

# Axial-Ligand-Cleavable Silicon Phthalocyanines Triggered by Near-Infrared Light toward Design of Photosensitizers for Photoimmunotherapy

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## Article

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1 **Axial-Ligand-Cleavable Silicon Phthalocyanines Triggered by Near-Infrared Light**

2 **toward Design of Photosensitizers for Photoimmunotherapy**

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18

## 19 **Abstract**

20           Near-infrared photoimmunotherapy (NIR-PIT) is a novel phototherapy for the  
21 treatment of cancer that uses NIR light and conjugates of antibody-IR700, a silicon  
22 phthalocyanine photosensitizer. A key feature of NIR-PIT is light-induced axial ligand  
23 cleavage of IR700, which finally causes cytotoxicity. Here, we focused on protonation of  
24 the axial ligand on the IR700 anion radical during the photochemical process. The Gibbs  
25 energy in the protonation reaction of IR700 derivatives with different axial ligands was  
26 calculated. These calculations suggested the order of the cleavage efficiency corresponds  
27 to the basicity of the axial ligand (*i.e.* alkoxy > siloxy (IR700) > phenoxy  $\approx$  oxycarbonyl),  
28 which was confirmed by the photoirradiation experiments with synthesized compounds.  
29 Thus, axial ligand cleavage is significantly dependent on the basicity of the axial ligand.  
30 Our findings suggest that PIT reagent with an IR700 derivative bearing alkoxy group  
31 would show better efficacy than IR700.

32

## 33 **Introduction**

34           IRDye700DX (IR700) is a silicon phthalocyanine (SiPc) derivative that absorbs  
35 near-infrared (NIR) light and has been used not only as a fluorescent imaging reagent but  
36 also as a photosensitizer for NIR photoimmunotherapy (NIR-PIT) (Fig. 1a).<sup>1,2</sup> In NIR-

37 PIT, IR700-antibody conjugate and NIR light are used to treat cancer. Because the  
38 cytotoxicity is induced only when the conjugate is bound to the cell membrane and  
39 irradiated with NIR light, NIR-PIT can specifically kill cancer cells. To date, various  
40 cancer antigens, such as epidermal growth factor receptor (EGFR) and human epidermal  
41 growth factor receptor 2 (HER2), have been targeted. In pre-clinical experiments, NIR-  
42 PIT has shown efficacy against gastric,<sup>3,4</sup> lung,<sup>5</sup> prostate,<sup>6</sup> and breast<sup>7</sup> cancers. In addition,  
43 successful first-in-human phase I and II studies in patients with unresectable locally  
44 advanced head and neck cancer were completed (NCT02422979). More recently,  
45 Cetuximab-IR700, a PIT conjugate for EGFR, was approved in Japan. These studies  
46 suggest PIT will be a valuable new form of cancer treatment.

47         Because light is attenuated by tissue absorption and scattering, PIT utilizes NIR  
48 light delivered through a thin fiber, such as a catheter or needle, to increase the efficiency  
49 of light delivery at deep sites. Moreover, cytotoxicity should be further enhanced by using  
50 a light-sensitive photosensitizer that is more responsive to NIR light. However, only  
51 IR700 has been reported as a photosensitizer for PIT. Therefore, it remains unclear  
52 whether IR700 is the optimal photosensitizer for this treatment. Recently, the mechanism  
53 of cytotoxicity in PIT has been investigated both experimentally and theoretically. As  
54 shown in Fig. 1b, IR700 is excited to the singlet excited state by NIR light, and then

55 transitions to the triplet state via intersystem crossing (ISC). However, the quantum yield  
56 of ISC is not high ( $\Phi_{ISC} = 0.019$ ).<sup>8</sup> IR700 in the triplet state receives electrons from  
57 electron donors to form the anion radical. Next, the anion radical is protonated by a  
58 hydronium ion, followed by axial ligand cleavage and the coordination of a hydroxy ion,  
59 which occur spontaneously at room temperature due to the lower activation energy of the  
60 photochemical process ( $E^\ddagger < 92.4 \text{ kJ mol}^{-1}$ ).<sup>9,10</sup> The photodegraded compound loses its  
61 hydrophilic functional moiety (*i.e.* six sulfonate groups in all), which drastically changes  
62 its physicochemical properties from water-soluble to insoluble. As a result, the conjugate  
63 aggregates on the cell membrane, leading to physical damage and ultimately cell  
64 death.<sup>11,12</sup> Thus, axial ligand cleavage of the photosensitizer is a crucial step in PIT. The  
65 development of a light-sensitive photosensitizer involves the design of SiPcs in which the  
66 axial ligand is more readily cleaved by NIR light.

67         Previously, we conducted experiments that showed the efficiency of axial ligand  
68 cleavage by NIR light is dependent on the pH of the solution.<sup>9</sup> At higher pH, light-induced  
69 cleavage of the axial ligand was inhibited because protonation of the SiPc anion radical  
70 did not occur. These results suggested that axial ligand cleavage is dependent on the  
71 basicity of axial ligand. Thus, in order to develop an SiPc derivative that is more light-  
72 sensitive than IR700, we focused on the basicity of the axial ligands. Here, the basicity

73 of SiPcs with various axial ligands was estimated by performing quantum chemical  
74 calculations. The compounds were then synthesized, and light-induced axial ligand  
75 cleavage was compared.

76

## 77 **Results and discussions**

### 78 **Quantum chemical calculations of SiPcs with different axial ligands in photoinduced**

79 **axial ligand cleavage.** We reported that in the presence of an electron donor under  
80 hypoxic conditions, the axial ligand of the IR700 derivative cleaves at pH 7.0, while at  
81 pH 11.0 no cleavage of the axial ligand occurs despite the generation of an anion radical.<sup>9</sup>

82 This result motivated us to investigate the reactivity of SiPcs having other axial ligands  
83 with a different basicity. Rather than a siloxy group as the axial ligand, we chose alkoxy,  
84 oxycarbonyl and phenoxy groups because they show a different basicity and are  
85 functional groups capable of forming an Si-O bond (Fig. 2a). As in our previous study,  
86 symmetric SiPc derivatives were used as model compounds for the calculations  
87 (compounds **1'-4'**, Fig. 2b).<sup>9</sup> Density functional theory (DFT) calculations were  
88 performed to determine the change in Gibbs energy ( $\Delta G$ ) of each compound (**1'-4'**) for  
89 the following protonation reaction of the axial ligand at 298.15 K:



91 where Y = OSiMe<sub>3</sub> (**1'**), OEt (**2'**), OCOEt (**3'**), and OPh (**4'**). The calculated  $\Delta G$  is also  
92 summarized in Fig. 2b. As for the oxycarbonyl axial ligand, protonation of the carbonyl  
93 oxygen was considered because it is more basic than the ester oxygen. All  $\Delta G$  values are  
94 negative, indicating that the reaction (1) is exergonic. The order of  $\Delta G$  for each compound  
95 was compound **2'** (alkoxy) < compound **1'** (siloxy) < compound **4'** (phenoxy)  $\approx$   
96 compound **3'** (oxycarbonyl). If we assume equilibration between the anion radical form  
97 ([SiPcY<sub>2</sub>]<sup>•-</sup>) and the protonated form ([SiPc(YH)Y]<sup>•</sup>) in solution, these results suggest the  
98 protonated form of the alkoxy ligand is favored over the other ligands and has more  
99 opportunity to proceed with the ligand cleavage reaction. However, the result differs from  
100 a previous report that investigated SiPcs having an asymmetric axial ligand, alkoxy and  
101 phenoxy groups.<sup>13</sup> The previous study concluded that the axial ligand of a phenoxy group  
102 cleaves more readily upon NIR light irradiation than an alkoxy group. This apparent  
103 discrepancy might arise because (i) the concerted hydrolysis reaction pathway has an  
104 activation energy of approximately 30 kJ mol<sup>-1</sup> higher than the stepwise pathways  
105 considered in this study, and (ii) symmetrical differences in the axial ligand (*i.e.*  
106 symmetric versus asymmetric). Our results suggest the anticipated efficiency of axial  
107 ligand cleavage to be: compound **2** > compound **1** > compound **4**  $\approx$  compound **3**. The  
108 detailed energy diagram for the formation of [SiPc(OH)Y]<sup>•-</sup> from [SiPcY<sub>2</sub>]<sup>•-</sup> in neutral

109 water solution (summarized in our previous report for the case of Y = OSiMe<sub>3</sub>) is shown  
110 in supporting information (see Supplementary Fig. 1).

111

### 112 **Synthesis and photochemical properties of the SiPcs with different axial ligands.**

113 Given the above results, we synthesized compounds **2-4** (Fig. 2a) to experimentally  
114 evaluate the predictions based on the theoretical calculations. As shown in Figure 3, the  
115 axial ligands were conjugated via SiPc dichloride (**6**) using sodium hydride or K<sub>2</sub>CO<sub>3</sub> as  
116 a base. Final products containing a water-soluble group at the end of the axial ligand were  
117 synthesized in MeOH with sultone. Compounds **2-4** were characterized by <sup>1</sup>H NMR and  
118 MS. To investigate whether the axial ligands of SiPc affect their photochemical properties,  
119 we measured the absorption/emission spectra and determined the fluorescence quantum  
120 yields ( $\Phi_{FL}$ ) and the molar extinction coefficients ( $\epsilon$ ). All the compounds showed almost  
121 identical photochemical properties (see Supplementary Fig. 2 and Table 1). Compounds  
122 **1-4** displayed similar shaped absorption and emission spectra with a sharp Q-band  
123 absorption peak in the range of 677-691 nm and a sharp emission peak in the range of  
124 684-699 nm. The relative  $\Phi_{FL}$  of compounds **2-4** were calculated with reference to the  
125 absolute  $\Phi_{FL}$  of compound **1** ( $\Phi_{FL} = 0.31$ ).<sup>8</sup> As a result,  $\Phi_{FL}$  of compounds **2-4** were 0.30,  
126 0.27 and 0.29, respectively. Furthermore,  $\epsilon$  at the Q-band of compounds **1-4** in water were

127  $1.7\text{-}1.9 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ . These results indicate the axial ligands do not significantly  
128 affect the photochemical properties of the SiPc derivatives.

129

### 130 **Comparison of photoinduced axial ligand cleavage reaction of synthesized SiPcs.**

131 With these compounds in hand, we investigated the axial ligand photocleavage of  
132 compounds **1-4**. The compounds in a phosphate buffer with dithiothreitol (DTT) as an  
133 electron donor were irradiated with an NIR laser (690 nm) at different light doses. After  
134 irradiation, the solutions were analyzed by HPLC with methylene blue as an internal  
135 standard. NIR light irradiation of compounds **1-4** caused the peaks of the original  
136 compound to decrease and other peaks to appear (Fig. 4a-d). Indeed, as the light dose  
137 increased, the peaks of the original compounds disappeared. The degradation products of  
138 compound **1**, named degrade 1a and 1b, were previously identified as SiPc with one or  
139 both ( $\text{SiPc}(\text{OH})_2$ ) of the axial ligands cleaved, respectively (Fig. 4e).<sup>10</sup> The other  
140 degradation products, degrade 2b, 3a and 4a, were also analyzed by double injection with  
141 authentic compound  $\text{SiPc}(\text{OH})_2$ . In each case the degradation product was identified as  
142  $\text{SiPc}(\text{OH})_2$ , as with compound **1** (see Supplementary Fig. 3). Based on these findings,  
143 degrade 2a was expected to be SiPc with uniaxial ligand. Thus, we synthetically prepared  
144 the compound (compound **2-half**) from compound **2** (Fig. 4e and see Supplementary

145 Scheme 1). Using the authentic compound, degrade 2a was identified as compound **2**-half  
146 (see Supplementary Fig. 3). In the case of no NIR irradiation, compounds **1-4** did not  
147 degrade at all (see supplementary Fig. 4). These results show that the axial ligands of  
148 compounds **2-4** are cleaved by NIR irradiation in the presence of an electron donor, as in  
149 compound **1**.

150           The cleavage of the axial ligands was categorized into two types. For compounds  
151 **1** and **2**, peaks corresponding to SiPc with uniaxial ligand (degrades 1a, 2a) was observed,  
152 which disappeared upon increasing light dose together with a concomitant increase in the  
153 appearance of SiPc(OH)<sub>2</sub> (degrades 1b, 2b) (Fig. 4a,b). By contrast, for compounds **3** and  
154 **4**, light irradiation resulted in the formation of SiPc(OH)<sub>2</sub> (degrades 3a, 4a), but no peaks  
155 corresponding to SiPc with uniaxial ligand (Fig. 4c,d). When compounds **3** and **4** were  
156 hydrolyzed in aqueous solution at pH11-12, peaks of compounds **3** and **4** with uniaxial  
157 ligand were not detected but that of SiPc(OH)<sub>2</sub> was detected (see Supplementary Fig. 5).  
158 On the other hand, in the hydrolysis of compounds **1** and **2**, peaks of compounds **1** and **2**  
159 with uniaxial ligand were detected. These results suggest compounds **3** and **4** with  
160 uniaxial ligand might be unstable in aqueous solution. Another possibility is that in the  
161 case of compounds **3** and **4**, the two axial ligands were sequentially cleaved in the  
162 photochemical reaction, whereas cleavage of the axial ligand of compounds **1** and **2**

163 occurred one by one. Thus, the change in  $\Delta G$  for the protonation reaction to the second  
164 axial ligand, namely,



166 was obtained from the DFT calculations (see Supplementary Fig. 6). Comparison with  
167 Fig. 2(b) shows the  $\Delta G$  of eq (2) for oxycarbonyl and phenoxy ligands are 7.8 and 0.3 kJ  
168 mol<sup>-1</sup> lower than those of eq (1), respectively. By contrast, the  $\Delta G$  of eq (2) for siloxy and  
169 alkoxy ligands are 4.5 and 4.6 kJ mol<sup>-1</sup> higher than eq (1), respectively. This observation  
170 supports the sequential cleavage of compounds **3** and **4**.

171 Next, we compared the efficiency of axial ligand cleavage for the synthesized  
172 compounds **1-4**. Because SiPc(OH)<sub>2</sub> is hydrophobic with limited solubility in aqueous  
173 solution, most of the reaction product precipitated. Thus, although the dissolved portion  
174 of SiPc(OH)<sub>2</sub> was detected by HPLC, we could not quantify axial ligand cleavage from  
175 the peak areas of SiPc(OH)<sub>2</sub>. Therefore, comparison of the reaction efficiency was  
176 performed from the decrease in the peak areas of compounds **1-4**. As anticipated from the  
177 theoretical calculations, the order of efficiency was compound **2** > compound **1** >  
178 compound **4**  $\approx$  compound **3**, consistent with the order of  $\Delta G$  of the anion radicals upon  
179 protonation (Fig. 4f). These results show that the  $\Delta G$  value correlated with the efficiency  
180 of axial ligand cleavage. Specifically, the basicity of the axial ligand was related to the

181 dissociation efficiency. Thus, we successfully developed compound **2** with an axial ligand  
182 comprising an alkoxy group that is more light-sensitive than compound **1**.

183 Finally, the electron donor was investigated. Previously, it was reported that axial  
184 ligand cleavage depended on the electron donor.<sup>14</sup> Thus, we used a variety of electron  
185 donors and compared the axial ligand cleavage of compounds **1-4** by NIR light in the  
186 presence of these donors (see Supplementary Table 1). In addition to DTT ( $E_{\text{red}} = -0.33$   
187 V),<sup>15,16</sup> we used nicotinamide adenine dinucleotide (NADH,  $E_{\text{red}} = -0.32$  V),<sup>17,18</sup> cysteine  
188 (Cys,  $E_{\text{red}} = -0.20$ - $-0.23$  V),<sup>16,19</sup> glutathione (GSH,  $E_{\text{red}} = -0.20$ - $-0.26$  V)<sup>16,19,20</sup> and  
189 sodium ascorbate (NaAA,  $E_{\text{red}} = 0.35$ - $0.39$  V).<sup>21,22</sup> As a result, the efficiencies of axial  
190 ligand cleavage varied, and was independent of the redox potential and the structure of  
191 the electron donating group (see Supplementary Fig. 7). For example, although GSH and  
192 Cys have similar redox potentials and the same functional group for electron donation,  
193 axial ligands of compounds **3** and **4** were efficiently cleaved in the presence of Cys, but  
194 not GSH. Nonetheless, the efficiency of the axial ligand cleavage for each electron donor  
195 was in the order of the basicity of the axial ligand. Hence, basicity had a significant effect  
196 on light-induced axial ligand cleavage.

197

198 **Discussion about development of new PIT reagents.** In NIR-PIT, it is thought that

199 aggregation of the conjugates, which causes physical damage to the cell membrane, is  
200 induced by axial ligand cleavage of the photosensitizer. Here, we performed theoretical  
201 calculations and experiments that show the basicity of the axial ligand is involved in  
202 efficiency of the cleavage. Moreover, axial ligand cleavage of compound **2** was more  
203 light-responsive than that of compound **1**. Therefore, compound **2** having a linker for  
204 conjugation with an antibody should be a better photosensitizer than IR700. Moreover,  
205 based on the results of this study, an even better photosensitizer could be developed by  
206 modifying the axial ligand. Thus, our analysis provides useful guidelines that will assist  
207 in the development of improved photosensitizers for PIT.

208

## 209 **Conclusions**

210         Herein, we examined the photoinduced ligand release of IR700 derivatives with  
211 different axial ligands, such as alkoxy, siloxy, oxycarbonyl and phenoxy groups.  
212 Theoretical and experimental investigations revealed the order of basicity of the axial  
213 ligands coincided with the order of efficiency of photoinduced axial ligand cleavage.  
214 These results indicate that axial ligand cleavage is significantly influenced by protonation  
215 of the axial ligand of the anion radical. Based on the results of this study it should also be  
216 possible to design a compound whose axial ligand is cleaved with high reactivity to light.

217 Thus, novel PIT reagents with improved properties will be developed in the near future.

218

## 219 **Materials and methods**

220 **Reagents and general information.** General chemicals were of the best grade available,  
221 supplied by FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), Tokyo  
222 Chemical Industries Co., Ltd (Tokyo, Japan), Sigma-Aldrich Co. LLC (St. Louis, MO,  
223 USA) and KANTO CHEMICAL Co., INC. (Tokyo, Japan), and were used without further  
224 purification. NMR spectra were recorded on a JNM-ECX400P or JMN-ECS400 (JEOL  
225 Ltd., Tokyo, Japan) instrument at 400 MHz for  $^1\text{H}$  NMR.  $\delta$  values are given in ppm  
226 relative to tetramethylsilane or deuterated solvent signals. ESI and MALDI-TOF mass  
227 spectra (MS) were measured with a JMS-T100LP (JEOL Ltd.) and an Ultraflex II  
228 TOF/TOF (Bruker Corporation, Billerica, MA, USA), respectively. High performance  
229 liquid chromatography (HPLC) analyses and purification were performed using an HPLC  
230 system (Shimadzu Corporation, Kyoto, Japan) with reverse-phase columns, Inertsil ODS-  
231 3 (4.6 mm  $\times$  150 mm for analysis, 10 mm  $\times$  150 mm for purification) (GL Sciences Inc.,  
232 Tokyo, Japan), with eluent A (0.1 M triethylammonium acetic acid (TEAA)) and eluent  
233 B (99%  $\text{CH}_3\text{CN}$ /1%  $\text{H}_2\text{O}$ ).

234

235 **Synthesis**

236 Compound **1** and SiPc(OH)<sub>2</sub> were synthesized according to literature reports.<sup>10</sup>

237 **Silicon Phthalocyanine Dichloride (6)**. The compound was synthesized according to a  
238 protocol described in the literature.<sup>23</sup> Briefly, 1,3-diiminoisoindoline **5** (176 mg, 1.2  
239 mmol) and silicon tetrachloride (300 mg, 1.8 mmol) were dissolved in quinoline (2 mL),  
240 and the mixture was refluxed for 2 h under an Ar atmosphere. After cooling to room  
241 temperature, MeOH was added to the reaction mixture. The precipitate was collected by  
242 filtration, washed with MeOH, and dried in vacuo. The green-blue crude product was  
243 obtained and used for the next synthesis without further purification (123 mg).

244 **Bis(4-aminobutyloxy) Silicon Phthalocyanine (7)**. Crude compound **6** (50 mg, ca.  
245 0.082 mmol), 4-aminobutanol (73 mg, 0.82 mmol), and sodium hydride (39 mg, 1.6  
246 mmol) were dissolved in toluene (40 mL) and the mixture was refluxed for 8 h under an  
247 Ar atmosphere. After cooling to room temperature, the reaction mixture was evaporated  
248 below 35°C. The residue was suspended with H<sub>2</sub>O-EtOH solution (2:1), collected by  
249 filtration, washed with H<sub>2</sub>O-EtOH solution (2:1), and dried in vacuo. A blue product was  
250 obtained (31 mg, 0.043 mmol, 52% (2 steps)). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ -2.10 (t, *J*  
251 = 6.1 Hz, 4H), -1.64 (tt, *J* = 6.1, 7.2 Hz, 4H), -1.25 (tt, *J* = 7.2, 7.2 Hz, 4H), 0.94 (t, *J*  
252 = 7.2 Hz, 4H), 8.34 (dd, *J* = 2.9, 5.6 Hz, 8H), 9.64 (dd, *J* = 2.9, 5.6 Hz, 8H). HRMS (ESI<sup>+</sup>)

253 m/z: calcd for C<sub>40</sub>H<sub>38</sub>N<sub>10</sub>O<sub>2</sub>Si: 359.1470 [M + 2H]<sup>2+</sup>; found: 359.1469.

254 **Bis[(6-aminohexanoyl)oxide] Silicon Phthalocyanine (8)**. Crude compound **6** (300 mg,  
255 ca. 0.49 mmol), 6-aminohexanoic acid (640 mg, 4.9 mmol), and K<sub>2</sub>CO<sub>3</sub> (200 mg, 1.5  
256 mmol) were dissolved in toluene (20 mL) and the mixture was refluxed for 21 h under an  
257 Ar atmosphere. After cooling to room temperature, the reaction mixture was evaporated  
258 below 35°C. The residue was suspended in CH<sub>2</sub>Cl<sub>2</sub>, collected by filtration, washed with  
259 CH<sub>2</sub>Cl<sub>2</sub>, and dried in vacuo. A blue product was obtained (76 mg, 0.094 mmol, 14% (2  
260 steps)). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ -0.92--0.89 (m, 4H), -0.79--0.71 (m, 4H),  
261 -0.64--0.62 (m, 4H), 0.15-0.23 (m, 4H), 1.94 (t, *J*=6.9 Hz, 4H), 8.39 (dd, *J* = 2.7, 5.7  
262 Hz, 8H), 9.70 (dd, *J* = 2.7, 5.7 Hz, 8H). HRMS (ESI<sup>+</sup>) m/z: calcd for C<sub>44</sub>H<sub>42</sub>N<sub>10</sub>O<sub>4</sub>Si:  
263 401.1574 [M + 2H]<sup>2+</sup>; found: 401.1574.

264 **Bis(4-aminomethylphenoxide) Silicon Phthalocyanine (9)**. The compound was  
265 synthesized using the same method as described for compound **7**. 4-  
266 (Aminomethyl)phenol was used as the reagent for the axial ligand. The product was used  
267 for the next synthesis without further purification.

268 **General procedure for alkylsulfonation**. Silicon phthalocyanine with two axial ligands  
269 (30-75 mg, 0.042-0.094 mmol), 1,3-propanesultone (23-25 eq) and *N,N*-  
270 diisopropylethylamine (DIEA, 46-50 eq) were dissolved in MeOH (4 mL), and the

271 mixture was stirred at 50 °C for 72 h under an Ar atmosphere. The product was purified  
272 by HPLC (eluent A/eluent B = 80/20 to 50/50 in 15 min, 50/50 to 0/100 in 5 min). The  
273 product was then desalted with a Sep-Pak C18 cartridge (Waters Corp., Milford, MA,  
274 USA) and cation-exchange resin.

275 **Bis{4-[tris(3-sulfopropyl)]ammoniobutyloxy} Silicon Phthalocyanine (2).** The  
276 reaction yield was 20%. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ -1.93 (t, *J* = 6.1 Hz, 4H), -1.46-  
277 -1.37 (m, 4H), -1.01--0.91 (m, 4H), 1.32-1.50 (m, 16H), 2.53-2.64 (m, 24H), 8.53 (dd,  
278 *J* = 2.9, 5.7 Hz, 8H), 9.79 (dd, *J* = 2.9, 5.6 Hz, 8H). HRMS (ESI<sup>-</sup>): *m/z* calcd for  
279 C<sub>58</sub>H<sub>68</sub>N<sub>10</sub>Na<sub>3</sub>O<sub>20</sub>S<sub>6</sub>Si: 1513.2403 [M-Na]<sup>-</sup>; found: 1513.2417.

280 **Bis({6-[tris(3-sulfopropyl)]ammoniohexanoyl}oxide) Silicon Phthalocyanine (3).** The  
281 reaction yield was 20%. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ -0.69--0.66 (m, 8H), -0.57-  
282 -0.54 (m, 4H), 0.63-0.66 (m, 4H), 1.88-1.91 (m, 12H), 2.36-2.38 (m, 4H), 2.78 (t, *J* = 6.3  
283 Hz, 12H), 3.13-3.18 (m, 12H), 8.57 (dd, *J* = 3.0, 6.0 Hz, 8H), 9.80 (dd, *J* = 3.0, 6.0 Hz,  
284 8H). HRMS (ESI<sup>-</sup>): *m/z* calcd for C<sub>62</sub>H<sub>72</sub>N<sub>10</sub>Na<sub>3</sub>O<sub>22</sub>S<sub>6</sub>Si: 1597.2615 [M-Na]<sup>-</sup>; found:  
285 1597.2615.

286 **Bis{4-[tris(3-sulfopropyl)]ammoniomethylphenoxide} Silicon Phthalocyanine (4).**  
287 The reaction yield was 7% (3 steps). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ 2.00-2.09 (m, 12H),  
288 2.59 (d, *J* = 8.3 Hz, 4H), 2.70-2.81 (m, 24H), 3.71 (s, 4H), 5.84 (d, *J* = 8.3 Hz, 4H), 8.57

289 (dd,  $J = 2.8, 5.5$  Hz, 8H), 9.81 (dd,  $J = 2.8, 5.5$  Hz, 8H). HRMS (ESI<sup>-</sup>):  $m/z$  calcd for  
290 C<sub>64</sub>H<sub>64</sub>N<sub>10</sub>Na<sub>3</sub>O<sub>20</sub>S<sub>6</sub>Si: 1581.2090 [M-Na]<sup>-</sup>; found: 1581.2101.

291

292 **Measurement and determination of photochemical properties.** 0.5-1.0 μM SiPc dyes  
293 were dissolved in sodium phosphate buffer (pH 7.0), and the absorbance and fluorescence  
294 spectra were measured using a UV spectrophotometer UV-1800 (Shimadzu Corporation)  
295 and a spectrofluorometer FP-8600 (JASCO Corporation, Tokyo, Japan), respectively. The  
296 relative fluorescence quantum yield ( $\Phi_{FL}$ ) was obtained with the following equation:

$$297 \quad \Phi_{FL}^{sample} = \Phi_{FL}^{standard} \times \frac{F^{sample}}{F^{standard}} \times \left( \frac{n^{sample}}{n^{standard}} \right)^2 \times \frac{Abs^{standard}}{Abs^{sample}}$$

298  $F$  denotes the area under the fluorescence band ( $F = \sum I_{FL}(\lambda)$ , where  $I_{FL}(\lambda)$  is the  
299 fluorescence intensity at each emission wavelength).  $n$  denotes the refractive index of the  
300 solvent.  $Abs$  denotes the absorbance at the excitation wavelength. Compound **1** was used  
301 as a reference ( $\Phi_{FL} = 0.31$ ) of the fluorescence quantum yield.

302

303 **NIR photolysis of phthalocyanine derivatives.** A solution of 5 μM phthalocyanine  
304 derivatives in sodium phosphate buffer (pH 7.0) containing 1 mM DTT was prepared in  
305 a vial. The solution was bubbled with Ar through the septum cap of the sealed vial before  
306 being irradiated with a laser MLL-III-690 (Changchun New Industries Optoelectronics

307 Technology Co., Ltd, Changchun, China) (690 nm, 40 mW cm<sup>-2</sup>) for 3-20 min (7.2-48 J  
308 cm<sup>-2</sup>). The irradiated solution was analyzed by HPLC (eluent A/eluent B = 75/25 to 60/40  
309 in 5 min, 60/40 to 0/100 in 10 min for compound **2**, eluent A/eluent B = 80/20 to 0/100  
310 in 10 min for compounds **1**, **3** and **4**). The detection wavelength was 670 nm. Methylene  
311 blue (MB) was used as an internal standard. The power densities were measured with an  
312 optical power meter PM200 (Thorlabs Inc., Newton, NJ, USA). All the experiments were  
313 carried out at room temperature.

314

315 **Computational details.** DFT calculations were performed for the silicon phthalocyanine  
316 model compounds, **1'**-**4'**, and their protonated forms using Gaussian16.<sup>24</sup> The LC-BLYP  
317 functional,<sup>25</sup> which combines the long-range corrected Becke exchange functional<sup>26</sup> and  
318 the Lee-Yang-Parr correlation functional<sup>27</sup> was employed. Spin-unrestricted Kohn-Sham  
319 self-consistent field calculations were also performed. The aug-cc-pVDZ basis set<sup>28</sup> was  
320 utilized for N and O atoms, while the cc-pVDZ basis set<sup>29,30</sup> was adopted for the other  
321 atoms. The water solvent effect was incorporated using the integral equation formalism  
322 variant of the polarizable continuum model (IEFPCM).<sup>31</sup> All geometries were optimized  
323 at the same level of theory and normal mode analysis was performed at the optimized  
324 structures to obtain the thermally corrected Gibbs energy at 298.15 K and 1 atm.

325

326 **Data availability**

327 The data that support the findings of this study are available from the corresponding  
328 author upon reasonable request.

329

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338 Okazaki and at Research Institute for Information Technology, Kyushu University, Japan.

339

340 **Author contributions**

341 H.T. and M.O. conceived and designed the experiments. S.M. and Y.G. performed  
342 the experiments, collected and analyzed the data. M.K., M.H. and T.T. performed

343 computational calculations. H.T. and M. K. wrote the paper and all authors contributed to  
344 manuscript revision and approved the final version.

345

346 **Competing interests**

347 M.O. has received research grants and consultation fees from Rakuten Medical,  
348 Inc.

349

350 **Additional information**

351 **Supplementary information** The online version contains supplementary material  
352 available.

353 **Correspondence** and requests for materials should be addressed to M.O.

354

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439 **Figures**

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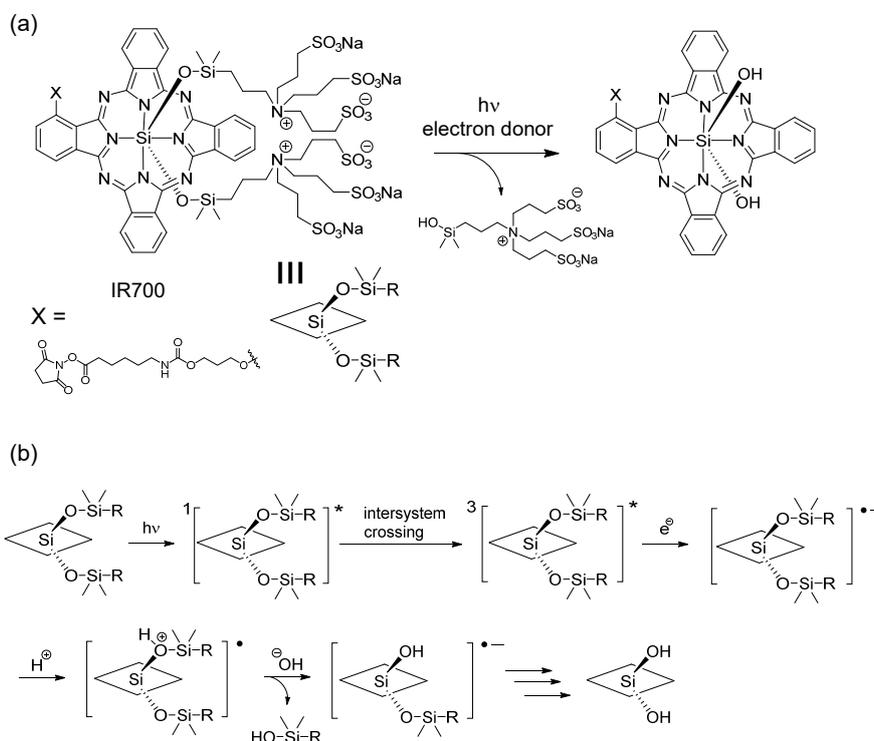
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448 **Fig. 1 Photochemical reaction in photoimmunotherapy** (a) Axial ligand cleavage of

449 IRDye700DX (IR700), a photosensitizer for photoimmunotherapy. Cleavage is induced

450 by NIR light in the presence of an electron donor, and the hydrophobic compound is

451 generated by loss of the hydrophilic axial ligands. (b) Plausible mechanism of axial ligand

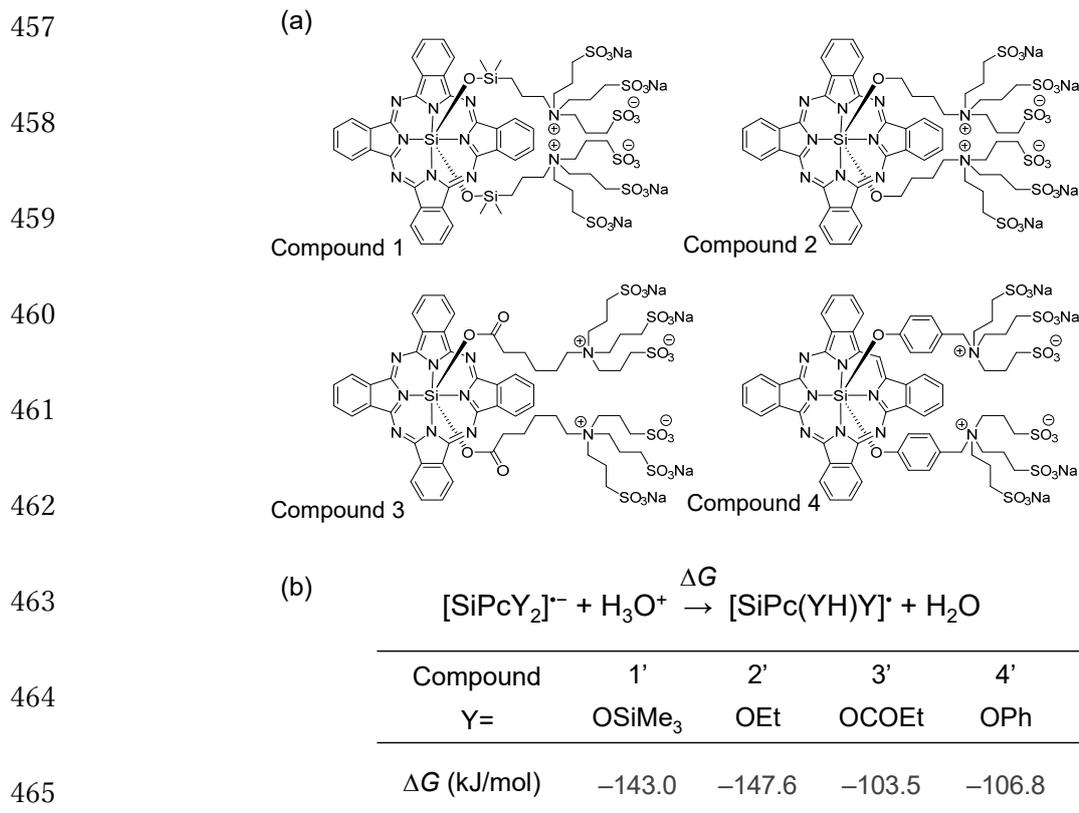
452 cleavage of IR700. Upon absorbance of excitation light, IR700 is excited to the singlet

453 state. Some of the singlet state are transferred to the triplet state, resulting in generation

454 of an anion radical by electron transfer from an electron donor. Next, stepwise reaction

455 of the axial ligand involving water, such as protonation and ligand exchange, occur

456 spontaneously. Finally, IR700 derivatives with the axial ligand cleaved are generated.



466 **Fig. 2 Computational calculation of silicon phthalocyanines with different axial**  
 467 **ligands** (a) Structures of compounds 1-4. (b) Calculated  $\Delta G$  (at 298.15 K, 1 atm) of  
 468 protonation to the oxygen atom on the Si-O bond (ULC-BLYP/cc-pVDZ, diffuse  
 469 functions were augmented to N and O atoms, in water solvent).

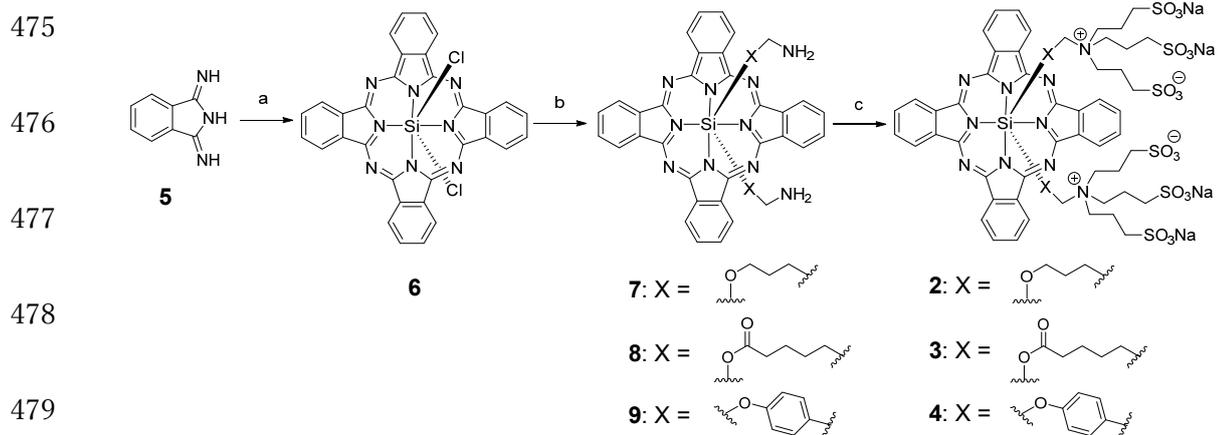
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480 **Fig. 3 Synthetic scheme for compounds 2-4.** (a)  $\text{SiCl}_4$ , quinoline, (b) appropriate amines,  
 481  $\text{NaH}$  or  $\text{K}_2\text{CO}_3$ , toluene, (c) sultone,  $i\text{Pr}_2\text{EtN}$ ,  $\text{MeOH}$ .

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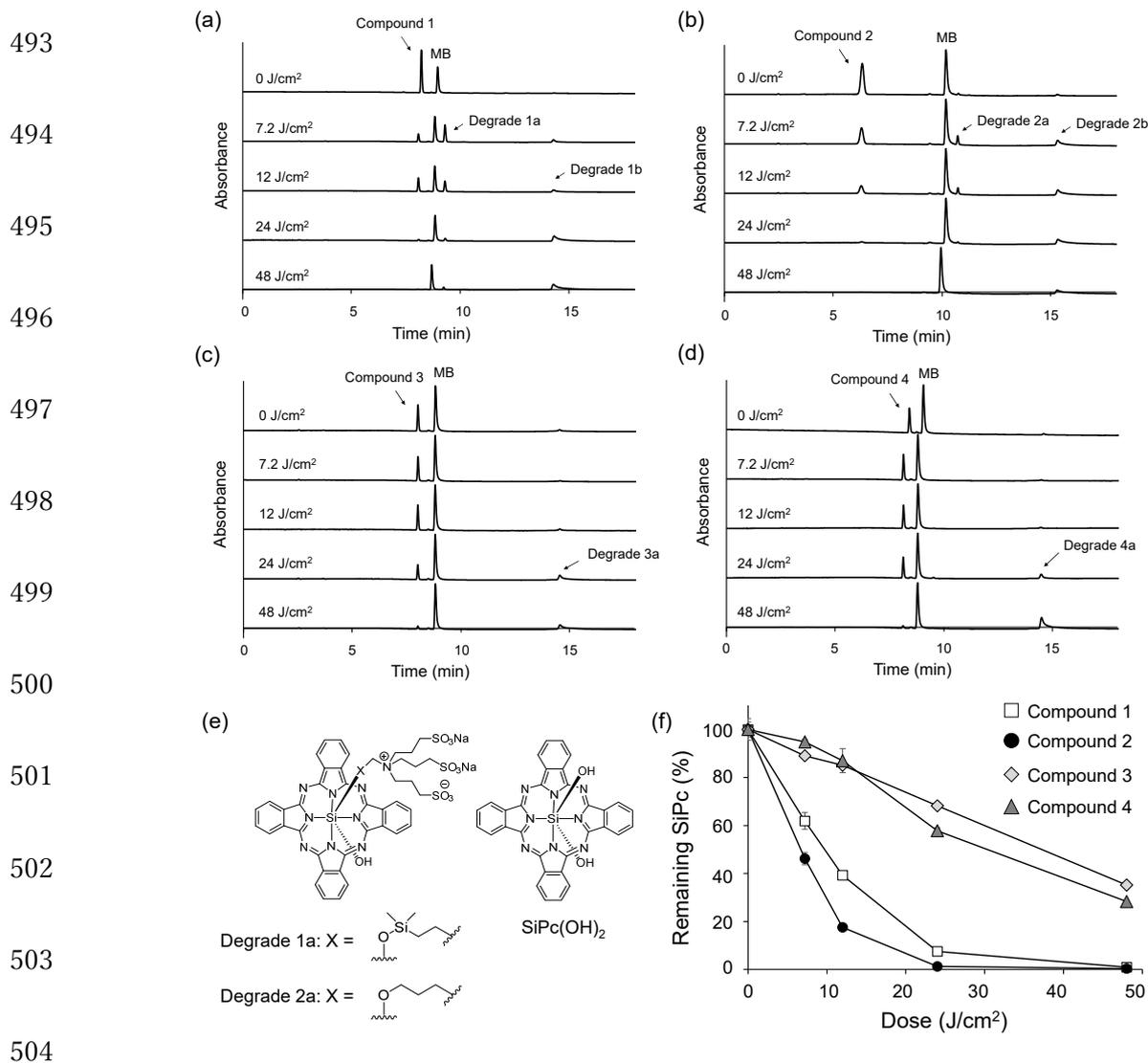
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505 **Fig. 4. HPLC analysis of photolysis by NIR light.** Compounds 1-4 in the deoxygenated  
 506 solution with 1 mM DTT were irradiated by NIR light (690 nm) for the indicated times  
 507 and the solution was analyzed by HPLC with methylene blue (MB) as an internal standard.  
 508 (a) compound 1, (b) compound 2, (c) compound 3 and (d) compound 4. (e) Chemical  
 509 structures of the degradation products after photolysis. (f) The ratio of absorbance peak  
 510 area compared to the sample before irradiation. Data represents the mean  $\pm$ SEM (n = 3).

511

512 **Table 1. Absorbance/emission wavelengths ( $\lambda_{\text{abs}}/\lambda_{\text{em}}$ ), molar extinction coefficient ( $\epsilon$ )**  
513 **and fluorescence quantum yields ( $\Phi_{\text{FL}}$ ) of compounds 1-4.**

514

Compound	$\lambda_{\text{abs}}/\text{nm}^{\text{a}}$	$\lambda_{\text{em}}/\text{nm}^{\text{a}}$	$\epsilon \times 10^5/\text{L mol}^{-1} \text{cm}^{-1}$		$\Phi_{\text{FL}}$
			H <sub>2</sub> O	DMSO	
<b>1</b>	677	684	1.9	2.4	0.31 <sup>b</sup>
<b>2</b>	684	690	1.7	2.0	0.30
<b>3</b>	691	699	1.9	2.5	0.27
<b>4</b>	689	695	1.7	2.0	0.29

515

516 <sup>a</sup> Absorption/emission wavelengths were obtained from Fig. S2. <sup>b</sup> Ref 8.

517

518

519

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