# Developing potent LC3-targeting AUTACs tools for protein degradation using selective autophagy

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# Electronic Supplementary Information

## Contents

## Materials and Methods

### Cell culture and reagents

Cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The HL60 and BT549 cell lines were cultured in Roswell Park Memorial Institute medium (RPMI) 1640 medium and the A549, Hela, MCF-7, MDA-MB-231 and MDA-MB-468 were maintained in Dulbeccos Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin streptomycin (Life Technologies) in 5% $CO_2$ at 37 $^oC$. Cells were grown to 70-80% confluence in cell culture dishes or plates and all the experiments were performed on logarithmically growing cells.

Rapamycin (V900930), 3-MA (M9281), CQ (C6628), JQ1 (SML1524), DAPI (D9542) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bafilomycin A1 (ab120497) and was purchased from Abcam (Cambridge, UK). Antibodies used in this study were as follow: LC3B (51520, Abcam), GAPDH (5174, CST), c-MYC (56, Abcam), PARP (9542, CST), BRD4 (13440, CST), BRD4 (128874, Abcam), LC3B (83506, CST), Caspase3 (9662, CST), LAMP1(15665, CST).

### Protein expression and purification

Human LC3B was cloned into the pET-28a vectors with a N-terminal His-tag followed by tobacco etch virus (TEV). All proteins were expressed in E.coli BL21(DE3) at 16 ℃ overnight and induced by 0.3 mM IPTG at an $OD_{600}$ of 0.6. Bacteria were harvested and lysed in lysis buffer consisting of 200 mM NaCl, 20 mM Tris, pH7.5 and 5 mM imidazole by high pressure cell cracker. The bacteria lysate was centrifuged at 12000 rpm/min for 30 minutes at 4 ℃, and the supernatant was transferred to the Ni-NTA Column. The LC3B protein was eluted by elution buffer consisting of 0.2 M NaCl, 20 mM Tris, pH 7.5 and 500 mM imidazole. Then the LC3B protein was further purified by SuperdexTM 75 Increase 10/300 GL size-exclusion chromatography (GE Healthcare). Finally, the purified proteins were concentrated to approximately 9 mg ml−1 in 20 mM HEPES buffer (pH = 7.5) with 100 mM NaCl for further analysis.

### Cell viability assay

The cells were plated in 96-well plates at a density of $1.5 \times 10^4$ cells/mL. After

incubation at 37 °C for 24 h, cells were treated with different concentrations of AUTACs for 96 h. Cell viability was measured by MTT assay.

**GFP/mRFP - LC3 transfection**

Cells were seeded into 24-well culture plates ($2.5 \times 10^4$ cells/well). After incubation of 24 h, cells were transfected with GFP/mRFP-LC3 (HB-AP2100001, HANBIO, China) for 6 h. Then the transfected cells were used for subsequent experiments 36 h later and were analyzed under a confocal laser scanning microscopy (Leica) of Targeted Tracer Research and Development Laboratory of West China Hospital.

**Immunofluorescence (IF) analysis**

Cells were seeded onto the glass cover slips in 24-well plates. After treatment, cells were fixed with 4% paraformaldehyde in PBS for 30 min. The slides were then washed three times with PBS and incubated with 0.2% Triton X-100 (Sigma-Aldrich, 9002-93-1) and 5% goat serum (Sigma-Aldrich, G9023) for 30 min. Cells were incubated with indicated primary antibody overnight at 4°C and subsequently incubated with secondary antibody (Cy3, A0516; Alexa Fluor 488, A0428) at room temperature for 1 h. Nuclei were finally stained with DAPI for 5 min. Images were captured using a confocal laser scanning microscopy (Leica) of Targeted Tracer Research and Development Laboratory of West China Hospital.

**Immunoblotting (IB) analysis**

All cells and animal tumors as well as lung tissues were collected and lysed by lysis buffer at 4 °C for 30 min. After 12000 rpm centrifugation for 10 min, the protein level of the supernatant was quantified by Bio-Rad DC protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of the total protein were separated by 12% or 10% SDS-PAGE and electrophoretically transferred to PVDF membranes. Subsequently, membranes were blocked with 5% nonfat dried milk. Proteins were detected using primary antibodies, followed by HRP-conjugated secondary antibodies, and visualized by employing ECL as the HRP substrate. Quantifications of immunoblot were performed by Image lab.

**Surface plasmon resonance (SPR)**

LC3B was immobilized to 5000-7000 response units on flow cells 1, 2, 3 and 4 of

Biacore series S CM5 sensor chip on a Biacore 8K instrument after predilution to HBS buffer containing 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% w/v Surfactant P20, pH7.4. After changing to assay buffer (137 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, 0.05% w/v Surfactant P20, pH (7.2~7.4), the sensor chip was predilution before used for KD measurements. The flow rate for assay was 10 μL /min, and the contact time and dissociation time were 60s and 70s, respectively. Data reported in this study are means of at least two independent experiments ± standard deviations.

**Colony formation assay**

The proliferation potential of cells was assessed by plating 500 cells in 6-well plates and treated with the indicated concentration of **10f** or vehicle control. After 2 weeks, cells were fixed with methanol and stained with crystal violet. The number of colonies was counted. Data represent the mean ± SD from 3 independent experiments performed in triplicate wells.

**Flow cytometry assay.** According to the manufacturer's instructions, the cell cycle and apoptosis analysis kit (Beyotime Biotechnology, Jiangsu, China) and Annexin-V-FLUOS staining kit (Roche, Germany) were used to evaluate the cell cycle process and the apoptotic ratio after compound **10f** treatment, respectively. Then, cell-cycle distribution and cell apoptosis were measured on a flow cytometer (LSRFortessa) of Core Facility of West China Hospital. Finally, statistical analysis was quantified using Flowjo 6.0 software.

**Statistical analysis**

All the presented data and results were confirmed by at least three independent experiments. The data were expressed as means ± SEM and analyzed with GraphPad Prism 7.0 software. Statistical differences between two groups were determined using Student's t test, while between multiple groups were determined using one-way analysis of variance. $P < 0.05$ was considered statistically significant.

**Hoechst 33258 stain**

The cells ($5 \times 10^4$ per well) were treated with compound **10f** for 24 h. After washing twice with cold PBS, the cells were stained with Hoechst 33258 for 30 min in the dark

at 37 ºC. Then observe the morphological changes of the cells under a fluorescence microscope (Nikon Ti-2U) of Core Facility of West China Hospital.

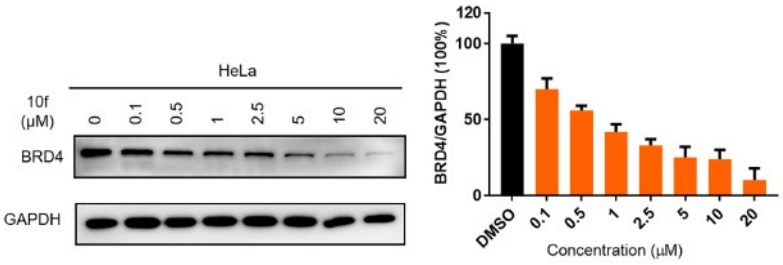



**Fig. S1** Immunoblotting analysis of BRD4 in HeLa cells treated with the indicated concentrations of AUTAC **10f** for 24 h. GAPDH was used as a loading control. Quantifications of immunoblotting analysis were shown. Data are expressed as mean ± SEM. All data were representative of at least three independent experiments.

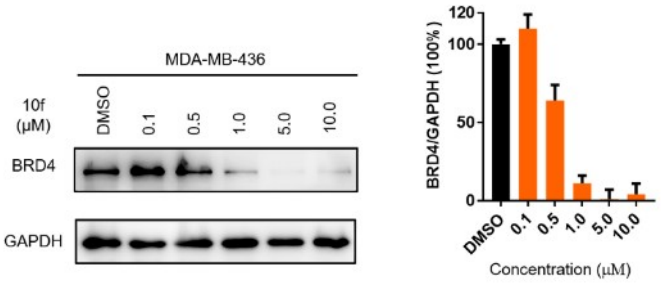



**Fig. S2** Immunoblotting analysis of BRD4 in MDA-MB-436 cells treated with the indicated concentrations of AUTAC **10f** for 24 h. GAPDH was used as a loading control. Quantifications of immunoblotting analysis were shown. Data are expressed as mean ± SEM. All data were representative of at least three independent experiments.

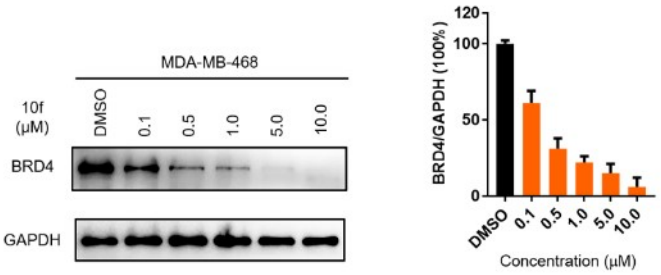



**Fig. S3** Immunoblotting analysis of BRD4 in MDA-MB-468 cells treated with the

indicated concentrations of AUTAC **10f** for 24 h. GAPDH was used as a loading control. Quantifications of immunoblotting analysis were shown. Data are expressed as mean ± SEM. All data were representative of at least three independent experiments.

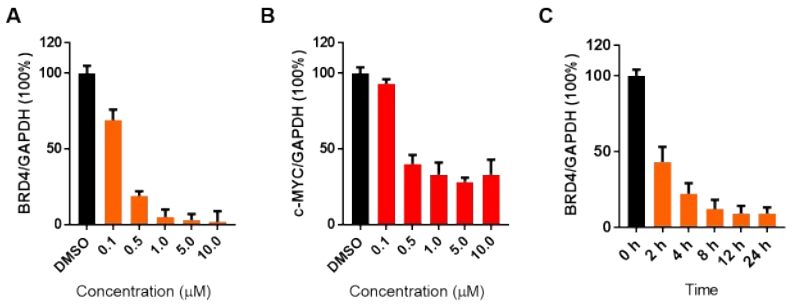



**Fig. S4** (A-B) Immunoblotting quantitative analysis of BRD4, c-MYC in MDA-MB-468 cells treated with the indicated concentrations of AUTAC **10f** for 24 h. (C) Immunoblot quantitative analysis of BRD4 after treatment of MDA-MB-231 cells with 0.5 μM AUTAC **10f** for the indicated exposures. Data are expressed as mean ± SEM. All data were representative of at least three independent experiments.

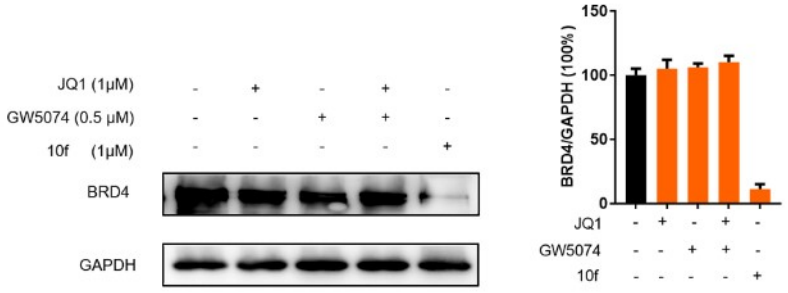



**Fig. S5** Immunoblotting analysis of BRD4 in MDA-MB-231 cells treated with the indicated concentrations of AUTAC **10f**, JQ1, GW5074 for 4 h. GAPDH was used as a loading control. Quantifications of immunoblotting analysis were shown. Data are expressed as mean ± SEM. All data were representative of at least three independent experiments.

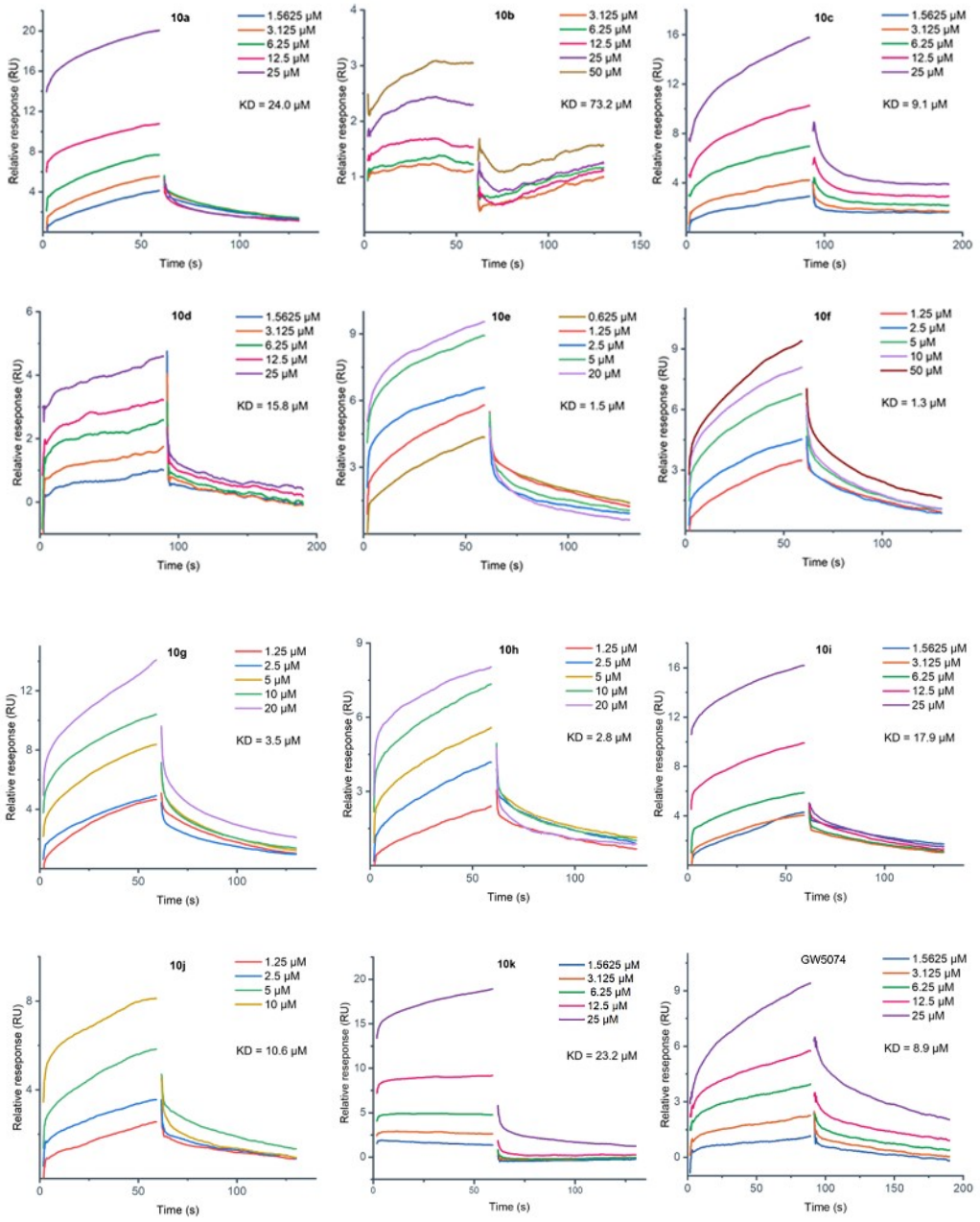



**Fig. S6** SPR analysis of **10a-10k** and GW5074

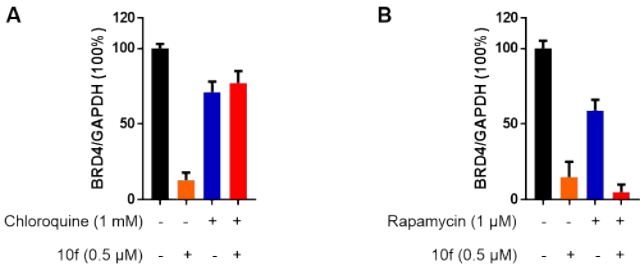



**Fig. S7** (A) Immunoblot quantitative analysis of BRD4 after a 2-hour pretreatment with

DMSO, Chloroquine (1 mM) followed by a 4-hour **10f** (0.5 μM) treatment in MDA-MB-231 cells. (B) Immunoblot quantitative analysis of BRD4 after a 2-hour pretreatment with DMSO, Rapamycin (1 μM) followed by a 4-hour **10f** (0.5 μM) treatment in MDA-MB-231 cells. Data are expressed as mean ± SEM. All data were representative of at least three independent experiments.

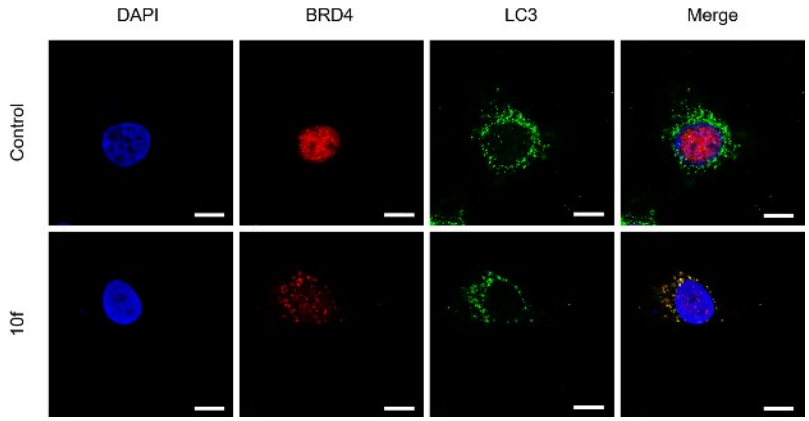



**Fig. S8** Immunofluorescence analysis of the colocalization of endogenous LC3 with BRD4 after treatment of 10f (1 μM) for 24 h in MDA-MB-231. Scale bar, 10 μm.

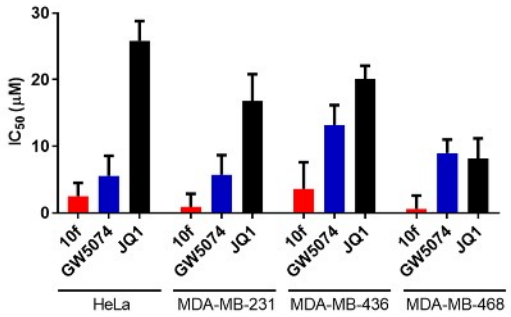



**Fig. S9** Cells were treated with serially diluted **10f**, GW5074 or JQ1 for 4 days, cell viability was measured by MTT assay. Data represent the mean of >3 determinations.

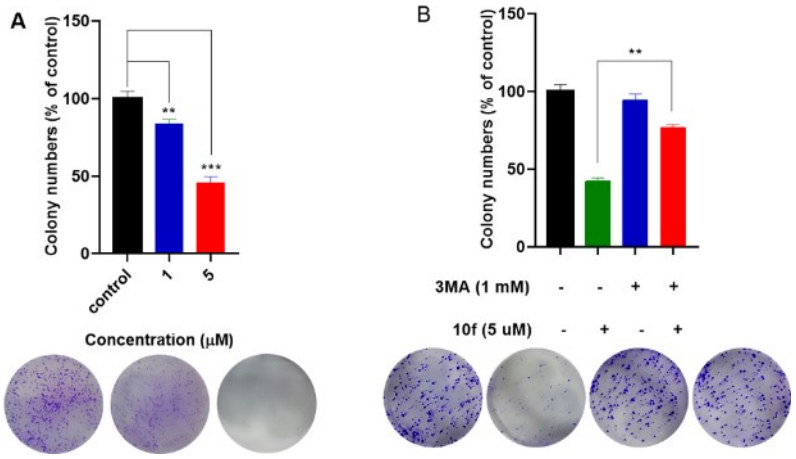



**Fig. S10** (A) Colony formation assay of MDA-MB-231 cells treated with or without **10f** (1 μM, 5 μM). (B) Colony formation assay of MDA-MB-231 treated with **10f** (5 μM) alone or in combination with 3-MA (1 mM). Representative images and quantifications of colonies were shown. 3-MA was added 1 h before treatment of **10f**. Data are expressed as mean ± SEM. All data were representative of at least three independent experiments. *, $P < 0.05$, **, $P < 0.01$, ***,$P < 0.001$. Statistical significance compared with respective control groups.

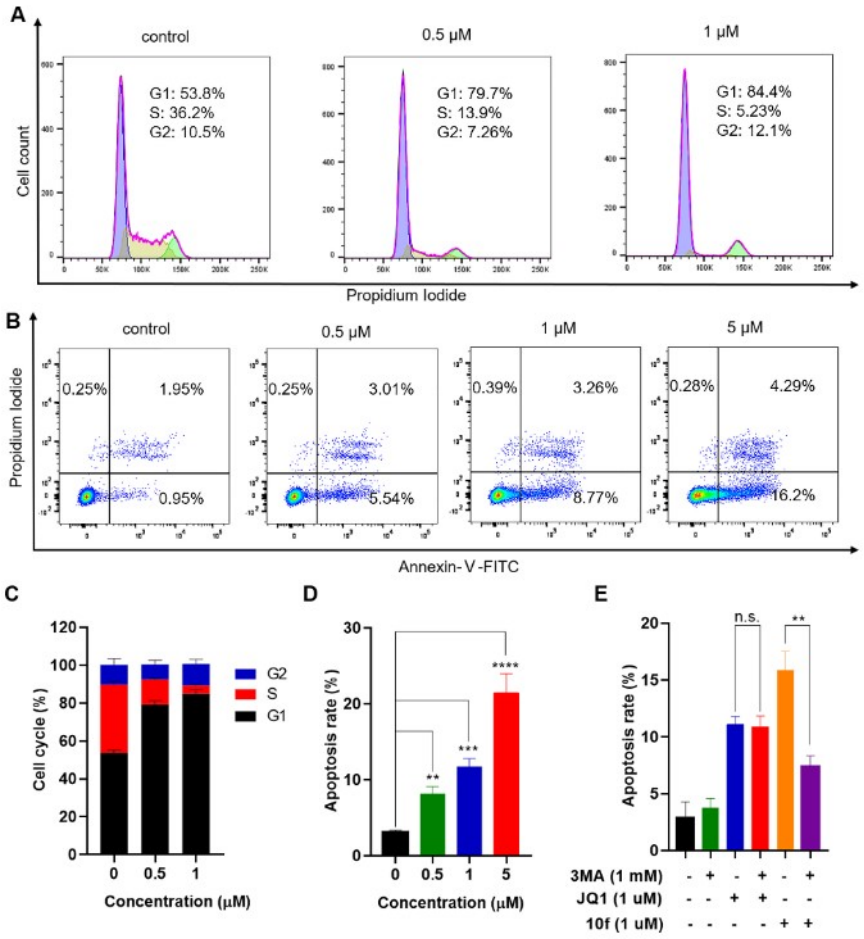



**Fig. S11** (A) Flow cytometry analysis of cycle distribution for the cells treated with **10f** (0, 0.5, 1 μM). Representative images of cycle distribution were shown. **10f** induced cell cycle arrest in the G1 phase. (B) MDA-MB-231 cells were treated with indicated concentrations of **10f** for 24 h, apoptosis ratios were determined by flow cytometry analysis of Annexin-V/PI double staining. Representative images of apoptosis were shown. (C) Flow cytometry quantifications analysis of cycle distribution for the cells treated with **10f** (0, 0.5, 1 μM). (D) Flow cytometry quantifications analysis of apoptosis for the cells treated with **10f** (0, 0.5, 1 μM). (E) MDA-MB-231 cells were treated with **10f** (1 μM), JQ1 (1 μM) alone or in combination with 3-MA (1 mM) for 24 h, and apoptosis ratios were determined by flow cytometry analysis of Annexin-V/PI double staining. 3-MA was added 2 h before treatment of **10f**. Representative quantifications analyses of apoptosis were shown. Data are expressed as mean ± SEM. All data were representative of at least three independent experiments. *, $P < 0.05$, **, $P < 0.01$, ***,$P < 0.001$. Statistical significance compared with respective control

groups.

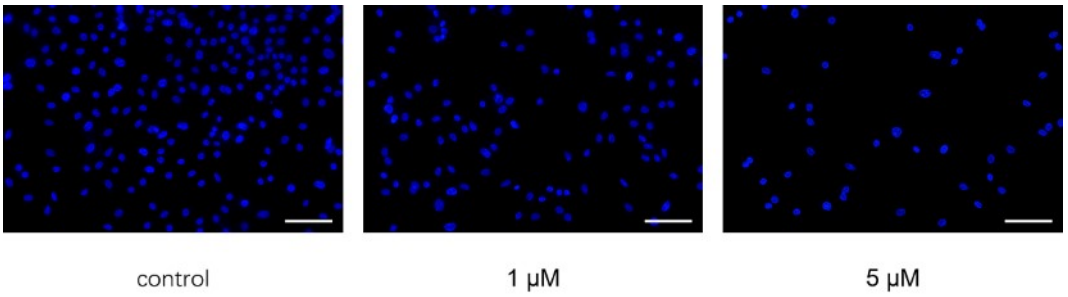



**Fig. S12** Hoechst 33258 staining assay of MDA-MB-231 treated with or without **10f** (1μM, 5 μM) with Scale bar: 100 μm.

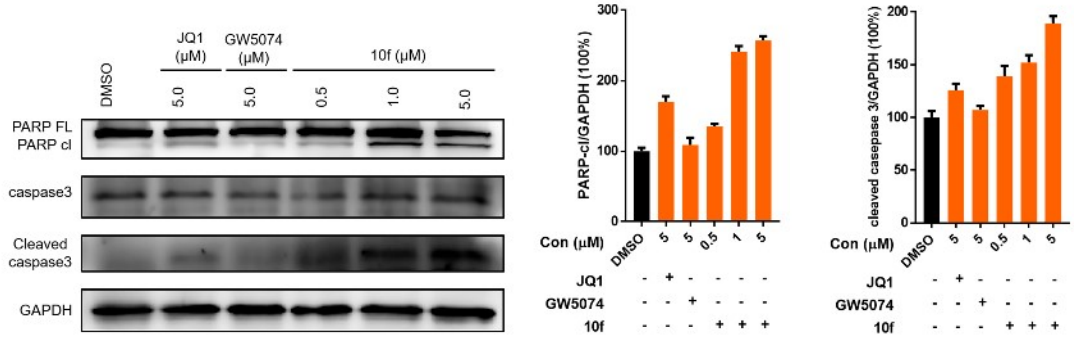



**Fig. S13** Immunoblotting analysis of PARP FL, PARP cl, caspase3, cleaved caspase 3 in MDA-MB-231 cells treated with the indicated concentrations of AUTAC **10f** for 12 h. GAPDH was used as a loading control.

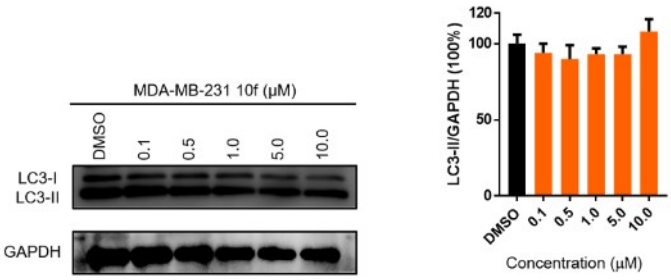
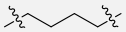
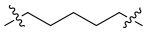
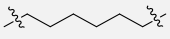
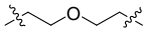
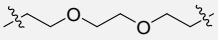
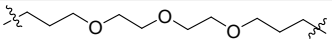
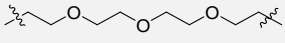
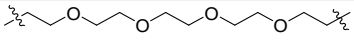
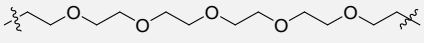
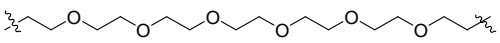
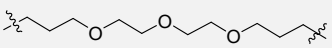



**Fig. S13** Immunoblotting analysis of LC3-I and LC3-II in MDA-MB-231 cells treated with the indicated concentrations of AUTAC **10f** for 12 h. GAPDH was used as a loading control.

**Table 1.** SAR study for AUTACs on Degradation Activity in MDA-MB-231 cells

| compound | R | Linker | Dmax (%) | $IC_{50}$ (μM) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| GW5074 | NA | NA | ND | 5.7 |
| JQ1 | NA | NA | ND | 16.8 |
| **10a** | I |  | ND | >50.0 |
| **10b** | I |  | 4 | >50.0 |
| **10c** | I |  | 23 | 16.8 |
| **10d** | I |  | 53 | 5.7 |
| **10e** | I |  | 82 | 1.5 |
| **10f** | I |  | 92 | 0.9 |
| **10g** | I |  | 85 | 2.0 |
| **10h** | I |  | 60 | 2.8 |
| **10i** | I |  | 57 | 2.6 |
| **10j** | I |  | 30 | 25.3 |
| **10k** | Br |  | 65 | 2.4 |

NA: not applicable. ND: not determined. Dmax: maximal degradation observed. Data represent the mean of >3 determinations.

**Chemistry Methods**

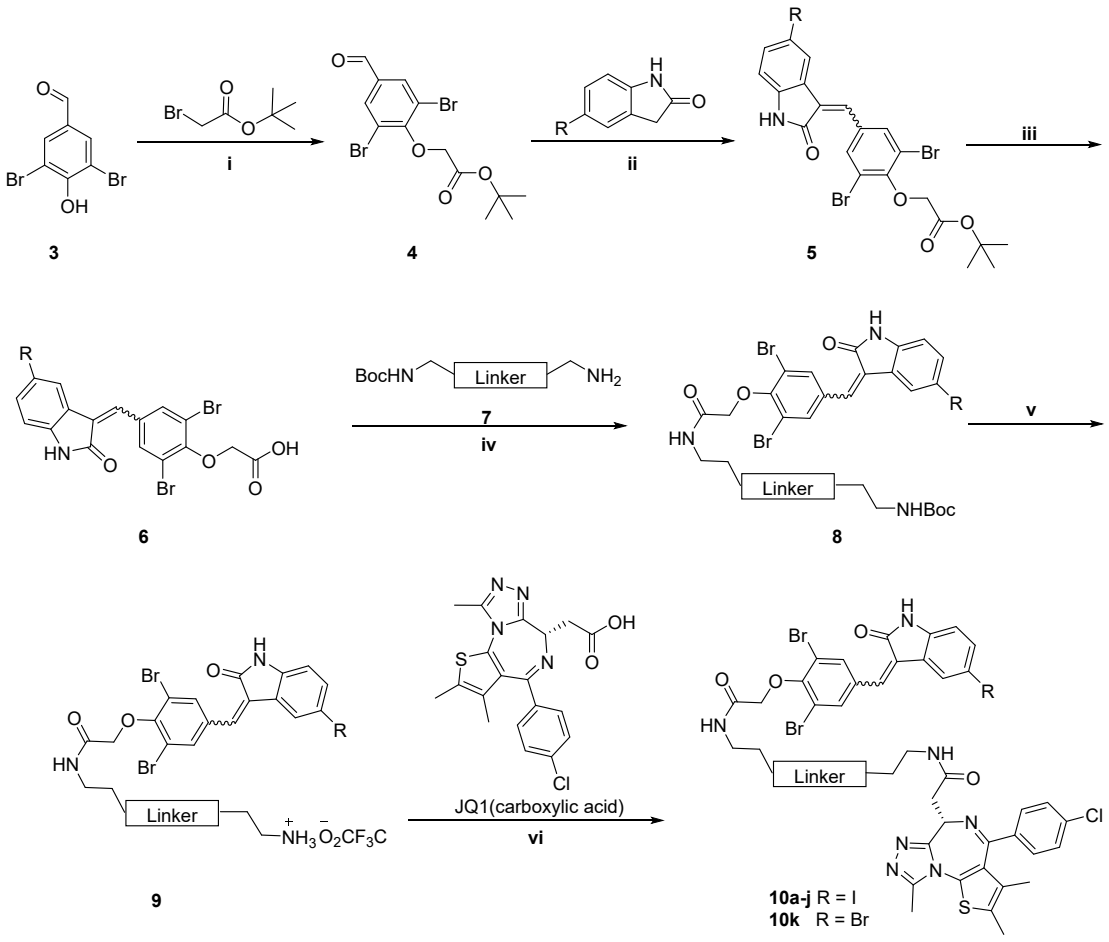
Commercial reagents and solvents were purchased from Sigma Aldrich, Bidepharm Ltd. (Shanghai, China), Fluka, and Alfa Aesar used as received, without further purification. The $^{1}H$ and $^{13}C$ NMR spectra were recorded at 400 MHz, 600 MHz for $^{1}H$-NMR and at 125 MHz, 150 MHz, 200 MHz for $^{13}C$-NMR. The chemical shifts ($\delta$) for $^{1}H$ and $^{13}C$ are given in ppm relative to residual signals of the solvents ($CDCl_3$ at 7.26 ppm $^{1}H$ NMR, 77.16 ppm $^{13}C$ NMR. $d_6$-DMSO at 2.50 ppm $^{1}H$ NMR, 39.52 ppm $^{13}C$ NMR). Coupling constants are given in Hz. The following abbreviations are used to indicate the multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. High-resolution mass spectra (HRMS) were obtained from the Waters Q-Tof Ultima Global.

Note: NMR signals containing common solvent contaminants were list. $H_2O$ in $CDCl_3$ at 1.56 ppm $^{1}H$ NMR, and in $d_6$-DMSO at 3.33 ppm $^{1}H$ NMR; Ethyl acetate in $CDCl_3$ at 2.05 (s), 4.12 (q), 1.26 (t) ppm $^{1}H$ NMR, and in $d_6$-DMSO at 1.99 (s), 4.03

(q), 1.17 (t) ppm $^1$H NMR; Dichloromethane in $CDCl_3$ at 5.30 (s) ppm $^1$H NMR, and in $d_6$-DMSO at 5.76 (s) ppm $^1$H NMR; Methanol in $CDCl_3$ at 3.49 (s), 1.09 (s) ppm $^1$H NMR, and in $d_6$-DMSO at 3.16 (s), 4.01 (s) ppm $^1$H NMR; Dimethylformamide in $CDCl_3$ at 8.02 (s), 2.96 (s), 2.88 (s) ppm $^1$H NMR, and in $d_6$-DMSO at 7.95 (s), 2.89 (s), 2.73 (s) ppm $^1$H NMR.

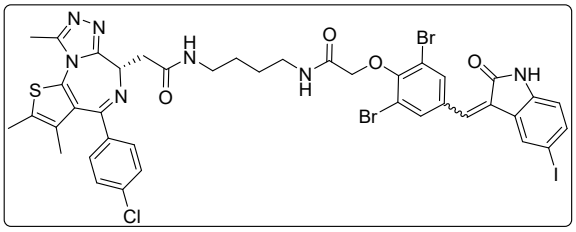
All the reactions were set up under air and using freshly distilled solvents, without any precautions to exclude moisture, unless otherwise noted open air chemistry on the bench-top. Chromatographic purification of products was accomplished using force-flow chromatography (FC) on silica gel (300-400 mesh). For thin layer chromatography (TLC) analysis throughout this work, Merck pre-coated TLC plates (silica gel 60 GF254, 0.25 mm) were used, using UV light as the visualizing agent and basic aqueous potassium permanganate ($KMnO_4$) as stain developing solutions.

**The synthesis of 10a-10k**



Scheme 1. The synthesis of AUTACs **10a**-**10k**. (i) $K_2CO_3$, DMF, 80 °C; (ii) piperidine, $CH_3CH_2OH$, reflux; (iii) TFA, DCM, r.t.; (iv) DMF, HATU, DIPEA, r.t.; (v) TFA, DCM, r.t.; (vi) DMF, HATU, DIPEA, r.t..

**The synthetic method of (*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno [3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(4-(2-(2,6-dibromo-4-((5-iodo-2-oxoindolin-3-ylidene)methyl)phenoxy)acetamido)butyl)acetamide (10a)**



A glass vial equipped with a magnetic stirring bar was charged with 3,5-dibromo-4-hydroxybenzaldehyde **3** (560 mg, 2.0 mmol), tert-butyl 2-bromoacetate (468.1 mg, 2.4 mmol), $K_2CO_3$ (552 mg, 4.0 mmol) in DMF (6.0 mL) at 80 °C. The resulting reaction mixture was kept under vigorous stirring until the consumption of 3,5-dibromo-4-hydroxybenzaldehyde **3** (monitored by TLC analysis). After completion of the reaction, the mixture was extracted with ethyl acetate. The combined organic layers were dried over $Na_2SO_4$ and the solvent evaporated. The product was purified by column chromatography on a silica gel (petroleum ether/ethyl acetate = 20:1 to 10:1) to afford **4** a white solid (704 mg, 89%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.86 (s, 1H), 8.03 (s, 2H), 4.60 (s, 2H), 1.53 (s, 9H) ppm.

To a solution of **4** (394 mg, 1.00 mmol) in $CH_3CH_2OH$ (5.0 mL) was added 5-iodoindolin-2-one (224 mg, 1.30 mmol) and piperidine at room temperature. Then return the reaction to 80 °C and reflux for 3 h. After completion of the reaction, the reaction mixture was purified by filtration to afford **5** (471.6 mg, 74%) after washed twice with ethanol. Then, compound **5** a yellow solid (471.6 mg, 0.743 mmol) was dissolved in anhydrous $CH_2Cl_2$ (7 mL) at 25 °C. TFA (2 mL) was added to the reaction mixtures. After full conversion of the second step, the reaction mixture was concentrated. The product was purified by column chromatography on a silica gel

($CH_2Cl_2$/MeOH = 5:1 to 3:1) to afford **6a** a yellow solid (306.9 mg, 53%).**$^1$H NMR** (600 MHz, DMSO-$d_6$) $\delta$ 10.89 (s, 1H), 8.77 (s, 1H), 8.06 (s, 1H), 7.99 (s, 1H), 7.86 (s, 2/3H), 7.74 (s, 1/3H)7.61 – 7.52 (m, 1H), 6.78 (d, $J$ = 8.1 Hz, 1/3H), 6.72 (d, $J$ = 8.1 Hz, 2/3H), 4.27 (s, 2H) ppm.

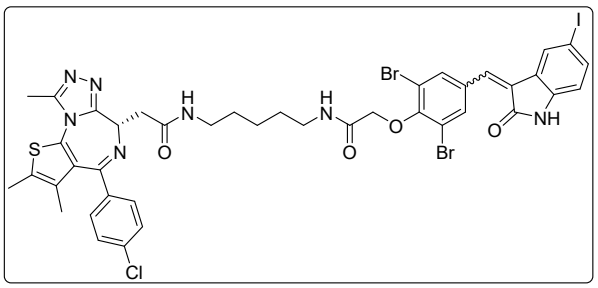
A mixture of **6a** (43.3 mg, 0.08 mmol), HATU (37.1 mg, 0.10 mmol), *tert*-butyl (4-aminobutyl)carbamate **7a** (15.1 mg, 0.08 mmol) and DIPEA (38.7 mg, 0.3 mmol) in 2 mL of DMF was stirred at room temperature for 3 h. After completion of the reaction, the mixture was extracted with ethyl acetate. The combined organic layers were dried over $Na_2SO_4$ and the solvent evaporated. **8a** was obtained as a yellow solid 39.9 mg in 66% yield which was used the next step directly.

A glass vial equipped with a magnetic stirring bar was charged with **8a** (37.5 mg, 0.05 mmol) in anhydrous $CH_2Cl_2$ (2.0 mL) at 25 $^o$C. TFA (1 mL) was added to the reaction mixtures. The mixture was stirred at room temperature for 3 h to get the product **9a** which was used the next step directly. A mixture of **9a** (38.2 mg, 0.05 mmol), HATU (26.6 mg, 0.07 mmol), JQ1 (carboxylic acid) (20.0 mg, 0.05 mmol) and DIPEA (25.8 mg, 0.2 mmol) in 2 mL of DMF was stirred at room temperature for 3 h. After completion of the reaction, the mixture was extracted with ethyl acetate. The combine organic layers were dried over $Na_2SO_4$ and the solvent evaporated. The product was purified by column chromatography on a silica gel ($CH_2Cl_2$/MeOH = 20:1 to 10:1) to afford **10a** a yellow solid (35.4 mg, over two steps 69%). **$^1$H NMR** (600 MHz, DMSO-$d_6$) $\delta$10.86 (s, 2/3H), 10.80 (s, 1/3H), 8.78 (s, 1H), 8.29 – 8.24 (m, 1H), 8.19 (dt, $J$ = 17.7, 6.0 Hz, 1H), 8.03 (s, 1H), 8.00 (s, 1H), 7.85 (s, 2/3H), 7.68 (s, 1/3H), 7.56 (q, $J$ = 7.9 Hz, 1H), 7.50 – 7.46 (m, 2H), 7.41 (d, $J$ = 8.3 Hz, 2H), 6.74 (d, $J$ = 8.1 Hz, 1/3H), 6.68 (d, $J$ = 8.1 Hz, 2/3H), 4.52 (dd, $J$ = 8.3, 6.0 Hz, 1H), 4.49 – 4.39 (m, 2H), 3.30 – 3.18 (m, 5H), 3.14 – 3.06 (m, 1H), 2.57 (s, 3H), 2.38 (s, 3H), 1.60 (s, 3H), 1.51 (dq, $J$ = 21.5, 7.4, 7.0 Hz, 4H) ppm. **$^{13}$C NMR** (150 MHz, DMSO-$d_6$) $\delta$ 169.5, 167.6, 166.5, 166.2, 163.1, 155.2, 152.8, 152.3, 149.9, 142.8, 140.6, 138.7, 137.6, 136.8, 136.2, 135.3, 134.4, 133.7, 133.6, 133.5, 133.3, 132.3, 130.7, 130.2, 129.9, 129.6, 128.5, 128.2, 127.1, 123.1, 117.8, 117.1, 112.1, 84.3, 84.0, 71.0, 55.0, 53.9, 38.4, 38.2, 37.7,

30.4, 29.1, 26.7, 26.6, 14.1, 12.7, 11.4 ppm. **HRMS**: $[M+H]^+$ *calcd*. For $C_{40}H_{36}Br_2ClIN_7O_4S^+$ 1029.96440, found 1029.96228.

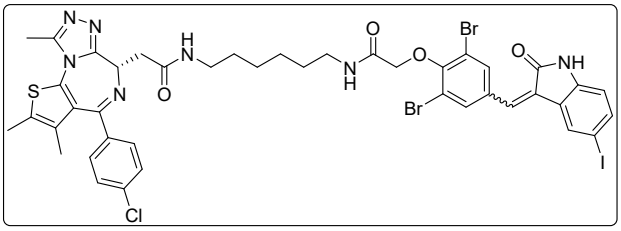
**Following the same synthetic methods to get other compounds 10b-10k, except using different types and lengths mono-Boc protected diamines.**

**(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1, 4]diazepin-6-yl)-*N*-(5-(2-(2,6-dibromo-4-((5-iodo-2-oxoindolin-3-ylidene)Methyl) phenoxy)acetamido)pentyl)acetamide (10b)**



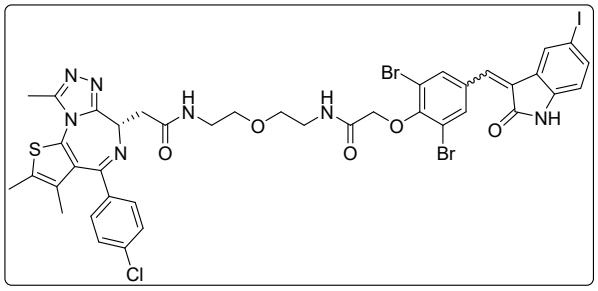
**10b** was obtained as a yellow solid 34.3 mg in 58% yield for two steps. **$^1$H NMR** (600 MHz, DMSO-$d_6$) $\delta$ 10.85 (s, 2/3H), 10.79 (s, 1/3H), 8.78 (s, 1H), 8.21 (t, $J$ = 5.6 Hz, 1H), 8.18 – 8.14 (m, 1H), 8.03 (d, $J$ = 1.7 Hz, 1H), 8.00 (s, 1H), 7.84 (s, 2/3H), 7.67 (s, 1/3H), 7.58 – 7.53 (m, 1H), 7.49 – 7.45 (m, 2H), 7.41 (d, $J$ = 8.3 Hz, 2H), 6.74 (d, $J$ = 8.1 Hz, 1/3H), 6.69 (d, $J$ = 8.1 Hz, 2/3H), 4.50 (td, $J$ = 6.1, 3.0 Hz, 1H), 4.45 (s, 2/3H), 4.42(s, 4/3H), 3.28 – 3.17 (m, 4H), 3.15 – 3.05 (m, 2H), 2.57 (s, 3H), 2.38 (s, 3H), 1.60 (s, 3H), 1.57 – 1.41 (m, 6H) ppm. **$^{13}$C NMR** (150 MHz, DMSO-$d_6$) $\delta$ 169.8, 168.0, 166.9, 166.6, 163.5, 155.6, 153.2, 152.7, 150.3, 143.2, 141.0, 139.1, 138.0, 137.2, 136.6, 135.7, 134.8, 134.2, 134.1, 134.0, 133.9, 133.7, 132.7, 131.1, 130.6, 130.3, 130.0, 128.9, 128.6, 127.5, 123.5, 118.2, 117.5, 113.2, 112.5, 84.7, 71.4, 54.4, 40.8, 38.8, 38.1, 29.3, 29.1, 24.2, 14.5, 13.1, 11.8 ppm. **HRMS**: $[M+H]^+$ *calcd*. For $C_{41}H_{38}Br_2ClIN_7O_4S^+$ 1043.98005, found 1043.97805.

**(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1, 4]diazepin-6-yl)-*N*-(6-(2-(2,6-dibromo-4-((5-iodo-2-oxoindolin-3-ylidene)methyl)p henoxy)acetamido)hexyl)acetamide (10c)**



**10c** was obtained as a yellow solid 43.2 mg in 71% yield for two steps.**$^1$H NMR** (400 MHz, DMSO-$d_6$) $\delta$ 10.84 (s, 2/3H), 10.79 (s, 1/3H), 8.78 (s, 1H), 8.23 – 8.11 (m, 2H), 8.05 – 7.99 (m, 1H), 7.85 (s, 2/3H), 7.68 (s, 1/3H), 7.55 (ddd, $J$ = 10.0, 7.5, 1.7 Hz, 1H), 7.51 – 7.38 (m, 5H), 6.75 (d, $J$ = 8.1 Hz, 1/3H)6.70 (d, $J$ = 8.1 Hz, 2/3H), 4.57 – 4.48 (m, 1H), 4.46 (s, 2/3H), 4.43 (s, 4/3H), 3.26 – 3.15 (m, 4H), 3.14 – 3.04 (m, 2H), 2.58 (s, 3H), 2.39 (s, 3H), 1.61 (s, 3H), 1.53 – 1.43 (m, 4H), 1.32 (p, $J$ = 3.6 Hz, 4H) ppm. **$^{13}$C NMR** (150 MHz, DMSO-$d_6$) $\delta$ 169.4, 166.5, 166.1, 163.0, 162.3, 155.1, 152.8, 149.8, 142.8, 140.6, 137.6, 136.8, 136.2, 135.3, 134.4, 133.5, 133.3, 132.3, 130.7, 130.1, 129.9, 129.6, 128.5, 127.1, 117.8, 117.1, 112.0, 84.3, 71.0, 53.9, 38.5, 38.3, 37.7, 29.3, 29.0, 26.1, 14.1, 12.7, 11.3 ppm. **HRMS**: $[M+H]^+$ *calcd*. For $C_{42}H_{40}Br_2ClIN_7O_4S^+$ 1057.99570, found 1057.99451.

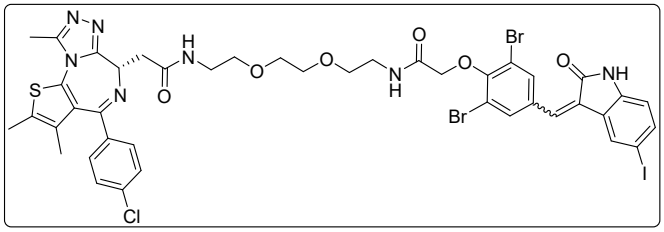
**(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(2-(2-(2-(2,6-dibromo-4-((5-iodo-2-oxoindolin-3-ylidene)methyl)phenoxy)acetamido)ethoxy)ethyl)acetamide (10d)**



**10d** was obtained as a yellow solid 38.2 mg in 64% yield for two steps. **$^1$H NMR** (400 MHz, DMSO-$d_6$) $\delta$ 10.83 (s, 2/3H), 10.78 (s, 1/3H), 8.76 (s, 1H), 8.30 (t, $J$ = 5.5 Hz, 1H), 8.22 – 8.11 (m, 1H), 8.02 (s, 1H), 7.98 (s, 1H), 7.82 (s, 2/3H), 7.67 (s, 1/3H), 7.53 (d, $J$ = 6.4 Hz, 1H), 7.49 – 7.37 (m, 4H), 6.74 (d, $J$ = 8.1 Hz, 1/3H), 6.69 (d, $J$ = 8.1 Hz, 2/3H), 4.69 – 4.38 (m, 3H), 3.62 – 3.47 (m, 4H), 3.41 (s, 2H), 3.28 (ddd, $J$ = 19.7, 12.2, 5.9 Hz, 4H), 2.57 (s, 3H), 2.38 (s, 3H), 1.60 (s, 3H) ppm. **$^{13}$C NMR** (150 MHz, DMSO-

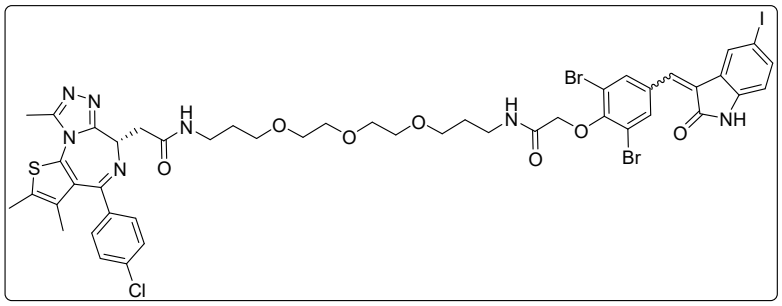
$d_6$) $\delta$ 170.2, 168.0, 166.9, 166.9, 163.5, 155.5, 153.2, 152.7, 150.2, 143.2, 141.0, 139.1, 138.0, 137.2, 136.6, 135.7, 134.8, 134.1, 133.9, 133.7, 132.7, 131.1, 130.6, 130.3, 130.0, 128.9, 128.6, 127.5, 123.4, 118.2, 117.5, 113.2, 112.4, 84.7, 84.4, 71.4, 69.4, 69.0, 54.3, 39.1, 38.8, 38.0, 14.5, 13.1, 11.8 ppm. **HRMS**: $[M+H]^+$ *calcd*. For $C_{40}H_{36}Br_2ClIN_7O_5S^+$ 1045.95931, found 1045.95874.

**(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(2-(2-(2-(2-(2,6-dibromo-4-((5-iodo-2-oxoindolin-3-ylidene)methyl)phenoxy)acetamido)ethoxy)ethoxy)ethyl)acetamide (10e)**



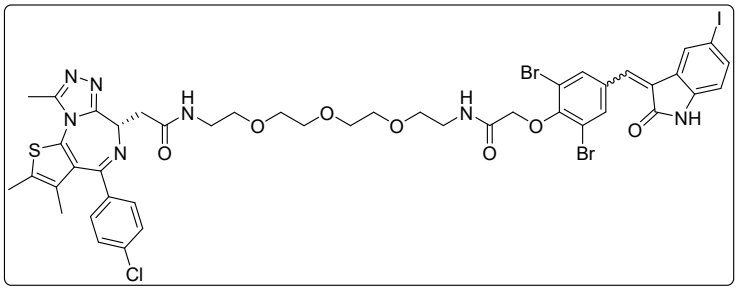
**10e** was obtained as a yellow solid 44.5 mg in 73% yield for two steps.**$^1$H NMR** (400 MHz, DMSO-$d_6$) $\delta$ 10.85 (s, 2/3H), 10.79 (s, 1/3H), 8.79 (s, 1H), 8.30 (s, 1H), 8.13 (dt, $J$ = 20.6, 5.8 Hz, 1H), 8.01 (s, 1H), 7.84 (s, 2/3H), 7.69 (s, 1/3H) 7.58 – 7.54 (m, 1H), 7.53 – 7.43 (m, 3H), 7.44 – 7.40 (m, 2H), 6.75 (d, $J$ = 8.1 Hz, 1/3H) 6.70 (d, $J$ = 8.1 Hz, 2/3H), 4.58 – 4.46 (m, 3H), 3.59 – 3.53 (m, 5H), 3.49 (t, $J$ = 5.9 Hz, 2H), 3.34 – 3.25 (m, 3H), 3.18 (d, $J$ = 5.1 Hz, 3H), 3.09 (q, $J$ = 7.3 Hz, 1H), 2.59 (s, 3H), 2.40 (s, 3H), 1.67 – 1.57 (m, 3H) ppm. **$^{13}$C NMR** (150 MHz, DMSO-$d_6$) $\delta$ 170.2, 168.0, 166.9, 163.5, 155.5, 153.2, 152.7, 150.2, 143.2, 141.0, 139.1, 138.0, 137.2, 136.6, 135.7, 134.8, 134.2, 134.1, 133.9, 133.7, 132.7, 131.1, 130.6, 130.3, 130.0, 128.9, 128.6, 127.5, 127.5, 123.4, 118.1, 117.4, 113.2, 112.4, 84.7, 84.3, 71.5, 70.1, 70.1, 69.7, 69.2, 54.3, 54.0, 46.1, 39.1, 38.8, 38.0, 14.5, 13.1, 11.8, 9.0 ppm. **HRMS**: $[M+H]^+$ *calcd*. For $C_{42}H_{40}Br_2ClIN_7O_6S^+$ 1089.98553, found 1089.98621.

**(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(1-(2,6-dibromo-4-((5-iodo-2-oxoindolin-3-ylidene)methyl)phenoxy)-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)acetamide (10f)**



**10f** was obtained as a yellow solid 45.2 mg in 63% yield for two steps. **$^1$H NMR** (400 MHz, $CDCl_3$) $\delta$ 10.67 (s, 2/3H), 9.36 (s, 1/3H), 8.51 (s, 1H), 7.77 (s, 1H), 7.76 – 7.65 (m, 2H), 7.54 (s, 1H), 7.46 (dd, $J$ = 8.2, 1.7 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.31 (dd, $J$ = 8.6, 2.7 Hz, 2H), 7.24 (s, 1H), 6.86 (d, $J$ = 8.1 Hz, 1/3H), 6.75 (d, $J$ = 8.1 Hz, 2/3H), 4.77 (t, $J$ = 8.1, 6.2 Hz, 2/3H), 4.67 (t, $J$ = 8.1, 6.2 Hz, 1/3H), 4.58(s, 2/3H), 4.56 (s, 4/3H), 3.72 – 3.40 (m, 16H), 3.40 – 3.18 (m, 2H), 2.69 (s, 3H), 2.41 (s, 3H), 1.90 (t, $J$ = 6.0 Hz, 2H), 1.80 (q, $J$ = 6.0, 5.6 Hz, 2H), 1.67 (s, 3H)ppm. **$^{13}$C NMR** (200 MHz, $CDCl_3$) $\delta$ 170.3, 170.3, 167.4, 167.1, 164.2, 163.9, 155.7, 152.8, 152.6, 150.0, 141.9, 140.6, 139.0, 138.2, 136.9, 136.8, 136.5, 136.5, 136.1, 133.8, 133.5, 133.4, 132.9, 132.1, 131.9, 131.8, 131.1, 131.1, 131.0, 131.0, 130.7, 130.6, 129.9, 128.8, 128.7, 128.3, 128.2, 127.9, 126.6, 123.1, 118.3, 117.5, 113.0, 112.8, 84.2, 84.1, 71.2, 71.0, 70.9, 70.6, 70.6, 70.5, 70.4, 70.1, 70.0, 69.8, 69.5, 69.0, 54.4, 38.9, 38.1, 37.6, 37.5, 36.9, 29.7, 29.2, 29.1, 29.1, 28.6, 14.5, 13.2, 11.9 ppm. **HRMS**: $[M+H]^+$ *calcd*. For $C_{46}H_{48}Br_2ClIN_7O_7S^+$ 1162.04304, found 1162.04346. Purity, 97.6%

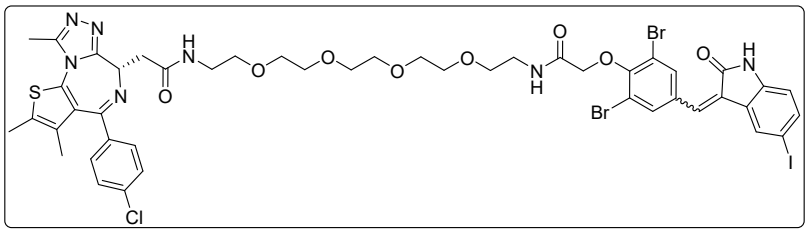
**(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(1-(2,6-dibromo-4-((5-iodo-2-oxoindolin-3-ylidene)methyl)phenoxy)-2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)acetamide (10g)**



**10g** was obtained as a yellow solid 35.4 mg in 55% yield for two steps. **$^1$H NMR** (400 MHz, DMSO-$d_6$) $\delta$ 10.89 (s, 1H),8.85(s, 1/3H), 8.79 (s, 2/3H), 8.35 – 8.25 (m, 1H),

8.18 – 8.09 (m, 1H), 8.07 – 8.00 (m, 1H), 7.86 (s, 1H), 7.71 – 7.53 (m, 2H), 7.51 – 7.45 (m, 2H), 7.42 (dt, *J* = 8.6, 2.1 Hz, 2H), 6.77 (dd, *J* = 8.2, 3.1 Hz, 1/3H), 6.72 (dd, *J* = 8.2, 3.1 Hz, 2/3H), 4.66 – 4.34 (m, 3H), 3.83 – 3.60 (m, 3H), 3.56 (s, 6H), 3.50 – 3.42 (m, 5H), 3.33 – 3.20 (m, 4H), 2.58 (s, 3H), 2.39 (s, 3H), 1.60 (s, 3H) ppm. **$^{13}$C NMR** (150 MHz, DMSO-$d_6$) $\delta$ 171.4, 170.2, 170.2, 166.8, 163.5, 162.8, 155.5, 150.3, 137.2, 136.2, 135.7, 132.7, 131.2, 130.6, 130.3, 130.0, 128.9, 125.7, 125.0, 125.0, 112.6, 84.8, 79.6, 70.2, 70.2, 70.1, 70.0, 69.6, 68.5, 61.1, 54.3, 49.1, 39.1, 38.0, 36.3, 31.2, 14.5, 13.1, 11.8 ppm. **HRMS**: $[M+Na]^+$ *calcd*. For $C_{44}H_{43}Br_2ClIN_7NaO_7S^+$ 1155.9937, found 1155.9938.

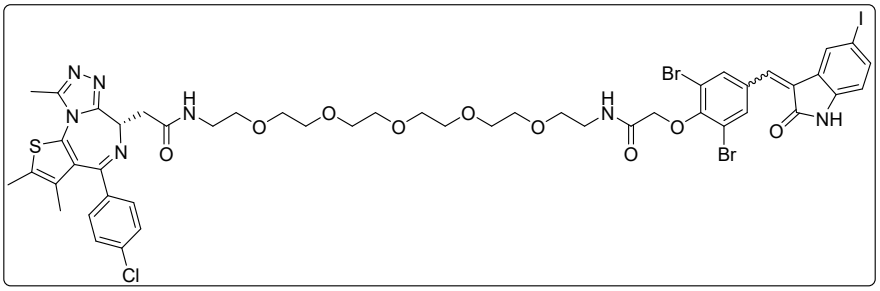
**(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)-*N*-(1-(2,6-dibromo-4-((5-iodo-2-oxoindolin-3-ylidene)methyl)phenoxy)-2-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-17-yl)acetamide (10h)**



**10h** was obtained as a yellow solid 35.6 mg in 66% yield for two steps. **$^1$H NMR** (400 MHz, $CDCl_3$) $\delta$ 9.15 (s, 4/5H), 8.52 (s, 1/5H), 7.82 (d, *J* = 1.7 Hz, 1H), 7.78 (s, 2H), 7.56 (s, 1H), 7.53 – 7.46 (m, 2H), 7.44 – 7.38 (m, 2H), 7.36 – 7.29 (m, 2H), 7.26 – 7.21 (m, 1H), 6.85 (d, *J* = 8.2 Hz, 1/5H), 6.76 (d, *J* = 8.2 Hz, 4/5H), 4.68 (t, *J* = 7.0 Hz, 1H), 4.61 (s, 2H), 3.86 – 3.64 (m, 10H), 3.63 (d, *J* = 4.4 Hz, 5H), 3.56 (ddd, *J* = 10.8, 8.5, 4.7 Hz, 4H), 3.50 (q, *J* = 4.8 Hz, 2H), 3.41 (dd, *J* = 14.7, 7.2 Hz, 1H), 2.66 (s, 3H), 2.39 (s, 3H), 1.66 (s, 3H)ppm. **$^{13}$C NMR** (100 MHz, $CDCl_3$) $\delta$ 170.7, 168.6, 167.4, 164.0, 155.8, 153.0, 150.0, 142.0, 139.2, 136.9, 136.7, 136.2, 133.9, 133.8, 133.5, 132.2, 131.9, 131.1, 130.9, 130.7, 130.0, 128.8, 128.4, 123.2, 118.3, 112.8, 84.3, 71.5, 70.7, 70.6, 70.5, 70.4, 70.0, 69.8, 54.5, 39.5, 39.0, 39.0, 14.5, 13.2, 12.0 ppm. **HRMS**: $[M+Na]^+$ *calcd*. For $C_{46}H_{47}Br_2ClIN_7NaO_8S^+$ 1200.0199, found 1200.0197.

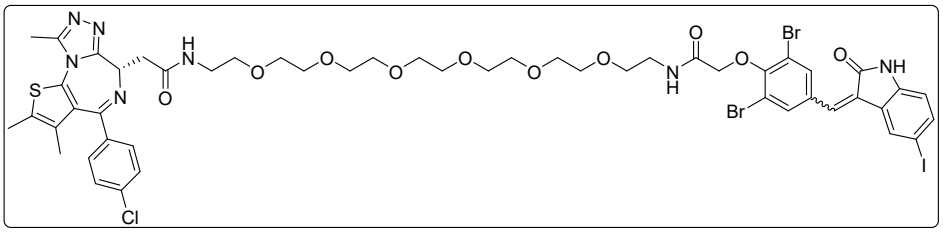
**(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,**

**4]diazepin-6-yl)-*N*-(1-(2,6-dibromo-4-((5-iodo-2-oxoindolin-3-ylidene)methyl)ph enoxy)-2-oxo-6,9,12,15,18-pentaoxa-3-azaicosan-20-yl)acetamide (10i)**



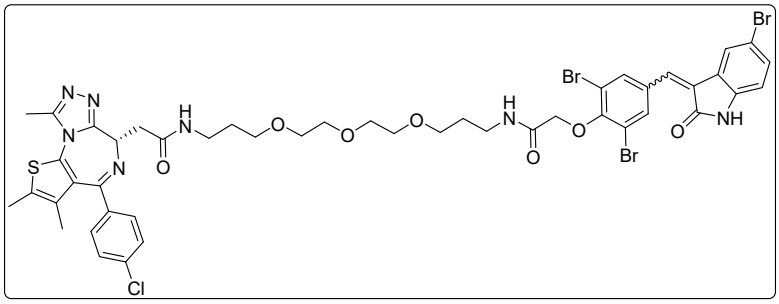
**10i** was obtained as a yellow solid 29.5 mg in 55% yield for two steps. **$^1$H NMR** (600 MHz, $CDCl_3$) $\delta$ 10.01 (s, 1/2H), 9.86 (s, 1/2H), 8.48 (s, 1H), 7.78 (d, $J$ = 1.6 Hz, 1H), 7.75 (s, 1H), 7.71 (t, $J$ = 5.5 Hz, 1H), 7.57 (dt, $J$ = 24.0, 5.6 Hz, 1H), 7.52 (d, $J$ = 5.7 Hz, 1H), 7.46 (dd, $J$ = 8.2, 1.7 Hz, 1H), 7.39 (d, $J$ = 8.2 Hz, 2H), 7.30 (d, $J$ = 8.6 Hz, 2H), 6.79 (d, $J$ = 8.1 Hz, 1/2H), 6.72 (d, $J$ = 8.1 Hz, 1/2H), 4.69 (dt, $J$ = 9.0, 7.1 Hz, 1H), 4.59 (s, 1H), 4.54 (s, 1H), 3.65 – 3.62 (m, 8H), 3.61 – 3.55 (m, 15H), 3.51 – 3.45 (m, 2H), 3.42 (td, $J$ = 14.5, 13.5, 6.2 Hz, 1H), 2.66 (s, 3H), 2.38 (s, 3H), 1.64 (s, 3H) ppm. **$^{13}$C NMR** (200 MHz, DMSO-$d_6$) $\delta$ 170.8, 168.8, 167.7, 167.6, 167.1, 164.1, 164.0, 155.7, 153.1, 152.8, 150.0, 142.4, 140.4, 139.1, 138.1, 136.9, 136.6, 136.2, 133.8, 133.6, 133.5, 133.0, 132.1, 131.6, 131.1, 131.0, 130.6, 129.9, 128.8, 128.5, 128.2, 127.7, 126.7, 123.1, 118.2, 117.4, 113.1, 112.5, 84.2, 71.4, 70.4, 70.2, 70.0, 69.8, 54.4, 39.4, 39.0, 38.8, 14.5, 12.0, 11.8 ppm. **HRMS**: $[M+Na]^+$ *calcd*. For $C_{48}H_{51}Br_2ClIN_7NaO_9S^+$ 1244.0461, found 1244.0463.

**(S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1, 4]diazepin-6-yl)-N-(1-(2,6-dibromo-4-((5-iodo-2-oxoindolin-3-ylidene)methyl)phe noxy)-2-oxo-6,9,12,15,18,21-hexaoxa-3-azatricosan-23-yl)acetamide (10j)**



**10j** was obtained as a yellow solid 36.7 mg in 70% yield for two steps. **$^1$H NMR** (400 MHz, $CDCl_3$) $\delta$ 10.02 (s, 2/3H), 9.92 (s, 1/3H), 8.51 (s, 1H), 7.78 (s, 2H), 7.74 – 7.54 (m, 2H), 7.53 – 7.44 (m, 2H), 7.43 – 7.38 (m, 2H), 7.34 – 7.30 (m, 2H), 6.83 – 6.79 (m, 2/3H), 6.72 – 6.69 (m, 1/3H) 4.77 – 4.66 (m, 1H), 4.61 (s, 4/3H), 4.57 (s, 2/3H) 3.75 (tt, $J$ = 4.4, 2.1 Hz, 1H), 3.65 (t, $J$ = 4.4 Hz, 8H), 3.61 (q, $J$ = 6.8, 6.2 Hz, 18H), 3.50 (q, $J$ = 5.6 Hz, 2H), 3.43 (dd, $J$ = 14.8, 6.9 Hz, 1H), 2.68 (s, 3H), 2.40 (s, 3H), 1.66 (s, 3H) ppm. **$^{13}$C NMR** (200 MHz, $CDCl_3$) $\delta$ 170.5, 168.5, 167.3, 167.2, 166.8, 163.9, 156.3, 155.5, 152.8, 152.5, 149.8, 142.5, 140.4, 138.9, 137.9, 136.6, 136.4, 136.0, 133.8, 133.7, 133.2, 133.1, 132.9, 131.8, 131.3, 130.9, 130.7, 130.3, 129.8, 128.5, 128.0, 127.6, 126.5, 122.8, 118.7, 117.9, 117.1, 113.0, 112.3, 83.8, 83.7, 71.1, 71.0, 71.0, 70.4, 70.3, 70.3, 70.3, 70.2, 70.2, 70.1, 70.0, 69.7, 69.7, 69.5, 69.5, 69.4, 54.1, 53.9, 42.2, 39.2, 38.8, 38.4, 18.3, 17.1, 14.3, 13.0, 11.9, 11.6 ppm. **HRMS**: $[M+Na]^+$ *calcd*. For $C_{50}H_{55}Br_2ClIN_7NaO_{10}S^+$ 1288.0723, found 1288.0729.

**(*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1, 4]diazepin-6-yl)-*N*-(1-(2,6-dibromo-4-((5-bromo-2-oxoindolin-3-ylidene)methyl)phenoxy)-2-oxo-7,10,13-trioxa-3-azahexadecan-16-yl)acetamide (10k)**

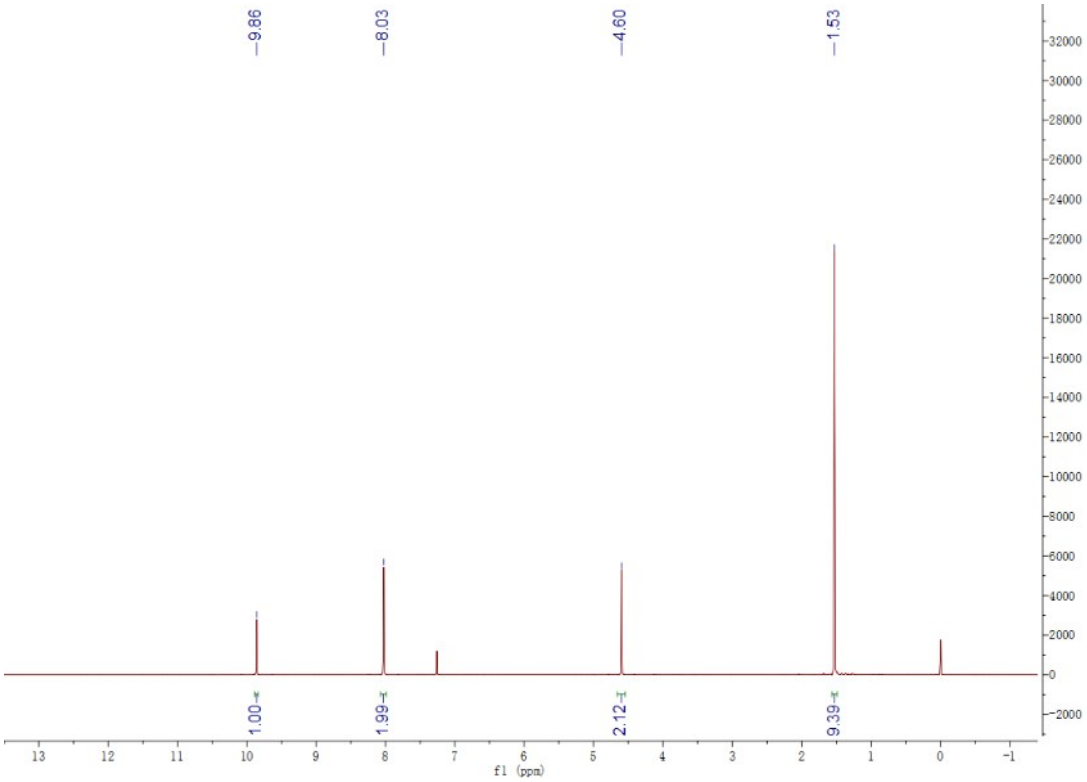



**10k** was obtained as a yellow solid 36.7 mg in 57% yield for two steps. **$^1$H NMR** (400 MHz, DMSO-$d_6$) $\delta$ 10.86 (s, 2/3H), 10.81 (s, 1/3H), 8.78 (s, 1H), 8.16 (dt, $J$ = 13.9, 5.5 Hz, 2H), 8.02 (s, 1H), 7.89 (t, $J$ = 1.9 Hz, 1H), 7.87 (d, $J$ = 2.4 Hz, 1H), 7.47 (d, $J$ = 8.4 Hz, 2H), 7.44 – 7.33 (m, 3H), 6.86 (d, $J$ = 8.3 Hz, 1/3H), 6.81 (d, $J$ = 8.3 Hz, 2/3H), 4.51 (dd, $J$ = 8.0, 6.2 Hz, 1H), 4.45 (s, 2/3H), 4.43 (s, 4/3H), 3.50 (dt, $J$ = 5.7, 3.4 Hz, 6H), 3.48 – 3.44 (m, 4H), 3.42 (d, $J$ = 6.6 Hz, 2H), 3.26 (q, $J$ = 4.8, 3.4 Hz, 2H), 3.23 – 3.18 (m, 2H), 3.18 – 3.08 (m, 2H), 2.58 (s, 3H), 2.39 (s, 3H), 1.79 – 1.64 (m, 4H), 1.60

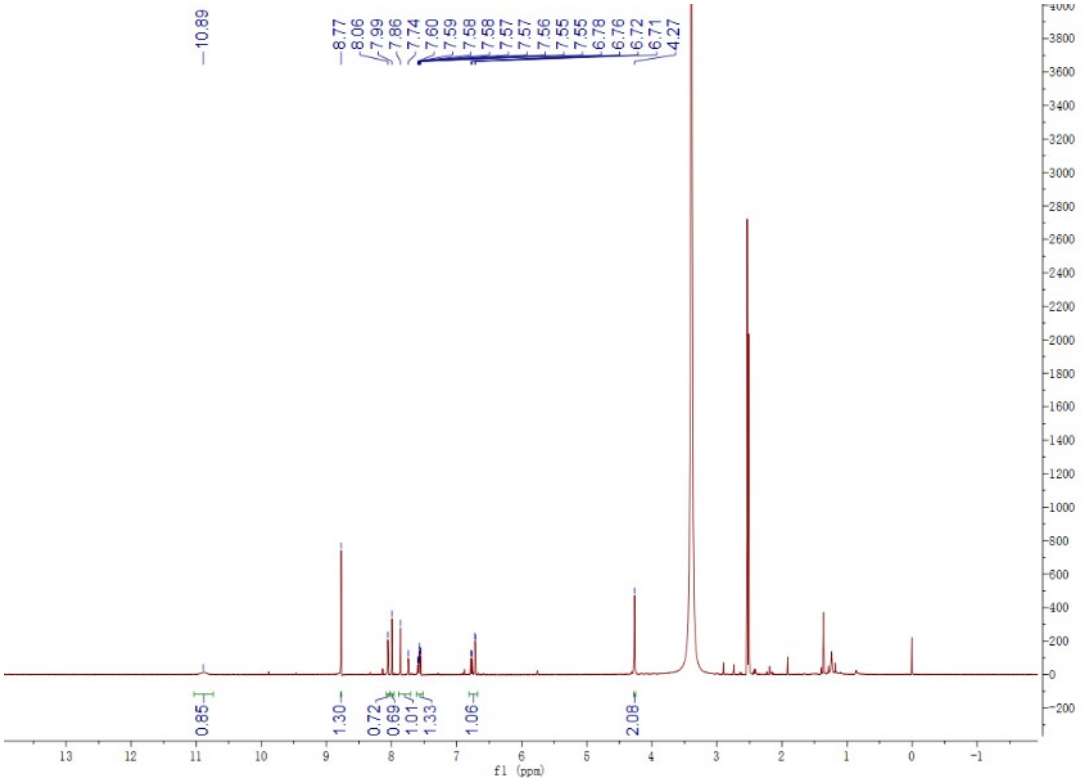
(s, 3H) ppm. **$^{13}$C NMR** (150 MHz, DMSO-$d_6$) $\delta$ 169.9, 168.2, 167.1, 166.7, 166.6, 163.5, 162.8, 155.5, 153.2, 152.8, 150.2, 142.9, 140.6, 137.2, 136.6, 135.7, 135.1, 134.3, 134.1, 133.9, 133.7, 133.4, 132.7, 132.2, 131.1, 130.6, 130.3, 130.0, 128.9, 128.8, 127.7, 127.2, 125.3, 123.4, 123.0, 118.2, 117.5, 113.7, 113.2, 112.0, 71.4, 70.2, 70.1, 70.0, 68.9, 68.5, 54.3, 38.1, 36.6, 36.3, 29.9, 29.6, 14.5, 13.1, 11.8 ppm. **HRMS**: $[M+Na]^+$ *calcd*. For $C_{46}H_{47}Br_3ClN_7NaO_7S^+$ 1136.0389, found 1136.0391.

**The $^{1}$H NMR and $^{13}$C NMR spectra**

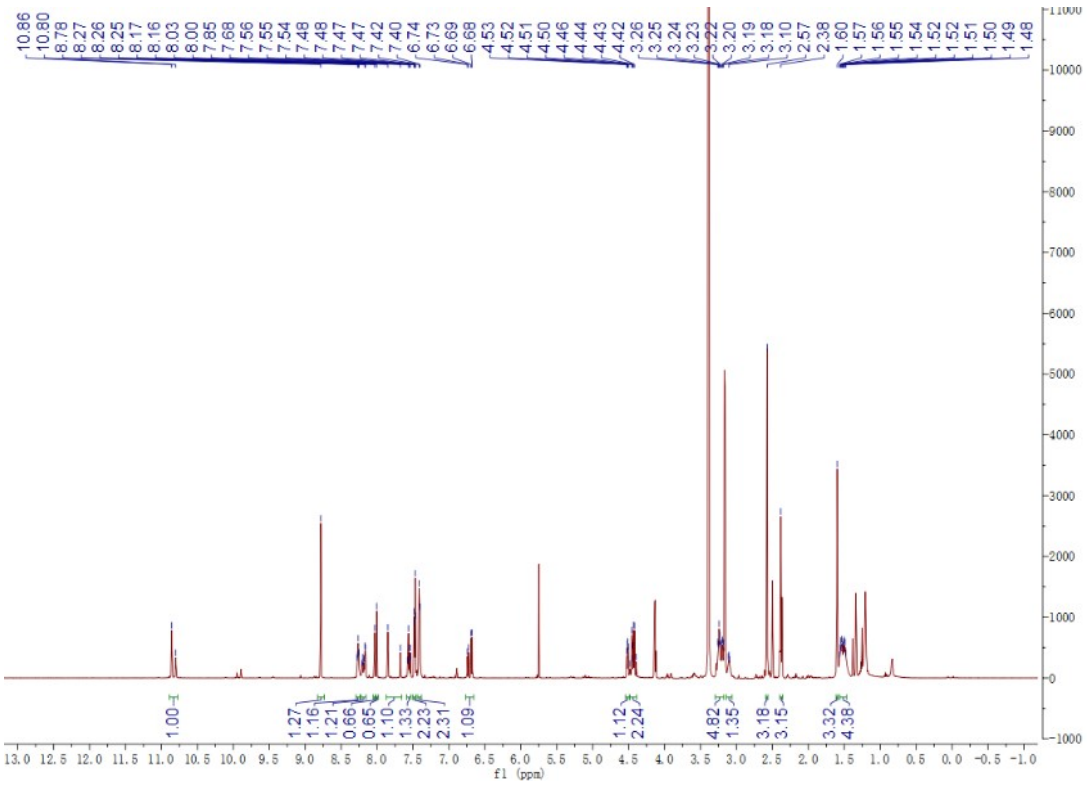
**The $^{1}$H NMR spectrum of 4**



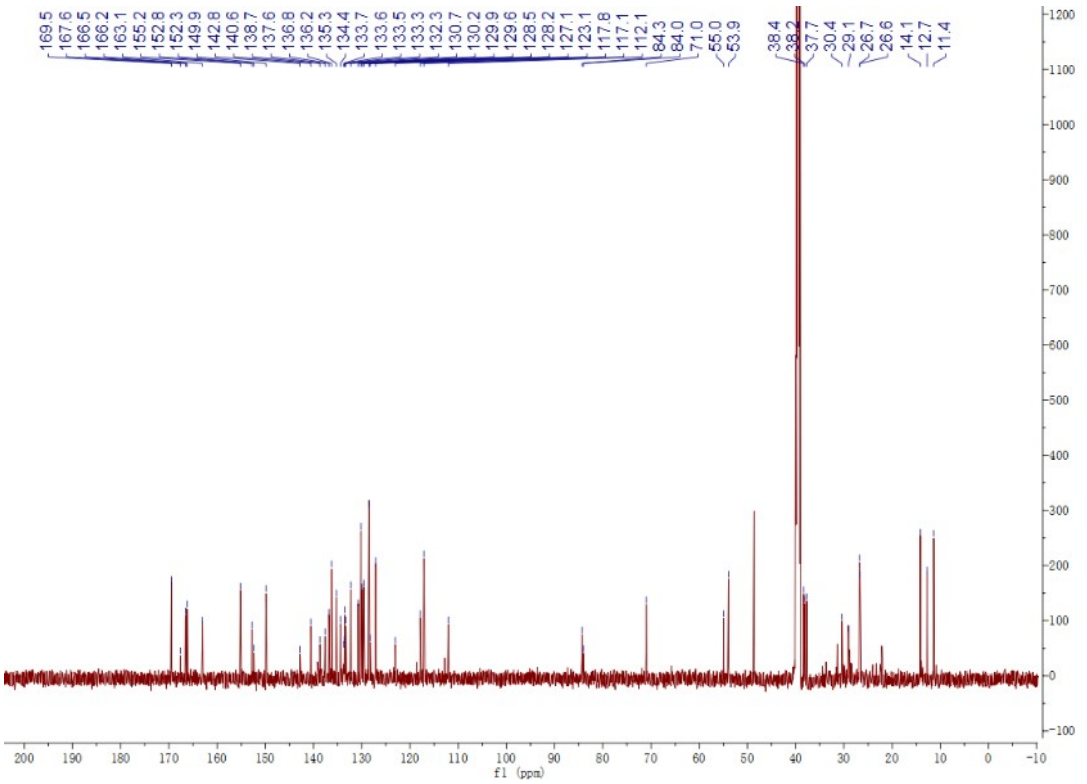

**The $^{1}$H NMR spectrum of 6a**



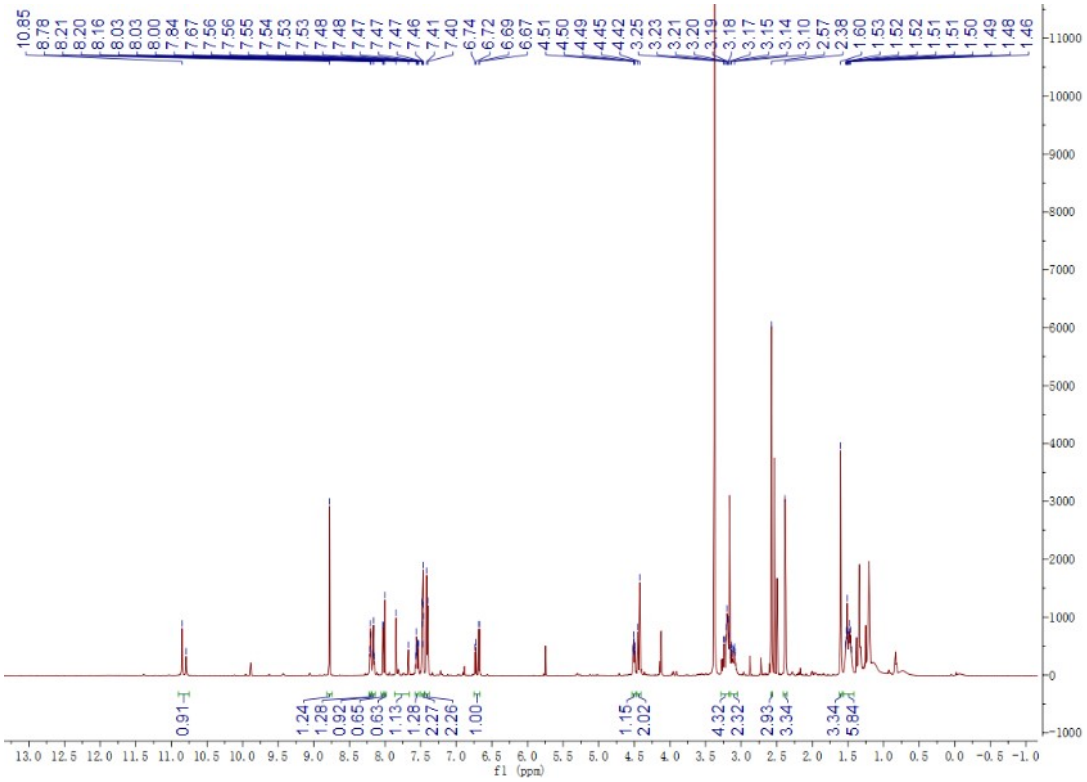
**The $^{1}$H NMR spectrum of 10a**



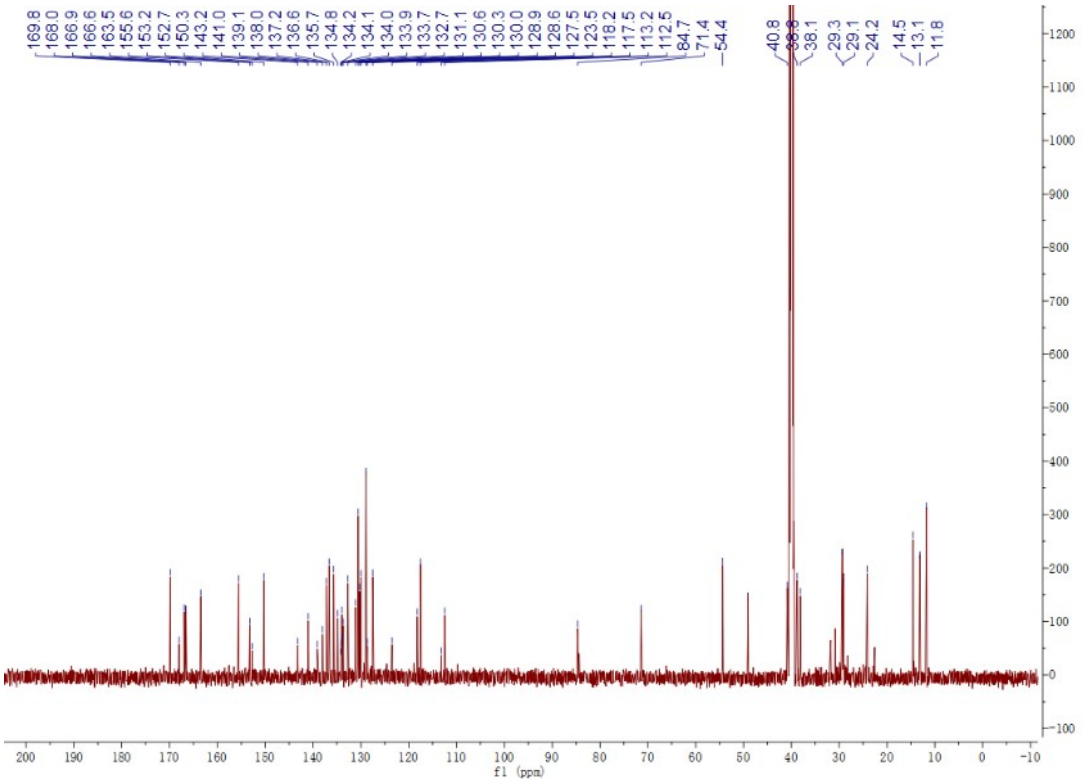

**The $^{13}$C NMR spectrum of 10a**



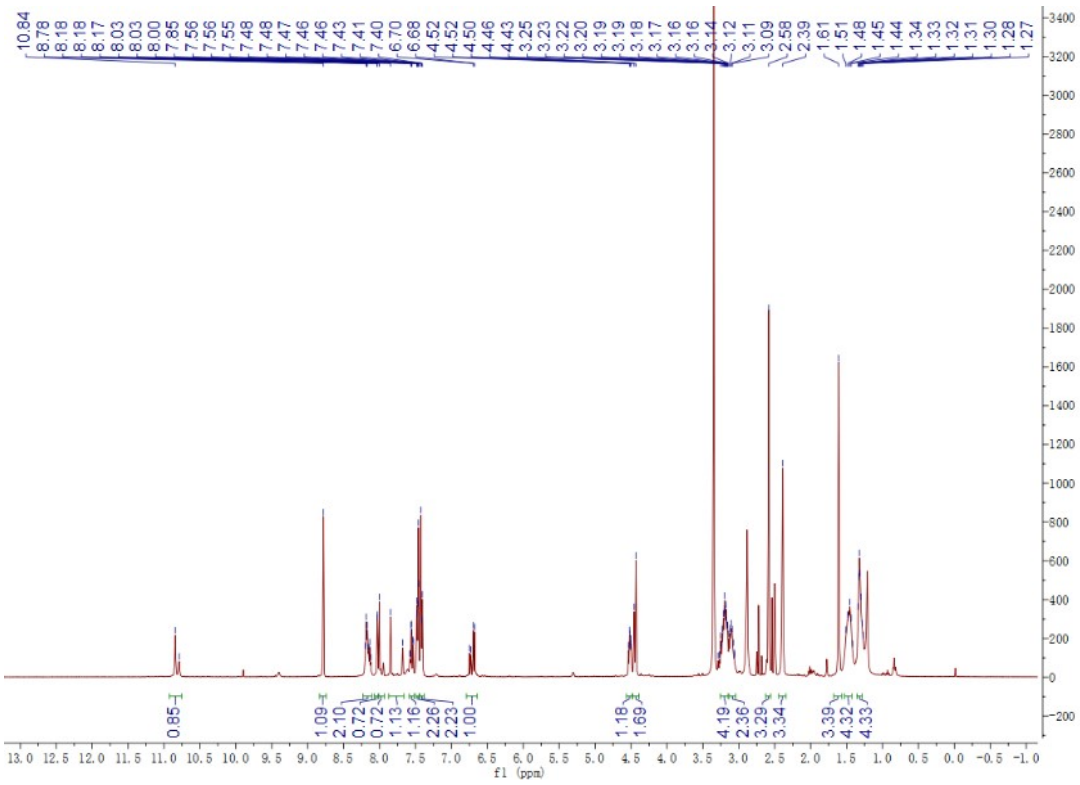
**The $^{1}$H NMR spectrum of 10b**



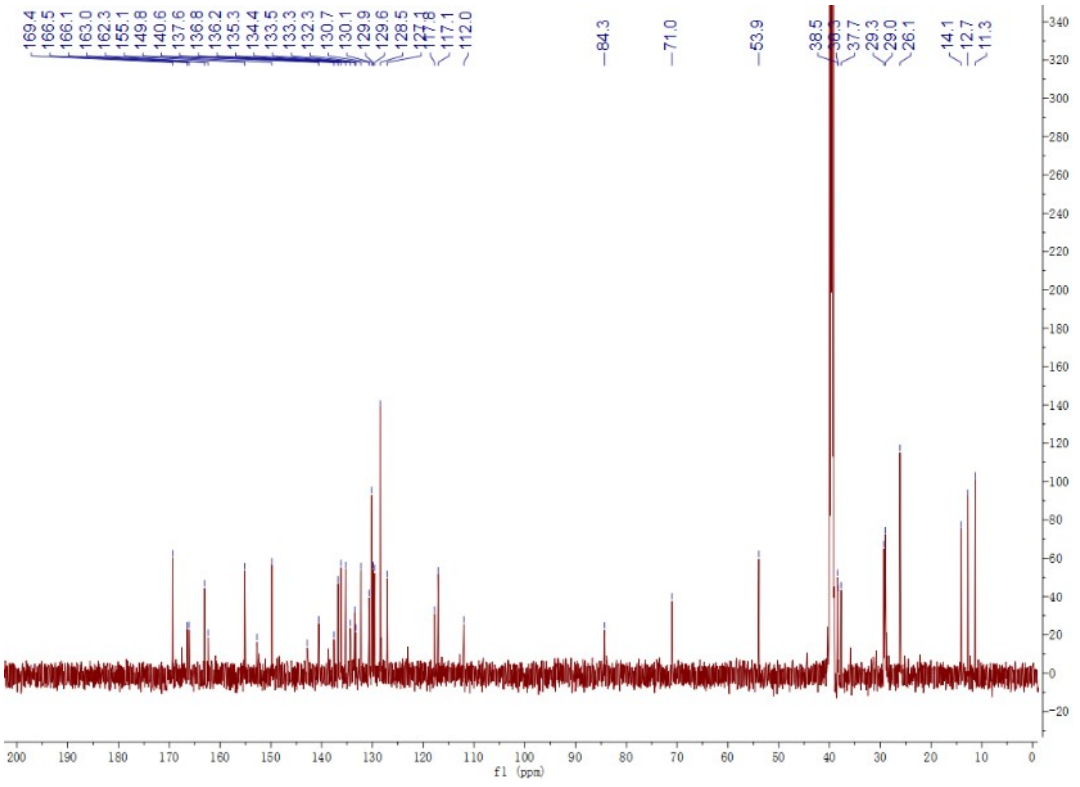
**The $^{13}$C NMR spectrum of 10b**



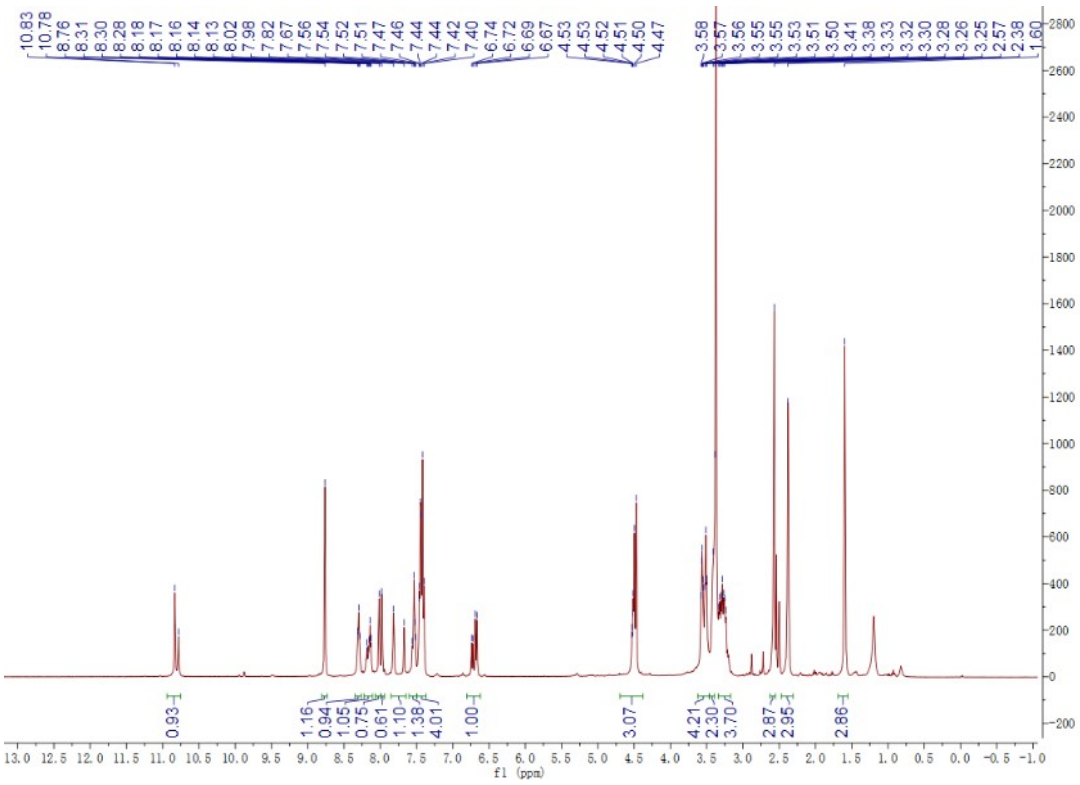
**The $^{1}$H NMR spectrum of 10c**



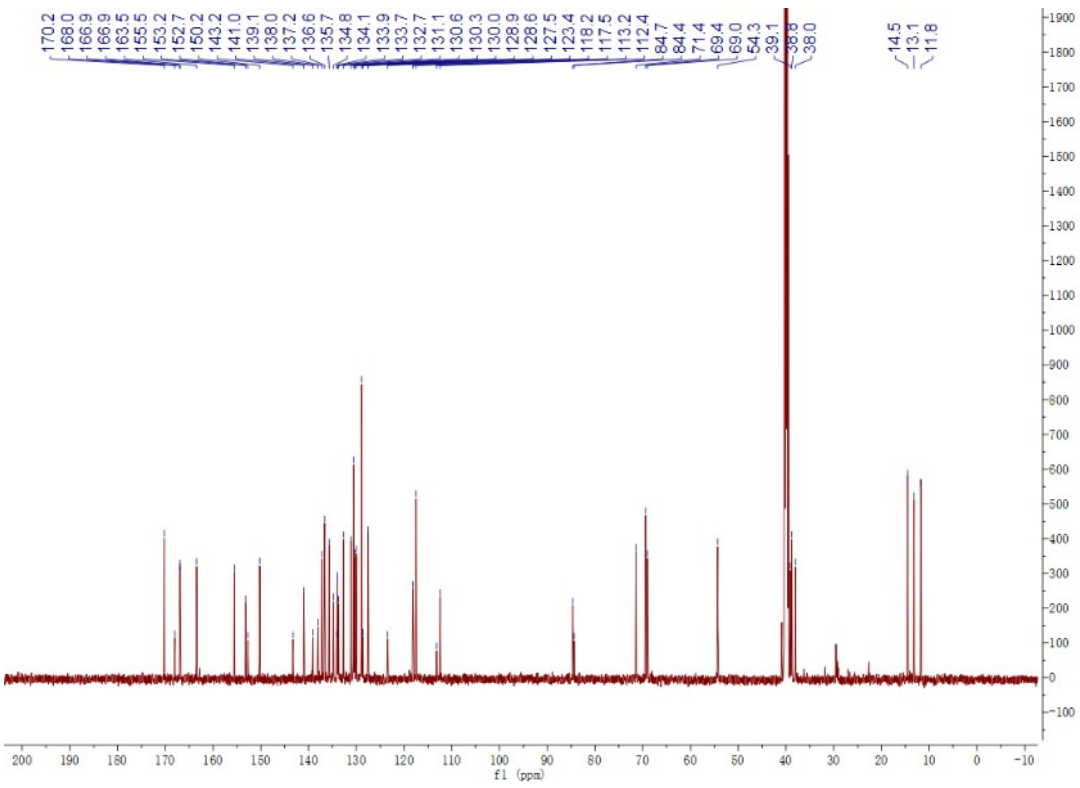
**The $^{13}$C NMR spectrum of 10c**



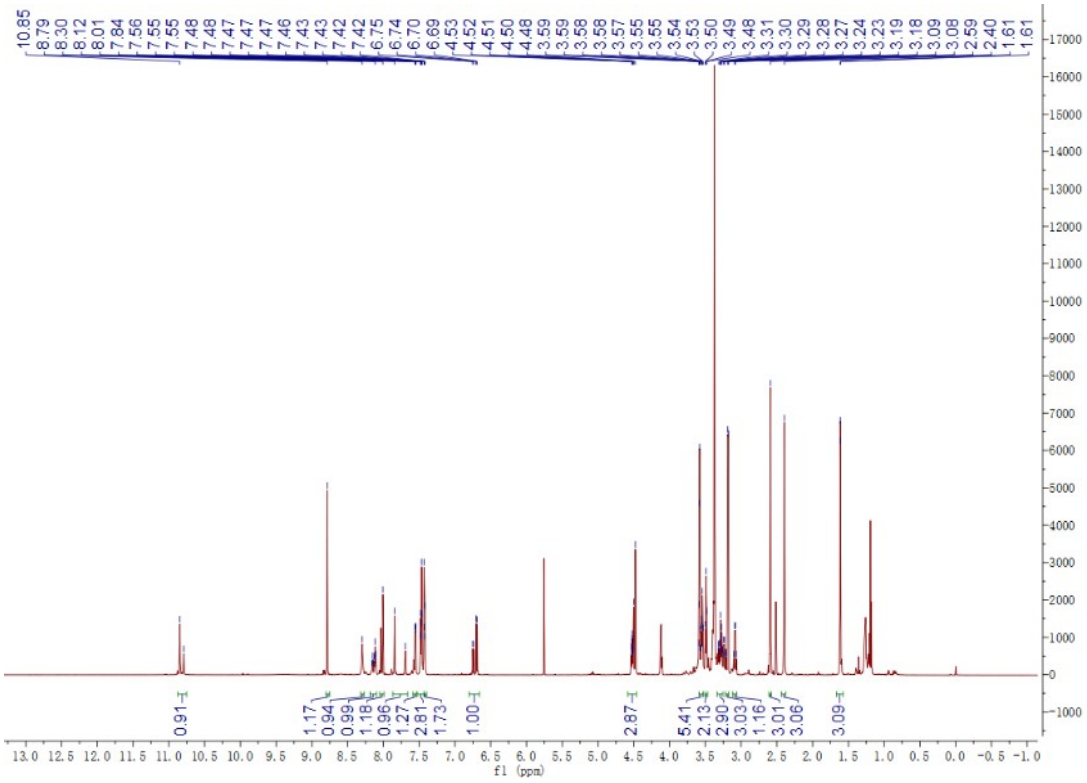
**The $^{1}$H NMR spectrum of 10d**



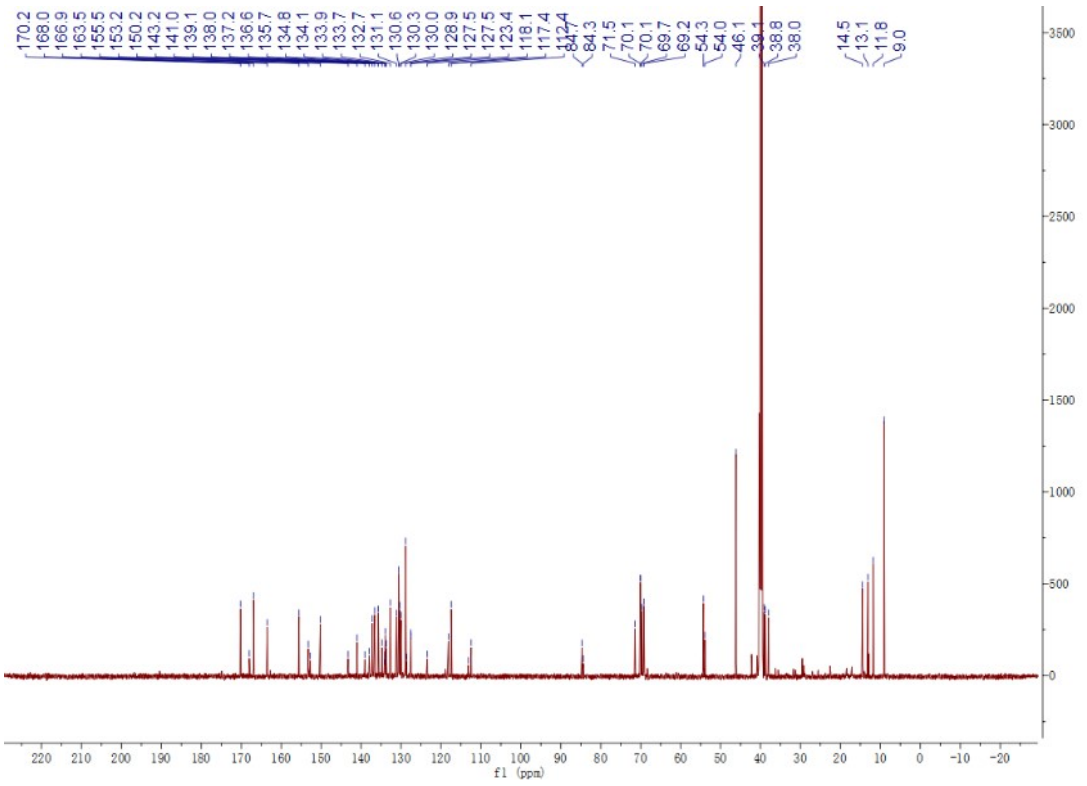
**The $^{13}$C NMR spectrum of 10d**



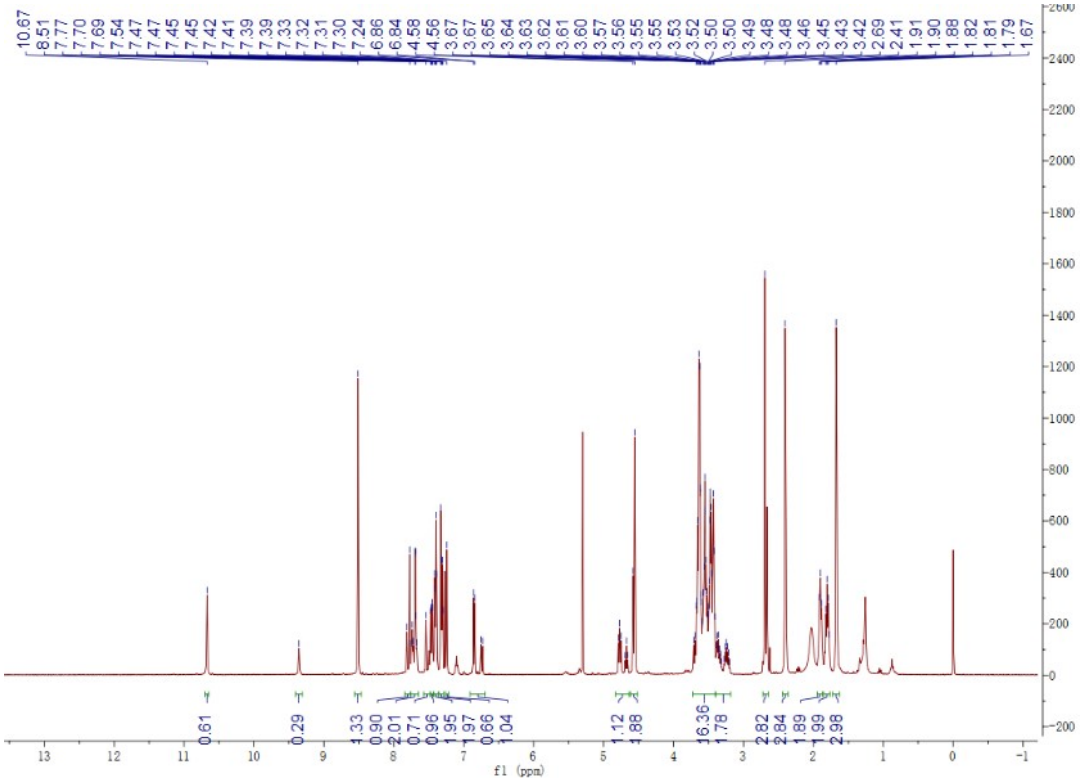
**The $^{1}$H NMR spectrum of 10e**



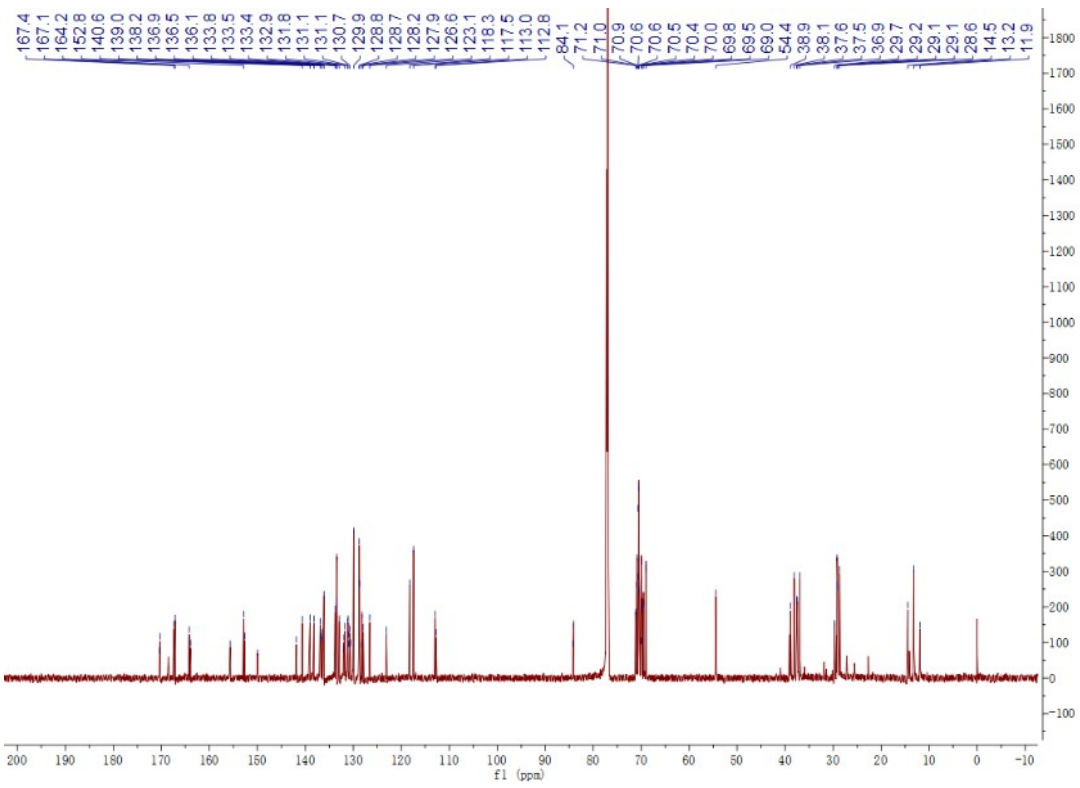
**The $^{13}$C NMR spectrum of 10e**



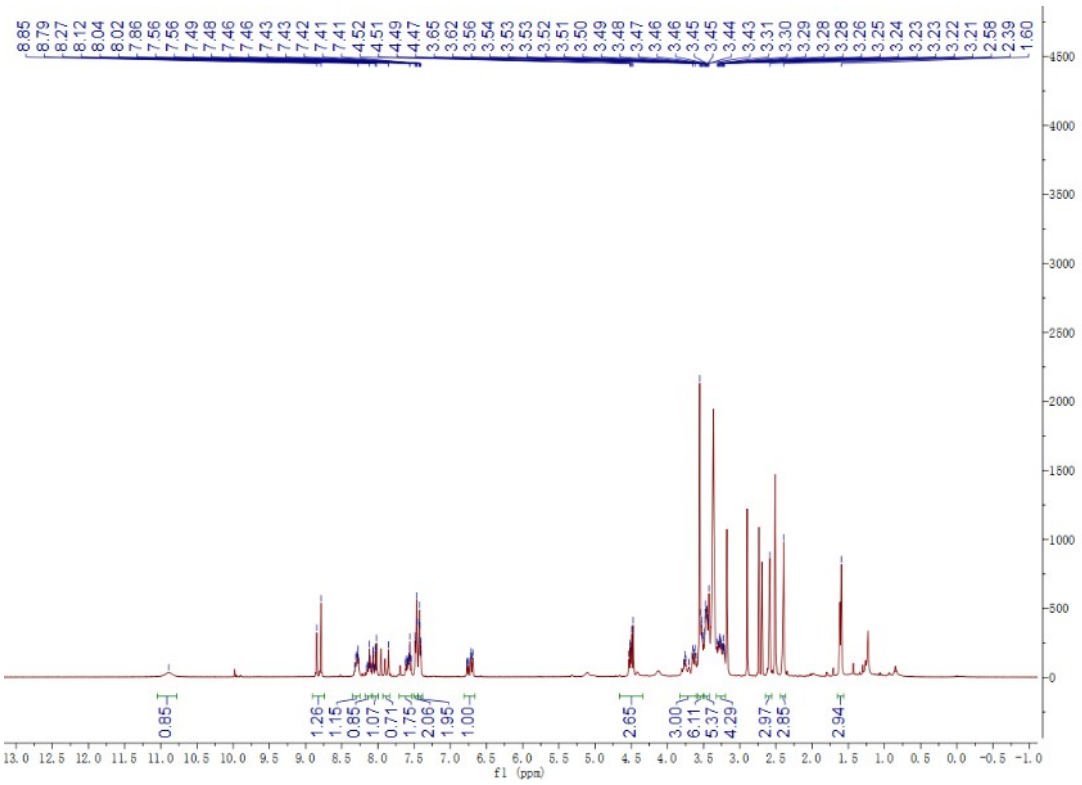
**The $^{1}$H NMR spectrum of 10f**



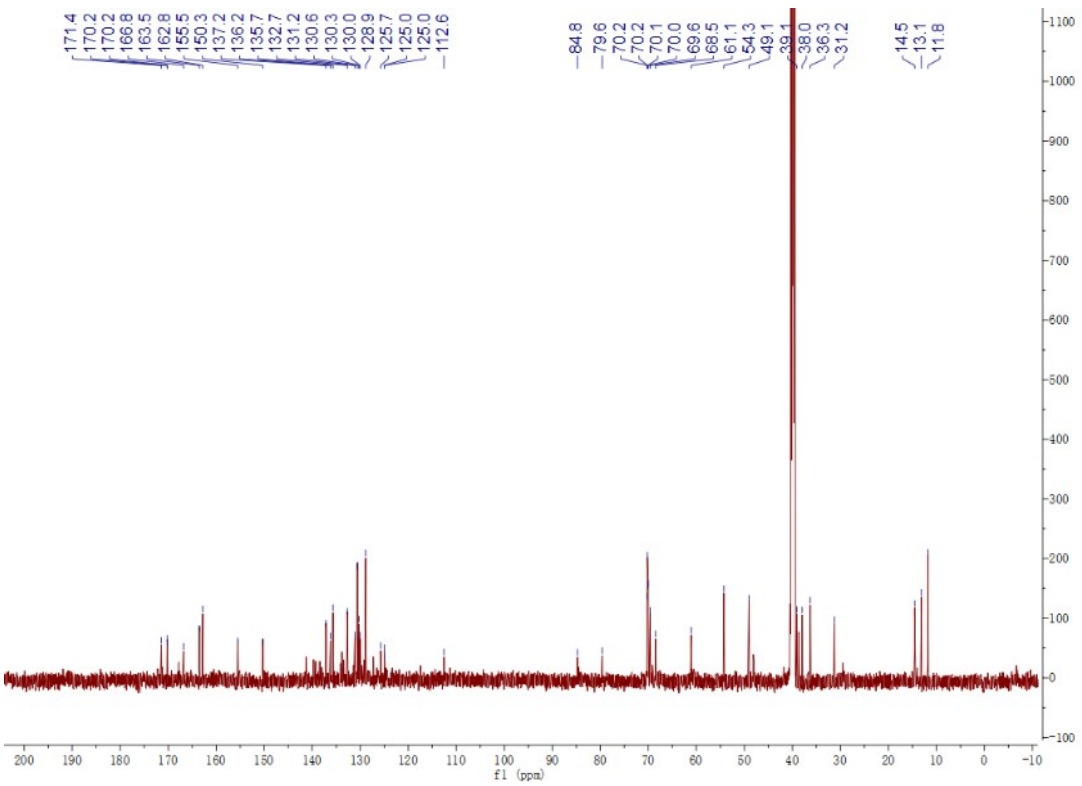
**The $^{13}$C NMR spectrum of 10f**



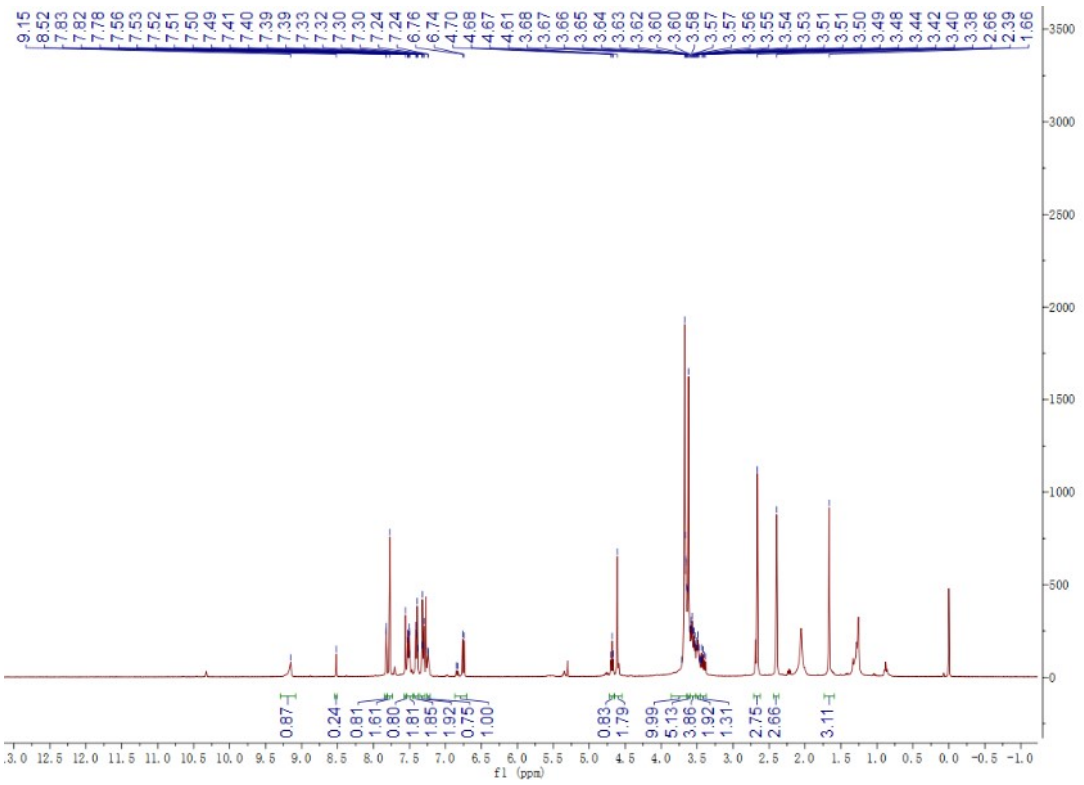
**The $^{1}$H NMR spectrum of 10g**



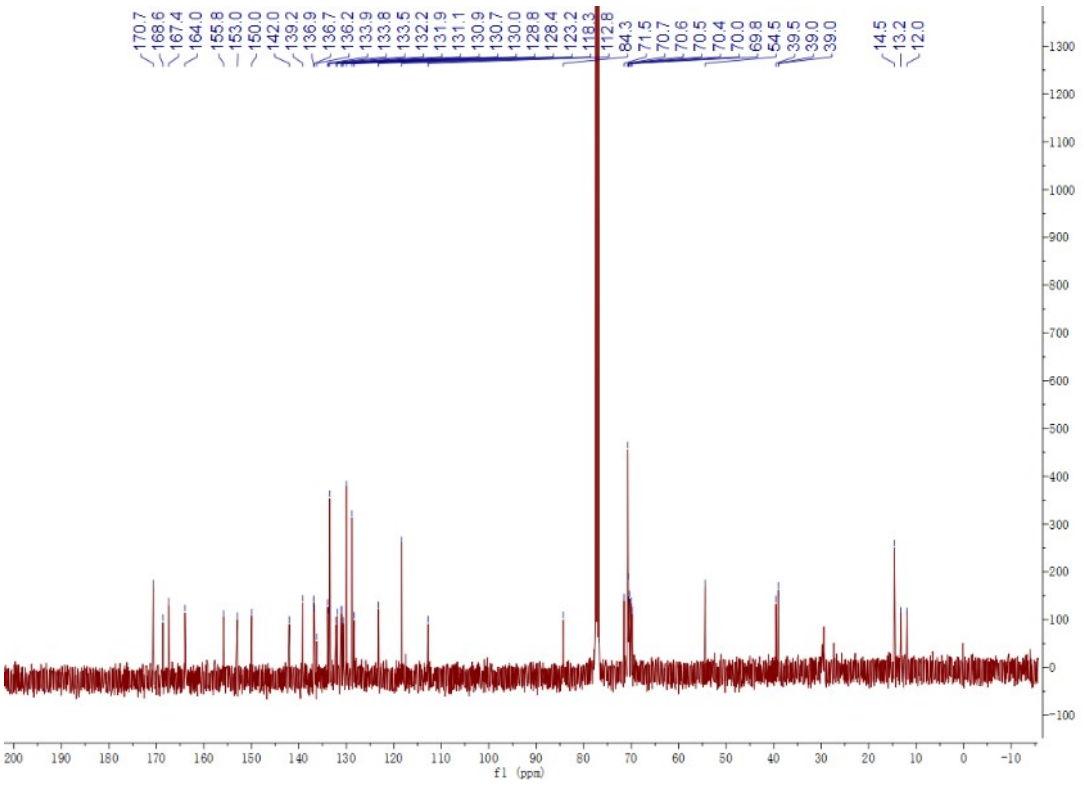
**The $^{13}$C NMR spectrum of 10g**



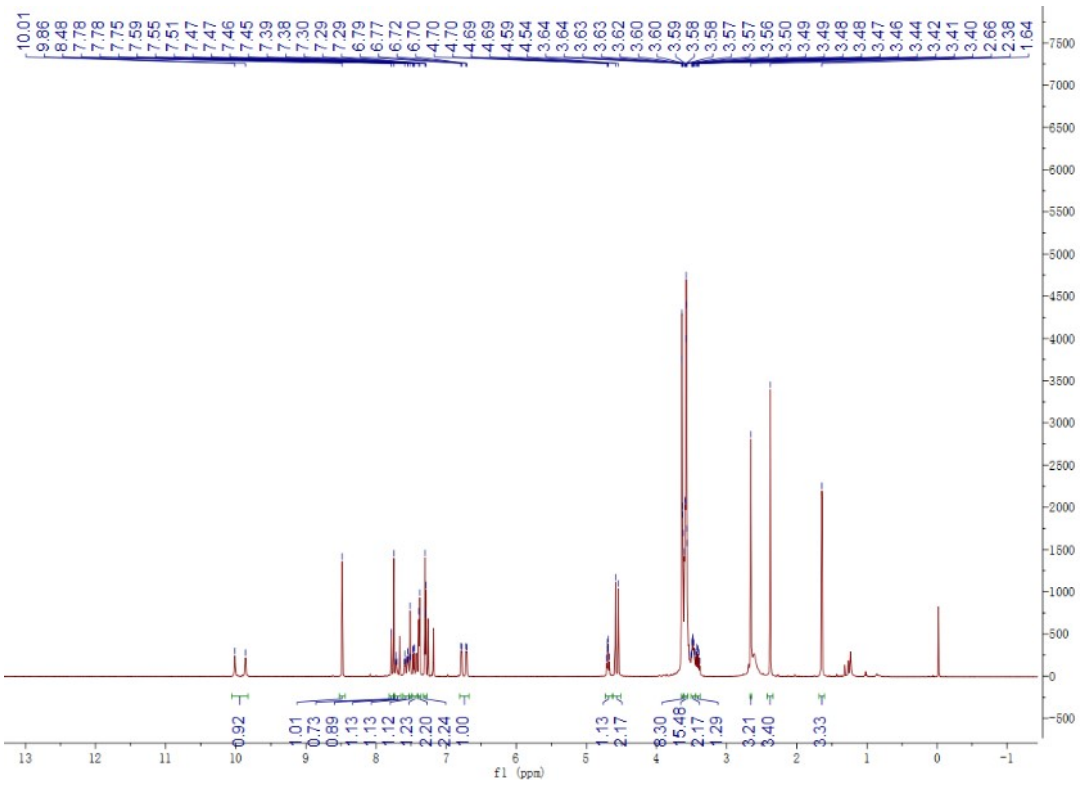
**The $^{1}$H NMR spectrum of 10h**



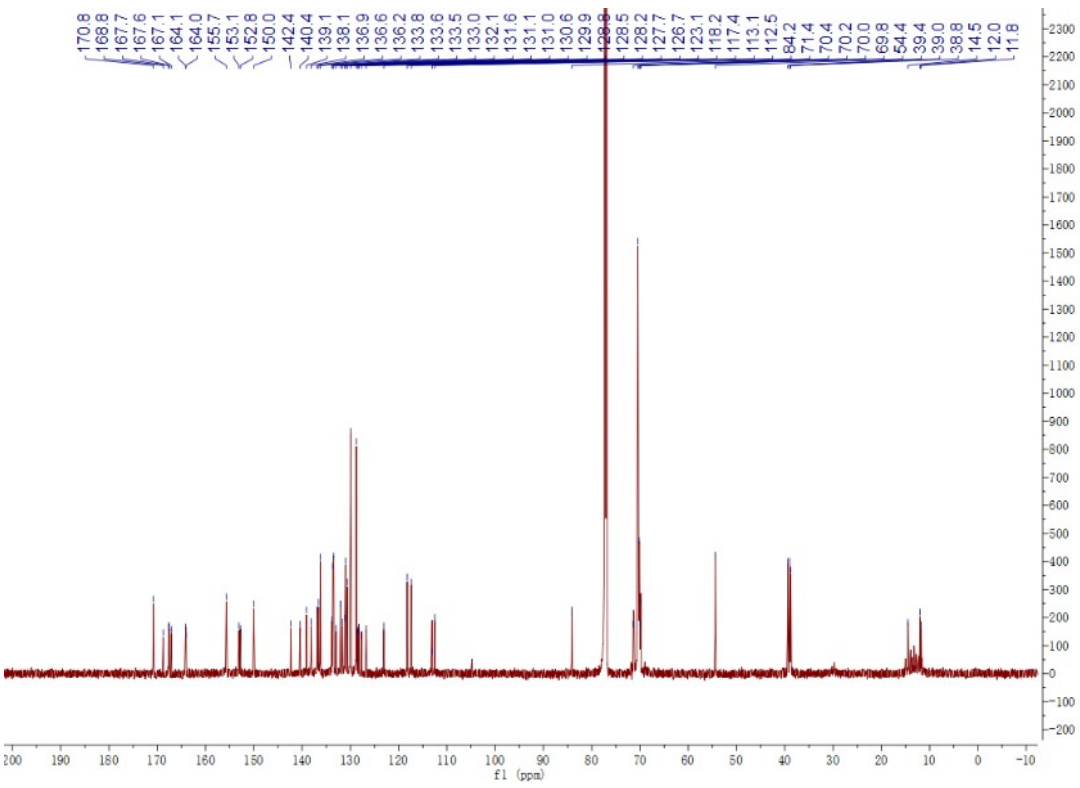

**The $^{13}$C NMR spectrum of 10h**



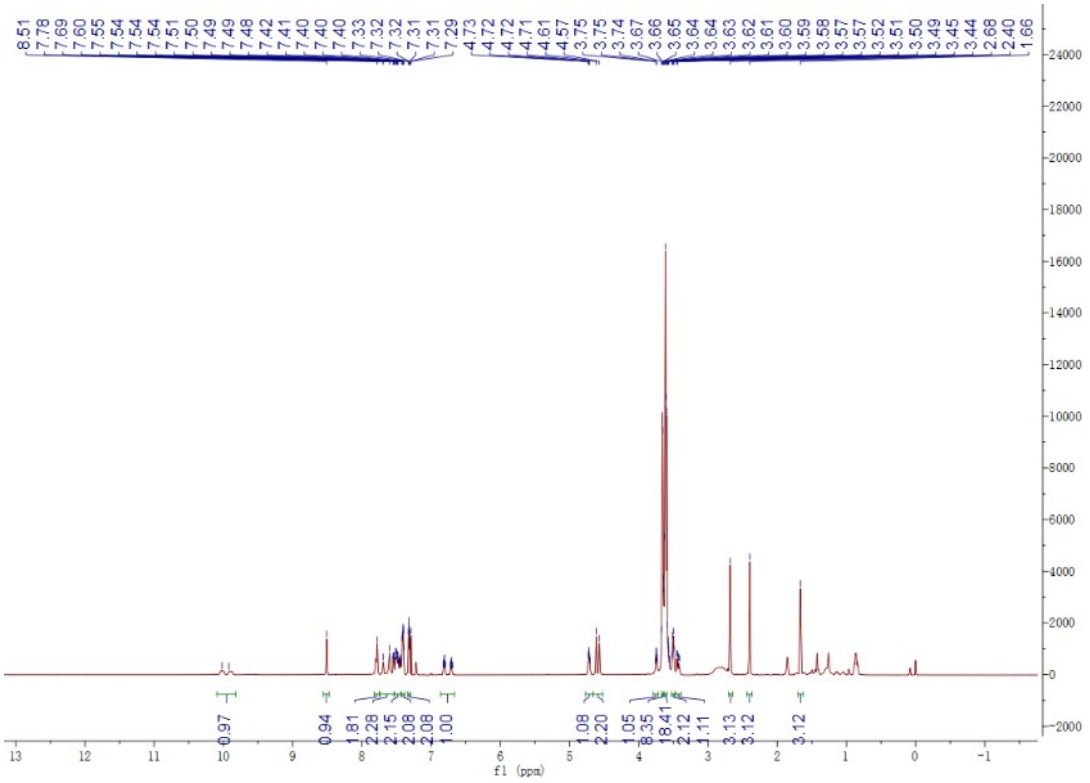
**The $^{1}$H NMR spectrum of 10i**



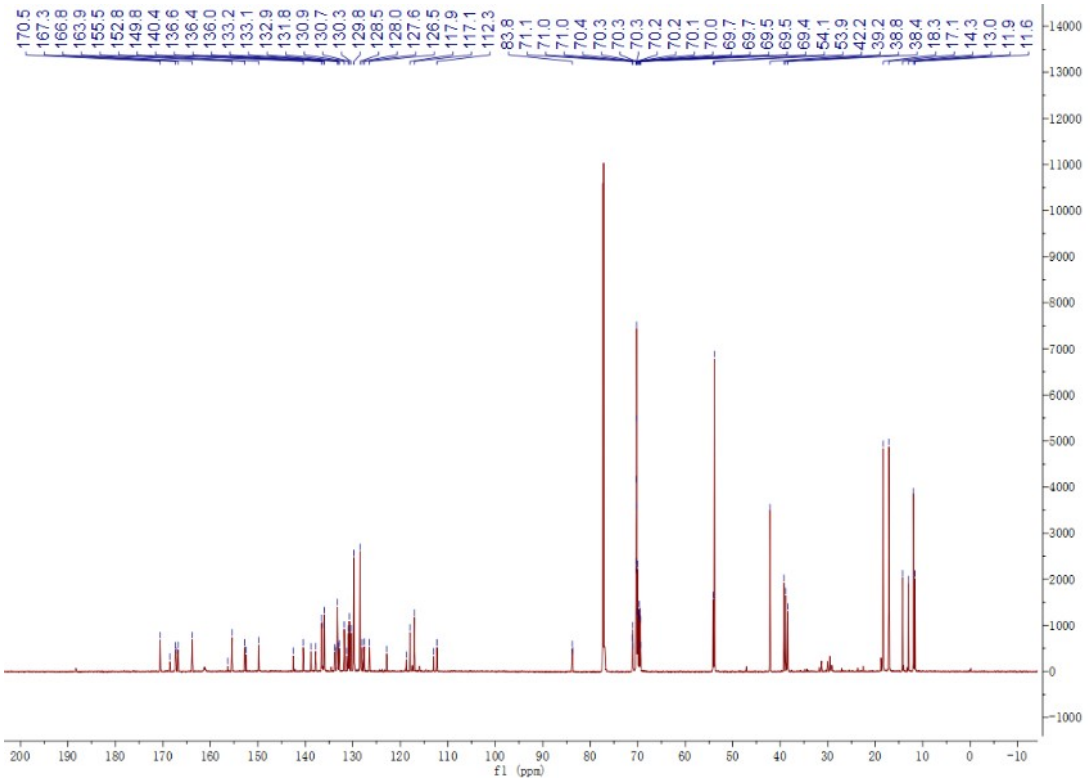
**The $^{13}$C NMR spectrum of 10i**



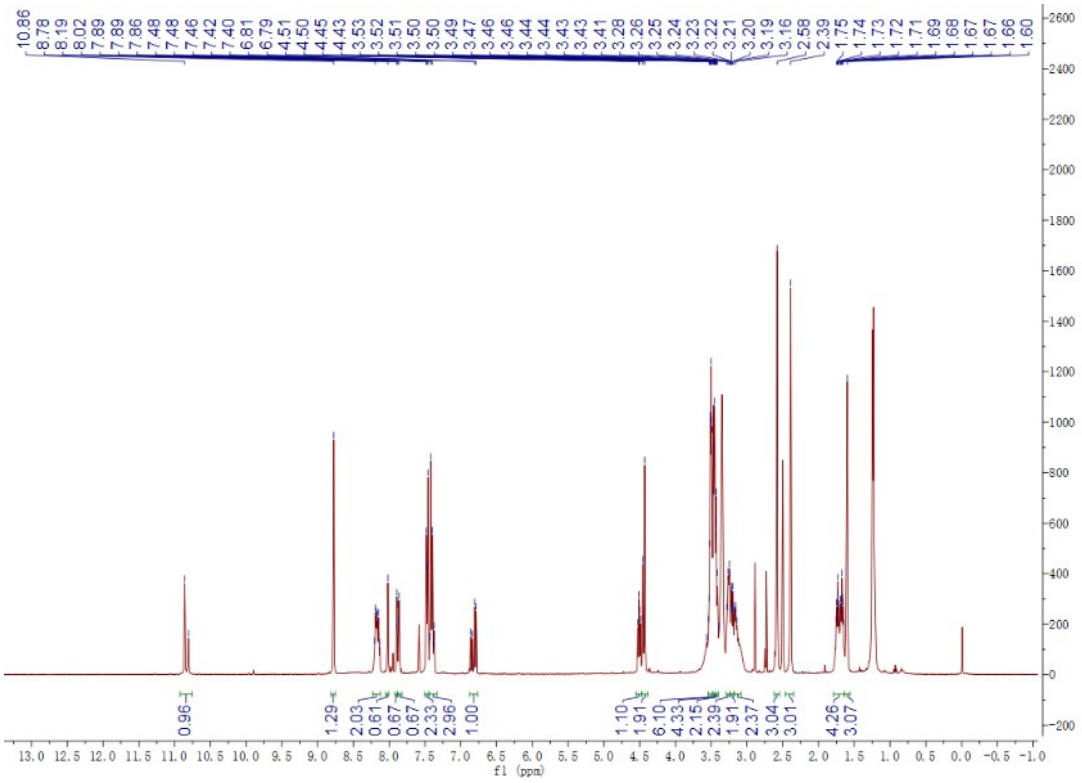
**The $^{1}$H NMR spectrum of 10j**



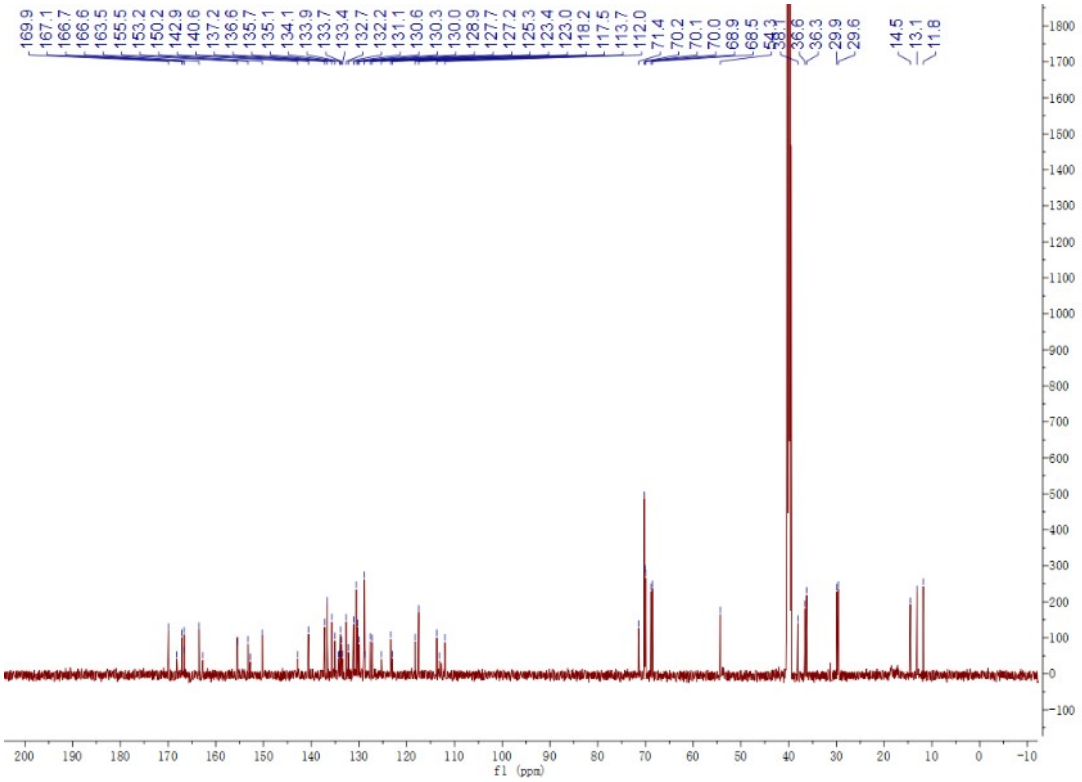

**The $^{13}$C NMR spectrum of 10j**



**The $^{1}$H NMR spectrum of 10k**

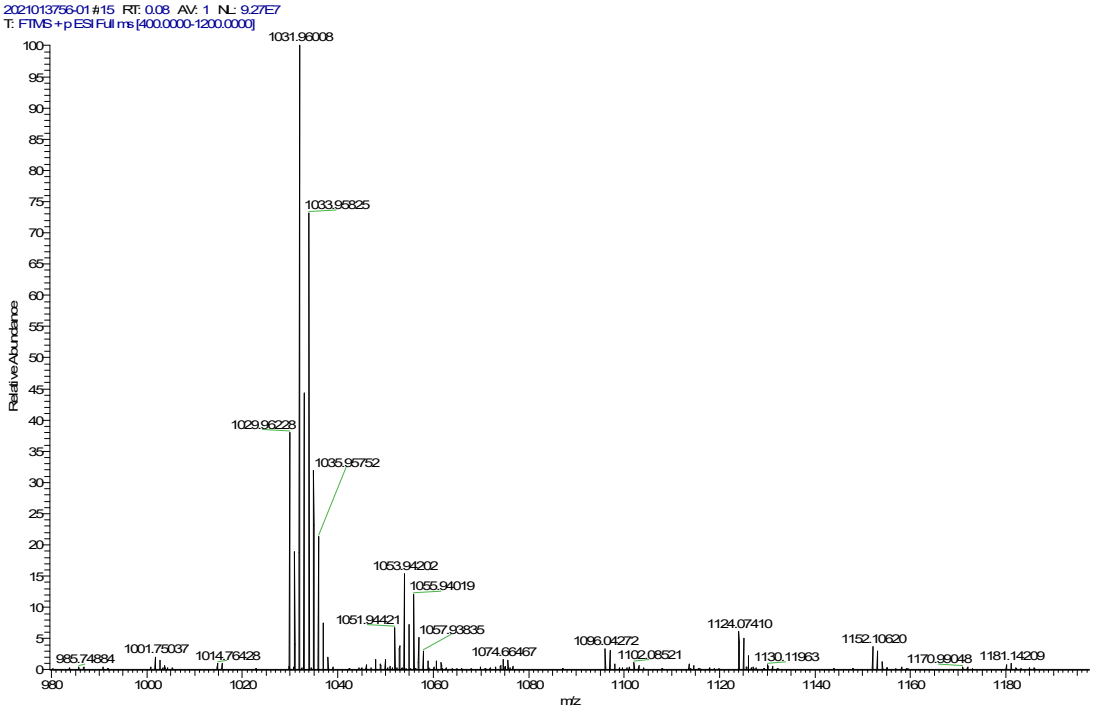


**The $^{13}$C NMR spectrum of 10k**

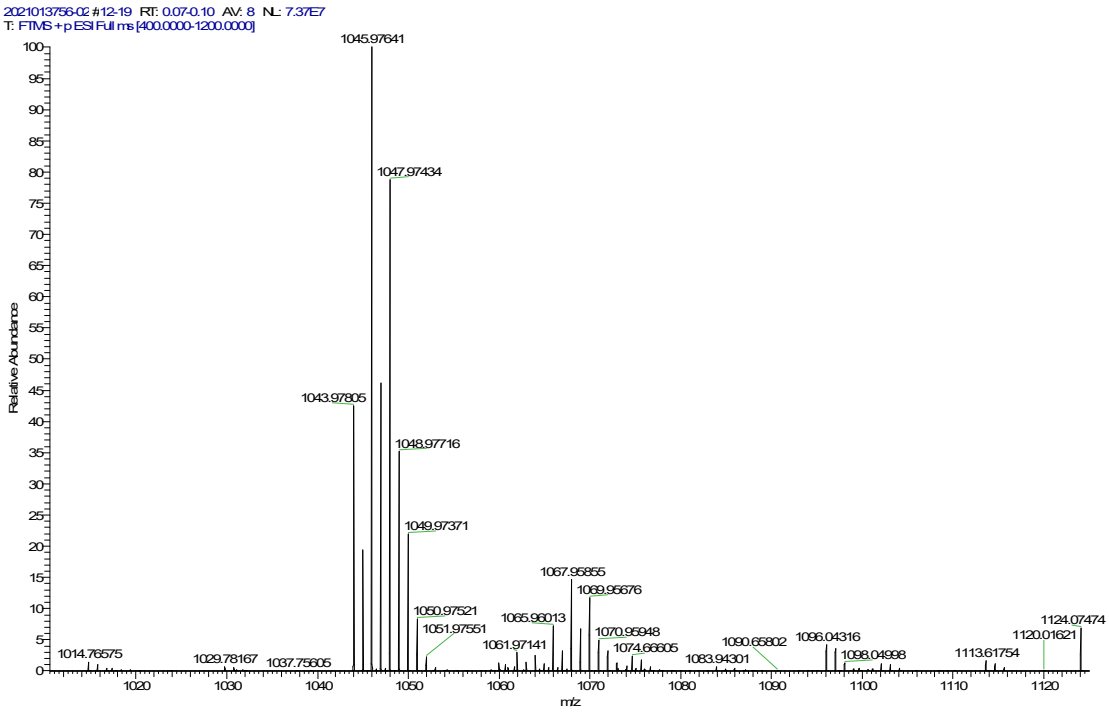


## The HR-MS (ESI) spectra

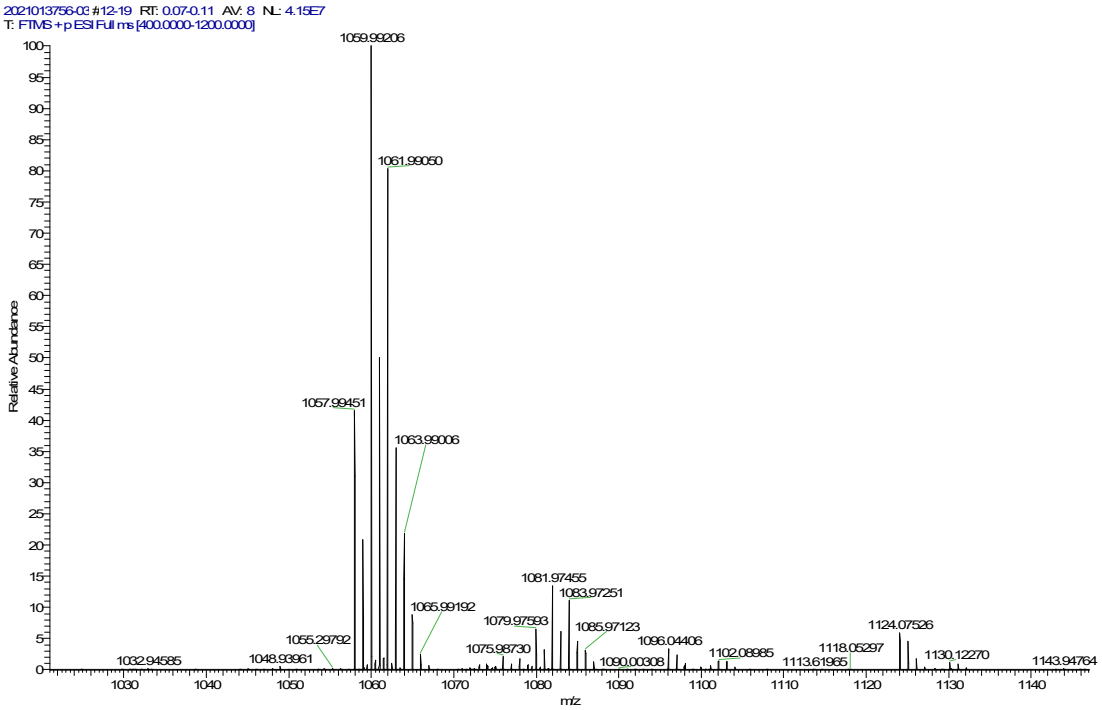
## The HR-MS (ESI) of 10a



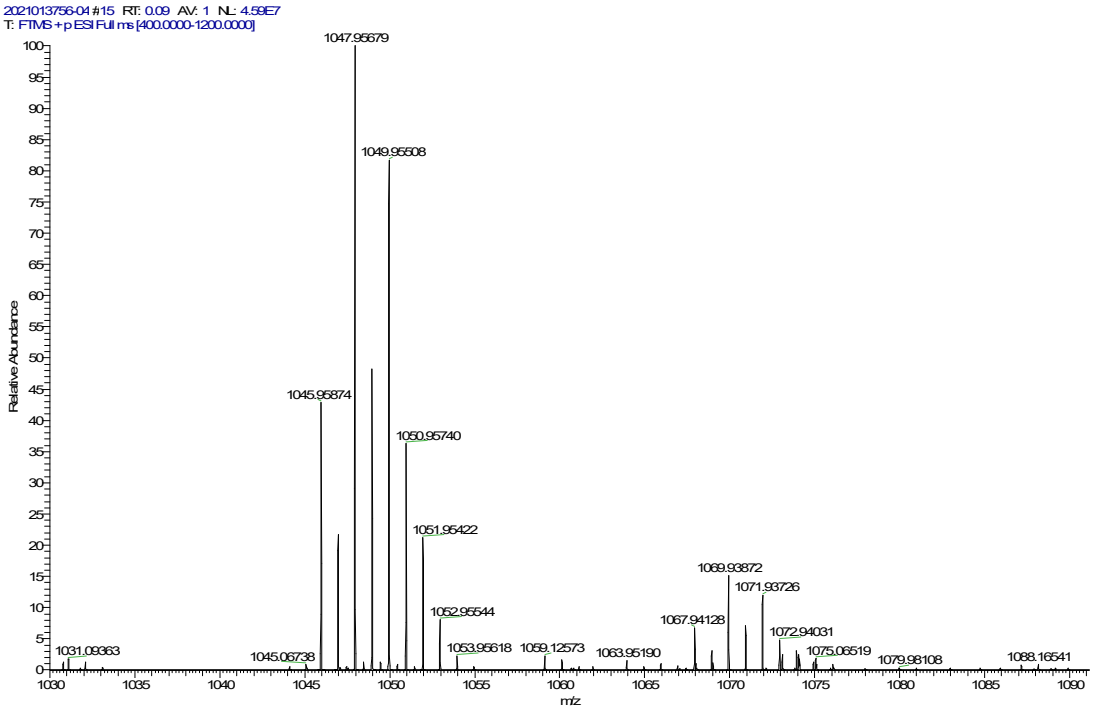

## The HR-MS (ESI) of 10b



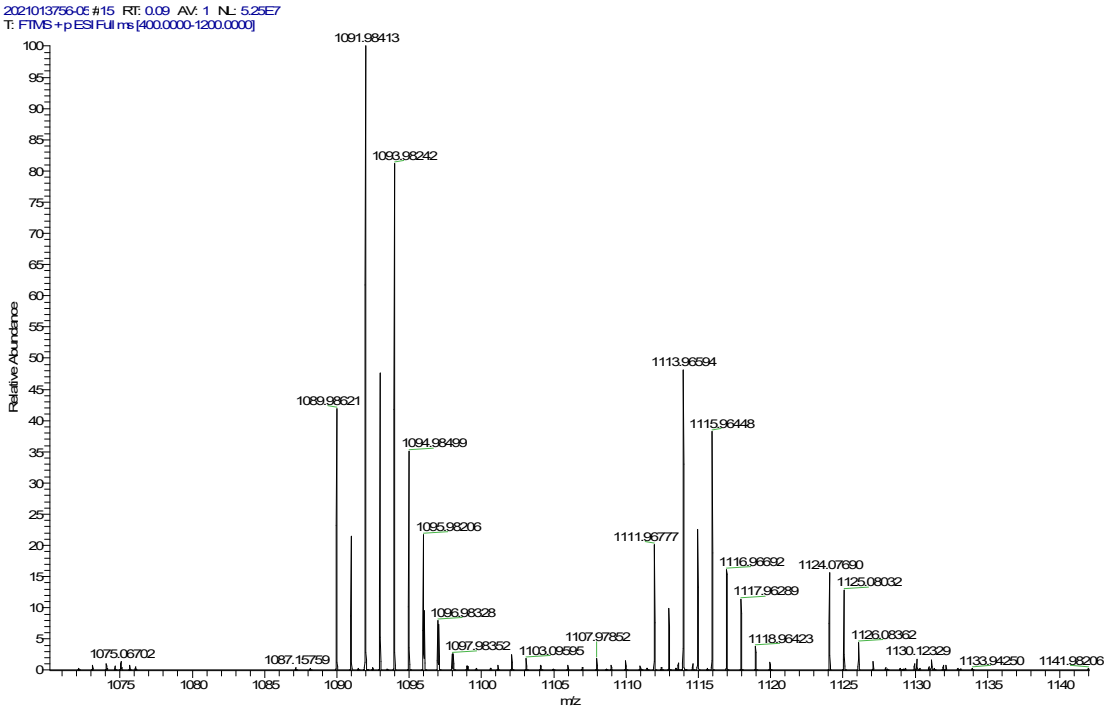
## The HR-MS (ESI) of 10c



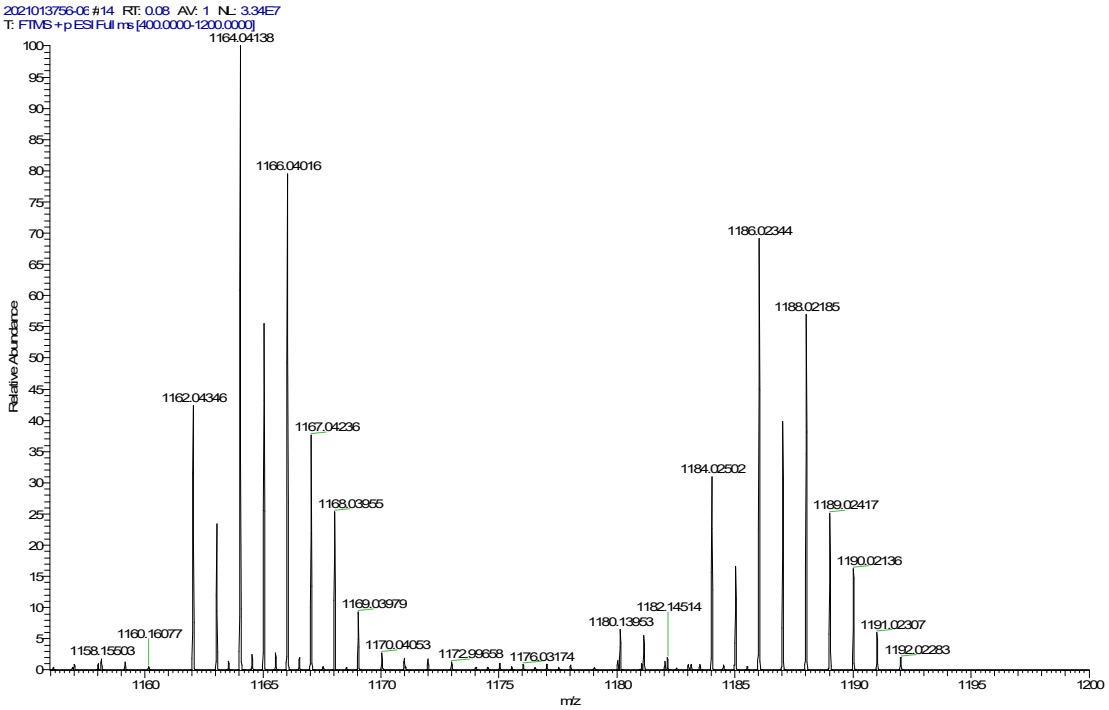

## The HR-MS (ESI) of 10d



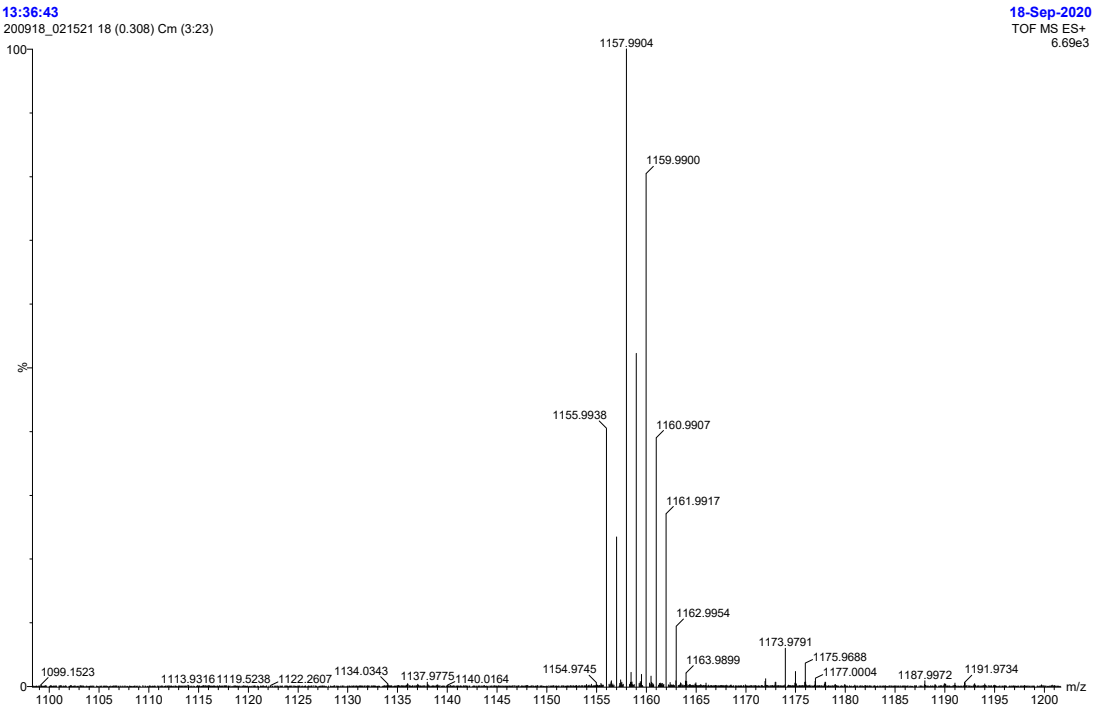
## The HR-MS (ESI) of 10e



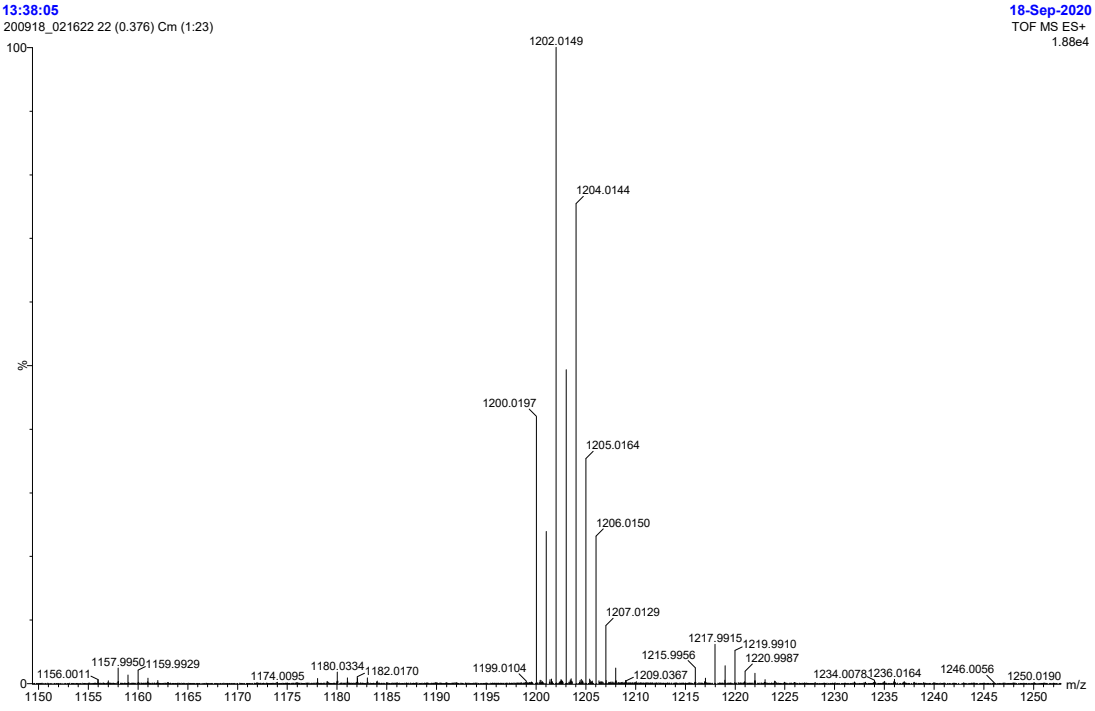

## The HR-MS (ESI) of 10f



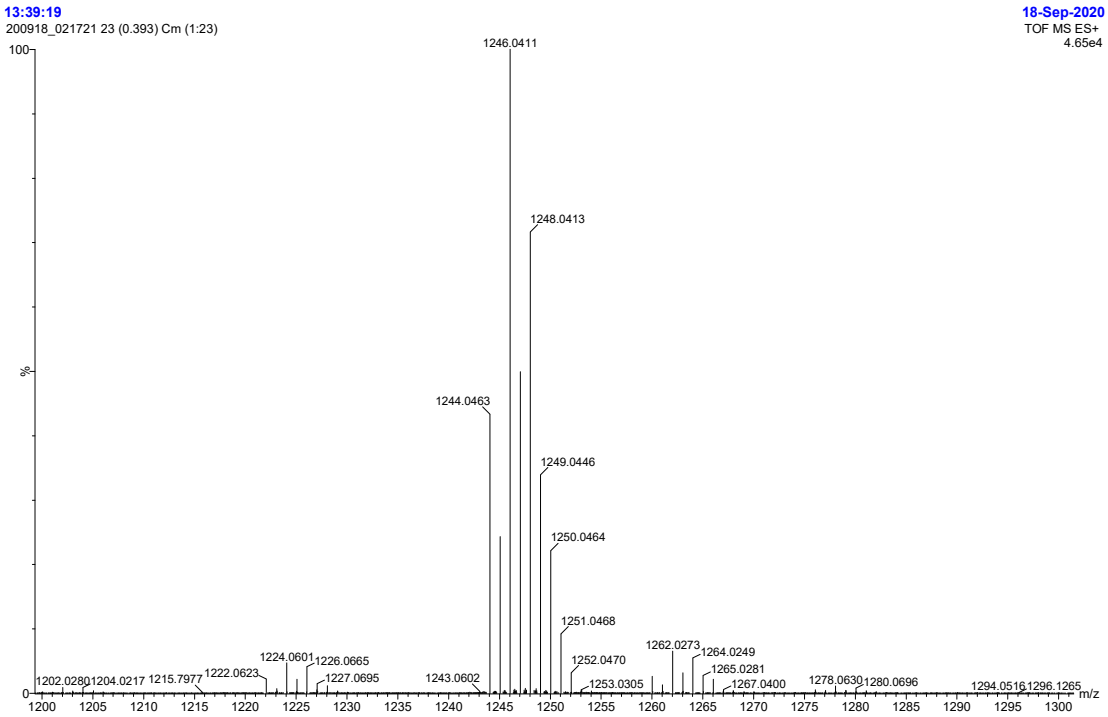
# The HR-MS (ESI) of 10g




# The HR-MS (ESI) of 10h



# The HR-MS (ESI) of 10i

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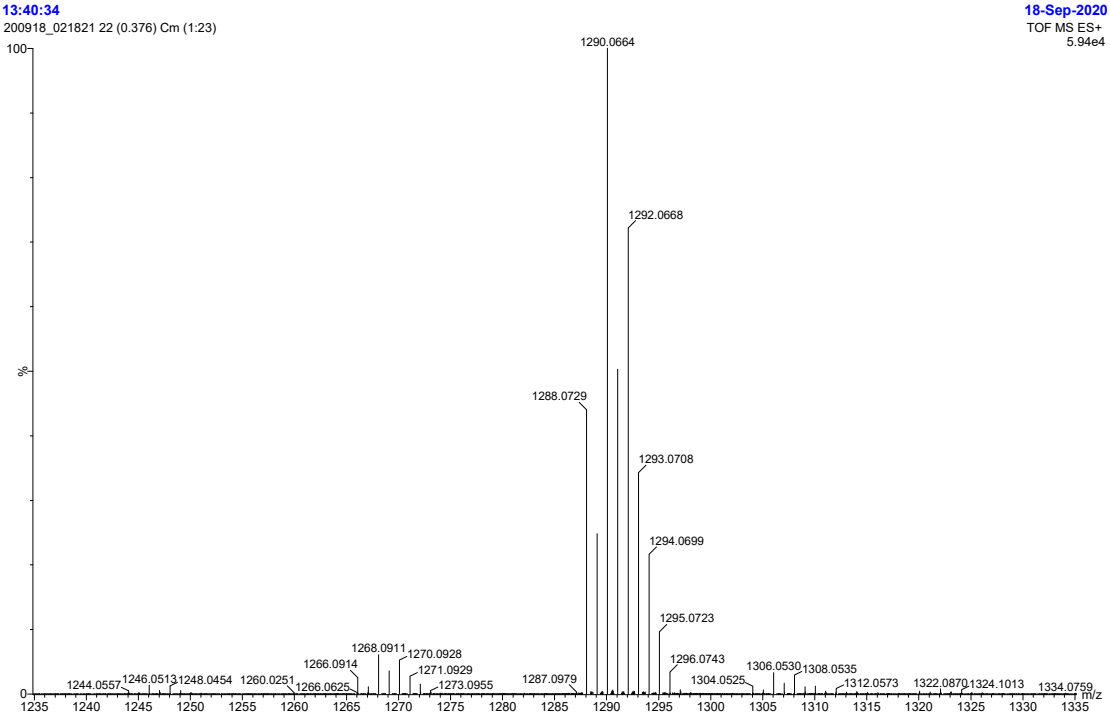

# The HR-MS (ESI) of 10k