Synthesis and Characterization of New Style of Water-Soluble Glycosylated Porphyrins as a Spectrophotometric Reagent for Metal Ions

Katsunori KOHATA,* Hisao HIGASHIO, Yuichi YAMAGUCHI, Mamoru KOKETSU,† and Tsugikatsu ODASHIMA††
National Research Institute of Vegetables, Ornamental Plants and Tea, Ano, Age, Mie 514-23
†Basic Research Laboratories, Taiyo Kagaku Co., Ltd, Takaramachi, Yokkaichi, Mie 510
††Institute for Chemical Reaction Science, Tohoku University, Katahira, Sendai 980

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Six new styles of water-soluble glycosylated porphyrins (5, 10, 15, 20-tetrakis[2-, 3- or 4-(β-D-glucopyranosyl)phenyl]porphine) were synthesized and studied concerning their structures by ^{1}H and ^{13}C NMR. Tetrakis (*o*-substituted phenyl)porphine, consisting of four atropisomers (αβαβ, ααββ, αααβ, and αααα), was clearly assigned based on the ^{13}C NMR peak-splitting pattern. It was especially noteworthy that water-soluble picket-fence porphyrin (αααα) was obtained. Furthermore, their characterization as a chromogenic reagent for metal ions was investigated. The introduction of highly water-soluble glucose successfully improved the aggregration and adsorption characteristics of the anionic or cationic porphyrins so far prepared without any marked change in the other important analytical properties.

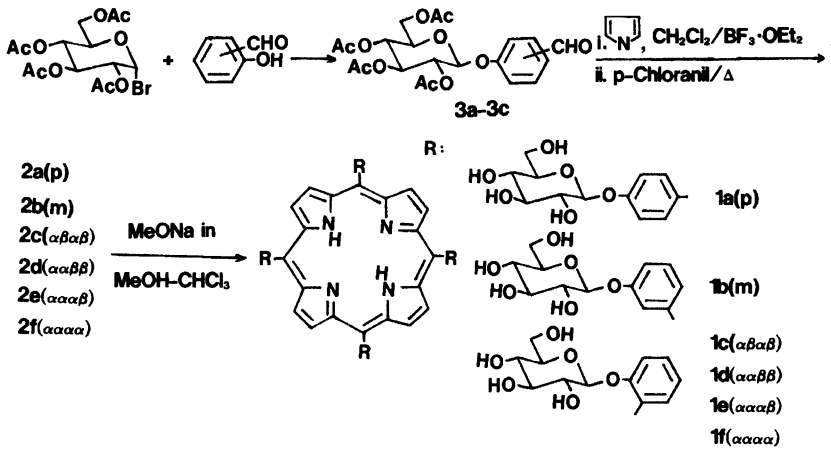
Porphyrins and their metal complexes have been extensively studied in biological and other fields of chemistry, and the synthesis of porphyrins having various functions has become increasingly important. In recent years water-soluble *meso*-substituted porphyrins have been studied as highly sensitive spectrophotometric reagents for metal ions having a very large molar absorptivity ($\varepsilon=2$—6×10^5 $mol^{-1}\,dm^3\,cm^{-1}$, at 400—500 nm).[1] Every water-soluble spectrophotometric reagent for metal ions so far prepared has been a cationic, anionic or amphoteric ion type.[2] We directed our attention to the high water-solubility of sugars, previously developed a new style water-soluble glycosylated hydrazone, bis(D-glucose) 1,1′-oxalylbishydrazone, and used it for the spectrophotometric determination of copper.[3] Based on this background, we recently succeeded in preparing six kinds of neutral-type glycosylated porphyrins, which have the β-D-glucopyranose moiety at the *para*-, *meta*- or *ortho*-position of 5,10,15,20-phenyl substituents.[4] These are 5,10,15,20-tetrakis-[4-(β-D-glucopyranosyl)phenyl]porphine (**1a**), 5,10,15,20-tetrakis[3-(β-D-glucopyranosyl)phenyl]porphine (**1b**) and 5,10,15,20-tetrakis[2-(β-D-glucopyranosyl)phenyl]-porphine, containing four atropisomers (αβαβ (**1c**), ααββ (**1d**), αααβ (**1e**), and αααα (**1f**)), as shown in Scheme 1. In these porphyrins tetrakis(*o*-substituted phenyl)porphine (*o*-STP) comprises of four atropisomers; it is very tedious and time-consuming to separate these. However, since this porphyrin has notably different properties from those of tetrakis(*p*- and *m*-substituted phenyl)porphines (*p*- and *m*-STP) concerning the rate of metallation, it is not only a useful spectrophotometric reagent, but also an interesting compound for kinetic studies of metallation. Furthermore, *o*-STP has been studied regarding thermal and photoatropisomerism;[5] especiallly the αααα-isomer (picket-fence porphyrin) has been preferably employed to model the function of the hemoproteines.[6]

As for a linked porphyrin to sugars, a tetraarylporphine chemically bonded to a β-cyclodextrin has been prepared as a model to mimic the light-induced electron-transfer process between the porphyrin chromophore and acceptors of varying reduction potential held in the cyclodextrin cavity.[7] Recently, Maillard et al. published information concerning glycoconjugated porphyrins, including *o*-STP as a superstructured model of the active site of hemoprotein,[8] and reported on the synthesis and characterization of these porphyrins.[9] However, their studies were mainly restricted to the acetyl-protected water-insoluble glycosylated porphyrins. They neither referred to the unexpected atropisomerization during the conversion of the acetates of glycosylated porphyrins (**2c**—**2e**) to free ones (**1c**—**1e**), nor obtained the αααα-isomer. Until now, these glycosylated porphyrins have not yet been used as a spectrophotometric reagent for metal ions.

In this report we describe the preparation of water-soluble glycosylated porphyrins in detail, and our study of their structure, chromogenic properties and complexation. An example with copper(II) is used to elucidate them as a reagent for the spectrophotometric determination of trace amounts of metal ions.

Experimental

Apparatus. The spectra of ^{1}H and ^{13}C NMR were measured in $CDCl_3$ or dimethyl-d_6 sulfoxide (DMSO-d_6) at 400 MHz (^{1}H) or 100 MHz (^{13}C) using a JEOL GSX-400 instrument operated in the pulsed Fourier-transfer mode. Tetramethylsilane was used as the internal standard. The mass spectra were obtained on a JEOL JMS HX-110 instrument by using a fast atom bombardment (FAB) method. The collision gas used was Xe and the matrix was glycerol. For measuring the absorption spectra, a Hitachi 200-10 spectrophotometer was used. Thin-layer chromatography (TLC) was carried out using 0.2 mm E. Merk 60F-254 pre-coated silica-gel plates. For column chromatography, E. Merk silica gel 60 (70—230 mesh) was used.



Scheme 1.

Reagents. Porphyrin Solution: Prepared by dissolving the required amount of each porphyrin in 40%(v/v) 1,4-dioxane in water. These solutions were further diluted with water, when necessary.

Standard Copper(II) Solution: A stock solution containing about 1 mg of copper per cm^3 was prepared by dissolving copper(II) sulfate pentahydrate in water, and adding a sufficient amount of nitric acid to give a pH of about 1. This stock solution was standardized by titration with ethylenediaminetetraacetic acid (EDTA) and appropriately diluted with water to prepare working solutions.

Buffer Solutions: 0.2 M (1 M=1 mol dm^{-3}) hydrochloric acid-0.2 M potassium chloride, 0.2 M chloroacetic acid–0.2 M sodium chloroacetate, 0.2 M acetic acid–0.2 M sodium acetate, 0.2 M 2-morpholinoethanesulfonic acid–0.2 M sodium hydroxide, 0.2 M tris(hydroxymethyl)aminomethane–0.2 M hydrochloric acid or 0.2 M boric acid–0.2 M sodium carbonate system was used according to the pH value required.

The Other Reagents: Dichloromethane was distilled from calcium hydride and stored over 4 Å molecular sieves. Dry chloroform and methanol were purchased from (Dojindo). Stock solutions (0.5 M) for BF_3·Et_2O (Tokyo Kasei) were prepared in dry dichloromethane and were used for approximately 1 week. Pyrrole and hydroxybenzaldehydes obtained from commercial sources were used (Tokyo Kasei). The solution of sodium methanolate (Wako) was prepared in dry methanol just before use. The other reagents were of analytical-reagent grade. All water solutions were prepared with distilled and deionized water.

Synthesis of Formylphenyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosides (3a—3c). 2-, 3-, or 4-Hydroxybenzaldehyde (8.8 g, 72.1 mmol) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide (11.6 g, 28.2 mmol) were dissolved in acetone (60 cm^3). After the addition of 5% (g/v) sodium hydroxide (20 cm^3) the solution was stirred intensively for 24 h at room temperature, poured into iced water (200 cm^3) and stirred for 1 h. In the meantime crystallization started slowly. The crystals were filtered off and recrystallized from ethanol. More target aldehydes were recovered from the remaining reaction filtrate by extraction with chloroform and purification by column chromatography using chloroform as the eluent. Total yields (**3a**, 5.1 g (40.0%); **3b**, 5.7 g (44.7%); **3c**, 6.2 g (48.6%)).

3a: mp 125—126 °C; ^{1}H NMR ($CDCl_3$) δ=2.0 (12H, m, CH_3COO), 7.11 (2H, d, benzene(2,6)), 7.86 (2H, d, benzene (3, 5)), 9.93 (1H, s, CHO). Found: C, 55.76; H, 5.28%.

3b: mp 108—109 °C, ^{1}H NMR ($CDCl_3$) δ=2.1 (12H, m, CH_3COO), 7.26 (1H, dd, benzene(5)), 7.47 (1H, d, benzene (6)), 7.51 (1H, s, benzene (2)), 7.59 (1H, d, benzene(4)), 9.98 (1H, s, CHO). Found: C, 55.50; H, 5.37%.

3c: mp 149—150 °C, ^{1}H NMR ($CDCl_3$) δ=2.1 (12H,m, CH_3COO), 7.13 (1H, d, benzene (6)), 7.19 (1H, m, phenyl(4)), 7.57 (1H, m, benzene(5)), 7.86 (1H, d, benzene(3)), 10.3 (1H, s, CHO). Found: C, 55.60; H, 5.64%.

Synthesis of the Acetates of Glycosylated Porphyrins. 5,10,15,20-Tetrakis[3- or 4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)phenyl]porphine (2a or 2b): A 1-dm^3 two-necked, round-bottomed flask, wrapped in aluminium foil and equipped with a reflux condenser and N_2 inlet was charged with dry dichloromethane (370 cm^3), 0.1 M pyrrole in dichloromethane (46 cm^3) and *p*- or 0.1 M *m*-formylphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**3a** or **3b**) in dichloromethane (46 cm^3). The mixture was stirred and purged with N_2 for 15 min, after which a 0.5 M BF_3·Et_2O solution in dichloromethane (0.19 cm^3) was added. This mixture was stirred for 24 h at room temperature. Then *p*-chloranil (0.82 g, 3.3 mmol) was added. After refluxing for 1 h, a dark-purple solution was concentrated to about 20 cm^3 and filtered in order to remove insoluble by-products. The filtrate was concentrated, loaded on to a silica-gel column (4×40 cm, chloroform) and then eluted with chloroform. The fractions of the main purple band were collected and evaporated to give red-purple crystals, which were recrystallized from chloroform–methanol.

2a: Yield 1.20 g (53.2%); FABMS *m/z* (rel intensity) 2000 ($(M+1)^+$; 100); ^{1}H NMR ($CDCl_3$)δ=−2.8 (2H, s, pyrrolic NH), 2.1—2.3 (48H, m, CH_3COO), 4.0—4.5 (12H, m, glucose), 7.4 (8H, d, benzene (2, 6), 8.9 (8H, s, pyrrole). Found C, 59.98; H, 5.09; N, 2.83%. Calcd for $C_{100}H_{102}N_4O_{40}$: C, 60.06; H, 5.14; N, 2.80%.

2b: Yield 0.59 g (26.0%); FABMS *m/z* (rel intensity) 2000 ($(M+1)^+$, 100); ^{1}H NMR ($CDCl_3$) δ=−2.9 (2H, s, pyrrolic NH), 1.2—1.5 (12H, m, CH_3COO), 1.8—2.1 (36H, m, CH_3COO), 3.8—4.2 (12H, m, glucose), 5.1—5.4 (16H, m, glucose), 7.5 (4H, d, benzene(4)), 7.7 (4H, m, benzene(5)), 7.9—8.0 (8H, m, benzene(3+6)), 8.9 (8H, s,pyrrole). Found: C, 59.96; H, 5.00; N, 2.54%. Calcd for $C_{100}H_{102}N_4O_{40}$: C,

60.06; H, 5.14; N, 2.80%.

5,10,15,20-Tetrakis[2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)phenyl]porphine (2c—2f): The above-mentioned condensation reaction was repeated twice using *o*-formylphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**3c**) instead of **3a** or **3b**. A mixture of atropisomers was chromatographed on a silica-gel column by elution with a solvent gradient consisting of 0—20% (v/v) acetone in chloroform. The four bands were separately collected and purified by recrystallization from chloroform–methanol or methanol. Yields (**2c**, 0.36 g (7.9%); **2d**, 0.30 g (6.6%); **2c**+**2d**, 0.28 (6.1%); **2e**, 1.15 g (25.3%); **2f**, 0.30 g (6.6%)).

2c: FABMS *m/z* (rel intensity) 2000 ($(M+1)^+$, 100); ^{1}H NMR ($CDCl_3$) $\delta=-3.0$ (2H, s, pyrrolic NH), −1.3 (3H, s, CH_3COO), 0.8—2.2 (45H, m, CH_3COO), 3.7—5.0 (28H, m, glucose), 7.2—7.9 (16H, m, benzene), 8.6 (4H, s, pyrrole), 8.8 (4H, s, pyrrole). Found: C, 60.15; H, 5.08; N, 2.88%. Calcd for $C_{100}H_{102}N_4O_{40}$: C, 60.06; H, 5.14; H, 2.80%.

2d: FABMS *m/z* (rel intensity) 2000 ($(M+1)^+$, 100); ^{1}H NMR($CDCl_3$) $\delta=-2.8$ (2H, s, pyrrolic NH), −2.3—−0.5 (9H, m, CH_3COO), 1.2—2.2 (39H, m, CH_3COO), 3.4—4.9 (28H, m, glucose), 7.4—8.0 (16H, m, benzene), 8.7—8.9 (8H, m, pyrrole). Found: C, 60.12; H, 5.04; N, 2.83%. Calcd for $C_{100}H_{102}N_4O_{40}$: C, 60.06; H, 5.14; N, 2.80%.

2e: FABMS *m/z* (rel intensity) 2000 ($(M+1)^+$, 100); ^{1}H NMR ($CDCl_3$) $\delta=-2.8$ (2H, s, pyrrolic NH), −0.88 (9H, d, CH_3COO), 1.2—2.2 (36H, m, CH_3COO), 3.4—5.3 (28H, m, glucose), 7.4—8.0 (16H, m, benzene), 8.5—8.8 (8H, m, pyrrole). Found: C, 59.83; H, 5.06; N, 2.54%. Calcd for $C_{100}H_{102}N_4O_{40}$: C, 60.06; H, 5.14; N, 2.80%.

2f: FABMS *m/z* (rel intensity) 2000 ($(M+1)^+$, 100);^{1}H NMR ($CDCl_3$) $\delta=-2.8$ (2H, m, pyrrolic NH), −0.8—−0.2 (3H, m, CH_3COO), 1.2—2.2 (45H, m, CH_3COO), 3.5—5.5 (28H, m, glucose), 7.3—8.2 (16H, m, benzene), 8.4—8.9 (8H, m, pyrrole). Found: C, 59.38; H, 5.12; N, 2.86%. Calcd for $C_{100}H_{102}N_4O_{40}\cdot H_2O$: C, 59.52; H, 5.20; N, 2.78%.

Deacetylation of the Glycosylated Porphyrins. 5,10,15,20-Tetrakis[3- or 4-(β-D-glucopyranosyl)phenyl]porphine (1a or 1b): Acetylated porphyrin (0.5 g, 0.25 mmol) was dissolved in dry chloroform (40 cm^3). To this solution were added dry methanol (40 cm^3) and 0.22 M sodium methanolate in dry methanol (2.5 cm^3). The mixture was stirred at around 55 °C for 0.5 h, neutralized with a few drops of acetic acid and concentrated to dryness. The obtained crude solid was dissolved in a minimum amount of ethanol-water and loaded onto a silica-gel column. Elution with 2:6:1 followed 3:6:2 (v/v) 2-propanol–ethyl acetate–water gave the only one band. The fractions of this band were collected and evaporated to yield pure crystals.

1a: Yield 310 mg (90.9%); FABMS *m/z* (rel intensity) 1327 ($(M+1)^+$, 84), 1328 ($(M+2)^+$, 100), 1329 ($(M+3)^+$, 81); ^{1}H NMR (DMSO-d_6) $\delta=-2.9$ (2H, s, pyrrolic NH), 3.3—5.5 (glucose), 7.5 (8H, d, benzene(3, 5)), 8.1 (8H, d, benzene (2, 6)), 8.9 (8H, s, pyrrole). Found: C, 59.92; H, 5.34: N, 4.08%. Calcd for $C_{68}H_{70}N_4O_{24}\cdot 2H_2O$: C, 59.91; H, 5.47; N, 4.11%.

1b: Yield 305 mg (83.9%); FABMS *m/z* (rel intensity) 1327 ($(M+1)^+$, 87), 1328 ($(M+2)^+$, 100), 1329 ($(M+3)^+$, 84); ^{1}H NMR (DMSO-d_6) $\delta=-2.9$ (2H, s, pyrrolic NH), 3.2—5.4 (glucose), 7.5 (4H, m, benzene (5)), 7.8—7.9 (8H, m, benzene (2+6)), 8.9 (8H, d, pyrrole). Found: C, 56.17; H, 5.32; N, 4.01%. Calcd for $C_{68}H_{70}N_4O_{24}\cdot 2H_2O$: C, 56.20; H, 5.83; N, 3.85%.

5α,10β,15α,20β-Tetrakis[2-(β-D-glucopyranosyl)-phenyl]porphine (1c): Porphyrin **1c** was obtained by the deacetylation of **2e** with atropisomerization. Porphyrin **2e** (550 mg, 27.5 mg) was dissolved in dry chloroform (50 cm^3) and methanol (50 cm^3). After the addition of 1.0 M sodium methanolate in dry methanol (1.1 cm^3), the mixture was stirred at around 55°C for 15 h. The reaction time (10—17 h) gave the best yield of **1c**. In the meantime crystallization started slowly. The crystals were filtered off, dissolved in 1,4-dioxane-water and stirred with a Dowex H8 (H^+) cation-exchange resin to be neutralized. The remaining filtrate was also neutralized with a Dowex H8(H^+) resin. After the resin was removed by filtration, both filtrates were mixed and concentrated until crystallization started. This crystals contained mainly **1c** and a small amount of **1e**; there was no **1c** in the filtrate, which consisted of a mixture of **1d** and **1e**. The crystals were filtered off, dissolved again in a minimum amount of 1,4-dioxane–water, loaded onto a silica-gel column (4×40 cm, ethyl acetate) and eluted with 8:24:3 followed 2:3:2 (v/v) 2-propanol–ethyl acetate–water. The fractions containing **1c** and **1e** were collected and concentrated to yield pure red-purple crystals. The other fractions containing **1c** and **1e** were collected, concentrated and chromatographed again to give more **1c**. Total yield (130 mg, 33.4%).

1c: FABMS *m/z* (rel intensity) 1326 (M^+, 34), 1327 ($(M+1)^+$, 100), 1328 ($(M+2)^+$, 74); ^{1}H NMR (DMSO-d_6) $\delta=-2.9$ (2H, s, pyrrolic NH), 1.7 (18H, s, glucose), 2.3—5.0 (m, glucose), 7.4—7.9 (16H, m, benzene), 8.7 (8H, d, pyrrole). Found: C, 57.44; H, 5.36; N, 3.89%. Calcd for $C_{68}H_{70}N_4O_{24}\cdot 5H_2O$: C, 57.62; H, 5.68; N, 3.65%.

5α,10α,15β,20β-Tetrakis[2-(β-D-glucopyranosyl)-phenyl]porphine (1d): As analogous to the procedure for **1a** and **1b**, **2d** (280 mg, 0.14 mmol) was deacetylated. The obtained crude solid was recrystallized from ethanol–water to give pure red-purple crystals (**1d**, 110 mg). The mother liquor was evaporated, loaded onto a silica-gel column (4×40 cm, ethyl acetate) and eluted with 4:12:1 followed 8:2:3 (v/v) 2-propanol–ethyl acetate –water. Each fraction containing **1d** or **1e** was collected and concentrated to give red-purple crystals, respectively. Total yield (**1d**, 116 mg (60.0%); **1e**, 46 mg (23.5%)). Porphyrin **2d** was converted to **1d** and **1e** in the ratio of 5 to 2. Since the reaction time was longer, or the amount of added sodium methanolate was more, the ratio of **1e** to **1d** increased.

1d: FABMS *m/z* (rel intensity) 1326 (M^+, 61), 1327 ($(M+1)^+$, 100), 1328 ($(M+2)^+$, 69); ^{1}H NMR (DMSO-d_6) $\delta=-2.9$ (2H, s, pyrrolic NH), 1.0—1.1 (2H, m, glucose), 2.1—5.2 (m, glucose), 7.4—8.0 (16H, m, benzene), 8.6—8.8 (8H, m, pyrrole). Found: C, 59.19; H, 5.76; N, 3.81%. Calcd for $C_{68}H_{70}N_4O_{24}\cdot 3H_2O$: C, 59.13; H, 5.55: N, 4.06%.

5α,10α,15α,20β-Tetrakis[2-(β-D-glucopyranosyl)-phenyl]porphine (1e): Porphyrin **1e** was prepared by the following two different procedures.

Procedure 1. Porphyrin **2c** (300 mg, 0.15 mmol) was converted to **1e** completely. The conditions of deacetylation and purification were almost the same as those used for **1d**. Red-purple crystals (176 mg, 83.8%).

Procedure 2. Porphyrin **2e** (420 mg, 0.21 mmol) was deacetylated by the same conditions as that of Procedure 1

and converted to **1e** almost completely. The obtained crude solids, which contained a small amount of **1c** and **1d**, were recrystallized from ethanol–water to give pure red-purple crystals (144 mg). More **1e** was recovered from the mother liquor and purified by silica-gel column chromatography using 2:6:1 followed 3:6:2 (v/v) 2-propanol–ethyl acetate–water as the eluent. Total yield (224 mg, 76.2%).

1e: FABMS *m/z* (rel intensity) 1326 (M^+, 67), 1327 ($(M+1)^+$, 100), 1328 ($(M+2)^+$, 69); ^{1}H NMR (DMSO-d_6) δ = −2.8 (2H, s, pyrrolic NH), 1.2 (2H, s, glucose), 2.0—5.1 (m, glucose), 7.4—8.1 (16H, m, benzene), 8.6—8.8 (8H, m, pyrrole). Found: C, 58.67; H, 5.58; N, 3.83%. Calcd for $C_{68}H_{70}N_4O_{24}\cdot 4H_2O$: C, 58.37; H, 5.62; N, 4.00%.

5α,10α,15α,20α -Tetrakis[2-(β-D-glucopyranosyl)-phenyl]porphine (1f): As analogous to the procedure for **1d**, **2f** (240 mg, 0.12 mmol) was deacetylated and purified by silica-gel column chromatography using 8:24:3 followed 2:6:1(v/v) 2-propanol–ethyl acetate–water as the eluent to yield dark-green solids (92.0 mg, 53.4%).

1f: FABMS *m/z* (rel intensity) 1326 (M^+, 50), 1327 ($(M+1)^+$, 100), 1328 ($(M+2)^+$, 86); ^{1}H NMR (DMSO-d_6) δ=−2.9 (2H, s, pyrrolic NH), 0.8—1.9 (m, glucose), 2.8—5.0 (m, glucose), 7.3—8.8 (24H, m, benzene+pyrrole). Found: C, 56.92, H, 5.43, N, 4.03%. Calcd for $C_{68}H_{70}N_4O_{24}\cdot 6H_2O$: C, 56.90, H, 5.76; N, 3.90%.

Measurement of Atropisomerism. The change in the isomer distribution in acetylated *o*-STP was measured by a method described in the literature.[10] Porphyrin (65 mg) was dissolved in toluene (30 cm^3); the solution was heated at around 120 °C in an oil bath under N_2. A portion of the solution was sampled with a pipette at appropriate intervals and spotted on a 10×20 cm TLC plate (Merck silica gel 60). The four isomers were separated on the TLC plate with 10% (v/v) acetone in chloroform by developing twice, and then 20% (v/v) ethyl acetate in dichloromethane by developing once. The quantity was measured by a spectrophotometric method after the extraction of each isomer from the TLC plate with acetone. The relative abundance of the isomers was photometrically determined using the Soret-band.

Results and Discussion

Synthesis of Formylphenyl β-D-Glucopyranosides. There has recently been a fair number of utilizations of the phase-transfer-catalyzed (PTC) method for the synthesis of arylglycosides[11,12] including thioglycosides[13] and sialic acid α-glycosides.[14] Although this PTC glycosylation of 4-hydroxybenzaldehyde has also been reported by Jain et al.[15] and Roy et al.,[16] the yield in this method was significantly influenced by the kinds of catalysts and aldehydes. Here, we employed a slight modification of the procedure of Schuster et al.,[17] which is different from the PTC method in reacting in a water-acetone homogeneous solution and not using any phase-transfer catalyst. Although our modified method required a longer reaction time and was not suitable for milligram-scale preparations, it was simple, and gave a slightly higher yield than did the PTC method and almost the same yield, regardless of the kinds of aldehydes (**3a**, 40%; **3b**, 44.7%; **3c**, 48.6%).

Condensation of Formylphenyl β-D-Glucopyranosides with Pyrrole. Although many *meso*-substituted porphyrins have been prepared according to the Alder–Longo procedure,[18] this method is beset with certain vexing problems. For example, the harsh reaction conditions result in a complete failure with benzaldehydes bearing sensitive functional groups; further, the work-up is very tedious. A few years ago Lindsey et al. developed a new synthetic strategy for preparing *meso*-substituted porphyrins,[19] in which the cyclization of pyrrole and aldehyde in an acid-catalyzed reaction to porphyrinogen is favored over lined polymerization. Once equilibration is complete, the porphyrinogen is oxidized by *p*-chloranil (PCA) or 2, 3-dichloro-5, 6-dicyano-1, 4- benzoquinone (DDQ). This Lindsey's method is complementary to the Alder–Longo procedure, allowing small quantities of porphyrins to be prepared from sensitive aldehydes in 30—40% yield without any difficult purification problems. Thereafter, Lindsey et al.[20] and van der Made et al.[21] improved Lindsey's method and succeeded in preparing sterically hindered porphyrins in high yield, for example 5,10,15, 20-tetramesitylporphine or 5,10,15,20-tetrakis (pentafluorophenyl)porphine. Specially, van der Made's procedure was suitable for gram-scale preparations. In this method $BF_3\cdot Et_2O$ gave better yields as a catalyst than did trifluroacetic acid, and PCA was superior to DDQ in the oxidation step.

Here, we employed the procedure of van der Made et al. for the condensation of formylphenyl β-D-glucopyranoside with pyrrole. As a result, the target glycosylated porphyrins were obtained in reasonably high yields wihtout any difficult or tedious purification problems (**2a**, 53.0%; **2b**, 26.0%; **2c**—**2f**, 52.5%). The yield for **2b** was about half that for **2a** or **2c**—**2f**. It is assumed that an electron-donating *m*-glucosyl group of **3b** releases electrons and, thus, destabilizes the transition state by intensifying the negative charge developing on the carbonyl oxygen.

Tetrakis(*o*-substituted phenyl)porphine consisted of four atropisomers ($\alpha\beta\alpha\beta$ (**2c**), $\alpha\alpha\beta\beta$ (**2d**), $\alpha\alpha\alpha\beta$ (**2e**), and $\alpha\alpha\alpha\alpha$ (**2f**) in the order of R_f values). They were identified by the NMR spectra and assigned by the NMR peak-splitting patterns, which are discussed later. Fortunately, we could obtain $\alpha\alpha\alpha\alpha$-isomer (**2f**), which Mailard et al. could not obtain.[8] Tables 1 and 2 show the isomer distribution of atropisomerization in toluene at 120 °C as well as the rel-

Table 1. Isomer Distribution of Atropisomerization in Toluene Heating at 120 °C

Heating time	**2f**	**2e**	**2d**	**2c**
h	%	%	%	%
0	6.3	41.1	26.3	26.3
48	11.0	46.1	24.2	18.7
100	10.8	50.9	22.7	15.6

Table 2. Relative Ratios in the Equilibrium State

Porphine	$\frac{\alpha\alpha\alpha\alpha}{\alpha\alpha\alpha\beta}$	$\frac{\alpha\alpha\beta\beta}{\alpha\alpha\alpha\beta}$	$\frac{\alpha\beta\alpha\beta}{\alpha\alpha\alpha\beta}$
o-Substituted tetraphenylporphine	0.21	0.45	0.30
Statistical abundance	0.25	0.50	0.25

ative ratio in the equilibrium state. It has been reported that the four isomers of 5,10,15,20-tetrakis(2-hydroxyphenyl)porphine,[22] 5,10,15,20-tetrakis(4-*t*-butyl-2-aminophenyl)porphine,[23] 5,10,15,20-tetrakis(2-aminophenyl)porphine,[24] and "picket-fence" porphyrin[24] have been equilibrated in statistical abundance ($\alpha\beta\alpha\beta : \alpha\alpha\beta\beta : \alpha\alpha\alpha\beta : \alpha\alpha\alpha\alpha = 1:2:4:1$). Although our relative-yield ratio ($\alpha\beta\alpha\beta : \alpha\alpha\beta\beta : \alpha\alpha\alpha\beta : \alpha\alpha\alpha\alpha = 1:1:1.6:0.66$, toluene heating for 0 h) was slightly different from the statistical abundance, the relative ratio in the equilibrium state (toluene heating for 100 h) was almost the same as that of conventional porphyrins.

Conversion of the Acetates of Glycosylated Porphyrins to Free Ones. Protected glycosylated porphyrins (**2a**—**2f**) were deacetylated to free ones (**1a**—**1f**) by a catalytic amount of sodium methanolate in dry chloroform–methanol (1 : 1, v/v) at around 55 °C. Although this reaction was completed within 30 min in *p*- and *m*-STP, unexpected atropisomerization was accompanied in *o*-STP. Porphyrin **2d** was converted to a mixture of **1d** and **1e**, while **2e** was converted to a mixture of **1c**, **1d**, and **1e**. The rate of these isomerizations was dependent on the reaction conditions. As the reaction time became longer or the amount of added sodium methanolate was increased, the ratio of **1e** to **1d** increased; similarly, the ratio of **1c** and **1d** to **1e** increased. In addition, **2c** was easily converted to only **1e** completely. On the other hand, **2f** gave only **1f** completely in spite of varying the reaction conditions. Since **1e** could be sufficiently obtained from **2c** or **2d**, we chose the best conditions which gave the maximum yield of **1d** in the conversion of **2d** to a mixture of **1d** and **1e**, and **1c** in the conversion of **2e** to a mixture of **1c**, **1d**, and **1e**, respectively. The details are described in the Experimental section.

Structural Analysis of Porphyrins by NMR Studies. Determination of Anomeric Configuration of Glucose in Porphyrins: The configuration of the anomeric center of glucose was confirmed by a comparison of the ^{1}H and ^{13}C NMR data of **1a** with those of *p*-nitrophenyl *β*-D-glucopyranoside (*β*-NPG) and *p*-nitrophenyl *α*-D-glucopyranoside (*α*-NPG),[25] which resembled the phenyl glucopyranoside moiety of **1a** in structure. Table 3 shows the ^{1}H NMR chemical shifts and coupling constants for the anomeric proton of **1a** (see Fig. 1) and a comparison with those of *β*- and *α*-NPG. The ^{13}C NMR chemical shift of the glucose of **1a** and a comparison with those of *β*-and *α*-

Table 3. ^{1}H NMR Chemical Shifts and Coupling Constants for the Anomeric Proton of **1a** and Comparison with Those of *β*-NPG and *α*-NPG

Compound	Chemical shift	Coupling constant
	ppm	Hz
1a	5.26	6.6
β-NPG	5.06	6.6
α-NPG	5.61	3.4

Solvent: DMSO-d_6 containing one drop of D_2O. *β*-NPG: *p*-Nitrophenyl *β*-D-glucopyranoside. *α*-NPG; *p*-Nitrophenyl *α*-D-glucopyranoside.

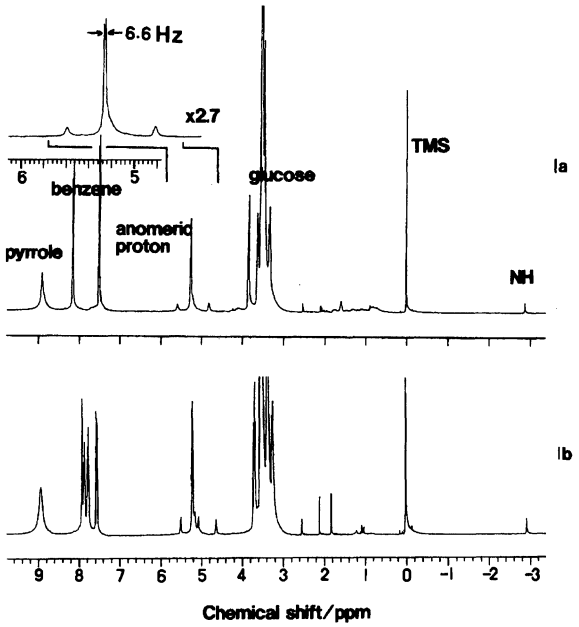


Fig. 1. ^{1}H NMR (400 MHz) spectra of **1a** and **1b** in DMSO-d_6 containing one drop of D_2O.

NPG are shown in Table 4. All of the NMR data of **1a** are very similar to those of *β*-NPG, indicating that the porphyrin ring is a strong electon-attracting like nitro group and *β*-configuration is maintained without any change in the course of the synthesis.

Identification of the Water-Soluble *p*- and *m*-STP (1a and 1b): Figure 1 shows the ^{1}H NMR spectra of **1a** and **1b**. The signals at $\delta = 8.9$, 7.5—8.2, 5.2, 3.2—3.8, and −2.9 were assigned to those of pyrrole, benzene, the anomeric proton, the other protons of glucose and pyrrolic NH, respectively. The peaks for pyrrole and pyrrolic NH underwent line broadening and a decrease in the intensity by the addition of one drop of D_2O to the solvent (DMSO-d_6). Porphyrin **1a** was identified clearly by the two characteristic doublet peaks at $\delta = 8.2$ (2- and 6-positions) and 7.5 (3- and 5- positions) derived from *p*-substituted benzene. It was also possible to identify **1b** by the three peaks at $\delta = 7.8$—7.9, 7.6, and 7.5, which were assigned to the protons at the (2+6)-, 4-, and 5-positions of *m*-substituted benzene. These porphyrins were also identified by FABMS

Table 4. ^{13}C NMR Chemical Shifts for the Glucose of **1a** and Comparison with Those of β-NPG and α-NPG

Compound	Chemical shift /ppm					
	C-1	C-2	C-3	C-4	C-5	C-6
1a	100.5	73.4	77.1	69.7	76.6	60.7
β-NPG	101.2	74.3	78.4	70.8	77.4	61.9
α-NPG	99.0	72.5	75.6	71.0	74.1	61.9
$\Delta\delta_1$	0.7	0.9	1.3	1.1	0.8	1.2
$\Delta\delta_2$	−1.5	−0.9	−1.5	1.3	−2.5	1.2

Solvent: DMSO-d_6. β-NPG: *p*-Nitrophenyl β-D-glucopyranoside. α-NPG: *p*-Nitrophenyl α-D-glucopyranoside. $\Delta\delta_1$: Chemical shift for β-NPG minus that for **1a**. $\Delta\delta_2$: Chemical shift for α-NPG minus that for **1a**.

and elementary analysis (shown in the Experimental section).

Identification and Assignment of the Water-Soluble *o*-STP (1c—1e): Unexpected atropisomerization occurred in the conversion of the acetates of glycosylated porphyrins (**2c**—**2e**) to free ones (**1c**—**1e**). Porphyrin **2d** was converted to a mixture of **1d** and **1e**, **2e** to a mixture of **1c**, **1d**, and **1e**, while **2c** to **1e** completely. The rate of isomerization was dependent on the reaction conditions. We needed to identify and assign these atropisomers. As reported by Maillard et al.,[8] the acetates of glycosylated porphyrins (**2c**—**2e**) could be assigned by ^{1}H NMR studies based on the isomer symmetries. Each type of proton in the glucose of **2c** should be equivalent in the ^{1}H NMR spectra; **2d** has, instead, two distinct resonances of two chemically equivalent protons, while **2e** has three distinct resonances. In short, **2c** gives the simplest peak-splitting pattern; on the other hand, **2e** gives the most complicated one. This rule was also the case for the corresponding glucose of free glycosylated porphyrins. Figure 2 shows the ^{1}H NMR spectra of **1c**—**1f**. The solvent (DMSO-d_6) also contained one drop of D_2O. Three porphyrins (**1c**—**1e**) were identified as the *o*-STP by the four peaks at 7.9—8.1, 7.8, 7.7, and 7.4 ppm, which were assigned to the protons at the 6-, 3-, 4-, and 5-positions of the *o*-substituted benzene. Although they gave almost the same spectral patterns as **1a** and **1b**, their resonances of protons of glucoses were shifted upfield to 1.0—3.0 ppm. This suggests that the glucose molecule at the *o*-position of the benzene ring bends over the porphyrin ring. The properties of **1f** are discussed later. By a comparison of the signals for the anomeric proton at about 5.0 ppm and the other protons at 2.0—3.5 ppm in glucose, it was proved that **1c** gave the simplest peak-splitting pattern, while **1e** gave the most complicated one. These results imply that **1c**, **1d**, and **1e** can be assigned to the $\alpha\beta\alpha\beta$-, $\alpha\alpha\beta\beta$-, and $\alpha\alpha\alpha\beta$-isomers.

However, this ^{1}H NMR assignment was not distinct because there was only a slight difference among three peak-splitting patterns. To justify the ^{1}H NMR assignment we also measured the ^{13}C NMR spectra, which was likely to reflect the structural difference of atropisomers in its peak-splitting pattern because of a wider chemical shift range than that of the ^{1}H NMR spectra. Figure 3 shows the ^{13}C NMR spectra of **1c**—**1e** and Table 5 shows the ^{13}C NMR chemical shift for four atropisomers together with those of **1a** and **1b**. The signals at δ=114—157 were assigned to those of 5,10,15, 20-positioned carbons, benzene and pyrrole. Six peaks at δ=100.1—100.7, 76.9—77.1, 76.3—76.6, 72.2—73.4, 69.2—69.7, and 60.6—60.7 were assigned to those for C-1, C-3, C-5, C-2, C-4, and C-6 of the glucose according to our previous data.[25] From these results, all of the porphyrins were identified as being the target ones. Undoubtedly, ^{13}C NMR gave a more distinctive difference in its peak-splitting pattern than did ^{1}H NMR. Attention should be paid to the chemical shifts of three isomers (**1c**—**1e**), especially to those for C-1 and C-2 and at around δ=156, 135, 130, and 115. The spectral patterns of all these signals became more complicated in the order **1c**<**1d**<**1e**, indicating the same result as that of the ^{1}H NMR studies. This apparently demonstrates that **1c**, **1d**, and **1e** can be assigned to the $\alpha\beta\alpha\beta$-, $\alpha\alpha\beta\beta$-, and $\alpha\alpha\alpha\beta$-isomers. It is noteworthy that the

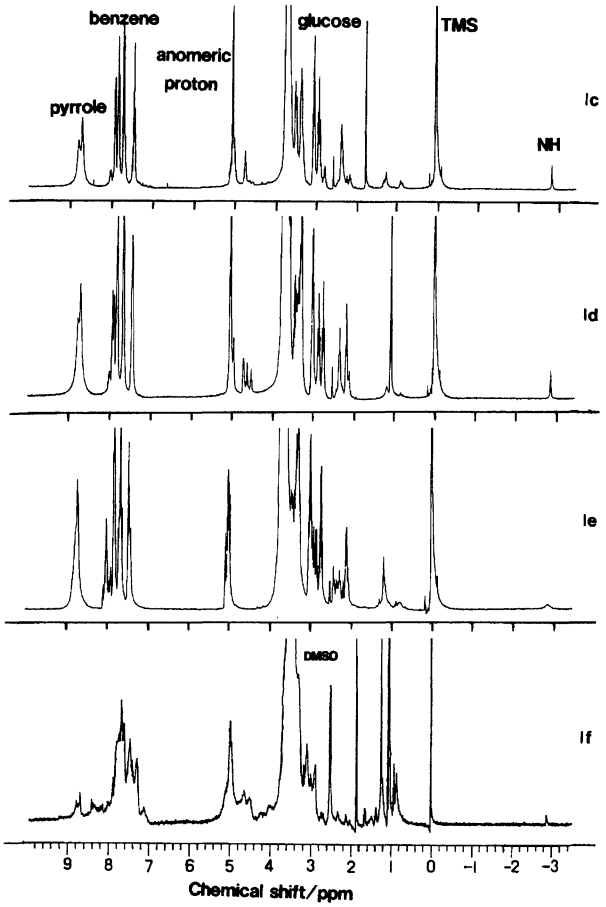


Fig. 2. ^{1}H NMR (400 MHz) spectra of **1c**, **1d**, **1e**, and **1f** in DMSO-d_6 contaning one drop of D_2O.

Table 5. ^{13}C NMR Chemical Shifts for the Glycosylated Porphyrins (**1a**—**1f**)

Compound	Chemical Shift/ppm												
	C-1	C-2	C-3	C-4	C-5	C-6	Benzene + Pyrrole + meso-positioned carbons						
1a	100.5	73.4	77.1	69.7	76.6	60.7	157.4	135.1				119.5	114.5
1b	100.3	73.3	76.9	69.6	76.5	60.6	155.8	142.2	128.3	127.8	122.3	119.5	114.5
1c	100.5	72.2	77.0	69.2	76.3	60.6	156.6	135.6	130.4			120.4	115.4
									129.9				115.1
1d	100.6	72.5	76.9	69.3	76.4	60.6	156.7	135.9	130.6			120.4	115.4
	100.4	72.2					156.5	135.6	130.2				115.2
									129.9				114.9
1e	100.7	72.6	76.9	69.3	76.4	60.6	156.9	136.0	130.6			120.4	115.4
	100.6	72.4			76.3		156.8	135.7	130.4				115.2
	100.4	72.2					156.6	135.5	130.3				115.0
							156.3	135.2	129.9				114.9
1f	100.2	72.7	77.0	69.4	76.5	60.6	156.2*	134.9	130.7			120.6	114.9
	100.1	72.3					156.0	134.5	130.4				
							155.7		130.2				
							152.8		129.3				

Solvent: DMSO-d_6 *: Signals are smaller than the other ones.

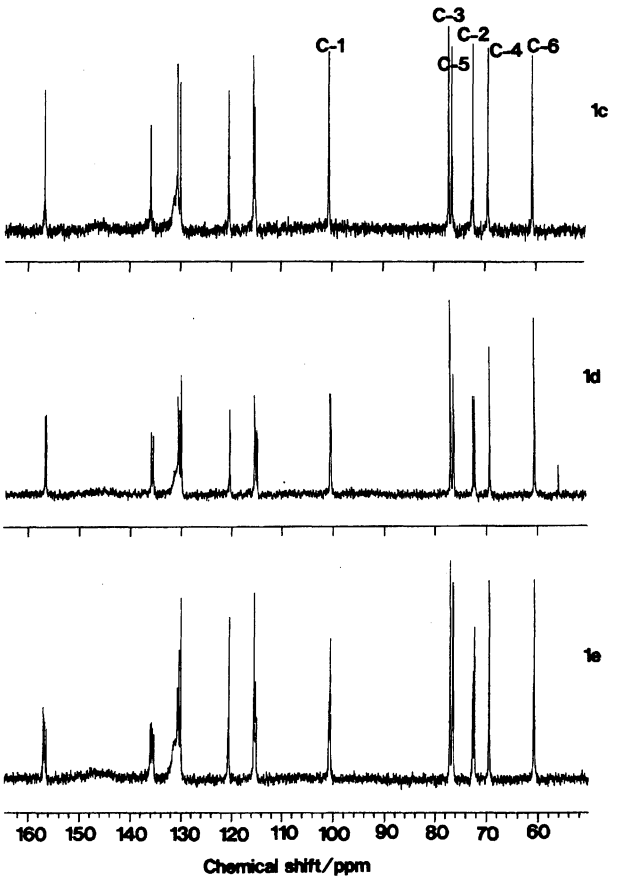


Fig. 3. ^{13}C NMR spectra of **1c**, **1d**, and **1e** in DMSO-d_6.

three atropisomers can be clearly assigned according to ^{13}C NMR peak-splitting pattern.

Identification and Assignment of the Water-Soluble Picket-Fence Porphyrin (1f): The ^{1}H NMR spectrum of **1f** is shown in Fig. 2. The characteristic signals for pyrrole and pyrrolic NH were clearly detected at δ=8.8 and −2.9, respectively. Furthermore, as described in the Experimental section and shown in Table 5, FABMS (Fig. 4), an elementary analysis and the ^{13}C NMR spectrum (Fig. 5) strongly substantiates that **1f** is one of the four atropisomers of *o*-STP. Since porphyrins **1c**, **1d**, and **1e** could be assigned to $\alpha\beta\alpha\beta$-,$\alpha\alpha\beta\beta$-, and $\alpha\alpha\alpha\beta$-isomers, the remaining **1f** could necessarily be assigned to the $\alpha\alpha\alpha\alpha$-isomer (picket-fence porphyrin). This assignment is substantiate by the following two facts: (1) The resonance of the proton for pyrrole at 8.8 ppm is split into a few peaks at around 8.3 ppm (see Fig. 2), which can explain the disorder of the porphyrin plane induced by the strong hydrogen bonds among the four glucose molecules (protic cavity), as shown in Fig. 6. This hydrogen bond is discussed later. (2) The peaks for the protons of glucose are shifted upfield (δ=1.9 and 0.8—1.2), as compared with the other *o*-STP, because of the magnetic anisotoropy effect of the porphyrin ring. As for this effect, it is presumed that the glucose moiety of **1f** is likely to bend over the porphyrin ring due to the above-mentioned strong hydrogen bonds among the glucose molecules.

It is important to have succeeded in the synthesis of a water-soluble picket-fence porphyrin, which has only been reported by Valiotti et al.[26]

General Properties of the Glycosylated Porphyrins. Although porphyrins (**1a**—**1f**) are weakly soluble in water and methanol, they are very soluble in DMSO or an aqueous solution containing a small percentage (5—20%, v/v) of methanol or 1,4-dioxane. They are gradually hydrolyzed below pH 1.0, but are very stable in a neutral solution. When stored in a desiccator the solid porphyrins are stable to isomerization. However, **1c** and **1e** are very gradually isomerized to **1d** in water. Picket-fence porphyrin (**1f**) did not isomerize in water at room temperature for at least 4 months.

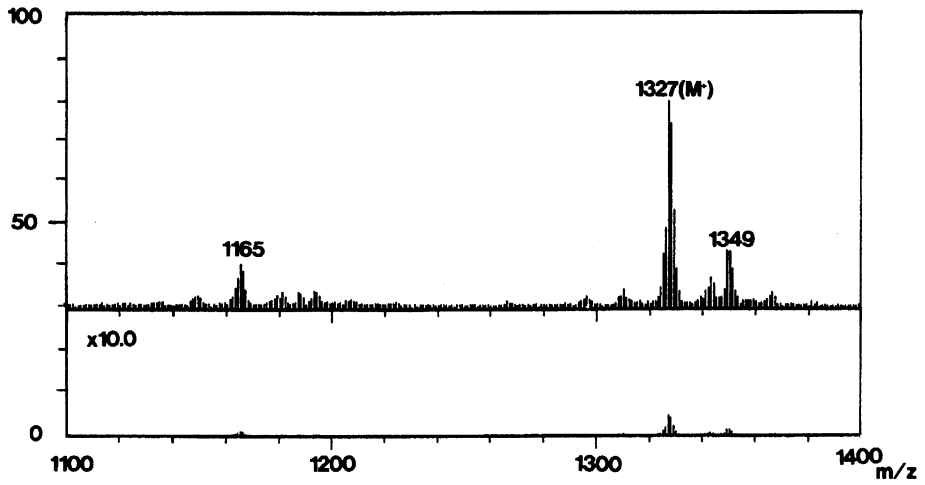


Fig. 4. FABMS spectrum of $\alpha\alpha\alpha\alpha$-isomer (**1f**).

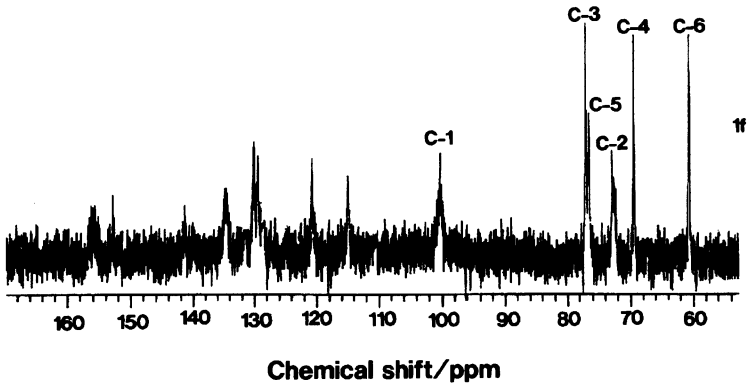
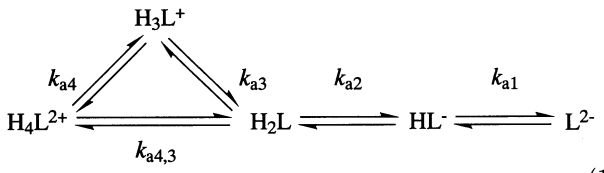


Fig. 5. ^{13}C NMR spectrum of $\alpha\alpha\alpha\alpha$-isomer (**1f**) in DMSO-d_6.

Because of the neutral type of porphyrins they did not show any adsorption on the surface of the glassware, or any aggregation over its concentration range up to 10^{-4} mol dm^{-3}.

The solubilities of porphyrins in water increased in the order **1d**<**1e**<**1c**<**1f**≃**1b**≃**1a**. This order was the same as that of the R_f values (**1d**, 0.51; **1e**, 0.49; **1c**, 0.45; **1f**, 0.31; **1b**, 0.30; **1a**, 0.27 in 2-propanol–ethyl acetate–water (3 : 6 : 2, v/v)). The smaller the R_f value became, the more the solubility increased. There was no remarkable difference in the solubility among **1d**, **1e**, and **1c** or among **1f**, **1b**, and **1a**. It is assumed that the difference in the solubility is based on the hydrogen bond between the hydroxyl groups in the adjacent glucose molecules which are located on the same side of the porphyrin ring plane. For example, **1d** has two sets of such glucose molecules. Similarly, although **1e** has one set, there is no set in **1a**, **1b** or **1c**. The structures of the four atropisomers (**1c**—**1f**) are shown in Fig. 6. As the number of the set increases, the solubility decreases. This implies that the hydrogen bonds between the two adjacent glucose molecules cannot be easily replaced by the hydrogen bonds between the water and glucose molecules. In spite of having two such sets, **1f** is very soluble in water. Picket-fence porphyrin has four very hydrophilic glucose moleucles which create a very protic cavity on one side of the porphyrin ring plane so that the solubility into water increases.

Acid Dissociation Constants and Spectral Data of the Glycosylated Porphyrins. The proton-dissociation equilibria can be expressed as follows:



(1)

Here, L denotes the undissociable part of the porphyrin and k_{a4}, k_{a3}, k_{a2}, and k_{a1} are the acid-dissociation constants. The $k_{a4,3}$ is the acid-dissociation constant when two protons are released simultaneously in the process of H_4L^{2+} to H_2L. The k_{a4} and k_{a3} were sufficiently close that the isobestic point for the separate process could not be distinguished, except for **1f**. On the other hand, the k_{a1} and k_{a2} were not obtained, because these glycosylated porphyrins were not sufficiently stable in an alkaline solution to permit their exact determinations. We could thus obtain only the $k_{a4,3}$, which was

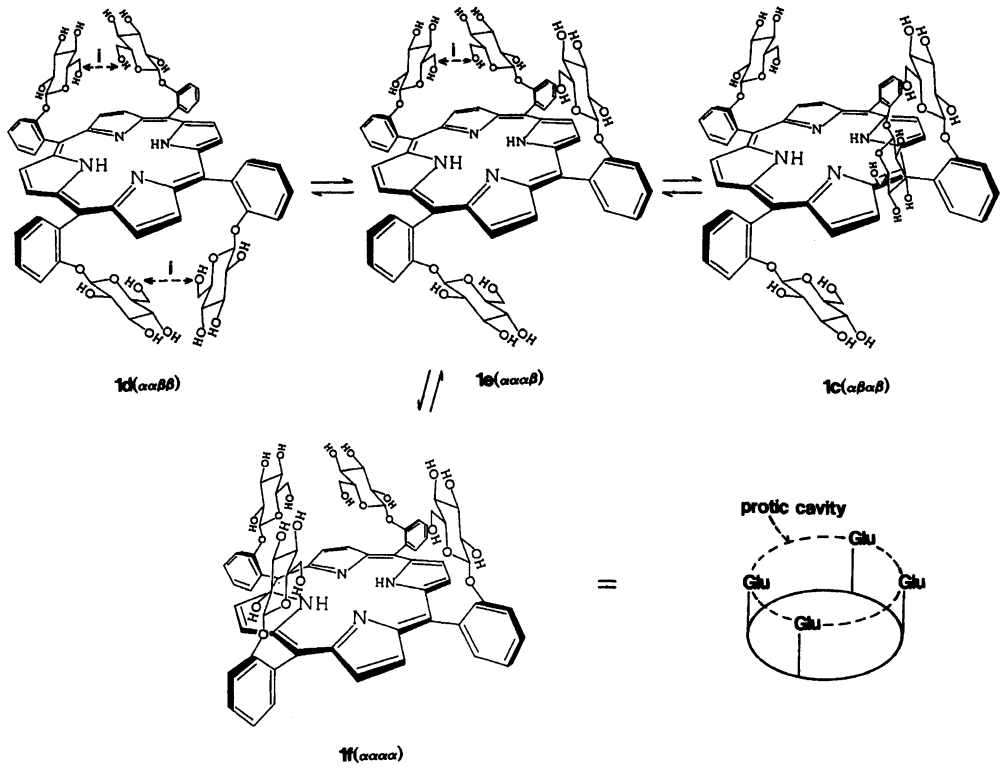


Fig. 6. Structures for the four atropisomers of tetrakis(*o*-substituted phenyl)porphine. i, interaction based on the hydrogen bonds; Glu, glucose.

determined spectrophotometrically at an ionic strength of 0.2 (NaCl) and at 25±0.1°C. For a representative example, the absorption spectra of **1a** at pH 8.0 and and 1.0 are shown in Fig. 7. Spectra A at pH 1.0 and B at pH 8.0 correspond to the H_4L^{2+} and H_2L species, and the Soret bands, which are useful for the determination of trace amounts of metals, are at 442 and 418 nm, respectively. These Soret bands have very large molar absorptivities (A: 4.07×10^5, $mol^{-1}\,dm^3\,cm^{-1}$), similarly to conventional *meso*-substituted porphyrins. At the region of 500—700 nm there are a few smaller band, the so-called Q bands ($\varepsilon=10^3$—10^4 $mol^{-1}\,dm^3\,cm^{-1}$). The absorption maxima (λ_{max}), apparent molar absorptivities (ε) of Soret bands of H_4L^{2+} and H_2L species and acid dissociation constants ($pK_{a4,3}$) of six glycosylated porphyrins are summarized in Table 6 in comparison with those of conventional anionic porphyrin(5,10,15,20-tetrakis(4-sulfophenyl)porphine, T(4-SP)P)[27] and cationic porphyrin (5,10,15,20-tetrakis(1-methyl-4-pyridinio)porphine, TMPyP).[28] In every glycosylated porphyrins, except for **1f**, λ_{max}, ε, and $pK_{a4,3}$ are almost intermediate between those of T(4-SP)P and TMPyP. It is very reasonable that spectral properties of the neutral-type porphyrins are intermediate between those of the cationic- and anionic-type porphyrins. The $pK_{a4,3}$ values for *p*- and *m*-STP (**1a** and **1b**) were larger than those for *o*-STP (**1c**, **1d**, and **1e**), being assumed that the disorder of the porphyrin plane was induced by a steric hindrance and an intramolecular hydrogen bond of the glucose moiety at the *o*-position of 5,10,15,20-phenyl substituents. However, this disorder of the porphyrin plane gave no remarkable difference in λ_{max} and ε among the glycosylated porphyrins. The specific spectral properties of **1f** also might be explained by the disorder of the porphyrin plane induced by the "protic cavity".

Reactivities of the Glycosylated Porphyrins with Copper (II) Ion. Figure 8 shows the spectra of **1a**, **1b**, and their complexes with copper(II) ion. The complexation reaction was completed within a few minutes by using 1.0 cm^3 of a 0.5% (g/v) hydroxylamine hydrochloride solution as an accelerator. After the formation of the complex in a neutral solution the pH was lowered to 1.0 in order to separate the Soret band of porphyrin from that of its complex. The complex remained stable at this pH value. The λ_{max} and ε of the glycosylated porphyrin-copper(II) complex are summarized in Table 7 along with those of T(4-SP)P and TMPyP. These complexes gave the almost same λ_{max}, which were comparable to those of T(4-SP)P, though the **1f**–copper(II) complex was not sufficiently stable to permit a constant measurement of its λ_{max} and ε. The reactivity of **1a** and **1b** with various metal ions was investigated, the results being summarized in Table 8. Both porphyrins reacted with cadmium(II), copper(II), and palladium(II) to form colored stable complexes with high molar absorptivities (ε), but with silver(I), palladium(II), mercury(II), and

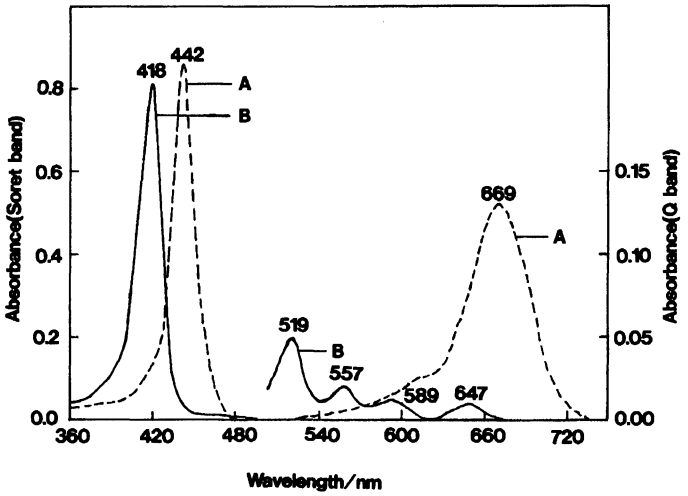


Fig. 7. Absorption spectra of **1a**. **1a**, 2.1×10^{-6} mol dm^{-3}; A, H_4L^{2+}, pH, 1.0; B, H_2L, pH, 8.0; reference, water.

Table 6. Spectral Properties of the Glycosylated Porphyrins

Porphyrin	$\frac{\lambda_{max}}{nm}$, $\left(\frac{\varepsilon}{10^{-5}mol^{-1}dm^3cm^{-1}}\right)$[a]		Acid dissociation constant[b]		
	H_2L (pH8.0)	H_4L^{2+} (pH1.0)			
	Soret band		pK_{a4}	$pK_{a4,3}$	pK_{a3}
1a(para)	418	442		4.75	
	(3.8)	(4.1)			
1b(meta)	416	439		4.05	
	(4.3)	(3.9)			
1c($\alpha\beta\alpha\beta$)	416	434		2.90	
	(4.9)	(4.1)			
1d($\alpha\alpha\beta\beta$)	414	433		3.32	
	(4.0)	(2.5)			
1e($\alpha\alpha\alpha\beta$)	415	434		3.34	
	(4.4)	(3.4)			
1f($\alpha\alpha\alpha\alpha$)	438	458	4.16		6.78
	(1.0)	(1.2)			
T(4-SP)P[c]	413	434	4.86		4.96
	(5.1)	(5.0)			
TMPyP[d]	423	444	0.80		2.06
	(2.6)	(3.2)			

a) ε: Molar absorptivity. b) H_3L^+ (pK_{a4}, pK_{a3}), H_4L^{2+} ⇌ H_2L ($pK_{a4,3}$), I=0.2 (NaCl), at 25±0.1 °C. c) 5,10,15,20-Tetrakis (4-sulfophenyl) porphine.[27] d) 5,10,15,20-Tetrakis (1-methyl-4-pyridinio) porphine.[28]

Table 7. Absorption Maxima and Apparent Molar Absorptivities of the Porphyrin–Cu(II) Complexes

	1a	**1b**	**1c**	**1d**	**1e**	**1f**	T(4-SP)P[a]	TMPyP[b]
$\frac{\lambda_{max}}{nm}$	417	415	415	415	415	—	413	434
$\frac{\varepsilon}{10^{-5}mol^{-1}dm^3cm^{-1}}$	4.2	3.8	4.3	4.1	4.3	—	4.8	2.3

a) 5,10,15,20-Tetrakis(4-sulfophenyl)porphine. b) 5,10,15,20-Tetrakis(1-methyl-4-pyridinio)porphine.

zinc(II) to form unstable complexes or to give a very low ε. Mercury(II) underwent a catalytic reaction. This reactivity had almost the same tendency as that of T(4-SP)P.

Comparison of 1a with T(4-SP)P as the Spectrophotometric Reagent for Copper(II). Ta-

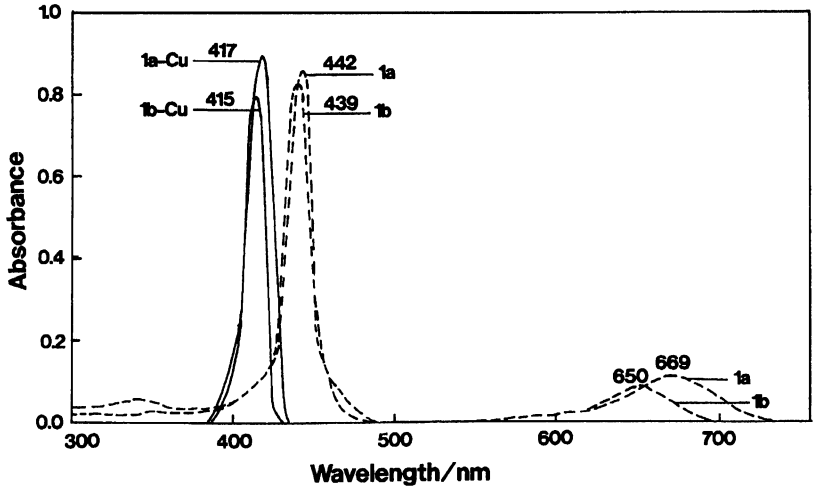


Fig. 8. Absorption spectra. **1a** and **1b**, 2.1×10^{-6} mol dm^{-3}; complexes, 4.2×10^{-6} mol dm^{-3}; pH, 1.0; reference, water (**1a**, **1b**) and reagent blank (complexes).

Table 8. Reactivities of **1a** and **1b** with Various Metal Ions

Metal ion	**1a** pH	**1a** λ_{max} / nm	**1a** ε / 10^{-5}mol^{-1} dm^3 cm^{-1}	**1b** pH	**1b** λ_{max} / nm	**1b** ε / 10^{-5}mol^{-1} dm^3 cm^{-1}
Ag (I)	>10	430	0.2	>10	428	0.1
Cd (II)	ca. 10	437	4.5	ca. 10	436	3.4
Cu (II)	4–7	417	4.2	3.5—8	415	3.8
Hg (II)	ca. 11	Inconstant	Inconstant[a)]	ca. 11	Inconstant	Inconstant[a)]
Pb (II)	ca. 11	466—467	1.0	ca. 11	466	2.0
Pd (II)	3–5	416—417	2.0	2—5	414—415	2.4
Zn (II)	>10	429	0.5	>10	428	0.2

a) Catalytic reaction.

Table 9. Comparison of the Conditions for the Spectrophotometric Determination of Copper in **1a** and T(4-SP)P[a)]

Porphine	pH	λ / nm	ε / mol^{-1} dm^3 cm^{-1}	Copper(II) concentration / ppb	Interference of diverse ion
1a	6→<1.0	417	4.2×10^5	8—240	
T(4-SP)P	4→2.5	434	4.8×10^5	6—60	Zn(II)

a) 5,10,15,20-Tetrakis(4-sulfophenyl)porphine.

ble 9 shows a comparison of the conditions for the spectrophotometric determination of copper using **1a** and T(4-SP)P. For a determination of copper using T(4-SP)P, it was preferred to use the absorption band of the free H_4L^{2+}at 434 nm, since the band of the copper(II) complex at 413 nm overlapped with the shoulder peak of H_4L^{2+}, which showed a somewhat poor reproducibility.[27)] In the case of **1a**, the band of the copper(II) complex at 417 nm could be used due to the larger difference of λ_{max} between the bands of the free H_4L^{2+} and copper(II) complex. The difference was 25 nm in **1a**, but 21 nm in T(4-SP)P. On the other hand, the concentration of copper(II) was determined in the 8—240 ppb range in **1a** and 6—60 ppb in T(4-SP)P. The former had about a 4-times wider dynamic range. Furthermore, upon the influence of diverse ions there was little difference between **1a** and T(4-SP)P, except for zinc(II). In T(4-SP)P only zinc(II) interfered with the determination of copper, because T(4-SP)P formed molecular aggregates under acid conditions where the zinc(II) complex was decomposed quantitatively; the absorbance did not conform to Beer's law. However, since the neutral type of glycosylated porphyrins did not show any aggregration, even under acid conditions, there was no interference of zinc(II). These characteristics strongly show that **1a** is a more excellent spectrophotometric reagent for copper(II) than T(4-SP)P. A report on the sensitive and selective spectrophotometric determination of copper using **1a** is being prepared.

In conclusion, these neutral-type glycosylated porphyrins improved the aggregation and adsorption char-

acteristics of the anionic or cationic porphyrin so far prepared without any marked change in the other analytical important properties, and can be used as an excellent reagent for the spectrophotometric determination of trace amounts of ions. Especially, **1a**, giving a moderate high yield and very large ε, but no containing any atropisomers, was a very useful spectrophotometric reagent for copper(II) because of a wider dynamic range of copper(II) concentration and no influence of zinc(II). New applications of these water-soluble glycosylated porphyrins can also be expected.

References

1) T. Yotsuyanagi, *Kagaku*, **35**, 233 (1980); H. Ishii, *Bunseki*, **1981**, 865.

2) S. Igarashi and T. Yotsuyanagi, *Chem. Lett.*, **1984**, 1871.

3) K. Kohata, T. Odashima, and H. Ishii, *Anal. Sci.*, **2**, 417 (1986).

4) K. Kohata, Y. Yamaguchi, H. Higashio, T. Odashima, and H. Ishii, *Chem. Lett.*, **1992**, 477.

5) R. A. Freitag, J. A. Mercer-Smith, and D. G. Whitten, *J. Am. Chem. Soc.*, **103**, 1229 (1981); R. A. Freitag and D. G. Whitten, *J. Phys. Chem.*, **87**, 3918 (1983); M. J. Crossley, L. D. Field, A. J. Forster, M. M. Harding, and S. Sterhelt, *J. Am Chem. Soc.*, **109**, 341 (1987); K. Hatano, K. Anzai, N. Nishino, and K. Fujii, *Bull. Chem. Soc. Jpn.*, **58**, 3653 (1985).

6) M. Momenteau, J. Mispelter, B. Look, and E. Bisagni, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 189; A. R. McIntosh, A. Siemiarczuk, J. R. Bolton, M. J. Stillmann, T. -F. Ho, and A. C. Weedon, *J. Am. Chem. Soc.*, **105**, 7215 (1983); J. T. Groves and R. S. Myers, *J. Am. Chem. Soc.*, **105**, 5791 (1983); D. Mansuy, P. Battioni, J. -P. Renald, and P. Guerin, *J. Chem. Soc., Chem. Commun.*, **1985**, 155; J. Weiser and H. A. Staab, *Tetrahedron Lett.*, **26**, 6059 (1985); J. P. Collmann, J. I. Brauman, J. P. Fitzgerald, P. D. Hampton, Y. Naruta, J. W. Sparapany, and J. A. Ibers, *J. Am. Chem. Soc.*, **110**, 3477 (1988); Y. Naruta, F. Tani, and K. Maruyama, *Chem. Lett.*, **1989**, 1269.

7) M. C. Gonzalez and A. C Weedon, *Can. J. Chem.*, **63**, 602 (1984).

8) P. Maillard, J-L. G. Kern, and M. Momenteau, *J. Am. Chem. Soc.*, **111**, 9125 (1989).

9) P. Maillard, J-L. G. Kern, C. Huel, and M. Momenteau, *J. Org. Chem.*, **58**, 2774 (1993).

10) T. Ken Miyamoto, S. Takagi, T. Hasegawa, S. Tsuzuki, E. Takahashi, K. Okude, I. Banno, and Y. Sasaki, *Bull. Chem. Soc. Jpn.*, **60**, 1649 (1987).

11) R. Roy and F. D Tropper, *Synth. Commun.*, **20**, 2097 (1990).

12) D. Ress, H. P. Kleine, D. V. Weinberg, R. J. Kaufman, and R. S. Sidhu, *Synthesis*, **1981**, 883.

13) J. Bogusiak and W. Szeja, *Pol. J. Chem.*, **59**, 693 (1985).

14) J. Rothermel and H. Faillard, *Carbohydr. Res.*, **196**, 29 (1990).

15) S. M. Jain, S. Devi, S. Bani, S. Singh, and G. B. Singh, Indian *J. Chem., Sect. B*, **27B**, 1019 (1988).

16) R. Roy, F. D. Tropper, A. Romanowska, M. Letellier, L. Cousineau, S. J. Meunier, and J. Boratynski, *Glycoconjugate J.*, **8**, 75 (1991).

17) B. Schuster, M. Winter, and K. Herrmann, *Z. Naturforsch., Sect. C: Biosci.*, **41**, 511 (1986).

18) A. D. Adler, F. R. Longo, J. D. Finarelli, J. Goldmacher, J. Assour, and L. Korsakoff, *J. Org. Chem.*, **32**, 476 (1967).

19) J. S. Linalsey, I. C. Schreiman, H. C. Hsu, P. C. Kearney, and A. M. Marguerettaz, *J. Org. Chem.*, **52**, 827 (1987).

20) R. W. Wagner, D. S. Lawrence, and J. S. Lindsey, *Tetrahedron Lett.*, **28**, 3069 (1987).

21) A. W. van der Made, E. J. H. Hoppenbrouwer, R. J. M. Nolte, and W. Drenth, *Recl. Trav. Chim. Pays-Bas*, **107**, 15 (1988).

22) L. K. Gottwald and E. F. Ullman, *Tetrahedron Lett.*, **36**, 3071 (1969).

23) S. Takagi, T. K. Miyamoto, and Y. Sasaki, *Bull. Chem. Soc. Jpn.*, **58**, 447 (1985).

24) K. Hatano, K. Anzai, A. Nishino, and K. Fujii, *Bull. Chem. Soc. Jpn.*, **58**, 3653 (1985).

25) K. Kohata, E. Ohtaki, C. Suzuki, and H. Meguro, *Nippon Kagaku Kaishi*, **1982**, 1609.

26) A. Valiotti, A. Adeyeno, R. F. X. Williams, L. Ricks, J. North, and P. Hambright, *J. Inorg. Nucl. Chem.*, **43**, 2653 (1984).

27) J. Itoh, T. Yotsuyanagi, and K. Aomura, *Anal. Chim. Acta*, **74**, 53 (1975).

28) T. Yotsuyanagi and J. Itoh, *Kagaku no Ryoiki*, **31**, 146 (1977).