Supplementary material

An HDAC8-Selective Fluorescent Probe for Imaging in Living Tumor Cell Lines and Tissue Slices

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Contents

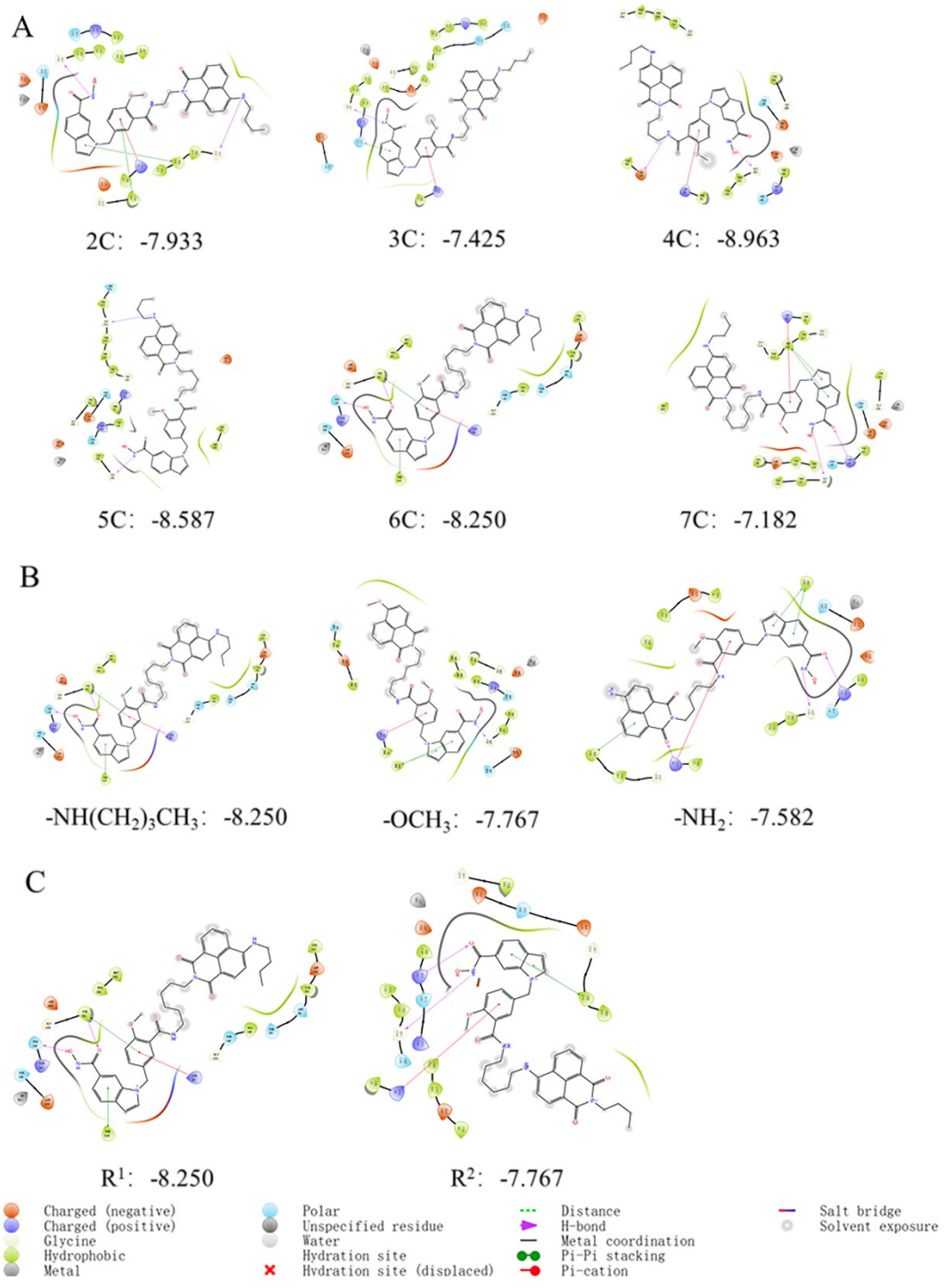

Figure S1: Molecular docking analysis of designed selective HDAC8 probes. (A) 2D docking diagrams of carbon chains of different lengths; (B) 2D docking diagrams of different naphthalene substituents; (C) 2D docking images of different splicing sites

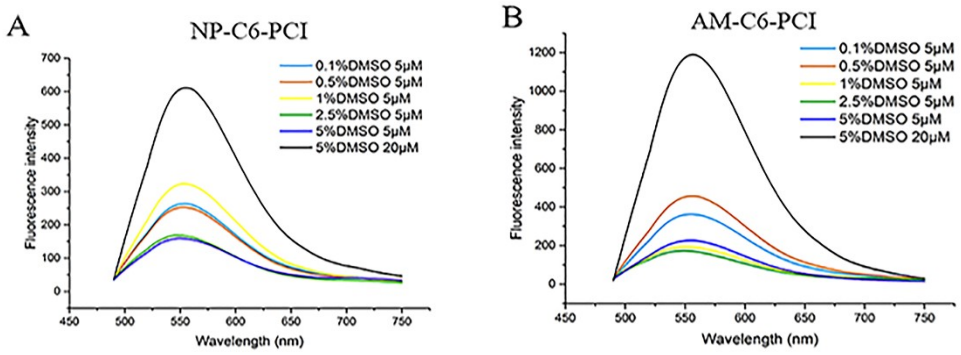

Figure S2: Fluorescence spectra of **NP-C6-PCI (**A**)** and **AM-C6-PCI (**B**)** in PBS solvent containing different proportions of DMSO (λex=450nm).

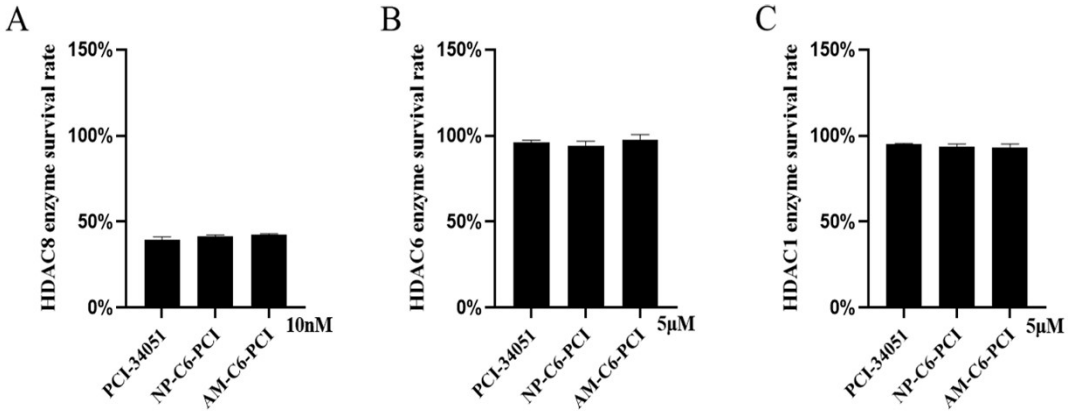

Figure S3: HDAC enzyme inhibitory activity assay. (A) Inhibitory activity of 10 nM tested compounds (**PCI-34051**, **NP-C6-PCI** and **AM-C6-PCI**) on HDAC8; (B) Inhibitor activity of 5 μM compounds (**PCI-34051**, **NP-C6-PCI** and **AM-C6-PCI**) against HDAC6; (C) Inhibitor activity of 5 μM compounds (**PCI-34051**, **NP-C6-PCI** and **AM-C6-PCI**) against HDAC1. The data was expressed as the mean ± SD, n = 3.

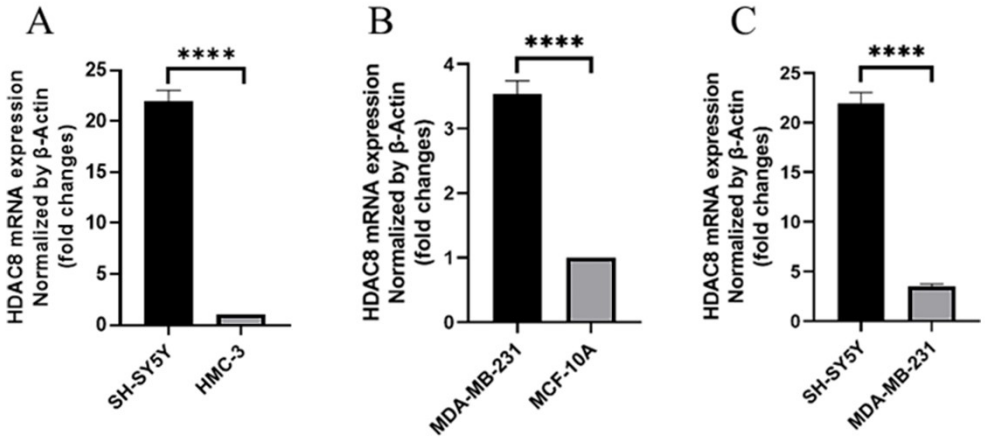

Figure S4: HDAC8 mRNA expression levels in cells. (A) HDAC8 mRNA expression in SH-SY5Y tumor cells and HMC-3 normal cells; (B) HDAC8 mRNA expression in MDA-MB-231 tumor cells and MCF-10A normal cells; (C) Comparison of HDAC8 mRNA expression between SH-SY5Y tumor cells and MDA-MB-231 tumor cells. The statistical difference of each cell was compared by analysis of variance, $p < 0.05$ was regarded as a significant difference (**** $P < 0.0001$), the data was expressed as the mean ± SD, n = 3.

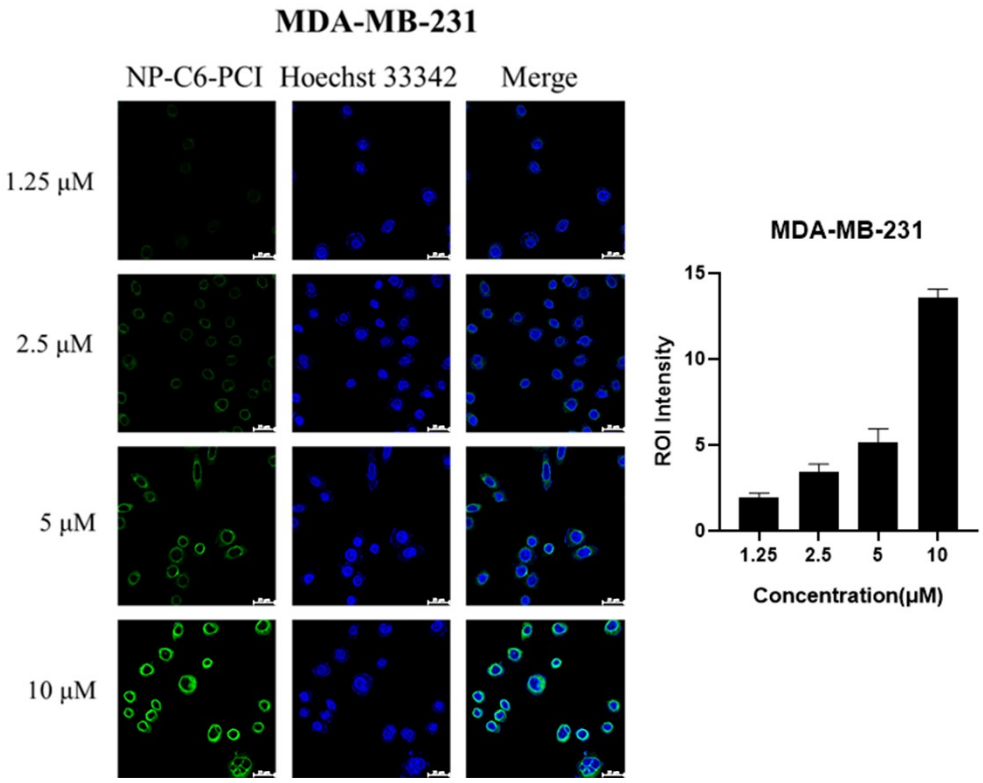

Figure S5: Fluorescence imaging of MDA-MB-231 cells at different uptake concentrations. NP-C6-PCI (green channel: λex = 458 nm, λem = 500-550 nm). Hochest 33342 (blue channel: λex = 405 nm, λem = 461 nm). Data are expressed as the mean SD of determinations in triplicate from at least three distinct experiments (The data was expressed as the mean ± SD, n = 3.), the scale bar represents 25 μm.

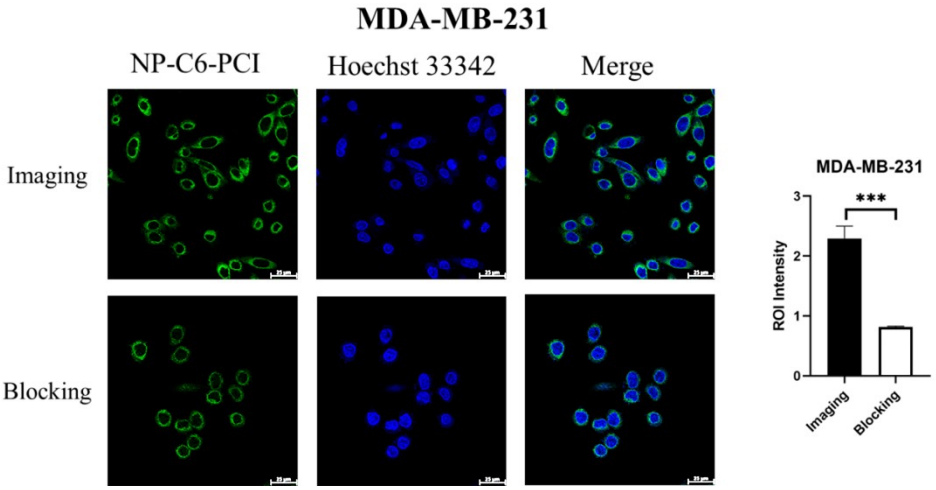

Figure S6: MDA-MB-231 cells incubated with **NP-C6-PCI** at 5μM (Imaging), **NP-C6-PCI** at 5μM in the presence of 100-fold excess **PCI-34051** (Blocking). NP-C6-PCI (green channel: λex = 458 nm, λem = 500-550 nm). Hochest 33342 (blue channel: λex = 405 nm, λem = 461 nm). Data are expressed as the mean SD of determinations in triplicate from at least three distinct experiments (Analysis of variance *** P <0.001, the data was expressed as the mean ± SD, n = 3.), the scale bar represents 25 μm.

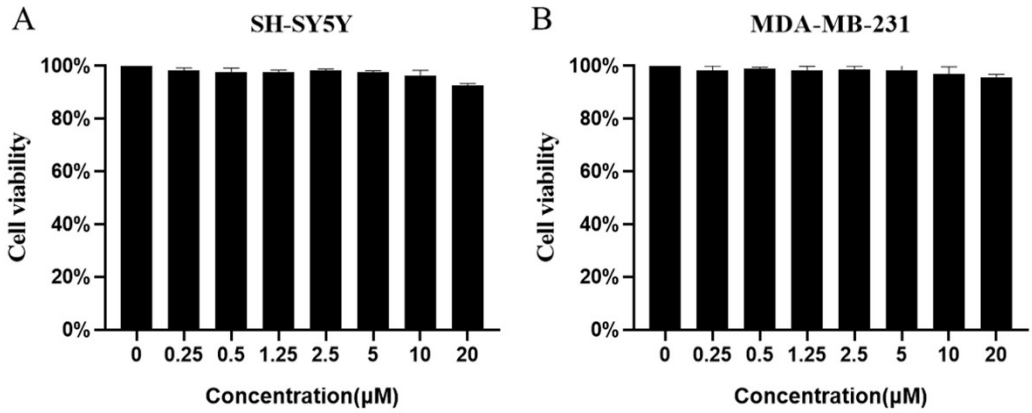

Figure S7：Cell viability after incubation of **PCI-34051** and **NP-C6-PCI** at different concentrations with SH-SY5Y(A) and MDA-MB-231(B) by MTT assay at 37℃ for 12 h (each sample was tested using three replicates, and the results are reported as the mean standard deviation); The data was expressed as the mean ± SD, n = 3.

HR-MS spectrum of **Compounds**

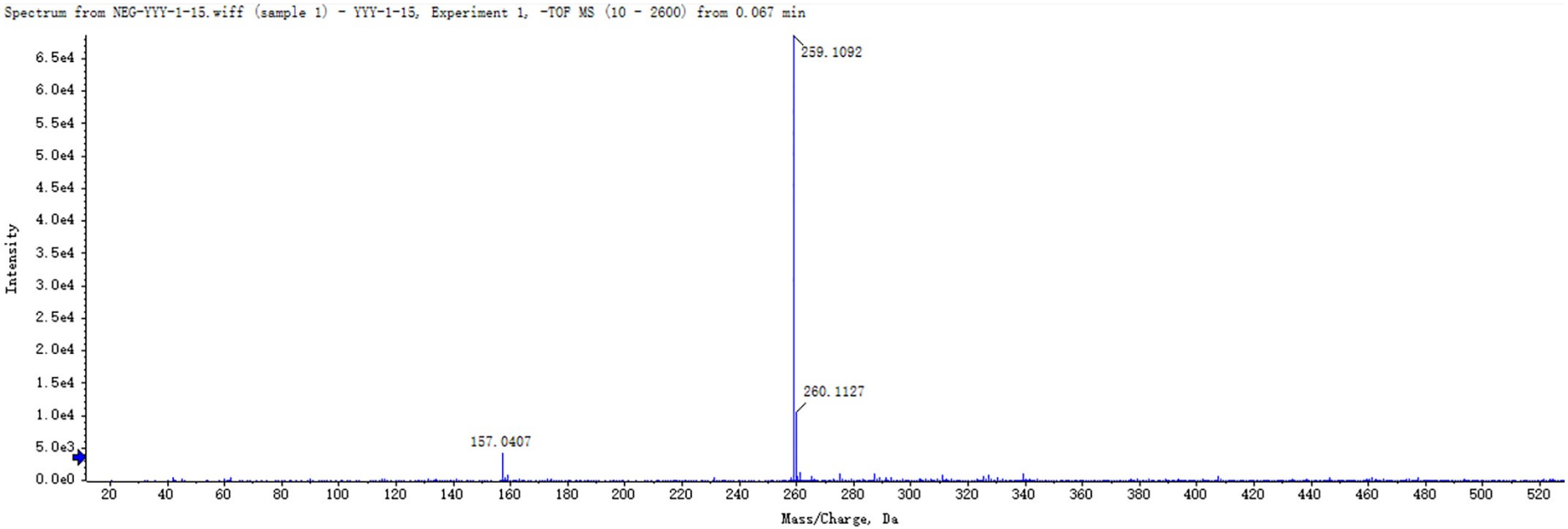

Figure S8：HR-MS spectrum of **Compound 3**

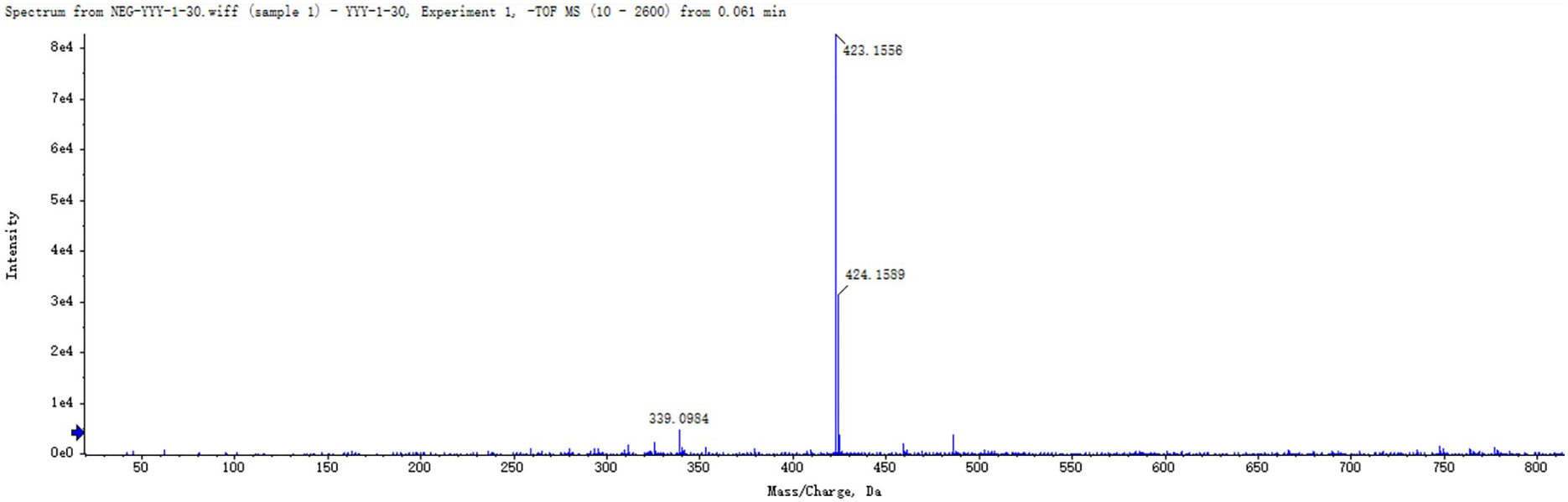

Figure S9：HR-MS spectrum of **Compound 6**

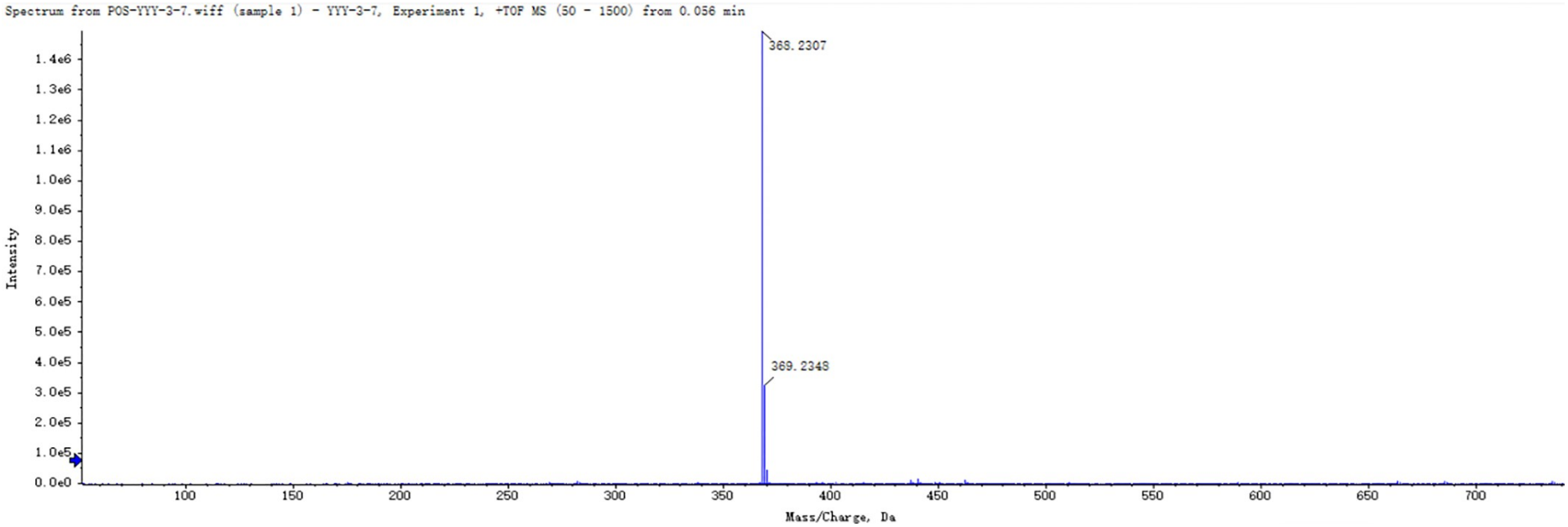

Figure S10：HR-MS spectrum of **Compound 9**

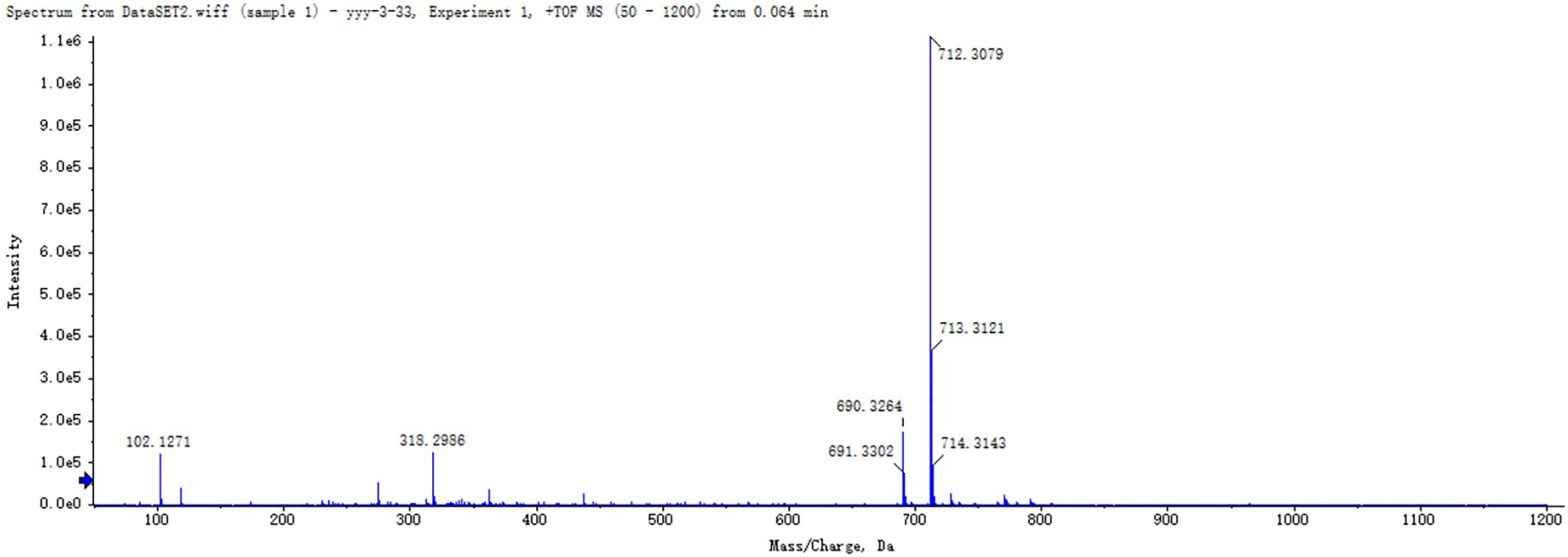

Figure S11：HR-MS spectrum of **Compound 10 (NP-C6-PCI)**

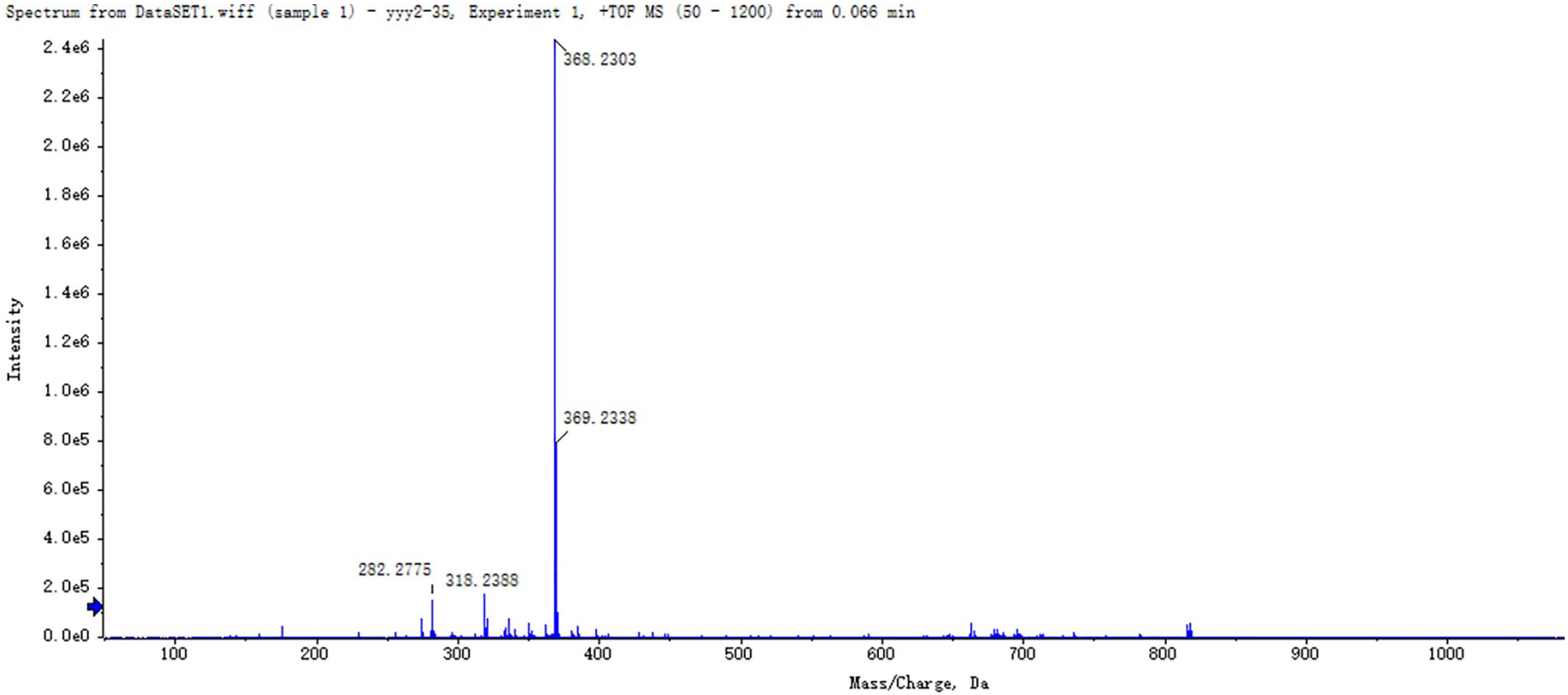

Figure S12：HR-MS spectrum of **Compound 12**

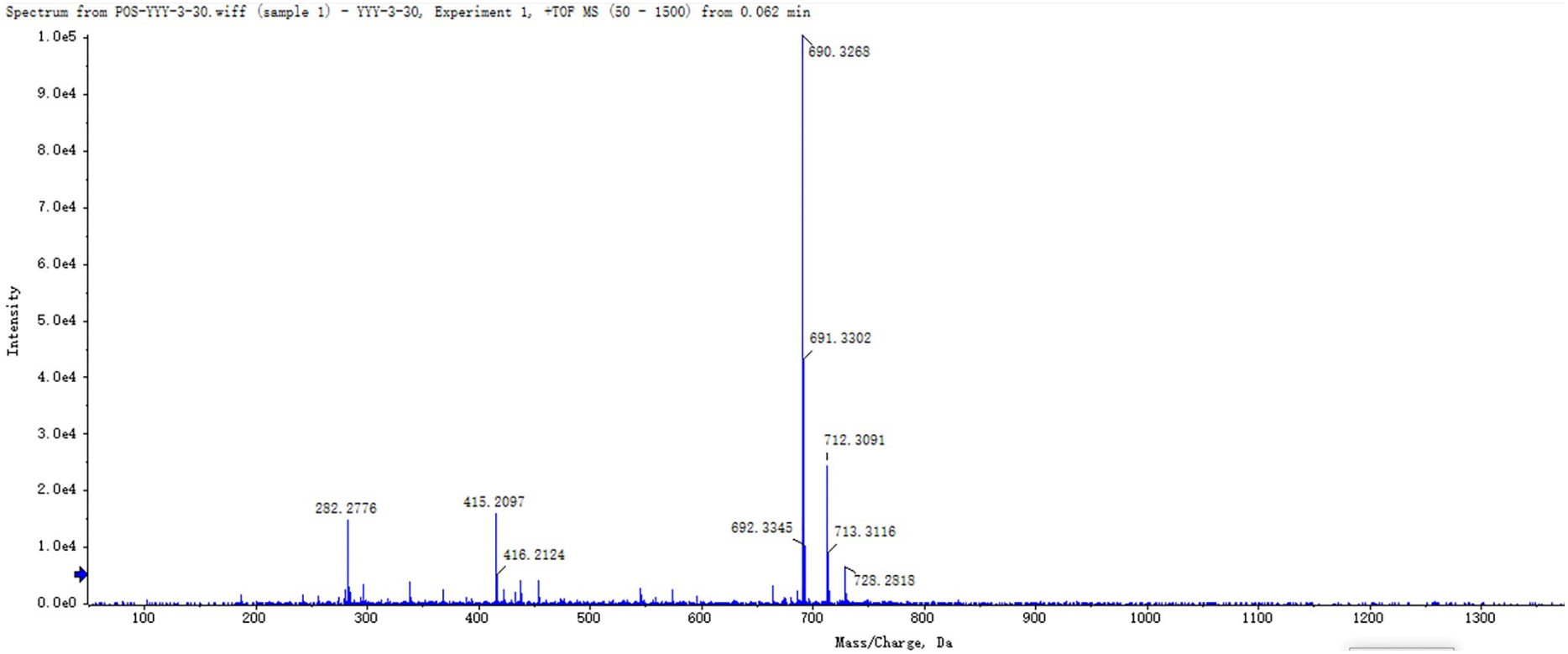

Figure S13：HR-MS spectrum of **Compound 13 (AM-C6-PCI)**

^{1}H NMR spectrum of **Compounds**

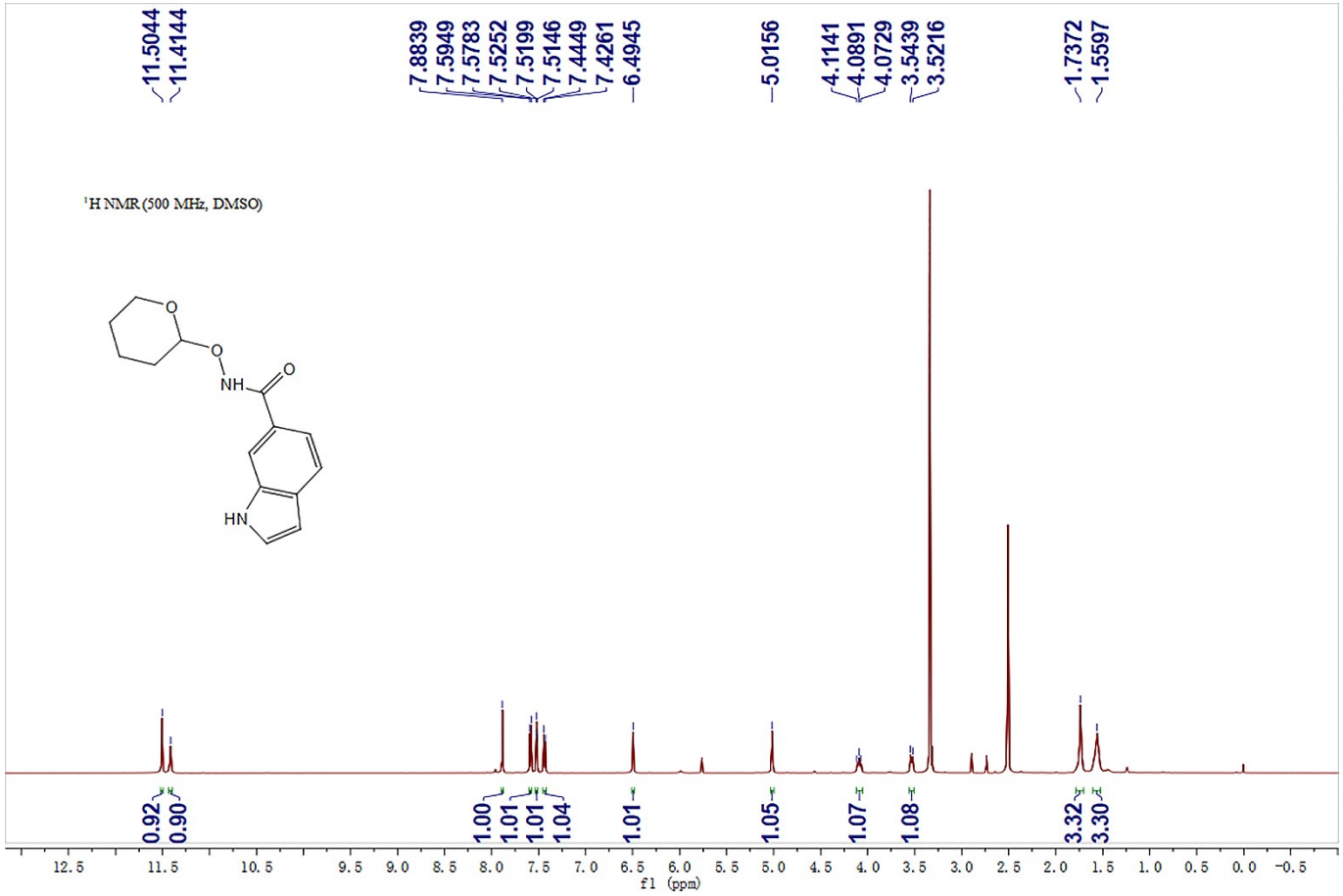

Figure S14：^{1}H NMR spectrum of **Compound 3** (500 MHz, DMSO-d_6)

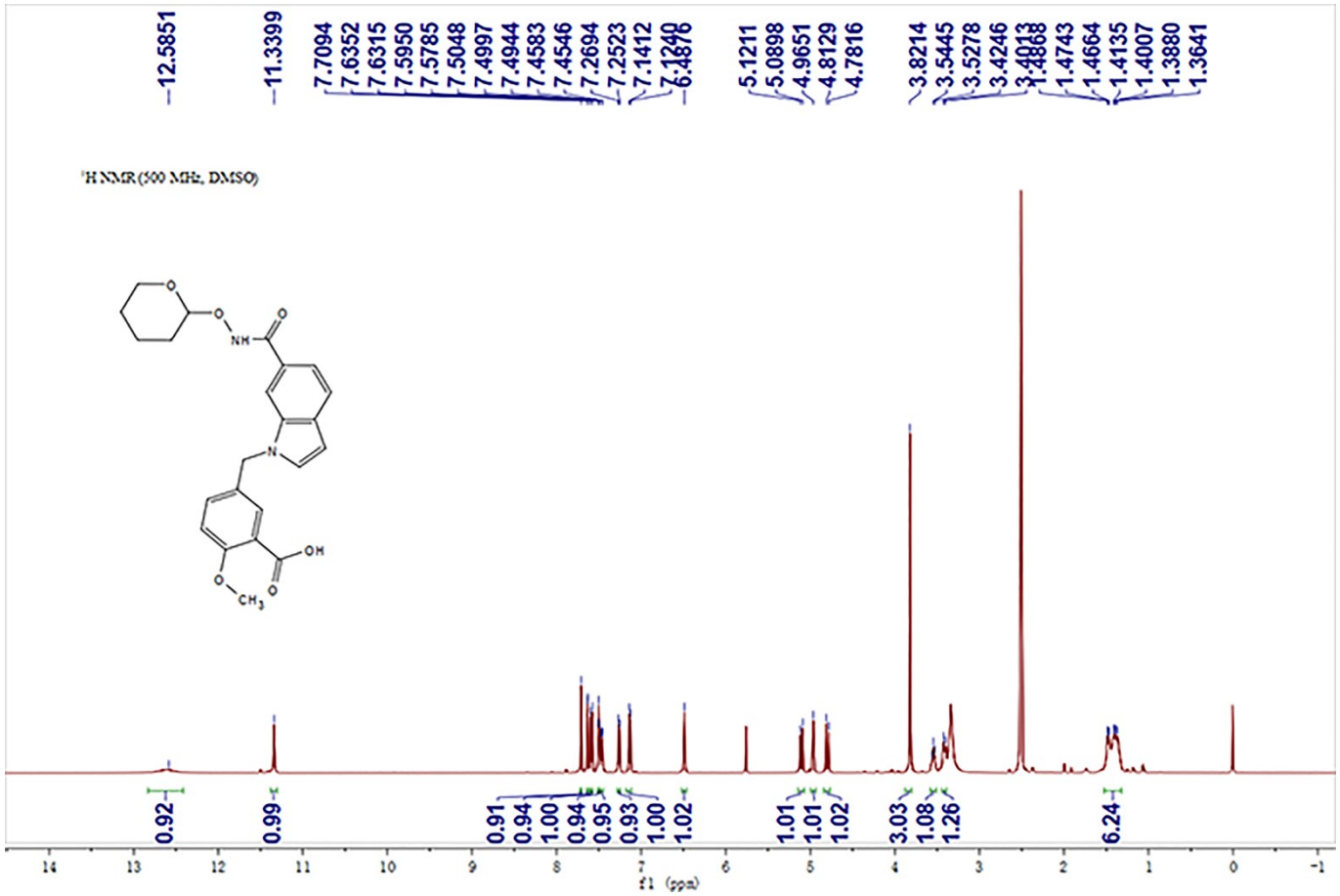

Figure S15：^{1}H NMR spectrum of **Compound 6** (500 MHz, DMSO-d_6)

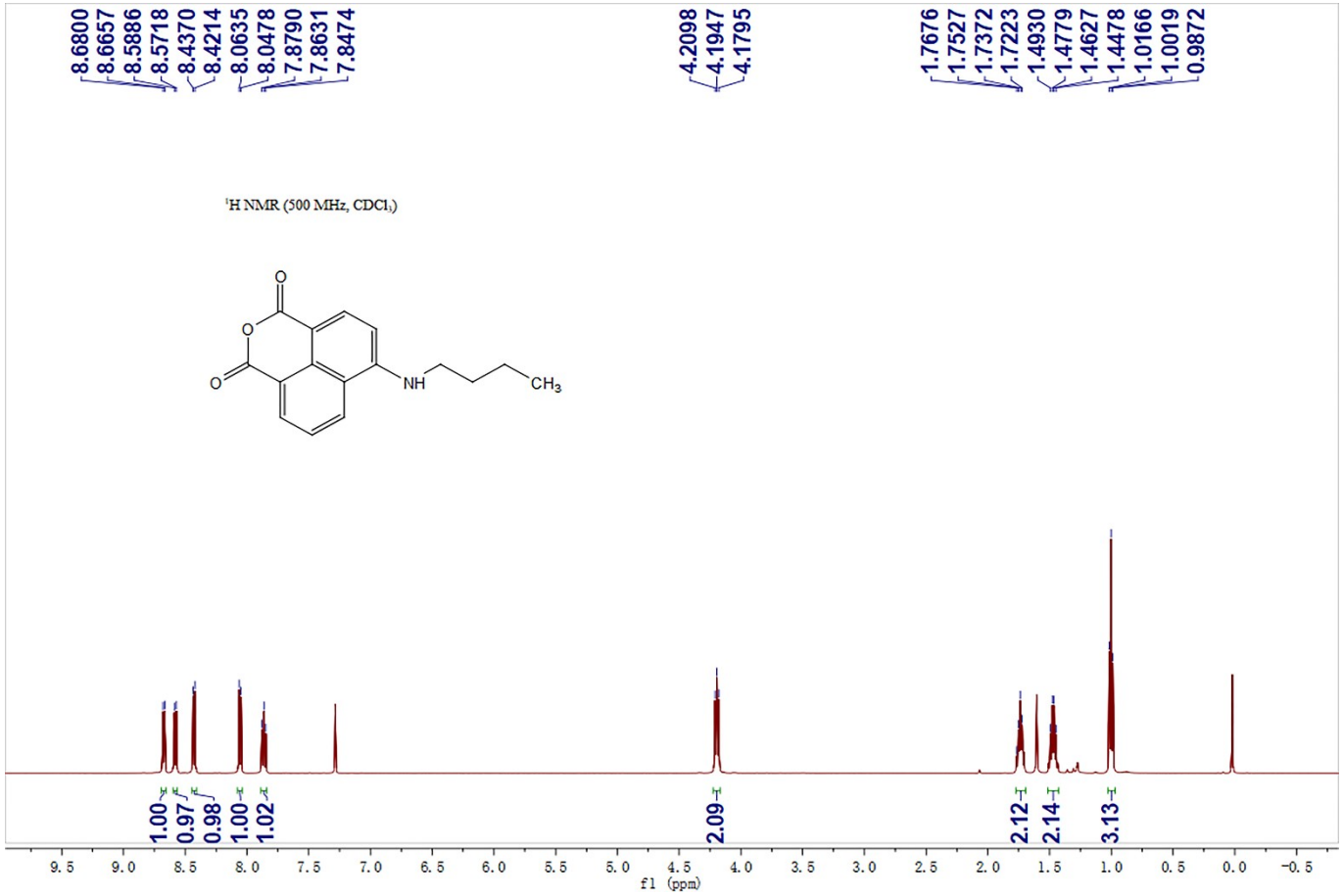

Figure S16：^{1}H NMR spectrum of **Compound 8** (500 MHz, $CDCl_3$)

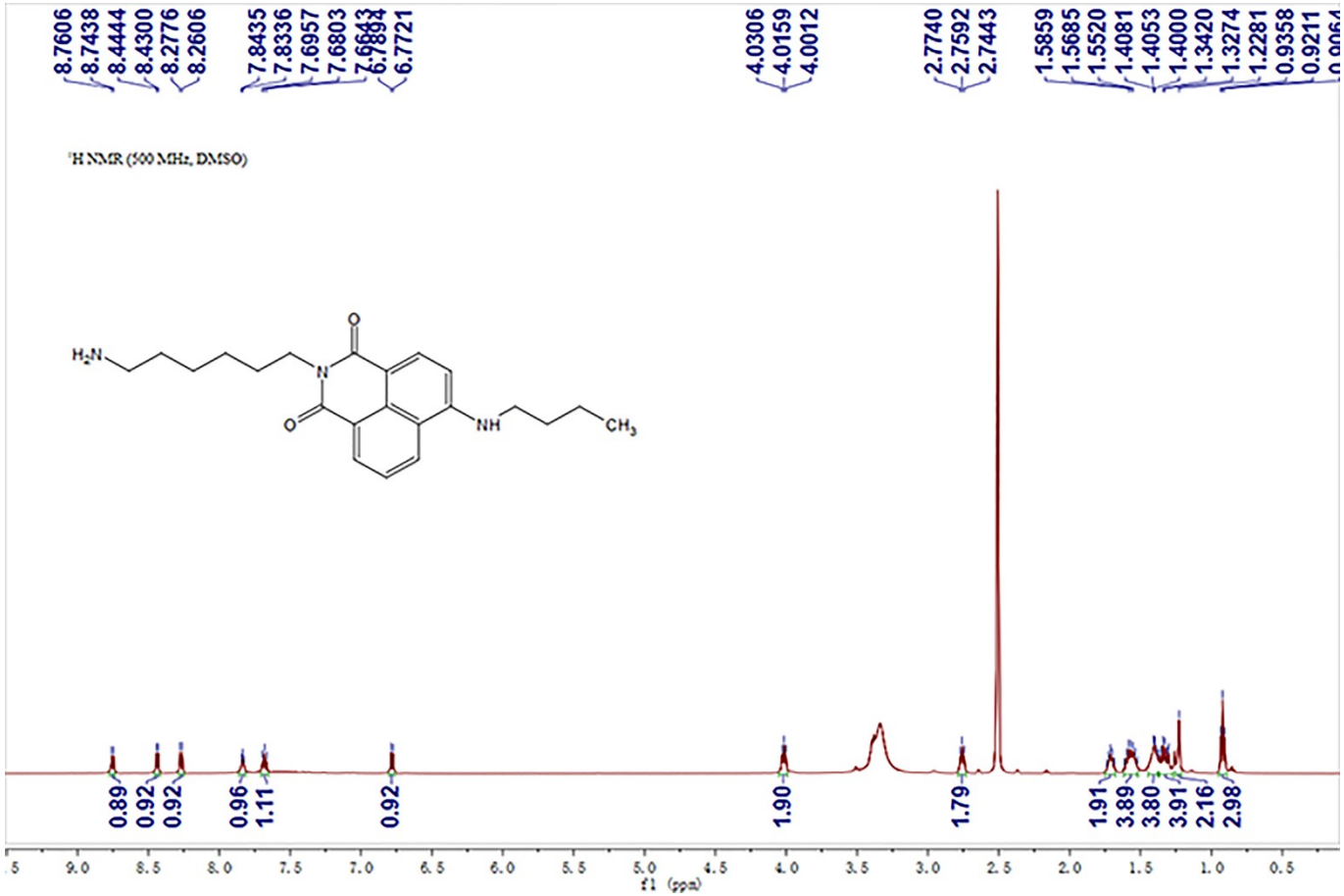

Figure S17：^{1}H NMR spectrum of **Compound 9** (500 MHz, DMSO-d_6)

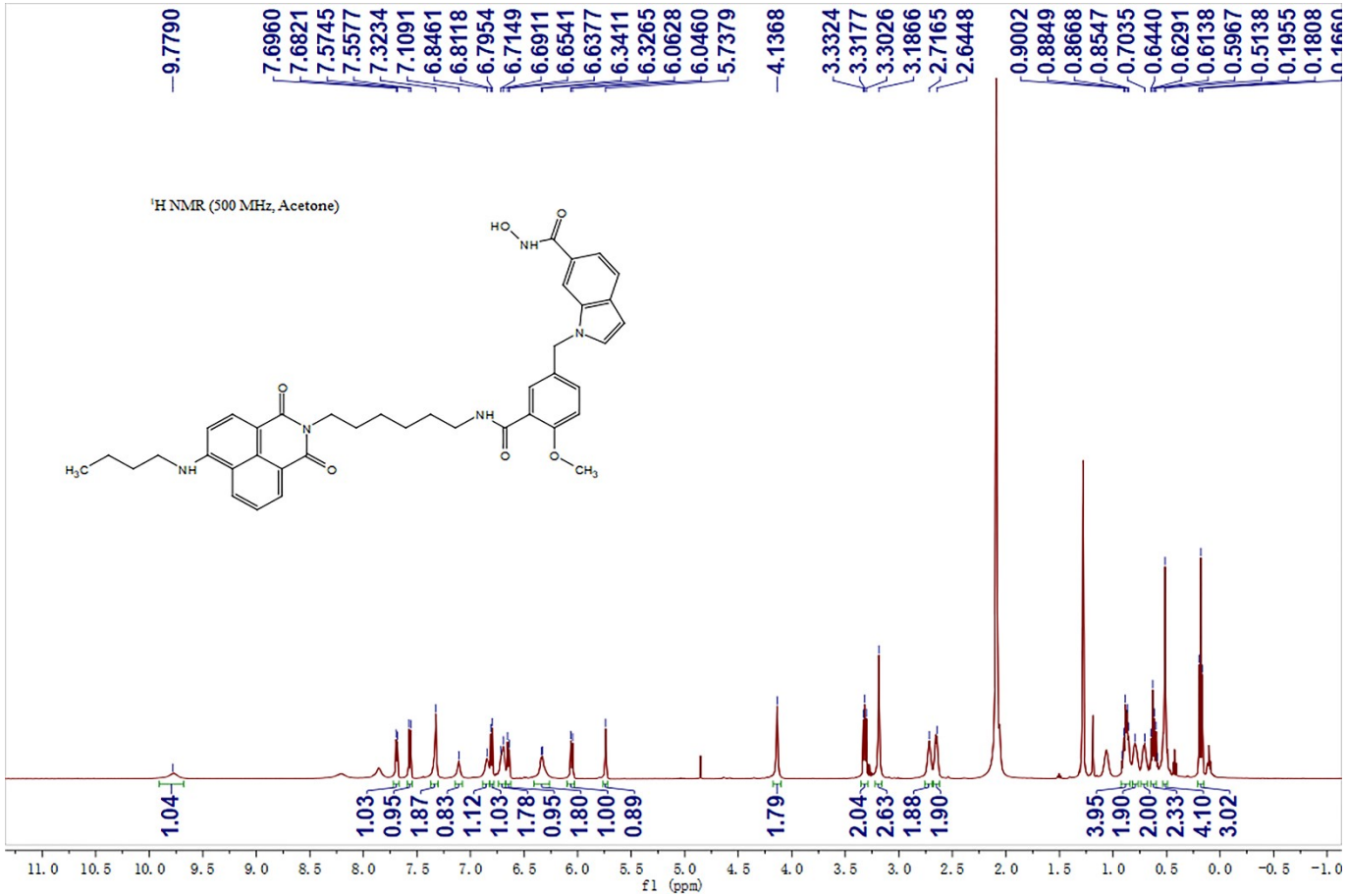

Figure S18：^{1}H NMR spectrum of **Compound 10 (NP-C6-PCI)** (500 MHz, Acetone-d_6)

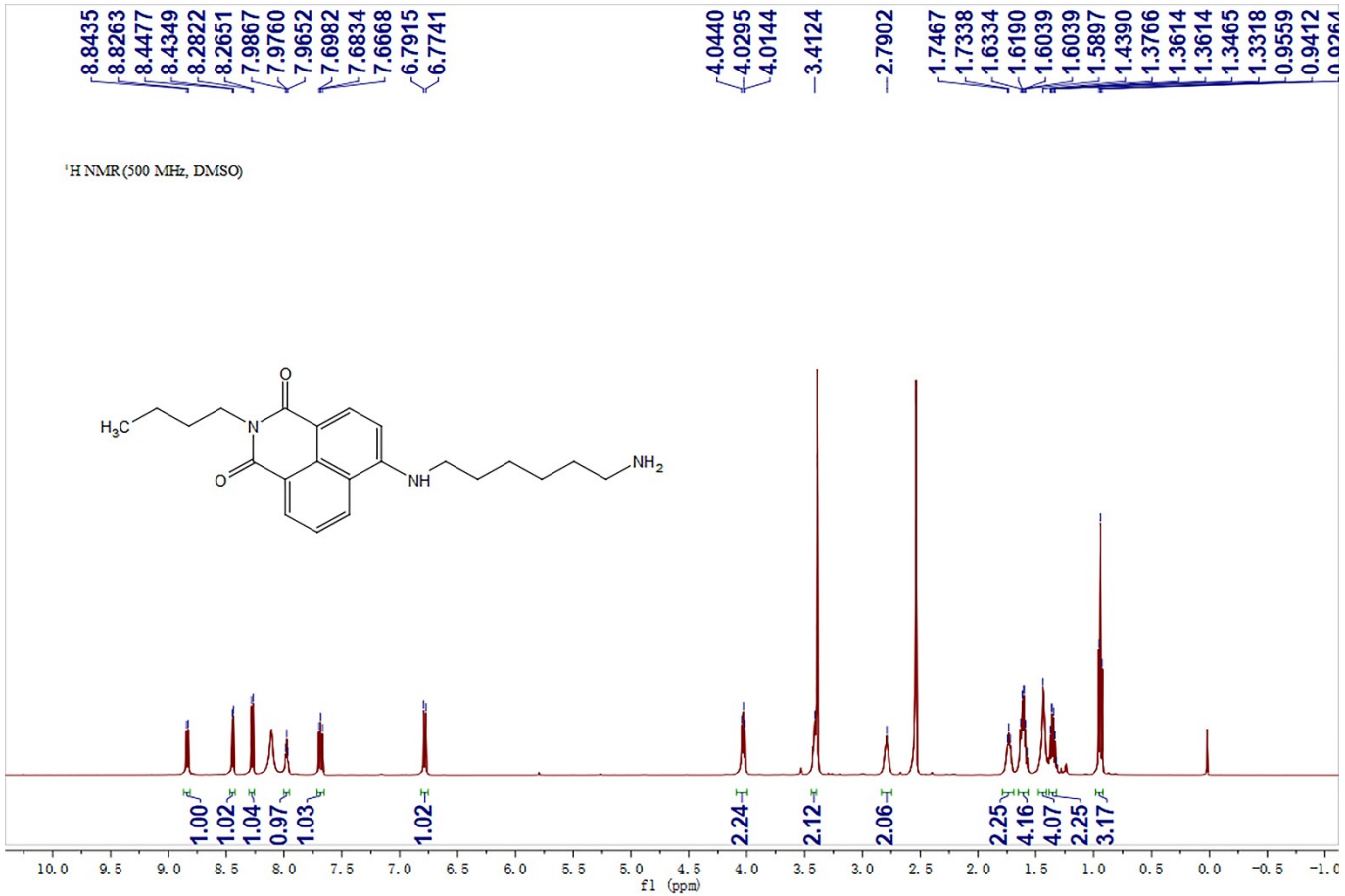

Figure S19：^{1}H NMR spectrum of **Compound 12** (500 MHz, DMSO-d_6)

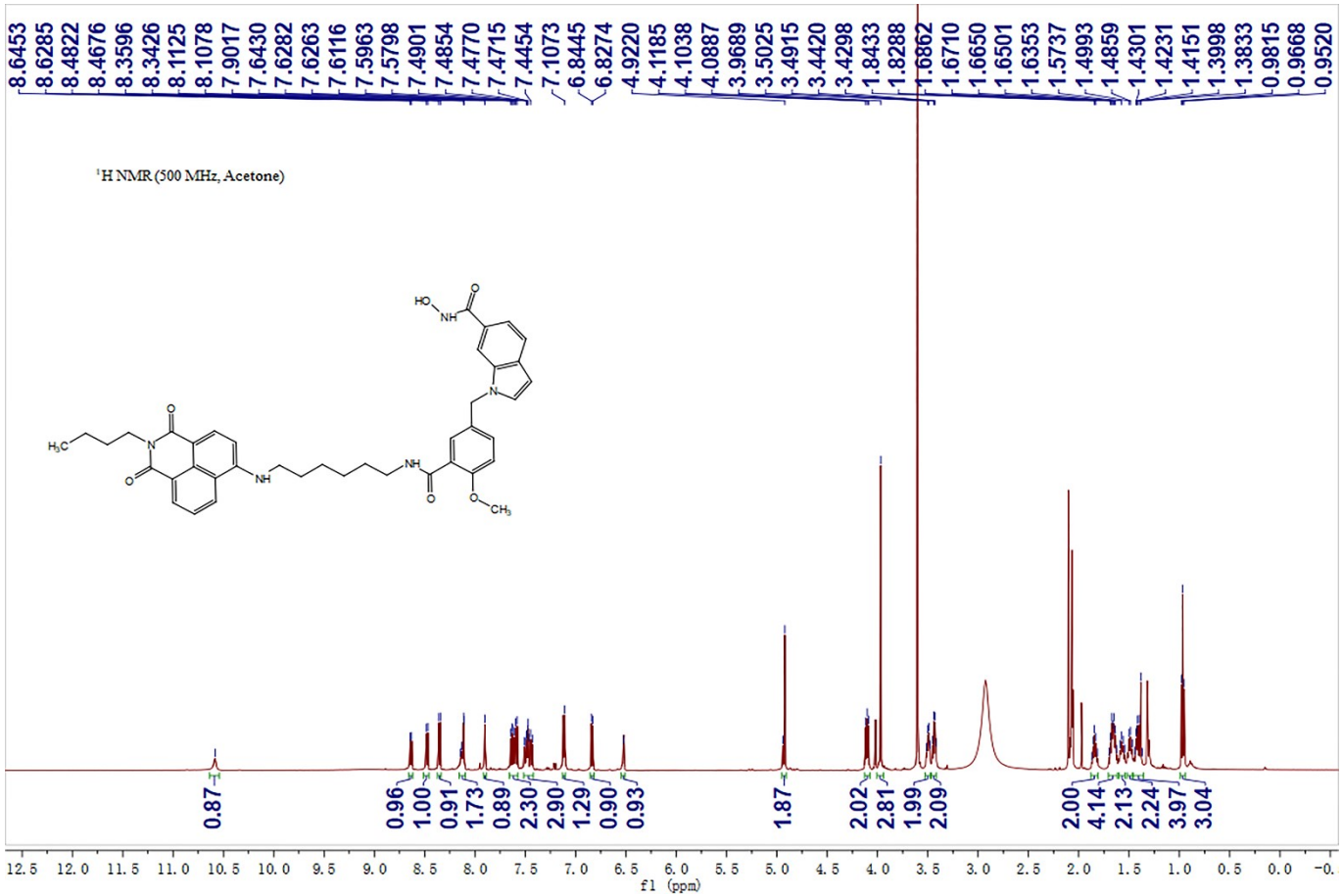

Figure S20：^{1}H NMR spectrum of **Compound 13 (AM-C6-PCI)** (500 MHz, Acetone-d_6)

^{13}C NMR spectrum of **Compounds**

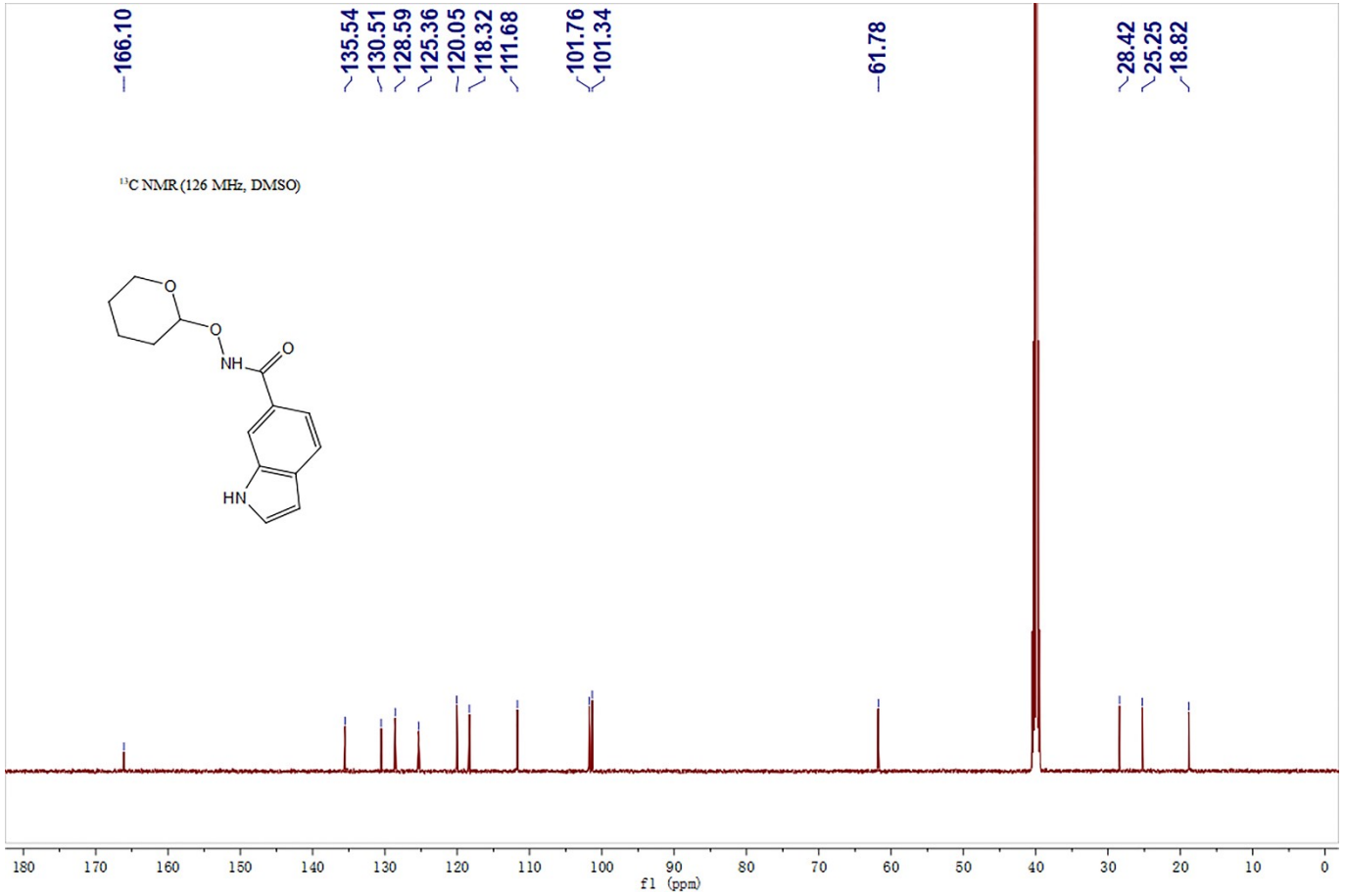

Figure S21：^{13}C NMR spectrum of **compound 3** (126 MHz, DMSO-d_6)

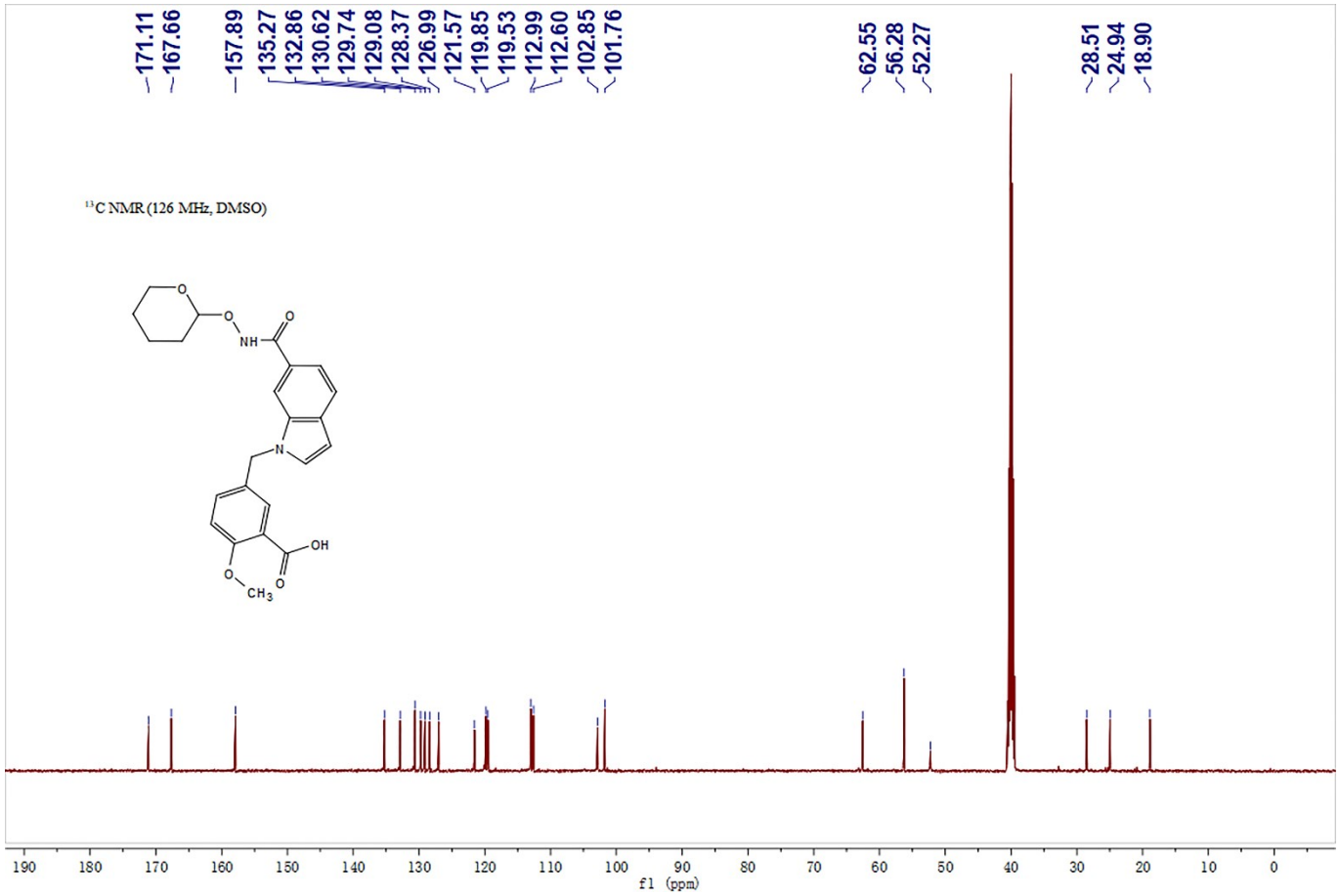

Figure S22：^{13}C NMR spectrum of **compound 6** (126 MHz, DMSO-d_6)

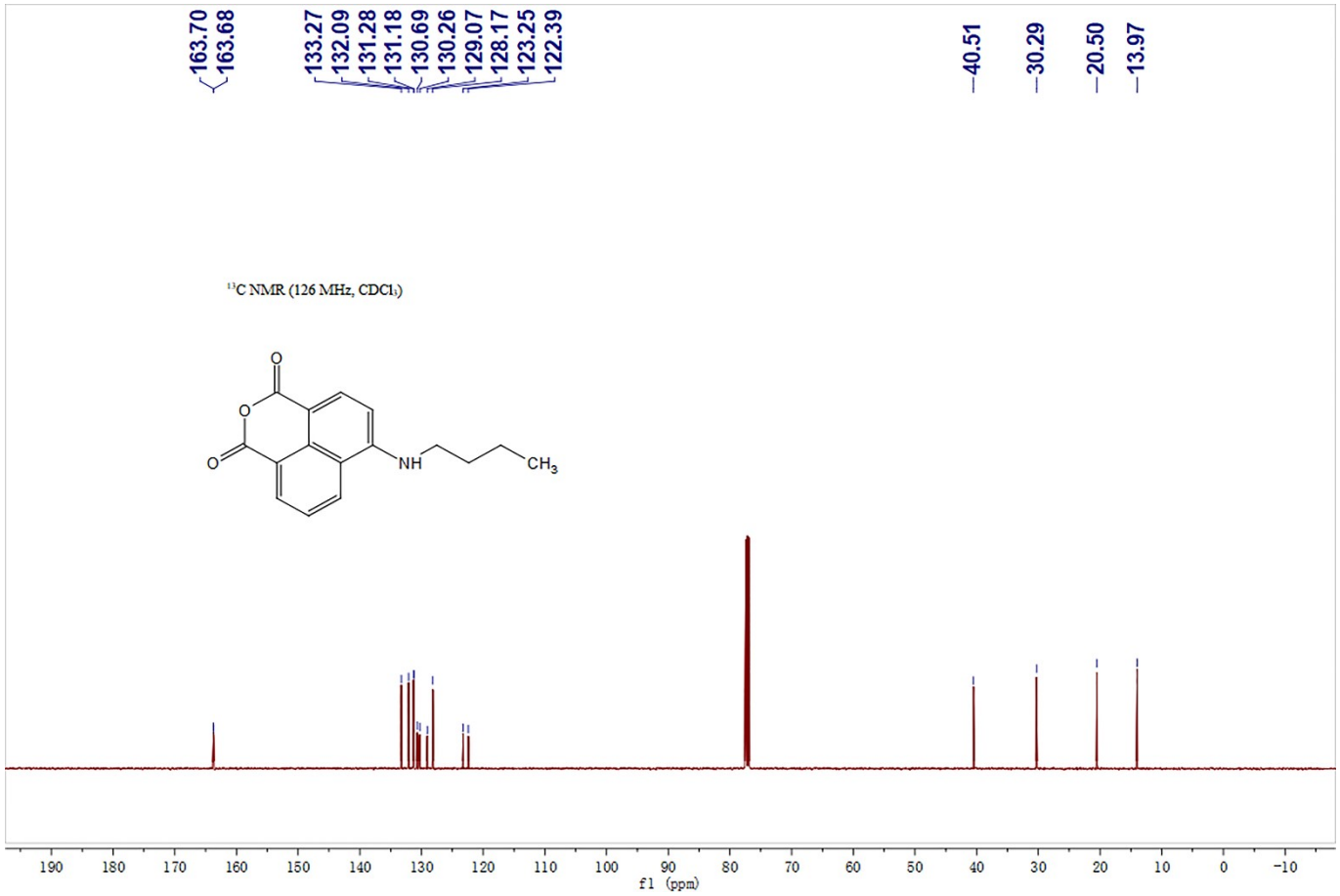

Figure S23：^{13}C NMR spectrum of **Compound 8** (500 MHz, $CDCl_3$)

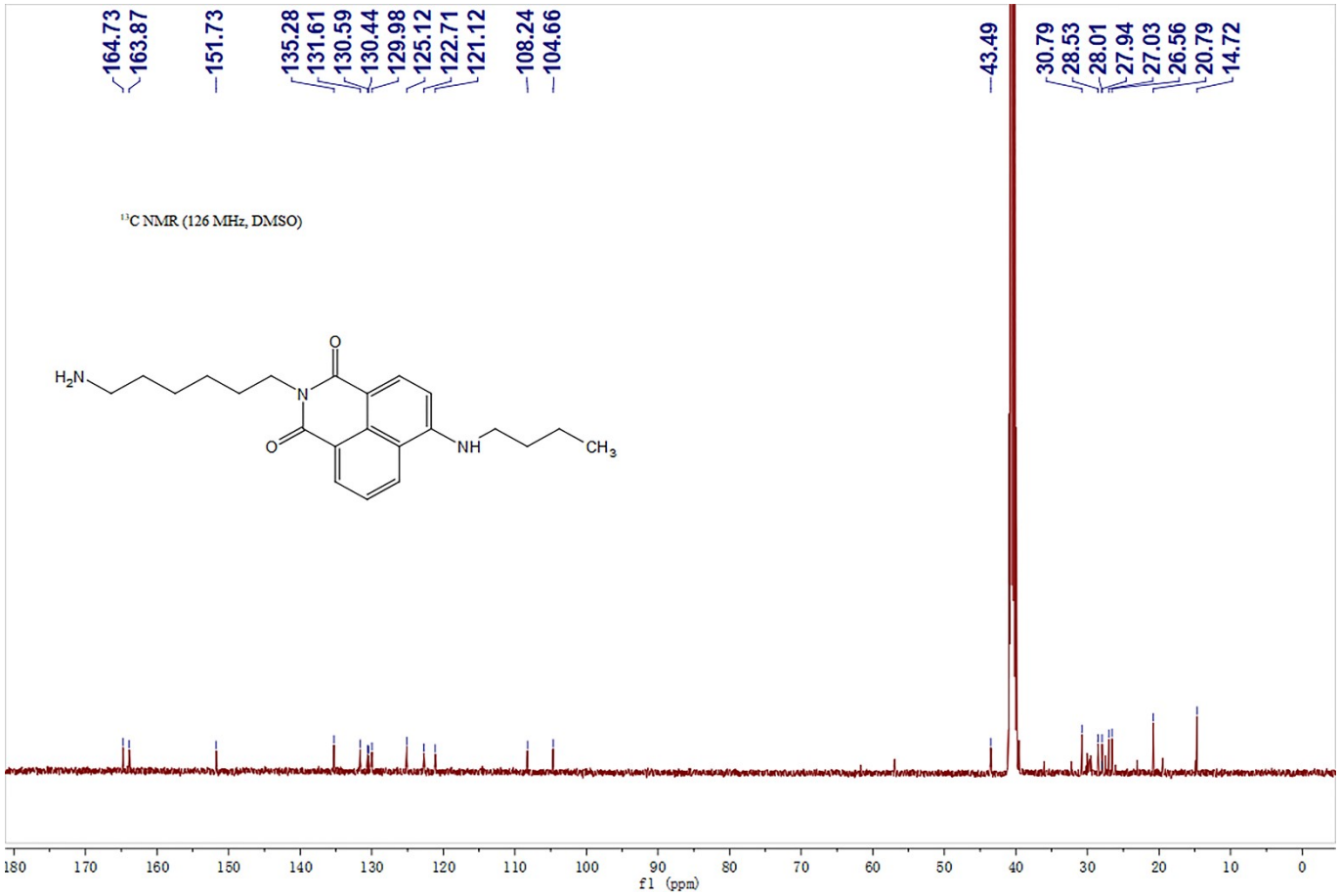

Figure S24：^{13}C NMR spectrum of **compound 9** (126 MHz, DMSO-d_6)

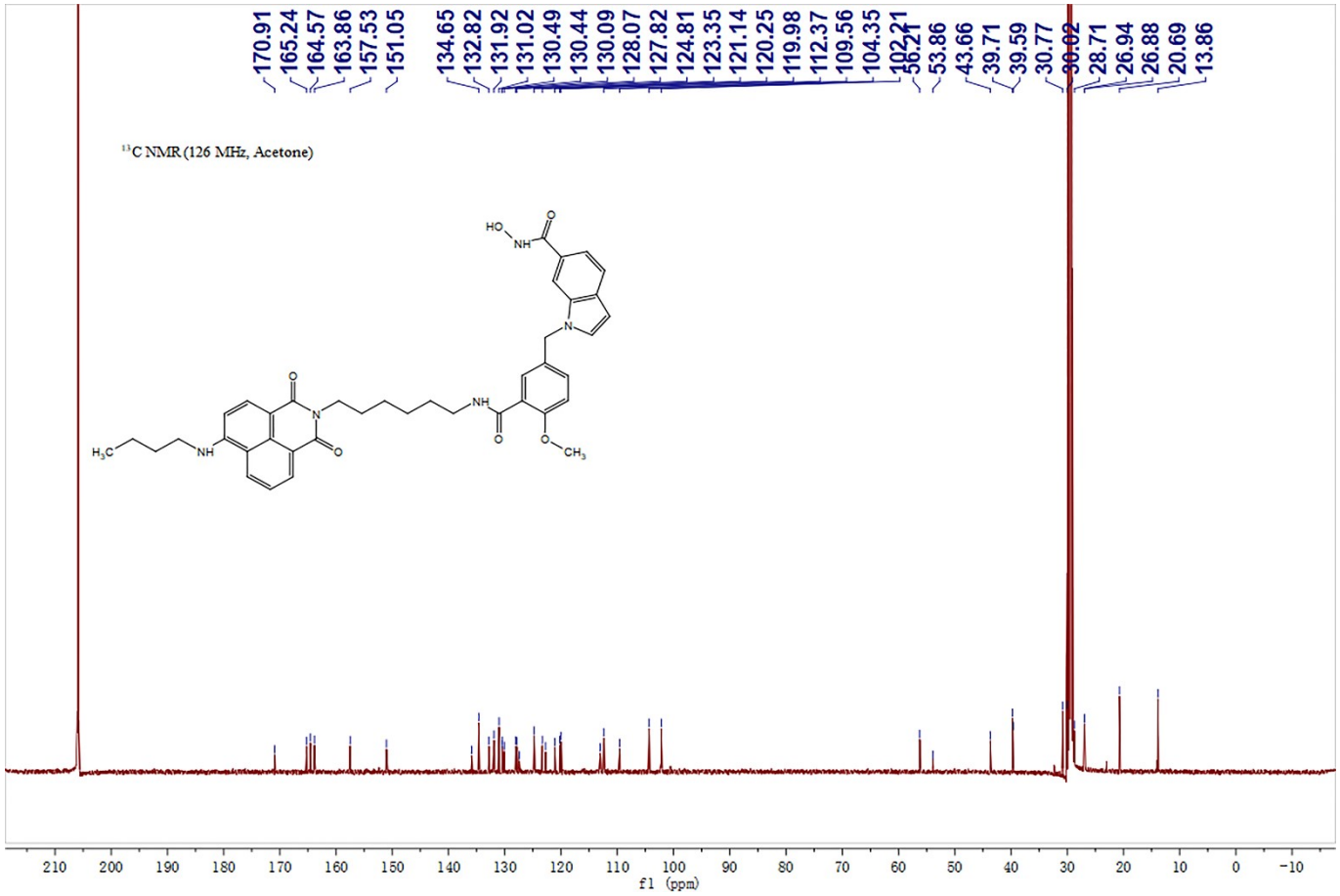

Figure S25：^{13}C NMR spectrum of **compound 10 (NP-C6-PCI)** (126 MHz, Acetone-d_6)

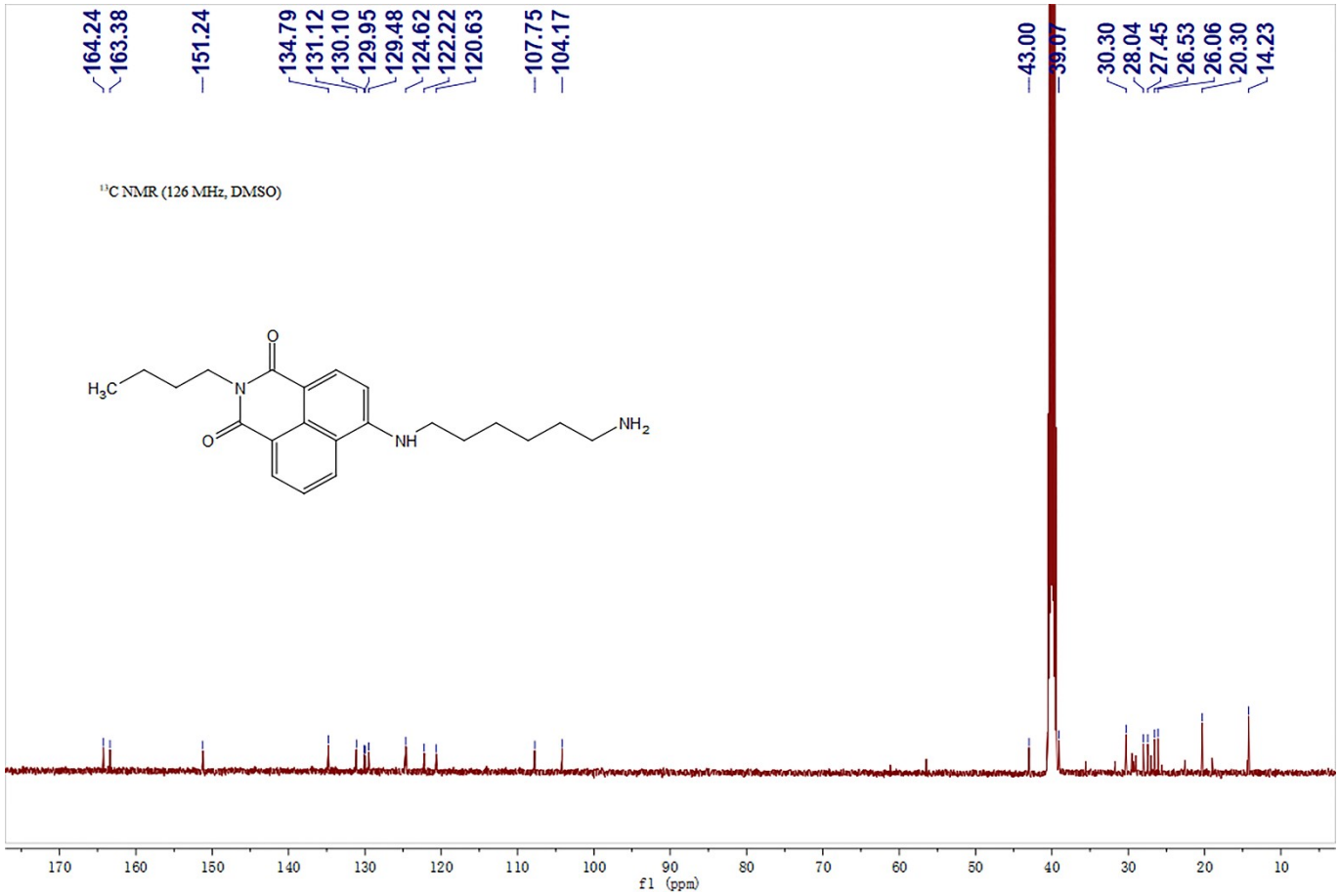

Figure S26：^{13}C NMR spectrum of **compound 12** (126 MHz, DMSO-d_6)

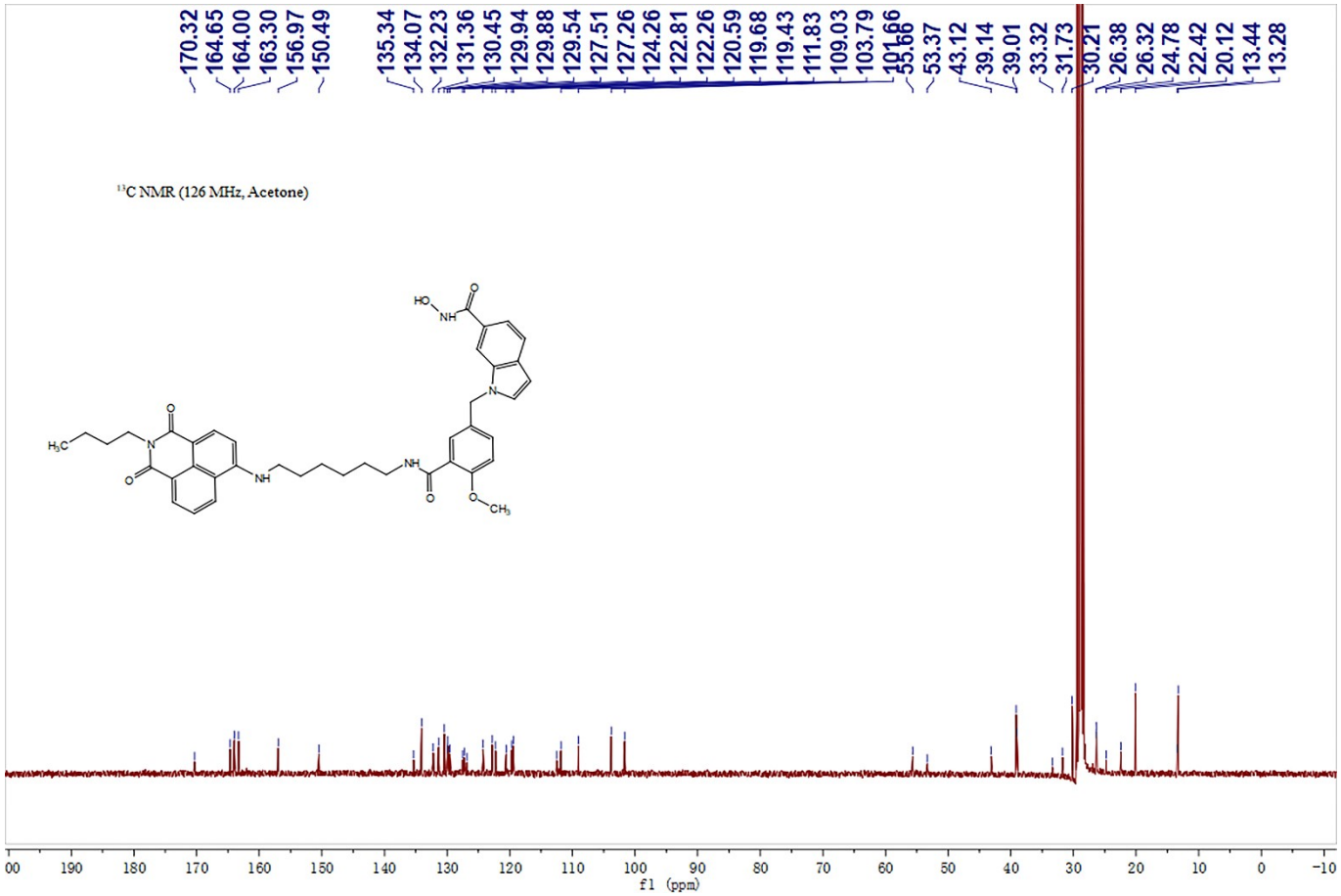

Figure S27：^{13}C NMR spectrum of **compound 13 (AM-C6-PCI)** (126 MHz, Acetone-d_6)

Table S1 Antitumor activities of tested compounds on SH-SY5Y cell (inhibition, %).

	PCI-34051	**NP-C6-PCI**	**AM-C6-PCI**
0.25μM	1.81	8.63	3.01
0.5μM	0.96	9.90	2.70
1.25μM	6.52	13.06	3.36
2.5μM	33.65	25.83	6.12
5μM	73.18	73.00	11.63
10μM	84.49	77.56	35.24
20μM	85.71	81.56	62.10

Table S2 Antitumor activities of tested compounds on MDA-MB-231 cell (inhibition, %).

	PCI-34051	**NP-C6-PCI**	**AM-C6-PCI**
0.25μM	2.72	3.48	5.98
0.5μM	1.52	1.04	3.54
1.25μM	12.37	10.16	5.95
2.5μM	20.65	10.52	8.16
5μM	18.12	14.62	15.78
10μM	38.49	20.96	24.08
20μM	48.87	36.04	39.22