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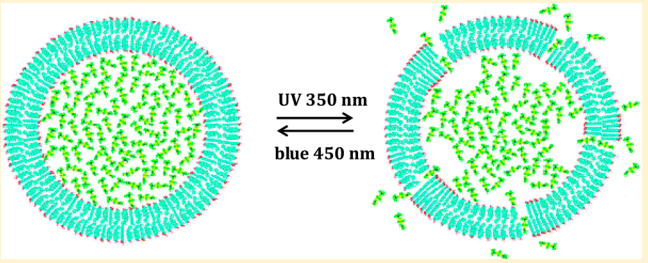
Nonphospholipid Fluid Liposomes with Switchable Photocontrolled Release

Zhong-Kai Cui, Thida Phoeung, Pierre-Antoine Rousseau, Gaulthier Rydzek, Qian Zhang, C. Geraldine Bazuin, and Michel Lafleur*

Department of Chemistry, Center for Self-Assembled Chemical Structures (CSACS), Université de Montréal, C.P. 6128, Succ. Centre Ville, Montréal, Québec, Canada H3C 3J7

ABSTRACT: We created novel nonphospholipid photosensitive liposomes from a mixture of a monoacylated azobenzene amphiphile ($AzoC_{10}N^+$) and cholesterol sulfate (Schol). This system belongs to the family of sterol-enriched nonphospholipid liposomes that were shown to form stable large unilamellar vesicles (LUVs) with enhanced impermeability. Fluid bilayers were successfully prepared from $AzoC_{10}N^+$/Schol (25/75 molar ratio) mixtures, and LUVs could be derived at room temperature using standard extrusion methods. The isomerization process of the bilayer-inserted $AzoC_{10}N^+$ was characterized. Leakage from these liposomes could be induced by the photoconversion of $AzoC_{10}N^+$ from its *trans* form to its *cis* form. This photocontrolled release from fluid liposomes contrasts with the case of phospholipid-based azo-containing liposomes, which are generally required to be in the gel phase to be photosensitive. It is proposed that the very high degree of conformational order of the monoalkylated amphiphile and the tight packing of the hydrophobic core of the $AzoC_{10}N^+$/Schol liposomes make them responsive to the presence of the bulky *cis* azo isomer. Interestingly, the liposome impermeability could be fully restored by the photoisomerization of the *cis* form back to the *trans* form, providing a sharp on-and-off control of payload release. In addition, these nonphospholipid liposomes display a very limited passive release. Therefore, it is shown that $AzoC_{10}N^+$/Schol LUVs can be used as nanocontainers, whose content can be released by light in a controlled and switchable manner.



1. INTRODUCTION

Light is a versatile stimulus for controlling the release of entrapped materials from nanocontainers. A broad range of parameters, such as frequency, quantity, and duration, can be straightforwardly optimized for a given application. In addition, this approach confers local control of the nanocontainer permeability, because of the use of light sources such as lasers. Various strategies (e.g., photopolymerization[1,2] and photooxidation[3,4]) have been exploited to achieve photocontrolled release from nanovectors. Among these, the isomerization of photosensitive groups, such as spiropyran,[5−7] spirooxazine,[8] and azobenzene,[9−11] constitutes an interesting approach to inducing photocontrolled release from vesicles, as it has the advantage of being reversible. The azobenzene group is a functional group that can undergo reversible *trans*−*cis* isomerization under UV or visible light irradiation.[9−11] Thermodynamically, the *trans* isomer is favored because of the minimized steric hindrance. Because of the greater stability of the *trans* form, there is thermal relaxation of the system from the *cis* isomer to the *trans* isomer; this relaxation process is generally much slower than the photoconversion from one isomer to the other.[12] It has been shown that when an azobenzene derivative is inserted into phospholipid liposomes, the *trans*−*cis* isomerization of the azobenzene group can induce defects in bilayers that lead to the release of the entrapped payload, including K^+,[10] calcein,[13−16] and doxorubicin.[17] This controlled release is typically obtained with gel-phase liposomes in which tight chain packing cannot accommodate the defects associated with the bulky *cis* conformers. It was shown that fluid bilayers prepared with a phospholipid would remain impermeable despite the photoformation of bent *cis* isomers because of the flexibility of a fluid hydrophobic core.[14,18]

In this paper, we demonstrate the possibility of making fluid-phase photosensitive nonphospholipid liposomes formed by a monoacylated amphiphile, decyl-azobenzyl-triethylammonium [$AzoC_{10}N^+$ (Figure 1)], and a sterol, cholesterol sulfate (Schol). Mixtures of a single-chain amphiphile (between 25 and 50 mol %) and a very large quantity of sterol (75 to 50 mol %) form stable liquid-ordered (lo) phases under certain conditions (for a review, see ref 19), and large unilamellar vesicles (LUVs) can be prepared from these mixtures by a simple extrusion process. This distinct composition confers to these nonphospholipid LUVs distinct advantages for the formation of photosensitive liposomes. First, these nanovectors show enhanced impermeability compared to that of conventional phospholipid-based liposomes. For example, LUVs

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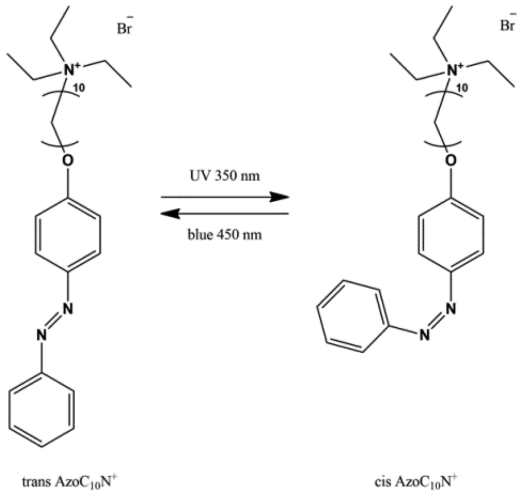

Figure 1. Chemical structure and photoisomerization of $AzoC_{10}N^+$.

prepared from palmitic acid (PA) and cholesterol, in a 30/70 molar ratio, release only 30% of the entrapped calcein after 18 months.[20] Second, the high sterol content leads to a very high degree of chain order, and very tight chain packing, while maintaining the fluid character of the bilayers.[21,22] It is hypothesized that the high degree of order should make the liposome bilayers responsive to the *trans*−*cis* isomerization of the azobenzene group. According to this hypothesis, these liposomes would be photoresponsive in the fluid but highly ordered phase, a feature that presents advantages with respect to their use as drug nanovectors, including the possibility of extruding liposomes at room temperature,[23] and efficient drug loading.[23,24] Third, single-chain amphiphile/sterol mixtures can display an advantageous chemical stability compared to that of phospholipids, which are degraded by indigenous phospholipases and whose ester links are susceptible to hydrolysis under extreme pH conditions.[25] Thus, our aim is to obtain the combined properties of very stable yet fluid liposomes, because of the high sterol content, and phototriggered delivery of encapsulated materials, because of the azobenzene-containing single-chain amphiphile.

$AzoC_{10}N^+$ was designed in such a way that its length matches that of Schol, a prerequisite for ensuring the stability of fluid bilayers.[26] The azobenzene moiety is located at the end of the acyl chain and, as a consequence, should be located mainly at the center of the self-assembled bilayers. The presence of the positively charged ammonium group is required to provide proper lipid mixing with the sterol and to ensure suitable interfacial hydration.[27,28] The design of the $AzoC_{10}N^+$/Schol system is based on comparable cetylpyridinium chloride (CPC)/sterol[28] and stearylammonium/sterol[27] mixtures, which were shown to form fluid bilayers. On the basis of the behavior of the well-characterized CPC/Schol systems, we explored the behavior of the mixture composed of 25 mol % $AzoC_{10}N^+$ and 75 mol % Schol, because this ratio is predicted to be a good candidate for forming fluid lamellar phases with very tight chain packing. First, we determined whether the $AzoC_{10}N^+$/Schol system forms fluid bilayers and whether LUVs could be generated. Subsequently, we characterized the kinetics of the *trans*−*cis* isomerization process of the azobenzene derivative inserted in $AzoC_{10}N^+$/Schol bilayers as well as solubilized in $CHCl_3$ as a control. Finally, we conducted release experiments to assess the efficiency of the photocontrol of the payload release that can be obtained from the reversible photoisomerization of the azobenzene group. We specifically investigated the possibility of exploiting the photoreversible isomerization for switching the payload release on and off. As shown below, this mixture provides a very tight switchable control of the release of the entrapped material to an extent that, to our knowledge, has never before been reported.

2. EXPERIMENTAL SECTION

2.1. Materials. The monoacylated azobenzene derivative, $AzoC_{10}N^+$, was synthesized according to a procedure published elsewhere.[29] Schol, PA (99%), Triton X-100, ethylenediaminetetraacetic acid (EDTA, 99%), and tris(hydroxymethyl)aminomethane (TRIS, 99%) were purchased from Sigma-Aldrich (St. Louis, MO). Sulforhodamine B (SRB) was purchased from Invitrogen (Eugene, OR). 2-(*N*-Morpholino)ethanesulfonic acid (MES, >99%) and NaCl (>99%) were obtained from EMD Chemicals (Gibbstown, NJ) and Anachemia (Montréal, QC), respectively.

2.2. Methods. We prepared $AzoC_{10}N^+$/Schol and PA/Schol mixtures by mixing weighed quantities of each compound solubilized in a benzene/methanol [75/25 (v/v)] mixture and freeze-drying the mixture for at least 16 h.

For UV−vis experiments, a stock liposome dispersion was prepared by hydrating the lipid mixtures in a MES/TRIS buffer [140 mM NaCl, 50 mM MES, 50 mM TRIS, and 0.5 mM EDTA (pH 7.4 for the $AzoC_{10}N^+$/Schol mixture and pH 7.0 for the PA/Schol mixture)]. The dispersions were then extruded 15 times through two stacked polycarbonate filters with a 100 nm pore size, using a hand-held Liposofast extruder (Avestin Corp., Ottawa, ON) at room temperature. The size of the resulting LUVs was determined at 25 °C, using a Malvern Zetasizer. The lipid concentration was adjusted to ∼20 μM for these measurements.

The isomerization of the azobenzene group was initiated using the xenon lamp (75 W) of a fluorimeter (QuantaMaster, Photon Technology International Inc.) with slit widths of 10 mm providing a power density of 500 $\mu W/cm^2$ at the sample. The samples were constantly stirred and equilibrated at 25 °C throughout the irradiation. UV−vis measurements were taken using a CaryWin spectrometer. Typically, the $AzoC_{10}N^+$ concentration in the samples was adjusted to ∼0.05 mM. The conversion from *trans* to *cis* isomers was induced using light at 350 nm, a wavelength at which the molar absorptivity coefficient of the *trans* isomer is much larger than that of the *cis* isomer. Photoisomerization from *cis* to *trans* was effected using light at 450 nm. The proportion of $AzoC_{10}N^+$ in the *trans* form at time *t* ($AzoC_{10}N^+_{trans}$) was determined using eq 1:

$$AzoC_{10}N^+_{trans} = \left[\frac{A(t) - A_c}{A_t - A_c}\right] \times 100 \quad (1)$$

where $A(t)$ is the absorbance at 350 nm at time *t* and A_t and A_c are the absorbances of the pure *trans* and *cis* forms, respectively, at 350 nm. It was assumed that there were only *trans* isomers when the absorbance at 350 nm was constant for samples kept in the dark and only *cis* isomers when the absorbance of a sample illuminated at 350 nm reached a constant value. Measurements were taken in at least triplicate. The percentages and uncertainties reported are the average values and standard deviations, respectively.

The isomerization kinetics were fitted according to an exponential process. The *trans*−*cis* and *cis*−*trans* isomerizations are described by eqs 2a and 2b, respectively.

$$AzoC_{10}N^+_{trans} = AzoC_{10}N^{+0}_{trans} \times e^{-t/\tau} \quad (2a)$$

$$AzoC_{10}N^+_{trans} = AzoC_{10}N^{+0}_{trans} + (1 - e^{-t/\tau})(AzoC_{10}N^{+\infty}_{trans} - AzoC_{10}N^{+0}_{trans}) \quad (2b)$$

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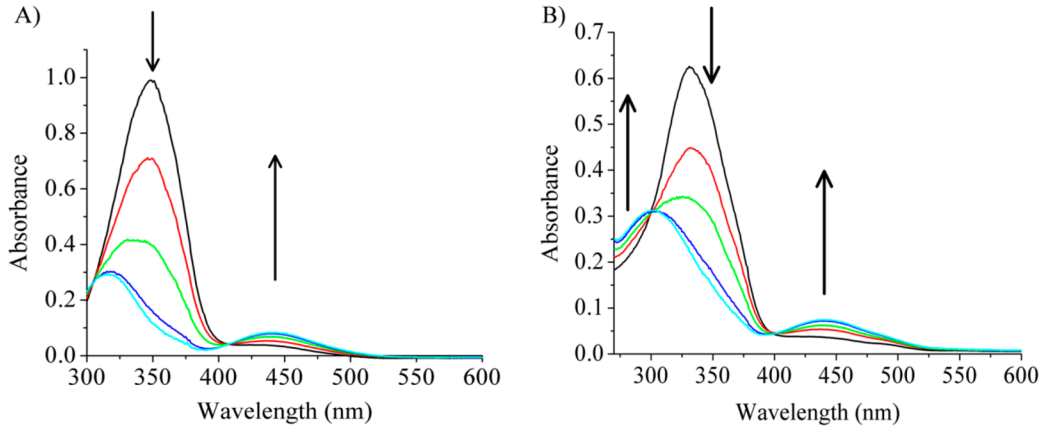

Figure 2. UV−vis spectra of $AzoC_{10}N^+$ recorded during the *trans*−*cis* photoisomerization process. Black lines are the spectra obtained at $t = 0$ (before irradiation). Turquoise lines are the spectra obtained at $t = \infty$ (at the end of the irradiation process, i.e., when no further evolution of the spectrum was observed). The arrows show the intensity change directions upon irradiation at 350 nm: (A) $AzoC_{10}N^+$ solubilized in $CHCl_3$ and (B) $AzoC_{10}N^+$ inserted into bilayers formed with Schol.

where $AzoC_{10}N^{+0}_{trans}$ and $AzoC_{10}N^{+\infty}_{trans}$ are the percentages of the $AzoC_{10}N^+$ *trans* isomer at $t = 0$ and ∞, respectively, and τ is the characteristic time of the exponential isomerization process.

For the release experiments, SRB, a fluorescent probe, was added to the buffer used for lipid hydration: 10 mg of lipid mixtures was hydrated in 300 μL of a MES/TRIS buffer containing 50 mM SRB (70 mM NaCl, 50 mM MES, 50 mM TRIS, and 0.5 mM EDTA), termed the internal buffer. At this concentration, SRB fluorescence is essentially self-quenched.[30] After the extrusion of the LUVs, free SRB was separated from the probe-loaded LUVs by gel exclusion chromatography using a column (diameter, 1.5 cm; length, 25 cm) filled with Sephadex G-50 medium (Pharmacia Biotech, Uppsala, Sweden) equilibrated with an iso-osmotic MES/TRIS buffer; this external buffer consisted of 140 mM NaCl, 50 mM MES, 50 mM TRIS, and 0.5 mM EDTA. The pH of both the internal and external buffers was set to 7.4 for the $AzoC_{10}N^+$/Schol system and 7.0 for the control PA/Schol LUVs. This exclusion chromatography step also ensured that $AzoC_{10}N^+$ was completely inserted into the collected LUVs.

Fluorescence measurements were taken using a Photon Technology International QuantaMaster fluorimeter. LUV suspensions were diluted with the external buffer to yield a lipid concentration of ~70 μM. The excitation and fluorescence wavelengths of SRB were 567 and 583 nm, respectively. The bandwidths of the excitation and emission monochromators were set to 1.5 and 2 nm, respectively. The percentage of SRB release was calculated according to eq 3:

$$\%\ \text{of release} = 100 - \left[\frac{(I_T - I_F)/I_T}{(I_{T,0} - I_{b,0})/I_{T,0}}\right] \times 100 \quad (3)$$

where $I_{b,0}$ and $I_{T,0}$ are the fluorescence intensities measured from the collected SRB-loaded LUVs immediately after the gel exclusion chromatography step, before and after the addition of Triton X-100 (final concentration of ~0.1 vol %), respectively. Triton X-100 led to the complete release of the probe, and the resulting fluorescence intensity was used to normalize the release curves. I_F and I_T are the SRB fluorescence intensities measured at a given time t, before and after the addition of Triton X-100, respectively. The experiments were conducted in independent triplicate. The reported release percentages and their uncertainties correspond to the average values and standard deviations, respectively.

3. RESULTS AND DISCUSSION

The first step was to establish that the $AzoC_{10}N^+$/Schol (25/75 molar ratio) mixture forms fluid bilayers. To this end, we examined if LUVs can be produced. We also determined if the putative LUVs can encapsulate SRB, a hydrophilic fluorophore, when the lipid mixture was hydrated with a buffer containing SRB and the resulting suspension was extruded. Dynamic light scattering measurements of this preparation indicated that LUVs were formed. Their size distribution was unimodal with an average diameter of 140 nm. In addition, SRB fluorescence measurements showed that the fluorophore was encapsulated in the LUVs with a self-quenching efficiency of ~90%, a value consistent with trapped SRB at a concentration of 50 mM. From these results, we concluded that the $AzoC_{10}N^+$/Schol (25/75 molar ratio) mixture behaves like other mixtures of a long acyl chain ammonium and a sterol,[27,28] and that it also forms fluid bilayers that can be extruded to provide LUVs.

The variations in the UV−vis spectrum associated with the isomerization of $AzoC_{10}N^+$ in a chloroform solution and of the bilayer-inserted $AzoC_{10}N^+$ are presented in Figure 2. Prior to irradiation, the spectrum of $AzoC_{10}N^+$ in chloroform was dominated by a peak at 348 nm accompanied by a weak component at 440 nm (Figure 2A). The most intense band corresponds to the $\pi \rightarrow \pi^*$ transition of the azobenzene *trans* form, whereas the weak component is assigned to its $n \rightarrow \pi^*$ transition.[29,31−34] When the sample was irradiated at 350 nm, the intensity of the band at 348 nm decreased, whereas the intensity of two weak bands located at 315 and 440 nm increased. These two bands correspond to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively, of the *cis* isomer.[9] A similar pattern was observed for the bilayer-inserted $AzoC_{10}N^+$ (Figure 2B). The intense band for the $\pi \rightarrow \pi^*$ transition of the *trans* form was slightly blue-shifted to 330 nm. This observation has been related to the less polar environment of the azobenzene group when it is inserted into the hydrophobic core of the bilayers compared to when it is solubilized in chloroform.[35] It has also been attributed to weak H-aggregation[13] or dimerlike formation[36] of the azobenzene chromophores. Therefore, this shift is a strong indication that $AzoC_{10}N^+$ was inserted into the self-assembled structures formed in the presence of Schol.

Upon illumination at 350 nm, the intensity of the band associated with the azobenzene *trans* isomer decreased, whereas that of the *cis* isomer bands increased. The spectra of free and bilayer-inserted $AzoC_{10}N^+$ before any irradiation (corresponding to the *trans* form) and at the end of the 350 nm irradiation period ($t = \infty$, corresponding to the *cis* form) (Figure 2) were fitted using two Gaussian functions that were representative of the two isomer bands. The spectra were fitted between 300 and

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377 nm and between 276 and 377 nm for the $CHCl_3$-solubilized and bilayer-inserted $AzoC_{10}N^+$, respectively. Prior to illumination, the spectra could be essentially reproduced by the high-wavelength component associated with the *trans* form, the low-wavelength component contributing <10% of the total area. Analogously, at $t = \infty$, the spectra could be simulated by the low-wavelength band representing the *cis* form, the band associated with the *trans* form representing <5% of the total area. These results suggest that, under both conditions, a single isomer is predominant. The presence of clearly defined isosbestic points (λ = 410 nm for the solubilized $AzoC_{10}N^+$, and λ = 300 and 397 nm for the bilayer-inserted form) demonstrates the transition between the *trans* and *cis* forms, without the accumulation of significant quantities of any intermediate form.

The kinetics of the *trans* to *cis* conversion, photoinduced by irradiation at 350 nm, is presented in Figure 3. It is clearly

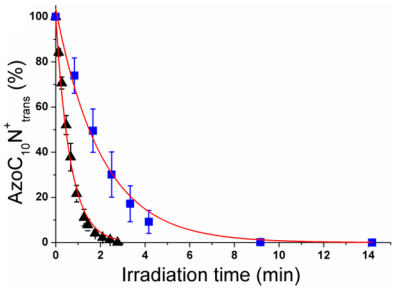

Figure 3. Kinetics of the *trans*−*cis* photoisomerization, induced by irradiation at 350 nm, of $AzoC_{10}N^+$ solubilized in $CHCl_3$ (▲) and bilayer-inserted $AzoC_{10}N^+$ (blue squares). The red lines represent the fitted exponential functions.

observed that the *trans* to *cis* conversion is more rapid for the single-chain azobenzene derivative solubilized in $CHCl_3$ than when it is inserted into Schol bilayers. The variation of the absorbance as a function of illumination time can be reproduced well using single-exponential functions. The extracted characteristic times, listed in Table 1, indicate that

Table 1. Characteristic Times, τ, for the *trans*−*cis* Photoisomerization, *cis*−*trans* Photoisomerization, and *cis*−*trans* Thermal Relaxation of $CHCl_3$-Solubilized and Bilayer-Inserted $AzoC_{10}N^+$

	$AzoC_{10}N^+$ in $CHCl_3$	$AzoC_{10}N^+$/Schol
trans−*cis* photoisomerization (min)	0.65 ± 0.02	2.1 ± 0.1
cis−*trans* photoisomerization (min)	2.3 ± 0.1	3.0 ± 0.1
cis−*trans* thermal relaxation (days)	3.3 ± 0.1	1.3 ± 0.1

the photoconversion of $AzoC_{10}N^+$ is ∼3 times slower in the bilayer environment than in the organic solution. This phenomenon can be partly associated with the fact that 350 nm corresponds more closely to the maximum of the absorption band of the *trans* form for the compound solubilized in $CHCl_3$ compared to that inserted into bilayers. The tight chain packing existing in the bilayers[28] can also inhibit the isomerization process, as has been reported for similar azobenzene derivatives inserted into bilayers.[10,16,37] The free volume accessible to $AzoC_{10}N^+$ when it is inserted into bilayers is expected to be considerably more limited than that of the form solubilized in an organic solution. It was previously reported that the mobility of the chromophore is crucial for efficient isomerization.[9] It is therefore a positive step that $AzoC_{10}N^+$ inserted into the highly ordered bilayers prepared with Schol could undergo a *trans*−*cis* isomerization at an acceptable rate.

The kinetics of the *cis*−*trans* isomerization induced by irradiation at 450 nm was also examined (Figure 4A) and was found to be slightly slower than the kinetics observed for *trans*−*cis* photoisomerization (Table 1). A stationary state was reached after illumination for ∼10 min. Furthermore, the *cis*−*trans* isomerization was slightly slower for bilayer-inserted $AzoC_{10}N^+$ (3.0 min) than for free form (2.3 min), which can be attributed to the structured environment of the apolar core of the bilayers and/or to different absorption efficiencies of the azobenzene moiety in different environments.

At the stationary phase reached after the 10 min illumination period, ∼70% of the initial *trans* $AzoC_{10}N^+$ content was observed for $AzoC_{10}N^+$ solubilized in $CHCl_3$, whereas it was ∼90% for the bilayer-inserted $AzoC_{10}N^+$. This indicates that the photoisomerization process was not completely reversible, a phenomenon that has also been observed for other azobenzene systems.[31,38] This might be due to the proximity of the absorption bands associated with each isomer that may result in a steady-state photoexchange between the two isomers. On the basis of this rationale, the greater recovery of the *trans* isomer for bilayer-inserted $AzoC_{10}N^+$ compared to the recovery of $CHCl_3$-solubilized $AzoC_{10}N^+$ is consistent with its increased energy separation between the $\pi \rightarrow \pi^*$ band maximum and the light wavelength used to induce the *cis*−*trans* isomerization (450 nm).

Figure 4B shows the thermal relaxation of the system, leading to the recovery of the *trans* isomers when the samples with the photoinduced *cis* form were kept in the dark. The kinetics, on the order of days (Table 1), is clearly considerably slower than that observed for photoisomerization at 450 nm. These results demonstrate that the *cis* $AzoC_{10}N^+$ resulting from 350 nm irradiation remained stable for several hours, a feature that was subsequently exploited in the photocontrolled release of the payload as discussed below.

Figure 5 illustrates that it is possible to cycle the isomerization process for $AzoC_{10}N^+$. In the case of $AzoC_{10}N^+$ solubilized in $CHCl_3$, the sample was irradiated for 3 min at 350 nm to induce the *trans*−*cis* isomerization, the proportion of the remaining *trans* form was measured, the reverse process was achieved by a 10 min irradiation at 450 nm, and the proportion of the *trans* form was measured again. This cycle was repeated three times. A similar cycling protocol was used for bilayer-inserted $AzoC_{10}N^+$ except that the period of illumination at 350 nm was 5 min. The results show that it is possible to photocycle between the *trans* and *cis* conformers of $AzoC_{10}N^+$ for both systems. A similar reversibility was reported for a monoacylated azobenzene derivative inserted into dihexadecyl phosphate micelles.[39] It was possible to photoconvert the *trans* isomer to the *cis* isomer almost completely, whereas the level of photoconversion from the *cis* form to the *trans* form, which is constant after the first cycle, is consistent with the photokinetics displayed in Figure 2, supporting the hypothesis of steady-state photoconversion between the two isomers. It should also be noted that our results indicate that illumination during several consecutive short periods led to an extent of conversion that is very similar to that resulting from continuous illumination (for example, in Figure 3, the 3 min illumination leading to the *trans*−*cis* photoisomerization was divided into 15

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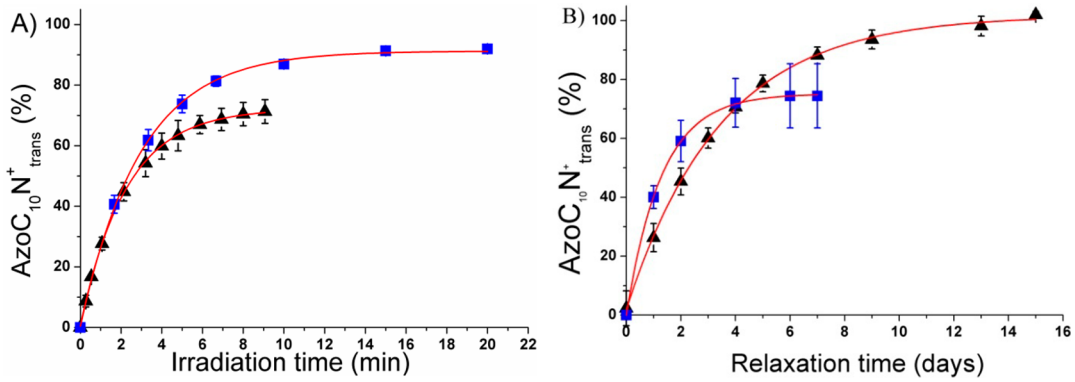

Figure 4. Kinetics of the *cis*–*trans* isomerization of $AzoC_{10}N^+$ solubilized in $CHCl_3$ (▲) and bilayer-inserted $AzoC_{10}N^+$ (blue squares): (A) photoinduced by illumination at 450 nm and (B) induced by thermal relaxation. The red lines represent the fitted exponential functions.

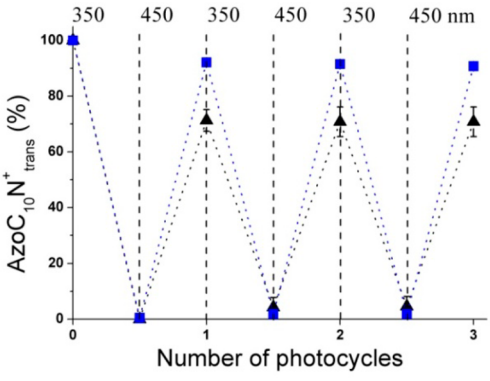

Figure 5. Photocycling of the *cis*–*trans* isomerization of $CHCl_3$-solubilized $AzoC_{10}N^+$ (▲) and bilayer-inserted $AzoC_{10}N^+$ (blue squares). One cycle included illumination at 350 nm to generate the *cis* isomer (3 min for $CHCl_3$-solubilized $AzoC_{10}N^+$ and 5 min for bilayer-inserted $AzoC_{10}N^+$), followed by a 10 min irradiation at 450 nm to generate the *trans* form.

s periods, separated by the recording of the UV–vis spectrum, whereas it was continuous for each period in the experiments depicted in Figure 5). This observation is associated with the slow *cis*–*trans* thermal relaxation described above, as well as the photostability of the azobenzene derivative under these illumination conditions.

After characterizing the isomerization behavior of the bilayer-inserted azobenzene, we proceeded to examine the possibility of triggering the release of the entrapped payload from the $AzoC_{10}N^+$/Schol LUVs by photoconversion of the azobenzene group. Figure 6A displays the extent of release of SRB from the LUVs upon irradiation at 350 nm, with $t = 0$ corresponding to the beginning of the illumination. The samples were illuminated for 30 s periods, and the level of material release was measured between these periods. No release of the probe was observed during the first 60 s. After this time lag, a progressive release was measured and ~75% of the SRB had leaked out from the LUVs after 5 min. It was inferred, on the basis of the isomerization kinetics, that a threshold amount of azobenzene groups should be in the *cis* form to initiate leakage. In our system, ~40% of the azobenzene groups are in the *cis* form after a 60 s illumination period. The conclusion that the release observed for $AzoC_{10}N^+$/Schol LUVs is due to the presence of the inserted azobenzene derivative and is effectively phototriggered was validated using PA/Schol LUVs as a negative control; in this control, the photosensitive Azo amphiphile was replaced with a linear saturated fatty acid. As expected, 350 nm irradiation of PA/Schol LUVs did not lead to any significant payload release (Figure 6A).

Time lags for photoinduced leakage were also observed for other azobenzene derivatives inserted into phospholipid liposomes.[10,14,40] To rationalize these lags between the isomerization and the release, it was proposed that the bulky *cis* isomers need to rearrange in the plane of the bilayers to create leaky defects.[14,40] Laser-induced photoisomerization and release studies of azobenzene-containing liposomes[14,40] showed that, despite very rapid *trans*–*cis* isomerization caused by a high laser light intensity, the release of the entrapped payload displayed a time lag. It was inferred that the release was not associated with the isomerization process per se, but with the lateral reorganization of the bulky *cis* azobenzene conformers in the liposome walls to create a defect.

It was also established previously that the *trans*–*cis* isomerization in phospholipid liposomes more generally induced leakage of the entrapped payload in gel-phase bilayers. It was concluded that the fluid bilayers prepared from phospholipids, whose chain packing is relatively loose, can accommodate the bent *cis* isomers in such a way that bilayer permeability is preserved and no release is observed despite the photoisomerization of the Azo group.[14,40] Likewise, it was reported recently that gel-phase domains or a large fraction of liquid-ordered domains were required for *cis* conformers to induce the bursting of giant unilamellar vesicles (GUVs).[11] In the case presented here, the single-chain amphiphile/sterol bilayers display a very high level of orientational order in the apolar core, which is a consequence of their high sterol content,[22,26] but nevertheless, the bilayers remain fluid, as exemplified by the fast rotational motion (relative to the ^2H NMR time scale) of the monoacylated amphiphile and the sterol.[22,27,28,41] The tight chain packing is probably essential for the enhancement of liposome permeability by the *cis* conformers reported here. Thus, the LUVs presented in this work represent a class of liposomes that combine fluidity and photosensitivity.

In a second series of experiments, we examined the impact of the presence of *cis* azobenzene conformers on bilayer permeability. First, we continuously illuminated the $AzoC_{10}N^+$/Schol LUVs at 350 nm for 120 or 150 s and then measured SRB release as a function of time (Figure 6B). The initial release levels, measured immediately after the illumination period, corresponded to those obtained when the samples were illuminated in a quasi-continuous manner (Figure 6A).

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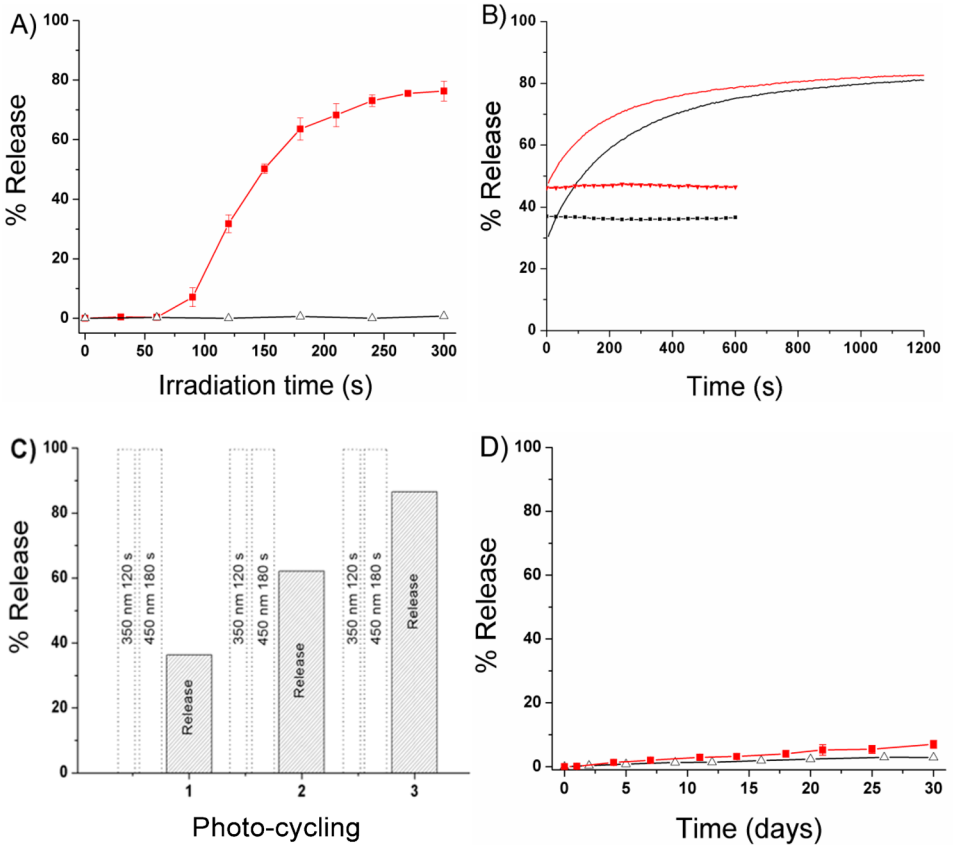

Figure 6. (A) Photoinduced release of SRB from $AzoC_{10}N^+$/Schol (red squares) and PA/Schol (△) LUVs by 350 nm irradiation. (B) Release of SRB from $AzoC_{10}N^+$/Schol LUVs induced by 350 nm irradiation for 120 s (black line) and for 150 s (red line). SRB release induced by 350 nm irradiation for 120 s (■) and 150 s (red triangles), followed by 450 nm irradiation for 180 s (the time was set to 0 at the end of this two-step irradiation process). (C) Release of SRB from $AzoC_{10}N^+$/Schol LUVs after 350−450 nm photocycling for 120−180 s. (D) Passive release of SRB from $AzoC_{10}N^+$/Schol (red squares) and PA/Schol (△) LUVs.

Despite the fact that the samples were no longer illuminated at 350 nm, the SRB release continued in a fashion similar to that obtained with continuous 350 nm illumination. These results indicate that the leaks introduced by the *trans*−*cis* photoconversion remained, even after illumination was stopped. This finding is consistent with the fact that thermal relaxation to the *trans* form is much slower than the time scale of the release measurements (days vs seconds). Therefore, once "static" leaky structures associated with the *cis* isomer were formed, the release of the material could be observed over the 20 min period. A similar observation was made for the permeability of K^+ from phospholipid liposomes containing an azobenzene derivative, where the release of K^+ from the liposomes continued at the same rate after turning off the light causing *trans*−*cis* isomerization.[10]

We showed above that it was possible to restore the *trans* form rapidly by illumination at 450 nm. With this in mind, we examined the possibility of stopping payload release by restoring the *trans* isomers. The experiment consisted of continuously illuminating the $AzoC_{10}N^+$/Schol LUVs at 350 nm for 120 or 150 s (to form the *cis* conformer), followed by illumination at 450 nm for 180 s (to re-form the *trans* conformer), and then measuring SRB release as a function of time (Figure 6B). The initial release levels were typical of those obtained for similar illumination periods at 350 nm. However, with this illumination protocol, further release was completely arrested, which can only be a result of the re-formation of tight vesicle walls with *trans* azobenzene conformers. Therefore, we can conclude that the photocontrol of the azobenzene conformation in the LUVs is an efficient approach for switching on as well as for turning off the delivery of the encapsulated material.

In addition, we successfully achieved precisely controlled release of the encapsulated SRB by exploiting photocycling. During a cycle, the $AzoC_{10}N^+$/Schol LUVs were irradiated first at 350 nm for 120 s and then at 450 nm for 180 s, after which the probe release was measured for a 300 s period. Figure 6C shows that this allows the release of a specific amount of the material (determined by the duration of the illumination period at 350 nm, after which it is stopped by photoinduced restoration of the *trans* azobenzene form due to illumination at 450 nm). Moreover, the delivery of the material can be performed in multidose format by applying several cycles, as illustrated in Figure 6C, which shows three distinct amounts of release of SRB from the LUVs.

It should be pointed out that the $AzoC_{10}N^+$/Schol LUVs are very efficient nanocontainers, because only ∼7% of the encapsulated SRB had leaked out passively after 30 days (Figure 6D). This impressive impermeability is due to the high content of cholesterol sulfate in the bilayer. This sterol has a strong ability to order neighboring acyl chains[22,28] and, consequently, to reduce the passive permeability of bilayers. A similar limited permeability was reported for analogous nonphospholipid liposomes prepared from a single-chain amphiphile and a sterol.[20,41] The restricted passive release compares advantageously with that of other photosensitive

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formulations. For example, photosensitive dimyristoylphosphatidylcholine/dicetyl phosphate/4-octyl-4′-(5-carboxypentamethyleneoxy)azobenzene (DMPC/DCP/8A5) liposomes showed 10% leakage over 20 h,[10] whereas those prepared with egg phosphatidylcholine and cholesterol onto which an azobenzene moiety had been grafted displayed passive release between 10 and 30% over 40 h.[16,42] Because of their very limited passive permeability, the on-and-off release provided by $AzoC_{10}N^+$/Schol LUVs is sharper than those previously reported with phospholipid-based Azo-containing LUVs for which residual leakage could be observed after the illumination restoring the *trans* Azo conformers.[10,16]

4. CONCLUSION

We demonstrated that it is possible to prepare fluid-phase liposomes from $AzoC_{10}N^+$ and Schol that combine very low passive permeability and photocontrol of the entrapped payload. The high proportion of sterol in the bilayers, which is similar to that reported for other nonphospholipid liposomes prepared from monoacylated amphiphiles and sterols,[19−22,27,28,43] leads to very limited permeability. The presence of the azobenzene derivative makes these LUVs sensitive to light and allows precise control of the release of the encapsulated material. It thus appears that the *trans* form of the azobenzene is compatible with the molecular packing of the bilayer, giving impermeable membranes. On the other hand, the *cis* form introduces defects into these fluid bilayers because of the tightly packed alkyl chains, thereby allowing the photoinduced leakage of the encapsulated material. Because the azobenzene conformer can be photoselected by illumination with the proper energy, it is possible to begin as well as to arrest the release of the entrapped solutes by photoinducing the *cis* and *trans* azobenzene conformers, respectively. Therefore, it is feasible to deliver, at will, a defined quantity of solute from these liposomes. Furthermore, multidose release can be controlled in a straightforward manner by photocycling between the *cis* (to initiate the release) and *trans* (to stop the release) azobenzene isomers. Passive release was found to be minimal. These distinct characteristics make $AzoC_{10}N^+$/Schol LUVs a very versatile type of photosensitive nanocontainer.

AUTHOR INFORMATION

Corresponding Author

*E-mail: michel.lafleur@umontreal.ca.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Mueller, A.; Bondurant, B.; O'Brien, D. F. Visible-light-stimulated destabilization of PEG-liposomes. *Macromolecules* **2000**, *33*, 4799−4804.

(2) Bondurant, B.; Mueller, A.; O'Brien, D. F. Photoinitiated destabilization of sterically stabilized liposomes. *Biochim. Biophys. Acta* **2001**, *1511*, 113−122.

(3) Thompson, D. H.; Gerasimov, O. V.; Wheeler, J. J.; Rui, Y.; Anderson, V. C. Triggerable plasmalogen liposomes: Improvement of system efficiency. *Biochim. Biophys. Acta* **1996**, *1279*, 25−34.

(4) Wymer, N. J.; Gerasimov, O. V.; Thompson, D. H. Cascade liposomal triggering: Light-induced Ca^{2+} release from diplasmenylcholine liposomes triggers PLA2-catalyzed hydrolysis and contents leakage from DPPC liposomes. *Bioconjugate Chem.* **1998**, *9*, 305−308.

(5) Khairutdinov, R. F.; Hurst, J. K. Photocontrol of ion permeation through bilayer membranes using an amphiphilic spiropyran. *Langmuir* **2001**, *17*, 6881−6886.

(6) Koçer, A.; Walko, M.; Meijberg, W.; Feringa, B. L. A light-actuated nanovalve derived from a channel protein. *Science* **2005**, *309*, 755−758.

(7) Ohya, Y.; Okuyama, Y.; Fukunaga, A.; Ouchi, T. Photo-sensitive lipid membrane perturbation by a single chain lipid having terminal spiropyran group. *Supramol. Sci.* **1998**, *5*, 21−29.

(8) Khairutdinov, R. F.; Giertz, K.; Hurst, J. K.; Voloshina, E. N.; Voloshin, N. A.; Minkin, V. I. Photochromism of spirooxazines in homogeneous solution and phospholipid liposomes. *J. Am. Chem. Soc.* **1998**, *120*, 12707−12713.

(9) Shimomura, M.; Kunitake, T. Fluorescence and photoisomerization of azobenzene-containing bilayer membranes. *J. Am. Chem. Soc.* **1987**, *109*, 5175−5183.

(10) Sato, T.; Kijima, M.; Shiga, Y.; Yonezawa, Y. Photochemically controlled ion permeability of liposomal membranes containing amphiphilic azobenzene. *Langmuir* **1991**, *7*, 2330−2335.

(11) Diguet, A.; Yanagisawa, M.; Liu, Y. J.; Brun, E.; Abadie, S.; Rudiuk, S.; Baigl, D. UV-induced bursting of cell-sized multicomponent lipid vesicles in a photosensitive surfactant solution. *J. Am. Chem. Soc.* **2012**, *134*, 4898−4904.

(12) Moss, R. A.; Jiang, W. Cis/trans isomerization in azobenzenecain liposomes. *Langmuir* **1995**, *11*, 4217−4221.

(13) Kuiper, J. M.; Engberts, J. B. F. N. H-aggregation of azobenzene-substituted amphiphiles in vesicular membranes. *Langmuir* **2004**, *20*, 1152−1160.

(14) Bisby, R. H.; Mead, C.; Mitchell, A. C.; Morgan, C. G. Fast laser-induced solute release from liposomes sensitized with photochromic lipid: Effects of temperature, lipid host, and sensitizer concentration. *Biochem. Biophys. Res. Commun.* **1999**, *262*, 406−410.

(15) Bisby, R. H.; Mead, C.; Morgan, C. G. Photosensitive liposomes as 'cages' for laser-triggered solute delivery: The effect of bilayer cholesterol on kinetics of solute release. *FEBS Lett.* **1999**, *463*, 165−168.

(16) Liu, X.-M.; Yang, B.; Wang, Y.-L.; Wang, J.-Y. Photoisomerisable cholesterol derivatives as photo-trigger of liposomes: Effect of lipid polarity, temperature, incorporation ratio, and cholesterol. *Biochim. Biophys. Acta* **2005**, *1720*, 28−34.

(17) Bisby, R. H.; Mead, C.; Morgan, C. G. Active uptake of drugs into photosensitive liposomes and rapid release on UV photolysis. *Photochem. Photobiol.* **2000**, *72*, 57−61.

(18) Morgan, C. G.; Yianni, Y. P.; Sandhu, S. S.; Mitchell, A. C. Liposome fusion and lipid exchange on ultraviolet irradiation of liposomes containing a photochromic phospholipid. *Photochem. Photobiol.* **1995**, *62*, 24−29.

(19) Cui, Z.-K.; Lafleur, M. Lamellar self-assemblies of single-chain amphiphiles and sterols and their derived liposomes: Distinct compositions and distinct properties. *Colloids Surf., B* **2014**, *114*, 177−185.

(20) Phoeung, T.; Aubron, P.; Rydzek, G.; Lafleur, M. pH-triggered release from nonphospholipid LUVs modulated by the pKa of the included fatty acid. *Langmuir* **2010**, *26*, 12769−12776.

(21) Carbajal, G.; Cui, Z.-K.; Lafleur, M. Non-phospholipid liposomes with high sterol content display a very limited permeability. *Sci. China: Chem.* **2013**, *56*, 40−47.

(22) Bastiat, G.; Lafleur, M. Phase behavior of palmitic acid/cholesterol/cholesterol sulfate mixtures and properties of the derived liposomes. *J. Phys. Chem. B* **2007**, *111*, 10929−10937.

(23) Nayar, R.; Hope, M. J.; Cullis, P. R. Generation of large unilamellar vesicles from long-chain saturated phosphatidylcholines by extrusion technique. *Biochim. Biophys. Acta* **1989**, *986*, 200−206.
(24) Dos Santos, N.; Cox, K. A.; McKenzie, C. A.; van Baarda, F.; Gallagher, R. C.; Karlssond, G.; Edwards, K.; Mayer, L. D.; Allen, C.; Bally, M. B. pH gradient loading of anthracyclines into cholesterol-free liposomes: Enhancing drug loading rates through use of ethanol. *Biochim. Biophys. Acta* **2004**, *1661*, 47−60.
(25) Cottenye, N.; Carbajal, G.; Cui, Z.-K.; Dauphin Ducharme, P.; Mauzeroll, J.; Lafleur, M. Formation, stability, and pH sensitivity of free-floating, giant unilamellar vesicles using palmitic acid−cholesterol mixtures. *Soft Matter* **2014**, *10*, 6451−6456.
(26) Ouimet, J.; Lafleur, M. Hydrophobic match between cholesterol and saturated fatty acid is required for the formation of lamellar liquid ordered phases. *Langmuir* **2004**, *20*, 7474−7481.
(27) Cui, Z.-K.; Bouisse, A.; Cottenye, N.; Lafleur, M. Formation of pH-sensitive cationic liposomes from a binary mixture of monoalkylated primary amine and cholesterol. *Langmuir* **2012**, *28*, 13668−13674.
(28) Phoeung, T.; Morfin Huber, L.; Lafleur, M. Cationic detergent/sterol mixtures can form fluid lamellar phases and stable unilamellar vesicles. *Langmuir* **2009**, *25*, 5778−5784.
(29) Zhang, Q.; Bazuin, C. G. Liquid crystallinity and other properties in complexes of cationic azo-containing surfactomesogens with poly(styrenesulfonate). *Macromolecules* **2009**, *42*, 4775−4786.
(30) Chen, R. F.; Knutson, J. R. Mechanism of fluorescence concentration quenching of carboxyfluorescein in liposomes: Energy transfer to nonfluorescent dimers. *Anal. Biochem.* **1988**, *172*, 61−77.
(31) Xue, X.; Zhu, J.; Zhang, W.; Zhang, Z.; Zhu, X. Preparation and characterization of novel main-chain azobenzene polymers via step-growth polymerization based on click chemistry. *Polymer* **2009**, *50*, 4512−4519.
(32) Bandara, H. M. D.; Friss, T. R.; Enriquez, M. M.; Isley, W.; Incarvito, C.; Frank, H. A.; Jose, G.; Burdette, S. C. Proof for the concerted inversion mechanism in the *trans*→*cis* isomerization of azobenzene using hydrogen bonding to induce isomer locking. *J. Org. Chem.* **2010**, *75*, 4817−4827.
(33) Wang, A.; Li, Y.-Y.; Ru, J. The mechanism and application of the electro-Fenton process for azo dye Acid Red 14 degradation using an activated carbon fibre felt cathode. *J. Chem. Technol. Biotechnol.* **2010**, *85*, 1463−1470.
(34) Diau, E. W.-G. A new trans-to-cis photoisomerisation mechanism of azobenzene on the S1(n,π^*) surface. *J. Phys. Chem. A* **2004**, *108*, 950−956.
(35) Song, X.; Perlstein, J.; Whitten, D. G. Supramolecular aggregates of azobenzene phospholipids and related compounds in bilayer assemblies and other microheterogeneous media: Structure, properties, and photoreactivity. *J. Am. Chem. Soc.* **1997**, *119*, 9144−9159.
(36) Shimomura, M.; Ando, R.; Kunitake, T. Orientation and spectral characteristics of the azobenzene chromophore in the ammonium bilayer assembly. *Ber. Bunsenges. Phys. Chem.* **1983**, *87*, 1134−1143.
(37) Liang, X.; Yue, X.; Dai, Z.; Kikuchi, J.-i. Photoresponsive liposomal nanohybrid cerasomes. *Chem. Commun.* **2011**, *47*, 4751−4753.
(38) Qu, J.; Qiu, Z.; Chen, H.; Ogata, N.; Masuda, T. DNA-lipid complexes carrying azobenzene moieties: Preparation, characterization, and photoisomerization. *Polymer* **2009**, *50*, 5398−5405.
(39) Lei, Y.; Hurst, J. K. Photoregulated potassium ion permeation through dihexadecyl phosphate bilayers containig azobenzene and stilbene surfactants. *Langmuir* **1999**, *15*, 3424−3429.
(40) Morgan, C. J.; Bisby, R. H.; Johnson, S. A.; Mitchell, A. C. Fast solute release from photosensitive liposomes: An alternative to 'caged' reagents for use in biological systems. *FEBS Lett.* **1995**, *375*, 113−116.
(41) Cui, Z.-K.; Bastiat, G.; Jin, C.; Keyvanloo, A.; Lafleur, M. Influence of the nature of the sterol on the behavior of palmitic acid/sterol mixtures and their derived liposomes. *Biochim. Biophys. Acta* **2010**, *1798*, 1144−1152.
(42) Liu, X.-M.; Yang, B.; Wang, Y.-L.; Wang, J.-Y. New nanoscale pulsatile drug delivery system. *Chem. Mater.* **2005**, *17*, 2792−2795.
(43) Bastiat, G.; Oliger, P.; Karlsson, G.; Edwards, K.; Lafleur, M. Development of non-phospholipid liposomes containing a high cholesterol concentration. *Langmuir* **2007**, *23*, 7695−7699.