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Biosourced 3-formyl chromenyl-azo dye as Michael acceptor type of chemodosimeter for cyanide in aqueous environment

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ABSTRACT

A simple water-soluble aldehyde functionalized chromone **5** was utilized as a doubly activated Michael acceptor type of chemodosimeter for cyanide in water. The water solubility of the probe **5** is due to the incorporation of two glycerol units on the starting prepared chemodosimeter. This sensory system is able to selectively distinguish cyanide among fluoride and many other anions at micromolar concentrations and instantly detect cyanide in water at ambient temperatures with a detection limit down to 1.0 mM. Thus, the chemodosimeter **5** was applied to the quantitative determination of cyanide anion in drinking water sample (drinking water from commence).

1. Introduction

Anion recognition is an area of growing interest in supra molecular chemistry due to its important role in a wide range of environmental, clinical, chemical, and biological applications, and considerable attention has been focused on the design of artificial receptors that are able to selectively recognize and sense anion species.[1,2] Among various anions, cyanide is one of the most concerned. Although substances containing cyanide have been used as poisons for centuries, it was not until 1782 that this anion was first isolated by the Swedish chemist Scheel[3] The extreme toxicity of cyanide in physiological systems, as well as the continuing environmental concern caused by its widespread industrial use, has led to considerable research into the development of methods for cyanide detection. Due to high toxicity in certain forms of cyanide, the acceptable levels of cyanide compounds in water and soil are generally very low. For example, the drinking water maximum contaminant level for free cyanide is about 2 μM according to the World Health Organization (WHO), while the criterion for ambient water quality is 22 mg/L for cyanide in freshwater systems.[4] For this purpose, many methods for cyanide detection have been reported in the literature: the complexation of free cyanide with transition metals,[5–7] CdSe quantum dots,[8,9] and boronic acid derivatives[10–12] to generate the sensory signals. For example, fluorescent and colorimetric sensing of free cyanide based on the formation of cyanide–boron complexes were achieved with the detection limit down to physiologically lethal levels (>20 mM) in a mixture of organic solvent and water. The interaction with cyanide via hydrogen bonding has also been utilized for cyanide detection.[13,14] Owing to strong nucleophilicity of cyanide anion; chemosensors are best designed by utilizing the nucleophilic addition reaction of cyanide with electron-deficient chromophores. For example, based on the nucleophilic addition reaction between an acridinium salt and cyanide anion, a colorimetric and fluorescent chemosensor was recently reported to have a high sensitivity (e.g., 2 mM cyanide) and high selectivity among many anions including fluoride.[15] Other electron-deficient compounds have also been explored for cyanide detection, such as pyrylium salt,[16] oxazine,[17–19] triazene,[20] trifluoroacetophenone,[21,22] dipyrrole carboxamide,[23] salicylaldehyde,[24–26] squaraine,[27] Near infrared (NIR) dye,[28,29] activated coumarin[30,31] Surfactant-assisted chromogenic sensing material[32] and others.[33,34] The majority of these chemodosimeters present many advantages, such as high sensitivity, low cost, easy detection, and suitability as a diagnostic tool but in organic solvent. There are, however, a few chemical probes that are operating in water and show both the colorimetric and fluorescence changes upon the complexation of cyanide anions. Recently, we are developed a new class of water soluble dyes as dyeing agents in water.[35–39] The water solubility was obtained by incorporation of the biosourced water soluble molecules such carbohydrates on the starting organic dyes such

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lactose or with its monosaccharidic components, glucose or galactose. In this regard, we used this water solubility strategy to develop a glycoconjugated chemodosimeter for cyanide in pure water by the naked eye[40–42] or also by the incorporation of the starting chemodosimeter on poly vinyl alcohol as water soluble polymer.[43] Motivated by this approach for a cyanide probe, we have developed and report herein our new water soluble cyanide chemodosimeter **5** based on the 3-chromone aldehyde as a Michael acceptor type of cyanide and its water solubility has been given by the incorporation of two glycerol units on the starting azo formyl chromenyl azo dye (Fig. 1).

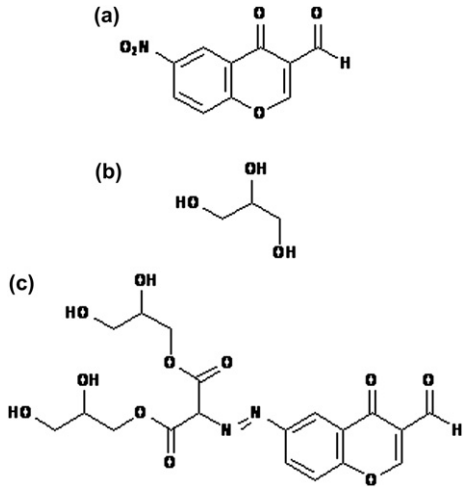


Fig. 1. General structure of chemosensor **5** and its starting materials (a) 3-formyl chromone derivative (b) glycerol (c) glycerylated 3-formyl chromenyl-azo dye **5**.

2. Results and discussion

2.1. Chemosensor conception

Our approach to design the chemosensor consists in utilizing the strong nucleophilicity of cyanide anion in pure water toward a chromophore having an adequate cyanide receptor. For a given chromophore having an electron acceptor that can react with a nucleophile, the stronger the acceptor the more reactive the chomophore will be toward the nucleophilic anions. Although the sensitivity is high, the selectivity could be low as many nucleophilic anions in addition to cyanide anion, such as carbonate, acetate, and phosphate, would also react. On the other hand, a chromophore with a weaker acceptor has a lower reactivity toward nucleophilic anions but could be more selective. Therefore, considering a possible trade-off between the selectivity and sensitivity, our strategy for rational design of a chemosensor system is to use the doubly activated 3-chromone aldehyde as Michael acceptor type of water soluble chromophore, and it is known that the nucleophilic character of different species is solvent dependent and that protic solvents decrease the anion's nucleophilicity by hydrogen bonding to the nucleophile's lone pair (solvation effects). Therefore, the cyanide detection will be carried out in water, the suitable and real medium for testing chromo- sensing probes for anions of environmental interest. The insertion of the glycerol on the final chemodosimeter affords the dye **5** with interesting water solubility at room temperature (Fig. 2).

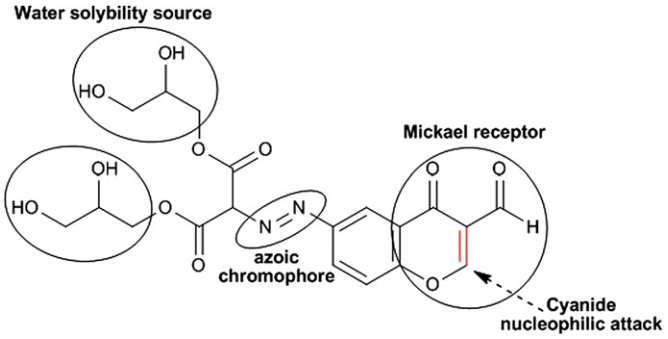
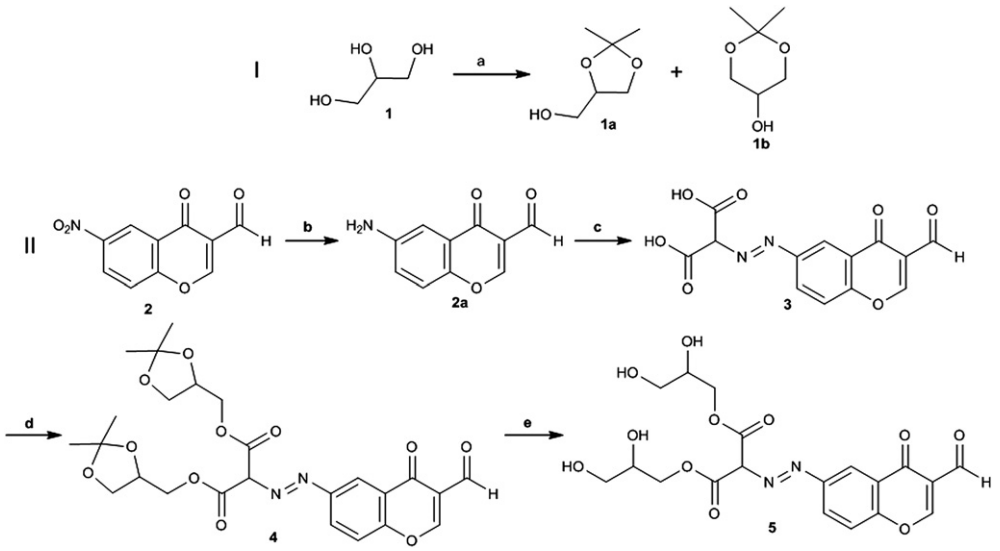


Fig. 2. General structure of cyanide chemodosimeter **5**.

2.2. Chemodosimiter synthesis

Scheme 1 reports the synthesis of the azoic dye **5** possessing 3-formyl chromenyl group as cyanide receptor. The 6-nitro-4-oxo-4*H*-chromene-3-carbaldehyde **2** was treated by S_nCl_2 In methanol at 70 °C to reduce the nitro group to its corresponding amine derivative **2a** in 92% as yield. The synthesis of the dye **3** was carried out by treating the derivative **2a** with sodium nitrite in acidic medium (15 mL of HCl 1 N solution) at 0 °C to form the corresponding diazonium salt, followed by treatment with the diethyl malonate di-ester sodium salt to yield the di-estereal dye **3a** in 89%.



Scheme 1. Synthesis of chemosensor **5**. Reagents and conditions; (a) Ref. 21; (b) S_nCl_2, MeOH, 70 °C; (c) $NaNO_2$, HCl/H_2O, 0 °C to rt, diethyl malonate; (d) KOH, water/THF, rt; (e) **1a**, DMTMM, NMM, THF, rt; (f) TFA, rt.

This step was followed by saponification with 2 equiv of potassium hydroxide in a water/THF mixture to provide the corresponding di-acid **3b** in quantitative yield. To obtain the desirated water soluble chemodosimeter **5**, the protected glycerol derivative **1a** was prepared as reported in the literature (Scheme 1).[44] Then, the coupling of the dye **3b** and 2 equiv of the derivative **1a** in THF as solvent and in the presence of the DMTMM[45] as activist and the NMM as a base afford the protected di-glycerylated chemodisimeter **4** in 84% as yield. The derivative **4** was eventually deprotected in 90% TFA to provide **5** in quantitative yield.

The structure of **5** was confirmed by mass spectroscopy, ^{13}C NMR, and. ^{1}H NMR data (Fig. 3).

not exhibit any significant shift after the double glycerylation with the two glycerol units. Also, molar extinction coefficient values can be considered almost constant, only very small differences being detected (Table 1).

Table 1
UV/vis absorption spectra of the dyes **3b** and of their glycerylated derivatives **4** and **5**.

Chemosensors	λ_{max} (DMSO) [nm]	Log (ε_{max}/M−1 cm^{-1})
3a	371	4.5678
4	371	4.5661
5	370	4.5666

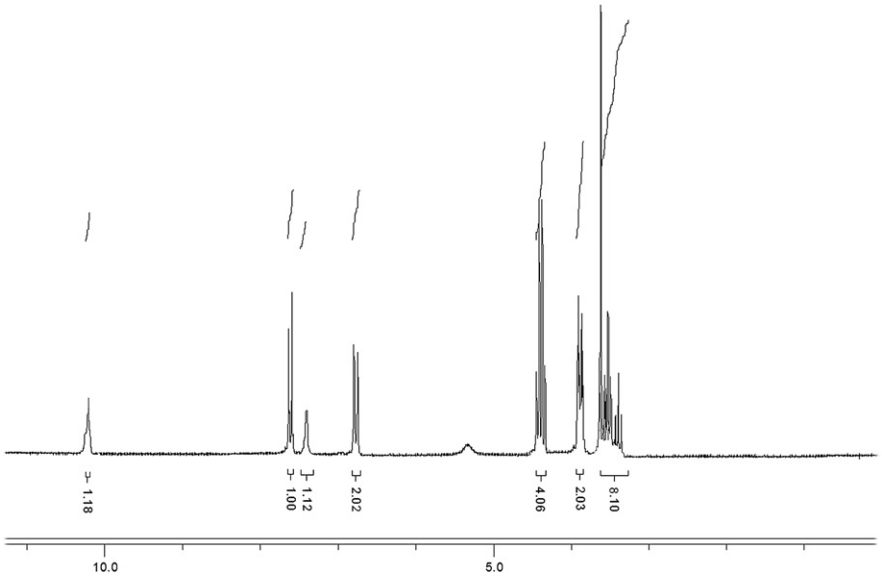


Fig. 3. ^{1}H NMR spectrum of chemosensor **5**.

2.3. UV/vis spectra studies

UV/vis absorption spectra of the newly generated glycerylated chemosensor is crucial because the modified chemosensor **5** should display the same color as the starting materials if the chromophore is not affected by the glycerylation process, as in dyes **3b**, whereas the di-acid groups are used to attach two glycerol units. The UV/vis spectra of the starting chemosensor **3b**, its protected glycerol derivative **4** and the deprotected glycerol chemosensor **5** in DMSO are shown in Fig. 4. The results reported in Fig. 3 show that λ_{max} does

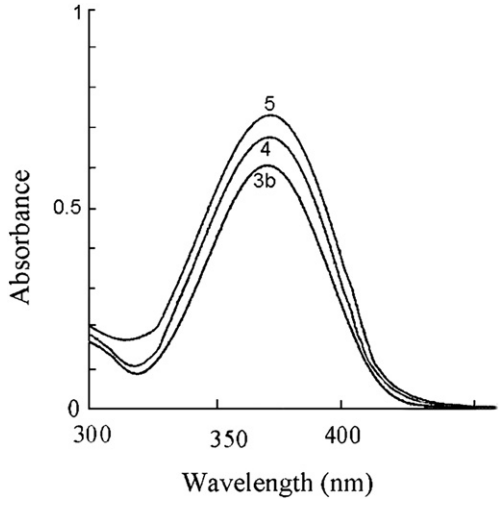


Fig. 4. UV/vis absorption spectra of the dyes **3b**, **4**, and **5** in DMSO.

2.4. Interaction of 3b with anions^{-} in a biphasic system

The interaction of receptor **3b** with different anion (CN^-, F^-, Cl^-, Br^-, I^-, N_3^-, $H_2PO_4^-$, HSO_4^-, and AcO^-) was investigated in DMSO solution through spectrophotometric titration experiments. In particular, a standard solution of anions as its metallic salts (10 equiv) was added stepwise to 100 μM solution of **3b** at 25 °C. Upon addition of anions, a red/orange solution of **3b** was observed with only CN^-, F^-, $H_2PO_4^-$, HSO_4^-, and AcO^-. Fig. 5 shows the UV/

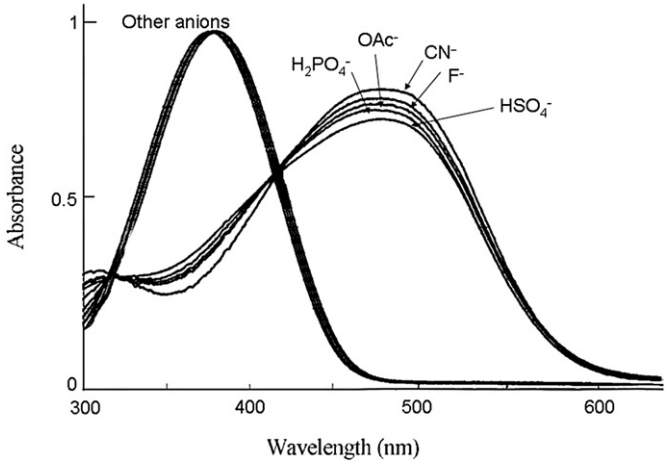


Fig. 5. UV/vis spectra of **3b** (10^{-5}M) in DMSO upon the addition of 10 equiv of anions (CN^-, F^-, Cl^-, Br^-, I^-, N_3^-, S^{2-}, $H_2PO_4^-$, HSO_4^-, AcO^-, and CO_3^{2-} as metallic salt) at pH=7.

vis spectra taken during the titration. Upon addition of anions (CN^-, F^-, $H_2PO_4^-$, HSO_4^-, and AcO^-), the band at 377 nm progressively decreases, while a new band with a peak at 483 nm forms and develops.

The cyanide chemosensor **3b** presents a poor selectivity toward CN^- in DMSO. In order to improve the cyanide selectivity of **3b**, the response of **3b** toward CN^-, F^-, $H_2PO_4^-$, HSO_4^-, and AcO^- have been investigated in aqueous solution of DMSO by increasing the amount of water in DMSO as reported in Fig. 6, we have found that when 2.5% water in water/DMSO mixture was chosen as solvent, only CN^-, F^-, and AcO^- showed obvious interaction with the sensor **3b**. It is not surprising considering the solvent effect. Namely, anions, such as F^-, AcO^-, $H_2PO_4^-$, and HSO_4^- should interact with water through hydrogen-bonding leading to a large decrease in their nucleophilicity. While cyanide has weaker hydrogen-bonding ability and stronger nucleophilicity toward the chromone aldehyde Mickeal receptor. Our thought was confirmed with further water increase. Water (5.0% vol) resulted in selective CN^- and F^- response. When 10.0% vol of water in DMSO was used as solvent, the selectivity of CN^- to F^- became higher with 1 equiv of anions F^- and CN^-, but when F^- was in large excess, the response of **3b** toward F^- was observed (Fig. 6).

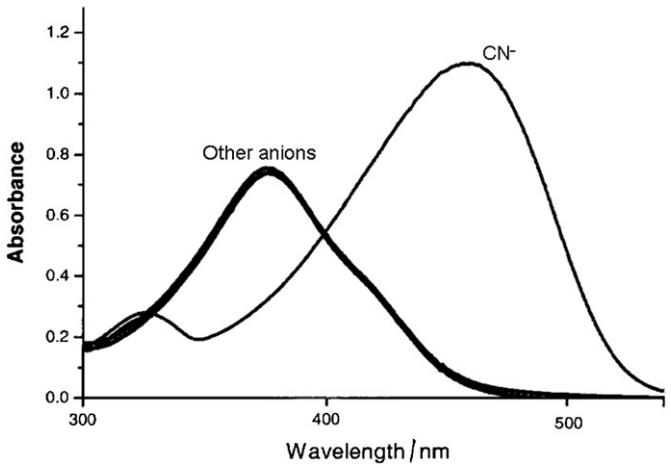


Fig. 7. UV/vis spectra of **5** (100 μM) in water upon the addition of 1 equiv of CN^- and 100 equiv of anions (F^-, Cl^-, Br^-, I^-, N_3^-, S^{2-}, $H_2PO_4^-$, HSO_4^-, AcO^-, and CO_3^{2-} as metal salt) at pH=7.

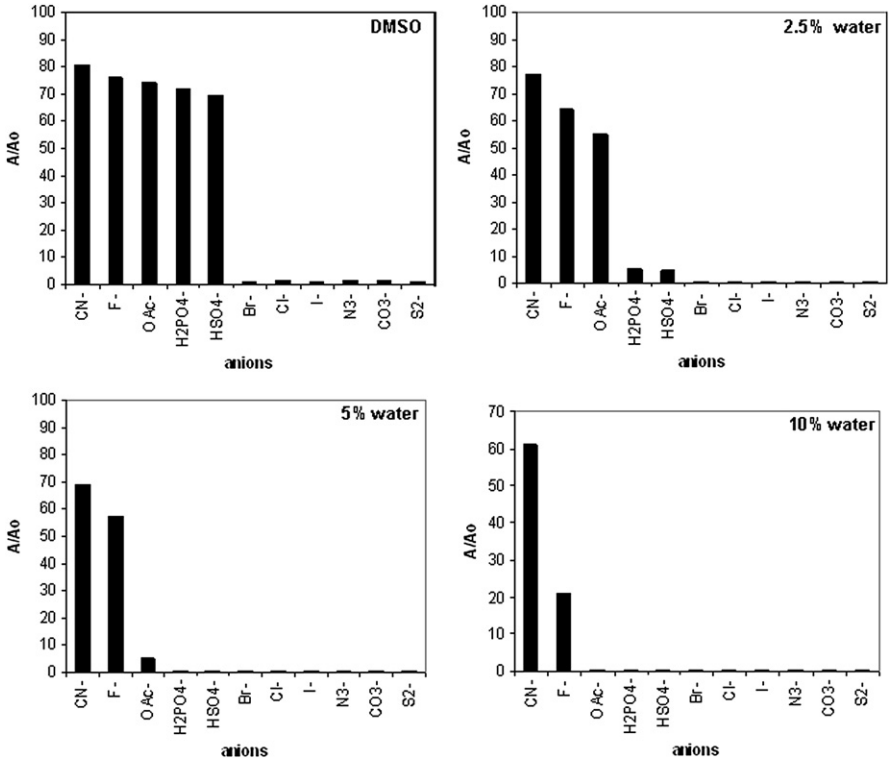


Fig. 6. Comparison of the percentage A/A_0 decrease of absorbance of **3b** (100 μM) in DMSO/H_2O (v/v) mixture at 483 nm in the presence of 10.0 equiv of anions at pH=7.

To overcome the cyanide selectivity limitation of **3b** and to develop a chemodosimeter able to detect easily and only CN^- in pure water, a series of titrations using UV/vis spectroscopy will be carried out on chromogenic probe **5** in pure water. When cyanide ions were added to 100 μM of **5** (1 equiv in pure water), we have found the appearance of a new absorbance at 490 nm and the disappearance of the starting absorbance bond at 380 nm. However, other anions, such as F^-, Cl^-, Br^-, I^-, N_3^-, $H_2PO_4^-$, HSO_4^-, CO_3^{2-}, and AcO^- did not cause any significant changes in their absorbance bond, even in the presence of 100 equiv of guests (Fig. 7). These spectral changes could be observed by the naked eye. The light orange color of chemosensor **5** in water changed into pink as soon as cyanide ions were added.

In order to get an insight into the reaction, we investigated a thin layer chromatogram of **5** by adding cyanide and compared it with that of the probe itself. Upon the addition of the cyanide to **5**, a new spot on the TLC plate appeared and the reaction was almost complete within 0.5 h. The ^1H NMR spectral analysis showed that an aromatic proton at 7.51 ppm of **5** was highly up field shifted to 4.59 ppm, while the other aromatic protons and an aldehyde proton are slightly up field shifted (Fig. 8).

These ^1H NMR spectra indicate that the cyanide anion was added to the aromatic region rather than the aldehyde group of **5** via a simple addition reaction of the cyanide ion to **5**. Due to the electron-withdrawing aldehyde group, **5** can play the role of a good Michael acceptor. Cyanide will be added to the β-position of the

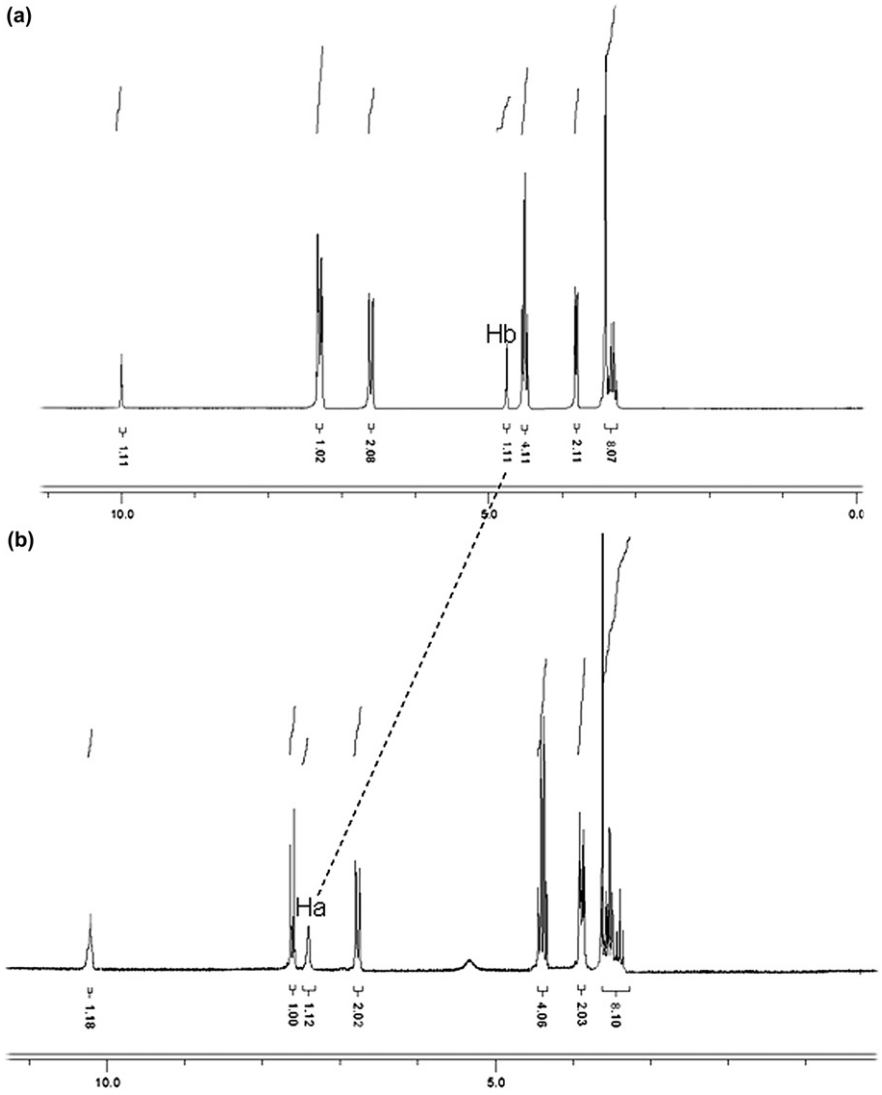


Fig. 8. ^{1}H NMR spectra of (a) chromophore **5** and (b) an equimolar mixture of chromophore **5** and CN^{-} in water.

aldehyde group, where it is doubly activated by both the aldehyde and the chromenyl carbonyl group. The Michael addition to the chromenyl aromatic group will lead to a bathochromic shift in the UV–vis spectra of the chemosensor **5** (Fig. 9). This suggestion was confirmed by the measurement of the mass data of the adduct **5**–**CN** (m/z=518.18 $[M+1]^{+}$).

meant that the enhanced absorbance should originate from the interaction of cyanide anion with its receptor on the chemosensor **5**.

To evaluate the detection limit[46] of cyanide anions in aqueous solution, we measured the absorbance changes by increasing the amount of cyanides (from 0 to 1 equiv). The absorbance changes of

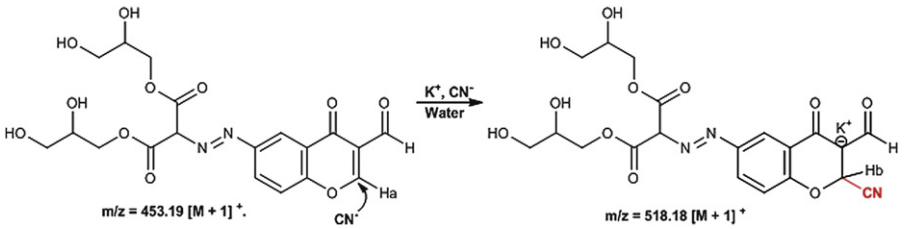


Fig. 9. Proposed reaction mechanism of **5** toward CN^{-} as KCN salt.

The Fig. 10 reports the absorbance recovery of the water soluble chemosensor **5** (concentration about 100 μM) in the presence of increasing amounts of cyanide. As shown, the absorbance was enhanced gradually with increasing the cyanide concentration from 0 to 3 equiv. This control experiment indicated no distinguishable effect of CN^{-} to the absorbance of **5** only, which the chemosensor **5** at λ_{max}=490 nm were monitored using 10^{-5} M of **5** in pure water. The absorbance intensity of **5** was completely quenched in the presence of 1 equiv of cyanide. To determine the binding stoichiometry, a Job's plot of $(A-A_0)$ versus equivalent of the cyanide ion was established. Fig. 10 shows a near linear correlation between intensity difference absorption $(A-A_0)$ and CN^{-}

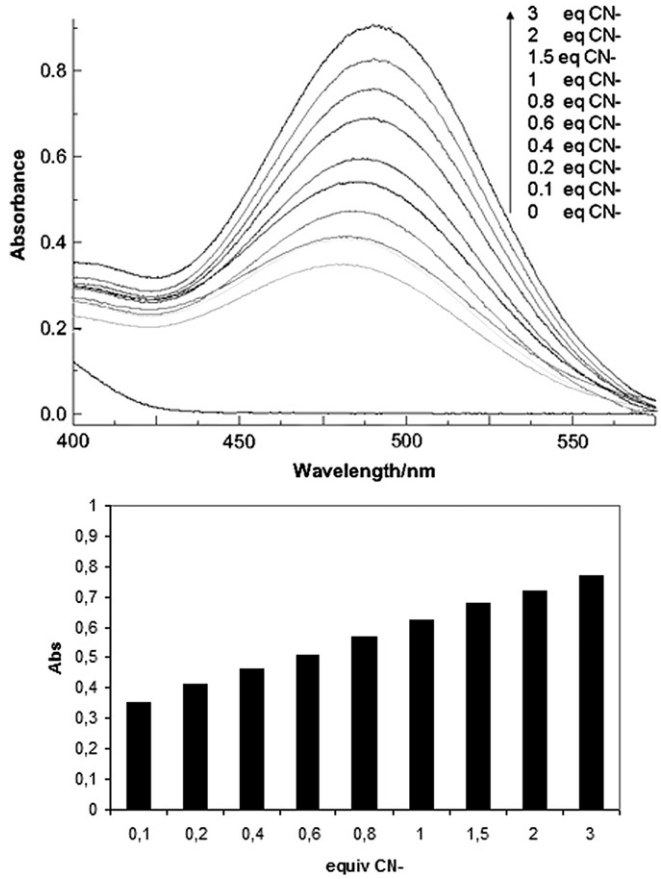


Fig. 10. UV–vis titration of chemosensor **5** (10^{-5} M) with CN^- (100 μM, 0–3.0 equiv) in water at 25 °C.

concentration in water at room temperature, which indicates one to one binding between the probe and cyanide (Fig. 11).

The detection limit was calculated to be 0.276 μM (see Supplementary data). According to the World Health Organization (WHO), cyanide concentrations lower than 1.9 μM are acceptable in drinking water,[4] which meant that our water soluble chemosensor **5** based colorimetric method was sensitive enough to monitor cyanide concentration in drinking water.

To verify the sensibility of our chromogenic probe **5**, a solution of cyanide anion (C=0.1 μM) was prepared in pure water, and analyzed by the chemosensor **5**, we have found a very clear response

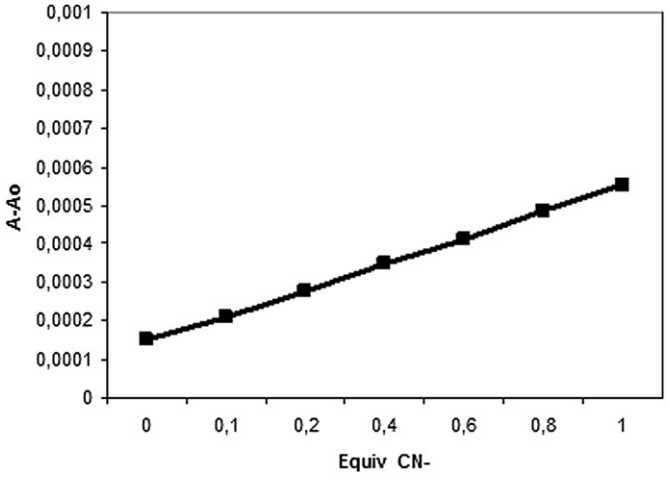


Fig. 11. The plot of $A-A_0$ versus equivalent of [CN^-] for the titration of **5** (100 μM) with CN^- (0–1 equiv) in water at 25 °C.

of **5** toward the cyanide anion at this concentration by color change of the starting prepared cyanide solution from the yellow to pink. Then the reproducibility of the chemodosimeter **5** was evaluated. The Table 2 reports the relative standard deviation for four repeated measurements of 0.1 μM, 3 μM and 50 μM of cyanide in pure water. These results illustrate that the response of chemosensor **5** toward cyanide was highly reproducible, which suggested the accuracy and reliability of the present method for cyanide determination in practical applications.

Table 2
The reproducibility of chemosensor **5**

[CN^-] μM	Test 1	Test 2	Test 3	Test 4	Error %
0.1	0.12	0.10	0.098	0.11	8.11
3	2.88	3.05	2.94	3.08	**7.75**
50	51.11	49.93	49.88	50.17	4.16

To test the anionic selectivity of the cyanide response on the Chromogenic probe **5**, we investigated the absorbance changes in the presence of other common anions, such as BrO_3^-, Cl^-, ClO_4^-, SCN^-, $C_2O_4^{2-}$, IO_3^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_3^{2-}, SO_4^{2-}, and $S_2O_8^{2-}$. As shown in Fig. 12, with the identical concentration as cyanide, there was nearly no variation on the absorbance of **5** by the addition of most anions. Thus the selectivity profile of the present system for cyanide was high.

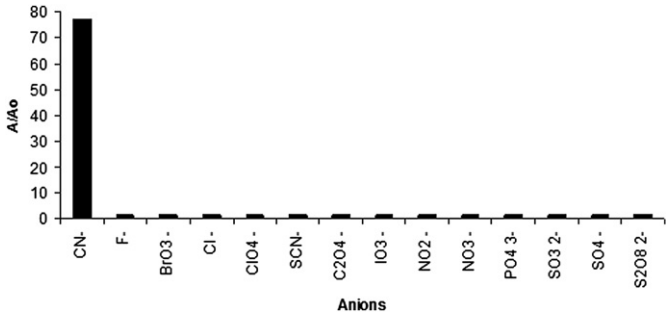


Fig. 12. The absorbance response of the solution containing chemosensor **5** in pure water (100 μM) at room temperature in the presence of 3×10^{-4} M various anions.

The potential interferences of common ions on the cyanide detection were then evaluated. The results showed that the coexistence of BrO_3^-, Cl^-, ClO_4^-, SCN^-, $C_2O_4^{2-}$, IO_3^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_3^{2-}, SO_4^{2-}, $S_2O_8^{2-}$, and F^- with concentrations up to 1 mM in pure water did not the affect the detection of 10^{-5} M cyanide solution.

As reported above, the equimolar cyanide addition into the chromogenic probe **5** causes the complete disappearance of the earlier mentioned band at 380 nm and the appearance of the new band at 490 nm. It is important to evaluate the kinetic of adduct **5**–**CN**$^-$ formation. For this purpose, 100 μM of **5** was treated by 1 equiv of CN^-, an immediate color change was observed and the absorbance of the adduct **5**–**CN**$^-$ was measured immediately to be 0.764. After 30 s and 30 min, no change in the absorbance value was observed. Therefore, the reaction between the chromogenic probe **5** and the cyanide anion is total and instant reaction.

3. Conclusion

We have developed a new generation of water soluble chromogenic prob for cyanide detection based on formyl chromenyl as Michael cyanide receptor, and two units of clycerol as a source of the water solubility. This chromogenic probe presents a very high selectivity toward the cyanide anions in pure water with a detection limit down to 1.0 μM. Thus, this analytical colorimetric

method can be applied to the quantitative and qualitative determination of cyanide anion in drinking water sample with high precision.

4. Experimental

4.1. Materials and instrumentation

All chemicals and solvents obtained from commercial sources were analytical pure and used without further purification. TLC was carried out on silica gel pre-coated plates (Merck; 60 Å F_{254}) and spots located with (a) UV light (254 and 366 nm), (b) ninhydrin (solution in acetone), (c) fluorescamine, (d) I_2 or (e) a basic solution of permanganate [$KMnO_4$ (3 g), K_2CO_3 (20 g), and NaOH (0.25 g) in water (300 mL)]. Flash column chromatography (FCC) was carried out on Merck silica gel 60 (230–400 mesh) according to Still et al.[47] ^1H and ^{13}C NMR spectra were recorded at 200 MHz with Varian spectrometers in deuterated solvents and are reported in parts per million (ppm) with the solvent resonance used as the internal reference. Mass spectra were recorded with a Thermo Fisher LCQ fleet ion-trap instrument (the spectra exported were also using the ESI $+c$ 85 technique). Elemental analysis was carried out with a Perkin–Elmer 240C Elemental Analyzer. UV/vis spectra were recorded with a Cary-4000 Varian spectrophotometer.

4.2. Detection procedure of cyanide in water samples

Water samples were obtained locally from the tap water in our laboratory. Since the water samples were found to be free from cyanide, known amounts of Bu_4NCN salt were spiked into the samples. An aliquot of KCN-spiked water sample was added into the solution containing the chromogenic probe in pure water at room temperature and then incubated for 10 min before spectral measurements. Each sample was repeated three times.

4.3. Synthesis and characterization

*4.3.1. 6-Amino-4-oxo-4H-chromene-3-carbaldehyde (**2**).* To a solution of the **2** (1 mol) in methanol (10 ml), $SnCl_2$ (2 mol)] was added and the resulting mixture was stirred under reflux. After the completion of the reaction (monitored by TLC), the reaction mixture was filtered through Celite. The filtrate was evaporated under vacuum and the residue was taken into chloroform, washed twice with 80% saturated brine solution and finally with water. The organic layer was dried over anhydrous sodium sulfate and evaporation of the organic layer was followed by purification by column chromatography (AcOEt/PE: 10/3, R_f=0.48) to yield **2a** in 84% as yield. ^1H NMR (200 MHz, $CDCl_3$): δ=10.35 (s, 1H), 7.56 (s, 1H), 6.89–6.79 (m, 3H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=187.3, 177.4, 170.6, 147.8, 143.5, 127.9, 124.6, 123.8, 114.4, 113.6 ppm. MS (ESI): m/z=190.13 $[M+1]^+$. $C_{10}H_7NO_3$ (189.04): calcd C, 63.49; H, 3.73, found, C, 63.53; H, 3.77.

*4.3.2. 2-(3-Formyl-4-oxo-4H-chromen-6-ylazo)-malonic acid diethyl ester (**3a**).* To a solution of **2a** (1 mol) in 15 mL of water/HCl 37%: 15/1.5, a solution of $NaNO_2$ (2.4 mol) in water (2 mL) was added drop wise at 0 C° and the resulting mixture was stirred for 30 min. The diazonium salt solution previously prepared was added drop wise to the solution of diethyl malonate (2 mmol) in methanol (3 ml) and the combined solution was maintained at 0 C° for 6 h with stirring. After this time the resulting mixture was diluted with petroleum ether (20 ml) and water (40 ml) and the product formed was isolated by filtration. The organic layer was diluted with chloroform, washed with water and dried with anhydrous MgSO4. The dried solution was evaporated to afford the corresponding azo dyes **3a** in 89% as yield. ^1H NMR (200 MHz, $CDCl_3$): δ=10.36 (s, 1H), 7.66 (s, 1H), 7.76 (m, 1H), 6.88–6.79 (m, 2H), 4.24–4.19 (q, J=7.8 Hz, 4H), 1.27–1.21 (t, J=7.7 Hz, 6H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=187.5, 177.6, 170.4, 163.5, 147.5, 137.5, 124.5, 123.4, 122.9, 120.1, 114.5, 113.6, 61.5, 13.6 ppm. MS (ESI): m/z=190.13 $[M+1]^+$. $C_{17}H_{16}N_2O_7$ (360,10): calcd C, 56.67; H, 4.48, found, C, 56.71; H, 4.52.

*4.3.3. 2-(3-Formyl-4-oxo-4H-chromen-6-ylazo)-malonic acid (**3b**).* To a solution of **3a** (1 mol) in THF (10 mL), an aqueous solution of KOH 2 N (10 mL) was added and the resulting mixture was stirred at room temperature for 2 h. The crud was diluted with water (20 mL) and extracted with dichloromethane (3×20 mL), the organic solutions were collected, dried over Na_2SO_4 and concentrated under reduce pressure to afford **3b** in 97% as yield. ^1H NMR (200 MHz, $CDCl_3$): δ=10.35 (s, 1H), 7.65 (s, 1H), 7.77 (m, 1H), 6.88–6.77 (m, 2H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=187.6, 177.7, 170.3, 163.7, 147.8, 137.4, 124.7, 123.7, 122.8, 120.5, 114.7, 113.4 ppm. MS (ESI): m/z=305.17 $[M+1]^+$. $C_{13}H_8N_2O_7$ (304.03): calcd C, 51.33; H, 2.65, found, C, 51.38; H, 2.619.

*4.3.4. 2-(3-Formyl-4-oxo-4H-chromen-6-ylazo)-malonic acid bis-(2,2-dimethyl-[1,3] dioxolan-4-ylmethyl) ester (**4**).* *N*-Methyl morpholine (2 mol) was added to a solution of **4** (1 mol) in THF (15 mL), and the mixture stirred at room temperature for 5 min. The resulting solution was cooled to 0 °C, DMTMM (2.4 mol) was added, and the mixture was again stirred at room temperature for 2 h. At this time TLC (dichloromethane/methanol: 10:0.2) indicated the formation of the activated intermediate (R_f 0.89) and the disappearance of the starting acid (R_f 0.1). protected glycerol derivative **1b** (2 mol) was added, and the reaction mixture was left under stirring at room temperature for 12 h. TLC showed the formation of one major spot at (R_f 0.53, dichloromethane/methanol: 10:0.20). The reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in chloroform (20 mL) and washed with a solution of HCl 5% (20 mL) and water (3×20 mL). The organic solution was dried over Na_2SO_4 and filtered, the filtrate was concentrated under the reduce pressure and the residue was purified by Flash chromatography (dichloromethane/methanol: 10:0.20) to afford **4** in 71% as yield. ^1H NMR (200 MHz, $CDCl_3$): δ=10.35 (s, 1H), 7.64 (s, 1H), 7.76 (m, 1H), 6.89–6.77 (m, 2H), 4.47 (m, 2H), 4.41–4.37 (m, 4H), 3.97–3.76 (m, 4H), 1.28 (s, 6H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=187.4, 177.5, 170.3, 163.8, 147.5, 137.6, 124.7, 123.5, 122.5, 120.4, 118.6, 114.6, 113.5, 75.6, 67.5, 63.6, 26.4 ppm. MS (ESI): m/z=533.21 $[M+1]^+$. $C_{25}H_{28}N_2O_{11}$ (532.17): calcd C, 56.39; H, 5.30, found, C, 56.43; H, 5.35.

*4.3.5. 2,3-Dihydroxypropyl 2-((3-formyl-4-oxo-4H-chromen-6-yl) diazenyl) malonate (**5**).* A solution of **4** (1 mol) in 90% aqueous CF_3COOH (15 mL) was stirred at room temperature over night. The TLC (dichloromethane/methanol: 10:0.20) indicates that the starting material (R_f 0.55) was completely reacted and the formation of the deprotected compound **5** (R_f 0.26) had taken place. The solvent was evaporated to dryness and repeatedly co-evaporated with toluene (5×25 mL) at reduced pressure to give the final product **5** in 99% as a yield. ^1H NMR (200 MHz, $CDCl_3$): δ=10.36 (s, 1H), 7.65 (s, 1H), 7.74 (m, 1H), 6.89–6.78 (m, 2H), 4.40–4.31 (m, 4H), 3.97 (m, 2H), 3.88–3.67 (m, 4H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ=187.5, 177.5, 170.7, 163.9, 147.7, 137.8, 124.6, 123.4, 122.8, 120.3, 118.6, 114.7, 113.57, 70.5, 67.4, 63.8 ppm. MS (ESI): m/z=453.19 $[M+1]^+$. $C_{19}H_{20}N_2O_{11}$ (452.11): calcd C, 50.45; H, 4.46, found, C, 50.49; H, 4.51.

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Supplementary data

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