

1 **Supplementary Data for**

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4 **A fluorogenic ROS-triggered hydrogen sulfide donor for alleviating cerebral ischemia-**
5 **reperfusion injury**

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18 [#]: These authors contributed equally.

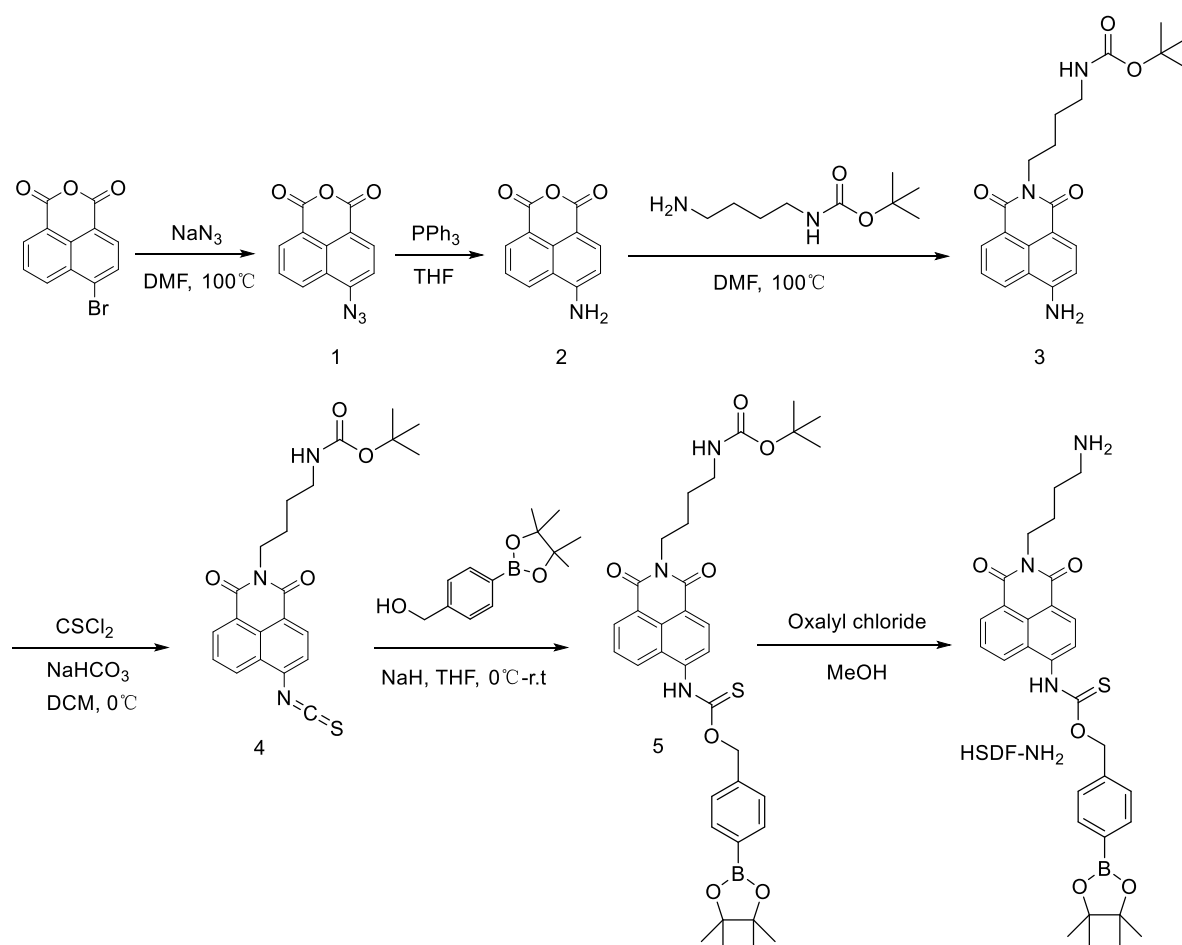
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24 **S1. Synthesis of Compound 1-7, HSDF-NH₂, CODF-NH₂ and HSDG-NH₂**



25
26 **Scheme S1.** Synthesis of HSDF-NH₂

27

28 **Synthetic procedures and characterizations:**

29 **Synthesis of compound 1**

30 Add 4-Bromo-1,8-naphthalic anhydride (13 g, 46.9 mmol) to 70 mL DMF, stir for 30 min
31 until dissolved. Dissolve sodium azide (3.5 g, 53.8 mmol) in 3 mL of water and add to the DMF
32 solution. The mixture was heated to 100°C and the reaction was stopped after 15 min. Add water
33 and collect the precipitate by filtration, wash with water and dry in vacuum to obtain compound 1.
34 Yield: 10.09g, 90%. ¹H NMR (400 MHz, Chloroform-*d*) δ 8.70 – 8.52 (m, 3H), 7.86 – 7.77 (m,
35 1H), 7.54 (d, J = 8.0 Hz, 1H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 160.47, 159.93, 145.20,
36 134.37, 133.94, 131.37, 130.33, 127.32, 124.51, 118.81, 115.12, 114.60.

37 Synthesis of compound 2

38 Compound 1 (3.68 g, 15.385 mmol) was suspended in a mixture of 30 mL THF and 60 mL
39 0.5 M HCl, and triphenylphosphine (5.25 g, 20 mmol) was slowly added to the suspension under
40 stirring. After stirring for an additional 30 min, the mixture was basified with 20 mL of 2 M
41 aqueous NaOH and the THF was removed by rotary evaporation. The resulting mixture was diluted
42 with ethyl acetate and collect the precipitate by filtration. The product was washed with ethyl
43 acetate and water, and dried under vacuum to obtain compound 2. Yield: 2.62g, 80%. ¹H NMR
44 (400 MHz, DMSO-*d*₆) δ 8.62 (d, J = 8.4 Hz, 1H), 8.36 (d, J = 7.2 Hz, 1H), 8.12 (d, J = 8.5 Hz,
45 1H), 7.73 (s, 2H), 7.62 (t, J = 7.9 Hz, 1H), 6.82 (d, J = 8.5 Hz, 1H). ¹³C NMR (101 MHz, DMSO-
46 *d*₆) δ 162.42, 160.73, 154.33, 136.25, 133.37, 132.94, 131.09, 124.72, 119.70, 118.58, 109.16,
47 102.63.

48

49 Synthesis of compound 3

50 Compound 2 (639.57 mg, 3 mmol) and tert-Butyl N-(4-aminobutyl)carbamate (1.129 g, 6
51 mmol) were heated to 100°C in 15 mL DMF overnight. Add cold water and collect the precipitate
52 by filtration. The residue was purified by flash column chromatography to obtain compound 3.
53 Yield: 804mg, 70%. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.60 (d, J = 8.4 Hz, 1H), 8.41 (d, J = 7.2
54 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.63 (t, J = 7.8 Hz, 1H), 7.42 (s, 2H), 6.84 (d, J = 8.4 Hz, 1H),
55 6.77 (t, J = 5.8 Hz, 1H), 3.99 (t, J = 7.1 Hz, 2H), 2.98 – 2.86 (m, 2H), 1.64 – 1.53 (m, 2H), 1.47 –
56 1.37 (m, 2H), 1.35 (s, 9H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 164.24, 163.37, 156.03, 153.14,
57 134.38, 131.42, 130.14, 129.72, 124.40, 122.24, 119.83, 108.62, 108.03, 77.79, 28.71, 27.70, 25.78.

58

59 Synthesis of compound 4

60 To an anhydrous DCM solution of compound 3 (1.43 g, 3.73 mmol) and NaHCO₃ (630 mg,
61 7.50 mmol), thiophosgene (0.858 mg, 7.45 mmol) in anhydrous DCM was added dropwise over
62 30 min at 0°C. The reaction mixture was stirred overnight at r.t. After removing the solvent, the
63 residue was purified by silica-gel chromatography to obtain compound 4. Yield: 951 mg, 60%. ¹H
64 NMR (400 MHz, Chloroform-*d*) δ 8.64 (dd, J = 7.3, 1.1 Hz, 1H), 8.52 (d, J = 7.9 Hz, 1H), 8.45

65 (dd, $J = 8.4, 1.1$ Hz, 1H), 7.85 (dd, $J = 8.4, 7.4$ Hz, 1H), 7.63 (d, $J = 7.9$ Hz, 1H), 4.67 (s, 1H), 4.18
66 (t, $J = 7.4$ Hz, 2H), 3.21 (q, $J = 6.7$ Hz, 2H), 1.82 – 1.73 (m, 2H), 1.68 – 1.58 (m, 2H), 1.44 (s, 9H).
67 ^{13}C NMR (101 MHz, Chloroform-*d*) δ 163.63, 163.12, 155.93, 133.96, 132.17, 131.26, 128.88 (d,
68 $J = 8.1$ Hz), 127.90, 127.48, 124.44, 123.11, 121.02, 40.05, 28.42, 27.59, 25.41. MS(ESI) m/z :
69 426.14 $[\text{M}+\text{H}]^+$.

70

71 Synthesis of compound 5

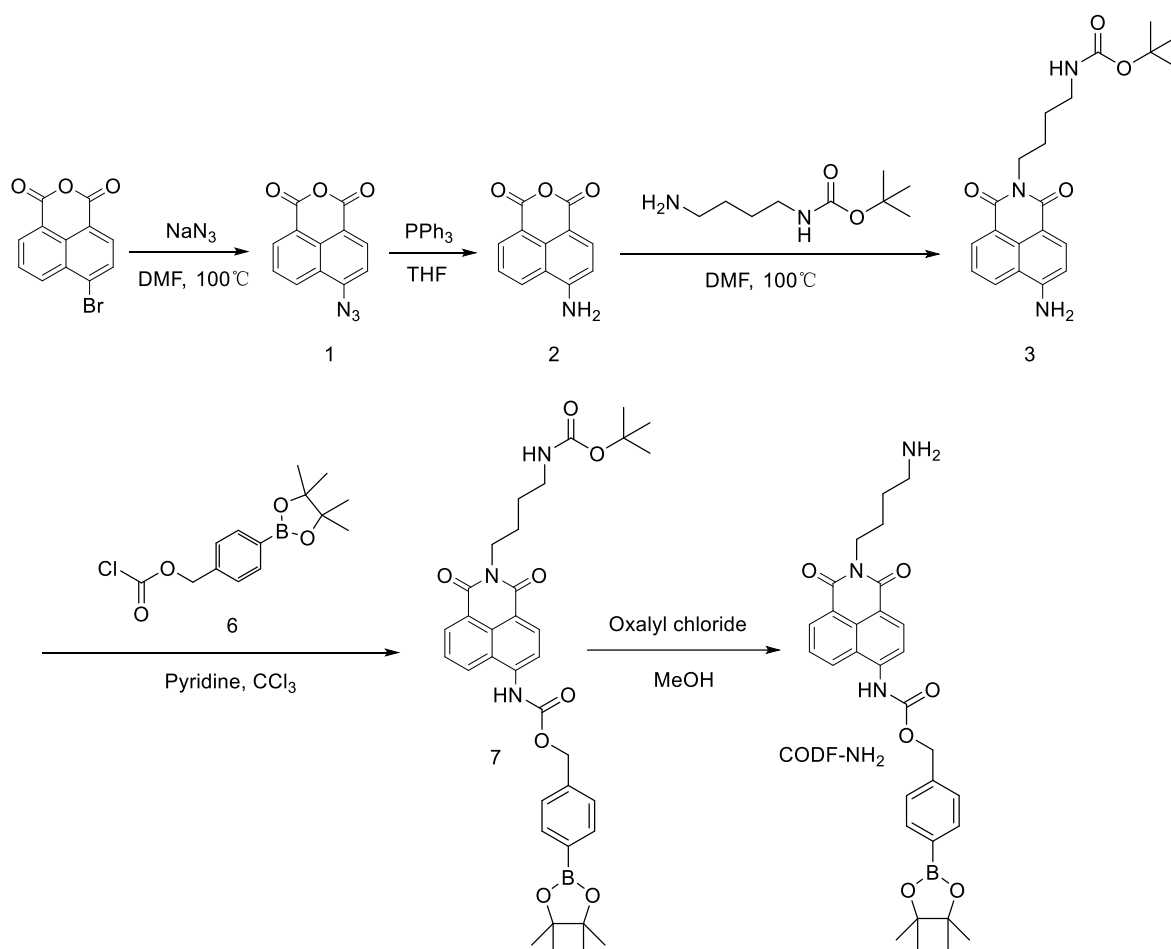
72 NaH (37 mg, 1.61 mmol, 60% in paraffin liquid) was dissolved in 15 mL anhydrous THF,
73 phenylboronic acid pinacol ester (377 mg, 1.61 mmol) was added to this solvent at 0°C. Then
74 compound 4 (684 mg, 1.61 mmol) was dissolved in 10 mL THF and added to the mixture dropwise.
75 The resultant mixture was stirred at 0°C for 30 min, then stirred at r.t. overnight. The reaction was
76 quenched by adding brine and the reactant was extracted with ethyl acetate. The organic layers
77 were combined and dried over MgSO_4 , filtered and evaporated under reduced pressure to obtain
78 the crude product, which was purified by silica gel flash chromatography to obtain compound 5.
79 Yield: 160 mg, 15%. ^1H NMR (400 MHz, Chloroform-*d*) δ 9.29 (s, 1H), 8.54 (dd, $J = 20.2, 7.6$ Hz,
80 2H), 8.30 (d, $J = 8.5$ Hz, 1H), 7.91 (s, 1H), 7.74 (dd, $J = 24.6, 7.8$ Hz, 3H), 7.38 – 7.26 (m, 2H),
81 5.61 (s, 2H), 4.71 (s, 1H), 4.14 (t, $J = 7.4$ Hz, 2H), 3.16 (q, $J = 6.8$ Hz, 2H), 1.79 – 1.68 (m, 2H),
82 1.61 – 1.54 (m, 2H), 1.41 (s, 9H), 1.35 (s, 12H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 189.47,
83 163.95, 163.45, 137.66, 135.04, 131.58, 131.39, 128.90, 128.31, 127.47, 127.18, 122.99, 120.65,
84 83.97, 73.48, 39.91, 28.41, 27.52, 25.39, 24.87. MS(ESI) m/z : 660.28 $[\text{M}+\text{H}]^+$.

85

86 Synthesis of HSDF-NH₂

87 Compound 5 (131.8 mg, 0.2 mmol) was dissolved in methanol, then oxalyl chloride (51.18
88 μl , 0.6 mmol) was added dropwise to the above solution, and stirred at room temperature for 1
89 hour. After the reaction was completed, the crude product was obtained by evaporation under
90 reduced pressure. The crude material was then extracted with dichloromethane and washed with
91 brine. The organic layer was dried over anhydrous MgSO_4 and filtered. After concentration, the
92 final product was purified by silica gel flash chromatography. Yield: 33.54 mg, 30%. ^1H NMR

93 (400 MHz, DMSO-*d*₆) δ 8.51 (dd, *J* = 10.0, 7.5 Hz, 2H), 8.38 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.93 – 7.87
 94 (m, 2H), 7.68 (d, *J* = 7.5 Hz, 2H), 7.42 (d, *J* = 7.5 Hz, 2H), 5.58 (s, 2H), 4.14 (s, 2H), 4.08 (t, *J* =
 95 6.7 Hz, 2H), 2.82 (t, *J* = 7.4 Hz, 2H), 1.71 (p, *J* = 6.7 Hz, 2H), 1.61 (q, *J* = 8.4, 7.6 Hz, 2H), 1.29
 96 (s, 12H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 163.94, 163.47, 158.43 (q, *J* = 31.4 Hz), 139.49,
 97 134.99, 131.56, 131.32, 130.35, 128.76, 127.71, 127.49, 122.84, 120.62, 118.63, 116.65, 114.66,
 98 84.19, 73.99, 71.79, 49.05, 39.09, 25.41, 25.13 (d, *J* = 3.5 Hz). HRMS(ESI) *m/z*: [M+H]⁺, calcd
 99 for C₃₀H₃₅BN₃O₅S⁺: 560.2312, found: 560.2383.



100
 101 **Scheme S2.** Synthesis of CODF-NH₂

102

103 **Synthetic procedures and characterizations:**

104 Synthesis of compound 6

105 Place Na₂CO₃ (7 g, 66 mmol) into a dry round-bottomed flask and add triphosgene (4.4 g,
 106 14.8 mmol) in 30 mL of toluene at 0°C. Dissolve (4-(4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-

107 yl)phenyl)methanol (1.74 g, 7.4 mmol) in 10 mL toluene, add it to the above system at 0°C and
108 stir for 1 hour. After stirring at r.t. for 6 hours, the mixture was filtered through a Celite pad. The
109 solvent was removed in vacuo without further purification. Yield: 658.4 mg, 30%.

110

111 Synthesis of compound 7

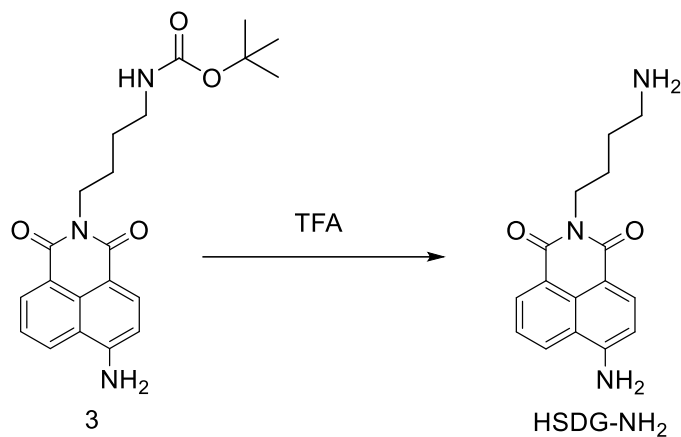
112 Compound 3 (2.276 g, 5.94 mmol) was dissolved in 30 mL of anhydrous THF, and Na₂CO₃
113 (3.5 g, 33 mmol) was added. Compound 6 (1.8 g, 6.069 mmol) was dissolved in 60 mL DCM
114 solution and added dropwise to the above solution. After stirring at room temperature overnight,
115 the solvent was removed in vacuo to obtain the crude product, which was purified by silica gel
116 flash chromatography to obtain compound 7. Yield: 649.61 mg, 17%. ¹H NMR (400 MHz,
117 Chloroform-*d*) δ 8.55 (dd, J = 12.0, 7.7 Hz, 2H), 8.32 (d, J = 8.3 Hz, 1H), 8.23 (d, J = 8.5 Hz, 1H),
118 7.93 (s, 1H), 7.84 (d, J = 7.9 Hz, 2H), 7.70 (dd, J = 8.5, 7.3 Hz, 1H), 7.43 (d, J = 7.7 Hz, 2H), 5.30
119 (s, 2H), 4.72 (s, 1H), 4.16 (t, J = 7.4 Hz, 2H), 3.18 (q, J = 6.5 Hz, 2H), 1.80 – 1.71 (m, 2H), 1.63
120 – 1.55 (m, 2H), 1.43 (s, 9H), 1.36 (s, 12H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 164.13, 163.62,
121 156.04, 153.26, 144.12, 139.25, 138.35, 135.13, 135.00, 132.45, 131.25, 127.61, 126.49, 126.05,
122 123.13, 83.98, 83.80, 67.73, 65.15, 40.22, 39.84, 28.42, 27.53, 25.42, 24.86. MS(ESI) m/z: 644.30
123 [M+H]⁺.

124

125 Synthesis of CODF-NH₂

126 Compound 7 (192 mg, 0.298 mmol) was dissolved in methanol, then oxalyl chloride (76.37
127 μL, 0.9 mmol) was added dropwise to the above solution, and stirred at room temperature for 1
128 hour. After the reaction was completed, the crude product was obtained by evaporation under
129 reduced pressure. The crude material was then extracted with dichloromethane and washed with
130 brine. The organic layer was dried over anhydrous MgSO₄ and filtered. After concentration, the
131 final product was purified by silica gel flash chromatography. Yield: 48.5 mg, 30%. ¹H NMR (400
132 MHz, DMSO-*d*₆) δ 8.75 – 8.69 (m, 1H), 8.53 – 8.43 (m, 2H), 8.19 (d, J = 8.2 Hz, 1H), 7.83 (dd, J
133 = 8.6, 7.3 Hz, 1H), 7.75 – 7.69 (m, 2H), 7.51 (d, J = 7.7 Hz, 2H), 5.31 (s, 2H), 4.06 (t, J = 6.7 Hz,
134 2H), 3.36 (s, 2H), 2.80 (t, J = 7.3 Hz, 2H), 1.77 – 1.66 (m, 2H), 1.66 – 1.55 (m, 2H), 1.30 (s, 12H).

135 ^{13}C NMR (101 MHz, DMSO- d_6) δ 164.01, 163.45, 154.37, 141.22, 140.07, 135.07, 132.21, 131.44,
136 129.89, 128.79, 127.73, 126.86, 124.31, 122.59, 118.68, 117.47, 84.19, 66.74, 49.05, 39.02, 25.43,
137 25.14. HRMS(ESI) m/z : $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{30}\text{H}_{35}\text{BN}_3\text{O}_6^+$: 544.2541, found: 544.2609.



138

139 **Scheme S3.** Synthesis of HSDG-NH₂

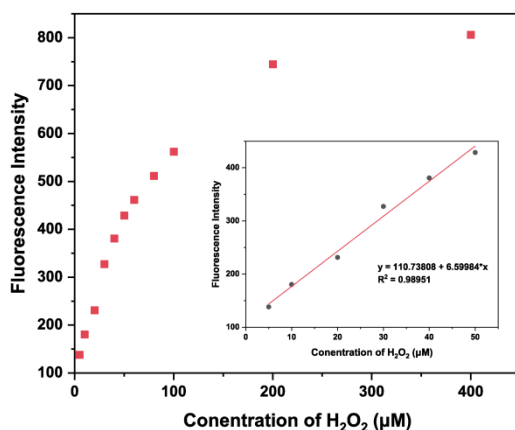
140

141 **Synthetic procedures and characterizations:**

142 Synthesis of HSDG-NH₂

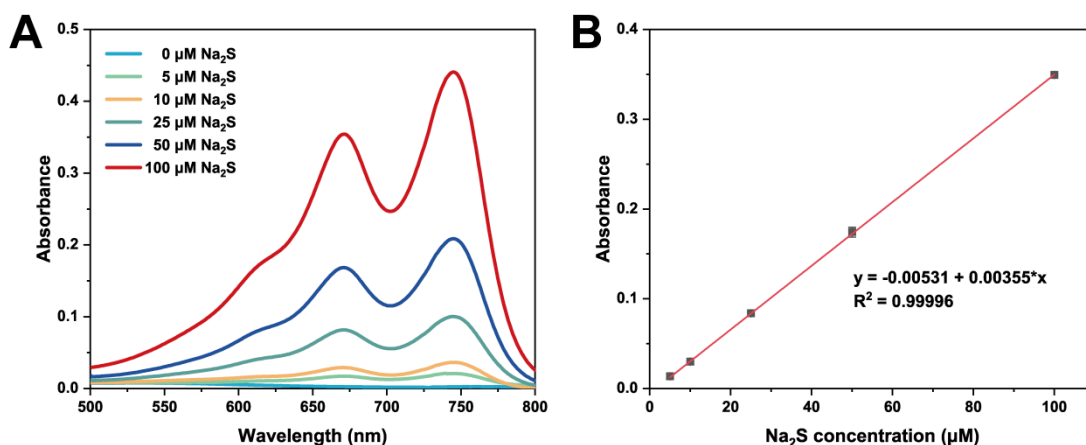
143 Compound 3 (383.18 mg, 1 mmol) was dissolved in methanol, then oxalyl chloride (225.9 μl ,
144 3 mmol) was added dropwise to the above solution, and stirred at room temperature for 1 hour.
145 After the reaction was completed, the crude product was obtained by evaporation under reduced
146 pressure. The crude material was then extracted with dichloromethane and washed with brine. The
147 organic layer was dried over anhydrous MgSO_4 and filtered. After concentration, the final product
148 was purified by silica gel flash chromatography. Yield: 90.6 mg, 32%. ^1H NMR (400 MHz,
149 DMSO- d_6) δ 8.70 (d, $J = 8.4$ Hz, 1H), 8.42 (d, $J = 7.2$ Hz, 1H), 8.18 (d, $J = 8.4$ Hz, 1H), 8.00 (s,
150 2H), 7.64 (t, $J = 7.8$ Hz, 1H), 7.61 (s, 2H), 6.87 (d, $J = 8.4$ Hz, 1H), 4.03 (t, $J = 6.6$ Hz, 2H), 2.77
151 (t, $J = 7.2$ Hz, 2H), 1.71 – 1.56 (m, 4H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 164.36, 163.45, 159.11,
152 158.80, 153.36, 134.47, 131.51, 129.92, 124.39, 119.07, 108.65, 49.02, 25.33, 25.16. HRMS(ESI)
153 m/z : $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{16}\text{H}_{18}\text{N}_3\text{O}_2^+$: 284.1321, found: 284.1390.

154 **S2. Spectroscopic Analysis**



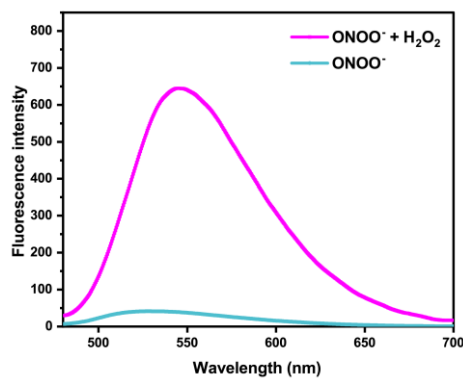
155
156 Figure S1. Fluorescence intensity changes with various concentration of H₂O₂ recorded after 40 min of reaction,
157 inset: the linear fitting curve in the range of 0–50 μM H₂O₂.

158

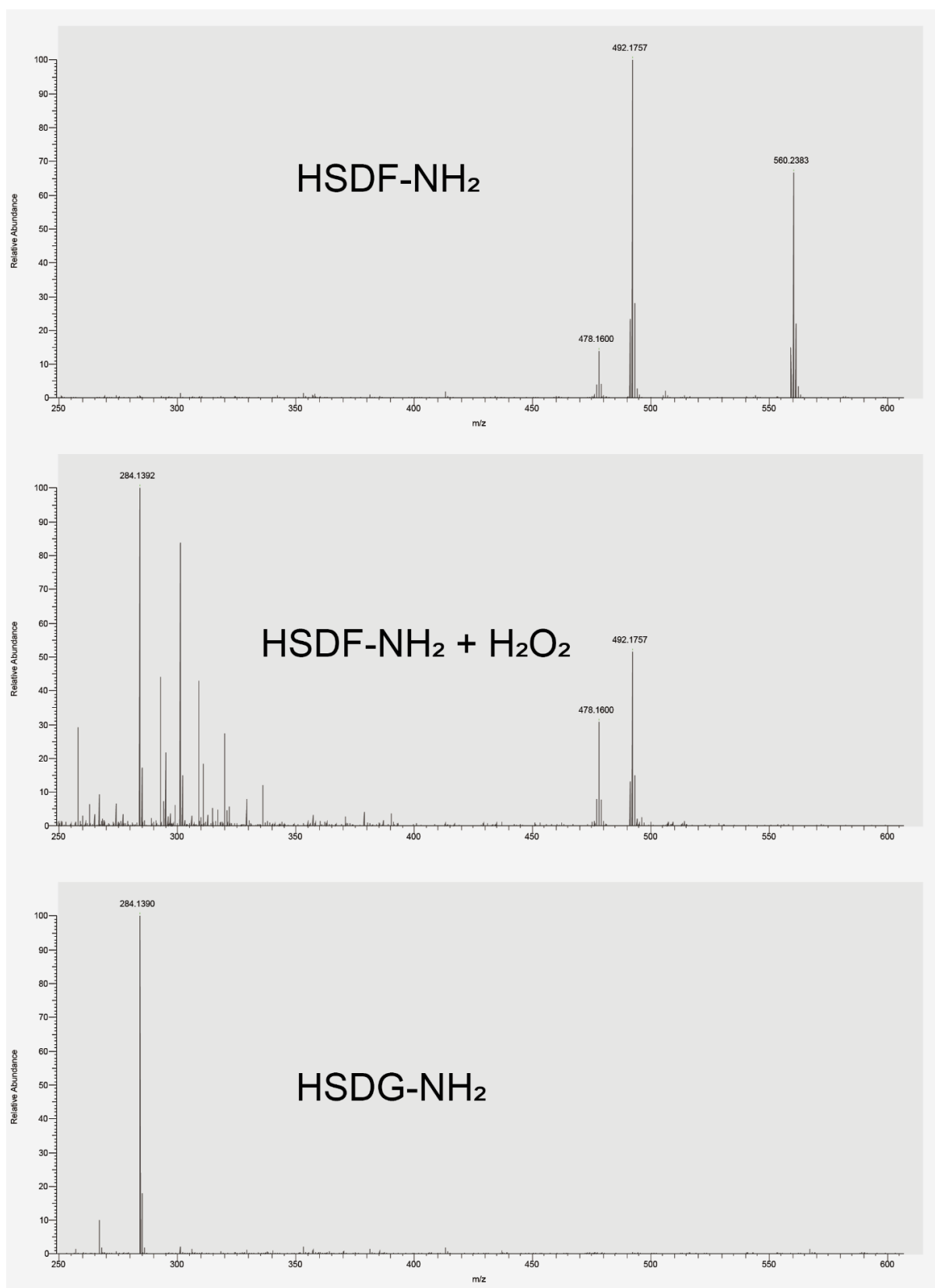


159
160 Figure S2. (A) UV-Vis absorption spectrum of Na₂S at different concentrations via MB assay. (B) H₂S calibration
161 curve obtained with Na₂S.

162

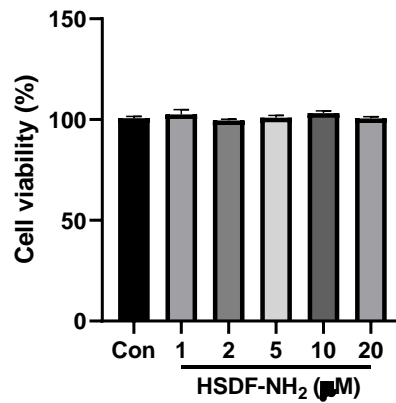


163
164 Figure S3. Fluorescence intensity of 10μM HSDF-NH₂ reacting first with ONOO⁻ (100 μM) and then with H₂O₂
165 (100 μM).

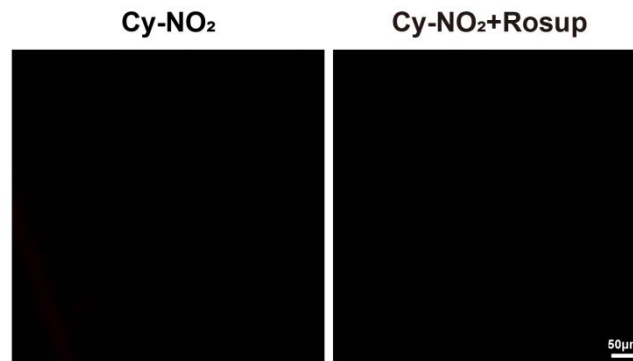


167
168 Figure S4. HRMS traces of the samples: HSDF-NH₂, HSDF-NH₂ after reacting with H₂O₂ in PBS buffer for 120
169 min, and HSDG-NH₂.

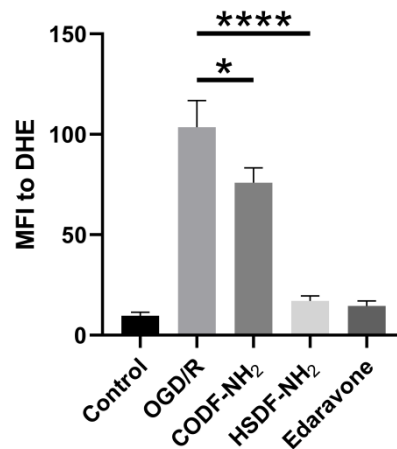
170 **S3. Cells Assay**



171
172 Figure S5. *In vitro* cytotoxicity evaluation of HSDF-NH₂ in PC-12 cells after incubation for 24 h. Data are
173 expressed as means ± SD (n = 5).
174

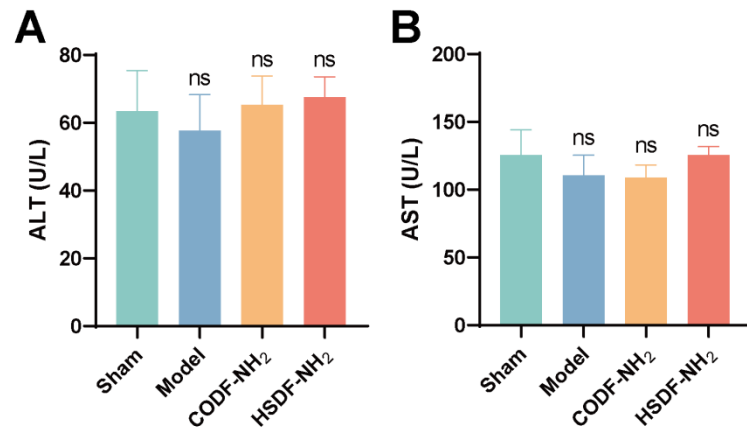


175
176 Figure S6. Confocal microscopy images of PC-12 cells treated with Rosup in Cy-NO₂ channel in the absence of
177 HSDF-NH₂.
178

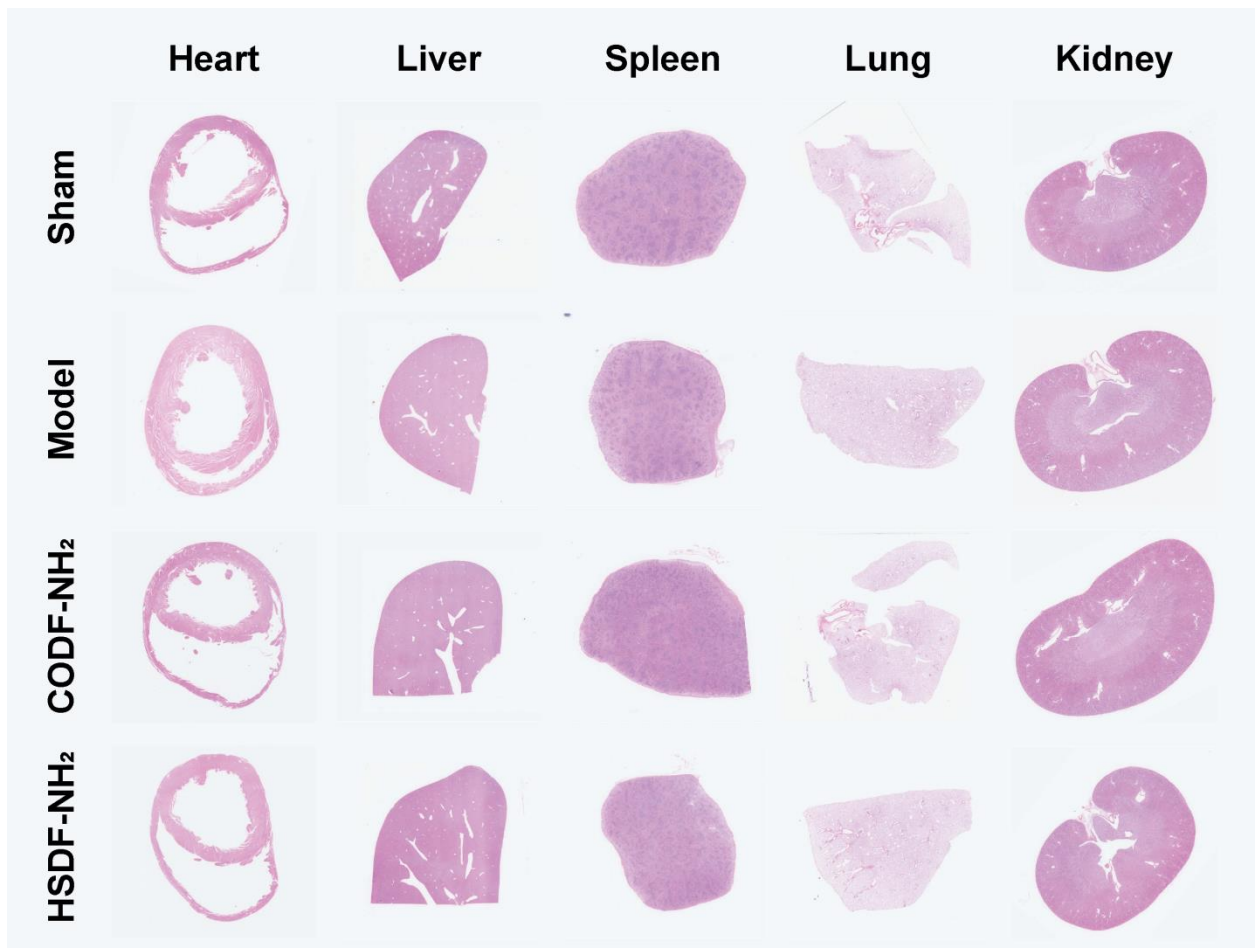


179
180 Figure S7. The quantified results of fluorescence in Figure 3B. Data are expressed as means ± SD (n = 3).
181

182 **S4. Biological Data**

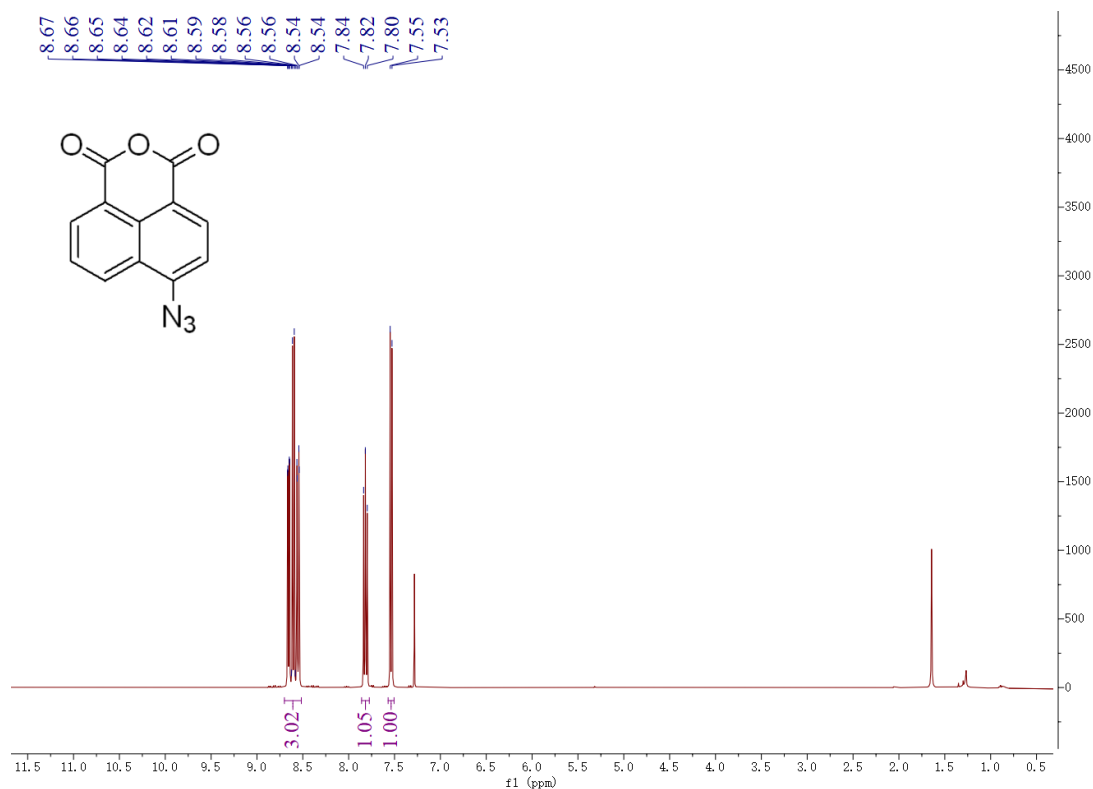


183
184 Figure S8. (A) The level of (a) ALT and (b) AST in rats after treated with different drugs. Data are expressed as
185 means \pm SD (n = 3).
186



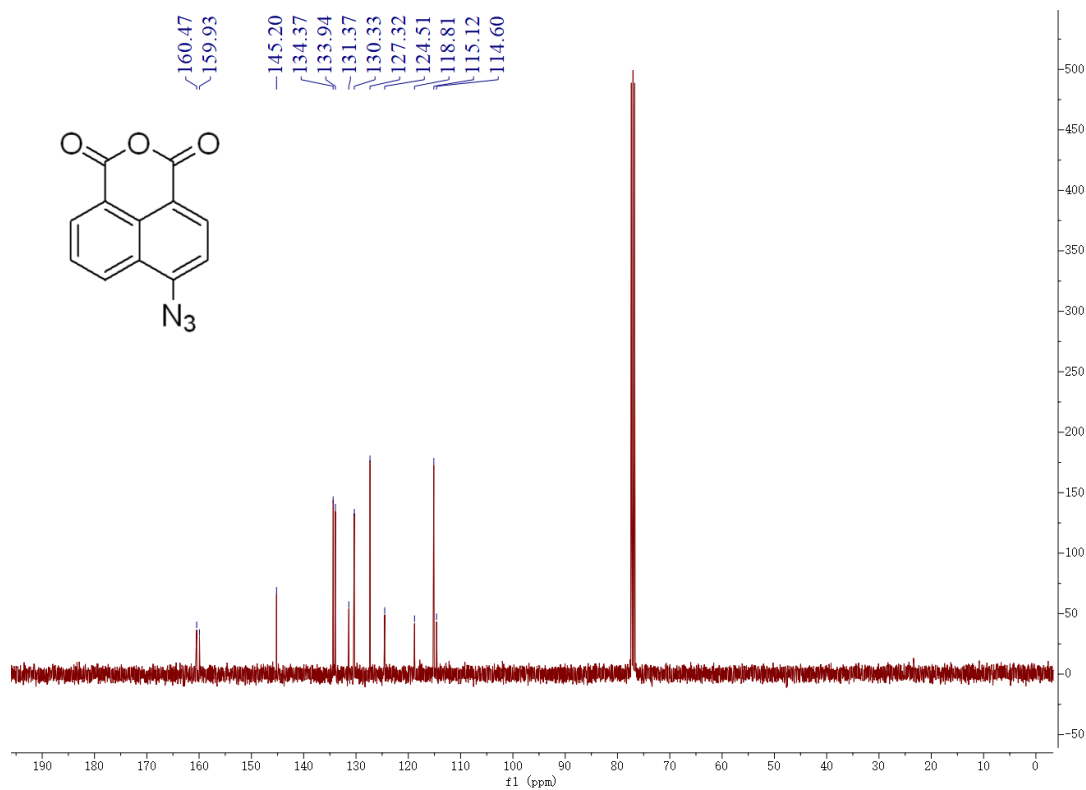
187
188 Figure S9. The H&E staining images of main organs with full view.

189 **S5. NMR Spectra and HRMS Spcctrum**



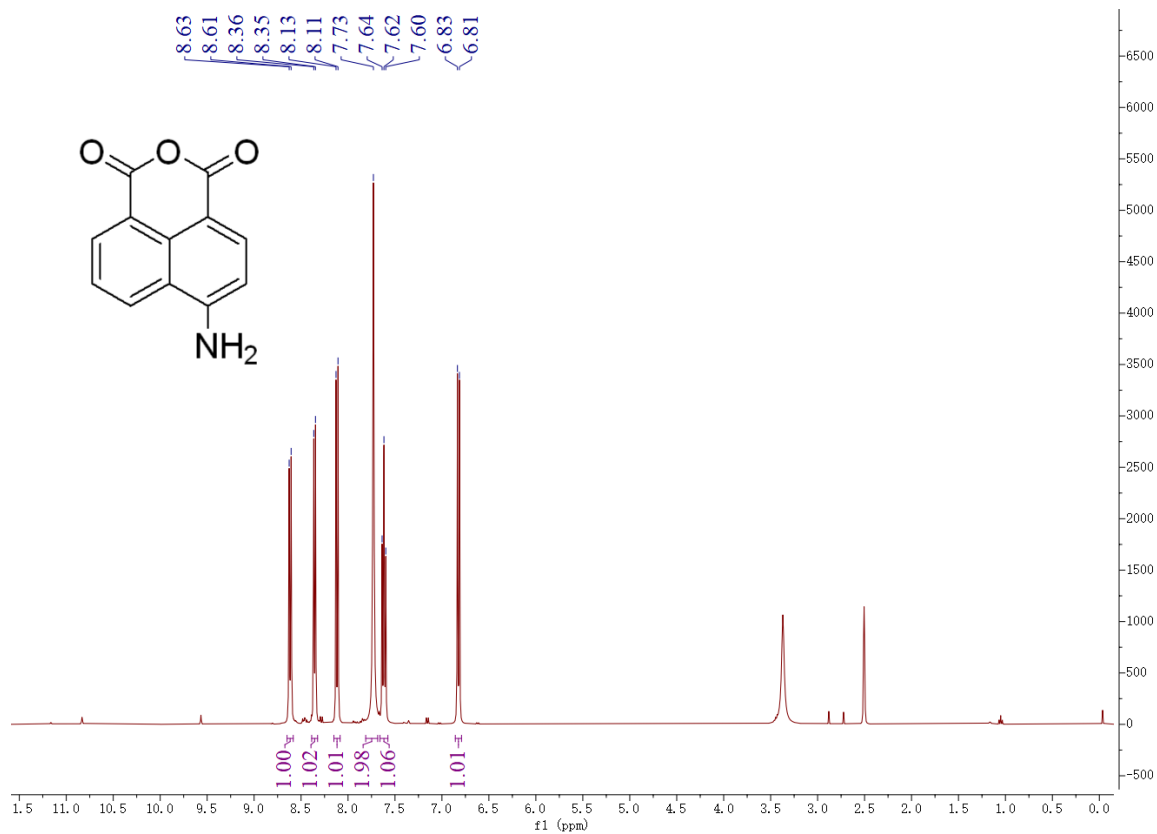
190

191 Figure S10. ¹H NMR spectrum (400 MHz, Chloroform-d) of compound 1.



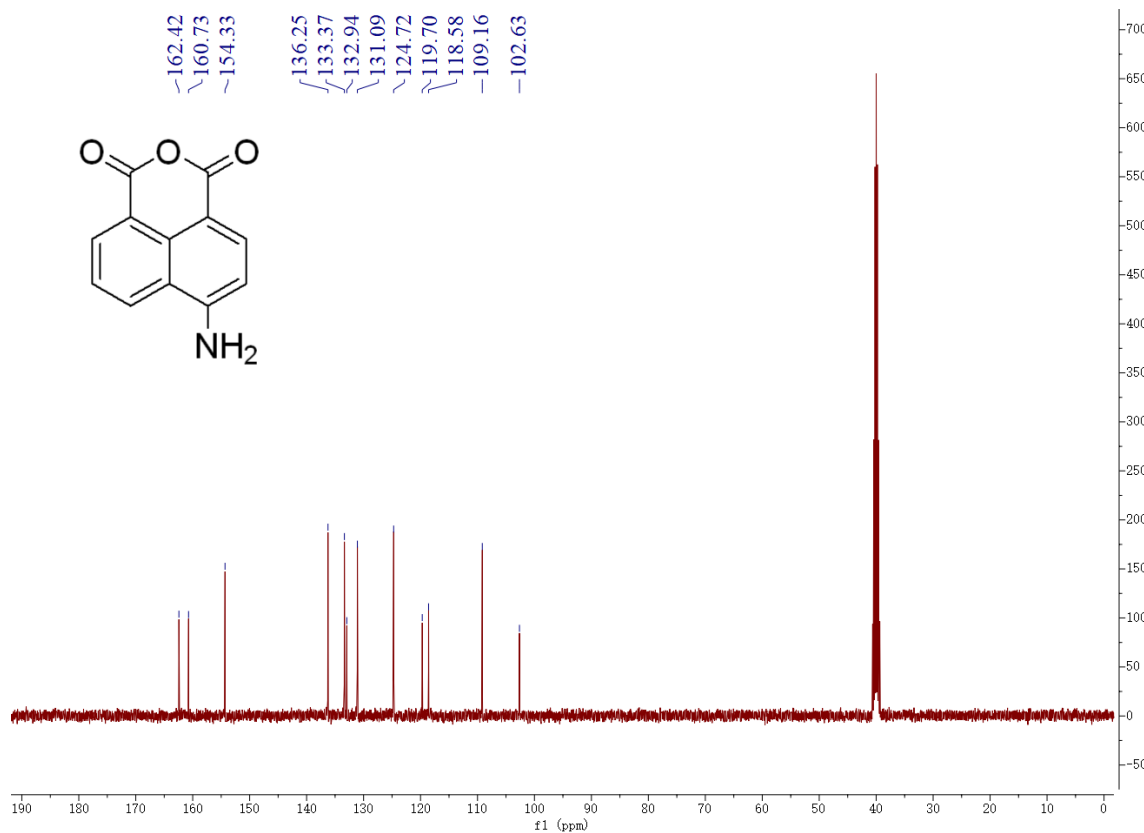
192

193 Figure S11. ¹³C NMR spectrum (101 MHz, Chloroform-d) of compound 1.



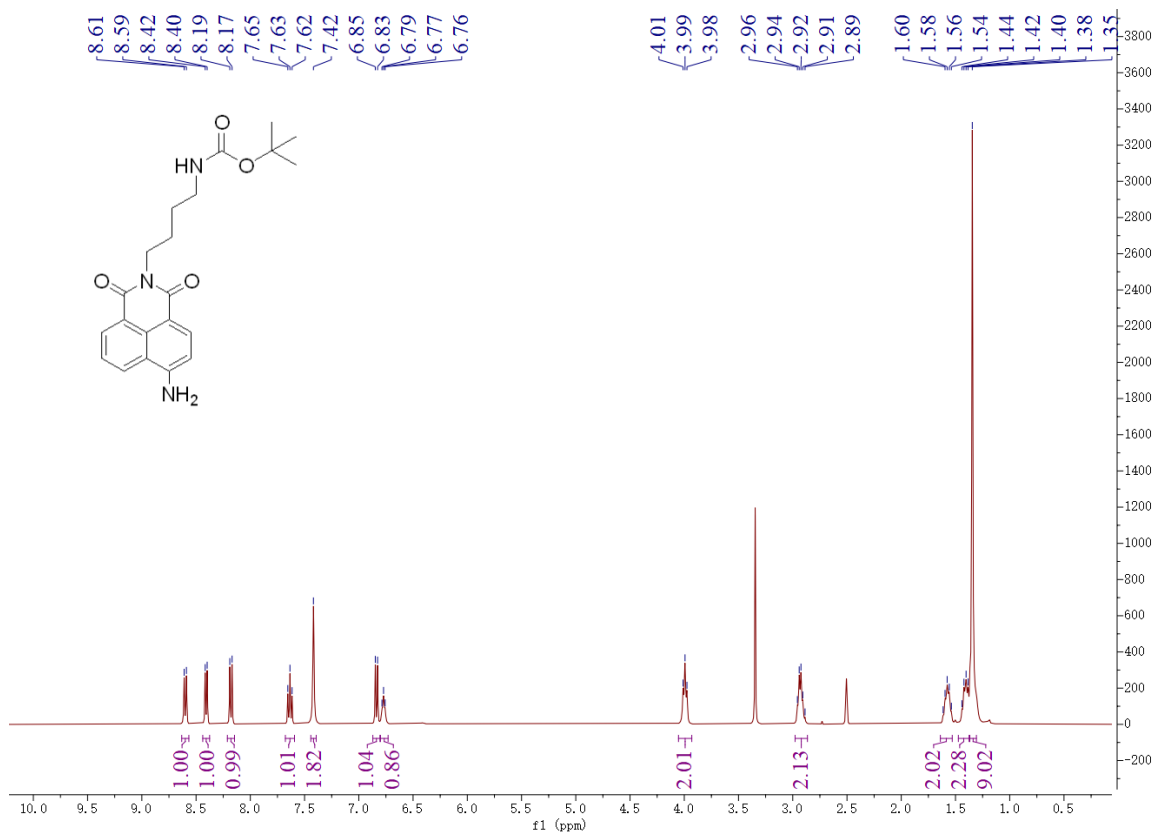
194

195 Figure S12. ¹H NMR spectrum (400 MHz, DMSO-d₆) of compound **2**.



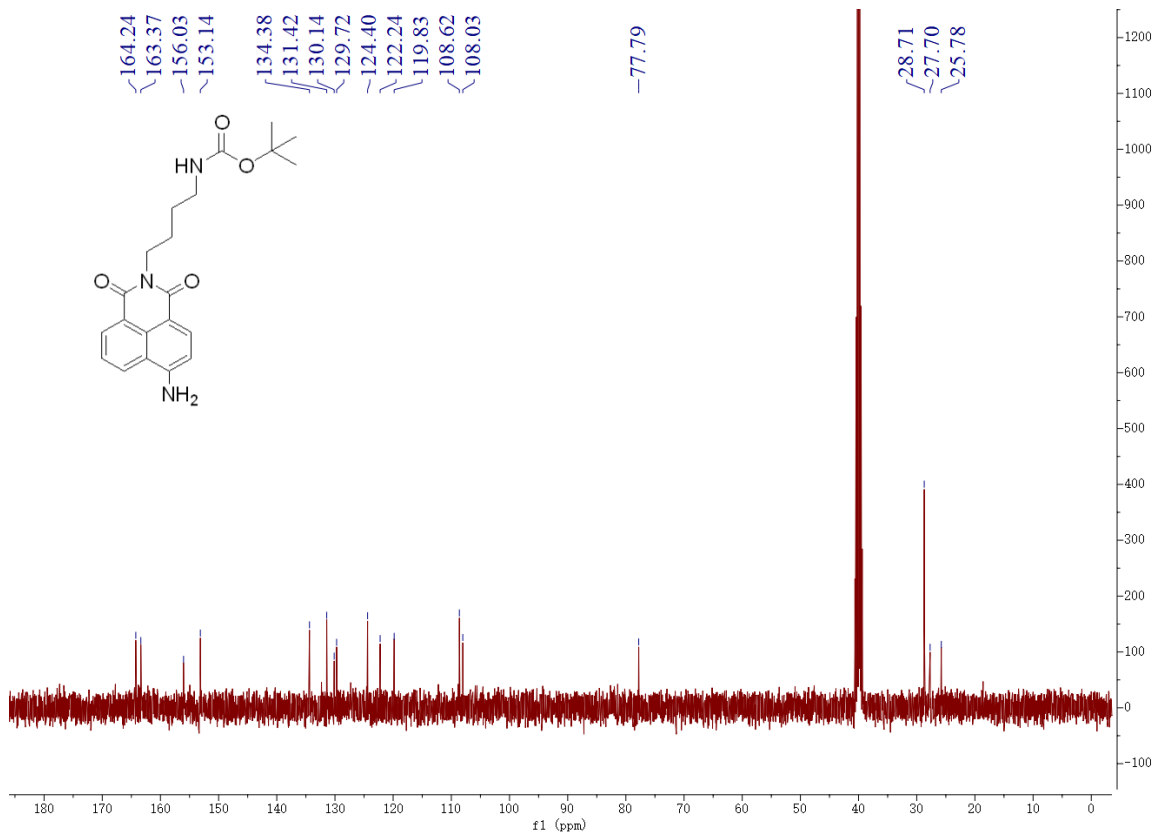
196

197 Figure S13. ¹³C NMR spectrum (101 MHz, DMSO-d₆) of compound **2**.



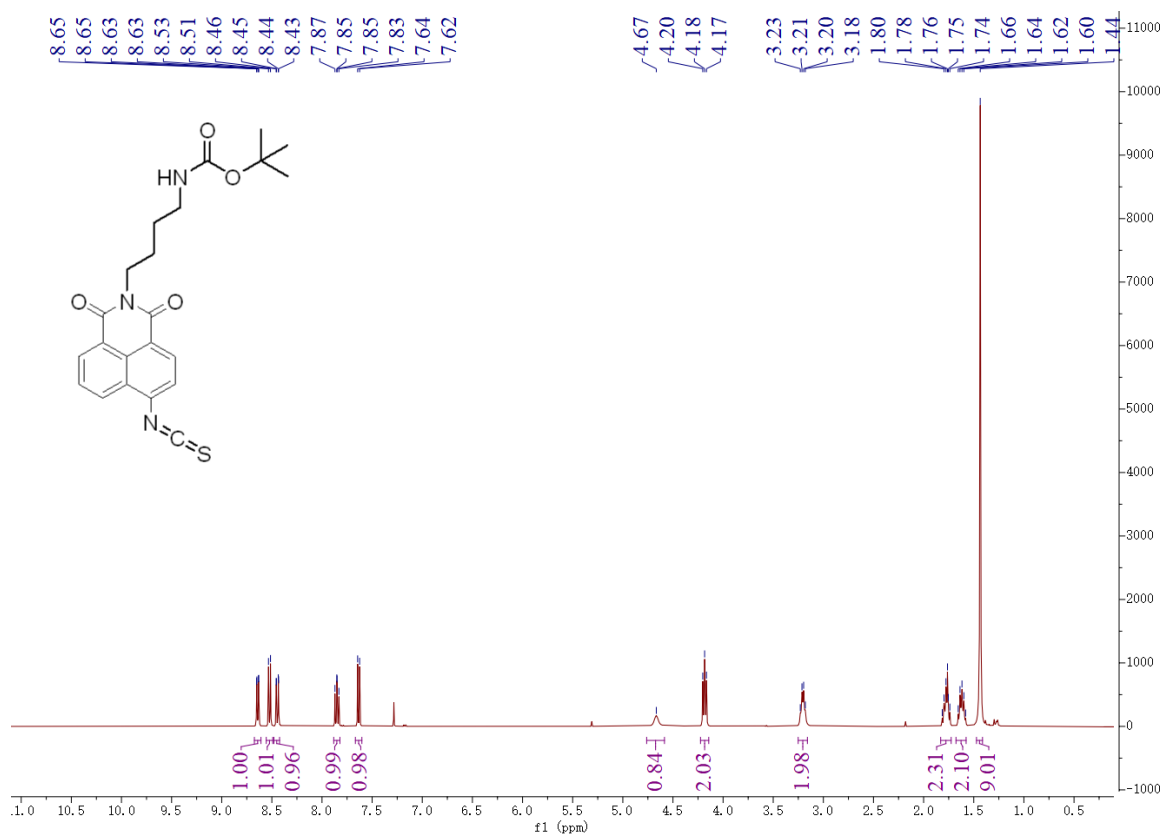
198

199 Figure S14. ¹H NMR spectrum (400 MHz, DMSO-d₆) of compound 3.



200

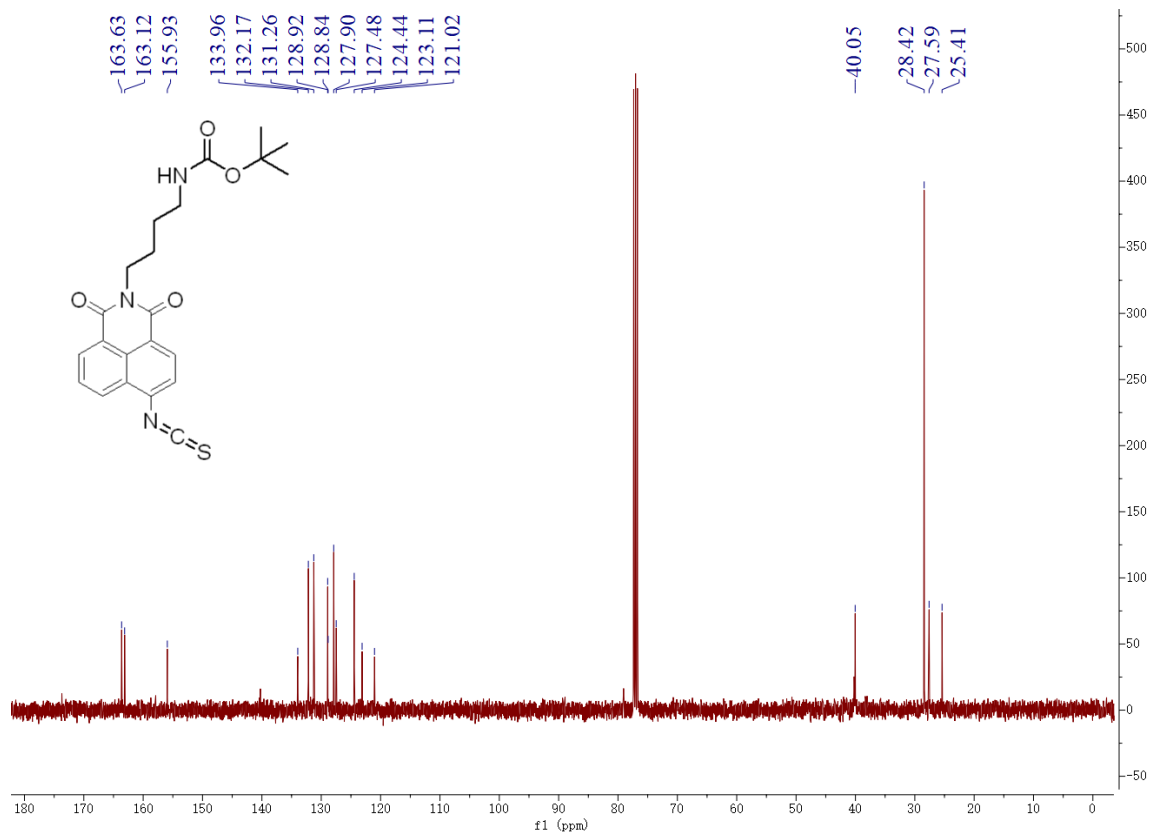
201 Figure S15. ¹³C NMR spectrum (101 MHz, DMSO-d₆) of compound 3.



202

203

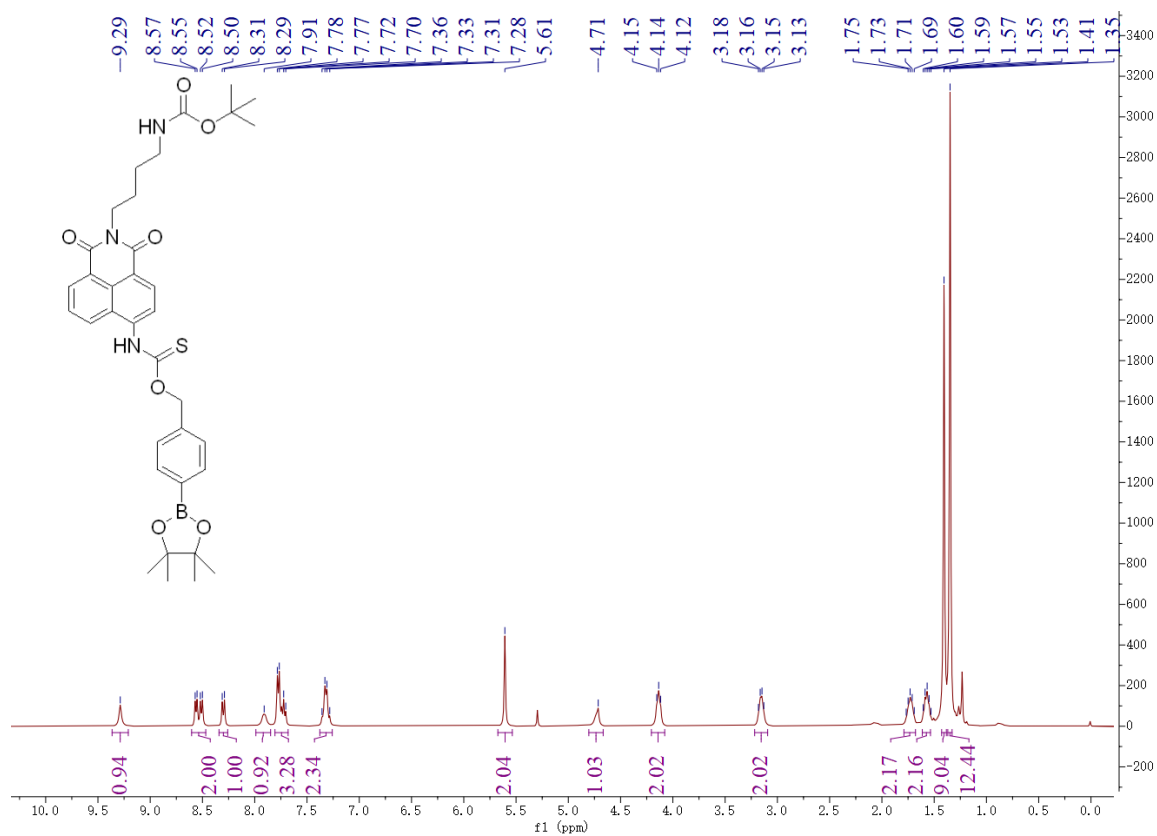
Figure S16. ¹H NMR spectrum (400 MHz, Chloroform-d) of compound 4.



204

205

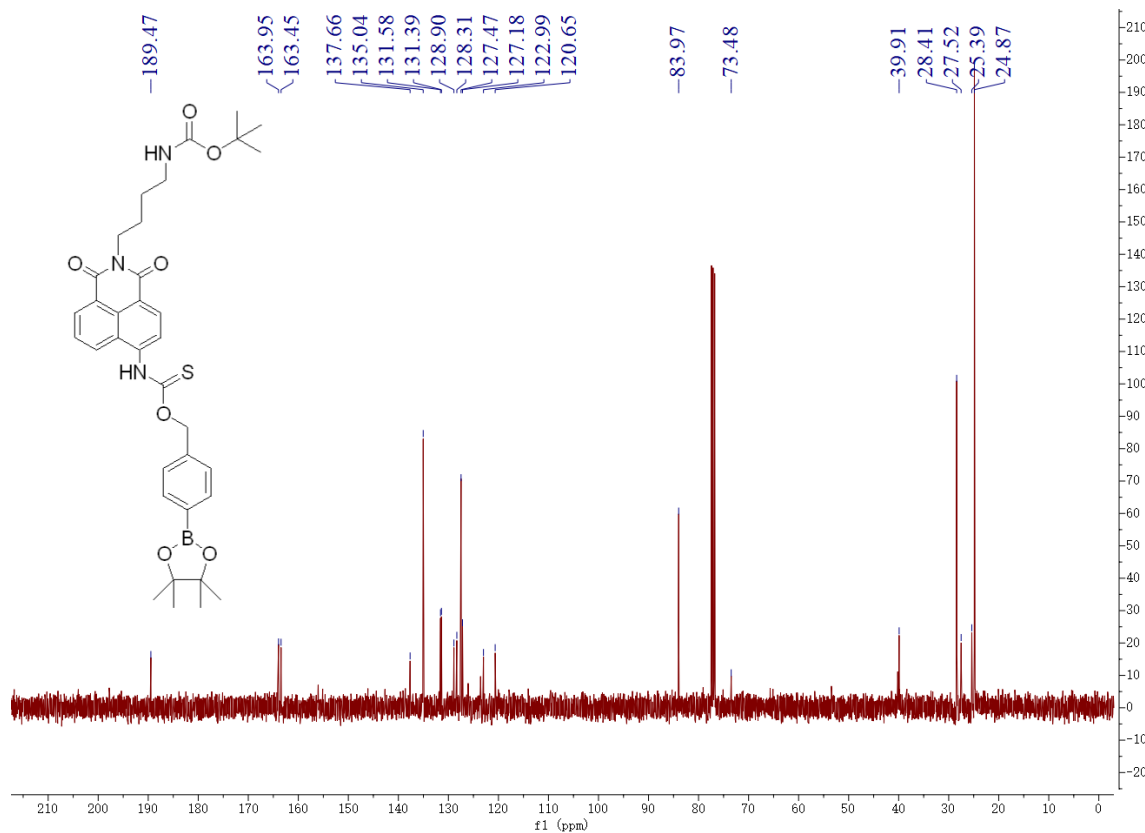
Figure S17. ¹³C NMR spectrum (101 MHz, Chloroform-d) of compound 4.



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207

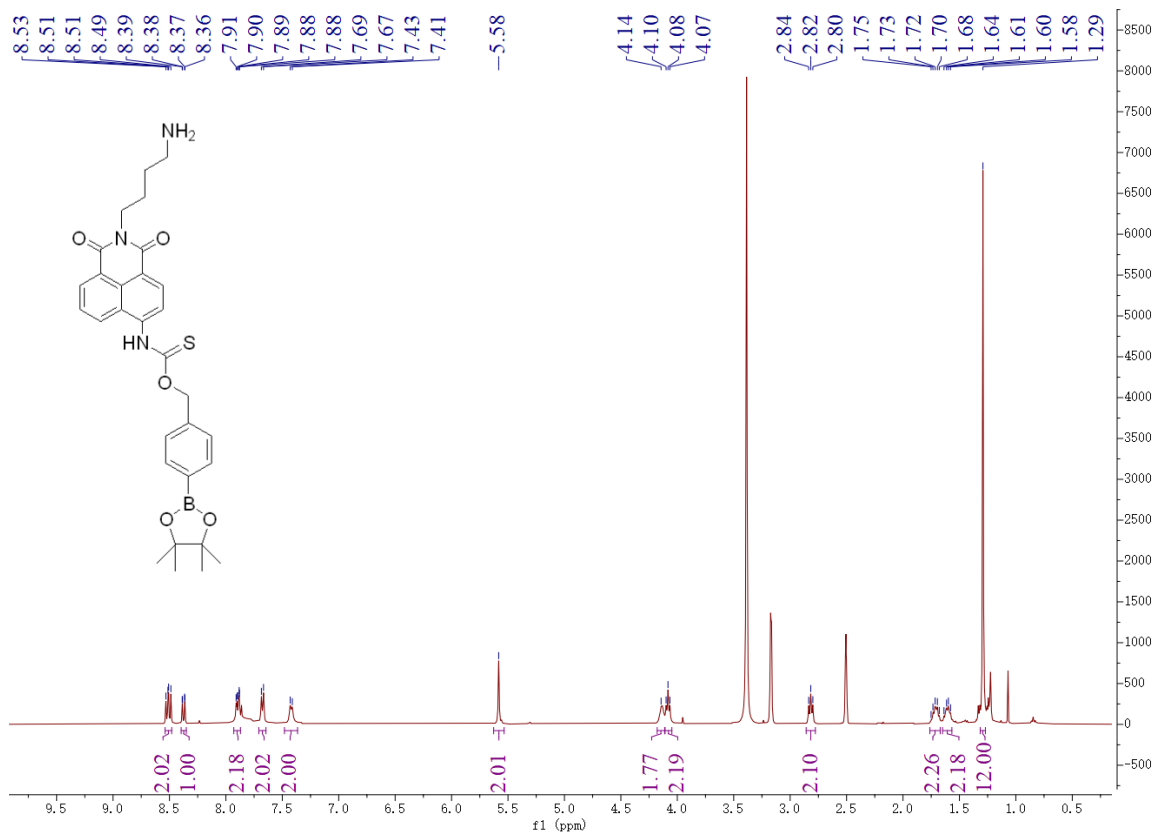
Figure S18. ¹H NMR spectrum (400 MHz, Chloroform-d) of compound 5.



208

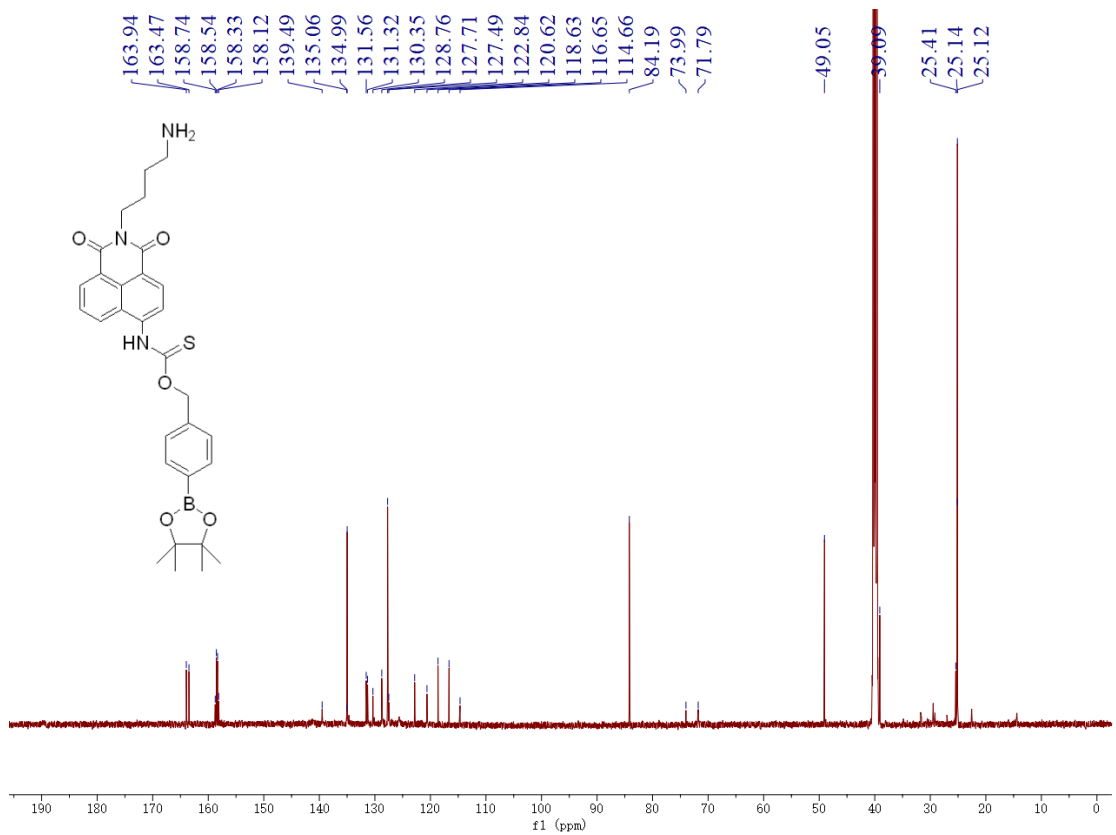
209

Figure S19. ¹³C NMR spectrum (101 MHz, Chloroform-d) of compound 5.



210

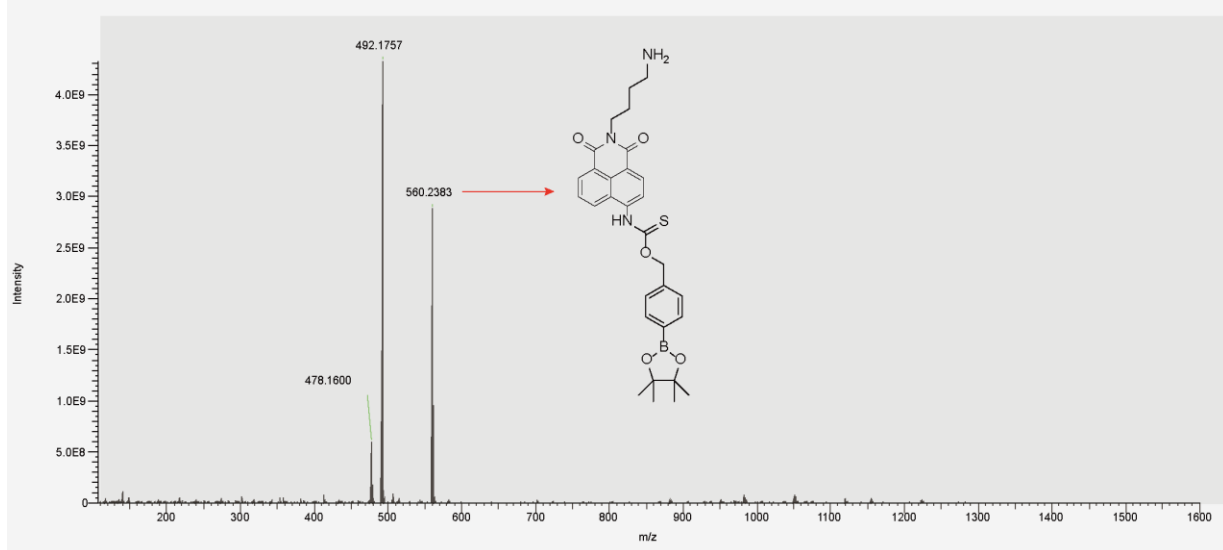
211 Figure S20. ¹H NMR spectrum (400 MHz, DMSO-d₆) of HSDF-NH₂.



212

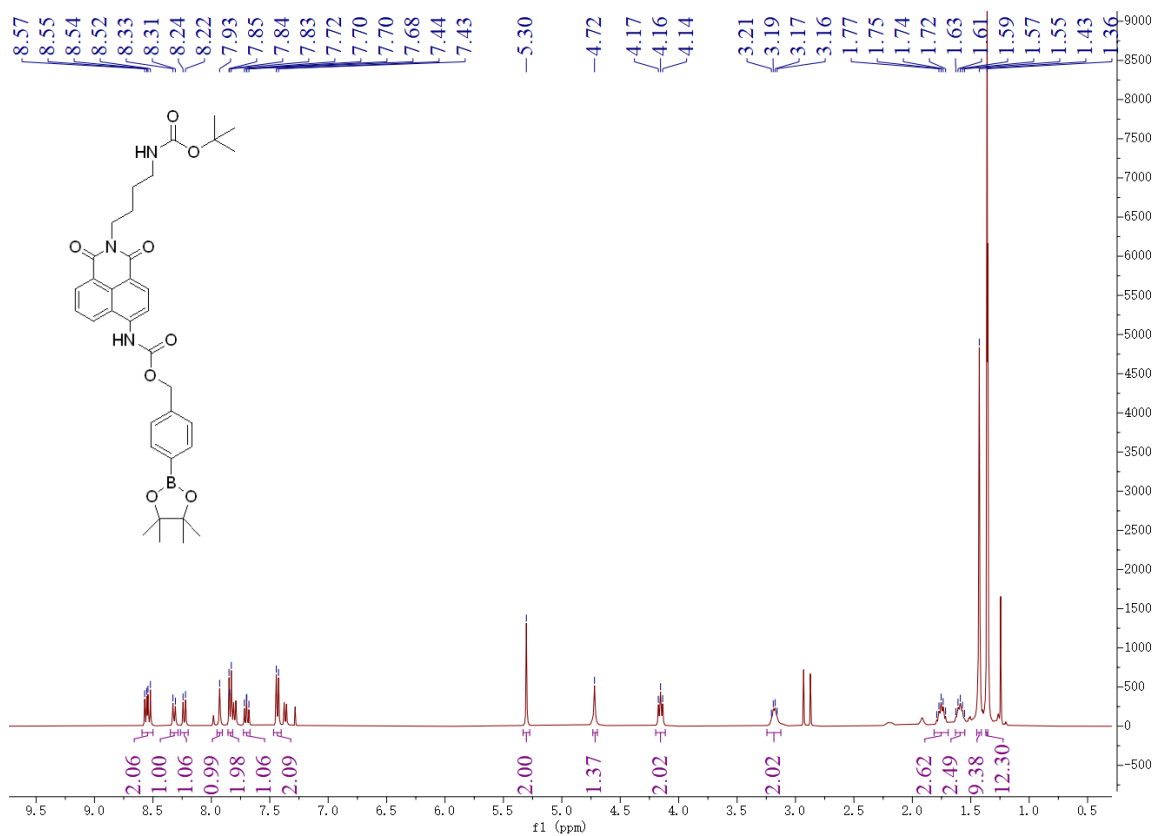
213 Figure S21. ¹³C NMR spectrum (151 MHz, DMSO-d₆) of HSDF-NH₂.

RT: 0.08 AV: 1 NL: 4.32E+009
T: FTMS + p ESI Full ms [106.7000-1600.0000]



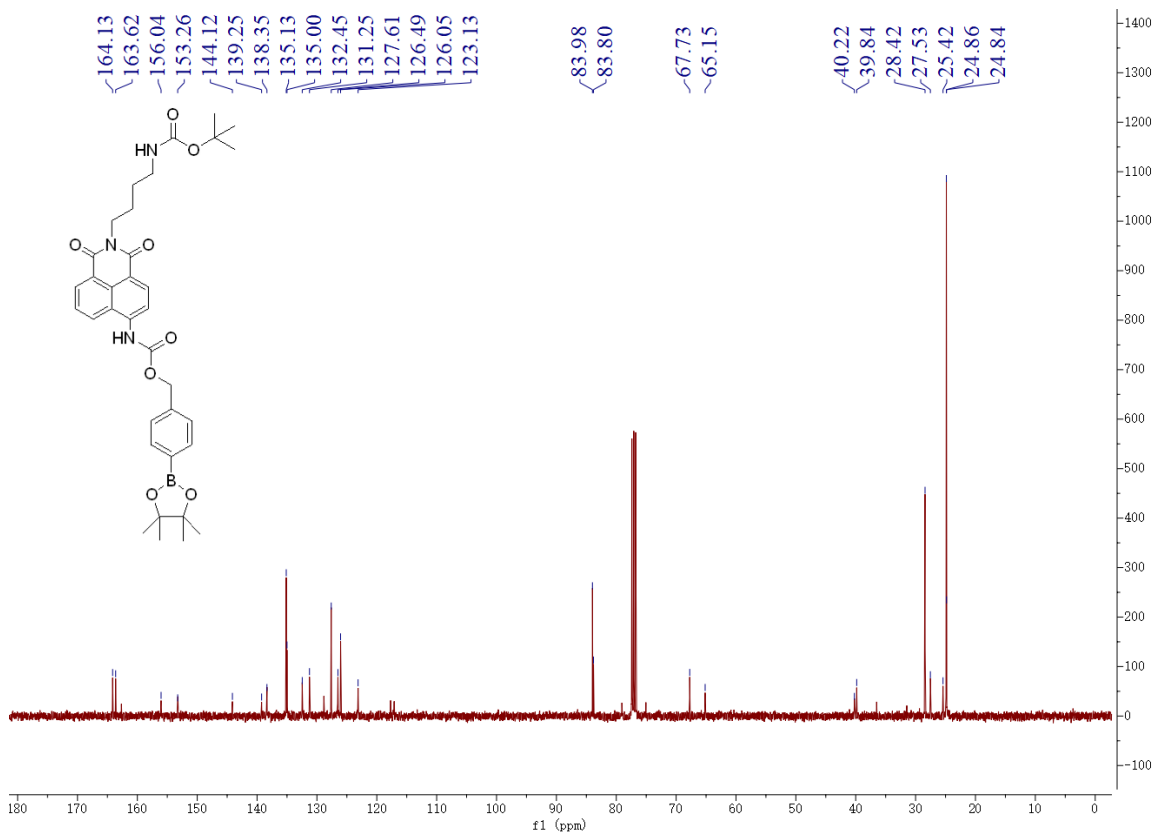
214

215 Figure S22. High-resolution mass spectrum of HSDF-NH₂.



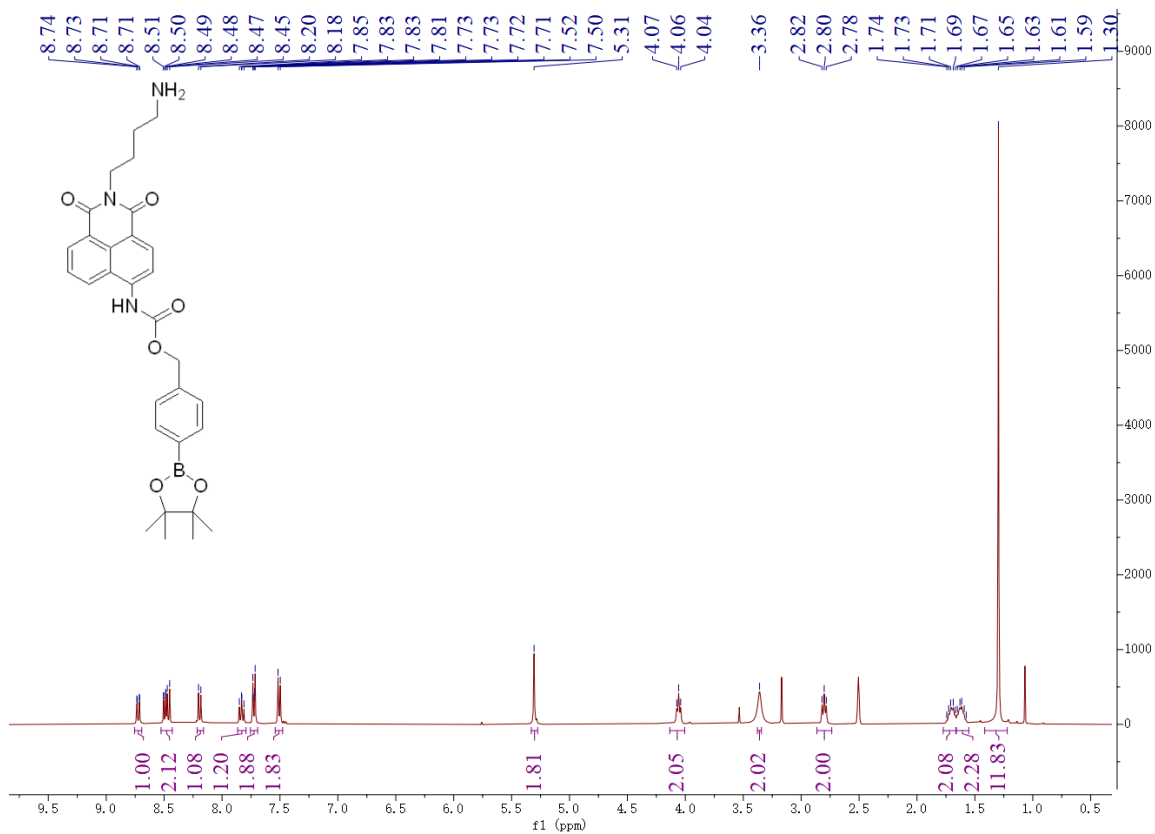
216

217 Figure S23. ¹H NMR spectrum (400 MHz, Chloroform-d) of compound 7.



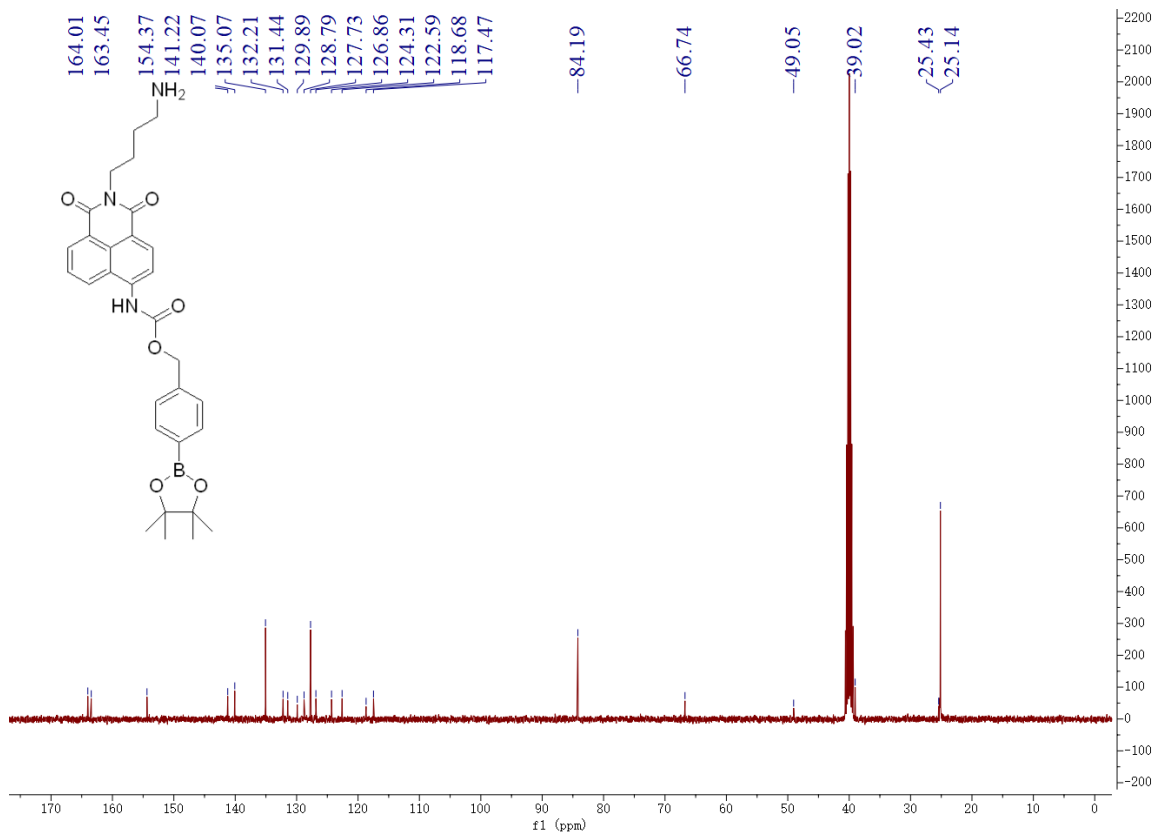
218

219 Figure S24. ¹³C NMR spectrum (101 MHz, Chloroform-d) of compound 7.



220

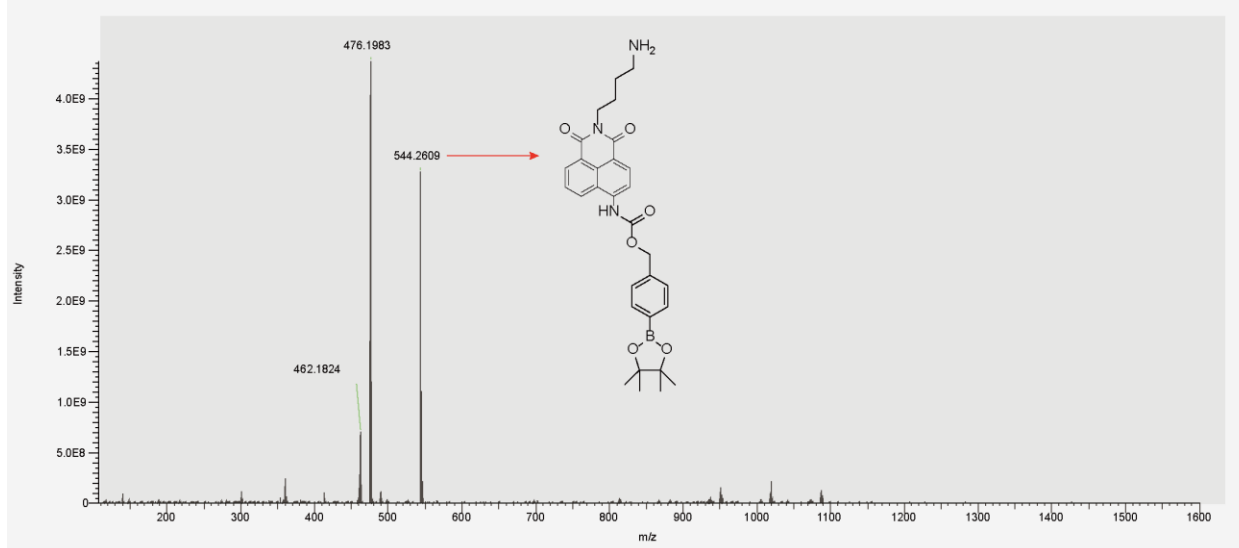
221 Figure S25. ¹H NMR spectrum (400 MHz, DMSO-d₆) of CODF-NH₂.



222

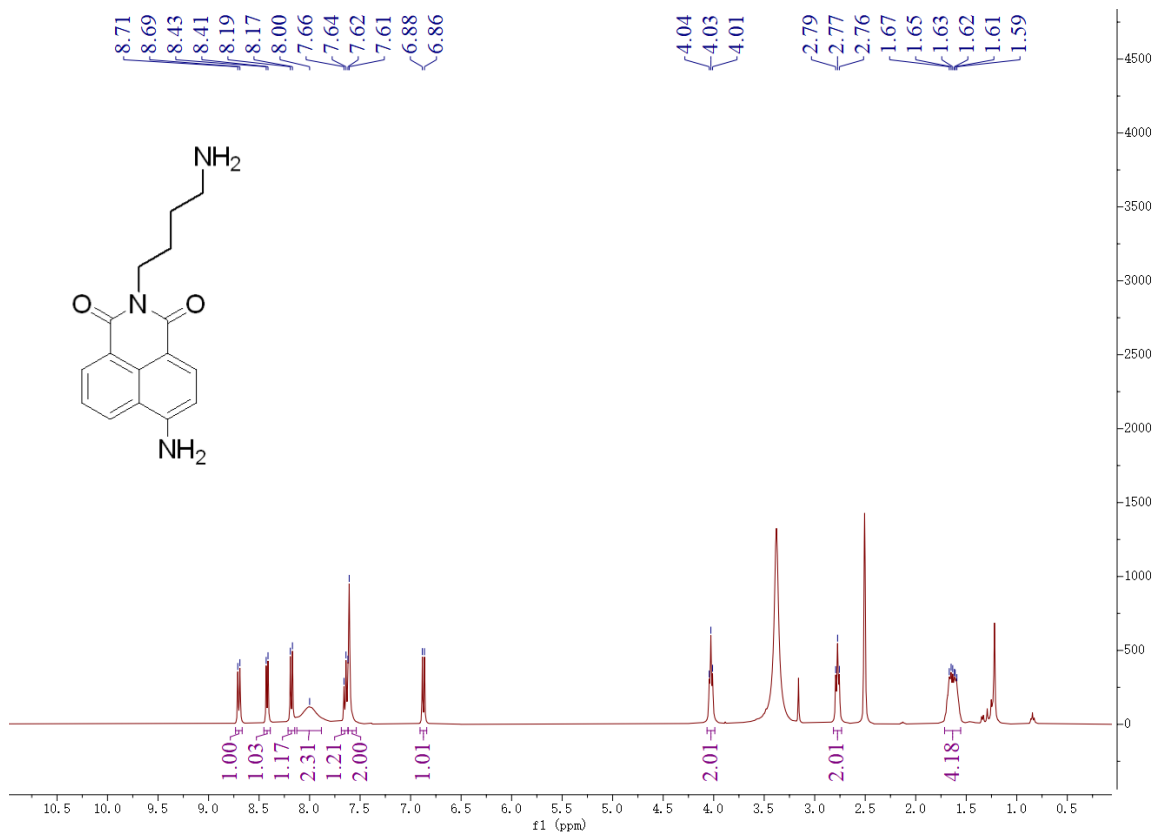
223 Figure S26. ¹³C NMR spectrum (101 MHz, DMSO-d₆) of CODF-NH₂.

RT: 0.08 AV: 1 NL: 4.37E+009
T: FTMS + p ESI Full ms [106.7000-1600.0000]



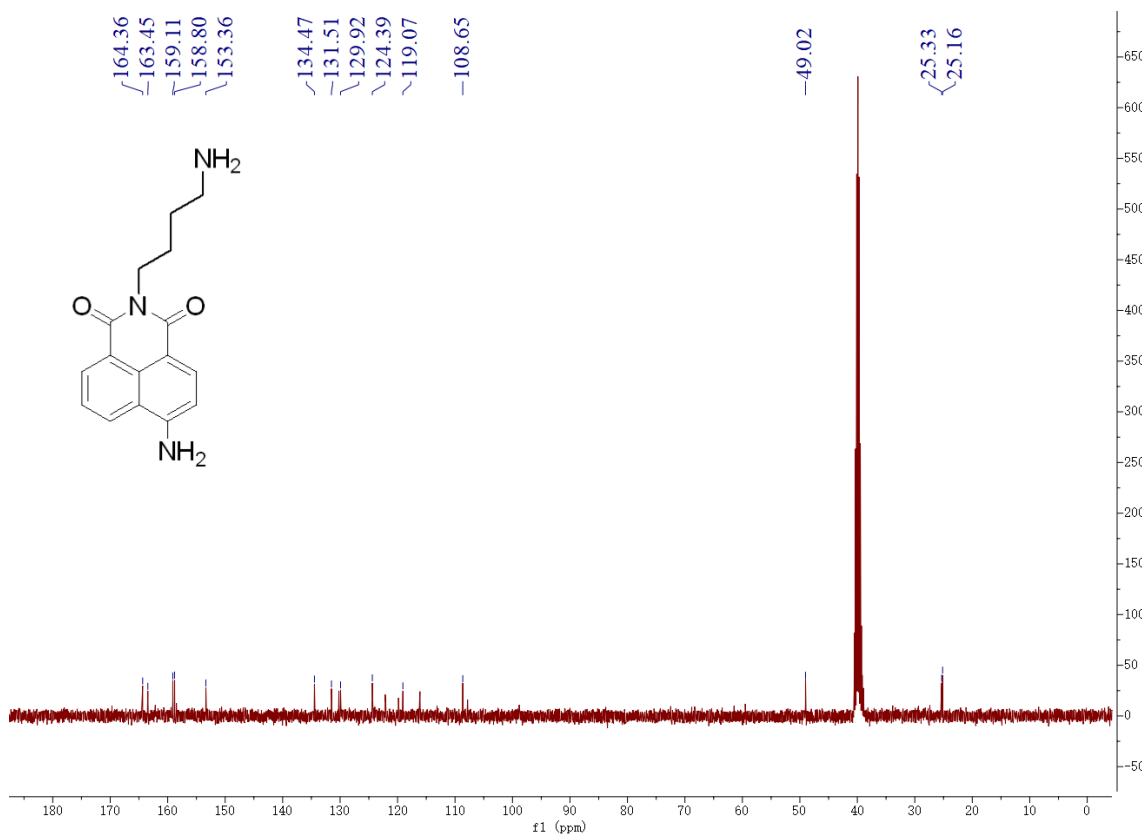
224

225 Figure S27. High-resolution mass spectrum of CODF-NH₂.



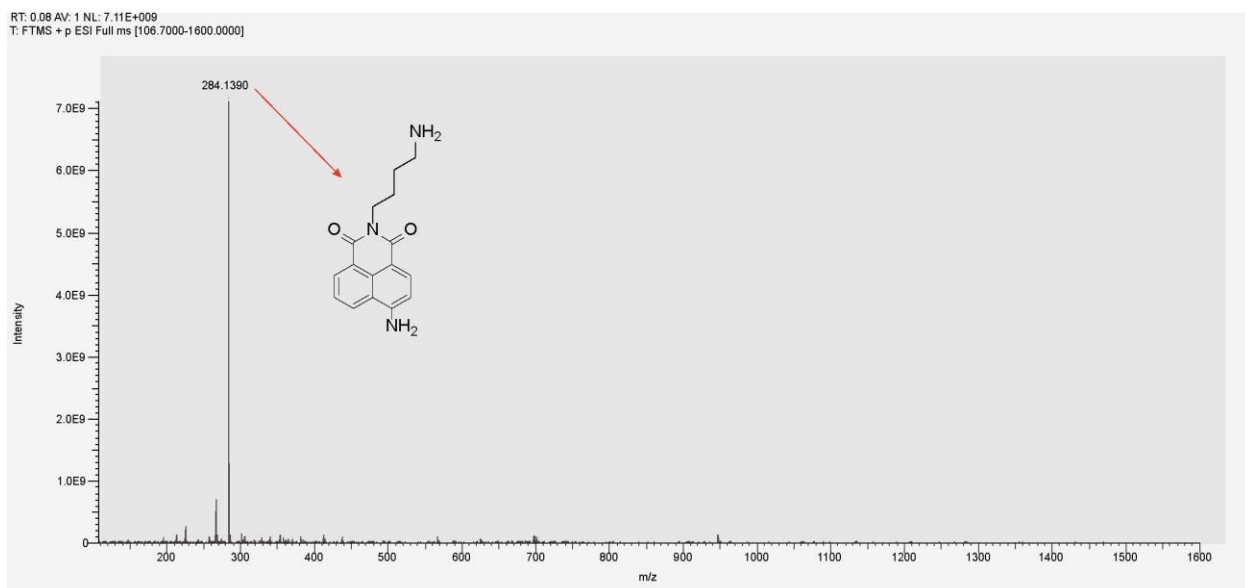
226

227 Figure S28. ¹H NMR spectrum (400 MHz, DMSO-d₆) of HSDG-NH₂.



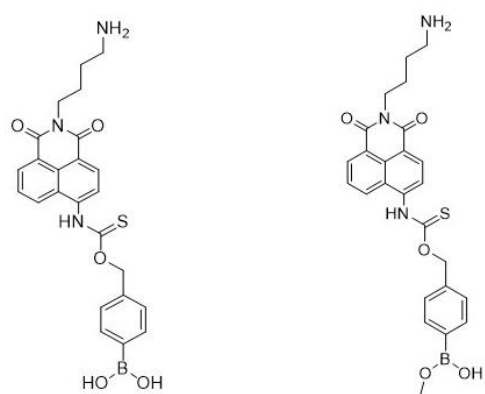
228

229 Figure S29. ¹³C NMR spectrum (101 MHz, DMSO-d₆) of HSDG-NH₂.



230

231 Figure S30. High-resolution mass spectrum of HSDG-NH₂.



232 M/Z: 478

M/Z: 492

233 Figure S31. Possible structures of the ionized fragments of HSDG-NH₂ in mass spectrometry.

234

235 **S6 Data supplementation**236 Table S1. Precision of the data (A: model/ OGD/R B: CONF-NH₂ C: HSDF-NH₂)

Figure 3G	A	B	C	P	
	42.13	37.08	18.75	A vs B	0.3423
	43.91	41.36	20.82	A vs C	0.0001
	51.01	44.92	26.22		
Figure 3H	A	B	C	P	
	3	2	1	A vs B	0.3802
	2	3	2	A vs C	0.0017
	3	2	1		
	3	3	2		
	3	2	2		
Figure 5F	A	B	C	P	
	24	22	12	A vs B	0.4943
	11	23	18	A vs C	0.0074
	31	26	10		
	25	18	14		
	37	18	13		
Figure 5G	A	B	C	P	
	51	42	34	A vs B	0.1469
	47	41	27	A vs C	< 0.0001
	57	54	32		
	59	50	35		
	45	40	37		
Figure 5H	A	B	C	P	
	23.67	18.68	11.28	A vs B	0.5248
	25.21	29.06	10.66	A vs C	0.0055
	32.00	31.08	15.00		
	29.00	29.67	13.83		
	25.00	3.82	13.04		
Figure 5I	A	B	C	P	
	0.206	0.244	0.124	A vs B	0.1594
	0.296	0.183	0.141	A vs C	< 0.0001
	0.291	0.267	0.145		
	0.335	0.183	0.163		
	0.253	0.277	0.091		
Figure S6	A	B	C	P	
	105.065	67.986	14.443	A vs B	0.0110
	115.969	77.131	17.478	A vs C	< 0.0001
	89.466	82.692	19.317		

