Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for Comment on 'Fluorescence sensing of arsenate at nanomolar level in a greener way: naphthalene based probe for living cell imaging'

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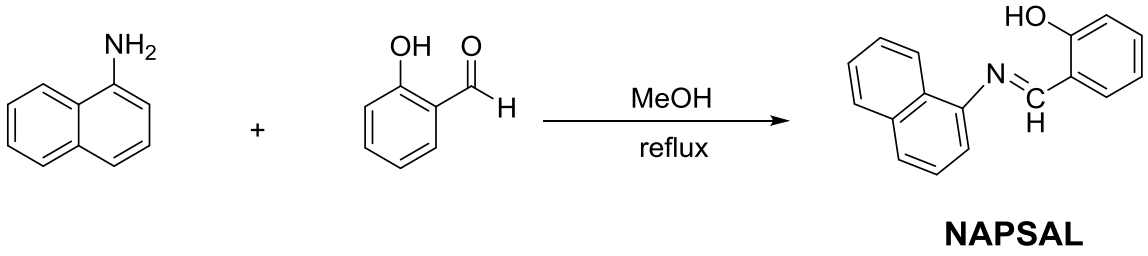
1. **General procedures**

NAPSAL was prepared according to the procedure reported in the literature.[1] Potassium arsenate monobasic salt was purchased from Sigma Aldrich, and sodium hydrogen arsenate heptahydrate was purchased from Alfa Aesar to provide two independent arsenic sources for testing. Other materials were purchased and used as received. ^{1}H NMR spectra were recorded with the use of a Jeol ECA 500 (500 MHz) spectrometer. The chemical shifts (δ ppm) are referenced to the respective solvent, and splitting patterns are designed as s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). ^{13}C NMR spectra were recorded with the use of a Varian VX 500 (500 MHz) spectrometer. UV-vis spectra were recorded with the use of a Perkin Elmer LAMBDA 35 UV/Vis spectrometer. Fluorescence measurements were performed with the use of a Perkin Elmer LS45 Luminescence Spectrometer. pH controlled experiments were performed with a Fisher Scientific accumet® AB15 pH Meter and pH values were adjusted using HCl (0.5 M), NaOH (0.5 M) or KOH (0.5 M) aqueous solutions. The titrations used either NaOH or KOH and yielded identical results.

2. Synthesis and characterization of NAPSAL



Scheme 1 Synthesis of N**APSAL** (same as suggested in the literature[1])

α-Napthyl amine (0.287 g, 2 mmol) and salicylaldehyde (0.25 g, 2 mmol) were combined in fresh distilled methanol (30 mL) and refluxed for 6 hours. After purification by silica-gel column chromatography (solvent: chloroform), a yellow solid was obtained in 72% yield. M. P., 45^{o}C (± 2^{o}C). CHN analysis Found: %C: 82.27; %H: 5.25; %N: 5.68%. Calculated: %C: 82.49; %H: 5.26; N%:5.66.

^{1}H NMR (500 MHz, CD_3OD) (ESI, Fig. S1): 6.98 (1H, td, J =7.5, 1.0 Hz, a2); 7.01 (1H, d, J = 8.5 Hz, a1); 7.30 (1H, d, J = 7.3Hz, b); 7.42 (1H, td, J = 8.5, 7.0 Hz, c); 7.51-7.54 (3H, m, d1,d2,d3); 7.58 (1H, dd, J = 7.8, 1.8 Hz, e); 7.79 (1H, d, J = 8.0 Hz, f); 7.89 (1H, m, g); 8.18 (1H, m, h); 8.87 (1H, s, i). Spectral parameters match those reported for NAPSAL by A. Sahana et al.[1]

^{1}H NMR (500 MHz, $(CD_3)_2SO$) (ESI, Fig. S2):7.03-7.05 (2H, m, a1,a2); 7.44 (1H, dd, J = 7.5, 1.0 Hz, b); 7.48 (1H, ddd, J = 8.0, 7.5, 2.0 Hz, c); 7.58-7.65 (3H, m, d1,d2,d3); 7.77 (1H, dd, J = 7.8, 1.8 Hz, e); 7.86 (1H, d, J = 8.5 Hz, f); 8.00 (1H, m, g); 8.16 (1H, m, h); 9.03 (1H, s, i); 13.07 (1H, s, j).

^{13}C NMR (500 MHz, CD_3OD) (ESI, Fig. S3): δ = 115.1 CH, 117.8 CH, 120.4 C, 121.0 C, 123.7 CH, 127.2 CH, 127.5 CH, 127.6C, 128.0 CH, 129.1 CH, 129.6 CH, 133.8 CH, 134.4 CH, 135.5 C, 147.3 C, 162.2 CH, 165.3C.

^{13}C NMR (500 MHz, $CDCl_3$) (ESI, Fig. S4): δ = 114.1 CH, 117.4 CH, 119.3 C, 119.6 C, 123.4 CH, 126.1 CH, 126.6 CH, 126.8, 127.1 CH, 128.0 CH, 128.4 CH, 132.5 CH, 133.5 CH, 134.0 C, 146.4 C, 161.2 CH, 163.9 C.

2.1 ^{1}H NMR and ^{13}C NMR spectra of NAPSAL in different solvents (CD_3OD, $(CD_3)_2SO$, $CDCl_3$) with proposed assignments

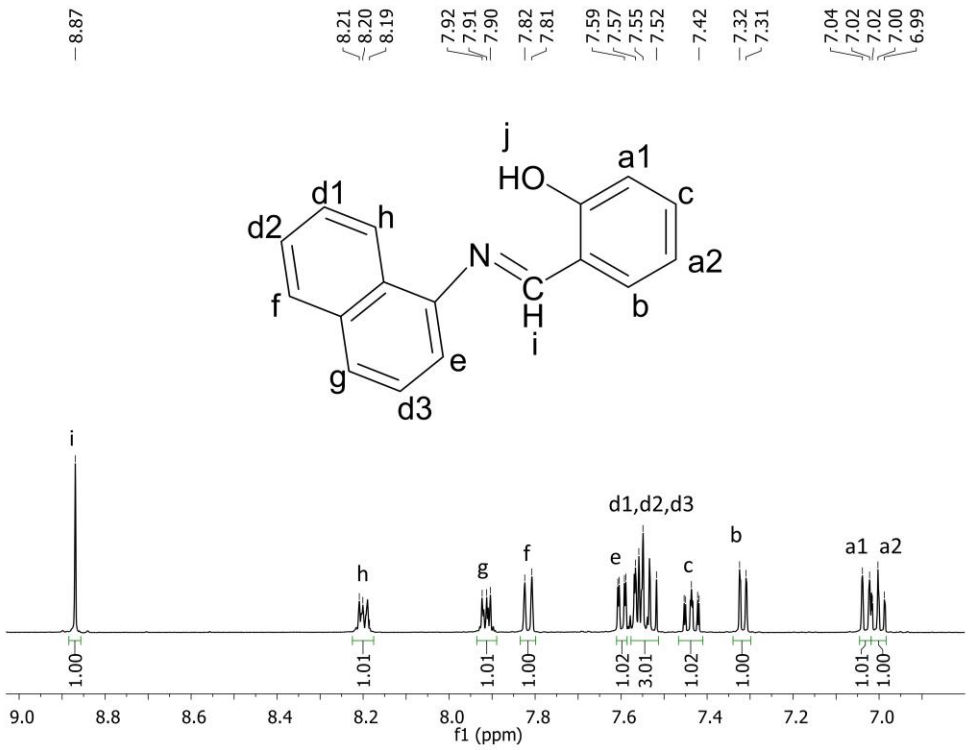


Fig. S1 (a) ^{1}H NMR (500 MHz) of **NAPSAL** in CD_3OD with chemical shifts from 6.8-9.0.

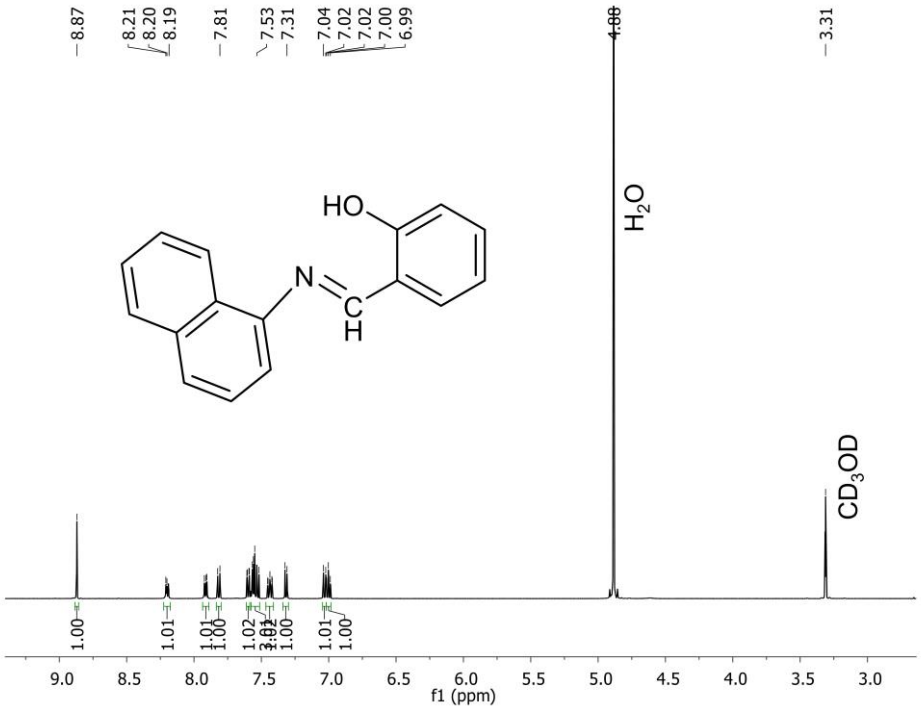


Fig. S1 (b) ^{1}H NMR (500 MHz) of **NAPSAL** in CD_3OD (full spectra).

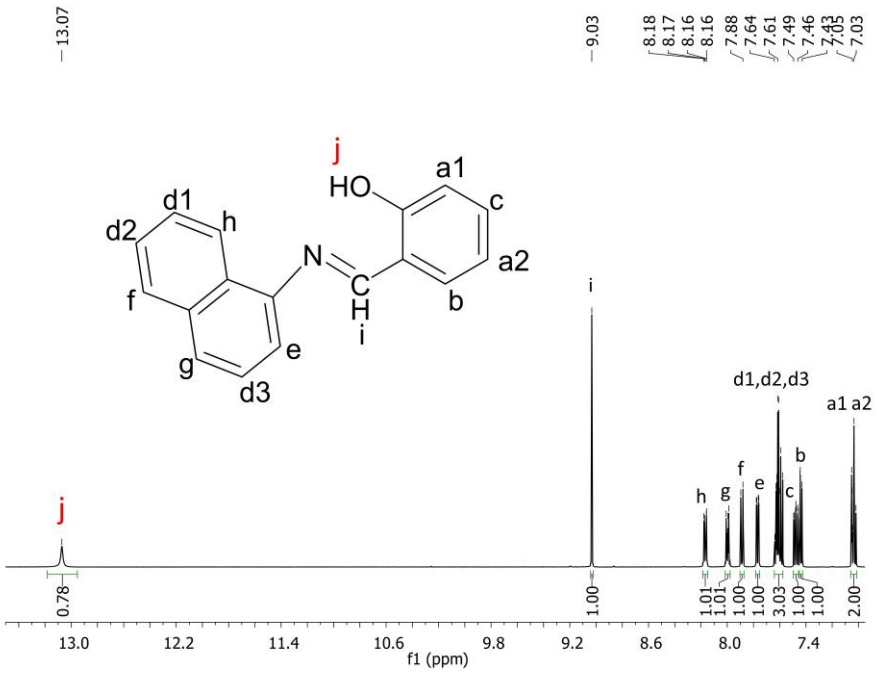


Fig. S2 (a) ^{1}H NMR (500 MHz) of **NAPSAL** in $(CD_3)_2SO$ with chemical shifts from 6.8-13.5. Peak j disappears immediately when D_2O is added, which confirms that peak j is a labile OH proton.

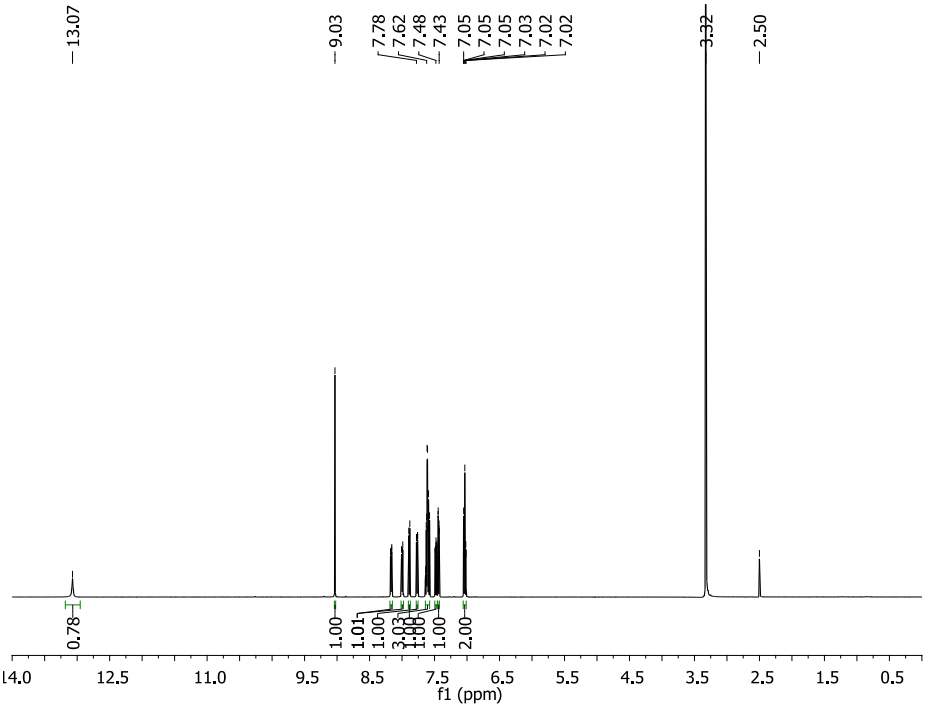


Fig. S2 (b) ^{1}H NMR (500 MHz) full spectrum of **NAPSAL** in $(CD_3)_2SO$ with chemical shifts.

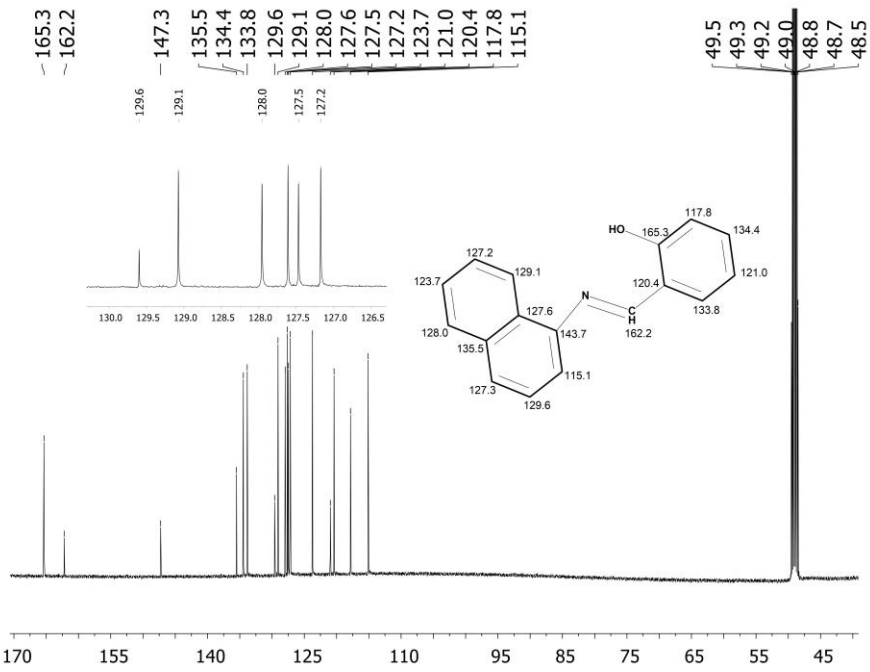


Fig. S3 ^{13}C NMR (500 MHz) spectrum of **NAPSAL** in CD_3OD with suggested assignments.

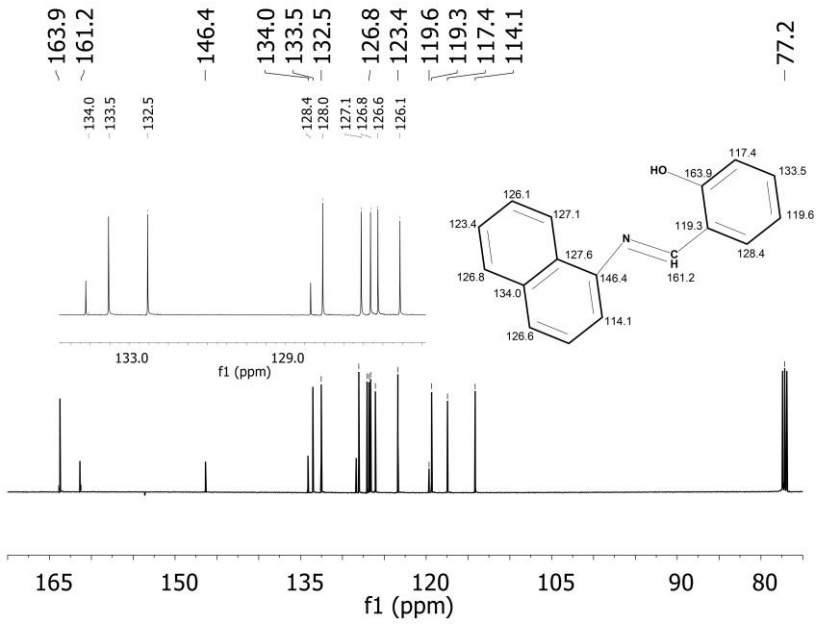


Fig. S4 ^{13}C NMR (500 MHz) spectrum of **NAPSAL** in $CDCl_3$ with suggested assignments.

2.2 pH effect on the hydrolysis of **NAPSAL**

T. K. Chondhekar, et al. have reported that pH plays an important role in the hydrolysis of Schiff bases of salicylaldehyde and that significant hydrolysis may occur near neutral pH.[2] We have tested the effect of pH on the hydrolysis of NAPSAL in this study. As shown in Fig S5, the hydrolysis process is slow in the presence of arsenate because the acidity of KH_2AsO_4 is weak (Fig S5 (b) and (c)). In contrast, the hydrolysis process is much faster in the presence of 0.5 equivalent of TFA (Fig S6 (b)), and some unidentified peaks appear in the presence of 1 equivalent of TFA (Fig S6 (c)) due to secondary decomposition. These NMR experiments were done in mixed solvents with lower concentrations of water, due to the low solubility of NAPSAL. UV-vis and fluorescence experiments described below show that hydrolysis is more extensive and faster in the 90% aqueous media used in the reported arsenate analyses.[1]

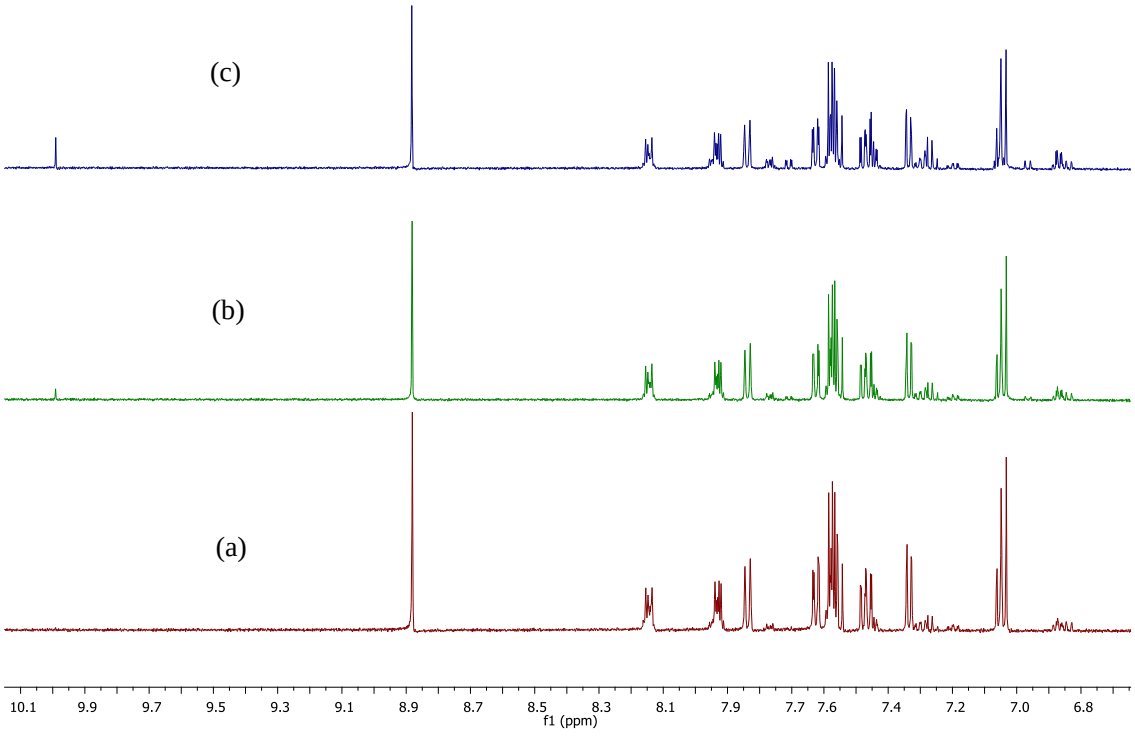


Figure S5 ^{1}H NMR spectra of 2 mM NAPSAL in D_2O and CD_3OD solvent mixtures (1:5), (a) spectra measured immediately after sample preparation; (b) addition of approximately 1equivalent of KH_2AsO_4 to the NAPSAL solution; (c) spectra measured 2 hours after addition of KH_2AsO_4. The sharp downfield peak that appears is attributed to salicylaldehyde and has been confirmed by addition of authentic sample (see Fig.2 in manuscript).

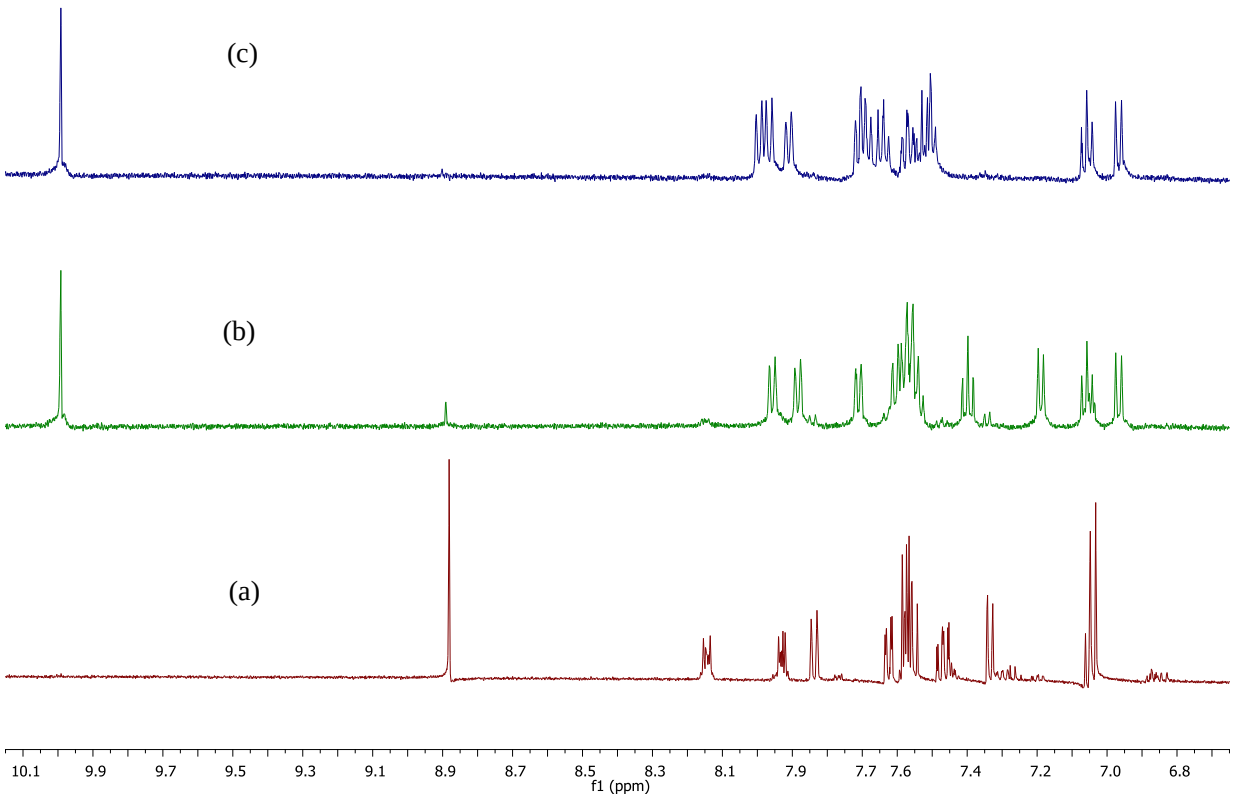


Figure S6 ^{1}H NMR spectra of 2 mM NAPSAL in D_2O and CD_3OD solvent mixtures (1:5), (a) spectra measured immediately after sample preparation; (b) addition of approximately 0.5 equivalent of TFA to the NAPSAL solution causing immediate and nearly complete hydrolysis; (c) addition of approximately 1 equivalent of TFA to the NAPSAL solution, which causes further reactions of the hydrolysis products.

2.3 HR-ESI-TOFMS spectra of NAPSAL

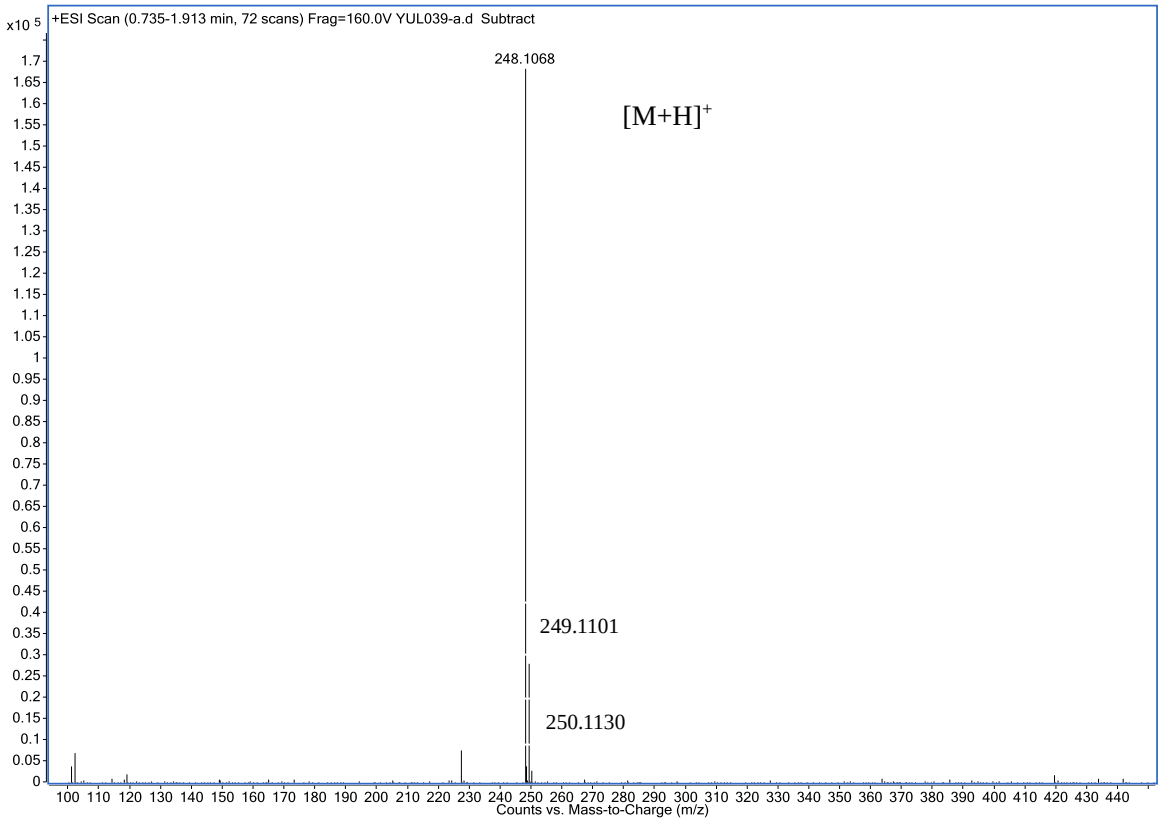


Fig. S7. HR-ESI-TOFMS spectra of **NAPSAL** in MeOH. Calculated M+1 = 248.10

3. Change of photophysical properties of NPSAL in aqueous solution

Besides ^{1}HNMR spectra, we also examined the hydrolysis process of NAPSAL in the 90% aqueous conditions used in the reported arsenate analyses by using UV-vis and fluorescence spectra. In an acidic environment, 10 μM of NAPSAL decomposed in less than one minute due to high ratio of water (90%) in the solvent mixtures, as shown in the UV-Vis spectra (Fig. S8) by the prominent UV absorption (~325 nm) of salicylaldehyde. At a near neutral pH 7.4 buffered environment, 10 μM of NAPSAL decomposed significantly in less than two minutes in the solvent mixtures, as shown by the growth of fluorescence due to salicylaldehyde (Fig. S9). This further confirms that the conditions reported in the literature[1] are unsuitable for using NAPSAL as a fluorescent sensor in aqueous solution due to hydrolysis, which produces a product (salicylaldehyde) that is much more fluorescent than NAPSAL itself.

3.1 Time dependent hydrolysis of NAPSAL monitored by UV-vis spectroscopy (at pH 5.2) and fluorescence spectroscopy (pH 7.4).

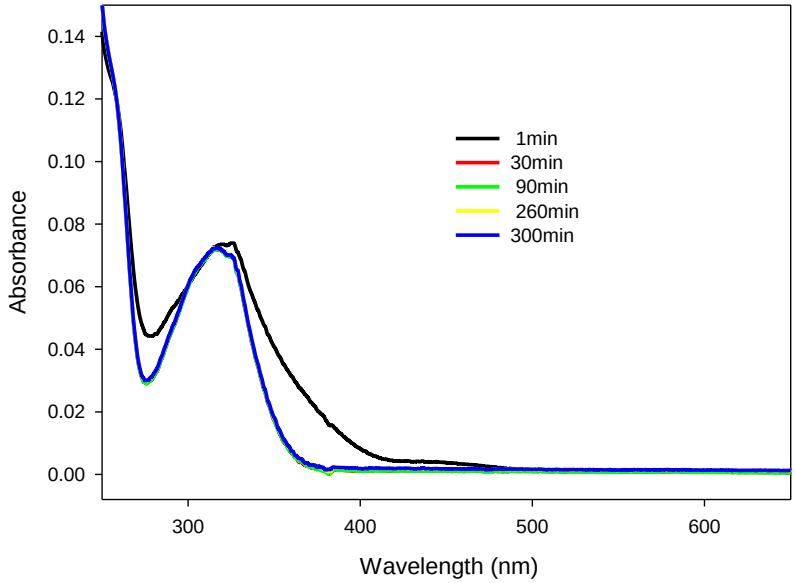


Fig. S8 Time dependent change of absorption spectra of NAPSAL (10 μM) in aqueous solution (ethanol/water = 1/9, v/v pH=5.2). After 1 min the spectrum shows nearly complete conversion to salicylaldehyde. The curves after 30 min are essentially superimposed due to the end of the reaction.

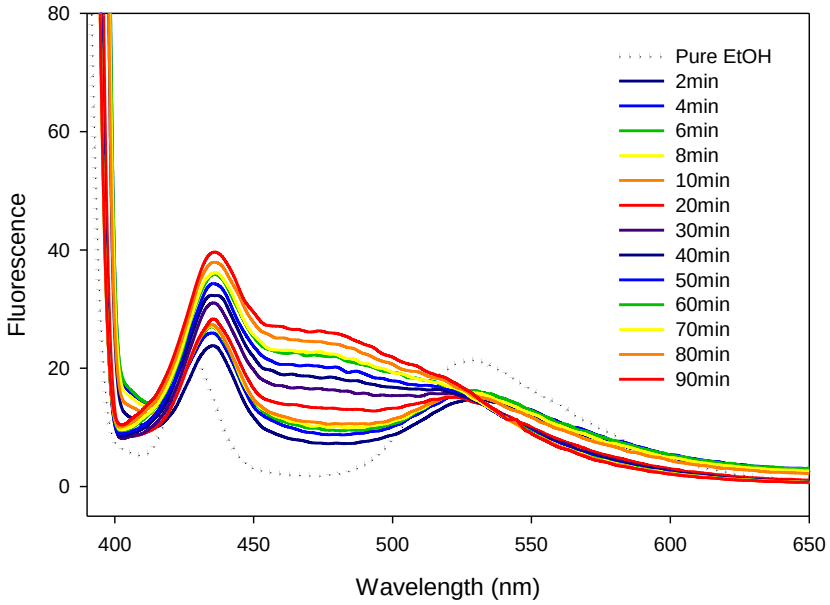


Fig. S9 Time dependent change of the fluorescence spectra of NAPSAL (10 μM) in aqueous solution (ethanol/water = 1/9, v/v pH=7.4 buffer) as hydrolysis occurs. The spectrum in pure ethanol is also shown, which reflects the intrinsic emission of NAPSAL in the absence of water and significant hydrolysis.

3.2 Emission spectral profile of a NAPSAL aqueous solution in the presence of a different source of arsenate.

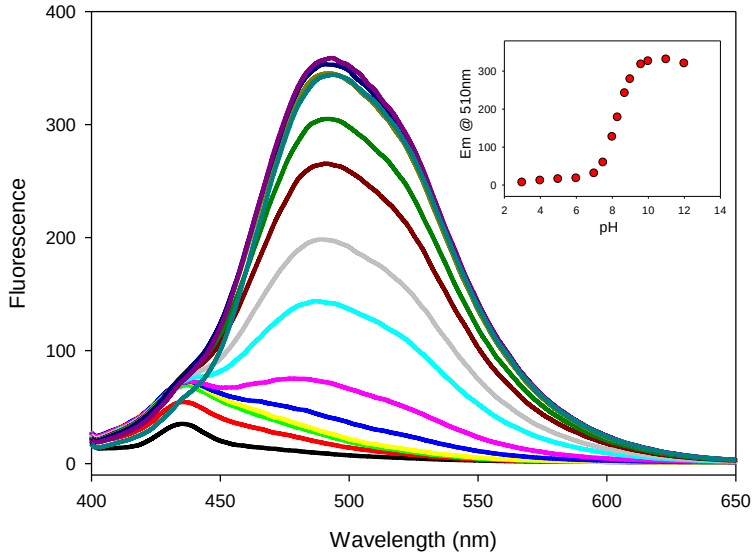


Fig. S10(a) Change of fluorescence spectra of NAPSAL (10 μM) in the presence of 0.8mM KH_2AsO_4 (supplier Sigma Aldrich) upon variation of pH from 3 to 12. Spectra were measured immediately after sample preparation. The pH was initially adjusted with HCl (0.5M) and then titrated with KOH (0.5M). Hydrolysis is nearly complete by 1 min.

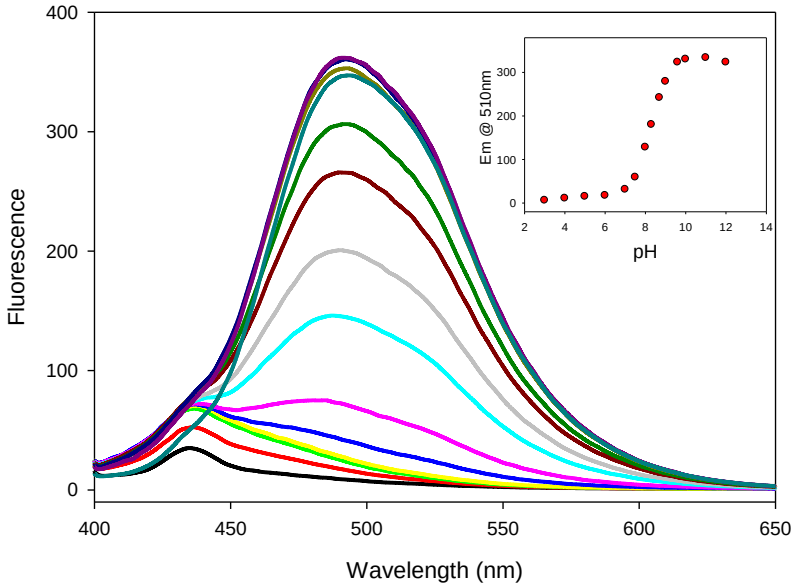


Fig. S10 (b) Change of fluorescence spectra of NAPSAL (10 μM) in the presence of 0.8mM $Na_2HAsO_4{\cdot}7H_2O$ (supplier Alfa Aesar) upon variation of pH from 3 to 12. Spectra measured immediately after sample preparation. The pH was initially adjusted by HCl (0.5M) and then titrated with KOH (0.5M). Again hydrolysis is nearly complete by the first spectrum.

3.3 Reproducing the pH dependent emission profile of NAPSAL aqueous solution with a mixture of authentic hydrolysis products.

The emission profile of NAPSAL aqueous solution can be reproduced by mixing a 1:1 ratio of commercial salicylaldehyde and 1-naphthylamine (Fig. S11), which provides further evidence that NAPSAL primarily undergoes hydrolysis to starting materials in aqueous solution at the starting value of the titration. In addition, the addition of phosphate to a NAPSAL aqueous solution (Fig. S12) also generates emission profiles similar to the literature, even though no arsenate is present.[1]

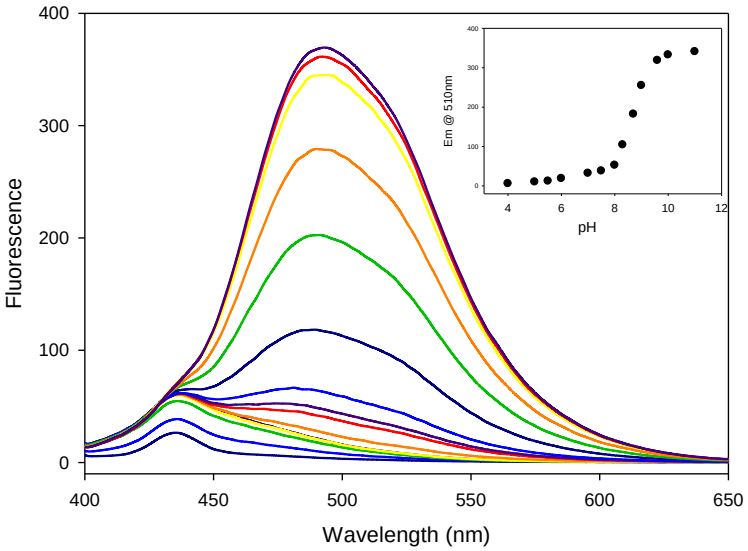


Fig. S11 Emission spectra of a mixture of salicylaldehyde and 1-naphthylamine (1:1 ratio) (10 μM) on varying the pH from 3 to 12; inset shows emission at 510 nm. Spectra were measured immediately after sample preparation.

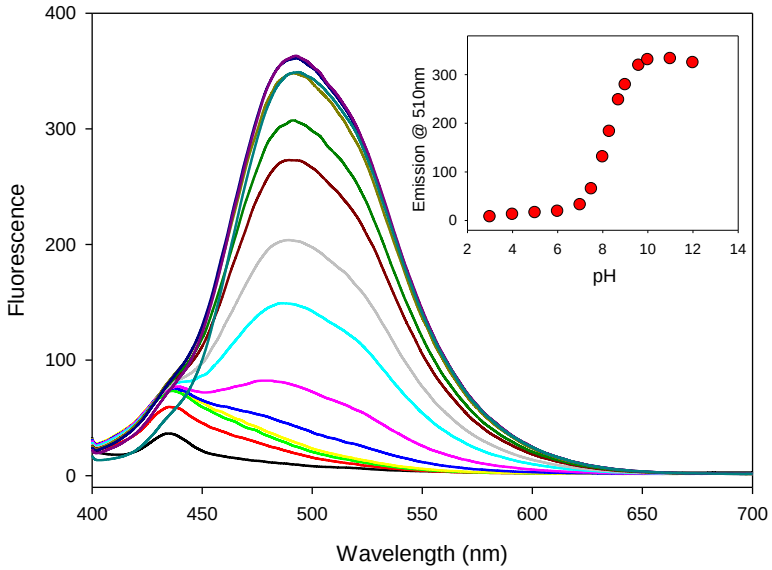


Fig. S12 Change of fluorescence spectra of NAPSAL (10 μM) in the presence of **NaH_2PO_4 (**0.8mM**)** on varying the pH from 3 to 12. The pH was initially adjusted with HCl (0.5M) and then titrated with KOH (0.5M). Spectra were measured immediately after sample preparation.

3.4. pH dependent absorption of NAPSAL

The absorption spectra of a NAPSAL solution was measured as the solution's pH is varied from 5 to 12. As is shown in Fig. S13, the isosbestic points in the spectra indicated that there is an acid/base equilibrium in NAPSAL solution as pH varies from acidic to basic. It is possible to reverse the process with negligible hysteresis by adding acid and reversing the titration. The titrated spectra are similar as reported in the supporting information of the original paper (Fig. S10 in ESI).[1]

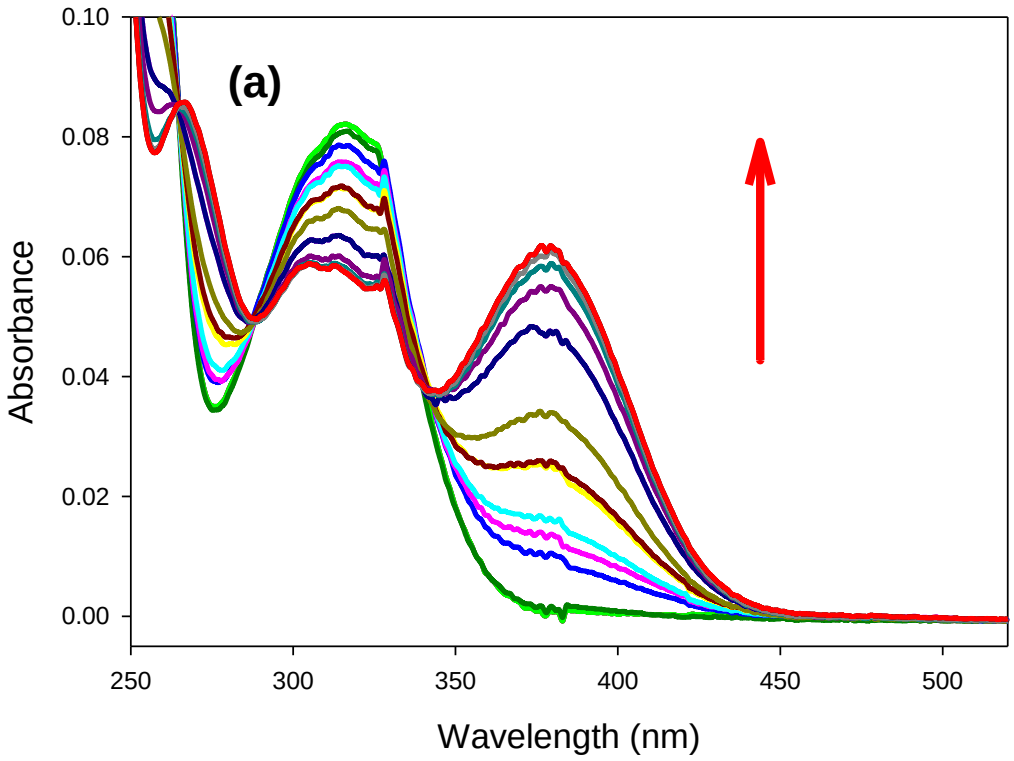


Fig. S13 Change of absorption spectra of NAPSAL (10 μM) in aqueous solution (ethanol/water = 1/9, v/v) on varying the pH from 5 to 12. The pH was initially adjusted with HCl (0.5M) and then titrated with KOH (0.5M). Recall that at pH 5 the NAPSAL is hydrolyzed by the time the first spectrum could be obtained (about 1-2 minutes).

4. Determination of the pKa of the NAPSAL hydrolysis solution

The fluorescence titration curve was modeled using the Henderson–Hasselbalch equation: $pKa = pH+\log[(F_{max}-F)/(F-F_{min})]$ where F is the fluorescence intensity at a certain wavelength, and F_{max} and F_{min} are the corresponding maximum and minimum values, respectively.[3] This yielded a pKa of 8.3 (±0.1) for the NAPSAL hydrolysis solution, which is within experimental error of the value of 8.2 (±0.1) obtained in Figure

S11 for the 1:1 mixture of salicylaldehyde and 1-naphthylamine. These values in 90% water are close to the reported pKa of aqueous salicylaldehyde itself (8.4).[4] This further confirms that NAPSAL undergoes hydrolysis to starting materials (salicylaldehyde and 1-naphthylamine), and that the pH spectral behavior is dominated by the acid/base equilibrium of salicylaldehyde.

5. References

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