Electronic Supplementary Material (ESI) for New Journal of Chemistry.

SUPPORTING INFORMATION

Easy and rapid estimation of ammonia in cold storage potatoes: Precautions in environment

Shampa Kundu[a†], Himadri Sekhar Sarkar[b†], Sujoy Das[a] and Prithidipa Sahoo*[a]

[a]Department of Chemistry, Visva-Bharati University, Santiniketan-731235, India
Email: prithidipa@hotmail.com

[b]Institute of Multidisciplinary Research for Advanced Materials (IMRAM)
2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan
† Both the authors contributed equally

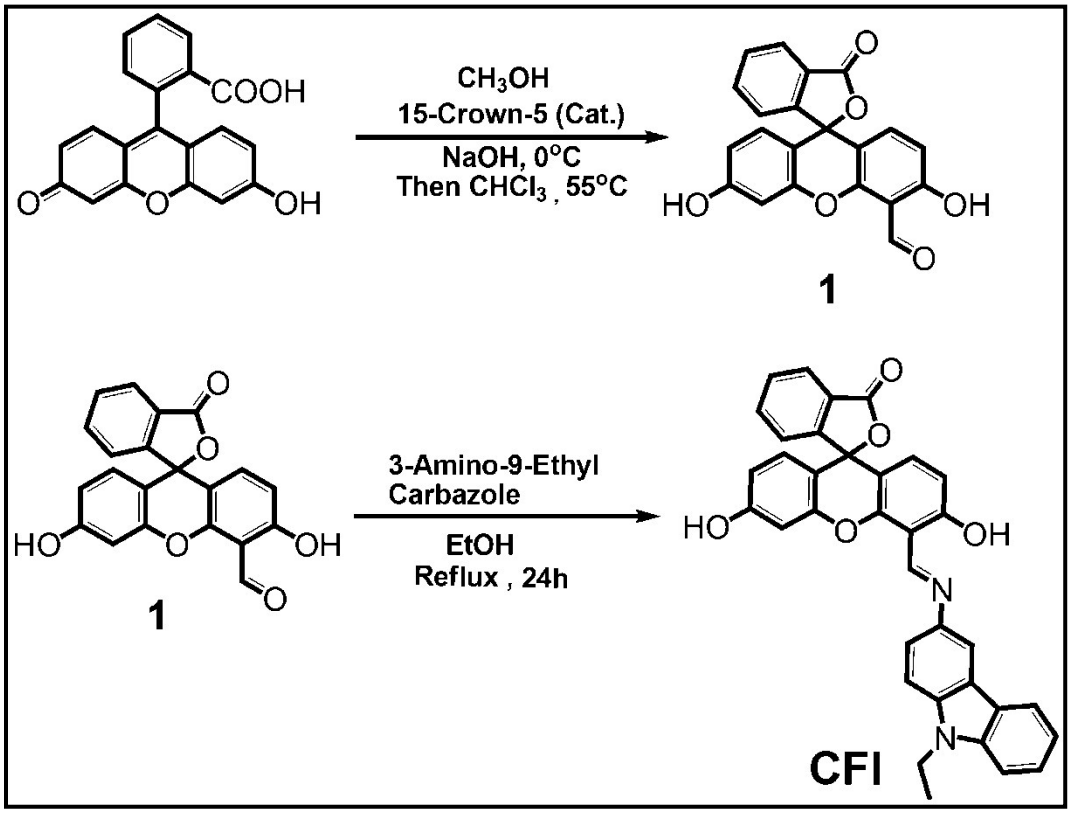
1. Table S1. Performance comparison of existing methods and present method (fluorescence based chemosensor) for detection of **NH_3**

Analytes	**Method of detection**	**Sensor type**	**Detection limit**	**Detection state**	**Sensitvity & Selectivity**	**Response Time**	**Estimation**	**Reference**
NH_3	Fluorescence based chemosensor	Fluorescein-carbazole	4.3nM	**Liquid, Gas**	**High**	**Instant**	**Yes**	**Current method**
NH_3	colorimetric gas sensors	Porphyrin cobalt(II)–dansyl	40nM	Liquid	Moderate	Not mentioned	No	*Tetrahedron Letters,* **2011**, 52, 2645–2648
NH_3	Optical sensor	Eosin ethylester and 2,7-dichlorofluorescein methylester	1µg/l	Liquid	Low	20-30min	No	*Talanta*, **2008**, 77, 66–72
NH_3	Optical sensor	Coumarin 545T, Coumarin 30	~1 ppb	Liquid	Moderate	2 min	No	*Chemistry Central Journal,* **2012**, 6, 124
NH_3	Optical sensor	NaYF4: YbEr, phenol red	400 μM	Liquid	Low	5-7 min	No	*Anal. Chem.* **2010,** 82, 5002–5004
NH_3	Optical chemical sensor	Eosin dye	0.1-5ppm	Liquid	Low	20-200min	No	*PHOTONIC SENSORS*, **2016**, 6, 2, 107–114
NH_3, amines	Optical Gas Sensor	MMPyP, TMPyP	0.05, 0.16ppm	Liquid	Moderate	4- 5 minutes	No	*Sensors*, **2017**, 17, 24

EXPERIMENTAL SECTION

Materials and Instruments:

All the reagents were purchased from Sigma-Aldrich Pvt. Ltd. (India). Unless otherwise mentioned, materials were obtained from commercial suppliers and were used without further purification. Solvents were dried according to standard procedures. Elix Millipore water was used in all respective experiments. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz instrument. For NMR spectra, DMSO-d_6 and for NMR titration DMSO-d_6 and D_2O were used as solvent using TMS as an internal standard. Chemical shifts are expressed in δ ppm units and ^{1}H–^{1}H and ^{1}H–C coupling constants in Hz. The mass spectrum (HRMS) was carried out using a micromass Q-TOF Micro™ instrument by using methanol as a solvent. Fluorescence spectra were recorded on a PerkinElmer Model LS55 spectrophotometer. UV spectra were recorded on a SHIMADZU UV-3101PC spectrophotometer. The following abbreviations are used to describe spin multiplicities in ^{1}H NMR spectra: s = singlet; d = doublet; t = triplet; m = multiplet.



Scheme S1. Step-wise Synthesis of the chemosensor **CFI**.

Compound 1 was synthesized from a known literature.[1] Fluorescein (2.5 g, 7.5 mmol) and CH_3OH (6 mL) were taken in a 100 mL three-neck round-bottom flask at room temperature. The whole system was then cooled to 0ºC, followed by the addition of a mixture of NaOH aqueous solution (50%) and 15-crown-5 (30 μL) within 5 min. The resulting mixture was stirred for 10 min, and then allowed to warm gradually in an oil bath. $CHCl_3$ (10 mL) was added dropwise while the reaction temperature was maintained at 55ºC. The reaction mixture was further stirred for 10 h at this temperature, and then cooled to room temperature. The mixture is acidified with H_2SO_4 (15 mL, 10 M), and the purple-black precipitate appeared. This solid was filtered and dried and purified by column chromatography ($CHCl_3$: EtOAc =3:1) on silica gel to get the crude monoaldehyde-functionalized fluorescein. ^{1}H NMR (400 MHz, DMSO-d_6): d (ppm) =11.88 (s, 1H), 10.63 (s, 1H), 10.26(s, 1H), 8.00-8.02 (d, 1H, J=8 Hz), 7.71-7.82 (m, 2H, J=44 Hz), 7.30-7.31 (d, 1H, J=4 Hz), 6.93-6.96 (d, 1H, J=12 Hz), 6.84-6.85 (d, 1H, J=4 Hz), 6.69-6.72 (d, 1H, J=12 Hz), 6.60-6.61 (d, 2H, J=4 Hz). ^{13}C NMR (400 MHz, DMSO-D_6): d (ppm)= 192.84, 168.54, 162.92, 159.62, 152.37, 152.16, 150.85, 136.52, 135.80, 130.32, 129.00, 125.90, 124.79, 123.99, 113.52, 113.38, 109.69, 109.21, 109.14, 102.64, 81.80.

CFI was synthesized from compound 1 by one-step imine formation reaction. compound 1 (2.0 g, 5.5 mmol), 3-amino-9-ethyl carbazole (1.40 g, 6.67 mmol), EtOH (40 ml) were taken in a 100 ml RB flask and reflux the mixture at 83ºC for 24 hrs. The product was extracted with $CHCl_3$ after evaporation of EtOH. After drying it over anhydrous Na_2SO_4, the organic layer was evaporated completely. The residue was purified by column chromatography with the eluent $CHCl_3$: EtOAc (6:1,v/v) to get the product **CFI** with 75% yield (Fig.). ^{1}H NMR (400 MHz, DMSO-d_6): d (ppm) =10.18 (s, 1H), 9.69(s, 1H), 8.51(s, 1H), 8.30-8.32 (d, 1H, J=8Hz), 8.01-8.03 (d, 1H, J=8Hz), 7.80-7.82 (d, 2H, J=8Hz), 7.73-7.77 (m, 3H, J=16Hz), 7.65-7.67 (d, 1H, J=8Hz), 7.51-7.53 (t, 1H, J=8Hz), 7.32-7.34 (d, 1H, J=8Hz), 7.24-7.28 (t, 1H, J=16Hz), 7.06-7.07 (d, 1H, J=4Hz), 6.69-6.76 (m, 2H, J=28Hz), 6.61-6.62 (t, 2H, J=4Hz), 4.50-4.52 (d, 2H, J=8Hz), 1.34-1.37 (t, 3H, J=12Hz). ^{13}C NMR (400 MHz, DMSO-D_6): d (ppm)= 168.62, 164.07, 159.52, 154.31, 152.28, 151.29, 150.59, 140.28, 139.00, 135.70, 132.27, 130.21, 128.88, 126.08, 124.70, 124.01, 122.94, 122.29, 120.93, 120.16, 119.07, 113.84, 113.22, 109.88, 109.47, 108.20, 106.83, 102.97, 82.61, 37.18, 13.73. HRMS (TOF MS): (m/z, %): Calcd. for $C_{35}H_{24}N_2O_5$: 552.1685. Found: m/z = 553.1755 ($M+H^+$).

2. NMR Studies:

^{1}H NMR of Compound-1 in DMSO-d_6 :

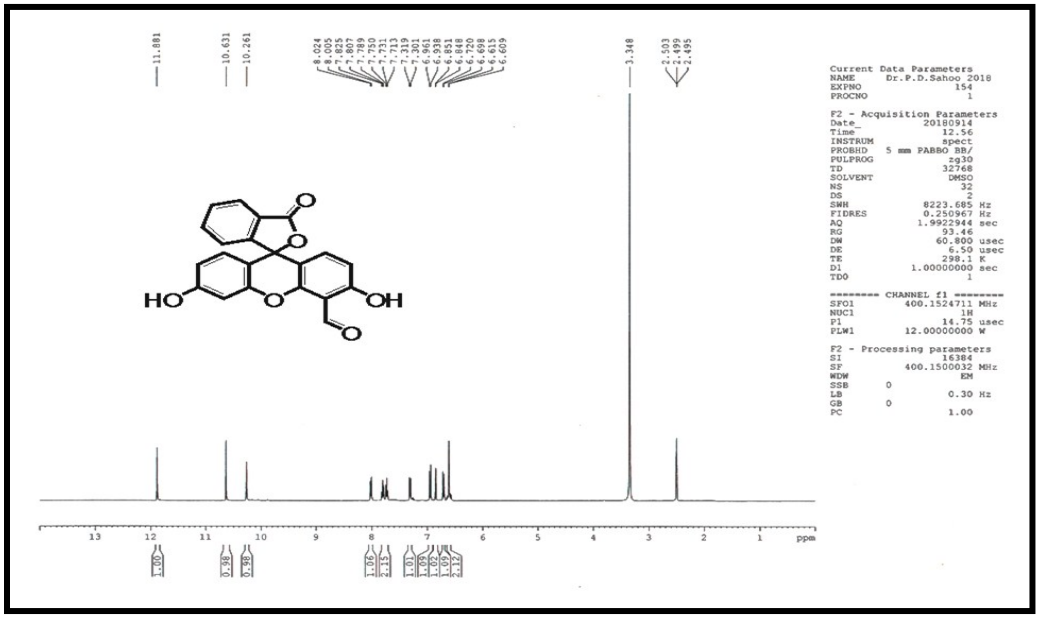


Fig.S1 ^{1}H NMR of **Compound 1** in DMSO-d_6 (400 MHz).

^{13}C NMR of Compound-1 in DMSO-d_6:

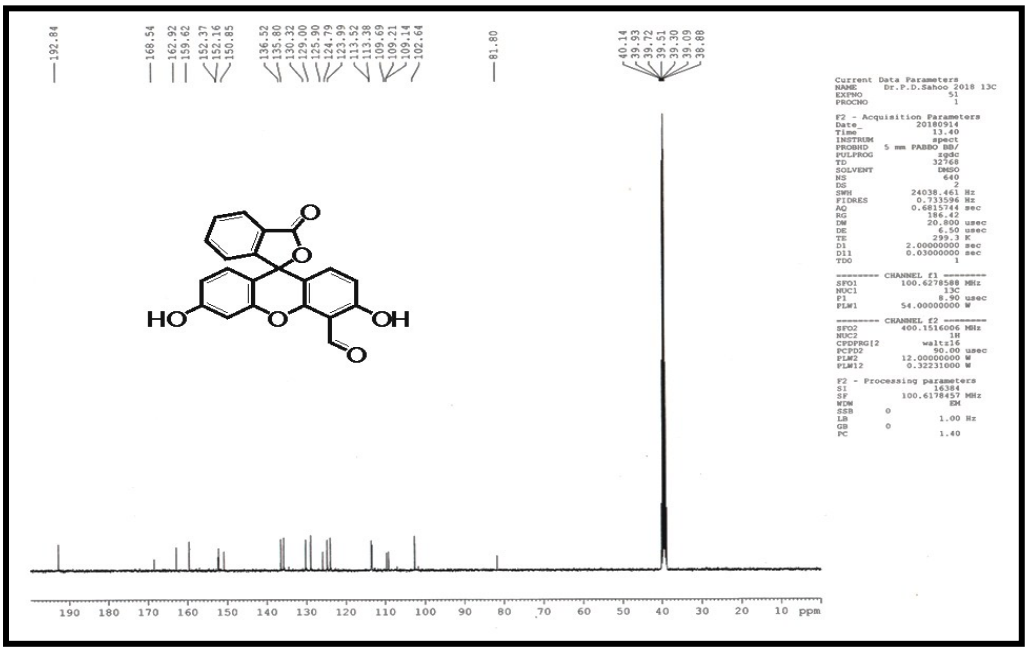


Fig. S2 ^{13}C NMR of **Compound 1** in DMSO-d_6 (400 MHz).

^{1}H NMR of CFI in DMSO-d_6:

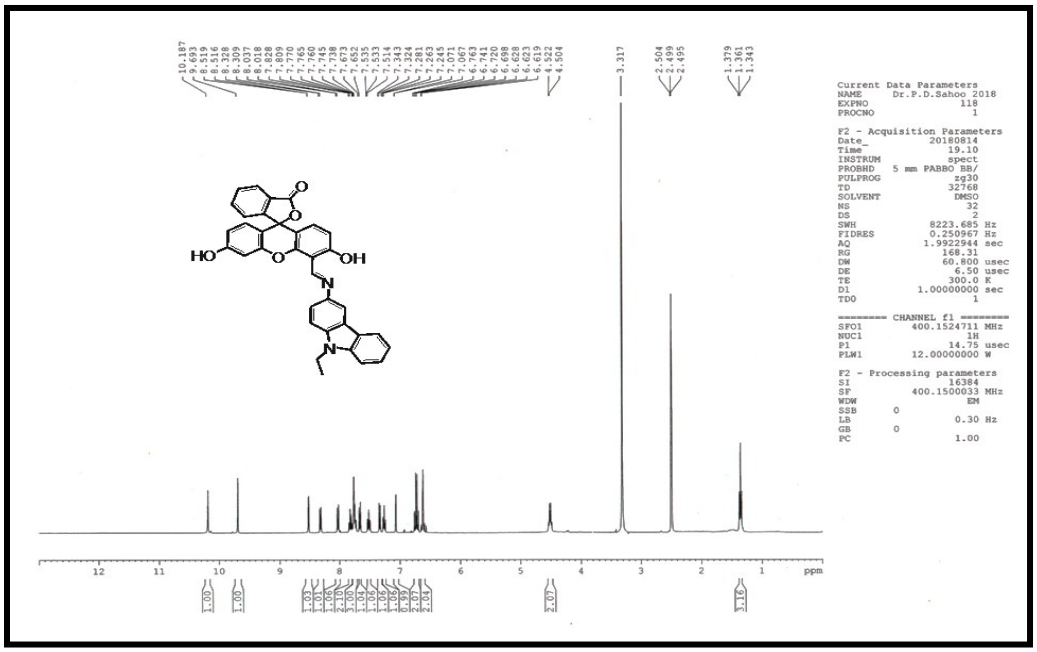


Fig. S3 ^{1}H NMR of **CFI** in DMSO-d_6 (400 MHz).

^{13}C NMR of CFI in DMSO-d_6:

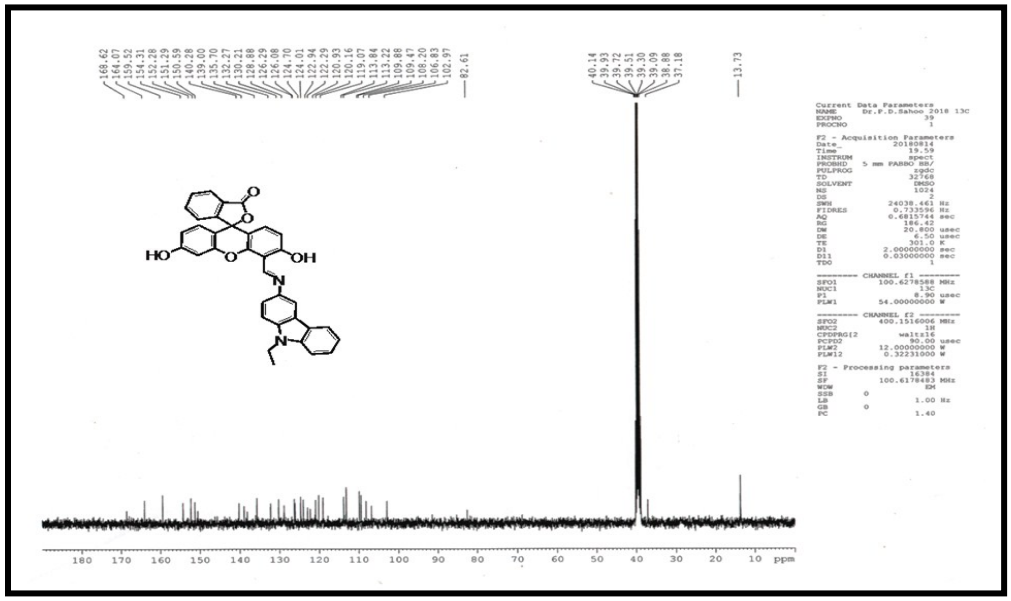


Fig. S4 ^{13}C NMR of **CFI** in DMSO-d_6 (400 MHz).

3. Mass spectrum of CFI:

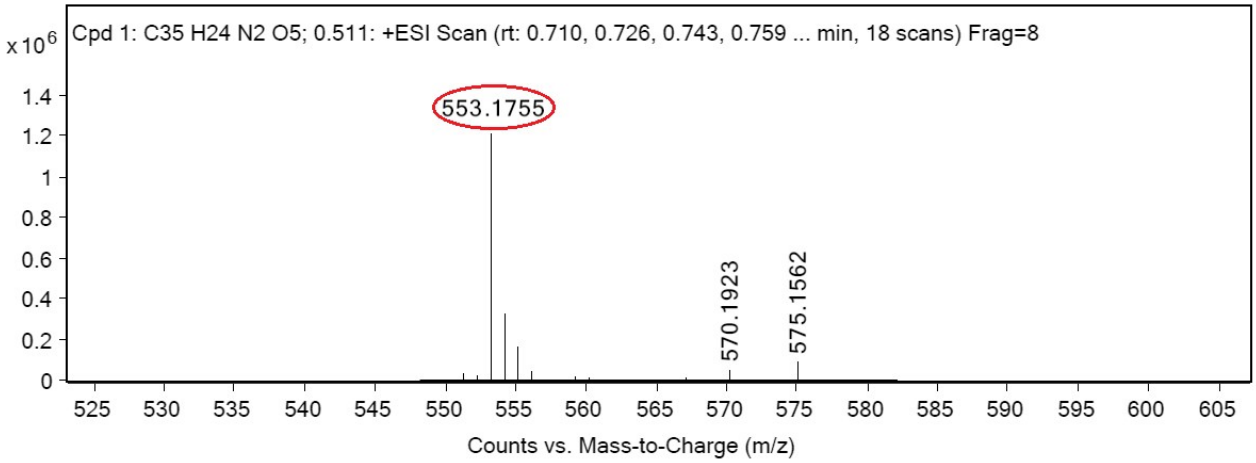


Fig. S5 HRMS of **CFI**

4. Probable mechanistic pathway of the interaction between CFI and NH_3:

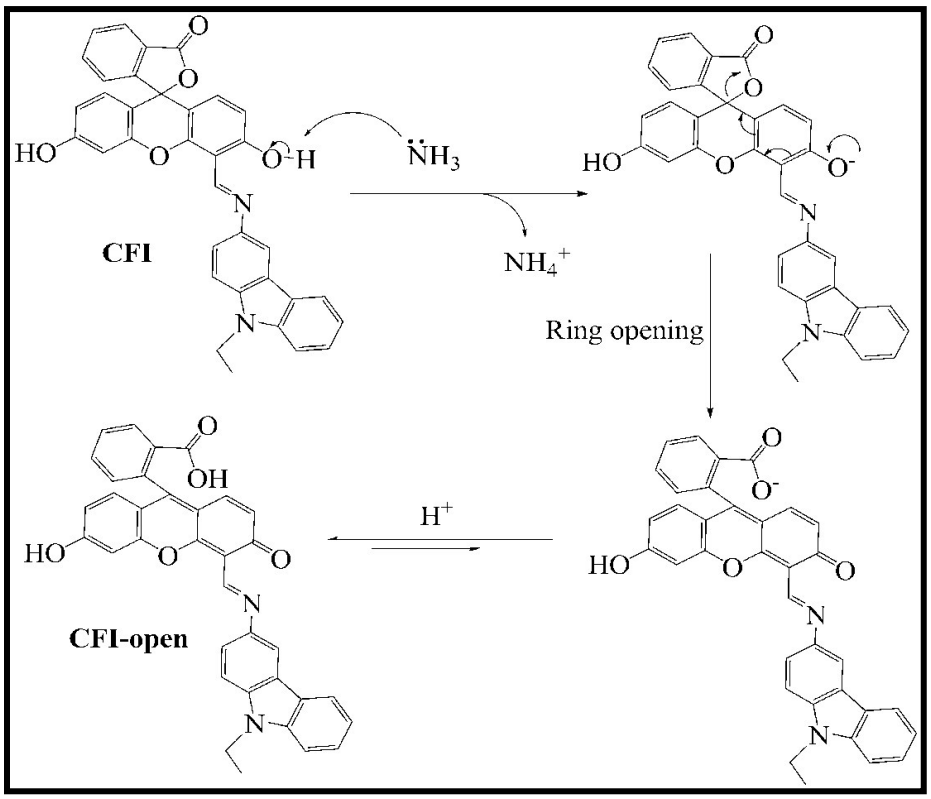


Fig. S6 Probable mechanistic pathway of the reaction between **CFI** and **NH_3**

5. Job's plot for determining the stoichiometry of interaction by fluorescence method:

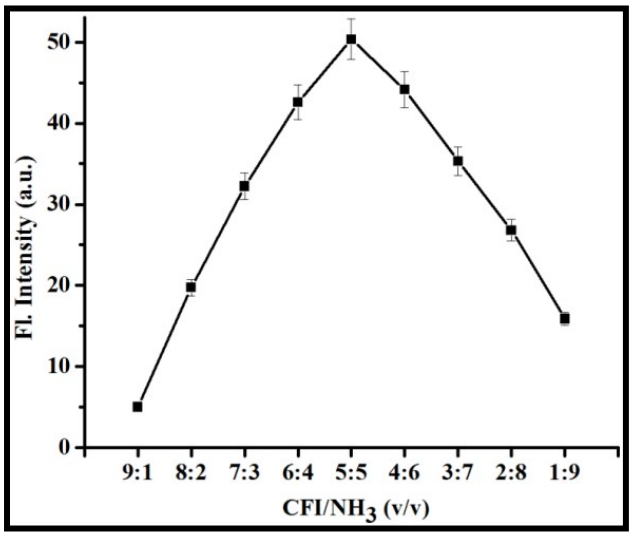


Fig. S7 Job's plot of **CFI** (c =1μM) with **NH_3** (1μM) in acetonitrile:water (1:10, v/v) at pH 7.0, (10 mM phosphate buffer) by fluorescence method, which indicate 1:1 stoichiometry for **CFI** with **NH_3**. Standard deviations are represented by error bar (n=3).

6. Calculation of limit of detection (LOD) of CFI with NH_3:

The detection limit of the chemosensor **CFI** for **NH_3** was calculated on the basis of fluorescence titration. To determine the standard deviation for the fluorescence intensity, the emission intensity of four individual receptors without **NH_3** was measured by 10 times and the standard deviation of blank measurements was calculated.

The limit of detection (LOD) of **CFI** for sensing **NH_3** was determined from the following equation[2].

$$LOD = K \times SD/S$$

Where K = 2 or 3 (we take 3 in this case); SD is the standard deviation of the blank receptor solution; S is the slope of the calibration curve.

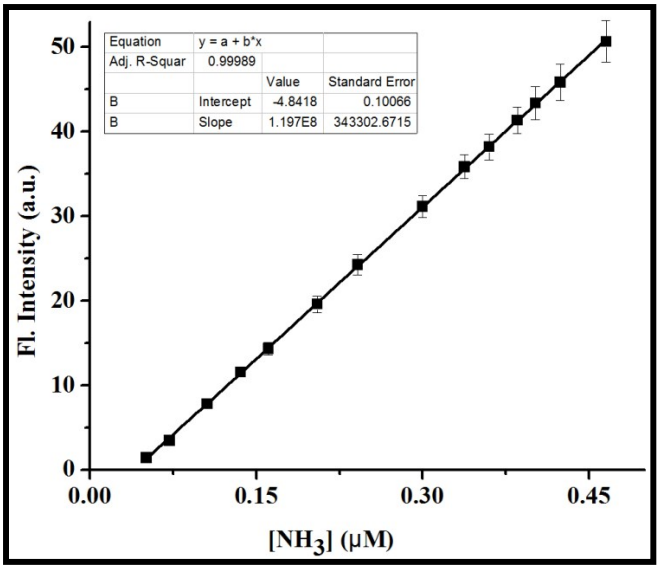


Fig. S8 Linear fit curve of **CFI** at 516 nm with respect to NH_3 concentration. Standard deviations are represented by error bar (n=3).

From the linear fit graph we get slope = 1.197×10^8, and SD value is 0.17305

Thus using the above formula we get the Limit of Detection = 4.33×10^{-9} M, i.e 4.33 nM. Therefore **CFI** can detect NH_3 up to this very lower concentration by fluorescence technique.

7. pH titration curve of CFI upon gradual addition of NH_3:

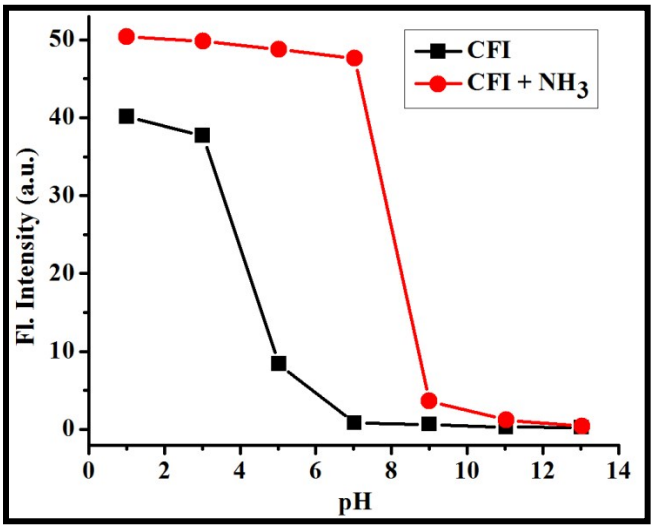


Fig. S9 Effect of pH on the fluorescence intensity of **CFI** (1 μM) in the absence of NH_3 (black line) and in the presence of NH_3 (10 μM, red line).

8. Competitive fluorescence studies of CFI with various analytes:

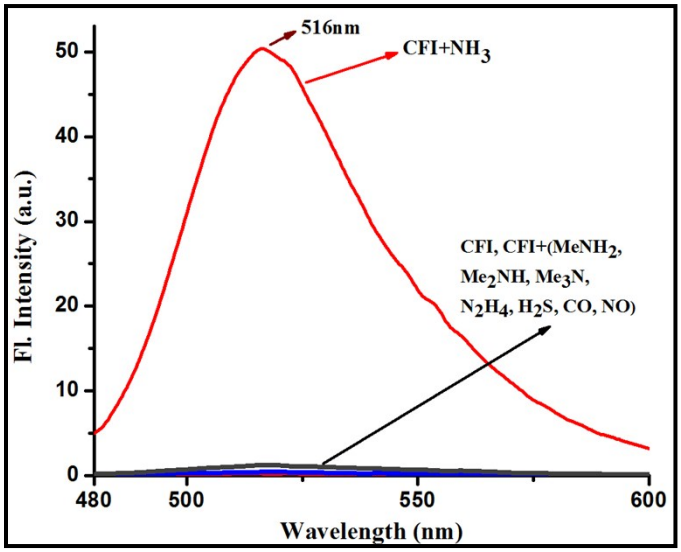


Fig. S10 Fluorescence emission spectra of **CFI** (1 μM) upon addition of different analytes at 516 nm (λ_{ex}= 450 nm) in CH_3CN:H_2O (1:10, v/v) at pH 7.0 (10 mM phosphate buffer) (Guests conc. = 10 μM).

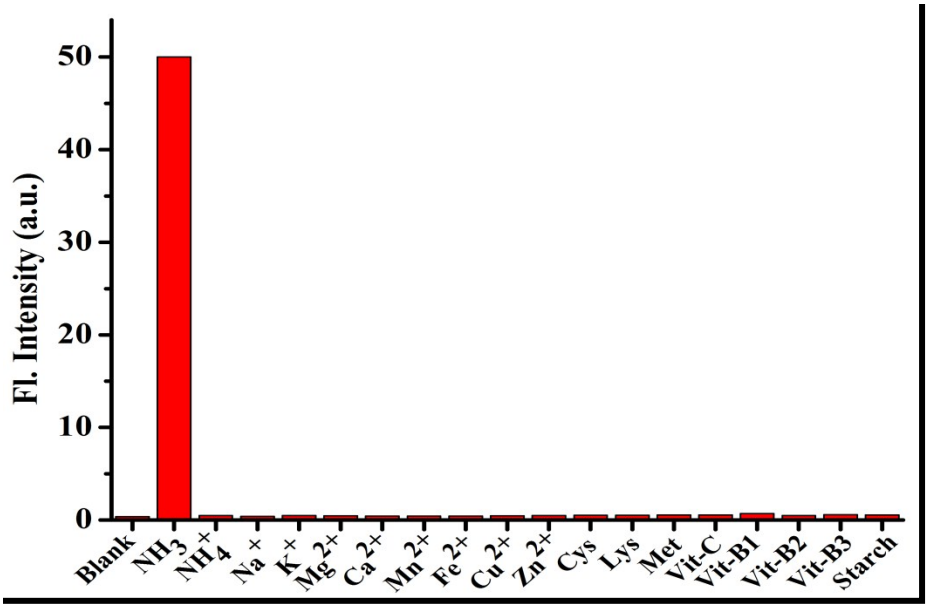


Fig. S11 Histogram representing competitive fluorescence spectra of **CFI** with other interfering analytes at 516 nm (λ_{ex}= 450 nm) in CH_3CN: H_2O (1:10, v/v) at pH 7.0 (10 mM phosphate buffer).

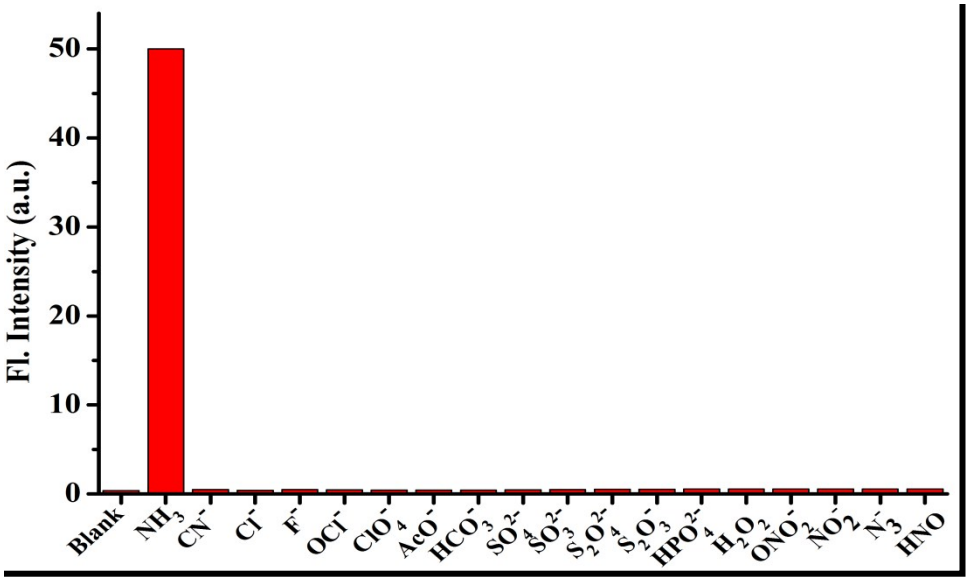


Fig. S12 Histogram representing competitive fluorescence spectra of **CFI** with ROS, RNS and various anions at 516 nm (λ_{ex}= 450 nm) in CH_3CN: H_2O (1:10, v/v) at pH 7.0 (10 mM phosphate buffer).

9. ^{1}H NMR titration spectrum of CFI with NH_3:

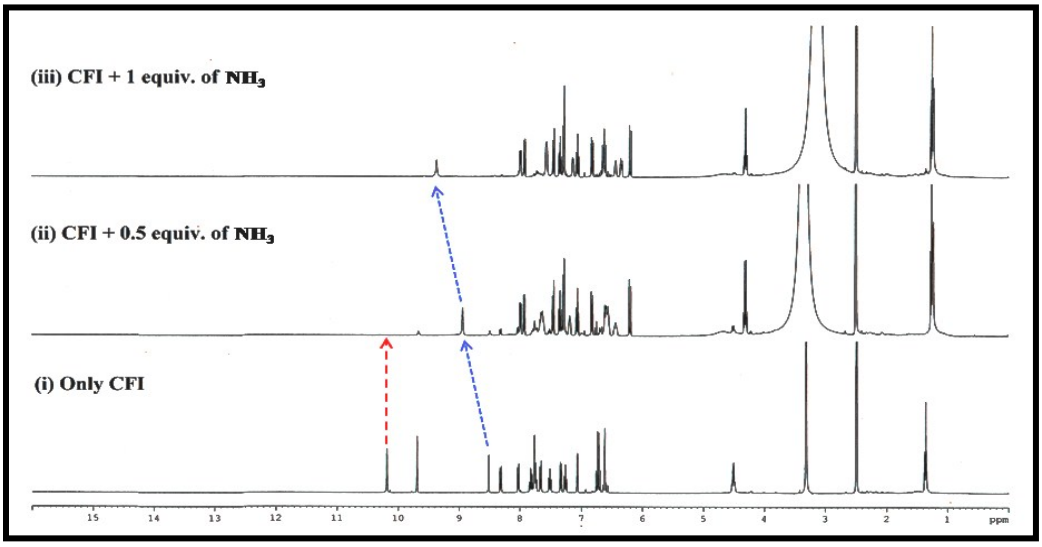


Fig. S13 ^{1}H NMR titration [400 MHz] of **CFI** in DMSO-d_6 at 25^{0}C and the corresponding changes after the gradual addition of 1 equiv. of **NH_3** in D_2O.

10. ^{13}C NMR titration spectrum of CFI with NH_3:

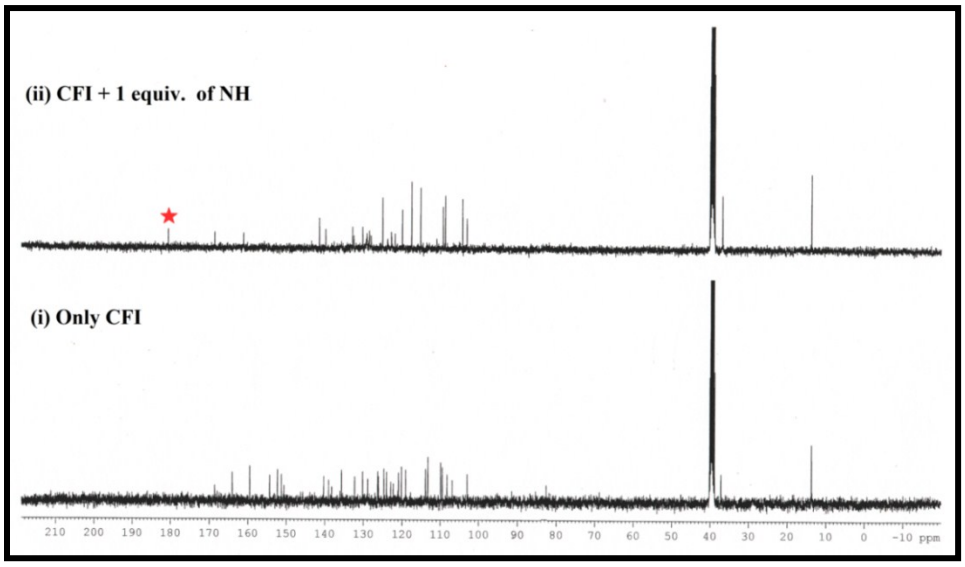


Fig. S14 ^{13}C NMR titration [400 MHz] of **CFI** in DMSO-d_6 at 25^{0}C and the corresponding changes after addition of one equiv. of **NH_3** in D_2O. The red spot indicates the formation of aromatic keto group at 182 ppm.

11. Partial HRMS of the mixed assay system:

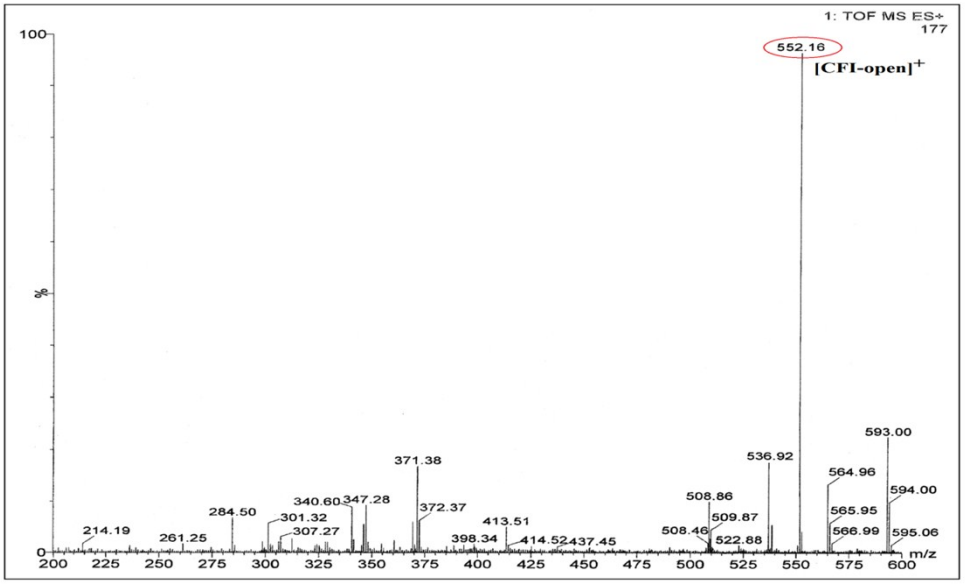


Fig. S15 Partial HRMS spectra of **CFI-NH_3** mixture in acetonitrile, taken after two hours of mixing.

12. Details of energy calculations using Density Functional Theory (DFT):

Table S2. Details of the geometry optimization in Gaussian 09 program

Details	CFI	CFI-open
Calculation method	B3LYP	B3LYP
Basis set	6-311G**	6-311G**
E(CAM-B3LYP) (a.u.)	-1834.296	-1834.302
Charge, Multiplicity	0, 1	0, 1
Solvent (CPCM)	Water	Water

Table S3. Selected electronic excitation energies (eV), oscillator strengths (f), main configurations of the low-lying excited states of all the molecules and complexes. The data were calculated by TDDFT//B3LYP/6-311G(d,p) based on the optimized ground state geometries.

Molecules	Electronic Transition	Excitation Energy[a]	f[b]	Composition[c] (%)
CFI	$S_0 \rightarrow S_1$	3.01 eV 399.93 nm	0.6751	H → L (69%)
	$S_0 \rightarrow S_{13}$	4.35 eV 284.76 nm	0.5768	H -1 → L+2 (42%)
CFI-Open	$S_0 \rightarrow S_3$	2.9731 eV 450.02 nm	0.3837	H -1 → L (68.6%)
	$S_0 \rightarrow S_4$	3.1155 eV 397.96 nm	0.1727	H -2 → L (65.7%)

[a]Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength. [b]Oscillator strength. [c]H stands for HOMO and L stands for LUMO.

Table S4. Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)

Species	E_{HOMO} (a.u)	E_{LUMO} (a.u)	ΔE(a.u)	ΔE(eV)	ΔE(kcal/mol)
CFI	-0.20148	-0.06945	0.13203	3.59	82.79
CFI-Open	-0.22212	-0.10152	0.1206	3.28	75.63

13. Paper strip detection:

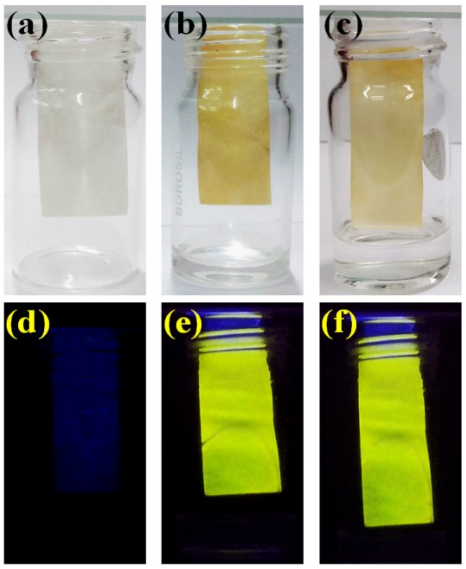


Fig. S16 Display of paper strip sensing of **NH_3** using a **CFI** coated paper strip in visual (top) and under a UV lamp (bottom): (a) only an **CFI** coated filter paper (b) after 15 seconds of incubation of **NH_3** (c) after 15seconds of dipping in **NH_3** solution.

NH_3 preparation in liquid and gas phase. An aqueous solution of liq. NH_3 (25%) was prepared by dissolving liquor ammonia in distilled water. Gaseous NH_3 was generated from concentrated liquor ammonia in a closed container.

Potato extraction:

One fresh potato (P1) sample was collected from our own garden (harvested without any pesticide) and the other three samples (P2, P3, P4) were collected from nearby cold storage. Then the potato samples were cut into thin slices and the slices were ground thoroughly in a pestle mortar to get potato juice. This potato juice was then filtered and the filtrate treated as potato extract. The potato extract from different potato samples were subjected to fluorimetric analysis at pH 7.0 (10 mM phosphate buffer) to quantify the amount of **NH_3** present therein.

14. Reference:

1. Z. H. Fu, L. B. Yan, X. Zhang, F. F. Zhu, X. L. Han, J. Fang, Y. W. Wang and Y. Peng, *Org. Biomol. Chem*., 2017, **15,** 4115–4121

2. (a) L. Long, D. Zhang, X. Li, J. Zhang, C. Zhang and L. Zhou, *Anal. Chim. Acta,* 2013*,* **775**, 100. (b) M. Zhu, M. Yuan, X. Liu, J. Xu, J. Lv, C. Huang, H. Liu, Y. Li, S. Wang, and D. Zhu, *Org. Lett.,* 2008, **10**, 1481.