesized (detailed procedure will be described elsewhere) by condensation of *N*-CBz-L-glutamic acid and docosa-10,12-diyn-1-ol, removal of the protecting group with iodotrimethylsilane, reaction of the free amino group with 6-bromohexanoyl chloride, and finally amination with triethylamine.[13]

Bilayer membrane vesicles of **1** were prepared from a thin film of 5 μmol of **1**, which was hydrated with 1 mL of water (Milli-Q, Millipore Corp) and subsequently sonicated for 30 s at room

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(13) Compound **1** was characterized by IR, NMR, TLC, and elemental analysis: IR (KBr) 3302, 2922, 2849, 2180, 2140, 1726, 1644, 1532, 1463 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 6 H), 1.26-1.85 (m, 75 H), 2.24 (t, 8 H), 2.53 (t, 2 H), 3.55 (q, 8 H), 4.04 (t, 2 H), 4.06 (t, 2 H), 4.45 (m, 1 H), 7.92 (d, 1 H). Anal. Calcd for $C_{61}H_{105}N_2O_5Br$: C, 71.41; H, 10.24; N, 2.73. Found: C, 71.64; H, 10.77; N, 2.74. Mp: t_m 35 °C, t_i 85 °C (thermotropic liquid crystalline behavior observed at this temperature range on a hot-stage polarizing microscope).

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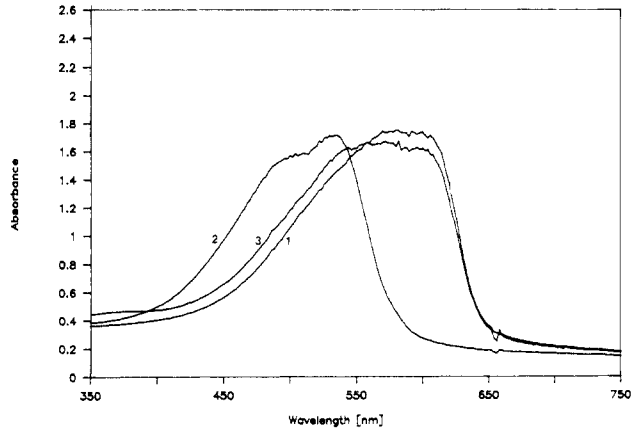

Figure 2. Absorption spectra of photopolymerized multibilayer film of **1** at 22 °C (1), at 50 °C (2), and after cooling back to 22 °C.

temperature in a thermostated cup-horn sonicator. Differential scanning calorimetry (Microcal MC-2) of membranes of **1** indicates the T_c is 14.6 °C. Hydrated bilayer membranes of **1** were not photosensitive unless they were cooled below room temperature with an ice bath. As noted previously lipid diacetylene bilayers are photopolymerizable only in the solid analogous phase ($<T_c$).

A few drops of the bilayer membranes of **1** (5 mM in lipid) were spread on a clean glass slide (75 × 25 mm) and allowed to dry slowly. After 2 days a thin transparent film was formed. The film on glass was further dried under vacuum for 2 h. It was then irradiated at room temperature by a low-pressure mercury lamp (Pen Ray) at a distance of 8 cm. The film immediately became blue, and the color intensified with continued irradiation in a manner typical of diacetylene polymerizations. After irradiation the PDA film was stripped off the glass to give a 10-μm thick, dark purple-blue, flexible free standing film. The absorption was too intense for spectrophotometric measurement; therefore, a thinner film was prepared from 0.25 mM lipid. Figure 1 shows the absorption spectra obtained by irradiation of this film with 254 nm light for various times. Visible PDA absorbance is readily detected after a few seconds. Note that photopolymerization of the film can occur at room temperature, because the T_c of lipid multilayers shifts to higher temperatures as they are dehydrated.[14] Thus the cast multilayer film of **1** has sufficient molecular order to allow the topotactic polymerization to proceed. We estimate the extent of polymerization of the diacetylenic lipids to be about 75% after 180 s exposure. At this point about 20% monomeric lipid may be extracted from the photopolymerized film. Although monomer may be extracted from the films, the free standing polymer film was not disrupted by treatment at room temperature, with any of the following organic solvents: chloroform, chlorobenzene, THF, DMSO, and DMF.

The absorption maxima of PDAs are indicative of the length of the polymer chain and/or the order of the polymer structure. Longer and/or more highly ordered PDAs, e.g., fatty acid diacetylene monolayers, exhibit absorption maxima at 650 nm or longer (blue form),[11] whereas shorter and/or less ordered PDAs, e.g., phosphatidylcholine diacetylene bilayers, show absorption maxima at 540 nm (red form).[15] The absorption maxima of poly-**1** in extended bilayers is about 640 nm and shifted to somewhat shorter wavelength (610 nm) for poly-**1** formed in cast multilayers. The film of poly-**1** shows reversible thermochromic behavior. As illustrated in Figure 2, if the film of poly-**1** was warmed from room temperature to 50 °C, the color changes to orange-red (534 nm) and then back on cooling.

Cast films were also prepared from the bilayers of poly-**1** in a manner similar to that described above for the bilayers of **1**. After drying a red-purple film was obtained. This prepolymerized film was significantly less stable to treatment with organic solvents, e.g., chloroform, than the PDA film obtained by first casting followed by polymerization of **1**.

Ordered multibilayer structures formed by lipid hydration and careful casting appear to be required for the formation of stable free standing films from **1**. As a control crystals of **1** were dissolved in chloroform and spread on a glass surface. After drying, a thin layer of lipid crystals was formed. Ultraviolet irradiation produced the typical PDA color, but films could not be obtained.

In conclusion, these data substantiate the retention of bilayer characteristics in cast multibilayer films as suggested by Kunitake and co-workers. These observations indicate that in some circumstances cast multibilayer are a possible alternate to Langmuir–Blodgett films for the formation of ordered thin film molecular assemblies. Moreover we have prepared the first insoluble, free standing PDA films. Investigations of the thermochromic, electrical conducting, and nonlinear optical properties of these PDA films will be described elsewhere.

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Design and Chemical Synthesis of a Sequence-Specific DNA-Cleaving Protein†

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We report the design and chemical synthesis of a sequence-specific DNA-cleaving protein consisting wholly of naturally occurring α-amino acids. The tripeptide, H-glyglyhis-OH (GGH), which is a consensus sequence for the copper-binding domain of serum albumin, was attached to the amino terminus of the DNA-binding domain of Hin recombinase (residues 139–190) to afford a new 55-residue protein, GGH(Hin 139–190) with two structural domains each with distinct functions, sequence-specific recognition, and cleavage of double helical DNA (Figure 1). The designed protein was synthesized by solid-phase techniques and shown, by footprinting, to be competent to bind at μM concentrations to four Hin sites, each 13 base pairs in length. In the presence of Cu(II), hydrogen peroxide, and sodium ascorbate, strong cleavage of DNA by GGH(Hin 139–190) occurs at one of the four sites by oxidative degradation of the deoxyribose backbone.

A 52-residue peptide identical with the carboxy terminal domain of Hin recombinase (190 amino acids) has been shown to bind to Hin recombination half sites (13 bp) and to inhibit Hin activity.[1] We recently described the conversion of this sequence-specific DNA-binding protein, Hin(139–190), into a sequence-specific DNA-cleaving protein by covalent attachment of an iron chelator, ethylenediaminetetraacetic acid (EDTA), to the amino-terminus.[2,3] In the presence of Fe(II) and reducing agent, EDTA-Hin(139–190) oxidatively cleaves DNA at Hin binding sites, revealing the base position and minor groove location of the amino terminus of the peptide when bound to DNA.[2] The issue arises whether the unnatural amino acid, EDTA, could be replaced by a sequence of α-amino acids that bind transition metals capable of facilitating oxidative cleavage of DNA.

The tripeptide GGH binds Cu(II) in a 1:1 complex over the pH range 6.5–11 with a dissociation constant of 1.2×10^{-16} M^{-1}.[4] A crystal structure of Cu(II)·GGH reveals square-planar complexation of the Cu(II) by an imidazole nitrogen, two deprotonated

† Dedicated to Professor E. T. Kaiser.

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