Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Construction of Glycosylated Hemoprotein by Reconstitutional Method

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Instruments. ^{1}H-NMR spectra were collected using a Bruker AVANCE500 (500 MHz) NMR spectrometer. The ^{1}H NMR chemical shifts are reported in ppm relative to the residual solvent resonances. The UV-visible experiments were conducted using a Shimadzu UV-3150 double beam spectrometer. The mass analysis of the reconstituted myoglobin was carried out using a time-of-flight (TOF) mass (MS) spectrometer equipped with electronspray ionization on an Applied Biosystems Mariner API-TOF Workstation.

Materials. All reagents and chemicals were obtained from commercial sources and used as received unless otherwise noted. Galactohemin **1** was synthesized by the methods described below. Wild-type myoglobin was expressed from *E. Coli*, and purified by column chromatography through CM-52 (Whatman) and Sephadex G-25 (Amersham Biosciences) columns. Biotin-labelled lectin from *Arachis Hypogaea* (peanut) (L6135) was purchased from Sigma Co., Ltd. Streptavidin-modified sepharose (17-5113-01) was purchased from Amersham Biosciences Co., Ltd. The reconstituted myoglobin was prepared by the method described below.

Synthesis of galactohemin 1.

Synthesis of 3. The compound **2** was prepared from 2,2'-iminodiethanol by the reported method.[1] In a 50- mL round-flask, **2** (3.83 g, 19.8 mmol) and *tert*-butyl acrylate (9.18 g, 71.7 mmol) were dissolved in the mixed solvent of dioxane (25 mL) and water (1 mL). Solid NaOH (0.32 g, 8.00 mmol) was added, and the solution was stirred at room temperature for 24 h. After diluted with water (150 mL), the organic materials were extracted with CH_2Cl_2 (50 mL x 4). The organic phase was dried over Na_2SO_4. The evaporation of the solvent gave yellow oil. δ_H (500 MHz; $CDCl_3$; residual undeuterated $CHCl_3$) 7.24-7.29 (5H, m), 3.67 (2H, s), 3.60 (4H, q, J = 6.5 Hz), 3.50 (4H, br), 2.70 (4H, br), 2.44 (4H, q, J = 6.5 Hz) and 1.41 (18H, s); δ_c (135.6 MHz; $CDCl_3$; residual undeuterated $CHCl_3$) 171.04, 140.22, 129.77, 129.30, 127.25, 80.93, 70.15, 67.56, 67.14, 60.0, 54.1, 36.83 and 28.57. In a 100- mL two-neck flask equipped with a condenser, the oil obtained above was dissolved in MeOH (25 mL) and 10 % palladium on carbon (60 mg) was added. The solution was gently pumped off and N_2 was introduced. This procedure was repeated 5 times. Under N_2 stream, ammonium formate (6.20 g, 98.3 mmol) was slowly added, and the reaction mixture was stirred at 45–50 ˚C for 7 h under a N_2 atmosphere. After cooling to room temperature and filtration, the solvent was evaporated. The white semi-solids were triturated with 2M NaOH*aq*. (150 mL) and the organic materials

were extracted with CH_2Cl_2 (50 mL x 4). The organic solution was dried over Na_2SO_4 and concentrated to afford **3** as yellow oil with the yield of 71% in 2 steps from **2**. δ_H (500 MHz, $CDCl_3$, residual undeuterated $CHCl_3$) 3.65 (4H, q, J = 6.5 Hz), 3.53 (4H, q, J = 5.5 Hz), 2.76 (4H, q, J = 5.5 Hz), 2.45 (4H, q, J = 6.5 Hz), 1.95 (1H, br) and 1.42 (18H, s); δ_c (135.6 MHz; $CDCl_3$; residual undeuterated $CHCl_3$) 171.6, 81.18, 71.02, 67.32, 49.91, 36.93 and 28.77.

Synthesis of 4. In a 100-mL flask, Z-β-alanine (1.88 g, 8.0 mmol) and **3** (2.89 g, 8.0 mmol) were dissolved in 25 mL of CH_2Cl_2 and cooled with an ice bath. DCC (1.79 g, 8.7 mmol) was slowly added and the solution was stirred in the ice bath for 3 h and at room temperature for 16 h. The reaction solution was filtered and concentrated. The obtained oily residue was dissolved in AcOEt (150 mL) and washed with 5 % citric acid (50 mL x 2), 5 % Na_2CO_3 (50 mL x 2) and brine. During this procedure, insoluble materials formed were removed by filtration. The organic phase was dried over Na_2SO_4, and the solvent was evaporated to give yellow viscous oil (4.1 g). δ_H (500 MHz, $CDCl_3$, residual undeuterated $CHCl_3$) 7.25-7.35 (5H, m), 5.6 (1H, br), 5.06 (2H, s), 3.61 (4H, q, J = 6.5 Hz), 3.40-3.60 (10H, m), 2.57 (2H, m), 2.42 (4H, q, J = 6.5 Hz) and 1.41 (18H, s); δ_c (135.6 MHz; $CDCl_3$; residual undeutrated $CHCl_3$) 172.53, 171.10, 156.83, 137.30, 128.84, 128.37, 81.10, 69.86, 69.20, 67.46, 67.12, 66.81, 49.03, 46.34, 37.35, 36.66, 31.24 and 28.55. In a 50- mL two-neck flask equipped with a condenser, the above-mentioned oil was dissolved in 30 mL of MeOH, and 10 % palladium on carbon (70 mg) was added. The solution was gently pumped off and N_2 was introduced. This procedure was repeated 5 times. Under a N_2 stream, ammonium formate (2.33 g, 37.1 mmol) was slowly added, and the reaction mixture was stirred at 45–50 ˚C for 5 h. After cooling at room temperature, the solution was concentrated, and the oily residue was triturated with 1 % citric acid (35 mL) and washed with ether twice. The water phase was bought to pH ~ 12 by adding 5 M NaOH*aq*. and extracted with CH_2Cl_2 (20 mL x 4). The organic phase was dried over Na_2SO_4. The solvent was evaporated to afford **4** as pale yellow oil (3.04 g) with the yield of 87% in 2 steps. δ_H (500 MHz, $CDCl_3$, residual undeuterated $CHCl_3$) 3.60 (4H, m), 3.48-3.60 (8H, m), 2.94 (2H, q, J = 6.5 Hz), 2.49 (2H, q, J = 6.5 Hz), 2.41 (4H, m), 1.80 (2H, br) and 1.40 (18H, s); δ_c (135.6 MHz; $CDCl_3$; residual undeuterated $CHCl_3$) 172.9, 171.2, 81.10, 70.02, 69.38, 67.46, 67.16, 49.10, 46.45, 38.61, 36.78, 36.68 and 28.55.

Synthesis of 5. In a 100-mL two-neck flask equipped with a stopper and a 3-way cock, protoporphyrin IX (140 mg, 249 mmol), 1-hydoxybenzotriazole monohydrate (80 mg, 523 mmol) and **4** (279 mg, 647 mmol) were dissolved in dry DMF (6 mL) under a N_2 atmosphere and cooled with an ice bath. EDC·HCl (191 mg, 996 mmol) was slowly added under a N_2 stream, and the reaction mixture was stirred in the ice bath for 2.5 h and at room temperature for 22 h. Water (100 mL) was added to the reaction mixture, and the product was extracted with CH_2Cl_2 (15 mL x 5). The organic extracts were combined and washed with 5% Na_2CO_3 (20 mL x 2), 5% citric acid (20 mL x 2) and brine. The organic phase was dried over Na_2SO_4. The solvent was evaporated to give purple oily residue. The residue was purified by silica gel chromatography with elution of CH_2Cl_2/MeOH = 9/1. The first leading purple band was collected, and the solvent was

evaporated to afford purple solid. The solid was further purified by GPC to give **5** with the yield of 92 % (320 mg). δ_H (500 MHz, $CDCl_3$, residual undeuterated $CHCl_3$) 9.80 (4H, m), 8.17 (2H, m), 7.22 (2H, br), 6.31 (2H, m), 6.15 (2H, m), 4.30 (4H, m), 3.54 (16H, m), 3.20 (8H, m), 3.00(6H, m), 2.92 (4H, m), 2.79 (4H, m), 2.60 (4H, m) 2.45 (6H, m), 2.30 (4H, m), 2.20 (4H, m), 1.37 (9H, s), 1.28 (9H, s), 1.80 (2H, br) and –4.5 (2H, s); λ_{max} (CH_2Cl_2)/nm 401, 502, 537, 574 and 629.

Synthesis of 7. The compound **5** (195 mg, 0.14 mmol) was dissolved in the mixture of HCO_2H (5 mL) /TFA (8 mL). The solution was stirred at room temperature for 22 h and concentrated. After the solvent was removed by azeotropy with toluene three times, the purple solid was obtained. The solid was dissolved in dry DMF (6 mL) together with β-1-amino-2,3,4,6-tetraacetylgalactopyranose (292 mg, 0.84 mmol) under a N_2 atmosphere and cooled in the ice bath. Et_3N (170 mg, 1.68 mmol) and BOP reagent (743 mg, 1.68 mmol) were sequentially added under a nitrogen stream, and the reaction mixture was stirred in the ice bath for 3 h and at room temperature for 40 h. After water (100 mL) was added to the mixture, the extractions with CH_2Cl_2 were carried out (20 mL x 5). The organic phases were combined and washed with 5% citric acid (20 mL x 3), 5% Na_2CO_3 (20 mL x 3) and brine. The organic phase was dried over Na_2SO_4. The solvent was evaporated to give purple semi-solid. The solid was subjected to silica gel chromatography (CH_2Cl_2/MeOH = 9/1) and recycle GPC to afford 146 mg of the purified **7** with 42%. λ_{max} (CH_2Cl_2)/nm 402, 503, 537, 574 and 628; *m/z* (ES, positive mode) 2484.0011(M + H^+, $C_{116}H_{154}N_{12}O_{48}$ requires 2482.9978).

Synthesis of 1. The compound **7** (16 mg, 6 μmol) was dissolved in 5 mL of N_2-purged $CHCl_3$/MeCN (1/1) under a N_2 atmosphere, and solid $NaHCO_3$ (5 mg) and $FeCl_2 \cdot nH_2O$ (85 mg) were added under a nitrogen stream. The reaction mixture was stirred at 60 ºC for 3 h. The solution was cooled to room temperature, exposed to the air and diluted with CH_2Cl_2 (40 mL). The mixture was washed with 1M HCl(aq) and brine. The organic phase was dried over Na_2SO_4 and concentrated. The obtained residue was dissolved in 5 mL of 20 mM NaOH in MeOH. The solution was stirred at room temperature for 4 h, and 10 μl of conc. HCl was added. After the solvent was evaporated, the green residue was dissolved in 100 μl of 0.01 M HCl(aq.) containing 1 M KCl and subjected to LH-20 gel filtration with elution of water. The third red band was collected, and the solvent was evaporated to yield 12 mg of galactohemin **1** (80% yield calculated as the Cl-coordinated compound). λ_{max} (100 mM NaCl*aq*.)/nm 398, 497 and 616; *m/z* (ES, positive mode) 1864.7930 ($M^+ - Cl^-$, $C_{84}H_{120}FeN_{12}O_{32}$ requires 1864.7881), 932.8795 (double charge for $M^+ + H^+ - Cl^-$; The doubly charged species for $C_{84}H_{121}FeN_{12}O_{32}$ requires 932.8780) and 943.8687 (double charge for $M^+ + Na^+ - Cl^-$; The doubly charged species for $C_{84}H_{120}FeN_{12}O_{32}Na$ requires 943.8689.

Preparation of the reconstituted myoglobin. The removal of the native heme from wild-type myoglobin to obtain apomyoglobin was carried out according to Teale's 2-butanone method.[2] Apomyoglobin was dissolved in 100 mM potassium phosphate (KPi) buffer (pH = 7.0) at 4 ºC. To the solution was slowly added galactohemin **1** in 100 mM KPi buffer (pH = 7.0) with slow shaking at 4 ºC. The mixture was slowly shaken at 4 ºC for 24 h and concentrated by ultrafiltration. The crude protein was

purified by Sephadex G-25 gel filtration.

Procedure of immunoprecipitation. The reconstituted or wild-type myoglobin (9 μM) and biotin-labelled peanut lectin (100 μM) were mixed in 10 mM PBS buffer (0.9% NaCl, pH = 7.0) in the presence of $CaCl_2$ (0.2 mM) and $MnCl_2$ (0.2 mM) (Solution A). As a reference, a myoglobin solution untreated with lectin was also prepared (Solution B). Solution A was slowly shaken at 4 ºC for 2 h, and streptavidin-modified Sepharose was added. The resultant mixture was shaken at 4 ºC for 1 h and centrifuged. The content of the residual heme in the supernatant was quantified by measuring the differential UV-visible spectrum between the supernatant and Solution B. The negative spectrum in the Soret region is indicative of the binding of the sugar units to the lectin (See Fig. S-2).

References

[1] Y. Shen, X. Feng, Y. Li, G. Zhang, Y. Jiang, *Tetrahedron*, 2003, **59**, 5667.

[2] F. W. Teale, *Biochim. Biophys. Acta.*, 1959, **35**, 543.

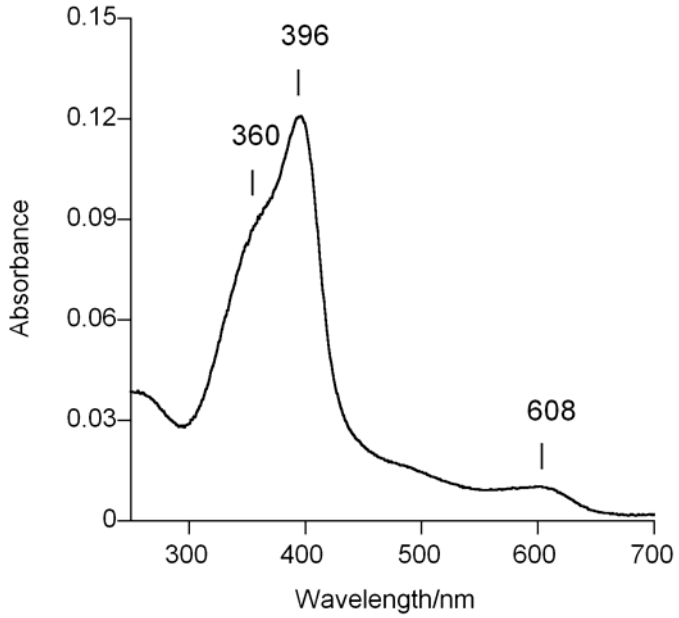

Fig. S-1. UV-Vis spectrum of galactohemin **1** in 100 mM phosphate buffer (pH = 7.0).

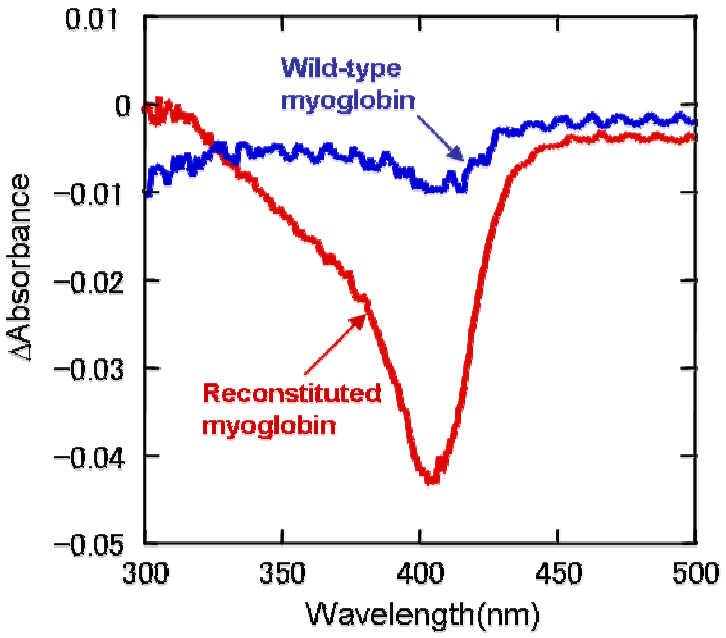

Fig. S-2. Differential UV-vis spectra in the immunoprecipitation experiments for wild-type myoglobin (blue line) and the reconstituted myoglobin (red line).