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.

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1. Introduction

Porphyran and their derivatives are ubiquitous in nature as essential components of various bio-macromolecules, such as hemoglobin and cytochromes, and various porphyran-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 (\( ^1{\text{O}}_2 \)) [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.
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 cyclization 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 Lindlsey 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 CuBr2. MALDI-TOF-MS analysis of the product was again carried out the same coupling reaction with 0.2 eq. of CuBr2. The MALDI-TOF-MS analysis of the product showed two peaks assignable to the substrate and Cu2+-inserted substrate, respectively, and no peak assignable to PorCu-Lac8 was observed. These data indicate that the Cu2+ is firstly consumed by insertion reaction, and then excess Cu2+ is reduced by ascorbic acid to catalyze the coupling reaction.

To elucidate water solubility of PorCu-Lac8, we measured its UV-vis spectra in water/DMSO mixed solvent system with various water contents. As shown in Figure 3, PorCu-Lac8 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-Lac8. Although the UV-vis spectrum of PorCu-Lac8 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-Lac8 simultaneously appears at 405 nm. We would like to, however, emphasize that the original peak arising from monomeric PorCu-Lac8 is still observed even in pure water, indicating good water solubility of PorCu-Lac8. This water solubility of PorCu-Lac8 should arise from eight β-lactoside-appendages introduced onto meta-position of 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-Lac4), in which each β-lactoside-appendage is attached onto para-position of phenyl-appendages. Although this tetracyglycosylated analogue is also well soluble in DMSO, an addition of 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-Lac8 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 aggreates was observed, showing the good solubility of PorCu-Lac8 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-Lac8 having thin porphyrin center and bulky carbohydrate-periphery, supporting that the each dot-like object observed in AFM images is arising from single PorCu-Lac8 molecule.

Spatial structure of PorCu-Lac8 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-\(\beta\)-lactosylated porphyrinocopper (PorCu-Lac8): (i) propargyl bromide, K\(_2\)CO\(_3\), DMF, r.t., (ii) pyrrole, TFA, CH\(_2\)Cl\(_2\), r.t., then \(p\)-chloranil, r.t., (iii) CuBr\(_2\), ascorbic acid, \(\beta\)-lactosyl azide, propylamine, r.t.
The most stable conformation during the dynamics process is shown in Figure 5, in which PorCu-Lac₈ 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-Lac₈.

Lectin-affinity of PorCu-Lac₈ was assessed based on the fluorescence titration assay using fluorescein isothiocyanate (FITC)-labeled lectins (Figure 6). Fluorescent intensity of FITC-RCA₁₂₀ (Recinus communis agglutinin, Lac-specific) was suppressed by addition of PorCu-Lac₈, and the dissociation constant \( K_d \) was estimated to \( 3.2 \times 10^{-5} \text{M} \) 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-Lac₈. The well-shielded porphyrin core of PorCu-Lac₈ is also important for its specific lectin-affinity: that is, PorCu-Lac₄ having an exposed hydrophobic porphyrin core interacts with not only RCA₁₂₀ but also ConA in nonspecific manner, presumably owing to hydrophobic interaction with hydrophobic protein residues.
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 (Accelrys 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, 75.988.

4.3. Synthesis of 5,10,15,20-Tetra-3′,5′-Dipropargyloxyphe-nyl-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′-dipropargyloxyporphyrin 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, 77.122, 77.208, 76.235.

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 Cu2+ into the porphyrin scaffold was simultaneously occurs. Porphyrinatozinc-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 Zn2+ prior to the Cu+-catalyzed introduction of oligosaccharide units. The resultant porphyrinatozinc-based glyoclusters 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.
4.4. Synthesis of Porphyrinatocopper Having Eight β-Lactoside Appendages (PorCu-Lac8).

However, 5,10,15,20-Tetrac-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 14.7% yield: [M+Na]+ = 4045.13 (calc. 4045.23); IR (KBr, cm⁻¹) 2970.73 (−OH).

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References


