Supporting Information

Tetrazines-Mediated Bioorthogonal Removal of 3-Isocyanopropyl Groups Enables the Controlled Release of Nitric Oxide *In Vivo*

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1. Supplementary figures and table

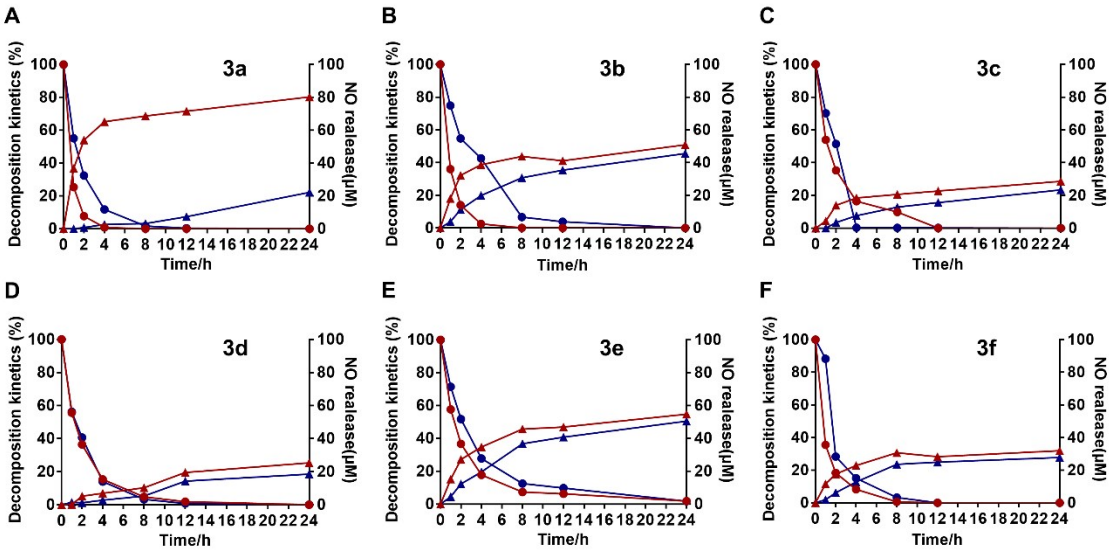


Fig. S1 Decomposition kinetics (●) and NO release (▲) from **3a** (A), **3b** (B), **3c** (C), **3d** (D), **3e** (E) or **3f** (F) triggered by BTZ (red) or STZ (blue).

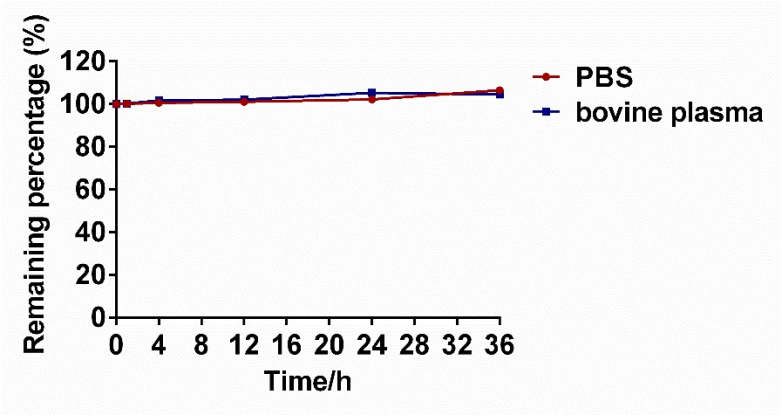


Fig. S2 Stability of **3a** in PBS (pH = 7.4) (red) and bovine plasma (blue).

Table S1. IC_{50} values of compounds m-TZ, BTZ, acrolein, **2a** and acrolein plus **2a** against HCT-116 cells [a]

Compounds	IC_{50} (μM)
BTZ	>50
m-TZ	>50
Acrolein	11.94 ± 1.03
2a	78.51 ± 4.11
Acrolein+**2a**	8.03 ± 0.64

[a]Data were expressed as the mean from five individual experiments.

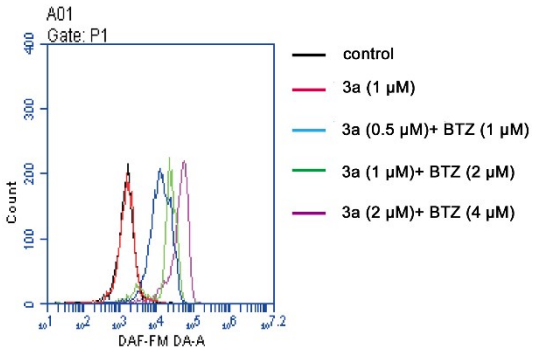
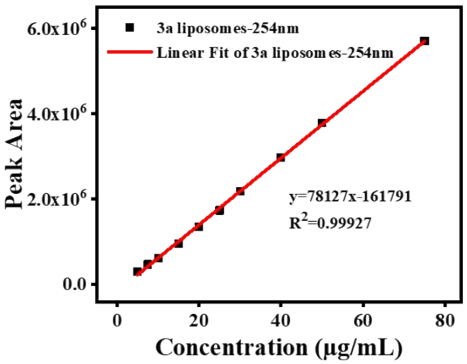
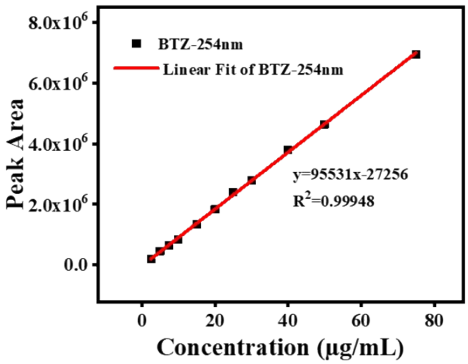


Fig. S3 NO released from **3a** in HCT-116 cells without or with the pretreatment of BTZ by using a NO probe (DAF-FM DA).



Drug loading of **3a** liposomes: 2.55%



Drug loading of BTZ liposomes: 1.38%

Fig. S4 The drug loading of **3a** liposomes and BTZ liposomes were measured by high performance liquid chromatography, respectively.

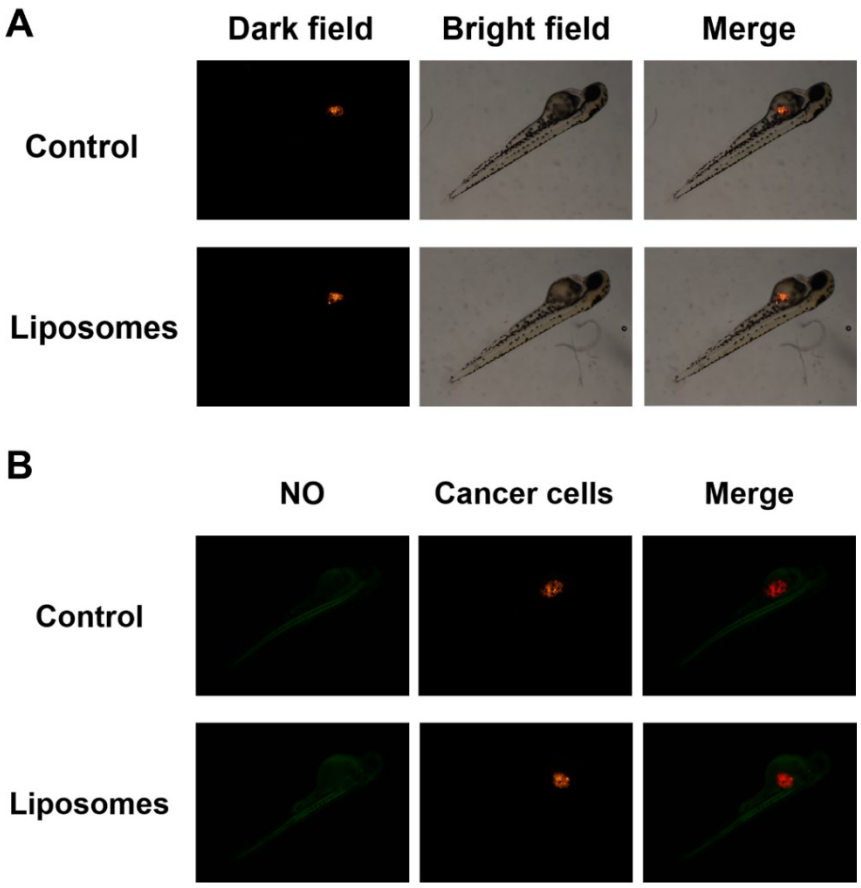
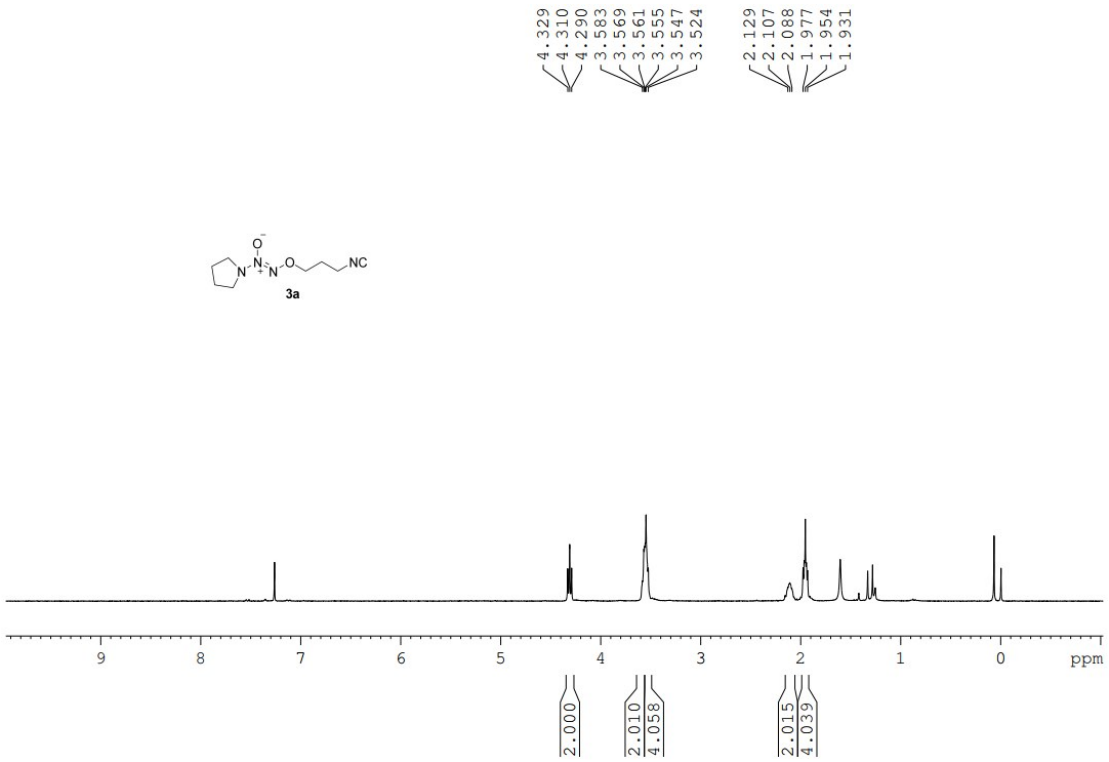


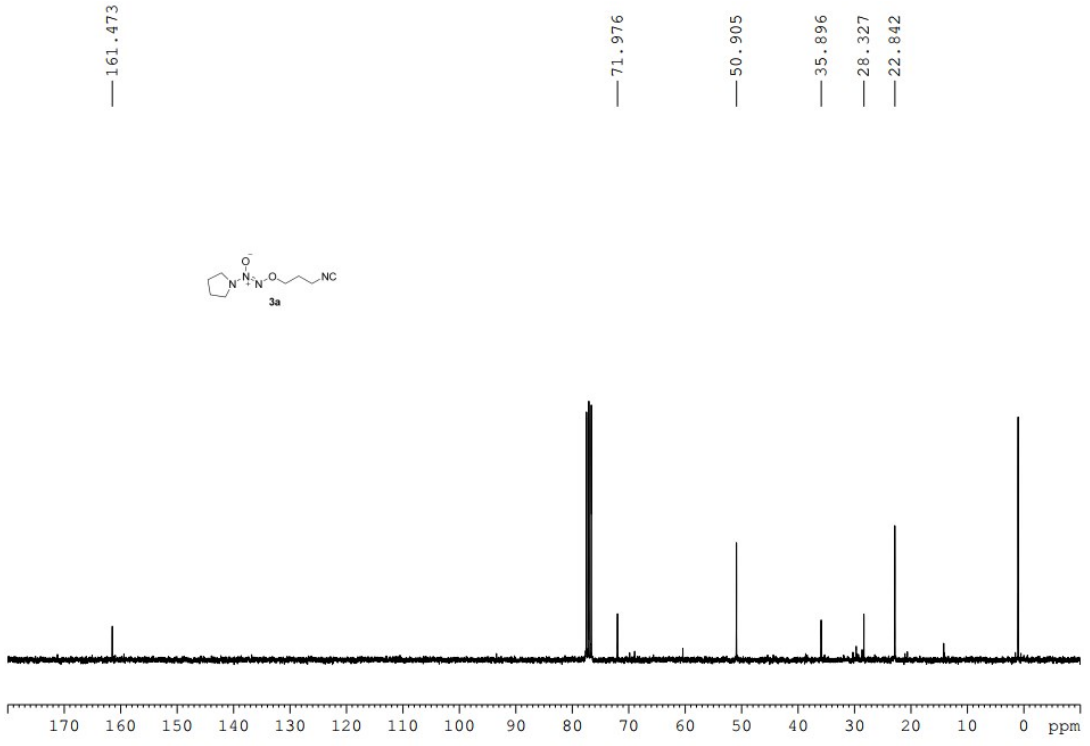
Fig. S5 In the assay to evaluate the anticancer activity of liposome in zebrafish implanted with CM-Dil-labeled HCT-116 cells (illustrated in Figure 6A), the representative confocal microscopy images of zebrafish for each group at 0 h before administration of the test compounds. B) In the dual-imaging assay (illustrated in Figure 6D), the representative confocal microscopy images of xenografts model zebrafish for each group at 0 h before administration of the test compounds.

2. NMR and HRMS Spectra for target compounds

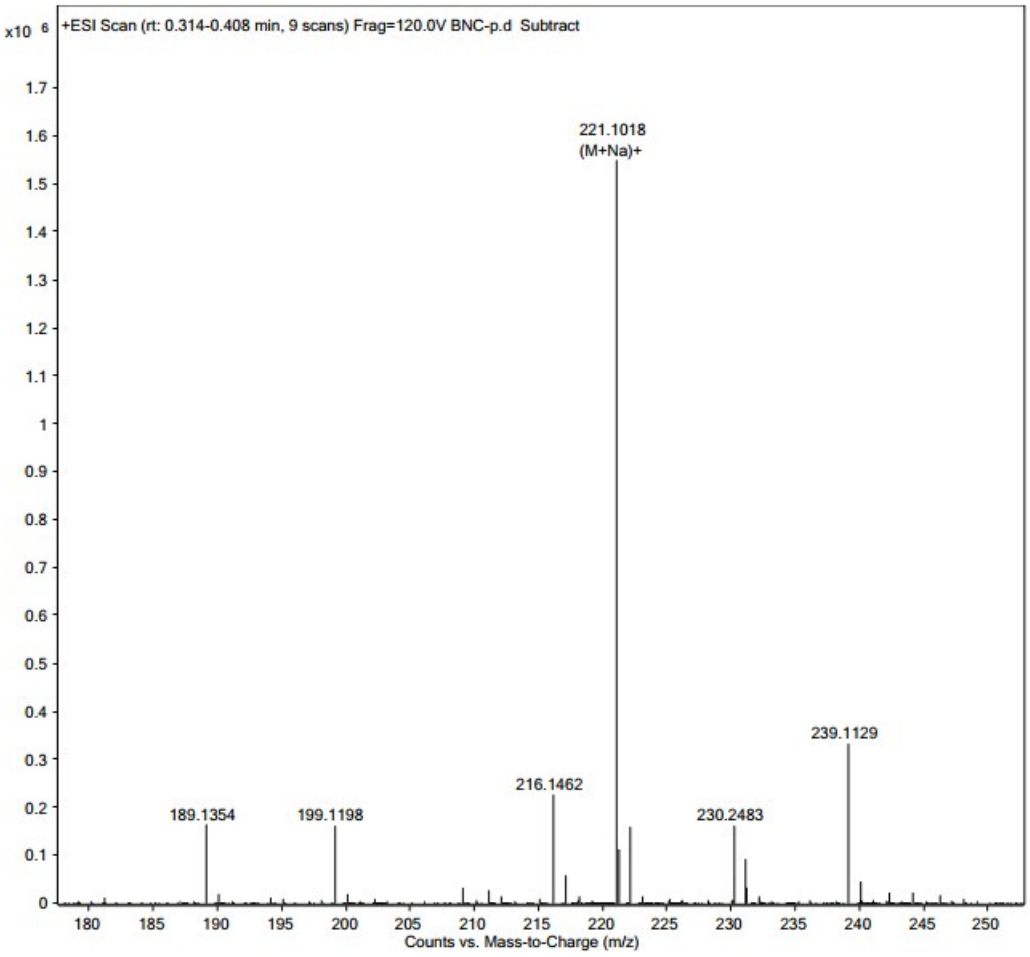
3a ^{1}H NMR $CDCl_3$ 303K AV-300



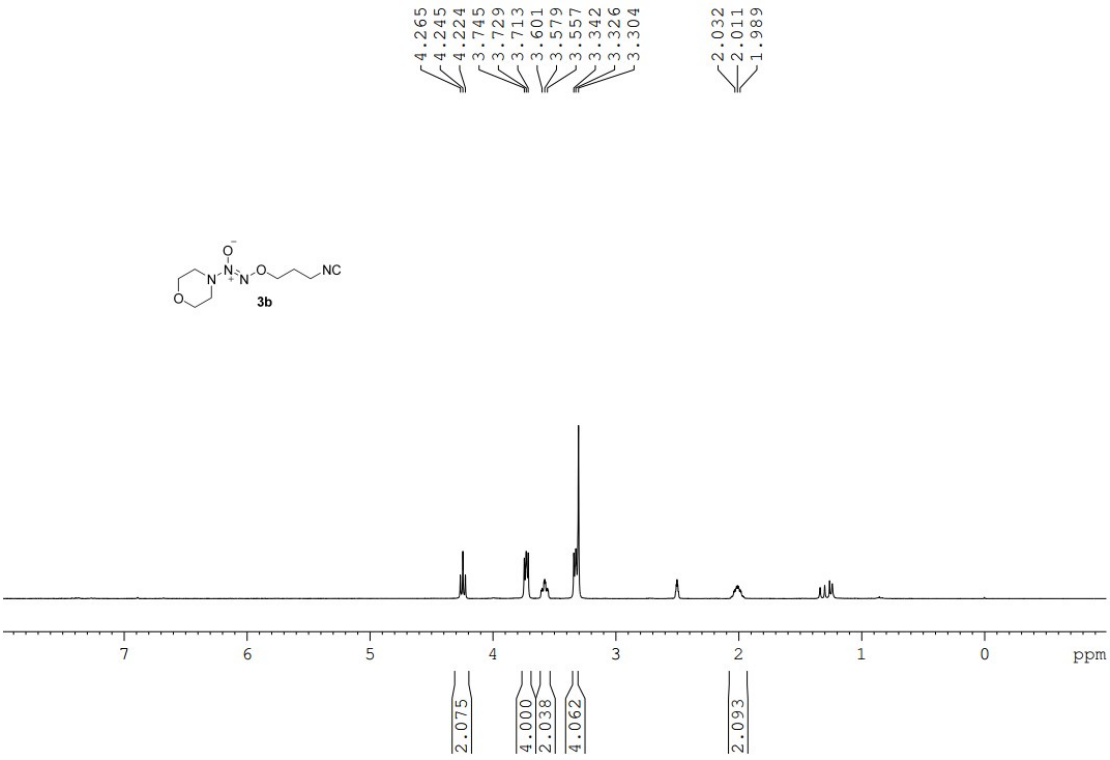
3a ^{13}C NMR $CDCl_3$ 303K AV-300



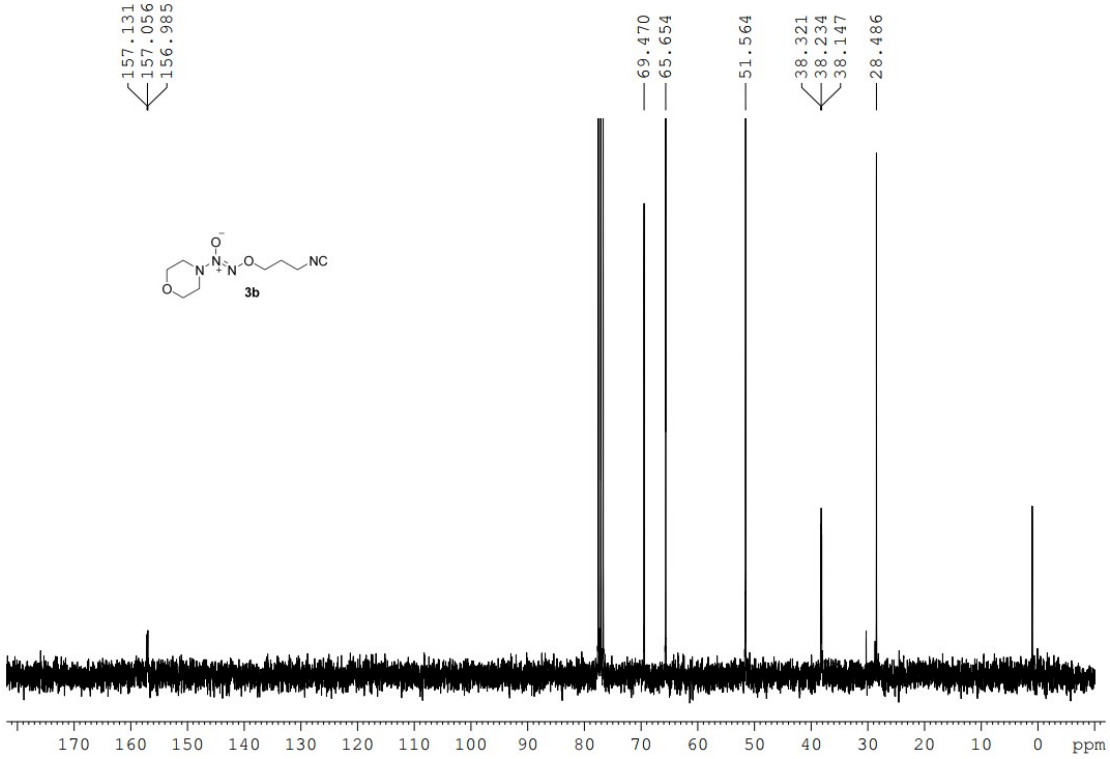
3a HRMS (ESI)


x10 6
+ESI Scan (rt: 0.314-0.408 min, 9 scans) Frag=120.0V BNC-p.d Subtract
221.1018
(M+Na)+
239.1129
216.1462
189.1354
199.1198
230.2483
Counts vs. Mass-to-Charge (m/z)

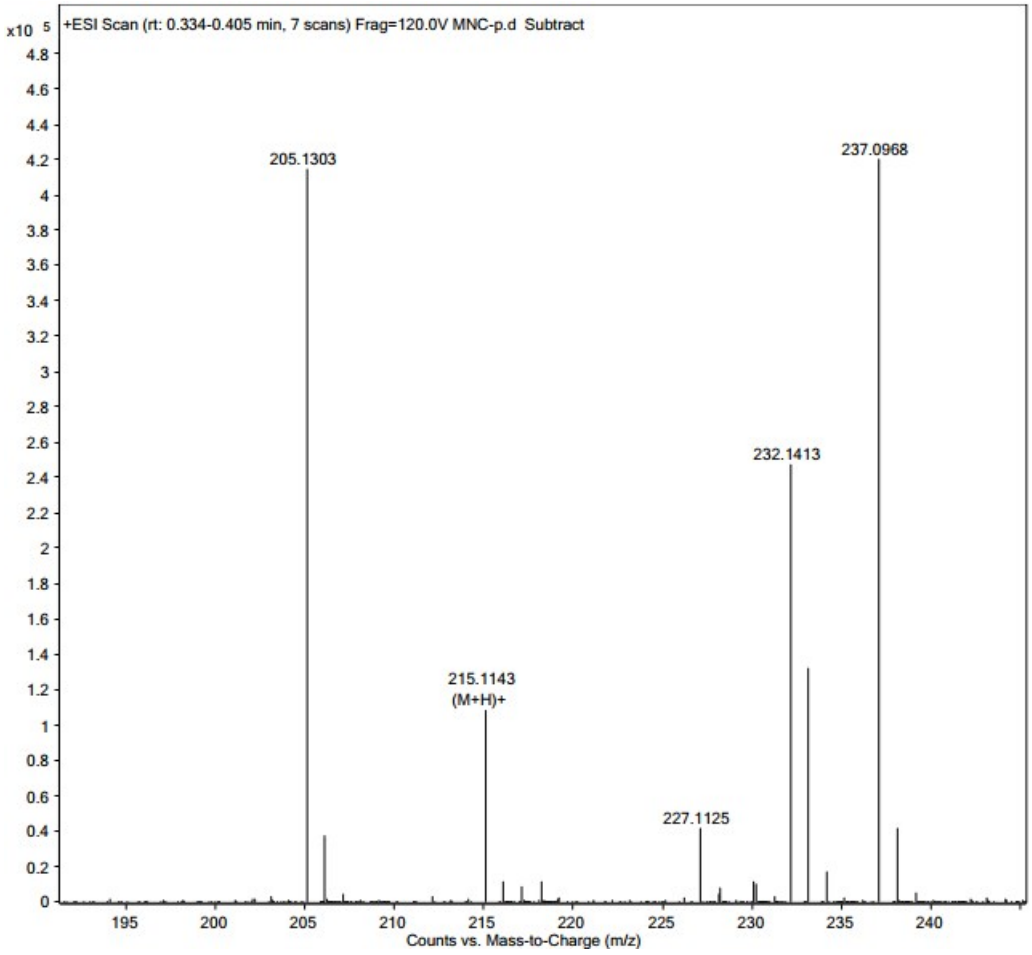
3b ^{1}H NMR DMSO-d_6 303K AV-300



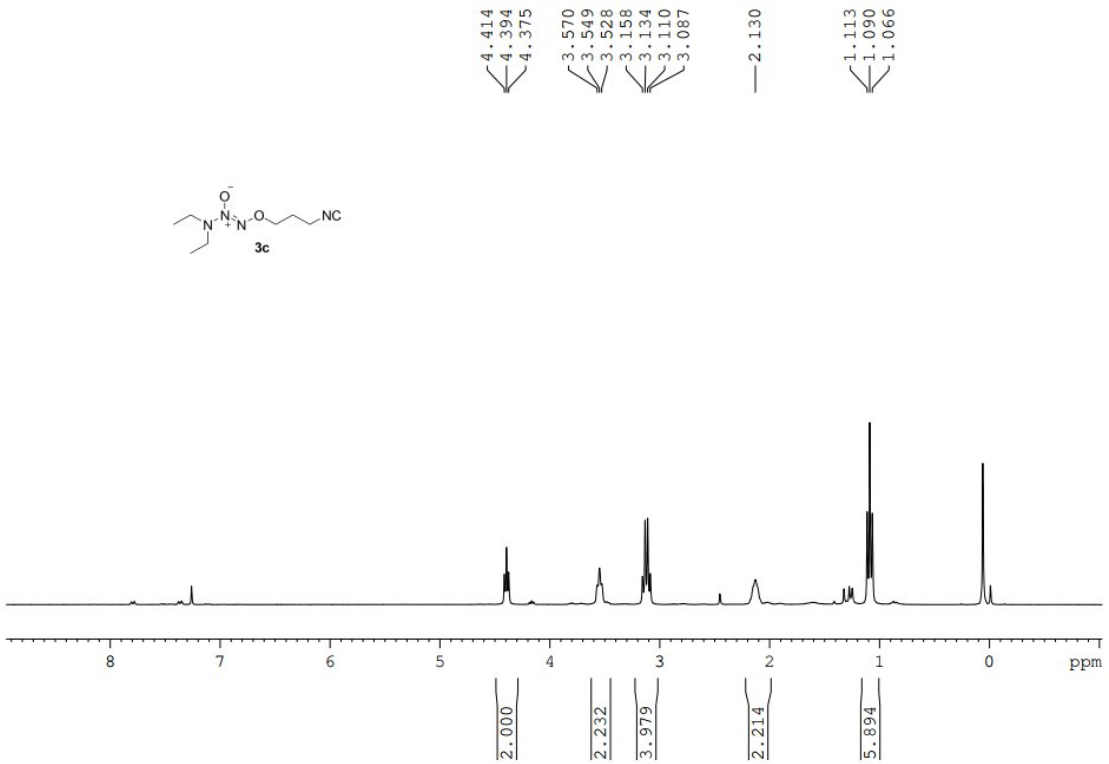
3b ^{13}C NMR $CDCl_3$ 303K AV-300



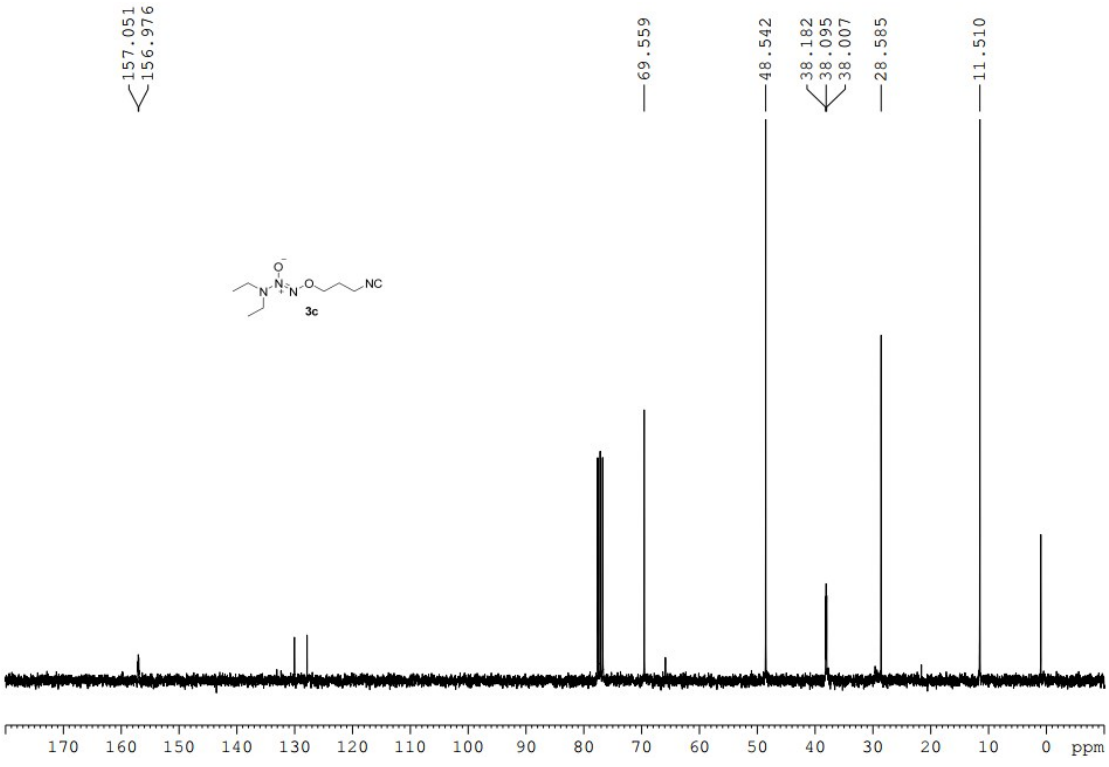
3b HRMS (ESI)


x10 5
+ESI Scan (rt: 0.334-0.405 min, 7 scans) Frag=120.0V MNC-p.d Subtract
205.1303
237.0968
232.1413
215.1143
(M+H)+
227.1125
Counts vs. Mass-to-Charge (m/z)

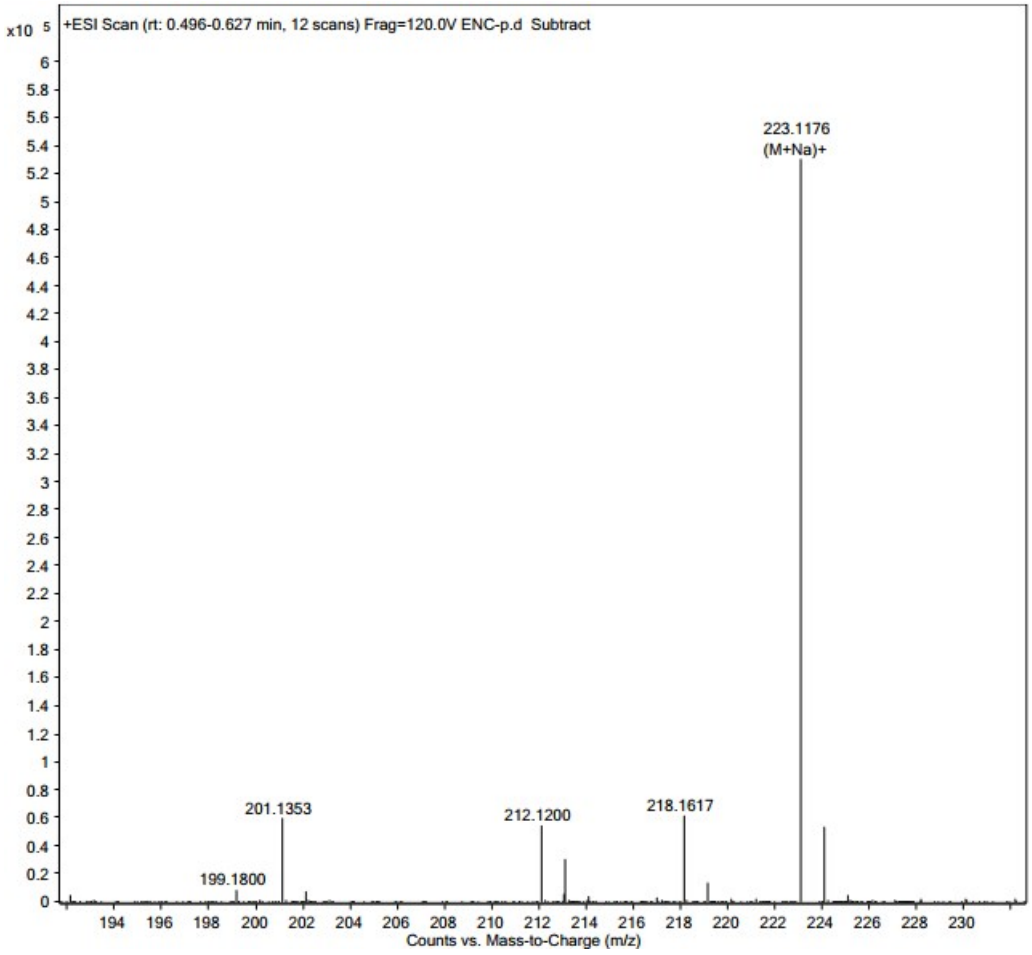
3c ^{1}H NMR $CDCl_3$ 303K AV-300



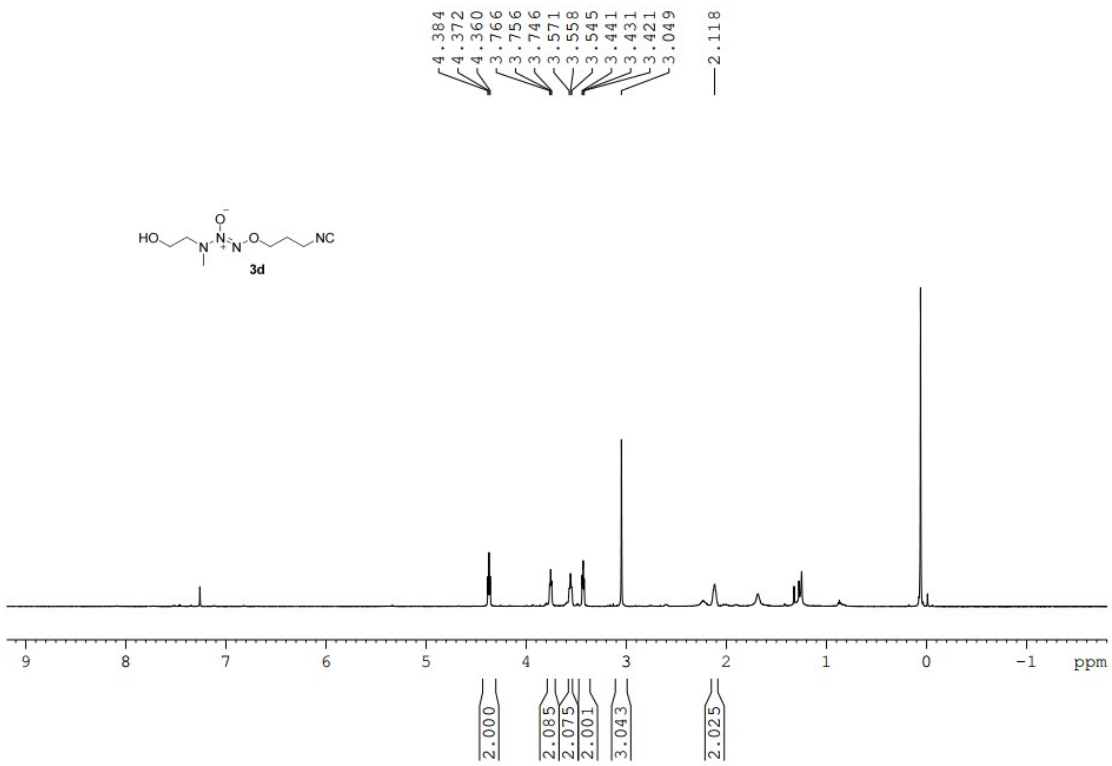
3c ^{13}C NMR $CDCl_3$ 303K AV-300



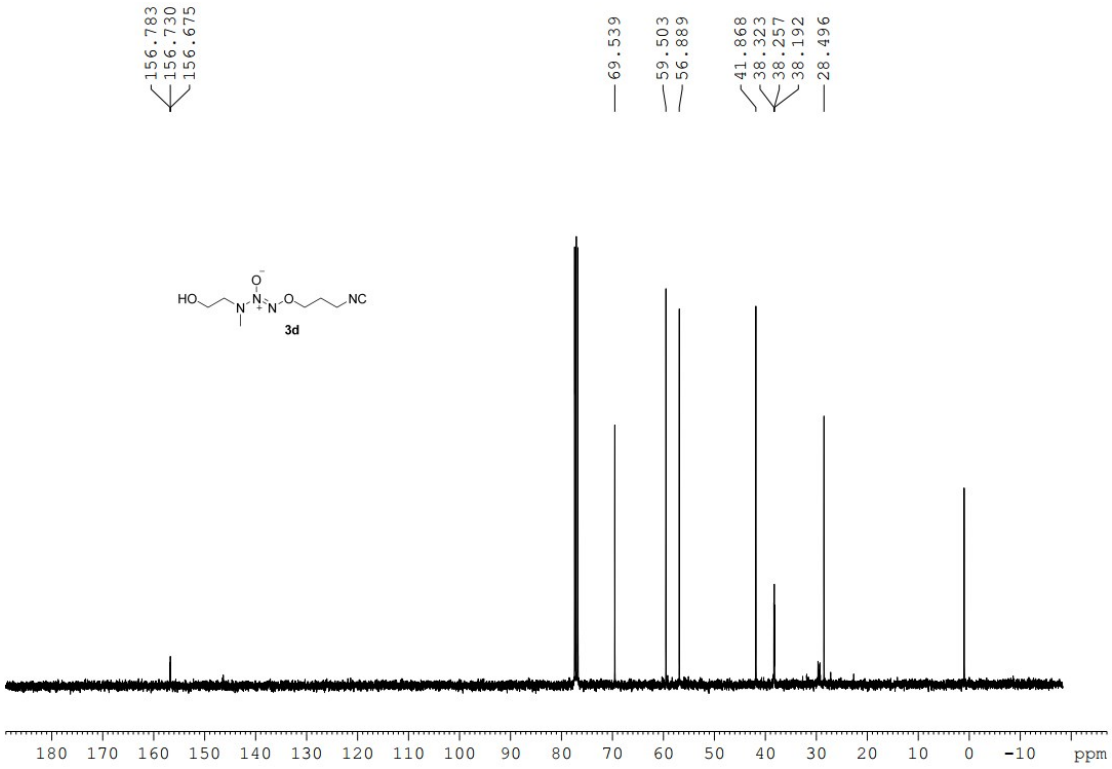
3c HRMS (ESI)


+ESI Scan (rt: 0.496-0.627 min, 12 scans) Frag=120.0V ENC-p.d Subtract
x10 5
223.1176
(M+Na)+
201.1353
212.1200
218.1617
199.1800
Counts vs. Mass-to-Charge (m/z)

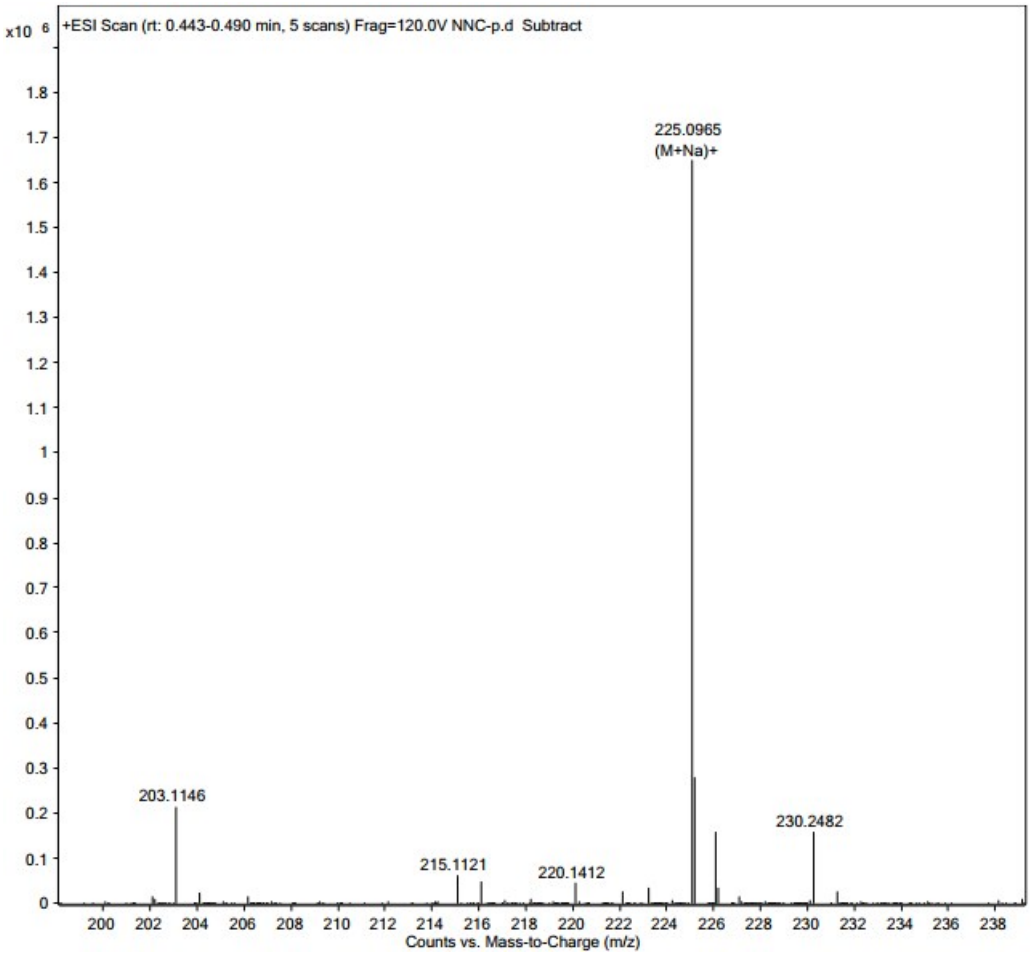
3d ^{1}H NMR $CDCl_3$ 303K AV-300



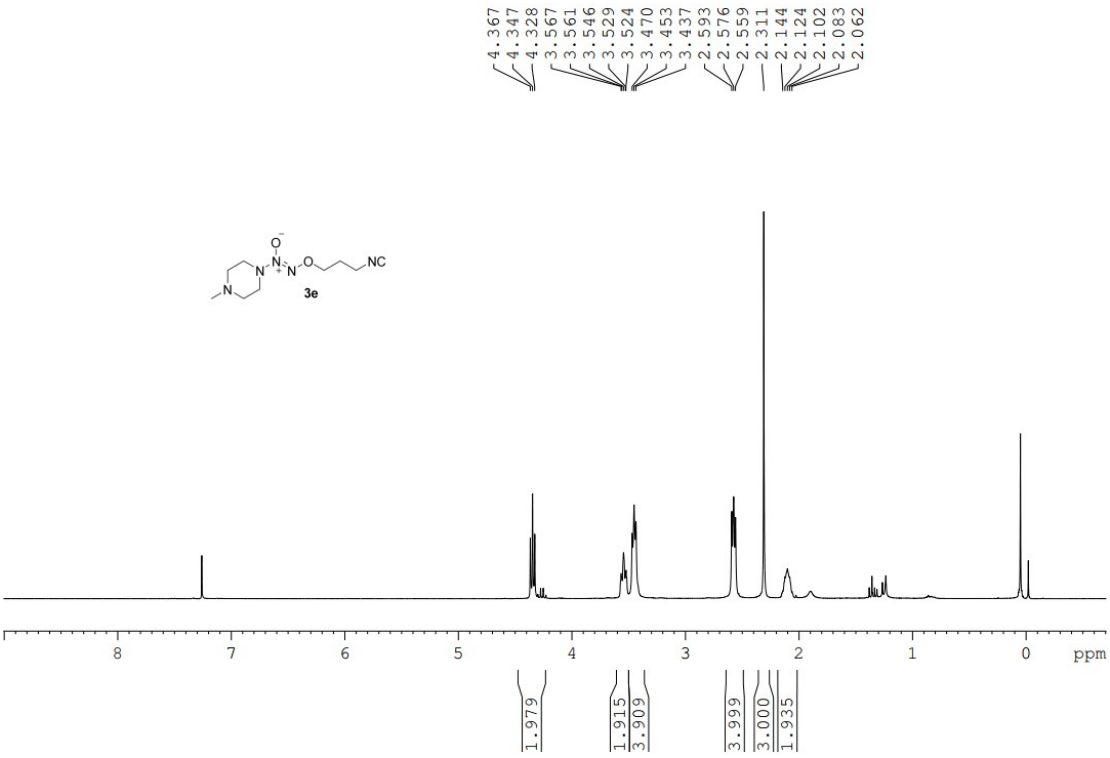
3d ^{13}C NMR $CDCl_3$ 303K AV-300



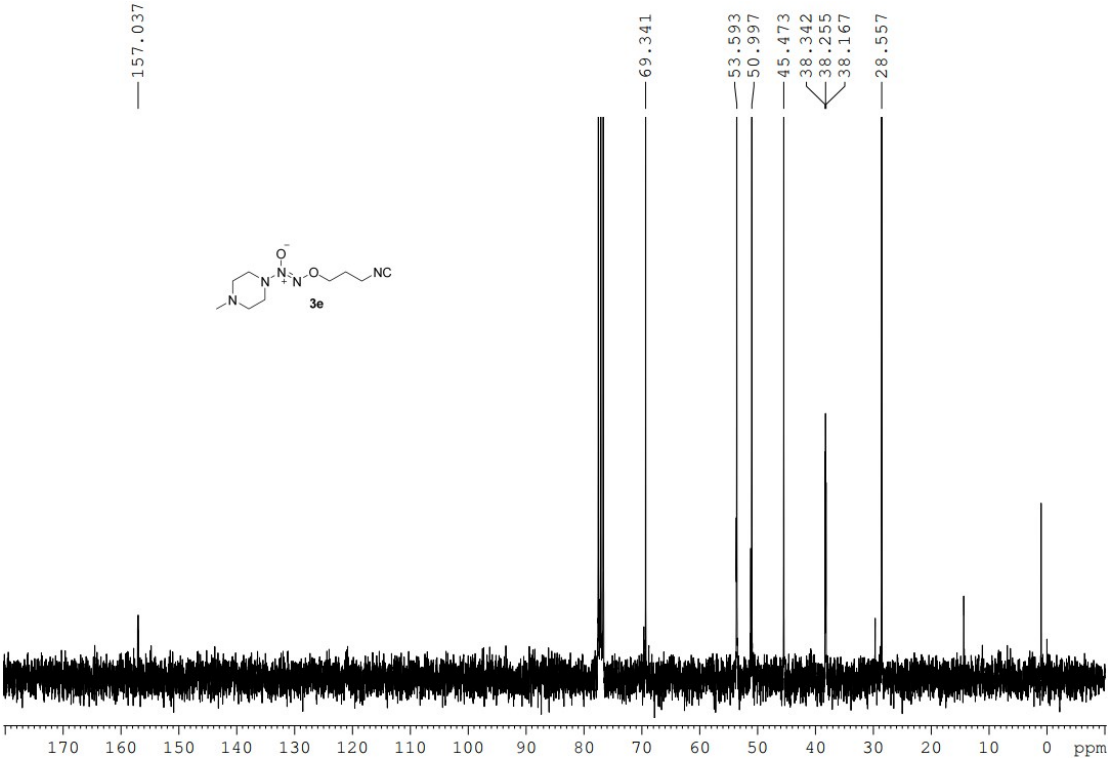
3d HRMS (ESI)


x10 6
+ESI Scan (rt: 0.443-0.490 min, 5 scans) Frag=120.0V NNC-p.d Subtract
225.0965
(M+Na)+
203.1146
215.1121
220.1412
230.2482
Counts vs. Mass-to-Charge (m/z)

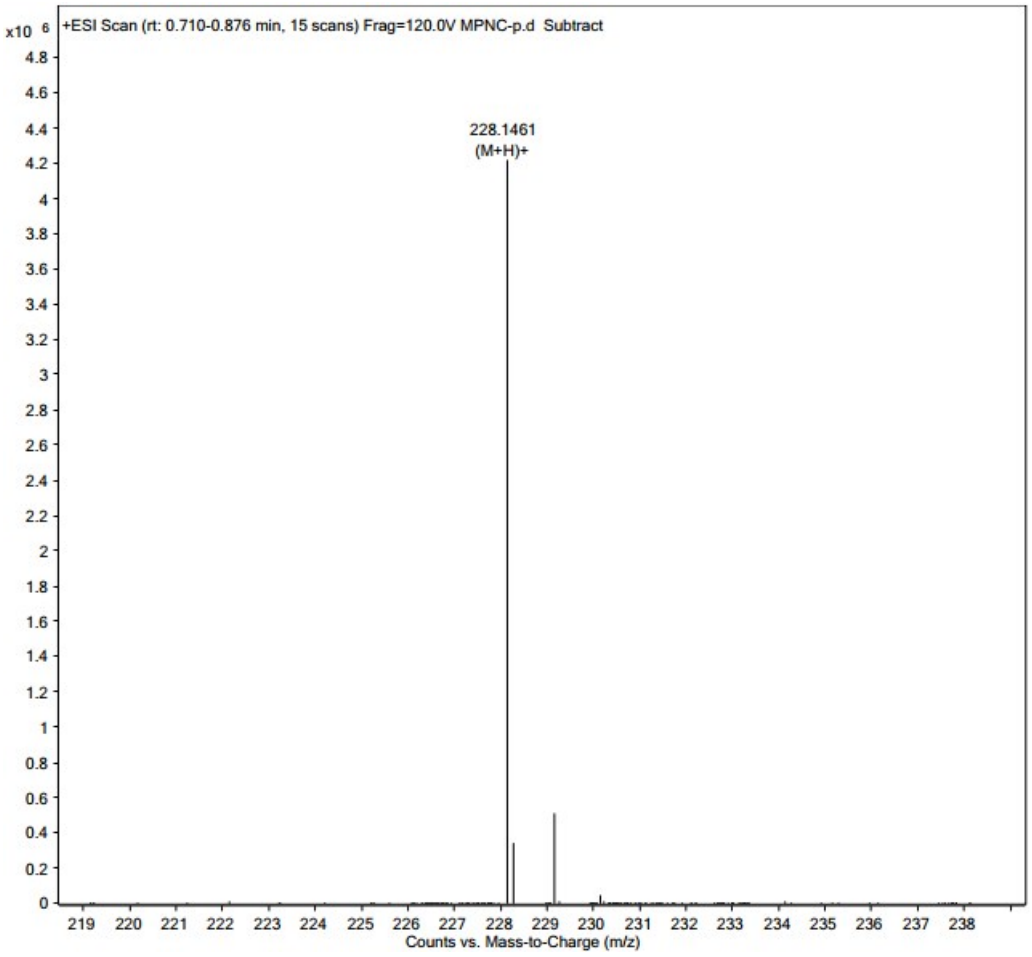
3e ^{1}H NMR $CDCl_3$ 303K AV-300



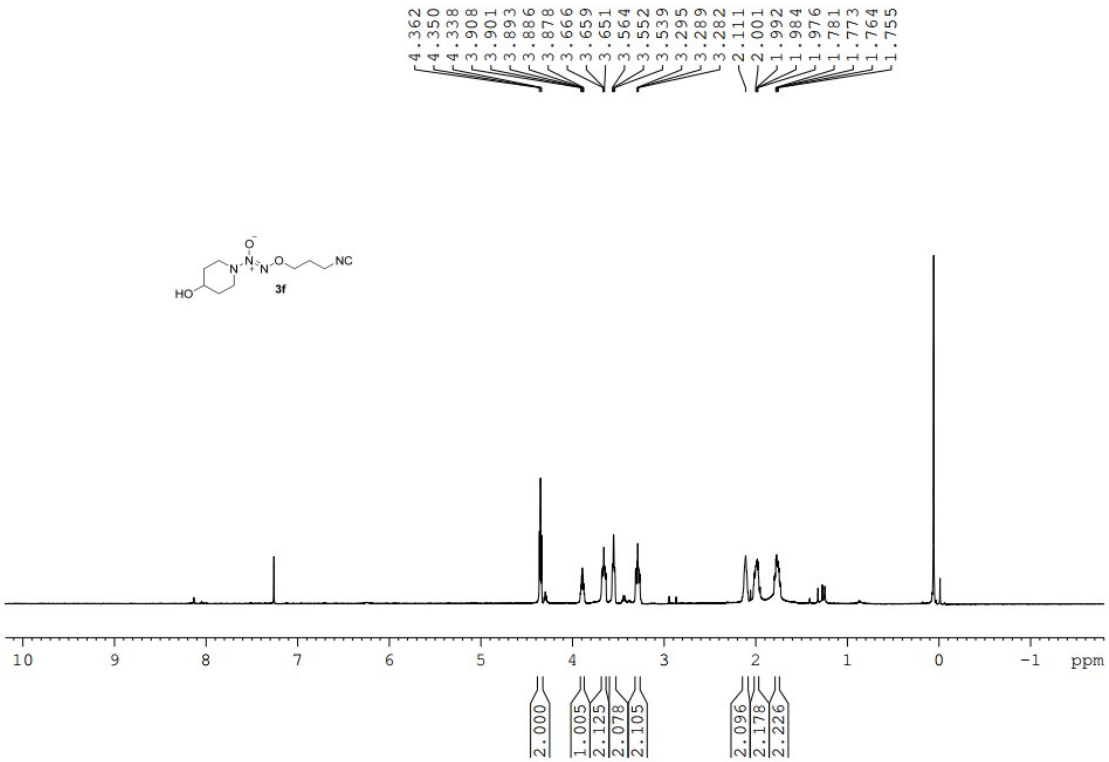
3e ^{13}C NMR $CDCl_3$ 303K AV-300



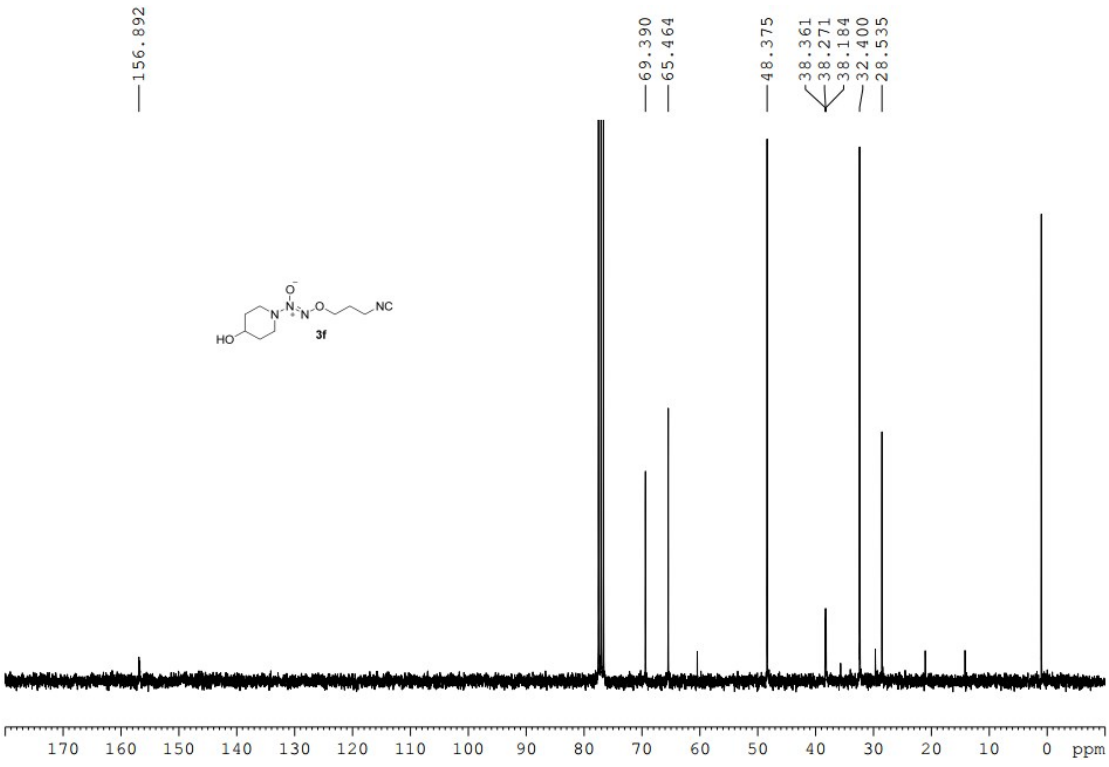
3e HRMS (ESI)


+ESI Scan (rt: 0.710-0.876 min, 15 scans) Frag=120.0V MPNC-p.d Subtract
x10 6
228.1461
(M+H)+
Counts vs. Mass-to-Charge (m/z)

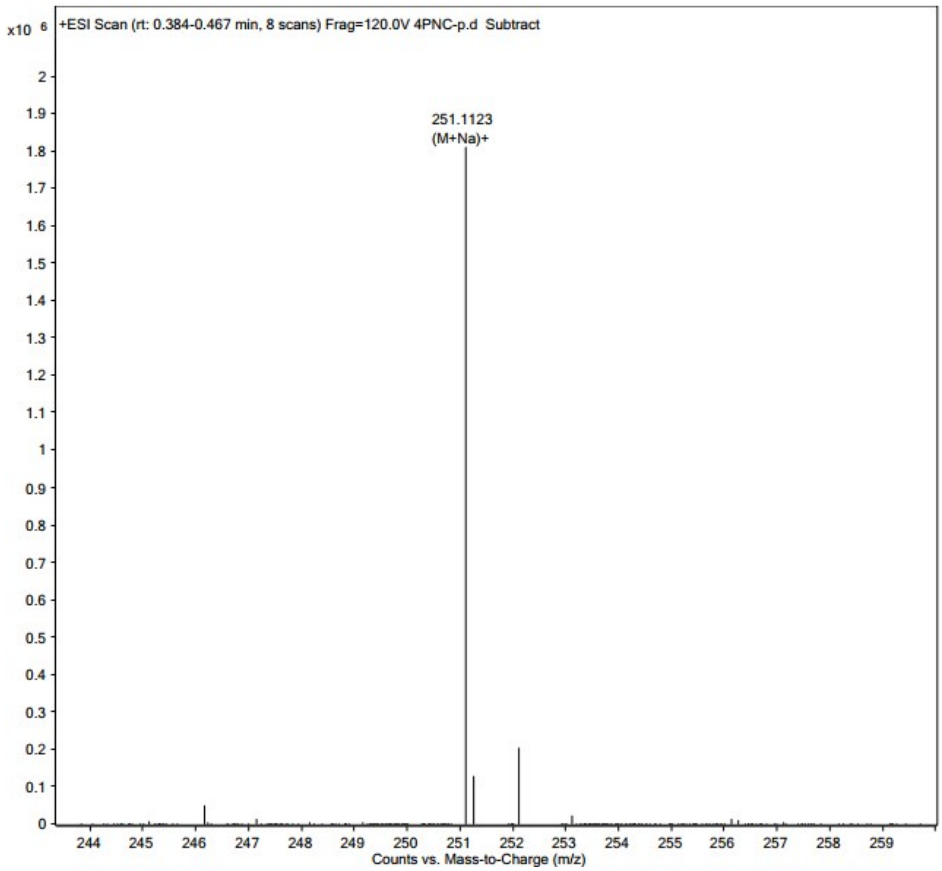
3f ^{1}H NMR $CDCl_3$ 303K AV-300



3f ^{13}C NMR $CDCl_3$ 303K AV-300



3f HRMS (ESI)


+ESI Scan (rt: 0.384-0.467 min, 8 scans) Frag=120.0V 4PNC-p.d Subtract
x10 6
251.1123
(M+Na)+
Counts vs. Mass-to-Charge (m/z)

3. HPLC assessment of compounds purity

Compounds **3a-f** with purities of >97% were used for further biological assays. We provided the spectra of HPLC assays as below.

Column: Innovai ODS2 (4.6 × 150 mm, 5 μm, 100 Å);

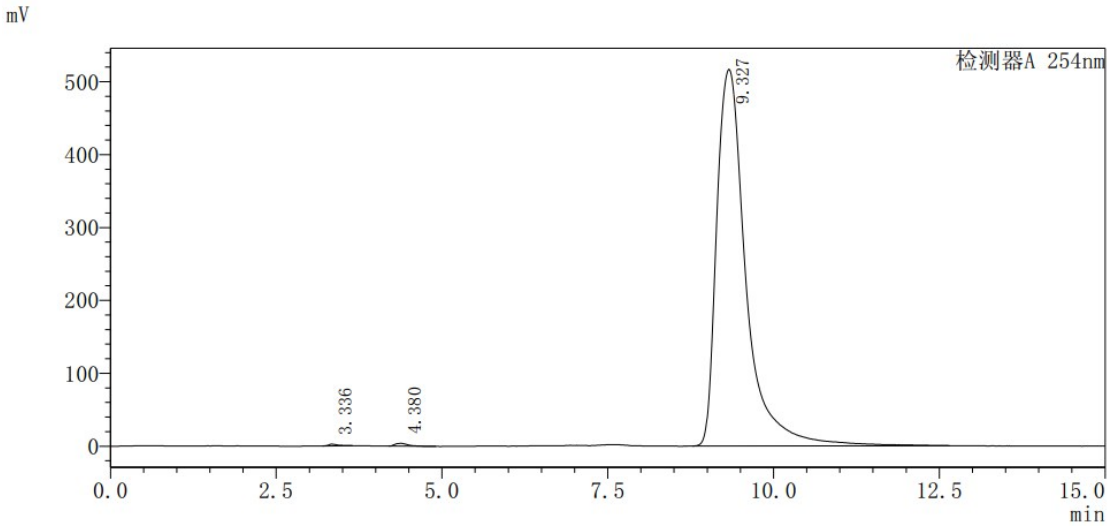
Mobile phase: Methanol-Water (70: 30 to 40: 60, v/v);

Detection Wavelength: 254 nm;

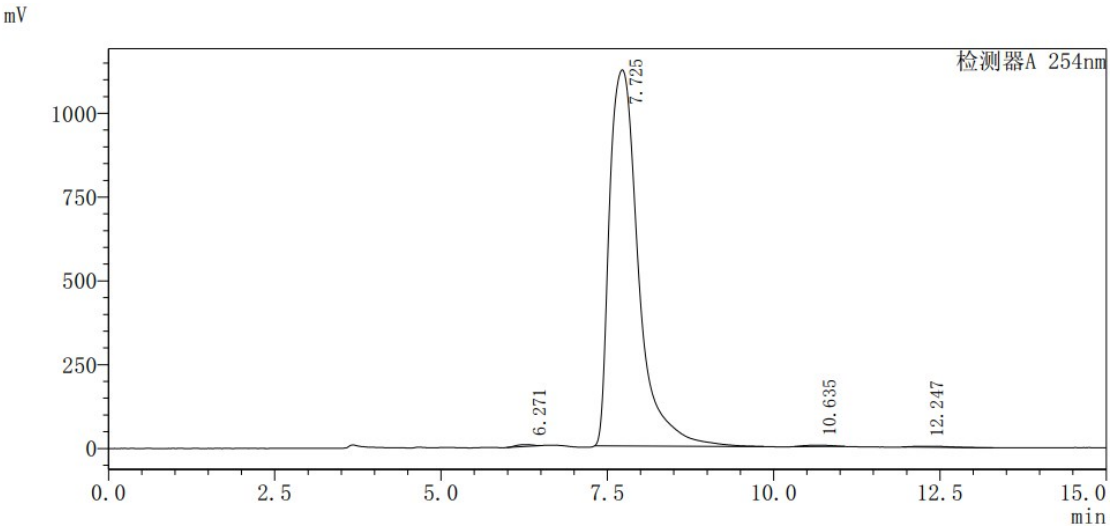
Rate: 0.5 ~ 1.0 mL/min;

Temperature: 25 °C;

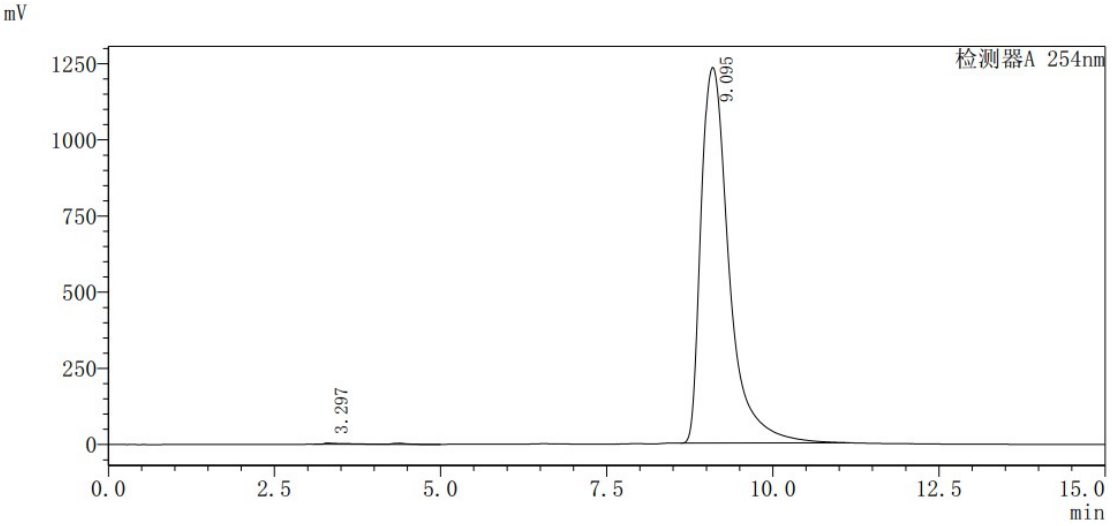
3a, purity 99.53%



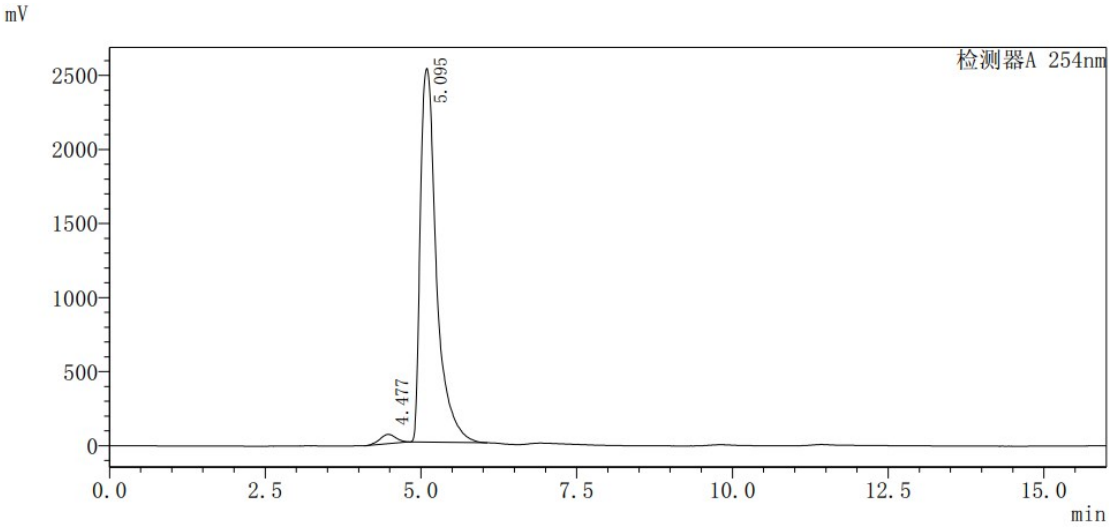
3b, purity 99.08%



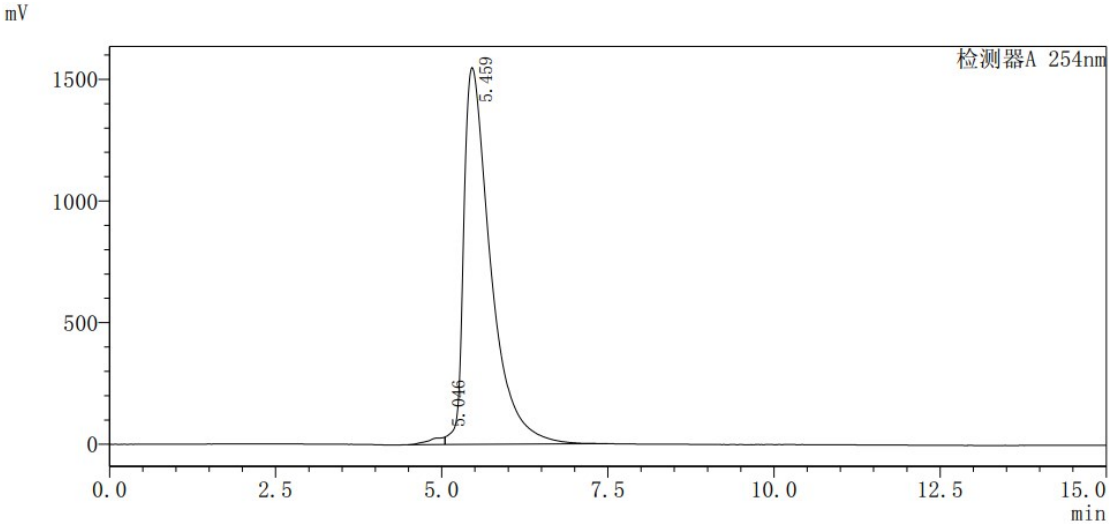
3c, purity 99.57%



3d, purity 97.54%



3e, purity 98.88%



3f, purity 98.53%