Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.


**Supporting Information for**

# Construction and Bioimaging Application of Novel Indole Heptamethine Cyanines Containing Functionalized Tetrahydropyridine Ring

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# 1. TD-DFT calculation

**Table S1** Major electronic excitations for **Cy-NH**, **Cy-NMe**, **Cy-MP**, **Cy-NNBD** and **Cy-MPM**

| Compound | Excited state | $\lambda$/nm [eV] | Osc. Str (f) | Major contributions |
|---|---|---|---|---|
| **Cy-NH** | $S_0 \rightarrow S_1$ | 619.47 [2.0014] | 2.4463 | HOMO→LUMO (71%) |
| **Cy-NMe** | $S_0 \rightarrow S_1$ | 619.84 [2.0003] | 2.4321 | HOMO→LUMO (71%) |
| **Cy-MP** | $S_0 \rightarrow S_1$ | 617.82 [2.0068] | 2.4045 | HOMO→LUMO (71%) |
| **Cy-NNBD** | $S_0 \rightarrow S_1$ | 615.53 [2.0143] | 2.3578 | HOMO→LUMO (69%) |
| | $S_0 \rightarrow S_4$ | 437.55 [2.8336] | 0.3285 | HOMO→LUMO (64%) |
| **Cy-MPM** | $S_0 \rightarrow S_1$ | 561.78 [2.2070] | 1.9264 | HOMO→LUMO (71%) |
| | $S_0 \rightarrow S_8$ | 326.92 [3.7925] | 0.3473 | HOMO→LUMO (61%) |

## 2. Photophysical property

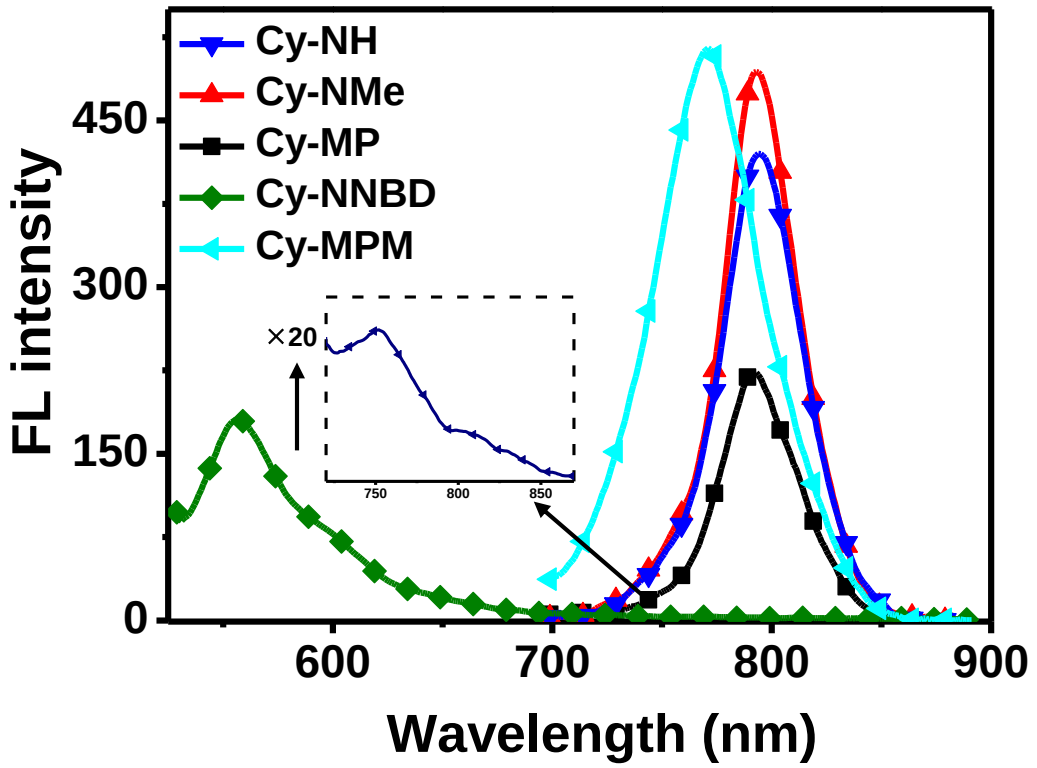



**Figure S1**. Fluorescence spectra of **Cy-NH** and its derivatives (10 μM) in PBS buffer (PH=7.4) at the same condition ($\lambda_{ex}$ = 680 nm for for **Cy-NH**, **Cy-NMe**, **Cy-MP**, **Cy-MPM**, $\lambda_{ex}$ = 500 nm for **Cy-NNBD**, the insert image was excited at 680 nm of **Cy-NNBD**).

## 2. Photophysical property

**Table S2** Optical properties of IR780, **Cy-NH**, **Cy-NMe**, **Cy-MP**, **Cy-NNBD** and **Cy-MPM**

| | **IR780** | **Cy-NH** | **Cy-NMe** | **Cy-MP** | **Cy-NNBD** | **Cy-MPM** |
|---|---|---|---|---|---|---|
| $\lambda_{abs}$ (nm) | 780 | 756 | 764 | 766 | 680 | 683 |
| $\lambda_{em}$ (nm) | 798 | 794 | 793 | 791 | 750 | 770 |
| Stoke shift (nm) | 18 | 38 | 29 | 25 | 70 | 83 |
| $\varepsilon_{max}$ ($cm^{-1}$ $mol \cdot L^{-1}$) | 79760 | 138540 | 95836 | 53954 | 44162 | 27294 |

# Photo stability

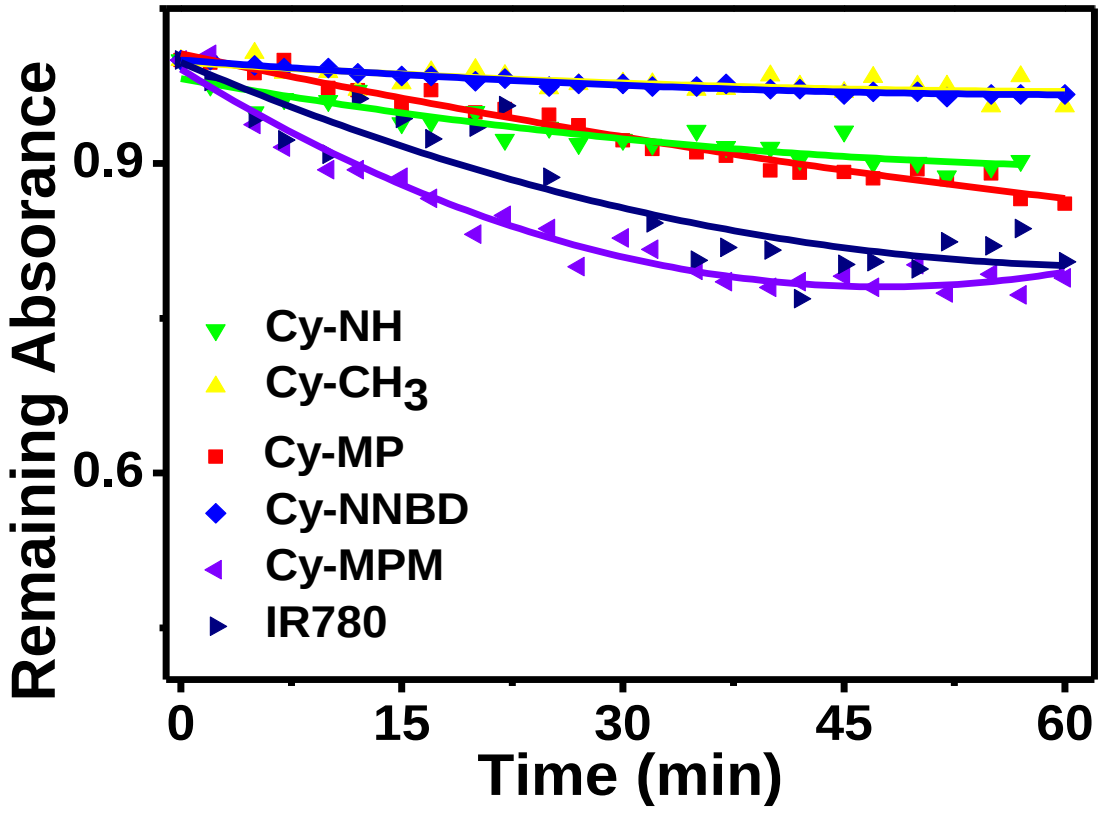



**Figure S2.** The photo stability of **Cy-NH** and its derivatives according to the changes of absorption spectra in PBS buffer (PH = 7.4) upon irradiation with tungsten lamp (7 w) for 1 h.

## 3. Water solubility
## Cy-NH

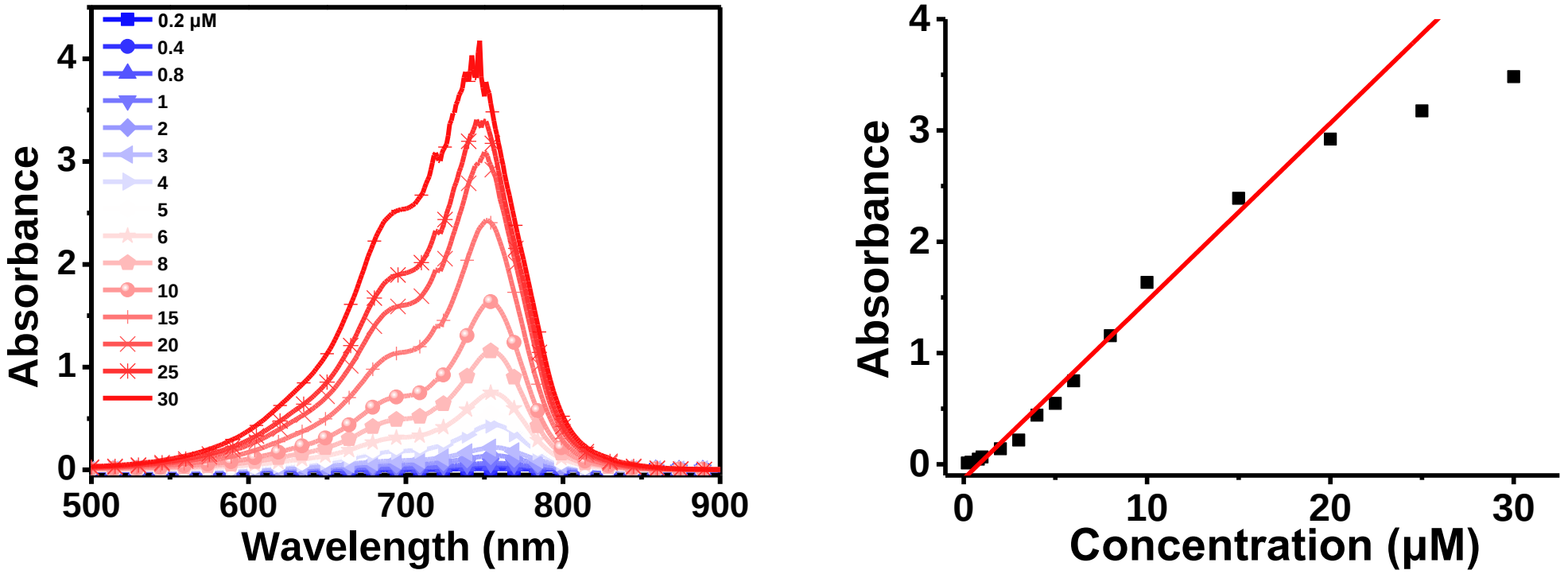



**Figure S3.** (A) Absorbance spectra and (B) plot of absorbance against the concentration of the dye for **Cy-NH** in $H_2O$.

# Water solubility
# Cy-NMe

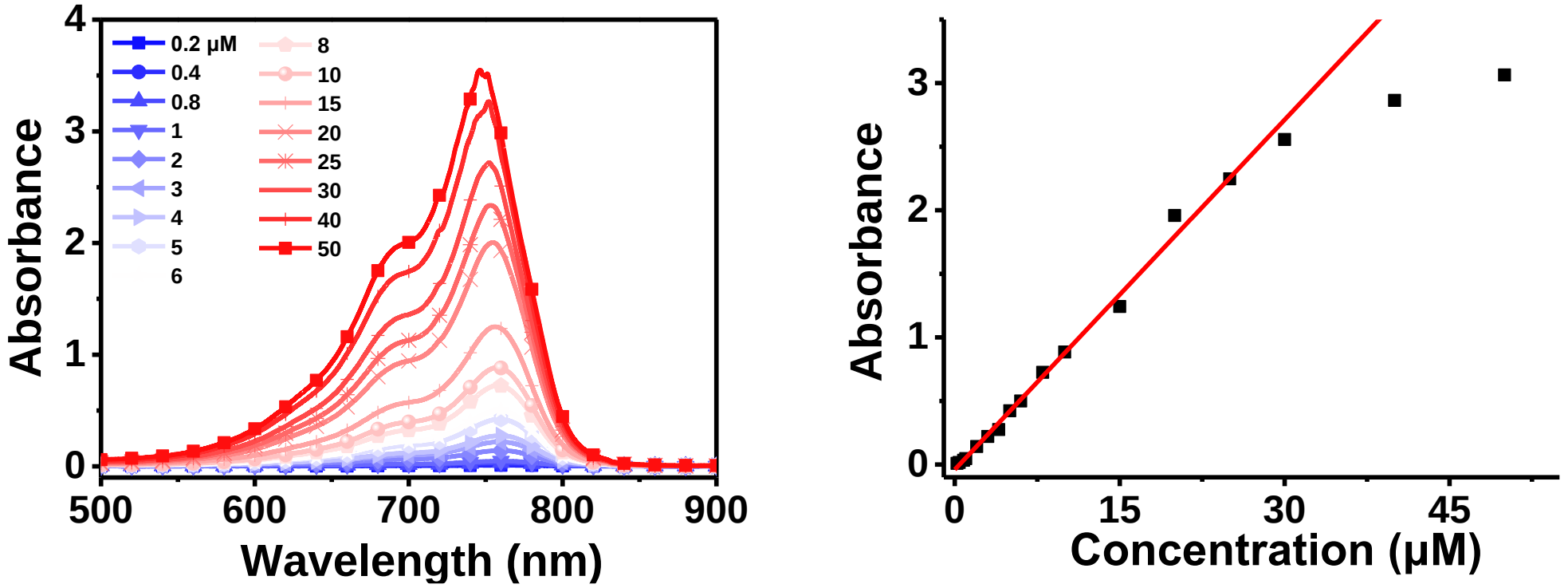



**Figure S4.** (A) Absorbance spectra and (B) plot of absorbance against the concentration of the dye for **Cy-NMe** in $H_2O$.

# Water solubility
# Cy-MP

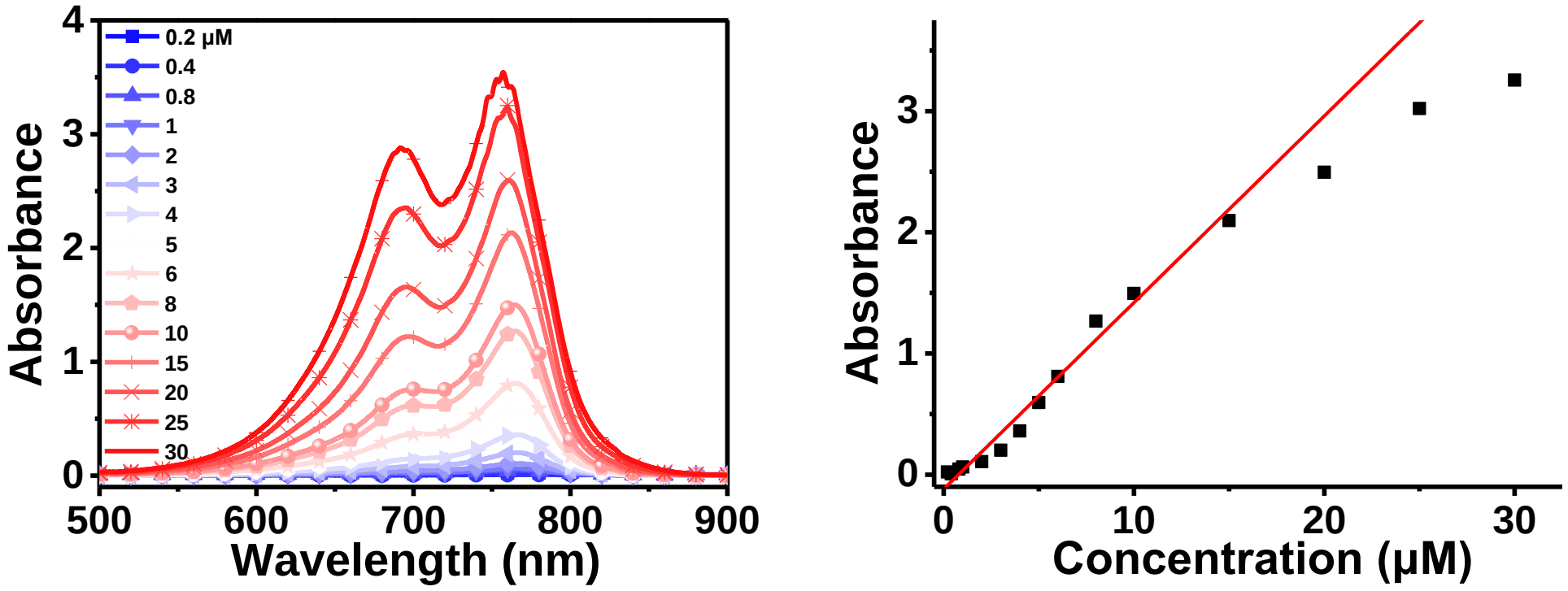



**Figure S5.** (A) Absorbance spectra and (B) plot of absorbance against the concentration of the dye for **Cy-MP** in $H_2O$.

# Water solubility
# Cy-NNBD

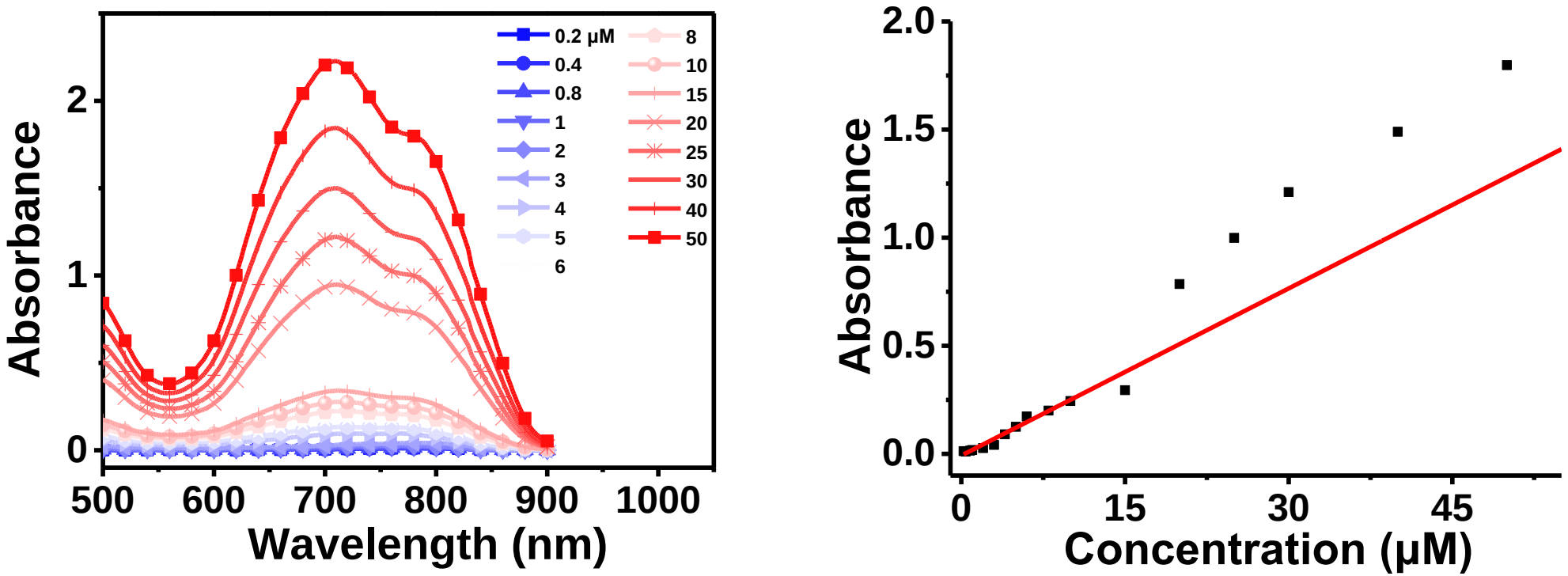



**Figure S6.** (A) Absorbance spectra and (B) plot of absorbance against the concentration of the dye for **Cy-NNBD** in $H_2O$.

# Water solubility
# Cy-MPM

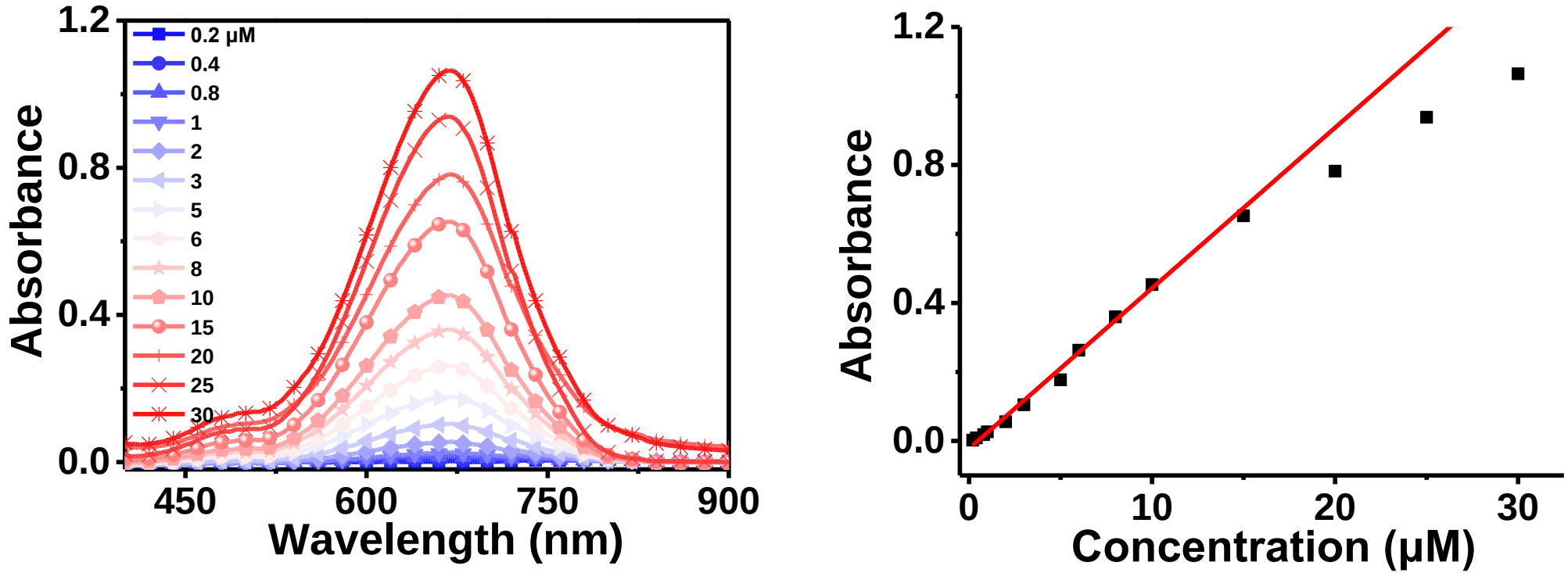



**Figure S7.** (A) Absorbance spectra and (B) plot of absorbance against the concentration of the dye for **Cy-MPM** in $H_2O$.

## 4. Cell viability
## Cy-NH

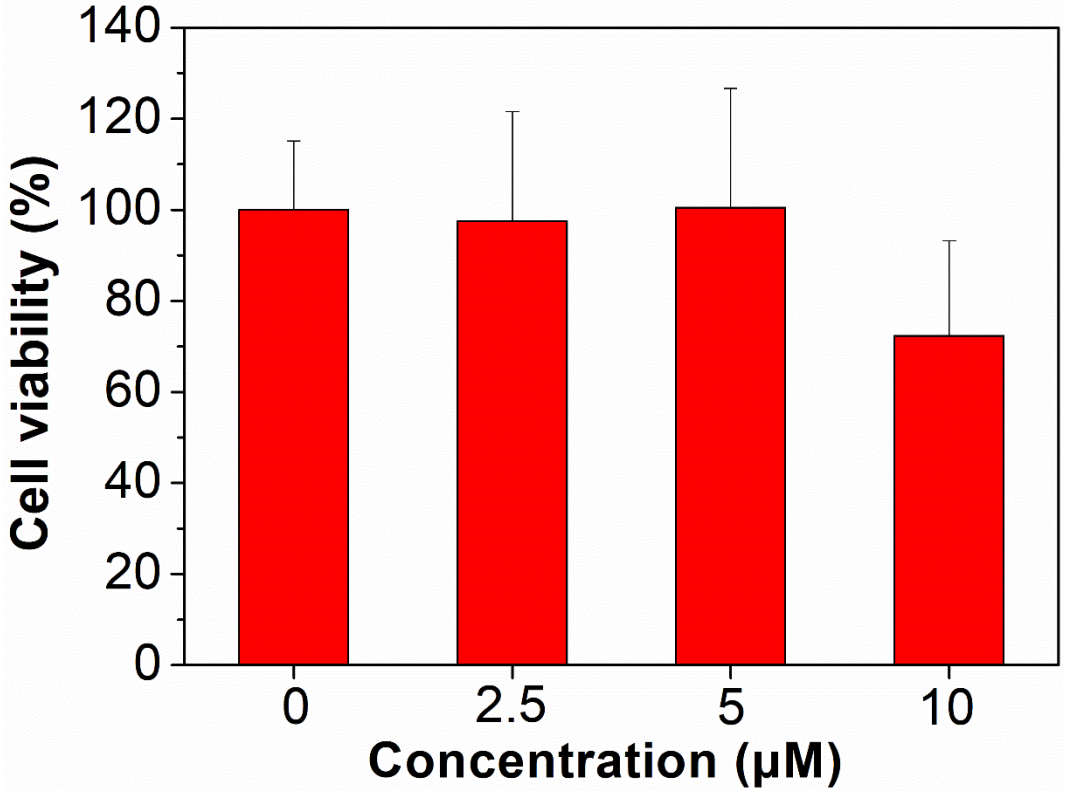



**Figure S8.** Viability of HeLa cells in the presence of **Cy-NH** as measured by using CCK-8 kit. The cells were incubated with **Cy-NH** for 24 h.

## 4. Cell viability
## Cy-NMe

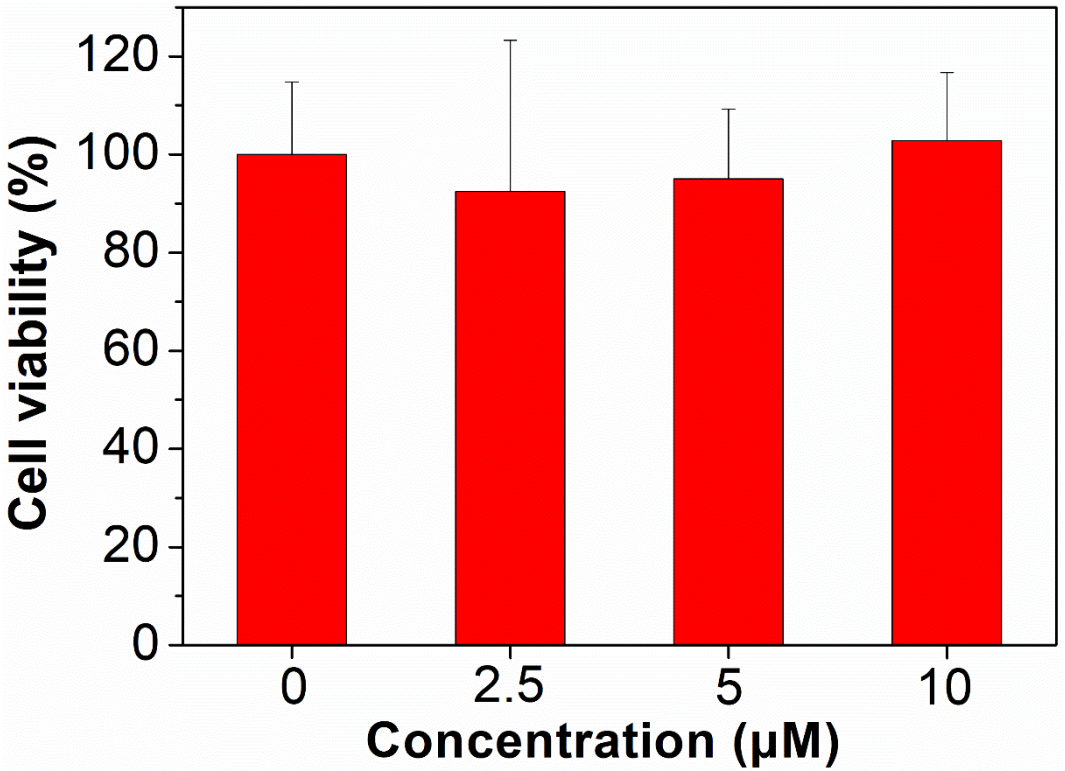



**Figure S9.** Viability of HeLa cells in the presence of **Cy-NMe** as measured by using CCK-8 kit. The cells were incubated with **Cy-NMe** for 24 h.

## 4. Cell viability
## Cy-MP

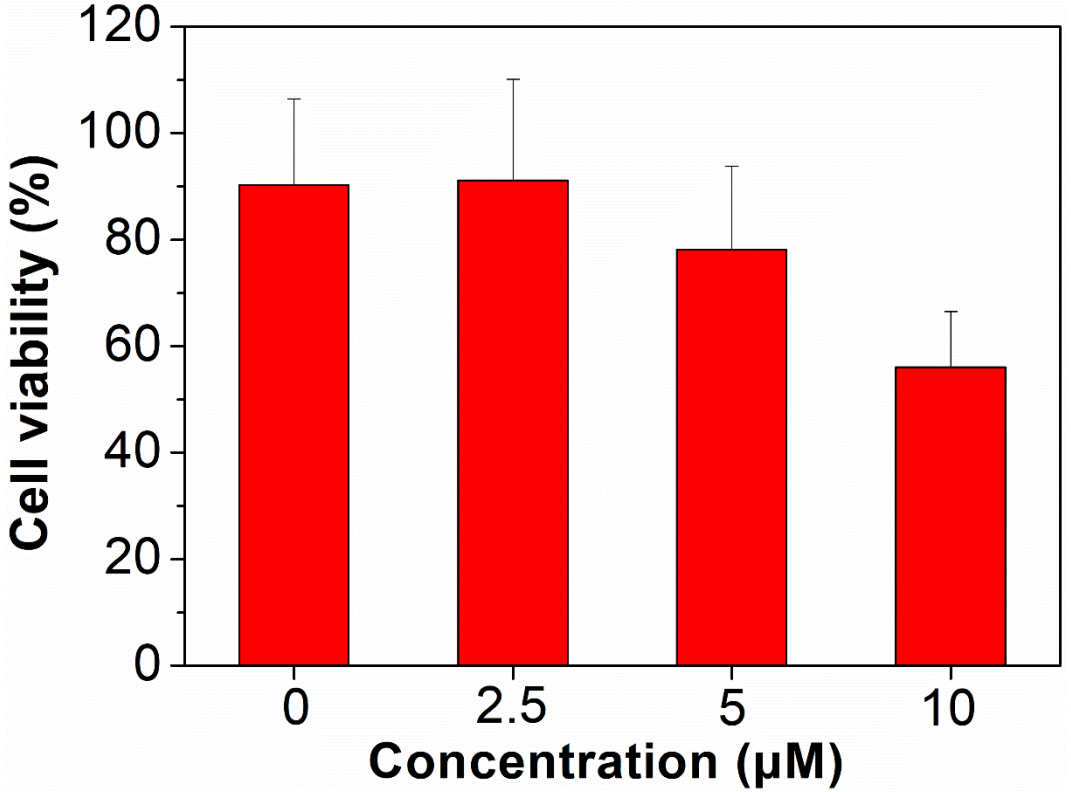



**Figure S10.** Viability of HeLa cells in the presence of **Cy-MP** as measured by using CCK-8 kit. The cells were incubated with **Cy-MP** for 2 h.

## 4. Cell viability
## Cy-NNBD

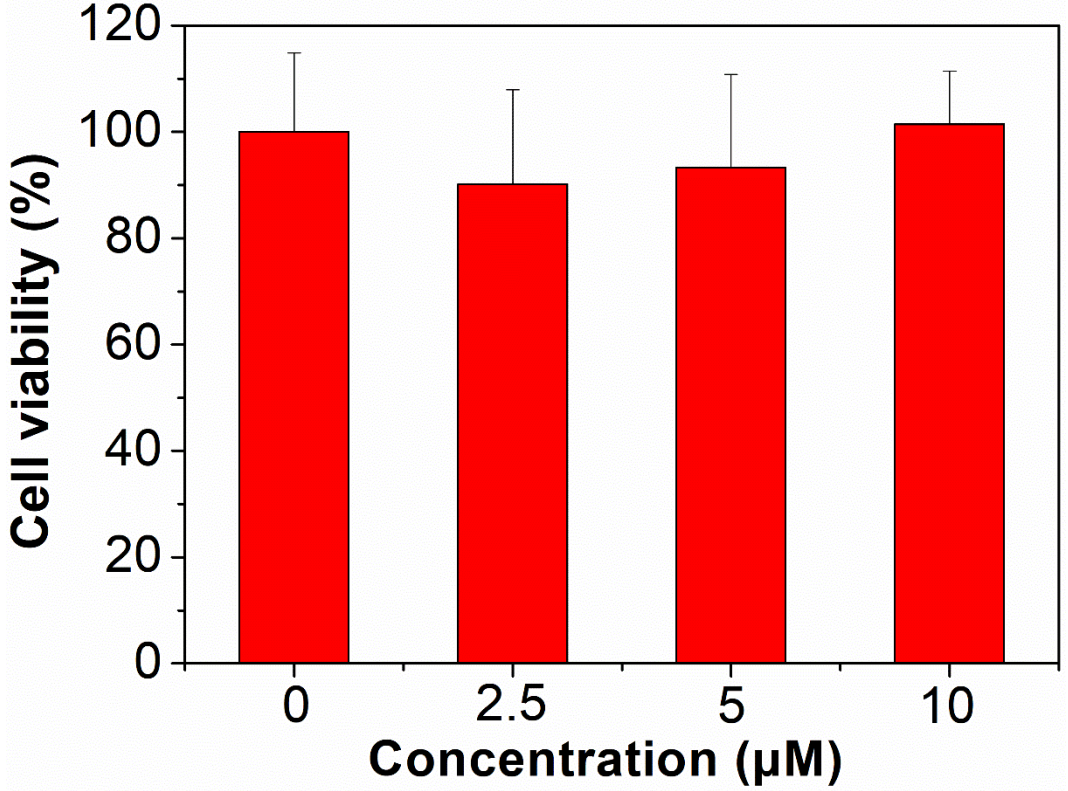



**Figure S11.** Viability of HeLa cells in the presence of **Cy-NNBD** as measured by using CCK-8 kit. The cells were incubated with **Cy-NNBD** for 24 h.

# 4. Cell viability
# Cy-MPM

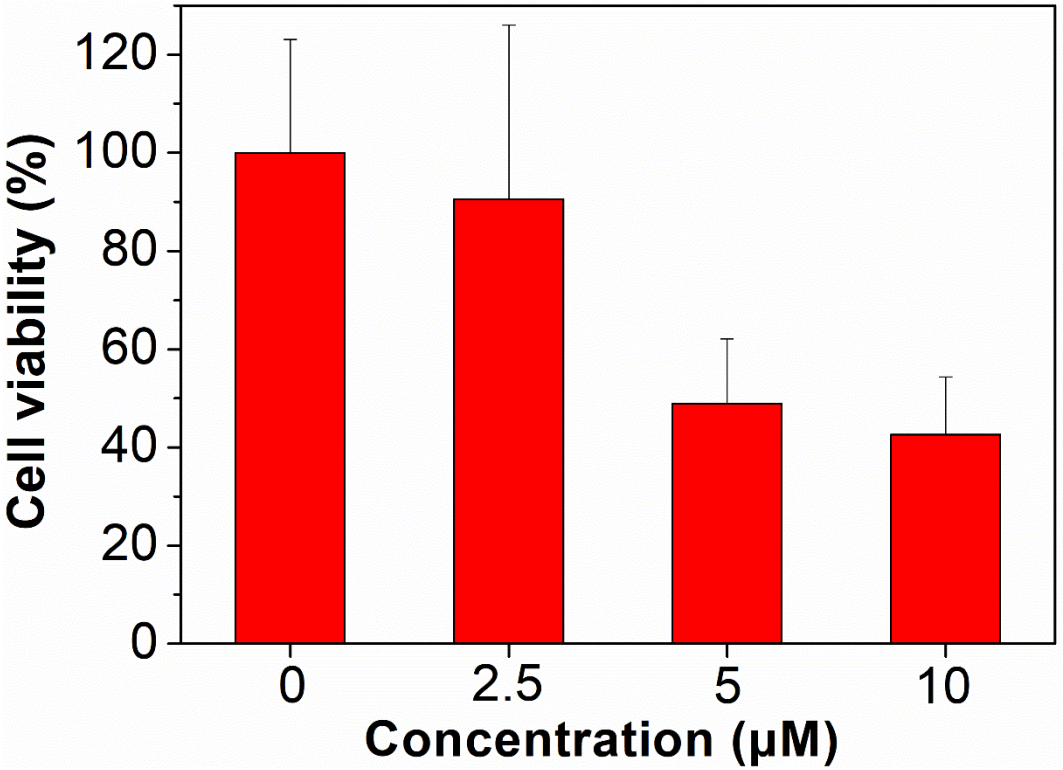



**Figure S12.** Viability of HeLa cells in the presence of **Cy-MPM** as measured by using CCK-8 kit. The cells were incubated with **Cy-MPM** for 24 h.

# 5. Imaging of Hela cells and zebrafish Cy-MPM

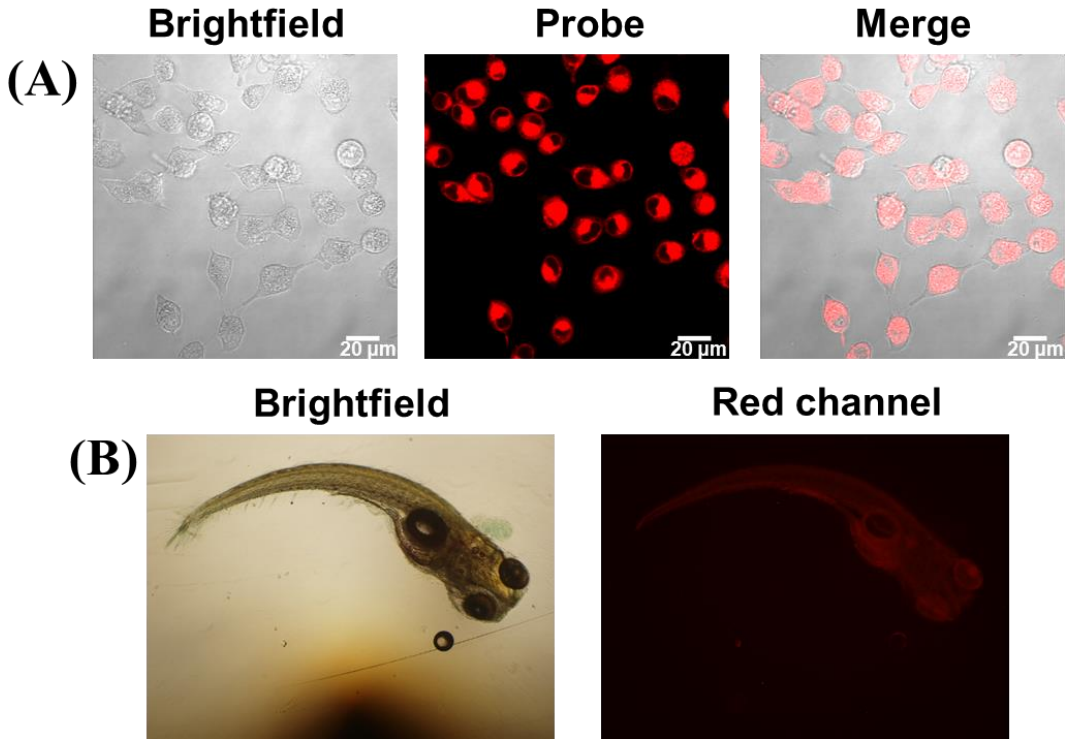



**Figure S13.** Confocal fluorescence image (A) of Hela cells with probe **Cy-MPM** (10 μM) in brightfiled, red channel and merged after incubation for 1h. (red channel: $\lambda_{ex}$ = 647 nm, $\lambda_{em}$ = 700-800 nm, scale bar: 20 μm); zebrafish fluorescence imaging (B) with probe **Cy-MPM** (10 μM) in brightfield and red channel.

# 5. Imaging of Hela cells
# Cy-NNBD

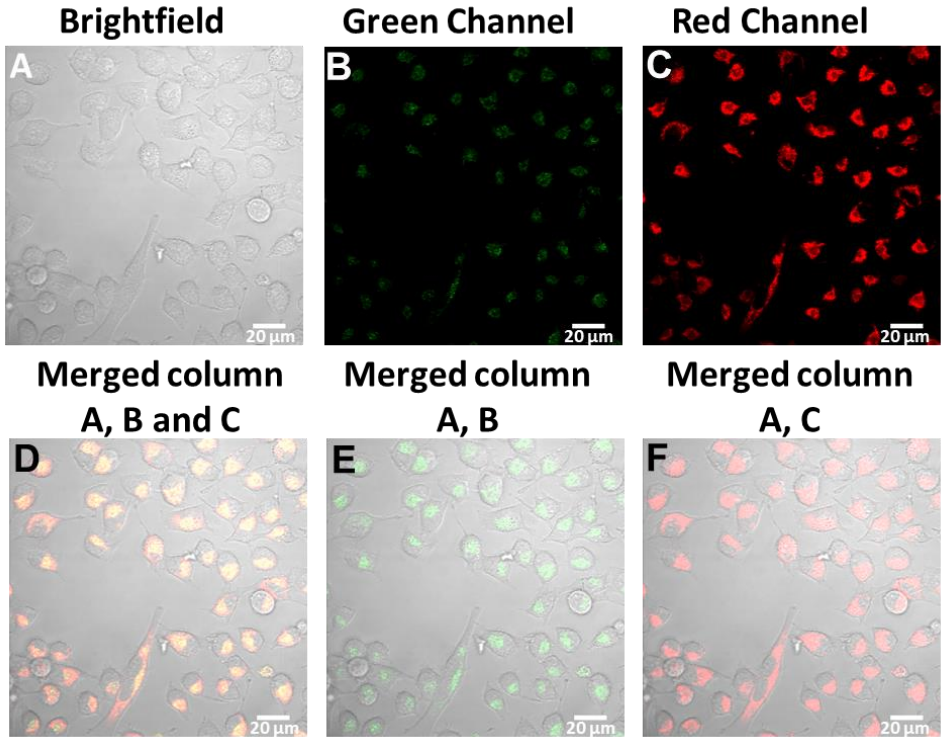



**Figure S14.** Confocal fluorescence image of Hela cells with probe **Cy-NNBD** (10 μM). (A) in brightfield; (B) in green channel ($\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-600 nm); (C) in red channel $\lambda_{ex}$ = 647 nm, $\lambda_{em}$ = 700-800 nm); (D) merged image in brightfiled, green channel and red channel; (E) merged image in brightfield and green field; (F) merged image in brightfield and red image. (incubation time: 1 h; scale bar: 20 μm).

## 5. Imaging of zebrafish
## Cy-NNBD

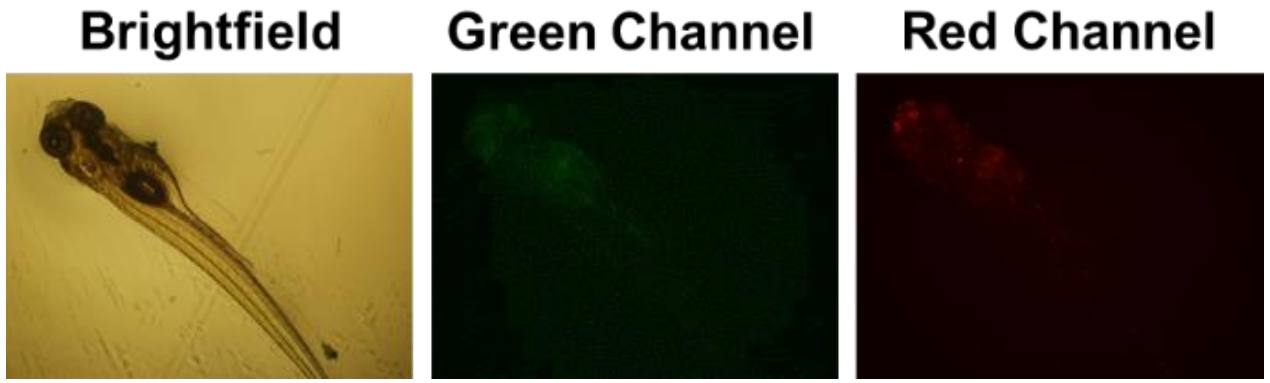
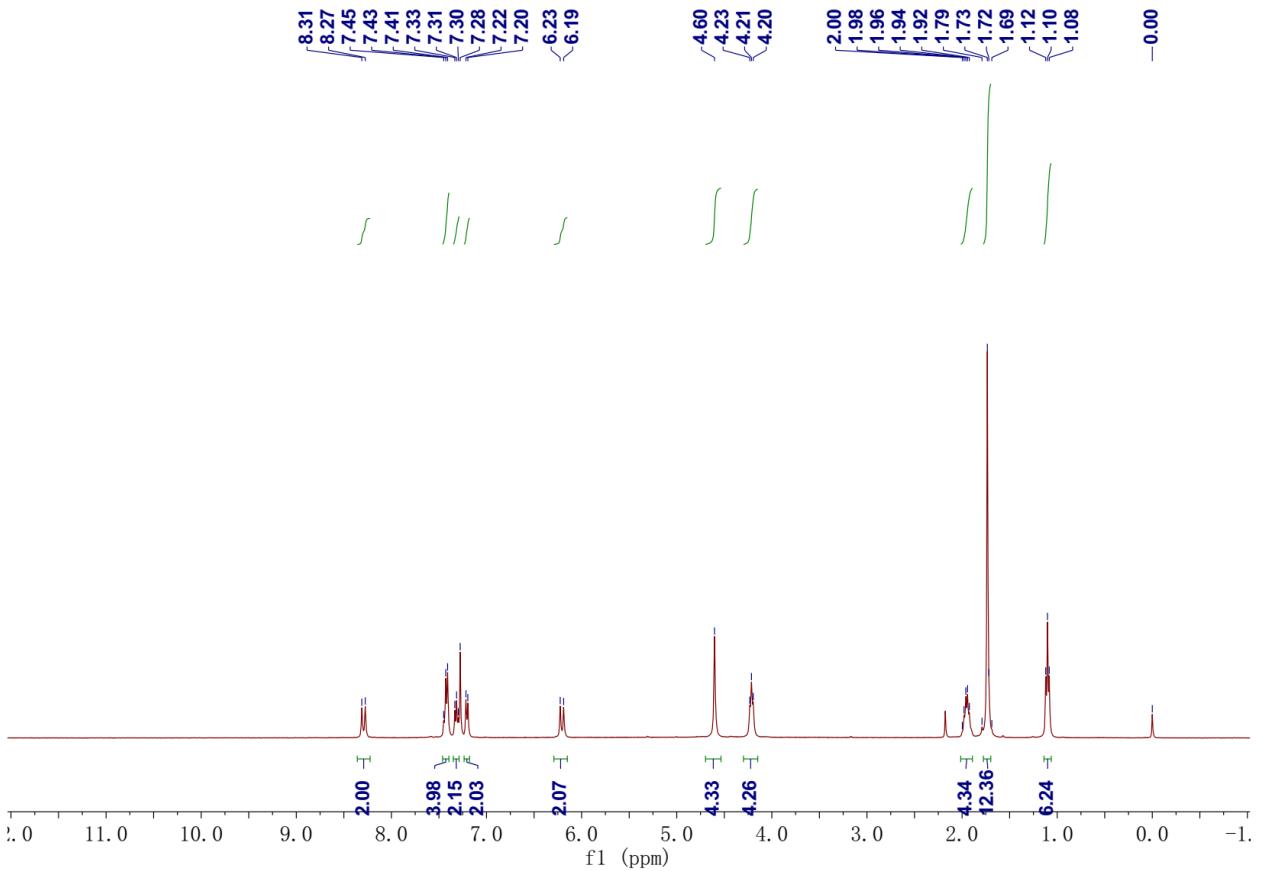


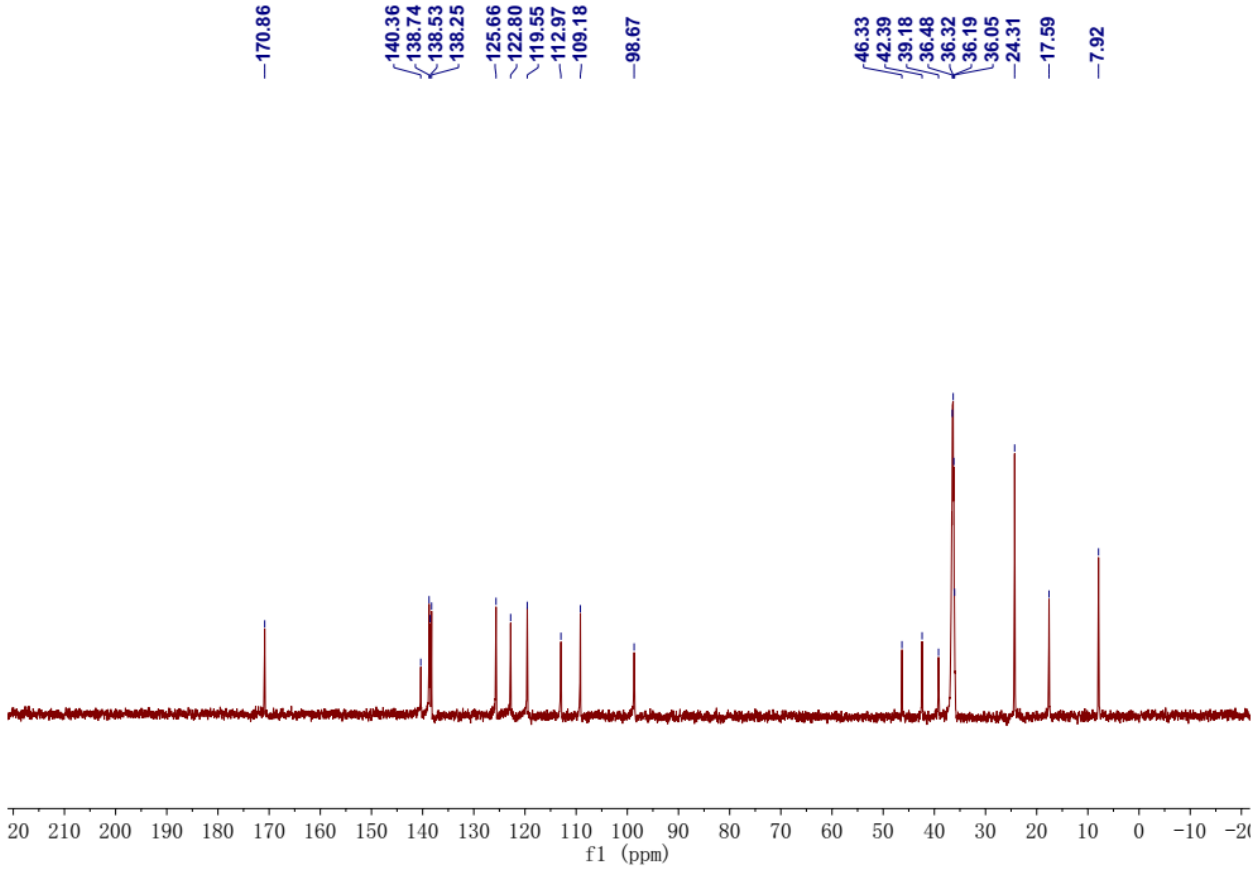

**Figure S15.** Zebrafish fluorescence imaging of **Cy-NNBD** (10 μM) in brightfield and green chanel and red channel.

# 6. $^{1}H$, $^{13}C$ NMR and MALDI-MS spectra

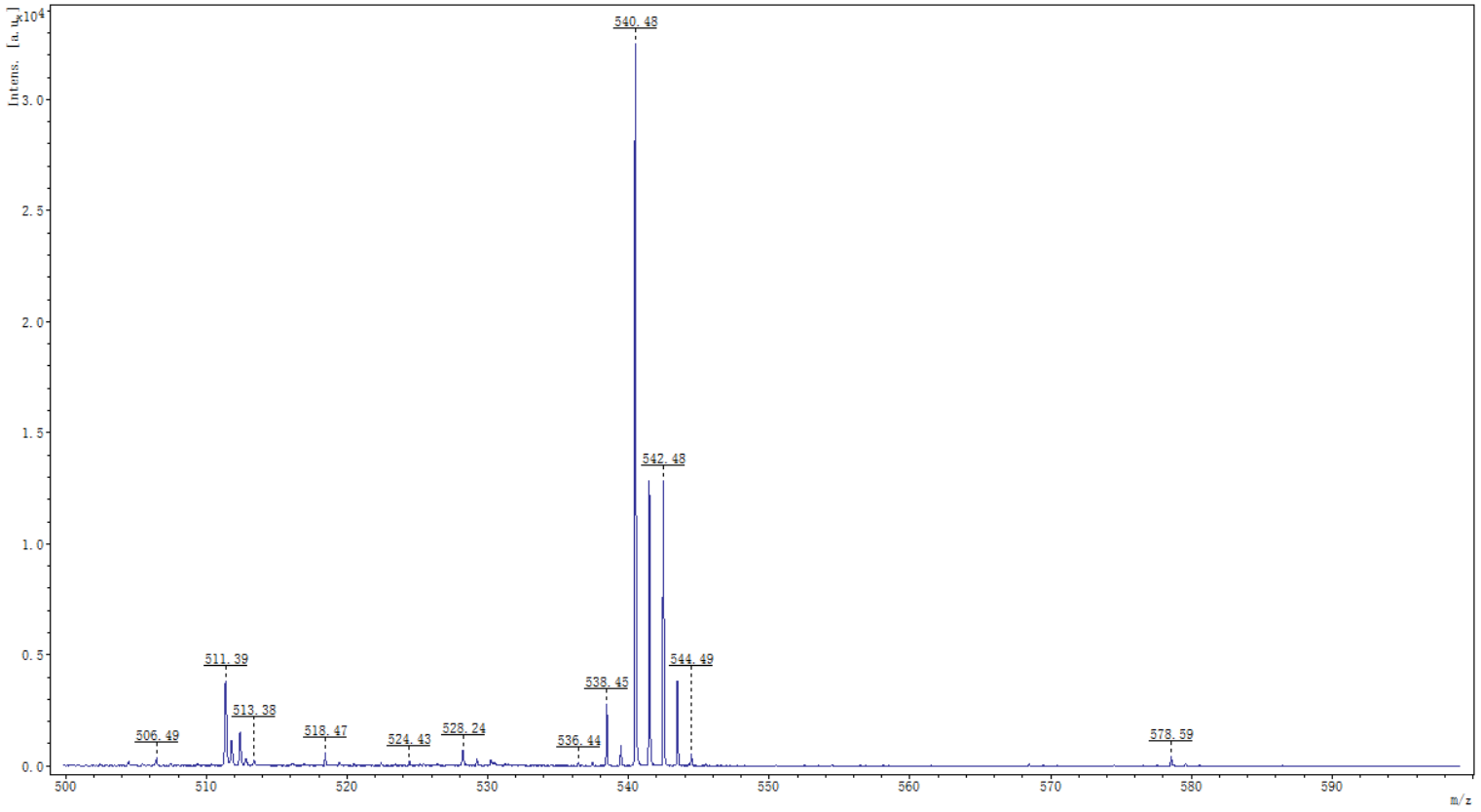



$^{1}H$ NMR spectra of **Cy-NH** in $CDCl_3$ (600 M)

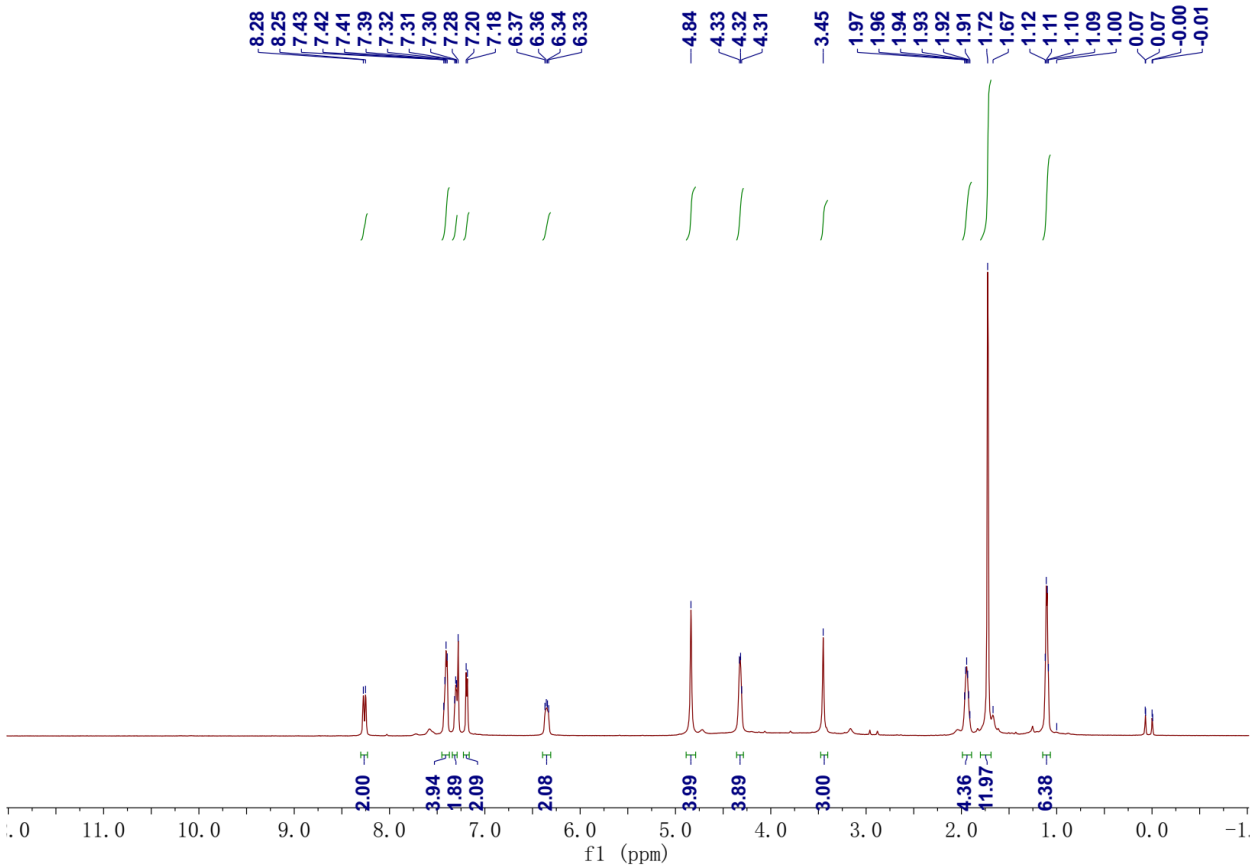
# $^{13}$C NMR spectra of **Cy-NH** in DMSO-d6 (600 M)



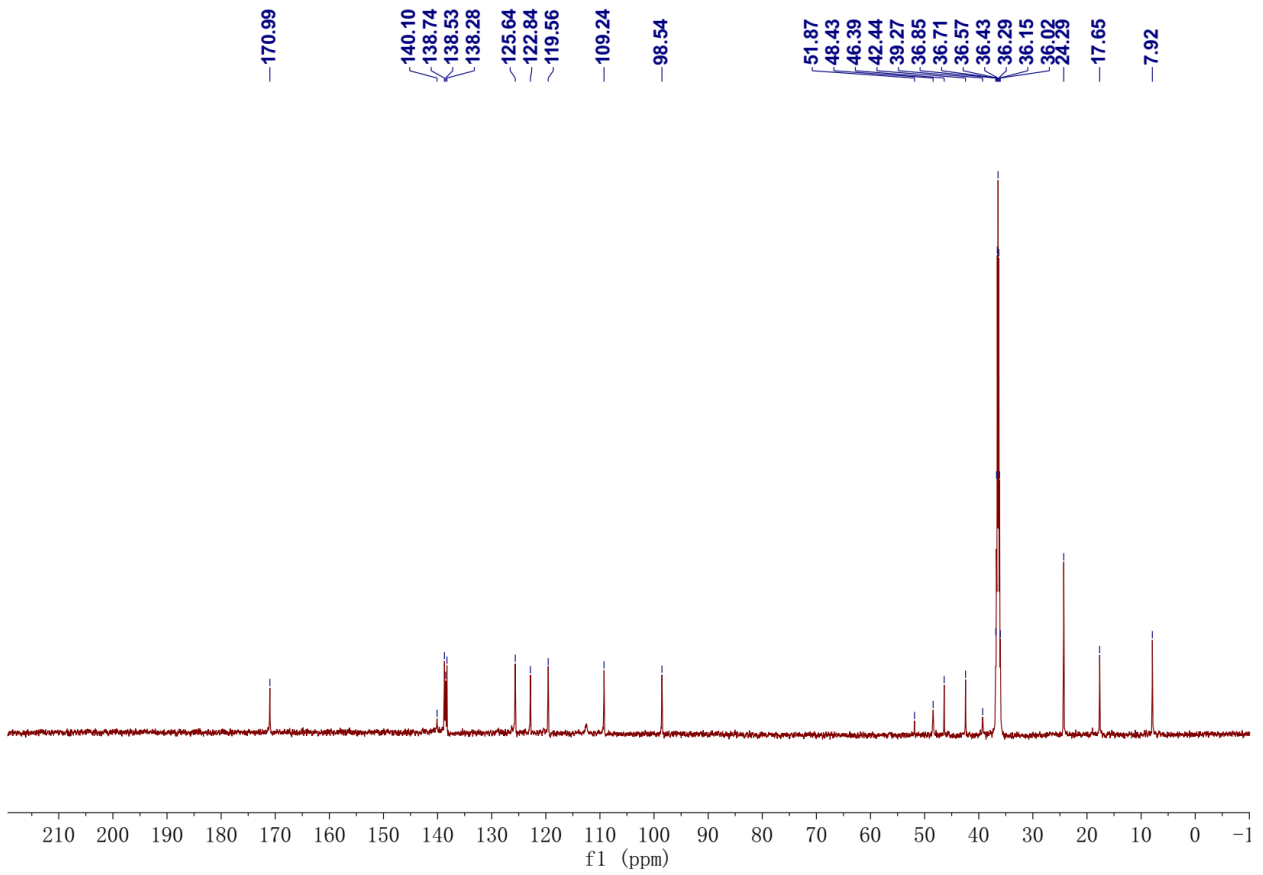
# MALDI-MS spectra of **Cy-NH**



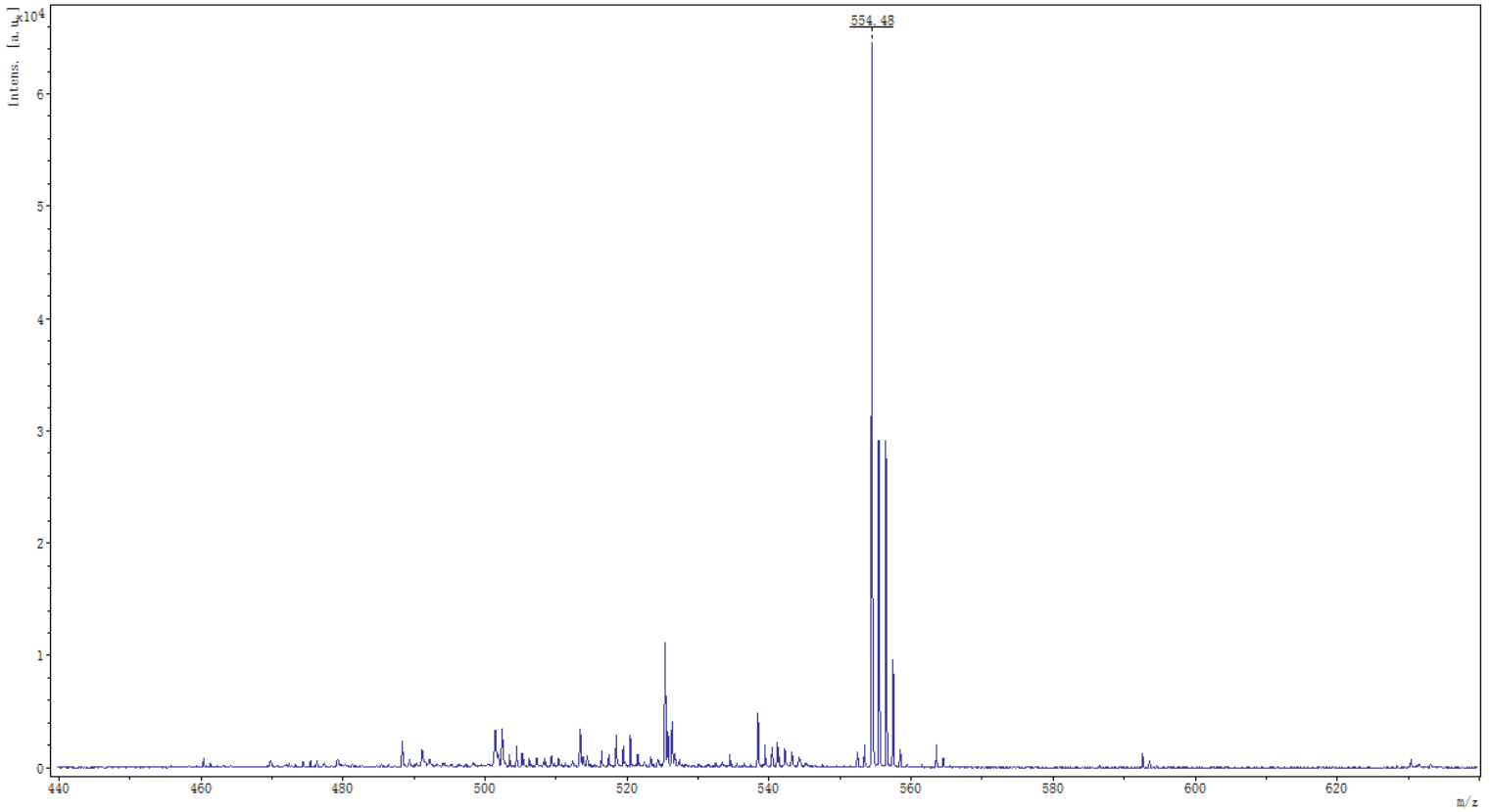
# $^{1}$H NMR spectra of **Cy-NMe** in $CDCl_3$ (600 M)



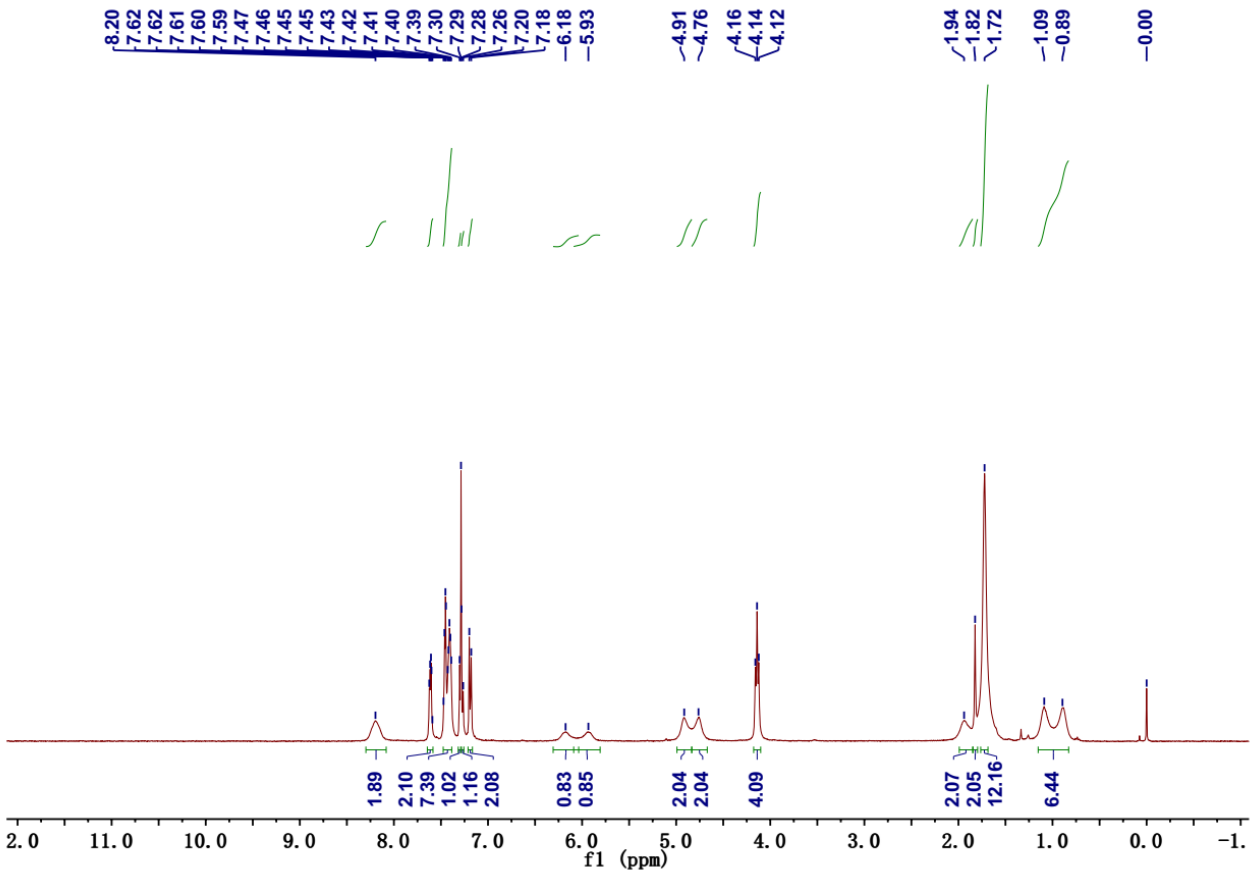
# $^{13}$C NMR spectra of **Cy-NMe** in DMSO-d6 (600 M)



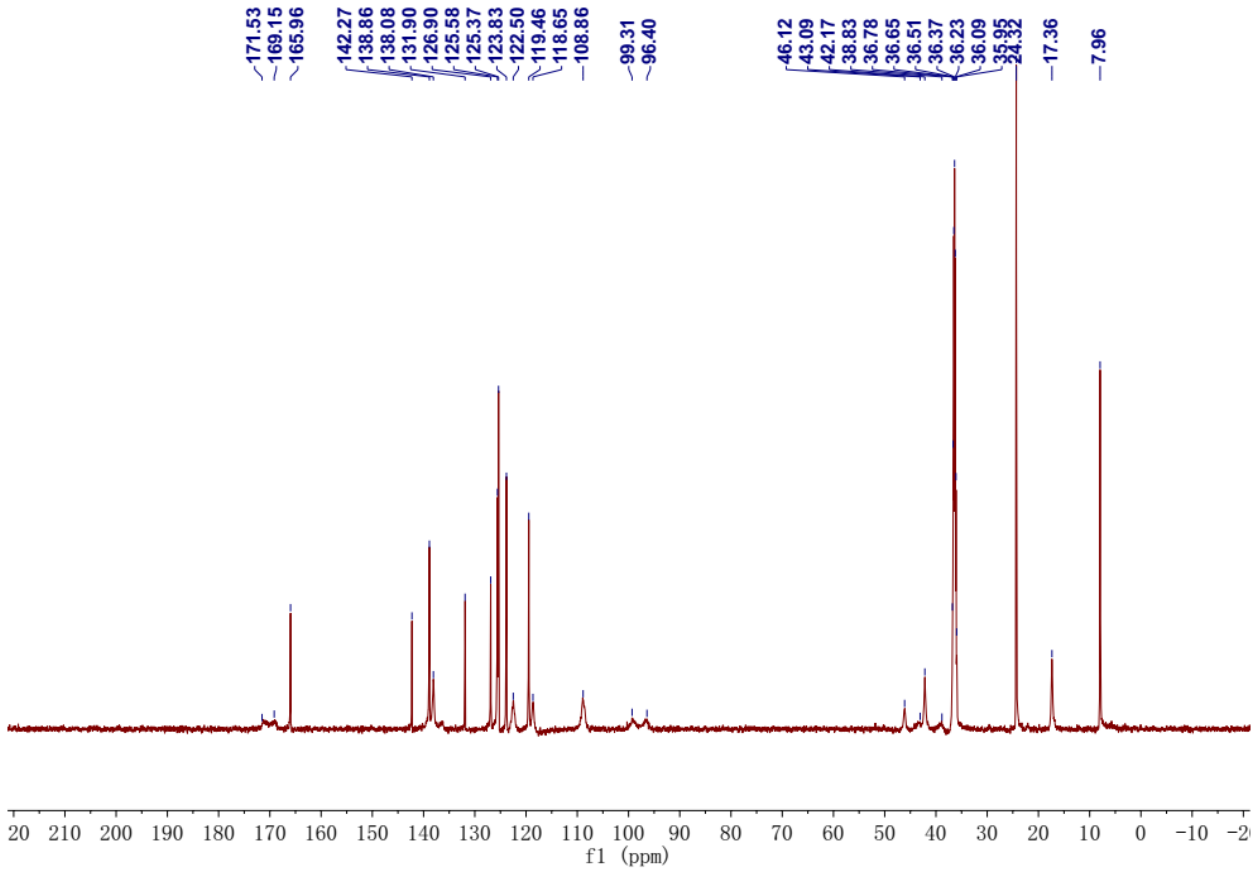
# MALDI-MS spectra of **Cy-NMe**



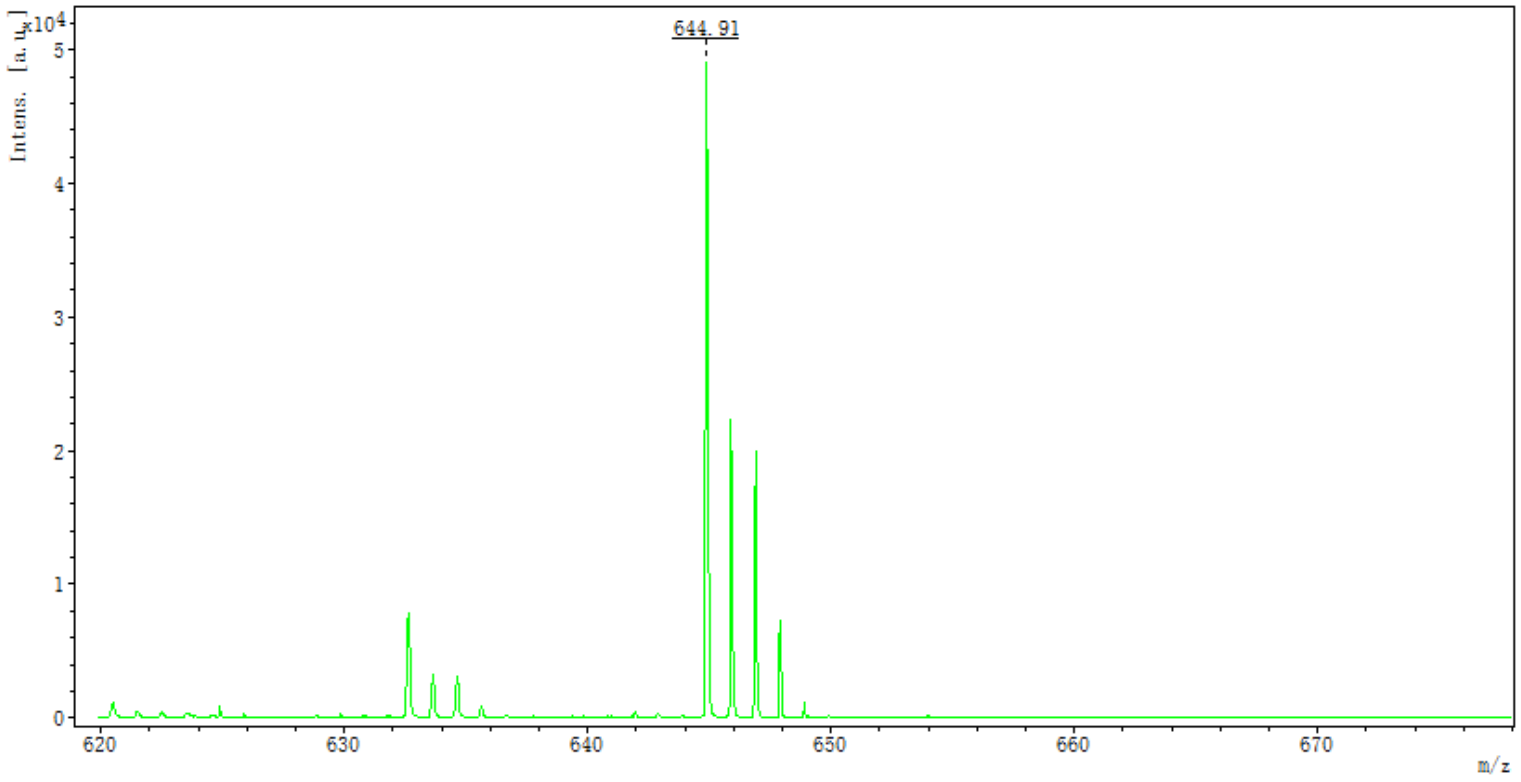
# $^{1}$H NMR spectra of **Cy-MP** in $CDCl_3$ (400 M)



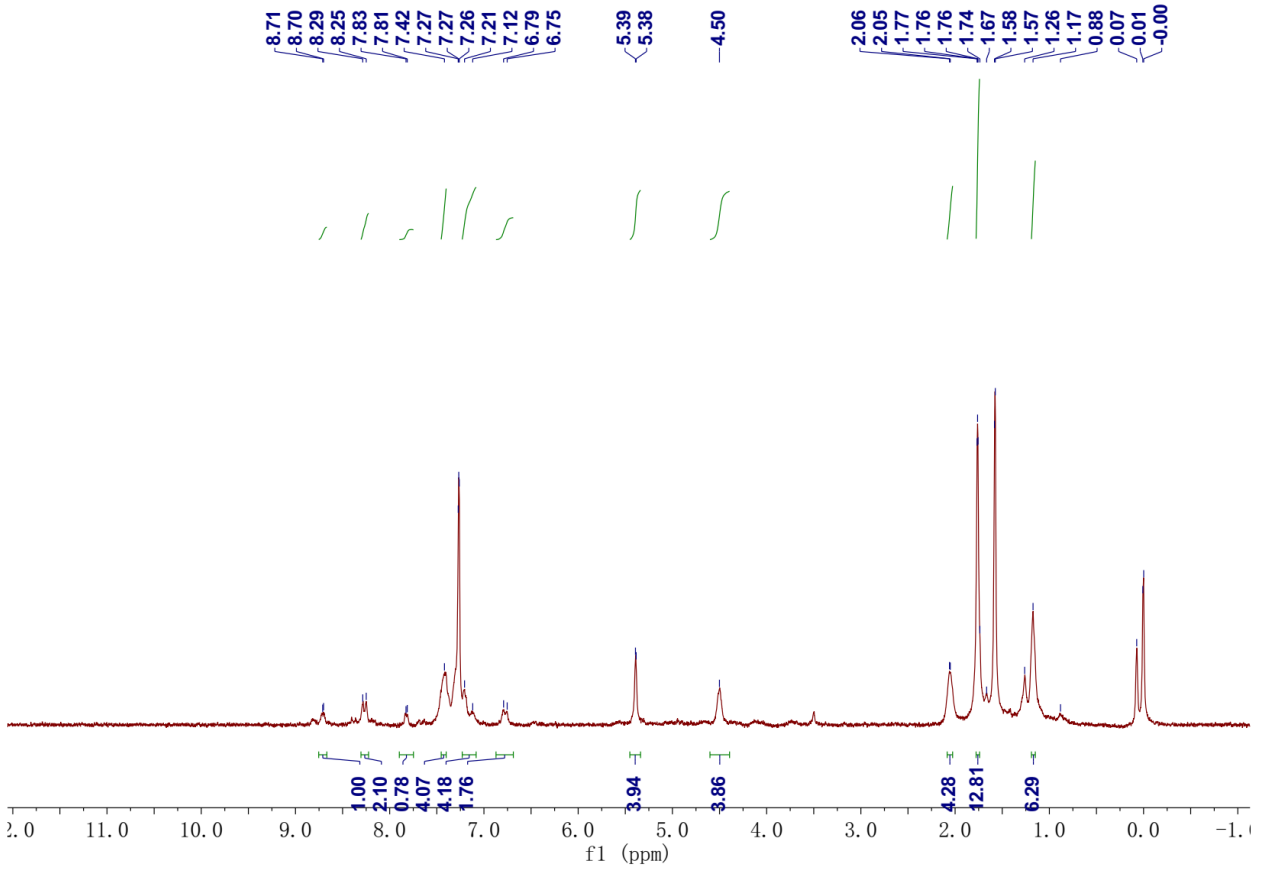
# $^{13}$C NMR spectra of **Cy-MP** in DMSO-d6 (600 M)



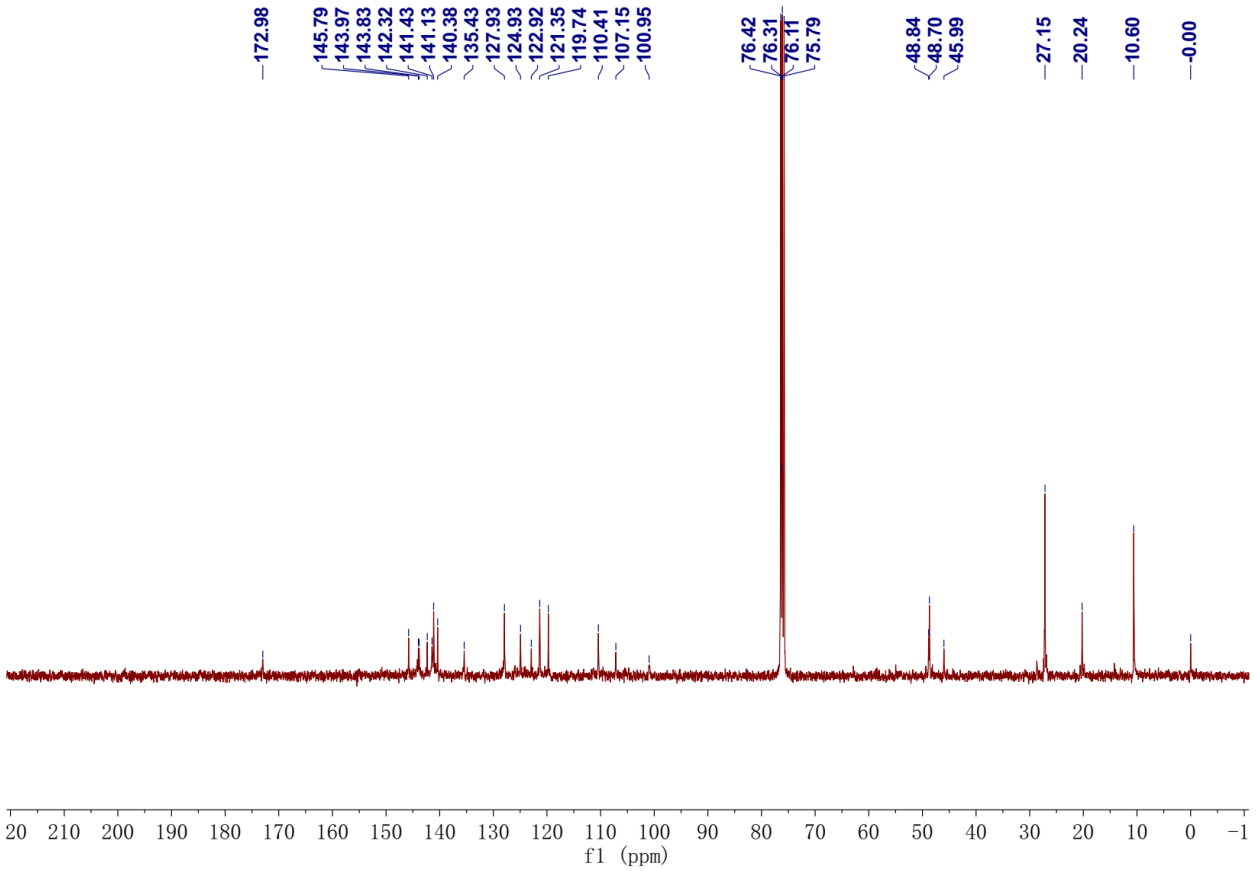
# MALDI-MS spectra of **Cy-MP**



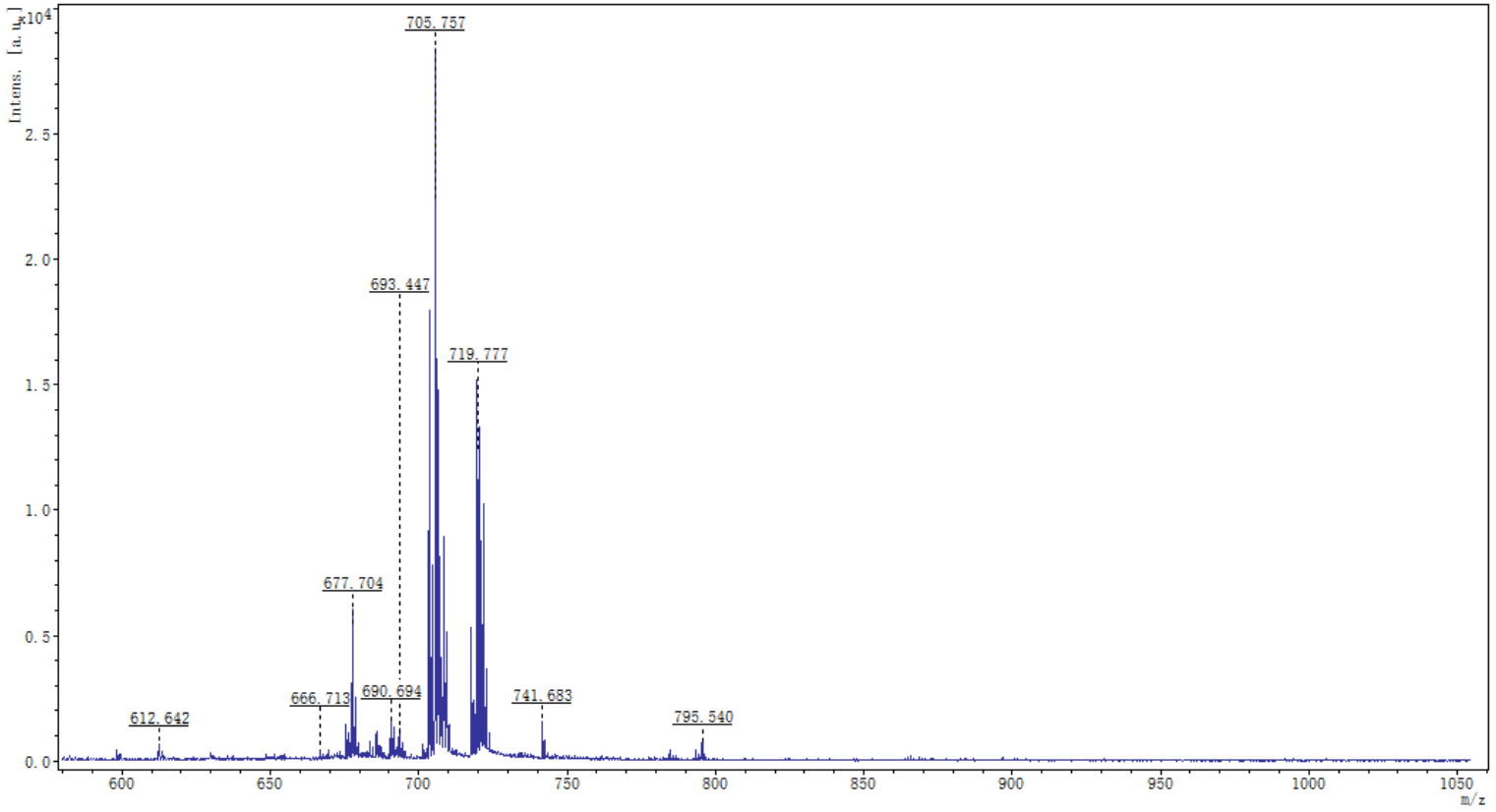
# $^{1}$H NMR spectra of **Cy-NNBD** in $CDCl_3$ (400 M)



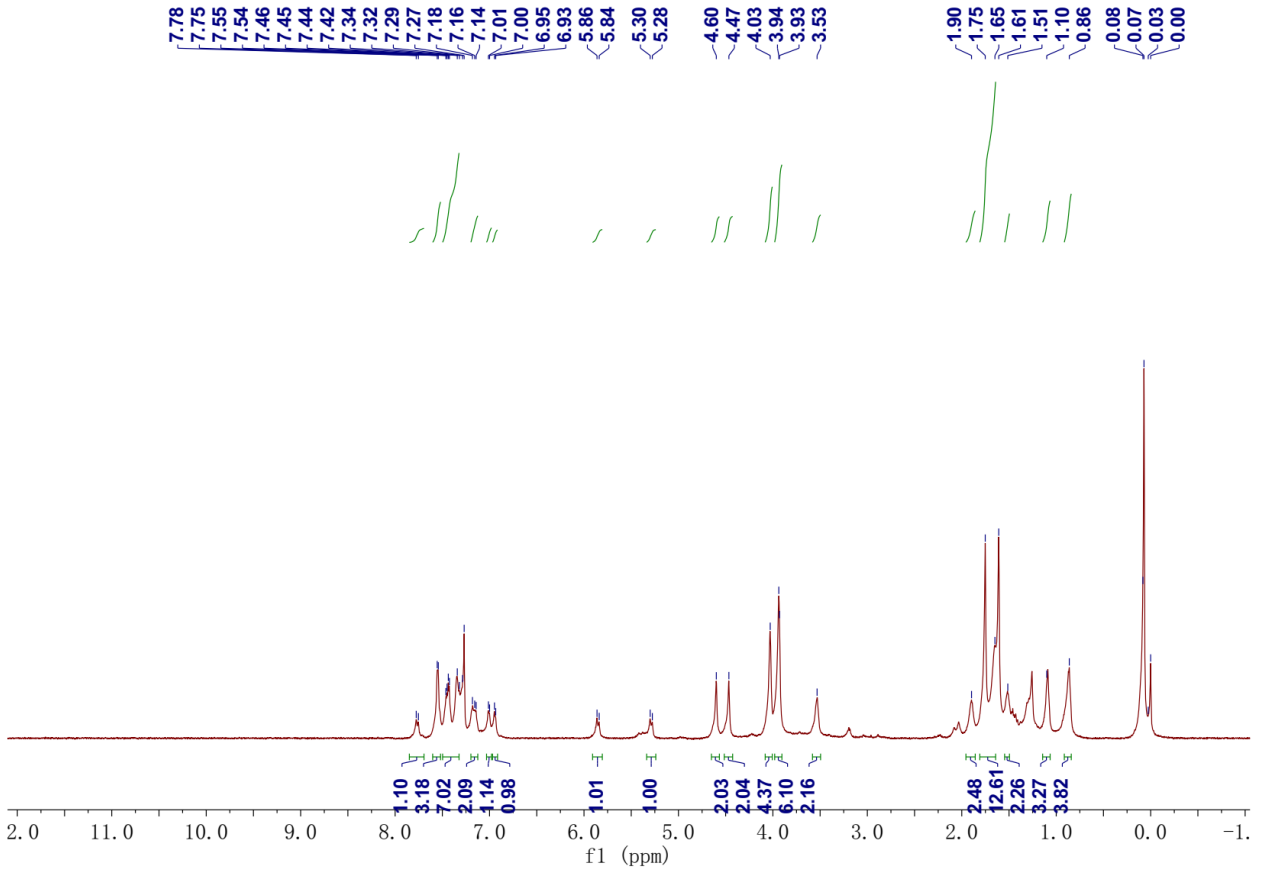
# $^{13}C$ NMR spectra of **Cy-NNBD** in $CDCl_3$ (400 M)



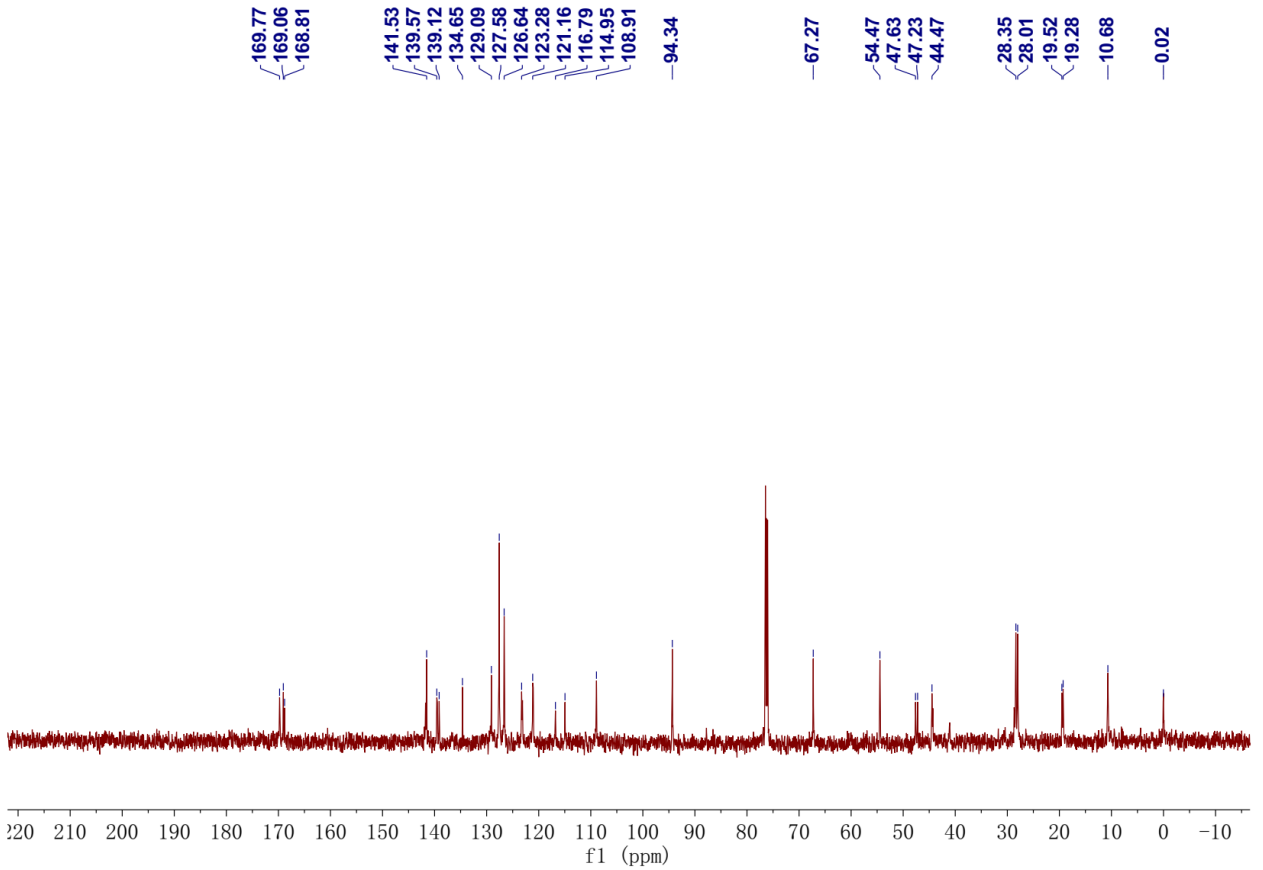
# MALDI-MS spectra of **Cy-NNBD**



# $^{1}$H NMR spectra of **Cy-MPM** in $CDCl_3$ (600 M)



# $^{13}C$ NMR spectra of **Cy-MPM** in $CDCl_3$ (600 M)



# MALDI-MS spectra of **Cy-MPM**