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Photochromism of spirooxazines with elements of lipid complementarity in solution and liposomes

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Abstract

Two new phenanthryl-based spirooxazines that contain elements of lipid complementarity have been synthesized. The effect of substitution on their photochromic properties and relative orientation within the lipid bilayer of dipalmitoylphosphatidylcholine large unilamellar vesicles (LUVs) has been examined. Absorption spectroscopy was used to determine the rate constants for thermal ring closure in LUVs, and solvents of varying polarity and viscosity. The rate constant for thermal ring closure was shown to increase with increasing solvent polarity. However, when solvent polarity was kept relatively constant, a slight decrease in this rate constant with increasing solvent viscosity was observed for a tetradodecoxy-substituted spirooxazine. Upon inclusion of these lipid-like spirooxazine analogues in LUVs, the rate constant for thermal ring closure was significantly smaller when the degree of substitution was high. As well, the relative orientation of these photochromes in the lipid bilayer was determined based on their solvatochromic properties, and also correlated to differences in substitution.

Keywords: Photochromism; Spirooxazine; Liposome

1. Introduction

Photochromic spirooxazines have been extensively studied for their potential application in optoelectronic molecular devices. Their photochromism has been investigated in solution and various other matrices such as liquid crystals and liposomes [1–4]. Spirooxazines are more robust than the structurally similar spiropyrans, which enhances their practical applicability. These photochromes undergo isomerization from the closed-ring spiro (SP) form to the open-ring merocyanine (MC) form upon photoexcitation with ultraviolet (UV) light (Chart 1). As a result of the extended conjugation length in the MC form, its absorption is observed in the visible region well separated from that of the SP form.

Photoinduced isomerization of the SP to MC form occurs on the picosecond time scale [5], whereas the half-life for thermal ring-closure is typically much slower, over a period of milliseconds to seconds [6,7]. The ring-closing reaction can be accelerated photochemically upon excitation of the MC form with visible light (Vis). In general, the introduction of substituents, which destabilize the zwitterionic resonance structure of the MC form through electron withdrawal from the indoline moiety, or electron donation into the oxazine moiety, has been shown to increase the rate constant for thermal ring closure. Further, decreasing the polarity of the local environment has been shown to increase this rate constant and cause a hypsochromic shift in the wavelength of maximum absorption of the MC form (λ_{MC}). The steric bulk of substituents in either the indoline or oxazine moieties has been shown to affect the rate of thermal ring closure as well. In particular, bulky substituents can hinder bond rotation required for the various stereoisomers of the MC form prior to ring closure (e.g. *cis–trans–cis* to *trans–trans–cis*) [3,4,8,9].

Phospholipids are amphiphilic because they possess hydrophobic and hydrophilic regions within the same molecule. Consequently, these amphiphiles can form self-assembled structures such as liposomes. In this study, two new phenanthryl-based spirooxazines that contain elements of lipid complementarity have been synthesized for their potential application in photoregulated liposomal drug delivery. These spirooxazines contain different substituents that influence their relative hydrophobicity. As a result, we expect these photochromes will

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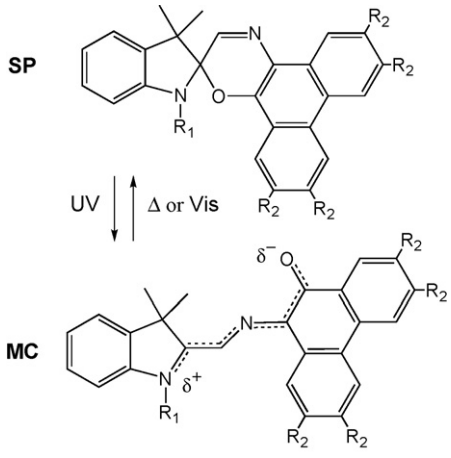


1: R_1=CH_3 R_2=$O(CH_2)_{11}CH_3$
2: R_1=$(CH_2)_{12}Br$ R_2=H
3: R_1=$(CH_2)_{12}N^+(Et)_3$ R_2=H
4: R_1=CH_3 R_2=H

Chart 1.

be included in different regions of the lipid bilayer. As prospective membrane disruptors with reversible photocontrols, it is important we demonstrate that they are included in the lipid bilayer of liposomes and that their photochromism is conserved. In particular, the effects of substitution, and the polarity and viscosity of the local environment on the photochromic properties of these spirooxazines have been examined. We have shown that the kinetics for thermal ring closure is sensitive to the polarity of the local environment, and viscosity when the degree of substitution is high. In addition, based on the solvatochromic properties of these photochromes, their relative orientation in the lipid bilayer of dipalmitoylphosphatidylcholine (DPPC) large unilamellar vesicles (LUVs) was determined, and also shown to be dependent on the type of substitution. These spirooxazine analogues, with improved lipid complementarity, will be used in the development of liposomes with reversible photocontrols over membrane permeability, and may have interesting implications in the development of optoelectronic devices based on liquid crystals.

2. Experimental

2.1. Synthesis

2.1.1. Instrumentation

^{1}H and ^{13}C NMR spectra were recorded at 200 MHz on a Bruker AC200 QNP spectrometer (Milton, ON, Canada) or at 300 MHz on a Varian Mercury plus spectrometer (Palo Alto, CA, USA). High-resolution mass spectra 1 analyses were carried out by the University of Saskatchewan on a VG 70SE mass spectrometer (Manchester, UK) that was operated in electrospray ionization mode. Elemental analyses were carried out by the University of Alberta on a Carlo Erba CHNS-O EA1108 elemental analyzer (Lakewood, NJ, USA).

2.1.2. Materials

All reactants (>99%, Aldrich, Oakville, ON, Canada), deuterated solvents (99.9 atom% D, Aldrich) were used as received. Preparative thin-layer chromatography (TLC) was carried out on aluminum-backed ALUGRAM SIL G/UV254 plates (Rose Scientific, Edmonton, AB, Canada) and visualized by a 6 W UVAC-18 dual wave (365 nm/254 nm) ultraviolet handheld lamp from UltraLum (Claremont, CA, USA). Flash column chromatography was performed on silica gel (200–400 mesh, 60 Å, Aldrich).

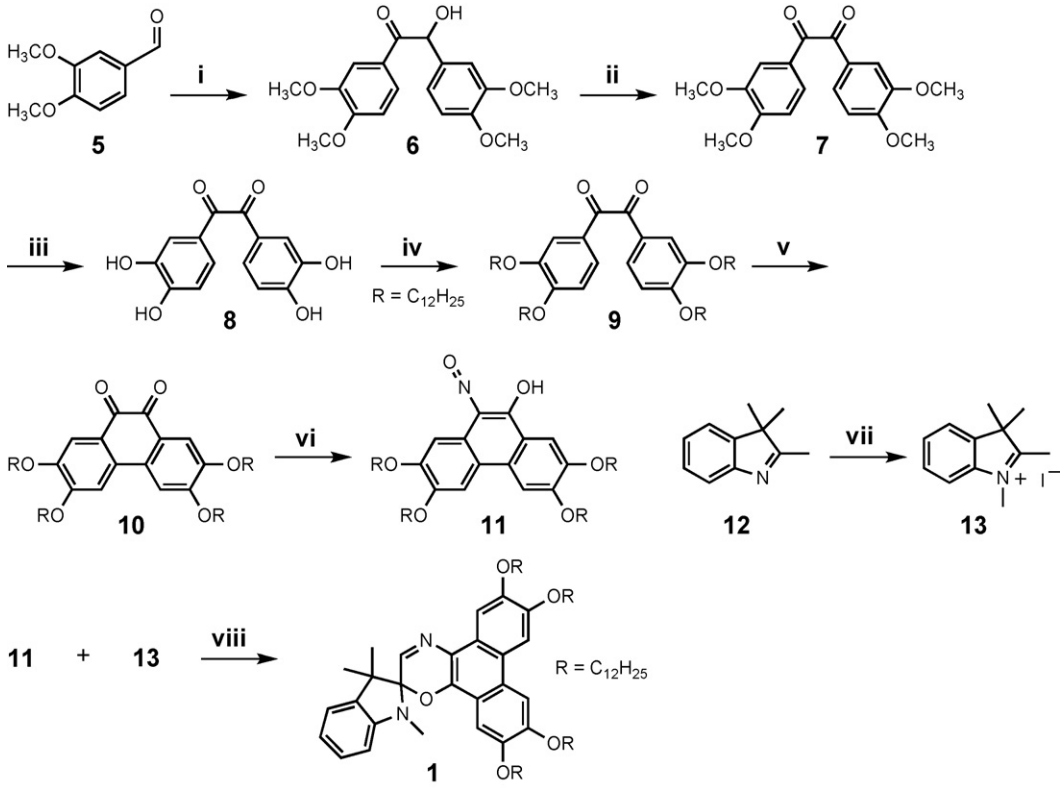
2.1.3. Procedures (Schemes 1 and 2)

*2.1.3.1. 2-Hydroxy-(2,2′,3,3′-tetramethoxy)-1,2-diphenylethanone (**6**).* The benzoin condensation was adapted from known procedures [10,11]. A solution of KCN (3.40 g, 52.2 mmol) in H_2O (20 mL) was added to a solution of 3,4-dimethoxybenzaldehyde (**5**, 20.0 g, 120 mmol) in EtOH (36 mL) and refluxed under N_2 for 50 h. The organic layer was extracted with $CHCl_3$ (3 × 150 mL), washed with H_2O (75 ml), and dried over Na_2SO_4. Compound **6** was obtained in a 90% yield (17.9 g) as a viscous yellow-orange liquid. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 3.6–4.0 (d, J = 12.3 Hz, OCH_3, 12H), 4.6 (d, J = 1.57 Hz, HCOH, 1H), 5.8 (s, OH, 1H), 6.7–6.9 (m, H-5,5′, H-6,6′, 4H), 7.5 (s, H-2,2′, 2H) ().

*2.1.3.2. (2,2′,3,3′-Tetramethoxy)-1,2-diphenylethanedione (**7**).* The oxidation of **6** was adapted from known procedures [10,11]. A solution of **6** (17.9 g, 53.8 mmol) in pyridine (97 mL) was added to a solution of $CuSO_4 \cdot 5H_2O$ (40.3 g, 161 mmol) in H_2O (73 mL). The reaction mixture was refluxed for 7.25 h, H_2O (120 mL) was added and the resulting precipitate was vacuum filtered. Compound **7** was obtained in a 30% yield (5.32 g) as needle-like, yellow crystals. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 3.9 (s, OCH_3, 12H), 6.8–6.9 (d, J = 8.4 Hz, H-5,5′, 2H), 7.4–7.5 (dd, J = 1.9, 8.4 Hz, H-6,6′, 2H), 7.6 (d, J = 1.8 Hz, H-2,2′, 2H).

*2.1.3.3. (2,2′,3,3′-Tetrahydroxy)-1,2-diphenylethanedione (**8**).* Adapted from known procedures [10,11], a solution of **7** (5.35 g, 16.2 mmol) in glacial AcOH (290 mL) and 48% HBr (290 mL) was refluxed under N_2 for 9.5 h. The reaction mixture was extracted with Et_2O, and the organic phase washed with H_2O (30 mL) and 5% $NaHCO_3$ (20 mL). The solution was dried over Na_2SO_4 and the solvent evaporated under vacuum. Compound **8** was obtained in a 67% yield (2.99 g) as a black-yellow solid. ^{1}H NMR (200 MHz, DMSO-d_6, δ): 6.9 (d, J = 8.3 Hz, H-5,5′, 2H), 7.2 (m, H-1,1′,4,4′, 4H), 8.1 (s, OH, 1H).

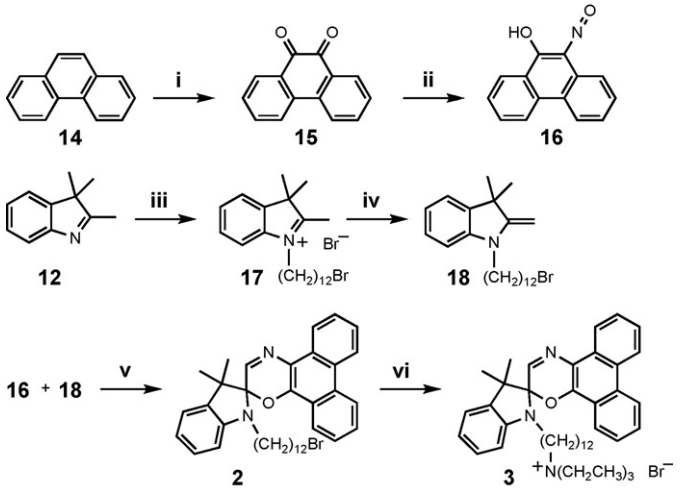
*2.1.3.4. (2,2′,3,3′-Tetradodecyloxy)-1,2-diphenylethanedione (**9**).* The alkylation of **8** was adapted from known procedures [10,11]. A solution of ground anhydrous K_2CO_3 (4.03 g, 29.2 mmol) in 1-bromododecane (7.26 g, 29.2 mol) was added to a solution of **8** (2.00 g, 7.29 mmol) in *N,N*-dimethylacetamide (45 mL). The reaction mixture was refluxed under N_2 for 17 h. The mixture was extracted with $CHCl_3$ (3 × 170 mL) and the organic phase washed with H_2O. The mixture was dried over Na_2SO_4 and the solvent evaporated under vacuum. The resulting precipitate was recrystallized twice from EtOAc



Scheme 1. Reaction conditions: (i) KCN, H_2O; (ii) $CuSO_4{\cdot}5H_2O$, pyridine; (iii) AcOH, HBr; (iv) K_2CO_3, $C_{12}H_{25}Br$, *N*,*N*-dimethylacetamide; (v) VOF_3, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (vi) $H_2NOH{\cdot}HCl$, EtOH, $CHCl_3$; (vii) CH_3I, 2-butanone; (viii) piperidine, 2-propanol.

(150 mL). Compound **9** was obtained in a 38% yield (2.63 g) as yellow crystals. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.8–1.0 (m, CH_2CH_3, 12H), 1.2–1.9 (m, $CH_2(CH_2)_{10}CH_3$, 80H), 4.0 (t, J = 6.5 Hz, $CH_2(CH_2)_{11}CH_3$, 8H), 6.8 (d, J = 8.5 Hz, H-6,6′, 2H), 7.4 (dd, J = 8.3, 1.8 Hz, H-5,5′, 2H), 7.6 (d, J = 1.9 Hz, H-2,2′, 2H).

*2.1.3.5. 2,3,6,7-Tetra-n-dodecyloxyphenanthrene-9,10-dione (**10**).* Adapted from known procedures [12,13], oxidative coupling was performed via the addition of VOF_3 (0.233 g, 1.88 mmol) to a solution of **9** (0.778 g, 0.821 mmol) and $BF_3{\cdot}Et_2O$ (0.245 g, 1.72 mmol) in anhydrous CH_2Cl_2 (4.1 mL) under Ar. The mixture was stirred for 0.5 h and quenched with 10% aqueous solution of citric acid (8.3 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phases were washed with H_2O (2 × 10 mL) and dried over Na_2SO_4. The product was purified by column chromatography (CH_2Cl_2). Compound **10** was obtained in a 53% yield (0.410 g) as a red solid. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.8–1.0 (m, $CH_2(CH_2)_{10}CH_3$, 12H), 1.1–2.0 (m, $CH_2(CH_2)_{10}CH_3$, 80H), 4.06 (t, J = 6.6 Hz, $CH_2(CH_2)_{10}CH_3$, 4H), 4.2 (t, J = 6.4 Hz, $CH_2(CH_2)_{10}CH_3$, 4H), 7.08 (s, H-4,5, 2H), 7.50 (s, H-1,8, 2H). ^{13}C NMR (200 MHz, $CDCl_3$, δ): 14.3, 22.9–32.1, 69.4, 69.8, 107.5, 113.3, 124.7, 131.4, 149.7, 155.8, 179.4.



Scheme 2. Reaction conditions: (i) H_5IO_6, CrO_3, CH_3CN; (ii) $H_2NOH{\cdot}HCl$, EtOH, $CHCl_3$; (iii) $Br(CH_2)_{12}Br$, $CHCl_3$; (iv) Na_2CO_3, H_2O; (v) EtOH; (vi) $N(CH_2CH_3)_3$, C_6H_6.

*2.1.3.6. 9-Hydroxy-10-nitroso-2,3,6,7-tetra-n-dodecyloxyphenanthrene (**11**).* Adapted from a known procedure [14], a solution of **10** (0.500 g, 0.529 mmol) and $H_2NOH{\cdot}HCl$ (0.0368 g, 0.529 mmol) in $CHCl_3$ (12.9 mL) and EtOH (14 mL) was refluxed for 2 h. The reaction mixture was distilled (13 mL) and the product precipitated upon cooling of the reaction mixture. The product was purified twice by column chromatography (1:1 $CHCl_3$:hexanes). Compound **11** was obtained in a 83% yield (0.424 g) as a red solid. mp = 100.4–101.6 °C. IR (KBr) ν_{max}: 3250–3050 (br, OH), 2956–2849 (CH, aliphatic), 1597 (N=O), 1525 (C=C, aromatic), 1505 (C=C, aromatic), 1467 (CH), 1428 (CH), 1080 (s, COR) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.92–2.1 (m,

$OCH_2(CH_2)_{10}CH_3$, 92H), 4.0–4.3 (m, $OCH_2(CH_2)_{10}CH_3$, 8H), 7.2 (d, $J = 7.3$ Hz, H-4,5, 2H), 7.7 (d, $J = 11.5$ Hz, H-1,8, 2H), 17.3 (s, O*H*, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 14.1, 22.6, 26.0, 26.1, 29.1–29.7, 31.9, 68.8, 69.0, 69.2, 69.8, 105.5, 106.7, 107.8, 110.4, 121.8, 121.8, 122.7, 133.7, 143.5, 148.7, 150.1, 150.5, 156.4, 180.5. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{62}H_{105}NO_6$, 960.8015; found, 960.8122. Anal. Calcd for $C_{62}H_{105}NO_6$: C, 77.53; H, 11.02; N, 1.46. Found: C, 77.23; H, 11.04; N, 1.74.

*2.1.3.7. N,2,3,3-Tetramethylindolium iodide (**13**).* Adapted from known procedures [15,16], a solution of 2,3,3-trimethylindolenine (**12**) (2.00 g, 12.6 mmol) and CH_3I (1.78 g, 12.6 mmol) in 2-butanone (14 mL) was refluxed for 12 h. The precipitate was filtered and recrystallized from EtOH (90 mL). Compound **13** was obtained in a 68% yield (2.55 g) as long white crystals. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.41 (s, $C(CH_3)_2$, 6H), 2.65 (s, CCH_3, 3H), 3.85 (s, NCH_3, 3H), 7.5–7.8 (m, Ar, 4H).

*2.1.3.8. 1,3-Dihydro-3,3-dimethyl-6′,7′,10′,11′-tetra-n-dodecyloxy-1-methylspiro[2H-indole-2,3′-[3H]phenanthr[9,10b][1,4]oxazine] (**1**).* Adapted from a known procedure [2], a solution of piperidine (0.030 g, 0.354 mmol) in 2-propanol (1.35 mL) was added to a refluxing mixture of **11** (0.340 g, 0.354 mmol) and **13** (0.107 g, 0.354 mmol) in 2-propanol (16.5 mL) and $CHCl_3$ (1 mL) over 5 min. The reaction mixture was refluxed for 3 h and then cooled. The precipitate was vacuum filtered and the product was purified by column chromatography (3:1 $CHCl_3$:hexanes). Compound **1** was obtained in a 13% yield (0.050 g) as a viscous blue oil. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.79–0.83 (m, $OCH_2(CH_2)_{10}CH_3$, 12H), 1.20–1.89 (m, $OCH_2(CH_2)_{10}CH_3$, $C(CH_3)_2$, 86H), 2.70 (s, NCH_3, 2H), 3.89–4.19 (m, $OCH_2(CH_2)_{10}CH_3$, 8H), 6.49 (d, $J = 7.8$ Hz, H-4, 1H), 6.81 (t, $J = 7.5$ Hz, H-5, 1H), 7.02 (d, $J = 6.0$ Hz, H-7, 1H), 7.11 (t, $J = 7.2$ Hz, H-6, 1H), 7.27 (s, H-3′, 1H), 7.66 (m, H-8′,9′, 2H), 7.95 (s, H-12′, 1H). ^{13}C NMR (300 MHz, $(CD_3)_2CO$, δ): 14.2, 21.2. 23.1, 25.4, 26.6, 26.8, 26.8, 26.8, 29.9–30.4, 31.9, 32.5, 51.7, 69.1, 69.5, 69.5, 70.0, 99.5, 104.9, 105.2, 106.5, 107.4, 107.7, 118.3, 119.7, 120.2, 121.0, 122.1, 125.3, 127.0, 128.5, 136.8, 138.5, 148.3, 148.8, 149.7, 150.0, 150.7, 151.4. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{74}H_{119}N_2O_5$, 1115.9113; found, 1115.9121. UV–vis (toluene) λ_{max}, nm (ε): 364 (740), 568 ($(9.2 \pm 4.6) \times 10^3$). Note that the ε at λ_{MC} was calculated using equilibrium constants determined by NMR spectroscopy ($K(C_6D_6) = [MC]/[SP] = 0.008 \pm 0.004$) [2,17–19]. Satisfactory elemental analysis was not obtained because the product was an oil that we were unable to recrystallize. However, the structure was confirmed by high-resolution mass spectrometry.

*2.1.3.9. Phenanthrene-9,10-dione (**15**).* Adapted from a known procedure [20], a solution of H_5IO_6 (44.8 g, 197 mmol) and CrO_3 (0.561 g, 5.61 mmol) in CH_3CN (650 mL) was combined with vigorous stirring and cooled to 5 °C. A solution of phenanthrene (5.00 g, 28.0 mmol) in CH_3CN (50 mL) was added to the CrO_3 solution and stirred for 1 h at 5 °C. The solvent evaporated under vacuum and dissolved in CH_2Cl_2 (150 mL) and H_2O (120 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were washed with 1 M NaOH (3 × 20 mL), brine (25 mL), and dried over $MgSO_4$. The solvent was evaporated under vacuum and compound **15** was recrystallized from EtOH and obtained in a 55% yield (3.23 g) as yellow crystals. mp 208.5–210.6 °C. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.47 (td, $J = 0.95$, 7.83 Hz, H-2,7, 2H), 7.71 (td, $J = 1.53$, 7.70 Hz, H-3,6, 2H), 8.01 (dt, $J = 0.53$, 8.13 Hz, H-1,8, 2H), 8.19 (dd, $J = 1.54$, 7.78 Hz, H-4,5, 2H).

*2.1.3.10. 9-Hydroxy-10-nitrosophenanthrene (**16**).* Adapted from a known procedure [14], a solution of **15** (3.50 g, 16.8 mmol) and $H_2NOH{\cdot}HCl$ (3.27 g, 47.1 mmol) in EtOH (70 mL) and $CHCl_3$ (30 mL) was refluxed for 2.5 h. The precipitate was filtered and recrystallized from EtOH. Compound **16** was obtained in a 53% yield (2.00 g) as yellow-orange crystals. mp 155.6–157.3 °C (lit. 157 °C) [14]. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.51 (m, 3H), 7.79 (t, $J = 7.10$ Hz, 1H) 8.10 (d, $J = 7.92$ Hz, 1H), 8.17 (d, $J = 8.13$ Hz, 1H) 8.34 (dd, $J = 1.68$, 7.92 Hz, 1H) 8.38 (dd, $J = 1.53$, 7.93 Hz, 1H).

*2.1.3.11. N-(12-Bromododecyl)-2,3,3-trimethylindolenium bromide (**17**).* Adapted from a known procedure [21], **12** (2.73 g, 17.1 mmol) was added to a refluxing solution of 1,12-dibromododecane (6.25 g, 19.1 mmol) in $CHCl_3$ (3.39 mL) over 2 h. The solution was refluxed for 24 h and precipitated from Et_2O (3 × 25 mL). Compound **17** was obtained in a 56% yield (4.68 g) as a pink-purple solid. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.20–1.52 (m, $CH_2CH_2CH_2$, 16H), 1.64 (s, $C(CH_3)_2$, 6H), 1.82 (m, NCH_2CH_2, 2H), 1.91 (m, $BrCH_2CH_2$, 2H), 3.15 (s, CH_3, 3H), 3.38 (t, $J = 6.81$ Hz, CH_2Br, 2H), 4.74 (t, NCH_2, 2H), 7.45–7.71 (m, Ar, 4H). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 16.5, 23.2, 26.8, 28.2, 28.8–29.5, 32.9, 34.2, 49.8, 54.7, 115.4, 123.4, 129.6, 130.1, 141.2, 196.1. HRMS-ESI (*m/z*): $[M]^+$ calcd for $C_{23}H_{37}NBr$, 406.2104; found, 406.2110.

*2.1.3.12. N-(12-Bromododecyl)-3,3-dimethyl-2-methylene indoline (**18**).* Adapted from a known procedure [21], a solution of Na_2CO_3 (1.54 g, 14.5 mmol) in H_2O (34 mL) was added to a solution of **17** (4.62 g, 9.48 mmol) in H_2O (60 mL) over 1 h. The reaction mixture was stirred for 1 h and then extracted with Et_2O (3 × 50 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated under vacuum. The oil was dissolved in hexanes, the grey precipitate was filtered, and the oil was purified twice by column chromatography (1:25 EtOAc:hexanes). Compound **18** was obtained in a 56% yield (2.17 g) as a red oil. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.25–1.49 (m, $CH_2CH_2CH_2$, 16H), 1.36 (s, $C(CH_3)_2$, 6H), 1.66 (m, CH_2CH_2N, 2H), 1.87 (p, CH_2CH_2Br, 2H), 3.41 (t, $J = 6.86$ Hz, CH_2Br, 2H), 3.50 (t, $J = 7.38$ Hz, CH_2N, 2H), 3.87 (d, $J = 10.71$ Hz, C=CH_2, 2H), 6.54 (d, $J = 7.74$ Hz, H-4, 1H), 6.76 (t, H-6, 1H), 7.10 (d, H-7, 1H), 7.13 (t, H-5, 1H). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 26.2, 27.3, 28.3–30.2, 32.9, 34.0, 42.4, 44.2, 72.9, 105.1, 118.2, 121.8, 127.5, 137.6, 146.1, 161.7. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{23}H_{37}NBr$, 406.2104; found, 406.2147.

*2.1.3.13. N-(12-Bromododecyl)-3,3-dimethylspiro[indoline-2,3′-[3H]phenanthr[9,10b](1,4)oxazine (**2**).* Adapted from a known procedure [21], a solution of **16** (1.19 g, 5.33 mmol) in EtOH (105 mL) was added to a solution of **18** (2.17 g, 5.34 mmol) in EtOH (85 mL) over 3.5 h. The mixture was refluxed for 3.5 h and the solvent removed under vacuum. The product was purified four times by column chromatography (2 × 1:10 EtOAc:hexanes, and 2 × CH_2Cl_2). Compound **2** was obtained in a 17% yield (0.563 g) as a blue solid. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.10–1.80 (m, ($CH_2CH_2CH_2$, $C(CH_3)_2$), 24H), 1.87 (m, $BrCH_2CH_2$, 2H), 3.21 (m, NCH_2, 2H), 3.40 (t, J = 10.26 Hz, $BrCH_2$, 2H), 6.61 (d, J = 11.46 Hz, H-7, 1H), 6.89 (td, J = 11.09, 1.22 Hz, H-5, 1H), 7.08 (d, H-4, 1H), 7.22 (t, H-6, 1H), 7.61 (m, H-6′,7′,10′,11′, 4H), 7.81 (s, H-3′, 1H), 8.12 (d, J = 12.54 Hz, H-12′,1H), 8.65 (m, H-5′8′9′, 3H). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 21.0, 25.5, 27.2, 28.2, 28.7, 28.7, 29.2–29.4, 32.8, 34.0, 44.5, 51.8, 99.4, 106.8, 119.2, 119.6, 121.7, 122.1, 122.4, 122.6, 122.7, 124.5, 124.8, 126.4, 126.6, 127.3, 127.6, 127.8, 129.9, 131.3, 135.7, 139.3, 147.1, 151.0. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{37}H_{44}ON_2Br$, 611.2632; found, 611.2637. Anal. Calcd for $C_{37}H_{43}BrN_2O$: C, 72.65; H, 7.09; N, 4.58. Found: C, 72.77; H, 7.09; N, 4.61.

*2.1.3.14. N-(12-Triethylammoniododecyl)-3,3-dimethyl-2,3′-[3H]phenanthr[9,10b](1,4)oxazine (**3**).* Adapted from a known procedure [21], triethylamine (1.53 g, 15.1 mmol) was added to a refluxing solution of **2** (0.463 g, 0.757 mmol) in benzene (1.39 mL). The reaction refluxed for 5.5 h. The solvent was removed under vacuum and the product was precipitated from hexanes (3 × 5 mL). The product was purified twice by column chromatography (50:10:1 $CHCl_3$:MeOH:H_2O). Compound **3** was obtained in a 7% yield (0.038 g) as a viscous blue oil. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.20–1.40 (m, ($CH_2CH_2CH_2$, $N(CH_2CH_3)_3$, $C(CH_3)_2$), 31H), 1.50–1.80 (m, NCH_2CH_2, 2H), 3.10–3.30 (m, (NCH_2, NCH_2CH_2), 4H), 3.50 (q, J = 7.28 Hz, $N(CH_2CH_3)_3$, 6H), 6.60 (d, J = 7.74 Hz, H-7, 1H), 6.88 (td, J = 7.37, 0.71 Hz, H-5, 1H), 7.08 (dd, J = 7.23, 0.93 Hz, H-4, 1H), 7.21 (td, J = 7.59, 1.25 Hz, H-6, 1H), 7.50–7.70 (m, H-6′, 7′,10′,11′, 4H), 7.79 (s, H-3′, 1H), 8.11 (dd, J = 8.15, 1.07 Hz, H-12′, 1H), 8.60 (t, J = 7.94 Hz, H-8′9′, 2H), 8.66 (dd, J = 8.19, 1.11 Hz, H-5′,1H). ^{13}C NMR (300 MHz, $CDCl_3$, δ): 8.1, 21.0, 22.1, 25.5, 26.4, 27.3, 28.8–29.4, 44.5, 51.9, 53.6, 57.6 99.4, 106.7, 119.2, 119.6, 121.7, 122.1, 122.4, 122.6, 122.7, 124.4, 124.8, 126.4, 126.7, 127.3, 127.7. 127.8, 129.9, 131.3, 135.7, 139.3, 147.1, 151.1. HRMS-ESI (*m/z*): $[M]^+$ calcd for $C_{43}H_{58}N_3O$, 632.4574; found 632.4581. Satisfactory elemental analysis was not obtained because the product was an oil that we were unable to recrystallize. However, the structure was confirmed by high-resolution mass spectrometry.

2.2. Absorption studies

2.2.1. General

A SB20 pH meter with a saturated calomel electrode from VWR (Edmonton, AB, Canada) was used to measure the pH of buffer stock solutions at constant temperature (20.0 ± 0.5 °C). The Mini-Extruder, 1 mL gas-tight syringes, 0.1, 0.2, and 0.4 μm polycarbonate membrane filters, and filter supports used for liposome preparation were purchased from Avanti Polar Lipids (Alabaster, AL, USA). PD-10 desalting columns containing Sephadex G-25 M medium were used to remove unincorporated compounds and purchased from Amersham Biosciences (Baie d'Urfe, QC, Canada).

2.2.2. Instrumentation

Steady-state absorption spectra were obtained at constant temperature (e.g. 16.0 ± 0.1 °C) on a Cary 100 Bio UV–vis spectrophotometer (Mississauga, ON, Canada) equipped with a dual cell Peltier circulator accessory. The absorption spectra were referenced to the solvent used, and recorded at a scan rate, step size, and integration time of 300 nm min^{-1}, 1 nm, and 0.1 s, respectively. All samples were irradiated with the 6 W UVA (365 nm) UltraLum lamp source, and were measured in 10 mm × 10 mm quartz SUPRASIL absorbance cells from Hellma (Concord, ON, Canada).

2.2.3. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (>99%, Avanti Polar Lipids), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) (>99.5%, Aldrich), ethylenediaminetetraacetic acid, disodium salt (EDTA·2Na) (>99%, Aldrich), NaCl (99%, EMD Chemicals, Gibbstown, NJ, USA), and Triton X-100 (scintillation grade, Eastman Kodak Company, Rochester, NY, USA) were used as received. All aqueous solutions were prepared in pH 7.25 HEPES buffer (10 mM HEPES, 145 mM NaCl, 0.1 mM EDTA·2Na, $NaHCO_3$), and deoxygenated with Ar (UHP grade, Praxair, Mississauga, ON, Canada) for at least 15 min. Deionized water was obtained from a D8991 NANOpure Infinity water system from Barnstead (Dubuque, IA, USA). All organic solvents were of the highest available commercial grade.

2.2.4. Procedures

2.2.4.1. Preparation of spirooxazine solutions. Stock solutions of **1**, **2**, and **4** (10 mM) and **3** (8.9 mM) were prepared in $CHCl_3$. An aliquot of the stock solution (3–4 μL) was added to a 10 mL round bottom flask. The solvent was evaporated and the respective solvent (2.00 mL) was added to give a final concentration in spirooxazine of ca. 1.75 × 10^{-5} M so to achieve an absorbance of ca. 0.1 at λ_{MC} after irradiation with UVA light for 15 s. The solution was transferred to a quartz cell, sealed with a septum, and deoxygenated with Ar for at least 15 min.

2.2.4.2. Preparation of liposomes containing spirooxazines. The preparation of LUVs containing **1** and **3** for absorption studies was adapted from known procedures [22,23]. An aliquot of DPPC stock solution (2.57 mL, 1.36 mM) in $CHCl_3$ was added to a 10 mL round bottom flask. To prepare LUVs incorporating a 1:10 mole ratio (**1**:DPPC) an aliquot from a stock solution of **1** (35.0 μL, 10 mM) in $CHCl_3$ was added to the 10 mL flask. To prepare LUVs incorporating a 1:37 mole ratio (**3**:DPPC) an aliquot from a stock solution of **3** (10.5 μL, 8.9 mM) in $CHCl_3$ was added to the flask. The solvent was evaporated and allowed to dry under vacuum (ca. 100 mmHg) for 24 h to deposit a dry

lipid film. The sample was hydrated with HEPES buffer (ca. 0.7 mL). Both buffer and the flask containing the dry lipid film were preheated to 50 °C. The sample was sealed and allowed to hydrate for at least 3 h at 50 °C. The sample was then covered and left to stand at ca. 4 °C for 14 h. The hydrated lipid suspension underwent 4–5 freeze and thaw cycles by placing the sample in a dry ice-acetone bath followed by a warm water bath. Extrusion was preformed at constant temperature (50.0 ± 0.5 °C) using membrane filters in the following sequence: 0.4, 0.2, and 0.1 μm. The gas-tight syringes were prewetted with buffer solution and the sample was extruded 10 times to give ca. 0.7 mL of sample. Following extrusion, all samples were diluted with buffer (2.5 mL), loaded onto buffered desalting columns, and eluted with 3.5 mL of buffer to remove unincorporated spirooxazine. Each column was equilibrated with 25 mL of buffer before use. The first 10 drops of eluent were discarded to give a purified LUV sample, which was then diluted with buffer (ca. 2.5 mL, ca. 0.5 mM). The sample was transferred to a quartz cell, sealed with a septum, and deoxygenated with Ar for at least 15 min.

2.2.4.3. Kinetic measurements. A sealed quartz cell was placed in the sample holder of the UV–vis spectrophotometer and allowed to equilibrate to constant temperature for 10–15 min. The sample was irradiated with UV light for 15 s, and the absorbance was monitored at λ_{MC} until a constant baseline was obtained. For samples that required >3 min to reach baseline, absorbance values were recorded every 1 s. For samples that took <3 min to reach baseline, absorbance values were recorded every 0.1 s. Integration time was set to 0.1 s. All reported values are the means and standard deviations determined from a minimum of three independent experiments.

3. Results and discussion

3.1. Photochromism in solution

Absorption spectra were recorded for **1**–**4** in solvents of varying polarity and viscosity. Photoexcitation of spirooxazine solutions with UV light caused absorption in the visible region to increase. Upon thermal equilibration, a decrease in absorbance at λ_{MC} was observed to give back the absorption spectrum prior to excitation (Fig. 1), indicating that photoisomerization is reversible. In addition, isosbestic points were observed for the spirooxazine solutions (e.g. 407 nm for **1** in toluene), which is a strong indication that only two interconverting species are present in solution [24].

The effective rate constants for thermal equilibration (k_T) were determined from exponential fits of the absorption decays at λ_{MC} following UV irradiation (Fig. 2). Further, the decay kinetics were monitored at various temperatures depending on the magnitude of the thermal rate constants. The activation energy for the thermal ring closure of **1** in toluene was determined from its Arrhenius behavior (inset of Fig. 2, $E_a = 8.6\,kJ\,K^{-1}\,mol^{-1}$). This value was used to extrapolate all values of k_T for **1** to 25 °C. Whereas for **2**–**4**, reported activation energies for **4** in toluene and acetonitrile were employed

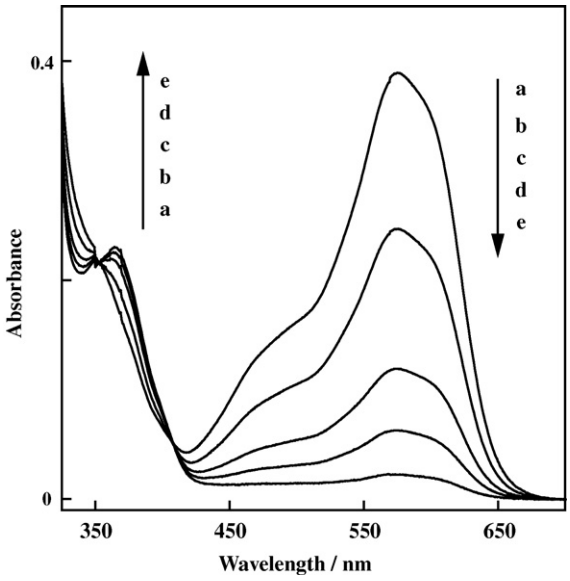


Fig. 1. Absorption spectra of **1** in toluene at 2 °C after a 15 s irradiation with UVA light. Spectra were recorded at (a) 120 s, (b) 190 s, (c) 380 s, (d) 570 s, and (e) 1020 s following irradiation as the MC form thermally reverts back to the SP form.

($E_a = 7.0$ and $6.8\,kJ\,K^{-1}\,mol^{-1}$, respectively) [25]. The former E_a was used to extrapolate k_T for **2**–**4** in hexane and DPPC. Note that $k_T = k_1 + k_{-1}$, where k_1 and k_{-1} are the respective thermal rate constants for the ring-opening and ring-closing steps. Due to the detection limits of our instrumentation, we were unable to determine k_1 (i.e. $k_1 \gg k_{-1}$). It has been shown previously for spirooxazines containing phenanthrene moieties that $k_{-1} \approx k_T$ [2]. Thus to a good approximation, we will specifically refer to

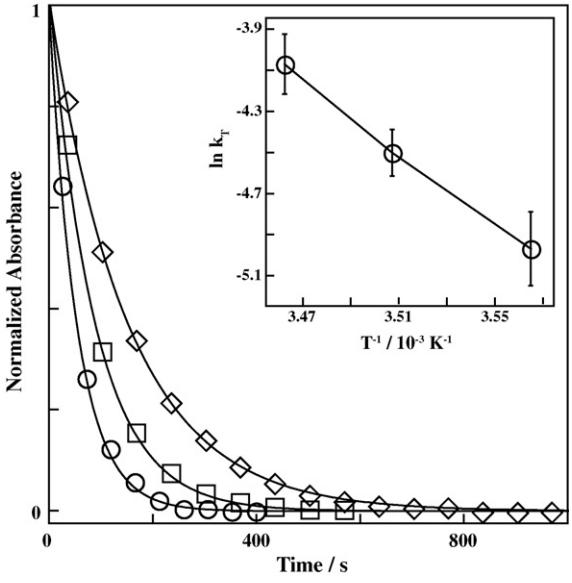


Fig. 2. Normalized decay kinetics of the MC form of **1** in toluene monitored at λ_{MC} following excitation with 15 s of UVA light, and recorded at various temperatures: 7 °C (◊), 12 °C (□), and 16 °C (○). For clarity not all data points are shown. The inset is an Arrhenius plot of the thermal rate constants obtained from exponential fits (solid lines). Error bars represent the standard deviation of the mean from a minimum of three independent experiments.

Table 1
Absorption maxima of the SP (λ_{SP}) and MC forms (λ_{MC}), and the rate constants for thermal ring closure (k_T) of **1**–**4** in solution and liposomes

Compound	Solvent	λ_{SP} (nm)[a]	λ_{MC} (nm)[a]	k_T (10^{-2} s^{-1})[b]
1	Hexane	363	557	1.36 ± 0.03[c]
	Octane	363	557	1.24 ± 0.05[c]
	Decane	364	557	1.19 ± 0.01[c]
	Dodecane	364	559	1.10 ± 0.02[c]
	Toluene	364	568	5.18 ± 0.30[c]
	DPPC	–	575	1.44 ± 0.20[c,e,f]
2	Toluene	345	587	9.14 ± 0.67[d]
	Acetonitrile	342	593	257 ± 12[d]
3	Toluene	344	590	35.7 ± 2.3[d]
	Acetonitrile	338	592	198 ± 10[d]
	DPPC	–	573	95.1 ± 16.6[d]
4	Hexane	343	573	9.02 ± 1.10[d]
	Toluene	344	582	14.6 ± 0.1[d]
	Acetonitrile	342	587	222 ± 14[d]

[a] Recorded at 25 °C and the errors are ±2 nm.
[b] Extrapolated to 25 °C and the errors are the standard deviations for the mean taken from a minimum of three independent measurements.
[c] Recorded at 16 °C.
[d] Recorded at 7 °C.
[e] Recorded at 12 °C.
[f] Recorded at 20 °C.

k_T as the rate constant for thermal ring closure.

$$SP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} MC \quad (1)$$

We have measured λ_{SP}, λ_{MC} and k_T for **1**–**4** in solvents of varying polarity and viscosity to determine the effect of substitution on these photophysical parameters (Table 1). Consistent with reports on similar spirooxazines [2,25,26], λ_{MC} for **1**–**4** undergo bathochromic shifts with increasing solvent polarity, whereas there is little effect on λ_{SP}. The shift in λ_{MC} to longer wavelengths is expected as the lowest excited state of the more polar MC form is better stabilized by a polar microenvironment. However, the increase in k_T is contrary to that observed for typical spiropyrans and spirooxazines [3,4]. It has been suggested that for phenanthrene systems the transition state is more polar than either the SP or MC forms because of a loss in pseudo π-conjugation between the indoline and oxazine moieties [2]. As a result, the electronic charge on the nitrogen and oxygen atoms increases, as does the molecular dipole moment. The k_T for **1**–**4** were observed to increase with increasing solvent polarity. For example, in solvents of similar viscosity, such as toluene and octane, k_T of **1** was 4-fold larger in the more polar toluene. A large solvent effect is observed for **2** and **4**, in which k_T is at least 15–28-fold larger in acetonitrile than toluene. Once again, these trends show that the transition state is relatively more polar than the SP and MC forms.

In toluene, k_T of **1** and **2** were ca. 2-fold smaller than **4**, which suggests that the four dodecoxy substituents on the oxazine moiety of **1** and the bromododecyl substituent on the indoline moiety of **2** are influencing the rate of ring closure through either electronic or steric effects. The presence of electron-donating alkoxy substituents would generally destabilize the MC form by increasing the negative charge on the oxazine oxygen atom and k_T. Although, given that the substitution on **2** will have relatively no influence via electronic effects and its k_T is similar to **1**, we suggest that steric effects are more significant on the relative magnitude of k_T. It has been shown that the rate of ring closure can be significantly slowed down through the addition of bulky groups on the indoline nitrogen [8]. Hobley et al. demonstrated that as the alkyl chain length increases, the change in entropy decreases, suggesting that the alkyl chain has to adopt a specific orientation prior to ring closure, moving out of the way of the advancing oxazine moiety. A similar argument can also be made for the dodecoxy chains of **1**. We observe a slight decrease in k_T for **1** as the viscosity of the solvent increases, when polarity is kept relatively constant (i.e. hexane to dodecane). These results show that the viscosity of the microenvironment has a greater effect on k_T of **1** in solvents of similar polarity. The dodecoxy substituents are responsible for this slight decrease in k_T as ring closure is most likely inhibited by a reduction in conformational mobility due to favorable interactions with the alkane solvents through dispersion forces. However, when the viscosity of the medium is kept constant (e.g. octane and toluene), the polarity of the microenvironment has a dominant effect.

Interestingly, k_T of **3** was ca. 4-fold larger than **2** in toluene, whereas in acetonitrile this rate constant was slightly smaller. One might expect that the charged tether may interact with the partially negative oxazine oxygen thereby stabilizing the polar MC form and decreasing the rate of ring closure. As this is not observed, this comparison may suggest that the MC form of **3** has less quinoidal and more zwitterionic character than similar spirooxazines such as **2** and **4**. As a result, the MC form will be more polar and destabilized in nonpolar solvents like toluene. In any case, to determine the exact origin of this substituent effect additional studies on compounds like **2** with varying alkyl chain lengths will be required.

3.2. Photochromism in liposomes

Compounds **1** and **3** were included in the lipid bilayer of DPPC LUVs to study the effect of the microenvironment on k_T and to determine their relative orientation in the membrane. Absorption spectra were recorded for **1** and **3** in LUVs at a mole ratio of 1:10 (e.g. **1**:DPPC). Although LUVs were prepared with a diameter of ca. 100 nm to reduce background absorbance due to light scattering, only absorption in the visible region was clearly observed upon photoexcitation with UV light (Fig. 3). Photoisomerization of **1** and **3** was reversible as the original absorption spectrum prior to excitation was obtained following thermal equilibration. The magnitude of k_T for **3** was 66-fold larger than **1**, which suggests that the higher degree of substitution on **1** greatly reduces the conformational mobility required for thermal ring closure. As well, at low temperature (7 °C) a kinetic decay with biexponential character was observed for **1** in DPPC LUVs. We were unable to accurately fit the data because the lifetime of the short-lived species was very close to the time resolution of the equipment. However, biexponential decays have been observed from time-resolved studies on **4**, and were attributed to the *cis–trans* isomerization of the MC

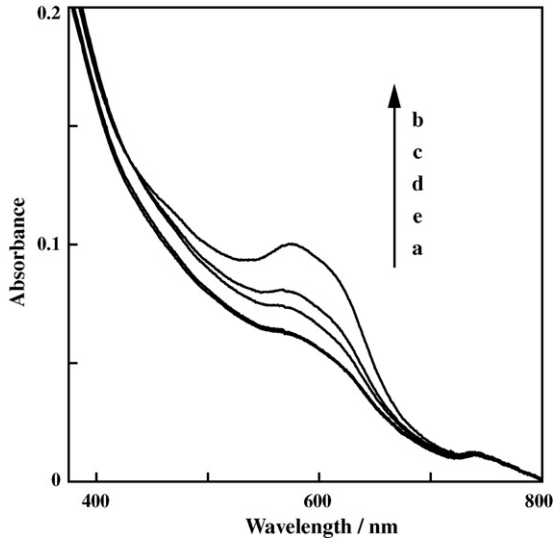


Fig. 3. Absorption spectra of **1** in 100 nm DPPC LUVs at 20 °C before (a) and after a 20 s irradiation with UVA light. Spectra were recorded at (b) 10 s, (c) 140 s, (d) 275 s, and (e) 3755 s following irradiation as the MC form thermally reverts back to the SP form.

form prior to ring closure [25]. This qualitative observation provides further support for our hypothesis that the substituents on **1** greatly reduce the conformational mobility required for thermal ring closure.

The solvatochromic properties **1** and **3** were used to determine their relative orientation in the lipid bilayer. The λ_{MC} of **1** was slightly red-shifted when compared to toluene, suggesting that the photochromic moiety of **1** is in a more polar region than toluene. Whereas for **3**, λ_{MC} was significantly blue-shifted from toluene, indicating that **3** is located in a region of lower polarity. These results suggest that relative orientation of these lipid-like analogues in the bilayer is different owing to differences in substitution. In particular, the photochromic moiety of **1** is most likely positioned near the slightly polar glycerol backbone of the lipids with the alkoxy chains primarily interacting in the nonpolar methylene region of the bilayer. In contrast, **3** is most likely oriented such that the charged moiety interacts with the polar headgroup region, thereby placing the photochromic moiety in the nonpolar methylene region. We are currently evaluating the effect of isomerization of these photochromes on the membrane permeability of LUVs. By embedding the photochromic moiety in the methylene region as suggested for **3**, we propose that photoisomerization will cause greater disruption in the local lipid order allowing for increased water penetration and enhanced permeability.

In summary, we have synthesized new phenanthryl-based spirooxazines with elements of lipid complementarity to determine the effect of substitution on their photochromic properties and relative orientation within the lipid bilayer of DPPC LUVs. We have show that the kinetics for thermal ring closure is sensitive to the polarity of the local environment, and viscosity when the degree of substitution is high. As well, the relative orientation of these photochromes in the lipid bilayer was determined based on their solvatochromic properties, and shown to be due to differences in substitution.

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