Electronic Supporting Information (ESI)

Strategic Engineering of Alkyl Spacer Length for pH-tolerant Lysosome Marker and Dual Organelle Localization

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1. Experimental section:

1.1 Materials and methods:

1.1.1. General Method:

All the reagents and solvents were brought from commercial source and used without further purification. Water used for PBS preparation and other spectroscopic measurements is of Milli-Q grade with resistivity 18.2 MΩ. Thin layer chromatography (TLC) was performed using Merck Silica gel 60 F-254 pre-coated plates and visualized using UV-irradiation (λ=254/365 nm). Silica gel from Merck (particle size 100–200 mesh) was used for column chromatography. ^{1}H and ^{13}C-NMR spectra were recorded on Bruker 400 MHz spectrometers with operating frequencies of 100 MHz for ^{13}C. Chemical shifts (δ) are reported in ppm relative to the residual solvent signal (δ=7.26 for ^{1}H NMR and δ = 77.0 for ^{13}C NMR). High-resolution mass spectrometry (HRMS) data were recorded on Bruker MicrOTOF-Q-II mass spectrometer using chloroform as the solvent. All absorption spectra and fluorescence measurements were carried out using SHIMADZU (UV-1800) spectrophotometer and HORIBA Jobin Yvon fluorimeter (fluorolog 3-2) using 1 cm path length quartz cuvettes. Fluorescence decay time was measured using a Hamamatsu MCP photomultiplier (R-3809U-50) in time-correlated single photon counting (TCSPC) setup consists of an Ortec 9327 pico-timing amplifier and using pulse Diode laser (λ_{ex} =440 nm) with fwhm ~143 ps with a setup target 10,000 counts. The instrument response function (IRF) was measured before and after fluorescence lifetime measurement using a dilute suspension of Ludox (purchased from Sigma) colloidal silica. All the measurements were carried out at ambient temperature (298K) using 5 μM dye concentration. For the pH-dependent study, we measured the pH of the solution using Oakton pH 700 Benchtop Meter.

SCXRD. Good quality single crystals of NIMC2, NIMC3, NIMC4, NIMC5 and NIMC10 suitable for X-Ray single crystal analysis were obtained by slow evaporation method at low temperature (4°C). Single Crystal X-ray Diffraction data were collected on Bruker AXS Kappa APEXII diffractometer using monochromated Mo Kα radiation (λ = 0.71073 Å) at 100 K using an Oxford Cryostream low-temperature device. Unit cell measurements, data integration, scaling and absorption corrections for the crystals were done with Bruker APEXII software.[1] Data reduction were carried out with Bruker SAINT suite.[2] Absorption correction was performed by multi-scan method implemented in SADABS.[3] All the crystal structures were solved by direct methods using SIR 2014.[4] The crystal structure refinements were done in the program package OLEX2,[5] and all non-hydrogen atoms were refined anisotropically by full matrix least-squares calculations

based on F^2 with SHELXL-2018.[6] Some hydrogen atoms were located from the difference Fourier Map, while the remaining hydrogen atoms were included in calculated positions as riding atoms. Details of crystal data, data collection, and refinement details are given in **Table S8**. Geometrical calculations were carried out using PARST[7] and PLATON.[8] MERCURY[9] program was used for structure analysis and also molecular and crystal structure drawings preparation.

1.1.2 Analysis of Topological Parameters: The *ab initio* calculations for the selected monomers and dimers at the crystal geometry were performed at the B3LYP/6-311G(d,p) level of theory using Gaussian 09[10] using AIMALL.[11] The selected topological parameters like electron densities (ρ), Laplacian ($\nabla^2 \rho$), local potential energy (V_b), and kinetic energy density (G_b) at the bond critical points (BCPs) for the relevant interactions were obtained (See **Table S10**).

1.1.3 Cell culture and imaging:

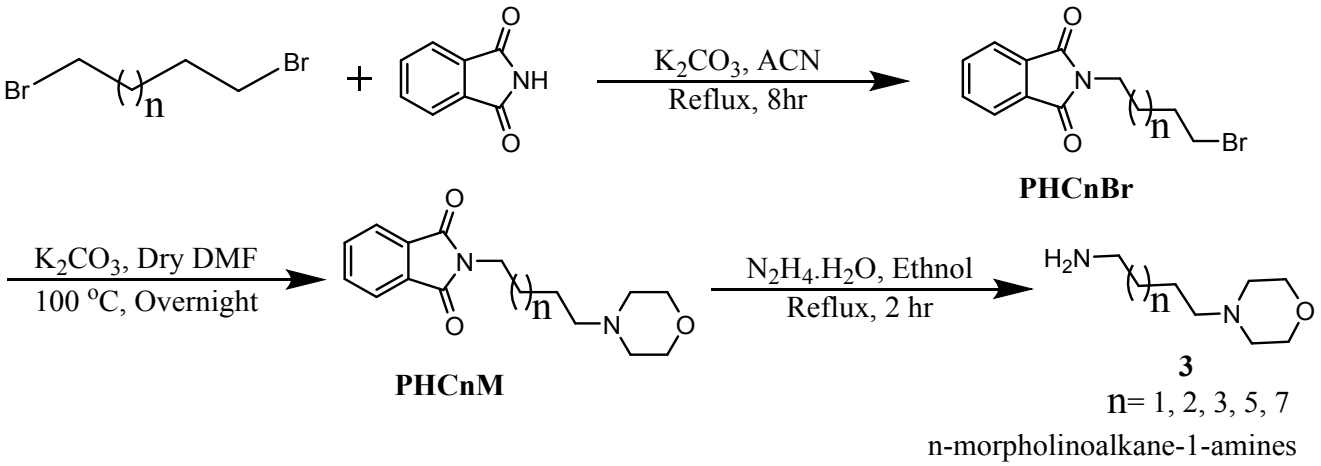
Dulbecco's Modified Eagle Medium (DMEM), Trypsin, Antibiotic cocktail and Fetal Bovine Serum (FBS) were purchased from HiMedia (USA). Lyso-Tracker Deep red and ER-Tracker Red were purchased from Thermo Fisher Scientific (USA). The 35 mm glass bottom imaging dishes were obtained from Ibidi (Germany, Cat# 81158). All the confocal microscopy imaging was performed with an Olympus FV3000 Confocal Laser Scanning Microscope (LSM). The image processing was done with the help of cell-Sens 3.1 software (Olympus). BHK-21 ells were obtained from NCCS Pune, India and were grown in a 25 cm^2 cell culture flask (Corning, USA) using DMEM (phenol red free) containing 10% (v/v) FBS and 1% (v/v) antibiotic cocktail in 5% CO_2 at 37 °C in a CO_2 incubator. For imaging purpose, cells were grown to 75% - 80% confluency in the 35 mm glass bottom imaging dishes (170 ± 5 μm) in DMEM with 10% FBS. The cells were washed twice with PBS (pH 7.4) containing 5 mM $MgCl_2$. Then the cells were co-incubated with 0.2 μM of the dye of interest and 0.3 μM of Lyso-Tracker Deep Red / ER-Tracker Red (whichever applicable) for 20 minutes and washed with PBS (pH 7.4) twice before imaging.

1.1.4. General synthetic procedure

1.1.4.1 Synthesis of NICBr: In a round bottom flask 8-Bromo-1,8-naphthalic anhydride (500 mg, 1.80 mmol) and 1-butylamine was suspended in ethanol and the reaction mixture was refluxed at 100 °C for 4 hours. The reaction was monitored by TLC and after the completion of the reaction the whole reaction mixture was poured in ice-cold water. A light-yellow precipitate appeared which was collected by filtration. Finally, a white solid pure product was isolated by column chromatography using 20 % EtOAc/ Hexane mixture with 73 % yield.

1.1.4.2 General synthetic procedure of NICM: NICM compounds were synthesized by following literature reported procedure.[12] Where NIBr (1 equivalent), N-aminoalkylmorpholine (5 equivalent) were taken in a Biotage microwave vial (2-5 mL capacity) and the tube was sealed with aluminum cap fitted with Teflon. Then to the reaction mixture dry DMSO (2 mL) was added under nitrogen environment and the nitrogen atmosphere was maintained throughout the reaction. The completion of the reaction was monitored by TLC and after the completion of reaction the reaction mixture was cooled down and whole reaction mixture was poured in ice-cold water. The organic phase was collected by $CHCl_3$ and washed three times with brine. The organic phase was treated with Na_2SO_4. Finally, a deep yellow pure compound was isolated by silica gel column chromatography. using 5% MeOH/ $CHCl_3$.

1.1.4.3 Synthesis of n-aminoalkylmorpholine: To synthesize the NIMCs with varying alkyl spacer, N-morpholinoalkane-1-amine is required. In case of **NIMC2** and **NIMC3** the required n-aminoalkylmorpholines are 2-morpholinoethane-1-amine, 3-morpholinopropane-1-amine, which are commercially available and we bought from TCI chemical Japan. The rest of higher n-morpholinoalkane-1-amines required for **NIMC4, NIMC5, NIMC6, NIMC8, NIMC10** were synthesized using literature reported procedure with suitable modification as mentioned herein.[13]



1.1.4.4 General Synthetic method of Compound PHCnBr: In a round bottle flask phthalimide (1 equivalent), K_2CO_3 (1.05 equivalent), Dibromoalkane (2 equivalent) were poured and the reaction flask was made inert by N_2. Then to the R.B dry MeCN was added and the reaction was allowed to reflux for 8 hours under N_2 atmosphere. After completion of the reaction the

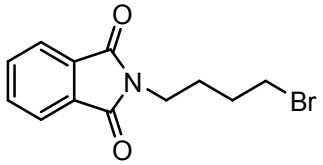
MeCN was evaporated off under reduced pressure and the reaction mixture was washed with water and the product was extracted with EtOAc. Finally, the pure product was isolated by column chromatography using 10% EtOAc/Hexane mixture.

1.1.4.5 General Synthetic method of Compound PHCnM: In a round bottle flask compound **PHCnBr** (1 equivalent), morpholine (1.5 equivalent), K_2CO_3 (1.25 equivalent) was added and the environment was made inert by nitrogen. Then to the reaction mixture dry DMF was added and the reaction mixture was allowed to react at 100 °C for overnight under N_2 atmosphere. After the completion of the reaction ice-chilled water was added to the reaction mixture and the compound was extracted with EtOAc. Then the product was washed with ice-cold water 3 times and finally, with brine solution to completely remove DMF. The EtOAc extract was treated with Na_2SO_4 and finally, the pure product was obtained by evaporating the EtOAc under reduced pressure.

1.1.4.6 General synthetic method of compound n-morpholinoalkane-1-amines: Compound **PHCnM** (1 equivalent), N_2H_4. H_2O (2.5 equivalent) ware taken in RB and to it EtOH was added. The reaction mixture was refluxed for 2 hours. After the reaction, a solid white precipitate was appeared. The precipitate was filtered and the filtrate was kept. Then ethanol was evaporated and again some precipitate appeared the precipitate was dissolved in minimum amount of ethanol and then kept for few hours in ice bath. Again, precipitate appeared and again the precipitate was washed with cold ethanol and filtrate was kept. After doing this precipitation and filtration step for 4 times, pale yellow viscous amine compound was isolated which used in the next step without further purification.

1.1.5 Synthetic description of the compounds:

1.1.5.1 Synthesis of PHC4Br:

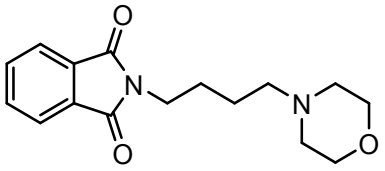


2-(4-bromobutyl)isoindoline-1,3-dione
(PHC4Br)

5 g (33.98 mmol) of phthalimide, 8.11 mL (67.96 mmol) 1,4-dibromobutane, 4.931 g (35.68 mmol) K_2CO_3, and 100 mL dry MeCN were used as a reaction mixture and after purification 6.81 g of pure white solid product was obtained 71 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 7.83 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.71 (dd, *J* = 5.4, 3.0 Hz, 2H), 3.69 (t, *J* = 7.2 Hz, 2H), 3.39 (t, *J* = 6.7 Hz, 2H), 1.90 (m, *J* = 6.9 Hz, 2H), 1.71 (m, *J* = 7.4 Hz, 2H),). ^{13}C NMR (101 MHz, $CDCl_3$) *δ*

168.39, 133.92, 132.10, 123.21, 37.66, 33.38, 32.19, 27.73. ESI-HRMS M/z [M+H$^+$] calculated for $C_{12}H_{13}BrNO_2$ is 282.0130 Da and found 282.0128 Da.

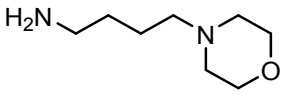
1.1.5.2 Synthesis of PHC4M:



2-(4-morpholinobutyl)isoindoline-1,3-dione
(PHC4M)

5 g (17.72 mmol) of 2-(4-bromobutyl)isoindoline-1,3-dione, 2.3 mL (26.58 mmol) morpholine, 3.06 g (22.15 mmol) K_2CO_3, and 15 mL dry DMF were used as a reaction mixture and after purification 3.10 g of pure white solid product was obtained 62 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 7.82 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.69 (dd, *J* = 5.5, 3.0 Hz, 2H), 3.70 – 3.64 (m, 6H), 2.39 (t, *J* = 4.7 Hz, 4H), 2.33 – 2.26 (m, 2H), 1.68 (m, *J* = 7.5 Hz, 2H), 1.52 (m, *J* = 7.5 Hz, 2H), ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 168.39, 133.89, 132.11, 123.16, 66.93, 58.36, 53.68, 37.80, 26.50, 23.83. ESI-HRMS M/z [M+H$^+$] calculated for $C_{16}H_{21}N_2O_3$ is 289.1552 Da and found 289.1574 Da.

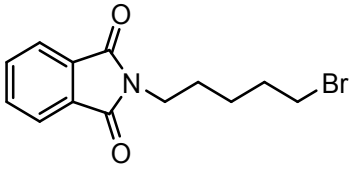
1.1.5.3 Synthesis of 4-morpholinobutan-1-amine:



4-morpholinobutan-1-amine

1.7 g (5.9 mmol) of 2-(4-morpholinobutyl) isoindoline-1,3-dione, 0.71 mL (14.75 mmol) hydrazine monohydrate, and 20 mL ethanol were used as a reaction mixture and after purification a viscous yellow liquid was obtained quantitative amount which was directly used for the next step without further purification.

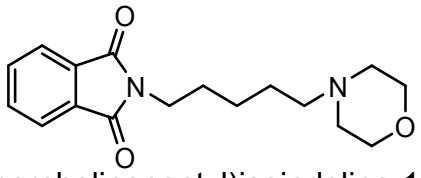
1.1.5.4 Synthesis of PHC5Br:



2-(5-bromopentyl)isoindoline-1,3-dione
(PHC5Br)

2.8 g (19.14 mmol) of phthalimide, 5.20 mL (38.28 mmol) 1,4-dibromopantane, 2.76 g (20 mmol) K_2CO_3, and 50 mL dry MeCN were used as a reaction mixture and after purification 4.5 g of pure white solid product was obtained 81 % yield. ^{1}H NMR (400 MHz, Chloroform-d) *δ* 7.84 (dd, J = 5.4, 3.1 Hz, 2H), 7.71 (dd, J = 5.5, 3.0 Hz, 2H), 3.70 (t, J = 7.2 Hz, 2H), 3.40 (t, J = 6.8 Hz, 2H), 1.95 – 1.87 (m, 2H), 1.76 – 1.67 (m, 2H), 1.54 – 1.46 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 168.33, 133.83, 132.07, 123.09, 37.82, 33.38, 32.19, 27.73, 25.39. ESI-HRMS M/z [M+H$^+$] calculated for $C_{13}H_{15}BrNO_2$ is 296.0286 Da and found 296.0269 Da.

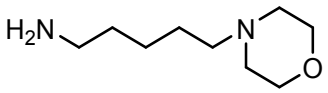
1.1.5.5 Synthesis of PHC5M:



2-(5-morpholinopentyl)isoindoline-1,3-dione
(PHC5M)

6.3218 g (21.35 mmol) of 2-(5-bromopentyl)isoindoline-1,3-dione, 2.78 mL (32.02 mmol) morpholine, 3.68 g (26.68 mmol) K_2CO_3, and 15 mL dry DMF were used as a reaction mixture and after purification 4 g of pure white solid product was obtained 62.50 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 7.82 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.69 (dd, *J* = 5.4, 3.0 Hz, 2H), 3.67 (dd, *J* = 6.2, 2.9 Hz, 6H), 2.39 (t, *J* = 4.7 Hz, 4H), 2.32 – 2.27 (m, 2H), 1.68 (m, *J* = 7.5 Hz, 2H), 1.52 (m, *J* = 7.5 Hz, 2H), 1.35 (m, *J* = 7.5, 6.9, 3.4 Hz, 2H).^{13}C NMR (101 MHz, $CDCl_3$) *δ* 168.33, 133.83, 132.07, 123.09, 66.87, 58.74, 53.68, 37.82, 28.37, 25.94, 24.62. ESI-HRMS M/z [M+H$^+$] calculated for $C_{17}H_{23}N_2O_3$ is 303.1709 Da and found 303.1695 Da.

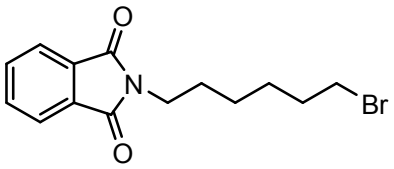
1.1.5.6 Synthesis of 5-morpholinopentan-1-amine:



5-morpholinopentan-1-amine

3 g (9.93 mmol) of 2-(4-morpholinobutyl) isoindoline-1,3-dione, 1.2 mL (24.82 mmol) hydrazine monohydrate, and 20 mL ethanol were used as a reaction mixture and after purification a viscous yellow liquid was obtained quantitative amount which was directly used for the next step without further purification.

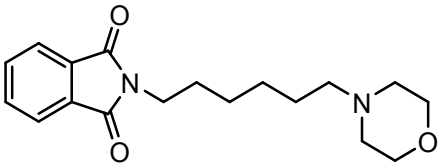
1.1.5.7 Synthesis of PHC6Br:



2-(6-bromohexyl)isoindoline-1,3-dione
(PHC6Br)

5 g (34 mmol) of phthalimide, 10.5 mL (68 mmol) 1,6-dibromohexane, and 4.92 mg (35.7 mmol) K_2CO_3, and 75 mL dry MeCN were used as a reaction mixture and after purification 8.54 g of pure white solid product was obtained 81 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 7.83 (dd, *J* = 5.4, 3.0 Hz, 2H), 7.70 (dd, *J* = 5.4, 3.1 Hz, 2H), 3.68 (t, *J* = 7.2 Hz, 2H), 3.39 (t, *J* = 6.8 Hz, 2H), 1.85 (m, *J* = 7.0 Hz, 2H), 1.69 (m, *J* = 7.4 Hz, 2H), 1.54 – 1.43 (m, 2H), 1.36 (m, *J* = 7.8, 7.4, 3.3 Hz, 2H) ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 168.44, 133.89, 132.13, 123.19, 37.84, 33.69, 32.61, 28.42, 27.72, 26.03. ESI-HRMS M/z [M+H$^+$] calculated for $C_{14}H_{17}BrNO_2$ is 310.0443 Da and found 310.0422 Da.

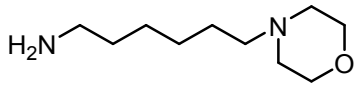
1.1.5.8 Synthesis of PHC6M:



2-(6-morpholinohexyl)isoindoline-1,3-dione
(PHC6M)

3 g (9.67 mmol) of 2-(5-morpholinobutyl) isoindoline-1,3-dione, 1.25 mL (14.5 mmol) morpholine, 1.66 g (12.08 mmol) K_2CO_3, and 15mL dry DMF were used as a reaction mixture and after purification 2.7 g of brownish white solid product was obtained 88 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 7.85 (dt, *J* = 5.6, 2.6 Hz, 2H), 7.72 (dd, *J* = 4.9, 2.6 Hz, 2H), 3.75 – 3.64 (m, 6H), 2.44 (q, *J* = 4.5 Hz, 4H), 2.32 (m, *J* = 7.8, 5.0, 3.0 Hz, 2H), 1.74 – 1.63 (m, 2H), 1.49 (q, *J* = 6.8 Hz, 2H), 1.38 (d, *J* = 6.1 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 168.46, 133.88, 132.13, 123.17, 66.96, 58.99, 53.74, 37.94, 28.52, 27.03, 26.76, 26.37. ESI-HRMS M/z [M+H$^+$] calculated for $C_{18}H_{25}N_2O_3$ is 317.1865 Da and found 317.1885 Da.

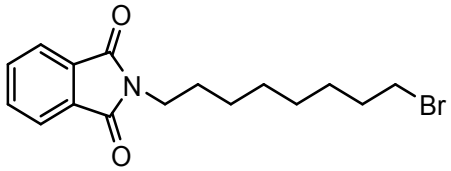
1.1.5.9 Synthesis of 6-morpholinohexan-1-amine:



6-morpholinohexan-1-amine

2.5 g (7.9 mmol) of 2-(4-morpholinobutyl) isoindoline-1,3-dione, 0.978 mL (19.75 mmol) hydrazine monohydrate, and 20 mL ethanol were used as a reaction mixture and after purification a viscous yellow liquid was obtained quantitative amount which was directly used for the next without further purification.

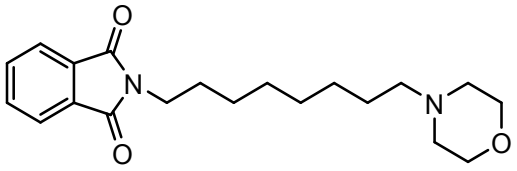
1.1.5.10 Synthesis of PHC8Br:



2-(8-bromooctyl)isoindoline-1,3-dione
(PHC8Br)

2.5 g (17 mmol) of phthalimide, 9.24 g (34 mmol) 1,8-dibromooctane, and 2.6 g (17.8 mmol) K_2CO_3, and 50 mL dry MeCN were used as a reaction mixture and after purification 4.5 g of pure white solid product was obtained 78 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 7.83 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.70 (dd, *J* = 5.5, 3.0 Hz, 2H), 3.67 (t, *J* = 7.3 Hz, 2H), 3.38 (t, *J* = 6.8 Hz, 2H), 1.83 (m, *J* = 7.0 Hz, 2H), 1.66 (d, *J* = 8.3 Hz, 2H), 1.44 – 1.29 (m, 8H) ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 168.45, 133.85, 132.17, 123.15, 37.98, 33.93, 32.73, 28.94, 28.58, 28.52, 28.05, 26.71. ESI-HRMS M/z [M+H$^+$] calculated for $C_{16}H_{21}BrNO_2$ is 338.0756 Da and found 338.0750 Da.

1.1.5.11 Synthesis of PHC8M:

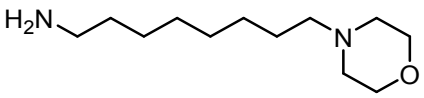


2-(8-morpholinooctyl)isoindoline-1,3-dione
(PHC8M)

3.8 g (11.23 mmol) of 2-(8-bromooctyl)isoindoline-1,3-dione, 1.45 mL (16.84 mmol) morpholine, and 1.93 g (14.03 mmol) K_2CO_3, and 15 mL dry DMF were used as a reaction mixture and after purification 3.42 g of pure white solid product was obtained 88 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 7.82 (dd, *J* = 7.4, 3.7 Hz, 2H), 7.73 – 7.67 (dd, 2H), 4.10 (broad s, 4H), 3.66 (t,

J = 7.2 Hz, 2H), 3.39 – 2.78 (m, 6H), 1.86 (m, 2H), 1.71 – 1.57 (m, 2H), 1.40-1.30 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 168.46, 133.90, 132.13, 123.17, 63.82, 58.02, 51.93, 37.83, 28.73, 28.68, 28.40, 26.58, 26.52, 23.21. ESI-HRMS M/z [M+H$^+$] calculated for $C_{20}H_{29}N_2O_3$ is 345.2178 Da and found 345.2195 Da.

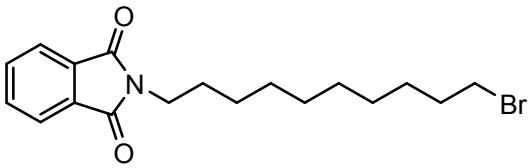
1.1.5.12 Synthesis of 8-morpholinooctan-1-amine:



8-morpholinooctan-1-amine

3 g (8.7 mmol) of 2-(4-morpholinobutyl) isoindoline-1,3-dione, 1.08 mL (21.75 mmol) hydrazine monohydrate, and 20 mL ethanol were used as a reaction mixture and after purification a viscous yellow liquid was obtained quantitative amount which was directly used for the next without further purification.

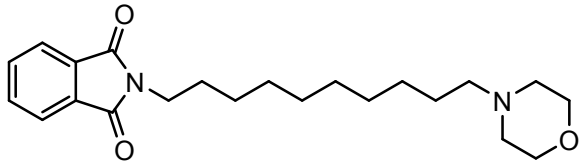
1.1.5.13 Synthesis of PHC10Br:



2-(10-bromodecyl)isoindoline-1,3-dione
(PHC10Br)

2.5 g (17 mmol) of phthalimide, 7.66 mL (34 mmol) 1,10-dibromodecane, and 2.4 g (17.8 mmol) K_2CO_3, and 50 mL dry MeCN were used as a reaction mixture and after purification 5.7 g of pure white solid product was obtained 91 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 7.84 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.70 (dd, *J* = 5.5, 3.0 Hz, 2H), 3.67 (t, *J* = 7.3 Hz, 2H), 3.40 (m, *J* = 6.9, 3.9 Hz, 2H), 1.90 – 1.79 (m, 2H), 1.66 (m, *J* = 7.4 Hz, 2H), 1.40 (m, *J* = 7.1 Hz, 4H), 1.35-1.28 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 168.43, 133.82, 132.18, 123.13, 38.03, 34.01, 32.82, 29.33, 29.31, 29.10, 28.70, 28.56, 28.13, 26.81. ESI-HRMS M/z [M+H$^+$] calculated for $C_{18}H_{25}BrNO_2$ is 366.1069 Da and found 366.1072 Da.

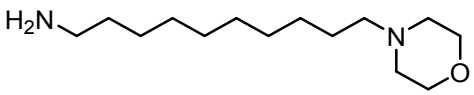
1.1.5.14 Synthesis of PHC10M:



2-(10-morpholinodecyl)isoindoline-1,3-dione
(PHC10M)

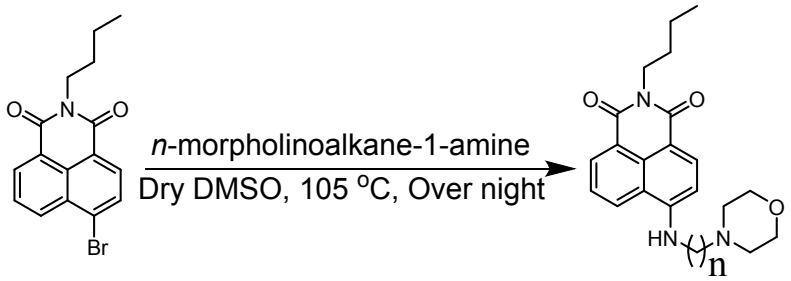
3.6 g (9.8 mmol) of 2-(10-bromodecyl)isoindoline-1,3-dione, 1. 28 mL (14.7 mmol) morpholine, and 1.7 g (12.25) mmol) K_2CO_3, and 15 mL dry DMF were used as a reaction mixture and after purification 3.03 g of pure white solid product was obtained 83 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 7.84 (dd, *J* = 5.4, 3.1 Hz, 2H), 7.70 (dd, *J* = 5.5, 3.1 Hz, 2H), 3.74 – 3.64 (m, 6H), 2.42 (t, *J* = 4.7 Hz, 4H), 2.33 – 2.26 (m, 2H), 1.46 (t, *J* = 7.6 Hz, 2H), 1.29 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 168.42, 133.81, 132.18, 123.12, 66.99, 59.22, 53.79, 38.05, 29.49, 29.45, 29.40, 29.14, 28.58, 27.48, 26.84, 26.53. ESI-HRMS M/z [M+H$^+$] calculated for $C_{22}H_{33}N_2O_3$ is 373.2491 Da and found 373.2462 Da.

1.1.5.15 Synthesis of 10-morpholinodecan-1-amine:



10-morpholinodecan-1-amine

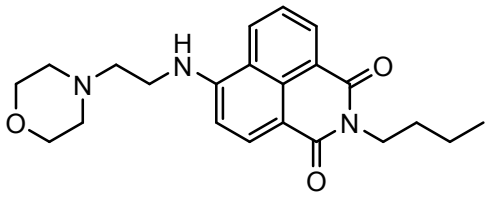
2.5 g (3.72 mmol) of 2-(4-morpholinobutyl) isoindoline-1,3-dione, 0.84 mL (16.8 mmol) hydrazine monohydrate, and 20 mL ethanol were used as a reaction mixture and after purification a viscous yellow liquid was obtained quantitative amount which was directly used for the next without further purification.



NICBr

NIMCn
n= 2, 3, 4, 5, 6, 8, 10

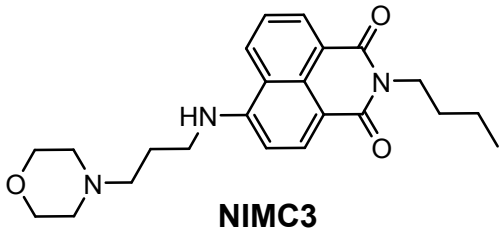
1.1.5.16 Synthesis of NIMC2:



NIMC2

64 mg (0.19 mmol) of 6-bromo-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione, 0.13 mL (0.98 mmol) 2-morpholinoethyl-1-amine, and 1.5 mL dry DMSO was used as reaction mixture after purification 41.8 mg yellow pure product was obtained, 57 % yield ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 8.63 (d, *J* = 7.2 Hz, 1H), 8.49 (d, *J* = 8.4 Hz, 1H), 8.12 (d, *J* = 8.3 Hz, 1H), 7.68 (dd, *J* = 8.4, 7.3 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 1H), 6.27 (s, 1H), 4.22 – 4.16 (m, 2H), 3.81 (t, *J* = 4.6 Hz, 4H), 3.48 – 3.43 (m, 2H), 2.86 (t, *J* = 5.9 Hz, 2H), 2.60 (s, 4H), 1.74 (m, *J* = 7.7 Hz, 2H), 1.47 (m, *J* = 7.4 Hz, 2H), 0.99 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 164.69, 164.17, 149.34, 134.47, 131.10, 129.77, 125.87, 124.82, 123.24, 120.40, 110.49, 104.53, 77.27, 67.09, 55.98, 53.11, 40.00, 38.96, 30.33, 20.45, 13.90. APCI-HRMS M/z [M+H^{+}] calculated for $C_{22}H_{28}N_3O_3$ is 382.2131 Da and found 382.2139 Da.

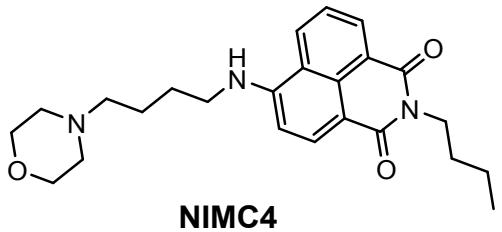
1.1.5.17 Synthesis of NIMC3:



NIMC3

50 mg (0.15 mmol) of 6-bromo-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione, 0.11 mL (0.75 mmol) 3-morpholinoproply-1-amine, and 1.5 ml dry DMSO was used as reaction mixture after purification 32.7 mg yellow pure product was obtained, 55 % yield ^{1}H NMR (400 MHz, Chloroform-*d*) *δ* 8.59 (d, *J* = 7.3 Hz, 1H), 8.45 (d, *J* = 8.5 Hz, 1H), 8.27 (d, *J* = 8.4 Hz, 1H), 7.63 (t, *J* = 7.7 Hz, 2H), 6.64 (d, *J* = 8.5 Hz, 1H), 4.16 (t, *J* = 7.7 Hz, 2H), 3.88 (m, *J* = 4.6 Hz, 4H), 3.50 (d, *J* = 5.2 Hz, 2H), 2.70 – 2.65 (m, 2H), 2.61 (s, 4H), 2.01 (m, *J* = 5.8 Hz, 2H), 1.71 (m, *J* = 7.7 Hz, 2H), 1.45 (m, *J* = 7.6 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 164.74, 164.20, 150.32, 134.68, 131.01, 129.84, 126.70, 124.21, 123.20, 120.48, 109.78, 103.75, 66.99, 59.13, 54.08, 44.69, 39.97, 30.35, 23.23, 22.41, 20.46, 13.91. APCI-HRMS M/z [M+H^{+}] calculated for $C_{23}H_{30}N_3O_3$ is 396.2287 Da and found 396.2308 Da.

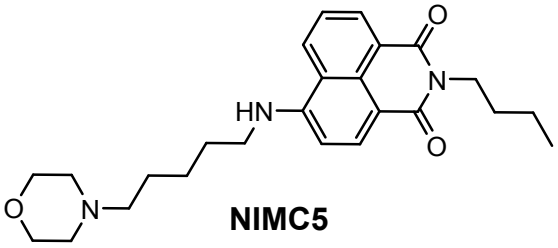
1.1.5.18 Synthesis of NIMC4:



NIMC4

40 mg (0.12 mmol) of 6-bromo-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione, 94 mg (0.60 mmol) 4-morpholinobutyl-1-amine, and 1.5 mL dry DMSO was used as reaction mixture after purification 30 mg yellow pure product was obtained, 60.9 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.61 (d, *J* = 7.2 Hz, 1H), 8.49 (d, *J* = 8.4 Hz, 1H), 8.16 (d, *J* = 8.2 Hz, 1H), 7.67 – 7.62 (m, 1H), 6.74 (d, *J* = 8.4 Hz, 1H), 5.65 (s, 1H), 4.22 – 4.15 (m, 2H), 3.80 (s, 4H), 3.50 – 3.43 (m, 2H), 2.54 (s, 6H), 1.90 (q, *J* = 7.0 Hz, 2H), 1.74 (m, *J* = 15.3, 8.9, 7.9 Hz, 4H), 1.47 (m, *J* = 7.3 Hz, 2H), 0.99 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.67, 164.15, 149.37, 134.43, 131.07, 129.80, 125.84, 124.57, 123.28, 120.23, 110.37, 104.30, 66.95, 58.11, 53.74, 43.62, 40.01, 30.33, 26.47, 24.31, 22.34, 20.45, 13.90. APCI-HRMS M/z [M+H^{+}] calculated for $C_{24}H_{32}N_3O_3$ is 410.2444 Da and found 410.2461 Da.

1.1.5.19 Synthesis of NIMC5:

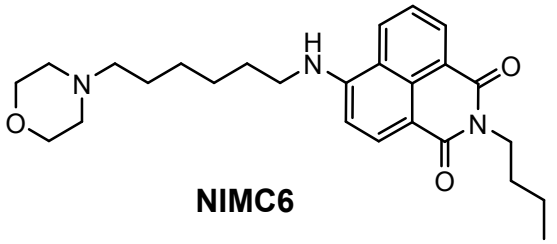


NIMC5

50 mg (0.15 mmol) of 6-bromo-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione, 129 mg (0.75 mmol) 5-morpholinopentyl-1-amine, and 1.5 mL dry DMSO was used as reaction mixture after purification 40 mg yellow pure product was obtained, 63 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.59 (dd, *J* = 7.3, 1.1 Hz, 1H), 8.47 (d, *J* = 8.4 Hz, 1H), 8.10 (d, *J* = 8.2 Hz, 1H), 7.62 (dd, *J* = 8.4, 7.3 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 5.28 (broad s, 1H), 4.19 – 4.13 (t, 2H), 3.75 (m, 4H), 3.43 (q, *J* = 6.6 Hz, 2H), 2.47 (Broad, *J* = 26.8 Hz, 6H), 1.84 (m, *J* = 7.3 Hz, 2H), 1.71 (m, *J* = 7.6 Hz, 2H), 1.44 (m, *J* = 14.9, 7.4 Hz, 4H), 0.97 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.67, 164.14, 149.25, 134.39, 131.07, 129.80, 125.62, 124.70, 123.32, 120.19, 110.50,

104.34, 66.96, 58.79, 53.80, 43.61, 40.00, 34.13, 30.33, 28.89, 25.00, 20.45, 14.06. APCI-HRMS M/z [M+H$^+$] calculated for $C_{25}H_{34}N_3O_3$ is 424.2600 Da and found 424.2607 Da.

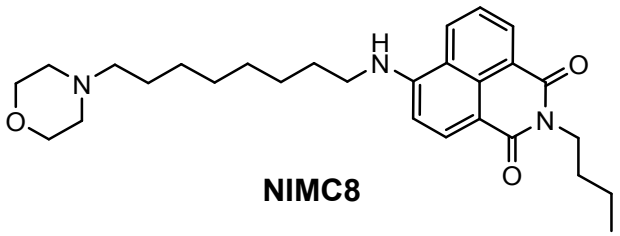
1.1.5.20 Synthesis of NIMC6:



NIMC6

75 mg (0.22 mmol) of 6-bromo-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione, 210 mg (1.12 mmol) 6-morpholinohexyl-1-amine, and 1.5 mL dry DMSO was used as reaction mixture after purification 38.8 mg yellow pure product was obtained, 59 % yield. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.61 (dd, *J* = 7.3, 1.1 Hz, 1H), 8.49 (d, *J* = 8.4 Hz, 1H), 8.11 (d, *J* = 8.4 Hz, 1H), 7.65 (dd, *J* = 8.4, 7.3 Hz, 1H), 6.75 (d, *J* = 8.4 Hz, 1H), 5.27 (s, 1H), 4.21 – 4.17 (m, 2H), 3.77 (s, 4H), 3.44 (q, *J* = 6.7 Hz, 2H), 2.47 (d, *J* = 42.3 Hz, 6H), 1.85 (m, *J* = 7.4 Hz, 2H), 1.77 – 1.69 (m, 2H), 1.51 – 1.42 (m, 5H), 0.99 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 164.68, 164.16, 149.33, 134.42, 131.07, 129.80, 125.69, 124.68, 123.26, 120.16, 110.40, 104.32, 66.80, 58.89, 53.69, 43.64, 40.00, 30.33, 28.92, 27.18, 27.06, 26.27, 20.46, 13.91. APCI-HRMS M/z [M+H$^+$] calculated for $C_{26}H_{36}N_3O_3$ is 438.2757 Da and found 438.2735 Da.

1.1.5.21 Synthesis of NIMC8:

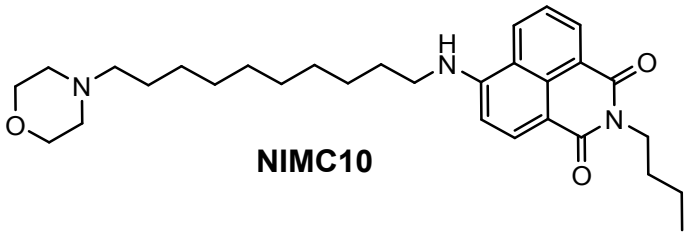


NIMC8

100 mg (0.30 mmol) of 6-bromo-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione, 316 mg (1.5 mmol) 8-morpholinooctyl-1-amine, and 1.5 mL dry DMSO was used as reaction mixture after purification 68.6 mg yellow pure product was obtained, 49 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.61 – 8.56 (m, 1H), 8.47 (d, *J* = 8.4 Hz, 1H), 8.07 (d, *J* = 8.5 Hz, 1H), 7.65 – 7.58 (m, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 5.20 (s, 1H), 4.20 – 4.12 (m, 2H), 3.72 (t, *J* = 4.7 Hz, 4H), 3.40 (dd, *J* = 6.6 Hz, 2H), 2.44 (s, 4H), 2.33 (t, *J* = 7.8 Hz, 2H), 1.80 (dd, *J* = 7.2 Hz, 2H), 1.71 (m, *J* = 7.6 Hz, 2H), 1.52 – 1.32 (m, 6H), 0.97 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 164.68, 164.15, 149.35, 134.42, 131.05, 129.80, 125.66, 124.65, 123.27, 120.16, 110.35, 104.32, 66.93, 59.15, 53.77, 43.72, 39.99, 30.33, 29.46, 29.30, 29.01, 27.43, 27.13, 26.48,

20.45, 13.90. APCI-HRMS M/z [M+H$^+$] calculated for $C_{28}H_{40}N_3O_3$ is 466.3070 Da and found 466.3039 Da.

1.1.5.22 Synthesis of NIMC10:



NIMC10

75 mg (0.22 mmol) of 6-bromo-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione, 275 mg (1.12 mmol) 10-morpholinodecyl-1-amine, and 1.5 mL dry DMSO was used as reaction mixture after purification 51.2 mg yellow pure product was obtained, 46 % yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.59 (dd, *J* = 7.4, 1.1 Hz, 1H), 8.47 (d, *J* = 8.4 Hz, 1H), 8.07 (d, *J* = 8.3 Hz, 1H), 7.62 (dd, *J* = 8.4, 7.3 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 5.20 (s, 1H), 4.19 – 4.13 (m, 2H), 3.72 (t, *J* = 4.7 Hz, 4H), 3.40 (q, *J* = 6.5 Hz, 2H), 2.43 (s, 4H), 2.35 – 2.29 (m, 2H), 1.81 (m, *J* = 7.3 Hz, 2H), 1.71 (m, *J* = 7.7 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 164.66, 164.18, 149.47, 134.41, 130.99, 129.76, 125.82, 124.55, 123.13, 120.14, 110.14, 104.24, 66.96, 59.20, 53.78, 43.73, 39.95, 30.33, 29.52, 29.49, 29.36, 28.96, 27.49, 27.18, 26.52, 20.44, 13.89. APCI-HRMS M/z [M+H$^+$] calculated for $C_{30}H_{44}N_3O_3$ is 494.3383 Da and found 494.3352 Da.

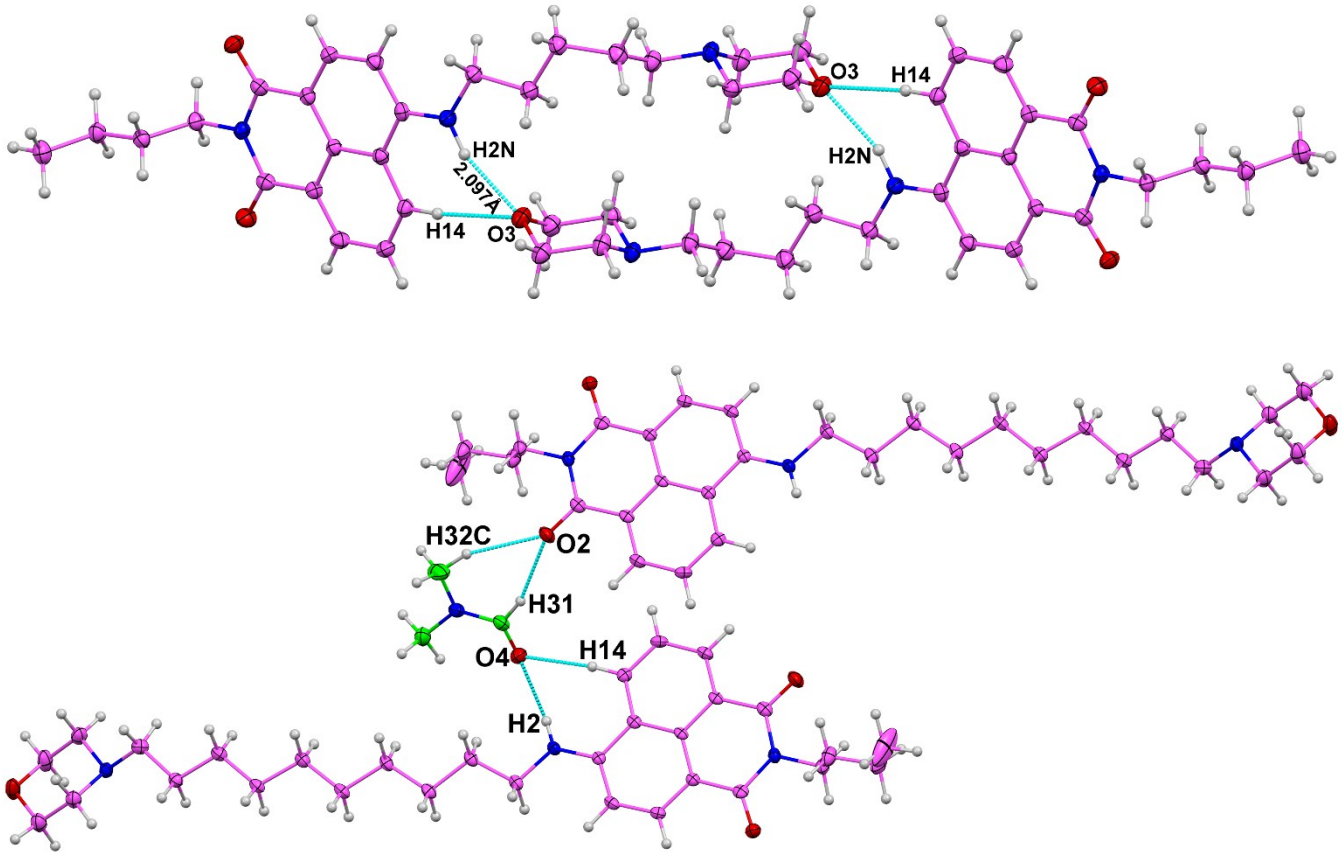


Fig. S1: Intramolecular H-bonding interactions in (a) **NIMC5** & **NIMC10**

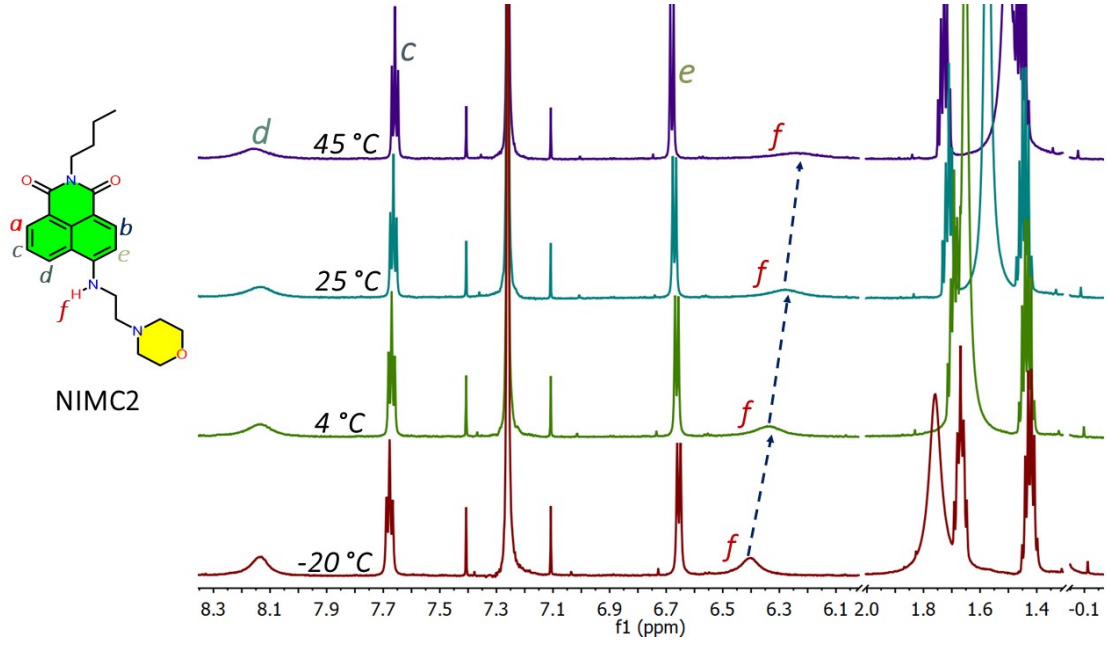


Fig. S2: Variable temperature ^{1}H-NMR of **NIMC2** in $CDCl_3$

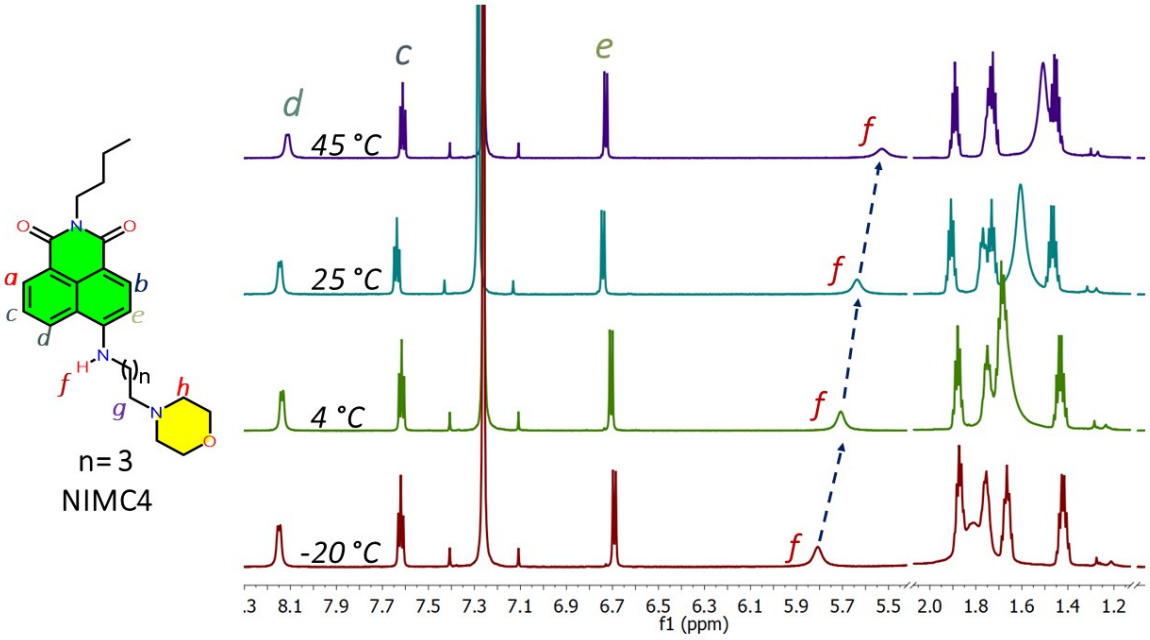


Fig. S3: Variable temperature ^{1}H-NMR of **NIMC4** in $CDCl_3$

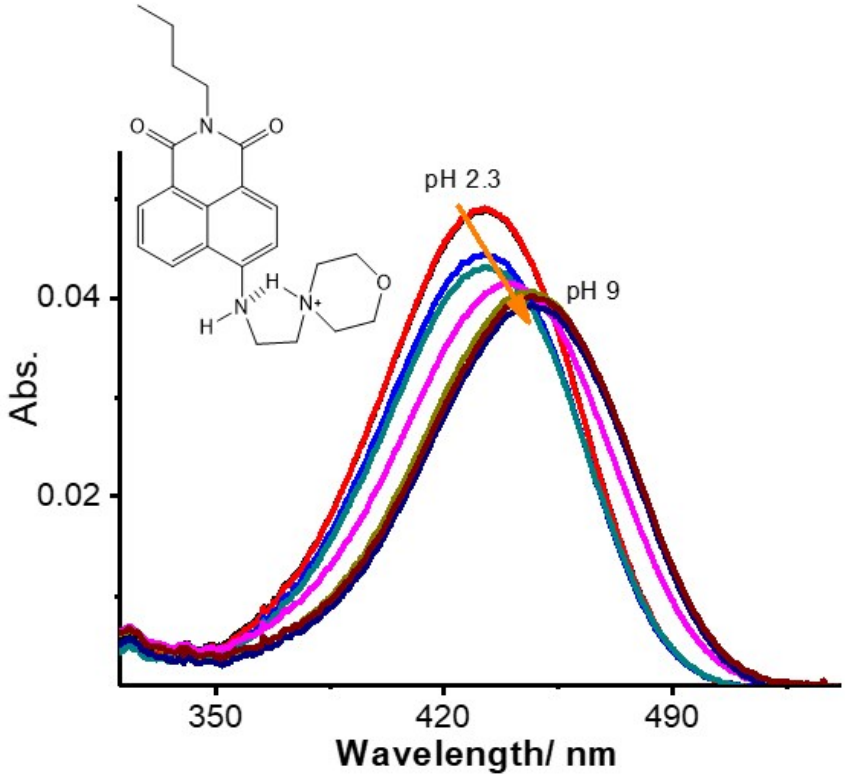


Fig. S4: Change in absorption spectra of **NIMC2** (10 μM) with the variation of pH, where with decreasing pH the absorption maxima show a blue shift, which is due the interaction of secondary amine lone pair with protonated morpholine hydrogen (inset).

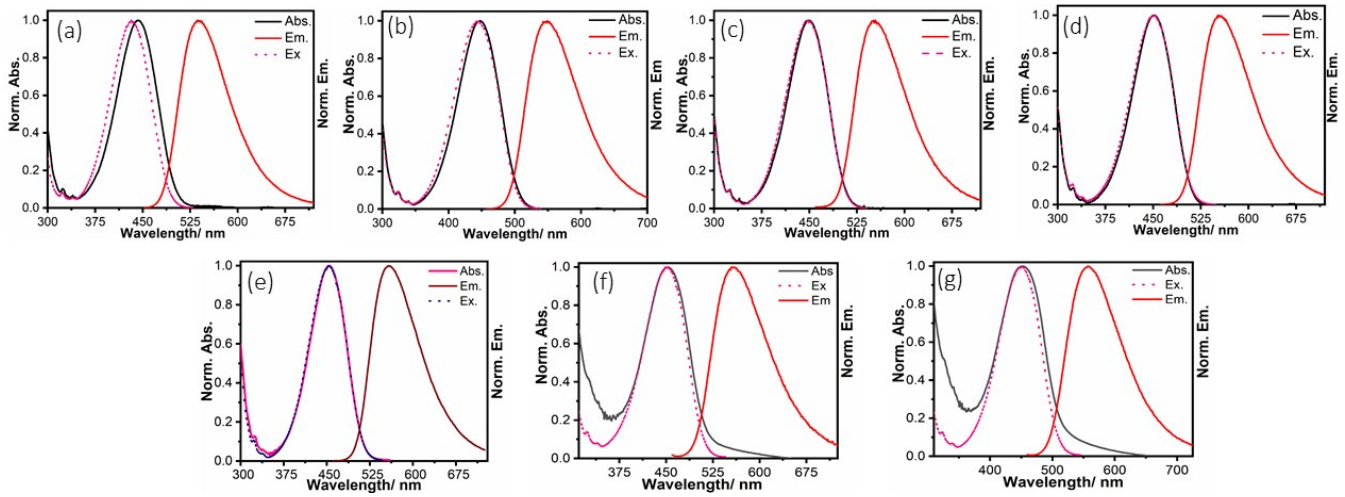


Fig. S5: Optical purity of the synthesized compound in water (pH ~6.0) (a) **NIMC2**, (b) **NIMC3**, (c) **NIMC4**, (d) **NIMC5**, (e) **NIMC6**, (f) **NIMC8**, and (f) **NIMC10**

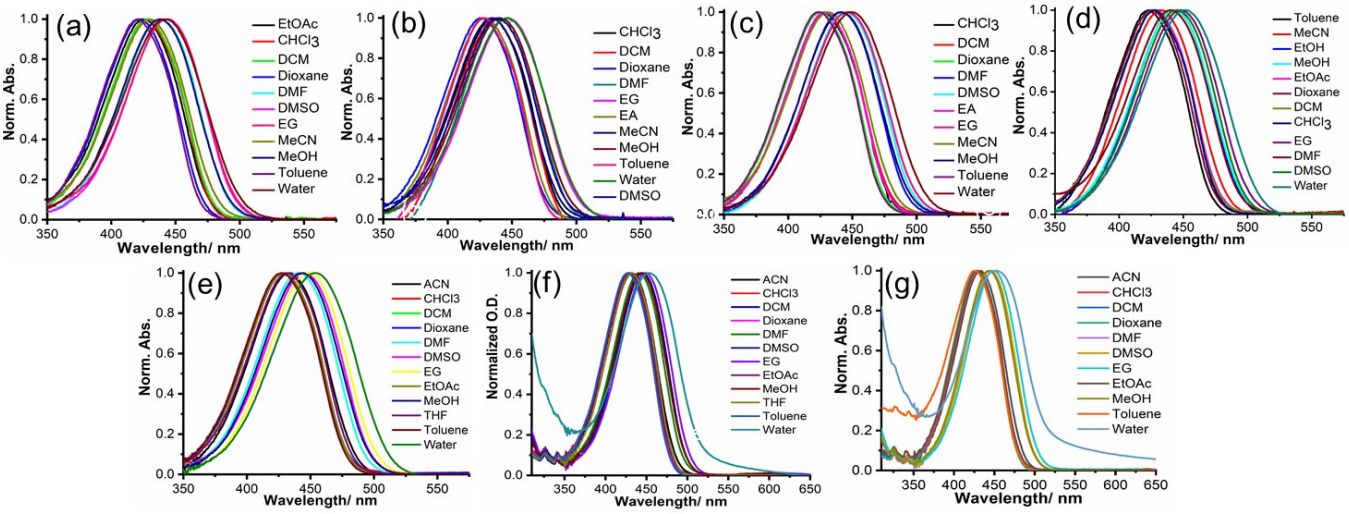


Fig. S6: Solvent-dependent absorption spectra of (a) **NIMC2**, (b) **NIMC3**, (c) **NIMC4**, (d) **NIMC5**, (e) **NIMC6**, (f) **NIMC8** and (f) **NIMC10**.

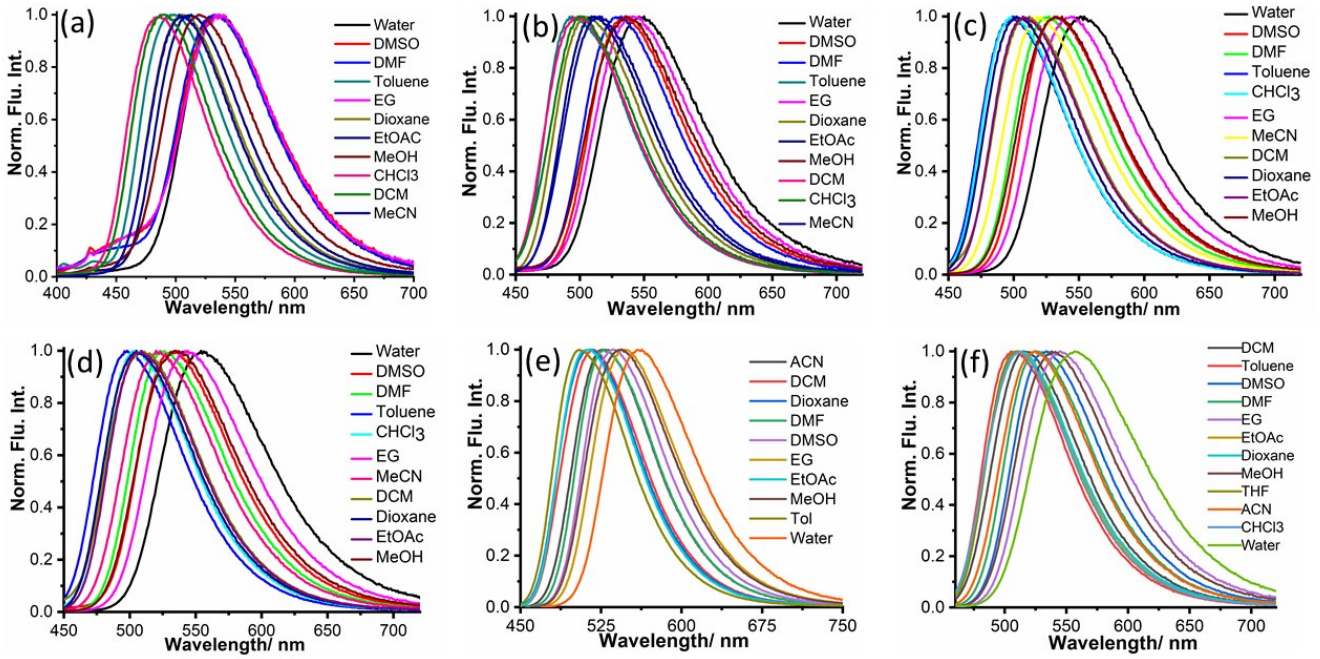


Fig. S7: Solvent-dependent emission spectra of (a) **NIMC2**, (b) **NIMC3**, (c) **NIMC4**, (d) **NIMC5**, (e) **NIMC8**, and (f) **NIMC10**.

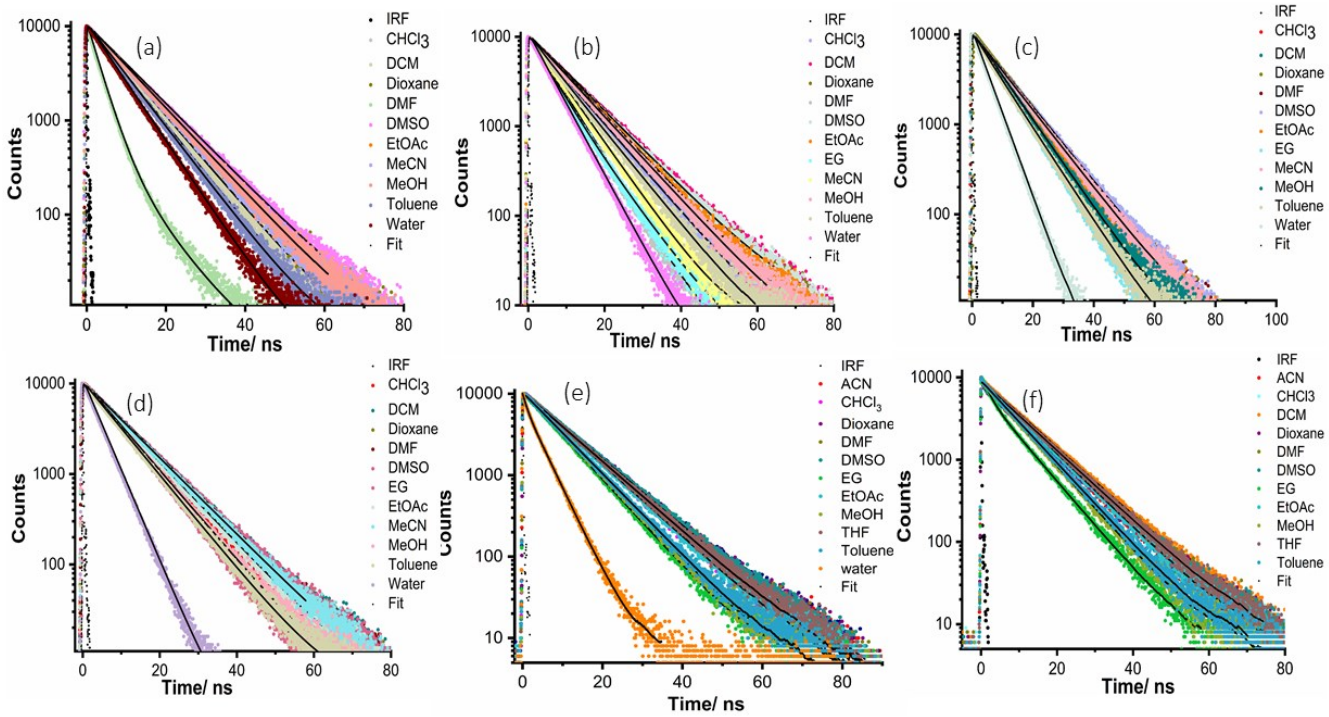


Fig. S8: Solvent-dependent emission lifetime of (a) **NIMC2**, (b) **NIMC3**, (c) **NIMC4**, (d) **NIMC5**, (e) **NIMC8**, and (f) **NIMC10**, using 440 nm LASER as excitation source.

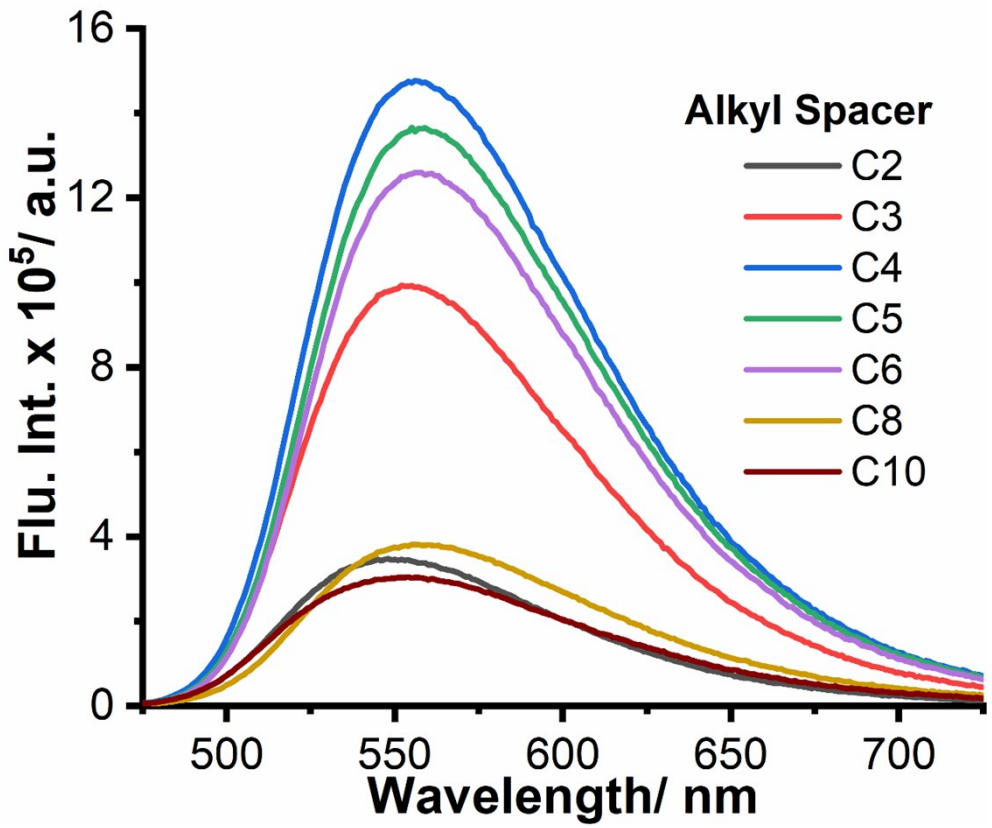


Fig. S9: Fluorescence intensity of **NIMCn** (n=2-6, 8, 10) with variation of alkyl chain length at pH 9.3 (λ_{ex}= 450 nm)

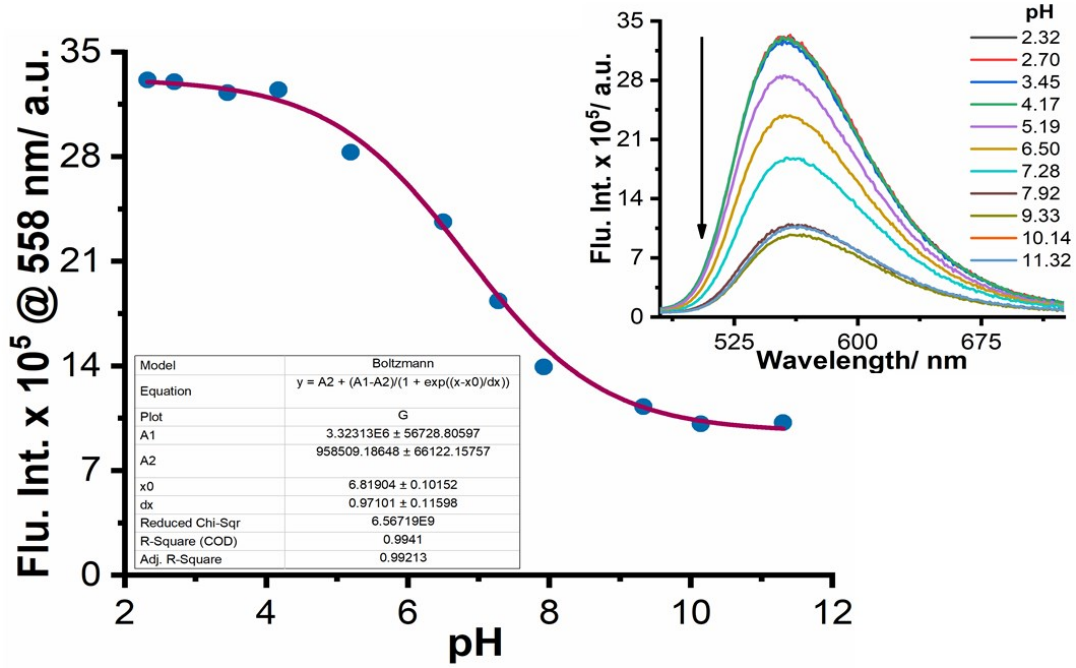


Fig. S10: Plot of pH-dependent fluorescence intensity of **NIMC3**, solid line shows the fitting using sigmoidal equation. The obtained pK_a value is 6.8±0.1. Inset shows the fluorescence spectra with the variation of pH of the solution (λ_{ex}= 450 nm).

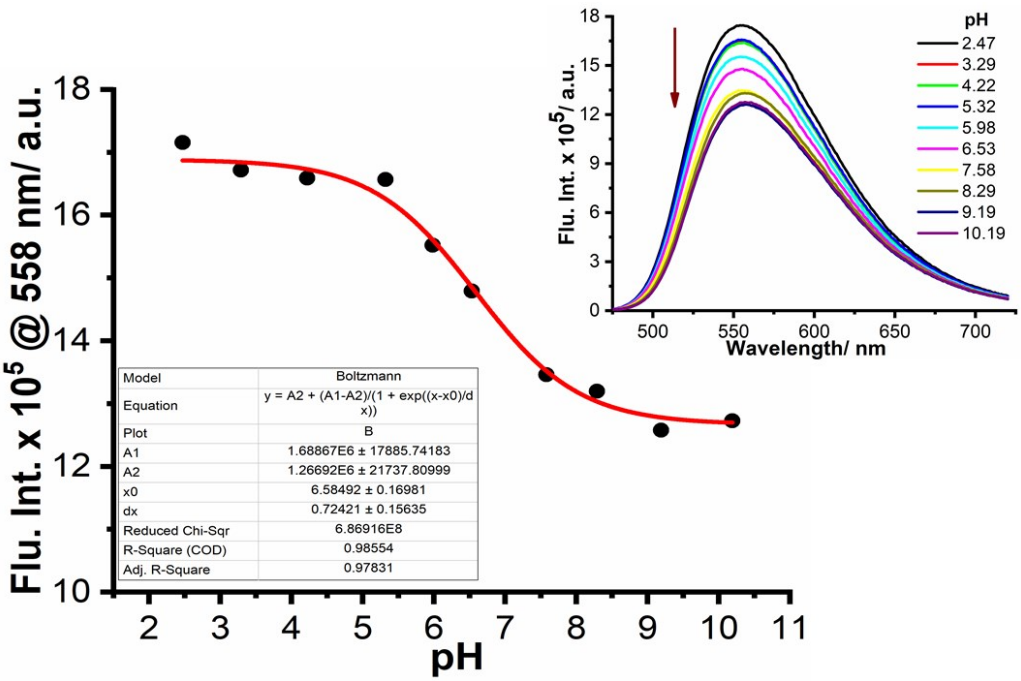


Fig. S11: Plot of pH-dependent fluorescence intensity of **NIMC4**, solid line shows the fitting using sigmoidal equation. The obtained pK_a value is 6.6±0.2. Inset shows the fluorescence spectra with the variation of pH of the solution (λ_{ex}= 450 nm).

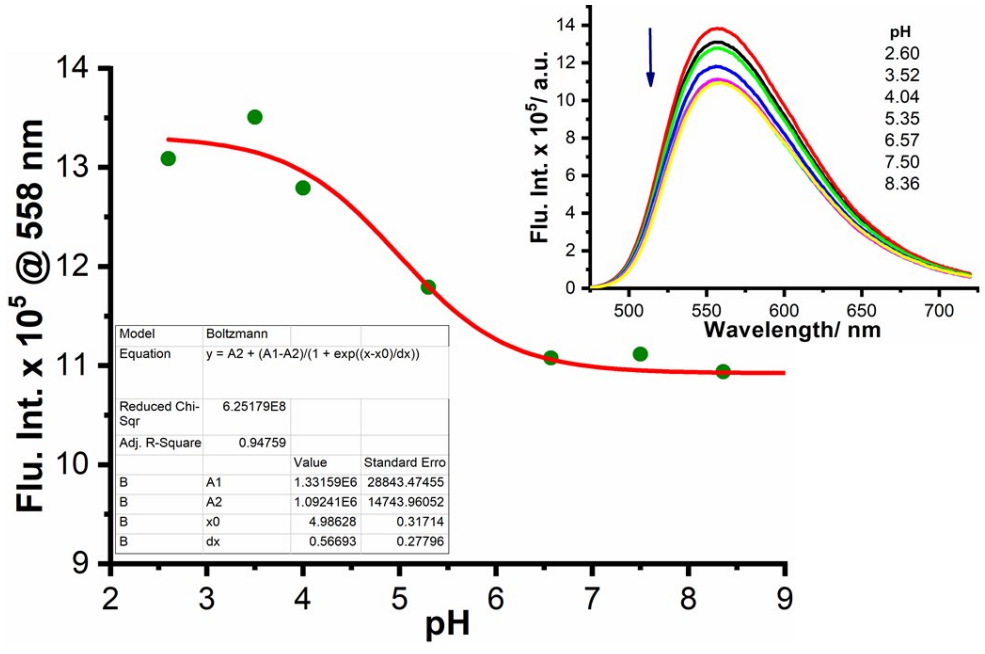


Fig. S12: Plot of pH-dependent fluorescence intensity of **NIMC5**, solid line shows the fitting using sigmoidal equation. The obtained pK_a value is 5.0±0.3. Inset shows the fluorescence spectra with the variation of pH of the solution (λ_{ex}= 450 nm).

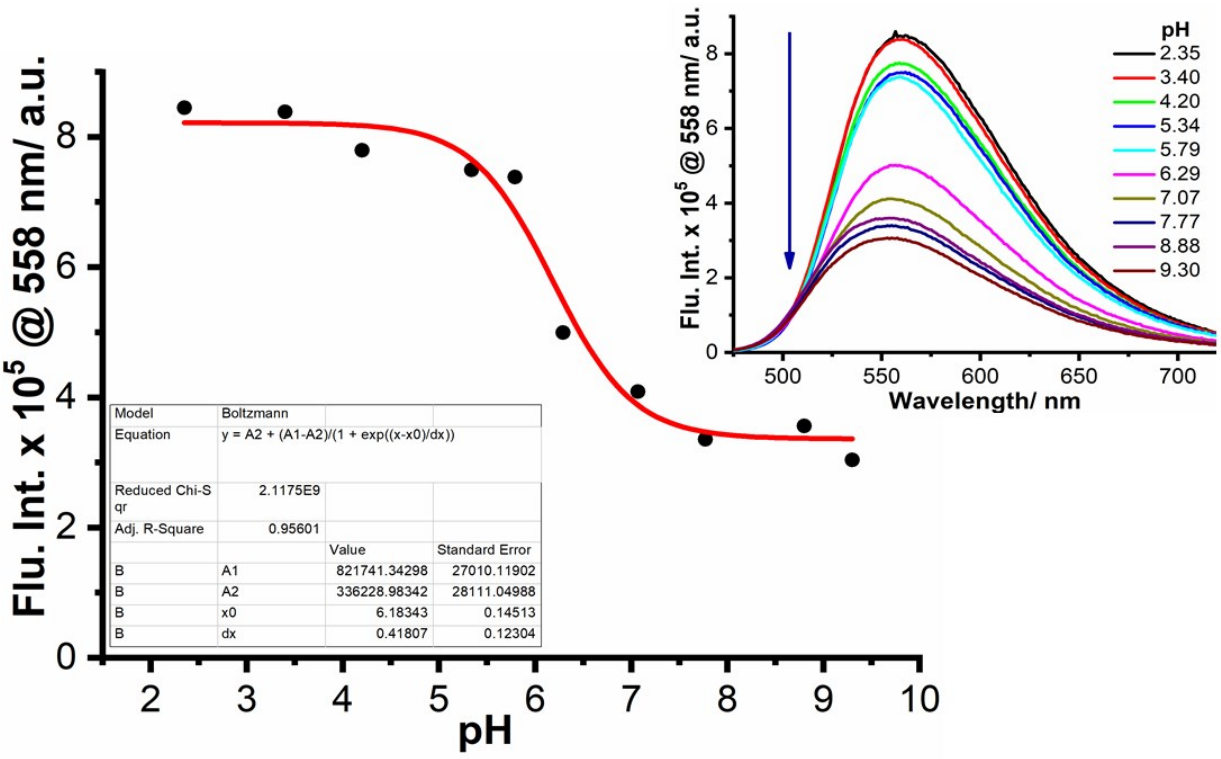


Fig. S13: Plot of pH-dependent fluorescence intensity of **NIMC10**, solid line shows the fitting using sigmoidal equation. The obtained pK_a value is 6.2±0.15. Inset shows the fluorescence spectra with the variation of pH of the solution (λ_{ex}= 450 nm).

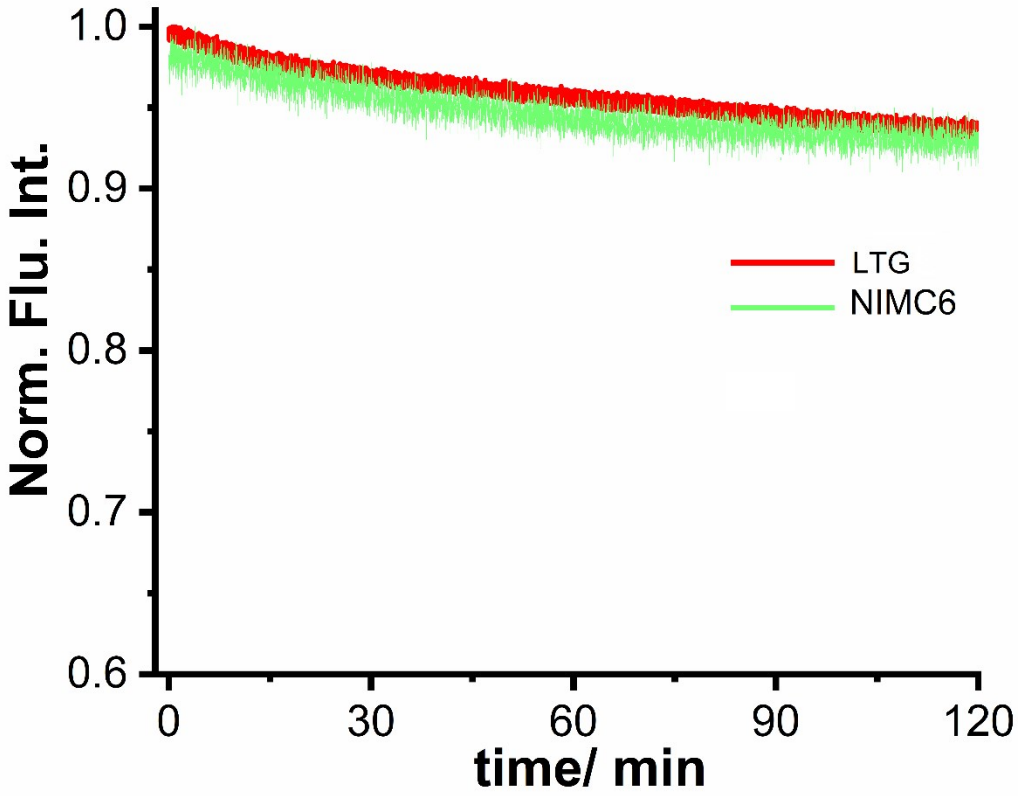


Fig. S14: Monitoring the photostability of **NIMC6** with respect to Lyso-tracker green (LTG), by exciting (λ_{ex}= 450 nm) light from 450 W Xenon lamp from Fluorolog instrument (lux).

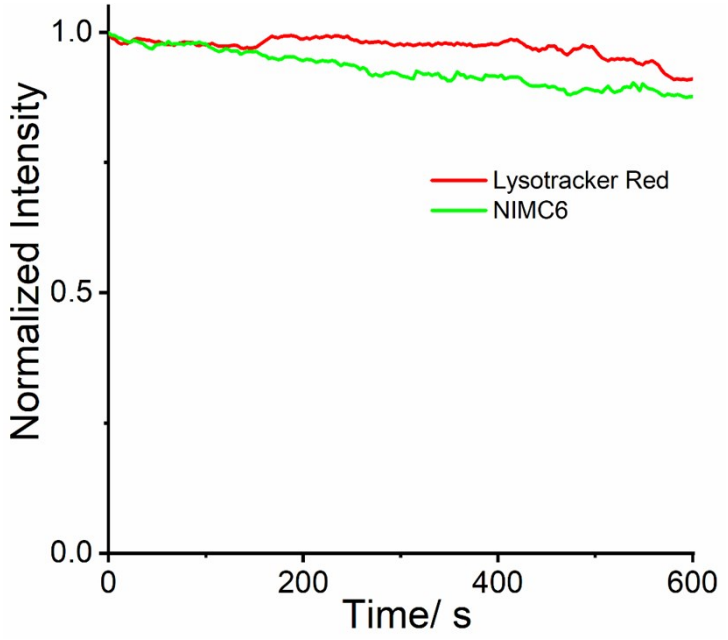


Fig. S15: Comparison of the photostability between Lysotracker red (red line) and **NIMC6** (green line) inside live BHK-21 cells.

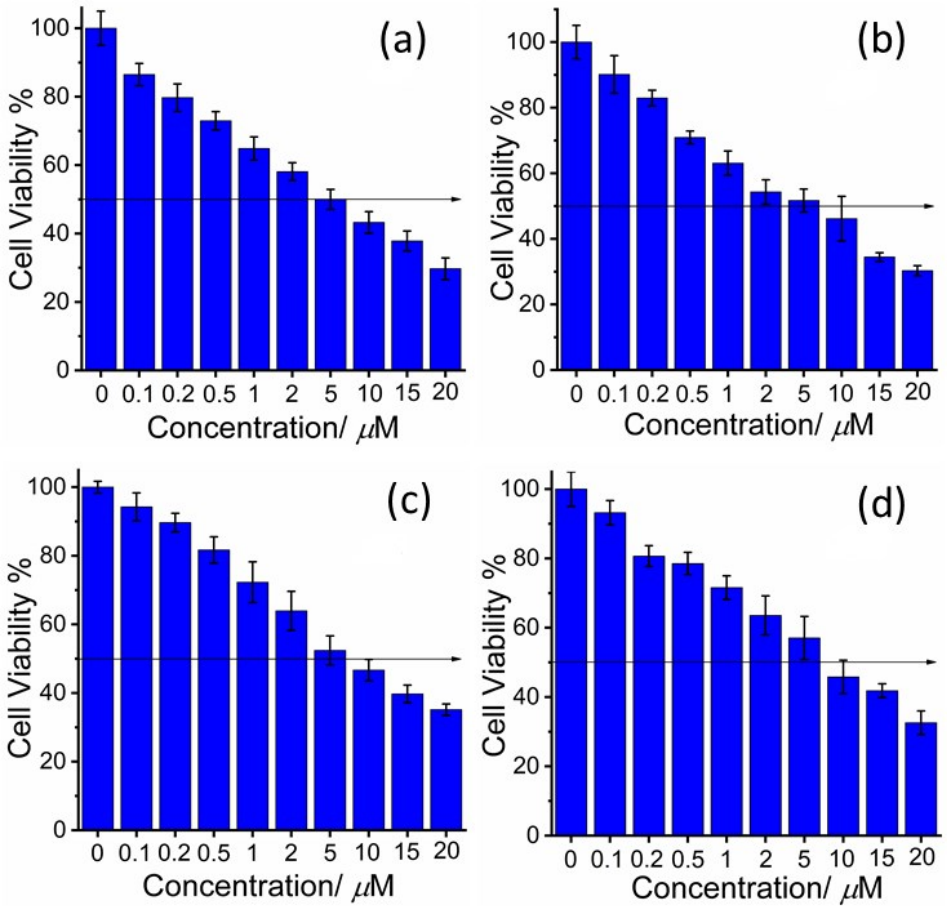


Fig. S16: Cell viability plot with increasing concentration of **NIMCn** using MTT assay of (a) **NIMC2**, (b) **NIMC4**, (c) **NIMC8**, and (d) **NIMC10** in BHK21 cells.

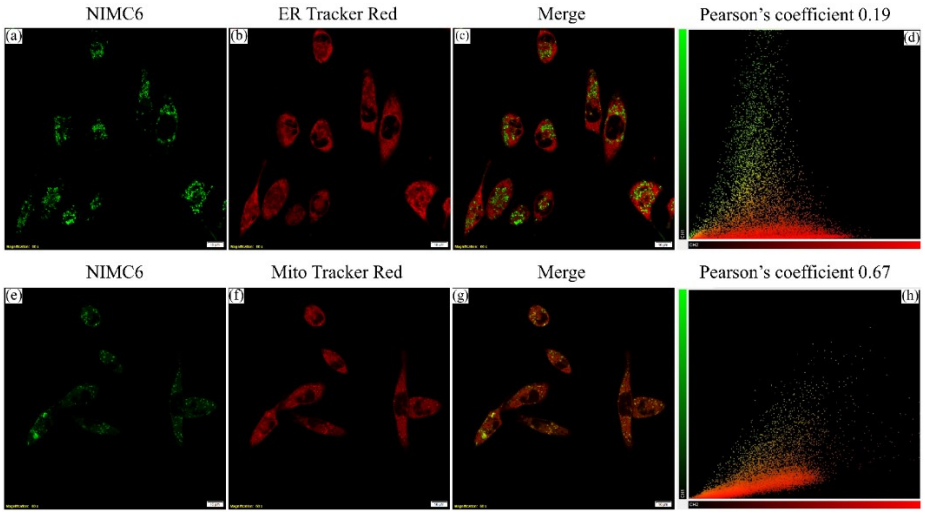


Fig. S17. CLSM images of BHK-21 cells stained with (a) 0.2 μM **NIMC6**, (b) 0.3 μM of ER-Tracker Red, (c) merge image of (a) and (b), (d) scatter plot showing Pearson's correlation coefficient of 0.19 ± 0.04, (e) 0.2 μM NIMC6, (f) 0.3 μM of Mito-Tracker Red, (g) merge image of (e) and (f), (h) scatter plot showing Pearson's correlation coefficient of 0.67 ± 0.02. (scale bar: 10 μm)

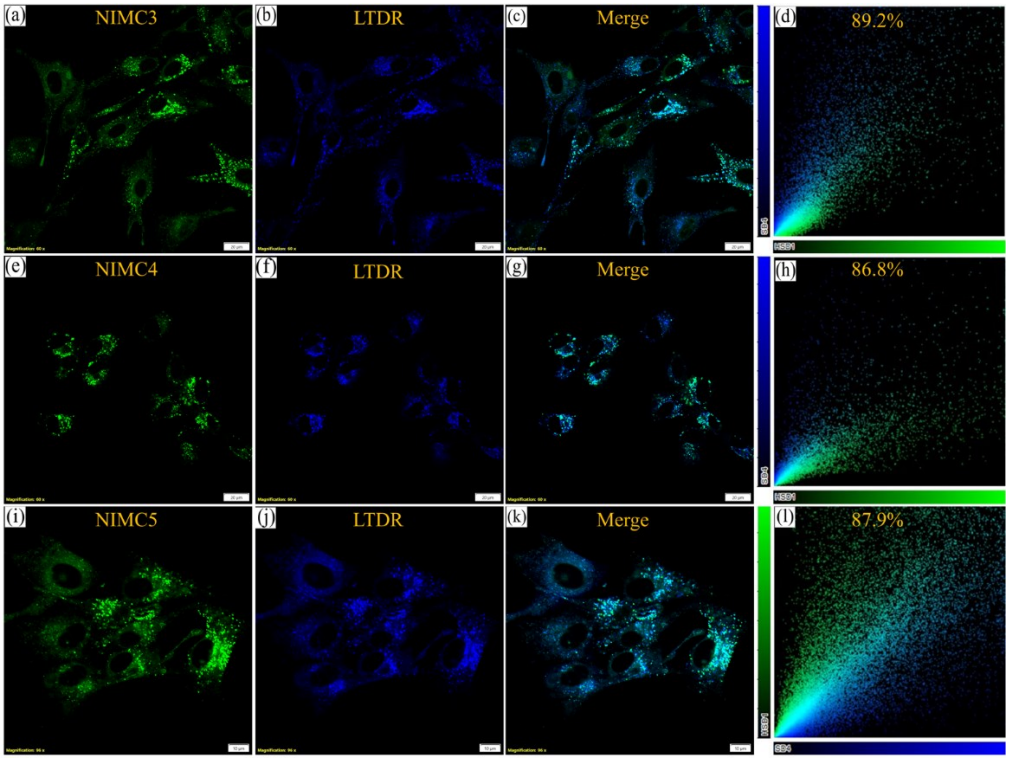


Fig. S18. CLSM images of BHK-21 cells stained with 0.2 μM (a) **NIMC3**, (e) **NIMC4** and (i) **NIMC5** where (b), (f), and (j) were treated with 0.3 μM of LysoTracker Deep Red. (c), (g) and (k) provides merge images. The scatter plots (d), (h) and (i) showing Pearson's correlation coefficient values of 0.89 ± 0.04, 0.86 ± 0.02 and 0.87 ± 0.04 for NIMC3, NIMC4, and NIMC5 respectively (scale bar: 10 μm)

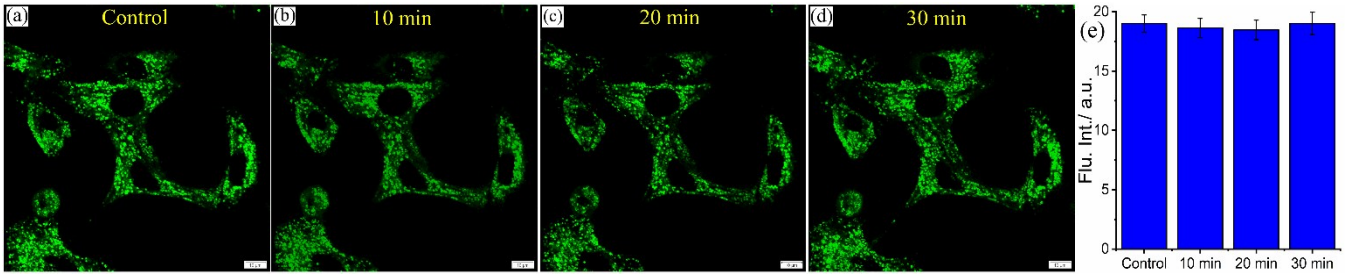


Fig. S19a. CLSM images of BHK-21 cells stained with (a) 0.2 μM **NIMC6** and stimulated with 20 μM chloroquine for (b) 10 min, (c) 20 min, and (d) 30 min; (e) shows the average fluorescence intensity in different ROIs **in same FOV** at the aforementioned time-points of the experiment. (scale bar: 10 μm) Under the stressed condition, the fluorescence intensity is intact over time indicating the pH-independency of NIMC6 in live-cell.

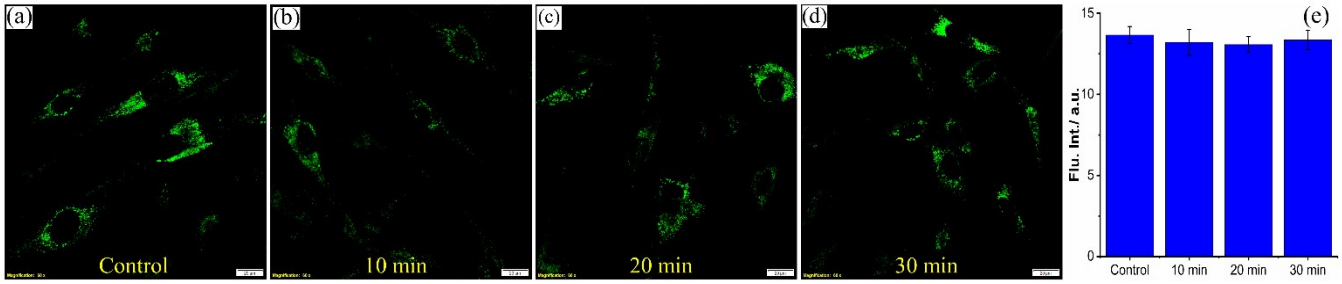


Fig. S19b. CLSM images of BHK-21 cells stained with (a) 0.2 μM **NIMC6** and stimulated with 20 μM chloroquine for (b) 10 min, (c) 20 min, and (d) 30 min; (e) shows the average fluorescence intensity in different ROIs **in different FOVs** at the aforementioned time-points of the experiment. (scale bar: 20 μm) Under the stressed condition, the fluorescence intensity is intact over time indicating the pH-independency of NIMC6 in live-cell.

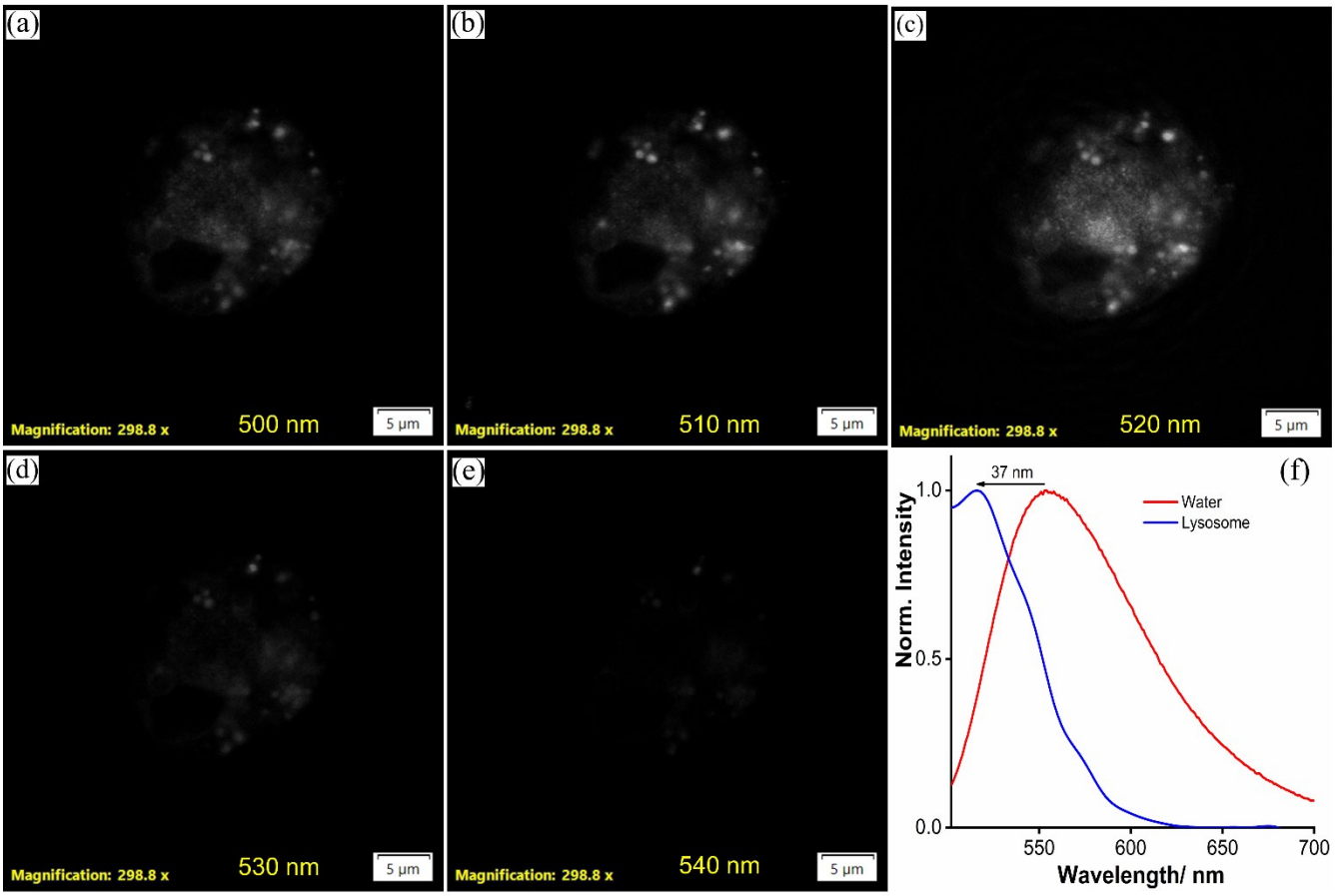


Fig. S20. (a-e) Series of individual images in different wavelength range recorded using confocal lambda scanning technique, (f) emission spectra of **NIMC6** inside lysosome in HEK-293 cells (λ_{max} = 518 nm) shows 37 nm blueshift compared to water (λ_{max} = 554 nm).

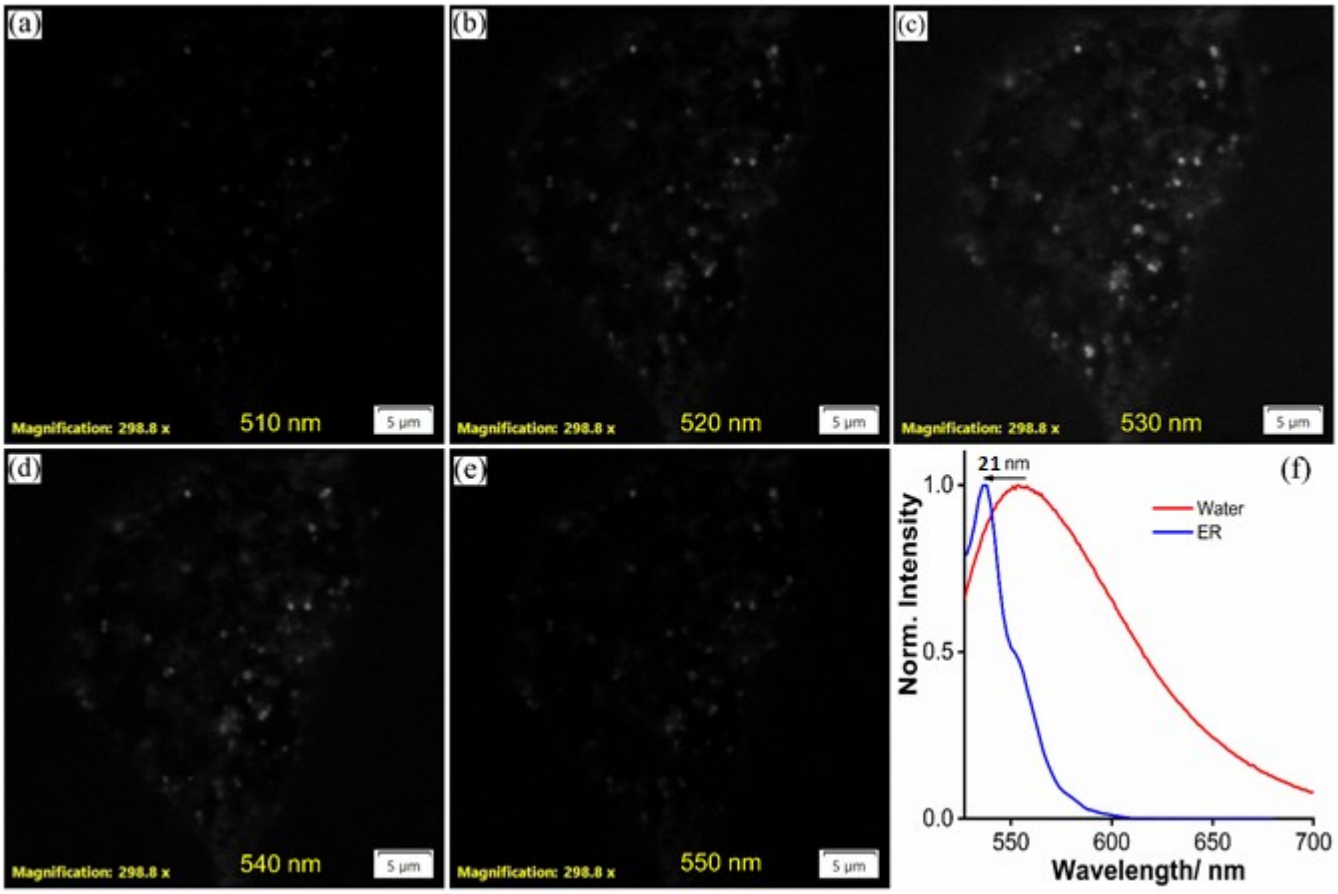


Fig. S21. (a-e) Series of individual images in different wavelength range recorded using confocal lambda scanning technique, emission spectra of **NIMC10** inside ER in BHK21 cells (λ_{max} = 537 nm) shows 21 nm blueshift compared to water (λ_{max} = 558 nm).

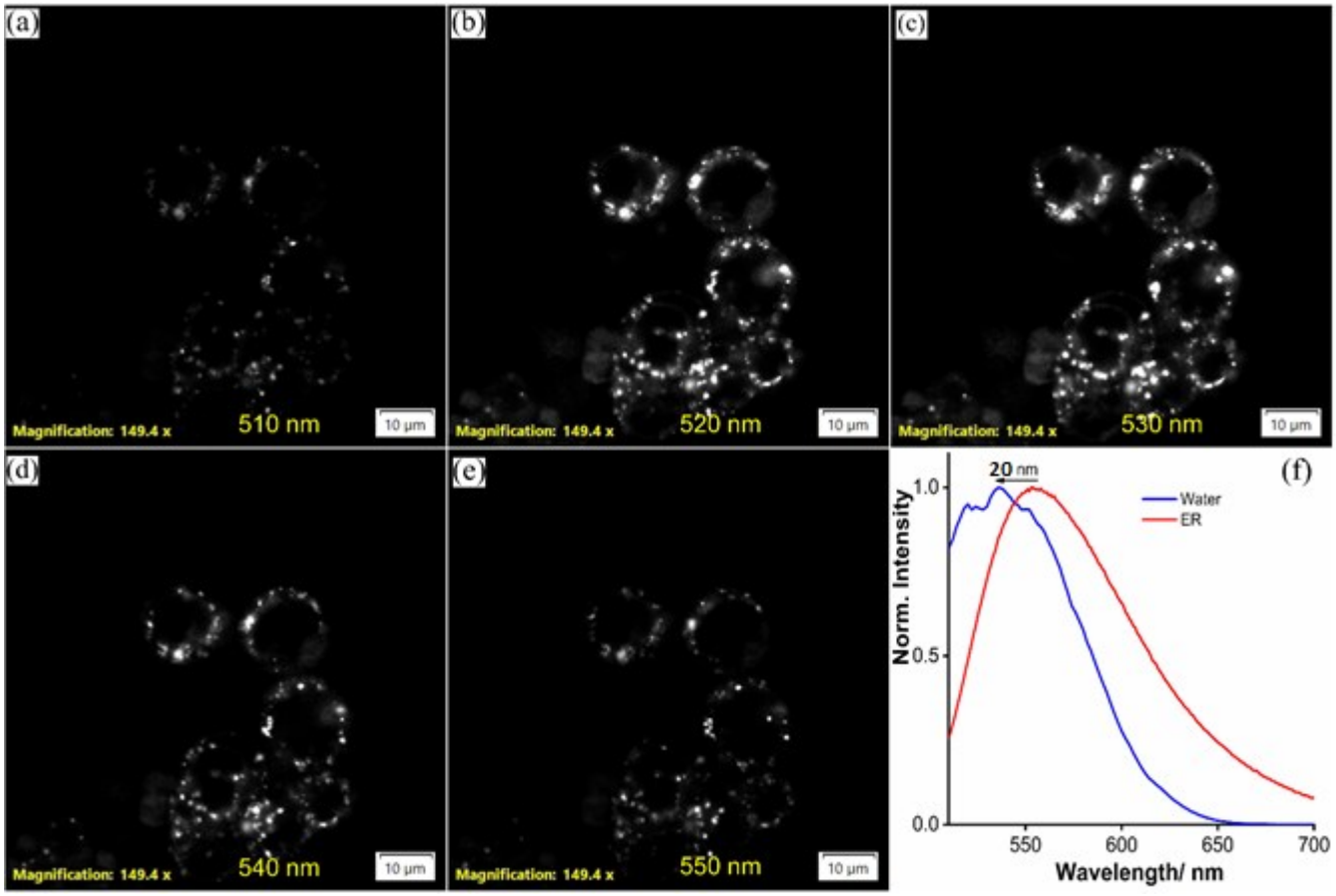


Fig. S22. (a-e) Series of individual images in different wavelength range recorded using confocal lambda scanning technique, (f) emission spectra of **NIMC10** inside ER in HEK-293 cells (λ_{max} = 536 nm) shows 20 nm blue shift compared to water (λ_{max} = 558 nm).

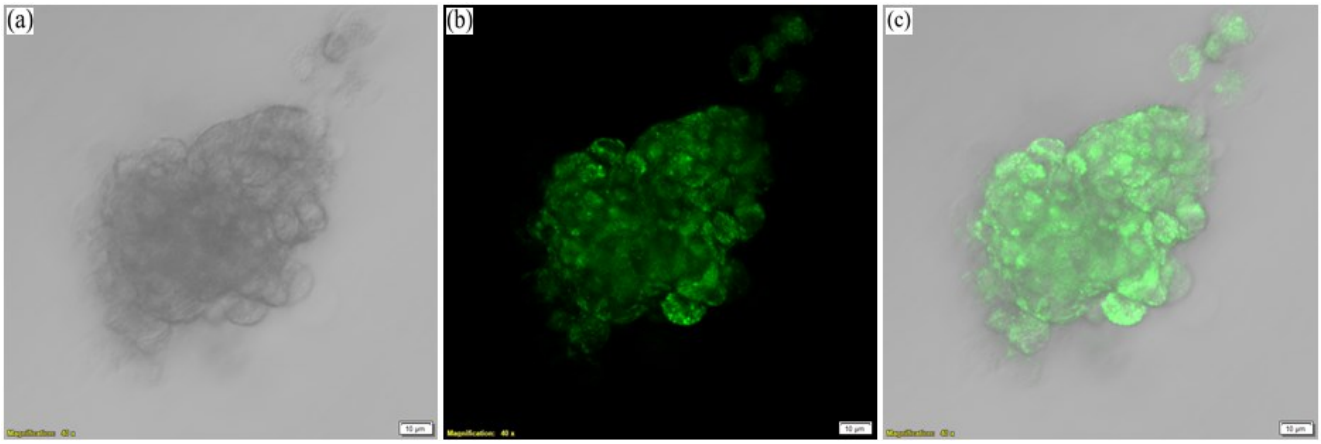


Fig. S23. Live-cell 3D spheroid **i**maging is better able to recapitulate not only the *in vivo* morphology, but the cell connectivity, polarity, gene expression. Hence, we performed a 3D-spheroids imaging derived from BHK-21 cells and incubated with 2 μM of **NIMC6**, where (a) bright field, (b) with NIMC6 and (c) merged images (scale bar 10 μm) suggested the applicability of **NIMC6** in 3D spheroid imaging and further imaging tissue samples.

Table S1. Solvent dependent photophysical properties of **NIMC2**, fluorescence quantum yield is calculated with respect to fluorescein in 0.1 N NaOH.[14]

Solvent	Absorption Max. (nm)	Extinction coefficient ε ($M^{-1}cm^{-1}$)	Emission Max. (nm)	Stokes shift (nm)	Lifetime (ns)	Quantum Yield ϕ_f
Water (pH 6.0)	441	16000	538	97	6.9	0.22
EG	442	6970	535	93	6.8	0.02
Methanol	438	7880	519	81	9.7	0.08
MeCN	430	5980	528	98	10.3	0.19
DMSO	443	7840	533	90	10.2	0.01
DMF	438	7620	531	93	10.3	0.02
DCM	428	6390	505	77	8.8	0.16
$CHCl_3$	427	6730	496	69	8.1	0.20
EtOAc	426	6390	512	86	9.4	0.09
Dioxane	422	7520	504	82	9.7	0.10
Toluene	420	5570	497	77	7.9	0.14

Table S2. Solvent dependent photophysical properties of **NIMC3**, fluorescence quantum yield is calculated with respect to fluorescein in 0.1 N NaOH.

Solvent	Absorption Max. (nm)	Extinction coefficient ε ($M^{-1}cm^{1}$)	Emission Max. (nm)	Stokes shift (nm)	Lifetime (ns)	Quantum Yield ϕ_f
Water (pH 6.0)	446	10800	548	102	5.4	0.47
EG	447	5400	541	94	6.4	0.15
Methanol	441	5160	536	95	9.3	0.47
MeCN	434	5300	520	86	7.1	0.44
DMSO	443	5270	535	92	10.1	0.10
DMF	441	4900	532	91	6.2	0.34
DCM	436	4400	510	74	10.3	0.46
$CHCl_3$	435	6000	503	68	8.7	0.64
EtOAc	429	4600	509	80	9.6	0.34
Dioxane	426	5200	505	79	9.9	0.29
Toluene	427	5320	494	67	8.1	0.52

Table S3. Solvent dependent photophysical properties of **NIMC4**, fluorescence quantum yield is calculated with respect to fluorescein in 0.1 N NaOH.

Solvent	Absorption Max. (nm)	Extinction coefficient ε ($M^{-1}cm^{1}$)	Emission Max. (nm)	Stokes shift (nm)	Lifetime (ns)	Quantum Yield ϕ_f
Water (pH 6.0)	449	12000	554	105	4.6	0.34
EG	447	8800	544	97	8.0	0.30
Methanol	442	7400	532	90	8.8	0.29
MeCN	431	8600	520	89	10.0	0.26
DMSO	445	8600	532	87	10.4	0.37
DMF	441	6700	526	85	10.0	0.41
DCM	429	7900	509	80	10.4	0.33
$CHCl_3$	429	5700	498	69	9.0	0.60
EtOAc	428	8600	509	81	9.6	0.30
Dioxane	425	6400	504	79	10.0	0.48
Toluene	424	7800	498	74	8.2	0.34

Table S4. Solvent dependent photophysical properties of **NIMC5**, fluorescence quantum yield is calculated with respect to fluorescein in 0.1 N NaOH.

Solvent	Absorption Max. (nm)	Extinction coefficient ε ($M^{-1}cm^{1}$)	Emission Max. (nm)	Stokes shift (nm)	Lifetime (ns)	Quantum Yield ϕ_f
Water (pH 6.0)	452	11600	553	101	4.3	0.27
EG	448	6000	544	96	8.1	0.73
Methanol	443	5560	534	91	8.8	1.16
MeCN	432	5820	520	88	10.2	1.19
DMSO	445	8833	533	88	10.4	1.00
DMF	440	8675	526	86	10.1	0.66
DCM	429	5610	508	79	10.4	1.03
$CHCl_3$	428	5800	502	74	9.4	1.09
EtOAc	428	5380	508	80	9.6	1.06
Dioxane	426	5600	506	80	10.1	1.21
Toluene	423	4940	498	75	8.4	1.00

Table S5. Solvent dependent photophysical properties of **NIMC6**, fluorescence quantum yield in water is calculated with respect to fluorescein in 0.1 N NaOH and for other solvents the calculation was done with respect to the quantum yield in water.

Solvent	Absorption Max. (nm)	Extinction coefficient ε ($M^{-1}cm^{1}$)	Emission Max. (nm)	Stokes shift (nm)	Lifetime (ns)	Quantum Yield ϕ_f
Water (pH 6.0)	453	13064	556	103	4.2	0.32
EG	448	13633	543	95	6.9	0.73
Methanol	444	13737	538	94	8.4	0.73
MeCN	433	13686	524	91	10.4	0.92
DMSO	446	13893	535	89	10.5	0.99
DMF	441	13494	524	83	10.3	1.00
DCM	427	10611	517	90	10.6	0.99
$CHCl_3$	429	12452	512	83	9.8	1.17
EtOAc	429	12279	513	84	9.7	1.03
Dioxane	428	12334	513	85	10.3	1.06
Toluene	426	12069	502	76	8.6	0.99

Table S6. Solvent dependent photophysical properties of **NIMC8**, fluorescence quantum yield in water is calculated with respect to fluorescein in 0.1 N NaOH and for other solvents the calculation was done with respect to the quantum yield in water.

Solvent	Absorption Max. (nm)	Extinction coefficient ε ($M^{-1}cm^{1}$)	Emission Max. (nm)	Stokes shift (nm)	Lifetime (ns)	Quantum Yield ϕ_f
Water (pH 6.0)	453	11000	559	106	4.1	0.23
EG	448	12240	545	97	8.1	0.77
Methanol	443	11700	541	98	8.4	0.75
MeCN	432	11800	526	94	10.3	0.95
DMSO	444	11560	535	91	10.5	0.99
DMF	439	12250	527	88	10.3	0.01
DCM	428	10190	517	89	10.5	0.92
EtOAc	429	12240	513	84	9.8	1.02
Dioxane	428	11158	516	88	10.5	1.09
Toluene	426	10935	503	77	8.6	0.98

Table S7. Solvent dependent photophysical properties of **NIMC10**, fluorescence quantum yield in water is calculated with respect to fluorescein in 0.1 N NaOH and for other solvents the calculation was done with respect to the quantum yield in water.

Solvent	Absorption Max. (nm)	Extinction coefficient ε ($M^{-1}cm^{1}$)	Emission Max. (nm)	Stokes shift (nm)	Lifetime (ns)	Quantum Yield ϕ_f
Water (pH 6.0)	452	11400	558	107	4.2	0.08
EG	449	13834	545	96	7.1	0.75
Methanol	443	13921	538	95	8.4	0.75
MeCN	433	13338	524	91	9.5	0.92
DMSO	444	13621	535	90	10.2	1.03
DMF	444	13096	525	81	10.2	0.98
DCM	429	12408	516	87	10.4	0.99
$CHCl_3$	428	14109	511	83	9.8	0.91
EtOAc	428	13158	512	84	9.6	1.00
Dioxane	428	12596	513	85	10.0	1.13
Toluene	423	12708	507	84	8.6	1.03

Table S8. Crystal data and structure refinement details.

NIMCs	NIMC2	NIMC3	NIMC4	NIMC5	NIMC10
Formula	C_{22} H_{22} N_3 O_3	C_{23} H_{29} N_3 O_3	C_{24} H_{31} N_3 O_3	C_{25} H_{33} N_3 O_3	C_{33} H_{50} N_4 O_4
Formula weight	381.46	395.49	409.52	423.54	566.77
Temperature/ K	100(2)	100(2)	100(2)	100(2)	100(2)
Wavelength/ Å	0.71073	0.71073	0.71073	0.71073	0.71073
Solvent system for crystallization	DCM + MeOH + DMF	DCM + MeOH + DMF	DCM + MeOH + DMF	DCM + MeOH + DMF	DCM + MeOH + DMF

Crystal size / mm	0.380 x 0.070 x 0.040	0.380 x 0.210 x 0.120	0.400 x 0.080 x 0.040	0.350 x 0.180 x 0.080	0.180 x 0.060 x 0.040
Morphology	Plate	Block	Plate	Block	Needle
Crystal system	Orthorhombic	Orthorhombic	Triclinic	Triclinic	Triclinic
Space group	$P2_12_12_1$	*Pbcn*	*P*-1	*P*-1	*P*-1
***a*/ Å**	6.9422(9)	24.1436(7)	8.6541(15)	9.5249(5)	4.9200(4)
***b*/ Å**	18.635(2)	9.2164(3)	9.2597(18)	9.5481(6)	16.6625(17)
***c*/ Å**	29.823(3)	18.0242(5)	13.842(3)	12.5232(7)	18.9799(17)
***α*/ º**	90	90	104.249(8)	90.010(4)	87.245(3)
***β*/ º**	90	90	97.838(8)	96.492(4)	87.143(3)
***γ*/ º**	90	90	96.127(8)	105.918(4)	86.580(3)
V/ Å^3	3858.1(8)	4010.7(2)	1053.6(3)	1087.64(11)	1549.7(2)
Z	8	8	2	2	2
ρ (calcd)/ g cm^{-3}	1.313	1.310	1.229	1.293	1.215
F (000), μ / mm^{-1}	1632, 0.088	1696, 0.088	440, 0.086	456, 0.085	616, 0.080
θ (min, max)/ °	2.186, 26.661	2.260, 28.281	2.294, 29.126	1.637, 29.129	2.45, 29.65
$h_{min,max}$, $k_{min,max}$, $l_{min,max}$	(-8, 8), (-22, 23), (-36, 36)	(-32, 30), (-12, 9), (-22, 24)	(-11, 11), (-12, 12), (-18, 18)	(-13, 12), (-13, 13), (-17, 17)	(6, -6), (23, -23), (26, -26)
Treatment of hydrogens	Located	Located	Located	Located	Located
No. of unique ref./ obs. refls.	3983, 2337	4933, 3972	5665, 4456	5806, 3192	8557, 4527
No. of	361	378	272	412	570

parameters					
R_1, wR_2 ($I>2\sigma(I)$)	0.0686, 0.1574	0.0470, 0.1089	0.0462, 0.1142	0.0591, 0.1241	0.0679, 0.1123
R_1, wR_2 (all data)	0.1247, 0.1931	0.0615, 0.1159	0.0640, 0.1242	0.1185, 0.1504	0.1653, 0.1405
$\Delta\rho_{min,\ max/}$ eÅ^3	-0.326, 0.248	-0.229, 0.338	-0.393, 0.339	-0.275, 0.292	-0.297, 0.374
goodness-of-fit, S	1.030	1.028	1.050	0.968	1.016
CCDC No.	2053600	2053601	2053602	2053603	2053604

Table S9. Intermolecular hydrogen bonds and other interactions in **NIMCs**

Intermolecular Interactions	Symmetry Code	H···A/ Å	D···A/ Å	D–H···A/ °
NIMC2				
H2N···H14	x, y, z	2.35	2.7457(3)	103
C19–H19A···O2	-x, -1/2+y, 1/2-z	2.65	3.5805(3)	152
C17–H17A···O2	-x, -1/2+y, 1/2-z	2.60	3.5919(3)	175
C18–H18A···O1	-1/2+x, y, 1/2-z	2.67	3.5310(3)	149
C20–H20B···O1	-1/2+x, y, 1/2-z	2.50	3.4142(2)	152
C8(π)···C13(π)	-1/2+x, y, 1/2-z		3.3893(4)	
NIMC3				
N2–H2N···N3	x, y, z	2.11	2.8938(1)	143
C20–H20A···O2	-x, y, 1/2-z	2.41	3.2780(1)	146
C1–H1B···O2	1/2-x, -1/2+y, z	2.48	3.4726(1)	175
C23–H23A···C5(π)	-x, -y, 1-z	2.88	3.8326(1)	169
C6(π)···C13(π)	-x, y, 1/2-z		3.2877(1)	
C15(π)···C16(π)	-x, y, 1/2-z		3.3708(1)	
C16(π)···C16(π)	-x, y, 1/2-z		3.3516(1)	

C12(π)···C12(π)	-x, -y, 1-z		3.3961(1)	
NIMC4				
N2–H2N···O3	x, -1+y, z	2.051	2.8881(1)	159
C14–H14···O3	x, -1+y, z	2.504	3.4324(1)	166
C15–H15···O1	x, -1+y, z	2.462	3.3194(2)	150
C21–H21A···O2	-x, 1-y, 1-z	2.497	3.4761(2)	170
C22–H22B···O1	-x, 2-y, 1-z	2.700	3.6242(2)	155
C23–H23B···O1	1-x, 2-y, 1-z	2.691	3.5829(2)	150
C24–H24A···C16(π)	1-x, 1-y, 1-z	2.791	3.7606(2)	167
NIMC5				
C24–H24B···O1	-1+x, y, -1+z	2.50	3.4523(2)	157
C15–H15···O1	-1+x, y, z	2.51	3.4261(2)	165
C22–H22A···O2	x, -1+y, 1+z	2.55	3.2598(2)	127
C14–H14···O3	-1-x, 1-y, -1-z	2.29	3.2693(2)	168
N2–H2N···O3	-1-x, 1-y, -1-z	2.10	3.0405(2)	163
C1–H1B···O2	-x, -y, 1-z	2.57	3.5474(2)	160
C24–H24B···C10(π)	-1+x, y, -1+z	2.89	3.7238(2)	140
C4–H4A···C12(π)	-x, -y, -z	2.82	3.2849(2)	111
C17–H17B···C8(π)	-x, 1-y, -z	2.84	3.5048(2)	124
C6(π)···C10(π)	-x, -y, -z		3.3998(2)	
C12(π)···C12(π)	-x, 1-y, -z		3.3345(2)	
NIMC10				
C27–H27B···O1	1-x, 1-y, 1-z	2.43	3.4243(3)	167
C23–H23A···O1	2-x, 1-y, 1-z	2.64	3.5654(2)	158
C14–H14···O4	x, y, z	2.27	3.2498(2)	172
N2–H2···O4	x, y, z	2.21	3.0534(2)	173
C33–H33A···O3	-x, 1-y, -z	2.50	3.4387(3)	155
C33–H33C···O4	-1+x, y, z	2.68	3.5653(3)	148
C17–H17A···C12(π)	-1+x, y, z	2.85	3.6461(2)	139

C17–H17A···C13(π)	-1+x, y, z	2.83	3.4700(3)	124

Table S10. Topological parameters for the intra and inter-molecular hydrogen bonds present in **NIMC2**, **NIMC3**, and **NIMC4**

Code	**Interaction**	**BPL (Å)**	ρ_{BCP} **(eÅ^{-3})**	$\nabla^2\rho_{BCP}$ **(eÅ^{-5})**	**V_b (a.u.)**	**G_b (a.u.)**	$\lvert V_b\rvert/G_b$
NIMC2	H2N···H14	1.949	0.099	1.521	-0.009591	0.012688	0.756
NIMC3	H2N···N3	2.014	0.159	1.931	-0.016218	0.018126	0.895
	H2N···H14	2.266	0.082	1.349	-0.008087	0.011044	0.732
NIMC4	H11···N3	2.593	0.052	0.479	-0.003854	0.004415	0.873
	H2N···O3	1.961	0.135	2.063	-0.015133	0.018267	0.828
	H14···O3	2.387	0.063	0.715	-0.005618	0.006519	0.862
	H15···O1	2.346	0.058	0.735	-0.005068	0.006349	0.798

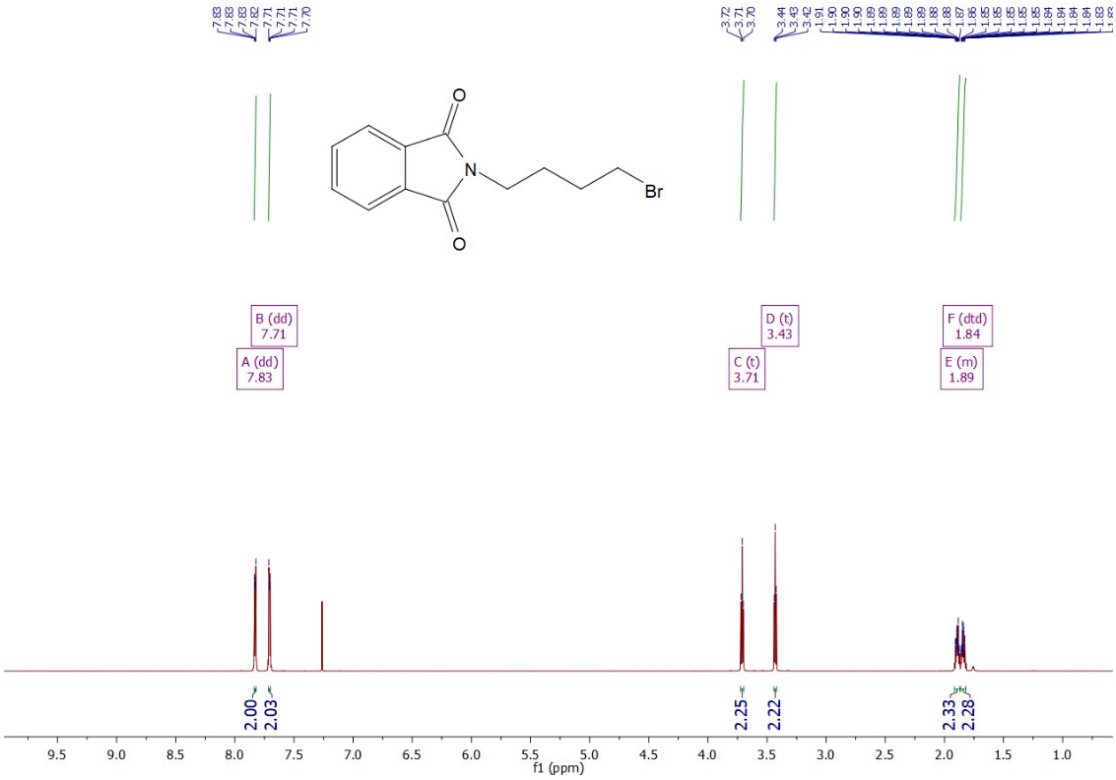


Fig. S24a: ^{1}H NMR of **PHC4Br** in $CDCl_3$

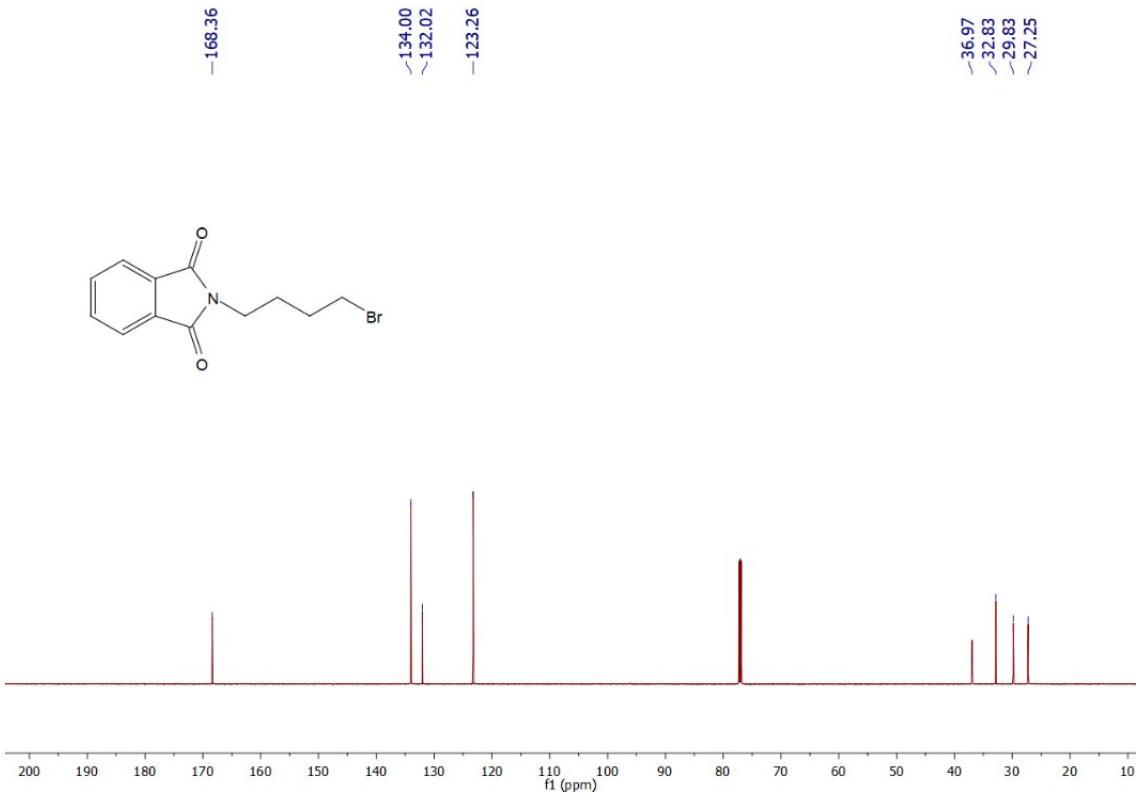


Fig. S24b: ^{13}C NMR of **PHC4Br** in $CDCl_3$

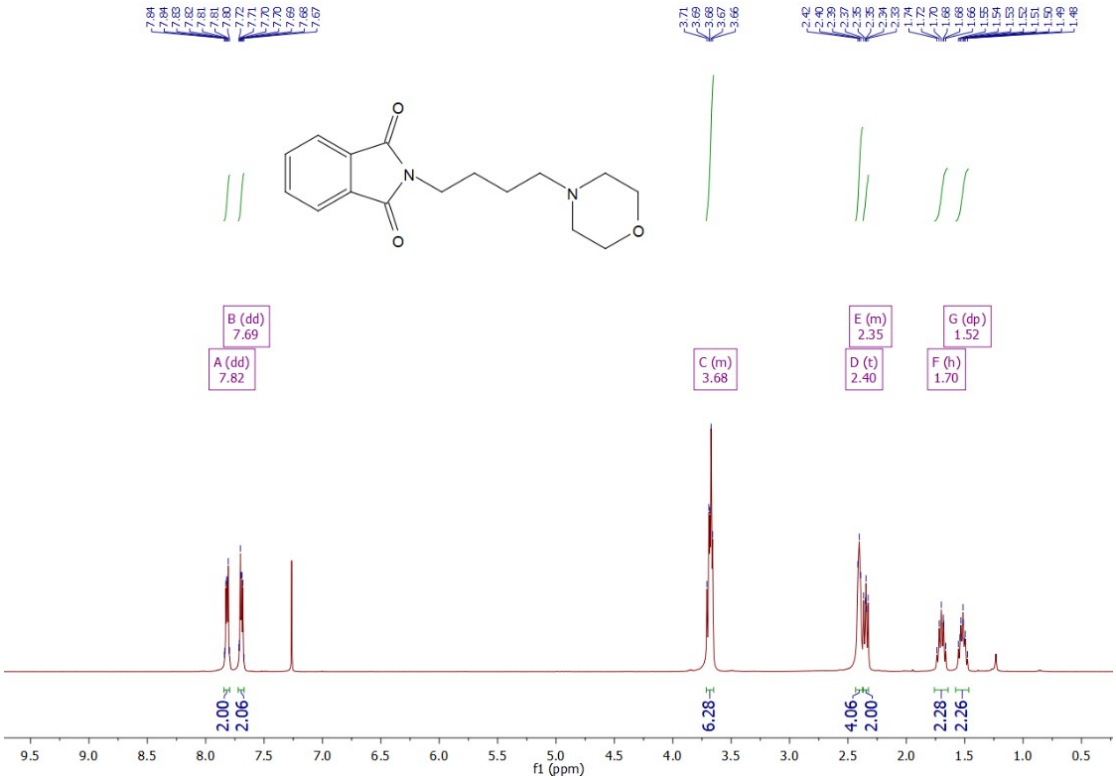


Fig. S25a: ^{1}H NMR of **PHC4M** in $CDCl_3$

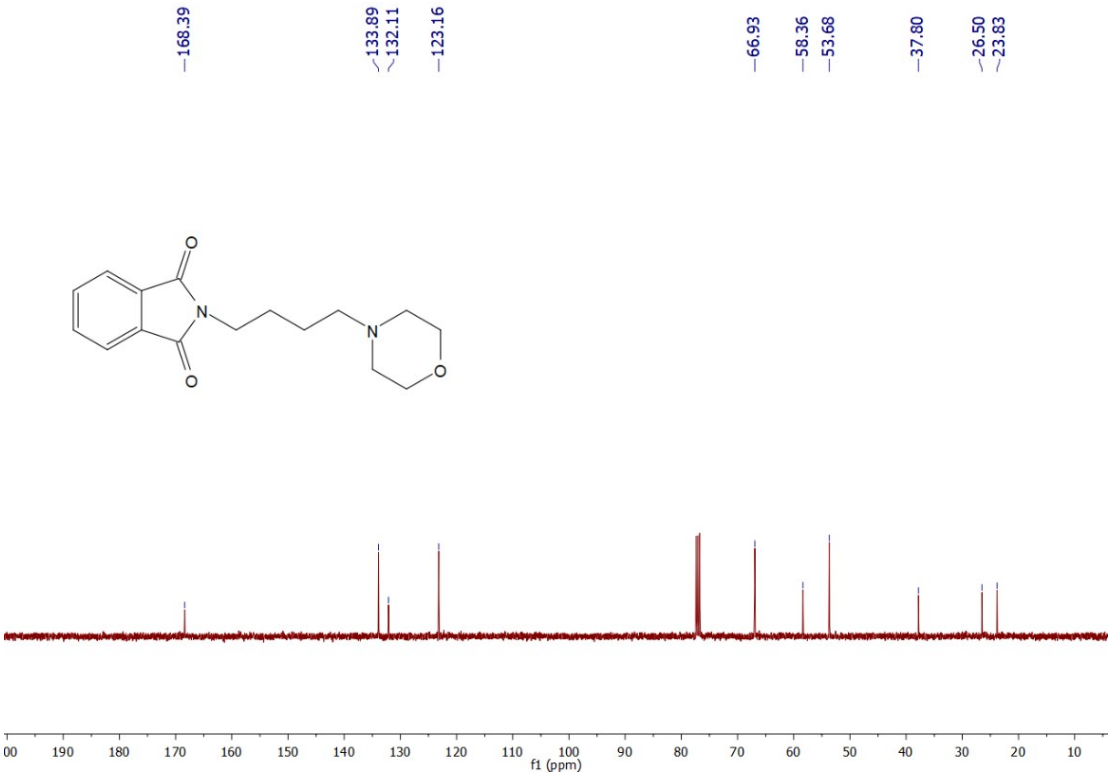


Fig. S25b: ^{13}C NMR of **PHC4M** in $CDCl_3$

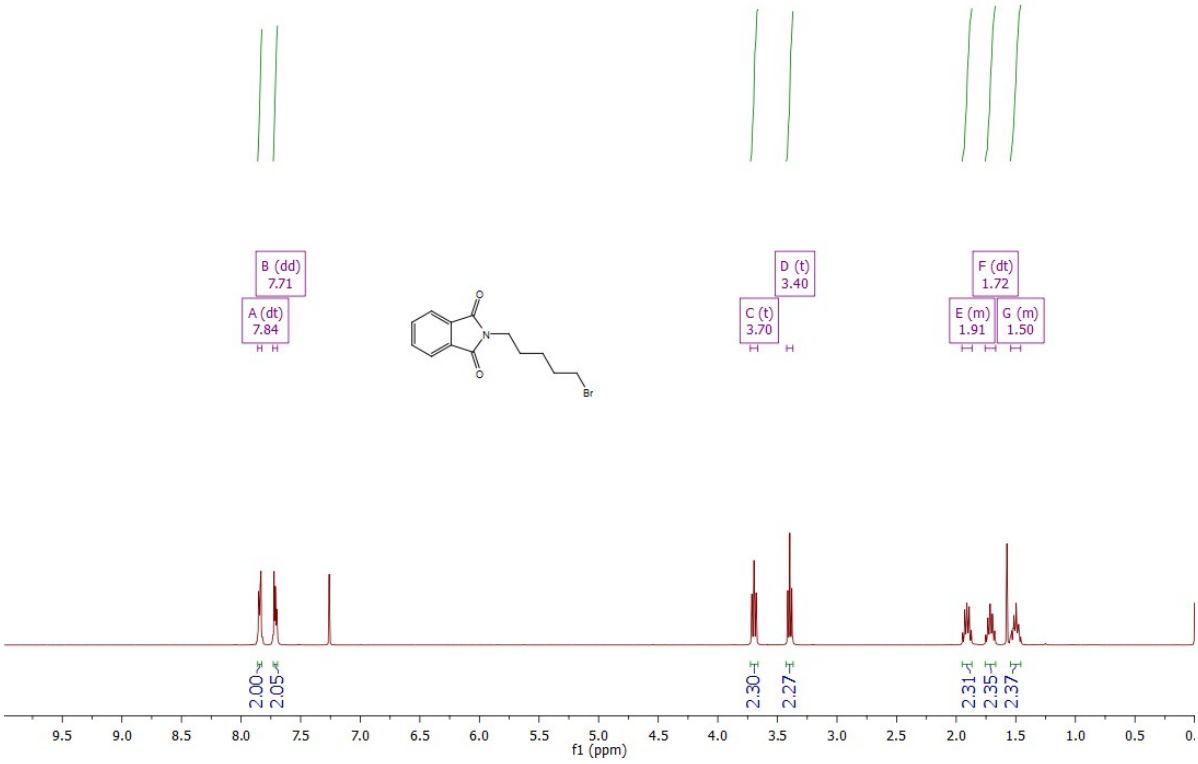


Fig. S26a: ^{1}H NMR of **PHC5Br** in $CDCl_3$

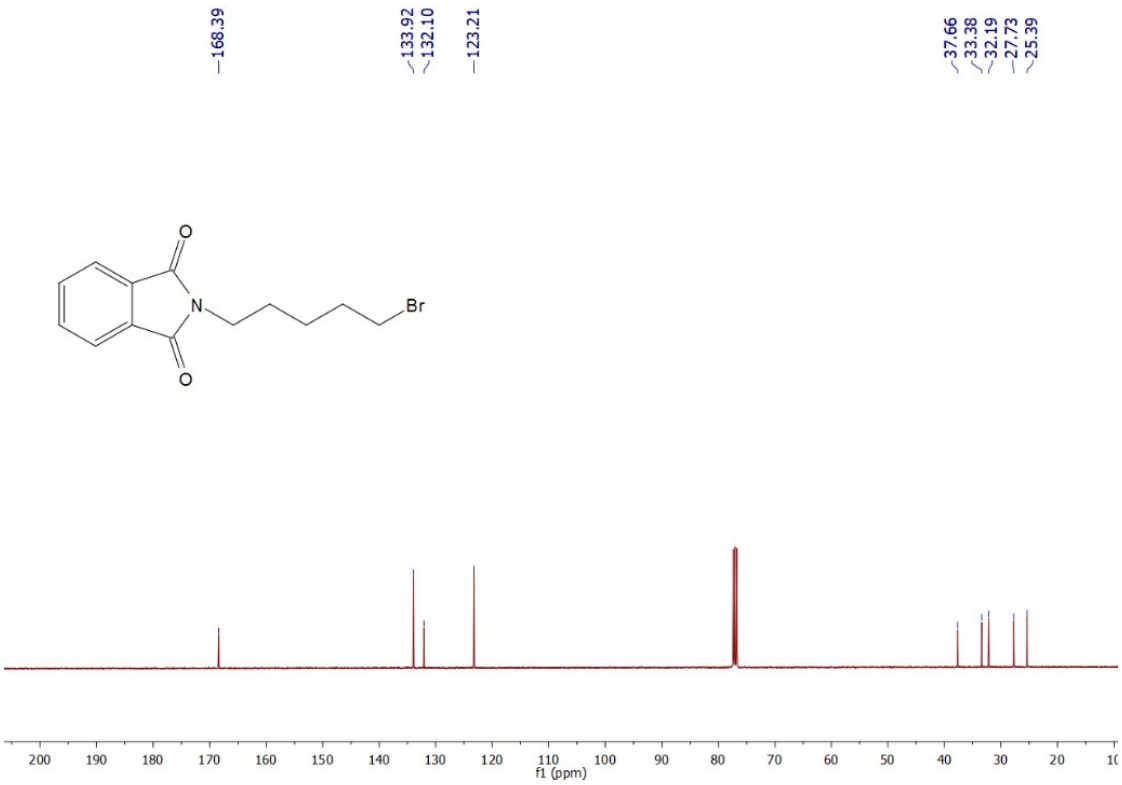


Fig. S26b: ^{13}C NMR of **PHC5Br** in $CDCl_3$

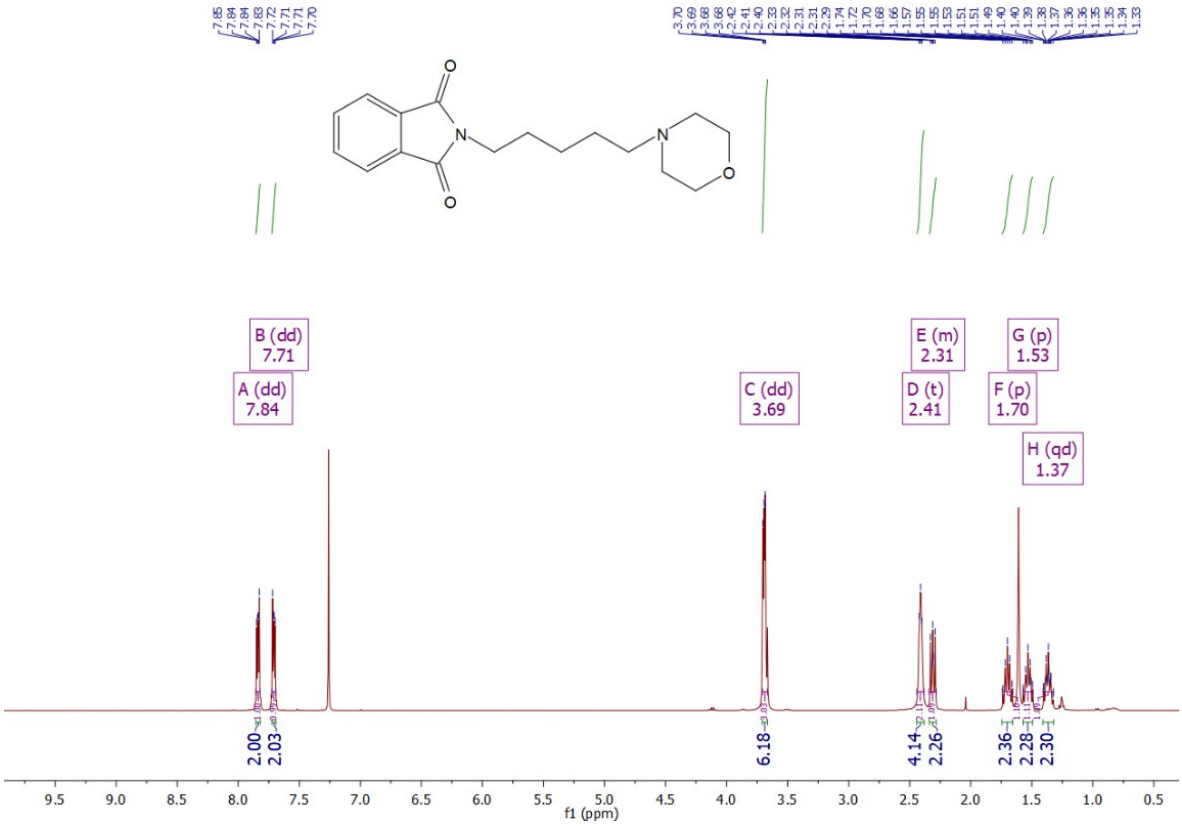


Fig. S27a: ^{1}H NMR of **PHC5M** in $CDCl_3$

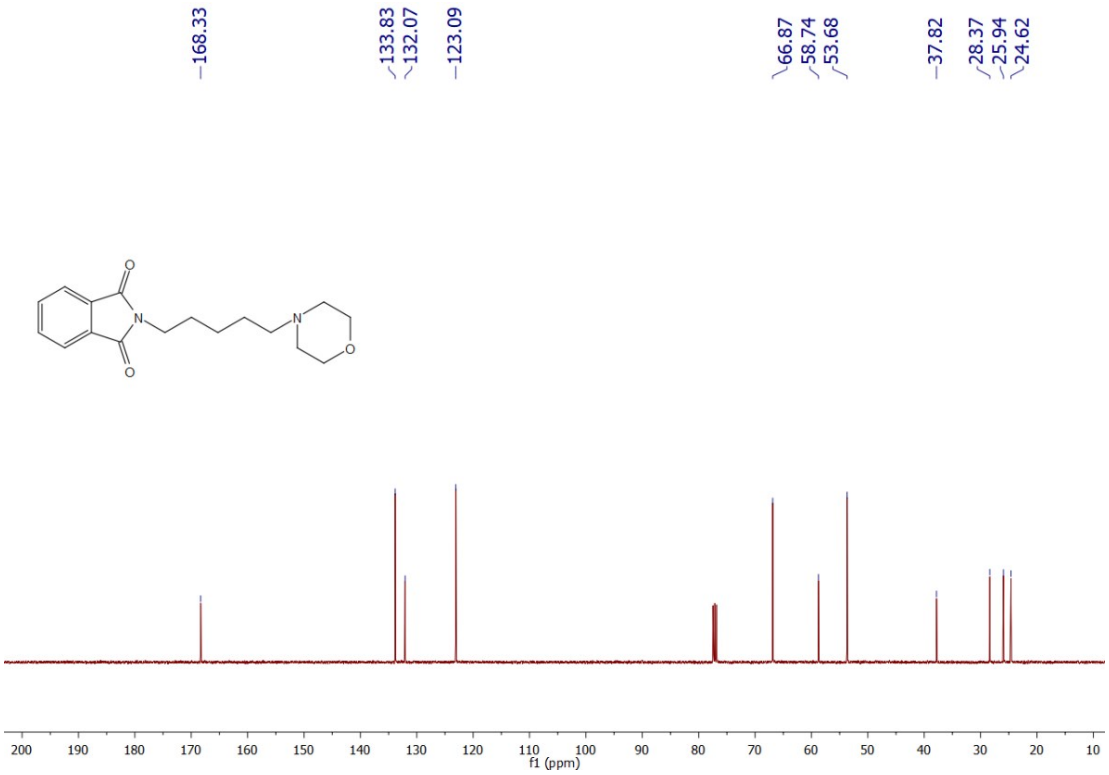


Fig. S27b: ^{13}C NMR of **PHC5M** in $CDCl_3$

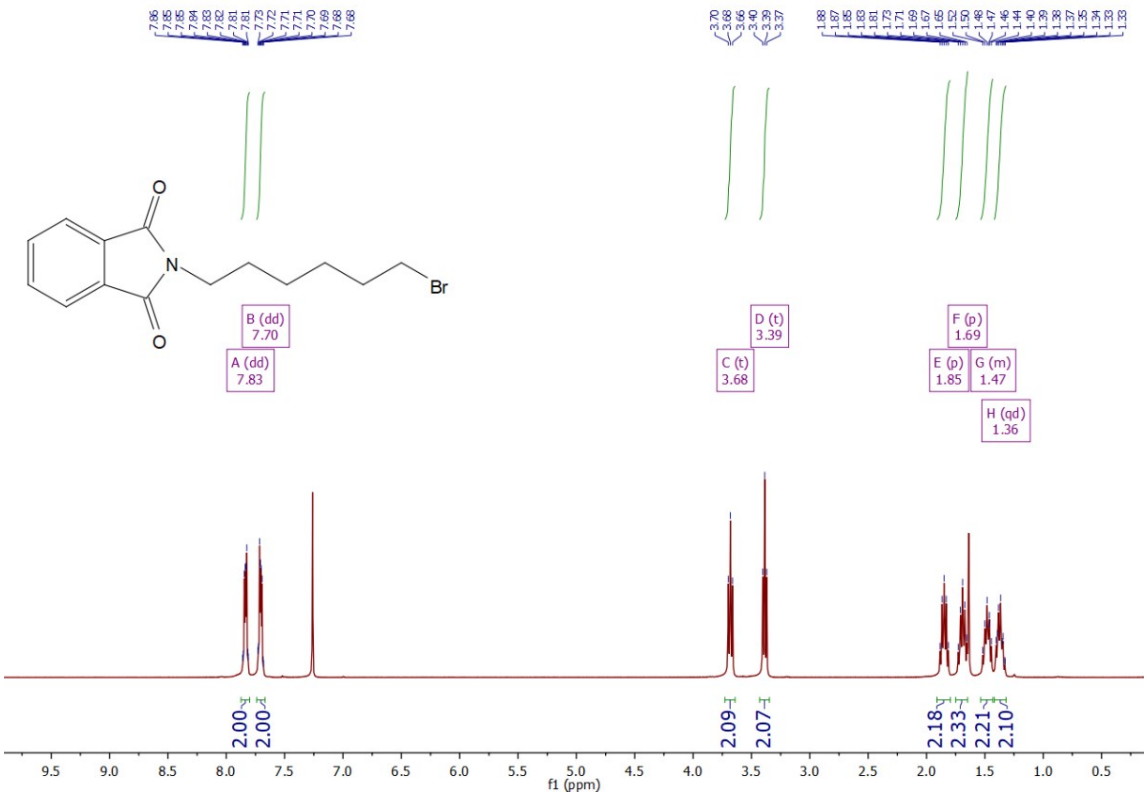


Fig. S28a: ^{1}H NMR of **PHC6Br** in $CDCl_3$

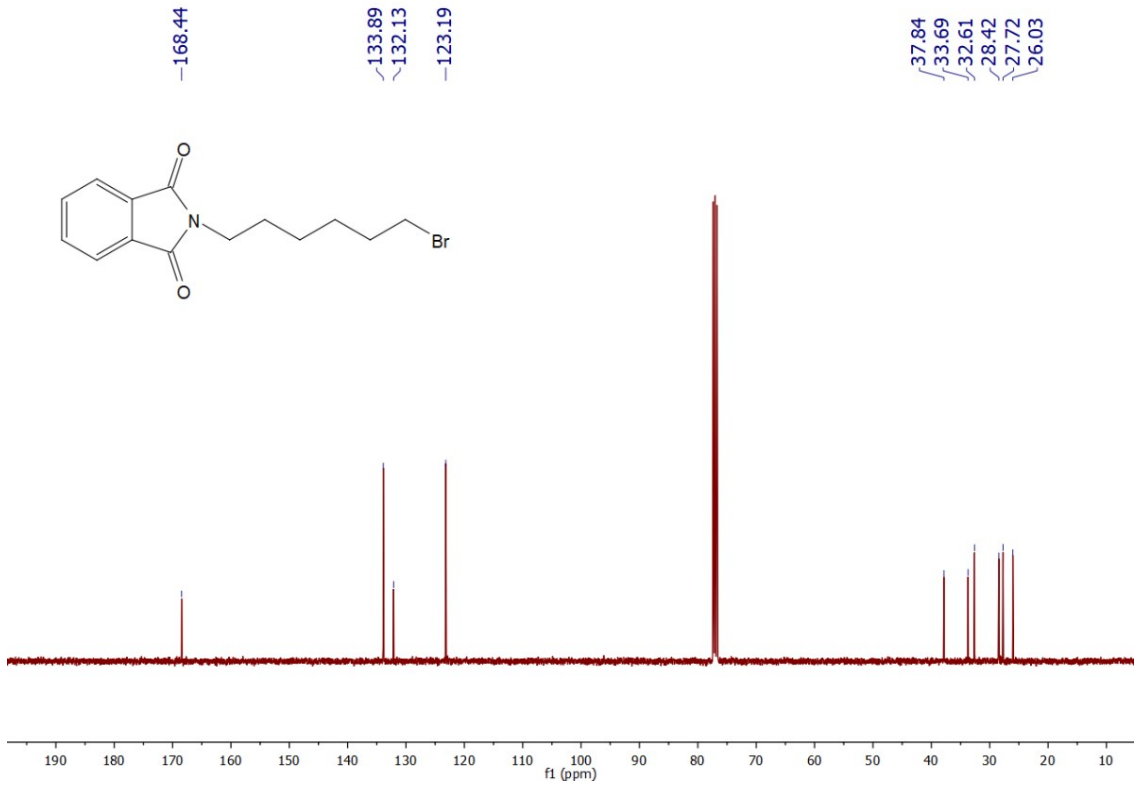


Fig. S28b: ^{13}C NMR of **PHC6Br** in $CDCl_3$

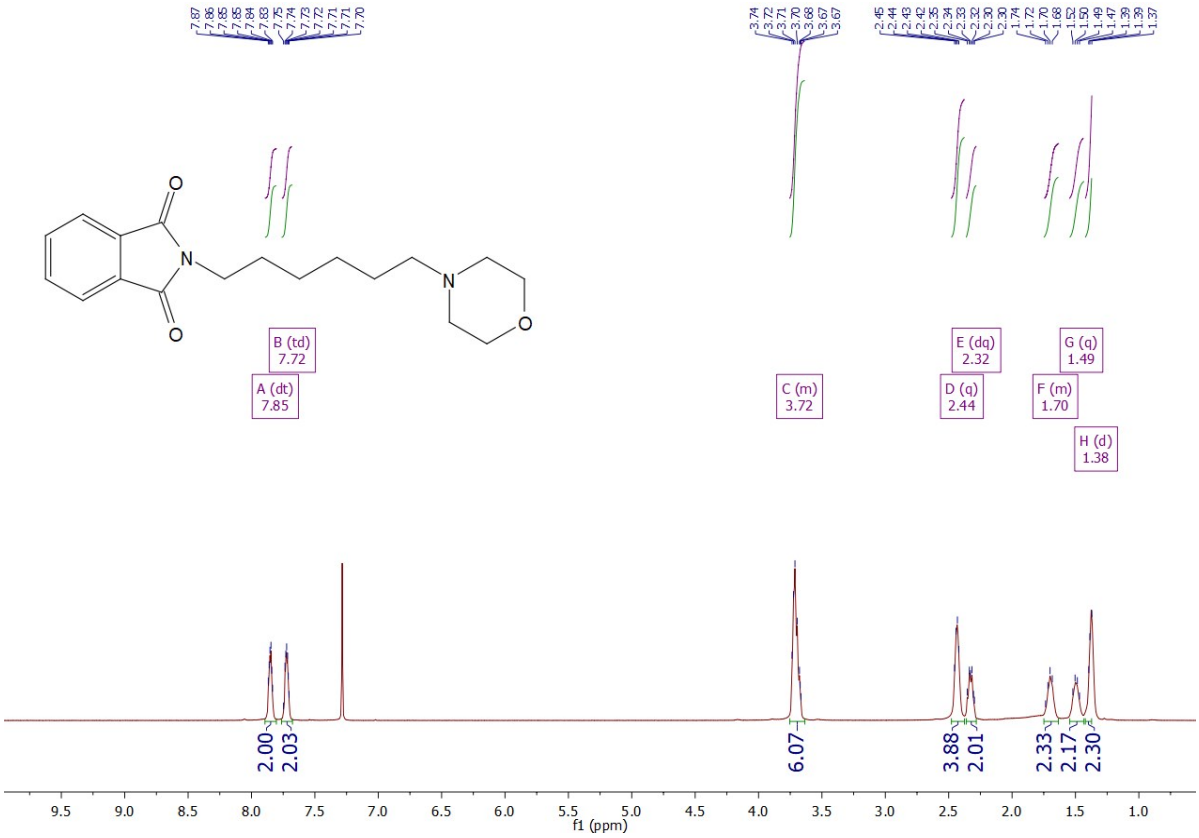


Fig. S29a: ^{1}H NMR of **PHC6M** in $CDCl_3$

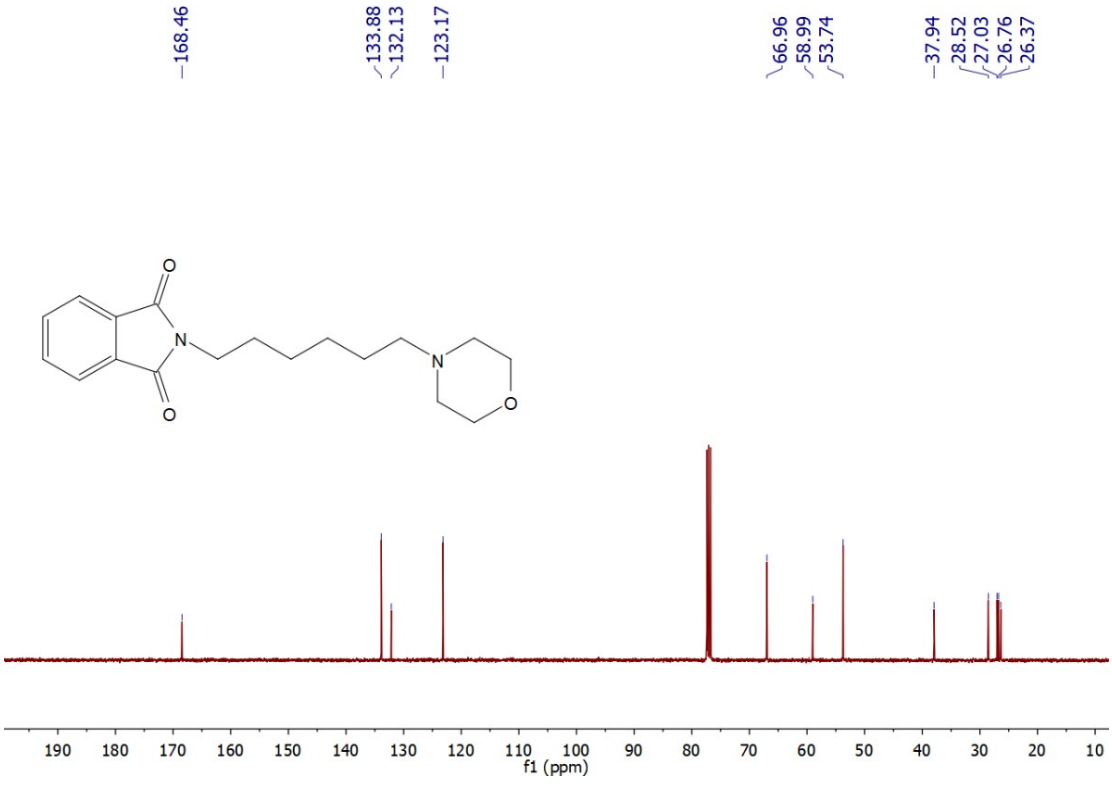


Fig. S29b: ^{13}C NMR of **PHC6M** in $CDCl_3$

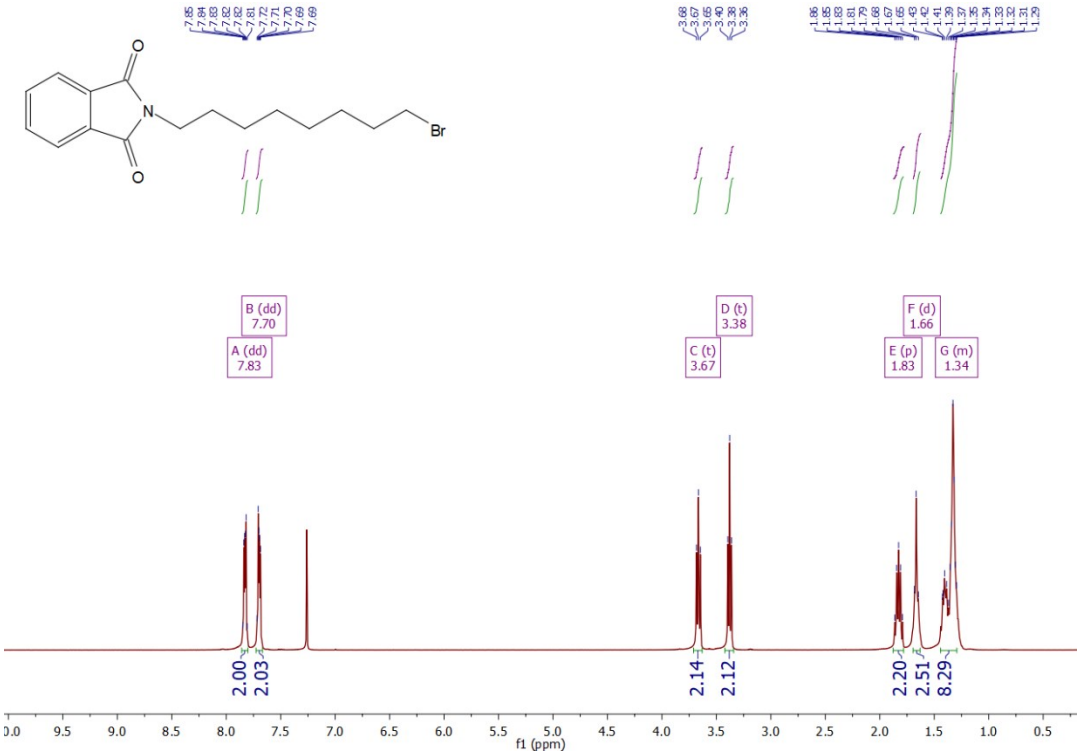


Fig. S30a: ^{1}H NMR of **PHC8Br** in $CDCl_3$

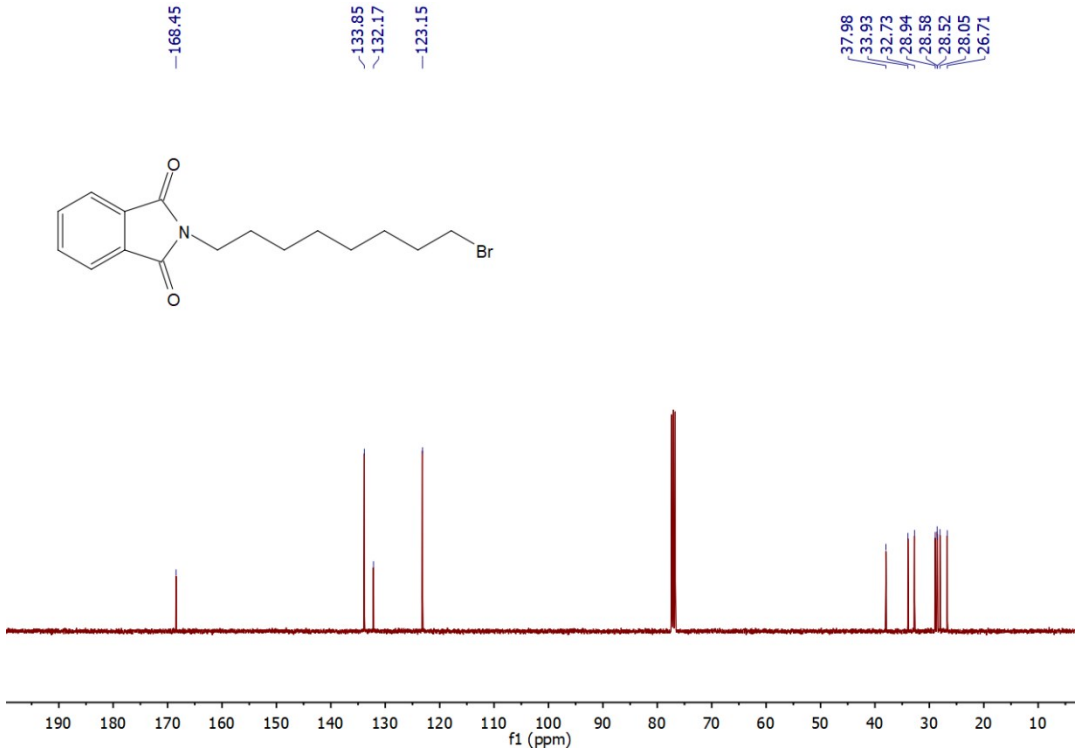


Fig. S30b: ^{13}C NMR of **PHC8Br** in $CDCl_3$

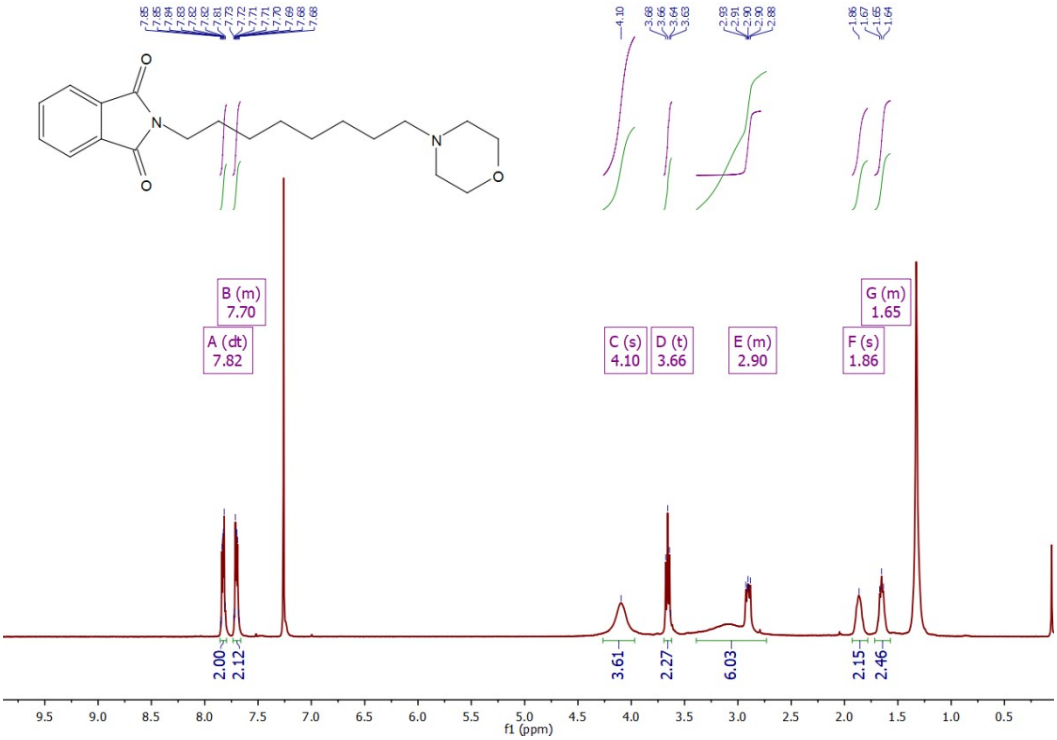


Fig. S31a: ^{1}H NMR of **PHC8M** in $CDCl_3$

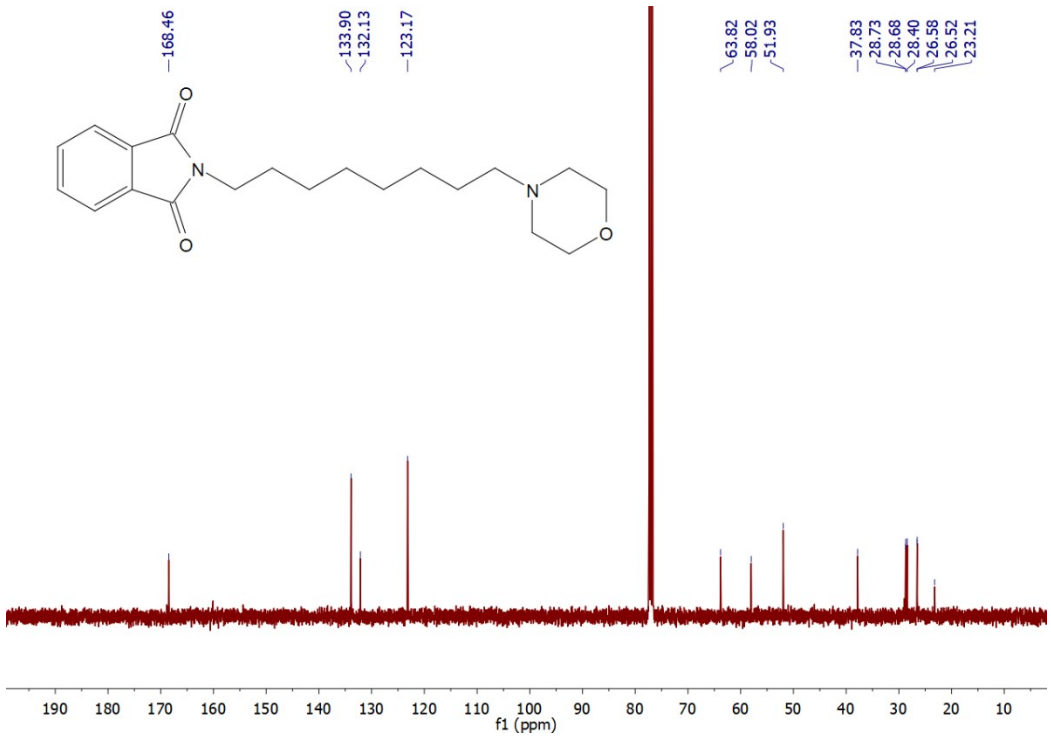


Fig. S31b: ^{13}C NMR of **PHC8M** in $CDCl_3$

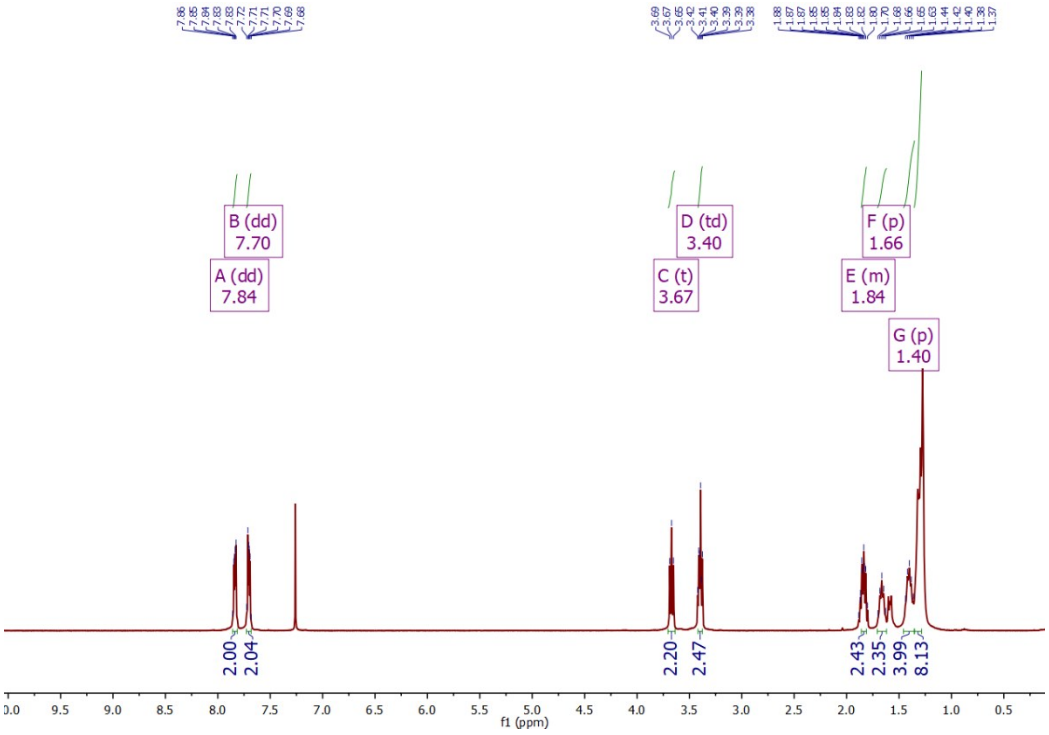


Fig. S32a: ^{1}H NMR of **PHC10Br** in $CDCl_3$

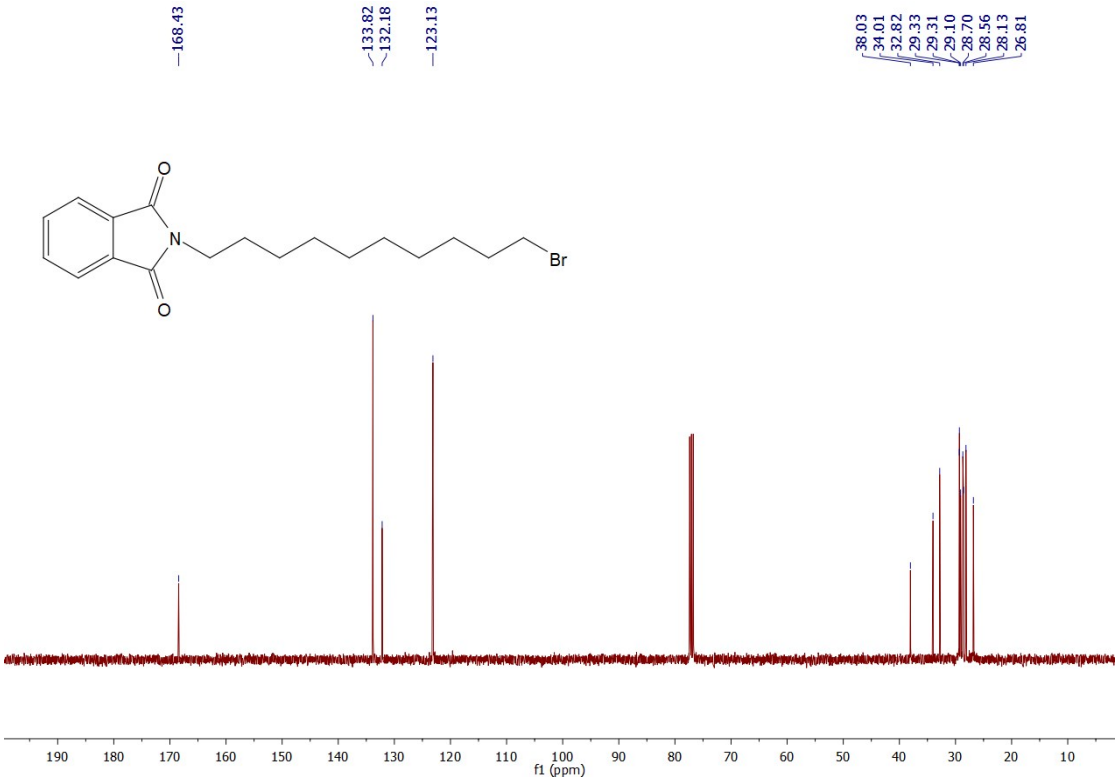


Fig. S32b: ^{13}C NMR of **PHC10Br** in $CDCl_3$

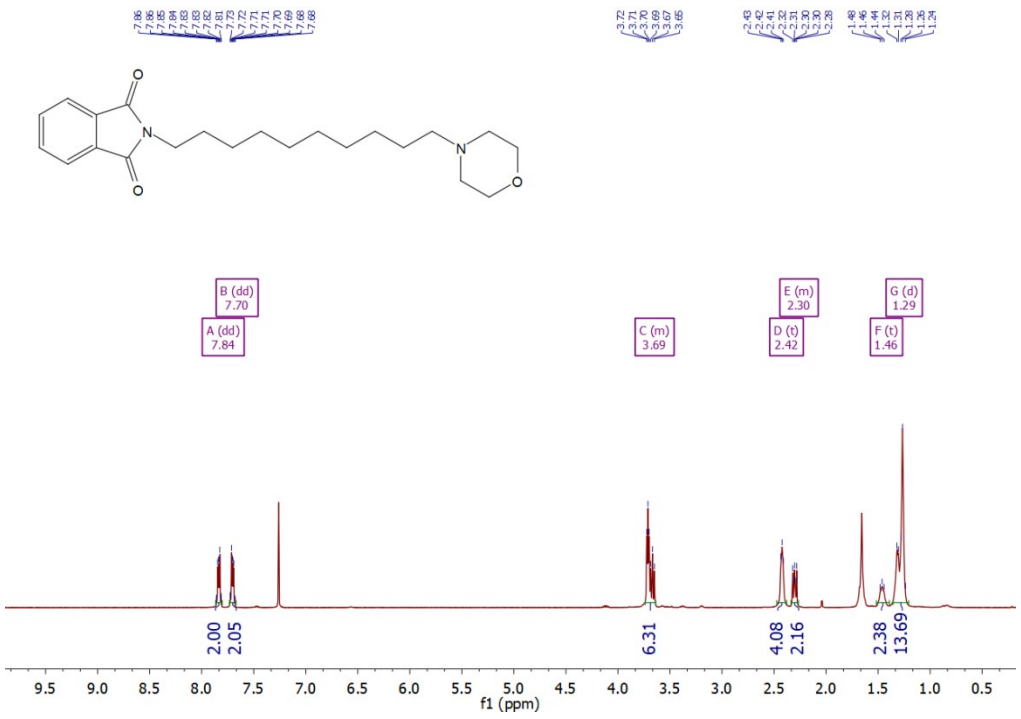


Fig. S33a: ^{1}H NMR of **PHC10M** in $CDCl_3$

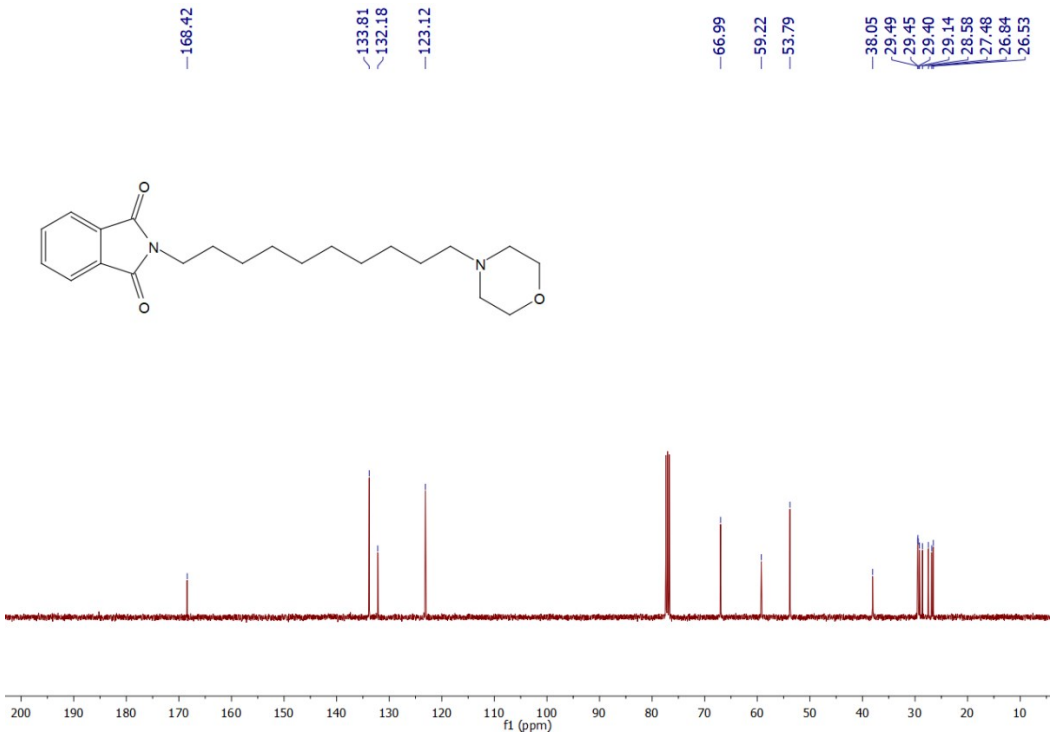


Fig. S33b: ^{13}C NMR of **PHC10M** in $CDCl_3$

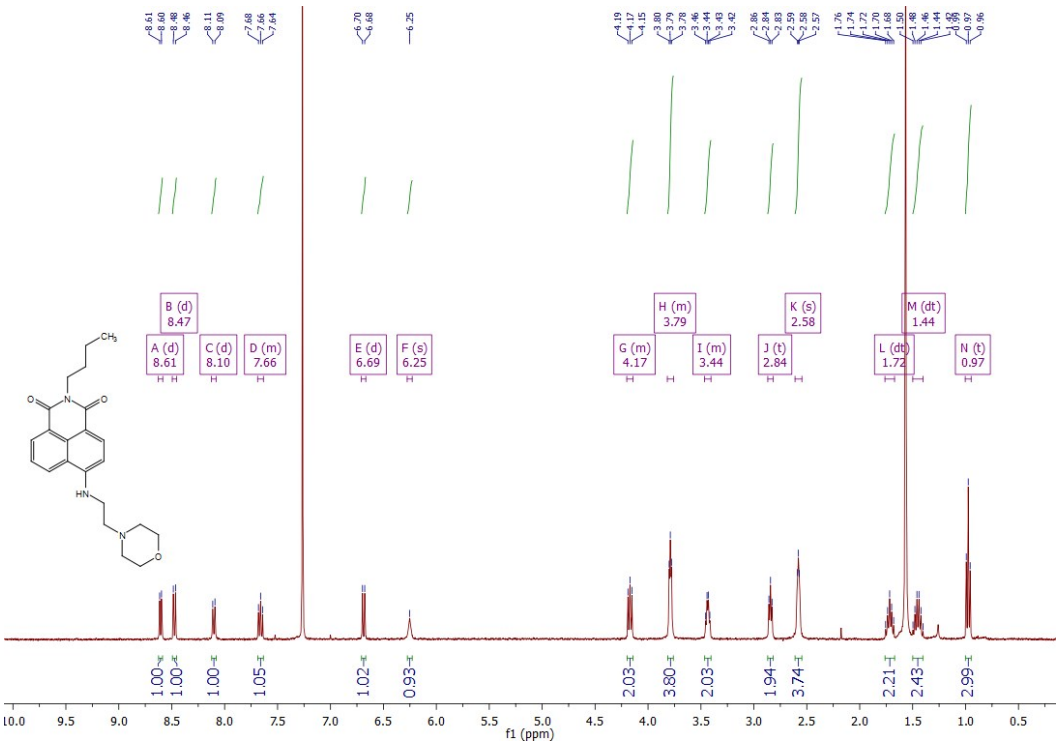


Fig. S34a: ^{1}H NMR of **NIMC2** in $CDCl_3$

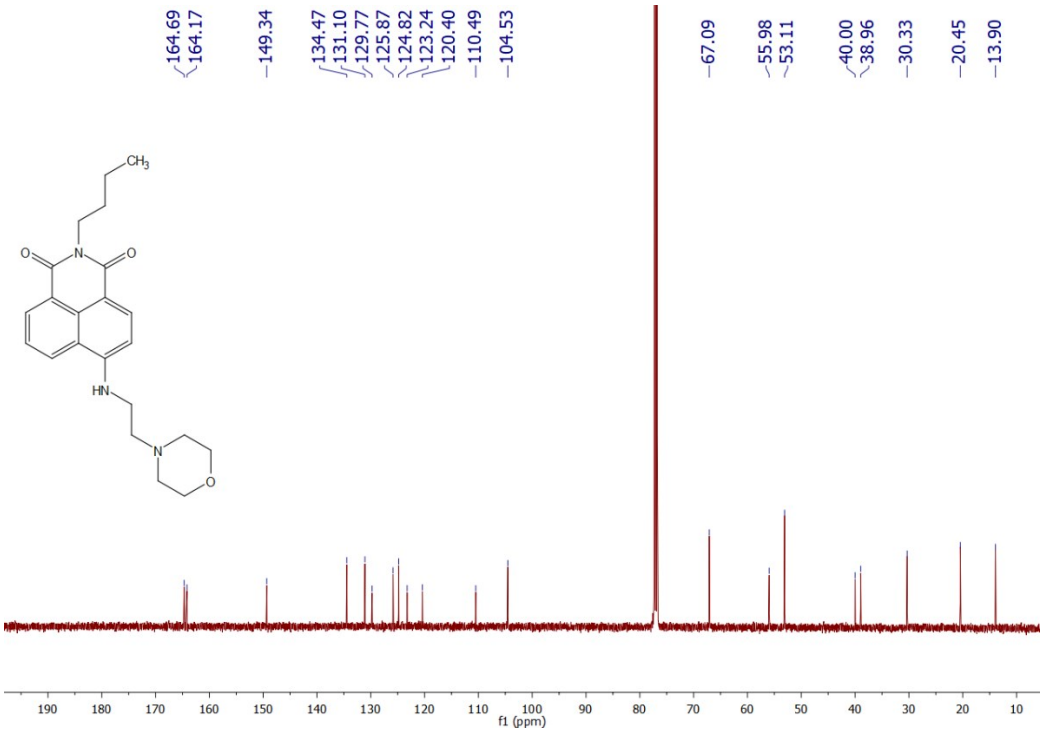


Fig. S34b: ^{13}C NMR of **NIMC2** in $CDCl_3$

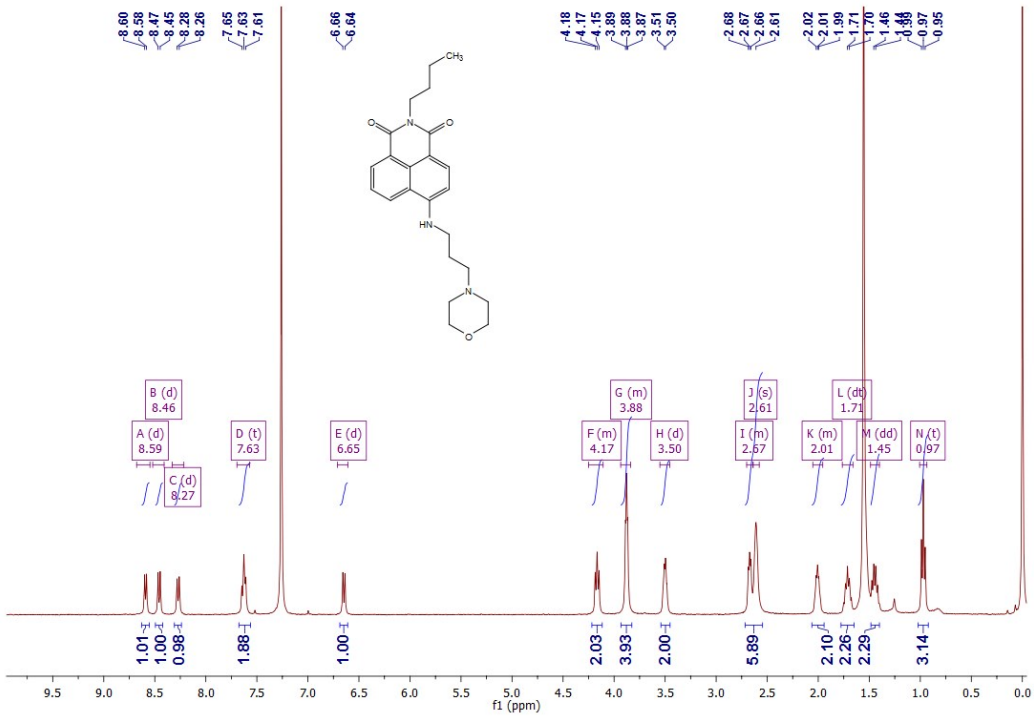


Fig. S35a: ^{1}H NMR of **NIMC3** in $CDCl_3$

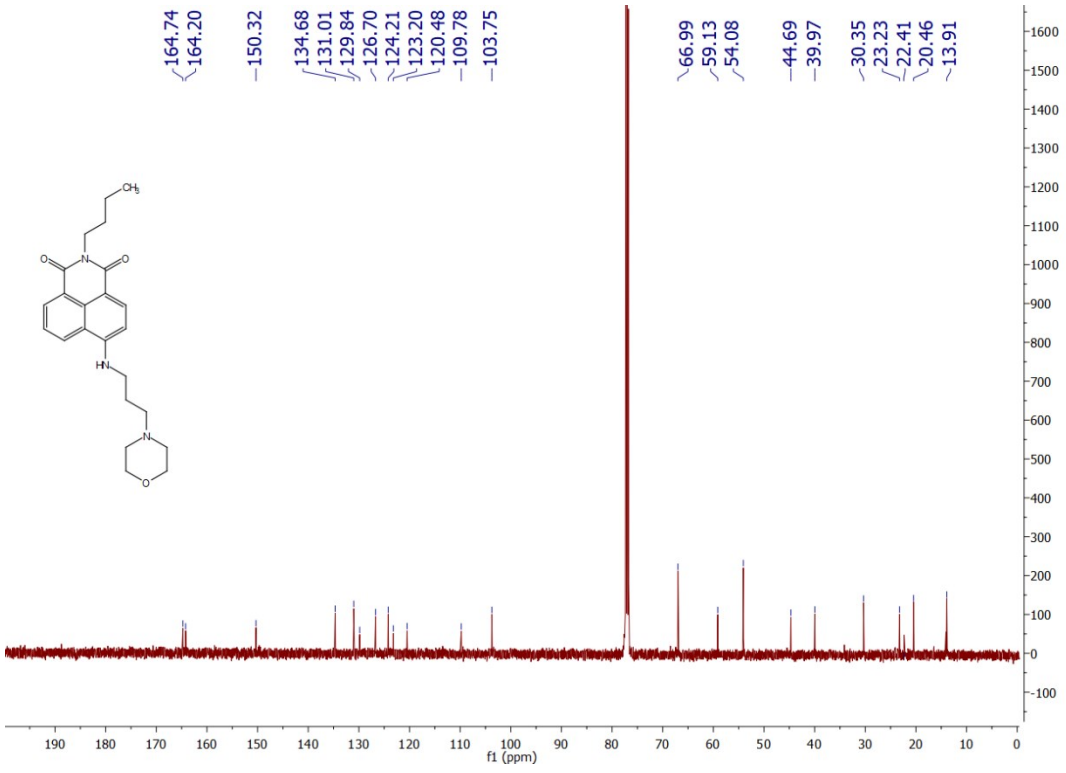


Fig. S35b: ^{13}C NMR of **NIMC3** in $CDCl_3$

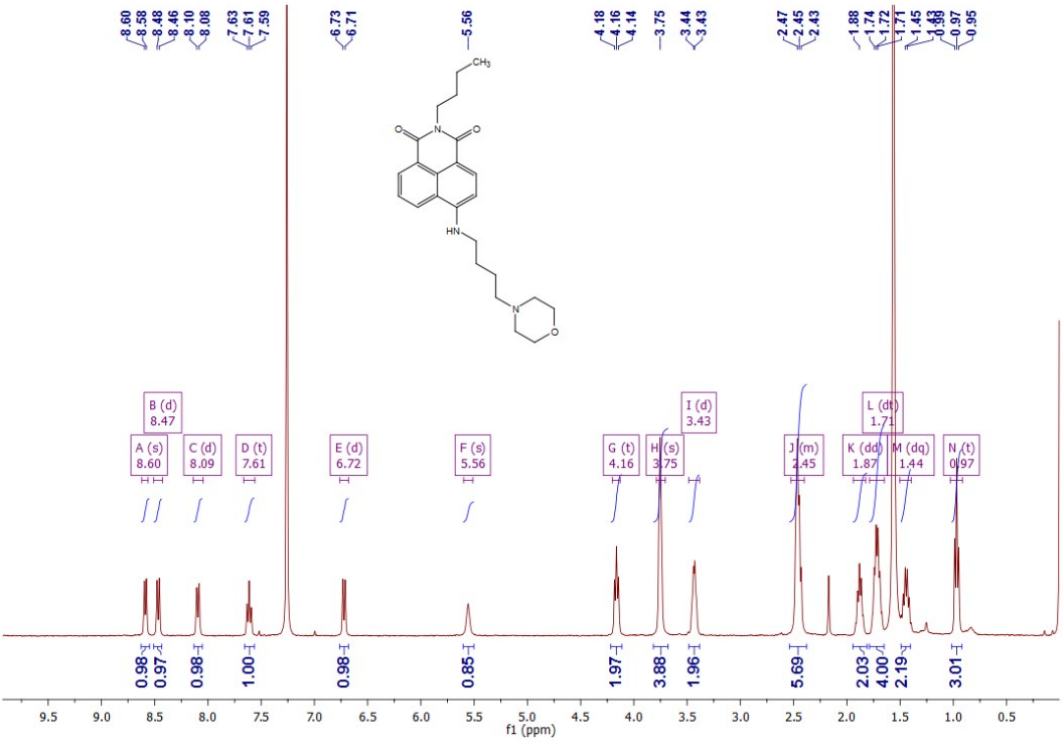


Fig. S36a: ^{1}H NMR of **NIMC4** in $CDCl_3$

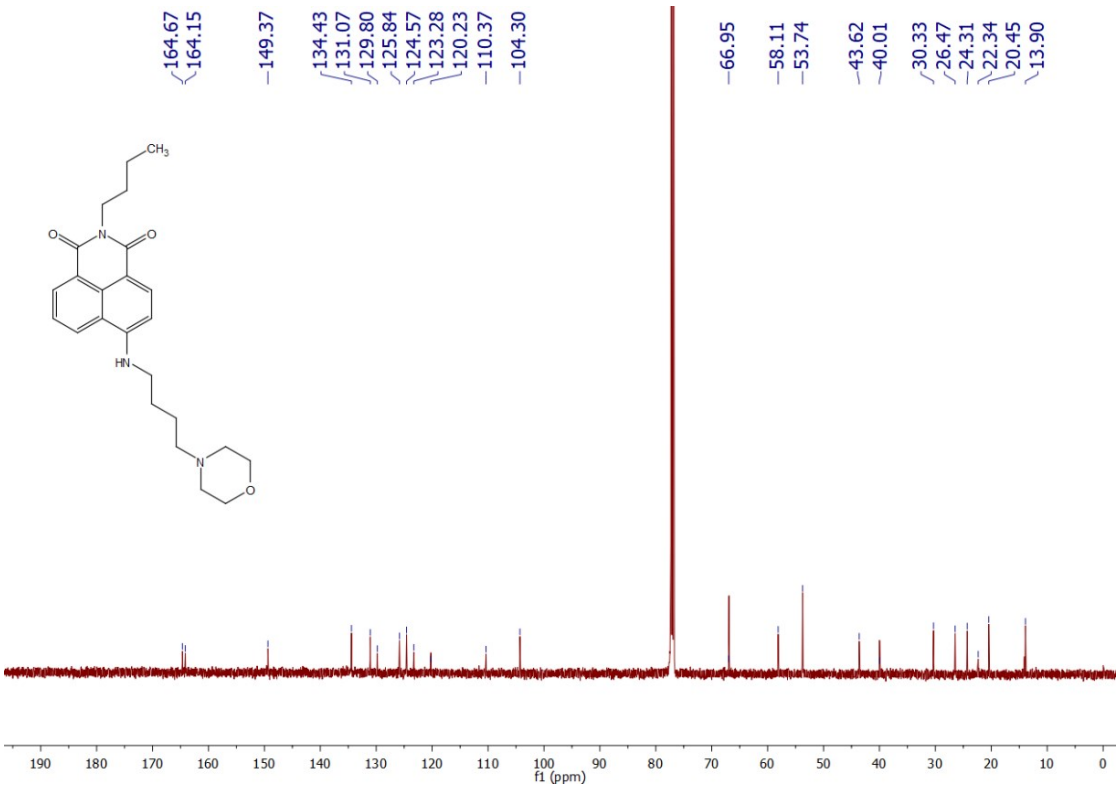


Fig. S36b: ^{13}C NMR of **NIMC4** in $CDCl_3$

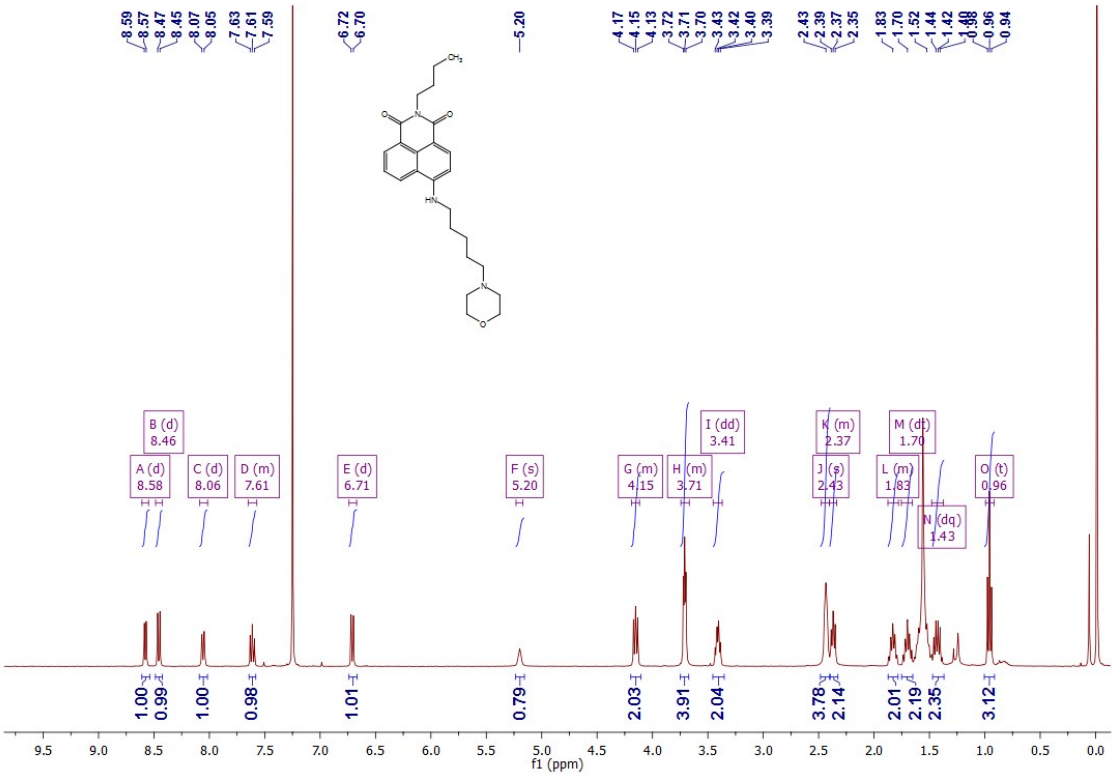


Fig. S37a: ^{1}H NMR of **NIMC5** in $CDCl_3$

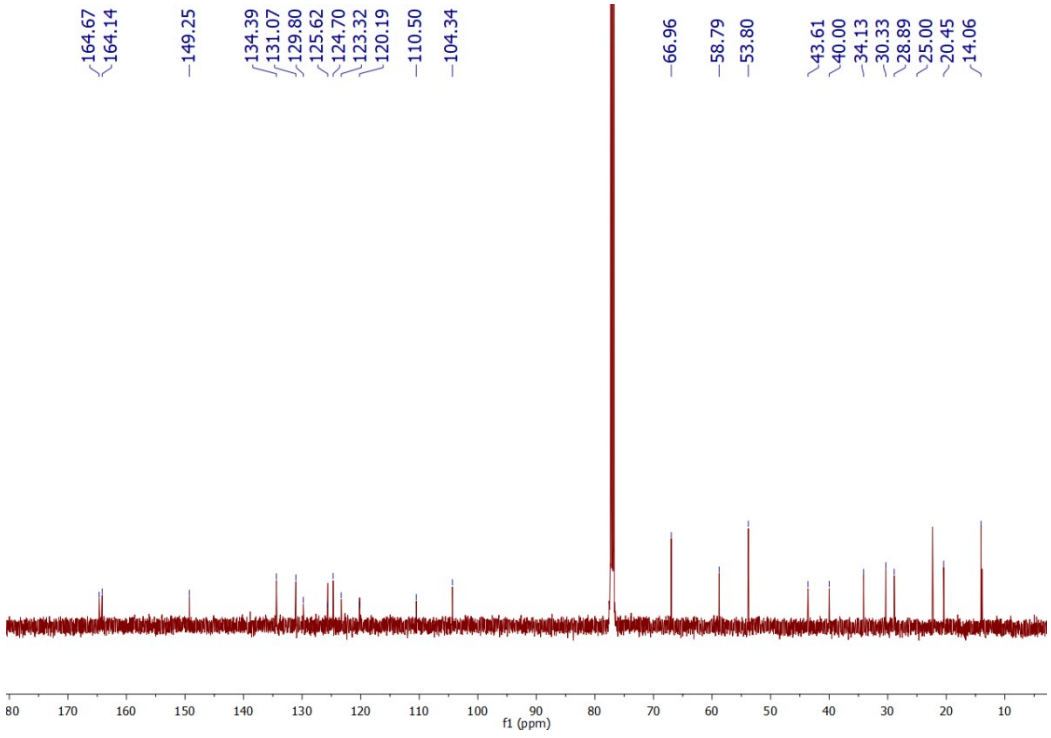


Fig. S37b: ^{13}C NMR of **NIMC5** in $CDCl_3$

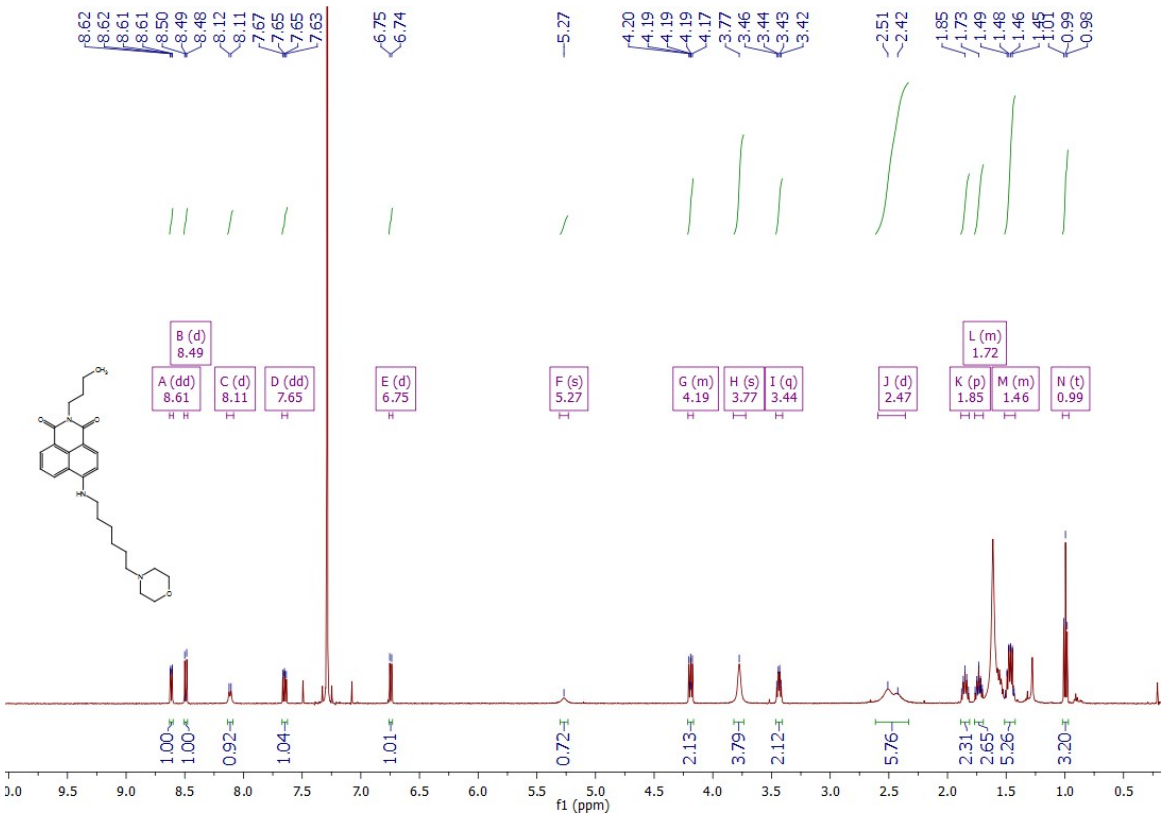


Fig. S38a: ^{1}H NMR of **NIMC6** in $CDCl_3$

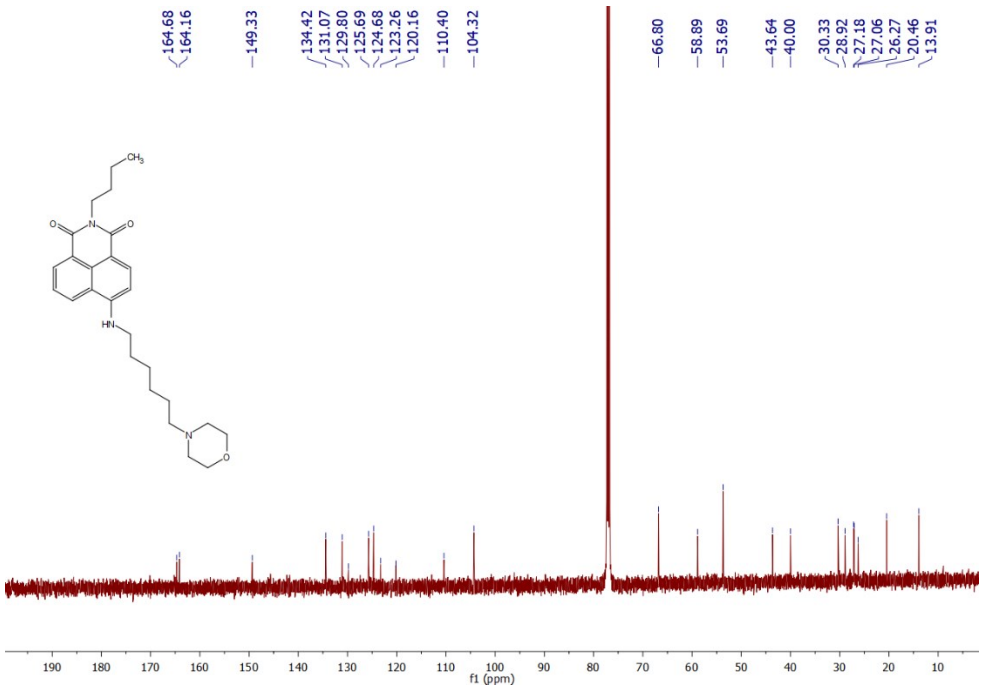


Fig. S38b: ^{13}C NMR of **NIMC6** in $CDCl_3$

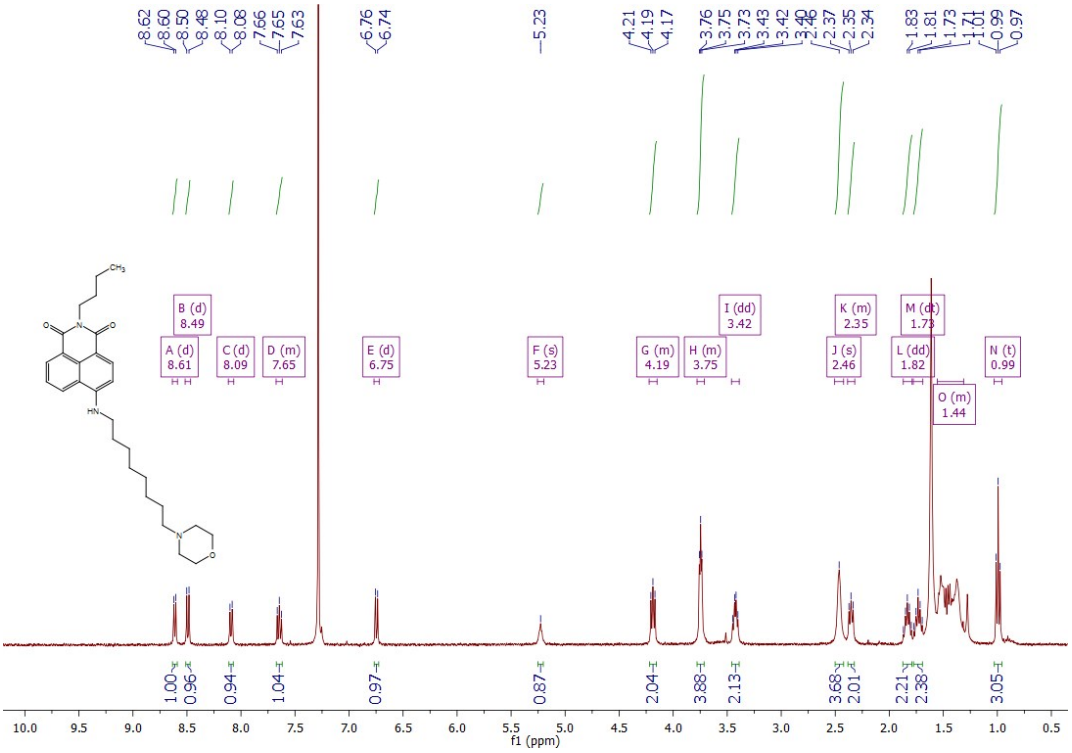


Fig. S39a: ^{1}H NMR of **NIMC8** in $CDCl_3$

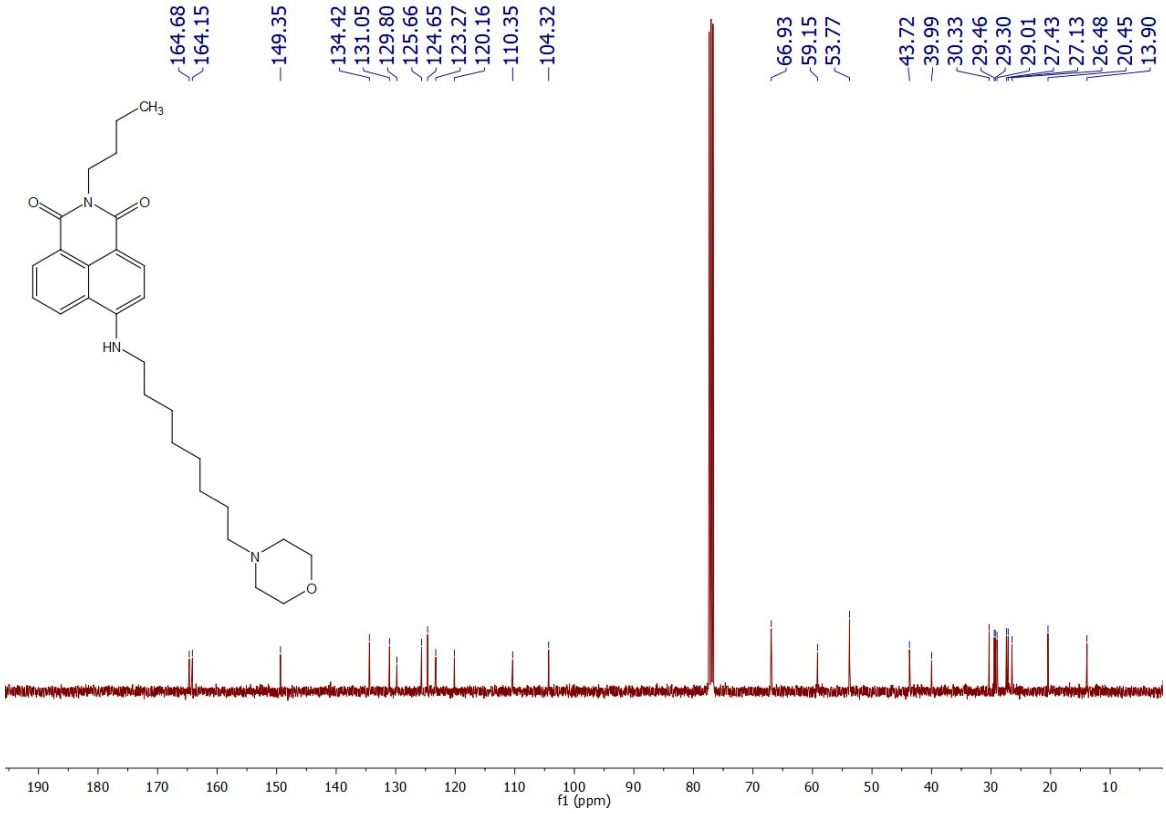


Fig. S39b: ^{13}C NMR of **NIMC8** in $CDCl_3$

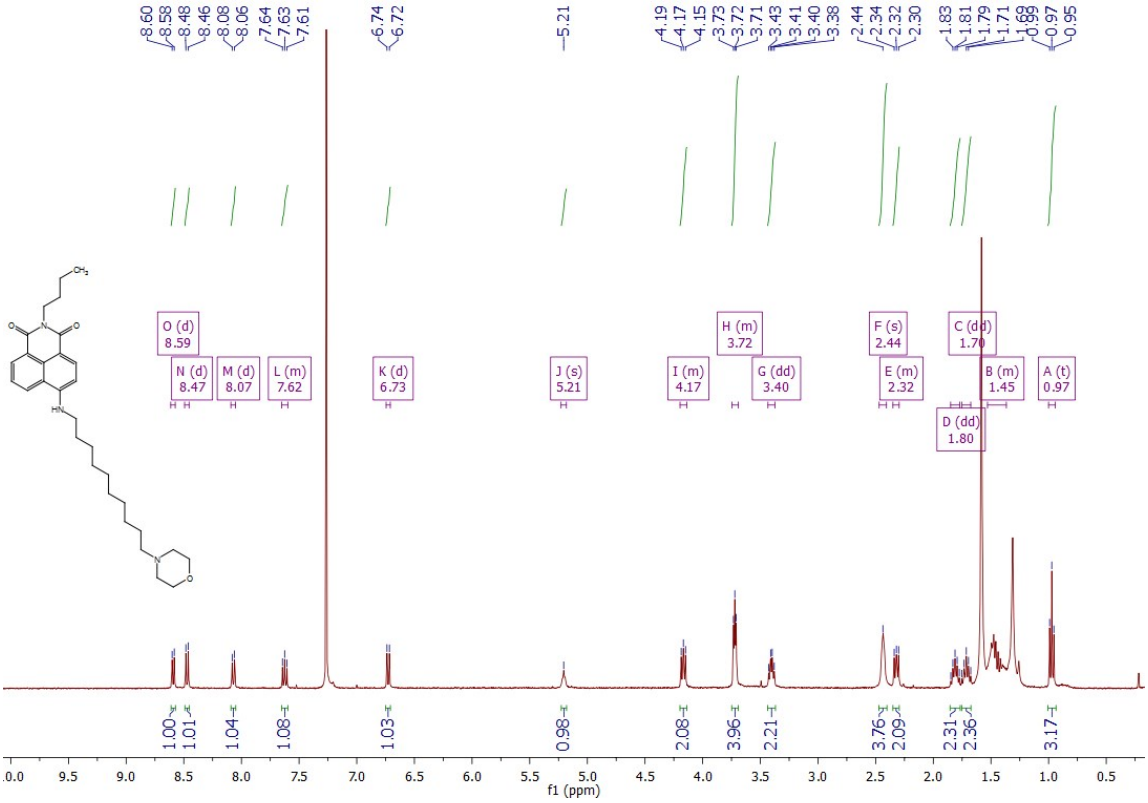


Fig. S40a: ^{1}H NMR of **NIMC10** in $CDCl_3$

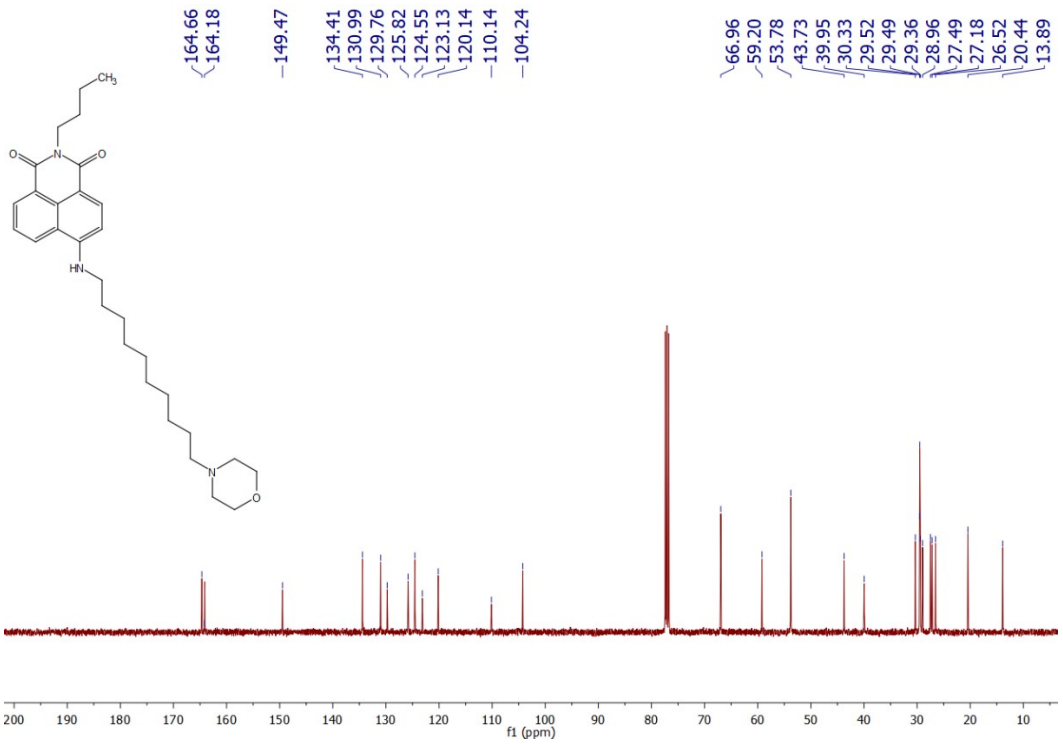


Fig. S40b: ^{13}C NMR of **NIMC10** in $CDCl_3$

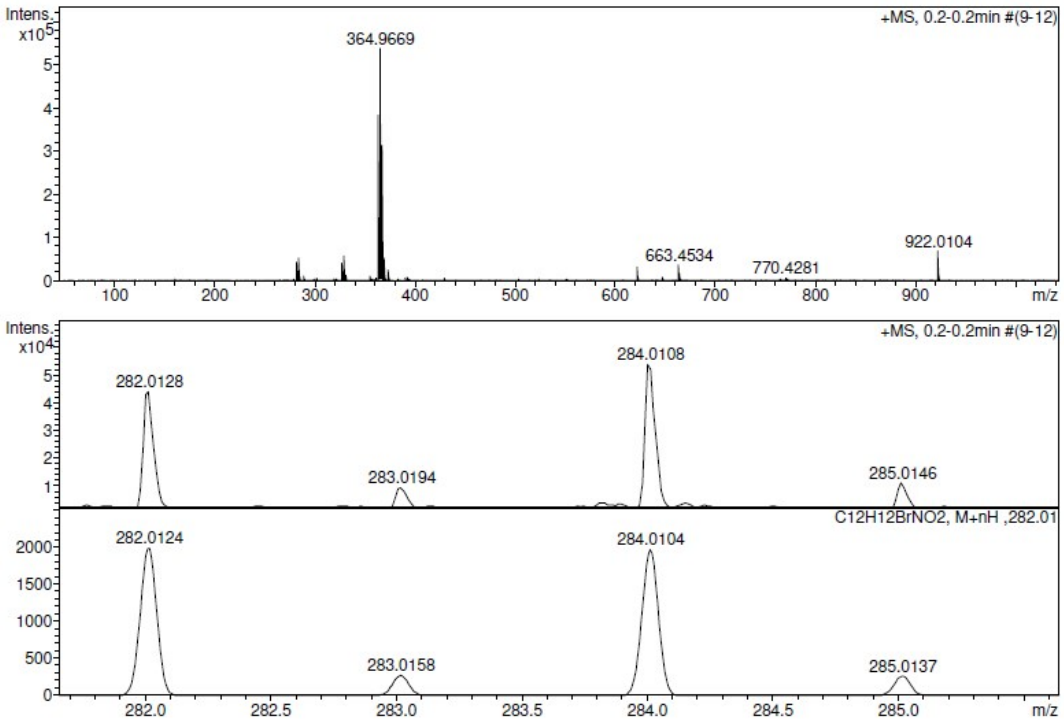


Fig. S41: ESI Mass of **PHC4Br**

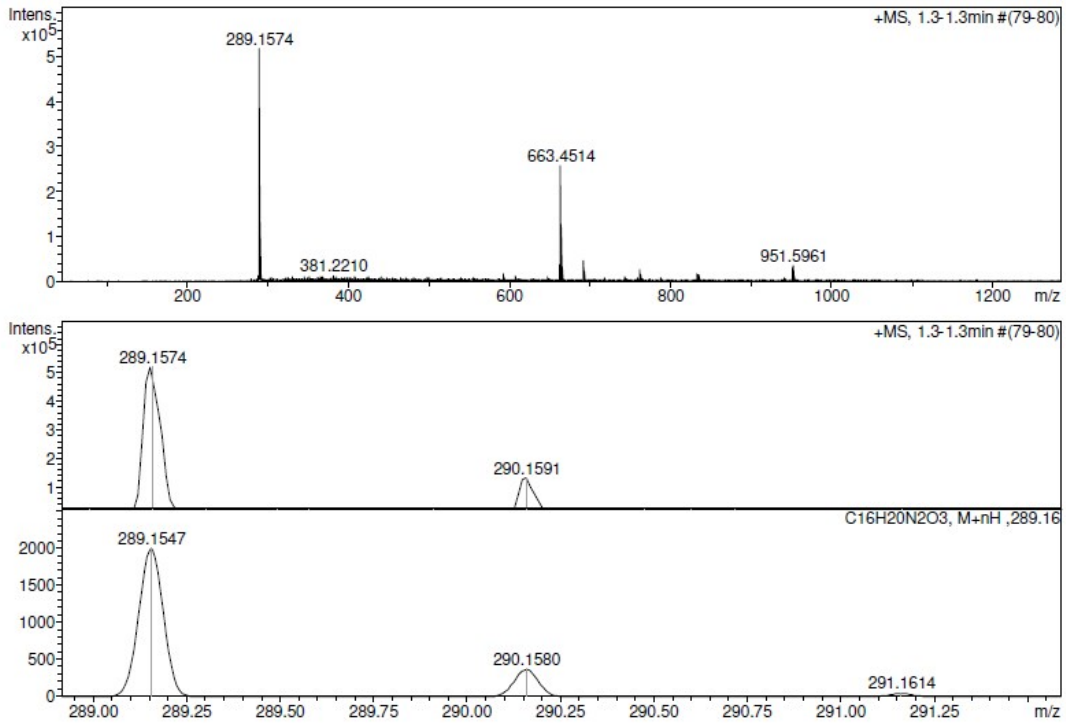


Fig. S42: ESI Mass of **PHC4M**

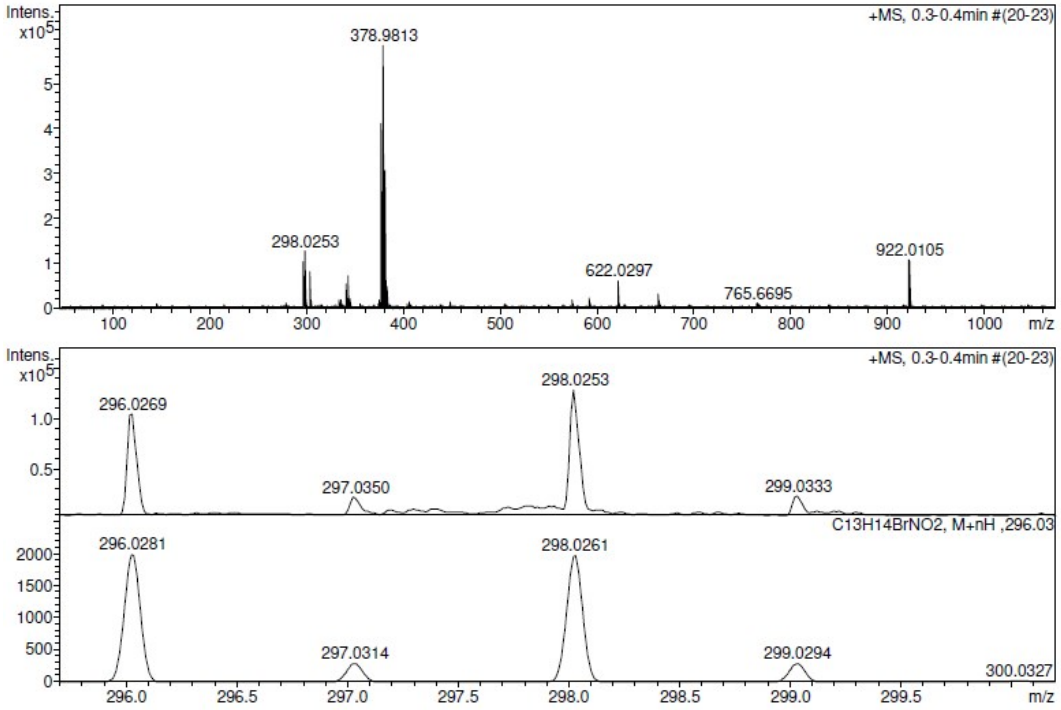


Fig. S43: ESI Mass of **PHC5Br**

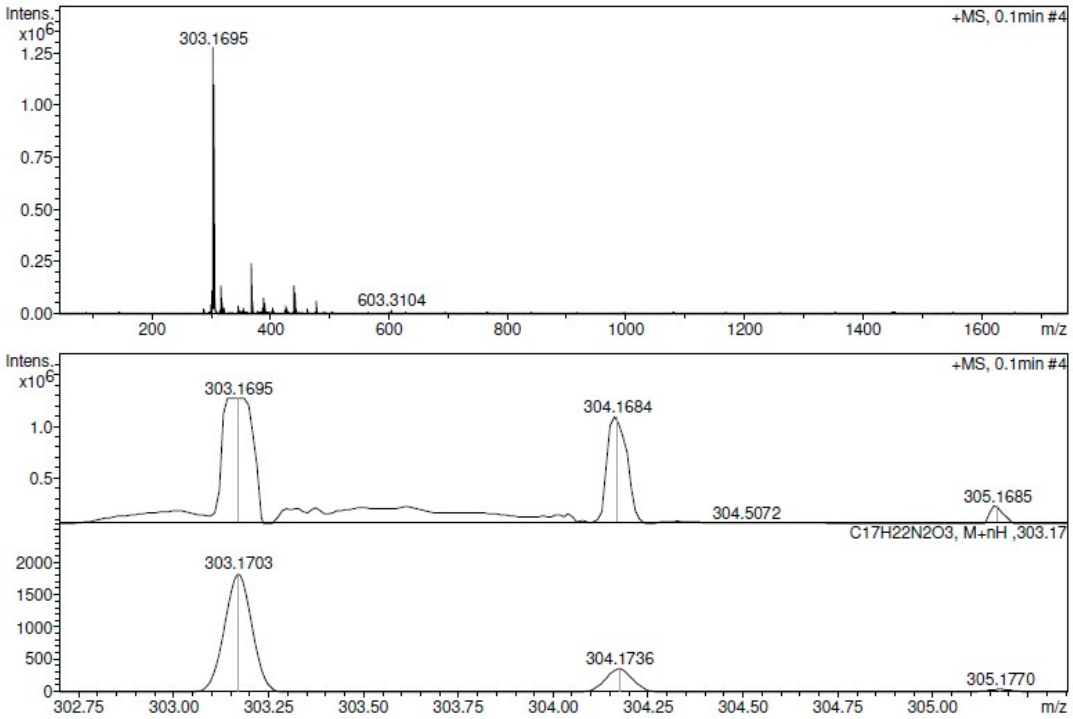


Fig. S44: ESI Mass of **PHC5M**

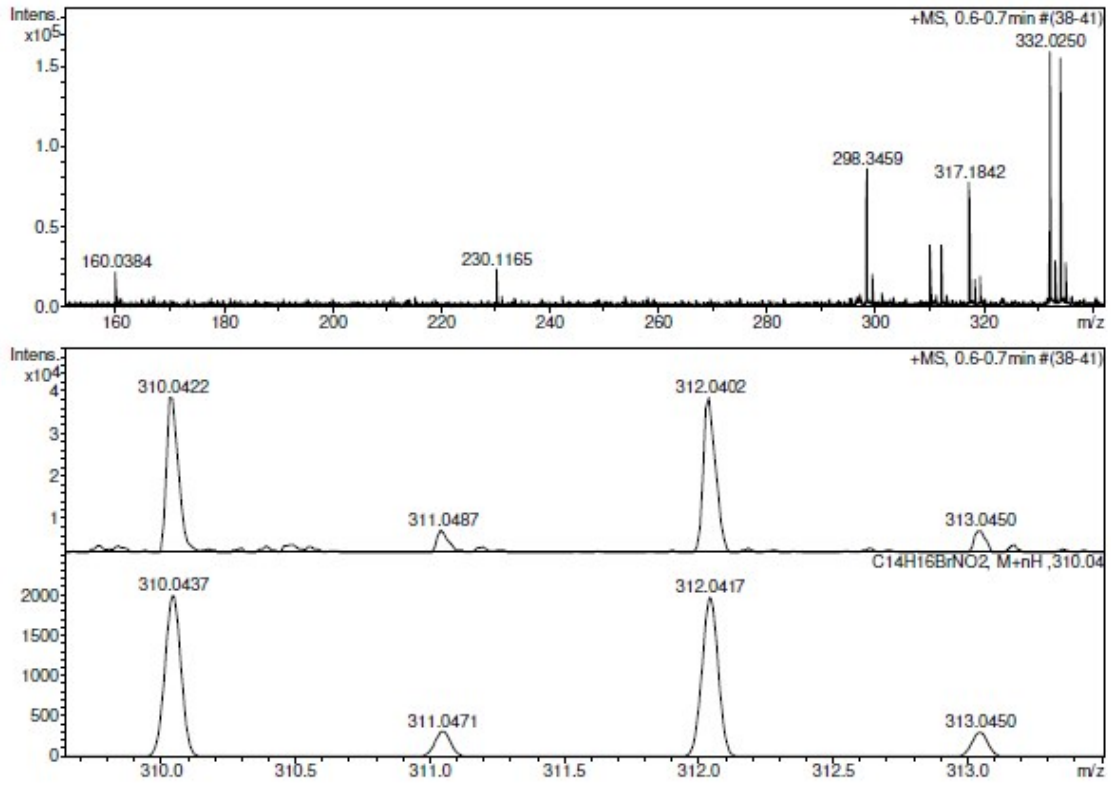


Fig. S45: ESI Mass of **PHC6Br**

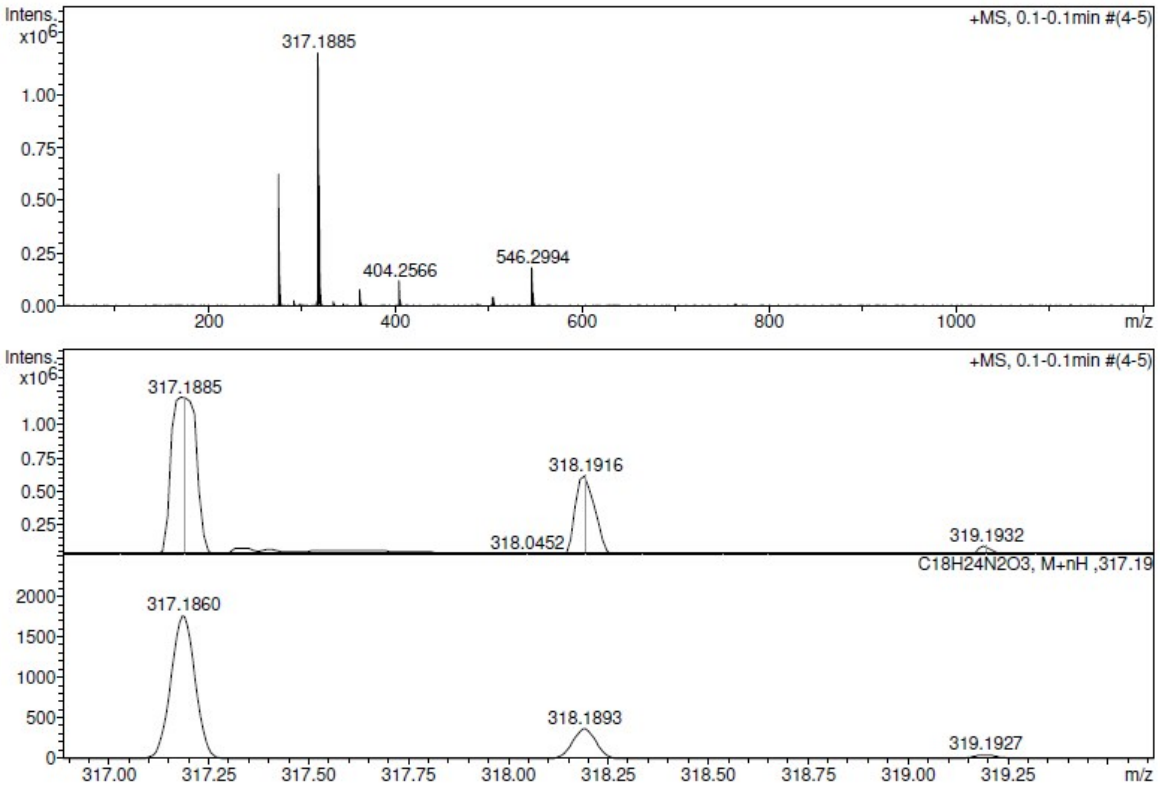


Fig. S46: ESI Mass of **PHC6M**

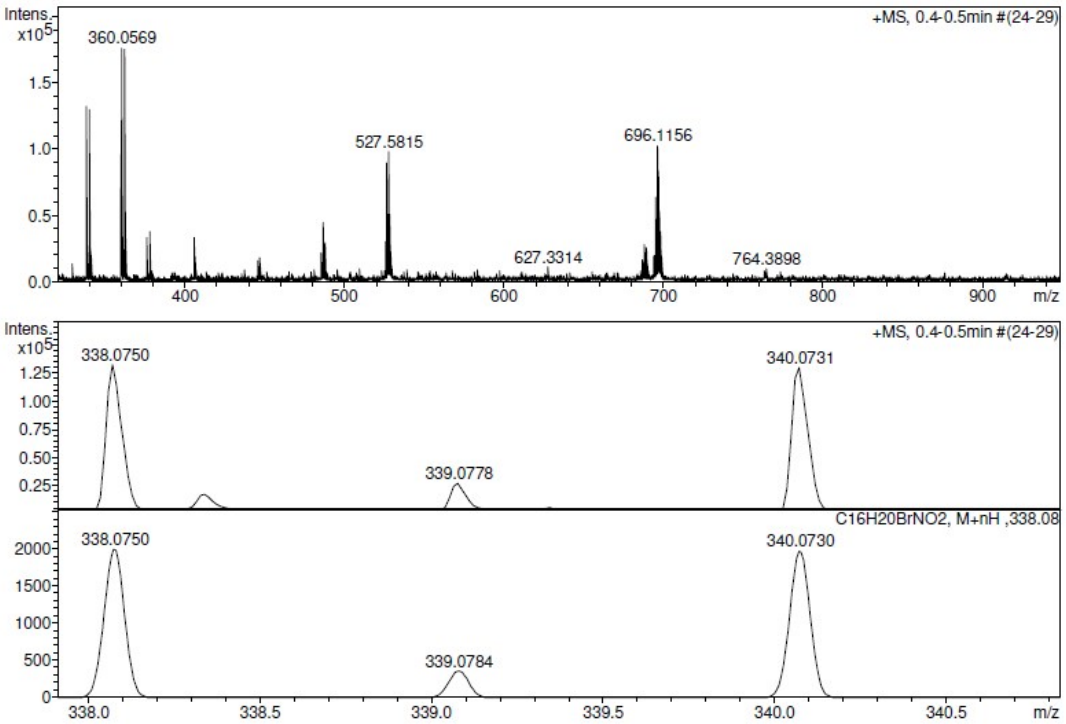


Fig. S47: ESI Mass of **PHC8Br**

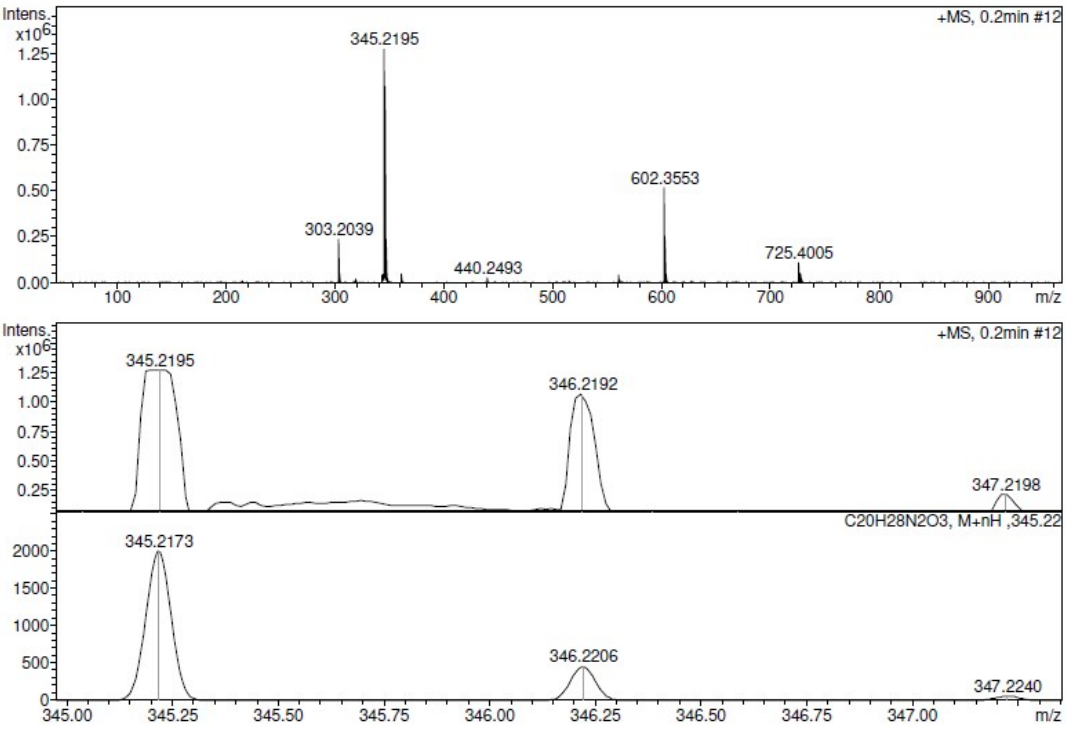


Fig. S48: ESI Mass of **PHC8M**

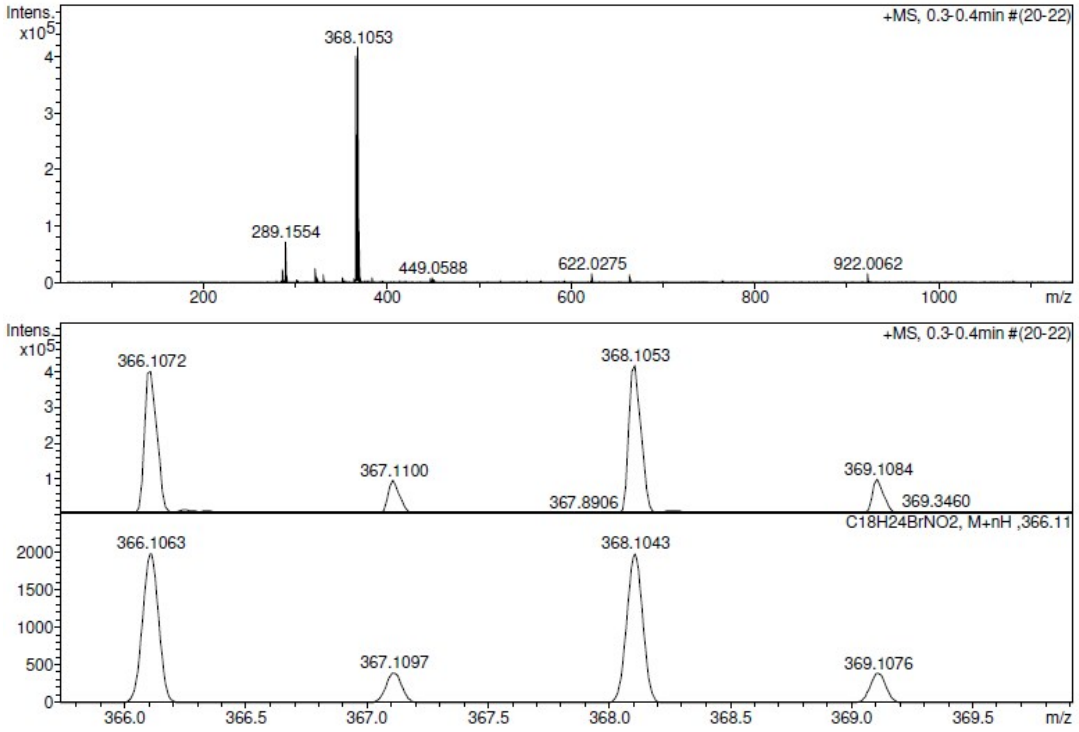


Fig. S49: ESI Mass of **PHC10Br**

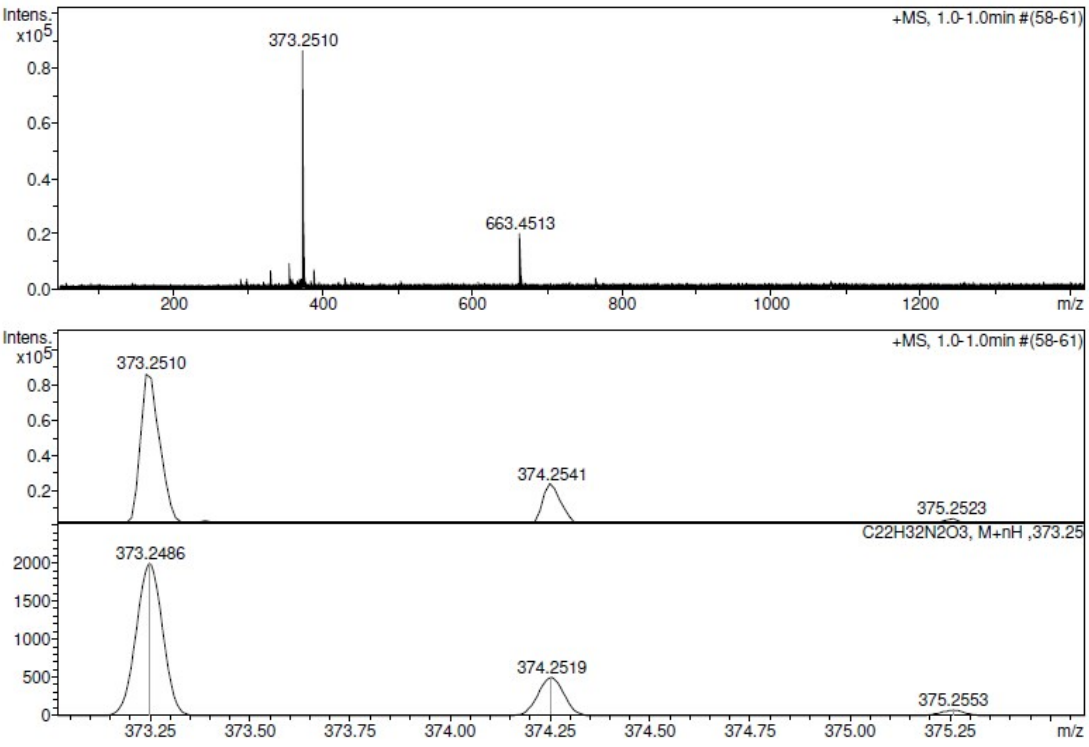


Fig. S50: ESI Mass of **PHC10M**

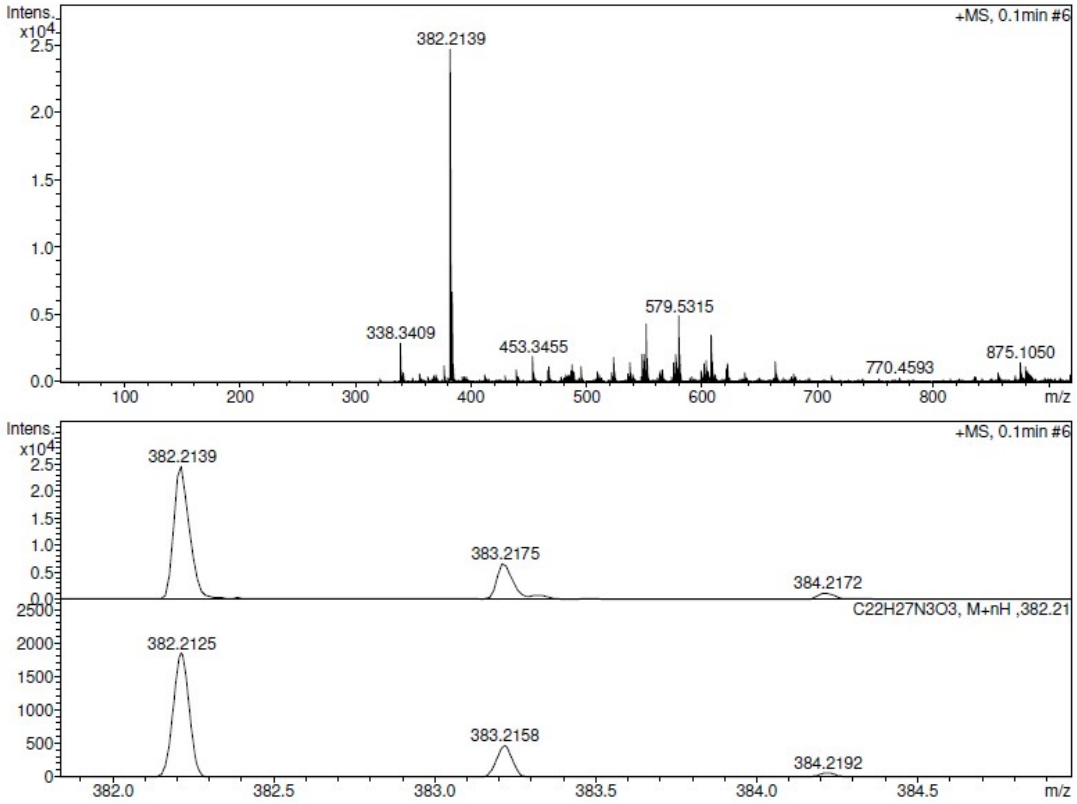


Fig. S51: APCI Mass of **NIMC2**

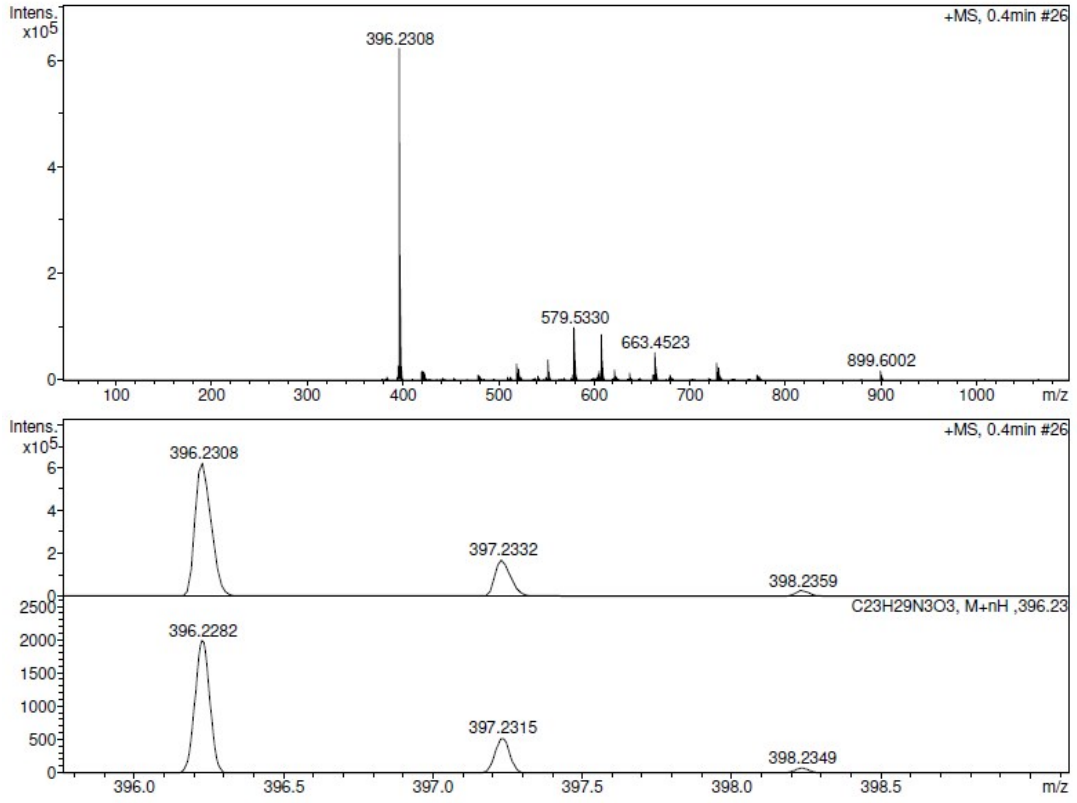


Fig. S52: APCI Mass of **NIMC3**

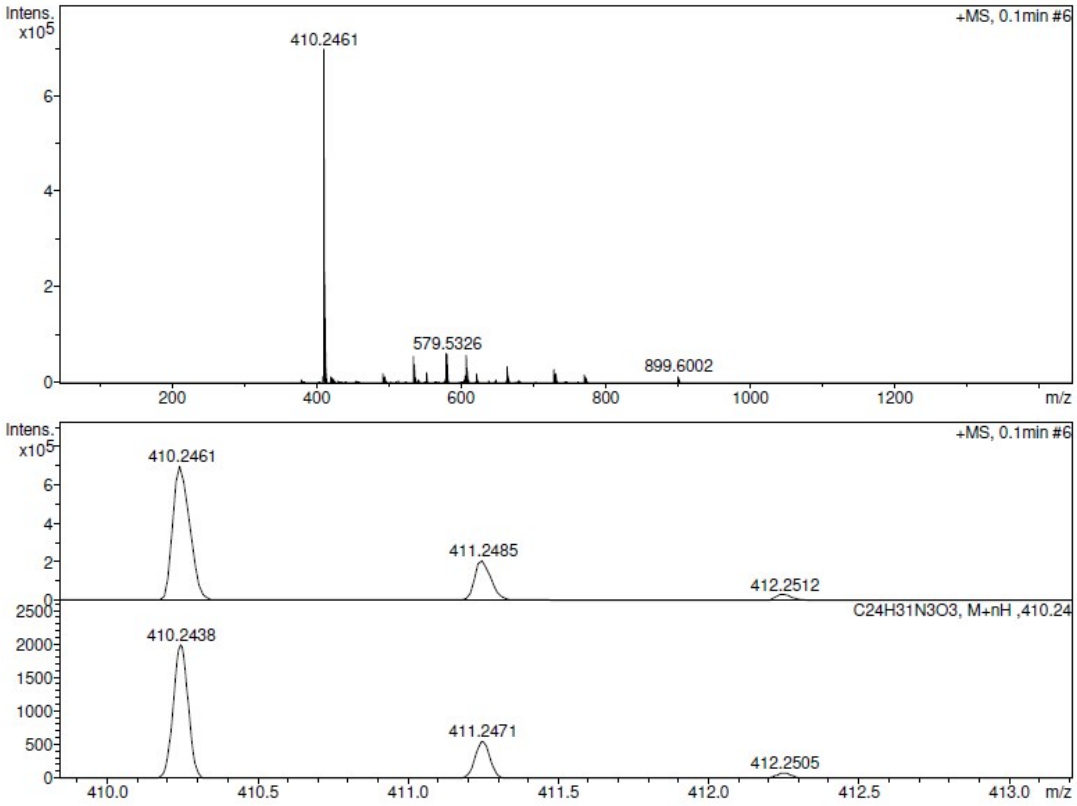


Fig. S53: APCI Mass of **NIMC4**

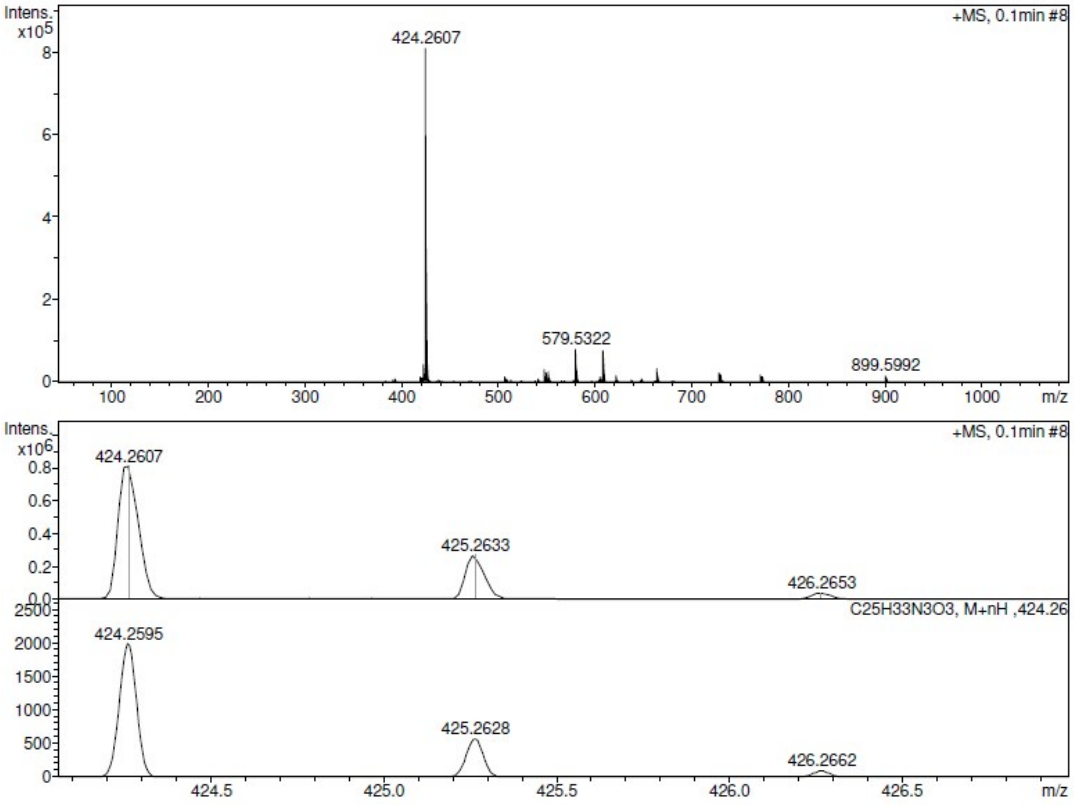


Fig. S54: APCI Mass of **NIMC5**

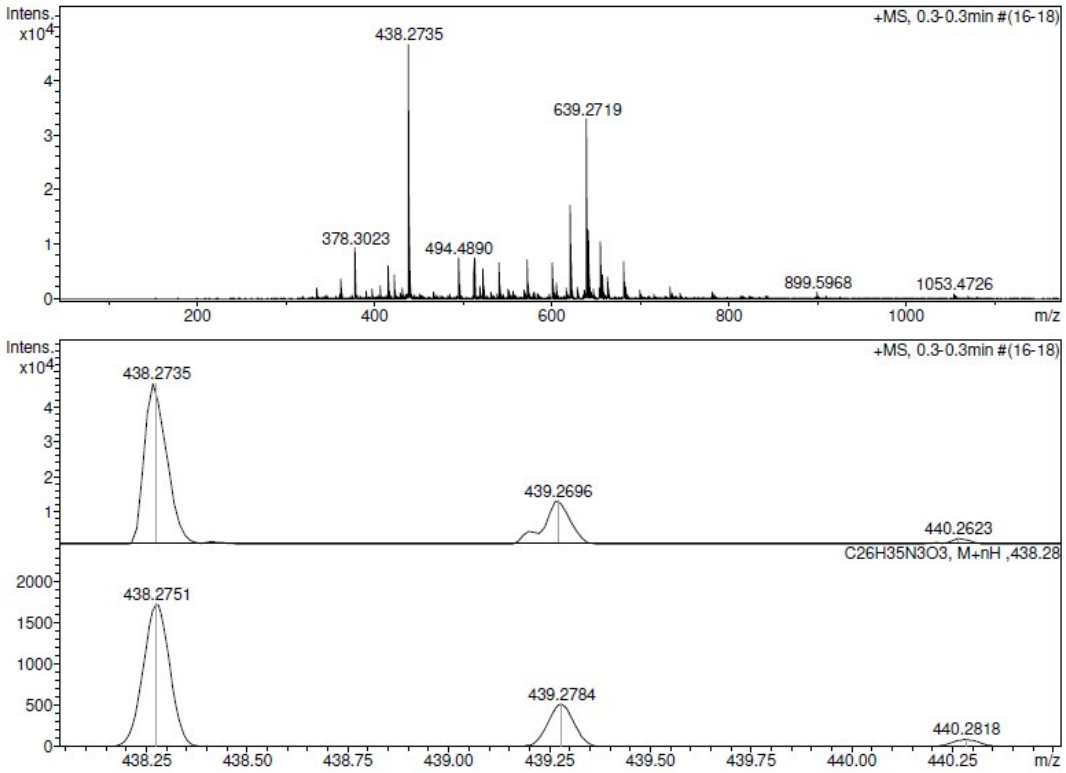


Fig. S55: APCI Mass of **NIMC6**

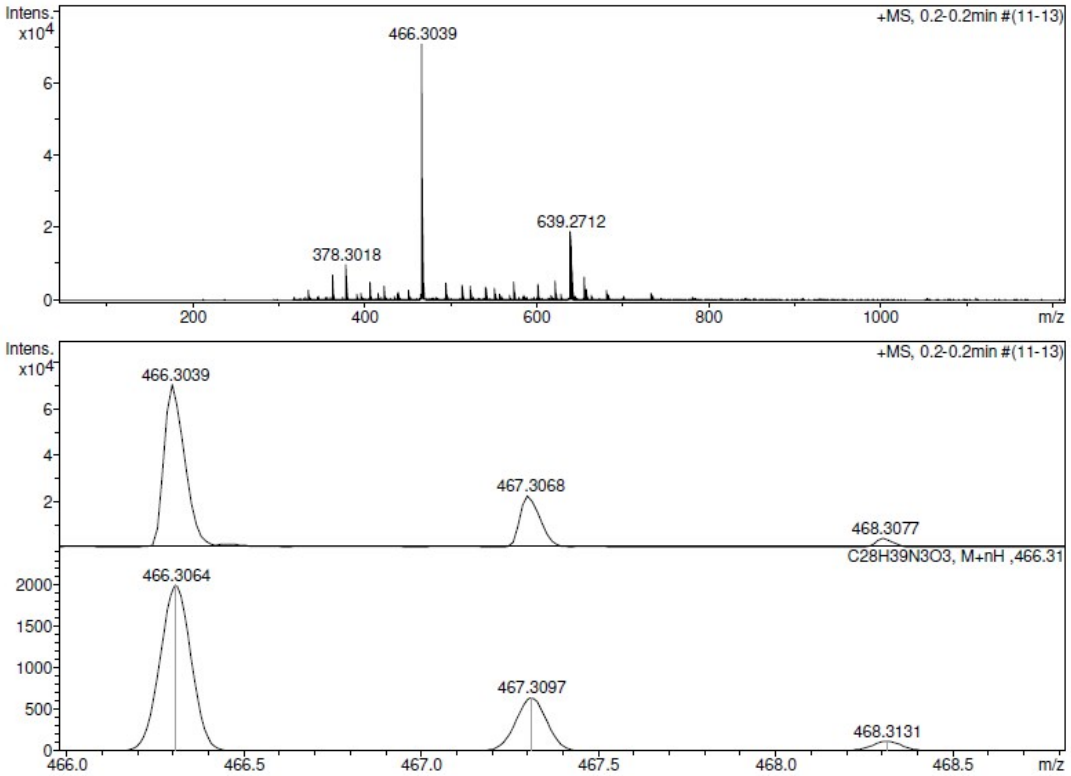


Fig. S56: APCI Mass of **NIMC8**

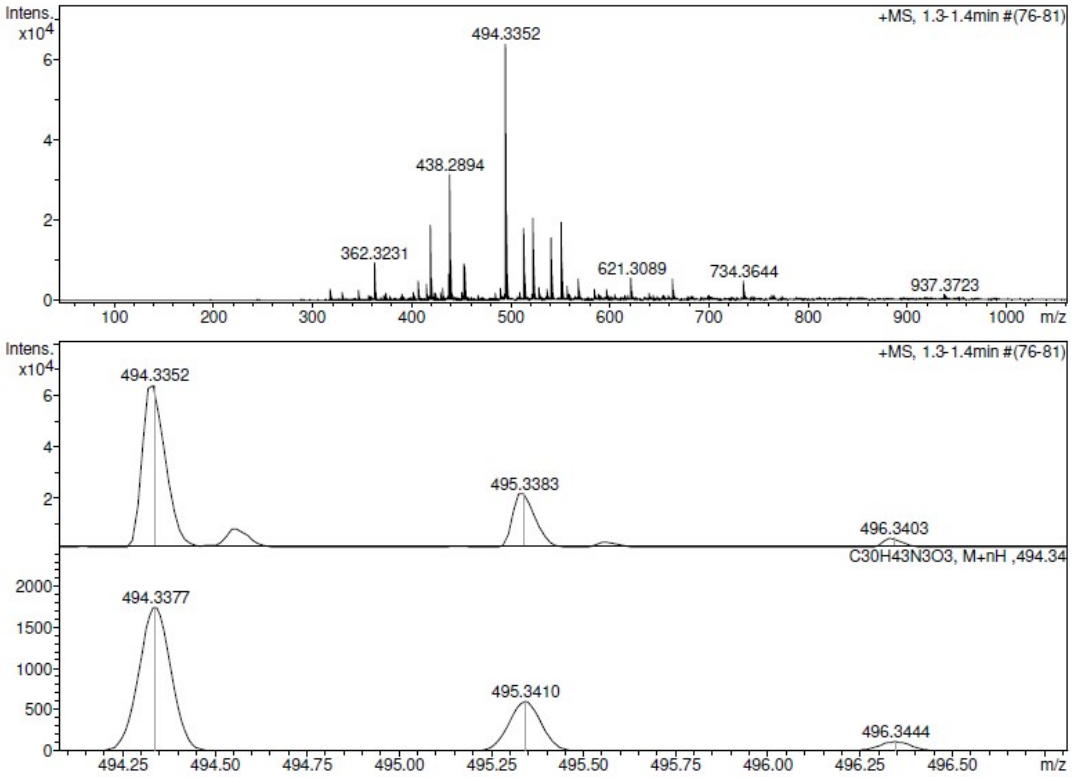


Fig. S57: APCI Mass of **NIMC10**

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