Apr 24th, 2:50 PM - 3:05 PM

Synthesis of ZnTPP-IL as a Potential Photodynamic Therapy Agent

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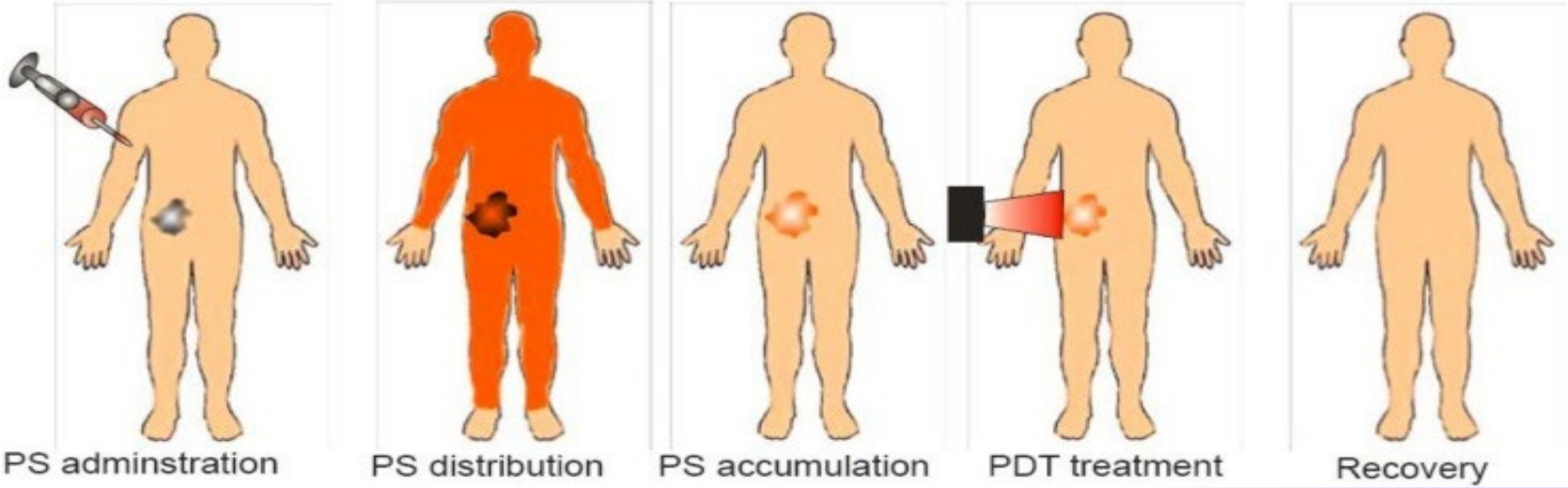
Outline

What is Photodynamic Therapy?



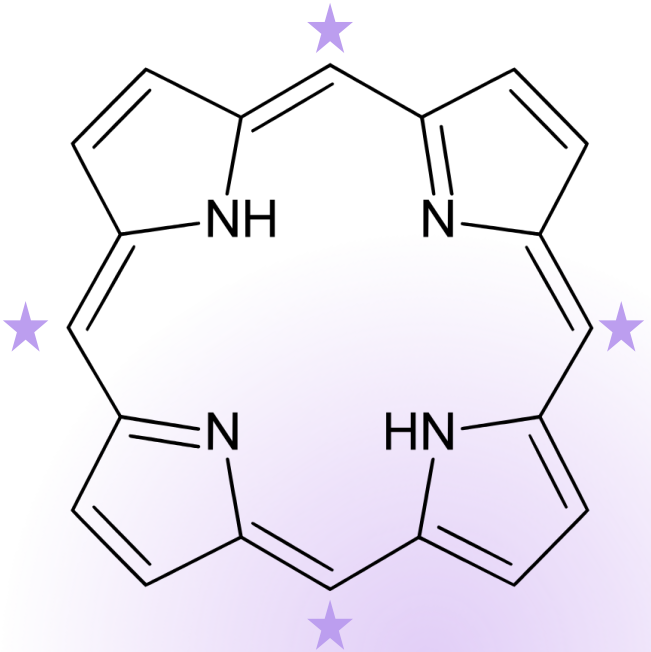
Miller, Jennifer. Photodynamic Therapy: The Sensitization of Cancer Cells to Light. Journal of Chemical Education, 76, 5. (1999, 592-594)

Photosensitizers- Porphyrins

PDT based on idea that photosensitizer will absorb certain photon of light

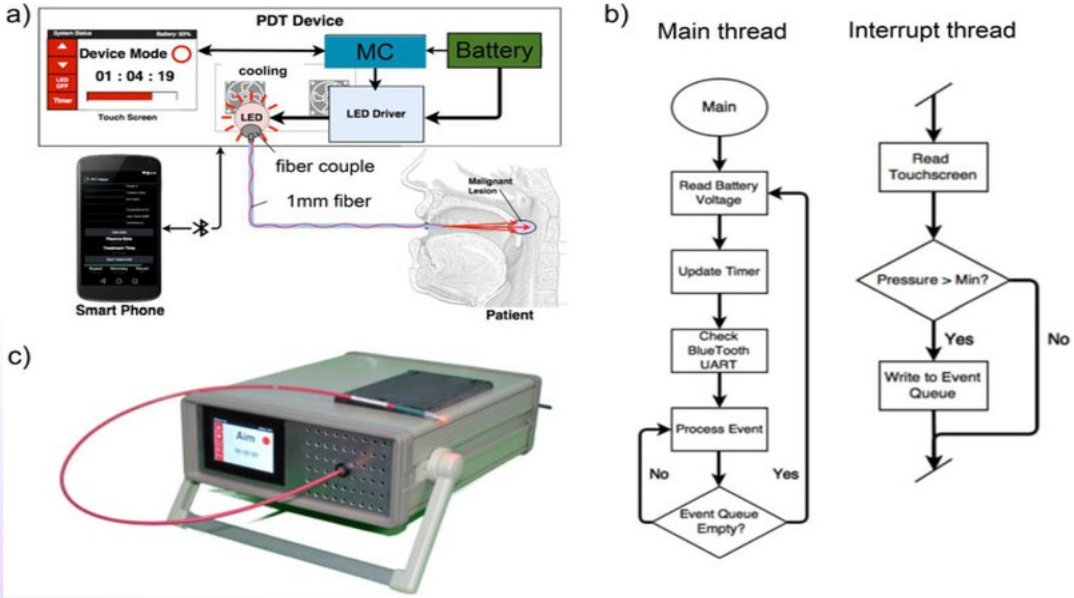
Photosensitizer: any molecule that uses radiant energy or light to elicit a specific response

Porphyrins: macrocyclic structures that differ in substituents and central metal atom



Michael R. Detty, Scott L. Gibson, Stephen J. Wagner. Current Clinical and Preclinical Photosensitizers for Use in Photodynamic Therapy. Journal of Medicinal Chemistry V. 47, 16. (July 2004, 3897-3911)

Considerations for PDT in rural/medically underserved areas



Portable PDT

- Portable, battery-operated, LED device for PDT treatment.
- Solves problem of electricity
- Continuity of care locally or through telemedicine if applicable

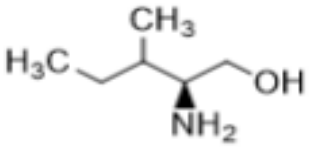
Hui Liu, Liam Daly, Grant Rudd, et al. Development and Evaluation of a Low-Cost, Portable, LED-Based Device for PDT Treatment of Early-Stage Oral Cancer in Resource-Limited Settings. *Lasers Surg Med*, 51(4). (2019). Doi:10.1002/lsm.23019

Project goals

To synthesize a novel porphyrin that is:

- Water soluble
- Minimally toxic to cells in the absence of light

Selection of Isoleucinol



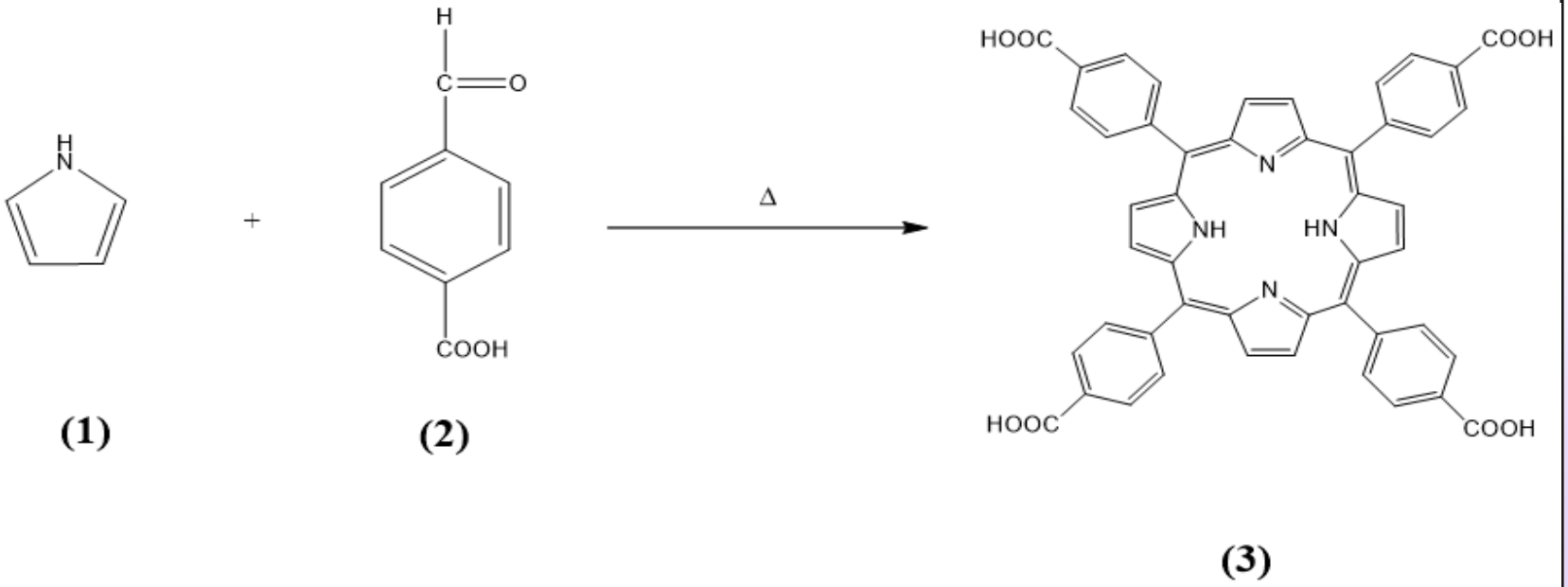
Isoleucinol is a derivative of amino acid, isoleucine

Isoleucinol:

1. Thought to have low cytotoxicity in healthy cells
2. High solubility in water due to alcohol and amine groups

Synthesis of ZnTPP- IL

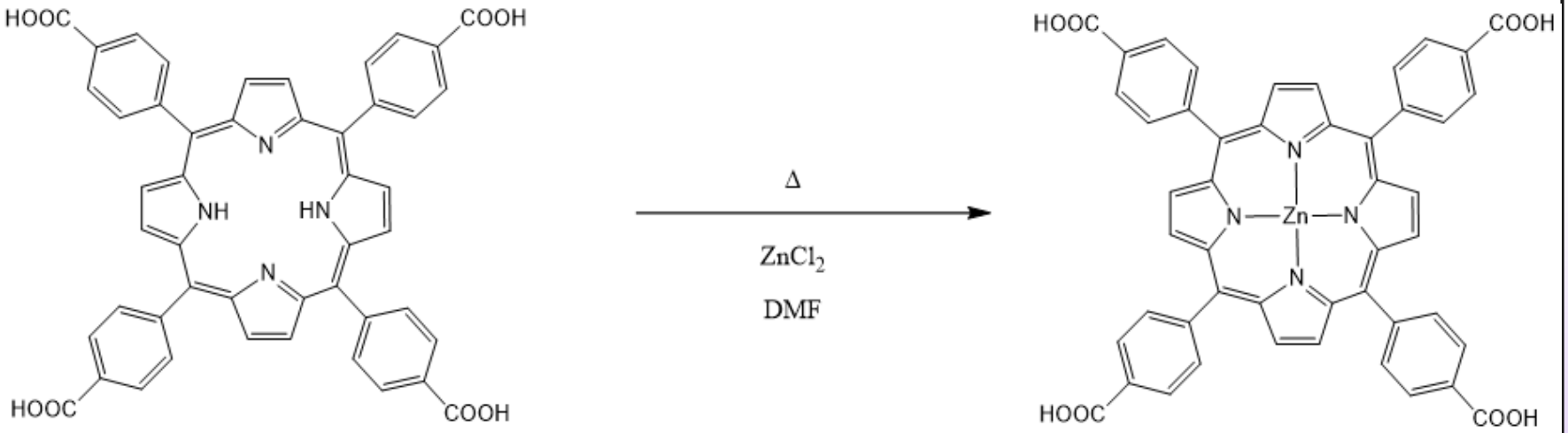
Reaction 1- reaction of pyrrole (1) and 4-formylbenzoic acid (2) to create H_2TPPC (3)



(1) (2) (3)

Synthesis of ZnTPP- IL

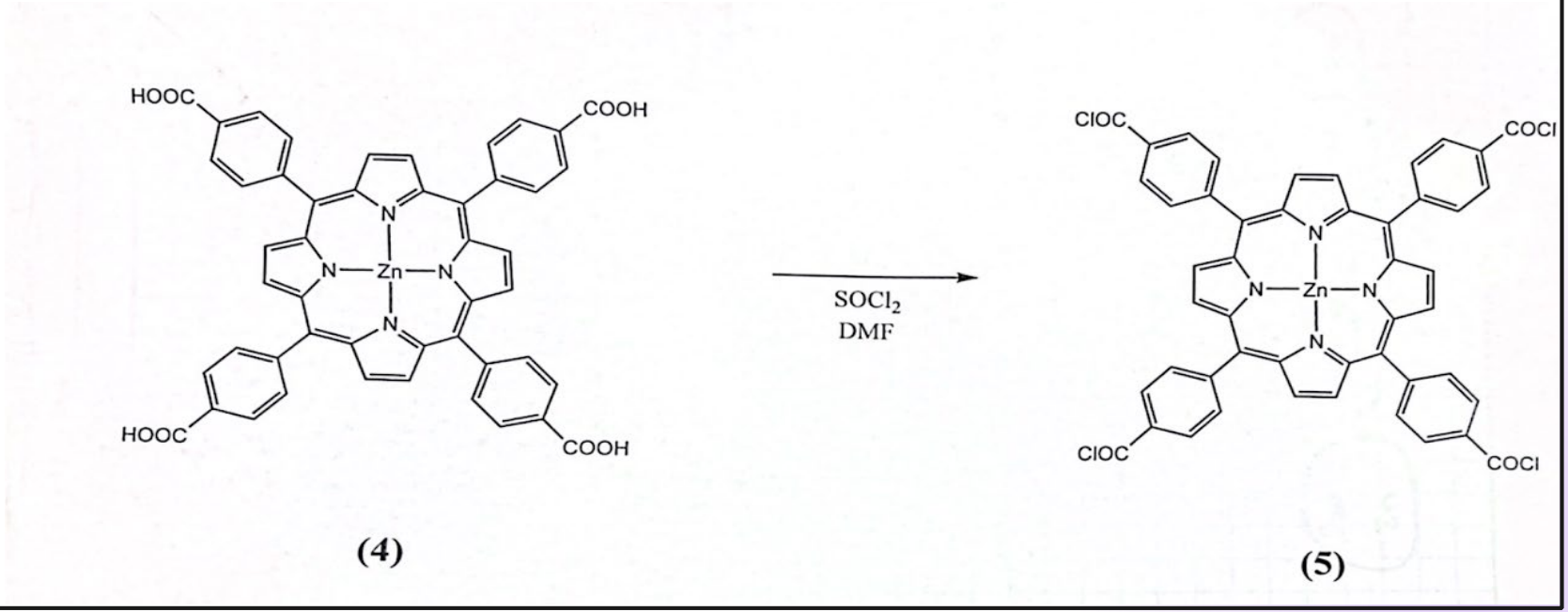
Reaction 2- reaction of H_2TPPC (3) and zinc chloride in DMF solution to create ZnTPPC (4)



(3) (4)

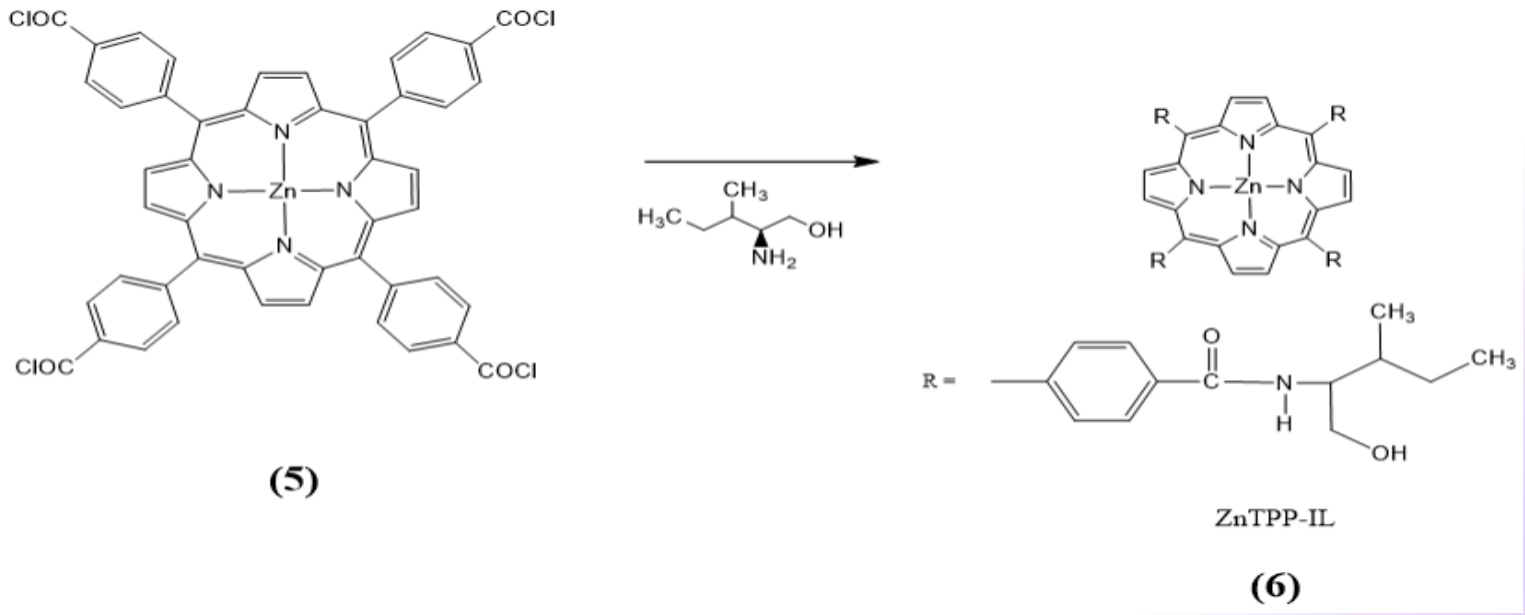
Synthesis of ZnTPP-IL

Reaction 3- reaction of ZnTPPC (4) and $SOCl_2$ in DMF solution to create acid chloride intermediate (5)



Synthesis of ZnTPP-IL

Reaction 4- reaction of acid chloride intermediate (5) and isoleucinol in DMF solution to create ZnTPP-IL (6)



(5)

ZnTPP-IL

(6)

Purification

Syringe Filtration

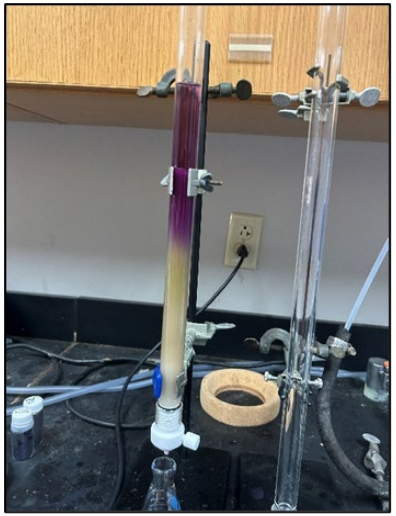
To remove large impurities in solution

Sephadex G-50

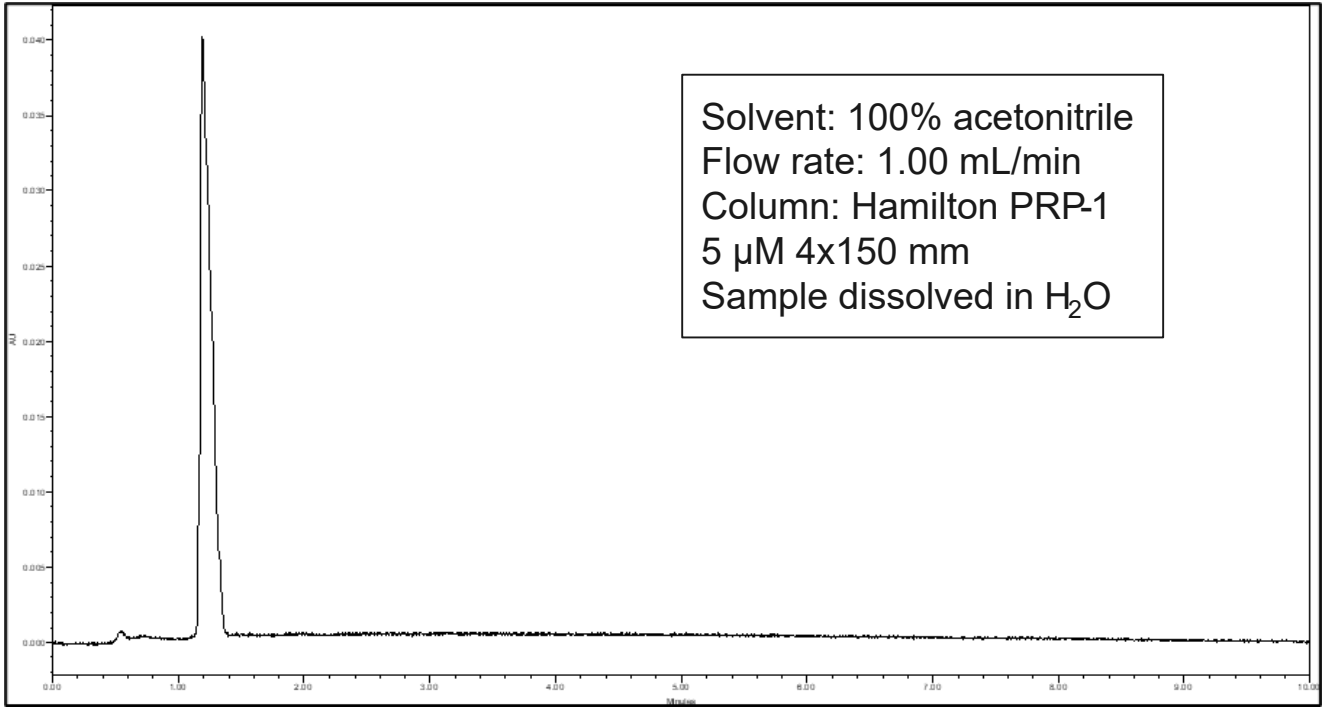
Size exclusion

Sephadex LH-20

Based on lipophilicity, polarity
Excess amine eluted first

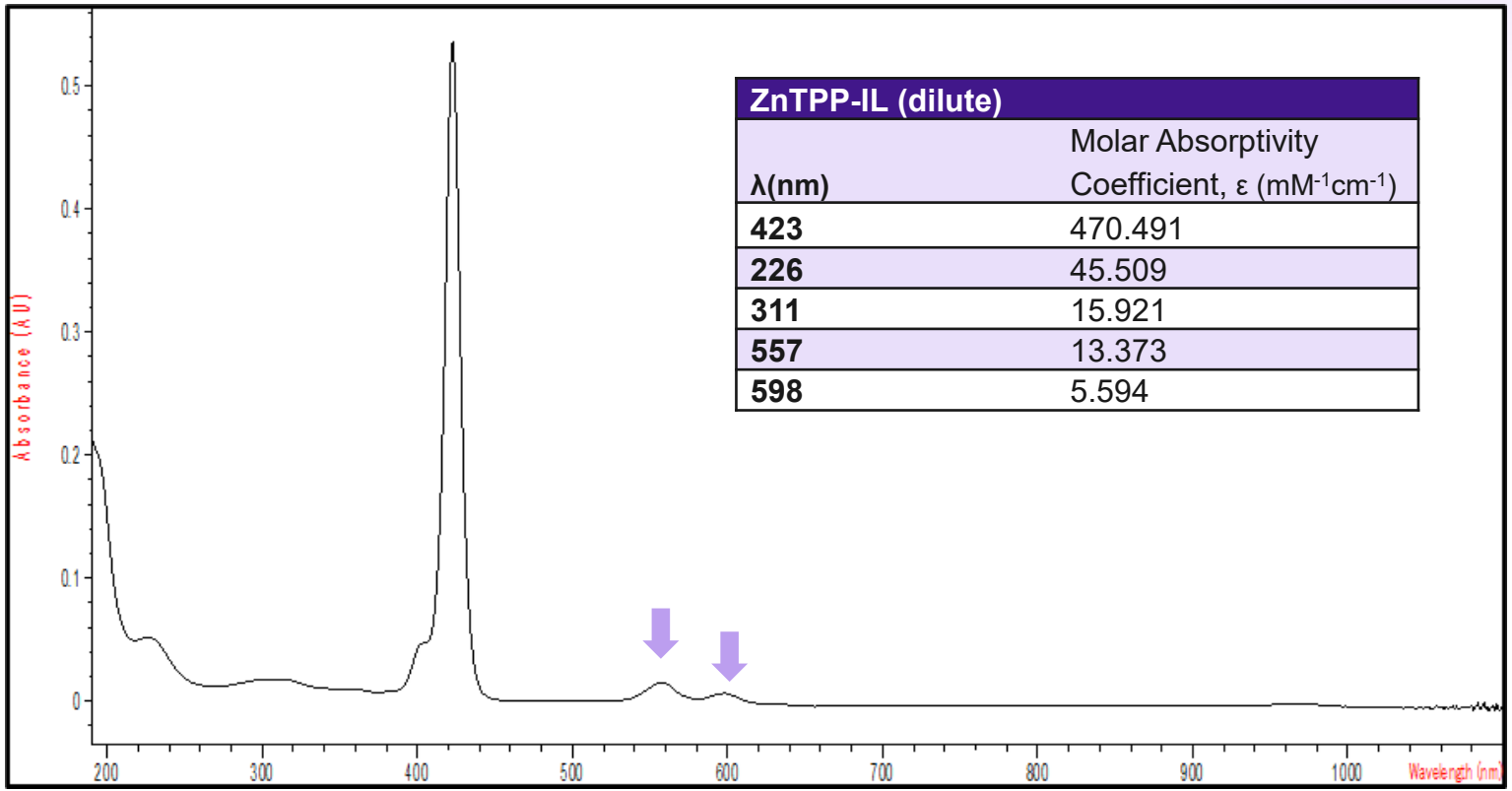


HPLC- 96% purity



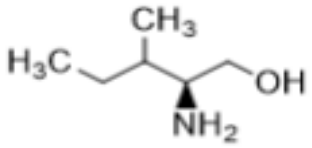
Solvent: 100% acetonitrile
Flow rate: 1.00 mL/min
Column: Hamilton PRP-1
5 µM 4x150 mm
Sample dissolved in H_2O

UV- Vis

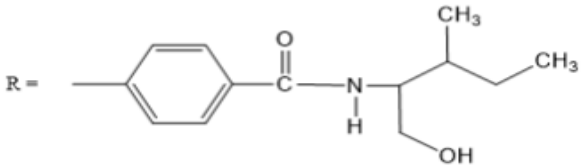


ZnTPP-IL (dilute)	
λ(nm)	Molar Absorptivity Coefficient, ε ($mM^{-1}cm^{-1}$)
423	470.491
226	45.509
311	15.921
557	13.373
598	5.594

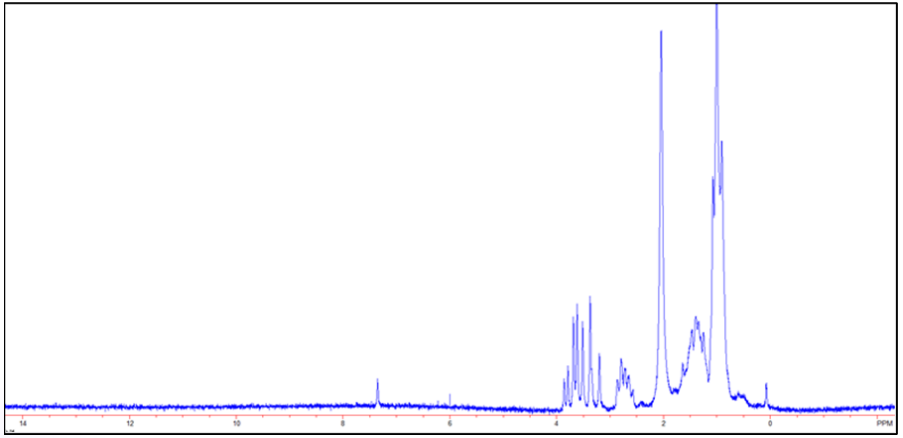
NMR

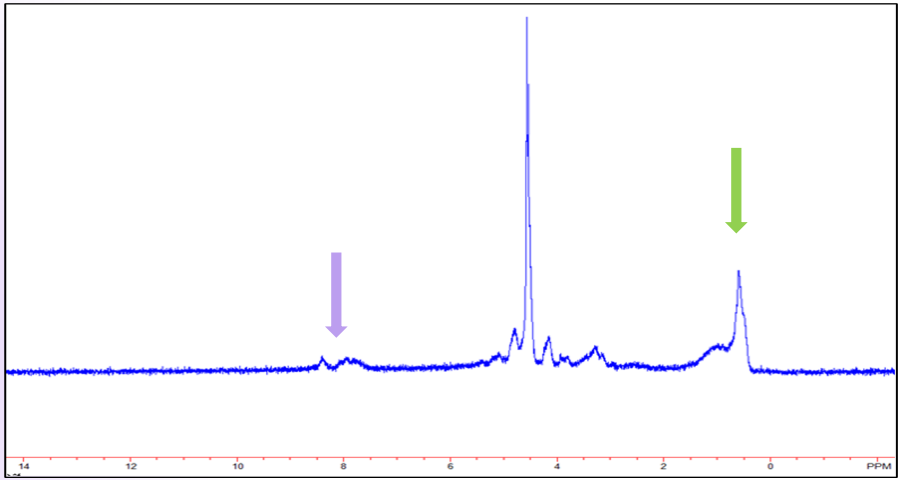

CH3
H3C
OH
NH2


R
R
N
N
Zn
N
N
R
R

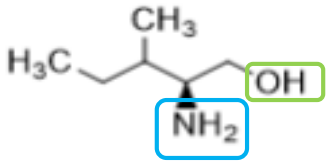

R =
O
C
N
H
CH3
CH3
OH

ZnTPP-IL

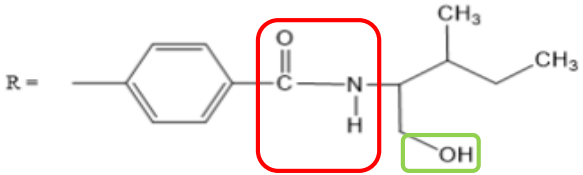

14
12
10
8
6
4
2
0
PPM


14
12
10
8
6
4
2
0
PPM

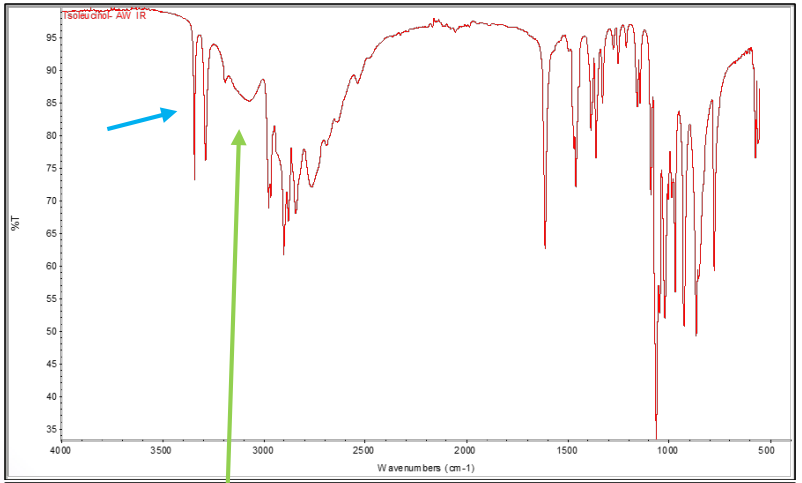
IR

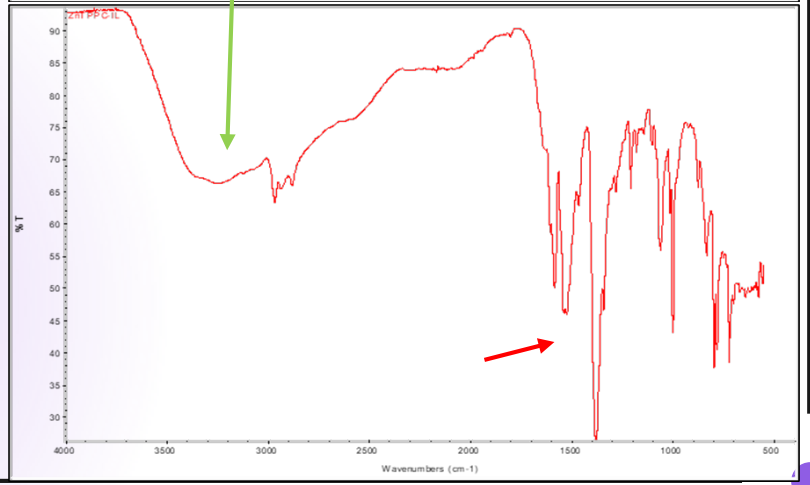

CH3
H3C
OH
NH2


R
R
N
N
Zn
N
N
R
R

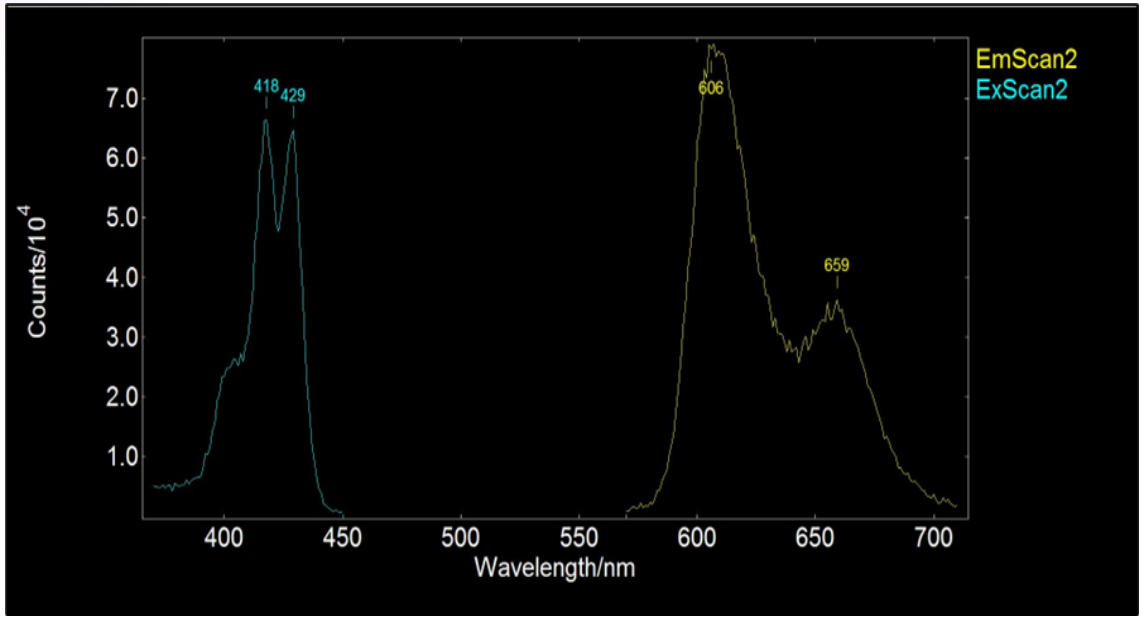

R =
O
C
N
H
CH3
CH3
OH

ZnTPP-IL


Isoleucinol- AW IR
%T
Wavenumbers (cm-1)


ZnTPPCIL
%T
Wavenumbers (cm-1)

Fluorescence Spectroscopy



ROS Assay

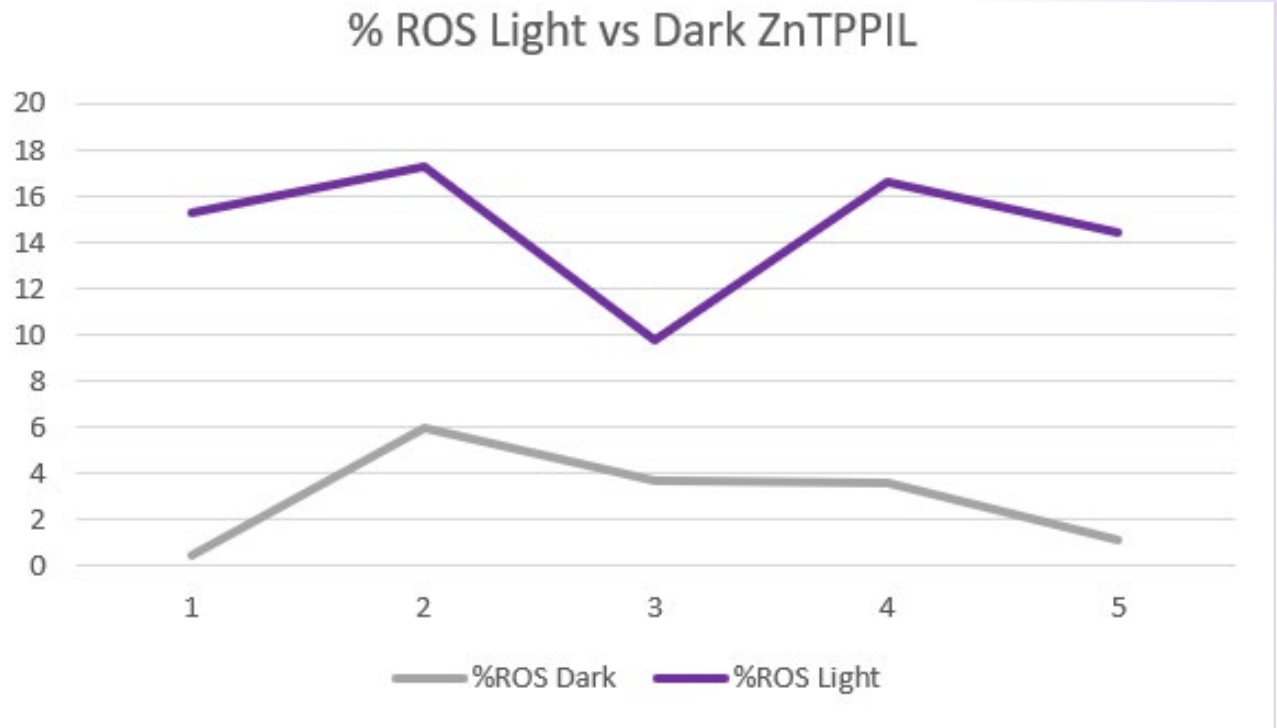
Why?

Photosenstizer will absorb a photon of energy and transfer that energy to oxygen. The photosensitizer becomes excited and reacts with ground state oxygen to produce singlet oxygen.

Methods

- NIH 3T3 fibroblast cells were plated and porphyrin was added to cells in concentrations of 1, 3, 10, 30, and 100 μM
- After incubation, plates exposed to white light for 22 minutes or kept in the dark
- 10 μM CDCHF-DA in HBS was added before using microplate reader

ROS Assay Results



MTT Assay

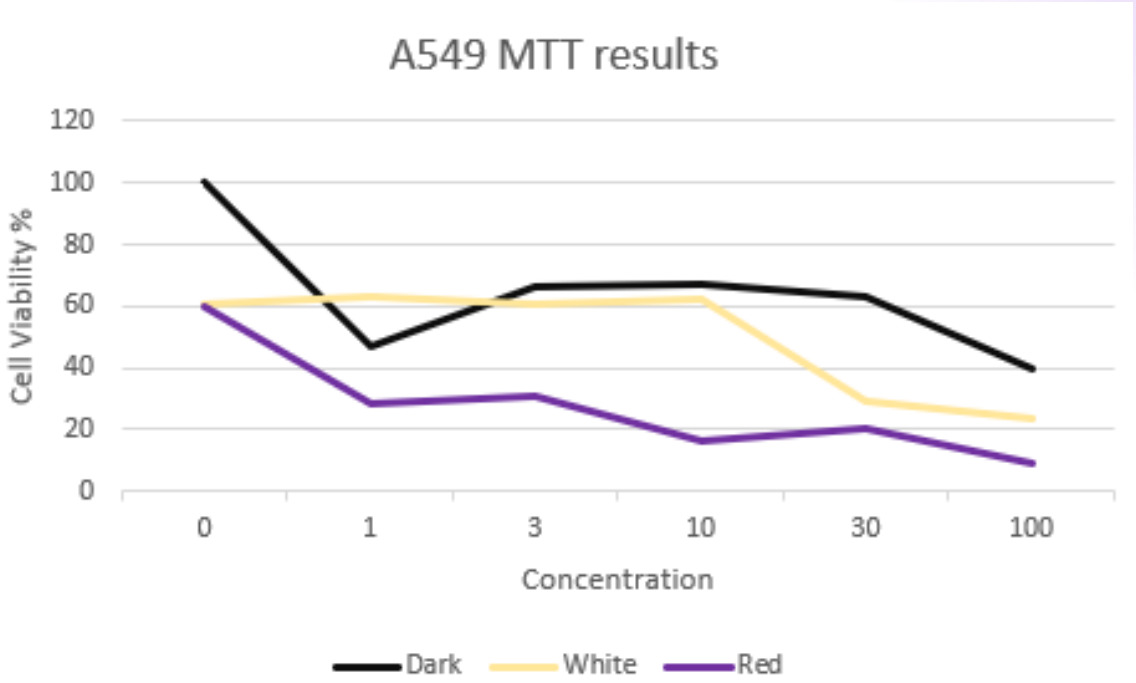
Goal:

Determine LD_{50} in both red and white light conditions

Methods

- A549 lung cancer cells plated in 96 well plate. Porphyrin added in concentrations of 1, 3, 10, 30, 100 μM.
- Plates exposed to red or white light for 22 minutes or kept in dark

MTT Assay Results



Conclusions

- ZnTPP-IL was successfully synthesized, purified, and retained internal structure
- Minimal toxicity when kept in the dark at low concentrations
- LD_{50} of 1.0 μM for red and 30.0 μM for white light conditions

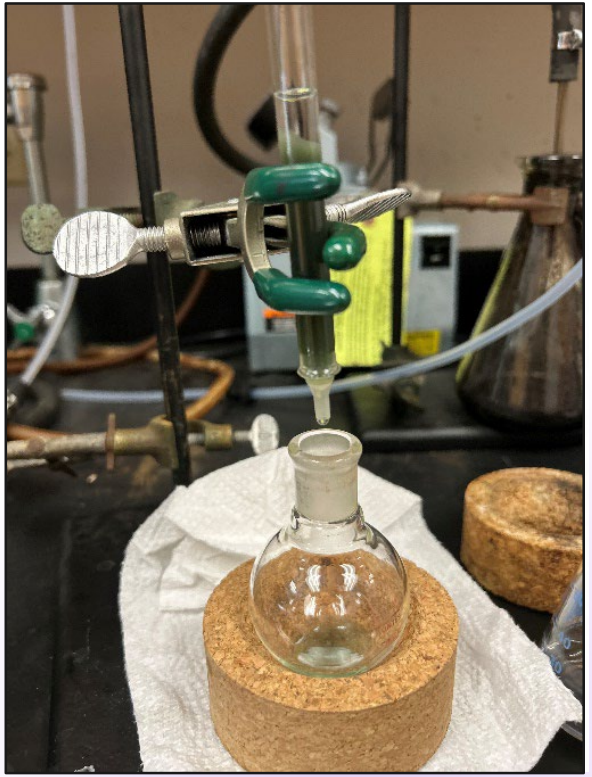
Future Work

- Further cytotoxicity testing with a wider concentration range and/or light exposure times and/or hypoxic conditions
- *In-vivo* testing to examine tumor recurrence
- Cyanine porphyrin synthesis and testing

Cyanine Porphyrin

Synthesized according to same 4 reactions
Purified with G-50 and LH-20
Cyanine compound is dark green dye

- Increased tumor penetrating depth



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J.D. Patterson School of Natural Sciences

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Questions?