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Photolabile Protecting Group Bonded to Aminopropyl Silica-Gel Beads

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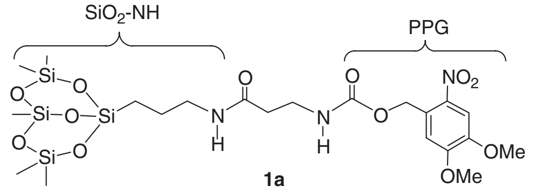
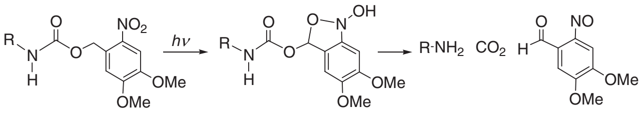
4,5-Dimethoxy-2-nitrobenzyloxycarbonyl group acting as a photolabile protecting group was bonded on 3-aminopropyl silica gels through a covalent bond in a 46% yield. The deprotection was performed under UV-irradiation. The resulting amino group was analyzed through the use of a confocal laser-scanning microscope using 4-(10,15,20-triphenylporphyrinyl)phenyl fluorophore.

Photolabile protecting groups (PPG) have been widely used in organic synthesis in solutions in order to be transformed to a variety of functional group after deprotection under irradiation.[1] Also PPG is often bonded to a solid matrix such as polystyrene beads[2] or a glass surface[3] to prepare functional micro-beads by controlling the site and amount. Functional micro-beads have been used as catalysts and scavengers in organic synthesis, carriers of solid-phase synthesis, and selective sensors for biomolecules. However, the introduction of PPG on to silica gel (SiO_2) has been scarcely reported.[4] In order to facilitate the site-controlled functionalization of silica gel, 3-aminopropyl silica gel (SiO_2–NH_2) was N-protected with PPG (Scheme 1).

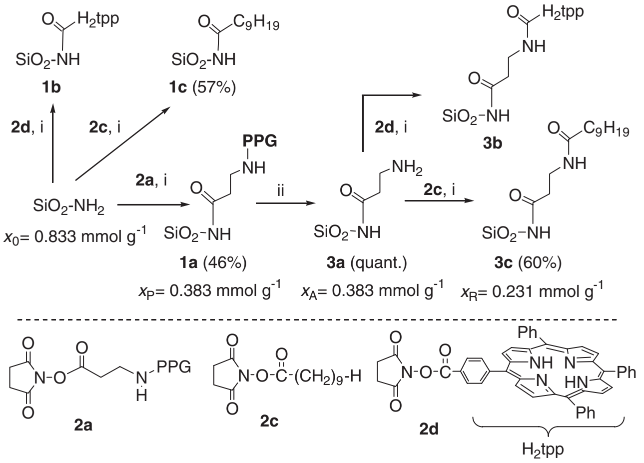
The 4,5-dimethoxy-2-nitrobenzyloxycarbonyl group was selected as it is a PPG which can generate an amino group under UV irradiation (Scheme 2).[5] Using succinimidyl ester of *N*-PPG-3-aminopropanoic acid **2a**, the PPG was bonded on micrometer SiO_2–NH_2 (average diameter: 95.4 μm, area: 229 $m^2\,g^{-1}$, pore volume: 1.08 $cm^3\,g^{-1}$, quantity of NH_2 (x_0): 0.833 $mmol\,g^{-1}$) (Scheme 3). *N*-PPG-protected SiO_2–NH_2 **1a** was prepared by the reaction of SiO_2–NH_2 (1 g) with **2a** (1 mmol) in CH_2Cl_2 (8 mL) for 3 days under a consistent gentle shaking in the presence of imidazole (Im; 1.67 mmol) acting as base to give free amino group as SiO_2–NH_2. The beads were separated by filtration and washed with $CHCl_3$/MeOH (5:1) to obtain **1a**. IR spectra of **1a** is shown in Figure 1. *N*-PPG protection of SiO_2–NH_2 was confirmed by the appearance of characteristic absorption of the PPG at 1709 cm^{-1} for C=O



Scheme 1. 3-Aminopropyl silica gel **1a** protected by 4,5-dimethoxy-2-nitrobenzyloxycarbonyl group acting as PPG.



Scheme 2. Deprotection scheme of PPG.



Scheme 3. The reaction routes of **1a** and its transformation. Reagent: i) Im, CH_2Cl_2, room temp, 3 days and ii) $h\nu$, $CHCl_3$–TFA, 2 h.

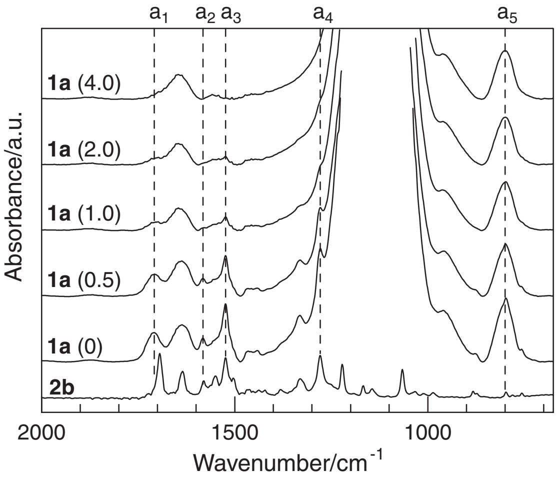


Figure 1. IR spectral changes of **1a** during UV-irradiation for a given time (t/h) shown in parenthesis along with IR spectra of **2b**. After irradiation for 4 h, **1a** was completely transformed to **3a**. The assignment of a_1 through a_5 are shown in text.

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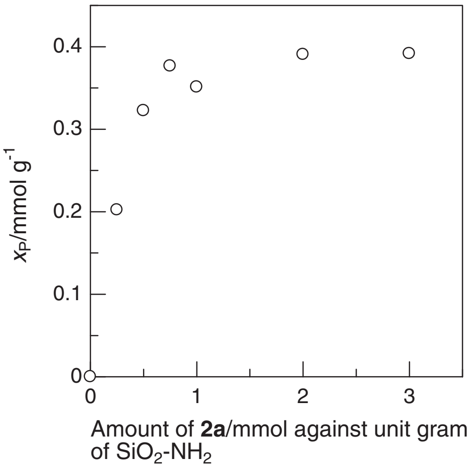

Figure 2. Dependence of the PPG amount (x_P) in **1a** on the amounts of **2a** used.

stretching of the urethane bond (a$_1$), 1582 cm^{-1} for the C=C stretching of the benzene ring (a$_2$), 1523 cm^{-1} for NO stretching of the nitro group (a$_3$), and 1279 cm^{-1} for the C–O stretching of the MeO group (a$_4$) in addition to the absorption of the Si–O stretching (a$_5$) at 800 cm^{-1}. These assignments were performed by comparison with the IR spectrum of a model compound, *N*-propyl-3-[(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)amino]propanamide (**2b**). The amounts of the PPG (x_P/mmol g^{-1}) in **1a** were determined by IR absorption spectrophotometry. The x_P was determined for **1a** which was prepared by the reaction of SiO$_2$–NH$_2$ with a specific amount of **2a**. In Figure 2, x_P was plotted against the amounts of **2a** added. As the amount of **2a** increased, the x_P also gradually increased until reaching a maximum level at approximately 0.8 mmol g^{-1} of **2a**, nearly equal to the amounts of x_0 (0.833 mmol g^{-1}). At the maximum point, 0.383 mmol g^{-1} x_P was formed in **1a**. This value corresponds to 46% of the protection yield (=100x_P/x_0) based on x_0.

As a consequence, 0.450 mmol g^{-1} of the amino groups remained in **1a**, however, the residual amino groups of **1a** did not react with acylation reagents such as Ac$_2$O and succinimidyl decanoate (**2c**). It was suggested that the residual amino groups remained deep within narrow channels of the silica gel to prevent the approach of any of the reagents. Therefore, the following deprotection was performed without the protection of the residual amino groups. The cleavage of the PPG from **1a** was carried out under 366 nm irradiation in CHCl$_3$–trifluoroacetic acid (TFA; 50 mM). The addition of TFA was effective in the decomposition of the acetal intermediate generated by the irradiation (Scheme 2). Removal of PPG was confirmed by the disappearance of a$_3$ in the IR spectra (Figure 1). The absorption of a$_3$ disappeared entirely upon irradiation for 4 h, resulting in complete transformation from **1a** to the PPG-deprotected SiO$_2$–NH$_2$ **3a**. Therefore, the quantity (x_A) of the free amino groups formed was assumed to be equal to the quantity of NO$_2$ consumed. i.e., $x_P = x_A$.

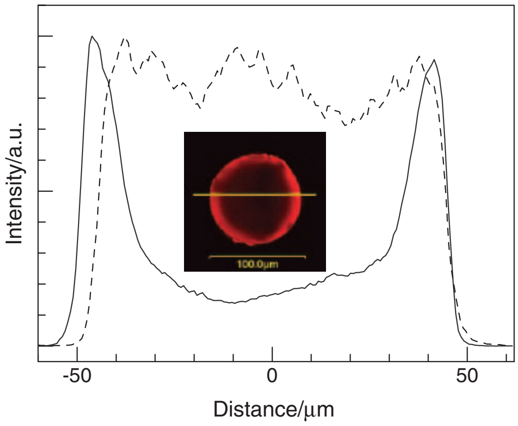

Figure 3. Distribution of the fluorescence intensity of **1b** (dotted line) and **3b** (solid line) at cross section located at the 25 µm depth from the surface. Inset: the cross sectional fluorescence image of **3b** at the 25 µm depth from surface. Measurement was performed by a CLSM under irradiation at 543 nm.

In order to analyze the distribution of the formed amino groups, **3a** were treated with 5-[4-(succinimidyloxycarbonyl)-phenyl]-10,15,20-triphenylporphyrin (**2d**).[6] The distribution of 4-(10,15,20-triphenylporphyrinyl)phenyl chromophore (H$_2$tpp)-bonded silica gel **3b** was examined through use of a fluorescence image measured by a confocal laser-scanning microscope (CLSM) under excitation at 543 nm. As shown in Figure 3, the fluorescence of **3b** was coming from the area between the surface and a 10 µm depth. It showed that the formed amino groups located at shallow site in the silica gel beads. In the fluorescence image of H$_2$tpp-incorporated SiO$_2$–NH$_2$ **1b** which was prepared by the reaction of SiO$_2$–NH$_2$ with **2d** (Figure 3), the fluorescence was coming from the entire inside of the SiO$_2$–NH$_2$ beads. These results demonstrate that **2a** cannot reach the deeper parts of the SiO$_2$–NH$_2$ beads possibly due to the adsorption of the polar nitro group in shallow sites, as has been reported for *o*-hydroxybenzophenone.[7]

The reactivity of the resulting amino group on **3a** was checked by decanoylation with **2c** which was performed by the reaction of **3a** with **2c** in the presence of Im. The decanolyated silica gel **3c** was subjected to IR absorption spectrophotometry in order to determine the amount of the decanoyl group (x_R). The yield (100x_R/x_A) for the formation of **3c** from **3a** was determined to be 60%. This value is similar to the value of the yield (57%) for **1c** which was prepared by the reaction of SiO$_2$–NH$_2$ with **2c**.

In conclusion, easy deprotection of PPG from **1a** was achieved, since SiO$_2$ is highly transport to visible light. Therefore, **1a** will be extensively applicable to the preparation of micro-beads with multifunctionalities such as catalysts, scavengers, and biosensors because of large surface area and high adsorption ability.[8]

Experimental

Instruments. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were taken on a Bruker AV 400M spectrometer for CDCl$_3$ solutions with tetramethylsilane used as an internal standard. Matrix-assisted laser desorption/ionization mass spectra (MALDI-MS) were measured on a Bruker Daltonics Autoflex III TOF/TOF in the positive ion mode. FT-IR was measured on a JASCO Herscel FT/IR-300 with a Micro-20 spectrometer. UV–vis spectra of the solution were obtained with a JASCO V-550 spectrophotometer.

Absorption and fluorescence microscopic spectroscopy of silica gel beads was performed on an Olympus FV-300 confocal laser scanning microscope (CLSM) equipped with spectrophotometer (STFL 250, Seki Technotron) linked to CLSM using an optical fiber.

Preparation of Succinimidyl 3-[(4,5-Dimethoxy-2-nitrobenzyloxycarbonyl)amino]propanoate (2a). 4,5-Dimethoxy-2-nitrobenzyl alcohol (5 mmol) was reacted with *p*-nitrophenyl chloroformate (6.0 mol) in dimethylacetamide (DMA, 20 mL) in the presence of Et_3N (10 mmol) at room temperature for 2 days to give *p*-nitrophenyloxycarbonyl 4,5-dimethoxy-2-nitrobenzyl carbonate (**4a**) in a 71% yield.[9] The reaction of **4a** (1.0 mmol) with ethyl 3-aminopropanoate (2.0 mol) was performed in the presence of 4-(dimethylamino)pyridine (DMAP, 1.0 mmol) in DMA (20 mL) at room temperature for 2 days. *p*-Nitrophenol was removed from the reaction mixture by extraction with an aqueous solution of $NaHCO_3$ and $CHCl_3$. The $CHCl_3$ solution was evaporated and then the residual oil was poured into hexane (200 mL) to give ethyl 3-[(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)amino]propanoate (**4b**) as a yellow precipitate in 83% yield. **4b** (1 mmol) was hydrolyzed in $CHCl_3$–EtOH (18 mL v/v 1:2) in the presence of an aqueous solution of Et_4NOH (10%, 3 mL) to give 3-[(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)amino]propanoic acid (**4c**) in 81% yield. The reaction of **4c** (1.0 mmol) with *N*-hydroxysuccinimide (HOSu; 1.20 mol) was performed in CH_2Cl_2 (15 mL) in the presence of DMAP (1.0 mmol) and *N*,*N′*-dicyclohexylcarbodiimide (DCC, 1.44 mmol) for 3 days at room temperature. After the filtration of the dicyclohexylurea, the CH_2Cl_2 solution was evaporated and then the residual oil was poured into hexane (200 mL) to give **2a** as a pale yellow precipitate in 79% Yield.

2a: ^{1}H NMR: δ 2.88 (t, $J = 6.2$ Hz, 2H), 2.89 (s, 4H), 3.64 (q, $J = 6.2$ Hz, 2H), 3.95 (s, 3H), 3.98 (s, 3H), 5.52 (s, 2H), 5.71 (br t, $J = 6.2$ Hz, 1H), 7.03 (s, 1H), 7.71 (s, 1H); ^{13}C NMR: δ 25.53, 32.17, 36.73, 56.35, 56.41, 63.69, 108.09, 109.79, 128.45, 139.54, 147.97, 153.59, 155.72, 167.29, 169.04. HRMS (MALDI-TOF) Found: *m*/*z* 448.1061. Calcd for $C_{17}H_{19}N_3O_{10}Na$: $[M + Na]^+$, 448.0963.

Preparation of *N*-Propyl-3-[(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)amino]propanamide (2b). A CH_2Cl_2 solution (10 mL) containing **2a** (100 mg), propylamine (21 mg), and Im (32 mg) was mixed at room temperature for 1 h. After the solution was evaporated, the precipitate was dissolved in $CHCl_3$ and the solution was washed with a dilute aqueous solution of HCl and water. After evaporation, the crude product was purified by column chromatography in SiO_2 to give **2b**.

2b: Yield 43%. ^{1}H NMR: δ 0.92 (t, $J = 7.4$ Hz, 3H), 1.52 (sext, $J = 7.4$ Hz, 2H), 2.43 (t, $J = 5.8$ Hz, 2H), 3.19–3.24 (m, 2H), 3.51 (q, $J = 5.8$ Hz, 2H), 3.95 (s, 3H), 3.98 (s, 3H), 5.50 (s, 2H), 5.61 (brs, 1H), 5.68 (brs, 1H), 7.01 (s, 1H), 7.71 (s, 1H); ^{13}C NMR: δ 11.30, 22.80, 35.76, 37.23, 41.27, 56.39, 56.44, 63.46, 108.17, 109.84, 128.37, 139.68, 148.03, 153.61, 155.94, 171.17. HRMS (MALDI-TOF) Found: *m*/*z* 392.1665. Calcd for $C_{16}H_{23}N_3O_7Na$: $[M + Na]^+$, 392.1428.

Preparation of Succinimidyl Decanoate (2c). Decanoic acid (**2e**; 5.0 mmol) was reacted with HOSu (6.0 mmol) in CH_2Cl_2 (20 mL) in the presence of DMAP (5.0 mmol) and DCC (7.5 mmol) for 2 days at room temperature. After the filtration of the dicyclohexylurea, the CH_2Cl_2 solution was evaporated and then the residual oil was purified by column chromatography in silica gel to give **2c**.

2c: Yield 79%. ^{1}H NMR: δ 0.88 (t, $J = 6.8$ Hz, 3H), 1.27 (m, 12H), 1.74 (quint, $J = 7.4$ Hz, 2H), 2.60 (t, $J = 7.4$ Hz, 2H), 2.84 (s, 4H); ^{13}C NMR: δ 14.06, 22.64, 24.54, 25.54, 28.75, 29.04, 29.30, 29.50, 29.58, 30.90, 31.86, 168.64, 169.14. HRMS (MALDI-TOF) Found: *m*/*z* 292.1446. Calcd for $C_{14}H_{23}NO_4Na$: $[M + Na]^+$, 292.1519.

Quantitative Analysis of Micro-Beads by IR Absorption Spectrophotometry. In order to determine x_P, a mixture of **2b** with SiO_2–NH_2 was prepared in a given molar ratio ($m_{\mathbf{2b}}/m_S$) where $m_{\mathbf{2b}}$ and m_S denoted the molar amounts of **2b** and SiO_2–NH_2. In the IR spectra of the mixed samples, the area ratios of a_3 of **2b** to a_5 of SiO_2–NH_2 were plotted against $m_{\mathbf{2b}}/m_S$ to form calibration curves. In order to determine x_R, a mixture of decanoic acid (**2e**) with SiO_2–NH_2 was prepared in a specified molar ratio ($m_{\mathbf{2e}}/m_S$) where $m_{\mathbf{2e}}$ and m_S represented the molar amounts of **2e** and SiO_2–NH_2. In the IR spectra of the mixed samples, the area ratio of the C–H stretching (a_6) at 3000–2830 cm^{-1} of **2e** to a_5 absorption of SiO_2–NH_2 were plotted against $m_{\mathbf{2e}}/m_S$ to make calibration curves.

Deprotection of 1a. The irradiation of **1a** (10 mg) was carried out in a $CHCl_3$–TFA (50 mM) solution (8 mL) by an Eikosha high-pressure mercury lamp through a Pyrex filter. The beads were then separated by filtration and washed with $CHCl_3$–MeOH (5:1) to produce **3a**.

Supporting Information

The spectral data of **4a**, **4b**, and **4c** are available free of charge on the web at http://www.csj.jp/journals/bcsj/.

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