Cyclodextrin Dimers as Cleavable Carriers of Photodynamic Sensitizers

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Abstract: Several phthalocyanines carrying hydrophobic components have been synthesized and shown to bind to a group of cyclodextrin dimers with a carbon−carbon double bond in the linker. The complexes are soluble in water. On irradiation in the presence of oxygen, the singlet oxygen produced cleaves the olefinic linkers in the complexes, resulting in precipitation of the sensitizers. This process concentrates the sensitizers in the light beam, a process that has useful potential in photodynamic therapy.

Introduction

In photodynamic therapy (PDT), a dye such as a porphyrin or a phthalocyanine is administered to a patient along with irradiation.[1−3] The excited-state dye converts triplet oxygen to the singlet form, which is lethal to cells. Thus, light directed into the area of a tumor can lead to the destruction of cancer cells if the photosensitizer is present. One problem with PDT is the accessibility of the tumors to light. Another issue has to do with the desirability of localizing the photosensitizer at the tumor site. Patients with a photosensitizer distributed throughout their system can have serious toxic effects in sunlight, for instance. One can try to target the photosensitizer to the cancer cells by using cancer-specific antibodies,[4] but the use of a foreign protein may cause its own problems.

Möser and Ruebner suggested that a cyclodextrin[5] (CD) dimer could play a useful role in PDT if the dimer were able to bind[6] and solubilize an otherwise insoluble photosensitizer.[7] The solubilized hydrophilic complex might not easily be taken up by cells, including cancerous cells. If the dimer had a linkage that was cleavable by singlet oxygen, the complex of the CD dimer with the photosensitizer could liberate the hydrophobic photosensitizer in a light beam when the linker cleaved. The photosensitizer could then enter cells—or at least bind to proteins—in the region and not be diffusible throughout the body. However, Möser and Ruebner did not suggest a linker that could function in this way.

If this process occurred, it would be a mechanism for concentrating the photosensitizer at the tumor site. That is, conversion of a soluble complex of the photosensitizer into a liberated photosensitizer in the light beam would probably lead to binding of the photosensitizer to proteins at the irradiation site. Then further diffusion of the complex into the light beam, and its conversion to a cleaved CD dimer and a localized photosensitizer, could result in removing the sensitizer from the rest of the body and fixing it in the light beam. Since convergent beams of light could give the tumor region a particularly high light intensity, the result would be an appealing approach to PDT. It would require lower amounts of the photosensitizer, and would direct it into the tumor site and away from other parts of the body.

As we have reported elsewhere, our early test of aspects of this idea was successful.[8] We synthesized and examined the β-cyclodextrin dimer **1** and the zinc phthalocyanine **2**, and saw that indeed they formed a complex **3** that was soluble in water. Then, on irradiation of complex **3** in the presence of oxygen, the double bond of **1** was cleaved by singlet oxygen to form 2 mol of thioformate **4**, Scheme 1. Singlet oxygen adds to double bonds to form dioxetanes, which spontaneously fragment to generate carbonyl groups.[9−11] The addition is particularly favorable for double bonds with electron donor substituents, as in **1**.

Since dimeric binding is stronger than the monomeric binding that occurs once the linker is cleaved, **4** then dissociated from **2**. Furthermore, we saw that the chain of **4** almost certainly lowers the affinity of the cyclodextrin for the phthalocyanine by tucking back into the cyclodextrin cavity. An analogue of **4** with a methyl group in place of the formyl group had an order

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(2) For a review on photodynamic therapy, and in particular the use of porphyrins as photosensitizers, see: Sternberg, E. D.; Dolphin, D.; Bruckner, C. *Tetrahedron* **1998**, *54*, 4151−4202.

(3) For a review on the biological aspects of photodynamic therapy see: Dougherty, T. J.; Gomer, C. J.; Henderson, B. W.; Jori, G.; Kessel, D.; Korbelik, M.; Moan, J.; Peng, Q. *J. Natl. Cancer Inst.* **1998**, *90*, 889−905.

(4) Möser, J. G.; Reubner, A.; Vervoorts, A.; Wagner, B. *J. Incl. Phenom.* **1996**, *25*, 29−34.

(5) For a thorough review on numerous aspects of cyclodextrin chemistry see: D'Souza, V. T.; Lipkowitz, K. B., Eds. *Chem. Rev.* **1998**, *98*, 1741−2076.

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(7) Möser, J. G.; Ruebner, A.; Vervoorts, A.; Wagner, B. In *Proceedings of the Eighth International Symposium on Cyclodextrins*; Szetli, J., Ed.; Kluwer: Boston, 1996; pp 71−76.

(8) Ruebner, A.; Yang, Z.; Leung, D.; Breslow, R. *PNAS* **1999**, *96*, 14692−14693.

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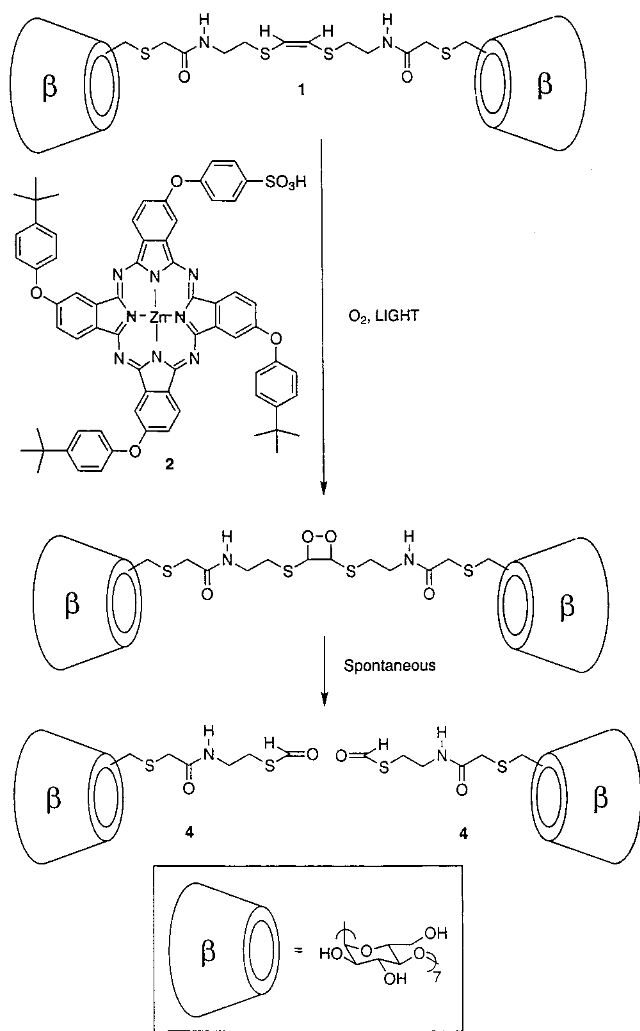
Scheme 1



of magnitude lower affinity for 4-*tert*-butylbenzoic acid than does simple β-cyclodextrin.

We also examined the reaction of **1** with externally generated singlet oxygen, produced by irradiation of methylene blue in the solution. Again we saw cleavage of the double bond of **1** to form **4**, but in addition there were significant amounts of other products formed from oxidation of the cyclodextrin units. Apparently, when singlet oxygen forms internally in complex **3** it is directed to the double bond of component **1** specifically, and does not carry out the random oxidations seen with externally generated singlet oxygen.

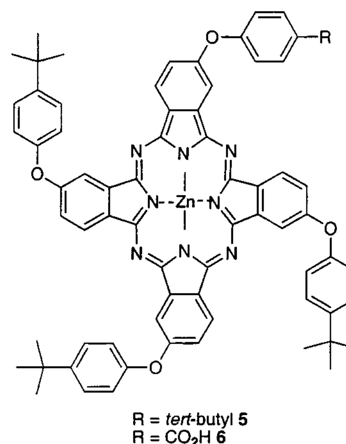
A key experiment involved directed irradiation of part of the solution of complex **3**. A tube was wrapped with aluminum foil so that only a small portion of it was exposed to light, and we saw that precipitation of **2** occurred only in the exposed region. This suggested that the soluble complex would indeed diffuse into the light beam and then be photolytically cleaved, but no quantitative studies were performed to indicate that all of the dissolved complex was transformed and concentrated into the light beam in this way.

Our preliminary experiments described above were strongly indicative, but had several problems. One is that the phthalocyanine used was in fact a mixture of eight compounds, in which the substituents may be attached 3333 to the four phthalocyanine benzene rings (this is the structure shown, with the first number assigned as position 3 for the position of the sulfonate substituent on **2**), 3233, 3323, 3332, 3223, 3322, 3232, or 3222.[12,13] Thus we wanted to examine phthalocyanines with single well-defined structures. (We chose them over porphyrins because they are more stable to oxidation.)

We also wanted to examine more than one CD dimer, to optimize the system. In particular, we wanted to see whether the binding of the phthalocyanine to the CD dimer, and the rate of the photolytic cleavage reaction, could be optimized. Finally, we wanted to show that with the appropriate cases we could indeed direct *all* of the photosensitizer into the light beam, mimicking the proposed biological concentration at the cancer site.

Results and Discussion

Synthesis of the Zinc Phthalocyanines. Using the standard phthalocyanine synthetic procedure,[14] then incorporation of zinc, we prepared **5**, the analogue of **2** with four equivalent substituents (it is a mixture of only four isomers since the 3333 isomer is the same as the 2222 isomer). We also prepared **6**, the analogue of **2** with a carboxyl instead of a sulfonate solubilizing group (again a mixture of eight isomers).[15]



To achieve the symmetry that would give us a single isomeric zinc phthalocyanine, we synthesized phthalonitrile **7**. This was made by brominating the catechol acetonide,[16] then displacing the bromines with CuCN (the Rosenmund−von Braun reaction).[17] Conversion to the phthalocyanine with Li in pentanol, then treatment with zinc acetate, afforded compound **8**. The

(9) For reviews see: Adam, W.; Cliento, G. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 529−542. Schaap, A. P.; Zaklika, K. A. In *Singlet Oxygen*; Wasserman, H. H., Murray, R. W., Eds.; Academic Press: New York, 1979; pp 173−242. Bartlett, P. D. *Chem. Soc. Rev.* **1976**, *79*, 149−163.

(10) For discussions on the mechanism see: Frimer, A. A. *Chem. Rev.* **1979**, *79*, 359−387. Clennan, E. L.; Nagraba, K. *J. Am. Chem. Soc.* **1988**, *110*, 4312−4318.

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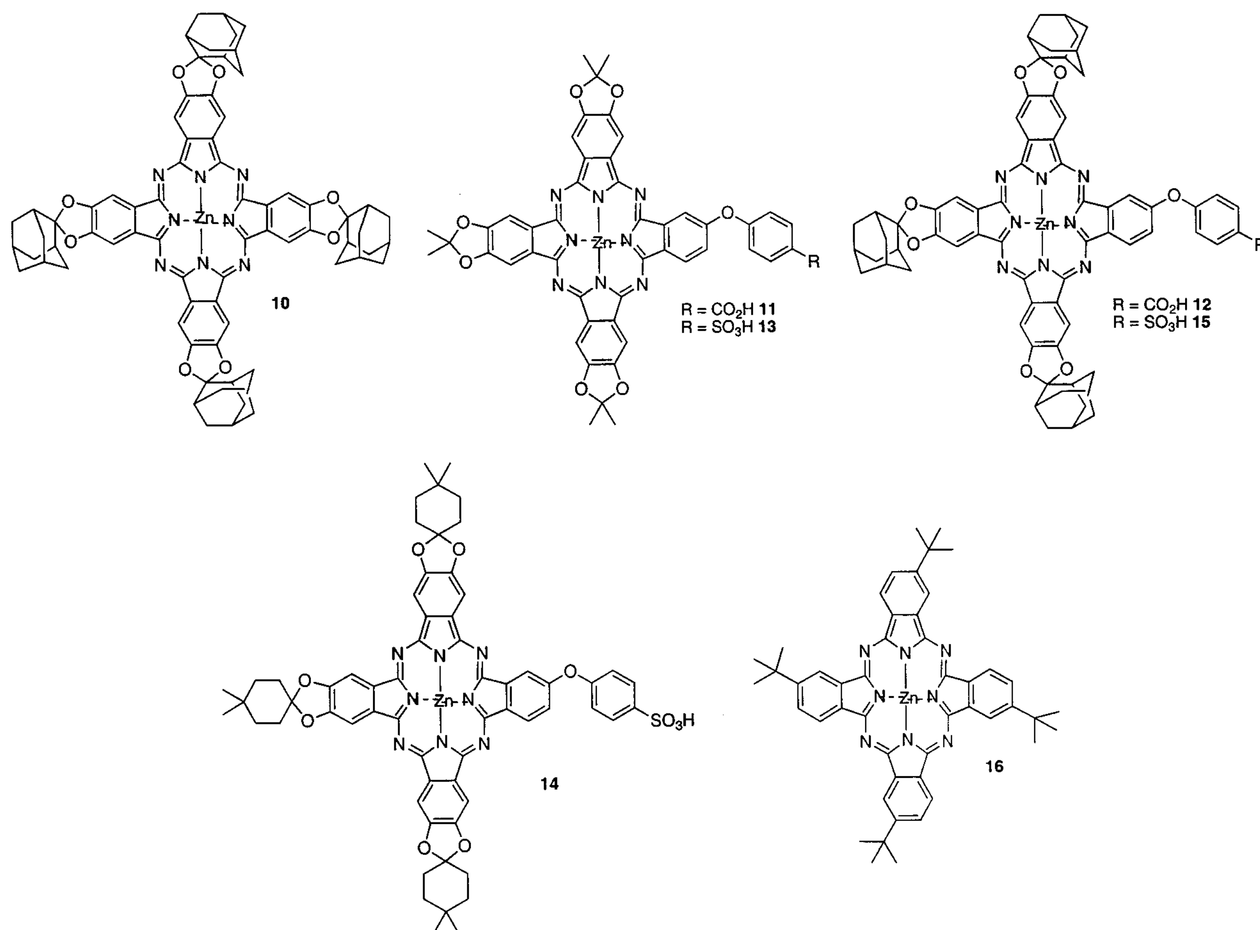
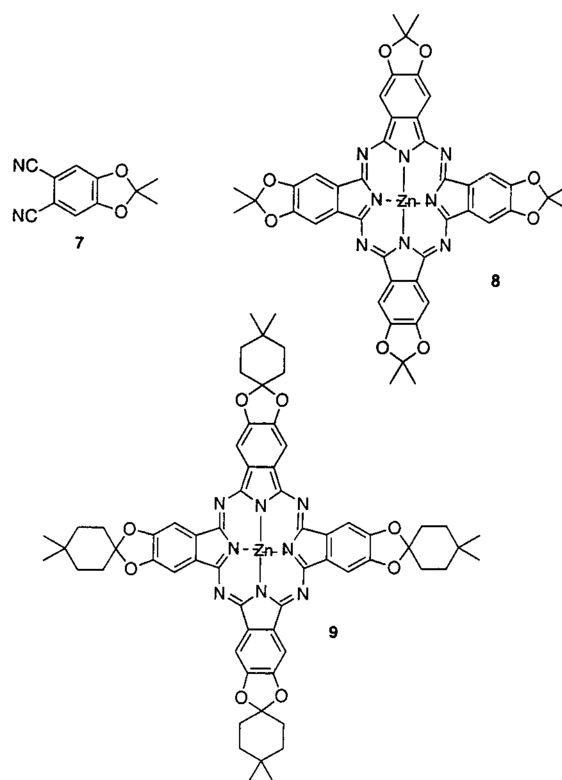
Chart 1



elongated hydrophobically substituted **9** was synthesized from the corresponding phthalonitrile, synthesized by converting 3,4-dibromocatechol[18] to its ketal with 4,4-dimethylcyclohexanone[19] then displacing with CuCN. This was converted to the fully symmetrical zinc phthalocyanine **9** in a one-pot procedure with Li and zinc acetate.[20]

Adamantane derivatives bind well to β-cyclodextrin in water,[21] so we also prepared zinc phthalocyanine **10** from the ketal of catechol and 2-adamantanone[22] by the bromination[23] and cyanide displacement sequence described above, with the one-pot Li and Zn^{2+} method of cyclization. We also prepared two further compounds with one carboxyl solubilizing group, **11** and **12**, by using a mixture of phthalonitriles and then separating the products. In the same manner we prepared the three monosulfonated analogues **13**−**15**. These compounds are of course all single isomers. Finally, we had the commercially available zinc phthalocyanine **16**,[24] which is smaller than the others.

Synthesis of the β-Cyclodextrin Dimers. Dimer **1** was synthesized as described previously.[8] Dimers **17** and **18** were prepared by using linker **19**, but attached to the primary and secondary faces of β-cyclodextrin respectively, Scheme 2. To make **19**, disulfide **20** was reduced with sodium in ammonia, and then reacted with *cis*-1,2-dichloroethylene to afford diacid



(18) Kohn, M. *J. Am. Chem. Soc.* **1951**, *73*, 480.

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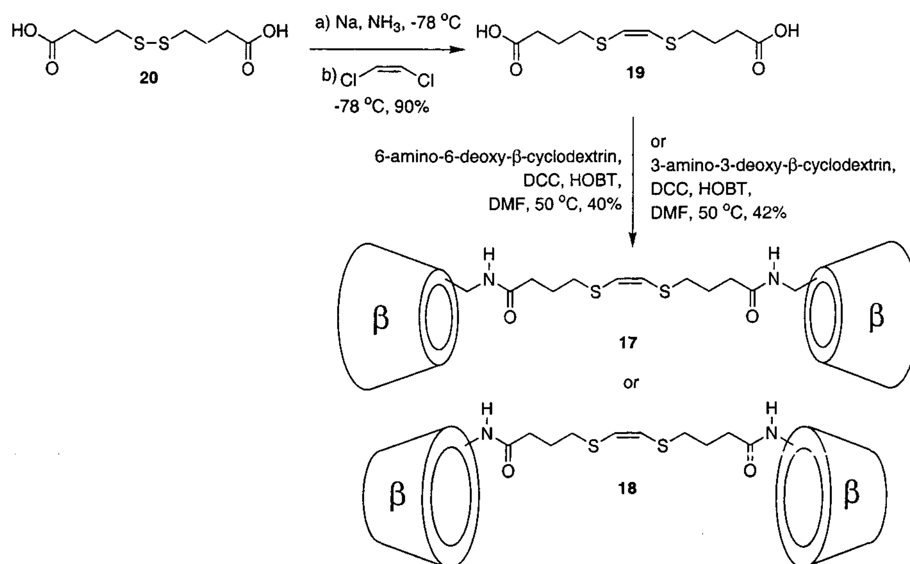
Scheme 2



19. This was then coupled with 6-deoxy-6-amino-β-cyclodextrin, using dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt), to afford **17**, and coupled with 3-deoxy-3-amino-β-cyclodextrin under the same conditions to afford **18**.

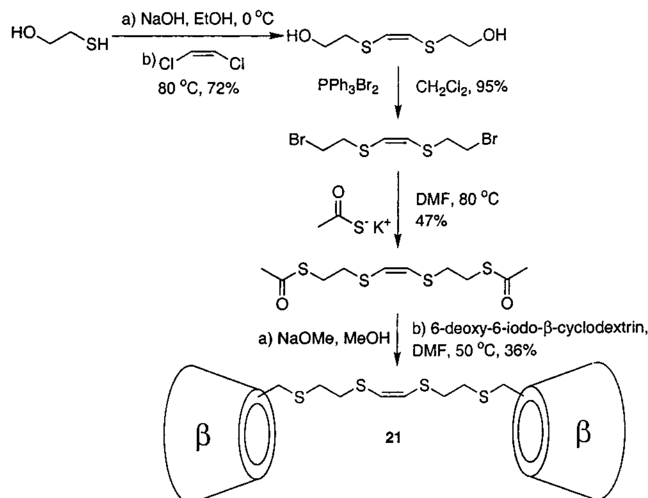
The 3-deoxy-3-amino-β-cyclodextrin was prepared as previously described[25] by preparing the 3-naphthalenesulfonate of β-cyclodextrin, closing it to the 2,3-alloepoxide with base and opening this with sodium azide. Reduction with triphenylphosphine then afforded 3-deoxy-3-amino-β-cyclodextrin, which was coupled with **19** to afford **18**. The ^{1}H NMR spectrum of the azide showed that the attachment was on carbon 3 of the cyclodextrin, thus affording the product with overall retention of configuration. A small amount of the 2-azido compound was also formed, which was easily removed by chromatography.

Finally, a CD dimer with a shorter linker was synthesized, compound **21**. 2-Mercaptoethanol was coupled with *cis*-1,2-dichloroethylene, the hydroxyls then converted to bromides with triphenylphosphine dibromide, and the bromines replaced with potassium thioacetate. Then the acetate groups were removed with NaOMe, and the dithiolate was directly coupled with 6-deoxy-6-iodo-β-cyclodextrin to afford **21**.

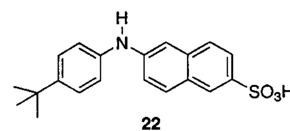
Binding Studies. Some estimates of likely binding pairs were obtained by MacroModel simulations of the dimers and the phthalocyanines. The distances between the carbons of the two β-cyclodextrins to which the linkers are attached are as follows: **1**, 22 Å; **17**, 20 Å; **18**, 18 Å; **21**, 16 Å. For the phthalocyanines, distances were measured from the methylated carbons across the ring for **5** (25 Å), **8** (17 Å), and **9** (23 Å). For the adamantane derivative **10**, the distance across the entire system, including all the adamantane atoms, was 23 Å. The MacroModel simulations also suggested that the alkene in the linker would be positioned directly above the metal center when the phthalocyanine is bound into the dimer. This would be expected to facilitate our desired site-specific oxidation.

Binding constants for a few of the possible pairs were determined by a fluorescence competition method we have used previously for cyclodextrin dimers, competing the substrate of interest with 2-(p-*tert*-butylanilino)naphthalene-6-sulfonic acid **22**, which we have called BNS.[6b] BNS is fluorescent when bound into a hydrophobic cavity such as that of a cyclodextrin,

Scheme 3



but only weakly fluorescent in water solution. Only the sulfonated phthalocyanines were soluble enough for this method.



The binding constant of BNS **22** to each dimer was determined as previously[6b,8] by titrating BNS into a dimer solution in a fluorescence cell, then exciting this solution at 330 nm and measuring the fluorescent emission at 438 nm. The binding constant of phthalocyanine to dimer was measured by titration of BNS into a 1:1 mixture of dimer and phthalocyanine. The double reciprocal plot of change in fluorescent intensity and BNS concentration gives straight lines (for an example of such a plot, see Figure 1). The straight lines found for each run show that the dimer and BNS were forming 1:1 complexes.[26] The binding constant of BNS to each dimer is given by K = intercept/slope, and the binding constant of the phthalocyanine can be calculated as $K_I = (K/K' - 1)/[I]$, where K' is the

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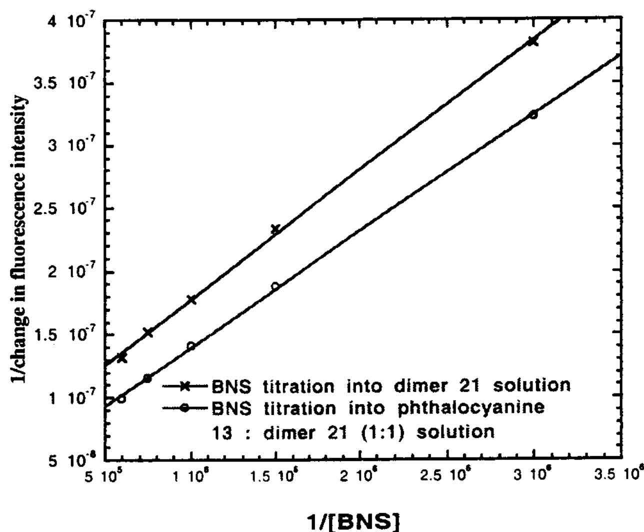


Figure 1. Determination of binding constant for dimer **21** with BNS and phthalocyanine **13**.

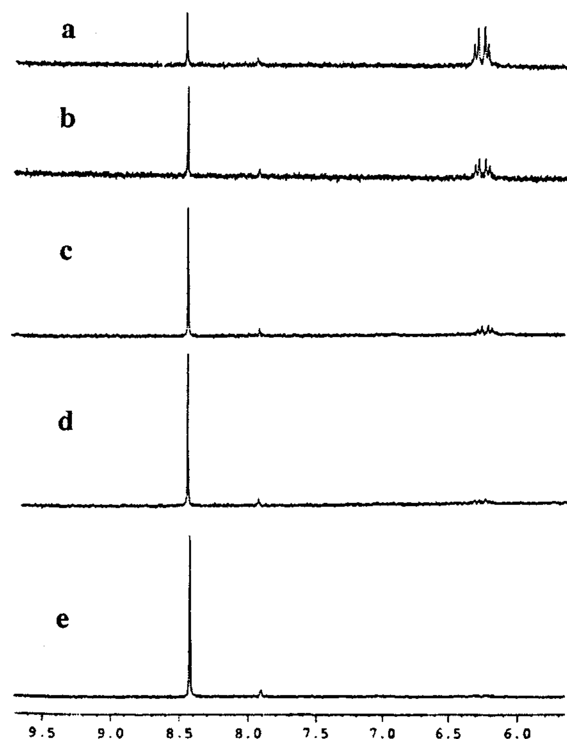


Figure 2. Conversion of **21** to its cleavage product on irradiation with **11**. Reaction time: (a) 30, (b) 60, (c) 90, (d) 120, and (e) 150 min.

apparent binding constant of BNS to the dimer in the presence of phthalocyanine, and [I] is the concentration of phthalocyanine.[26]

The binding constants (M^{-1} at ca. 25 °C) of BNS to the dimers used were as follows: **1**, 1.93×10^5 (reported 1.9×10^5);[8] **17**, 1.07×10^6; **18**, 4.18×10^5; **21**, 7.01×10^5. For the dimers with the phthalocyanines the binding constants (M^{-1} at ca. 25 °C) were the following: **1**/**2**, 2.00×10^6 (reported 2×10^6);[8] **17**/**15**, 6.05×10^5; **18**/**14**, 1.94×10^6; **18**/**15**, 1.76×10^6; **21**/**13**, 1.30×10^6.

As we reported earlier,[8] there is some evidence that the linker chain in **1** is partly tucked into one cyclodextrin cavity in water solution, so these binding constants are somewhat diminished as a result. In the ^{1}H NMR spectrum of **1**, the two vinyl protons are equivalent in DMSO solution, but nonequivalent in water. They become equivalent in water when hyodeoxycholic acid is added; this is known to bind strongly into β-cyclodextrin,[27] and would thus be expected to displace the chain. Partial binding of the linker chain of **1** into one of the cyclodextrins in water leads to the nonequivalence, which apparently equilibrates slowly on the NMR time scale. This phenomenon was seen in the ^{1}H NMR spectra of all four of the cyclodextrin dimers we synthesized.

Photochemical Cleavage. All of the photocleavage reactions were carried out with the same apparatus, as described earlier.[8] A halogen lamp (50 W) was set up with a 540 nm cutoff filter and a focusing lens. The NMR tube in which the reactions were run was placed in the most strongly focused area, and oxygen was bubbled continuously through the solution while the photocleavage reactions were run. To monitor the reactions, ^{1}H NMR spectra were taken at regular intervals, whereby the disappearance of the alkene peaks and the appearance of the single formyl peak could be observed.

The dimers were at a concentration of 2.5 mM while the photosensitizers were at 0.14 mM in 5% DMSO-d_6 in D_2O. Typical ^{1}H NMR traces following the progress of a photocleavage reaction are shown in Figure 2. Here dimer **21** was cleaved with phthalocyanine **11**. It can clearly be seen that the amount of alkene (doublet at ~6.2 ppm) decreases and the formyl peak (singlet at ~8.4 ppm) appears as the reaction progresses, and that no other peaks are seen in this area.

(27) Yang, Z.; Breslow, R. *Tetrahedron Lett.* **1997**, *38*, 6171−6172.

Table 1. Times for 50% Cleavage of the Dimers (min)[a]

	2	5	6	8	11	12	13	14	15	16
dimer **1**	7					60			6	
dimer **17**		80	40							
dimer **18**			55					6		
dimer **21**			85	180	55		5.3; 5.4			22% in 180 min

[a] The dimers were at a concentration of 2.5 mM while the photosensitizers were at 0.14 mM in 5% DMSO-d_6 in D_2O.

The plots of percent cleavage vs time were all linear, indicating that the phthalocyanines remain active during the reactions and the light intensity is essentially constant. Furthermore, the NMR tubes were repeatedly removed from the apparatus, wrapped in aluminum foil, and taken to the NMR machine, then returned to the apparatus. The consistency of data indicates that the light flux was consistent throughout the runs. Also, repeats of the **21**/**13** experiment more than three weeks apart gave values of 5.3 and 5.4 min for 50% cleavage, showing that the photolysis apparatus is rather stable.

We compared the relative times needed for 50% cleavage of the dimers by various bound phthalocyanines under the conditions above, at ambient temperature (25 °C). These are not perfect kinetic studies, of course, but give a general idea of the efficiency with which our various possible systems undergo the dioxetane formation and cleavage. The data are listed in Table 1.

As the data indicate, there is considerable variation in the effectiveness of the photolytic cleavage process. Dimer **1** is rapidly cleaved by the previously made sulfonate sensitizer **2**, a mixture of eight isomers, and also by the well-defined adamantyl sulfonate sensitizer **15**, but not as rapidly by the corresponding carboxylate **12**. Dimer **17** was not rapidly cleaved by either **6**, the carboxylate version of **2**, or **5**, the version of **2** with no solubilizing group.

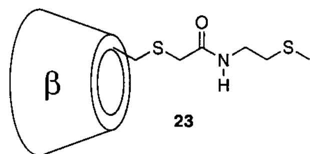
The importance of a solubilizing group is clear. Sensitizers **5**, **8**, and **16** have no sulfonate or carboxylate group, and all

their cleavage reactions are slow. Sensitizers **6**, **11**, and **12** have only carboxylate solubilizing groups, and are not as effective as the sulfonates in **2**, **13**, **14**, and **15**. As mentioned above, the high reproducibility of the data for the **21**/**13** cleavage reaction, data collected more than three weeks apart, supports the idea that these data are generally reliable. Since the compounds were apparently in solution during these runs, it seems likely that the better solubilizing groups produce a faster off rate once the linker is cleaved, permitting the turnovers that are involved in these processes with their excess of dimer over photosensitizer.

Some good agreement of the experimental data with the MacroModel information we described previously (vide supra) can also be seen here. According to the MacroModel calculations, phthalocyanine **6** would be expected to bind most effectively with dimer **17** and least effectively with dimer **21**. This agrees very well with our experimental data, where the times for 50% cleavage of dimers **17**, **18**, and **21** by phthalocyanine **6** are 40, 55, and 85 min, respectively.

Several control reactions were performed. When the oxygen was replaced by argon there was no cleavage, nor any in the absence of the sensitizer under the normal conditions. When the sensitizer was replaced by methylene blue, dimer **1** was cleaved to product **4** with its ^{1}H NMR peak at 8.4 ppm for the formyl group, but additional peaks were also seen at 10.2 and 7.8 ppm. Apparently singlet oxygen generated this way, rather than in complex **3**, is able to attack the cyclodextrin ring also. As we pointed out previously,[8] this indicates that the singlet oxygen generated in complex **3** is selectively taken up by the nearby olefin linkage of **1**. After dimer **21** had been completely cleaved by sensitizer **13**, in ca. 10 min, irradiation was continued for an additional 20 min but produced no further change in the ^{1}H NMR of the solution. In accordance with this result, when the cyclodextrin dimer was replaced by β-cyclodextrin and the reaction was run under normal conditions, no oxidation products were observed.

The cleavage of dimers diminishes their affinity for the sensitizers, and not just because the chelate effect is gone. We examined the binding of compound **23**, which mimics the



cleavage product **4**, except with a methyl group replacing the somewhat hydrolytically labile formyl group. With 4-*tert*-butylbenzoic acid, β-cyclodextrin has a binding constant of 1.7 $\times$ 10^4 M^{-1}, while **23** had a binding constant of only 1.8 $\times$ 10^3 M^{-1}, an order of magnitude less. We ascribe this difference to the competitive binding of the chain in **23** into the cyclodextrin cavity, and propose that the same interaction occurs with cleavage product **4** and related cleavage products from the other dimers.

We were also interested in achieving concentration of the sensitizer complex into a light beam. To achieve this we repeated the experiment described previously,[8] in which a tube was shielded with aluminum foil so that only a small section could be irradiated through a window in the foil. This time we used phthalocyanine **13** and dimer **21**, and made up a 1:1 solution in D_2O. A small amount of **13** was insoluble, and removed. The solution was then irradiated through the window, and after 40 h sensitizer **13** had precipitated solely in the window, and the ^{1}H NMR of the solution indicated that all the dimer **21** had been cleaved; all the vinyl protons were gone, replaced by formyl protons of the monomer. Thus, as expected, the dissolved components do diffuse into the light beam, where they undergo the cleavage reaction.

Of course only biological experiments will indicate whether the same concentrating effect is seen in an animal. In particular, it will be important to see whether biological components interfere with the bindings we have observed, and whether the precipitation of the photosensitizer near a tumor is sufficient to promote selective tumor destruction. Such studies are projected.

Conclusions

1. Cyclodextrin dimers can solubilize various phthalocyanines in water.

2. If the dimers have carbon−carbon double bonds in the linkers, irradiation of the resulting complexes causes the linkers to be cleaved by singlet oxygen generated in the complex.

3. The singlet oxygen is directly delivered to the double bond in the complex, and is thus more selective than singlet oxygen generated in solution by methylene blue.

4. Cleavage of the linkers in the dimers causes dissociation of the cyclodextrins, and resultant precipitation of the phthalocyanines in water.

5. This dissociation occurs both because the chelate binding is gone and because the cleaved chain fragments bind into the cyclodextrins, diminishing their affinity for the phthalocyanines.

6. When the irradiation is directed to a small section of the solution, the phthalocyanines precipitate in the light beam, and are concentrated there as more complexes diffuse into the beam.

7. This concentration effect could have useful applications in the photodynamic therapy of cancer.

Experimental Section

Complete synthesis details are described in the Supporting Information. Thus, here we report only the characterization of the compounds synthesized and details of the binding and photolysis experiments.

Characterization of the Dimers and the Phthalocyanines. (a) Dimer 1: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.09 (t, 2H), 6.22 (s, 2H), 5.90−5.60 (m, 28H), 4.95−4.75 (m, 14H), 4.55−4.40 (m, 12H), 3.90−3.45 (m, 56H), 2.77 (t, 4H); MS (FAB) 2560 (M + 2$^+$, 10). Dimer **17**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.68 (bs, 2H), 6.16 (s, 2H), 5.80−5.67 (m, 28H), 4.81 (m, 14H), 4.47 (m, 14H), 3.62−3.32 (m, 84H), 2.72−2.66 (m, 4H), 2.21−2.10 (m, 4H), 1.84−1.66 (m, 4H); MS (MALDI) *m/z* (%) 2517 (M + 1 + Na$^+$, 5). Dimer **18**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.90 (bs, 2H), 6.20 (s, 2H), 5.99 (d, J = 6.1, 2H), 5.79−5.61 (m, 26H), 4.82−4.75 (m, 14H), 4.50−4.45 (m, 14H), 3.62−3.32 (m, 84H), 2.73 (m, 4H), 2.26−2.23 (m, 4H), 1.82−1.79 (m, 4H); MS (MALDI) *m/z* (%) 2516 (M + Na$^+$, 15). Dimer **21**: TLC R_f 0.13 7:7:5 i-PrOH:ethyl acetate:water; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.24 (s, 2H), 5.78−5.67 (m, 28H), 4.84 (s, 14H), 4.49−4.43 (m, 12H), 3.87−3.30 (m, 80H), 3.05−2.64 (m, 12H).

(b) Zinc phthalocyanine 2: TLC R_f 0.24 20% MeOH (10% NH_3):$CHCl_3$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.45−9.30 (m, 4H), 8.92−8.73 (m, 4H), 7.89−7.81 (m, 3H), 7.70−7.61 (m, 8H), 7.45−7.35 (m, 8H), 1.47−1.41 (m, 27H)); MS (FAB) *m/z* (%) 1193 (M + H$^+$, 5); λ_{max} (nm) 678. Zinc phthalocyanine **5**: TLC R_f 0.67 10% MeOH (10% NH_3):$CHCl_3$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.72−7.78 (m, 8H), 7.60−7.51 (m, 4H), 7.38−7.07 (m, 16H), 1.55−1.42 (m, 36H); MS (FAB) *m/z* (%) 1170 (M + H$^+$, 2); λ_{max} (nm) 680. Zinc phthalocyanine **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.22−8.60 (m, 8H), 8.25−7.35 (m, 20H), 1.46−1.35 (m, 27H); MS (FAB) *m/z* (%) 1157 (M + H$^+$, 5). Zinc phthalocyanine **8**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15−7.05 (m, 8H), 1.81−1.73 (m, 24H); MS (APCI) *m/z* (%) 864 (M$^+$, 4); λ_{max} (nm) 667. Zinc phthalocyanine **9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (bs, 8H), 2.35 (bs, 16H), 1.88 (bs, 16H), 1.24 (s, 26H); MS (FAB) *m/z* (%) 1137 (M + H$^+$, 6), 1136 (M$^+$, 5); λ_{max} (nm) 669. Zinc phthalocyanine **10**: TLC R_f 0.80 10% MeOH (10% NH_3):$CHCl_3$; ^{1}H NMR (300 MHz,

$CDCl_3$) δ 8.60 (bs, 8H), 1.61−2.67 (m, 56H); MS (FAB) *m/z* (%) 1235 ($M + H^+$, 5); λ_{max} (nm) 668. Zinc phthalocyanine **11**: TLC R_f 0.24 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO-d_6) δ 8.71 (m, 4H), 8.13−8.01 (m, 5H), 7.71−7.23 (m, 4H), 5.76 (s, 1H), 1.96 (s, 18H); MS (FAB) *m/z* (%) 930 ($M + H^+$, 2) 929 (M^+, 2); λ_{max} (nm) 666. Zinc phthalocyanine **12**: TLC R_f 0.25 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO-d_6) δ 9.38−9.33 (m, 1H), 8.94−8.90 (m, 1H), 8.58−8.42 (m, 4H), 8.16 (d, J = 8.4, 2H), 8.04−7.92 (m, 1H), 7.58 (s, 1H), 7.49 (d, J = 8.7, 2H), 7.27 (s, 1H), 2.33−1.73 (m, 42H); MS (FAB) *m/z* (%) 1205 (M^+, 0.5); λ_{max} (nm) 667. Zinc phthalocyanine **13**: TLC R_f 0.20 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO-d_6) δ 9.42−9.36 (m, 1H), 8.72−8.50 (m, 4H), 8.41−8.21 (m, 1H), 7.78 (d, J = 8.7, 2H), 7.64−7.53 (m, 1H), 7.32 (d, J = 9.0, 2H), 7.25 (s, 1H), 7.11 (s, 1H), 1.98−1.95 (m, 6H), 1.71−1.65 (m, 12H); MS (FAB) *m/z* (%) 966 ($M + H^+$, 1.5), 965 (M^+, 1.5); λ_{max} (nm) 665. Zinc phthalocyanine **14**: TLC R_f 0.21 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO-d_6) δ 9.38−9.28 (m, 1H), 8.89−8.82 (m, 1H), 8.67−8.49 (m, 4H), 7.82 (d, J = 7.5, 2H), 7.37 (d, J = 8.1, 2H), 7.25 (d, J = 7.2, 1H), 7.03 (s, 1H), 6.87 (d, J = 7.5, 1H), 2.27 (bs, 4H), 1.96 (bs, 4H), 1.74 (bs, 4H), 1.49 (bs, 4H), 1.22−0.74 (m, 26H); MS (FAB) *m/z* (%) 1171 (M^+, 30); λ_{max} (nm) 666. Zinc phthalocyanine **15**: TLC R_f 0.25 20% MeOH (10% NH_3):$CHCl_3$; ^1H NMR (300 MHz, DMSO-d_6) δ 9.378 (bs, 2H), 8.93−8.85 (m, 1H), 8.70−8.52 (m, 3H), 7.80 (d, J = 7.5, 2H), 7.54 (s, 1H), 7.50 (d, J = 9.9, 1H), 7.37 (d, J = 7.5, 2H), 7.05 (s, 1H), 2.26−1.72 (m, 42H); MS (FAB) *m/z* (%) 1243 (M^+, 10); λ_{max} (nm) 667.

Binding Studies. (a) Monomer 23 with *p-tert*-butylbenzoic acid: β-CD monomer **23** (6.4 mg, 0.005 mmol) was dissolved in 0.20 M pH 9.0 $Na_2CO_3/NaHCO_3$ buffer (5.00 mL) to make a 1.0 mM solution. *p-tert*-Butylbenzoic acid (11.0 mg, 0.062 mmol) was dissolved in 6.16 mL of the same buffer to make a 10.0 mM solution. Both solutions were degassed under reduced pressure with a sonicator for 5 min immediately prior to the binding study. The β-CD monomer **23** solution (2.50 mL) was put into the sample cell compartment of an Omega microcalorimeter, whereas the *p-tert*-butylbenzoic acid was loaded in a 250 μL syringe and then assembled onto the calorimeter. The system was equilibrated until root-mean-square error was less than 5×10^{-3} with the syringe spinning at 400 rpm. The *p-tert*-butylbenzoic acid solution was then injected into the cell in 25 injections (10 μL, 7 s per injection). The time interval between injections was set to be 4 min. Injection data were automatically collected by the computer, and the data were analyzed by ORIGIN software with the single-binding-site model. Two trials were performed: trial 1, binding constant $1.8 \pm 0.2 \times 10^3$ M^{-1}, binding ratio 0.91 ± 0.07; trial 2, binding constant $1.8 \pm 0.2 \times 10^3$ M^{-1}, binding ratio 0.99 ± 0.07.

(b) Binding of β-cyclodextrin to *p-tert*-butylbenzoic acid: β-Cyclodextrin (4.0 mg, 0.0035 mmol) was dissolved in 0.20 M pH 9.0 $Na_2CO_3/NaHCO_3$ buffer (3.53 mL) to make a 1.0 mM solution. *p-tert*-Butylbenzoic acid (10.0 mg, 0.056 mmol) was dissolved in 5.60 mL of the same buffer to make a 10.0 mM solution. Both solutions were degassed under reduced pressure with a sonicator for 5 min immediately prior to the binding study. The β-Cyclodextrin solution (2.50 mL) was put into the sample cell compartment of the microcalorimeter, whereas the *p-tert*-butylbenzoic acid was loaded in a 250 μL syringe and then assembled onto the calorimeter. The whole setup was equilibrated until root-mean-square error was less than 5×10^{-3} with the syringe spinnning at 400 rpm. The *p-tert*-butylbenzoic acid solution was then injected into the cell in 25 injections (10 μL, 7 s per injection). The time interval between injections was set to be 4 min. Injection data were automatically collected by the computer, and the data were analyzed by ORIGIN software with the single-binding-site model.

The binding constant was $1.7 \pm 0.2 \times 10^4$ M^{-1}.

(c) Representative binding study of a phthalocyanine to a cyclodextrin dimer: Three solutions were made: (i) β-cyclodextrin dimer **21** (0.20 mg, 8.18×10^{-8} mol) was dissolved in degassed water (250 mL); (ii) BNS **22** (0.14 mg, 3.94×10^{-7} mol) was dissolved in degassed water (4.00 mL); and (iii) β-cyclodextrin dimer **21** (0.40 mg, 1.64×10^{-7} mol) and phthalocyanine **13** (1.64×10^{-7} mol) were dissolved in a mixture of methanol (1 mL) and water (0.1 mL). Mixture iii was stirred in the dark for 1 h. The solution was then concentrated under vacuum and placed under high-vacuum for 18 h. The resulting material was dissolved in degassed water (500 mL).

The binding constant for BNS to cyclodextrin dimer **21** was determined by the fluorescence emission method. Cyclodextrin dimer **21** solution (3.00 mL) was added to a fluorescence cell and was excited at 330 nm and the emission at 418 nm was measured. Five additions of BNS solution (10 μL) were made, with a measurement being taken after each one. The area under the peak between 400 and 500 nm was measured for each addition. The experiment was run in duplicate.

The binding constant for phthalocyanine **13** to dimer **21** was determined by the fluorescence emission method. Dimer:phthalocyanine solution (1:1, 3.00 mL) was added to a fluorescence cell. This solution was excited at 330 nm and the emission at 418 nm was measured. Five additions of BNS solution (10 μL) were made, with a measurement being taken after each one. The experiment was run in duplicate.

The data are plotted in Figure 1.

Representative photocleavage procedure: To a solution of β-cyclodextrin dimer **17** (5.9 mg, 2.35×10^{-6} mol) and potassium carbonate (2 mg) in D_2O (1.00 mL) was added a 3 mM solution of phthalocyanine **6** (0.17 mg, 1.50×10^{-7} mol) in DMSO-d_6 (50 μL). This solution was transferred to an NMR tube and was irradiated with a halogen lamp (50 W) with a cutoff filter to exclude wavelengths below 540 nm. During irradiation, oxygen was bubbled through the solution. The reaction was monitored by ^1H NMR, by observance of the disappearance of the alkene peaks and the appearance of the formyl peak. Whenever the ^1H NMR was taken, the NMR tube was completely shielded from light by using an aluminum foil cover during transportation to the NMR room. The results of a typical run are shown in Figure 2.

Representative precipitation experiment procedure: β-Cyclodextrin dimer **21** (2.4 mg, 1.00×10^{-6} mol), potassium carbonate (0.5 mg), and phthalocyanine **13** (0.97 mg, 1.00×10^{-6} mol) were dissolved in a mixture of methanol (1 mL) and D_2O (0.1 mL). The mixture was stirred in the dark for 1 h. The solvents were removed under vacuum and the resulting solid was placed under high vacuum for 18 h. The residue was treated with D_2O (1.00 mL) and stirred in the dark for 2 h, during which time everything appeared to have dissolved. The solution was then filtered through a cotton wool plug and transferred to an NMR tube. The tube was covered in aluminum foil except for an area approximately 0.5 cm wide, which was left uncovered, through which the solution could be seen. Oxygen was bubbled through the solution for 5 min while the solution was kept in the dark. The solution was then placed on its side and was irradiated with a halogen lamp (50 W) with a cutoff filter to exclude wavelengths below 540 nm. The solution was re-saturated with oxygen, in the same manner as above, after 18 h.

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Supporting Information Available: Additional experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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