Electronic Supplementary information for

Novel bodipy—cellulose nanohybrids for production of singlet oxygen

by

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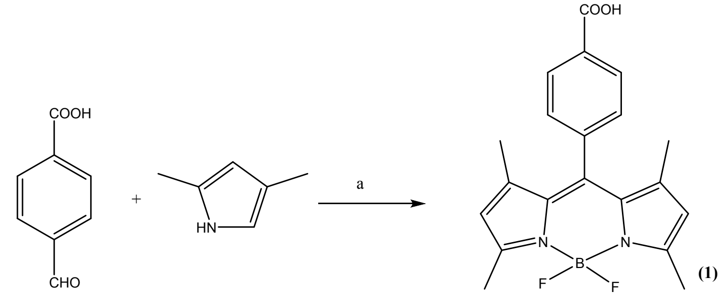
1. General Information

All the reagents and chemicals were purchased from Sigma Aldrich® Canada. Z-potential experiments were conducted on a Brookhaven Instruments Corporation instrument (Zeta Plus). Thermal analysis were performed on a (TGA Q500) TA instrument with a programme tuned to temperature ranging from 10 to 100 °C at a rate of 10°C/min, stable for 5 min at 100 °C, again temperature increased upto 800 °C at a rate of 10°C/min. Ultra-centrifugations were carried out with a Beckman Coulter centrifuge. TEM images were captured on a Hitachi H7000 transmission electron microscope and the images where analysed with the ImageJ software for crystal size measurements. The image length data were evaluated with a Origin 8 software. A 100 W Hg bulb was used as light source. Inductive coupled plasma atomic emission spectroscopy (ICP-AES) analysis was performed on Perkin Elmer Optima 7300 DV instrument. XPS analysis were performed on a Thermo Scientific K-Alpha X-ray Photoelectron Spectrometer (XPS) having a monochromated Al Kα X-ray source.

2. Synthesis of Bodipy dye derivative

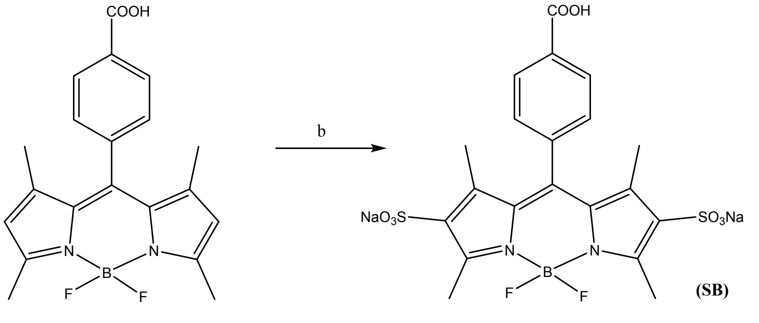
1. Synthesis of carboxylated bodipy (1)



Scheme S1: Preparation of carboxylated bodipy: a) TFA, dry CH_2Cl_2, DDQ, Et_3N, $BF_3.Et_2O$

In a 3-neck (1 L) round bottom flask, 2.7 g of p-formyl benzoic acid (18.0 mmol) was added. Then, 500 mL dichloromethane was added to the flask. The flask was degassed with Nitrogen. After 30 minutes, 3.7 g of 2,4-dimethyl pyrrole was added (38.8 mmol). After 15 minutes, two drops of Tri-fluoroacetic acid was added to reaction mixture. The flask was covered with aluminium foil and stirred for 20 hours at room temperature. Then, a solution of 4.08 g of DDQ (18.0 mmol) was added in 100 mL dichloromethane. The reaction mixture was stirred for further 4 hours at room temperature. After this, 10 ml of each triethyl amine and boron trifluoride diethyl etherate were successfully added. After stirring for 12 hours at reaction mixture was treated with water (500 mL) and extracted with dichloromethane. The organic layer was treated with anhydrous sodium sulfate and evaporated over rota vapour. The residue was purified over silica gel column chromatography using DCM/MeOH (30:1, v/v) as solvent system, giving the red colour solid bodipy dye (1) in 12 % yield. ^1H NMR (500 MHz, MeOD): 8.18 (d, 2H), 7.41 (d, 2H), 6.07 (s, 2H), 2.49 (s, 6H), 1.40 (s, 6H). MALDI: m/z $[C_{20}H_{19}BF_2N_2O_2]^-$: 368.15.

2. Synthesis of sulfonated bodipy (SB)



Scheme S2: Preparation of sulfonated bodipy: a) chlorosulfonic acid, -40° C, $NaHCO_3$

In 50 mL round bottom flask, 0.1 g of bodipy dye (1) (0.2 mmol) was added to 20 mL dichloromethane. The reaction mixture was placed in an ice bath having temperature around -40 °C. To this 50μL of chlorosulfonic acid (0.75 mmol) and stirred for 20 minutes. Then, the ice bath was removed and the reaction mixture was allowed to attain the room temperature. The solvent was removed over rota vapour and re-dissolved in a 10 mL aqueous solution of sodium bicarbonate (40 mM). The solvent was removed over rota vapour. The product purified over reverse phase silica column chromatography using methanol as solvent, giving the desired sulfonated bodipy (SB) as red solid in 40 % yield. ^{1}H NMR (500 MHz, MeOD): 8.18 (d, 2H), 7.49 (d, 2H), 2.66 (s, 6H), 1.94 (s, 6H). MALDI: m/z $[C_{20}H_{17}BF_2N_2Na_2O_8S_2]^{2-}$: $[(m/z)-2Na]^{2-}$: 311.15. ^{11}B NMR (400 MHz, MeOD): 0.71. . ^{19}F NMR (400 MHz, MeOD): -144.24.

NMR Spectra

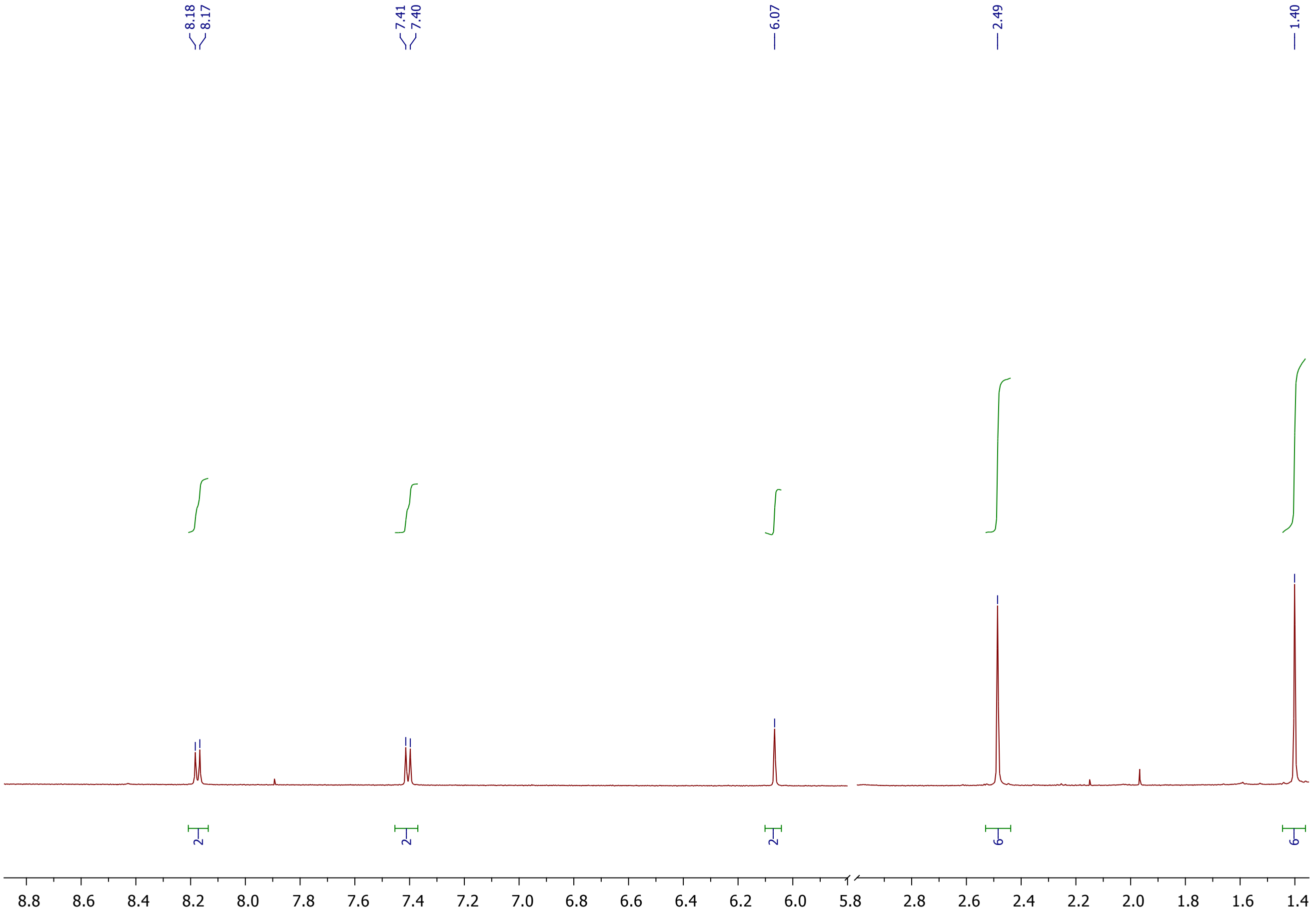


Figure 1: ^{1}H NMR spectra of carboxylated bodipy (1) (500 MHz in MeOD)

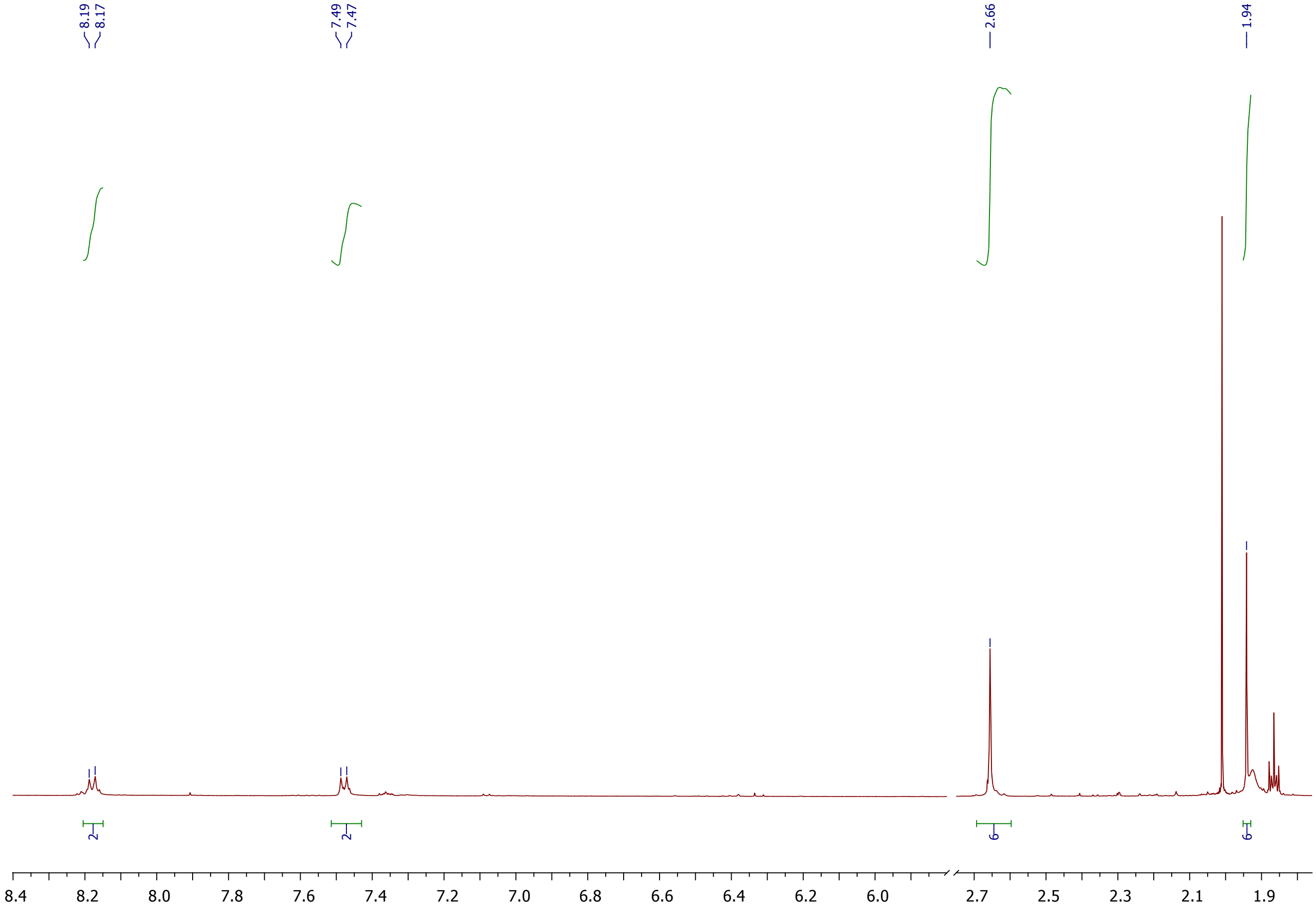


Figure 2: ^1H NMR spectra of sulfonated bodipy (SB) (500 MHz in MeOD)

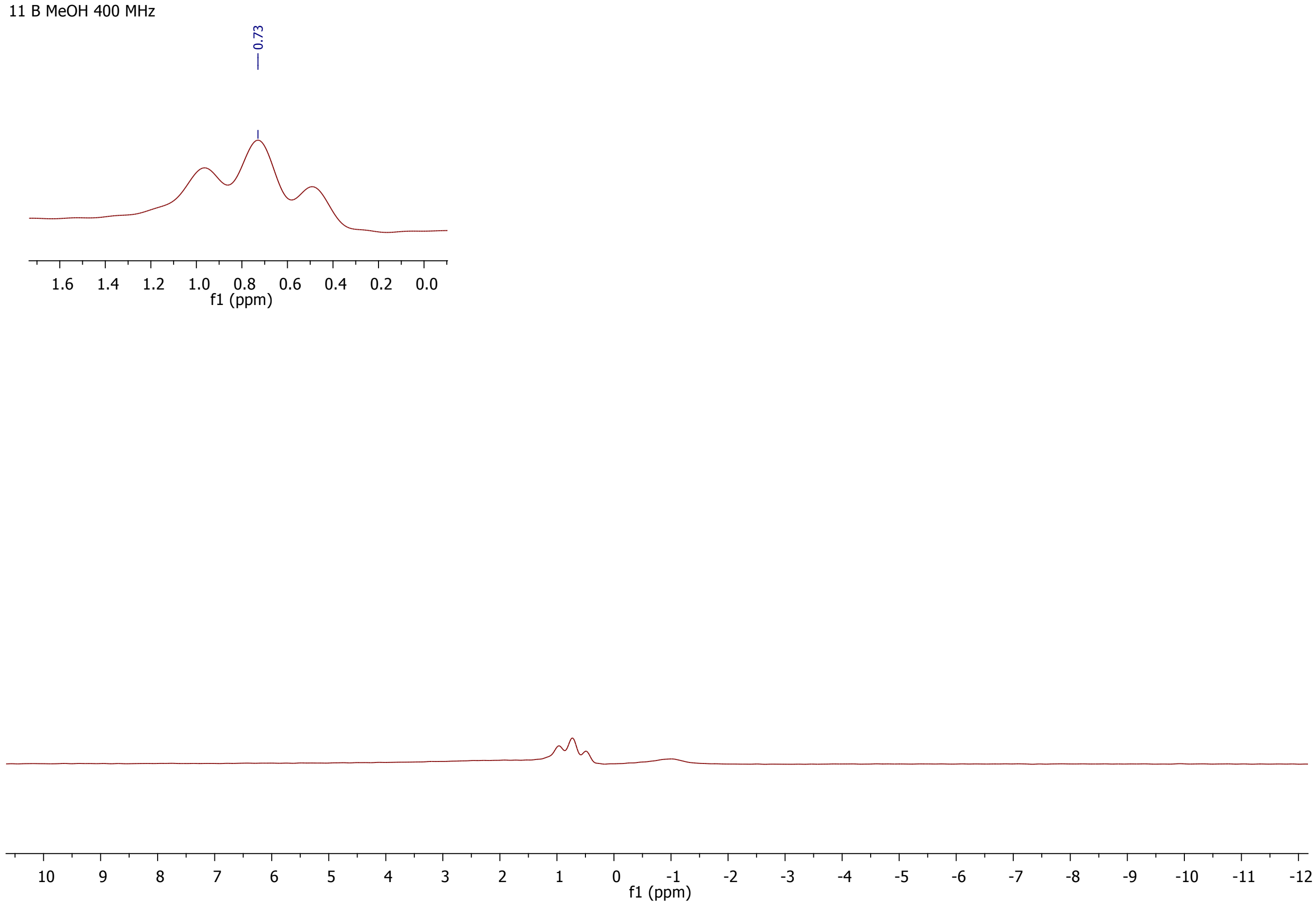


Figure 3: ^{11}B NMR spectra of sulfonated bodipy (SB) (400 MHz in MeOD)

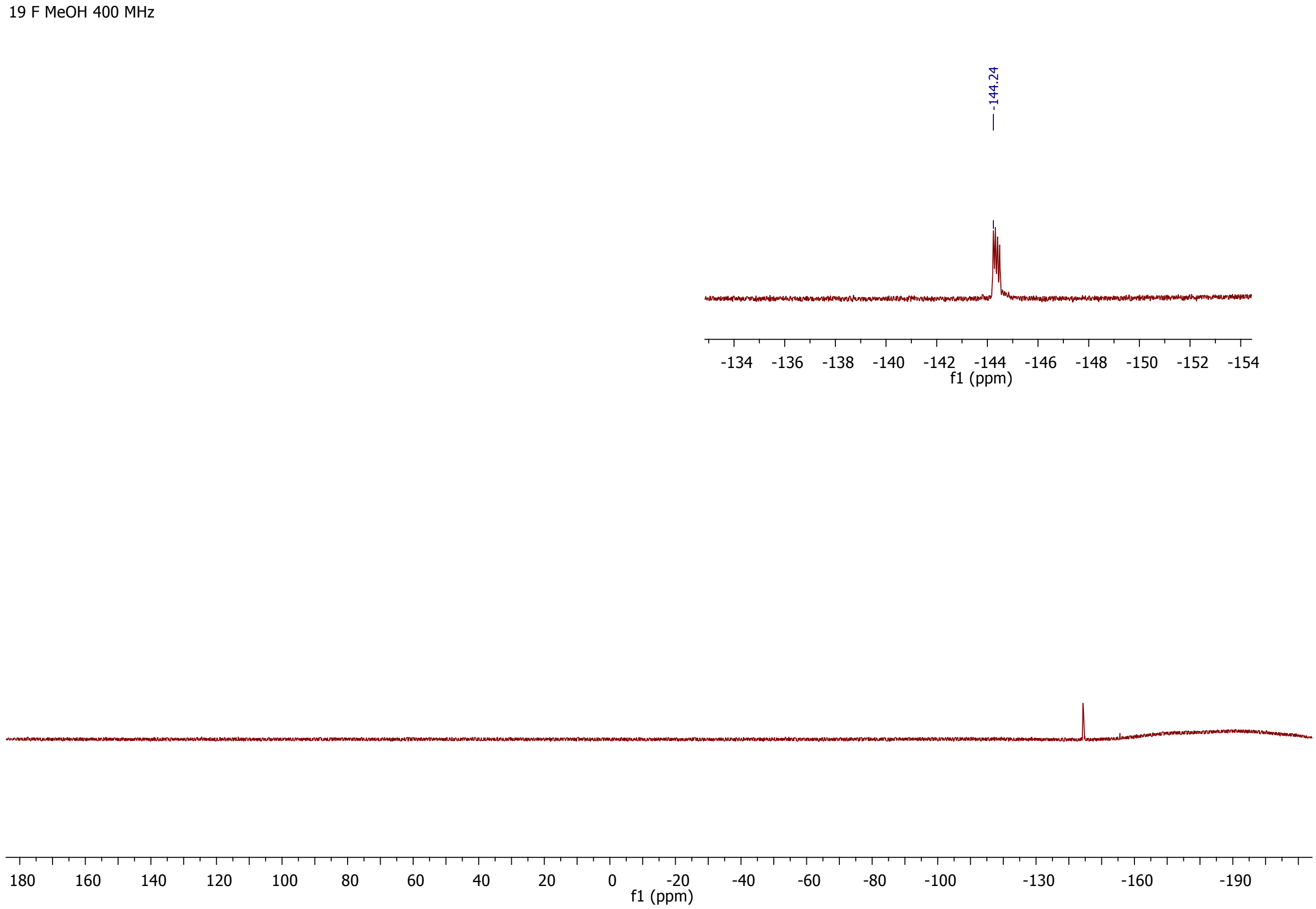


Figure 4: ^{19}F NMR spectra of sulfonated bodipy (SB) (400 MHz in MeOD)

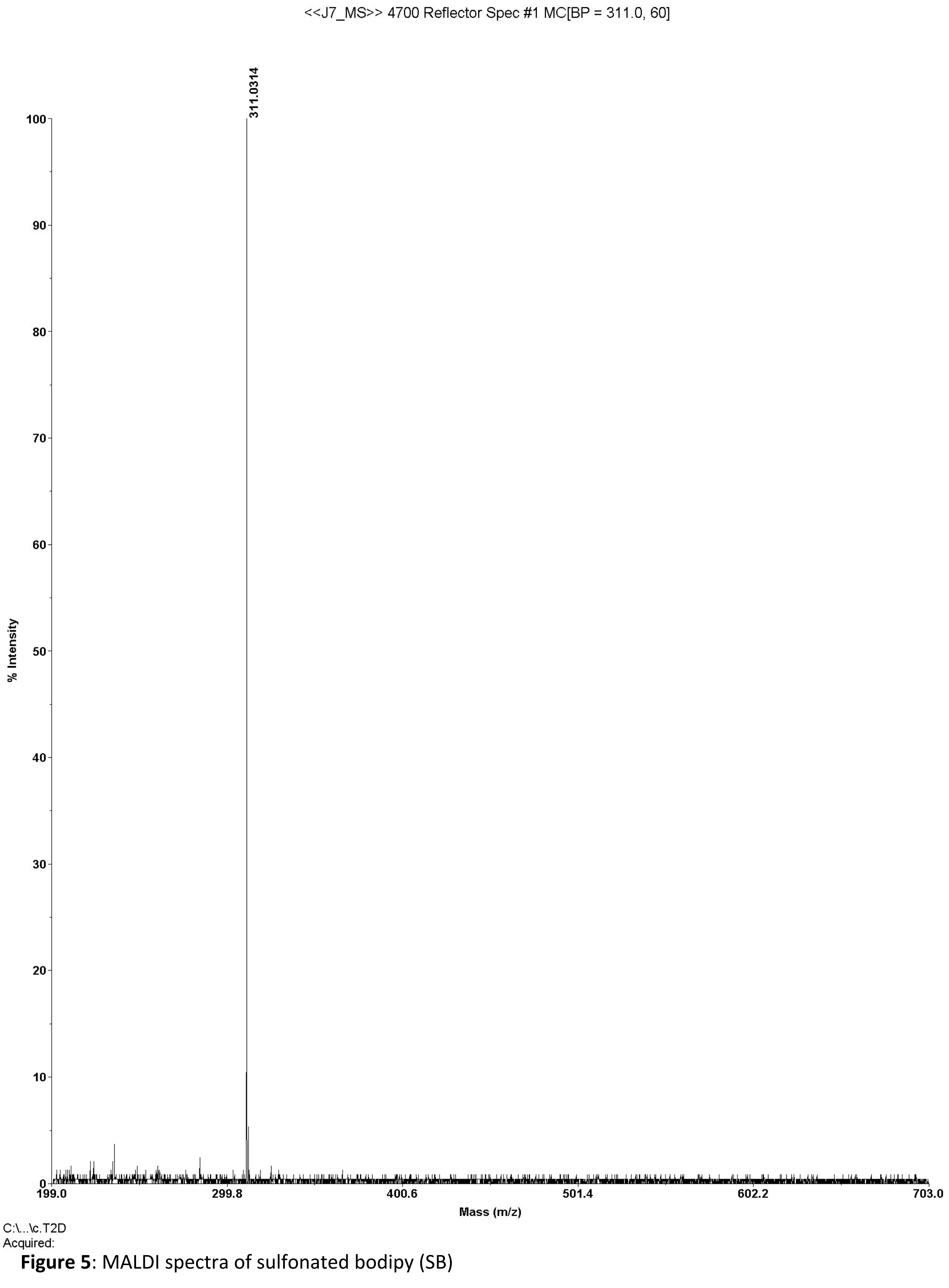


Figure 5: MALDI spectra of sulfonated bodipy (SB)

3. Synthesis of Cellulose Nanocrystals (NC)

To a preheated (at 45 °C) 37 mL solution of (64%) sulfuric acid, 5 gm of MCC (microcrystalline cellulose) was added. The white suspension thus formed was stirred for 2hours. The hydrolysis was stopped by adding the 10 fold of water to the suspension. The suspension was subjected to centrifugation treatment at 5K rpm for 5 minutes to remove the most of acidic supernatant. The remaining white solid was again washed with water until neutral pH was achieved. Finally, the NC was recovered by centrifugation at 10K rpm for 5 minutes. The NC was transferred in dialysis tubes (12kD cut off) and dialysed against milliQ water for 3 days. The NC was recovered by lyophilization in ~50 % yield.

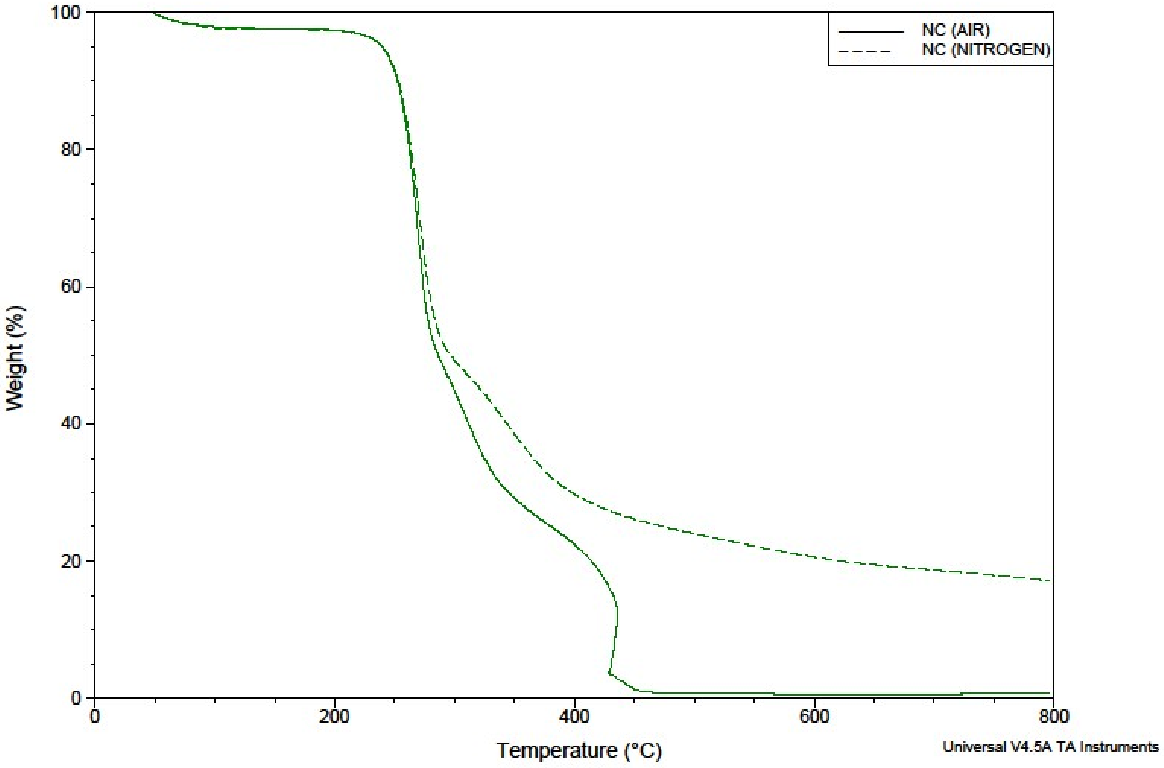


Figure 6: TGA curves of NC in presence of air and nitrogen

NC aqueous suspension (1mg/mL)	ζ-Potential (mV)
1st reading	-27.0
2nd reading	-26.4
3rd reading	-26.6
4th reading	-26.4
Arithmetic Average	-26.6

Table S1: ζ-potential values for NC

4. Synthesis of Amino Cellulose Nanocrystals (NC-NH2)

To a 40 mL NaOH (1M) solution, 0.5 g of NC was added and sonicated for 15 minutes. To this suspension 231.25 µL of epichlorohydrin (3.0 mmol) was added and was heated at 60 °C for 2 hours. The suspension was transferred in dialysis tube (Spectra/Por 4 dialysis tubing) and dialysed against water until pH below 12 was achieved. After readjusting the pH again to 12 with 50% (w/v) NaOH, 2.5 mL of NH_4OH solution was added to the reaction mixture and was stirred for 2 hours at 60 °C temperature. The reaction mixture was transferred in dialysis tube and dialysed against milliQ water until the pH 7 was obtained. The solid product in 0.25 g yield was recovered by lyophilization.

NC-NH2 aqueous suspension (1mg/mL)	ζ-Potential (mV)
1st reading	-29.5
2nd reading	-29.5
3rd reading	-30.0
4th reading	-29.0
Arithmetic Average	-29.5

Table S2: ζ-potential values for NC-NH2

5. Degree of substitution of primary amino groups

Kaiser test was employed for detection of primary amino group in NC. It is a simple Ninhydrin test which is used to quantify presence of primary amino group.

The presence of a primary amino group can be detected by formation of blue colour. By exploiting the absorption peak in the UV- visible spectrum at 570 nm, we can estimate the amino groups. According to Lambert-Beer law, absorbance can be associated with concentration of free amino as follows:

$$\frac{mmol}{g} = \frac{(Abs\ sample - Abs\ blank) \times dilution\ (mL) \times\ 10^3}{Extinction\ coefficient\ \times Sample\ weight\ (mg)}$$

Where Abs sample is absorbance of the ninhydrin complex, Abs blank is the absorbance of the blank (ethanolic soln. of phenol, KCN soln. in pyridine and ethanolic soln. of ninhydrin) recorded at 570 nm. The extinction coefficient is 15000 m^{-1} cm^{-1}, as reported in literature.[R1]

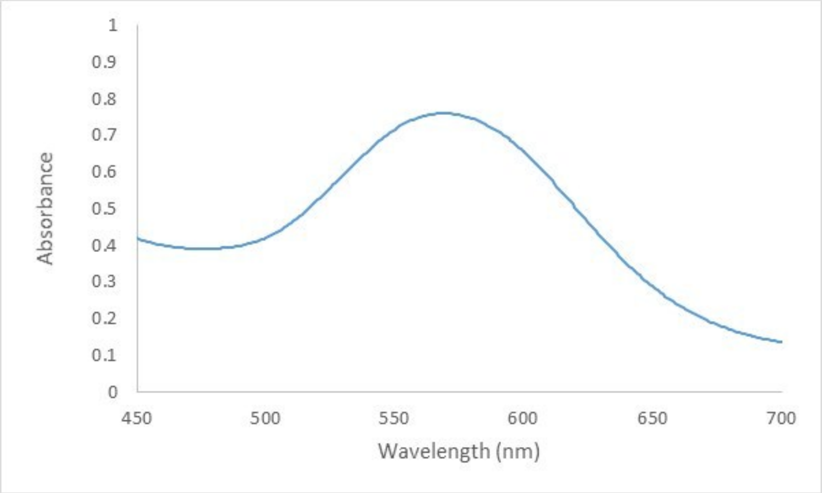


Figure 7: UV visible spectrum of blue colour suspension from Kaiser test at 570nm

Abs sample: 0.760 Abs blank: 0.020 Dilution: 3 mL Sample weight: 0.25 mg

The degree of substitution of primary amino group was calculated to be 0.60 mmol per gram of NC.

6. Synthesis of Nanocellulose-bodipy conjugate (NC-SB)

0.1 g of SB bodipy (0.17 mmol) was added to a 20 mL (50 mM, pH-4) MES buffer solution. After 5 minutes, 0.33 g of EDC.HCl (1.7 mmol) and 0.20 g of NHS (1.7 mmol) were added and stirred for 3 hours. To this, 0.1 g NCC-NH2 was added. The suspension was stirred for 4 days at room temperature. The suspension was subjected to centrifugation treatment at 10k rpm for 10 minutes. The solid was re-suspended in water and centrifuged again until supernatant was colourless. The suspension was transferred in dialysis tube against brine for 1 day and further 3 days against milliQ water. The red colour solid of NC-SB was recovered by lyophilization in ~ 40 % yield.

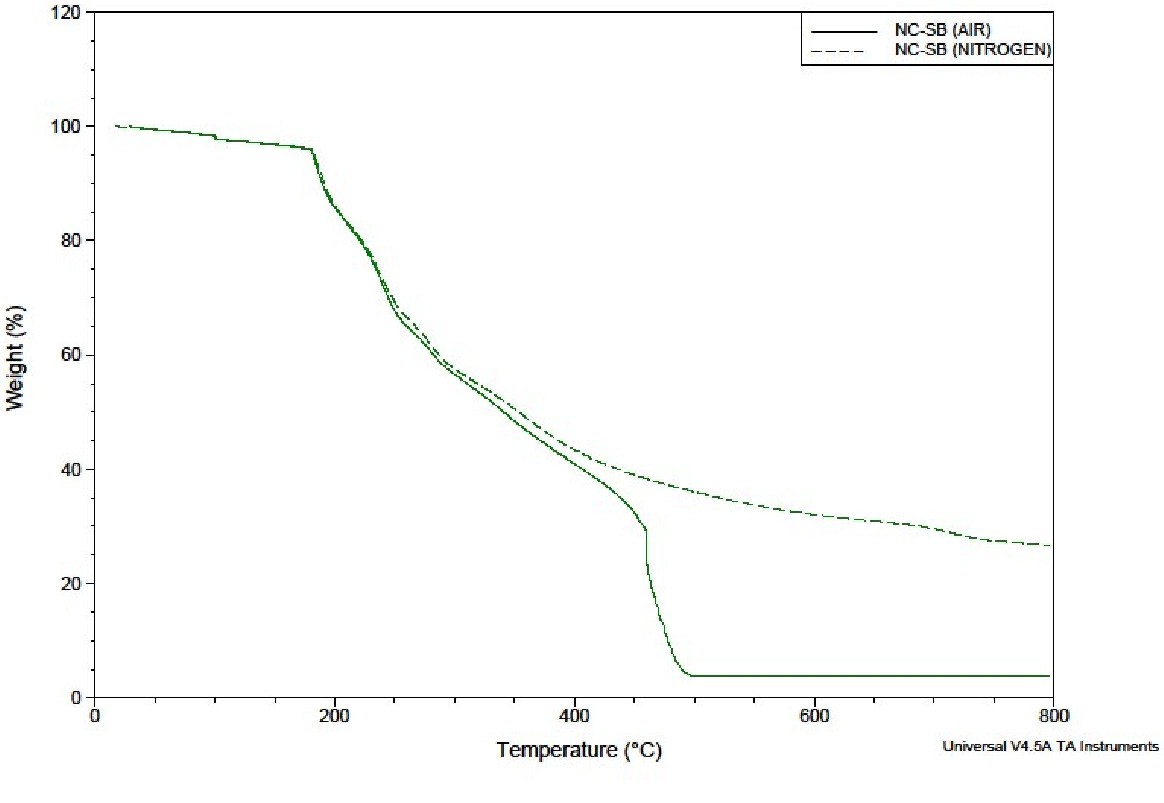


Figure 8: TGA curves of NC-SB in presence of air and nitrogen

NC-SB aqueous suspension (1mg/mL)	ζ-Potential (mV)
1st reading	-33.0
2nd reading	-32.0
3rd reading	-32.5
4th reading	-32.5
Arithmetic Average	-32.5

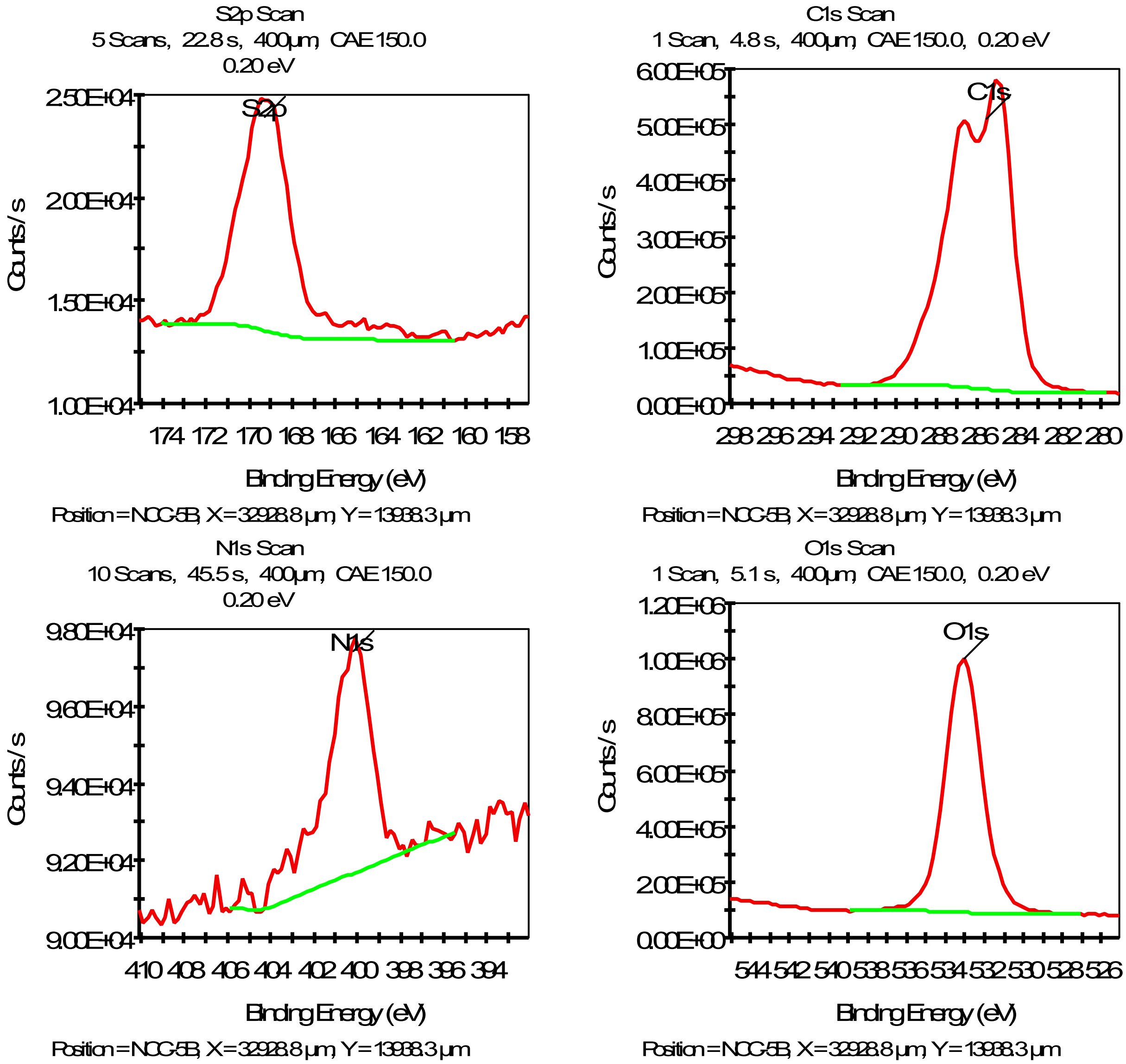
Table S3: ζ-potential values for NC-SB

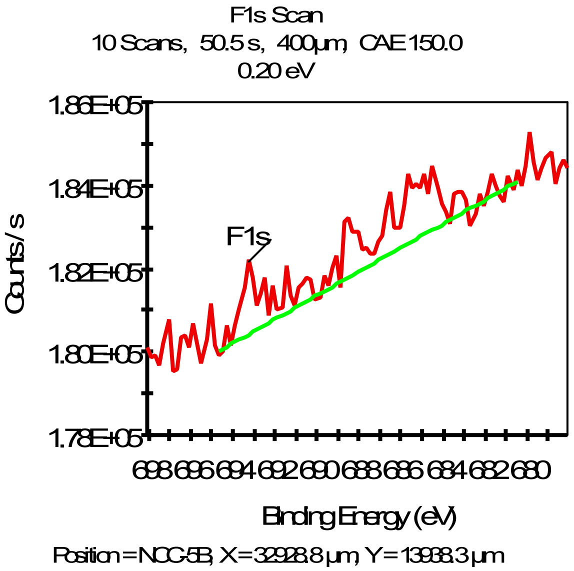
7. Inductive coupled plasma atomic emission spectroscopy (ICP-AES) analysis

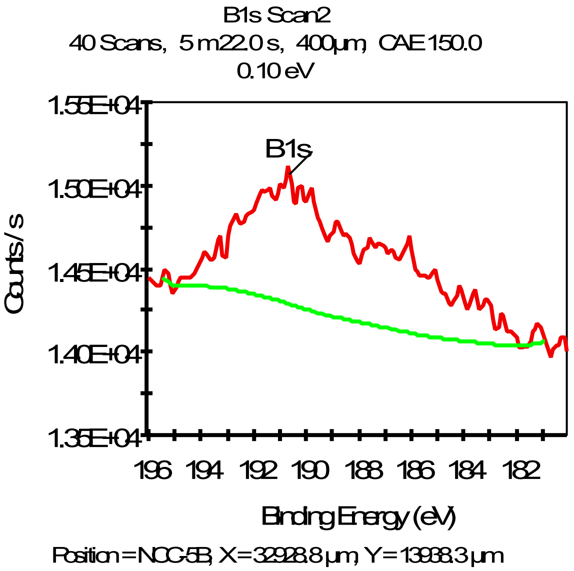
30 mg of NC-SB sample was accurately weighed in a small beaker. To this 10 mL of aqua-regia solution of HCl: HNO_3 (3:1) was added. The resultant mixture was covered by a watch glass and allowed to heat at 50 °C for 2 hours. The resultant solution was cooled, filtered and then diluted to 50 mL with 18.2 Mω deionized water. The resultant solution was then analyzed by ICP-AES. The Boron and Fluorine content was found to be in ratio of 1:2 which concomitant with the bodipy dye SB.

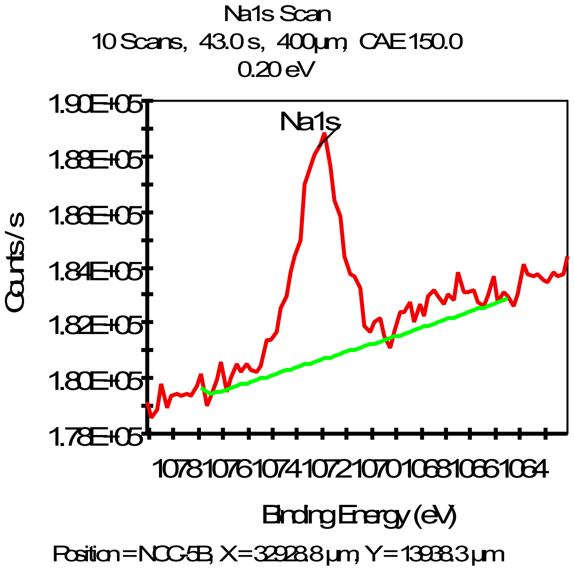
8. X-ray photoelectron spectroscopy (XPS) analysis

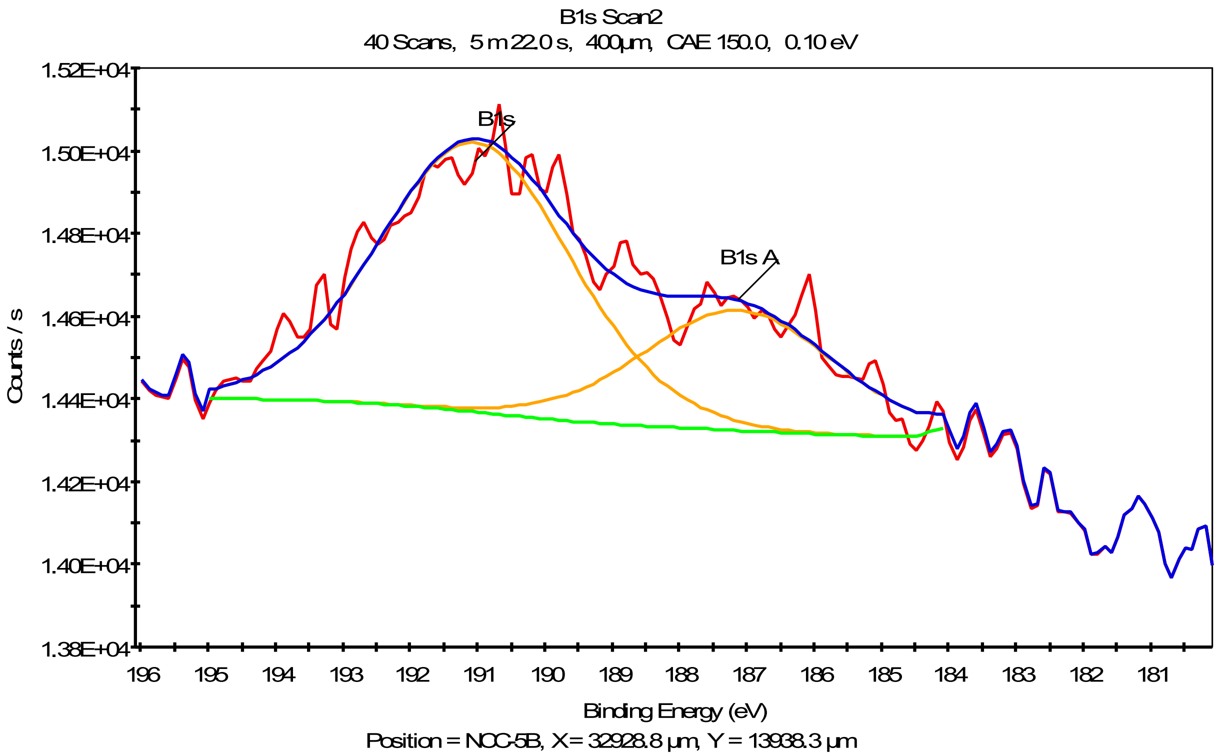
XPS absorption spectrum of NCC-SB for different metals




F1s Scan
10 Scans, 50.5 s, 400µm, CAE 150.0
0.20 eV
1.86E+05
1.84E+05
1.82E+05
1.80E+05
1.78E+05
Counts / s
F1s
698 696 694 692 690 688 686 684 682 680
Binding Energy (eV)
Position=NCC-5B, X=32928.8 µm, Y=13938.3 µm


B1s Scan2
40 Scans, 5 m 22.0 s, 400µm, CAE 150.0
0.10 eV
1.55E+04
1.50E+04
1.45E+04
1.40E+04
1.35E+04
Counts / s
B1s
196 194 192 190 188 186 184 182
Binding Energy (eV)
Position=NCC-5B, X=32928.8 µm, Y=13938.3 µm

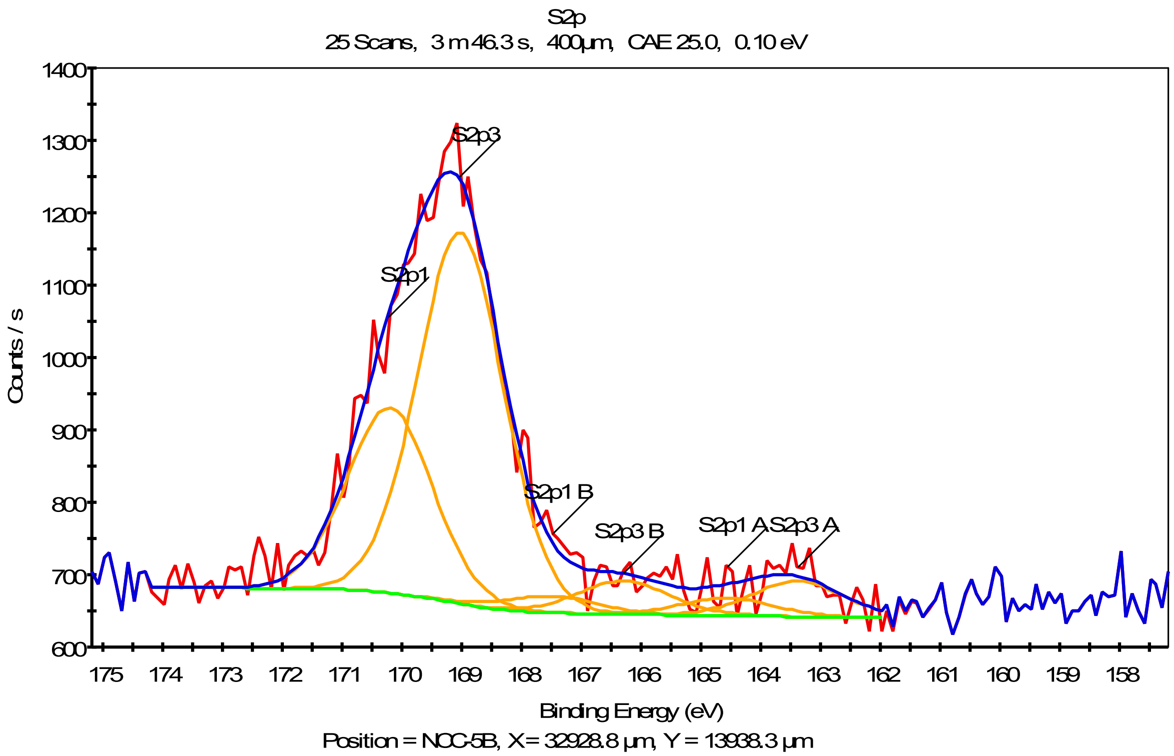

Na1s Scan
10 Scans, 43.0 s, 400µm, CAE 150.0
0.20 eV
1.90E+05
1.88E+05
1.86E+05
1.84E+05
1.82E+05
1.80E+05
1.78E+05
Counts / s
Na1s
1078 1076 1074 1072 1070 1068 1066 1064
Binding Energy (eV)
Position=NCC-5B, X=32928.8 µm, Y=13938.3 µm


B1s Scan2
40 Scans, 5 m 22.0 s, 400µm, CAE 150.0, 0.10 eV

Position = NCC-5B, X= 32928.8 µm, Y = 13938.3 µm

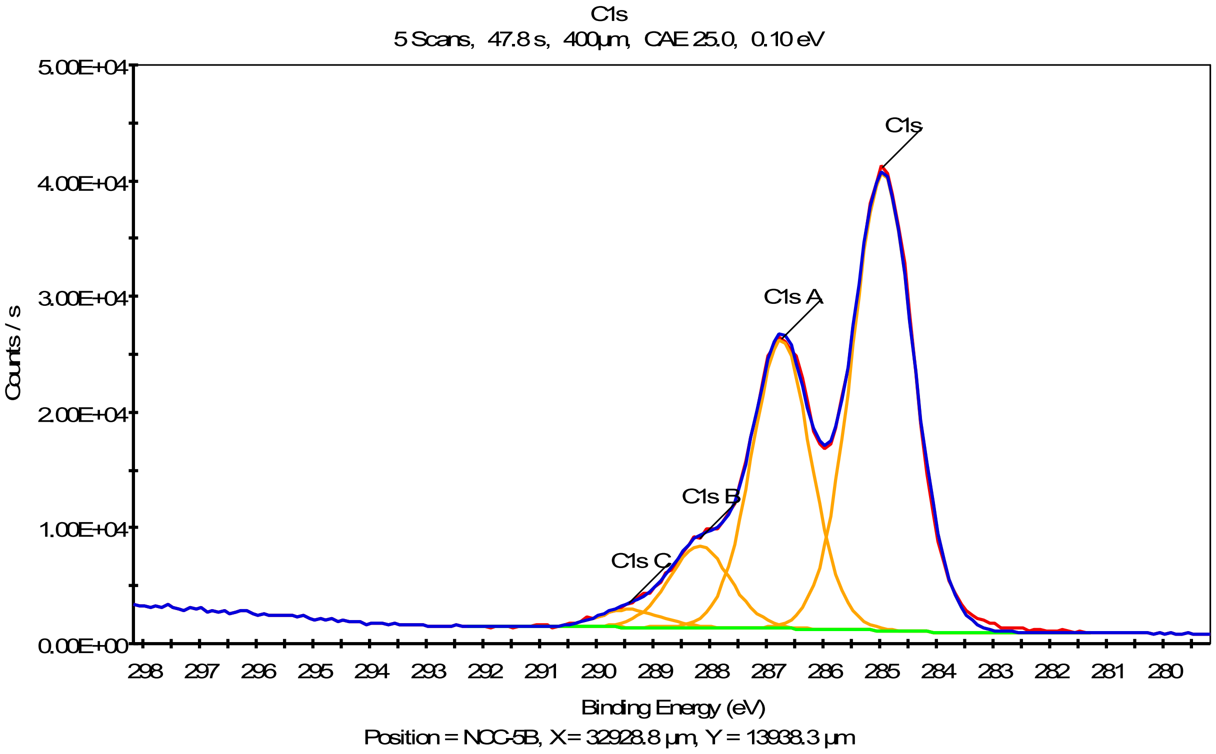
Elemental ID and Quantification

Name	***Peak BE***	***FWHM eV***	***Area (P) CPS.eV***	***Atomic %***	***Q***
B1s	191.03	3.37	2371.05	69.15	1
B1s A	187.11	3.37	1060.39	30.85	1



Elemental ID and Quantification

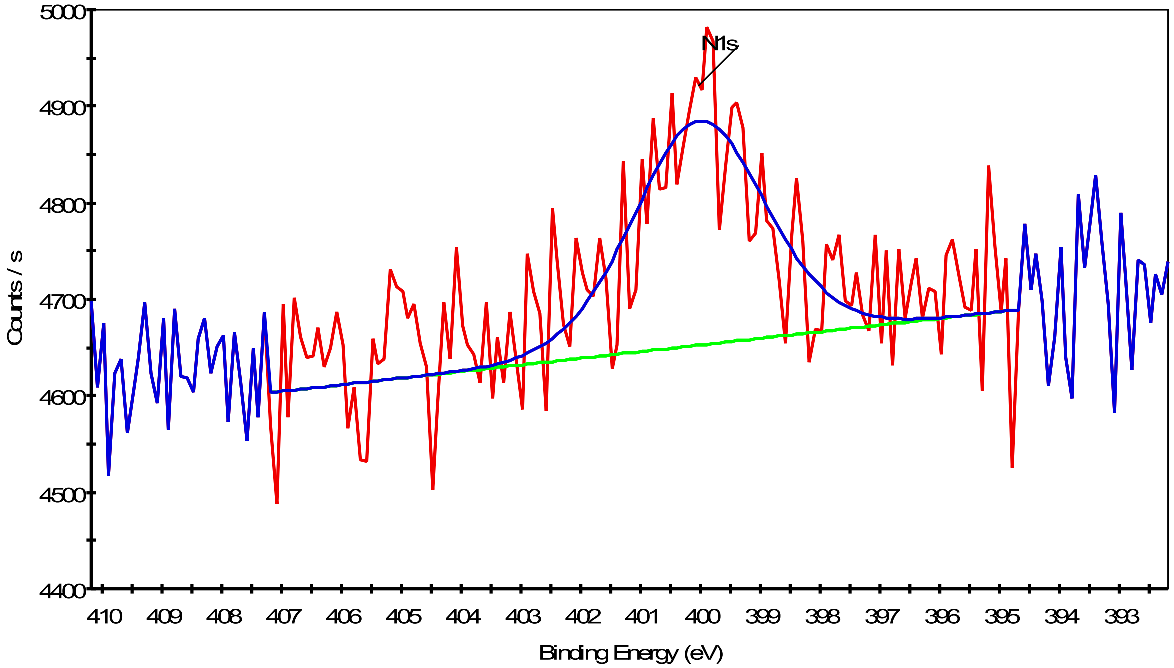
Name	*Peak BE*	*FWHM eV*	*Area (P) CPS.eV*	*Atomic %*	*Q*
S2p3	169.01	1.68	931.67	83.56	1
S2p1	170.19	1.68	465.83	0.00	0
S2p3 A	163.38	1.79	94.65	8.46	1
S2p1 A	164.56	1.79	47.32	0.00	0
S2p3 B	166.30	1.79	89.07	7.98	1
S2p1 B	167.48	1.79	44.54	0.00	0



Elemental ID and Quantification

Name	*Peak BE*	*FWHM eV*	*Area (P) CPS.eV*	*Atomic %*	*Q*
C1s	284.96	1.28	54783.25	55.11	1
C1s A	286.75	1.22	32946.64	33.18	1
C1s B	288.19	1.25	9542.62	9.62	1
C1s C	289.44	1.25	2066.74	2.09	1

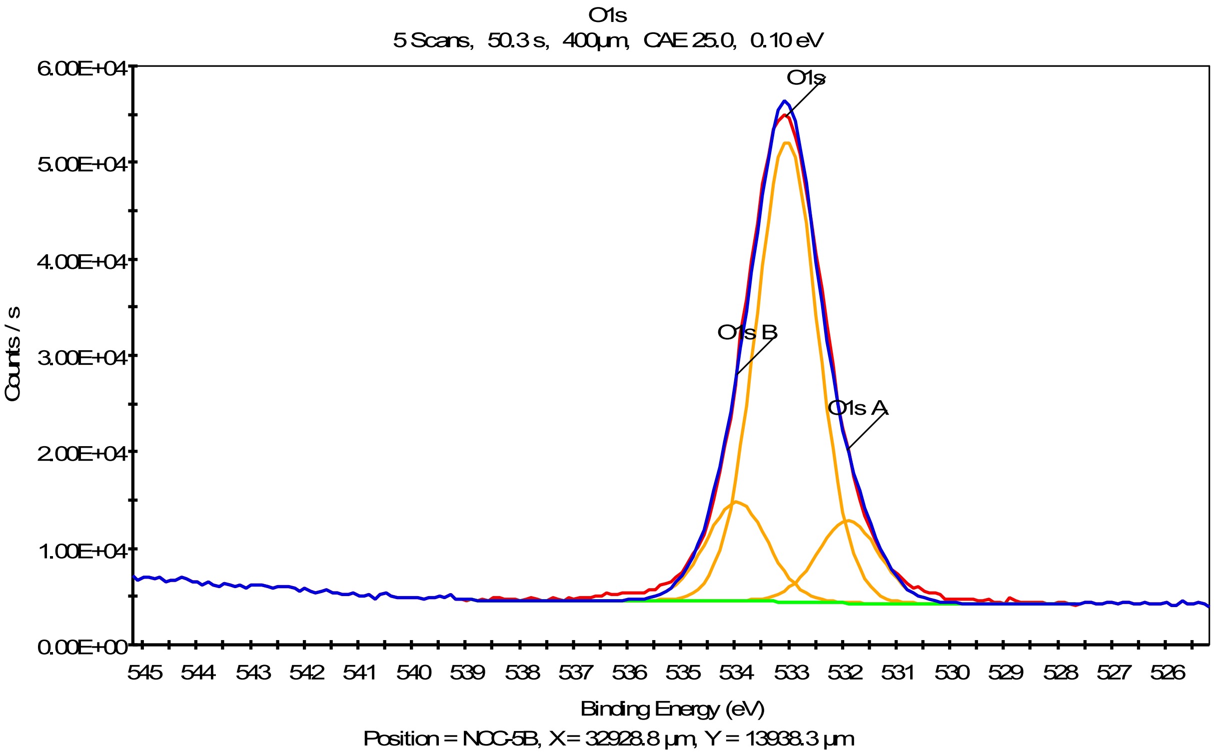
N1s
25 Scans, 3 m 46.3 s, 400µm, CAE 25.0, 0.10 eV



Position = NCC-5B, X= 32928.8 µm, Y = 13938.3 µm

Elemental ID and Quantification

Name	***Peak BE***	***FWHM eV***	***Area (P) CPS.eV***	***Atomic %***	***Q***
N1s	400.02	2.60	653.48	100.00	1



Elemental ID and Quantification

Name	***Peak BE***	***FWHM eV***	***Area (P) CPS.eV***	***Atomic %***	***Q***
O1s	533.03	1.34	69358.73	71.74	1
O1s A	531.89	1.34	12444.13	12.86	1
O1s B	533.96	1.34	14877.28	15.40	1

9. Singlet Oxygen Production test with ABDA

0.25 mM solution of 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA), was prepared in phosphate buffer (0.001 M, ph -7, D_2O). The solution was saturated with oxygen for 5 minutes. It was illuminated for 5 minutes in front of white light source (75±5 mW/cm2) lamp (filter >375 nm). It was found that the UV-visible of Spectrum of ABDA was not affected.

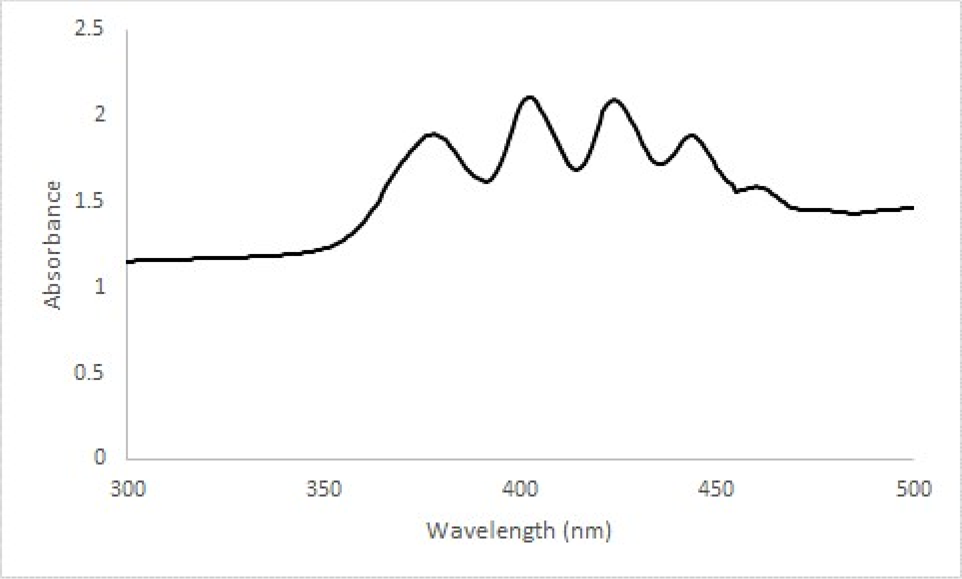


Figure 9: UV visible spectrum in the absence of NC-SB

On the contrary, when the same experiment was carried out in in the presence of NC-SB (0.3 mg), it was found that there is 10% decrease in ABDA spectrum, when illuminated for 5 minutes.

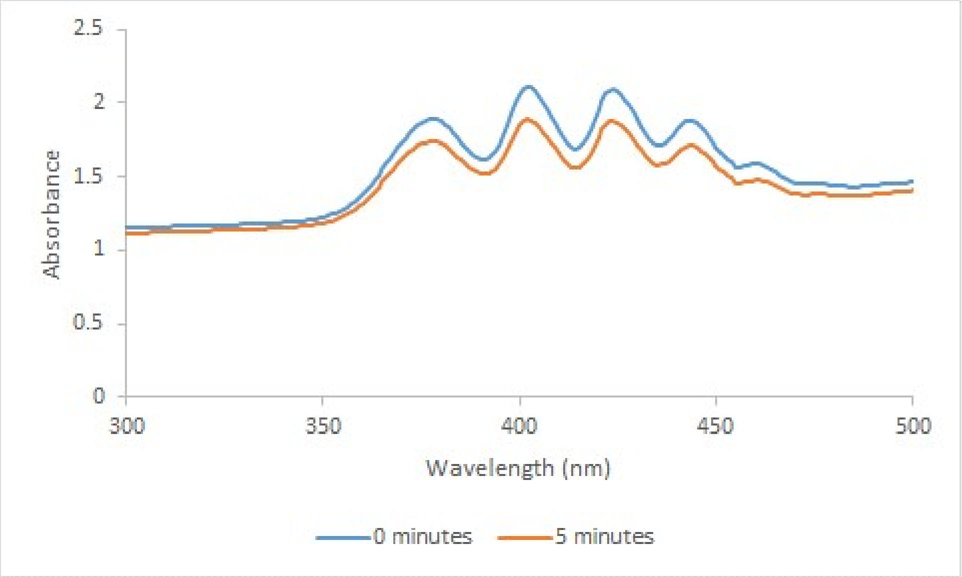


Figure 10: UV- visible spectrum in the presence of NC-SB

absorbance at 380 nm in 0 minutes = 2.084

absorbance at 380nm

after 5 minutes = 1.870

X= 2.084-1.870 = 0.214/2.084= 10.26 %

10. Photooxidation of L-methionine methyl ester

20 mg of L-methionine methyl ester was dissolved in 1.5 mL deutaratted water. To this 1mg of NC-SB was added. The suspension was sonicated for 5 minutes. The suspension was transferred to a quartz cuvette, capped with a rubber septum and saturated for 5 minutes. It was illuminated for 210 minutes in front of white light source (75±5 mW/cm2 Hg) lamp (filter >375 nm).[R2] Finally, the suspension was centrifuged for 5 minutes at 10k to remove NC-SB and analysed by NMR spectroscopy.

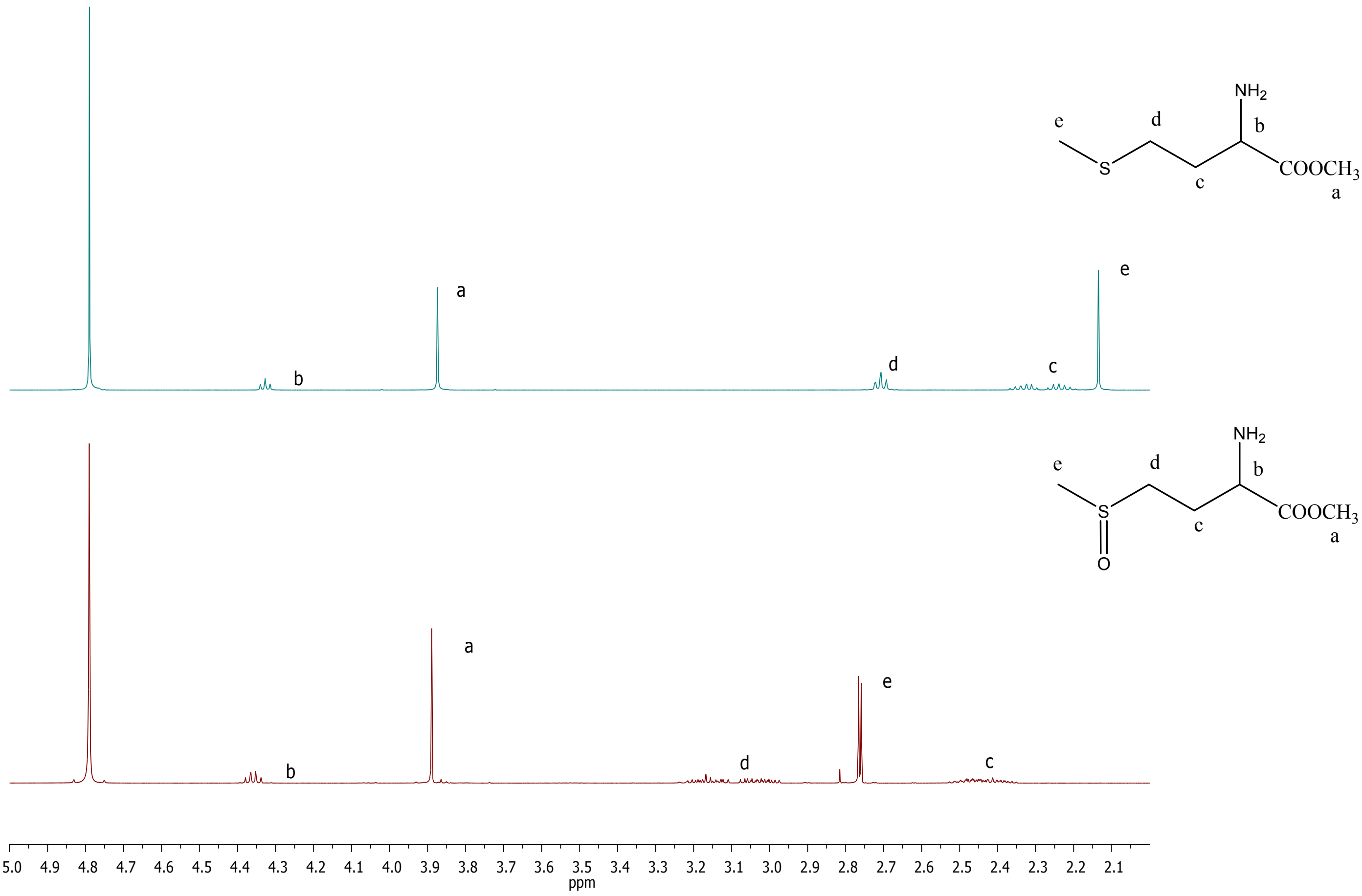


Figure 11: ^{1}H-NMR spectra of L-methione methyl ester and its sulfoxide in D_2O

11. References

R1 E. Kaiser, R. L. Colescott, C. D. Bossinger and P.I. Cook, *Anal. Biochem.*, 1970, **34**, 595-598.

R2 T. Carofiglio, P. Donnola, M. Maggini, M. Rossetto, and E. Rossi, *Adv. Synth. Catal.*, 2008, **350**, 2815.