Green light activated BODIPY based Hydrogen sulfide donor

Thesis

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By

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CERTIFICATE

This is to certify that this dissertation entitled "**Green light activated BODIPY based Hydrogen Sulfide donor**" towards the partial fulfilment of the BS-MS dual degree programme at the Indian institute of Science Education and Reasearch, Pune represents study/work carried out by Mr.Aswin PK, under the supervision of Dr.Harinath Chakrapani, Associate professor, chemeistry department during the academic year 2017-2018

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Dr. Harinath Chakrapani

Date : 20/03/2018

Place : Pune

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(20131114)

DECLARATION

I hereby declare that the matter embodied in the report entitled "**Green light activated BODIPY based Hydrogen Sulfide donor**" are the work carried out by me at the Department of Chemistry, Indian Institute of Science Education and Research, Pune under the supervision of Dr. Harinath Chakrapani, Associate professor, Chemistry Department and the same has not been submitted elsewhere for any other degree.

C. Harinath

Dr. Harinath Chakrapani

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(20131114)

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CONTENTS

1. ABBREVIATIONS

NMR	Nuclear Magnetic Resonance
HRMS	High Resolution Mass Spectrometry
J	Coupling Constant
Hz	Hertz
MHz	Megahertz
EtOAc	Ethyl Acetate
DCM	Dichloromethane
DMSO	Dimethyl Sulfoxide
LED	Light emitting diode
mg	Milligram
g	Gram
mL	Millilitre
µL	Microlitre
rt	Room temperature
mmol	Millimoles
min	Mintues
h	Hours
ACN	Acetonitrile

2. Abstract

Hydrogen sulfide (H_2S) is a small gaseous molecule which is known to be a toxic air pollutant. It is now widely recognised as a gasotransmitter, which has a vital role in many physiological processes. It protects human tissues from inflammation, helps in vasodilation, H_2S secreted by enteric bacteria in small concentration helps in smooth functioning of gastrointestinal processes. However, delivery of H_2S to biological systems is a cumbersome process, it is a small gaseous molecule, and hence it is highly diffusible. Sulfide salts like Na_2S and NaSH are widely used in the delivery of H_2S in laboratories. But it's rapid, uncontrolled release of H_2S is a severe drawback, and hence it cannot be used efficiently in biological systems. To overcome these difficulties, researchers realised the need for localised delivery of H_2S in a controlled manner. Henceforth researchers have developed several H_2S donors with different triggers. Considering that a light source can be directed to specific targets, light-activated delivery of H_2S can be envisaged as an ideal strategy. This may provide spatiotemporal control over the release of the gaseous molecule. Different groups have reported molecule which releases H_2S when exposed to light, all these reported molecules account for the spatiotemporal release of the molecule, but a significant drawback of these designs was, the wavelength they used has low tissue penetration and has phototoxic effects. Here we are introducing a new H_2S donor which cleaves under green light, which has no phototoxic effects reported.

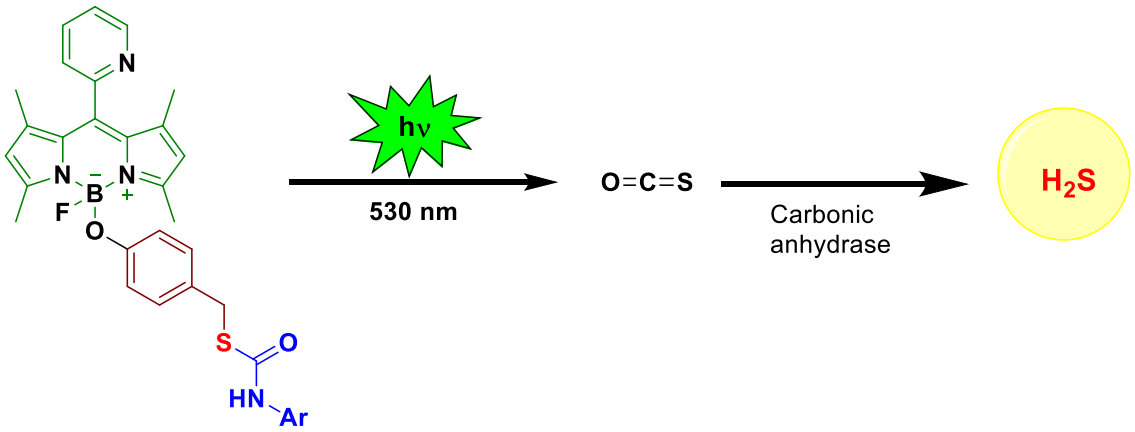


Fig 1: Design of BODIPY based green light activated COS/ H_2S donor.

3. Introduction

Hydrogen sulfide is now widely considered as an essential signalling molecule along with nitric oxide and carbon monoxide. Studies have shown that H_2S has a significant effect on human physiology[1], which makes it an important molecule to the scientific world.

3.1. Biosynthesis and metabolism

Pathways involved in the endogenous production of H_2S are regulated by the enzymes cystathionine γ-lyase (CSE)[2], cystathionine β-synthase (CBS)[3] and mercaptopyruvate sulfurtransferase (MPST)[4]. Both CSE and CBS are pyridoxal 5′-phosphate (PLP)–dependent enzymes and catalyse the production of H_2S from L-cysteine and homocysteine, respectively. MPST act upon 3-mercaptopyruvate produced by cysteine aminotransferase (CAT). CBS is predominantly expressed in brain[5] whereas CSE mostly regulates the H_2S production in the cardiovascular system and MPST is expressed chiefly in the central nervous system[6].

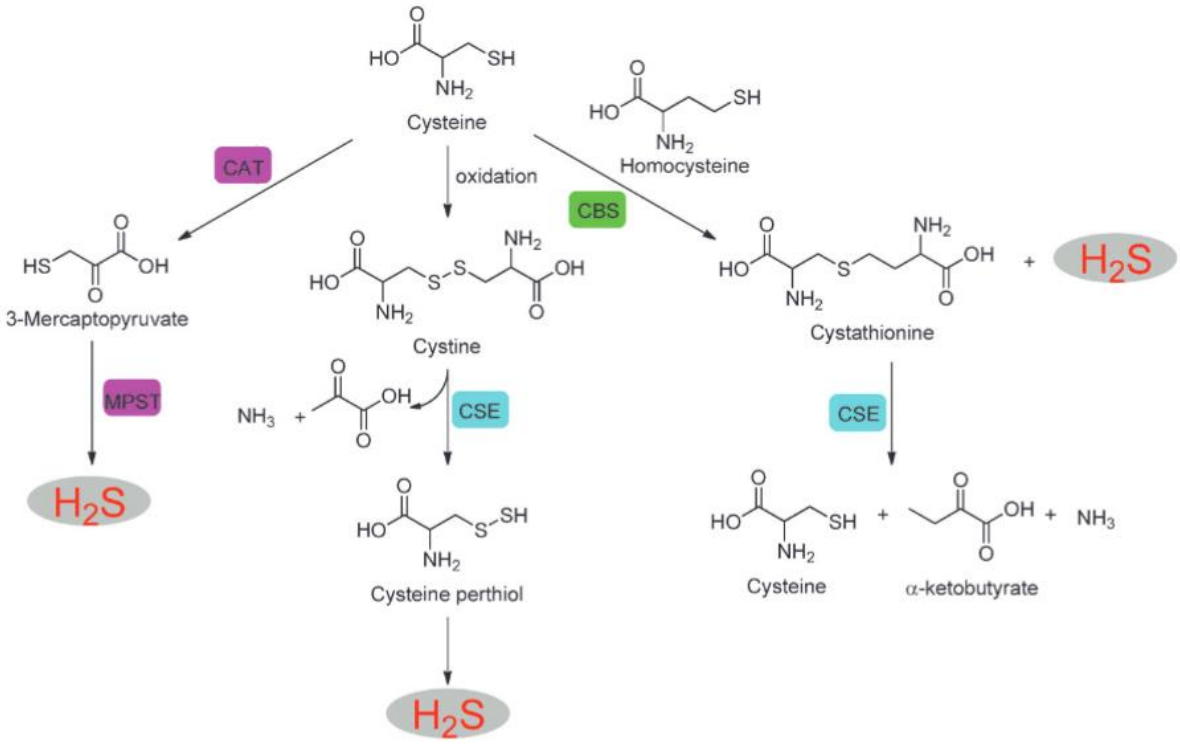


Fig 2: Biosynthesis of H_2S[7]

Two mechanisms by which H_2S is metabolised in our body are oxidation and methylation. Oxidation mainly occurs in the mitochondria. H_2S is oxidised to thiosulfate and then to sulfite, with the end-product under physiological conditions being sulfate[8]. The mitochondrial enzyme sulfide-quinone reductase helps in the oxidation. The major site of methylation is cytosol which is catalysed by *S*-methyltransferase to give methanethiol and dimethylsulfide[6]. Methaemoglobin also helps in the metabolism by scavenging the free H_2S in the cellular cytoplasm and circulatory system[9].

3.2 H_2S in physiology

H_2S is an important gasotransmitter which intervenes in numerous biological pathways. The lipid-soluble nature of the molecule helps to reach its molecular targets with ease. Role of H_2S in those pathways are indispensable.

One of the major pathways by which H_2S mediate the cellular process is by interacting with the ion channels. Studies have shown that H_2S interact with The ATP-sensitive potassium (K_{ATP}) channels which in turn induces vasorelaxation[10]. There are reports which indicate that H_2S interacts with Voltage-dependent calcium channels (VDCCs) of different cell types and regulates the intracellular calcium concentration[11]. H_2S interacts with different secondary messengers and affect many biological pathways. It is known that it monitors the level of secondary messengers like cyclic GMP (cGMP) and cyclic AMP (cAMP) by interacting with phosphodiesterases[12]. H_2S also takes part in the s-sulfhydration of proteins, for instance, *S*-sulfhydration of the cysteine residue, Cys38, of the p65 subunit of NF-κB, a transcription factor, improves the binding of NF-κB to ribosomal protein, which enhances the transcriptional activity[13]. H_2S also plays a vital role in regulating the redox homeostasis of the cell.[14]

3.3 H_2S donors

H_2S donors are small molecules which releases H_2S under specific conditions. Highly diffusible nature of H_2S demand for molecules which can ensure localised delivery of the gas to the target sites. There are many donors known to the scientific world, and rigorous research is going on in this field to improve the efficiency of these

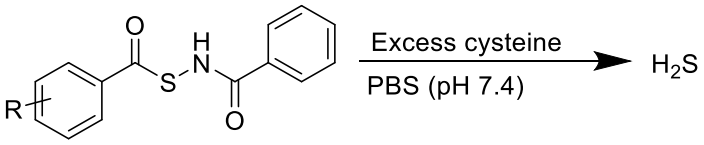
donors regarding permeability and cytotoxicity. Some of the donors are summarised below.

3.3.1 Inorganic sulfide salts as H_2S donors

Inorganic sulfide salts like Na_2S and NaSH are widely used in the delivery of H_2S in laboratories. Researchers have found that the treatment of cells, tissues, or animals with sulfide salts have shown protective effects against some disease conditions like osteoarthritis, ischemic heart failure etc[15]. It is very effective at delivering H_2S, but lack of control over the release of the gas remains as a severe drawback. Its rapid uncontrolled release can damage cells, and hence it cannot be used efficiently in biological systems.

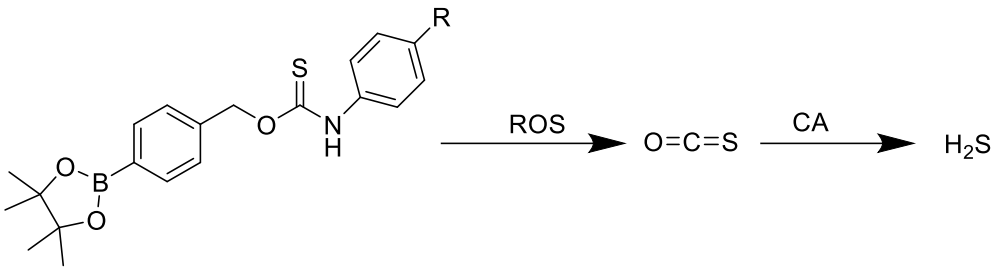
3.3.2 Thiol activated H_2S donors

The idea of thiol activated H_2S donors was first introduced by Ming Xiang. They have reported a series of cysteine-activated H_2S donors based on the N-mercapto (N-SH) template[16]. These compounds are stable in buffers. They also proved that H_2S release rates from these compounds could be controlled through structural modifications. They have also synthesised perthiol-based donors. A significant flaw in this method was the ubiquitous nature of thiols. This makes the compound less target specific.



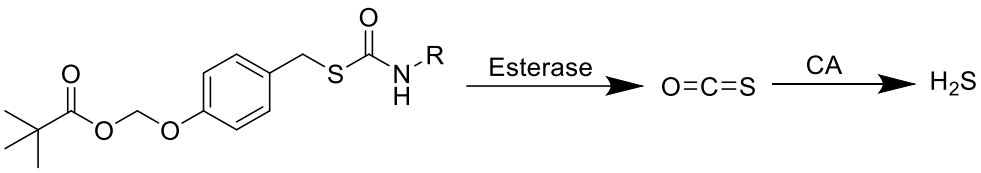
3.3.3 ROS activated H_2S donors

This class of molecules are H_2S donors which release H_2S when reacted with reactive oxygen species. M.Pluth and coworkers have reported the first example of ROS triggered H_2S donors[17]. Their studies reveal that, these compounds have cytoprotective effects against H_2O_2 induced oxidative stress



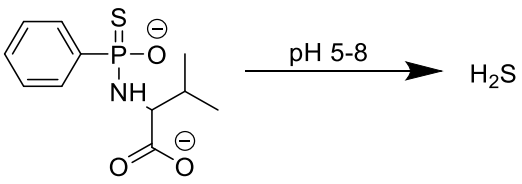
3.3.4 Enzymatically activated H_2S donors

This class of donor molecules consists of molecules that release hydrogen sulfide with an enzymatic trigger. There are many enzymatic activated donors reported[18,19]. B.Wang et al. introduced a hydrogen sulfide donor that release hydrogen sulfide upon esterase-mediated cleavage of an ester group[20]. Here also the ubiquitous nature of esterase remains a problem and it affects the specificity of the molecule. Other class of enzymatically activated H_2S donors are molecules which are triggered by the enzyme nitroreductases. Slow metabolism of nitro group results in the slow release of H_2S, which can be pointed out as a minor limitation of this approach.



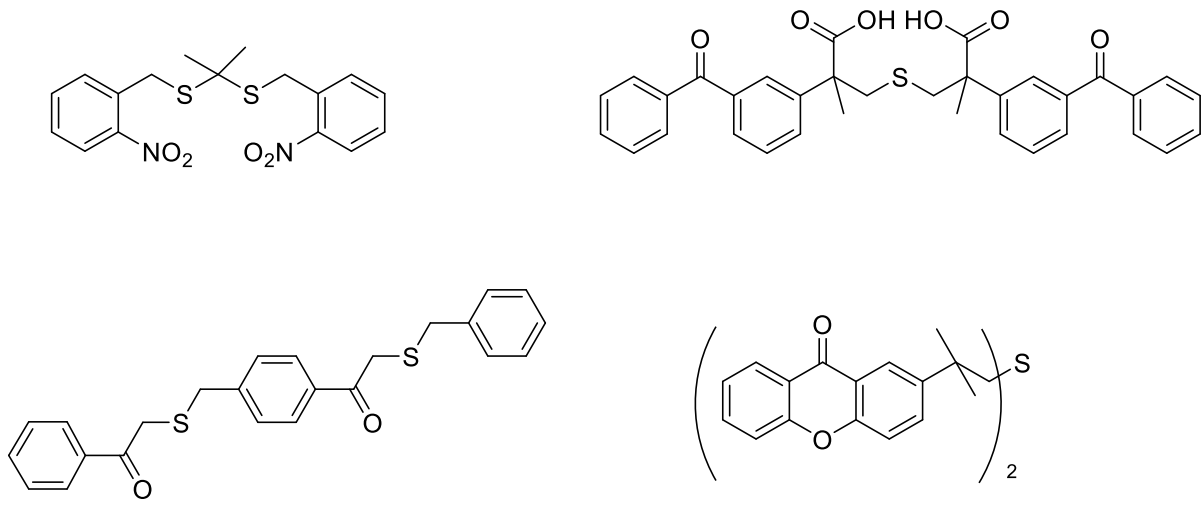
3.3.5 pH modulated H_2S donors

M.Xian and coworkers have developed a molecule in which pH-dependent intramolecular cyclization occurs and it, in turn, promotes H_2S release from the donors[21]. These donors based on the phosphonamidothioate template. These compounds are highly water-soluble and showed slow, controllable, and pH-sensitive production of H_2S in aqueous solutions. Its high water solubility makes the molecule less cell permeable.



3.3.6 Light activated H_2S donors

Xian and co-workers have reported a molecule which releases H_2S when exposed to 365 nm light[22]. Similarly, Nakagawa's[23,24] and Connal's[25] group have reported H_2S donors which are activated by light. All these reported molecules account for the spatiotemporal release of the molecule, but a significant drawback of these designs was, the wavelength they used for activation lies in the UV region. UV light has low tissue penetration and has phototoxic effects which may result in conditions like melanoma[26].



Examples of different light activated H_2S donors

3.4 Design of new visible light activated H_2S donor

Recently our lab has introduced a new BODIPY scaffold which generates H_2S when exposed to blue light[27]. Here we have used a boron dipyrromethene-based carbamothioate, which produces carbonyl sulfide (COS), upon irradiation by visible light of 470 nm, which is rapidly hydrolysed to H_2S in the presence of carbonic anhydrase to release H_2S.

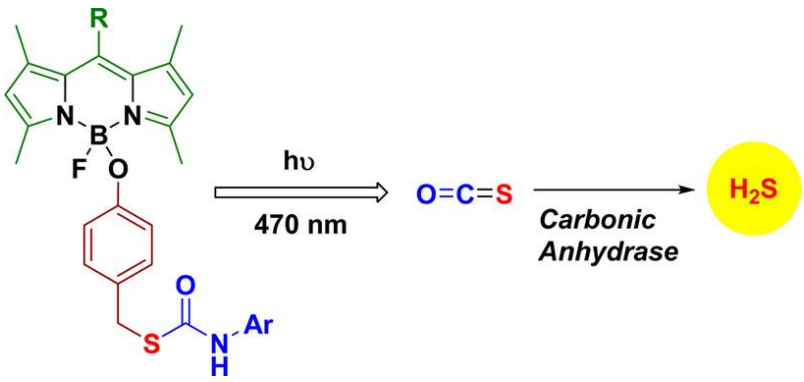
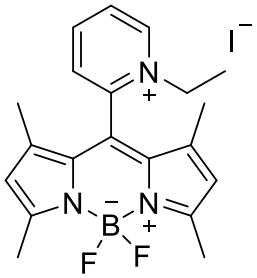


Fig: 3 Blue light activated BODIPY based COS/H_2S donor.

Even though it solves the issues with the UV light, there are recent reports which indicate irradiation with blue light can induce marginal oxidative stress[28]. For this scaffold, we have used 470 nm light for the release of H_2S, which can be pointed out as an impediment. Low water solubility is another issue with this molecule which may hinder further cellular and animal studies.

In 2012, Xiaojun Peng and co-workers reported a BODIPY dye which acts as a mitochondrial fluorescence probe. It has an absorption maxima at 520 nm (green light)[29].



In the light of above scaffolds, we decided to design a new BODIPY based scaffold which might undergo photocleavage in green light to produce phenolate intermediate which in turn self-immolates to give COS. This COS gets hydrolysed in the presence of carbonic anhydrase to produce H_2S. This scaffold has pyridine moiety which can be functionalised to improve water solubility and may solve the issues with the current scaffold.

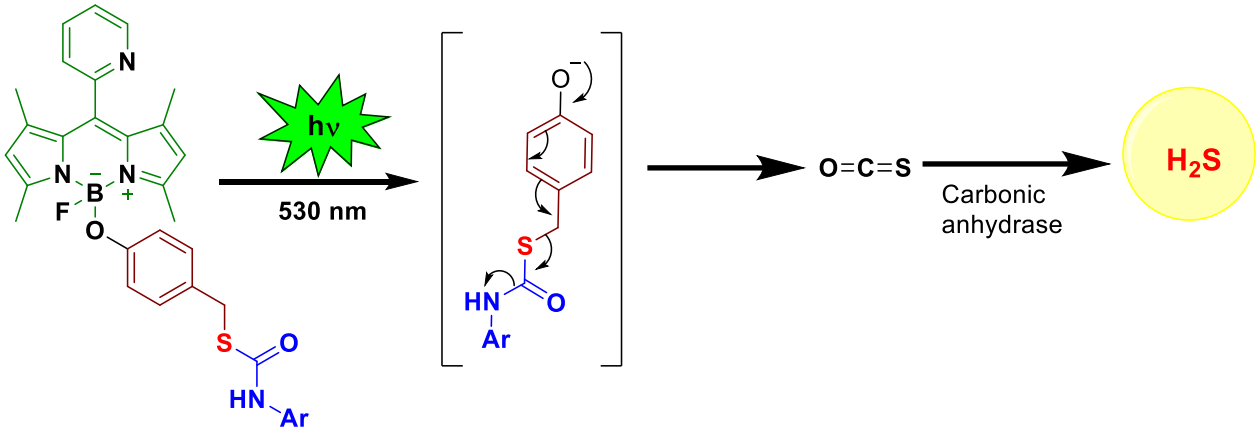
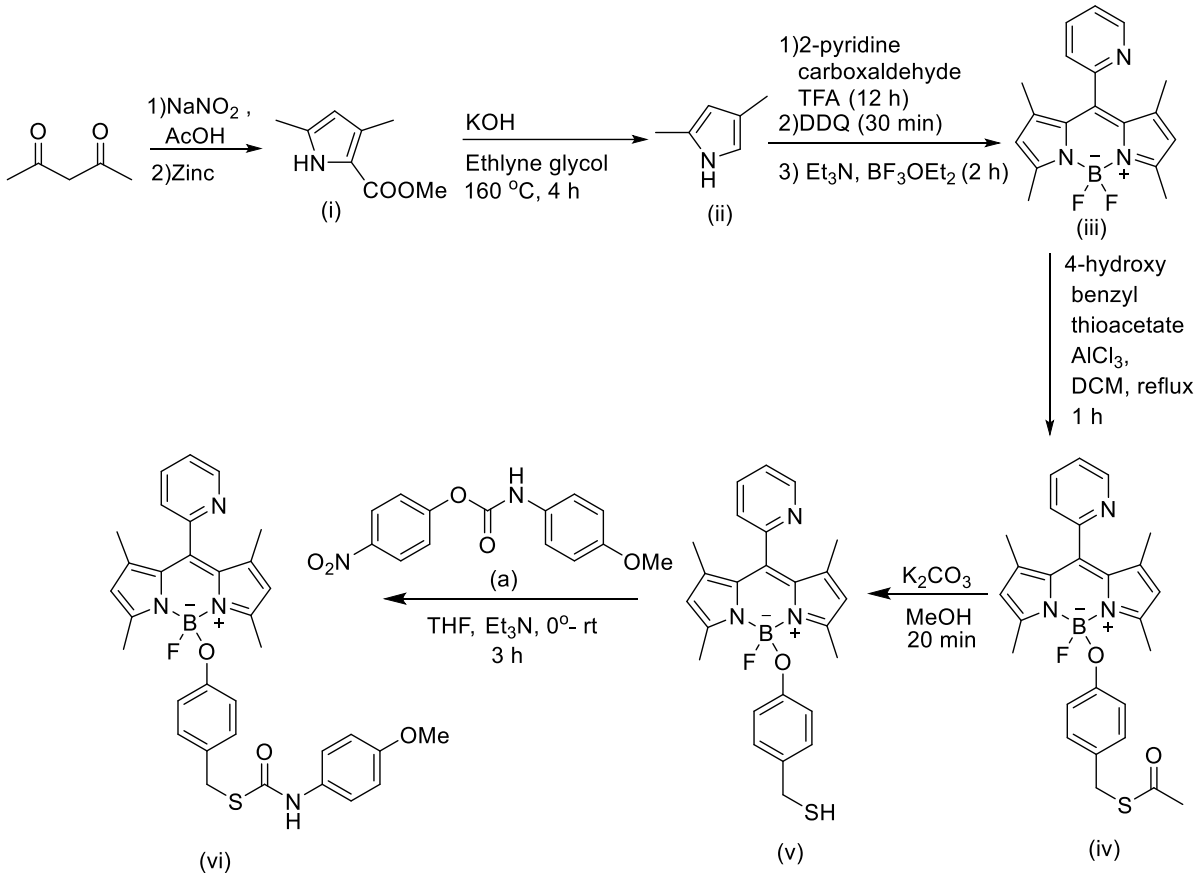


Fig 4: Design of new green light activated BODIPY based H_2S donor.

4. Materials and Methods

All reactions were carried out under nitrogen atmosphere. All the chemicals were purchased from commercial sources and used as received unless stated otherwise. Rankem silica gel (60–120 mesh) or silica gel Spectrochem (100-200 mesh) or Rankem neutral alumina was used as stationary phase for column chromatography. ^{1}H and ^{13}C spectra were recorded on JEOL 400 MHz (100 MHz for ^{13}C) spectrometers using either residual solvent signals as an internal standard ($CHCl_3$ δ_H, 7.26 ppm, δ_C 77.2 ppm) or an internal tetramethylsilane (δ_H = 0.00, δ_C = 0.00). Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. The following abbreviations are used: s (singlet), m (multiplet), d (doublet), t (triplet), td (triplet of doublet) dd (doublet of doublet). High-resolution mass spectra (HRMS) were obtained from HRMS-ESI-Q-Time of Flight LC/MS. FT-IR spectra were recorded using NICOLET 6700 FT-IR spectrometer. Agilent Technologies 1260 infinity with Phenomenex® C-18 reversed phase column (250 mm × 4.6 mm, 5 µm) was used for high-performance liquid chromatography (HPLC). Irradiation was done using green LED (530nm, 3W) Luxeon star LED and intensity was calibrated using GENTEC-EO-UNO laser power meter. Photometric measurements were performed using a Thermo Scientific Varioscan microwell plate reader. Optical properties of BODIPY derivatives (2.5 µM, MeOH) were determined by Shimadzu UV-2600 UV-VIS spectrophotometer and Fluoromax-4 Spectrofluorometer (Horiba Scientific).

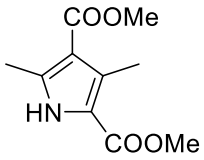
4.1 Synthesis and characterisation



Scheme 1: Synthesis of compound (vi)

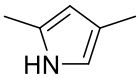
A detailed synthesis of compounds and spectroscopic data are given below.

Dimethyl 3,5-dimethyl-1H-pyrrole-2,4-dicarboxylate (Compound i)



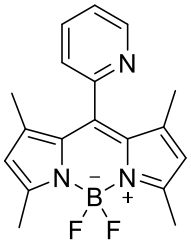
In a 250 mL RB a mixture of acetic acid (40 mL) and methyl aceto-acetate(20 mL, 0.185 moles) was made and was kept at 0 ^{0}C. To this, a previously prepared Sodium nitrite (6.38 g, 0.092 moles) dissolved in 10 mL of water was added dropwise. After 10 minutes the ice bath was removed, and the mixture was allowed to stir for 3 h at rt. After 3 h the reaction mixture was again brought down to 0 ^{0}C and to this zinc was added very slowly with vigorous stirring. After 10 minutes the temperature was raised to 45^{o}C then to 110^{o}C. A clear homogeneous solution was observed. The reaction was stopped after 1.5 h. The reaction mixture was then poured into ice cold water and then filtered using a sintered funnel to obtain yellowish white solid (18.3 g, 47%). The crude compound was taken to next step without further purification.

2,4-dimethyl-1H-pyrrole (compound ii)



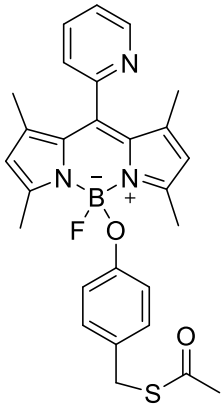
KOH (13.18 g, 0.23 moles) was mixed thoroughly with ethylene glycol (40 mL) in a 250 mL RB and to this (10 g, 0.047 moles) of compound (i) was added and stirred at 160 ^{0}C for 4 h. Once the TLC indicated the completion of the reaction, 250 mL of water was added to the reaction mixture and extracted with DCM. Then the DCM layer was passed through anhydrous sodium sulfate and evaporated under reduced pressure to obtain a brownish black liquid (3.1 g, 68.8%). This compound was carried to next step immediately without any further purification.

5,5-difluoro-1,3,7,9-tetramethyl-10-(pyridin-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (compound iii)



Compound (ii) (3.55 g, 0.037 mol) was taken in a 500 mL RB and was completely dissolved in 120 mL of dry DCM. To this 2- Pyridine carboxaldehyde (1.77 mL, 0.018 mol) and a catalytic amount of TFA were added and allowed to stir overnight (16 h) at rt. Once the starting material was completely consumed, which was monitored by TLC, DDQ (6.3 g, 0.028 moles) was added to the reaction mixture and stirred it for 1 hour. Once the TLC showed completion of the reaction, the reaction mixture was taken to 0 ^{0}C, and then triethylamine (12.89 mL, 0.095 mol), and Boron trifluoro etherate (14.86 mL, 0.12 mol) were added to it. After 10 min of stirring, the ice bath was removed, and the reaction mixture was allowed to stir at rt for 1 h. Once TLC shows the formation of new fluorescent spot the reaction was stopped and the solvent was evaporated under reduced pressure. This was dissolved in 150 mL of EtOAc and then filtered through cotton. The filtrate was then washed with (2x300) mL of brine solution. The organic layer was dried over Na_2SO_4. The crude product obtained after the evaporation of the solvent under reduced pressure was further purified by column chromatography (Neutral Alumina as a stationary phase) with EtOAc/hexane as eluent, to obtain a brownish orange sparkly solid (Yield: 250 mg, 14.7%). ^{1}H NMR (400 MHz, $CDCl_3$): ∂ 8.79 - 8.77 (m, 1H), 7.83 (td, *J* = 2.0 Hz, 7.7 Hz, 1H), 7.42 (m, 2H), 5.98 (s, 2H), 2.55 (s, 6H), 1.31 (s, 6H)

5-fluoro-1,3,7,9-tetramethyl-5-(4-((methylsulfinyl)methyl)phenoxy)-10-(pyridin-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (compound iv)



Compound (iii) (107 mg, 0.329 mmol) was taken in a 25 mL RB and was completely dissolved in 6 mL of dry DCM. To this $AlCl_3$ (65.80mg, 0.493 mmol) was added and was refluxed. After 40 mins TLC indicates the formation of the product. The reaction was stopped, since further reaction was giving uncharacterised products along with the expected product. The solvent was evaporated under reduced pressure and the crude was subjected to column (neutral alumina as a stationary phase) mixture obtained was purified by preparatory column (yield = 51mg, 32%). FT- IR (γ_{max}, cm-1): 1737, 1463, 1374, 1246. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.83 – 8.79 (m, 1H), 7.87 – 7.83 (m, 1H), 7.47 – 7.45 (m, 3H), 7.01 (t, *J* = 8 Hz, 2H), 6.56 (d, *J*=8.5 Hz, 1H), 6.48 (8.5 Hz, 1H), 5.93 (s, 2H), 4.00 (d, 2H, *J* = 4.5 Hz), 2.52 (s, 3H), 2.48 (s, 3H), 2.31 (s, 3H), 1.33 (s, 3H), 1.31 (s, 3H) ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.0, 156.8, 154.6, 150.2, 142.9, 137.0, 136.0, 129.7, 127.8, 124.6, 124.5, 124.0, 121.7, 118.3, 117.3, 96.1, 77.4, 77.3, 77.1, 76.8, 33.18, 30.47, 15.6, 13.9. HRMS m/z $[M+H]^+$ calcd. for $C_{22}H_{27}BFN_3O_2S$, 488.1979; found 488.1999

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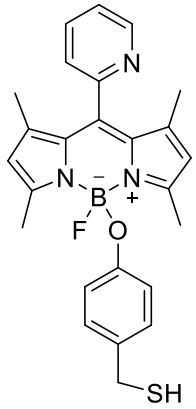
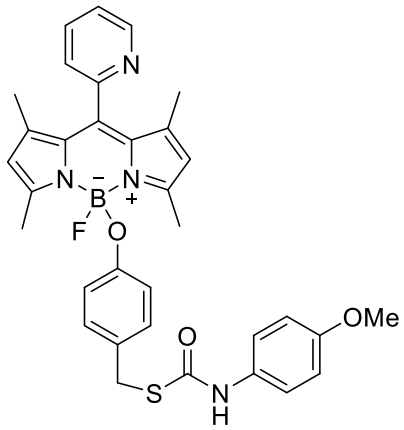
5-fluoro-1,3,7,9-tetramethyl-5-(4-((methylsulfinyl)methyl)phenoxy)-10-(pyridin-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (compound v)



In a 25 mL RB compound (iv) (30 mg, 0.063 mmol) was taken, and it was dissolved in 6 mL of dry methanol. K_2CO_3 (26.16 g, 0.189 mmol) was added to the solution and stirred at rt. After 20 min TLC indicates the completion of the reaction. 10 mL of EtOAc was added to the reaction mixture and was washed with (2x5 mL) water. The organic layer was passed over Na_2SO_4 and evaporated under reduced pressure to obtain crude product. Without any further purification, the crude product was taken to next step.

S-(4-((5-fluoro-1,3,7,9-tetramethyl-10-(pyridin-2-yl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-5-yl)oxy)benzyl) (4-methoxyphenyl)carbamothioate (compound vi)



Crude of Compound (v) was taken in a 25 mL RB and was dissolved in 6 mL of dry THF and kept at 0^{o}C. To this triethylamine (28 µL, 0.0002 mmol) and previously made solution of (a) (28.96 mg, 0.10 mmol) in THF (1 mL) was added dropwise. The reaction was stopped after 3 h, once the TLC indicated the consumption of starting material. 10 mL of water was added to the reaction mixture and was extracted with EtOAc (2x10 mL) water. The organic layer was passed over Na_2SO_4 and evaporated under reduced pressure. Crude product obtained was further purified using preparatory column to obtain orange solid (16 mg, yield = 45%). FT- IR (γ_{max}, cm-1): 1674, 1465, 1413, 1264. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.82 – 8.77 (m, 1H), 7.83 (dd, *J* = 7 Hz,14.3 Hz, 1H), 7.445 – 7.40 (m, 2H), 7.34 – 7.26 (m, 2H), 7.07 (t, *J*=8Hz, 2H), 6.99 (s, 1H), 6.82 (d, 2H, *J* = 7Hz) 6.5 (d, *J*=8.2 Hz, 1H), 6.49 (d, J =8.2 Hz, 1H), 5.91 (s, 2H), 4.08 (d, 2H, J=6.4Hz), 3.7(s, 3H), 2.51(s, 2H), 2.47(s, 3H), 1.32(s, 3H), 1.30(s, 3H) ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.7, 153.9, 150.1, 142.7, 142.5, 138.6, 138.5, 137, 131.6, 131.5, 130.0, 129.7, 128.5, 128.1, 124.4, 123.9, 123.8, 121.7, 121.6, 118.2, 117.6, 114.2, 55.4, 34.1, 14.9, 13.8. . HRMS m/z $[M+H]^+$ calcd. for $C_{33}H_{32}BFN_4O_3S$, 595.2354; found 595.2360.

4.2 Procedure for irradiation

Methenolic solution of compound (vi) was taken in a quartz cuvette and was irradiated at 530 nm (30 mW/cm^2), using a green LED at room temperature in a closed container. This solution was used for further studies.

4.3 Decomposition study by HPLC

200 µL of compound (vi) (50 µM) was irradiated for 5, 10, 15, 20 and 25 min and HPLC analysis of the aliquots was conducted. A diode array detector (DAD) operating at 250 nm and 500 nm was used. Water: acetonitrile was used as a mobile phase with a run time of 22 min. A multistep gradient was used with a flow rate of 1 mL/min starting with 60: 40 →0 min, 60:40 → 0 - 15 min, 60:40 to 10: 90 → 15 - 20 min, 10:90 to 60: 40 →20 – 21 min.

4.4 H_2S detection by Methylene blue assay

10 mM stock of compound (vi) prepared in DMSO was diluted to 200 µM in MeOH. 100 µL of irradiated sample was taken in 1.5 mL microcentrifuge which contains 292 µL of phosphate buffer of pH 7.4, 4 µL of $Zn(OAc)_2$, (40 mM stock in H_2O) and 4 µL of carbonic anhydrase (1% stock in phosphate buffer). Similar protocol was followed for preparing the sample of the non-irradiated compound. Both these samples were kept for incubation at 37 °C for 120 min. After incubation 400 µL of N,N–dimethyl-*p*- phenylenediamine sulfate (20 mM stock in 7.2 M HCl) and 400 µL of $FeCl_3$ (30 mM stock in 1.2 M HCl) were added to the reaction mixture. These reaction mixtures were further incubated at 37 °C for 30 min to allow the formation of methylene blue complex. Then these aliquots were transferred to a 96 well plate (250 µL/well). The absorbance spectra were collected from 550 to 700 nm using microplate reader (Thermo Scientific Varioskan), and the absorbance values were measured at 676 nm.

4.5 H_2S detection using electrode.

Calibration of the instrument (SO-H2S-100 Microsensor for H_2S measurement) was done by freshly prepared 2.5 mM of $Na_2S.9H_2O$. 50 µM of compound (vi) was prepared by adding 3 µL of 10 mM stock of compound into a vial containing 591 µL of phosphate buffer (pH 7.4), 6 µL of carbonic anhydrase and a small magnetic bead. The sensor was then dipped into the solution, and it was irradiated for 30 minutes under green light (530 nm) and simultaneously H_2S release was recorded using Labscribe software.

4.6 Photochemical experiments

Optical properties of BODIPY derivatives (2.5 µM, MeOH) were determined by Shimadzu UV-2600 UV-VIS spectrophotometer and Fluoromax-4 Spectrofluorometer (Horiba Scientific). Quantum efficiency (φ) of fluorescence for BODIPY derivatives were determined by using fluorescein (φ_{fl}= 0.85, 2.5 µM, 0.1 M NaOH aq.) as a standard with the following equation

$$\varphi = \varphi_{ref} \frac{\eta^2}{\eta_{ref}^2} \times \frac{I\, A_{ref}}{A\, I_{ref}}$$

Where, φ_{ref} quantum yield of the reference compound

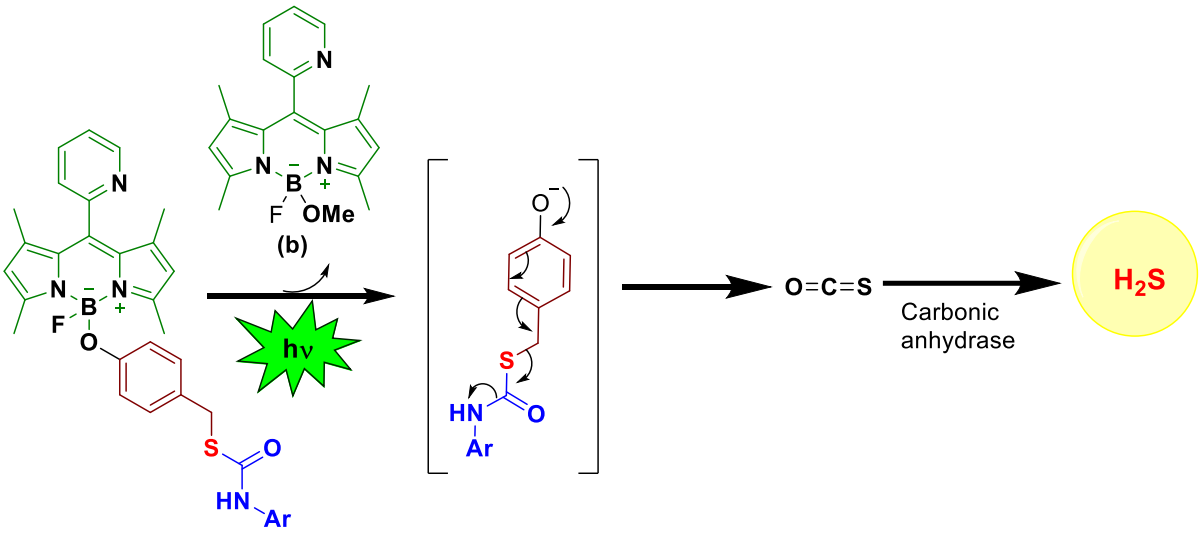
η Refractive index of the solvent used

I Integrated fluorescence intensity

A Absorbance at the excitation wavelength

5. Results and discussions

Proposed mechanism of the photocleavage.



5.1 HPLC studies.

50 µM of compound (vi) was injected at predetermined time points for irradiation. HPLC traces observed clearly indicates almost complete decomposition of starting material within 25 min of irradiation under green light (530 nm) to give (b) and phenolate intermediate as expected. This phenolate intermediate further self-immolates to produce COS/H_2S. Retention time observed for compound (vi) is 16.3 min and for the new peak formed for compound (b) after irradiation was observed at 10.5 min (at 502 nm). The kinetics study suggests that the reaction follows first order kinetics. Rate constant (k) value obtained for the exponential rise of the formation of (b) is 0.16 min^{-1} with an R^2 value 0.995 and for the exponential decay of compound (vi) k, and R^2 values are 0.12 min^{-1} and 0.994 respectively. This HPLC studies suggests that the compound can be photo-cleaved under visible light since we did not observe

any non-specific peaks suggests that the photo-cleavage of this compound (vi) is a very efficient process.

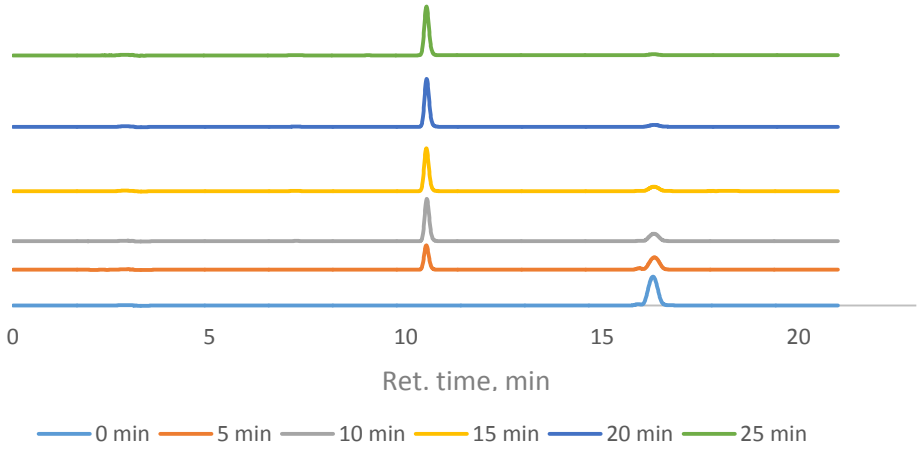


Fig: 5 50 µM of compound (vi) was injected at different time points (0, 5, 10, 15, 20, 25) minutes. 25 minutes of irradiation completely cleave the compound to give the product with retention time 10.5 min. Diode Array Detector was operated at 502 nm.

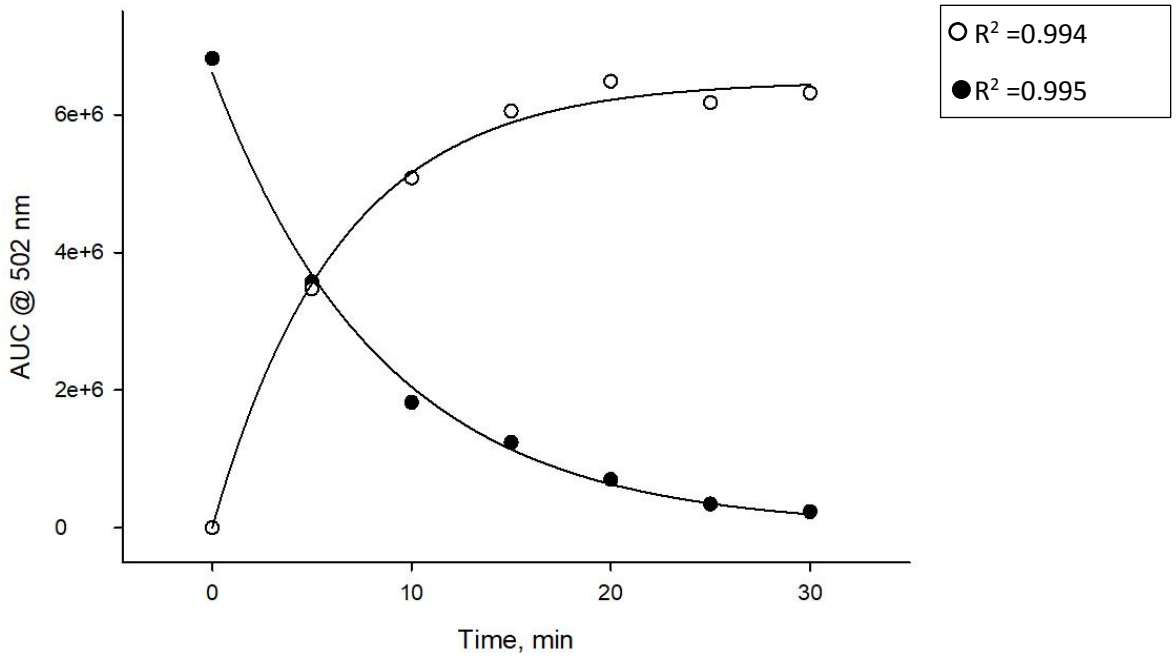


Fig: 6 Kinetics of decomposition of compound (vi) and the formation of the product. 50 µM of compound was injected at different time points (5,10,15,20,25 and 30 mins). Curve fitting for first-order kinetics gave rate constant = 0.16 min^{-1} and 0.12 min^{-1} for the formation of compound (b) and decomposition of compound (vi) respectively.

5.2 Detection of H_2S by Methylene Blue Assay

Methanolic solution of Compound (vi), when irradiated with green light (530 nm), HPLC study revealed the formation of phenolate intermediate which should self-immolate in buffer to generate COS. In the presence of carbonic anhydrase, this COS hydrolyses to produce H_2S. To test this hypothesis methylene blue assay was conducted and the characteristic peak for the formation of methylene blue was observed at 676 nm only in the irradiated samples. The yield of H_2S production was measured, and it was found to be 30%. However, there was no significant signal observed for methylene blue formation in the non-irradiated samples. This experiment suggests that the compound (vi) produces H_2S only in the presence of visible light.

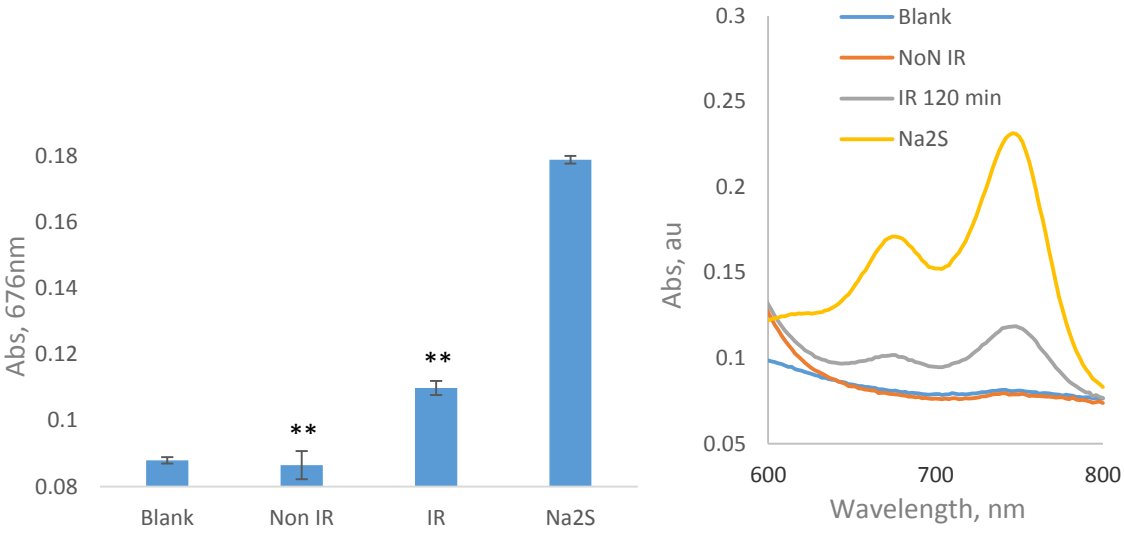


Fig: 7 A) Comparison of production of H_2S from irradiated, non-irradiated, blank and Na_2S. Blank and non-irradiated samples show no production of H_2S whereas irradiated samples indicate the release of H_2S from the molecule. (** indicates the P value ≤ 0.01) B) Characteristic increase in absorbance observed at 676 nm suggests the production of H_2S.

5.3 H_2S detection using electrode experiment

H_2S sensitive electrode can be used for real-time monitoring of H_2S production, which can further validate the production of H_2S. The compound (vi) in PBS along with carbonic anhydrase was irradiated with the sensor dipped in the solution. This real-

time monitoring helped us to observe a gradual rise in the H_2S production which gradually saturated around 25 minutes of irradiation. There was no signal observed when we did the same experiment without irradiating the sample. This experiment confirms that, the compound (vi) generates H_2S only when irradiated with green light.

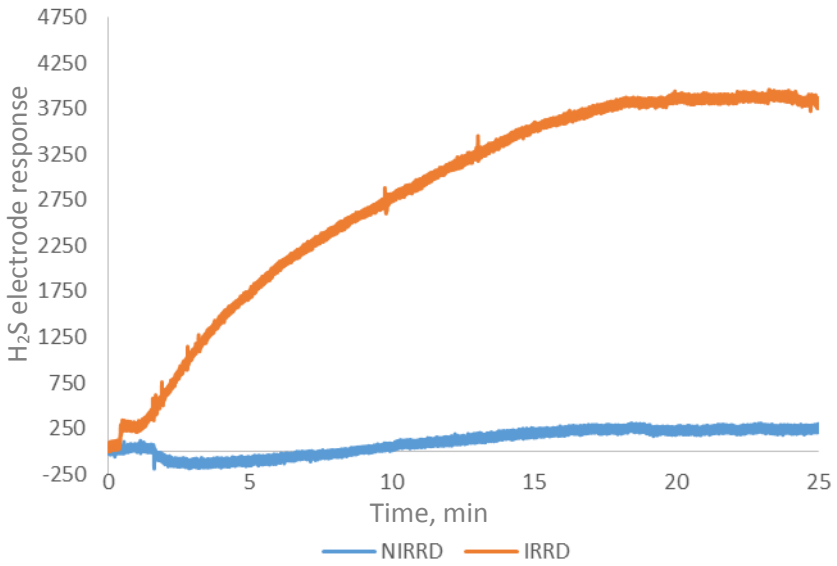


Fig: 8 Production of H_2S was observed when the electrode was dipped in the solution containing the compound in buffer (pH 7.4) along with carbonic anhydrase, irradiated with green light of wavelength 530 nm.

5.4 Photo-physical experiments

Compound (vi) have an aryl ring attached with the BODIPY through the B-O bond. This aryl ring may act as fluorescence quencher, which may result in lower quantum yield (Photo induced electron transfer). Irradiation of the compound (vi), should then cleave the B-O bond to form methoxy BODIPY and phenolate intermediate. This methoxy BODIPY may have higher quantum yield compared compound (vi). To check the change in quantum yield before and after irradiation, we carried out the photophysical experiments with fluorescein as reference compound. Absorption maxima (λ_{max}) of irradiated and non-irradiated samples of compound (vi) was found to be 505 nm, and quantum yields (φ) were calculated. The quantum yield of irradiated sample was 0.2, and for the non-irradiated sample was 0.008 as

expected. The low quantum yield of non-irradiated sample suggests that the fluorophore is getting quenched by the aryl ring and hence indicates the labile nature of the B-O bond.

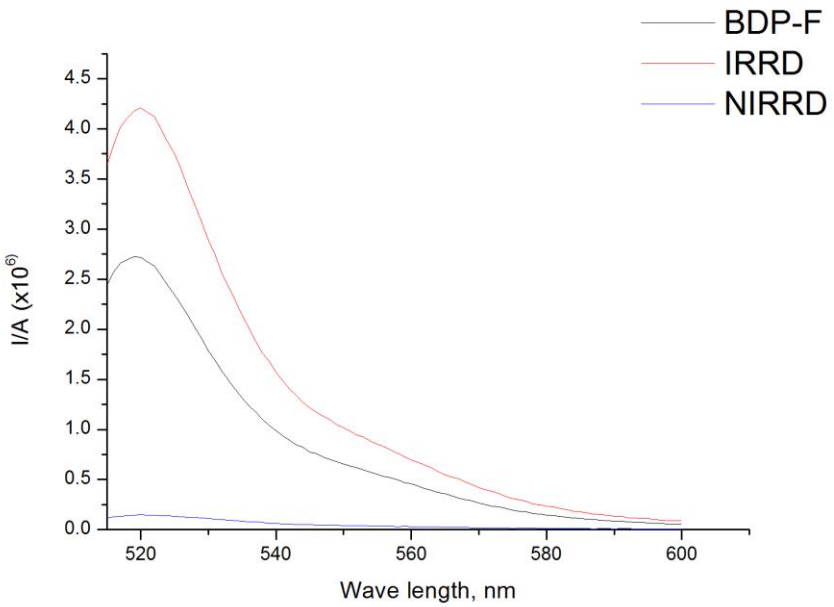


Fig: 9 Fluorescence spectra of irradiated (IRRD) and non-irradiated (NIRRD) samples of compound (vi) along with the difluoro BODIPY compound (BDP-F).

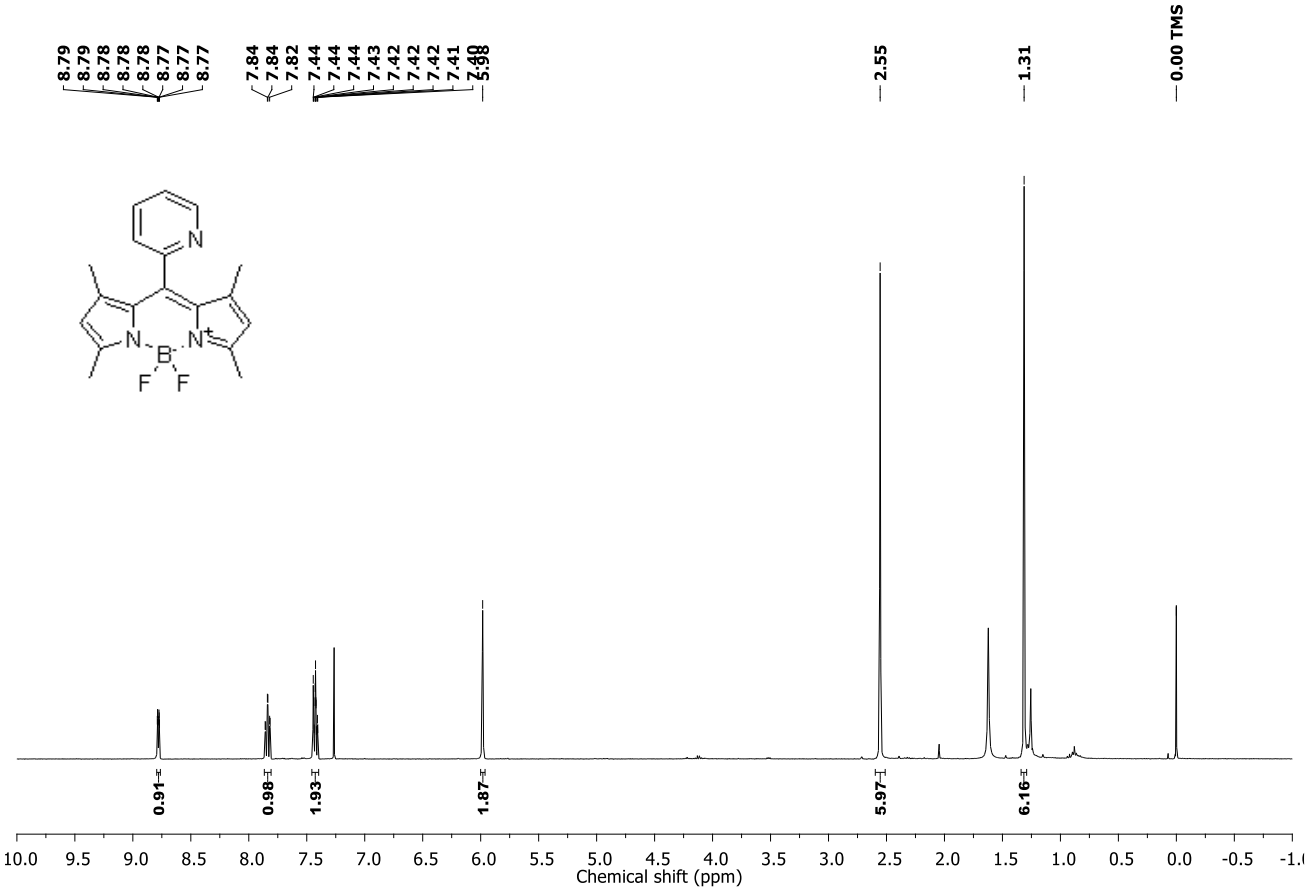
6. Conclusion and future outlook

We have developed a novel H_2S donor which is green light activated and have improved water solubility compared to previously reported phenyl analogue. The compound was stable in buffer (pH 7.4) and generates H_2S upon irradiating with visible light of wavelength 530 nm.

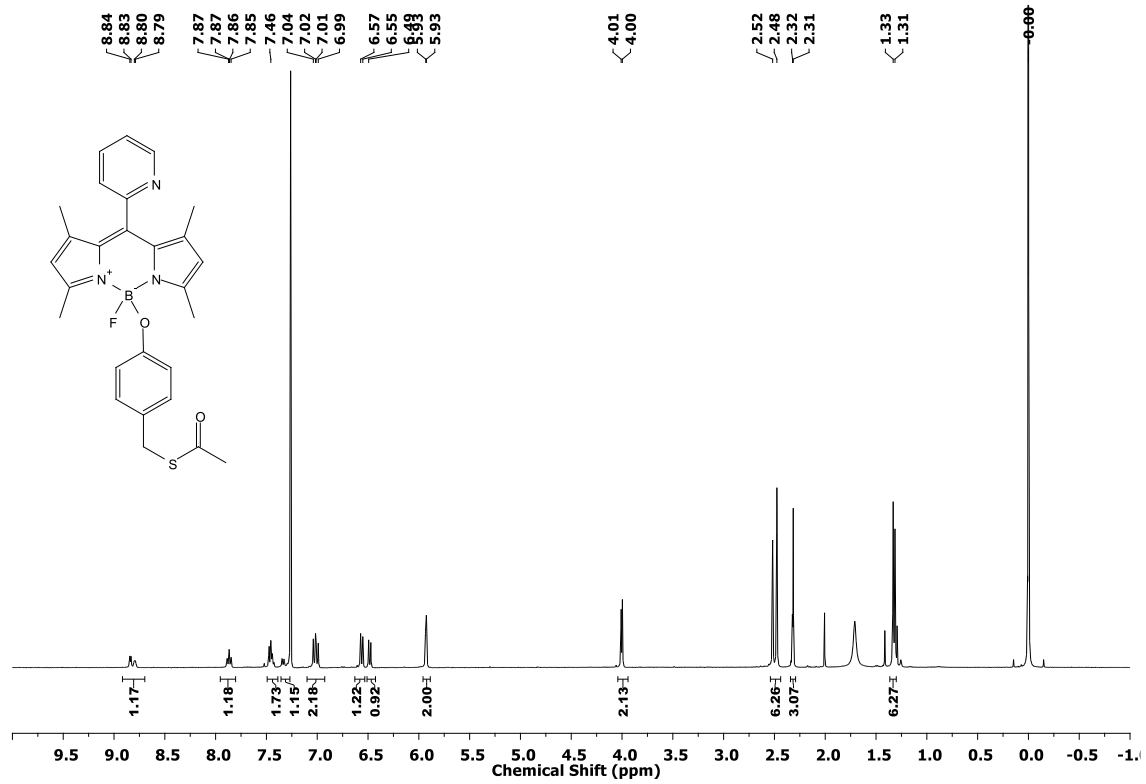
There are reports where compound (iii) was used as a mitochondrial fluorescence probe[29]. Incorporating an ethyl group on the pyridine moiety of compound (vi) could profoundly improve the water solubility and help in the localised delivery of H_2S to the mitochondria which may help in furthers studies of effects of H_2S on mitochondria.

NMR SPECTRA

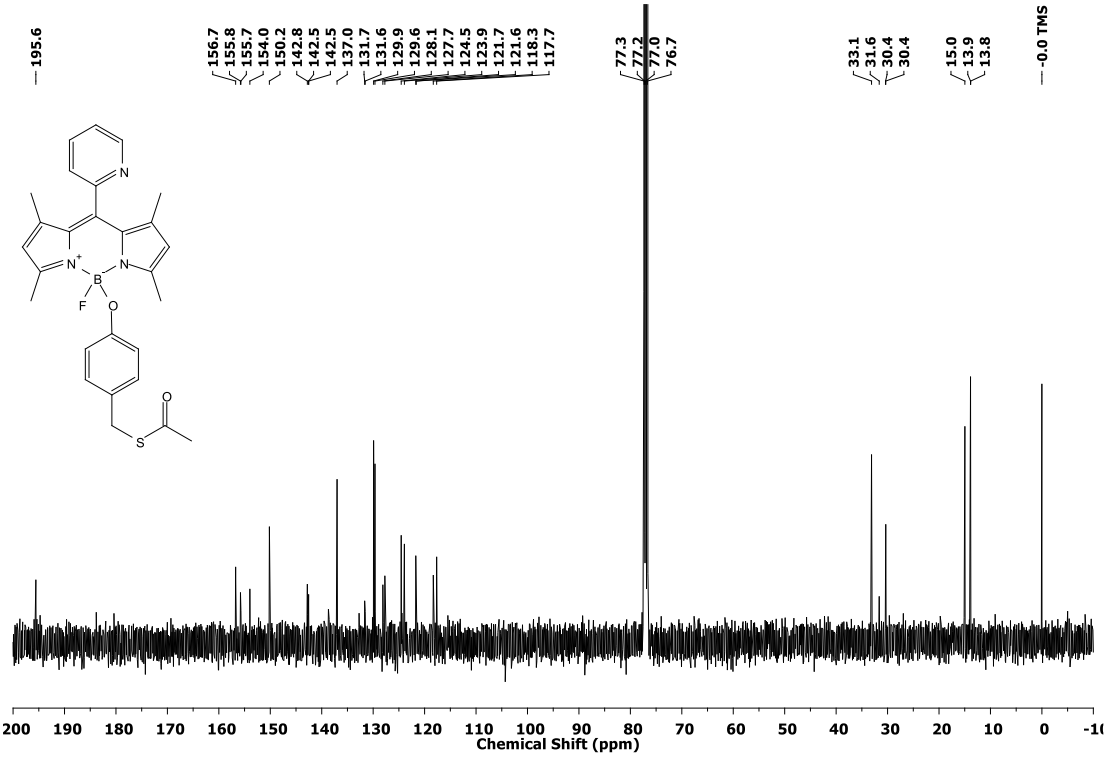
^{1}H NMR of compound (iii)



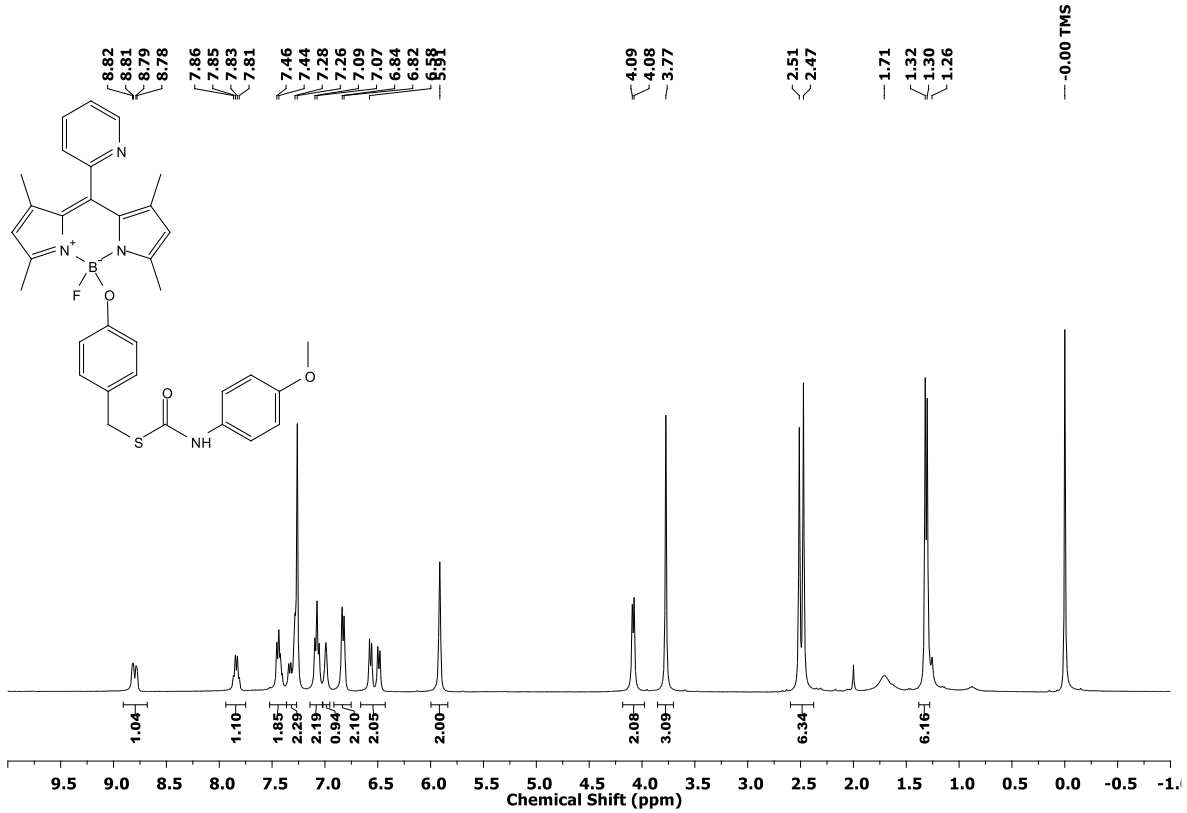
^{1}H NMR of compound (iv)



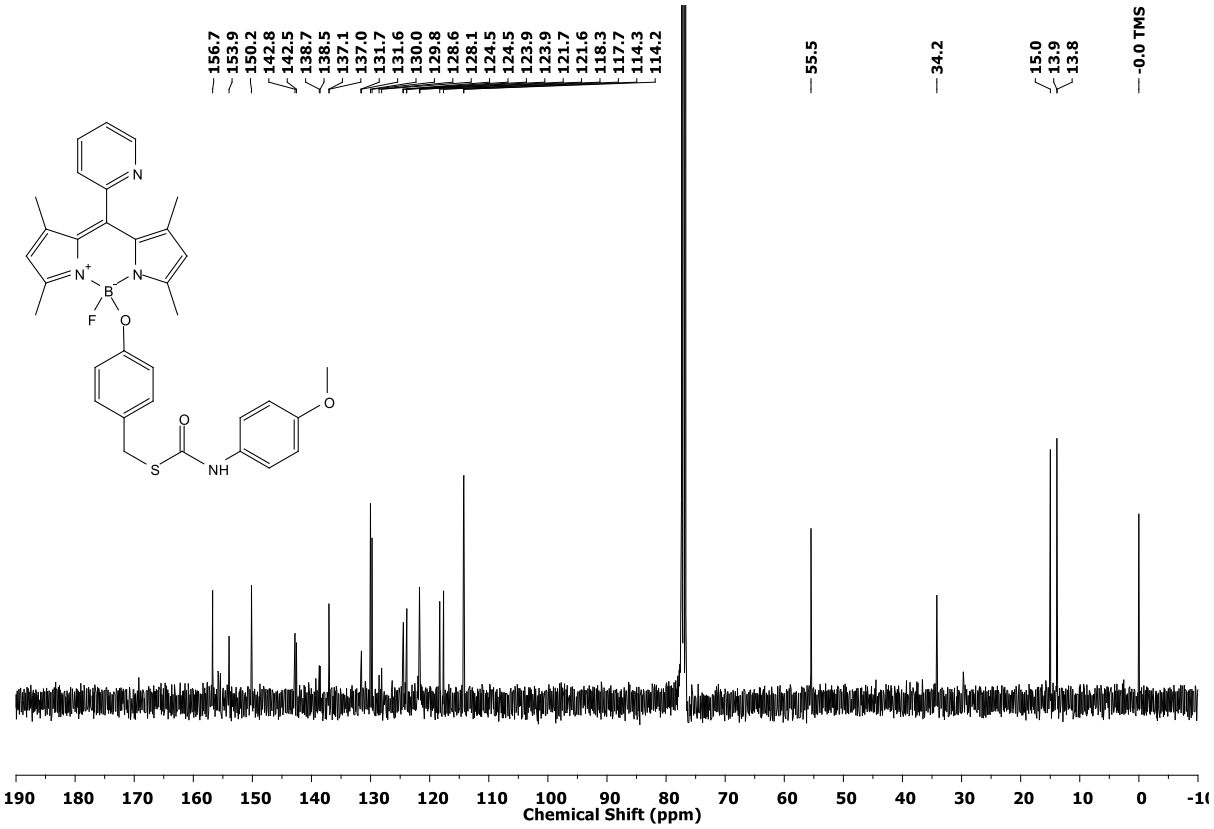
^{13}C NMR of compound (iv)



^{1}H NMR of compound (vi)



^{13}C NMR of compound (vi)



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