

# Synthesis of Iridium(III) Complex Bearing 1-Thio- $\beta$ -D-glucose Tetraacetate Moieties and its Photogeneration of Singlet Oxygen

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**Abstract:** Investigation of effective photodynamic therapy (PDT) reagents needs to observe  $^1\text{O}_2$ . The observation of the amount of photogenerated  $^1\text{O}_2$  emission at 1,270 nm is one of the engaged method for  $^1\text{O}_2$  generation evolution quantitatively, however it is convenient to observe fluorescent spectra relevant to a phosphorescence of singlet molecular oxygen due to the energy transfer from PDT reagents to  $^3\text{O}_2$ . Ir(III) complex containing a thioglucose tetraacetate-conjugated bipyridyl ligand were synthesized newly, and the photogeneration of  $^1\text{O}_2$  was confirmed by measurement of a phosphorescence of singlet molecular oxygen.

**Key words:** Photosensitizer, photodynamic therapy (PDT) reagents, singlet oxygen, glucose, iridium(III) complex, warburg effect.

## 1. Introduction

Recently, photodynamic therapy (PDT) has attracted much attention because it is a promising non-invasive treatment for cancer [1-4]. PDT involves the administration of a photosensitizer, and photoexcitation of the photosensitizer kills the cancer cells. Generation of singlet oxygen is an important pathway of PDT. Generally, it has been reported that the activation of the photosensitizer by photoirradiation converts triplet molecular oxygen ( $^3\text{O}_2$ ) to reactive singlet oxygen species ( $^1\text{O}_2$ ), which damages cancer cells (Scheme 1). This activation occurs selectively at the photoirradiated tumor site, thus PDT is relatively non-invasive compared to the conventional methods used for cancer treatment [5, 6].

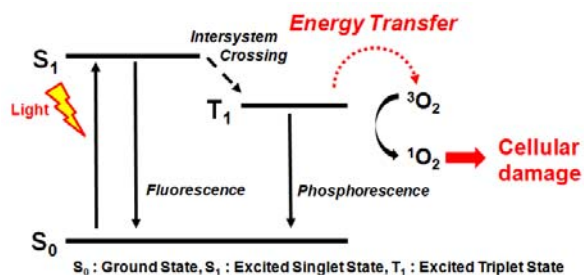
However, photosensitizers have several disadvantages such as skin photosensitivity; therefore, more effective photosensitizers need to be developed. Hence, it is believed that the conjugation of bio-competitive molecules can improve the photosensitizer properties.

Cancer cells uptake glucose in higher levels than normal cells; this phenomenon is called the Warburg effect [7]. For example, the glucose derivative  $^{18}\text{F}$ -fluorodeoxyglucose ( $^{18}\text{F}$ -FDG) has been used for positron emission tomography (PET) imaging [2, 8]. By the conjugation of sugar and by taking advantage of this property, the required amount of photosensitizer can be reduced.

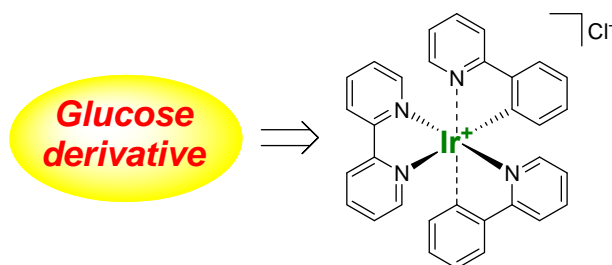
To investigate more effective PDT reagents, it is convincing to observe  $^1\text{O}_2$ . The amount of photogenerated  $^1\text{O}_2$  can be detected by measuring its emission at 1,270 nm. We focused on Ir(III) complexes because they are well-known singlet-oxygen sensitizers,

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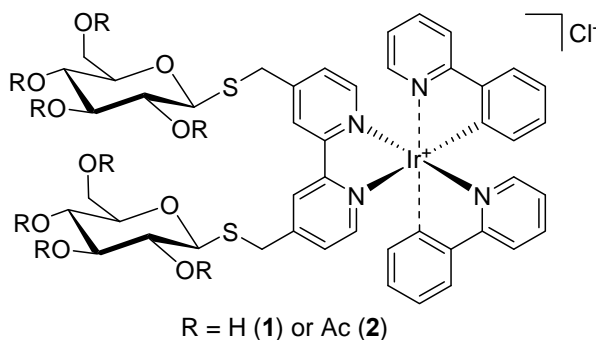
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Scheme 1 Schematic diagram for the formation of singlet oxygen.



Scheme 2 Glucose-conjugated Ir(III).



Scheme 3 Thioglucose-Ir(III) (1) and thioglucose tetraacetate-Ir(III) (2) complexes.

and synthesized iridium(III) complexes conjugated with glucose derivatives (Scheme 2).

We already established the synthetic method of acetyl-protected bipyridyl ligands and their complexes with rhenium(I), technetium(I), and ruthenium [9, 10], followed by deprotection of acetyl groups affording sugar-conjugated metal complexes. Ir(III) has also been reported in a pioneering synthetic study [11, 12]. For photogeneration of singlet oxygen, iridium complexes having 2-phenylpyridinato ligand (ppy) are noteworthy that they emit phosphorescence in the visible light region with high quantum yields and their absorption properties ( $\lambda_{\text{max}}$ ) can be tuned by modifying the ligands [13].

In this study, Ir(III) complexes containing a

thioglucose tetraacetate-conjugated bipyridyl ligand and a thioglucose-conjugated bipyridyl ligand were synthesized (Scheme 3), and their oxygen-responsiveness properties were investigated.

## 2. Experiment

### 2.1 Instrumentation

$^1\text{H-NMR}$  spectra were recorded on a JEOL JNM-AL-300 or JEOL JNM-AL-400 spectrometer using  $\text{CDCl}_3$  as the solvent with tetramethylsilane (TMS) as an internal standard. Chemical shifts in  $^{13}\text{C-NMR}$  were measured relative to  $\text{CDCl}_3$  by using  $\delta$  77.0 ppm. IR spectra were determined on a Shimadzu FTIR 8400 infrared spectrometer. Melting points were determined on a Yanagimoto micro melting point apparatus. Elemental analysis was carried out on a YANACO MT-5 and MALDI-TOF-mass spectra were measured on a JEOL JMS-T100LC and Bruker Autoflex II. All materials were obtained from commercial supplies and purified by distillation or recrystallization.

Absolute phosphorescence quantum yields of singlet oxygen were determined by an high sensitivity NIR quantum efficiency measurement system OTSUKA QE-5000.

### 2.2 Iridium(III) Complex Bearing 1-thio- $\beta$ -D-glucose 1 [11]

Thioglucose-conjugated bipyridyl ligand was synthesized according to our previously reported synthetic method. The solution of  $[\text{Ir}(\text{ppy})_2-\mu\text{-Cl}]_2$  (140 mg, 0.13 mmol) and bpy-thioglucose (150 mg, 0.26 mmol) in  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (60 mL, 1:1 (v/v)) was heated at 50 °C for 12 h under  $\text{N}_2$ . The solution was evaporated and then the residue was dissolved in about 5 mL of methanol. The yellow precipitate (1) was obtained after filtration: 210 mg (73%):  $^1\text{H NMR}$  (400 MHz, DMSO):  $\delta_{\text{H}}$  8.79 (s, 2H, H3-bpy), 8.26 (d, 2H,  $J = 8.4$  Hz, H6-bpy), 7.96-7.90 (m, 4H, H5-bpy, H3-ph), 7.77-7.74 (m, 2H, H3-py), 7.65-7.61 (m, 4H, H4-py, H6-py), 7.17 (t, 2H,  $J = 5.9$  Hz, H5-py), 7.00

(t, 2H,  $J = 7.6$  Hz, H4-ph), 6.89 (t, 2H,  $J = 7.3$  Hz, H5-ph), 6.17-6.19 (m, 2H, H6-ph), 5.25 (s, 2H, OH), 5.10 (s, 2H, OH), 5.02 (s, 2H, OH), 4.74-4.68 (m, 2H, OH), 4.16-4.10 (m, H1-sugar, CH2-S), 3.96-3.91 (m, 2H, CH2-S), 3.67-3.60 (m, 2H, H6-sugar), 3.36 (s, 2H, H6'-sugar, overlap with solvent peaks), 3.08-3.00 (m, 8H, H2-sugar, H3-sugar, H4-sugar, H5-sugar).

### 2.3 $\Lambda/\Delta$ -iridium(III) Complex Bearing 1-thio- $\beta$ -D-glucose Tetraacetate 2

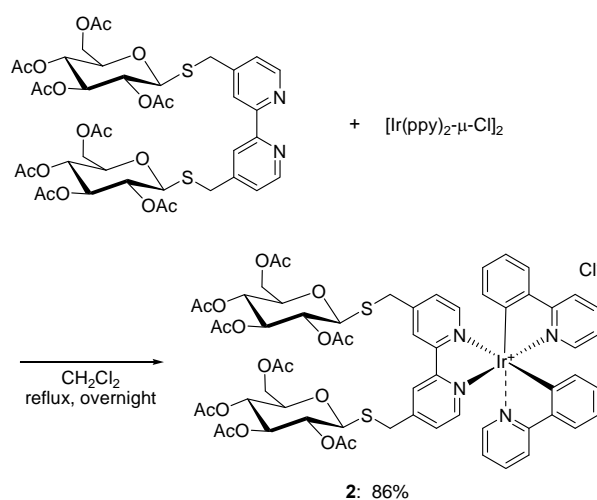
The solution of  $[\text{Ir}(\text{ppy})_2-\mu\text{-Cl}]_2$  (107 mg, 0.11 mmol) and bpy-thioglucose tetraacetate (200 mg, 0.22 mmol) in  $\text{CH}_2\text{Cl}_2$  (7 mL) was heated over night at 50 °C under  $\text{N}_2$ . The solution was evaporated and then the residue was washed with  $\text{Et}_2\text{O}$ /hexane (1/1), chromatographed ( $\text{CHCl}_3/\text{MeOH} = 8 : 1$ ), and recrystallized from  $\text{CH}_2\text{Cl}_2$ . The isolated products are mixtures of the  $\Lambda$ - and  $\Delta$ -isomeric forms of the octahedrally coordinated Ir(III) center, and this renders the complex 2 diastereomers as well as complex 1: Yellow solid, 273 mg (86%):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta_{\text{H}}$  10.00 (s, 1H, H3-bpy), 9.87 (s, 1H, H3'-bpy), 7.89-7.92 (m, 2H, H3-py), 7.72-7.80 (m, 4H, H6-bpy, H4-ph), 7.67 (m, 2H, H3-ph), 7.55 (d, 1H,  $J = 6.0$  Hz, H6-py), 7.50 (d, 1H,  $J = 6.0$  Hz, H6'-py), 7.40 (t, 2H,  $J = 7.0$  Hz, H5-bpy), 6.96-7.04 (m, 4H, H4-ph, H5-py), 6.88-6.91 (m, 2H, H5-ph), 6.27 (t, 2H,  $J = 8.8$  Hz, H6-ph), 5.25-5.33 (m, 3H, H6-sugar, CH2-S), 4.89-5.11 (m, 3H, H5-sugar, CH2-S), 4.93 (q, 2H,  $J = 9.6$  Hz, H6'-sugar), 4.26-4.38 (m, 4H, H3-sugar, CH2-S), 4.16-4.22 (m, 3H, H1-sugar, H4-sugar), 4.05-4.11 (m, 2H, H2-sugar), 3.72 (d, 1H,  $J = 9.6$  Hz, H1'-sugar), 1.97-2.04 (m, 12H, Ac), 1.91 (s, 6H, Ac), 1.75 (s, 6H, Ac);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.5, 170.4, 170.0, 169.9, 169.8, 169.7, 168.2, 168.1 (CO), 156.0 (C2-bpy), 153.9 (C2-py), 150.6 (C4-bpy), 149.9 (C1-ph), 149.7 (C6-bpy), 149.0 (C6-py), 143.5 (C2-ph), 138.0 (C4-py), 132.0 (C6-ph), 130.8 (C5-py), 128.1 (C5-bpy), 127.4 (C3-bpy), 124.8 (C3-ph), 123.2 (C5-ph), 122.6 (C4-Ph), 119.6 (C3-py), 85.2

(C6-sugar), 84.5 (C5-sugar), 75.8 (C4-sugar), 74.9 (C2-sugar), 74.1 (S-CH<sub>2</sub>), 70.7 (C6'-sugar), 69.0 (C5'-sugar), 68.5 (S-C'H<sub>2</sub>), 62.2 (C1-sugar), 61.9 (C2'-sugar), 34.9 (C4'-sugar), 34.3 (C3-sugar), 20.8, 20.7, 20.7, 20.6 (CH<sub>3</sub>). Mp. 185 °C (decomp); IR (KBr,  $\text{cm}^{-1}$ ): 601, 732, 762, 911, 1041, 1228, 1368, 1419, 1478, 1583, 1608, 1751, 2943, 3045, 3412; MS (MALDI-TOF):  $m/z$  (%): 1409.3 (100)  $[(\text{M}-\text{Cl})^+]$ . Anal. Calcd(%) for  $\text{C}_{62}\text{H}_{64}\text{ClIrN}_4\text{O}_{18}\text{S}_2\cdot\text{CH}_2\text{Cl}_2$ : C 49.46, H 4.35, N 3.66. Found: C 49.36, H 4.64, N 3.78.

### 3. Results and Discussion

To observe the remarkable emission of  $^1\text{O}_2$  at 1,270 nm,  $\text{C}_6\text{D}_6$  is the preferred solvent [14, 15]. However, the reported sugar-containing Ir(III) complex (1) is hardly soluble in benzene; thus, we synthesized a novel Ir(III) complex coordinated with bipyridyl ligands bearing acetyl-protected glucose by following a modified version of our reported method (Scheme 4) [9-11].

The starting bipyridyl ligands bearing acetyl-protected glucose were prepared according to a reported method [9]. Bromomethyl-2,2'-bipyridine (160 mg, 0.47 mmol) was dissolved in DMF (2 mL), followed by the addition of 1-thio- $\beta$ -D-glucose tetraacetate (350 mg, 0.97 mmol) and  $\text{Na}_2\text{CO}_3$  (350 mg, 3.3 mmol) at room temperature. After stirring for



Scheme 4 Synthesis of thioglucose tetraacetate-Ir(III) (2).

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two days, the reaction mixture was extracted with ethyl acetate, and the desired ligand was obtained by removing solvent in vacuo (354 mg, 90%).

The corresponding thioglucose tetraacetate-Ir(III) complex (2) was obtained in good yield and was soluble in common organic solvents and H<sub>2</sub>O.

At first, we tried to predict the energy transfer based on the emission spectra of the Ir(III) complex. Complexes 1 and 2 gave the same UV-vis and emission spectra in H<sub>2</sub>O under N<sub>2</sub>. Ir(III)-ppy complexes showed the same type of spectra in general

[16, 17]; therefore, the introduction of a sugar moiety does not influence their photoabsorption and emission properties. In chloroform, almost the same UV-vis spectrum was obtained for complex 2 as that obtained in water (Fig. 1).

We next measured the reduced emission in various solvents upon saturation with oxygen. In organic solvents chloroform, DMF, and DMSO (Figs. 2a, 2b and 2c, respectively), remarkable changes were observed due to energy transfer from the Ir(III) complex to <sup>3</sup>O<sub>2</sub>. The fluorescence intensity under the

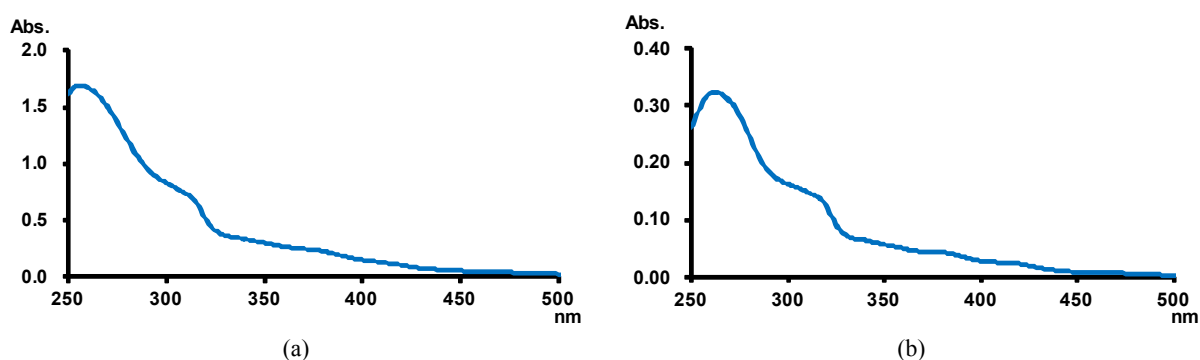


Fig. 1 UV-vis. spectra of 2 in (a) H<sub>2</sub>O ( $3.0 \times 10^{-5}$  M) and (b) CHCl<sub>3</sub> ( $6.0 \times 10^{-6}$  M).

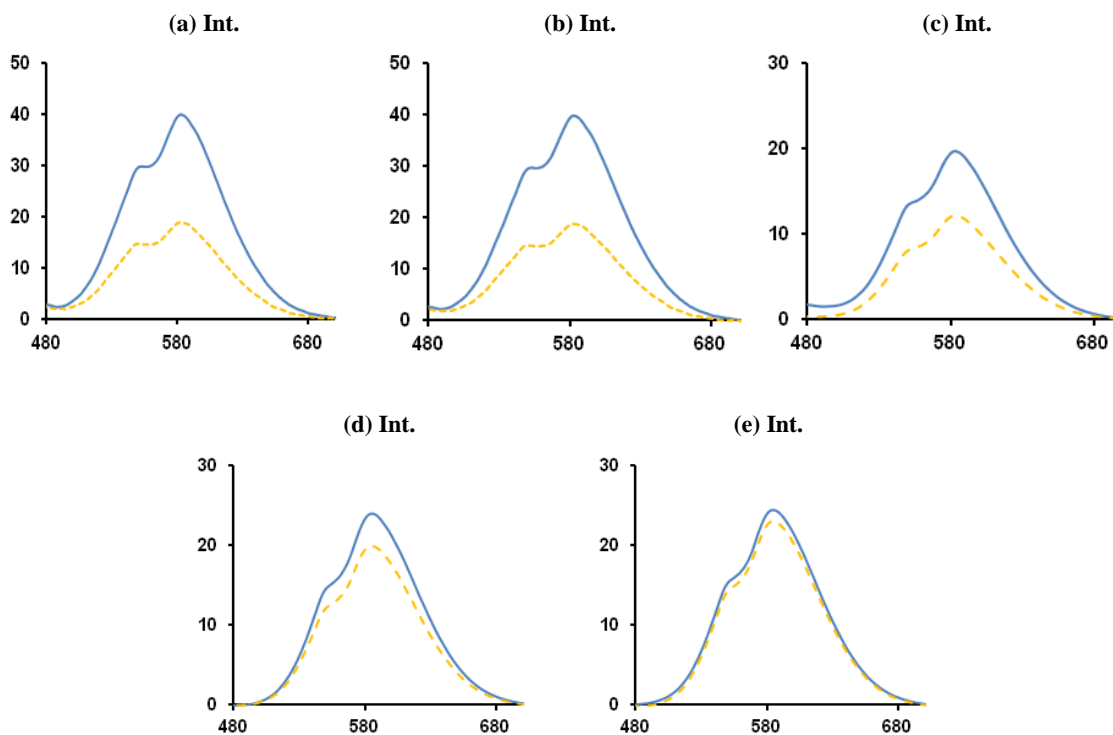


Fig. 2 Emission spectra of (2) excited at 371 nm in (a) CHCl<sub>3</sub> ( $6.0 \times 10^{-6}$  M), (b) DMF ( $6.0 \times 10^{-6}$  M), (c) DMSO ( $6.0 \times 10^{-6}$  M), (d) H<sub>2</sub>O ( $3.0 \times 10^{-5}$  M), and (e) (1) in H<sub>2</sub>O ( $3.0 \times 10^{-5}$  M). Blue lines show under N<sub>2</sub> and dashed lines show after O<sub>2</sub> bubbling.

oxygen-saturated condition was less than that under N<sub>2</sub>.

This result indicates that reactive oxygen species were generated by energy transfer from the photosensitizer to molecular oxygen. In contrast to this, it is difficult to observe the decrease in the emission of complexes 1 and 2 in water (Figs. 2d and 2e).

The emission spectra of <sup>1</sup>O<sub>2</sub> generated by photoirradiation were measured on complexes 2, which were dissolved in C<sub>6</sub>D<sub>6</sub> and excited at 450 nm to observe the emission spectra of <sup>1</sup>O<sub>2</sub> (Fig. 3) [18]. A comparison of the spectra under N<sub>2</sub> with that obtained after O<sub>2</sub> bubbling showed that the phosphorescence of singlet molecular oxygen decreased.

Emission intensities of complexes 1, 2, and Ir(bpy)(ppy)<sub>2</sub><sup>+</sup>(Cl<sup>-</sup>) (3) were also measured; the results are summarized in Table 1. Complexes 1 and 2 dissolve in D<sub>2</sub>O; however, the intensities were weak (entries 1, 2, 5, 6, 9 and 10). In the C<sub>6</sub>D<sub>6</sub> solvent, remarkable intensities were observed (entries 3, 4, 7 and 8). These results show that the phosphorescence of singlet molecular oxygen observed in the organic solvent is clearly relevant to the results of the fluorescence spectra for <sup>1</sup>O<sub>2</sub> generation.

The absolute phosphorescence quantum yields of 2 were measured in C<sub>6</sub>D<sub>6</sub> on the QE-5000 apparatus. In the D<sub>2</sub>O solvent, the intensity was too weak to estimate the quantum yield; however, in the C<sub>6</sub>D<sub>6</sub> solvent, the quantum yield was 0.0255% on the

QE-5000 apparatus.

In summary, an Ir complex containing a thioglucose tetraacetate-conjugated bipyridyl ligand was synthesized. This complex can dissolve in common organic solvents and the emission spectra of <sup>1</sup>O<sub>2</sub> generated by photoirradiation can be observed. The quantum yields are not very high; however, it is revealed that the measurement of the fluorescence spectra is a convenient method to generate <sup>1</sup>O<sub>2</sub> for exploring effective PDT reagents.

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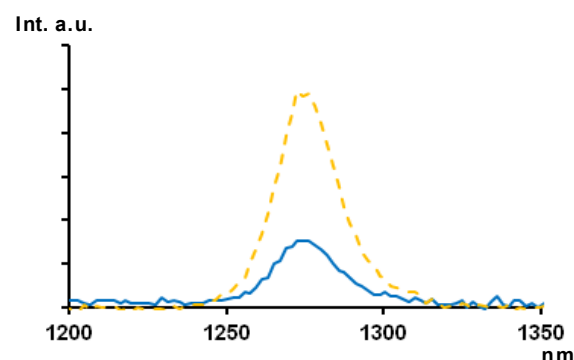


Fig. 3 Emission spectra of <sup>1</sup>O<sub>2</sub> in C<sub>6</sub>D<sub>6</sub> solution of 2 (3 × 10<sup>-4</sup> M). Blue lines show under N<sub>2</sub> and dashed lines show after O<sub>2</sub> bubbling.

Table 1 Emission intensities.

Entry	Complex	Solv	Condition	Intensity (A. U.)
1	1	D <sub>2</sub> O	N <sub>2</sub>	9
2			O <sub>2</sub>	9
3	2	C <sub>6</sub> D <sub>6</sub>	N <sub>2</sub>	153
4			O <sub>2</sub>	484
5		D <sub>2</sub> O	N <sub>2</sub>	0
6			O <sub>2</sub>	87
7	3	C <sub>6</sub> D <sub>6</sub>	N <sub>2</sub>	1,863
8			O <sub>2</sub>	2,662
9		D <sub>2</sub> O	N <sub>2</sub>	10
10			O <sub>2</sub>	13

Yoshihiro Osawa for measurements of emission spectra of  $^1\text{O}_2$ .

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