

Preparation and characterization of porphyrin chromophores immobilized on micro-silica gel beads

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Abstract

Micro-silica gel beads (**1a–c**) immobilizing dihydroxoantimony(V) tetraphenylporphyrin (SbTpp), free base tetraphenylporphyrin (H₂Tpp), and zinc(II) tetraphenylporphyrin (ZnTpp) chromophores were prepared by the reactions of 3-aminopropyl silica gel (SiO₂-NH₂) with the dihydroxoantimony(V) complex (**2a**), the free base (**2b**), and the zinc complex (**2c**) of 5-[4-(succinimidylcarbonyl)phenyl]-10,15,20-triphenylporphyrin, respectively. Absorption spectrophotometry was performed on **1a–c** using a confocal laser scanning microscope (CLSM) and determined that the yields for the immobilization of SbTpp, H₂Tpp, and ZnTpp on SiO₂-NH₂ were 31.7, 95.9, and 45.1%, respectively. Moreover, **1a** was converted into its acetyl analog (**1a'**) by the acetylation of the axial hydroxo ligands and aminopropyl of **1a** with Ac₂O, since the interactions of the SbTpp chromophore with the residual amino group on SiO₂ was observed by spectroscopic analysis using CLSM. In micro-channel reactors (MCR), the photoreaction of **1a'** with Et₂NH induced demetallation to produce **1b** immobilizing H₂Tpp chromophore. Moreover, the reaction of **1b** with Zn(OAc)₂ produced **1c** immobilizing ZnTpp chromophore. Thus, micro-silica gel beads (**1a**) were transformed into **1c** in a micro-region of a silica gel surface by way of photochemical reaction with Et₂NH and subsequent metallation with Zn(OAc)₂.

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Keywords: Porphyrin chromophore; Absorption spectrophotometry; Confocal laser scanning microscope; Micro-channel reactor

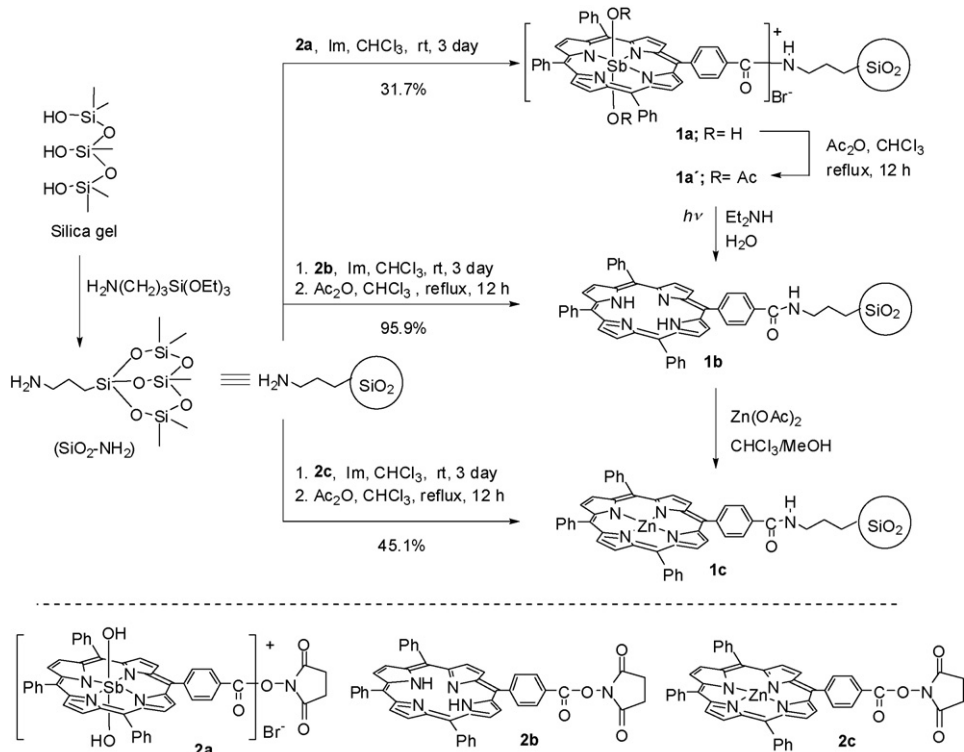
1. Introduction

Micro-beads have been used extensively as catalysts and scavengers in organic synthesis, carriers of solid phase synthesis, and selective sensors for bio-molecules (e.g. DNA chips) [1]. Additionally, the use of micro-beads to immobilize photosensitizers has been advantageous in separating sensitizers from the reaction mixture [2]. In these cases, silica gel (SiO₂) has been widely used as the carrier, because of its wide surface area and ability to be immobilized [3–7]. As photosensitizers, much attention has been paid to the porphyrin chromophores, which integrally function as energy-harvest pigments in natural photosynthesis [8,9] and as photocatalysts when operating under visible light irradiation [10]. Recently, the micro-SiO₂

beads immobilizing free base porphyrin have been prepared to act as photocatalysts in micro-channel chips [11]. Also, we have already reported on the photocatalytic abilities of a dihydroxoantimony tetraphenylporphyrin absorbed on SiO₂ beads in dechlorination [12], epoxidation [13], and sterilization [14] under visible light irradiation.

The surface and ground samples of micro-beads have been qualitatively characterized using FT-IR and UV–vis spectroscopies, diffuse reflectance spectroscopy [15], fluorescence decays [16], and quenching experiments [17]. However, quantitative characterizations of the micro-beads have been poorly analyzed. Therefore, it is desirable to develop a method that can be used confidently to precisely and quantitatively analyze the chromophores of the micro-beads in the preparation and the reaction of the micro-beads. Here, we will report on a characterization of the micro-SiO₂ beads (**1**) as immobilizers of porphyrin chromophores as well as the modification of **1** in micro-channel reactors (MCR) by using

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Scheme 1. Micro-silica gel beads (**1a–c**) immobilizing porphyrin chromophores.

a confocal laser scanning microscope (CLSM) (Scheme 1) [18].

2. Experimental

2.1. Instruments

^1H and ^{13}C NMR spectra were taken on a Bruker AC 250P spectrometer for CDCl_3 solutions, with SiMe_4 used as an internal standard. SIMS spectra were taken on a Hitachi M2000A. Matrix-assisted laser desorption/ionization mass spectra (MALDI-MS) were measured on a Bruker Daltonics Autoflex II TOF/TOF in the positive ion mode at Miyazaki Prefectural Industrial Support Foundation. FT-IR was measured on a JASCO Herschel FT/IR-300 with a Micro-20 spectrometer. UV-vis spectra of the solutions were obtained on a JASCO V-550 spectrophotometer. Reduction potentials were measured for an MeCN solution of **3f** (1×10^{-2} M) in the presence of a supporting electrolyte (Et_4NBF_4 ; 0.1 M) at a scan rate of 0.3 V s^{-1} at 23°C on a BAS cyclic voltammeter using a platinum disk working electrode, a carbon counter electrode, and an Ag/AgNO_3 reference electrode. On the ^1H NMR spectra, the proton peaks for the axial hydroxo ligands of **2a**, **3c**, and **3e** and the carboxylic group of **3b–d** were not observed due to extremely broadening.

2.2. Preparation of 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin (**3b**)

Preparation of 4-(methoxycarbonyl)phenyltriphenylporphyrin (**3a**) was performed using the Lindsey method [19]. Benzaldehyde (0.61 ml; 6.0 mmol) and $\text{BF}_3 \cdot \text{OEt}_2$

(0.1 ml) were added to a CHCl_3 solution (500 ml) of *p*-methoxycarbonylbenzaldehyde (328 mg; 2.0 mmol). A CHCl_3 solution (300 ml) of pyrrole (0.56 ml; 8.0 mmol) was then added. After the solution turned from pale orange to red-violet, it was confirmed that the Soret band appeared at 410 nm. NEt_3 (0.2 ml; 1.43 mmol) and chloranil (2.2 g; 9.0 mmol) were added to the solution and then heated at $80\text{--}90^\circ\text{C}$ for 1 h under dark conditions. After evaporation, the condensed solution was filtrated and extracted with CH_2Cl_2 /hexane (2:1, v/v). The extracted crude products were subjected to column chromatography on silica gel (Fuji Silysia BW 300) to produce **3a**.

3a: Yield 33%. ^1H NMR $\delta = -2.77$ (s, 2H), 4.09 (s, 3H), 7.70–7.76 (m, 9H), 8.18–8.22 (m, 6H), 8.23 (d, $J = 8.1$ Hz, 2H), 8.43 (d, $J = 8.1$ Hz, 2H), 8.79 (d, $J = 4.8$ Hz, 2H), 8.85 (s, 4H), 8.86 (d, $J = 4.8$ Hz, 2H).

3a (1.0 g) was dissolved in $\text{CF}_3\text{CO}_2\text{H}$ (50 ml) and aqueous HCl solution (35%; 25 ml) and then heated at $80\text{--}90^\circ\text{C}$ for 36 h. The reaction mixture was extracted with EtOAc and water. An EtOAc layer was dried with Na_2SO_4 and evaporated to produce 4-carboxyphenyltriphenylporphyrin (**3b**).

3b: Yield 95%. ^1H NMR $\delta = -2.77$ (s, 2H), 7.70–7.76 (m, 9H), 8.18–8.22 (m, 6H), 8.37 (d, $J = 8.1$ Hz, 2H), 8.52 (d, $J = 8.1$ Hz, 2H), 8.77 (d, $J = 4.8$ Hz, 2H), 8.85 (s, 4H), 8.89 (d, $J = 4.8$ Hz, 2H).

2.3. Preparation of dihydroxoantimony (V) 5-[4-(succinimidylloxycarbonyl)phenyl]-10,15,20-triphenylporphyrin bromide (**2a**)

3b (200 mg) in *N,N*-dimethylformamide (DMF; 30 ml) was added to a pyridine solution (30 ml) of SbBr_3 (542 mg) and

heated at 180 °C until the Soret band shifted from 410 to 465 nm. The solution was treated with Br₂ (5 ml) at room temperature for 30 min to give dibromoantimony(V) 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin bromide (203 mg) in a 66% yield.

An MeCN/H₂O/pyridine solution (2:2:1, v/v, 75 ml) of dibromoantimony(V) 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin bromide (200 mg; 0.20 mmol) was heated at 100 °C until the Soret band shifted from 425.6 to 417.2 nm. After evaporation, the crude dihydroxoantimony(V) 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin bromide (**3c**) was purified by column chromatography on SiO₂ using CH₂Cl₂/MeOH (9:1, 5:1, v/v).

3c: Yield 63.3%. ¹H NMR δ = 7.98 (m, 9H), 8.39 (m, 6H), 8.47 (d, *J* = 8.3 Hz, 2H), 8.51 (d, *J* = 8.3 Hz, 2H), 9.55 (d, *J* = 4.8 Hz, 2H), 9.58 (s, 4H), 9.60 (d, *J* = 4.8 Hz, 2H); MS 811 [M⁺].

A CH₂Cl₂ solution (20 ml) of **3c** (200 mg; 0.22 mmol) was treated with *N*-hydroxysuccinimide (HOSu; 34 mg; 0.27 mmol) in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC; 33 mg; 0.27 mmol) and 4-dimethylaminopyridine (DMAP; 46 mg; 0.22 mmol) for 3 days at room temperature. The resulting dicyclohexylurea was removed from the solution by filtration. After evaporation, crude **2a** was purified by column chromatography on SiO₂ using CH₂Cl₂/MeOH (9:1 and 5:1) as eluents.

2a: Yield 78%. ¹H NMR (CDCl₃) δ = 3.04 (brs, 4H), 7.81–7.92 (m, 9H), 8.30–8.33 (m, 6H), 8.66 (d, *J* = 8.3 Hz, 2H), 8.72 (d, *J* = 8.3 Hz, 2H), 9.64 (d, *J* = 4.8 Hz, 2H), 9.71 (s, 4H), 9.75 (d, *J* = 4.8 Hz, 2H); ¹³C NMR δ = 25.79, 119.47, 122.25, 122.39, 125.93, 127.51, 127.51, 129.29, 129.29, 129.50, 132.41, 133.27, 133.31, 133.69, 134.92, 134.92, 135.17, 138.98, 139.03, 144.66, 145.33, 145.35, 145.53, 145.86, 166.43, 171.08. Exact mass (MALDI-MS) calcd. for C₄₉H₃₃N₅O₆Sb [M⁺]: 908.1469. Found: 908.1638.

2.4. Preparation of 5-[4-(succinimidylloxycarbonyl)phenyl]-10,15,20-triphenylporphyrin (**2b**)

3b (330 mg; 0.5 mmol) was reacted with HOSu (70 mg; 0.6 mmol) in CH₂Cl₂ (10 ml) in the presence of DCC (155 mg; 0.75 mmol) and DMAP (62 mg; 0.5 mmol) at room temperature for 3 days. After the filtration of the dicyclohexylurea, the CH₂Cl₂ solution was evaporated and then the crude product was purified by column chromatography on SiO₂ to produce **2b**.

2b: Yield 68%. ¹H NMR δ = -2.77 (s, 2H), 2.94 (brs, 4H), 7.72–7.76 (m, 9H), 8.20–8.24 (m, 6H), 8.37 (d, *J* = 7.5 Hz, 2H), 8.52 (d, *J* = 7.5 Hz, 2H), 8.77 (d, *J* = 4.8 Hz, 2H), 8.86 (s, 4H), 8.90 (d, *J* = 4.8 Hz, 2H); ¹³C NMR δ = 25.78, 117.61, 120.55, 120.82, 124.46, 126.75, 126.75, 127.84, 128.94, 131.29, 131.29, 131.29, 131.29, 134.54, 134.54, 134.54, 134.91, 134.91, 134.91, 141.98, 141.98, 142.04, 142.04, 149.36, 162.13, 169.31. Exact mass (MALDI-MS) calcd. for C₄₉H₃₃N₅O₄ [M⁺]: 755.2308. Found: 755.2533.

2.5. Preparation of zinc 5-[4-(succinimidylloxycarbonyl)phenyl]-10,15,20-triphenylporphyrin (**2c**)

Zn(OAc)₂·2H₂O (280 mg) in MeOH (5 ml) was added to a CHCl₃ (20 ml) solution of **3b** (200 mg) and reacted at reflux

temperature for about 3 h until the Soret band shifted from 413 to 420.5 nm. After evaporation, crude zinc 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin (**3d**) was obtained.

3d: Yield 94%. ¹H NMR δ = 7.70–7.74 (m, 9H), 8.20–8.23 (m, 6H), 8.32 (d, *J* = 8.0 Hz, 2H), 8.46 (d, *J* = 8.0 Hz, 2H), 8.86 (d, *J* = 4.7 Hz; 2H), 8.90 (s, 4H), 8.91 (d, *J* = 4.7 Hz, 2H).

A CH₂Cl₂ solution of **3d** (600 mg; 0.83 mmol) was treated with HOSu (138 mg; 0.99 mmol) in the presence of DCC (258 mg; 1.2 mmol) and DMAP (102 mg; 0.83) for 3 days at room temperature. The resulting dicyclohexylurea was removed from the solution by filtration. After evaporation, the crude product of **2c** was purified by column chromatography on SiO₂ using CH₂Cl₂/MeOH (5:1) as an eluent.

2c: Yield 76%. ¹H NMR δ = 2.92 (brs, 4H), 7.72–7.78 (m, 9H), 8.20–8.24 (m, 6H), 8.38 (d, *J* = 8.3 Hz, 2H), 8.50 (d, *J* = 8.3 Hz; 2H), 8.86 (d, *J* = 4.7 Hz; 2H), 8.96 (s, 4H), 9.00 (d, *J* = 4.7 Hz, 2H); ¹³C NMR δ = 25.69, 116.05, 120.96, 121.22, 123.71, 126.34, 127.27, 128.51, 130.87, 131.72, 131.72, 131.81, 131.81, 132.15, 134.43, 134.43, 134.87, 143.06, 143.06, 149.08, 149.99, 150.06, 150.22, 150.67, 162.26, 169.62. Exact mass (MALDI-MS) calcd. for C₄₉H₃₃N₅O₄ [M⁺]: 817.1575. Found: 817.1667.

2.6. Preparation of dihydroxoantimony(V) 4-(propylcarbamoyle)phenyltriphenylporphyrin hexafluorophosphate (**3e**)

A CH₂Cl₂ solution (25 ml) containing **2b** (300 mg), propylamine (108 mg) and imidazole (Im; 135 mg) was reacted at room temperature for 17 h. The solution was extracted with CHCl₃ (100 ml) and washed with dilute aqueous HCl solution and 5% NaHCO₃ solution. After evaporation, the crude product was purified by column chromatography on SiO₂ to produce 4-(propylcarbamoyle)phenyltriphenylporphyrin in a 90% yield.

4-(Propylcarbamoyle)phenyltriphenylporphyrin (300 mg) in DMF (40 ml) was added to a pyridine solution (40 ml) of SbBr₃ (776 mg) and heated at 140 °C until the Soret band shifted from 413 to 467.2 nm. The solution was treated with Br₂ (5 ml) at room temperature for 30 min to produce dibromoantimony(V) 4-(propylcarbamoyle)phenyltriphenylporphyrin bromide in a 96% yield. Dibromoantimony 4-(propylcarbamoyle)phenyltriphenylporphyrin bromide (100 mg) was heated in a MeCN/H₂O/pyridine solution (16:12:1, v/v, 29 ml) at 100 °C until the Soret band shifted from 423.6 to 417.0 nm. After evaporation, the crude dihydroxoantimony 4-(propylcarbamoyle)phenyltriphenylporphyrin bromide was purified by column chromatography on SiO₂ using CH₂Cl₂/MeOH (9:1 and 5:1, v/v).

The counter anion was exchanged in order to enhance its solubility in organic solvents. An aqueous solution (10 ml) of AgPF₆ (55 mg) was added to an MeCN/MeOH solution (1:1, 20 ml) of dihydroxoantimony(V) 4-(propylcarbamoyle)phenyltriphenylporphyrin bromide (25 mg) and stirred at room temperature for 17 h. After the removal of AgBr by filtration, the solvent was evaporated to produce a crude **3e**. Purification was performed by column chromatography on SiO₂.

3e: Yield 49%. $^1\text{H NMR}$ δ = 1.01 (t, J = 7.3 Hz, 3H), 1.69 (q, J = 7.3 Hz, 2H), 2.99 (brs, 1H), 3.39 (t, J = 7.3 Hz, 2H), 7.85–7.98 (m, 9H), 8.14 (d, J = 8.0 Hz, 2H), 8.31–8.36 (m, 8H), 9.44 (d, J = 4.5 Hz, 2H), 9.48 (s, 4H), 9.49 (d, J = 4.5 Hz, 2H); $^{13}\text{C NMR}$ δ = 11.25, 22.46, 41.87, 121.07, 122.17, 122.17, 126.30, 127.65, 127.65, 129.49, 129.49, 133.15, 133.25, 133.25, 133.48, 134.56, 134.56, 134.58, 135.53, 138.57, 138.63, 141.30, 144.96, 145.20, 145.26, 145.29, 167.77. Exact mass (MALDI-MS) calcd. for $\text{C}_{48}\text{H}_{37}\text{N}_5\text{O}_3\text{Sb}$ [M^+]: 852.1935. Found: 852.1917.

2.7. Preparation of di(acetyloxo)antimony(V) tetraphenylporphyrin bromide (**3f**)

Dihydroxoantimony(V) tetraphenylporphyrin bromide (**3g**; 200 mg) [20] was treated with Ac_2O (4.4 ml) in MeCN (40 ml) and pyridine (0.2 ml) at refluxing temperature for 4 h. After evaporation, di(acetyloxo)antimony(V) tetraphenylporphyrin bromide (**3f**) was purified by reprecipitation of the CHCl_3 solution of **3f** with hexane.

3f: Yield 79%. $^1\text{H NMR}$ δ = -0.90 (s, 6H), 7.90–8.00 (m, 12H), 8.31–8.40 (m, 8H), 9.62 (s, 8H); $^{13}\text{C NMR}$ δ = 19.55, 123.08, 128.04, 130.13, 134.01, 134.65, 137.80, 146.02, 164.72; UV-vis λ_{max} (nm) (log ϵ) 420 (5.66), 552 (4.30), 593 (4.00).

2.8. Preparation of 3-aminopropyl silica gel ($\text{SiO}_2\text{-NH}_2$)

Micrometer-sized silica gel beads were purchased from Fujii Silysia Chemical Ltd. Silica gel beads (10 g) were reacted with $\text{H}_2\text{N}(\text{CH}_2)_3\text{Si}(\text{OEt})_3$ (5.78 g) in toluene (30 ml) for 1 day to produce the 3-aminopropyl silica gel ($\text{SiO}_2\text{-NH}_2$). The beads were separated by filtration and washed with CHCl_3 and MeOH. The physical properties of the prepared $\text{SiO}_2\text{-NH}_2$ were as follows: average diameter = 56.7 μm , area = 272 $\text{m}^2 \text{g}^{-1}$, pore volume (P) = 0.49 $\text{cm}^3 \text{g}^{-1}$. Density (W/V) was calculated to be 1.06 g cm^{-3} by Eq. (1), where the number 2.2 is the density of quartz and W and V represent the mass and the volume of $\text{SiO}_2\text{-NH}_2$ beads, respectively.

$$\frac{W}{V} = \frac{1}{2.2^{-1} + P} \quad (1)$$

2.9. General procedure for the immobilization of **2** on $\text{SiO}_2\text{-NH}_2$

A typical procedure is the reaction of $\text{SiO}_2\text{-NH}_2$ with 11.9 meq of the molar equivalent (a) of **2a** against the amino content of $\text{SiO}_2\text{-NH}_2$. A CH_2Cl_2 solution (300 μl) of **2a** (1 mM; 0.99 mg ml^{-1}) and a 300 μl CH_2Cl_2 solution of Im (10 mM; 0.68 mg ml^{-1}) were added into the $\text{SiO}_2\text{-NH}_2$ (10 mg) suspended in CH_2Cl_2 (9.4 ml) and stood for 3 days while being gently shaken. The beads (**1a**) were separated by filtration and washed with CHCl_3 and MeOH. The beads (**1a**; 6 mg) were treated with Ac_2O (2 ml) in CHCl_3 (30 ml) at refluxing temperature for 12 h for acetylation. After the reaction, the beads were separated by filtration and washed with $\text{CHCl}_3/\text{MeOH}$ to produce **1a'**. The absorption and fluorescence spectra

of the prepared micro-beads (**1**) were then measured using a CLSM.

2.10. Absorption and fluorescence microscopic spectroscopy using a CLSM

Absorption and fluorescence microscopic spectroscopy were performed on the micro-beads using an Olympus FV-300 CLSM equipped with a spectrophotometer (STFL 250, Seki Technotron) linked to the CLSM by an optical fiber. The CLSM had a positional resolution of $\pm 1 \mu\text{m}$ in the vertical direction and $\pm 1 \mu\text{m}$ in the horizontal direction. Absorption spectrophotometry was performed using a back-light as the light source. The control experiments, conducted in order to prove that the absorption spectrophotometry was working in the micro-region, were performed using the micro-silica gel beads needed to support a given concentration of dihydroxoantimony(V) tetraphenylporphyrin (**3g**) [21].

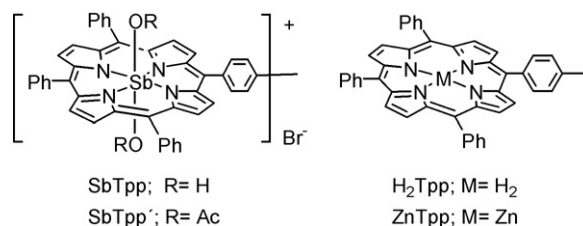
2.11. The reaction in micro-channel reactors

A MCR (ICC-IR01) was purchased from the Institute of Microchemical Technology (Kanagawa, Japan). A slurry of **1a'** in water was introduced to the inlet of the MCR under reduced outlet pressure. The **1a'** was packed on a neck located at the middle point of the MCR and was washed with water (100 μl) and an aqueous solution (100 μl) of Et_2NH (1–50 mM) using a microsyringe pump. Then, a 543 nm He–Ne laser was used to irradiate **1a'** in squares of area of 30 μm using a CLSM under an Et_2NH solution (1–50 mM) running at 50 $\mu\text{l min}^{-1}$. After 3 min of irradiation, the micro-channel was washed with MeOH/ H_2O (1:2, v/v; 100 μl) and MeOH (100 μl). Furthermore, a $\text{CHCl}_3/\text{MeOH}$ (5:1, v/v) solution of $\text{Zn}(\text{OAc})_2 \cdot 5\text{H}_2\text{O}$ (22.7 mM) was introduced into the MCR at 50 $\mu\text{l min}^{-1}$ for 25 min. The reaction of the micro-beads (**1**) was followed by using the CLSM to measure the absorption and fluorescence spectra at regular intervals.

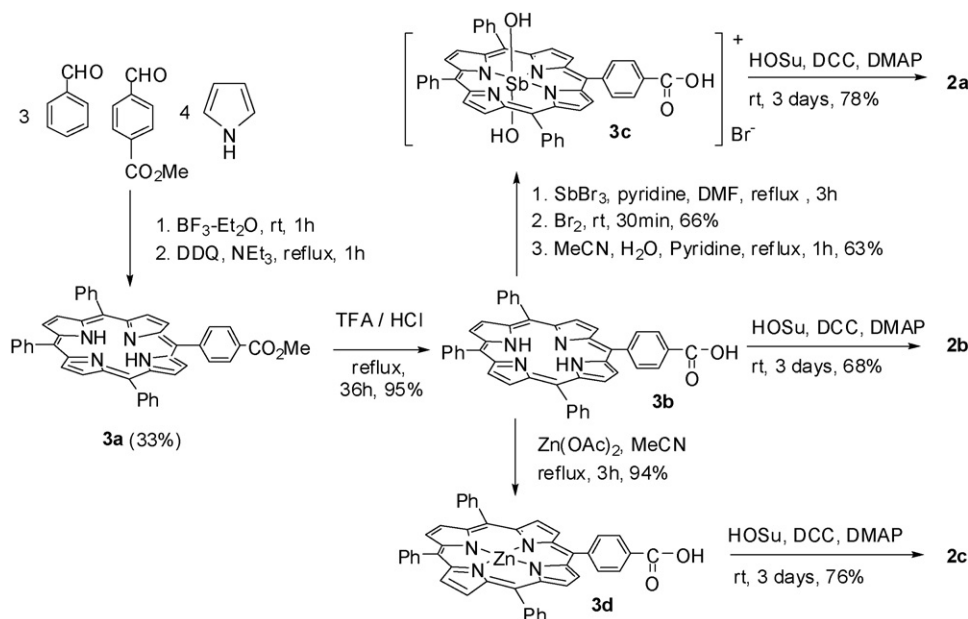
3. Results and discussion

3.1. Preparation of porphyrin chromophore (**2** and **3**)

Our initial efforts were focused on the preparation of the following porphyrin chromophores: the 4-(dihydroxoantimonytriphenylporphyrinyl)phenyl (SbTpp), the 4-(triphenylporphyrinyl)phenyl (H_2Tpp), and the 4-(zinctriphenylporphyrinyl)phenyl (ZnTpp) groups (Scheme 2).



Scheme 2. Porphyrin chromophores.

Scheme 3. Preparation of the porphyrin chromophores (**2a**–**c**).

5-(4-Methoxycarbonylphenyl)-10,15,20-triphenylporphyrin (**3a**) was hydrolyzed with TFA to produce 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin (**3b**). Using **3b** as the starting material, the dihydroxoantimony(V) complex (**2a**), the free base (**2b**), and the zinc complex (**2c**) of 5-[4-(succinimidylxycarbonyl)phenyl]-10,15,20-triphenylporphyrin were prepared as Scheme 3. The reaction of **3b** with SbBr₃ followed by treatment with Br₂ and subsequent hydrolysis produced dihydroxo[5-(4-carboxyphenyl)-10,15,20-triphenylporphyrinato]antimony(V) bromide (**3c**). The esterification of **3c** with HOSu in the presence of DCC and DMAP produced **2a**. Similarly, **2b** was prepared by the esterification of **3b** with HOSu in the presence of DCC and DMAP. **2c** was prepared by reacting **3b** with Zn(OAc)₂ and subsequently esterifying it with HOSu. Dihydroxoantimony(V) (4-propylcarbamoilphenyl)triphenylporphyrin hexafluorophosphate (**3e**) and di(acetyloxo)antimony(V) tetraphenylporphyrin bromide (SbTpp'-H, **3f**) were used as reference samples (Scheme 4).

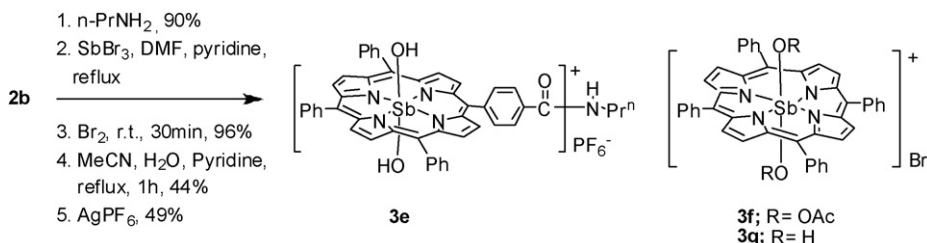
3.2. Immobilization of porphyrin chromophore on micro-silica gel beads

The 3-aminopropyl silica gel (SiO₂-NH₂) was selected as the carrier because of its high transparency of visible light,

wide surface area, and ability to immobilize the functional groups through a covalent bond. Micrometer-sized SiO₂-NH₂ (area: 272 m² g⁻¹, density: 1.06 g cm⁻³) beads were prepared by reacting silica gel with 3-aminopropyl(triethoxy)silane (H₂N(CH₂)₃Si(OEt)₃) [22]. The contents of the amino group were measured to be 2.53 mmol g⁻¹ by way of elemental analysis.

The immobilization of SbTpp, H₂Tpp, and ZnTpp chromophores on SiO₂-NH₂ was performed by the usual method [23] using **2a**–**c**, respectively. **1a** was prepared by the reaction of SiO₂-NH₂ with given amounts of **2a** set by molar equivalent (*a* meq) to the content of an amino group in SiO₂-NH₂ (2.53 mmol g⁻¹) in the presence of Im for 3 days (Scheme 1). The bond formation was confirmed by the appearance of an amide bond at 1650 and 1539 cm⁻¹ in the FT-IR spectra (see Supplementary data).

The absorption spectra are shown in Fig. 1. Q bands of the porphyrin of **1a** appeared at 552 and 592 nm, similar to those of **3e** in the MeOH solution ($\lambda_{\text{max}} = 552$ and 595 nm). However, the fluorescence spectra of **1a** were remarkably different from those of **3e** in the solution; additional new broad emissions appeared near 630 nm, as shown in Fig. 2A. We therefore deduce that other chromophores interacted with the SbTpp in its excited state. When **1a** was acetylated with Ac₂O to form the acetylated **1a** (**1a'**), the new emission at 630 nm disappeared

Scheme 4. Preparation of the reference samples (**3e**–**g**).

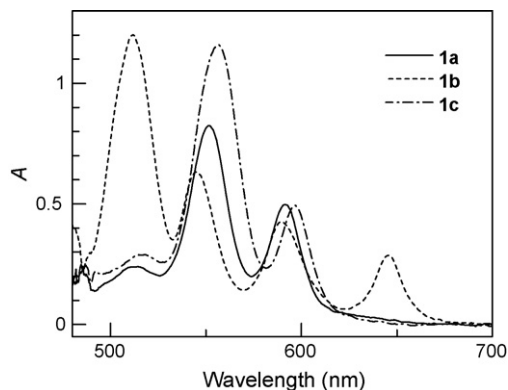


Fig. 1. Absorption spectra of the micro-beads **1a** ($a = 11.9$ meq), **1b** ($a = 5.9$ meq), and **1c** ($a = 5.9$ meq).

(Fig. 2B). Therefore, these results show that residual aminopropyl moieties can interact with the SbTpp chromophore. Since the λ_{\max} of the SbTpp chromophore shifted slightly to short wavelengths from 552 and 592 to 550 and 590 nm when treated with Ac_2O , not only the aminopropyl group but also the axial hydroxo ligands of the SbTpp chromophore were acetylated. The micro-beads (**1b** and **c**) were prepared by the reaction of $\text{SiO}_2\text{-NH}_2$ with **2b** and **c** followed by the acetylation in the similar method to the case of **1a'**, respectively. Moreover, spectral changes were observed in the fluorescence spectra of **1a–c** when large amounts of the porphyrin chromophores were loaded on

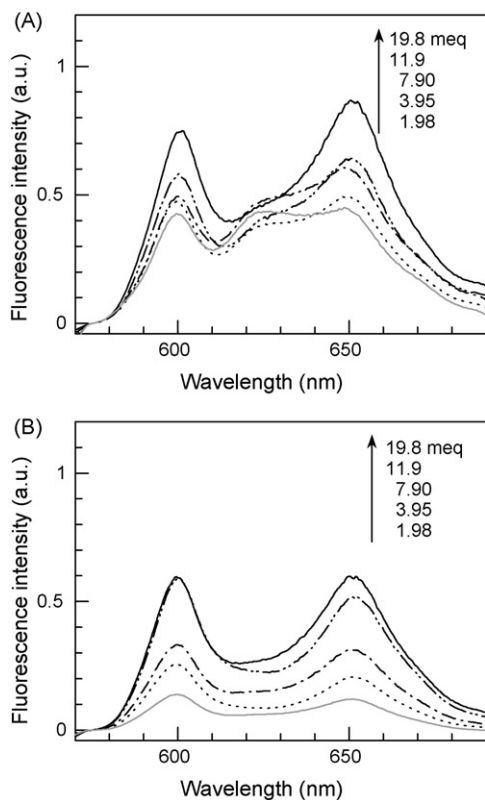


Fig. 2. Fluorescence spectra of (A) **1a**, which was prepared by the reaction of $\text{SiO}_2\text{-NH}_2$ with a given molar equivalent ($a = 1.98$ – 19.8 meq) of **2a**, and (B) the corresponding **1a'** after acetylation with Ac_2O .

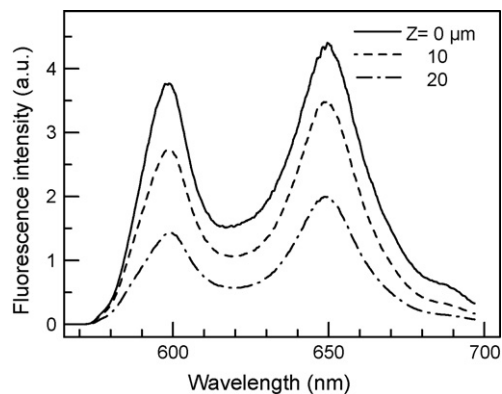


Fig. 3. Fluorescence spectra of **1a'** ($a = 11.9$ meq; $x = 9.54 \mu\text{mol g}^{-1}$) at depth from the surface ($z = 0 \pm 1$, 10 ± 1 , and $20 \pm 1 \mu\text{m}$).

the beads. Thus, the porphyrin chromophores tend to form the aggregates of one another in order to deactivate the catalytic reactivities [24]. Therefore, it is important to control the number of immobilized porphyrin chromophores.

Fig. 3 shows the CLSM fluorescence spectra at given depths ($z = 0, 10$, and $20 \mu\text{m}$) from the surface of **1a'** beads: average diameter = $56.7 \mu\text{m}$, $a = 11.9$ meq, the immobilized amounts of SbTpp' on $\text{SiO}_2\text{-NH}_2$ ($x = 9.54 \mu\text{mol g}^{-1}$). Since the absorbance of **1a'** beads was less than 0.18, more than 85% of incident light reached the SbTpp, even at $z = 20 \mu\text{m}$. As z was increasing, however, the fluorescence intensity was gradually decreasing, revealing that the concentration of SbTpp inside the beads was lower than that at the surface. Similarly, the concentrations of H_2Tpp and ZnTpp were decreasing from the surface to the interior of the beads in the cases of **1b** and **c**, respectively (see Supplementary data).

3.3. Determination of immobilization yields

In order to determine the yield of the reaction of **2a** with $\text{SiO}_2\text{-NH}_2$, absorption spectrophotometry was performed on a micro-bead (**1a**). The absorbance (A) and diameters (b , cm) of **1a** beads were measured using a CLSM. Lambert-Beer's law (Eq. (2)) can be written as Eq. (3) because the concentration of SbTpp chromophores (c) on $\text{SiO}_2\text{-NH}_2$ is equal to $1000Wx/V$ (Eq. (4)), where W and V represent the mass and volume of $\text{SiO}_2\text{-NH}_2$ beads and x denotes the immobilized amount (mol g^{-1}) of SbTpp on $\text{SiO}_2\text{-NH}_2$. Since the density (W/V) was 1.06 g cm^{-3} and x can be written as $2.53 \times 10^{-3}\gamma a$ using the immobilization yield (γ) (Eq. (5)), the slope for the plots of A/b against a will be written as $2.68\epsilon\gamma$ (Eq. (6)).

$$A = \epsilon bc \quad (2)$$

$$\frac{A}{b} = \epsilon c = 2.68\epsilon\gamma a \quad (3)$$

$$c = \frac{1000Wx}{V} = \frac{2.53W\gamma a}{V} = 2.68\gamma a \quad (4)$$

$$x = 2.53 \times 10^{-3}\gamma a \quad (5)$$

$$\text{slope} = 2.68\epsilon\gamma \quad (6)$$

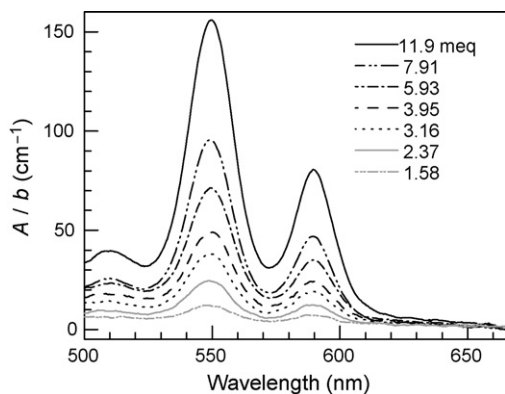


Fig. 4. Absorption spectra of a micro-bead (**1a'**) at given a values.

Fig. 4 shows the change in absorption spectrum appearing when **1a** was prepared by reacting $\text{SiO}_2\text{-NH}_2$ with a given amount of a (1.58–11.9 meq) of **2a**. Fig. 5 shows the plots of the values (A/b) at 552 nm against a , producing a good linear correlation. The γ for the reaction of **2a** with $\text{SiO}_2\text{-NH}_2$ was determined to be 31.7% by Eq. (3) using the slope of Fig. 5 ($1.26 \times 10^4 \text{ eq}^{-1} \text{ cm}^{-1}$) and ϵ_{552} (**3e**, in MeOH) = $1.48 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. After treatment with Ac_2O , the slope remained at $1.26 \times 10^4 \text{ eq}^{-1} \text{ cm}^{-1}$ for **1a'**. Thus, x can be precisely controlled by a .

The Q bands of **1b** appeared at 513, 548, 590, and 646 nm whereas those of **1c** appeared at 560 and 595 nm (Fig. 1). Therefore, the γ values for the reaction of **2b** and **c** with $\text{SiO}_2\text{-NH}_2$ were determined by plotting A/b against a at 548 and 560 nm, respectively (Fig. 5). In the case of **2b**, γ was determined to be 95.9% using the slope of $2.05 \times 10^4 \text{ eq}^{-1} \text{ cm}^{-1}$ and ϵ_{551} (**2b**, in MeOH) = $7.97 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$. In the case of **2c**, γ was determined to be 45.1% using the slope of $2.26 \times 10^4 \text{ eq}^{-1} \text{ cm}^{-1}$ and ϵ_{562} (**2c**, in MeOH) = $1.87 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$.

3.4. Photochemical reaction of micro-beads

Our attention was turned next to the reaction of micro-beads. The **1a'** beads ($x=9.54 \mu\text{mol/g}$, average diameter = $56.7 \mu\text{m}$)

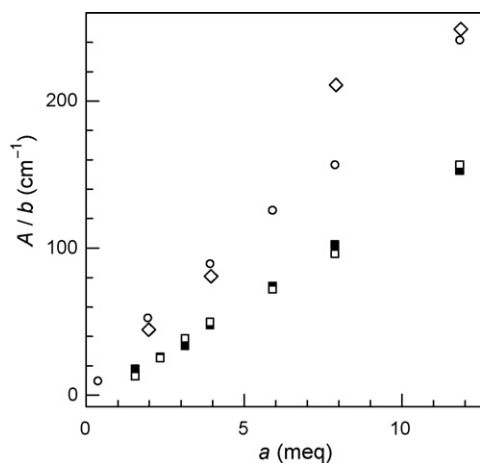


Fig. 5. Plots of A/b vs. a in the preparation of **1a** (■), **1a'** (□), **2b** (○), and **2c** (◇): slope = $1.26 \times 10^4 \text{ eq}^{-1} \text{ cm}^{-1}$ (**1a**), $1.26 \times 10^4 \text{ eq}^{-1} \text{ cm}^{-1}$ (**1a'**), $2.05 \times 10^4 \text{ eq}^{-1} \text{ cm}^{-1}$ (**2b**), and $2.26 \times 10^4 \text{ eq}^{-1} \text{ cm}^{-1}$ (**2c**).

were placed inside a MCR [25,26] that consisted of a narrow channel (width $190 \mu\text{m}$, depth $85 \mu\text{m}$, length 6 cm), with a neck (width $20 \mu\text{m}$, depth $20 \mu\text{m}$) located at the middle point of the channel, as shown in Scheme 5.

Photoinduced electron transfer between **1a'** and the electron donors was attempted in the MCR. Et_2NH was selected as an electron donor by the photoreaction of **1a'** with a few amines (Et_3N , Et_2NH , and $n\text{-PrNH}_2$). An aqueous solution of Et_2NH (1–50 mM) was continuously pumped into the MCR from an inlet with a micro-syringe pump. Then, a 543 nm He–Ne laser was used to irradiate **1a'** by the CLSM in squares of area $30 \mu\text{m}^2$, which was comparable in size to the micro-beads. Fig. 6A shows the fluorescence spectra from the surface of the beads during photo-irradiation under a running Et_2NH solution (50 mM). The emissions decreased at 600 nm and marked new emissions appeared at 650 nm as irradiation time increased. The new emissions at 650 nm can be safely assigned to the H_2Tpp group by comparing the fluorescence spectra with those of the authentic sample of **1b**. The photo-irradiation was stopped for 180 s when the emissions at 600 nm disappeared entirely. In this time, **1a'** was quantitatively transformed into **1b** in the surface of beads (Fig. 6A). However, no spectral changes were observed in the non-irradiated beads under dark conditions, even in the presence of Et_2NH .

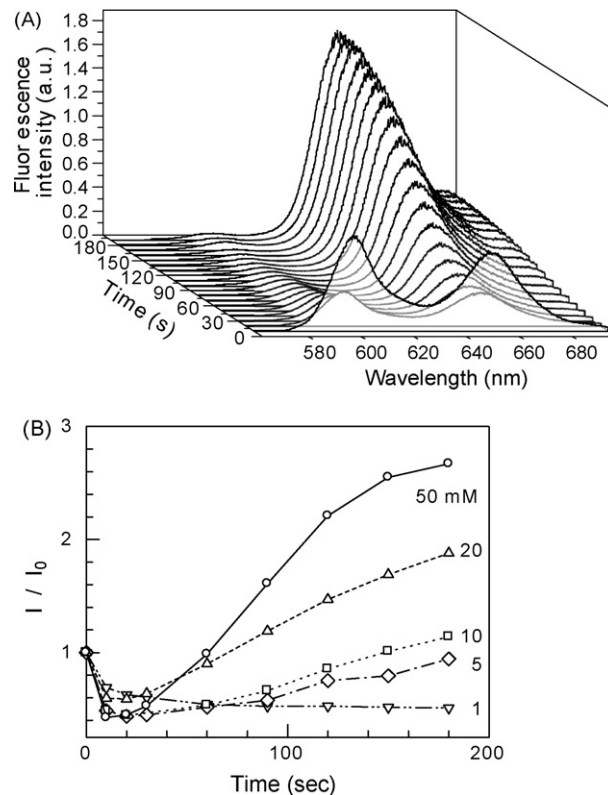


Fig. 6. (A) Fluorescence spectral changes from 550 to 700 nm at the surface of micro-silica gel beads (**1a'**) during photochemical reaction with Et_2NH (50 mM) in the MCR and (B) the time-conversion of the fluorescence intensities at 650 nm during the photoreaction of **1a'** with a given concentration of Et_2NH (1–50 mM). Fluorescence intensity ratio (I/I_0) means the ratio of intensity (I) at the given reaction time to the initial **1a'** (I_0).

Sb^VTpp' to Sb^{III}Tpp' by way of double photoreaction with Et₂NH.

4. Conclusions

Micro-silica gel beads (**1**) that immobilized porphyrin chromophores were prepared using controlled amounts of porphyrin chromophores. Moreover, **1** was modified in a micro-region on the silica gel surface using a combination of an MCR and a CLSM. **1** will be applicable as a photocatalyst for a continuous flow-type MCR since silica gel can catch and condense the reactants and has a high transparency of visible light.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2007.12.010.

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