

## Simple Fluorescence Assay for Triethylamine Based on the Palladium Catalytic Dimerization of Benzofuran-2-boronic Acid

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Although benzofuran-2-boronic acid hardly emits fluorescence, it can be rapidly converted to a highly fluorescent benzofuran dimer after mixing with a palladium catalyst and amine. We found that a fluorescence enhancement accompanying dimerization was quantitatively promoted upon increasing the concentration of amine. In the present study, we developed a simple fluorescence assay for amines based on the promotive effect. As the result of a fluorescence measurement of the reaction mixture of 19 kinds of typical amines, it was found that tertiary amines including triethylamine (TEA) provided a significant fluorescence enhancement. Finally, the fluorogenic reaction could be applied to develop a high-throughput fluorescent microplate assay for TEA with the limit of detection (blank + 3SD) of 0.091  $\mu\text{M}$ .

**Keywords** Triethylamine, fluorescence assay, benzofuran-2-boronic acid, fluorogenic dimerization reaction, palladium

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### Introduction

Palladium-catalyzed cross-coupling reactions are considered to be efficient to construct carbon-carbon bonds.<sup>1-3</sup> The Suzuki-Miyaura coupling reaction is a palladium-catalyzed cross-coupling process between aryl halides and aryl boronic acids, and has been widely used to synthesize various chemicals including pharmaceuticals due to the stability of the reagents.<sup>4-6</sup> In the case of the Suzuki-Miyaura coupling reaction, bases including amines are frequently added to activate aryl boronic acids in order to achieve a higher reaction yield.<sup>7</sup>

On the other hand, we previously reported that benzofuran-2-boronic acid could be used as a fluorescent sensor to detect palladium.<sup>8</sup> Although benzofuran-2-boronic acid hardly emits fluorescence, a significant fluorescence enhancement can be observed upon the addition of palladium in the presence of amine, such as triethylamine (TEA). As shown in Fig. 1, this phenomenon could be attributed to the conversion of weakly fluorescent benzofuran-2-boronic acid to a highly fluorescent benzofuran dimer by the catalytic activity of palladium. Furthermore, in the course of related work, we found that the fluorescence quantitatively increased upon increasing the concentration of TEA. Therefore, we considered that the

fluorogenic dimerization reaction could also be utilized to determine the amine concentration. In the present study, we attempted to develop a simple fluorometric assay for amines with benzofuran-2-boronic acid and palladium as reagents. Firstly, to investigate the applicability of the proposed fluorogenic reaction, the fluorescence of 19 kinds of typical amines was measured after the reaction with benzofuran-2-boronic acid in the presence of the palladium catalyst. Next, we developed a simple and rapid microplate assay for TEA based on the proposed fluorogenic reaction.

### Experimental

#### Materials and reagents

Benzofuran-2-boronic acid, diethylamine, pyrrole, ethanolamine and benzyloethanolamine were purchased from Tokyo Chemical Industries (Tokyo, Japan). Palladium(II) acetate ( $\text{Pd}(\text{OAc})_2$ ), palladium(II) nitrate ( $\text{Pd}(\text{NO}_3)_2$ ), hexylamine and choline chloride were purchased from Nacalai Tesque (Kyoto, Japan). Ethylamine, aniline, diphenylamine, trimethylamine, TEA, triphenylamine, acetylcholine chloride, L-carnitine, pyridine, piperidine, 1,3-diaminopropane, spermidine, spermine, tris[tris(3,5-bis(trifluoromethyl)phenyl)phosphine]palladium (Superstable Pd(0)), tetrakis(triphenylphosphine)palladium(0) ( $\text{Pd}(\text{PPh}_3)_4$ ), and bis(dibenzylideneacetone)palladium(0) ( $\text{Pd}(\text{dba})_2$ ) were purchased from Wako (Osaka, Japan). Acetonitrile (HPLC grade) and palladium(II) chloride ( $\text{PdCl}_2$ ) were from Kanto Chemical (Tokyo, Japan). Water was distilled and passed through a Pure Line WL21P system (Yamamoto, Tokyo, Japan). All other chemicals were of the highest purity and quality available. Stock solutions of amine were prepared in acetonitrile at the concentration of 500  $\mu\text{M}$  each.

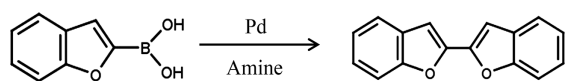


Fig. 1 Fluorogenic dimerization of benzofuran-2-boronic acid by the addition of amine and palladium.

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### Fluorescence spectra of TEA after reacting with benzofuran-2-boronic acid and Pd(OAc)<sub>2</sub>

To 100  $\mu\text{L}$  of TEA in acetonitrile, 100  $\mu\text{L}$  each of 10  $\mu\text{M}$  Pd(OAc)<sub>2</sub> and 5.0 mM benzofuran-2-boronic acid in acetonitrile were added; the mixture was then kept at room temperature for 15 min. After it was diluted 10 times with acetonitrile, the fluorescence emission spectrum excited at 315 nm was recorded on a RF-1500 spectrofluorometer (Shimadzu, Kyoto, Japan).

### Fluorescence measurement by microplate reader

In each well of a 96-well black microplate (Corning, NY, USA), 50  $\mu\text{L}$  of amines in acetonitrile, 50  $\mu\text{L}$  of 10  $\mu\text{M}$  Pd(OAc)<sub>2</sub> in acetonitrile, and 50  $\mu\text{L}$  of 5.0 mM benzofuran-2-boronic acid in acetonitrile were successively added. The microplate was immediately placed into a Spectra Max M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA), and shaken for 30 s. After keeping the microplate for 15 min at room temperature in the microplate reader, the fluorescence was measured at excitation and emission wavelengths of 315 and 360 nm, respectively.

## Results and Discussion

### Fluorescence response of amines after reacting with benzofuran-2-boronic acid and Pd(OAc)<sub>2</sub>

Figure 2 shows the emission spectra excited from benzofuran-2-boronic acid after the addition of TEA in the presence of Pd(OAc)<sub>2</sub>. As the TEA concentration increased, the fluorescence with emission maxima at 360 nm increased at an excitation wavelength of 315 nm. The results meant that the fluorogenic dimerization of benzofuran-2-boronic acid was promoted upon the addition of TEA, depending to its concentration. Then, to investigate the applicable range of the proposed fluorogenic reaction, the fluorescence of 19 kinds of typical amines after the reactions with benzofuran-2-boronic acid and Pd(OAc)<sub>2</sub> was measured. As shown in Fig. 3, among the tested amines, TEA, trimethylamine, triphenylamine and diphenylamine provided a significant fluorescence enhancement resulting in a signal-to-

blank (S/B) ratio > 2. Also, it was found that TEA had the highest fluorescence intensity. Considering the results obtained by the present investigation, the proposed reaction would be useful to determine tertiary amines.

### Fluorescence microplate assay for TEA

TEA is a volatile organic compound, and is used in a variety of fields, such as pharmaceuticals, agricultural chemicals, surfactants and raw materials for paints, *etc.*<sup>9,10</sup> It is known that TEA has a strong ammonia smell, and can cause eye and lung irritations.<sup>11,12</sup> Also, TEA is designated as a hazardous air pollutant. Although several gas chromatographic methods have been developed, these methods require relatively complicated instruments.<sup>13-15</sup> Further, although the fluorescence method has advantages in terms of simplicity, sensitivity and rapidness,<sup>16</sup> TEA does not exhibit intrinsic fluorescence, and it should be difficult to apply fluorescence derivatization because TEA does not have a derivatizable moiety.<sup>17</sup> From these aspects, we attempted to develop a simple and rapid fluorescence microplate

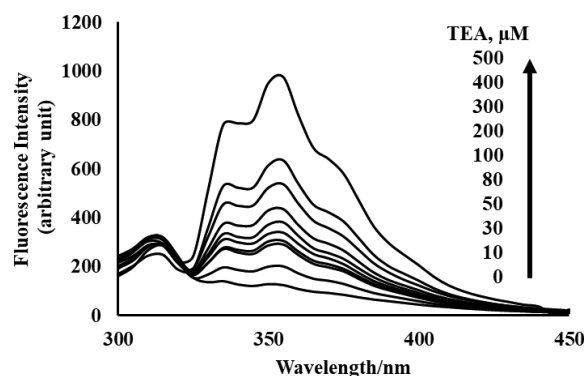


Fig. 2 Fluorescence emission spectra of benzofuran-2-boronic acid (5.0 mM) after the addition of different concentrations of TEA (0, 10, 30, 50, 80, 100, 200, 300, 400 and 500  $\mu\text{M}$ ) in the presence of Pd(OAc)<sub>2</sub>.

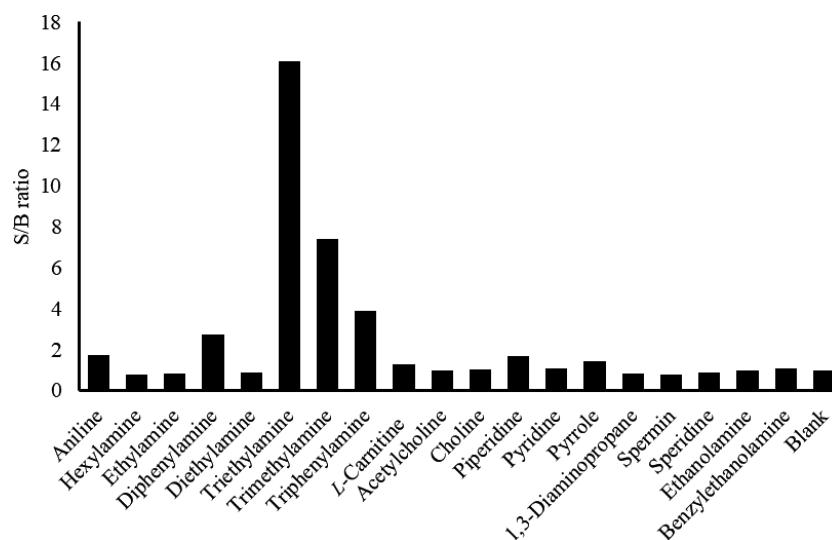


Fig. 3 S/B ratio obtained from fluorescence measurements of benzofuran-2-boronic acid after the addition of various amines and Pd(OAc)<sub>2</sub>. The concentrations of amines, benzofuran-2-boronic and Pd(OAc)<sub>2</sub> in acetonitrile were 500  $\mu\text{M}$ , 5.0 mM and 10  $\mu\text{M}$ , respectively. The reaction temperature was ambient temperature.

assay for TEA based on promoting the dimerization of benzofuran-2-boronic acid.

In order to achieve high reactivity, the reaction conditions were optimized using a standard solution of TEA. The measurement results are shown as the average value of triplicate measurements. The optimum reaction time and temperature were 15 min and ambient temperature (25°C), respectively, in consideration that the maximum S/B ratio could be achieved under these conditions (Figs. S1 and S2). The S/B ratio decreased with the reaction time by more than 15 min, because the signal with TEA reached the maximum value at approximately 15 min, and kept contained, while a blank without TEA slightly increased with increasing the reaction time. Then, the concentration of benzofuran-2-boronic acid was investigated over the range of 0 – 20, and 5 mM was selected because it gave the maximum S/B ratio (Fig. S3). The effect of the Pd species on the fluorescence was investigated using superstable Pd(0), Pd(PPh<sub>3</sub>)<sub>4</sub>, Pd(dba)<sub>2</sub>, Pd(NO<sub>3</sub>)<sub>2</sub>, Pd(OAc)<sub>2</sub>, and PdCl<sub>2</sub>, because different Pd species could induce different dimerization reactivity (Fig. S4). Among the tested Pd species, the highest fluorescence intensity was obtained with Pd(OAc)<sub>2</sub>. Subsequently, the concentration of Pd(OAc)<sub>2</sub> was investigated over the range of 0 – 50 μM and the maximum S/B ratio was achieved with 10 μM Pd(OAc)<sub>2</sub>, thus it was selected (Fig. S5).

The calibration curve was prepared with the standard TEA solution under optimized conditions. A good linear relationship ( $r = 0.993$ ) between the TEA concentration and the fluorescence intensity was obtained in the range of 1 to 400 μM. The limit of detection (LOD), defined as the mean blank signal plus 3-times the standard derivation of the blank (blank + 3SD), was 0.091 μM. Table S1 summarizes a comparison of the LOD values between the proposed assay and previously reported methods. The proposed assay was more than 10 times more sensitive than gas chromatography with flame ionization detection,<sup>18</sup> capillary electrophoresis,<sup>19</sup> and chemiluminescence assay triggered by a TEA free-radical reduction of carbon dots.<sup>20</sup> Also, the LOD of the proposed assay was similar to that of a fluorescence quenching assay based on the quenching of amphiphilic fluorescent micelles formed from polyethylene glycol and tetraphenylene ethylene.<sup>17</sup>

The repeatability of the proposed assay was evaluated using three different concentrations (5, 50 and 400 μM) of the standard TEA solution in the calibration range. The relative standard deviations (RSD) for intra-day ( $n = 5$ ) assays were 4.4, 5.5 and 3.1%, respectively, and for inter-day ( $n = 5$ ) assays were 6.2, 6.0 and 4.3%, respectively. Therefore, the sufficient repeatability of the proposed microplate assay could be proved.

## Conclusion

We found that the fluorescence enhancement was observed from a solution of weakly-fluorescent benzofuran-2-boronic acid after the mixing with amine and palladium. The fluorescence enhancement could be attributed to the promotion of a palladium catalytic fluorogenic dimerization of benzofuran-2-boronic acid by the addition of amine. Remarkable fluorescence was

obtained from a mixture containing tertiary amines including TEA. Finally, the fluorogenic reaction could be successfully applied to develop a fluorescent microplate assay for TEA. Since the developed assay allowed a high-throughput measurement of TEA without derivatization, it should become a powerful tool to determine tertiary amines in environmental, biological and food samples.

## Supporting Information

This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>. Supporting information includes the results of optimization studies.

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