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Research Article

Convenient Approach to Access Octa-Glycosylated Porphyrins via "Click Chemistry"

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Easy, quantitative, and one-pot introduction of eight β-lactoside-modules onto a porphyrin-core was achieved through Cu^{+}-catalyzed chemoselective coupling (click chemistry) between a porphyrin carrying eight alkyne-terminals and β-lactosyl azides. The obtained porphyrin-based glycocluster shows not only good water-solubility but also strong/specific lectin-affinity.

1. Introduction

Porphyrin and their derivatives are ubiquitous in nature as essential components of various bio-macromolecules, such as hemoglobin and cytochromes, and various porphyrin-based artificial materials have been developed so far for optical, electrical, chemical, and therapeutic purposes [1–7]. In the potential applications of the artificial porphyrins, the porphyrin-based photodynamic therapeutic (PDT) systems are of quite interest, since porphyrins are nontoxic under dark condition and efficient photosensitizer to produce singlet oxygen ($^1O_2$) [8–13]. The porphyrin-based PDT photosensitizers, however, include some obstacles: that is, their low water solubility and no cell specificity.

To overcome these problems, various porphyrin derivatives having carbohydrate-appendages (glycoporphyrins) have been developed so far [14–22]. Since carbohydrates have many hydroxy groups and the resultant excellent water solubility, introduction of carbohydrate units is expected to improve the water solubility of porphyrin derivatives. Since increasing number of introduced carbohydrate units should result in further improvement of the water solubility, porphyrin-derivatives having multiple carbohydrate-appendages (glycoclusters: the number of introduced carbohydrate units is usually up to 4 in the case of porphyrins) have been developed by many research groups. However, these limited numbers of carbohydrate-appendages still cannot compensate hydrophobicity of porphyrins and only few examples of water-soluble glycoporphyrin have been reported so far. To increase water solubility of the glycoporphyrins, much larger number of carbohydrate units should be introduced into porphyrin scaffolds.

In addition to the increasing water-solubility, introduction of carbohydrate units has another aspect, that is, enhanced cell specificity. It is now well known that carbohydrates exist as components of glycoproteins and glycolipids on cell surfaces and play substantial roles in various molecular recognition events (fertilization, differentiation, cell-cell adhesion, etc.) via specific carbohydrate-protein interactions [23–25]. Many researchers have studied the carbohydrate-protein interactions and revealed that clustered, or multivalent, carbohydrates interact with carbohydrate-binding proteins in a stronger and more specific manner than monomeric carbohydrates do [26]. This phenomenon is now widely recognized as carbohydrate cluster effect and various artificial compounds having clustered carbohydrates have been developed so far [27–32]. For example, oligosaccharide-appended polystyrene is commercially available as a coating reagent of polystyrene dishes for hepatocyte cultivation [33, 34] and oligosaccharide-appended cyclodextrins/cyclophanes are developed as cell-specific drug carriers [35–39]. The porphyrin derivatives having multivalent carbohydrates should, therefore, acquire both well water solubility and excellent cell specificity.

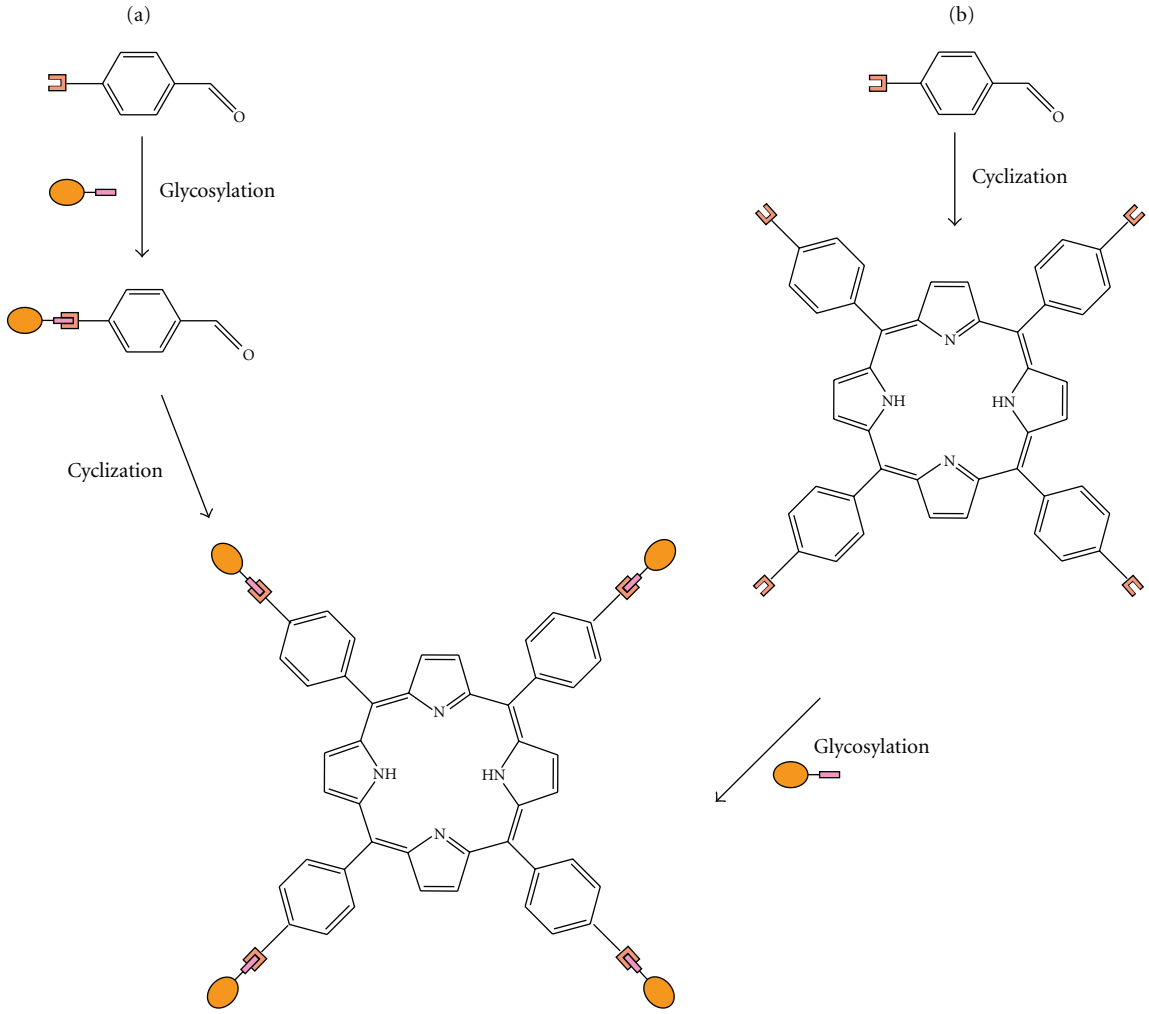


Figure 1: Schematic illustration of traditional synthetic routes toward multiglycosylated porphyrins: (a) cyclization of glycosylated benzaldehydes and (b) glycosylation of porphyrins.

As mentioned earlier, much larger number of carbohydrate units should be introduced into porphyrin scaffolds to increase their water solubility and cell specificity. This strategy, however, also increases synthetic difficulty. Synthetic strategy to access glycoporphyrins can be divided into two categories: (1) cyclization of glycosylated benzaldehydes [14–19] and (2) glycosylation of porphyrins [20–22] (Figure 1). In the first strategy, benzaldehyde derivatives having carbohydrate-appendages are synthesized and then coupled with pyrrole to be cyclized into the corresponding glycoporphyrins. This strategy suffers from low yields in the cyclization step owing to steric hindrance of carbohydrate-appendages. Larger number of introduced carbohydrates results in enhanced steric hindrance and the resultant lower yields. On the other hand, the later strategy includes cyclization of benzaldehyde having reaction points (hydroxy-, carboxy-, haloalkyl-groups, etc.) and the following multiglycosylation onto the multiple reaction points. This strategy is free from the steric hindrance in the cyclyzation step, but one-pot introduction of a large number of carbohydrate units also suffers from low total coupling yields. To develop easy, general, and quantitative strategy for introducing multiple copies of carbohydrate units is essential to establish practical PDT systems.

Recently, Cu^{+}-catalyzed chemoselective couplings between organic azides and terminal alkynes have attracted increasing research interest owing to its convenient, quick, and quantitative reaction [40–42]. This chemoselective coupling is now, therefore, recognized as "click chemistry" and is used for various applications including chemical modifications of self-assembled monolayers (SAMs), ligation between polymer strands, and so forth [43–45]. These excellent works encouraged us to establish an easy two-step strategy based on the click chemistry for one-pot introduction of large number of carbohydrate units into porphyrin scaffolds: (1) synthesis of porphyrins carrying multiple but small alkyne-terminals and (2) chemoselective and quantitative introduction of sugar azide onto the porphyrins. Since the click chemistry is

highly chemoselective and tolerant for various reaction media, this approach should accelerate the development of glycoporphyrin library to establish practical porphyrin-based photosensitizers. We, herein, report not only synthetic details of octa-β-lactosylated porphyrin but also its water solubility, spatial structure, and lectin affinity.

2. Results and Discussion

The porphyrin core having eight alkyne-terminals was synthesized from 3,5-dihydroxy-benzaldehyde via Williamson coupling with propargyl bromide and the subsequent Lindsey condensation with pyrrole (Scheme 1). The introduction of eight β-lactoside modules was attained by treating the porphyrin core with β-lactosyl azide in DMSO-containing $CuBr_2$, ascorbic acid, and propylamine. After dialysis (water, MWCO500) followed by lyophilization, octa-β-lactosylated porphyrinato-copper (PorCu-Lac_8) was obtained as brownish purple powder. We firstly tried to obtain the chemical evidence through NMR measurements, ^{1}H NMR spectrum of PorCu-Lac_8 was, however, too noisy to be assignable, possibly owing to its insufficient solubility in pure D_2O and/or the magnetic property of inserted Cu^{2+}. The chemical evidence was, therefore, obtained from MALDI-TOF-MS showing a predominant peak at 4045.13 ($[M+Na]^+$, calc. 4045.23 for PorCu-Lac_8, Figure 2(a)). No peak assignable to porphyrins having smaller numbers of β-lactoside-appendage (by-products resulting from incomplete coupling) was observed in the spectrum. These spectral data clearly indicate that the porphyrin having eight alkyne-terminals is quantitatively converted into PorCu-Lac_8. Such quantitative introduction of carbohydrate-modules onto presynthesized porphyrin was never obtained through the conventional coupling strategies, such as amide- and ether-couplings. We would like to also to emphasize the great advantage of this strategy; that is, the simple dialysis of the reaction mixture is enough to produce the porphyrin having large number of carbohydrate-appendages in a chemically pure form.

We also tried to support this quantitative conversion through chromatographic analysis of the product. The RP-HPLC analysis of PorCu-Lac_8, however, gave multiple broad peaks, possibly arising from the aggregation of PorCu-Lac_8 in the HPLC condition. We, therefore, carried out per acetylation of PorCu-Lac_8 and the per acetylated PorCu-Lac_8 was analyzed on RP-HPLC column. As shown in Figure 2(b), one main peak (c) along with two minor peaks (a) and (b) is observed in the RP-HPLC chart, supporting the high conversion yield.

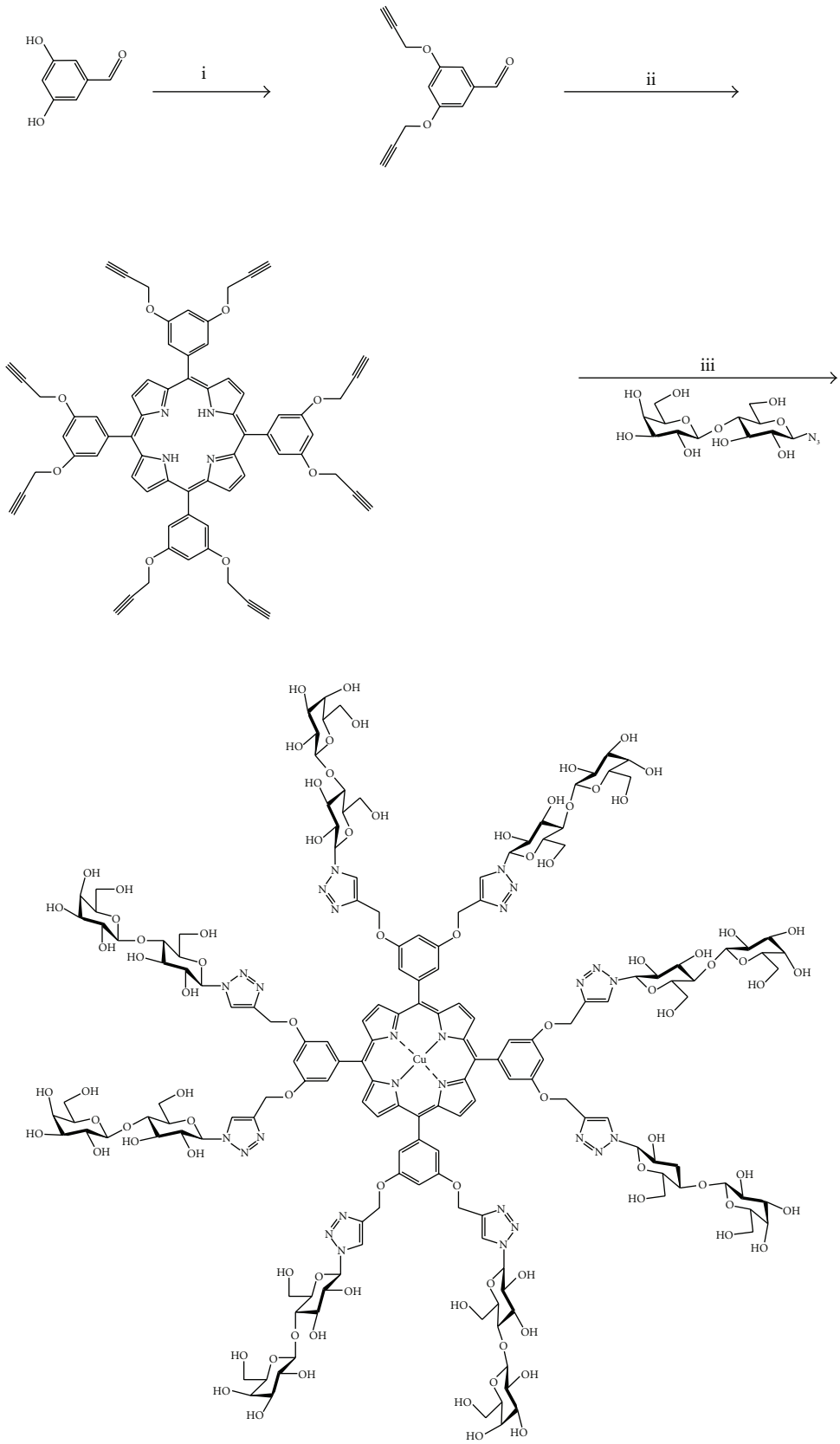
Through this chemoselective coupling, insertion of Cu^{2+} into the porphyrin ring was simultaneously occurred and no peak assignable to the corresponding free-base porphyrin carrying eight β-lactoside-appendages was observed. To clarify which process (i.e., coupling or insertion) firstly occurs, we again carried out the same coupling reaction with 0.2 eq. of $CuBr_2$. The MALDI-TOF-MS analysis of the product showed two peaks assignable to the substrate and Cu^{2+}-inserted substrate, respectively, and no peak assignable to PorCu-Lac^8 was observed. These data indicate that the Cu^{2+} is firstly consumed by insertion reaction, and then excess Cu^{2+} is reduced by ascorbic acid to catalyze the coupling reaction.

To elucidate water solubility of PorCu-Lac_8, we measured its UV-vis spectra in water/DMSO mixed solvent system with various water contents. As shown in Figure 3, PorCu-Lac_8 showed sharp Soret-band peak at 422 nm in DMSO-rich solvent system (water = 2.0% v/v), indicating the single molecular dispersion of PorCu-Lac_8. Although the UV-vis spectrum of PorCu-Lac_8 remains unchanged when water contents are below 50%, intensity of the Soret band gradually decreases with an increasing water-content when water content is above 50%, and a new peak attributable to H-aggregated PorCu-Lac_8 simultaneously appears at 405 nm. We would like to, however, emphasize that the original peak arising from monomeric PorCu-Lac_8 is still observed even in pure water, indicating good water solubility of PorCu-Lac_8. This water solubility of PorCu-Lac_8 should arise from eight β-lactoside-appendages introduced onto metapositions that form a thick hydrophilic shell structure to effectively shield the hydrophobic surface of porphyrin-core. (We also synthesized another octa-β-lactosylated porphyrin with a different spacer structure through the Cu^+-catalyzed coupling between porphyrin having eight azide-terminals and propargyl β-lactoside. Although this compound was used to investigate whether structural variation at the spacer moiety has any affects on properties (water solubility and lectin affinity) of glycosylated porphyrins, negligible differences can be observed between the properties of these two octa-β-lactosylated porphyrins (data now shown)).

To confirm our assumption, we synthesized a porphyrin derivative having four β-lactoside-appendages (PorCu-Lac_4), in which each β-lactoside-appendage is attached onto para-position of phenyl-appendages. Although this tetraglycosylated analogue is also well soluble in DMSO, an addition of only small amount of water induces strict broadening and blue-shift of Soret band of its UV-vis spectrum, suggesting its strong aggregation via hydrophobic interactions between the exposed porphyrin cores.

Atomic force microscopic (AFM) images of PorCu-Lac_8 cast from aqueous solution onto mica substrates also strongly support its good water-solubility. As shown in Figure 4(a), dot-like objects having a similar diameter are observed in the AFM images. No extremely large-dot arising from the porphyrin aggregates was observed, showing the good solubility of PorCu-Lac_8 in aqueous media. It should be noted that cross section profiles of the dot-like objects (Figures 4(b) and 4(c)) have height of ca. 2 nm with trapezoidal or hollow shapes. This unique trapezoidal/hollow shape resembles the spatial structure of PorCu-Lac_8 having thin porphyrin center and bulky carbohydrate-periphery, supporting that the each dot-like object observed in AFM images is arising from single PorCu-Lac_8 molecule.

Spatial structure of PorCu-Lac_8 was also assessed in detail through molecular dynamics (MD) calculation. The molecule was constructed and then, dynamics (100 000 steps, CHARMm, 300 K, NVT, GBSW solvent model) was carried out after minimization, heating, and equilibration



Scheme 1: Synthesis of octa-β-lactosylated porphyrinatocopper (PorCu-Lac$_8$): (i) propargyl bromide, K_2CO_3, DMF, r.t., (ii) pyrrole, TFA, CH_2Cl_2, r.t., then *p*-chloranil, r.t., (iii) $CuBr_2$, ascorbic acid, β-lactosyl azide, propylamine, r.t.

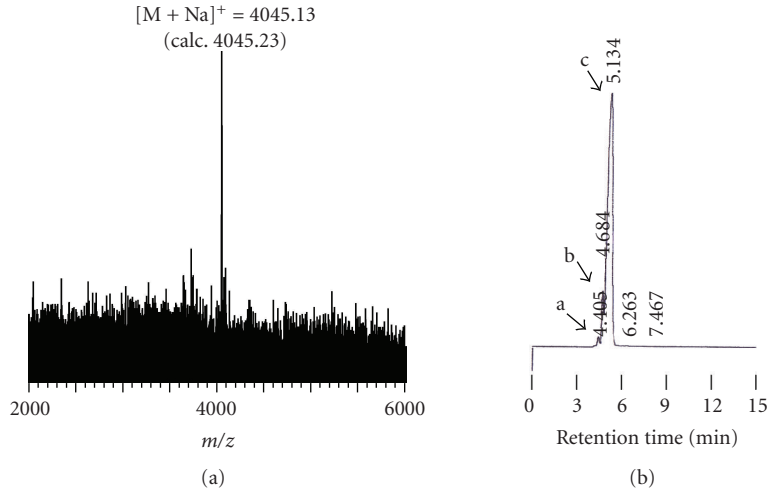


FIGURE 2: (a) MALDI-TOF-MS of PorCu-Lac$_8$: obs. = 4045.13, calc. ([M+Na]$^+$) = 4045.23, and (b) RP-HPLC chart of the per-acetylated PorCu-Lac$_8$ on SHISEIDO CAPCELL PAK C18 (Conditions: 40°C, Flow rate = 2 mL/min, H_2O/CH_3CN = 13/87 isocratic, λ = 254 nm).

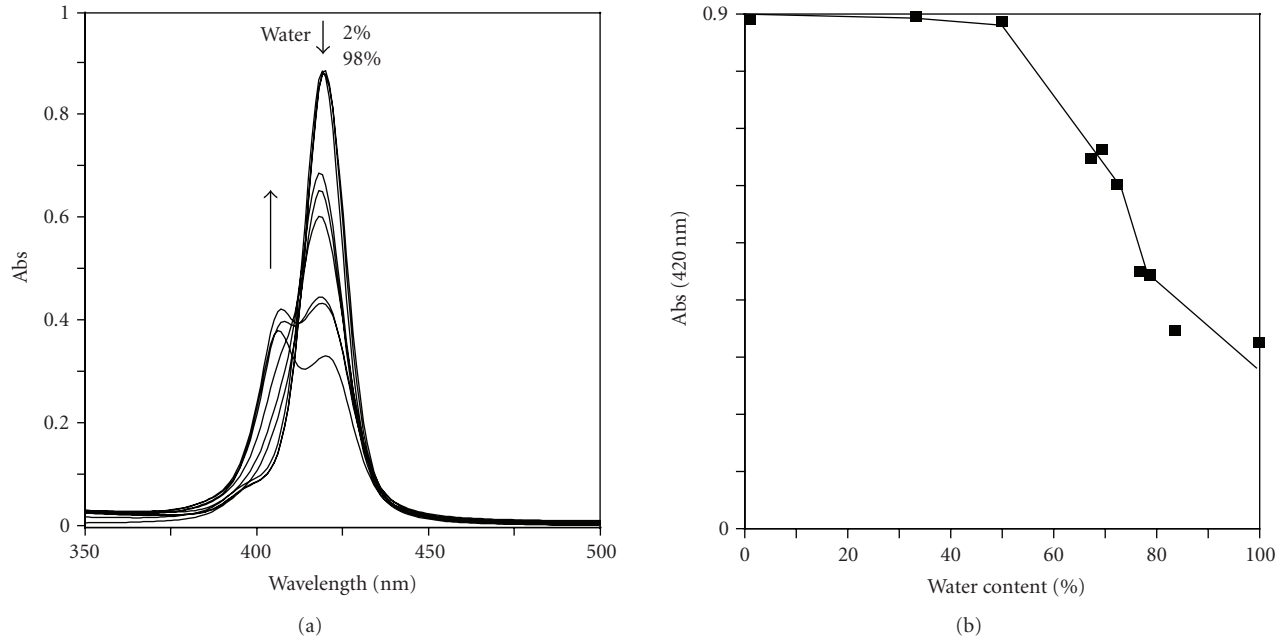


FIGURE 3: (a) UV-vis spectra of PorCu-Lac$_8$ in DMSO/water mixed solution systems and (b) relationship between absorption intensity (420 nm) and water content of the media: [PorCu-Lac$_8$] = 4.9×10^{-7} M, 20°C, d = 1 cm.

processes. The most stable conformation during the dynamics process is shown in Figure 5, in which PorCu-Lac$_8$ takes spherical structure with the porphyrin-core embedded into the densely packed carbohydrate shell. This closely packed carbohydrate-appendages should effectively shield the hydrophobic porphyrin-core from the media and then, improve the water solubility of PorCu-Lac$_8$.

Lectin-affinity of PorCu-Lac$_8$ was assessed based on the fluorescence titration assay using fluorescein isothiocyanate (FITC)-labeled lectins (Figure 6). Fluorescent intensity of FITC-RCA$_{120}$ (*Recinus comunus agglutinin*, Lac-specific) was suppressed by addition of PorCu-Lac$_8$, and the dissociation constant (K_d) was estimated to 3.2×10^{-5} M by computational curve fitting, indicating their strong binding. On the other hand, no such fluorescence spectral change was observed for FITC-ConA (Concanavalin A, α-Glc/Man-specific). These fluorescent spectroscopic data indicate strong and specific lectin-affinity of PorCu-Lac$_8$. The well-shielded porphyrin core of PorCu-Lac$_8$ is also important for its specific lectin-affinity: that is, PorCu-Lac$_4$ having an exposed hydrophobic porphyrin core interacts with not only RCA$_{120}$ but also ConA in nonspecific manner, presumably owing to hydrophobic interaction with hydrophobic protein residues.

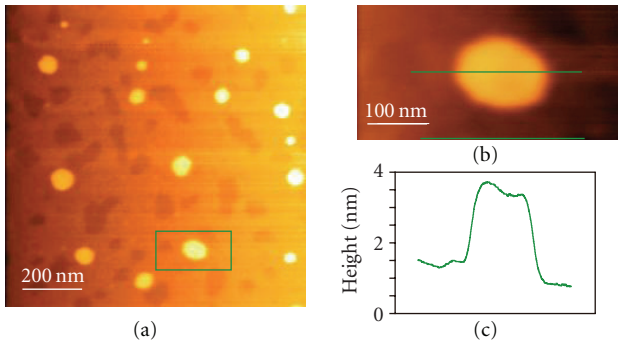


Figure 4: (a) AFM image of PorCu-Lac$_8$ cast on mica substrate from aqueous solution and (b) the expanded image and (c) cross-section profile of the dot-like object.

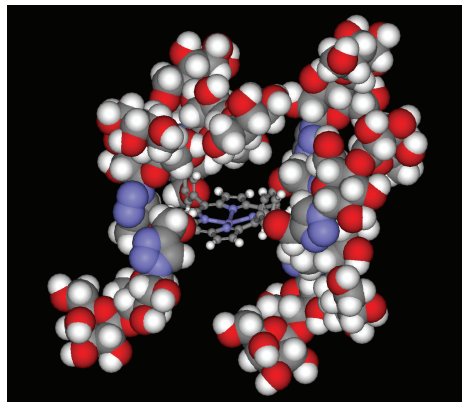


Figure 5: The most stable conformation of PorCu-Lac$_8$ during the dynamics (CHARMm, 300 K, NVT, GBSW): in this figure, porphyrin-core including four phenyl spacers and carbohydrate-appendages including triazole-spacers are depicted in ball-and-stick and CPK models, respectively, for clear presentation.

3. Conclusion

We established easy and quick strategy toward multiglycosylated porphyrins by using click chemistry. Through this strategy, not only the Cu^+-catalyzed introduction of multiple oligosaccharide units but also the insertion of Cu^{2+} into the porphyrin scaffold was simultaneously occurs. Porphyrinatocopper derivatives have, however, poor singlet oxygen productivity and, therefore, are not suitable for PDT photosensitizers. Our research effort is now paid to a metallation of the porphyrin having eight alkyne-terminals by Zn^{2+} prior to the Cu^+-catalyzed introduction of oligosaccharide units. The resultant porphyrinatozinc-based glycoclusters would be easily converted by treating with dilute acids into the corresponding free base porphyrins with high singlet oxygen productivity. The results will be reported elsewhere in due course.

4. Experimental

4.1. General. 1H and 13C NMR spectra were acquired on a JEOL AL300 (JEOL DATUM, Ltd) in CDCl3 at 300 MHz. The chemical shifts were reported in ppm (δ) relative to Me4Si. IR spectra were recorded on a JASCO FT/IR-4100 Fourier transform infrared spectrometer (JASCO Co., Ltd). Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were recorded on SHIMAZU AXIMA-CFR+ (SHIMAZU, Ltd). Fluorescence spectra were recorded on FP-6200 spectrofluorometer (JASCO, Co. Ltd.). Atomic force microscopic (AFM) images were acquired on SII Nanonavi E-sweep (SII nanotechnology, Inc.) using SI-DF40 as a cantilever. Molecular dynamic calculation was carried out on Discover Studio 2.1 (Accerlys Software Inc.). Silica gel 60 N (particle size 63–210 μm) for column chromatography was purchased from KANTO CHEMICAL Co., INC. Thin layer chromatography (TLC) was carried out with Merck TLC aluminum sheets precoated with silica gel 60 F254. All other chemicals were purchased from Wako Pure Chemical Industries Ltd. or Kishida Chemicals Co. Ltd.

4.2. Synthesis of 3,5-Dipropargyloxy-Benzaldehyde. However, 3,5-Dihydroxy-benzaldehyde (0.10 g, 0.74 mmol) and K2CO3 (3.5 g, 25 mmol) were added to *N*,*N*-dimethylformamide (DMF) and then the resultant mixture was stirred at 60∘C for 30 minutes. After cooling down to the ambient temperature, propargyl bromide (0.26 g, 2.2 mmol) was added and the reaction mixture was stirred for overnight. The resultant mixture was diluted with ethyl acetate and washed with water and NaCl-saturated water several times. The organic layer was dried over anhydrous MgSO4, filtered, and concentrated to dryness to obtain 3,5-dipropargyloxy-benzaldehyde as white powder in 42% yield: [M+H]+ = 215.12 (calc. 215.06); 1H NMR (CDCl3, TMS): 9.92 (s, 1H), 7.14 (d, J = 2.4 Hz, 2H), 6.88 (t, J = 2.3 Hz, 1H), 4.74 (d, J = 2.4 Hz, 4H), 2.56 (t, J = 2.3 Hz, 2H); 13C NMR (CDCl3, TMS): 191.503, 159.105, 138.377, 108.732, 78.122, 77.208, 76.235.

4.3. Synthesis of 5,10,15,20-Tetra-3′,5′-Dipropargyloxyphenyl-Porphyrin. To 3,5-dipropargyloxy-benzaldehyde (67 mg) and pyrrole (21 mg) in dry CH2Cl2 (200 mL), trifluoroacetic acid (30 μL) was added. The stirring was continued for 15 hours at room temperature and then *p*-chloranil (92 mg) was added. After additional 1 hour stirring, triethylamine (65 μL) was added and the resulting mixture was evaporated to dryness. Insoluble materials were separated through silica-gel-packed column using chloroform as an eluent. The fractions were combined, evaporated, and purified by chromatography on a silica-gel column (30 cm long; 3 cm i.d.; hexane-ethyl acetate = 2 : 1 in v/v) to give 5,10,15,20-tetra-3′,5′-dipropargyloxyphenyl-porphyrin as a purple powder (7.9%): [M+Na]+ = 1048.88 (calc. 1048.11); 1H NMR (CDCl3, TMS): 8.96 (s, 8H), 7.52 (d, J = 2.4 Hz, 8H), 7.07 (t, J = 2.3 Hz, 4H), 4.86 (d, J = 2.4 Hz, 16H), 2.60 (t, J = 2.4 Hz, 8H), −2.87 (brs, 2H); 13C NMR (CDCl3, TMS): 156.805, 144.028, 131.133, 119.413, 115.343, 102.205, 78.394, 77.216, 75.988, 56.224.

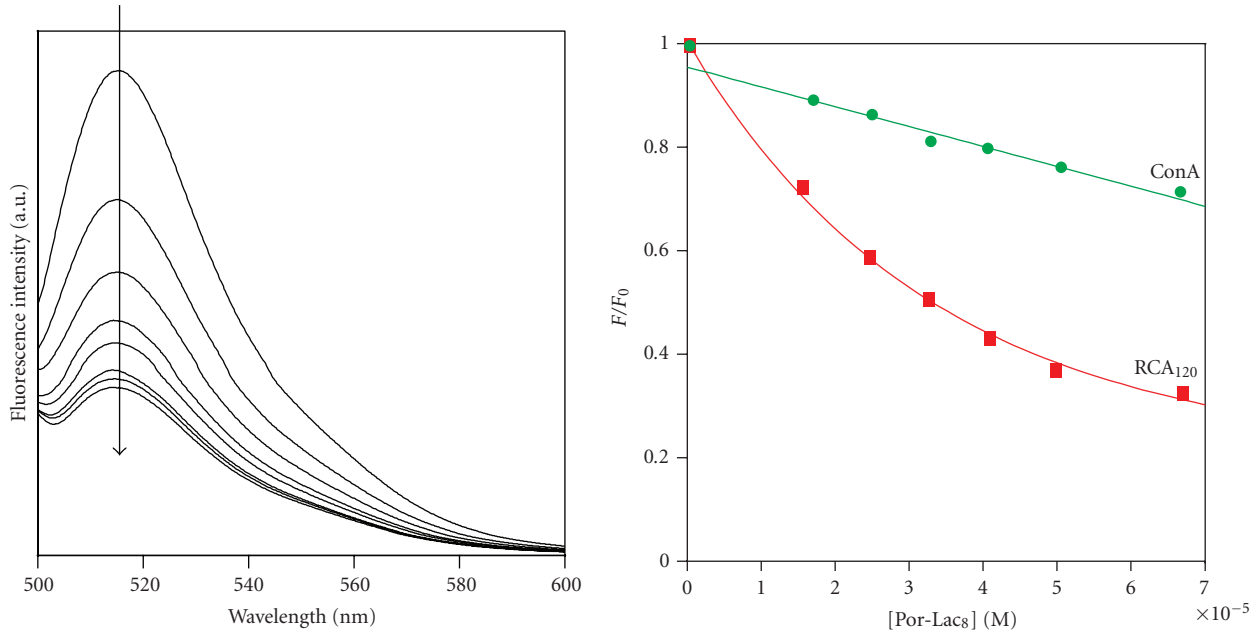


FIGURE 6: (a) Fluorescence spectra of FITC-RCA_{120} with increasing concentration of PorCu-Lac_8 and (b) plots of maximum fluorescent intensity of FITC-RCA_{120} and FITC-ConA against the concentration of PorCu-Lac_8: Temp = 25°C, Ex = 490 nm, 20 mM Tris-HCl (pH = 7.2), [$CaCl_2$] and [$MnCl_2$] = 100 mM.

4.4. Synthesis of Porphyrinatocopper Having Eight β-Lactoside Appendages (PorCu-Lac_8). However, 5,10,15,20-Tetra-3′,5′-dipropargyloxyphenyl-porphyrin (10 mg), β-lactosyl-azide (68 mg), CuBr2 (5.0 mg), ascorbic acid (6.0 mg), *n*-propylamine (1.0 mg) were added into DMSO in small vessel and the resultant reaction mixture was kept at room temperature for overnight. The resultant mixture was dialyzed (MWCO500, water) and lyophilized to give PorCu-Lac8 as brownish purple powder in 14.6% yield: [M+Na]+ = 4045.13 (calc. 4045.23); IR (KBr, cm-1) 2970.73 (−OH).

Acknowledgment

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