Updated 09 August 2024

Functionalized Cyanostilbene-based Nano-AIEgens: Multipoint Binding Interactions for Improved Sensing of Gallic Acid in Real-Life Food Samples

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Experimental Section

General Information: All chemicals, including starting materials, reagents, and substances, were purchased from local chemical suppliers and used as received without additional purification. Solvents were distilled and dried before their use.

FTIR Spectroscopy: FTIR spectra were recorded using a Perkin-Elmer FT-IR Spectrum BX system, and the results are reported in wave numbers (cm^{-1}).

NMR Spectroscopy: ^{1}H NMR and ^{13}C NMR spectra were acquired with a Bruker Advance DRX 400 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectroscopy, respectively. Chemical shifts are reported in ppm relative to the internal standard, tetramethylsilane (TMS).

Mass Spectrometry: Mass spectra were obtained using a Micromass Q-TOF Micro TM spectrometer.

Spectroscopic Studies: UV-Vis Spectroscopy: UV-Vis spectroscopic measurements were conducted on a Shimadzu model 2100 spectrometer. The slit-width used for the experiment was set to 5 nm.

Scanning Electron Microscopy. The samples (**1,2** and **1.** GA) were made under dust-free conditions and drop-cast over double-sided tapes attached to the brass stubs. Then the stubs were air-dried for 48 h. The coatings with gold vapor were done before analyzing the samples on a Quanta 200 SEM operated at 15 kV.

Fluorescence Decay Experiment: Fluorescence lifetime values were measured by using a time-correlated single photon counting fluorimeter (Horiba Jobin Yvon). The system was excited with nano LED of Horiba - Jobin Yvon with pulse duration of 1.2 ns. Average fluorescence lifetimes (τ_{av}) for the exponential iterative fitting were calculated from the decay times (τ_i) and the relative amplitudes (a_i) using the following relation

$$\tau_{av} = (a_1\tau_1^2+a_2\tau_2^2+a_3\tau_3^2)/(a_1\tau_1+a_2\tau_2+a_3\tau_{3)}$$

Where a_1, a_2 and a_3 are the relative amplitudes and τ_1, τ_2, and τ_3 are the lifetime values, respectively. For data fitting, a DAS6 analysis software version 6.2 was used.

Dynamic Light Scattering Studies (DLS): DLS measurements were done using a Malvern Zetasizer NanoZS particle sizer (Malvern Instruments Inc., MA) instrument. Samples (compound **1**, **2,** and **1**.GA) were prepared in water and examined under dust-free conditions. Reported mean hydrodynamic diameters were obtained from Gaussian analysis of the intensity-weighted particle size distributions.

Detection limit determination: The method used for the calculation of the detection limit is known as the blank variability method. In this method, the calibration curve was prepared by recording the fluorescence spectra of compound **1** in a water medium with different amounts of Gallic acid. From the equation obtained from the calibration plot, the added GA concentrations were calculated. Then another calibration curve was drawn between the C_{real} (added GA, μM) vs. $C_{calc.}$ (Calculated amount of GA, μM). This afforded a value of the slope (b). The fluorescence of compound **1** in water without

added GA were taken as blank reading. A total 10 replicates of the blank were measured. The standard deviation from the blank readings was calculated by fitting the fluorescence reading into the equation obtained from the first calibration curve (titration spectra). Using this standard deviation value, we calculated decision limit by this following equation.

$$L_C = t_C \times s \times (1 + 1/N)1/2 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (1)$$

where, N = the number of blank replicates taken; the value of tc for 10 blank readings is 1.833; and s = the standard deviation. The detection limit (L_D) was calculated as the double of the decision limit obtained,

$$L_D = 2\ L_c \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots. \quad (2)$$

In concentration term, the detection limit appeared as,

$$x_D = 2 \times C = 2\ L_C\ /b \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (3)$$

where, b = slope of the calibration curve (C_{real} vs. C_{calc}.).

Chemical modification of paper strips: Cellulose filter papers were cut into half and immersed in a freshly prepared aqueous solution of 10% NaOH (100 mL). This mixture was left overnight for complete interactions. The cellulose samples were washed thrice with EtOH and dried. This process of chemical modification was done to break hydrogen bonds and enhance the reactivity of hydroxyl groups (Barsbay et al., 2007). The pre-treated cellulosic papers were then dipped in the developed solution of compound **1** for an hour followed by drying. Various characterization techniques such as FTIR, and FE-SEM were carried out to confirm the successful coating of compound **1** on the cellulose paper. The chemically modified strips were then exposed to different concentration of Gallic acid (0 – 120 μM) in aqueous solution. After 1 hr, the modified paper strip was removed from the aqueous solution of Gallic acid and dried. The fluorescent color changes of the strips were analysed upon irradiation with UV lamp at 365 nm.

SYNTHESIS AND CHARACTERIZATION
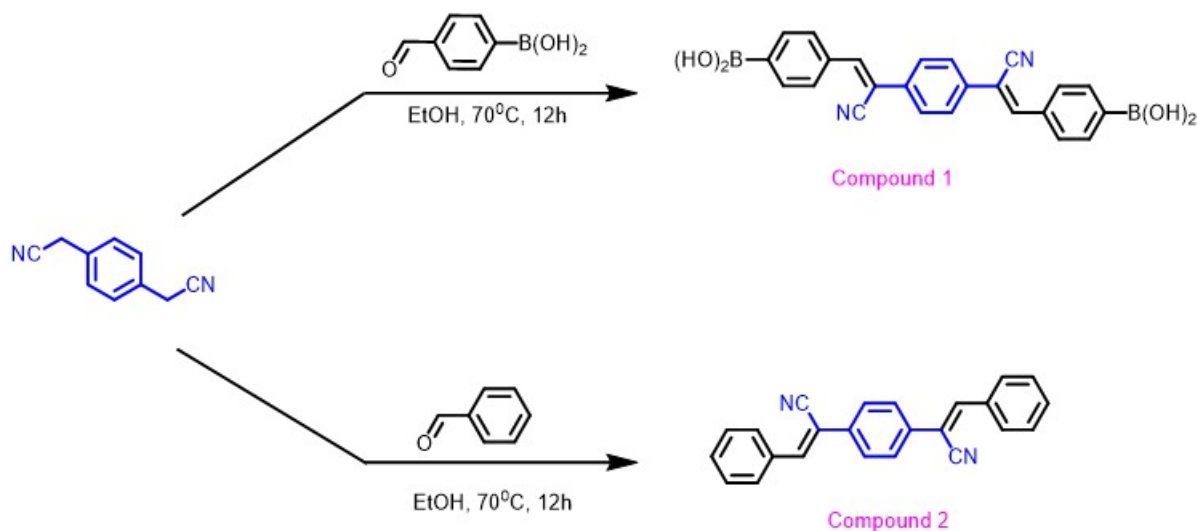
The compounds involved in the present study were synthesized following the procedure reported in the literature [11].

Synthesis of compound 1: (4-formylphenyl) boronic acid (150 mg, 0.960 mmol) and 1,4-phenylenediacetonitrile (30 mg, 0.192 mmol) were dissolved in ethanol (3 mL). A solution of NaOH (3 mg) in ethanol (3 mL) was added dropwise to the mixture under an argon atmosphere. The reaction mixture was stirred at 70^{0}C for 12 h. The product precipitates, was filtered off and washed with water to give compound **1** as a yellow powder.

 ^{1}H NMR (400 MHz, DMSO-d6) δ 10.03 (s, 2H), 8.33 (s, 4H), 7.98 (d, 4H), 7.88 – 7.84 (m, 4H), 7.38 (s, 4H). IR (KBr, cm^{-1}) 3422.2, 2223.2, 1600.2, 1395.8, 1208.7, 1013.3, 903.2. Chemical Formula: $C_{24}H_{18}B_2N_2O_4$ (M+H)$^+$: 421.14, found: 421.88.

Synthesis of compound 2: Benzaldehyde (40 mg, 0.384 mmol) and 1,4-phenylenediacetonitrile (20 mg, 0.128 mmol) were dissolved in ethanol (3 mL). A solution of NaOH (3 mg) in ethanol (3 mL) was added dropwise to the mixture under an argon atmosphere. The reaction mixture was stirred at 70^{0}C for 12 h. The product precipitates were filtered off and washed with water to give compound **2** as a brown powder.
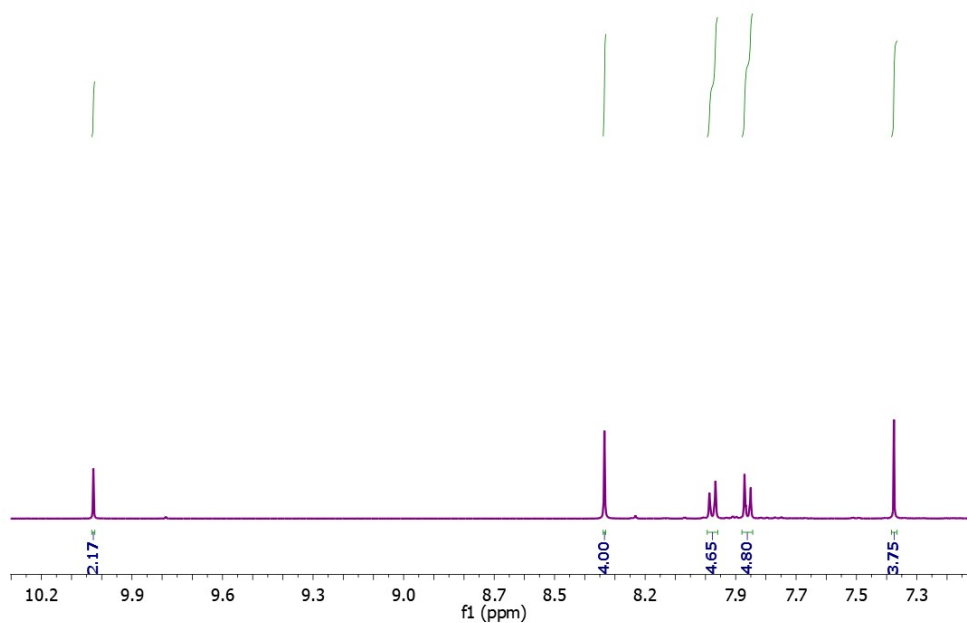
Additional Spectral Data

Figure S1. Partial ^{1}H-NMR spectra of compound **1** and in DMSO-d6 medium.

System	a1	a2	a3	T1(ns)	T2(ns)	T3(ns)	Avg Lifetime (ns)	Chi.Sq
Compound-**1**	0.29	0.06	0.65	1.75	6.08	0.49	1.73	1.12
Compound **1** + GA	0.22	0.05	0.74	1.61	5.55	0.44	1.59	1.12

Table S1: Time-Correlated Single Photon Counting (TCSPC) experiment (λ_{ex} =320 nm) of compound **1** and **1.GA** in water medium at 525 nm.