Two-Photon Absorption in Tetraphenylporphycenes: Are Porphycenes Better Candidates than Porphyrins for Providing Optimal Optical Properties for Two-Photon Photodynamic Therapy?

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Abstract: Porphycenes are structural isomers of porphyrins that have many unique properties and features. In the present work, the resonant two-photon absorption of 2,7,12,17-tetraphenylporphycene (TPPo) and its palladium(II) complex (PdTPPo) has been investigated. The data obtained are compared to those from the isomeric compound, *meso*-tetraphenylporphyrin (TPP). Detection of phosphorescence from singlet molecular oxygen, $O_2(a^1\Delta_g)$, produced upon irradiation of these compounds, was used to obtain two-photon excitation spectra and to quantify two-photon absorption cross sections, δ. In the spectral region of 750–850 nm, the two-photon absorption cross sections at the band maxima for both TPPo and PdTPPo, δ = 2280 and 1750 GM, respectively, are significantly larger than that for TPP. This difference is attributed to the phenomenon of so-called resonance enhancement; for the porphycenes, the two-photon transition is nearly resonant with a comparatively intense one-photon Q-band transition. The results of quantum mechanical calculations using density functional quadratic response theory are in excellent agreement with the experimental data and, as such, demonstrate that comparatively high-level quantum chemical methods can be used to interpret and predict nonlinear optical properties from such large molecular systems. One important point realized through these experiments and calculations is that one must exercise caution when using qualitative molecular-symmetry-derived arguments to predict the expected spectral relationship between allowed one- and two-photon transitions. From a practical perspective, this study establishes that, in comparison to porphyrins and other tetrapyrrolic macrocyclic systems, porphycenes exhibit many desirable attributes for use as sensitizers in two-photon initiated photodynamic therapy.

Introduction

The porphycene macrocycle is an isomer of the naturally occurring porphyrin macrocycle. Since the synthesis of the first porphycene in 1986,[1] a variety of substituted derivatives have been prepared, including 2,7,12,17-tetraphenylporphycene, TPPo, which is an isomer of *meso*-tetraphenylporphyrin, TPP (Chart 1).[2,3] Likewise, several metal–ion complexes of porphycenes have been prepared, including the palladium(II) complex of tetraphenylporphycene, PdTPPo.[4]

Porphyrin derivatives have arguably been the most studied class of molecules for application as sensitizers in photodynamic

therapy, PDT.[5-7] Indeed, one of the few compounds approved for medical use in this regard, Photofrin, is a composite of porphyrin derivatives.[5] Given the structural similarities between porphycenes and porphyrins, it is reasonable to consider that porphycenes would likewise be viable sensitizers for PDT. In this regard, it is important to note that, upon irradiation, porphycenes generally produce singlet molecular oxygen, $O_2(a^1\Delta_g)$, in moderate to high yields.[8,9] This is pertinent in that singlet oxygen plays a critical role in the mechanisms of cell death associated with PDT.[5,10] Several studies have examined the behavior of TPPo, PdTPPo, and other porphycenes in cells,

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Chart 1. Structures of 2,7,12,17-Tetraphenylporphycene, TPPo, Its Palladium(II) Complex, PdTPPo, and *meso*-Tetraphenylporphyrin, TPP

TPPo (x = 2H)

PdTPPo (x = Pd)

TPP
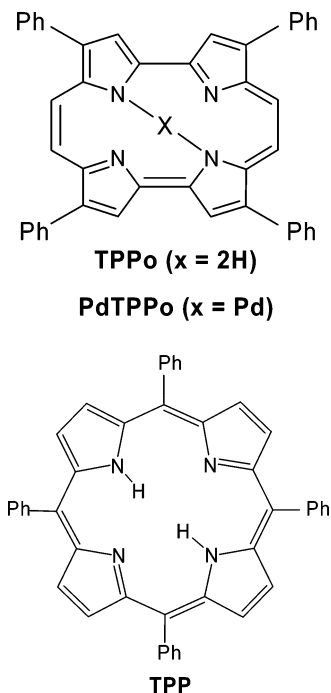
and it has been demonstrated that these molecules are indeed PDT photosensitizers.[4,11−14] It has specifically been shown, for example, that TPPo is effective in mediating lysosome destruction,[11] which is a pathway to induce apoptotic cell death.[15]

Linear photophysical and photosensitizing parameters for TPPo and PdTPPo have been quantified and compared with corresponding values for the much studied naturally occurring porphyrin isomer TPP.[9,16,17] For the porphycenes, one-photon absorption in the so-called Q-region is significantly enhanced and shifted toward longer wavelengths in comparison with their porphyrin counterparts (vide infra). Nevertheless, despite this red shift, direct one-photon absorption of the porphycene in the wavelength range of ∼700−900 nm still does not occur. The latter is pertinent from the perspective of one interested in PDT simply because 700−900 nm is the wavelength range over which tissue is most transparent.[5,18] This wavelength-dependent limitation in both porphyrins and porphycenes, however, can potentially be overcome via nonlinear, two-photon excitation of the PDT sensitizer over the range of 700−900 nm. Although the absorbance of tissue is comparatively large over the range

of ∼900−1050 nm due to a water absorption band with λ_{max} at 970 nm,[18] one could also potentially excite a PDT sensitizer at a wavelength slightly longer than 1050 nm. As such, we set out to study the two-photon absorption properties of the porphycenes TPPo and PdTPPo in the spectral region of 750−850 nm and also at 1100 nm and to compare the results obtained to those obtained from the porphyrin TPP.

From a fundamental perspective, we first note that a comparatively large number of studies have focused on the nonlinear optical properties of porphyrins and porphyrin derivatives.[19−27] To our knowledge, however, corresponding nonlinear studies have not been performed on the isomeric porphycenes. As such, a simple comparison between the data sets obtained from these respective isomers should provide unique insight into the effect that molecular structure has on defining nonlinear optical properties. Such insight is a prerequisite in any attempt to exert control over these chromophores in the design, for example, of a viable drug for PDT.

In recent years, the level of sophistication applied to experimental studies of nonlinear optical phenomena has increased dramatically. Much of this derives from the relative ease with which well-characterized femtosecond laser pulses can now be obtained. The development and application of independent techniques to quantify two-photon absorption cross sections has also been a boon to the field.[28,29] With respect to the latter, we have established the viability and advantages of using the intensity of the photosensitized $O_2(a^1\Delta_g) \rightarrow O_2(X^3\Sigma_g^-)$ phosphorescence signal as a tool to characterize nonlinear light absorption by a given molecule,[25,30,31] and it is this approach we use in the present study on porphycenes. Finally, and perhaps most importantly, it is now established that two-photon excitation of a sensitizer can be advantageous, not only in PDT but in studying the mechanisms of cell death pertinent to PDT.[32,33]

Although the level of sophistication with which computational methods can be applied to model electronic excitations is likewise constantly improving, the calculation of nonlinear phenomena such as two-photon absorption has been a significant challenge to the theoretical community. Almost all of the

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approaches that have been used thus far on molecules of this size have relied on explicit sum-over-states methods that are generally limited by problems such as slow convergence when considering a large number of states.[19,34−36] Nevertheless, significant improvements in the calculation of two-photon absorption cross sections and transition energies have recently become possible due to advances in response theory, and this newer methodology has been applied to comparatively large molecular systems with reasonable success.[25,37−39] Although the characteristic features of response theory are described in detail elsewhere,[40−42] one issue pertinent for molecules of the size considered here is that response theory implicitly sums over all states in the system without explicitly constructing these states. This is particularly important for the problem at hand because the two-photon transition proceeds through a virtual state which is a linear combination of all real eigenstates in the system. Furthermore, recent advances in density functional theory (DFT), higher-order density functional response theory, and functionals for use in response theory[43−45] mean it is now possible to more readily perform accurate response computations of both one- and two-photon absorption spectra for molecules as large as TPP and TPPo. We have recently shown that, when used with density functional quadratic response theory, the CAM-B3LYP functional is capable of obtaining excitation energies and two-photon transition strengths which compare favorably with those obtained using high-level coupled-cluster response theory (i.e., CC3).[46] In the present report, we show that this DFT methodology can be used to compute two-photon parameters of comparatively large molecules that agree well with those experimentally obtained. Our quantum chemical approach thus represents progress in the application of theory to nonlinear optical problems.

Experimental Details

All experiments were performed using a femtosecond (fs) excitation source and optical detection apparatus that have been described in detail elsewhere.[25,31] Briefly, the output of a Ti:sapphire laser (Spectra Physics, Tsunami 3941) was regeneratively amplified (Spectra Physics, Spitfire), resulting in tunable fs pulses over the wavelength range of ∼765−850 nm. In addition, an optical parametric amplifier (Spectra Physics, OPA-800CF) pumped by the Spitfire delivered fs pulses that could be tuned over the range of 300−3000 nm. For the present experiments, the laser polarization at the sample was linear, and the spectral output characteristics were measured using a fiber-optic spectrometer (Avantes AVS-USB2000). The laser power was controlled by adjusting a Glan-Taylor polarizer, and for experiments

performed at 1100 nm, a long-pass filter (Schott, 830 nm cutoff) was inserted to eliminate higher harmonics from the OPA and residuals from the Spitfire pump beam. For all experiments, the pulse repetition rate was 1 kHz.

The sample was contained in a 1 cm path length quartz cuvette mounted in a light-tight housing with a small entrance hole for the laser beam. Singlet oxygen phosphorescence from the sample was collected and focused onto the active area of an IR photomultiplier tube, PMT (Hamamatsu model R5509-42). The 1270 nm phosphorescence of singlet oxygen was spectrally isolated using an interference filter (Barr Associates, 50 nm fwhm). The output of the PMT was amplified (Stanford Research Systems model 445 preamplifier) and sent to a photon counter (Stanford Research Systems model 400), operated using a program written in LabView (National Instruments, Inc.).

The approach to quantify two-photon absorption cross sections of a given molecule using the amount of singlet oxygen sensitized by that molecule has likewise been discussed elsewhere.[25,30,31] Briefly, to obtain two-photon parameters from a given molecule, the intensity of singlet oxygen phosphorescence produced by that molecule was compared to that produced by a standard molecule. For the TPPo and PdTPPo two-photon experiments performed over the wavelength range of 750−850 nm, the standard used was 2,5-dicyano-1,4-bis(2-(4-diphenylaminophenyl)vinyl)benzene, CNPhVB, for which the two-photon absorption spectrum and absolute values of the two-photon absorption cross section, δ, have been determined.[31] For the TPPo and PdTPPo two-photon experiments performed at 1100 nm, TPP was used as the reference molecule. As long as the respective experiments are performed under identical conditions, this relative procedure eliminates the need to characterize the temporal and spatial properties of the laser beam at each excitation wavelength.

In these comparative singlet oxygen experiments, data were collected upon irradiation of the sensitizers in incremental wavelength steps of 10 nm, which is roughly the spectral width of our fs laser pulses.[25,31] Values for the two-photon absorption cross section, δ, at a given wavelength, λ, were obtained through the use of eq 1. Here the subscript r refers to the reference compound, CNPhVB, with S, P, C, and Φ_Δ being the observed singlet oxygen signal, irradiation power at the sample, sensitizer concentration, and singlet oxygen quantum yield, respectively.

$$\delta(\lambda) = \frac{S(\lambda)\Phi_{\Delta,r}C_r P_r^2}{S_r(\lambda)\Phi_\Delta C P^2}\delta_r(\lambda) \qquad (1)$$

Typical peak intensities of the irradiating laser were 1−10 GW/cm^2, with a pulse duration of ∼120 fs and laser beam waist of ∼500 μm. One-photon absorption spectra in the UV−vis region were recorded using a Hewlett-Packard/Agilent model 8453 diode array spectrometer, while the near-IR spectrum of toluene was recorded on a Shimadzu UV-3600 spectrophotometer. Concentrations of the porphycenes used in the two-photon experiments (∼2 × 10^{-4} M) were calculated from known molar extinction coefficients (Table 1). It was confirmed that, over the course of our experiments, laser irradiation caused no photodegradation of the sensitizers.

Materials. TPPo and PdTPPo were synthesized and purified as described previously,[2−4] while TPP was purchased from Porphyrin Systems. Spectroscopic grade toluene was purchased from Aldrich and used as received. Experiments were performed using air-equilibrated samples.

Computational Details

Ground-state geometries of both TPP and TPPo were optimized using density functional theory with the B3LYP functional and the 6-31G*

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Table 1. Selected Photophysical Parameters for TPPo, PdTPPo, and TPP in Toluene[a]

	$\log \epsilon_{Soret}$[b] (λ_{max})	$\log \epsilon_Q$[b] ($\lambda_{max, 0-0}$)	Φ_{ic}	Φ_T	Φ_Δ	δ/GM[c]	(λ)
TPPo	5.05 (378 nm)	4.70 (659 nm)	0.52 ± 0.07	0.33 ± 0.04	0.23 ± 0.02	2280 ± 350 14 ± 3[d]	(770 nm) (1100 nm)
PdTPPo	4.92 (395 nm)	4.92 (632 nm)	0.22 ± 0.06	0.78 ± 0.06	0.78 ± 0.04	1750 ± 265 19 ± 3[d]	(770 nm) (1100 nm)
TPP	5.62 (419 nm)	3.60 (648 nm)	0.05 ± 0.10	0.82 ± 0.10	0.66 ± 0.08[e]	24[f] 6[g]	(760 nm) (1100 nm)

[a] One-photon parameters for TPPo and PdTPPo taken from Rubio et al.[9] Singlet oxygen quantum yields, Φ_Δ, were independently verified in the present study. [b] ϵ values in M^{-1} cm^{-1}. [c] 1 GM = 10^{-50} cm^4 s $photon^{-1}$ $molecule^{-1}$. [d] These numbers are based on a value of 6 GM for TPP at 1100 nm reported by Kruk et al.[22] (see discussion in text). [e] From Wilkinson et al.[53] [f] Value published by Kruk et al.[22] Error limits for their number were not reported. [g] Value reported by Kruk et al.[22] and used as a reference for the TPPo and PdTPPo experiments at 1100 nm.

basis set, as implemented in the Gaussian program.[47] At each of the minimum energy geometries, we computed one- and two-photon absorption spectra and associated absorption cross sections using density functional response theory as implemented in a local version of the Dalton program.[48]

In the response calculations, we used the Coulomb-attenuated version of the B3LYP functional, CAM-B3LYP, because this has been shown to give reliable two-photon results compared to high-accuracy coupled-cluster response calculations.[46] This is mainly due to the increased flexibility in the exchange functional caused by "switching on" and increasing the amount of "exact" Hartree−Fock exchange into the Kohn−Sham orbitals as the interelectronic distance increases. This can be important for the accurate prediction of electronic excitations[44,45] and properties such as two-photon absorption strengths that formally include a sum over intermediate states. We used the 6-31G* basis set which, in computations that are tractable, is sufficiently flexible to yield meaningful one- and two-photon properties for molecules as large as TPP and TPPo.[25,37]

In the response theory calculations, the one-photon spectra were obtained from the linear response function, whereas the two-photon spectra were obtained from the quadratic response function, the poles giving the excitation energies and the residues the transition moments.[40] More specifically with respect to the latter, we obtain the spatially dependent components of the two-photon transition tensor T from the first residue of the quadratic response function.[40] To facilitate discussion and data interpretation, it is sometimes convenient to describe these components of the tensor T in a sum-over-states expression such as that shown in eq 2.[37]

$$T_{\alpha\beta} = \sum_j \frac{\langle 0|\mu_\alpha|j\rangle\langle j|\mu_\beta|f\rangle}{\omega_j - \omega} + \frac{\langle 0|\mu_\beta|j\rangle\langle j|\mu_\alpha|f\rangle}{\omega_j - \omega} \quad (2)$$

In eq 2, T is expressed in terms of the transition moments between ground, 0, intermediate or virtual, j, and final, f, states, where ω_j denotes the excitation frequency of the jth state, ω the frequency of the irradiating light, and μ_α and μ_β are the spatially dependent components of the electric dipole operator (i.e., α and β refer to the Cartesian coordinates x, y, and z).[19,49−51] Note that the summation is carried over all intermediate states j, including the ground state. However, it is important to stress that eq 2 is not required in the response calculation, and that the summation over all states j is implicit in the value of T obtained through the response calculation.

Calculated values of T can then be used to obtain the two-photon absorption cross section, δ. As shown in eq 3, the latter is reported as a rotationally averaged quantity,

$$\delta = F\delta^F + G\delta^G + H\delta^H$$

$$\delta^F = \frac{1}{30} \sum_{\alpha,\beta} T_{\alpha\alpha} T_{\beta\beta}^*$$

$$\delta^G = \frac{1}{30} \sum_{\alpha,\beta} T_{\alpha\beta} T_{\alpha\beta}^*$$

$$\delta^H = \frac{1}{30} \sum_{\alpha,\beta} T_{\alpha\beta} T_{\beta\alpha}^* \quad (3)$$

where the summations are performed over the molecular axes (i.e., x, y, and z in Cartesian coordinates), and F, G, and H depend on the polarization of the incident photons.[19,49,52] Under our conditions in which excitation is achieved using linearly polarized light, $F = G = H = 2$. The two-photon calculations were carried out with identical photon energies equal to half the vertical excitation energy of the final state, a condition consistent with the experiments that were performed.

The calculated two-photon absorption cross sections are reported in atomic units (au), not in the so-called Göppert−Mayer units (GM) pertinent to experimental results (1 GM = 10^{-50} cm^4 s $photon^{-1}$ $molecule^{-1}$). A more complete discussion of the conversion from atomic units to GM is presented elsewhere.[37] In this conversion, it is pertinent to note that the lifetime-dependent broadening of the excited quantum levels is sometimes treated phenomenologically in the final expression for the absorption cross section through the inclusion of a band shape function.[19,34,35,37,38] Although some attempts have been made to include molecule-specific band shape functions,[37] the use of a constant, molecule-independent multiplicative scaling factor is commonly employed.[34,35] We have chosen not to include such a scaling factor in the present work.

Results and Discussion

Because porphycenes and porphyrins are both tetrapyrrolic systems, they have many common electronic-structure-based properties. This is readily seen in the one-photon absorption spectra, which for the three molecules considered in this study, TPPo, PdTPPo, and TPP, are shown in Figure 1.

Briefly, all molecules show strong one-photon absorption in the Soret region ($\sim 350-430$ nm). For the porphycenes, however, the Soret band is less intense, broadened, and blue-shifted in comparison with the Soret band for TPP. Also, for both porphycenes, one-photon transitions in the Q-region between ~ 500 and 700 nm are significantly enhanced compared

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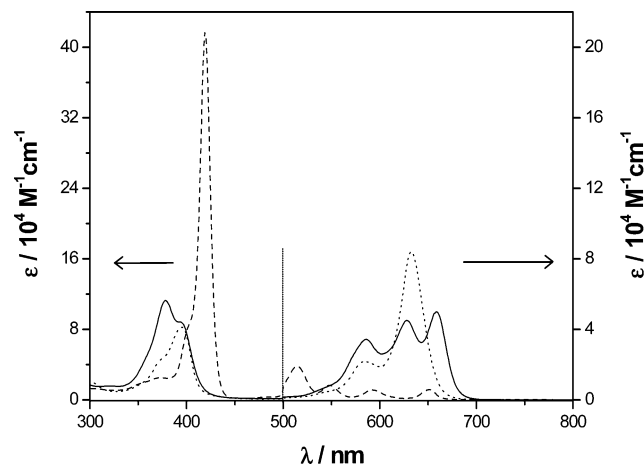
Figure 1. Absorption spectra of TPPo (—), PdTPPo (· · ·), and TPP (- - -) in toluene. Note the change of scale in the ordinate for wavelengths longer than 500 nm.

with those for TPP. Moreover, of the bands in the Q-region, the 0−0 transition is the weakest in TPP, whereas this transition is the strongest for TPPo and PdTPPo.

A more detailed discussion of these spectroscopic features is presented elsewhere, along with a discussion of the significant photophysical properties of these molecules.[9,17] One point to be noted here is that data from a number of sources, including our own computations (vide infra), indicate that the macrocycle in TPPo is not planar and is characterized by an appreciable amount of flexibility. This is reflected, for example, in a quantum yield for TPPo internal conversion, $\Phi_{ic} = 0.52$, that is much larger than that for TPP, $\Phi_{ic} = 0.05$ (Table 1).[9] For PdTPPo, the introduction of the metal has other consequences on the photophysical behavior of the porphycene. Of greatest interest here is the effect of the metal on intersystem crossing, resulting in a comparatively large triplet yield, Φ_T, and, in turn, a large quantum yield of singlet oxygen production, Φ_Δ (Table 1).

A. Two-Photon Absorption Spectra. As already outlined, two-photon absorption parameters for a given molecule were obtained by monitoring the phosphorescence intensity of singlet oxygen produced by energy transfer from that molecule. Experiments were performed using, as a reference standard, the singlet oxygen signal obtained from a molecule for which two-photon parameters had been independently quantified.

Upon irradiation of TPPo and PdTPPo at wavelengths longer than 740 nm, the intensity of the singlet oxygen signal observed scaled quadratically with the incident laser power, as required for a two-photon process (eq 1). This is illustrated in Figure 2 for TPPo in a double logarithmic plot of the observed $O_2(a^1\Delta_g)$ emission intensity as a function of incident laser power for irradiation at 800 nm.

Upon irradiation of TPPo and PdTPPo at 740 nm and shorter wavelengths, however, plots of the singlet oxygen signal intensity against the incident laser power deviated from this quadratic dependence and approached a linear dependence instead. Keeping in mind that the spectral profile of our fs laser pulse is comparatively broad (i.e., fwhm of ∼10−15 nm over the range of 750−850 nm),[31] this observation is consistent with the onset of the more dominant one-photon transitions in the Q-region that competitively absorb the incident light (see Figure 1). The masking of two-photon transitions by interfering one-
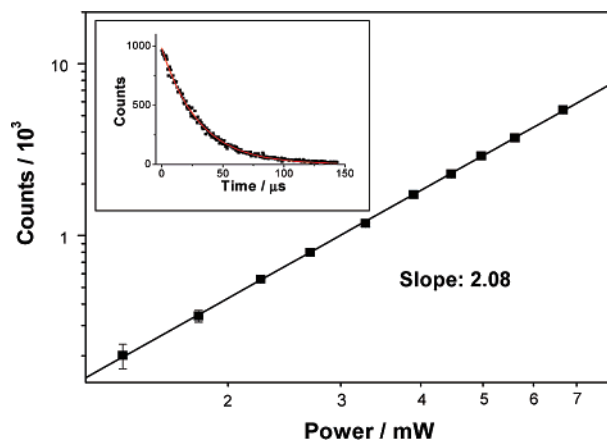
Figure 2. Double logarithmic plot of the singlet oxygen phosphorescence intensity (data from a photon counting experiment) as a function of the incident laser power used to irradiate TPPo at 800 nm in air-saturated toluene. A linear fit to the data yields a slope of 2 as expected for a two-photon process. With the exception of the data points for the two lowest powers, the error on each point falls within the mark used to denote that point. Inset: time-resolved trace of the singlet oxygen phosphorescence signal. A single exponential decay function was fitted to the data, resulting in a lifetime of $\tau = 30.4 \pm 0.2$ μs.

photon absorption is a common phenomenon observed in many molecules, including TPP and other porphyrins.[21,22]

For all experiments, the time-resolved singlet oxygen signal observed followed first-order decay kinetics, yielding a lifetime consistent with that expected for an experiment performed in toluene [τ_Δ(toluene) ≈ 30 μs,[54] inset of Figure 2]. This result demonstrates the absence of competing photoinduced processes that result in the creation of transients that, in turn, can quench any singlet oxygen produced.[55−58] Finally, and arguably most importantly, it was verified that irradiation of neat toluene under our conditions did not give rise to a singlet oxygen signal, a phenomenon which can adversely influence similar two-photon experiments.[31]

Two-photon excitation spectra for TPPo and PdTPPo, obtained through the use of eq 1, are shown in Figure 3. These data, recorded over the spectral range of 750−850 nm, are plotted together with the corresponding one-photon spectra. Note that these spectra are plotted as a function of both the total transition energy (in eV) and total transition wavelength (in nm), an approach that facilitates comparison with calculated spectra (vide infra). Thus, it is important to recognize that the two-photon experiments were actually performed at wavelengths twice as long as those shown in the plots.

Corresponding one- and two-photon absorption spectra for TPP are shown in Figure 4. In this case, we have re-plotted the two-photon fluorescence excitation profile originally published by Kruk et al.[22] (It is important to note here that, in the report of Kruk et al.,[22] the scale published for the TPP molar extinction coefficient is incorrect in their Figure 1a. The published scale should indicate a value of ∼4.7 × 10⁵ M⁻¹ cm⁻¹ for the extinction coefficient at the 419 nm maximum of the TPP Soret

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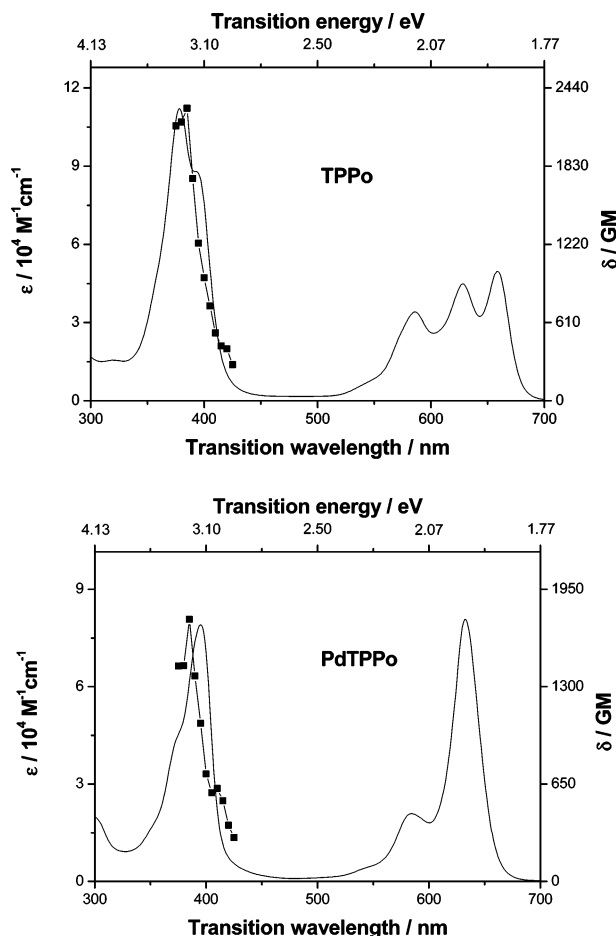
Figure 3. Two-photon absorption spectra for TPPo and PdTPPo in toluene (■, right axes). As outlined in the text, the data were recorded as singlet oxygen excitation spectra through the use of eq 1. The corresponding one-photon spectra are also plotted (—, left axes). The x-axes display the total transition energy and wavelength, and, as such, it is important to recognize that the two-photon experiments were performed at wavelengths twice as long as those shown. The scale for the two-photon absorption cross section, δ, was established using values independently determined for the reference compound CNPhVB in toluene.[31] The error on each data point (not shown for clarity) is estimated to be 15%.

band.[60] This latter extinction coefficient is consistent with a value of 4.2×10^5 M^{-1} cm^{-1} obtained in our own experiments and with published data.[59] However, the scale published by Kruk et al.[22] for the TPP two-photon cross sections in their Figure 1a is correct.[60])

For TPPo, it is clear from Figure 3 that the spectral profile for two-photon absorption matches the corresponding one-photon absorption profile for experiments performed in the region of the Soret band. Specifically, the two-photon spectrum has a band maximum at twice the wavelength of the band maximum observed in the one-photon spectrum. Thus, the data indicate that, for the porphycene TPPo, (a) the state initially populated upon two-photon excitation is nearly degenerate with the state initially populated upon one-photon excitation, or (b) the two-photon transition, in fact, populates the same state as the one-photon transition. These data are in stark contrast to those observed from the isomeric porphyrin, TPP (Figure 4). In this latter case, although one is unable to discern a band
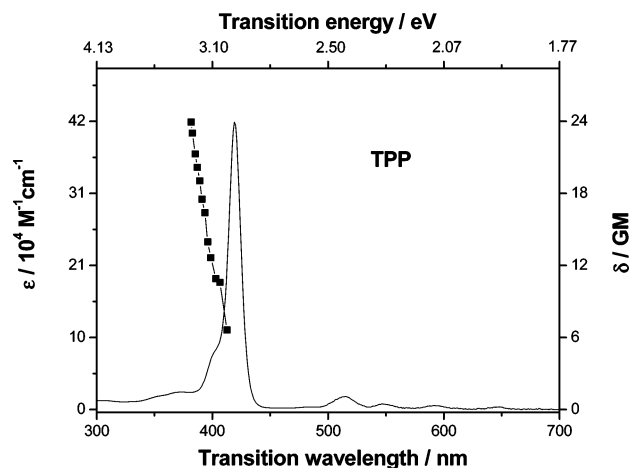
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Figure 4. Two-photon excitation spectrum (■, right axis) and one-photon absorption spectrum (—, left axis) of TPP in toluene. The two-photon spectrum uses data published by Kruk et al.,[22] using an average for the individual data sets reported at a given wavelength. The x-axes display the total transition energy and wavelength, and, as such, it is important to recognize that the two-photon experiments were performed at wavelengths twice as long as those shown. Two-photon data could not be collected at wavelengths shorter than those shown due to competing absorption by one-photon transitions (see text).

maximum in the two-photon spectrum of TPP, the data clearly indicate that this maximum will occur at a transition energy that is significantly higher than that observed in the one-photon spectrum. Thus, for TPP, the state initially populated upon two-photon excitation is higher in energy than that initially populated upon one-photon excitation.

The one- and two-photon data in TPP have been discussed in terms of parity selection rules applied to a centrosymmetric molecular geometry wherein transitions allowed as one-photon electric dipole processes are forbidden as two-photon processes.[22] On this same basis, one would thus expect that the one- and two-photon spectra recorded for TPPo would likewise show the same behavior as that observed for TPP. The data obtained, however, clearly show otherwise. Indeed, the similarity between the one- and two-photon spectra shown in Figure 3 suggests that, on the basis of these selection rules, the equilibrium geometry of TPPo does not have a center of inversion. In part, this phenomenon could reflect the increased flexibility of the porphycene macrocycle (vide supra) through which, due to state mixing mediated by vibronic coupling, two-photon character could be imparted into a transition that would otherwise contain only one-photon character. We will return to these issues when discussing our calculations on these systems (section D, vide infra).

B. Two-Photon Absorption Cross Sections. Pertinent values of the two-photon absorption cross section, δ, are listed in Table 1. At their band maxima, both TPPo and PdTPPo have comparatively large absorption cross sections: $\delta(770 \text{ nm}) = 2280 \pm 350$ and 1750 ± 265 GM, respectively. These data indicate that incorporation of the metal into the porphycene macrocycle has only a minor effect on the two-photon transition probability. On the other hand, the porphycene δ values are significantly larger than the maximum value reported for TPP in the same spectral region, $\sim$24 GM. Admittedly and as clearly seen in Figure 4, this δ value for TPP may not represent the value at the maximum of the two-photon absorption band. Nevertheless, given the spectra shown, it is fairly safe to surmise

that the maximum δ values for the porphycenes will be significantly larger than that for TPP. This issue is discussed further in section C, vide infra.

To further study the nonlinear optical behavior of TPPo and PdTPPo, and to compare this behavior to that of TPP, two-photon absorption into the Q-region was performed by tuning the excitation wavelength to 1100 nm. Upon irradiation of these respective molecules at this wavelength, we indeed observed a singlet oxygen phosphorescence signal that scaled quadratically with incident power. To quantify δ values for the porphycenes at this wavelength, experiments were performed using TPP as the two-photon reference. Specifically, Kruk et al. have reported that $\delta = 6$ GM for irradiation of TPP at 1100 nm in toluene.[22] Against this δ value for TPP, we ascertained that $\delta = 14 \pm 3$ and 19 ± 3 GM for TPPo and PdTPPo, respectively.

First, it is clear that, for all three molecules, values for the absorption cross section at 1100 nm are significantly smaller than those recorded upon irradiation in the Soret region (vide supra, Table 1). Moreover, and as with the data recorded upon irradiation in the Soret region, these data obtained upon irradiation in the Q-region indicate that the two-photon absorption cross sections for the porphycenes are larger than that for the corresponding porphyrin. However, care must be exercised in this latter assessment simply because data were only recorded at one wavelength. Rather, one should ideally record data over a range of wavelengths to accommodate molecule-dependent spectral shifts. Unfortunately, upon irradiation of these molecules at wavelengths longer than 1100 nm, we observed a large near-IR emission signal that scaled linearly with the incident power. Although this observation might reflect direct absorption by oxygen (i.e., $O_2(X^3\Sigma_g^-)_{v=0} \rightarrow O_2(a^1\Delta_g)_{v=1}$),[61] it more likely derives from the increased transmittance of the silicon-based optics used to isolate the 1270 nm singlet oxygen phosphorescence. As such, our detector may be responding to light from a number of different sources. It is also important to note that, in this spectral region, toluene has an appreciable one-photon absorption band that is assigned to a second vibrational overtone (Figure 5).[62] We have previously demonstrated that both overtone and two-photon excitation of toluene itself can cause appreciable interference when performing two-photon experiments on a sensitizer dissolved in this solvent.[31] (Indeed, in a private communication, we have been told that δ values for TPP at wavelengths longer than ∼1100 nm in toluene published by Kruk et al.[22] may be incorrect due to the interfering effects of solvent in their measurement.[60])

C. Resonance Enhancement in Two-Photon Transitions. The observation that, in the Soret region, the porphycenes have a much larger two-photon absorption cross section than the corresponding porphyrin can be qualitatively explained using the concept of resonance enhancement. The latter has been invoked in discussing the nonlinear optical behavior of other molecules,[34,37,49,63] but, as outlined below, it is particularly relevant for porphyrins and related systems.[21,22,64]

In describing the qualitative features of the two-photon transitions in the porphycenes as compared to that in the

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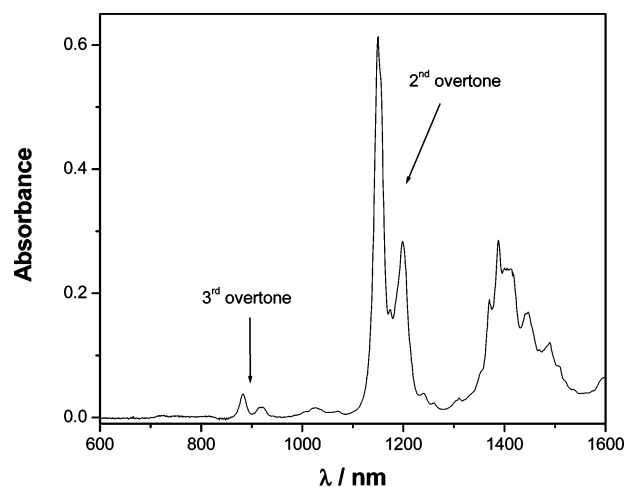
Figure 5. Absorption spectrum of neat toluene over the spectral region pertinent to the present two-photon experiments. The second and third overtones of C−H stretching modes are marked. Data were recorded using a 1 cm path length cell.
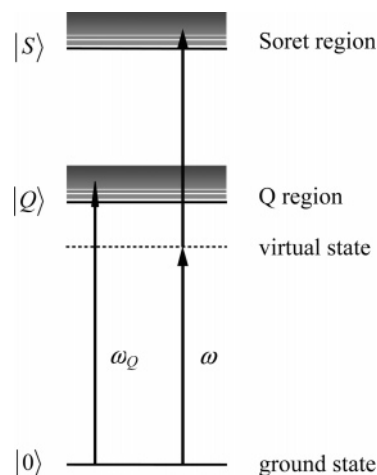

Figure 6. Generalized three-level energy diagram describing resonance-enhanced two-photon excitation in porphyrins and porphycenes. The laser frequency used in the two-photon experiment is ω, and ω_Q is the frequency of an allowed one-photon transition in the Q-region.

porphyrin TPP, we consider the condensed three-level scheme in Figure 6 that shows the pertinent electronic eigenstates and transition frequencies (with no formal reference to state parity). Here 0 represents the ground state, and S and Q represent states in the Soret and Q-regions, respectively. Simultaneous absorption of two photons is shown to occur via a virtual state.

The salient features of this model are perhaps most easily understood in terms of the sum-over-states expression shown in eq 2. Given that the virtual state is accurately described as a linear combination of all real eigenstates in the molecule, it is important to note that included in eq 2 are the transition dipole moments for the one-photon processes between the ground and jth state, $\langle 0|\mu|j\rangle$, and between the jth and final state, $\langle j|\mu|f\rangle$, summed over all real states j. Moreover, the denominator in eq 2 is a term, $\omega_j - \omega$, whose magnitude depends on the difference between the excitation frequency of the jth state and the frequency of the irradiating laser. Referring to Figure 6 and eq 2, and recognizing that ω_Q denotes the frequency of an allowed one-photon transition to a state in the Q-region, it is readily seen that a special case arises when the laser frequency ω approaches ω_Q. Under these conditions, the cross section for

Table 2. Parameters for Optimized Conformers of Ground State TPP and TPPo

molecule	point group	Dihedral Angles[a]				energy (au)	ΔE (kJ/mol)[b]
		a	**b**	**c**	**d**		
TPP	D_{2h}	90°	90°	90°	90°	−1913.74947	4.20
	C_{2h}	69.1°	68.9°	110.9°	111.1°	−1913.75075	0.84
	D_2	71.2°	71.2°	71.2°	71.2°	−1913.75034	1.92
	C_2	66.1°	113.4°	66.1°	113.4°	−1913.75107	0
TPPo	C_{2h}	90°	90°	90°	90°	−1913.77265	30.56
	C_i	47.5°	134.2°	134.3°	44.0°	−1913.78411	0.47
	C_2	134.8°	134.6°	134.8°	134.6°	−1913.78429	0

[a] Dihedral angle between the plane of the pyrrole ring and the adjacent phenyl substituent in TPPo and between the plane of the macrocycle and the phenyl ring in TPP, measured clockwise around the macrocycle such that **a**, **c** and **b**, **d** are on opposite sides (see Figure 7). [b] Energy difference, ΔE (kJ/mol), relative to the lowest energy conformer. Perpendicular geometries are fourth-order saddle points.

two-photon absorption into the Soret region will be dominated by terms in which the virtual state principally reflects states in the Q-region. Although exact resonance is not achieved because $\omega_Q > \omega$, this condition of near resonance with an allowed one-photon transition can result in a significant enhancement of the two-photon transition in the Soret domain. A similar situation will not arise for two-photon absorption into the Q-region because there are no allowed one-photon transitions to a state j where $\omega_j \sim \omega$. Thus, on this basis, one can generally rationalize why two-photon absorption cross sections in both porphyrins and porphycenes are greater in the Soret region than in the Q-region.

Application of these same arguments also explains why the Soret two-photon absorption cross sections for the porphycenes TPPo and PdTPPo are greater than that for TPP. In this case, the transition moments for one-photon absorption in the Q-region, $\langle 0|\mu|Q\rangle$, are far greater for the porphycenes than for TPP. This is clearly seen in the linear absorption spectra (Figure 1). Moreover, for both TPPo and PdTPPo, the most intense Q-band is the 0−0 transition, thereby resulting in the greatest enhancement when the detuning term $\omega_Q − \omega$ is the smallest. In contrast, in TPP, the 0−0 transition in the Q-domain is comparatively weak (Figure 1).

D. DFT Calculations. To go beyond the qualitative arguments presented above, and to address the relationship between molecular symmetry and the observed spectra, one- and two-photon quantum chemical calculations were performed on TPP and TPPo. As outlined in the section on computational details (vide supra), this was accomplished using density functional response theory.

1. Geometry Optimization. Ground-state geometries of both TPP and TPPo were optimized using density functional theory with the B3LYP functional and 6-31G* basis set, as implemented in the Gaussian program.[47] Key parameters are collected in Table 2, while the structures are shown in Figure 7 (complete coordinates are in the Supporting Information).

Through these calculations, three minima were found for TPP with geometries belonging to the point groups C_{2h}, D_2, and C_2. In all cases, the TPP macrocycle itself was planar, and the three geometries differ only in the dihedral angles between the plane of the macrocycle and the planes of the pendant phenyl rings. The geometry in which the pendant phenyl rings are perpendicular to the plane of the TPP macrocycle (D_{2h} point group) is a fourth-order saddle point having normal modes with imaginary vibrational frequencies. In general, these results are consistent

with those of Kruk et al.,[22] for example, who likewise found minima in which the pendant phenyl rings are twisted through a dihedral angle of about 60° with respect to the plane of the macrocycle. On the other hand, Kruk et al. identified two minimum energy conformers (i.e., C_{2h} and C_2), whereas we have identified three.

The energy differences between these respective minima in TPP are small (<2 kJ/mol), and, as such, the actual geometry of the molecules studied will be a combination of all conformers (i.e., our data would reflect a Boltzmann weighted average of these conformers). With this in mind, and with respect to our present study on two-photon transitions, it is important to recognize that, of the three TPP conformers shown, only one has a center of inversion (C_{2h} point group); the others are non-centrosymmetric.

Likewise, two minima were found for TPPo with geometries that belong to the point groups C_i and C_2. In these cases, the TPPo macrocycle was slightly twisted out of planarity, but this twisting occurs such that the molecules still transform according to the point groups indicated. These geometries differ in the dihedral angles between the plane of a given pyrrole ring and the adjacent phenyl group. The energy difference between these conformers is likewise small (0.47 kJ/mol), and facile interconversion between the two will occur at room temperature. As with TPP, the geometry in which the phenyl rings are perpendicular to the pyrrole rings (C_{2h} point group in this case) is a fourth-order saddle point. Moreover, the conclusion that the TPPo macrocycle is not planar is consistent with the photophysical behavior ascribed to a macrocycle that is more flexible than that in TPP (vide supra). Finally, and again with respect to our present study on two-photon transitions, it is important to recognize that, of the TPPo conformers shown, only one has a center of inversion; the other is non-centrosymmetric.

In concluding this section, we reiterate the significant point that both TPP and TPPo are equally characterized by conformers that are centrosymmetric and conformers that are not. Thus, when interpreting the correspondence, or lack thereof, between one- and two-photon spectra such as those shown for TPP and TPPo in Figures 3 and 4, one must exercise caution when invoking a qualitative discussion based on symmetry-derived parity selection rules. Rather, as outlined below, it is prudent to carry out a more rigorous calculation.

2. Response Calculations. For each of the TPP and TPPo conformations shown in Figure 7, density functional response theory was used to obtain the energies and probabilities for one- and two-photon transitions. Importantly, for each molecule, there is not a significant difference between the spectra calculated for the individual conformers (i.e., the spectrum for the C_{2h} TPP is almost identical with those for D_2 and C_2 TPP; see Supporting Information). Thus, we can effectively compare a single TPP spectrum with a single TPPo spectrum, and this is done in Figure 8 using conformers for which there is no inversion symmetry. Keeping in mind that the spectra calculated for TPP and TPPo correspond to rigid, non-vibrating gas-phase systems, these spectra agree quite well with the experimental data.

We first focus on the one-photon transitions. The calculated spectrum of TPP readily reproduces the fact that the Q-bands in the spectral domain of 2.0−2.4 eV are much less intense
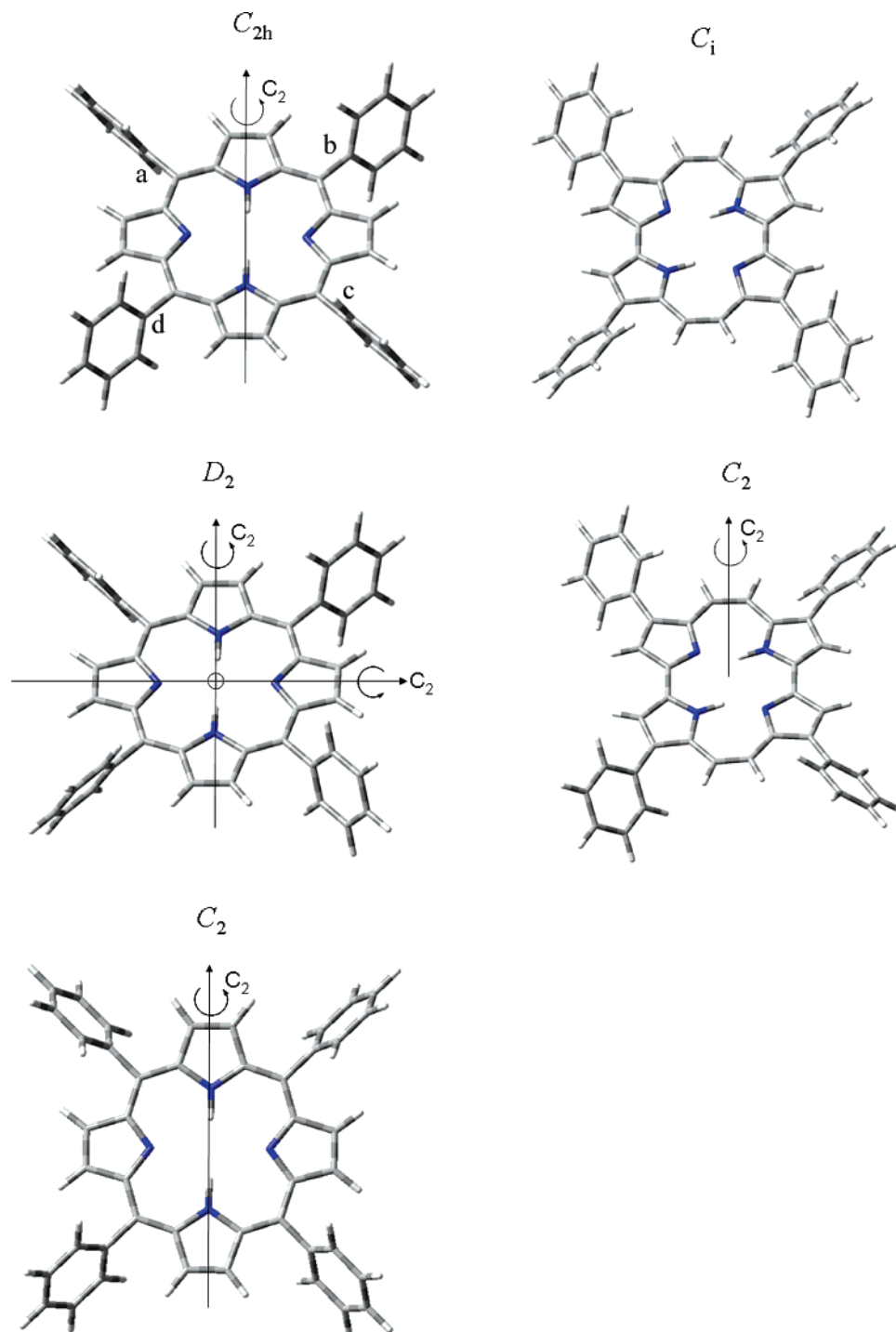
Figure 7. Minimum energy conformers for TPP (left column) and TPPo (right column). The largest energy difference between the conformers for TPP, $E(D_2) - E(C_2)$, is 1.92 kJ/mol, whereas for TPPo, $E(C_i) - E(C_2)$, it is 0.47 kJ/mol. The letters shown on the upper left-hand structure correspond to the columns shown in Table 2 in which dihedral angles are designated. This labeling holds for all of the structures.

than the Soret transitions in the spectral range of 3.3−3.5 eV. Likewise, the calculated spectrum of TPPo indicates that the Q-band transitions for this molecule are more intense, not only relative to the TPPo Soret band but also relative to the Q-band transitions in TPP. Finally, the transition energy of the TPPo Soret band is calculated to be slightly higher than that of TPP and the intensity of the TPPo Soret transition to be less than that of TPP, as indeed observed experimentally.

The calculated two-photon spectra are equally as impressive. For TPP, the calculations indeed indicate that the principal two-photon absorption band will occur at a transition energy higher than that for the one-photon band. For TPPo, the calculations correctly indicate that, in the Soret region, the principal two-photon absorption band will be co-incident with the one-photon band, just as we have observed experimentally (Figure 3). The calculations also correctly reproduce a δ value for TPPo in the Soret domain that is significantly larger than the corresponding value for TPP. With respect to this latter point, however, it is important to note that the calculated ratio of TPPo/TPP δ values is not as large as the ratio of TPPo/TPP δ values experimentally
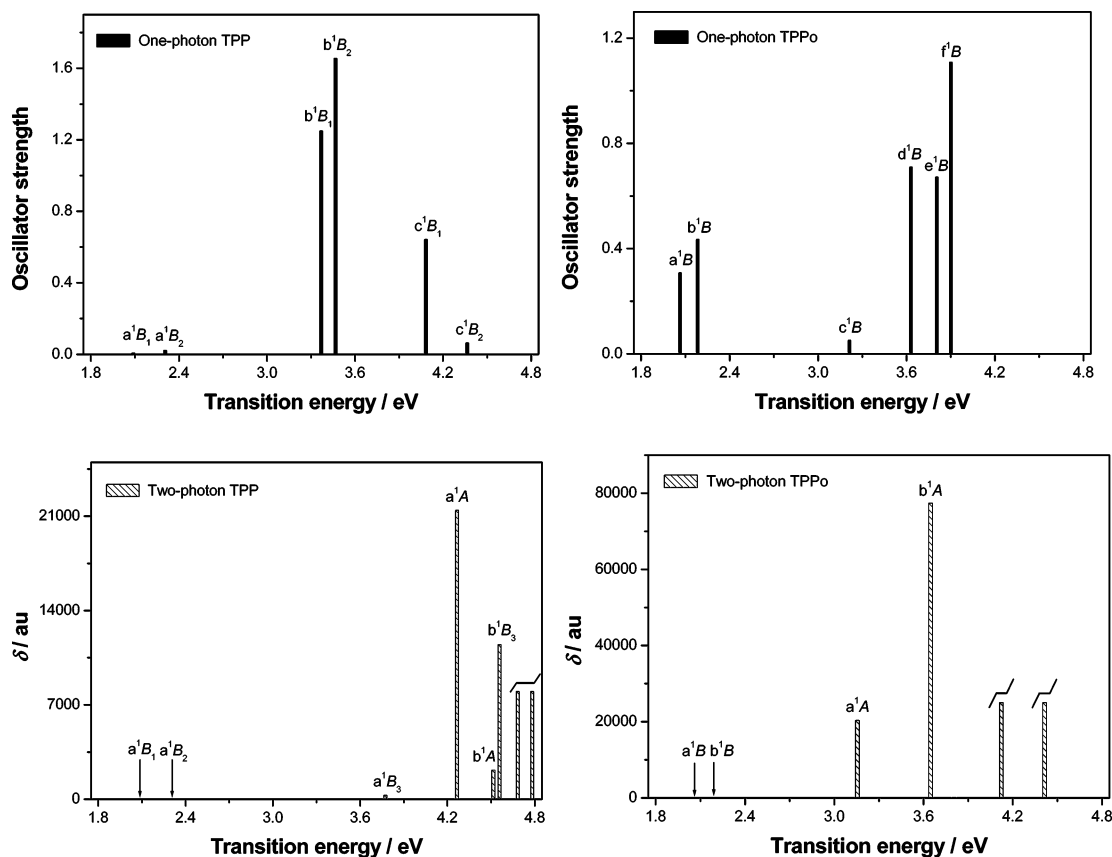
Figure 8. Calculated one- and two-photon spectra for TPP (left) and TPPo (right). Transition probabilities are given in terms of oscillator strength for the one-photon spectra and rotationally averaged δ values for the two-photon spectra (the latter are given in atomic units; see discussion in the text). The TPP spectrum shown is for the conformer that transforms according to the point group D_2, whereas the TPPo spectrum is for the conformer that transforms according to the C_2 point group. The irreducible representation to which a given transition transforms in the corresponding point group is shown. A complete list of the calculated transitions and transition probabilities is shown in tabular form in the Supporting Information.

obtained. In part, this discrepancy likely reflects the fact that, in the experimental spectrum of TPP, the band maximum of the two-photon absorption profile is not obtained at the shortest wavelength used (see Figure 4).

There are several other more subtle points in the calculated spectra that deserve comment. First, for TPPo, the two-photon transitions shown at 4.12 and 4.35 eV are so close to being in resonance with the one-photon Q-band transitions at 2.07 and 2.18 eV that reliable two-photon matrix elements cannot be safely calculated for these specific transitions, but they are expected to be very large. Second, although the calculations yield two-photon TPPo Q-band transitions at 2.07 and 2.18 eV, respectively, they are not seen in the spectrum shown in Figure 8 because, relative to the two-photon Soret transition, these calculated Q-band transitions are significantly less intense. Similarly, the calculated two-photon Q-transitions for TPP at 2.09 and 2.31 eV are not seen in the spectrum because they, too, are small compared to the Soret transition (as with TPPo, there are also one-photon transitions in TPP at these same energies). Nevertheless, despite the fact that these calculated two-photon Q-transition probabilities are small relative to the Soret transitions, the TPPo Q-band transition probabilities are indeed calculated to be larger than those for TPP (see Supporting Information).

Arguably, the most important point that is realized through these calculations is that one must exercise caution when using qualitative molecular-symmetry-derived arguments to predict the expected relation between allowed one- and two-photon

transitions. From a simplistic perspective, one might say that, because TPP appears to be centrosymmetric, it is expected that the first allowed two-photon transition will populate a state higher in energy than the state populated by a one-photon transition.[50] It is thus reassuring to observe that this expectation is indeed met in the experimental data (Figure 4). By extension, because TPPo also appears to be centrosymmetric, one would likewise expect the two-photon transition to populate a state higher in energy than that populated in a one-photon process. This latter expectation is not realized in the experimental data (Figure 3). Rather, on the basis of the experimental data, one would infer that TPPo is, in fact, non-centrosymmetric. In reality, both TPP and TPPo are arguably best described in terms of a Boltzmann distribution of centrosymmetric and non-centrosymmetric conformers. Thus, attempts to predict the expected relation between allowed one- and two-photon transitions should ideally incorporate the results of reasonably rigorous computations; one can be misled by arguments based solely on apparent molecular symmetry. As illustrated in the case of TPPo, such computations can point to "accidental degeneracies" in which a state populated in a two-photon transition is energetically very close to a state populated in a one-photon transition (Figure 8). Of course, our computations do not include the effects of vibronic coupling, and, in reality, there may be some mixing between states (e.g., for TPPo, the one-photon d^1B state could mix with the two-photon b^1A states).

3. *cis*-Porphycene Tautomers. Our presentation thus far has been based on the framework that TPPo exists as the *trans*

tautomer, as shown in Chart 1 and Figure 7. At this point, however, it is pertinent to note that, in our room-temperature samples, the TPPo population could also contain some of the higher-energy *cis* tautomers that result as a consequence of changes in the position of the N−H bonds within the macrocycle.[65] Calculated minimum energy structures for these *cis* tautomers are shown in the Supporting Information, and the energies obtained are indeed consistent with the notion that a room-temperature population of TPPo will contain a mixture of *cis* and *trans* tautomers. On the other hand, corresponding calculations for TPP indicate, as expected,[65] that the *cis* tautomer is significantly higher in energy than the *trans* (see Supporting Information). As such, it is only necessary for us to consider the *trans* tautomer of TPP, as shown in Chart 1 and Figure 7.

Of course, these *cis* tautomers of TPPo do not have inversion symmetry, and, as such, the associated one- and two-photon spectra are expected to show similarities. Our response calculations indicate that this is indeed the case (spectra provided in the Supporting Information). Most importantly, if our ground-state TPPo population is indeed accurately described as a weighted mixture of *cis* and *trans* tautomers, our central thesis developed in the previous sections is only further substantiated. Specifically, our spectroscopic data reflect a distribution of both centrosymmetric and non-centrosymmetric structures.

Conclusions

Porphycenes are isomers of porphyrins and, as such, can be used to yield fundamental information about the relationship between structure and reactivity in tetrapyrrolic macrocycles found in a wide range of ubiquitous compounds. In their own right, porphycenes have unique properties that distinguish them from the commonly found porphyrins. In the present study, we have established that, in the spectral domain of 750−850 nm (i.e., the two-photon Soret region), the two-photon absorption cross sections for two porphycenes, TPPo and PdTPPo, are large compared to that from the porphyrin analogue, TPP. These observations are attributed to the fact that, for the porphycenes, the two-photon transition is nearly resonant with a comparatively intense one-photon Q-band transition. In this regard, our data corroborate a model based on resonance enhancement that can be quite useful in qualitatively predicting the relative magnitudes of two-photon transitions in tetrapyrrolic systems. We have also established, however, that qualitative symmetry-based arguments used to predict the spectral relationship between one- and two-photon transitions can be misleading, and that one should ideally rely on more rigorous quantum chemical calculations to predict nonlinear optical properties. To this end, we have demonstrated that density functional response theory with appropriate functionals can provide useful results, even for these comparatively large porphycene and porphyrin systems. Furthermore, such calculations show that one can now apply fairly rigorous quantum mechanical methods to such nonlinear optical phenomena as two-photon absorption in large chemical systems without having to use arbitrary sum-over-states expressions.

In the Soret spectral region (∼750−850 nm), the two-photon absorption cross sections obtained for these fundamental porphycenes are not only larger than that for TPP but they are also larger than those for other analogous tetrapyrrolic macrocyclic systems. For example, although tetraazaporphyrins (i.e., por-

phyrazines) can have large two-photon absorption cross sections,[25,66] the data reported herein indicate that the basic porphycene, TPPo, still has a larger two-photon transition probability in the Soret region.

Porphycenes sensitize the production of singlet molecular oxygen, $O_2(a^1\Delta_g)$, upon two-photon irradiation, and with such large two-photon absorption cross sections in the spectral region around 800 nm, these molecules are good candidates for use in two-photon initiated photodynamic therapy. With this in mind, it is important to note that the efficiency of the two-photon absorption process in porphyrins can be enhanced by structural modifications,[24] including the generation of porphyrin dimers.[23,26] Similar structural modification to the porphycene framework could likewise prove to be quite beneficial for use in PDT, particularly when combined with modifications that might enhance the selectivity with which these compounds either target a malignant cell or localize in a specific subcellular domain. To this end, a recently published diversity-oriented synthesis of porphycenes should facilitate such developments.[67] In any event, even in the absence of such modifications, the two-photon absorption cross sections reported herein for TPPo and PdTPPo, 2280 and 1750 GM, are significantly greater than the absorption cross sections for the porphyrin-based PDT drugs Photofrin (δ < 10 GM over the spectral range of 800−900 nm)[68] and protoporphyrin IX (δ < 3 GM over the spectral range of 760−790 nm)[20] and other analogous tetrapyrrolic macrocyclic systems.

At this juncture, it is pertinent to mention the ingenious approach of using a two-chromophore conjugate to increase the amount of singlet oxygen produced upon two-photon excitation of a sensitizer.[69,70] In this approach, a chromophore that efficiently absorbs light in a two-photon process is covalently linked to a second chromophore designed principally to sensitize the production of singlet oxygen in high yield. Upon nonlinear photoexcitation of the first chromophore, energy transfer to the second chromophore ensues with the resultant production of singlet oxygen. Although this approach is indeed clever, it has the potential limitation of a cumbersome synthetic procedure to make the rather sophisticated conjugate. On the other hand, working solely with the porphycene framework, one can combine a reasonably large two-photon absorption cross section, efficient singlet oxygen production, and biocompatibility in a potentially simpler and more easily delivered molecular package.

The comparatively large two-photon absorption cross sections in the porphycenes can also be exploited for other nonlinear optical applications. Still within the spirit of this present paper, one such application is as a sensitizer in the so-called two-photon singlet oxygen microscope wherein a focused laser is used to create a discrete and localized population of singlet oxygen in subcellular spatial domains.[71,72] General application of this tool

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is currently limited, in part, by the lack of biologically compatible singlet oxygen sensitizers that have large two-photon absorption cross sections.[72] To this end, derivatives of the porphycenes described herein could prove to be beneficial in the use of this microscope to further elucidate spatially resolved mechanisms of oxygen-dependent photoinduced cell death.

Supporting Information Available: Cartesian coordinates for the optimized TPP and TPPo structures used in the computations, tabulated transition energies and probabilities calculated for the D_2 conformer of TPP and the C_2 conformer of TPPo, calculated one- and two-photon spectra for all TPP and TPPo conformers studied, calculated geometries for the *cis* isomers of TPP and TPPo, calculated one- and two-photon spectra for the *cis* TPPo tautomers, and the full author lists for refs 47 and 48. This material is available free of charge via the Internet at http://pubs.acs.org.

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