SUPPORTING INFORMAION

Visualizing the cellular internalization of therapeutic antibodies via pH-sensitive release of AIEgen

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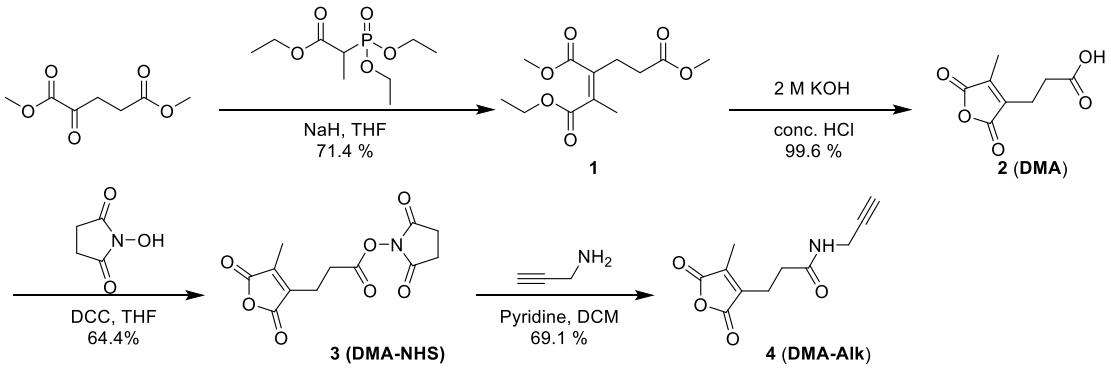
1. Materials and reagents

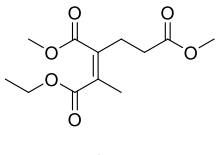
All reagents were purchased from commercial suppliers and used without further purification. All reactions were carried out under an ambient atmosphere. For thin layer chromatography (TLC), Qingdao Bangkai Hi-tech Materials Co., Ltd TLC plates (HSGF 254) were used, and compounds were visualized with a UV light or stained by $KMnO_4$. Flash column chromatography was carried out using silica gel 60F (Qingdao Haiyang Chemical co., Ltd). **^{1}H** and **^{13}C NMR** spectra were recorded on a Bruker AVIII 500M (500 MHz) or WNMR-I 400M (400 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: ^{1}H (chloroform δ 7.26; DMSO δ 2.50), ^{13}C (chloroform δ 77.16; DMSO δ 39.52). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets), coupling constants (Hz) and integration. Mass spectra were measured on Agilent 1290/6460 Series LC-MS. High resolution mass spectra (HRMS) were obtained on an Agilent G6545 spectrometer using an electron spray ionization time-of flight (ESI-TOF) source. Phosphate buffered saline solution (PBS, pH 7.4, containing 0.138 M NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4). Cetuximab (Erbitux®, 5mg/mL) was purchased from Merck.

2. Chemical synthesis

(1) Chemical synthesis of DMA-Alk



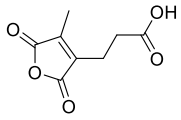
Scheme S1. Synthesis of **DMA-Alk**.



1

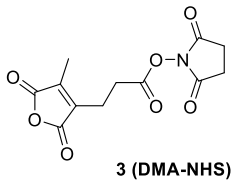
Synthesis of DMA was performed as previously reported.[1] To triethyl-2-

phosphonopropionate (0.420 g, 17.4 mmol) in anhydrous tetrahydrofuran (THF; 30 mL) under nitrogen was slowly added sodium hydride (0.334 g, 14.5 mmol) on ice, followed by stirring until hydrogen gas generation completed. Dimethyl 2-oxopentanedioate (1.68 mL, 11.6 mmol) was added to the above solution and stirred for 30 minutes on ice. Saturated ammonium chloride aqueous solution (45 mL) was poured into the reaction solution, and the resulting product was extracted using ethyl acetate (EA) for three times. The combined organic layers were washed with water and saturated brine sequentially, before being dried over anhydrous Na_2SO_4. Finally, the solvent was removed *in vacuo* and the crude product was purified by silica gel column chromatography (petroleum ether:EA= 5:1) to obtain **1** (2.140 g, 71.4%) as colorless oil. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 4.20 (q, *J* = 7.2 Hz, 2H), 3.74 (s, 3H), 3.67 (s, 3H), 2.66 (t, *J* = 7.9 Hz, 2H), 2.47 (dd, *J* = 8.8, 6.9 Hz, 2H), 1.99 (s, 3H), 1.31-1.26 (m, 3H).



2 (**DMA**)

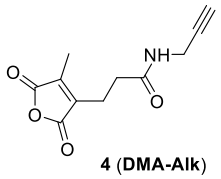
1 (2.140 g, 8.29 mmol) was dissoveld in the mixture of ethonal (37 mL) and KOH aqueous solution (2 M, 20 mL), and refluxed for 1 hour. After cooling to room temperature, ethonal was removed *in vacuo* and the remaining solution was acidified with HCl aqueous solution to pH 1~2, and extracted using ethyl acetate for three times. The combined organic layers were dried over anhydrous Na_2SO_4 to give desired DMA (1.520 g, 99.6%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 12.30 (s, 1H), 2.64 (ddt, *J* = 8.2, 7.2, 1.0 Hz, 2H), 2.54-2.51 (m, 2H), 2.02 (d, *J* = 0.9 Hz, 3H).



3 (DMA-NHS)

To a solution of **2** (0.700 g, 3.8 mmol) in anhydrous THF (17 mL) at 0 °C was added *N*-hydroxysuccinimide (0.430 g, 3.7 mmol) in anhydrous THF (3.5 mL) and dicyclohexylcarbodiimide (DCC; 0.875 g, 4.24 mmol) in anhydrous THF (7 mL) sequentially. The reaction was stirred for 3 hours at 0 °C before warming up to room

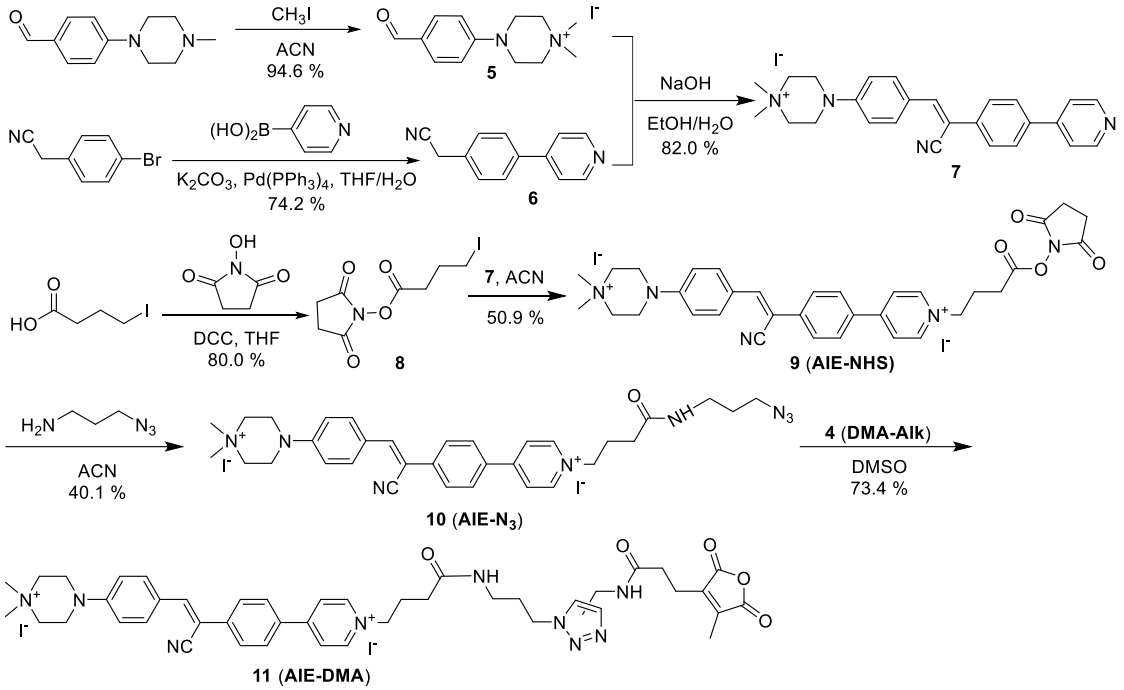
temperature and reacted overnight. The mixture was filterd with celite pad and the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography (petroleum ether:EA= 2:1) to obtain **3** (0.670 g, 64.4%) as white solid. **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 3.02 (t, J = 6.7 Hz, 2H), 2.88 (t, J = 7.0 Hz, 2H), 2.83 (s, 4H), 2.10 (d, J = 0.9 Hz, 3H).



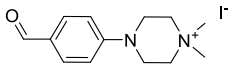
4 (**DMA-Alk**)

3 (0.300 g, 1.067 mmol) was dissolved in CH_2Cl_2 (2 mL) under a nitrogen atmosphere and cooled to 0 ^oC. To the solution was added propargyl amine (58.5 μL, 0.85 mmol) and pyridine (180 μL) in CH_2Cl_2 (4 mL), stirred for 15 minutes at 0 ^oC before warming up to room temperature and reacted overnight. The reaction mixture was washed with saturated $CuSO_4$ aqueous solution and brine, sequentially. The crude product was purified by silica gel column chromatography (CH_2Cl_2:EA= 5:1) to obtain **4** (0.130 g, 69.1%) . **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 5.91 (s, 1H), 4.01 (dd, J = 5.4, 2.5 Hz, 2H), 2.80 (t, J = 7.0 Hz, 2H), 2.57 (t, J = 7.0 Hz, 2H), 2.24 (t, J = 2.5 Hz, 1H), 2.12 (d, J = 1.0 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.35, 166.06, 165.97, 142.70, 142.42, 79.39, 71.91, 32.74, 29.42, 20.37, 9.84.

(2) Chemical synthesis of AIE-NHS and AIE-DMA

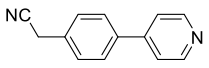


Scheme S2. Synthesis of **AIE-NHS** and **AIE-DMA**.



5

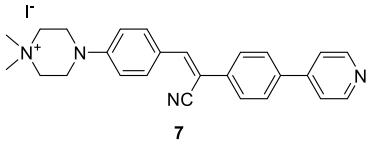
AIE-NHS was synthesized according published procedures.[2] Into a two-necked round bottom flask equipped with a condenser was dissolved 4-(4-methylpiperazin-1-yl)benzaldehyde (1.000 g, 4.9 mmol) in 20 mL of acetonitrile. Iodomethane (0.75 mL, 12 mmol) was then added and the mixture was heated to reflux for 3.5 h. After cooling to room temperature, the mixture was poured into diethyl ether. The pale yellow precipitates formed were filtered by suction filtration to give **5** (1.600 g, 94.6%).



6

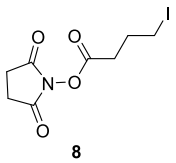
Into a two-necked round bottom flask equipped with a condenser were added 2-(4-bromophenyl)acetonitrile (2.000 g, 10.20 mmol), 4-pyridinylboronic acid (1.240 g, 10.20 mmol), potassium carbonate (14.080 g, 102.00 mmol) and $Pd(PPh_3)_4$ (0.140 g, 0.12 mmol) in 200 mL of THF and 40 mL of water under nitrogen. The mixture was stirred and heated to reflux overnight. After cooling to room temperature, the mixture was extracted with CH_2Cl_2 for three times. The organic phase was collected, washed with water and dried over anhydrous Na_2SO_4. After solvent evaporation, the crude

product was purified by silica-gel column chromatography (CH_2Cl_2:EA = 10:1) to give **6** (1.470 g, 74.2%) as white solid.



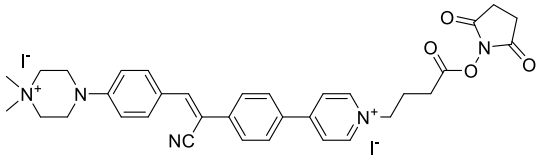
7

Into a round bottom flask were dissolved **6** (0.84 g, 4.33 mmol) and **5** (1.500 g, 4.33 mmol) in a mixture of ethanol (34 mL) and water (8.4 mL). NaOH (0.172 g, 4.3 mmol) in 8.4 mL of ethanol was then added slowly into the mixture. After stirring for 2.5 hours, the pale yellow precipitates were filtered, washed with cold ethanol and dried under reduced pressure to give **7** (1.860 g, 82.0%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.97 (d, J = 6.2 Hz, 2H), 8.43 (d, J = 6.1 Hz, 2H), 8.18 (d, J = 8.3 Hz, 2H), 8.14 (s, 1H), 7.98 (dd, J = 14.9, 8.5 Hz, 4H), 7.19 (d, J = 8.7 Hz, 2H), 3.74 (d, J = 5.2 Hz, 4H), 3.57 (t, J = 5.2 Hz, 4H), 3.23 (s, 6H).



8

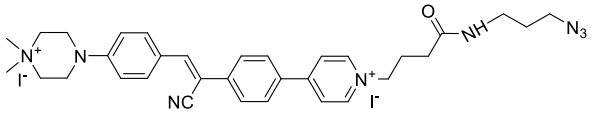
To 4-iodobutanoic acid (0.960 g, 4.48 mmol) in anhydrous THF at 0 ^{o}C was added *N*-hydroxysuccinimide (0.500 g, 4.34 mmol) and DCC (1.04 g, 5.00 mmol) in anhydrous THF (12 mL). The reaction was stirred for 3 hours at 0 ^{o}C before warming up to room temperature and reacted overnight. The mixture was filterd with celite pad and the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography (*n*-hexane:EA= 2:1) to obtain **8** (1.080 g, 80.0%). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 3.29 (t, J = 6.7 Hz, 2H), 2.84 (s, 4H), 2.77 (t, J = 7.2 Hz, 2H), 2.25 (p, J = 6.9 Hz, 2H).



9 (AIE-NHS)

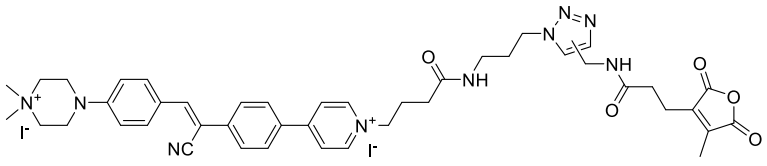
Into a two-necked round bottom flask equipped with a condenser was dissolved **7** (0.390 g, 0.74 mmol) in 40 mL of acetonitrile. **8** (1.150 g, 3.69 mmol) was then added

and the mixture was heated to reflux until **7** was completely consumed as analyzed by LCMS. After cooling to room temperature, the mixture was poured into diethyl ether. The orange precipitates formed were filtered by filtration and washed by cold ethanol to give **9** (**AIE-NHS**; 0.310 g, 50.9%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 9.11 (d, J = 6.5 Hz, 2H), 8.69 – 8.52 (m, 2H), 8.24 (d, J = 8.4 Hz, 2H), 8.18 (s, 1H), 7.99 (dd, J = 10.6, 8.6 Hz, 4H), 7.20 (d, J = 8.6 Hz, 2H), 4.67 (t, J = 7.1 Hz, 2H), 3.76 (t, J = 5.1 Hz, 4H), 3.57 (t, J = 5.2 Hz, 4H), 3.23 (s, 6H), 2.90 (t, J = 7.6 Hz, 2H), 2.81 (s, 4H), 2.34 (t, J = 7.4 Hz, 2H).



10 (AIE-N_3)

Into a round bottom flask was dissolved **9** (**AIE-NHS**; 0.200 g, 0.24 mmol) in acetonitrile (50 mL). Then 3-azidopropan-1-amine (0.360 g, 0.36 mmol) was added slowly at 0 ^{o}C. The mixture was stirred at 0 ^{o}C for 1 hour before warming up to room temperature and reacted overnight. To the mixture was poured diethyl ether and the orange precipitates formed were filtered by filtration to give **10** (**AIE-N_3**; 0.787 g, 40.1%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 9.10 (d, J = 6.6 Hz, 2H), 8.60 (d, J = 6.5 Hz, 2H), 8.24 (d, J = 8.2 Hz, 2H), 8.17 (s, 1H), 7.99 (dd, J = 10.7, 8.5 Hz, 5H), 7.20 (d, J = 8.6 Hz, 2H), 4.62 (d, J = 6.4 Hz, 2H), 3.75 (d, J = 5.2 Hz, 4H), 3.58 (d, J = 5.1 Hz, 4H), 3.38 (d, J = 7.0 Hz, 2H), 3.22 (s, 6H), 3.07 (q, J = 6.5 Hz, 2H), 2.20 (s, 4H), 1.62 (t, J = 6.8 Hz, 2H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 170.65, 153.46, 151.10, 144.88, 144.21, 137.94, 132.85, 131.38, 128.85, 126.17, 124.17, 123.85, 118.43, 114.44, 103.75, 64.87, 59.78, 50.35, 48.39, 40.68, 35.85, 31.38, 28.32, 15.13. ESI-MS $[M]^{2+}$ calculated: 282.1657; found: 282.2.



11 (AIE-DMA)

Into a round bottom flask was dissolved **4** (**DMA-Alk**; 0.222 g, 0.10 mmol) and **10** (**AIE-N_3**; 0.545 g， 0.067 mmol) in DMSO (1 mL). CuI (0.215 g, 0.11 mmol) was then added to the above mixture. The reaction was monitored by HPLC. DMSO was

removed by freeze drying and the residue was crystalized by methonal to give **11** (**AIE-DMA**; 0.516 g, 73.4%) as yellow solid. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.21 (s, 2H), 8.70 (s, 2H), 8.34 (s, 2H), 8.26 (s, 1H), 8.17-7.99 (m, 5H), 7.41-7.18 (m, 2H), 4.74 (s, 2H), 4.56-4.42 (m, 2H), 4.38 (s, 1H), 3.87 (s, 4H), 3.69 (s, 4H), 3.48 (s, 2H), 3.34 (s, 6H), 3.17 (d, J = 15.2 Hz, 2H), 2.74 (t, J = 7.3 Hz, 2H), 2.62 (s, 2H), 2.33 (s, 4H), 2.20 (s, 1H), 2.05 (s, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 170.63, 170.00, 166.13, 165.69, 153.29, 150.91, 144.72, 144.07, 142.55, 142.50, 140.93, 137.74, 132.65, 131.25, 128.69, 126.02, 124.06, 123.68, 118.25, 114.31, 103.57, 59.63, 59.41, 50.28, 40.54, 35.61, 34.35, 32.01, 31.22, 30.54, 29.23, 26.35, 19.84, 19.75, 9.10. ESI-HRMS $[M]^{2+}$ calculated: 392.7001; found: 392.7003.

3. Supplementary figures

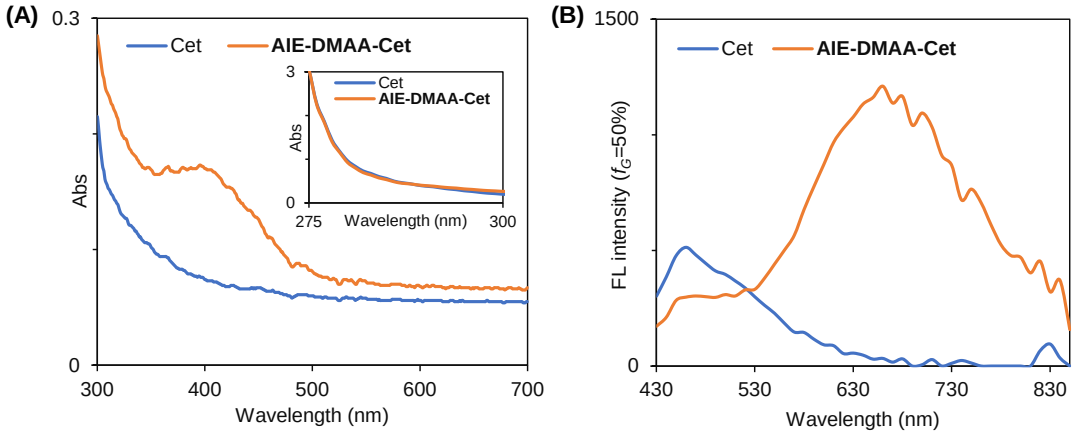


Fig. S1 Charactering the successful labeling of AIEgen in **AIE-DMAA-Cet** according to the (A) absorption (in PBS) and (B) emission spectra (PBS/glycerol = 1:1) in comparison with the non-labeled cetuximab (Cet for short). **AIE-DMAA-Cet** and Cet at the same protein concentration (with identical absorption at 280 nm as shown in the inset of (A)) were analyzed in parallel.

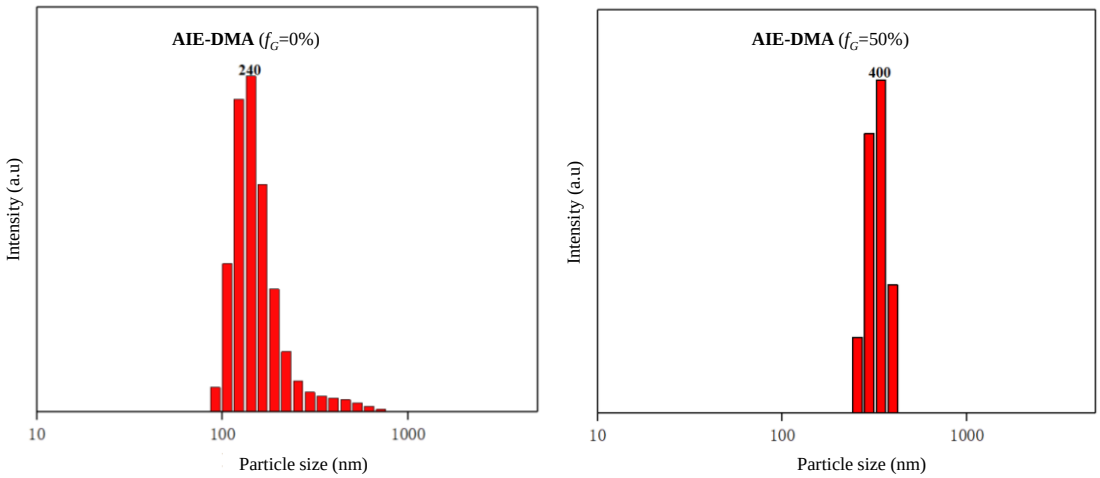
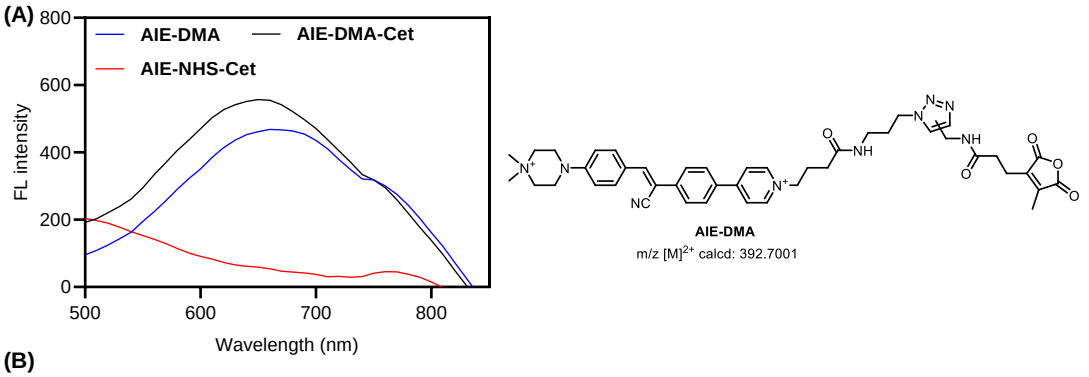


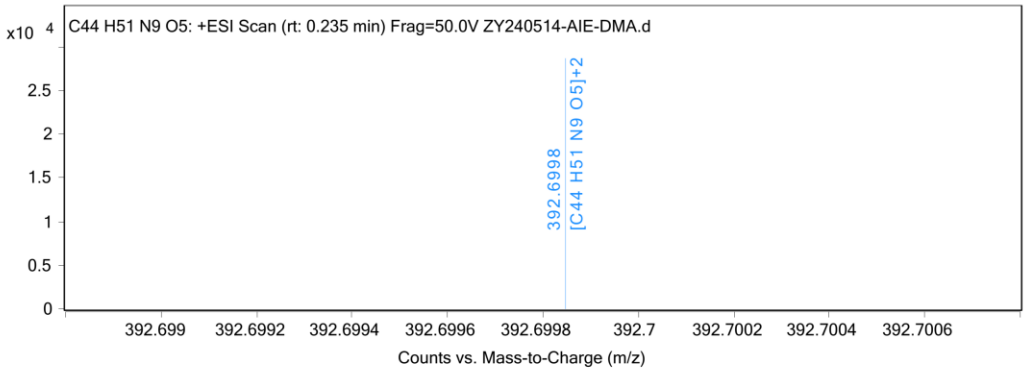
Fig. S2 Hydrodynamic particle size of **AIE-DMA** in the water/glycerol (G) mixture with f_G = 0% or 50% as measured by the method of dynamic light scattering (DLS).



(B)

Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
50	0	ESI



Formula Calculator Element Limits

Element	Min	Max
C	0	100
H	0	110
N	0	10
O	0	5

Formula Calculator Results

Formula	Best	Measured Mass	Tgt Mass	Diff (ppm)	Score
C44 H51 N9 O5	True	392.6998	392.7001	-0.42	99.85

Fig. S3 Analysis of the cleavage between the AIEgen and the antibody in **AIE-DMAA-Cet**/**AIE-NHS-Cet** at pH 4.0 by the (A) emission spectra (excited at 400 nm) or (B) ESI-HRMS (for **AIE-DMAA-Cet**). To be specific, **AIE-DMAA-Cet**/**AIE-NHS-Cet**/**AIE-DMA** (5 μM, 20 μL) were incubated at pH 4.0 (PBS) for 4 h at 37 ºC. Afterward, cold acetonitrile (100 μL) was added to the solution to precipitate the protein. The supernatant of these three samples was collected for emission spectra analysis (glycerol was added to be f_G=50% before recording) and the supernatant from **AIE-DMAA-Cet** was also analyzed by HRMS.

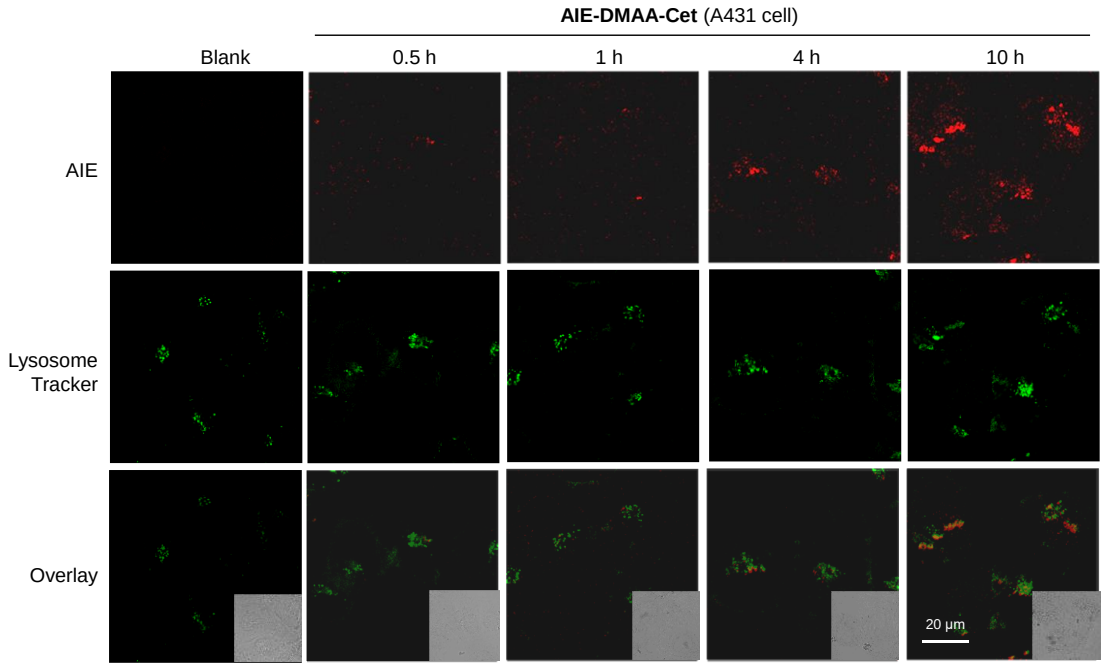


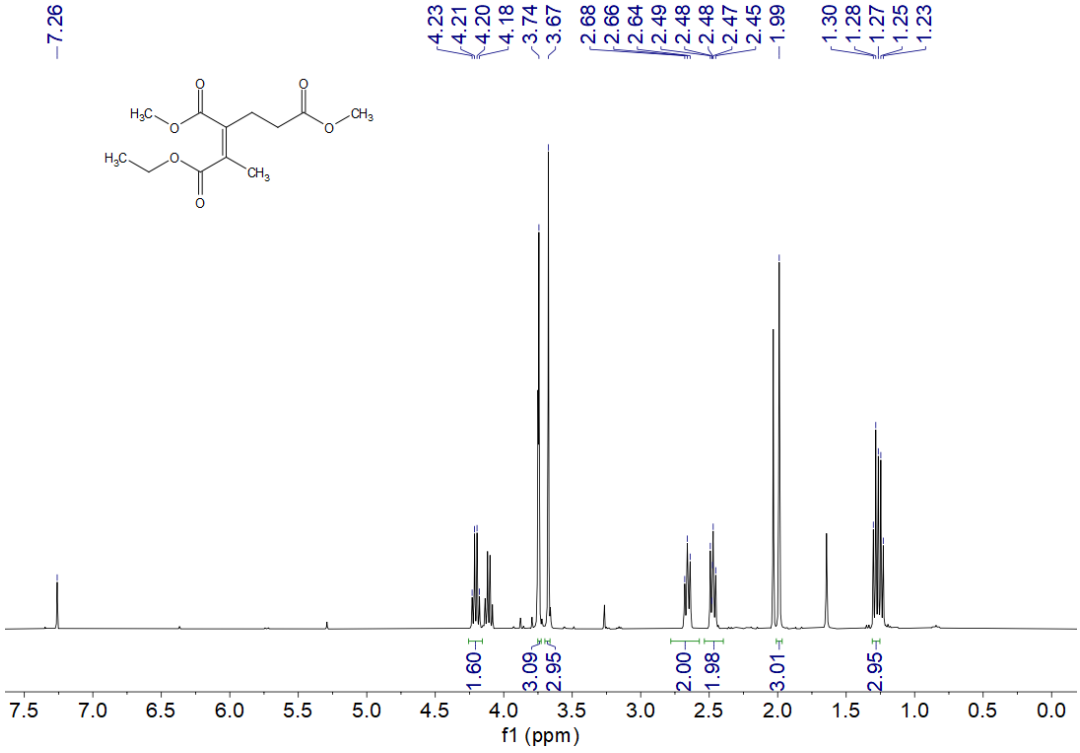
Fig. S4 Live-cell imaging of A431 cells incubated with 200 nM of **AIE-DMAA-Cet** for different time without washing (red; AIE channel: λ_{ex} = 405 nm, λ_{em} = 550-700 nm). Cells were co-stained with Lysosome Tracker (LysoTracker; green, λ_{ex} = 488 nm, λ_{em} = 519 nm) for colocalization analysis.

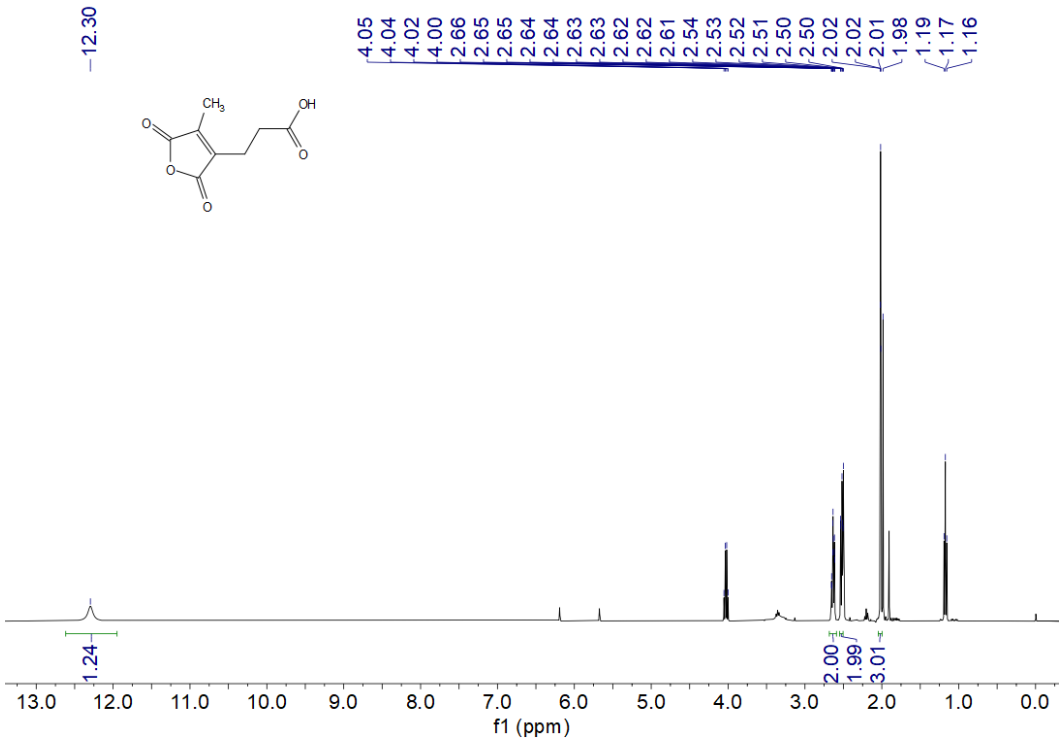
4. References

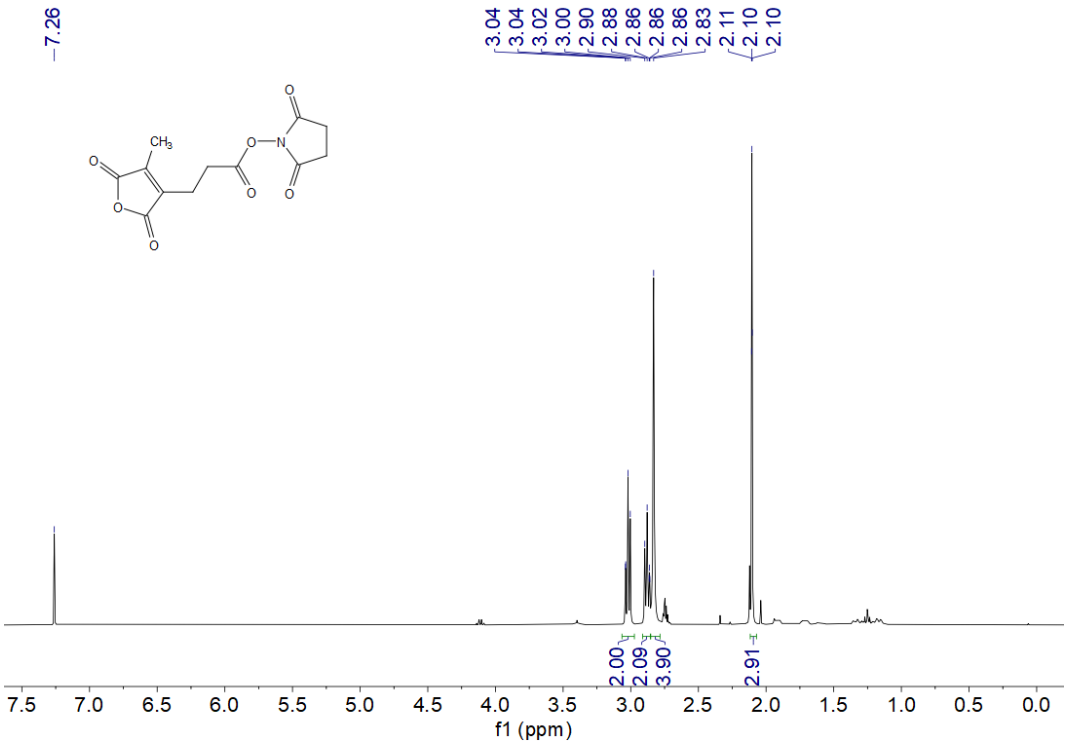
[1] H. Takemoto, K. Miyata, S. Hattori, T. Ishii, T. Suma, S. Uchida, N. Nishiyama, K. Kataoka, Acidic pH-Responsive siRNA Conjugate for Reversible Carrier Stability and Accelerated Endosomal Escape with Reduced IFNa-Associated Immune Response, Angew. Chem. Int. Ed. 52 (2013) 6218-6221.

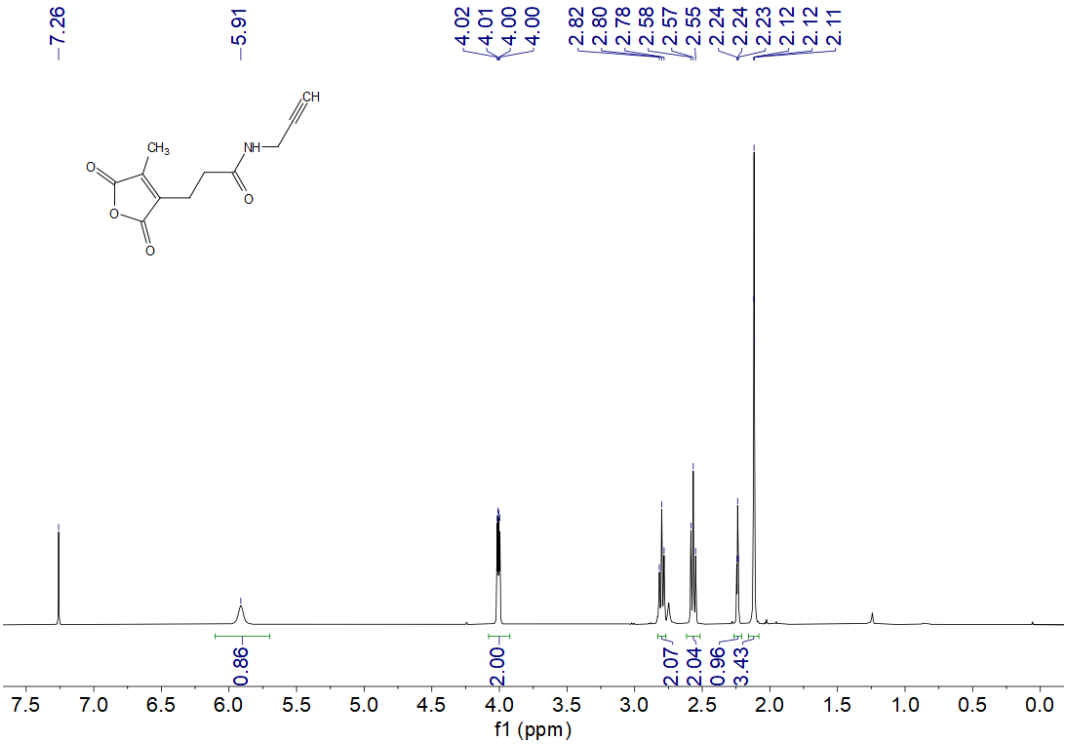
[2] X. Shi, C. Y. Y. Yu, H. Su, R. T. K. Kwok, M. Jiang, Z. He, J. W. Y. Lam, B. Z. Tang, A red-emissive antibody–AIEgen conjugate for turn-on and wash-free imaging of specific cancer cells, Chem. Sci. 8 (2017) 7014-7024.

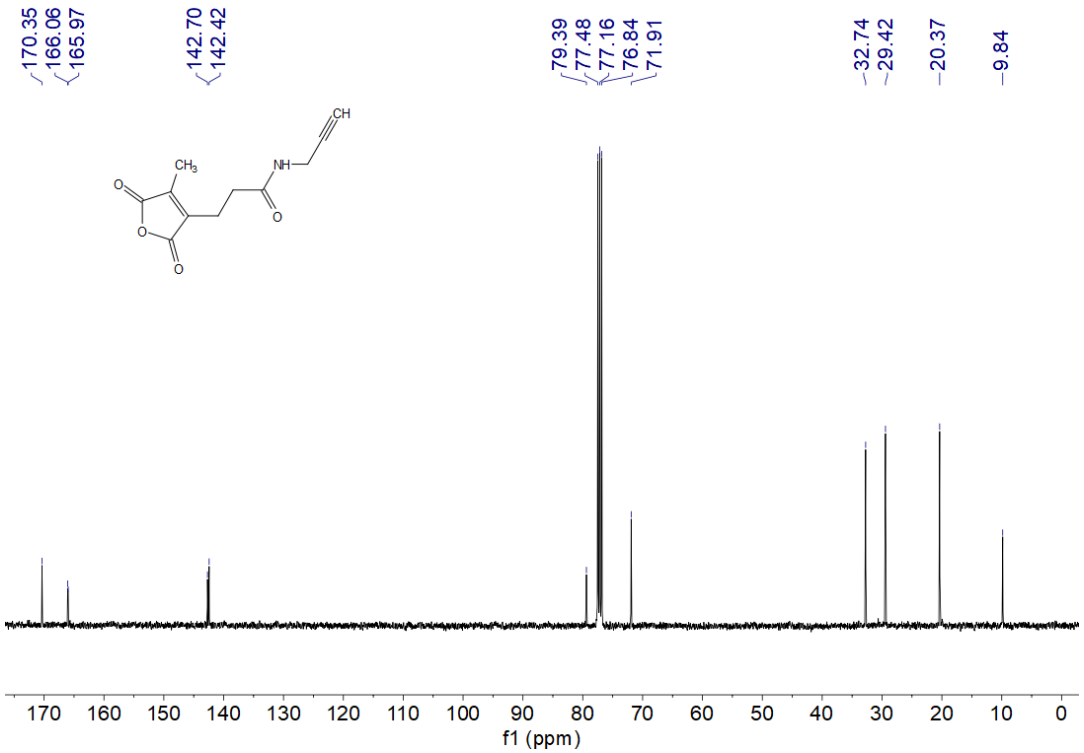
5. Appendix ($^{1}H/^{13}C$ NMR spectra & MS Data)






7.26
3.04
3.04
3.02
3.00
2.90
2.88
2.86
2.86
2.86
2.83
2.11
2.10
2.10
CH3
O
O
O
O
O
O
N
O
2.00
2.09
3.90
2.91
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


7.26
5.91
4.02
4.01
4.00
4.00
2.82
2.80
2.78
2.58
2.57
2.55
2.24
2.24
2.23
2.12
2.12
2.11
CH3
NH
CH
O
O
O
O
0.86
2.00
2.07
2.04
0.96
3.43
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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166.06
165.97
142.70
142.42
79.39
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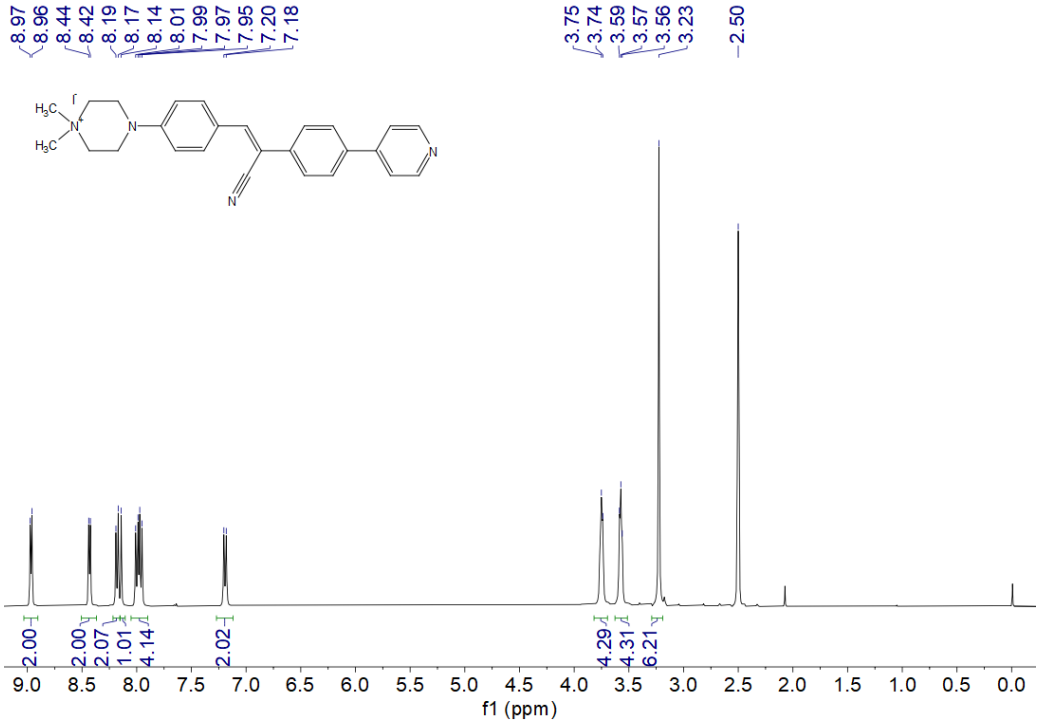
77.16
76.84
71.91
32.74
29.42
20.37
9.84
CH
NH
CH3
O
O
O
O
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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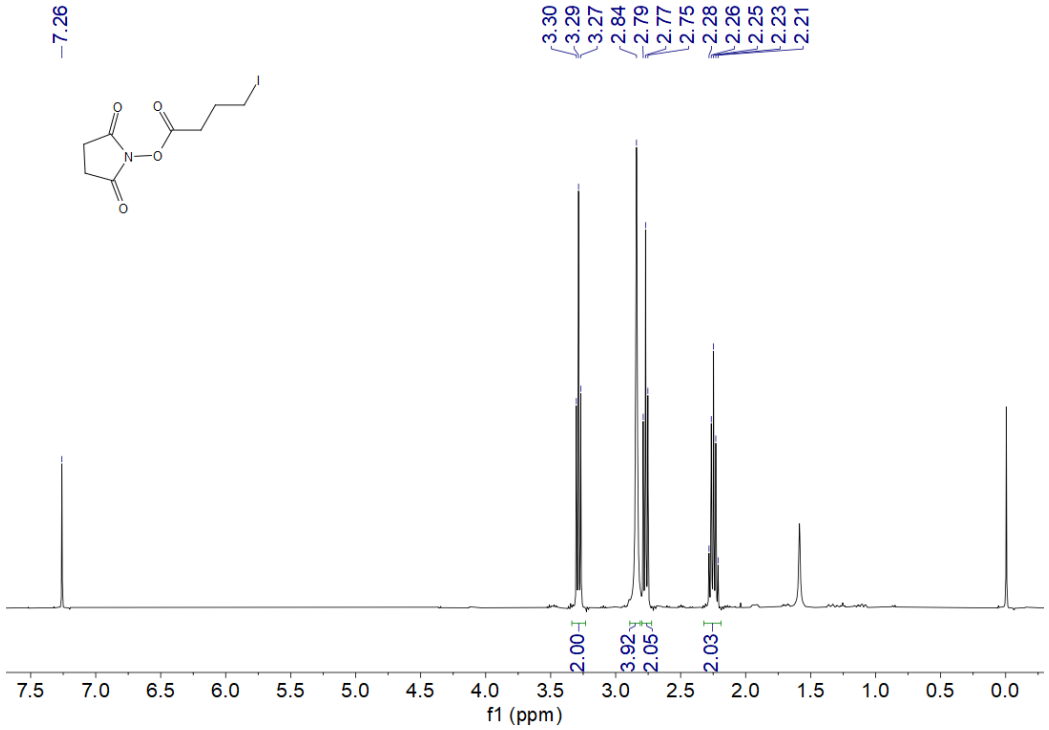
8.44
8.42
8.19
8.17
8.14
8.01
7.99
7.97
7.95
7.20
7.18
3.75
3.74
3.59
3.57
3.56
3.23
2.50
H3C
H3C
N
I
N
N
2.00
2.00
2.07
1.01
4.14
2.02
4.29
4.31
6.21
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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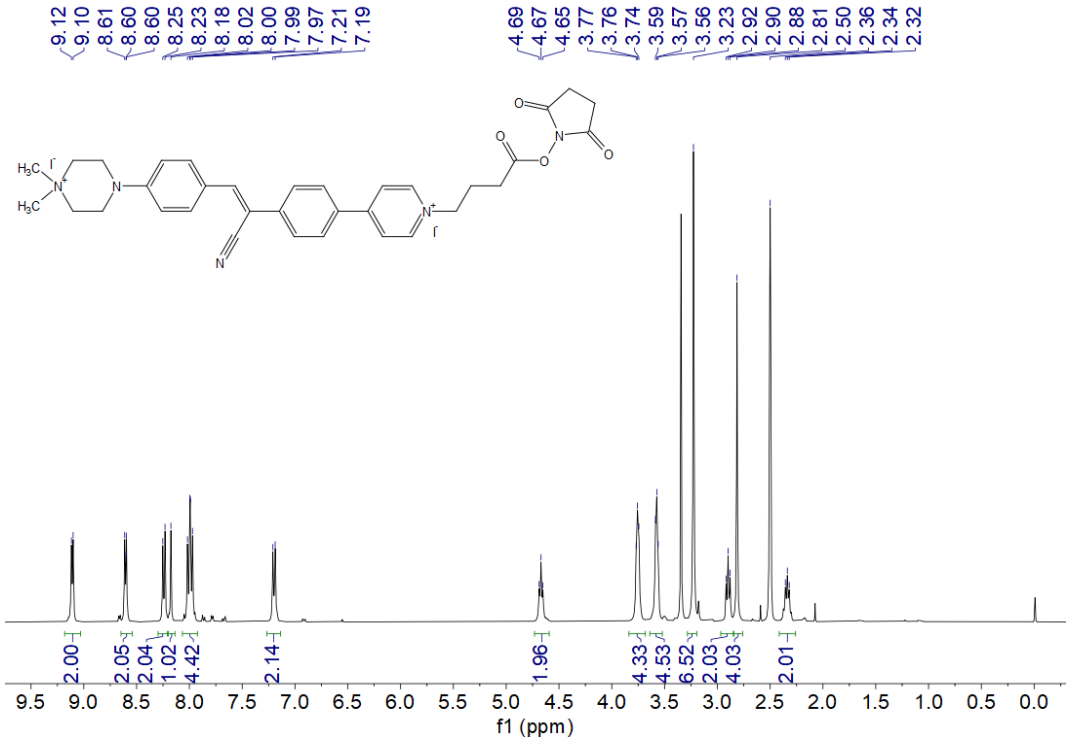
3.30
3.29
3.27
2.84
2.79
2.77
2.75
2.28
2.26
2.25
2.23
2.21
2.00
3.92
2.05
2.03
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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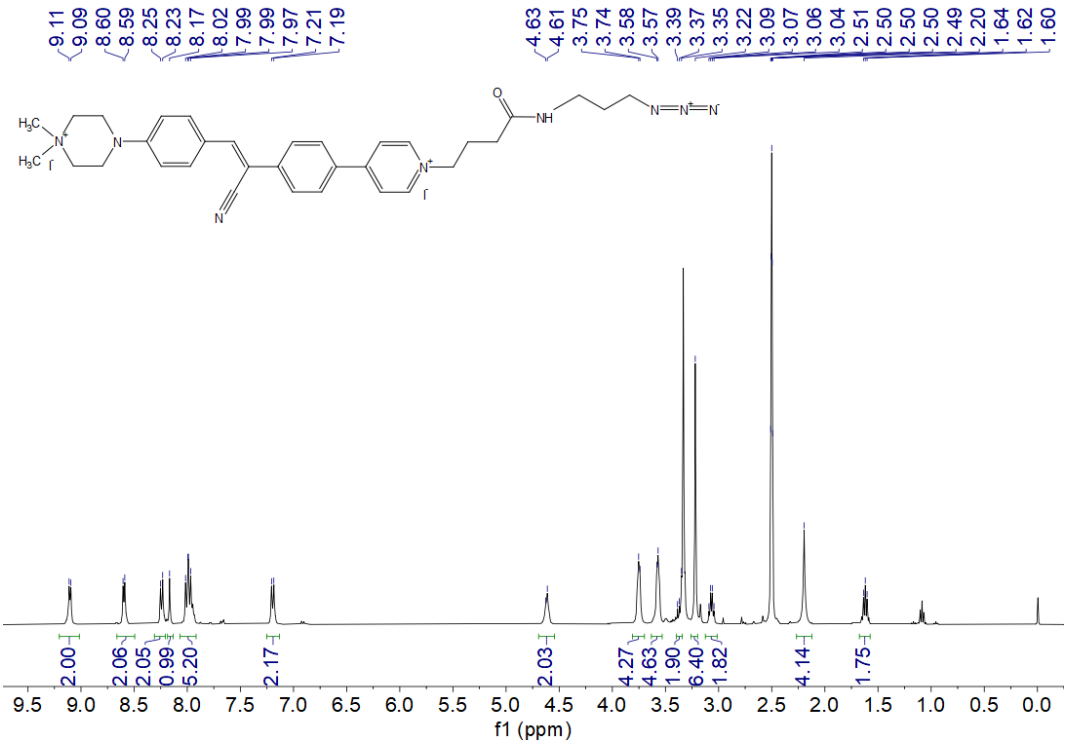
8.61
8.60
8.60
8.25
8.23
8.18
8.02
8.00
7.99
7.97
7.21
7.19
4.69
4.67
4.65
3.77
3.76
3.74
3.59
3.57
3.56
3.23
2.92
2.90
2.88
2.81
2.50
2.36
2.34
2.32
2.00
2.05
2.04
1.02
4.42
2.14
1.96
4.33
4.53
6.52
2.03
4.03
2.01
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


9.11
9.09
8.60
8.59
8.25
8.23
8.17
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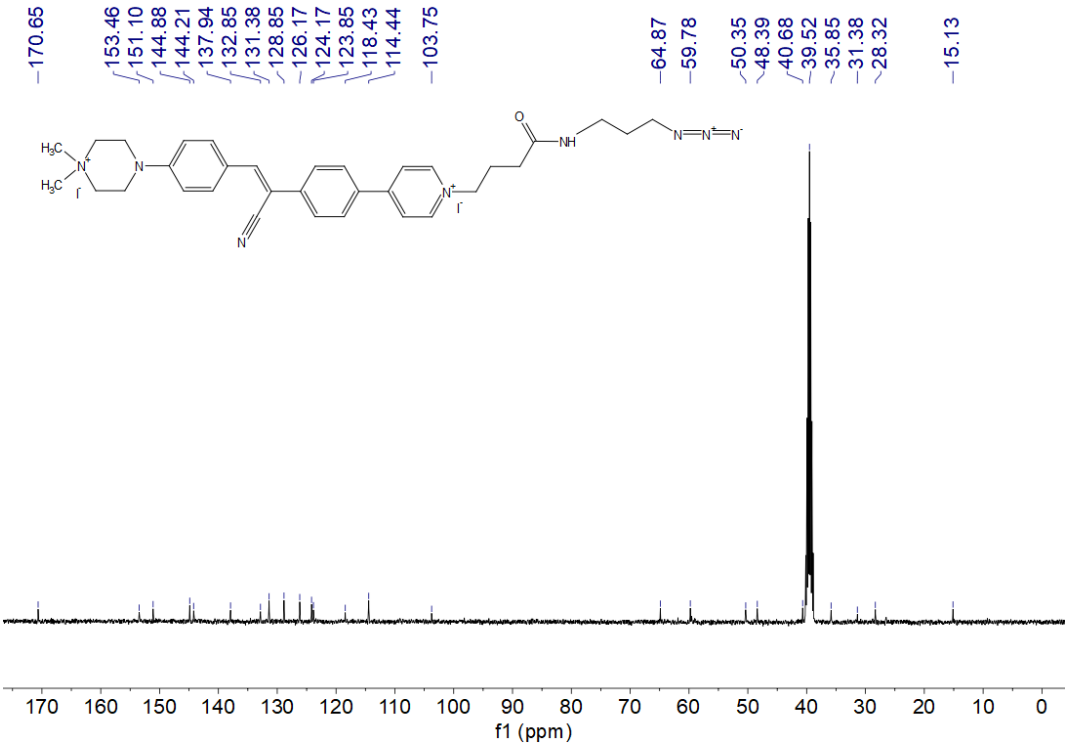
7.99
7.99
7.97
7.21
7.19
4.63
4.61
3.75
3.74
3.58
3.57
3.39
3.37
3.35
3.22
3.09
3.07
3.06
3.04
2.51
2.50
2.50
2.50
2.49
2.20
1.64
1.62
1.60
2.00
2.06
2.05
0.99
5.20
2.17
2.03
4.27
4.63
1.90
6.40
1.82
4.14
1.75
f1 (ppm)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131.38
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124.17
123.85
118.43
114.44
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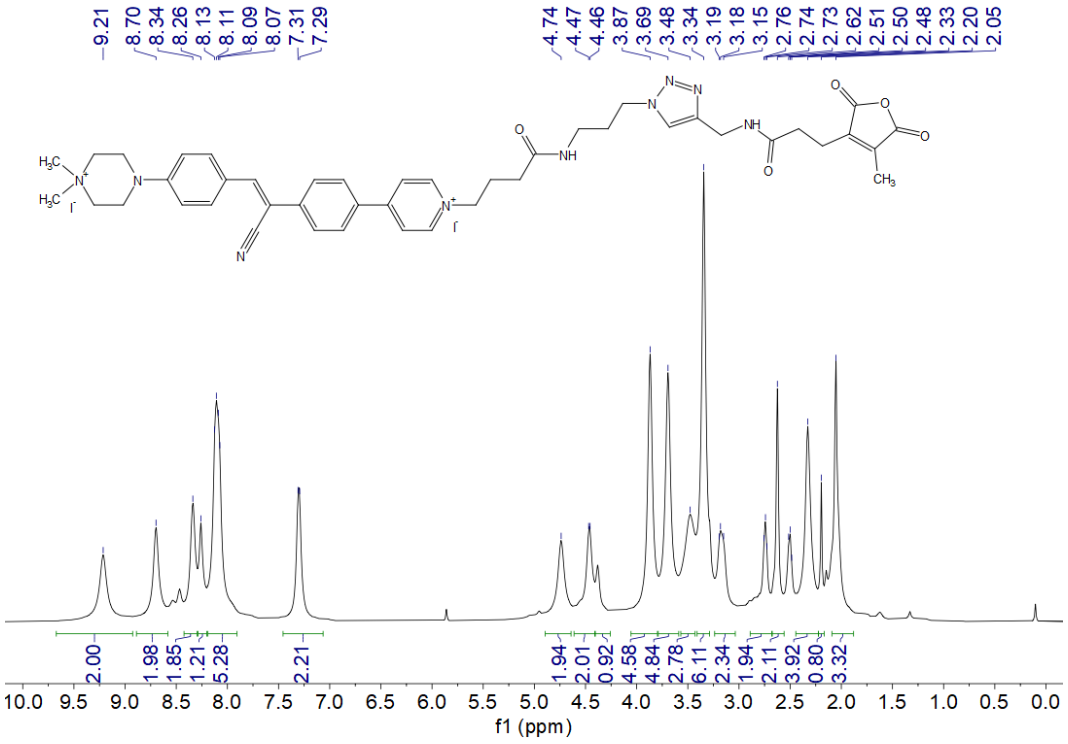
59.78
50.35
48.39
40.68
39.52
35.85
31.38
28.32
15.13
f1 (ppm)


9.21
8.70
8.34
8.26
8.13
8.11
8.09
8.07
7.31
7.29
4.74
4.47
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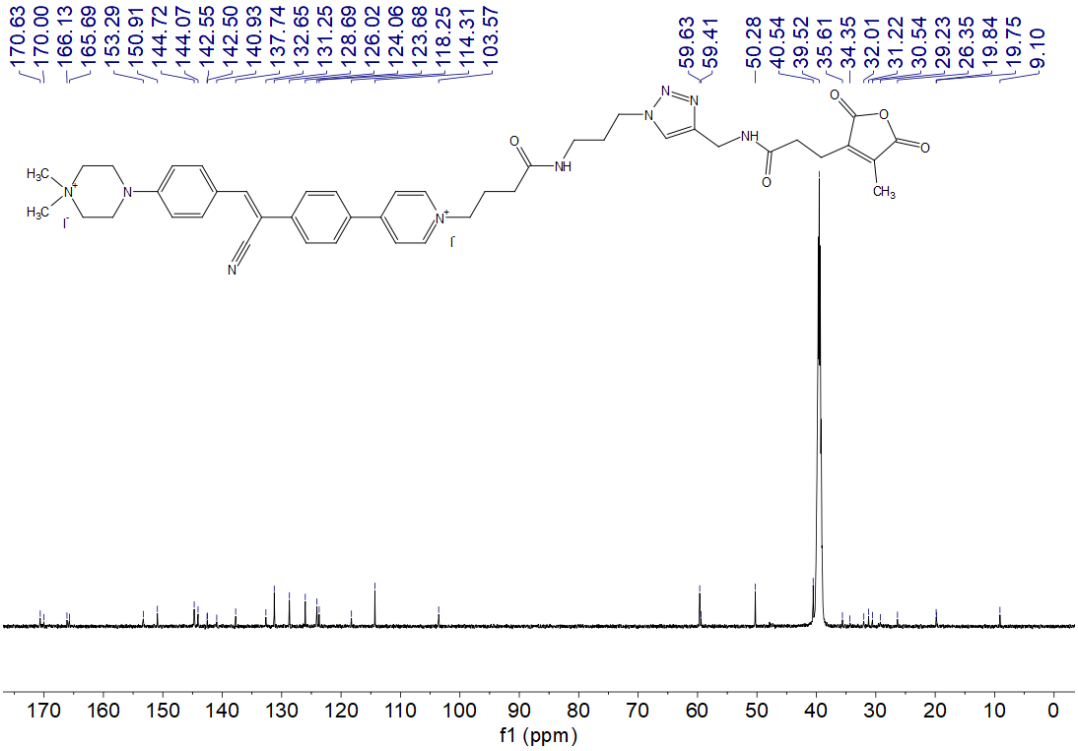
3.87
3.69
3.48
3.34
3.19
3.18
3.15
2.76
2.74
2.73
2.62
2.51
2.50
2.48
2.33
2.20
2.05
2.00
1.98
1.85
1.21
5.28
2.21
1.94
2.01
0.92
4.58
4.84
2.78
6.11
2.34
1.94
2.11
3.92
0.80
3.32
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


170.63
170.00
166.13
165.69
153.29
150.91
144.72
144.07
142.55
142.50
140.93
137.74
132.65
131.25
128.69
126.02
124.06
123.68
118.25
114.31
103.57
59.63
59.41
50.28
40.54
39.52
35.61
34.35
32.01
31.22
30.54
29.23
26.35
19.84
19.75
9.10
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

Spectra

Fragmentor Voltage	Collision Energy	Ionization Mode
50	0	ESI