Electronic Supplementary Material (ESI) for Analyst.

Assembly of BODIPY-carbazole Dye with Liposome to fabricate Fluorescent Nanoparticles for Lysosomal Bioimaging in Living Cells

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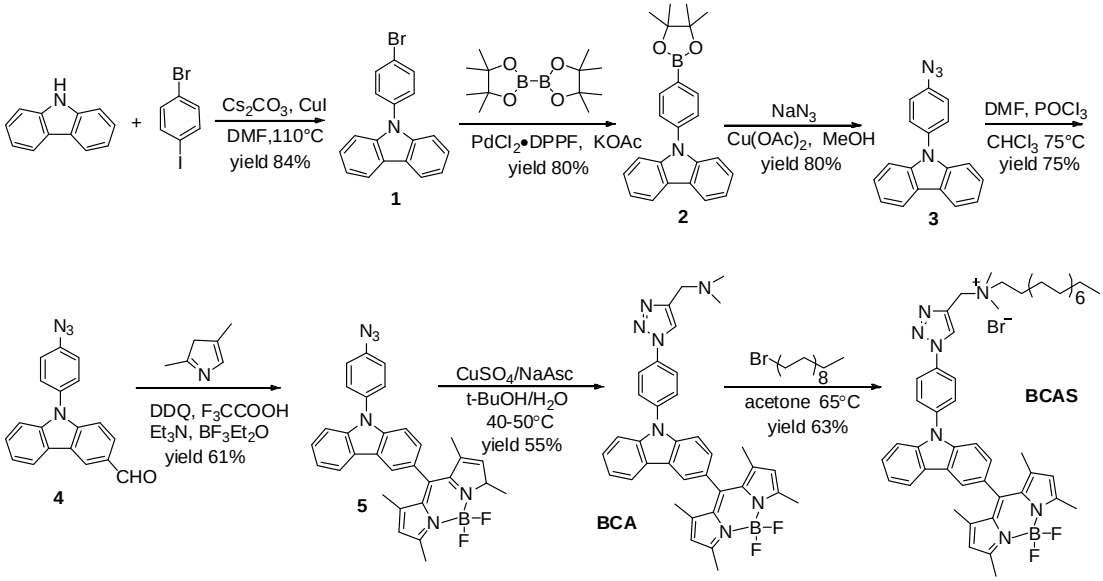
Contents

1. General Information

All of the materials were purchased as reagent grade and used without further purification. DMF was vacuum distilled over calcium hydride (CaH_2). $CHCl_3$was redistilled. Reactions were monitored with analytical thin-layer chromatography (TLC) on silica gel F254 glass plates and visualized under UV light (254 nm/365 nm). Flash column chromatography was performed on silica gel (200-300 mesh). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were taken on a Bruker Avanced III spectrometer with tetramethylsilane as an internal standard and chloroform ($CDCl_3$) as the solvent. High resolution electrospray ionization mass spectra (HRMS-ESI) were recorded with a Waters LCT Premier XE mass spectrometer. Absorption spectra were recorded by on a Shimadzu UV2600 spectrophotometer. Zeta potentials and particle sizes were performed using a Malvern nano zs 90 Zeta Potential Meter. Fluorescence microscopy photos were recorded by Olympus FV1200 confocal fluorescence microscopy. All cuvette experiments were carried out at room temperature.

2. Synthesis of BCA, BCAS and NPs



Scheme S1 Synthetic route of **BCA** and **BCAS**.

9-(4-bromophenyl)-9H-carbazole (1)

To a solution of 9H-carbazole (800 mg, 4.8 mmol) in DMF (10 mL), 1-bromo-4-iodobenzene (1.765 g, 6.24 mmol) was added, followed by addition of CuI (274 mg, 1.44 mmol). The reaction mixture was stirred in a 110°C of oil bath under argon for about 30 h until the starting material had been completely consumed as detected by TLC. The solution was then allowed to cool to room temperature, and the DMF was evaporated under vacuum. After the removal of the solvent, the mixture was purified by column chromatography (Hexanes/EtOAc = 150/1) to give compound 1 (1.299 g, 4.05 mmol, 84%) as yellow solid; mp: 144-145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, J = 7.8 Hz, 2H), 7.74 (d, J = 8.6 Hz, 2H), 7.46 (m, 2H), 7.40 (dd, J = 13.5, 4.4 Hz, 4H), 7.32 - 7.28 (td, J = 7.3, 1.32 Hz, 2H).

9-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) phenyl)-9H-carbazole (2).

A Schlenk flask was charged with compound **1** (600 mg, 1.87 mmol), then bis(pinacolato)diboron (522 mg, 2.06 mmol), $PdCl_2$·DPPF (113 mg, 0.14 mmol), KOAc (550 mg, 5.61 mmol) and dry 1, 4-dioxane (15 mL) were added to the flask under argon. The mixture was preactivated for 1 h under room temperature followed by immersing in an oil bath at 80 °C for about 20 h until the starting material had completely disappeared as judged by TLC. The solvent was evaporated under reduced pressure and purified by column chromatography (Hexanes / EtOAc = 200/1) to give compound **2** (546.4 mg, 1.48 mmol, 80%) as pale yellow solid; mp: 173-174 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, J = 7.7 Hz, 2H), 8.05 (d, J = 8.2 Hz, 2H), 7.59 (d, J = 8.2 Hz, 2H), 7.48 - 7.36 (m, 4H), 7.33 - 7.25 (m, 2H), 1.40 (s, 12H).

9-(4-azidophenyl)-9H-carbazole (3).

To a solution of compound **2** (228 mg, 0.617 mmol) in methanol (10 mL), NaN_3 (60.22 mg，0.926 mmol) and $Cu(OAc)_2$ (12.32 mg，0.0617 mmol) were added. The mixture was stirred in a 55°C of oil bath under air for about 3 h until the starting material had been completely consumed as detected by TLC. The crude yellow oil was then diluted with EtOAc (150 mL), washed with saturated NaCl solution, and dried with $MgSO_4$. After removal of the solvent, the mixture was purified by column chromatography (Hexanes/EtOAc = 150/1) to give **3** (126 mg, 0.44 mmol, 80%) as a yellow crystals; mp: 111-112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, J = 7.7 Hz, 2H), 7.56 (d, J = 8.8 Hz, 2H), 7.40 (td, J = 8.0, 1.2 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 7.30 - 7.25 (m, 4H).

9-(4-azidophenyl)-9H-carbazole-3-carbaldehyde (4).

A Schlenk flask was charged with dry DMF (5 mL), $POCl_3$ (5 mL) was then dropping to the flask in ice bathe. The mixture was stirred for 20 min at 0 ℃ and for another 1h under room temperature. Then $CHCl_3$ (5 mL) and compound **3** (254.19 mg, 0.89 mmol) was added to reflux for about 10 h. The mixture was neutralized by NaOH solution in ice bath to pH = 10. Then the crude product was diluted with DCM (150 mL), washed with saturated NaCl solution (30 mL), and dried with $MgSO_4$. After removal of the solvent by vacuum distillation, the mixture was purified by column chromatography (Hexanes/EtOAc = 50/1) to give compound **4** (234 mg, 0.746 mmol, 83%).Dark yellow crystals; mp: 131-132 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.13 (s, 1H), 8.68 (d, J = 1.2 Hz, 1H), 8.21 (d, J = 7.7 Hz, 1H), 7.96 (dd, J = 8.5, 1.5 Hz, 1H), 7.55 (d, J = 8.7 Hz, 2H), 7.49 (t, J = 7.1 Hz, 1H), 7.42 - 7.33 (m, 3H), 7.30 (d, J = 8.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.64, 144.45, 141.84, 133.30, 129.58, 128.66, 127.53, 127.10, 123.82, 123.61, 123.24, 121.35, 120.75, 120.64, 110.21, 109.91, 77.37, 77.05, 76.73. HRMS (ESI): m/zcalcd for $C_{19}H_{13}N_4O$ $[M+H]^+$: 313.1089, found: 313.1095.

10-(9-(4-azidophenyl)-9H-carbazol-3-yl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (5)

To 40 mL of CH_2Cl_2, compound 4 (200 mg, 0.62 mmol) was added, followed by the addition of the 2, 4-dimenthylpyrrole (0.2 mL, 2.8mmol) and trifluoroacetic acid (0.1 mL). The reaction mixture was stirred at room temperature for 5 h. Then DDQ (175 mg, 0.76mmol) in CH_2Cl_2 (15 mL) was added and the reaction mixture was stirred at room temperature for another 30 min.Finally, triethylamine (3 mL) and $BF_3 \cdot Et_2O$ (3 mL) were added to the mixture in turn. The reaction mixture was further stirred at room temperature overnight. The crude product was washed with waterfor 3 times (3×30 mL), and dried with $MgSO_4$.After removal of the solvent by vacuum distillation,the crude product was purified by column chromatography (Hexanes/EtOAc = 75/1) to give compound 5 (171.7 mg, 0.32mmol, 52.14%) as orange solid; mp: 88-90 °C; 1H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 7.7 Hz, 1H), 8.05 (d, J = 1.1 Hz, 1H), 7.62 (d, J = 8.7 Hz, 2H), 7.47 (dd, J = 7.7, 2.8 Hz, 2H), 7.42 (d, J = 8.1 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.30 (dd, J = 9.4, 2.1 Hz, 3H), 5.99 (s, 2H), 2.58 (s, 6H), 1.35 (s, 6H); 13C NMR (100 MHz, $CDCl_3$) δ 155.25, 143.27, 142.72, 141.30, 140.88, 139.62, 133.95, 132.18, 128.53, 126.71, 126.55, 125.78, 123.89, 123.02, 121.11, 120.57, 120.53, 120.09, 110.26, 109.95, 29.69, 14.72, 14.59;HRMS (ESI): m/z calcd for $C_{31}H_{26}BF_2N_6$ $[M+H]^+$: 531.2356, found: 531.2356.

BCA

To a mixture of water and *t*-BuOH (v/v=3:1), compound 5 (70mg, 0.132mmol) and dimethylpropargylamine (0.2mL, 0.154mmol) was added. Freshly prepared sodium ascorbate solution (0.6ml, 0.2mol/L) and $CuSO_4$ solution (1.2ml, 0.05mol/L) was mixed rapidly, and then added to above mixture. The heterogeneous mixture was stirred overnight at 40 ℃. After completion of the reaction, the row product was washed with distilled water, extracted with DCM, and dried with $MgSO_4$. After removing the solvent with vacuum distillation, the mixture was purified by column chromatography (CH_2Cl_2/MeOH=40:1) to give compound BCA (44.2mg, 0.07mmol, 54.6%) as orange solid; mp: 140-145 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, J = 7.8 Hz, 2H), 8.06 (t, J = 4.5 Hz, 3H), 7.82 (d, J = 8.1 Hz, 2H), 7.56 (d, J = 8.4 Hz, 1H), 7.50 (d, J = 3.8 Hz, 2H), 7.34 (ddd, J = 10.0, 8.2, 2.9 Hz, 2H), 5.99 (s, 2H), 3.81 (s, 2H), 2.58 (s, 6H), 2.42 (s, 6H), 1.36 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.33, 143.23, 142.52, 141.00, 140.56, 137.61, 132.14, 128.31, 126.95, 126.91, 125.97, 124.17, 123.25, 122.07, 121.15, 120.94, 120.68, 120.22, 110.23, 109.92, 14.73, 14.59; HRMS (ESI): m/z calcd for $C_{36}H_{34}BF_2N_7$ $[M+H]^+$: 614.3016, found: 614.3017.

BCAS

Compound BCA (70mg, 0.114mmol) and octadecyl bromide (190mg, 0.57mmol) was dissolved in 15mL acetone solution. The mixture was activated at room temperature for about 3h, and then refluxed until the starting material had been completely consumed as detected by TLC.After removal of the solvent under vacuum distillation, the mixture was purified by column chromatography (DCM/MeOH = 30/1) to give BCAS (67.6mg, 0.07mmol, 63.2%) as orange solid; mp: 138-140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (s, 1H), 8.17 (dd, J = 8.4, 2.3 Hz, 2H), 8.10

(d, J = 7.7 Hz, 1H), 8.04 (s, 1H), 7.81 (d, J = 8.4, 2H), 7.54 (d, J = 8.3Hz, 1H), 7.47 (s, 2H), 7.36 - 7.27 (m, 2H), 5.97 (s, 2H), 5.32 (s, 2H), 3.63 - 3.51 (m, 2H), 3.41 (s, 6H), 2.56 (s, 6H), 1.94 (s, 2H), 1.34 (s, 6H), 1.23 (s, 30H), 0.87 (t, 1.6Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.25, 143.22, 142.46, 140.83, 140.39, 138.21, 136.69, 135.09, 132.08, 128.20, 127.32, 126.94, 126.92, 125.94, 124.18, 123.23, 122.21, 121.12, 120.97, 120.63, 120.16, 110.21, 109.86, 65.31, 57.95, 50.79, 31.86, 29.64, 29.59, 29.53, 29.40, 29.32, 29.29, 29.14, 26.26, 22.92, 22.62, 14.71, 14.55, 14.06; HRMS (ESI): m/z calcd for $C_{54}H_{71}BF_2N_7$ $[M+H]^+$: 866.5838, found: 866.5838.

FNPs

A THF solution (0.5 mL) containing 1.1 mg of BCA (or 1.7 mg of BCAS) and 3 mg of DSPE-$mPEG_{2000}$ was poured into 10 mL of 90% (v/v) water/THF solution. The mixture was followed by sonicating for 60 seconds and then stirred at room temperature overnight to evaporate THF and obtained **BCA-FNP** (or **BCAS-FNP**).

3. HR-SEM, UV and Fluorescence Spectra

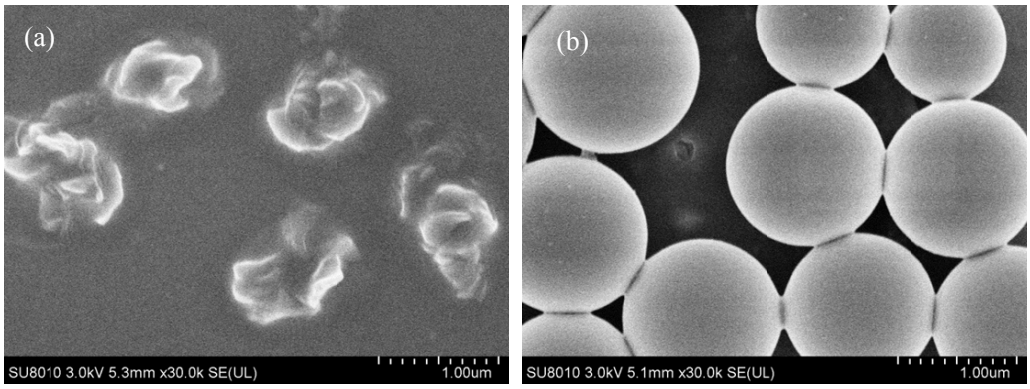


Fig. S1 HR-SEM images of (a) BCA and (b) BCAS.

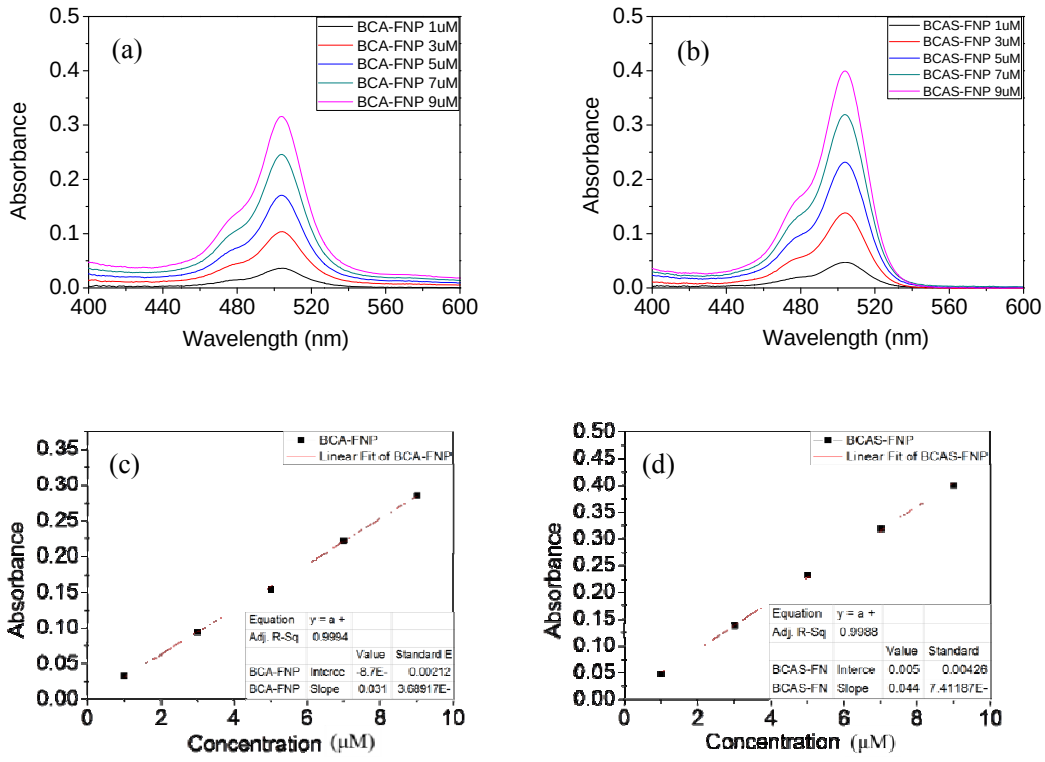


Fig. S2 UV absorbance change of (a) BCA-FNP and (b) BCAS-FNP in HEPES buffer (10 mM, pH = 7.4) *via* different concentration. Absorbance at 504 nm as functions of (c) BCA-FNP and (d) BCAS-FNP concentrations: μM

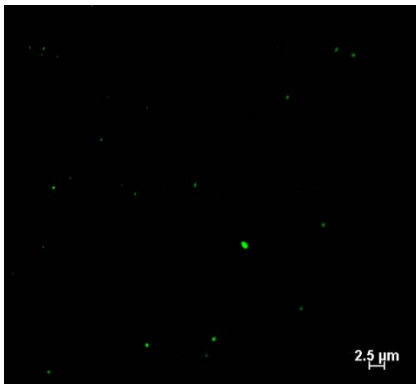


Fig. S3 Fluorescence microscopy photos of the fluorescent nanoparticles BCAS-FNP**.**

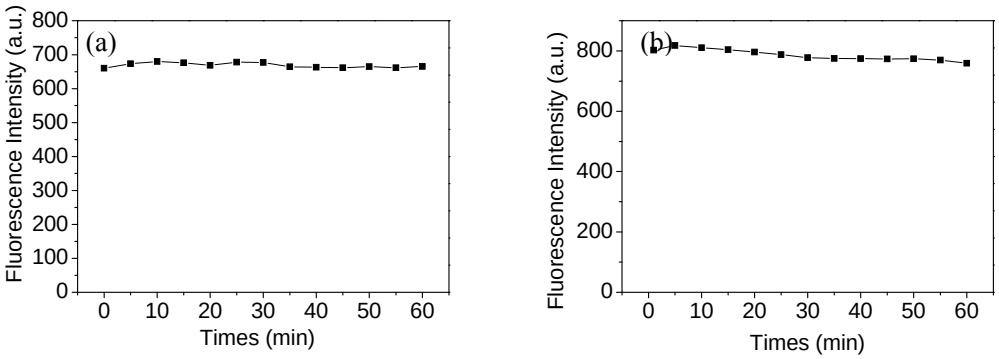


Fig. S4 Fluorescence stability of (a) 5 μM BCA-FNP and (b) 5 μM BCAS-FNP in HEPES buffer (10 mM, pH = 7.4)

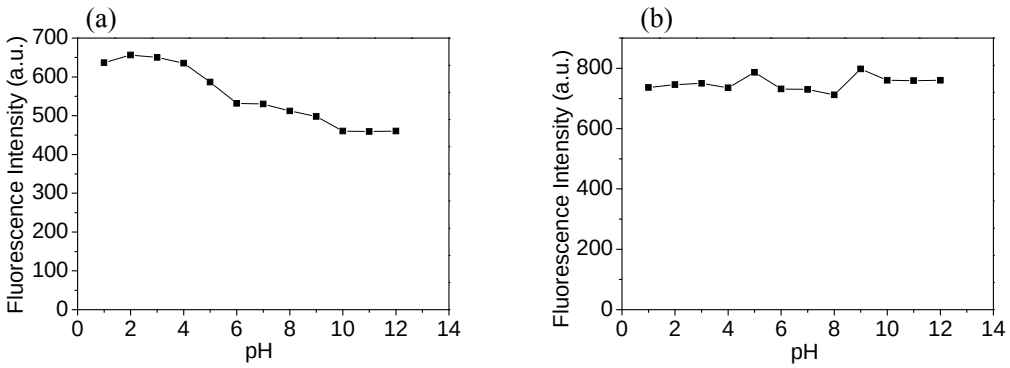


Fig. S5 Fluorescent intensities of (a) 5 μM BCA-FNP and (b) 5 μM BCAS-FNP when pH changed from 1 to 12.

4. Cell Culture and Confocal Imaging

HeLa cells (purchased from Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences) were cultured in modified DMEM medium (HyClone Corporation) supplemented with 10% fetal bovine serum. Fluorescence imaging of cells was performed using a confocal microscope (Olympus FV1200 with a 250 × oil objective).

HeLa cells were incubated in humidified incubator (37℃, 5% CO_2) overnight to make them adhere to the glass bottom of culture vessels (Nest Corporation, Φ 20 mm). After washing once with phosphate buffer solution (PBS, pH = 7.4), the cells were treated with probes and incubated for necessary time. The final concentration of target compounds was 1 μM. Then the cells were washed thrice with PBS, and added new culture medium to observe the fluorescence. Corresponding parameters of optical path were set as below: excitation: 488nm; emission range: 510-550 nm.

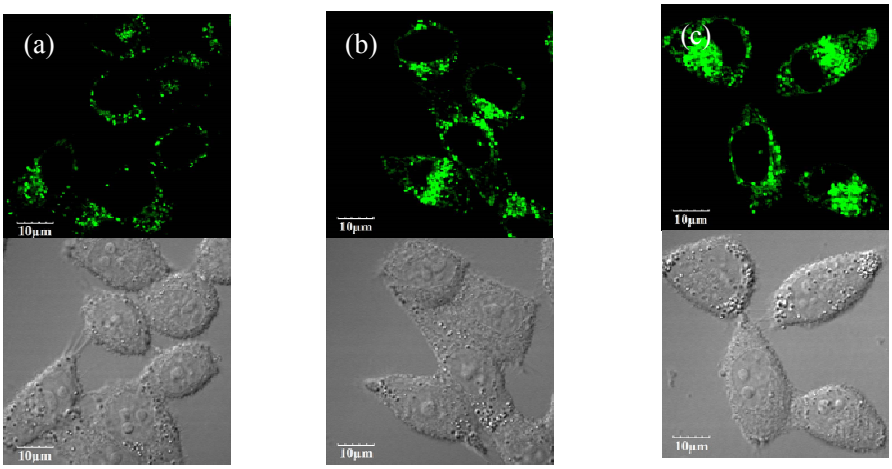


Fig S6 Confocal fluorescence images of HeLa cells stained with 1 μM BCA-FNP for (a) 5 min (b) 15 min and (c) 30 min. bottom: bright field.

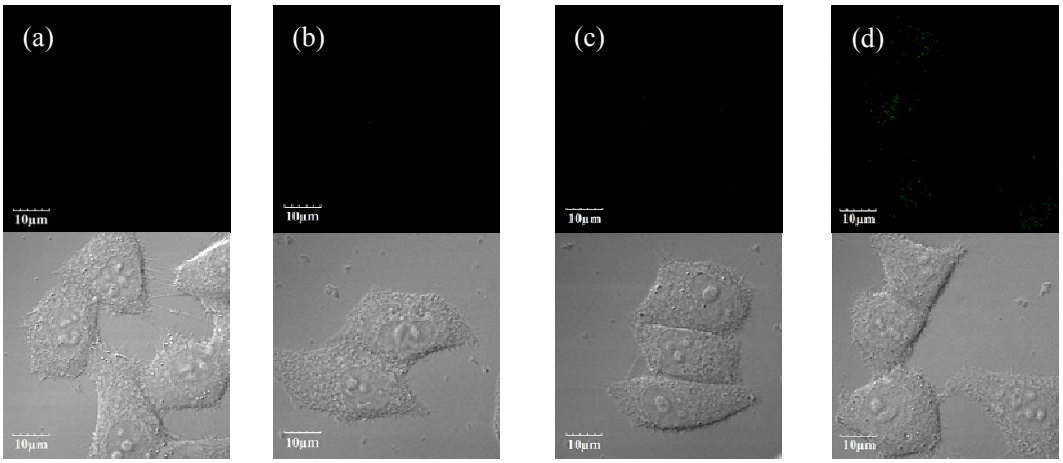
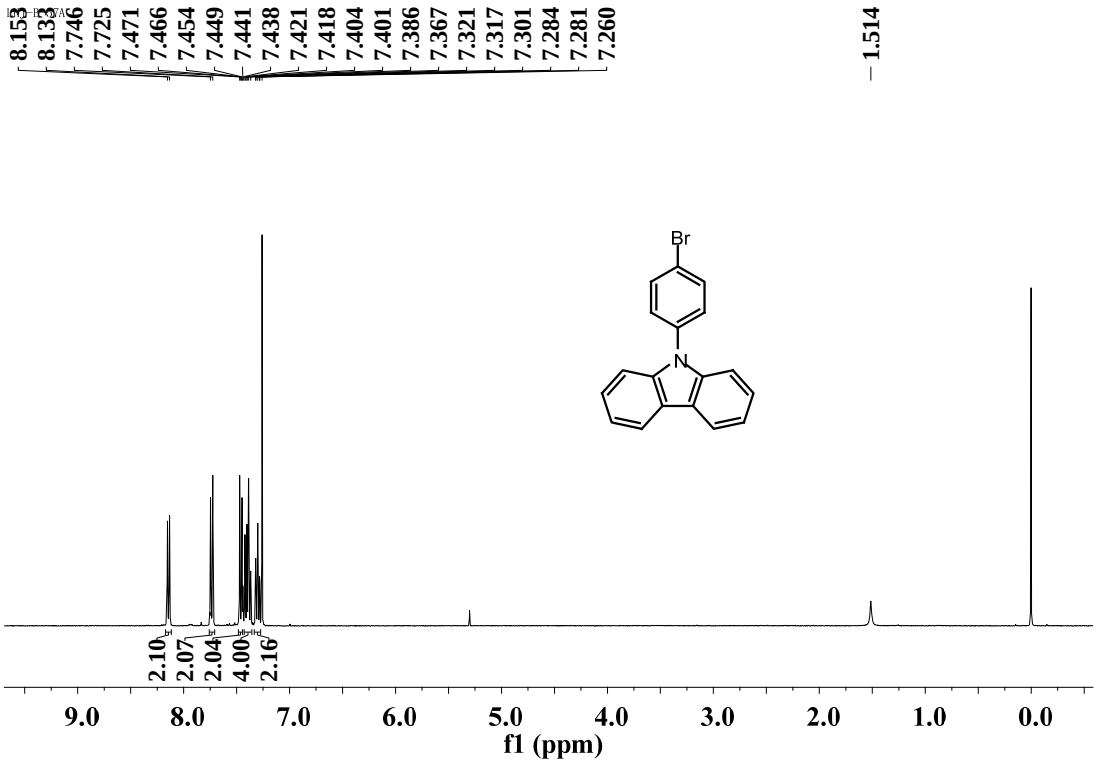


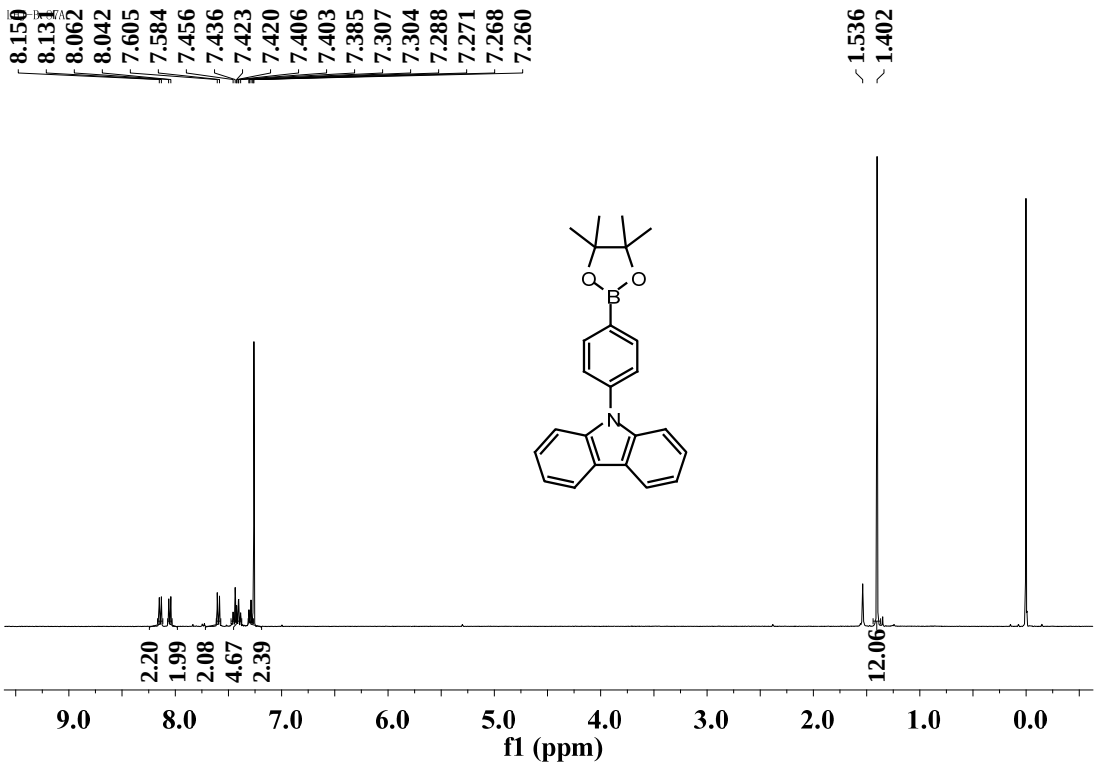
Fig. S7 Confocal images of HeLa cell incubated with 1 μM of BCAS-FNP for (a) 5 min and (b) 30 min. Confocal images of HeLa cell incubated with 5 μM of BCAS-FNP for (c) 5 min and (d) 30 min

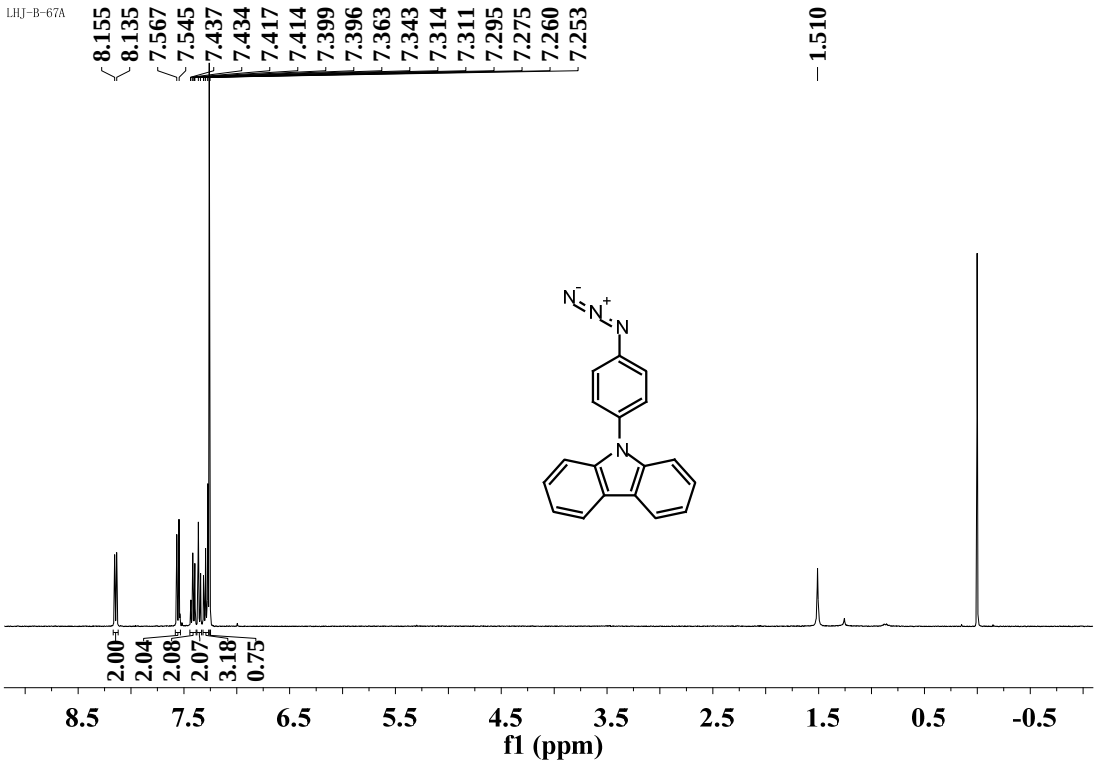
Table S1 Zeta potentials of the BCA-FNP and BCAS-FNP in HEPES buffer and culture medium.

	HEPES buffer (mV)	**Culture Medium (mV)**
BCA-FNP	-18.55±0.21	-4.37±0.12
BCAS-FNP	14.67±0.91	-2.11±0.16

5. NMR Spectra for Compounds



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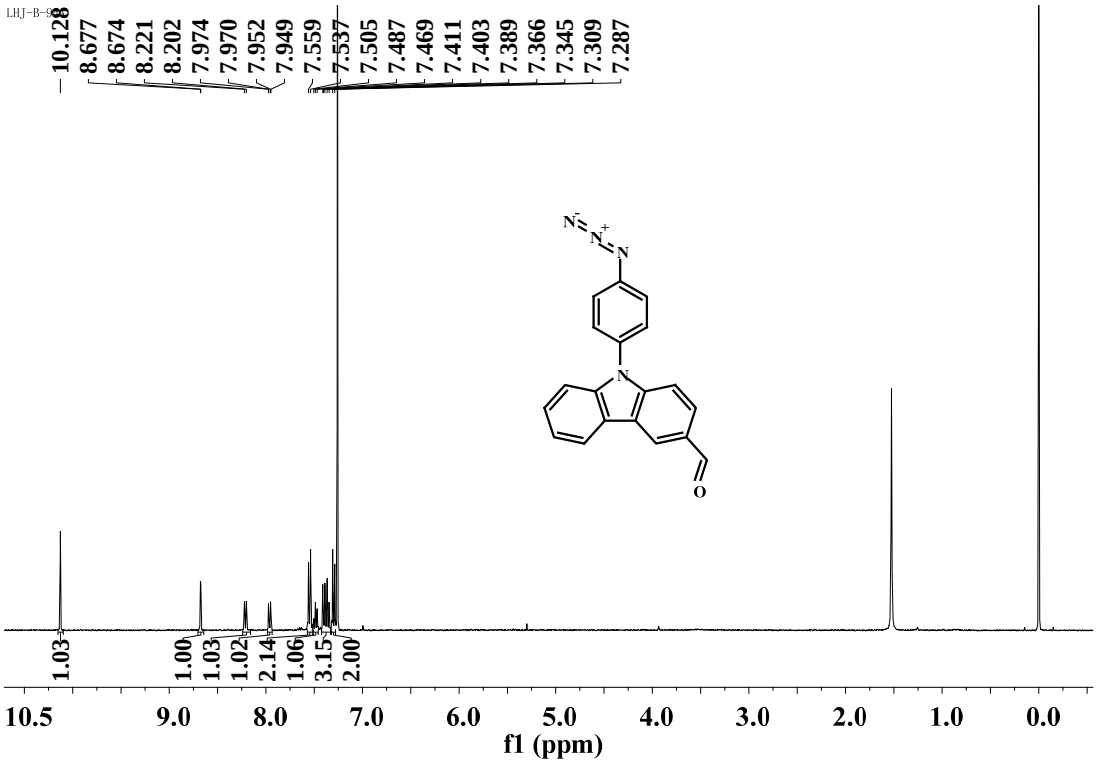
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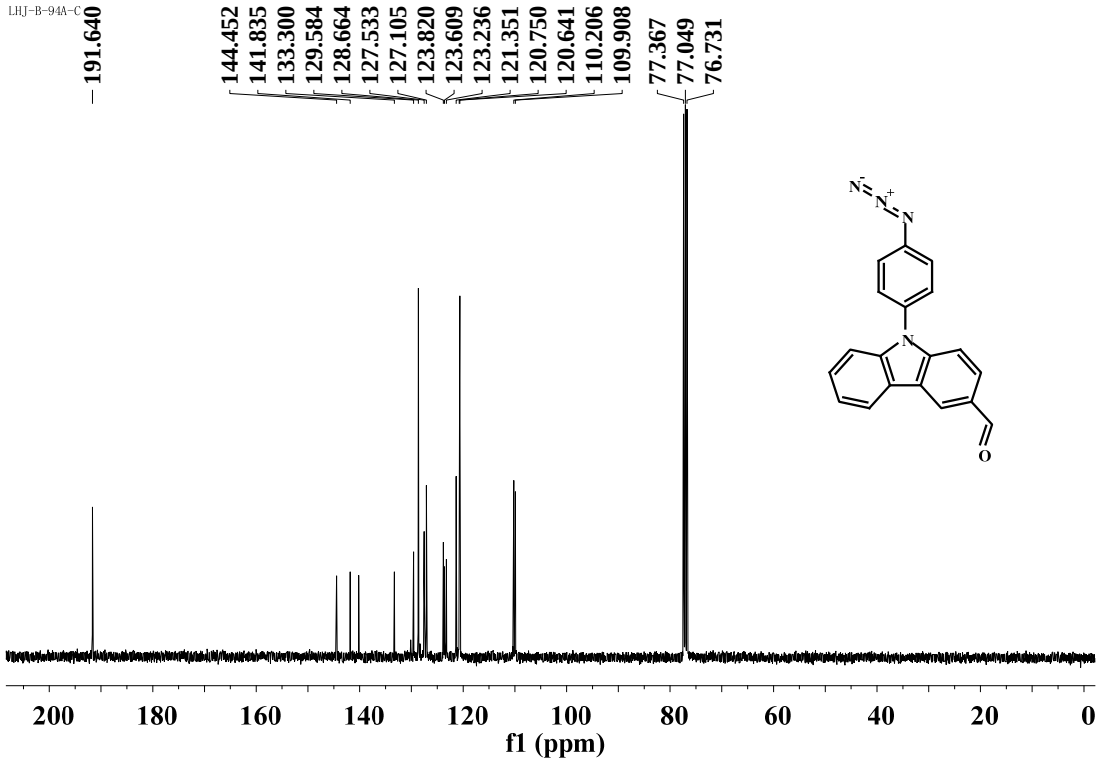
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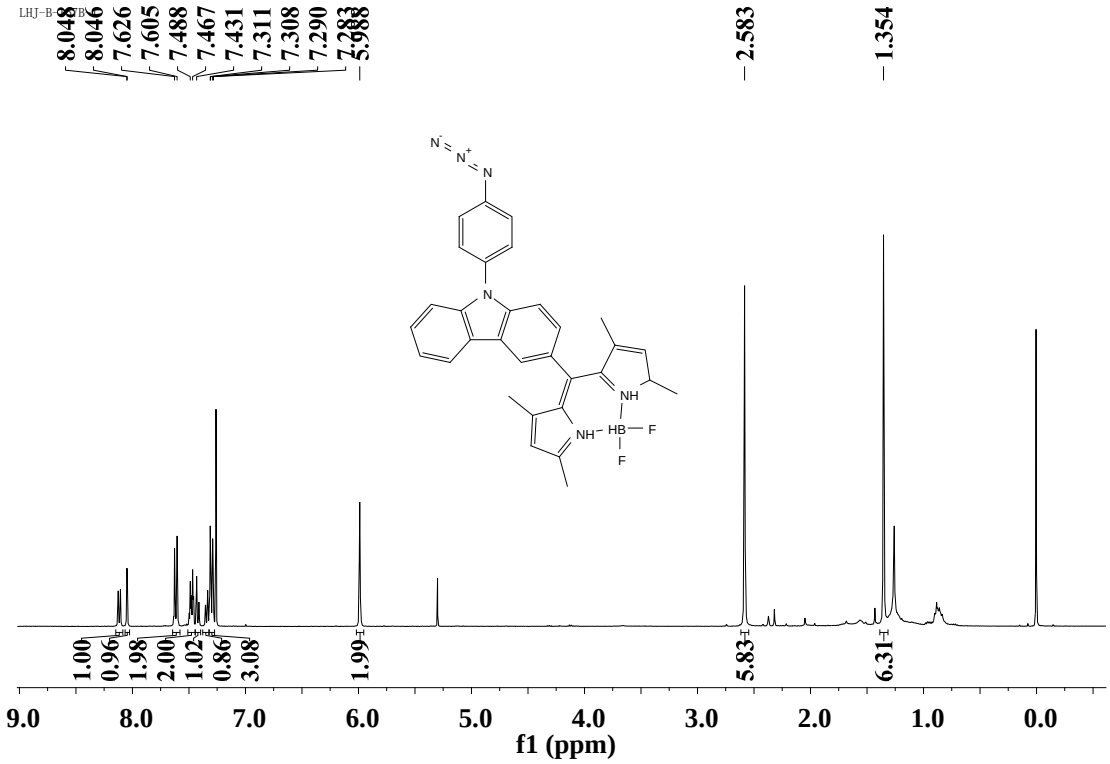
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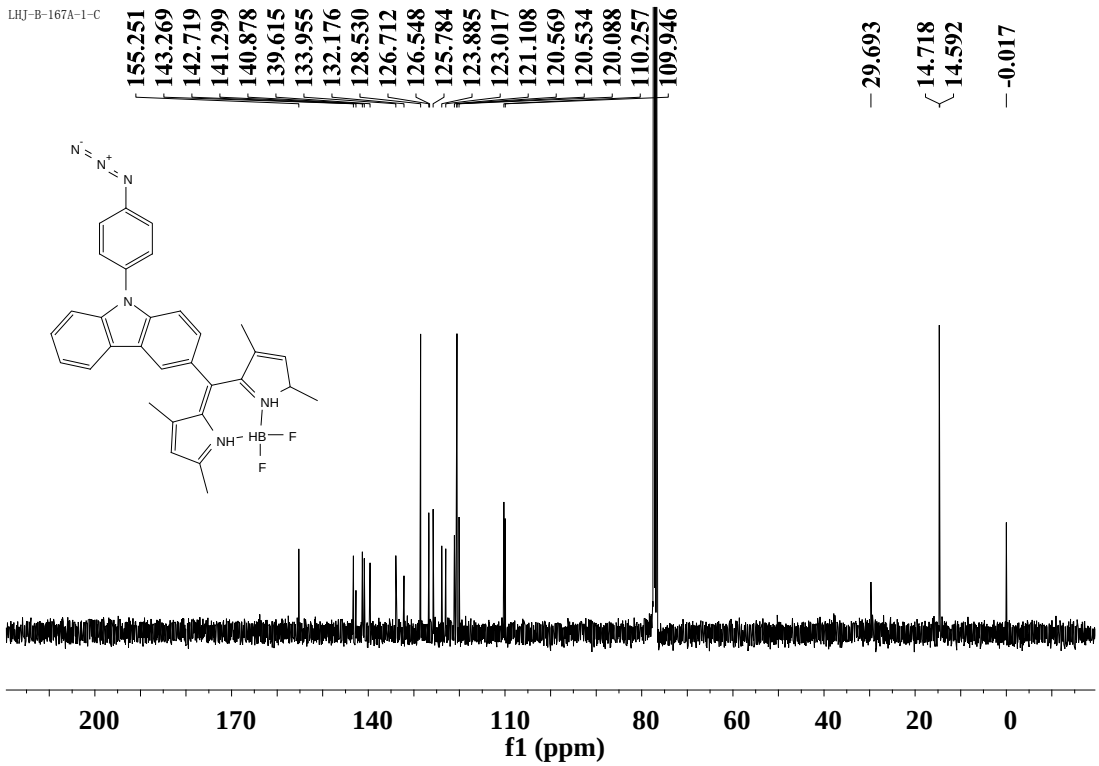
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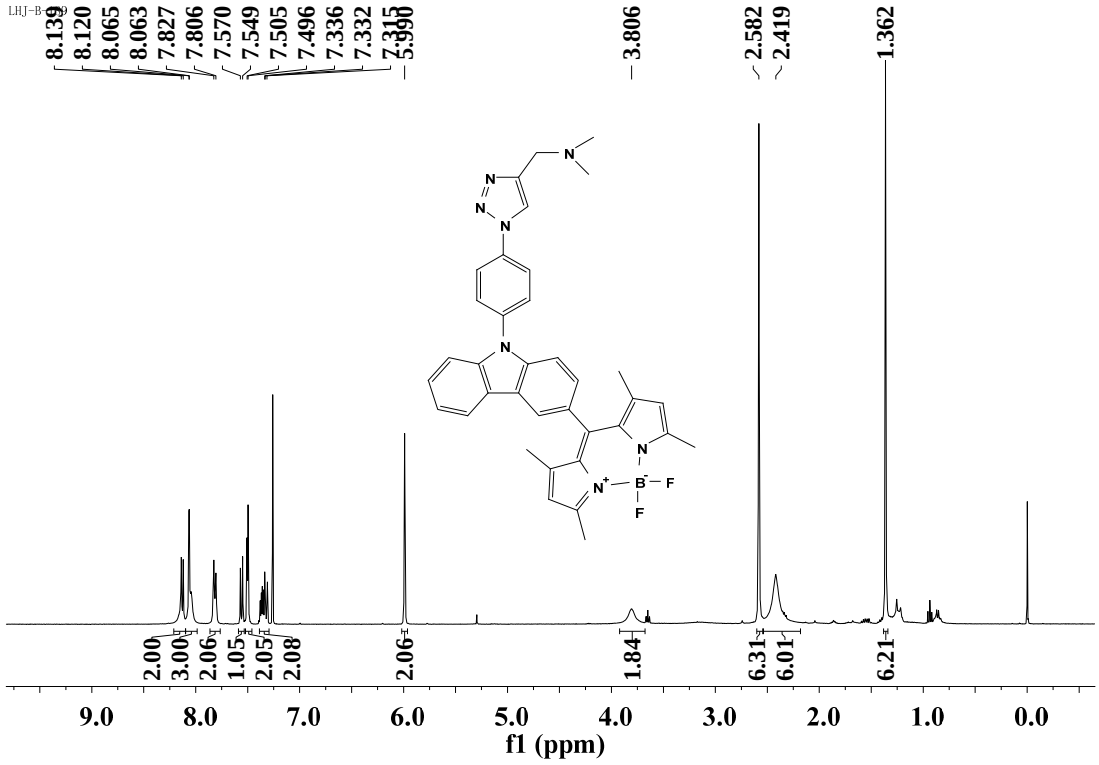
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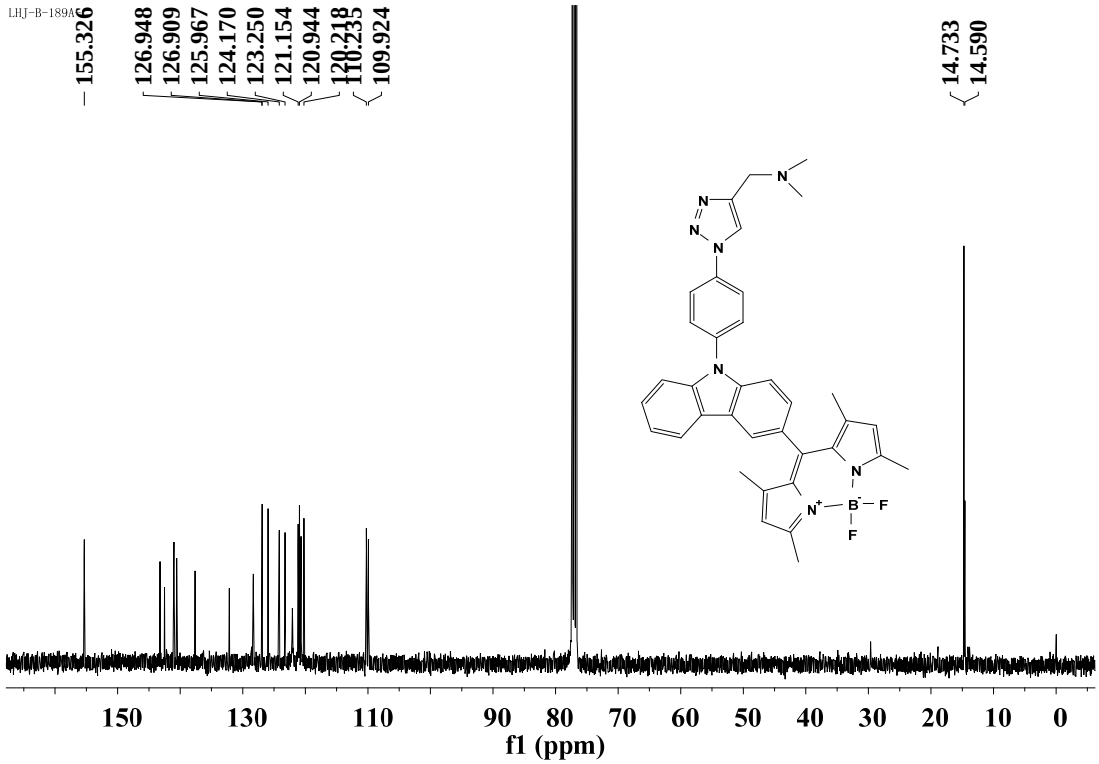
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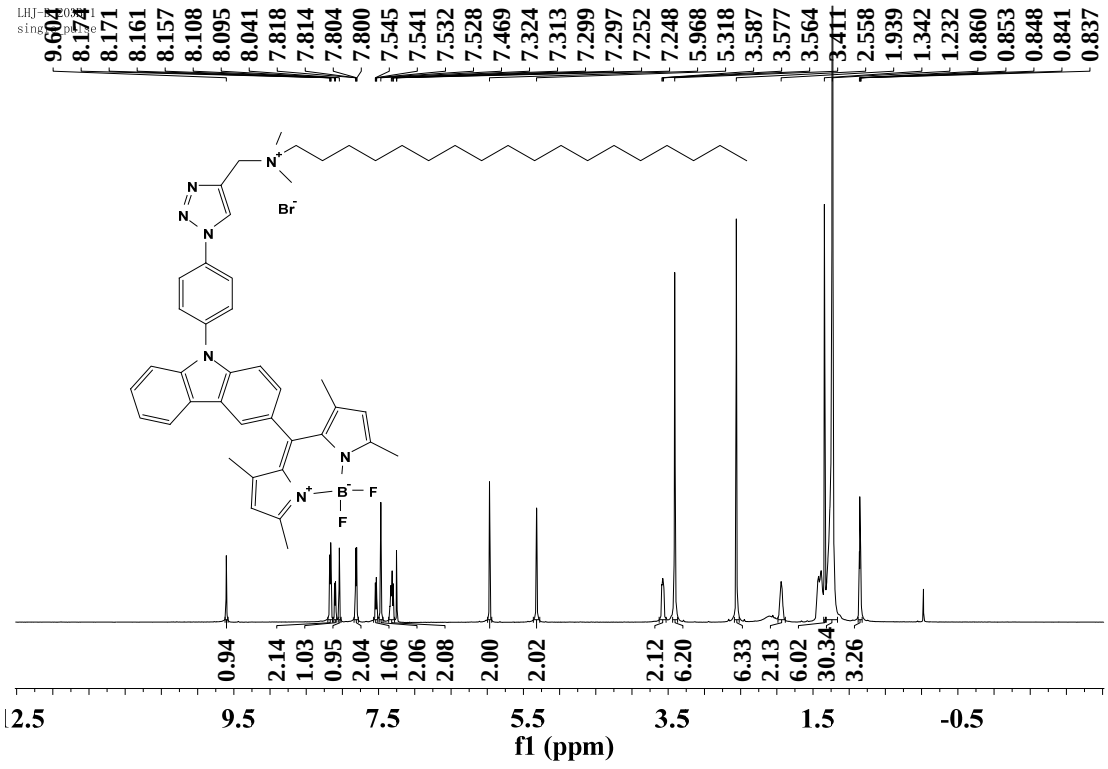
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3.587
3.577
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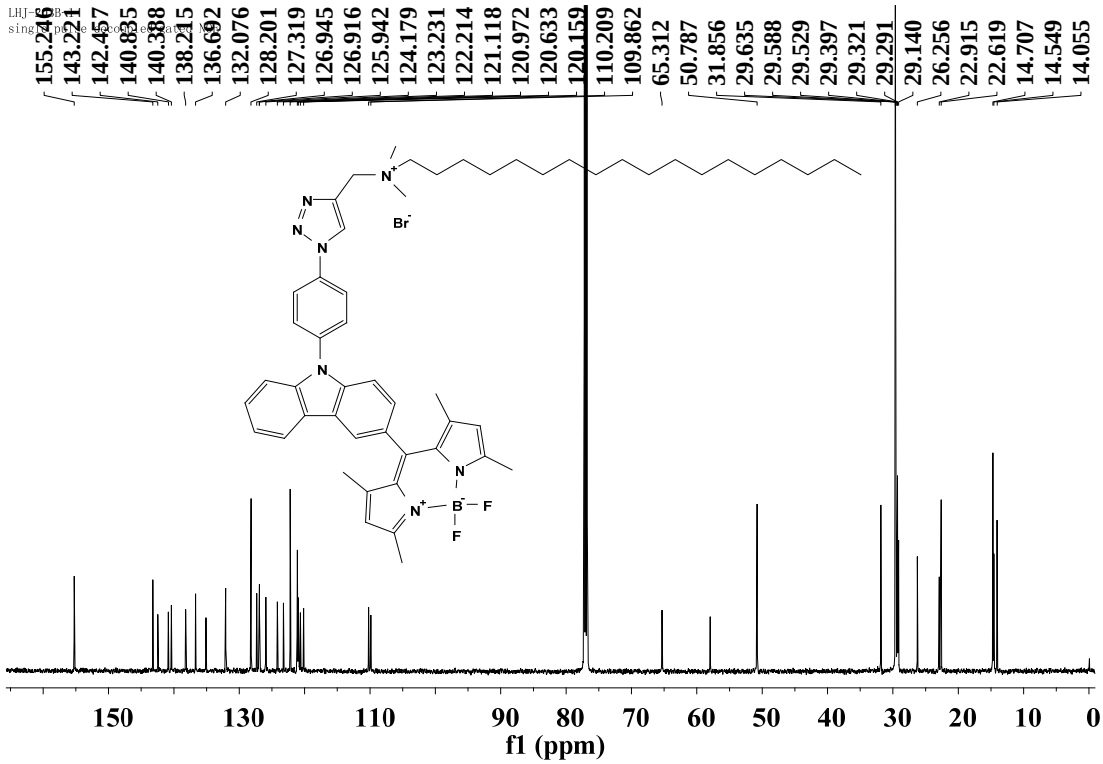
3.411
2.558
1.939
1.342
1.232
0.860
0.853
0.848
0.841
0.837
Br
0.94
2.14
1.03
0.95
2.04
1.06
2.06
2.08
2.00
2.02
2.12
6.20
6.33
2.13
6.02
30.34
3.26
12.5
9.5
7.5
5.5
3.5
1.5
-0.5
f1 (ppm)


155.246
143.221
142.457
140.855
140.388
138.215
136.692
132.076
128.201
127.319
126.945
126.916
125.942
124.179
123.231
122.214
121.118
120.972
120.633
120.159
110.209
109.862
65.312
50.787
31.856
29.635
29.588
29.529
29.397
29.321
29.291
29.140
26.256
22.915
22.619
14.707
14.549
14.055
Br
F
F
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)