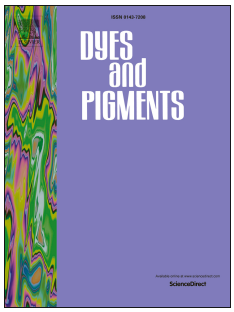

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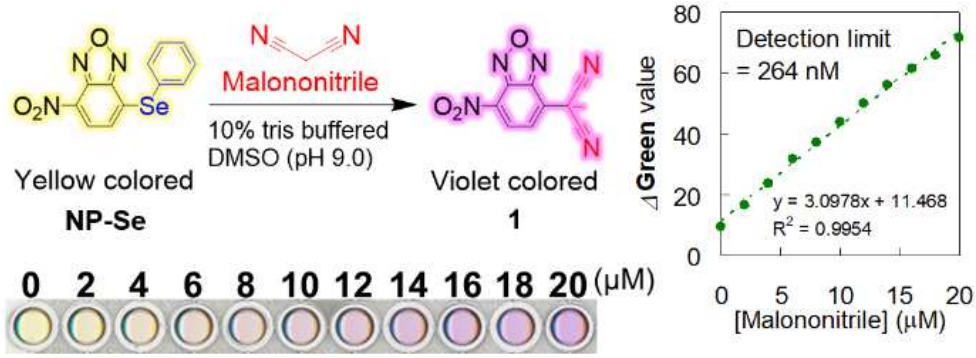
Graphical Abstract



Colorimetric analysis of malononitrile via the formation of a novel NBD-based CH-acidic dye

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Abstract:

Malononitrile is a key starting material used in synthetic organic, medical, and industrial chemistry, but it is also a notorious cyanogenic toxicant. However, selective and sensitive analytical methods for this key chemical are rare. In this research, a novel reaction-based colorimetric signaling probe for the selective analysis of malononitrile via the S_NAr-type nucleophilic substitution reaction of an NBD-based phenyl selenoether was investigated. The probe exhibited a pronounced colorimetric response, with a color change from yellow to violet due to its CH-acidity. The interference of sulfide ions and several transition metal ions was

effectively circumvented with the use of copper foil as a sulfide scavenger and EDTA as a masking agent for the metal ions. The detection limit of the probe for malononitrile was found to be 264 nM. It was also demonstrated that this convenient colorimetric analysis of malononitrile in chemical and industrial applications using the probe can be conducted with an office scanner.

Keywords: Malononitrile sensing; 4-Nitrobenzoxadiazole; Colorimetric signaling; CH-acidic dye; Masking agent; Office scanner.

1. Introduction

Malononitrile is a versatile compound with exceptional reactivity that acts as a key starting material for a number of synthetic organic, medical, and industrial chemistry applications [1]. For example, it is frequently used in the synthesis of various heterocyclics, pharmaceuticals, pesticides, fungicides, and charge-transfer salts [2]. It is also used in the manufacture of lubricating oil additives [3], dyes and acrylic fibers [4], thiamine and anti-cancer agents [5], 2-chlorobenzalmalononitrile (CS-gas, also known as tear gas), which is used for self-defense purposes [6], and as a

leaching agent for gold [7]. In analytical chemistry, malononitrile has been used in the detection of 1,2-quinones in color tests via paper chromatography and spot plates [8,9], the spectrophotometric determination of vitamin K_3 [10], and the analysis of sialic acids using postcolumn fluorescence labeling in high-performance liquid chromatography [11].

Despite these practical uses, malononitrile is a notorious cyanogenic toxicant [3] that is characterized by a number of serious symptoms, such as irritation of the eyes and skin, headaches, dizziness, convulsions, dyspnea, nausea, and vomiting [12]. Therefore, particular care should be taken in handling malononitrile because it exhibits extensive chemical reactivity and has a low LD_{50} (66 mg/kg) [13]. As such, the National Institute for Occupational Safety and Health (NIOSH) has established a recommended exposure limit (REL) of 3 ppm (8 mg/m^3) as an 8-hour time-weighted average [14]. Therefore, the development of a selective and sensitive analytical method for the detection of malononitrile is very important not only for the health of workers in various industrial activities but also for analytical assays involving this key reagent.

Despite this need, only a few studies have analyzed malononitrile and its derivatives from this perspective (Table S1, Supplementary Data). Malononitrile has primarily

been analyzed using gas chromatography [15] and gas chromatography–mass spectrometry [16]. Polarographic analysis based on its electroactivity compared to the closely related compounds cyanoacetic acid, malonic acid, and succinic acid dinitrile has also been reported [17]. In addition, due to their greater convenience, several colorimetric detection methods have been reported. For instance, malononitrile and its derivatives have been detected and quantified according to their reaction with benzofuran oxide in an alkaline medium, which produces an intense violet color [18]. In addition, the reaction of malononitrile with 4-(4′-nitrobenzyl)pyridine leads to the generation of a yellow color [19], while it also reacts with conventional coloring reagents (e.g., diazotized *p*-nitroaniline) to produce a blue color [20]. Colorimetric spot tests using nitroprusside ($Fe(CN)_5NO_2$) [21] and the 2,4-dinitrobenzenediazonium coupling reaction [22] have also been reported. However, a more selective and sensitive analytical method is required because most of these colorimetric methods, except the benzofuran oxide method, essentially use a qualitative approach involving TLC visualization or spot testing and thus cannot provide practical and analytically useful quantitative information.

For this reason, we designed a new colorimetric reaction-based probe for the selective and sensitive signaling of malononitrile using the nucleophilic aromatic

72 substitution (S_NAr) reaction with a selenoether derivative of 7-nitrobenz-2-oxa-1,3-
73 diazole (NBD) dye. Recently, selective colorimetric probes for hydrogen sulfide
74 based on the S_NAr-type reaction of NBD thioethers through the thiol extrusion and
75 formation of a uniquely colored nitrobenzofurazan-thiol (λ_{max} = 534 nm) have been
76 reported [23–26]. Following these reports, a variety of NBD derivatives based on
77 amines [27], ethers [28], and thioethers [29] with several dye subunits, such as
78 rhodamine and fluorescein, have been designed through the thiolysis-type cleavage
79 of C–N, C–O, and C–S bonds to detect hydrogen sulfide and various biothiols,
80 including cysteine (Cys) and reduced glutathione (GSH); these have been
81 summarized in a recent review [30].
82

83 **2. Materials and methods**

84 **2.1 General.** Malononitrile, 4-chloro-7-nitro-1,2,3-benzoxadiazole (NBD-Cl),
85 phenol, thiophenol, benzeneselenol, and diphenyl diselenide were purchased from
86 Merck KGaA. Other chemicals and solvents were obtained from commercial sources
87 at spectroscopic grade. ^{1}H (300 MHz and 600 MHz) and ^{13}C (150 MHz) NMR
88 spectra were measured on Varian Gemini 2000 and VNS NMR spectrometers, using

residual solvent signals as a reference. UV–vis spectra were recorded using a Scinco S-3100 spectrophotometer. Fluorescence spectra were measured with a Scinco FluoroMate FS-2 fluorescence spectrophotometer. Mass spectra were acquired using a Micromass Autospec mass spectrometer.

2.2 Preparation of malononitrile-selective NP-O, NP-S, and NP-Se probes. NBD-based malononitrile-selective probes (**NP-O**, **NP-S**, and **NP-Se**) were prepared according to a previously reported procedure [31]. Phenol (0.28 g, 3.0 mmol, for **NP-O**), thiophenol (0.33 g, 3.0 mmol, for **NP-S**), or benzeneselenol (0.47 g, 3.0 mmol, for **NP-Se**) was dissolved in ethanol (20 mL), and triethylamine (0.42 mL, 3.0 mmol) was added to the solution. A solution of NBD-Cl (0.20 g, 1.0 mmol) in 5 mL of ethanol was slowly added to the reaction mixture and stirred at room temperature overnight. The reaction mixture was diluted with distilled water and extracted with dichloromethane. The organic phase was separated, washed with water several times, and then evaporated. The residue was purified using short-column chromatography (silica gel, dichloromethane). **NP-O**: 65%. Yellow crystal. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.64 (d, J = 8.4 Hz, 1H), 7.61 (dd, J = 8.5, 7.3 Hz, 2H), 7.48–7.40 (m, 3H), 6.69 (d, J = 8.4 Hz, 1H); ^{13}C (150 MHz, DMSO-d_6) δ 153.7, 153.3, 145.8, 144.8, 136.0, 131.3, 130.6, 127.4, 121.3, 109.9; LRMS (EI$^+$); *m/z* calcd for $C_{12}H_8N_3O_4$

[M+H]$^+$: 258.0, found 258.1. **NP-S**: 69%. Yellow crystal. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.51 (d, J = 8.0 Hz, 1H), 7.79–7.74 (m, 2H), 7.71–7.63 (m, 3H), 6.83 (d, J = 8.0 Hz, 1H); ^{13}C (150 MHz, DMSO-d_6) δ 149.0, 143.3, 140.4, 135.7, 133.3, 133.0, 131.6, 131.2, 126.4, 123.8; LRMS (EI$^+$); *m/z* calcd for $C_{12}H_8N_3O_3S$ [M+H]$^+$: 274.1, found 274.0. **NP-Se**: 72%. Amber crystal. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.45 (d, J = 7.8 Hz, 1H), 7.83–7.78 (m, 2H), 7.67–7.56 (m, 3H), 7.03 (d, J = 7.8 Hz, 1H); ^{13}C (150 MHz, DMSO-d_6) δ 150.2, 143.0, 136.9, 134.2, 132.5, 131.05, 131.04, 127.7, 124.2; LRMS (EI$^+$); *m/z* calcd for $C_{12}H_8N_3O_3Se$ [M+H]$^+$: 321.8, found 321.9.

2.3 Preparation of NBD malononitrile derivative 1. Signaling product **1** was independently prepared via the reaction of NBC-Cl with malononitrile in ethanol. NBD-Cl (0.20 g, 1.0 mmol) was dissolved in ethanol (10 mL), and sodium carbonate (0.11 g, 1.0 mmol) was added to the solution. Malononitrile (0.07 g, 1.0 mmol) was added to the reaction mixture, and the solution was stirred at 50 °C. After stirring for 3 h, the solvent was removed under reduced pressure. Acetone (10 mL) was added to the residue, and the precipitate was filtered and dried in a vacuum oven. **1**: 95%. Dark violet crystal. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.19 (d, J = 9.0 Hz, 1H), 6.49 (d, J = 9.0 Hz, 1H); ^{13}C (150 MHz, DMSO-d_6) δ 146.8, 144.7, 142.6, 134.3, 119.9,

118.8, 118.6, 108.6, 51.1; HRMS (ESI⁻); *m/z* calcd for $C_9H_2N_5O_3^-$ [M–H]⁻: 228.0163, found 228.0159.

2.4 Preparation of stock solutions. Stock solutions (5.0×10^{-4} M) of **NP-O**, **NP-S**, and **NP-Se** were prepared in spectroscopic-grade DMSO, and a stock solution of malononitrile (1.0×10^{-2} M) was prepared in distilled water. Other stock solutions of metal ions and anions (1.0×10^{-2} M) were prepared in distilled water using the perchlorate and sodium salts of metal ions and anions, respectively. A solution of ethylenediaminetetraacetic acid (EDTA, 1.0×10^{-1} M) was also prepared in deionized water.

2.5 Measurement of signaling behavior. All measurements were carried out under optimal conditions with a 10% aqueous DMSO solution buffered with tris buffer (pH 9.0). The solutions used to measure malononitrile signaling were prepared by mixing a stock solution of the probe (**NP-O**, **NP-S**, or **NP-Se**; 60 μL, 5.0×10^{-4} M), malononitrile or the tested metal ions and anions (30 μL, 1.0×10^{-2} M), and tris buffer solution (150 μL, pH 9.0, 0.20 M) in a vial. The sample solution was diluted using deionized water and DMSO to give a final volume of 3.0 mL with a 1:9 (*v*/*v*) ratio of water to DMSO. The final concentrations of the probe, analyte (malononitrile

141 or metal ions/anions), and buffer in the solution were 1.0×10^{-5} M, 1.0×10^{-4} M, and
142 1.0×10^{-2} M, respectively.

143 **2.6 Suppression of sulfide and transition metal ion interference.**

144 **A. Suppression of sulfide interference.** To prevent sulfide interference, copper foil
145 (battery electrode grade) was used. A square piece of copper foil (10.0 mm × 10.0
146 mm, 18 mg) was fully soaked in the prepared sample solution for 1 min and was then
147 removed. The **NP-Se** probe was added to the solution treated with copper foil, and
148 changes in the UV–vis spectrum or green channel of the sample solution were
149 measured.

150 **B. Suppression of transition metal ion interference.** The interference of transition
151 metal ions in malononitrile signaling was successfully prevented using an EDTA as a
152 masking agent. After the signaling was complete, 15.0 μL of EDTA solution ($1.0 \times$
153 10^{-1} M) was added to the solution, and changes in the UV–vis spectrum or green
154 channel of the sample solution were measured.

155 **2.7 Determination of the detection limit.** The detection limit for malononitrile was
156 calculated according to IUPAC recommendations using the equation $3s_{bl}/m$, where s_{bl}
157 is the standard deviation of the blank responses (number of measurements = 15), and
158 m is the slope of the obtained titration curve [32].

2.8 Calculation of p*K*a for CH-acidic dye 1. To calculate the acid dissociation constant (K_a) for CH-acidic dye **1**, changes in the absorbance at 577 nm of CH-acid **1** as a function of the solution pH in water were measured. The pH of the sample solution was adjusted using HCl solution. From the obtained pH-dependent plot, the pK_a of CH-acid **1** was calculated using the Henderson-Hasselbalch equation [33]:

$$pK_a = pH - \log\left(\frac{[A^-]}{[HA]}\right)$$

2.9 Evidence for the signaling process. A deuterated phosphate buffer solution (0.10 mL, pH 7.0) containing malononitrile (6.6 mg, 0.10 mmol) was added to a solution of **NP-Se** (3.2 mg, 0.010 mmol) in 0.90 mL of DMSO-d_6. After mixing, the ^{1}H NMR spectrum of the mixture (**NP-Se** + malononitrile) was obtained. As a reference, the ^{1}H NMR spectra of independently prepared compound **1** and diphenyl diselenide were acquired in the same deuterated phosphate-buffered DMSO-d_6 (1:9, *v/v*).

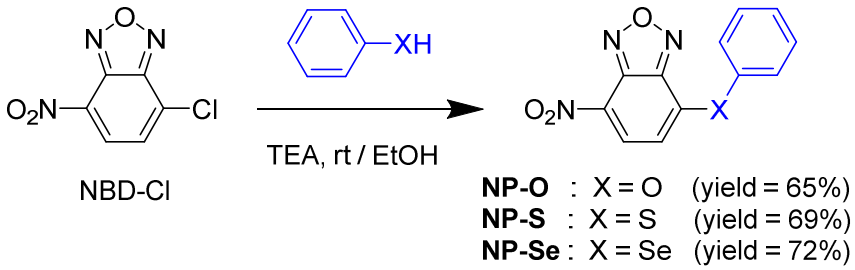
2.10 Colorimetric analysis using an office scanner [34]**.** The red, green, and blue channel values of the signaling solutions were recorded using an office scanner (Perfection V550, Epson Inc.). To obtain images of the samples, solutions with varying concentrations of malononitrile were treated with the probe, and 0.30 mL of the solution was placed in a 96-well plate. Images of the plate were captured using a

transmittance-type scanner (resolution: 1200; image type: 24-bit color). The color channels of the scanned images were estimated using a color analysis program (Adobe, Photoshop CS6). Because the change in the green channel levels was more pronounced than the red or blue channels, the ***Δ*Green** value (255 – green channel level) was used when plotting the results.

3. Results and discussion

A malononitrile-selective probe was designed based on the nucleophilic substitution capability of malononitrile on NBD phenyl ether derivatives. We expected that the reaction of a reactive malononitrile anion at the ether-substituted C-4 atom of the malononitrile moiety of the probe would result in the formation of a novel CH-acidic dye. The produced compound exhibited pronounced chromogenic behavior owing to the deprotonation of the methine proton of the malononitrile moiety and to the extended cross-conjugation of the anionic species through the nitro group of the NBD moiety. To achieve this, three candidate NBD-based compounds containing ether, thioether, and selenoether functions (**NP-O**, **NP-S**, and **NP-Se**) were prepared via the reaction of 4-chloro-7-nitrobenzofurazan (NBD-Cl) with phenol, thiophenol,

193 and benzeneselenol, respectively (Scheme 1). Preliminary research demonstrated that
194 all three compounds readily reacted with malononitrile to generate intensely violet-
195 colored solutions within 1 h at 20 ^{o}C. However, the reaction speed of the probes with
196 malononitrile differed, decreasing in the order of **NP-O** > **NP-Se** > **NP-S** (Fig. S1,
197 Supplementary Data). The ether derivative **NP-O** exhibited the most favorable
198 signaling speed; however, the stability of the probe in the measurement solution was
199 not satisfactory. The nearly colorless **NP-O** solution progressively became more
200 yellow due to the formation of a hydroxylated NBD derivative (7-nitro-2,1,3-
201 benzoxadiazol-4-ol) [35] via a hydrolysis reaction (Fig. S2, Supplementary Data).
202 The thioether **NP-S** also showed a similar undesirable response but to a lesser extent.
203 On the other hand, the selenoether **NP-Se** showed relatively good stability, remaining
204 stable for up to 12 h after preparation. After further optimization, **NP-Se** was
205 selected as the probe for the sensing of malononitrile because it demonstrated
206 satisfactory selectivity, reaction speed, and probe stability under the target
207 measurement conditions.
208



Scheme 1. Preparation of NBD-based malononitrile signaling probes (**NP-O**, **NP-S**, and **NP-Se**).

The **NP-Se** probe solution was lightly yellow-colored and exhibited a UV–vis absorption maximum at 434 nm in tris-buffered DMSO (pH = 9.0, 1:9, *v*/*v*). The mixing of the **NP-Se** solution with malononitrile resulted in the development of a violet-colored solution. In addition, a 250-fold increase in absorbance (A/A_0) at 577 nm was measured, while there were no discernible changes in the absorption spectra in the presence of other tested common metal ions (Fig. 1). However, of the tested anions, significant responses to sulfide ions were observed (Fig. S3, Supplementary Data). Sulfide-induced interference was not unexpected because the NBD phenyl selenoether derivative has previously been used as a colorimetric sulfide-signaling probe [23–26]. We attempted to suppress sulfide interference to allow exclusive malononitrile-selective signaling using several transition metal ions, metal powders, and metal foils. Subsequently, it was found that, in the presence of copper foil

225 (battery electrode grade), sulfide ion interference was completely suppressed without
226 significantly affecting malononitrile signaling (Fig. S4, Supplementary Data). The
227 sulfide ions were found to be removed from the solution via deposition on the
228 surface of the copper foil, which was confirmed by energy-dispersive X-ray analysis
229 (Fig. S5, Supplementary Data). In fact, copper or copper naphthenate has been used
230 as a sulfide scavenger for corrosion prevention via the deactivation of corrosive
231 sulfur [36]. In contrast to this prominent colorimetric response, no practically useful
232 changes in the fluorescence of the **NP-Se** probe were observed for any of the tested
233 species (Fig. S6, Supplementary Data).

234

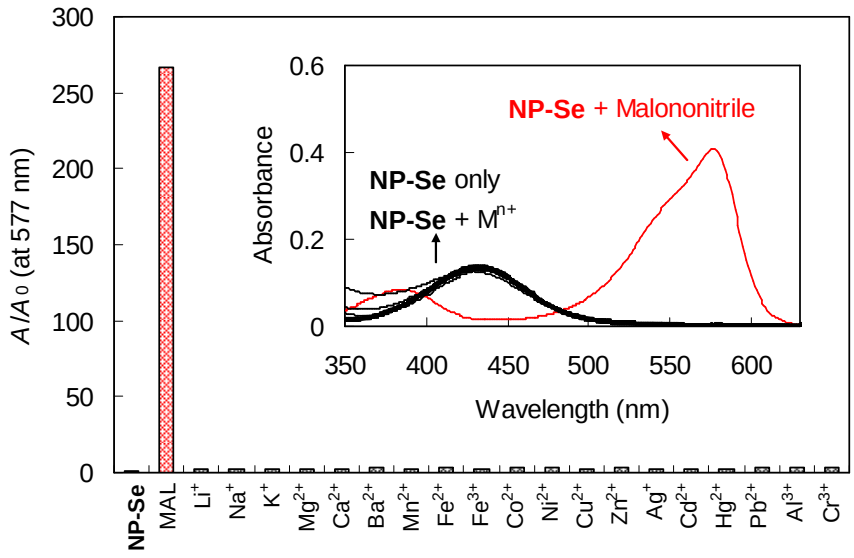


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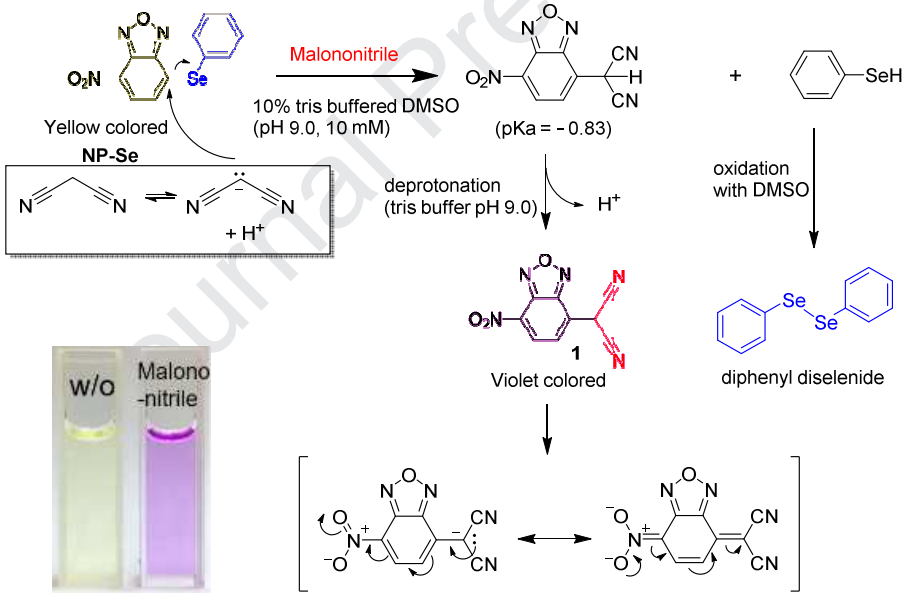
Fig. 1. Selective signaling of malononitrile by **NP-Se** compared to common metal ions as expressed by the absorbance ratio (A/A_0) at 577 nm. Inset: Changes in the absorption spectra of the probe in the presence of malononitrile or the metal ions shown in the main graph. [**NP-Se**] = 1.0×10^{-5} M, and [Malononitrile] = [M^{n+}] = 1.0×10^{-4} M in a mixture of tris buffer (pH = 9.0, 0.10 M) and DMSO (1:9, *v*/*v*). MAL = malononitrile.

The prominent colorimetric response of the **NP-Se** probe is due to the S_NAr-type reaction with malononitrile to form the strongly colored CH-acidic dye **1** (Scheme 2). The postulated conversion of **NP-Se** to dye **1** via the well-established nucleophilicity of malononitrile anions was supported by NMR and mass spectroscopy. As shown in Fig. 2, in the presence of 1 equiv of malononitrile, the ^{1}H NMR resonance ascribable to **NP-Se** disappeared completely, and only new resonances for compound **1** and diphenyl diselenide were observed. The nucleophilic substitution of the **NP-Se** probe with malononitrile produced dye **1** and benzeneselenol, which was subsequently oxidized by the DMSO in the signaling solution to diphenyl diselenide as shown in Scheme 2. It is known that DMSO works well as a mild oxidant in many organic synthetic applications [37,38]. The postulated conversion was corroborated by the

observation that the ^{1}H NMR spectra of benzeneselenol and diphenyl diselenide in deuterated phosphate-buffered DMSO-d_6, as well as the mass spectra of these compounds in DMSO solution, were nearly identical (Figs. S7 and S8, Supplementary Data). In addition, the mass spectrum of the purified reaction product also confirmed the postulated structure of compound **1** by revealing a peak at *m/z* = 228.0159 (calcd. for $C_9H_2N_5O_3^-$ [M–H]$^-$ = 228.0163; Fig. S9, Supplementary Data).



Scheme 2. Colorimetric signaling of malononitrile using the **NP-Se** probe.

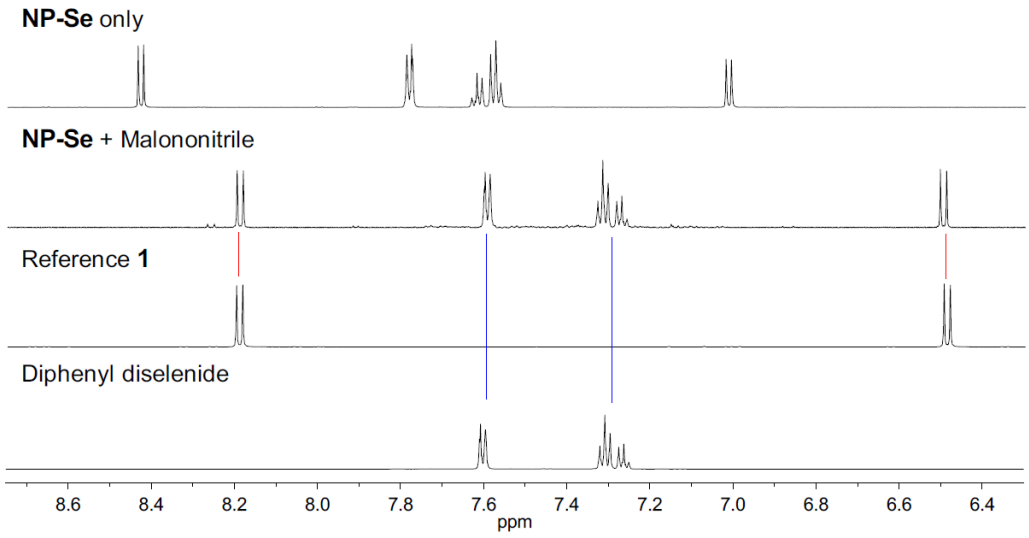


Fig 2. Partial ^{1}H NMR spectra of **NP-Se** in the presence and absence of malononitrile, reference **1**, and diphenyl diselenide in a mixture of deuterated phosphate buffer (pH 7.0) and DMSO-d_6 (1:9, *v/v*). [**NP-Se**] = [**1**] = [Diphenyl diselenide] = 1.0×10^{-2} M. The ^{1}H NMR spectrum of **NP-Se** + malononitrile was obtained by mixing **NP-Se** (10 mM) and malononitrile (10 mM) without further purification.

The violet color of the signaling product is due to the strong CH acidity of the methine proton in signaling product **1**, which is ascribable to the presence of efficient electron-withdrawing substituents of an NBD moiety and two cyano groups. Malononitrile itself is an inherently CH-acidic compound with a pK_a of 11.2 [6]. The presence of the NBD moiety, which has strong electron-withdrawing and resonance-stabilizing properties, on the malononitrile subunit would further enhance the acidity

of the resulting compound [39]. The effective stabilization of anionic structure **1** can be demonstrated using resonance structures (Scheme 2). The acid dissociation constant (pK_a) of the resulting CH-acid **1** was estimated using UV–vis spectroscopy with the Henderson–Hasselbalch equation and found to be –0.83 (Fig. 3).

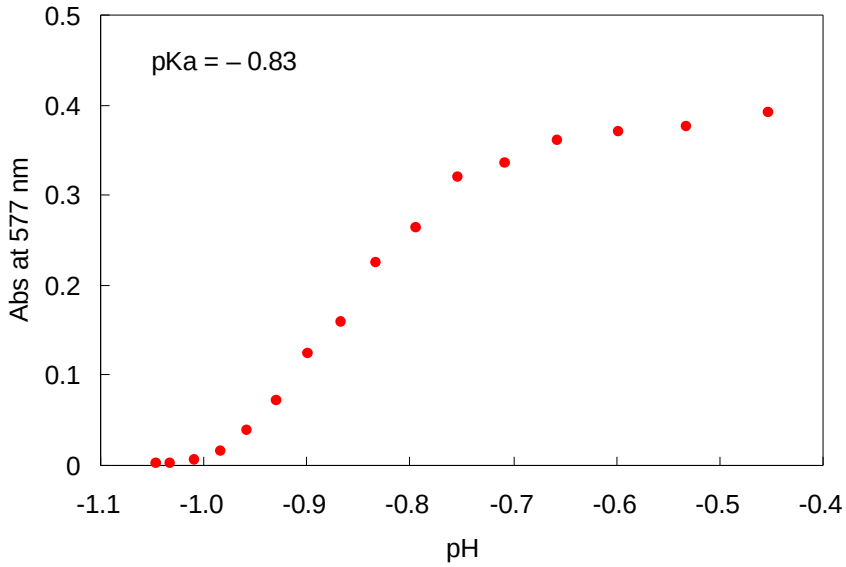
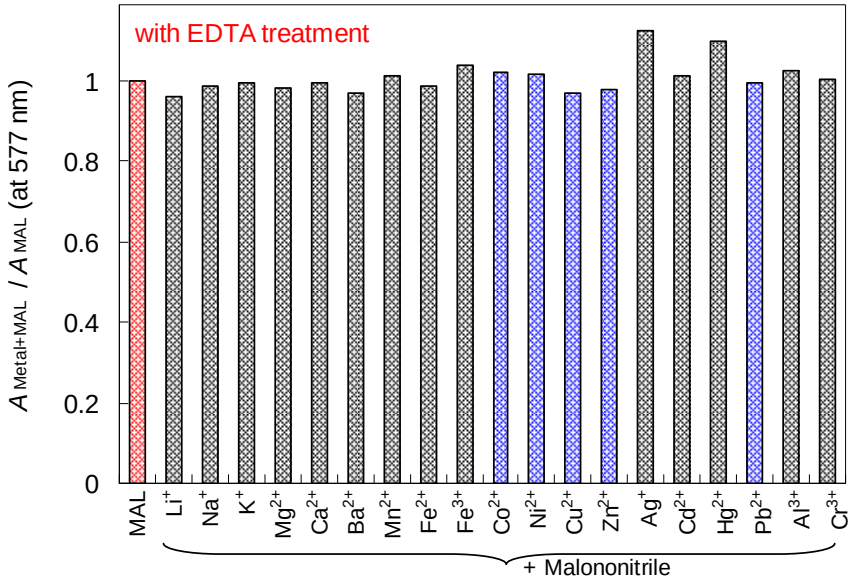


Fig 3. Changes in the absorbance at 577 nm of CH-acid dye **1** as a function of solution pH in water. [**1**] = 1.0×10^{-5} M in distilled water. The pH of the solution was adjusted with HCl solution.

Based on these promising results, we carried out further experiments to characterize the colorimetric signaling properties of **NP-Se** with regards to

malononitrile in detail. To confirm possible interference from other commonly encountered ionic species, the effect of common metal ions and anions on malononitrile signaling was investigated. We found that some metal ions, particularly the transition metal ions Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Pb^{2+}, led to an undesirable negative deviation in absorbance, possibly due to the interaction with the phenolate anion and the adjacent nitrogen ligand of the oxadiazole moiety of signaling product **1** (Fig. S10, Supplementary Data). Therefore, to expand the applicability of the probe for use in practical applications where these interfering ions might be present, we attempted to suppress unwanted changes using a chelating agent, which could sequester the interfering transition metal ions. To achieve this, after the signaling had been completed, EDTA solution (5 equiv for the analyte) was added to the signaling mixture. As a result, most of the interference from the transition metal ions was found to be effectively suppressed; the absorbance ratio $A_{Metal+MAL}/A_{MAL}$ observed at 577 nm varied between 0.96 and 1.12 (Fig. 4). On the other hand, for anions, no measurable interference was observed, with a nearly constant $A_{Anion+MAL}/A_{MAL}$ ratio (Fig. S11, Supplementary Data).



306

307 **Fig. 4.** Competitive signaling of malononitrile by **NP-Se** in the presence of common
308 metal ions in the background expressed by the absorbance ratio ($A_{Metal+MAL}/A_{MAL}$) at
309 577 nm. [**NP-Se**] = 1.0×10^{-5} M, and [Malononitrile] = [M^{n+}] = 1.0×10^{-4} M,
310 [EDTA] = 5.0×10^{-4} M in a mixture of tris buffer (pH = 9.0, 0.10 M) and DMSO
311 (1:9, *v*/*v*). All measurements were carried out after treatment of the signaling solution
312 with EDTA solution. MAL = malononitrile.

313

314 The signaling speed of **NP-Se** for malononitrile was relatively slow, with complete
315 signaling requiring about 40 min (Fig. S12, Supplementary Data). However, the
316 signaling process could be sped up by increasing the temperature of the solution. As
317 the temperature rose, the signaling speed progressively increased; at 20 °C, a

saturated signal was attained after about 40 min, which fell to about 20 min and 10 min at 30 ^{o}C and 40 ^{o}C, respectively.

Next, to determine the detection limit of **NP-Se** for malononitrile, UV–vis titration was carried out (Fig. S13, Supplementary Data). The absorbance of **NP-Se** at 577 nm steadily increased with an increase in the concentration of malononitrile, whereas the absorbance at 434 nm simultaneously decreased. The malononitrile concentration-dependent change in absorbance for **NP-Se** exhibited a linear calibration trend up to 1.0×10^{-5} M. From this plot, the detection limit of **NP-Se** for malononitrile was calculated to be 3.66×10^{-7} M according to IUPAC recommendations ($3s_{bl}/m$) [33]. The observed sensitivity of the probe for malononitrile detection was thus found to be superior to other reported analytical methods (Table S1, Supplementary Data).

Finally, the rapid and convenient detection of malononitrile with a daily-use IT device was investigated. Recently, colorimetric analysis using smartphones and scanners that do not rely on complex and/or heavy instruments has attracted significant research interest [40]. The proposed probe is particularly suitable for this purpose because the colorimetric response to malononitrile is clearly evident to the naked eye. We tested the use of an office scanner as a signal-capturing device for the rapid and convenient analysis of malononitrile. Using the red, green, and blue

channel levels of images of the signaling solution attained using a color analysis program (Adobe, Photoshop CS6), linear calibration curves were plotted as a function of the concentration of malononitrile up to 2.0×10^{-5} M, with satisfactory R^2 values (0.9814 – 0.9954; Fig. S14, Supplementary Data). Based on the most sensitive plot using the green channel, the detection limit of malononitrile measured by the scanner was calculated to be 2.64×10^{-7} M (Fig. 5).

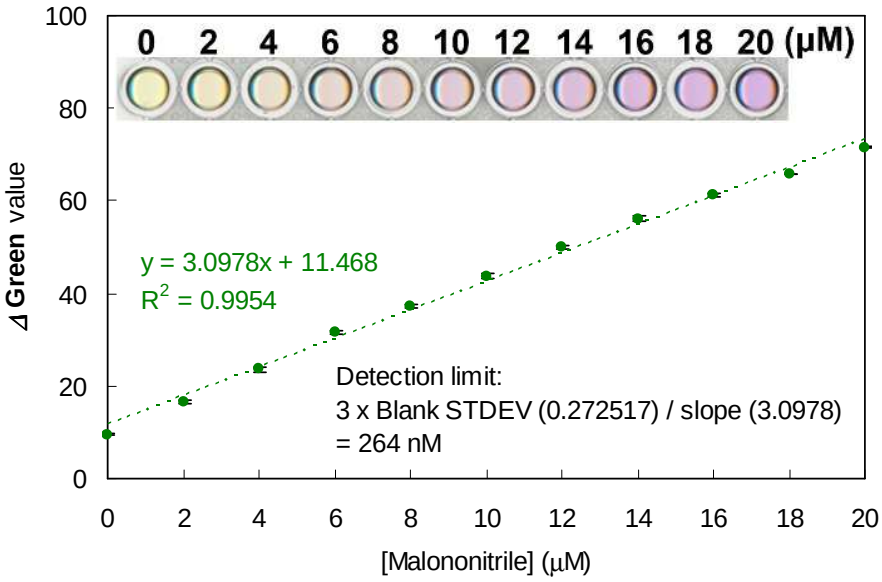


Fig. 5. Malononitrile concentration-dependent changes in ***Δ*Green** values (***Δ*Green** value = 255 – green channel level) of **NP-Se**. Inset: photographic images of the signaling solutions in the presence of varying concentrations of malononitrile. [**NP-Se**] = 1.0×10^{-5} M, and [Malononitrile] = 0 – 1.0×10^{-4} M in a mixture of tris buffer

348 (pH = 9.0, 0.10 M) and DMSO (1:9, *v*/*v*). The inset images were obtained using a
349 transmittance-type office scanner, and the error bars were estimated from three
350 independent experiments.
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355 **4. Conclusions**

356 In this study, we developed a novel reaction-based colorimetric probe for the
357 synthetically important chemical malononitrile. The selenoether-based probe
358 demonstrated a marked change in color from yellow to violet via an S_NAr-type
359 reaction with malononitrile to form a novel CH-acidic dye. Interference from sulfide
360 ions and some transition metal ions was readily suppressed using copper foil and
361 post-treatment with the chelating agent EDTA, respectively. By elevating the
362 temperature of the signaling solution, the signaling speed was noticeably enhanced.
363 Based on colorimetric behavior of the probe, which can be readily detected by the

naked eye, malononitrile monitoring and analysis can be successfully conducted using an office scanner with a detection limit of 2.64×10^{-7} M.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:**.****.

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Highlights:

- Colorimetric malononitrile probe based on NBD-phenyl selenoether was investigated.
- The probe manifested a color change from yellow to violet via an S_NAr-type reaction.
- Interferences from sulfide and transition metals were suppressed by Cu foil and EDTA.
- Colorimetric analysis of malononitrile was easily conducted with an office scanner.