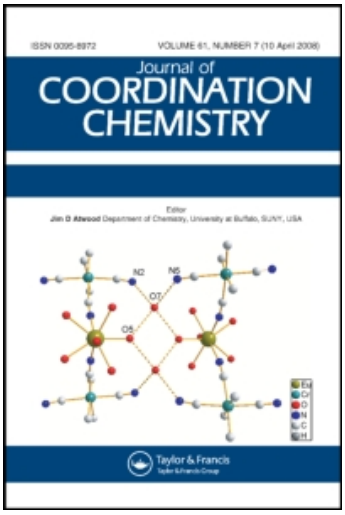

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NEW LIPOPHILIC BISPOCKET-PORPHYRINS FOR CHEMOTHERAPY OF CANCER (PART I); PREPARATION AND PROPERTIES OF 5,10,15,20-TETRAKIS(3',5'-DI-*t*-BUTYLPHENYL) PORPHYRIN METAL COMPLEXES[1]

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NEW LIPOPHILIC BISPOCKET-PORPHYRINS FOR CHEMOTHERAPY OF CANCER (PART I); PREPARATION AND PROPERTIES OF 5,10,15,20-TETRAKIS(3',5'-DI-*t*-BUTYLPHENYL) PORPHYRIN METAL COMPLEXES[1]

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A series of highly lipophilic bis-pocket porphyrins, 5,10,15,20-tetrakis(3', 5'-di-*t*-butylphenyl)porphyrin with five metals (copper(II), nickel(II), zinc(II), cobalt(II), and iron(III)), have been prepared and characterized on the basis of electronic, infrared and ^{1}H NMR spectra. Although electronic spectra of these porphyrins are substantially the same as their prototype tetraphenylporphyrin complexes of the respective metals, their solubility in common organic solvents such as chloroform, benzene, and hexane is dramatically enhanced.

KEYWORDS: porphyrin, bis-pocket, lipophilic, cancer, chemotherapy, anti-tumor

INTRODUCTION

Synthetic metalloporphyrins possess a number of attractive properties for bioclinical applications.[2] One of the properties is the tendency of tetraphenylporphyrin derivatives to accumulate in malignant tissues,[3] and this enables, for example, radioactive metal porphyrins with γ-emitters to be utilized as tumor markers.[4] This effect is presently attributed to the lipophilic nature of solid tumors and their activated metabolism.[7] On the other hand, the artificial methylation of DNA, RNA and histones inhibited cell replication which is considered to be among the key factors in the chemotherapy. We focused upon bispocket porphyrins[6–12] as possible methyl carriers[13] because of their expected highly lipophilic nature as well as their proper protection towards their metal centers. In spite of their potential importance,

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only very few moderately protected porphyrins were know when we began this work; for example, no simple 3,5-di-*t*-butylphenyl porphyrins were reported, though 4-methoxy and 4-hydroxy derivatives were known.[14–18] We have hence prepared the title porphyrins and their basic chemistry was studied prior to chemotherapy experiments. These porphyrins have enhanced solubility toward many organic solvents without significant changes of their electronic spectra, compared to the corresponding tetraphenylporphyrins.

EXPERIMENTAL

Chemicals

Alumina for column chromatography was obtained from Aldrich. All the other reagents were purchased from Wako Chemicals or Tokyo Kasei Kogyo. Organic solvents were purified according to standard procedures[21] and stored under an N_2 atmosphere.

Instrumentation and Analytical Methods

UV-vis spectra were obtained with a Shimazu spectrophotometer. Infrared spectra were obtained on a JEOL spectrophotometer. Proton NMR spectra were obtained by using Hitachi-R24B or Bruker WM-270 with TMS as an internal reference. Elemental analyses were carried out at the Laboratory of Organic Analyses, Department of Chemistry, Faculty of Science, the University of Tokyo. The existence of Cl was checked by Beilstein test before sending the sample of the laboratory.

PREPARATION OF COMPOUNDS

5,10,15,20-tetrakis(3',5'-di-t-butylphenyl)porphyrin (1)

The porphyrin-free base was synthesized by condensation of 3,5-di-*t*-butylphenyl aldehyde (**0**) and pyrrole. Forty-three grams of **0** were dissolved in 300 mL of glacial acetic acid in a three-necked round bottomed flask fitted with Allihn condensers and a dropping funnel. The solution was heated and stirred with a magnetic bar. Pyrrole (14 mL) was poured down into the boiling solution. The resulting black mixture was allowed to reflux for 120 min and then left to stand over night. The purple brown crystals thus precipitated were collected over a frit and washed with methyl alcohol. Finally, the crude product was purified by almina chromatography with chloroform as eluent.

Yield: 7.0 g (16% from **2**). UV-vis λmax/nm (chloroform) ($\varepsilon/10^3$): 421 (561), 515(21), 551 (11). IR(KBr): NH vibrates at 980 cm^{-1}. ^1H NMR in $CDCl_3$ (25°C); δ = 8.9 (8 H, s, pyrrole β-H), 8.1 (8 H, s, ortho phenyl H), 7.8 (4H, t, para phenyl H), 1.5 (72 H, s, t-butyl H), −2.6 (2 H, s, internal pyrrole H). *Anal.* Calcd. for $C_{76}H_{94}N_4$(%): C, 85.82; H, 8.91; N, 5.27; Found: C, 85.83; H, 9.07; N, 5.16.

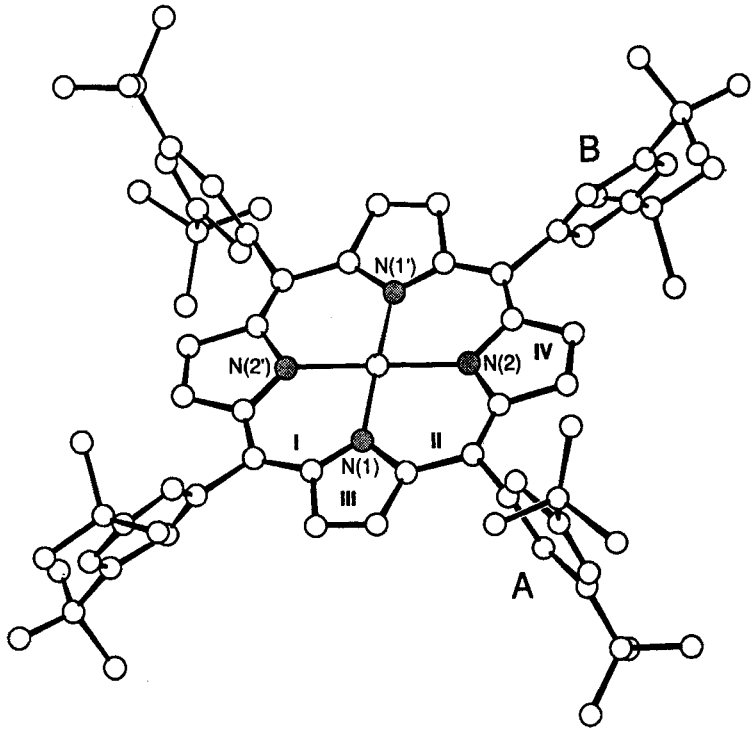

Figure 1 Structure of 5,10,15,20-tetrakis(3',5'-di-*t*-butylphenyl)porphyrin metal complex

5,10,15,20-tetrakis(3', 5'-di-t-butylphenyl)porphyrin Fe(III) chloride (2)

To a mixed solvent of toluene (45 mL) and acetic acid (450 mL) was added 0.60 g (0.67 mmol) of **1**. The solution was purged with Ar for thirty minutes to expel oxygen. Under an Ar atmosphere, $FeSO_4 \cdot 7H_2O$ (1.2 g), anhydrous sodium acetate (1.7 g), and pyridine (2 mL) were added to the solution. The resulting solution was heated to reflux (383 K in vapor) for 2 h. The color changed from dark brown-green to brown. After air was passed through the solution for 30 min, toluene was added and stirred well. The organic layer was then separated and washed subsequently with water, HCl (0.4 N), water and then with a dilute sodium hydroxide solution. This compound was purified by alumina chromatography; the chloroform solution of the product was placed in a silica-gel column (Wako gel C-200) prepared as a slurry in chloroform. The first eluate was the remaining free base. (**1**). The second eluate was collected and washed with hydrochloric acid. The organic layer was separated and the solution was rotary evaporated to form purple solid. UV-vis λmax/nm (chloroform) (ε/100): 383(60.7), 420 (113), 512 (14.3), 578 (3.42), 666 (2.92), 696 (3.54). IR (KBr): NH vibration at 980 cm^{-1} disappeared. ^1H NMR in $CDCl_3$; δ 13.46 (8 H, s, β-pyrrole H), 1.28 (36 H, s, *t*-butyl H),

0.96 (36 H, s, *t*-butyl H). *Anal.* Calcd. for $C_{76}H_{92}N_4FeCl$(%): C, 79.18; H, 8.22; N, 4.86; Cl, 3.08; Found: C, 79.16; H, 7.94; N, 4.76; Cl, 3.17.

5,10,15,20-tetrakis(3',5'-di-t-butylphenyl)porphyrin Fe(III) hydroxide bishydrate (3)

To a toluene solution of **2**, sodium hydroxide (10 N) was added. After stirring for 3 days, the solution was placed in an alumina column (Merck Alumina 90) and chromatographed: the second brown eluate was separated and the solution was rotary evaporated. Further purification was performed by alumina chromatography. Yield was about 80%.

UV-vis λmax/nm (chloroform): 420, 580, 630. ^{1}H NMR in $CDCl_3$; δ ~80 (br), 2.00 (s, *t*-butyl H), 1.75 (s. *t*-butyl H). *Anal.* Calcd. for $C_{152}H_{188}N_8Fe_2O_3$(%): C, 79.83; H, 8.29; N, 4.90; Cl, 0.0, Found: C, 79.61; H, 7.89; N, 4.62; Cl, 0.00.

μ-oxo-bis[5,10,15,20-tetrakis(3',5'-di-t-butylphenyl)porphyrin Fe(III)] (4)

To a toluene solution of **2**, sodium hydroxide (10 N) was added. After stirring for 3 days, the solution was placed in an alumina column (Merck Almina 90) and chromatographed: the first green eluate was separated and the solution was rotary evaporated. Furhter purification was performed by alumina chromatography. Yield was about 20%. UV-vis λmax/nm (chloroform): 411, 574, 615. ^{1}H NMR in $CDCl_3$; δ~14 (br), 1.28 (s, t-butyl H), 0.96 (s, *t*-butyl H). *Anal.* Calcd. for $C_{76}H_{93}N_4FeO$(%) C, 80.46; H, 8.26; N, 4.94; Cl, 0.0; Found: C, 80.26; H, 7.88; N, 4.78; Cl, 0.00.

5,10,15,20-tetrakis(3',5'-di-t-butylphenyl)porphyrin Co(II) (5)

Cobalt was inserted into (**1**) by the conventional acetic acid method.[20] UV-vis λmax/nm (toluene) (ε/10^3): 415 (240), 529 (110), 607 (1.8), 649 (0.4). ^{1}H NMR in C_6D_6; δ 16.8 (8 H, pyrrole H), 14.05 (8 H, ortho phenyl H), 10.17 (4 H, para phenly H), 2.75 (36 H, t-butyl H). *Anal.* Calcd. for $C_{76}H_{92}N_4Co$(%): C, 81.47; H, 8.28; N, 5.00; Found: C, 81.38; H, 8.43; N, 5.11.

5,10,15,20-tetrakis(3',5'-di-t-butylphenyl)porphyrin Zn(II) (6)

The conventional acetic acid method was applied. In this case, large excess of zincacetate dihydride was treated with (**1**) in acetic acid. Column chromatography was done using $CHCl_3$-hexane (1:4) mixed solvent.

UX-vis λmax/nm (toluene) (ε/10^3): 404 (42, shoulder), 424 (527), 476 (3.0), 508 (3.9), 550 (22), 588(5.2). ^{1}H NMR in C_6D_6; δ 9.03 (8 H, pyrrole H), 8.42 (8 H, ortho phenyl H), 7.98 (4 H, para phenyl H), 1.50 (36 H, *t*-butyl H). *Anal.* Calcd. for $C_{76}H_{92}N_4Zn$(%): C, 81.00; H, 8.23; N, 4.97; Found: C, 81.14; H, 8.48; N, 4.88.

5,10,15,20-tetrakis(3',5'-di-t-butylphenyl)porphyrin Ni(II) (7)

The conventional acetic acid method was also applied. While we obtained **7** when $Ni(CH_3COO)_2 \cdot xH_2O$ in acetic acid was refluxed with **1**, our trial with $NiSO_4 \cdot xH_2O$ instead of the acetate salt did not work. Column chromatography was done

using hexane as the eluent. UV-vis λmax/nm (toluene) (ε/10^3): 418 (262), 490 (3.8), 528 (19.3), 614 (1.0). ^{1}H NMR in C_6D_6; δ 8.74 (8 H, pyrrole H), 7.79 (8 H, ortho phenyl H), 7.59 (4 H, para phenyl H), 1.11 (36 H, *t*-butyl H). *Anal.* Calcd. for $C_{76}H_{92}N_4Ni$(%): C, 81.48; H, 8.28; N, 5.00; Found: C, 81.14; H, 8.48; N, 4.88.

5,10,15,20-tetrakis (3',5'-di-t-butylphenyl)porphyrin Cu(II) (8)

In this case, $Cu_2(CH_3COO)_4 \cdot xH_2O$(2.0 g) was allowed to react with **1** (0.4 g) in acetic acid (150 mL). UV-vis λmax/nm (toluene) (ε/10^3): 418 (499), 470 (4.2), 500 (3.9), 540 (22.7), 576 (2.9). ^{1}H NMR in C_6D_6; δ 7.4 (broad), 1.97 (*t*-butyl H). *Anal.* Calcd. for $C_{76}H_{92}N_4Cu$(%): C, 81.13; H, 8.24; N, 4.98. Found: C, 81.16; H, 8.51; N, 5.01.

Results and Discussion

We have prepared a series of lipophilic porphyrins with moderately protected groups toward the metal centers. We chose tertiary butyl as the protecting group because its effectiveness was well known in organic chemistry.[21]

Conventional methods[22] were applied to synthesize cobalt(II), iron(III), nickel(II), zinc(II), and copper(II) compounds. The purity and structure of these porphyrins were determined by elemental analysis IR spectra and ^{1}H NMR.

The observation of CPK models of these porphyrins shows proper protection of the metal center. In general, protection of the central metals prevent them from many reactions. Using the famous picket-fence porphyrins, Collman and his coworkers showed that hydrogen complexes were stabilized over corresponding oxo dimers when the metal centers were well protected.[23] We have used the stability of hydroxy complexes as a rough indicator of protection toward metal centers.[24] While TPP and 5,10,15,20-tetrakis(3,5-dimethylphenyl)porphyrin give no hydroxy complexes in normal conditions, the framework of **1** has enough protection toward the center to form a relatively stable hydroxy complex (**3**). The treatment of **2** with sodium hydroxide gives both **3** and **4** (relative weight of products: hydroxy/oxo dimer = 5/1). A dynamic experiment shows that **1** is stable in organic solvents at room temperature (*ca.* 298 K) for at least 6 days. At higher temperatures, however, **3** is gradually converted to **4**; for example, after 8 h of healing in toluene (343 K), **3** is completely turned into **4**.

These porphyrins (**1–8**) also show amazing solubility toward many organic solvents. For example, **1** is readily soluble in chloroform; very soluble in benzene, THF, decaline and toluene; soluble in hexane, dioxane, N,N-dimethyl formamide, ether, cyclohexane and carbon tetrachloride; and sparingly sobluble in methyl and ethyl alcohol. The solubility of **1** in pentane is enhanced by a factor of 390 compared to tetraphenylporphyrin at 298 K. This enhancement is attributed to the lipophilicity of the substituted groups. The solubility factors of related porphyrins, 5,10,15,20-tetrakis(3',5'-di-(1'',1''-dimethypropyl)phenyl)porphyrin[25] and 5,10,15,20- tetrakis(4'-*t*-butylphenyl)porphyrin,[26] are 1100 and 30, respectively. This indicates we can further control the solubility, which is sometimes the key factor in synthetic chemistry, by simply substituting the phenyl groups.

In all cases, the UV-visible spectra of these porphyrins (**1–8**) show basically the same character compared to their parent TPP derivatives; therefore, we concluded

that the tert-butyl groups do not interfere with the electric distribution of the metal centers.

The anticancer activities of related compounds *in vivo* and *in vitro*[27,28] will be published elsewhere.

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