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Light-mediated and H-bond facilitated liposomal release: the role of lipid head groups in release efficiency

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ABSTRACT

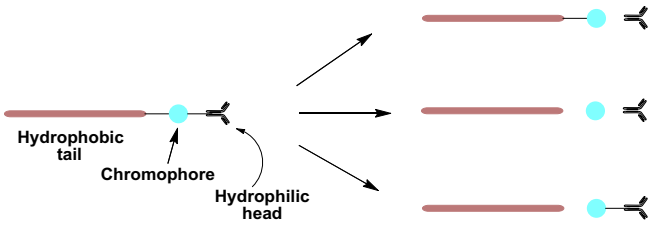
Syntheses of coumarin-containing lipids and liposomal formulations incorporating these lipids are studied. The influence of the lipid head groups in enhancing the release efficiency of these liposomes under light irradiation is studied and a molecular mechanism is provided.

Liposomes are self-assembled lipid bilayers enclosing an aqueous interior. Liposomes are versatile drug carriers approved by the US Food and Drug Administration (FDA) and are amenable to incorporation of other lipid derivatives for targeting/anchoring functionalities.[1] Even though liposomes formulated with phosphatidylcholines by themselves are non-toxic, the passive release of the drug from the currently employed formulations can lead to the development of drug resistance and side effects due to inefficient and incomplete drug release.[2] Several triggered release mechanisms, for example, enzymes,[3] pH,[2c] heat,[4] metal ions[5] and redox potential[2c] have been developed for efficient and active release of liposomal contents.[2–5]

We have been investigating light-mediated release of liposomal contents as this could potentially provide an external spatiotemporal control over the drug release.[6] These liposomes typically incorporate a phosphatidylcholine and a photosensitive lipid. The ideal photosensitive lipid amphiphile is comprised of three parts: (i) a polar hydrophilic head group, (ii) the chromophoric group responsible for light absorption and subsequent photochemistry and (iii) a hydrophobic tail (Scheme 1). Upon irradiation, liposomes incorporating these lipids are rendered unstable due to the photochemistry of the chromophore. In order to regain stability, liposomes will reorganize the lipids and in the process, the liposomal contents are released.

Whereas the rate of photochemical cleavage is primarily determined by the chromophore, the head and tail groups of the lipid amphiphile can potentially influence the release profiles from the liposomes. The chemical nature of the head group may allow it to have secondary interactions within its microenvironment, affecting the light-mediated cleavage process of the lipid amphiphile.[7] On the other hand, the lipid tail can influence the distribution of photosensitive lipids within the liposomal bilayer, influencing the release efficiency of the liposomes.[3a] Thus, the ideal photosensitive liposomal formulation needs to incorporate the photosensitive lipid amphiphile that is comprised of the optimum head group and lipid tail at concentrations that will yield minimum background release and efficient release with short durations of irradiation.

The coumarin chromophore has been extensively studied as a photolabile protective group for carboxylates,[8,12] alcohols,[9] diols,[10] carbonyl groups,[11] amines,[12] etc.[8,12] We envisaged that lipid amphiphiles of coumarins can be utilized to prepare photosensitive liposomes. Herein, we report our results on the release of liposomal contents from coumarin-containing lipids under single photon irradiation. We synthesized lipid derivatives of 6-bromo-7-hydroxycoumarin-4-methyl (Bhc) group (Scheme 3)[13] for incorporation into liposomes. Bhc-carbamates were previously shown to be photolabile protective groups for amines (Scheme 2).[12] For the optimization of the lipid head group, we intentionally prepared the phenolic ester derivatives of the coumarin moiety (Scheme 3). We anticipated that the resulting lipid will not have any significant absorption at near visible wavelengths resulting in minimal (or non-existent) photocleavage by the visible light. The synthesized lipids had absorption maxima at 272 and 322 nm.[13] In order to verify if the synthesized lipids followed a similar photolysis pattern,



Scheme 1. Potential outcomes upon irradiation of a photosensitive lipid.

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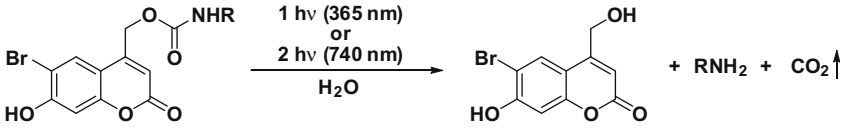
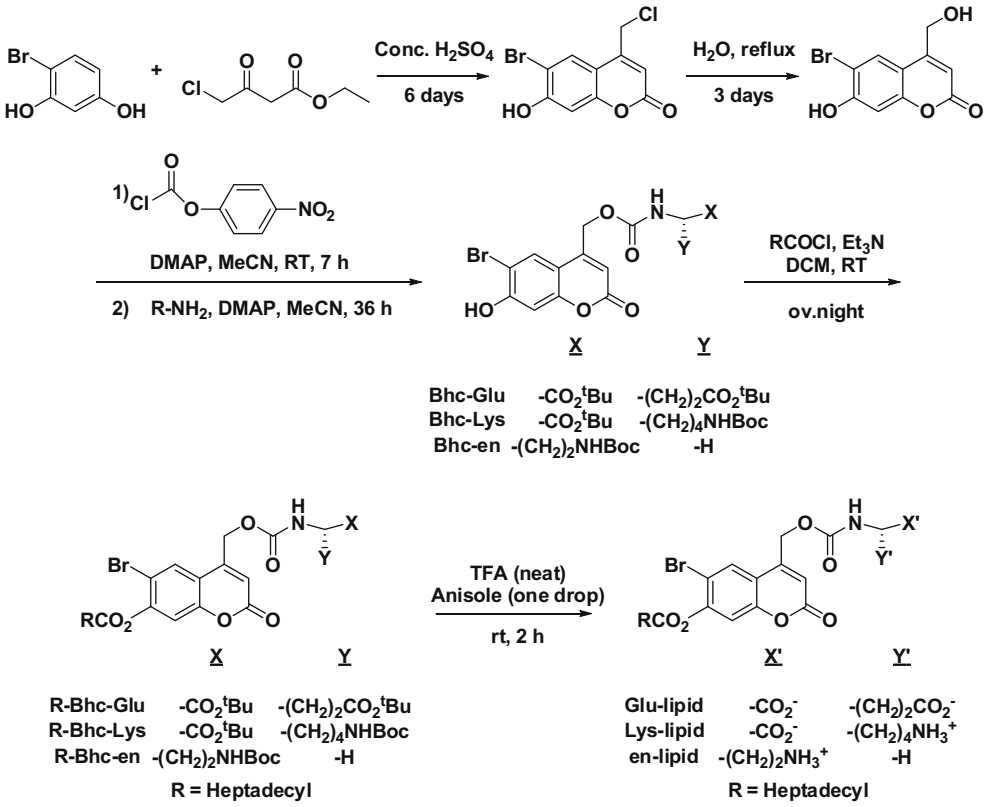


Scheme 2. Photolysis of Bhc-carbamates.



Scheme 3. Synthesis of photocleavable lipids.

the **Glu-lipid** was subjected to 254 nm and 365 nm irradiation. The photoproducts of the **Glu-lipid** were characterized by mass spectroscopy and indicated the cleavage of the lipid amphiphile at the carbamate group releasing the amino acid among other unidentifiable products.[13]

Initially, we prepared the liposomes formulated with 20 mol % of **Glu-lipid** and 80 mol % of different phosphatidylcholines encapsulating the self-quenching dye 5(6)-carboxyfluorescein (HEPES buffer, 25 mM, pH 7.0). The liposomes were irradiated at 254 nm and the release of the encapsulated dye was monitored by the enhancement of emission intensity at 520 nm (λ_{ex} = 490 nm) as a function of irradiation time. The results are shown in Table 1. We observed that the release efficiency of the liposomes depends on the transition temperature (T_m) of the major phosphatidylcholines. For example, 1,2-distearoyl-*sn*-glycero-3-phospocholine (DSPC) has a T_m of 55 °C and the liposomes released about 11% of the contents against a 2% background (non-irradiated) release. However, the release efficiency improved appreciably with 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (T_m = 41 °C, 40% irradiated release vs 2% background release) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphochloine (POPC) (T_m = −2 °C, 71% irradiated

Table 1
Release summary for various liposomal formulations under 254 nm irradiation for 2 h.[a]

Head group (20 mol %)	Diluent (80 mol %)	Irradiated Release (%) (254 nm)	Background release (%)
Glu	DSPC	11	2
	DPPC	40	2
	POPC	71	2
Lys	DSPC	21	3
	DPPC	63	4
	POPC	85	1

[a] Average of duplicate experiments.

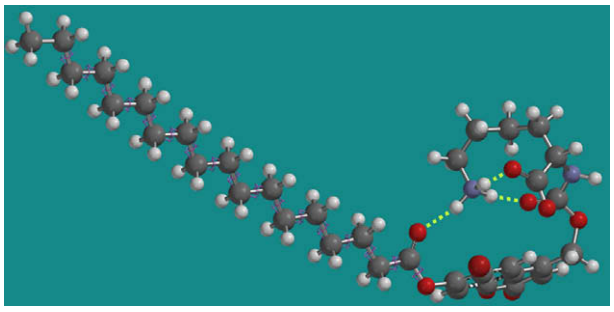


Figure 1. Energy-minimized structure of the **Lys-lipid** conformer showing the hydrogen bonds.

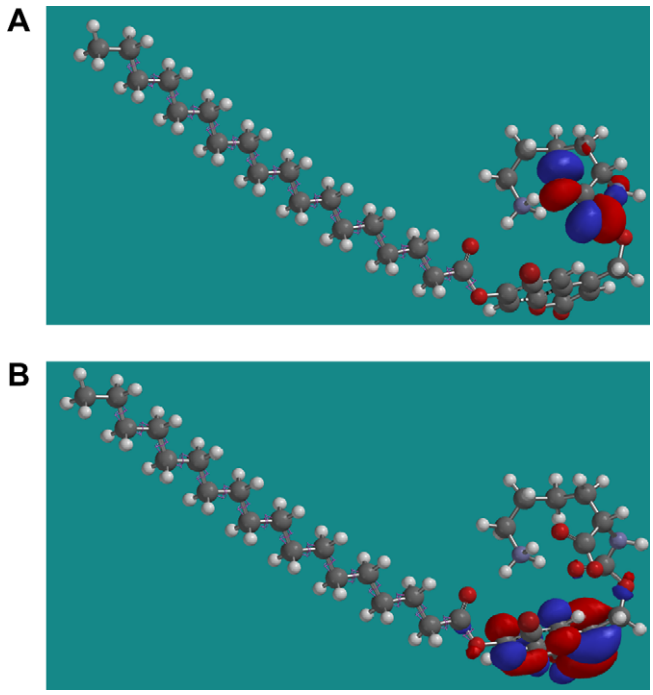


Figure 2. (A) HOMO, (B) LUMO of the **Lys-lipid** conformer shown in Figure 1.

release vs 2% background release). This was anticipated since the liposomal contents release are favored at temperatures higher than T_m (liquid crystalline phase) compared to temperatures below T_m (gel phase).[14] Interestingly, when different liposomes formulated with the **Lys-lipid** (20 mol %) and various phosphocholines (80 mol %) were irradiated with 254 nm light, the release efficiency was consistently higher than that of the **Glu-lipid** liposomes (Table 1).

Apart from the number of the methylene moieties in the amino acid head groups, the only distinguishing feature between the two lipids is the charge on the head group, and hence on the resulting liposomes. At pH 7.0, while the dianionic **Glu-lipid** is expected to give negatively charged liposomes, the zwitterionic **Lys-lipid** will generate neutral liposomes. In order to understand if the positive charge on the ε-amino group of lysine would in any way assist the photocleavage process, we performed DFT (B3LYP) calculations using the Spartan® software (Wavefunction, Inc.). We found that the ε-ammonium group of the lysine in the **Lys-lipid** folded back to simultaneously hydrogen bond the α-carboxylate, the phenolic ester and the carbamate groups (Fig. 1).

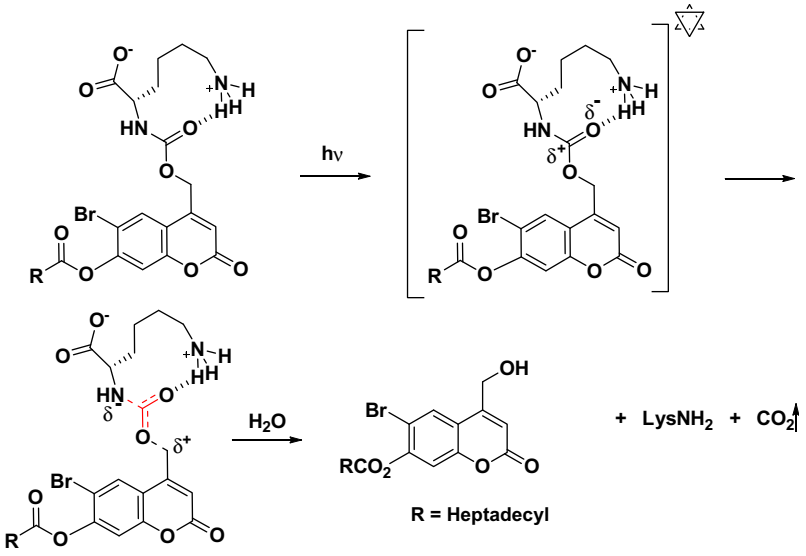
Upon calculating the HOMO and LUMO for the conformer shown in Figure 1, we observed that the HOMO is localized on the α-carboxylate moiety whereas the LUMO is distributed over the coumarin ring with a small coefficient on the carbamate group (Fig. 2).

Based on the results of the calculations, we hypothesized that the ε-ammonium group is likely acting as a general acid to protonate and stabilize the developing negative charge on the carbamate group upon photoexcitation (Scheme 4). The protonated carbamate will be a better leaving group, and thus the head group from the **Lys-lipid** is possibly assisting in the photocleavage, and the subsequent release of the lysine from the lipid (Scheme 4). This would result in liposomal destabilization, releasing the encapsulated contents upon lipid reorganization.

In order to verify our hypothesis, we synthesized a Bhc lipid derivative with an ethylenediamine head group (**en-lipid**, Scheme 3). We formulated liposomes containing 20 mol % **en-lipid** and 80 mol % POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine). These liposomes were irradiated with 254 nm light, and the release was monitored for 2 h. It was found that the release efficiency for the **en-lipid** was higher than that of the **Lys-lipid** (100% vs 85%) under identical conditions (Fig. 3). The improved release efficiency in the case of **en-lipid** compared to the **Lys-lipid** is probably due to the smaller ring formed during the hydrogen bonding in the case of **en-lipid** compared to the **Lys-lipid**.

Particle size of the liposomes incorporating 20 mol % **Lys-lipid** and 80 mol % POPC was measured before and after irradiation employing dynamic light scattering. While particles before irradiation measured 38–40 nm in diameter, after 2 h of irradiation, the particle diameter measured decreased to about 20 nm—suggesting that liposomes underwent reorganization and leakage of contents from the lumen following irradiation. In contrast, the non-irradiated sample did not show any change in particle size even after 5 h.

In conclusion, we have identified an optimal head group for the Bhc-based photocleavable lipids and provided a molecular explanation for the enhanced release efficiency demonstrated by these head groups. The studies for identifying the role of the lipid tail, the effect of these lipids on the phosphatidylcholines and the



Scheme 4. Proposed mechanism of photolysis facilitation by a general acid in the head group.

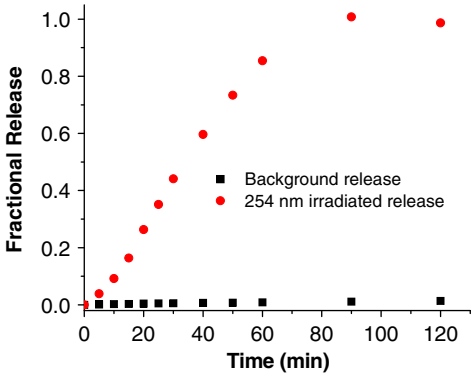


Figure 3. Photorelease profiles for a liposomal formulation containing 20 mol % **enlipid** and 80 mol % POPC in HEPES buffer (25 mM, pH 7.0).

two-photon-mediated liposomal release are currently under progress, and will be reported shortly.

Acknowledgments

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Supplementary data

Supplementary data (the synthesis, ^{1}H and ^{13}C NMR spectra of the lipids and the photolysis conditions) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.11.084.

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