The final refined model of 1956 protein atoms and 218 water molecules[12] gives a crystallographic R value of 0.164 for the 21 779 reflections between 10- and 1.8-Å resolution. The *upper limit* for the average error in atomic positions, from the variation of R with resolution, is 0.15 Å, but for well-ordered parts of the structure, such as the copper site, the error is much less. Our estimate of the standard deviations in the copper site geometry, based on the level of agreement between the two molecules,[13] is ~0.05 Å for Cu–ligand bond distances and ~3.0° for ligand–Cu–ligand angles.

The copper site is shown diagrammatically in Figure 1, and relevant bond distances and angles in Table I. The copper atom makes three strong bonds, with the thiolate sulfur of Cys-112 (mean Cu–S 2.13 Å) and the imidazole nitrogens of His-46 and His-117 (mean Cu–N 2.06 and 1.96 Å, respectively). These distances, including the unusually short Cu–S bond, agree very closely with those deduced from X-ray absorption spectroscopy on azurin[14] and X-ray crystallographic and X-ray absorption spectroscopy[15] measurements on plastocyanin. The three ligands form a distorted trigonal-planar arrangement about the copper atom. Much longer approaches are made to the thioether–sulfur of Met-121 on one side of this plane (Cu–S 3.13 Å) and the peptide carbonyl oxygen of Gly-45 on the other side (Cu–O 3.11 Å), making overall a distorted axially elongated trigonal-bipyramidal arrangement.

Whether the coordination geometry is best described as trigonal planar, trigonal pyramidal, or trigonal bipyramidal depends on whether the Cu–S(Met) and Cu–O approaches are regarded as weak bonds or not. The Cu–S(Met) interaction almost certainly is, while the Cu–O distance of 3.1 Å is probably right at the limit for a weak Cu–O bond.[16] We note, however, that this carbonyl oxygen is buried in a nonpolar environment, with no possibility of interaction with any other polar or polarizable atom, and that this copper–oxygen (carbonyl) interaction has been detected by NMR spectroscopy.[17] The relative positions of the copper atom and these more distant axial groups probably has a role in tuning the redox potential in different proteins.

The copper atom lies very close to the N_2S plane formed by the three strong ligands, its deviation (toward Met-121) being 0.13 Å in molecule **1** and 0.08 Å in molecule **2**. During the refinement, and again at the end, we tried artificially moving the copper about 0.3 Å toward Met-121 (decreasing the Cu–S distance and increasing Cu–O). In each case, refinement brought it right back to its "in-plane" position. The bond angles within the trigonal plane are quite irregular (mean values 103°, 119°, and 136°) emphasizing the low symmetry of this site. This low symmetry must clearly be taken into account in any theoretical analyses of (for example) ESR spectra. The long approaches to S_δ(121) and O(45) are both bent away from a precise axial disposition. The copper site groups are tightly constrained by the surrounding protein structure, through hydrogen bonds (to His-46 $N_{\epsilon i}$ and Cys-112 S_γ) and van der Waals contacts.

Finally, the structure described above is entirely consistent with that determined earlier for *Pseudomonas aeruginosa* azurin[18] (although the latter has not yet been refined) and this, together with the high level of consistency between the two independent molecules in our crystal asymmetric unit, further validates the above results.

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(12) Coordinates and thermal parameters have been deposited with the Brookhaven Protein Data Bank, Brookhaven National Laboratory, Upton, NY 11973.

(13) Superposition of the two copper sites and their immediate surrounds (112 atoms) gives an rms difference in atomic positions of 0.14 Å. A better guide to the likely error in copper site geometry, however, is given by comparison of distances and angles that were not restrained during refinement. For copper bond lengths and angles the rms *differences* between the two molecules are 0.05 Å and 3.6° and for hydrogen bond lengths and angles 0.10 Å and 6.2° (for 55 pairs of hydrogen bonds). Standard deviations should be less than these values.

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Reversible Thermochromism in Photopolymerized Phosphatidylcholine Vesicles

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There is substantial interest in modified phospholipids that can be polymerized. Adding a polymer backbone to noncovalent lipid assemblies such as liposomes and Langmuir/Blodgett films should make them more rugged and useful in biotechnology.[1-4] Perhaps the best known have been phosphatidylcholines incorporating diacetylenic moieties within their acyl side chains, which can be polymerized by ultraviolet light or γ radiation.[5,6] The solid-state polymerization is accompanied by the development of a characteristic blue color, which can be converted to red upon heating or treatment with solvents;[7] in some cases, cooling reverses the change. We report here that an aqueous suspension of polydiacetylenic phospholipid vesicles can exhibit reversible thermochromism and examine the implications of this phenomenon for models of polydiacetylene thermochromism.[6,8,30]

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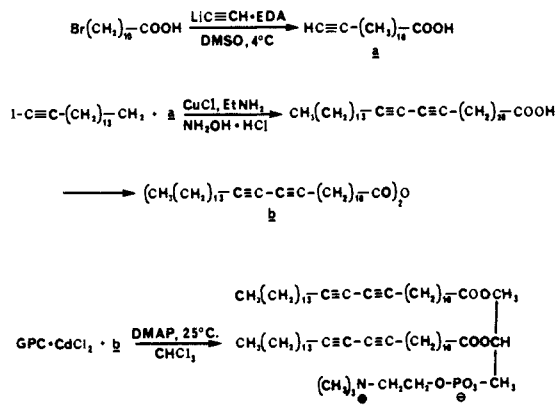
Scheme I



To understand the influence of the diacetylene position within the acyl side chains on bilayer structure and the formation of novel lipid microstructures,[9,10] we synthesized 1,2-bis(12,14-nonacosadiynoyl)-*sn*-glycero-3-phosphorylcholine, as described in Scheme I.

12,14-Nonacosadiynoic acid was made from 11-bromoundecanoic acid and 1-iodohexadec-1-yne by a new, general method.[11] The diacetylenic fatty acids were converted to their anhydride form by DCC/$CHCl_3$ and esterified with egg lecithin derived *sn*-glycero-3-phosphorylcholine/$CdCl_2$ complex as previously described.[12] The compounds were characterized by TLC on silica gel, IR, NMR, melting point, mass spectroscopy, and elemental analysis.[13]

An aqueous dispersion of multilamellar vesicles of 1,2-bis(12,14-nonacosadiynoyl)phosphatidylcholine was prepared by drying 2 mg of the lipid (in $CHCl_3$) in a quartz vial and resuspension in 0.5 mL of water by vortexing at room temperature, under subdued light. The dispersion[14] was polymerized at 3 °C

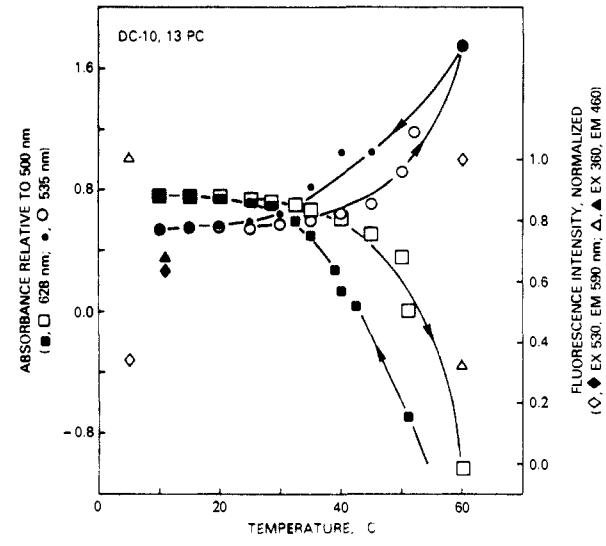


Figure 1. Effect of temperature on absorption and fluorescence of aqueous dispersions of polymerized 1,2-bis(12,14-nonacosadiynoyl)-phosphatidylcholine. The peak absorbance of the red form at 535 nm minus the absorbance at 500 nm is depicted as the temperature is increased (○) then decreased (●); similarly, the peak absorbance of the blue form (628 nm) minus the absorbance at 500 nm is shown as the temperature is raised (□) and lowered (■). Normalized fluorescence intensities are shown for the red form at low and high temperatures (◇) and after return to low temperature (◆); they are also depicted for the ultraviolet-excited fluorescence of the blue form at low and high temperatures (△) and upon return to low temperature (▲).

(well below the 64 °C main phase transition of the unpolymerized lipid[8]) by 30-s illumination with 25 W of 254-nm light in a Rayonette reactor. The resulting dark blue suspension contained less than 1% intact monomer by TLC and turned orange upon warming above 50 °C, as has been previously observed with other polydiacetylenes.[7] However, unlike many other polydiacetylenes,[15] the poly(bis(12,14-nonacosadiynoyl)) lipid returns to its original blue color upon cooling. This is depicted in Figure 1, where the temperature dependence of the absorbance peaks of the blue and red forms (628 and 535 nm, respectively) relative to absorbance at 500 nm are shown. The thermochromic cycle can be repeated if the sample is not further photopolymerized. The fluorescence of the two forms is also reversibly temperature-dependent, suggesting that the polymer electronic structure is the same before and after cooling. In particular, the very weak UV-excitable violet fluorescence of the blue form[16] decreases with temperature, while the reddish emission characteristic of the red form[17] increases with temperature; the relative strength of the two emission bands reverses upon cooling (see Figure 1). This effect does not appear fully reversible, probably because the strong illumination in the fluorimeter further polymerized the sample;[18] note that each phospholipid molecule has two diacetylenes and thus there may be groups available to cross-link the polymer, even though no unincorporated lipid remains after the original polymerization.

Several theories have been advanced to explain the thermochromism of polydiacetylenes.[7,19-21] The facts that our polydiacetylene is contained within a phospholipid bilayer and is reversibly thermochromic within a modest temperature range impose important constraints on any explanation. The diradicals and carbenes observed in low-temperature solid-phase photopolymerization[22] are probably transient intermediates in aqueous solution near room temperature and thus do not produce the observed colors. Moreover, our absorbance and fluorescence data suggest that the polymer does revert to the original electronic structure upon cooling, which would require these high-energy species to be created and destroyed or interconverted by a modest thermal change. Since the polymer is confined roughly within a plane parallel to the bilayer lamellae, it is unlikely to undergo a coil-to-rod transition[19] or form three-dimensional aggregates[20] without grossly distorting the bilayer as well. However, optical

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microscopy of the vesicles showed no distortion. An overt diene-yne–butatriene transition might explain the data,[21,29] but it thus would be coincidental that an electronic isomerization would be thermally induced near a temperature where the bilayer structure is known to change, according to DSC.[8] Evidently, these models fit the data poorly.

The data above, along with the known temperature-dependent changes in structure of other phosphatidylcholines,[23,24] suggest that thermally induced changes in bilayer structure alter the polymer conformation, which controls the thermochromism. More precisely, the increased motional freedom of the acyl side chains caused by raising the temperature might allow a more disordered (and less coplanar[25]) polymer with a lower characteristic conjugation length and thus a blue-shifted absorbance spectrum. Reversible thermochromism is observed because the bilayer morphological changes are thermally reversible.

Our proposal is consistent with other observations of thermochromism in polydiacetylenes,[6,7,30] particularly given the similarity of the absorbance and/or fluorescence spectra of the polymer in crystals,[26] in solution[27] and as mono- and multilayers on substrates,[7,17] to those in an aqueous bilayer dispersion.[6,8,16,28,30] Moreover, the greatest effect occurs (Figure 1) near the principal phase transition temperature of the polymerized phospholipid[8] (64.3 °C), suggesting that the electronic change is correlated with the structural change. The fact that reversible thermochromism is much less apparent or absent in other polymerized diacetylenic phosphatidylcholines,[6,30] even those with the same chain length fatty acid (but with differing placement of the diacetylene moiety), suggests the influence subtle changes in the bilayer structure can have on the polymer conformation. Efforts are under way to understand the influence of structure on this phenomenon in greater detail.

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Free Radical Macrocyclization

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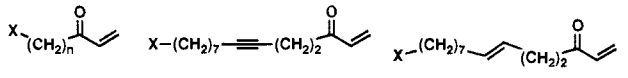
Free radical reactions have been used increasingly in recent years for the synthesis of organic molecules.[1-4] The development of radical synthetic methodology is primarily the result of mechanistic studies of the past 40 years that have identified the important pathways available and have provided detailed reactivity guidelines[5-7] for free radical reactions. Mechanistic studies provided a framework for understanding free radical cyclization,[7-9] for example; and this approach has been extensively utilized for the construction of natural products in subsequent elegant investigations.[10,11] The use of radical cyclization has been limited primarily to the construction of five- and six-membered rings and has not been used for larger ring systems. In fact, the rate of cyclization decreases from 2×10^5 s^{-1} for 5-exo cyclization (5-hexenyl at 25 °C) to <70 s^{-1} for the corresponding 7-exo reaction.[6] It occurred to us that radical cyclization might prove to be possible for larger rings if substrates were chosen that gave consideration to steric and polar effects in the cyclization reaction.[5,13,14] Carbon radicals are nucleophilic and electron-withdrawing substituents activate alkenes toward addition of such radicals. Furthermore, substrates chosen that minimize steric effects in the cyclization transition state are preferred, since this can be a dominant factor in addition reactions. We report here the results of our studies with several substrates for radical macrocyclization. The reaction proves to be a useful one, with yields as high as 75–80% being possible for some substrates.

Table I. Formation of Macrocyclic Ketones via Radical Cyclization

substrate, mM	Bu_3SnH, mM	products (% yield)[a] cyclic	acyclic
1c, 3.3	3.6	**7** (63)	**1f** (22)
1b, 14	26[b]	**7** (30)	**1f** (37)
1d, 6.7	7.4	**9**[c] (54)	**1g** (16)
1a, 3.0	4.0	**8** (15)	**1c** (27)
2a, 5.0	5.5	**11** (76)	**2b** (6)
3a, 5.0	5.5	**10** (78)	**3b** (8)

[a] Yields based on GC. GC yields were confirmed by HPLC isolation on silica gel (94:4, hexane/ethyl acetate). [b] Syringe addition of alkyl halide and tin hydride over 3 h. [c] Reference 22.

The acyclic substrates **1–3** were available by straightforward procedures from readily available starting materials. The compounds **1b** and **1c**, for example, were prepared from 12-bromo-1-dodecanol[15] by pyridinium chlorochromate[16] oxidation to the aldehyde (74%) followed by addition of vinylmagnesium bromide affording an allylic alcohol **4**, $Br(CH_2)_{11}CHOHCH{=}CH_2$ (76%). Oxidation of **4** with Jones reagent[17] gave the ketone **1b** (93%) which was converted to the iodide **1c** by reaction with NaI in methyl ethyl ketone (96%).[18] In a similar manner, **2** was prepared from the bromo alcohol **5**, $Br(CH_2)_7C{\equiv}C(CH_2)_3OH$, and **3** from



1 a; X=I, n=7
b; X=Br, n=11
c; X=I, n=11
d; X=I, n=15
e; X=H, n=7
f; X=H, n=11
g; X=H, n=15

2 a; X=I
b; X=H

3 a; X=I
b; X=H

the bromo aldehyde **6**, $Br(CH_2)_7CH{=}CH(CH_2)_2CHO$. Compound **5** was synthesized by acetylenic coupling of 1,7-dibromoheptane and pent-4-yn-1-ol ($LiNH_2$, 30%) and **6** was constructed by a Claisen rearrangement (120 °C, 24 h, 90%) of the vinyl ether prepared from the alcohol $Br(CH_2)_7CHOHCH{=}CH_2$ by reaction with ethyl vinyl ether and mercuric trifluoroacetate (75%).[18]

Several attempts were made to cyclize the bromide precursor **1b**, utilizing tributyltin, triphenyltin, or tributylgermanium hydrides. Reactions were carried out at concentrations ranging from 1 to 100 mM of bromide and with equivalent or excess hydride

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