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'Click chemistry' on polysaccharides: a convenient, general, and monitorable approach to develop (1→3)-β-D-glucans with various functional appendages

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Abstract—(1→3)-β-D-Glucans having various functional appendages (lactoside, ferrocene, pyrene, and porphyrin) can be prepared in an convenient, quantitative, and regioselective manner through regioselective bromination–azidation of curdlan to afford 6-azido-6-deoxycurdlan followed by chemoselective [3+2]-cycloadditions with various functional modules bearing a terminal alkyne group. The ability to monitor reaction conversions is an additional advantage of this synthetic approach over the conventional direct modifications on polysaccharides; the reaction can be readily monitored based on the intensity of azido peaks in the in situ attenuated total reflection infrared spectra.

Keywords: (1→3)-β-D-Glucan; Click chemistry; Direct modification; Regioselective modification; Attenuated total reflection infrared spectroscopy

1. Introduction

Polysaccharides are potential candidates for 'eco-materials', since they are biodegradable and abundant in Nature. Their inherent chirality and helical superstructures also emphasize their potential as scaffolds for chiral materials. Polysaccharides are also attractive research targets as 'bio-materials', and many polysaccharides found in herbal medicines exhibit pharmaceutical effects.[1,2] Investigations on polysaccharide-based materials are thus of interest in various fields (material science, pharmaceutics, 'green' chemistry, and others). Chemical modification of native polysaccharides, a prerequisite for access to polysaccharide-based functional materials, is still troublesome. The hydroxyl groups of polysaccharides have similar reactivity toward electrophiles, making regioselective and quantitative reactions difficult. Much research effort has been devoted to exploit chemical/enzymatic strategies to obtain polysaccharide derivatives having various functionalities at desired positions. For example, enzymatic polymerization of chemically modified monosaccharides to afford the corresponding polysaccharides (bottom-up approach) has high potential,[3,4] but tedious synthetic routes for the modified monosaccharides and their lowered affinity (especially, those having large substituents) toward synthase enzymes hinder their wide application. Convenient, general, quantitative, and regioselective methods useful for direct modifications of native polysaccharides are, therefore, sought for obtaining suitable polysaccharide-based materials.

Recently, a Cu(I)-catalyzed chemoselective coupling between organic azides and terminal alkynes has attracted attention owing to its convenient, quick, and

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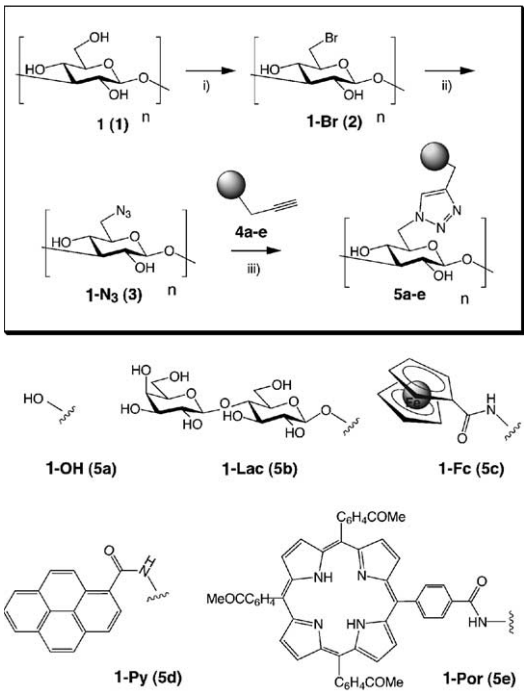

Scheme 1. Chemoselective coupling between 6-azido-6-deoxycurdlan and alkyne-terminated functional modules: (i) triphenylphosphine, DMF, LiCl, rt, 3 h, and then, carbon tetrabromide, 60 °C, 24 h, (ii) sodium azide, Me_2SO, 80 °C, 36 h, (iii) alkyne-terminated functional modules, $CuBr_2$, ascorbic acid, propylamine, rt, 12 h, Me_2SO or NMP.

quantitative reaction.[5–7] This chemoselective coupling is termed 'click chemistry' and is used for various applications, including chemical modifications of self-assembled monolayers (SAMs), and ligation between polymer strands.[8–10] Such applications encouraged us to establish 'click chemistry with polysaccharides', namely, chemoselective coupling between azido-appended polysaccharides and alkyne-terminated functional modules, to develop various polysaccharide-based materials. Herein we report one such successful example that allows various functional modules (β-lactoside, ferrocene, pyrene, and porphyrin) to be readily introduced into a linear (1→3)-β-D-glucan through our protocol, in a regioselective and quantitative manner (Scheme 1). Since (1→3)-β-D-glucans have interesting structural features (such as a rigid and triple-stranded helical structure),[11–13] pharmaceutical effects (anticancer activity),[14] and binding properties (with polynucleotides, single-walled carbon nanotubes),[15–17] developments of these (1→3)-β-D-glucan derivatives should be useful for preparing various chiral or bio-materials and materials of pharmacological interest.

2. Results and discussion

Native linear (1→3)-β-D-glucan (curdlan, **1**) was first converted into 6-bromo-6-deoxycurdlan (**1**-Br) according to the literature, by activation of primary hydroxyl groups with triphenylphosphine followed by bromination with carbon tetrabromide (Scheme 1).[18] Subsequent azidation using sodium azide in Me_2SO (80 °C, 36 h) afforded 6-azido-6-deoxycurdlan (**1**-N_3). Quantitative and exclusive conversion of primary hydroxyl groups into azido groups was confirmed by the ^{13}C NMR spectrum of the product (Fig. 1), in which peaks assignable to the hydroxymethyl-group (–CH_2OH, 60.90 ppm) and bromomethyl-group (–CH_2Br, 44.27 ppm) had disappeared and that of the azidomethyl-group (–CH_2N_3, 50.72 ppm) appeared. Furthermore, no peak arising from an elimination product (5,6 alkene) was observed in the ^{13}C NMR spectrum, indicating the highly homogeneous structure of **1**-N_3.

The chemoselective couplings between **1**-N_3 and alkyne-terminated functional modules were successfully carried out in Me_2SO containing $CuBr_2$, ascorbic acid, and bases (triethylamine, propylamine, ammonia) at ambient temperature, where we used various alkyne-terminated modules having (1) a β-lactoside (a strong ligand for asialo-glycoprotein receptors on hepatocytes, **4b**), (2) ferrocene (a redox-active unit, **4c**), (3) pyrene (a chromophore with strong fluorescence, **4d**), and (4) porphyrin (an essential component of native light-harvesting and oxygen-binding systems, **4e**). Subsequent dialysis and lyophilization, followed by washing with solvents afforded various **1**-derivatives [**1**-Lac (**5b**), **1**-Fc (**5c**), **1**-Py (**5d**), and **1**-Por (**5e**), respectively]. Along with IR spectra showing no residual azide peak (Fig. 2), ^{13}C NMR spectra clearly showed quantitative conversion of **1**-N_3 into the resultant derivatives (Fig. 3), as demonstrated by (1) no residual signal from azidomethyl groups, (2) new peaks assigned to the appended functional modules, (3) two new peaks (at 145 and 124 ppm) assigned to a 1,4-triazole-linker, and (4) no other unassignable peak. The peaks assigned to the (1→3)-β-D-glucan main chain are significantly broadened, presumably owing to restricted molecular motion of these polysaccharides bearing bulky appendages.

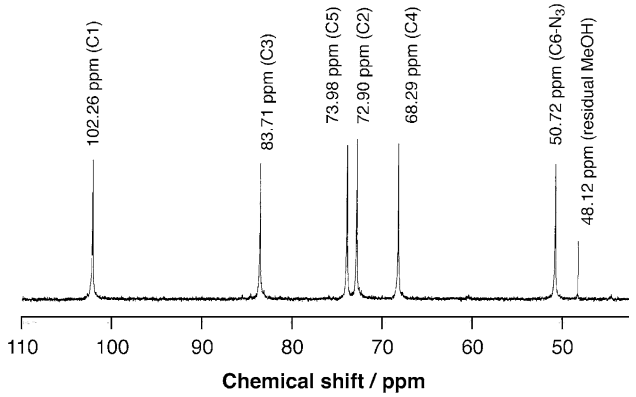

Figure 1. ^{13}C NMR spectrum of **1**-N_3: Me_2SO-d_6, 60 °C.

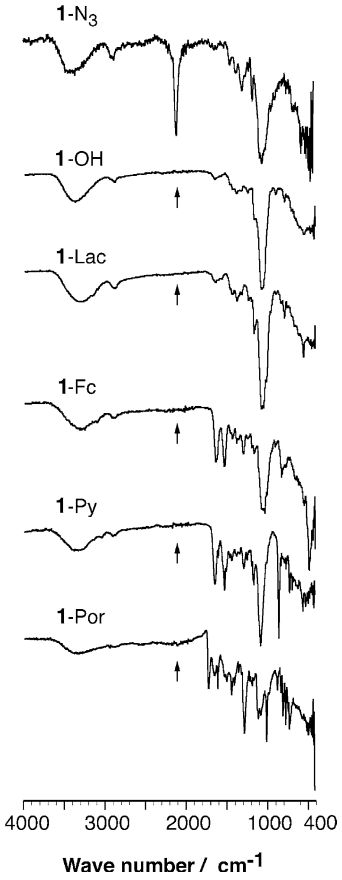

Figure 2. FTIR spectra of **1**-N_3, **1**-OH, **1**-Lac, **1**-Fc, **1**-Py, and **1**-Por: dry powder.

Although we could not obtain a good ^{13}C NMR spectrum of **1**-Por due to poor solubility, (1) no residual azido peak in its IR spectrum and (2) a molecular ion-peak (obsd 1076.01) of a porphyrin-modified glucose unit (calcd 1075.36) in a MALDI-TOF-MS spectrum after acidic degradation of **1**-Por strongly support quantitative coupling. It should be emphasized that these extremely bulky modules (Lac, Fc, Py, and Por) are unlikely to be introduced into polysaccharides through the enzymatic bottom-up strategies using modified monosaccharides having these substituents. Furthermore, it should be noted that this reaction can be carried out in various polar organic solvents (Me_2SO, DMF, NMP), emphasizing the advantages of this approach for developing various functionalized polysaccharide derivatives, since they are excellent solvents for a wide range of organic reagents, so that various functional modules can be, used in this approach (Chart 1).

Furthermore, facile in situ monitoring of the reaction by using attenuated total reflection-infrared (ATR-IR) spectroscopy is feasible, since azido functionalities show a clear and strong IR peak at around 2100 cm^{-1}. This is a clear advantage of this approach over conventional chemical modification of polysaccharides, where monitoring is difficult. For example, the coupling between

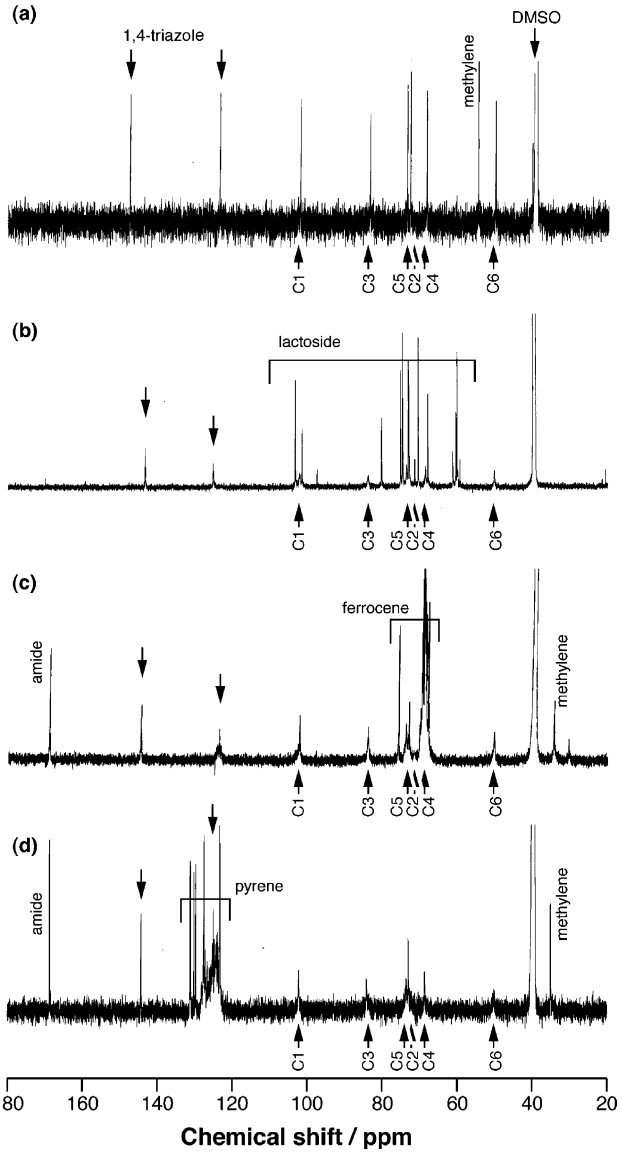

Figure 3. ^{13}C NMR spectra of (a) **1**-OH, (b) **1**-Lac, (c) **1**-Fc, and (d) **1**-Py: Me_2SO-d_6, 60 °C.

1-N_3 and an alkyne-terminated lactoside can be readily monitored based on disappearance of the azide peak, as shown in Figure 4a.

This monitorability allows easy tuning (or optimizing) of the reaction conditions. For example, we optimized the reaction conditions using various amines (aqueous ammonia, propylamine, triethylamine), finding that ammonia and propylamine strongly accelerate the triazole formation, and quantitative conversion was achieved within 1 h (Fig. 4b).

In conclusion, linear (1→3)-β-D-glucan derivatives having various functional modules at only the C-6 positions of each glucoside units were readily prepared through chemoselective coupling between alkyne-terminated functional modules and 6-azido-6-deoxycurdlan. The advantages of this strategy include quick (~1 h),

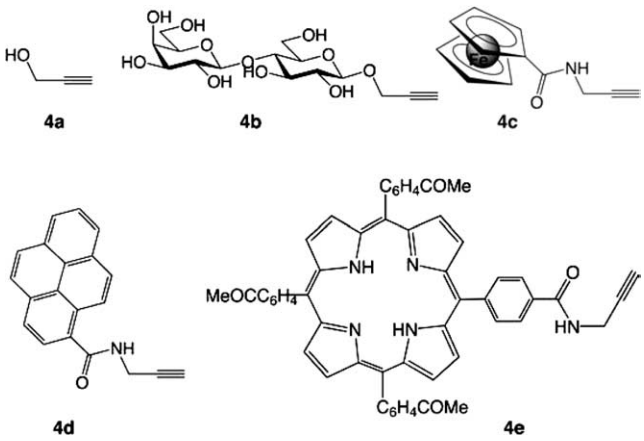

Chart 1. Chemical structures of the functional modules (**4a**, **4b**, **4c**, **4d**, and **4e**) used for the click chemistry.

quantitative (~100%), regioselective (C-6 position), and monitorable (ATR-IR) reaction as well as applicability for bulky modules carrying various functionalities. These advantages should facilitate access to various polysaccharide-based materials. Continued efforts are focused on developments of such polysaccharide-based functional materials as well as applications of the concept toward other polysaccharides (amylose, cellulose, chitin). It has already been confirmed, in preliminary experiments, that the (1→3)-β-D-glucan derivatives are useful for gene carriers (**1**-Lac) as well as redox-active (**1**-Fc) and fluorescent (**1**-Py) 'green' materials.

3. Experimental

3.1. General

^{1}H NMR spectra were acquired on a Bruker DRX600 instrument, using $CDCl_3$, Me_2SO-d_6, or D_2O at 600 MHz. Chemical shifts are reported in ppm (δ) relative to Me_4Si. Matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectra were recorded on PerSeptive Biosystems Voyager-DERP Biospectrometry Workstation. IR spectra were recorded on a Perkin–Elmer Spectrumone Fourier-transform infrared spectrometer with a Universal ATR Sampling Accessory. Silica gel 60 N (particle size 40–50 μm) for column chromatography was purchased from Kanto Chemical Co. Inc. Thin layer chromatography (TLC) was carried out with Merck TLC aluminum sheets pre-coated with silica gel 60 F_{254}. The other chemicals were purchased from Aldrich or Wako.

3.1.1. Hepta-*O*-acetyl-1-*O*-(2-propargyl)-β-lactoside.[19]

Boron trifluoride etherate (10 mL) was added to octa-*O*-acetyl-lactose (10.2 g) and propargyl alcohol (3.0 mL) in anhydrous CH_2Cl_2 (50 mL) at room temperature, and stirring was continued for 40 h under nitrogen. The resulting mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$), and concentrated to dryness. Although the residue was subjected to purification by silica gel column chromatography [hexane only–hexane–EtOAc (1:1)], the R_f values of the starting material and the product were so similar that a pure product could not be obtained. Deacetylation (in a mixture of aqueous ammonia and THF) of this product was thus performed for purification (silica gel, $CHCl_3$–MeOH–water 4:5:1). Reacetylation of the compound was carried out by treating with a pyridine (200 mL)–Ac_2O (150 mL) mixture. An excess of EtOH was added to the resultant mixture to quench the reaction, and the solution was concentrated in vacuo. The mixture was diluted with EtOAc and the organic layer was washed with aqueous 0.5 N HCl and saturated aqueous $NaHCO_3$ several times. The organic layer was dried ($MgSO_4$)

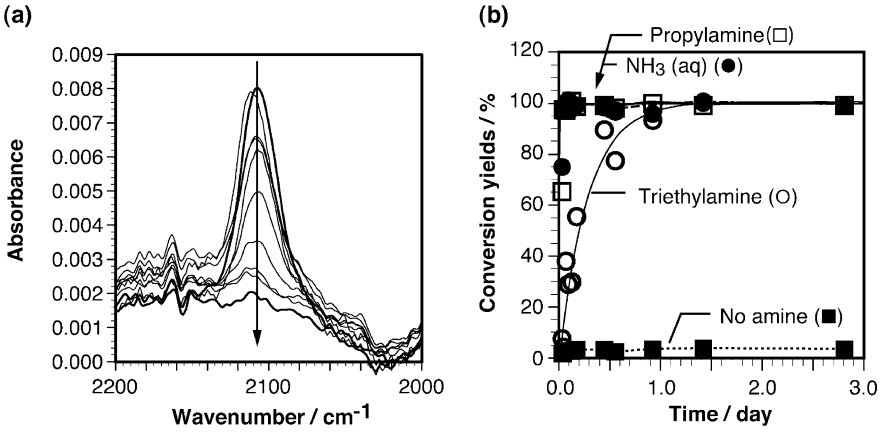

Figure 4. (a) Normalized ATR-IR spectra of the reaction mixture containing triethylamine as a base (a solvent peak (1437 cm^{-1}) was used as a reference (absorbance = 0.04)) and (b) time courses of the conversion ratios estimated from the IR peak intensity at 2100 cm^{-1}: [**1**-N_3] = 30 mg/mL, [propargyl lactoside] = 300 mg/mL, [$CuBr_2$] = 0.45 mg/mL, [ascorbic acid] = 1.35 mg/mL, [bases] = 2.75 v/v%, [H_2O] = 2.75 v/v%, Me_2SO, rt.

and concentrated to dryness. The residue was subjected to purification by silica gel column chromatography [hexane only–hexane–EtOAc (1:4)] to give hepta-*O*-acetyl-1-*O*-(2-propargyl)-β-lactoside as white powder after concentration (4.66 g, 46%). ^{1}H NMR ($CDCl_3$, Me_4Si): 5.35 (d, *J* 2.81 Hz, 1H), 5.23 (t, *J* 9.34 Hz, 1H), 5.11 (dd, *J* 8.31 and 10.3 Hz, 1H), 4.96 (dd, *J* 3.40 and 10.4 Hz, 1H), 4.92 (t, *J* 8.4 Hz, 1H), 4.74 (d, *J* 7.98, 1H), 4.50 (dd, *J* 1.52 and 12.8 Hz, 1H), 4.49 (d, *J* 8.08 Hz, 1H), 4.34 (s, 1H). 4.34 (s, 1H), 4.15–4.07 (m, 3H), 3.88 (t, *J* 6.80 Hz, 1H), 3.82 (t, *J* 9.49 Hz, 1H), 3.64 (m, 1H), 2.47 (s, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.06 (s, 3H), 2.05 (s, 6H), 1.97 (s, 3H); ^{13}C NMR ($CDCl_3$): 170.74, 170.72, 170.54, 170.46, 170.15, 170.13, 169.44, 101.43, 98.27, 78.48, 76.53, 75.88, 73.15, 73.09, 71.70, 71.38, 71.09, 69.49, 67.01, 62.22, 61.22, 56.27, 21.85, 21.26, 21.20, 21.12, 21.04, 21.03, 20.91; $[M+Na]^+ = 697.85$ (calcd 697.21); IR (KBr, cm^{-1}) 1751 (acetyl).

3.1.2. 1-*O*-(2-Propargyl)-β-lactoside (4b). Aqueous ammonia (50 mL) was added to crude hepta-*O*-acetyl-1-*O*-(2-propargyl)-β-lactoside (crude, 6.03 g) in a THF–MeOH mixture (200 mL, 1:1 v/v) and stirring was continued for 2 h at room temperature. The solvent was evaporated and the resultant residue was subjected to purification by silica gel column chromatography ($CHCl_3$–MeOH (4:1)–$CHCl_3$–MeOH–H_2O (4:5:1)) to afford 1-*O*-(2-propargyl)-β-lactoside as white powder (2.50 g, 95%) after a lyophilization. $[M+Na]^+ = 403.62$ (calcd 403.13); ^{1}H NMR (D_2O, HOD): 4.53 (d, *J* 7.93 Hz, 1H), 4.35 (dd, *J* 1.90 and 8.66 Hz, 1H), 4.32 (d, *J* 7.72 Hz, 1H), 3.85 (d, *J* 11.5 Hz, 1H), 3.80 (d, *J* 3.03 Hz, 1H), 3.70–3.59 (m, 5H), 3.55–3.48 (m, 3H), 3.42 (t, *J* 8.11 Hz, 1H), 3.22 (t, *J* 8.28 Hz, 1H). 2.82 (s, 1H); IR (KBr, cm^{-1}) 2973.

3.1.3. *N*-(2-Propargyl)amidoferrocene (4c). *N*,*N*′-Dicyclohexylcarbodiimide (DCC, 1.03 g) and *N*,*N*-dimethylaminopyridine (DMAP, 0.47 g) were added to ferrocenecarboxylic acid (1.00 g) in CH_2Cl_2 (60 mL). The resultant mixture was stirred for 10 min and then, propargylamine (340 μL) was added. After stirring for 1 day, the solvent was removed by evaporation and the residue was dissolved in EtOAc. The organic layer was washed with aqueous 0.5 N HCl and saturated aqueous $NaHCO_3$ several times. The resultant organic layer was dried ($MgSO_4$), and concentrated to dryness. The residue was subjected to purification by silica gel column chromatography [hexane only–hexane–EtOAc (1:4)] to give *N*-(2-propargyl)amidoferrocene as a yellow powder (0.80 g, 69%) after evaporation. ^{1}H NMR ($CDCl_3$, Me_4Si): 5.79 (br s, 1H), 4.69 (s, 2H), 4.37 (s, 2H), 4.24 (s, 5H), 4.18 (d, *J* 5.46, 2H), 2.28 (s, 1H); IR (KBr, cm^{-1}) 1632 and 1543; $[M+Na]^+ = 268.31$ (calcd 268.03).

3.1.4. *N*-(2-Propargyl)amidopyrene (4d). Pyrenecarboxylic acid (0.99 g) was dissolved in thionyl chloride (100 mL) and the resultant mixture was refluxed for 30 min. The excess thionyl chloride was evaporated off and the residue was dried in vacuo for 1 h. The dry residue was dissolved in THF and propargylamine (0.35 mL) and Et_3N (3 mL) were added. After stirring for 30 min, the resultant mixture was diluted with EtOAc and the organic layer was washed with 0.5 N aqueous HCl and saturated aqueous $NaHCO_3$ several times. The resultant organic layer was dried ($MgSO_4$), and evaporated to dryness. The residue was subjected to purification by silica gel column chromatography [hexane only–hexane–EtOAc (1:4)] to afford *N*-(2-propargyl)amidopyrene a pale-yellow powder (0.82 g, 72%) after evaporation. ^{1}H NMR (Me_2SO-d_6, Me_4Si): 10.32 (br s, 1H), 9.66–9.21 (m, 9H), 4.58 (br s, 2H), 2.29 (s, 1H); IR (KBr, cm^{-1}) 1629 and 1524; $[M+Na]^+ = 283.51$ (calcd 284.10).

3.1.5. 5,10,15-Tris(methoxycarbonylphenyl)-20-[*N*-(2-propargyl)amidophenyl]porphyrin (4e). Trifluoroacetic acid (9 mL) was added to methyl formylbenzoate (13.1 g) *p*-*N*-(2-propargyl)amidobenzaldehyde (5.0 g), and pyrrole (7.35 mL) in dry CH_2Cl_2 (1000 mL) and the resultant mixture was stirred under nitrogen for 12 h. *p*-Chloranil (20 g) was added and stirring was continued for 1 h. Triethylamine (10 mL) was added and the mixture was evaporated and the resultant residue was subjected to column chromatography ($CHCl_3$ only, five times) followed by recrystallization from MeOH to give 5,10,15-tris(methoxycarbonylphenyl)-20-(*N*-(2-propargyl)amidophenyl)porphyrin (1.39 g, 6.0%) as purple powder. ^{1}H-NMR ($CDCl_3$, Me_4Si): 8.81 (m, 8H), 8.44 (d, *J* 7.24, 2H), 8.43 (d, *J* 7.52, 4H), 8.29–8.27 (m, 8H), 8.18 (d, *J* 7.83, 2H), 6.63 (br s, 1H), 4.45 (d, *J* 4.98, 2H), 4.11 (s, 9H), 2.39 (s, 1H), −2.82 (s, 2H); IR (KBr, cm^{-1}) 1717, 1665, 1537, and 1273; $[M+H]^+ = 871.08$ (calcd 870.92).

3.1.6. Chemoselective coupling between 6-azido-6-deoxycurdlan and functional modules. Copper(II) bromide (2.7 mg), ascorbic acid (25 mg), compounds (**4a**, **4b**, **4c**, **4d**, or **4e**) (400 mg), and propylamine (100 μL) were added to 6-azido-6-deoxycurdlan (50 mg) in Me_2SO (2 mL) and the mixture was incubated at room temperature for 12 h. In the case of the alkyne-terminated porphyrin, NMP was used instead of Me_2SO for the coupling. The mixture was dialyzed (water, MWCO 8000) followed by lyophilization and washing with solvents (MeOH for **1**-Fc and **1**-Py or $CHCl_3$ for **1**-Por) to give the desired curdlan derivatives as white (**1**-OH (**5a**) and **1**-Lac (**5b**)), yellow (**1**-Fc (**5c**)), pale-yellow (**1**-Py (**5d**)) or purple (**1**-Por (**5e**)) powders with yields of 63–78%.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2005.10.009.

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