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Goutam Ghosh
Graduate Center, City University of New York

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SYNTHESIS AND EVALUATION OF SENSITIZER DRUG PHOTORELEASE CHEMISTRY: MICRO-OPTIC METHOD APPLIED TO SINGLET OXYGEN GENERATION AND DRUG DELIVERY

by

GOUTAM GHOSH

A DISSERTATION SUBMITTED TO THE GRADUATE FACULTY IN CHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, THE CITY UNIVERSITY OF NEW YORK

2014
This manuscript has been read and accepted by the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy

PROFESSOR ALEXANDER GREER
(Chair of Examining Committee)

PROFESSOR MARIA C. TAMARGO
(Executive Officer)

PROFESSOR ALEXANDER GREER

PROFESSOR SHENPING ZHENG

PROFESSOR ROBERT ENGEL
(Supervisory Committee)

THE CITY UNIVERSITY OF NEW YORK
Abstract

SYNTHESIS AND EVALUATION OF SENSITIZER DRUG PHOTORELEASE CHEMISTRY: MICRO-OPTIC METHOD APPLIED TO SINGLET OXYGEN GENERATION AND DRUG DELIVERY

BY

GOUTAM GHOSH

Adviser: Professor Alexander Greer

Abstract: This thesis summarizes a new micro-optic method for singlet oxygen generation and sensitized drug delivery, which include i) synthesis and evaluation of a first generation device for drug delivery from native and fluorinated silica probe tips, ii) synthesis of PEG conjugated sensitizers to study phototoxicity in ovarian cancer cells, and iii) synthesis and evaluation of tris-PEGylated chlorin conjugated fluorinated silica for its future integration into the device to use as a 2nd generation device. A first generation micro-optic device was developed that works by sparging O₂ gas and light generating cytotoxic singlet oxygen that cleaves the covalently attached drug (sensitizer) from the probe tip at the distal end of the fiber. The aim is to develop a 1st and 2nd generation device for site specific delivery of photosensitizer and singlet oxygen to overcome the challenges involved in systemic administration of the sensitizer.

Synthesis and evaluation of drug (pheophorbide-α) delivery applying micro-optic method from native and fluorinated silica probe tip was achieved. The amount of sensitizer photocleavage depends on the loading level of sensitizer onto the probe tips. We also found that photorelease efficiency depends on the nature of the solvents where sensitizer is photocleaved. For example, no photorelease was observed in an aqueous solvent where sensitizer remained
adsorbed to the native silica probe-tip. But, 90% photocleavage was obtained in octanol. A significant amount of photosensitizer (formate ester of pyropheophorbide-\(d\)) diffused into the liposome when photocleavage study was carried out in liposome. Substantial increase of photorelease was observed in organic solvent when pyropheophorbide-\(d\) (PPa) sensitizer was attached to the partially fluorinated porous Vycor glass. We also explored sensitizer photorelease from the fluorinated silica surface at various temperatures and we found that autocatalytic photorelease happened at room temperature and above. No photorelease was observed at low temperature.

Chlorin e\(_6\) and its one, two and three short chain methoxy triethylene glycol (PEG) conjugated derivatives were synthesized. A comparative study of photocytotoxicity and cellular uptake between each showed that \(17^3,15^2,13^1\)-chlorin e\(_6\) methoxy triethylene glycol triester has the highest photocytotoxic activity and uptake by ovarian OVCAR-5 cancer cells.

Therefore, we decided to load three short chain PEG conjugated chlorin e\(_6\) onto the silica surface through spacer alkene for delivery via a fiber-optic probe tip. In order to load chlorin e\(_6\)-triPEG ester conjugate, in chapter 4, we explored different synthetic strategies. We have been successful in synthesizing spacer alkene succinate linker conjugated chlorin e\(_6\)-tri PEG ester, which was attached to the fiber-optic probe tip. Reactions were carried out in mild conditions to avoid detachment of the PEG ester from the carboxylic acid sites of chlorin. Photocleavage of the triPEG modified fluorinated probe tip system was studied in \(n\)-butanol.
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detecting mass spectra of some chlorin sensitizers. I really appreciate the help from my friend, Joshua Jones for numerous discussions regarding synthesis. I am thankful to all the staff members in the Department of Chemistry of Brooklyn College and Graduate Center for helping me in many aspects during the five years of my Ph.D path.

My last, but not least, appreciation goes to my parents, who always stand next to me and support me. They provide me with the deepest love, the continuous support and encouragement. It is them who always believed in me and encouraged me to do my best. Without their love and confidence in my abilities, I would have never been able to achieve what I achieved so far in life.
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<tr>
<td>Å</td>
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<tr>
<td>brine</td>
<td>Saturated aqueous sodium chloride solution</td>
</tr>
<tr>
<td>br</td>
<td>Broad</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>CCl₄</td>
<td>Carbon tetrachloride</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
</tr>
<tr>
<td>CDCl₃</td>
<td>Deuterated chloroform</td>
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<tr>
<td>DCM</td>
<td>Dichloromethane</td>
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<tr>
<td>DMF</td>
<td>Dimethyl formamide</td>
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<td>DMAP</td>
<td>4-Dimethyl amino pyridine</td>
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<tr>
<td>D₂O</td>
<td>Deuterium oxide</td>
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<tr>
<td>d</td>
<td>Doublet</td>
</tr>
<tr>
<td>dd</td>
<td>Doublet</td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift in ppm</td>
</tr>
<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>Et₂O</td>
<td>Diethyl ether</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>¹H NMR</td>
<td>Proton nuclear magnetic resonance</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant</td>
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<td>Symbol</td>
<td>Name</td>
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<td>-------------------------------</td>
</tr>
<tr>
<td>LiAlH₄</td>
<td>Lithium aluminum hydride</td>
</tr>
<tr>
<td>m</td>
<td>Multiplet</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
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<td>mg</td>
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<td>NaH</td>
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<td>Sodium borohydride</td>
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<tr>
<td>NBS</td>
<td>n-Bromosuccinimide</td>
</tr>
<tr>
<td>NaI</td>
<td>Sodium iodide</td>
</tr>
<tr>
<td>n-BuLi</td>
<td>n-Butyllithium</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PVG</td>
<td>Porous vycor glass</td>
</tr>
<tr>
<td>q</td>
<td>Quartet</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet and visible spectroscopy</td>
</tr>
<tr>
<td>DPPC</td>
<td>Dipalmitoyl phosphatidil choline</td>
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<tr>
<td>EL</td>
<td>Egg lecithin</td>
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Chapter 1: Background

1.1 The Need for Point-source Device in Precise Tumor Removal. Precise removal of tumor possesses a great challenge to surgeons. With the current medical technology, removal of tumor close to the vital organs\(^1,2,3\) is extremely difficult. Although surgery, chemotherapy and radiation therapy are the mainstays treatments,\(^4,5,6\) advancement in this field is highly required. Therefore, therapies in combination are desired for high-precision tumor excision.

One attractive method is photodynamic therapy (PDT), in which cytotoxic singlet oxygen (\(^1\)O\(_2\)) is among the key reactive species generated from molecular oxygen and can kills cancer cells. Although PDT is an efficient method of tumor destruction, some limitations in conventional PDT exist, such as (i) uniform delivery of light to the diseased region, and (ii) difficulty of employing the technique under hypoxic conditions in tumor tissue,\(^7,8,9\) as the yield of singlet oxygen depends on the concentration of ground-state oxygen (triplet oxygen).

A significant advancement in PDT methodology would be the “point-source” delivery of singlet oxygen. The word point-source refers to the level of precision in photorelease of sensitizer and generation of singlet oxygen to a target area using the hand-held phototherapy device (Fig 1), which was first reported by our research group.\(^10,11\) The generated cytotoxic singlet oxygen kills tumor cells rapidly in the target area. Our device has a potential in oxygenating the tumor. Therefore, it could be used in biomedical application for the treatment of hypoxic tumors. The research projects presented here involve the use of this integrative device with inserted hollow optical fiber carrying light and oxygen, and includes singlet oxygen generation by optical excitation of sensitizer (chlorin) released from silica probe tip.
Figure 1. Fiber-optic coupled sensitizer modified porous silica cap for singlet oxygen ($^1$O$_2$) and sensitizer-drug delivery. Porous silica cap (native or fluorinated porous Vycor glass) is covalently attached to spacer alkene-sensitizer conjugate. Hollow fiber optic, which is inserted into the porous silica cap carry oxygen and red light. Sensitizer attached to the probe tip (native or fluorinated porous Vycor glass) produce singlet oxygen and photodetaches sensitizer molecules.


1.2 Overview on the Point-source Device. Key components of our device are: oxygen, sensitizer, fiber optic cable and light source. Sensitizer, which plays an important role in PDT and in our device, is covalently attached to the silica cap to generate cytotoxic singlet oxygen in presence of light and oxygen. We have used chlorin compounds (pheophorbide-$a$ and chlorin e$_6$) as sensitizers. Chlorins are unique class of sensitizer with slightly different in structure from porphyrin. As shown in the Figure 2 below, porphyrin core is aromatic and 22\pi electronic system. However, only 18\pi electrons take part in one conjugated pathway and still maintain the aromaticity.$^{12}$ Thus, the remaining two isolated \pi bonds can be easily reduced. Reduction of one such \pi bond converts porphyrin into chlorin. This saturation of one of the C=C \pi bonds brings dramatic change in their absorption spectra. For example, weak Q-absorption bands for porphyrins are observed in between 450-700 nm,$^{13}$ whereas, for chlorins intense Q-band appears
at or beyond 650 nm. Intense absorption at this wavelength allows a greater optical penetration depth in tissue. This specific difference opens up the utility of chlorins over porphyrins as a near IR sensitizer in photodynamic therapy to treat tumor deep inside the body.

Figure 2. Structural differences are shown between tetrapyrolic moiety of porphyrin 1 and chlorin 2. Isolated double bonds are marked in red color in porphyrin. One of such double bonds are absent in chlorin (marked in blue color).

Chlorins such as temoporfin and mono-L-aspartyl chlorin e6 (referred as NPε6) are in advanced stage of clinical trial for FDA approval.14,15 Chlorin-based photosensitizer (Chlorin e6, monoethylene diamine monoamide) has been used to treat murine model of ovarian cancer.16 It has been reported that polyethylene glycol modified poly-L-lysine chlorin e6 conjugate improves tumor targeting based on PDT study at ovarian cancer cell line (OVCAR-5)17 in vitro. In chapter 3, we have discussed in details about chlorin e6 and its PEG conjugates for in vitro ovarian cancer phototherapy. In chapter 4, synthetic route for attachment of chlorin e6-triPEG ester onto a fluorinated silica probe-tip to integrate ovarian cancer photokilling using our device has been reported.

Second component of our device is fiber, we have inserted fiber into the silica cap to carry laser light for precise light delivery to the targeted site. Fiber is very efficient light carrier when it comes to deliver light in very small areas (~1 mm).18 Core-diameter of the optical fiber
can be varied between 20-600 µm depending on the area of the treatment site. In all our studies we have used optical fiber of core-diameter 1.1 mm.

Another component in the device is red laser light. It excites sensitizer, which thereby reaches to its singlet state followed by triplet state and mix with ground state oxygen to generate singlet oxygen. To excite a dye at its absorption maxima, drug and its corresponding light source of specific bandwidth needs to be selected together. Most recent light sources, which have been used in photodynamic therapy, are argon pumped dye laser and semiconductor diode laser. Argon-pumped dye laser combined with fiber-optic has been used to treat cancers such as early stage lung cancer, esophagus cancer. Diode lasers were also used extensively combining with fiber-optic for endoscopic PDT application.

We used continuous wave diode laser as a light source, which was connected to the custom made fiber-optic cable. The distal end of fiber-optic cable was inserted into the porous Vycor glass cap and glued by ethyl cyanoacrylate for the generation of singlet oxygen and drug delivery.

1.3 Advancement of the Device. The aim of the work presented in this thesis is advancement of the device towards its use as an alternative PDT device in the future. Towards this development, this thesis works mainly focuses on synthesis of sensitizer and sensitizer-alkene conjugates followed by attachment of them to the native and fluorinated silica probe tip, and photocleavage study of the sensitizer from those probe-tips. In chapter 2, we reported the synthesis of PPa-spacer alkene conjugates and its covalent attachment to the native and fluorinated silica probe-tip, which was used to study: (i) effect of surface fluorination on sensitizer photorelease, and (ii) autocatalytic photorelease of sensitizer attached to the fluorinated silica. In chapter 3, we synthesized chlorin e6-PEG conjugates to explore chemistry: (i) philicity, (ii) relative stability,
and (iii) computed conformations of PEG groups. We also explored biology of chlorin e₆-PEG conjugates such as (i) cellular uptake, (ii) phototoxicity, and (iii) the mechanistic differences in their photochemistry in *in vitro* ovarian cancer cell. These studies were important to decide what sensitizer would work well to integrate into our device for killing ovarian cancer cell. The results obtained in chapter 3 enabled us to find out the best sensitizer. Therefore, in chapter 4, we designed synthetic strategies and successfully attached that sensitizer to the fluorinated silica for its future application in the area of ovarian cancer phototherapy.
1.4 References


Chapter 2. Singlet Oxygen Generation and Photosensitizer Drug Delivery  
Applying Micro-Optic Method

2.1 Introduction. Photosensitizers in photodynamic therapy (PDT) are used as drugs that generate cytotoxic singlet oxygen ($^1$O$_2$) upon irradiation of light.$^1$ Singlet oxygen damage cancer cells by apoptosis or necrosis. PDT involves systemic administration of the sensitizer followed by visible light irradiation to the diseased tissue after its accumulation (the usual time of sensitizer accumulation is 3-5 days).$^1$

We have developed fiber-optic guided unique sensitizer drug delivery system. Many groups have used UV-light for their drug delivery applications. Perhaps the most widely used drug delivery systems employing the use of photo-labile protecting groups are 6-nitroveratryloxycarbonyl (NVOC) and 2-nitrobenzyloxy carbonyl.$^2$ Ester bonded 3,5-dimethyl benzoin has also been used for UV-light drug delivery.$^3$ Because of the lower penetration depth and harmful effects of UV-light in the \textit{in vivo} environment, the use of visible light is often encouraged. We developed a system that employs visible light and oxygen for drug delivery. Silica Gel, polymers, alumina have been used as a solid support for photosensitizer in singlet oxygen generation, as reported in the earlier literature. Dolphine$^4$ and Breslow$^5$ have used visible light and oxygen for solution phase photocleavage and drug delivery. We have used singlet oxygen as a reagent, which is generated \textit{in situ} by sensitizer and reacts with the electrophilic alkene, conjugated to the sensitizer by an ester bond. Singlet oxygen can diffuse through the solution. Therefore, it can react with the substrate moiety away from its site of generation. However, diffusion distance and the lifetime of singlet oxygen are very small. They depend on the nature of the solvent singlet oxygen is produced in. For example, in H$_2$O the lifetime of singlet oxygen is 3.5 $\mu$s$^6$ and diffusion distance is 150 nm, whereas in D$_2$O the lifetime is 65 $\mu$s and diffusion distance is 62 $\mu$m.$^7$ The first report of $^1$O$_2$ as a diffusible intermediate came from
Kautsky and de Bruijn in 1931, in which trypaflavine (a sensitizer) and leucomalachite green (an oxygen-acceptor compound) were adsorbed separately on silica gel beads. Upon irradiation of light in the presence of O$_2$, ¹O$_2$ was generated on sensitizer coupled SiO$_2$ bead and diffused to another (separate) SiO$_2$ bead where it was trapped by leucomalachite green. The first covalently bound heterogeneous photosensitizer was polymer Rose Bengal, where chloromethylated styrene-divinylbenzene copolymer beads were used as a heterogeneous surface. This was achieved by Neckers et al.

Our hypothesis was that sensitizer molecules, which are covalently attached to the silica surface would be photoreleased by the singlet oxygen (which is generated in situ), upon reacting with electron rich spacer alkene, conjugated by an ester bond with the sensitizer. This covalently attached system enables generation of singlet oxygen away from the surface. Therefore, it is less likely to be quenched by surface functional group (Si-OH). O$_2$ gas was purged from a compressed oxygen tank to a T-valve in the custom optical fiber, which was connected to the sensitizer cap via a Teflon inner flow tube. Pheophorbide was selected as the ¹O$_2$ sensitizer, and Z-enol ether was used as an electron rich spacer group bridging the sensitizer and the glass tip, which can react with ¹O$_2$ and be cleaved via formation of dioxetane intermediate.

We have used pheophorbide-$a$ as a photosensitizer since it exhibits a Q-band absorption maximum at 660 nm. Therefore, it will be ideal for use in the tissue environment. It is known from literature that light within 600-700 nm region penetrates 50-200% more (higher optical penetration depth) than light within 400-500 nm region. In PDT, phephorbide-$a$ is known to be localized in mitochondria and induce cell death by apoptosis or necrosis. We have used Z-spacer alkene as a substrate for singlet oxygen, as rate of reaction of singlet oxygen with the electron rich spacer alkene is higher than the E-alkene.
In this chapter we will discuss the synthesis of PPa-spacer alkene conjugates and its covalent attachment to the native and fluorinated silica probe-tip to study: (i) determination of loading of the sensitizer and how it affects photorelease of the sensitizer, (ii) influence of solvents on photorelease, (iii) effect of surface fluorination on sensitizer photorelease, (iv) surface quenching effect of native and fluorinated silica on singlet oxygen, and (v) autocatalytic photorelease of sensitizer attached to the fluorinated and native silica at various temperatures.

2.2 Results and discussion

2.2.1 Covalent Attachment of Sensitizer to the Porous Vycor Glass (Synthetic Scheme 1 was designed and first carried out by Dr. Matibur Zamadar. This scheme was repeated many times for my research project: determination of sensitizer loading and photorelease in various solvents). We synthesized photocleavable system that delivers hydrophobic sensitizer pyropheophorbide-\(a\) formate ester 3 upon cleavage of the electron rich alkene via the formation of dioxetane intermediate 2. Hydrophobic sensitizer pyropheohorbide-\(a\) was chosen in order to avoid the re-adsorption to the hydrophilic porous Vycor glass surface after its photorelease. Electron rich Z-alkene 8 was used as a precursor in the synthesis of photocleavable alkene 10, and 8 was synthesized from meso-7. Meso-7 was synthesized by the two step procedure obtained from literature and is described in scheme 1.16 Electron rich alkene 8 was reacted with butyl lithium (nBuLi) and dimethyl formamide (DMF) to form bis-aldehyde 9. It was reduced by sodium borohydride (NaBH₄) to get spacer alkene 10. No decomposition of spacer alkene was found when its stability was studied within pH 2.0-8.0.
Spacer alkene was linked by ester bond with pheophorbide to get pheophorbide monoester 11. Pheophorbide monoester was reacted with iodopropyl trimethoxy silane to get sensitizer silane compound 12. Porous Vycor glass tip (which has free Si-OH groups) was added in situ and refluxed in toluene for 24 h to get sensitizer coated on the porous Vycor glass. Coated porous Vycor glass was washed with various solvents, such as tetrahydrofuran (THF), dichloromethane (DCM), acetone, dimethylsulfoxide (DMSO), ethanol and then placed for soxhlet extraction using methanol in order to remove adsorbed dye from the PVG surface. No sensitizer leaching from the coated PVG was observed at low and high pH in aqueous solution and no leaching was observed in dark when kept in an organic solvent.

We have chosen porous Vycor glass (PVG) as a silica solid support. It has free silanol groups which can be functionalized very easily. It has 40 Å pore size; therefore, oxygen can be diffused through the pores. Significant oxygen diffusion is important for us as sensitizer bound on the surface is cleaved by singlet oxygen, which is generated in the presence of light, oxygen and sensitizer. Moreover, PVG is a hard solid material and holes can be drilled in it without any unwanted damage of measurable size to fit the fiber into it. Significant light from the fiber can also be transmitted through the PVG surface. Therefore, singlet oxygen could be generated at the sensitizer site and diffused through the solution for photocleavage to take place. Photocleavage study was carried out in different solvent. Determination of loading and photocleavage study are discussed in next section.
Fig 1. Concept of singlet oxygen fiber-optic: (a) Photocleavable sensitizer 1, sensitizer attached to the PVG cap coupled to the fiber carrying O$_2$ and light. (b) Dioxetane 2 formed by [2+2] cycloaddition reaction with singlet oxygen. (c) Cleavage of dioxetane to get photocleaved compound 3. (d) Hydrolysis products.
Scheme 1. Synthesis of Sensitizer Functionalized Cap 1

Reagents and conditions: (i) BrCH₂CH₂Br, NaOH, 100°C, 6 h; (ii) NBS, benzoyl peroxide, CCl₄, 80°C, 6 h (meso-7 was carried on to step iii); (iii) NaI, acetone, 25°C, 2 h; (iv) n-BuLi, DMF, -78°C, 3.5 h; (v) NaBH₄, CH₃OH, 25°C, 14 h; (vi) pyropheophorbide-a, EDC, DMAP, CH₂Cl₂, 25°C, 24 h; (vii) (CH₃O)₃SiCH₂CH₂I, NaI, THF, under N₂, 70°C, 24 h; (viii) porous Vycor glass (pre-heated at 500°C), toluene, reflux at 110°C, 24 h.

2.2.2 Loading of the Sensitizer. Loading amount of the sensitizer onto the porous Vycor glass (PVG) after covalent attachment was determined based on the spectroscopic method and hydrofluoric acid (HF) stripping method. In spectroscopic method absorbance of the dye in solution before (initial value) and after (final value) its covalent attachment to the PVG was determined. Amount of dye attached to the porous Vycor glass is equal to a corresponding
concentration of reduced absorbance after its attachment, which was determined from the calibration plot. However, spectroscopic method was abandoned because suspension of the dye after attachment was found. This gave erroneous absorbance and thereby gave erroneous loading amount.

HF stripping method for the determination of loading amount is a more straightforward and more accurate method. We stripped off ~ 100% dye from the PVG into the aqueous HF solution and then extracted in CHCl₃ to measure its absorbance. Calibration plot of known concentrations of 11 with respect to its absorbance in CHCl₃ was made. We hypothesized that dye coming off into the CHCl₃ has almost identical solubility with 11. Therefore, it was possible to know the concentration of the dye in CHCl₃, which was identical with the amount loaded on the surface.

Although the determination of loading amount onto the silica surface turned out to be easy, tunable loading to the glass for the controlled release of dye 3 was difficult to achieve. Control on the release of dye in the media was important as we have planned of applying this device for PDT in the future where the exact amount of dye released gives maximum therapeutic effect. Silane 12 quantities of 0.06-1.1 μmol per gram of PVG were loaded resulting in sensitizer sites separated by 8.9-38.4 nm. The loading of 0.3 μmol (0.33%) sensitizer onto the fiber caps resulted in the maximal photocleavage of 3 in toluene-d₈. Higher or lower sensitizer loading reduced the photocleavage efficiency and was attributed to less availability of sensitizer and self-quenching, respectively.

2.2.3 Photocleavage Study of Sensitizer in Toluene, D₂O and Petrolatum. After loading sensitizer onto the PVG, we checked photocleavage of dye in various organic solvents (toluene and n-octanol) and deuterated water. Initially toluene-d₈ was used to study the
photocleavage of the dye from the porous Vycor glass as the lifetime of the singlet oxygen in deuterated solvent is higher than its analogous protonated solvent. Therefore, higher amount of photocleavage was expected to be obtained. Table 1 summarizes the results of photocleavage study in different media.

**Figure 2.** Time course of photorelease of 3 into toluene-$d_8$ solution arising from photooxidative cleavage and departure from the fiber-optic device tip. The absorption spectra show the fourth Q-band of 3 and were normalized at 640 nm: (a) orange 0.0 h, (b) turquoise 0.5 h, (c) blue 1.0 h, (d) green 1.5 h, (e) red 2.0 h, and (f) black 4.0 h.


Figure 2 shows the amount of sensitizer 3, photocleaved into toluene-$d_8$ solution with time. There was an increase of absorbance with time, which gave a maximum of 7300 nM of 3. Significant quantities of sensitizer 3 remained adsorbed on the probe tip even though the alkene bond bridging the sensitizer and glass was broken. The sensitizer photorelease in D$_2$O has not been observed, as indicated by the fact that 3 were not detected in D$_2$O solution (entry 8, Table 1). A mechanism has been proposed on how dioxetane cleaves after its formation is facilitated by the neighboring silanol group present in PVG surface. Adsorbed dye 3, remaining on the surface
was taken off by the soxhlet extraction with MeOH. We observed significant photorelease of dye 3 in petrolatum (soft paraffin, mixture of hydrocarbons) at 65°C. We assumed petrolatum is very similar to the lipophilic biological media. After 30 min the sensitizer diffused away from the probe tip for 1.03 mm, when the spot geometry was considered approximately circular. After 4 h, quantitative release of the sensitizer was observed. However, no adsorption of dye to the probe-tip was observed after 4 h. Therefore, it is quite evident that surrounding media influences the photorelease.

**Table 1. Photorelease of sensitizer 3 in different media.**

<table>
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<tr>
<th>Entry</th>
<th>Medium</th>
<th>Irradiation Time (h)</th>
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<th>Free 3 (nmol)</th>
<th>Free 3 (nM)</th>
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<td>1</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>in dark</td>
</tr>
<tr>
<td>2</td>
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<td>0.5</td>
<td>960</td>
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<tr>
<td>3</td>
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<td>1.6</td>
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<td>75,000</td>
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2.2.4 Photocleavage Study in \textit{n}-octanol. So far we have seen that efficiency of photocleavage from the fiber-optic probe tip depends upon the lipophilicity of the surrounding media. We observed that fiber-optic probe tip that sparged O\textsubscript{2} gas can photo-detach pheophorbide molecules for the pigmentation of petrolatum (a semisolid hydrocarbon used as a model of lipophilic biological media).\textsuperscript{17} In this study, the fiber-optic implement was inserted into various media so their effects on sensitizer photorelease could be determined. A qualitative understanding was desired on how the sensitizer photorelease depends on the surrounding environment. Thus, we used octanol, a lipophilic solvent, and water for this study. As in many biological studies, octanol-water partition coefficient was measured to determine lipophilicity of the compound. Measuring partition coefficient is one of the pre-requisites for any kind of cellular studies. Once medium effects are known, steps could be undertaken for the development of a medical device for precise, site-specific delivery of photosensitizer and \textsuperscript{1}O\textsubscript{2}, as an alternative technique of PDT.

Photocleavage study has been conducted with the fiber-optic device delivering excitation light and oxygen gas to the device tip in octanol or H\textsubscript{2}O solutions. The samples were irradiated at 669 nm (irradiance of 4.8 mW cm\textsuperscript{-2}) for 4 h. During the photo-irradiation, the green color of the tip slowly became transparent and the surrounding octanol solution turned green.
Pyropheophorbide-\(a\) and its derivatives have been shown to produce singlet oxygen in high yield,\(^{18,19}\) Here sensitizer 3 had a similar absorption spectrum with a strong Soret band at 410 nm and weaker Q-bands, such as the red absorbing 4\(^{th}\) Q-band at \(\lambda_{\text{ex}} = 673\) nm. For irradiance of up to 2 h and a fluence of 34 J cm\(^{-2}\) light intensity, the plot of absorption of the 4\(^{th}\) Q-band vs time was linear, indicating the release of 3 into octanol (inset, Fig. 3). With 155 nmol of 12 loaded onto the fiber tip 1, the photorelease of 3 into octanol reached 154 nmol (344 \(\mu\)M, 99%), which is an approximate value because porphyrins can aggregate in organic solvents in the hundreds of \(\mu\)M range. For example, in DMF pyropheophorbide-\(a\) methyl ester (PPME) was shown to follow the Beer-Lambert Law up to 46 \(\mu\)M suggesting it was monomeric up to that point.\(^{20}\) Unlike octanol solution, the probe tip did not photorelease 3 into H\(_2\)O. Although the lifetime of \(^{1}\)O\(_2\) in neat H\(_2\)O (3.5 \(\mu\)s)\(^{21}\) is 5 times shorter than in octanol (19 \(\mu\)s),\(^{22}\)

**Figure 3.** Time-course of photorelease of 3 into 1-octanol arising from photo-oxidative cleavage and departure from the fiber tip. The absorption spectra show the fourth Q-band of 3 and were normalized at 770 nm. The inset is a plot of the concentration of 3 photocleaved away from the fiber tip into octanol (circles) and H\(_2\)O (diamonds) at room temperature.

3 was not detected by UV-Vis in H\(_2\)O after an 8 h irradiation period. The reason that photoreleased compound 3 in H\(_2\)O was not detected is that it remained adsorbed on the probe tip,
even after $^1$O$_2$ cleaved the covalent alkene spacer bonds as evidenced from subsequent polar solvent washings and the resulting desorption and detection of 3. Figure 4 shows a plot of $\ln \left( \frac{\text{Sens}_t}{\text{Sens}_0} \right)$ vs time that gave a linear correlation up to 2 h of irradiance in octanol. After 2 h, a saturation line appeared, signifying that the probe tip was $\sim 100\%$ depleted of sensitizer.

Because the readsorption of 3 back onto the probe tip did not take place within the irradiation time course (4 h), a first order photocleavage rate constant of 1.13 h$^{-1}$ was measured. The alkene bridge photo-oxidation and dioxetane cleavage steps were fast in octanol, allowing the first order rate constant for sensitizer departure to be measured accurately.

**Fig 4.** Time profile for the photocleavage of 154 nmol sensitizer 3 from the probe tip into 1.0 mL octanol at 25°C. The data were recorded by absorption spectroscopy and the fourth Q-band of 3 at 673 nm was monitored. The plateau region represents quantitative sensitizer cleavage and no detectable readsorption.

The water solubility of 3 was low enough that we could not determine its water-octanol partition coefficient (P) experimentally. Computed log P values of 6.7 ± 1.5 for PPa and 8.0 ± 1.5 for 3 with Advanced Chemistry Development program (ACD, version 12.01)$^{23}$ predicted low solubilities in water. Log P values greater than ca 4 indicate high hydrophobicity of the
compound and therefore partitioning into octanol is favored, which is consistent with PPa compounds known to aggregate in buffer solution and localize in lipophilic media \(^{20, 24–28}\). Hydrophobicity of the dye and preferential partitioning of 3 in octanol suggest that dye 3 might incorporate into biological membranes, which led us to study the photocleavage in liposomes and binding of photocleaved dye 3 with the liposome. Photocleavage in liposome was carried out by Dr. Yasemin Kopkalli and Dr. Adaikapillai Mahendran in collaboration with Prof. Lesley Davenport. We found that photocleaved dye 3 binds with DPPC and EL liposome with binding constants of 66 and 59 (mg mL\(^{-1}\))\(^{-1}\) respectively.

2.3 Sensitizer Conjugated Fluoroalkane Modified Porous Vycor Glass Cap in the Study of Photorelease and Singlet Oxygen Production.

Earlier in this chapter we discussed about photocleavage of the sensitizer from sensitizer conjugated spacer alkene covalently attached to the porous Vycor glass probe tip using fiber-optic. We found that adsorption of dye to the silica surface took place after scission of the photolabile (Z)-1,2-dioxyethylene spacer. This adsorption inhibited the release of the sensitizer to the surrounding media (like toluene, D\(_2\)O). Therefore, we observed about 6% photorelease in toluene and almost no photorelease in D\(_2\)O. It was important to develop a system that would help us in discharging the sensitizer from the probe tip. Fluoroalkylsilane (3,3,4,4,5,5,6,6,6-nonafluorohexyltrimethoxysilane) coating on porous silica have repellant and self-cleaning properties, \(^{29,30}\) and thus, a modification could be introduced into the fiber-optic probe tip device in order to enhance the sensitizer photorelease. Scheme 2 below describes the design of fluoroalkyl silane coated silica conjugated to the pheophorbide-\(a\) sensitizer.
We explored a fluorinated fiber-optic tip bound to a photolabile ethene-sensitizer in an attempt to minimize adsorption of the sensitizer once the ethene bond was cleaved. Specifically, we (1) quantified sensitizer repulsion at the fluorinated probe surface, (2) synthesized hybrid tips by covalent attachment of the pyropheophorbide-α monoester photosensitizer via a stable linkage to the fluorinated glass bound to the end of a hollow fiber, (3) determined the efficiency with which the sensitizer molecules bound to the fluorinated tips cleaved free in toluene solution and
bovine tissue as an in vivo model, (4) quantitated the quenching of the silica surface and ethene linkage by $^{1}$O$_{2}$ by measuring the total bimolecular quenching rate constant kT, and (5) utilized $^{1}$O$_{2}$ quenching and lifetime ($\tau$) data for bond types in silica containing C-H and C-F bonds, particularly that replaced the Si-OH bonds to identify loading and quenching parameters that influence photosensitizer turnout yield.

In order to perform covalent attachment, I performed synthesis of compound 11 many times, which is depicted in Scheme 1. Our postdoctoral fellow Dr. Dorota Bartusik and Dr. David Aebisher 1) characterized the fluoroalkane attached sensitizer modified heterogeneous silica surface (shown in Scheme 2), 2) performed kinetic studies related to singlet oxygen quenching, and 3) established the efficiency of photorelease in toluene and bovine tissue.

2.4 Autocatalytic Sensitizer Drug Photorelease Bound to Silica Support. With the sensitizer conjugated fluoroalkyl silane modified silica, we explored autocatalytic release of the photosensitizer. Photorelease of the sensitizer from the fluoroalkyl modified silica surface was initiated by the reaction with singlet oxygen. Irradiation of the released sensitizer also generated singlet oxygen, which accelerated the release of the sensitizer in an autocatalytic manner. Proposed autocatalytic mechanism is described in the Fig 5 below.
Scheme 2 was repeated to attach spacer alkene-pheophorbide conjugate to the fluorinated silica probe-tip. Photorelease of sensitizers was carried out at high temperatures (50°C, and 100°C), room temperature and low temperatures (0°C, -25°C, -50°C). At room temperature and higher temperatures (20-100°C), when photorelease was plotted against time, a sigmoidal curve was obtained (shown in Fig 6). At these temperatures, the surface-bound dioxetane was short-lived. However, sigmoidal behavior was not retained at lower temperatures, which we attribute to increases in the stability of the surface-bound dioxetane, and thus the retention of the sensitizer.
drug on the fluorinated silica surface. The ethene was first converted to the dioxetane and then to the final carbonyl products after scission of the dioxetane.

![Graph](image)

**Fig 6.** The concentration of Sens U photoreleased free from the fluorinated silica sensitizer as a function of time in n-butanol at 20°C. The fluorinated silica was removed, and the concentration of Sens U by UV-Vis was measured at the indicated time. “Reprinted with permission from (Bartusik, D.; Minnis, M.; Ghosh, G.; Greer, A. *J. Org. Chem.* 2013, 78, 8537-8544). Copyright (2013) American Chemical Society.”

### 2.4.1 Photorelease Study at Low Temperature and Detection of Dioxetane by Trapping Agent.

At 10, 0, -25 and -50°C, the percent of the sensitizer U photoreleased was progressively diminished in n-butanol. Indirect evidence for the surface-bound dioxetane was established by GC/MS for O=PPh₃ after a reaction with PPh₃ in the dark (Scheme 3). (Peroxides are known to be readily trapped by phosphines, often through phosphorane intermediates.⁴¹,⁴²). Trapping with PPh₃ accounted for the surface-bound dioxetane in the amount of ~70% at -35°C and ~20% at 10°C. The lower percent at 10°C reflected a lower quantity of the dioxetane on the silica surface.
The above result was anticipated since dioxetane stability was expected to increase at lower temperatures. Small molecules such as di and tetra-O-vinyl ethers are known to react with $^{1}\text{O}_2$ to form dioxetanes, which can be detected at very low temperature (-78°C). In our case, the dioxetane is residing on a solid surface and the release of Sens U into solution is a phase transition.

**Scheme 3**

**Scheme 4**

Since dioxetanes are high energy intermediates, ethene cleavage of the fluorinated silica sensitizer may be caused in part by the decomposition of dioxetane molecule in the dark. Therefore, we conducted control reactions as represented schematically in Scheme 4, which
showed that dioxetane diol 13 (9.2 mM), prepared by Rose Bengal photosensitization at -35°C in
n-butanol, when added to 0.2g of fluorinated silica sensitizer in the dark and warmed to room
temperature, did not release sensitizer U after 24 h. Similarly, surface-bound dioxetane generated
at -35°C was incapable of cleaving ethene 10 in the dark. As might have been anticipated, the
amount of light emitted by surface bound dioxetane was too small to have any significant effect.
As reported in the literature, the thermal decomposition of the adamantylideneadamantane 1,2-
dioxetanes only yields ~6×10¹⁹ photons/mole which is insufficient as a light source in our case.
Tens or hundreds million times more photons were produced by the diode laser per mole of
reagent in our reaction. Furthermore, the heat produced by decomposition of those dioxetane was
too small to account for any significant release of the sensitizer.

2.4.2 Photocleavage Study at Room Temperature or Higher to Explore Autocatalytic
Sensitizer Photorelease (This Study was Carried Out by Dr. Dorota Bartusik). At room
temperature or higher (20-100°C) sigmoidal behavior was retained. Autocatalytic mechanism of
the released sensitizer to help release more surface bound sensitizer was also proved by
externally adding sensitizer to the solution prior to photocleavage. When photocleavage was
conducted in that solution, no slow photorelease period was observed. Therefore, the sensitizer
which was photocleaved into the solution catalyzed the release of the surface bound sensitizer.

2.5 Conclusion. A porous fiber-optic cap modified by the photo-detachable sensitizer has been
synthesized. Photorelease study in toluene-d₈ has been carried out and it was found that 60 nmol
sensitizer (0.33% surface coverage) loaded caps gave a maximum photocleavage (photocleavage
efficiency of 6%). However, quantitative photocleavage (photocleavage efficiency ~ 100%) was
observed in petrolatum. Poor photocleavage efficiency in D₂O was observed because the
sensitizer 3 remained adsorbed onto the surface after photorelease. Photocleavage efficiency in
petrolatum indicated that lipophilicity of the surrounding medium is important. Therefore, we carried out photocleavage study in hydrophobic solvent, octanol and found that photocleavage efficiency was ~ 100%. This result encouraged us to do photocleavage study with our fiber-optic probe tip in liposome. We found that significant amount of photocleaved dye transferred into the liposome. Mass transform (photorelease) in DPPC liposome was higher compared to the egg lecithin liposome. These results showed us the way to employ our device for phototherapy in cell. In vitro ovarian cancer cellular studies with sensitizers have been reported in chapter 3.

We explored photocleavage of the sensitizer from fluoroalkyl conjugated silica surface. Attachment of fluoroalkylsilane improved self-repelling property of the surface. Therefore, sensitizer photocleavage increased in an organic solvent (the results are not discussed here). Fluorine also reduced quenching of singlet oxygen and enhanced oxygen solubility. Therefore, with the sensitizer conjugated fluorinated PVG, we observed autocatalytic release of the sensitizer at room temperature or higher. But, no autocatalytic dye photorelease was observed at low temperatures as dioxetane is stable at low temperature on the surface.

Significant amount of sensitizer photorelease in aqueous media is important to integrate our device for cellular studies. Therefore, with the advancement made by modifying the silica surface using fluoroalkyl silane, designing of hydrophilic sensitizer is also required. Therefore, next we designed short chain PEG conjugated sensitizers to dampen its hydrophobicity. We evaluated their photochemistry and photocytotoxicity in the next chapter in order to employ them in fiber-optic guided drug delivery application.
2.6 References


3.1 Introduction

The following study was done to develop silica probe tips for fiber-optic based photosensitizer and singlet oxygen delivery. The question considered was: What sensitizer would work well for the photodynamic killing of human ovarian cancer cells? Chlorin derivatives are well known as a promising class of sensitizers for photodynamic therapy and ovarian cancer cell killing. However, substitution to the chlorin core is often required to overcome problems with aggregate formation and poor aqueous solubility. A pegylated polymer (PEG 8000 molecular weight) conjugated to chlorin e_6 was studied, where the attachment of large PEG led to inverting philicity from hydrophobic to hydrophilic. Subcellular localization has been observed in N-(2-hydroxypropyl) methacrylamide copolymer chlorin e_6 monoethylenediamine conjugates in a human ovarian carcinoma. Chlorin e_6 and its derivative have been extensively studied for PDT and thus shown to have low dark toxicities while able to effectively produce singlet oxygen upon light irradiation.

Our hypothesis was that PEG substituent numbers are adjustable for the photo-killing activity of human ovarian cancer cell in relation to each other. Therefore, we have synthesized PEG conjugated chlorin e_6 photosensitizers, where one, two, and three of the carboxylic acid groups were modified by a short 160 amu triethylene glycol chain \([\text{CH}_3(\text{OCH}_2\text{CH}_2)_3\text{OH}]\) to fine tune solubility and aggregation (Figure 1). Our hypothesis behind the PEGylation of chlorin e_6 is that it reduces hydrophobicity and that there is an ovarian cancer photo-killing dependence on the number of PEGs in chlorin e_6 applicable for controlled-release of a pegylated sensitizer from a \(^1\text{O}_2\) fiber-optic implement. With the idea of eventual integration in optical fiber-based
sensitizer delivery systems in mind, the aims of the present work were to determine the chemistry including: (1) whether chlorins could be functionalized with increasing numbers of attached PEG groups from 0 to 3 conjugated to the three different carboxylic acid sites, (2) stability of the PEG-chlorin ester groups towards acid base hydrolysis, (3) the extent to which PEG groups could enhance solubility, (4) the computed conformation of PEG groups using density functional theory and molecular mechanics calculations; and the photobiology including: (5) cellular uptake of PEGylated chlorins and their subcellular localization, (6) whether photocytotoxicity is influenced by PEG attachment, and (7) mechanistic consideration of photocytotoxicity based on H₂O and D₂O solvent effects on the singlet oxygen lifetime. The results obtained here showed that triPEG conjugated chlorin is a potent ovarian cancer phototherapeutic agent.

Figure 1. Parent chlorin e₆ and PEG conjugated chlorin e₆ 1, 2 and 3.
3.2 Results and Discussion

3.2.1 Synthesis and Characterization. The addition of the [CH₃(OCH₂CH₂)₃OH] PEG to chlorin e₆ relied on an EDC condensation reaction in the formation of ester groups. Chlorin e₆ reacted with EDC, DMAP, and CH₃(OCH₂CH₂)₃OH to produce 1 in 50% and 2 in 48% overall yields in 24 h, while 48 h periods produced 3 in 33% overall yield. According to HPLC, the purity of 1 was 94.7%, of 2 was 99.2%, and of 3 was 99.9%. Higher soluble chlorins were easier to purify, as has been reported by others as well. LCMS data indicated that 1 contained one PEG group (MS calcd for C₄₁H₅₁N₄O₉ [M+H]+ = 743.3651, found 743.3673), 2 contained two PEG groups (MS calcd for C₄₈H₆₅N₄O₁₂ [M+H]+ = 889.4593, found 889.4586), and 3 contained three PEG groups (MS calcd for C₅₅H₇₉N₄O₁₅ [M+H]+ = 1035.5536, found 1035.5538). Although LCMS provided mass identification for 1-3, it gave no information about structure.

1D ¹³C and ¹H along with 2D NMR experiments enabled the regiochemical assignments of the PEGs to the chlorin carboxy sites. For 1 and 2, the ¹³C NMR spectra indicated 20 sp² chlorin core carbons and 3 carbonyl carbons for the total of 23 signals within 93.5-174.7 ppm. The 23 carbon signals for each indicate that 1 and 2 formed as single isomers and not as mixture of isomers. Mono, di, and tri-PEG attachments to 1, 2, and 3, respectively, were also evident due to the observation of 7, 14, and 21 ¹³C NMR signals, respectively, coming in the region of 58-73 ppm. Figure 2 is the expanded portion of HMBC spectra for 1 and 2. For 1, a portion of the HMBC spectrum is shown in Figure 2(a) where three sets of signals detected for the 1⁷³ (172.7 ppm) carbonyl carbon coupled to protons attached to the 1⁷¹ (1.51 ppm), 1⁷² (2.18 ppm), and 1⁷⁴ (3.82 ppm) carbons suggesting a linkage between the 1⁷³ carbonyl carbon and the PEG. In accordance with the previous study, ¹H NMR assigned protons of chlorin e₆ trimethyl ester
attached to $17^1$ and $17^2$ carbons at 1.75 and 2.19 ppm, respectively as a multiplet, assisted us in assigning the $17^1$ and $17^2$ carbons of 1. Earlier work with a mono-amide chlorin e₆ conjugate had shown the $\delta$ values for 1.7 and 2.4 ppm for the protons connected to $17^1$ and $17^2$ carbons, respectively. Thus, we assigned the PEG to be attached at the $17^3$ site in 1. In 1, other regioisomers were ruled out by analyzing the coupling between $17^3$ carbonyl carbon and protons attached to the $17^1$, $17^2$ and $17^4$ carbons. Correlations between $15^2$ carbonyl carbon and protons attached to $15^1$ chlorin e₆ core carbon and $15^3$ PEG carbons were not found, and a correlation between PEG hydrogens and $13^1$ carbonyl carbon was also not found. For 2, the HMBC spectrum in Figure 2(b) showed two sets of signals for the $15^2$ (173.1 ppm) carbonyl carbon coupled to the protons attached to the $15^1$ (5.61-5.37 ppm) and $15^3$ (4.16 ppm) PEG carbons. Another two sets of signals for the $17^3$ (173.3 ppm) carbonyl carbon to the protons attached to $17^2$ (2.63, 2.29 ppm) carbon and $17^4$ (4.11 ppm) PEG carbon suggesting a linkage between the $15^2$ and $17^3$ carbonyl carbons and two PEGs in 2. In 2, other regioisomers were ruled out by analyzing the coupling between $15^2$ carbonyl carbon and protons attached to the $15^1$ and $15^3$ carbons. A correlation between PEG hydrogens and the $13^1$ carbonyl carbon was not observed. For 3, evidence for the attachment of three PEGs to all vacant chlorin e₆ carboxylic acid sites was given by HSQC experiments. Twenty-one PEG carbon signals were observed (7 from each PEG) bearing protons that appeared as multiplets ranging from 3.29-4.08 ppm.
3.2.2 Hydrolytic Stability. Table 1 shows the results of solvolysis studies of the pegylated chlorins 1-3. The solvent conditions were CH3OH:H2O (9:1), where the pH was adjusted to 2.0 or 8.0 by formic acid or ammonium hydroxide. The results suggest that the triethylene glycol
chains covalently attached to chlorin e6 do not spontaneously hydrolyze. Although, the solvolysis rates were increased as the number of PEG groups increased. After 4h at pH 2.0, the solvolysis of 1 was 28%, of 2 was 57%, and of 3 was 100%. After 4h at pH 8.0, the solvolysis of 1 was 21%, of 2 was 29%, and of 3 was 100%. In 3, solvolysis was increased compared to 2 and 1. In acid or alkaline methanol/water of chlorin 3 led to a mixture of products, where there was the formation of 1, 2, and native chlorin e6.

Table 1. Stability of Pegylated Chlorins 1-3.

<table>
<thead>
<tr>
<th>pH</th>
<th>Time</th>
<th>% Disappearance of compounds †</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2.0</td>
<td>5 min</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>4 h</td>
<td>28</td>
</tr>
<tr>
<td>8.0</td>
<td>5 min</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>4 h</td>
<td>21</td>
</tr>
</tbody>
</table>

† LCMS was used to follow the reaction (retention time \( t_R \) for 1, 2, and 3 was 7.10, 8.09, and 12.20 min, respectively). Values are an average of 3-4 measurements.

3.2.3. Intrinsic Solubilities. The intrinsic solubilities of chlorin e6 and 1-3 were determined in 1% DMSO water (Table 2). Aliquots of 1% DMSO water were added to 50 μg quantities of chlorin e6 or 1-3, and the solutions were stirred for 1 h at room temperature and then allowed to stand for 5 h. Solution was filtered to separate insoluble compounds and the amount of compound in the filtrate was determined by monitoring the soret bands of chlorin e6 and 1-3. The 1, 2, and 3 conjugates were increasingly soluble as the number of PEG conjugation was increased. By comparison to the nonderivatized chlorin e6, chlorins 1-3 had an enhanced
solubility of 1.6-3.6-fold. Similar factors that make the pegylated chlorins more soluble in 1% DMSO water affected their octanol/water partition coefficients. Computed log $P$ values were obtained with the ACD algorithm, which performed reasonably well in predicting log $P$ values of drugs.\textsuperscript{9} We obtained Clog$P$ values to decrease by about 2 orders of magnitude as the number of conjugated PEG groups increased from 0 to 3.

**Table 2. Effect of Increasing the Number of PEG Groups on Chlorin e$_6$ on the Solubility and Computed Octanol-Water Partition Coefficients**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of PEG groups\textsuperscript{a}</th>
<th>Solubility in 1% DMSO-H$_2$O (\textgreek{ug/mL})\textsuperscript{a}</th>
<th>CLog $P$\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorin e$_6$</td>
<td>0</td>
<td>11±0.8</td>
<td>6.59±1.74</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>18±0.8</td>
<td>5.61±1.65</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>29±0.8</td>
<td>5.56±1.67</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>40±0.8</td>
<td>4.7±1.68</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Measurements were conducted three times, and the solubility value was averaged.  
\textsuperscript{b} The Clog $P$ values were calculated with the ACD program (Advanced Chemistry Development, Inc., Toronto, ON, Canada).

### 3.3 Experimental Section

#### 3.3.1 General Information. Methanol, dichloromethane, 1-octanol, chloroform-$d_1$, deuterium oxide-$d_2$, chlorin e$_6$, $N$-(3-dimethylaminopropyl)-$N'-$ethylcarbodiimide hydrochloride (EDC), $N,N$-dimethyl-4-aminopyridine (DMAP), triethylene glycol monomethyl ether (TGEE, MW=164.20) were used as received from commercial suppliers. Deionized water was purified using a deionization system. An HPLC chromatogram suggested the commercial chlorin e$_6$ to be of 99.9% purity. Purification of the sensitizing mixtures was conducted by column chromatography using 200–400 mesh silica gel. TLC was carried out using silica gel 60F254 TLC plates. Proton NMR data were acquired at 400 MHz, and $^{13}$C NMR data were acquired at
100.6 MHz on a Bruker DPX400 MHz instrument. HRMS, LCMS, GC/MS, HPLC, and melting point data were collected. UV-VIS spectra were collected on an Agilent 8453 Spectrophotometer. Steady-state fluorescence measurements were performed using Photon Technology International (PTI) spectrofluorimeter (Birmingham, NJ). HPLC and LCMS instruments were used as has been described in our previous work. Light was delivered from either a 669 nm or a 670 nm CW diode laser (Intense, North Brunswick, NJ), or a Minilase 10-Hz Nd:YAG Q-switched laser (New Wave Research, Fremont, CA).

3.3.2 Synthesis of \( \text{17}^3 \)-Chlorin e\(_6\) Methoxy Tri(ethylene glycol) Ester (1). Yield 0.056 g (50%); monomeric purity: 94.7%. Chlorin e\(_6\) (90.0 mg, 0.15 mmol) was reacted with TGME (178.20 mg, 1.10 mmol), EDC (28.0 mg, 0.15 mmol), and DMAP (18.3 mg, 0.15 mmol) in CH\(_2\)Cl\(_2\) (10.0 mL), which was stirred for 24 h under N\(_2\) at room temperature. Purification of the residue was done by silica gel column eluting with 10% CH\(_3\)OH in CHCl\(_3\) yielding 1 as a blue solid. \( R_f = 0.10 \). \(^1\)H NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 9.79 (s, 1H), 9.67 (s, 1H), 9.11 (s, 1H), 8.36 (dd, \( J = 17.6 \) Hz, 11.6 Hz, 1H), 6.45 (d, \( J = 18 \) Hz, 1H), 6.16 (d, \( J = 11.6 \) Hz, 1H), 6.00 (d, \( J = 19.6 \) Hz, 1H), 5.81 (d, \( J = 5.6 \) Hz, 2H), 5.45 (d, \( J = 11.6 \) Hz, 1H), 4.57 (d, \( J = 7.6 \) Hz, 2H), 4.35 (q, \( J = 8.8 \) Hz, 2H), 4.14 (m, 4H), 3.82 (m, 4H), 3.25 (m, 4H), 3.09 (s, 3H), 2.98 (m, 4H), 2.18 (m, 3H), 1.69 (m, 3H), 1.63 (d, \( J = 6.8 \) Hz, 2H), 1.51 (m, 3H), 1.48 (m, 3H), -1.97 (s, 1H), -2.5 (s, 1H). \(^{13}\)C NMR (100.6 MHz, DMSO-\( d_6 \)) \( \delta \) 174.7, 172.7, 170.7, 170.2, 168.4, 157.1, 153.7, 148.8, 144.9, 140.1, 138.6, 136.5, 135.3, 134.4, 134.3, 130.7, 129.7, 122.4, 107.3, 103.1, 101.6, 98.8, 94.6, 72.8, 72.7, 71.4, 70.2, 70.0, 69.8, 58.3, 53.1, 49.0, 38.1, 31.2, 30.0, 23.4, 19.3, 18.2, 12.5, 12.4, 11.4. HRMS (ESI) m/z calcd. for [C\(_{41}\)H\(_{50}\)N\(_4\)O\(_9\)+Na\(^+\)] 765.3470, found 765.3500. (ESI) m/z calcd. for C\(_{41}\)H\(_{51}\)N\(_4\)O\(_9\) [M+H\(^+\)] 743.3651, found 743.3673. UV-Vis (CHCl\(_3\)): \( \lambda_{\text{max}} (\varepsilon / \text{M}^{-1}\text{cm}^{-1}) \): 665 nm (87868), 404 nm (272083).
3.3.3 Synthesis of 17\textsuperscript{3,15}\textsuperscript{2}-Chlorin e\textsubscript{6} Methoxy Tri(ethylene glycol) Diester (2). Yield 0.035 g (48%); monomeric purity: 99.2%. Chlorin e\textsubscript{6} (50.0 mg, 0.08 mmol) was reacted with TGME (32.0 mg, 0.4 mmol), EDC (30.0 mg, 0.16 mmol), and DMAP (19.5 mg, 0.16 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (10.0 mL), which was stirred for 24 h under N\textsubscript{2} at room temperature. Purification of the residue was done by silica gel column eluting with 5% CH\textsubscript{3}OH in CH\textsubscript{2}Cl\textsubscript{2} yielding 2 as a blue solid. R\textsubscript{f} = 0.21. \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}\textsubscript{6}) \(\delta\) 9.74 (s, 1H), 9.73 (s, 1H), 9.09 (s, 1H), 8.31 (dd, \(J = 17.6\) Hz, 11.6 Hz, 1H), 6.65 (d, \(J = 6.8\) Hz, 1H), 6.45 (d, \(J = 18\) Hz, 1H), 5.61 (d, \(J = 19.0\) Hz, 1H), 5.37 (d, \(J = 19.0\) Hz, 1H), 4.61 (q, \(J = 7.21\) Hz, 2H), 4.40 (m, 2H), 4.16 (m, 4H), 4.11 (m, 4H), 3.81 (m, 4H), 3.57 (m, 4H), 3.23 (s, 4H), 3.22 (s, 4H), 3.10 (s, 3H), 3.07 (s, 3H), 2.98 (s, 3H), 2.63 (m, 1H), 2.29 (m, 3H), 2.13 (m, 3H), 1.66 (t, \(J = 8.0\) Hz, 3H), 1.64 (d, \(J = 8.0\) Hz, 3H), -1.72 (s, 1H), -2.10 (s, 1H). \textsuperscript{13}C NMR (100.6 MHz, DMSO-\textit{d}\textsubscript{6}) \(\delta\) 173.3, 173.1, 172.7, 168.3, 168.1, 152.2, 149.1, 144.0, 137.1, 136.5, 136.5, 133.4, 133.3, 133.0, 131.5, 130.0, 129.2, 121.7, 103.7, 99.8, 99.0, 94.3, 72.7, 71.5, 71.3, 70.0, 70.0, 69.9, 69.8, 69.8, 69.6, 68.7, 68.5, 64.2, 63.6, 60.7, 58.3, 53.3, 48.2, 37.9, 31.1, 29.9, 23.6, 19.4, 18.2, 12.5, 11.4. HRMS (ESI) m/z calcd. for [C\textsubscript{48}H\textsubscript{64}N\textsubscript{4}O\textsubscript{12}\textsubscript{Na}\textsuperscript{+}]\textsuperscript{+} 911.4411, found 911.432. (ESI) m/z calcd. for C\textsubscript{48}H\textsubscript{65}N\textsubscript{4}O\textsubscript{12} [M+H]\textsuperscript{+} 889.4593, found 889.4586. UV-Vis (CHCl\textsubscript{3}): \(\lambda_{\text{max}}\) (\(\varepsilon / M^{-1}cm^{-1}\)) 664 nm (94342), 404 nm (285333).

3.3.4 Synthesis of 17\textsuperscript{3,15,13}\textsuperscript{1}-Chlorin e\textsubscript{6} Methoxy Tri(ethylene Glycol) Triester (3). Yield 0.014 g (33.0%); monomeric purity: 99.9%. Chlorin 2 (36.0 mg, 0.04 mmol) was reacted with TGME (0.50g, 3.04 mmol), EDC (23.04 mg, 0.12 mmol), and DMAP (14.64 mg, 0.12 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (10.0 mL), which was stirred for 24 h under N\textsubscript{2} at room temperature. Purification of the residue was done by silica gel column eluting with 1% CH\textsubscript{3}OH in CH\textsubscript{2}Cl\textsubscript{2} yielding 3 as a blue solid. R\textsubscript{f} = 0.5. \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}\textsubscript{6}) \(\delta\) 9.76 (s, 1H), 9.65 (s, 1H), 9.06 (s, 1H), 8.27 (dd, \(J = 17.6\) Hz, 11.6 Hz, 1H), 6.63 (d, \(J = 18\) Hz, 1H), 6.44 (d, \(J = 11.6\) Hz, 1H), 6.18 (d, \(J = 12.5\) Hz, 1H), 5.97 (d, \(J = 11.6\) Hz, 1H), 5.37 (d, \(J = 11.6\) Hz, 1H), 4.61 (q, \(J = 7.21\) Hz, 2H), 4.40 (m, 2H), 4.16 (m, 4H), 4.11 (m, 4H), 3.81 (m, 4H), 3.57 (m, 4H), 3.23 (s, 4H), 3.22 (s, 4H), 3.10 (s, 3H), 3.07 (s, 3H), 2.98 (s, 3H), 2.63 (m, 1H), 2.29 (m, 3H), 2.13 (m, 3H), 1.66 (t, \(J = 8.0\) Hz, 3H), 1.64 (d, \(J = 8.0\) Hz, 3H), -1.72 (s, 1H), -2.10 (s, 1H). \textsuperscript{13}C NMR (100.6 MHz, DMSO-\textit{d}\textsubscript{6}) \(\delta\) 173.3, 173.1, 172.7, 168.3, 168.1, 152.2, 149.1, 144.0, 137.1, 136.5, 136.5, 133.4, 133.3, 133.0, 131.5, 130.0, 129.2, 121.7, 103.7, 99.8, 99.0, 94.3, 72.7, 71.5, 71.3, 70.0, 70.0, 69.9, 69.8, 69.8, 69.6, 68.7, 68.5, 64.2, 63.6, 60.7, 58.3, 53.3, 48.2, 37.9, 31.1, 29.9, 23.6, 19.4, 18.2, 12.5, 11.4. HRMS (ESI) m/z calcd. for [C\textsubscript{48}H\textsubscript{64}N\textsubscript{4}O\textsubscript{12}\textsuperscript{+}]\textsuperscript{+} 911.4411, found 911.432. (ESI) m/z calcd. for C\textsubscript{48}H\textsubscript{65}N\textsubscript{4}O\textsubscript{12} [M+H]\textsuperscript{+} 889.4593, found 889.4586. UV-Vis (CHCl\textsubscript{3}): \(\lambda_{\text{max}}\) (\(\varepsilon / M^{-1}cm^{-1}\)) 664 nm (94342), 404 nm (285333).
19.6 Hz, 1H), 5.23 (m, 2H), 4.82 (m, 1H), 4.59 (q, \( J = 8.8 \) Hz, 2H), 4.08 (m, 4H), 3.73 (m, 4H), 3.61 (m, 4H), 3.54 (m, 4H), 3.50 (s, 3H), 3.49 (s, 3H), 3.48 (s, 3H), 3.42 (m, 4H), 3.41 (m, 4H), 3.29 (m, 4H), 3.24 (s, 3H), 3.19 (m, 4H), 3.11 (s, 3H), 3.06 (s, 3H), 3.04 (m, 4H), 2.66 (m, 1H), 2.27 (m, 2H), 2.15 (m, 2H), 1.67 (m, 3H), 1.65 (m, 3H), -1.64 (s, 1H), -1.66 (s, 1H). \(^{13}\)C NMR (100.6 MHz, CDCl\(_3\)) \( \delta \) 173.5, 173.1, 172.5, 169.7, 168.8, 154.7, 148.8, 142.9, 139.4, 136.4, 135.9, 135.5, 135.3, 134.7, 130.5, 129.3, 127.8, 123.5, 121.7, 102.4, 102.1, 98.6, 93.5, 72.5, 71.9, 71.7, 70.7, 70.5, 70.4, 70.3, 70.1, 70.0, 69.2, 68.9, 68.8, 66.8, 65.0, 64.3, 63.5, 61.7, 59.0, 58.9, 58.7, 53.0, 49.3, 41.5, 38.6, 31.0, 29.7, 23.8, 19.6, 17.6, 14.0, 12.4. HRMS (ESI) m/z calcd. for [C\(_{55}\)H\(_{78}\)N\(_4\)O\(_{15}\)+Na\(^+\)] 1057.535, found 1057.540. (ESI) m/z calcd. for C\(_{55}\)H\(_{79}\)N\(_4\)O\(_{15}\) [M+H]\(^+\) 1035.5536, found 1035.5538. UV-Vis (CHCl\(_3\)): \( \lambda_{\text{max}} (\varepsilon / \text{M}^{-1}\text{cm}^{-1}) \): 665 nm (89000), 404 nm (309333).

### 3.3.5 Hydrolytic Stabilities and Intrinsic Solubilities

Stock solutions of 1-3 were prepared in 9:1 methanol:water and were adjusted to pH = 2 or 8. Acidic pH = 2 was adjusted by 0.01 M formic acid and alkaline pH = 8 was adjusted by 0.01 M NH\(_4\)OH solution. The samples were injected in the LC/MS at 5 min, 1 h and 4 h after adding acid/alkali to each of them. The percent of starting material decomposed was reported based on the reduction in LC/MS chromatogram peak area and total ion abundance for 1-3. For the intrinsic solubilities, aliquots of water were added to 50 \( \mu \text{g} \) quantities of chlorin e\(_6\) or 1-3, and the solutions were stirred for 1 h at room temperature and then allowed to stand for 5 h. Solution was filtered to separate insoluble compounds and the amount of compound in the filtrate was determined by monitoring the soret bands of chlorin e\(_6\) and 1-3.
Figure 3. UV-Vis spectra of chlorin e₆, 1, 2, 3 in CHCl₃.
Figure 4. Fluorescence spectra of chlorin e$_6$, 1, 2, 3 in CHCl$_3$. 
Figure 5. $^{1}$H NMR spectrum of 1 in DMSO-$d_6$. 
Figure 6. $^{13}$C NMR spectrum of 1 in DMSO-$d_6$. 

![NMR Spectrum of Compound 1 in DMSO-$d_6$](image)
Figure 7. 2D HMBC spectrum of 1 in DMSO-$d_6$. 
Table 3. Cross peak observed in the 2D HMBC spectrum of 1

<table>
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<th>Proton No</th>
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<th>Correlated $^{13}$C peaks ppm</th>
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<td>9.67, s</td>
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<td>20</td>
<td>9.09, s</td>
<td>130.7, 129.7</td>
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<td>3&lt;sup&gt;1&lt;/sup&gt;</td>
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Figure 8. The expanded 2D HSQC spectrum of 1 in DMSO-$d_6$. 
Table 4. Cross peak observed in the HSQC spectrum of 1.

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<th>$^{13}$C δ (ppm)</th>
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<td>58.3</td>
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Figure 9. LCMS of 1 was carried out in gradient mixture of 90% MeOH in 10% H₂O containing 0.1% formic acid for 8min. \( t_R = 6.98 \) min.
Figure 10. HRMS of 1.
Figure 11: HPLC of 1 was carried out in gradient mixture of 90% MeOH in 10% H2O for 10 min and 98% MeOH in 2% H2O for 10 min. $t_R = 10.85$ min.
Figure 12. $^1$H NMR spectrum of 2 in DMSO-$d_6$. 
Figure 13. $^{13}$C NMR spectrum of 2 in DMSO-$d_6$. 

[Diagram of molecule 2]
Figure 14. 2D HMBC spectrum of 2 in DMSO-$d_6$. 
Figure 15. LCMS of 2 was carried out in gradient mixture of 90% MeOH in 10% H₂O containing 0.1% formic acid for 15min. \( t_R = 8.11 \) min.
**Qualitative Compound Report**

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**MS Spectrum**

Cpd 1: C48 H64 N4 O12; 0.25. + Scan (0.24-0.35 min, 8 scans) AM20120447.d Subtract 889.45858 (M+H)+

**MS Spectrum Peak List**

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Figure 16. HRMS of 2.
Table 5. Cross peak observed in the 2D HMBC spectrum of 2.

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<th>Correlated $^{13}$C peaks (ppm)</th>
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<td>9.73, s</td>
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<td>3²</td>
<td>6.56, d; 6.45, d</td>
<td>133.4-131.5</td>
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<td>15¹</td>
<td>5.61, d; 5.37, d</td>
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<td>8¹</td>
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<td>72.7-70.0; 69.8-58.3</td>
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</table>
Figure 17. HPLC of 2 was carried out in gradient mixture of 90% MeOH in 10% H₂O for 10 min and 98% MeOH in 2% H₂O for 10 min. $t_R = 12.21$ min.
Figure 18. $^1$H NMR spectrum of 3 in CDCl$_3$. 
Figure 19. $^{13}$C NMR spectrum of 3 in CDCl$_3$. 
Figure 20. The expanded 2D HSQC spectrum of 3 in CDCl$_3$. 
Table 6. Cross peak observed in the 2D HSQC spectrum of 3.

<table>
<thead>
<tr>
<th>$^{13}$C $\delta$ (ppm)</th>
<th>Correlated Proton ($\delta$ ppm)</th>
<th>2D HSQC cross signal</th>
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Figure 21. LCMS of 3 was carried out in gradient mixture of 90% MeOH in 10% H₂O containing 0.1% formic acid for 15min. $t_R = 12.24$ min.
Figure 22. HRMS of 3.
Figure 23. HPLC of 3 was carried out in gradient mixture of 90% MeOH in 10% H2O for 10 min and 98% MeOH in 2% H2O for 10 min. $t_R = 16.7$ min.
3.4 Conclusion

(1) Short-chained PEGs were coupled to the carboxylic acid sites of chlorin e6. The synthesis showed mono (1) PEG attached to 1 at the 173-position, di (2) PEG attached to 152- and 172-positions, tri (3) PEG attached to 131-, 152-, and 173-positions of chlorin e6. The regioselectivity of PEG linker attachment was confirmed by 2D NMR and mass spectrometry data. (2) Chlorins 1-3 were increasingly hydrolytically unstable as the number of PEG groups conjugated to the carboxylic acid site increased. (3) The mono, di, and tri-PEG chlorin conjugates were increasingly soluble in aqueous DMSO solution. Computed log P values suggest successive diminished lipophilicity in chlorins 1-3 compared to chlorin e6 by one to two log units. (4) Aggregation of 3 was less pronounced than chlorin e6, 1, and 2. MM+ and DFT calculations predicted a PEG association with the porphyrin ring where an increasing numbers of PEG groups increasingly resist formation of aggregates held together by π-π stacking forces. (5) Biological studies of this PEG conjugated chlorin compounds (performed by Dr. Stanley Kimani in collaboration with Prof. Tayyaba Hassan at Harvard Medical School) showed that increased numbers of PEG groups led to enhanced phototoxicity in an in vitro model of human ovarian cancer with an MTT assay. Pegylated chlorins had greater cellular uptake than chlorin e6, but the phototoxicity was not increased in parallel with cellular uptake. (6) For 2 and 3, the quenching study pointed to Type-II photo-oxidation, whereas for chlorin e6 and 1, Type-I mechanism was evident.

Our results indicated that introduction of the three PEG groups into chlorin e6 had improved phototoxicity and dampened its hydrophobicity. Therefore, we decided to incorporate tri-PEG conjugated chlorin 3 into the fiber-optic device to use in ovarian cancer photodynamic
therapy. In the next chapter we explored different synthetic strategies to covalently attach tri-PEG chlorin e₆ to the silica probe tip.
3.5 References


Chapter 4. A Tris-PEGylated Chlorin as a Photosensitizer for Use in a Fiber-Optic Based Phototherapy Device

4.1 Introduction

The study in this chapter was done to develop synthetic methodology for covalent attachment of chlorin-triPEG ester ($17^3,15^2,13^1$-chlorin methoxy triethylene glycol triester) $3$ to the fluorinated silica probe-tip. The purpose was to utilize the phototoxicity of $17^3,15^2,13^1$-chlorin methoxy triethylene glycol triester on photokilling of ovarian cancer cells by integrating the sensitizer $3$ into our device. In order to photorelease sensitizer from the fluorinated silica surface, electron rich spacer alkene alcohol linker was conjugated with chlorin e$_6$ tri-PEG ester. Fluorinated porous Vycor glass probe tip has repelling surface property and thereby inhibits the adsorption and improve the photorelease of the sensitizer after alkene bond cleavage.$^1$

A couple of synthetic strategies were developed and shown in the schemes below for tri (3) PEG conjugated chlorin attachment to the fluorinated silica probe tip for visible light and oxygen induced delivery from the probe tip.

We used two different chlorins, rhodin G7 $1$ and chlorin e$_6$ $2$ as the sensitizer to synthesize spacer alkene conjugated chlorin-triPEG ester. Rhodin G7 is structurally very similar to chlorin e$_6$, as shown in Fig 1. Both of them have very similar spectral properties ($\lambda_{max} (Q_y) = 653$ nm).$^2$ Therefore, our assumption was that both would show similar photophysical properties. In this chapter we have reported couple of proposed synthetic strategies to make fiber-optic probe tip with photocleavable spacer alkene bridged chlorin e$_6$-triPEG ester or rhodin G7-triPEG ester for ovarian cancer phototherapy.
Fig 1. Structures of rhodin G7 1, chlorin e₆ 2 and 17³,15²,13¹-chlorin methoxy triethylene glycol trimester 3.

To attach chlorin e₆-triPEG ester to the fiber-optic probe tip, attempts were made to attach spacer alkene linker to the 3¹-number carbon. Many attempts have been made to functionalize 3¹-3² vinyl moiety of chlorin e₆, such as hydro-bromination by HBr, hydroxylation by epoxidation. However, we were unsuccessful in all of these attempts.

In the strategies discussed below, we have not accomplished synthesis of spacer alkene conjugated rhodin G7-triPEG ester. Therefore, we decided to use linker (1,3-diol or succinic acid) in between sensitizer and spacer alkene to ease synthetic difficulty of attaching spacer alkene to the chlorin e₆-triPEG ester. We explored that conversion of ³¹-³² vinyl bond to ³¹-
hydroxyl could be easily achieved by reacting chlorin-triPEG ester with acidic OsO$_4$ solution followed by the reduction of $3^1$-formyl by sodium borohydride. It was then easy to attach succinic acid as a linker between photocleavable spacer alkene and chlorin. The yield of each step was moderate to good. Chemistry involved in the associated steps of each strategy was studied.

Photocleavage of the tri-PEG chlorin modified fluorinated silica probe tip was carried out in $n$-butanol. However, the photocleavage results reported here is preliminary. The studies on effect of surface adsorption of the sensitizer and oxygen solubility on photorelease efficiency are currently undergoing.

### 4.2 Results and Discussion

In Scheme 1, the strategy of synthesizing spacer alkene conjugated rhodin G7-triPEG ester in an attempt to attach it to the partially fluorinated porous Vycor glass was described. In schemes 3, 4, 6 and 7, the strategy of attaching spacer alkene conjugated chlorin e$_6$-triPEG ester was described. However, we were not successful in conjugating spacer alkene to the rhodin G7-triPEG ester and $3^1$-etherate propanol chlorin e$_6$-triPEG ester. Therefore, spacer alkene conjugated rhodin G7-triPEG ester and spacer alkene-dietherate-propane chlorin e$_6$-triPEG ester were not attached to the silica solid support (Schemes 3 and 4). We eventually became successful in attaching spacer alkene to the $3^1$-succinate chlorin triPEG ester (Schemes 6 and 7). All of the strategies and schemes are discussed below.
4.2.1 Strategy 1

Scheme 1. Synthesis of Spacer Alkene Conjugated Rhodin G7 Methoxy Tri(ethylene glycol) Triester and its Attachment to the Partially Fluorinated Porous Vycor Glass.

1. Hydrolysis of methyl ester by resin
2. Acedification by dil HCl
3. mono methoxy triethylene glycol, EDC, DMAP, 24h, N₂ atm, DCM

(MeO)₃Si-···OH
NaH, THF, N₂ atm, reflux

Toluene, reflux, 24h
4.2.1.1 Synthesis and characterization. Commercially available rhodin G7 sodium salt was used and treated with dil HCl to get acidic form of Rhodin G7. Rhodin G7 was treated with trimethyl silyl diazomethane to get trimethyl ester of rhodin G7 1 with 50% yield. Tri-methyl ester of rhodin G7 1 was reduced by NaBH₄ to get 7₁-hydroxyl-Rhodin G7-trimethyl ester 2 with 80% yield.

Characterization of product 1 was confirmed by ¹H NMR spectra. In ¹H NMR, 4 signals in the downfield region for three meso hydrogens and one aldehydic hydrogen at 11.05 ppm, 10.27 ppm, 9.58 ppm, 8.65 ppm were observed. 5 singlets were also found within 3.2-4.4 ppm for three methyl groups attached as an ester bond and two methyl groups attached to the chlorin core. 1 was reduced to 7₁-hydroxyl-Rhodin G7-trimethyl ester 5 by NaBH₄ with 80% yield. Characterization of compound 2 was confirmed by 1D and 2D NMR.

In ¹H NMR, 2 showed two peaks at 9.71 and 8.76 ppm for three hydrogens. 2D HSQC (Fig 2) spectrum shows that three meso-hydrogens were attached to three different carbons. It
showed that two protons at 9.71 ppm were correlated with the carbon at 103.0 and 99.3 ppm respectively. Whereas, proton at 8.76 ppm was correlated with the carbon appears at 93.6 ppm. 

Aldehyde was reduced upon reaction with NaBH₄. This reaction was monitored by proton NMR. We found no aldehyde peak at 11.21 ppm and new peak was appeared as a singlet at 5.78 ppm for 7¹-hydrogens. Identity of these 7¹-hydrogens in 2 was also confirmed by correlation between carbon at 56.7 ppm and protons which appeared as a singlet at 5.78 ppm in 2D HSQC spectra (shown in Fig 2).

Comparison of carbon NMR of Rhodin G7-trimethyl ester 1 with 7¹-hydroxyl Rhodin G7-trimethyl ester 2 showed that a signal at 187.4 (for carbonyl carbon) for 1 was absent in 2, which further confirmed the reduction of aldehyde.
Fig 2. Expanded 2D HSQC of 2 shows correlation between protons at 5.78 ppm and carbon at 56.7 ppm. Singlet for 2H at 9.71 ppm correlates with the carbon at 99.3 ppm and 103.0 ppm respectively.
Spacer alkene alcohol 6 was converted into its dihalide (scheme 2). A dibromo spacer alkene 7 was synthesized from spacer alkene alcohol using PBr₃ with 20% yield and diiodo spacer alkene 8 was synthesized from spacer alkene alcohol using catalytic amount of p-TSA and excess NaI with 40% yield. However, diiodo spacer alkene was not stable at rt, and decomposed immediately after its formation. Therefore, it was not used in step 3 for conjugation with 2. However, dibromo spacer alkene was stable at room temperature. Therefore, we used it for conjugation with compound 2. Reaction of spacer alkene with 2 was performed in the presence of NaH at room temperature under nitrogen atmosphere for 24 h, but no product formation was found, as observed by TLC. Reaction mixture was then heated by reflux and kept for 24 h and monitored by TLC. A complex reaction mixture was formed, which did not move in TLC plate. ¹H NMR of the crude reaction mixture didn’t show any spacer alkene signal. Therefore, the expected product was not obtained in step 3.

Conversion of dibromo and diiodo was very poor and the diiodo spacer alkene was found to decompose at room temperature after its formation. We carried out step 3 thrice with variation in the amount of NaH, and temperature, but we were not been able to get our expected product. Since we were not successful in making 3 by the above synthetic route, we decided to change the strategy. Alternative strategy is discussed below.
4.2.2 Strategy 2

Scheme 3. Synthesis of Spacer Alkene-3\textsuperscript{1}-Etherate Propanol Chlorin e\textsubscript{6} Methoxy Tri (ethylene glycol) Triester and its Attachment to the Partially Fluorinated Porous Vycor Glass.

1. LiOH, 60\% THF-H\textsubscript{2}O, H\textsubscript{2}O\textsuperscript{+} 24h
2. monomethoxy tri-ethylene glycol, EDC, DMAP, N\textsubscript{2} atm, 24h, DCM
Scheme 4. Attachment of Spacer Alkene-3¹-Etherate Propanol-Chlorin e₆-Methoxy Tri(ethylene glycol) Triester to the Fluorinated Silica Probe Tip.

4.2.2.1 Synthesis and characterization. In Scheme 2, we have shown an alternative synthetic strategy to attach chlorin e₆-triPEG ester to the probe tip. Chlorin e₆ has been converted to chlorine e₆-trimethyl ester 9 to reduce its polarity in order to facilitate the separation of chlorin by column chromatography. Chlorin e₆ was treated with trimethyl silyl diazomethane to get chlorin e₆-trimethyl ester with 97% yield. 9 was treated with HBr-HOAc³ to brominate vinyl bond. 1,3-propane diol was added in excess in situ to get 3¹-diol-chlorin e₆-trimethyl ester 10 with 45% yield.

Identity of compound 10 was confirmed by ¹H NMR spectroscopy. In ¹H NMR, 8¹-vinyl protons were absent and a new quartet found at 6.01 ppm for the protons attached to 3¹-carbon, which confirmed attachment with 1,3-propane diol to the 3¹- of chlorin. Terminal –OH of the propyl alcohol in 10 was tosylated using TsCl to get tosyl propanol-chlorin e₆-trimethyl ester 11 with 54% yield. Tosylation was confirmed by identification of the protons of the phenyl ring at
7.56 ppm and 6.92 ppm. Tosylation was carried out to facilitate nucleophilic substitution reaction to form ether linkage with spacer alkene in the next step.

3\(^{1}\)-tosyl propanol–chlorin e\(_{6}\)-trimethyl ester 11 was then treated with spacer alkene in the presence of NaH in THF to get spacer alkene conjugated-1,3-diol-chlorin e\(_{6}\)-trimethyl ester 12. The reaction was first carried out at room temperature for 24 h and monitored by TLC, but no product was formed. It was then kept under reflux condition for 16 h. After 16 h, TLC of the reaction mixture indicated the formation of product. The crude product was taken into a silica column and eluted with 5-10% MeOH-DCM. \(^{1}\)H NMR of the isolated compound didn’t show any evidence of conjugation of spacer alkene. The expected product was not obtained, and thus, effort was not made to identify the compound. Step 4 was carried out thrice with minute variation in the amount of reactant and reaction conditions, but we were not successful in the synthesis of the expected compound 12.

As nucleophilic substitution reaction between 11 and spacer alkene didn’t take place, we decided to do control hydrolysis of chlorin e\(_{6}\)-trimethyl ester under mild condition to see how efficient the conversion is. It was our priority, because, even if we had achieved 12 by alternative synthetic route (For example, by converting terminal hydroxyl group of 10 into bromide or iodide, followed by nucleophilic substitution with spacer alkene alcohol), we would still need to carry out the hydrolysis of tri-methyl ester to reinstall tri-carboxylic acid in mild condition. Mild hydrolysis condition would necessary to avoid any side reaction to the vinyl bond of the spacer alkene moiety. Reinstallation of tri-carboxylic acid was required to attach tri (3) PEG as an ester bond to get the final compound 13. Controlled hydrolysis study of chlorin e\(_{6}\)-trimethyl ester is discussed below.

**4.2.2.2 Hydrolysis Study of Chlorin e\(_{6}\)-Trimethyl Ester by HPLC.**
Scheme 5.

Hydrolysis of chlorin e₆-trimethyl ester 9 was carried out using LiOH, H₂O at room temperature for 24 h followed by heating at 40°C for 48 h. Conversion of hydrolysis reaction was 25% as obtained by HPLC. Signal for chlorin e₆ appeared at \( t_R = 8.36 \) and a new signal at \( t_R = 14.54 \) was also appeared.

Presumably, in mild condition all of the methyl ester was not hydrolyzed, as methyl ester at 13¹-position is more stable compared to the 17³- and 15²-position. More vigorous conditions (higher temperature or stronger base) are required to hydrolyze 13. However, we have not tried base catalyzed hydrolysis of the trimethyl ester of chlorin at high temperature, as structural changes to the chlorin core might take place. Moreover, if we had accomplished the synthesis of compound 12, base catalyzed hydrolysis of the same compound at high temperature would harm vinyl ether moiety of spacer alkene.

Therefore, we decided that it is not worth trying to pursue nucleophilic substitution chemistry by an alternative pathway to get spacer alkene conjugated chlorin e₆-trimethyl ester 12. However, we thought that it would be desirable to synthesize PEG ester with the carboxylic acids of chlorin e₆ prior to do make any change to the 3¹-3² vinyl bonds in order to attach spacer
alkene. Therefore, we developed a succinic acid linker assisted synthesis, which is discussed below.

4.2.3 Strategy 3

Scheme 7. Photocleavage of 22.

4.2.3.1 Synthesis and characterization. Tri (3) PEG ester of chlorin e₆ 16 was synthesized from chlorin e₆ by the previously mentioned procedure in chapter 3. 16 was treated with OsO₄ followed by acidic solution of sodium periodate to convert 3¹-3² vinyl bond to –CHO. Conversion of vinyl bond to –CHO was confirmed by ¹H NMR and UV-Vis spectroscopy. In ¹H NMR, we found four signals in the downfield region for one aldehyde hydrogen and three meso hydrogens (appears at 11.56, 10.28, 9.70 and 8.97 ppm respectively). A dd for 3¹- hydrogen at 8.08 ppm and two doublets for 3²- hydrogens appeared at 6.37 ppm and 6.16 ppm respectively for 16, and were absent in 17, due to the presence of –CHO in its β-pyrrolic position. UV-Vis spectrum showed characteristic redshift of the Qₚ absorption maxima from 664 nm in 16 to 694 nm in 17 due to the change in transition dipole moment along the y-axis, which in turn reduced the energy gap between the energy states involved in the transition. Similar observation was
made by Tamiaki. H. Carbon NMR of 17 showed 21 PEG carbons for three PEG’s within 71.9-58.8 ppm confirming that PEG ester bond was stable under reaction conditions.

17 was reduced to 3¹-alcohol 18 by sodium borohydride. Three signals in the downfield region in ¹H NMR at 9.72, 9.55, 8.76 ppm and a new peak at 5.87 ppm for two methylene protons attached to 3¹- carbon (2D HSQC NMR of very similar compound: 7¹-hydroxyl rhodin G7-trimethyl ester showing correlation with proton appeared at 5.78 ppm and with carbon at 56.7 ppm), confirming the reduction of aldehyde to alcohol. UV-Vis spectrum showed further evidence as Qₛ absorption maximum blue-shifted to 659 nm.

In order to conjugate spacer alkene to the sensitizer, succinic acid linker was used. Presumably linker attachment would keep phenyl ring of the spacer alkene away from the sensitizer core. Therefore, photo-physical property of the sensitizer was not likely to be influenced. Conjugation of succinic acid was achieved by EDC-DMAP coupling. In order to get mono carboxylate ester of succinic acid 19, succinic acid was used about 5 times in excess of 18, which gave us 74% yield of 19. Identification of 19 was confirmed by proton NMR, where two hydrogens attached to the 3¹- carbon shifted more downfield (6.46 ppm) due to esterification of 3¹- OH with –COOH of succinic acid.

19 was coupled with spacer alkene alcohol using EDC and DMAP to get 20. Similarly to an earlier reaction step, spacer alkene was used in excess to get mono ester 20, which gave us 52% yield of 20. Identity of the compound 20 was confirmed by 1D and 2D NMR spectroscopy. In ¹H NMR, we found four doublets at 7.32, 7.01, 6.97 and 6.71 ppm for phenyl protons (J = 8.4 Hz) and two doublets at 5.91 and 5.71 ppm (J = 3.2 Hz) for the two olefinic hydrogens attached to the vinyl ether moiety. In HSQC, thus four doublets for aromatic protons and two doublets for
olefinic proton correlate with 7 carbon within 129.8-115.8 ppm. We also found that 21 PEG carbons of three PEGs within 71.9-58.8 ppm, indicating that three PEGs remain attached via an ester bond to the carboxylic acid sites of chlorin.

Spacer alkene conjugated chlorin 20 was then reacted with 3-iodopropyl trimethoxy silane to get 21. Sensitizer silane 21 was not purified by column chromatography as its formation of Si-O-Si bonds inside the column could prevent it from elution. Moreover, -Si(OMe)_3 moiety is very labile towards hydrolysis to –Si(OH)_3. Therefore, nonafluorohextrimethoxy silane attached PVG was added in situ to the sensitizer silane to get 22. It was repeatedly washed with THF, DCM, toluene, methanol, and hexane followed by soxhlet extraction in MeOH for 24h to remove physically adsorbed sensitizer. No sensitizer leaching was observed in chloroform and methanol, when 22 was dipped for about 1 h at room temperature. Therefore, we concluded that 22 contained siloxane bond, where sensitizer was chemically bound to the Si-OH bond in the silica matrix of the partially fluoroalkane modified porous Vycor glass. In our previous study, covalent bond formation of the sensitizer to the porous Vycor glass was confirmed by FTIR spectra from the peaks at 2851 and 2954 cm⁻¹ for C-H stretching of the spacer methylene group. Loading amount of the sensitizer to the conjugated fluoroalkane modified porous Vycor glass 22 was determined by previously described HF stripping method (in Chapter 1) to quantify % photorelease of the sensitizer in organic media. When aqueous HF solution was extracted by chloroform and mass spectra of the organic extract was checked, a peak for fluorosilane conjugated spacer alkene was observed, which further indicated covalent attachment of the sensitizer.
4.2.3.2 Photocleavage of spacer alkene conjugated chlorin e₆-triPEG ester modified cap.

4.50 µM (15%) of dye was photocleaved into n-butanol after 90 min. In the Q-band region of photocleaved dye, a small hump at 694 nm has been observed along with the expected band at 665 nm. The time course of photocleavage is shown in Fig 3. After photocleavage of dye for 90 min, when the amount of dye in solution reached its saturation, glass didn’t turn colorless. Therefore, it was presumed that the significant amount of dye remained attached to the surface after its photocleavage, which was not an unexpected result, as our previous study⁶,⁷ suggests that at room temperature surface bound dioxetane could be stable enough. Another reason could be that dye, after its photocleavage, remained adsorbed onto the surface. Photorelease amount with time in n-butanol are given in table 1.

![Sensitizer photorelease profiles for fluorinated silica in n-butanol solution at 25 °C. The lines are present to help guide the eye. Error bars represent the standard deviation obtained from 3 measurements.](image)

Fig 3. Sensitizer photorelease profiles for fluorinated silica in n-butanol solution at 25 °C. The lines are present to help guide the eye. Error bars represent the standard deviation obtained from 3 measurements.
Table 1. Yield of Photorelease by Fluorinated Silica Photooxidation$^{a,b}$

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<th>Solid Support</th>
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<th>Time (min)</th>
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<td>2.57±0.20</td>
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<td>Vycor</td>
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<td></td>
<td>3.76±0.14</td>
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<td>4.17±0.33</td>
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<td>90</td>
</tr>
</tbody>
</table>

$^a$External irradiation of tip via a fiber optic connected to 669 nm diode laser and operated at 4.0 psi O$_2$ pressure, 0.2-0.3 ppm/min O$_2$ flow rate through the 0.328 g fluorinated silica. Fiber tip dimensions: cylinder shape with a length of 8.0 mm, diameter of 5.0 mm, and hole (2.0 length x 3.0 mm diameter). Experiments were repeated thrice. $^b$Absorption spectroscopy was used for the quantitation of x in n-butanol.

4.3. Experimental Section

4.3.1 7-Formyl-Rhodin G7-Trimethyl Ester (1). Yield 50.0 mg (50 %). To a 16 ml mixed solution (10 ml MeOH and 6 ml Toluene) 94.0 mg Rhodin G7 (0.154 mmol) was added and stirred for 5 min under nitrogen. 460 µl (0.924 mmol) 2M ethereal solution of trimethyl silyl diazomethane was added to the reaction mixture drop wise. Reaction mixture was stirred under N$_2$ for 5 h. AcOH (10% aqueous, 10 mL) was added to the reaction mixture to quench excess diazomethane. MeOH was evaporated under reduced pressure. Reaction mixture was diluted with 20 ml dichloromethane and organic layer was washed with 10 ml saturated Na$_2$CO$_3$. Organic layer was dried on Na$_2$SO$_4$ and evaporated to get crude product. Product was separated by column chromatography using 0.25-0.30% MeOH-DCM to get pure brown solid. $R_f$ = 0.7 in 1% MeOH-DCM. $^1$H NMR (400.0 MHz, CDCl$_3$) δ 11.05 (s, 1H), 10.27 (s, 1H), 9.58 (s, 1H),
8.65 (s, 1H), 7.95 (dd, J = 17.6 Hz, 11.6 Hz, 1H), 6.37 (d, J = 18 Hz, 1H), 6.15 (d, J = 11.6, 1H), 5.32 (d, J = 19.2 Hz, 1H), 5.24 (d, J = 18.8, 1H), 4.42 (m, 2H), 4.30 (s, 3H), 3.94 (m, 2H), 3.83 (s, 3H), 3.70 (s, 3H), 3.52 (s, 3H), 3.40 (s, 3H), 2.64 (m, 1H), 2.31 (m, 2H), 1.82 (m, 3H), 1.73 (m, 3H), 1.31 (m, 2H), -0.85 (br s, 1H), -0.93 (br s, 1H); ¹³C NMR (100.6 MHz, CDCl₃) δ 187.4, 173.5, 172.8, 172.1, 169.4, 168.9, 158.3, 150.6, 144.8, 141.6, 138.6, 138.8, 137.8, 136.5, 136.4, 132.3, 131.1, 129.3, 128.7, 124.2, 122.5, 104.2, 102.5, 102.2, 93.6, 53.1, 53.0, 52.2, 51.7, 49.4, 38.2, 31.1, 29.5, 22.9, 19.4, 19.0, 12.3, 11.9. (+ESI) m/z calculated for C₃₇H₄₁N₄O₇ [M+H]⁺ 653.29698, found: 653.29751.

4.3.2 7¹-Hydroxyl-Rhodin G7-Trimethyl Ester (2). Yield 25 mg (83%). To a 10 ml MeOH, 30 mg (0.046 mmol) of 1 and 9.0 mg (0.23 mmol) of NaBH₄ was added in ice cold temperature. Color of the solution was changed from red to bluish due to the loss of extended conjugation that occurred due to the reduction of 7-formyl group and therefore, transition of dipole moment change along the axis.⁸ Reaction was stirred at room temperature for 15 h. MeOH was evaporated by reduced pressure. Reaction mixture was diluted with 20 ml DCM. Organic layer was washed with 10 ml 10% AcOH followed by saturated sodium bicarbonate. Organic layer was dried on Na₂SO₄ and evaporated on rotavapor to get green solid. Crude product was purified by column chromatography using 0.5-0.6% MeOH-DCM. Rᵣ = 0.5 in 1% MeOH-DCM. ¹H NMR (400.0 MHz, CDCl₃) δ 9.71 (s, 2H), 8.76 (s, 1H), 8.01 (dd, J = 17.6, 11.2, 1H), 6.34 (d, J = 17.6, 1H), 6.12 (d, J =11.2, 1H), 5.75 (s, 2H), 5.36 (d, J = 18.8, 1H), 5.25 (d, J = 18.8, 1H), 4.45 (m, 2H), 4.29 (s, 3H), 3.82 (m, 5H), 3.67 (s, 3H), 3.58 (s, 3H), 3.45 (s, 3H), 2.59 (m, 1H), 2.22 (m, 2H), 1.78 (m, 3H), 1.75 (m, 4H), -1.20 (br s, 1H), -1.40 (br s, 1H); ¹³C NMR (100.6 MHz, CDCl₃) δ 173.5, 172.9, 170.2, 169.3, 167.6, 152.7, 147.6, 147.5, 140.0, 137.7, 137.1, 136.0, 135.5, 135.3, 130.7, 129.3, 129.1, 123.6, 122.1, 103.0, 102.3, 99.3, 93.6, 56.7, 53.0, 52.1, 51.6,
49.4, 38.5, 31.0, 29.7, 29.5, 22.9, 19.5, 19.0, 12.3, 12.1. (+ESI) m/z calculated for C₃₇H₄₂N₄O₇Na [M+Na]⁺ 677.29493, found: 677.29457

4.3.3 (Z)-1,2-Bis(4-bromomethylphenoxy) Ethene (7). Yield 10 mg (23%). To a solution of 6 (30.0 mg, 0.110 mmol) in anhydrous THF (5 mL), at 0 °C and under a nitrogen atmosphere, was slowly added PBr₃ (12 μL, 0.043 mmol). The reaction solution was stirred for 5 min at 0 °C, the ice bath was then removed and stirring was continued for 25 min at room temperature. THF was evaporated at reduced pressure. 15 ml CH₂Cl₂ was added and it was washed with NaHCO₃ (8 mL) and brine (8 mL) and then dried with Na₂SO₄ and concentrated in vacuo. Crude reaction mixture was assessed by flash chromatogram using 50:50 hexane-dichloromethane mixture to get pure 7.

¹H NMR (400.0 MHz, CDCl₃) δ 7.38 (d, J = 8.4 Hz, 4H), 7.07 (d, J = 8.4 Hz, 4H), 6.18 (s, 2H), 4.52 (s, 2H).

4.3.4 (Z)-1,2-Bis(4-iodomethylphenoxy) Ethene (8). Yield 40.0 mg (22 %) To a vigorously stirred solution of NaI (505 mg, 3.67 mmol) and 6 (100 mg, 0.367 mmol) in acetonitrile (10mL) under nitrogen was added paratoluene sulfonic acid (253 mg, 1.47 mmol) in 5 mL of acetonitrile with a syringe at room temperature. The reaction mixture was allowed to stir for 2 h, quenched with water, and extracted with ether (50 mL). The organic layer was washed with 10% sodium thiosulphate solution and dried over anhydrous sodium sulphate. Upon evaporation, crude diiodide was obtained. This was purified by silica gel column chromatography by eluting with 10% hexane in ethyl acetate. ¹H NMR (400.0 MHz, CDCl₃) δ 7.37 (d, J = 8.4, 4H), 7.02 (d, J = 8.8, 4H), 6.17 (s, 2H), 4.48 (s, 4H). ¹³C NMR (100.6 MHz, CDCl₃) δ 156.7, 133.8, 130.3, 130.1, 128.3, 116.4, 5.5
4.3.5 Chlorin e₆-Trimethyl Ester (9). Yield 100.0 mg (93.4%). To a 10 ml mixed solution (6 ml MeOH and 4 ml Toluene), 100.0 mg (0.167 mmol) of chlorin e₆ was added and stirred for 5 min under nitrogen. 460 µl (0.924 mmol) 2M hexane solution of trimethyl silyl diazomethane was added to the reaction mixture drop wise. Reaction mixture was stirred under N₂ for 5 h. AcOH (10 mL 10% aqueous solution) was added to the reaction mixture to quench excess diazomethane. MeOH was evaporated under reduced pressure. Reaction mixture was diluted with 20 ml dichloromethane and organic layer was washed thrice with 10 ml water, dried on Na₂SO₄ and evaporated to get crude product. Crude product was separated by column chromatography using 0.2% MeOH in DCM. Rᵣ = 0.85. ¹H NMR and ¹³C matches with the literature value.⁸ Purity of the compound was checked by HPLC and it is 99%: tᵣ = 19.2 min in gradient mixture of MeOH and H₂O. ¹H NMR (400.0 MHz, CDCl₃) δ 9.72 (s, 1H), 9.54 (s, 1H), 8.80 (s, 1H), 8.03 (dd, J = 18.0 Hz, 11.6 Hz, 1H), 6.34 (d, J = 18.0 Hz, 1H), 6.13 (d, J = 11.2 Hz, 1H), 5.44 (d, J = 18.8 Hz, 1H), 5.33 (d, J = 13.6 Hz, 1H), 4.51 (m, 2H), 4.33 (s, 3H), 3.84 (s, 3H), 3.77 (m, 2H), 3.70 (s, 3H), 3.64 (s, 3H), 3.48 (s, 3H), 3.29 (s, 3H), 2.63 (m, 1H), 2.26 (m, 2H), 1.83 (d, J = 7.6 Hz, 4H), 1.75 (t, J = 11.2 Hz, 3H), -1.22 (br s, 1H), -1.37 (br s, 1H).

4.3.6 3¹-Etherate Propanol-Chlorin e₆-Trimethyl Ester (10). Yield 25.0 mg (45%). To the 50.0 mg of chlorin e₆-trimethyl ester 9, 1.5 ml of 33% HBr in HOAc was added and kept stirring at room temperature for 2.0 h. Acid was removed in high vacuum within 35-40°C. Solid residue was dissolved in 10 ml of dry dichloromethane, 2.0 ml of 1,3-diol and 250 mg of anhydrous K₂CO₃ was added and kept stirring for 1.0 h at room temperature under nitrogen atmosphere. K₂CO₃ was removed by filtration. Dichloromethane was evaporated. Residue was put directly into the column and separated using 0.5-0.6% MeOH-DCM. Rᵣ = 0.55 in 1% MeOH-DCM. Purity of the compound was checked by HPLC and it was 97%: tᵣ = 5.57 min in gradient mixture.
of MeOH and H₂O. ¹H NMR (400.0 MHz, CDCl₃) δ 9.85 (s, 1H), 9.76 (s, 1H), 8.77 (s, 1H), 5.99 (q, J = 6.8 Hz, 1H), 5.37 (d, J = 18.8 Hz, 1H), 5.25 (d, J = 18.8 Hz, 1H), 4.50 (m, 2H), 4.31 (s, 3H), 3.89 (m, 5H), 3.82 (s, 3H), 3.67 (s, 3H), 3.63 (s, 3H), 3.48 (d, J = 2 Hz, 3H), 3.36 (s, 3H), 2.58 (m, 2H), 2.28 (m, 2H), 2.19 (t, J = 5.6 Hz, 3H), 1.98 (m, 2H), 1.77 (q, J = 7.6 Hz, 7H), -1.28 (br s, 1H), -1.46 (br s, 1H). (+ESI) m/z calculated for C₄₀H₅₁N₄O₈ [M+H]⁺ 715.36, found: 715.25

**4.3.7 3¹-Etherate Propane Tosylate-Chlorin e₆-Trimethyl Ester (11).** Yield 7.0 mg (54%). To the 11.0 mg (0.015 mmol) of 3¹-diol-chlorin e₆-trimethyl ester 10, 0.025 mg (0.021 mmol) of DMAP and 50 ul (0.840 mmol) of pyridine was added in 10 ml dry dichloromethane under nitrogen atmosphere and reaction mixture was stirred at room temperature for 15 min. 20.0 mg (0.10 mmol) of tosyl chloride was added at 0-4°C. Reaction mixture was stirred at room temperature for 36 h under nitrogen atmosphere. Reaction mixture was washed with dilute sodium bicarbonate followed by water several times. Organic layer was separated and dried in vacuum and directly put into the column and separated using 0.27% MeOH-DCM. Rᶠ = 0.9 in 1% MeOH-DCM. ¹H NMR (400.0 MHz, CDCl₃) 9.74 (s, 1H), 9.73 (s, 1H), 8.72 (s, 1H), 7.56 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.4 Hz, 2H), 5.87 (m, 1H), 5.37 (d, J = 18.8 Hz, 1H), 5.26 (d, J = 18.8 Hz, 1H), 4.40 (m, 3H), 4.28 (s, 3H), 4.13 (m, 1H), 3.82 (m, 5H), 3.65 (s, 3H), 3.60 (s, 3H), 3.42 (s, 3H), 3.20 (s, 3H), 2.59 (m, 1H), 2.19 (m, 2H), 2.09 (m, 5H), 2.02 (s, 3H), 1.77 (t, J = 3.6 Hz, 3H), 1.73 (m, 4H), -1.49 (br s, 1H), -1.59 (br s, 1H); ¹³C NMR (100.6 MHz, CDCl₃) δ 173.5, 173.0, 169.6, 169.5, 166.8, 154.5, 148.9, 145.0, 144.4, 139.2, 139.1, 138.2, 136.4, 136.0, 135.3, 134.5, 134.4, 132.9, 131.2, 131.1, 129.5, 129.3, 127.6, 123.4, 102.2, 102.1, 99.0, 93.2, 73.2, 73.1, 67.8, 64.9, 53.0, 52.9, 52.1, 51.6, 49.4, 38.5, 31.0, 29.7, 24.6, 22.9, 21.2, 19.6, 17.7, 12.3, 11.3, 11.1.
4.3.8 3-Formyl-17\(^3\),15\(^2\),13\(^1\)-Chlorin e\(_6\) Methoxy Tri(ethylene glycol) Triester (17). Yield 12.0 mg (60%). To the 20.0 mg (0.019 mmol) of 16 in 15 ml THF, 7.68 mg (0.03 mmol) of OsO\(_4\) in 20 µL CCl\(_4\) was added at 0°C under N\(_2\) atmosphere. Reaction mixture was stirred within 0-5°C temperature for 25 min. 82.8 mg (0.38 mmol) of NaIO\(_4\) dissolved in 1% AcOH solution and added to the reaction mixture. Reaction was stirred overnight at room temperature. A drop of saturated sodium bicarbonate was added to neutralize acid. Reaction mixture was extracted with 50 ml of dichloromethane and washed with water. Organic layer was dried over sodium sulfate. After evaporating organic solvent, residue was purified by column chromatography using 1.1-1.2% MeOH-DCM. R\(_f\) = 0.69 in 3% MeOH-DCM. Purity of the compound was checked by HPLC and it was 98%: \(t_R = 12.2\) min in gradient mixture of MeOH and H\(_2\)O.

\(^1\)H NMR (400.0 MHz, CDCl\(_3\)) \(\delta\) 11.56 (s, 1H), 10.28 (s, 1H), 9.70 (s, 1H), 8.97 (s, 1H), 5.48 (d, \(J = 18.4\) Hz, 1H), 5.30 (d, \(J = 18.4\) Hz, 1H), 4.96 (m, 1H), 4.89 (m, 1H), 4.51 (m, 2H), 4.35 (m, 2H), 4.16 (m, 4H), 3.86 (m, 3H), 3.84 (s, 3H), 3.82 (m, 3H), 3.71 (t, \(J = 4.8\) Hz, 3H), 3.65 (m, 5H), 3.56 (m, 10H), 3.44 (t, \(J = 4.8\) Hz, 3H), 3.37 (s, 3H), 3.34 (m, 4H), 3.30 (s, 3H), 3.23 (s, 3H), 3.22 (s, 3H), 3.18 (t, \(J = 4.8\) Hz, 2H), 2.60 (m, 1H), 2.25 (m, 2H), 1.77 (m, 8H), -1.24 (br s, 1H), -1.76 (br s, 1H); \(^{13}\)C NMR (100.6 MHz, CDCl\(_3\)) \(\delta\) 188.3, 173.0, 172.2, 168.9, 168.5, 167.6, 155.1, 151.5, 144.9, 138.3, 138.1, 137.9, 136.6, 136.0, 134.0, 131.9, 128.6, 125.8, 103.4, 101.3, 100.7, 95.5, 71.9, 71.8, 71.6, 70.7, 70.6, 70.5, 70.4, 70.2, 70.1, 69.2, 69.0, 68.9, 65.2, 64.3, 63.6, 59.0, 58.9, 58.8, 53.4, 48.7, 38.5, 31.0, 29.7, 29.5, 23.3, 19.6, 17.5, 12.4, 11.4, 11.3. HRMS (+ESI) m/z calculated for C\(_{54}\)H\(_{77}\)N\(_4\)O\(_{16}\) [M+H]\(^+\) 1037.5335, found: 1037.5357

**UV-Vis Spectral analysis:** Characteristic red shift in UV-Vis spectrum was observed due to its conversion to 3\(^1\)-Formyl (\(\lambda_{sorret} = 427\) nm, \(Q_y = 694\) nm) from 3\(^1\)-3\(^2\) vinyl bond in chlorin e\(_6\)-triPEG ester (\(\lambda_{sorret} = 400\) nm, \(Q_y = 664\) nm).
4.3.9 31-Hydroxyl-17,15,13-Chlorin e6 Methoxy Tri(ethylene glycol) Triester (18). Yield 10.0 mg (77%). To the 13.0 mg (0.012 mmol) of 17 in 4:1 ml Methanol-dichloromethane at 5-10°C 1.0 mg (0.025 mmol) of NaBH4 was added. A sudden color change from brown to green was observed. Reaction was monitored by TLC. It was completed in 0.5 h. Reaction mixture was quenched with water and extracted by dichloromethane. Organic solvent was dried over sodium sulfate and evaporated in rotavapor. Rf = 0.50 in 3% MeOH-DCM. Purity of the compound was checked by HPLC and it was 92%: tR = 7.79 min in gradient mixture of MeOH and H2O.

1H NMR (400.0 MHz, CDCl3) δ 9.72 (s, 1H), 9.55 (s, 1H), 8.76 (s, 1H), 5.87 (s, 2H), 5.44 (d, J = 18.8 Hz, 1H), 5.29 (d, J = 19.6 Hz, 1H), 4.93 (m, 1H), 4.88 (m, 1H), 4.47 (m, 2H), 4.32 (m, 2H), 4.11 (m, 4H), 3.85 (m, 3H), 3.79 (m, 3H), 3.70 (t, J = 4.8 Hz, 2H), 3.60 (m, 5H), 3.55 (m, J = 4.8 Hz, 3H), 3.48 (m, 11H), 3.40 (m, 2H), 3.36 (s, 3H), 3.30 (s, 3H), 3.28 (s, 3H), 3.20 (m, 4H), 3.16 (m, 3H), 3.11 (m, 2H), 3.02 (m, J = 4.8 Hz, 2H), 2.27 (m, 1H), 2.16 (m, 1H), 2.09 (m, 2H), 1.86 (m, 2H), 1.75 (m, 7H), -1.58 (br s, 1H). 13C NMR (100.6 MHz, CDCl3) δ 173.0, 172.4, 169.7, 168.8, 166.8, 154.5, 148.9, 145.0, 139.1, 136.6, 136.0, 135.7, 135.5, 135.2, 132.5, 129.4, 123.7, 102.6, 102.0, 98.2, 93.7, 71.9, 71.7, 71.5, 70.7, 70.6, 70.3, 70.1, 70.0, 69.2, 68.8, 65.0, 64.3, 63.5, 59.0, 58.9, 58.7, 56.3, 53.4, 52.9, 49.3, 38.6, 30.9, 29.7, 29.6, 23.0, 19.6, 17.6, 14.1, 12.4, 11.3, 11.1.

HRMS (+ESI) m/z calculated for C54H79N4O16 [M+H]+ 1039.5491, found: 1039.5490

4.3.10 31-Succinate-17,15,13-Chlorin e6-Methoxy Tri(ethylene glycol) Triester (19). Yield 8.0 mg (73.4%). To the 10.0 mg (0.0096 mmol) of 18 in 10 ml of dry dichloromethane under nitrogen atmosphere, 5.65 mg (0.048 mmol) of succinic acid, 4.57 mg (0.024 mmol) of EDC and 2.9 mg (0.024 mmol) of DMAP was added. Reaction was stirred for 36 h under N2 at room temperature. 15 ml dichloromethane was added. Organic layer was washed with water and dried
over sodium sulfate. Organic layer was evaporated and compound was purified by 2% MeOH-DCM. \( R_f = 0.37 \) in 3% MeOH-DCM. Purity of the compound was checked by HPLC and it was 90%: \( t_R = 7.79 \) min in gradient mixture of MeOH and H\(_2\)O in gradient mixture of MeOH and H\(_2\)O.

\(^1\)H NMR (400.0 MHz, CDCl\(_3\)) \( \delta \) 9.73 (s, 1H), 9.58 (s, 1H), 8.80 (s, 1H), 6.46 (s, 2H), 5.44 (d, \( J = 18.4 \) Hz, 1H), 5.29 (d, \( J = 19.6 \) Hz, 1H), 4.92 (m, 2H), 4.48 (m, 2H), 4.30 (t, \( J = 4.8 \) Hz, 2H), 3.80 (m, 6H), 3.70 (t, \( J = 4.8 \) Hz, 2H), 3.62 (s, 3H), 3.55 (m, 4H), 3.51 (s, 3H), 3.47 (s, 3H), 3.45 (m, 4H), 3.36 (m, 8H), 3.23 (s, 3H), 3.11 (s, 3H), 3.07 (m, 2H), 2.99 (t, \( J = 4.8 \) Hz, 2H), 2.88 (m, 2H), 2.82 (m, 2H), 2.75 (m, 2H), 2.69 (m, 2H), 2.48 (m, 2H), 2.29 (m, 4H), 1.86 (m, 2H), 1.74 (dd, \( J = 16.4 \) Hz, 11.6 Hz, 6H), -1.67 (br s, 1H). HRMS (+ESI) m/z calculated for C\(_{58}H_{83}N_4O_{19}\) \([\text{M+H}]^+\) 1139.5652, found: 1139.5634.

4.3.11 **Spacer Alkene-3\(^1\)-Succinate-17\(^3\),15\(^2\),13\(^1\)-Chlorin \( \varepsilon_6 \) Methoxy Tri(ethylene glycol) Triester (20).** Yield 0.0019 (52%). To the 3.0 mg (0.0026 mmol) of 19 in dry dichloromethane in nitrogen atmosphere, 2.0 mg (0.0073 mmol) of spacer alkene alcohol, 1.06 mg (0.0052 mmol) of EDC and 1.0 mg (0.0078 mmol) of DMAP was added. Reaction was stirred for 36 h under N\(_2\) at room temperature. 15 ml dichloromethane was added. Organic layer was washed with water and dried over sodium sulfate. Organic layer was evaporated and compound was purified by 1.5% MeOH-DCM. \( R_f = 0.64 \) in 3% MeOH-DCM. Purity of the compound was checked by HPLC and it was 91%: \( t_R = 9.24 \) min in gradient mixture of MeOH and H\(_2\)O.

\(^1\)H NMR (400.0 MHz, CDCl\(_3\)) \( \delta \) 9.73 (s, 1H), 9.58 (s, 1H), 8.80 (s, 1H), 7.32 (d, \( J = 8.4 \) Hz, 2H), 7.01 (d, \( J = 8.4 \) Hz, 2H), 6.97 (d, \( J = 8.4 \) Hz, 2H), 6.71 (d, \( J = 8.4 \) Hz, 2H), 6.45 (s, 2H), 5.91 (d, \( J = 3.2 \) Hz, 1H), 5.71 (d, \( J = 3.2 \) Hz, 1H), 5.44 (d, \( J = 18.8 \) Hz, 1H), 5.27 (d, \( J = 18.4 \) Hz, 1H), 4.89 (m, 4H), 4.61 (s, 2H), 4.47 (m, 2H), 4.33 (m, 2H), 4.14 (m, 5H), 3.83 (m, 3H), 3.77
(m, 3H), 3.70 (t, J = 4.8, 4H), 3.62 (m, 5H), 3.56 (m, 10H), 3.50 (s, 3H), 3.43 (m, 3H), 3.36 (s, 3H), 3.33 (s, 3H), 3.29 (s, 3H), 3.21 (m, 7H), 3.14 (t, J = 4.8 Hz, 2H), 2.80 (m, 2H), 2.74 (m, 2H), 2.55 (m, 1H), 2.19 (m, 3H), 1.73 (m, 7H), -1.40 (br s, 1H), -1.65 (br s, 1H). 13C NMR (100.6 MHz, CDCl3) δ 173.5, 173.0, 172.4, 172.2, 172.0, 169.5, 168.7, 167.1, 157.0, 156.7, 145.0, 138.6, 136.7, 136.2, 135.6, 135.5, 135.2, 134.0, 130.7, 129.8, 129.7, 129.6, 128.4, 127.6, 123.9, 116.1, 115.8, 101.9, 98.3, 94.0, 71.9, 71.8, 71.5, 70.7, 70.6, 70.5, 70.4, 70.1, 69.2, 68.9, 66.8, 66.0, 65.0, 64.7, 64.3, 63.5, 59.0, 58.9, 58.8, 57.6, 53.4, 53.0, 49.2, 38.7, 38.6, 34.0, 31.0, 30.4, 29.7, 29.6, 29.3, 29.2, 28.9, 24.4, 23.8, 23.0, 22.9, 19.6, 17.7, 14.1, 14.0, 12.4, 11.3, 11.0.

HRMS (+ESI) m/z calculated for C74H97N4O22 [M+H]+ 1393.6594, found: 1393.6592.

4.3.12 Fluoroalkyl Silane Modified Glass. Twelve pieces of Vycor (ea. 0.41 g) were added to the 5.52 g (15.9 mmol) of nonafluorohexatrimethoxy silane in 30 ml toluene and refluxed for 24 h under N2. Any nonafluorohexatrimethoxy silane, which was not covalently attached, was washed away by soxhlet extraction in methanol for 24h. Evidence of covalent attachment of nonafluorohexatrimethoxy silane was found by FTIR, mass and NMR (after dissolution of the glass).1

4.3.12 Chlorin e6-TriPEG Ester Modified Fluorinated Glass. To the 18.0 mg (0.013 mmol) of 20 in dry THF, 3-iodopropyltrimethoxy silane (0.50 mmol), NaH (0.015 mmol) was added in 100 ml round bottom flask and refluxed for 24 h under nitrogen atmosphere. THF was evaporated by purging nitrogen in the reaction mixture. To the solid residue, 30 ml of dry toluene and twelve pieces (average weight 0.41g) of fluoroalkyl silane loaded (1.45 mmol/g)1 porous Vycor glass cap were added. Reaction mixture was refluxed for 24 h under nitrogen atmosphere. Glass turned into green color, which was washed with several solvents (DCM, THF, MeOH,
toluene, hexane) and soxhlet extracted using methanol for 24 h to remove any physically adsorbed sensitizer.

No fluorosilane or sensitizer leaching was observed from the solids after soxhlet extraction in methanol. After dissolution of solid 22 by the HF treatment, mass spectra was taken. Peak observed in GC/MS was tentatively assigned to (i) HOCH$_2$C$_6$H$_4$OCH=CHOC$_6$H$_4$CH$_2$OCH$_2$CH$_2$CH$_2$SiF$_3$ based on MS (+ESI) calculated for C$_{19}$SiH$_{30}$O$_4$F$_3$ = 398.12, found = 399.25 and (ii) HOCH$_2$C$_6$H$_4$OCH=CHOC$_6$H$_4$CH$_2$OCH$_2$CH$_2$CH$_2$Si(OH)$_3$ based on MS (+ESI) calculated for C$_{19}$H$_{24}$O$_7$Si = 392.12, found = 391.28.

4.3.13 Determination of Loading of the Sensitizer. Amount of covalently attached sensitizer (loading) to the porous Vycor glass was determined by HF stripping method. 22 was placed in 50% HF for 24 h. Suspended dye in aqueous solution was extracted into CHCl$_3$. Amount of loading of the sensitizer was calculated from the calibration plot (calibration plot of absorbance of soret band and known concentration of 20 in CHCl$_3$ was generated). Amount of loaded sensitizer was calculated by the above mentioned procedure is 40 nmol in 0.42g cap or 90 nmol/g of silica.

4.3.14 Photocleavage of Chlorin e$_6$-TriPEG Ester Modified Glass. In the 800 µl n-butanol, 0.335 g cap was dipped in a 7.4 cm test-tube (diameter 1.0 cm) and oxygen was bubbled and laser light (Power 2.5 Amp) was irradiated from 7.4 cm distance continuously. Photocleavage was checked every 15 min until it reached a saturation point at 75 min. The amount of photocleavage was determined from the calibration of 20 in n-butanol by monitoring the soret absorption band of dye.

4.3.15 Homogeneous Photooxidation of Spacer Alkene Conjugated Chlorin e$_6$-triPEG Ester 20. 0.71 mM oxygen saturated acetone-$d_6$ solution of 20 was irradiated by 669 nm red laser light
for 25 minutes. Photocleavage of the compound was confirmed by mass spectra. The mass spectra revealed an intense peak for the expected photocleaved compound 24. HRMS (+ESI) m/z calculated for $C_{66}H_{89}N_4O_{21}$ [M+H]$^+$ 1273.6014, found: 1273.6018.
Fig. 4. $^1$H NMR (400 MHz) of 7-formyl rhodin G7 trimethyl ester 1 in CDCl$_3$. 
Fig. 5. $^{13}$C NMR (100.6 MHz) of 7-formyl rhodin G7 trimethyl ester 1 in CDCl$_3$. 
Fig. 6. HPLC of 7-formyl rhodin G7-trimethyl ester 1 was carried out in gradient mixture of 90% and 98% MeOH in H₂O for 20 minutes each respectively.
Fig 7. HRMS (+ESI) of 7-formyl rhodin G7-trimethyl ester 1
Fig. 8. $^1$H NMR (400 MHz) of 7'-hydroxyl rhodin G7-trimethyl ester 2 in CDCl$_3$. 
Fig 9. $^{13}$C NMR (100.6 MHz) of 7-hydroxyl rhodin G7-trimethyl ester 2 in CDCl$_3$
Fig 10. HPLC of $^1$-hydroxyl rhodin G7-trimethyl ester 2 was carried out in gradient mixture of 90% and 98% MeOH in H2O for 20 minutes each respectively.
Fig 11. HRMS (+ESI) of 7-hormyl rhodin G7-trimethyl ester 2
Fig 12. $^1$H NMR (400 MHz) (Z)-1,2-Bis(4-bromomethylphenoxy) ethene 7
Fig 13. $^1$H NMR (400 MHz) (Z)-1,2-Bis(4-iodomethyl)phenoxo) ethene 8
Fig 14. $^{13}$C NMR (100.6 MHz) (Z)-1,2-Bis(4-iodomethylphenoxy) ethene 8
Fig 15. $^1$H NMR (400 MHz) of Chlorin e$_6$-trimethyl ester 9 in CDCl$_3$. 
Fig 16. HPLC of chlorine e₆-trimethyl ester 9 was carried out in gradient mixture of 95% and 98% MeOH in H₂O for 10 minutes each respectively.
Fig 17. $^1$H NMR (400 MHz) of 3'-propanediol-chlorin e$_6$-trimethyl ester 10 in CDCl$_3$
Fig 18. HPLC of $3^1$-propanediol-chlorin e$_6$-trimethyl ester 10 was carried out in gradient mixture of 95% and 98% MeOH in H$_2$O for 10 minutes each respectively.
Fig 19. MS (+ESI) of 3\textsuperscript{1}-etherate propanol-chlorin e\textsubscript{6}-trimethyl ester 10
Fig 20. $^1$H NMR (400 MHz) of 3$^{1}$-tosyl propanol-chlorin $e_6$-trimethyl ester 11 in CDCl$_3$. 
Fig 21. $^{13}$C NMR (100.6 MHz) of $3^1$-tosyl propanol-chlorin e$_6$-trimethyl ester 11 in CDCl$_3$
**Fig 22.** $^{13}$C NMR (400 MHz) 3-Formyl-17$^{3},15^{2},13^{1}$-Chlorin e$_6$ Methoxy Tri(ethylene glycol) Triester 17
Fig 23. $^{13}$C NMR (100.6 MHz)3-Formyl-17$^3$,15$^5$,13$^1$-Chlorin e$_6$ Methoxy Tri(ethylene glycol) Triester 17
Fig 24. HPLC of 3-Formyl-17\textsuperscript{3}, 15\textsuperscript{2}, 13\textsuperscript{1}-chlorin e\textsubscript{6}-methoxy tri(ethylene glycol) triester 17 in was carried out in gradient mixture of 90% and 98% MeOH in H\textsubscript{2}O for 10 minutes each respectively.
Fig 25. Blue line is UV-Vis spectrum of Chlorin \( e_6 \)-triPEG ester 16. Red line is UV-Vis spectrum of 3-formyl chlorin \( e_6 \)-triPEG ester 17 in CHCl\(_3\).
Fig 26: HRMS (+ESI) of 17

Measured Mass: 1037.5357

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Fig 27. $^1$H NMR (400 MHz) 3$^1$-hydroxyl-17$^2$,15$^2$,13$^1$-Chlorin e$_6$ Methoxy Tri(ethylene glycol) Triester 18
Fig 28. $^{13}$C NMR (100.6 MHz) of $^{3^1}$-hydroxyl-$17^3, 15^2, 13^1$-chlorin e$_6$-methoxy tri(ethylene glycol) triester 18 in CDCl$_3$
Fig 29. HPLC of $^{31}$-Hydroxyl-17$^3$, 15$^2$, 13$^1$-chlorin e$_6$-methoxy tri(ethylene glycol) triester 18 in was carried out in gradient mixture of 90% and 98% MeOH in H$_2$O for 10 minutes each respectively respectively.
**Fig 30.** Blue line is UV-Vis spectrum of 3\textsuperscript{1}-hydroxyl chlorin e\textsubscript{6}-triPEG ester 18. Red line is UV-Vis spectrum of 3-formyl chlorin e\textsubscript{6}-triPEG ester 17 in CHCl\textsubscript{3}. 

![UV-Vis spectra](image-url)
Fig 31. HRMS (+ESI) of 18

Measured Mass: 1039.549

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Fig 32. $^1$H NMR (400 MHz) of 3$^1$-succinate-17$^3$, 15$^2$, 13$^1$-chlorin e$_6$-methoxy tri(ethylene glycol) triester 19 in CDCl$_3$
Fig 33. HPLC of 3'-succinate-17', 15', 13'-chlorin e₆-methoxy tri(ethylene glycol) triester 19 in gradient mixture of 90% and 98% MeOH in H₂O for 10 minutes each respectively.
**Fig 34.** HRMS (+ESI) of 19

Measured Mass: 1139.5634

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Fig 35. $^1$H NMR (400 MHz) 3-methanol succinate-spacer alkene-17,15,13-chlorin e$_6$ methoxy tri(ethylene glycol) triester 20
Fig 36. $^{13}\text{C}$ NMR (100.6 MHz) 3-methanol succinate-spacer alkene-$17^3,15^2,13^1$-chlorin e$_6$ methoxy tri(ethylene glycol) triester 20
Fig 37. 2D HSQC of compound 20 showed that 8 phenyl proton (red color) coupled with 3 carbon within 129.8-129.6 and 2 carbon at 102.7 and 101.9. 2 olefinic proton (black color) couples with 2 carbon at 128.4 and 127.6.
Fig 38. HPLC of Spacer alkene-3¹-Succinate-17³, 15², 13¹-chlorin e₆-methoxy tri(ethylene glycol) triester 20 was carried out in gradient mixture of 90% and 98% MeOH in H₂O for 10 minutes each respectively.
Fig 39. HRMS (+ESI) of 20

Measured Mass: 1393.6592

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Fig 40. HRMS (+ESI) of photocleaved dye 24
Fig 41. MS (+ESI) of chloroform extract of dissolute glass in aqueous HF.
4.5 Conclusion

Synthesis of spacer alkene-3\textsuperscript{1}-succinate-17\textsuperscript{3}, 15\textsuperscript{2}, 13\textsuperscript{1}-chlorin \textit{e}_6-methoxy tri(ethylene glycol) triester was achieved in six steps. In order to achieve this synthesis, 3\textsuperscript{1}-3\textsuperscript{2} vinyl bond of chlorin \textit{e}_6 was utilized to install 3-formyl, which was reduced to alcohol and coupled with linker (succinic acid) to attach with spacer alkene. Formylation was carried out in mild acidic condition in presence of OsO\textsubscript{4} and NaIO\textsubscript{4} to avoid dePEGylation.

Chlorin-triPEG was successfully attached to the fluorinated silica cap and succinate-PTFE-PVA (succinate-polytetrafluoroethylene-polyvinyl alcohol) polymer to evaluate photocleavage, oxygen solubility and surface repelling property of two surfaces. Synthesis and photocleavage results of chlorin \textit{e}_6-triPEG modified succinate-PTFE-PVA polymer probe tip have not been mentioned here.
4.6 References


Bibliography

Chapter 1.


**Chapter 2.**


Chapter 3.


Chapter 4.


