Synthesis and Evaluation of Hypervalent Iodine Compounds as Antibacterials

A Thesis

submitted to
Indian Institute of Science Education and Research Pune
in partial fulfilment of the requirements for the
BS-MS Dual Degree Programme

by

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Suraj Sharma 20141092

Certificate

This is to certify that this dissertation entitled **"Synthesis and Evaluation of Hypervalent Iodine Compounds as Antibacterial"** towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Suraj Sharma at the Indian Institute of Science Education and Research, Pune under the supervision of Dr.Harinath Chakrapani, Associate Professor, Department of chemistry, IISER Pune during the academic year 2018-19.

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Dr. Harinath Chakrapani

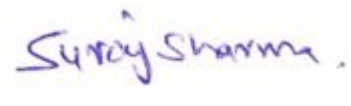
Committee:

Dr. Harinath Charkrapani

Dr. S.G Srivatsan

DECLARATION

I hereby declare that the matter embodied in the report entitled **"Synthesis and Evaluation of Hypervalent Iodine Compounds as Antibacterial"** are the results of the work carried out by me at the Indian Institute of Science Education and Research, Pune under the supervision of Dr.Harinath Chakrapani, Associate Professor, Department of chemistry, IISER Pune and the same has not been submitted elsewhere for any other degree.



Suraj Sharma

Date: 20 March

Place: IISER Pune

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Suraj Sharma

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
ACN	Acetonitrile
CYT b	Cytochrome b
DPI	Diphenyliodonium
IDP	Iodonium Diphenyl
FAD	Flavin adenine dinucleotide
PPI	Phenylpyridene Iodonium

2. Abstract

In past two decades, there has been a global surge in the number of drug resistant pathogens. This can be accounted to the indiscriminate use of antibiotics. The increased global drug resistant bacterias pose a serious health hazard in upcoming decades while global pharmaceutical companies just try to maximise their profits. In this context, Diphenyleneiodonium chloride (DPIC) a known inhibitor of NADH/NADPH has been reported as a potent antibacterial molecule against *M. Tb* and *S.aureus.* Based on this fact a series of pyridine derivatives of DPI (PPI) compounds were synthesised and were evaluated against gram-negative and gram-positive bacteria. All the compounds exhibit strong antimicrobial activity against gram-negative, gram-positive bacteria. Diphenyl iodonium (DPI) has considerably less antibacterial activity against gram-negative bacteria compared to phenylpyridine iodonium (PPI) which showed very good antibacterial activity against gram-positive as well as gram-negative bacteria. PPI shows very promising antibacterial activity it has the potential of being an antibacterial agent.

3. Introduction

Modern healthcare medication over the course of the past century has used antibacterial drugs to treat bacterial infection. But over time especially in recent decades, these drugs have been less effective to treat a given infection. This can be accounted for the increasing resistance of the bacteria towards these drugs.

Antimicrobial resistance is a growing concern in the context of global health systems.[1] All across the world, there are millions of cases reported of people suffering from infectious diseases, like tuberculosis. Every year more than 23000 people die due to infection of antibiotic-resistant bacteria in the United States.[1] The emergence of antibiotic-resistant microbial pathogens has made the task of effectively addressing infectious diseases more challenging. Due to antimicrobial resistance, the advancement in the field of medical science has decreased, not only in terms of human cost but antimicrobial resistance is a mammoth economic burden as well. Since there are only a few novel drugs in the pipeline, new strategies to address drug resistance are necessary.

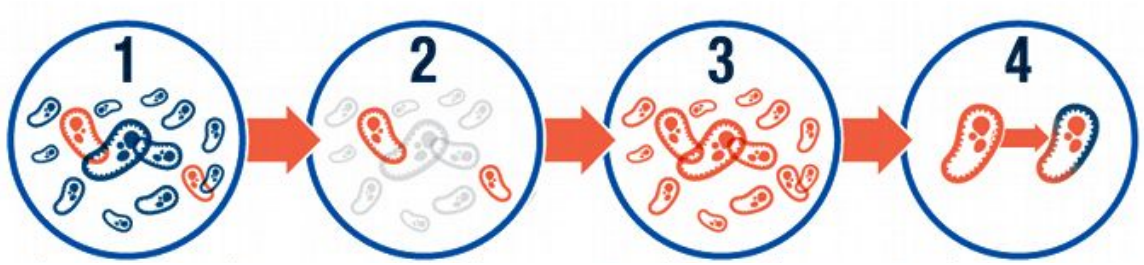


Figure- 1. The process of antibacterial resistance (*Illustration by the World Health Organization).*

For a given population of bacteria, there might be a small percentage of them which might be resistant to the drug administered. Over the course of time, natural selection comes into action and population of these drug-resistant bacteria increases. With overuse or over-exploitation of antibacterial drugs, the bacteria evolve, adapt and transfer their ability to the other bacteria as well. (Figure 1)

To resolve the problem of the antibiotic resistance several pharmaceutical active small molecules have been tested. Recently, Diphenyleneiodonium chloride (DPIC)

a known inhibitor of NADH/NADPH has been reported as a potent antibacterial agent against *M. Tb* and *S.aureus.*[2,3,6]

Diphenyleneiodonium chloride (DPIC) is a known inhibitor of NADH/NADPH in the mammalian cells and DPI bounds to a flavin-based protein (Figure.2).[4]

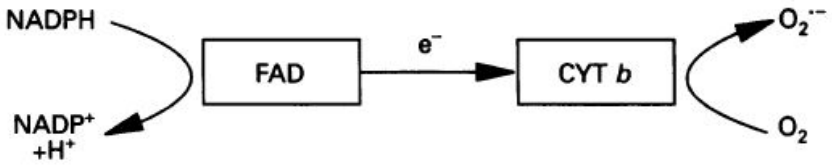


Figure 2: Arrangement of electron carriers in neutrophil NADH oxidase.

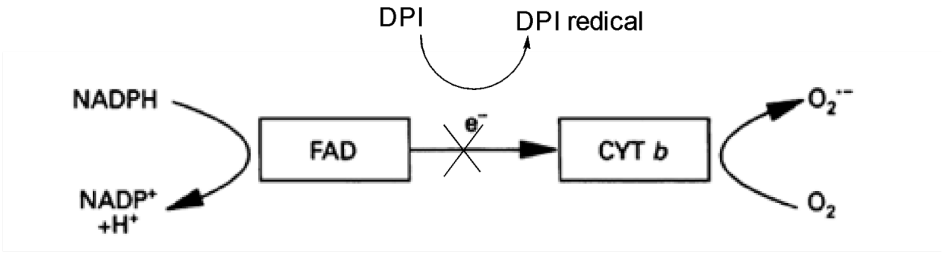


Figure 3: Proposed mechanism of NADH/NADPH inhibition by DPI.

Recently, DPIC has been found to have strong antimicrobial activity against gram negative, gram positive bacteria including *Mycobacterium tuberculosis*.[5-6] Even though it is very effective, it has shortcomings such as poor solubility and systemic toxicity. However, since this compound is active against multidrug-resistant strains of *Mycobacterium tuberculosis*, we propose to make systematic structural changes in order to improve its selectivity. In order to achieve this, various parameters like toxicity and solubility need to be taken care of.

4. Aim and hypothesis

Results for DPI against gram positive bacteria and *Mycobacterium tuberculosis* have been promising but for gram negative bacteria, they are not so good, due to its poor solubility and also toxicity of DPIC. We hypothesise a pyridine derivative of DPI (PPI) (Figure 4). Various drugs, vitamins, insecticides and herbicides contain pyridine moiety and due to its high polarity, it increases the solubility.[7] Due to this enhanced solubility, it is expected to show potent activity against gram-negative bacteria as well. Due to its benefits of increasing solubility and widespread use in various antibiotics, it would be wise to synthesise pyridine derivatives of DPI Synthesis of these compounds will be followed by evaluation against gram-negative and gram-positive bacteria.

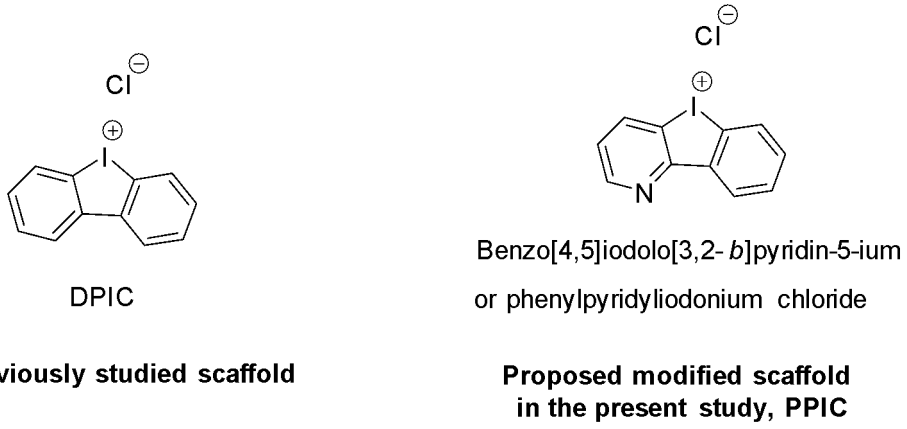


Figure 4: DPIC and its derivative

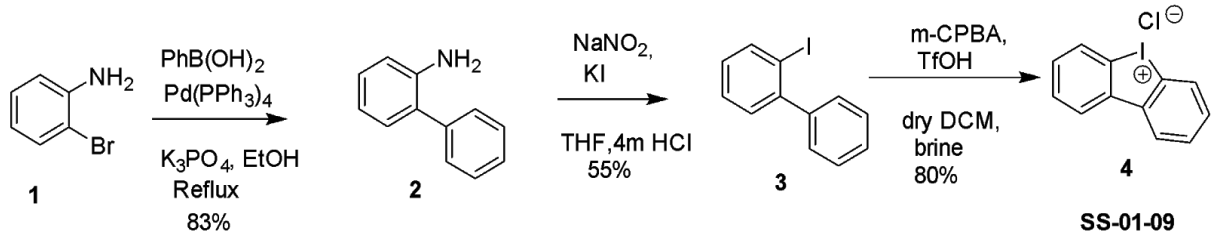
5. Material and method:

All the chemical were purchased from the Sigma Aldrich, TCI Chemicals. All the solvents were purchased from the commercial sources. Renkem silica gel (60-120 mesh) or (100-200 mesh) and neutral alumina were used to perform column chromatography. Sometimes solvents were filtered for purity. ^{1}H and ^{13}C were recorded on JEOL 400 MHz or a Bruker 400 MHz(or 100 MHz for ^{13}C). $CDCl_3$, DMSO-d_6 and MeOH-d_4 were used as a solvent to record the spectra with the small impurities of TMS in it. Solvents signals ($CDCl_3$, δ_H,= 7.26 ppm, δ_C = 77.2 ppm) and for internal TMS (TMS, δ_H,= 0.0 ppm, δ_C = 0.0 ppm). Chemical shifts ($\delta_)$ are reported in the ppm and coupling constant (*J*) in Hz. Abbreviations follow: s (singlet), d (doublet), dd (doublet of doublet), m (multiplet), t (triplet) and td (triplet of doublet). HRMS –ESI-Q-Time of Flight LC/MS was used for recording for HRMS (High-resolution mass spectra). NICOLET FT-IR spectrometer was used for recording of FT-IR spectra. Phenomenex ® C-18 reversed phase column (450 nm x 4.6 nm, 5µm) was used for HPLC (High performance liquid chromatography).

6. Synthesis and characterisation

6.1 Synthesis of DPIC

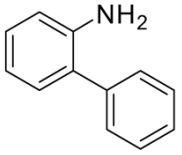
Compound **4** was synthesized using previously reported procedures and analytical data was consistent with reported values.[8]



Scheme 1: Synthesis of compound 4

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

6.1.1 Synthesis of [1,1'-biphenyl]-2-amine(2):



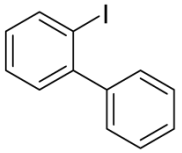
2

Compound **1** (500 mg, 2.91 mmol) was taken in an RB and dissolved in 40 mL of EtOH. $PhB(OH)_2$ (389.84 mg, 3.20 mmol) and K_3PO_4 (1.54 g, 7.27 mmol) were added to this solution. Under nitrogen atmosphere $Pd(PPh_3)_4$ (167.94 mg, 0.143 mmol) was added to the reaction mixture. Then the reaction was stirred overnight at reflux under the nitrogen atmosphere. After completion of the reaction as monitored by TLC, around 75% EtOH was evaporated. Then EtOAc was added and filtered through celite and the filtrated cake was washed with ethyl acetate and water. The organic components were extracted with EtOAc (3 X 15 mL), and collected organic phases were dried over anhydrous Na_2SO_4, concentrated under rota. The crude product as the yellow coloured residue was obtained. This residue was purified by column chromatography using neutral alumina as the stationary phase and EtOAc:

Hexane(0:100 to 6:94) as the mobile phase to afford **2** (408 mg, 83%) as a yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ= 7.45-7.44 (m, 4H), 7.36 - 7.33 (m, 1H), 7.17-7.12 (m, 2H), 6.84 (td, *J* = 7.34, 1.24Hz, 2H), 6.78-6.76 (m, 1H), 3.35 (s, 2H).

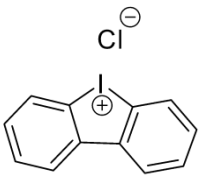
6.1.2 Synthesis of 2-iodo-1-1'-biphenyl (3):



3

Compound **2** (402 mg, 2.36 mmol) was taken in an RB and dissolved in THF (12 mL) and 4M HCl (16 mL). Then dropwise solution of $NaNO_2$ (246 mg, 3.55 mmol) dissolved in the minimum amount of water was added to the reaction mixture at 0 °C. Then the reaction mixture was allowed to stir for the next 20 min at 0 °C. Then a solution of KI (1.2g, 7.09 mmol) in water was added to the reaction mixture dropwise. Then the reaction allowed to stir for 12 h at rt. After completion of the reaction as monitored by TLC, EtOAc and H_2O were added to the reaction mixture and all organic components were extracted with EtOAc (3 X 15 mL). Collected organic phases were dried over anhydrous Na_2SO_4, concentrated under vacuum, to obtain the crude product as a yellow coloured residue. The residue was purified by column chromatography using silica as the stationary phase and EtOAc: Hexane(0:100 to 2:98) as the mobile phase to afford **3** (360 mg, 55%).

6.1.3 Synthesis of dibenzo[b,d]iodol-5-ium (4):



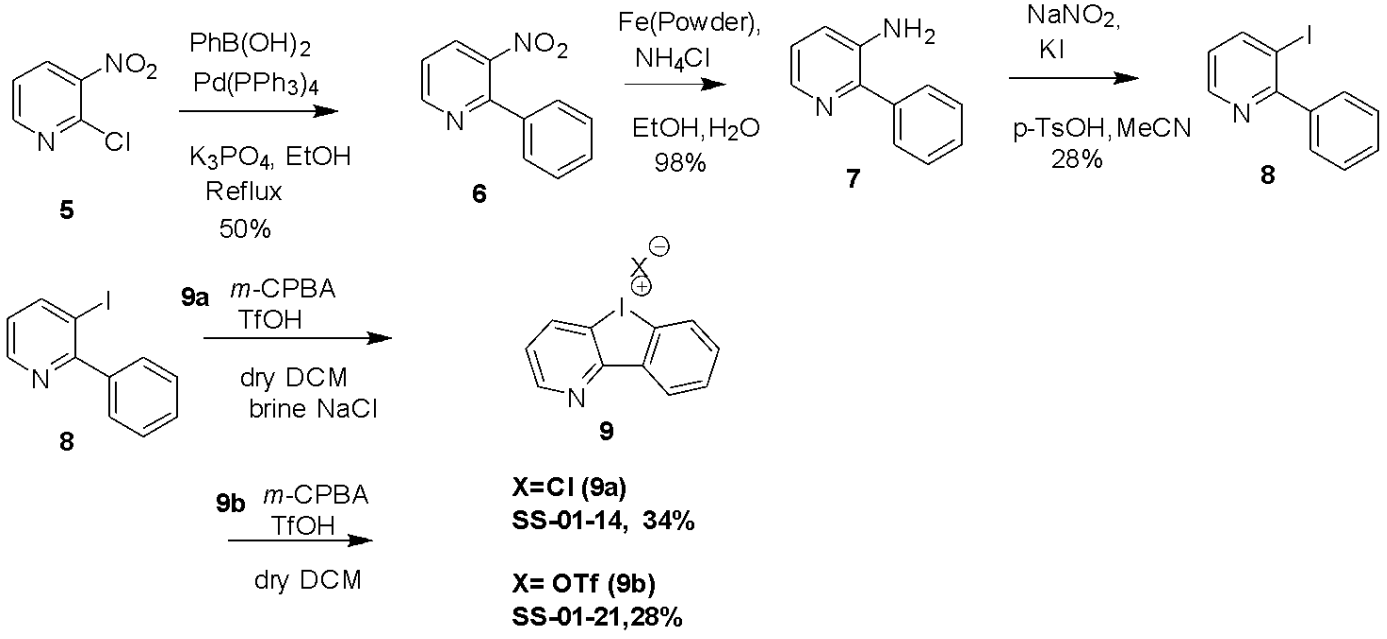
4

Compound **3** (100 mg, .357 mmol) was taken in an RB and dissolved in anhydrous DCM (5 mL). Then m-CPBA (65 mg, 364 mmol) and TfOH (94.7 µL, 1.07 mmol) were added under nitrogen condition at 0 °C. Then the resulting mixture allowed to stirred for 2h at rt. Then the solvent was evaporated and then formic acid was added dropwise to the pale-yellow residue. Then brine NaCl solution was added to the mixture. After a few minutes, the white solid was filtered off and washed several times with cold Et_2O and H_2O to afford the pure white solid **4** (80 mg, 80%).

^{1}H NMR (400 MHz, DMSO-d_6), δ= 8.57 (dd, *J* = 8.16,0.96Hz, 2H), 8.43 (dd, *J* = 7.94, 1.32 Hz, 2H), 7.83 - 7.79 (m, 2H),7.69 – 7.65 (m,1H); ^{13}C NMR (100 MHz, , $CDCl_3$) δ 141.10, 131.16, 130.93, 130.84, 126.84, 124.66.

6.2 Synthesis of derivative of DPI

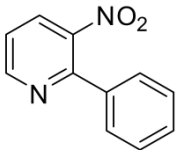
Compound **9b** was synthesized using previously reported procedures and analytical data were consistent with reported values for **9b** and as expected for **9a.**[8]



Scheme - 2: Synthesis of compound 9

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

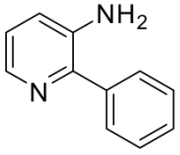
6.2.1 Synthesis of 3-nitro-2-phenylpyridine (6):



6

Compound **5** (882 mg, 5.56 mmol) was taken in an RB and dissolved in 50 mL of EtOH. $PPhB(OH)_2$ (746.16 mg, 6.12 mmol) and K_3PO_4 (2.95 g, 13.96 mmol) were added to this solution. Under nitrogen condition $Pd(PPh_3)_4$ (321.44 mg, 0.278 mmol) was added to the reaction mixture. Then the reaction allowed to stir for 36 hours at reflux under the nitrogen atmosphere. After completion of the reaction as monitored by TLC, around 75% EtOH was evaporated then EtOAc was added and then filtered through celite and the filtrated cake was washed with ethyl acetate and water. The organic components were extracted with EtOAc (3 X 15 mL) and collected organic phases were dried over anhydrous Na_2SO_4, concentrated under vacuum, obtain the crude product as a yellow coloured residue. The residue was purified by column chromatography using neutral silica as the stationary phase and EtOAc: Hexane (5:95 to 28:72) as the mobile phase to afford **6** (556 mg, 50%) as a yellow liquid.
^1H NMR (400 MHz, $CDCl_3$) δ 8.86 (dd, *J* = 3.24, 1.52Hz, 1H), 8.14(dd, *J* = 6.6, 1.56 Hz, 2H), 7.58 - 7.56 (m, 2H), 7.48 - 4.42 (m,4H);

6.2.2 Synthesis of 2-phenylpyredin-3-amine (7):



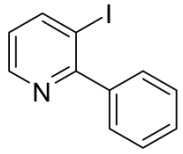
7

Compound **6** (556 mg, 2.72 mmol) was taken in an RB and dissolved in EtOH: H_2O [4:1] (35 mL). Fe (Powder) (464 mg 8.30 mmol) and NH_4CL (445 mg, 8.30 mmol) were added to this solution. Then the reaction allowed to stir for 15h reflux. After completion of the reaction as monitored by TLC then EtOAc was added to the

reaction mixture and filtered through celite and the filtrated solvent was evaporated by vacuum. The organic components were extracted with EtOAc (3 X 15 mL) and quenched with $NaHCO_3$. Then collected organic phases were dried over anhydrous Na_2SO_4, concentrated under vacuum to obtain pure product 7 (460 mg, 98%) as a yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (dd, *J* = 3.82, 1.56Hz, 1H), 7.68 - 7.66 (m, 2H), 7.50 - 7.46 (m, 2H), 7.50 - 4.46 (m, 2H), 7.42 - 7.38 (m, 1H), 7.09 - 7.08 (m, 2H).

6.2.3 Synthesis of 3-iodo-2-phenylpyredine (8):

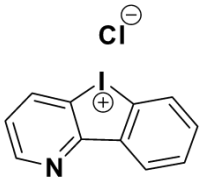


8

Compound **7** (250 mg, 2.06 mmol) was taken in an RB and dissolved in anhydrous MeCN (10 mL). p-TsOH (1.05g, 6.17mmol) was added under the nitrogen atmosphere at 0 ^{o}C. A solution of $NaNO_2$ (283.74 mg, 4.11 mmol) and KI (1.05g, 5.14 mmol) (dissolved in minimum amount of water) were added dropwise to the reaction mixture. Then the reaction mixture was allowed to stir for the next 10 min at 0 ^{o}C and 12 h at rt. After completion of the reaction as monitored by TLC, H_2O was added to the reaction mixture and quenched with $NaHCO_3$ until pH became 9. Then $Na_2S_2O_3$ was added and the colour of the solution became light orange. Organic components were extracted with Et_2O (3 X 15 mL) and collected organic phases were dried over anhydrous Na_2SO_4, concentrated under vacuum to obtain the crude product as a yellow coloured residue. The residue was purified by column chromatography using neutral alumina as the stationary phase and EtOAc: Hexane(0:100 to 5:95) as the mobile phase to afford (162 mg, 28%) as a yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (dd, *J* = 4.68, 1.52Hz, 1H), 8.26 (dd, *J* = 7.96, 1.52Hz, 1H), 7.60 - 7.57 (m, 2H), 7.47 - 7.42 (m, 3H), 6.99 (dd, *J* = 7.96,4.68 Hz, 1H).

6.2.4 Synthesis of benzo[4,5]iodolo-[3,2-b]pyridin-5-ium chloride (9a):

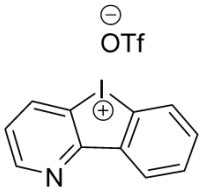


9a

Compound **8** (150 mg, 0.533 mmol) was taken in an RB and dissolved in anhydrous DCM (5 mL). Then m-CPBA (93.8mg, 0.544 mmol) and TfOH (141.65µL, 1.60 mmol) were added under the nitrogen atmosphere at 0 °C. The resulting mixture was allowed to stir for 2h at rt. Then the solvent was evaporated and formic acid was added dropwise to the pale-yellow residue. Brine NaCl solution was added to the mixture. After a few minutes, the white solid was filtered off and washed several times with cold Et_2O and H_2O to afford the pure white solid **9a** (51 mg, 34%). FT-IR (ν_{max} ,cm^{-1}); 3042, 1568, 1398, 1012, 739.

^1H NMR (400 MHz, DMSO-d_6) δ 8.94 (dd, *J*=4.63, 1.44Hz, 1H), 8.89 (dd, *J*=8.1, 1.16Hz, 1H), 8.64 (d, *J* = 7.6 Hz, 1H), 8.42 (dd, *J* = 7.58, 1.4H, 1H), 7.87 - 7.78 (m, 2H), 7.70 (dd, *J* = 6.78,4.64 Hz, 1H), ^{13}C NMR (400 MHz, DMSO-d_6) δ 157.85, 151.54, 139.81, 139.61, 132.81, 130.95, 130.69, 128.08, 125.82, 125.111, 122.31; DEPT-135 NMR (400 MHz, DMSO-d_6) δ 151.73, 139.80, 133, 131.14, 130.88, 128.27, 126.01. HRMS m/z $[M+H]^+$calcd for $C_{11}H_7IN^+$, 279.9618, found 279.9626.

6.2.5 Synthesis of benzo[4,5]iodolo-[3,2-b]pyridin-5-ium triflate (9b):



9b

Compound **8** (120 mg, 0.426 mmol) was taken in an RB and dissolved in anhydrous DCM (8 mL). Then m-CPBA (75.14mg, 0.432 mmol) and TfOH (113.32 µL, 1.28

mmol) were added under nitrogen atmosphere at 0 °C. Then the resulting mixture allowed to stir for 2 hours at rt. Then the solvent was evaporated and anhydrous Et_2O(20 mL) was added to the pale-yellow residue. After a few minutes, the white solid was filtered off and washed several times with cold Et_2O to afford the pure **9b** as a white solid; yield: 50mg (42%).

^{1}H NMR (400 MHz, MeOH-d_4) δ 9.00 (dd, *J*=4.6, 1.32Hz, 1H), 8.59(dd, *J* = 8.32, 1.32Hz, 1H) 8.46(dd, J = 7.64, 1.64Hz,1H) 8.29(dd, *J* = 7.92, 0.8 Hz,1H), 7.94 - 7.83 (m, 2H), 7.76 (dd, *J* = 8.32,4.6Hz, 1H); ^{13}C NMR (400 MHz, MeOH-d_4) δ 159.58, 153.06, 141.12, 140.07, 134.43, 132.35, 131.09, 130.50, 127.00, 121.38, 119; DEPT-135 NMR (400 MHz, MeOH-d_4) δ 152.78, 139.79, 134.15, 132.07, 130.81, 130.22, 126; HRMS m/z $[M+H]^+$ calcd for $C_{11}H_7IN^+$, 279.9618, found, 279.9627.

6.3 Synthesis of derivative of PPI

We synthesized analogues **9a, 9b, 14a, 14b, 15a ,15b, 16a, 16b, 17a,** and **17b**of PPI using the general scheme **2**.[8]

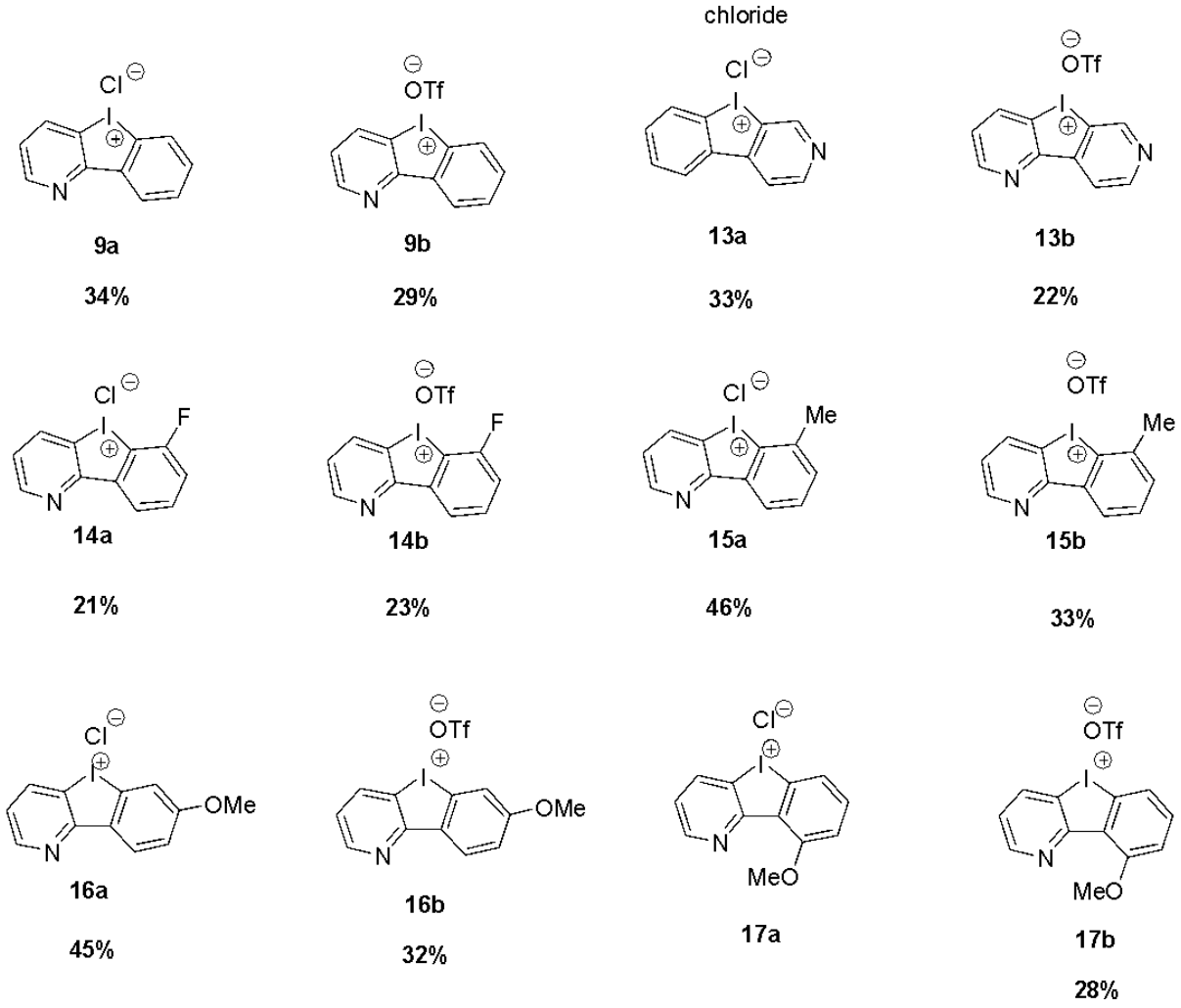
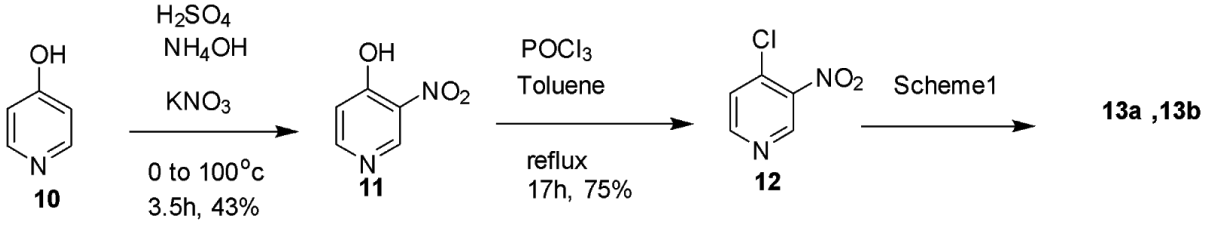


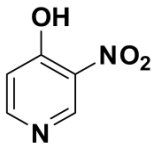
Figure 5: Analogues of PPI

The synthesis of analogues **13a** and **13b** required some extra steps. We started with the commercially available compound **10** and synthesised compound **12** which was used for the synthesis of **13a** and **13b** following Scheme-**2.**[9-10]



Scheme 3: Synthesis of compound 13a, 13b

Synthesis of 3-nitropyridin-4-ol(11):

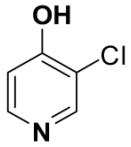


11

15 mL of sulphuric acid was taken in an RB and cooled to 0^0C. Compound **10** (2 g, 21.03 mmol) and KNO_3(4.25 g, 42.06 mmol) were added very slowly to it at the same temperature. Then the temperature was raised to 100 ^{0}C and the reaction was stirred for 1.5 hours. Then the temperature was brought down to 0 ^{0}C and the reaction mixture was poured into ice water. The mixture was neutralized using ammonia solution (pH~6.5) and kept overnight. A yellow precipitate formed was filtered and dried in vacuum to provide compound **11** (570 mg, 20%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (d, *J*=1.44,1H), 7.84(dd, *J*=7.48, 1.52Hz, 1H), 6.49 (d, *J*=7.44, 1H).

Synthesis of 4-chloro-3-nitropyridine(12).

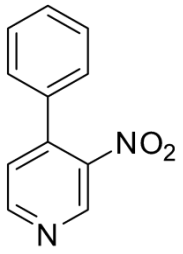


12

Compound **11** (560 mg, 4 mmol) was taken in an RB and dissolved in toluene (15 mL). Then $POCl_3$ (1.12 ml, 11.99 mmol) was added under a nitrogen atmosphere at 0 ^{o}C. Then the temperature was raised to rt and the reaction was stirred under reflux at 110 ^{o}C. After 17 hours the reaction completed and the residue was poured into ice water and then basified using K_2CO_3 up to (pH~10). Organic components were extracted with EtOAc and collected organic phases were dried over anhydrous Na_2SO_4, concentrated under vacuum to obtain the product **12** as a brown oil coloured liquid (470mg, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.72 (d, *J* = 5.32Hz, 1H), 7.57 (d, *J* = 5.32Hz, 1H).

Synthesis of 3-nitro-4-phenylpyridine (18):

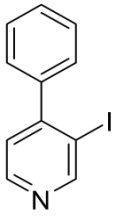


18

Using the above general scheme **2**, compound **18** was obtained as a yellow liquid with 20% yield. (neutral silica EtOAc : Hexane (1:99 to 16:84)).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s,1H), 8.80(d, *J* = 5.04Hz, 1H), 7.48 - 7.47 (m, 3H), 7.42(d, *J* = 5.04 Hz, 1H) 7.36 - 4.33 (m, 2H);

Synthesis of 3-iodo-4-phenylpyridine (19):

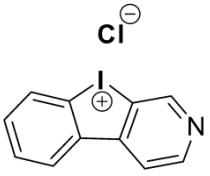


19

Using the above general scheme **2**, compound **19** was obtained as a yellow solid with 47% yield. (neutral alumina EtOAc:Hexane (0:100 to 5:95)).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (s,1H), 8.54(d,*J* = 4.92Hz, 1H), 7.48-7.46 (m, 3H), 7.37 - 7.35 (m, 2H), 7.27 (dd, *J* = 4.94, 0.48 Hz, 1H);

Synthesis of benzo[4,5]iodolo-[3,2-c]pyridin-9-ium chloride (13a):

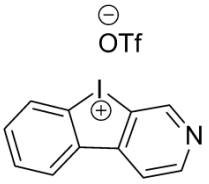


13a

FT-IR (ν_{max} ,cm^{-1}); 3049, 1567, 1384, 979, 606.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s,1H), 8.926 (d,J=5.2Hz, 1H), 8.707 (dd, *J* = 8.14, 0.96 Hz, 1H), 8.56 (dd, *J* = 7.78, 1.4Hz, 1H), 8.43 (dd, *J* = 5.32, 0.56Hz, 1H) 7.89 (td, *J* = 7.78, 1.12Hz, 1H) 7.82-7.78 (m, 1H). ^{13}C NMR (400 MHz, DMSO-d_6) δ 150.42, 149.64, 148.53, 139.51, 132.29, 130.69, 127.32, 125.74, 121.66, 120.13. DEPT-135 NMR (400 MHz, DMSO-d_6) δ 150.38, 149.60, 132.25, 130.65, 130.27, 127.28, 120.09. HRMS m/z $[M+H]^+$calcd for $C_{11}H_7IN^+$, 279.9618, found, 279.9625.

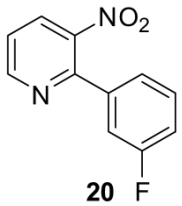
Synthesis of benzo[4,5]iodolo-[3,2-c]pyridin-9-ium triflate (13b):



13b

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.29 (s,1H), 8.98 (d, *J* = 5.2Hz, 1H), 8.66 (dd, *J* = 7.86, 1.44 Hz, 1H), 8.50 (dd, *J* = 5.28, 0.56Hz, 1H), 8.27(dd, *J* = 8.22, 0.92Hz, 1H) 7.96 - 7.78 (m, 2H). ^{13}C NMR (400 MHz, DMSO-d_6) δ150.58, 150.54, 149.43, 140.41, 133.49, 131.13, 130.99, 128.41, 123.78, 121.28, 119.49.^{19}F NMR (400 MHz, DMSO-d_6) δ -77.76. HRMS m/z $[M+H]^+$calcd for $C_{11}H_7IN^+$, 279.9618, found, 279.9625.

Synthesis of 2-(3-fluorophenyl)-3-nitropyridine(20).

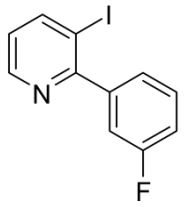


20

Using the above general scheme **2**, compound **20** was obtained with 43% yield as a yellow liquid. (neutral silica EtOAc:Hexane(1:99 to 10:90)).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (dd, *J* = 4.8, 1.84Hz, 1H), 8.18 (dd, *J* = 8.22, 1.44Hz, 1H), 7.50 - 7.40 (m, 2H), 7.34 – 7.28 (m, 2H) 7.20-7.16 (m,1H);

Synthesis of 2-(3-fluorophenyl)-3-iodopyridine (21).

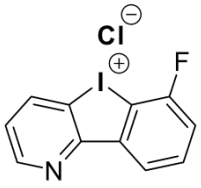


21

Using the above general scheme **2**, compound **21** was obtained with a 43% yield as a yellow solid. (neutral alumina EtOAc:Hexane(0:100 to 10:95)).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (dd, *J* = 4.62, 1.52Hz, 1H), 8.27 (dd, *J* = 8.04, 1.72Hz, 1H), 7.45-7.36 (m, 2H), 7.32 - 7.29 (m, 1H), 7.15-7.10 (m,1H) 7.0 (dd, *J* = 8, 4.6 Hz, 1H);

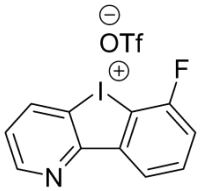
Synthesis of 6-fluorobenzo[4,5]iodolo-[3,2-b]pyridin-5-ium chloride (14a):



14a

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97-8.87 (m,2H), 8.67 (dd, *J* = 8.94, 5.12Hz, 1H), 8.15 (dd, *J* = 9, 2.88 Hz, 1H) 7.75-7.67 (m, 2H). HRMS m/z $[M+H]^+$calcd for $C_{11}H_6FIN^+$, 297.9523, found, 297.9527.

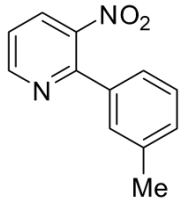
Synthesis of 6-fluorobenzo[4,5]iodolo-[3,2-c]pyridin-9-ium triflate (14b):



14b

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.035 (dd, *J* = 4.62, 1.32Hz, 1H), 8.62 (dd, *J* = 8.4, 1.36Hz, 1H), 8.31(dd, *J* = 9, 4.88 Hz, 1H), 8.23 (dd, *J* = 8.88, 2.92 Hz, 1H), 7.80 - 7.73 (m,2H). HRMS m/z $[M+H]^+$calcd for $C_{11}H_6FIN^+$, 297.9523, found, 297.9533.

Synthesis of 2-(3-methylphenyl)-3-nitropyridine (22).

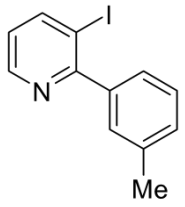


22

Using the above general scheme **2**, compound **22** was obtained with 57% yield as a yellow liquid.(neutral silica EtOAc:Hexane(1:99 to 11:89)).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (dd, J=4.74,1.48Hz,1H), 8.13 (dd,J=8.14,1.52Hz,1H), 7.45 - 7.41 (m, 2H), 7.37 – 7.27 (m,3H),2.42(s,3H)

Synthesis of 2-(3-methylphenyl)-3-iodopyridine (23).

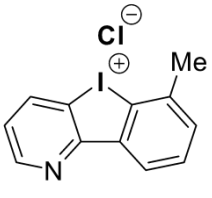


23

Using the above general scheme **2**, compound **23** was obtained with a 32% yield as a yellow solid. (neutral alumina EtOAc:Hexane(0:100 to 5:95)).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (dd, *J* = 4.8,1.52Hz, 1H), 8.25(dd, *J*=8.12, 1.52Hz, 1H), 7.40-7.32 (m, 3H), 7.23 (dd, *J* = 7.42, 0.6Hz, 1H), 2.42 (s, 3H);

Synthesis of 6-methylbenzo[4,5]iodolo-[3,2-b]pyridin-5-ium chloride (15a):

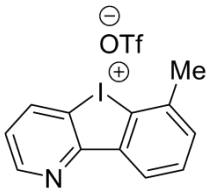


15a

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (dd, *J* = 4.6,1.4Hz,1H)8.86 (dd,*J* = 8.24, 1.4Hz, 1H), 8.48 (d, *J* = 8.32Hz, 1H), 8.26 (d, *J* = 1.04Hz, 1H) 7.69 (dd, *J* = 8.24, 0.56 Hz, 1H), 7.63(dd, *J*=8.66, 1.44Hz, 1H), 2.52 (s,3H). ^{13}C NMR (400 MHz, DMSO-d_6). Not

soluble properly in DMSO. HRMS m/z $[M+H]^+$calcd for $C_{11}H_6MeIN^+$, 293.9774, found, 293.9764.

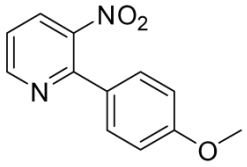
Synthesis of 6-methylbenzo[4,5]iodolo-[3,2-c]pyridin-9-ium triflate (15b)



15b

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (dd, *J* = 4.6, 1.32Hz, 1H), 8.57 (dd, *J* = 8.34,1.36Hz, 1H), 8.29(d, *J* = 1.48 Hz, 1H), 8.14(d, *J* = 8.4 Hz, 1H), 7.74(dd, *J* = 8.34, 4.64 Hz, 1H), 7.68 (dd, *J* = 8.38,1.64Hz, 1H), 2.55 (s,3H) ^{13}C NMR (400 MHz, MeOH-d_4) δ159.52, 153.0, 143.60, 141.03, 140.11, 135.54, 130.80, 130.66, 126.91, 119.44, 117.66, 21.15. DEPT-135 NMR (400 MHz, MeOH-d_4) δ152.74, 139.85, 135.28, 130.55, 130.41, 126.65, 20.90. HRMS m/z $[M+H]^+$calcd for $C_{11}H_6MeIN^+$, 293.9774, found, 293.9785.

Synthesis of 2-(4-methoxyphenyl)-3-nitropyridine (24)

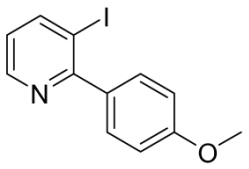


24

Using the above general scheme **2**, compound **24** was obtained in 57% yield as a yellow liquid.(neutral silica EtOAc:Hexane(2:98 to 12:88)).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (dd, *J* = 4.74, 1.44Hz, 1H), 8.08 (dd, *J*=7.96, 1.44Hz, 1H), 7.56 - 7.52 (m, 2H), 7.38(dd, *J* = 8.1, 4.76 Hz, 1H), 7.00 – 6.97 (m,2H), 3.86 (s,3H).

Synthesis of 2-(4-methoxyphenyl)-3-iodopyridine (25).

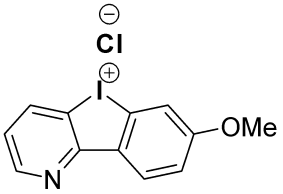


25

Using the above general scheme **2**, compound 25 was obtained in 33% yield as a yellow solid.(neutral alumina EtOAc:Hexane(0:99 to 9:91)).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (dd,J=4.62,1.44Hz,1H), 8.24(dd, J=7.96,1.28Hz, 1H), 7.60-7.56 (m, 2H),6.99-6.96(m,2H),6.94 (dd, *J* = 9.32,4.64Hz, 1H),3.87(s,3H);

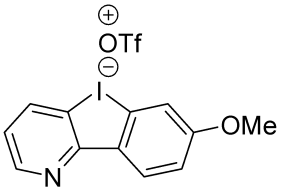
Synthesis of 7-methoxybenzo[4,5]iodolo-[3,2-b]pyridin-5-ium chloride (16a):



16a

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24(dd, *J* = 8.34, 0.96 Hz, 1H) 8.84 (dd, *J* = 4.62, 1.44 Hz, 1H), 8.70 (d, *J* = 2.28Hz, 1H), 7.27 (d, *J* = 8.64 Hz, 1H) 7.57 (dd, *J* = 8.96, 4.64 Hz, 1H), 7.38(dd, *J* = 8.64, 2.4Hz, 1H), 3.89 (s,1H). ^{13}C NMR (400 MHz, DMSO-d_6). Not soluble properly in DMSO. HRMS m/z $[M+H]^+$ calcd for $C_{11}H_6OMeIN^+$, 309.9723, found, 309.9734.

Synthesis of 7-methoxylbenzo[4,5]iodolo-[3,2-c]pyridin-9-ium triflate (16b):

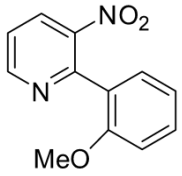


16b

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93(dd, *J* = 4.64, 1.32Hz, 1H) 8.53 (dd, *J* = 8.32, 1.32Hz, 1H), 8.32 (d, *J* = 8.68 Hz,1H), 7.81 (d,*J* = 2.4Hz, 1H) 7.67 (dd, *J* = 8.32,4.64

Hz, 1H), 7.48 (dd, *J* = 8.72, 2.4Hz, 1H), 3.94 (s,1H). ^{13}C NMR (400 MHz, DMSO-d_6) δ 162.51, 157.32, 151.40, 139.13, 131.97, 129.15, 126.36, 124.84, 123.22, 118.91, 118.19, 114.55, 56.33. ^{19}F NMR (400 MHz, DMSO-d_6) δ -77.76. HRMS m/z $[M+H]^+$ calcd for $C_{11}H_6OMeIN^+$, 309.9723, found, 309.9734.

Synthesis of 2-(2-methoxyphenyl)-3-nitropyridine (26):

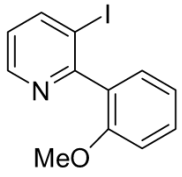


26

Using the above general scheme **2**, compound **26** was obtained with 57% yield as a yellow liquid.(neutral silica EtOAc:Hexane(2:98 to 12:88)).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (dd, *J* = 4.72, 1.56 Hz, 1H), 8.21 (dd, *J* = 8.14, 1.56 Hz, 1H), 7.68 (dd, *J* = 7.56, 1.72 Hz, 1H) 7.44 - 7.40 (m, 2H), 7.15 (dd, *J* = 7.52, 0.96 Hz, 1H), 6.91 (dd, *J* = 8.83, 0.64Hz, 1H) 3.72 (s,3H).

Synthesis of 2-(2-methoxyphenyl)-3-iodopyridine (27).

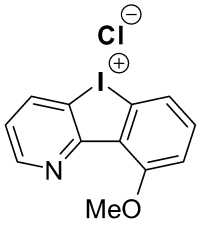


27

Using the above general scheme **2**, compound **27** was obtained with a 33% yield as a yellow solid.(neutral alumina EtOAc:Hexane(0:100 to 9:91)).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (dd, *J* = 4.7, 1.52Hz, 1H), 8.20 (dd, *J* = 7.98, 1.56Hz, 1H), 7.41 (ddd, *J* = 7.88, 1.8, 0.84 Hz, 1H) 7.23 (dd, *J* = 7.46, 1.72 Hz, 1H), 7.06 (td, *J* = 7.4, 0.96 Hz, 1H) 6.99 - 6.96 (m, 2H), 3.80 (s,3H).

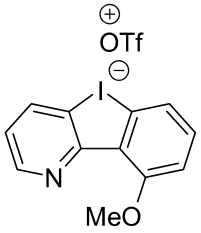
Synthesis of 9-methoxybenzo[4,5]iodolo-[3,2-b]pyridin-5-ium chloride (17a):



17a

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22(dd, *J* = 8.18, 1.24 Hz, 1H) 8.98 (dd, *J* = 4.8, 1.48 Hz, 1H), 8.41(d, *J* = 8 Hz, 1H), 7.72 - 7.66 (m, 2H), 7.52 (d, *J* = 8.28 Hz, 1H), 4.05 (s,3H). ^{13}C NMR (400 MHz, DMSO-d_6) Not soluble properly in DMSO. HRMS m/z $[M+H]^+$ calcd for $C_{11}H_6$ OMeIN$^+$, 309.9723, found, 309.9734.

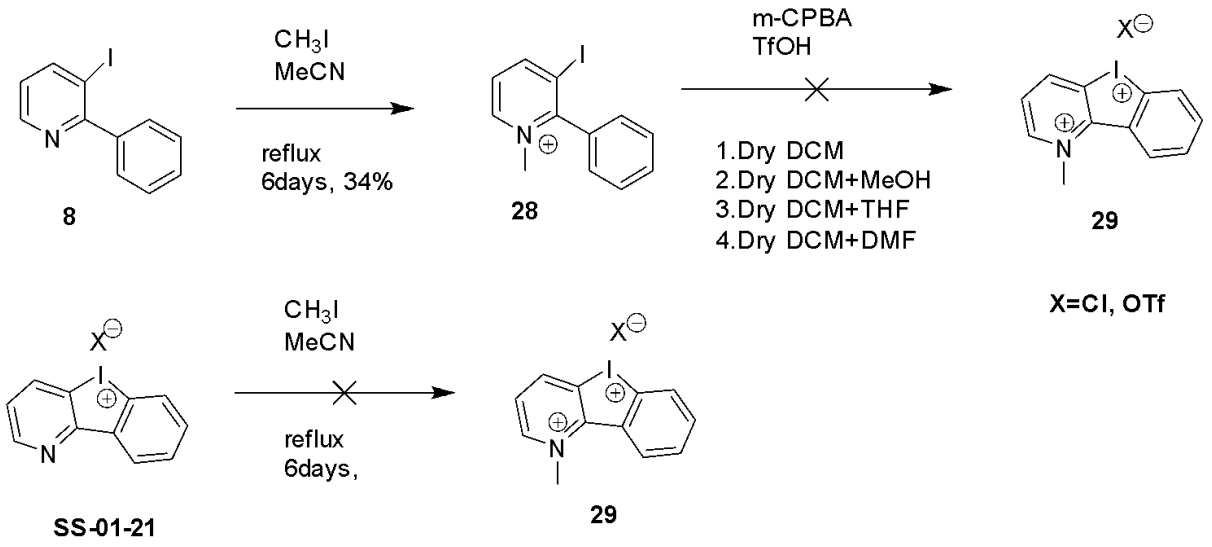
Synthesis of 9-methoxylbenzo[4,5]iodolo-[3,2-c]pyridin-9-ium triflate (17b):



17b

^{1}H NMR (400 MHz, MeOH-d_4) δ 8.93 (dd, *J* = 4.64, 1.32 Hz, 1H) 8.53 (dd, *J* = 8.32, 1.32 Hz, 1H), 8.32 (d, *J* = 8.68 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H) 7.67 (dd, *J* = 8.32, 4.64 Hz, 1H), 7.48 (dd, *J* = 8.72, 2.4Hz, 1H), 3.94 (s,1H).^{13}C NMR (400 MHz, MeOH-d_4) δ 163.01, 159.92, 152.07, 140.04, 134.28, 127.75, 125.57, 122.89, 121.76, 117.52, 115.77, 57.14; DEPT-135 NMR (400 MHz, MeOH-d_4) δ 152.07, 140.02, 134.28, 1125.58, 122.89, 115.77, 57.14 ^{19}F NMR (400 MHz, MeOH-d_4) δ -77.76. HRMS m/z $[M+H]^+$ calcd for $C_{11}H_6$ OMeIN$^+$, 309.9723, found, 309.9730.

6.4 Synthesis of N-methylated derivative of PPI.



8 28 29

SS-01-21 29

Scheme 4: Failed synthesis

6. Results and discussions

Several compounds were synthesized and MIC (minimum inhibitory concentration is the lowest concentration of a chemical, usually a drug, which prevents visible growth of bacterium.)[11] data were obtained for four of them, it is observed that some analogues of PPI showed more antibacterial activity compared to DPI as we had originally proposed. (Table 1) DPI has considerably less antibacterial activity against gram-negative bacteria compared to PPI which showed very good antibacterial activity against gram-positive as well as gram-negative bacteria. However, cytotoxicity data suggest that PPI (9a and 9b) is very cytotoxic because these compounds shows very less selective index (Table 2) which is very undesirable and hence we have to tune the compound so as to obtain a compound with low cytotoxicity but still retaining the same high antibacterial activity.

Table 1: MIC (Minimum Inhibitory Concentration) data table

		MIC(µg/ml)				
S.No.	**Compound code**	***E.coli* ATCC 25922**	***S.aureus* ATCC 29213**	***K.pneumoniae* BAA 1705**	***A.baumannii* BAA 1605**	***P.aeruginosa* ATCC 27853**
1	DPI	4	1	16-32	4	4 to 8
2	MOXI.HCl	<0.0625	<0.0625	64	8	2
3	Levofloxacin	<0.03	0.25	64	16	1
4	9a	0.25	0.5	2	0.125	0.25
5	13a	0.5	0.5	4	2	2
6	13b	0.5	0.5	2	2	2
7	14a	0.25	1	1	0.25	1
8	14b	0.125	0.25	2	0.125	0.5
9	15a	0.25	0.25	8	0.25	1
10	15b	0.25	0.5	8	0.25	1
11	16a	0.5	0.5	16	0.5	8
12	16b	0.25	0.25	8	0.25	2
13	17a	0.5	1	2	4	4
14	17b	0.5	1	2	8	4

Credit: Dr. Sidharth Chopra's Lab CDRI, Lucknow

Table 2: Cytotoxicity Data value

Compound	MIC(µg/ml)	CC50	SI (Selectivity index)
9a	0.125	0.25 to 0.125	2 to 1
9b	0.125	0.25	2
9a(second batch)	0.125	< 0.125	< 1

Credit: Dr. Sidharth Chopra's Lab CDRI, Lucknow

Then we synthesised some other analogues of PPI, first we changed the position of nitrogen on pyridine ring (**13a**) and (**13b**) it also showed very good antibacterial activity although its cytotoxicity data hasn't been obtained yet looking at the MIC data and comparing it with the previous compounds (**9a** and **9b**). Since MIC values of **13a** and **13b** are similar to that of **9a** and **9b**, we argue that even these analogues will have high cytotoxicity.

We then also synthesised fluoro and methyl substituted PPI (**14a**, **14b, 15a, and 15b**), to see what are the effects of the addition of an electron withdrawing and electron donating group on the PPI. Though we observed that the methyl substituted PPIC **(15a)** is has a solubility problem. Compounds **14a**, **14b, 15a, and 15b** showed very good antibacterial activity against all the pathogens. The cytotoxicity data of the compounds **(14a**, **14b, 15a, and 15b)** are awaited.

We then tried to synthesize an N-methylated derivative of PPI **(16)** in hope of higher activity due to increased polarity resulting from the methyl group attached to the nitrogen but we were unsuccessful in the synthesis.[12]

Jonathan Baell's group has synthesised various derivatives of DPI and screened with various pathogens and observed that if methoxy group is attached on the DPI on the 5th position then this analogue has activity pretty low compared to all other analogues they had synthesised. Keeping these results in mind we have synthesised four more derivatives of PPI **16a, 16b, 17a and 17b**. Compounds **16a, 16b, 17a and 17b** also showed very good antibacterial activity. But the cytotoxicity data of these compounds are awaited.

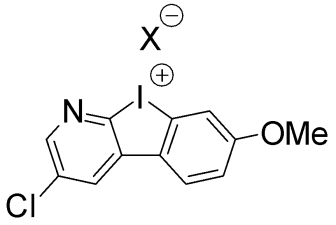
Since PPI shows very promising antibacterial activity it has the potential of being an antibacterial agent.

7. Conclusion and future outlook

We have successfully synthesised various derivatives of PPI and have obtained MIC data for all the synthesized compounds. MIC value of derivatives of PPI is much better than the DPI as we expected in the starting.

The cytotoxicity of PPI is higher as compared to DPI. For some analogues of PPI data is still awaited. We expect these analogues would show good activity and have low cytotoxicity.

We are still trying to synthesise a disubstituted derivative of PPI (Figure 5). Once we are able to synthesise this we will get the idea that how di substitution effects in the MIC and cytotoxicity.

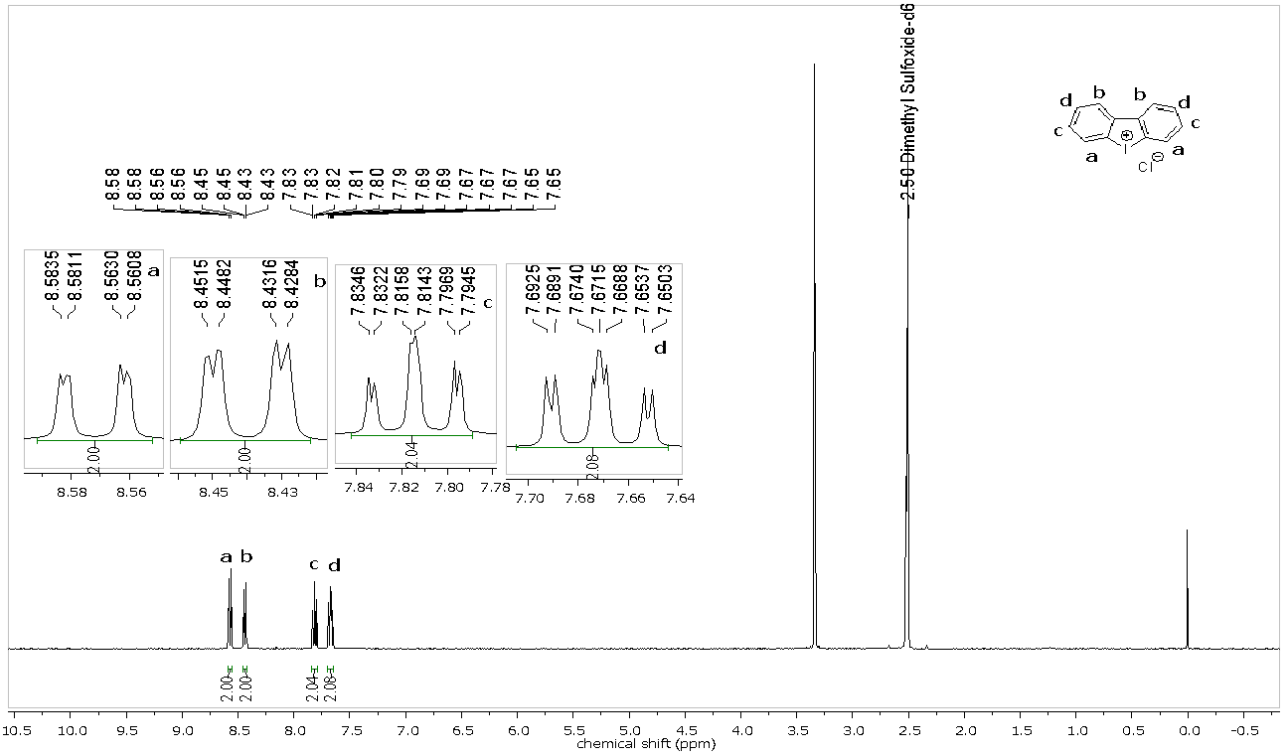


X=Cl, OTf

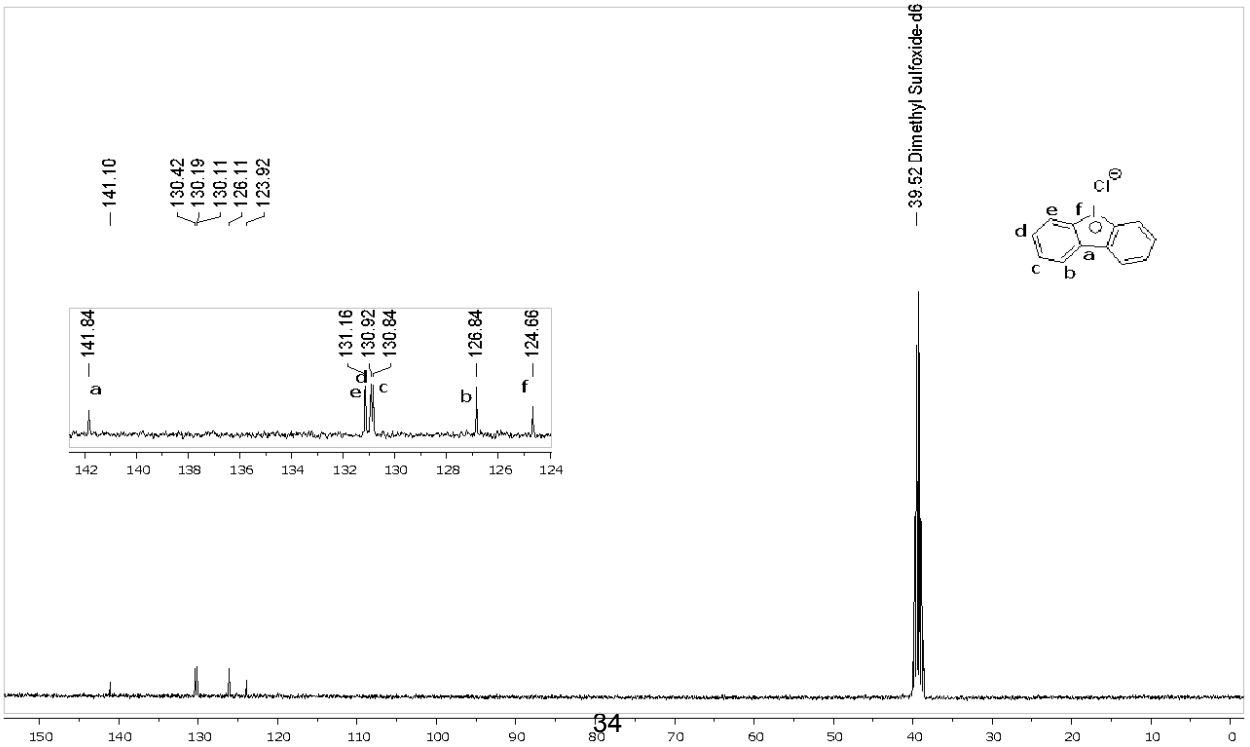
Figure 5: Disubstituted PPI

8. NMR SPECTRAS

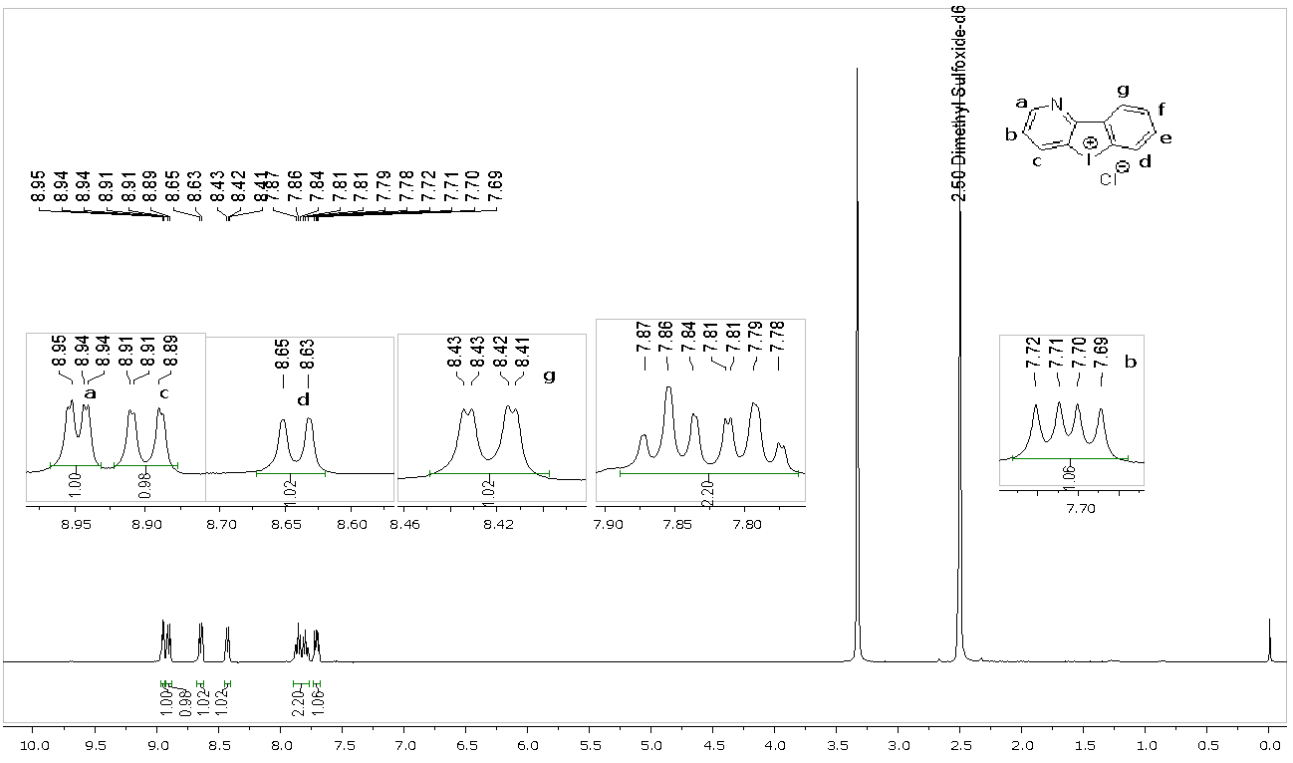
^{1}H NMR of compound 4



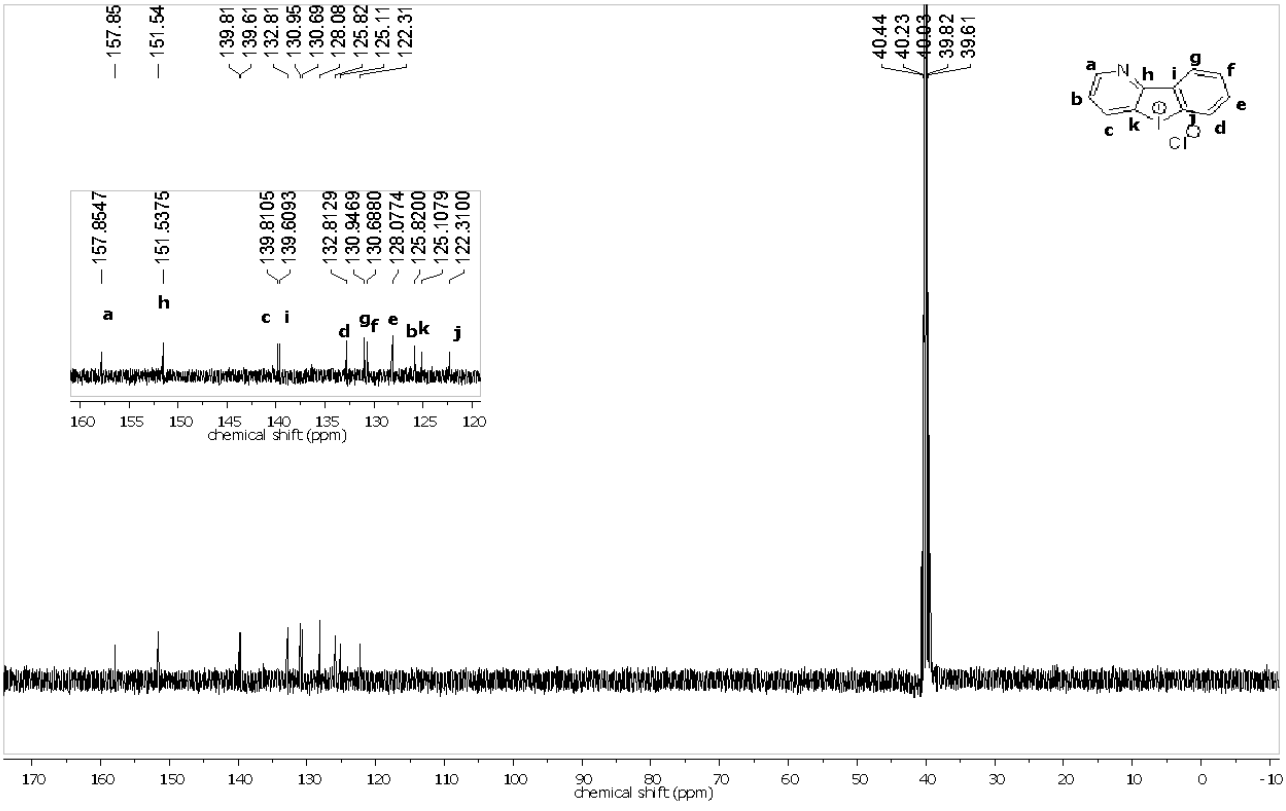
^{13}C NMR of compound 4



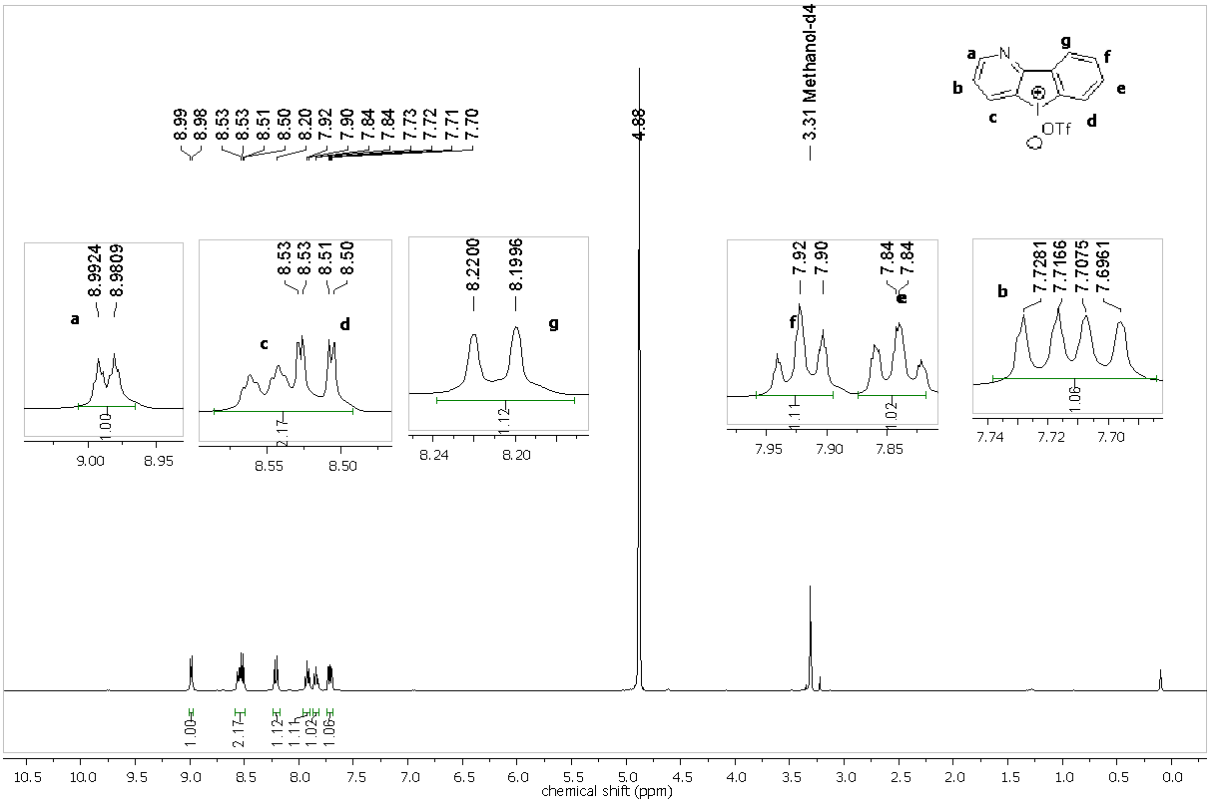
^{1}H NMR of compound 9a



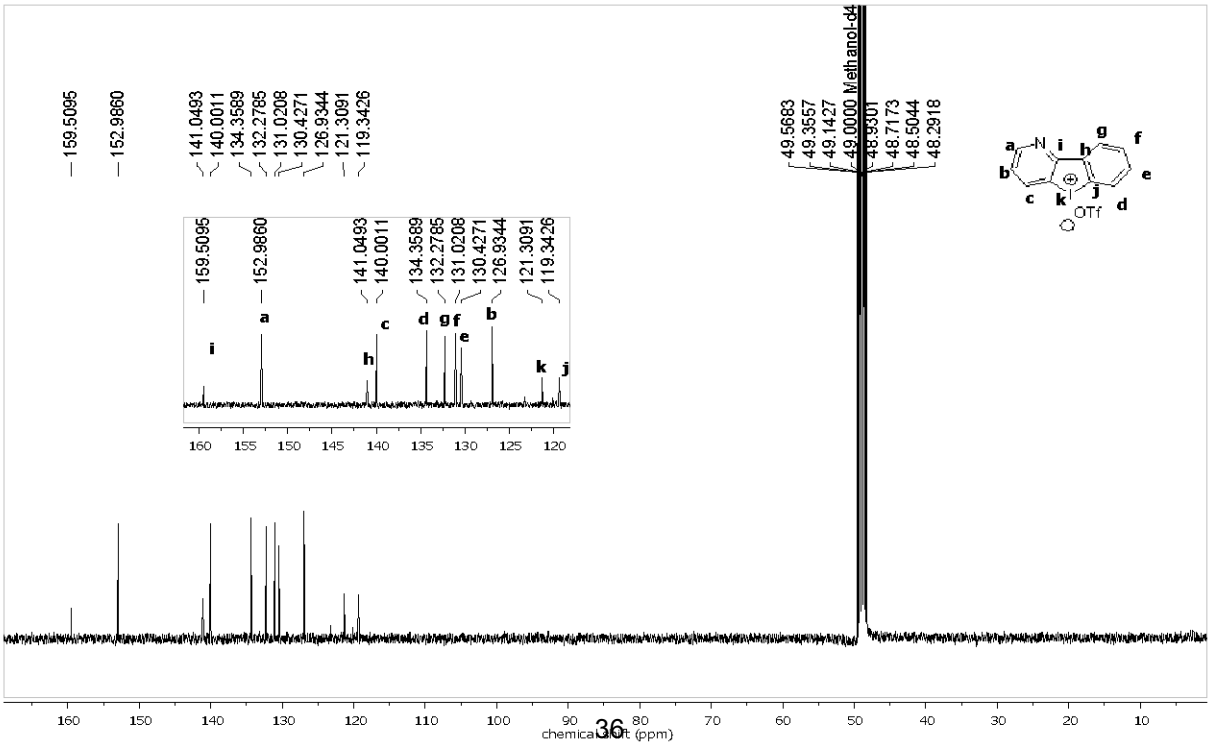
^{13}C NMR of compound of 9a



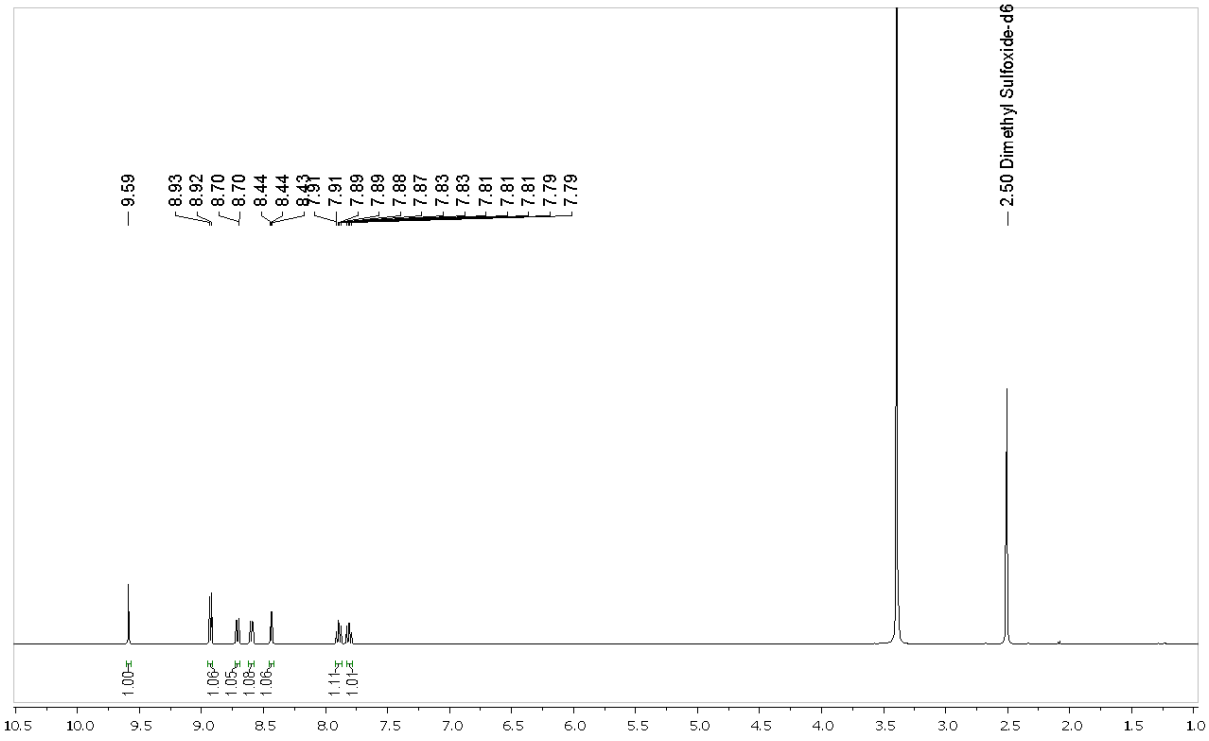
^{1}H NMR of compound 9b



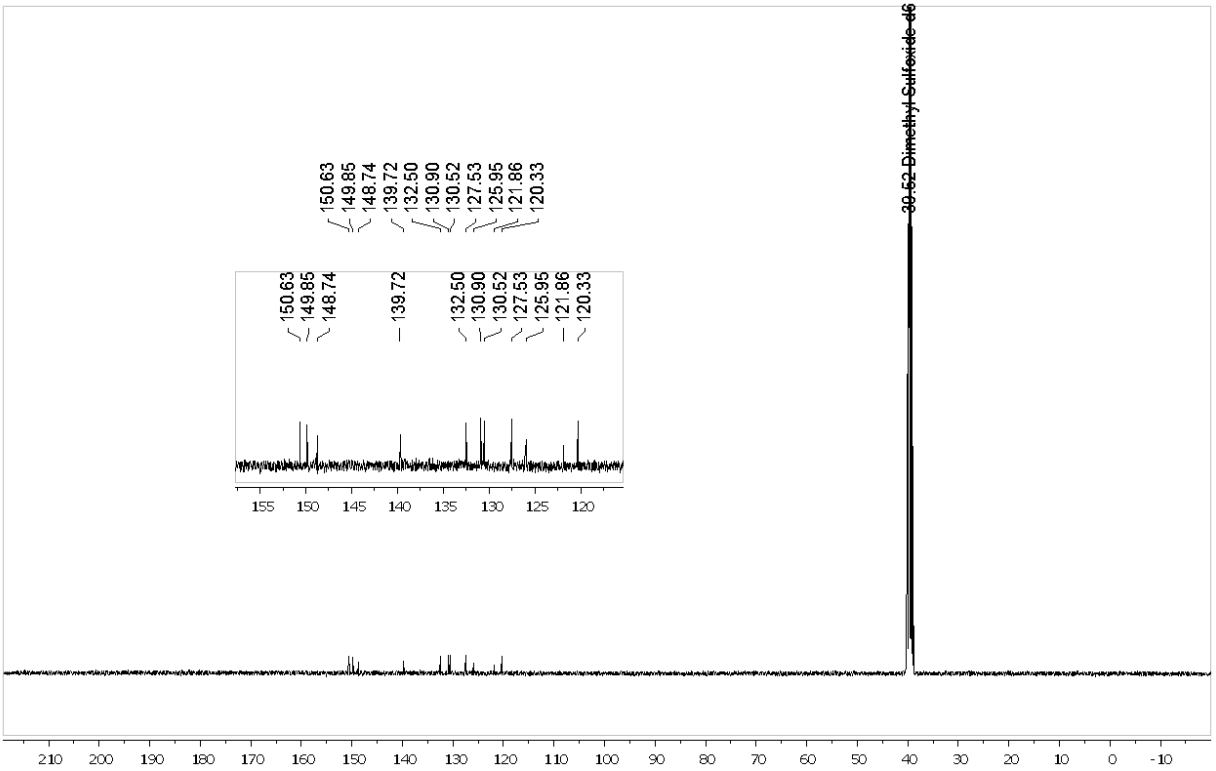
^{13}C NMR of compound 9b



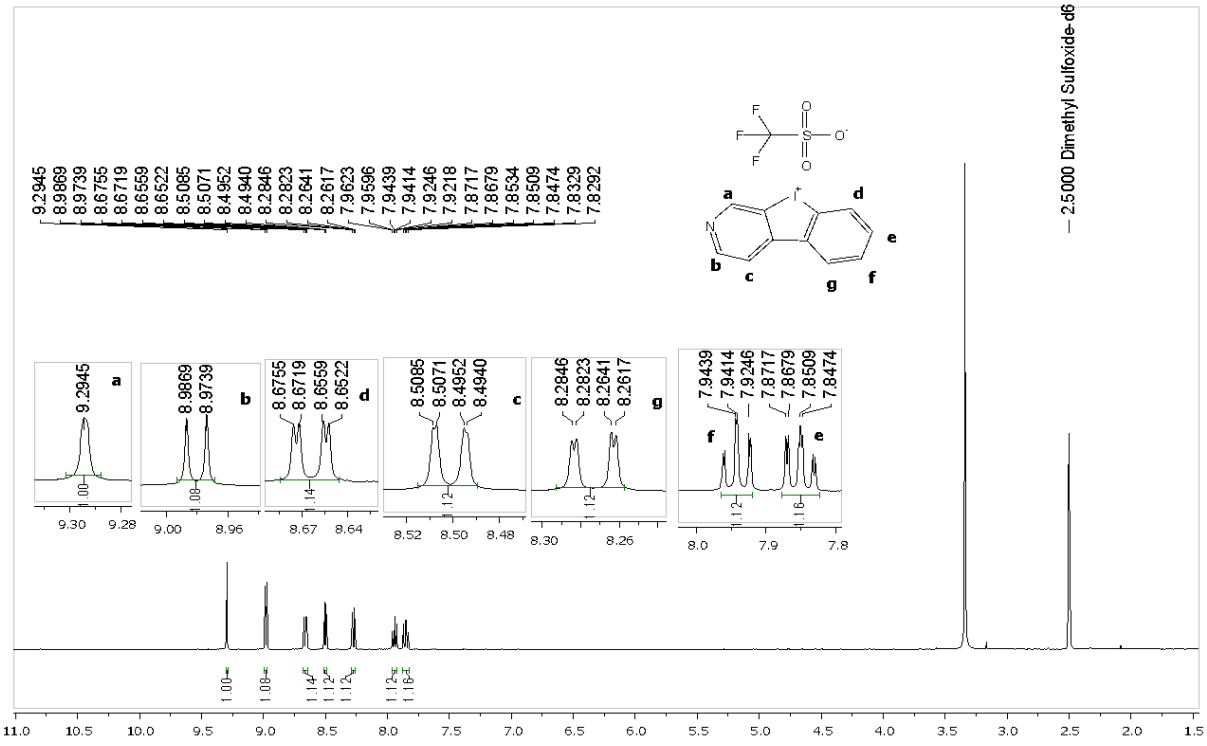
1H NMR of compound 13a



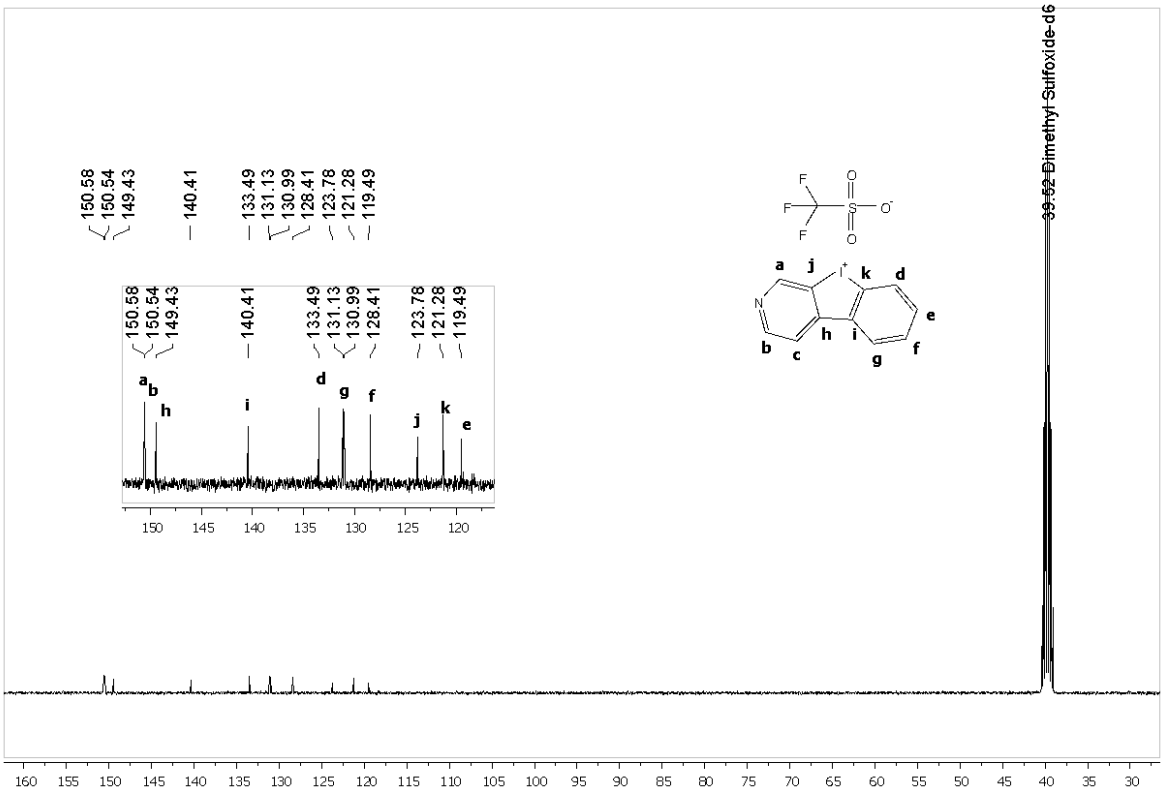
^{13}C NMR of compound 13a



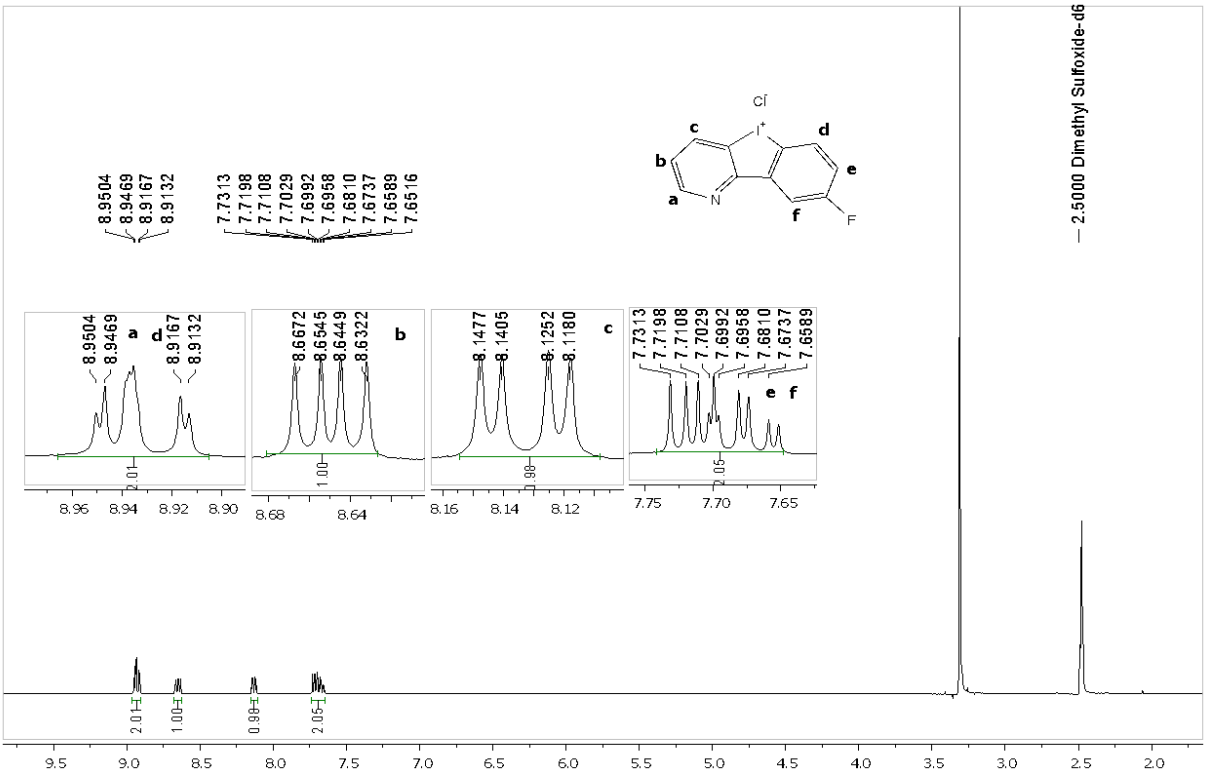
^{1}H NMR of compound 13b



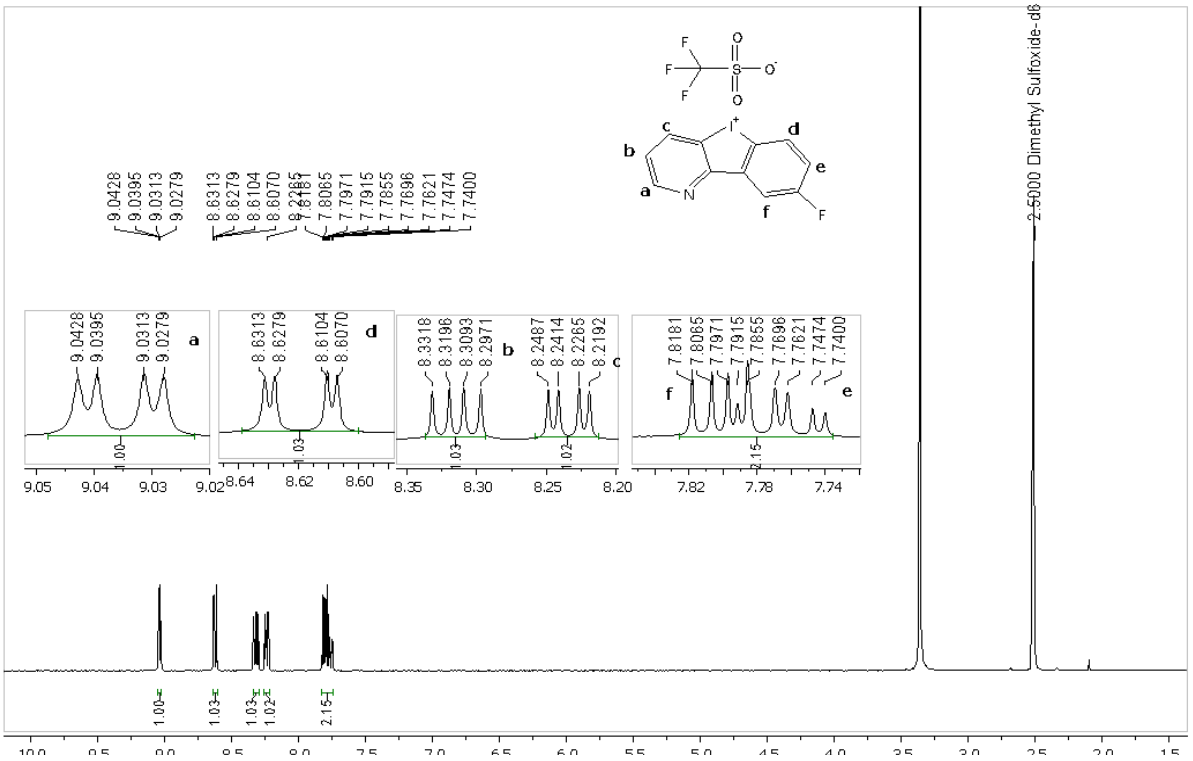
^{13}C NMR of compound 13b



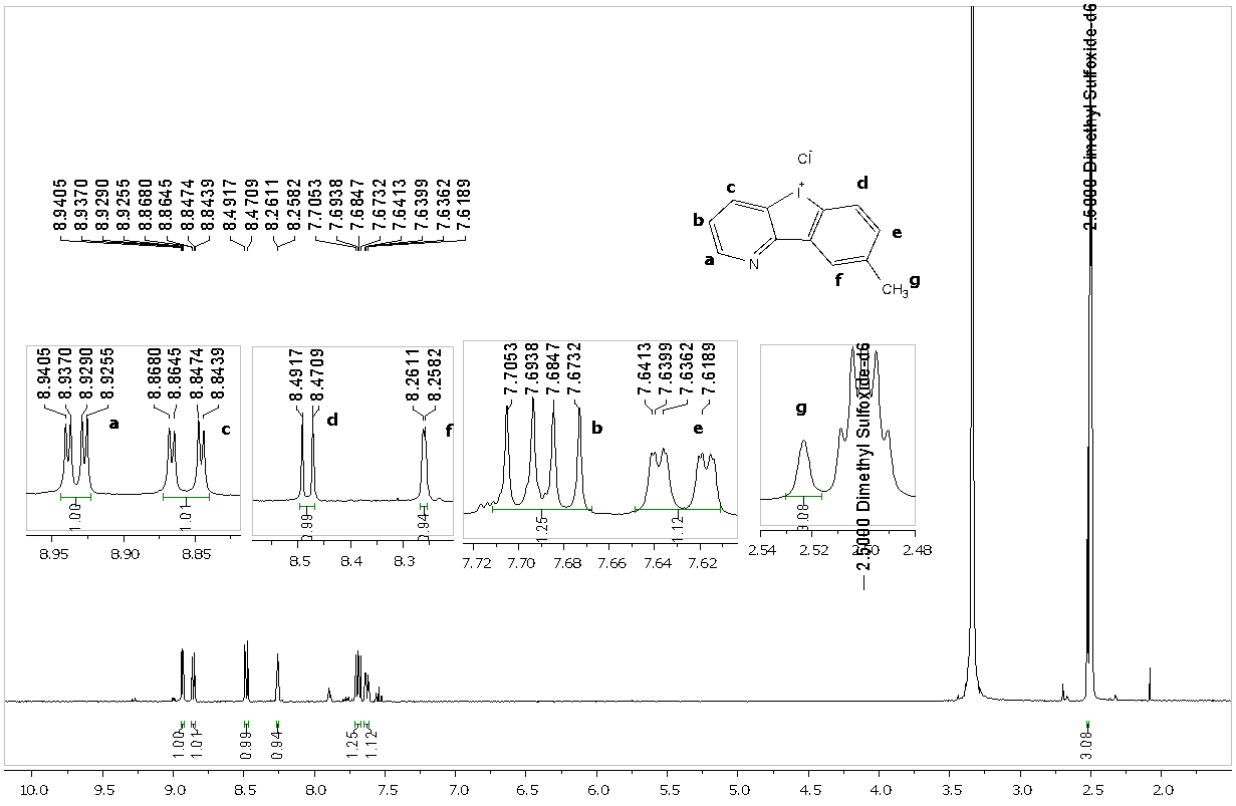
1H NMR of compound 14a



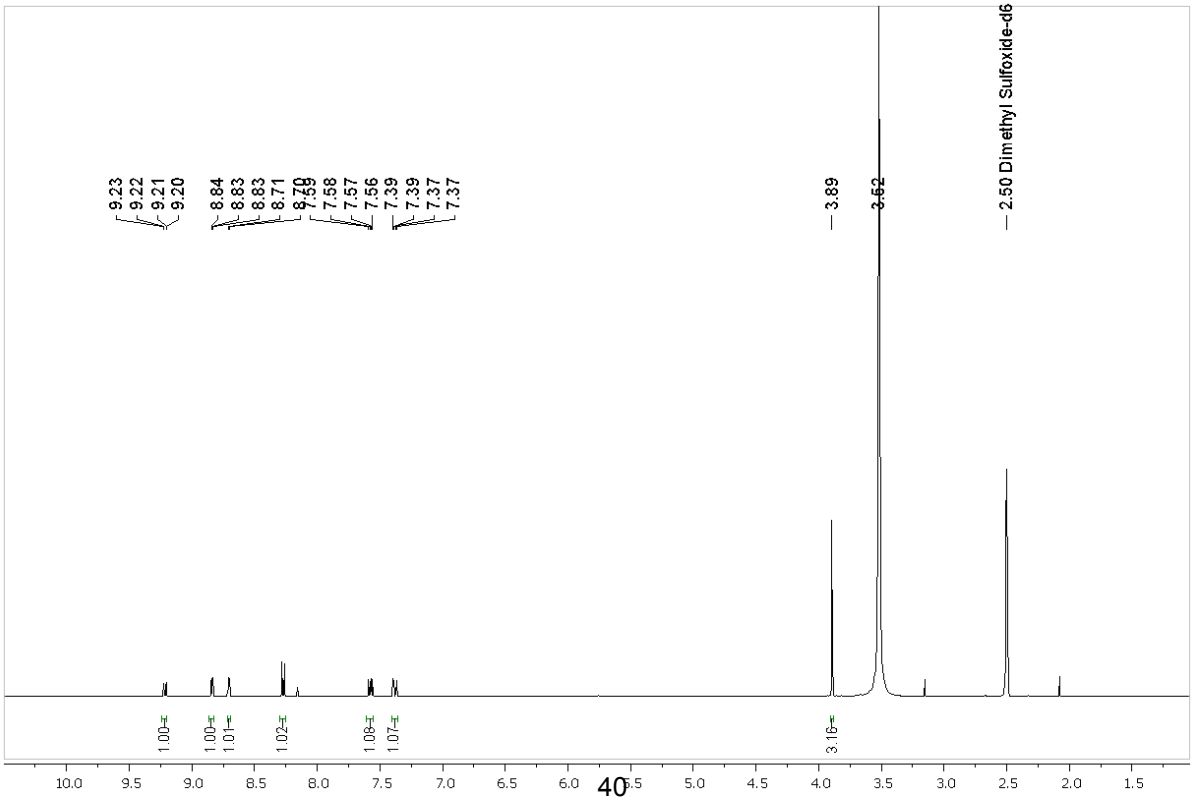
1H NMR of compound 14b



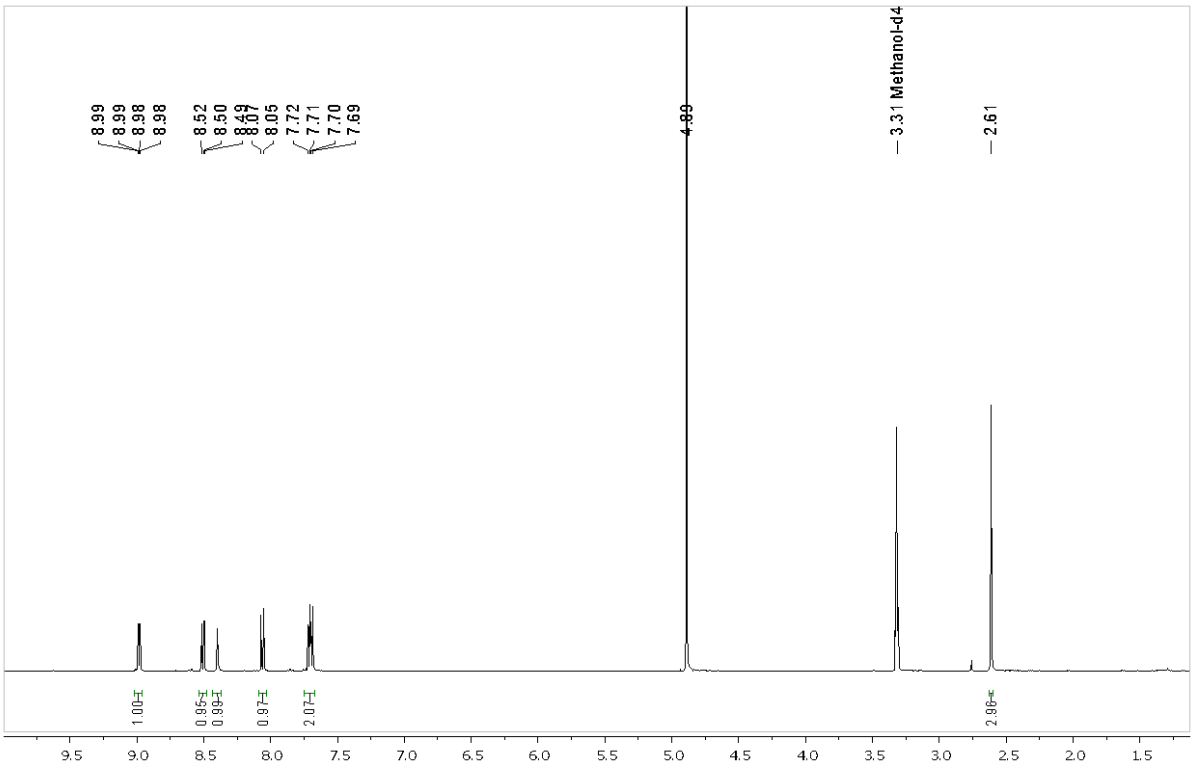
1H NMR of compound 15a



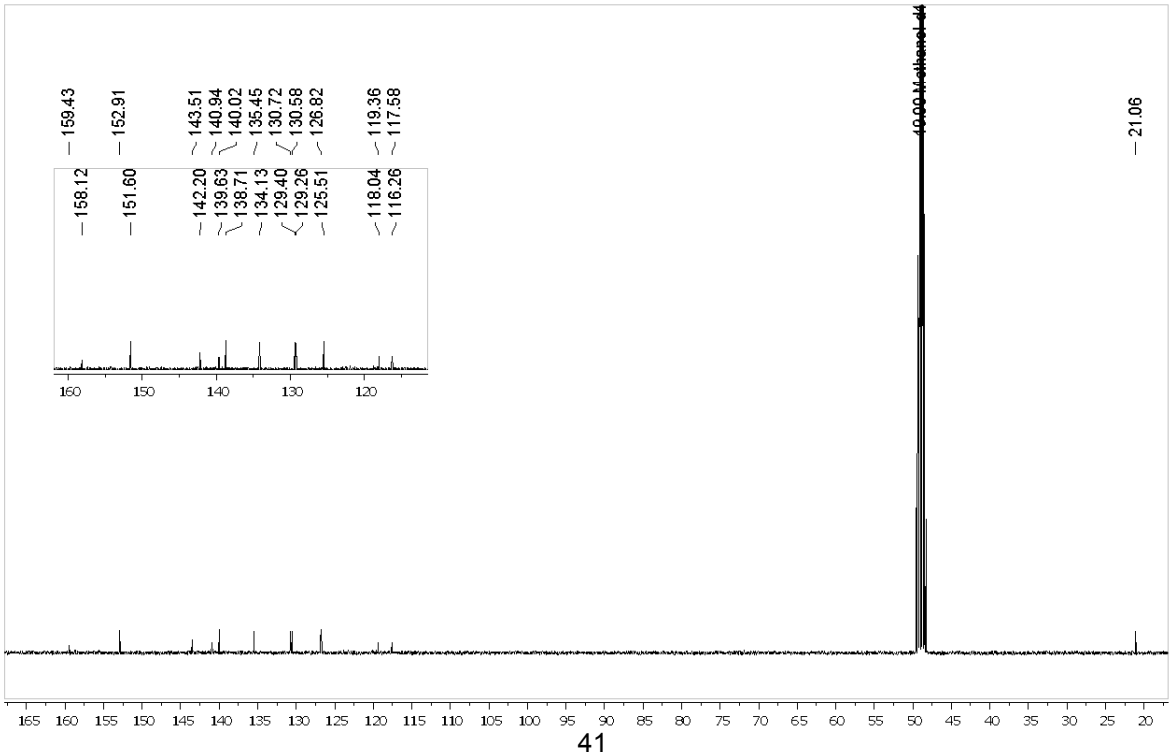
1H NMR of compound 16a



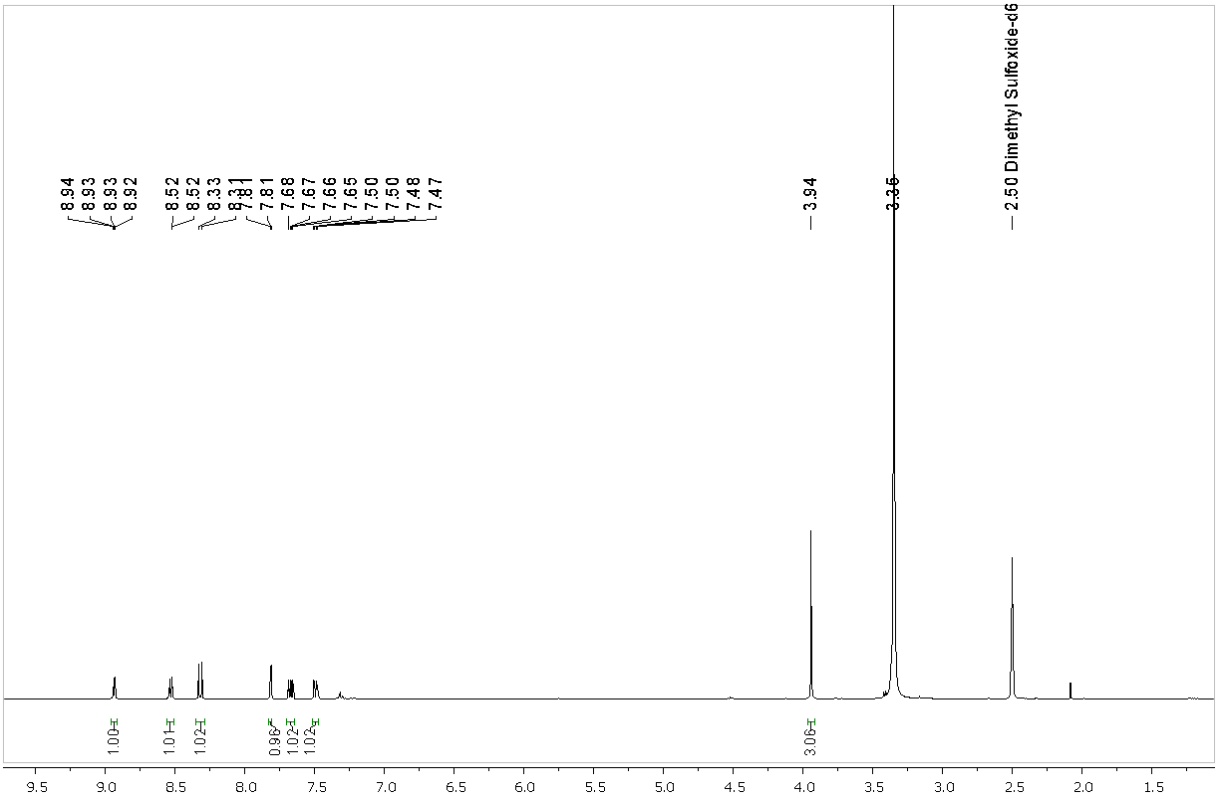
1H NMR of compound 15b



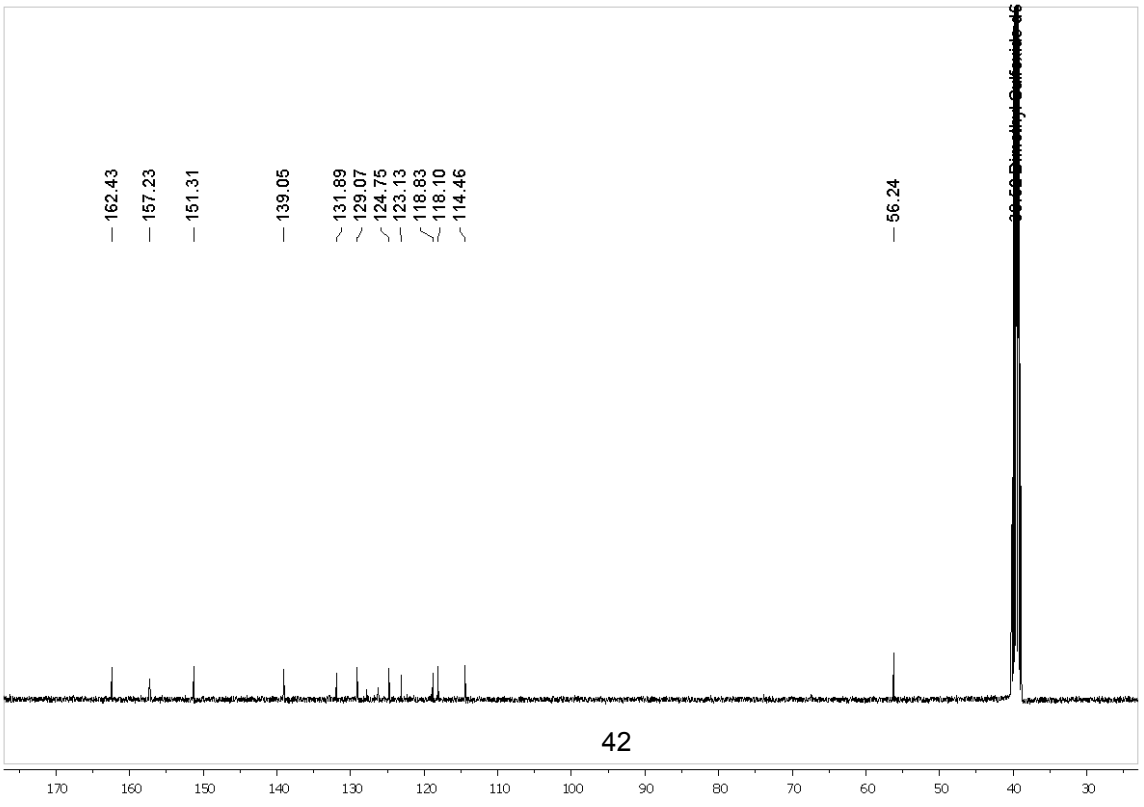
^{13}C NMR of compound 15b



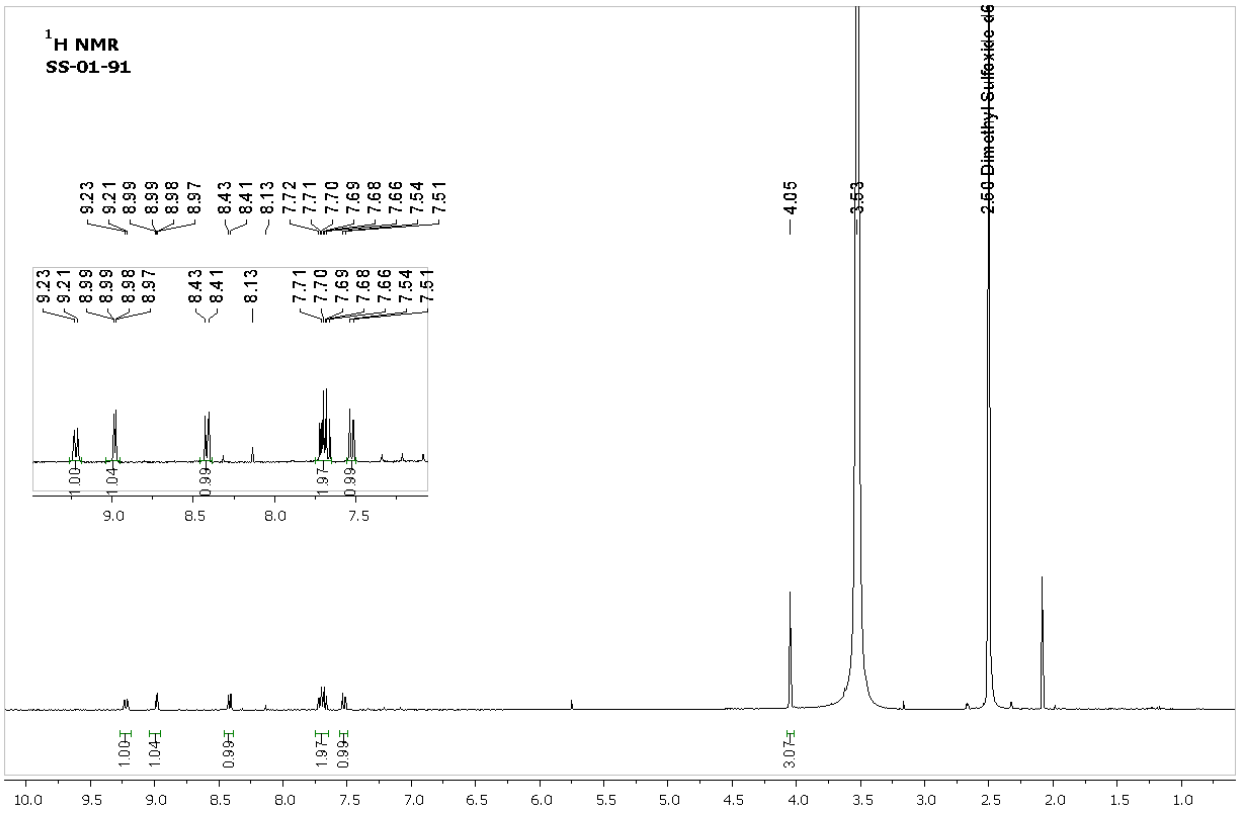
^{1}H NMR of compound 16b



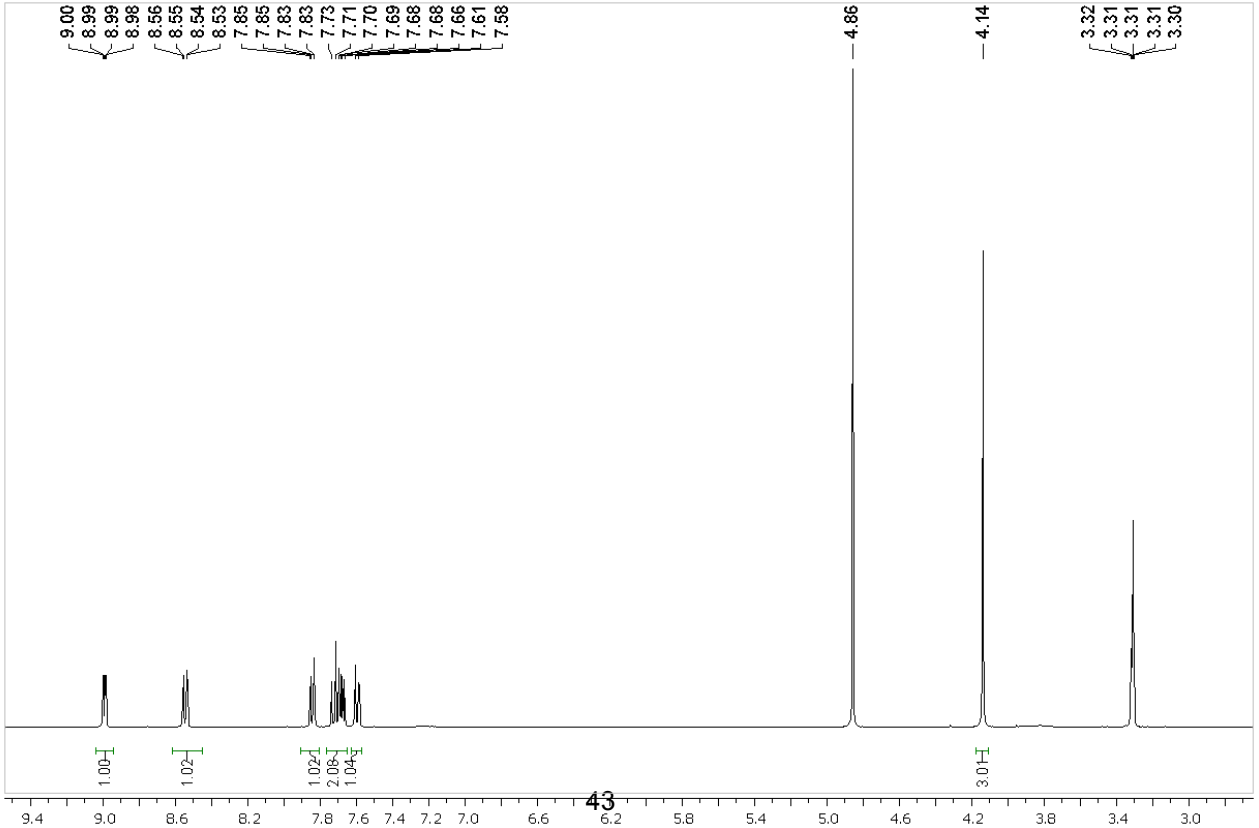
^{13}C NMR of compound 16b



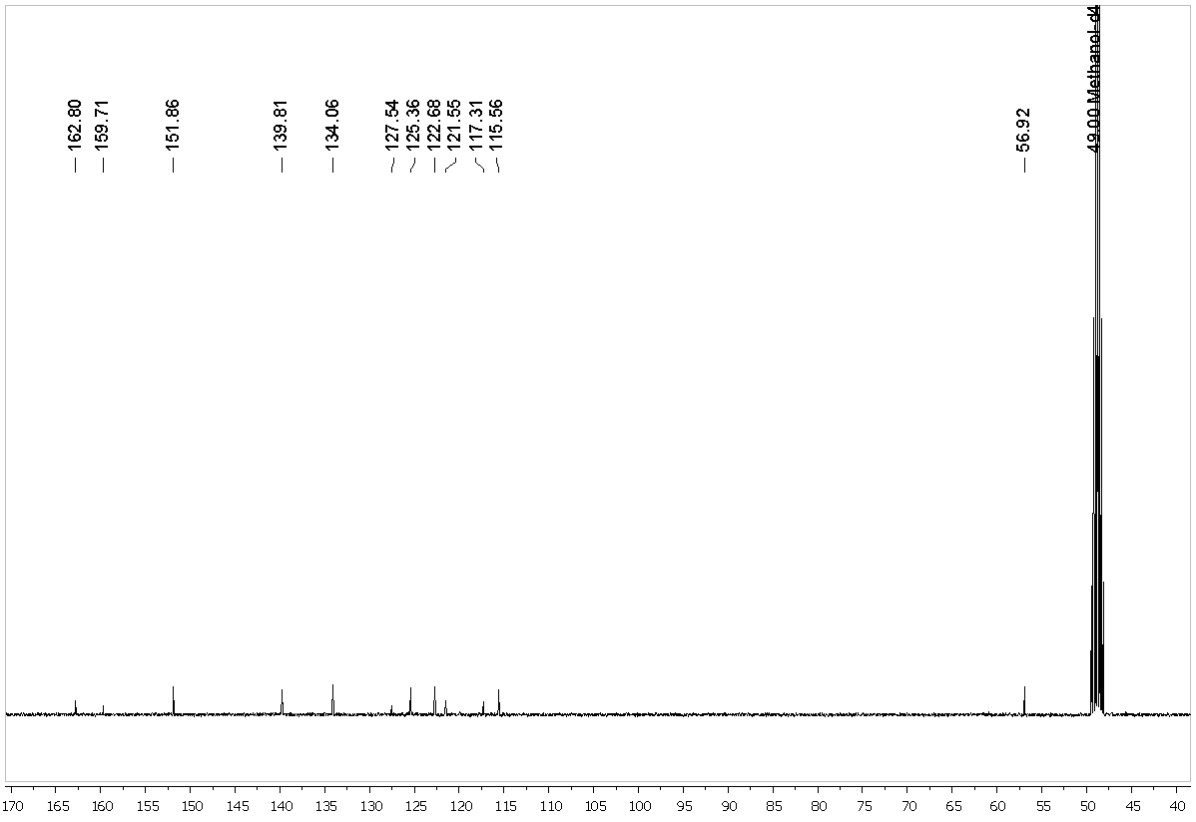
1H NMR of compound 17a



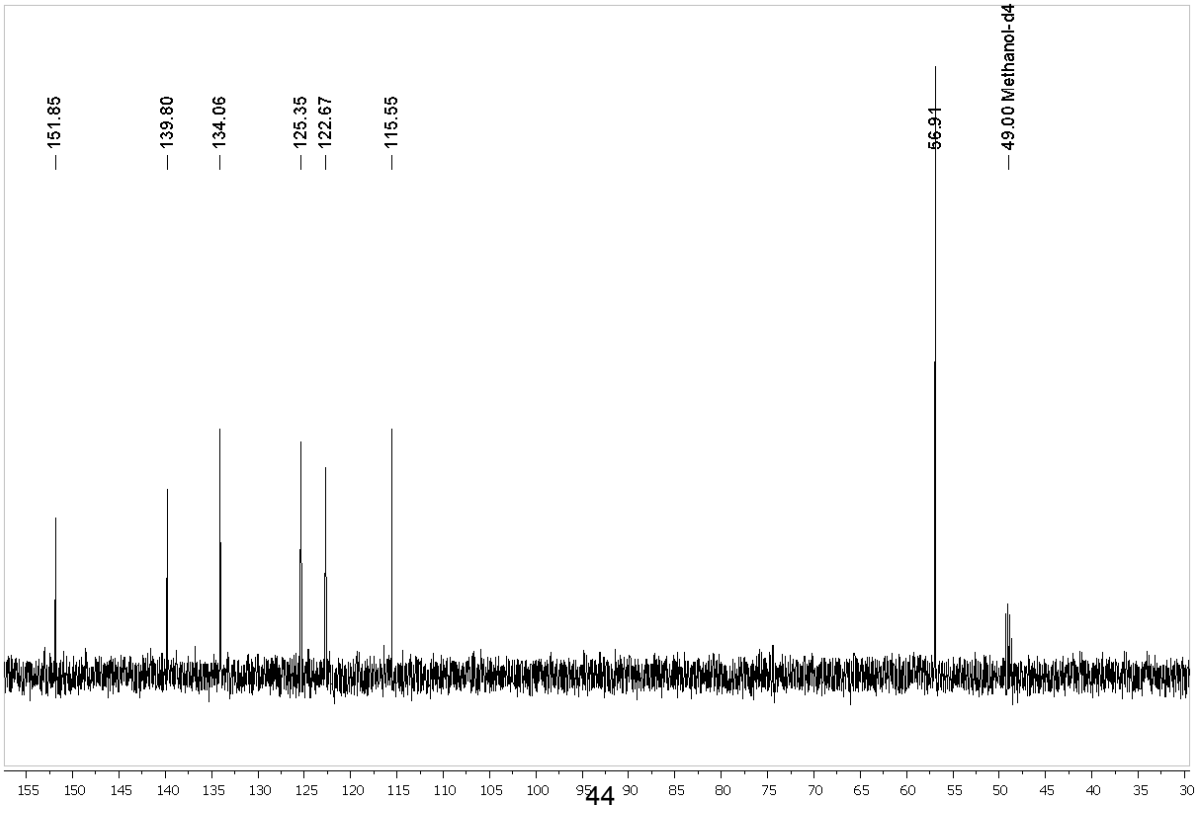
^{1}H NMR of compound 17b



^{13}C of compound 17b



DEPT-135 of compound 17b



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