

Supplementary Information

Design and synthesis of phosphonate functionalized naphthalenediimide: Application to induce mitochondria mediated apoptosis in the human skin melanoma cells (SKMEL2)

Kiran R Kharat^a, Rajesh S Bhosale^b, Kamalakar P Nandre^c, Madan R Biradar^{c,d}, Sheshanath V Bhosale^c & Sidhanath V Bhosale^{c,d,*}

^a KETs V. G. Vaze College, Mulund, Mumbai 400 081, Maharashtra, India

^b Department of Chemistry, Indrashil University, Rajpur, Mesana 382 470, Gujarat, India

^c Polymers and Functional Materials Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, Telangana, India

^d Academy of Scientific and Innovative Research (AcSIR), CSIR-HRDC Campus, Postal Staff College Area Sector 19, Kamla Nehru Nagar, Ghaziabad 201 002, Uttar Pradesh, India

^e School of Chemical Sciences, Goa University, Taleigao Plateau, Goa 403 206, India

*E-mail: bhosale@iict.res.in

Sl. No.	Contents	Pg. No.
1	Fig. S1 — HPLC analysis of NDI-1 and NDI-2	2
2	Fig. S2 — Analysis of exposure of Phosphatidylserine on SKMEL2 cells by flow cytometry. (A) NDI-1 without phosphonate in SKMEL2 cells (B) NDI-2 without phosphonate in SKMEL2 cells. Flow cytometry analysis of apoptosis induced by the NDI derivatives using the Annexin V-FITC/PI double staining method	3
3	Table S1— Primers used for the gene expression by Real Time PCR	3
4	Fig. S3 — ¹ H NMR spectrum of NDI-1	4
5	Fig. S4 — ¹³ C NMR spectrum of NDI-1	4
6	Fig. S5 — ¹ H NMR spectrum of NDI-2	5
7	Fig. S6 — ¹³ C NMR spectrum of NDI-2	5
8	Fig. S7— HRMS spectrum of NDI-1	6
9	Fig. S8— HRMS spectrum of NDI-2	7

Experimental Section

HPLC analysis:

The HPLC analysis of the NDI 1 and NDI 2 was performed by Agilent ProStar C-18 HPLC (equipped with Agilent and UV detector) using Agilent HPLC C18 column (250x 4.6mm, 5 μ m). The mobile phase, Acetonitrile 40% (v/v), 60% (v/v) and 80 % (v/v) in water was used separately as mobile phase for NDI 1 and NDI 2. Samples were analysed by Agilent software, and UV absorbance scans of the resultant peaks were measured on 220 nm (Fig. S1)

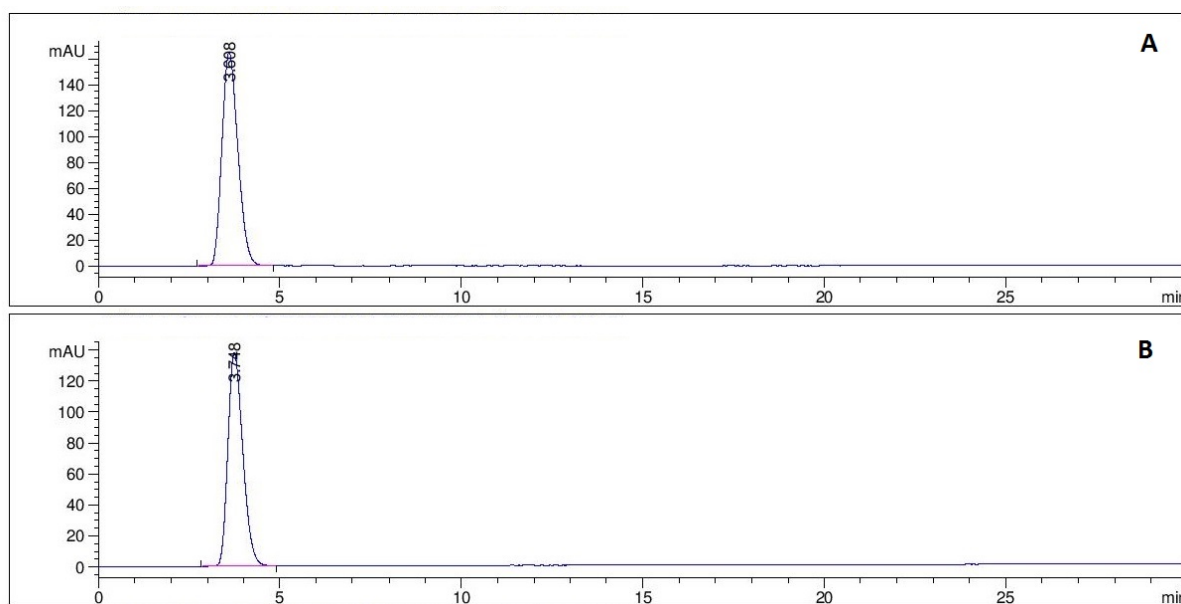


Fig. S1 — HPLC analysis of **NDI-1** and **NDI-2**

Where, **A** is the **NDI-1** and **B** is **NDI-2** compounds. The mobile phase, Acetonitrile 40% (v/v), 60% (v/v) and 80 % (v/v) in water was used separately as mobile phase for **NDI-1** and **NDI-2**. Samples were analysed by Agilent software, and UV absorbance scans of the resultant peaks were measured on 220 nm.

Detection of Phosphatidylserine on the membranes of the apoptotic cells

In order to confirm the presence of apoptotic cells in the population, flow cytometric analysis of the phosphatidylserine on the membranes of the apoptotic cells was undertaken. The SKMEL2 cells were used for the study. The cells incubated with various concentrations

of 4 mM **NDI-1** and **NDI-2** compounds without phosphonates. We found that only 11% apoptotic cells were present in **NDI-1** treated cells, whereas 6.5% cells were found apoptotic in **NDI-2** treated cells.

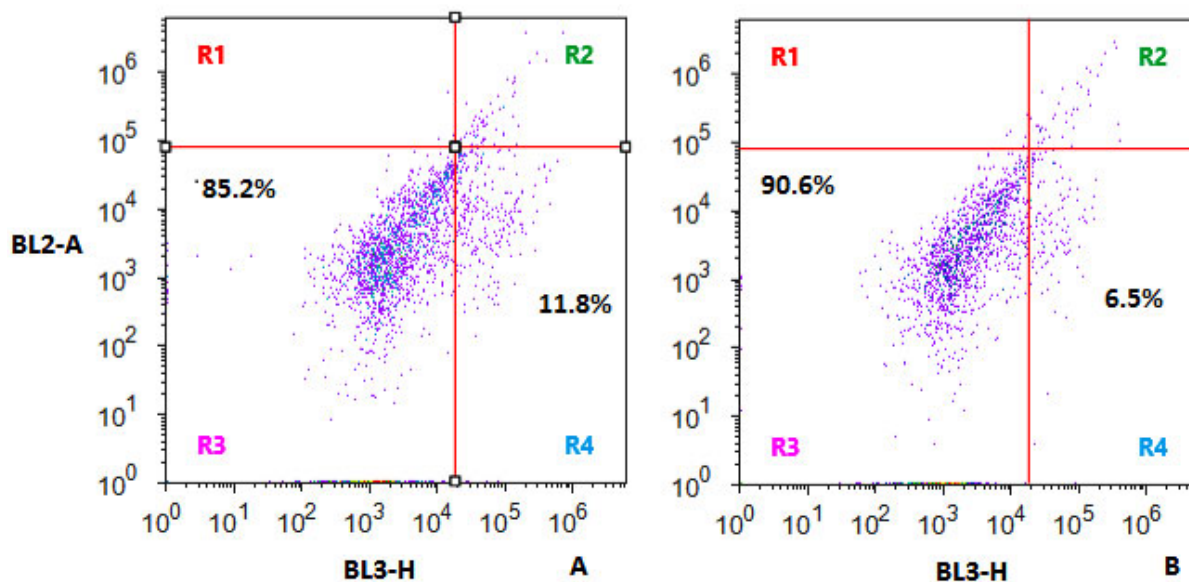


Fig. S2 — Analysis of exposure of Phosphatidylserine on SKMEL2 cells by flow cytometry. (A) NDI-1 without phosphonate in SKMEL2 cells (B) NDI-2 without phosphonate in SKMEL2 cells. Flow cytometry analysis of apoptosis induced by the NDI derivatives using the Annexin V-FITC/PI double staining method

Table S1 — Primers used for the gene expression by Real Time PCR

Name of the primer	Forward	Reverse
Bcl2	ATGTGTGTGGAGAGCGTCAA	ACAGTTCCACAAAGGCATCC
Bax	TTTTGCTTCAGGGTTTCATC	CAGTTGAAGTTGCCGTCAGA
Bak1	GCCTTGCAGTTGGACTCTC	GGGTTGGGAGCAAGTGTCTA
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

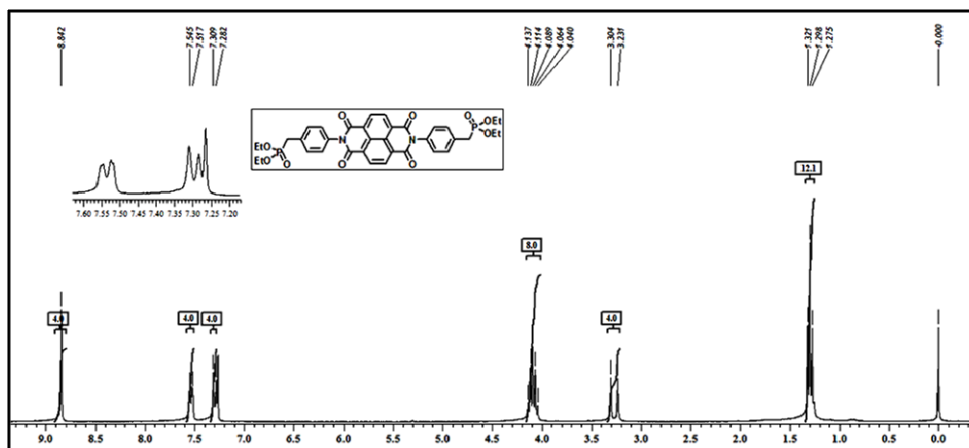


Fig. S3 — ^1H NMR spectrum of **NDI-1** in CDCl_3

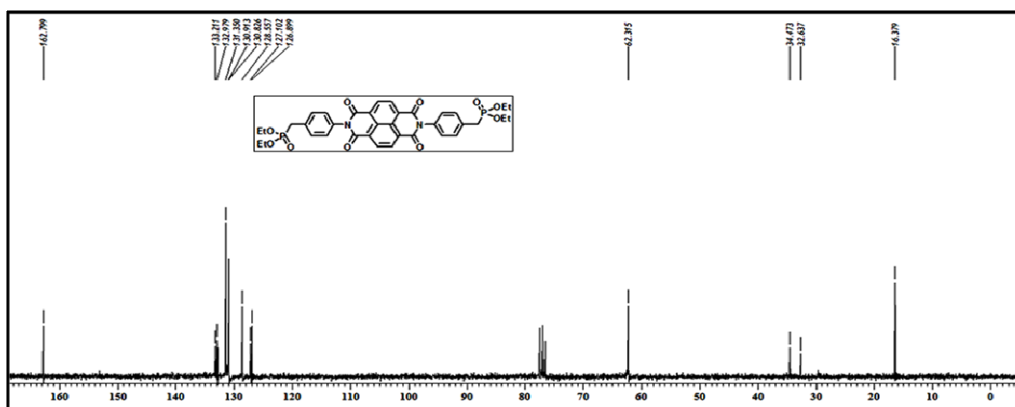


Fig. S4 — ^{13}C NMR spectrum of **NDI-1** in CDCl_3

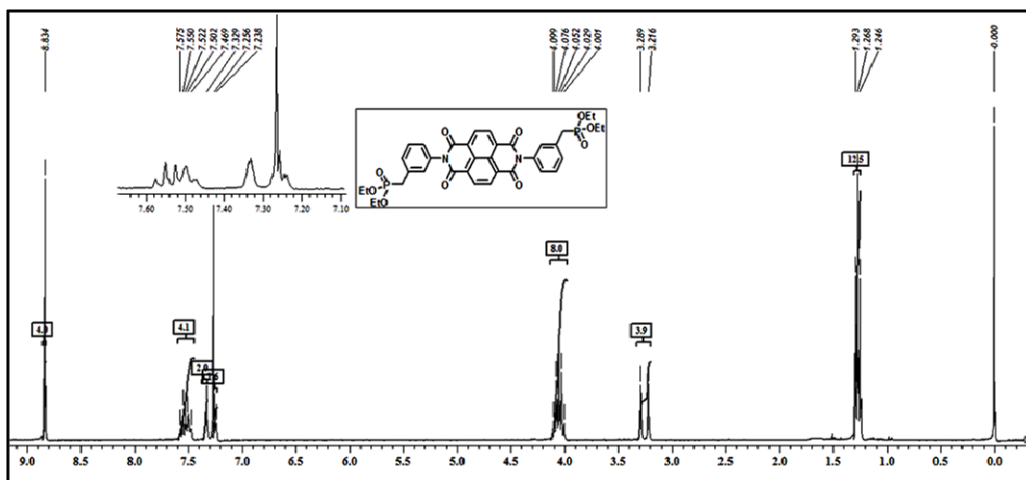


Fig. S5 — ^1H NMR spectrum of **NDI-2** in CDCl_3

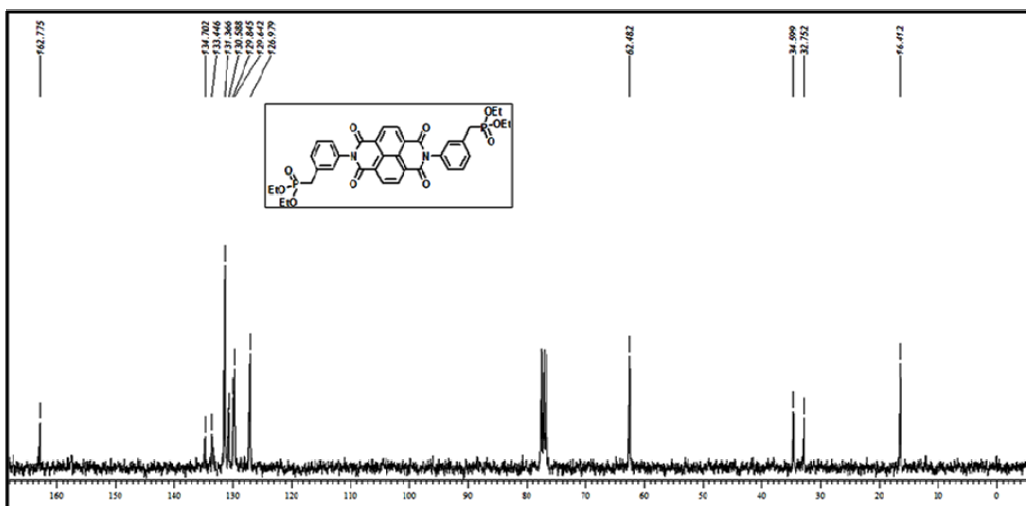


Fig. S6 — ^{13}C NMR spectrum of **NDI-2** in CDCl_3

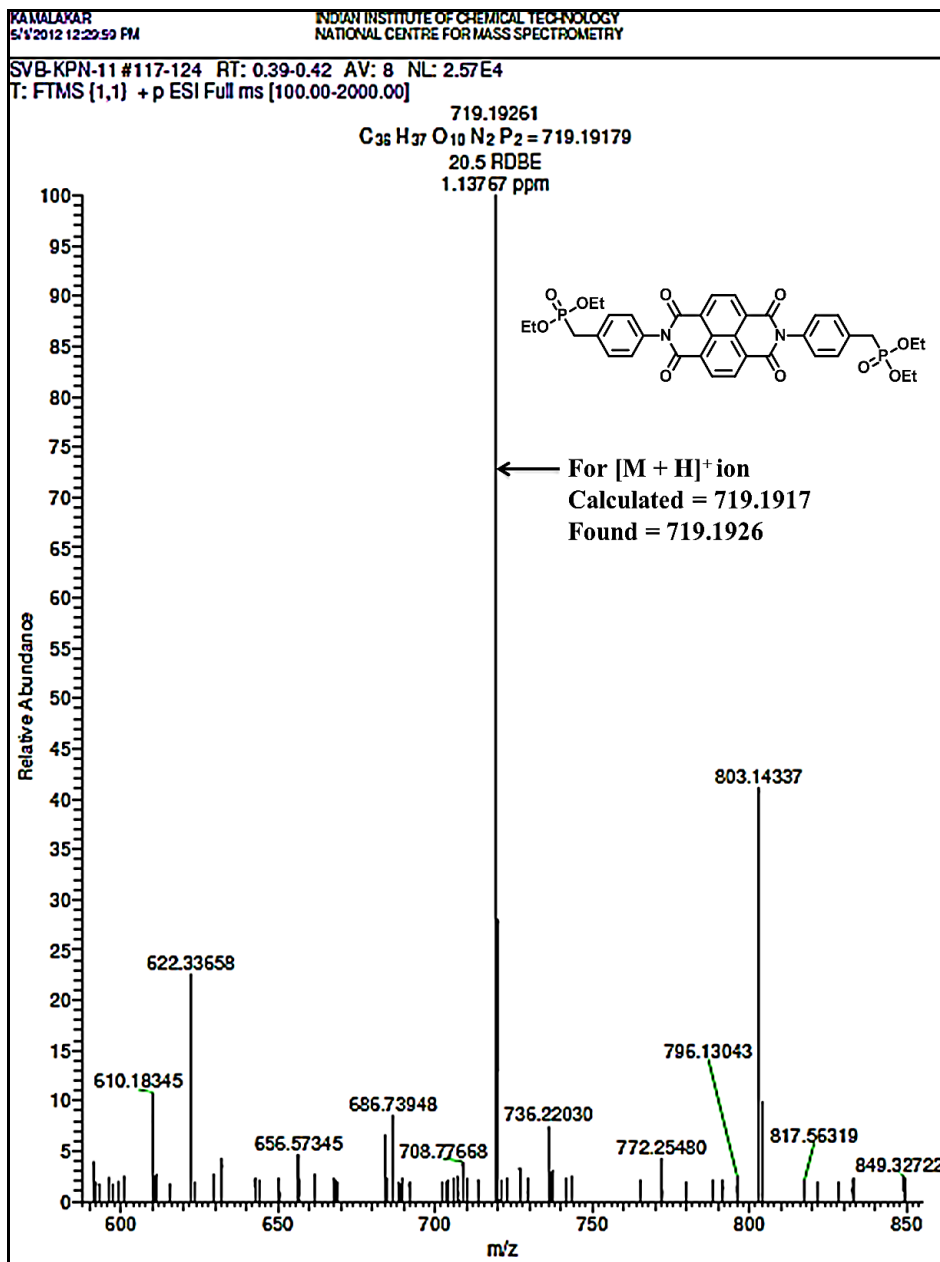


Fig. S7— HRMS spectrum of NDI-1

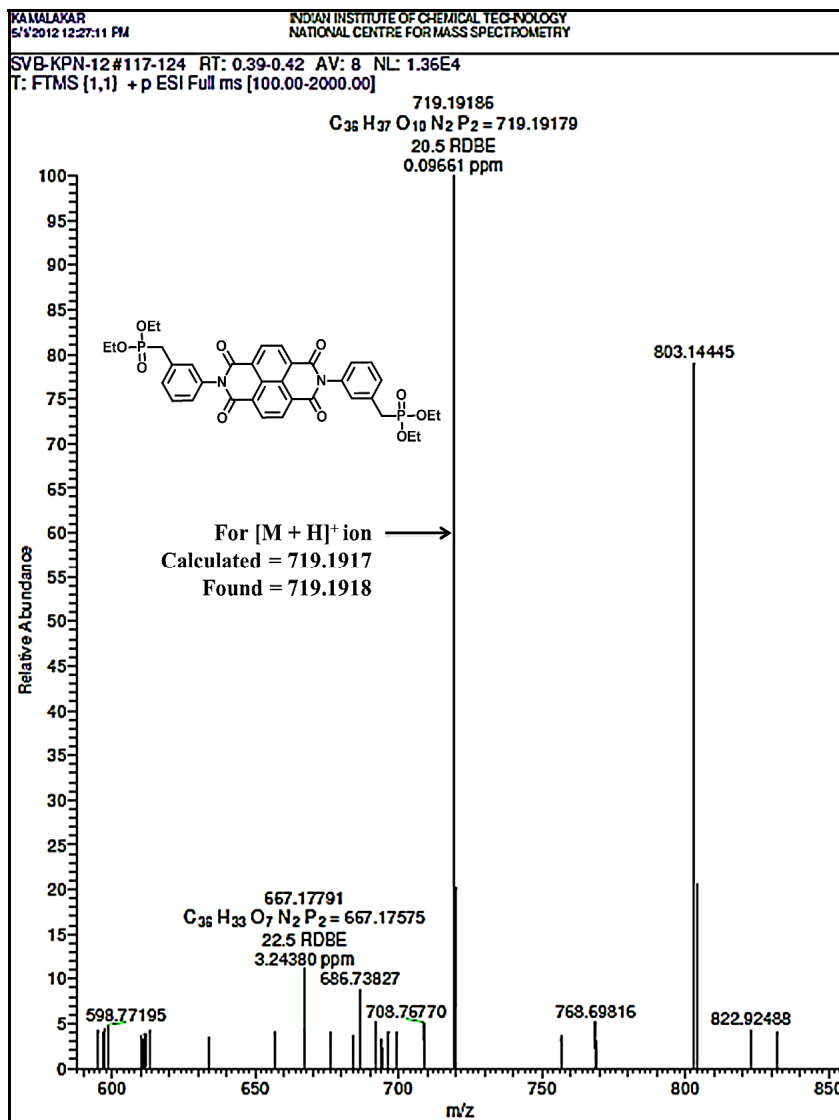
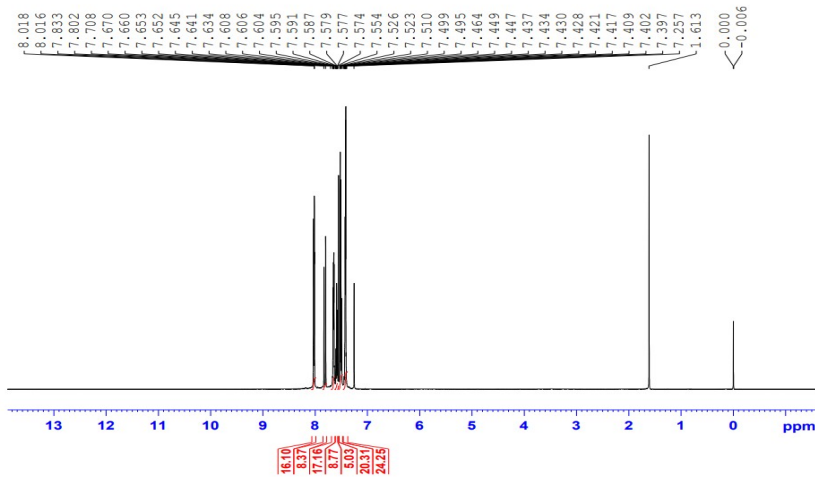


Fig. S8— HRMS spectrum of NDI-2

NJ-01
 CIF_Proton CDCl3 (E:\BHARATI VIDYAPEETH) CIF 14



Current Data Parameters
 NAME Jan13-2022
 EXPNO 1
 PROCNO 1
 F2 - Acquisition Parameters
 Date_ 20220113
 Time_ 11:53 h
 INSTRUM E119470_0152
 PROBRD E119470_0152
 PULPROG zgpg30
 SU 83.26
 SOLVENT CDCl3
 NS 16
 DS 2
 SWH 10000.002 Hz
 FIDRES 0.305176 Hz
 AQ 3.2167099 sec
 RG 509.52
 DM 30.000 usec
 DE 6.50 usec
 TE 296.4 K
 D1 1.00000000 sec
 FID 500.130083 MHz
 SFO1 500.130083 MHz
 NUCL1 1H
 P1 9.50 usec
 PL1 24.68000031 W
 F2 - Processing parameters
 SI 65336
 SF 500.1300137 MHz
 MW 3M
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

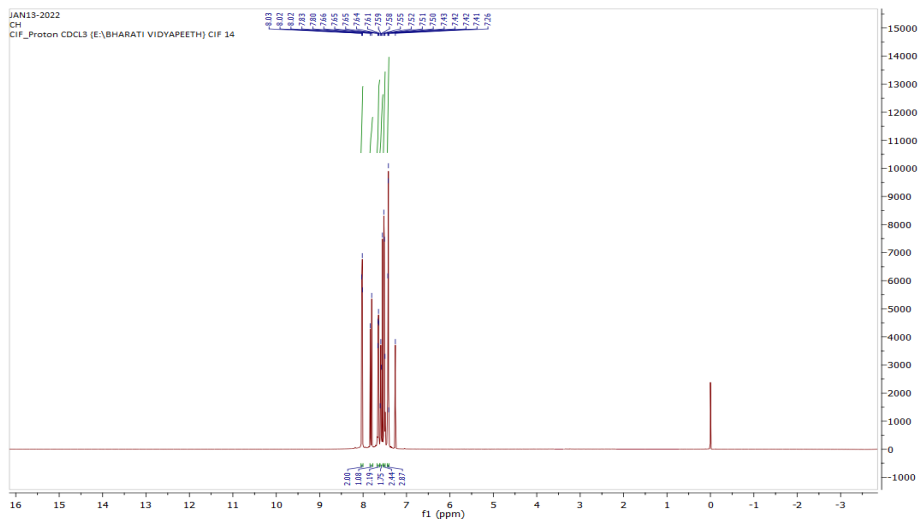
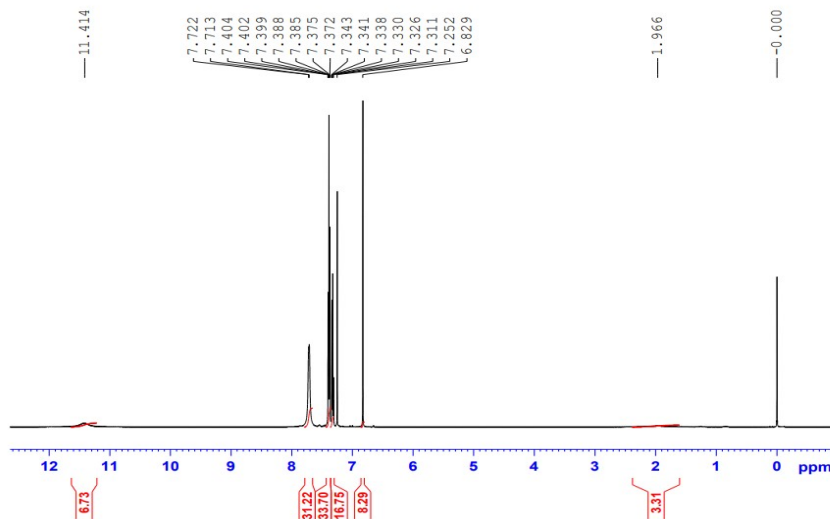


Fig. S4 — ¹H NMR spectra of CH

NJD2
CIF_Proton CDCl3 (E:\POONA COLLEGE) CIF 17



Current Data Parameters
NAME May06-2022
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220506
Time_ 19.08 h
INSTRUM spect
PROBHD Z119470_0152 (C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 98.03
DW 50.000 usec
DE 6.50 usec
TE 298.0 K
D1 1.0000000 sec
TDD 1
SFO1 500.130083 MHz
NUC1 1H
PC 1.17 usec
F1 9.30 usec
F2 24.6800031 M

F3 - Processing parameters
SI 65536
SF 500.130082 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

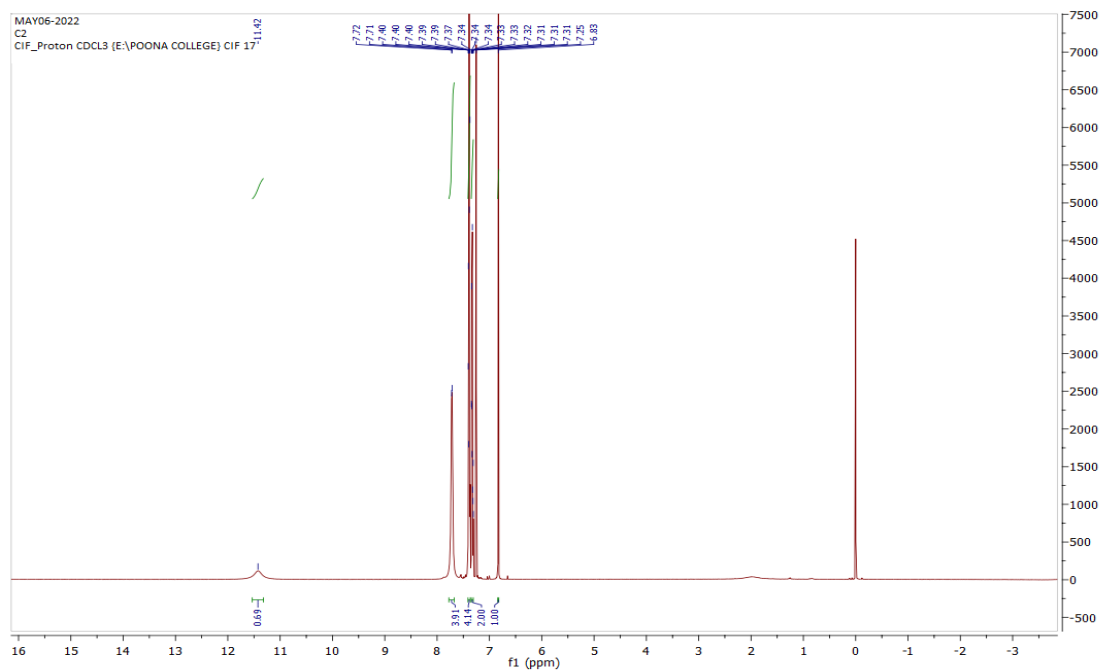


Fig. S6 — ¹H NMR spectra of NJD2

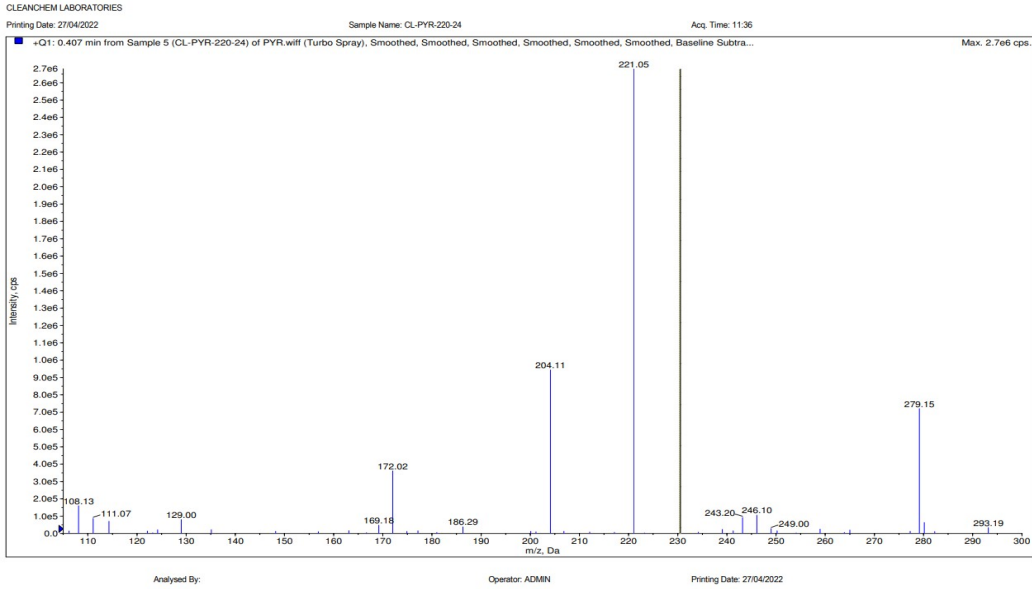
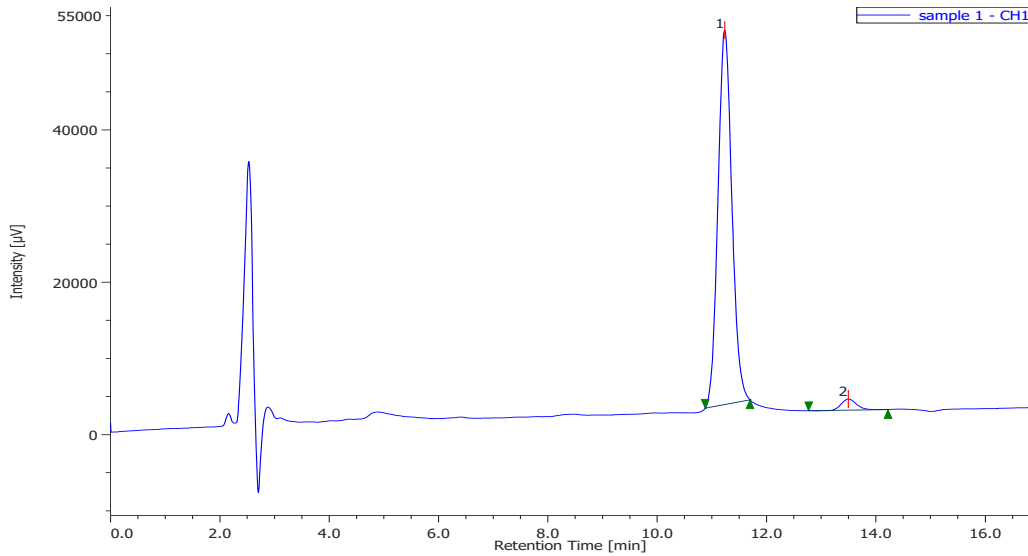
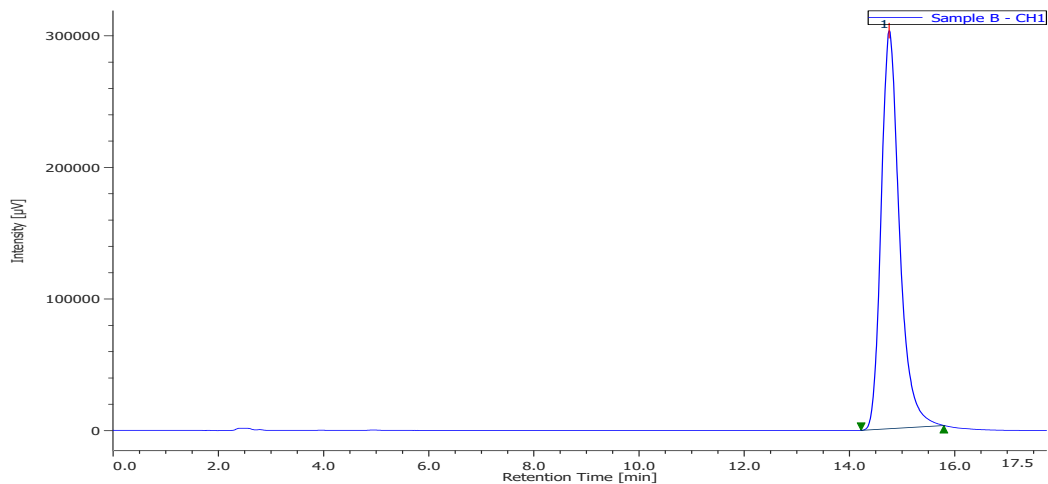


Fig. S9 — Mass spectrum of NDJ2



RT	Area	Height	Area%	Height% (Purity)	Quantity
11.233	868454	49108	96.816	97.104	N/A
13.492	28562	1465	3.184	2.896	N/A

Fig. S10 — HPLC percent purity graph of NDJ1



RT	Area	Height	Area%	Height% (Purity)	Quantity
14.7	868454	28108	99.121	99	N/A

Fig. S11 — HPLC percent purity graph of **NDJ2**