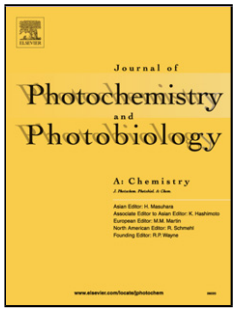


Title: Triggered emission for rapid detection of hydrogen sulfide chaperoned by large Stokes shift

Authors: Bidisha Biswas, M. Venkateswarulu, Pankaj Gaur, Yamini Sharma, Sougata Sinha, Subrata Ghosh

PII: S1010-6030(18)31300-5
DOI: https://doi.org/10.1016/j.jphotochem.2018.11.011
Reference: JPC 11583

To appear in: *Journal of Photochemistry and Photobiology A: Chemistry*

Received date: 5 September 2018
Revised date: 12 October 2018
Accepted date: 7 November 2018

Please cite this article as: Biswas B, Venkateswarulu M, Gaur P, Sharma Y, Sinha S, Ghosh S, Triggered emission for rapid detection of hydrogen sulfide chaperoned by large Stokes shift, *Journal of Photochemistry and amp; Photobiology, A: Chemistry* (2018), https://doi.org/10.1016/j.jphotochem.2018.11.011

Triggered emission for rapid detection of hydrogen sulfide chaperoned by large Stokes shift

Bidisha Biswas,[a] M. Venkateswarulu,[a] Pankaj Gaur,[a] Yamini Sharma,[a] Sougata Sinha,[b,*] and Subrata Ghosh[a,*]

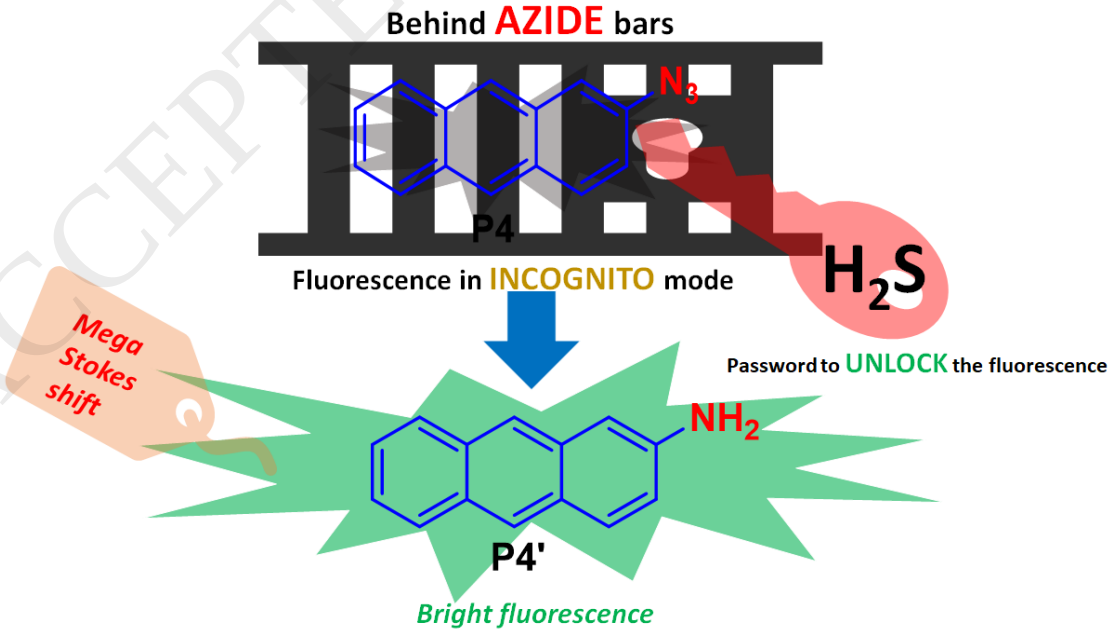
[a]*School of Basic Sciences, Indian Institute of Technology Mandi, Mandi-175001, H.P, India*

[b]*Department of Chemistry, Nalanda College of Engineering, Chandi- 803108, Bihar, India*

[*]Corresponding author: Tel. +91-1905-267069; Fax: +91-1905-237924 (S. Ghosh)

E-mail address: subrata@iitmandi.ac.in (S. Ghosh)sinhasougatachemistry@gmail.com (S. Sinha)

Graphical Abstract



Highlights:

- Reaction-based 'turn-on' fluorescent probe for optical detection of H_2S;
- One step synthesis with good yield;
- Guided by mega-Stokes shift;
- Low limit of detection and limit of quantification;
- High selectivity over biothiols, bio-relevant cations and anions;

Abstract

Current manuscript demonstrates the development of a reaction-based "turn-on" green emissive molecular probe (**P4**) for rapid detection of H_2S in physiological condition. The probe was found to exhibit low limit of detection (1.19 μM), along with high selectivity, large Stokes shift andgood quantum efficiency. The change in the optical behaviour of the probe was also theoretically scrutinized employing density functional theory calculations which stand in the strong agreement with the photophysical outcomes. Given the demand for large Stokes dyes for sensing applications mainly because of their special photophysical properties, and as the reported number is very limited, the present probe with 110 nm Stokes shift is a good addition in the area of large Stokes dyes for H_2S recognition in physiological conditions.

Keywords: Fluorescent probe, Hydrogen sulfide, large Stokes shift, Anthracene, Azide reduction

1. Introduction

The volatile organosulfur species are well identified for their characteristic obnoxious odour and considered to be toxic to human health as well as to the environment [1-3]. Amongst these, hydrogen sulfide is the most commonmalodorousgas and often found to be associated with numerous biological/physiological activities [1-14]. Literature reports also evidenced the close attachment of diseases such as Alzheimer, Down's Syndrome, liver cirrhosis, diabetes, pulmonary hypertension etc. with H_2S concentration [8, 13-16]. Hence, efforts are being made to get better insights of its physio-pathological importance and in this context, development of simple cost-effective tools for monitoring H_2S level in environmental/biological sample is on urgent demand.

Even though, a number of techniques are currently existing for detection of H_2S, such as colorimetric [11, 17, 18], metal induced sulfide precipitation [19], electrochemical [20-22] and chromatographic [23, 24] assays but the latter require a lot of sample processing, often found to be destructive and not been able to provide much spatiotemporal profiling in native biological system. As a result of continuous efforts, fluorescence based biomolecular detection has grown as an efficient and attractive technique owing to its ease of handling, more accuracy and better sensitivity. Recently, a number of excellent azide, nitro, azamacrocyclic copper (II) complex and Michael acceptors based fluorescent probes have been developed and used for detection of H_2S [25-59]. However, the selective tracking and real-time analysis of H_2S in environmental/biological samples is still a hurdle. This is because of some of the reported probes are wrapped up with disadvantages such as lengthy multi-step synthesis procedures with low yields, strong background fluorescence, low quantum yield and notable affinity toward other biorelevant analytes such as cysteine, homocysteine, glutathione etc. It has been well established that for being a smart molecular probe, small

molecular markers should not only display bright and fast fluorescence response but at the same time should also exhibit long-range excitation/emission wavelengths (excitation wavelength >400 nm and emission wavelength above 500 nm) to avoid awful issues associated with cell damage and autofluorescence. Furthermore, large Stokes shift is another crucial desirability that a smart molecular probe should own. It substantially minimizes/diminishes the overlap between excitation and emission spectra leading to the depletion of reabsorption of emitted photons which in consequence helps to get rid of background interferences. Another advantage of having large Stokes shift is better sensitivity and more accuracy in detection [27, 60-64]. Although several H_2S markers have been witnessed in literature but the markers having large Stokes shift (> 100 nm) are very small in number and hence demands more attention [27, 30, 32-36, 39, 40, 46].

Carrying forward our research interests in developing smart molecular markers for H_2S,[40, 41] herein, we wish to report our recent study on facile fabrication of an azide-based fluorescent probe for the detection of H_2S chaperoned by 110 nm Stokes shift. 2-azidoanthracene along with few of derivatives were synthesized and utilized for developing various fluorophores or fluorescent chemical sensors, however, to our knowledge, application of 2-azidoanthracene as a molecular marker for H_2S detection has not yet been reported [65-68]. It is worth mentioning that the developed probe exhibited excellent selectivity toward H_2S over various competitive sulfide derivatives.

2. Experimental

2.1.General information:

All chemicals were purchased from Merck, S.D. Fine and Sigma Aldrich, and were used without any further purification. HPLC gradeDMSOwas used for the spectral studies.Freshly prepared solutions of anions (Cl^-, F^-, Br^-, NO_3^-, PO_4^{3-} and AcO^- as tetrabutylammonium salt, I^-, HSO_3^-, SCN^-, $S_2O_3^{2-}$ and $S_2O_5^{2-}$as sodium salt), cations (Na^+, K^+,

Ca^{2+} and Mg^{2+} as nitrate salt), cysteine, homocysteine, glutathione and H_2S (as NaHS) in H_2O (1 mM HEPES, pH = 7.2) were used as stock solutions to record the UV-vis and fluorescence spectra. FT-IR spectra were acquired in a Carry-660 FT-IR spectrometer in ATR mode. ^1H and ^{13}C NMR spectra in DMSO-d_6 were recorded on Jeol-ECX-500 MHz spectrometer using tetramethylsilane as an internal standerd. Absorption spectra and fluorescence spectra were recorded on PerkinElmer Lambda 750and Cary Eclipse spectrophotometer respectively. HRMS-ESI spectra were recorded on a Bruker impact-HD spectrometer.

2.2. Synthesis:

Synthetic procedure for ***P4:*****P4** was synthesized following a previously known literature report [65].2-Aminoanthracene (0.730 g, 3.77 mmol) was taken in water (10 mL) in a two-necked round-bottom flask and stirred at room temperature for 5 min. The reaction mixture was cooled to 0 °C and concentrated H_2SO_4 (5 mL) was added to the suspension very carefully with constant stirring. The whole reaction mixture was allowed to stir at 0 °C for 20 min followed by dropwise addition of $NaNO_2$ (0.332 g, 4.82 mmol) in water (10 mL). The resultant red coloured solution was stirred at 0 °C for 30 min. Next, NaN_3 (439 mg, 6.76 mmol) was dissolved in water (10 mL) and added dropwise over 10 min to the previously stirred mixture. The resulting solution mixture was then slowly warmed to room temperature and stirred for another 3 h. Brown precipitation was observed, filtered through a sintered funnel, washed with water (20 mL) and air dried for 1 h. The crude product was purified by column chromatography (ethyl acetate/hexane = 2:8, v/v) to afford a yellow solid.

Yield: 59% (0.500 g). Melting point: 156-160 °C; FT-IR (ATR, ν in cm^{-1}): 2112-2130;^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.58 (s, 1H), 8.52 (s, 1H), 8.15 (d, J = 8.9 Hz, 1H), 8.07- 8.03 (m, 2H), 7.79 (s, 1H), 7.52 - 7.50 (m, 2H), 7.26 (dd, J = 8.9, 2.05 Hz, 1H);^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 136.6, 131.9, 131.4, 130.9, 130.7, 129.1, 128.3, 127.9,

126.6, 126.3, 125.6, 125.1, 119.5, 115.1; HRMS: *m/z* calculated for $C_{14}H_9N_3$ $[M]^+$ 219.0796, found 219.0795;$C_{14}H_9N_3Na$ $[M+Na]^+$ 242.0694, found 242.2885.

*Synthesis procedure for **P4'**:*Compound **P4** (67 mg, 0.32 mmol) was dissolved in THF followed by addition of NaHS solution (119 mg, 2.14 mmol) in 3 mL H_2O dropwise with constant stirring at room temperature. The reaction mixture was further stirred at room temperature for 2 h and the solvent was removed under reduced pressure. The obtained residue was purified by column chromatography (ethyl acetate /hexane = 4:96, v/v)to furnish**P4'** as yellow solid.

Yield: 61% (35 mg); FT-IR (ATR, ν in cm^{-1}): 3444, 3361 ;^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 8.27 (s, 1H), 8.02 (s, 1H), 7.90 - 7.85 (m, 2H), 7.80 (d, J = 8.9 Hz, 1H), 7.37 - 7.34 (m, 1H), 7.29 -7.26 (m, 1H), 7.04 (dd, J = 8.95, 2.05 Hz, 1H), 6.88 (s, 1H), 5.57 (s, 2H); ^{13}C NMR (DMSO-d_6, 125 MHz, ppm): δ 146.09, 133.69, 131.94, 128.99, 128.43, 128.13, 127.07, 126.57, 125.83, 125.17, 123.12, 120.96, 120.90, 102.72; HRMS: *m/z* calculated for $C_{14}H_{12}N$ $[M+H]^+$ 194.0970, found 194.0964.

2.3.UV-vis and fluorescence titrations:

UV-vis and fluorescence titrations were conducted using 50 µM solutions of probe (**P4**) in DMSO: H_2O solution (1:1, v/v).H_2O was used in form of 1 mM HEPES buffer of pH = 7.2. All measurements were performed using 395 nm as an excitation wavelength and keeping both excitation and emission slit widths as 5at 37 °C. UV-vis and fluorescence spectra were recorded using 3 mL quartz cuvette (path length 1 cm) by addition of aliquots of freshly prepared stock solutions of various analytes such as Cl^-, F^-, Br^-, I^-, NO_3^-, PO_4^{3-}, AcO^-, HSO_3^-, SCN^-, $S_2O_3^{2-}$, $S_2O_5^{2-}$, Na^+, K^+, Ca^{2+}, Mg^{2+}, cysteine, homocysteine and glutathione. NaHS solution was used as a source of H_2S (50 mM stock solution).UV-vis and fluorescence

titrations were conducted taking aliquots of highly concentrated stock solutions of various analytes to avoid dilution error.

2.4.DFT calculations

The geometry of the compounds (**P4** and amine **P4′**) was optimized at density functional theory (DFT) with B3LYP/6-31G(d) basis set [69, 70] with no symmetry constraint using Gaussian 09 suite of programmes [71]. Frequency calculation at the same level with the same basis set was performed to ensure that the geometries correspond to real minima. The solvent effect was incorporated employing conducting polarisable continuum model (CPCM model) [72]. Water was chosen as solvent to optimize the geometry and for TD-DFT calculation. Gauss view software along with Chemcraft was used for data visualization and preparation purpose [73].

3. Results and discussion

3.1. Synthesis

Probe **P4** was synthesized in a single step by using 2-aminoanthracene following the experimental protocols as outlined in Scheme 1. The chemical structures of **P4** and **P4′**were characterized using various spectroscopic techniques such as FT-IR, NMR spectroscopy and high-resolution mass spectrometry (HRMS).

3.2. Photo-physical studies

The photophysical properties of**P4** were investigated by absorption/emission spectroscopy in 50% aqueous DMSO solution buffered with HEPES (1 mM, pH = 7.2). As shown in Fig. 1, initially **P4**(5 x 10^{-5} M) showed one strong absorption band centred at 276nmand a broad band ranging from 314 nm to 431 nm having peaks at 341, 357, 373, 394and 418 nm with variable intensity due to π-π* and n-π* transitions of aromatic rings and the azide (-N_3) group respectively. Upon introduction of H_2S (50 x 10^{-5} M), 13 nm blue shift in 276 nm was observed along with even broader absorption band ranging from 315 to 470

nm (absorption peaks at 321 nm, 338 nm, 355 nm, 374 nm, 394 nm and 418 nm) (Fig. 1, S1). On the contrary, the absorption profile of **P4** remained almost unaltered in the presence of various bio-analytes such as Cl^-, F^-, Br^-, I^-, NO_3^-, PO_4^{3-}, AcO^-, HSO_3^-, SCN^-, $S_2O_3^{2-}$, $S_2O_5^{2-}$, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cys, Glu and Homo Cys (Fig. 1a).

In the absence of H_2S, **P4** was almost non-fluorescent in nature (Fig. 2). The silent optical behaviour was attributed to the photoinduced electron transfer (PET) from excited fluorophore (anthracene) to the strong electron-withdrawing group (azide unit) which is also popularly known as donor-excited- PET (d-PET)[28].Upon exposure to H_2S,a significant fluorescence turn-on response was observed at 505 nm (quantum yield: 0.274, quinine sulphate was used as standard)due to the stoppage of d-PET process. The fluorescence response was guided by 110 nm Stokes shift (Fig. 3).

H_2S instigated fluorescence enhancement and occurrence of multiple isobestic points (268, 291, 341, 384 and 398 nm) (inset Fig. 1b) directed us to envision the reduction of azide (**P4**) to amine (2-aminoanthracene, **P4′**) in the presence of H_2S.It is important to note that **P4** was allowed to react with H_2S at 37 °C for 40 minutes to achieve optimum response (Fig. S2). This is due to the slower rate of H_2S induced reduction at room temperature (25 °C) (Fig. S2).

Further, time dependent fluorescence response experiment was conducted to monitor the reduction kinetics of azide group of **P4**in the presence of NaHS (50 x 10^{-5} M). Although enhancement in fluorescence intensity was instantaneous to the addition of NaHS (~10% enhancement), it took around 40 min to obtain a stable/saturated fluorescence signaling (Fig. 2b). The pseudo first order rate constant (k_{obs}) and time required for 50% completion ($t_{1/2}$) of the reaction was found to be 1.26 x 10^{-3} sec^{-1} and 550 sec respectively (Fig. S3). This data revealed the quick response time of **P4** toward H_2S along with total time required for complete reduction of azide group.

Fascinated by the initial photophysical outcomes, we became curious to check whether any other relevant analyte has the similar potential of displaying the fluorescence enhancement brought by H_2S. In this regard, probe **P4** was treated with wide variety of anions (Cl^-, F^-, Br^-, I^-, NO_3^-, PO_4^{3-}, AcO^-, HSO_3^-, SCN^-, $S_2O_3^{2-}$ and $S_2O_5^{2-}$), cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) and biothiols (homocysteine, glutathione and cysteine).Satisfyingly, probe **P4** remained silent in the presence of all those analytes, however it displayed bright green emission once H_2S was added to the solution (Fig. 4, S4). Therefore, probe **P4** established its high selectivity toward H_2S over other various relevant competitors. The mechanistic pathway for H_2S instigated reduction of 2-azidoanthracene (**P4**) was adopted from a recent report by Pluth Group (Scheme 2) [56]. Reduction of azide functionality to an amine one is chiefly controlled by high nucleophilicity of HS^- and its subsequent attack at the electrophilic group like $-N_3$ [56]. Besides being the smallest unit of thiol community, HS^-could also approach the electrophilic centre overlooking any kind of steric hindrance unlike other thiols such as cysteine, homocysteine and glutathione [59]. Next, limit of detection (LOD) and limit of quantification values (LOQ) were calculated using 3σ/slope and 10σ/slope methods [74] and those values were found to be 1.19 µM and 3.99 µM respectively (Fig. S5), which strongly validated the sensitivity of **P4** toward H_2S.

3.3.Spectroscopic investigations for azide reduction

Further to corroborate our assumption that exposure of H_2S led to the reduction of **P4** to **P4′**, ^{1}H-NMR and mass spectroscopic studies were carried out. During ^{1}H-NMR experiments, appearance of a sharp resonance signal at 5.56 ppm in the isolated product after exposure of $H_2S$was attributed to the $–NH_2$ protons of **P4′**(Fig. 5).It was further supported by the similar NMR spectra of anthracene amine(Fig. S6, S7).

Similarly, the mass spectra of **P4** in the presence of H_2S showed the disappearance of molecular ion peak at 219.0795, sodium adduct peak at 242.2855 and the appearance of distinct peak corresponding to anthracene amine at 194.0964 $[M+H]^+$(Fig. S8). In combination, the outcomes of ^{1}H-NMR and mass experiments strongly supported our speculation that the azide functionality of **P4** underwent reduction to produce the amine derivative **P4′** (Scheme 2).

3.4.Theoretical studies

Finally, to understand the probable structures of **P4, P4′** and simulate their absorption spectra in water as a polar medium, theoretical analyses were carried out employing density functional theory (DFT). Optimized geometry of **P4** and the frontier molecular orbitals are depicted in Fig. 6a. While the HOMO-1, HOMO and LUMO are distributed throughout the **P4** derivative, LUMO+1 is located on the azido functionality (Fig. 6a). This is due to the electron withdrawing effect of azido functionality which facilitates electron transfer from the anthracene moiety to the azido functionality resulting in generation of non-fluorescent azido derivative. In the continuation, geometry of the amine derivative was also optimized (Fig. 6b) and the contribution of molecular orbitals was evaluated. HOMOs and LUMOs are distributed throughout the amine containing anthracene system (Fig. 6b). Time dependent-DFT calculations (TD-DFT) have been performed to correlate the absorption maxima of the azide and amino derivative (Fig. S9, S10 and Table S1). Theoretically simulated absorption profiles of **P4**/**P4′** were in close agreement with the experimentally observed absorption spectra (Fig. S9, S10 and Table S1).

4. Conclusions

To conclude, we have successfully developed following a simple one step synthesis process an anthracene-based molecular probe (**P4**) for the detection of H_2S. LOD and LOQ values for H_2S sensing were found to be 1.19 µM and 3.99 µM respectively. The probe **P4** was found to be non-fluorescent initially due to d-PET process. Interaction with H_2S led to the reduction of azide functionality to the amine which in turn blocked the fluorescence quenching through d-PET process and generated the concealed emission. This emerging bright green emission was also chaperoned by 110 nm Stokes shifts. The H_2S instigated azide reduction was evidenced by different spectroscopic studies and photo-physical outcomes were further validated by TD-DFT calculations. **P4** being a non-emissive molecular material, background fluorescence issue was successfully eliminated. We strongly feel that the probe **P4** could be very handy, highly selective and efficient for monitoring H_2S level in various environmental and biological samples.

Acknowledgement

Financial support was received from the Department of Science and Technology, India (Grant No. SERB/F/2408/2012-13). SS is thankful to the National Project Implementation Unit, MHRD for TEQIP-III faculty programme. We thankfully acknowledge the Director, IIT Mandi for research facilities. The support of Advanced Materials Research Center (AMRC), IIT Mandi, for sophisticated instrument facility is thankfully acknowledged. We are thankful to the reviewers for their valuable suggestions.

References

[1] R. Wang, The gasotransmitter role of hydrogen sulfide, Antioxid. Redox Signal. 5 (2003) 493-501.

[2] Y. Han, J. Qin, X. Chang, Z. Yang, J. Du, Hydrogen sulfide and carbon monoxide are in synergy with each other in the pathogenesis of recurrent febrile seizures, Cell. Mol. Neurobiol. 26 (2006) 101-107.

[3] L. Li, P. Rose, P.K. Moore, Hydrogen sulfide and cell signaling, Annu. Rev. Pharmacol. Toxicol. 51 (2011) 169-187.

[4] J.W. Elrod, J.W. Calvert, J. Morrison, J.E. Doeller, D.W. Kraus, L. Tao, X. Jiao, R. Scalia, L. Kiss, C. Szabo, H. Kimura, C.-W. Chow, D.J. Lefer, Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 15560-15565.

[5] C. Szabó, Hydrogen sulphide and its therapeutic potential, Nat. Rev. Drug Discov. 6 (2007) 917-935.

[6] H. Kimura, Hydrogen sulfide: its production, release and functions, Amino Acids 41 (2011) 113-121.

[7] K. Abe, H. Kimura, The possible role of hydrogen sulfide as an endogenous neuromodulator, J. Neurosci. 16 (1996) 1066-1071.

[8] Y. Wei, Y. Guangdong, J. Xuming, W. Lingyun, W. Rui, Activation of KATP channels by H_2S in rat insulin secreting cells and the underlying mechanisms, J. Physiol. 569 (2005) 519-531.

[9] G. Yang, L. Wu, B. Jiang, W. Yang, J. Qi, K. Cao, Q. Meng, A.K. Mustafa, W. Mu, S. Zhang, S.H. Snyder, R. Wang, H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine γ-Lyaseas, Science 322 (2008) 587-590.

[10] Y.-J. Peng, J. Nanduri, G. Raghuraman, D. Souvannakitti, M.M. Gadalla, G.K. Kumar, S.H. Snyder, N.R. Prabhakar, H_2S mediates O_2 sensing in the carotid body, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 10719-10724.

[11] M.N. Hughes, M.N. Centelles, K.P. Moore, Making and working with hydrogen sulfide: The chemistry and generation of hydrogen sulfide in vitro and its measurement in vivo: A review, Free Radic. Biol. Med. 47 (2009) 1346-1353.

[12] N. Dufton, J. Natividad, E.F. Verdu, J.L. Wallace, Hydrogen sulfide and resolution of acute inflammation: A comparative study utilizing a novel fluorescent probe, Sci. Rep. 2 (2012) 499.

[13] Y. Zhang, Z.-H. Tang, Z. Ren, S.-L. Qu, M.-H. Liu, L.-S. Liu, Z.-S. Jiang, Hydrogen sulfide, the next potent preventive and therapeutic agent in aging and age-associated diseases, Mol. Cell. Biol. 33 (2013) 1104-1113.

[14] A.B. Salmina, Y.K. Komleva, I.A. Szijártó, Y.V. Gorina, O.L. Lopatina, G.E. Gertsog, M.R. Filipovic, M. Gollasch, H_2S- and NO-signaling pathways in Alzheimer's amyloid vasculopathy: synergism or antagonism?, Front. Physiol. 6 (2015) 361.

[15] K. Pierre, B. Maria- Cristina, C. Allel, L. Karim, C.V. Bernadette, Endogenous hydrogen sulfide overproduction in Down syndrome, Am. J. Med. Genet. A 116A (2003) 310-311.

[16] F. Stefano, A. Elisabetta, M. Andrea, O. Stefano, R. Barbara, R. Giovanni, D. Eleonora, S. Vijay, M. Antonio, The third gas: H_2S regulates perfusion pressure in both the isolated and perfused normal rat liver and in cirrhosis, Hepatology 42 (2005) 539-548.

[17] W. Lei, P.K. Dasgupta, Determination of sulfide and mercaptans in caustic scrubbing liquor, Anal. Chim. Acta 226 (1989) 165-170.

[18] D. Jiménez, R. Martínez-Máñez, F. Sancenón, J.V. Ros-Lis, A. Benito, J. Soto, A new chromo-chemodosimeter selective for sulfide anion, J. Am. Chem. Soc. 125 (2003) 9000-9001.

[19] M. Ishigami, K. Hiraki, K. Umemura, Y. Ogasawara, K. Ishii, H. Kimura, A source of hydrogen sulfide and a mechanism of its release in the brain, Antioxid. Redox Signal. 11 (2009) 205-214.

[20] C.J. Richardson, E.A.M. Magee, J.H. Cummings, A new method for the determination of sulphide in gastrointestinal contents and whole blood by microdistillation and ion chromatography, Clin. Chim. Acta 293 (2000) 115-125.

[21] D.G. Searcy, M.A. Peterson, Hydrogen sulfide consumption measured at low steady state concentrations using a sulfidostat, Anal. Biochem. 324 (2004) 269-275.

[22] J.E. Doeller, T.S. Isbell, G. Benavides, J. Koenitzer, H. Patel, R.P. Patel, J.R. Lancaster, V.M. Darley-Usmar, D.W. Kraus, Polarographic measurement of hydrogen sulfide production and consumption by mammalian tissues, Anal. Biochem. 341 (2005) 40-51.

[23] J. Radford-Knoery, G.A. Cutter, Determination of carbonyl sulfide and hydrogen sulfide species in natural waters using specialized collection procedures and gas chromatography with flame photometric detection, Anal. Chem. 65 (1993) 976-982.

[24] P.R. Bérubé, P.D. Parkinson, E.R. Hall, Measurement of reduced sulphur compounds contained in aqueous matrices by direct injection into a gas chromatograph with a flame photometric detector, J. Chromatogr. A 830 (1999) 485-489.

[25] H. Peng, Y. Cheng, C. Dai, A.L. King, B.L. Predmore, D.J. Lefer, B. Wang, A fluorescent chemoprobe for fast and quantatative detection of hydrogen sulfide in blood, Angew. Chem. Int. Ed. 50 (2011) 9672-9675.

[26] S. Chen, Z.-J. Chen, W. Ren, H.-W. Ai, Reaction-based genetically encoded fluorescent hydrogen sulfide sensors, J. Am. Chem. Soc. 134 (2012) 9589-9592.

[27] L. Yang, Y. Su, Z. Sha, Y. Geng, F. Qi, X. Song, A red-emitting fluorescent probe for hydrogen sulfide in living cells with a large Stokes shift, Org. Biomol. Chem. 16 (2018) 1150-1156.

[28] N. Gupta, S.I. Reja, V. Bhalla, M. Gupta, G. Kaur, M. Kumar, A bodipy based dual functional probe for the detection of hydrogen sulfide and H_2S induced apoptosis in cellular systems, Chem. Commun. 51 (2015) 10875-10878.

[29] Z. Guo, G. Chen, G. Zeng, Z. Li, A. Chen, J. Wang, L. Jiang, Fluorescence chemosensors for hydrogen sulfide detection in biological systems, Analyst 140 (2015) 1772-1786.

[30] S. Chen, P. Hou, X. Song, A red-emitting fluorescent probe for imaging hydrogen sulphide with a large Stokes shift, Sens. Actuators B-Chem. 221 (2015) 951-955.

[31] J. Cheng, J. Song, H. Niu, J. Tang, D. Zhang, Y. Zhao, Y. Ye, A new rosamine-based fluorescent chemodosimeter for hydrogen sulfide and its bioimaging in live cells, New J. Chem. 40 (2016) 6384-6388.

[32] L. He, W. Lin, Q. Xu, H. Wei, A new strategy to construct a FRET platform for ratiometric sensing of hydrogen sulfide, Chem. Commun. 51 (2015) 1510-1513.

[33] P. Hou, H. Li, S. Chen, A highly selective and sensitive 3-hydroxyflavone-based colorimetric and fluorescent probe for hydrogen sulfide with a large Stokes shift, Tetrahedron 72 (2016) 3531-3534.

[34] D.-T. Shi, D. Zhou, Y. Zang, J. Li, G.-R. Chen, T.D. James, X.-P. He, H. Tian, Selective fluorogenic imaging of hepatocellular H_2S by a galactosyl azidonaphthalimide probe, Chem. Commun. 51 (2015) 3653-3655.

[35] F.-J. Huo, J. Kang, C. Yin, J. Chao, Y. Zhang, Highly selective fluorescent and colorimetric probe for live-cell monitoring of sulphide based on bioorthogonal reaction, Sci. Rep. 5 (2015) 8969.

[36] S.-A. Choi, C.S. Park, O.S. Kwon, H.-K. Giong, J.-S. Lee, T.H. Ha, C.-S. Lee, Structural effects of naphthalimide-based fluorescent sensor for hydrogen sulfide and imaging in live zebrafish, Sci. Rep. 6 (2016) 26203.

[37] N. Thirumalaivasan, P. Venkatesan, S.-P. Wu, Highly selective turn-on probe for H_2S with imaging applications in vitro and in vivo, New J. Chem. 41 (2017) 13510-13515.

[38] J. Wang, Y. Chen, C. Yang, T. Wei, Y. Han, M. Xia, An ICT-based colorimetric and ratiometric fluorescent probe for hydrogen sulfide and its application in live cell imaging, RSC Adv. 6 (2016) 33031-33035.

[39] K. Xiang, Y. Liu, C. Li, B. Tian, T. Tong, J. Zhang, A colorimetric and ratiometric fluorescent probe with a large stokes shift for detection of hydrogen sulfide, Dyes Pigm. 123 (2015) 78-84.

[40] M. Venkateswarulu, P. Gaur, S. Sinha, A. Pramanik, S. Ghosh, At the molecular level through photophysical studies: structural implications on the reactivity of dual-site sensitive positional isomers toward a gasotransmitter (H_2S), J. Phys. Chem. C 119 (2015) 19367-19375.

[41] M. Venkateswarulu, S. Kumar, S. Ghosh, Modified atomic orbital overlap: molecular level proof of the nucleophilic cleavage propensity of Dinitrophenol-based probes, J. Org. Chem. 82 (2017) 4713-4720.

[42] V.S. Lin, W. Chen, M. Xian, C.J. Chang, Chemical probes for molecular imaging and detection of hydrogen sulfide and reactive sulfur species in biological systems, Chem. Soc. Rev. 44 (2015) 4596-4618.

[43] Y. Qian, J. Karpus, O. Kabil, S.-Y. Zhang, H.-L. Zhu, R. Banerjee, J. Zhao, C. He, Selective fluorescent probes for live-cell monitoring of sulphide, Nat. Commun. 2 (2011) 495.

[44] C. Liu, J. Pan, S. Li, Y. Zhao, Y. Wu Lisa, E. Berkman Clifford, A.R. Whorton, M. Xian, Capture and visualization of hydrogen sulfide by a fluorescent probe, Angew. Chem. Int. Ed. 50 (2011) 10327-10329.

[45] K. Sasakura, K. Hanaoka, N. Shibuya, Y. Mikami, Y. Kimura, T. Komatsu, T. Ueno, T. Terai, H. Kimura, T. Nagano, Development of a highly selective fluorescence probe for hydrogen sulfide, J. Am. Chem. Soc. 133 (2011) 18003-18005.

[46] F. Yu, P. Li, P. Song, B. Wang, J. Zhao, K. Han, An ICT-based strategy to a colorimetric and ratiometric fluorescence probe for hydrogen sulfide in living cells, Chem. Commun. 48 (2012) 2852-2854.

[47] A.R. Lippert, E.J. New, C.J. Chang, Reaction-based fluorescent probes for selective imaging of hydrogen sulfide in living cells, J. Am. Chem. Soc. 133 (2011) 10078-10080.

[48] M.D. Hartle, M.D. Pluth, A practical guide to working with H_2S at the interface of chemistry and biology, Chem. Soc. Rev. 45 (2016) 6108-6117.

[49] T. Chen, Y. Zheng, Z. Xu, M. Zhao, Y. Xu, J. Cui, A red emission fluorescent probe for hydrogen sulfide and its application in living cells imaging, Tetrahedron Lett. 54 (2013) 2980-2982.

[50] V.S. Lin, A.R. Lippert, C.J. Chang, Cell-trappable fluorescent probes for endogenous hydrogen sulfide signaling and imaging H_2O_2-dependent H_2S production, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 7131-7135.

[51] T.-W. Wu, F.-H. Lee, R.-C. Gao, C.Y. Chew, K.-T. Tan, Fluorescent probe encapsulated in avidin protein to eliminate nonspecific fluorescence and increase detection sensitivity in blood serum, Anal. Chem. 88 (2016) 7873-7877.

[52] K. Zheng, W. Lin, L. Tan, A phenanthroimidazole-based fluorescent chemosensor for imaging hydrogen sulfide in living cells, Org. Biomol. Chem. 10 (2012) 9683-9688.

[53] J. Zhang, W. Guo, A new fluorescent probe for gasotransmitter H2S: high sensitivity, excellent selectivity, and a significant fluorescence off-on response, Chem. Commun. 50 (2014) 4214-4217.

[54] T. Saha, D. Kand, P. Talukdar, A colorimetric and fluorometric BODIPY probe for rapid, selective detection of H_2S and its application in live cell imaging, Org. Biomol. Chem. 11 (2013) 8166-8170.

[55] B. Deng, M. Ren, X. Kong, K. Zhou, W. Lin, An ESIPT based fluorescent probe for imaging hydrogen sulfide with a large turn-on fluorescence signal, RSC Adv. 6 (2016) 62406-62410.

[56] H.A. Henthorn, M.D. Pluth, Mechanistic insights into the H_2S-mediated reduction of aryl azides commonly used in H_2S detection, J. Am. Chem. Soc. 137 (2015) 15330-15336.

[57] B. Chen, C. Lv, X. Tang, Chemoselective reduction-based fluorescence probe for detection of hydrogen sulfide in living cells, Anal Bioanal Chem 404 (2012) 1919-1923.

[58] M.D. Hammers, M.J. Taormina, M.M. Cerda, L.A. Montoya, D.T. Seidenkranz, R. Parthasarathy, M.D. Pluth, A bright fluorescent probe for H_2S enables analyte-responsive, 3D imaging in live zebrafish using light sheet fluorescence microscopy, J. Am. Chem. Soc. 137 (2015) 10216-10223.

[59] Y. Zhao, X. Zhu, H. Kan, W. Wang, B. Zhu, B. Dua, X. Zhang, A highly selective colorimetric chemodosimeter for fast and quantitative detection of hydrogen sulfide, Analyst, 137 (2012) 5576-5580.

[60] Z. Gao, Y. Hao, M. Zheng, Y. Chen, A fluorescent dye with large Stokes shift and high stability: synthesis and application to live cell imaging, RSC Adv. 7 (2017) 7604-7609.

[61] J.F. Araneda, W.E. Piers, H. Belinda, P. Masood, M. Robert, High Stokes shift anilido-pyridine boron difluoride dyes, Angew. Chem. Int. Ed. 50 (2011) 12214-12217.

[62] J.C. Er, M.K. Tang, C.G. Chia, H. Liew, M. Vendrell, Y.-T. Chang, MegaStokes BODIPY-triazoles as environmentally sensitive turn-on fluorescent dyes, Chem. Sci. 4 (2013) 2168-2176.

[63] F. Schluter, K. Riehemann, N.S. Kehr, S. Quici, C.G. Daniliuc, F. Rizzo, A highly fluorescent water soluble spirobifluorene dye with a large Stokes shift: synthesis, characterization and bio-applications, Chem. Commun. 54 (2018) 642-645.

[64] A.C. Benniston, T.P.L. Winstanley, H. Lemmetyinen, N.V. Tkachenko, R.W. Harrington, C. Wills, Large Stokes shift fluorescent dyes based on a highly substituted terephthalic acid core, Org. Lett. 14 (2012) 1374-1377.

[65] F. Xie, K. Sivakumar, Q. Zeng, M.A. Bruckman, B. Hodges, Q. Wang, A fluorogenic 'click' reaction of azidoanthracene derivatives, Tetrahedron 64 (2008) 2906-2914.

[66] S. S. Bag, H. Gogoi, Design of "Click" fluorescent labeled 2′-deoxyuridines via C5-[4-(2-Propynyl(methyl)amino)]phenyl acetylene as a universal linker: synthesis, photophysical properties, and interaction with BSA, J. Org. Chem. 83 (2018) 7606−7621.

[67] H.A. Michaels, C.S. Murphy, R.J. Clark, M.W. Davidson, L. Zhu, 2-Anthryltriazolyl-containing multidentate ligands: zinc-coordination mediated photophysical processes and potential in live-cell imaging applications, Inorg. Chem. 49 (2010) 4278–4287.

[68] S. Huang, R.J. Clark, L. Zhu, Highly sensitive fluorescent probes for zinc ion based on triazolyl-containing tetradentate coordination motifs, Org. Lett. 9 (2007) 4999-5002.

[69] A.D. Becke, Density- functional thermochemistry. III. The role of exact exchange, J. Chem. Phys. 98 (1993) 5648-5652

[70] C. Lee, W. Yang, R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, Phys. Rev. B 37 (1988) 785-789.

[71] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A.M. Jr., J.E. Peralta, F.o. Ogliaro, M.J. Bearpark, J. Heyd, E.N. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A.P. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N.J. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ã.d.n. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Gaussian, Inc., Wallingford, CT, USA (2009).

[72] C. Maurizio, R. Nadia, S. Giovanni, B. Vincenzo, Energies, structures, and electronic properties of molecules in solution with the C- PCM solvation model, J. Comput. Chem. 24 (2003) 669-681.

[73] R. Dennington, T. Keith, J. Millam, Gauss View, Version 5, Semichem Inc., Shawnee Mission, KS, (2009).

[74] G.L. Long, J.D. Winefordner, Limit of detection A closer look at the IUPAC definition, Anal. Chem. 55 (1983) 712A-724A.

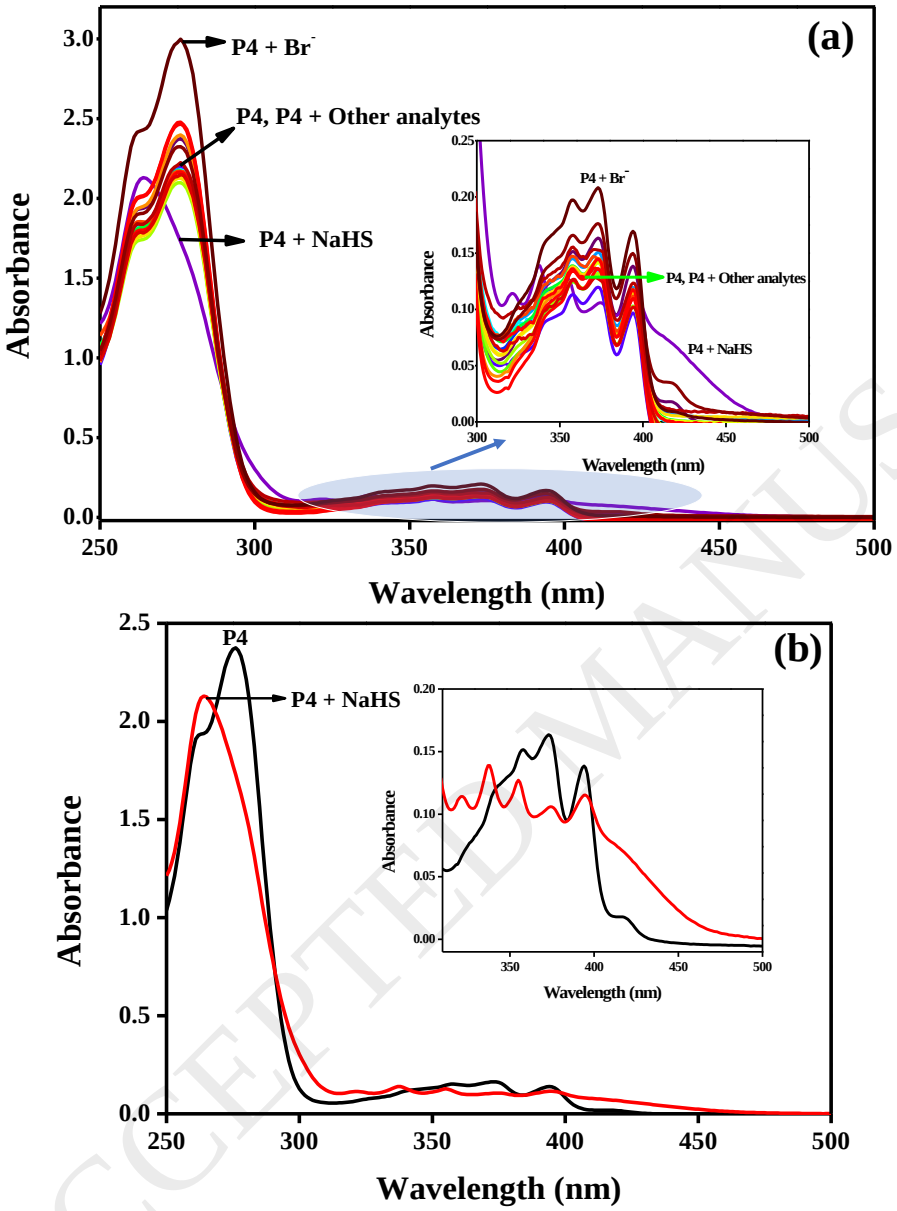


Fig. 1. (a) UV-vis spectra of probe **P4** (5 x 10^{-5} M) in DMSO : H_2O (1:1, v/v) upon addition of NaHS, Cl^-, F^-, Br^-, I^-, NO_3^-, PO_4^{3-}, AcO^-, HSO_3^-, SCN^-, $S_2O_3^{2-}$, $S_2O_5^{2-}$, Na^+, K^+, Ca^{2+}, Mg^{2+},Cys, Glu and Homo Cys (50 x 10^{-5} M); (b) UV-vis spectra of probe **P4** (5 x 10^{-5} M) in DMSO : H_2O (1:1, v/v) upon addition of NaHS (50 x 10^{-5} M).

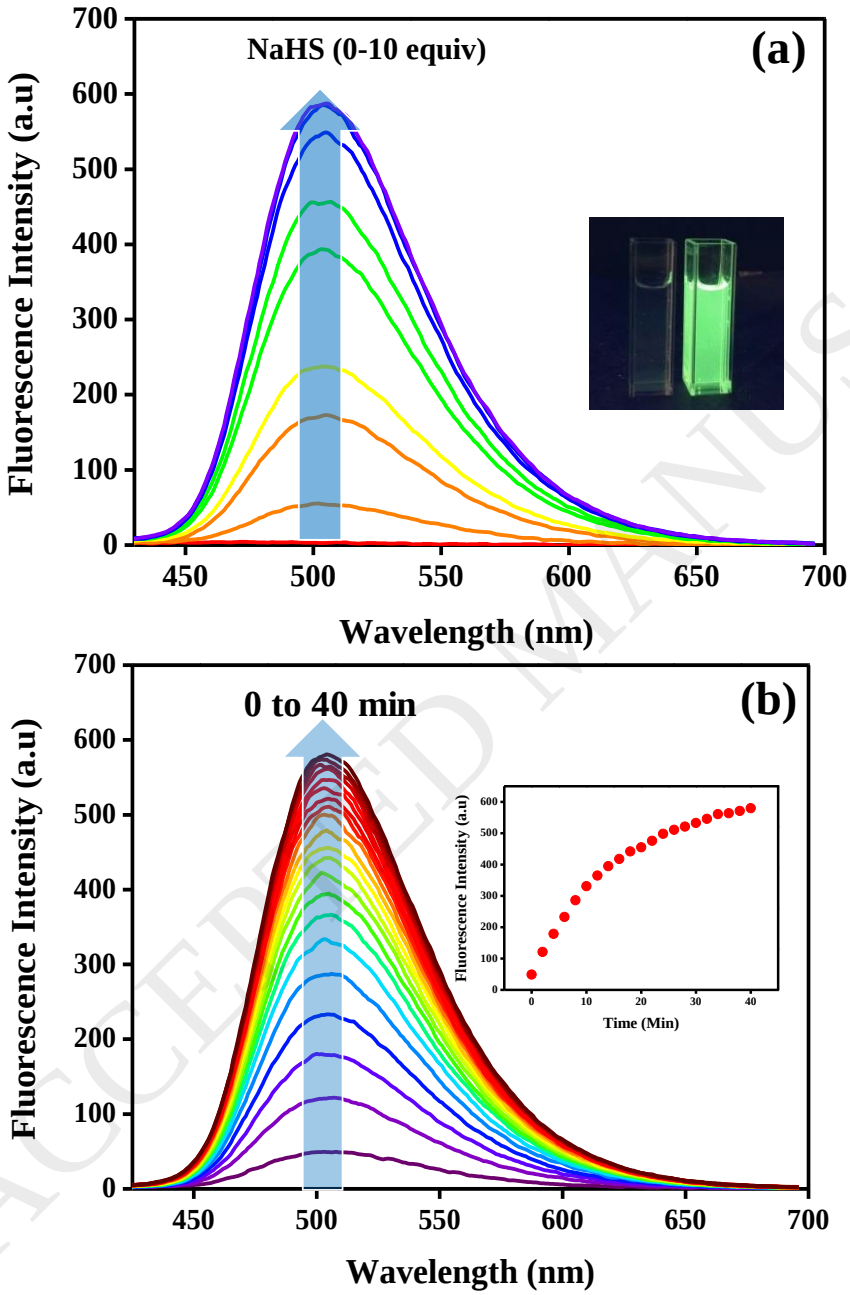


Fig. 2. (a)Fluorescence responses of probe **P4** (5 x 10^{-5} M) in DMSO : H_2O (1:1, v/v) upon addition of increasing quantities of NaHS (0 to 50 x 10^{-5} M). The solution was incubated for

40 min at 37 °C (λ_{ex}=395 nm, λ_{em}= 505 nm); (b) Fluorescence responses of probe **P4** (5 x 10^{-5} M) to NaHS (50 x 10^{-5} M) after incubation of 0 to 40 min in DMSO : H_2O (1:1, v/v).

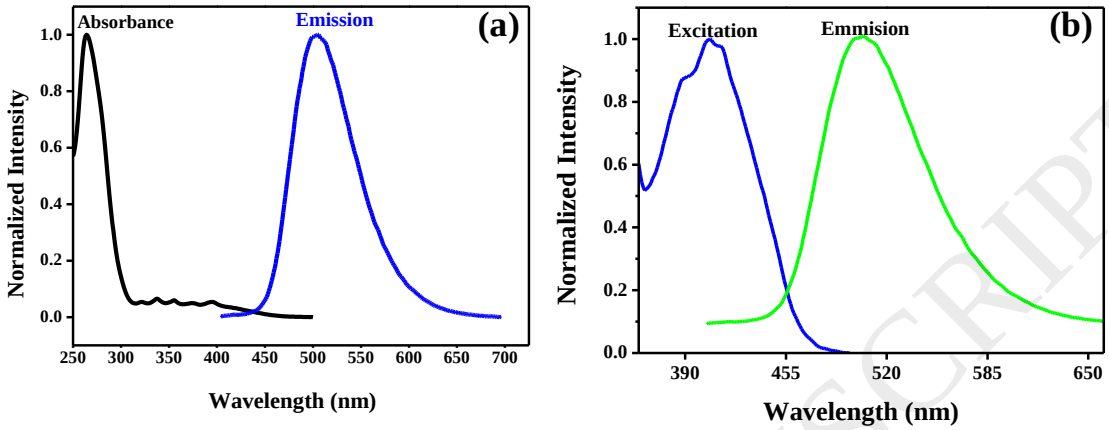


Fig. 3. (a) Normalized absorption (black) and emission spectra (blue) of probe **P4** (5 x 10^{-5} M) in DMSO: H_2O (1:1, v/v) upon addition of NaHS (50 x 10^{-5} M); (b) normalized excitation (blue) and emission spectra (green) upon addition of NaHS (50 x 10^{-5} M). The solution was incubated for 40 min at 37 °C(λ_{ex}=395 nm, λ_{em}= 505 nm).

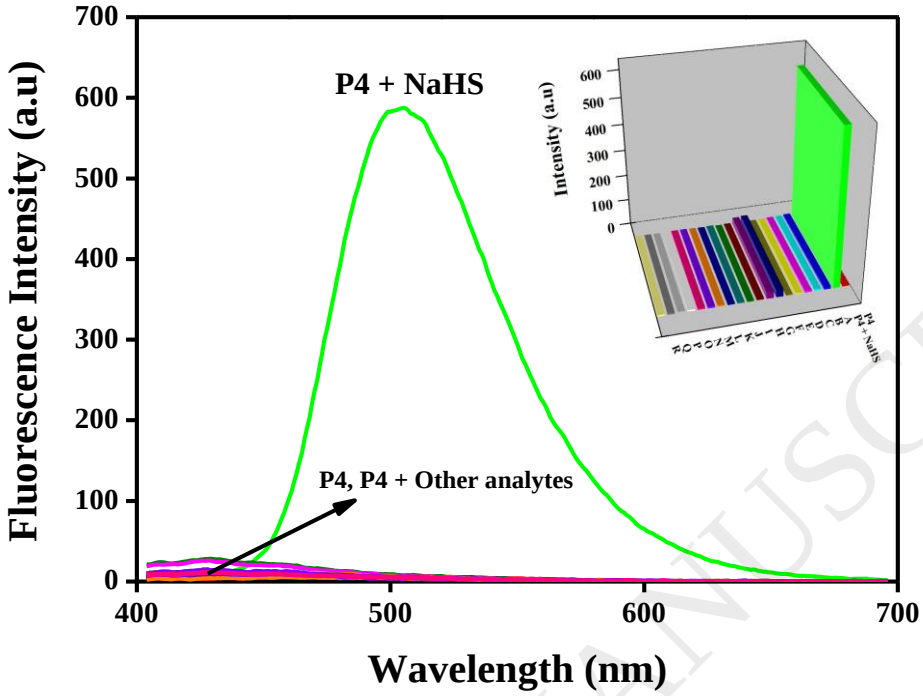


Fig. 4. Fluorescence responses of probe **P4** (5 x 10^{-5} M) in DMSO : H_2O (1:1, v/v) upon the addition of NaHS, Cl^-, F^-, Br^-, I^-, NO_3^-, PO_4^{3-}, AcO^-, HSO_3^-, SCN^-, $S_2O_3^{2-}$, $S_2O_5^{2-}$, Na^+, K^+, Ca^{2+}, Mg^{2+},Cys, Glu and Homo Cys (50 x 10^{-5} M). Inset: Fluorescence intensity of **P4** in the presence of different analytes. A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q and R representsCys, Glu, Homo Cys, F^-, Cl^-, Br^-, I^-, NO_3^-, PO_4^{3-}, AcO^-, $S_2O_3^{2-}$, SCN^-, HSO_3^-, $S_2O_5^{2-}$, Na^+, K^+, Ca^{2+} and Mg^{2+}. The solution was incubated for 40 min at 37 °C (λ_{ex} = 395 nm, λ_{em} = 505 nm).

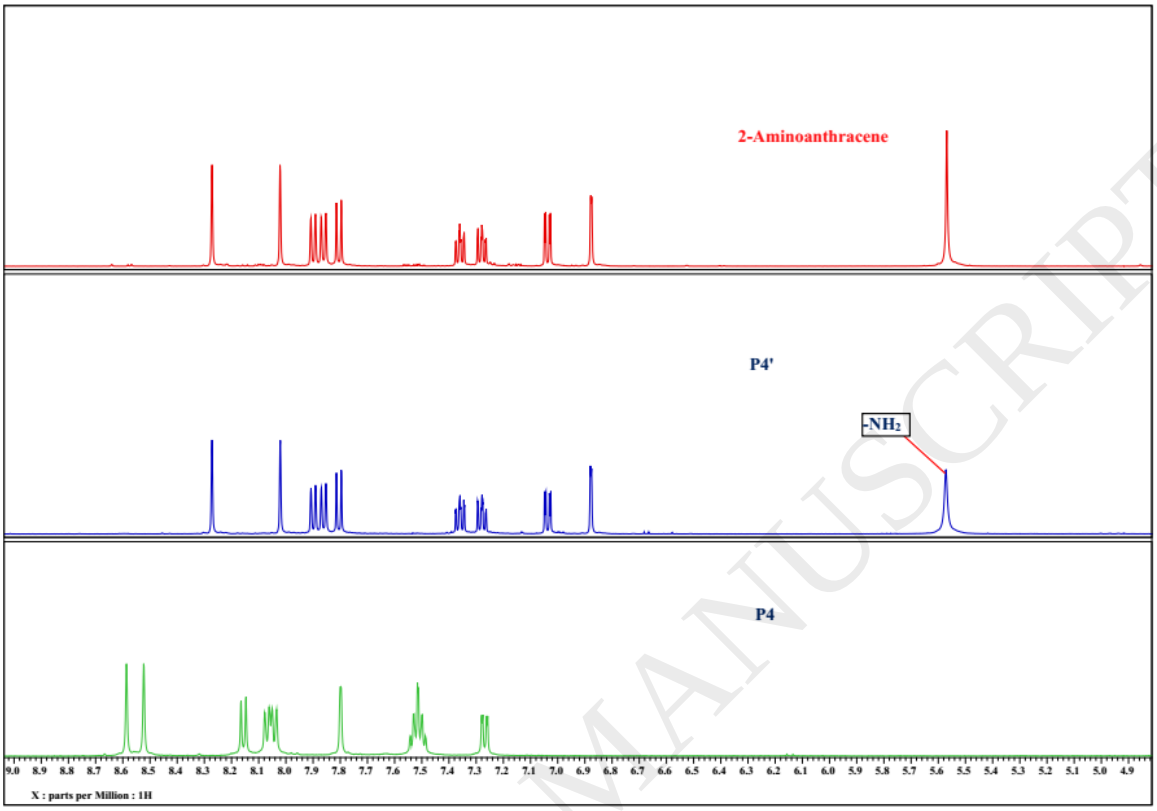


Fig.5. ^{1}H-NMR spectra of 2-aminoanthracene, **P4** and **P4′** (isolated product of **P4** with NaHS) in DMSO-d_6.

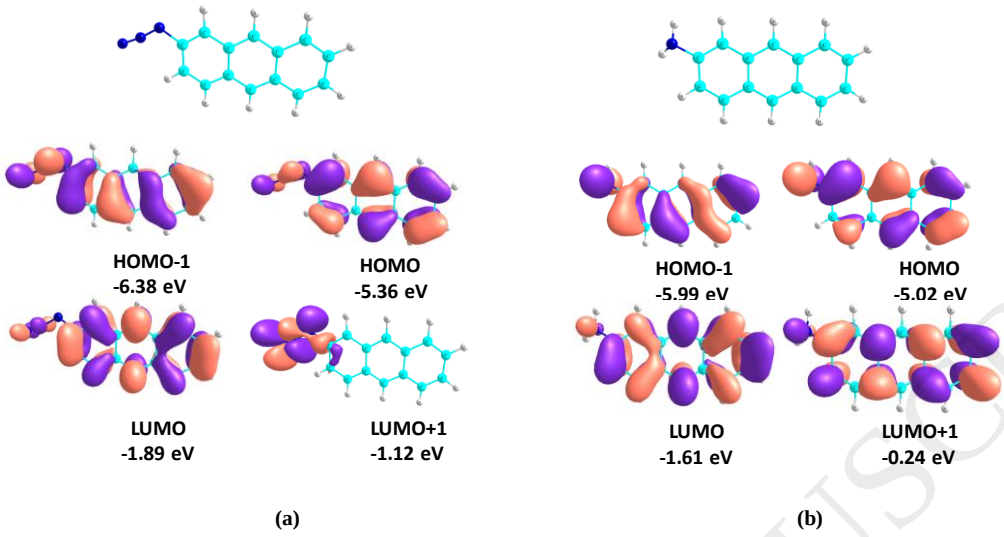
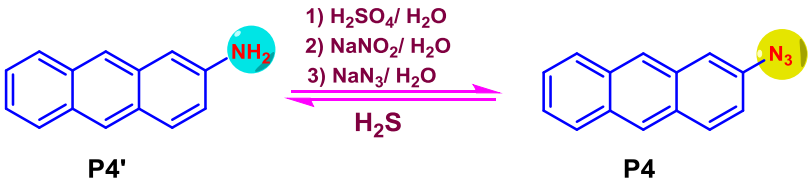
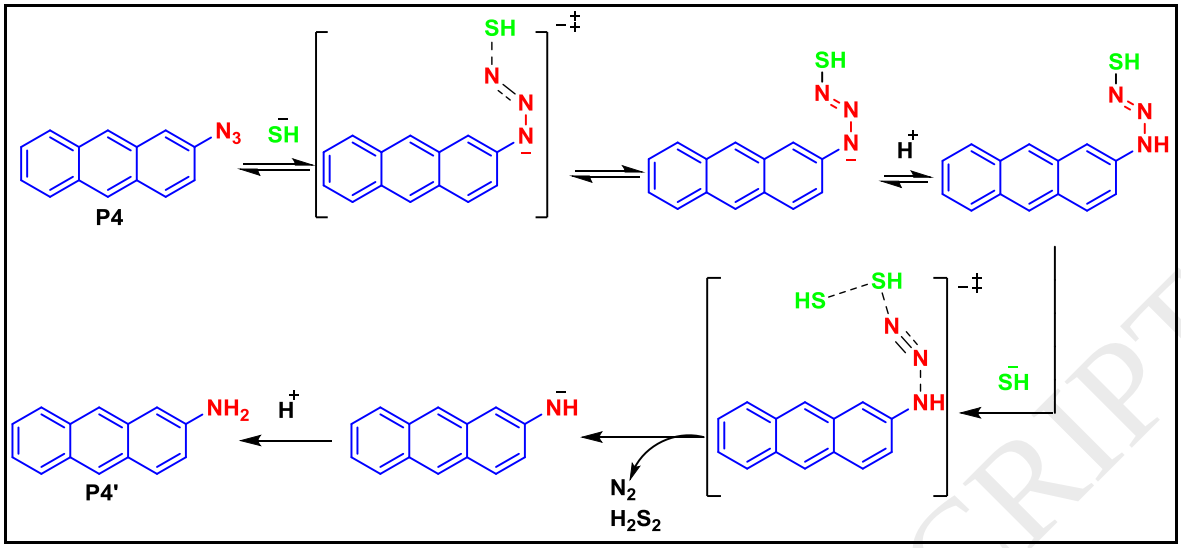


Fig.6. Optimized structures and frontier molecular orbitals of **P4** (a) and**P4′** (b) (isocontour at 0.03 au).



P4' P4

Scheme 1. Synthesis of **P4** and **P4'**



Scheme 2. Proposed mechanism of azidereduction by NaHS