



Title	Absorption, Fluorescence, and Two-Photon Excitation Ability of 5-o-Tolyl-11 (or 13)-o-tolylisoindolo[2,1-a]quinolines Prepared by Ring-Closing Metathesis and [2+3] Cycloaddition
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Absorption, Fluorescence, and Two-Photon Excitation Ability of 5-*o*-Tolyl-11 (or 13)-*o*-tolylisoindolo[2,1-*a*]quinolines Prepared by Ring-Closing Metathesis and [2 + 3] Cycloaddition

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We have successfully improved the fluorescence quantum yield of isoindolo[2,1-*a*]quinoline derivatives by suppressing the rotation of the phenyl groups at positions 5 and 11 (or 13).

Additionally, we found that the planarity of these phenyl groups at positions 5 and 11 (or 13) of isoindolo[2,1-*a*]quinoline derivatives is crucial for two-photon absorption properties.

Near-infrared absorbing dyes have been applied to medical diagnostics, photodynamic therapy, and near-infrared immunotherapy due to their ability penetrate to water and cells with near-infrared light at wavelengths ranging from 700 to 2,000 nm. Near infra-red light is also relatively non-invasive to the human body because of their long wavelength and low energy (Figure 1).^[1]

Furthermore, in recent years, with the development of laser technology, two-photon absorbing dyes have attracted considerable attention. Two-photon excitation is an excitation process in which two photons are simultaneously absorbed, with the probability of this occurring being proportional to the square of the light intensity. Therefore, in the field of bioimaging, two-photon absorbing dyes, which are excited only at the focal site using laser light,

have been actively investigated for deep and high-resolution imaging.^[2]

We have discovered a one-pot synthesis method for a novel nitrogen-containing polycyclic isoindolo[2,1-*a*]quinoline skeleton **1** (Scheme 1), and we have been conducting research on the absorption, fluorescence and two-photon excitation properties of its analogs.^[3] In this report, we tried to synthesize derivatives that increase the rotational energies of the aryl rings at positions 5 and 11 (or 13) based on our previous research results on fluorescein and information in the literature. First, we confirmed by calculation that the above rotational energies of the designed compounds were increased. Next, through actual chemical synthesis, we clarified the role of the aryl ring of the above-designed compound on the optical properties. The novelty of this paper is that we found which part of the isoindoloquinoline dye is the important structure and how these aryl substituents affect the optical properties such as ϵ , Φ , and GM values.

The highest fluorescence quantum yield of our previously prepared compound **1** is $\Phi = 0.18$. We envisioned that the fluorescent properties could be improved by introducing *ortho*-substituents to the phenyl groups at the 5- and 11 (or 13)-positions of isoindolo[2,1-*a*]quinoline, thereby increasing the dihedral angle and rotational energy, as in

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Table 1. HOMO/LUMO energy levels of xanthen, the pigment matrix of fluorescein, and isoindolo[2,1-*a*]quinoline, benzene, and toluene assumed to be the pigment matrix.

Compound	HOMO ^[a]	LUMO ^[a]
Xanthenes	−0.20598	−0.08821
Isoindolo[2,1- <i>a</i>]quinoline	−0.21531	−0.10011
Benzene	−0.24690	0.00267
Toluene	−0.23561	0.00297

[a] unit is hartree

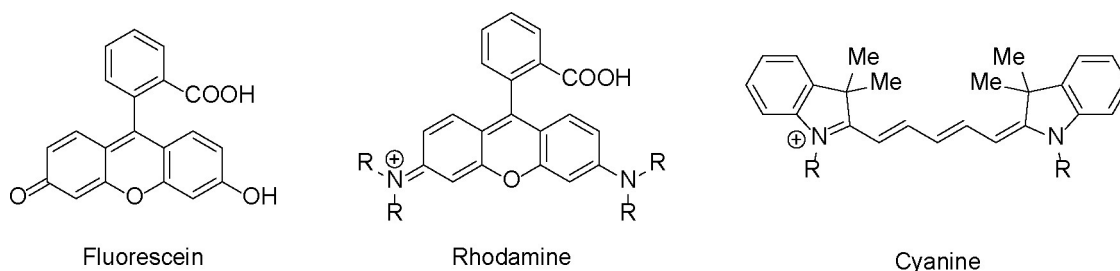
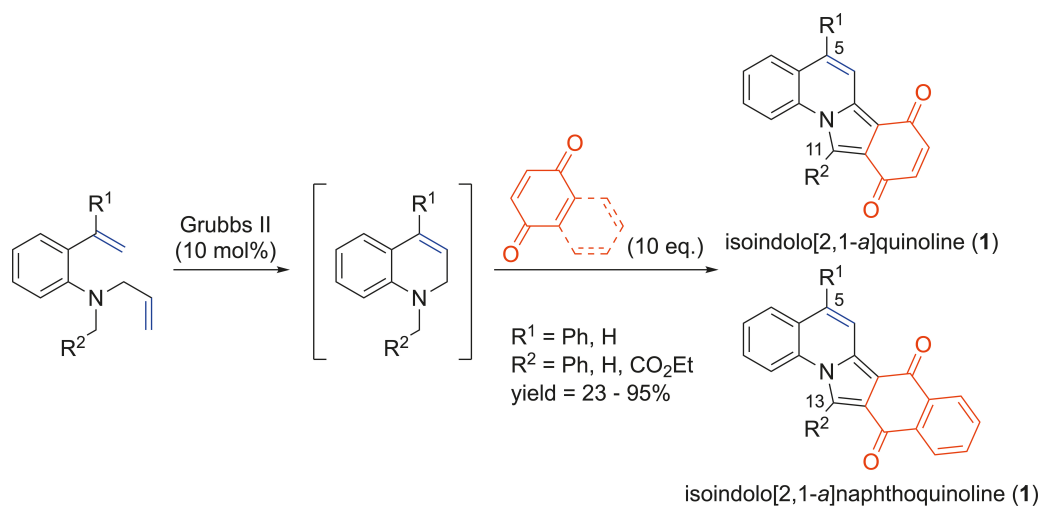


Figure 1. Typical examples of fluorescent dyes used as protein fluorescent labeling reagents.



Scheme 1. Synthesis method of isoindolo[2,1-a]quinoline skeleton 1, and numbering of positions.

fluorescein^[4] (Figure 2). We also wondered whether the planarity of these phenyl groups at positions 5 and 13 of isoindolo[2,1-a]quinoline derivatives is crucial for two-photon absorption properties.

In order to exhibit the same high fluorescence quantum yield as fluorescein, it is necessary to suppress intramolecular photoinduced electron transfer (PeT). If the HOMO/LUMO of the pigment matrix skeleton lies between the HOMO/LUMO of the benzene ring, the excited electrons of the pigment matrix skeleton can return to the ground state HOMO of the pigment matrix skeleton, thereby exhibiting strong fluorescent properties (Figure 3).^[5] Both energy levels of HOMO and LUMO of the pigment matrix structure should be between the HOMO/LUMO gap of benzene. The pigment matrix skeleton's HOMO level should be above the HOMO level of benzene. On the other hand, the pigment matrix skeleton's LUMO level should be below the LUMO level of benzene.

We used Gaussian 16 with B3LYP /6-31 G(d,p) basis set to calculate the HOMO/LUMO energy levels. In this calculation, the isoindolo[2,1-a]quinoline skeleton was assumed to be the pigment matrix skeleton for comparison (Table 1).

As a result, it was found that the HOMO/LUMO energy levels of the isoindolo[2,1-a]quinoline skeleton lies between those of the benzene ring, similar to the xanthene skeleton. In other words, the electrons in the isoindolo[2,1-

Table 2. Table of dihedral angles (a-b-c-d); hydroxyphenylfluorone, fluorescein, and Tokyo Green.

Compound	R	Dihedral angle (°)
Hydroxyphenylfluorone	H	67.8
Fluorescein	COOH	74.1
Tokyo Green	Me	85.8

Table 3. UV-visible absorption and fluorescence photophysical properties of compounds 1 a - 1 h.

	λ_{\max} (nm)	ϵ (M ⁻¹ cm ⁻¹)	λ_{em} (nm)	Φ
1 a	485.0	8504	609	0.001576
1 b	471.5	6262	596	0.001142
1 c	482.5	9091	599	0.002522
1 d	478.5	9637	595	0.003842
1 e	454.5	12318	553	0.1888
1 f	448.5	10880	547	0.1757
1 g	453.0	11797	545	0.2414
1 h	447.0	12143	541	0.2574

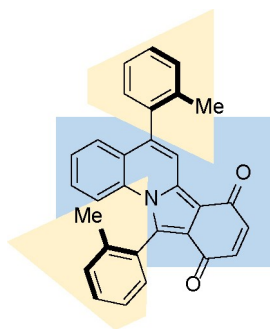


Figure 2. Conceptual diagram of the dihedral angle when *ortho*-methyl groups are introduced into the phenyl groups at positions 5 and 11 of the isoindolo[2,1-*a*]quinoline skeleton.

Table 4. Emission lifetime τ , radiative transition rate constant k_r , and nonradiative transition rate constant k_{nr} , of compounds 1 e - 1 h .			
	τ (ns)	k_r (ns ⁻¹)	k_{nr} (ns ⁻¹)
1 e	4.9	0.0394	0.169
1 f	6.8	0.0262	0.121
1 g	7.3	0.0330	0.104
1 h	7.6	0.0338	0.0978

a]quinoline skeleton are excited by light, transition through the singlet excited state, and return to the ground state without causing PeT. This suggests that, similar to fluorescein, increasing the dihedral angle may improve its fluorescence properties. In addition, we hypothesized introducing a methyl group, as in Tokyo Green, could further enhance its fluorescence characteristics, so we also examined the HOMO/LUMO energy levels of toluene.

Based on these results, we decided to introduce *ortho*-methyl groups to the phenyl groups at the 5th and 11th (or 13th) positions and compare the effects on changes in the dihedral angle and rotational energy. We then compared the dihedral angles and rotational energies of hydroxyphenylfluorone, fluorescein, and Tokyo Green (Table 2).^[6]

It was found that the dihedral angles in the most stable structure of Tokyo Green are almost orthogonal. However, when comparing the dihedral angles of hydroxyphenylfluorone and fluorescein, the difference was 6.3°. On the other hand, the dihedral angles of fluorescein and Tokyo Green differ by 10.7°, yet their fluorescence quantum yields are the same. This suggests that discussing fluorescence quantum yield based only on dihedral angles is insufficient. Therefore, we calculated the rotational energy of each compound using Gaussian 16 B3LYP/6-31 G(d,p) and compared the differences in rotational barriers (Figure 3). In this calculation, we used the most stable structure as a reference and compared it by rotating the molecule in 30° increments. For hydroxyphenylfluorone, a rotation of 180° was calculated based on the symmetry of its structure.

The rotational barrier of hydroxyphenylfluorone was found to be greater than 22.6 kcal/mol. Furthermore, the rotational barrier for fluorescein was 36.5 kcal/mol or more,

and for Tokyo Green, the rotational barrier was 49.4 kcal/mol or more. This suggests that the substituent on the phenyl group increases the rotational barrier and suppresses rotation.

Next, similar rotational energy calculations were performed for isoindolo[2,1-*a*]quinoline derivatives using Gaussian 16 B3LYP/6-31 G(d,p) (Figure 4).^[7]

The rotational barrier of the phenyl group at the 5-position of isoindolo[2,1-*a*]quinoline **1 a** was found to be considerably smaller than that of hydroxyphenylfluorone, measuring more than 6.1 kcal/mol (Figure 4a). However, the rotational barrier of the *ortho*-tolyl group at position 5 of isoindolo[2,1-*a*]quinoline **1 b** is greater than 24.1 kcal/mol, indicating that the rotational barrier becomes larger due to the presence of the methyl group, which is absent in **1 a** (Figure 4b). Notably, the rotational barrier of the *ortho*-tolyl group at the 5-position of **1 b** is close to that of hydroxyphenylfluorone, and is attributed to the difference between the xanthene skeleton and the isoindolo[2,1-*a*]quinoline skeleton. For clarity, the xanthene skeleton has three rings arranged horizontally in a row, whereas the isoindolo[2,1-*a*]quinoline skeleton has two rings arranged horizontally in a row and the remaining two rings oriented diagonally downward. This arrangement is thought to make the isoindolo[2,1-*a*]quinoline skeleton less susceptible to steric hindrance due to the phenyl group substituent at the 5th position.

In addition, the rotational barrier of the phenyl group at position 11 was more than 15.4 kcal/mol. In contrast, the rotational barrier of isoindolo[2,1-*a*]quinoline **1 c**, which has an *ortho*-methyl group on the phenyl group at position 11, was found to be significantly higher, exceeding 52.3 kcal/mol (Figure 4c). This suggests that the *ortho*-tolyl group at position 11 suppresses rotation to the same extent as Tokyo Green (49.4 kcal/mol).

Based on these results, we decided to synthesize isoindolo[2,1-*a*]quinolines **1 b** and **1 c** to verify whether increasing the rotational barrier leads to an improvement in the fluorescence quantum yield. We also aimed to verify the rotational barrier value required to improve the fluorescence quantum yield by comparing **1 b** and **1 c**.

Following previous reports,^[3] we synthesized isoindolo[2,1-*a*]quinoline derivatives **1 a** - **1 d**. We've tried other solvents like toluene and found that benzene is the best solvent in this catalytic system, as reported in reference 3a.

As shown in Scheme 2, a Grignard reagent was prepared from a bromobenzene derivative and reacted with *ortho*-aminoacetophenone to yield the corresponding alcohol **3**. When a solution of **3** and *p*-TsOH·H₂O in toluene was refluxed, dehydration proceeded to produce compound **4**. Compound **6** was prepared through reductive amination of **4** and a benzaldehyde derivative, and subsequent allylation on the amino group. The desired isoindolo[2,1-*a*]quinoline derivatives **1 a** - **1 d**, were synthesized by diene ring-closing metathesis of the corresponding compounds **6**, followed by oxidation of the 1,2-dihydroquinoline derivative to

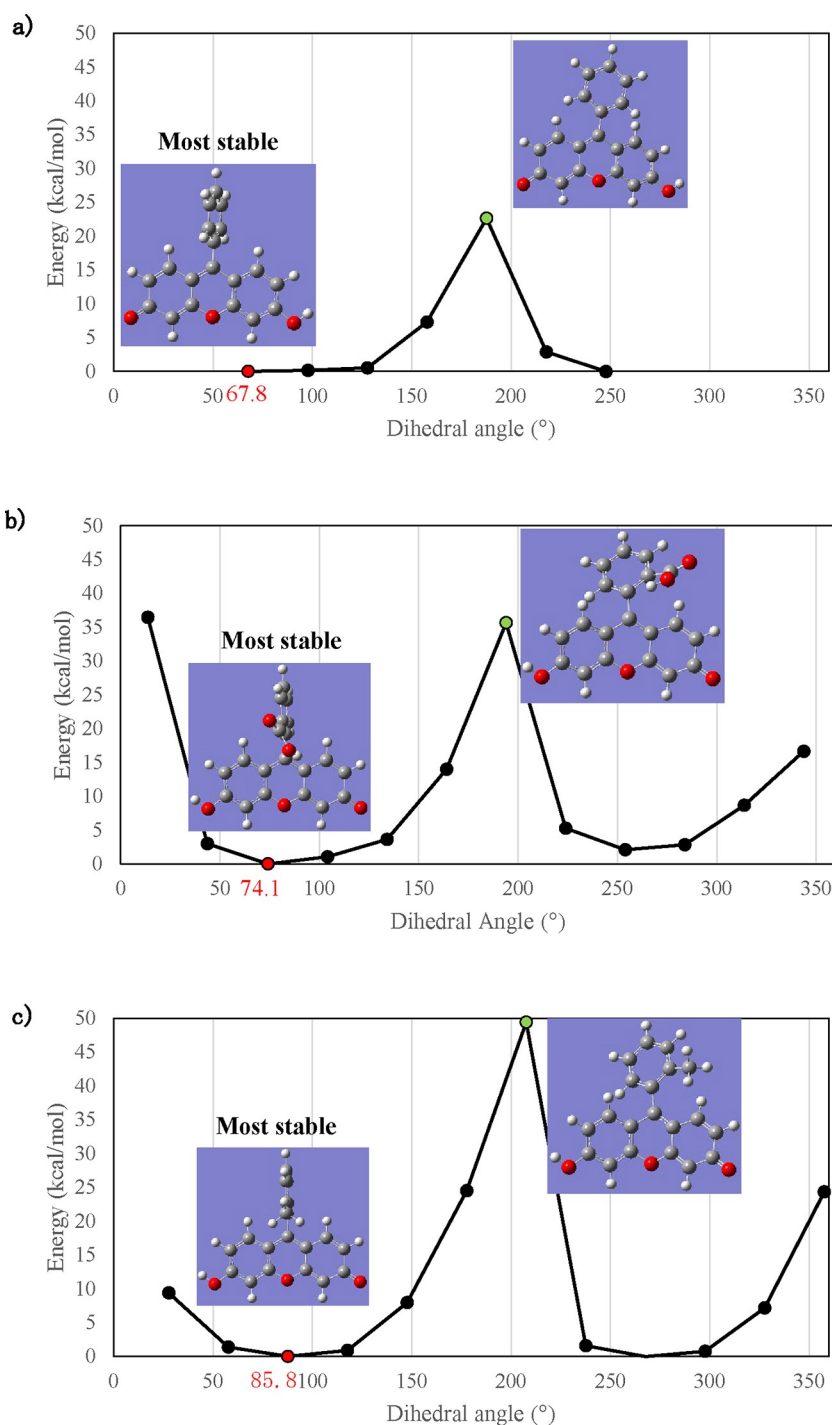


Figure 3. Rotational energy calculation results: most stable structure (red circle), most unstable structure (green circle); (a) hydroxyphenylfluorone, (b) fluorescein, (c) Tokyo Green.

quinoline with quinone, and 1,3-dipolar cycloaddition reaction. For compounds **6b** and **6d**, which have a methyl group at the 2'-position of the phenyl group at the 5-position, the yields of the corresponding **1b** and **1d** were significantly reduced. This is thought to be because the presence of the *ortho*-methyl group increased the bulk around the styrene, which is the reaction site, and the ring-

closing metathesis reaction competed with the intermolecular reaction.

Subsequently, a similar reaction was performed using naphthoquinone instead of benzoquinone, leading to the synthesis of compounds **1e-1h** with a naphthoquinone skeleton (Scheme 3).

Similar to **6b** and **6d**, compounds **6f** and **6h**, which have an *ortho*-methyl group at the 2'-position of the phenyl

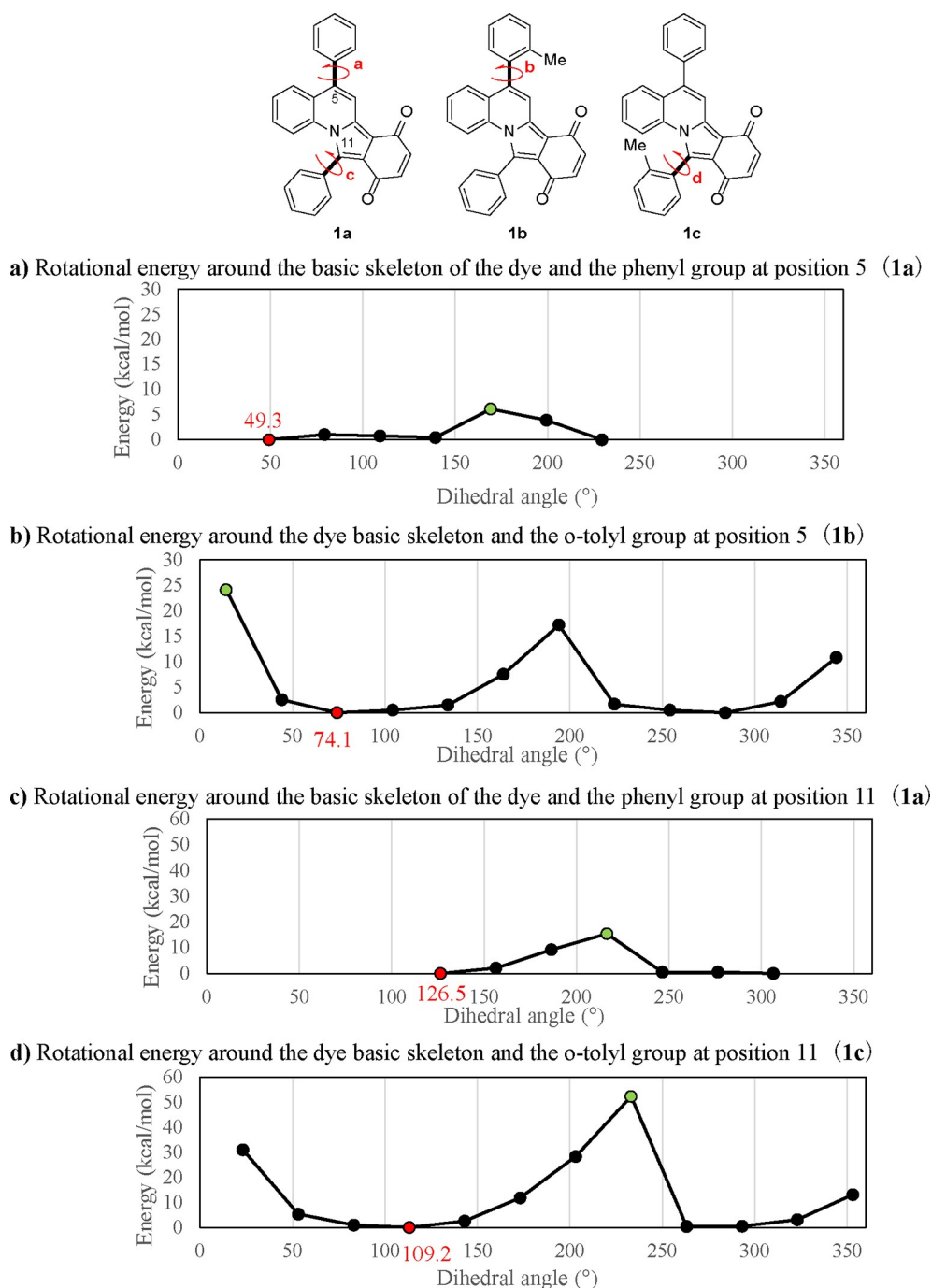


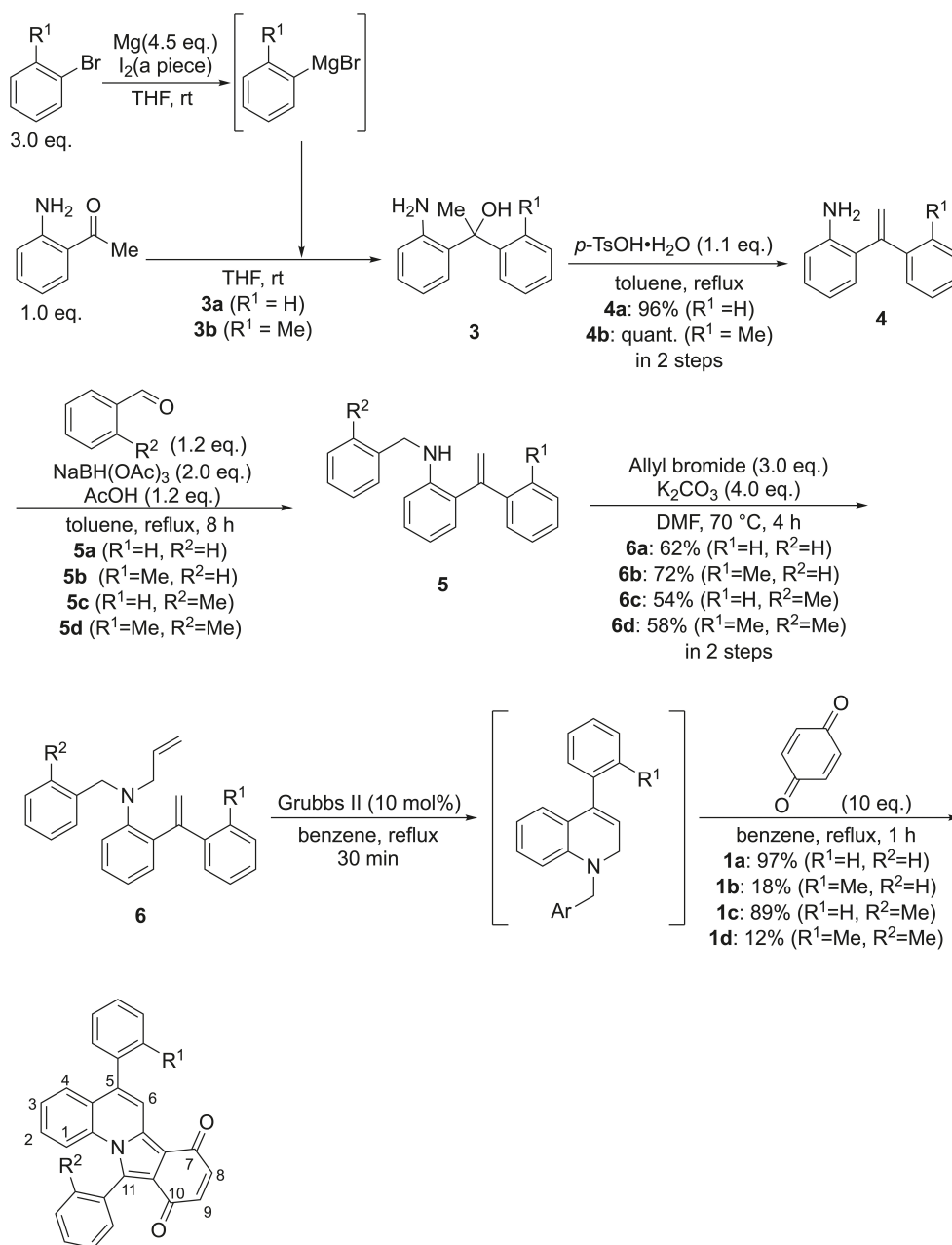
Figure 4. Calculation results of rotational energy between the isoindolo[2,1-*a*]quinoline skeleton and the substituent at the 5th or 11th position: Most stable structure (red circle), most unstable structure (green circle).

group at the 5-position, significantly reduced the yields of the corresponding **1f** and **1h**.

The ultraviolet and visible absorption spectra of synthesized **1a** - **1h** were measured in dimethylsulfoxide, DMSO,^[8] and the molar extinction coefficient $\epsilon(\lambda_{\max})$ at the maximum absorption wavelength λ_{\max} were measured (Figures 5 and 6). In addition, fluorescence spectra obtained by irradiating each compound with light at the maximum absorption wavelength λ_{\max} are shown. The fluorescence wavelength λ_{em} and fluorescence quantum yield Φ were measured with

the excitation wavelength λ_{ex} set to the maximum absorption wavelength of each compound.

First, compounds **1a** - **1d** were compared. Compounds **1b** - **1d**, which have *ortho*-methyl groups, did not show a significant difference in the maximum absorption wavelength λ_{\max} and fluorescence wavelength λ_{em} compared to **1a**. Compound **1b** showed a decrease in molar absorption coefficient ϵ of about $2,000 \text{ M}^{-1} \text{ cm}^{-1}$ compared to **1a**. Conversely, for compounds **1c** and **1d**, which have a methyl group on the phenyl group at the 11th position, the molar



Scheme 2. Synthesis of isoindolo[2,1-*a*]quinoline, **1a** - **1d**.

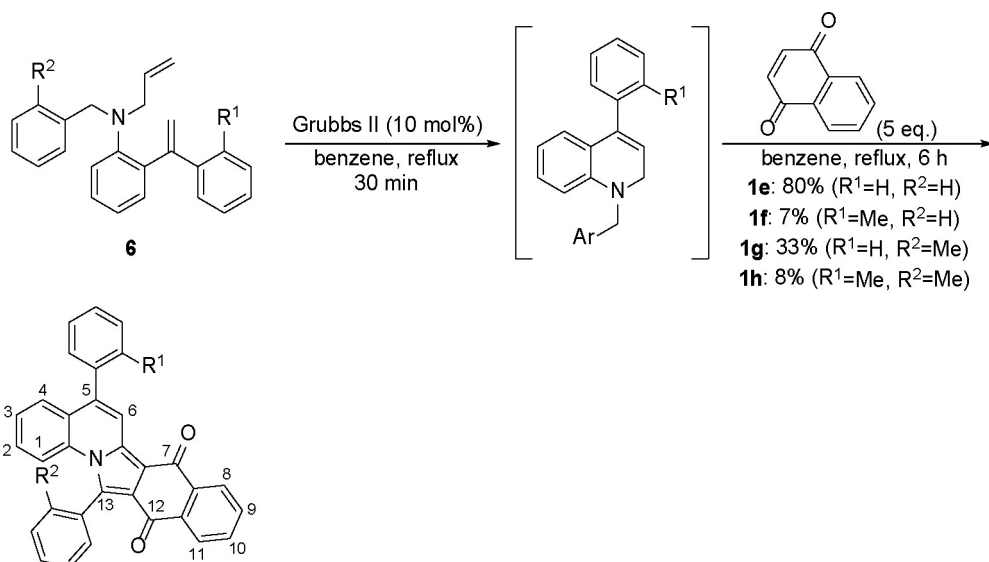
absorption coefficient ϵ was improved by about $1,000 \text{ M}^{-1} \text{ cm}^{-1}$ compared to **1a**.

Next, the fluorescence quantum yield Φ was compared. The introduction of an *ortho*-methyl group to the phenyl group at the 5th position did not show any improvement in the fluorescence quantum yield Φ . However, the introduction of an *ortho*-methyl group to the phenyl group at the 11th position more than doubled the fluorescence quantum yield Φ from 0.001576 to 0.003842. Despite the improvement, the value was small, so we decided to use **1e** - **1h** to further investigate the effect of the *ortho*-methyl group.

Compounds **1f** - **1h**, which have *ortho*-methyl groups, did not show any significant differences in the maximum

absorption wavelength λ_{max} and fluorescence wavelength λ_{em} when compared with **1e**, similar to the results observed with compounds **1a** - **1d**. The molar absorption coefficient ϵ of compound **1f** was reduced by about $1,500 \text{ M}^{-1} \text{ cm}^{-1}$ compared to **1a**. Furthermore, the molar absorption coefficient ϵ of **1g** and **1h**, which have an *ortho*-methyl group on the phenyl group at the 13th position, were almost the same as that of **1e**.

Next, the fluorescence quantum yield Φ was compared. No improvement in the fluorescence quantum yield Φ was observed for **1f**, where an *ortho*-methyl group was introduced on the phenyl group at the 5th position, and its value was almost the same as that of **1e**. However, the



Scheme 3. Synthesis of isoindolo[2,1-*a*]quinolines, **1e** - **1h**.

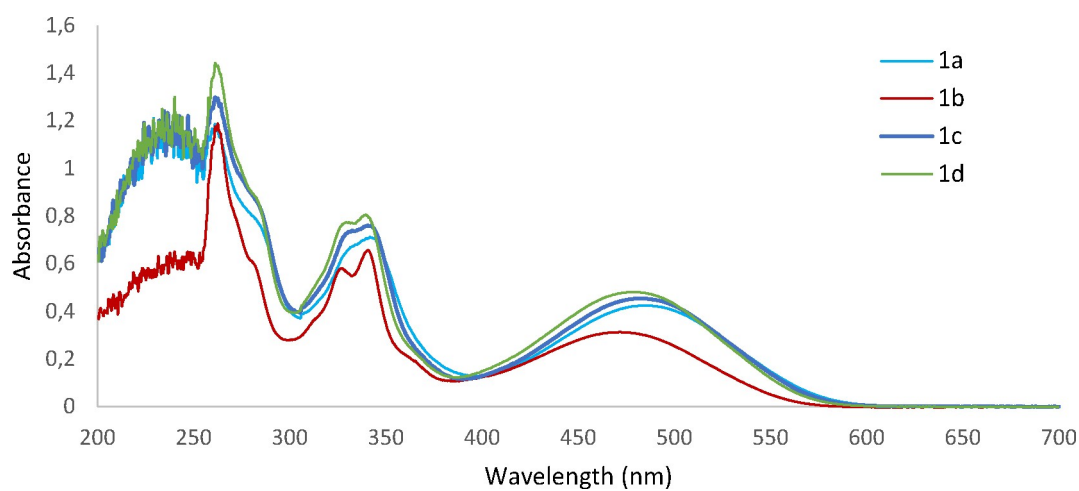


Figure 5. UV-visible absorption spectra of dyes **1a** - **1d** (50 μ M in DMSO).

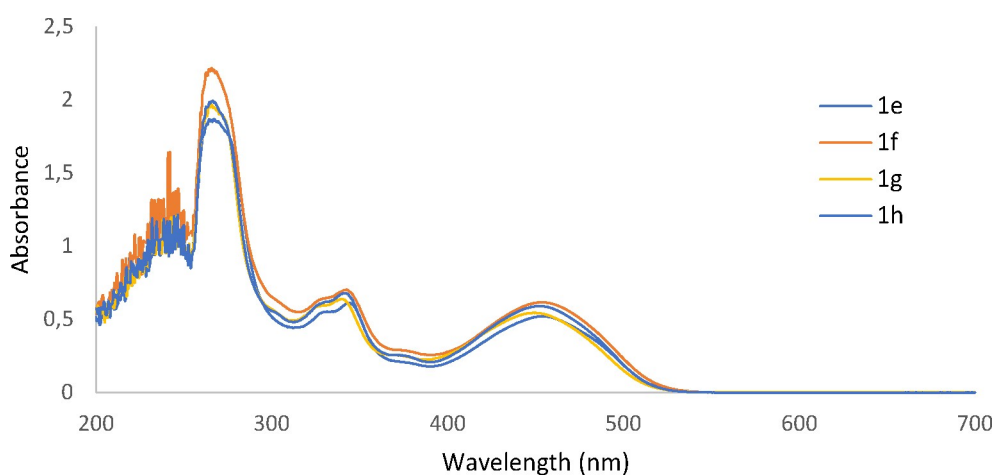


Figure 6. UV-visible absorption spectra of dyes **1e** - **1h** (500 nM in DMSO).

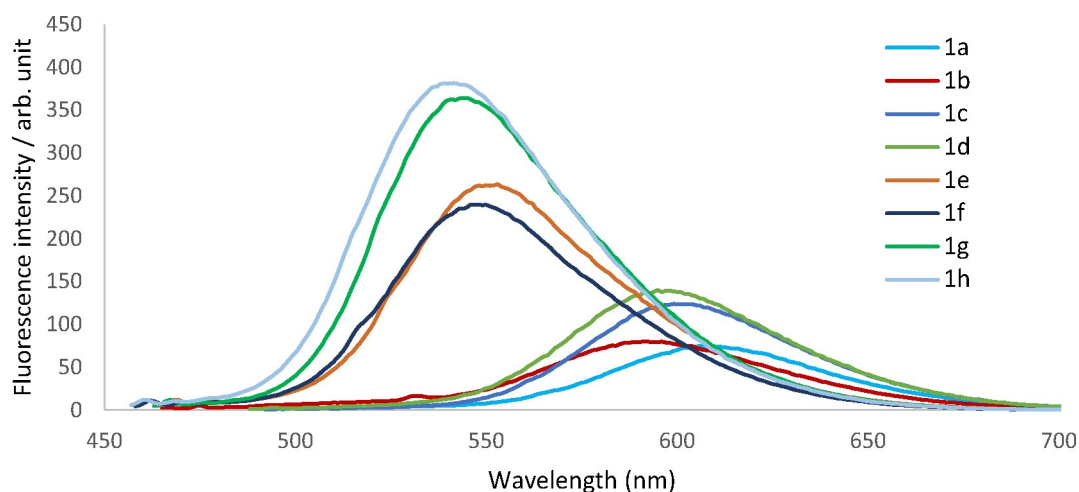


Figure 7. Fluorescence spectra of dyes **1 a - 1 h** (500 nM in DMSO).

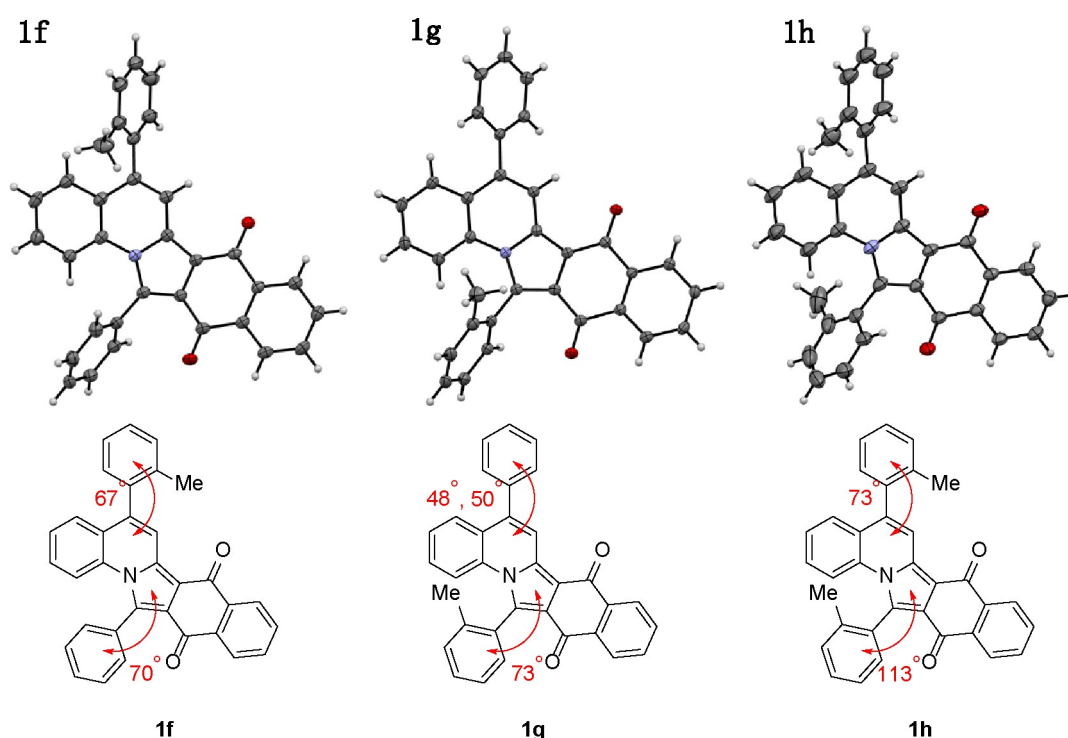


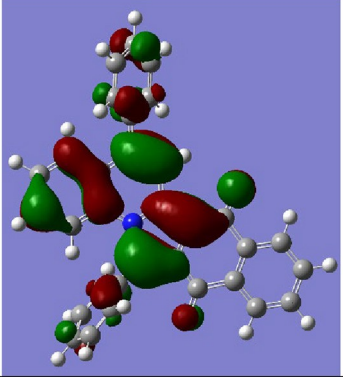
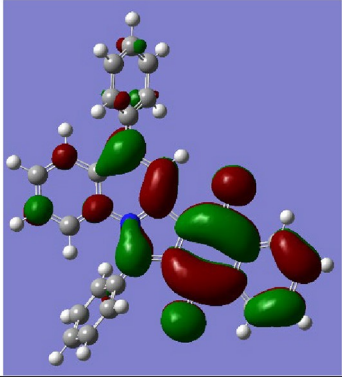
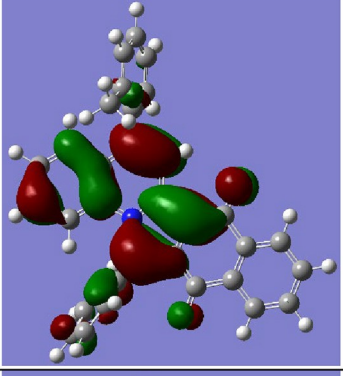
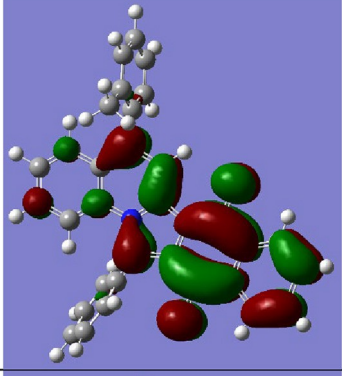
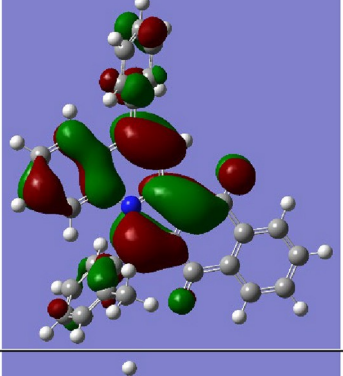
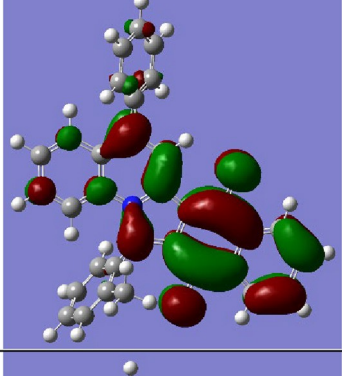
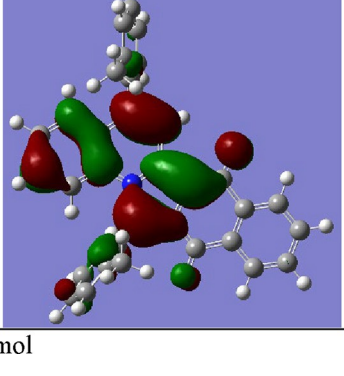
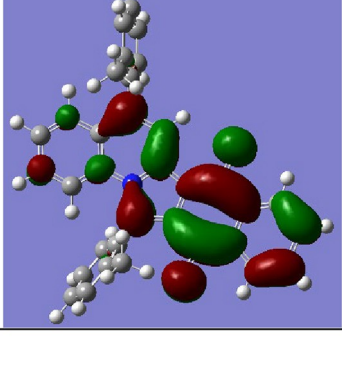
Figure 8. X-ray crystal structure of compounds **1 f - 1 h** and diagram of each dihedral angle (CCDC deposition number **1 f**: 2368638, **1 g**: 2368639, **1 h**: 2368640).

introduction of a methyl group on the phenyl group at the 13th position improved the fluorescence quantum yield to 24% for **1 g** and 26% for **1 h**, which is higher than the 19% of **1 e**.

In addition, the fluorescence lifetimes of **1 f-1 h** were measured (Table 4). It was found that the fluorescence lifetime was longer in all compounds. Furthermore, the radiative transition rate constant k_f and the nonradiative transition rate constant k_{nr} were calculated using the obtained fluorescence quantum yield and lifetime.^[9] For compound **1 f**, the radiative transition rate constant decreased significantly. In contrast, for compounds **1 g** and **1 h**,

the radiative transition rate constant did not decrease much, while the nonradiative transition rate constant decreased significantly.^[10]

Based on the above results, it is considered that the substituents were effective in suppressing rotation in all compounds, as the introduction of the substituents lowered the nonradiative transition rate. However, the nonradiative transition rate constant of **1 f** did not decrease as much as **1 g** and **1 h**, which is attributed to the smaller rotational energy of the phenyl group at position 5 compared to the phenyl group at position 11. Furthermore, when the rotation of the phenyl group at the 5-position was sup-

Compound	HOMO	LUMO	Optimization energy ^a
1e			-9.012×10^5
1f			-9.259×10^5
1g			-9.259×10^5
1h			-9.505×10^5

a: unit is kcal/mol

Figure 9. HOMO-LUMO orbital of the most stable structure for 1f - 1h.

pressed, the radiative transition rate was significantly reduced and the fluorescence quantum yield also decreased significantly. These results suggest that the phenyl group at position 5 could be a part of the fluorophore, suggesting

that the phenyl groups at position 5 and 11 have different roles Table 4.

In addition, the obtained compounds 1f - 1h were recrystallized in a mixed solution of chloroform/hexane, and

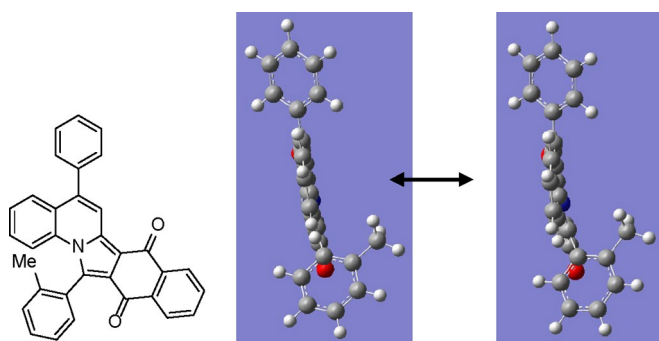


Figure 10. Diagram showing the distortion of the isoindolo[2,1-*a*]quinoline skeleton.

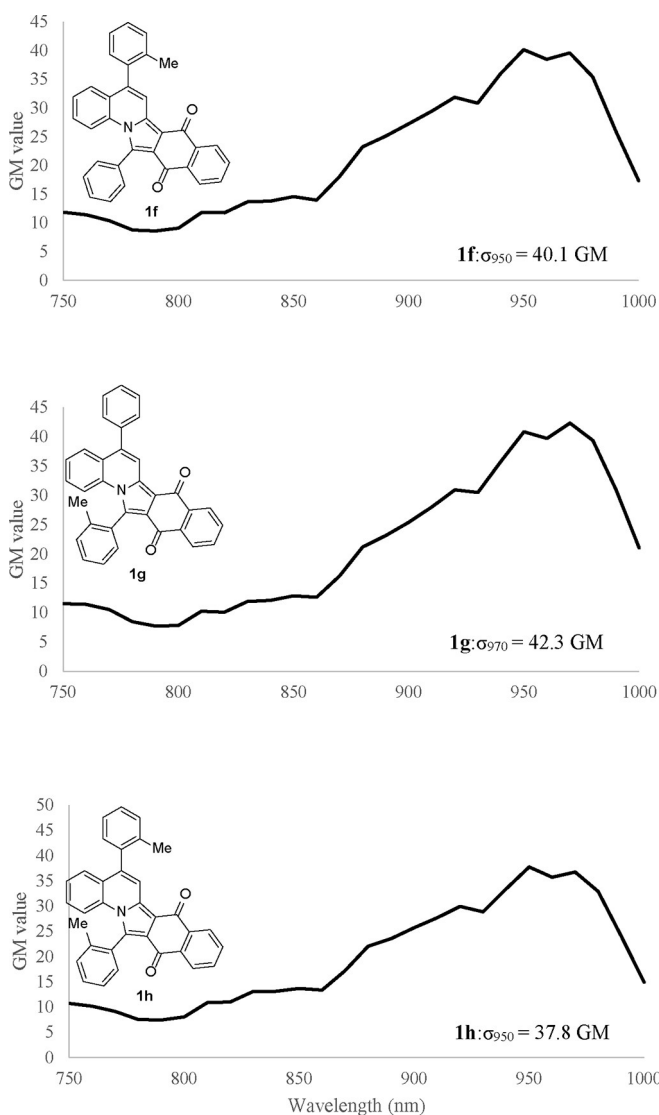


Figure 11. Two-photon properties of dyes **1f** - **1h** (in DMSO solution).

X-ray crystal structure analysis was performed on the resulting crystals. The dihedral angle results obtained by X-ray crystal structure analysis (Figure 8) showed no significant correlation between fluorescence quantum yield and

dihedral angle. In addition, the dihedral angles obtained from the X-ray crystal structure indicate that the 5- and 13-position aryl groups are stabilized by the adjacent molecules and the recrystallization solvent, since the observed molecules have a crystalline structure. Therefore, these crystal structure data are not considered suitable for comparison with the fluorescence quantum yield measured in solution, which is less affected by neighboring molecules.

Next, the most stable HOMO-LUMO orbitals of compounds **1f** - **1h** were analyzed using Gaussian 16 B3LYP/6-31 G(d,p). The results are illustrated in Figure 9. It was found that in the HOMO orbital, the electron density is biased toward the isoindolo[2,1-*a*]quinoline skeleton, while in the LUMO orbital, the electron density is biased toward the naphthoquinone skeleton. This polarization of electrons on the isoindolo[2,1-*a*]quinoline skeleton is essential for it to have a dye function.

Furthermore, when replacing the phenyl group at position 5 with a tolyl group, a comparison of the HOMO orbitals revealed that the phenyl group had a higher electron density than the tolyl group (**1e**, **1g** vs. **1f**, **1h**). Specifically, when comparing **1e** and **1f**, the fluorescence quantum yield was slightly lower in **1f**, suggesting that a change in electron density influenced the fluorescence quantum yield. Furthermore, since **1e** is larger than **1f** in terms of molar extinction coefficient, it is believed that this difference in electron density also affects the molar extinction coefficient. Therefore, in addition to the rotational barrier, it is possible that both the fluorescence quantum yield and the molar extinction coefficient could be improved if the phenyl group is not perpendicular to the basic skeleton and maintains its planarity. On the other hand, regarding the phenyl group at position 13, no significant difference in electron density was observed in the HOMO-LUMO orbital, even when it was replaced with a tolyl group.

When calculating the molecular vibration of the most stable structure using Gaussian 16 B3LYP/6-31 G(d,p), it was observed that for fluorescein and Tokyo Green, the vibration is only in the direction of rotation of the phenyl group bonded to the xanthene skeleton. In contrast, for isoindolo[2,1-*a*]quinoline derivatives, relatively large vibrations of energy in the direction of the distortion of the isoindolo[2,1-*a*]quinoline skeleton were observed. It is believed that the fluorescence quantum yield of the isoindolo[2,1-*a*]quinoline skeleton did not improve as much as that of fluorescein because molecular vibrations in the direction of distortion of the quinoline skeleton require excitation energy (Figure 10).

We also investigated two-photon properties of dyes **1f** - **1h** (Figure 11), as we had previously found that compound **1e** has two-photon properties with a GM value of σ_{948} is 165. However, the newly synthesized **1f** - **1h**, exhibited significant decreases in two-photon properties.

The cause of this decrease was considered. It was found that compounds with larger planar structures have higher GM values, which are crucial for strong two-photon

absorption properties. In other words, it is important that both the phenyl groups at positions 5 and 13 maintain planarity with the isoindolo[2,1-*a*]quinoline skeleton, which is the basic dye skeleton.

In conclusion, introducing a methyl group into the phenyl group at the 5th position of isoindolo[2,1-*a*]quinoline derivatives increased the rotational barrier from 6.1 kcal/mol to more than 24.1 kcal/mol, but this increase was found to be insufficient to suppress rotation. Conversely, introducing a methyl group into the phenyl group at the 11th position increased the rotation barrier from 15.4 kcal/mol to more than 52.3 kcal/mol, leading to an improvement in the fluorescence quantum yield. These findings suggest that clarification of the fluorophore is possible by examining the rotational suppression.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

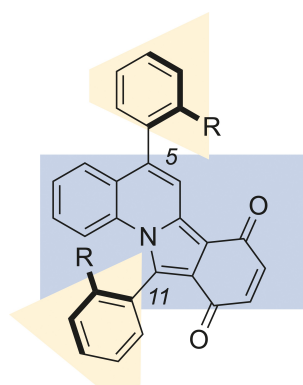
The data that support the findings of this study are available in the supplementary material of this article.

Keywords: Nitrogen heterocycles • Fluorescence • Conformation analysis

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- [6] For fluorescein, we used the results of X-ray crystal structure analysis, and for hydroxyphenylfluorone and Tokyo Green, we used the results of structure optimization using Gaussian 16 B3LYP/6-31 G(d,p) because there are no reports of X-ray crystal structure analysis.
- [7] As with fluorescein, the most stable structure was used as a reference, and the structures were rotated in 30° increments for comparison. If the structure has symmetry, calculations were made for a rotation of 180°. Furthermore, since isoindolo[2,1-*a*]quinoline derivatives have aryl groups at the 5th and 11th positions, there are two rotation axes. Therefore, the two aryl groups rotate simultaneously and can take on a variety of structures. In this calculation, for example, if the aryl group at position 5 is rotated by 30 degrees, the aryl group at position 11 can be freely rotated so that each of its structures can take the most stable structure.
- [8] We chose DMSO as a suitable solvent for the optical characterization of isoindolo[2,1-*a*]quinoline derivatives, based on the results of our previous studies, ref. 3a, regarding its solubility. Also, investigating the two-photon property and GM value of fluorescein in DMSO is the standard in this research field. We wanted to compare the data on our dye's GM values with the standards. So we needed to choose DMSO as a solvent.
- [9] The calculation formulas used are shown in equation 1 and 2). A radiative transition is a transition that releases excitation energy as fluorescence or phosphorescence, while a nonradiative transition is a transition that releases excitation energy as energy other than light.
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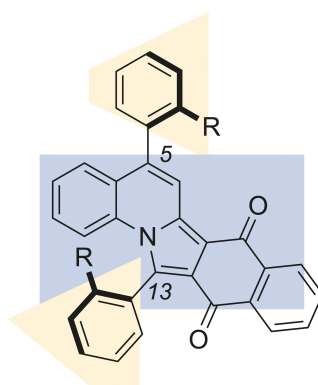
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RESEARCH ARTICLE



R = H; $\Phi = 0.001576$
R = Me; $\Phi = 0.003842$

The fluorescent properties was improved by introducing *ortho*-substituents to the phenyl groups at the 5- and 11 -positions of isoindolo[2,1-*a*]quinoline, thereby increasing the dihedral angle and rota-



R = H; $\Phi = 0.1888$, $\sigma_{948} = 165 \text{ GM}$
R = Me; $\Phi = 0.2574$, $\sigma_{950} = 37.8 \text{ GM}$

tional energy. The planarity of these phenyl groups at positions 5 and 11 (or 13) of isoindolo[2,1-*a*]quinoline derivatives is crucial for two-photon absorption properties.

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Absorption, Fluorescence, and Two-Photon Excitation Ability of 5-*o*-Tolyl-11 (or 13)-*o*-tolylisoindolo[2,1-*a*]quinolines Prepared by Ring-Closing Metathesis and [2 + 3] Cyclo-

