Photosensitization of Singlet Oxygen via Two-Photon-Excited Fluorescence Resonance Energy Transfer in a Water-Soluble Dendrimer

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A novel approach for the sensitization of singlet oxygen has been developed which utilizes indirect excitation of the photosensitizer by two-photon-excited fluorescence resonance energy transfer (FRET) from separate chromophores assembled into a dendrimer. This approach effectively enhances the two-photon excitation efficiency of a known photosensitizer, without the sort of chromophore modifications that could lead to loss of photosensitization and other desirable photophysical properties. Photosensitization of singlet oxygen via excitation wavelengths transmissive to human body tissue (750−1000 nm) could alleviate the depth limitations of photodynamic therapy. The dendritic photosensitizer was prepared by grafting two-photon-absorbing chromophores and water-solubilizing moieties to a known multivalent porphyrin photosensitizer. Efficient FRET (>99% quenching of donor emission) between the peripheral donor two-photon-absorbing chromophores and the central acceptor photosensitizer at the core of the dendrimer was demonstrated under two-photon excitation conditions in an aqueous medium. Photosensitized production of singlet oxygen was monitored through chemical trapping and oxygen luminescence. Both methods independently demonstrated enhanced two-photon-induced singlet oxygen generation upon incorporation of two-photon-absorbing chromophores capable of efficient FRET to the photosensitizer.

Introduction

The utilization of two-photon absorption (TPA) phenomena in aqueous media impacts many biological applications including bioimaging,[1−4] drug delivery,[5] and phototherapies.[6−11] For example, the generation of cytotoxic singlet oxygen via TPA could expand the scope of photodynamic therapy (PDT) to subcutaneous tumors. Current PDT photosensitizers absorb in the ultraviolet and visible regions of the spectrum, where skin is poorly transmissive, limiting treatment to topical ailments. Sensitizers capable of efficient TPA could employ 750−1000 nm light (near-infrared, NIR), where tissue is more transparent, allowing for deeper light penetration and reduced risk of laser hyperthermia.[8] The TPA process displays a quadratic dependence on laser intensity. As a result, treatment localization at the focal point of a laser beam provides increased spatial resolution of treatment as an additional advantage to PDT.

Porphyrins and similar photosensitizers have received the most attention as PDT agents due to their high singlet oxygen quantum yields and preferential accumulation in tumor tissue.[12−15] One of the goals of current research is to design porphyrins that can utilize NIR light. For example, many modifications are aimed at decreasing the band-gap energy, thus shifting the linear absorbance further into the NIR.[13−15] Another approach is to incorporate functionalities into the porphyrin that enhance the TPA cross-section.[8,15] Both methodologies require modification of the chromophore to allow excitation with longer wavelengths. However, their scope is limited since the desirable properties of the porphyrin must be preserved. Our goal is to indirectly excite

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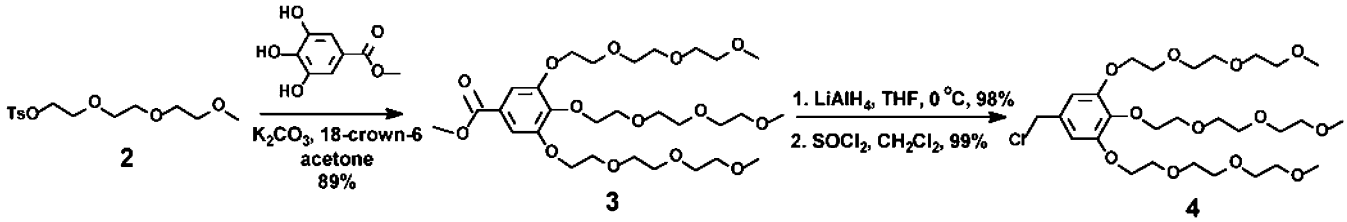
Scheme 1. Synthesis of the Water-Solubilizing TEG Moiety



the photoactive moiety through fluorescence resonance energy transfer (FRET) from donor two-photon-absorbing chromophores (TPACs). This approach permits retention of the established photophysical advantages afforded by the porphyrin while allowing excitation at wavelengths where living tissue is transparent. Our modular design permits incorporation of a variety of chromophores chosen specifically for their TPA cross-section, FRET efficiency, and photophysical properties in aqueous media. This methodology is versatile as it can readily be adapted to other systems that could also benefit from two-photon excitation.

Encouraged by the success achieved in preliminary work with an organic-soluble photosensitizer,[6] a water-soluble system was designed and synthesized (Figure 1). The donor

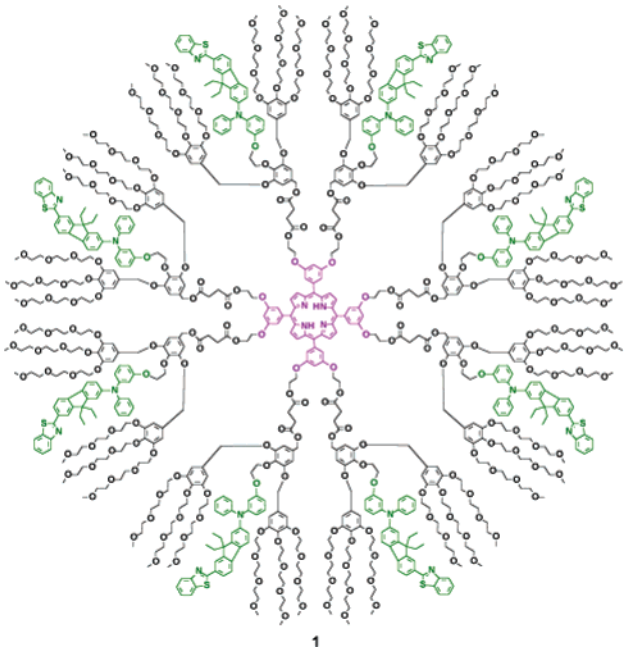


Figure 1. Molecular structure of the target dendritic photosensitizer **1**.

chromophore AF-343[17] (Scheme 1), chosen for its efficient TPA and spectral overlap with the porphyrin, was incorporated into a poly(benzyl ether) framework with a tri(ethylene glycol) monomethyl ether (TEG) periphery, which imparts water solubility to the entire molecular assembly.

Singlet oxygen generation via TPA begins with simultaneous absorption of two photons by the TPAC, which may populate an excited singlet state different (higher) from that produced by one-photon excitation as shown in Figure 2.

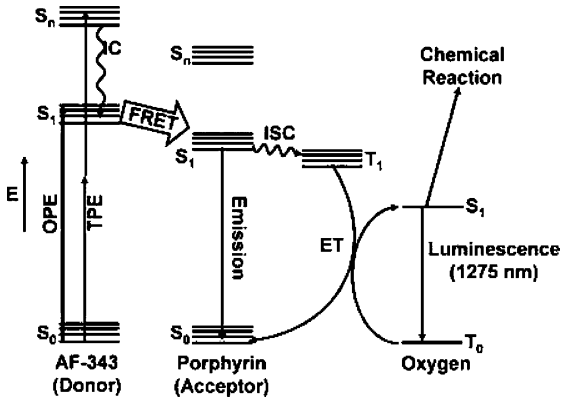


Figure 2. Jablonski diagram[9] illustrating the photophysical processes of the target photosensitizer: S_n, singlet energy level; OPE, one-photon excitation; TPE, two-photon excitation; IC, internal conversion; FRET, fluorescence resonance energy transfer; ISC, intersystem crossing; ET, energy transfer; T_n, triplet energy level.

This initially populated higher singlet state relaxes by internal conversion (IC) to populate its first excited singlet state (S_1). Subsequent FRET from the TPAC to the porphyrin allows indirect excitation of the porphyrin to its excited singlet state (S_1). Intersystem crossing (ISC) of the porphyrin to the triplet state (T_1) occurs, and then collisional deactivation by $^3O_2$ produces $^1O_2$* and a ground-state sensitizer. Described herein are the synthesis and evaluation of this water-soluble photosensitizer.

Results and Discussion

Synthesis of Target and Model Photosensitizers. Synthesis of the water-solubilizing TEG moieties began by alkylation of the three hydroxyl groups of methyl 3,4,5-trihydroxybenzoate by TEG-OTs **2** to produce **3** in 89% yield (Scheme 1). Reduction of the methyl ester of **3** (98% yield) and chlorination of the resulting benzyl alcohol by $SOCl_2$ produced **4** in 99% yield.

Synthesis of the target porphyrin **1** began with the selective alkylation at the 4-position of methyl 3,4,5-trihydroxybenzoate with the known TPAC AF-343 to afford **5** in 99% yield (Scheme 2). Alkylation of the remaining hydroxyl groups of **5** was accomplished using excess **4** to produce intermediate **6** in 90% yield. Reduction of the methyl ester of **6** with $LiAlH_4$ provided the benzyl alcohol derivative **7** in 99% yield. The alcohol functionality of **7** was then used in a nucleophilic addition to succinic anhydride, affording the acid derivative **8** in 84% yield. In our studies, intermediate **8** served as the model donor chromophore as well as the

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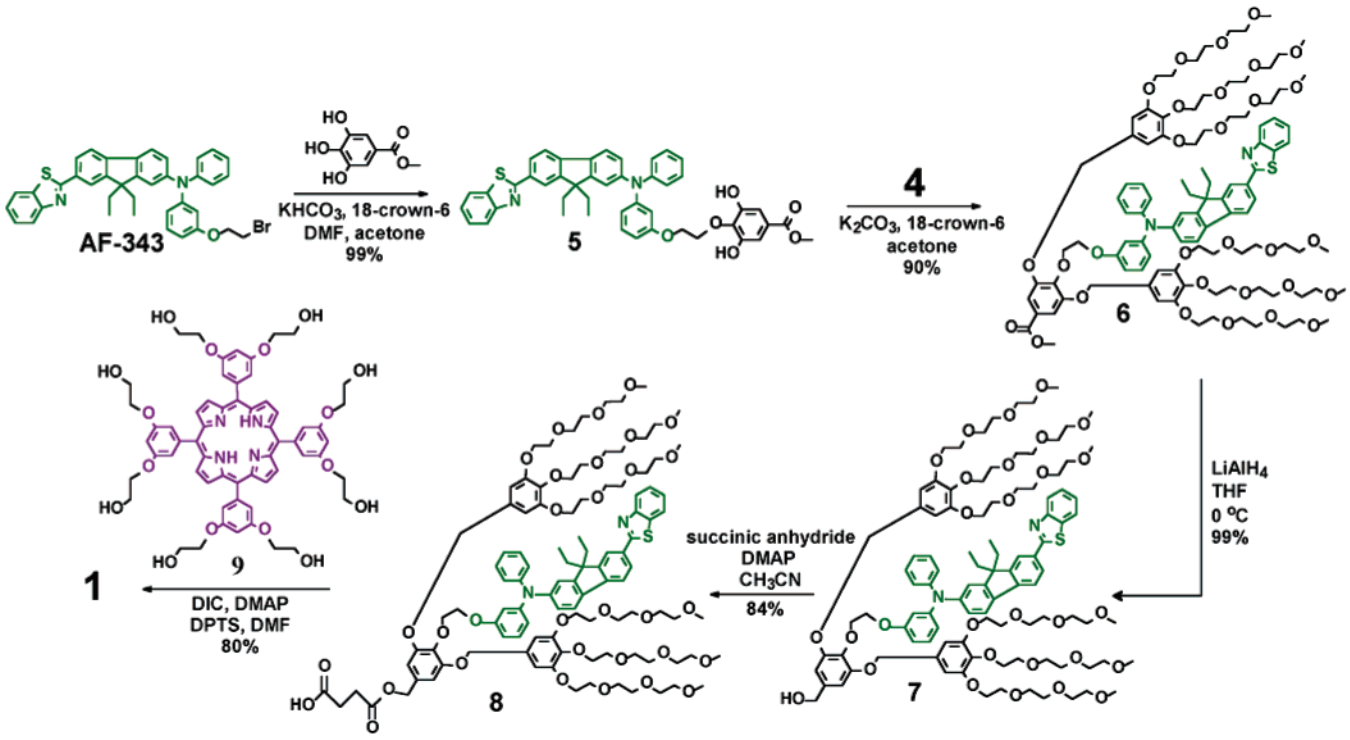
Scheme 2. Synthesis of Target Photosensitizer 1 and Model FRET Donor 8



synthetic precursor to the target **1**. Finally, the photosensitizer porphyrin **1** was produced in 80% yield through 1,3-diisopropylcarbodiimide (DIC)-mediated esterification between the tetrakis(3,5-bis(2′-hydroxy-1′-ethoxy)phenyl)-porphyrin[18] polyol **9** and the TPAC carboxylic acid **8**.

To fully characterize the photophysical capabilities of the biologically relevant target compound **1**, it was necessary to synthesize an analogous water-soluble porphyrin model **13** without the donor TPACs (Scheme 3). Synthesis of the model porphyrin **13** began with exhaustive alkylation of all three hydroxyl groups of methyl 3,4,5-trihydroxybenzoate by **4** to afford **10** in 89% yield. Reduction of the methyl ester of **10** to a benzyl alcohol with $LiAlH_4$ furnished the derivative **11** in 98% yield. After nucleophilic addition of **11** to succinic anhydride, acid derivative **12** was isolated in 85% yield. Finally, DIC-mediated esterification between **12** and porphyrin **9** provided model compound **13** in 30% yield.

Dendrimer Characterization. Dendrimers **1** and **13** were characterized by UV/vis spectroscopy, NMR spectroscopy, size exclusion chromatography (SEC), and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). All characterization methods indicated that the target and model compounds were nearly monodisperse. Some lower mass peaks were observed in the MALDI-TOF mass spectrum, which corresponded to porphyrins with seven full arms and one truncated arm. This arose from the trace amount of the benzyl alcohol intermediate carried on from the synthesis of **4**, which reacted with succinic anhydride and was subsequently coupled to the porphyrin.

Comparison by absorption spectroscopy to our previously reported singlet oxygen sensitizer[6] proved useful in confirming the number of donor chromophores surrounding each acceptor. Our previous organic-soluble photosensitizer shown in Figure 3 also contained eight AF-343 dyes surrounding a porphyrin ($(AF\text{-}343)_8TPP$) and was fully characterized as a monodisperse photosensitizer. The absorbance spectrum of **1** in chloroform was normalized at the porphyrin Q-band to that of $(AF\text{-}343)_8TPP$ as shown in Figure 4. Integration of the area underneath the overlapping Soret/AF-343 band was calculated for both photosensitizers. The integration ratio indicates that porphyrin **1** contains an average of 7.7 AF-343 dyes per porphyrin in contrast to the 8:1 ratio observed for our smaller and more readily accessed organic-soluble photosensitizer.

The refractive index traces obtained in SEC measurements show a polydispersity of 1.03 and 1.02 relative to polymethyl methacrylate (PMMA) for dendritic porphyrins **1** and **13**, respectively. Figure 5 shows the SEC traces obtained for **1** and **13**, while Figure 6 shows the 3D UV absorption chromatogram for **1**. Both the target compound **1** and its model **13** were soluble to at least 10^{-4} M in water as well as in a number of organic solvents such as methanol, acetonitrile, $CHCl_3$, *N*,*N*-dimethylformamide (DMF), THF, and benzene.

Photophysical Characterization. Preparative TLC purification of **1**, **8**, and **13** was done immediately prior to photophysical measurements to ensure their purity. Many of the spectroscopic experiments were conducted in D_2O because of the increased lifetime of singlet oxygen in deuterated solvents.[19] Steady-state absorption spectra of **1**, **8**, and **13** in D_2O are shown in Figure 7. The absorption spectrum of the dendrimer **1** is a composite of the donor **8** and acceptor **13** absorption spectra. This additive overlap indicates the absence of ground-state interactions or aggregation in D_2O.

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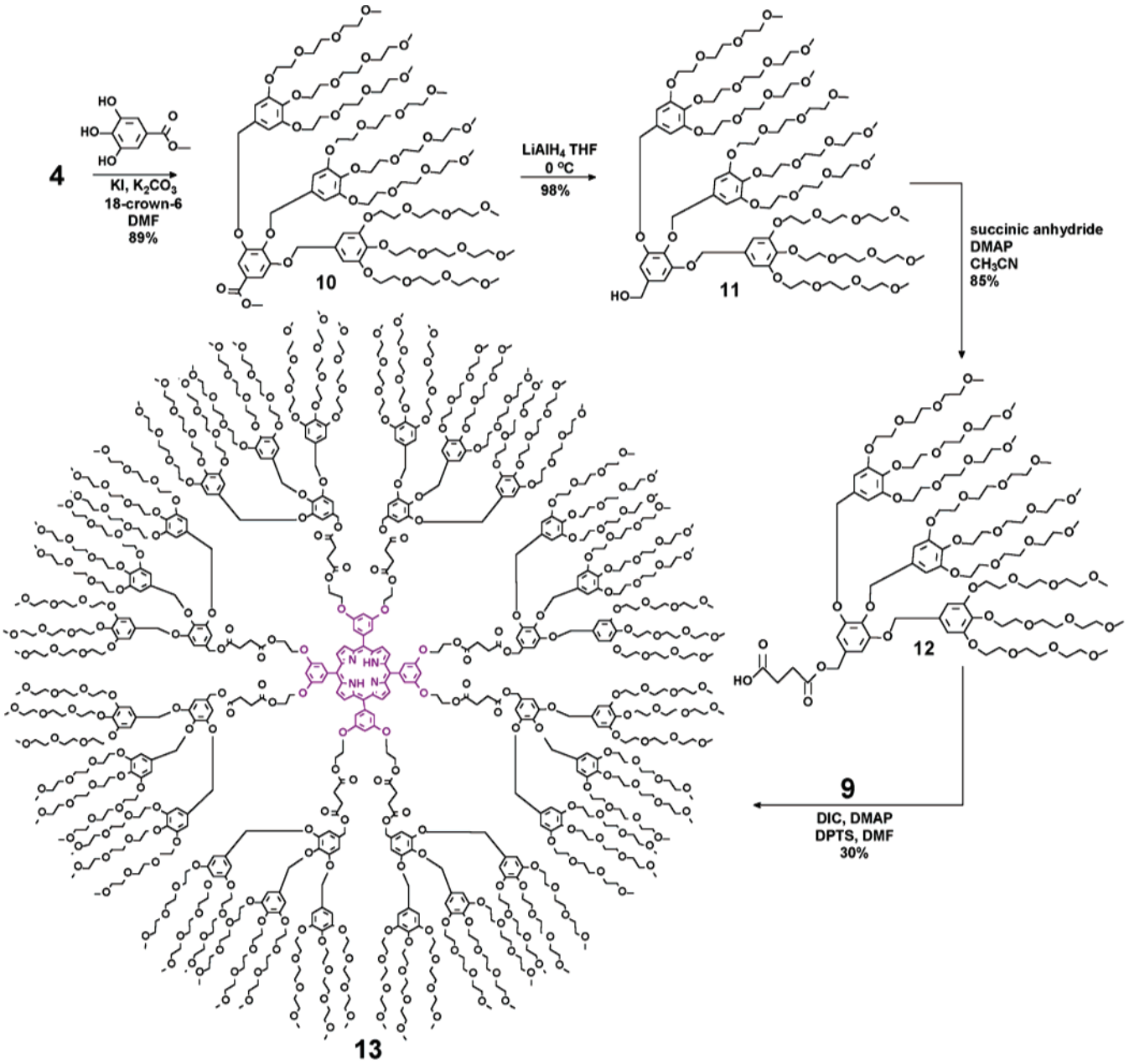
Scheme 3. Synthesis of a Model FRET Acceptor



A comparison was made between the fluorescence spectra for **1** and **8** in H_2O under one-photon excitation conditions (Figure 8). Irradiation of the donor **8** at its absorption maximum (390 nm) results in a broad emission with a maximum at 507 nm. When the donor chromophores in dendrimer **1** are excited under the same conditions, emission is observed exclusively from the acceptor; thus, quenching of donor emission in **1** can be attributed to efficient FRET (99%) to the porphyrin acceptor.

The emissions of **1**, **8**, and **13** were evaluated (Figure 9) under TPA conditions using 780 nm excitation from a femtosecond mode-locked Ti−sapphire laser (Spectra-Physics). As was the case for single-photon excitation, the donor emission in **1** is quenched, and emission is observed exclusively from the porphyrin. As expected, emission from dendrimer **1** is enhanced significantly compared to that of model compound **13**, due to the higher two-photon excitation efficiency of the donor chromophores and efficient FRET (99%). The emission spectrum observed mirrors that produced by single-photon excitation.

Chemical Oxidation. The ability of dendrimer **1** and model **13** to generate singlet oxygen in D_2O after excitation with a 780 nm laser was evaluated using 9,10-anthracenedipropionic acid (ADPA) as a chemical trap.[20] In this process, photobleaching of the anthracene absorbance can be used to effectively monitor singlet oxygen generation. Though the anthracene absorbs in the same region as the sensitizer, it exhibits a characteristic vibrational structure, which allows for resolution of each absorbance band. Equimolar solutions of ADPA containing **1**, **13**, and no sensitizer were irradiated at 780 nm (two-photon excitation conditions). In the presence of **1**, the anthracene absorbance intensity showed a continuous decrease over 3 h (Figure 10) while the porphyrin

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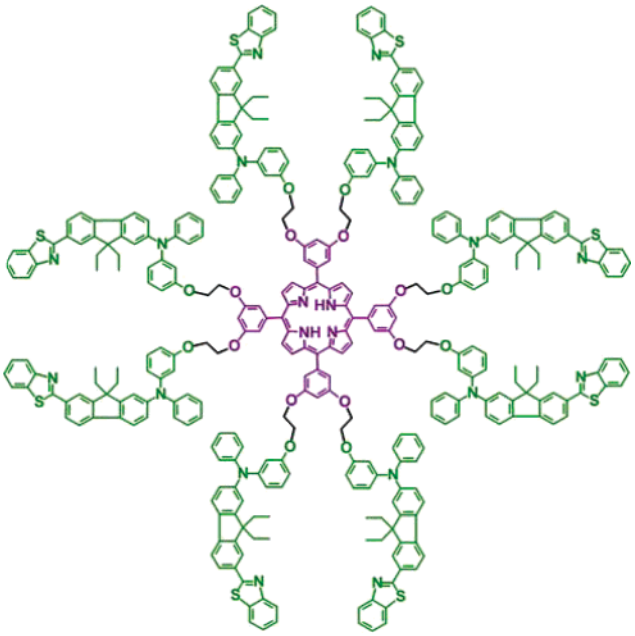

Figure 3. Structure of the organic-soluble photosensitizer.

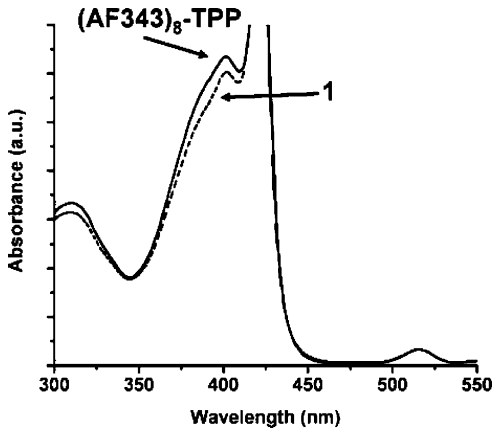

Figure 4. Absorption spectra of **1** and our previously reported[6] organic-soluble photosensitizer.

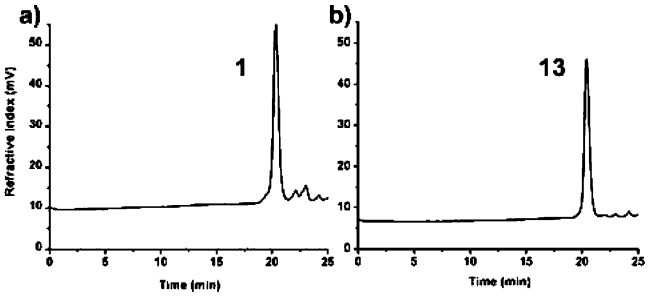

Figure 5. SEC refractive index traces of **1** and **13** in THF showing that both compounds are nearly monodisperse with PDI values of 1.03 and 1.02 (relative to PMMA), respectively.

Q-bands remained unchanged, suggesting that the porphyrin chromophore itself is photostable under these irradiation conditions.

After subtraction of the contributions of the target and model to each absorption spectrum, the extent of photobleached ADPA was determined (Figure 11). In contrast, only minimal photobleaching of ADPA was observed under 780 nm laser light illumination in the control experiments when model **13** was used as the sensitizer or when no sensitizer was added. This confirms that it is the presence

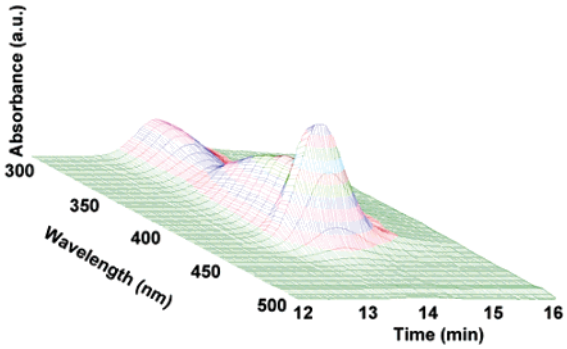

Figure 6. 3D UV SEC absorption chromatogram of porphyrin **1** in DMF demonstrating the nearly monodisperse nature of the chromophoric species.

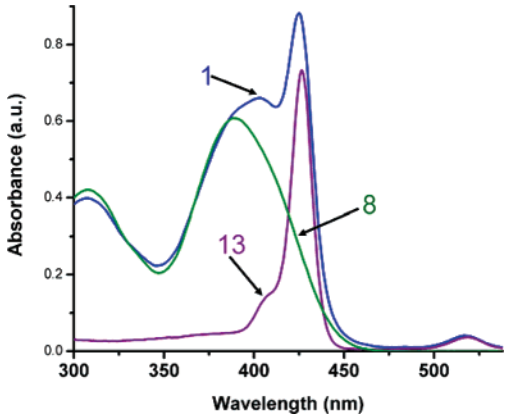

Figure 7. Absorption spectra of **1**, **8**, and **13** in D_2O at room temperature. Additive overlap of the appropriately normalized donor **8** and acceptor **13** spectra indicates the absence of aggregation in D_2O.

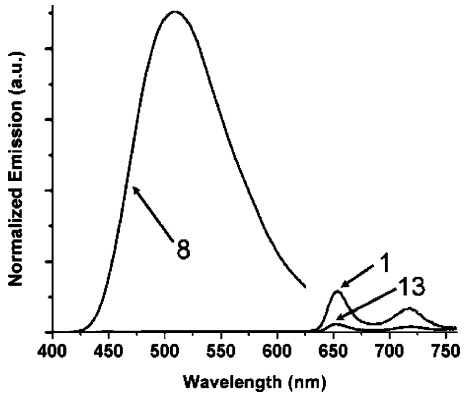

Figure 8. Single-photon-induced fluorescence spectra of **1**, **8**, and **13** upon 390 nm irradiation in H_2O at room temperature normalized to the absorption spectra (Figure 7). Efficient FRET is demonstrated by quenched donor emission and exclusive porphyrin emission in dendrimer **1**.

of the donor chromophores in **1** which significantly increases the ability of the dendrimer to utilize 780 nm light to generate singlet oxygen.

Oxygen Luminescence. Singlet oxygen can be observed spectroscopically by monitoring its emission at 1270 nm.[6,9] Singlet oxygen luminescence was observed from solutions of both **1** and **13** in D_2O upon single-photon excitation at 532 nm. Oxygen luminescence could not be observed under two-photon excitation conditions in D_2O due to the short lifetime of singlet oxygen in this solvent combined with the inherent low efficiency of two-photon absorption. However, when the solvent was changed to benzene-d_6, in which the lifetime of singlet oxygen is an order of magnitude longer,[19] weak emission could be observed under TPA conditions

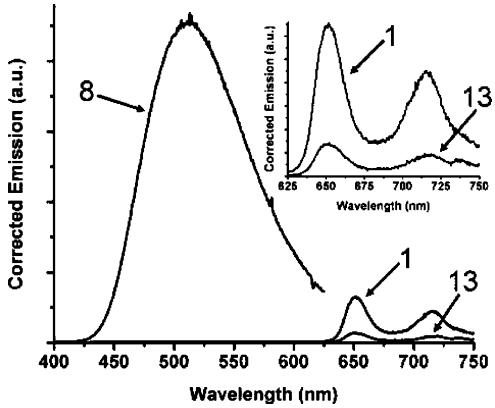

Figure 9. Two-photon-induced emission spectra of **1**, **8**, and **13** in D_2O at room temperature. Efficient two-photon-excited FRET is demonstrated by donor quenching and exclusive emission from the acceptor. Enhanced emission from the porphyrin in **1** is a result of the larger TPA cross-section imparted by the AF-343 donor chromophores. Inset: Magnification of the corrected two-photon-induced emission of **1** and **13** in D_2O at room temperature.

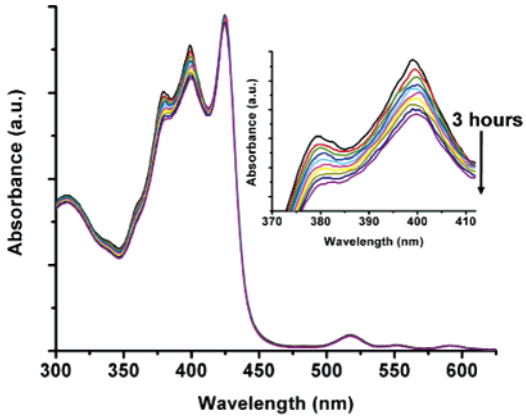

Figure 10. Photobleaching of ADPA by singlet oxygen generated upon two-photon excitation (780 nm) of **1** in D_2O at room temperature. The porphyrin appears to be photostable since no photobleaching is seen in the Q-band. Inset: Magnification of the consistent decrease in absorbance of ADPA over the course of 3 h of exposure.

(Figure 12). Under these conditions, the target **1** shows an enhanced ability to generate singlet oxygen relative to **13**, further confirming that the donor chromophores enhance the effective two-photon excitation efficiency of the porphyrin.

Conclusion and Outlook

A water-soluble photosensitizer incorporating TPACs has successfully been designed and synthesized. Two-photon-induced fluorescence spectroscopy measurements have demonstrated that efficient FRET occurs in dendrimer **1** as evidenced by donor quenching and enhanced porphyrin emission. Chemical trapping and singlet oxygen luminescence experiments have been used to detect singlet oxygen generation from **1** under IR irradiation. Both methods have independently demonstrated the occurrence of enhanced singlet oxygen generation under TPA conditions upon incorporation of TPACs in the dendritic porphyrin.

Sensitization of singlet oxygen via TPA has the potential to broaden the scope of PDT through increased depth of treatment and enhanced spatial resolution. Our modular design can be used in other aqueous systems where enhanced TPA could be beneficial. Future work in our laboratories will focus on improved sensitizers for two-photon-induced PDT and other applications of TPA in aqueous media.

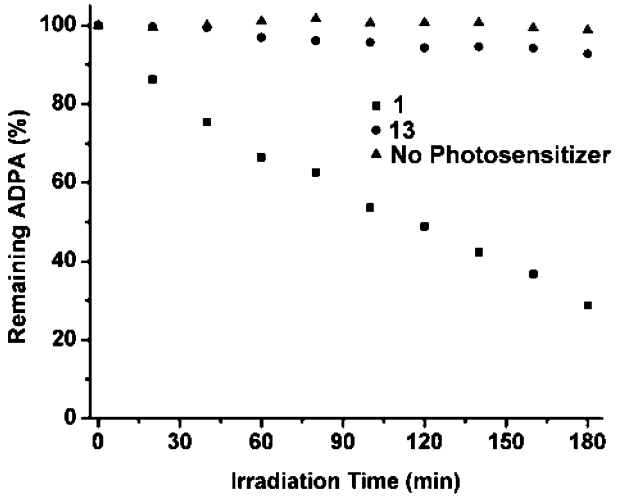

Figure 11. Percentage of unreacted ADPA in the presence of **1** and **13** and in the absence of photosensitizer after intervals of irradiation at 780 nm. ADPA shows negligible photobleaching under TPA conditions in the presence of **13** and if no photosensitizer is used. Photobleaching induced by singlet oxygen is drastically enhanced upon incorporation of TPACs as demonstrated by exposure of the ADPA solution containing dendrimer **1**.

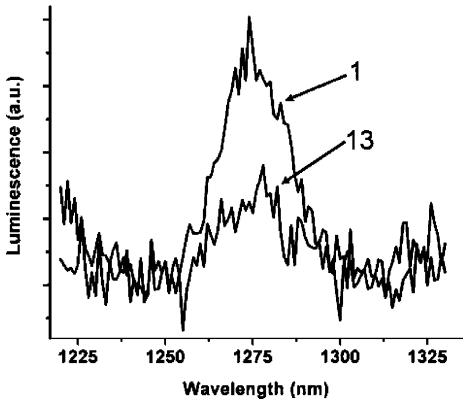

Figure 12. Luminescence of singlet oxygen in benzene-d_6 upon 780 nm irradiation of **1** and **13**. Enhanced oxygen luminescence reflects the greater TPA capability of dendrimer **1** when compared to model **13**.

Experimental Section

General Procedures. Unless otherwise noted, all reagents were used as received and without further purification, or were prepared according to literature procedures. Tetrahydrofuran (THF) and toluene were distilled under N_2 from sodium/benzophenone immediately prior to use. Acetonitrile was distilled under N_2 from CaH_2 immediately prior to use. Potassium carbonate was ground and stored in an oven (120 °C) prior to use. Chromatography was carried out with Merck silica gel for flash columns, 230−400 mesh, and preparative thin-layer chromatography (TLC) was conducted on 1000 μm Whatman plates. Unless otherwise specified, extracts were dried over $MgSO_4$ and solvents were removed with a rotary evaporator at aspirator pressure.

All NMR spectra were measured in $CDCl_3$ with TMS or solvent signals as the standards. High-resolution mass spectrometry (HRMS) was performed with a Micromass LCT using electrospray ionization (ESI) and Micromass ProSpec using fast atom bombardment (FAB). MALDI-TOF MS was performed on a PerSeptive Biosystems Voyager-DE, using *trans*-3-indoleacrylic acid as the matrix. Elemental analyses were performed by MHW Laboratories in

Phoenix, AZ, or by the staff at the University of California, Berkeley, Analytical Facility. SEC in THF solution was carried out at 1.0 mL/min using three 5 μm PLgel columns (7.5 × 300 mm) with pore sizes of 10^5, 10^3, and 500 Å, respectively, thermostated at 35 °C. The SEC system consisted of a Waters 510 pump, a Waters 717 autosampler, a Waters 486 UV/vis detector, a Wyatt DAWN-EOS light scattering detector, and a Wyatt Optilab differential refractive index detector. SEC in DMF solution was carried out at 1.0 mL/min using two 5 μm Plgel mixed-bed C columns (7.5 × 300 mm) thermostated at 70 °C. The SEC system consisted of a Waters 510 pump, a Waters U6K injector, a Waters 486 UV/vis detector, and a Waters 410 differential refractive index detector. In some cases a Waters 996 PDA detector was used with the same columns. The solvents used for absorbance and luminescence measurements were spectroscopic grade. Absorption spectra were recorded on a Cary 50 UV/vis spectrometer. Emission and excitation spectra were obtained using an ISA/SPEX Fluorolog 3.22 equipped with a 450 W Xe lamp, double excitation and double emission monochromators, and a digital photon-counting photomultiplier. Slit widths were set to 2 nm band-pass on both excitation and emission monochromators. Samples for absorbance and emission experiments were measured in standard 1 cm quartz cells. All measurements were performed at room temperature. Two-photon excitation at 780 nm was performed using a Ti−sapphire laser (Spectra-Physics-Tsunami) pumped by a frequency-doubled diode-pumped solid-state laser (Spectra-Physics-Millennia). It provided 90 fs pulses at an 82 MHz repetition rate with an average power of 1.3 W/cm^2. For single-photon fluorescence measurements, the optical density was kept below 0.1 (2×10^{-7} M in **1** or **13**) and the samples were degassed by bubbling N_2 into the cuvette for 5 min before measurement. Two-photon fluorescence measurements were obtained at optical densities of 0.9 (1.7×10^{-6} M porphyrin concentration). Solutions used for ADPA photobleaching experiments were 3×10^{-5} M in ADPA and 4×10^{-6} M in either **1** or **13**. Oxygen luminescence experiments were performed in solutions of benzene-d_6 using 10^{-4} M concentrations of **1** or **13**.

[G-1]-(TEG)$_3$-CO$_2$Me (3). To 34.58 g (108.6 mmol) of TEG-OTs in 300 mL of acetone were added 6.34 g (34.47 mmol) of methyl 3,4,5-trihydroxybenzoate, 23.83 g (172.4 mmol) of K_2CO_3, and 1.82 g (6.88 mmol) of 18-crown-6. The reaction mixture was stirred at reflux for 2 days under N_2. The crude reaction mixture was filtered, concentrated, and redissolved in chloroform (500 mL) before extraction with a saturated solution of Na_2CO_3 (5 × 500 mL) followed by a saturated solution of $NaHCO_3$ (3 × 250 mL) and then brine (1 × 250 mL). The organic phase was dried, concentrated, and chromatographed (eluting with 95:5 $CHCl_3$/MeOH) to yield 19.0 g of a colorless oil (89%). ^{1}H NMR (500 MHz): δ 3.35 (s, 9), 3.52, (m, 6), 3.63 (m, 12), 3.71 (m, 6), 3.74 (t, 2, J = 4.9 Hz), 3.85 (t, 4, J = 4.9 Hz), 3.86 (s, 3), 4.17 (m, 6), 7.27 (s, 2). ^{13}C NMR: δ 59.0, 67.9, 68.7, 69.5, 70.4, 70.5, 70.6, 70.8, 71.8, 72.3, 108.9, 124.9, 142.5, 152.2, 166.5. HRMS (EI): calcd for $C_{29}H_{50}O_{14}$, m/z = 622.3200; found, m/z = 622.3214. Anal. Calcd for $C_{29}H_{50}O_{14}$: C, 55.94; H, 8.09. Found: C, 55.88; H, 8.08.

[G-1]-(TEG)$_3$-CH$_2$OH. To an ice-cooled suspension of $LiAlH_4$ (1.21 g, 32 mmol) in 50 mL of dry THF was added a solution of **3** (16.60 g, 108.6 mmol) in 150 mL of dry THF dropwise over 1 h. The reaction mixture was allowed to warm to room temperature following the addition. After 6 h, the reaction mixture was diluted with 300 mL of THF and small portions of a 1:1 mixture of $Na_2SO_4 \cdot 10H_2O$/Celite until the hydride was fully quenched. The reaction mixture was filtered and concentrated to give the product as a colorless oil (15.55 g, 98%). ^{1}H NMR (500 MHz): δ 3.34 (s, 9), 3.51 (m, 6), 3.61 (m, 12), 3.69 (m, 6), 3.75 (t, 2, J = 4.9 Hz), 3.80 (t, 4, J = 4.9 Hz), 4.11 (m, 6), 4.53 (s, 2), 6.58 (s, 2). ^{13}C NMR: δ 58.9, 65.0, 68.7, 69.7, 70.4, 70.6, 70.7, 71.8, 72.1, 106.3, 136.7, 137.5, 152.5. HRMS (EI): calcd for $C_{28}H_{50}O_{13}$, m/z = 594.3251; found, m/z = 594.3244. Anal. Calcd for $C_{28}H_{50}O_{13}$: C, 56.55; H, 8.47. Found: C, 56.32; H, 8.41.

[G-1]-(TEG)$_3$-CH$_2$Cl (4). To a solution of [G-1]-(TEG)$_3$-CH$_2$-OH (9.80 g, 16.48 mmol) in 500 mL of dry CH_2Cl_2 was added a solution of $SOCl_2$ (12 mL, 165 mmol) in 200 mL of CH_2Cl_2 over 15 min. After 4 h, the reaction mixture was concentrated to give the product as a yellow oil (10.0 g, 99%). ^{1}H NMR (500 MHz): δ 3.35 (s, 9), 3.52 (m, 6), 3.62 (m, 12), 3.71 (m, 6), 3.76 (t, 2, J = 4.9 Hz), 3.82 (t, 4, J = 4.9 Hz), 4.14 (m, 6), 4.47 (s, 2), 6.60 (s, 2). ^{13}C NMR: δ 58.9, 68.8, 69.9, 70.40, 70.43, 70.45, 70.50, 70.59, 70.62, 70.7, 71.8, 72.2, 108.21, 132.68, 138.42, 152.6. HRMS (EI): calcd for $C_{28}H_{49}ClO_{12}$, m/z = 612.2912; found, m/z = 612.2903. Anal. Calcd for $C_{28}H_{50}O_{13}$: C, 54.85; H, 8.06. Found: C, 54.65; H, 8.24.

Methyl 4-(AF-343)-3,5-dihydroxybenzoate (5). To 20.0 mg (31.0 μmol) of AF-343 in 2 mL of acetone and 0.5 mL of DMF were added 114 mg (619 μmol) of methyl 3,4,5-trihydroxybenzoate, 52.0 mg (515 μmol) of $KHCO_3$, and 8 mg (30.3 μmol) of 18-crown-6. The reaction mixture was charged with N_2 and stirred at 50 °C for 5 days. Upon being cooled to room temperature, the solution was poured into a separatory funnel containing 30 mL of CH_2Cl_2 and washed sequentially with saturated $NaHCO_3$ (1 M), 30 mL of saturated $NaHCO_3$, and then 30 mL of brine. The resulting solution was dried over Na_2SO_4, concentrated, taken up in a minimum amount of CH_2Cl_2, and passed through a silica plug to yield 23.0 mg (99%) of a fluorescent yellow solid (**5**). ^{1}H NMR (500 MHz): δ 0.38 (t, 6, J = 7.5 Hz), 1.96 (m, 2), 2.11 (m, 2), 3.87 (s, 3), 4.19 (t, 2, J = 4 Hz), 4.36 (t, 2, J = 4 Hz), 6.41 (s, 2), 6.68 (d, 1, J = 6 Hz), 6.77 (d, 2, J = 2 Hz), 7.08 (t, 2, J = 7 Hz), 7.15−7.23 (m, 6), 7.30 (t, 2, J = 8 Hz), 7.39 (t, 1, J = 2 Hz), 7.51 (t, 1, J = 7.5 Hz), 7.64 (d, 1, J = 8 Hz), 7.72 (d, 1, J = 8 Hz), 7.92 (d, 1, J = 8 Hz), 8.03 (d, 1, J = 6 Hz), 8.09 (t, 2, J = 3.5 Hz). ^{13}C NMR (125 MHz): δ 8.59, 29.68, 32.60, 52.16, 56.44, 66.91, 72.57, 109.18, 109.47, 109.57, 117.41, 119.16, 119.46, 121.10, 121.47, 121.54, 122.90, 123.30, 123.70, 124.55, 125.00, 126.30, 126.91, 127.30, 129.34, 130.19, 131.54, 134.88, 135.76, 137.44, 144.39, 147.49, 147.75, 149.37, 150.72, 152.16, 154.15, 158.45, 162.64, 166.55. HRMS (FAB): calcd for $C_{46}H_{40}N_2O_6S$, m/z = 748.2607 (M^+); found, m/z = 748.2626.

[G-2]-4-(AF-343)-3,5-(TEG)$_6$-CO$_2$Me (6). To 23.0 mg (31.0 μmol) of methyl 4-(AF-343)-3,5-dihydroxybenzoate (**5**) in 3 mL of acetone were added 0.53 mg (86.4 μmol) of **4**, 8.50 mg (6.14 μmol) of K_2CO_3, and approximately 1 mg (1.54 μmol) of 18-crown-6. The reaction mixture was charged with N_2 and stirred at 55 °C for 60 h. After being cooled to room temperature, the mixture was added to water and extracted into 30 mL of $CHCl_3$/2-propanol (1:1). The extracted layer was dried, concentrated, and chromatographed (eluting with 95:5 CH_2Cl_2/MeOH) to yield 52.0 mg (90%) of a yellow solid (**6**). ^{1}H NMR (400 MHz): δ 0.31 (t, 6, J = 7.5 Hz), 1.87 (m, 2), 2.03 (m, 2), 3.32−4.12 (m, 95), 4.36 (t, 2, J = 4 Hz), 4.94 (s, 4), 6.43 (d, 1, J = 8.4 Hz), 6.59 (m, 6), 6.98 (t, 2, J = 7 Hz), 7.04−7.10 (m, 4), 7.19 (t, 2, J = 8 Hz), 7.25 (m, 2), 7.34 (t, 1, J = 7.6 Hz), 7.45 (t, 1, J = 8 Hz), 7.54 (d, 1, J = 8.4 Hz), 7.65 (d, 1, J = 7.6 Hz), 7.87 (d, 1, J = 8 Hz), 7.97 (d, 1, J = 8 Hz), 8.03−8.04 (m, 2). ^{13}C NMR (100 MHz): δ 8.57, 27.16, 29.65, 32.54, 46.58, 52.19, 56.33, 58.97, 67.37, 68.76, 68.83, 69.64, 70.44, 70.47, 70.48, 70.61, 70.63, 70.73, 71.24, 71.32, 71.85, 71.88, 72.28, 106.73, 108.22, 108.67, 109.32, 110.25, 116.50, 118.88, 119.38, 120.99, 121.34, 121.50, 122.87, 123.04, 123.42, 124.40, 124.92, 125.18, 126.23, 127.21, 129.22, 129.85, 131.42, 132.04, 134.85, 135.39, 137.96, 142.37, 144.41, 147.52, 147.83, 148.95, 150.62, 151.97, 152.09, 152.62, 152.70, 154.17, 159.48, 166.36, 168.77.

HRMS (FAB): calculated for $C_{102}H_{137}N_2O_{30}Sna$, $m/z = 962.4431$ ($M + H^+ + Na^+$); found, $m/z = 962.4495$ ($M + H^+ + Na^+$). Anal. Calcd for $C_{102}H_{136}N_2O_{30}S$: C, 64.40; H, 7.21; N, 1.47; S, 1.69. Found: C, 64.80; H, 7.15; N, 1.34; S, 1.46.

[G-2]-4-(AF-343)-3,5-(TEG)$_6$-CH$_2$OH (7). To 3 mL of THF was added 75.0 mg, (39.4 μmol) of **6**. The solution was cooled to 0 °C, and 1.50 mg (39.4 mmol) of $LiAlH_4$ was added. The solution was warmed to room temperature, where it remained for 1.5 h. Approximately 2 g of $Na_2SO_4 \cdot 10H_2O$/Celite was added to the solution, and the resulting solution was filtered and washed with CH_2Cl_2. The filtrate was concentrated, resulting in 74.5 mg (99%) of a viscous yellow oil (**7**). The product exhibited high purity as determined by NMR and was used without further purification. ^{1}H NMR (400 MHz): δ 0.34 (t, 6, $J = 7.5$ Hz), 1.93 (m, 2), 2.04 (m, 2), 3.32−4.12 (m, 92), 4.30 (t, 2, $J = 4$ Hz), 4.05 (s, 2), 4.94 (s, 4), 6.38 (d, 1, $J = 8.4$ Hz), 7.49−7.62 (m, 6), 7.00 (t, 2, $J = 7$ Hz), 7.07 (m, 4), 7.20−7.24 (m, 4), 7.36 (t, 1, $J = 7.6$ Hz), 7.46 (t, 1, $J = 8$ Hz), 7.55 (m, 1), 7.67 (m, 1), 7.89 (d, 1, $J = 8$ Hz), 7.98 (d, 1, $J = 8$ Hz), 8.05 (m, 2). ^{13}C NMR (100 MHz): δ 8.43, 29.48, 32.39, 46.41, 53.32, 56.18, 58.80, 58.82, 64.61, 67.13, 68.66, 68.74, 69.50, 70.29, 70.32, 70.35, 70.47, 70.49, 70.57, 70.62, 71.07, 71.14, 71.70, 71.74, 72.11, 74.45, 106.63, 108.13, 108.61, 110.24, 116.35, 118.72, 119.23, 120.85, 121.20, 121.35, 122.71, 122.87, 123.26, 124.24, 124.78, 126.08, 127.07, 129.07, 129.70, 131.27, 132.46, 132.52, 134.70, 135.20, 136.98, 137.38, 137.80, 138.40, 144.27, 147.42, 147.74, 148.78, 150.49, 151.83, 152.31, 152.49, 152.52, 154.02, 159.49, 168.58. HRMS (FAB): calcd for $C_{101}H_{137}N_2O_{29}SK$, $m/z = 956.4326$ ($M + H^+ + K^+$); found, $m/z = 956.4323$ ($M + H^+ + K^+$). Anal. Calcd for $C_{101}H_{136}N_2O_{29}S$: C, 64.73; H, 7.31; N, 1.49; S, 1.71. Found: C, 64.54; H, 7.53; N, 1.47; S, 1.69.

[G-2]-4-(AF-343)-3,5-(TEG)$_6$-CO$_2$H (8). Prior to use, 413 mg (220 μmol) of **7** was stored overnight at 40 °C under 0.2 Torr in the reaction vessel. To the vessel were added 30.9 mg (309 μmol) of succinic anhydride, 10.8 mg (88.2 μmol) of 4-(dimethylamino)-pyridine (DMAP), and 5 mL of acetonitrile. After being heated to reflux for 60 h, the solution was concentrated and loaded onto a silica plug. The plug was flushed with ethyl acetate to remove excess succinic anhydride, and the product was eluted with 9:1 CH_2Cl_2/MeOH. The eluent was dried, concentrated, and chromatographed (eluting with 9:1 CH_2Cl_2/MeOH) to yield 366 mg (84%) of a yellow viscous oil (**8**). ^{1}H NMR (400 MHz): δ 0.33 (t, 6, $J = 7.2$ Hz), 1.90 (m, 2), 2.04 (m, 2), 2.53 (m, 4), 3.34−4.14 (m, 91), 4.16 (t, 2, $J = 4$ Hz), 4.33 (t, 2, $J = 4$ Hz), 4.93 (s, 2), 4.96 (s, 4), 6.49−6.52 (m, 3), 6.61 (s, 4), 6.66 (m, 2), 6.99 (m, 2), 7.06−7.13 (m, 4), 7.21 (t, 2, $J = 8$ Hz), 7.36 (t, 1, $J = 8$ Hz), 7.48 (t, 1, $J = 8$ Hz), 7.56 (d, 1, $J = 8.4$ Hz), 7.67 (d, 1, $J = 8$ Hz), 7.89 (d, 1, $J = 8.4$ Hz), 7.98 (d, 1, $J = 8$ Hz), 8.04−8.07 (m, 2). ^{13}C NMR (100 MHz): δ 8.57, 28.72, 29.16, 29.64, 32.53, 56.33, 58.94, 65.99, 67.19, 68.66, 69.62, 70.40, 70.56, 70.66, 71.21, 71.81, 72.22, 106.44, 107.97, 108.78, 110.29, 116.48, 118.85, 119.38, 120.99, 121.36, 121.50, 122.84, 123.02, 123.40, 124.40, 124.94, 126.25, 127.24, 129.22, 129.84, 131.34, 131.66, 132.67, 134.80, 135.34, 137.65, 137.96, 144.43, 147.56, 147.87, 148.94, 150.62, 151.98, 152.42, 152.62, 154.10, 159.61, 168.85, 172.00, 173.71. HRMS (ESI): calcd for $C_{105}H_{141}N_2O_{32}SK$, $m/z = 1006.4407$ ($M + H^+ + K^+$); found, $m/z = 1006.4419$ ($M + H^+ + K^+$). Anal. Calcd for $C_{105}H_{140}N_2O_{32}S$: C, 63.88; H, 7.15; N, 1.42; S, 1.62. Found: C, 63.98; H, 7.21; N, 1.33; S, 1.77. UV/vis (water): λ_{max} (ϵ) = 389 (29700), 308 (19900). Fluorimetry (water, $\lambda_{ex} = 390$): $\lambda_{em} = 507$.

[G-2]-4-(AF-343)$_8$-3,5-(TEG)$_{48}$-TPP (1). Prior to use, 48.7 mg (24.7 μmol) of **8** was stored overnight at 40 °C under 0.2 Torr in the reaction vessel. To the vessel were added 2.25 mg (2.06 μmol) of tetrakis(3,5-bis(2′-hydroxy-1′-ethoxy)phenyl)porphyrin (**9**), 12.6 mg (103 μm) of DMAP, 7.26 mg (24.7 μmol) of 4-(dimethylamino)pyridine-*p*-toluenesufonate (DPTS), and 250 μL of DMF. The solution was stirred for 10 min before addition of 1.5 μL (9.58 μmol) of DIC. Every 12 h, 1 μL (6.39 μmol) of DIC was added until 40 h later when the product stopped increasing to higher molecular weight as determined by DMF SEC. The crude product was condensed, loaded onto a preparative TLC plate, and eluted with 9:1 CH_2Cl_2/MeOH. Silica from the TLC plate was placed on a fritted funnel and washed with 9:1 CH_2Cl_2/MeOH to remove the product. The red band corresponding to product was removed and further purified with a second preparative TLC plate. Condensation of the eluent yielded 27.4 mg (80%) of a red oil (**1**). ^{1}H NMR (500 MHz): δ 0.35 (br, 48), 1.91 (m, 24), 2.04 (m, 24), 2.60 (br, 32), 3.32−4.32 (m, 928), 4.50 (br, 16), 4.96 (br, 48), 6.47−6.68 (m, 72), 6.92 (br, 4), 7.01 (br, 16), 7.07 (br, 32), 7.22 (m, 16), 7.38 (m, 16), 7.47 (m, 8), 7.57 (m, 8), 7.68 (m, 8), 7.87−7.92 (m, 8), 8.0 (m, 8), 8.06 (m, 16), 8.91 (br, 8). MS (MALDI): calcd $m/z = 16913$ ($M + K^+$); found, $m/z = 16938$. UV/vis (water): λ_{max} (ϵ) = 308 (148000), 404 (264000), 425 (353000), 517 (16000). Fluorimetry (water, $\lambda_{ex} = 390$): $\lambda_{em} = 654$, 718. THF SEC: PDI = 1.03, $M_w = 10527$, $M_n = 10249$.

[G-2]-(TEG)$_9$-CO$_2$Me (10). To 5 mL of DMF were added 82.5 mg (448 μmol) of methyl 3,4,5-trihydroxybenzoate, 967 mg (1.58 mmol) of **4**, 186 mg (1.30 mmol) of K_2CO_3, 29.6 mg (110 μmol) of 18-crown-6, and 32.4 mg (195 μmol) of KI. The solution was heated to 60 °C for 5.5 h. The crude product was concentrated and chromatographed (eluting with 9:1 CH_2Cl_2/MeOH) to yield 760 mg (89%) of a brown oil (**10**). ^{1}H NMR (400 MHz): δ 3.35−4.155 (m, 138), 5.05 (s, 6), 6.66 (s, 2), 6.69 (s, 4), 7.36 (s, 2). ^{13}C NMR (100 MHz): δ 58.78, 68.48, 68.76, 69.47, 69.50, 70.33, 70.48, 70.52, 70.59, 71.16, 71.74, 71.76, 72.11, 72.16, 74.65, 106.58, 106.97, 109.32, 126.13, 131.88, 132.71, 137.73, 137.87, 142.05, 152.21, 152.30, 152.60, 166.15. HRMS (ESI): calcd for $C_{92}H_{152}O_{41}Na_2$, $m/z = 979.4797$ ($M + 2Na^+$); found, $m/z = 979.4832$ ($M + 2Na^+$). Anal. Calcd for $C_{92}H_{152}O_{41}$: C, 57.73; H, 8.00. Found: C, 57.84; H, 7.97.

[G-2]-(TEG)$_9$-CH$_2$OH (11). To 1.5 mL of THF was added 292 mg (152 μmol) of **10**, and the resulting solution was cooled to 0 °C. Directly to the solution was added 5.79 mg (152 μmol) of $LiAlH_4$. Approximately 2 g of $Na_2SO_4 \cdot 10H_2O$/Celite was added to the vessel after the solution was stirred for 35 min. The mixture was filtered and washed with CH_2Cl_2. Concentration of the solution yielded 283 mg (98%) of a colorless oil (**11**). No further purification was needed after ^{1}H NMR indicated a highly pure product. ^{1}H NMR (400 MHz): δ 3.35−4.15 (m, 136), 4.54 (s, 2), 4.97 (s, 2), 4.99 (s, 4), 6.64 (s, 2), 6.66 (s, 6). ^{13}C NMR (100 MHz): δ 58.83, 64.48, 67.77, 68.48, 68.66, 69.51, 69.54, 70.35, 70.41, 70.50, 70.54, 70.59, 71.13, 71.75, 71.79, 72.16, 106.49, 106.64, 107.179, 132.54, 133.15, 136.99, 137.36, 137.64, 137.74, 152.15, 152.57. HRMS (ESI): calcd for $C_{91}H_{152}O_{40}Na_2$, $m/z = 965.4822$ ($M + 2Na^+$); found, $m/z = 965.4828$ ($M + 2Na^+$). Anal. Calcd for $C_{91}H_{152}O_{40}$: C, 57.95; H, 8.12. Found: C, 58.15; H, 8.15.

[G-2]-(TEG)$_9$-CO$_2$H (12). To 4.5 mL of acetonitrile were added 979 mg (519 μmol) of **11**, 72.7 mg (727 μmol) of succinic anhydride, and 25.4 mg (208 μmol) of DMAP. The solution was refluxed for 10 h, and the crude product was concentrated. Chromatography (eluting with 9:1 CH_2Cl_2/MeOH) yielded 880 mg (85%) of a colorless oil (**12**). ^{1}H NMR (400 MHz): δ 2.50 (m, 4), 3.23−4.02 (m, 136), 4.85 (s, 2), 4.89 (s, 6), 6.48 (s, 2), 6.54 (s, 6). ^{13}C NMR (100 MHz): δ 28.65, 29.09, 58.90, 64.57, 66.13, 66.28, 68.61, 68.75, 69.64, 70.42, 70.58, 70.60, 70.68, 71.29, 71.84, 72.27, 74.84, 106.54, 107.23, 107.67, 108.14, 131.30, 131.67, 132.53, 133.24, 137.76, 137.83, 137.97, 138.22, 152.39, 152.68, 172.05, 173.88, 174.12. HRMS (ESI): calcd for $C_{95}H_{156}O_{43}Na_2$, $m/z = 1015.4902$ ($M + 2Na^+$); found, $m/z = 1015.4902$ ($M + 2Na^+$).

Anal. Calcd for $C_{95}H_{156}O_{43}$: C, 57.45; H, 7.92. Found C, 57.51; H, 7.88.

[G-2]-(TEG)$_{72}$-TPP (13). To 500 μL of DMF were added 171 mg (85.8 μmol) of **12**, 7.83 mg (7.15 μmol) of **9**, 43.7 mg (358 μmol) of DMAP, and 25.3 mg (85.8 μmol) of DPTS, and the resulting solution was allowed to stir for 10 min before addition of 7 μL (44.7 μmol) of DIC. After 12 and 24 h, 5 μL (31.9 μmol) of DIC was added. The reaction showed completion by DMF SEC 34 h after addition of the first aliquot of DIC. The solution was concentrated and chromatographed (eluting with 9:1 CH_2Cl_2/MeOH). The resulting product was further purified on a preparative TLC plate (eluting with 9:1 CH_2Cl_2/MeOH) to yield 35.8 mg (30%) of a red oil (**13**). ^{1}H NMR (400 MHz): δ −2.88 (s, 2), 2.72 (br, 32), 3.34−4.53 (m, 1112), 4.36 (br, 16), 4.53 (br, 16), 4.94 (s, 16), 5.00 (s, 48), 6.55 (br, 4), 6.65 (s, 50), 6.75 (d, 5, J = 8 Hz), 6.96 (br, 4), 7.05 (d, 5, J = 8 Hz), 7.42 (br, 8), 8.92 (s, 8). MS (MALDI): calcd for $C_{821}H_{1299}N_4O_{352}$, m/z = 16857 (M + H$^+$); found, m/z = 16831. UV/vis (water): λ_{max} (ϵ) = 427 (425000), 519 (21000). Fluorimetry (water, λ_{ex} = 425): λ_{em} = 651, 718. THF SEC: PDI = 1.02, M_n = 9663, M_w = 9836.

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