Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Catalyst free hydrazone ligation for protein labeling and modification using electron-deficient benzaldehyde reagents

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1. General Information

a. Materials

All chemical reagents and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd., Alfa Aesar China Co. Ltd., CS Bio Co. (shanghai), GL Biochem (shanghai), Aladdin-reagent Co. (shanghai), J&K Chemical Co. Ltd. and were purified when necessary. TLC was executed on plates pre-coated with silica gel 60 F254 (250 layer thickness). Visualization was achieved using UV light, iodine vapors, permanganate solution. Flash column chromatographic purification of products was achieved using forced-flow chromatography on Silica Gel (200-300 mesh on small-scale or 300-400 mesh on large-scale). Manual peptide-synthesis apparatus was using the peptide synthesis vessel and in a constant-temperature shaker at 30℃.Automated peptide-synthesis apparatus was using a CS Bio 136XT automated synthesizer conducting with a 0.25 mmol resin scale.

b. HPLC

Analytical HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using analytical column (Grace Vydac "Protein & Peptide C18", 250 × 4.6 mm, 5 μm particle size, flow rate1.5 mL/min, R.T.). Analytical samples were monitored at 214 nm and 254 nm. Semi-preparative HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using a semi preparative column (Grace Vydac "Peptide C18", 250 × 10 mm, 10 μm particle size, flow rate 5 mL/min, rt). Solution A was 0.08 % trifluoroacetic acid in acetonitrile, and solution B was 0.1 % trifluoroacetic acid in ddH_2O.

c. Mass spectrometry and NMR

ESI-MS (/MS) spectra were recorded on a Finnigan LCQ Advantage MAX ion trap mass spectrometer (Thermo Fisher Scientific. USA) equipped with a standard ESI ion source. Data acquisition and analysis were done with the Xcalibur (version 2.0, Thermo quest Finnigan) software package.

^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer in deuteriochloroform ($CDCl_3$) with the solvent residual peak ($CDCl_3$: 7.26 ppm (^{1}H)as internal reference unless otherwise stated. ^{13}C-NMR spectra were recorded with^{1}H-decoupling on a Brucker 101 MHz spectrometer. Data are reported in the following order: chemical shifts are given (δ); multiplicities are indicated as br (broadened), s(singlet), d (doublet), t (triplet), q (quartet), m (multiplet), app (apparent); coupling constants, J, are reported (Hz); integration is provided.

d. General procedures for SPPS of peptides following the Fmoc strategy

The peptide synthesis reaction vessels were attained from commercial sources. Hydrazine 2CTC resin was initially swelled with DCM/DMF (1/1, V/V) about 0.5 h. For pre-activation of the first protected amino acid, 3.6 eq. of HCTU, 8 eq. of DIEA were added to a solution of 4 eq. protected amino acid in DMF. After pre-activation for 1 min, the mixture was added to the resin. After 1 h the resin was washed with DMF (3 times), DCM (3 times) and DMF (3 times). Then treatment with 20% piperidine/DMF (2 min, 10min), the resin was washed again with DMF (3 times), DCM (3 times) and DMF (3 times). Amino acid residues were coupled by a pre-activated solution of 4 eq. protected amino acid using 3.6 eq. HBTU, 8 eq. DIEA to the resin. After 1 h, the resin was washed with DMF (3 times), DCM (3 times) and DMF (3 times). Removal of Fmoc roup:

piperidine (20% in DMF) was added to the resin for 12 min (twice: 2 min and 10 min). Couplings were checked by ninhydrin test. Cleavage: A mixture of 88%TFA, 5% water, 5% phenol and 2% TIPS was added. After 2.5 h, the resin was washed with TFA. The combined solutions were concentrated by blowing with N_2. The crude peptides were acquired by precipitation with cold ether and centrifugation. The residue was dissolved in water, purified by preparative HPLC and analyzed by High-resolution ESI mass spectra.

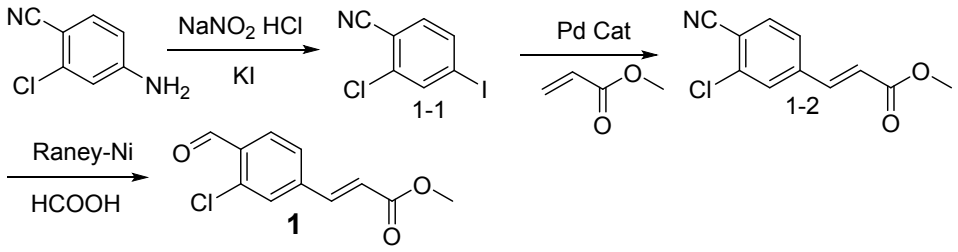
2. Experimental Section

a. Synthesis of hydrazine 2CTC resin and peptide hydrazides

Hydrazine 2CTC resin. 2-Chlorotrityl chloride resin (1 g, loading = 0.55 mmol/g) was swelled in 10 mL DCM/DMF (1/1, *v*/*v*) at 30℃. Then 10 mL $NH_2NH_2{\cdot}H_2O$/DMF (1/20, *v*/*v*) were added. The reaction was conducted overnight. 10 mL Methanol/DMF (1/20, *v*/*v*) was added to quench the remaining 2-Chlorotrityl chloride resin. After 30 min, the resin was washed with DMF, H_2O, Methanol, Ethylether and kept under high vacuum for 2 h.

Peptide synthisis. Hydrazine 2CTC resin was initially swelled with DCM/DMF (1/1, *v*/*v*) about. 30 min. Then 3.6 eq. of HCTU, 8 eq. of DIEA were added to a solution of 4 eq. protected amino acid in DMF for the pre-activation of the first protected amino acid. After pre-activation for 1 min, the mixture was added to the resin. After 1 h the resin was washed with DMF (3 times), DCM (3 times) and DMF (3 times). Removal of Fmoc roup: piperidine (20% in DMF) was added to the resin for 12 min (twice: 2 min and 10 min). The resin was washed again with DMF (3 times), DCM (3 times) and DMF (3 times). Cleavage: A mixture of 88%TFA, 5% water, 5% phenol and 2% TIPS was added. After 2.5 h, the resin was washed with TFA. The combined solutions were concentrated by blowing with N_2. The crude peptides were acquired by precipitation with cold ether and centrifugation. The residue was dissolved in water, purified by preparative HPLC and analyzed by LC-MS.

b. Synthesis of compound 1 and 2

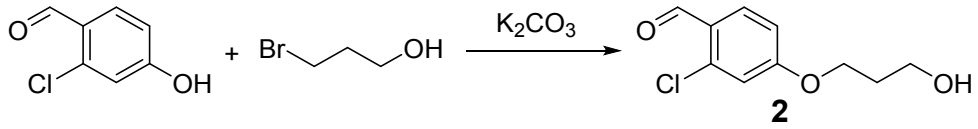


2-chloro-4-iodobenzonitrile (1-1). 4-amino-2-chlorobenzonitrile (1.5 g, 10 mmol) was dissolved in water (5ml) . Hydrochloric acid (12 mmol) was added dropwise and stirred for 30 mins. Then Sodium nitrite (0.76 g, 11 mmol) was added to the mixture and stirred for 10 mins. After that, potassium iodide (1.82 g, 11 mmol) was added. The mixture was stirred for another 1 h. Then the reaction was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by VLC to yield 2-chloro-4-iodobenzonitrile (2.24 g, 8.5 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 1.4 Hz, 1H), 7.74 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.37 (d, *J* = 8.2 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.73, 136.59, 134.40, 115.52, 112.86, 100.20. HRMS *m/z* $[M + H]^+$ calcd for $C_7H_4ClIN^+$ 263.90715, found 263.90649.

methyl (E)-3-(3-chloro-4-cyanophenyl)acrylate (1-2). 2-chloro-4-iodobenzonitrile (2.24 g, 8.5 mmol), palladium diacetate (190 mg, 0.85 mmol) , methyl acrylate (0.8ml, 8.93mmol) and potassium carbonate (1.79 g, 21.25 mmol) were dissolved in dry DMF (17mL) under nitrogen. The mixture was stirred for 10 h under 70℃. Then the reaction was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by VLC to yield methyl (E)-3-(3-chloro-4-cyanophenyl)acrylate (0.95 g, 4.3 mmol). ^{1}H

NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.0 Hz, 1H), 7.65 (s, 1H), 7.61 (d, *J* = 16.3 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 6.53 (d, *J* = 16.0 Hz, 1H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.21, 141.11, 140.12, 137.50, 134.34, 128.93, 126.33, 122.66, 115.61, 114.08, 52.17. HRMS *m/z* $[M + H]^+$ calcd for $C_{11}H_9ClNO_2^+$ 222.03163, found 222.03078.

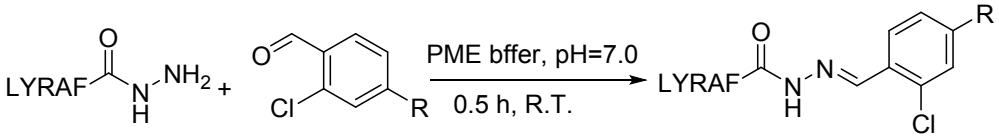
methyl (E)-3-(3-chloro-4-formylphenyl)acrylate (1). Then this compound was dissolved in formic acid (25 mL). Raney-Ni was added to the mixture. The reaction mixture was heated to reflux (105℃) for 4 h. The crude product was purified by VLC to yield compound 1 (0.44 g, 2 mmol). ^1H NMR (400 MHz, $CDCl_3$) δ 10.49 (dd, *J* = 12.1, 0.7 Hz, 1H), 7.94 (d, *J* = 8.1 Hz, 1H), 7.63 (d, *J* = 16.1 Hz, 1H), 7.59 (d, *J* = 1.5 Hz, 1H), 7.52 (dd, *J* = 8.1, 0.7 Hz, 1H), 6.55 (d, *J* = 16.0 Hz, 1H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.02, 166.44, 141.71, 141.05, 138.36, 132.96, 129.83, 129.81, 126.51, 122.12, 52.11. HRMS *m/z* $[M + H]^+$ calcd for $C_{11}H_{10}ClO_3^+$ 225.0313, found 225.0271.



2

2-chloro-4-(3-hydroxypropoxy)benzaldehyde (2). 2-chloro-4-hydroxybenzaldehyde (1.5 g, 10 mmol), potassium carbonate (1.65 g, 12 mmol) and 3-Bromo-1-propanol (1.2 mL, 13 mmol) was added to a round-bottom flask and stirred for 2 h. Then the mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography to yield compound 2 (1.1 g, 5,3 mmol). ^1H NMR (400 MHz, $CDCl_3$) δ 10.31 (t, *J* = 1.8 Hz, 1H), 7.88 (d, *J* = 8.7 Hz, 1H), 6.94 (t, *J* = 6.9 Hz, 1H), 6.89 (ddd, *J* = 7.0, 3.5, 2.9 Hz, 1H), 4.19 (t, *J* = 6.1 Hz, 2H), 3.86 (q, *J* = 6.1 Hz, 2H), 2.12 – 2.02 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.69, 163.98, 139.78, 131.03, 125.97, 115.70, 114.03, 65.83, 59.37, 31.71. HRMS *m/z* $[M + H]^+$ calcd for $C_{10}H_{12}ClO_3^+$ 215.0469, found 215.0391.

c. Conjugates between model peptides and different aldehydes



The peptide hydrazide (30 μM) were added to the aqueous PME buffer (100 mM PIPES, 1 mM $MgSO_4$, 2 mM EGTA at pH 7.0). Aldehyde derivatives firstly dissolved in ethanol (1g/mL) for stock solution and then added to reaction buffer in 37.5 μM final concentration. Then, the mixture was stirred at neutral pH and 25 ℃ for about 30 min before the yield was determined by RP-HPLC and LC-MS.

Conjugates **1a** (LYRAF-$NHNH_2$ + compound 1) was obtained according to the general procedure. LC-MS analysis: $[M + H]^+$ *m/z* calcd 889.43, found 889.55.

Conjugates **2a** (LYRAF-$NHNH_2$ + compound 2) was obtained according to the general procedure. LC-MS analysis: $[M + H]^+$ *m/z* calcd 879.84, found 879.40.

Conjugates **3a** (LYRAF-$NHNH_2$ + compound 3) was obtained according to the general procedure. LC-MS analysis: $[M + H]^+$ *m/z* calcd 805.79, found 805.80.

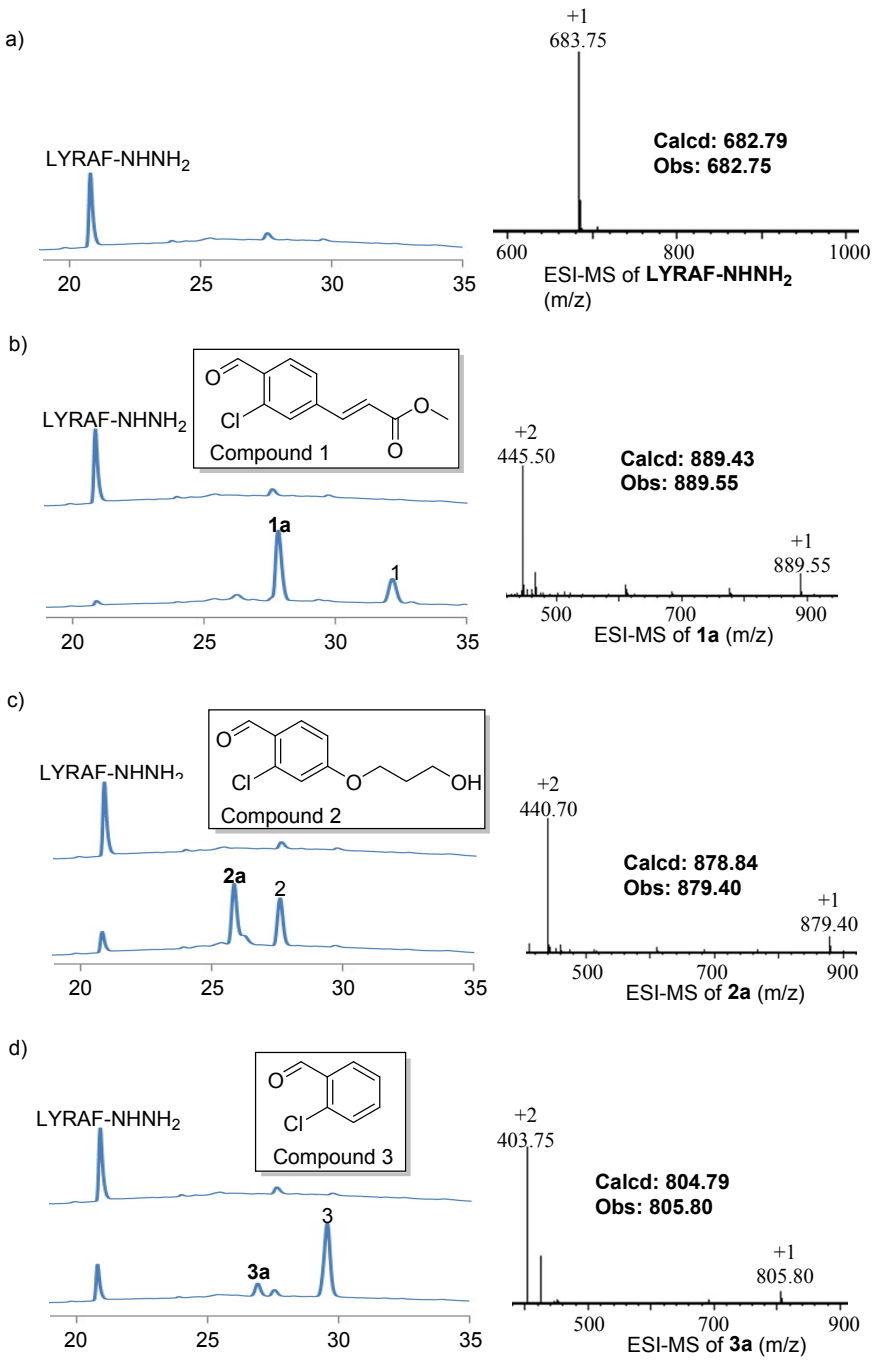


Figure S1: The chromatogram of the reactions between model peptide hydrazides Leu-Tyr-Arg-Ala-Phe-$NHNH_2$ and different aldehydes.

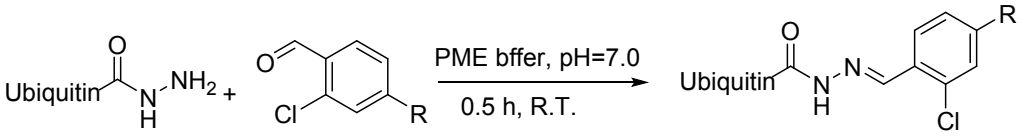
d. The expression of Ub-$NHNH_2$ and the reaction between Ub-$NHNH_2$ and different aldehydes

Ub sequence:

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRL RG

Plasmid pTYB2-Ub(1-75) was transformed into E. coli BL21(DE3) Chemically Competent Cell and monoclonal colony was selected to grow in 10 ml LB with 100 μg/ml ampicillin overnight at 37 ℃ under shaking at 220 rmp. The 10 ml LB with E.coli was transferred into 1 l LB with 100 μg/ml ampicillin and shaked at 37 ℃ until OD 600 reached 0.8. 0.2 mM IPTG was added to induce expression of protein. The cell was grown at 18 ℃for another 18 h and then could be harvest by

centrifugation at 4500g for 30 min at 4°C. After the cell resuspension and ultrasonification in lysis buffer (500 mM NaCl, 20 mM Hepes, and 1 mM EDTA, pH 7.5), the supernatant was separated from the pellet by centrifugation at 12000g for 30 min. Then it was loaded on about 10 ml chitin beads, which had been pre-equilibrated with lysis buffer. After about 1 h, the column was washed by lysis buffer for about 30 column volume. Finally, Ub(1–75)-$NHNH_2$ was cleaved from chitin beads by incubating the column in cleavage buffer (500 mM NaCl, 20 mM Hepes, 1 mM EDTA, 8% hydrazine hydrate, pH 7.5) for 3 h. After purified by HPLC and lyophilized, Ub(1-75)-$NHNH_2$ was obtained. The yield was about 3 mg/L. The protein hydrazine was characterized by ESI-MS.[1]



The aldehyde derivatives were firstly dissolved in ethanol (1g/mL) for stock solution and then added to reaction buffer in 100 μM final concentration. Ub-$NHNH_2$ was dissolved in 6 M Gn.HCl (1mg in 100 μL) for stock solution and then added to reaction buffer in 30 μM final concentration. 20% (v/v) ethanol was added for dissolving the insoluble compounds. The reaction was incubated for 0.5 h and detected by analytical RP-HPLC.

Conjugates **1b** (Ub-$NHNH_2$ + compound 1) was obtained according to the general procedure. LC-MS analysis: $[M + H]^+$ *m/z* calcd 8727.77, found 8726.80.

Conjugates **2b** (Ub-$NHNH_2$ + compound 2) was obtained according to the general procedure. LC-MS analysis: $[M + H]^+$ *m/z* calcd 8718.48, found 8717.50.

Conjugates **3b** (Ub-$NHNH_2$ + compound 3) was obtained according to the general procedure. LC-MS analysis: $[M + H]^+$ *m/z* calcd 8644.34, found 8643.53.

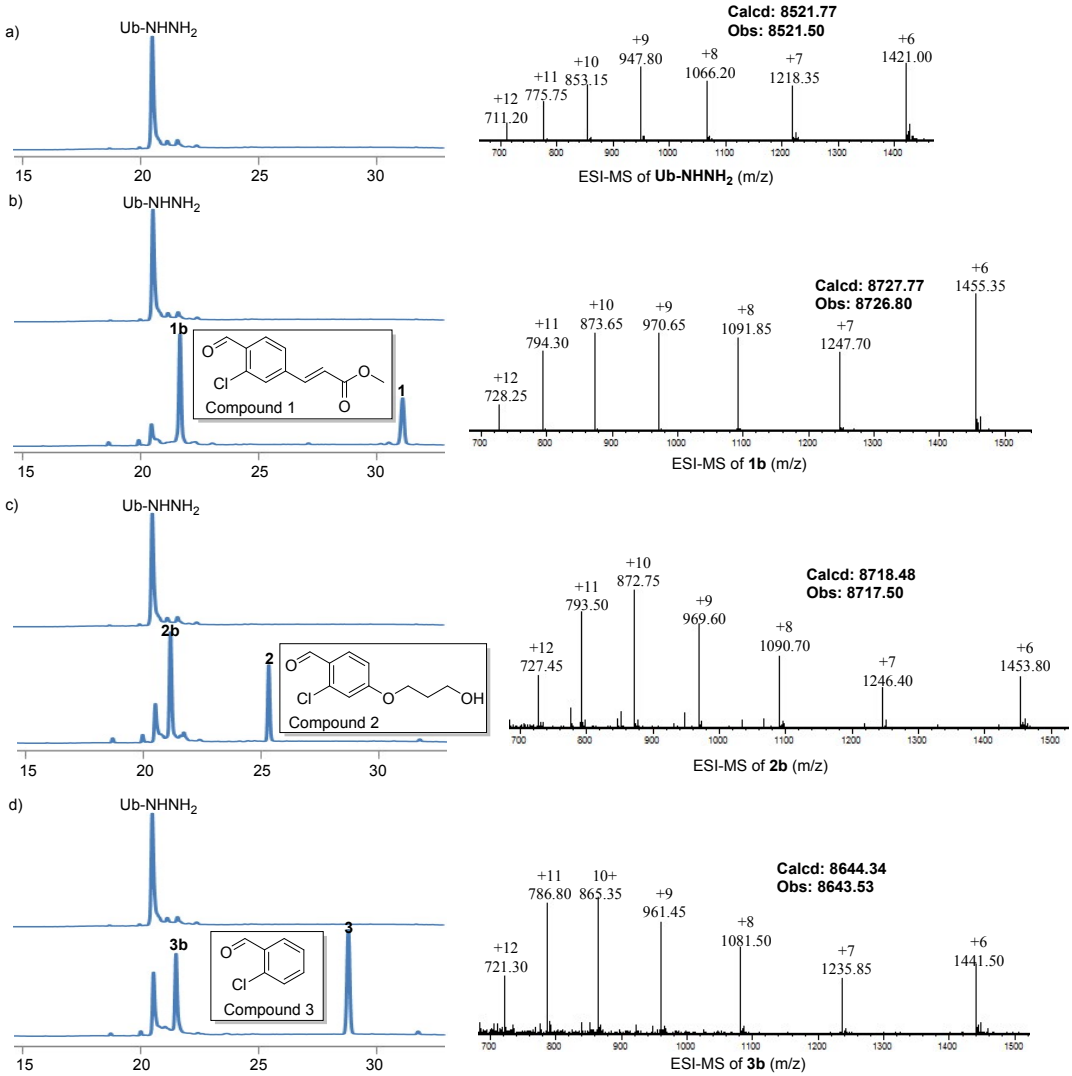
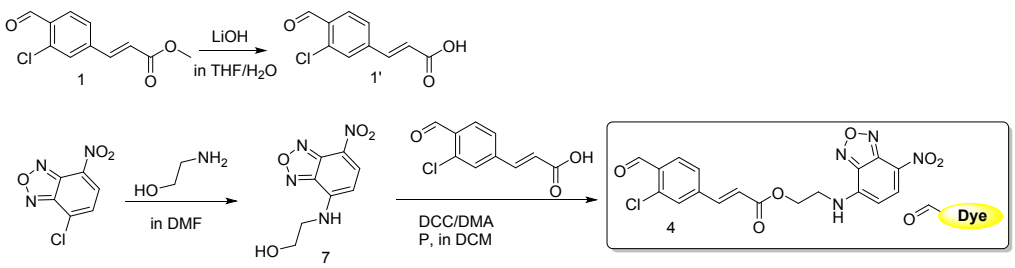


Figure S2: The chromatogram of the reactions between ubiquitin hydrazide and different aldehydes.

e. Conjugates between reagent 1 and model peptides with different C-terminal residues.

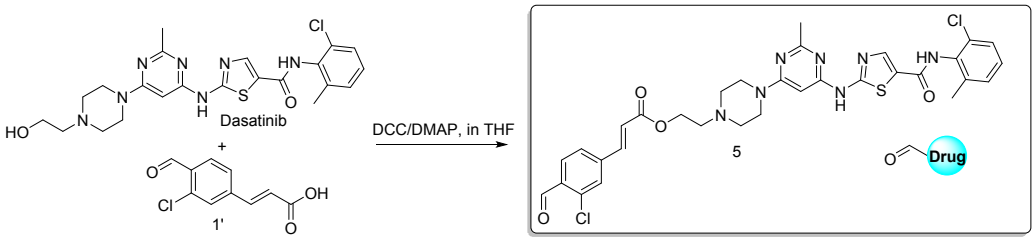
The peptide hydrazide (30 μM in final concentration) were added to the aqueous PME buffer (100 mM PIPES, 1 mM $MgSO_4$, 2 mM EGTA at pH 7.0). Reagent 2 was firstly dissolved in ethanol (10 mM) for stock solution and then added to reaction buffer in 200 μM final concentration. 20% (v/v) ethanol was added for dissolving the insoluble compounds. Then, the mixture was stirred at neutral pH and 25 ℃ for about 0.5 h before the yield was determined by RP-HPLC.

f. The functionalization of reagents

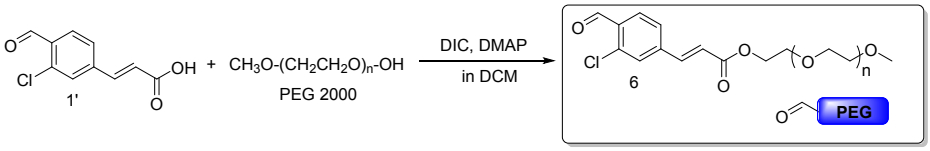


Compound 1 (0.45g, 2 mmol) and LiOH.H_20 (126 mg, 3 mmol) was dissolved in THF/H2O (1/1, *v/v*). The mixture was stirred for 2 h. Then the mixture was diluted with 2 M HCl and extracted with CH_2Cl_2, then dried over Na_2SO_4, filtrated and concentrated. The crude product 1' was obtained without purification. 4-chloro-7-nitrobenzo[c][1,2,5]oxadiazole (0.6g, 3mmol) were dissolved in ethanolamine (1.2ml, 0.02mol) and heated at 40 ℃ for 3 h. The mixture was diluted with H_2O and extracted with CH_2Cl_2, then dried over with Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography to yield 7 (0.20 g, 5.4 mmol, 30%) as brown solid. ^{1}H NMR (400 MHz, DMSO) δ 9.44 (s, 1H), 8.52 (d, *J* = 8.6 Hz, 1H), 6.46 (d, *J* = 8.9 Hz, 1H), 4.96 (s, 1H), 3.70 (q, *J* = 5.5 Hz, 2H), 3.56 (s, 2H). This product is a known compound, the detail characterization data was published in *Org. Biomol. Chem*., 2016, **14**, 11117.[2]

Compound 7 (62 mg, 0.28 mmol), compound 1' (58 mg, 0.28 mmol), DCC (72 mg, 0.35 mmol) and DMAP (3.8 mg, 0.028 mmol) was dissolved in THF. The reaction mixture was stirred overnight. The mixture was diluted with H_2O and extracted with CH_2Cl_2, then dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by column chromatography to yield compound 4 (82 mg, 0.20 mmol, 70%). However, compound 4 was insoluble in $CDCl_3$/DMSO-D6/DMF-D7/D_2O and it was difficult to achieve effective concentrations for NMR. Fortunately, HRMS proved the correctness of this compound. Moreover, subsequent fluorescence labeling experiments can also prove that the compound contains a benzofurazan. HRMS *m/z*: $[M + Na]^+$ calcd for $C_{18}H_{13}O_6N_4ClNa^+$ 439.04158, found 439.04129.

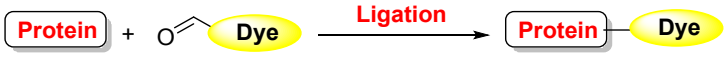


Dasatinib (136 mg, 0.28 mmol), compound 1' (59 mg, 0.28 mmol), DCC (72 mg, 0.35 mmol) and DMAP (3.8 mg, 0.028 mmol) was dissolved in THF. The reaction mixture was stirred overnight. The mixture was diluted with H_2O and extracted with CH_2Cl_2, then dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by column chromatography to yield compound 5 (135 mg, 0.20 mmol). ^{1}H NMR (400 MHz, DMSO) δ 11.48 (s, 1H), 10.33 (s, 1H), 9.88 (s, 1H), 8.31 (s, 1H), 8.22 (s, 1H), 8.07 (s, 1H), 7.93 – 7.84 (m, 2H), 7.71 (d, *J* = 16.0 Hz, 1H), 7.40 (d, *J* = 7.2 Hz, 1H), 7.28 (s, 1H), 6.93 (d, *J* = 16.0 Hz, 1H), 6.06 (s, 1H), 4.33 (s, 2H), 3.54 (s, 4H), 2.71 (s, 2H), 2.55 (s, 4H), 2.41 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 189.80, 170.80, 166.15, 165.64, 163.02, 162.80, 160.40, 157.40, 142.23, 141.44, 139.28, 137.19, 133.97, 133.03, 130.79, 130.40, 129.49, 128.64, 127.82, 127.47, 126.18, 123.01, 79.64, 60.23, 52.87, 43.98, 26.05, 21.23. HRMS *m/z*: $[M + H]^+$ calcd for $C_{32}H_{32}Cl_2N_7O_4S^+$ 680.16081; found 680.16144.

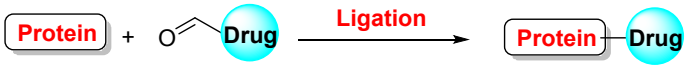


PEG 2000 (125mg, 0.0625mmol), DIC(34.7mg, 0.275mmol), DMAP(3.1mg, 0.025mmol) was dissolved in 1ml CH_2Cl_2. Compound 1' (53mg, 0.23mmol) was dissolved in 0.5ml DMF and added dropwise to the solution. The reaction was stirred for 6 h. Then the CH_2Cl_2 was removed by heating and the crude product was precipitated with ice-ether and purified by RP-HPLC (67 mg).

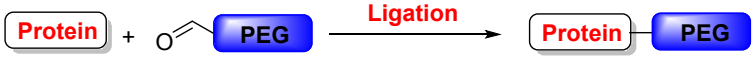
g. Conjugates between protein and different functionalized reagents



Fluorescence molecular 4 was firstly dissolved in DMF (1g/mL) for stock solution and then added to reaction buffer in 200 μM final concentration. Ub-$NHNH_2$ was dissolved in 6 M Gn-HCl (1mg in 100 μL) for stock solution and then added to reaction buffer in 30 μM final concentration. 30% (v/v) DMF was added for dissolving the insoluble compounds. The reaction was incubated for 0.5 h and detected by analytical SDS-PAGE, in-gel fluorescence analysis and ESI-HRMS.



Drug molecular 5 was firstly dissolved in DMF (1g/mL) for stock solution and then added to reaction buffer in 200 μM final concentration. Ub-$NHNH_2$ was dissolved in 6 M Gn.HCl (1mg in 100 μL) for stock solution and then added to reaction buffer in 30 μM final concentration. 40% (v/v) DMF was added for dissolving the insoluble compounds. The reaction was incubated for 0.5 h and detected by analytical ESI-HRMS.



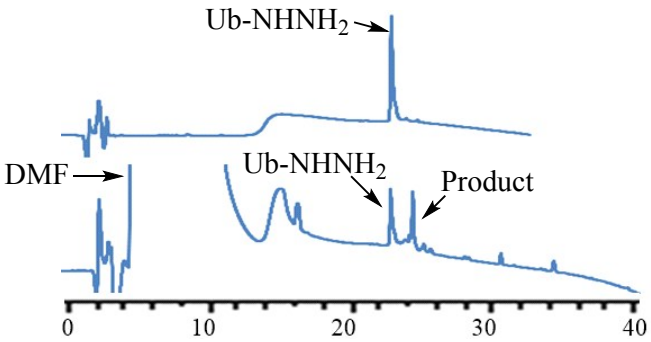
Reagent 6 was firstly dissolved in H_2O (1mg in 100 μL) for stock solution and then added to reaction buffer in 150- 250 μM final concentration. Ub-$NHNH_2$ was dissolved in 6 M Gn.HCl (1mg in 100 μL) for stock solution and then added to reaction buffer in 30 μM final concentration. The reaction was incubated for 0.5 h and detected by analytical SDS-PAGE, RP-HPLC and Maldi-Tof-MS.

For SDS-PAGE, samples were loaded onto 15% SDS-PAGE gelsand ran for 30 min at 80 V and 50 min at 150 V. All the protein samples were prepared in Tris buffer containing bromophenol blue. When samples were ran near the edge of gel, it was removed from electrophoresis chamber and first irradiated under 365 nm UV for fluorescence analysis. Then, the gel was stained in Coomassie brilliant blue for 1 h for SDS-PAGE analysis.

h. The supplementary note for compound 4

As we mentioned before, compound 4 was insoluble in $CDCl_3$/DMSO-D6/DMF-D7/D_2O and it was difficult to achieve effective concentrations for NMR. The HRMS proved the correctness of this compound (HRMS *m/z*: [M + Na]$^+$ calcd for $C_{18}H_{13}O_6N_4ClNa^+$ 439.04158, found 439.04129).

Moreover, subsequent fluorescence labeling experiments can also prove that the compound contains a benzofurazan. Unfortunately, compound 4 was not observed on the RP-HPLC over 40 minutes and may remain on the column (Gradient : A linear gradient of 1% to 90% B over 30 min, then 99% B over 20 min, solution A was 0.1% TFA in water and solution B was 0.1% TFA in MeCN). The following figure shows the monitoring of the reaction process.



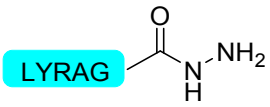
Reference:

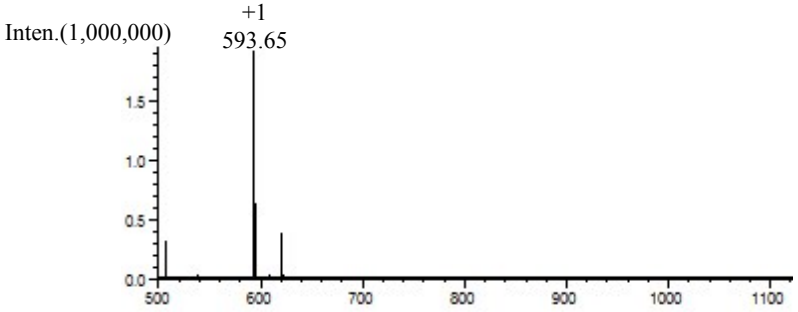
1. G. M. Fang, Y. M. Li, F. Shen, Y. C. Huang, J. B. Li, Y. Lin, H. C. Cui and L. Liu, *Angew. Chem., Int. Ed.*, 2011, **50**, 7645-7649.

2. F. B. Song, Z. F. Li, J. Y. Li, S. Wu, X. B. Q, Z. Xi and L. Yi, *Org. Biomol. Chem.*, 2016, **14**, 11117-11124.

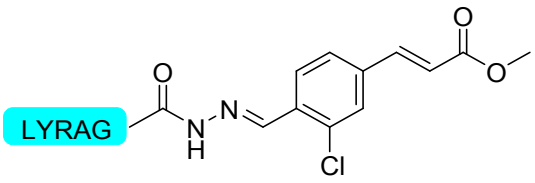
3. Spectra

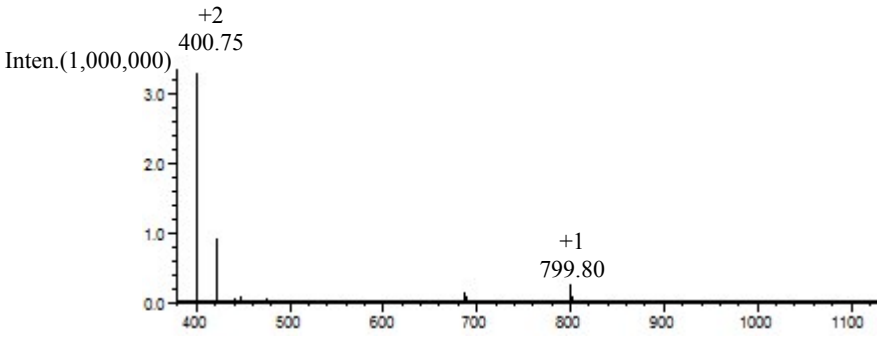
a. MS

1. 

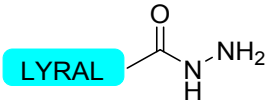


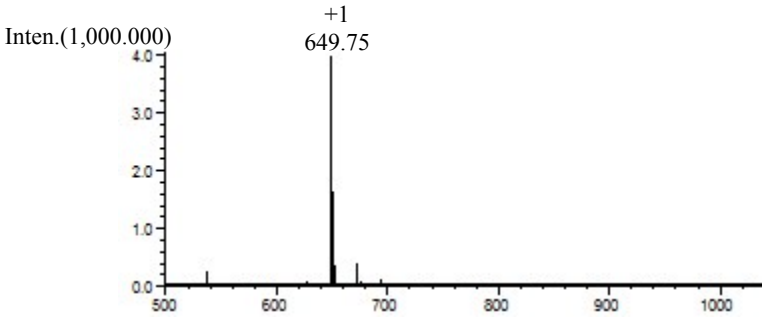
Mass observed: 592.65 Da, mass calculated: 592.67 Da

2. 



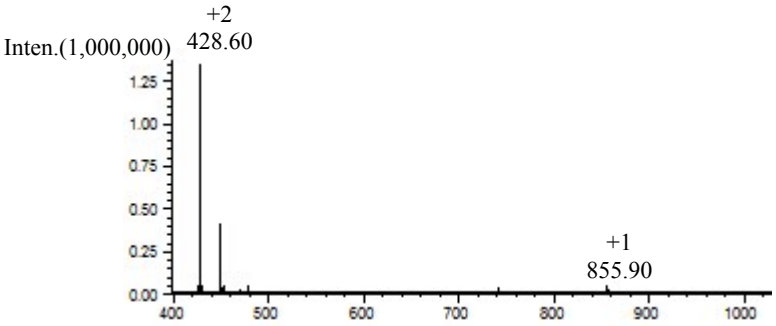
Mass observed: 799.50 Da, mass calculated: 799.31 Da

3. 



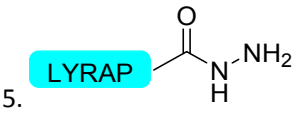
Mass observed: 649.75 Da, mass calculated: 648.77 Da

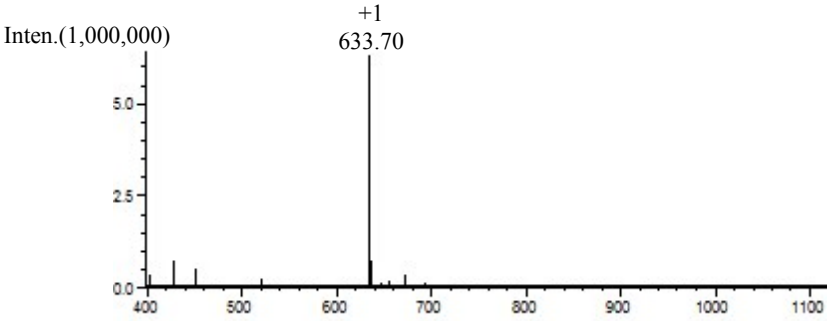
4. LYRAL



Mass observed: 855.20 Da, mass calculated: 855.41 Da

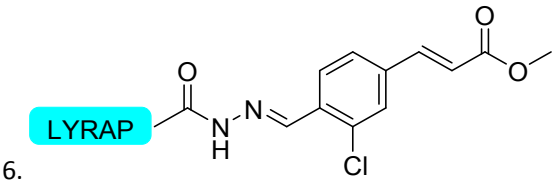
5. LYRAP

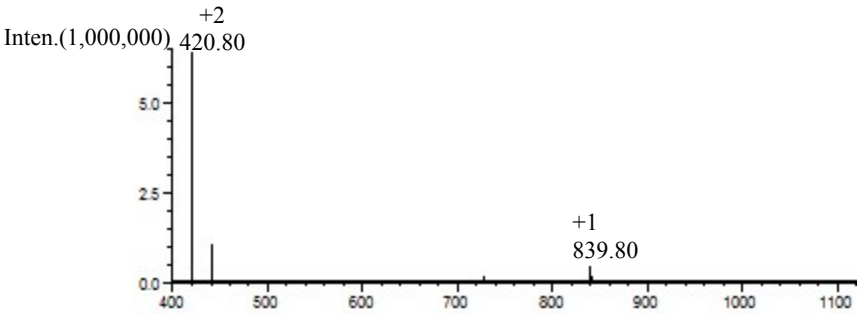




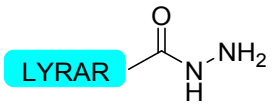
Mass observed: 632.70 Da, mass calculated: 632.73 Da

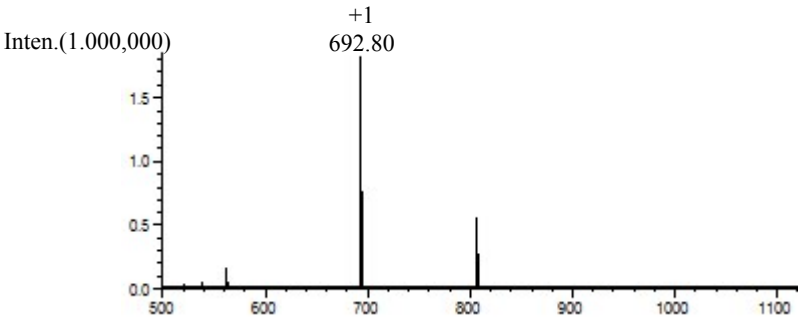
6. LYRAP



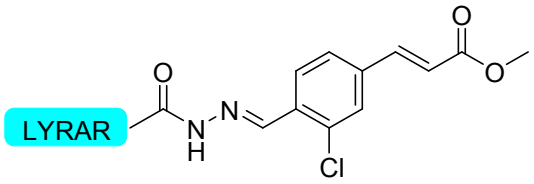


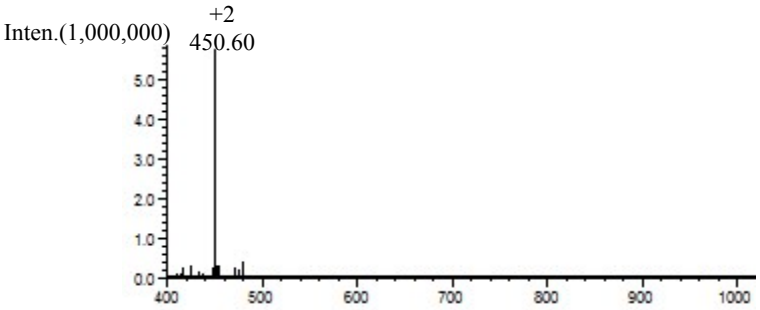
Mass observed: 839.60 Da, mass calculated: 839.37 Da

7. 

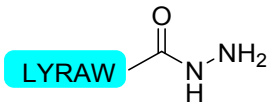


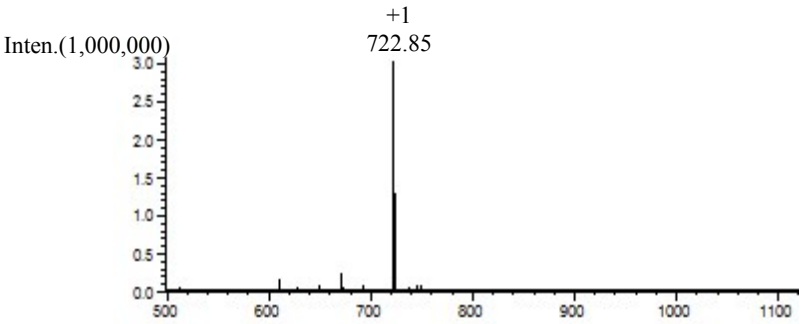
Mass observed: 691.80 Da, mass calculated: 691.80 Da

8. 



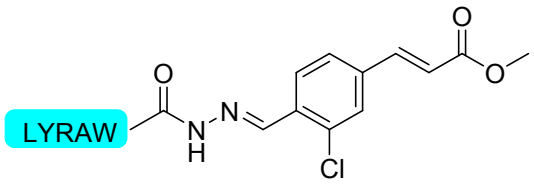
Mass observed: 899.20 Da, mass calculated: 898.44 Da

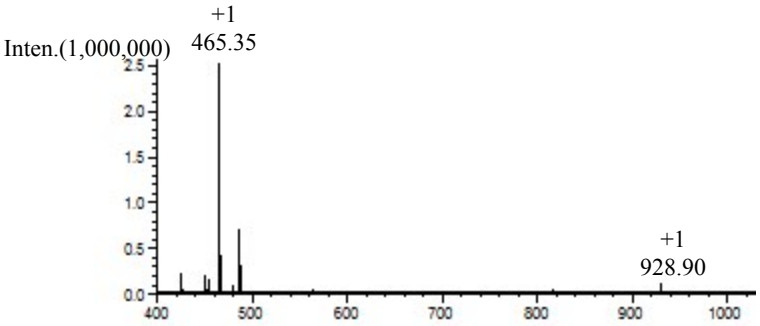
9. 



Mass observed: 721.85 Da, mass calculated: 721.83 Da

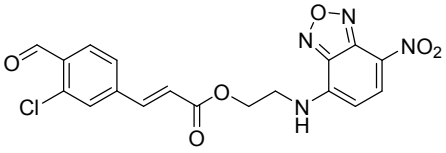
10.



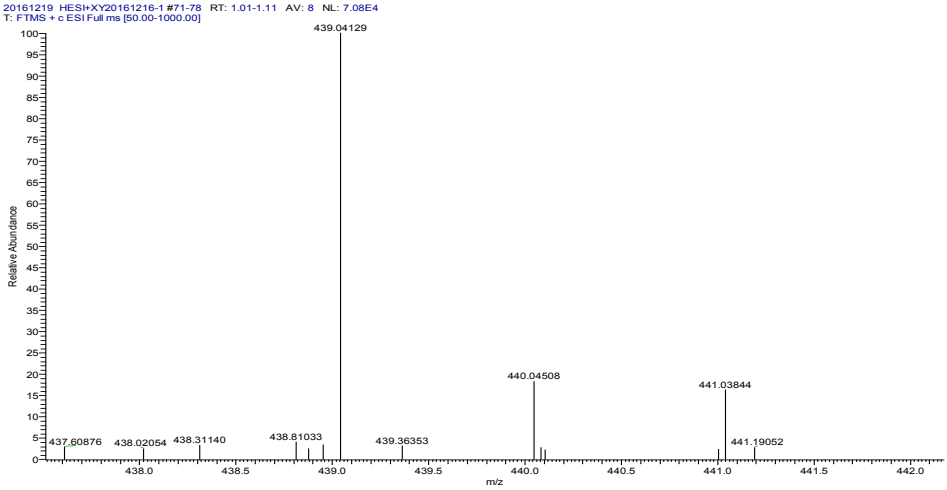


Mass observed: 928.70 Da, mass calculated: 928.47 Da

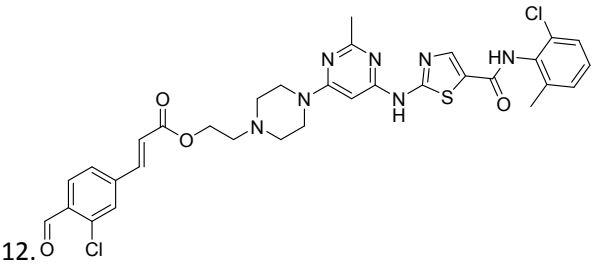
11.



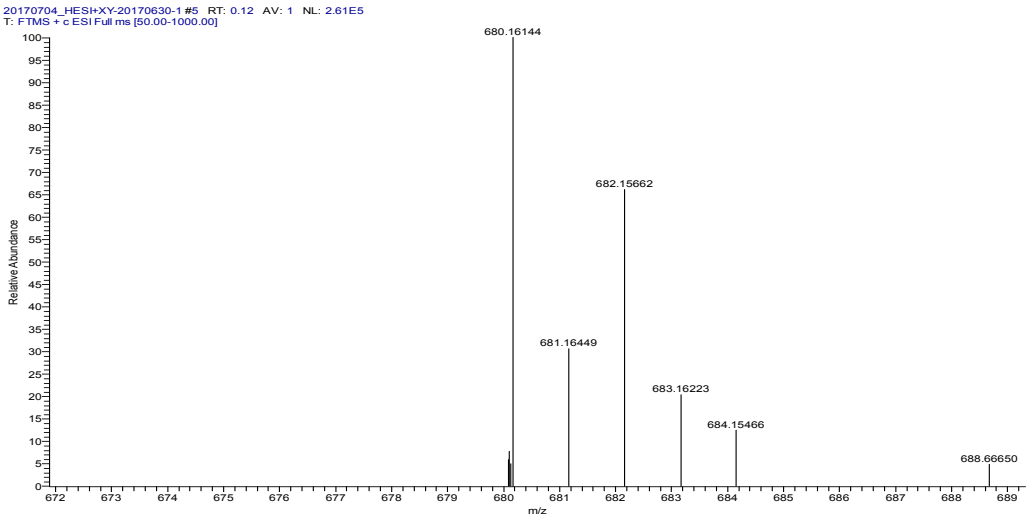
HRMS *m/z*: $[M + Na]^+$ calcd for $C_{18}H_{13}O_6N_4ClNa^+$ 439.04158; found 439.04129.



m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
439.0413	70836.6	100	439.0416	-0.29	C18 H13 O6 N4 Cl Na

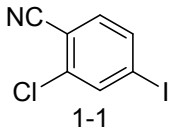
12. 

HRMS *m/z*: $[M + H]^+$ calcd for $C_{32}H_{32}Cl_2N_7O_4S^+$ 680.16081; found 680.16144.

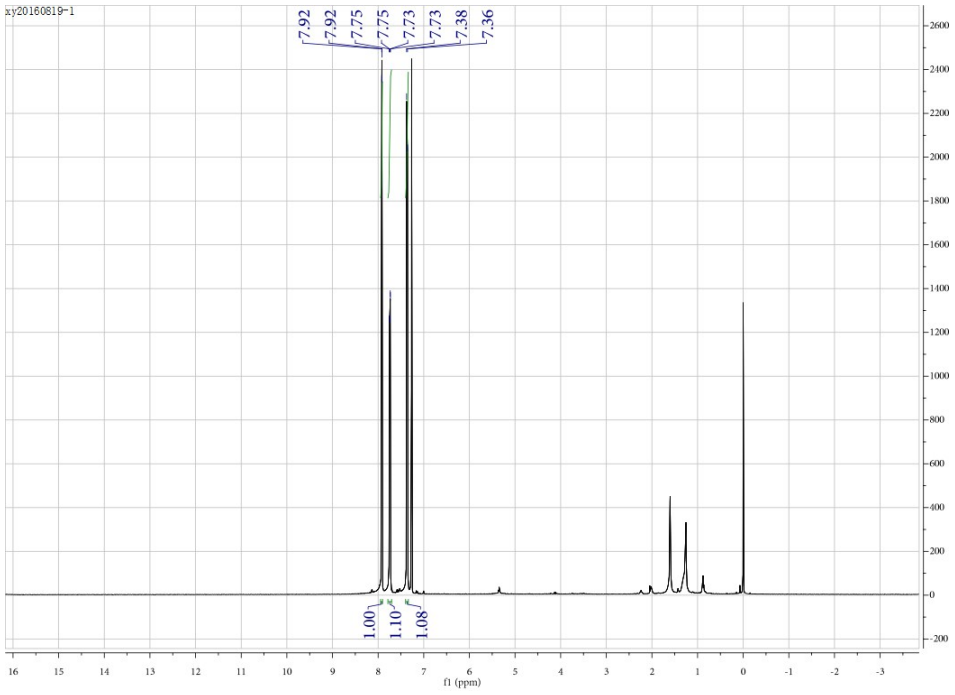


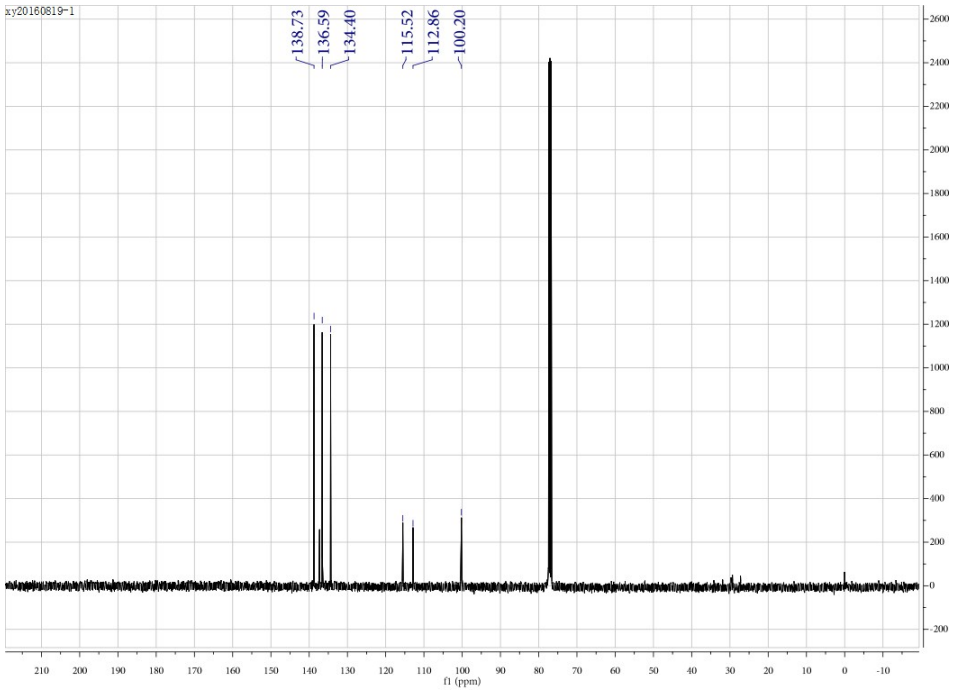
m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
680.1614	261160.6	100	680.16081	0.63	C32 H32 O4 N7 Cl2 S

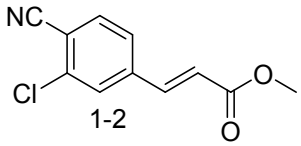
b. NMR


NC
Cl
I

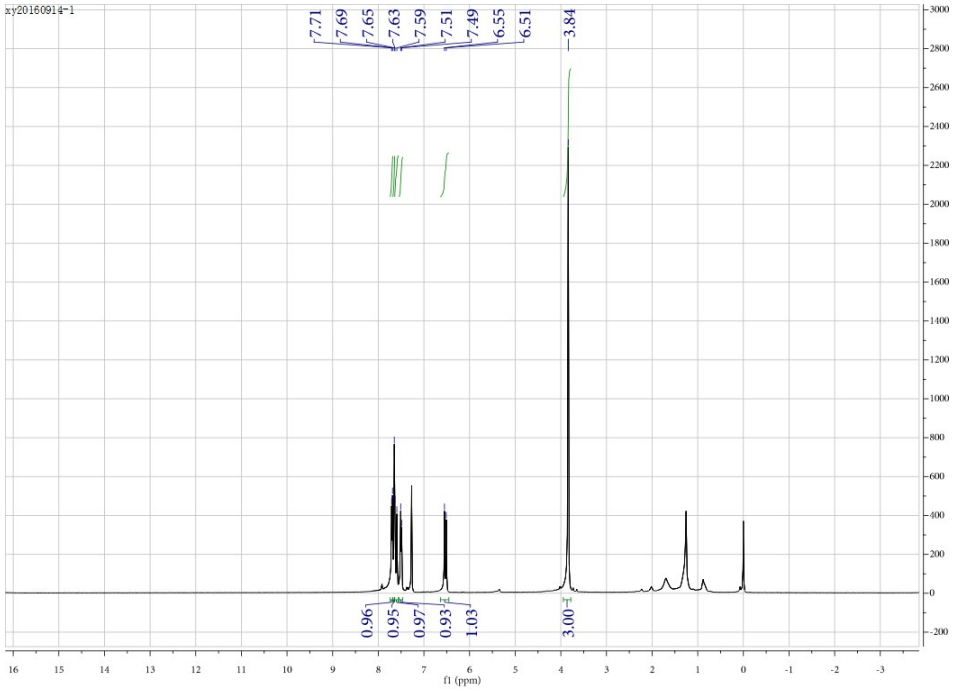
1-1

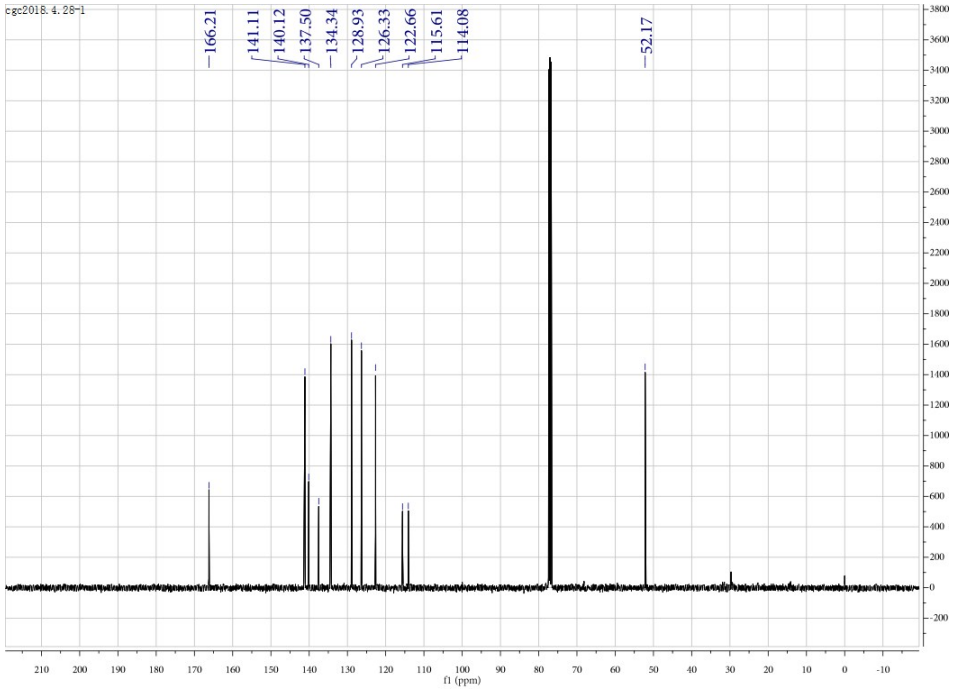

xy20160819-1
7.92
7.92
7.75
7.75
7.73
7.73
7.38
7.36
1.00
1.10
1.08
f1 (ppm)

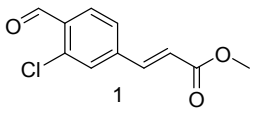

xy20160819-1
138.73
136.59
134.40
115.52
112.86
100.20
f1 (ppm)


NC
Cl
O
O

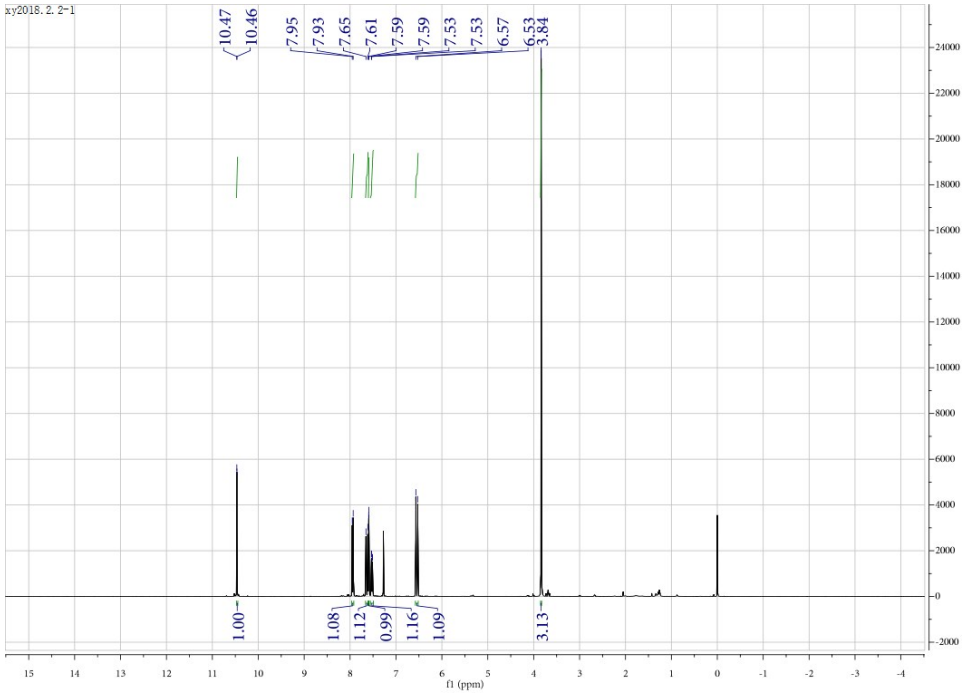
1-2

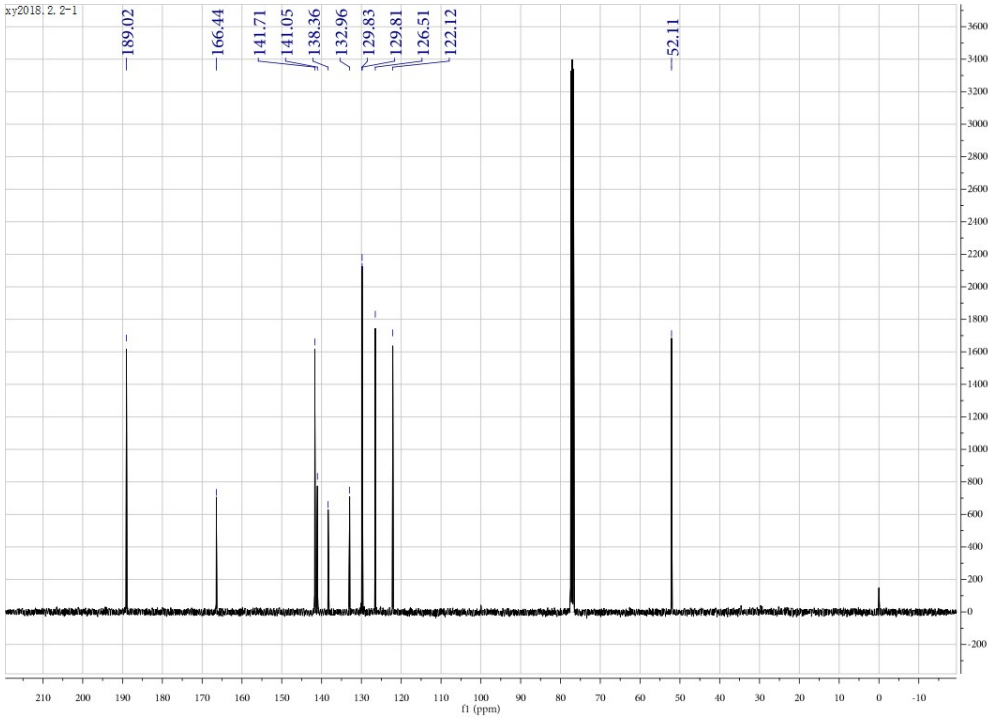

xy20160914-1
7.71
7.69
7.65
7.63
7.59
7.51
7.49
6.55
6.51
3.84
0.96
0.95
0.97
0.93
1.03
3.00
f1 (ppm)

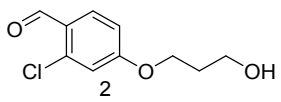

cgc2018.4.28-1
166.21
141.11
140.12
137.50
134.34
128.93
126.33
122.66
115.61
114.08
52.17
f1 (ppm)


O
Cl
O
O

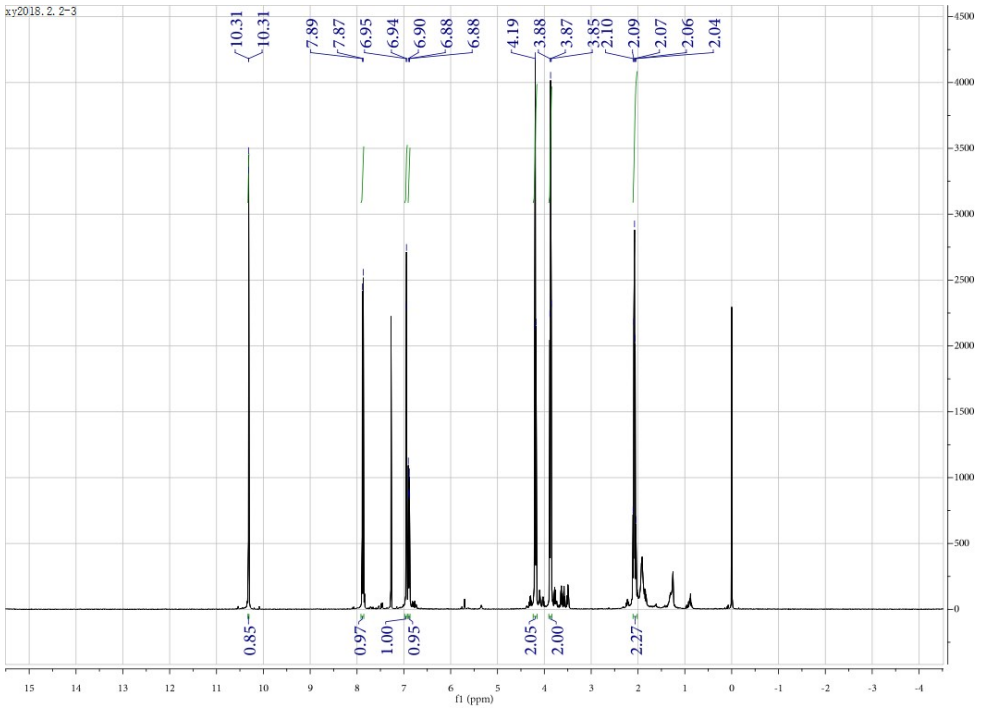
1

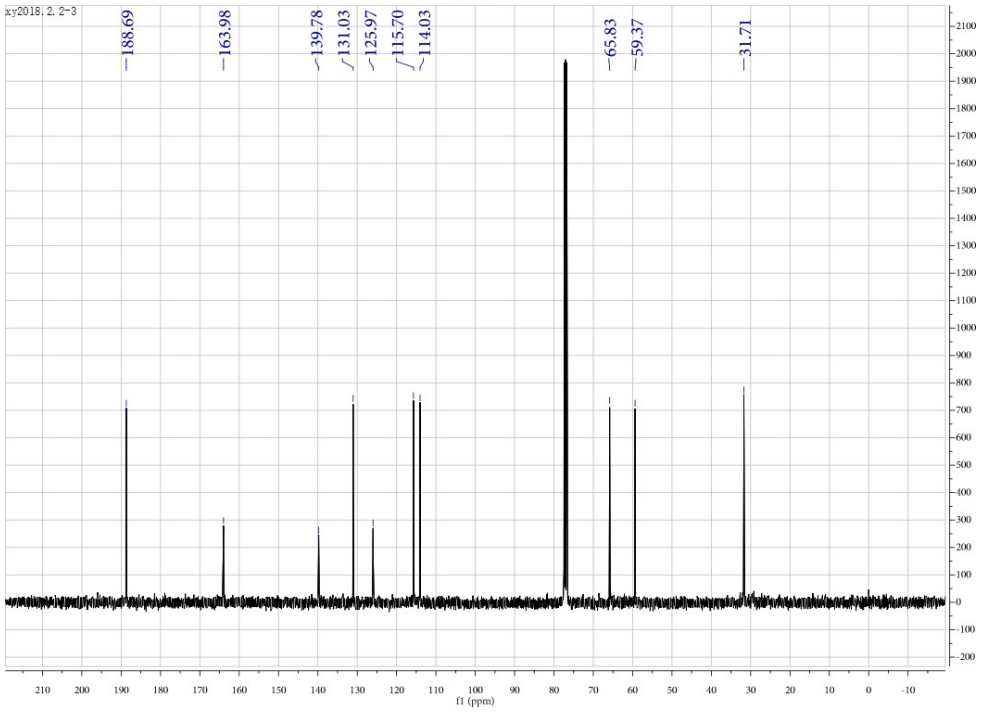

xy2018.2.2-1
10.47
10.46
7.95
7.93
7.65
7.61
7.59
7.59
7.53
7.53
6.57
6.53
3.84
1.00
1.08
1.12
0.99
1.16
1.09
3.13
f1 (ppm)

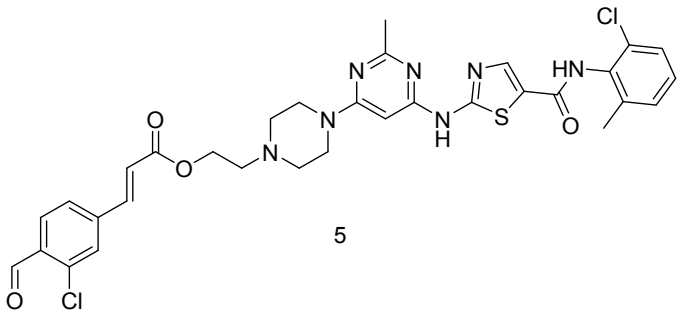

xy2018.2.2-1
189.02
166.44
141.71
141.05
138.36
132.96
129.83
129.81
126.51
122.12
52.11
f1 (ppm)


O
Cl
O
OH

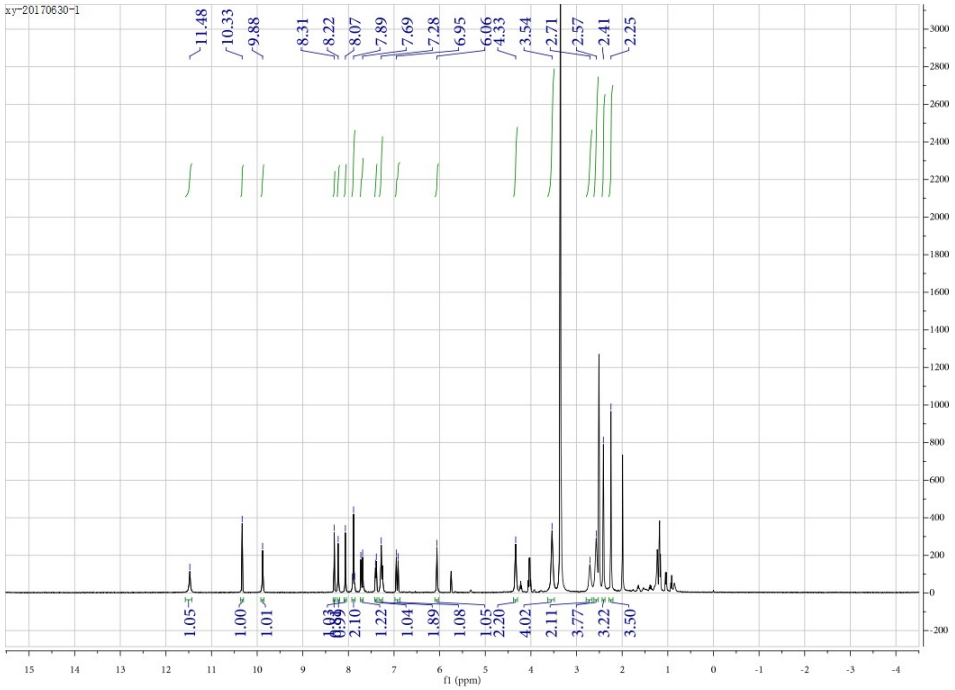
2

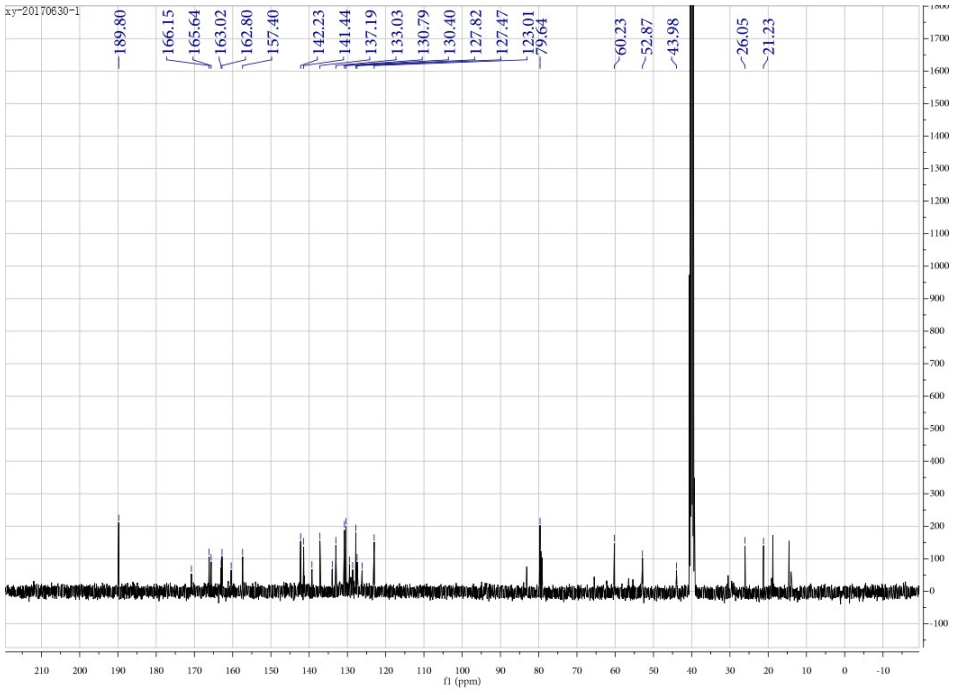

xy2018.2.2-3
10.31
10.31
7.89
7.87
6.95
6.94
6.90
6.88
6.88
4.19
3.88
3.87
3.85
2.10
2.09
2.07
2.06
2.04
0.85
0.97
1.00
0.95
2.05
2.00
2.27
f1 (ppm)


xy2018.2.2-3
188.69
163.98
139.78
131.03
125.97
115.70
114.03
65.83
59.37
31.71
f1 (ppm)


Cl
HN
N
N
N
S
N
H
O
O
O
N
N
O
Cl

5


xy-20170630-1
11.48
10.33
9.88
8.31
8.22
8.07
7.89
7.69
7.28
6.95
6.06
4.33
3.54
2.71
2.57
2.41
2.25
1.05
1.00
1.01
2.10
1.22
1.04
1.89
1.08
1.05
2.20
4.02
2.11
3.77
3.22
3.50
f1 (ppm)


xy-20170630-1
189.80
166.15
165.64
163.02
162.80
157.40
142.23
141.44
137.19
133.03
130.79
130.40
127.82
127.47
123.01
79.64
60.23
52.87
43.98
26.05
21.23
f1 (ppm)