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Progress Towards A Facile Synthesis of a Gadolinium (III) Octabrominated Porphyrin

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Abstract
Towards preparing porphyrin complexes that combine contrast agent and photodynamic therapy capabilities at tumor sites, the Gd(III) porphyrin derivative of the octabrominated *meso*-tetrakis(N-methyl-4-pyridyl)porphyrin, $GdTMPyPBr_8^{5+}$ was synthesized by refluxing Gd(III) ion with $H_3TMPyPBr_8^{5+}$ at pH 7. The metallocomplex was characterized by UV-visible spectroscopy and showed a Soret band shift from 503 nm to 463 nm at pH 7, suggesting that metal insertion of Gd(III) into the core of porphyrin ligand had occurred. However, synthesis of the Gd(III) complex required high temperatures and long reaction times. To circumvent these harsh conditions, the lithium porphyrin derivative was first prepared and subsequently combined with Gd(III) ion to yield the desired product at room temperature.

INTRODUCTION

Cancer is the number two killer in the U.S. just after heart disease. Recent statistics show that 1 in 4 deaths in the United States are due to cancer.[1] Current treatments for cancer and harmful tumors include chemotherapy, radiation, and surgery. However, these treatments are invasive in nature and may produce a number of negative side effects. Photodynamic therapy (PDT), a relatively new mode for cancer treatment, combines a light source and a photosensitizing agent to destroy targeted cells through a photodynamic effect. The photosensitizer is an organic compound that is activated when exposed to light as demonstrated in Figure 1.[2]

Photodynamic Therapy

Figure 1 shows that exposure of the photosensitizer to light of appropriate wavelengths causes energy transitions from the electronic ground state (S_0) to excited singlet energy states (S_1, etc). The fate of this excited energy depends on the photosensitizer - energy can be emitted as fluorescence (k_f) and in some cases, intersystem crossing (ISC) to triplet excited energy states can result in phosphorescence (k_p). The goal of PDT is for the transfer of excitation energy to molecules such as oxygen to form singlet oxygen ($^1O_2$) or other reactive species ($\bullet O^{2-}$) that are cytotoxic to cancer and tumor cells.[2]

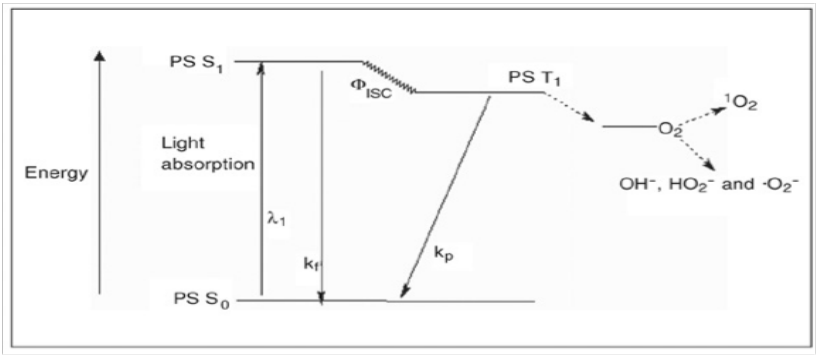


Figure 1. Photosensitization of oxygen represented as a Jablonski diagram

The photosensitizing agent in PDT is a critical component of the treatment. Consequently, porphyrins are currently under intense investigation as photosensitizing agents.

Porphyrins and Photodynamic Therapy

Porphyrins are cyclic organic compounds that possess four alternating pyrrole rings and two acidic core protons.[3] From a structural perspective, porphyrins are derived from the parent porphyrin "porphine" that is comprised of four alternating pyrrole rings with four methane bridges (Figure 2). Porphyrins consist of 22 π-electrons and 18 contribute to the aromatic system. Porphyrins are highly conjugated systems that strongly absorb visible light. These aromatic macrocyles exhibit a diagnostic optical spectrum due to π-π* transitions. At approximately 400 nm, porphyrins exhibit an intense Soret band (extinction coefficients ~ 10^5 $cm^{-1}M^{-1}$) and less intense Q-bands between 500 and 700 nm. A key characteristic of porphryin is that the conjugated structure allows for substitution at the β- and γ-carbons. In addition, metalloporphyrins can be prepared by substitution of metal ions for porphyrin's core protons.

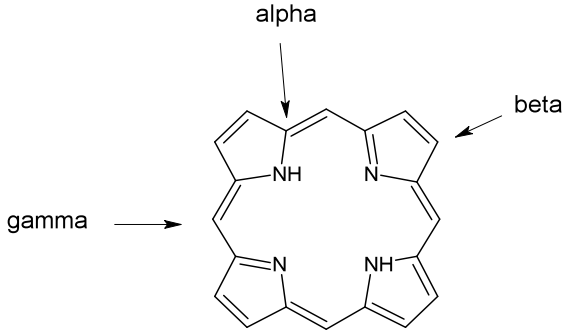


Figure 2. Porphine structure

Our interest in porphryins centers on their application as contrast agents in magnetic resonance imaging (MRI) and their potential as photosensitizers in the photodynamic therapy of tumor and cancer cells. Porphyrins absorb visible light strongly and at long wavelengths (> 600 nm) necessary for penetration of photons through deep tissue, they have demonstrated selective accumulation in tumors, low level or dark toxicity, and general lack of severe side effects and disfigurement of patients.[4] At the same time, Photofrin®, the first porphyrin-based PDT treatment to win approval in the U.S., Canada, Japan, and Europe, has one major limitation – it accumulates in the skin for up to six weeks, rendering patients photosensitive especially to strong sunlight.[5] Plus, the longest wavelength at which Photofrin® can be photo-activated is 630 nm which is within the general absorption profile of hemoglobin, an abundant pigment in blood. This limits Photofrin's® therapeutic application.

Porphyrins and Magnetic Resonance Imaging Gadolinium(III) Contrast Agents
At present, four non-porphyrinic gadolinium(III) MRI contrast agents have been approved for clinical use since the gadolinium(III) ion provides high quality enhancement of internal images in the human body.[6] However, Gd(III) contrast agents have been linked to negative health issues such as Nephrogenic Systemic Fibrosis, a rare disease that causes hardening of skin, joints, eyes, and internal organs.[7] Recent studies suggest that Zn(II), Cu(II), and Ca(II) ions *in vivo* compete for the binding site of the organic ligand to which the Gd(III) ion is bound via a transmetallation reaction that releases the acutely toxic lanthanide ion.[8] However, other reports demonstrate stability of the metal ion in other porphyrin-based ligands.[9]

MOTIVATION
We recently reported[10] the synthesis of the Gd(III) derivative of a novel

octabrominated water-soluble porphyrin, 2,3,7,8,11,12,17,18-octabromo-meso-tetrakis(N-methyl-4-pyridyl)porphyrin, $H_3TMPyPBr_8^{5+}$ because of our interest in combining the tumor accumulation and photosensitizing properties of porphyrins with the contrast agent enhancement capability of Gd(III) ions. While most porphyrins are near planar, the $H_3TMPyPBr_8^{5+}$ exhibited a saddle-shaped structure with a core that appears large enough to form a stable complex with the large ionic radius of the Gd(III) ion (~108 pm). Although we have successfully synthesized the $GdTMPyPBr_8^{5+}$ complex, reaction at pH 7 required long reflux times (100°C, > 24 h) (equation 1), reducing the cost-effectiveness of the product.

$$H_3TMPyPBr_8^{5+} + Gd^{3+} = GdTMPyPBr_8^{5+} + 3\,H^+ \quad (1)$$

Arnold and others[10] have demonstrated the ease of synthesizing lanthanide porphyrin complexes by reaction between lithium porphyrins and lanthanide ions such as ytterbium(III) ions (equation 2). However, manipulation of these materials required air-sensitive environments.

$$2\,Li_2P + Yb^{3+} = YbP_2^- + 4\,Li^+ \quad (2)$$

We were the first to show that a stable mono-lithium complex, $LiTMPyBr_8^{3+}$, could exist in aqueous basic media (equation 3).[11]

$$H_3TMPyPBr_8^{5+} + 2\,Li^+ = (LiTMPyPBr_8^{3+})\,Li^+ + 3\,H^+ \quad (3)$$

Therefore, transmetallation of the lithium complex should provide a pathway for the preparation of the Gd(III) porphyrin complex at room temperature (equation 4).

$$LiTMPyPBr_8^{3+} + Gd^{3+} = GdTMPyPBr_8^{5+} + Li^+ \quad (4)$$

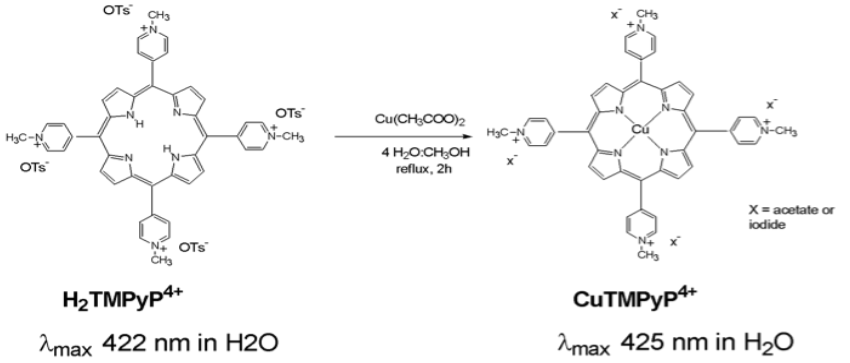
To enhance this reactivity in aqueous and nonaqueous solutions, we recently isolated the air-stable lithium ion porphyrin complex as a red solid at room temperature via metathesis of lithium hexafluorophosphate and the free base octabromoporphyrin.

MATERIALS AND METHODS

Synthesis of $GdTMPyPBr_8^{5+}$ was achieved via reaction of $H_3TMPyPBr_8^{5+}$ and Gd (III) ion using the methods of Richards *et al.*[10] The reaction progress was monitored by UV-visible spectroscopy using a Shimadzu UV2401PC spectrophotometer. *meso*-Tetrakis(N-methyl-4-pyridyl)porphyrin, H_2TMPyP^{4+}, was purchased as the 4-toluenesulfonate (OTs-) salt from Frontier Scientific, Inc. Ammonium hexafluorophosphate (NH_4PF_6), lithium hexafluorophosphate ($LiPF_6$), bromine (Br_2), dimethylformamide (DMF), gadolinium(III) chloride ($GdCl_3$) and acetate ($GdOAc_3$), and tetrabutylammonium chloride (NBu_4Cl) were purchased from Sigma Aldrich. All other reagents, including metal salts, were purchased from Sigma-Aldrich and used without further modification.

Synthesis of $H_3TMPyPBr_8^{5+}$

To 0.239 g (0.175 mmol) of H_2TMPyP^{4+} was added an equimolar quantity of cupric acetate (Cu(CH3COO)2; 0.0401 g) in 20 mL of methanol. The solution was refluxed for 2 hrs resulting in an initial color change of the solution from purple to dark red (Scheme 1). The mixture was cooled and the solvent removed via distillation. The red solid was collected by suction filtration and air-dried without further purification.



Scheme 1. Synthesis of $CuTMPyP^{4+}$

The reaction was monitored by UV-visible spectroscopy (Figure 3).

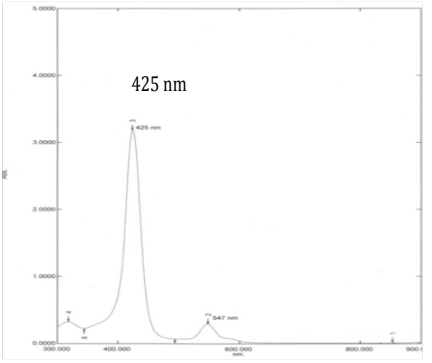
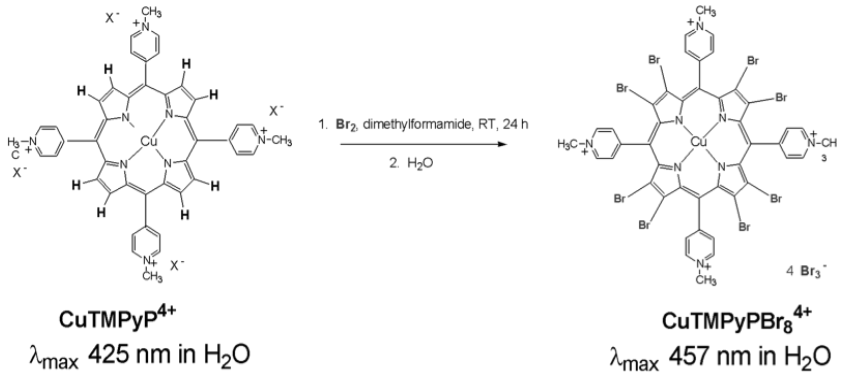


Figure 3. Absorption spectrum of CuTMPyP4+ (pH 7)

Next, approximately 0.35 g of the product was dissolved in 25 mL DMF. While stirring, 12 mL of Br_2 solution (1 mL Br_2 in 11 mL DMF) was added drop-wise over a time span of 30 minutes. Upon exposure to Br_2, the solution slowly transitioned from a red solution to a dark green solution and was allowed to stir for an additional 24 hrs as shown in Scheme 2.



Scheme 2. Bromination of CuTMPyP4+

The resulting solution was vacuum filtered, resulting in a green powder that was air-dried. The product was characterized via UV-Vis spectroscopy (Figure 4).

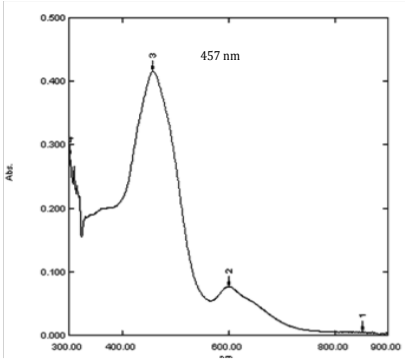
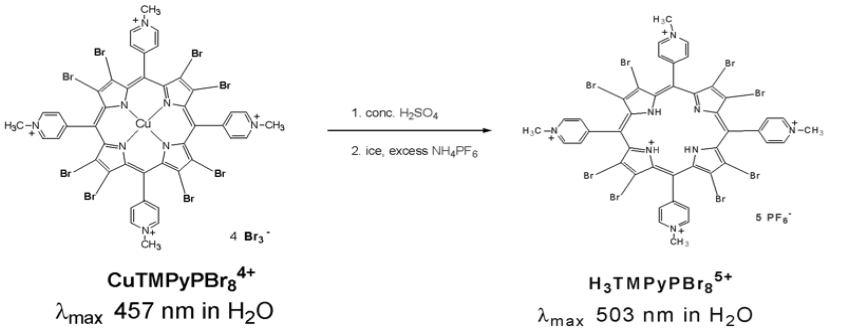


Figure 4. Absorption spectrum of $CuTMPyPBr_8^{4+}$ (pH 7)

The resulting $CuTMPyPBr_8^{4+}$ was dissolved in 10 mL of concentrated sulfuric acid to demetallate the Cu(II) ion. The solution changed to a dark brown color and red vapors were evident. After stirring for 30 min, approximately 50 mL of ice was poured into the solution and allowed to stir gently. Once the ice started to melt, excess NH_4PF_6 was added resulting in the formation of a precipitate (Scheme 3). Suction filtration produced a black-brown solid that was washed several times with deionized water and allowed to air-dry overnight.



Scheme 3. Demetallation of Cu(II) ion to prepare $H_3TMPyPBr_8^{5+}$

The product was observed by absorption spectroscopy (Figure 5).

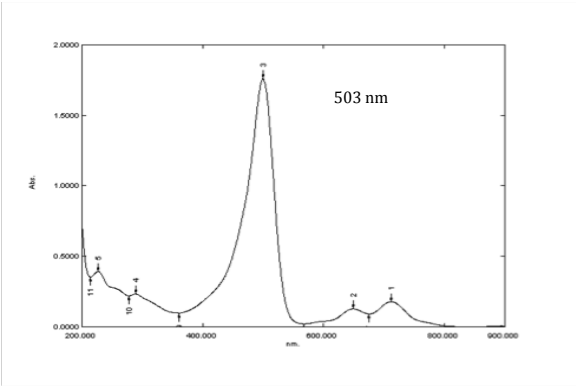
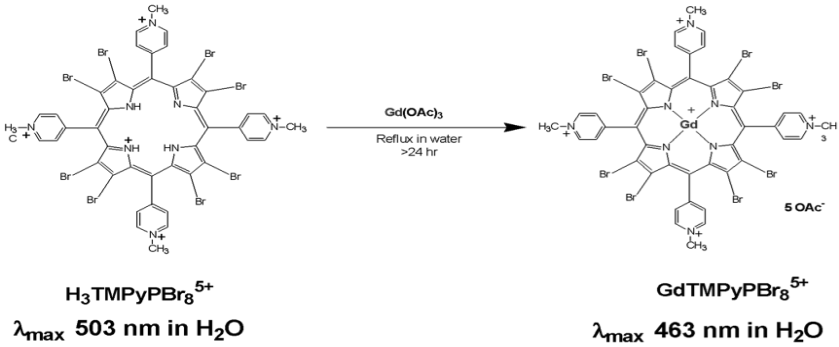


Figure 5. Absorption spectrum of $H_3TMPyPBr_8^{5+}$ (pH 7)

Synthesis of $GdTMPyPBr_8^{5+}$

To 0.352 g of $H_3TMPyPBr_8^{5+}$ was added an equimolar $GdCl_3$ (0.0103g) in 15 mL of water at pH 7. The reaction was refluxed for 24 h and the solution color changed from a black-brown solution to a dark green solution (Scheme 4) as previously reported.



Scheme 4. Synthesis of $GdTMPyPBr_8^{5+}$

The solution was filtered via suction filtration and the solid was collected and air dried. The absorption spectrum is shown in Figure 6.

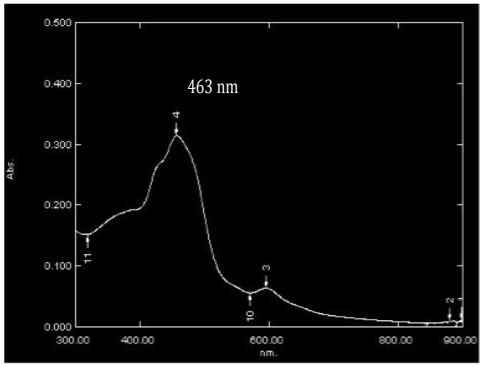
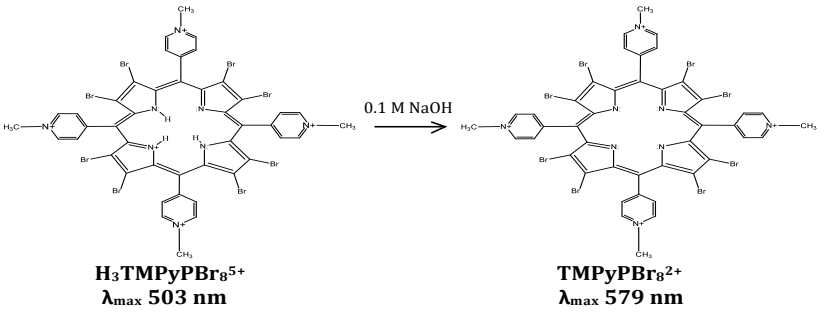


Figure 6. Absorption spectrum of target molecule, $GdTMPyPBr_8^{5+}$ (pH 7)

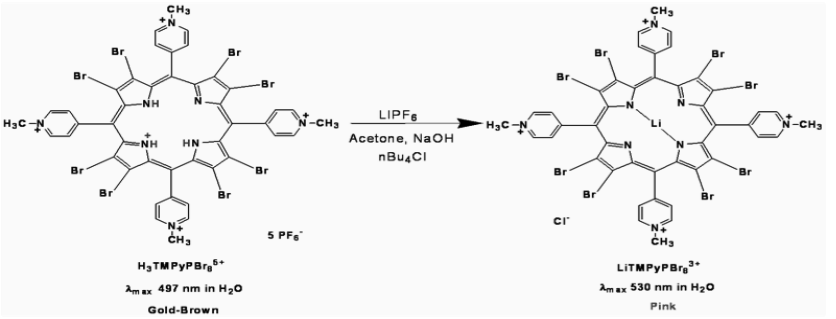
Synthesis of $GdTMPyPBr_8^{5+}$ via $LiTMPyPBr_8^{3+}$

The $LiTMPyPBr_8^{3+}$ was prepared via metathesis of the deprotonated freebase porphyrin, $TMPyPBr_8^{2+}$, with lithium ion in basic solution. To a clean glass vial charged with 5 mL of 0.1M NaOH (pH 11) was added approximately 1 mg of the chloride salt of $H_3TMPyPBr_8^{5+}$ forming a purple solution (Scheme 5).



Scheme 5. Synthesis of the deprotonated free base porphyrin at pH 11.

Thereafter, the LiTMPyPBr$_8^{3+}$ complex was prepared and isolated as the PF_6^- salt using two different routes. (1) Excess LiCl was added to the basic solution of the TMPyPBr$_8^{2+}$ followed by addition of excess NH_4PF_6. The solution color immediately changed from purple to red. Formation of the red precipitate was slow (~ 1 h). The solid was collected by passing the mixture through a glass pipette plugged with glass wool and air-dried (2). To the basic solution of the TMPyPBr$_8^{2+}$ in 0.1*M* NaOH was added excess $LiPF_6$. A red solution formed followed immediately by a precipitate. The solid was collected using a glass pipette filtration system and air-dried. The resulting PF_6^- salt was then dissolved in acetone by passing 1 mL aliquots of solvent through the glass-wool charged with dried porphyrin. The solution was collected in a glass vial (~ 5 mL) and transformed to the chloride salt by addition of excess nBu_4Cl. The resulting precipitate was collected using the glass pipette filtration and air-dried (Scheme 6).



Scheme 6. Synthesis of LiTMPyPBr$_8^{3+}$ under ambient conditions

The absorption spectrum of the LiTMPyPBr$_8^{3+}$ is shown in Figure 7.

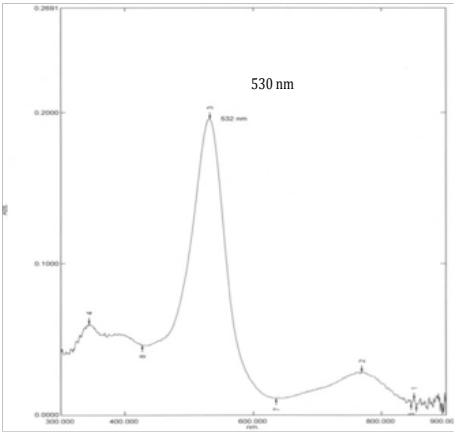
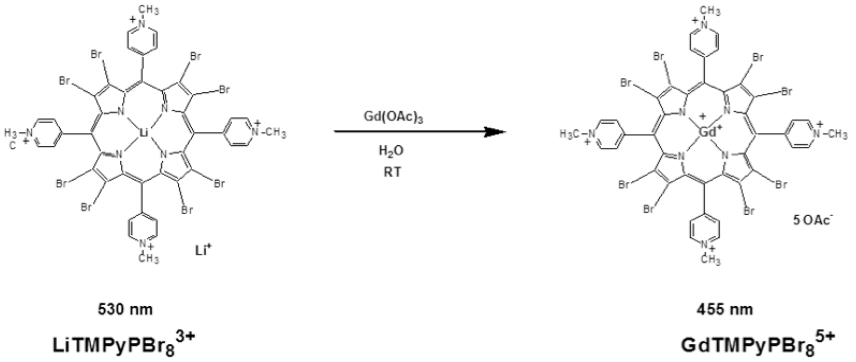


Figure 7. Absorption spectra of Li^+ porphyrin derivative (pH 11)

Approximately 2 mg of $LiTMPyPBr_8^{3+}$ in 1 mL of pH 7 solution was added to an aqueous solution of $GdCl_3$ at room temperature. The mixture immediately changed from red to yellow-green. Addition of excess NH_4PF_6 formed a green precipitate that was collected using the glass pipette filtration system. The solid was air-dried (Scheme 7).



Scheme 7. Reaction between Gd(III) ion and $LiTMPyPBr_8^{3+}$

The absorbance showed a Soret band at 455 nm (Figure 8).

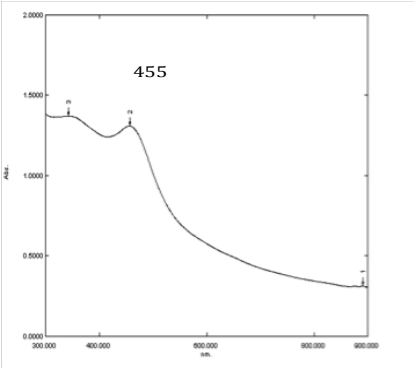


Figure 8. Absorbance spectrum of reaction product between Gd(III) ion and $LiTMPyPBr_8^{3+}$ (pH 7)

RESULTS AND DISCUSSION

The project design for a more facile synthesis of the $GdTMPyPBr_8^{5+}$ was based on a transmetallation reaction between the lithium porphyrin derivative and Gd(III) ion which was a modified reaction approach to our previous report on the synthesis of $GdTMPyPBr_8^{5+}$.[10] To advance this reactivity, the $LiTMPyPBr_8^{3+}$ precursor was prepared using a multistep synthetic scheme.

Table I demonstrates that bromination of the starting material, H_2TMPyP^{4+}, resulted in the shift of the porphyrin absorption spectrum from 425 nm to 503 nm. Deprotonation of $H_3TMPyPBr_8^{5+}$ in aqueous base resulted in the purple $TMPyPBr_8^{2+}$ species presumably due to delocalization of the negative charges at the core nitrogen atoms and solvation of the dianionic species in polar solvent. Subsequent metallation with lithium ion transformed the color from purple to pink, consistent with previous studies using curve-fitting analyses and ^7Li NMR that established the formation of a mono-lithium porphyrin species.[12] Bromination was likely required for the subsequent insertion of the Gd(III) ion since β-substitution afforded a saddle-shaped macrocycle with a core large enough to bind the Gd(III) ion.

Table I: Absorption spectral data of synthesized porphyrins and metal derivatives

Porphyrin	λmax (nm; pH 7)	Color
H_2TMPyP^{4+}	425	red
$CuTMPyP^{4+}$	425	red
$CuTMPyPBr_8^{4+}$	457	green
$H_3TMPyPBr_8^{5+}$	503	brown-black
$TMPyPBr_8^{2+}$	579	purple
$LiTMPyPBr_8^{3+}$	530-538	red-pink
$GdTMPyPBr_8^{5+}$	455-463	green

The UV-visible spectrum of the reaction product between the lithium porphyrin derivative and Gd(III) ion was consistent with our findings for the synthesis of $GdTMPyPBr_8^{5+}$ and resembled the absorption profile of transition metal derivatives of the $H_3TMPyPBr_8^{5+}$.[13] Our preliminary results indicate that we can efficiently prepare and use the $LiTMPyPBr_8^{3+}$ as a precursor for transition metal ion complexes and the Gd(III) porphyrin.

CONCLUSIONS AND OUTLOOK

We plan to further characterize the Gd(III) complex via NMR spectroscopy and powder diffraction analysis. Thereafter, the photophysical properties of the $GdTMPyPBr_8^{5+}$ will be investigated to determine its interaction with dioxygen. A scale-up of the $LiTMPyPBr_8^{3+}$ product as the solid hexafluorophosphate salt was confounded during the precipitation stage, presumably due to reactivity of the anion in aqueous base. Therefore, this reaction will be revisited.

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