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European Journal of Organic Chemistry

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Accepted Article

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To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201701277

Link to VoR: http://dx.doi.org/10.1002/ejoc.201701277

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Synthesis and Fluorescence Property of 1,1-Dimethyl-1,4-Dihydrodibenzo[b,h][1,6]naphthyridinium Iodides: Turn-on Type Detection of DNA

Kentaro Okuma,* Akinori Oba, Risa Kuramoto, Hidefumi Iwashita, Noriyoshi Nagahora, Kosei Shioji, Ryoma Noguchi, and Masatora Fukuda

Abstract: 1,4-Dihydrodibenzo[b,h][1,6]naphthyridines were synthesized by the reaction of 2-acetylaminobenzaldehyde with acetophenones in the presence of NaOH. The *N*-methylation of the dihydrodibenzonaphthyridines with MeI gave 1,1-dimethylammonium iodides. These salts showed UV/vis absorbance maxima at 235 and 418 nm and very weak fluorescence at 530 nm. The fluorescence intensities were enhanced 3–10 times when these salts were treated with double-stranded DNA via intercalation, which enabled the detection of DNA/RNA in PAGE gels.

It is necessary to acquire an insight into the selective staining of DNA to achieve a full understanding of biological functions and processes.[1] For example, ethidium bromide, a quinoline derivative, is widely used for the detection of DNA because of its ability to intercalate into the DNA double helix.[2] Ethidium bromide inserts itself into the spaces between the base pairs of the double helix (turn on). Generally, the binding of an analyte to a probe causes fluorescence enhancement (turn on) or fluorescence quenching (turn off).[3] Other quinolines, 1,8-naphthyridines, and 1,6-naphthyridines are also important compounds because of their interesting fluorescence properties and biological activities.[4] A cascade reaction in which a reactive intermediate from one step directly undergoes further transformations is very important in organic synthesis, because such sequential processes not only rapidly build up complex molecules but also efficiently enhance chemo- and regioselectivity of the overall transformation.[5] We have reported the synthesis of 6-methyl-1,6-dibenzonaphthyridinium triflates **1** via a cascade approach.[6] These compounds produce fluorescence at 420 nm when irradiated at 360 nm, which have the ability to intercalate into DNA. However, these compounds have some disadvantages compared with ethidium bromide. Compound **1** produces an emission at 440 nm in solution, but when it intercalates into DNA, its emission intensity decreases (turn off), rendering DNA detection difficult (Figure 1).

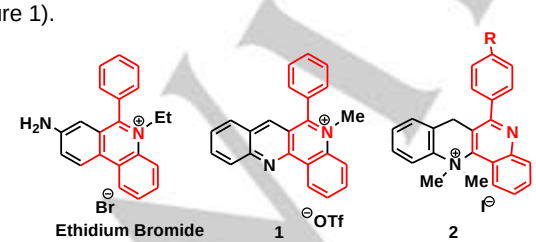

Figure 1. DNA intercalation reagents.

[*] K. Okuma, A. Oba, R. Kuramoto, H. Iwashita, N. Nagahora, K. Shioji, R. Noguchi, and M. Fukuda
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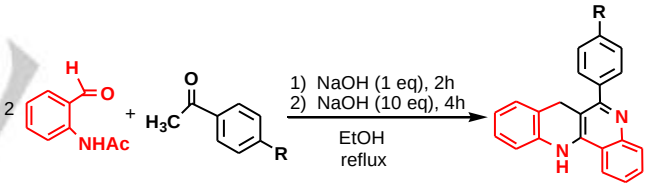
Thus, we focused on compounds that produce strong emission when intercalated into DNA (turn on type). In the course of synthesizing compound **1**, we found a new type of fluorescent reagent, the 1,4-dihydrodibenzonaphthyridine derivatives. The methylation of these compounds produced 1,1-dimethyldibenzonaphthyridinium salts **2**. We report herein a series of highly DNA staining quinoline-based fluorophores, aromatic substituted dihydrodibenzonaphthyridines, for high-contrast and high-brightness imaging in DEAE gels.

Firstly, we attempted to synthesize 1,4-dihydrodibenzonaphthyridines. Treatment of 2-acetylaminobenzaldehyde **3a** with acetophenone **4a** in the presence of NaOH (1 eq.) in refluxing ethanol for 2 h followed by the addition of NaOH (10 eq.) for an additional 4 h resulted in the formation of 1,4-dihydrodibenzonaphthyridine in 61% yield. 1,4-Dibenzodihydro[1,6]naphthyridine **5a** was easily oxidized in solution to afford dibenzonaphthyridine, the isolation of which could be accomplished in 10 minutes. The other reactions were carried out in a similar manner (Table 1).

Table 1. Synthesis of dihydrodibenzonaphthyridines **5**



Entry	4	5	Yield/% [a]
1	**4a**	**5a**	61
2	**4a**	**5a**	63
3	**4b**	**5b**	65
4	**4c**	**5c**	71
5	**4d**	**5d**	70
6	**4e**	**5e**	65

a) Reactions were carried out by using **3** (3.0 mmol), **4** (1.5 mmol), 5.0 M NaOH (0.3 mL, 1.5 mmol), and 20 M NaOH (0.75 mL, 15 mmol).

The structure of **5a** was confirmed by spectroscopic analysis (^{1}H NMR, ^{13}C NMR, MS) and elemental analysis. The ^{1}H NMR spectrum of **5a** shows signals for methylene (4.1 ppm) and aromatic groups (6.8–9.4 ppm). The reaction is speculated to proceed as follows: The aldol reaction of acetylaminobenzaldehyde **3** with acetophenone **4** gave unsaturated ketone **a**, which was hydrolyzed and condensed to give corresponding imine **b**. The intramolecular aza-Morita-Baylis-Hillman reaction and the subsequent intramolecular condensation gave 1,2-dihydronaphthyridine **c**. Proton transfer of this compound finally afforded 1,4-dihydronaphthyridine **5** (Scheme 1).

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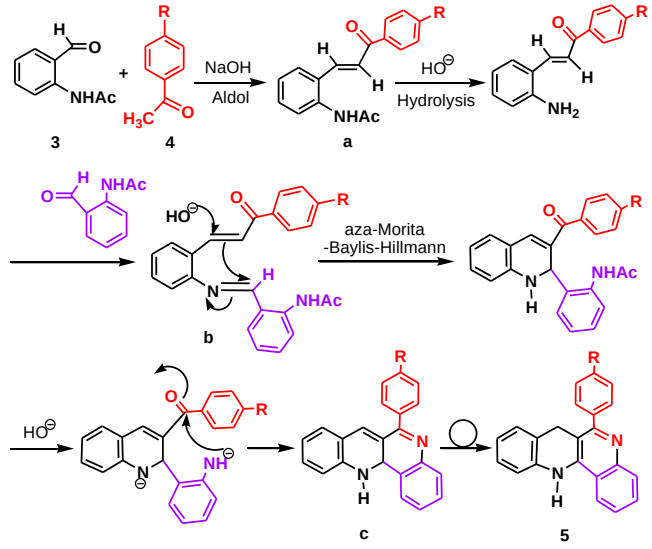


Scheme 1. Reaction Mechanism.

Dihydronaphthyridine **5a** was treated with an excess amount of methyl iodide in refluxing acetone for 15 h followed by the addition of aq. NaOH to afford 1-methyldihydrodibenzonaphthyridine (**6a**) in 68% yield. Further methylation by adding excess methyl iodide in refluxing acetone afforded dimethylammonium iodide **2a** in 57% yield. The other methylation reactions were carried out in a similar manner (Table 2).

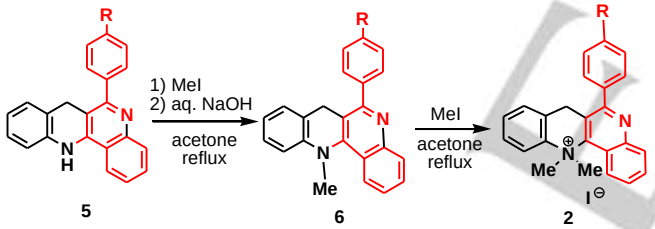


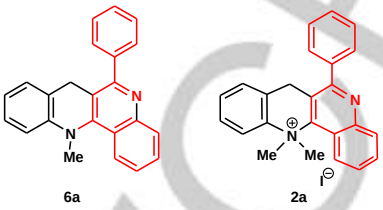
Table 2. Synthesis of *N*-methyl-1,4-dihydro[b,h][1,6]dibenzonaphthyridines **6** and *N,N*-dimethyl-1,4-dihydro[b,h][1,6]dibenzonaphthyridium iodides **2**

Entry	R	**6**	Yield/%	**2**	Yield/%
1	H	**6a**	68	**2a**	57
2	Me	**6b**	62	**2b**	69
3	OMe	**6c**	69	**2c**	42
4	Cl	**6d**	49	**2d**	50
5	Br	**6e**	54	**2e**	52

The structures of **6a** and **2a** were fully characterized by ^{1}H NMR and ^{13}C NMR measurements and elemental analysis. The ^{1}H NMR spectrum of **6a** shows signals for *N*-methyl (3.3 ppm) and aromatic protons (6.7-8.7 ppm), and that of **2a** shows *N*-methyl (4.0 and 4.1 ppm) and aromatic signals (6.9–8.3 ppm). Compound **2a-2e** are reddish orange stable crystals and can be stored on the shelf for more than 2 months. As can be seen in Table 2, yields of **2a-2e** were in the range of 42-69%, which indicated that no substituent effects of R were observed.

Because the product, 1,4-dihydro-1-methyldibenzonaphthyridine **6a**, produced strong flurorescence, we further investigated the photophysical properties of **6a** and **2a**.

Figure 2 shows the excitation and emission spectra of **6a** and **2a** in CH_2Cl_2 solution. *N*-methyldihydronaphthyridine **6a** was found to have an excitation wavelength at 420 nm, whereas upon excitation at its absorption maxima, 1,1-dimethylnaphthyridinium iodide **2a** exhibited relatively weak emission and low fluorescence quantum yield in CH_2Cl_2, which could be attributed to the very rapid nonradioactive decay caused by the strong and dynamic adhesive interactions with EtOH and water molecules (Figure 2).



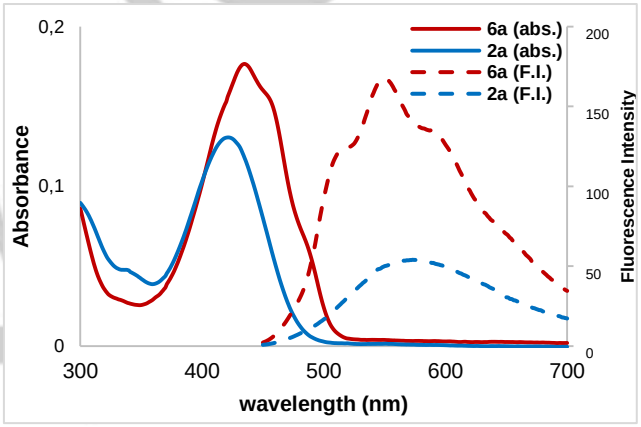


Figure 2. UV and fluorescence spectra of **6a** and **2a** in CH_2Cl_2.

Other ammonium iodides **2b–2e** showed a similar tendency as well (Table 3, Figure S-1).

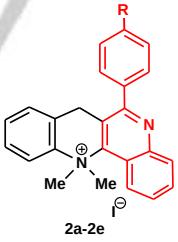


Table 3. UV/vis and fluorescence spectral data for **2a–2e**

2	R	λ	ε	λ	Stokes shift (cm^{-1})	θ
2a	H	418	11800	581	6710	0.32
2b	Me	415	11500	572	6610	0.36
2c	OMe	422	12300	577	6370	0.28
2d	Cl	424	11200	587	6550	0.32
2e	Br	416	12000	577	6710	0.31

We then examined the intercalation properties of **6a** and **2a** with the expectation that these compounds would behave as an ethidium bormide-like DNA staining reagent. As shown in Figure 3, a titration experiment revealed a decrease in the fluorescence intensity of **6a** with the addition of plasmid DNA, whereas another titration experiment showed a gradual increase in the fluorescence intensity of **2a** as plasmid DNA concentration was increased (Figure 4, also see Figure S-2). These results can be explained by considering the ability of the nitrogen-containing

planar ring to coordinate to DNA, because the structure of the ring system is very similar to that of ethidium bromide. Interestingly, such a positive effect of the intercalation of **2a** on the fluorescence is similar to that observed in ethidium bromide, whose fluorescence is strongly enhanced by the intercalation into DNA. The results indicate the successful development of a new turn-on-type DNA staining reagent. The mechanism for fluorescent signal induction is possibly due to the suppression of free rotation of the aromatic fragment and the vibrational motions of molecule when it is bound inside the pocket of the DNA substrate.

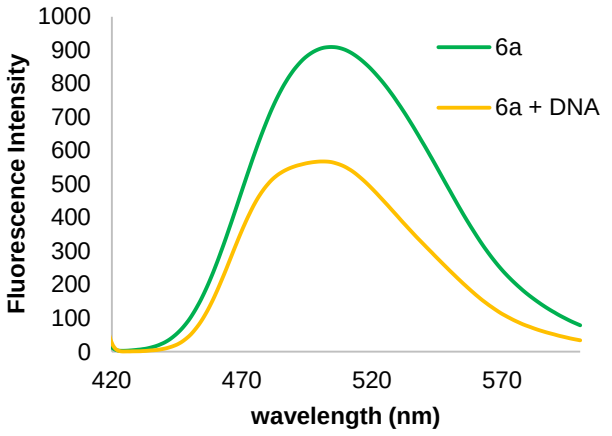
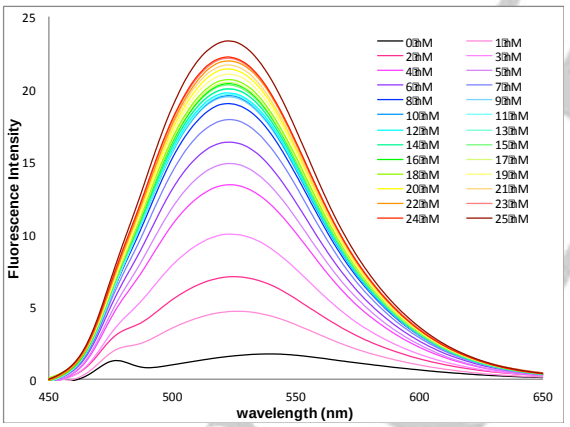


Figure 3. Titration experiment of **6a.** Plasmid DNA (1.5 μg/μL) in Tris buffer (0.01 mM, pH 7.6) was added to a solution of **6a** (1 μL) in Tris buffer (0.01 mM, pH 7.6) containing 0.1% DMSO.



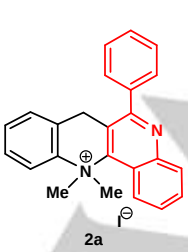


Figure 4. a) Titration experiment of **2a** with DNA. Plasmid DNA (1.5 μg/μL) in Tris buffer (0.01 mM, pH 7.6) was added to a solution of **2a** (1 μL) in Tris buffer (0.01 mM, pH 7.6) containing 0.1% DMSO. λ_{ex} = 410 nm. b) Excitation of **2a** with black light in the absence (left) or presence (right) of plasmid DNA.

The similarity of the fluorescence properties of **2a** and ethidium bromide was visually confirmed when DNA fragments electrophoresed on acrylamide gels were stained with those two reagents. When **2a**-stained gel was observed under UV irradiation, DNA fragments were visible as bright bands against the dark background of the gel (Figure 5). As typical DNA intercalators, such as ethidium bromide and acridinium derivatives, have planar aromatic rings, the interaction mode of **2a** is predicted to be an intercalation. The fluorescence of **2a** was enhanced with increasing DNA concentration, as was observed in several intercalators. Therefore, one of the possible mechanisms for this fluorescence enhancement is the energy transfer between the compound and nucleic bases and/or the immersion of ethidium bromide in a hydrophobic region.[7]

Figure 5. DNA staining of **2a**.100 bp DNA ladder fragments electrophoresed on 8% acrylamide gels were stained with ethidium bromide (left) and **2a** (right). Fluorescence was observed by excitation with 254 nm UV light.

To confirm that the fluorescence was a result of intercalation, we conducted a competition assay using ethidium bromide-stained DNA.[8] As shown in Figure 6, the fluorescence peak of ethidium bromide was gradually decreased by adding compound **2a** in buffer solution.

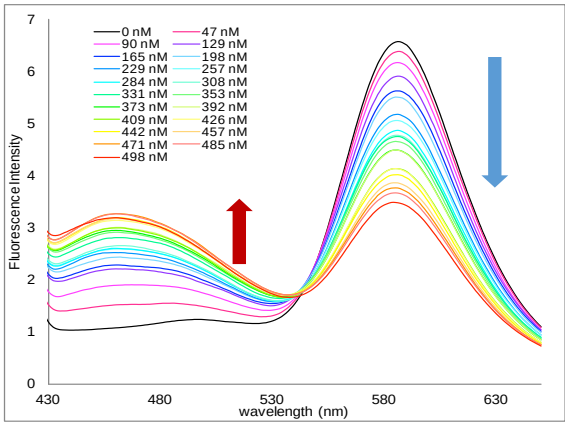


Figure 6. Fluorescence changes for the titration of **2a** with plasmid DNA-ethidium bromide system ([**2a**] = 0–498 nM). Arrows show the intensity change upon increasing **2a** concentration.

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We have synthesized 1,4-dihydrodibenzo[b,h][1,6]-naphthyridines **5** in one pot by reacting 2-acetylaminobenzaldehyde **3** with acetophenone **4** under basic conditions. 1,1-Dimethyl-1,6-dibenzonaphthyridinium iodides **2** were easily synthesized by treatment of **5** with methyl iodide in refluxing acetone, and showed significant fluorescence when treated with double-stranded DNA. A new turn-on type fluorescence reagents was developed. Further studies of the novel features of these reagents are in progress.

Keywords: Dibenzonaphthyridine • 2-acetylaminobenzaldehyde • acetophenone • DNA • intercalation

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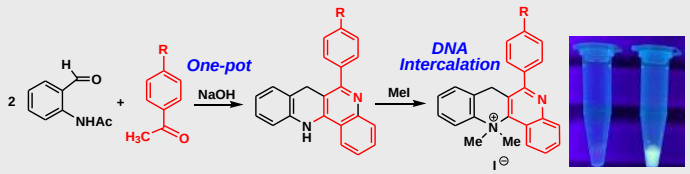

K. Okuma, A. Oba, R. Kuramoto, H. Iwashita, N. Nagahora, K. Shioji, R. Noguchi, M. Fukuda*

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