LIBERTY UNIVERSITY

Cholesterol Terminated Fluorescent Dyes

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Background

This experiment focuses on attaching a cholesterol group to the fluorophore, 9,10-anthraquinone core **1 (Fig.1)**. Cholesterol is a structural component of cell membranes, and its lipophilic properties allow it to pass through the phospholipid bilayer.[1] Anthraquinones are a class of compounds obtained from natural sources such as fungi, plants, animals, and bacteria and represent the second most important class of dyes. Anthraquinone dyes are composed of 9,10-anthraquinones and can be modified at various positions **3 (Fig. 2)**.[2] This reaction uses a cholesterol derivative which has been used as a lipid anchor for hydrophobization of other fluorophores.[3] Cholesterol-fluorophore conjugates have been used to examine the functions of cholesterol in cell membranes such as cholesterol organization, trafficking, and lipid interactions.[4] Fluorescence spectroscopy is a method of analyzing a compound's fluorescent properties and is used extensively in biotechnology, flow cytometry, medical diagnostics, DNA sequencing, and genetic analysis.[5] This experiment will attempt to synthesize a fluorescent dye that will be able to embed its lipid portion in the phospholipid bilayer while the fluorophore is left exposed.[6] Successfully attaching the cholesterol group to the fluorophore suggests the potential for fluorescent dye integration in the cell membrane and cell staining applications **(Fig. 9)**.

Research Question

Can cholesterol be successfully attached to anthraquinone to synthesize a cholesterol-fluorophore bioconjugate? Once accomplished, is this an effective fluorophore for fluorescent biomedical applications?

Methods

The EGB1 compound was synthesized using the starting material and a cholesterol derivative. The product was analyzed by thin-layer chromatography (TLC), infrared spectroscopy (IR), and fluorescence spectroscopy. TLC was performed using silica gel TLC plates and a mobile phase of chloroform and methanol (9:1) and visualized under visible light, short wavelength (254 nm) UV light, long wavelength (365 nm) UV light, and cerium ammonium molybdate stain (CAM stain) **(Fig. 3)**. Infrared spectroscopy was used to analyze the compound's purity and identity **(Fig. 4)**. Fluorescence spectroscopy was used to analyze the fluorescent properties of the starting material and EGB1. These properties include the excitation and emission spectra **(Fig. 5 & 6)** and Stokes shift. The step increment of the spectrofluorometer was 5 nm and the scans for the starting material and EGB1 were taken between 294-750 nm and 288-750 nm, respectively. Solubility tests of starting material **(Fig. 7)** and EGB1 **(Fig. 8)** were performed using 5mg of each compound along with the following solvents: diethyl ether, acetone, cyclohexane, and ethyl acetate.

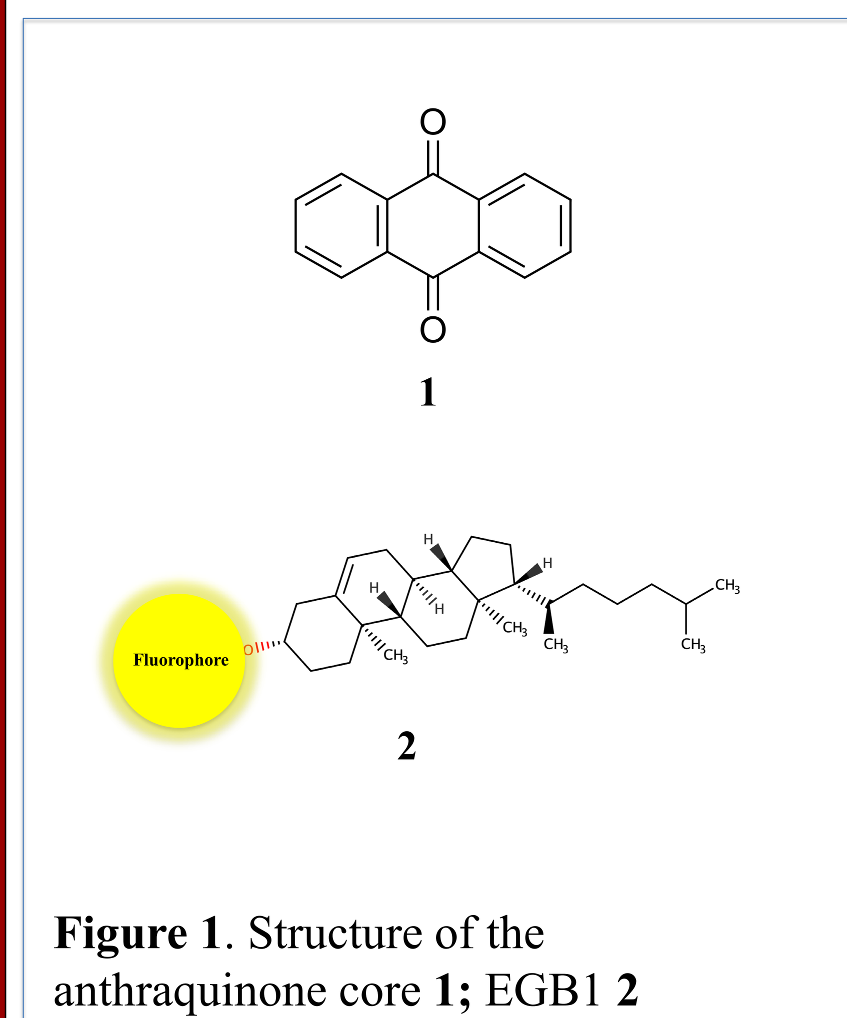


Figure 1. Structure of the anthraquinone core **1;** EGB1 **2**

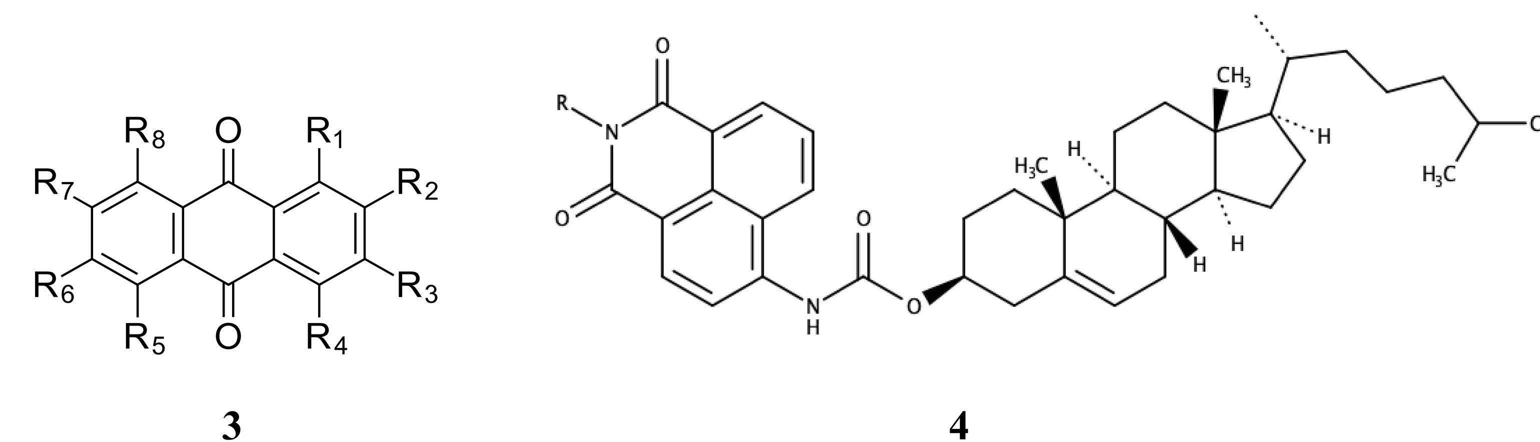


Figure 2. Structure of the anthraquinone core with numbered R-group positions **3;** cholesterol–naphthalimide conjugate **4** [6].

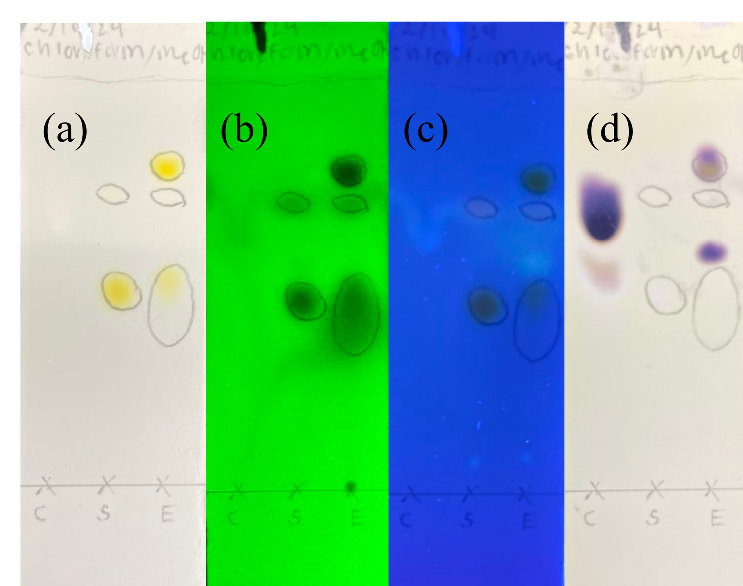


Figure 3. TLC plates of the cholesterol derivative (left lane), starting material (center lane), and the sediment portion of the EGB1 reaction sample (right lane); (a) daylight; (b) 254 nm UV light; (c) 365 nm UV light; (d) CAM stain.

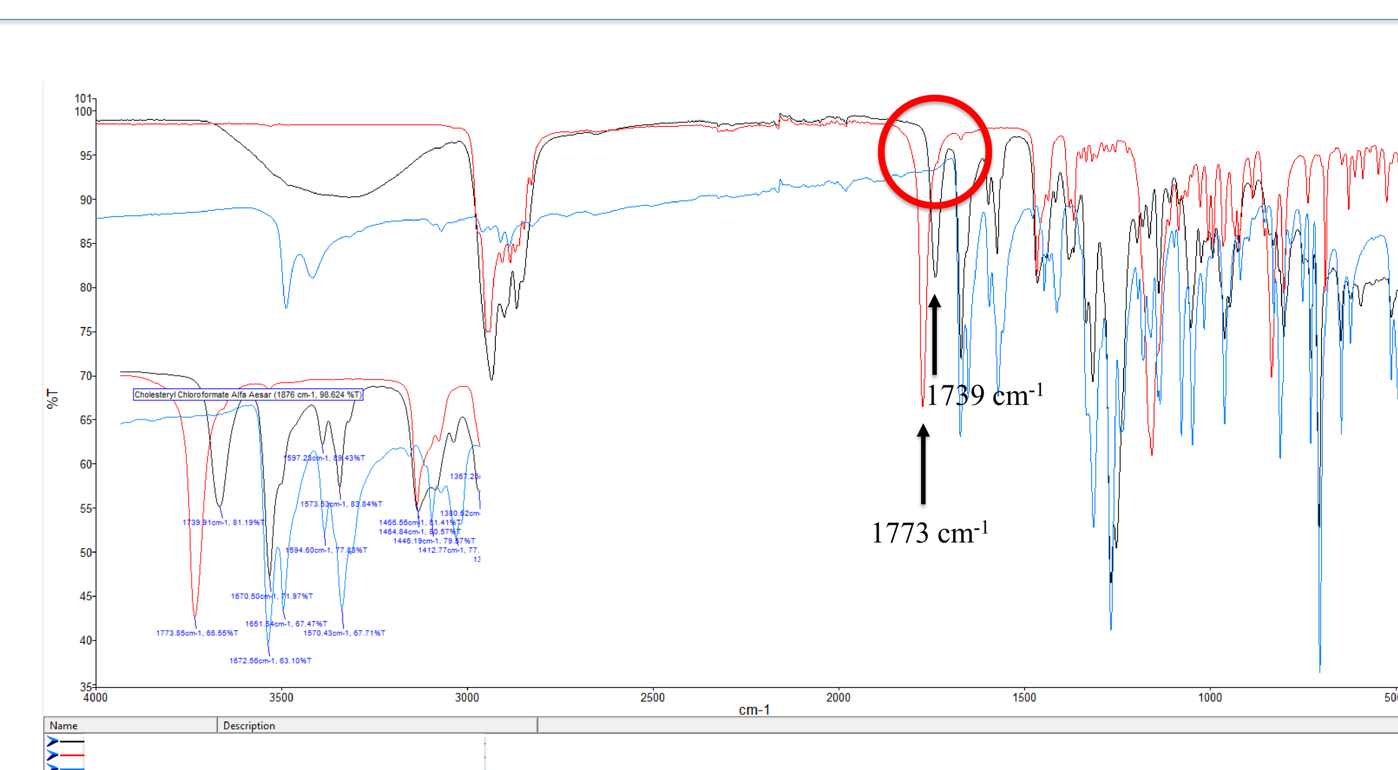
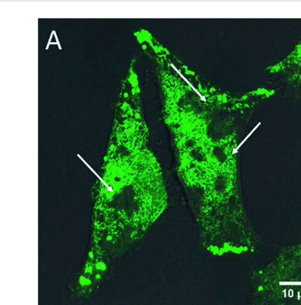


Figure 4. Infrared spectrum of EGB1(black), starting material (blue), and cholesterol reagent (red); insert shows magnified region from (1876-1380 cm^{-1}). The red circle indicates the region of focus on the IR spectrum. The black arrows point to the peaks of greatest interest.

Figure 9. Confocal fluorescence image of live Hela cells and CHO cells stained with a BODIPY-Ahx-Chol [7].



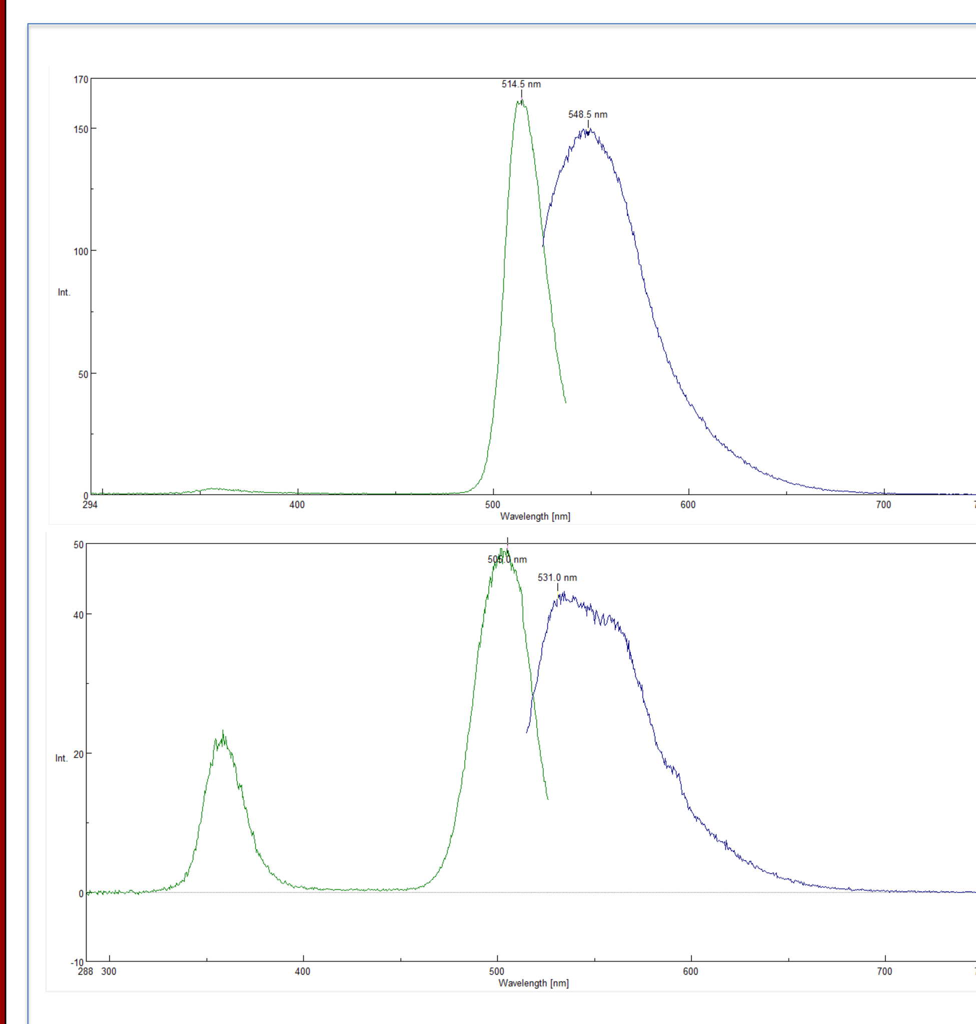


Figure 5. Excitation-emission spectrum of starting material in THF

Figure 6. Excitation-emission spectrum of EGB1 in THF

Figure 7. Starting material in various solvents. From left to right: diethyl ether, acetone, cyclohexane, and ethyl acetate.

Figure 8. EGB1 in various solvents. From left to right: diethyl ether, acetone, cyclohexane, and ethyl acetate.

Results and Conclusions

1. TLC: Under visible light, the spots in the EGB1 lane appear yellow, suggesting that the fluorophore is attached. The CAM stain suggests that cholesterol is present in EGB1.

2. IR: A close analysis of the IR spectrum suggests that the starting material is no longer present in the EGB1 compound.

3. Fluorescence Spectroscopy: The starting material exhibited a Stokes shift of 34 nm while EGB1 exhibited a Stokes shift of 26 nm.

4. Solubility: The starting material was insoluble in all solvents except for acetone. EGB1 was insoluble in all tested solvents thus far.

Future Work

The synthesis and analysis of a cholesterol-functionalized fluorescent dye is impactful within the culture at large because it has the potential to improve the efficacy, efficiency, and accessibility of biomedical applications.

Once the cholesterol-fluorophore bioconjugate is successfully synthesized, future work would entail applying this fluorophore to cell staining techniques such as flow cytometry and fluorescence microscopy. Future research could be conducted comparing EGB1 to commercially manufactured fluorescent dye.

References and Acknowledgments

References

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