Synthesis of β-Galactose-Conjugated Chlorins Derived by Enyne Metathesis as Galectin-Specific Photosensitizers for Photodynamic Therapy

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A first report on the synthesis and biological evaluation of the β-galactose-conjugated purpurinimides (a class of chlorins containing a six-membered fused imide ring system) as Gal-1 (galectin-1) recognized photosensitizers, prepared from purpurin-*N*-propargylimide via enyne metathesis, is discussed. On the basis of examination of the available crystal structure of the galectin-1 *N*-acetyllactose amine complex, it was considered that the chlorin-based photosensitizers could be introduced into a carbohydrate skeleton to expand the repertoire of the galectin-1-specific ligands. Preliminary molecular modeling analysis utilizing the modeled photosensitizers and the available crystal structures of galectin−carbohydrate complexes indicated that addition of the photosensitizer to the carbohydrate moiety at an appropriate position does not interfere with the galectin-carbohydrate recognition. Under similar drug and light doses, compared to the free purpurinimide analogue, the purpurinimides conjugated either with galactose or with lactose (Gal(β1−4)-Glc) produced a considerable increase in photosensitizing efficacy *in vitro*. This indicates the possibility for development of a new class of specific photosensitizers for photodynamic therapy (PDT) based on recognition of a cellular receptor.

Introduction

In recent years, photodynamic therapy (PDT) has received increasing attention as a new modality for selective treatment of solid tumors.[1] Systemic injection of the clinically used photosensitizer leads to relatively selective retention of the compound in tumors. Since in many cases greater amounts of photosensitizer are present within tumors, as compared to nonneoplastic tissues, the neoplasms are destroyed while surrounding normal tissues remain relatively uninjured.[2] While a variety of photosensitizers have been evaluated, the reasons for the accumulation of these compounds in tumors are not clearly understood. However, it has been demonstrated by us and others that overall lipophilicity of the molecule plays an important role in PDT efficacy.[3−5] Although the mechanism of tumor cure likely requires the interplay of several biological responses, a key point remains the ability of the photosensitizer to generate singlet oxygen after light exposure. In tumor destruction, desired cytotoxic effects on the cell membrane, mitochondria, and nuclear structures have been observed. These effects could relate to the demonstrated microcirculatory aberrations during and after PDT.[6]

For synthesizing various amphiphilic porphyrins, among various solubilizing substituents, sugars have attracted most attention. In addition to providing the molecule with polar hydroxy groups, it is possible that the sugar moiety might lead the conjugate to a cell surface target through specific binding to its receptor. Oligosaccharides play essential roles in various cellular activities as antigens,

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growth signals, targets of bacterial and viral infection, and glues in cell adhesion and metastasis, where the saccharide−receptor interactions are usually specific and multivalent.[7] This specificity suggests a potential utility of synthetic saccharide derivatives as carriers in directed drug delivery. Therefore, in recent years, a number of carbohydrate derivatives of various photosensitizers have been synthesized. Hombrecher et al.[8] reported the preparation of a galactopyranosyl-cholesteryloxy-substituted porphyrin by following the McDonald approach. Several tetra- and octaglycoconjugated tetraphenylporphyrins were also reported by Yano and co-workers,[9] Cornia et al.,[10] Momenteau and co-workers,[11] and Ono et al.[12] A glycosylated peptide porphyrin synthesized by Krausz and co-workers[13] showed promising in vitro PDT efficacy. Maillard et al.[14] reported the synthesis of a glycoconjugated *meso*-monoarylbenzochlorin. This compound displayed good in vitro PDT efficacy in tumor cell lines after irradiation with light. A few years ago, Bonnett and co-workers[15] showed that, compared to β-hydroxyoctaethylchlorin, the related glycosylated analogue was more effective as a photosensitizing agent in vitro as well as in vivo. Montforts et al.[16] further explored this approach by attaching hydrophilic carbohydrate structural units to certain chlorins. Such conjugation increased the water solubility of the parent chlorins by introducing an estradiol with a diethyl spacer. The chlorin−estrogen conjugate was then prepared with the hope that it would bind to an estrogen receptor that could induce destruction of mammalian carcinoma. In a recent report, Aoyama and co-workers[17] reported the synthesis of certain TPP-based saccharide-functionalized porphyrins and demonstrated the importance of hydrophobicity masking for the saccharide-directed cell recognition. Since the saccharide-receptor interactions are ubiquitous, well-defined/well-designed synthetic saccharides clusters may serve as a new tool in glycoscience and glycotechnology.

In our efforts to prepare target-specific photosensitizers for photodynamic therapy, we sought to target those proteins that are known for their overexpression in malignant tumors and show recognition for the sugar moieties. A literature survey revealed that among certain proteins the galectins are a family of animal lectins defined by a highly conserved 15-kDa carbohydrate recognition domain (CRD) known to show high affinity for β-galactoside.[18] Because galectins are involved in the modulation of cell adhesion, cell growth, immune response, and angiogenesis, it is clear that changes in their expression might have a critical role in tumor progression. Galectin-1 (Gal-1) is a prototype, dimeric galectin with two identical CRD, and its expression is known to correlate with the degree of malignancy in rat thyroid cell lines transformed with several cellular or viral oncogenes.[19] Gal-1 mRNA levels increase 20-fold in low tumorigenic and up to 100-fold in highly tumorigenic cells. These observations are consistent with those observed in human tumors.[20] Furthermore, Gal-1 null mutant mice are found to be relatively healthy.[8] Therefore, it seems that this may be a valuable cellular target for β-galactoside-based photosensitizers.

Analysis of the carbohydrate binding site in Gal-1 shows that Gal-1 has a pronounced specificity for the Gal(β1−4)- and Gal(β1−3)GlcNAc sequences with no apparent affinity for GlcNAc residues. The binding specificity for Gal moiety is due to hydrogen bond interactions between its C4-hydroxy group and His 44, Asn 46 and Arg 48 residues that are conserved in all galectins. The van der Waals contact of Trp 68 with the Gal moiety as well as the hydrogen bonding of the C6-hydroxy group to Gal-1 also contribute to binding.[21]

Since our objective has been to develop target specific photosensitizers as therapeutic agents for photodynamic therapy, it was desired to establish a general synthetic route for the preparation of β-galactoside based long wavelength absorbing photosensitizers as Gal-1 recognizing agents. Our study was aimed at determining the effect of the presence of the carbohydrate moieties on tumor selectivity and to explore the viability of this approach in converting an inactive compound with the required photophysical properties into an active therapeutic drug.

Results and Discussion

Rational Design of Carbohydrate Conjugates. The feasibility and specific positions to introduce the photosensitizers into carbohydrates without disrupting the sugar-receptor recognition were studied by examining the available crystal structure of galectin-ligand complexes. The interaction between bovine galectin-1 and *N*-acetyllactoseamine found in crystal structure (1SLT)[25] is shown in Figure 2. The galactose moiety of the ligand is interacting with galectin-1 binding residues, which form a shallow cleft. On the other hand, the glucoseamine moiety is far from these binding residues and points out toward solvents. Thus, it appears that the photosensi-

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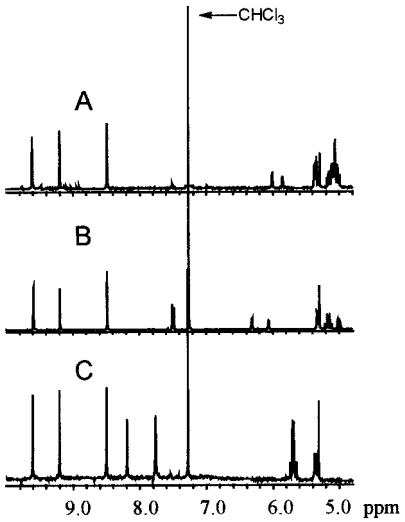

Figure 1. Partial ^{1}H NMR spectra (400 MHz, $CDCl_3$) of purpurinimide **9** (A), **10** (B), and **11** (C).

tizers can occupy the same regions as the glucose amine moiety of the ligand without causing any disruptions to the specific recognition of galactosides by the galectin-1 (Figure 2).

To retain the binding affinity of the carbohydrate conjugate at the target site, instead of directly introducing the carbohydrate moiety to the porphyrin skeleton, we thought it necessary to link a photosensitizer with a spacer at position-1 of the β-galactose. In our approach, purpuin-18 methyl ester **1**[22] was selected as a starting material due to its many advantages over TPP and other types of porphyrins: (a) its ready availability from chlorophyll *a* by following the methodology developed in our laboratory;[3e] (b) its strong absorption near 700 nm and, thus, the ability to treat more deeply seated tumors; (c) its high singlet oxygen yield (55%), a key cytotoxic agent for PDT; and (d) the presence of the vinylpropionic acid side chain and the fused anhydride ring systems that can be modified easily, thus avoiding multistep total syntheses. On the basis of molecular modeling, appropriate imide-based spacers can be introduced between the photosensitizer and the carbohydrate moiety through the fused anhydride ring system.

To construct the desired β-galactose conjugated photosensitizers, we extended our recent approach developed for the preparation of chlorindiene **4** via ruthenium-catalyzed enyne metathesis.[23] For our initial studies, purpurin-18 methyl ester **1** on hydrogenation over Pd/carbon was converted into mesopurpurin-18 methyl ester **2** in 90% yield. Reaction of **2** with propargylamine in refluxing benzene for 12 h produced the corresponding propargylimide derivative **3** in 80% yield and was used as the alkyne substrate for ynene cross metathesis. As for the alkene component, acetylated β-D-1-*O*-allylgalactopyranoside **6** was prepared from β-D-galactose pentaacetate **5** by BF_3−etherate-induced glycosylation.[24] Compounds **4** and **6** were then introduced to ynene cross-metathesis reaction by treatment with Grubbs' ruthenium catalyst in CH_2Cl_2 solution at room temperature for 48 h. The galactopyranose−chlorin conjugate **7** was obtained in 40% yield as the diastereomeric mixture, due to the incorporation of the *E*/*Z* mixtures of the carbohydrate-substituted 1,3-diene. To determine the effect of the rigidity of the spacer to the Gal-1 binding affinity and PDT efficacy, compound **7** on refluxing with dimethyl acetylenedicarboxylate (DMAD) in toluene afforded the corresponding Diels−Alder adduct **9**. As expected, deacetylation of diene **7** by NaOMe/MeOH−CH_2Cl_2 produced the expected conjugate **8**. The removal of the acetyl groups in **9** under similar reaction conditions produced chlorin **10** as the major product and the unexpected chlorin **11** as a minor component (Scheme 1). The structures of the minor and the major components were confirmed by 2D ROESY and COSY NMR spectroscopy. Partial ^{1}H NMR spectra (aromatic region only) of the cabohydrate−chlorin conjugates **9**−**11** are shown in Figure 1. A possible mechanism for the formation of these compounds via the base-catalyzed rearrangement is illustrated in Scheme 2. Following a similar approach as discussed for the preparation of conjugate **8**, diene **4** was reacted with **13** and the related photosensitizer containing a lactose moiety **15** was obtained in 30% yield (Scheme 1). ^{1}H NMR and mass spectrometry or elemental analyses confirmed the structures of all new compounds.

Molecular Modeling Studies. The ability of the chlorin−carbohydrate conjugates to bind to the cellular target was examined by molecular modeling of the galectin chlorin−carbohydrate conjugate complexes. The high-resolution crystal structures of bovine galectin-1 complexed with *N*-acetyllactosamine (Figure 2) was used as a template to model galectin-1 chlorin−carbohydrate conjugate complexes. It should be noted that the aim of this modeling study was to obtain a rationale for the positioning of the chlorin moiety that does not interfere with the carbohydrate recognition by galectin-1. The optimized structures of compounds **8**, **10**, and **15** are placed into the binding site. The six galactose ring atoms were used for the superposition of the galactose portion of *N*-acetyllactosemine with the galactose moiety of the compounds **8**, **10**, and **15**. No attempt was made to optimize the orientation of the chlorin or linker regions toward galectin-1.

The structures of compounds **8**, **10**, **15** are built from the crystal structure of benzimidazo[2,1-*n*]purpurin-18 13^1-imino-13^2-imide methyl ester.[27] Appropriate modifications were performed with the SYBYL modeling program version 6.6 (Tripos Inc., St. Louis, MO) using standard geometry and the SYBYL fragment library. The extended conformation was assumed for the linker region. The geometry of each compound was fully optimized with a semiempirical molecular orbital method, AM1, with the SPARTAN (Wavefunction Inc., Irvine, CA) program.

The superposition operations resulted in good fit for all the compounds with the root-mean-square deviation values ranging from 0.03 to 0.04 Å. (Figure 3). The resulting structures of the complex clearly indicated that the chlorin moiety is far from the galactose binding site. Thus, they should not interfere with the recognition of the carbohydrate moiety by galectin-1. The overall view of the complexes for the compound **15** are shown in Figure 4, while the close-up views of the binding site are shown in Figure 5. Since the aim of this study was to examine a feasibility of the chlorin−carbohydrate conjugate binding to galectin-1, only a limited conformational

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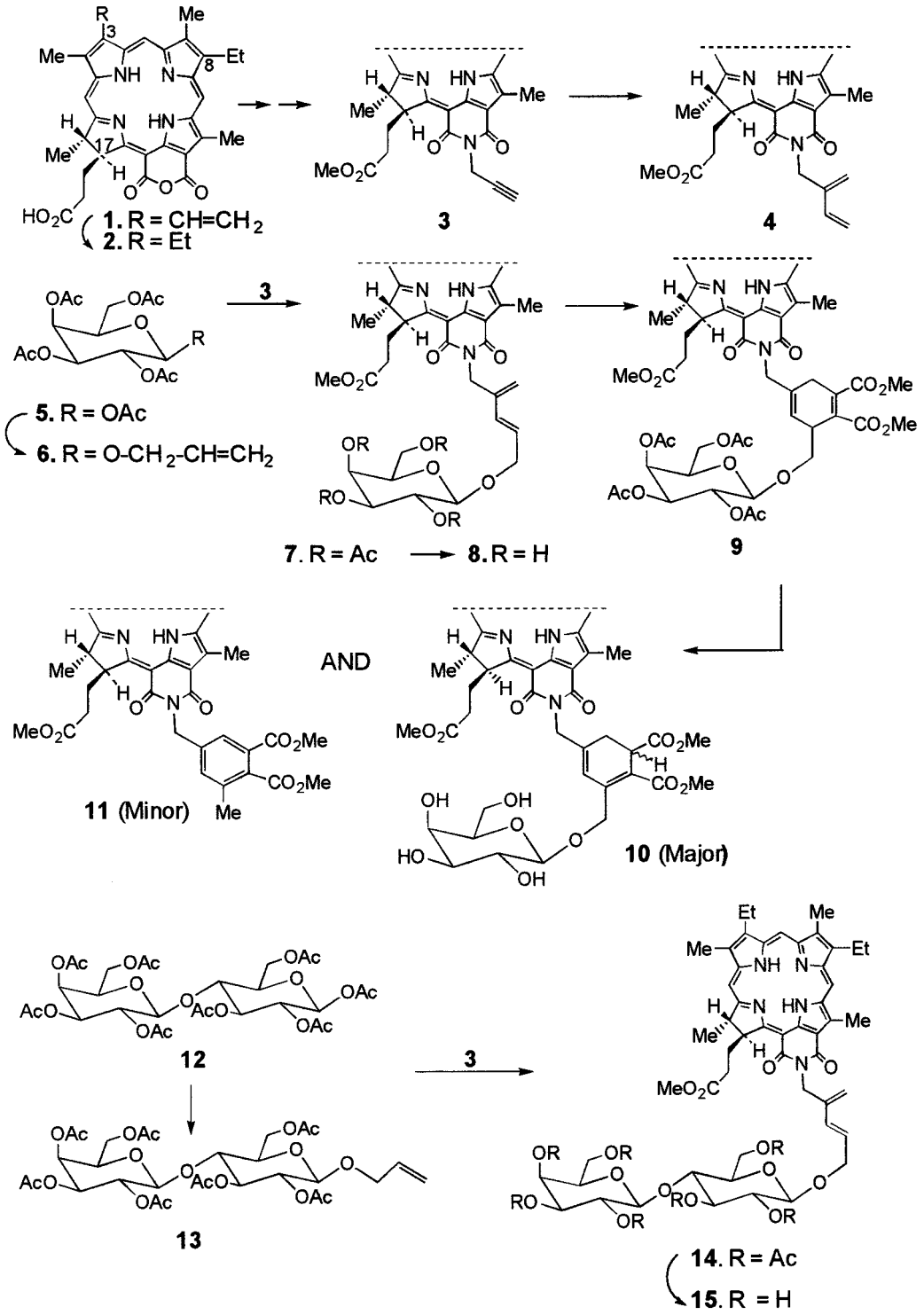
Scheme 1. Syntheses of Chlorin−Carbohydrate Conjugates

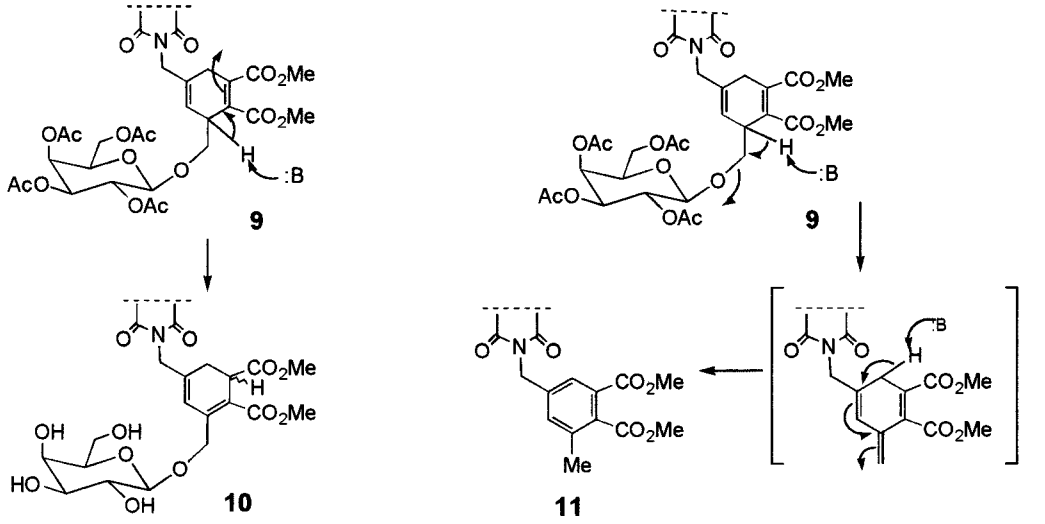


flexibility was examined for the ligand binding. More detailed studies with galectin-1 are currently underway.

Photosensitizing Efficacy.[26] The photocytoxicity of photosensitizer **10** in Molt-4 cells with variable drug concentrations is shown in Figure 6. Viability was measured by trypane blue exclusion. The drug conentration (3.25 μM) that produced the best results was used for evaluating other photosensitizers with and without carbohydrate conjugates. As can be seen from Figure 7, compared to the nongalactose analogue **4**, both chlorin−galactose conjugates **8** and **10** showed significant increase in photosensitizing efficacy. It was interesting to observe that decreasing the flexibility of the diene system (compound **8**) by converting it into the corresponding DMAD analogue **10** exhibited similar PDT efficacy. However, compared to photosensitizers **8** and **10**, the corresponding lactose conjugate **15** showed enhanced PDT efficacy.

Further detailed in vitro and in vivo studies indicated the superiority of the carbohydrate conjugates (**8**, **10**, and **15**) over the nonconjugated (**4**) photosensitizers. Cells incubated with lactose prior to the addition of the carbohydrate photosensitizers produced a significant decrease in in vitro PDT efficacy, indicated the target specificity of the carbohydrate analogues. In a prelimi-

Scheme 2. Possible Mechanisms for the Formation of 10 and 11



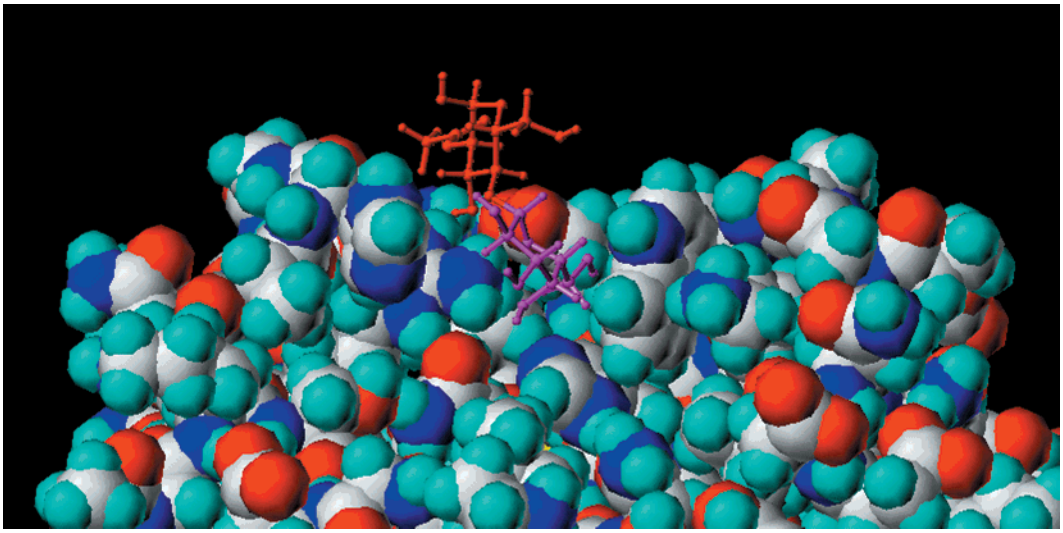


Figure 2. Interaction between the bovine galectin-1 and *N*-acetyllactosamine found in the crystal structure (ISLT). The protein is shown in the CPK model, while the ligand is shown in a stick and ball figure. The galactose portion of the ligand is shown in magenta and the lactose moiety is shown in orange.

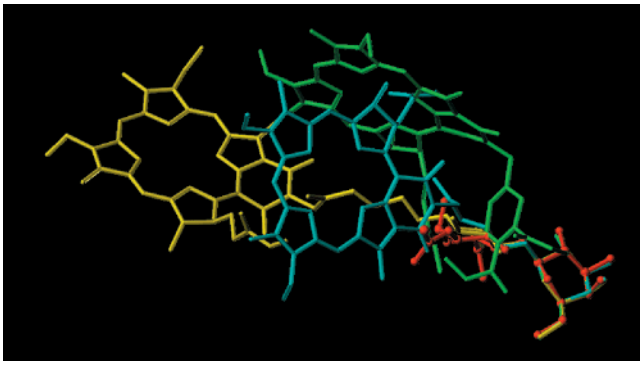


Figure 3. Superposition of the galactose ring atoms between the crystal structure of *N*-acetyllactosamine with the AM1-optimized geometries of compound **8** (cyan), **10** (yellow), and **15** (green). The crystal structure is drawn as a ball and stick figure (red), while the model compounds are shown in stick figures.

nary in vivo experiment, compared to the nonconjugated analogue which was found to be ineffective in vivo (C3H mice transplanted with RIF tumors, six mice/group, drug dose: 5.0 mm/kg, light dose: 130 J/cm^2, treated 24 h postinjection), the corresponding carbohydrate conjugates **10** and **15** under similar treatment conditions gave 50% tumor response at day 30 (50% mice were tumor free). These results along with those obtained from Gal-1 inhibition binding and intracellular localization studies, will be reported in a journal with a more biological/drug development orientation.

Conclusion

The present work demonstrates the use of enyne metathesis for the preparation of chlorin−carbohydrate conjugates as gal-1 recognized photosensitizers for PDT. This approach provides an opportunity for synthesizing a variety of photosensitizers conjugated with carbohydrates via a stable carbon−carbon bond. If desired, the length of the spacer joining the two moieties can be varied by selecting appropriate reagents and reaction conditions. The high PDT efficacy (in vitro) of the carbohydrate analogues over the related nonconjugated analogue demonstrate a great potential in converting a nonactive compound with the required photophysical characteristics into an effective photosensitizer. The molecular

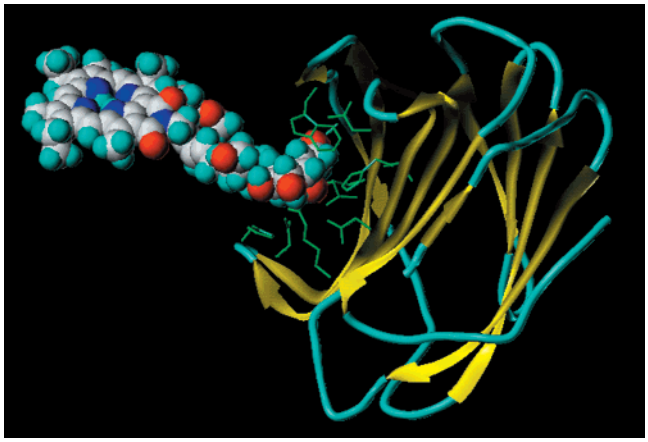


Figure 4. Overall view of the structures of galectin-1 and compound **15** complex. The chlorin−carbohydrate conjugate is shown as a space-filling model, while galectin-1 is shown with schematic presentation of the secondary structure. It is clear from this figure that the chlorin moiety is pointing out toward solvents and thus does not interfere with the carbohydrate−galectin recognition. The binding residues side chains are shown in green sticks.

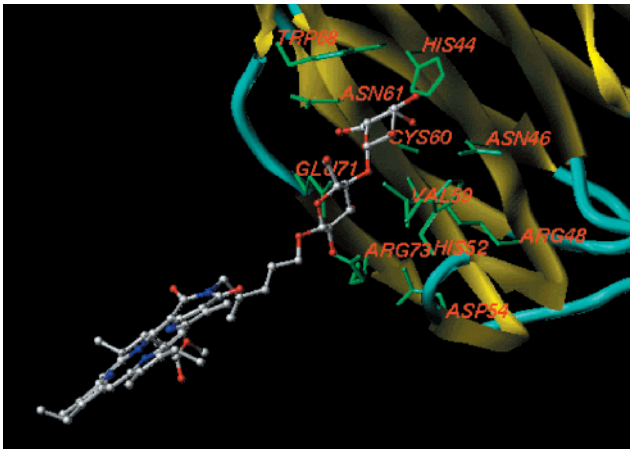


Figure 5. Close-up view of the carbohydrate binding site of the galectin-chlorin-carbohydrate conjugate complex for the compound **15**. The chlorin−carbohydrate conjugates are shown as ball and stick figures, while the suggested binding residues are shown as a stick (green) figure with residue labels in red. The protein backbone is shown in schematic drawing of the secondary structure.

modeling results suggest that the six membered ring of the linker diminishes the flexibility of the spacer and mimics the glucose moiety of βGal(1−4)-Glc. Replacing galactose with a lactose moiety (photosensitizer **15**), however, produced a significant increase in in vitro photosensitizing efficacy. In our goal to select the best candidate from this series, the detailed in vivo studies with these and other long-wavelength absorbing photosensitizers at different drug/light doses at various time intervals are currently in progress and will be reported elsewhere.

Experimental Section

General Methods. Unless otherwise noted, all reactions were run under argon atmosphere, and distilled solvents were transferred by syringe. The solvents were dried by following the standard procedures. Melting points are uncorrected. Mass spectral and HRMS analyses were carried out at the Mass

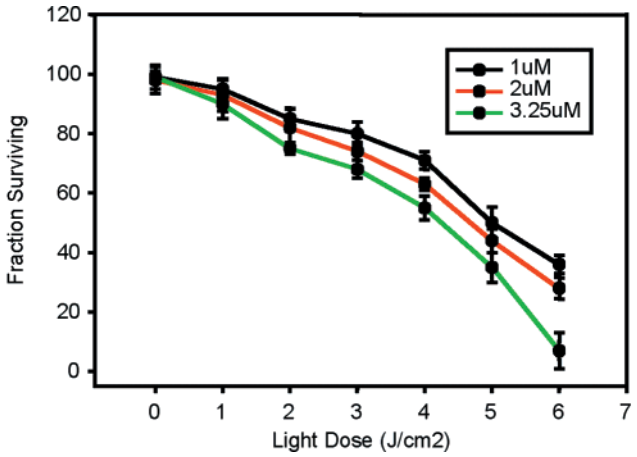


Figure 6. In vitro photosensitizing efficacy of purpurinimide−galactose conjugate **10** in Molt-4 cells at various concentrations.

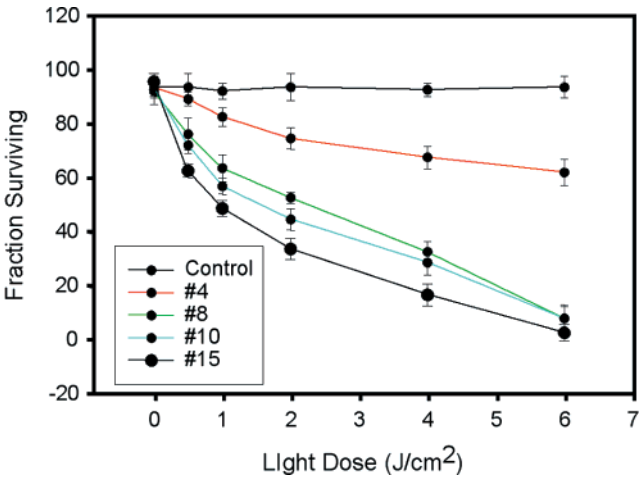


Figure 7. Comparative in vitro photosensitizing efficacy of photosensitizer **4**, its galactose conjugates **8** and **10**, and lactose conjugate **15** in Molt-4 cells. Control: No photosensitizer was added.

Spectrometry Facility, SUNY, Buffalo, and MSU−NIH mass spectrometry facility, East Lansing. The reactions were monitored by TLC. Preparative TLC was performed on 20 × 20 cm TLC plates (1 mm thick, Analtech).

Mesopurpurin-18-*N*-propargylimide Methyl Ester (3). A mixture of mesopurpurin-18 methyl ester **2** [475 mg (0.82 mmol)] and propargylamine [3.0 g (55 mmol)] was dissolved in benzene (40 mL), and the mixture was refluxed gently under an argon atmosphere overnight. After the mixture was cooled to room temperature, solvent and excess propargylamine were removed. The crude product was purified by silica column chromatography with 2% methanol in dichloromethane. Crystallization of the product with dichloromethane and hexanes afforded 425 mg (0.69 mmol) of the title compound as a purple solid. Yield: 85%. Mp: 235−237 °C. UV−vis in CH_2Cl_2, λ_{max} (nm, ϵ): 692 (4.50×10^4), 543 (2.05×10^4), 506 (7.66×10^3), 413 (1.40×10^5), 360 (5.22×10^4). ^{1}H NMR (400 MHz, 5.0 mg/mL $CDCl_3$, δ ppm): 9.56, 9.17, and 8.50 (each s, 1H, 5-H, 10-H and 20-H); 5.40 (dd, J = 8.9, 2.7 Hz, 1H, 17-H); 5.29 (dd, J = 6.5, 2.3 Hz, 2H, NCH_2); 4.35 (q, J = 7.4 Hz, 1H, 18-H); 3.81, 3.59, 3.24, and 3.17 (each s, 3H, 2-, 7-, 12- CH_3, and CO_2CH_3); 3.75 and 3.63 (each q, J = 7.9 Hz, 2H, 3- and 8-CH_2CH_3); 2.74 (m, 1H, 1 × 17$CH_2CH_2CO_2CH_3$); 2.43 (m, 2H, 1 × 17CH_2CH_2-CO_2CH_3 and 1 × 17$CH_2CH_2CO_2CH_3$); 2.32 (t, J = 2.2 Hz, 1H, C≡CH); 2.01 (m, 1H, 1 × 17$CH_2CH_2CO_2CH_3$); 1.76 (d, J = 7.2 Hz, 3H, 18-CH_3); 1.71 and 1.67 (each t, J = 8.0 Hz, 3H, 3-CH_2CH_3 and 8-CH_2CH_3); 0.19 and −0.01 (each br s, 1H, 2NH). MS (FAB) found m/z 618.0 (100, $M^+ + 1$). HRMS: calcd

for $C_{37}H_{39}N_5O_4$ 618.3077 (M + 1), found 618.3078 (MH^+). Anal. Calcd for $C_{37}H_{39}N_5O_4 \cdot {}^1/_2H_2O$: C, 70.89; H, 6.44; N, 11.18. Found: C, 71.25; H, 6.41; N, 10.91.

Per-O-acetylated 1-*O*-Allyl-β-D-galactopyranose (6). Boron trifluoride etherate [8.0 mL (63 mmol)] was added dropwise to a cooled solution of 5.0 g (12.8 mmol) of peracetylated galactose and 1.05 mL (15.4 mmol) allyl alcohol in dry dichloromethane (20 mL). The ice bath was removed after 0.5 h, and the reaction was allowed to warm to room temperature until the starting material had been consumed (monitored by TLC). The reaction mixture was then poured into 300 mL of aqueous saturated sodium bicarbonate solution, extracted with dichloromethane, and washed with water (2 × 200 mL). Drying (Na_2SO_4) and evaporation of the dichloromethane layer gave a crude residue that was chromatographed on silica column with 30% ethyl acetate in cyclohexane to afford 3.2 g (8 mmol) of the title compound. Yield: 60%. ^{1}H NMR (400 MHz, 5.0 mg/mL $CDCl_3$, δ ppm): 5.83 (m, 1H, $OCH_2CH{=}CH_2$); 5.33 (d, J = 3.5 Hz, 1H, 4-H); 5.27−5.12 (m, 3H, $OCH_2CH{=}CH_2$ and 2-H); 4.97 (dd, J = 10.0, 3.0 Hz, 1H, 3-H); 4.50 (d, J = 8.1 Hz, 1H, 1-H); 4.32 (dd, J = 13.3, 4.9 Hz, 1H, 1 × 6-H); 4.17−4.05 (m, 3H, 1 × 6-H and $OCH_2CH{=}CH_2$); 3.88 (t, J = 6.6 Hz, 1H, 5-H); 2.12, 2.03, 2.02 and 1.95 (each s, 3H, 4 × $COCH_3$). MS (FAB): found *m/z* 331.3 [M − 57.03 (*O*-allyl)].

Peracetylated 1-*O*-Allyl-β-D-galactopyranosyl-[1−4]-β-D-glucopyranose (13). Following the methodology as described above, the title compound was obtained from β-D-lactose octaacetate and allyl alcohol in the presence of boron trifluoride etherate in 50% yield. ^{1}H NMR (400 MHz, 5.0 mg/mL $CDCl_3$, δ ppm): 5.84 (m, 1H, $OCH_2CH{=}CH_2$); 5.50−3.59 (each m, total 18H, 14-Lac-H and 4H from $OCH_2CH{=}CH_2$); 2.15 and 2.12 (each s, 3H, 2 × $COCH_3$); 2.06 (s, 6H, 2 × $COCH_3$); 2.04 (s, 9H, 3 × $COCH_3$); 1.96 (s, 3H, $COCH_3$). MS (FAB): found *m/z* 676.5 (100, M).

Mesopurpurin-18-*N*-methyl-(2′-butadiene)imide (4). A mixture of propargylimide **3** [300 mg (0.48 mmol)] and Grubbs' catalyst [bis(tricyclohexylphosphine)benzylidine rutheniun-(IV)] (10 mol %) was dissolved in 40 mL of dry dichloromethane. The flask was equipped with a balloon filled with ethylene gas. The reaction mixture was stirred under ethylene atmosphere for 48 h. After evaporation of solvent, the crude was separated by silica column with 1.5% methanol in dichloromethane. The title compound was obtained in 30% yield, mp 126−128 °C (turned black). On the basis of the starting material recovered, the yield was quantitative. UV−vis in CH_2Cl_2, λ_{max} (nm, ϵ): 695 (4.5 × 10^4), 640 (9.1 × 10^3), 545 (2.2 × 10^4), 505 (7.8 × 10^3), 480 (4.5 × 10^3), 415 (1.4 × 10^5), 360 (5.4 × 10^4). ^{1}H NMR (400 MHz, 5.0 mg/mL $CDCl_3$, δ ppm): 9.60, 9.20 and 8.51 (each s, 1H, 5-H, 10-H, and 20-H); 6.69 (dd, J = 17.8, 11.1 Hz, 1H, 3′-H); 5.63 (d, J = 17.7 Hz, 1H, *trans*-4′-H); 5.40 (dd, J = 9.0, 2.5 Hz, 1H, 17-H); 5.31 (d, J = 11.0 Hz, 1H, *cis*-4′-H); 5.31 (s, 2H, N-CH_2); 5.21 (d, J = 15.2 Hz, 2H, 2 × 1′-H); 4.33 (q, J = 7.5 Hz, 1H, 18-H); 3.82, 3.55, 3.25, and 3.18 (each s, 3H, 2-, 7-, 12-CH_3 and CO_2CH_3); 3.76 and 3.64 (each q, J = 7.7 Hz, 2H, 3- and 8-CH_2CH_3); 2.67 (m, 1H, 1 × 17CH_2-$CH_2CO_2CH_3$); 2.38 (m, 2H, 1 × 17$CH_2CH_2CO_2CH_3$ and 1 × 17$CH_2CH_2CO_2CH_3$); 2.00 (m, 1H, 1 × 17$CH_2CH_2CO_2CH_3$); 1.76 (d, J = 7.2 Hz, 3H, 18-CH_3); 1.71 and 1.67 (each t, J = 8.0 Hz, 3H, 3-CH_2CH_3 and 8-CH_2CH_3); 0.14 and −0.07 (each br s, 1H, 2N−H). MS: calcd for $C_{39}H_{43}N_5O_4$ 645.3, found *m/z* 646.6 (100, M^+ + 1). HRMS: 646.3408 (M + 1).

Per-acetylated Galactose−Chlorin Conjugate Joined with 2′,4′-Diene Linkage (7). To a solution of propargylimide **3** [150 mg (0.24 mmol)] and *O*-allyl sugar **6** [350 mg (0.90 mmol)] in dichloromethane (10 mL) was added Grubbs' catalyst [25 mg (0.03 mmol)]. The reaction mixture was stirred under argon for 24 h. It was allowed to stir for another 24 h after addition of an additional 25 mg (0.03 mmol) of the catalyst. After evaporation the solvent, the crude residue was purified by silica plate preparative chromatography, eluting with 2% methanol in dichloromethane. The title compound was obtained in 30% yield (70 mg). Mp: 120−122 °C (turned black). ^{1}H NMR (400 MHz, 5.0 mg/mL $CDCl_3$, δ ppm) showing a diastereomeric mixture: 9.61 and 9.59 (each s, 2H, total 2 meso-H); 9.20 and 8.50 (each s, 2H, total 4 meso-H); 6.65−3.90 (each m, total 30H, 14 sugar H, 2 × 17-H, 2 × 18-H, 12H for (2′-butadiene)); 3.82 and 3.81 (each s, total 6H, 2 × CH_3); 3.76 and 3.65 (each q, J = 7.6 Hz, total 8H, 2 × 3- and 8-CH_2-CH_3); 3.71 and 3.55 (each m, total 4H, 2 × 4′-CH_2O); 3.57 and 3.54 (each s, total 6H, 2 × CH_3); 3.25 and 3.18 (each s, 6H, 4 × CH_3); 2.67 (m, 2H, 2 × 17$CH_2CH_2CO_2CH_3$); 2.37 (m, 4H, 2 × 17$CH_2CH_2CO_2CH_3$ and 2 × 17$CH_2CH_2CO_2CH_3$); 2.22−1.92 (m, total 26H, 2 × 17$CH_2CH_2CO_2CH_3$ and 8 × $COCH_3$); 1.76 (d, J = 7.2 Hz, 6H, 2 × 18-CH_3); 1.71 and 1.68 (each t, J = 8.0 Hz, 6H, 2 × 3-CH_2CH_3 and 8-CH_2CH_3); 0.18, 0.09, −0.04 and −0.10 (each br s, total 4H, 4 × N−H). MS (FAB) found: *m/z* 1006.9 (100, M + 1). HRMS: calcd 1006.4430 (M + 1), found 1006.4428 (MH^+). Anal. Calcd for $C_{54}H_{63}N_5O_{14} \cdot H_2O$: C, 63.31; H, 6.40; N, 6.84. Found: C, 62.92; H, 6.20; N, 6.48.

Galactose−Chlorin Conjugate Joined with Diene Linkage (8). To a solution of **7** [40 mg (0.04 mmol) in dichloromethane (20 mL) was added 1 M NaOMe in MeOH (200 μL), and the reaction mixture was stirred under argon for 1 h. After the standard workup, the residue was separated by silica plate chromatography with 8% MeOH/CH_2Cl_2. The title compound was obtained in 50% yield (17 mg). Mp: 134−137 °C (turned black). UV−vis in CH_2Cl_2, λ_{max} (nm, ϵ): 695 (4.5 × 10^4), 640 (8.9 × 10^3), 545 (2.2 × 10^4), 505 (7.8 × 10^3), 480 (4.5 × 10^3), 415 (1.4 × 10^5), 360 (5.4 × 10^4); MS (FAB): found *m/z* 839.1 (100, M^+ + 1); ^{1}H NMR (400 MHz, 5.0 mg/mL $CDCl_3$, δ ppm) showing a diastereomeric mixture in 4:1 ratio: 9.35, 9.14, 9.08, 9.02 and 8.46 (each s, total 6H, 2 × 5-, 10-, and 20-H); 6.67−3.55 (each m, total 34H, 14 sugar H, 2 × 17-H, 2 × 18-H, 16H for linker-H); 3.51, 3.46, 3.22, and 2.96 (each s, 6H, 2 × 2-, 7-, 12-CH_3 and 2 × CO_2CH_3); 3.31 and 3.04 (each m, 4H, 2 × 3- and 8-CH_2CH_3); 2.66 (m, 2H, 2 × 17$CH_2CH_2CO_2CH_3$); 2.36 (m, 4H, 2 × 17$CH_2CH_2CO_2CH_3$ and 2 × 17$CH_2CH_2CO_2CH_3$); 1.95 (m, total 2H, 2 × 17$CH_2CH_2CO_2CH_3$); 1.76 (d, J = 7.6 Hz, 6H, 2 × 18-CH_3); 1.68 and 1.48 (each t, J = 7.3 Hz, 6H, 2 × 3- and 8-CH_2CH_3); −0.12 (br s, total 4H, 4 × NH). MS: calcd for $C_{45}H_{55}N_5O_{10}$ 837.4, found *m/z* 839.1. HRMS: calcd 838.4047 (M + 1), found (FAB) 838.4046 (MH^+).

Peracetylated Galactose−Chlorin Conjugate Joined with 1,4-Cyclohexadiene Linkage (9). To a solution of **7** [160 mg (0.16 mmol)] in toluene (20 mL) was added 1.0 mL of dimethylacetylenedicarboxylate (DMAD). The reaction mixture was refluxed under argon for 3 h. After solvent was removed under high vacuum (for removing DMAD), the residue was purified by silica column chromatography, eluting with 2% MeOH/CH_2Cl_2 to afford 60 mg (0.05 mmol) of the title compound in 33% yield. Mp: 135−137 °C (turned black). ^{1}H NMR of the diastereomeric mixture (400 MHz, 5.0 mg/mL $CDCl_3$, δ ppm): 9.13 (s, 2H, 2 × 10-H); 9.22 (s, 2H, 2 × 5-H); 8.50 (s, 2H, 2 × 20-H); 6.02 and 5.88 (each s, total 2H, 2 × 13^8-H); 5.36 (m, 2H, 2 × 17-H); 5.00−5.24 (m, 4 × 13^3-H); 4.61 and 4.62 (each d, 2H, 13^5H); 4.29 (q, 2H, 2 × 18-H); 4.18 (m, 2H, 2 × Gal-H); 4.10 and 4.08 (each s, total 2H, 2 × 13^{10}-H); 4.01 (m, 4H, 4 × Gal-H); 3.76, 3.75, 3.74, 3.60, 3.25, 3.20 (each s, 36H 6 × CH_3 and 6 × CO_2CH_3); 3.76 (m, 4H, 2 × 3-CH_2-CH_3); 3.70 (m, 4H, 4 × Gal-H); 3.62 (m, 2H, 2 × Gal-H); 3.60 (m, 4H, 2 × 8- CH_2CH_3); 3.43 and 3.38 (each m, 4H, 4 × 13^9-H); 3.35 (m, 2H, 2 × 13^{10}-H); 2.64 (m, 2H, 2 × 17^1-H); 2.55 (m, 4H, 2 × 17^1-H) 2.22 (m, 2 × 17^2-H); 2.2−1.60 (8s, 8 × −OAc, 2t, 4 × CH_2CH_3, d, 2 × 18-CH_3); 0.17 and −0.12 (each brs, 2H, 4 × NH). HRMS: calcd for $C_{61}H_{69}N_5O_{18}$ 1148.4760 (M + 1), found 1148.4762 (MH^+).

Galactose−Chlorin Conjugate Joined with 1,3-Cyclohexadiene Linkage (10). To a solution of **9** [40 mg (0.035 mmol)] in dichloromethane (20 mL) was added 1 M NaOMe in MeOH (250 μL), and the reaction mixture was stirred under argon for 1 h. After standard workup, the residue was separated by silica plate chromatography, eluting with 8% MeOH/CH_2Cl_2, and the title compound was obtained in 44% yield (15 mg) along with 7 mg (0.009 mmol) of **11** in 25% yield: mp 73−76 °C (turned black); ^{1}H NMR (400 MHz, 5.0 mg/mL $CDCl_3$, δ ppm) showing a diastereomeric mixture: 9.53 (s, 2H, 2 × 10-H); 9.15 (s, 2H, 2 × 5-H); 8.46 (s, 2H, 2 × 20-H); 7.49 and 7.46 (each s, total 2H, 2 × 13^5-H); 6.29 and 6.04 (each s, total 2H, 2 × 13^8-H); 5.30 (m, 2H, 2 × 17-H); 5.14 (m, 2H, 2 × 13^3-H); 4.98 (m, 2H, 2 × 13^3-H); 4.29 (m, 2H, 2 × 18-H);

4.18 (m, 2H, 2 × Gal-H); 4.04 and 3.99 (each s, total 2H, 2 × 13^{10}-H); 3.85 (m, 4H, 4 × Gal-H); 3.77 (s, 6H, 2 × 12-CH_3); 3.75 (m, 4H, 2 × 3-CH_2CH_3); 3.70 (m, 4H, 4 × Gal-H); 3.63 (m, 2H, 2 × Gal-H); 3.60 (m, 4H, 2 × 8- CH_2CH_3); 3.53 (s, 6H, 2 × 17^3-CO_2CH_3); 3.43 and 3.38 (each m, 4H, 4 × 13^9-H); 3.35 (m, 2H, 2 × 13^{10}-H); 3.21 (s, 6H, 2 × 2-CH_3); 3.13 (s, 6H, 2 × 7-CH_3); 2.64 (m, 2H, 2 × 17^1-H); 2.61 (s, 6H, 2 × 13^7-CO_2CH_3); 2.33 (m, 4H, 2 × 17^1-H and 2 × 17^2-H); 2.15 (s, 6H, 2 × 13^8-CO_2CH_3); 1.93 (m, 2H, 2 × 17^2-H); 1.74 (d, 6H, 2 × 18-CH_3); 1.68 (t, 6H, 2 × 3-CH_2CH_3); 1.65 (t, 6H, 2 × 8-CH_2CH_3); 0.17 and −0.12 (each brs, 2H, 4 × NH). MS (FAB): calcd for $C_{52}H_{61}N_5O_{14}$ 979.4, found *m*/*z* 980.9 (100, $M^+ + 1$). HRMS: calcd 980.4305 (M + 1), found 980.4305 (MH^+).

Mesopurpurin-18-*N*-1′-(5-methyl-3,4-dicarboxylate methyl ester)phenylmethylimide (11). ^{1}H NMR (400 MHz, 5.0 mg/mL $CDCl_3$, δ ppm): 9.61, 9.20 and 8.50 (each s, 1H, 5-H, 10-H and 20-H); 8.18 and 7.76 (each s, 1H, 2 × phenyl-H); 5.70 (m, 2H, J = 9.8 Hz, NCH_2); 5.36 (d, J = 9.1 Hz, 1H, 17-H); 4.34 (q, J = 7.1 Hz, 1H, 18-H); 3.92, 3.87, 3.83, 3.56, 3.25, and 3.19 (each s, 3H, 4 × CH_3 and 2 × CO_2CH_3); 3.77 and 3.66 (each q, J = 8.4 Hz, 2H, 3- and 8-CH_2CH_3); 2.67 (m, 1H, 1 × 17$CH_2CH_2CO_2CH_3$); 2.39 (m, 2H, 1 × 17$CH_2CH_2CO_2CH_3$ and 1 × 17$CH_2CH_2CO_2CH_3$); 2.37 (s, 3H, phenyl-CH_3); 1.99 (m, 1H, 1 × 17$CH_2CH_2CO_2CH_3$); 1.77 (d, J = 7.2 Hz, 3H, 18-CH_3); 1.72 and 1.68 (each t, J = 8.0 Hz, 3H, 3- and 8-CH_2CH_3); 0.20 and −0.01 (each br s, 1H, 2NH). MS (FAB): calcd for $C_{46}H_{49}N_5O_8$ 799.4, found *m*/*z* 801.4 (100, $M^+ + 1$). HRMS: calcd 800.3687 (M + 1), found 800.3686 (MH^+).

Peracetylated Lactose−Chlorin Conjugate Joined with Diene Linkage (14). To a solution of **3** [220 mg (0.36 mmol)] and *O*-allyl sugar **13** [330 mg (0.50 mmol)] in dichloromethane (10 mL) was added Grubbs' catalyst [50 mg (0.06 mmol)]. The reaction mixture was stirred under argon for 48 h. After evaporation of the solvent, the residue was purified by silica plate chromatography, eluting with 60% ethyl acetate in cyclohexane. The title compound was obtained in 10% yield (50 mg) as a sticky solid. UV−vis in CH_2Cl_2, λ_{max} (nm, ϵ): 694 (4.5×10^4), 544 (2.1×10^4), 507 (7.6×10^3), 417 (1.5×10^5), 362 (4.5×10^4). ^{1}H NMR (400 MHz, 5.0 mg/mL $CDCl_3$, δ ppm) showing a diastereomeric mixture: 9.58, 9.18, and 8.48 (each s, 2H, 2 × 5-, 10-, and 20-H); 7.16−3.82 (each m, total 44H, 2 × 14 sugar H, 2 × 17-H, 2 × 18-H, 12H for (2′-butadiene)); 3.76 and 3.64 (each q, J = 7.4 Hz, total 8H, 2 × 3- and 8-CH_2CH_3); 3.77−3.60 (m, total 4H, 2 × 4′-CH_2O); 3.79, 3.56, 3.24 and 3.17 (each s, 6H, 2 × 2-, 7- and 12-CH_3 and 2 × CO_2CH_3); 2.65 (m, 2H, 2 × 17$CH_2CH_2CO_2CH_3$); 2.38 (m, 4H, 2 × 17$CH_2CH_2CO_2CH_3$ and 2 × 17$CH_2CH_2CO_2CH_3$); 2.18−1.99 (m, total 44H, 2 × 7 x $COCH_3$ and 2 × 1 × 17$CH_2CH_2CO_2CH_3$); 1.94 (m, 18H, 2 × 18-CH_3, 2 × 3-CH_2CH_3 and 8-CH_2CH_3); 0.21 (br s, total 4H, 4 × N−H). HRMS: calcd for $C_{66}H_{80}N_5O_{22}$ 1294.5340, found 1294.5338 (MH^+).

Lactose−Chlorin Conjugate Joined with Diene Linkage (15). To a solution of **14** [45 mg (0.035 mmol) in dichloromethane (20 mL)] was added 1 M NaOMe in MeOH (300 μL), and the reaction mixture was stirred under argon for 1 h. After the standard workup, the product so obtained was crystallized with CH_2Cl_2/hexanes to afford the title compound in 86% yield (30 mg) as a sticky solid. ^{1}H NMR (400 MHz, 5.0 mg/mL $CDCl_3$, δ ppm) showing a diastereomeric mixture in a very aggregated form: 9.57, 9.29, and 8.79 (each s, total 6H, 2 × 5-, 10-, and 20-H); 6.72−2.64 (each m, total 56H, 28 sugar H, 2 × 17-H, 2 × 18-H, 16H for linker-H and 8H for 2 × 3- and 8-CH_2CH_3); 3.66, 3.38, 3.23, and 3.09 (each s, 6H, 2 × 2-, 7-, 12-CH_3 and 2 × CO_2CH_3); 2.63−2.09 (m, total 8H, 2 × 17$CH_2CH_2CO_2CH_3$ and 2 × 17$CH_2CH_2CO_2CH_3$); 1.95 (m, total 2H, 2 × 17$CH_2CH_2CO_2CH_3$); 1.71 (m, 6H, 2 × 18-CH_3); 1.62 and 1.54 (each t, 6H, 2 × 3- and 8-CH_2CH_3); −0.11 and −0.31 (each br s, total 4H, 4 × NH). MS (FAB): found *m*/*z* 1000.4 (100, $M^+ + 1$). HRMS: calcd for $C_{52}H_{66}N_5O_{15}$ 1000.4520, found (FAB) 1000.4520 (MH^+).

In Vitro Studies. The photosensitizing activity of the conjugates **4**, **8**, **10**, and **15** was determined on Molt-4 human leukemic T-cells by following the methodology routinely used in our laboratories.[26] These cells were grown in RPCI 1640 5% FCS (Fetal calf serum) in 100% humidity with 5% CO_2. Cells were transferred to phenol red free (prf) RPMI 1640 media with 1% FCS and plated at 25×10^4 cells/mL in 96 well plates at 25×10^4 cells/well. After 3 h incubation in the dark, in one set of experiments, the cells were washed once with PBS and resuspended in prf RPMI 1640 with 1% FCS. These cells were then illuminated with a 1000 W Quartz Halogen Lamp with IR and band-pass dichroic filters to allow light between 400 and 700 nm, at a dose rate of 16mW/cm^2 at 695 nm.

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Supporting Information Available: Relevant spectral data (^{1}H NMR and mass spectrometry) for compounds **3**, **4**, **8**−**11**, **14**, and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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