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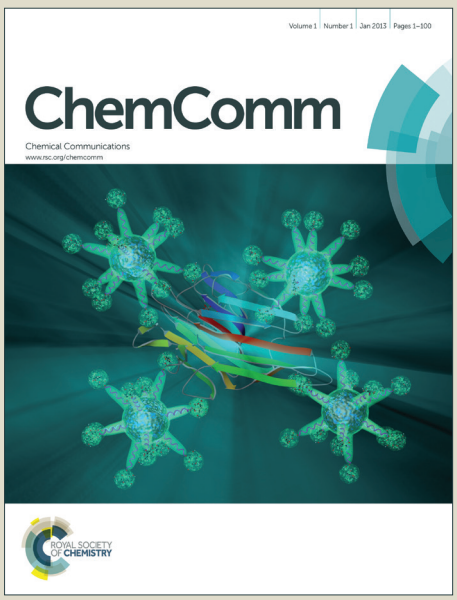

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ARTICLE TYPE

A highly selective phenothiazine-based fluorescence 'turn-on' indicator based on cyanide-promoted novel protection/deprotection mechanism†

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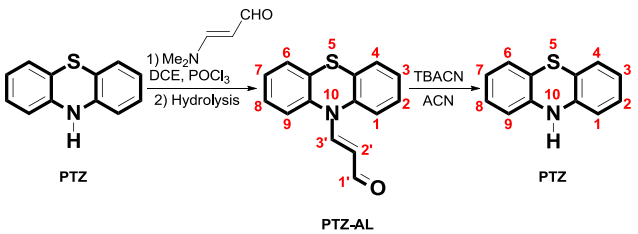
A cyanide anion (CN^-)-triggered deprotection of NH-protected phenothiazine, (*E*)-10-(*10H*-phenothiazin-3'-yl)propenal, has been discovered as a novel mechanism for the highly selective fluorescence detection of CN^- under ambient conditions. The present protocol may pave the way for its broad applications in organic synthesis in near future.

Cyanide anion (CN^-) is highly toxic to living organisms that damage by absorption through the lungs, gastrointestinal track, or skin and can kill mammals upon binding to a heme unit.[1] Despite this, the industrial use of mass quantities of cyanide is one of the most routine operations in many chemical processes, essential to a healthy economy, including gold mining, electroplating, and plastic production.[2] Nevertheless, this mechanization or industrialization, certainly, has resulted 'an unsweet smell' of growing threat of cyanide exposure into the environment. The third world is particularly at risk, because the flooding can result in a rapid rise in the concentration of cyanide in drinking water. Besides, the cyanide has also been used as a chemical warfare reagent and even as a terror material.[3] The importance of both these areas to health and security has made a cogent need for the development of cyanide-selective sensors or indicators.

Traditionally, hydrogen bonding or supramolecular interactions have been used for the design of CN^- sensors.[4] These approaches, however, usually display poor selectivities over other common anions. The shift towards an emphasis on selectivity issue has resulted in extensive research efforts, particularly, in recent years, devoted to the development of reaction-based indicators that take advantage of nucleophilic property of CN^- to effect chemical changes in a chemodosimetric fashion.[5] In this milieu, endeavours have been made using C-B bond formation,[6] C-S bond formation,[7] and predominantly C-C bond forming reactions,[8] utilizing systems based on benzyl,[8a,b] calix[4]pyrrole,[8c] acridinium salt,[8d] coumarin,[8e] BODIPY,[8f] oxazine,[8g] squaraine,[8h] phenothiazine,[8i] 2,4,6-triphenylthiopyrylium,[8j] and triazolopyridinium salt,[8k] to name but a few. Effective as these systems are, it would be advantageous to ripen a new cyanide-driven reaction that (*i*) has a vibrant mechanistic signature, (*ii*) exhibits high selectivity, (*iii*) operates at room temperature, and (*iv*) could be exploited for the development of off-the-shelf optical sensors for CN^-.

With these realizations as well as based on our experience in developing optical probes or method for detection of biologically important species,[8i,9] herein, we report a CN^--selective indicator, namely, (*E*)-10-(*10H*-phenothiazin-3'-yl)propenal (**PTZ-AL**), a



Scheme 1 Synthesis of (*E*)-10-(*10H*-phenothiazin-3'-yl)propenal (**PTZ-AL**) and its CN^--promoted deprotection reaction.

NH-protected phenothiazine (**PTZ**). The **PTZ-AL** contains an α,β-unsaturated aldehyde group as a putative CN^--triggered deprotection subunit and thus works in a chemodosimetric fashion.

PTZ-AL was synthesized following the route as outlined in Scheme 1. Briefly, a Vilsmeier-Hacck formylation reaction of **PTZ** employing 3-(dimethylamino)propenal as an amide in dichloroethane (DCE) solution followed by hydrolysis of the resulting intermediate in the presence of $NaHCO_3$ solution afforded **PTZ-AL** in appreciable yield. The structure and purity of **PTZ-AL** was confirmed using NMR spectroscopy and mass spectrometry (see ESI†). The unequivocal proof about the structure of **PTZ-AL** was accessed by X-ray diffraction analysis. Single crystals suitable for X-ray structure analysis were grown by layering *n*-hexane onto a THF solution followed by slow evaporation of solutions at room temperature over a period of 2 days. The **PTZ-AL** crystallizes in the monoclinic system, space group *P 1 21/c 1*, where the crystal structure shows a typical trans-configuration with respect to the C_1'-C_2' bond (see ESI†).

In order to evaluate the nature of the interaction of CN^- with **PTZ-AL**, we commenced our investigations by first monitoring the ^1H NMR spectral changes produced *via* the incremental addition of tetra-*n*-butylammonium cyanide (TBACN) to a solution of **PTZ-AL** in $(CD_3)_2SO$) at room temperature.

As shown in Fig. 1, upon the addition of ~ 1.5 equiv. of TBACN, the aldehyde, vinylic and aromatic protons of **PTZ-AL** at δ = 9.37 ($H_{1'}$), 8.01, 5.72 ($H_{3'}$, $H_{2'}$), and 7.35-7.62 ppm ($H_{1\text{-}4}$ and $H_{6\text{-}9}$), respectively, were almost completely disappeared. Concomitantly, however, new resonances at δ = 6.57-6.94 ppm emerged. On careful examination, the newly appeared resonances were found identical with those of the aromatic protons of standard compound **PTZ**, suggesting that, indeed, **PTZ-AL** was transformed into **PTZ** (Scheme 1) rather than the typical addition of CN^- to the α,β-unsaturated carbonyl moiety as reported previously.[10]

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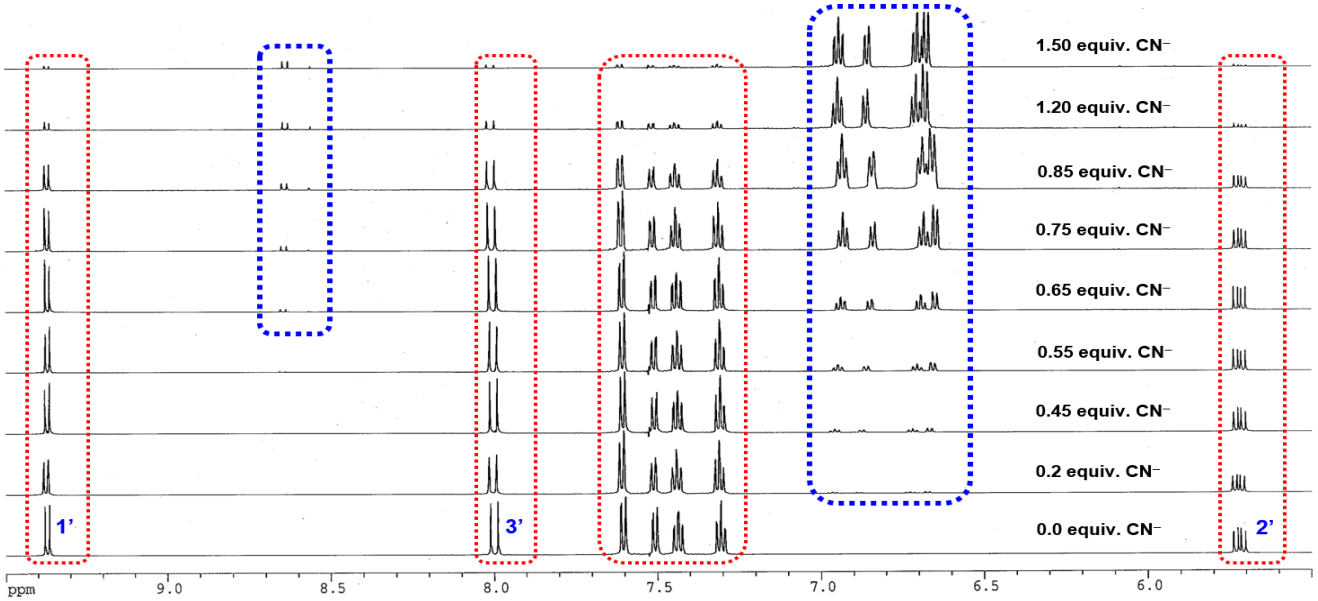

Fig. 1 Partial ^{1}H NMR (600 MHz, $(CD_3)_2SO$) spectral changes upon the addition of cyanide anion (CN^-) to **PTZ-AL** (10.0 mM). In red: signals disappearing. In blue: signals appearing during titration.

In order to evaluate the CN^--selective nature towards deprotection of **PTZ-AL**, the ^{1}H NMR titration was also performed with F^- which often obscures the detection of CN^-, especially, in organic solvents acting as a strong competitor of CN^-.[8i] In marked contrast to what was seen with CN^-, all the resonance lines shown within 5-10 ppm were totally unchanged after the addition of 5 equiv. of F^- (Fig. S6†). Furthermore, the ^{1}H NMR spectrum of resulting mixture did not show any noticeable changes even on keeping the solution for more than two weeks, indicating that F^- is completely silent for this particular reaction. All other studied anions ranging from Cl^-, Br^-, I^-, HSO_4^-, NO_3^- to $H_2PO_4^-$, and AcO^- gave similar responses as that of F^-, authenticating **PTZ-AL** to be exceptionally selective for CN^-. It is worth mentioning here that the deprotection mechanism of **PTZ-AL** fairly works even in aqueous solution or with inorganic CN^- (NaCN) as evidenced by ^{1}H NMR spectroscopy (Fig. S7†).

With these intriguing results in hand, the quantitative analytical behaviour of CN^--triggered transformation of **PTZ-AL** into **PTZ** was further examined by fluorescence emission spectroscopy. In this context, it should be noted that whereas **PTZ** exhibits blue fluorescence, **PTZ-AL** appears as a transparent and non-emissive compound in solution, presumably, due to the protection of 10N–*H* group. Therefore, a fluorescence change should ensue due to the CN^--triggered transformation of 10N–*C*=*C*-*CHO* into 10N–*H* group as confirmed above by ^{1}H NMR titrations. Upon incremental addition of CN^-, the acetonitrile solution of **PTZ-AL** resulted in a significant enhancement of emission intensity positioned at λ = 443 nm (Fig. 2). When 5 equiv. of CN^- was added, the emission intensity reached its maximum with ca. 43-fold enhancement factor validating the results of ^{1}H NMR spectroscopy. Notably, there was a linear dependence of the fluorescence intensity on the CN^- concentration in the range of 2.3×10^{-5} M to 2.3×10^{-4} M (R^2 = 0.9856) with a detection limit of 1.3×10^{-5} M. In line with this, upon gradual introduction of CN^-, a significant increase (relatively weak than that of only in acetonitrile) in the fluorescence emission was also observed in a mixture of acetonitrile-water (2:3, v/v; LOD = 3.0×10^{-5} M, λ_{em} = 443 nm) as well as acetonitrile-PBS buffer (7:3, v/v, pH 7.4; LOD = 2.7×10^{-5} M, λ_{em} = 439 nm) solutions, which demonstrate, that **PTZ-AL** can be effectively used in more realistic environments (Fig. S8 and S9†).

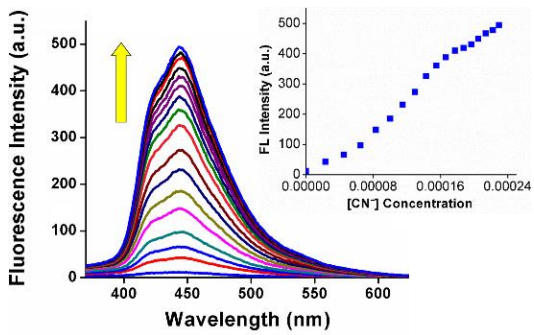

Fig. 2 Fluorescence titration (emission) spectra of **PTZ-AL** (5.0×10^{-5} M) in acetonitrile solution upon incremental addition of TBACN (0 → 5 equiv.) Inset (right): Changes in the emission intensity of **PTZ-AL** *vs.* equiv. of CN^- (λ_{ex} = 320 nm and λ_{em} = 443 nm).

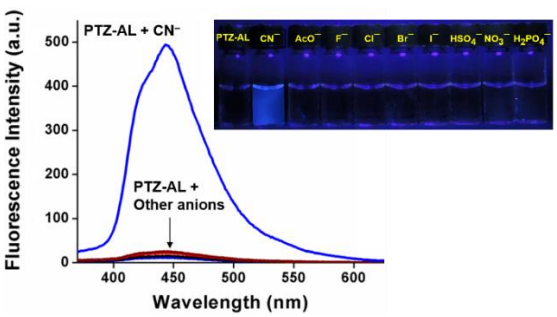

Fig. 3 Fluorescence emission intensity spectra of **PTZ-AL** (5.0×10^{-5} M) in acetonitrile solution recorded after the addition of 5 equiv. of the tetrabutylammonium salts of various anions (CN^-, AcO^-, F^-, Cl^-, Br^-, I^-, HSO_4^-, NO_3^- and $H_2PO_4^-$. Inset: Corresponding visual fluorescence responses of **PTZ-AL** to different anions at room temperature.

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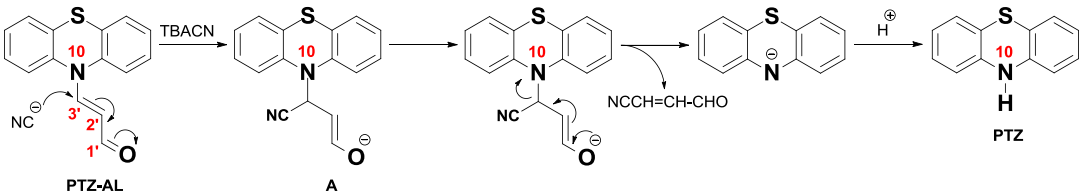

Scheme 2 Proposed mechanism for CN^--triggered transformation of **PTZ-AL** into **PTZ**.

To further evaluate the selectivity of **PTZ-AL** towards CN^-, we also measured fluorescence intensity changes upon addition of a variety of anions. Specifically, 5 equiv. of each anion were added to an acetonitrile solution of **PTZ-AL** (5.0×10^{-5} M) and incubated for 10 min at room temperature, before being subjected to spectral analysis. The fluorescence emission intensity of **PTZ-AL** enhanced remarkably only after the addition of CN^-. The other studied anions (AcO^-, F^-, Cl^-, Br^-, I^-, HSO_4^-, NO_3^-, and $H_2PO_4^-$), inorganic sulphide, and (bio)thiols did not cause any remarkable changes in the fluorescence emission intensity (Fig. 3a, S10-17†). The fact that **PTZ** exhibits blue fluorescence also allowed the CN^--driven transformation of **PTZ-AL** to be followed visually by making the use of a short UV hand lamp (UV-GL 25; UV-254/365 nm) (Fig. 3b).

The mechanism of interaction of CN^- with **PTZ-AL** is proposed in Scheme 2, which involves initial nucleophilic attack of CN^- on β-position of the aldehyde group (C_3') resulting in the formation of intermediate **A**. Under experimental conditions, intermediate A seems to be unstable and readily undergoes rearrangement, releasing 4-oxobut-2-enenitrile to produce **PTZ**.

In order to get an unequivocal proof that CN^- signalling is *via* selective CN^--induced deprotection of **PTZ-AL** (Scheme 2), the chemical reaction between **PTZ-AL** and TBACN (1.2 equiv.) was performed in acetonitrile solution under ambient conditions (see ESI†). The progress of the reaction could be easily followed by TLC. After completing the reaction, the solvent was evaporated and crude was column chromatographed. As expected, the isolated product turned out to be **PTZ** as confirmed by ^{1}H NMR and ^{13}C NMR spectroscopy.

In summary, a PTZ scaffold having an α,β-unsaturated aldehyde group (**PTZ-AL)** as a putative reacting subunit towards CN^- was synthesized. In the presence of CN^-, **PTZ-AL** revealed a highly selective 'turn-on' type fluorogenic signalling behaviour based on the selective and efficient cleavage of N-C bond (CN^--triggered deprotection), which was established by ^{1}H NMR titrations as well as the isolated product from a chemical reaction between **PTZ-AL** and TBACN. Effective as it is, however, it must be stressed that **PTZ-AL** does not serve as a typical signalling probe but can be classified as a chemodosimeter due to the irreversible nature of as-proposed chemical reaction to produce fluorescence response. Although several chemodosimetric probes for CN^- detection have been reported over the years, to the best of our knowledge, this is the first example enabling the use of a protection/deprotection mechanism for selective CN^- detection. The present approach holds great promises in organic synthesis and may be further developed as N–H protection/deprotection strategy. Further exploration of this approach, as is a search for other heterocyclic systems that might allow an exceptional CN^- recognition, are currently underway in our laboratory.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Synthesis and characterization data of **PTZ-AL**, spectra of ^{1}H NMR, ^{13}C NMR, and HRMS, ^{1}H NMR titrations and single crystal X-ray data for **PTZ-AL**. CCDC 1049885]. See DOI: 10.1039/b000000x/

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Graphical Abstract

A highly selective phenothiazine-based fluorescence 'turn-on' indicator based on cyanide-promoted novel protection/deprotection mechanism

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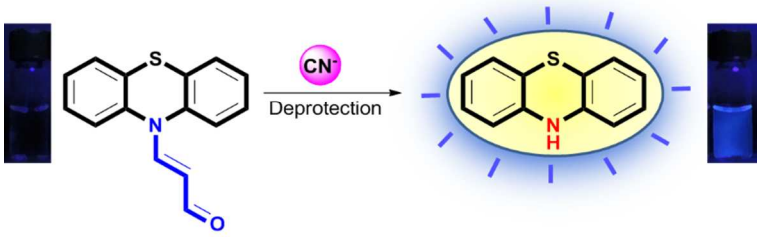

A CN^--triggered selective cleavage of N-C bond is described using (*E*)-10-(10*H*)-phenothiazin-3'-yl)propenal to produce selective fluorescence 'turn-on' response for CN^-.