Diels-Alder Adducts of Vinyl Porphyrins: Synthesis and in Vivo Photodynamic Effect against a Rat Bladder Tumor

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Benzoporphyrin derivatives have been proposed as potential photosensitizers for photodynamic therapy. We have prepared a number of benzoporphyrin derivatives and tested their effect, in combination with red light, on the *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) induced rat bladder tumor (AY-27) transplanted into Fischer CDF(F344)/CrlBr rats. Tetracyanoethylene (TCNE) adducts showed very little activity, which may be attributable to their poor tumor uptake or to chemical instability of the adduct under physiological conditions. However, dimethyl acetylenedicarboxylate (DMAD) adducts tested showed greater cytotoxic effect than hematoporphyrin derivative and similar activities to metallopurpurins when tested under the same protocol.

Photodynamic therapy (PDT) is a developing technique for the selective destruction of neoplasms.[1] The method involves selective incorporation (or retention) of a photosensitizer in the tissue to be destroyed, at which time light of the appropriate wavelength is directed at the target site. The photosensitizer absorbs this energy and, in combination with molecular oxygen, generates a photodynamic effect which leads to subsequent cell death.[2]

Currently, the most studied photosensitizer, Photofrin II is in stage III clinical trial. However, a number of disadvantages exist with the use of this material, including the difficulty in characterizing its various components[3,4] and its lack of absorption in the far-red region of the electromagnetic spectrum, the region where light penetration of tissue is optimal.[5]

Consequently, one field of intense research is the development of sensitizers of known composition with stronger absorptions in the therapeutically useful (red) region of the spectrum which also retain the useful properties of Photofrin II (e.g. low systemic toxicity, efficient photosensitization). Phthalocyanines[6] and purpurins[7] are two classes of compound which have been reported to have good in vivo photodynamic action; however, other sensitizers continue to be proposed.[8-10] More recently, a series of benzoporphyrin derivatives, derived from the Diels-Alder addition of dimethyl acetylenedicarboxylate (DMAD) to protoporphyrin IX dimethyl ester have been reported to be phototoxic in vitro to a number of human normal and malignant cell lines.[11]

We ourselves have developed a similar series of compounds from Diels-Alder additions of tetracyanoethylene (TCNE) or DMAD to protoporphyrin dimethyl ester (**1**) (**1** → **3**, **2**), heptaethylvinylporphyrin **4** (**4** → **5**, **6**) and triethyltetramethylvinylporphyrin **8** (**8** → **10**, **11**). We here report their synthesis and in vivo activities against the FANFT-induced urothelial cell carcinoma, transplanted into Fisher CDF(F344)/CrlBr rats.

Results and Discussion

Synthesis: (a) Porphyrin Dienes (Schemes I and II). Three porphyrin systems, each bearing at least one vinyl group, were chosen for these studies. Of these, protoporphyrin IX dimethyl ester (**1**) and 2-vinyl-3,7,8,12,13,17,18-heptaethylporphyrin (**4**) were conveniently prepared from hematoporphyrin and 2,3-dihydroxy-2,3,7,8,12,13,17,18-octaethylchlorin (**7a**) as previously reported.[12,13] The only reported preparation of 2-vinyl-7,12,17-triethyl-3,8,13,18-tetramethylporphyrin (**8**) however, involves a stepwise synthesis of the corresponding 2-hydroxyethylporphyrin, with dehydration as the final step.[14] As a part of this study we have developed an alternative two-step synthesis of **8** by dehydration of diol **7b** formed by addition of osmium tetroxide to etioporphyrin I.[13] Treatment of **7b** in vacuo at 140 °C for 5 h resulted in formation of two products. The faster moving band was identified as vinyl derivative **8** by its ^{1}H NMR spectrum. The slower moving fraction was characterized as ketone **9**. Formulation of the product as the isomer resulting from ethyl migration, rather than that derived from methyl migration, was made on the basis of the extensive survey of Chang et al. of migratory aptitudes in related systems.[18]

Diels-Alder Adducts of Heptaethylvinylporphyrin 4. [4 + 2] cycloaddition of TCNE to heptaethylvinylporphyrin **4** in chloroform (at reflux) led to isolation of 80% of the corresponding adduct **5**. In addition to the typical chlorin-like visible spectrum, product **5** also exhibited resonances, in its ^{1}H NMR spectrum, attributable to an sp^3 ethyl group and to the methylene and methine protons of the newly formed cyclohexyl ring. In addition, mass spectrometry also indicated cycloaddition.

As expected, reaction (of **4**) with DMAD proved slower.

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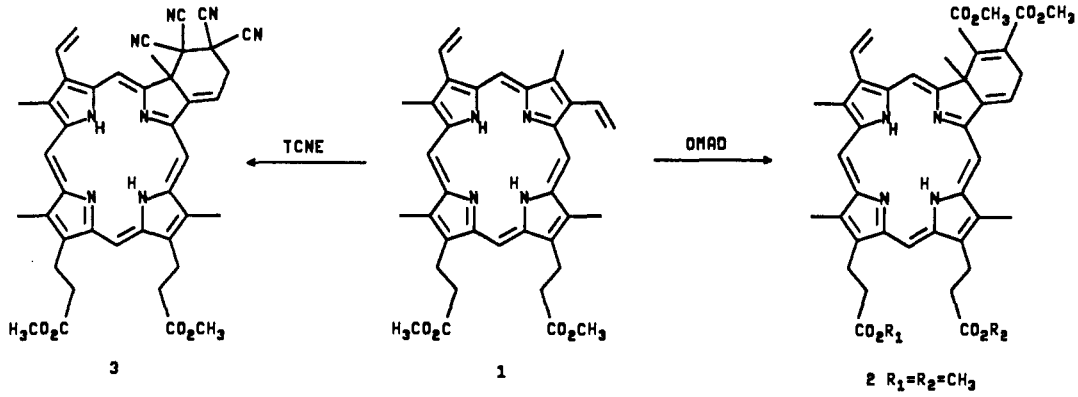
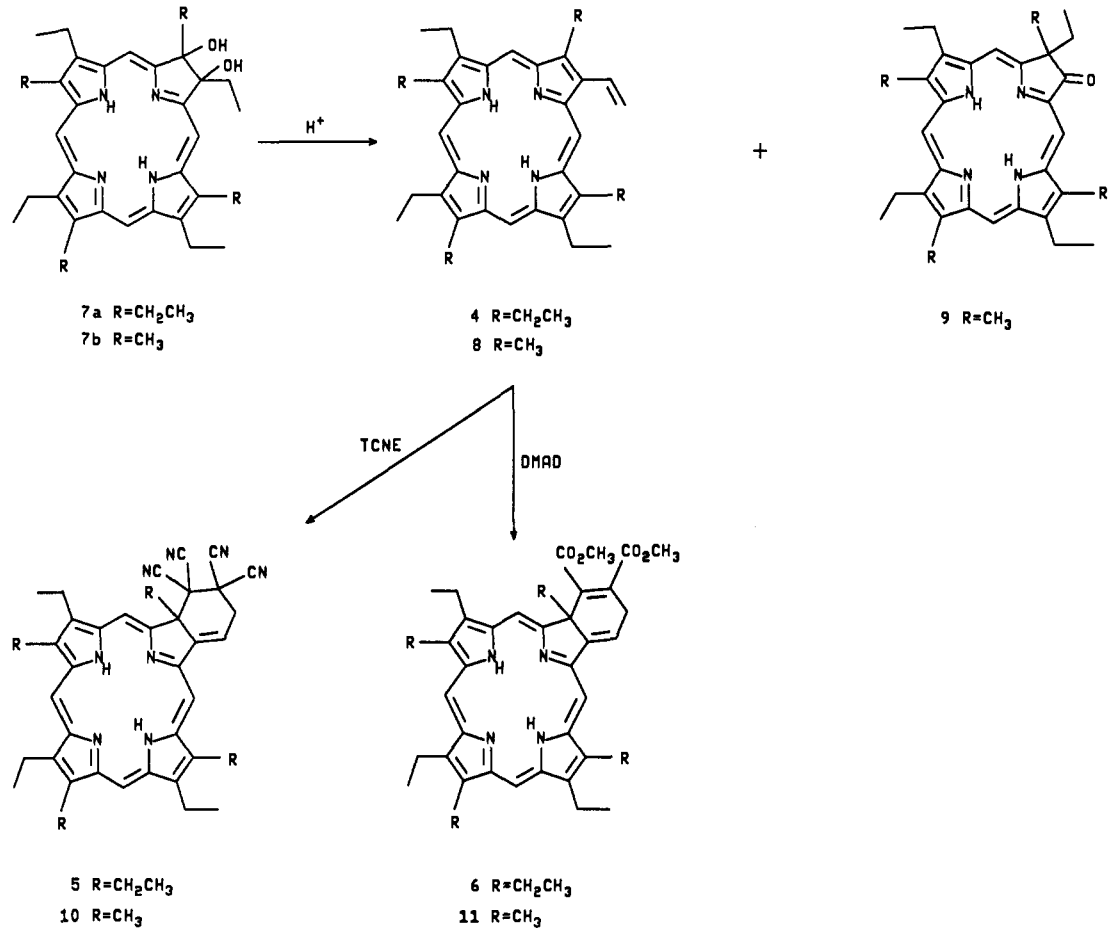
Scheme I. Synthesis of Diels–Alder Adducts of Protoporphyrin IX Dimethyl Ester (1)



Scheme II. Synthesis and Diels–Alder Reactions of Heptaethylvinylporphyrin 4 and Triethyltetramethylvinylporphyrin 8



After a 96-h reflux in toluene, the reaction, as monitored by visible spectroscopy, following appearance of the 653-nm band of the product and the disappearance of the 624-nm absorbance of the reactant, was still incomplete. Chromatography of the crude product resulted in isolation of two bands. The first, red band (30%) was shown to be the reactant heptaethylvinylporphyrin 4. The second band (brown) gave a visible spectrum typical of a chlorin and was shown, by ^1H NMR spectroscopy, to be the expected product (6) of [4 + 2] cycloaddition.

Diels-Alder Adducts of Triethyltetramethylvinylporphyrin 8. Treatment of 8 with TCNE as described above, led to isolation in good yield (70%) of the corresponding [4 + 2] adduct **10**, characterized by visible and ^1H NMR spectroscopy and mass spectrometry. Treatment (of 8) with DMAD again led to incomplete reaction even after 96 h. Cycloaddition product 11 was isolated by chromatography and characterized by NMR spectroscopy where the appropriate resonances for cycloaddition were observed.

Diels–Alder Adducts of Protoporphyrin IX Dimethyl Ester (1). Diels–Alder adducts 2 and 3 were prepared (as a 50:50 mixture of ring A and ring B isomers) as previously described.[15,16]

In Vivo Studies

We utilized the 12-day tumor dry weight protocol for these studies. In this protocol, the transplantable FANFT-induced rat bladder tumor (AY-27) was grafted sc into the flanks of Fischer CDF(F344)/CrlBr rats.[17] Once tumors had reached 1-cm transverse diameter, animals were injected with sensitizer under pentobarbital anesthesia. After 24 h, one tumor of each animal was shielded from light and the second tumor was given pho-

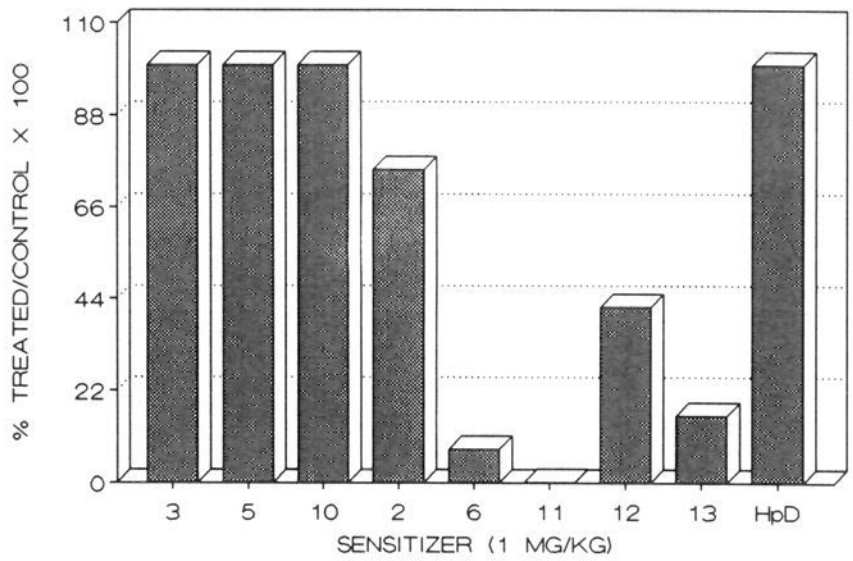


Figure 1. Twelve day tumor response, dry tumor weight 12 days after phototherapy. Statistical significance: **3**, **5**, **10**, **2**, HpD, NS (not statistically significant); **6**, $\rho < 0.006$; **12**, $\rho < 0.02$; **13**, $\rho < 0.02$. Animals free of palpable tumor: **3** (0%), **5** (0%), **10** (0%), **2** (0%), **6** (50%), **11** (100%), **12** (0%),[19] **13** (40%),[19] HpD (0%).

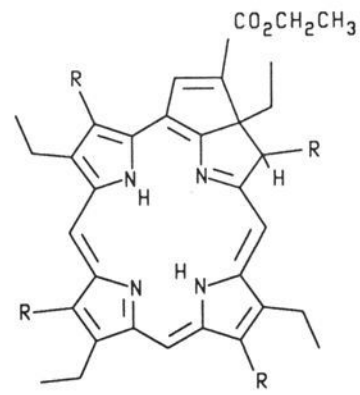
totherapy. Twelve days after treatment, control and treated tumors were harvested and desiccated to constant weight. In our experience, PDT treatment results in hemorrhagic necrosis, leading to formation of an eschar over the treatment site and edema. Consequently, the measurement of tumor volume becomes difficult to perform and the result may not accurately reflect tumor content. Dry-weight measurements eliminate these uncertainties. In addition, we have found that 12-day dry-weight results and 30-day tumor response studies are in good agreement, at least when purpurins and metallopurpurins are used as sensitizers.[19] Thus, with the FANFT induced tumor model, this protocol appears valid for the rapid assessment of sensitizer activity.

We began these studies with the DMAD adduct of protoporphyrin IX dimethyl ester (i.e. **2**) at a dose of 5.0 mg/kg of body weight. Twelve days after phototherapy the treated tumor could not be detected either by palpation or histologically, although the control tumor continued to grow. We therefore reduced the dose to 1.0 mg/kg, where all animals were found, twelve days after treatment, to still bear tumors although tumor reduction (to 73% control tumor size) had occurred. Replacing the dimethyl acetylene moiety by the tetracyano group lowered the activity of the sensitizer. Thus **3** was found to have little effect on tumor growth with treated and control tumors showing no statistical difference.

Both the TCNE derivatives **5** and **10** also showed little cytotoxicity at a dose of 1 mg/kg, however the DMAD adducts **6** and **11** proved to be powerful photodynamic agents (Figure 1). Treatment with adduct **6** resulted in 50% of animals free of palpable tumor 12 days after treatment, while use of derivative **11** raised this value to 100%. Reduction of the dose (of **11**) to 0.5 mg/kg resulted in a reduced effect, as expected, although significant tumor reduction was still observed, with 20% of treated animals free of palpable tumor.

We are unable at present to explain the marked difference in activity between TCNE adducts **3**, **5**, and **10** and DMAD adducts **2**, **6**, and **11** although factors such as tumor uptake and photodynamic efficiency have yet to be determined. One alternative explanation may be that of a retro-Diels–Alder reaction occurs in vivo, converting the TCNE adducts back to their reactant vinyl porphyrins. This would result in a loss of the strong red absorption band of the adducts and consequently a lack of excitation of the sensitizer. In support of this hypothesis is the observation that retro-Diels–Alder reactions of TCNE adducts occur under mild conditions[16] in which DMAD adducts are stable.

Of the three DMAD adducts **2**, **6**, and **11**, the most reactive appears to be **11** (derived from etioporphyrin I), followed by **6** (derived from octaethylporphyrin). Interestingly, we have previously reported a similar observation for purpurins derived from etioporphyrin I and octaethylporphyrin.[19] Both **6** and **11** appear to be more active than the corresponding metal-free purpurins **12** and **13**



12 R=CH_2CH_3
13 R=CH_3

(Figure 1) even though both absorb at shorter wavelengths (660 nm compared to 695 nm). In the purpurin series, we have shown that the activity of the sensitizer can be increased by incorporation of zinc or tin into the macrocycle cavity, which also shifts the red absorption band (from 695 nm) to 666 nm. Whether incorporation of metals into DMAD adducts **6** and **11** will lead to a similar increase in activity remains to be determined.

We have also previously reported a dose–response study with a hematoporphyrin derivative (HpD) using the same tumor model. In these studies, doses of 10 mg/kg of body weight of sensitizer were needed to obtain a significant response, although even at these high doses all animals carried palpable tumors (treated = 50% of control) 12 days after phototherapy.[17] Clearly, benzoporphyrin derivatives represent a significant improvement over HpD in terms of tumoricidal action.

In conclusion, we have developed synthetic routes for the preparation of Diels–Alder adducts of several vinylporphyrins. The use of either TCNE or DMAD as dienophile results in adducts with markedly different tumoricidal activities against the FANFT-induced rat bladder tumor (AY-27). This difference may be due to the greater stability of the DMAD adducts, to the photophysical or pharmacokinetic properties of each sensitizer, or to a combination of all of these parameters. In this tumor model at least, all DMAD adducts exhibited a tumoricidal effect greater than that of HpD and similar to that of the metallopurpurins. Further studies are in progress to elucidate the in vivo mechanism(s) of action of these sensitizers.

Experimental Section

Synthesis. Visible spectra were recorded on a Bausch and Lomb Spectronic 2000; absorptions are given in nanometers (solutions in dichloromethane). Proton nuclear magnetic resonance spectra (^{1}H NMR) were obtained on a Varian VXR400 spectrometer, chemical shifts are expressed in parts per million downfield from internal tetramethylsilane. Mass spectra were recorded (direct insertion probe) on a Hewlett-Packard 5987 mass spectrometer or (fast atom bombardment) on a JEOL HXIIO mass spectrometer. Analytical TLC was performed by using

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Merck silica gel 60F 254 precoated sheets (0.2 mm); preparative chromatography was performed on a Chromatotron (Harrison and Harrison, Paulo Alto, CA) using Merck silica gel 60F 254 with $CaSO_4 \cdot {}^1/_2H_2O$ (catalog no. 7749).

Vinyl Porphyrins. Protoporphyrin IX dimethyl ester (1) and 2-vinyl-3,7,8,12,13,17,18-heptaethylporphyrin (4) were prepared as previously reported.[12,13]

2-Vinyl-7,12,17-triethyl-3,8,13,18-tetramethylporphyrin (8). 2,3-Dihydroxy-2,7,12,17-tetraethyl-3,8,13,18-tetramethylchlorin (7b)[13] (25 mg) was placed in a 25-mL beaker and was placed in a vacuum oven containing phosphorus pentoxide. After 5 h at 140 °C × (10 mmHg) the solid was removed, cooled, and dissolved in the minimum amount of methylene chloride. This mixture was chromatographed on silica gel with 60% hexane in methylene chloride as eluant. Two bands were collected. Each was evaporated to dryness and recrystallized from hexane/dichloromethane (60:40).

Fraction 1 (15 mg, 60%), **2-vinyl-7,12,17-triethyl-3,8,13,18-tetramethylporphyrin (8)**: ^{1}H NMR ($CDCl_3$) δ 10.21, 10.12 (s, 2 H, meso), 10.05 (s, 2 H, meso), 8.30 (dd, 1 H, C*H*=CH_2, J_1 = 24 Hz, J_2 = 12 Hz), 6.34 (dd, 1 H, CH=C*H*$_2$, J_1 = 2 Hz, J_2 = 18 Hz), 6.14 (dd, 1 H, CH=C*H*$_2$, J_1 = 2 Hz, J_2 = 12 Hz), 4.09, 4.12 (q, 6 H, C*H*$_2$CH$_3$), 3.66, 3.63, 3.62, 3.60 (s, 12H, C*H*$_3$), 1.85 (t, 9 H, CH$_2$C*H*$_3$); λ_{max} 624, 569, 538, 500, 405 nm (ϵ 3175, 5279, 7880, 8266, 80193); mass spectrum calcd for $C_{32}H_{36}N_4$ m/e = 476, found (DIP) *m/e* = 476.

Fraction 2 (8 mg, 30%), **2-oxo-3,7,12,17-tetraethyl-3,8,13,18-tetramethylporphyrin (9)**: ^{1}H NMR ($CDCl_3$) δ 9.91 (s, 1 H, meso), 9.83 (s, 2 H, meso), 9.10 (s, 1 H, meso), 4.05 (m, 6 H, C*H*$_2$CH$_3$), 3.59, 3.55, 3.45 (s, all 3 H, C*H*$_3$), 2.77 (q, 2 H, C*H*$_2$CH$_3$ of reduced pyrrole), 2.04 (s, 3 H, C*H*$_3$ of reduced pyrrole), 1.79 (m, 9 H, CH$_2$C*H*$_3$), 0.40 (t, 3 H, CH$_2$C*H*$_3$ of reduced pyrrole), -2.86, -2.96 (s, both 1H, NH); λ_{max} 640, 585, 545, 506, 390 (ϵ 37604, 4928, 5917, 3137, 115655). mass spectrum calcd for $C_{32}H_{38}N_4O$ *m/e* = 494, found (DIP) *m/e* = 494.

Repetition of the above reaction, at 180 °C for 6 h, led to isolation of vinylporphyrin 8 (6 mg, 24%) and oxochlorin 9 (19 mg, 76%).

Diels-Alder Additions to Protoporphyrin IX Dimethyl Ester (1). DMAD adduct 2 and TCNE adduct 3 were prepared according to literature procedures[15,16] (Scheme I).

Diels-Alder Additions to 2-Vinyl-3,7,8,12,13,17,18-heptaethylporphyrin (4). Reaction with Tetracyanoethylene (TCNE). TCNE (30 mg) was added to the vinylporphyrin 4 (30 mg) in chloroform (25 mL) and the resulting solution was refluxed for 2 h. After cooling, the solvent was evaporated and the residue recrystallized from dichloromethane/methanol (1:10). [4 + 2] adduct 5 was obtained in 81% yield: ^{1}H NMR ($CDCl_3$) δ 9.86, 9.82, 9.44, 9.27 (all s, 4 X meso H); 7.19 (dd, 1 H, C=C*H*); 4.07 (m, 2 H, CHC*H*$_2$); 3.98 (m, 12 H, C*H*$_2$CH$_3$); 2.96 (q, 2 H, C*H*$_2$ of sp^3 ethyl); 1.85 (m, 18 H, CH$_2$C*H*$_3$); 0.13 (t, 3 H, C*H*$_3$ of sp^3 ethyl); -2.70, -2.80 (all s, 2 H, NH$_2$); λ_{max} 651, 594, 534, 499, 400 (ϵ 25201, 3046, 5999, 7062, 75951); mass spectrum calcd for $C_{42}H_{44}N_8$ *m/e* = 660, found (FAB) *m/e* = 661 (M + 1). Anal. ($C_{42}H_{44}N_8 \cdot 7H_2O$) C, H.

Reaction with Dimethyl Acetylenedicarboxylate (DMAD). Vinylporphyrin 4 (20 mg) in toluene (30 mL) was treated with DMAD (1 mL) and the resulting solution was refluxed for 5 days. The solution was cooled, the solvent was removed under reduced pressure, and the residue was chromatographed on silica gel using dichloromethane containing 2% ether and 2% toluene as eluent.

The first (red) band was collected, the solvent was removed, and the residue was recrystallized from dichloromethane/methanol to give recovered reactant 4 (7 mg).

The second (green) band was worked up as described above to give the expected Diels-Alder adduct 6 (30% yield): ^{1}H NMR ($CDCl_3$) δ 9.76, 9.70, 9.31, 9.06 (all s, all 1 H, 4 X meso); 7.43 (dd, 1 H, C=C*H*); 4.20-3.98 (m, 14 H, CHC*H*$_2$, 6 X C*H*$_2$CH$_3$); 3.95 (s, 3 H, CO$_2$C*H*$_3$); 3.87 (s, 3 H, CO$_2$C*H*$_3$); 2.68, 2.58 (both m, both 1 H, diastereotropic C*H*$_2$ of sp^3 ethyl); 1.80 (m, 18 H, 6 X CH$_2$C*H*$_3$); 0.29 (t, 3 H, C*H*$_3$ of sp^3 ethyl); λ_{max} 653, 626, 599, 532, 508, 497, 394 (ϵ 29315, 6983, 6312, 10994, 11025, 10980, 123412); mass spectrum calcd for $C_{42}H_{50}N_4O_4$ *m/e* = 674, found (DIP) *m/e* = 674. Anal. ($C_{42}H_{50}N_4O_4 \cdot 1.5H_2O$) C, H.

Diels-Alder Additions to 2-Vinyl-7,12,17-triethyl-3,8,13,18-tetramethylporphyrin (8). Reaction with TCNE. Vinylporphyrin 8 (20 mg) was treated with TCNE (20 mg) as described above. The crude product was recrystallized from dichloromethane/methanol (1:10) to give 71% yield of the product 10: ^{1}H NMR ($CDCl_3$) δ 9.81, 9.79, 9.38, 9.25 (all s, 4 X meso H); 6.96 (m, 1 H, C=C*H*); 3.98, 3.81 (m, 8 H, CC*H*$_2$, 3 X C*H*$_2$CH$_3$); 3.55, 3.46, 3.40 (all s, 9 H, C*H*$_3$); 2.37 (s, 3 H, sp^3 C*H*$_3$); 1.81 (t, 3 H, CH$_2$C*H*$_3$); 1.75 (t, 3 H, CH$_2$C*H*$_3$); 1.72 (t, 3 H, CH$_2$C*H*$_3$); -2.75, -2.8 (both s, 2 H, NH$_2$); λ_{max} 650, 594, 532, 496, 396 (ϵ 27944, 3127, 5783, 8035, 117677); mass spectrum calcd for $C_{38}H_{36}N_8$ *m/e* = 604, found (FAB) *m/e* = 605 (M + 1). Anal. ($C_{38}H_{36}N_8$) C, H.

Reaction with DMAD. Vinylporphyrin 8 (10 mg) in toluene (20 mL) was treated with DMAD (3 drops) and the resulting solution was refluxed for 5 days. After cooling, the solvent was removed under reduced pressure and the residue was chromatographed on silica gel using dichloromethane containing 2% ether and 2% toluene as eluant. The major (brown) band was collected, the solvent was evaporated, and the residue was recrystallized from dichloromethane/methanol to give product 11 in 30% yield: ^{1}H NMR ($CDCl_3$) δ 9.73, 9.70, 9.33, 9.09 (all s, 4 X meso H); 7.37 (dd, 1 H, C=C*H*); 3.85-4.01 (m, 8H, CHC*H*$_2$, 3 X C*H*$_2$CH$_3$); 3.99 (s, 3 H, OC*H*$_3$); 3.88 (s, 3 H, OC*H*$_3$); 3.63, 3.55, 3.52 (all s, 9 H, C*H*$_3$); 2.05 (s, 3 H, sp^3 C*H*$_3$); 1.76, 1.74, 1.72 (all t, 9 *H*, CH$_2$C*H*$_3$); -2.69 (br s, 2 H, NH$_2$); λ_{max} 654, 598, 531, 496, 400 (ϵ 28061, 5313, 10781, 10412, 129680); mass spectrum calcd for $C_{38}H_{42}N_4O_4$ *m/e* = 618, found (FAB) *m/e* 619 (M + 1). Anal. ($C_{38}H_{42}N_4O_4 \cdot 2H_2O$) C, H.

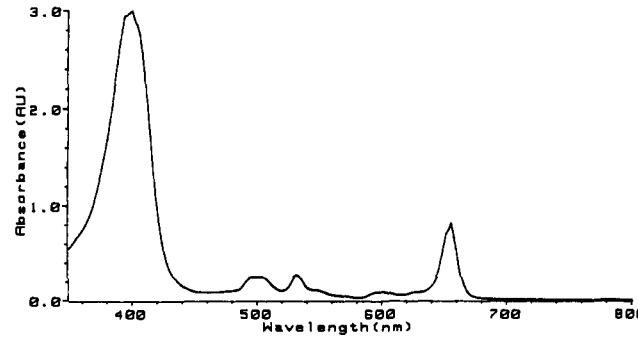

Figure 2. Visible absorption spectrum of Diels-Alder adduct 11 in a Cremophor EL based emulsion.

In Vivo Protocols. Delivery System. An oil-based emulsion (Cremophor EL, BASF Wyandotte, NY) prepared as previously described[17] was used to deliver each sensitizer. Freshly prepared solutions were sterilized by passage through a 0.45-μm filter (Nalgene) prior to injection. All preparations were checked by UV/visible spectroscopy of a diluted sample to ensure stability and to determine concentration of the sensitizer. Visible spectra were similar to those obtained in dichloromethane (Figure 2).

Tumor Model. The tumor model used was the transplantable *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide-induced urothelial tumor (AY-27) grafted subcutaneously into male Fischer CDF (F-344)/CrlBr rats (Charles River Breeding Laboratories, Boston, MA) as previously described.[17] Two tumors were implanted into each animal, one in each dorsal thigh. One tumor served as an internal control by being shielded during phototherapy. Tumors generally became visible within 1 week of implantation and reached 1-cm transverse diameter within 2 weeks.

Phototherapy Unit. A 500-W G.E. Quartzline lamp (GECBA, Cleveland, OH) in a Kodak slide projector equipped with a Kodak Ektanar lens served as a phototherapy unit. The output lens of the projector was fitted with a filter (#2418 Dow Corning, Corning, NY) to allow only light with a wavelength greater than 590 nm to pass. The light was reflected 90° by placing a 5 × 5 cm silver mirror 45° to the axis of the light beam, 24 cm from the output lens of the projector. The beam was then passed through a 6-cm-diameter double-convex lens with a focal length of 12 cm and focused to give a 1-cm-diameter light beam at the surface of the tumor. The irradiance of the light source was measured with a radiometer (UDT #351S, Culver City, CA) and has been previously reported.[6] Total light dose was 200 mW/cm^2 (360 J/cm^2).

Tumor temperature was monitored by a 24-gauge hypodermic thermistor probe (#524, Yellow Springs Instruments, Yellow

Springs, OH) placed percutaneously beneath the surface of the tumor. Core body temperature was monitored with a rectal probe (#401, Yellow Springs Instruments, Yellow Springs, OH). Tumor temperature was maintained within 2 °C of core temperature (35 °C) by a jet of cool air placed over the tumor.

Dose-Response Studies. Animals bearing two tumors, one in each flank, were divided into eight groups of five rats per group. Each group received either 0.5, 1.0, 2.5, or 5.0 mg/kg of body weight of sensitizer, administered under pentobarbital anesthesia (65 mg/kg), via the dorsal tail vein. Twenty four hours after injection, one tumor was shielded from light while the other was irradiated for 30 min as described above.

Twelve days after treatment, animals were sacrificed by saturated KCl injection intracardiacally. Both treated and control tumors were harvested, freed of surrounding fat and subcutaneous tissue, and placed in a vacuum dessicator for 72 h, after which, tumor dry weight was determined.

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Synthesis of Perfluoroalkylated Xylitol Ethers and Esters: New Surfactants for Biomedical Uses

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New, well-defined surfactants and cosurfactants were synthesized with the objective of enhancing the stability of fluorocarbon emulsions destined to serve as oxygen carriers for biomedical applications. Monoperfluoroalkylated ethers of xylitol were achieved by addition of perfluoroalkyl iodide on the double bond of a protected xylitol allyl ether in a one-step addition–elimination reaction. Monoesters were obtained specifically on position 5 by treating 1,2:3,4-di-*O*-isopropylidenexylitol with perfluoroalkylated acid chlorides of various chain lengths in pyridine at room temperature. The products display strong surface activity and produce a remarkable synergistic stabilization of a fluorocarbon/Pluronic F-68 type emulsion. Biocompatibility data are reported, which include in vitro toxicity tests on Namalva cell cultures and hemolysis tests on human blood cells; the latter was found to decrease as the length of the *F*-alkyl chain increased. IV injection in mice (n = 10) showed that these products were innocuous at 400–1000 mg/kg of body weight. Preliminary exchange–perfusion experiments on rats with an emulsion containing the *F*-octyl xylitol ether were encouraging.

Fluorocarbons to be administered intravenously as oxygen carriers must be in the form of emulsions,[1-3] which requires the presence of appropriate surfactants. Those used in the first generation of such "blood substitutes", Fluosol-DA (Japan), Ftorosan (Soviet Union), and Emulsion No. 2 (China),[4] are essentially polyoxypropylene/polyoxyethylene block polymers of the Pluronic F-68 type and are not particularly effective for emulsifying fluorocarbons, which makes it necessary to store and ship the emulsions in the frozen state. In order to improve on this situation, we undertook the synthesis of more fluorophilic surfactants, i.e. amphiphilic molecules with a perfluoroalkylated hydrophobic tail.[5] For the hydrophilic head, the crucial biocompatibility requirement oriented our choice toward sugars and natural polyols, and more particularly xylitol, an essentially nontoxic low-cost pentitol. Other desirable improvements over the polyoxyalkylene surfactants were to obtain monodisperse, better defined, purer products and to introduce the possibility of varying the total length of the surfactant molecule and its fluorophilic/hydrophilic balance so as to permit the optimization of the emulsion's characteristics for specific applications.[5] This was achieved by varying the length of the fluorocarbon tail and the number of methylene groups within the intermediate hydrocarbon segment, the junction unit between the hydrophilic and hydrophobic parts being either an ether or an ester group. As cosurfactants with Pluronic F-68, such perfluorinated xylitol derivatives were expected to exercise a synergistic effect, both by decreasing the fluorocarbon/water interfacial tension and by reinforcing the interfacial surfactant layer, owing to the possibility of hydrogen bonding between the polyols' hydroxy groups and the polyethers' oxygens.[6] Preliminary results indicate that these expectations are borne out.[7]

The approaches used and the compounds described in this paper are collected in Scheme I.

Results and Discussion

Synthesis of Perfluoroalkylated Xylitol Allyl Ethers. The linear perfluoroalkyl chain was grafted onto the polyol via the intermediate of a hydrocarbon segment acting from both an electronic standpoint as a stabilizing screen and structurally as a chain prolongator. The simplest and most readily available commercial perfluoroalkyl chains being the iodides R_FI and the addition of R_FI on double bonds being a well-established reaction, we chose to use the following sequence of reactions: formation of the allyl ether **3** from the 1,2:3,4-di-*O*-isopropylidenexylitol, subsequent addition of R_FI to give **4**, followed by the

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