<table>
<thead>
<tr>
<th>項目</th>
<th>内容</th>
</tr>
</thead>
<tbody>
<tr>
<td>题目</td>
<td>Development and Application of 1,3a,6a-Triazapentalene Derivatives as a Novel Fluorescent Molecule</td>
</tr>
<tr>
<td>作者(s)</td>
<td>大澤 歩</td>
</tr>
<tr>
<td>出版日期</td>
<td>2015-03-25</td>
</tr>
<tr>
<td>DOI</td>
<td>10.14943/doctoral.k11914</td>
</tr>
<tr>
<td>链接</td>
<td><a href="http://hdl.handle.net/2115/58993">http://hdl.handle.net/2115/58993</a></td>
</tr>
<tr>
<td>类型</td>
<td>theses (doctoral)</td>
</tr>
<tr>
<td>文件信息</td>
<td>Ayumi_Osawa.pdf</td>
</tr>
</tbody>
</table>

Hokkaido University Collection of Scholarly and Academic Papers: HUSCAP
DISSERTATION

Development and Application of 1,3a,6a-Triazapentalene Derivatives as a Novel Fluorescent Molecule

Ayumi Osawa

Hokkaido University

2015
Contents

Introduction p. 3

Chapter 1 Establishment of the New Preparative Method for 1,3a,6a-Triazapentalene Derivatives p. 8

Chapter 2 Investigation of the Optical Properties of 1,3a,6a-Triazapentalene Derivatives p. 13

Chapter 3 Application of 1,3a,6a-Triazapentalene as a Fluorescent Labeling Reagent p. 21

Experimental Section p. 30

References p. 61

Acknowledgement p. 66
Abbreviation

Ac  acetyl group
Bu  butyl
CASPT2  complete active space second-order perturbation theory
DAPI  4',6-diamidino-2-phenylindole
dba  dibenzylideneacetone
DBU  1,8-diazabicyclo[5.4.0]-7-undecene
DCM  dichloromethane
DFT  density functional theory
DMAP  4-([N,N-dimethylamino])pyridine
DMF  N,N-dimethylformamide
DMSO  dimethylsulfoxide
DNA  deoxyribonucleic acid
ECHO  exciton-controlled hybridization-sensitive oligonucleotide
EDCI  1-ethyl-3-(3-dimethylaminopropyl)-Carbodiimide
equiv  equivalent
Et  ethyl group
EWG  electron withdrawing group
GFP  green fluorescent protein
i-  iso
LG  leaving group
Me  methyl group
Mes  2,4,6-trimethylphenyl group
Ms  methanesulfonyl group
NHS  N-hydroxysuccinimide
NMR  nuclear magnetic resonance
PeT  photo-induced electron transfer
Ph  phenyl group
Pr  propyl group
quant  quantitative yield
RNA  ribonucleic acid
rt  room temperature

t-  tertiary
TAP  1,3a,6a-triazapentalene
TBAF  tetra-normal-butylammonium fluoride
TBS  tertiary-butylmethyisilyl group
TD-DFT  time-dependent density functional theory
TEA  triethylamine
TF  trifluoromethanesulfonyl group
TFA  trifluoroacetic acid or trifluoroacetyl group
TFAA  trifluoroacetic anhydride
TGA  thermogravimetry analysis
THF  tetrahydrofuran
TMS  trimethylsilyl group
Ts  (4-methylphenyl)sulfonyl group
UV  ultraviolet
Φ_f  fluorescence quantum yield
λ_abs  absorption maximum wavelength
λ_em  emission maximum wavelength
λ_ex  excitation maximum wavelength
σ_p  Hammett value at para-position
**Introduction**

All of life phenomena are caused by numerous reactions and interactions of biofunctional molecules such as protein, DNA, hormones and so on. Therefore, clarification of their biological role leads to understanding of life phenomena and development of cure of diseases and new drugs. For this purpose, various methods for study of their function have been developed. In particular, fluorescence imaging technologies is one of the most powerful tools for analysis of biofunctional molecules inside cells and tissues due to their high sensitive visualization. For example, fluorescent proteins such as green fluorescent protein (GFP) have played an important role to analyze amount and localization of focused proteins.\(^1\) The biogenetic protocol for the introduction of fluorescent protein into focused protein has been well established, and this reliable method become a routine operation in laboratory.

On the other hand, fluorescent small compounds are used for fluorescence imaging of other type of biologically active molecules such as inorganic active species\(^2,3\), nucleotides\(^4,5\), peptides\(^6\), and bioactive small molecules\(^7\) (Figure 1). For example, fura-2 (1), which was developed by Tsien, is the most popular probe for Ca\(^{2+}\) ion and frequently used to analyze concentration of Ca\(^{2+}\) ion by variation of absorbance and emission intensity *in vivo*.\(^2a,b\) Nagano and co-workers reported rationally designed fluorescent probes for reactive oxygen species such as singlet oxygen in the basis of a PeT mechanism.\(^3\) In nucleotide chemistry, exciton-controlled hybridization-sensitive oligonucleotide (ECHO) probe has received attention for detection of target sequence of DNA or RNA. Oligonucleotide including D\(_{514}\) (3) exhibits no fluorescence without a complementary nucleotide but strong fluorescence after hybridization with a target nucleotide because fluorescent chromophore can intercalate into resulting hybridized nucleotide.\(^4\)

![Figure 1. Structure of various probes](image-url)
In contrast, development of smaller molecules including oligopeptides and low-molecular-weight drugs as fluorescent probes are still limited although several achievements were reported. The main reason is that the introduction of fluorescent molecule to biologically active small compound readily induces the change of biological activities due to the structural modification. For example, although Alexa Fluor 405 (4), Cy3 (5), and Texas Red (6) are commonly used for fluorescence labeling, they are sometimes too large to ignore their molecular size compared to bioactive small compounds. Furthermore, their ionic structure, which makes their hydrophobic core skeleton soluble to water, also affects the polarity of their fluorescent probes. Therefore, preparation of their fluorescent probes has generally depended on trial and error. For example, screening of effective fluorescent chromophores and linkers is mainly adopted for the preparation of suitable probes.

![Fluorescent molecules](image)

Figure 2. Selected examples of reported fluorescent molecules.

Recently, fluorescent probes of mugineic acid have been developed in our group. Mugineic acid is a phytosiderophore as a natural iron chelator and secreted from the roots of barley, and its tracking inside cell and tissues were required for functional studies. The coumarin derivative 7a was demonstrated to be incorporated into cell through transporter (HvYS1) that was overexpressed on cell membrane, whereas stronger fluorescent acridine derivative 7b was not incorporated into cell (Figure 3). This result clearly showed that the properties of fluorescent chromophore affected biological activities.
Figure 3. The incorporation of fluorescent probes of mugineic acid into *Xenopus* oocyte cells through transporter HvYS1 overexpressed on cell membrane. The coumarin derivative 7a was incorporated into cell, whereas the acridine derivative 7b was not incorporated. The picture was fluorescence image of incorporation of 7a into *Xenopus* oocyte cell.

Because of above factors, the author planed to develop a new fluorescent chromophore. First of all, the author focused his attention on a heterocyclic aromatic compound 1,3a,6a-triazapentalene (TAP, 8a). TAP possesses a specific dipole structure within a compact 10π-electron system and exhibits a totally neutral character due to delocalization of electrical charge (Figure 3).\(^{13}\) Therefore, it was expected that TAP would be a quite small fluorescent chromophore although the fluorescent properties of 8a have not yet been elucidated. Thus, in order to investigate the optical properties of TAP, it was needed to develop a common and efficient method for the synthesis of TAP.

Figure 4. The structure and the numbering of 1,3a,6a-triazapentalene (TAP, 8a).

There have been several examples of the synthesis of 1,3a,6a-triazapentalene derivatives with aryl fused and heteroaryl fused systems such as benzotriazapentalene 11.\(^{14}\) For example, McRobbie and co-workers reported intramolecular N–N bond-forming reaction of nitrene intermediate 10 generated from pyrazole derivative 9 to afford 11 (Scheme 1(a)).\(^{14a}\) In addition, Park and co-workers reported that tri-substituted benzotriazapentalene 14 was obtained by cyclization reaction of benzotriazole derivatives 12 via Pummerer type intermediate 13 (Scheme 1(b)).\(^{14i,j}\)
Scheme 1. Synthesis of benzotriazapentalene derivatives via nitrene-intermediate (a) and Pummerer reaction (b).

In contrast, there have been very few examples of the smaller 1,3a,6a-triazapentalene derivatives without an aryl fused system, and the simple 1,3a,6a-triazapentalene (8a) has never been synthesized. Thus far, the only two examples of the synthesis of mono-substituted TAPs by different method were reported by Hirobe et al. One of them was shown in Scheme 2. The reaction of aminopyrazole (16), which was prepared by N-amination of pyrazole (15) in 52% yield, with 2-chloroacetylace tone (17) in benzene at reflux afforded 3-acetyl-2-methyl TAP (8b) in 58% yield via formation of imine intermediate 18. Then, treatment of acetylated TAP 8b with

Scheme 2. Synthesis of TAP derivative 8b and 8c reported by Hirobe et al.
concentrated hydrochloric acid under reflux condition followed by neutralization with potassium carbonate gave 2-methyl TAP (8c) in 95% yield. Another example was synthesis of 2-phenyl TAP (8d) as shown in Scheme 3. The reaction of α-pyrazolyl acetophenone (20) with a sulfonylehydrazide resulted in enamine 21 as an intermediate and the subsequent intramolecular substitution forming a N-N bond afforded 2-phenyl TAP (8d) in 66% yield. However, fluorescence properties of synthetic 8c and 8d have not been mentioned.

![Scheme 3. Synthesis of 2-phenyl TAP (8d) reported by Hirobe et al.](image)

In this context, the author first attempted to establish a versatile and common method for the synthesis of various TAPs without aryl fused systems and has successfully developed a new single-step synthesis of TAPs (Chapter 1). In Chapter 2, the optical properties of synthetic TAPs were investigated, and their interesting fluorescent characters were revealed. Finally, the utility of TAP as a fluorescent labeling reagent was evaluated in Chapter 3.16
Chapter 1

Establishment of the New Preparative Method for 1,3a,6a-Triazapentalene Derivatives

1-1. Synthetic Plan

The author has first attempted to establish a versatile and common method for the synthesis of simple mono-substituted TAPs without aryl fused systems to elucidate the properties of the TAP skeleton as a fluorescent chromophore. The author focused on the triazole structure included in TAP, and therefore, planned to apply a click reaction of alkyne with azide (Scheme 4). That is, the Cu(I)-catalyzed click reaction\textsuperscript{17} of alkyne \textit{23} with azide \textit{22}, which possesses two leaving groups at each of the C2 and C3 positions, would afford a triazole \textit{A}, which should undergo intramolecular cyclization to give a triazolium ion \textit{B}. Under basic conditions, the intermediate \textit{B} would be subsequently converted to \textit{8} by a sequential reaction of E2 elimination and deprotonation.

Scheme 4. A synthetic plan for TAP applying a click reaction.

1-2. Establishment of Preparative Method for TAPs

The author began the investigation of the click reaction with 3-azidopropane-1,2-diol bis(trifluoromethanesulfonate) (22\textit{a}) as an azide fragment because of its high reactivity and ready availability. The azide \textit{22a} was easily obtained from economical 3-chloro-1,2-propanediol (24)\textsuperscript{18} by azidation followed by triflation\textsuperscript{19} of two hydroxyl groups in 82\% two-step yield and it was stable enough to be purified by silica-gel column chromatography and stored for a year in a freezer.
To determine whether the click reaction of azide 22a proceeds uneventfully, the author first tried the click reaction without a base. Treatment of 1-pentadecyne (23e) with a 1.0 equiv of 22a in the presence of 5 mol% of copper(I) iodide and 5 mol% of bis[2-(N,N-dimethylamino)ethyl] ether\(^{20}\) as a ligand afforded a bicyclic triazolium ion 26e in 29% NMR yield, along with a trace amount of 8e (Table 1, entry 1). The formation of 26e suggested that the triazole generated in the click reaction underwent intramolecular substitution to form the bicyclic framework and this encouraged us to examine the coexisting bases. The similar click reactions in the presence of a 5 equiv of 2,6-lutidine or DBU as a base did not give the desired 8e because they reacted with 22a faster than the click reaction (entries 2, 3). In contrast, the use of diisopropylethylamine afforded the desired 8e in 66% yield (entry 4), and a higher yield (80%) was obtained with triethylamine (entry 5). Finally, 8e was obtained in quantitative yield when a 1.2 equiv of 22a was used (entry 6), although the isolated yield of 8e

![Scheme 5. Preparation of the azide fragment 22a.](image)

<table>
<thead>
<tr>
<th>entry</th>
<th>base</th>
<th>solvent</th>
<th>NMR yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none</td>
<td>THF</td>
<td>trace + 26e (29%)</td>
</tr>
<tr>
<td>2</td>
<td>2,6-lutidine</td>
<td>THF</td>
<td>trace</td>
</tr>
<tr>
<td>3</td>
<td>DBU</td>
<td>THF</td>
<td>0%</td>
</tr>
<tr>
<td>4</td>
<td>i-Pr(_2)EtN</td>
<td>THF</td>
<td>66%</td>
</tr>
<tr>
<td>5</td>
<td>Et(_3)N</td>
<td>THF</td>
<td>80%</td>
</tr>
<tr>
<td>6(^a)</td>
<td>Et(_3)N</td>
<td>THF</td>
<td>quant</td>
</tr>
<tr>
<td>7</td>
<td>Et(_3)N</td>
<td>ether</td>
<td>41%</td>
</tr>
<tr>
<td>8</td>
<td>Et(_3)N</td>
<td>t-BuOH</td>
<td>49%</td>
</tr>
<tr>
<td>9(^b)</td>
<td>Et(_3)N</td>
<td>H(_2)O</td>
<td>56% (isolated)</td>
</tr>
<tr>
<td>10</td>
<td>Et(_3)N</td>
<td>DMSO</td>
<td>0%</td>
</tr>
</tbody>
</table>

\(^a\) 1.2 equiv of 22a was used. \(^b\) Phenylacetylene (23c) was used.

Table 1. Optimization of the reaction condition for preparation of TAP.
was reduced to 71% because of partial generation of protonated blue product $8e(H^+)$, which strongly adsorbed on silica gel, during column chromatography. In addition, this reaction also proceeded in other solvents such as ether and t-BuOH. Furthermore, it is noteworthy that the reaction with phenylacetylene (23c) proceeded even in water, whereas the reaction in aprotic polar solvents such as DMSO did not proceed due to decomposition of 22a.

In addition, triflate groups were necessary in this reaction and the click reaction of the azides 22b and 22c possessing mesyloxy and toslyoxy groups as a leaving group gave the corresponding triazoles 27bc and 27cc in quantitative yields, respectively, and the triazolium ion 26c and TAP 8e were not detected.

Since the highly reactive azide 22a is potentially hazardous, the manipulation of 22a requires extreme caution. The multigram-scale preparation of 22a was actually conducted several times without any accident, and the author found that a solution of 22a in 1,4-dioxane was stable enough to be heated to 100 °C for 2 h. Observation of the thermal stability of 22a by thermogravimetry analysis (TGA) indicates slow thermal degradation at > 90 °C (Figure 5).21 Furthermore, microscopic observation of the thermolytic behavior of 22a using a melting point apparatus revealed that 22a was partially volatilized at 92 °C. Therefore, the azide 22a is basically stable below 90 °C. However, it is still best to handle this molecule with great care.

**Scheme 6.** The similar reaction of phenylacetylene (23c) with the azide fragments possessing mesylate 22b and tosylate 22c

![Scheme 6](image)

![Figure 5](image)
Having established the optimal condition for preparation of TAP, the reaction of various alkynes was examined. The click reaction of 1-hexyne (23f) with 22a afforded the desired 2-butyl-TAP (8f) in 84% isolated yield. The aliphatic acetylenes possessing a functional group such as methyl propargyl ether (23g) and methyl propiolate (23h) also gave the corresponding desired TAPs, 8g, and 8h in 80% and 90% yields, respectively. The reaction of phenyl acetylene (23c) and biphenyl acetylene (23i) afforded TAP 8c and 8i in 89% and 81% yields, respectively. While the reaction of electron-rich derivative 22j showed a slight decline in yield to give TAP 8j in 56% yield, the phenyl acetylene derivatives having an electron withdrawing group such as a chloro group (23k), an ester group (23l), and a nitro group (23m) also gave the desired TAPs 8k, 8l and 8m in 81%, 73% and 96% yields, respectively. The TAP derivatives possessing

<table>
<thead>
<tr>
<th>R</th>
<th>Isolated yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₆H₅</td>
<td>8c, 89%</td>
</tr>
<tr>
<td>Ph</td>
<td>8i, 81%</td>
</tr>
<tr>
<td>Cl</td>
<td>8k, 81%</td>
</tr>
<tr>
<td>CO₂Me</td>
<td>8l, 73%</td>
</tr>
<tr>
<td>NO₂</td>
<td>8m, 96%</td>
</tr>
<tr>
<td>CN</td>
<td>8n, 77%</td>
</tr>
<tr>
<td>CN</td>
<td>8o, 87%</td>
</tr>
<tr>
<td>OTf</td>
<td>8q, 88%</td>
</tr>
<tr>
<td>S</td>
<td>8r, 84%</td>
</tr>
</tbody>
</table>

*[a]isolated yield. [b]alkyne (1.2 equiv), azide (1.0 equiv), THF (0.01 M).

Table 2. The synthesis of TAPs possessing various functional groups. a
cyano group at para (8n), meta (8o), and ortho (8p) position on benzene ring were also obtained in good yields (77%, 87% and 93%, respectively). The reaction of triflate derivative 23q, which has a coupling site for further reactions, also afforded 8q in 88% yield. Furthermore, thiophene derivative as a heteroaromatic analog was also reactive to afford desired TAP (8r) in 84% yield. Thus, it was confirmed that the established preparative method for the TAPs 8 is applicable to various acetylenes.

The structure of 8n was unequivocally established by an X-ray crystallographic analysis, which elucidated that 8n has a 10π-electron resonance structure as an aromatic ring, as shown in Figure 6. The structures of the other TAPs were also confirmed by the correlation of spectral data with 8n.22

![Figure 6. ORTEP drawing of the molecular structure and bond lengths of 8n.](image)

Next, the first synthesis of unsubstituted TAP (8a) was examined in order to elucidate the physical and fluorescence properties of the TAP skeleton. A similar click reaction of 22a with (trimethylsilyl)acetylene (23s) afforded 8s in 71% NMR yield. It was found that 8s decomposed readily during silica-gel column chromatography. Therefore, the crude 8s was directly subjected to desilylation with TBAF to give 8a in 46% overall yield as a volatile oil (Scheme 7).

![Scheme 7. The first synthesis of unsubstituted TAP 8a.](image)

In summary, the single-step synthesis of TAPs via click-cyclization-aromatization cascade reaction has been established and successfully obtained TAP derivatives possessing various functional groups in good yields. Furthermore, the parent analog 8a was first synthesized by desilylation of 2-trimethylsilyl-TAP (8s).
Chapter 2

Investigation of the Optical Properties of 1,3a,6a-Triazapentalene Derivatives

2-1. Basic optical properties of 1,3a,6a-Triazapentalenes

Having prepared various TAP derivatives, the author next tried to investigate their optical properties. First, the measurement of absorption and fluorescence spectra of simple 8a exhibited obvious absorption ($\lambda_{abs} = 288$ nm) and fluorescence ($\lambda_{em} = 389$ nm) bands, respectively (Figure 7). This was a first example of experimental demonstration that TAP skeleton not possessing additional fused ring was fluorescent chromophore, although fluorescence quantum yield ($\Phi_F$) was not high ($\Phi_F = 0.014$). On the other hand, benzotriazapentalene (11), prepared by the procedure of Bettinetti,\textsuperscript{14b} exhibited almost no fluorescence ($\Phi_F < 0.001$).

Having been encouraged by the above result, the author next turned to investigate optical properties of the other selected TAPs 8c-m and the results were summarized in Table 3. In contrast to 8a, 2-substituted derivatives had characteristic absorption bands from 300 nm to 500 nm (see experimental section). In addition, noteworthy fluorescence was also observed. For example, 2-methoxycarbonyl TAP (8h) exhibited much stronger fluorescence ($\Phi_F = 0.18$) and longer fluorescence wavelength ($\lambda_{em} = 431$ nm) than those of 8a with large Stokes shift (89 nm) despite its compact molecular size. Furthermore, it was found that fluorescence maxima of
2-phenyl analogs possessing various functional groups at 4′-position on benzene ring varied widely. For example, methoxyphenyl (8j, \( \lambda_{em} = 413 \) nm), phenyl (8c, \( \lambda_{em} = 419 \) nm), biphenyl (8i, \( \lambda_{em} = 456 \) nm) and chlorophenyl (8k, \( \lambda_{em} = 432 \) nm) analogs emitted from violet to blue light (Figure 8). On the other hand, the derivatives possessing a strong electron-withdrawing group such as methoxycarbonyl (8l, \( \lambda_{em} = 456 \) nm), cyano (8n, \( \lambda_{em} = 456 \) nm) and nitro group (8m, \( \lambda_{em} = 456 \) nm) exhibited red-shifted fluorescence emitting from green to yellow light.

Table 3. The optical properties of selected TAPs.

<table>
<thead>
<tr>
<th></th>
<th>( \lambda_{abs} ) (nm)</th>
<th>( \lambda_{em} ) (nm)</th>
<th>Stokes Shift (nm)</th>
<th>( \sigma_{\phi} )</th>
<th>( \sigma_{\phi} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>-</td>
<td>342</td>
<td>330</td>
<td>326</td>
<td>345</td>
</tr>
<tr>
<td>8h</td>
<td>389</td>
<td>431</td>
<td>413</td>
<td>419</td>
<td>456</td>
</tr>
<tr>
<td>8j</td>
<td>-</td>
<td>89</td>
<td>83</td>
<td>93</td>
<td>111</td>
</tr>
<tr>
<td>8c</td>
<td>0.014</td>
<td>0.18</td>
<td>0.053</td>
<td>0.026</td>
<td>0.20</td>
</tr>
<tr>
<td>8k</td>
<td>-</td>
<td>-</td>
<td>-0.28</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>8l</td>
<td>0.059</td>
<td>0.22</td>
<td>0.22</td>
<td>0.71</td>
<td>0.81</td>
</tr>
<tr>
<td>8n</td>
<td>-</td>
<td>-</td>
<td>-0.28</td>
<td>0</td>
<td>0.02</td>
</tr>
<tr>
<td>8m</td>
<td>0.15</td>
<td>0.14</td>
<td>0.14</td>
<td>0.15</td>
<td>0.14</td>
</tr>
</tbody>
</table>

\( ^{a} \)in CH\(_2\)Cl\(_2\). \( ^{b} \)excited at 330 nm. \( ^{c} \)excited at 370 nm. \( ^{d} \)refered to 9,10-DPA.

Figure 8. Fluorescence spectra of phenyl analogs.
Therefore, since the author focused on the electric factor of a substituent at 4’-position on benzene ring, the correlation of fluorescence maxima with Hammett value ($\sigma_p$)\textsuperscript{23} was investigated (Figure 9). As a result, the interesting correlation was observed that emission maxima shifted from 413 nm ($8j$) to 556 nm ($8m$) with increasing the Hammett values of 4’-substituent. That is, the suitable choice of substituent on benzene ring enabled to design fluorescent molecules which displayed desired color.\textsuperscript{24}

![Figure 9. The correlation of fluorescence maxima ($\lambda_{em}$) with Hammett constant ($\sigma_p$).](image)

In addition, these phenyl analogs had large Stokes shift (83-144 nm)\textsuperscript{25}. In terms of practical fluorescence microscope observation, fluorescent molecules with large Stokes shift, which is represented by difference between emission and absorption maxima, are preferred for detection of fluorescence so that the overlap with exciting light would be suppressed.

Next, photo-stability of TAPs was examined in order to confirm the availability of TAPs for a dye. Although simple TAP derivatives $8a$ and $8h$ along with electron-rich analog $8j$ occurred degradation by irradiation of UV light (254 nm, 6 W) within several minutes, $8h$ was stable enough to observe fluorescence with longer wavelength light (380 nm) during measurement. In contrast, phenyl TAP analogs possessing cyano ($8n$) and nitro group ($8m$) as electron-withdrawing group have slightly decomposed but mainly remained even in 60 min. Therefore, it was revealed that TAPs having electron-deficient benzene ring was stable enough to use as a fluorescent probe even under UV irradiation. Further investigation and improvement of photo-stability of TAPs is currently underway in our group.
2-2. Solvent Effect: Positive Fluorosolvatochromism

Next, solvent effect of optical properties was investigated. In concrete term, absorption and fluorescence spectra of 4'-cyanophenyl derivative (8n) were measured in benzene, dichloromethane, acetone, acetonitrile, respectively (Figure 10). Notably, while absorption maxima did not change in each solvent, fluorescence maxima shifted to longer wavelengths with increasing solvent polarity. Thus, 8n was revealed to show strong positive fluorescence solvatochromism. In living cells, it is known that there are locally hydrophobic points such as surface of cell membrane and pocket of proteins in contrast to aqueous environment and some kinds of solvatochromic molecules change the fluorescence color depending on surrounding environment. Therefore, it is possible that TAP and its probe molecule also would be used for an environmentally sensitive probe.

![Figure 10](image)

Figure 10. (a) The optical properties of 8n in various solvents and (b) fluorescence spectra.

2-3. Fluorescence of TAPs in the Solid States

The author found that some of the present TAPs exhibited fluorescence even in the solid state as the data were shown in Figure 11 (next page). The solid 4-cyanophenyl analog 8n showed a long-wavelength shift ($\lambda_{em} = 526$ nm) as compared with the fluorescence in dichloromethane ($\lambda_{em} = 509$ nm). Although the $\Phi_F$ value of 8n in the solid state ($\Phi_F = 0.13$) is slightly smaller than that in dichloromethane ($\Phi_F = 0.15$), it is noteworthy that the solid compound still exhibits intense fluorescence despite its compact molecular size. The solid methoxycarbonyl analog 8l also exhibited remarkable fluorescence in the wavelength region similar to that in dichloromethane, whereas the $\Phi_F$ value substantially decreased ($\Phi_F = 0.06$).
In contrast, the solid state of nitrophenyl analog 8m showed no fluorescence despite its intense fluorescence in dichloromethane (Φ_F = 0.16). Elucidation of the mechanistic details of these solid-state fluorescence properties and their applications to light-emitting devices are currently underway in our laboratory.

<table>
<thead>
<tr>
<th>Compound</th>
<th>λ_{em}^{max} (nm)</th>
<th>Φ_F</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>8n</td>
<td>520</td>
<td>0.13</td>
<td>Green</td>
</tr>
<tr>
<td>8l</td>
<td>503</td>
<td>0.06</td>
<td>Green</td>
</tr>
<tr>
<td>8m</td>
<td>N/A</td>
<td>0.00</td>
<td>Black</td>
</tr>
</tbody>
</table>

Figure 11. Fluorescence behavior of selected TAPs in the solid state. (a) fluorescence parameters of TAPs 8l-n, (b) fluorescence spectra of 8l and 8n in the solid state.

2-4. pH response of TAPs

During development of preparative method for TAPs, it was observed that a solution of several derivatives in CDCl_3 changed color from colorless or pale yellow to green or blue. This interesting phenomena was expected to be caused by trace amount of hydrogen chloride (HCl or DCl) generated from degradation of CDCl_3, because the use of CDCl_3 treated with basic alumina suppressed change of color. In order to confirm this hypothesis, a solution of TAP 8n in dichloromethane (0.002 M) was treated with TFA as an acid. As a result, change of color from
pale yellow to blue was observed and new broad absorption band appeared in a range of 550 nm to 750 nm (Figure 12). By NMR analysis, it was determined that an acidified product was 3-protonated compound \( \text{8n(H}^+ \text{)} \). Furthermore, treatment of the blue solution with triethylamine as a base induced color change to give a pale yellow solution. Repeated treatment with TFA and \( \text{Et}_3\text{N} \) was revealed that this process was reversible. Further investigation of pH dependence is ongoing in our group.

![Figure 12. Acid-base behavior of 8n. (a) the structure of TAP 8n and 3-protonated TAP 8n(H^+). (b) absorption spectra of 8n and 8n(H^+)](image)

**2-6. Effect of Substituent at 4-position on TAP**

Since it has been elucidated that substituent at 2-position on TAP skeleton played an important role of fluorescence enhancement and wavelength control, effect of substituent at other positions has next attracted our attention. Herein, the author described the properties of 2,4-di- substituted TAPs.29

The syntheses of 2,4-di-substituted TAPs were shown in Scheme 8. Azidoditriflates possessing methyl (22d) or phenyl (22e) group were readily obtained from the corresponding epoxyalcohol 28d and 28e by regio-selective ring-opening reaction with azide30 followed by triflation of two hydroxyl groups. Prepared azide fragments were applied to our click reaction
with phenylacetylene (23c) and 4-cyano-phenylacetylene (23n) to afford desired 2,4-di-substituted TAPs in good yields.

Scheme 8. Synthesis of 2,4-di-substituted TAPs.

The optical properties of synthesized 2,4-di-substituted TAPs and the corresponding 2-mono-substituted TAPs were listed in Table 4. In comparison with 2-phenyl TAP (8c), 4-methyl analog (8t) showed 18 nm longer fluorescence wavelength shift and decreasing to 0.015 in $\Phi_F$, whereas 4-phenyl analog (8u) exhibited 38 nm longer absorption wavelength shift and 21 nm shorter fluorescence wavelength shift with decreased $\Phi_F$ value (0.014). On the other hand, 4-cyano-phenyl analog (8w) showed 26 nm shorter absorption wavelength shift and 17 nm shorter fluorescence wavelength shift with decreased $\Phi_F$ value (0.014).

Table 4. The optical properties of 2,4-di-substituted TAPs.

<table>
<thead>
<tr>
<th>TAP</th>
<th>$\lambda_{obs}$ (nm)</th>
<th>$\lambda_{em}$ (nm)</th>
<th>$\Phi_F^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8c</td>
<td>326</td>
<td>419</td>
<td>0.026$^b$</td>
</tr>
<tr>
<td>8t</td>
<td>330</td>
<td>437</td>
<td>0.015$^b$</td>
</tr>
<tr>
<td>8u</td>
<td>364</td>
<td>398</td>
<td>0.014$^b$</td>
</tr>
<tr>
<td>8n</td>
<td>381</td>
<td>509</td>
<td>0.15$^c$</td>
</tr>
<tr>
<td>8v</td>
<td>389</td>
<td>543</td>
<td>0.18$^c$</td>
</tr>
<tr>
<td>8w</td>
<td>387</td>
<td>542</td>
<td>0.46$^c$</td>
</tr>
</tbody>
</table>

$^a$ In CH$_2$Cl$_2$ and 9,10-DPA was used as standard. $^b$ excited at 340 nm. $^c$ excited at 370 nm.
hand, fluorescence maxima of 4-substituted-2-cyanophenyl TAPs shifted to 543 nm (8v) and 542 nm (8w) in contrast to 8n (λ_em = 509 nm), while absorption maxima displayed slightly longer shifts. Notably, fluorescence quantum yield of 8w dramatically increased from 0.15 (8n) to 0.46 (8w), whereas that of 8v exhibited slight increase. A general effect of an introduction of 4-substituent have been unclear yet but further investigation is ongoing in our group.

In summary of Chapter 2, optical properties, especially fluorescence character, of TAPs were revealed. First, it was found that the parent small TAP 8a showed fluorescence. TAPs exhibited intense fluorescence and large Stokes shift despite its compact molecular size. In particular, fluorescence wavelength of 2-phenyl TAP analogs shifted to longer wavelength with increasing Hammett value of substituent at 4’-position on benzene ring. Therefore, it would be possible to control fluorescence wavelength by the suitable choice of functional groups. In the next chapter, the author would describe an actual example of wavelength control by using this character and development of fluorescent labeling reagent based on TAP.
Chapter 3

Application of 1,3a,6a-Triazapentalene Derivatives as a Fluorescent Labeling Reagent

3-1. Strategy for Fluorescent Labeling of Bioactive Small Molecules

Since it was clarified that TAPs displayed interesting and useful fluorescence properties, the author next studied on application of TAPs for fluorescence labeling and planned three labeling methods; (i) labeling via an amino group by condensation with carboxylic acid to generate stable amide bond, (ii) labeling via a thiol group using Michael acceptor such as maleimide, and (iii) labeling of an alkyne via click reaction constructing TAP skeleton (Figure 13). In this chapter, the author focused on fluorescent labeling via an amino group (i) because amide bond linkage is most common in fluorescent labeling chemistry.7

First, fluorescence labeling of an amino acid with TAP was examined (Scheme 9). Methyl ester moiety of small TAP 8h emitting blue light was hydrolyzed with 1 equiv of lithium hydroxide to afford lithium carboxylate (8x), which emitted blue light (λ<sub>em</sub> = 449 nm) even in water although fluorescence quantum yield was not high (Φ<sub>F</sub> = 0.034). Then, condensation of 8x with glycine ethyl ester (30) under a usual condition<sup>31</sup> resulted in labeled product 8y in 52% two-step yield. Thus, it was successful to label glycine derivative with TAP and confirm fluorescence of 8y (λ<sub>em</sub> = 418 nm, Φ<sub>F</sub> = 0.033). However, although 8x was the most compact TAP derivatives that can be connected by amide linkage, fluorescence observation of 8y required excitation with UV light and fluorescence wavelength was not long enough to imaging inside cell. This result turned our attention for expansion of fluorescence wavelength to the
yellow and red color region.

In order to obtain TAPs with longer wavelength, it was assumed that the effect of substituent on benzene ring would be useful. As described in Chapter 2, fluorescence wavelength shifted from blue to lime-green with increasing the electron-withdrawing ability of 4'-substituent on benzene ring (Figure 14). However, since TAP (\(8m\)) possessing nitro group which is one of the strongest electron-withdrawing groups exhibited lime-green fluorescence, modification of substituent only at 4'-position was considered to be limited to extend further longer-wavelength shift. Therefore, an introduction of additional electron-withdrawing group at suitable position, for example, 2'- and/or 6'-position was seemed to be effective for further modification.

3-2. Orientational effect of substituent on 2-phenyl ring.

A cyano group was chosen as additional electron-withdrawing group due to its small size and stability against UV irradiation. Thus, in order to investigate a suitable position for introduction of the cyano group to the benzene ring, the optical properties of 2-phenyl TAPs possessing para- (\(8n\)), meta- (\(8o\)), and ortho-cyano group (\(8p\)) were investigated. In contrast to

![Diagram of fluorescent labeling of glycine derivative.](image)
8n, meta-analog 8o exhibited shorter shift of absorption ($\lambda_{abs} = 327$ nm) and fluorescence maxima ($\lambda_{em} = 493$ nm), whereas fluorescence quantum yield was increased to 0.24. On the other hand, ortho-cyano analog 8p showed similar absorption ($\lambda_{abs} = 376$ nm) and fluorescence maxima ($\lambda_{em} = 515$ nm) to those of 8n and higher fluorescence quantum yield ($\Phi_F = 0.24$). Therefore, introduction of electron-withdrawing group to ortho-position was expected to be more suitable for expansion of fluorescence wavelength to the yellow and red color region.

<table>
<thead>
<tr>
<th>TAP</th>
<th>$\lambda_{abs}$ (nm)</th>
<th>$\lambda_{em}$ (nm)</th>
<th>$\Phi_F$</th>
<th>color</th>
</tr>
</thead>
<tbody>
<tr>
<td>8n</td>
<td>381</td>
<td>509</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>8o</td>
<td>327</td>
<td>493</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>8p</td>
<td>376</td>
<td>515</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ excited at 370 nm in CH$_2$Cl$_2$ using 9,10-DPA as a standard.

Table 5. Oriented effect of cyano group on benzene ring.

3-3. Rational Design and Synthesis of TAPs with long wavelength

Based on the structure of TAP 8l bearing an ester group as a linking site, TAPs possessing one or two ortho-cyano groups were designed. That is, 8l and ortho-cyano analog 8p emitted green light, and then the introduction of a cyano group to 8l was expected to induce a further longer shift from 520 nm (green). Furthermore, 8aa, which has additional cyano group, was expected to exhibit greater shift.

Figure 15. Rational design of TAPs with long wavelength
The author first examined to synthesize an alkyne fragment 23z. After commercially available iodide 31 was converted into TBS-protected alkyne 32 by Sonogashira coupling with TBS-acetylene, 32 was treated with Pd(PPh₃)₄ and tributylamine in MeOH/DMF under a CO atmosphere to give methyl ester 33. Finally, treatment of 33 with TBAF and AcOH afforded desired alkyne 23z. Preparation of an alkyne fragment possessing two cyano groups was next examined. Although the Sonogashira coupling of the known iodide 34 under various conditions initially afforded not desired silyl alkyne 35 but mainly deiodide product, the combination of Pd₂(dba)₃·CHCl₃, tri(2-furyl)phosphine, and triethylamine in DMF at 50 °C was found to afford the desired coupling product 35. Then, removal of the TBS group gave the alkyne fragment 23aa.

With alkyne fragments in hand, the click reaction of 23z and 23aa with azide fragment 22a was investigated. As a result, these reactions smoothly proceeded to give the desired TAPs 8z and 8aa in 71% and 72% yields, respectively.
The optical properties of synthesized TAPs 8z and 8aa were investigated. As was expected, the mono-cyano analog 8z exhibited longer-wavelength shift of fluorescence from 510 nm of 8l to 572 nm, and it emitted yellow fluorescence resulting from 44 nm longer shifts of absorption maxima (λ<sub>abs</sub>: from 376 to 420 nm). Although fluorescence quantum yield was decreased to 0.34, this value was high enough to use as a fluorescent probe. Furthermore, TAP 8aa, which possessed two cyano groups, showed further longer wavelength shift of absorption (λ<sub>abs</sub> = 466 nm) and fluorescence (λ<sub>em</sub> = 632 nm) to emit a red light although fluorescence quantum yield decreased to 0.096. It was noteworthy that Stokes shifts of 8z (152 nm) and 8aa (166 nm) also became larger than that of 8l (134 nm). There have been a few examples of fluorescent molecules that exhibited such a large Stokes shift in the region over 550 nm of wavelength. In addition, 8l and 8z in the solid state also emitted green (λ<sub>em</sub> = 496 nm) and yellow (λ<sub>em</sub> = 549 nm) lights, whereas fluorescence quantum yields showed low values (Φ<sub>F</sub> = 0.06 in each case). On the other hand, di-cyano analog 8aa exhibited almost no fluorescence.

<table>
<thead>
<tr>
<th>TAP</th>
<th>state</th>
<th>λ&lt;sub&gt;abs&lt;/sub&gt;(nm)</th>
<th>λ&lt;sub&gt;em&lt;/sub&gt;(nm)</th>
<th>Φ&lt;sub&gt;F&lt;/sub&gt;</th>
<th>color</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="8l" /></td>
<td>solution&lt;sup&gt;a&lt;/sup&gt;</td>
<td>376</td>
<td>510</td>
<td>0.44</td>
<td>![image]</td>
</tr>
<tr>
<td></td>
<td>solid</td>
<td>N/A</td>
<td>496</td>
<td>0.06</td>
<td>![image]</td>
</tr>
<tr>
<td><img src="image" alt="8z" /></td>
<td>solution&lt;sup&gt;a&lt;/sup&gt;</td>
<td>420</td>
<td>572</td>
<td>0.34</td>
<td>![image]</td>
</tr>
<tr>
<td></td>
<td>solid</td>
<td>N/A</td>
<td>549</td>
<td>0.06</td>
<td>![image]</td>
</tr>
<tr>
<td><img src="image" alt="8aa" /></td>
<td>solution&lt;sup&gt;a&lt;/sup&gt;</td>
<td>466</td>
<td>632</td>
<td>0.096</td>
<td>![image]</td>
</tr>
</tbody>
</table>

<sup>a</sup>in CH<sub>2</sub>Cl<sub>2</sub>

Table 6. The optical properties of a basic ester derivative 8l, mono-cyano analog 8z, and di-cyano analog 8aa.

As described above, it was successful to develop TAPs which exhibited longer-wavelength fluorescence. However, extinction coefficient (ε) of these compounds within visible light region was not high although fluorescence quantum yield was acceptable value. For example, ε values of 8z presented 6.3x10<sup>2</sup> dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup> (420 nm) and 9.5x10<sup>3</sup> dm<sup>3</sup> mol<sup>-1</sup> cm<sup>-1</sup>
(285 nm), respectively (absorption spectra were shown in experimental section). Therefore, next subject was improvement of $\varepsilon$ value to obtain more bright fluorescence derivative. As one of the solution toward this problem, the author has found that 4-phenyl analogs enhanced extinction coefficient (Table 7). In the absorption spectra of 4-phenyl analogs $8ab$ and $8ac$, which were prepared by click reaction with the corresponding azide $22e$, new strong absorption bands appeared within a range from around 300 nm to 380 nm. Their $\varepsilon$ values were $2.3 \times 10^4$ ($\lambda_{abs} = 345$ nm, $8ab$) and $3.8 \times 10^4$ ($\lambda_{abs} = 336$ nm, $8ac$) dm$^3$ mol$^{-1}$ cm$^{-1}$. Furthermore, extinction coefficient existing in longer-wavelength region was increased to ca. $7.5 \times 10^3$ ($\lambda_{abs} = ca. 375$ nm, $8ab$) and $4.6 \times 10^3$ ($\lambda_{abs} = 432$ nm, $8ac$), respectively, although fluorescence quantum yields were decreased to 0.35 and 0.07. Therefore, further modification of substituent at 4-position on TAP skeleton might be effective to develop more bright TAPs.

![Image](image1.png)

In addition, fluorosolvatochromism of yellow fluorescent TAP $8z$ was investigated. Aprotic solvents such as benzene, dichloromethane, and acetone showed positive solvatochromism emitting green, yellow, and orange light, respectively. Interestingly, fluorescence spectra in protic solvents such as methanol and water shifted to shorter region and

![Image](image2.png)

Figure 16. Fluorescence spectra and color of $8z$ in various solvents

<table>
<thead>
<tr>
<th></th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$\varepsilon$</th>
<th>$\phi_y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$8l$</td>
<td>H</td>
<td>H</td>
<td>$1.2 \times 10^3$ (376)</td>
<td>0.44 (510)</td>
</tr>
<tr>
<td>$8ab$</td>
<td>Ph</td>
<td>H</td>
<td>ca. $7.5 \times 10^3$ (ca. 375)</td>
<td>0.35 (546)</td>
</tr>
<tr>
<td>$8z$</td>
<td>H</td>
<td>CN</td>
<td>$6.3 \times 10^3$ (420)</td>
<td>0.34 (572)</td>
</tr>
<tr>
<td>$8ac$</td>
<td>Ph</td>
<td>CN</td>
<td>$4.6 \times 10^3$ (432)</td>
<td>0.07 (613)</td>
</tr>
</tbody>
</table>

Table 7. The optical properties of $8l$, $8ab$, $8z$, and $8ac$. 

26
fluorescence quantum yields were decreased. Therefore, it was expected that fluorescent color would change depending on environmental change inside cell. For example, when a fluorescent probe labeled by TAP would move from hydrophilic environment to hydrophobic environment, fluorescent color might change from blue to yellow enhancing fluorescence intensity.

3-4. Cell Staining Study toward Application for a Fluorescent Probe

Next, in order to demonstrate the utility of TAPs for fluorescent probes, fluorescence observation of TAPs inside cell was attempted. Thus, HeLa cell was treated with a solution of yellow fluorescent TAP 8z (10 μM in 0.02% DMSO) without washing the cell medium, and monitored in the 572–642 nm wavelength region. As shown in figure 17(b), 8z was incorporated into cell within several minutes to observe fluorescence staining of its cytoplasm successfully, whereas treatment of HeLa cell with DMSO as a control was not stained (Figure 17(a)). In addition, the cytotoxicity of this incorporation of 8z was not detected during fluorescence observation. Interestingly, the incorporated 8z displayed clear yellow emission regardless of blue color in water as it was described in the previous section. Furthermore, the more bright points in cytoplasm were appeared. These phenomena might be caused by hydrophobic effect involving polarity inside cell and localization of 8z into specific organelle. Further detailed investigation is currently underway in collaborative research.

![Figure 17](image_url)

Figure 17. Observation of 8z in HeLa cells. Living cells were cultured in 0.02% DMSO as a control (a and b) or with 10 μM 8z in 0.02% DMSO (c and d). Uptake of 8z was monitored by using a fluorescence microscope (BZ-9000; Keyence) in a bright-field image (b and d) or in a fluorescence image with a BZ set (FF01-452/45 nm exciter, FF01-607/70 nm emitter, FF511 nn-Di01 dichroic mirror) (a and c).

Having prepared yellow and red fluorescent TAPs, the author next tried to synthesize a NHS ester as a fluorescent labeling reagent. Yellow fluorescent TAP 8z, which was adopted as a basic structure due to its high $\Phi_F$ value, was treated with lithium hydroxide at 50 °C followed by acidification to afford carboxylic acid 8ae (Scheme 12). The next condensation reaction of 8ad with N-hydroxysuccinimide (36) under usual conditions using DCC, EDCI, and other condensation agents was smoothly proceed to give desired NHS ester 8ae. However, excess amount of N-hydroxysuccinimide and urea derivatives generated as by-product were not able to separate from 8ae mainly because separation process induced degradation of 8ae. On the other hand, the use of polymer-supported DCC-type carbodiimide reagent, which was easily removed by filtration after completion of the reaction, was effective to obtain pure form of 8ae (gram scale) in 60% two-step yield after recrystallization.

![Scheme 12. Synthesis of the NHS ester 8ae](image)

Having prepared the NHS ester 8ae, fluorescence labeling of amino acids with 8ae was examined (Table 8). Treatment of glycine ethyl ester with 8ae in the presence of triethylamine in DMF afforded the labeled product 37a in 95% yield. The similar reaction of tripeptide smoothly proceeded to give the desired product 37b in 82% yield. Furthermore, mugineic acid derivative as a more complicated amino acid also readily reacted with 8ae, and fluorescent labeled mugineic acid 37c was obtained in 84% yield. Thus, it was found that TAP NHS ester derivative 8ae is useful as fluorescent labeling reagent for amines, and 8ae is soon-to-be commercial available from Tokyo Chemical Industry Co., Ltd (TCI).

In addition, it was confirmed that labeled compounds exhibited yellow fluorescence similar to ester derivative 8z. In particular, optical properties of the labeled glycine 37a and tri-peptide 37b were investigated (Table 9). Absorption maxima of 37a and 37b showed 397 nm and 389 nm, respectively, and fluorescence maxima exhibited very similar value ($\lambda_{em} = 567$ nm) in dichloromethane, each other. Additionally, fluorescence quantum yield of 37a showed high value ($\Phi_F = 0.37$), whereas that of 37b was slightly decreased in contrast to 37a, but high
enough to use as a fluorescent probe. Furthermore, the emissions of $37a$ and $37b$ were observed in water and showed similar trend to ester analog $8z$, which is described in section 3-3. Therefore, TAP labeled molecules would be useful fluorescent probes especially for recognition of environmental changes in vivo experiment.

**Table 8.** Fluorescent labeling of amino acids by TAP NHS ester $8ae$.

<table>
<thead>
<tr>
<th>labeled compound</th>
<th>$\lambda_{abs}$/nm</th>
<th>$\lambda_{em}$/nm</th>
<th>$\Phi_F$</th>
<th>color in CH$_2$Cl$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$37a$</td>
<td>397(383)</td>
<td>567(457)</td>
<td>0.37</td>
<td>(0.019)</td>
</tr>
<tr>
<td>$37b$</td>
<td>389(353)</td>
<td>567(459)</td>
<td>0.24</td>
<td>(0.077)</td>
</tr>
</tbody>
</table>

Each parameter was measured in CH$_2$Cl$_2$. A value in parenthesis was measured in water.

**Table 9.** The optical measurement of the labeled compounds $37a$ and $37b$.

$^a$ using THF as a solvent. Reaction details were shown in experimental section.
3-6. The Theoretical Explanation of Spectral Changed of TAPs by Quantum Chemical Calculations.

In order to investigate the origin of spectral change along with 2-substituent, quantum chemical calculation was attempted. First, geometry optimization of simple TAP $8a$ was performed by DFT calculation using B3LYP/6-31 + G(d,p) in ground state (($S_0$)$_{\text{min}}$) and by TD-DFT calculation using CAM- B3LYP/6-31 + G(d,p) in lowest excited state (($S_1$)$_{\text{min}}$)$^{35}$. As a result, it was found that the bond lengths of C4-N3a and N1-N6a were elongated as a result of photo-excitation (Figure 18).

![Figure 18. The equilibrium structure of TAP $8a$ in the $S_0$ and $S_1$ in the gas phase. The values indicated the bond lengths (Å).](image)

Next, vertical excitation energies were calculated by CASPT2 calculation in the reference SA-CASSCF (10, 8)$^{36, 37}$. The resulting molecular orbitals were shown in Figure 19. It was found that the lowest singlet excitation was HOMO-LUMO $\pi-\pi^*$ transition and the calculated excitation wavelength was 291 nm, which agreed with the experimental one (288 nm). On the other hand, the calculated fluorescence wavelength of $8a$ by CASPT2 was 82 nm shorter than the experimental one ($\lambda_{\text{em(cal.)}}$ / $\lambda_{\text{em(exp.)}} = 307$ nm / 389 nm).

![Figure 19. The molecular orbital of $8a$ calculated by CASPT2 calculation in the reference SA-CASSCF (10, 8).](image)
Similarly, the equilibrium structures of TAP 8n in the ground (S\textsubscript{0}) and excited (S\textsubscript{1}) states were estimated and summarized in Figure 20. The bond lengths of C2-C3 and C2-C1’ were elongated in the S\text sub{1} state.

Figure 20. The equilibrium structure of TAP 8n in the S\textsubscript{0} and S\textsubscript{1} in the gas phase. The values indicated the bond lengths (Å).

Figure 21. The molecular orbital of 8n calculated by CASPT2 calculation in the reference SA-CASSCF (12, 10).
The vertical excitation energies were calculated by CASPT2 calculation in the reference SA-CASSCF (12, 10)\textsuperscript{36,37}. The resulting molecular orbital was shown in Figure 21. The lowest singlet excitation was assigned to HOMO-LUMO $\pi-\pi^*$ transition, which was characterized by an intramolecular charge transfer (ICT) from TAP skeleton to the phenyl ring. This result revealed that absorption and fluorescence change of 2-substituted TAPs from simple TAP 8a caused by change from simple $\pi-\pi^*$ transition to ICT transition. Furthermore, red shift of fluorescence wavelength was observed since the electron-withdrawing ability of 2-substituent was stronger, CT character was larger. As well as the result of 8a, the calculated excitation wavelength was 380 nm, which agreed with the experimental one (381 nm), whereas the calculated fluorescence wavelength by CASPT2 was 79 nm shorter than the experimental one ($\lambda_{\text{em(cal.)}} / \lambda_{\text{em(exp.)}} = 430 \text{ nm} / 509 \text{ nm}$).

![Figure 22. The selected TAPs for theoretical calculation analysis.](image)

![Figure 23. The comparison of (a) the absorption and (b) the fluorescence wavelengths between the calculated and experimental values. The central broken line indicated a perfect cal./exp. match.](image)

Because calculated wavelengths of 8n exhibited the similar behavior to 8a, the difference between calculated and experimental wavelengths of selected TAPs (Figure 22) was investigated using TD-DFT and CASPT2 calculation in the gas phase and dichloromethane,
respectively. For the absorption wavelengths, respective calculation result showed linear graph and the calculated value by CASPT2 mostly agreed with the experimental one (Figure 23(a)). On the other hand, although the calculated fluorescence wavelengths were shorter than the experimental ones, the graphs showed a good correlation between the calculated and experimental values (Figure 23(b)). The overestimation of the fluorescence energies may be attributed to the insufficient treatment of the solvent environments because excitation involved a significant CT character. These results were expected to help to predict wavelengths of TAPs.

In summary, a novel fluorescent labeling reagent based on TAP was developed. Several amino acids were successfully labeled by this reagent and the labeled products also exhibited intense fluorescence. In addition, yellow fluorescent TAP 8z was incorporated into HeLa cells and stained its cytoplasm. Therefore, TAP is expected to be useful fluorescent probes in the field of life science.
Experimental Section

· Experimental Details for Synthesis of 1,3a,6a-Triazapentalene derivatives  p. 35

· Computational Details for Theoretical Calculation  p. 51

· Absorption and Fluorescence Spectra of Selected 1,3a,6a-Triazapentalene derivatives  p. 52

General Method and Procedure.

All the reactions were carried out under an argon atmosphere. Tetrahydrofuran (THF) was freshly prepared by distillation from benzophenone ketyl before use. Triethylamine was distilled from CaH₂ under argon atmosphere and stored over NaOH. Other anhydrous solvents and reagents were commercial grade and used as supplied.

NMR spectra were recorded on a JEOL JNM-ECA-500 (500 MHz) and a JEOL JNM-AL400 (400 MHz). Chemical shifts were reported in parts per million (ppm). For ¹H NMR spectra (CDCl₃ and D₂O), tetramethylsilane and the residual solvent peak were used as the internal reference (0.00, 7.26 and 4.65 ppm), whereas the central solvent peak and methanol were used as the reference (77.0 and 49.5 ppm) for ¹³C NMR spectra. Mass spectra were recorded on a JEOL JMS-T-100GCV (FD) a Thermo Scientific Exactive (ESI) or a Waters Micromass LCT Premier (ESI). Infrared (IR) spectra were recorded on a JASCO FT/IR-4100 and a JASCO FT/IR-4200 spectrometer using CaF₂ and NaCl plate. Analytical thin layer chromatography (TLC) was performed with E. Merck pre-coated TLC plates, silica gel 60F-254, layer thickness 0.25 mm. Flash chromatography was performed on Kanto Chemical 60 N (0.04–0.05 mm) mesh silica gel.

Absorption spectra were recorded on a Hitachi U-3300 and a JASCO V-600 spectrometer and corrected fluorescence spectra were recorded on a Hitachi F-4500 and a JASCO FP-8200 spectrofluorometer. Sample solutions were degassed thoroughly by purging with an Ar gas stream for 30 min prior to the experiments and then sealed in their cells. Fluorescence quantum yields were estimated by using 9,10-diphenylanthracene (9,10-DPA) in cyclohexane (Φᵢ = 0.91) or rhodamine B in ethanol (Φᵢ = 0.94) as a standard.

Thermogravimetry analyses (TGA) were performed on a SHIMADZU TGA-50 at scanning rate of 3.0 K min⁻¹ under nitrogen.
Experimental Details for Synthesis of 1,3a,6a-Triazapentalene derivatives

Preparation of Azide Reagent 22a

3-azidopropane-1,2 -diyl bis(trifluoromethanesulfonate) (22a)

To a solution of 3-chloro-1,2-propanediol (1.92 g, 17.3 mmol) in water (17 mL) was added sodium azide (1.39 g, 20.8 mmol) at room temperature. After the mixture was heated to reflux for 5.5 h, sodium chloride was added until saturation at 0 °C. The resulting mixture was extracted with ethyl acetate (x 5). The combined organic layers were dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The residue was dissolved in anhydrous CH₂Cl₂ (87 mL). To this solution were added 2,6-lutidine (10.1 mL, 86.5 mmol) and Tf₂O (5.82 mL, 34.6 mmol) in this order at -78 °C. The mixture was stirred for 5 min, quenched with saturated aqueous NH₄Cl, and extracted with hexane (x 3). The combined organic layers were washed with brine, dried over MgSO₄, filtered, and concentrated in vacuum. The residue was purified by silica gel column chromatography (hexane/ACOEt = 95/5, 90/10 to 70/30) to give 22a (5.38 g, 14.1 mmol, 82%) as a pale yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 5.10 (quint, J = 5.2 Hz, 1H), 4.71 (d, J = 5.2 Hz, 2H), 3.83 (dd, J = 13.7, 5.2 Hz, 1H), 3.77 (dd, J = 13.5, 5.4 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 118.5 (q, J = 319 Hz), 118.3 (q, J = 319 Hz), 81.4, 72.1, 50.1; IR (neat) 2114, 1457, 1418, 1220, 1139 cm⁻¹. TGA: (heat from 30 °C to 500 °C at 3.0 °C /min) The weight loss of 22a (8.646 mg) reached to 5% at 107 °C.

Synthesis of 2-substituted TAPs

Preparation of ligand·copper solution

To a solution of bis[2-(N,N-dimethylaminoethyl)]ether (19.0 µL, 0.10 mmol) in THF (10 mL) was added copper(I) iodide (19 mg, 0.10 mmol) at room temperature. The mixture was stirred until homogeneous.

Typical procedure of the click reaction

Method A:
To the flask containing azide 22a (217 mg, 0.568 mmol) were transferred 2.8 mL (0.028 mmol of ligand·copper complex) of above copper·ligand solution, Et₃N (400 µL, 2.84 mmol), and alkyne 23 (0.682 mmol) at room temperature, successively. The mixture was stirred for 1 h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 8.
Method B:

To a solution of 22a (74.7 mg, 0.196 mmol) and alkyne 23 (0.235 mmol) in THF (20 mL) were added Et₃N (138 µL, 0.980 mmol), and 980 µL of the ligand-copper solution (0.01 M, 9.80 µmol), successively at room temperature. The mixture was stirred for 1h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 8.

The method B is recommended for syntheses of 2-aryl derivatives, especially possessing electron-withdrawing group, in order to avoid generation of 1-allyl triazole derivatives as byproducts or precipitation of the products.

The synthetic TAPs mentioned in Chapter 1 except for 8m are yellow-colored materials just after purification by silica gel column chromatography. However, the yellow color readily changed to green by a trace amount of acids such as a derivation from CDCl₃.

2-<i>tridecyl</i>-1,3a,6a-triazapentalene (8e)

Yellow oil; ¹H NMR (500 MHz, CDCl₃)  δ 7.32 (d,  J = 2.3 Hz, 1H), 7.00 (d,  J = 2.3 Hz, 1H), 6.89 (s, 1H), 6.53 (t,  J = 2.9 Hz, 1H), 2.63 (t,  J = 7.7 Hz, 2H), 1.69 (quint,  J = 7.4 Hz, 2H), 1.41-1.26 (m, 20H), 0.88 (t,  J = 7.2 Hz, 3H); ¹³C  NMR (125 MHz, CDCl₃)  δ 149.4, 107.8, 101.7, 99.9, 94.4, 31.9, 29.7, 29.6 x3, 29.5, 29.4, 29.3, 29.3, 26.7, 22.7, 14.1; IR (neat) 3155, 2924, 2853, 1465, 1436, 1376, 1284, 1246, 1163, 1138 cm⁻¹; HRMS (EI) m/z [M⁺] calcd for [C₁₈H₃₁N₃]⁺ 289.2518, found 289.2524.

2-<i>butyl</i>-1,3a,6a-triazapentalene (8f)

Yellow oil; ¹H NMR (500 MHz, CDCl₃)  δ 7.32 (d,  J = 2.3 Hz, 1H), 7.00 (d,  J = 2.9 Hz, 1H), 6.90 (s, 1H), 6.53 (t,  J = 2.9 Hz, 1H), 2.64 (t,  J = 7.7 Hz, 2H), 1.68 (quint,  J = 7.4 Hz, 2H), 1.42 (sex,  J = 7.4 Hz, 2H), 0.94 (t,  J = 7.4 Hz, 3H); ¹¹C  NMR (125 MHz, CDCl₃)  δ 149.3, 107.9, 101.7, 99.9, 94.4, 31.4, 26.4, 22.4, 13.8; IR (neat) 3153, 2955, 2930, 2860, 1457, 1436, 1376, 1242, 1138 cm⁻¹; HRMS (EI) m/z [M⁺] calcd for [C₉H₁₃N₃]⁺ 163.1109, found 163.1104.

2-(methoxymethyl)-1,3a,6a-triazapentalene (8g)

Yellow oil; ¹H NMR (500 MHz, CDCl₃)  δ 7.37 (d,  J = 2.9 Hz, 1H), 7.13 (s, 1H), 7.08 (d,  J = 2.9 Hz, 1H), 6.59 (t,  J = 2.9 Hz, 1H), 4.50 (s, 2H), 3.45 (s, 3H); ¹³C  NMR (125 MHz, CDCl₃)  δ 145.4, 108.5, 102.1, 100.6, 95.6, 66.7, 58.4; IR (neat) 3148, 2977, 2926, 2897, 2817 cm⁻¹; HRMS (EI) m/z [M⁺] calcd for [C₇H₉N₃O]⁺  151.0749, found 151.0749.
2-(methoxycarbonyl)-1,3a,6a-triazapentalene (8h)

Yellow solid; mp 94-100 °C (recrystallized from ether); ¹H NMR (500 MHz, CDCl₃) δ 7.68 (d, J = 1.1 Hz, 1H), 7.46 (d, J = 2.9 Hz, 1H), 7.22 (d, J = 2.9 Hz, 1H), 6.75 (t, J = 2.9 Hz, 1H), 3.96 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 162.0, 139.0, 110.8, 102.7, 101.8, 100.3, 52.2; IR (neat) 3154, 3120, 3033, 3008, 2958, 2928, 1746, 1733, 1218 cm⁻¹; HRMS (EI) m/z [M+] calcd for [C₇H₇N₃O₂]+ 165.0538, found 165.0540. UV/Vis (CH₂Cl₂): λₘₐₓ (log ε) = 342 (3.43), 281 (4.20) nm. FL (CH₂Cl₂): λₑₘ = 431 nm; Φᵣ = 0.18 (reference to 9,10-DPA; excited at 370 nm).

2-phenyl-1,3a,6a-triazapentalene (8c)

Yellow solid; mp 75-77 °C (recrystallized from CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃) δ 7.77 (d, J = 6.9 Hz, 2H), 7.43-7.40 (m, 3H), 7.38 (d, J = 1.1 Hz, 1H), 7.35 (t, J = 7.2, 1.6 Hz, 1H), 7.10 (d, J = 2.3 Hz, 1H), 6.60 (t, J = 2.9 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 147.7, 131.4, 128.7, 128.3, 125.8, 108.7, 102.2, 100.7, 93.2; IR (neat) 3153, 3072, 3036, 2698, 2653 cm⁻¹; HRMS (EI) m/z [M+] calcd for [C₁₁H₉N₃]+ 138.0796, found 138.0779. UV/Vis (CH₂Cl₂): λₘₐₓ (log ε) = ~326 (3.48), 288 (3.97), 282 (3.97), 251 (4.17) nm. FL (CH₂Cl₂): λₑₘ = 419 nm; Φᵣ = 0.026 (reference to 9,10-DPA; excited at 340 nm).

2-(biphenyl-4-yl)-1,3a,6a-triazapentalene (8i)

Yellow solid; ¹H NMR (500 MHz, CDCl₃) δ 7.85 (d, J = 8.6 Hz, 2H), 7.64 (d, J = 7.4 Hz, 2H), 7.48-7.42 (m, 4H), 7.36 (t, J = 7.4 Hz, 1H), 7.13 (d, J = 2.8 Hz, 1H), 6.62 (t, J = 2.8 Hz, 1H); ¹³C NMR (125.8 MHz, CDCl₃) δ 147.4, 141.0, 140.6, 130.4, 128.8, 127.4, 127.4, 126.8, 108.8, 102.2, 100.8, 93.3; IR (neat) 3566, 3147, 3133, 3062, 3033, 2955, 2926, 2853 cm⁻¹; HRMS (EI) m/z [M⁺] calcd for [C₁₇H₁₃N₃]+ 259.1109, found 259.1107. UV/Vis (CH₂Cl₂): λₘₐₓ (log ε) = 345 (3.70), 289 (4.53) nm. FL (CH₂Cl₂): λₑₘ = 456 nm; Φᵣ = 0.20 (reference to 9,10-DPA; excited at 370 nm).

2-(4-methoxyphenyl)-1,3a,6a-triazapentalene (8j)

Yellow solid; ¹H NMR (500 MHz, CDCl₃) δ 7.70 (d, J = 8.6 Hz, 2H), 7.41 (d, J = 8.6 Hz, 2H), 7.31 (s, 1H), 7.09 (d, J = 2.9 Hz, 1H), 6.95 (d, J = 9.2 Hz, 2H), 6.58 (t, J = 2.6 Hz, 1H), 3.85 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 159.8, 147.6, 127.1, 124.1, 114.1, 108.5, 102.1, 100.6, 92.6, 55.3; IR (neat) 3154, 2992, 2960, 2937, 2837, 2109, 1251, 1028 cm⁻¹; HRMS (EI) m/z [M⁺] calcd for [C₁₂H₁₁N₃O]+ 213.0902, found 213.0889. UV/Vis (CH₂Cl₂): λₘₐₓ (log ε) = ~330 (3.71), 285 (4.22), 260 (4.30) nm. FL (CH₂Cl₂): λₑₘ = 413 nm; Φᵣ = 0.053 (reference to 9,10-DPA; excited at 370 nm).
at 340 nm).

### 2-(4-chlorophenyl)-1,3a,6a-triazapentalene (8k)

Blue solid; mp 133–135 °C; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.70 (d, $J = 8.6$ Hz, 2H), 7.42 (d, $J = 2.3$ Hz, 1H), 7.38 (d, $J = 8.6$ Hz, 2H), 7.36 (s, 1H), 7.11 (d, $J = 2.8$ Hz, 1H), 6.61 (t, $J = 2.8$ Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 146.5, 134.0, 130.0, 129.8, 127.0, 108.9, 102.3, 101.0, 93.2; IR (neat) 3153, 2922, 2852, 1413, 1242, 1142, 1038, 947, 838 cm$^{-1}$; HRMS (ESI) m/z [M+H]$^+$ calcd for [C$_{11}$H$_8$N$_3$O$_2$+H]$^+$ 218.0480, found 218.0481. UV/Vis (CH$_2$Cl$_2$): $\lambda_{max}$ (log $\varepsilon$) = ~324 (3.58), ~276 (4.12), 258 (4.22) nm. FL (CH$_2$Cl$_2$): $\lambda_{max}$ = 432 nm; $\Phi_F$ = 0.059 (reference to 9,10-DPA; excited at 370 nm).

### 2-(4-(methoxycarbonyl)phenyl)-1,3a,6a-triazapentalene (8l)

Green solid; mp 148–153 °C; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.09 (d, $J = 8.6$ Hz, 2H), 7.84 (d, $J = 8.6$ Hz, 2H), 7.47 (s, 1H), 7.45 (d, $J = 2.8$ Hz, 1H), 7.15 (d, $J = 2.8$ Hz, 1H), 6.64 (t, $J = 2.9$ Hz, 1H), 3.94 (s, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 166.8, 146.4, 135.8, 130.0, 129.6, 125.5, 109.2, 101.4, 52.1; IR (neat) 3152, 3134, 3120, 1710, 1433, 1417, 1280, 1104 cm$^{-1}$; HRMS (ESI) m/z [M+Na]$^+$ calcd for [C$_{13}$H$_{11}$N$_3$O$_2$+Na]$^+$ 264.0744, found 264.0745. UV/Vis (CH$_2$Cl$_2$): $\lambda_{max}$ (log $\varepsilon$) = 376 (3.09), 287 (4.14) nm. FL (CH$_2$Cl$_2$): $\lambda_{max}$ = 521 nm; $\Phi_F$ = 0.44 (reference to 9,10-DPA; excited at 370 nm).

### 2-(4-nitrophenyl)-1,3a,6a-triazapentalene (8m)

Red crystal; dec. 179 °C (recrystallized from ether) $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.28 (d, $J = 9.2$ Hz, 2H), 7.93 (d, $J = 8.6$ Hz, 2H), 7.52 (s, 1H), 7.19 (d, $J = 2.3$ Hz, 1H), 7.19 (d, $J = 2.9$ Hz, 1H), 6.68 (t, $J = 2.9$ Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 147.4, 145.3, 138.0, 126.3, 124.2, 109.6, 102.8, 101.5, 94.4; IR (neat) 3150, 3118, 1508, 1340, 1324 cm$^{-1}$; HRMS (EI) m/z [M$^+$] calcd for [C$_{11}$H$_8$N$_4$O$_2$]$^+$ 228.0647, found 228.0636. UV/Vis (CH$_2$Cl$_2$): $\lambda_{max}$ (log $\varepsilon$) = 412 (3.32), 300 (4.25) nm. FL (CH$_2$Cl$_2$): $\lambda_{max}$ = 556 nm; $\Phi_F$ = 0.14 (reference to 9,10-DPA; excited at 370 nm).

### 2-(4-cyanophenyl)-1,3a,6a-triazapentalene (8n)

Yellow solid; dec. 150 °C (recrystallized from CH$_2$Cl$_2$); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.87 (d, $J = 8.0$ Hz, 2H), 7.70 (d, $J = 8.0$ Hz, 2H), 7.47 (s, 1H), 7.46 (d, $J = 2.9$ Hz, 1H), 7.17(d, $J = 2.8$ Hz, 1H), 6.66 (t, $J = 2.8$ Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 145.7, 136.0, 132.5, 126.2, 118.8, 111.5, 109.5, 102.6,
2-(3-cyanophenyl)-1,3a,6a-triazapentalene (8o)
Green solid; mp 118–123 °C (recrystalized from ether); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.04 (t, $J$ = 1.8 Hz, 1H), 7.98 (ddd, $J$ = 8.0, 1.7, 1.1 Hz, 1H), 7.59 (ddd, $J$ = 8.0, 1.7, 1.2 Hz, 1H), 7.51 (t, $J$ = 8.0 Hz, 1H), 7.44 (d, $J$ = 2.3 Hz, 1H), 7.42 (s, 1H), 7.16 (d, $J$ = 2.9 Hz, 1H), 6.65 (t, $J$ = 2.9 Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 145.3, 132.8, 131.4, 129.8, 129.4, 129.2, 118.6, 112.8, 109.2, 102.6, 101.4, 93.5; IR (neat) 3150, 2227, 1435, 1378, 1144, 969, 844 cm$^{-1}$; HRMS (ESI) m/z [M+Na]$^+$ calcd for [C$_{12}$H$_8$N$_3$O$_2$+Na]$^+$ 231.0641, found 231.0642. UV/Vis (CH$_2$Cl$_2$): $\lambda_{\text{max}}$ (log $\varepsilon$) = 327 (3.45), 282 (4.15), 274 (4.16), 260 (4.20) nm. FL (CH$_2$Cl$_2$): $\lambda_{\text{max}}$ = 493 nm; $\Phi_F$ = 0.24 (reference to 9,10-DPA; excited at 370 nm).

2-(2-cyanophenyl)-1,3a,6a-triazapentalene (8p)
Light green solid; mp 85–87 °C (recrystalized from ether); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.09 (dd, $J$ = 8.0, 1.1 Hz, 1H), 7.93 (d, $J$ = 1.2 Hz, 1H), 7.74 (dd, $J$ = 8.0, 1.1 Hz, 1H), 7.66 (td, $J$ = 8.0, 1.2 Hz, 1H), 7.46 (d, $J$ = 2.9 Hz, 1H), 7.43 (td, $J$ = 8.0, 1.2 Hz, 1H), 7.19 (d, $J$ = 2.8 Hz, 1H), 6.68 (t, $J$ = 2.9 Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 143.4, 134.4, 133.9, 133.0, 128.4, 128.1, 119.1, 109.5, 109.0, 102.3, 101.4, 96.0; IR (neat) 3150, 2223, 1474, 1424, 949 cm$^{-1}$; HRMS (ESI) m/z [M+Na]$^+$ calcd for [C$_{12}$H$_8$N$_3$O$_2$+Na]$^+$ 231.0641, found 231.0642. UV/Vis (CH$_2$Cl$_2$): $\lambda_{\text{max}}$ (log $\varepsilon$) = 376 (3.11), 287 (4.19), ~270 (4.10), 260 (4.12) nm. FL (CH$_2$Cl$_2$): $\lambda_{\text{max}}$ = 515 nm; $\Phi_F$ = 0.24 (reference to 9,10-DPA; excited at 370 nm).

2-(4-(trifluoromethylsulfonyloxy)phenyl)-1,3a,6a-triazapentalene (8q)
Yellow solid; mp 106-114 °C (recrystalized from ether); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.84 (dt, $J$ = 9.2, 2.4 Hz, 2H), 7.44 (d, $J$ = 2.3 Hz, 1H), 7.33 (dt, $J$ = 9.4, 2.3 Hz, 1H), 7.15 (d, $J$ = 2.9 Hz, 2H), 6.64 (t, $J$ = 2.8 Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 149.2, 145.9, 132.1, 127.6, 121.7, 118.8 (q, $J$ = 320 Hz), 109.2, 102.6, 101.3, 93.6; IR (neat) 3151, 3052, 2924, 2853, 1420, 1252, 1213, 1139 cm$^{-1}$; HRMS (EI) m/z [M$^+$] calcd for [C$_{12}$H$_8$F$_3$N$_3$O$_2$S]$^+$ 331.0238, found 331.0227
2-(thiophen-2-yl)-1,3a,6a-triazapentalene (8r)

Red solid; mp 71–73 °C (recrystallized from ether); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.40 (d, \(J = 2.8\) Hz, 1H), 7.36 (d, \(J = 3.4\) Hz, 1H), 7.31 (brs, 1H), 7.30 (d, \(J = 5.2\) Hz, 1H), 7.10–7.04 (m, 2H), 6.58 (t, \(J = 2.8\) Hz, 1H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 142.6, 133.8, 127.4, 125.0, 124.1, 108.7, 102.4, 101.0, 92.9; IR (neat) 3147, 3101, 3069, 1437, 1414, 1381, 1238, 1222, 1034 cm\(^{-1}\); HRMS (ESI) m/z [M+H\(^+\)] calced for \([\text{C}_9\text{H}_7\text{N}_3\text{S}+\text{H}]^+\) 190.0433, found 190.0435. UV/Vis (CH\(_2\)Cl\(_2\)): \(\lambda_{\text{max}} (\log \varepsilon) = 341 (3.16), 285 (3.97)\) nm. FL (CH\(_2\)Cl\(_2\)): \(\lambda_{\text{em}} = 439\) nm; \(\Phi_F = 0.049\) (reference to 9,10-DPA; excited at 350 nm).

Synthesis of Unsubstituted TAP 8a

2-(trimethylsilyl)-1,3a,6a-triazapentalene (8s)

To a solution of azide 22a (165.0 mg, 0.433 mmol) in THF (2.2 mL) were added (trimethylsilyl)acetylene (73.4 μL, 0.519 mmol), TEA (304 μL, 2.16 mmol) and the ligand-Cu(I) complex solution (2.2 mL, 0.02 mmol), prepared according to the typical procedure, at room temperature. The mixture was stirred for 3 h and concentrated in vacuo. The residue was diluted with pentane, washed with water and brine, dried over anhydrous MgSO\(_4\), filtered, and concentrated in vacuo to give 2-trimethylsilyl derivative 8s (71% NMR yield using pyrazine as an internal standard) as a yellow oil. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.44 (dd, \(J = 2.8, 1.2\) Hz, 1H), 7.08 (d, \(J = 1.2\) Hz, 1H), 7.07 (d, \(J = 2.8\) Hz, 1H), 6.62 (t, \(J = 2.9\) Hz, 1H), 0.33 (s, 9H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 147.7, 109.4, 101.6, 101.6, 99.6, -1.4.

1,3a,6a-triazapentalene (8a)

To a solution of TBAF in THF (1.0 M, 0.65 mL, 0.650 mmol) was added the crude 8s at 0 °C. The mixture was stirred at room temperature for 3 h and directly subjected to silica gel column chromatography (pentane/ether = 5/1) to give unsubstituted triazapentalene 8a (22 mg, 46% in 2 steps) as a pale yellow volatile oil. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.47 (s, 1H), 7.43 (d, \(J = 2.8\) Hz, 1H), 7.12 (s, 1H), 7.12 (s, 1H), 6.63 (td, \(J = 2.8, 1.2\) Hz, 1H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 134.5, 108.9, 102.2, 100.4, 96.6; IR (neat) 3149, 2951, 2924, 2856, 2203, 2104, 1152 cm\(^{-1}\); HRMS (FI) m/z [M+] calced for \([\text{C}_5\text{H}_5\text{N}_3\text{]+} 107.0483, found 107.0491. UV/Vis (CH\(_2\)Cl\(_2\)): \(\lambda_{\text{max}} (\log \varepsilon) = 288 (4.17)\) nm. FL (CH\(_2\)Cl\(_2\)): \(\lambda_{\text{em}} = 389\) nm; \(\Phi_F = 0.014\) (reference to 9,10-DPA;
Preparation of 3-Protonated TAP

![image](image_url)

2-(4-cyanophenyl)-3H-pyrazolo[1,2-a][1,2,3]triazol-8-ium 2,2,2-trifluoroacetate (8n(H+))

To a solution of 8n (1.1 mg, 5.3 μmol) in CDCl₃ (0.6 mL) was added TFA (3.2 μL, 42 μmol) and the mixture was directly analyzed with NMR. ¹H NMR (500 MHz, CDCl₃) δ 8.64 (d, J = 2.8 Hz, 1H), 8.30 (d, J = 2.9 Hz, 1H), 8.11 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.6 Hz, 2H), 7.04 (t, J = 2.8 Hz, 1H), 6.18 (brs, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 169.5, 133.4, 132.0, 129.0, 129.0, 125.2, 118.2, 117.0, 112.4, 56.9.

Synthesis of the Azide Reagents 22d and 22e

![image](image_url)

rac-(2S,3S)-3-azidobutane-1,2-diol (29d)

To a solution of NaN₃ (263 mg, 3.92 mmol) in toluene (2.6 mL) was added Et₂AlCl (4.1 mL, 1.05 M solution in hexane, 4.31 mmol) at 0 °C. After the resulting mixture was stirred at room temperature for 4 h, the epoxide 28d (173 mg, 1.96 mmol) in toluene (1.3 mL) was added via cannula at –78 °C. The reaction mixture was stirred at –78 °C for 1 h and at room temperature for 16 h, then water (2 mL), potassium fluoride (5 g), THF (20 mL) were added carefully at 0 °C. After the suspension mixture was stirred at room temperature for 5 h, MgSO₄ was added. The mixture was stirred at room temperature for 1 h, then filtered through Celite and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 29d (184 mg, 1.40 mmol, 72%) as a colorless oil; ¹H NMR (500 MHz, CDCl₃) δ 3.79–3.53 (m, 4H), 2.56 (brs, 1H, OH), 2.05 (brs, 1H, OH), 1.33 (d, J = 6.3 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 74.3, 63.1, 58.7, 14.8; IR (neat) 3376, 2976, 2936, 2886, 2117, 1259, 1041 cm⁻¹.
**rac-(2S,3S)-3-azidobutane-1,2-diyl bis(trifluoromethanesulfonate) (22d)**

To a solution of diol 29d (75.0 mg, 0.572 mmol) in CH₂Cl₂ (2.9 mL) were added 2,6-lutidine (330 μL, 2.86 mmol) and Tf₂O (252 μL, 1.50 mmol) in this order at –78 °C. The mixture was stirred for 15 min, quenched with saturated aqueous NH₄Cl, and extracted with hexane (x 3). The combined organic layers were washed with water and brine, dried over MgSO₄, filtered and concentrated in vacuum. The residue was purified by silica gel column chromatography to give 22d (223 mg, 0.572 mmol, 99%) as a pale yellow oil. Yellow oil; ¹H NMR (500 MHz, CDCl₃) δ 4.85 (td, J = 5.7, 2.8 Hz, 1H), 4.75 (dd, J = 12.0, 5.2 Hz, 1H), 4.74 (dd, J = 8.0, 2.8 Hz, 1H), 4.05 (quint, J = 6.3 Hz, 1H), 1.48 (d, J = 6.3 Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 118.5 (q, J = 317 Hz), 118.3 (q, J = 318 Hz), 85.0, 71.6, 56.3, 15.7; IR (neat) 2987, 2950, 2925, 2851, 2131, 1246, 1209 cm⁻¹.

**rac-(2S,3S)-3-azido-3-phenylpropane-1,2-diyl bis (trifluoromethanesulfonate) (22e)**

This compound was prepared by the same manner as 22d from known diol (ref (30)). 68% (ca. 9:1 diastereomeric mixture); White solid; mp 54–55 °C (recrystallized from ether); ¹H NMR (500 MHz, CDCl₃) δ 7.52–7.45 (m, 3Ha+3Hb), 7.41–7.36 (2Ha+2Hb), 5.08 (td, J = 6.3, 2.3 Hz, 1Ha), 5.04 (d, J = 6.3 Hz, 1Ha), 4.95 (d, J = 8.0 Hz, 1Hb), 4.79 (dd, J = 12.0, 5.8 Hz, 1Ha), 4.64 (dd, J = 12.0, 2.3 Hz, 1Ha), 4.61 (dd, J = 12.0, 2.3 Hz, 1Hb), 4.21 (dd, J = 12.0, 4.0 Hz, 1Hb); ¹³C NMR (125 MHz, CDCl₃) δ 132.2 (a), 130.4 (b), 130.1 (a), 130.0 (b), 129.5 (a), 127.5 (b), 127.4 (a), 118.5 (q, J = 318 Hz), 118.1 (q, J = 317 Hz), 84.9 (a), 84.6 (b), 72.0 (b), 71.6, 64.5 (b), 64.5 (a); IR (neat) 3090, 3070, 3037, 3016, 2975, 2960, 2934, 2122, 1428, 1416, 1249, 1215, 1142 cm⁻¹.

**Synthesis of 2,4-di-substituted TAPs**

**4-methyl-2-phenyl-1,3a,6a-triazapentalene (8t)**

Yellow amorphous material; ¹H NMR (500 MHz, CDCl₃) δ 7.79 (d, J = 6.8 Hz, 2H), 7.43-7.48 (m, 3H), 7.34 (t, J = 7.4 Hz, 1H), 7.16 (br s, 1H), 6.35 (d, J = 1.7 Hz, 1H), 2.42 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 147.7, 131.7, 128.6, 128.2, 125.8, 109.8, 107.3, 102.2, 90.3, 11.2; IR (neat) 3152, 3062, 3032, 2954, 2924, 2853, 1360, 1391, 1360, 1234, 1165, 1071, 1034 cm⁻¹; HRMS (EI) m/z [M⁺] calcd for [C₁₂H₁₁N₃]⁺ 197.0953, found 197.0956. UV/Vis (CH₂Cl₂): λmax (log ε) = 252 (4.04), ~295 (3.77), ~330 (3.51), 436 (2.74) nm. FL (CH₂Cl₂): λem = 437 nm; ΦF = 0.015 (reference to 9,10-DPA; excited at 340 nm).
2,4-diphenyl-1,3,6a-triazapentalene (8u)

White solid; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.84 (dd, $J = 8.6, 1.2$ Hz, 2H), 7.81 (s, 1H), 7.61 (dd, $J = 8.6, 1.2$ Hz, 2H), 7.57 (d, $J = 2.8$ Hz, 1H), 7.48 (t, $J = 8.0$ Hz, 2H), 7.45 (t, $J = 7.4$ Hz, 2H), 7.37 (t, $J = 7.4$ Hz, 1H), 7.27 (t, $J = 7.4$ Hz, 1H), 6.87 (d, $J = 2.9$ Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$148.3, 131.3, 130.0, 129.2, 128.8, 128.6, 126.1, 126.0, 123.4, 115.2, 106.4, 104.3, 93.9; IR (neat) 3154, 3062, 3032, 3006, 2958, 2918, 1850, 1601, 1524, 1466, 1456, 1445, 1388, 1167, 951 cm$^{-1}$; HRMS (EI) m/z [M+] calcd for [C$_{17}$H$_{13}$N$_3$]+ 259.1109, found 259.1115. UV/Vis (CH$_2$Cl$_2$): $\lambda_{\text{max}}$ (log $\varepsilon$) = 250 (4.33), 364 (4.21) nm. FL (CH$_2$Cl$_2$): $\lambda_{\text{em}}$ = 398 nm; $\Phi_F$ = 0.014 (reference to 9,10-DPA; excited at 340 nm).

2-(4-cyanophenyl)-4-methyl-1,3,6a-triazapentalene (8v)

Green amorphous material; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.88 (d, $J = 8.6$ Hz, 2H), 7.69 (d, $J = 8.0$ Hz, 2H), 7.42 (d, $J = 2.9$ Hz, 1H), 7.23 (bs, 1H), 7.40 (d, $J = 2.8$ Hz, 1H), 2.44 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 145.6, 136.2, 132.5, 126.0, 118.9, 111.3, 110.6, 108.0, 102.6, 91.3, 11.2; IR (neat) 3158, 3147, 3134, 3123, 2917, 2223, 1609, 1417, 1251, 1172, 1038 cm$^{-1}$; HRMS (ESI) m/z [M+H]$^+$ calcd for [C$_{13}$H$_{10}$N$_4$+H]$^+$ 223.0978, found 223.0979. UV/Vis (CH$_2$Cl$_2$): $\lambda_{\text{max}}$ (log $\varepsilon$) = 389 (3.00), 286 (4.02) nm. FL (CH$_2$Cl$_2$): $\lambda_{\text{em}}$ = 543 nm; $\Phi_F$ = 0.18 (reference to 9,10-DPA; excited at 370 nm).

2-(4-cyanophenyl)-4-phenyl-1,3,6a-triazapentalene (8w)

Yellow solid; mp 175–177 ºC; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.92 (d, $J = 8.6$ Hz, 2H), 7.85 (brs, 1H), 7.71 (d, $J = 8.0$ Hz, 2H), 7.60 (d, $J = 7.4$ Hz, 2H), 1.78 (d, $J = 3.4$ Hz, 1H), 7.50 (t, $J = 7.4$ Hz, 2H), 7.30 (t, $J = 7.4$ Hz, 1H), 6.90 (d, $J = 2.8$ Hz, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 146.1, 135.8, 132.6, 129.5, 129.3, 126.6, 126.2, 123.6, 118.8, 115.9, 111.8, 107.0, 104.6, 94.6; IR (neat) 3152, 3060, 2959, 2924, 2855, 2226, 1669, 1611, 1526, 1454, 1389, 1278, 1240, 1171, 845 cm$^{-1}$; HRMS (ESI) m/z [M+H]$^+$ calcd for [C$_{18}$H$_{12}$N$_4$+H]$^+$ 285.1135, found 285.1138. UV/Vis (CH$_2$Cl$_2$): $\lambda_{\text{max}}$ (log $\varepsilon$) = ~387 (3.84), 345 (4.44), 281 (4.48) nm. FL (CH$_2$Cl$_2$): $\lambda_{\text{em}}$ = 542 nm; $\Phi_F$ = 0.46 (reference to 9,10-DPA; excited at 370 nm).
Condensation of TAP with Glycine Derivative 30

Labeled glycine (8y)

To a solution of 8h (57.1 mg, 0.35 mmol) in MeOH (1.3 mL) and H2O (0.4 mL) was added LiOH·H2O (14.5 mg, 0.35 mmol) at 0 °C. The mixture was stirred at room temperature for 16 h, concentrated under reduced pressure to give lithium salt 8x as a red amorphous material. The residue was dissolved in DMF (1.7 mL) and cooled to 0 °C. To the mixture were added EDCI (148 mg, 0.77 mmol), DMAP (14 mg, 0.11 mmol) and glycine ethyl ester hydrochloride (97 mg, 0.69 mmol). The mixture was stirred at room temperature for 18 h, quenched with 5% aqueous citric acid (~ pH 5), and extracted with EtOAc (~2). The combined organic layers were washed with brine, dried over anhydrous MgSO4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (hexane/ EtOAc = 3/2) to give 8y (42.1 mg, 0.178 mmol, 52%) as a yellow crystal. mp 96-98 °C (recrystalized from EtOAc/hexane); 1H NMR (500 MHz, CDCl3) δ 7.66 (d, J = 1.2 Hz, 1H), 7.40 (d, J = 2.8 Hz, 1H), 7.29 (m, 1H), 7.20 (d, J = 2.8 Hz, 1H), 6.72 (t, J = 2.8 Hz, 1H), 4.26 (q, J = 6.9 Hz, 2H), 4.22 (d, J = 5.8 Hz, 2H), 1.31 (t, J = 6.9 Hz, 3H); 13C NMR (125 MHz, CDCl3) δ 169.5, 161.1, 141.7, 110.2, 102.2, 101.8, 98.5, 61.4, 41.0, 14.1; IR (neat) 3352, 3152, 2982, 2938, 1744, 1667, 1577, 1524 cm⁻¹; HRMS (EI) m/z [M +] calcd for [C10H12N4O3]+ 236.09094, found 236.09095. UV/Vis (CH2Cl2): λmax (log ε) = 281 (4.16), 330 (3.47) nm. FL (CH2Cl2): λem = 418 nm; ΦF = 0.033 (reference to 9,10-DPA; excited at 340 nm).

Lithium carboxylate (8x)

Red amorphous material; 1H NMR (500 MHz, D2O) δ 7.40 (d, J = 1.1 Hz, 1H), 7.32 (d, J = 1.1 Hz, 1H), 7.17 (d, J = 2.9 Hz, 1H), 6.57 (t, J = 2.9 Hz, 1H); 13C NMR (125 MHz, D2O) δ 170.0, 145.2, 111.6, 104.8, 104.4, 101.5; IR (neat) 3156, 1603, 1496, 1406, 1320 cm⁻¹; HRMS (ESI) m/z [M⁻] calcd for [C6H4N3O2]⁻ 150.0309, found 150.0303. UV/Vis (H2O): λmax (log ε) = 272 (4.01), 317 (3.47) nm. FL (H2O): λem = 449 nm; ΦF = 0.034 (reference to 9,10-DPA; excited at 340 nm). The lithium carboxylate 8x was able to pass through the ion exchange resin (Dowex 50 W x 4) to give carboxylic acid form.
Preparation of Alkyne Fragment 23z and 23aa

5-bromo-2-((tert-butyldimethylsilyl)ethynyl)benzonitrile (32)

To a solution of iodide 31 (24.9 g, 81.0 mmol), TBS-acetylene (15.1 mL, 81.0 mmol) and Et₂NH (25.1 mL, 243 mmol) in anhydrous DMF (405 mL) were added CuI (771 mg, 4.05 mmol), Pd(PPh₃)₄ (936 mg, 0.81 mmol) under Ar atmosphere at room temperature. After stirred for 16 h, the mixture was quenched with 1 M HCl at 0 °C and extracted with hexane (twice). The combined organic layers were washed with water and brine, dried over MgSO₄, filtrated and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography to give the coupling product 32 (26.5 g, quant) as a yellow solid: mp 69–72 °C; \(^1\)H NMR (500 MHz, CDCl₃) \(\delta\) 7.75 (d, \(J = 2.0\) Hz, 1H), 7.65 (dd, \(J = 8.5, 2.0\) Hz, 1H), 7.42 (d, \(J = 8.0\) Hz, 1H), 1.01 (s, 9H), 0.22 (s, 6H); \(^{13}\)C NMR (125 MHz, CDCl₃) \(\delta\) 135.4, 135.0, 133.6, 125.8, 122.0, 117.1, 115.9, 102.2, 100.0, 25.9, 16.5, −5.0; IR (neat) 3099, 3070, 2927, 2888, 2859, 2230, 2165, 1469, 1249, 825 cm⁻¹; HRMS (ESI) m/z [M+Na]⁺ calcd for [C₁₅H₁₈NBrSi+Na]⁺ 342.0284, found 342.0284.

Methyl 4-((tert-butyldimethylsilyl)ethynyl)-3-cyanobenzoate (33)

To a solution of 32 (13.9 g, 43.5 mmol) and n-Bu₃N (31 mL, 130 mmol) in MeOH (36 mL) and DMF (181 mL) was added Pd(PPh₃)₄ (2.5 g, 2.18 mmol) under Ar atmosphere at room temperature. After the flask was substituted with CO gas, the reaction mixture was heated to 85 °C for 14 h. After cooled to room temperature, the mixture was quenched with 1 M HCl, diluted with hexane and filtrated through Celite. The filtrate was separated and the aqueous layer was extracted with hexane (x1). The combined organic layers were washed with water and brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography to give ester 33 (13.0 g, quant) as a yellow oil: \(^1\)H NMR (500 MHz, CDCl₃) \(\delta\) 8.29 (d, \(J = 1.5\) Hz, 1H), 8.16 (dd, \(J = 8.5, 1.5\) Hz, 1H), 7.64 (d, \(J = 9.0\) Hz, 1H), 3.95 (s, 3H), 1.03 (s, 9H), 0.24 (s, 6H); \(^{13}\)C NMR (125 MHz, CDCl₃) \(\delta\) 164.6, 133.6, 132.9, 132.7, 130.9, 130.0, 116.5, 116.0, 104.6, 100.4, 52.7, 26.0, 16.6, −4.9; IR (neat) 3072, 3006, 2952, 2928, 2894, 2858, 2234, 2162, 1727, 1601, 1299, 1257, 828 cm⁻¹; HRMS (ESI) m/z [M+Na]⁺ calcd for [C₁₅H₁₈O₂NSi+Na]⁺ 342.1234, found 322.1233.
Methyl 3-cyano-4-ethynylbenzoate (23z)

To a solution of 33 (12.9 g, 43.1 mmol) and acetic acid (3.0 mL, 51.7 mmol) in THF (216 mL) was added TBAF (47.4 mL, 1 M solution in THF, 47.4 mmol) at room temperature. After stirred for 5 min, the mixture was diluted with water, extracted with ether (x2). The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was recrystallized from EtOAc to give 23z (6.2 g, 77%) as orange crystals: mp 111–113 °C; ¹H NMR (500 MHz, CDCl₃) δ 8.30 (d, J = 1.5 Hz, 1H), 8.19 (dd, J = 8.0, 2.0 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 3.95 (s, 3H), 3.65 (s, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 164.5, 133.6, 133.1, 133.1, 130.7, 129.7, 116.3, 116.2, 86.8, 79.0, 52.8; IR (neat) 3249, 2236, 2110, 1714, 1298, 1287, 1258 cm⁻¹; HRMS (ESI) m/z [M+H]+ calcd for [C₁₁H₈NO₂]+ 186.0550, found 186.0552.

Methyl 3,5-dicyano-4-ethynylbenzoate (23aa)

To a solution of iodide 34 (511 mg, 1.64 mmol), TBS-acetylene (460 µL, 2.46 mmol) and Et₃N (691 µL, 4.92 mmol) in anhydrous DMF (16.4 mL) were added CuI (62.5 mg, 0.328 mmol), P(2-furyl)₃ (76.2 mg, 0.328 mmol), Pd₂(dba)₃·CHCl₃ (170 mg, 0.164 mmol) under Ar atmosphere at room temperature. After stirred for 2 h at 50 °C, the mixture was quenched with 1 M HCl at room temperature, extracted with ether (twice). The combined organic layers were washed with water (x2) and brine, dried over MgSO₄, bleached with activated charcoal, filtrated through Celite and concentrated under reduced pressure. The crude product was dissolved in THF (16.4 mL) and then acetic acid (940 µL, 16.4 mmol) and TBAF (1.8 mL, 1 M solution in THF, 1.80 mmol) were added to the solution. After stirred for 30 min at room temperature, the reaction mixture was quenched with 1 M HCl, extracted with ether (x3). The combined organic layers were washed with water (x2) and brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was recrystallized from EtOAc to give 23aa (192 mg, 56% for 2 steps) as orange crystals: dec. 157 °C; ¹H NMR (500 MHz, CDCl₃) δ 8.50 (s, 2H), 4.10 (s, 1H), 4.01 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 136.7, 132.6, 131.5, 117.9, 114.8, 93.7, 76.1, 53.4; IR (neat) 3261, 3070, 2231, 2109, 1729, 1319, 1228 cm⁻¹; HRMS (FD) m/z [M]⁺ calcd for [C₁₂H₆N₂O₂]⁺ 210.0429, found 210.0434.
Synthesis of Yellow and Red Fluorescent TAPs

2-(2-cyano-4-(methoxycarbonyl)phenyl) -1,3a,6a-triazapentalene (8z)

Light brown powder; \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta 8.40\) (d, \(J = 1.5\) Hz, 1H), \(8.28\) (dd, \(J = 8.5, 1.5\) Hz, 1H), \(8.22\) (d, \(J = 8.5\) Hz, 1H), \(8.06\) (d, \(J = 1.0\) Hz, 1H), \(7.48\) (d, \(J = 2.5\) Hz, 1H), \(7.23\) (d, \(J = 3.0\) Hz, 1H), \(6.71\) (dd, \(J = 3.0, 2.5\) Hz, 1H), \(3.97\) (s, 3H); \(^13\)C NMR (125 MHz, CDCl\(_3\)) \(\delta 164.9, 142.4, 138.2, 135.2, 133.7, 129.8, 128.5, 118.3, 110.0, 109.0, 102.5, 101.8, 96.7, 52.6; IR (neat) 3170, 3155, 2226, 1716, 1296 cm\(^{-1}\); HRMS (ESI) m/z [M+Na\(^+\)] calcd for [C\(_{14}\)H\(_{10}\)N\(_4\)O\(_2\)+Na\(^+\)] 289.0696, found 289.0698. UV/Vis (CH\(_2\)Cl\(_2\)): \(\lambda_{\text{max}}\) (log \(\varepsilon\)) = 420 (2.80), 285 (3.98) nm. FL (CH\(_2\)Cl\(_2\)): \(\lambda_{\text{max}} = 572\) nm; \(\Phi_F = 0.34\) (reference to rhodamine B; excited at 400 nm).

2-(2,6-dicyano-4-(methoxycarbonyl)phenyl) -1,3a,6a-triazapentalene (8aa)

Dark brown solid; dec. 195 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta 8.61\) (s, 2H), \(7.80\) (s, 2H), \(7.59\) (d, \(J = 2.0\) Hz, 1H), \(7.30\) (d, \(J = 2.5\) Hz, 1H), \(6.77\) (t, \(J = 2.5\) Hz, 1H), \(4.03\) (s, 3H); \(^13\)C NMR (125 MHz, CDCl\(_3\)) \(\delta 163.1, 140.5, 139.4, 138.5, 130.9, 116.3, 113.9, 110.3, 103.4, 102.2, 97.4, 53.3; IR (neat) 3179, 3158, 3141, 3064, 2231, 1732, 1717, 1558, 1541, 1521, 1507, 1315, 1232 cm\(^{-1}\); HRMS (ESI) m/z [M+Na\(^+\)] calcd for [C\(_{15}\)H\(_9\)N\(_5\)O\(_2\)+Na\(^+\)] 314.0648, found 314.0648. UV/Vis (CH\(_2\)Cl\(_2\)): \(\lambda_{\text{max}}\) (log \(\varepsilon\)) = 466 (3.20), 286 (4.42) nm. FL (CH\(_2\)Cl\(_2\)): \(\lambda_{\text{max}} = 632\) nm; \(\Phi_F = 0.096\) (reference to rhodamine B; excited at 430 nm).

Synthesis of 2,4-di-substituted TAPs 8ab and 8ac.

2-(4-(methoxycarbonyl)phenyl) -4-phenyl-1,3a,6a-triazapentalene (8ab)

Green solid; mp. 124–127 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 8.11\) (d, \(J = 10.8\) Hz, 2H), \(7.90\) (d, \(J = 10.8\) Hz, 2H), \(7.86\) (s, 1H), \(7.60\) (d, \(J = 10.0\) Hz, 2H), \(7.58\) (d, \(J = 3.6\) Hz, 1H), \(7.49\) (t, \(J = 10.0\) Hz, 2H), \(7.32–7.24\) (m, 1H), \(6.88\) (d, \(J = 4.0\) Hz, 1H), \(3.94\) (s, 3H); \(^13\)C NMR (125 MHz, CDCl\(_3\)) \(\delta 166.7, 147.0, 135.6, 130.0, 129.8, 129.7, 129.2, 126.3, 125.6, 123.4, 115.5, 106.7, 104.4, 94.5, 52.1; IR (neat) 3155, 1950, 1729, 1277 cm\(^{-1}\); HRMS (ESI) m/z [M+Na\(^+\)] calcd for [C\(_{13}\)H\(_{13}\)N\(_3\)O\(_2\)+Na\(^+\)] 340.1062, found 340.1064. UV/Vis (CH\(_2\)Cl\(_2\)): \(\lambda_{\text{max}}\) (log \(\varepsilon\)) = 345 (4.35), 279 (4.44) nm. FL (CH\(_2\)Cl\(_2\)): \(\lambda_{\text{max}} = 548\) nm; \(\Phi_F = 0.36\) (reference to rhodamine B; excited at 400 nm).
2-(2-cyano-4-(methoxycarbonyl)phenyl)-4-phenyl,3a,6a-triazapentalene (8ac)

Orange solid; dec. 216 °C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.44 (s, 1H), 8.39 (s, 1H), 8.31 (d, $J = 8.0$ Hz, 1H), 8.24 (d, $J = 8.0$ Hz, 1H), 7.66–7.59 (m, 3H), 7.50 (t, $J = 7.6$ Hz, 2H), 7.30 (t, $J = 7.6$ Hz, 1H), 6.95 (d, $J = 2.8$ Hz, 1H), 3.98 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 164.9, 143.1, 138.0, 135.3, 133.8, 130.2, 129.4, 129.4, 128.7, 126.8, 123.7, 118.3, 116.3, 109.3, 107.6, 104.5, 96.9, 52.7; IR (neat) 3155, 2960, 2228, 1733, 1300 cm$^{-1}$; HRMS (ESI) m/z [M+H]$^+$ calcd for [C$_{20}$H$_{15}$N$_4$O$_2$]+ 343.1195, found 343.1198. UV/Vis (CH$_2$Cl$_2$): $\lambda_{max}$ (log $\varepsilon$) = 432 (3.66), 336 (4.58), 282 (4.48) nm. FL (CH$_2$Cl$_2$): $\lambda_{max}$ = 613 nm; $\Phi_F$ = 0.070 (reference to rhodamine B; excited at 400 nm).

Synthesis of TAP NHS Ester 8ae

![Synthesis of TAP NHS Ester 8ae](image)

Typical procedure for large-scale synthesis of 8z.

To a solution of azide 22a (5.10 g, 13.4 mmol) and alkyne 23z (2.25 g, 12.2 mmol) in THF (610 mL) were added the homogeneous solution of CuI (116 mg, 0.610 mmol), bis-[2-(N,N-dimethylaminoethyl)]ether (115 $\mu$L, 0.610 mmol) and Et$_3$N (8.6 mL, 61.0 mmol) in THF (61 mL) at room temperature. After the resulting mixture was stirred for 24 h, the solvent was removed to less than one third under reduced pressure and then aq 5% NH$_3$ was poured to precipitate a powder. The powder was separated by filtration and dried $\textit{in vacuo}$ to afford 8ad (2.7 g, 83%) as a light brown powder.

2-(2-cyano-4-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)phenyl)-1,3a,6a-triazapentalene (8ae)

To a solution of 8z (2.70 g, 10.1 mmol) in H$_2$O (50 mL) and THF (101 mL) was added LiOH·H$_2$O (510 mg, 12.1 mmol) at room temperature. After stirred at 50 °C for 30 min, the
mixture was washed with ether (x1), acidified by 1M HCl to participate yellow solid. The solid was dissolved in THF/EtOAc mixture and the organic layer was separated. After the aqueous layer was extracted with THF/EtOAc (x2), the combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo. The residue was dehydrated by toluene azeotropy and dissolved in THF (54 mL). To the mixture were added N-hydroxysuccinimide (1.23 g, 10.7 mmol) and N-cyclohexylcarbodiimidomethyl polystyrene resin (8.7 g, 13.0 mmol, TCI No. C2141, 1.5 mmol/g loading) at room temperature. After the reaction mixture was stirred for 1 h, solid materials were removed by filtration and the residue was washed with THF. The filtrate was concentrated in vacuo to form red powder. This powder was dispersed to hexane, filtered, washed with ether and CH₂Cl₂ to give 8ae (2.1 g, 56% for 2 steps) as a red powder: dec. 200 °C; ¹H NMR (500 MHz, CD₂Cl₂) δ 8.50 (d, J = 1.5 Hz, 1H), 8.35 (dd, J = 8.0, 1.5 Hz, 1H), 8.31 (d, J = 8.5 Hz, 1H), 8.11 (s, 1H), 7.51 (d, J = 2.5 Hz, 1H), 7.29 (d, J = 3.0 Hz, 1H), 6.75 (dd, J = 3.0, 2.5 Hz, 1H), 2.91 (brs, 4H); ¹³C NMR (100 MHz, DMSO-d₆) δ 170.1, 160.2, 141.4, 139.5, 136.0, 134.2, 129.2, 124.1, 117.4, 110.5, 109.1, 103.3, 102.9, 97.4, 25.5; IR (neat) 3181, 3141, 2225, 1773, 1737, 1200 cm⁻¹; HRMS (ESI) m/z [M+Na]⁺ cale for [C₁₇H₁₂N₅O₄+Na]⁺ 350.0884, found 350.0886. UV/Vis (CH₂Cl₂): λ_max (log ε) = 446 (3.38), 287 (4.47) nm. FL (CH₂Cl₂): λ max = 624 nm (fluorescence quantum yield was not estimated because of gradual degradation of 8ae under the measurement condition.)

**Synthesis of Tripeptide II as a Substrate for Labeling Reaction**

![Scheme S4. Synthesis of tripeptide II](scheme.png)

To a solution of I (104.9 mg, 0.250 mmol) in MeOH (2.5 mL) was added TMSCHN₂ (1.3 mL, 2.60 mmol, 2.0 M ether solution) at room temperature. After stirred for 1 h, the mixture was concentrated in vacuo. The residue was dissolved in MeOH (2.5 mL) and to the resulting solution was added 10% Pd/C (12 mg). After stirred for 24 h under H₂ atmosphere, the suspension was diluted with CHCl₃, filtered through Celite and concentrated in vacuo. The residue was purified by silica-gel column chromatography to give II (86.7 mg, quant) as a colorless amorphous solid: ¹H NMR (400 MHz, CDCl₃) δ 7.96 (d, J = 7.2 Hz, 1H), 4.65 (dd, J = 7.6, 2.4 Hz, 1H), 4.42 (ddd, J = 10.0, 7.6, 5.2 Hz, 1H), 4.13 (d, J = 16.0 Hz, 1H), 3.98 (d, J = 15.6 Hz, 1H), 3.71 (s, 3H), 3.73–3.60 (m, 1H), 3.51 (dd, J = 16.8, 8.8 Hz, 1H), 2.23–1.85 (m,
5H), 1.72–1.50 (m, 2H), 0.93 (d, J = 6.4 Hz, 3H), 0.88 (d, J = 6.4 Hz, 3H); 13C NMR (400 MHz, CDCl3) δ 174.0, 172.1, 167.1, 60.8, 52.4, 51.4, 46.8, 41.8, 39.4, 29.8, 25.0, 24.3, 22.8, 21.4; IR (neat) 3148 (br), 3054, 2958, 2874, 1740, 1654, 1540, 1228, 1209, 669 (br) cm⁻¹; HRMS (ESI) m/z [M+Na⁺] calcld for [C14H25N3O4+Na⁺] 322.1743, found 322.1743.

**Condensation of amines with 8ae**

**Labeled glycine ethyl ester 37a**

To a solution of glycine ethyl ester hydrochloride (5.5 mg, 0.0394 mmol), Et₃N (27.7 µL, 0.197 mmol) in DMF (80 µL) was added NHS ester 8ae (15.2 mg, 0.0451 mmol) at room temperature. After stirred for 15 min, the mixture was filtered through silica-gel pad (CHCl₃/EtOAc = 1:1). The filtrate was concentrated under reduced pressure and contaminated DMF was removed by azeotrope with toluene to afford pure 12 (12.6 mg, 95%) as a yellow amorphous powder: 1H NMR (400 MHz, CDCl3) δ 8.22 (d, J = 6.8 Hz, 1H), 8.20 (s, 1H), 8.05 (dd, 1H), 8.04 (d, J = 1.2 Hz), 7.48 (d, J = 2.8 Hz, 1H), 7.22 (d, J = 3.2 Hz, 1H), 6.78 (brt, 1H), 6.71 (t, J = 2.8 Hz, 1H), 4.29 (q, J = 7.2 Hz, 2H), 4.26 (d, J = 4.8 Hz, 2H), 1.33 (t, J = 7.2 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 169.8, 164.9, 142.4, 137.3, 133.3, 133.0, 131.2, 128.7, 118.3, 109.9, 109.2, 102.5, 101.8, 96.6, 61.9, 42.0, 14.1; IR (neat) 3394, 3343, 3158, 2982, 2932, 2228, 1747, 1651, 1608, 1540, 1511, 1208 cm⁻¹; HRMS (ESI) m/z [M+Na⁺] calcld for [C17H15N5O3+Na⁺] 360.1073, found 360.1072. UV/Vis (CH2Cl2): λmax (log ε) = 387 (3.40), 284 (4.30) nm. FL (CH2Cl2): λmax = 563 nm; Φ_F = 0.37 (reference to rhodamine B; excited at 400 nm).

**Labeled tripeptide 37b**

To a solution of tripeptide II (11.0 mg, 0.0367 mmol), Et₃N (25.8 µL, 0.184 mmol) in DMF (73 µL) was added NHS ester 8ae (12.8 mg, 0.0367 mmol) at room temperature. After stirred for 10 min, the mixture was directly purified by silica-gel column chromatography to give 11 (16.1 mg, 82%) as a yellow amorphous solid: 1H NMR (400 MHz, CDCl3) δ 8.24 (s, 1H), 8.18 (d, J = 8.4 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 8.02 (s, 1H), 7.48 (d, J = 2.8 Hz, 1H), 7.45 (brs, 1H), 7.22 (d, J = 2.8 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.70 (t, J = 2.8 Hz, 1H), 4.64–4.50 (m, 2H), 4.30 (dd, J = 17.6, 4.4 Hz, 1H), 4.22 (dd, J = 18.0, 4.0 Hz, 1H), 3.74 (s, 3H), 3.73–3.63 (m, 1H), 3.54 (dd, J = 15.6, 8.0 Hz, 1H), 2.40–2.28 (m, 1H), 2.26–1.95 (m, 3H), 1.82–1.50 (m, 3H), 0.92 (d, J = 5.6 Hz, 3H),
0.92 (d,  J = 5.2 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 173.2, 170.5, 167.9, 164.7, 142.5, 137.2, 133.3, 133.1, 131.2, 128.6, 118.4, 109.9, 109.2, 102.5, 101.8, 96.6, 60.3, 52.3, 51.1, 46.5, 42.6, 41.3, 28.0, 25.0, 24.8, 22.7, 22.0; IR (neat) 3307, 3154, 3069, 2956, 2228, 1742, 1646, 1541, 1314, 1203 cm⁻¹; HRMS (ESI) m/z [M+Na]^+ cale d for [C22H31N7O5+Na]^+ 556.2284, found 556.2283. UV/Vis (CH2Cl2): λmax (log ε) = 379 (3.34), 283 (4.26) nm. FL (CH2Cl2): λmax = 561 nm; ΦF = 0.24 (reference to rhodamine B; excited at 400 nm).

**Computational Details for Theoretical Calculation**

The equilibrium geometry in the electronic ground state (S0) was determined by the density functional theory (DFT) calculations using the B3LYP functionals, while the geometry optimization in the lowest ππ* excited state S1 (ππ*) was performed by the time-dependent DFT (TD-DFT) calculations employing the coulomb attenuated B3LYP (CAM-B3LYP) functionals. The C₃ symmetry constraint was imposed for 8a, 8l, 8n and 8z while no constraint was applied for 8aa because the twisted structure was more stable due to the steric hindrance. The choice to employ the CAM-B3LYP functionals was due to the significant charge-transfer character involved in excitation to the S1 state. The 6-31 + G(d,p) basis set was used in the DFT calculations and the equilibrium geometries were determined both in the gas phase and in dichloromethane. The solvent effects were taken into account by the polarizable continuum solvation model (PCM), where the radii are taken from the universal force field. After the geometry optimization, the vertical excitation and fluorescence energies were calculated at the S0 and S1 equilibrium structures (denoted as (S0)min and (S1)min), respectively, by the TD-DFT(CAM-B3LYP) method. In PCM calculations, the linear-response method with a non-equilibrium solvation was employed to obtain the vertical excitation energies at (S0)min, while the equilibrium solvation was adapted for the calculation of the excitation energies during the S1 geometry optimization. The excitation energy was also refined at the DFT-optimized geometries by the CASPT2 method in order to obtain more reliable excitation energies. A level shift with a value of 0.3 is applied for the CASPT2 calculations. The notation of CASPT2 (m,n) was occasionally used, in which case the active space for a reference state-averaged complete active space self-consistent field (SA-CASSCF) wavefunction was composed of m electrons and n orbitals (SA-CASSCF (m,n)). The augmented correlation-consistent polarized double-zeta basis set (denoted as aug-cc-pVDZ) was employed in the CASPT2 calculations. For obtaining the oscillator strengths, the vertical excitation energies calculated by CASPT2 and the transition dipole moments calculated by SA-CASSCF were used.

For 8a, the active space for the reference SA-CASSCF wavefunction was comprised of six π orbitals (four π orbitals were doubly-occupied and two were unoccupied in the
closed-shell configuration), and it was therefore denoted as SA-CASSCF (8,6). \(8a\) possessed ten \(\pi\) orbitals and the lowest and highest \(\pi\) orbitals were excluded from the active space. This was justified by the larger active space calculation, which includes all \(\pi\) orbitals, where only a difference of \(\sim 0.01\) eV was observed in the \(S_1\) vertical excitation energies. The active space for the other chromophores was composed of twelve electrons distributed in ten \(\pi\) orbitals (SACASSCF (12,10)), and the active orbitals of \(8n\) at \((S_0)_{\text{min}}\) were shown in Figure 20. As seen in the figure, the active space of the SA-CASSCF (12,10) wavefunction included orbitals that corresponded to the active orbitals of SA-CASSCF (8,6) in \(8a\). For all chromophores, the \(S_0\) and \(S_1\) states were averaged with equal weights in the SA-CASSCF calculations, except where otherwise noted. The DFT and TD-DFT calculations were performed using the Gaussian09 program package\(^{40}\) while the CASPT2 calculations were carried out using the MOLPRO2010.1 program package\(^{41}\).

<table>
<thead>
<tr>
<th></th>
<th>TD-DFT (gas)</th>
<th>TD-DFT (DCM)</th>
<th>CASPT2 (gas)</th>
<th>CASPT2 (DCM)</th>
<th>Exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(8a)</td>
<td>259</td>
<td>268</td>
<td>291</td>
<td>300</td>
<td>288</td>
</tr>
<tr>
<td>(8n)</td>
<td>322</td>
<td>339</td>
<td>380</td>
<td>397</td>
<td>381</td>
</tr>
<tr>
<td>(8l)</td>
<td>317</td>
<td>332</td>
<td>374</td>
<td>389</td>
<td>376</td>
</tr>
<tr>
<td>(8z)</td>
<td>353</td>
<td>372</td>
<td>418</td>
<td>437</td>
<td>420</td>
</tr>
<tr>
<td>(8aa)</td>
<td>387</td>
<td>398</td>
<td>450</td>
<td>461</td>
<td>466</td>
</tr>
</tbody>
</table>

* the value was an estimate in DCM

**Table E1.** The excitation energies (wavelength in nm) for the \(S_1\) state calculated by TD-DFT and CASPT2 at \((S_0)_{\text{min}}\).

<table>
<thead>
<tr>
<th></th>
<th>TD-DFT (gas)</th>
<th>TD-DFT (DCM)</th>
<th>CASPT2 (gas)</th>
<th>CASPT2 (DCM)</th>
<th>Exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(8a)</td>
<td>268</td>
<td>287</td>
<td>307</td>
<td>326</td>
<td>389</td>
</tr>
<tr>
<td>(8n)</td>
<td>358</td>
<td>390</td>
<td>430</td>
<td>462</td>
<td>509</td>
</tr>
<tr>
<td>(8l)</td>
<td>356</td>
<td>389</td>
<td>426</td>
<td>459</td>
<td>510</td>
</tr>
<tr>
<td>(8z)</td>
<td>394</td>
<td>425</td>
<td>471</td>
<td>502</td>
<td>572</td>
</tr>
<tr>
<td>(8aa)</td>
<td>440</td>
<td>463</td>
<td>533</td>
<td>556</td>
<td>632</td>
</tr>
</tbody>
</table>

* the value was an estimate in DCM

**Table E2.** The fluorescence energies (wavelength in nm) for the \(S_1\) state calculated by TD-DFT and CASPT2 at \((S_1)_{\text{min}}\).

Absorption and Fluorescence Spectra of Selected 1,3a,6a-Triazapentalene Derivatives

![Absorption and Fluorescence Spectra of Selected 1,3a,6a-Triazapentalene Derivatives](image_url)
References


(18) The starting material, 3-chloro-1,2-propanediol was purchased by Tokyo Chemical Industry (TCI). The price was in 2014.

(19) In the triflation reaction, although i-Pr$_2$EtN was also available as a base, use of 2,6-lutidine resulted in better yield. This reaction required low temperature because it was possible to generate hazardous azido-epoxide under higher temperature.

(20) We found that bis[2-(N,N-dimethylamino)ethyl] ether was an effective ligand to click reaction of benzyl azide with 1-hexyne as a result of screening of various ligands (unpublished result).


(22) The spectral data of 8c agreed with reported one in ref. 10 (a)


(29) We also investigated the properties of 2,5-di-substituted TAPs: Namba, K.; Mera, A.; Osawa, A.; Sakuda, E.; Kitamura, N.; Tanino, K. Org. Lett. 2012, 14, 5554–5557.


(34) Other palladium source and ligand such as Pd(acac)$_2$, Pd(OAc)$_2$, Pd(OCCF$_3$)$_2$, PdCl$_2$(CH$_3$CN)$_2$, PdCl$_2$(PhCN)$_2$, Pd(PPh$_3$)$_4$, Pd$_2$(dba)$_3$, P(3-Bu)$_3$, dppf, xanthphos, P(Oi-Pr)$_3$, and P(o-tol)$_3$ gave 35 in trace to poor yield.


(41) Werner, H.-J.; Knowles, P. J.; Knizia, G.; Manby, F. R.; Schütz, M. *et al. MOLPRO, version 2010.1, a package of ab initio programs.*
Acknowledgement

All the studies described in this thesis were carried out under the supervision of Professor Dr. Keiji TANINO, Department of Chemistry, Faculty of Science, Hokkaido University. The author would like to express deeply sincere appreciation to Professor Tanino for his guidance and constant encouragement and helpful discussion in the preparation of this thesis.

Grateful acknowledgement is made to Professor Dr. Kosuke NAMBA for his considerable advice, valuable discussions, and constant encouragement throughout the course of this work. It was a great honor to get an opportunity for his guidance. The author also expresses his sincere thanks to Assistant Professor Dr. Fumihiko YOSHIMURA for practical advice and helpful discussion.

The author would like to show his greatest appreciation to Professor Dr. Takanori SUZUKI, Professor Dr. Hideaki OIKAWA, Professor Dr. Masaya SAWAMURA, and Professor Dr. Hajime ITO for serving the author’s dissertation committee at Hokkaido University.

Professor Dr. Noboru KITAMURA, Associate Professor Dr. Shoji ISHIZAKA, and Assistant Professor Dr. Eri SAKUDA gave practical suggestion for optical measurement of TAPs to the author. Associate Professor Dr. Hidetoshi KAWAI made a contribution to X-ray crystallographic analysis of TAP 8n. The thermogravimetry analysis of azide 22a was performed by Professor Dr. Kazuki SADA, Assistant Professor Dr. Kenta KOKADO, and Mr. Takumi ISHIWATA. Tremendous contributions to fluorescence observation of TAPs inside cells were made by Professor Dr. Kazuyasu SAKAGUCHI, Associate Professor Dr. Yoshiro Chuman, and Ms. Fumi TANO. The quantum chemical calculation for the structure and the molecular orbital of TAPs was performed by Professor Dr. Tetsuya TAKETSUGU and Associate Professor Dr. Akira NAKAYAMA. The author would like to offer his special thanks to these contributors.

A part of this work was accomplished at Professor Namba’s laboratory, Faculty of Pharmaceutical Sciences, Tokushima University. The author would like to thank Associate Professor Dr. Masahiro YOSHIDA and Assistant Professor Dr. Atsushi NAKAYAMA for useful discussion and comments, sincerely. The author is grateful to all colleagues at Tanino group, Hokkaido University and Namba group, Tokushima University. Special Thanks to Mr. Ryohei UEMATSU, Ms. Ayano KOWATA, Mr. Ryusuke SAKOH, Mr. Takashi TOYOTA, and Ms. Shoko OHNO for extensive discussion and considerable encouragement. The author also expresses his appreciation to the member of the TAP project, Ms. Akane MERA, Ms. Michika KANAKI, Mr. Akira OTANI, Ms. Tsukiho HAYASHI, Ms. Masami ITO, and Mr. Satoshi NISHIO and the member of second laboratory at Namba group, Mr. Ryota KOTAKA and Mr. Akira SAKAMOTO for invaluable contributions and encouragement.

This work was partially supported by Grant-in-Aid for JSPS fellow. The author sincerely
thanks to Research Fellowship for Young Scientists from JSPS and scholarship from The Mitsubishi UFJ Trust Scholarship Foundation.

Finally, the author would like to thank sincerely his family, Kazuhiro, Fumiko, Shusuke, and late Yuuki and Sumiko for their encouragement and understanding.