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Synthesis of boradiazaindacene–imidazopyrazinone conjugate as lipophilic and yellow-chemiluminescent chemosensor for superoxide radical anion

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ABSTRACT

As a chemiluminescent chemosensor that emits yellow light on reacting with a superoxide radical anion ($O_2^{\bullet-}$) and has a lipophilic character, a 6-phenylimidazo[1,2-*a*]pyrazin-3(7*H*)-one derivative possessing a boradiazaindacene (BODIPY) at the *para* position of 6-phenyl (**1**) was synthesized. The lipophilicity of **1** was investigated by reversed-phase liquid chromatography, and its log P_{ow} value was found to be 3.57. This value was much higher than that of 2-methyl-6-(4-methoxypheyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one (MCLA, log P_{ow}=1.19) and 6-[4-[2-{*N*′-(5-fluoresceinyl)thioureido}ethoxy]phenyl]-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (FCLA, log P_{ow}=−0.08), and it was comparable to that of benzenoid hydrocarbons. The $O_2^{\bullet-}$-induced chemiluminescence of **1** was investigated using the hypoxanthine/xanthine oxidase system as the source of $O_2^{\bullet-}$, and as a result, yellow emission was observed. The maximum wavelength was observed at 542 nm, and it was longer than that of FCLA.

1. Introduction

A superoxide radical anion ($O_2^{\bullet-}$) is a highly reactive free radical formed by the univalent reduction of oxygen during normal metabolism, and it plays important roles in biological systems. In mammalian cells, for example, $O_2^{\bullet-}$ acts as a signaling molecule that regulates important biological events such as protein phosphorylation,[1] cell growth,[2] and apoptosis.[3] Polymorphonuclear leukocytes and other phagocytes also produce $O_2^{\bullet-}$ as a chemical bomb to kill infections and ingested bacteria, respectively,[4] and to maintain healthy bodies. However, the increased production or insufficient extinction of $O_2^{\bullet-}$ causes cellular or tissue damage, inducing serious disease states such as ischemia reperfusion injury, Alzheimer's disease, and carcinogenesis.[4] Thus, the detection of production, distribution, and quantity of $O_2^{\bullet-}$ in cells and tissues is of particular clinical relevance.

2-Methyl-6-(4-methoxyphenyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one (MCLA) has been widely recognized as the most popular probe used for this purpose. MCLA can specifically react with $O_2^{\bullet-}$ immediately after its production to emit visible light (λ_{max}=465 nm in buffers at physiological pH conditions) without any light sources for excitation, accordingly enabling sensitive and real-time detection of $O_2^{\bullet-}$.[5] A fluorescein-linking MCLA derivative, 6-[4-[2-{*N*′-(5-fluoresceinyl) thioureido}ethoxy]phenyl]-2-methylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (FCLA), has been developed to detect $O_2^{\bullet-}$ at longer wavelength than MCLA.[6] On reacting with $O_2^{\bullet-}$ at physiological pH conditions, FCLA emits green light (λ_{max}=532 nm). This feature makes FCLA more useful than MCLA, because the blue light emitted by MCLA is absorbed by some biomaterials.[7] FCLA has the additional advantage of better water solubility over MCLA owing to the hydrophilicity of the fluorescein moiety, which is charged at the physiological pH conditions. However, because of the hydrophilic property, fluorescein-linking small molecules cannot easily permeate cell membranes. Therefore, the development of MCLA-based lipophilic chemiluminescent probes with emission at a longer wavelength than MCLA is still required for the successful detection of $O_2^{\bullet-}$ inside cells. For this purpose, we have previously synthesized imidazo[1,2-*a*]pyrazin-3(7*H*)-ones that have two lipophilic phenyl groups at the 6- and 8-positions of the imidazopyrazinone skeleton. Although these compounds exhibited $O_2^{\bullet-}$-induced chemiluminescence with an emission maxima around 500–530 nm in buffer solutions under basic conditions, their chemiluminescence spectra measured under neutral conditions showed peaks at around 400 nm.[8] In the present study, as part of our ongoing efforts to develop long-wavelength-light emitting imidazopyrazinones, we have designed a BODIPY-attached imidazopyrazinone, **1**. BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene) fluorophores are currently of special interest as substitutes for fluoresceins because of their superior photostability to fluorescein,[9] insensitivity toward solvent polarity and pH,[10] and sufficiently high lipophilicity to permeate cell membranes, as well as high extinction coefficients and fluorescence quantum yields approaching unity.[11–14]

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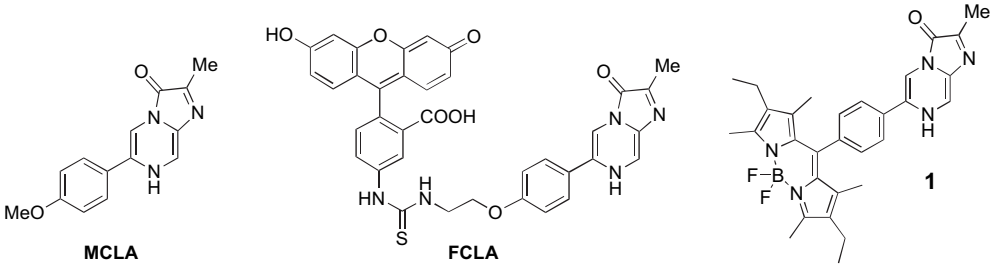
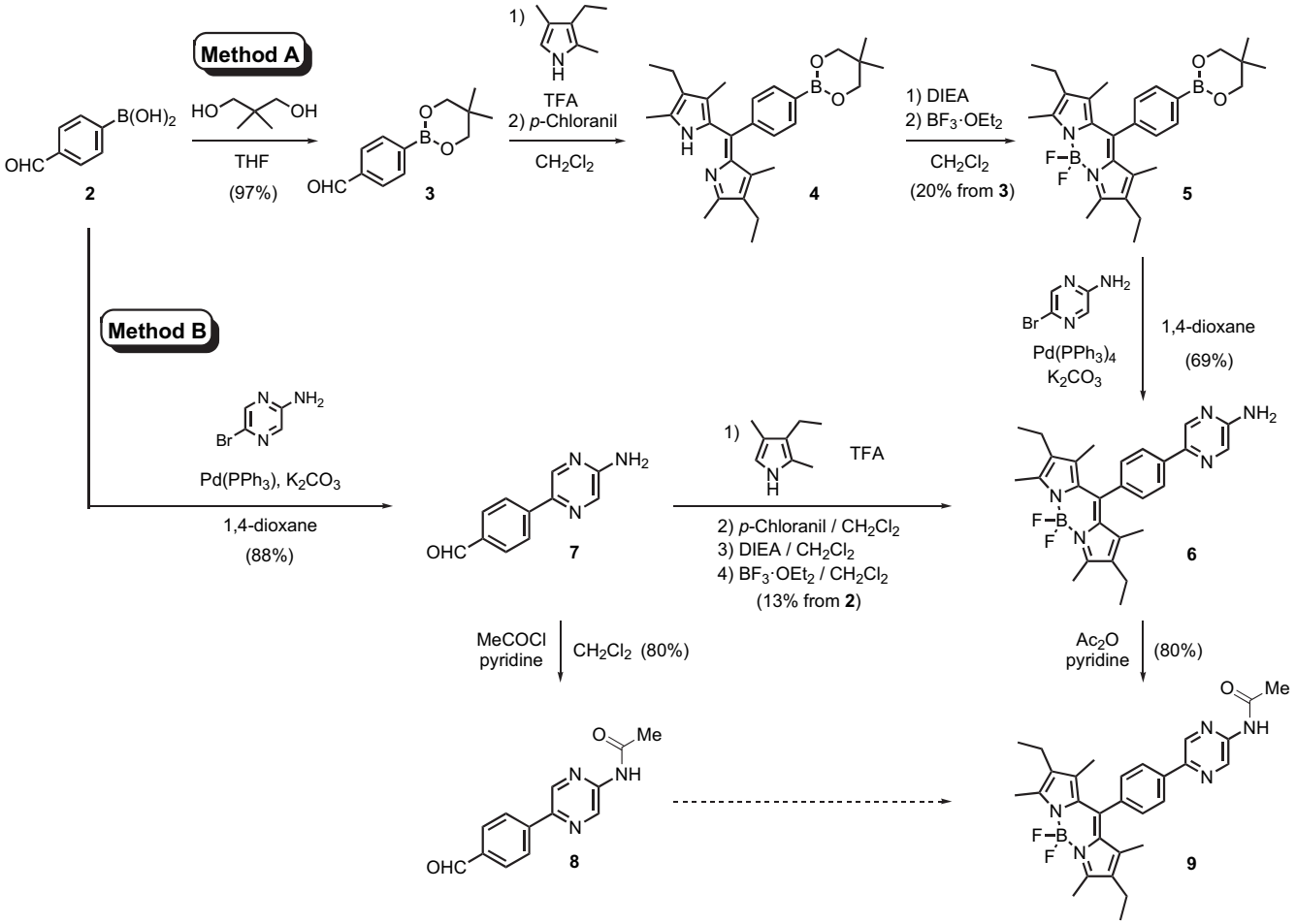
Herein, we describe the synthesis, lipophilicity, and chemiluminescence of the BODIPY-attached imidazopyrazinone that emits yellow light both in an aprotic organic solvent and in a buffer with an $O_2^{\cdot-}$-generating system at a physiological pH condition.



2. Results and discussion

2.1. Synthesis

The synthesis of the BODIPY-attached imidazopyrazinone **1** was achieved as shown in Scheme 1. First, boronic acid **2** was protected with 2,2-dimethylpropan-1,3-diol to give boronate **3**.[15] Compound **3** was then reacted with 3-ethyl-2,4-dimethylpyrrole to give **4**, followed by successive treatment with chloranil and boron trifluoride etherate to afford borondipyrromethene **5**. The palladium-catalyzed cross-coupling reaction of **5** with 2-amino-5-bromopyrazine, prepared by the bromination of 2-aminopyrazine, gave BODIPY–aminopyrazine conjugate **6** (method A). We also followed an alternative reaction procedure (method B) that involves shorter steps than method A, beginning with the same starting material **2**. In this method, **2** was reacted with 2-amino-5-bromopyrazine to give 2-amino-5-(4-formylphenyl)pyrazine **7**, which was then successively reacted with pyrrole, chloranil, and boron trifluoride etherate to give **6**. The total yield of **6** from **2** via method A was 13%, while that via method B was 3%, apparently indicating that method A is superior to method B even though the number of steps in method A is more than method B. We considered that the existence of free amino group in **7** was unfavorable for the conversion of **7** to **6** and lead to the low yield via method B. To confirm this, we attempted the same reaction with *N*-acetyl derivative of **7** (**8**) to obtain **9** (in Scheme 1) but failed because **8** was hardly soluble in conventional organic solvents including dimethyl sulfoxide (DMSO).



Scheme 1.