Supplementary Information (SI) for Organic Chemistry Frontiers.

Supplemental Information

One pot Photoenzymatic Synthesis of β-chiral malononitrile derivatives

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Context

1 General Information

1.1 Sequence information

OYE1 (from *Saccharomyces pastorianus*)

Gen Bank: CP049012.1

Protein sequence：

MSFVKDFKPQALGDTNLFKPIKIGNNELLHRAVIPPLTRMRALHPGNIPNRDWAVEYYTQ
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AKNSIAAGADGVEIHSANGYLLNQFLDPHSNTRTDEYGGSIENRARFTLEVVDALVEAIG
HEKVGLRLSPYGVFNSMSGGAETGIVAQYAYVAGELEKRAKAGKRLAFVHLVEPRVTNP
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DNA sequence：

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OYE3 (from *Saccharomyces cerevisiae* S288C)

Gen Bank: NP_015154.1

Protein sequence：

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RVGLRLSPYGTFNSMSGGAEPGIIAQYSYVLGELEKRAKAGKRLAFVHLVEPRVTDPSLV
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GluER (from *Gluconobacter oxydans*)

Gen Bank: WP_011252080.1

Protein sequence：

MPTLFDPIDFGPIHAKNRIVMSPLTRGRADKEAVPTPIMAEYYAQRASAGLIITEATGISRE
GLGWPFAPGIWSDAQVEAWKPIVAGVHAKGGKIVCQLWHMGRMVHSSVTGTQPVSSSA
TTAPGEVHTYEGKKPFEQARAIDAADISRILNDYENAARNAIRAGFDGVQIHAANGYLID
EFLRNGTNHRTDEYGGVPENRIRFLKEVTERVIAAIGADRTGVRLSPNGDTQGCIDSAPET
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CACCACCACTGA

NCR (from *Zymomonas mobile*)

Gen Bank: WP_011241612.1

Protein sequence：

MPSLFDPIRFGAFTAKNRIWMAPLTRGRATRDHVPTEIMAEYYAQRASAGLIISEATGISQ
EGLGWPYAPGIWSDAQVEAWLPITQAVHDAGGLIFAQLWHMGRMVPSNVSGMQPVAPS
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1.2 Materials and analytical methods

All chemicals and reagents were purchased from Okinno, Aladdin and Tansoole. Chemicals were obtained from authentic suppliers at least of reagent grade and used without further purification. All biological reagents are purchased from Fisher Scientific, Solarbio (Beijing, China) and Sangon Biotech (Shanghai, China). The recombinant plasmids, pET-22B-GluER, pET-22B-NCR, pET-28a(+)-OYE1, pET-28a(+)-OYE3, respectively, containing the gene of GluER (from *Gluconobacter* *oxydans*, GenBank: WP_011252080.1)/ NCR (from *Zymomonas mobile*, GenBank: WP_011241612.1)/ OYE1 (from *Saccharomyces pastorianus*, GenBank: CP049012.1)/ OYE3 (from *Saccharomyces cerevisiae* S288C, GenBank: NM_001183985.1), were provided by Hunan Keai Medical Equipment Co., Ltd. and Beijing Qingke Biotechnology Co., Ltd. The competent cells BL21 (DE3) and Rosetta (DE3) were provided by Beijing Solarbio Technology Co., Ltd. The Escherichia coli strain containing the glucose dehydrogenase (GDH) gene was provided by Wu Zhongliu researcher's group from Chengdu Institute of Biology, Chinese Academy of Sciences. Racemic product standards were synthesized based on previous studies.[1,2] NMR spectra were obtained by an Agilent 400-MR DD2 spectrometer (400 MHz). The chemical shifts (ppm) were recorded with respect to TMS in $CDCl_3$ or DMSO-d_6. Analytical high-performance liquid chromatography (HPLC) was carried out using an Agilent 1260 Infinity instrument equipped with ChiralCel® OD-3 chiral columns (4.6×250 mm, 3 μm), ChiralPak® IH chiral columns (4.6×250 mm, 5 μm), ChiralCel® OJ chiral columns (4.6×250 mm, 10 μm), isopropanol and hexane were used as an isocratic mobile phase system. The temperature was set at 30 °C.

2 Expression and purification of ene reductase

2.1 Methods

The expression and purification of ERs referred to our previous work. The recombinant plasmids were transformed into E. coli BL21(DE3) or Rosetta(DE3) competent cells and selected on Luria–Bertani (LB) agar plates containing 100 μg/mL kanamycin or ampicillin. Single colonies were grown overnight at 37 °C in LB medium containing 100 μg/mL kanamycin or ampicillin. The overnight culture was then inoculated into Terrific-broth (TB) medium containing 100 μg/mL kanamycin or ampicillin. Isopropyl-beta-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM when OD_{600} of the culture reached 0.6-0.8, and the cultivation was continued at 20-28 °C for 18-22 h. Cells were harvested by centrifugation at 4000 rpm for 10 min at 4 °C, washed with phosphate buffered saline (PBS). After disruption with an ultrasonic cell disruptor (SCIENTZ-IID, SCIENTZ Inc., China), the cell debris was removed by centrifugation at 12000 rpm for 30 min at 4 °C. The obtained supernatant was first filtered using a 0.45 μm aqueous needle filter and then loaded onto Ni^{2+}-nitrilotriacetic acid column (Bio-Rad, USA) equilibrated with buffer A. The enzyme was eluted with buffer A containing a gradient of imidazole from 10 to 250 mM at a flow rate of 1 mL/min, and five column volumes were used for the gradient elution. The fractions containing the target protein were collected and dialyzed against 10 mM PBS (pH 7.2-7.4). Purified enzymes were analyzed by SDS-PAGE and used for enzymatic assays. Protein estimations were carried out with the Bradford method with bovine serum albumin as a standard.

2.2 SDS-PAGE of crude and purified enzymes

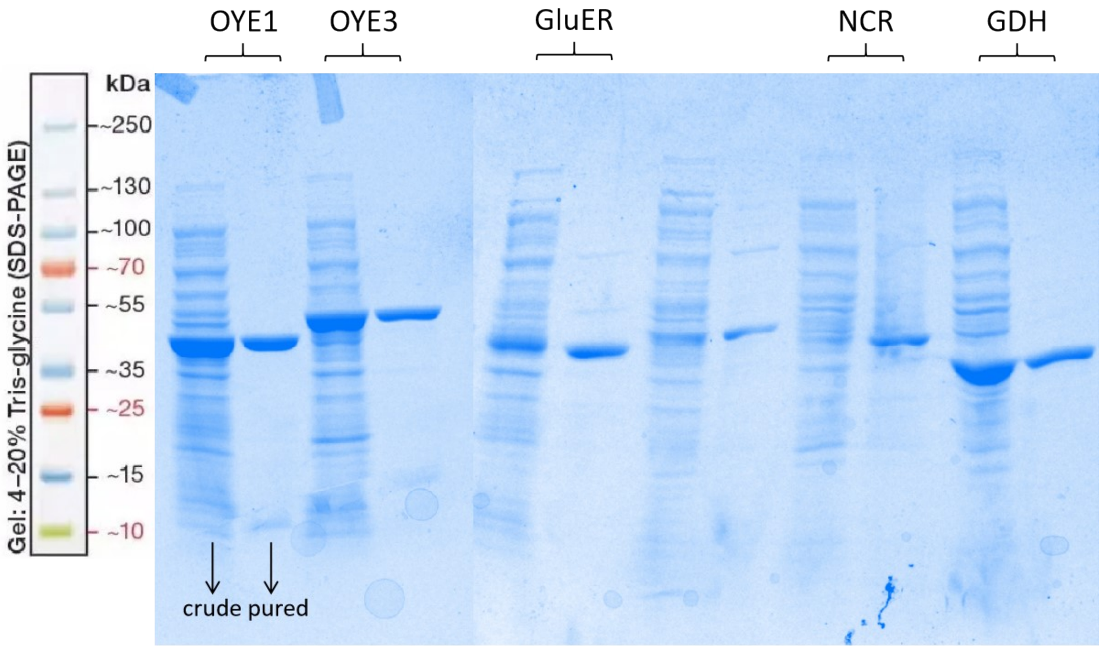


Fig. S1 SDS-PAGE of crude and purified enzymes

3 Experimental Procedures

3.1 General procedures for photoenzymatic synthesis of malononitrile alkylated derivatives

1) 1mL reaction system contains acetophenone (0.20 mmol), malononitrile (0.40 mmol), MO (8 mol%), 10 %v/v ethanol and H_2O. The mixture is stirred in a quartz tube irradiated by white light (36 W) at room temperature in air for 36 h.

2) After turning off the light source, DMSO (2 mL) is added and dissolved uniformly under ultrasound. Then take 300 μL system into a new conical flask, which contains NCR (0.4 mg/mL), GDH (0.5 mg/mL), $NADP^+$ (0.1 mM), glucose (20 mM), PBS (10 mM, pH 7.2-7.4), with a final volume of 2 mL. The enzymatic reaction is carried out in a constant temperature shaker, 32 ℃, 200 rpm, 50 min. Yield and enantiomeric excess (ee) values were determined by chiral HPLC.

3.2 Docking models

Automated docking models were obtained using Autodock1.5.7, OYE1 (PDB:3TX9), OYE3 (PDB:5V4V), NCR (PDB:4A3U) were used as receptors and prepared using Autodock Tools. The ligand structures were prepared by GaussView 6.0, and the energy minimizations of ligand structures were carried out in Chem3D.

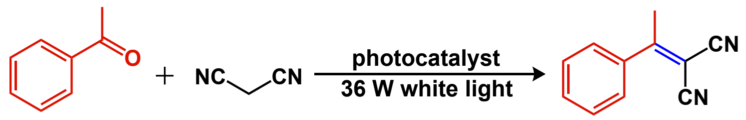
3.3 Molecular dynamics simulation

Molecular dynamics simulation was performed using GROMACS, a widely used software package for simulating the behavior of biomolecules. The parameters of the substrate 2-(1-phenylethylidene)malononitrile and coenzyme FMN in the MD process were generated by the combination of Antechamber tool with the GAFF force field, which is an extensively used force field for small organic molecules. Amber 99sb-ildn was chosen as the protein force field, which is a frequently used force field for simulating proteins.

To simulate the behavior of the protein substrate complex in a solvent environment, the complex was embedded into a triclinic box with a boundary distance of 0.8 nm from the protein surface. The protein and the substrate were immersed in a rectangular box of TIP3P water.

The complete OYE1 system ultimately contained 65192 atoms, while the OYE3 system contained 54560 atoms. After a series of pre-treatments, such as balancing charges, the two systems underwent energy minimization of 1000 steps, using the conjugate gradient method, followed by NPT simulations of 100 ps with the Berendsen method. The Berendsen method is a commonly used method for controlling the temperature and pressure of a simulation system. Molecular dynamics simulations of 100 ns were conducted for both systems, which allowed for the investigation of the dynamic behavior of the protein substrate complex over a long time scale.

3.4 Optimization of photocatalytic reaction conditions



1a 2 3a

Table S1 Screening of photosensitizers and loadings [a]

Entry	Photosensitizer	Photosensitizer Loading (mol%)	Yield(%) [b]
1	Rose Bengal	2	32.9
2	Erythrosine B	2	29.8
3	Eosin Y	2	37.8
4	Crystal Violet	2	12.4
5	Phloxine B	2	38.4
6	Methyl Orange	2	48.6
7	Methyl Orange	1	44.3
8		4	50.9
9		8	63.3
10		12	47.4

11	16	50.3

[a] Reaction condition: **1a** (0.24 mmol), **2** (0.48 mmol), photosensitizer (x mol%), H_2O, total reaction volume 1 mL, 24 h, 36 W white light. [b] Yield determined by HPLC analysis with **3a** sample.

Table S2 Screening of co-solvents [a]

Entry	Cosolvent	Yield(%) [b]
1	Dioxane	63.1
2	EtOH	**80.6**
3	MeCN	62.8
4	Ethyl Acetate	43.3
5	DMSO	49.9
6	THF	51.5

[a] Reaction condition: **1a** (0.24 mmol), **2** (0.48 mmol), MO (8 mol%), H_2O, co-solvent (10 % v/v), total reaction volume 1 mL, 24 h, 36 W white light. [b] Yield determined by HPLC analysis with **3a** sample.

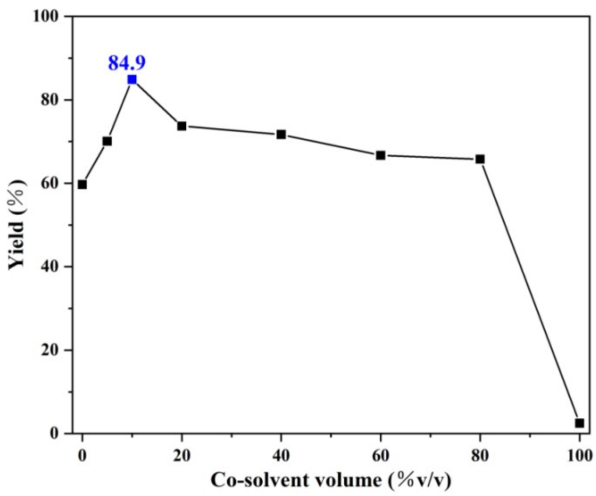


Fig. S2 Screening of co-solvent (EtOH) volume. Reaction condition: **1a** (0.20 mmol), **2** (0.40 mmol), MO (8 mol%), EtOH (x % v/v), H_2O, total reaction volume 1 mL, 36 h, 36 W white light. Yield determined by HPLC analysis with **3a** sample.

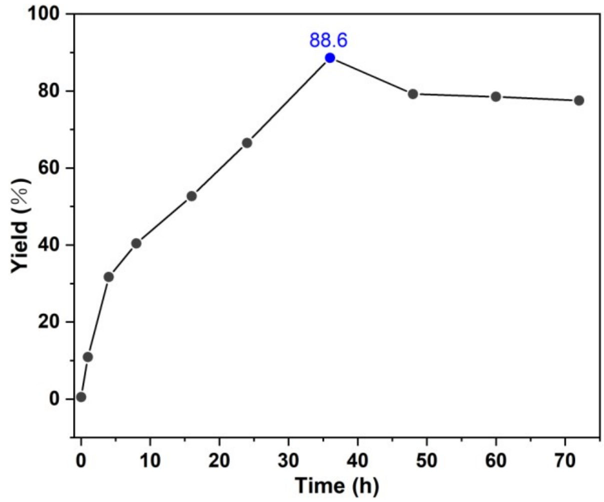


Fig. S3 Photoreaction time curve. Reaction condition：**1a** (0.24 mmol), **2** (0.48 mmol), MO (8 mol%), H_2O (900 μL), ethanol (100 μL), 36 W white light. Yield determined by HPLC analysis with **3a** sample.

Table S3 Control experiments [a]

Entry	Deviation from standard conditions	Yield (%) [b]
1	None	48.6
2	Without photocatalyst	28.4
3	Without photocatalyst[c]	32.0
4	Without light	29.3
5	Without photocatalyst and light	22.3

[a] Reaction condition: **1a** (0.24 mmol), **2** (0.48 mmol), MO (2 mol%), H_2O, total reaction volume 1 mL, 24 h, 36 W white light. [b]Yield determined by HPLC analysis with **3a** sample. [c] 46 hours

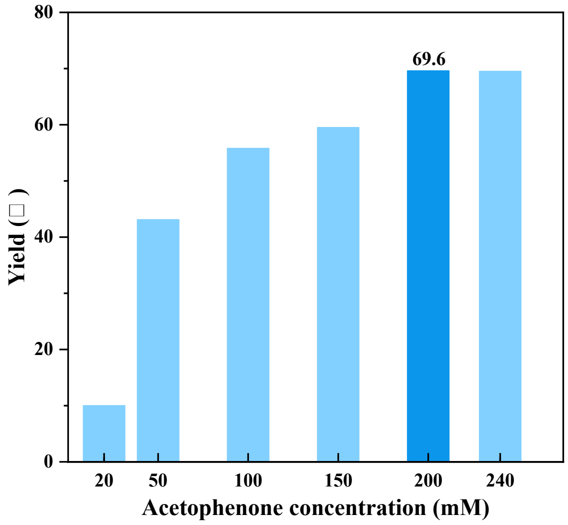


Fig. S4 Screening of acetophenone concentration (maintain acetophenone to malononitrile=1:2). Reaction condition: **1a** (1.0 eq.), **2** (2.0 eq.), MO (8 mol%), EtOH (10 % v/v), H_2O, total reaction volume 1 mL, 36 h, 36 W white light. Yield determined by HPLC analysis with **3a** sample.

High concentration of organic substrates can disrupt the three-dimensional structure of enzyme proteins, thus, we investigated the effect of substrate concentration on yield (with acetophenone concentration as the standard, the molar ratio of acetophenone to malononitrile maintained at 1:2). As the substrate concentration decreased, the yield significantly decreased. To ensure a high yield of the photocatalytic reaction and take into account the damage of high-concentration substrates to the enzymes, we chose 200 mM acetophenone as the optimal substrate concentration.

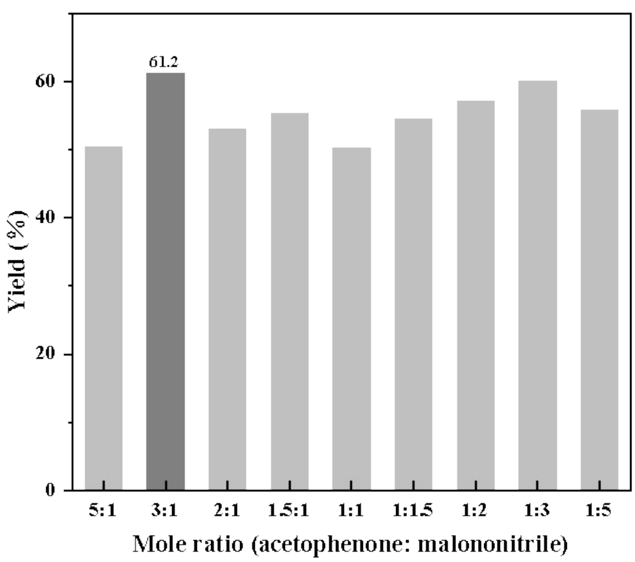


Fig. S5 Screening of substrate molar ratio. Reaction condition: **1a** (0.24 mmol), **2** , MO (8 mol%), EtOH (10 % v/v), H_2O, total reaction volume 1 mL, 36 h, 36 W white light. Yield determined by HPLC analysis with **3a** sample.

The molar ratio of substrates may affect the equilibrium of the reaction and the selectivity of the product, therefore, we investigated the effect of substrate molar ratio (acetophenone: malononitrile) on yield. Varying the molar ratio (acetophenone: malononitrile) from 5:1 to 1:5 had little contribution to betterment. So we still used acetophenone: malononitrile=1:2 as the optimal reaction condition.

Table S4 Screening of wavelength [a]

Entry	Wavelength (nm)	Yield (%) [b]
1	385	55.3
2	410	63.0
3	460	57.6
4	White light	**85.6**
5	Dark	31.5

[a] Reaction condition：**1a** (0.20 mmol), **2** (0.40 mmol), MO (8 mol%), H_2O (900 μL), EtOH (100 μL), 36 h, 36 W. [b] Yield determined by HPLC analysis with **3a** sample.

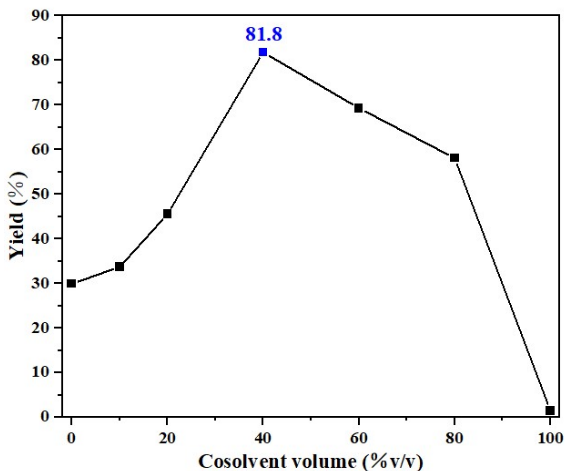
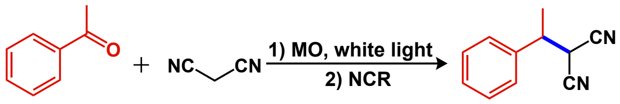


Fig. S6 Screening of co-solvent (EtOH) volunme. Reaction condition: **1c** (0.20 mmol), **2** (0.40 mmol), MO (8 mol%), EtOH (x % v/v), H_2O, total reaction volume 1 mL, 36 h, 36 W white light. Yield determined by HPLC analysis with **3c** sample.

3.5 Optimization of photoenzymatic reaction conditions

Table S5 Screening of dilution ratio[a]



1a **2** **4a**

Entry	Final substrate concentration (mM)	Yield (%) [b]	ee (%) [b]
1	10	50.2	98.3
2	20	25.4	99.4
3	30	19.9	98.4
4	40	14.9	96.5
5	50	15.1	92.4

[a] Reaction condition: **1a** (0.20 mmol), **2** (0.40 mmol), MO (8 mol%), H_2O (900 μL), EtOH (100 μL), 36 h, 36 W white light. Then added crude NCR (0.4 mg/mL), GDH (0.5 mg/mL), Glucose (20 mM), $NADP^+$ (0.1 mM), DMSO (10 % v/v) and diluted to different multiples with PBS (0.01 M, pH 7.2-7.4) , 37 °C, 20 h. [b] Yield and ee value were determined by Chiral HPLC analysis with racemic **4a** sample.

After determining the optimal enzyme catalyst, we attempted two tandem strategies: 1) adding all components to the system and conducting both photocatalytic and enzymatic reactions simultaneously; 2) conducting the photoreaction first, followed by adding ERs and cofactor cycling systems for enzymatic reaction. Unfortunately, neither strategy was feasible, due to the disruption of enzymes by high concentrations of substrates. However, a decrease in substrate concentration significantly reduced the yield of olefin intermediates (Fig. S4). Therefore, we propose a dilution strategy to cascade photoreaction and enzyme reaction. After the photo reaction is completed, dilute the reaction system and add the required components for enzyme catalysis for enzymatic reaction. This strategy reduces the damage of high-concentration substrates to enzymes while maintaining a high photoreaction yield. The results showed that the higher the dilution ratio, the higher the yield of enzyme-catalyzed reactions (Table S5). However, excessive dilution increases production costs and makes post-processing operations more complex. Therefore, we chose a final substrate concentration of 10 mM to achieve photo-enzymatic cascade catalysis.

Table S6 Comparing the catalytic effects of purified enzymes, crude enzymes, and whole cell [a]

Entry	Enzyme (0.3 mg)	Yield (%)[b]
1	Whole cells	34.0
2	Crude enzymes	51.6
3	Purified enzymes	48.8

[a] Reaction condition: **1a** (0.20 mmol), **2** (0.40 mmol), MO (8 mol%), H_2O (900 μL), EtOH (100 μL), 36 h, 36 W white light. Then added NCR (20 mg), GDH (0.618 mg), Glucose (72.2 mg), $NADP^+$ (0.001 mmol), DMSO (10 % v/v) and dilute the system to 20 mL with PBS (0.01 M, pH 7.2-7.4), 37 °C, 24 h. [b] Yield determined by Chiral HPLC analysis.

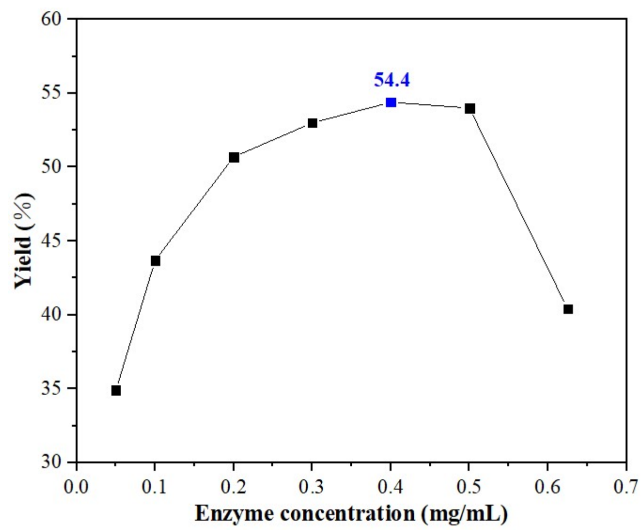


Fig. S7 Screening of enzyme concentration. Reaction condition: **1a** (0.20 mmol), **2** (0.40 mmol), MO (8 mol%), H_2O (900 μL), EtOH (100 μL), 36 h, 36 W white light. Then 100 μL of the reaction system was added crude NCR, GDH (0.3 mg), Glucose (20 mM), $NADP^+$ (0.1 mM), DMSO (10 % v/v) and diluted to 2 mL with PBS (0.01 M, pH 7.2-7.4), 37 °C, 20 h. Yield determined by HPLC analysis.

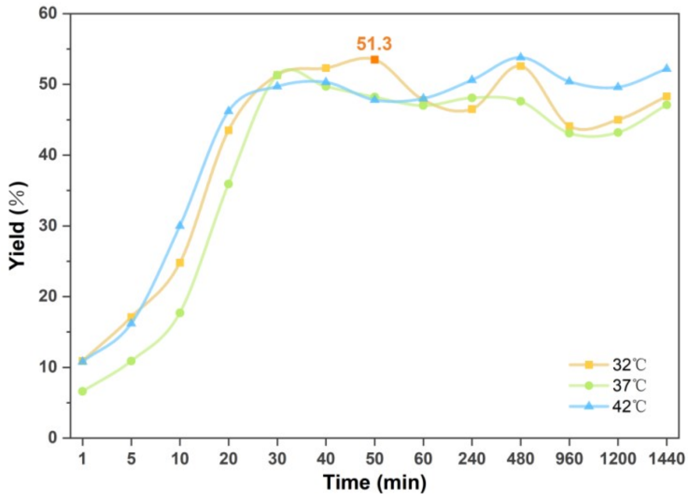


Fig. S8 Time-temperature curve. Reaction conditions: **1a** (0.20 mmol), **2** (0.40 mmol), MO (8 mol%), H_2O (900 μL), EtOH (100 μL), 36 h, 36 W white light. Then 100 μL of the reaction system was added crude NCR (0.4 mg/mL), GDH (0.5 mg/mL), Glucose (20 mM), $NADP^+$ (0.1 mM), DMSO (10 % v/v) and diluted to 2 mL with phosphate buffer (0.01 M, pH 7.2-7.4), 160 rpm. Yield determined by Chiral HPLC analysis with **4a** sample.

Table S7 Screening of buffer [a]

Entry	Buffer	Yield (%) [b]
1	PBS	55.2
2	MOPS	65.7
3	Tris-HCl	65.5
4	Tricine	64.2
5	TEOA	**68.0**

[a] Reaction condition：**1a** (0.20 mmol), **2** (0.40 mmol), MO (8 mol%), H_2O (900 μL), EtOH (100 μL), 36 h, 36 W white light. Then 100 μL of the reaction system was added crude NCR (0.4 mg/mL), GDH (0.5 mg/mL), Glucose (20 mM), $NADP^+$ (0.1 mM), DMSO (10 % v/v) and diluted to 2 mL with buffer (0.01 M, pH 7.2-7.4), 32 ℃, 200 rpm, 50 min. [b] Yield was determined by Chiral HPLC analysis with racemic product samples.

Table S8 Screening of pH [a]

Entry	pH	Yield (%) [b]	ee (%) [b]
1	5.5	66.8	83.0
2	6.5	65.5	82.2
3	7.2-7.4	**66.6**	**97.7**
4	8.5	66.0	97.6
5	9.5	64.6	97.8

[a] Reaction condition：**1a** (0.20 mmol), **2** (0.40 mmol), MO (8 mol％), H_2O (900 μL), EtOH (100 μL), 36 h, 36 W white light. Then 100 μL of the reaction system was added crude NCR (0.4 mg/mL), GDH (0.5 mg/mL), Glucose (20 mM), $NADP^+$ (0.1 mM), DMSO (10 % v/v) and diluted to 2 mL with TEOA buffer (0.01 M), 32 ℃, 200 rpm, 50 min. [b] Yield and ee value determined by Chiral HPLC analysis with racemic product samples.

Table S9 Screening of co-solvent [a]

Entry	Co-solvent	Yield (%) [b]
1	DMSO	**70.6**
2	ACN	65.7
3	EtOH	59.2
4	IPA	65.7
5	THF	63.4
6	H_2O	45.2

[a] Reaction condition：**1a** (0.20 mmol), **2** (0.40 mmol), MO (8 mol%), H_2O (900 μL), EtOH (100 μL), 36 h, 36 W white light. Then 100 μL of the reaction system was added crude NCR (0.4 mg/mL), GDH (0.5 mg/mL), Glucose (20 mM), $NADP^+$ (0.1 mM), co-solvent (10 % v/v) and diluted to 2 mL with TEOA buffer (0.01 M, pH 7.2-7.4), 32 °C, 200 rpm, 50 min. [b] Yield determined by Chiral HPLC analysis with racemic product samples.

3.6 Enzyme kinetics

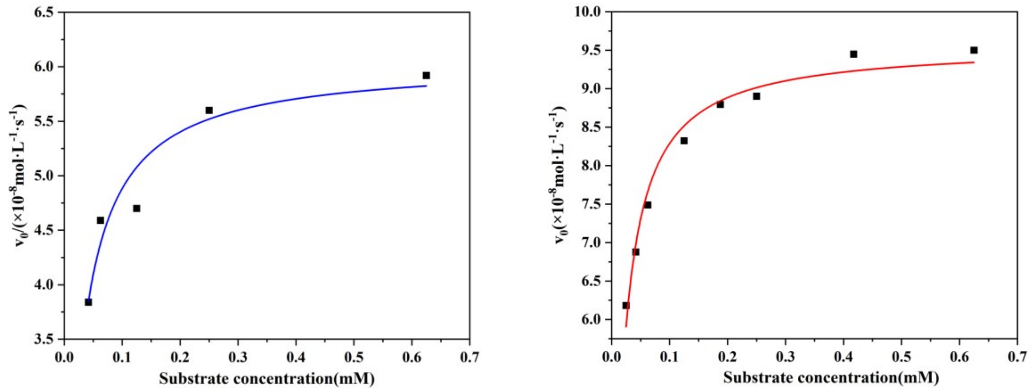


Fig. S9 Enzymatic reaction kinetics curves of OYE1-3a (left) and NCR-3a (right). Reaction condition: 1c (0.025-2.5 mM) , NADPH (0.25 mM), PBS (10 mM, pH 7.2-7.4), purified enzymes (20 μg), total reaction volume 200 μL. Measured the change in absorbance value at 340 nm.

3.7 Gene sequence comparison

Use DNAMAN and DNAstar bioinformatics software for analysis. Based on the sequence comparison results (Fig. S10), we found that the gene sequences of OYE1 and OYE3 have a high consistency of 80.25%, and the amino acids at the active sites are highly conserved (only different in the 296 position). The sequence consistency between GluER and NCR reached 67.85%, and the amino acids at the active sites also showed high conservatism. The yellow highlighted amino acids are identified as active site amino acids in the literature [3].

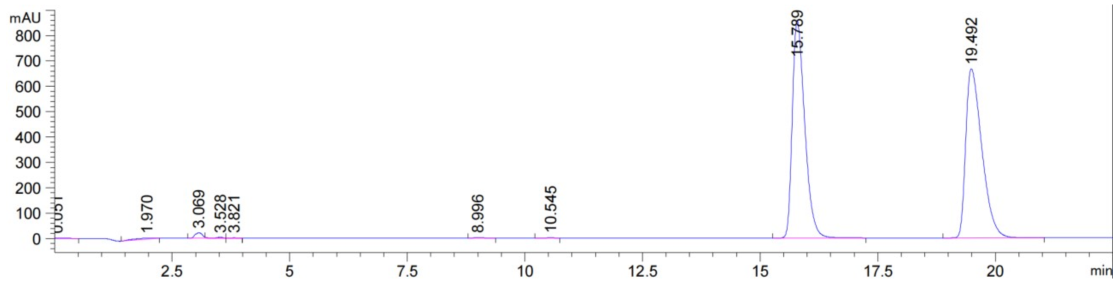
```
GluER.pro       .......HHH HHHMPTLFDP IDFGPIHAKN RIVMSPLTRG RADKEAVP..
NCR.pro         .......... ...MPSLFDP IRFGAFTAKN RIWMAPLTRG RATRDHVP..
OYE1.pro        MSFVKDFKPQ ALGDTNLFKP IKIGNNELLH RAVIPPLTRM RALHPGNIPN
OYE3.pro        MPFVKGFEPI SLRDTNLFEP IKIGNTQLAH RAVMPPLTRM RATHPGNIPN

GluER.pro       TPIMAEYYAQ RASAG..LII TEATGISREG LGWPFAPGIW SDAQVEAWKP
NCR.pro         TEIMAEYYAQ RASAG..LII SEATGISQEG LGWPYAPGIW SDAQVEAWLP
OYE1.pro        RDWAVEYYTQ RAQRPGTMII TEGAFISPQA GGYDNAPGVW SEEQMVEWTK
OYE3.pro        KEWAAVYYGQ RAQRPGTMII TEGTFISPQA GGYDNAPGIW SDEQVAEWKN

GluER.pro       IVAGVHAKGG KIVCQLWHMG RMVH...SSV TGTQPVSSSA TTAPGEVHTY
NCR.pro         ITQAVHDAGG LIFAQLWHMG RMVP...SNV SGMQPVAPSA SQAPGLGHTY
OYE1.pro        IFNAIHEKKS FVWVQLWVLG WAAFPDNLAR DGLRYDSASD NVFMDAEQEA
OYE3.pro        IFLAIHDCQS FAWVQLWSLG WASFPDVLAR DGLRYDCASD RVYMNATLQE

GluER.pro       EGKK...... PFEQARAIDA ADISRILNDY ENAARNAIRA GFDGVQIHAA
NCR.pro         DGKK...... PYDVARALRL DEIPRLLDDY EKAARHALKA GFDGVQIHAA
OYE1.pro        KAKK...... ANNPQHSLTK DEIKQYIKEY VQAAKNSIAA GADGVEIHSA
OYE3.pro        KAKD...... ANNLEHSLTK DDIKQYIKDY IHAAKNSIAA GADGVEIHSA

GluER.pro       NGYLIDEFLR NGTNHRTDEY GGVPENRIRF LKEVTERVIA AIGADRTGVR
NCR.pro         NGYLIDEFIR DSTNHRHDEY GGAVENRIRL LKDVTERVIA TIGKERTAVR
OYE1.pro        NGYLLNQFLD PHSNTRTDEY GGSIENRARF TLEVVDALVE AIGHEKVGLR
OYE3.pro        NGYLLNQFLD PHSNKRTDEY GGTIENRARF TLEVVDALIE TIGPERVGLR

GluER.pro       LSPNG..... .......... ...DTQGCID SAPETVFVPA AKLLQDLGVA
NCR.pro         LSPNG..... .......... ...EIQGTVD SHPEQVFIPA AKMLSDLDIA
OYE1.pro        LSPYGVFNSM SGGAETGIVA QYAYVAGELE KRAKAGKRLA FVHLVEPRVT
OYE3.pro        LSPYGTFNSM SGGAEPGIIA QYSYVLGELE KRAKAGKRLA FVHLVEPRVT

GluER.pro       WLELREPGPN GTFGKTDQPK LSPQIRKVFL RPLVLNQDYT FEAAQTALAE
NCR.pro         FLGMREGAVD GTFGKTDQPK LSPEIRKVFK PPLVLNQDYT FETAQAALDS
OYE1.pro        NPFLTEGEGE YEGGSNDFVY SIWKGPVIRA GNFALHPEVV REEVK...DK
OYE3.pro        DPSLVEGEGE YSEGTNDFAY SIWKGPIIRA GNYALHPEVV REQVK...DP

GluER.pro       GKADAIAFGR KFISNPDLPE RFARGIALQP DDMKTWYSQG PEGYTDYPSA
NCR.pro         GVADAISFGR PFIGNPDLPR RFFEKAPLTK DVIETWYTQT PKGYTDYPLL
OYE1.pro        ..RTLIGYGR FFISNPDLVD RLEKGLPLNK YDRDTFYQMS AHGYIDYPTY
OYE3.pro        ..RTLIGYGR FFISNPDLVY RLEEGLPLNK YDRSTFYTMS AEGYTDYPTY

GluER.pro       TSGPN..... ......
NCR.pro         GD........ ......
OYE1.pro        EEALKLGWDK K.....
OYE3.pro        EEAVDLGWNK N.....
```

Fig. S10 Sequence comparison results

4 Chiral HPLC data for enantiomers and reaction systems

No.	product	Column	Solvent system (hexane/i-PrOH)	Flow rate (mL/min)	Detection wavelength	Retention times
1	4a	OD-3	90:10	1.0	210 nm	15.789 min; 19.492 min
2	4b	OJ	80:20	1.0	210 nm	9.746 min; 11.219 min
3	4c	OJ	80:20	1.0	210 nm	8.076 min; 9.058 min
4	4d	OD-3	93:7	1.0	210 nm	15.588 min; 18.444 min
5	4e	IH	95:5	1.0	210 nm	15.235 min; 16.334 min
6	4f	OJ	80:20	1.0	210 nm	10.162 min; 11.630 min
7	4g	IH	90:10	1.0	210 nm	14.495 min; 16.270 min
8	4h	OJ	98:2	1.0	220 nm	30.419 min; 32.769 min
9	4i	OD-3	90:10	1.0	210 nm	18.253 min; 19.268 min
10	4j	OD-3	90:10	1.0	220 nm	19.508 min; 20.996 min
11	4k	IH	95:5	1.0	210 nm	18.752 min; 20.405 min
12	4l	OD-3	95:5	1.0	210 nm	24.082 min; 26.926 min
13	4m	IH	90:10	1.0	210 nm	21.653 min; 24.289 min
14	4n	OJ	80:20	1.0	220 nm	14.996 min; 18.683 min
15	4o	IH	99:1	1.0	210 nm	18.538 min; 19.841 min

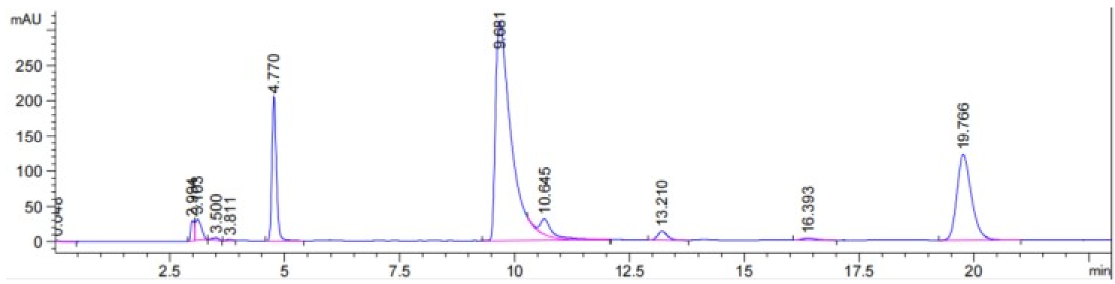
HPLC trace of *rac*-**4a:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	15.789	1.57640e4	856.89081	48.9195
2	19.492	1.59354e4	667.27185	49.4513

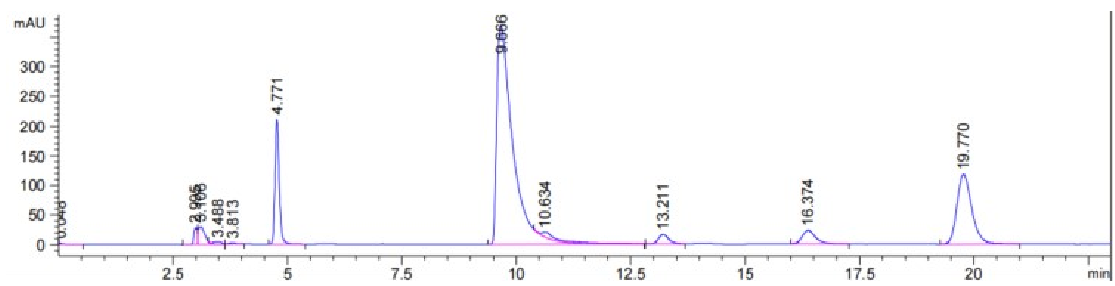
HPLC trace of **4a**, obtained with photoenzymatic reaction**:**

NCR**:**



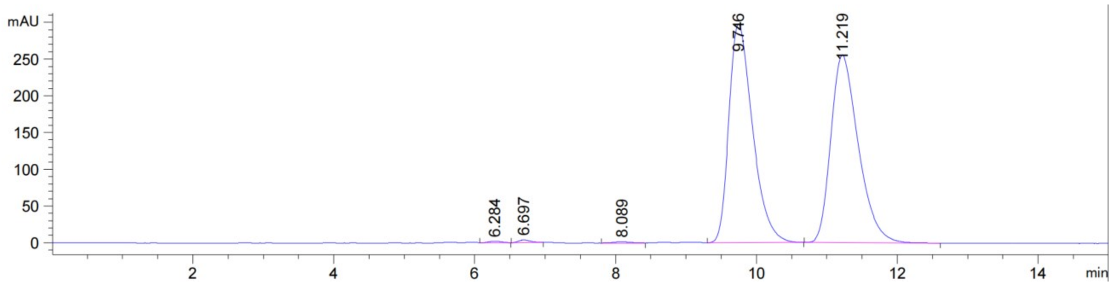
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	16.393	60.92949	2.90903	0.4762
2	19.766	2737.77466	121.53600	21.3963

GluER**:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	16.374	458.72461	23.09771	3.1309
2	19.770	2651.01074	117.57581	18.0937

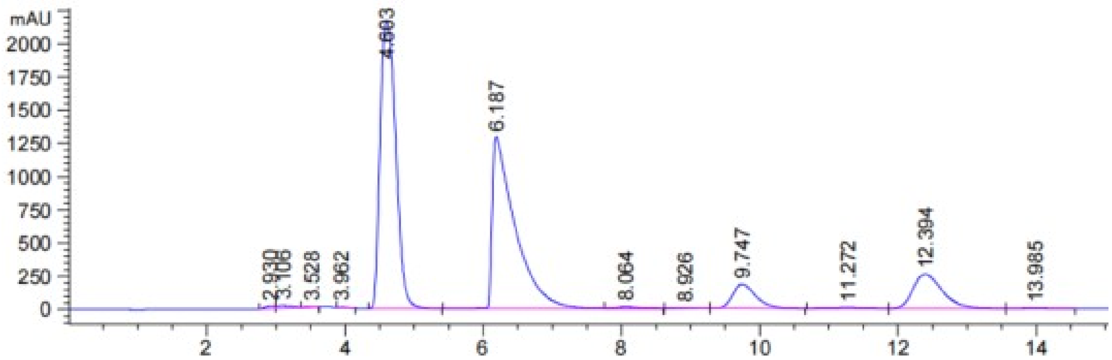
HPLC trace of *rac*-**4b:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	9.746	6941.44189	297.70920	48.3570
2	11.219	6930.19385	255.54117	48.2786

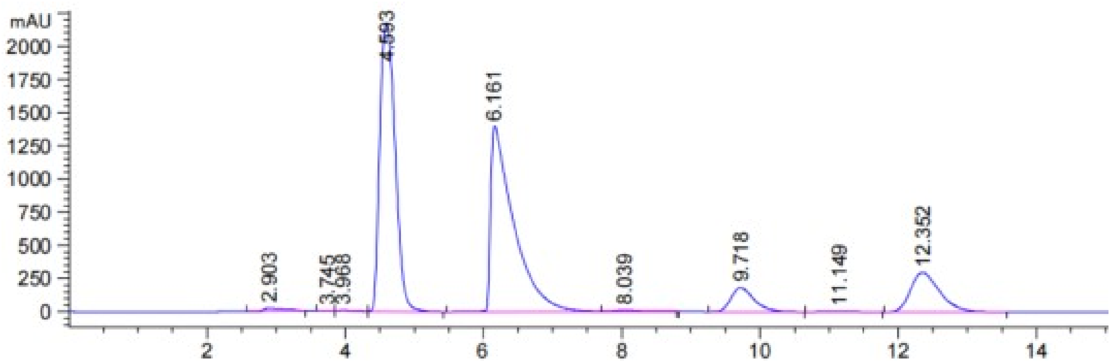
HPLC trace of **4b**, obtained with photoenzymatic reaction**:**

NCR**:**



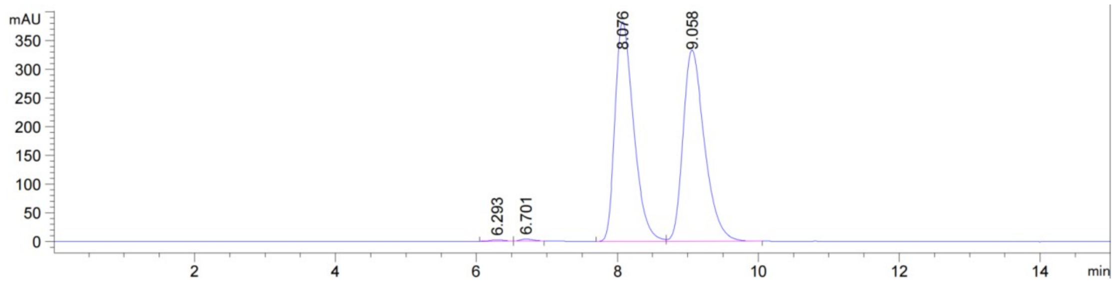
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	9.747	4226.45947	181.16693	5.3964
2	11.272	213.20457	6.82493	0.2722

GluER**:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	9.718	4232.58936	181.00433	5.2383
2	11.149	94.93866	2.47643	0.1175

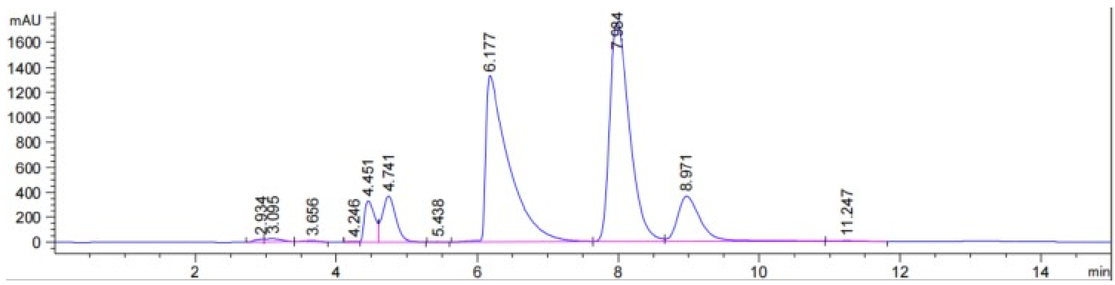
HPLC trace of *rac*-**4c:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	8.076	7030.05469	382.98389	48.3649
2	9.058	7052.33594	333.38864	48.5182

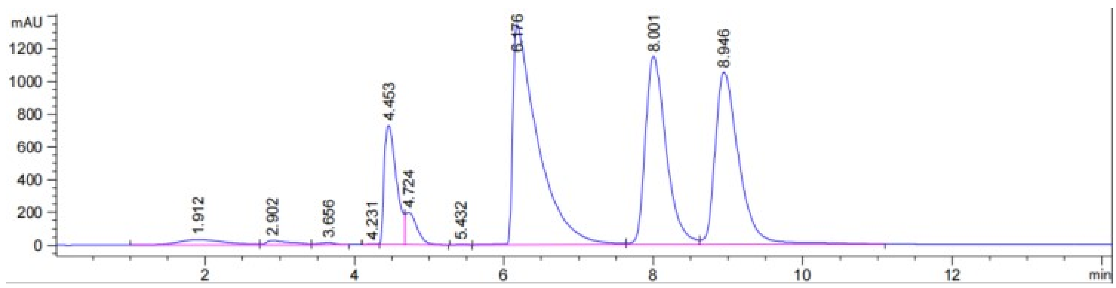
HPLC trace of **4c**, obtained with photoenzymatic reaction**:**

NCR**:**



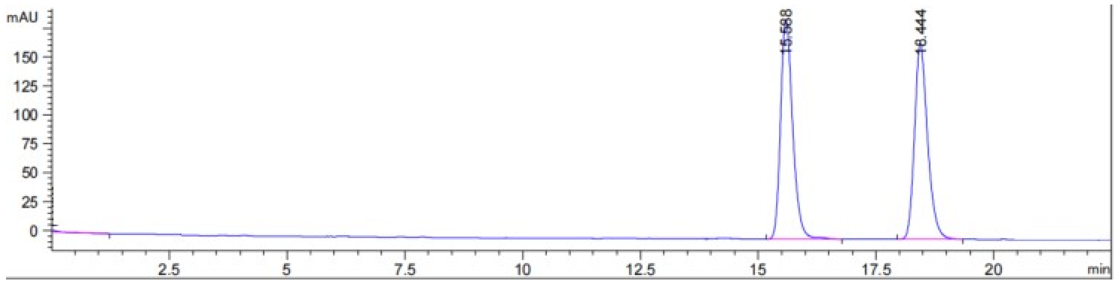
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	7.984	3.45265e4	1753.48865	42.0470
2	8.971	8148.20020	361.23303	9.9230

GluER**:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	8.001	2.21883e4	1149.22314	24.9269
2	8.946	2.30237e4	1050.80701	25.8655

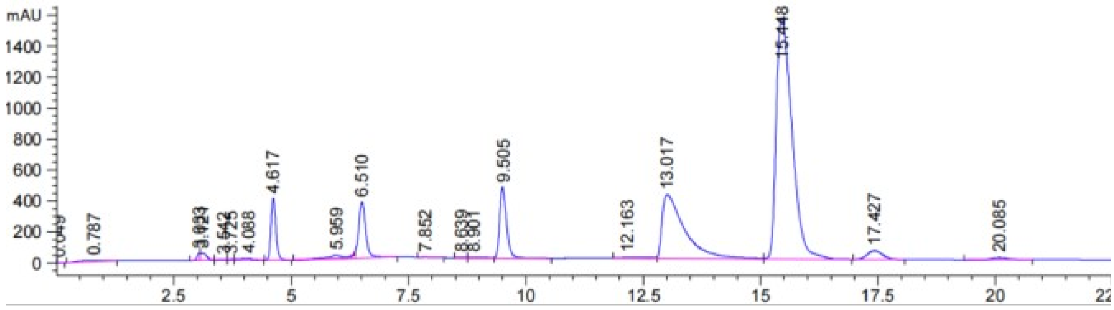
HPLC trace of *rac*-**4d:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	15.588	3363.63892	190.08977	50.2782
2	18.444	3326.41479	169.09909	49.7217

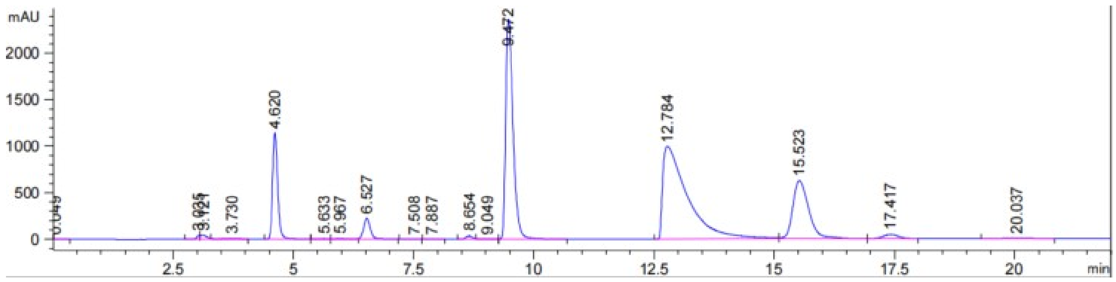
HPLC trace of **4d**, obtained with photoenzymatic reaction**:**

NCR**：**



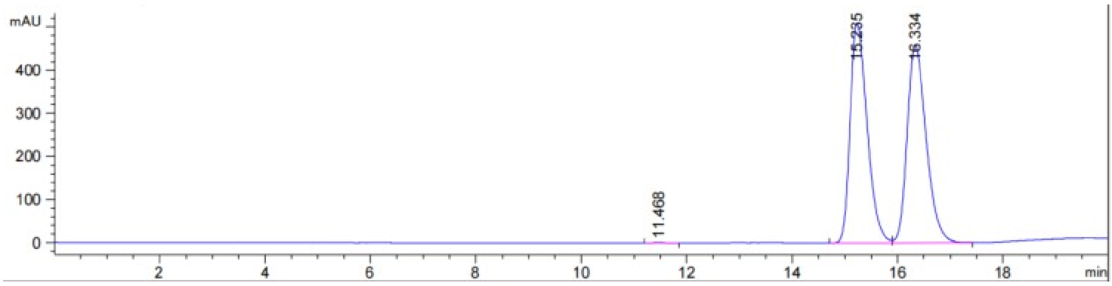
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	15.448	3.64929e4	1555.23462	55.7185

GluER**：**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	15.523	1.45240e4	626.25342	16.2623

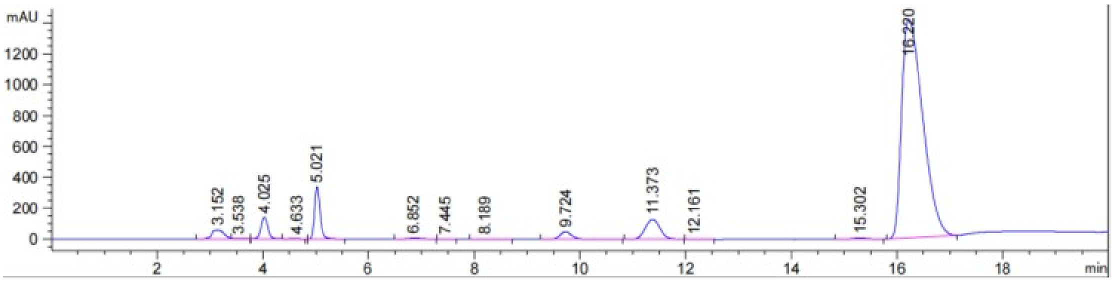
HPLC trace of *rac*-**4e:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	15.235	1.14580e4	508.50076	49.7050
2	16.334	1.15731e4	457.55530	50.2044

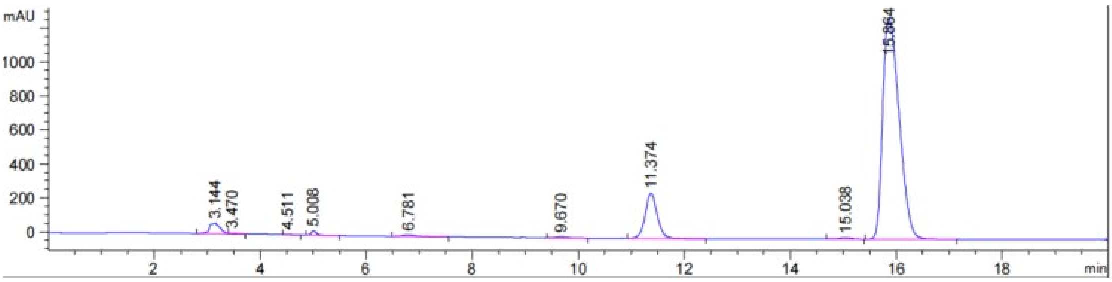
HPLC trace of 4e, obtained with photoenzymatic reaction:

NCR：



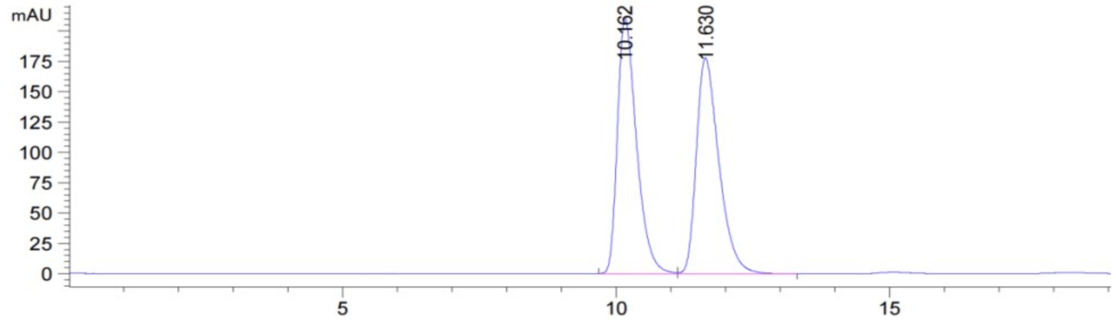
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	15.302	149.80655	7.56626	0.3135
2	16.220	3.93613e4	1416.40027	82.3595

GluER：



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	15.038	127.69012	7.63333	0.3811
2	15.864	2.78980e4	1297.89941	83.2548

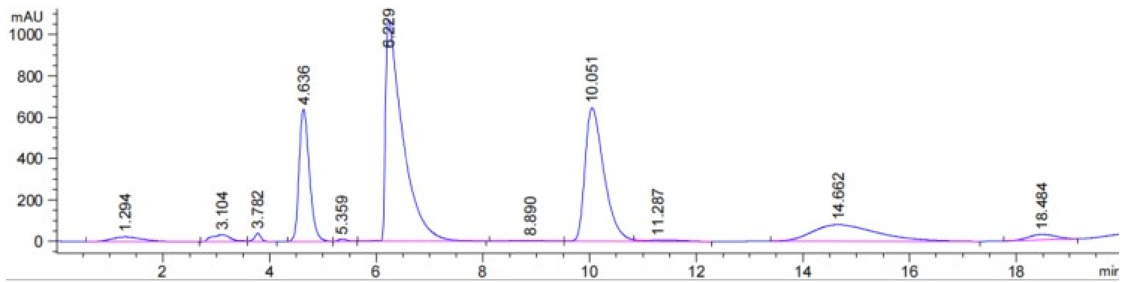
HPLC trace of *rac*-**4f:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	10.162	5102.08691	210.82014	49.8963
2	11.630	5123.28564	177.94373	50.1037

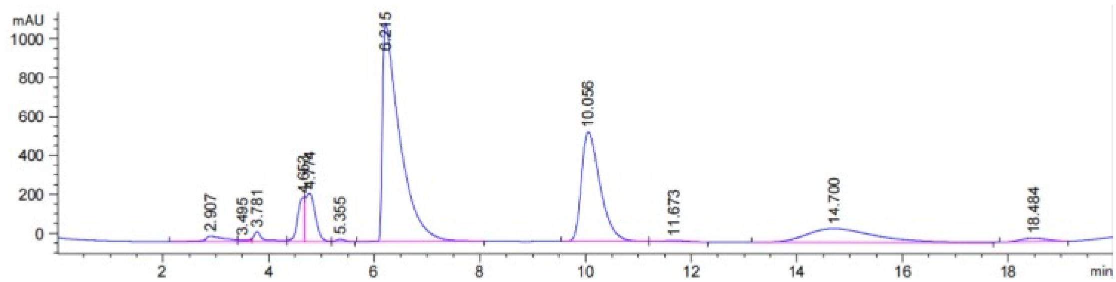
HPLC trace of **4f**, obtained with photoenzymatic reaction**:**

NCR**:**



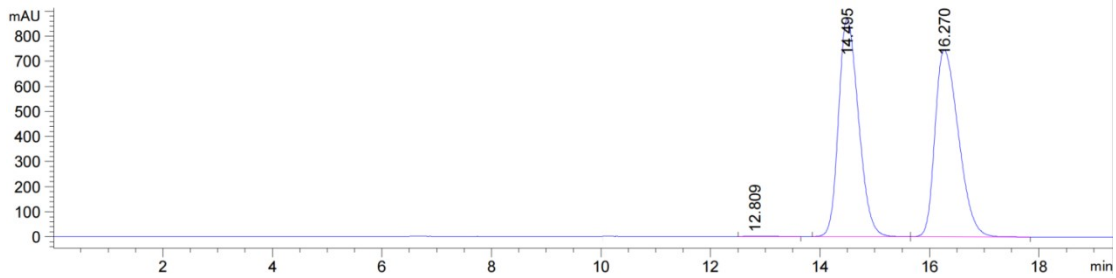
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	10.051	1.59094e4	646.27936	26.9675
2	11.287	263.17883	5.71406	0.4461

GluER**:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	10.056	1.37798e4	561.82489	26.0119
2	11.673	158.51068	5.90323	0.2992

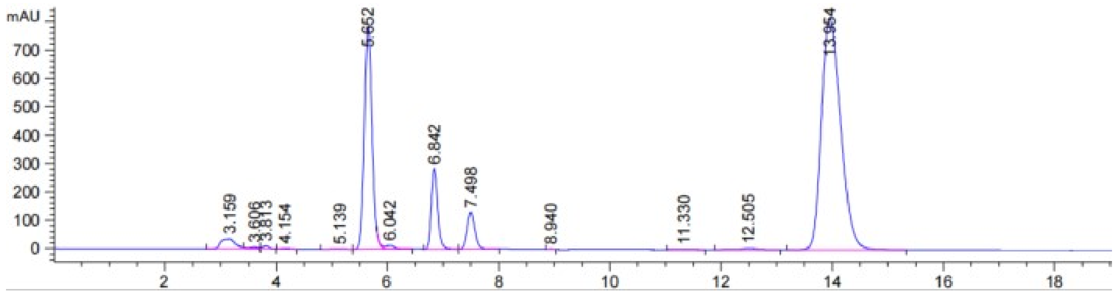
HPLC trace of *rac*-**4g:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	14.495	2.20161e4	869.40277	49.8851
2	16.270	2.21175e4	744.71246	50.1149

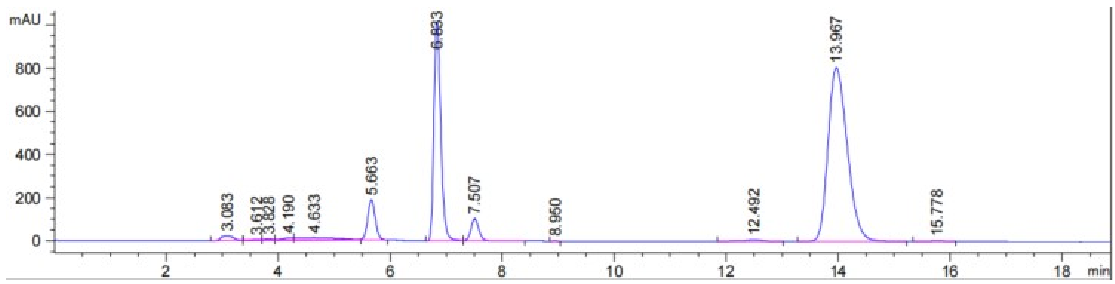
HPLC trace of **4g**, obtained with photoenzymatic reaction**:**

NCR**：**



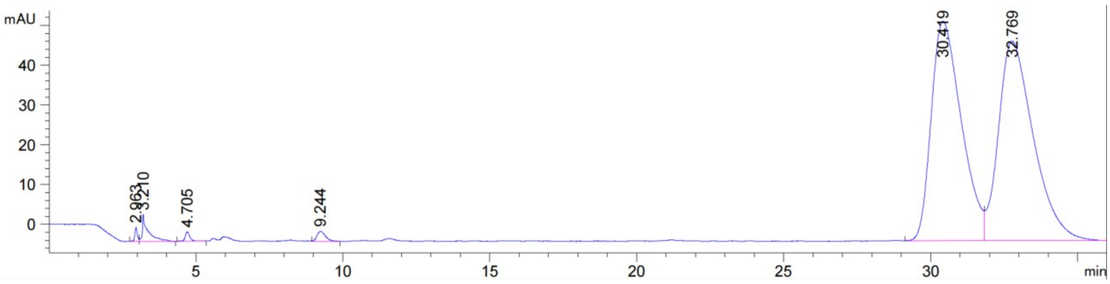
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	13.954	1.96055e4	815.05640	61.2883

GluER**：**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	13.967	1.93042e4	804.71729	60.1333
2	15.778	29.56886	1.36888	0.0921

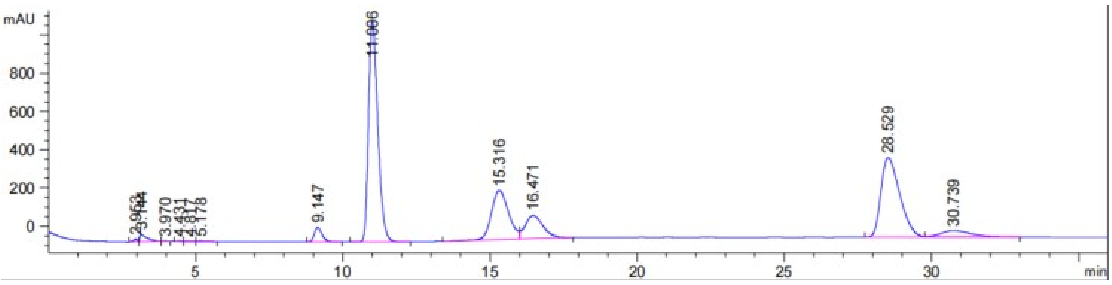
HPLC trace of *rac*-**4h:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	30.419	3929.84253	55.13811	47.5448
2	32.769	4145.77637	50.16341	50.1573

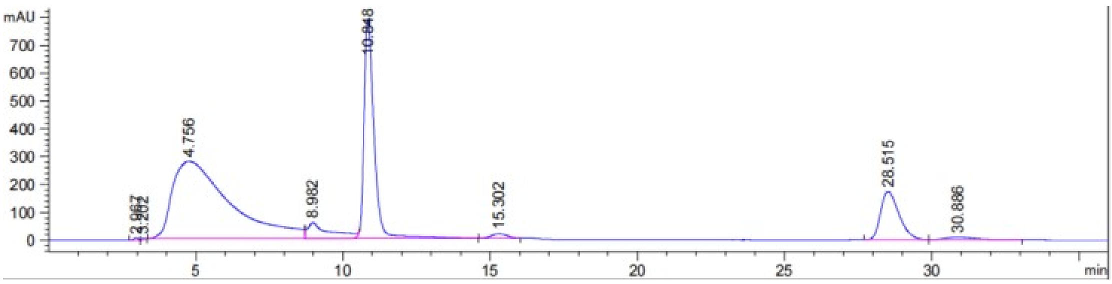
HPLC trace of **4h**, obtained with photoenzymatic reaction**:**

NCR**:**



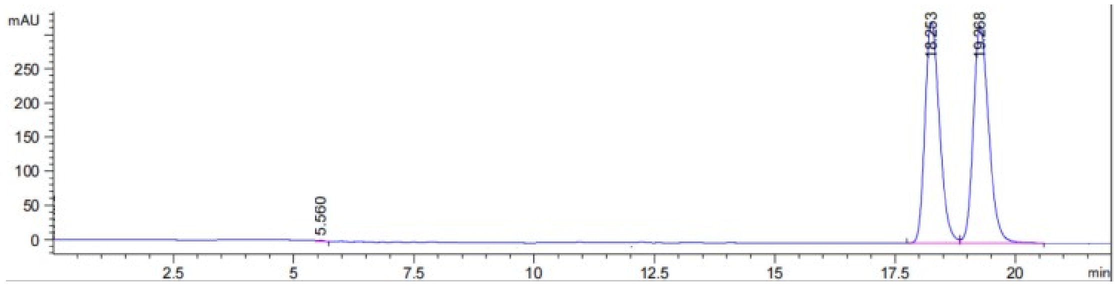
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	30.738	1490.11157	21.80930	4.3955

GluER**:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	30.886	659.06970	9.77491	0.9556

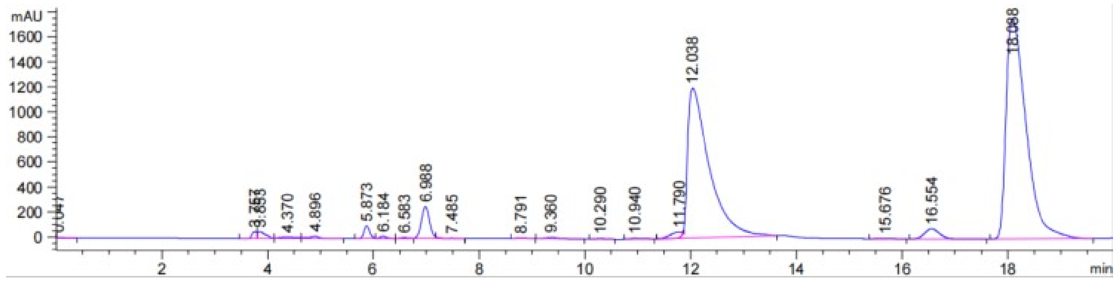
HPLC trace of *rac*-**4i:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	18.253	6719.63574	322.92419	48.7767
2	19.268	7039.95361	318.33133	51.1018

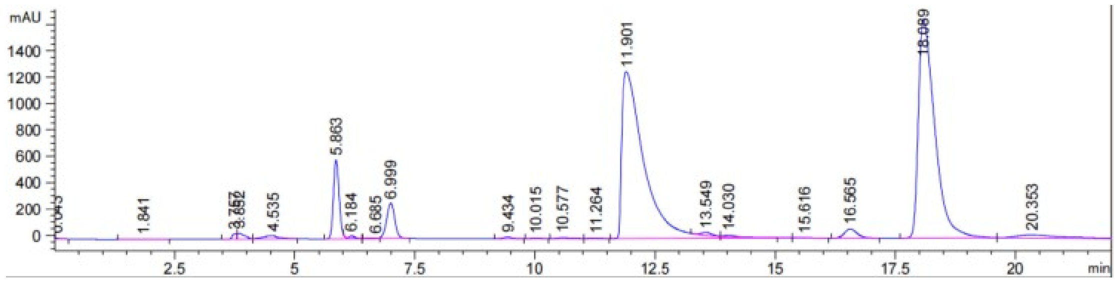
HPLC trace of **4i**, obtained with photoenzymatic reaction**:**

NCR**:**



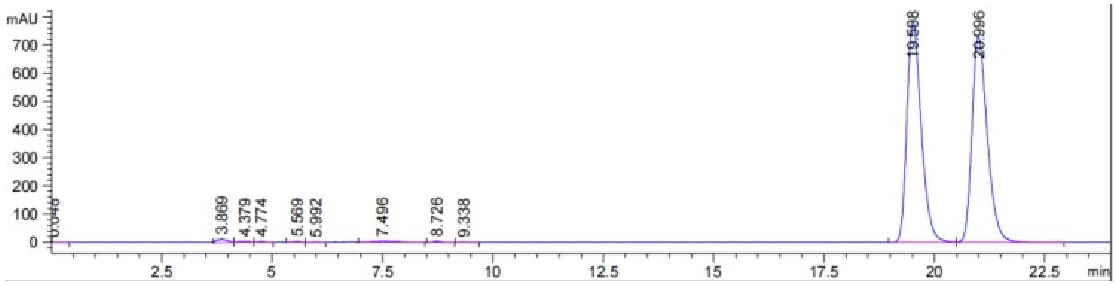
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	18.088	4.61496e4	1764.75989	52.3042

GluER**:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	18.089	3.98015e4	1655.46509	42.3998

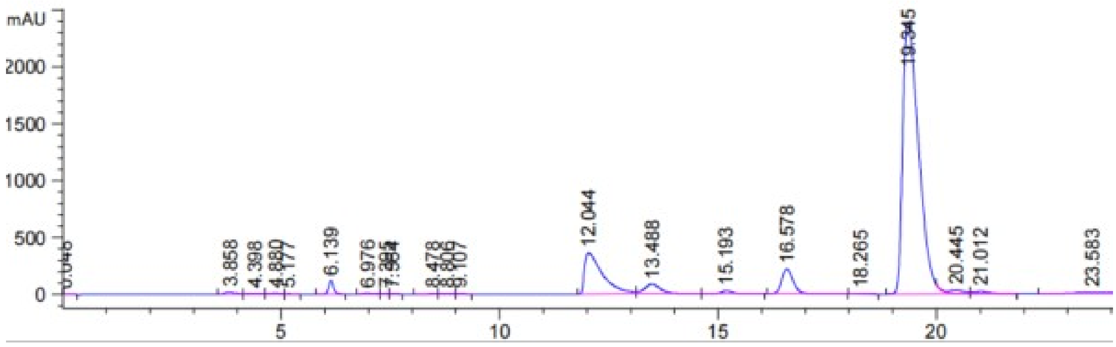
HPLC trace of *rac*-**4j:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	19.508	1.77560e4	782.56616	49.0597
2	20.996	1.78110e4	730.10089	49.2117

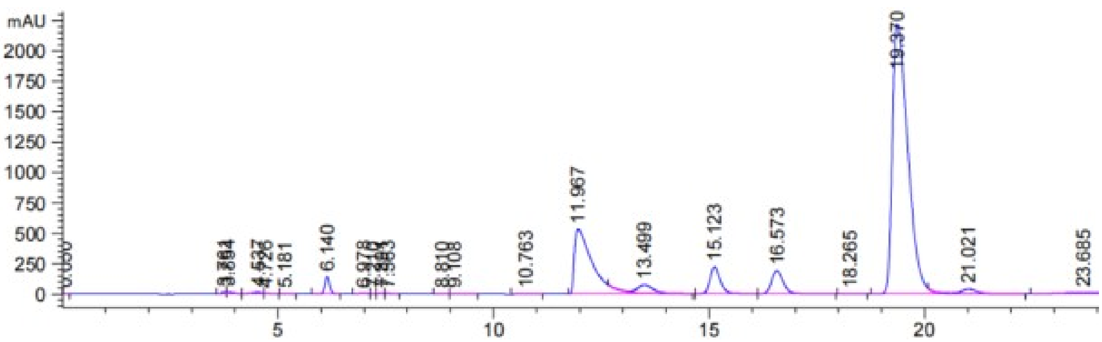
HPLC trace of **4j**, obtained with photoenzymatic reaction**:**

NCR：



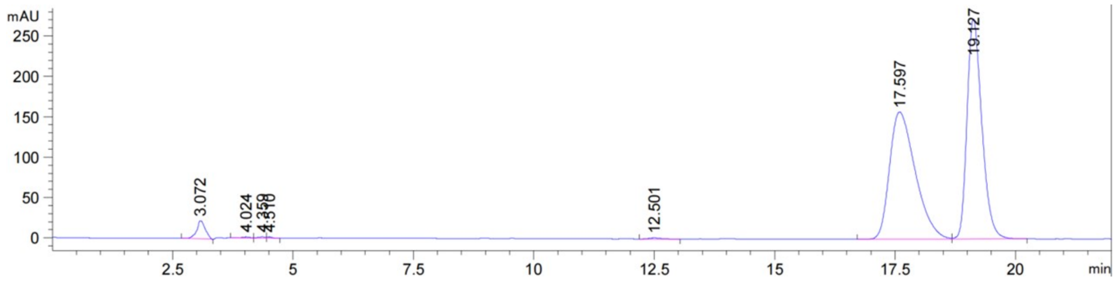
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	19.345	6.08409e4	2388.93457	72.2577
2	21.012	554.55219	21.46774	0.6586

GluER：



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	19.370	5.53249e4	2213.53857	64.6561
2	21.021	1190.23193	38.06504	1.3910

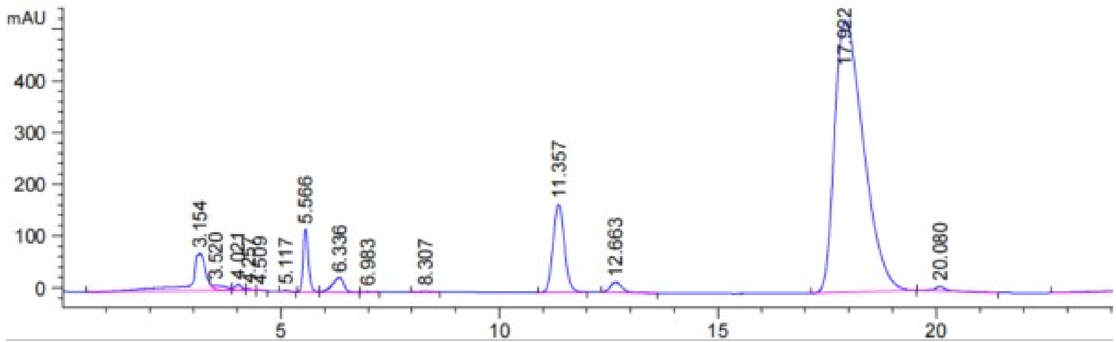
HPLC trace of *rac*-**4k:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	17.597	5850.75342	157.60025	48.6189
2	19.127	5837.43506	272.56689	48.5082

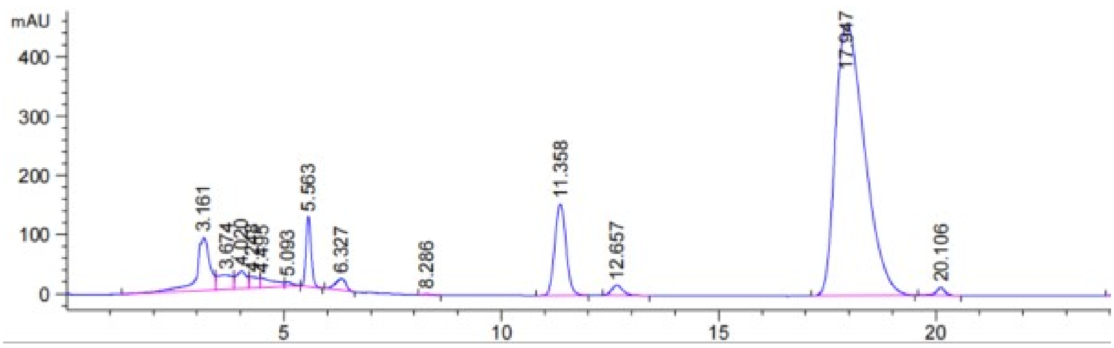
HPLC trace of **4k**, obtained with photoenzymatic reaction**:**

NCR**:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	17.922	2.42161e4	525.24359	66.4198
2	20.080	163.54437	8.74254	0.4486

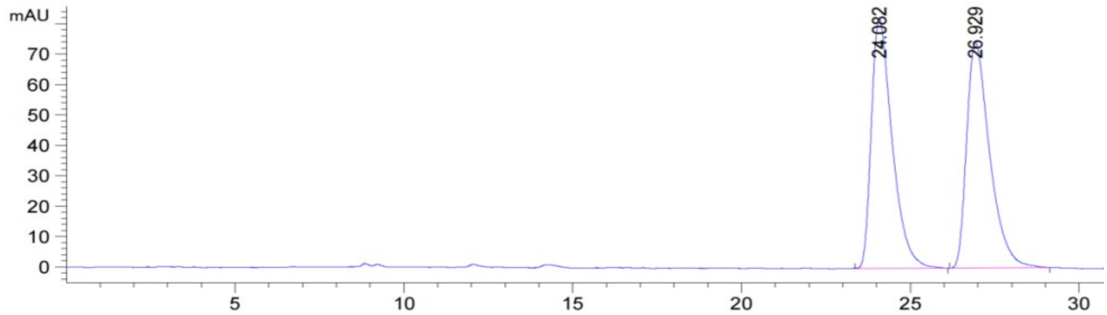
GluER**:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	17.947	2.14341e4	458.18457	63.4352

2	20.106	198.81239	13.95777	0.5884

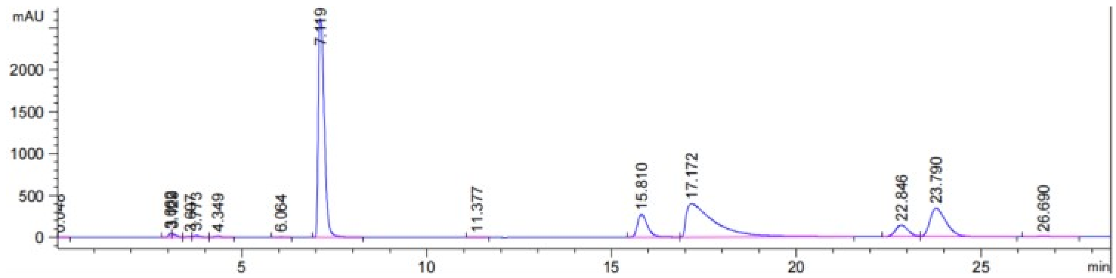
HPLC trace of *rac*-**4l:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	24.082	3532.67236	82.18550	49.9976
2	26.929	3533.01367	74.33914	50.0024

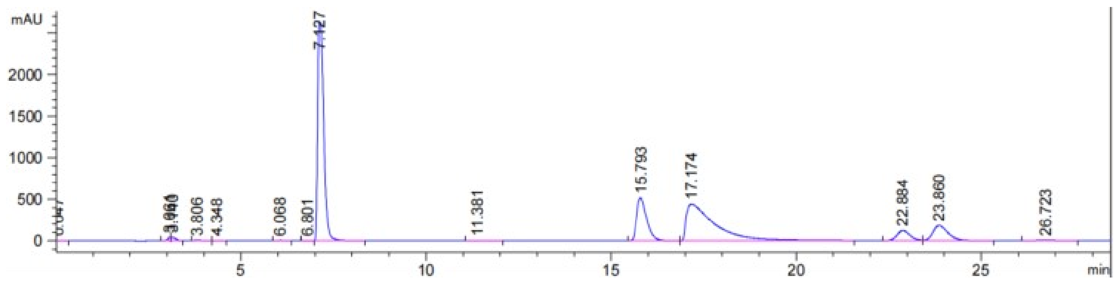
HPLC trace of **4l**, obtained with photoenzymatic reaction**:**

NCR**：**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	23.790	1.01904e4	342.11456	14.4829
2	26.690	172.28575	5.85135	0.2449

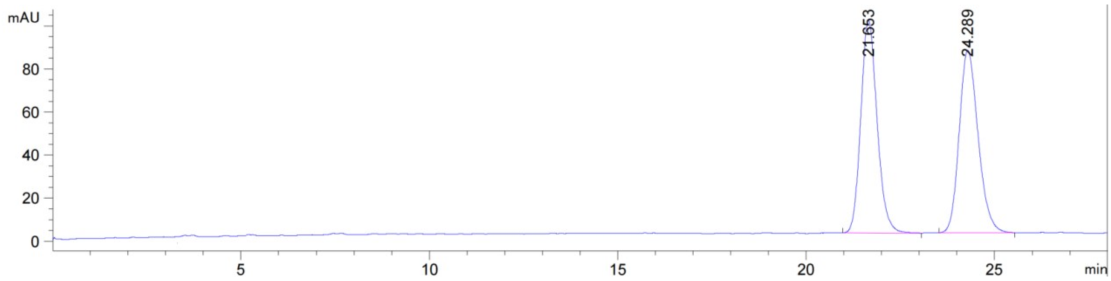
GluER**：**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	23.860	5201.79688	182.08836	7.1928

2	26.723	243.14815	8.13967	0.3362

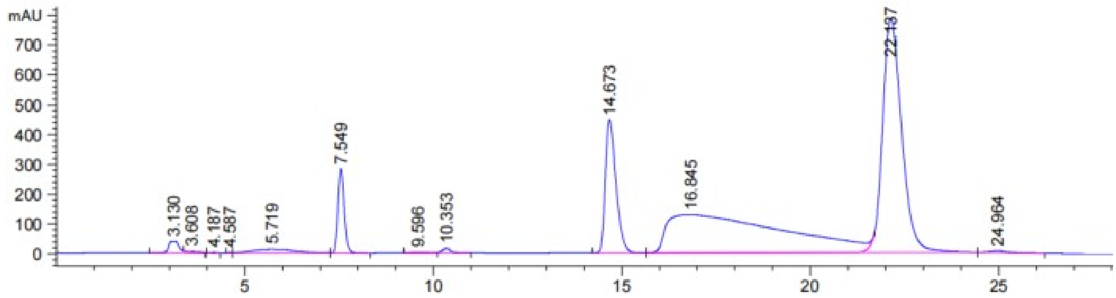
HPLC trace of *rac*-**4m:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	21.653	2966.98560	98.84965	50.0583
2	24.289	2960.07520	84.74799	49.9417

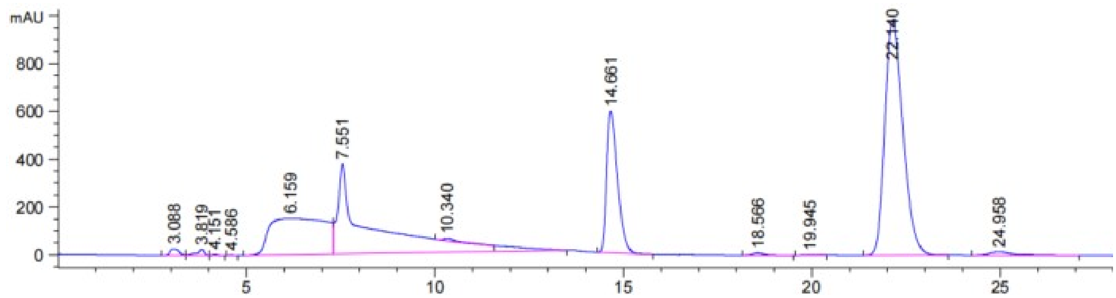
HPLC trace of **4m**, obtained with photoenzymatic reaction**:**

NCR**:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	22.137	2.77590e4	786.59204	39.9104
2	24.964	214.83688	5.87770	0.3089

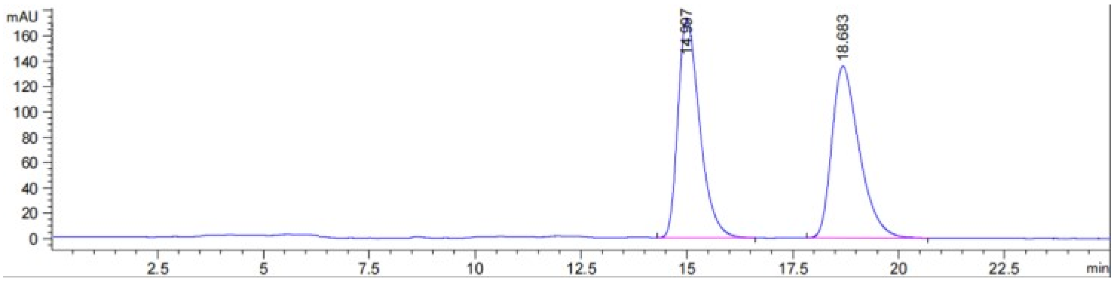
GluER**:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	22.140	3.28805e4	982.14142	37.1066

2	24.958	698.80994	14.92718	0.7886

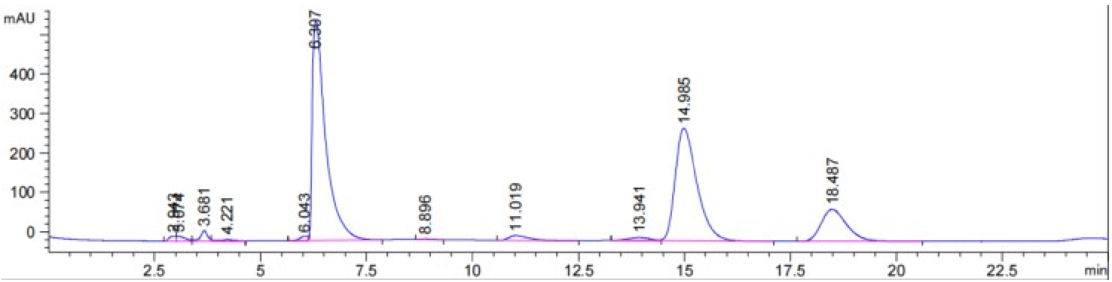
HPLC trace of *rac*-**4n:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	14.997	6142.64307	172.56667	49.9962
2	18.683	6143.57764	135.55336	50.0038

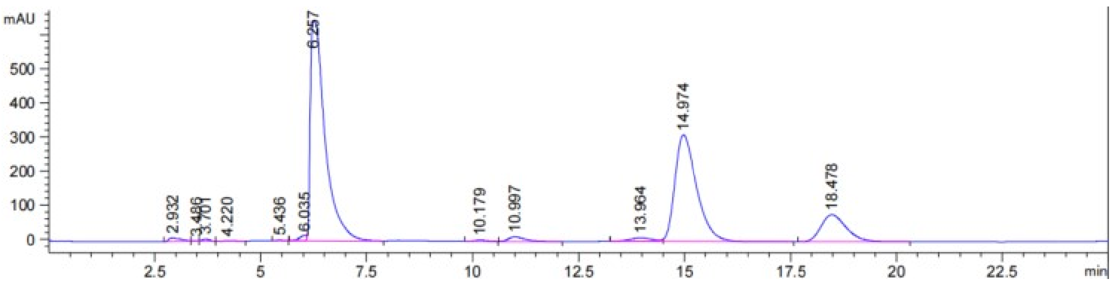
HPLC trace of **4n**, obtained with photoenzymatic reaction**:**

NCR**:**



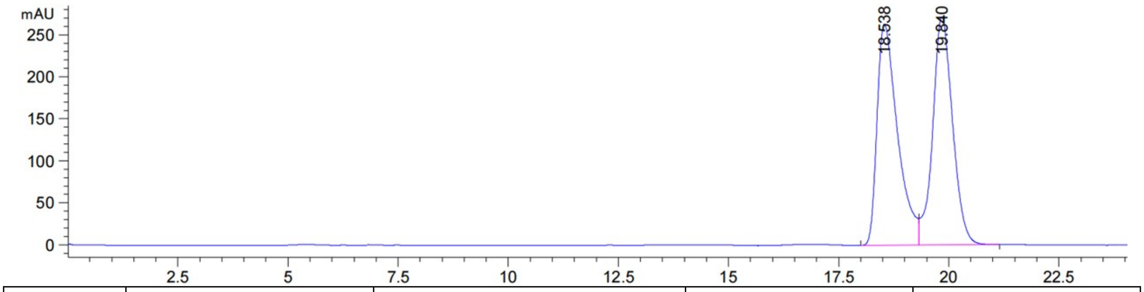
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	14.985	1.01677e4	284.84781	37.3009
2	18.487	3386.05225	80.53845	12.4219

GluER**:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	14.974	1.11670e4	311.94879	35.7363
2	18.478	3293.35913	78.98102	10.5393

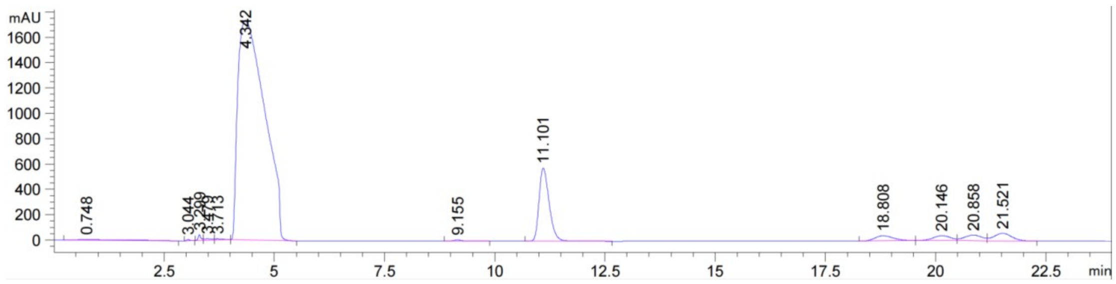
HPLC trace of *rac*-**4o:**



No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	18.538	8539.57422	263.33539	50.0419
2	19.840	8525.28809	271.54053	49.9581

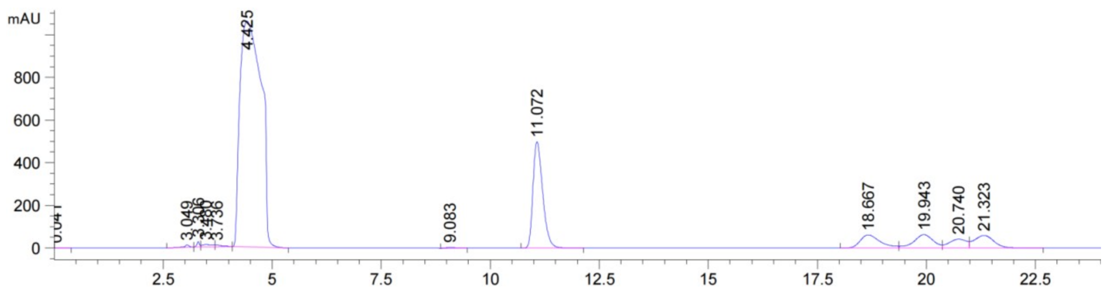
HPLC trace of **4o**, obtained with photoenzymatic reaction**:**

NCR**：**



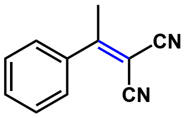
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	18.808	1192.88708	41.04874	1.3431
2	20.146	1103.00354	39.96597	1.2419

GluER**：**



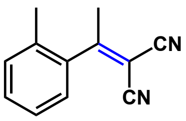
No.	Ret. Time (min)	Area (mAU*min)	Height (mAU)	Area (%)
1	18.667	1957.34180	61.58783	3.8366
2	19.943	1925.85596	62.73369	3.7748

5 ^{1}H NMR and ^{13}C NMR



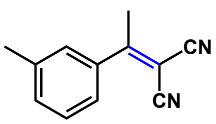
2-(1-phenylethylidene)malononitrile (3a)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.24 (m, 5H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.59, 135.85, 132.32, 129.13, 127.36, 112.82, 112.75, 84.66, 24.33, 24.32; light yellow solid



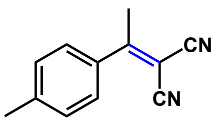
2-(1-(o-tolyl)ethylidene)malononitrile (3b)

^{1}H NMR (400 MHz, CDCl3) δ 7.37 – 7.24 (m, 3H), 7.07 (d, *J* = 8.0 Hz, 1H), 2.53 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 179.06, 136.59, 133.62, 131.22, 130.43, 126.46, 125.99, 111.90, 111.75, 88.27, 25.36, 19.26; white solid



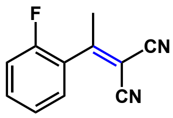
2-(1-(m-tolyl)ethylidene)malononitrile (3c)

^{1}H NMR (400 MHz, CDCl3) δ 7.41 – 7.33 (m, 4H), 2.62 (s, 3H), 2.42 (s, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 175.71, 139.06, 135.93, 133.02, 128.99, 127.77, 124.46, 112.82, 112.78, 84.47, 24.28, 21.35; white solid



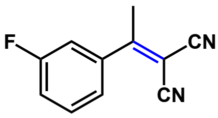
2-(1-(p-tolyl)ethylidene)malononitrile (3d)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 2.62 (s, 3H), 2.42 (s, *3*H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.21, 143.36, 133.02, 129.77, 127.49, 113.13, 112.99, 83.62, 24.07, 21.56; white solid



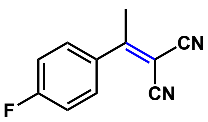
2-(1-(2-fluorophenyl)ethylidene)malononitrile (3e)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.50 (m, 1H), 7.38 (td, J = 8.0, 4.0 Hz, 1H), 7.30 (dd, J = 8.0, 4.0 Hz, 1H), 7.25-7.20 (m, 1H), 2.63 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.81, 171.80, 159.74, 157.23, 133.64, 133.55, 128.81, 124.87, 124.84, 124.29, 124.15, 117.00, 116.79, 111.87, 111.82, 88.52, 88.35, 24.34, 24.31; light yellow solid



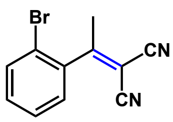
2-(1-(3-fluorophenyl)ethylidene)malononitrile (3f)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.47 (m, 1H), 7.36 – 7.34 (m, 1H), 7.28 – 7.21 (m, 2H), 2.63 (s, *3*H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.70, 163.81, 161.32, 137.72, 137.64, 131.07, 130.99, 123.15, 123.12, 119.25, 119.04, 114.59, 114.36, 112.27, 85.88, 24.29; light brown oily liquid



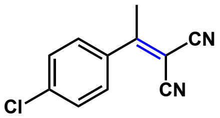
2-(1-(4-fluorophenyl)ethylidene)malononitrile (3g)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.57 (m, 2H), 7.24 – 7.18 (m, 2H), 2.64 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.91, 166.11, 163.57, 131.86, 131.83, 129.95, 129.86, 116.62, 116.40, 112.75, 112.59, 84.70, 84.69, 29.69, 24.28; light yellow solid



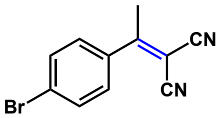
2-(1-(2-bromophenyl)ethylidene)malononitrile (3h)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, J = 8.0 Hz, 1H), 7.44 (t, J = 16.0 Hz, 1H), 7.34 (t, J = 16.0 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H), 2.58 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.98, 137.71, 133.69, 131.82, 128.15, 127.77, 119.31, 111.52, 111.25, 89.65, 24.80; yellow oily liquid



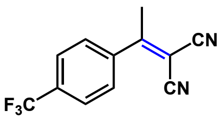
2-(1-(4-chlorophenyl)ethylidene)malononitrile (3i)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.47 (m, 4H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.83, 138.69, 134.13, 129.50, 128.76, 112.56, 112.45, 85.16, 29.68, 24.16; yellow solid



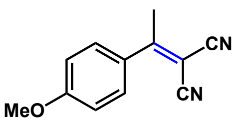
2-(1-(4-bromophenyl)ethylidene)malononitrile (3j)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 – 7.63 (m, 2H), 7.45 – 7.40 (m, 2H), 2.61 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.97, 134.60, 132.47, 128.86, 127.10, 112.56, 112.46, 85.16, 24.13; white yellow



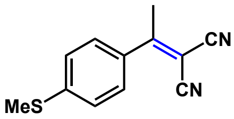
2-(1-(4-(trifluoromethyl)phenyl)ethylidene)malononitrile (3k)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, 2H), 7.65 (d, 2H), 2.67 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.84, 139.18, 127.72, 126.30, 126.26, 126.22, 126.19, 112.07, 112.02, 109.99, 86.72, 24.41; light brown oily liquid



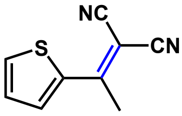
2-(1-(4-methoxyphenyl)ethylidene)malononitrile (3l)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (dt, *J* = 12.0, 4.0 Hz, 2H), 6.99 (dt, *J* = 8.0, 4.0 Hz, 2H), 3.88 (s, 3H), 2.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.93, 163.08, 129.79, 127.88, 114.45, 113.62, 113.35, 81.96, 55.58, 23.80; light yellow solid



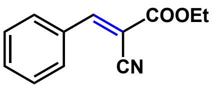
2-(1-(4-(methylthio)phenyl)ethylidene)malononitrile (3m)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 2.62 (s, 3H), 2.53 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.02, 145.83, 131.50, 127.94, 125.40, 113.27, 113.09, 83.05, 29.70, 23.83, 14.73; bright yellow solid



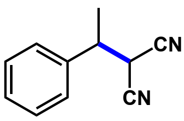
2-(1-(thiophen-2-yl)ethylidene)malononitrile (3n)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 4.0 Hz, 1H), 7.79 (d, J = 4.0 Hz, 1H), 7.25 (d, J = 4.0 Hz, 1H), 2.71 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.54, 138.27, 134.35, 133.54, 129.15, 114.00, 113.51, 78.80, 23.63; light yellow solid



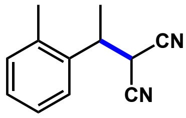
ethyl (E)-2-cyano-3-phenylacrylate (3o)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.99 (d, J = 8.0 Hz, 2H), 7.58 – 7.49 (m, 3H), 4.39 (q, J = 8.0 Hz, 2H), 1.40 (t, J = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.45, 154.99, 133.26, 131.47, 131.03, 129.25, 115.44, 103.05, 62.71, 14.14; white solid



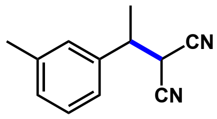
2-(1-phenylethyl)malononitrile (4a)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.31 (m, 5H), 3.84 (d, J = 4.0 Hz, 1H), 3.47 – 3.41 (m, 1H), 1.64 (d, J = 4.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.14, 129.23, 128.83, 127.20, 111.94, 111.65, 41.16, 31.19, 17.74; ESI calculated for [C11H10N2+H] 171.0917. Found 171.0916; light yellow liquid



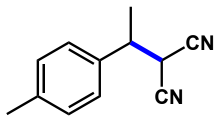
2-(1-(o-tolyl)ethyl)malononitrile (4b)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.26 (m, 2H), 7.25 – 7.21 (m, 2H), 3.81 (d, J = 8.0, 1H), 3.79 – 3.72 (m, 1H), 2.40 (s, 3H), 1.62 (d, J = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.69, 135.57, 131.24, 128.41, 127.05, 125.34, 112.16, 111.74, 36.34, 29.99, 19.48, 18.04; ESI calculated for [C12H12N2+H] 185.1073. Found 185.1088; light yellow liquid



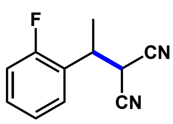
2-(1-(m-tolyl)ethyl)malononitrile (4c)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.27 (m, 1H), 7.17 （d, J = 8.0 Hz, 1H), 7.12 – 7.11 (m, 2H), 3.84 (d, J = 4.0, 1H), 3.44 – 3.38 (m, 1H), 2.37 (s, 3H), 1.64 (d, J = 4.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.01, 138.18, 129.56, 129.10, 127.86, 124.16, 112.02, 111.70, 41.17, 31.19, 21.44, 17.80; ESI calculated for [C12H12N2+H] 185.1073. Found 185.1088; light yellow liquid



2-(1-(p-tolyl)ethyl)malononitrile (4d)

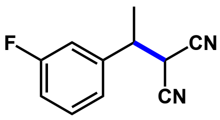
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (s, 4H), 3.82 (d, J = 8.0 Hz, 1H), 3.46 – 3.39 (m, 1H), 2.36 (s, 3H), 1.63 (d, J = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.70, 135.17, 129.87, 127.05, 112.01, 111.73, 40.89, 31.32, 21.09, 17.81; ESI calculated for [C12H12N2+H] 185.1073. Found 185.1088; white solid



2-(1-(2-fluorophenyl)ethyl)malononitrile (4e)

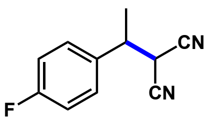
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.34 (m, 2H), 7.22 (t, J = 8.0 Hz, 1H), 7.15 –

7.10 (m, 1H), 4.04 (d, *J* = 8.0, 1H), 3.84 – 3.77 (m, 1H), 1.68 (d, *J* = 4.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.60, 159.15, 130.58, 130.49, 128.34, 125.06, 116.14, 115.92, 111.90, 111.34, 34.86, 34.84, 29.44, 29.41, 16.42, 16.41; ESI calculated for [C11H10FN2+H] 189.0823. Found 189.0814; light yellow liquid



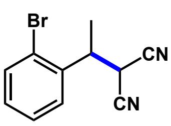
2-(1-(3-fluorophenyl)ethyl)malononitrile (4f)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (td, *J* = 16.0, 8.0 Hz, 1H), 7.15 – 7.03 (m, 3H), 3.86 (d, *J* = 4.0 Hz, 1H), 3.49 – 3.42 (m, 1H), 1.65 (d, *J* = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.23, 161.76, 140.45, 140.45, 130.96, 130.87, 123.04, 123.01, 116.05, 115.84, 114.47, 114.25, 111.61, 111.35, 40.83, 30.98, 17.74; ESI calculated for [C11H10FN2+H] 189.0823. Found 189.0814; light yellow liquid



2-(1-(4-fluorophenyl)ethyl)malononitrile (4g)

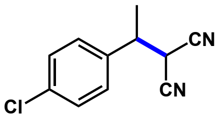
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.30 (m, 2H), 7.14 – 7.08 (m, 2H), 3.84 (d, *J* = 8.0 Hz, 1H), 3.50 – 3.43 (m, 1H), 1.64 (d, *J* = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.01, 161.54, 133.83, 129.09, 129.00, 116.35, 116.13, 111.74, 111.55, 40.47, 31.31, 17.90; ESI calculated for [C11H10FN2+H] 189.0823. Found 189.0814; yellow liquid



2-(1-(2-bromophenyl)ethyl)malononitrile (4h)

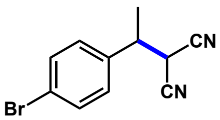
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.0 Hz, 1H), 7.46 – 7.39 (m, 2H), 7.26 – 7.22 (m, 1H), 4.13 (d, *J* = 4.0 Hz, 1H), 4.03 – 3.96 (m, 1H), 1.70 (d, *J* = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.79, 133.54, 130.31, 128.43, 128.12, 124.40, 112.01, 110.90, 39.51, 29.21, 15.92; ESI calculated for [C11H9BrN2+H] 249.0022.

Found 249.0480; light yellow liquid



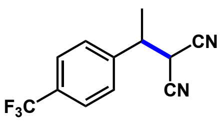
2-(1-(4-chlorophenyl)ethyl)malononitrile (4i)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.38 (m, 2H), 7.30 – 7.26 (m, 2H), 7.83 (d, J = 8.0 Hz, 1H), 3.48 – 3.41 (m, 1H), 1.64 (d, J = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.49, 134.88, 129.46, 128.64, 111.61, 111.42, 40.61, 31.08, 17.77; ESI calculated for [C11H9ClN2+H] 205.0527. Found 205.0561; light yellow liquid



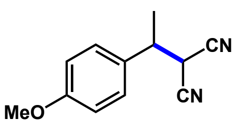
2-(1-(4-bromophenyl)ethyl)malononitrile (4j)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, J = 8.0 Hz, 2H), 7.22 (m, J = 8.0 Hz, 2H), 3.83 (d, J = 8.0 Hz, 1H), 3.47 – 3.40 (m, 1H), 1.63 (d, J = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.00, 132.42, 128.94, 123.00, 111.59, 111.40, 40.68, 30.99, 17.71; ESI calculated for [C11H9BrN2+H] 249.0022. Found 249.0480; light brown liquid



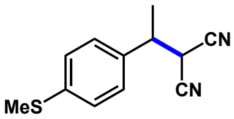
2-(1-(4-(trifluoromethyl)phenyl)ethyl)malononitrile (4k)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 8.0 Hz, 2H), 3.80 (d, J = 8.0 Hz, 1H), 3.57 – 3.51 (m, 1H), 1.68 (d, J = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.87, 133.34, 131.02, 127.85, 126.33, 126.29, 126.25, 126.21, 125.05, 122.35, 111.47, 111.29, 40.88, 40.86, 30.87, 30.86, 17.72; ESI calculated for [C12H9F3N2+H] 239.0791. Found 239.0784; light yellow liquid



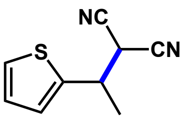
2-(1-(4-methoxyphenyl)ethyl)malononitrile (4l)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, J = 12.0 Hz, 2H), 6.92 (m, J = 8.0 Hz, 2H), 3.81 (s, 3H), 3.45 – 3.38 (m, 1H), 1.62 (d, J = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.81, 130.10, 128.38, 114.52, 112.04, 111.81, 55.31, 40.53, 31.47, 17.87; ESI calculated for [C12H12N2O+H] 201.1022. Found 201.1008; light yellow liquid



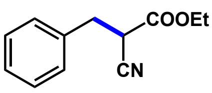
2-(1-(4-(methylthio)phenyl)ethyl)malononitrile (4m)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-7.23 (m, 4H), 3.84 (d, J = 4.0 Hz, 1H), 3.46 – 3.39 (m, 1H), 1.63 (d, J = 4.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.64, 134.55, 127.69, 126.74, 111.91, 111.69, 40.71, 31.23, 17.73, 15.46; ESI calculated for [C12H12N2S+H] 217.0794. Found 217.0776; light yellow liquid



2-(1-(thiophen-2-yl)ethyl)malononitrile (4n)

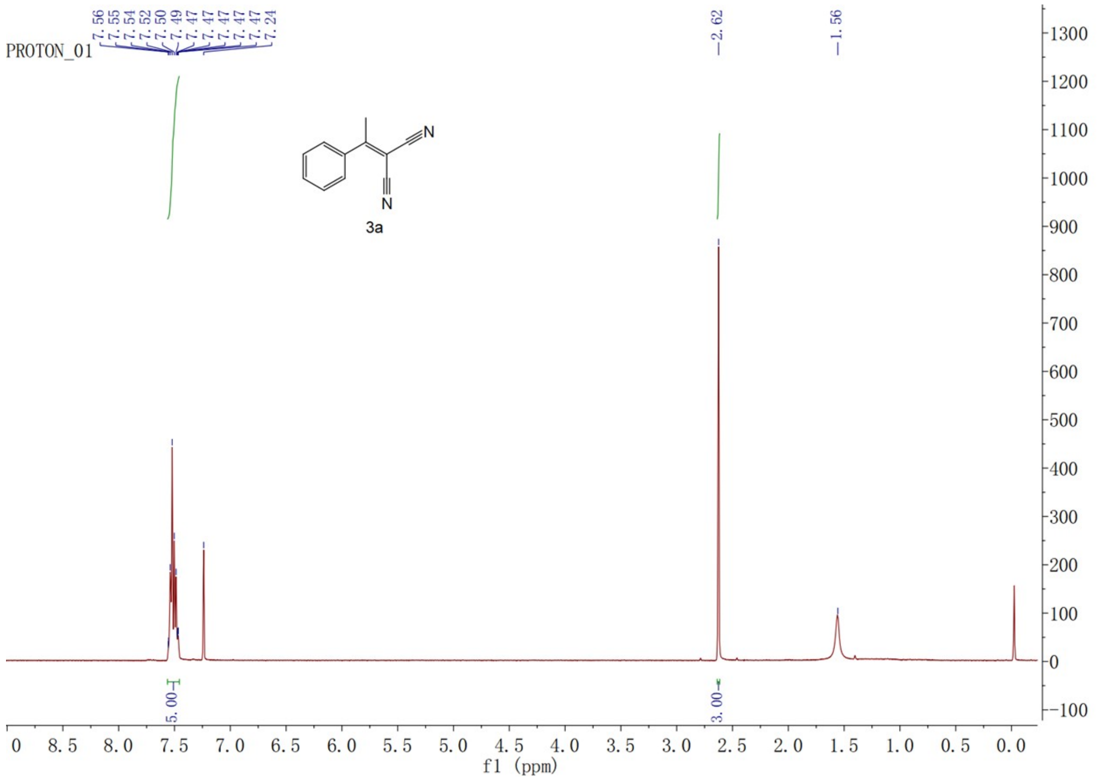
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, J = 4.0 Hz, 1H), 7.12 (d, J = 4.0 Hz, 1H), 7.03 (t, J = 4.0 Hz, 1H), 3.93 (d, J = 4.0 Hz, 1H), 3.81 – 3.75 (m, 1H), 1.72 (d, J = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.80, 127.40, 126.20, 125.62, 111.65, 111.35, 36.95, 32.01, 19.07; ESI calculated for [C9H8N2S+H] 177.0481. Found 177.0498; light yellow liquid

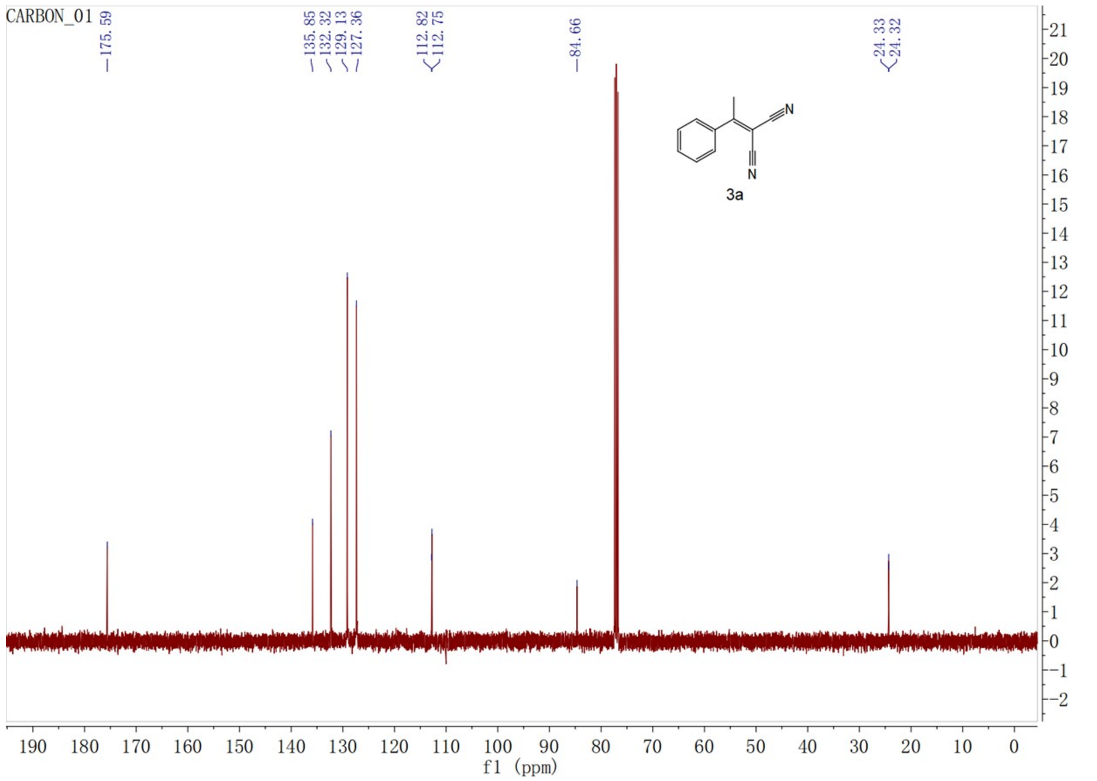


ethyl 2-cyano-3-phenylpropanoate (4o)

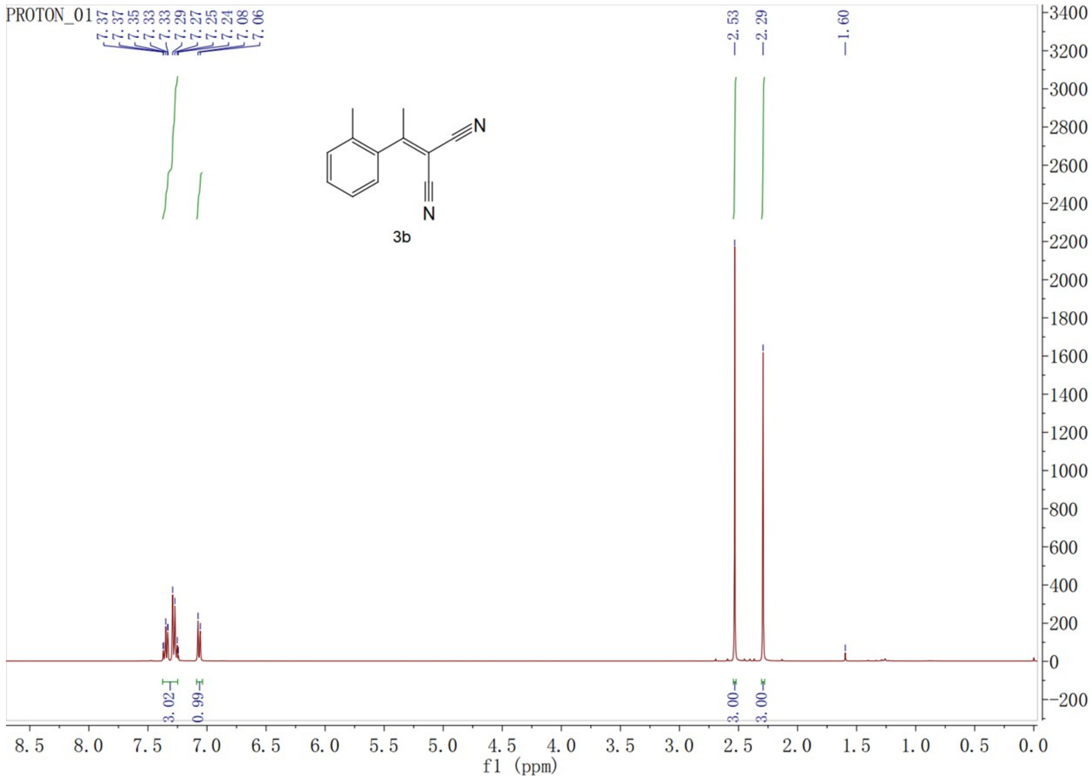
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H), 4.24 (q, J = 8.0 Hz, 2H), 3.72 (dd, J = 8.0, 4.0 Hz, 1H), 3.31 – 3.17 (m, 2H), 1.27 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.52, 135.25, 129.02, 128.87, 127.79, 116.16, 62.96, 39.70, 35.75, 13.94; ESI calculated for [C12H13NO2+H] 204.1019. Found 249.1036; light yellow liquid

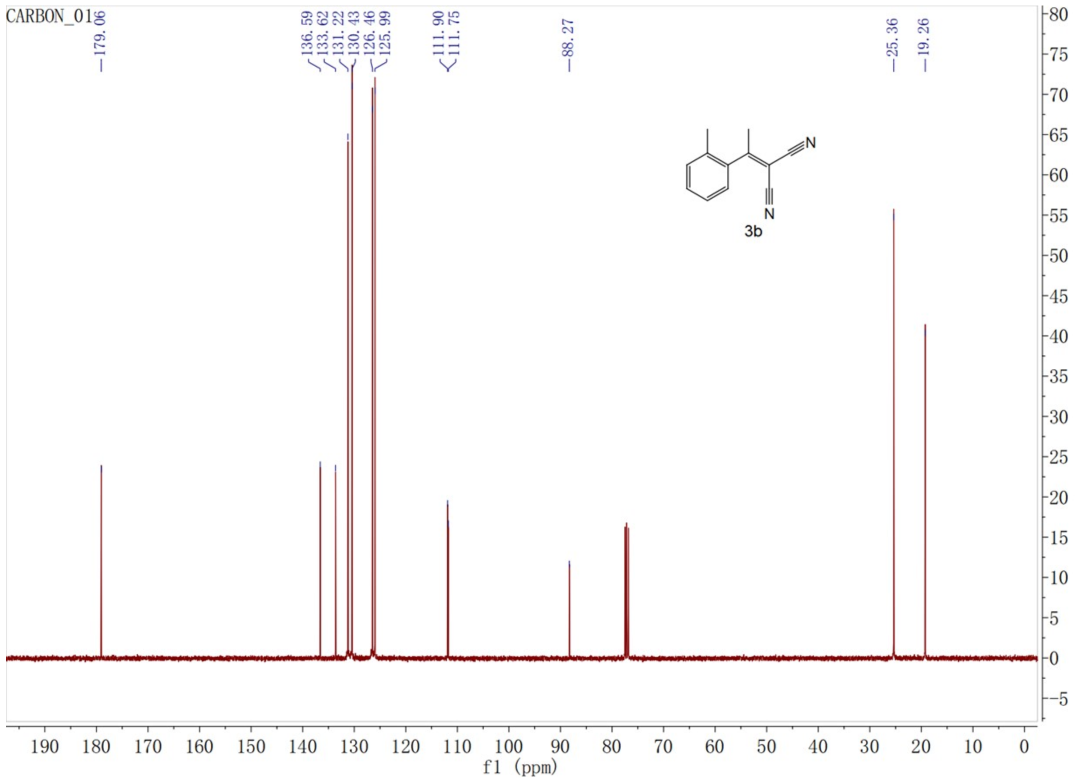
2-(1-phenylethylidene)malononitrile (3a)



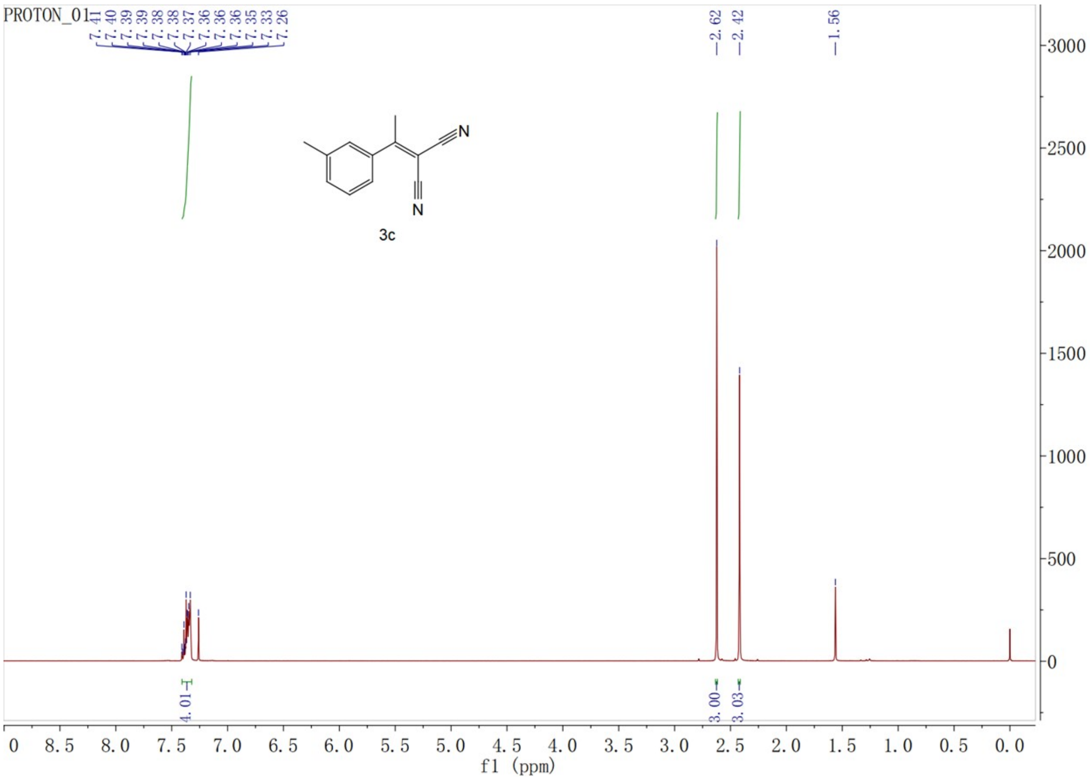


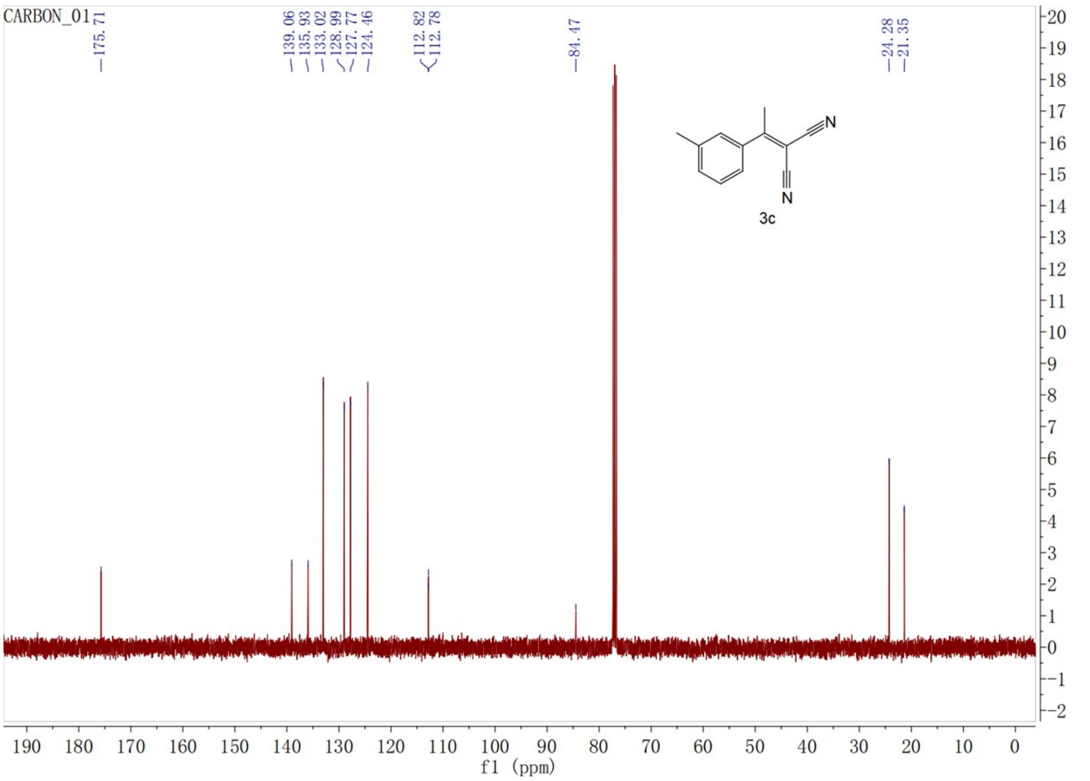
2-(1-(o-tolyl)ethylidene)malononitrile (3b)



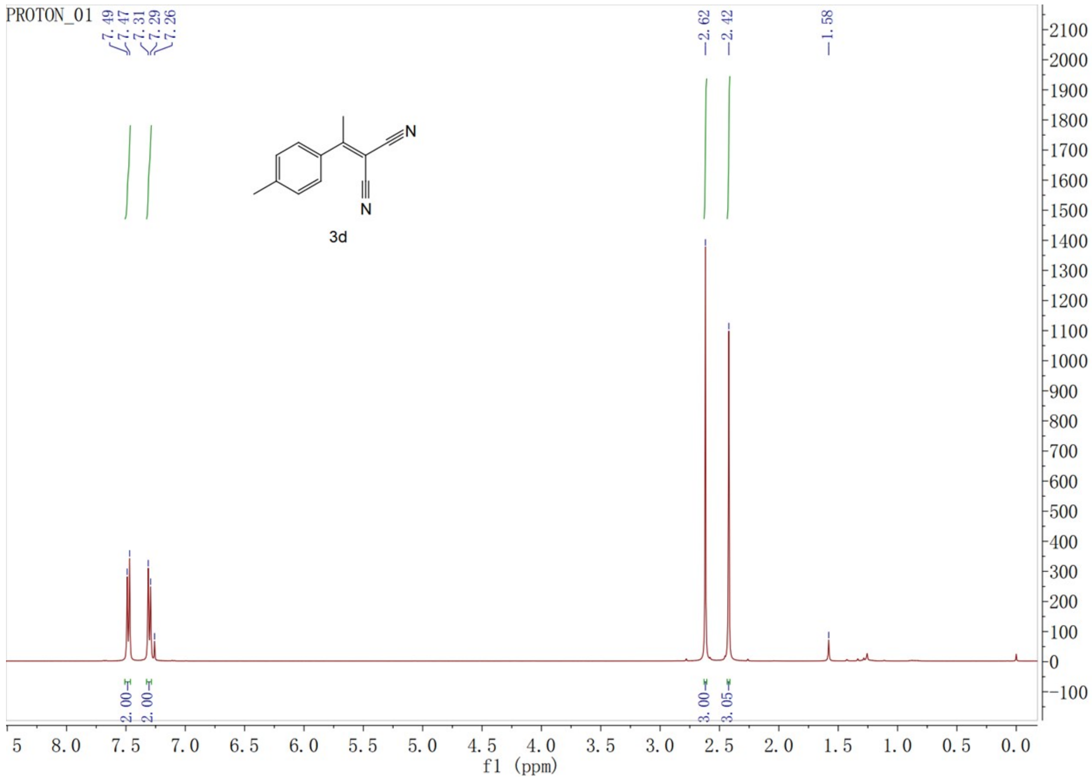


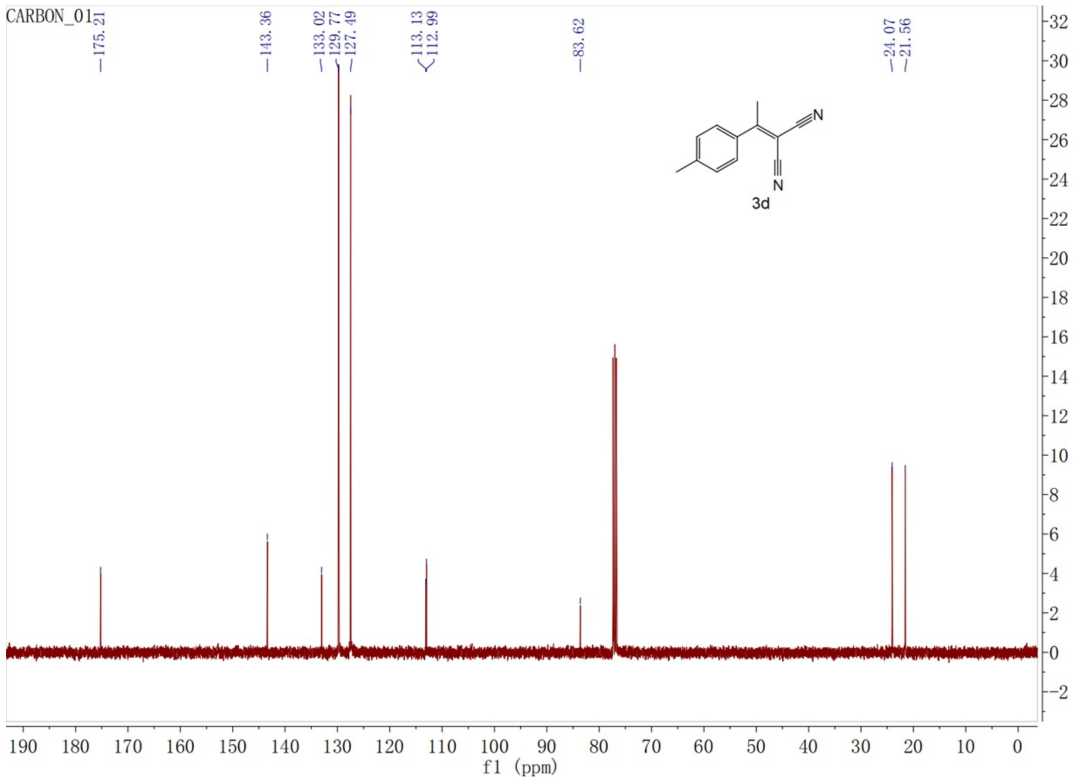
2-(1-(m-tolyl)ethylidene)malononitrile (3c)



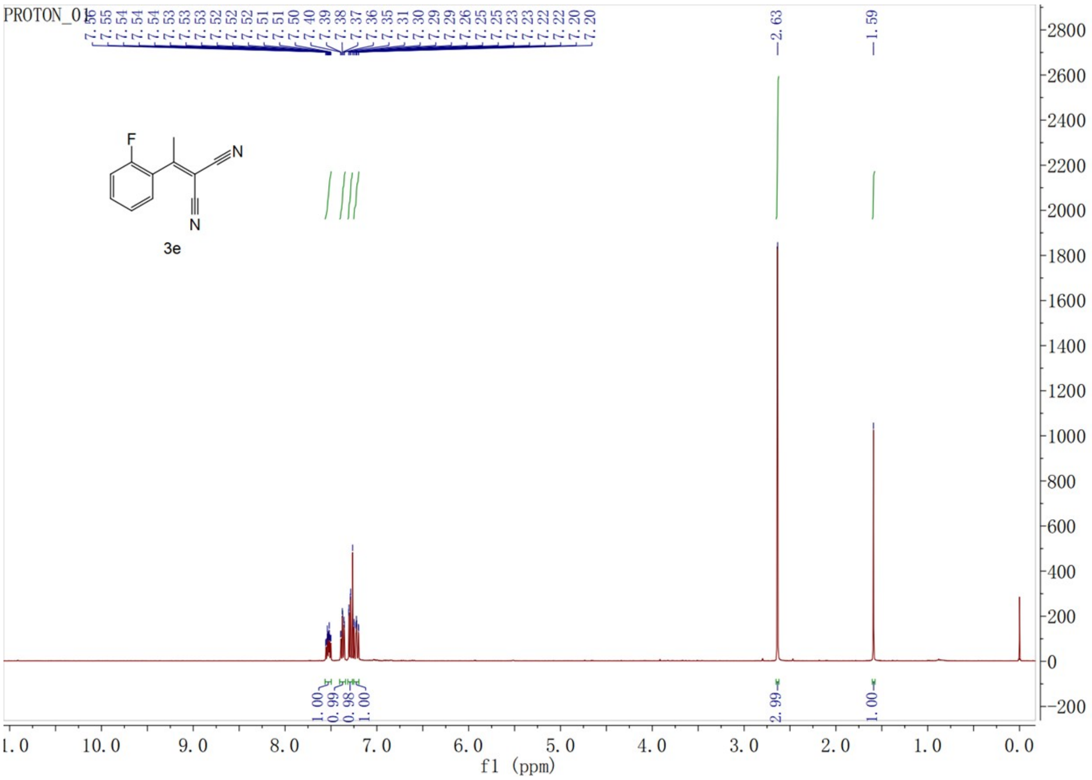


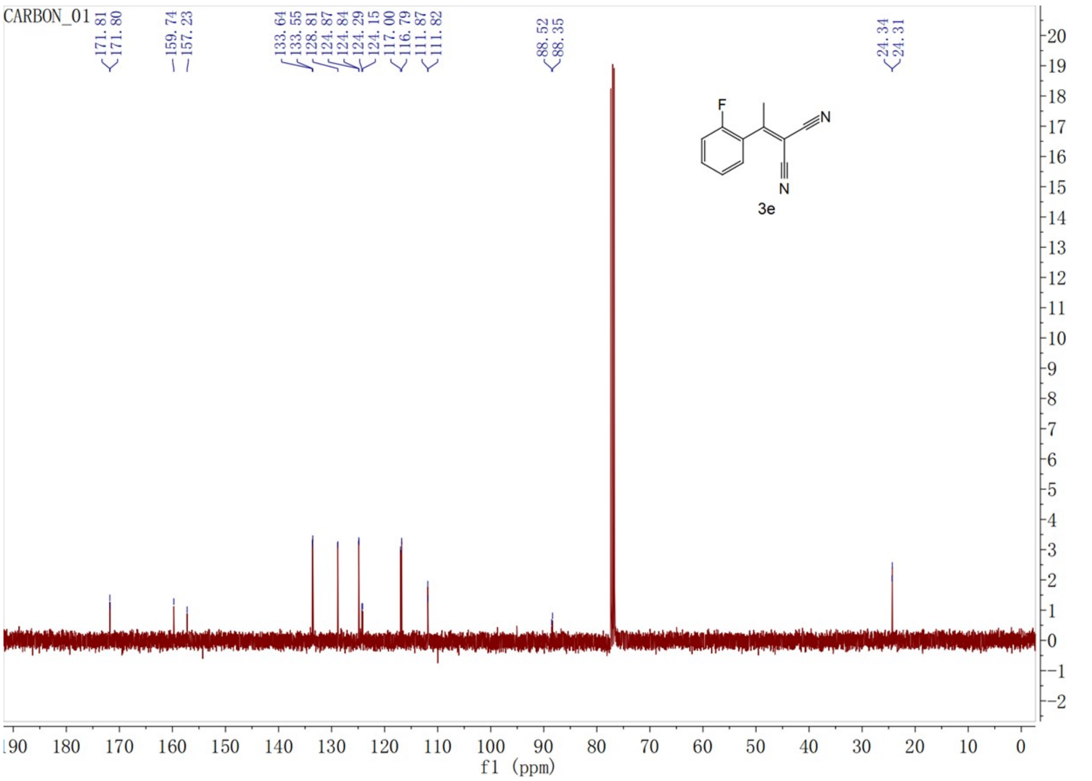
2-(1-(p-tolyl)ethylidene)malononitrile (3d)



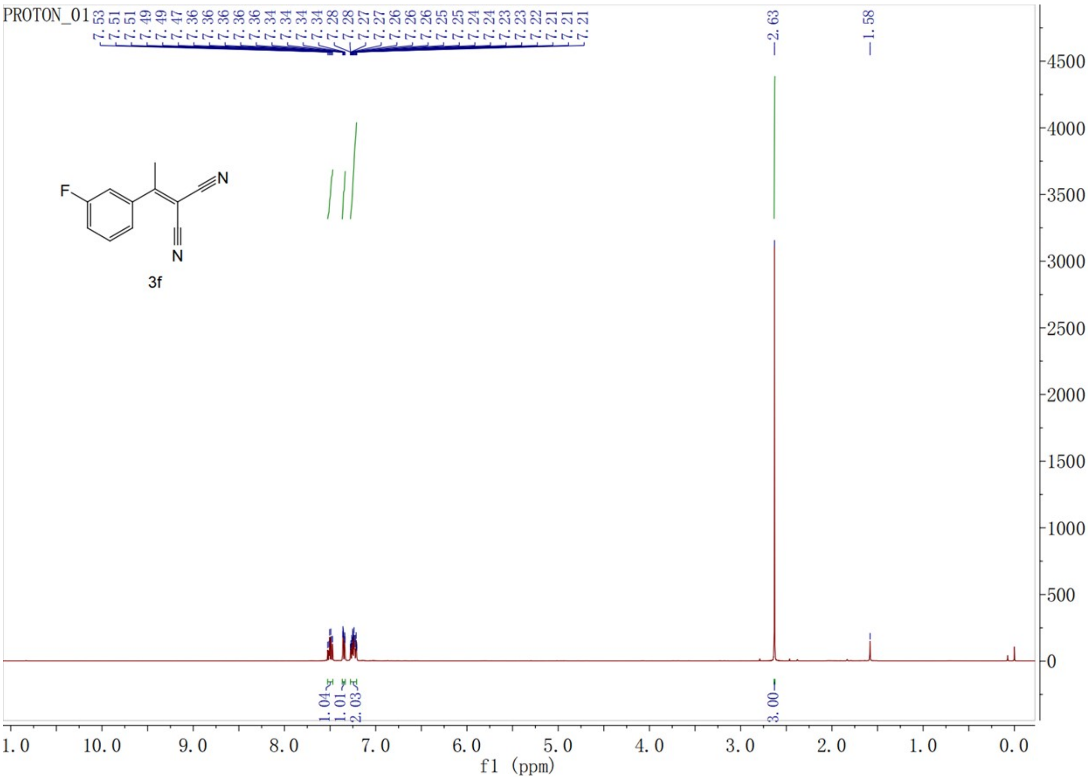


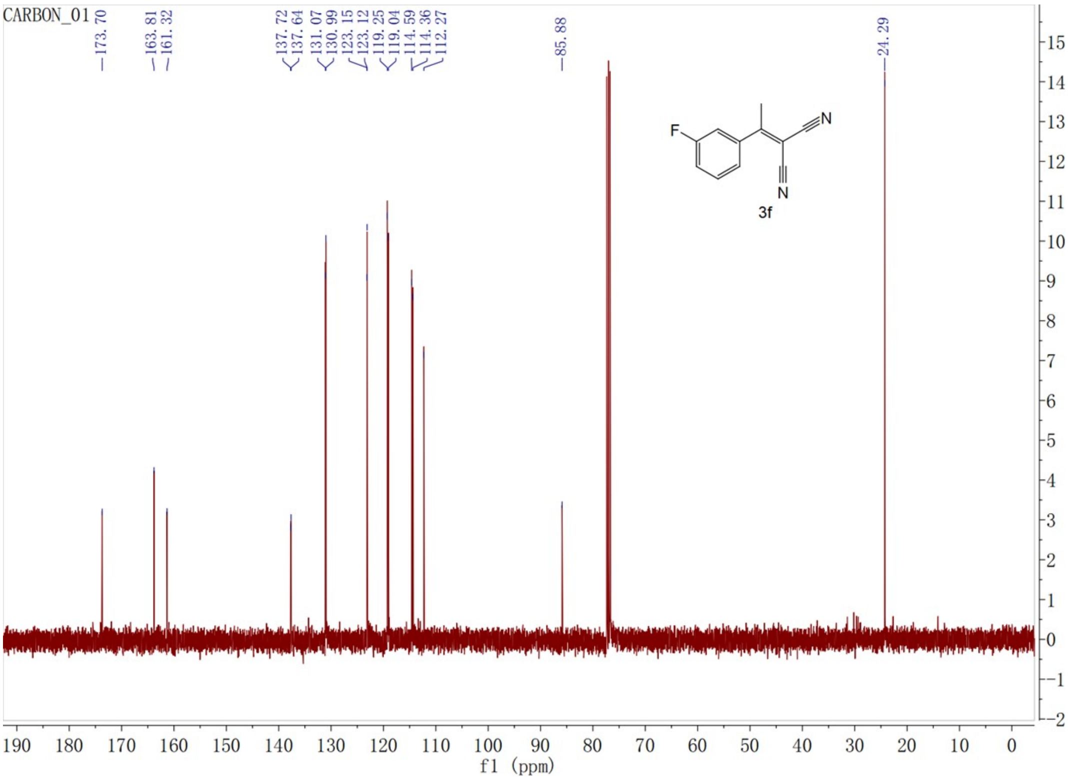
2-(1-(2-fluorophenyl)ethylidene)malononitrile (3e)



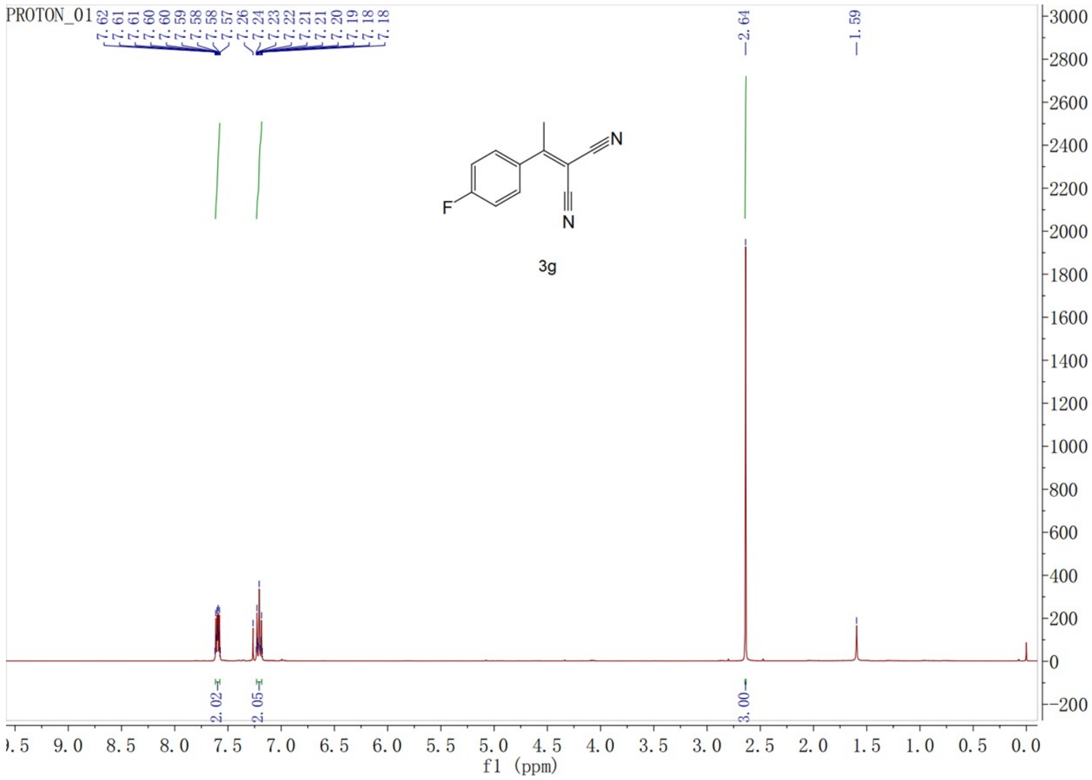


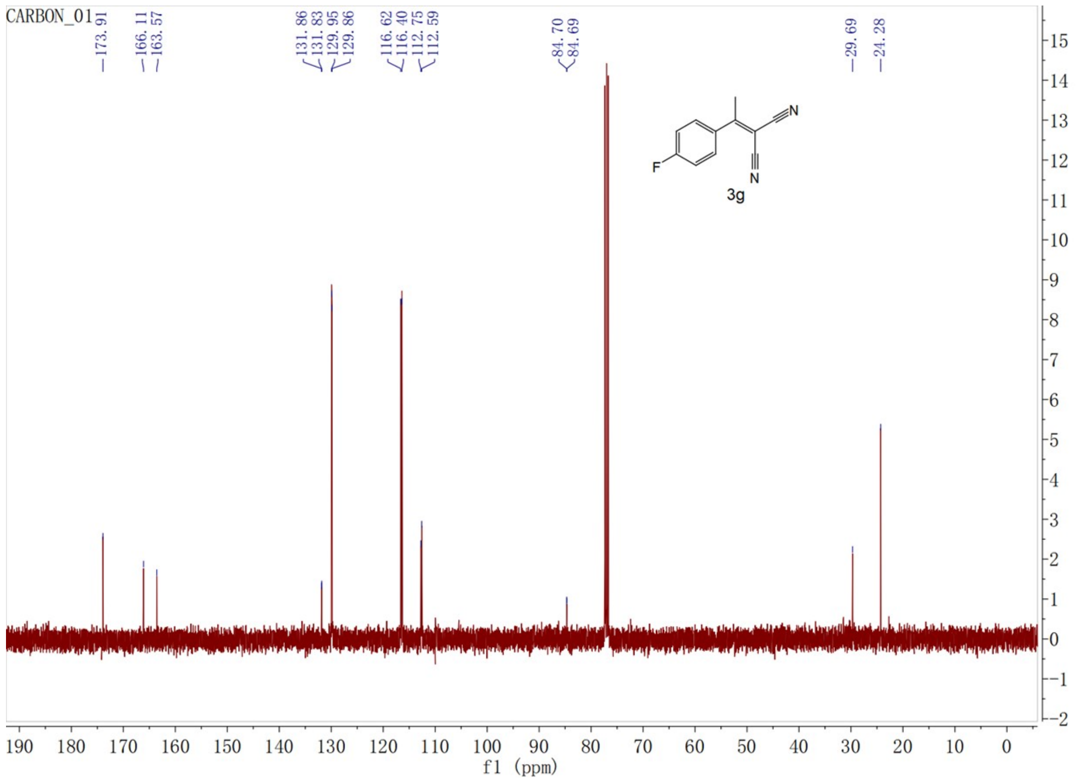
2-(1-(3-fluorophenyl)ethylidene)malononitrile (3f)



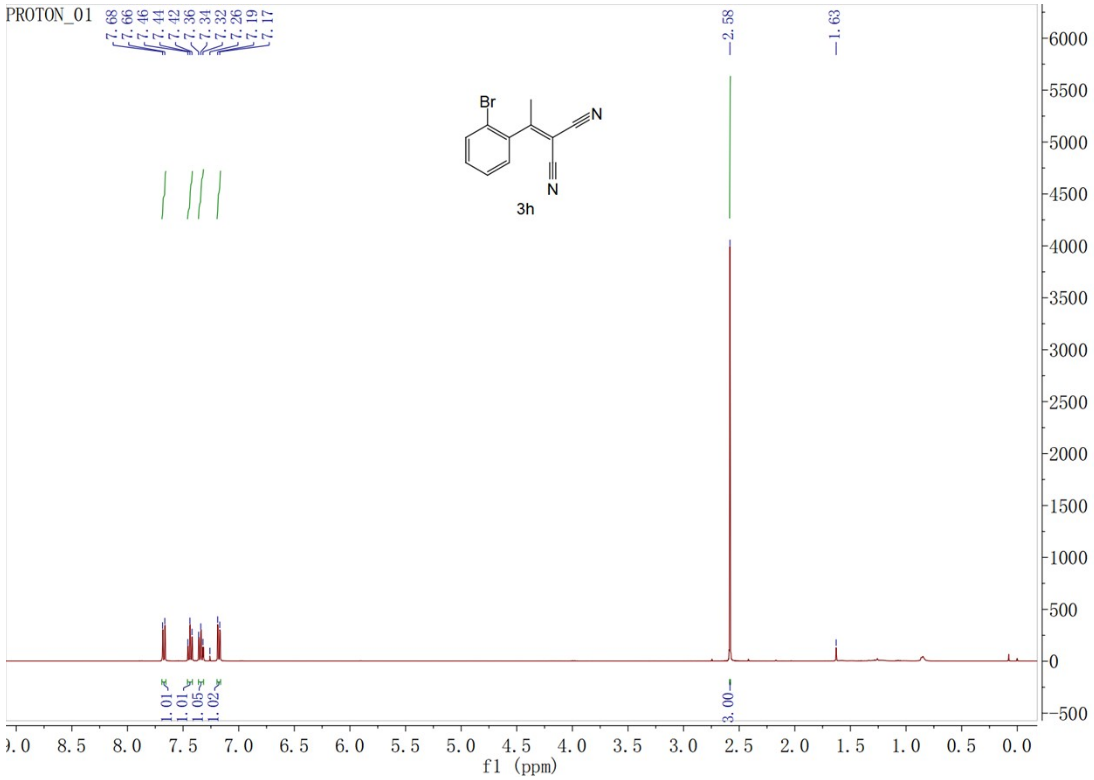


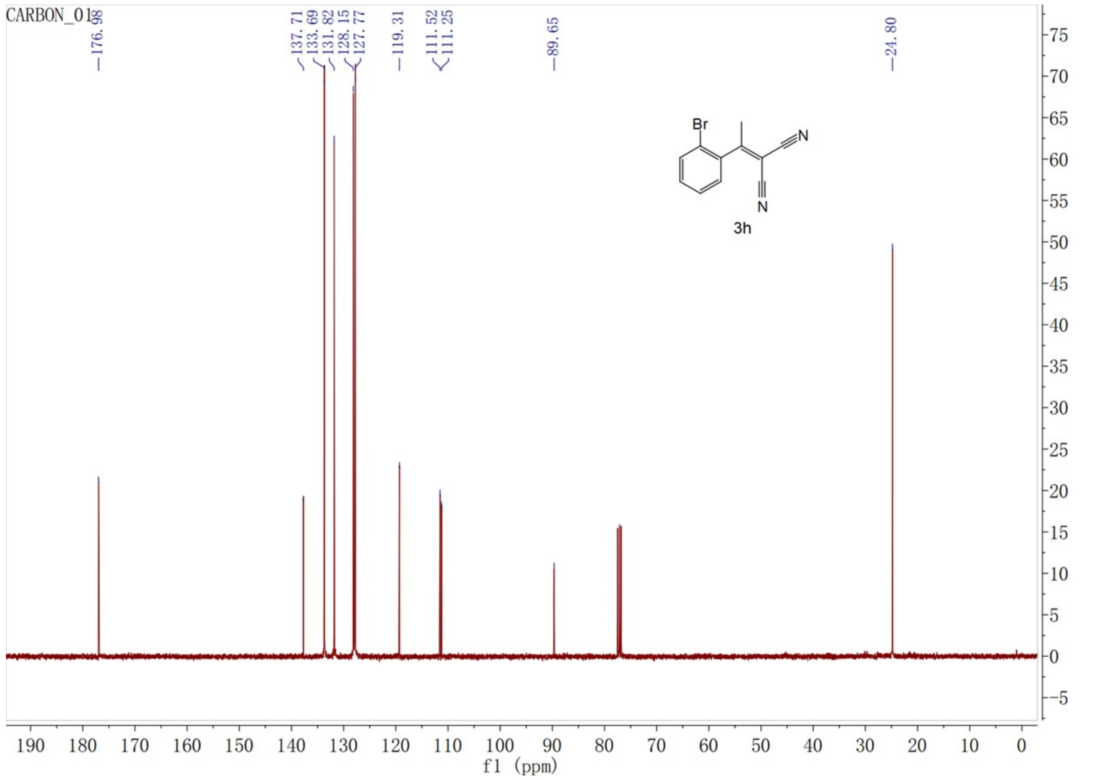
2-(1-(4-fluorophenyl)ethylidene)malononitrile (3g)



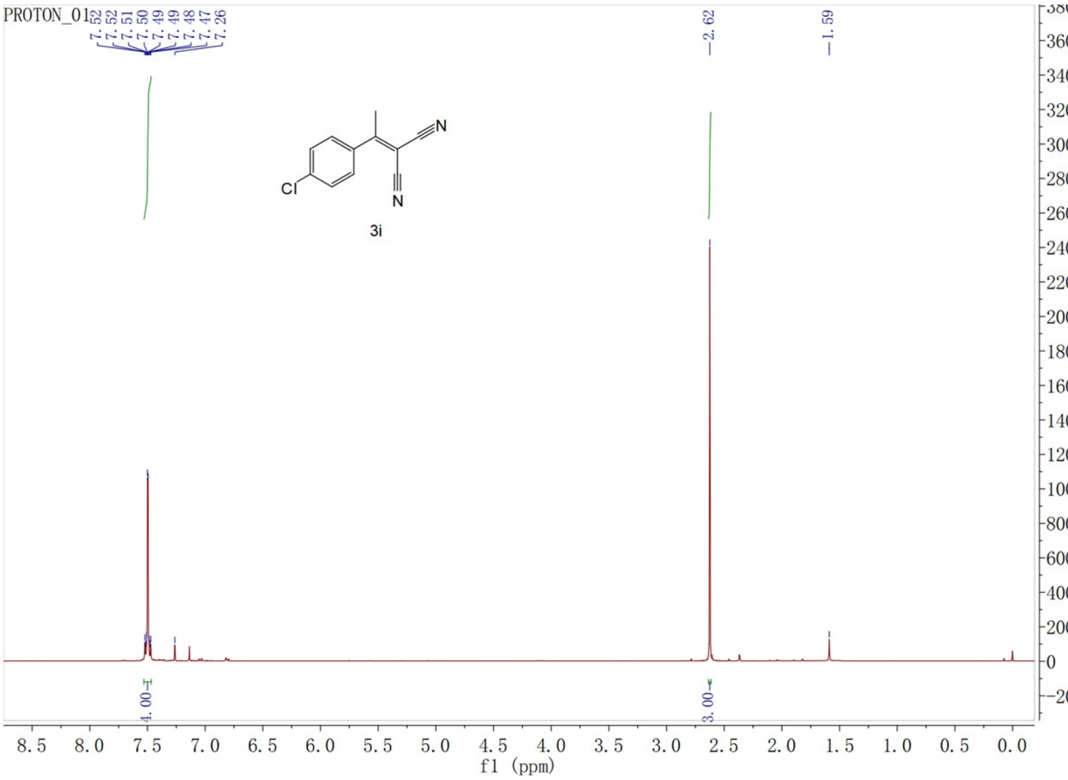


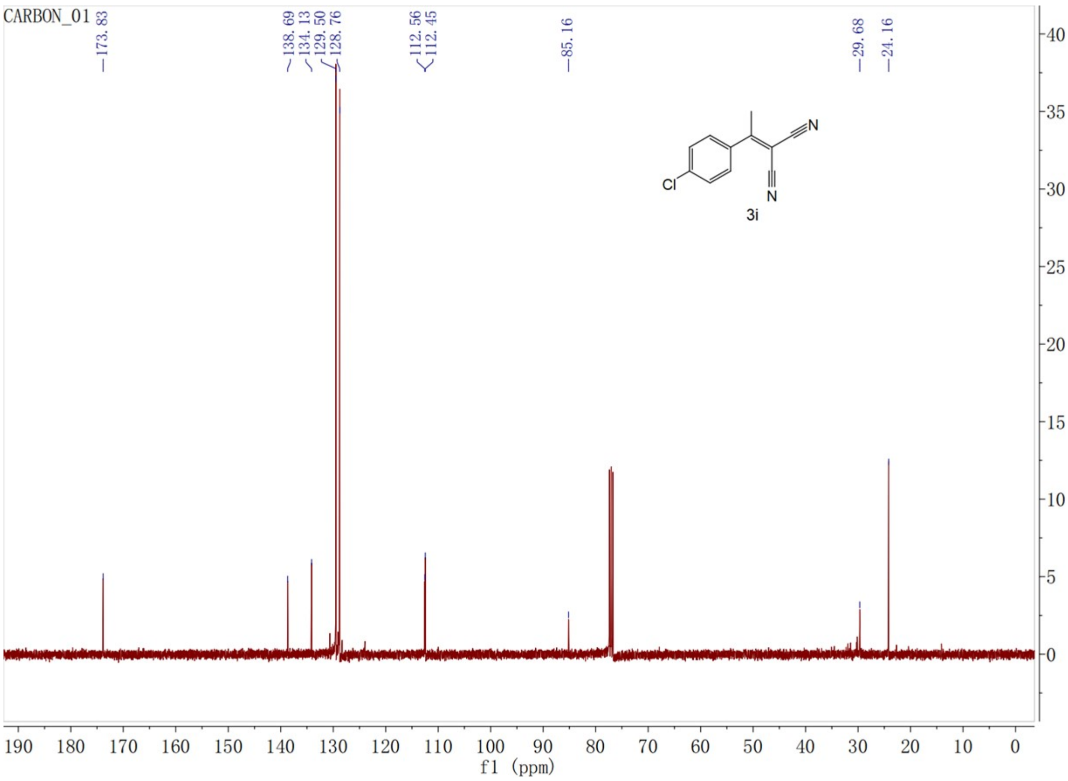
2-(1-(2-bromophenyl)ethylidene)malononitrile (3h)



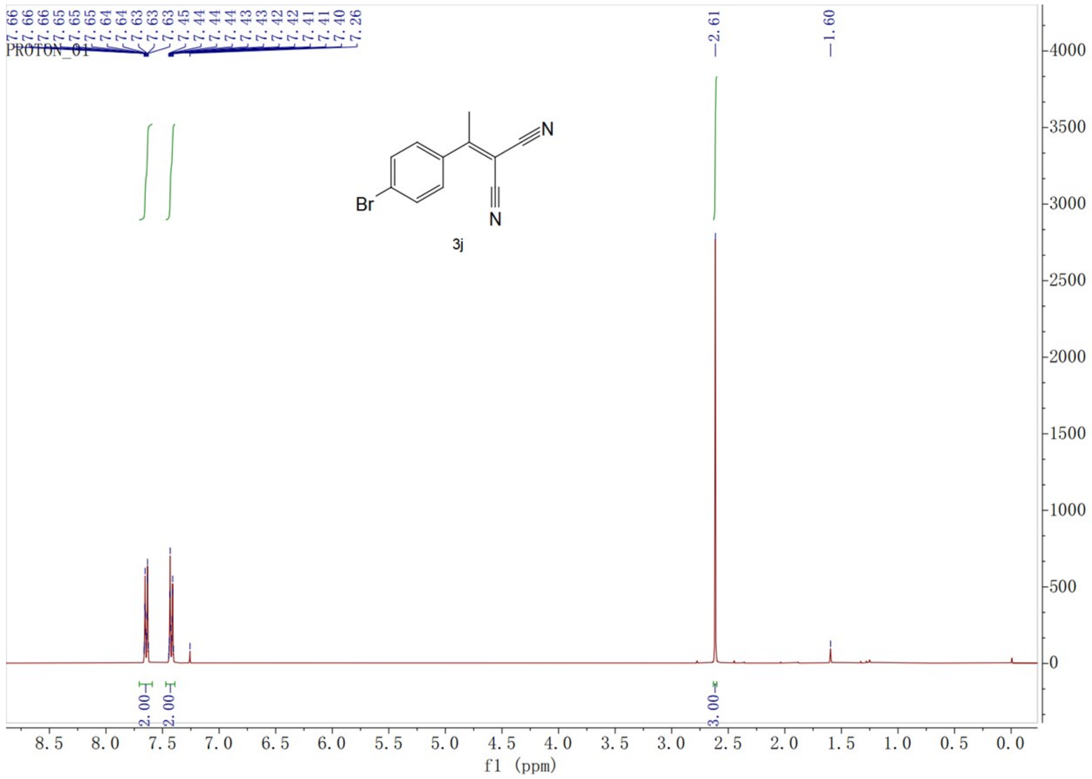


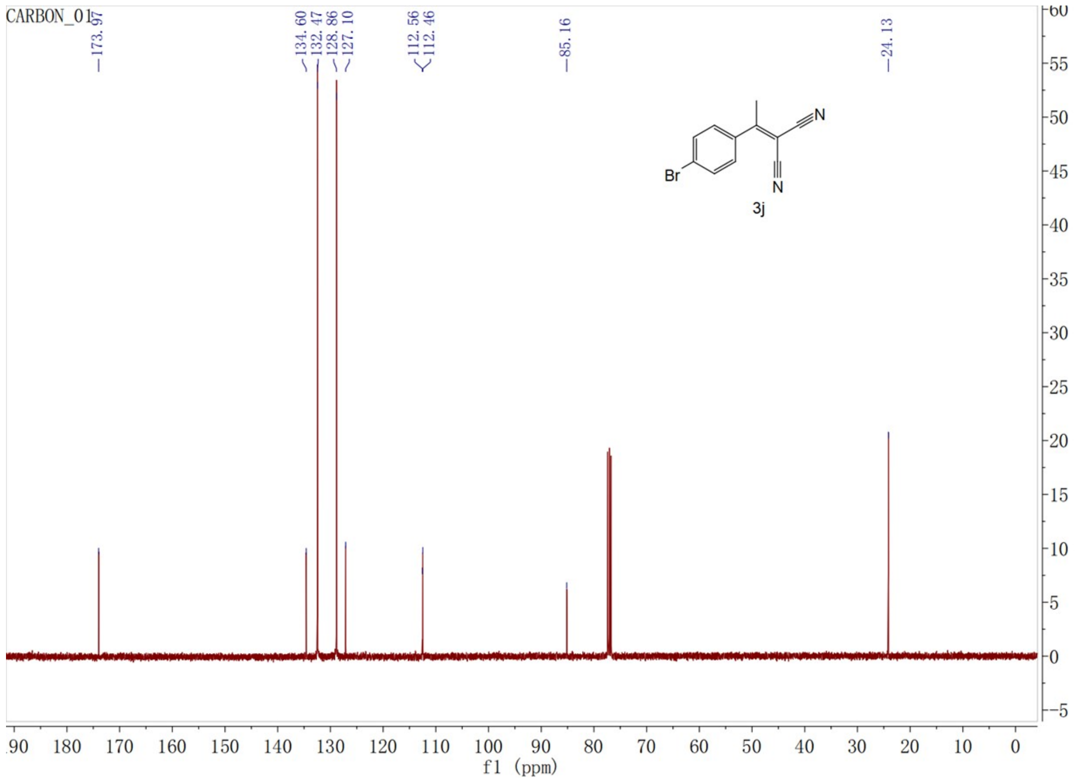
2-(1-(4-chlorophenyl)ethylidene)malononitrile (3i)



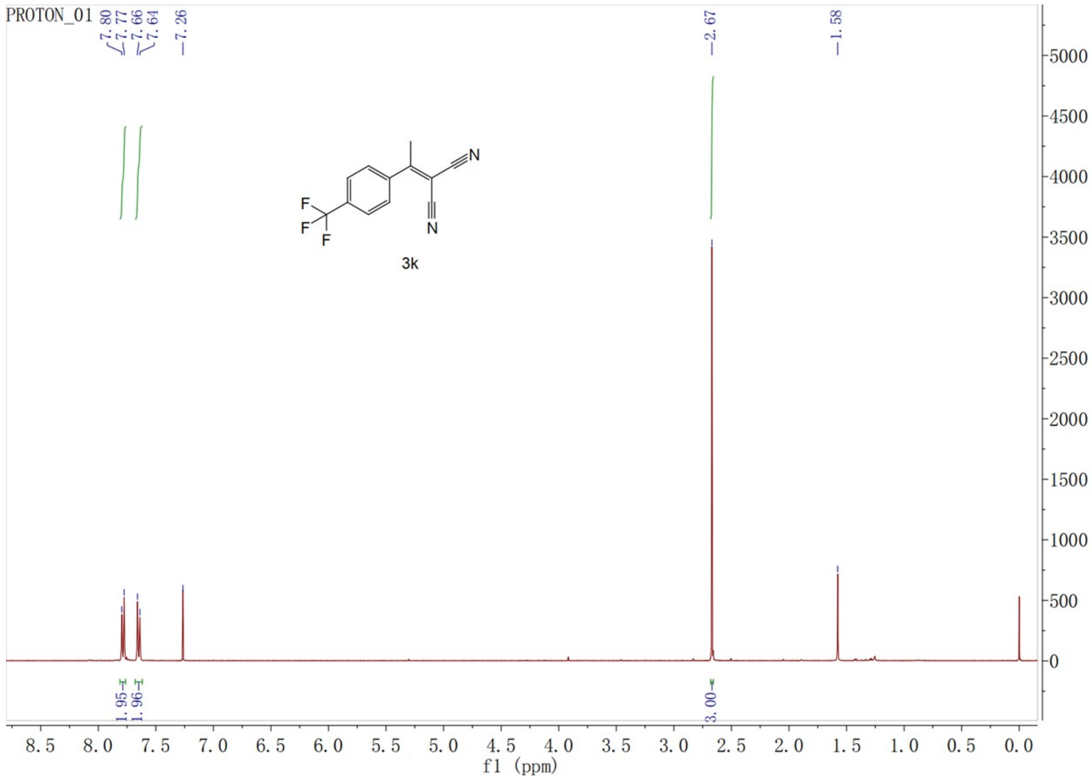


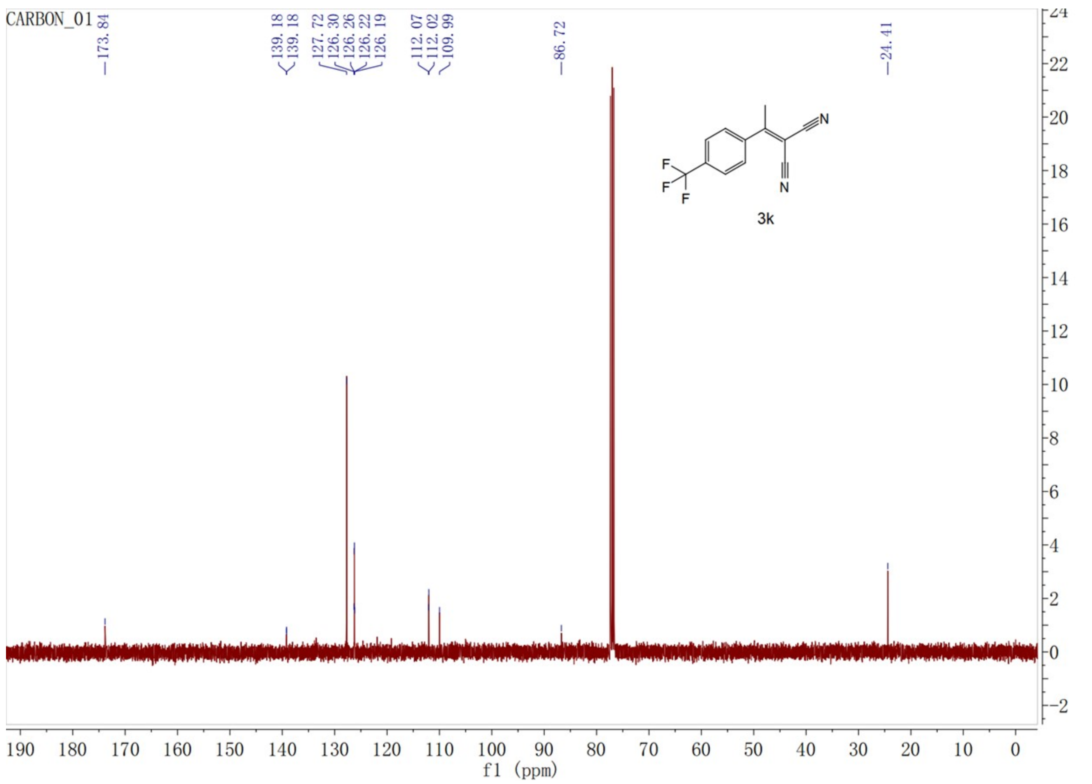
2-(1-(4-bromophenyl)ethylidene)malononitrile (3j)



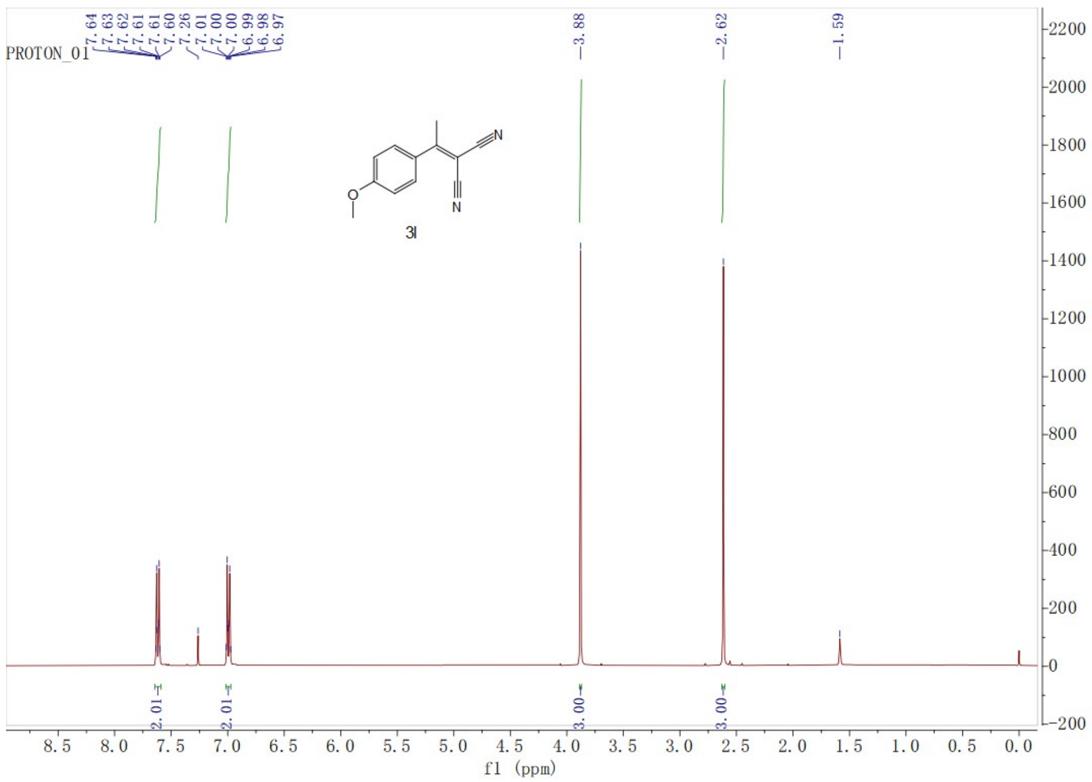


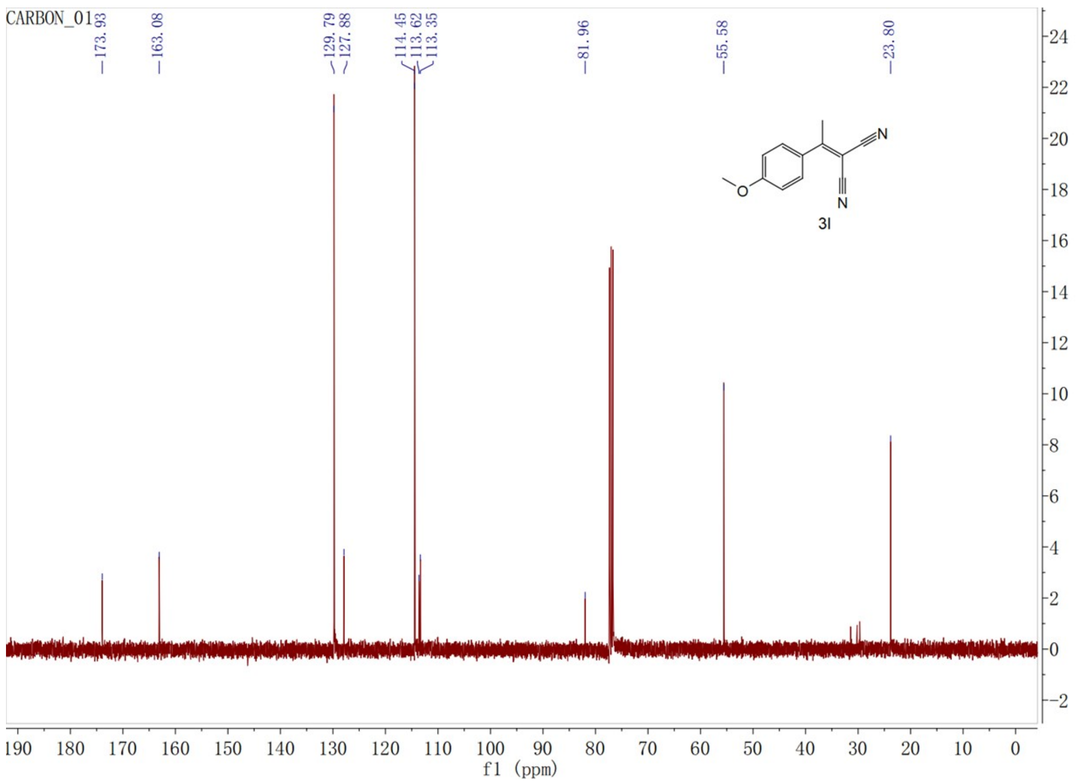
2-(1-(4-(trifluoromethyl)phenyl)ethylidene)malononitrile (3k)



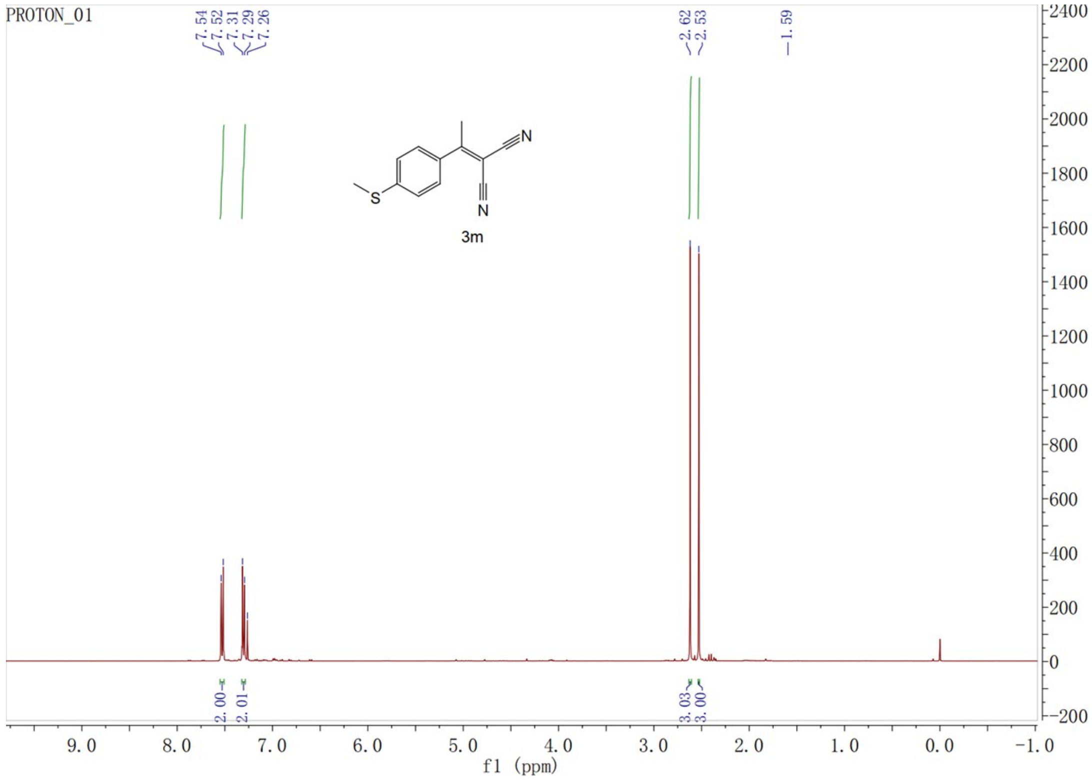


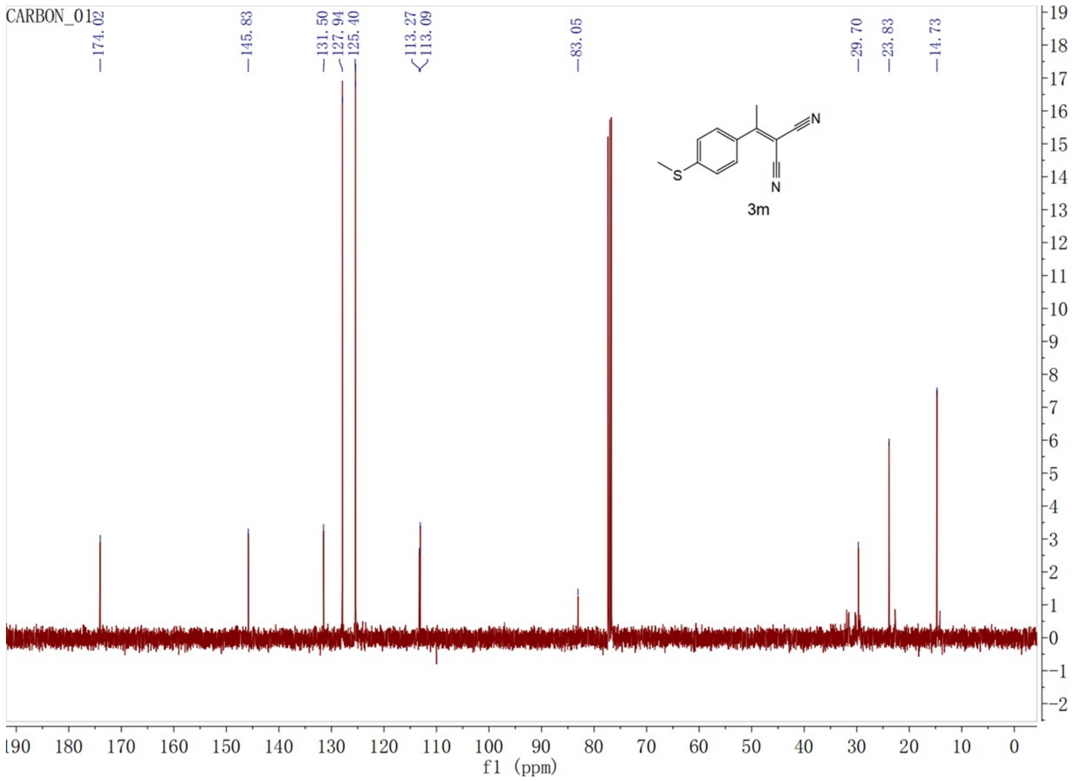
2-(1-(4-methoxyphenyl)ethylidene)malononitrile (3l)



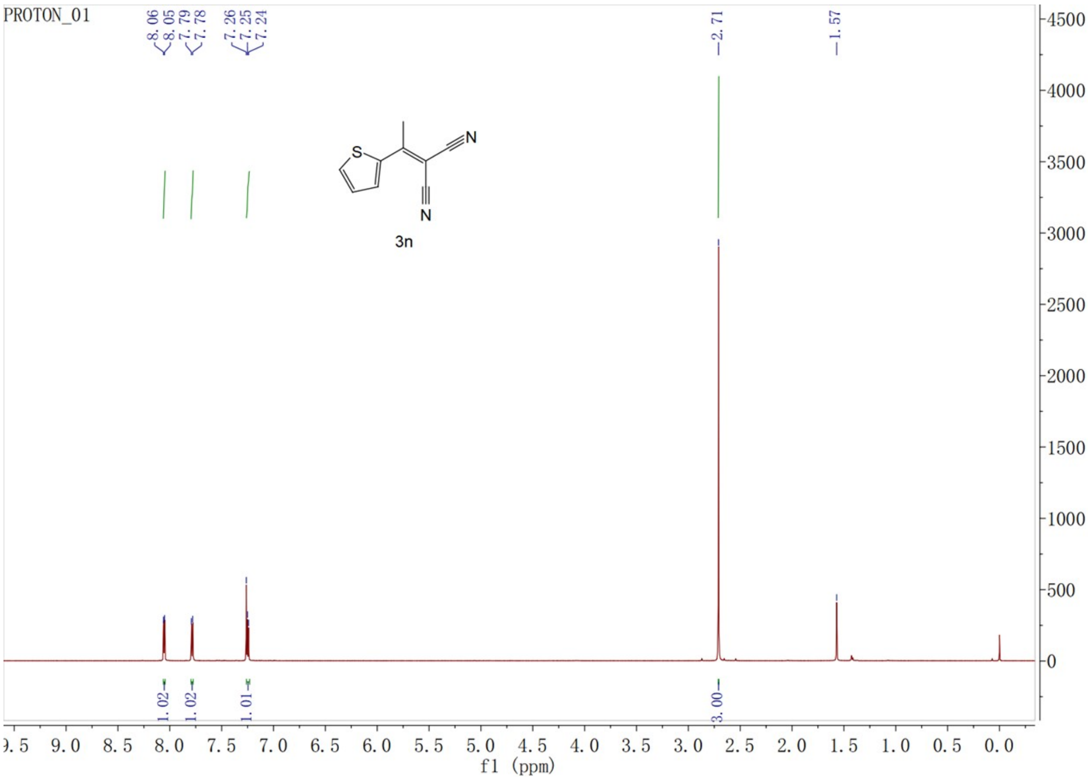


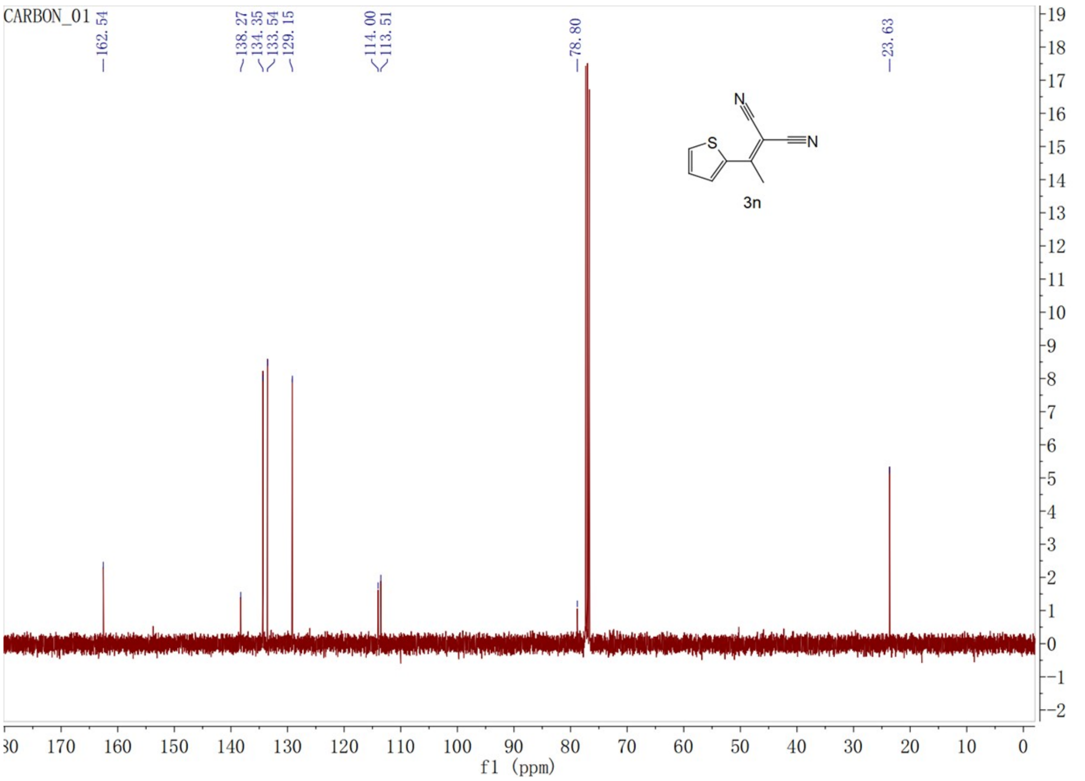
2-(1-(4-(methylthio)phenyl)ethylidene)malononitrile (3m)



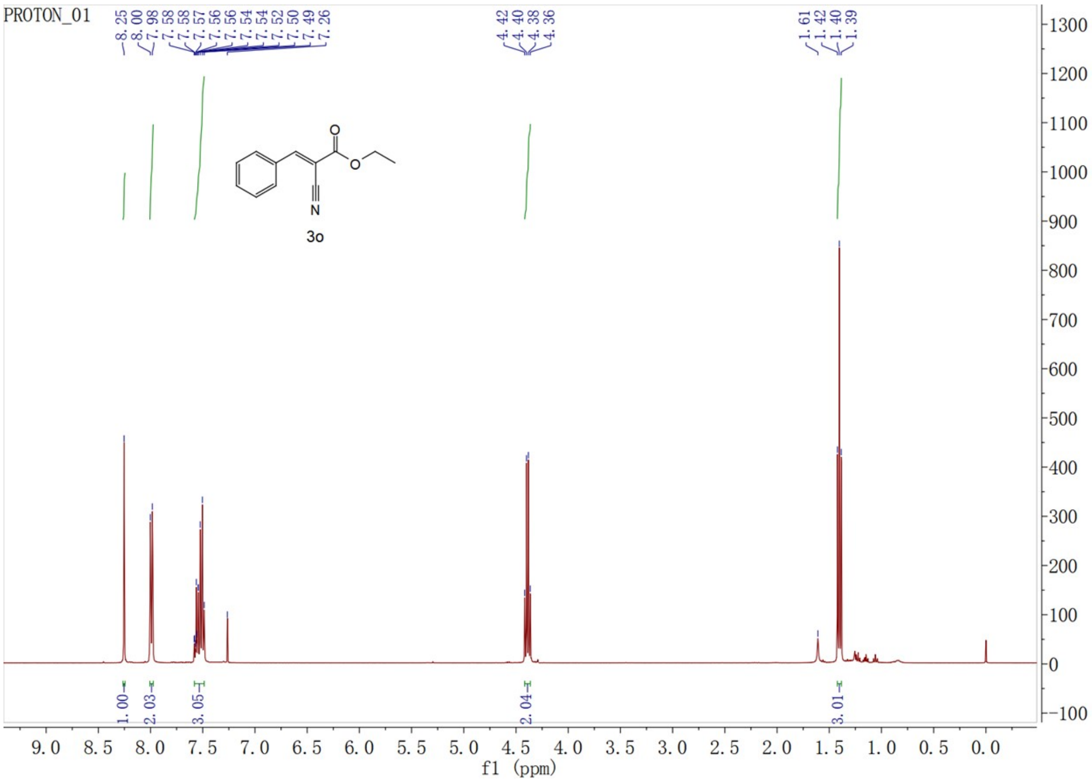


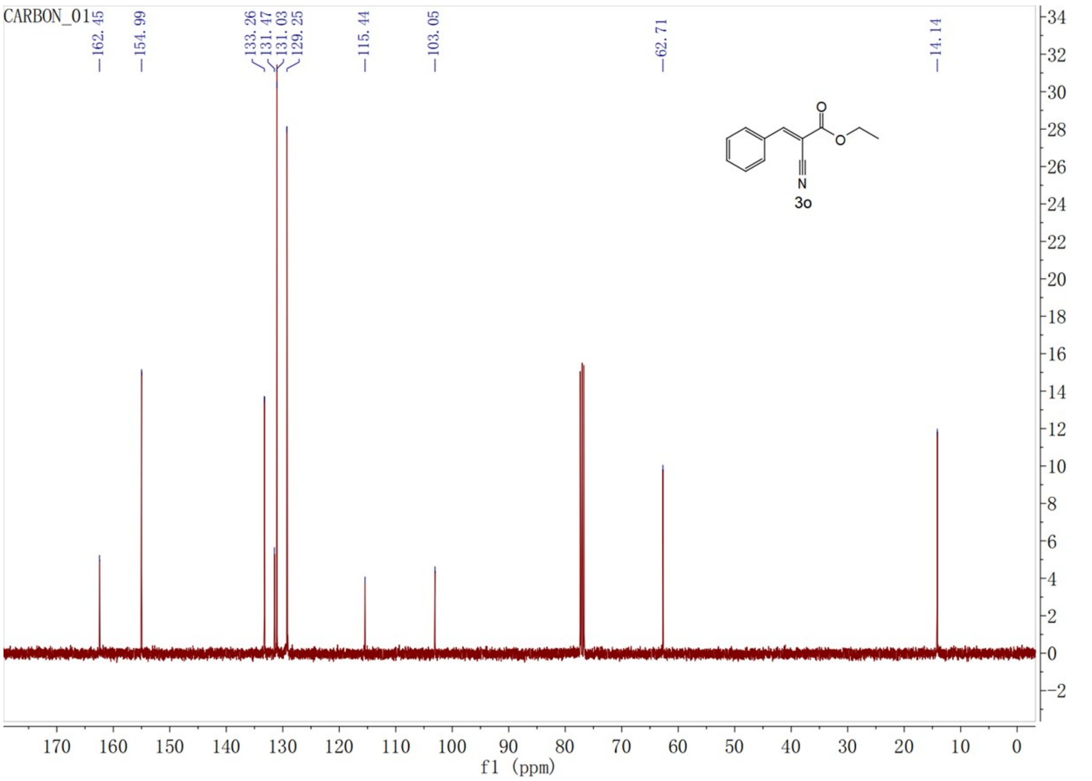
2-(1-(thiophen-2-yl)ethylidene)malononitrile (3n)



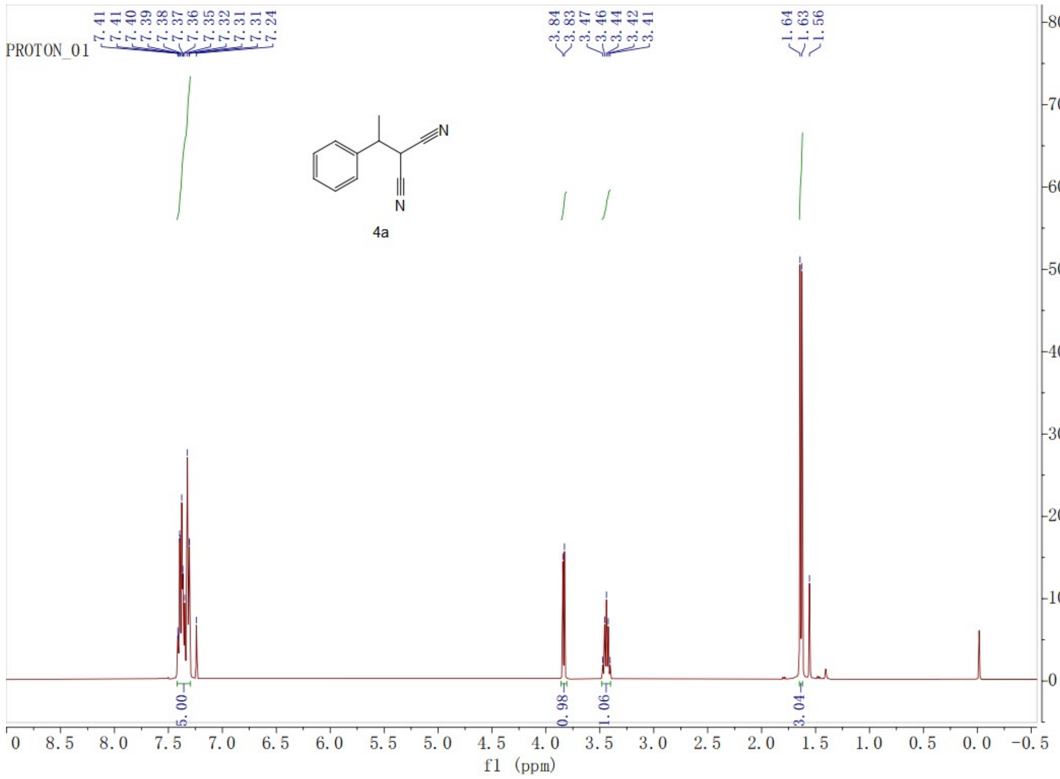


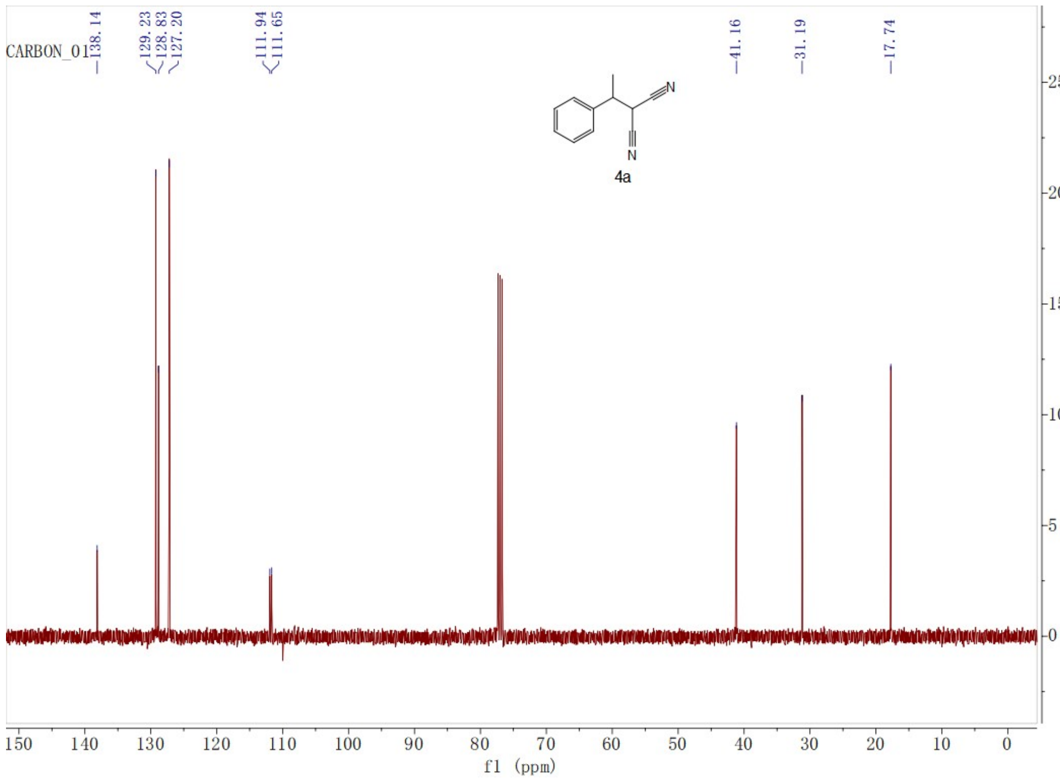
ethyl (E)-2-cyano-3-phenylacrylate (3o)



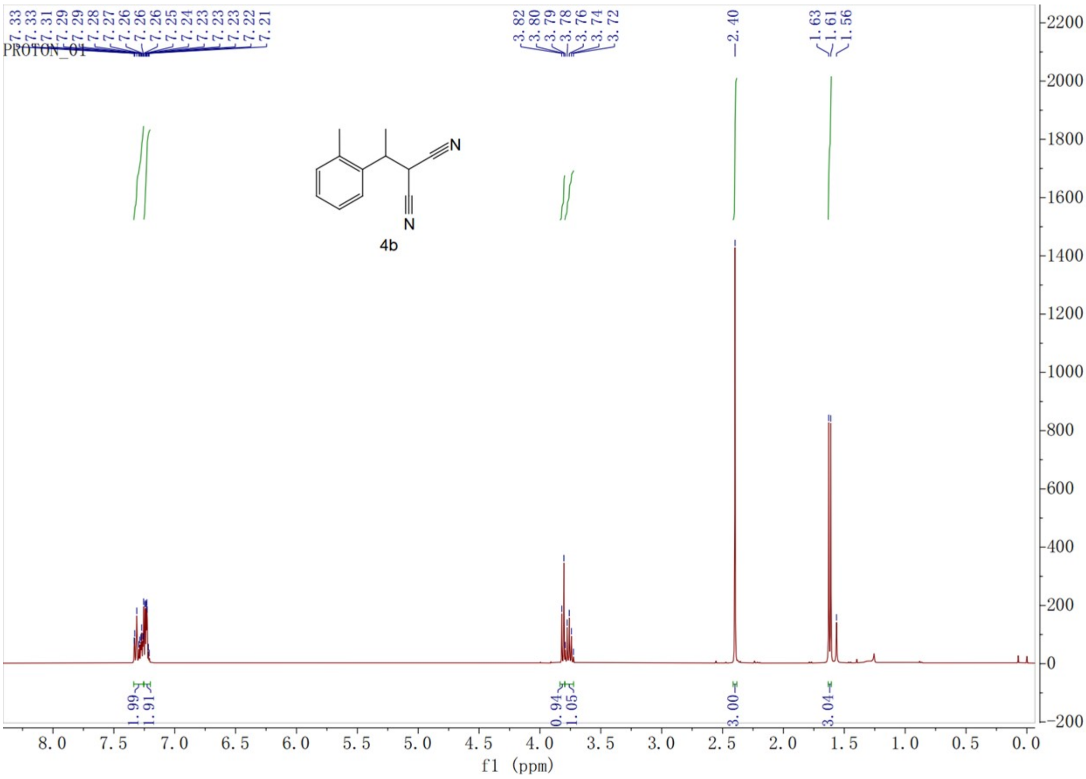


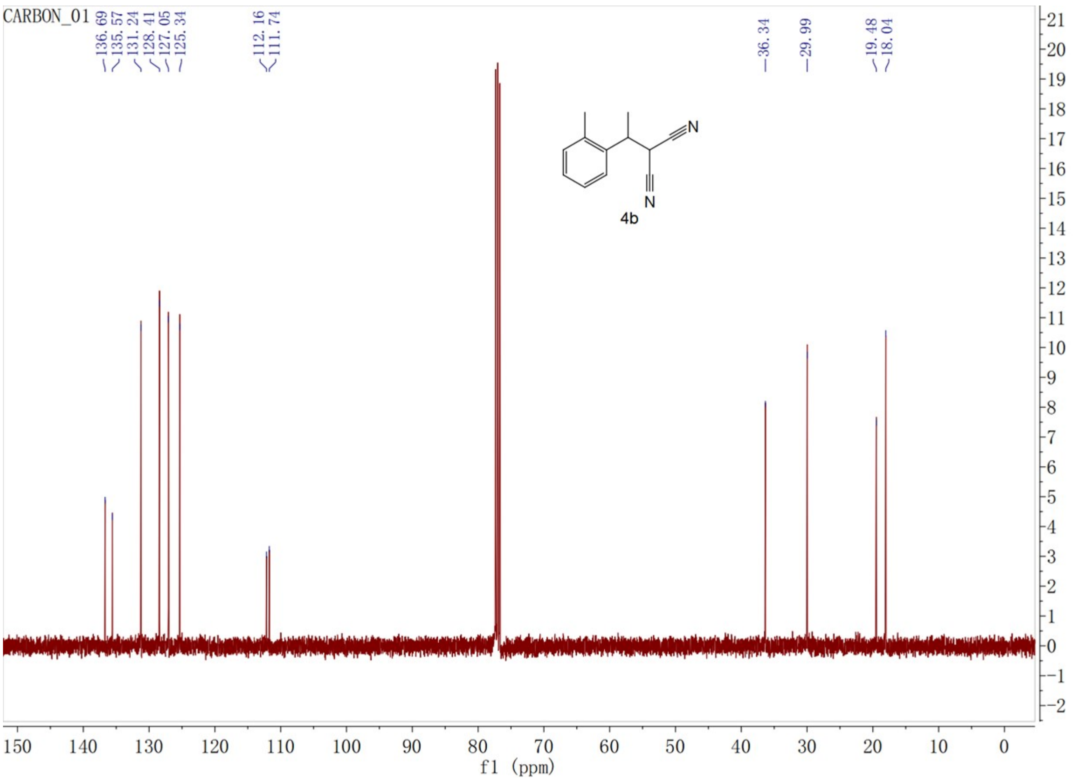
2-(1-phenylethyl)malononitrile (4a)





2-(1-(o-tolyl)ethyl)malononitrile (4b)





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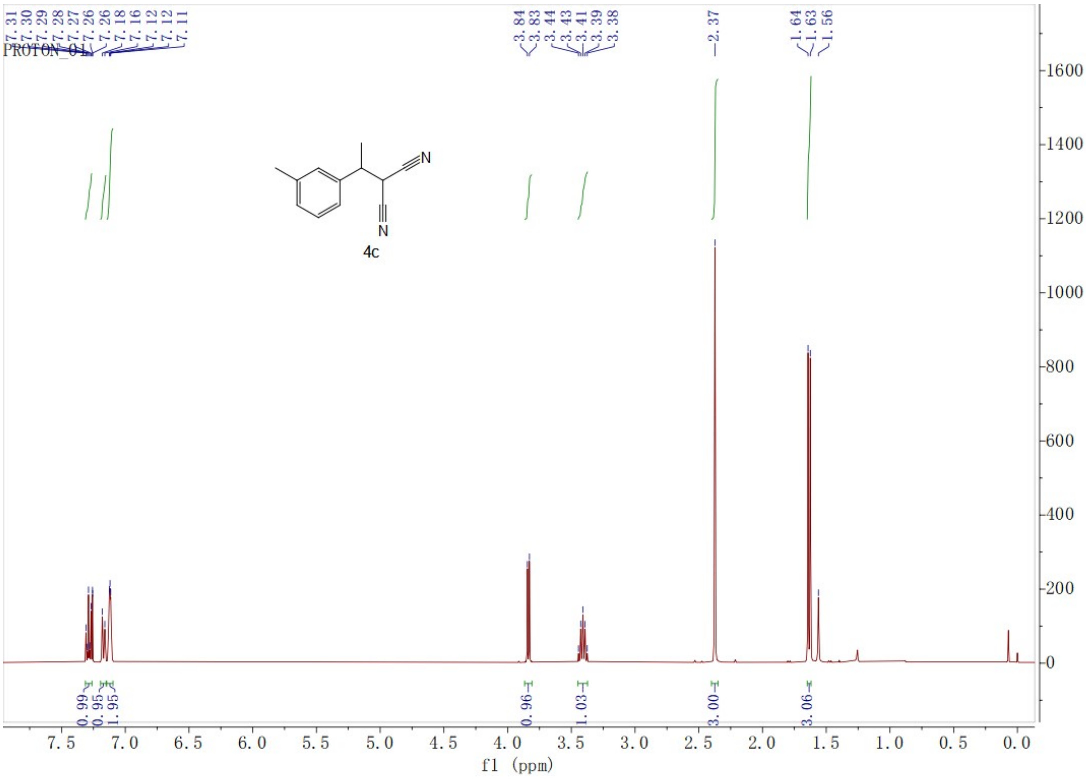

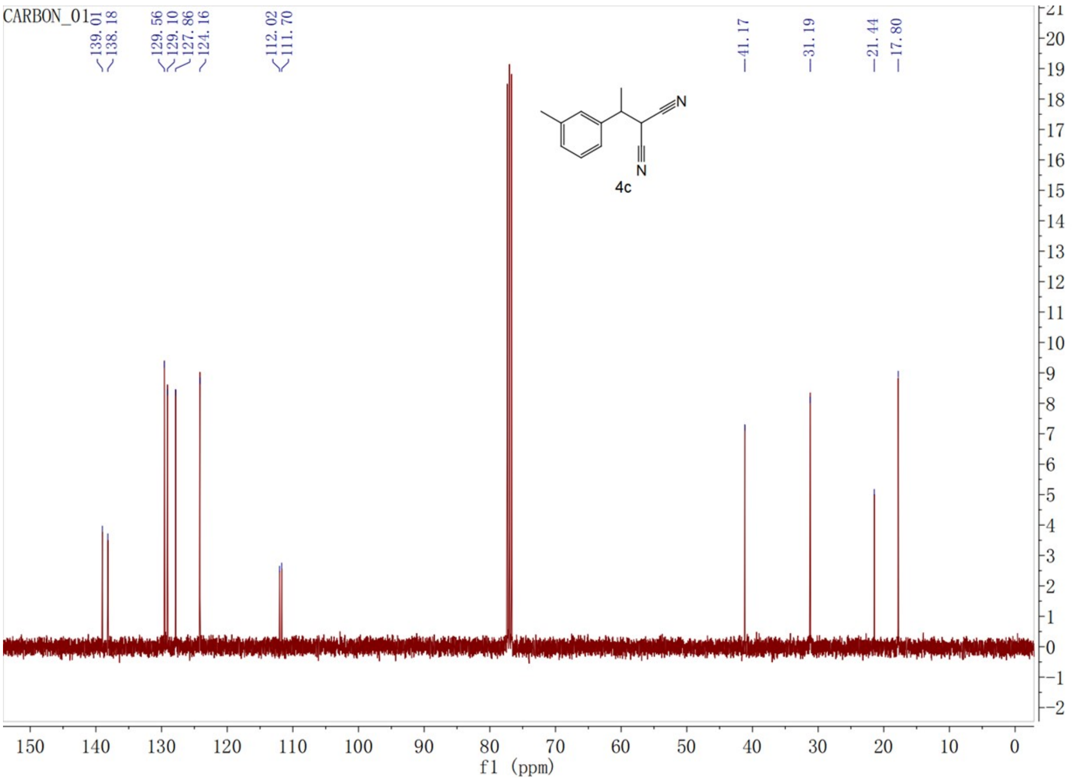

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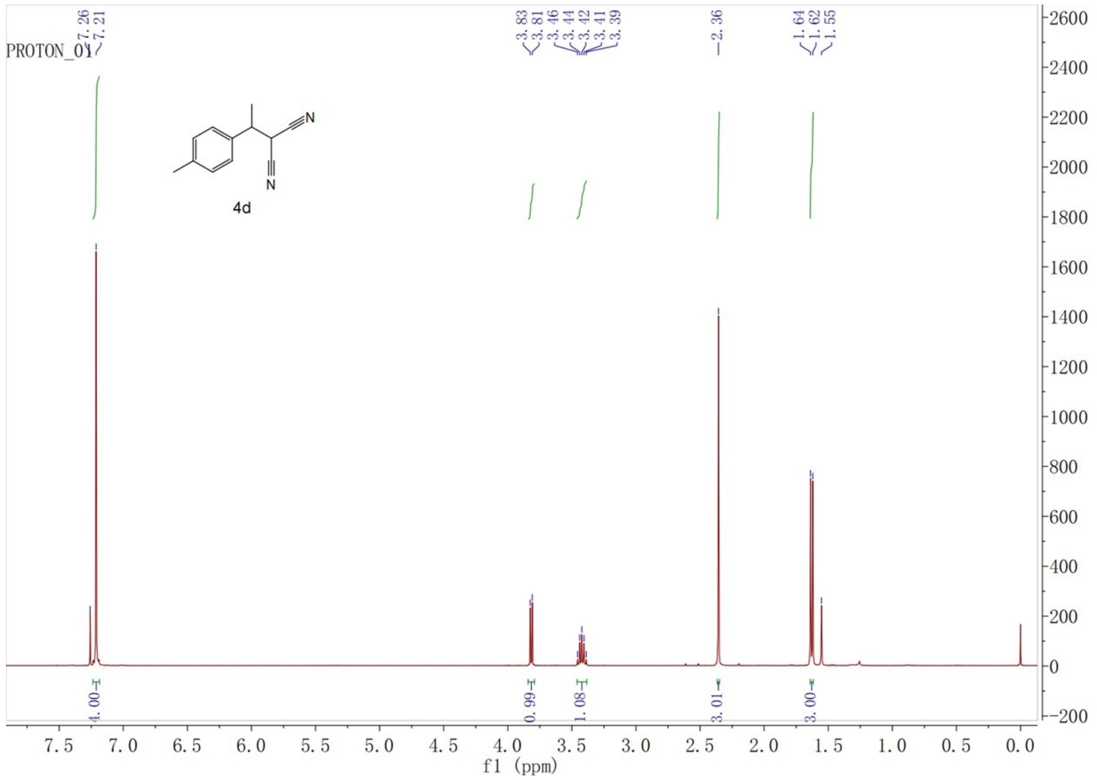
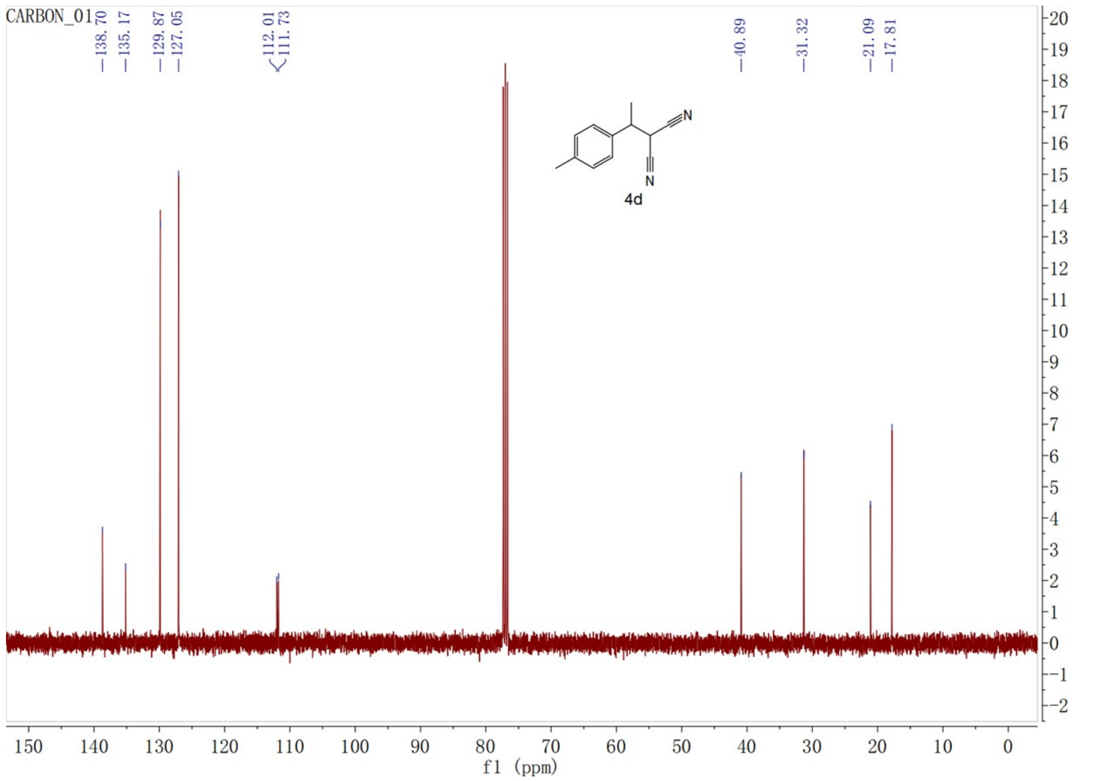
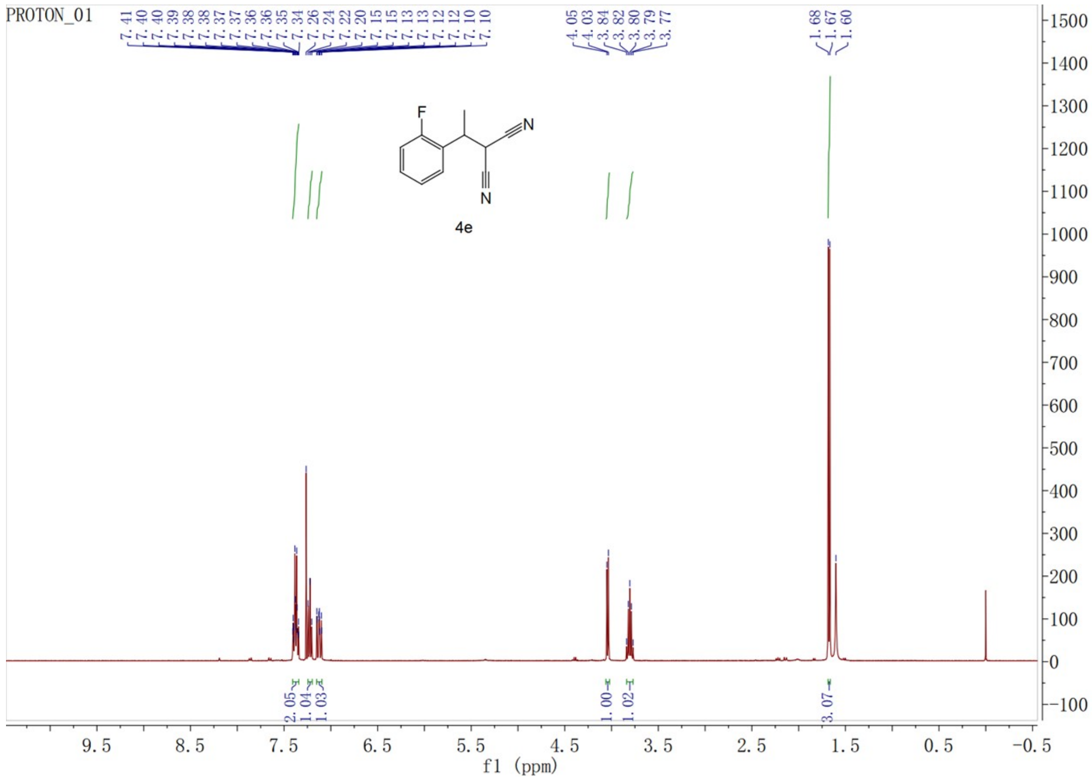
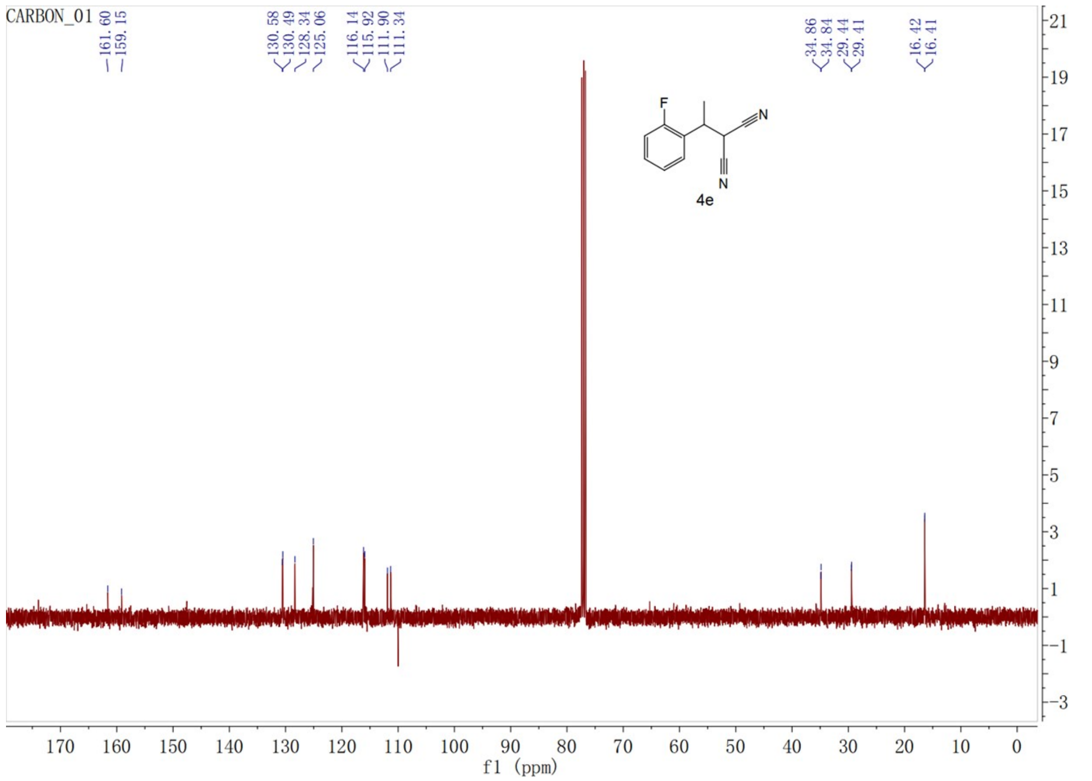


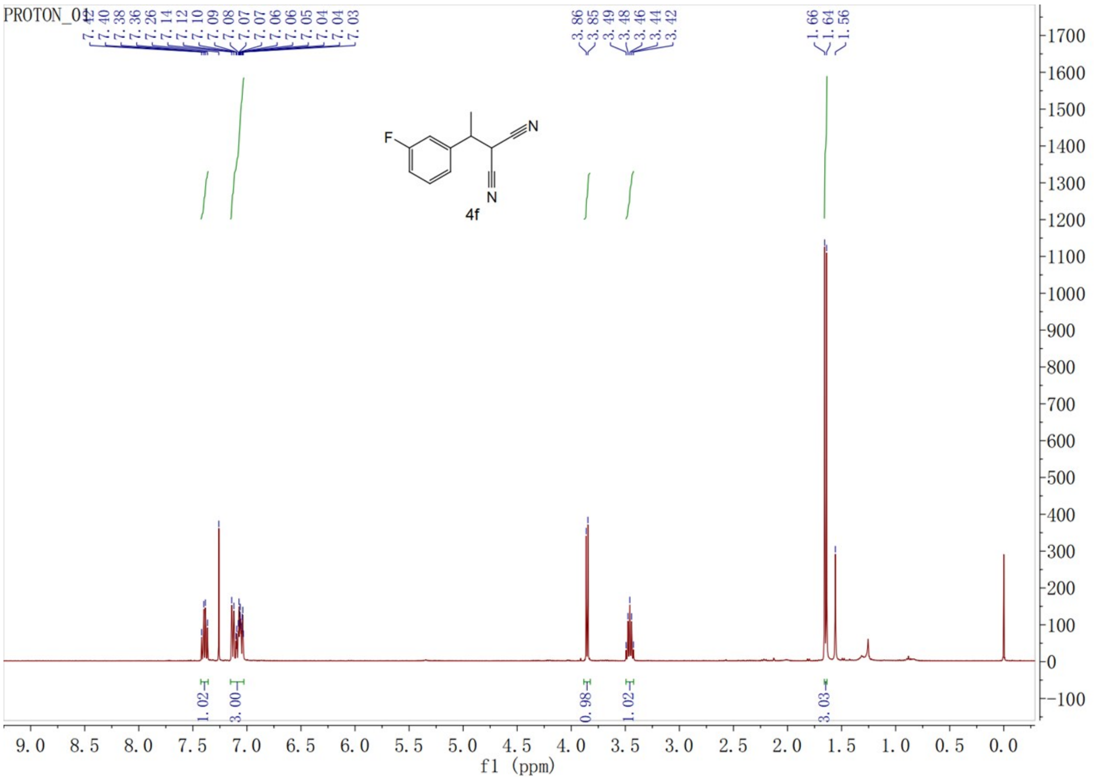


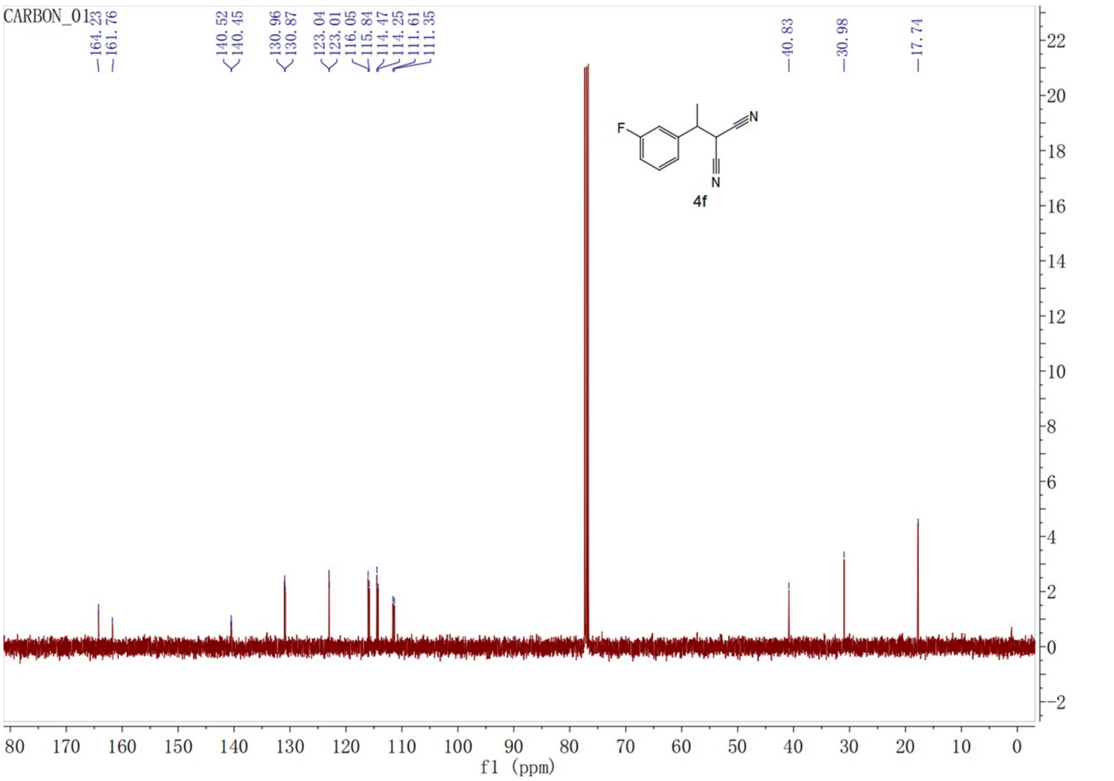
2-(1-(2-fluorophenyl)ethyl)malononitrile (4e)



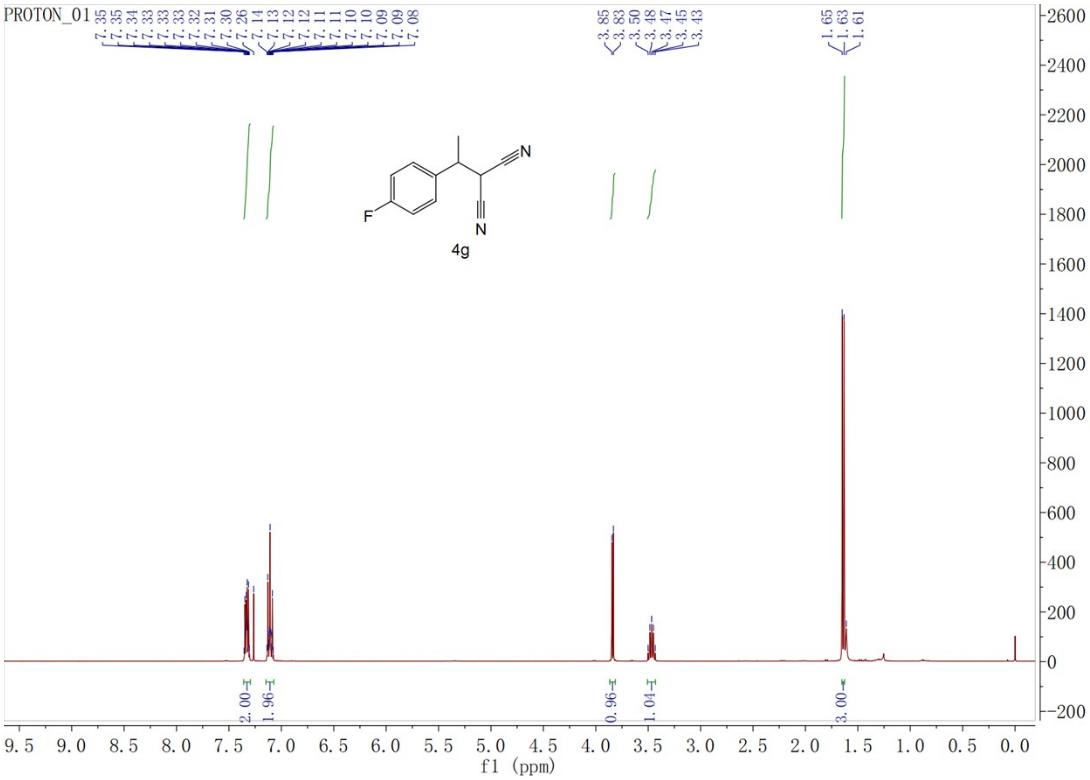


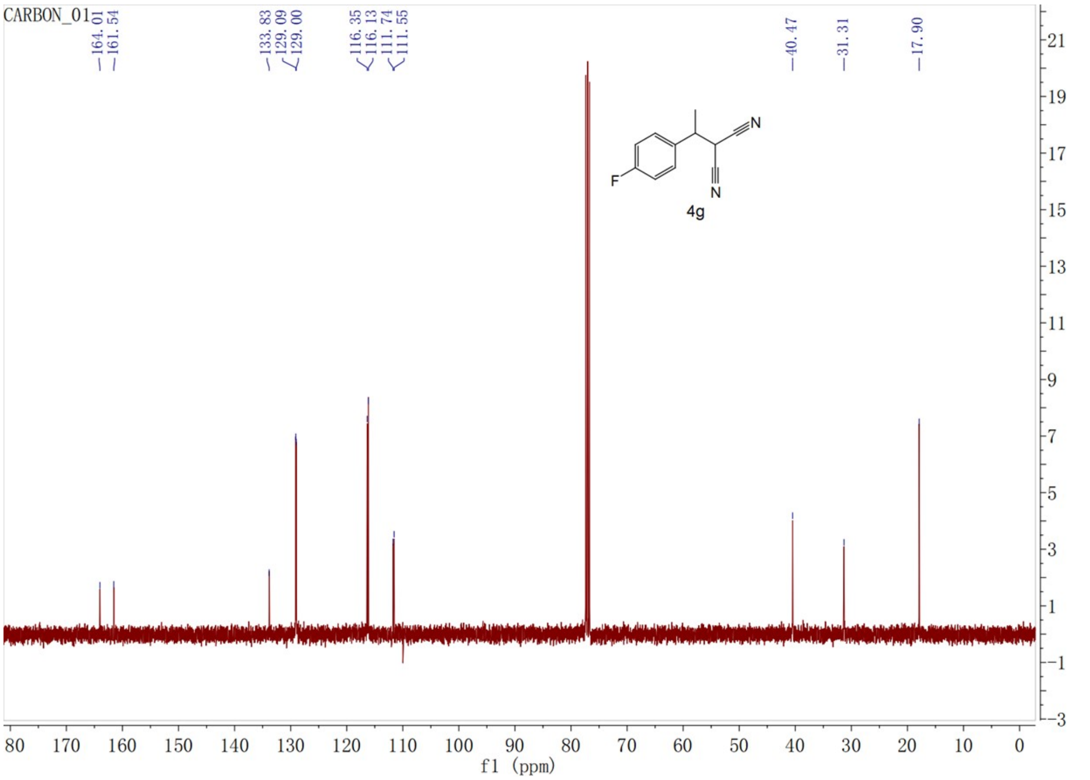
2-(1-(3-fluorophenyl)ethyl)malononitrile (4f)



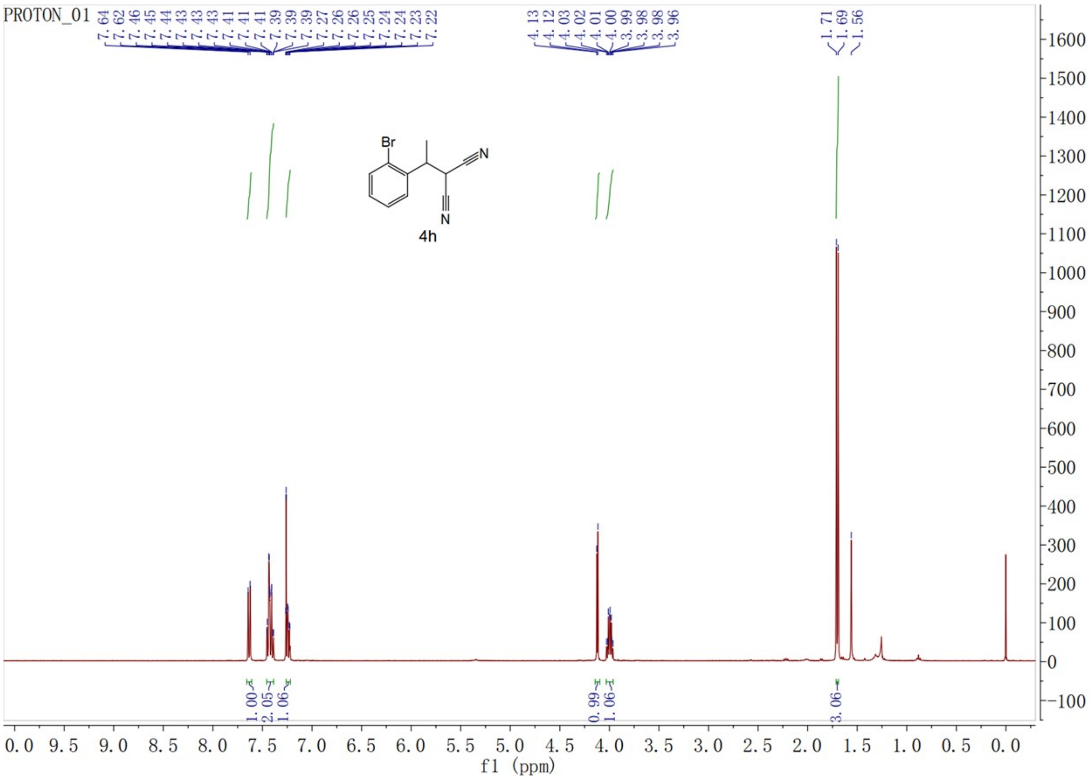


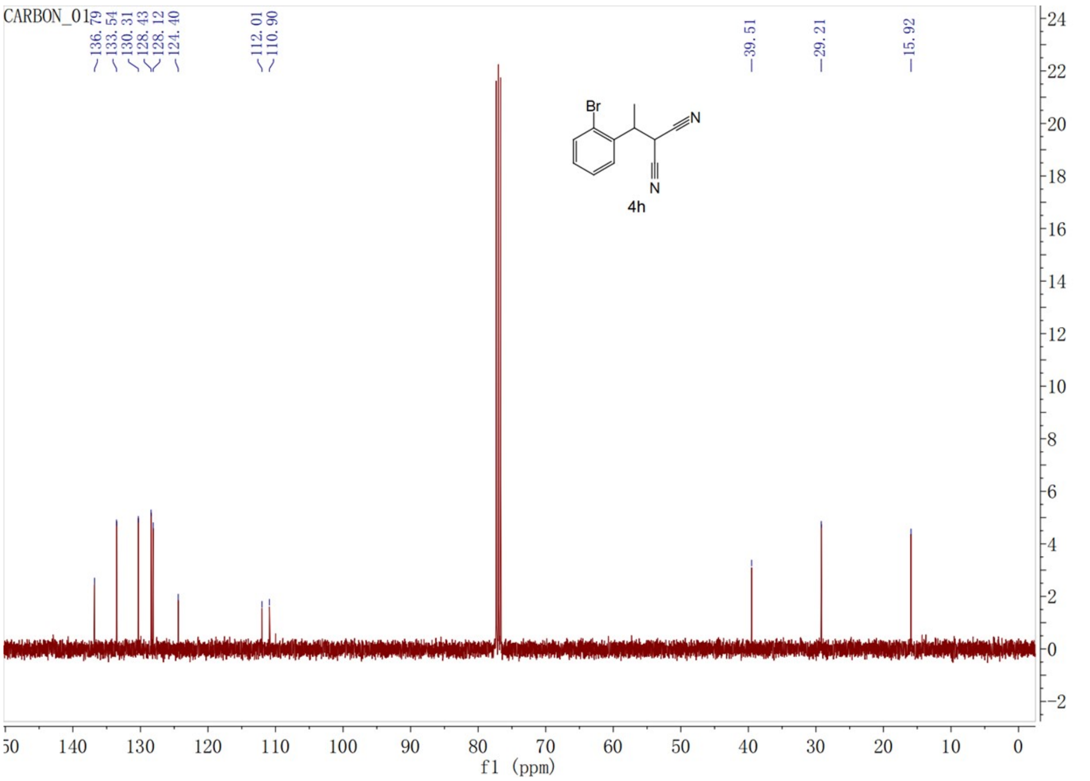
2-(1-(4-fluorophenyl)ethyl)malononitrile (4g)



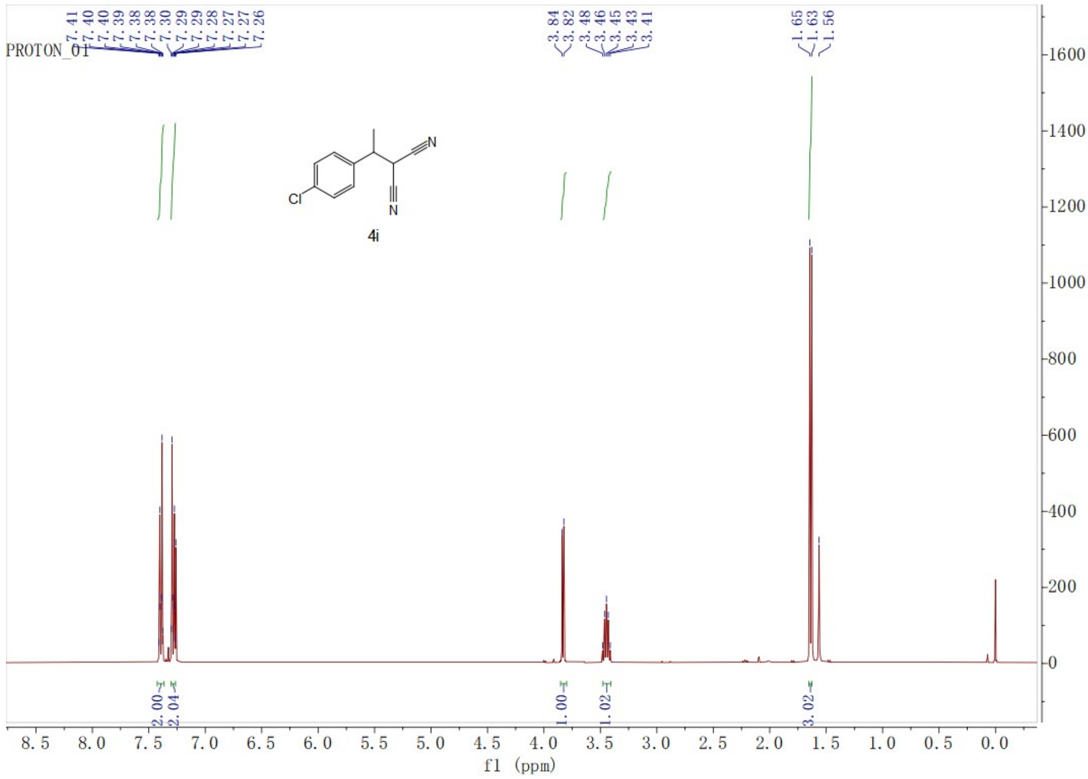


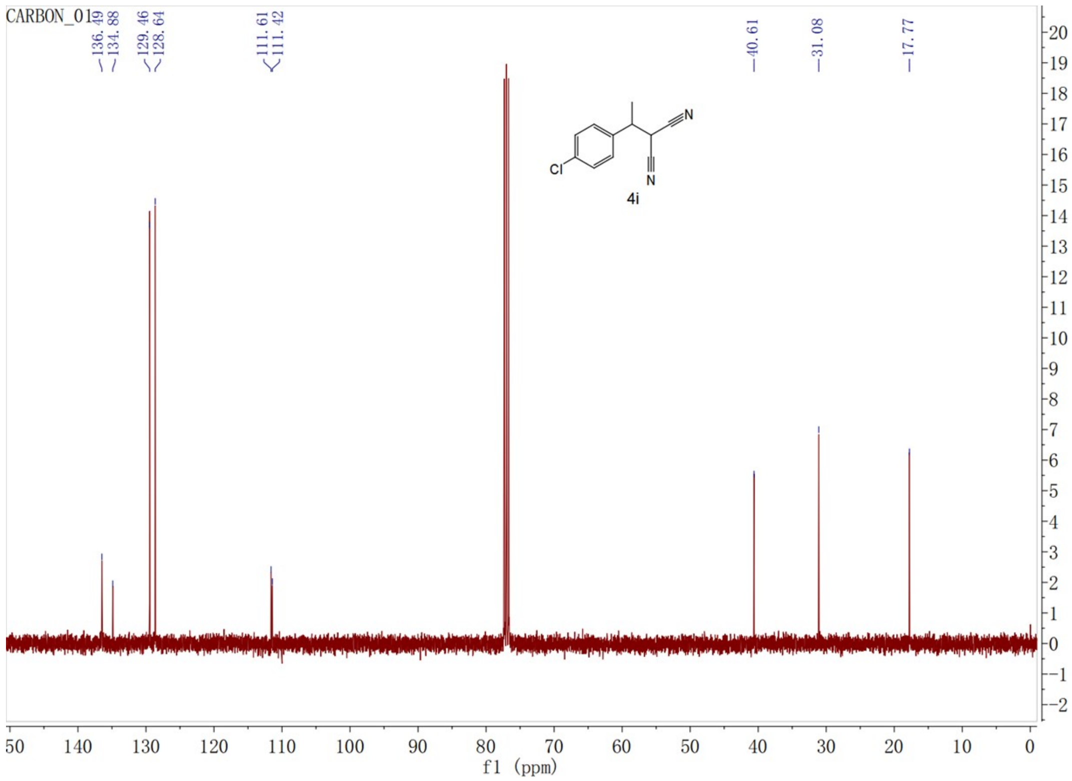
2-(1-(2-bromophenyl)ethyl)malononitrile (4h)



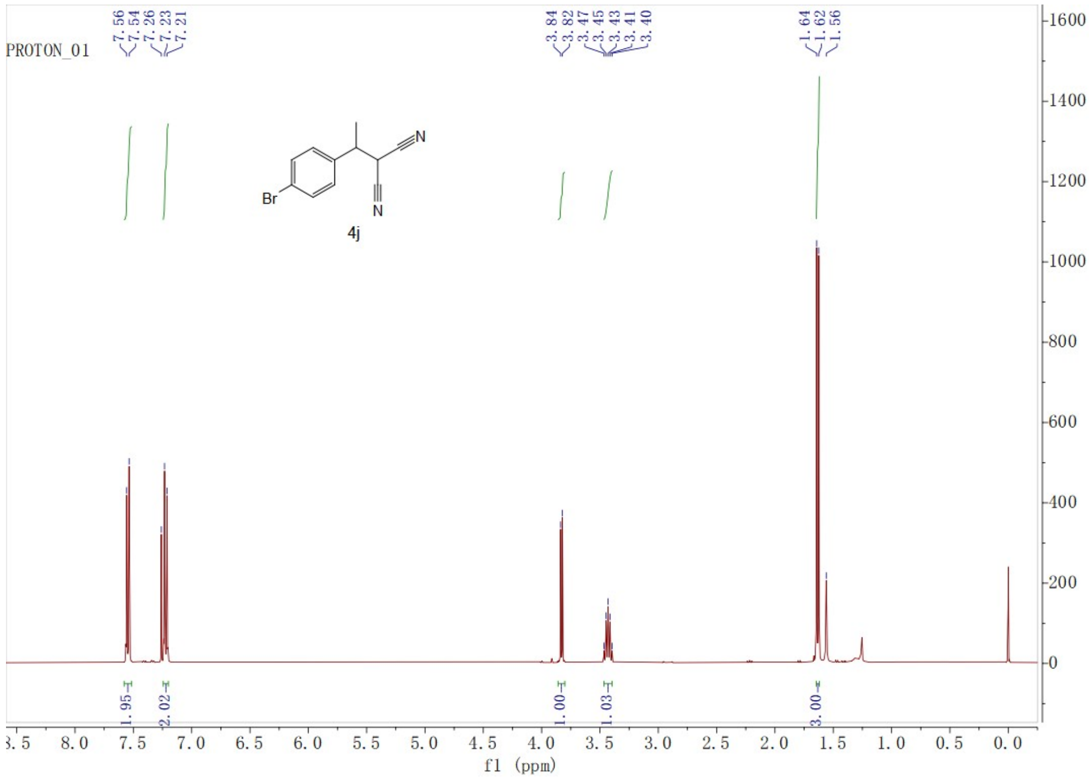


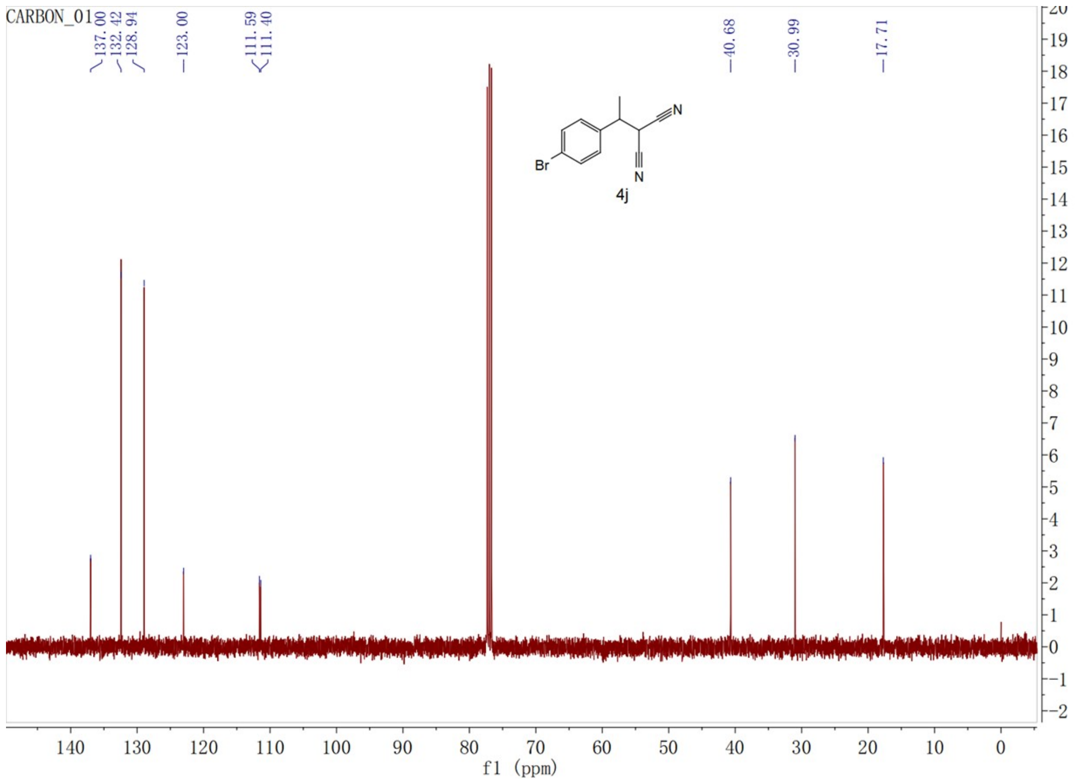
2-(1-(4-chlorophenyl)ethyl)malononitrile (4i)



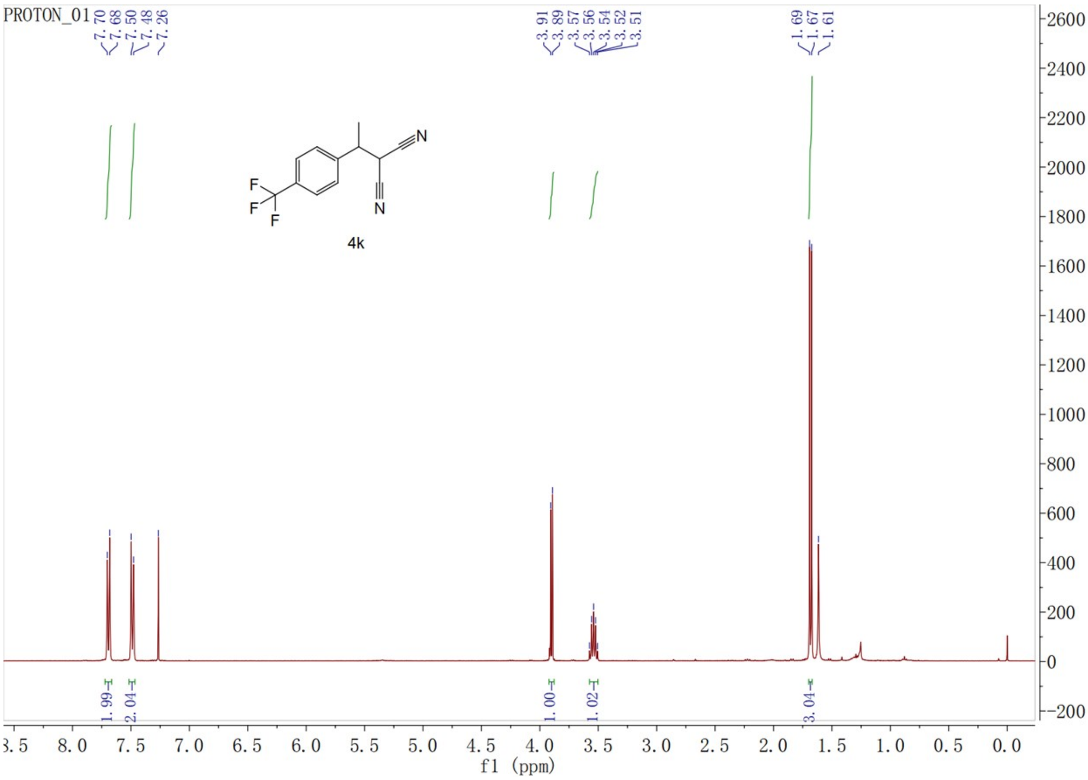


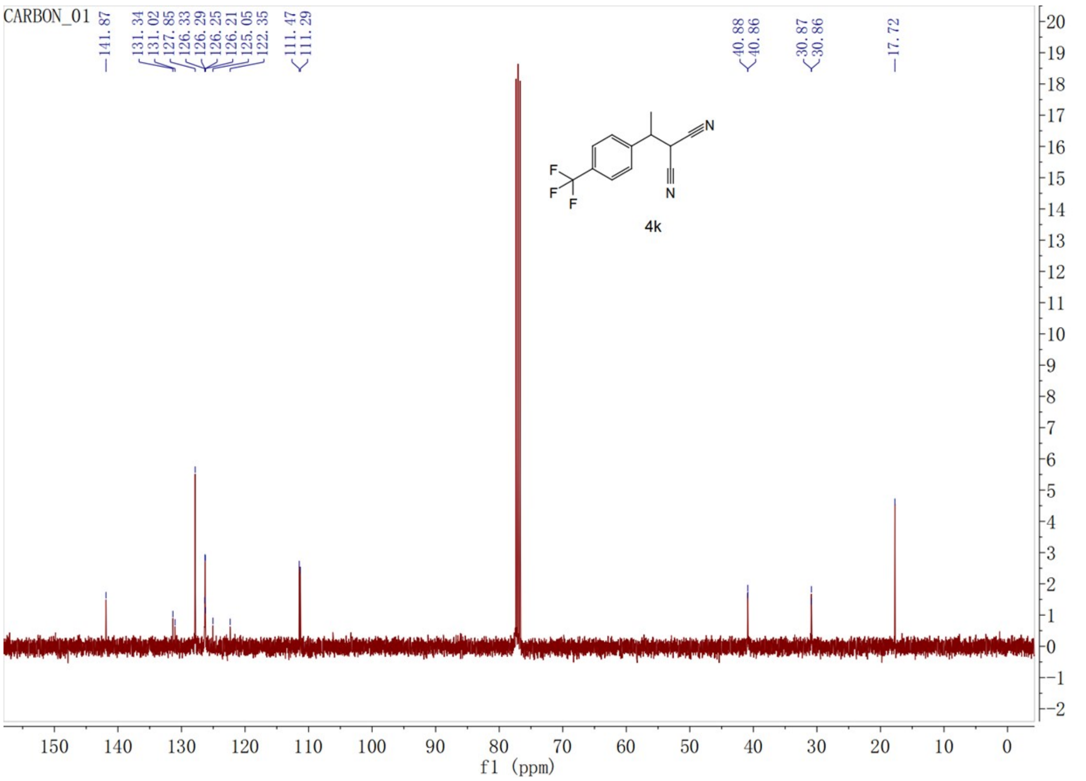
2-(1-(4-bromophenyl)ethyl)malononitrile (4j)



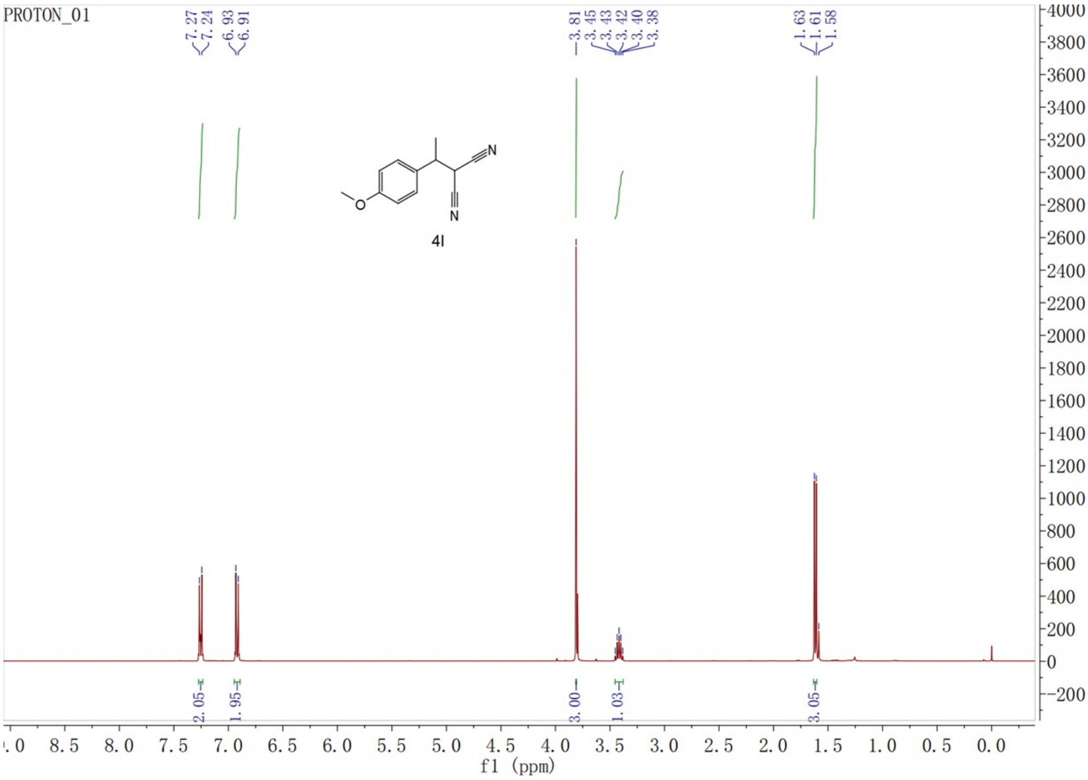


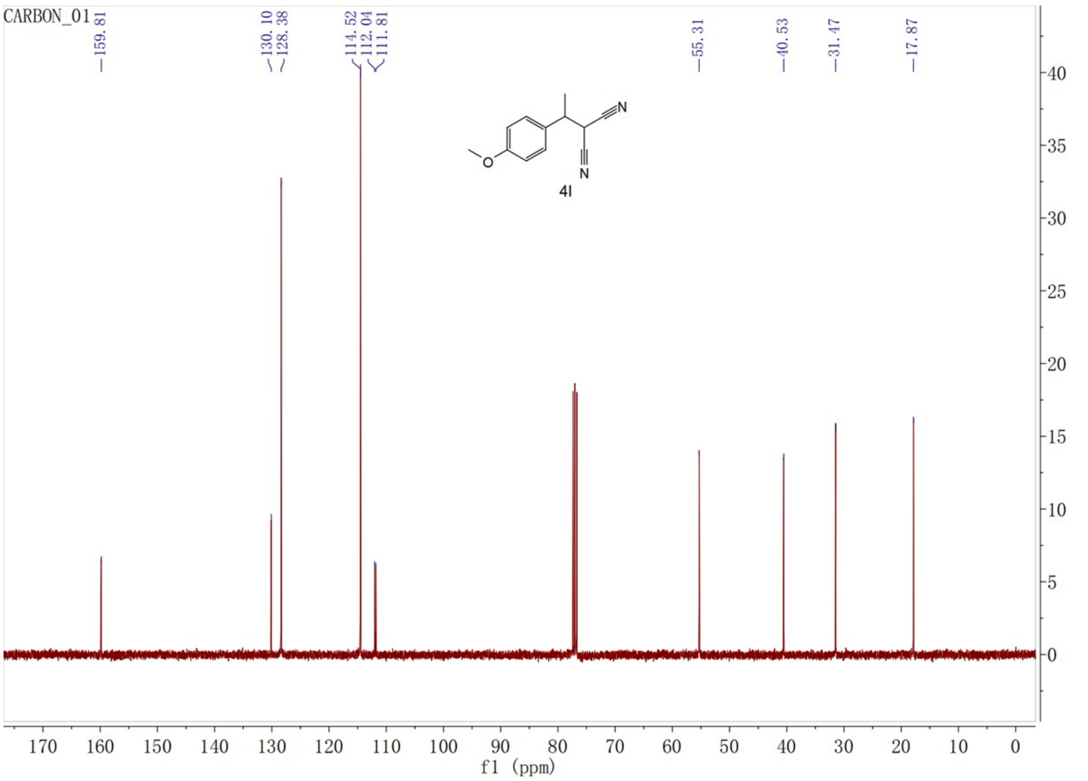
2-(1-(4-(trifluoromethyl)phenyl)ethyl)malononitrile (4k)



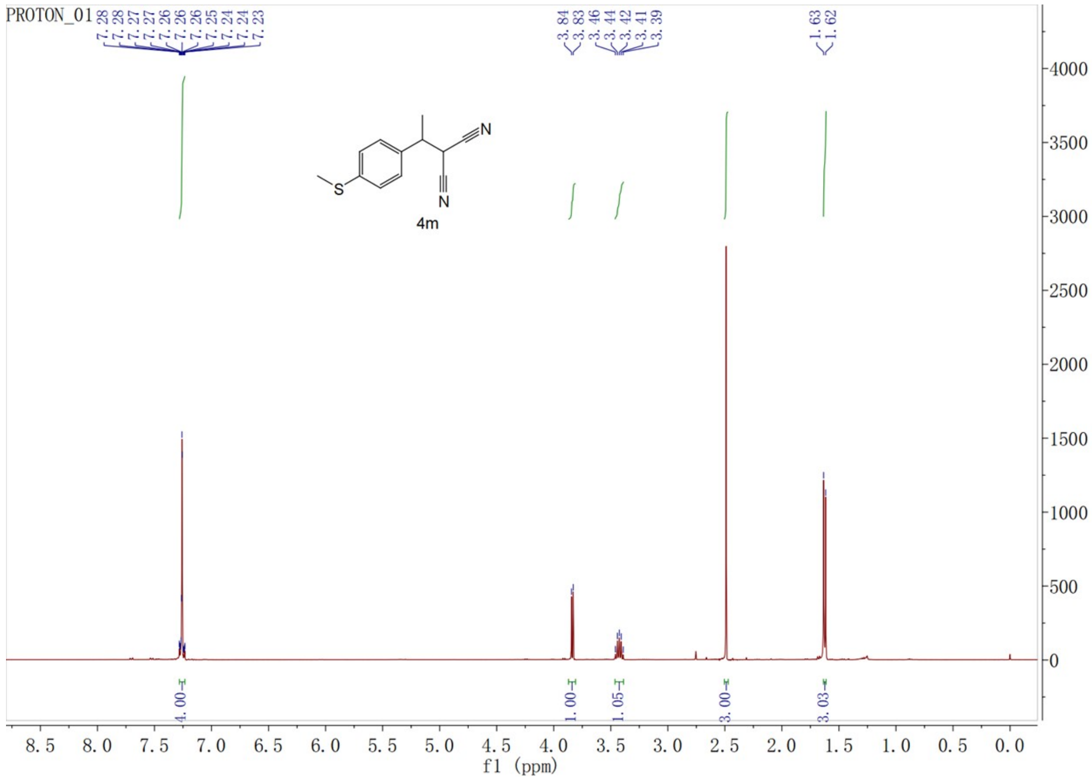


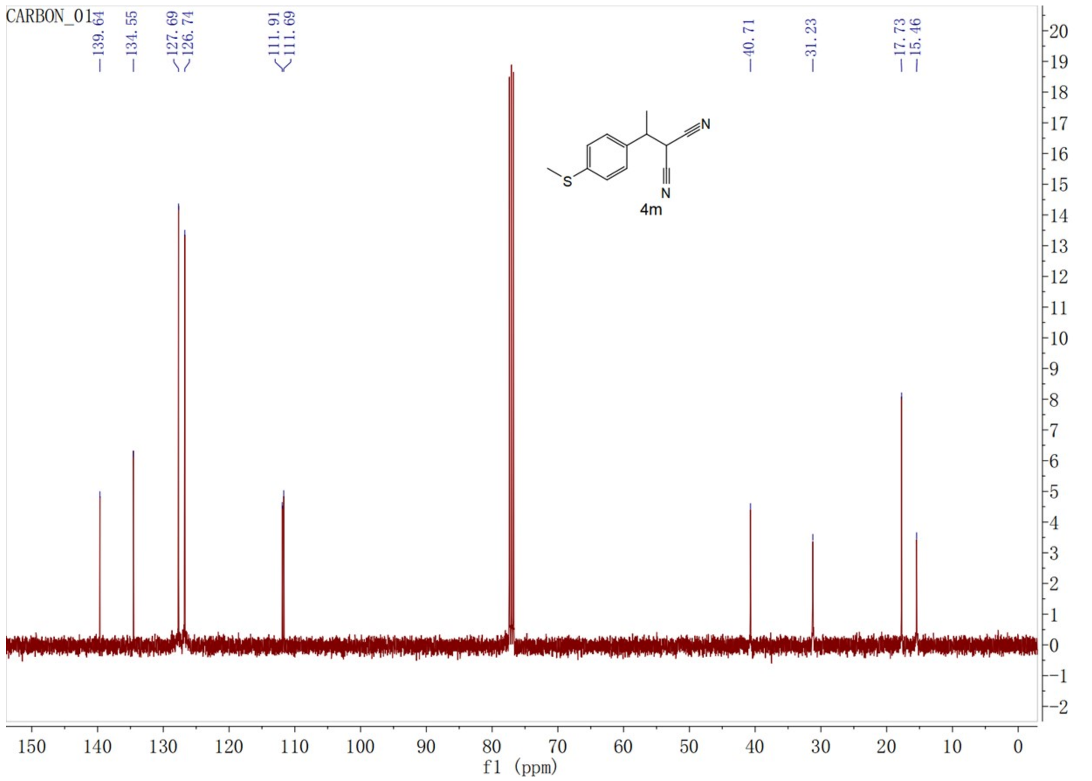
2-(1-(4-methoxyphenyl)ethyl)malononitrile (4l)



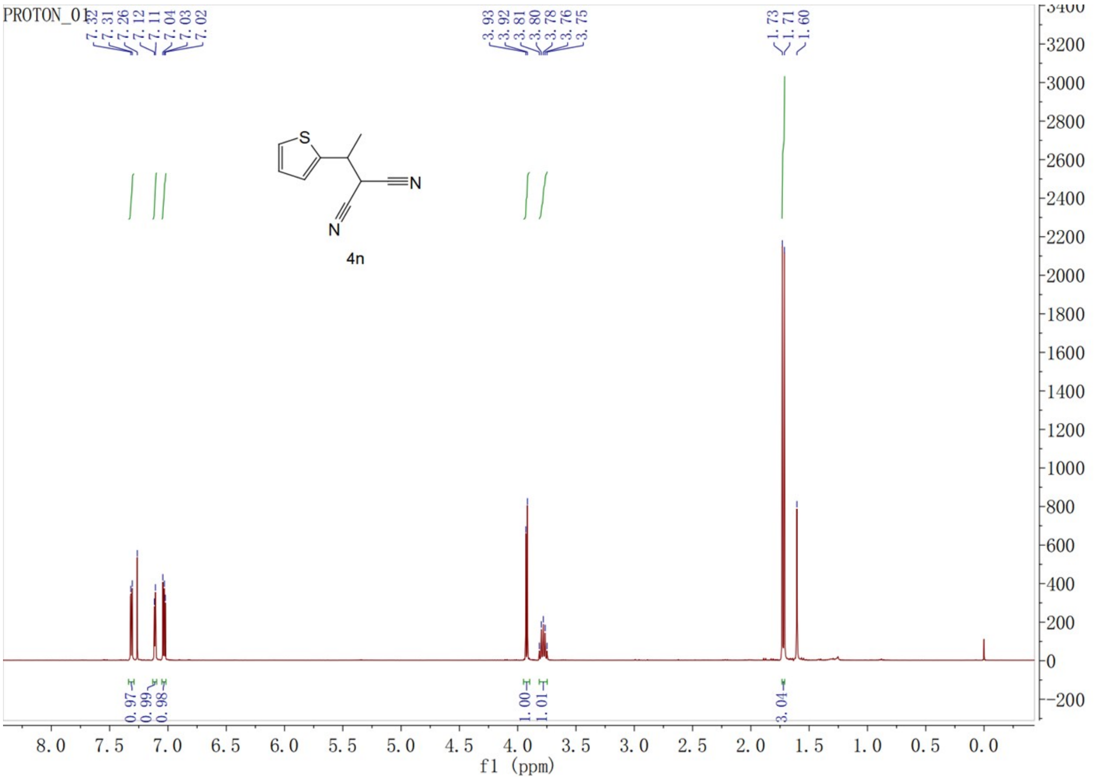


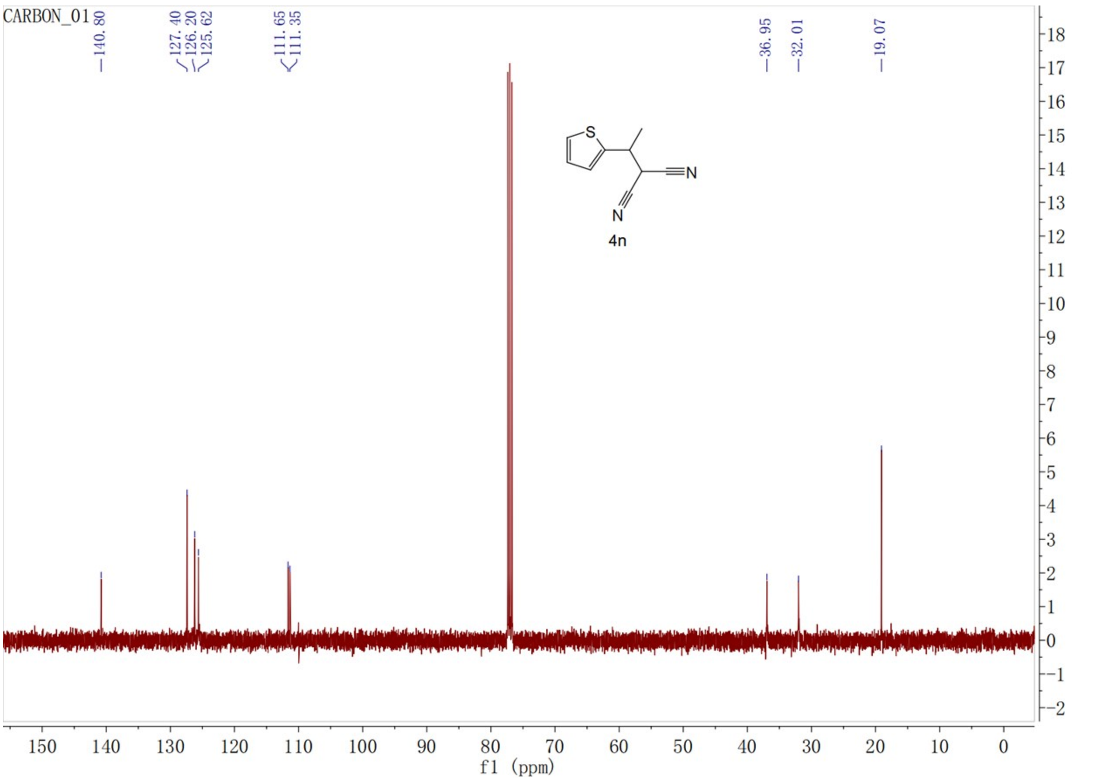
2-(1-(4-(methylthio)phenyl)ethyl)malononitrile (4m)



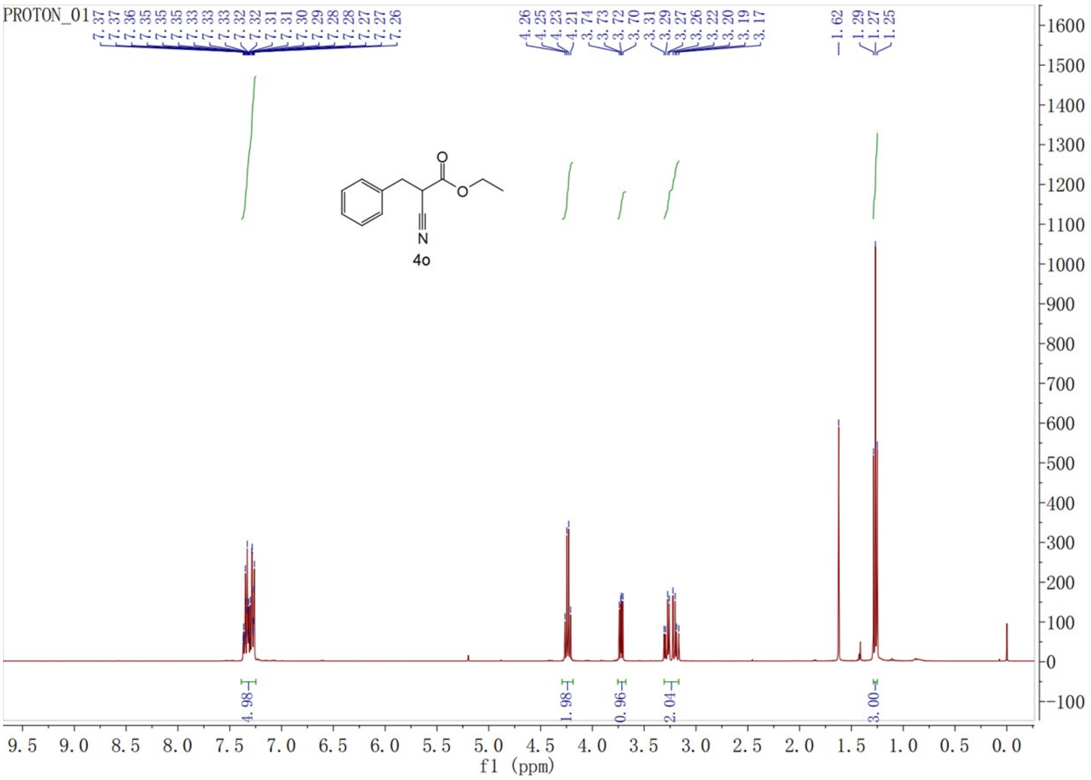


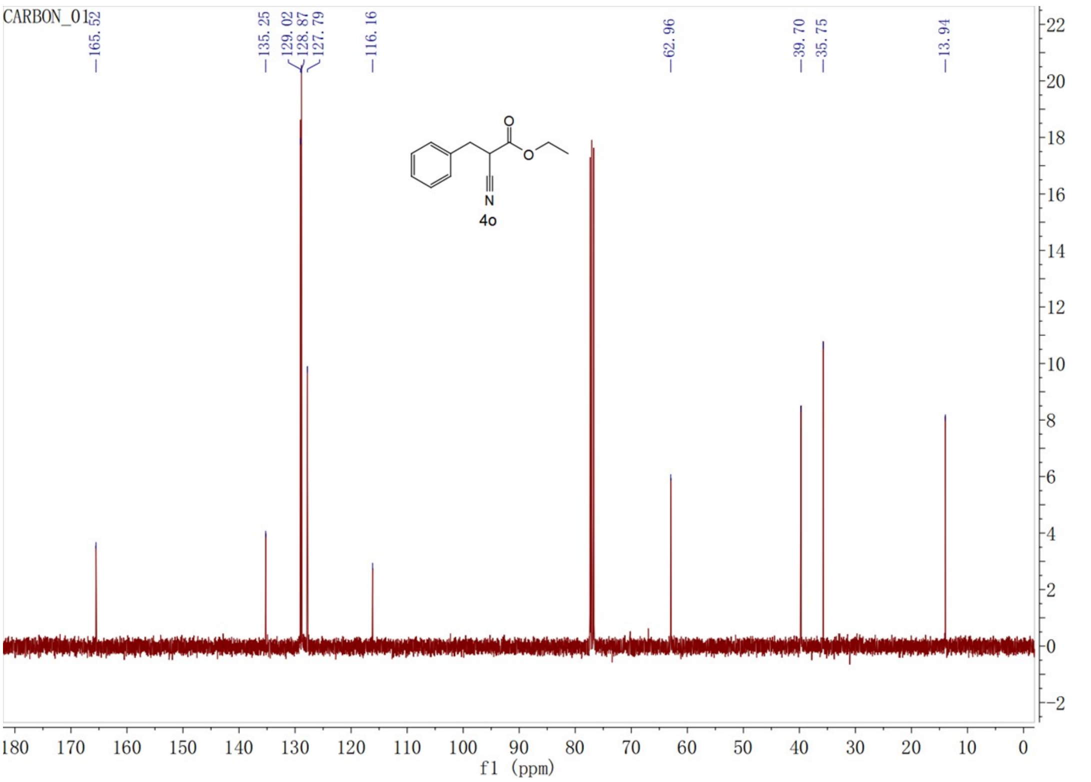
2-(1-(thiophen-2-yl)ethyl)malononitrile (4n)





ethyl 2-cyano-3-phenylpropanoate (4o)





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2 B. C. Ranu and S. Samanta, Use of indium hydride (Cl_2InH) for chemoselective reduction of the carbon-carbon double bond in conjugated alkenes, *Tetrahedron Lett.*, **2002**, 43, 7405-7407.

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