JOC Article

Functionalization of OEP-Based Benzochlorins To Develop Carbohydrate-Conjugated Photosensitizers. Attempt To Target β-Galactoside-Recognized Proteins±

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Received September 2, 2003

meso-(2-Formylvinyl)octaethylporphyrin on reaction with cyanotrimethylsilane in the presence of various catalysts {copper triflate [$Cu(OTf)_2$], indium triflate [$In(OTf)_3$], or magnesium bromide diethyl etherate ($MgBr_2 \cdot Et_2O$)} produced a mixture of the intermediate 3-hydroxy-3-cyanopropenoporphyrin, the corresponding trimethylsilyl ether derivative, and the unexpected propenochlorins. The yields of the reaction products were found to depend on the reaction conditions and the catalysts used. The intermediate porphyrins on treatment with concentrated sulfuric acid yielded the free-base cyanobenzochlorins in major quantity along with several other novel benzochlorins as minor products. Reduction of ethyl-3-hydroxy-1-pentenoate-porphyrin with DIBAL-H/$NaBH_4$ and subsequent acid treatment provided the corresponding free-base 10^3-(2-hydroxyethyl)benzochlorin, which upon a sequence of reactions gave a free-base benzochlorin bearing a carboxylic acid functionality in good yield. It was then condensed with a variety of carbohydrates (glucosamine, galactosamine, and lactosamine), and the related conjugates were screened using the galectin-binding-ability assay. Among the carbohydrate conjugates investigated, the lactose and galactose analogues displayed the galectin-binding ability with an enhancement of about 300−400-fold compared to lactose. In preliminary studies, all photosensitizers (with or without carbohydrate moieties) were found to be active in vitro [radiation-induced fibrosarcoma (RIF) tumor cells]. However, the cells incubated with lactose (known to bind to β-galactoside-recognized proteins) prior to the addition of the photosensitizers containing the β-galactose moiety (e.g., galactose and lactose) produced a 100% decrease in their photosensitizing efficacy. Under similar experimental conditions, benzochlorin without a β-galactoside moiety or the related glucose conjugate did not show any inhibition in its photosensitizing efficacy. These results in combination with the galectin-binding data indicate a possible β-galactoside-recognized protein specificity of the galactose− and lactose−benzochlorin conjugates.

Introduction

In recent years photodynamic therapy (PDT) has received increasing attention as a new modality for the selective treatment of solid tumors.[1] The mechanism of the tumor destruction is likely to require the interplay of several biological responses. However, a key point remains the ability of the photosensitizing agent to be retained to some extent by the tumor and upon light exposure to generate cytotoxic species (e.g., singlet oxygen).[2] So far, Photofrin (a porphyrin-based preparation) is the only photosensitizer that has been approved all over the world for the treatment of various types of cancer by PDT. One of the major problems associated with Photofrin is its long-term skin phototoxicity. Therefore, considerable efforts are underway in various laboratories to develop photosensitizers with improved tumor selectivity.[3]

± A part of this work was previously published as a communication in *Chem. Commun.* **2002**, 1172−1173.

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10.1021/jo030280b CCC: $27.50
Published on Web 12/12/2003

For designing new photosensitizers, the balance between hydrophobicity and hydrophilicity is recognized as an important factor.[4] In this regard, various research groups synthesized a variety of porphyrin analogues,[5] including porphyrin−carbohydrate conjugates.[6−10] It was believed that the presence of the carbohydrate moiety could enhance the membrane interaction, which may increase their tumor selectivity. However, compared with the parent molecules, none of the related carbohydrate conjugates were evaluated for their affinity to target a particular protein. Therefore, 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 the family of lectins defined by a highly conserved carbohydrate recognition domain and exhibit high affinity for β-galactoside.[11] Because galectins are involved in the modulation of cell adhesion,[12] cell growth,[13] immune response,[14] and angiogenesis,[15] it is clear that changes in their expression play a critical role in tumor progression. To confirm a "proof of principle" concept, we recently reported an efficient approach for the preparation of certain β-galactose-conjugated purpurinimides (a type of chlorin).[16] In preliminary in vitro and in vivo studies, compared to the nonconjugated analogues, the related carbohydrate conjugates produced an enhanced galectin-binding ability with a remarkable increase in the photosensitizing ability.[17] Interestingly, compared to Photofrin, the purpurinimide−lactose conjugate also produced an enhanced tumor selectivity (no skin phototoxicity was observed at 72 h postinjection).[17] Currently, the synthesis and detailed biological studies with a series of purpurinimides and bacteriochlorin conjugates are in progress, and the preliminary results are promising.

Encouraged with our previous findings, we were interested in extending this approach to other compounds known for their photosensitizing action and that could serve as a prototype or lead molecule for the synthesis of analogues for further biological testing. In general, for designing an improved drug, besides increased potency, greater selectivity, increased or decreased duration of action, low toxicity, and increased stability, economics is also considered a prime reason. Therefore, among the second generation of photosensitizers, we were especially interested in those benzochlorins, which could be derived from the commercially available octaethylporphyrin (OEP).[18,19] One of the major problems associated with OEP-based benzochlorin preparation is the difficulty in the demetalation at the final step of the synthesis.[18] We have previously reported a simple and efficient approach for the preparation of a series of fluorinated and nonfluorinated OEP-based benzochlorins (free-base and related metal complexes) with variable lipophilicity.[20] To investigate the effect of substituents in their photosensitizing efficacy, these compounds were then evaluated for their biological efficacy and, in general, compared to free-base analogues. The related Zn(II) complexes were found to be more effective.[21] Our next objective was to extend this methodology for introducing such functionalities that could be easily modified to prepare a series of corresponding carbohydrate conjugates for investigating their galectin-binding ability.

Results and Discussion

Chemistry. Our initial approach was to introduce a cyano group at the fused benzene ring of the benzochlorin system, which could then be converted into the related carboxylic acid or aminomethyl functionality under appropriate reaction conditions. On a subsequent reaction with various carbohydrates containing either an amino acid or carboxylic acid functionality, the functionalized benzochlorins should produce the desired conjugates. Therefore, to achieve our goal, Ni^{II}OEP (**1**) was converted

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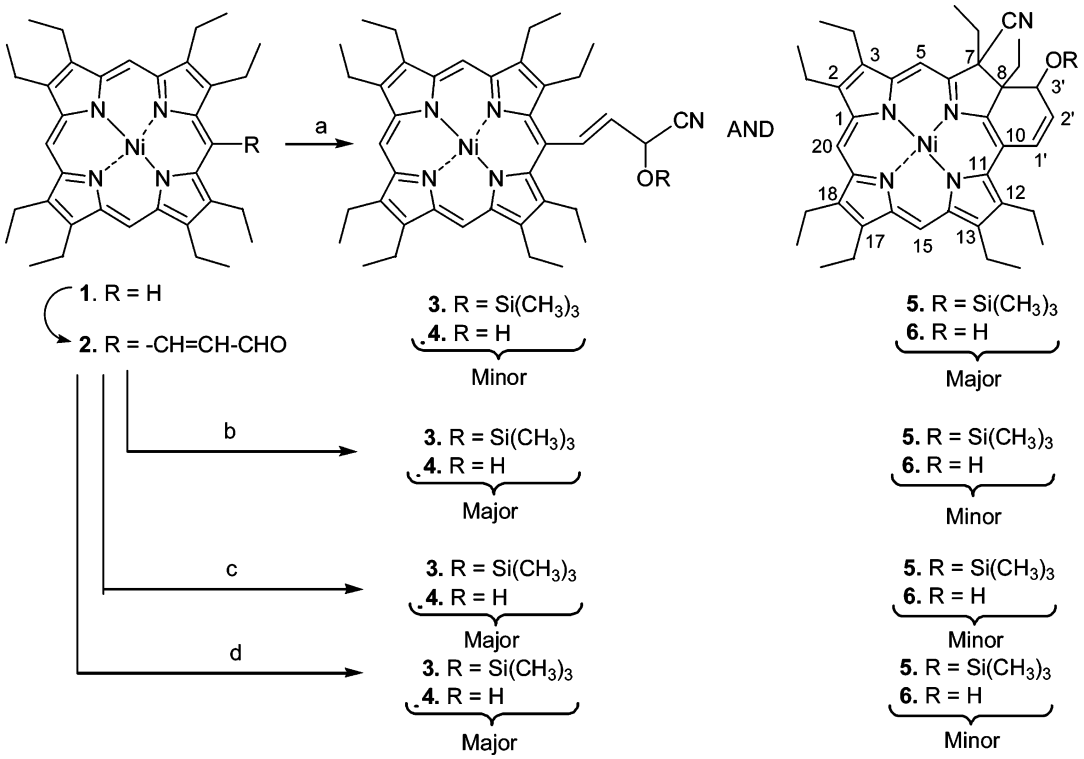
SCHEME 1[a]



[a] Reagents: (a) $(CH_3)_3SiCN$, $Cu(OTf)_2/CH_2Cl_2$; (b) $(CH_3)_3SiCN$, $Cu(OTf)_2$/THF; (c) $(CH_3)_3SiCN$, $In(OTf)_3$/THF or CH_2Cl_2; (d) $(CH_3)_3SiCN$, $MgBr_2 \cdot Et_2O$/THF or CH_2Cl_2.

into the corresponding *meso*-(2-formylvinyl)octaethylporphyrin **2** in excellent yield by following the Vicenta and Smith approach.[22] The reaction of **2** with cyanotrimethylsilane in combination with copper(II) triflate in CH_2Cl_2 mainly produced a mixture of novel-fused propenochlorins **5** and **6** in a combined yield of 76%, and the intermediate porphyrins **3** and **4** were obtained as minor products. The formation of the reaction products was found to depend on the reaction conditions used. For example, the reaction was quite slow in dichloromethane, and a relatively large amount of catalyst (50−75% mmol) was required for the completion of the reaction. Replacement of dichloromethane with tetrahydrofuran produced mainly porphyrins **3** and **4**, whereas chlorins **5** and **6** were isolated as minor products. During the workup (washing the organic layer with water), porphyrin **3** was found to be quite unstable and converted to porphyrin **4** as a major product. Attempts to purify porphyrin **4** by silica or alumina (grade III) column chromatography were unsuccessful, and the resulting product was identified as the starting porphyrin **2**. The reaction mixture containing mainly **4** was also found to be unstable on alumina plates. However, we were able to isolate it in pure form by silica gel G preparative TLC using hexanes/CH_2Cl_2/EtOAc (v/v, 4:4:1) as the developing solvent. However, during this process, a significant part (∼50%) of it was converted into the starting porphyrin **2**.

With the replacement of the catalyst copper(II) triflate with indium(III) triflate or with magnesium bromide diethyl etherate in both THF and CH_2Cl_2, the same reaction produced a mixture of porphyrin intermediates **3** and **4** as major products, and porphyrins **5** and **6** were isolated in minor quantities (∼20%) [Scheme 1]. In this particular reaction, both indium(III) triflate and magnesium bromide diethyl etherate were found to be more effective than copper(II) triflate. The literature survey revealed[23] that a number of Lewis acids [such as copper(II) triflate, magnesium bromide diethyl etherate, titanium complexes, aluminum alkoxides, bismuth bromide, indium fluoride, indium chloride, indium bromide, zinc iodide, tin(II) triflate, and scandium(III) triflate] have been reported as catalysts for the reaction of a carbonyl compound with cyanotrimethylsilane to form cyanohydrin. However, to the best of our knowledge, this is the first example that reports the utility of indium(III) triflate as a catalyst for the reaction of a carbonyl compound with cyanotrimethylsilane to form cyanohydrin under mild reaction conditions.

The formation of ketochlorin **7** and acetoxychlorin **8** by reacting chlorin **6** with TPAP/NMO[24] (TPAP, tetra-

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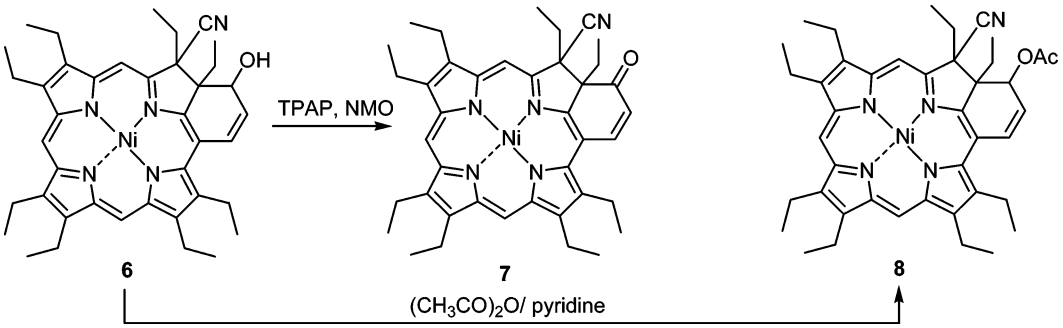
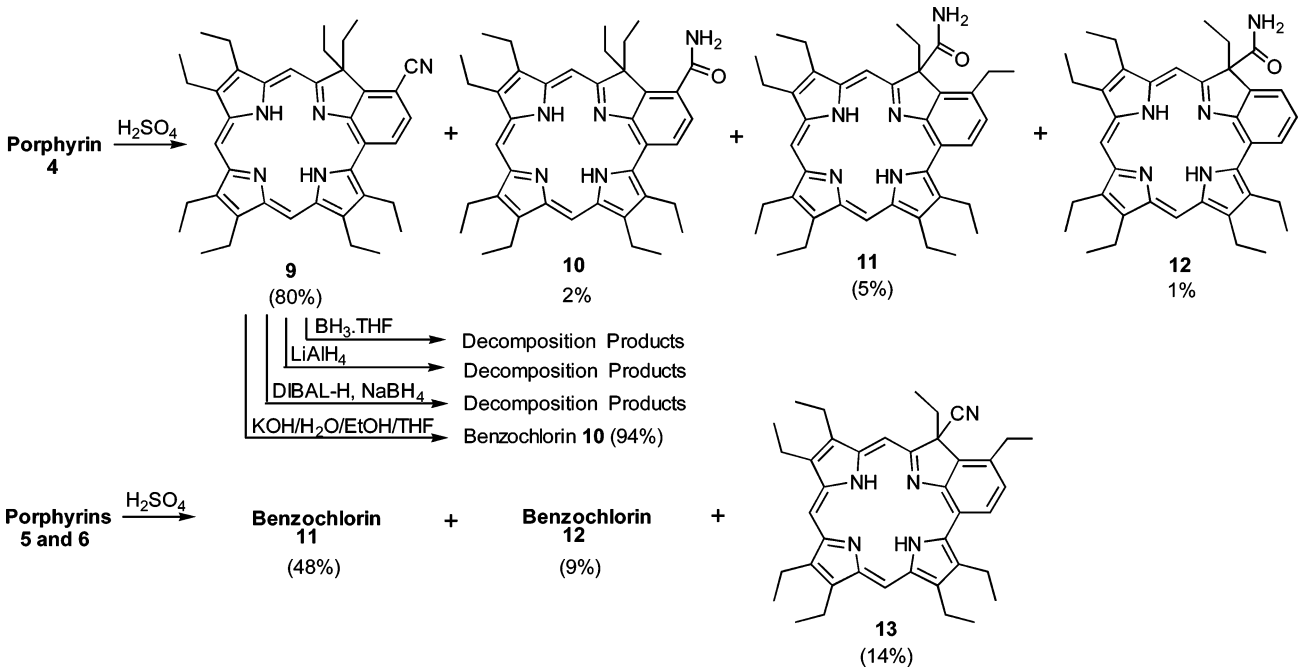
SCHEME 2



SCHEME 3



propylammonium perruthenate; NMO, 4-methylmorpholine *N*-oxide) and acetic anhydride in pyridine, respectively, confirmed the presence of the hydroxy group in the molecule (Scheme 2). Our attempts to obtain suitable crystals of **5** and **6** for X-ray crystallographic studies were unsuccessful. However, a high quality of crystals of compound **7** was obtained by using hexanes/CH_2Cl_2 as the crystallizing solvent, and the X-ray crystallographic studies confirmed the proposed structures.[25] The X-ray crystallographic studies indicated that porphyrin macrocycle **7** exhibits a nonplanar distortion that was mainly saddled with a mean deviation of the 24 macrocyclic atoms from their least-squares plane of 0.283 Å. The average Ni−N bond length was 1.935[6] Å (the number in the square brackets indicates the deviation from the mean) that is within the normal range for Ni porphyrinoids. The ^{1}H and ^{13}C NMR assignments for chlorins **6** and **7** were achieved by analyzing their H−H correlation spectroscopy (COSY), rotating-frame Overhauser enhancement spectroscopy (ROESY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) spectra (see the Supporting Information).

The reaction of a crude mixture of porphyrins **3** and **4** with concentrated sulfuric acid produced the desired 3′-cyanobenzochlorin **9** (71%) along with a mixture of three other benzochlorins **10** (2%), **11** (5%), and **12** (1%) bearing an amide group at various positions of the macrocycle (Scheme 3). The formation of these benzochlorins derived from porphyrins **3** and **4** was confirmed by treatment of pure **4** with concentrated sulfuric acid. Interestingly, the reaction of a mixture of propenochlorins **5** and **6** with concentrated sulfuric acid under similar conditions produced a mixture of benzochlorins with amide substituents **11** (48%) and **12** (9%) and a 7-cyano-3′-ethylbenzochlorin **13** (14%). The mixture was separated into individual compounds by column chromatography (alumina grade III) and preparative silica TLC. The structures were confirmed by extensive NMR studies (^{1}H, ^{13}C, H−H COSY, and ROESY). The structure of **11** was also confirmed with an X-ray crystallographic study.[25]

Several attempts to convert the cyanobenzochlorin **9** into the corresponding aminomethyl analogue by reacting

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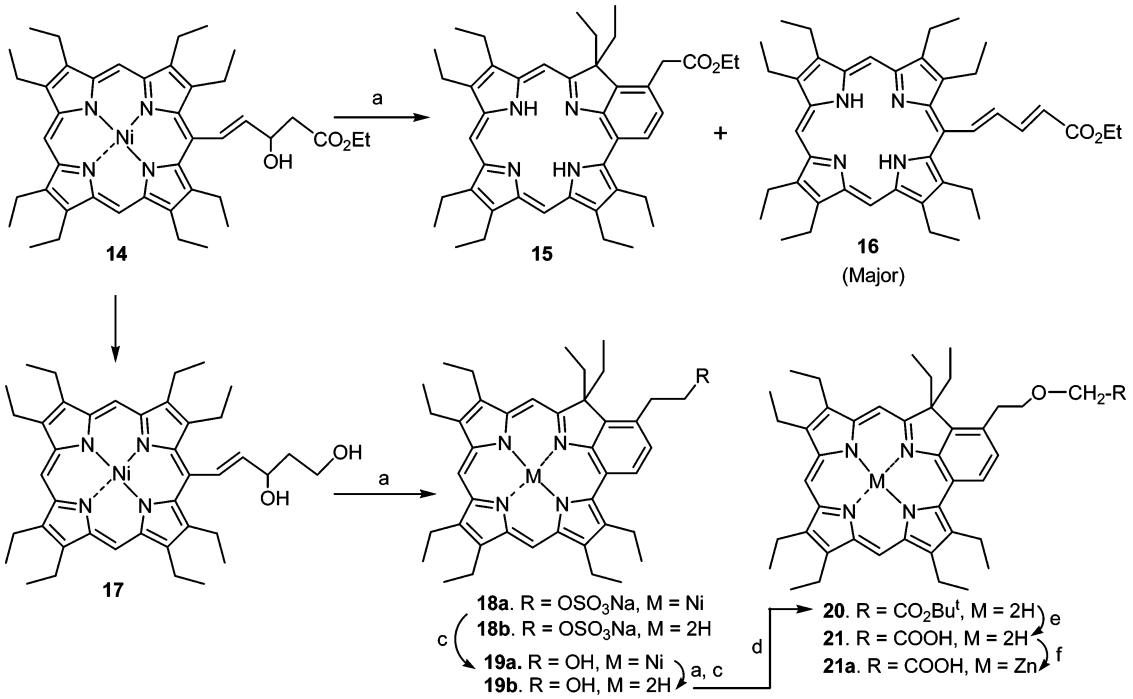
SCHEME 4[a]



[a] Reagents: (a) H_2SO_4; (b) (i) DIBAL-H, (ii) $NaBH_4$; (c) 3 N HCl/EtOH; (d) (i) $BrCH_2CO_2Bu^t$, 25% NaOH, Bu_4NHSO_4; (e) TFA.

it with various reducing agents including BH_3·THF, $LiAlH_4$, and DIBAL-H/$NaBH_4$ were unsuccessful. Reaction of **9** under strong basic conditions did not produce the desired benzochlorin with carboxylic acid functionality; instead, benzochlorin **10** containing an amide group at the 3′ position was isolated as a sole product. Attempts to convert the cyano group in **9** into the corresponding carboxylic acid with HCl or H_2SO_4 at variable reaction conditions were unsuccessful and resulted in the recovery of the starting material.

Because of our unsuccessful attempt to prepare a benzochlorin with aminomethylene and carboxylic acid functionalities at the 3′ position, we turned our attention to our previous approach where porphyrin **14** in the presence of concentrated sulfuric acid produced mainly the corresponding dehydrated free-base porphyrin **16**, and the expected benzochlorin **15** was obtained in low yield. To circumvent the formation of the dehydrated product problem, **14** was first reduced with DIBAL-H/$NaBH_4$ to give the corresponding porphyrin **17** in 85% yield, which on treatment with concentrated sulfuric acid provided a mixture of the sulfonated benzochlorin **18b** and its Ni(II) complex **18a** in good yield. The separation and purification of **18a** and **18b** were quite difficult, and the pure sample of **18b** was obtained by using a Sephadex LH-20 column eluted with MeOH/CH_2Cl_2 (v/v, 1:1). The treatment of the crude mixture of **18a** and **18b** with 3 N HCl in EtOH at a refluxing temperature gave a mixture of **19a** and **19b** in a combined yield of 78% (from **17**), which was readily separated by silica column chromatography. Reaction of **19a** with sulfuric acid and then with 3 N HCl/EtOH provided **19b** in quantitative yield. Benzochlorin **19b** was then reacted with a large excess of *tert*-butyl bromoacetate in the presence of NaOH and Bu_4HSO_4, and benzochlorin **20** was obtained in quantitative yield. Treatment of **20** with TFA produced **21**, bearing a carboxylic acid functionality in 100% yield (Scheme 4).

For investigating the galectin specificity and photosensitizing efficacy of benzochlorin−carbohydrate conjugates, benzochlorin **21** was individually reacted with acetylated galactosamine **22**,[26] glucosamine **25**,[27] and lactosamine **28**,[26] and the corresponding benzochlorin−carbohydrate conjugates **23**, **26**, and **29** were obtained in 40%, 97%, and 43% yields, respectively (see the Experimental Section). These compounds, when subjected to the sodium methoxide treatment, produced the corresponding hydroxy derivatives **24**, **27**, and **30** in 67−95% yields depending on the substrates (Scheme 5). Alternately, compound **27** was also prepared in 45% yield by the reaction of **21** with glucosamine **32** using a mixed anhydride method (isobutyl chloroformate and Et_3N;[28] Scheme 5). The basic idea for the preparation of the benzochlorin−glucose conjugate was to investigate the utility of β-galactoside (galactose and lactose) over other carbohydrates (e.g., glucose).

From the NMR spectrum, an interesting phenomenon was observed for the benzochlorin−glucosamine conjugate **27**, which was isolated as a mixture of two isomers (α-OH and β-OH at the 1″ position of the glucosamine part) in a ratio of 4:1 (α/β). The 2″-H at the glucosamine part of the conjugate was found to be easily exchanged by deuterium to produce **27a** in a mixture of CD_3OD and $CDCl_3$ (NMR solvent: 6 drops of CD_3OD and 0.6 mL of $CDCl_3$) during the evaporation of the solvent. The electrospray ionization mass spectrometry (ESIMS) spectrum of **27a** produced a MH^+ peak at 837.8 (100) cm^{-1} and [M

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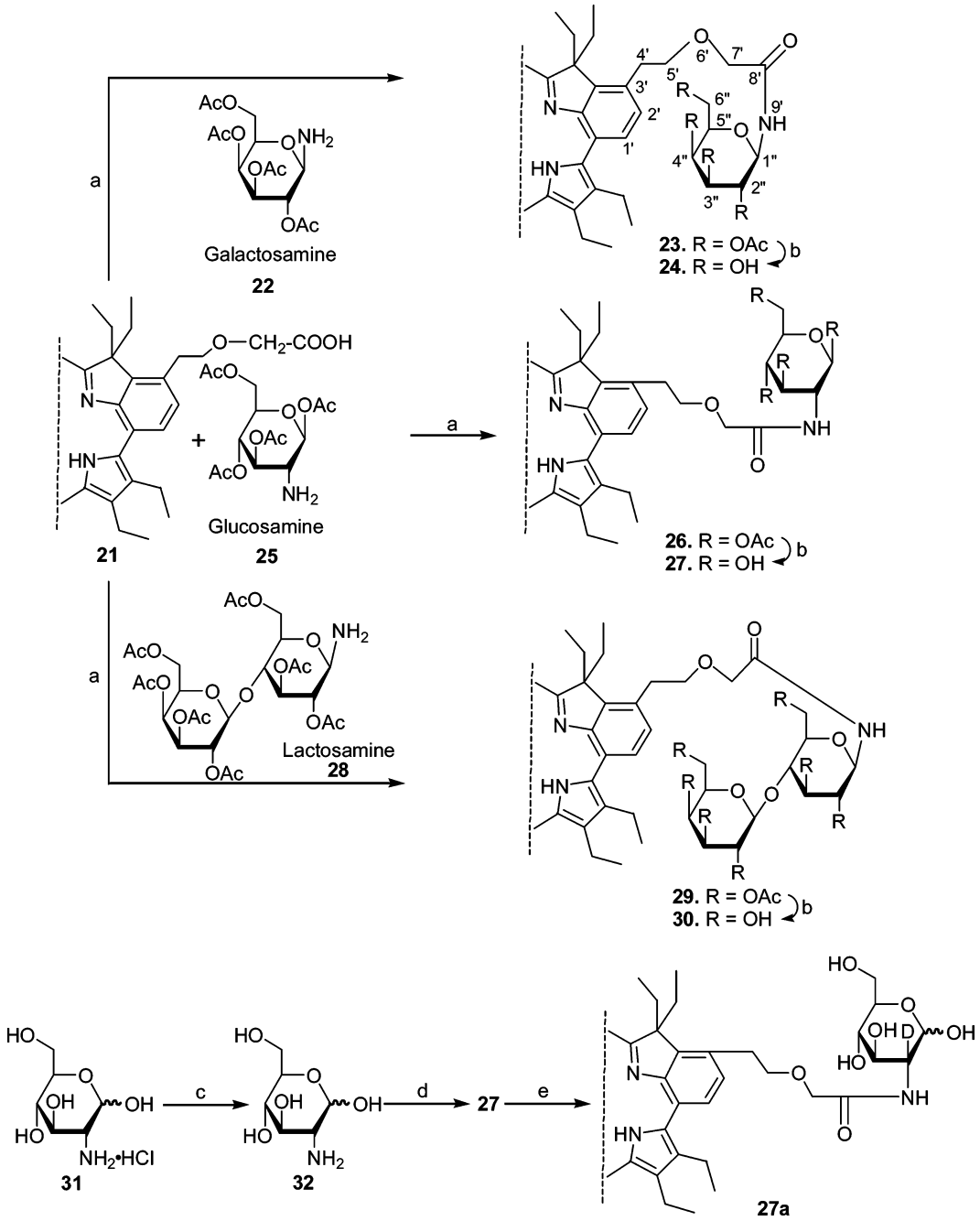
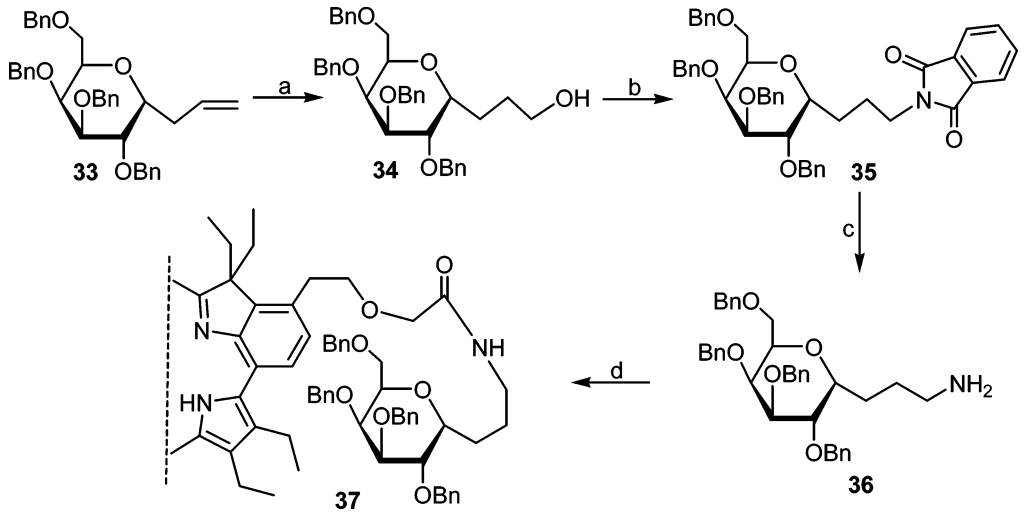
SCHEME 5[a]



[a] Reagents: (a) BOP/Et_3N/CH_2Cl_2; (b) NaOMe/MeOH/CH_2Cl_2; (c) NaOH/H_2O/EtOH; (d) **21**, isobutyl chloroformate/Et_3N/THF; (e) CD_3OD/$CDCl_3$.

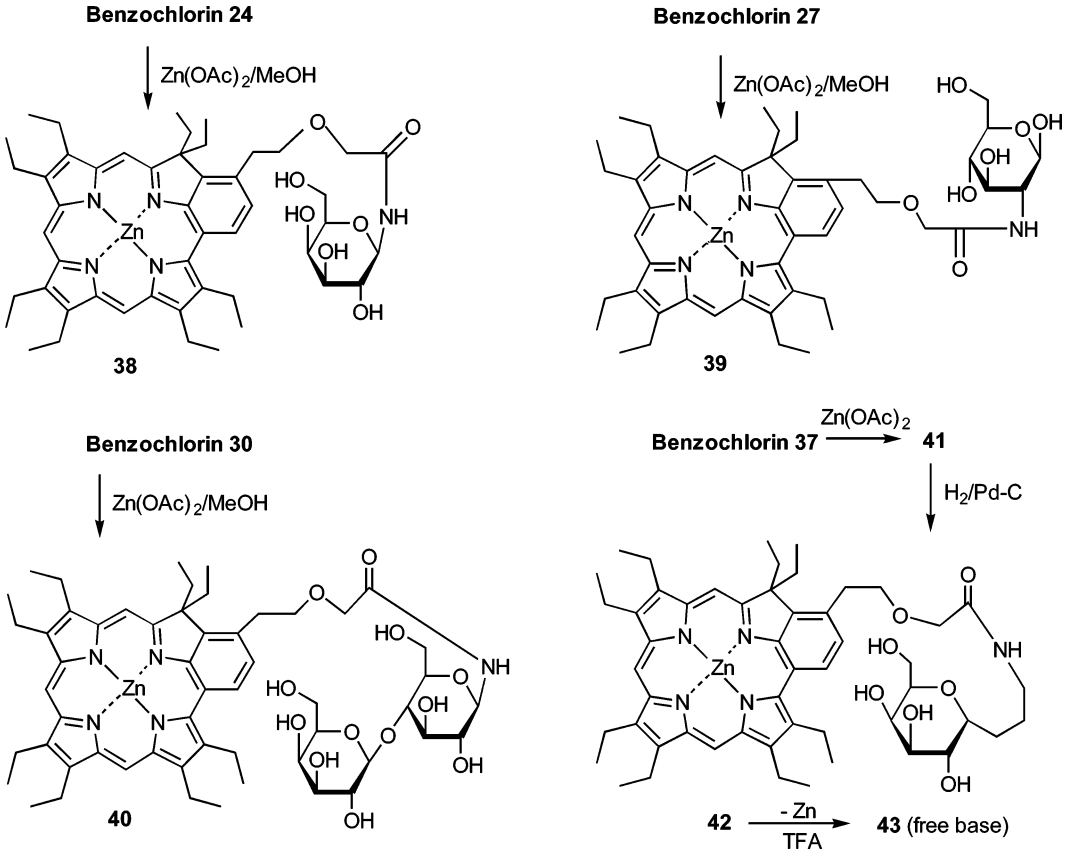
+ Na]$^+$ at 859.7 cm^{-1}. The FAB HRMS of **27a** exhibited an exact mass of 837.5039 (M + 1), indicating that the molecular weight for **27a** should be 836.5 g/mol and was not consistent with the initially assigned structure. However, the ESIMS spectrum of **27** (without treating with 6 drops of CD_3OD and 0.6 mL of $CDCl_3$) showed a molecular ion peak (M^+) at 835.9 cm^{-1}, which matched with the molecular formula of $C_{49}H_{65}N_5O_7$. Upon further analysis of the NMR results of **27** and **27a** in pyridine-d_5 (assigned undoubtedly by H−H COSY and ROESY NMR studies), it was observed that the resonance at δ 4.97 (1H, ddd, J = 12.7, 9.0, 3.2 Hz, 2″-H) for compound **27** was almost nonexistent for **27a**. Besides, the resonances of **27a** at δ 8.08 (1H, s, 9′-H) and 5.96 (1 H, s, 1″-H) appeared as singlets, while they were observed as doublets with coupling constants (8.9 Hz for 9′-H and 3.2 Hz for 1″-H) in the spectrum of **27**. This is certainly due to the exchange of hydrogen by deuterium at the 2″ position of the glucose moiety (see Scheme 5). The above findings were also confirmed by ^{13}C NMR and DEPT-135 experiments (acquired in pyridine-d_5), where the resonance at δ 55.9 ppm in the ^{13}C NMR spectrum of **27a** produced much less intensity than the corresponding carbon resonance in that of **27**. All of these experiments were performed under similar conditions, using the same relaxation delay (see the Supporting Information).

SCHEME 6[a]



[a] Reagents: (a) (i) 9-BBN, (ii) NaOH, H_2O_2; (b) Ph_3P/phthalimide/DEAD; (c) NH_2NH_2/EtOH; (d) **21**, DCC/DMAP.

SCHEME 7



It has been reported that the *C*-glycosyl derivatives have a higher stability toward both chemical and enzymatic deglycosylation than the corresponding *O*- or *N*-glycosyl compounds.[29] To investigate its impact on PDT efficacy, benzochlorin−*C*-galactose conjugates **42** and **43** were synthesized by following the reaction sequences as depicted in Schemes 6 and 7. The benzylated galactose derivative **34** was prepared by following the literature procedure.[30] Reaction of **34** with phthalimide in the presence of Ph_3P and diethyl azodicarboxylate gave **35** in 79% yield, which on subsequent treatment with hydrazine monohydrate in EtOH afforded **36** in 91% yield. Condensation of **21** with **36** by the dicyclohexylcarbodiimide (DCC) method gave **37** in excellent yield.

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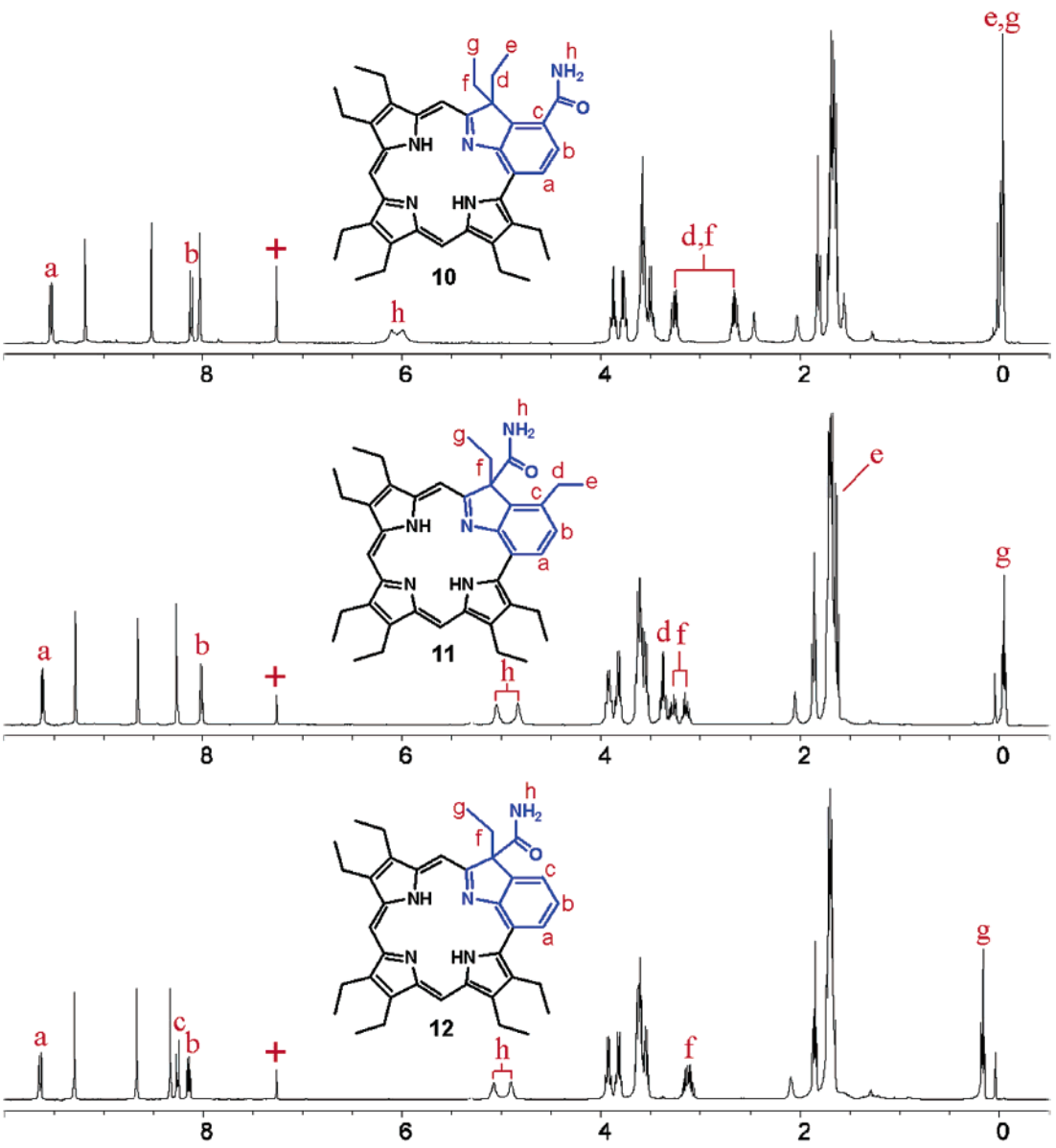

FIGURE 1. ^{1}H NMR spectra of benzochlorins **10**−**12** (the signals labeled with + are the resonances of the residual $CHCl_3$ in $CDCl_3$).

For the removal of the benzyl groups in the galactose moiety by hydrogenation, compound **37** was first converted into the corresponding Zn(II) complex **41**, which on hydrogenation provided **42** in 75% yield. Treatment of **42** with TFA produced the free-base benzochlorin−galactose conjugate **43** in 97% yield.

In our previous studies, compared to free-base benzochlorins, the corresponding Zn(II) analogues were found to be more effective for PDT efficacy.[21] Therefore, benzochlorins **21**, **24**, **27**, and **30** were transformed into the corresponding Zn(II) complexes **21a**, **38**, **39**, and **40**, respectively (see the Experimental Section).

The structures of the newly synthesized benzochlorins and the intermediates were confirmed by mass spectrometry, elemental analyses, and extensive NMR studies (^{1}H, ^{13}C, COSY, and ROESY). Among all of the compounds, the characterization of benzochlorins **10**, **11**, and **12** that were formed after the sulfuric acid reaction was much more challenging. Both compounds **10** and **11** showed the molecular ion peak at 616.6 cm^{-1} (MH^+), whereas benzochlorin **12** produced the molecular ion peak 588.6 cm^{-1} (MH^+) (−28), indicating a loss of an ethyl group. In the electronic absorption spectra, all of these compounds produced a characteristic absorption pattern of a typical benzochlorin system, exhibiting the long wavelength absorption near 660 nm. However, the NMR spectra of these compounds were quite different (Figure 1). For example, in the ^{1}H NMR spectrum of benzochlorin **10**, the two protons of the formamide group were observed at δ 6.12 and 5.98, and the resonances of the two ethyl groups at the 7 position were observed at δ 3.25 (2H, m, ABX system), 2.65 (2H, m, ABX system), and −0.04 (6H, t, J = 7.6 Hz). For compound **11**, the two broad singlets at δ 5.00 and 4.83 were assigned to two protons of the formamide group and produced about a 1.1 ppm upfield shift as compared to the corresponding benzochlorin **10**. This is possibly due to the tilting of the formamide group toward the benzochlorin system, causing an anisotropic

TABLE 1. Determination of the IC_{50} Values and the Relative Inhibitory Capacity (Relative Potency) of the Benzochlorin Derivatives in a Solid-Phase Assay with Surface-Immobilized Asialofetuin and Gal-1 in Solution

compound	IC_{50} (μM)	relative potency
lactose	1200	1
21a	6.2	194
38	5.1	235
39	8.5	141
40	4.3	279
42	3.1	387

effect by the benzochlorin ring current. The ROESY spectrum of **11** showed strong interactions among the resonances at δ 8.00 (1H, d, J = 8.5 Hz, 2′-H), 3.35 (2H, q, J = 7.7 Hz, 3′-CH_2CH$_3$), and 1.63 (3H, J = 7.6 Hz, 3′-CH_2CH$_3$, partially overlapped with other signals), indicating the presence of an ethyl group at the 3′ position, which possibly migrated during the intramolecular cyclization from the 8 position of the starting porphyrin **3** or **4**. Compared to **10** and **11**, in benzochlorin **12**, the resonances for one of the ethyl groups were missing. In the H−H COSY spectrum, the interactions between the resonances at δ 9.62 (1H, d, J = 8.2 Hz, 1′-H) and 8.13 (1H, dd, J = 7.3, 6.0 Hz, 2′-H) and the cross peak between the resonances at δ 8.13 (1H, dd, J = 7.3, 6.0 Hz, 2′-H) and 8.25 (1H, d, J = 6.7 Hz, 3′-H) indicated the absence of the ethyl group in **12** that was observed at the 3′ position in benzochlorin **11**. Further, the ROESY analysis produced a strong interaction between 3′-H and 7-CH_2CH$_3$, which along with the ^{13}C NMR data, mass spectrometry, and elemental analyses confirmed the proposed structure (Scheme 3).

A possible mechanism for the formation of **5** or **6** from porphyrin **2** is possibly due to the cyanide displacement (catalyzed by copper triflate or indium triflate) in intermediate **3** or **4**, followed by the Woodward−Hoffmann [1,6] electrocyclization as suggested in our previous paper.[25] However, the mechanism of the migration of the ethyl group to form **10** and **11** and its elimination to produce **12** is not clear.

For drug development, a proper balance between hydrophobicity and hydrophilicity is believed to be an important factor, which influences the in vivo biodistribution and clearance of the drug. The standard octanol/water approach ("shake flask" method) that is generally used to determine the overall lipophilicity of the molecule (partition coefficient) was found to be unsuccessful for the benzochlorin analogues. All photosensitizers with or without a carbohydrate moiety stayed in the octanol phase, suggesting an extremely high hydrophobic nature for this class of compounds. The PALLAS program (CompuDrug Chemistry Ltd., Budapest, Hungry), which has been used successfully for calculating the overall lipophilicity of other free-base photosensitizers,[5g] also has some limitations and is not suitable for calculating the log P values of the metalated analogues and the charged molecules.

Galectin-Binding Studies. The enzyme-linked immunosorbent assay (ELISA) confirmed that the compounds (**21a**, **38**−**40**, and **42**) bind to galectin with a much higher affinity (141−387 times) than that of lactose 50% inhibitory concentration (IC_{50}) = 1.2 mM, a standard β-galactoside used as a reference] (Table 1 and Figures 2 and 3). Among the above compounds, the benzochlorin−galactose conjugates (**38**, IC_{50} = 5.1 μM, and **42**, IC_{50} = 3.1 μM) and the benzochlorin−lactose conjugate (**40**, IC_{50} = 4.3 μM) have a slight edge (~2 times) over the benzochlorin−glucose conjugate (**39**, IC_{50} = 8.5 μM) and the nonconjugate precursor (**21a**, IC_{50} = 6.2 μM). This further enforces the fact that the presence of the galactose subunit is playing an important role. In addition to β-galactoside, the benzochlorin unit has significant galectin-binding affinity, which is not surprising because porphyrins are known to interact with almost all proteins because of their lipophilic nature (presumably because of the combination of the weak binding forces at the microscopic level such as hydrogen bonding, $\pi-\pi$, and other hydrophobic interactions). Therefore, relative to the lactose moiety, the benzochlorin subpart in the lactose and galactose conjugates is definitely a contributing factor for the enhanced binding affinity of these compounds to galectin.

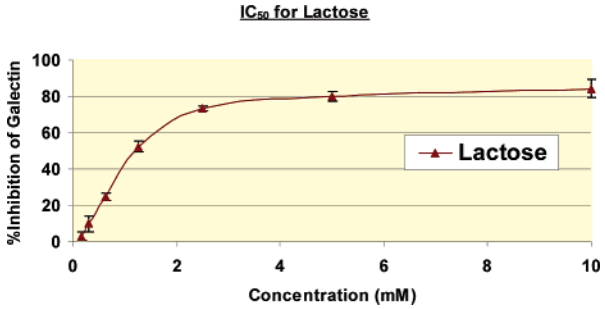

FIGURE 2. Titration of lactose by ELISA. The symbol on the line with the error bars shows the standard error in the mean based on three different measurements.

In Vitro Photosensitizing Efficacy. Benzochlorins with and without carbohydrate moieties were evaluated for their in vitro efficacy in radiation-induced fibrosarcoma (RIF) tumor cells known for their galectin-3 (Gal-3) expression.[31] A standard MTT assay was performed at a fixed drug concentration (1.0 μM), and the cells were exposed to light at variable light doses (1−5 J/cm^2) after a 4 h incubation.[5i] The in vitro results obtained from benzochlorin **21a** and the related carbohydrate analogues **38**−**40** and **42** are summarized in Figure 4. As can be seen from Figure 4A, all compounds produced a similar efficacy. To confirm the binding affinity of the galactose− and lactose−benzochlorin conjugates (**38**, **40**, and **42**, respectively) to the β-galectoside-recognized proteins, the RIF tumor cells were preincubated with lactose alone to block the β-galactoside binding sites before their treatment with photosensitizers and light exposure. In a typical experiment, lactose (100 μM) was added to RIF tumor cells and incubated for 1 h. Photosensitizers with or without the β-galactoside moiety (1.0 μM) were added in an individual set of experiments. The cells were incubated for an additional 3 h. After the treatment with light (see Figure 4), the cells were resuspended in fresh media. A total of 48 h later MTT was added, and the cells were incubated for an additional 4 h. DMSO (100 μL) was then added to each well to dissolve the formazine crystals. The plates were read on a 96-well plate reader at an absorbance of 560 nm. As can be seen from Figure 4B,C,

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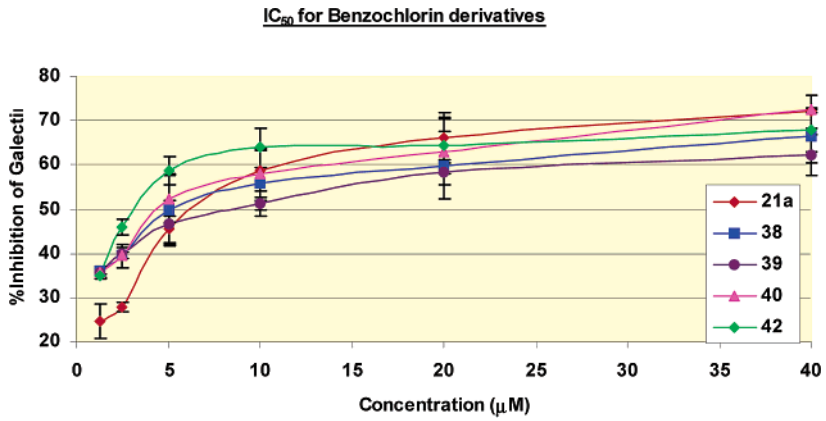

FIGURE 3. Titration of benzochlorin **21a** and its carbohydrate conjugates (**38**−**40** and **42**) by ELISA. The symbol on the line with the error bars shows the standard error in the mean based on three different measurements.

the galactose− and lactose−benzochlorin conjugates (**38**, **40**, and **42**, respectively) in the presence of free lactose produced 100% inhibition in their photosensitizing efficacy, whereas the related non-β-galactoside−benzochlorin conjugate **21a**, which was equally effective (Figure 4C), did not show any inhibition in the presence of free lactose.

Incubation of the cells with free glucose (100 μM) instead of lactose (Figure 4D) did not produce any inhibition in the PDT efficacy of benzochlorin−carbohydrate conjugates **40** and **42**. The related glucose conjugate **39** in the presence of either free glucose or lactose did not diminish its photosensitizing efficacy. These results suggest that benzochlorins conjugated with galactose and lactose moieties bind to the β-galactose binding site of the protein. No such specificity was observed with non-carbohydrate benzochlorin **21a** and the related glucose conjugate **39**.

Conclusion

We have developed a facile approach for functionalizing OEP-based free-base benzochlorins. For determining the effect of the nature of the carbohydrate moiety in developing β-galectoside-recognized protein-specific photosensitizers for PDT, we extended our approach by introducing a carboxylic acid functionality at the fused exocyclic benzene ring system. It was then converted into a series of carbohydrate conjugates and evaluated for the galectin specificity. Among the conjugates subjected for galectin-binding affinity (IC_{50}), the galactose− and lactose−benzochlorin conjugates produced the best binding affinity, with a 235−387-fold enhancement compared to that of lactose. These results suggest the specificity of the galactose and lactose conjugates to proteins known for their β-galectoside recognition sites and overexpression in tumor cell surfaces (Gal-1 to Gal-12).[11,32,33] Because of the commercial availability of Gal-1, all binding studies were performed with this particular protein. Interestingly, the benzochlorin without a β-galactoside moiety also produced a significant binding to Gal-1, and it could be possibly due to a nonspecific interaction(s) of the chromophore with the aromatic residues of various proteins, including galectins present in the cell surface. Currently, the isolation and purification of other galectins known for overexpression in various tumors (especially Gal-3, which is highly expressed in brain tumors compared to a normal brain[34] and is a presurgical marker of human thyroid carcinoma[35]) is in progress. Attempts are also underway to conjugate those carbohydrates to benzochlorins and other porphyrin-based photosensitizers (containing flexible or rigid linkers with variable carbon units) known for their enhanced binding to Gal-3 or other tumor-specific galectins.

Experimental Section

Melting points are uncorrected. Unless otherwise stated, chemical shifts are reported in ppm and referenced to residual solvent resonance peaks ($CDCl_3$: for ^1H, 7.26 ppm and for ^{13}C, 77.2 ppm; and pyridine-d_5: for ^1H, 8.74 ppm and for ^{13}C, 150.3 ppm, both referenced to the most downfield resonance; methanol-d_4: for ^1H, 3.31 ppm). Hydrogen connectivity (C, CH, CH_2, and CH_3) information was obtained from DEPT-135 experiments. Proton and carbon peak assignments were based on 2D NMR analysis (COSY, ROESY, HMQC, and HMBC). Column chromatographic separations were performed over silica gel 60 (70−230 mesh) or neutral alumina. Preparative TLC was performed on silica 20 × 20 cm TLC plates.

Porphyrin 4, Chlorins 5−8, and Benzochlorins 9−13. For the synthetic details and characterization of these compounds including the detailed NMR data, please see the Supporting Information.

Porphyrin 17. To a solution of compound **14** (350 mg) in dry toluene (30 mL) was dropwise added DIBAL-H (2.0 mL, 1 M in toluene) at −78 °C under N_2. The mixture was maintained at this temperature for 45 min until the TLC showed that all starting material was consumed. Water (1 mL) was then added to quench the reaction. Solvent was removed with rotavapor, and the residue was redissolved in a mixture of CH_2Cl_2 (80 mL) and MeOH (20 mL). $NaBH_4$ (500 mg) was added to the reaction mixture in three portions (about 1 min between each portion). This mixture was stirred at room temperature for 10 min before water (100 mL) was added. It was extracted with CH_2Cl_2, washed with water, dried over Na_2SO_4, and concentrated. The residue was purified over an alumina column, eluting with CH_2Cl_2/EtOAc (v/v, 5:1) and MeOH/CH_2-

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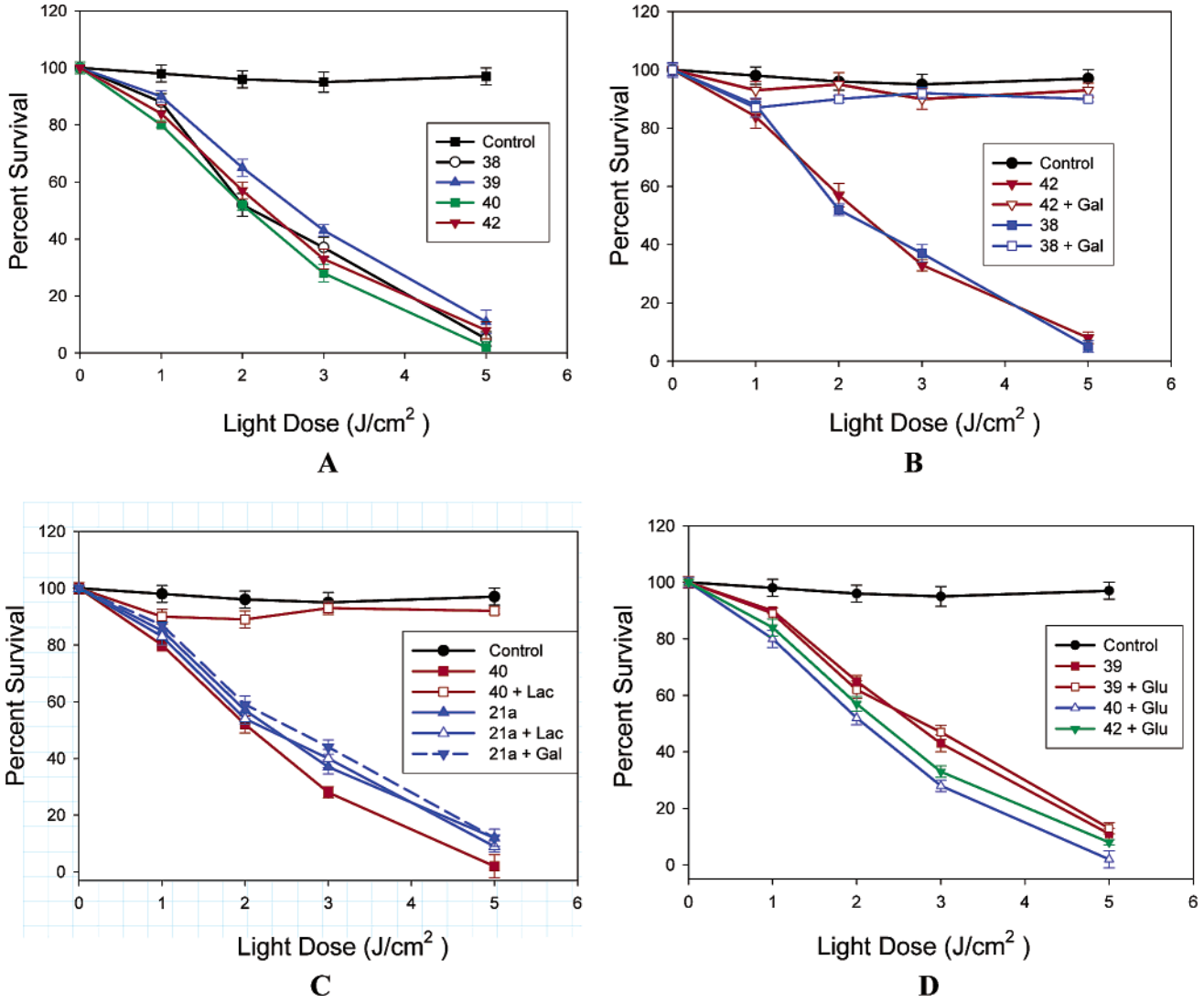

FIGURE 4. Comparative in vitro photosensitizing efficacy of various benzochlorins with and without carbohydrate moieties (1.0 μM) in RIF tumor cells. (A) Photosensitizers (**38**−**40** and **42**) incubated for 4 h (48 h MTT). Control: the cells incubated with photosensitizers but not exposed to light. (B) Galactose conjugates **38** and **42** with and without free lactose (100 μM). (C) Benzochlorin **21a** and the related lactose conjugate **40** with and without free lactose. (D) Carbohydrate conjugate **39** with and without free glucose (100 μM) and **40** and **42** with glucose (100 μM). (*Note*: The cells incubated with galactose and lactose conjugates (**38**, **42**, and **40**) in the presence of free glucose did not produce any inhibition in their photosensitizing efficacy, whereas replacing glucose with lactose at the same concentration showed 100% inhibition in their efficacy, suggesting the binding of the lactose and galactose conjugates to those proteins known for recognizing β-galactosides. No inhibition in the photosensitizing efficacy of benzochlorin **21a** (without a galactose or a lactose moiety) in the presence of free lactose further suggests its nonspecificity to the β-galactoside-recognized protein.)

Cl_2 (5%) to provide **17** (280 mg, 85%) as red needles. Mp: 216−218 °C. ^{1}H NMR ($CDCl_3$) δ: 9.47 (2H, s, 2 × *meso*-H), 9.45 (1H, s, 1 × *meso*-H), 9.05 (1H, d, J = 15.1 Hz, 1′-H), 4.86 (1H, dd, J = 15.3, 4.5 Hz, 2′-H), 4.71 (1H, m, 3′-H), 4.04−3.68 (18H, m, 8 × −CH_2CH$_3$ and 5′-H), 2.76 (1H, br s, −OH), 2.11 (1H, br s, −OH), 1.90−1.68 (26H, m, 8 × −CH$_2$CH_3 and 4′-H). MS (ESI) m/z: 690.5 (M^+, 42), 713.6 (MNa^+, 100). Anal. Calcd for $C_{41}H_{52}N_4NiO_2$: C, 71.21; H, 7.58; N, 8.10. Found: C, 71.32; H, 7.59; N, 8.16.

Benzochlorins 18a and 18b. Porphyrin **17** (300 mg) was dissolved in sulfuric acid (40 mL, 95−98%), and the mixture was stirred at room temperature for 2 h. It was then poured into ice, extracted with CH_2Cl_2, washed with water and 5% $NaHCO_3$, dried over Na_2SO_4, and filtered. The solvent was removed to provide a mixture of benzochlorins **18a** and **18b** in a ratio of 1:4 approximately based on TLC (silica TLC, 10% MeOH/CH_2Cl_2). The mixture was used for the next step without further purification. An analytical sample of **18b** was purified with Sephadex LH-20 column using MeOH/CH_2Cl_2 (v/v, 1:1) as the eluant. Data for **18b**: dark blue solid. Mp: 220 °C (dec). UV−vis (MeOH) λ_{max}, nm (ϵ): 411 (88 027), 531 (5159), 564 (6695), 606 (8781), 660 (24 476). ^{1}H NMR (CD_3-OD) δ: 9.51 (1H, d, J = 8.7 Hz, 1′-H), 9.03 (1H, s, 15-H), 8.46 (1H, s, 20-H), 8.10 (1H, s, 5-H), 8.08 (1H, d, J = 8.7 Hz, 2′-H), 4.62 (2H, t, J = 7.7 Hz, 5′-H), 3.88 (2H, t, J = 7.7 Hz, 4′-H), 3.77 (2H, q, J = 7.5 Hz, 12-CH_2CH$_3$), 3.57 (4H, m, 3-CH_2CH$_3$ and 13-CH_2CH$_3$), 3.51 (2H, q, J = 7.6 Hz, 2-CH_2CH$_3$ or 18-CH_2CH$_3$), 3.40 (2H, q, J = 7.6 Hz, 17-CH_2CH$_3$), 3.36 (2H, q, J = 7.6 Hz, 2-CH_2CH$_3$ or 18-CH_2CH$_3$), 3.03 (2H, m, ABX system, 7-CH_2CH$_3$), 2.76 (2H, m, ABX system, 7-CH_2CH$_3$), 1.74 (3H, t, J = 7.6 Hz, 12-CH$_2$CH_3), 1.68 (3H, t, J = 7.6 Hz, 3-CH$_2$CH_3), 1.64 (3H, t, J = 7.6 Hz, 2-CH$_2$CH_3 or 18-CH$_2$CH_3), 1.57 (9H, m, 3-CH$_2$CH_3, 17-CH$_2$CH_3, 2-CH$_2$CH_3, or 18-CH$_2$CH_3), 0.03

(6H, t, J = 7.1 Hz, 2 × 7-CH_2CH_3). MS (ESI, negative) m/z: 695.6 ([M − Na]$^-$, 100).

Benzochlorins 19a and 19b. To a solution of the foregoing mixture of **18a** and **18b** in EtOH (30 mL) was added HCl (3 N, 45 mL). The mixture was refluxed for 4 h. After the neutralization with saturated $NaHCO_3$, the mixture was extracted with CH_2Cl_2, washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel, eluting with CH_2Cl_2/EtOAc (v/v, 40:1) to provide **19a** (45 mg, 15% from **17**) and **19b** (167 mg, 63% from **17**). Treatment of **19a** with sulfuric acid (following the procedure discussed at the preceding step) and then with 3 N HCl/EtOH provided **19b** in quantitative yield. Data for **19a**: dark blue solid. Mp: 288−290 °C. UV−vis (CH_2Cl_2) λ_{max}, nm (ϵ): 420 (69 615), 622 (8933), 673 (33 883). ^{1}H NMR ($CDCl_3$) δ: 8.94 (1H, d, J = 8.7 Hz), 8.86 (1H, s), 8.52 (1H, s), 7.78 (1H, s), 7.66 (1H, d, J = 8.7 Hz), 4.21 (2H, t, J = 7.0 Hz), 3.64 (2H, q, J = 7.6 Hz), 3.59 (2H, t, J = 7.7 Hz), 3.54 (2H, q, J = 7.7 Hz), 3.46 (6H, m), 3.38 (2H, q, J = 7.7 Hz), 2.76 (2H, m, ABX system), 2.53 (2H, m, ABX system), 1.75−1.50 (18H, m), 0.08 (6H, t, J = 7.2 Hz). MS (ESI) m/z: 672.6 (M^+, 100). Anal. Calcd for $C_{41}H_{50}N_4NiO\cdot{}^3/_2H_2O$: C, 70.29; H, 7.63; N, 8.00. Found: C, 70.07; H, 7.21; N, 7.93. Data for **19b**: dark blue solid. Mp: 275−277 °C. UV−vis (CH_2Cl_2) λ_{max}, nm (ϵ): 413 (111 381), 528 (7235), 563 (9044), 607 (10 948), 661 (31 225). ^{1}H NMR ($CDCl_3$) δ: 9.59 (1H, d, J = 8.7 Hz, 1′-H), 9.32 (1H, s, 15-H), 8.66 (1H, s, 20-H), 8.08 (1H, s, 5-H), 7.96 (1H, d, J = 8.7 Hz, 2′-H), 4.22 (2H, t, J = 7.4 Hz, 5′-H), 3.97 (2H, q, J = 7.6 Hz, 12-CH_2CH$_3$), 3.88 (2H, q, J = 7.6 Hz, 13-CH_2CH$_3$), 3.69 (8H, m, 3, 17, 18-CH_2CH$_3$ and 4′-H), 3.62 (2H, q, J = 7.6 Hz, 2-CH_2CH$_3$), 2.97 (2H, m, ABX system, 7-CH_2CH$_3$), 2.77 (2H, m, ABX system, 7-CH_2CH$_3$), 2.41 (1H, br s, −NH), 2.03 (1H, br s, −NH), 1.93 (3H, t, J = 7.6 Hz, 12-CH_2CH_3), 1.86−1.71 (15H, m, 2, 3, 13, 17, 18-CH_2CH_3), 0.08 (6H, t, J = 7.5 Hz, 2 × 7-CH_2CH_3). ^{13}C NMR ($CDCl_3$) δ: 175.6 (C), 157.2 (C), 153.6 (C), 146.8 (C), 145.4 (C), 142.7 (C), 140.5 (C), 140.0 (C), 139.9 (C), 139.4 (C), 139.2 (C), 138.2 (C), 134.8 (C), 130.3 (C), 129.0 (C), 127.2 (C), 125.7 (CH), 123.4 (CH), 114.0 (C), 107.1 (CH), 95.0 (CH), 87.1 (CH), 64.2 (CH_2), 63.4 (C), 35.1 (CH_2), 33.1 (CH_2), 21.5 (CH_2), 19.6 (CH_2), 19.4 (CH_2), 19.3 (CH_2), 19.1 (CH_2), 18.5 (CH_3), 18.4 (CH_3), 17.9 (CH_3), 17.44 (CH_3), 17.37 (CH_3), 16.0 (CH_3), 8.6 (CH_3). MS (ESI) m/z: 617.5 (MH^+, 100). Anal. Calcd for $C_{41}H_{52}N_4O\cdot{}^3/_2H_2O$: C, 76.48; H, 8.61; N, 8.70. Found: C, 76.35; H, 8.20; N, 8.62.

Benzochlorin 20. To a solution of **19a** (270 mg) in CH_2Cl_2 (20 mL) were added *tert*-butyl bromoacetate (3 mL), 25% NaOH (12 mL), and Bu_4NHSO_4 (500 mg). The mixture was stirred at room temperature for 20 h, and TLC (alumina TLC, CH_2Cl_2) confirmed the completion of the reaction. The mixture was diluted with CH_2Cl_2 (50 mL), washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on alumina, eluting with hexanes/CH_2Cl_2 (v/v, 2:1) to provide **20** (313 mg, 98%) as a dark blue solid. Mp: 124−126 °C. UV−vis (CH_2Cl_2) λ_{max}, nm (ϵ): 413 (116 258), 530 (7698), 564 (9707), 607 (11 492), 661 (32 244). ^{1}H NMR ($CDCl_3$) δ: 9.50 (1H, d, J = 8.7 Hz), 9.21 (1H, s), 8.56 (1H, s), 8.01 (1H, s), 7.96 (1H, d, J = 8.8 Hz), 4.17 (2H, s), 4.14 (2H, t, J = 7.7 Hz), 3.89 (2H, q, J = 7.5 Hz), 3.81 (4H, m), 3.61 (6H, m), 3.53 (2H, q, J = 7.6 Hz), 2.97 (2H, m, ABX system), 2.72 (2H, m, ABX system), 2.30 (1H, br s), 1.92 (1H, br s), 1.85 (3H, t, J = 7.5 Hz), 1.77−1.62 (15H, m), 1.55 (9H, s), 0.01 (6H, t, J = 7.5 Hz). MS (ESI) m/z: 730.8 (M^+, 100). Anal. Calcd for $C_{47}H_{62}N_4O_3$: C, 77.22; H, 8.55; N, 7.66. Found: C, 77.09; H, 8.71; N, 7.71.

Benzochlorin 21. Compound **20** (262 mg) was dissolved in TFA (15 mL). The resulting solution was stirred at room temperature for 1 h. It was poured into cold water, extracted with CH_2Cl_2, washed with water, dried with Na_2SO_4, and filtered. After the solvent was removed, **21** (240 mg, 100%) was obtained as a dark blue solid. Mp: 136−138 °C. UV−vis (CH_2Cl_2) λ_{max}, nm (ϵ): 413 (112 659), 530 (7313), 564 (9181), 607 (10 970), 661 (30 810). ^{1}H NMR ($CDCl_3$) δ: 9.49 (1H, d, J = 8.8 Hz), 9.20 (1H, s), 8.55 (1H, s), 7.98 (1H, s), 7.91 (1H, d, J = 8.7 Hz), 4.30 (2H, s), 4.18 (2H, t, J = 7.6 Hz), 3.86 (2H, q, J = 7.7 Hz), 3.79 (4H, m), 3.59 (6H, m), 3.51 (2H, q, J = 7.7 Hz), 2.90 (2H, m, ABX system), 2.72 (2H, m, ABX system), 2.26 (1H, br s), 1.82 (3H, t, J = 7.4 Hz), 1.75−1.60 (16H, m), −0.01 (6H, t, J = 7.5 Hz). MS (ESI) m/z: 675.5 (MH^+, 100). Anal. Calcd for $C_{43}H_{54}N_4O_3\cdot{}^1/_2H_2O$: C, 75.52; H, 8.11; N, 8.19. Found: C, 75.56; H, 7.88; N, 8.17.

Benzochlorin 21a. The title compound was obtained as a dark green solid in quantitative yield by treating compound **21** with zinc acetate dihydrate by following the standard procedure. UV−vis (MeOH) λ_{max}, nm (ϵ): 342 (23 438), 424 (92 815), 533 (4063), 579 (5000), 620 (10 078), 674 (46 876). ^{1}H NMR (pyridine-d_5) δ: 9.89 (1H, d, J = 8.8 Hz), 9.61 (1H, s), 9.13 (1H, s), 8.24 (1H, s), 8.17 (1H, d, J = 8.8 Hz), 4.69 (2H, s), 4.53 (2H, t, J = 7.5 Hz), 4.01 (2H, t, J = 7.6 Hz), 3.91 (2H, q, J = 7.5 Hz), 3.80 (2H, q, J = 7.6 Hz), 3.70 (6H, m), 3.60 (2H, q, J = 7.6 Hz), 3.13 (2H, m, ABX system), 2.82 (2H, m, ABX system), 1.84 (3H, t, J = 7.4 Hz), 1.80−1.64 (15H, m), 0.12 (6H, t, J = 7.4 Hz). MS (ESI) m/z: 736.6 (M^+, 100).

Compound 23. To a solution of **21** (35 mg, 0.05 mmol) and **22** (27 mg, 0.075 mmol) in dry CH_2Cl_2 (3 mL) were added Et_3N (6 drops) and the benzyl octyl phthalate (BOP) reagent (Chem. Abstr. 56602-33-6, 28 mg, 0.06 mmol). The mixture was stirred under N_2 at room temperature for 18 h. It was then diluted with CH_2Cl_2 (30 mL), washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified with preparative silica TLC using CH_2Cl_2/acetone (v/v, 8:1) as a developing solvent to provide crude **23**, which was further purified by preparative silica TLC using hexanes/EtOAc (v/v, 1:2) as a developing solvent to give pure **23** (21 mg, 40%) as a dark blue gummy solid. UV−vis (CH_2Cl_2) λ_{max}, nm (ϵ): 413 (117 733), 530 (7556), 563 (9564), 606 (11 381), 661 (32 135). ^{1}H NMR ($CDCl_3$) δ: 9.52 (1H, d, J = 8.7 Hz), 9.20 (1H, s), 8.55 (1H, s), 8.00 (1H, s), 7.94 (1H, d, J = 8.7 Hz), 7.54 (1H, d, J = 8.8 Hz), 5.48 (1H, d, J = 3.3 Hz), 5.39−5.16 (3H, m), 4.18 (2H, dd, AB system, J = 15.9 Hz), 4.11 (5H, m), 3.88 (2H, q, J = 7.7 Hz), 3.80 (4H, m), 3.60 (6H, m), 3.52 (2H, q, J = 7.6 Hz), 2.94 (2H, m, ABX system), 2.74 (2H, m, ABX system), 2.27 (1H, br s), 2.13 (3H, s), 2.07 (3H, s), 2.04 (3H, s), 2.03 (3H, s), 1.91 (1H, br s), 1.84 (3H, t, J = 7.6 Hz), 1.76−1.60 (15H, m), 0.02 (6H, t, J = 7.5 Hz). MS (FAB) m/z: 1004.3 (MH^+, 100). HRMS (FAB): calcd for $C_{57}H_{74}N_5O_{11}$ (M + H), 1004.5390; found, 1004.5390.

Compound 24. Compound **23** (17 mg) was dissolved in a mixture of CH_2Cl_2 (9 mL) and MeOH (1 mL). Freshly prepared sodium methoxide (0.3 mL, prepared from diluting 0.4 mL of 25% MeONa in MeOH with 5 mL of CH_2Cl_2) was added dropwise. The mixture was stirred at room temperature for 5 min. It was then neutralized with AcOH, washed with water and 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated. The residue was crystallized from hexanes/CH_2Cl_2 twice to provide **24**. The filtrate was concentrated and passed through a small short silica column, eluting with MeOH/CH_2Cl_2 (5%) to remove fast-moving impurities and then with MeOH/CH_2Cl_2 (30%) to provide **24** (total 13 mg, 92%) as a dark blue solid. Mp: 232−234 °C. UV−vis (MeOH/CH_2Cl_2, 30%) λ_{max}, nm (ϵ): 412 (114 538), 530 (7547), 564 (9471), 606 (11 543), 661 (32 704). ^{1}H NMR (a mixture of 0.6 mL of $CDCl_3$ and 6 drops of CD_3OD) δ: 9.45 (1H, d, J = 8.7 Hz, 1′-H), 9.16 (1H, s, 15-H), 8.51 (1H, s, 20-H), 7.95 (1H, s, 5-H), 7.88 (1H, d, J = 8.9 Hz, 2′-H), 4.92 (1H, d, J = 9.0 Hz, 1″-H), 4.17 (2H, s, 7′-H), 4.08 (2H, t, J = 7.5 Hz, 5′-H), 3.89 (1H, d, J = 2.6 Hz, 1 × sugar H), 3.81 (2H, q, J = 7.7 Hz, 12-CH_2CH$_3$), 3.78−3.64 and 3.63−3.40 (17H, m, 2-, 3-, 13-, 17-, and 18-CH_2CH$_3$, 4′-H, 5 × sugar H), 2.84 (2H, m, ABX system, 7-CH_2CH$_3$), 2.67 (2H, m, ABX system, 7-CH_2CH$_3$), 1.76 (3H, t, J = 7.6 Hz, 12-CH_2CH_3), 1.69−1.55 (15H, m, 2-, 3-, 13-, 17-, and 18-CH_2CH_3), −0.05 (6H, t, J = 7.2 Hz, 2 × 7-CH_2CH_3). ^{1}H NMR (pyridine-d_5) δ: 9.70 (1H, d, J = 8.7 Hz), 9.50 (1H, s), 9.24 (1H, d, J = 9.3 Hz), 8.88 (1H, s), 8.35 (1H, s), 8.14 (1H, d, J = 8.8 Hz), 7.18 (1H, br s), 6.87 (1H, br s), 6.51 (1H, br s), 6.37 (1H, br s),

6.06 (1H, t, J = 9.3 Hz), 4.70 (1H, t, J = 8.7 Hz), 4.66 (1H, br s), 4.48 (4H, br s), 4.25 (4H, m), 3.87 (2H, q, J = 7.4 Hz), 3.80 (4H, m), 3.64 (2H, q, J = 7.6 Hz), 3.57 (6H, m), 2.99 (2H, m, ABX system), 2.89 (3H, m), 2.55 (1H, br s), 1.79 (3H, t, J = 7.6 Hz), 1.76−1.62 (15H, m), 0.19 (6H, m). ^{13}C NMR (a mixture of 0.6 mL of $CDCl_3$ and 6 drops of CD_3OD) δ: 175.5, 171.8, 157.2, 153.4, 146.7, 145.4, 142.4, 140.5, 139.9, 139.6, 139.2, 138.1, 134.4, 129.6, 129.0, 126.9, 125.6, 123.4, 113.8, 106.9, 94.9, 86.9, 79.7, 76.6, 74.3, 73.0, 70.4, 70.3, 69.3, 63.3, 61.9, 32.9, 31.8, 21.3, 19.4, 19.2, 19.1, 18.9, 18.3, 17.7, 17.23, 17.17, 15.7, 8.4. MS (FAB) m/z: 836.3 (MH^+, 100). HRMS (FAB): calcd for $C_{49}H_{66}N_5O_7$ (M + H), 836.4963; found, 836.4960.

Compound 26. To a solution of **21** (91 mg, 0.13 mmol) and **25** (78 mg, 0.20 mmol) in dry CH_2Cl_2 (5 mL) were added Et_3N (15 drops) and the BOP reagent (71 mg, 0.16 mmol). The mixture was stirred under N_2 at room temperature for 2 h. It was then diluted with CH_2Cl_2 (30 mL), washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified with preparative silica TLC using hexanes/EtOAc (v/v, 1:2) as a developing solvent to provide **26** (130 mg, 97%) as a dark blue solid. Mp: 133−135 °C. UV−vis (CH_2Cl_2) λ_{max}, nm (ϵ): 413 (129 898), 530 (8554), 563 (10 693), 606 (12 646), 661 (35 520). ^{1}H NMR ($CDCl_3$) δ: 9.56 (1H, d, J = 8.8 Hz), 9.23 (1H, s), 8.57 (1H, s), 8.01 (1H, s), 7.94 (1H, d, J = 8.7 Hz), 6.68 (1H, d, J = 9.4 Hz), 5.79 (1H, d, J = 8.8 Hz), 5.30 (1H, dd, J = 10.0, 10.0 Hz), 5.17 (1H, dd, J = 9.8, 9.8 Hz), 4.36−4.21 (2H, m), 4.19−4.05 (5H, m), 3.91 (2H, q, J = 7.4 Hz), 3.80 (5H, m), 3.62 (6H, m), 3.54 (2H, q, J = 7.6 Hz), 2.93 (2H, m, ABX system), 2.75 (2H, m, ABX system), 2.32 (1H, br s), 2.12 (3H, s), 2.09 (3H, s), 2.07 (3H, s), 2.04 (3H, s), 1.95 (1H, br s), 1.86 (3H, t, J = 7.4 Hz), 1.77−1.63 (15H, m), 0.03 (6H, t, J = 7.2 Hz). MS (FAB) m/z: 1004.4 (MH^+, 100). HRMS (FAB): calcd for $C_{57}H_{74}N_5O_{11}$ (M + H), 1004.5390; found, 1004.5400.

Compound 27. Method A. When the same procedure as that described for the foregoing compound is followed, **26** (70 mg) was treated with excess MeONa for 5 min. After workup and purification with preparative silica TLC using MeOH/CH_2Cl_2 (10%) as a developing solvent, **27** (39 mg, 67%) was obtained as a dark blue solid. Mp: 149−151 °C. UV−vis (MeOH/CH_2Cl_2, 30%) λ_{max}, nm (ϵ): 413 (119 210), 530 (8015), 563 (10 023), 606 (11 790), 661 (32 544). **27** was a mixture of two isomers with a ratio of 4:1 (α/β, referred to −OH at the 1″ position). The NMR data was given for the α isomer. ^{1}H NMR (pyridine-d_5) δ: 9.75 (1H, d, J = 8.8 Hz, 1′-H), 9.49 (1H, s, 15-H), 8.89 (1H, s, 20-H), 8.34 (1H, s, 5-H), 8.25 (1H, d, J = 8.8 Hz, 2′-H), 8.09 (1H, d, J = 8.9 Hz, 9′-H), 5.97 (1H, d, J = 3.2 Hz, 1″-H), 5.86 (4H, very broad s, 4 × −OH), 4.97 (1H, ddd, J = 12.7, 9.0, 3.2 Hz, 2″-H), 4.80 (2H, m, 3″-H and 5″-H), 4.58 and 4.45 (each 1H, each m, 6″-H), 4.47 (2H, splitting s, 7′-H), 4.35 (1H, dd, J = 9.2, 9.2 Hz, 4″-H), 4.28 (2H, t, J = 7.1 Hz, 5′-H), 3.87 (4H, m, 4′-H and 12-CH_2CH$_3$), 3.77 (2H, q, J = 7.7 Hz, 13-CH_2CH$_3$), 3.64 (2H, q, J = 7.7 Hz, 17-CH_2CH$_3$), 3.58 (6H, m, 2-, 3-, and 18-CH_2CH$_3$), 3.03 (2H, m, ABX, 7-CH_2CH$_3$), 2.89 (2H, m, ABX, 7-CH_2CH$_3$), 2.84 (1H, br s, −NH), 2.54 (1H, br s, −NH), 1.81 (3H, t, J = 7.4 Hz, 12-CH$_2$CH_3), 1.77−1.62 (15H, m, 2-, 3-, 13-, 17-, and 18-CH$_2$CH_3), 0.21 (6H, m, 2 × 7-CH$_2$CH_3).^{13}C NMR (pyridine-d_5) δ: 176.8 (C), 170.7 (C), 158.1 (C), 154.2 (C), 147.9 (C), 146.2 (C), 143.3 (C), 141.4 (C), 141.0 (C), 140.7 (C), 140.1 (C), 139.9 (C), 139.2 (C), 135.4 (C), 132.0 (C), 130.1 (C), 127.9 (C), 126.5 (CH), 124.9 (CH), 114.7 (C), 107.9 (CH), 96.2 (CH), 93.1 (CH), 87.9 (CH), 74.5 (CH), 73.9 (CH), 73.7 (CH), 73.2 (CH$_2$), 71.9 (CH$_2$), 64.2 (C), 63.7 (CH$_2$), 55.9 (CH), 33.6 (CH$_2$), 32.7 (CH$_2$), 22.1 (CH$_2$), 20.0 (CH$_2$), 19.9 (CH$_2$), 19.6 (CH$_2$), 19.5 (CH$_2$), 19.1 (CH$_3$), 19.0 (CH$_3$), 18.6 (CH$_3$), 17.89 (CH$_3$), 17.86 (CH$_3$), 16.4 (CH$_3$), 9.22 (CH$_3$), 9.19 (CH$_3$). MS (ESI) m/z: 835.9 (M^+, 100). MS (FAB) m/z: 836.5 (MH^+, 100). HRMS (FAB): calcd for $C_{49}H_{66}N_5O_7$ (M + H), 836.4963; found, 836.4958.

Method B. To a solution of **21** (25 mg) in a mixture of dry THF (10 mL) and Et_3N (0.2 mL) was added the isobutyl chloroformate (0.2 mL) at 0 °C. The mixture was stirred at this temperature for 45 min. A solution of **32** in H_2O/EtOH [1.0 mL, prepared by treating D-glucosamine hydrochloride **31** (750 mg, 3.48 mmol) to a solution of NaOH (139 mg, 3.48 mmol) in a mixture of water (2 mL) and EtOH (11 mL)] and Et_3N (0.2 mL) were added successively. The resulting reaction mixture was stirred at room temperature for 2 h. The same solution of **32** in H_2O/EtOH (1.0 mL) was added. The final mixture was stirred at room temperature for 17 h, poured into water, and extracted with CH_2Cl_2 (2 × 100 mL). The organic layer was washed with water (2 × 100 mL), 5% $NaHCO_3$, and again with water (100 mL). The dichloromethane layer was separated, dried over Na_2SO_4, filtered, and concentrated. The residue was purified with preparative silica TLC using acetone/CH_2Cl_2/acetone (v/v, 1:9:10) as a developing solvent to provide **27** (14 mg, 45%) as a dark blue solid.

Compound 27a. For the preparation and characterization of this compound including the detailed NMR and MS data, please see the Supporting Information.

Compound 29. To a solution of **21** (96 mg, 0.14 mmol) and **28** (132 mg, 0.21 mmol) in dry CH_2Cl_2 (5 mL) were added Et_3N (10 drops) and the BOP reagent (75 mg, 0.17 mmol). The mixture was stirred under N_2 at room temperature for 17 h. It was then diluted with CH_2Cl_2 (30 mL), washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by preparative silica TLC using CH_2Cl_2/acetone (v/v, 7:1) as a developing solvent to provide **29** (79 mg, 43%) as a dark blue solid. Mp: 150−153 °C. UV−vis (CH_2Cl_2) λ_{max}, nm (ϵ): 413 (122 657), 530 (8078), 562 (10 097), 606 (12 011), 661 (33 800). ^{1}H NMR ($CDCl_3$) δ: 9.50 (1H, d, J = 8.7 Hz), 9.19 (1H, s), 8.54 (1H, s), 7.98 (1H, s), 7.91 (1H, d, J = 8.7 Hz), 7.36 (1H, d, J = 9.3 Hz), 5.42−5.23 (3H, m), 5.11 (1H, dd, J = 10.9, 7.6 Hz), 5.04−4.90 (2H, m), 4.43 (1H, d, J = 7.9 Hz), 4.39 (1H, d, J = 12.3 Hz), 4.24−3.99 (7H, m), 3.87 (3H, m), 3.76 (6H, m), 3.59 (6H, m), 3.51 (2H, q, J = 7.6 Hz), 2.90 (2H, m, ABX system), 2.72 (2H, m, ABX system), 2.26 (1H, br s), 2.16 (3H, s), 2.10 (3H, s), 2.08 (6H, s), 2.01 (6H, s), 1.97 (3H, s), 1.89 (1H, br s), 1.83 (3H, t, J = 7.5 Hz), 1.75−1.59 (15H, m), −0.01 (6H, t, J = 7.3 Hz). MS (FAB) m/z: 1292.5 (MH^+, 100). HRMS (FAB): calcd for $C_{69}H_{90}N_5O_{19}$ (M + H), 1292.6230; found, 1292.6240.

Compound 30. Compound **29** (41 mg) was added to a mixture of CH_2Cl_2 (9 mL) and MeOH (1 mL) and a MeONa solution (0.5 mL, prepared from diluting 1.0 mL of 25% MeONa in MeOH with 5 mL of CH_2Cl_2) dropwise. The mixture was stirred at room temperature for 5 min. AcOH (0.1 mL) was then added to the above mixture. Then 5% $NaHCO_3$ was added until the solution became slightly basic. The solvent was removed, and the residue was extracted with MeOH/CH_2Cl_2 (25%) repeatedly (typically, 5 × 30 mL). The extracts were combined, and then the solvent was removed. The residue was redissolved in MeOH/CH_2Cl_2 (25%, 10 mL). Hexane was added dropwise, and a dark blue solid was precipitated out. It was filtered, and compound **30** (30 mg, 95%) was obtained as a dark blue solid. Mp: 235−237 °C. UV−vis (MeOH/CH_2Cl_2, 30%) λ_{max}, nm (ϵ): 412 (117 551), 530 (7401), 563 (9380), 606 (11 617), 661 (33 217). ^{1}H NMR (pyridine-d_5) δ: 9.71 (1H, d, J = 8.9 Hz, 1′-H), 9.50 (1H, s, 15-H), 9.26 (1H, d, J = 9.2 Hz, 9′-H), 8.88 (1H, s, 20-H), 8.35 (1H, s, 5-H), 7.88 (1H, d, J = 8.8 Hz, 2′-H), 6.41 (7H, very broad s, 7 × −OH), 6.05 (1H, t, J = 9.2, 9.2 Hz, 1″-H), 5.12 (1H, d, J = 7.9 Hz, 1‴-H), 4.65−4.32 (10H, m, 7′-H and 8 × sugar H), 4.27 (3H, m, 5′-H and 2″-H), 4.16 (2H, m, 2 × sugar H), 4.06 (1H, m, 1 × sugar H), 3.94−3.73 (6H, m, 4′-H and 12, 13-CH_2CH$_3$), 3.64 (2H, q, J = 7.5 Hz, 17-CH_2CH$_3$), 3.57 (6H, m, 2-, 3-, and 18-CH_2CH$_3$), 2.99 (2H, m, ABX, 7-CH_2CH$_3$), 2.90 (2H, m, ABX, 7-CH_2CH$_3$), 2.87 (1H, br s, −NH), 2.55 (1H, br s, −NH), 1.80 (3H, t, J = 7.4 Hz, 12-CH$_2$CH_3), 1.77−1.62 (15H, m, 2-, 3-, 13-, 17-, and 18-CH$_2$CH_3), 0.19 (6H, t, J = 7.2 Hz, 2′- and 7-CH$_2$CH_3). ^{13}C NMR (pyridine-d_5) δ: 176.8, 171.5, 158.2, 154.2, 147.9, 146.2, 143.3, 141.4, 141.0, 140.7, 140.0, 139.9, 139.2, 135.3, 131.8, 130.0, 127.9, 126.4, 124.8, 114.7, 107.9, 106.3, 96.2, 87.9, 82.5, 81.2, 78.7, 78.2, 77.7, 75.6, 74.4, 73.3, 72.9, 71.8, 70.5, 64.2, 62.52, 62.49, 33.5, 32.6, 22.0, 20.0, 19.8, 19.6, 19.4, 19.1, 19.0, 18.5,

17.85, 17.82, 16.3, 9.2. MS (FAB) *m/z*: 998.3 (MH^+, 100). HRMS (FAB): calcd for $C_{55}H_{76}N_5O_{12}$ (M + H), 998.5491; found, 998.5494.

Compound 35. To a solution of compound **34** (14.04 g, 24.1 mmol) in dry THF (200 mL) were added Ph_3P (6.96 g, 26.5 mmol) and phthalimide (4.30 g, 29.2 mmol). The resultant solution was cooled to 0 °C, and diethyl azodicarboxylate (4.93 mL, 31.3 mmol) was added slowly (∼5 min) via a syringe. The mixture was stirred at room temperature under N_2 for 20 h. It was diluted with CH_2Cl_2 (300 mL), washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified with column chromatography on silica gel, eluting with hexanes/EtOAc (v/v, 2:1) first and then hexanes/EtOAc (v/v, 3:2) to provide **35** (13.65 g, 79%) as a colorless oil.^{1}H NMR ($CDCl_3$, TMS as ref, 0.00 ppm) δ: 7.82 (2H, m), 7.69 (2H, m), 7.36−7.19 (20H, m), 4.76−4.42 (8H, m), 3.97 (3H, m), 3.86−3.60 (6H, m), 1.88−1.48 (4H, m). ^{13}C NMR ($CDCl_3$) δ: 168.4 (C), 138.76 (C), 138.68 (C), 138.54 (C), 138.39 (C), 133.9, 132.3, 128.45 (CH), 128.38 (CH), 128.08 (CH), 127.99 (CH), 127.88 (CH), 127.78 (CH), 127.68 (CH), 127.61(CH), 123.2, 76.9 (CH), 76.8 (CH), 74.5 (CH), 73.4 (CH_2), 73.2 (CH_2), 73.1 (CH_2), 72.4 (CH), 70.9 (CH), 67.7 (CH_2), 37.9 (CH_2), 25.2 (CH_2), 24.7 (CH_2). MS (ESI) *m/z*: 734.6 (MNa^+, 100).

Compound 36. To a solution of **35** (13.30 g) in absolute EtOH (300 mL) was added $NH_2NH_2{\cdot}H_2O$ (6 mL) dropwise. The mixture was refluxed for 4 h (a white solid precipitated out after the mixture was refluxed for a half hour). It was then cooled to 0 °C with an ice bath. Concentrated HCl (37%, 60 mL) was slowly added to the reaction mixture (∼10 min). The solvent was removed to dry, and then EtOH/H_2O (v/v, 1:1, 200 mL) was added to the residue. The resulting mixture was neutralized with NaOH (20%), and the pH was adjusted to 14 to give a clear pale yellow solution, which was then extracted with Et_2O (4 × 100 mL), washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on alumina (grade III), eluting with MeOH/CH_2Cl_2 (2%) first and then with MeOH/CH_2Cl_2 (5%) to provide **36** (9.89 g, 91%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$, TMS as reference, 0.00 ppm) δ: 7.42−7.20 (20H, m, 4 × $-CH_2C_6H_5$), 4.78−4.42 (8H, m, 4 × $-CH_2C_6H_5$), 3.98 (2H, s, 1′-H), 3.92 (1H, m), 3.86−3.75 (2H, m), 3.72 (1H, dd, J = 7.1, 2.5 Hz), 3.63 (1H, dd, J = 10.7, 4.3 Hz), 2.67 (2H, m, 1-H), 1.86 (2H, br s, 1-NH_2), 1.66 (1H, m, one proton of 3-H), 1.54 (2H, m, one proton of 2-H and one proton of 3-H), 1.36 (1H, m, one proton of 2-H). ^{13}C NMR ($CDCl_3$) δ: 138.8 (C), 138.7 (C), 138.5 (C), 128.5 (CH), 128.4 (CH), 128.1 (CH), 128.04 (CH), 127.95 (CH), 127.87 (CH), 127.71 (CH), 127.68 (CH), 127.63 (CH), 77.1 (CH), 77.0 (CH), 74.6 (CH), 73.4 (CH_2), 73.3 (CH_2), 73.2 (CH_2), 72.3 (CH), 71.6 (CH), 67.9 (CH_2), 42.1 (CH_2), 30.4 (CH_2), 24.6 (CH_2). MS (APCI) *m/z*: 582.5 (MH^+, 100).

Compound 37. A reaction flask containing a mixture of **21** (73 mg, 0.11 mmol), **36** (100 mg, 0.17 mmol), DCC (27 mg, 0.13 mmol), and DMAP (14 mg, 0.11 mmol) was dissolved in CH_2Cl_2 (3 mL). The resulting solution was stirred at room temperature under N_2 for 15 h. Water (5 mL) was added, and the mixture was stirred at room temperature for 15 min. It was then diluted with CH_2Cl_2 (20 mL). The organic layer was collected, dried over Na_2SO_4, filtered, and concentrated. The residue was transferred into a 10 mL flask with CH_2Cl_2. The solvent was removed, and CH_2Cl_2 (2 mL) was added to the residue. The mixture was refrigerated for 1 h. It was filtered and washed with CH_2Cl_2 (most of the urea was removed by this process). The filtrate was concentrated, and the residue was purified by column chromatography on silica gel, eluting with CH_2Cl_2/acetone (v/v, 12:1) to provide **37** (112 mg, 83%) as a dark blue gummy solid. UV−vis (CH_2Cl_2) λ_{max}, nm (ϵ): 413 (124 784), 530 (8035), 563 (9971), 607 (11 907), 662 (34 173). ^{1}H NMR ($CDCl_3$) δ: 9.50 (1H, d, J = 8.7 Hz), 9.20 (1H, s), 8.54 (1H, s), 7.98 (1H, s), 7.90 (1H, d, J = 8.7 Hz), 7.40−7.15 (20H, m), 6.59 (1H, t, J = 5.8 Hz), 4.76−4.34 (8H, m), 4.12 (2H, s), 4.09 (2H, t, J = 7.5 Hz), 3.97−3.82 (5H, m), 3.82−3.68 (6H, m), 3.66−3.55 (8H, m), 3.51 (2H, q, J = 7.6 Hz), 3.26 (2H, m), 2.89 (2H, m, ABX system), 2.71 (2H, m, ABX system), 2.26 (1H, s), 1.88 (1H, s), 1.82 (3H, t, J = 7.6 Hz), 1.78−1.52 (17H, m), 1.46 (2H, m), 0.00 (6H, t, J = 7.2 Hz). MS (FAB) *m/z*: 1238.6 (MH^+, 100). HRMS (FAB): calcd for $C_{80}H_{96}N_5O_7$ (M + H), 1238.7310; found, 1238.7270.

Compound 38. The title compound was obtained as a deep green solid in 85% yield by treating compound **24** with zinc acetate dihydrate by following the standard procedure [the compound was purified with a Sephadex LH-20 column, eluting with MeOH/CH_2Cl_2 (v/v, 1:1)]. UV−vis (MeOH/CH_2Cl_2, 80%) λ_{max}, nm (ϵ): 343 (23 218), 425 (96 082), 535 (3964), 578 (5285), 621 (10 760), 675 (49 268). ^{1}H NMR (pyridine-d_5) δ: 9.88 (1H, d, J = 8.8 Hz), 9.61 (1H, s), 9.22 (1H, d, J = 9.4 Hz), 9.14 (1H, s), 8.24 (1H, s), 8.02 (1H, d, J = 8.8 Hz), 6.05 (1H, t, J = 9.1 Hz), 4.70 (1H, t, J = 9.1 Hz), 4.66 (1H, d, J = 2.9 Hz), 4.47 (4H, br s), 4.25 (4H, m), 3.92 (2H, q, J = 7.4 Hz), 3.81 (4H, m), 3.70 (6H, m), 3.60 (2H, q, J = 7.7 Hz), 2.97 (2H, m, ABX system), 2.81 (2H, m), 1.85 (3H, t, J = 7.4 Hz), 1.80−1.65 (15H, m), 0.09 (6H, t, J = 7.1 Hz). MS (FAB) *m/z*: 897.3 (M^+, 100).

Compound 39. The title compound was obtained as a deep green solid in 81% yield by treating compound **27** with zinc acetate dihydrate by following the standard procedure [the compound was purified with a Sephadex LH-20 column, eluting with MeOH/CH_2Cl_2 (v/v, 1:1)]. UV−vis (MeOH/CH_2Cl_2, 80%) λ_{max}, nm (ϵ): 342 (24 369), 424 (94 966), 534 (4706), 578 (5962), 620 (10 668), 674 (47 588). ^{1}H NMR (pyridine-d_5) δ: 9.92 and 9.89 (total 1H, d, J = 8.7 Hz), 9.62 (1H, s), 9.15 (1H, s), 8.24 (1H, s), 8.62 and 8.13 (total 1H, d, J = 8.9 Hz), 8.11 and 8.05 (1H, d, J = 8.9 Hz), 7.27 (1H, br s), 7.05 (1H, br s), 6.23 (1H, br s), 5.99 (1H, d, J = 3.0 Hz), 5.54 (1H, br s), 4.92 (1H, m), 4.81 (2H, m), 4.60 (1H, m), 4.47 (2H, s), 4.43 (1H, m), 4.35 (1H, dd, J = 9.1, 9.1 Hz), 4.28 (2H, t, J = 7.4 Hz), 3.91 (4H, m), 3.80 (2H, m), 3.70 (6H, m), 3.61 (2H, m), 3.00 (2H, m, ABX), 2.82 (2H, m, ABX), 1.85 (3H, m), 1.80−1.66 (15H, m), 0.09 (6H, m). MS (FAB) *m/z*: 898.3 (MH^+, 100).

Compound 40. The title compound was obtained as a deep green solid in 77% yield by treating compound **30** with zinc acetate dihydrate by following the standard procedure [the compound was purified with a Sephadex LH-20 column, eluting with MeOH/CH_2Cl_2 (v/v, 1:1)]. UV−vis (MeOH/CH_2Cl_2, 80%) λ_{max}, nm (ϵ): 341 (23 683), 425 (97 439), 532 (4060), 579 (5413), 621 (10 962), 675 (49 667). ^{1}H NMR (pyridine-d_5) δ: 9.88 (1H, d, J = 8.7 Hz), 9.61 (1H, s), 9.25 (1H, m), 9.14 (1H, s), 8.24 (1H, s), 8.03 (1H, d, J = 8.8 Hz), 6.04 (1H, dd, J = 9.3, 9.3 Hz), 5.11 (1H, d, J = 7.9 Hz), 4.60−4.31 (10H, m), 4.26 (3H, m), 4.15 (2H, m), 4.04 (1H, m), 3.92 (2H, q, J = 7.6 Hz), 3.82 (4H, m), 3.70 (6H, m), 3.60 (2H, q, J = 7.6 Hz), 2.97 (2H, m), 2.80 (2H, m), 1.85 (3H, t, J = 7.3 Hz), 1.80−1.64 (15H, m), 0.08 (6H, t, J = 7.2 Hz). MS (ESI) *m/z*: 1082.7 (MNa^+, 100).

Compound 41. The title compound was obtained as a deep green solid in quantitative yield by treating compound **37** with zinc acetate dihydrate by following the standard procedure. UV−vis (MeOH) λ_{max}, nm (ϵ): 425 (91 154), 531 (4652), 573 (5909), 620 (10 373), 675 (48 783). ^{1}H NMR ($CDCl_3$) δ: 9.70 (1H, d, J = 8.7 Hz), 9.26 (1H, s), 8.84 (1H, s), 7.95 (1H, s), 7.90 (1H, d, J = 8.7 Hz), 7.45−7.17 (10H, m), 7.16−7.04 (5H, m), 6.96−6.80 (5H, m), 5.84 (1H, br s), 5.77 (2H, br s), 4.73 (2H, s), 4.53 (1H, d, J = 11.2 Hz), 4.35 (2H, dd, AB system, J = 11.9 Hz), 4.23 (1H, m), 4.16 (1H, m), 4.09−3.89 (6H, m), 3.86 (1H, m), 3.75 (1H, m), 3.70−3.43 (11H, m), 3.36 (1H, m), 3.23 (2H, m), 3.11 (1H, m), 3.03 (1H, br s), 2.94 (1H, m), 2.84 (1H, m), 2.71 (2H, m), 2.57 (1H, m), 2.05 (1H, m), 1.91 (3H, t, J = 7.6 Hz), 1.77 (3H, t, J = 7.6 Hz), 1.74 (3H, t, J = 7.6 Hz), 1.70 (3H, t, J = 7.5 Hz), 1.67 (3H, t, J = 7.6 Hz), 1.56 (3H, t, J = 7.7 Hz), 1.06 (3H, m), 0.67 (1H, m), 0.24 (3H, t, J = 7.2 Hz), −0.17 (3H, t, J = 7.2 Hz). MS (FAB) *m/z*: 1300.7 (MH^+, 100).

Compound 42. To a solution of **41** (42 mg) in EtOH (15 mL) was added Pd/C (10%, 150 mg). The mixture was stirred at room temperature under a hydrogen atmosphere for 19 h.

It was then filtered through a pad of Celite and washed with MeOH/CH_2Cl_2 (10%). The filtrate was concentrated, and the residue was purified by preparative silica TLC using MeOH/CH_2Cl_2 (15%) as a developing solvent to afford pure **42** (23 mg, 75%) as a deep green solid. UV−vis (MeOH/CH_2Cl_2, 80%) λ_{max}, nm (ϵ): 343 (23 613), 425 (94 829), 531 (4584), 578 (5652), 620 (10 676), 674 (48 859). ^{1}H NMR (pyridine-d_5) δ: 9.91 (1H, d, J = 8.8 Hz), 9.61 (1H, s), 9.14 (1H, s), 8.25 (1H, s), 8.21 (1H, t, J = 5.7 Hz), 8.07 (1H, d, J = 8.8 Hz), 6.65 (1H, d, J = 4.7 Hz), 6.43 (1H, d, J = 5.4 Hz), 6.22 (1H, t, J = 6.1 Hz), 6.19 (1H, d, J = 4.5 Hz), 4.73 (1H, m), 4.66 (1H, br s), 4.52 (2H, m), 4.45 (1H, q, J = 5.7 Hz), 4.42 (2H, s), 4.33 (1H, m), 4.27 (2H, t, J = 7.4 Hz), 4.25 (1H, partially overlapped with the signal at δ 4.27), 3.94 (2H, q, J = 7.4 Hz), 3.88 (2H, t, J = 7.4 Hz), 3.81 (2H, q, J = 7.5 Hz), 3.69 (8H, m), 3.61 (2H, q, J = 7.6 Hz), 3.01 (2H, m, ABX system), 2.82 (2H, m, ABX system), 2.33−2.03 (3H, m), 1.97 (1H, m), 1.86 (3H, t, J = 7.4 Hz), 1.80−1.65 (15H, m), 0.09 (6H, t, J = 7.2 Hz). MS (ESI) m/z: 962.9 (MNa^+, 100).

Compound 43. 42 (32 mg) was treated with TFA (5 mL) for 20 min. It was then poured into cold water (30 mL), extracted with CH_2Cl_2, washed with water and $NaHCO_3$ (5%), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by preparative silica TLC using MeOH/CH_2Cl_2 (15%) as a developing solvent to provide **43** (29 mg, 97%) as a dark blue solid. Mp: 102−105 °C. UV−vis (MeOH/CH_2Cl_2, 30%) λ_{max}, nm (ϵ): 412 (113 419), 529 (7528), 564 (9592), 606 (11 351), 659 (32 097). ^{1}H NMR ($CDCl_3$) δ: 9.51 (1H, d, J = 8.7 Hz, 1′-H), 9.20 (1H, s, 15-H), 8.56 (1H, s, 20-H), 7.99 (1H, s, 5-H), 7.94 (1H, d, J = 8.6 Hz, 2′-H), 6.20 (1H, br s, 9-H), 4.10 (2H, t, J = 6.1 Hz, 5′-H), 4.02 (2H, splitting s, 7′-H), 3.85 (2H, q, J = 7.6 Hz, 12-CH_2CH$_3$), 3.76 (3H, q, J = 7.5 Hz, 13-CH_2CH$_3$ and one proton of 4′-H), 3.65 (1H, t, J = 6.0 Hz, one proton of 4′-H), 3.63−3.45 (9H, m, 2-, 3-, 17-, and 18-CH_2CH$_3$ and 1″-H), 3.36 (1H, m, 1 × sugar H), 3.21, 2.89, and 2.67 (4H, 4H, and 3H, all m, 5 × sugar H, 2 × 7-CH_2CH$_3$ and 10′-H), 2.20 (1H, br s, −NH), 2.01 (1H, br s, −NH), 1.84 (3H, t, J = 7.2 Hz, 12-CH$_2$CH_3), 1.74−1.59 (15H, m, 2-, 3-, 13-, 17-, and 18-CH$_2$CH_3), 1.07, 0.84, and 0.58 (2H, 1H, and 1H, all m, 11′-H and 12′-H), 0.06 (3H, t, J = 7.2 Hz, 7-CH$_2$CH_3), −0.09 (3H, t, J = 7.2 Hz, 7-CH$_2$CH_3). ^{1}H NMR (pyridine-d_5) δ: 9.73 (1H, d, J = 8.8 Hz), 9.49 (1H, s), 8.87 (1H, s), 8.36 (1H, s), 8.24 (1H, t, J = 5.7 Hz), 8.19 (1H, d, J = 8.8 Hz), 6.65 (1H, br s), 6.42 (1H, br s), 6.22 (1H, br s), 6.19 (1H, br s), 4.73 (1H, m), 4.66 (1H, br s), 4.53 (2H, m), 4.45 (1H, m), 4.43 (2H, s), 4.32 (1H, dd, J = 8.8, 3.0 Hz), 4.27 (2H, t, J = 7.4 Hz), 4.25 (1H, partially overlapped with the signal at δ 4.27), 3.87 (4H, m), 3.78 (2H, q, J = 7.7 Hz), 3.69 (2H, q, J = 7.4 Hz), 3.64 (2H, q, J = 7.6 Hz), 3.57 (6H, m), 3.03 (2H, m, ABX system), 2.91 (3H, m), 2.55 (1H, br s), 2.34−2.04 (3H, m), 1.98 (1H, m), 1.80 (3H, t, J = 7.4 Hz), 1.76−1.61 (15H, m), 0.19 (6H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$) δ: 175.9, 170.3, 157.7, 153.6, 146.9, 145.7, 142.8, 140.8, 140.3, 140.2, 139.7, 139.4, 138.5, 134.5, 130.7, 129.1, 127.1, 125.6, 123.4, 113.9, 106.9, 95.1, 87.3, 74.7, 73.0, 70.4, 70.3, 69.8, 69.6, 68.1, 63.5, 62.4, 38.6, 33.2, 33.1, 31.9, 25.9, 21.5, 20.8, 19.5, 19.4, 19.2, 19.1, 18.5, 17.8, 17.4, 17.3, 16.0, 8.8, 8.5. MS (ESI) m/z: 900.6 (MNa^+, 100). MS (FAB) m/z: 878.5 (MH^+, 100). HRMS (FAB): calcd for $C_{52}H_{72}N_5O_7$ (M + H), 878.5432; found, 878.5430.

Determination of Galectin Binding by ELISA. A 96-well microtiter plate were coated with asialofetuin (Sigma; 10 μg/mL; 100 μL/well) for 3 h at 37 °C and then washed three times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-T). The mixture containing equal volumes of the Gal-1 concentration (Sigma; 1:50-fold dilution in 1% bovine serum albumin (BSA) in PBS-T) and serially diluted glycoinhibitors (**21a**, **38**−**40**, **42**, and lactose), which had been preincubated for 30 min at room temperature, were added in duplicates to the wells (100 μL/well). The wells were incubated for 1 h and then washed three times with PBS-T at 37 °C. Anti-Gal-1 mouse monoclonal antibody (Novocastra Laboratories Ltd., U.K.; 1:100-fold dilution in 1% BSA−PBS-T; 100 μL/well) was added to each well and incubated for 1 h at 37 °C. After three washings with PBS-T, antimouse IgG (γ-chain specific) alkaline phosphate conjugate (Sigma; 1:1000-fold dilution in 1% BSA−PBS-T, 100 μL/well) was added to each well and kept for 1 h at room temperature. Each well was washed three times with PBS-T, and the substrate 4-nitrophenyl phosphate disodium salt hexahydrate (Sigma; 1 mg/mL dissolved in 10% diethanolamine buffer containing 0.5 mM magnesium chloride, pH 9.8, 100 μL/well) was added to each well. After 1 h at room temperature, the plate was read on a microplate autoreader (EL311s, Bio-Tek Instruments Inc.) at 405 nm. Experiments were repeated at three different time points, and the data were plotted on the Microsoft Excel 2000 software. The IC_{50} values for compounds (**21a**, **38**−**40**, and **42**)- and lactose were determined.

Acknowledgment. This work was supported by the National Institutes of Health (CA 55791) and the shared resources of the Roswell Park Cancer Center Support Grant P30CA16056. The mass spectrometry analyses were performed at the Biopolymer Facility of our institute and the Mass Spectrometry facility, Michigan State University, East Lansing, MI.

Supporting Information Available: The experimental details and the characterization (NMR, HRMS, and C, H, and N analyses) of porphyrin **4**, chlorins **5**−**8**, benzochlorins **9**−**13**, and **27a**; the ^{1}H NMR spectra of compounds **4**−**13**, **17**, **19a**, **19b**, **20**, **21**, **23**, **24**, **26**, **27**, **27a**, **29**, **30**, **35**−**37**, and **43**; ^{13}C NMR spectra of compounds **6**, **7**, **9**−**13**, **19b**, **24**, **27**, **27a**, **30**, **35**, **36**, and **43**; and DEPT-135 NMR spectra of compounds **19b**, **27**, **27a**, **35**, and **36**. The material is available free of charge via the Internet at the http://pubs.asc.org.

JO030280B