Stereoselective construction of chiral flavanones via enzymatic intramolecular C(sp3)-H activation

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This PDF file includes:

Other supplementary materials for this manuscript include the following:

Dataset. Original HDX data for Mn and Mn-triple mutant in the absence/presence of ligand.

Materials and Methods

Reagents. All chemicals and reagents were obtained from commercial suppliers with the highest level of purity. Restriction endonucleases, 2×Fast Pfu Master Mix, and T4 DNA ligase were purchased from New England Biolabs. QIAquick PCR purification kit and QIAprep Spin Miniprep Kit were from Qiagen (Hilden, Germany). The gene of Mn and its mutants were inserted into the plasmid pET-28a(+) (Novagen). The plasmid contains an isopropyl β-D-1-thiogalactopyranoside (IPTG) inducible T7 promoter and an kanamycin resistance gene. *E. coli* strains of DH5α and BL21(DE3) competent cells were used for plasmid propagation and protein expression, respectively. M9-N buffer contains 47.7 mM Na_2HPO_4, 22mM KH_2PO_4, 8.6mM NaCl, 2mM $MgSO_4$, and 0.1mM $CaCl_2$ at pH=7.4.

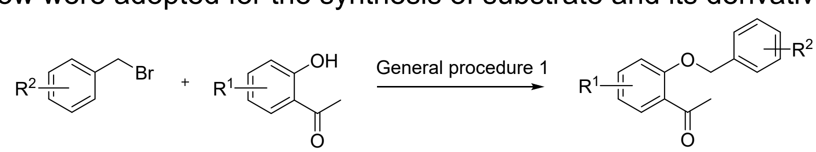
Cloning, mutagenesis, and expression of proteins. Plasmid pET-28a(+) (Novagen) was used as a cloning and expression vector for all variants described in this paper. Site-saturation mutagenesis was performed using a modified QuikChangeTM mutagenesis protocol using the 22-codon trick. The PCR products were digested with DMT, purified with E.Z.N.A. Gel Extraction kit. Purified mixture was transformed into 50 μL *Escherichia coli* BL21(DE3) (Transgen Biotech). The cells were grown overnight in 5 mL Luria-Bertani (LB) medium, supplemented with kanamycin, and 2 mL of this preculture were used to inoculate 200 mL of Terrific Broth (TB) medium. The expression culture was incubated at 37 °C and 200 rpm until OD_{600} reached 1.0-1.2. Then, the expression culture was cooled on ice for 20 minutes and was treated with 0.3 mM 5-aminolevulinic acid (5-ALA) and 0.25 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) (final concentrations). Cells were allowed to express at 20 °C and 150 rpm for 18 to 22 hours. Once expression was finished, the cultures were centrifuged (4,000 g, 15 minutes) and the precipitated bacteria were collected and stored at -80°C.

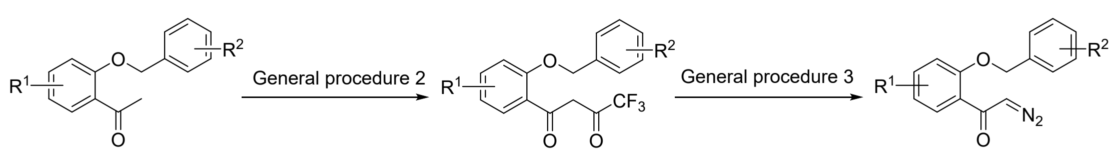
Reaction setup and product characterization. To prepare for the reaction, whole cells (OD_{600} of 40 in 4.75mL M9-N buffer) were degassed by sparging with argon in a sealed 10mL two-neck bottle for at least 30 minutes. Diazo substrate (0.25 mL of a DMSO stock) was injected into a 5mL whole cells reaction system with a final diazo substrate concentration of 5mM under argon atmosphere. The bottle was sealed by Vaseline and shaken at 25 ℃ and 200 rpm for 24 h. The reaction was then quenched by addition of acetonitrile (5mL). This mixture was transferred to a microcentrifuge tube and centrifuged at 12,000 rpm for 10 minutes. A total of 100 µl of supernatant was taken to mix with 100 µl of internal standard (0.5 mg/mL in methanol) and 800 µl methanol. This solution was subsequently analyzed by HPLC. Protein concentration in the cell was determined by performing hemochrome assay on the cell lysate. The cell debris was removed by centrifugation (5,000rpm, 30 minutes, 4 °C), and the supernatant was filtered through a 0.45 µm cellulose filter. All hemoprotein concentrations were determined in triplicate using the hemochrome assay. A solution of 1 M NaOH (0.4 mL) was mixed with pyridine (1 mL) in a 1.5 mL centrifuge tube. Centrifuged was performed (11000 rpm, 1 minute) to separate the excess aqueous layer and give basified pyridine solution on top. Separately, a solution of sodium dithionite (10 mg/mL) was prepared in M9-N buffer. A volume of 50 μL of dithionite solution and 150 μL pyridine-NaOH solution were added to a cuvette containing 800 μL protein solution in M9-N buffer and mixed. UV-vis spectrum of the reduced hemoprotein was recorded immediately. Hemoprotein concentrations were determined using $\varepsilon_{550-535}$ = 23.8 $mM^{-1}cm^{-1}$ for heme b.

Biocatalyst screening using 96-well plates. Single colonies from LB agar plates were picked using sterile toothpicks and cultured in deep-well 96-well plates containing LB (300 μL/well) and kanamycin at 37 ºC, 220 rpm shaking overnight. Then 80 μL of culture from each well were transferred to new deep 96 well plates with 1200 μL TB medium in each well. The deep 96 well plates incubated at 37 ºC, 220 rpm for 3h. The plates were then cooled on ice for 30 minutes and the cultures were induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 1.0 mM 5-aminolevulinic acid (final concentrations). Expression was conducted at 20 ºC, 150 rpm for 16 to 20 hours. The cells were pelleted (1,500 rpm, 10 min, 4 ºC) and resuspended in M9-N buffer (300 μL/well) by gentle vortexing after expression. Diazo substrate (30 μL/well, 50 mM in DMSO) was added into the wells. Then, the 96-well plate was quickly transferred to a box with an air valve, and the sealed box was degassed by sparging with argon for at least 30 min. After closing the air valve, the box was placed in the shaker and shaken at 30 ºC ,170 rpm. After 24h of incubation, the 96-well plate was taken out and acetonitrile (300 μL/well) was added and mixed, the plate was centrifuged (15,00rpm, 10 min) to separate the supernatants that were further filtered and transferred to centrifuge tubes for HPLC analysis.

NMR and HPLC measurements. ^1H and ^{13}C NMR spectra were recorded on Bruker Prodigy 400 MHz spectrometer (400 MHz ^1H and 100.6 MHz ^{13}C) at ambient temperature. Chemical shifts are referenced to chloroform (7.26 ppm for ^1H NMR and 77.16 ppm for ^{13}C NMR). Data for ^1H NMR are reported in the conventional form: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, hept = heptet, m = multiplet, br = broad), coupling constant (Hz), and integration. Chemical reactions were monitored using thin layer chromatography and a UV lamp for visualization. Reverse-phase high-performance liquid chromatography (HPLC) for analysis was carried out using Shimadzu (LC-20AT) instruments, and C18 (SilGreen®, 4.6 × 250 mm, 5 μm) columns. Water and acetonitrile containing 0.1% trifluoroacetic acid were used as eluents. Analytical chiral HPLC was performed using Daicel® Chiralcel OD-H columns (4.6 × 250 mm, 5 μm) with hexanes and isopropanol as the mobile phase.

Synthesis and characterization of substrate/derivatives. Three general procedures shown below were adopted for the synthesis of substrate and its derivatives.





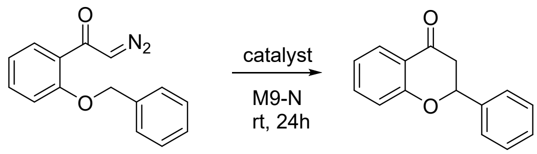
General procedure 1: To a solution of phenol (3.0 mmol) in acetone (6 mL) was added the K_2CO_3 (4.5 mmol), tetrabutylammonium iodide (3.0 mmol) and the alkyl halide (3.3 mmol), successively. The reaction mixture was vigorously stirred at 65 °C for overnight. Afterwards, the reaction mixture was cooled to room temperature, and water (20 mL) was added with stirring until all solids dissolved. The mixture was extracted with ethyl acetate (20×3 mL). The combined organic layer was washed with saturated sodium chloride solution and was dried over anhydrous Na_2SO_4. After filtration, the solvent was removed under reduced pressure and the crude product was directly used in the next step without purification. General procedure 2: To a solution of the acetophenone (1 mmol) in THF (3 mL) at 0 °C was added Sodium hydride (60% dispersed in mineral oil, 1.5 mmol) slowly over 10 min. The mixture was stirred at 0 °C for 10 minutes before 2,2,2-trifluoroethyl acetate (0.15 mL, 1.2 mmol) was added in one portion. Then the mixture was stirred at room temperature for 30 min before being quenched with 1 M HCl. The aqueous layer was extracted with ethyl acetate for three times and the combined organic layers were washed with saturated sodium chloride solution and was concentrated under reduce pressure to afford the crude ketone without purification. General procedure 3: The crude ketone (1 mmol) was redissolved in MeCN (5 mL) under nitrogen. To this solution was added Et_3N (1.5 mmol). Then, a MeCN solution of 4-acetamidobenzolsulfonyl azide (p-ABSA) (1.2 mmol) was slowly added over 5 min. The reaction mixture was stirred at room temperature for overnight. Afterwards, the reaction was quenched with aqueous $NaHCO_3$ solution, and the mixture was extracted with ethyl acetate for three times. The combined organic layers were washed with saturated sodium chloride solution, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure, and the crude product was purified by silica column chromatography.

General procedure for the catalytic asymmetric intramolecular cyclization:

While reacting, the whole cells (OD600 of 40-50 in 76mL Kpi buffer) were degassed by sparging with argon in sealed 100mL two-neck bottle for at least 30 minutes. Diazo substrate (4mL of a DMSO stock) was injected into the whole cells under argon atmosphere. Final concentration of diazo substrate was 5mM, and final reaction volume was 80mL. The bottle was sealed by vaseline, and shaken at 25-30℃ and 200 rpm for 24 h. The reaction solution was centrifuged, and the supernatant was extracted with 30 mL of ethyl acetate three times, the organic phases were combined, and the organic phases were washed with saturated sodium chloride solution and dried with anhydrous sodium sulfate. After filtration, the solvent was removed under reduced pressure and the crude product was purified by silica column chromatography.

Tables and Figures

Table S1. Intramolecular C-H activation of precursor 1a with hemin and various hemoproteins.



1a 2a

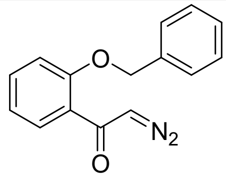
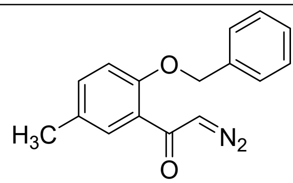
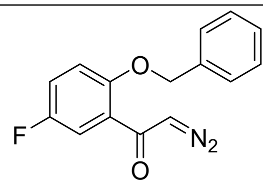
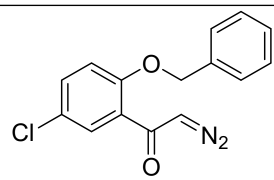
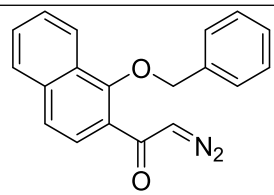
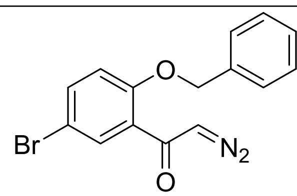
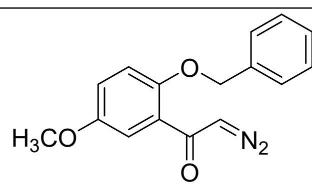
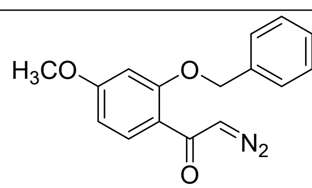
Catalyst	Note	Yield [%]
Hemin	Concentration: 0.5mM	11.79%
Hemin + sodium dithionite	0.5mM hemin + 10mM sodium dithionite	5.11%
ParPgb-HYA-5213[(a)]	Variant from *Pyrobaculum arsenaticam* protoglobin	trace
$P411_{CHA}$[(b)]	Variant from $P450_{BM3}$	5.61%
$P411_{CHF}$[(c)]	Variant from $P450_{BM3}$	8.45%
$P411_{HF}$[(d)]	Variant from $P450_{BM3}$	6.69%
Myoglobin H64V/V68A[(e)]	Variant from myoglobin	18.22%
Myoglobin H64V/V68A pure enzyme	Concentration: 20µM	trace
Myoglobin H64V/V68A pure enzyme + sodium dithionite	20µM pure enzyme + 10mM sodium dithionite	9.46%

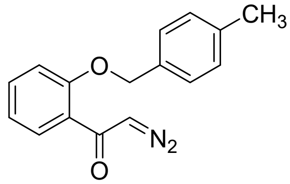
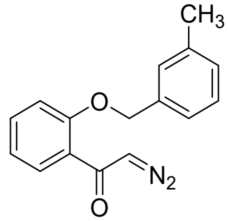
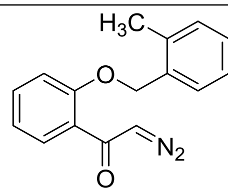
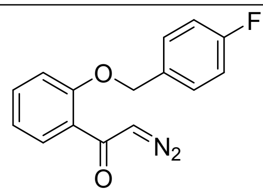
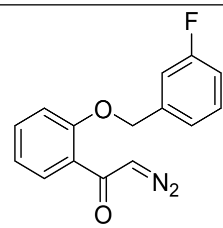
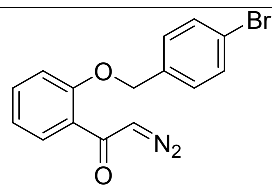
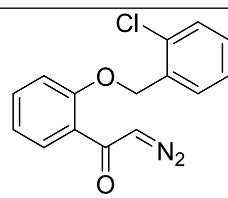
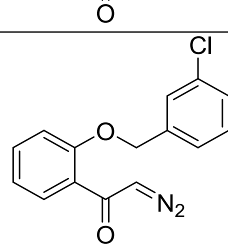
Trace=less than 0.1% yield.
All reactions except heme and pure enzyme were carried out under whole-cell conditions at OD_{600}=40. Results are the average of duplicate reactions.
(a) Variants identified for nitrene C-H insertion.[1]
(b) Variants identified for nitrene C-H insertion.[2]
(c) Variants identified for carbene C-H insertion.[3]
(d) Variants identified for functionalization of indoles.[4]
(e) Created for C-H functionalization of unprotected indoles.[5]

Table S2. Summary of optimization data for conversion of substrate 1a to flavanones 2a using Mn-triple mutant.

Optimization of OD_{600}						
Reaction buffer	Cosolvent	Substrate load [mM]	OD_{600}	Temperature	yield[%]	TTN
M9-N (pH=7.4)	DMSO(5%)	5mM	10	30℃	31.70%	191
M9-N (pH=7.4)	DMSO(5%)	5mM	20	30℃	83.95%	506
M9-N (pH=7.4)	DMSO(5%)	5mM	30	30℃	92.21%	555
M9-N (pH=7.4)	DMSO(5%)	5mM	40	30℃	93.04%	560
M9-N (pH=7.4)	DMSO(5%)	5mM	50	30℃	90.07%	542
M9-N (pH=7.4)	DMSO(5%)	5mM	60	30℃	84.83%	511
M9-N (pH=7.4)	DMSO(5%)	5mM	70	30℃	86.72%	522
Optimization of reaction buffer						
Reaction buffer	Cosolvent	Substrate load [mM]	OD_{600}	Temperature	yield[%]	TTN
PBS (pH=7.4)	DMSO(5%)	5mM	40	30℃	76.50%	461
Hepes (pH=7.4)	DMSO(5%)	5mM	40	30℃	60.23%	363
Optimization of temperature						
Reaction buffer	Cosolvent	Substrate load [mM]	OD_{600}	Temperature	yield[%]	TTN
M9-N (pH=7.4)	DMSO(5%)	5mM	40	20℃	96.17%	579
M9-N (pH=7.4)	DMSO(5%)	5mM	40	25℃	99.97%	602
M9-N (pH=7.4)	DMSO(5%)	5mM	40	30℃	91.80%	553
M9-N (pH=7.4)	DMSO(5%)	5mM	40	35℃	95.81%	577

Table S3. Structures of diazo substrates and NMR characterizations.

Compound name	Structure	NMR characterizations
(Benzyloxy)phenyl)-2-diazoethan-1-one (1a)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.72 (s, 1H), 7.53-7.32 (m, 6H), 7.21-7.23 (m, 1H), 7.07-7.03 (m, 1H), 6.63 (brs, 1H), 5.26 (s, 2H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 185.4, 157.3, 137.0, 133.9, 129.9, 129.0, 128.5, 128.1, 126.8, 121.2, 114.2, 70.4, 58.2 ppm.
1-(2-(Benzyloxy)-5-methylphenyl)-2-diazoethan-1-one (1b)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.54-7.50 (m, 2H), 7.43-7.26 (m, 5H), 7.12-7.09 (m, 1H), 6.64 (brs, 1H), 5.41 (s, 2H), 5.41 (s, 3H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 185.4, 155.4, 137.1, 134.2, 130.1, 130.0, 129.0, 128.4, 128.1, 126.5, 114.2, 70.5, 58.1, 20.3 ppm.
1-(2-(Benzyloxy)-5-fluorophenyl)-2-diazoethan-1-one (1c)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.51-7.33 (m, 7H), 7.26-7.23 (m, 1H), 6.68 (brs, 1H), 5.24 (s, 2H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 183.9, 157.8, 155.4, 153.7, 136.8, 129.1, 128.5, 128.2, 120.3, 120.1, 116.3, 116.2, 115.9, 115.7, 71.2, 58.8 ppm. **^{19}F NMR** (282 MHz, DMSO-d_6): δ −122.9 ppm.
1-(2-(Benzyloxy)-5-chlorophenyl)-2-diazoethan-1-one (1d)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.66 (s, 1H), 7.53-7.48 (m, 3H), 7.42-7.32 (m, 3H), 7.27-7.24 (m, 1H), 6.66 (brs, 1H), 5.26 (s, 2H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 183.8, 156.0, 136.6, 133.2, 129.1, 129.0, 128.6, 128.1, 125.3, 116.4, 70.9, 58.8 ppm.
1-(1-(Benzyloxy)naphthalen-2-yl)-2-diazoethan-1-one (1e)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.12-8.10 (m, 1H), 7.95-7.92 (m, 1H), 7.75-7.70 (m, 2H), 7.61-7.53 (m, 2H),7.50-7.49 (m, 2H), 7.40-7.33 (m, 3H),6.60 (brs, 1H), 5.02 (s, 2H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 186.0, 154.8, 136.9, 136.5, 129.0, 128.8, 128.6, 128.1, 127.5, 127.0, 125.4, 124.7, 123.4, 78.4, 58.4 ppm.
1-(2-(Benzyloxy)-5-bromophenyl)-2-diazoethan-1-one (1f)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.78 (s, 1H), 7.67-7.64 (m, 1H), 7.50-7.48 (m, 2H), 7.43-7.39 (m, 2H), 7.36-7.32 (m, 1H), 7.22-7.20 (m, 1H), 6.66 (brs, 1H), 5.26 (s, 2H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 183.8, 156.4, 136.6, 136.1, 132.0, 129.1, 128.6, 128.1, 116.8, 112.9, 70.8, 58.8 ppm.
1-(2-(Benzyloxy)-5-methoxyphenyl)-2-diazoethan-1-one (1g)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.51-7.49 (m, 2H), 7.43-7.39 (m, 2H), 7.36-7.32 (m, 1H), 7.25 (s, 1H), 7.18-7.16 (m, 1H), 7.09-7.06 (m, 1H),6.68 (brs, 1H), 5.20 (s, 2H), 3.73 (s, 3H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 184.9, 153.6, 151.4, 137.2, 129.0, 128.4, 128.1, 127.3, 119.6, 116.1, 114.0, 71.1, 58.4, 56.0 ppm.
1-(2-(Benzyloxy)-4-methoxyphenyl)-2-diazoethan-1-one (1h)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.76 (d, J= 8Hz, 1H), 7.54-7.52 (m, 2H), 7.44-7.41 (m, 2H), 7.37-7.35 (m, 1H), 6.74-6.73 (d, J= 4Hz, 1H), 6.66-6.63 (m, 1H),6.60 (brs, 1H), 5.28 (s, 2H), 3.81 (s, 3H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 164.1, 159.1, 151.4, 136.8, 131.8, 129.1, 128.5, 128.2, 127.7, 119.5, 106.6, 100.34, 70.5, 57.6, 56.0 ppm.

2-Diazo-1-(2-((4-methylbenzyl)oxy)phenyl)ethan-1-one (1i)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.72 (s, 1H), 7.51-7.46 (m, 1H), 7.41-7.39 (m, 2H), 7.23-7.21 (m, 3H), 7.06-7.02 (m, 1H), 6.62 (brs, 1H), 5.21 (s, 2H), 2.31 (s, 3H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 185.3, 157.3, 137.8, 133.9, 129.9, 129.6, 128.3, 126.7, 121.2, 114.2, 70.4, 58.2, 21.2 ppm.
2-Diazo-1-(2-((3-methylbenzyl)oxy)phenyl)ethan-1-one (1j)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.71 (s, 1H), 7.51-7.47 (m, 1H), 7.32-7.30 (m, 3H), 7.22-7.20 (m, 1H), 7.17-7.15 (m, 1H), 7.07-7.03 (m, 1H), 6.63 (brs, 1H), 5.22 (s, 2H), 2.33 (s, 3H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 185.4, 157.3, 138.2, 136.9, 133.9, 129.9, 129.1, 129.0, 128.6, 126.8, 125.2, 121.2, 114.2, 70.5, 58.3, 21.5 ppm.
2-Diazo-1-(2-((2-methylbenzyl)oxy)phenyl)ethan-1-one (1k)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.71 (s, 1H), 7.54-7.46 (m, 2H), 7.29-7.20 (m, 4H), 7.09-7.05 (m, 1H), 6.50 (brs, 1H), 5.23 (s, 2H), 2.36 (s, 3H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 185.5, 157.3, 136.9, 134.9, 133.9, 130.7, 129.8, 128.8, 128.6, 126.9, 126.4, 121.2, 114.2, 69.2, 58.0, 19.0 ppm.
2-Diazo-1-(2-((4-fluorobenzyl)oxy)phenyl)ethan-1-one (1l)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.72 (d, *J*=4.0 Hz, 1H), 7.59-7.48 (m, 3H), 7.27-7.21 (m, 3H), 7.08-7.04 (m, 1H), 6.61 (brs, 1H), 5.24 (s, 2H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 185.4, 163.5, 161.1, 157.1, 133.9, 133.2, 133.1, 130.5, 130.4, 129.9, 126.8, 121.3, 116.0, 115.8, 114.2, 69.7, 58.2 ppm. **^{19}F NMR** (282 MHz, DMSO-d_6): δ −114.2 ppm.
2-Diazo-1-(2-((3-fluorobenzyl)oxy)phenyl)ethan-1-one (1m)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.51-7.33 (m, 7H), 7.27-7.23 (m, 1H), 6.68 (brs, 1H), 5.24 (s, 2H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 183.9, 157.8, 155.4, 153.7, 136.8, 129.1, 128.5, 128.2, 120.3, 120.1, 116.3, 116.2, 116.0, 115.7, 71.1, 58.8 ppm. **^{19}F NMR** (282 MHz, DMSO-d_6): δ −122.9 ppm.
1-(2-((4-Bromobenzyl)oxy)phenyl)-2-diazoethan-1-one (1n)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.71 (s, 1H), 7.62-7.59 (m, 2H), 7.51-7.47 (m, 3H), 7.21-7.19 (m, 1H), 7.08-7.04 (m, 1H), 6.62 (brs, 1H), 5.24 (s, 2H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 185.3, 157.0, 136.4, 133.9, 132.0, 130.3, 129.9, 126.8, 121.6, 121.3, 114.1, 69.6, 58.3 ppm.
1-(2-((2-Chlorobenzyl)oxy)phenyl)-2-diazoethan-1-one (1o)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.70-7.64 (m, 2H), 7.55-7.50 (m, 2H), 7.43-7.39 (m, 2H), 7.25-7.23 (m, 1H), 7.11-7.07 (m, 1H), 6.56 (brs, 1H), 5.24 (s, 2H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 185.4, 157.0, 134.2, 133.9, 132.9, 130.6, 130.5, 130.0, 128.0, 126.9, 121.6, 114.1, 68.1, 58.1 ppm.
1-(2-((3-Chlorobenzyl)oxy)phenyl)-2-diazoethan-1-one (1p)		**^{1}H NMR** (400 MHz, DMSO-d_6): δ 7.70 (s, 1H), 7.59 (s, 1H), 7.52-7.39 (m, 4H), 7.21-7.19 (m, 1H), 7.08-7.05 (m, 1H), 6.65 (brs, 1H), 5.24 (s, 2H) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ 185.5, 156.9, 139.6, 133.8, 133.7, 131.0, 129.9, 128.4, 127.8, 127.0, 126.6, 121.4, 114.2, 69.5, 58.2 ppm.

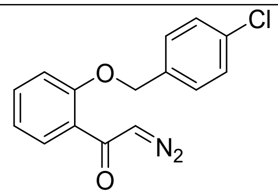
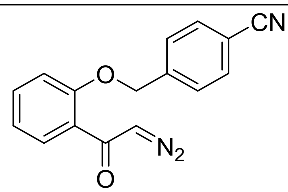
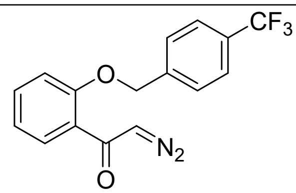
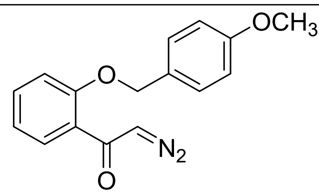
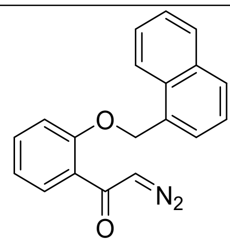
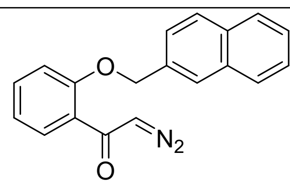
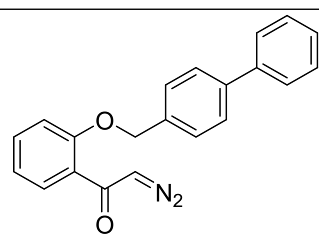
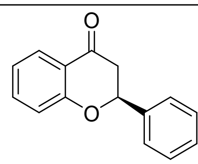
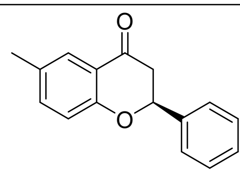
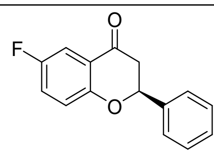
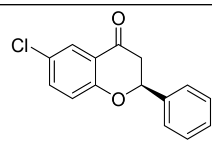
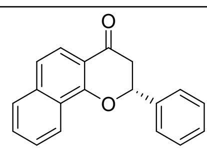
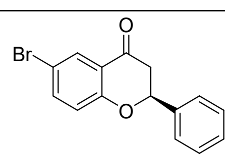
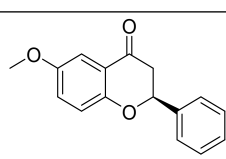
1-(2-((4-Chlorobenzyl)oxy)phenyl)-2-diazoethan-1-one (1q)		**¹H NMR** (400 MHz, DMSO-d_6): δ 7.70 (s, 1H), 7.56-7.47 (m, 5H), 7.22-7.20 (m, 1H), 7.08-7.04 (m, 1H), 6.62 (brs, 1H), 5.25 (s, 2H) ppm. **¹³C NMR** (101 MHz, DMSO-d_6): δ 185.4, 157.1, 136.0, 133.9, 133.1, 130.0, 129.9, 129.0, 126.8, 121.4, 114.2, 69.6, 58.3 ppm.
4-((2-(2-Diazoacetyl)phenoxy)methyl)benzonitrile (1r)		**¹H NMR** (400 MHz, DMSO-d_6): δ 7.90-7.88 (m, 2H), 7.72-7.70 (m, 3H), 7.52-7.48 (m, 1H), 7.20-7.18 (m, 1H), 7.09-7.05 (m, 1H), 6.56 (brs, 1H), 5.37 (s, 2H) ppm. **¹³C NMR** (101 MHz, DMSO-d_6): δ 185.2, 158.3, 142.8, 133.8, 133.0, 129.9, 128.6, 127.0, 121.5, 119.2, 114.1, 111.1, 69.5, 58.3 ppm.
2-Diazo-1-(2-((4-(trifluoromethyl)benzyl)oxy)phenyl)ethan-1-one (1s)		**¹H NMR** (400 MHz, DMSO-d_6): δ 7.79-7.73 (m, 5H), 7.52-7.48 (m, 1H), 7.22-7.20 (m, 1H), 7.09-7.05 (m, 1H), 6.66 (brs, 1H), 5.38 (s, 2H) ppm. **¹³C NMR** (101 MHz, DMSO-d_6): δ 185.4, 156.9, 141.9, 133.8, 129.9, 128.9 (d, JCF = 32.3 Hz), 128.5, 127.0, 125.9 (d, JCF = 3.4 Hz), 123.3, 121.4, 114.1, 69.5, 58.3 ppm.
2-Diazo-1-(2-((4-methoxybenzyl)oxy)phenyl)ethan-1-one (1t)		**¹H NMR** (400 MHz, DMSO-d_6): δ 7.72 (s, 1H), 7.55 – 7.35 (m, 3H), 7.24 (d, J = 8.0 Hz, 1H), 7.04 (td, J = 7.7, 0.8 Hz, 1H), 7.00 – 6.94 (m, 2H), 6.61 (s, 1H), 5.17 (s, 2H), 3.76 (s, 3H) ppm. **¹³C NMR** (101 MHz, DMSO-d_6): δ 185.3, 159.6, 157.4, 133.9, 130.0, 129.9, 128.7, 126.7, 121.1, 114.4, 114.3, 70.3, 58.2, 55.5 ppm.
2-Diazo-1-(2-(naphthalen-1-ylmethoxy)phenyl)ethan-1-one (1u)		**¹H NMR** (400 MHz, DMSO-d_6): δ 8.18 (d, J = 8.0 Hz, 1H), 8.01-7.94 (m, 2H), 7.74-7.52 (m, 6H), 7.45-7.42 (m, 1H), 7.10-7.06 (m, 1H), 6.48 (brs, 1H), 5.72 (s, 2H) ppm. **¹³C NMR** (101 MHz, DMSO-d_6): δ 185.4, 157.3, 133.9, 133.8, 132.4, 131.4, 129.9, 129.3, 129.0, 127.1, 127.0, 126.5, 125.9, 124.3, 121.4, 114.5, 69.1, 58.0 ppm.
2-Diazo-1-(2-(naphthalen-2-ylmethoxy)phenyl)ethan-1-one (1v)		**¹H NMR** (400 MHz, DMSO-d_6): δ 8.02 (s, 1H), 7.97-7.91 (m, 3H), 7.66-7.53 (m, 2H), 7.52-7.48 (m, 3H), 7.28-7.26 (m, 1H), 7.06-7.04 (m, 1H), 6.67 (brs, 1H), 5.41 (s, 2H) ppm. **¹³C NMR** (101 MHz, DMSO-d_6): δ 185.4, 157.3, 134.6, 133.3, 133.1, 129.9, 128.8, 128.3, 128.1, 126.9, 126.8, 126.7, 126.1, 121.3, 114.3, 70.6, 58.4 ppm.
1-(2-((1,1'-Biphenyl)-4-ylmethoxy)phenyl)-2-diazoethan-1-one (1w)		**¹H NMR** (400 MHz, DMSO-d_6): δ 7.77 – 7.65 (m, 5H), 7.61 (d, J = 8.1 Hz, 2H), 7.54 – 7.44 (m, 3H), 7.37 (t, J = 7.3 Hz, 1H), 7.26 (d, J = 8.3 Hz, 1H), 7.07 (dd, J = 11.1, 3.9 Hz, 1H), 6.68 (brs, 1H), 5.32 (s, 2H) ppm. **¹³C NMR** (101 MHz, DMSO-d_6): δ 185.4, 157.2, 140.3, 140.2, 136.2, 133.9, 129.9, 129.4, 128.7, 128.0, 127.3, 127.1, 126.8, 121.3, 114.2, 70.1, 58.3 ppm.

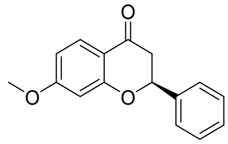
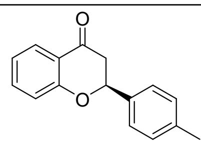
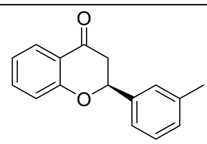
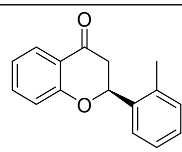
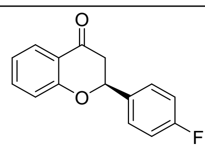
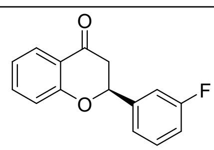
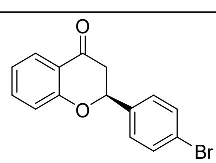
Table S4. Substrate scope data.

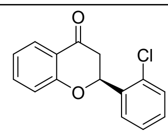
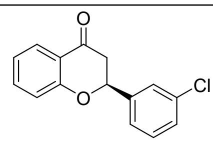
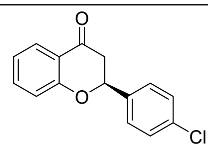
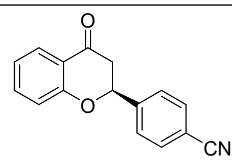
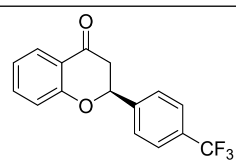
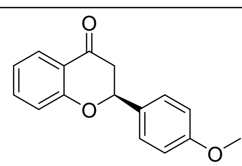
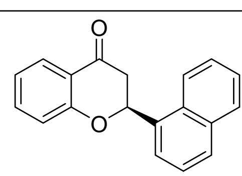
Product	Substrate load[mM]	OD_{600}	Temperature	ee% (S)	yield%	TTN
2b	5mM	40	25℃	93.8%	71.39%	430
2c	5mM	40	25℃	91.64%	80.76%	487
2d	5mM	50	30℃	87.25%	68.22%	411
2e	5mM	50	30℃	34.68% (R)	62.57%	377
2f	5mM	40	25℃	89.79%	75.02%	452
2g	5mM	40	25℃	86.74%	88.72%	534
2h	5mM	40	25℃	95.98%	81.25%	489
2i	5mM	40	25℃	80.43%	64.18%	387
2j	5mM	40	25℃	80.83%	80.66%	486
2k	5mM	40	25℃	51.15%	64.71%	390
2l	5mM	40	25℃	84.68%	86.82%	523
2m	5mM	40	25℃	90.06%	90.5%	545
2n	5mM	50	25℃	92.25%	43.58%	263
2o	5mM	50	30℃	83.9%	59.51%	358
2p	5mM	50	30℃	88.31%	40.62%	245
2q	5mM	50	30℃	25.43%	13.17%	79
2r	5mM	40	30℃	99.40%	89.18%	537
2s	5mM	40	30℃	90.26%	88.17%	531
2t	5mM	40	30℃	96.92%	35.91%	216
2u	5mM	40	30℃	>99%	23.10%	139
2v	5mM	40	30℃	>99%	27.43%	165
2w	5mM	40	30℃	>99%	12.72%	77

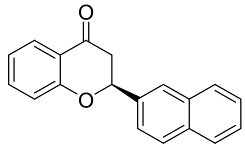
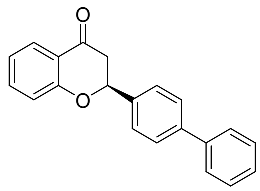
Analytical reactions were performed with 0.25 mL of substrates 1b–1q, in a 5 mL reaction system. Mn-triple mutant was used in whole *E. coli* cells resuspended typically to OD_{600} = 40 or 50 in M9-N (pH=7.4). The temperature and OD_{600} used for the analytical reactions were determined after some optimization. Reactions proceeded at specified temperatures for 24 hours. Reported numbers were the average of triplicate reactions.

Table S5. Structure and NMR characterizations of products.

Compound name	Structure	NMR characterizations
(*S*)-2-phenylchroman-4-one（2a）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=7.97（dd，1H），δ=7.47-7.53（m，6H），δ=7.07-7.11（m，2H），δ=5.52（dd，1H），δ=3.13（dd，1H），δ=2.93（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 191.98, 161.58, 138.77, 136.22, 128.88, 128.81, 127.08, 126.17, 121.65, 120.97, 118.16, 79.63, 44.17 ppm.
(*S*)-6-Methyl-2-phenylchroman-4-one（2b）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=7.76（d，1H），δ=7.51（dd，2H），δ=7.40-7.48（m，3H），δ=7.34（dd，H），δ=6.99（d，1H），δ=5.47（dd，1H），δ=3.09（dd，1H），δ=2.90（dd，1H），δ=2.35（s，3H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 192.21, 159.66, 138.92, 137.29, 131.08, 128.84, 128.73, 126.63, 126.17, 120.57, 117.93, 79.58, 44.73, 20.45 ppm.
(*S*)-6-Fluoro-2-phenylchroman-4-one（2c）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=7.61（dd，1H），δ=7.42-7.51（m，5H），δ=7.24-7.28（m，1H），δ=7.07（dd，1H），δ=5.49（dd，1H），δ=3.11（s，3H），δ=2.94（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 191.23, 158.59, 157.80, 156.18, 138.44, 128.91, 126.15, 123.86, 123.62, 119.88, 119.81, 112.16, 111.93, 79.86, 44.37 ppm.
(*S*)-6-Chloro-2-phenylchroman-4-one（2d）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=7.92（d，1H），δ=7.40-7.51（m，6H），δ=7.07（d，1H），δ=5.50（dd，1H），δ=3.11（s，3H），δ=2.94（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 190.82, 159.98, 157.80, 138.27, 136.04, 128.98, 128.93, 127.22, 126.41, 126.16, 121.73, 119.90, 79.84, 44.29 ppm.
(*R*)-2-Phenyl-2,3-dihydro-4H-benzo[*h*]chromen-4-one（2e）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=8.38（d，1H），δ=7.94（d，1H），δ=7.82（d，1H），δ=7.60-7.65（m，3H），δ=7.46-7.54（m，5H），δ=5.61（dd，1H），δ=3.21（dd，1H），δ=3.03（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 191.64, 159.85, 138.84, 137.62, 129.73, 128.91, 128.81, 127.89, 126.31, 126.15, 124.89, 123.70, 121.75, 121.28, 115.53, 80.35, 44.08 ppm.
(*S*)-6-Bromo-2-phenylchroman-4-one（2f）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=8.06（d，1H），δ=7.61（dd，1H），δ=7.41-7.49（m，5H），δ=6.99（d，1H），δ=5.50（dd，1H），δ=3.11（s，3H），δ=2.94（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 190.67, 160.42, 138.82, 138.24, 129.53, 128.99, 128.94, 126.17, 122.21, 120.25, 114.37, 79.80, 44.23 ppm.
(*S*)-6-Methoxy-2-phenylchroman-4-one（2g）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=7.38-7.52（m，6H），δ=7.15（dd，1H），δ=7.02（d，1H），δ=5.47（dd，1H），δ=3.85（s，3H），δ=3.10（dd，1H），δ=2.91（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 192.11, 156.31, 154.27, 138.87, 128.85, 128.76, 126.16, 125.43, 120.78, 119.47,

		107.38, 79.73, 55.84, 44.60 ppm.
(*S*)-7-Methoxy-2-phenylchroman-4-one（2h）		**¹H NMR** (400 MHz, $CDCl_3$): δ=7.90（d，1H），δ=7.47-7.52（m，5H），δ=6.65（dd，1H），δ=6.54（d，1H），δ=5.50（dd，1H），δ=3.86（s，3H），δ=3.07（dd，1H），δ=2.87（dd，1H）ppm. **¹³C NMR** (101 MHz, $CDCl_3$): δ 190.61, 166.23, 163.56, 138.82, 128.87, 128.79, 126.18, 114.86, 110.30, 100.94, 80.04, 55.68, 44.36 ppm.
(*S*)-2-(p-Tolyl)chroman-4-one（2i）		**¹H NMR** (400 MHz, $CDCl_3$): δ=7.96（dd，1H），δ=7.51-7.53（m，1H），δ=7.40（d，2H），δ=7.27（d，2H），δ=7.06-7.09（m，2H），δ=5.48（dd，1H），δ=3.13（dd，1H），δ=2.91（dd，1H），δ=2.41（t，3H）ppm. **¹³C NMR** (101 MHz, $CDCl_3$): δ 192.22, 161.66, 138.74, 136.19, 135.76, 129.52, 127.06, 126.22, 121.55, 120.94, 118.17, 79.56, 44.58, 21.23 ppm.
(*S*)-2-(m-Tolyl)chroman-4-one（2j）		**¹H NMR** (400 MHz, $CDCl_3$): δ=7.95-7.98（m，1H），δ=7.54（td，1H），δ=7.31-7.36（m，3H），δ=7.26（d，1H），δ=7.07-7.10（m，2H），δ=5.48（dd，1H），δ=3.13（dd，1H），δ=2.91（dd，1H），δ=2.43（s，3H）ppm. **¹³C NMR** (101 MHz, $CDCl_3$): δ 192.12, 161.63, 138.66, 136.20, 129.58, 128.78, 127.07, 126.88, 123.27, 121.60, 120.95, 118.16, 79.75, 44.71, 21.50 ppm.
(*S*)-2-(o-Tolyl)chroman-4-one（2k）		**¹H NMR** (400 MHz, $CDCl_3$): δ=7.99（dd，1H），δ=7.62-7.64（m，1H），δ=7.54（td，3H），δ=7.27-7.34（m，3H），δ=7.07-7.10（m，2H），δ=5.72（dd，1H），δ=3.11（dd，1H），δ=2.87（dd，1H），δ=2.42（s，3H）ppm. **¹³C NMR** (101 MHz, $CDCl_3$): δ 192.30, 161.90, 136.76, 136.20, 135.13, 130.86, 128.65, 127.14, 126.58, 125.77, 121.65, 120.94, 118.13, 76.83, 43.62, 19.05 ppm.
(*S*)-2-(4-Fluorophenyl)chroman-4-one（2l）		**¹H NMR** (400 MHz, $CDCl_3$): δ=7.96（dd，1H），δ=7.48-7.52（m，3H），δ=7.06-7.17（m，4H），δ=5.49（dd，1H），δ=3.09（dd，1H），δ=2.91（dd，1H）ppm. **¹³C NMR** (101 MHz, $CDCl_3$): δ 191.72, 161.41, 138.50, 136.29, 134.64, 134.61, 128.10, 128.01, 127.11, 121.78, 120.91, 118.11, 115.94, 115.72, 78.95, 44.69 ppm.
(*S*)-2-(3-Fluorophenyl)chroman-4-one（2m）		**¹H NMR** (400 MHz, $CDCl_3$): δ=7.96（dd，1H），δ=7.54-7.56（m，1H），δ=7.42-7.44（m，1H），δ=7.25-7.28（m，2H），δ=7.08-7.12（m，3H），δ=5.52（dd，1H），δ=3.07（dd，1H），δ=2.94（dd，1H）ppm. **¹³C NMR** (101 MHz, $CDCl_3$): δ 191.44, 164.24, 161.26, 141.34, 141.27, 136.34, 130.54, 130.46, 127.11, 121.87, 121.62, 120.93, 118.12, 115.75, 115.54, 113.33, 113.11, 78.76, 44.67 ppm.
(*S*)-2-(4-Bromophenyl)chroman-4-one（2n）		**¹H NMR** (400 MHz, $CDCl_3$): δ=7.96（dd，1H），δ=7.53-7.61（m，3H），δ=7.39（d，2H），δ=7.07-7.12（m，2H），δ=5.48（dd，1H），δ=3.07（dd，1H），δ=2.92（dd，1H）ppm. **¹³C NMR** (101 MHz, $CDCl_3$): δ 191.49, 161.30, 137.81,

		136.32, 132.03, 127.81, 127.11, 122.74, 122.25, 121.85, 118.11, 78.87, 44.59 ppm.
(*S*)-2-(2-Chlorophenyl)chroman-4-one（2o）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=7.99（dd，1H），δ=7.79（dd，1H），δ=7.56（td，1H），δ=7.42-7.45（m，2H），δ=7.35-7.37（m，1H），δ=7.09-7.13（m，2H），δ=5.91（dd，1H），δ=3.07（dd，1H），δ=2.92（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 191.58, 161.58, 136.78, 136.23, 131.68, 129.76, 129.63, 127.47, 127.25, 127.20, 121.87, 120.99, 118.11, 76.54, 43.54 ppm.
(*S*)-2-(3-Chlorophenyl)chroman-4-one（2p）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=7.96（d，1H），δ=7.54-7.58（m，2H），δ=7.35-7.42（m，3H），δ=7.08-7.12（m，2H），δ=5.49（dd，1H），δ=3.07（dd，1H），δ=2.93（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 191.40, 161.25, 140.79, 136.35, 134.85, 130.17, 128.89, 127.13, 126.36, 124.16, 121.90, 120.91, 118.12, 78.78, 44.67 ppm.
(*S*)-2-(4-Chlorophenyl)chroman-4-one（2q）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=7.96（dd，1H），δ=7.55（ddd，1H），δ=7.44-7.45（m，4H），δ=7.07-7.11（m，2H），δ=5.50（dd，1H），δ=3.07（dd，1H），δ=2.91（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 191.55, 161.33, 137.29, 136.33, 134.62, 129.08, 127.53, 127.12, 121.84, 120.92, 118.11, 78.85, 44.62 ppm.
(S)-4-(4-Oxochroman-2-yl)benzonitrile（2r）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=7.96（dd，1H），δ=7.75-7.78（m，2H），δ=7.63-7.65（m，2H），δ=7.55-7.59（m，1H），δ=7.09-7.14（m，2H），δ=5.58（dd，1H），δ=3.04（dd，1H），δ=2.95（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 190.79, 160.96, 143.91, 136.49, 132.71, 127.18, 126.64, 122.15, 120.88, 118.38, 118.06, 112.58, 78.51, 44.55 ppm.
(S)-2-(4-(Trifluoromethyl)phenyl)chroman-4-one（2s）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=7.97（dd，1H），δ=7.72-7.74（m，2H），δ=7.64-7.66（m，2H），δ=7.51-7.56（m，1H），δ=7.09-7.13（m，2H），δ=5.58（dd，1H），δ=3.09（dd，1H），δ=2.96（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 191.18, 161.17, 142.71, 136.40, 127.15, 126.36, 125.89, 125.86, 121.99, 120.92, 118.10, 78.77, 44.67 ppm.
(S)-2-(4-Methoxyphenyl)chroman-4-one（2t）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=7.94-7.97（m，1H），δ=7.50-7.55（m，1H），δ=7.43-7.45（m，2H），δ=7.05-7.09（m，2H），δ=6.97-7.00（m，2H），δ=5.45（dd，1H），δ=3.86（s，3H），δ=3.13（dd，1H），δ=2.89（dd，1H）ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ 192.27, 161.65, 159.98, 136.18, 130.77, 127.75, 127.04, 121.54, 120.92, 118.15, 114.22, 79.36, 55.38, 44.62 ppm.
(S)-2-(Naphthalen-1-yl)chroman-4-one（2u）		**^{1}H NMR** (400 MHz, $CDCl_3$): δ=8.03-8.09（m，2H），δ=7.91-7.96（m，2H），δ=7.81（d，1H），δ=7.54-7.61（m，4H），δ=7.11-7.15（m，2H），δ=6.26（dd，1H），δ=3.30（dd，1H），δ=3.13（dd，1H）ppm.

		¹³C NMR (101 MHz, $CDCl_3$): δ 192.27, 161.79, 136.25, 134.16, 133.89, 130.20, 129.41, 129.12, 127.20, 126.73, 126.00, 125.40, 123.89, 122.82, 121.79, 121.12, 118.24, 76.87, 43.99 ppm.
(S)-2-(Naphthalen-2-yl)chroman-4-one（2v）		**¹H NMR** (400 MHz, $CDCl_3$): δ=7.90-7.98（m，5H），δ=7.61-7.64（m，1H），δ=7.54-7.58（m，3H），δ=7.09-7.14（m，2H），δ=5.68（dd，1H），δ=3.22（dd，1H），δ=3.02（dd，1H）ppm. **¹³C NMR** (101 MHz, $CDCl_3$): δ 191.95, 161.57, 136.28, 136.07, 133.39, 133.18, 128.81, 127.79, 127.12, 126.58, 125.43, 123.68, 121.70, 121.01, 118.20, 78.85, 44.62 ppm.
(S)-2-([1,1'-biphenyl]-4-yl)chroman-4-one（2w）		**¹H NMR** (400 MHz, $CDCl_3$): δ=7.99（dd，1H），δ=7.69（d，2H），δ=7.60-7.65（m，5H），δ=7.55-7.58（m，2H），δ=7.40-7.51（m，1H），δ=7.10-7.12（m，2H），δ=5.57（dd，1H），δ=3.18（dd，1H），δ=2.98（dd，1H）ppm. **¹³C NMR** (101 MHz, $CDCl_3$): δ 192.00, 161.59, 141.84, 140.51, 137.65, 136.28, 128.89, 127.63, 127.19, 127.12, 126.69, 121.70, 120.99, 118.19, 78.85, 44.62 ppm.

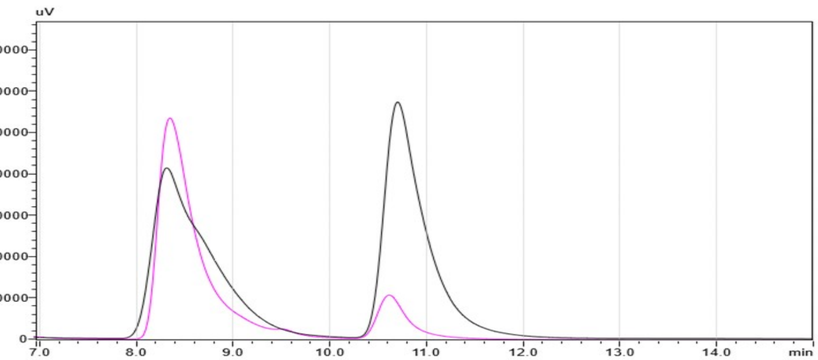


Figure S1. Determination of the enantioselectivity of Mn on substrate 1a. Black trace represents the chemically synthesized standards, while the pink trace represents the product from the enzymatic reaction. Myoglobin demonstrates *S*-type selectivity for the substrate, with an enantiomeric excess (ee) value of 72%.

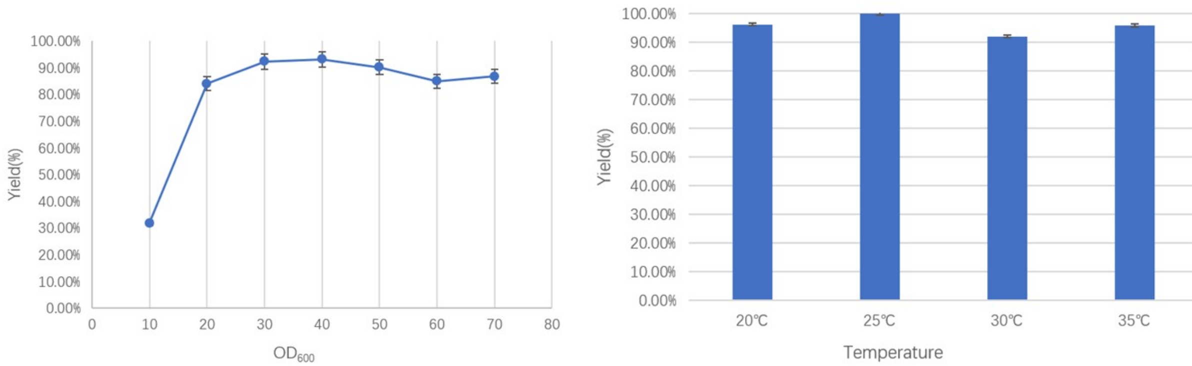
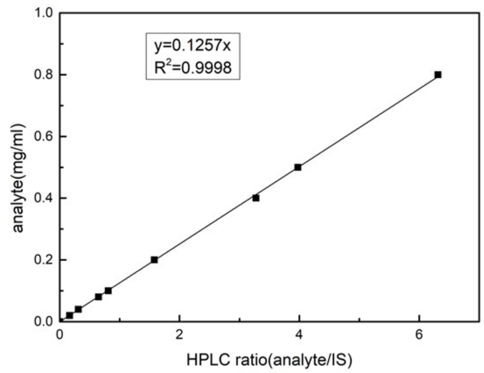
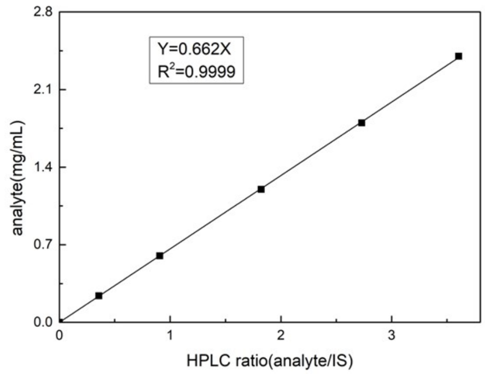


Figure S2. Effects of OD_{600} and temperature on the yield of the cyclization reaction catalyzed by Mn-triple mutant using 1a as substrate.

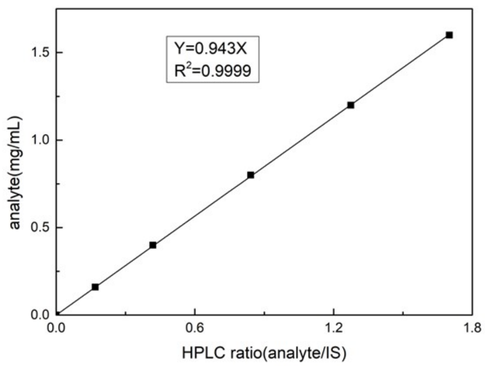
Flavanone 2a



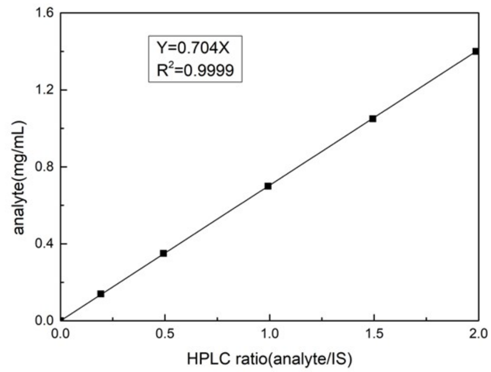
Flavanone 2b



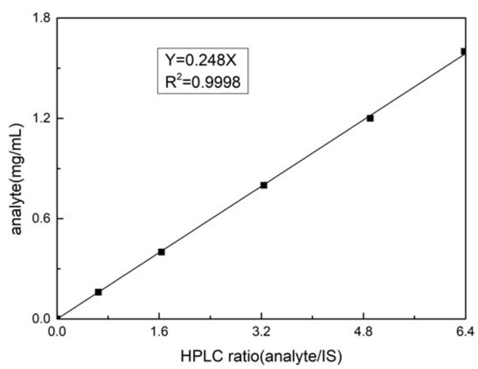
Flavanone 2c



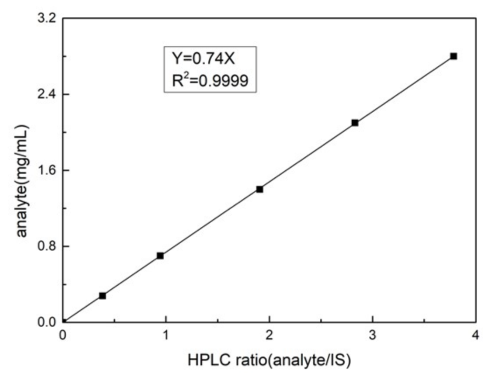
Flavanone 2d



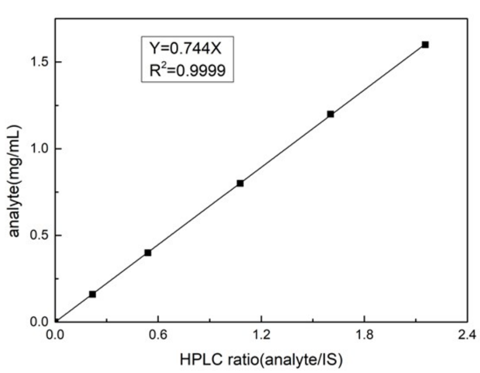
Flavanone 2e



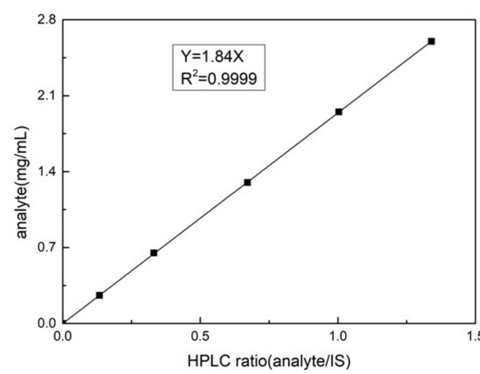
Flavanone 2f



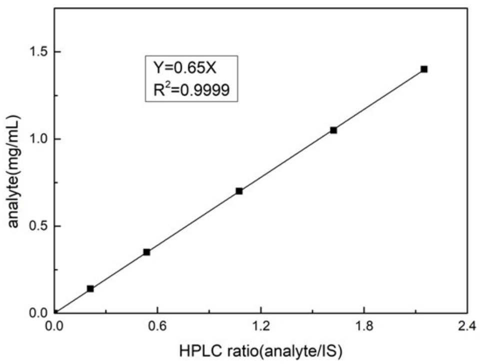
Flavanone 2g



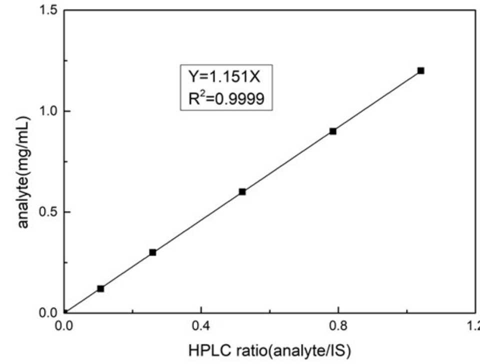
Flavanone 2h



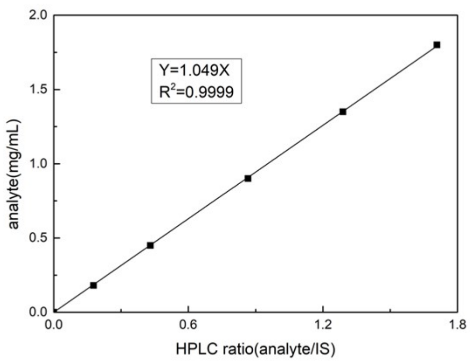
Flavanone 2i



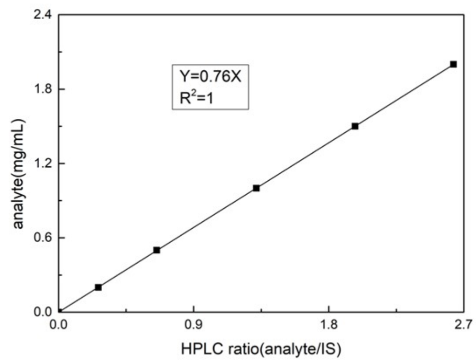
Flavanone 2j



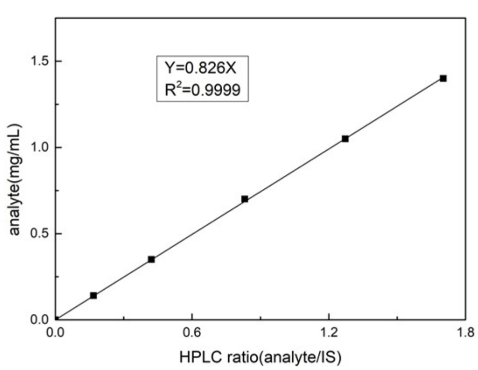
Flavanone 2k



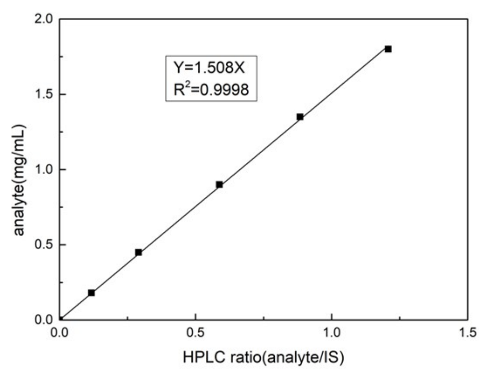
Flavanone 2l



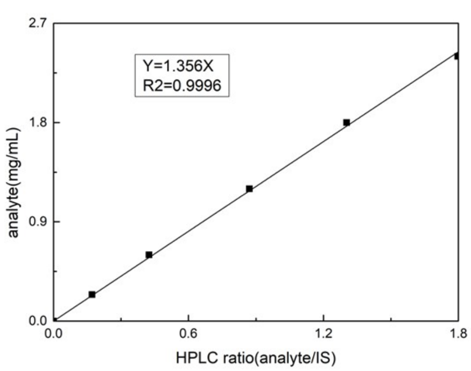
Flavanone 2m



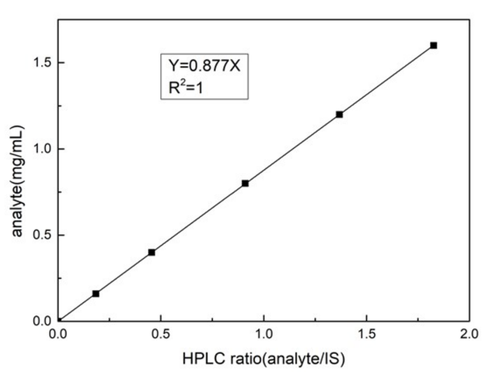
Flavanone 2n



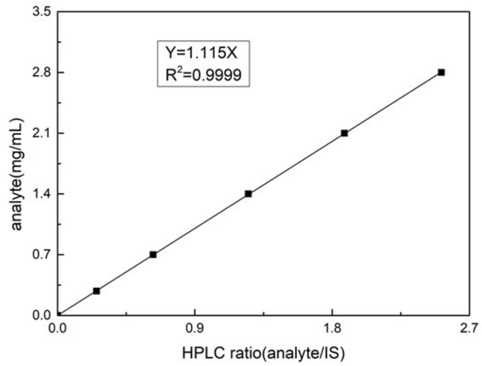
Flavanone 2o



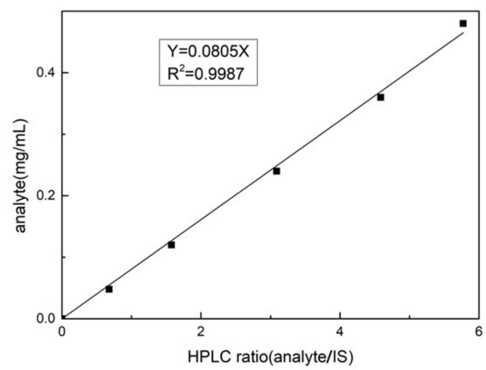
Flavanone 2p



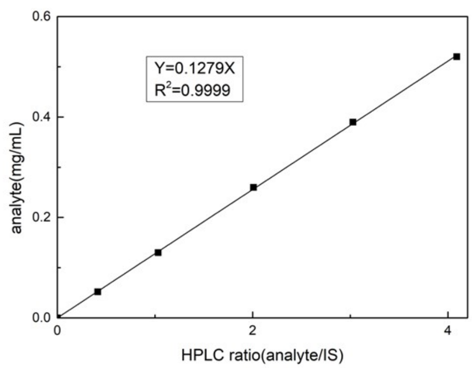
Flavanone 2q



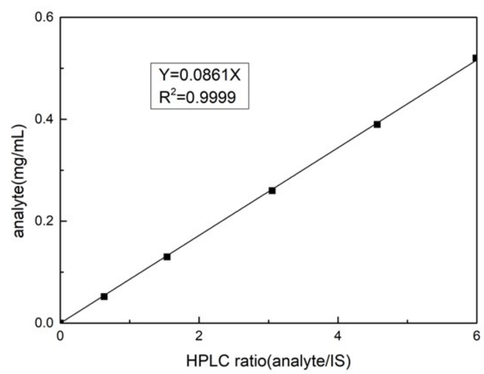
Flavanone 2r



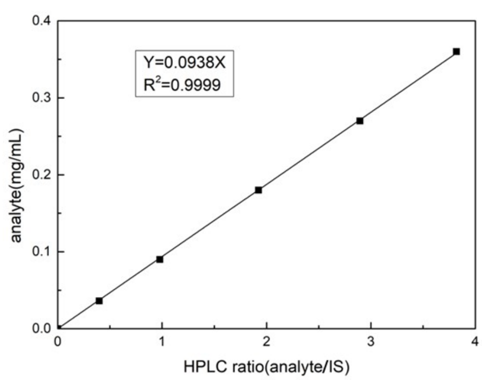
Flavanone 2s



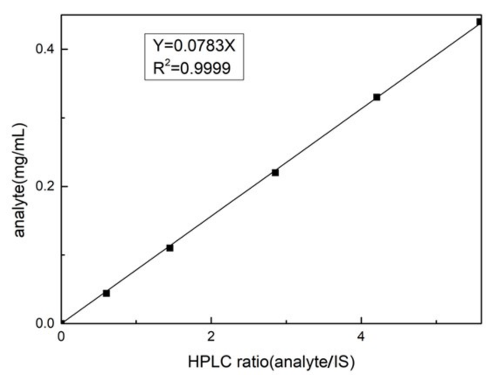
Flavanone 2t



Flavanone 2u



Flavanone 2v



Flavanone 2w

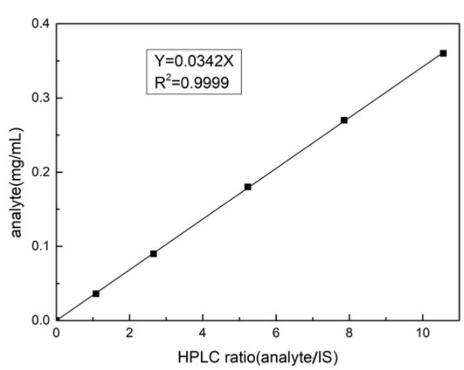
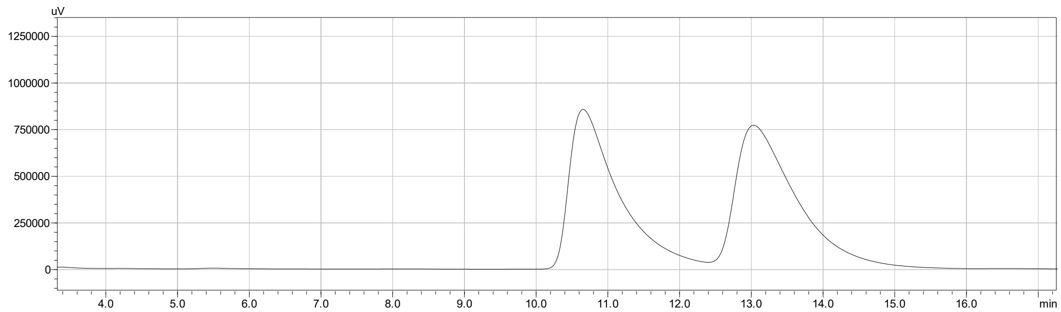
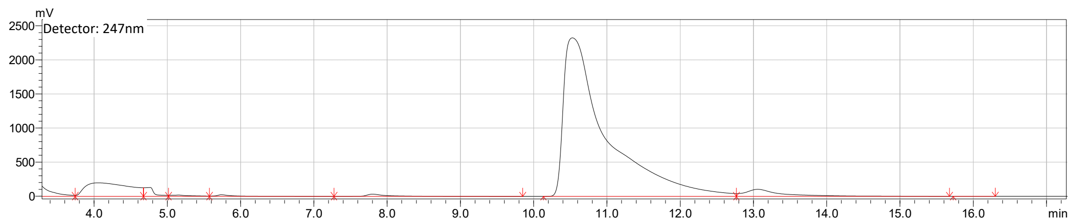


Figure S3. Calibration curves for flavanone products. Calibration curves with an internal standard were created for the determination of yield and TTN. The concentration of UV-active analytes was plotted as a function of the UV absorbance ratio of the analyte over an appropriate internal standard. Product standards analyzed based on absorbance at 247 nm were calibrated with 1-(4-ethylphenyl)ethanone (0.5mg/mL, final concentration) as the internal standard. Product formation in enzymatic reactions was quantified by HPLC based on standard curves. All data points represent the average of three replicate runs. For all analysis, water and acetonitrile containing 0.1% acetic acid were used as mobile phase for a C18 column. The method used 55% acetonitrile, using 10μL sample injections. The flow rate was 1mL/minute, and the column was maintained at 30 °C.

Rac 2a

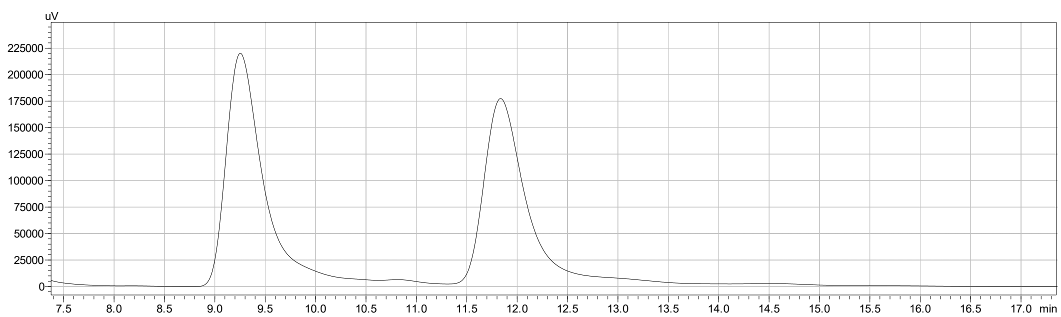


Flavanone 2a

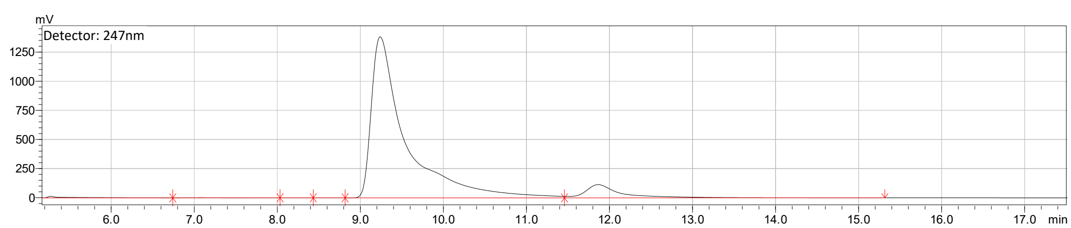


Hexanes : Isopropanol=0.97:0.03, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	10.543	62943626	98%
2	13.060	1280488	2%

Rac 2b

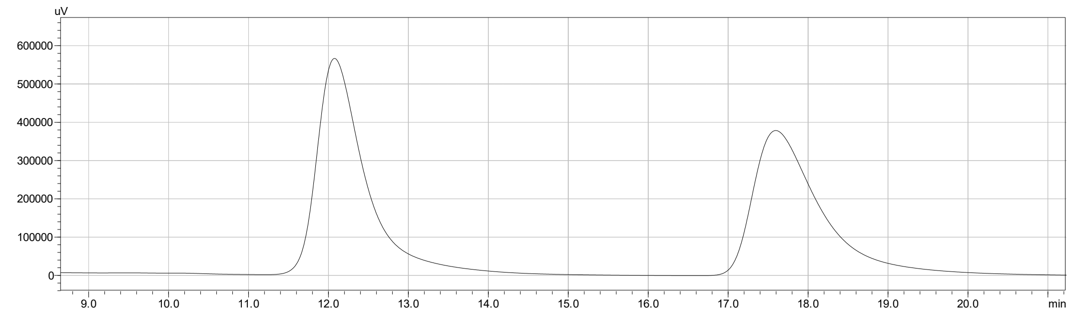


Flavanone 2b

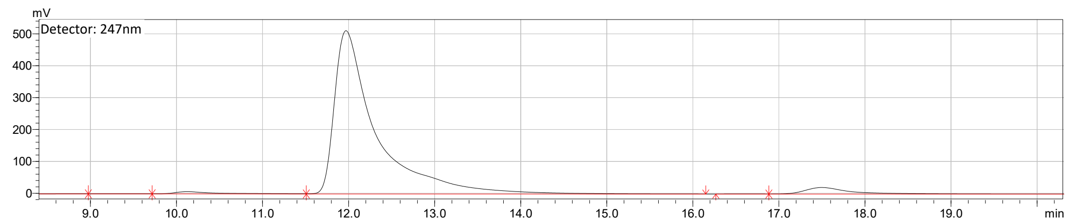


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	9.235	39532062	91.9%
2	11.860	3484320	8.1%

Rac 2c

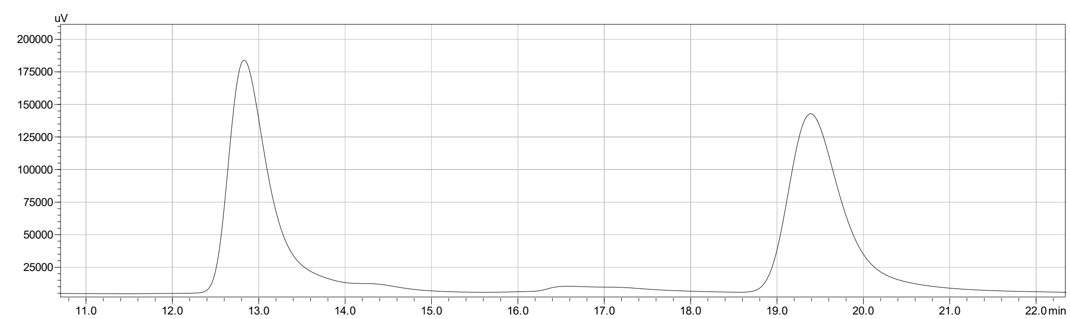


Flavanone 2c

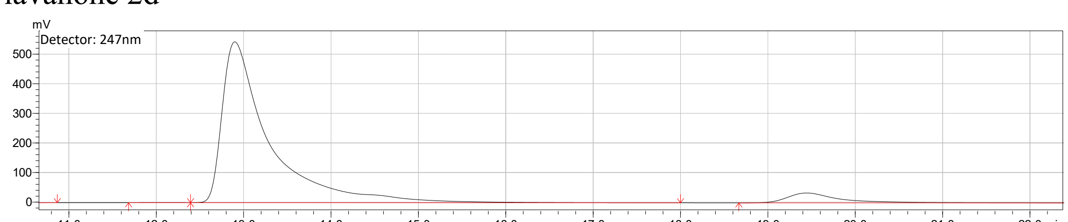


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	11.965	17192281	95.8%
2	17.494	749573	4.2%

Rac 2d

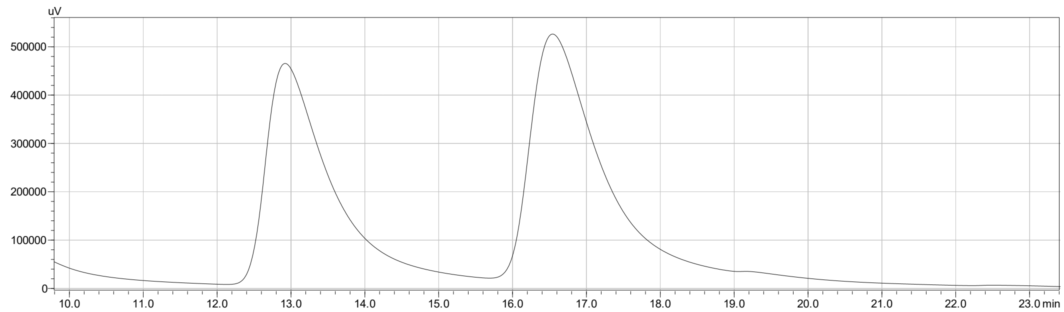


Flavanone 2d

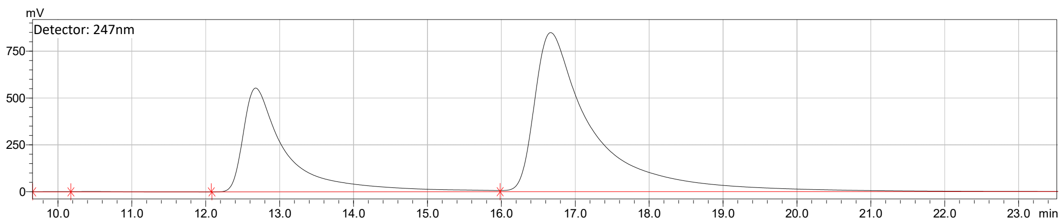


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	12.894	20353799	93.6%
2	19.439	1385920	6.4%

Rac 2e

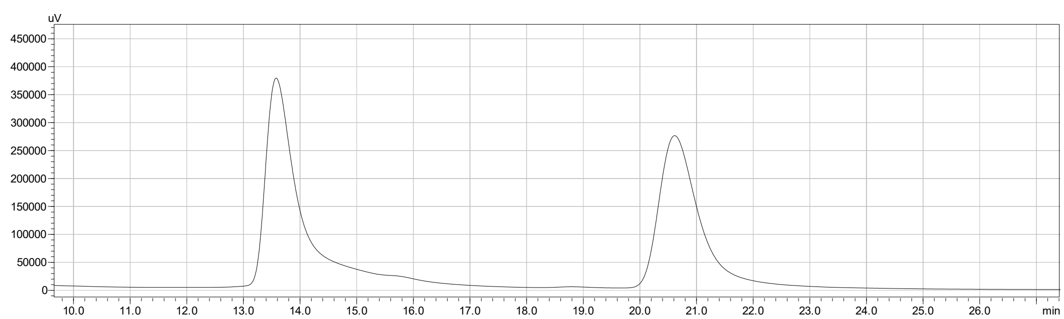


Flavanone 2e

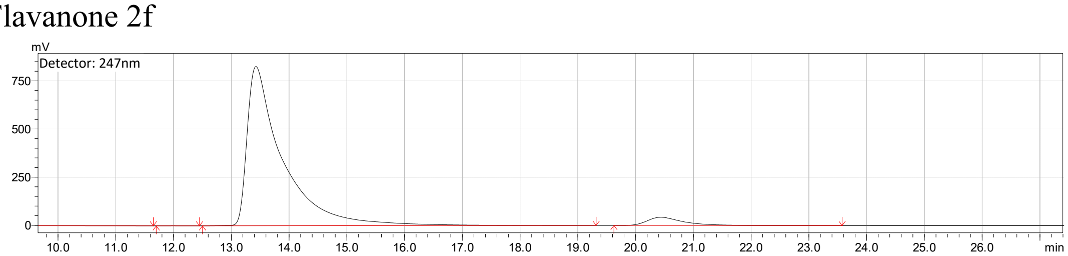


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	12.671	22752447	32.6%
2	16.663	46912830	67.4%

Rac 2f

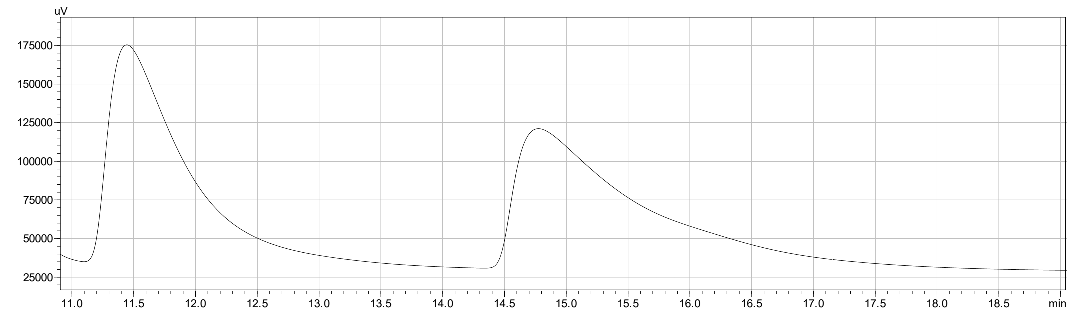


Flavanone 2f

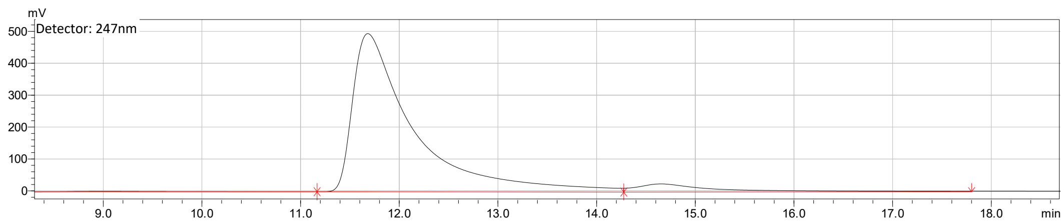


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	13.422	35396113	94.9%
2	20.433	1903394	5.1%

Rac 2g

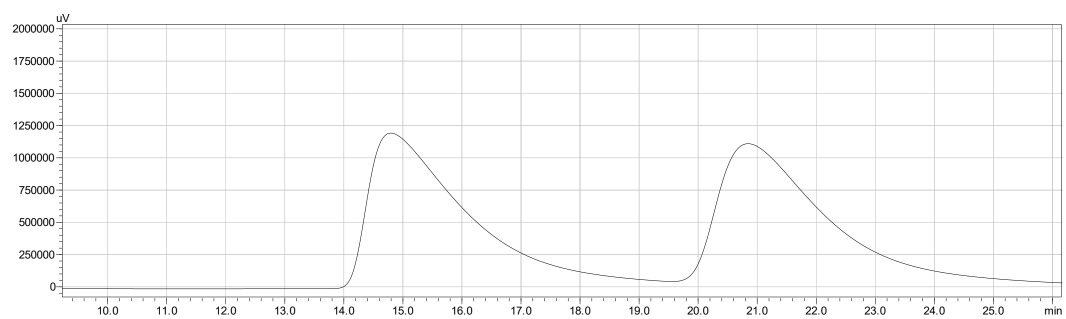


Flavanone 2g

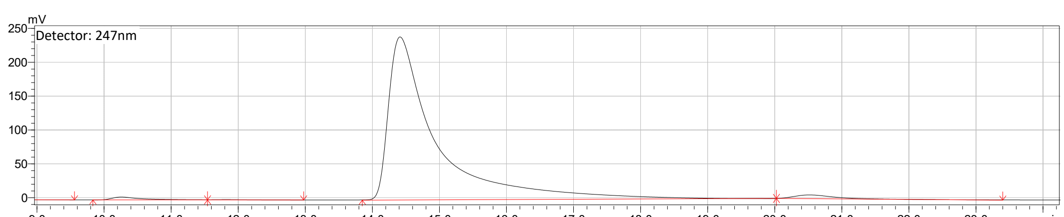


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	11.677	21093121	93.4%
2	14.647	1497692	6.6%

Rac 2h

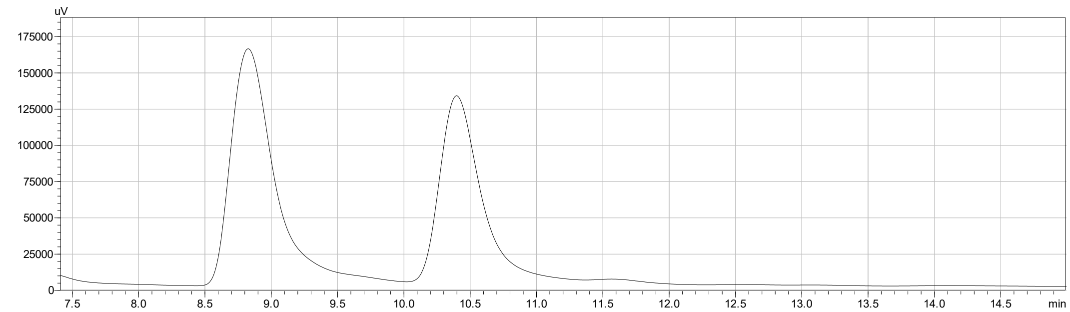


Flavanone 2h

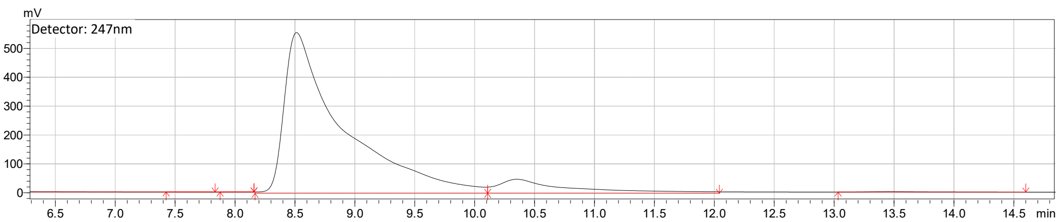


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	14.409	11906483	98%
2	20.514	244086	2%

Rac 2i

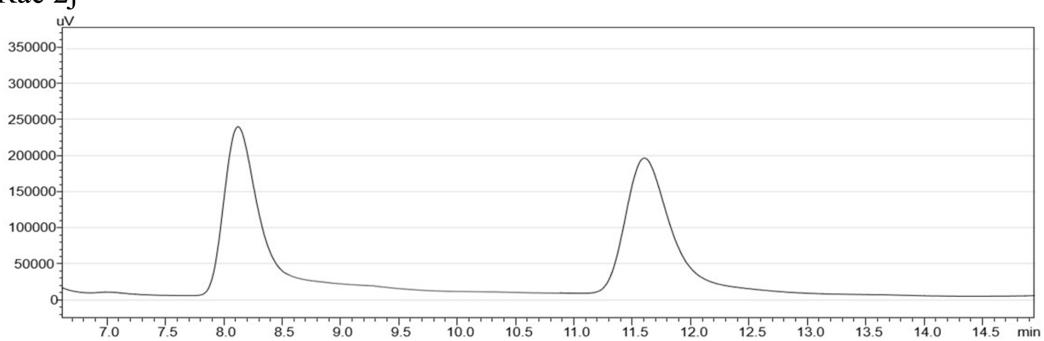


Flavanone 2i

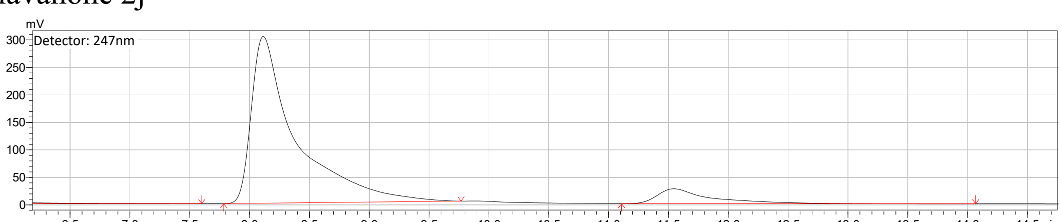


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	8.509	18949428	90.2%
2	10.347	2055282	9.8%

Rac 2j

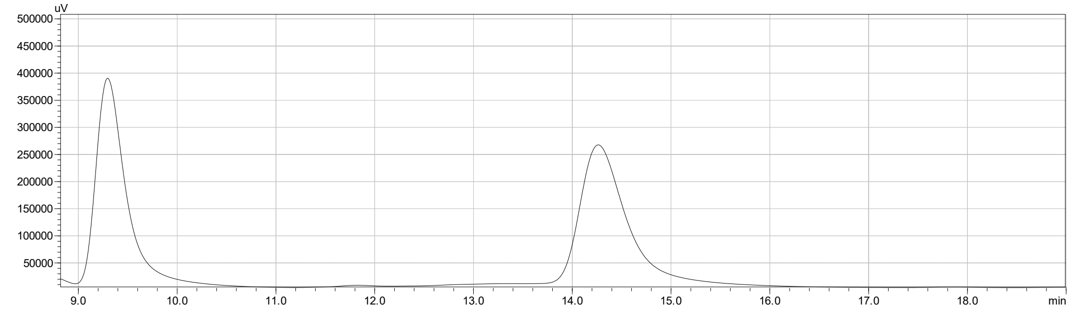


Flavanone 2j

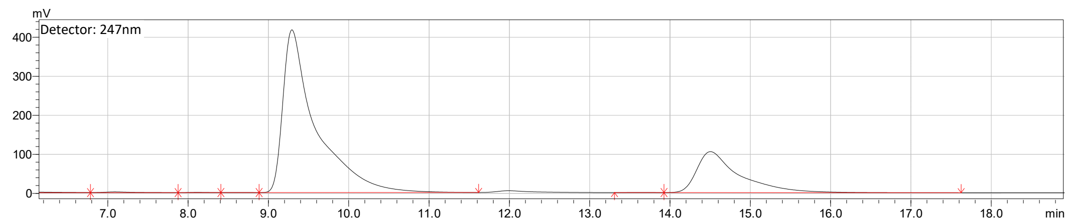


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	8.109	7853826	90.4%
2	11.541	832388	9.6%

Rac 2k

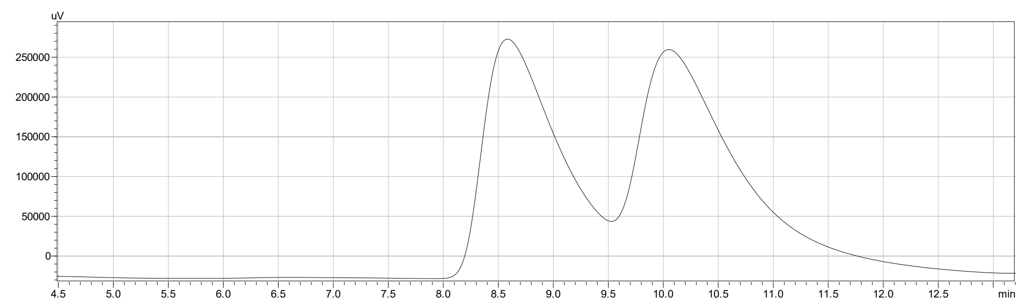


Flavanone 2k

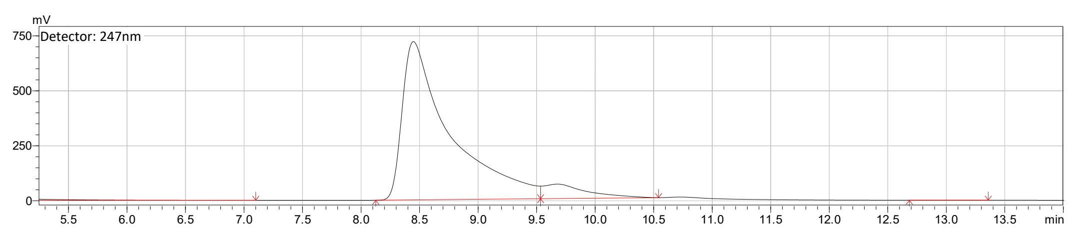


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	9.288	11896624	75.6%
2	14.500	3845195	24.4%

Rac 2l

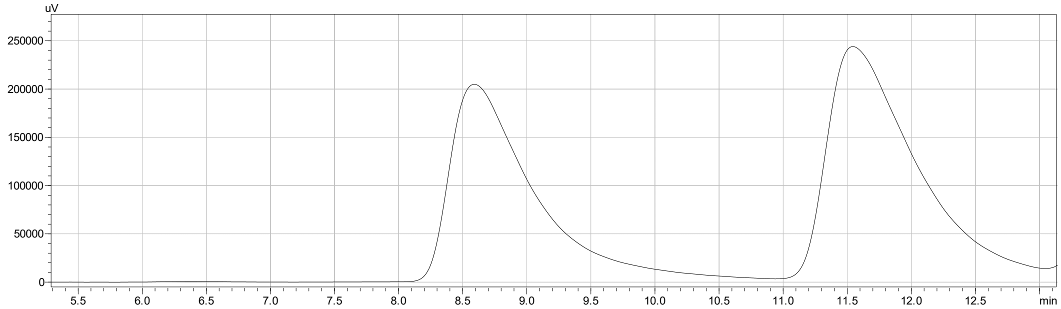


Flavanone 2l

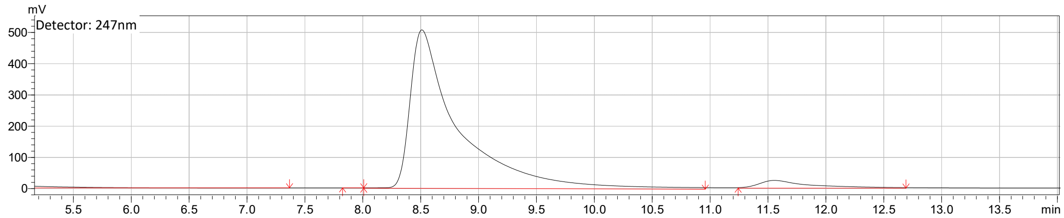


Hexanes : Isopropanol=0.93:0.07, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	8.443	20424660	92.3%
2	9.674	1694682	7.7%

Rac 2m

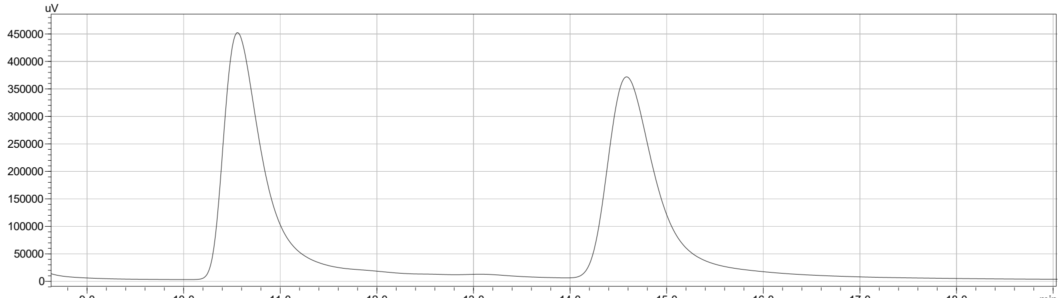


Flavanone 2m

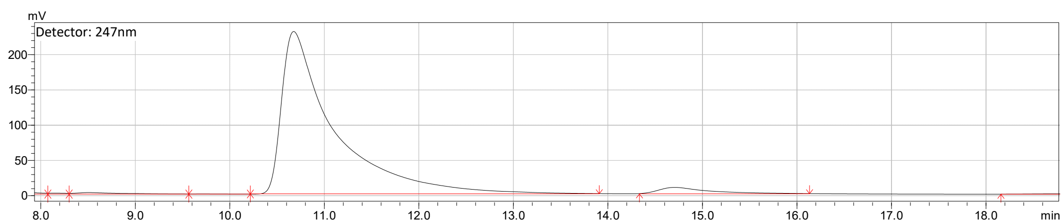


Hexanes : Isopropanol=0.93:0.07, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	8.506	14763837	95%
2	11.552	772373	5%

Rac 2n

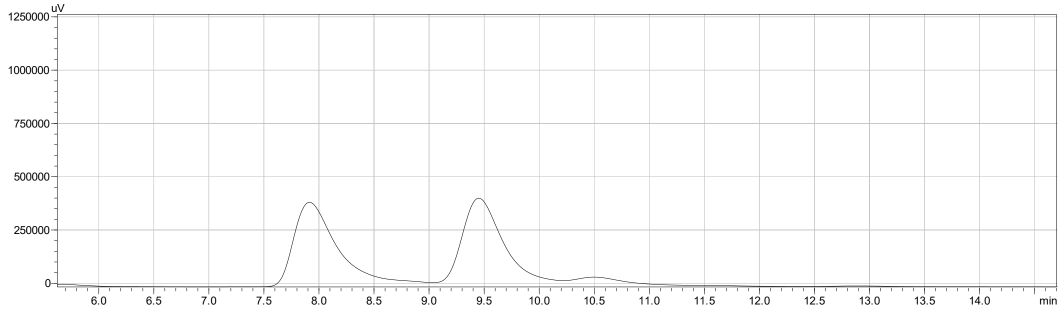


Flavanone 2n

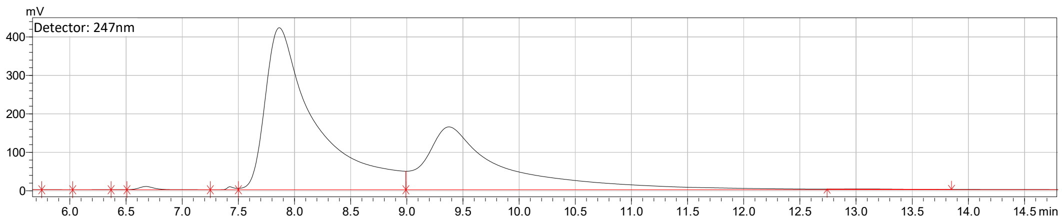


Hexanes : Isopropanol=0.93:0.07, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	10.671	8893376	96.1%
2	14.703	358395	3.9%

Rac 2o

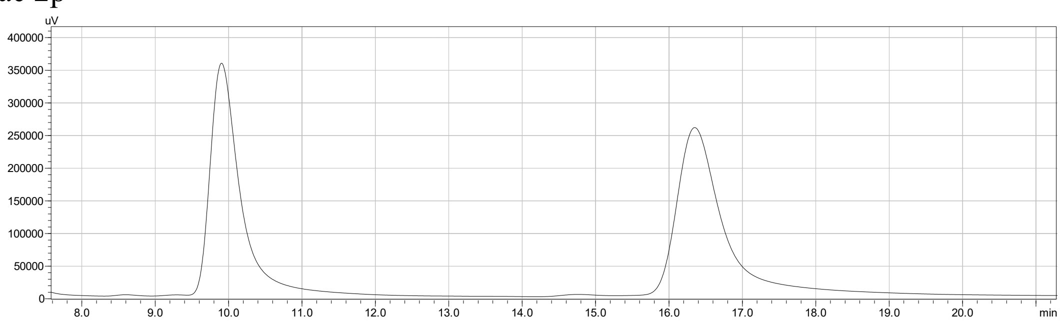


Flavanone 2o

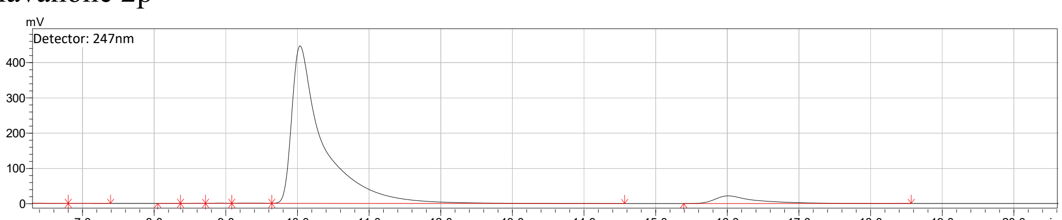


Hexanes : Isopropanol=0.93:0.07, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	7.860	13252513	62.7%
2	9.371	7878097	37.3%

Rac 2p

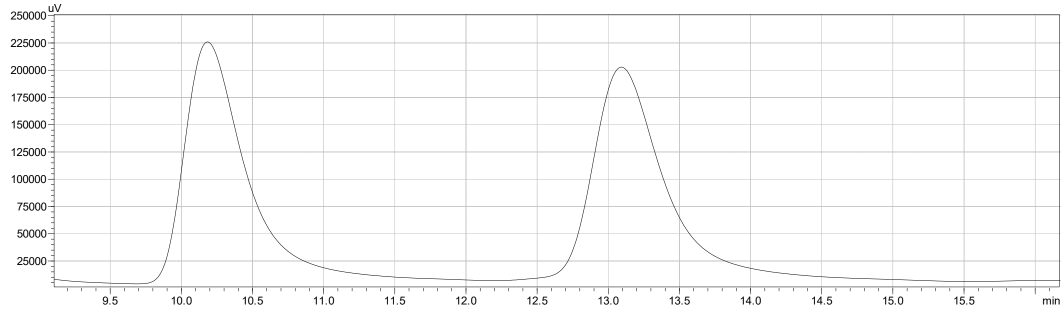


Flavanone 2p

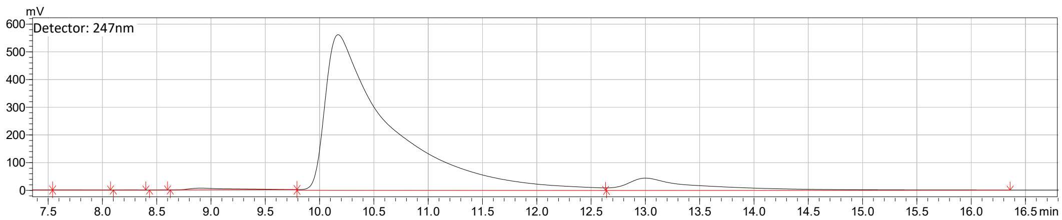


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	10.032	13631754	94.1%
2	16.009	845955	5.9%

Rac 2q

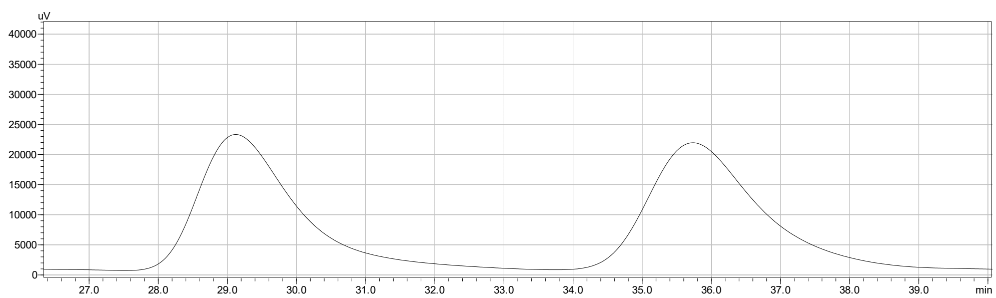


Flavanone 2q

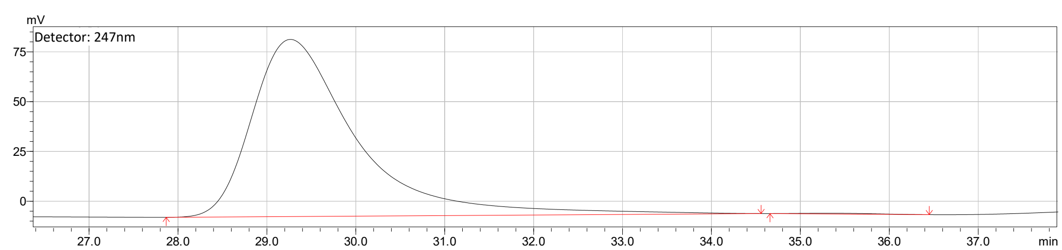


Hexanes : Isopropanol=0.94:0.06, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	10.166	23504389	91.8%
2	12.997	2099880	8.2%

Rac 2r

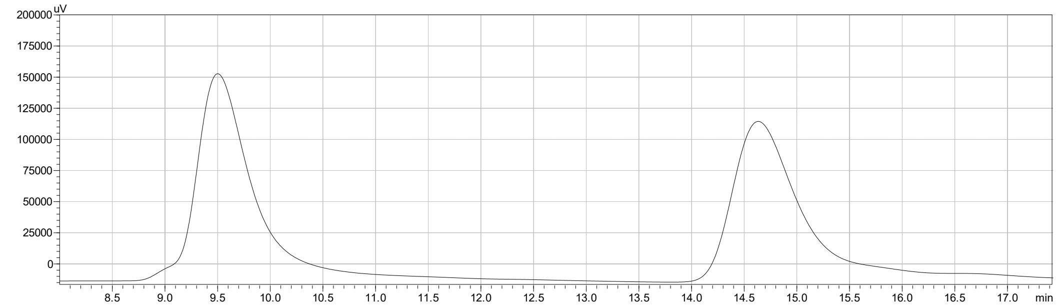


Flavanone 2r

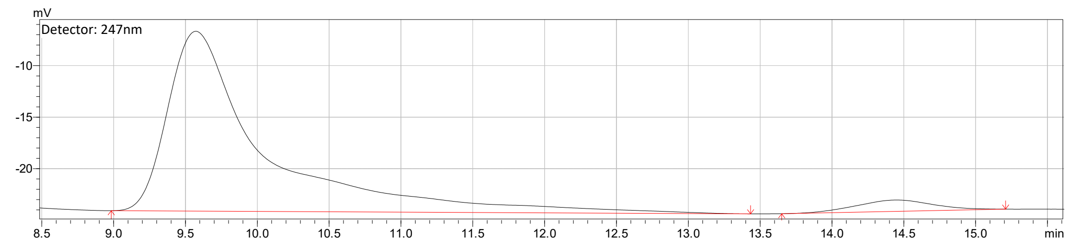


Hexanes : Isopropanol=0.85:0.15, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	29.261	7266263	99.7%
2	35.269	21783	0.3%

Rac 2s

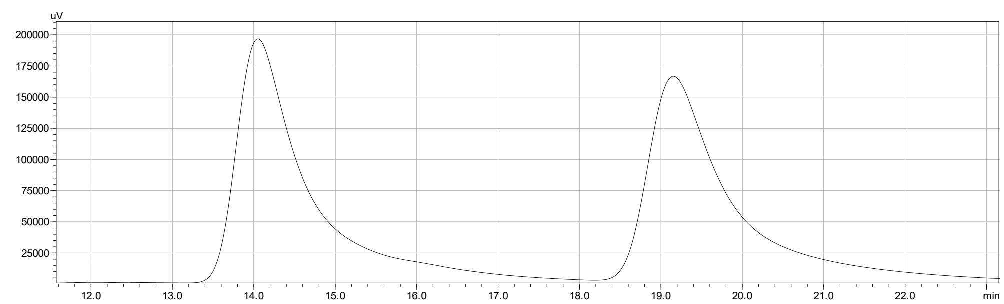


Flavanone 2s

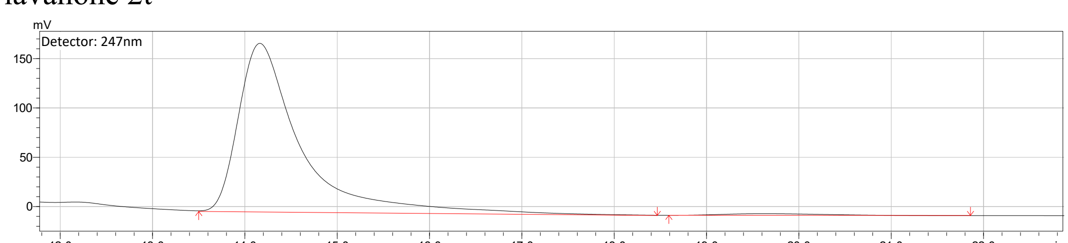


Hexanes : Isopropanol=0.97:0.03, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	9.568	806322	95.1%
2	14.441	41274	4.9%

Rac 2t

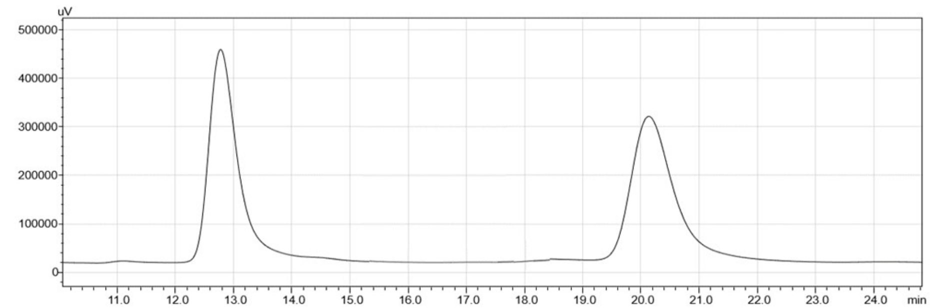


Flavanone 2t

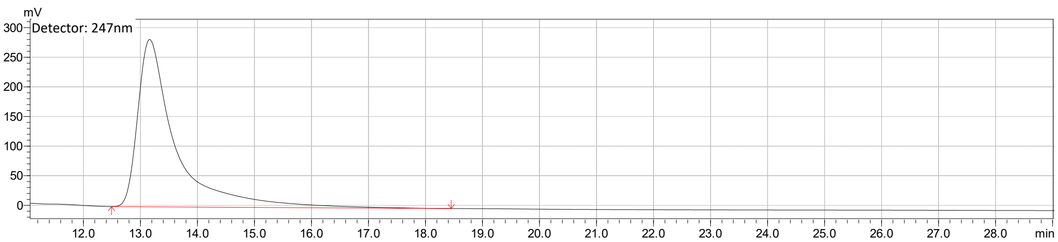


Hexanes : Isopropanol=0.9:0.1, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	14.158	8039223	98.5%
2	19.620	125596	1.5%

Rac 2u

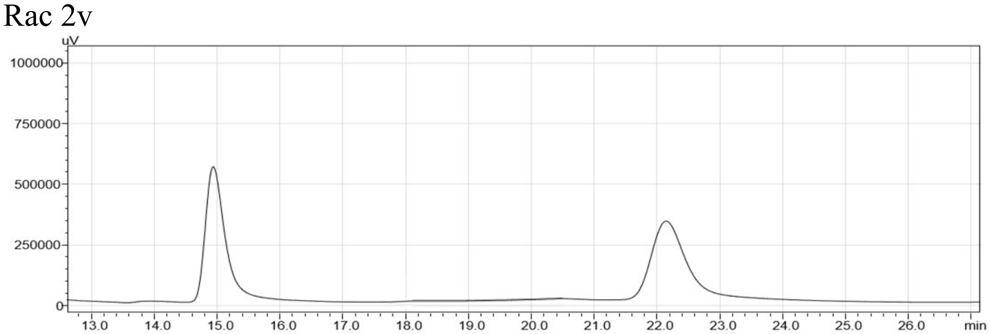


Flavanone 2u

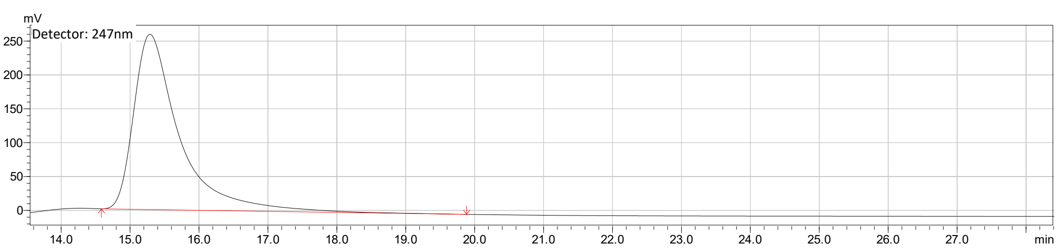


Hexanes : Isopropanol=0.85:0.15, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	13.157	12967350	100%
2	20.198	——	——

Rac 2v

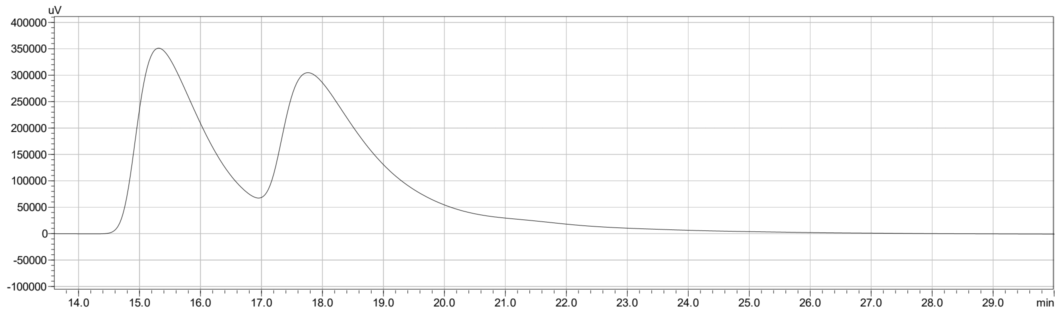


Flavanone 2v

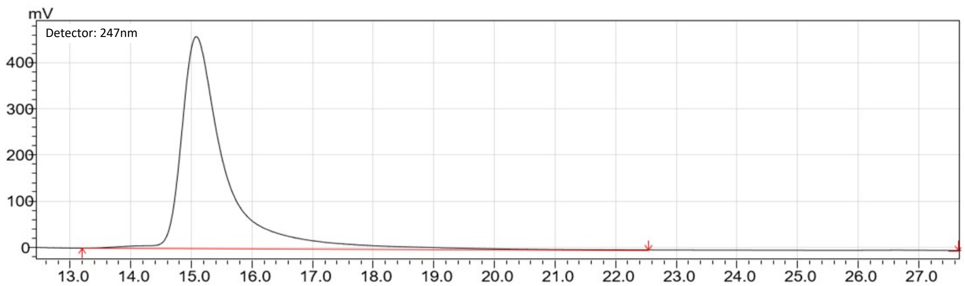


Hexanes : Isopropanol=0.85:0.15, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	15.284	11886051	100%
2	22.206	——	——

Rac 2w



Flavanone 2w

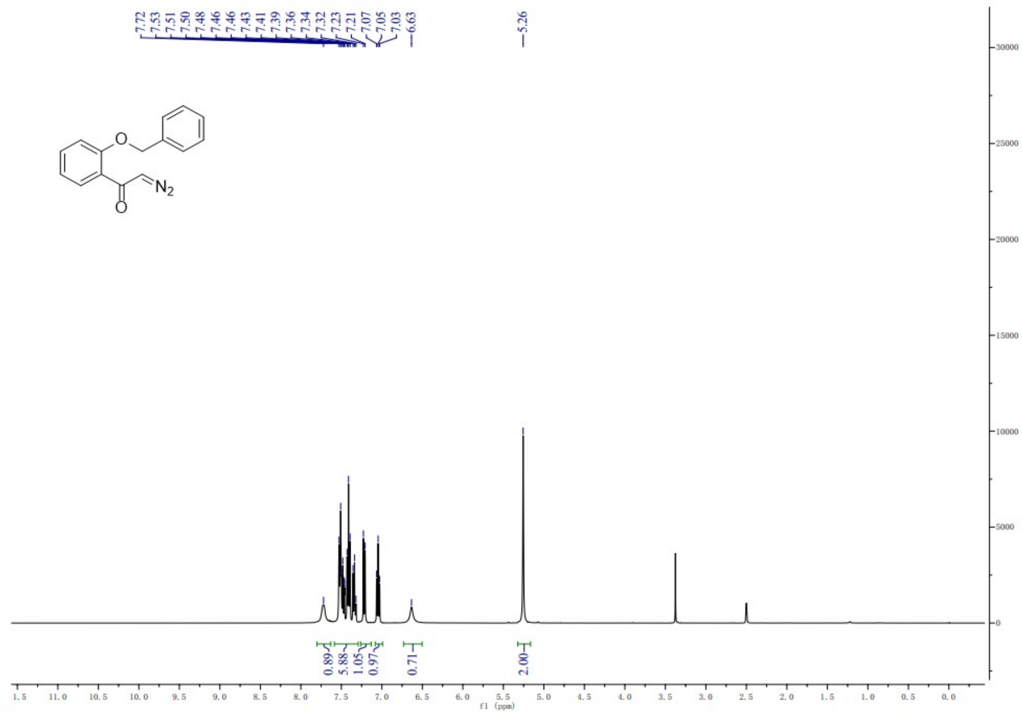


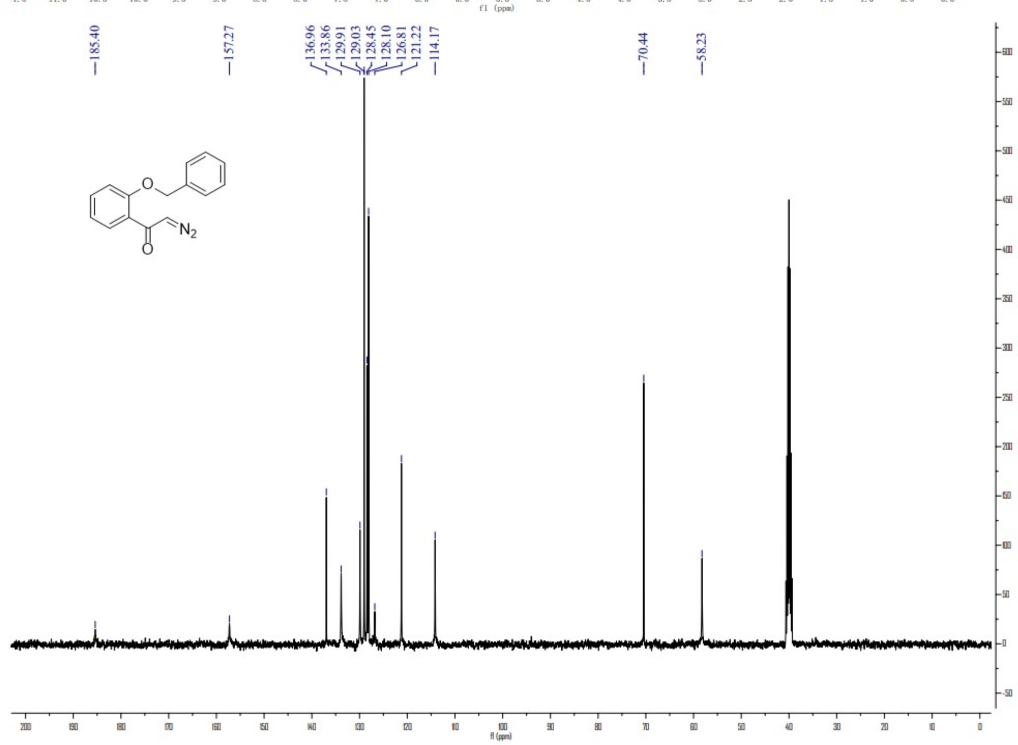
Hexanes : Isopropanol=0.85:0.15, 1.0 mL/min, 247nm			
Peak	Ret Time (min)	Area (mAU*S)	Area %
1	15.069	22703868	>99%
2	17.733	——	——

Figure S4. Enantioselectivities determined by Chiral HPLC. The target products purified after whole-cell reaction was used for selectivity determination by Chiralcel OD-H column, and the results obtained were compared with those previously reported in the literature to determine the *S*-enantiomer and *R*-enantiomer of the products. For all analysis, hexane and isopropanol were used as eluents for the Chiralcel OD-H column. The volume for sample injection was 10μL and the flow rate was 1mL/minute, and the column was maintained at 30 °C for the entire method.

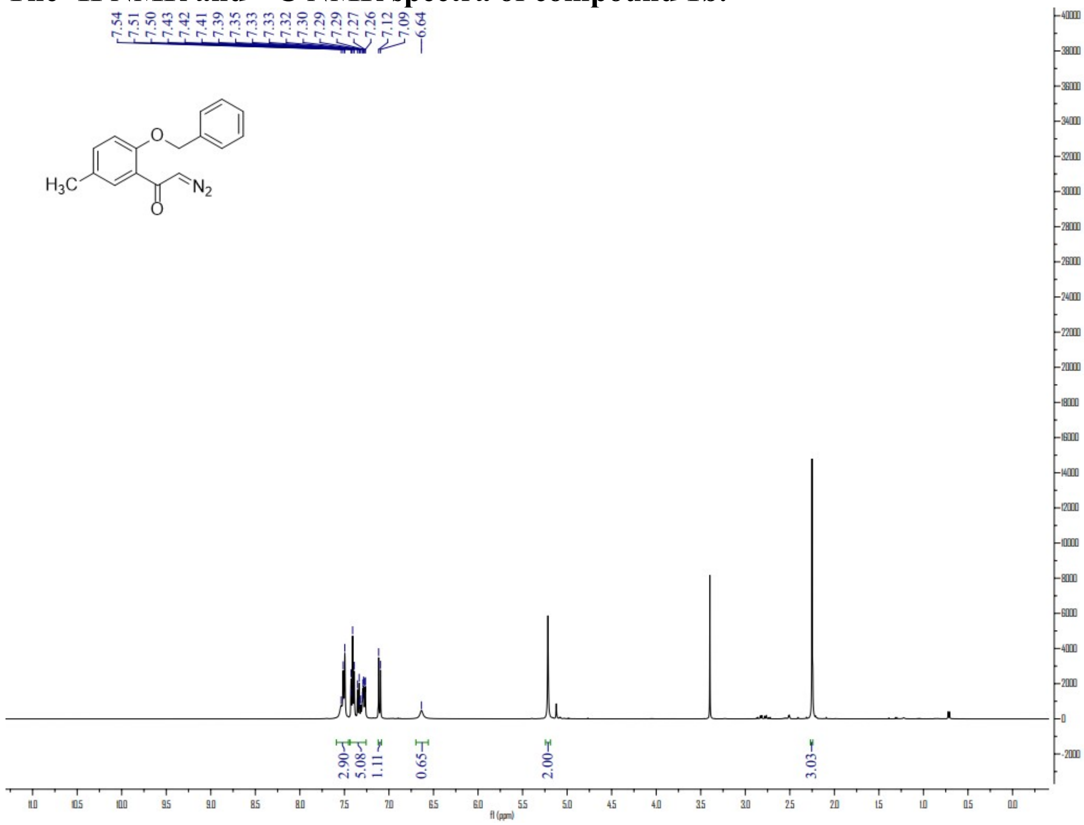
NMR Spectra

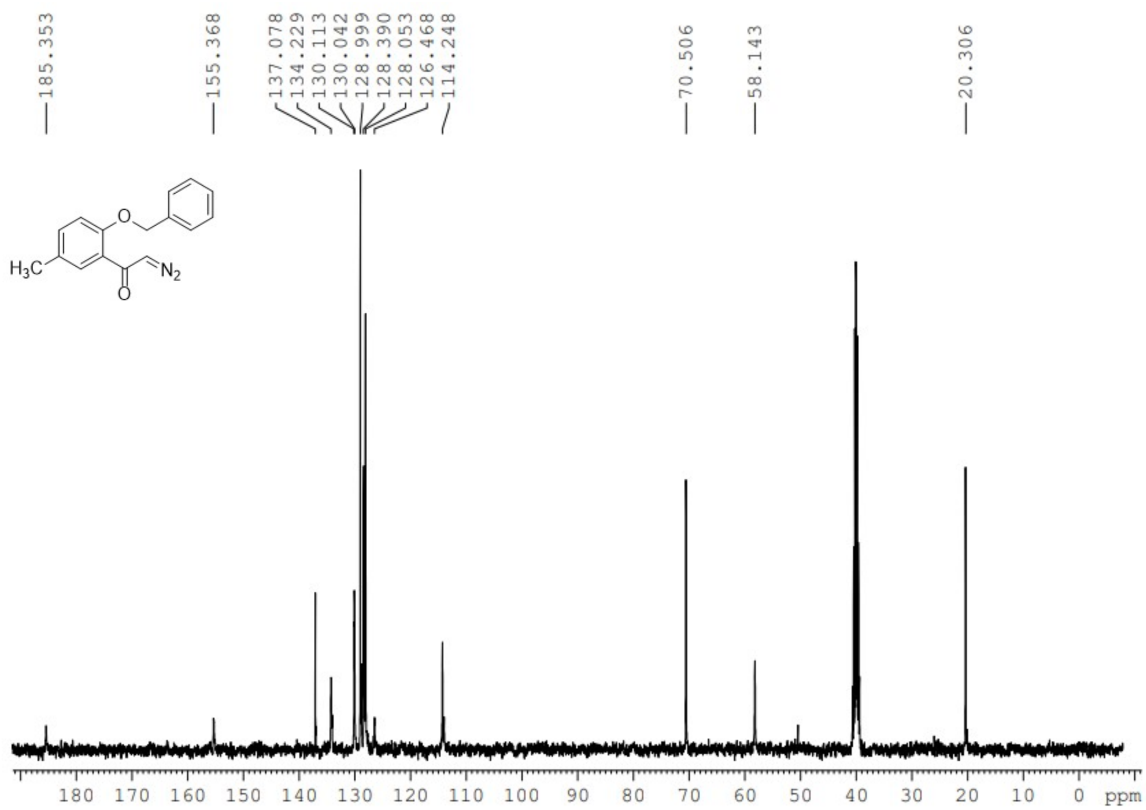
The ^{1}H NMR and ^{13}C NMR spectra of compound 1a.



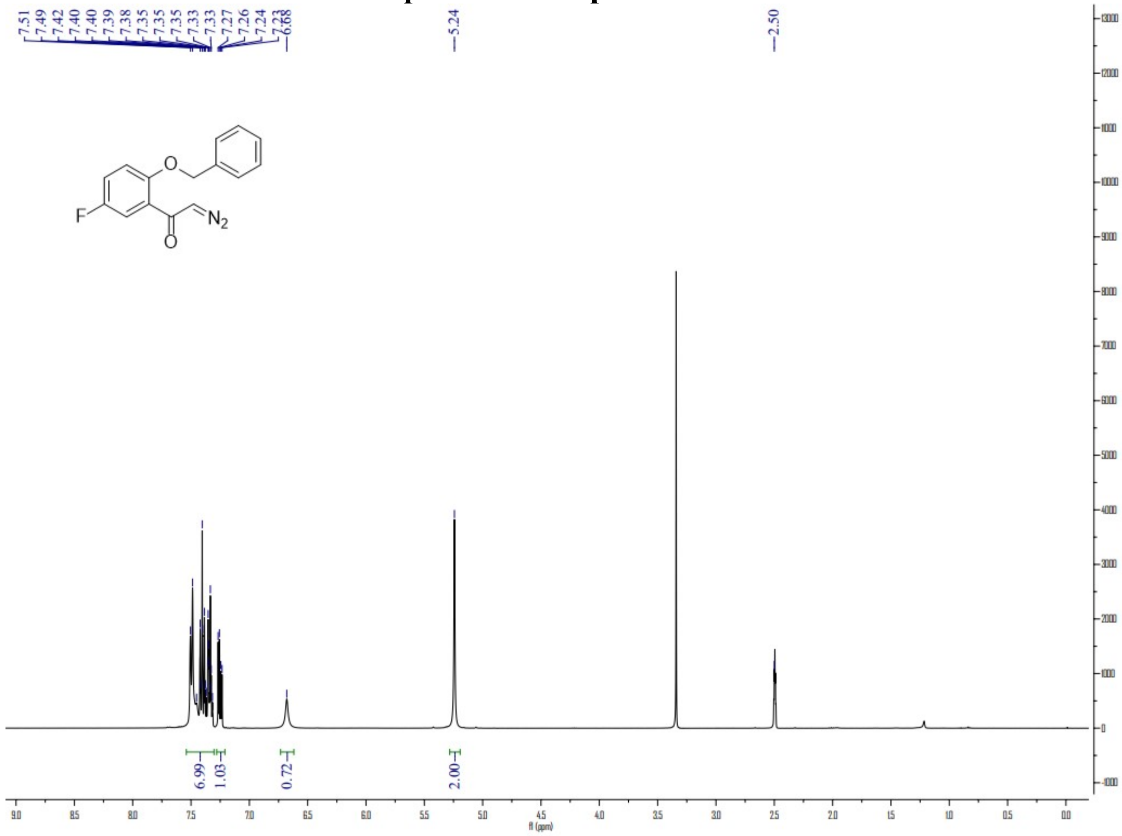


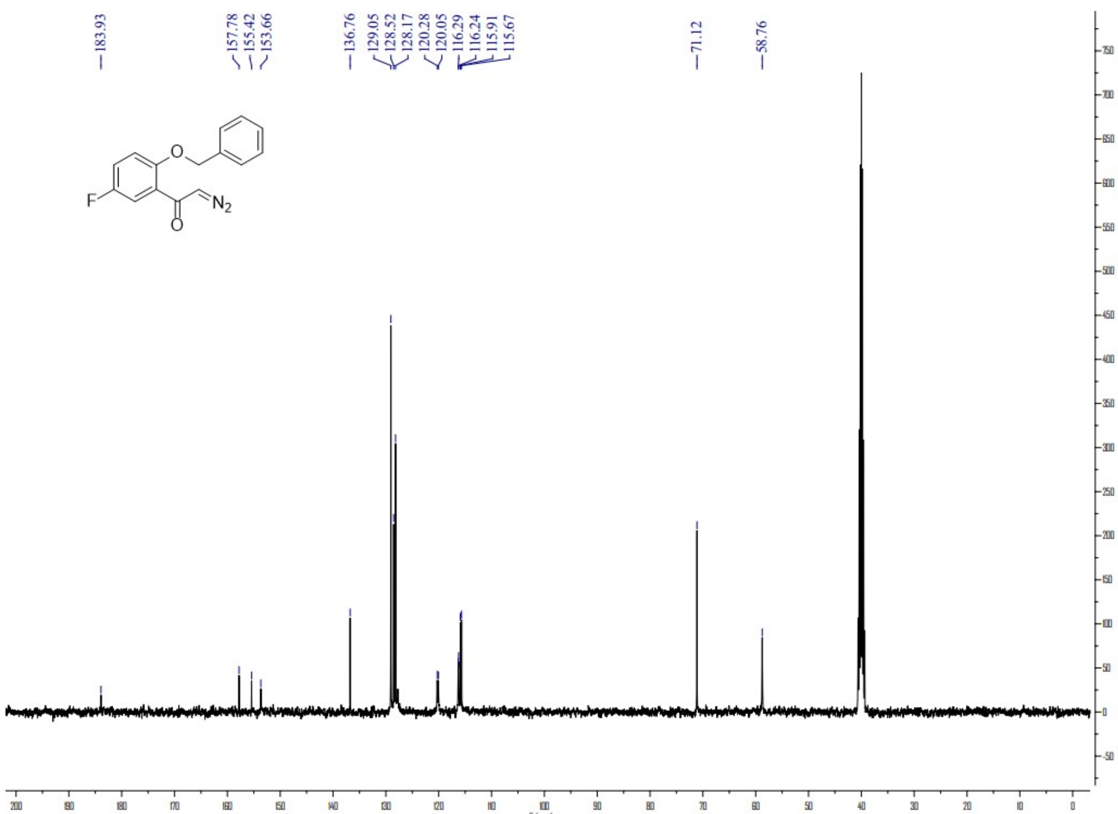
The ^{1}H NMR and ^{13}C NMR spectra of compound 1b.

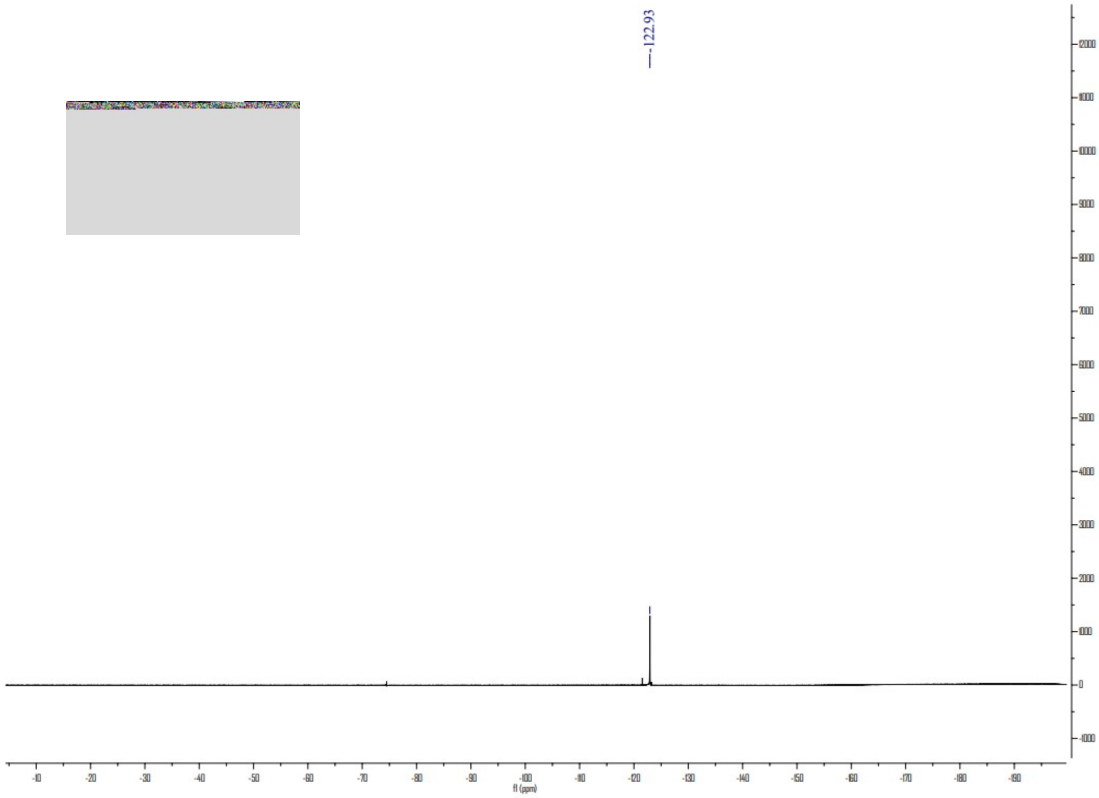




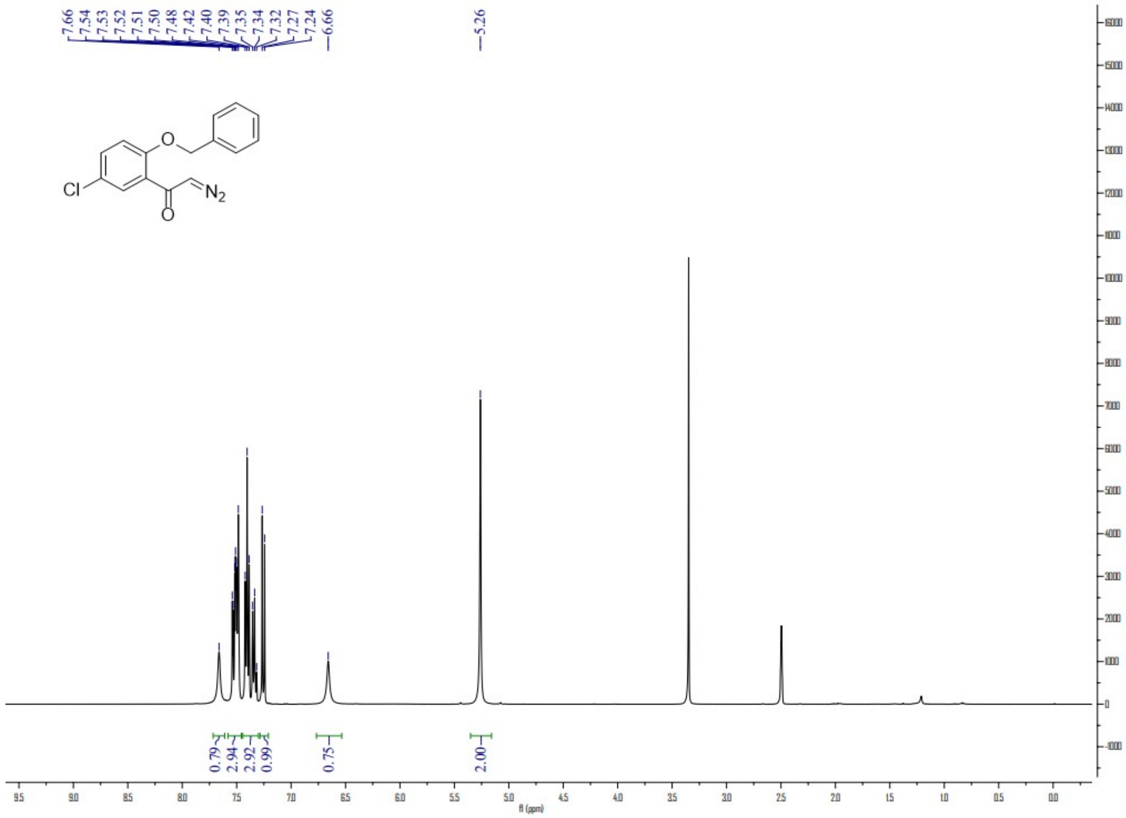
The ^{1}H NMR and ^{13}C NMR spectra of compound 1c.

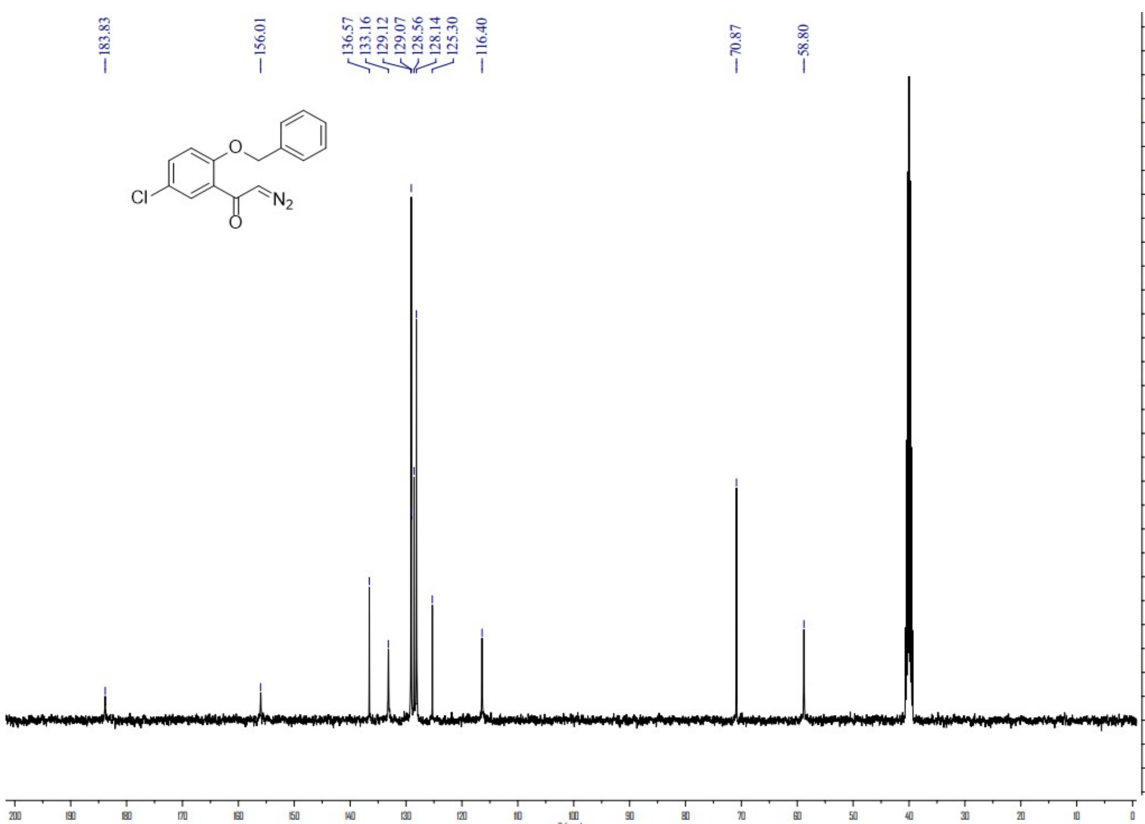




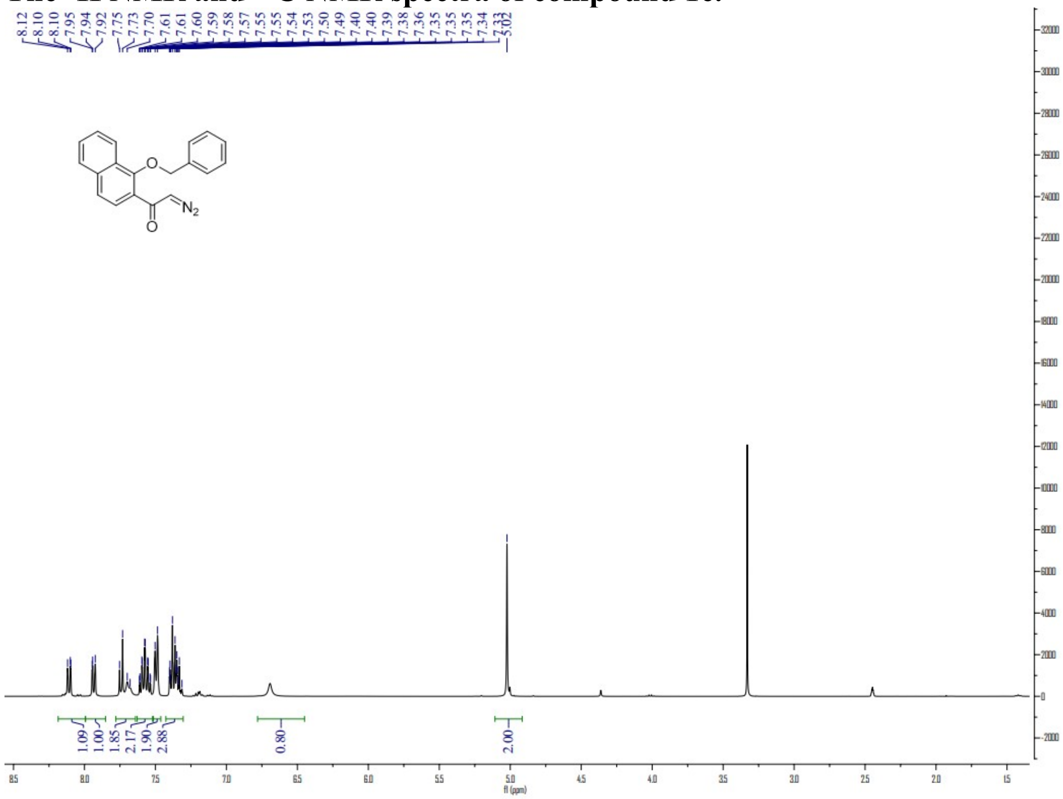

-122.93
f1 (ppm)

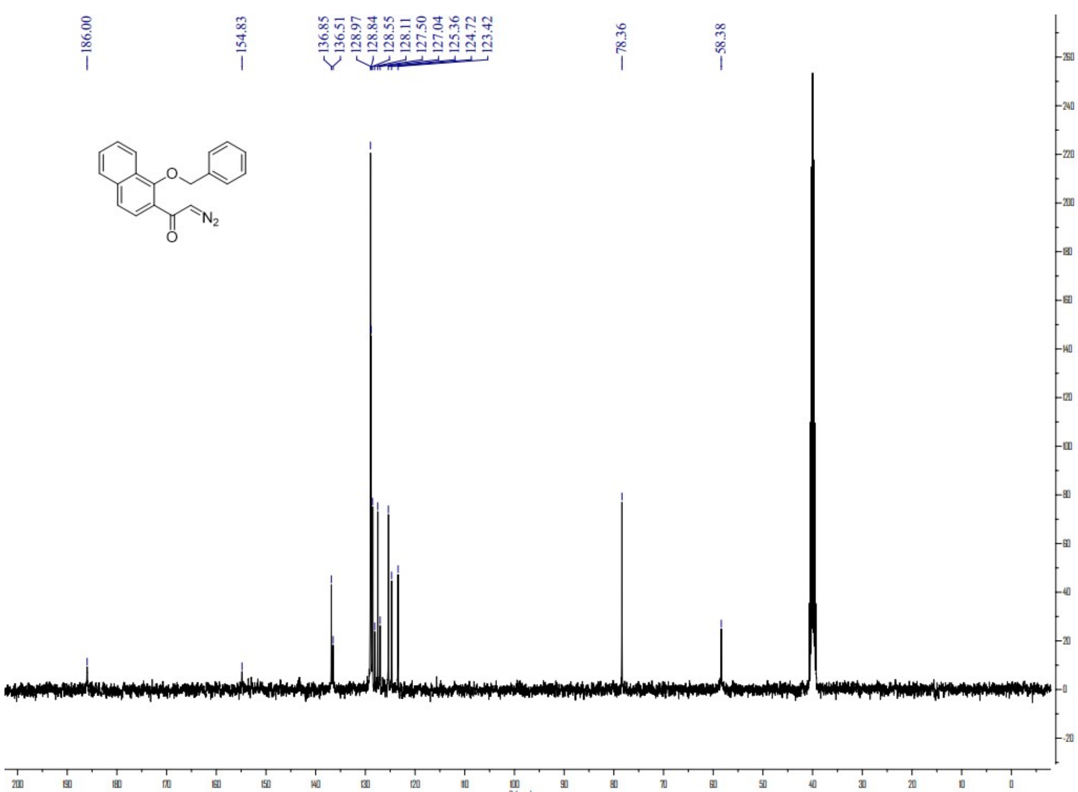
The ^{1}H NMR and ^{13}C NMR spectra of compound 1d.



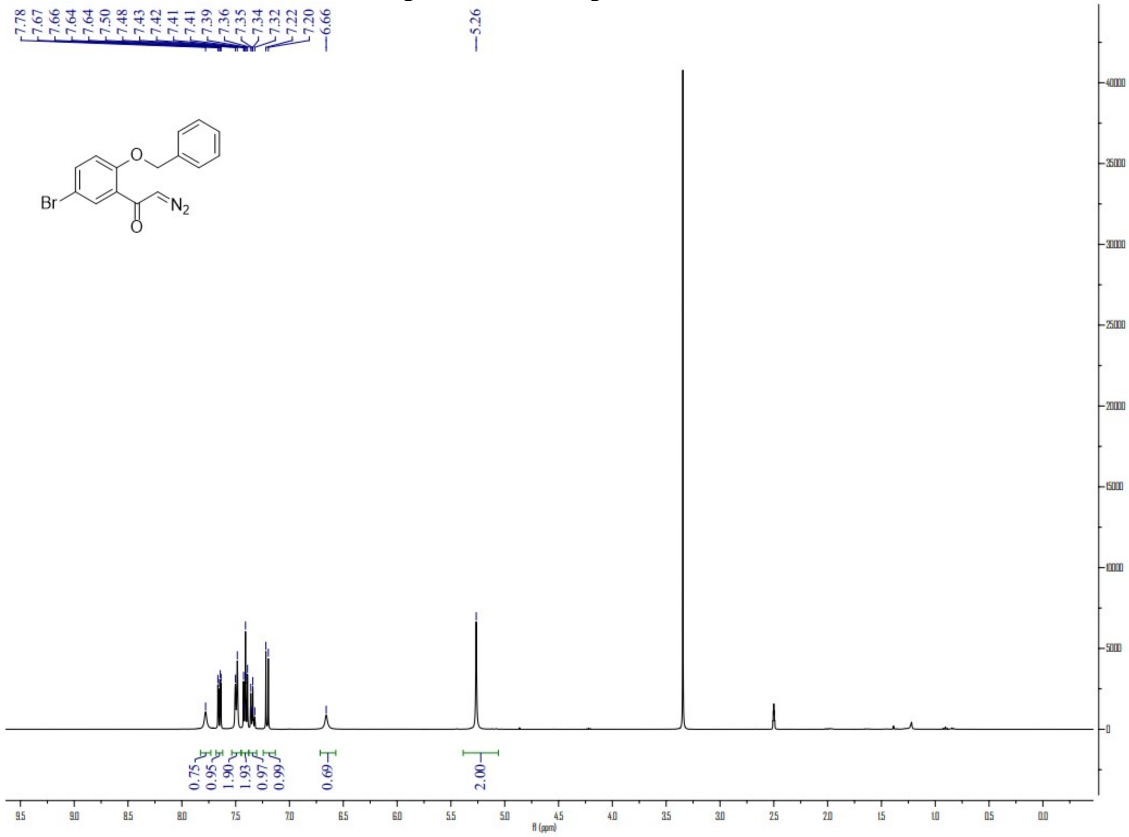


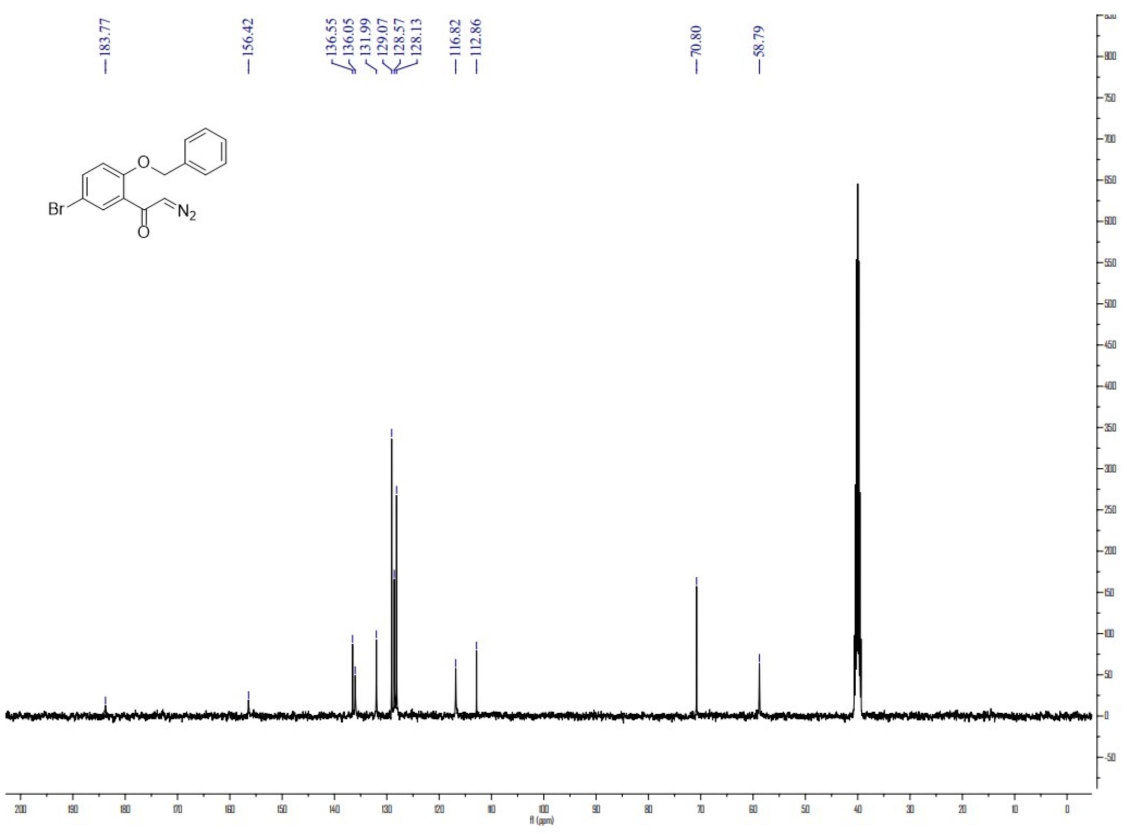
The ^{1}H NMR and ^{13}C NMR spectra of compound 1e.



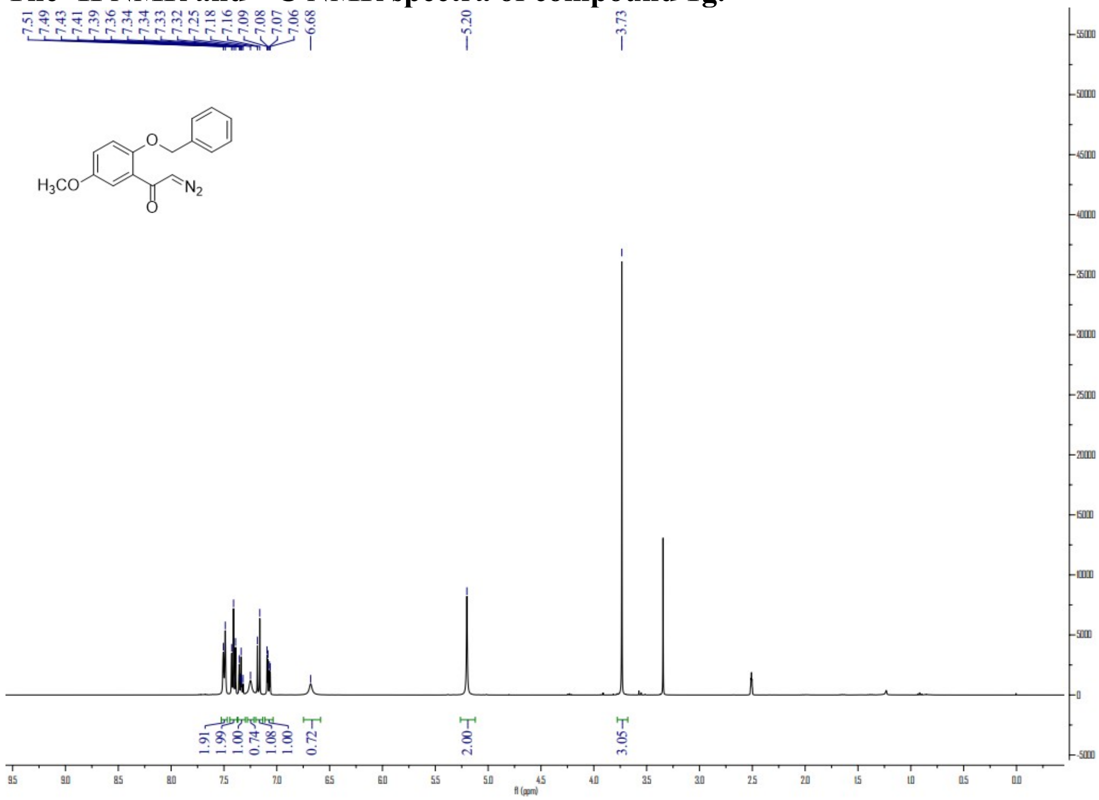


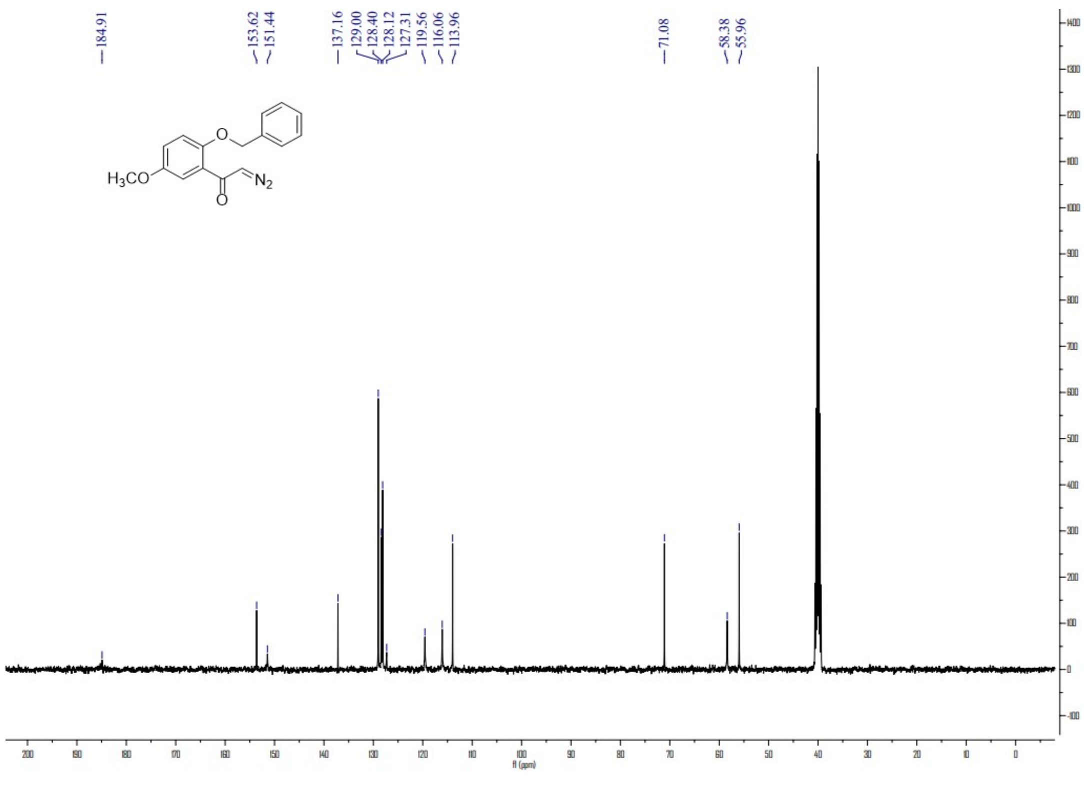
The ^{1}H NMR and ^{13}C NMR spectra of compound 1f.



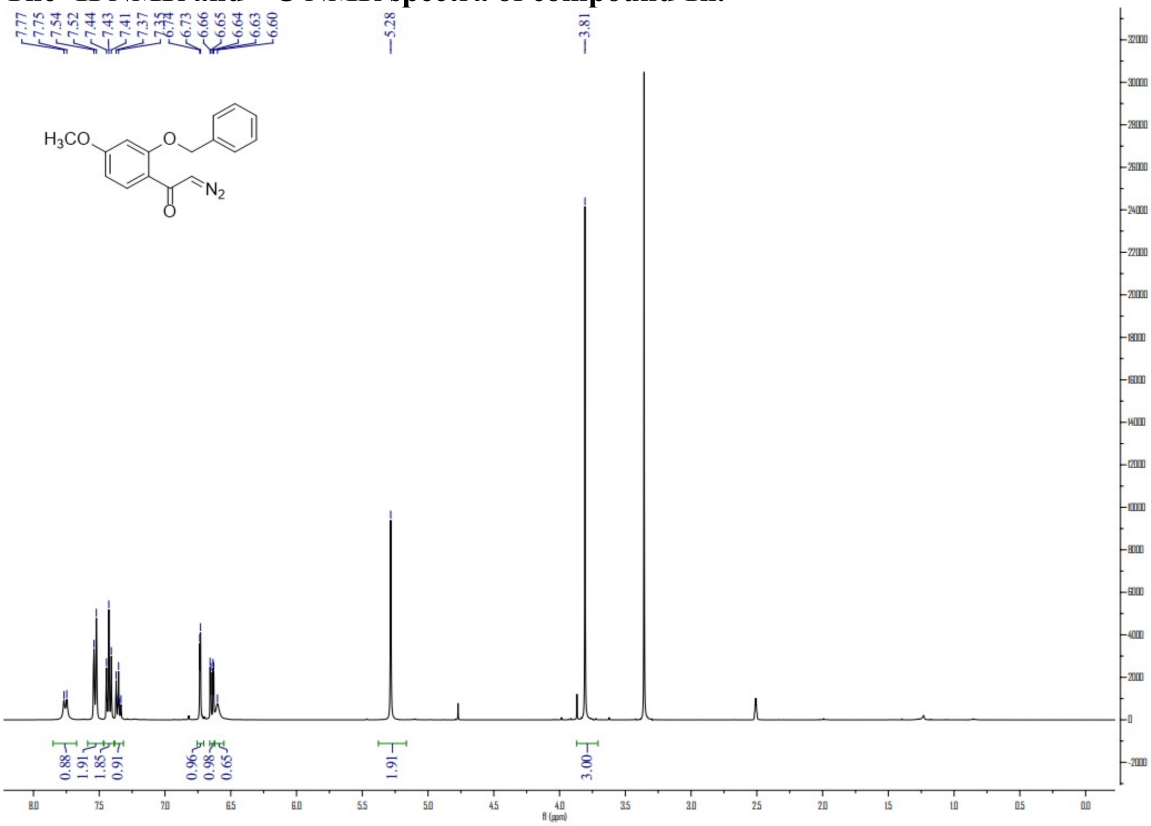


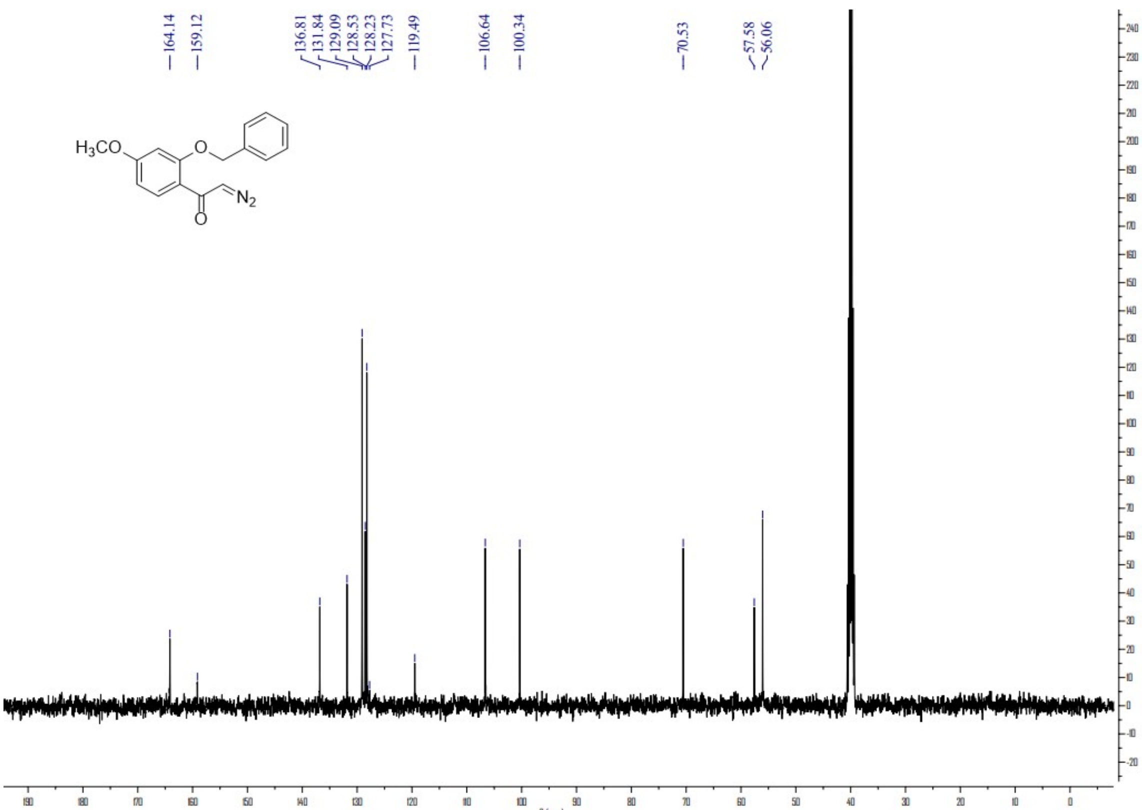
The ^{1}H NMR and ^{13}C NMR spectra of compound 1g.



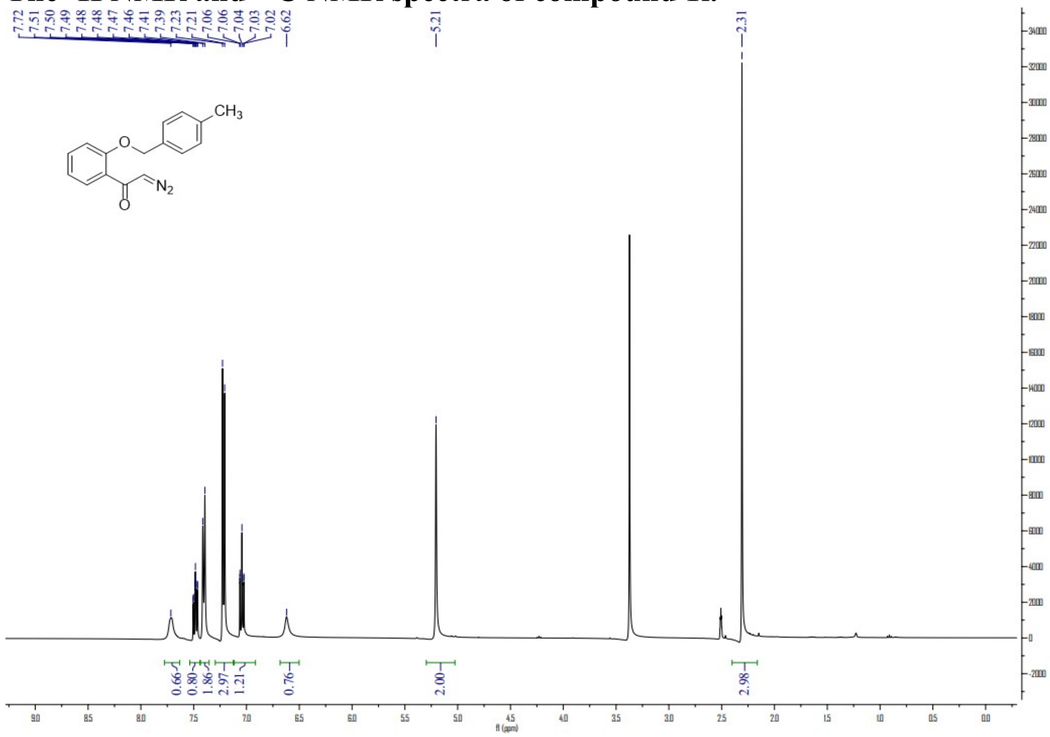


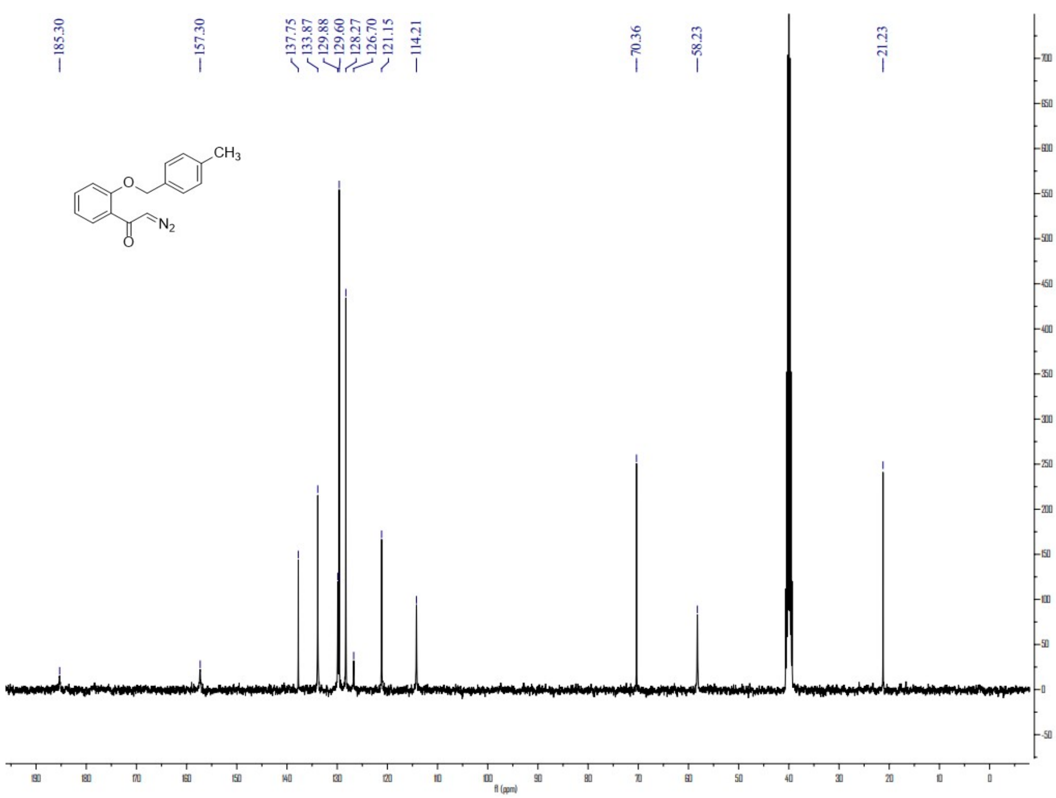
The ^{1}H NMR and ^{13}C NMR spectra of compound 1h.



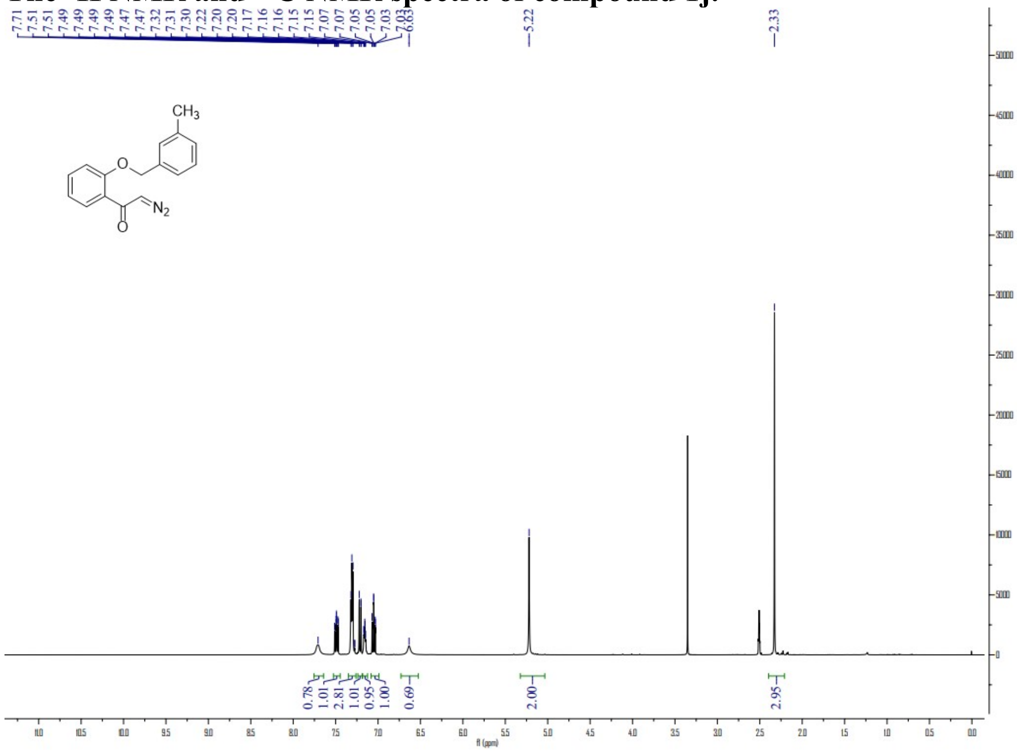


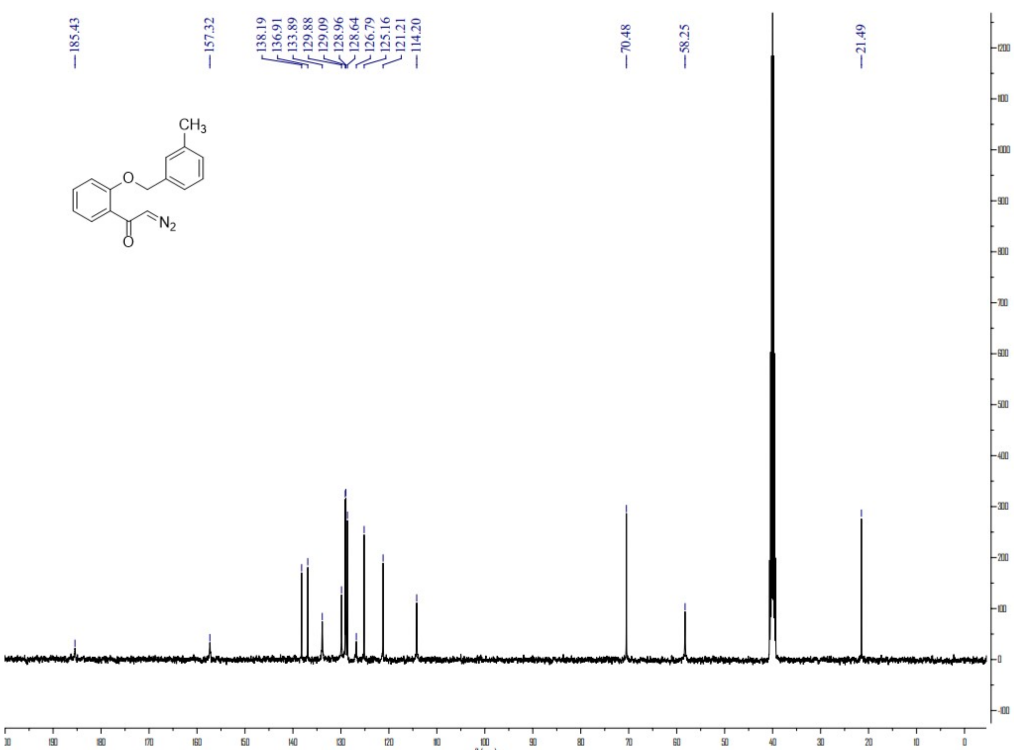
The ^{1}H NMR and ^{13}C NMR spectra of compound 1i.



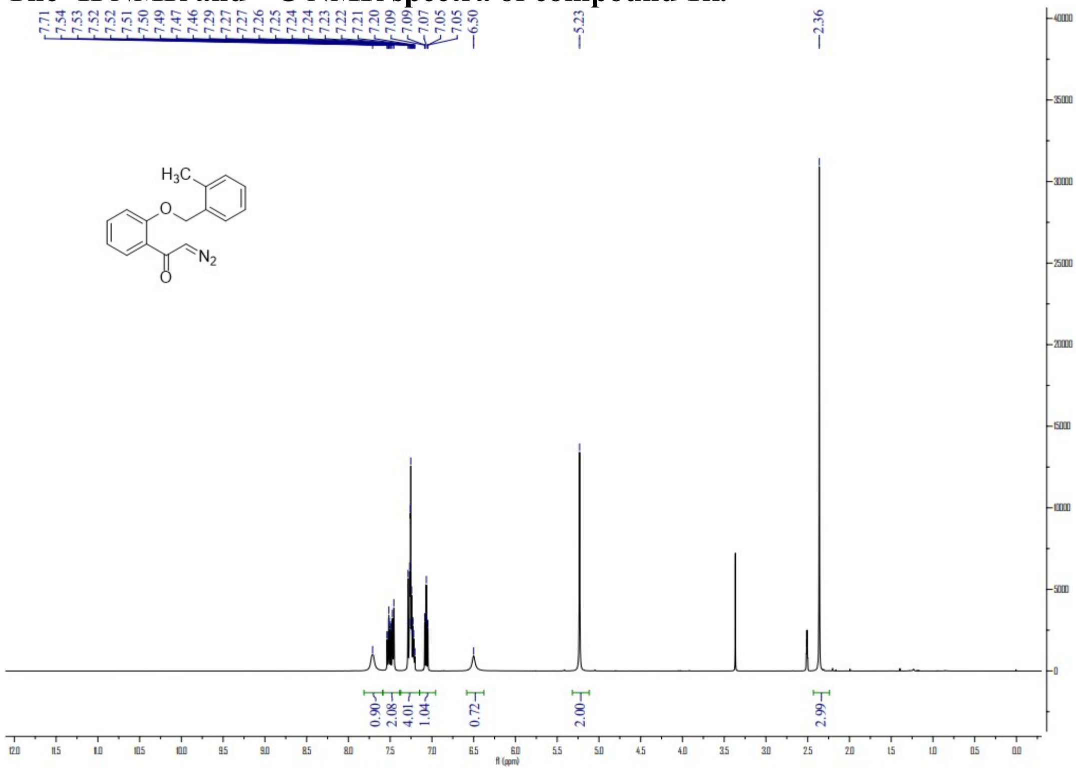


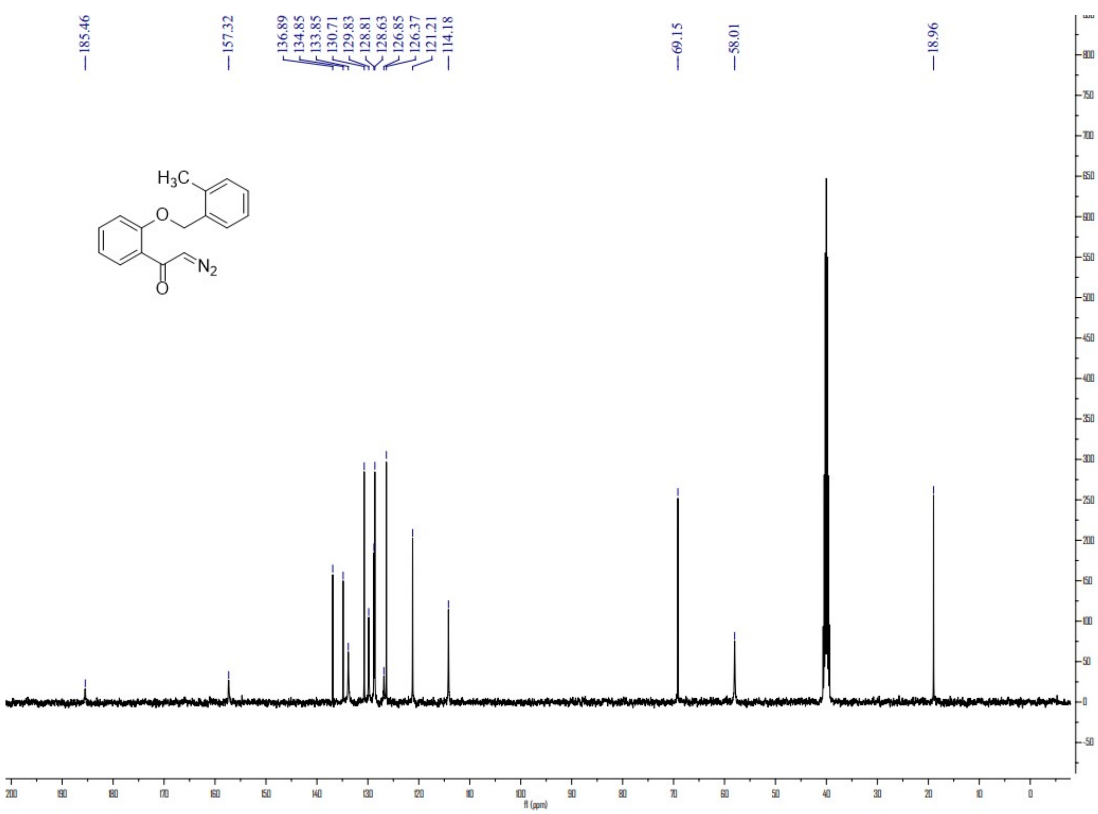
The ^{1}H NMR and ^{13}C NMR spectra of compound 1j.



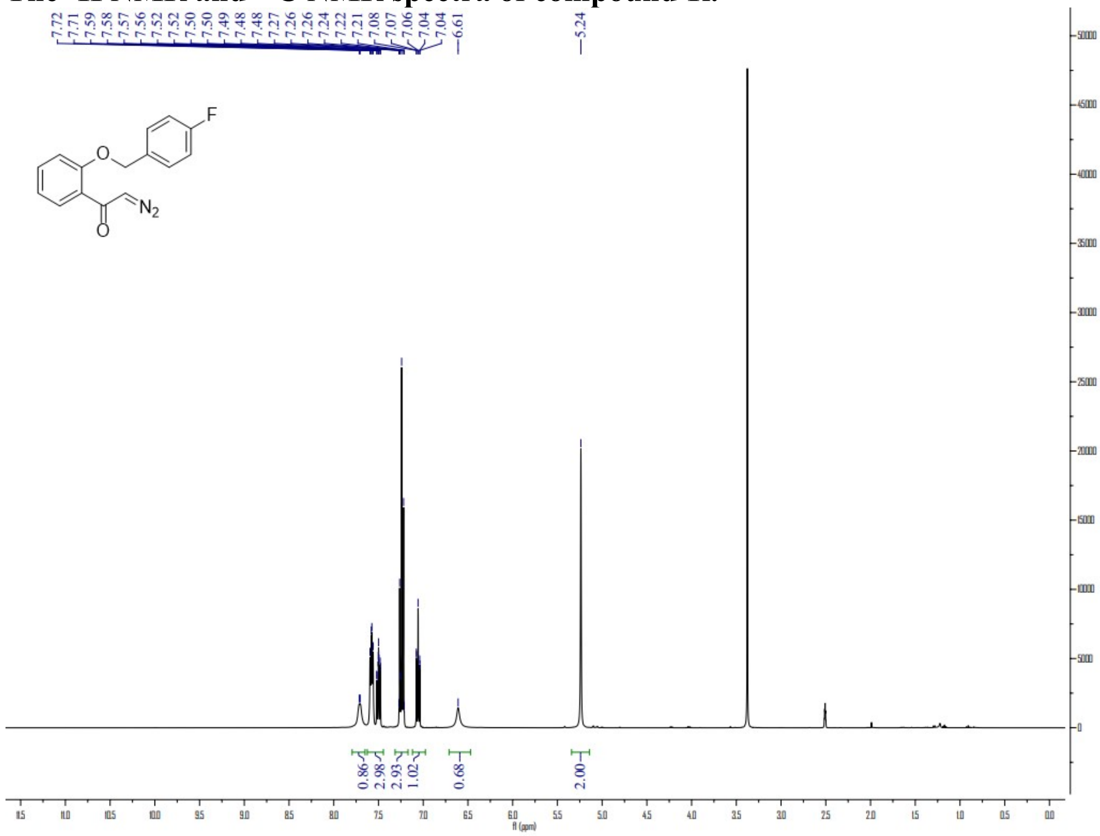


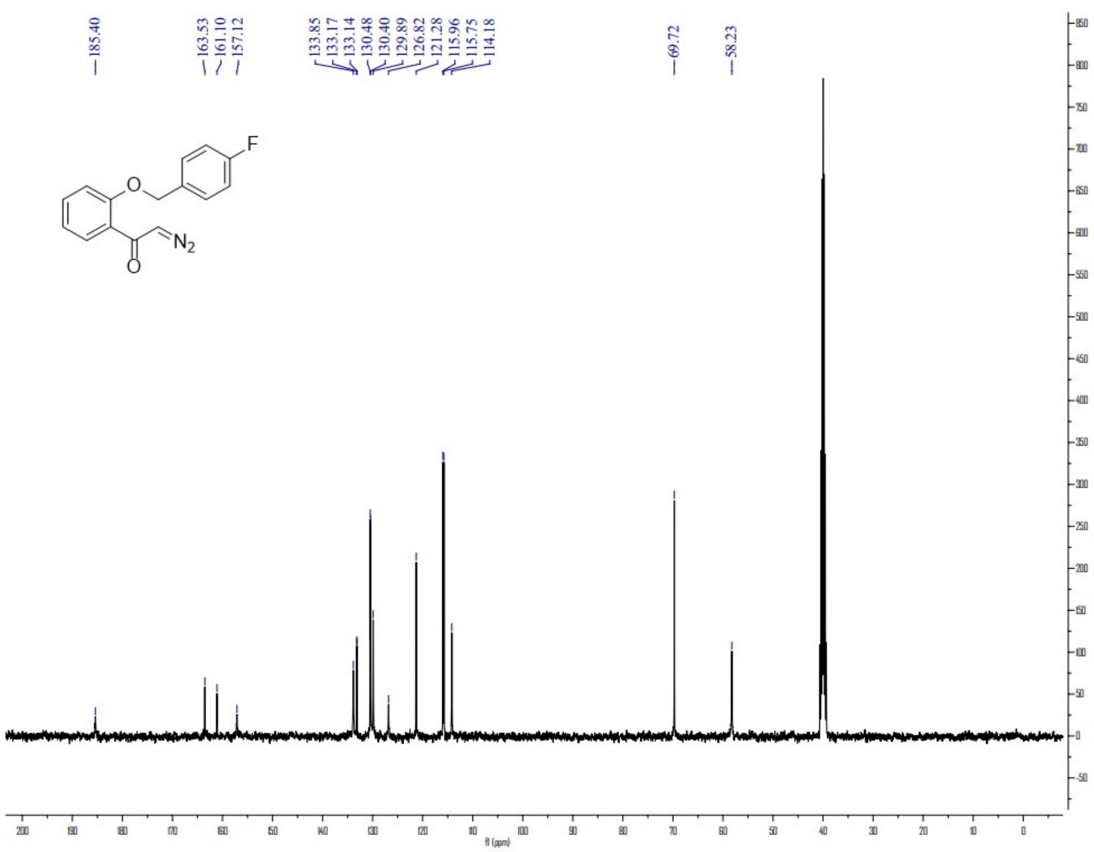
The ^{1}H NMR and ^{13}C NMR spectra of compound 1k.

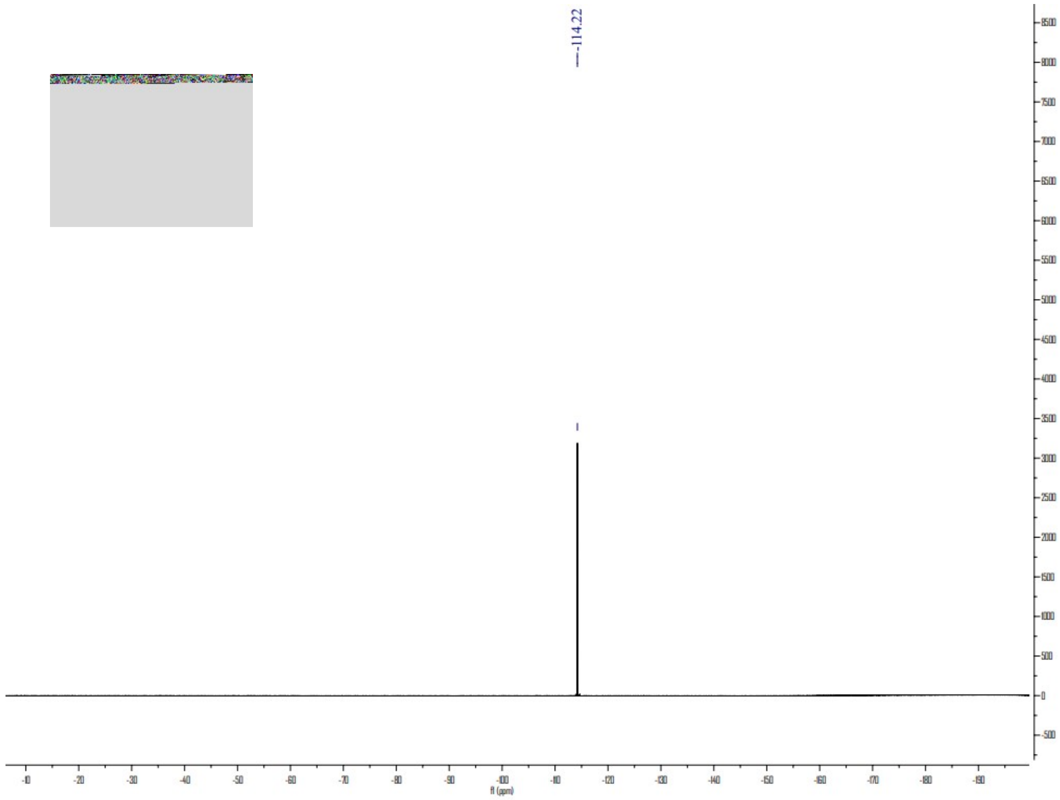




The ^{1}H NMR and ^{13}C NMR spectra of compound 1l.

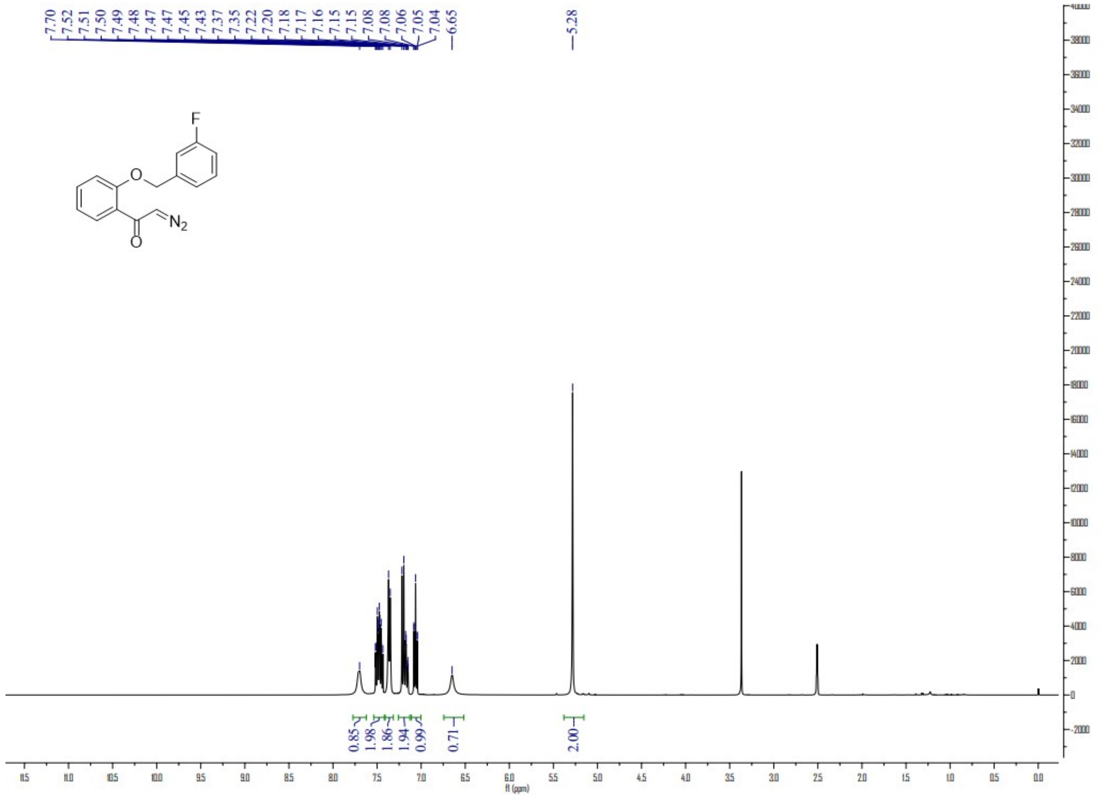


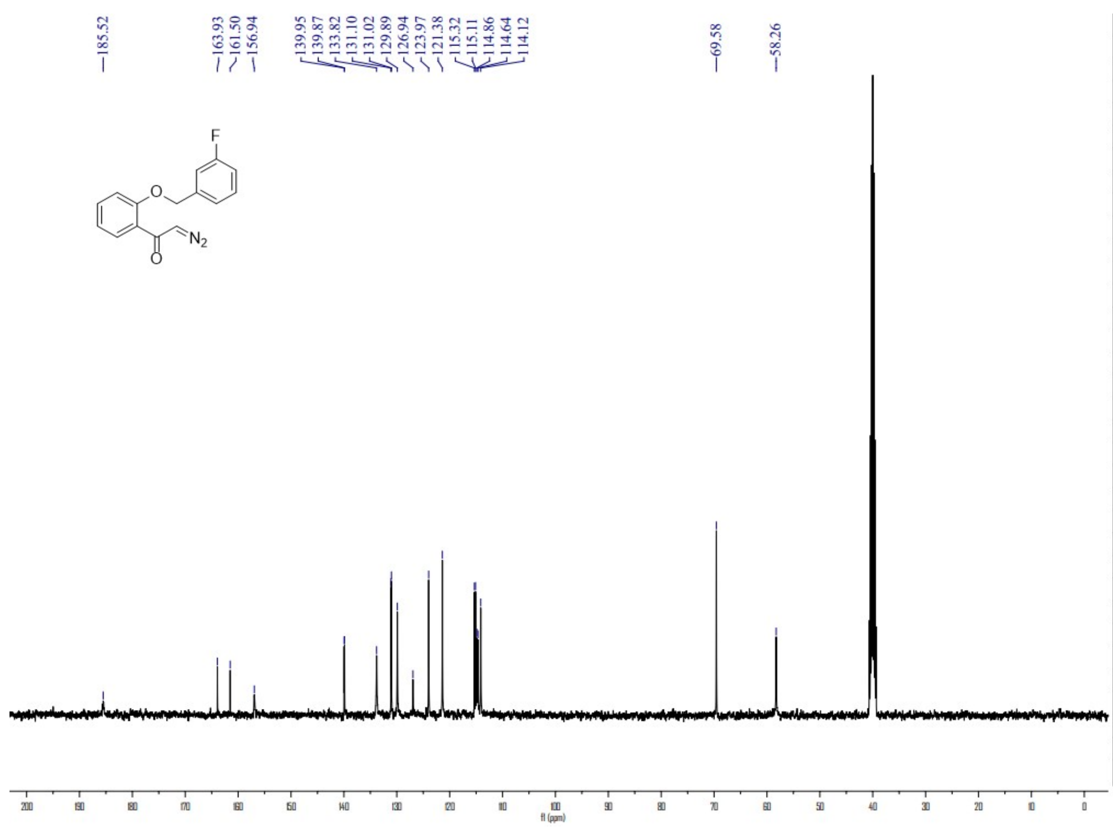


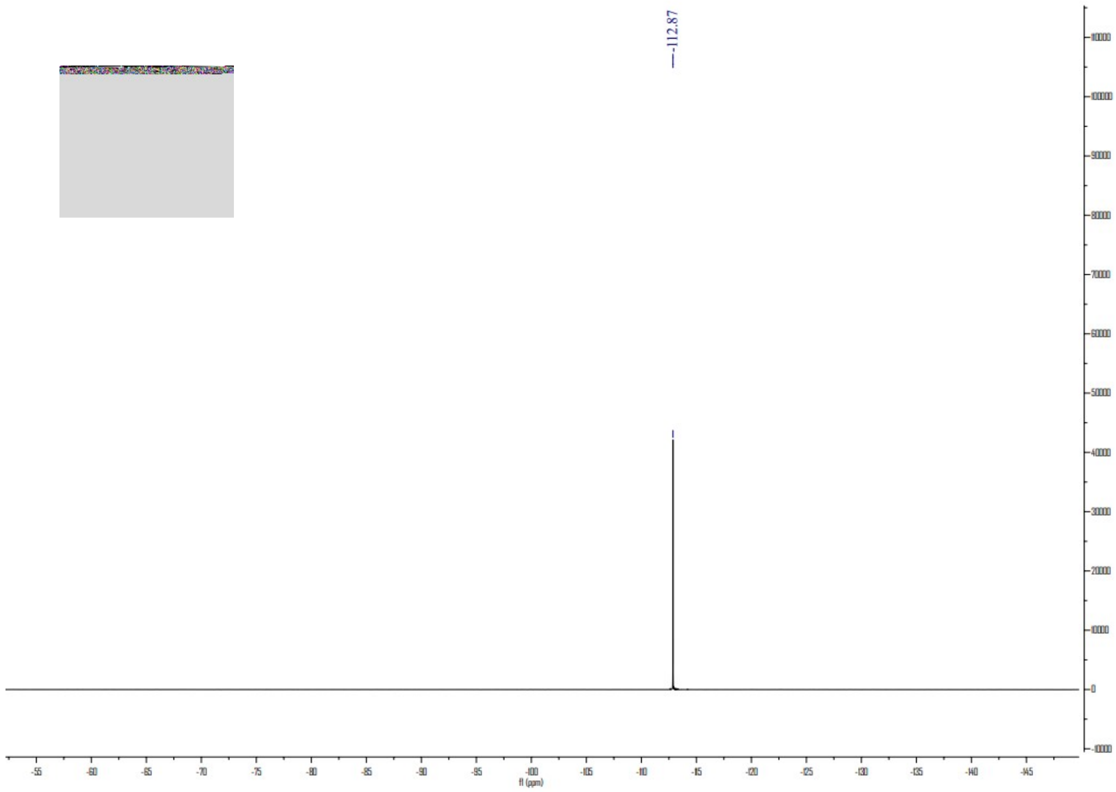


-114.22
f1 (ppm)

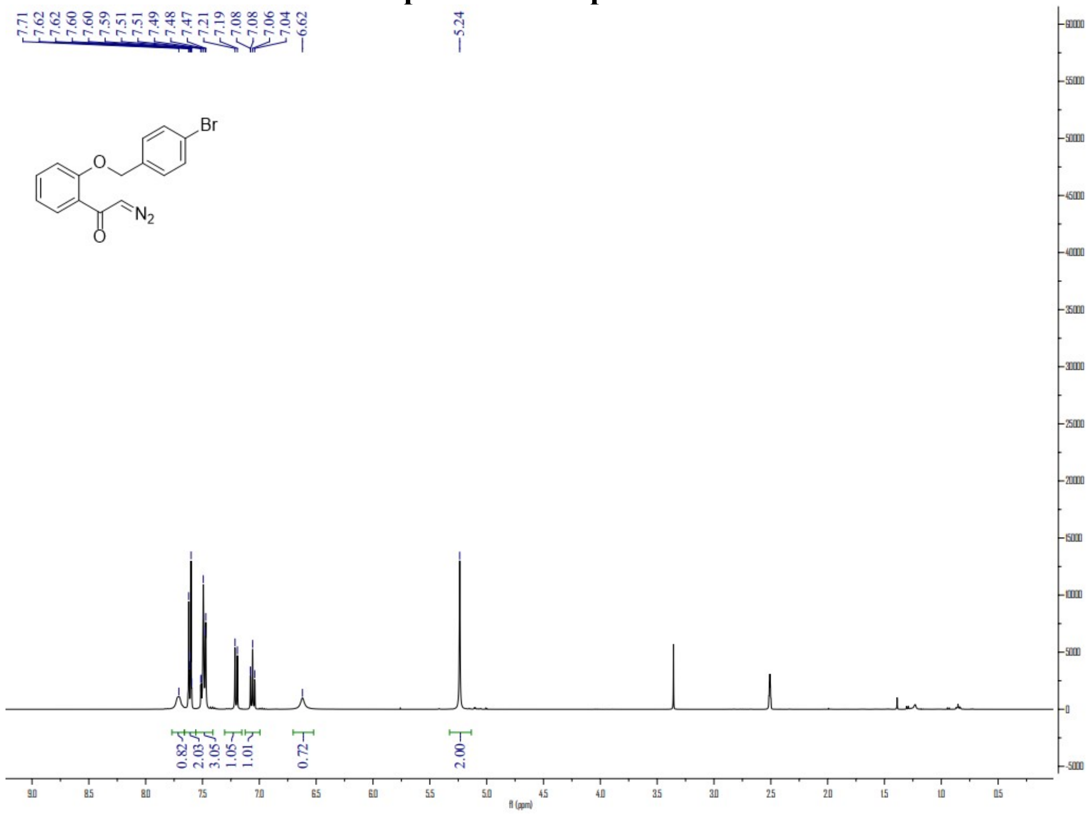
The ^{1}H NMR and ^{13}C NMR spectra of compound 1m.

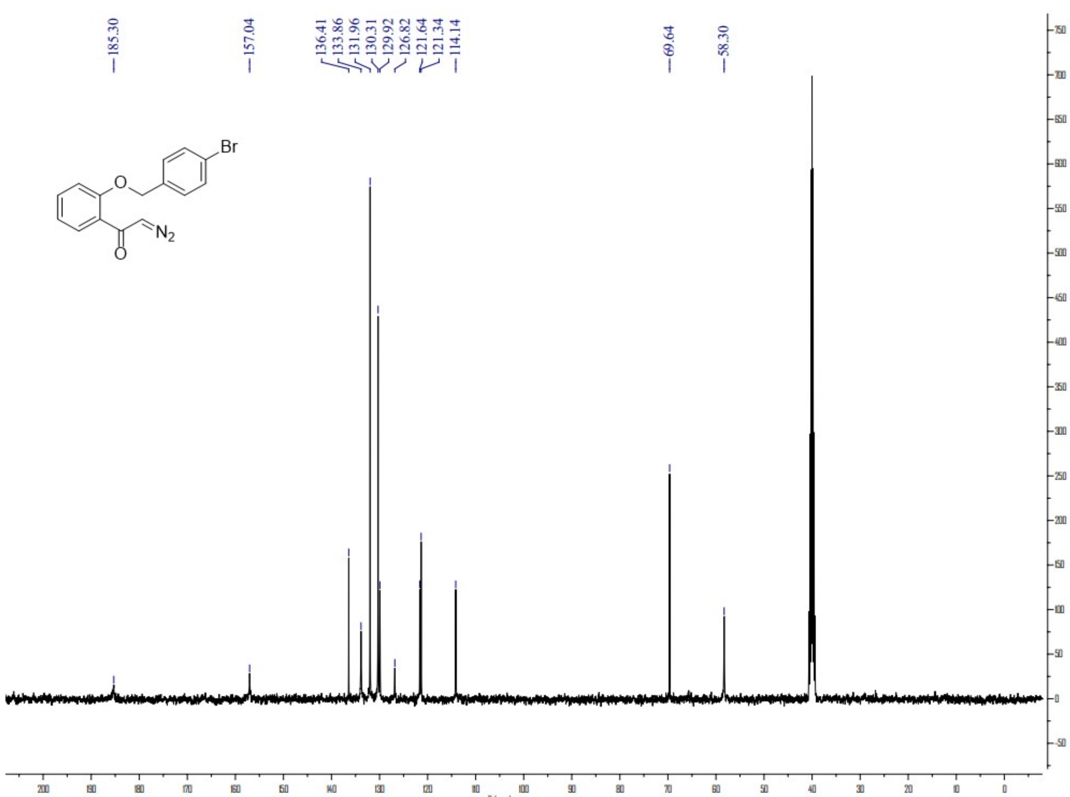




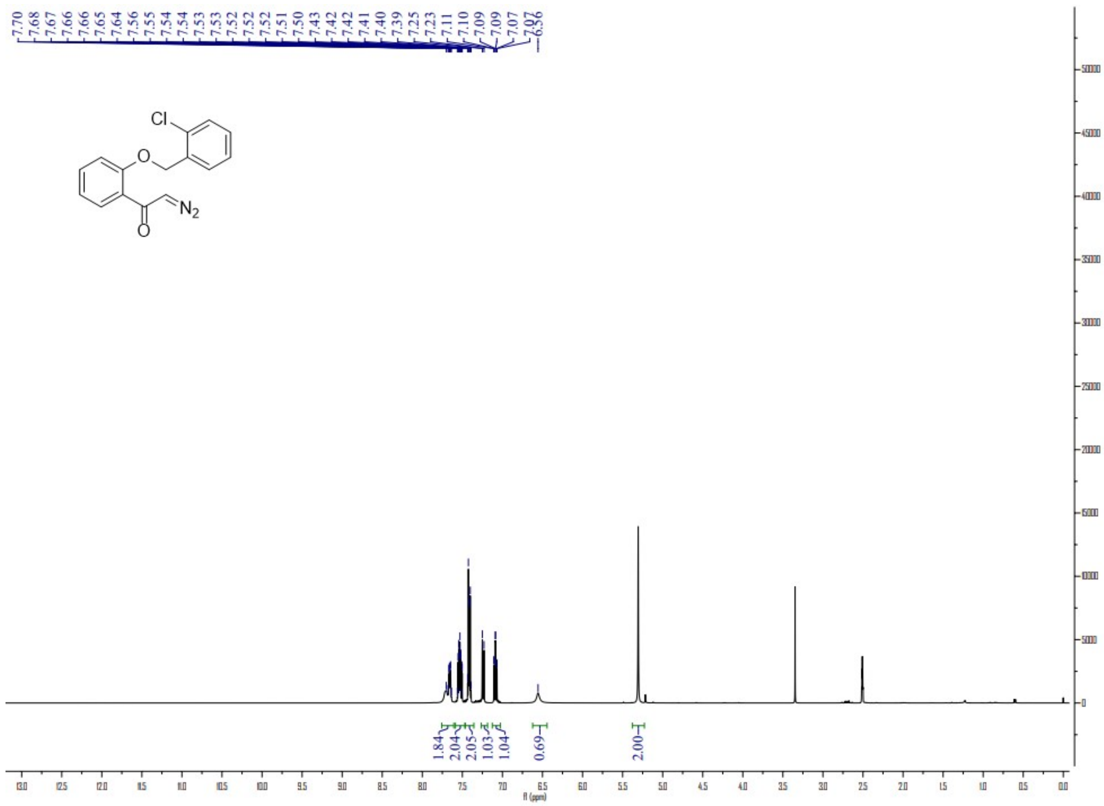

-112.87
f1 (ppm)

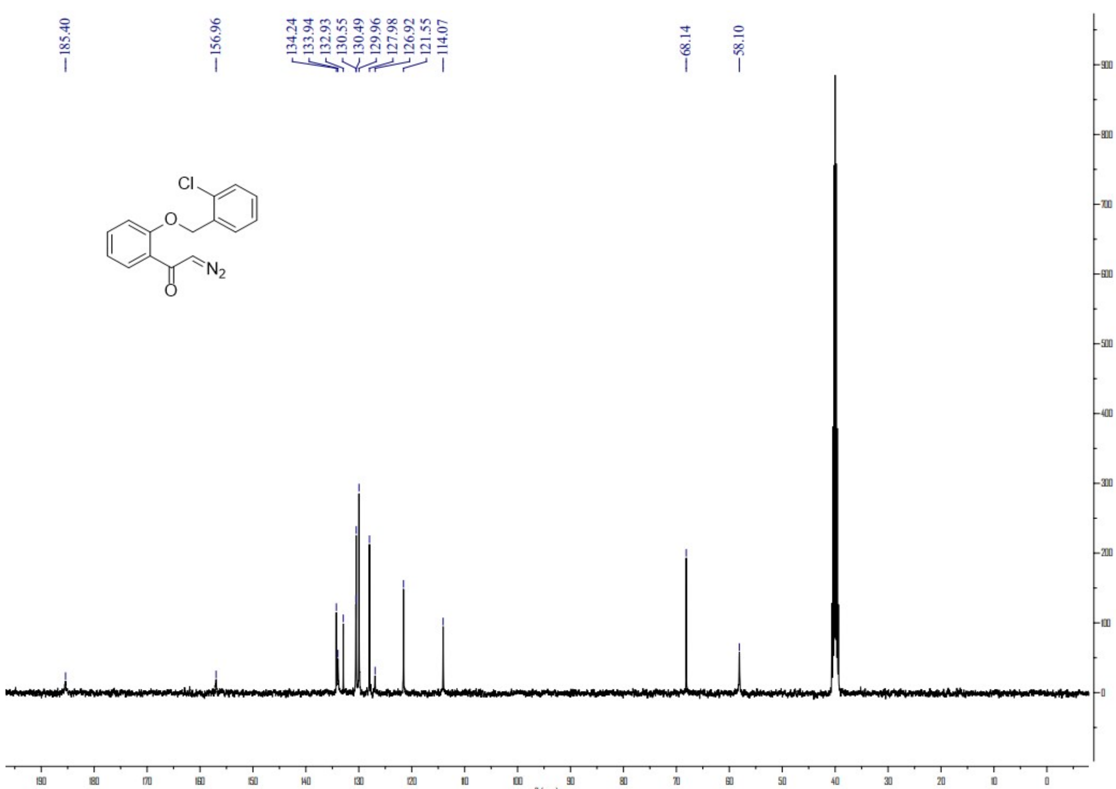
The ^{1}H NMR and ^{13}C NMR spectra of compound 1n.



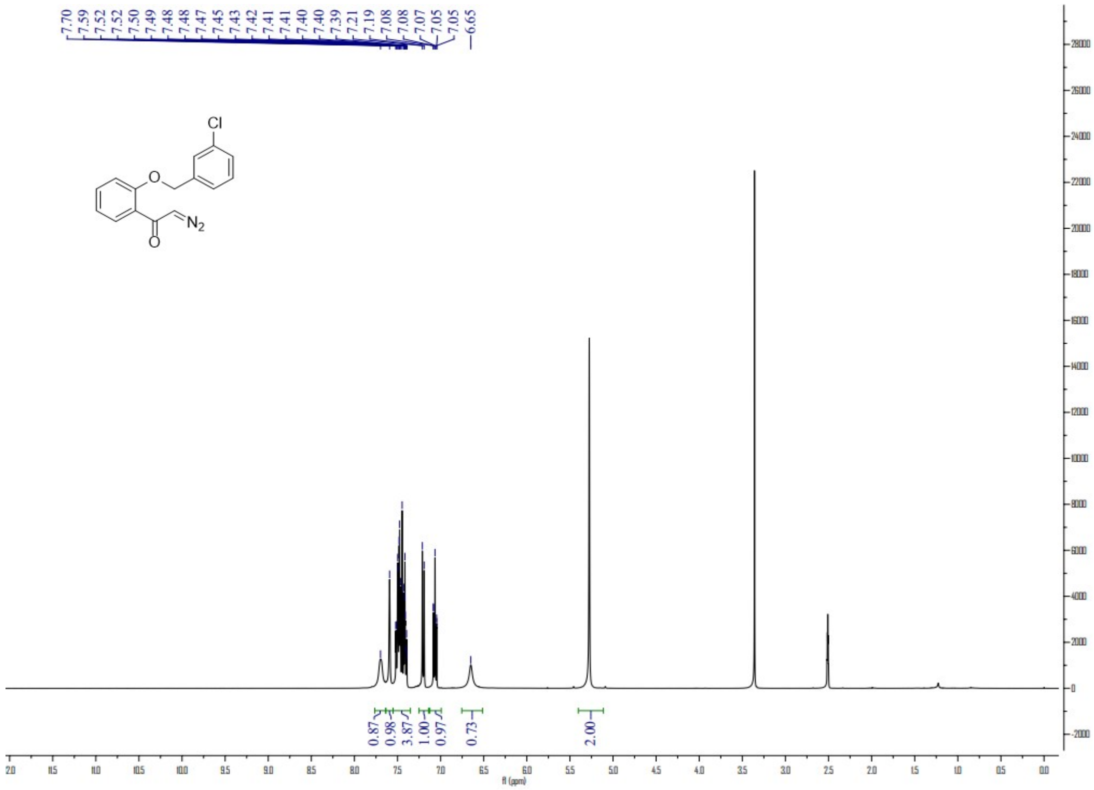


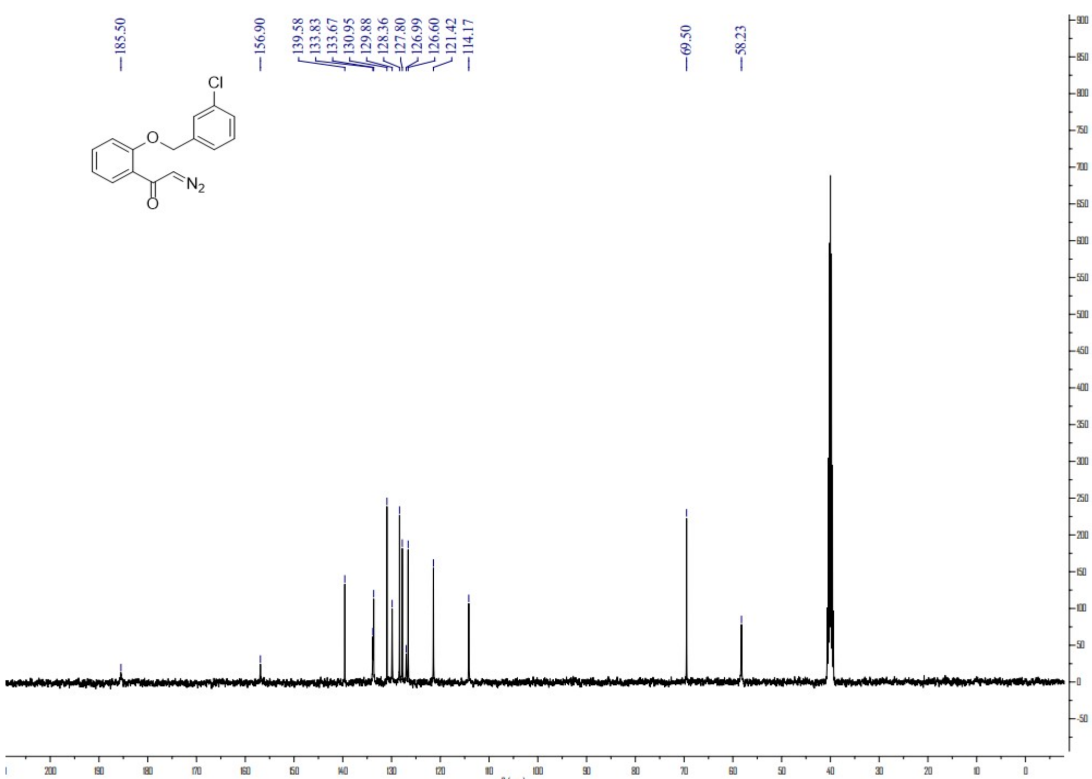
The ^{1}H NMR and ^{13}C NMR spectra of compound 1o.



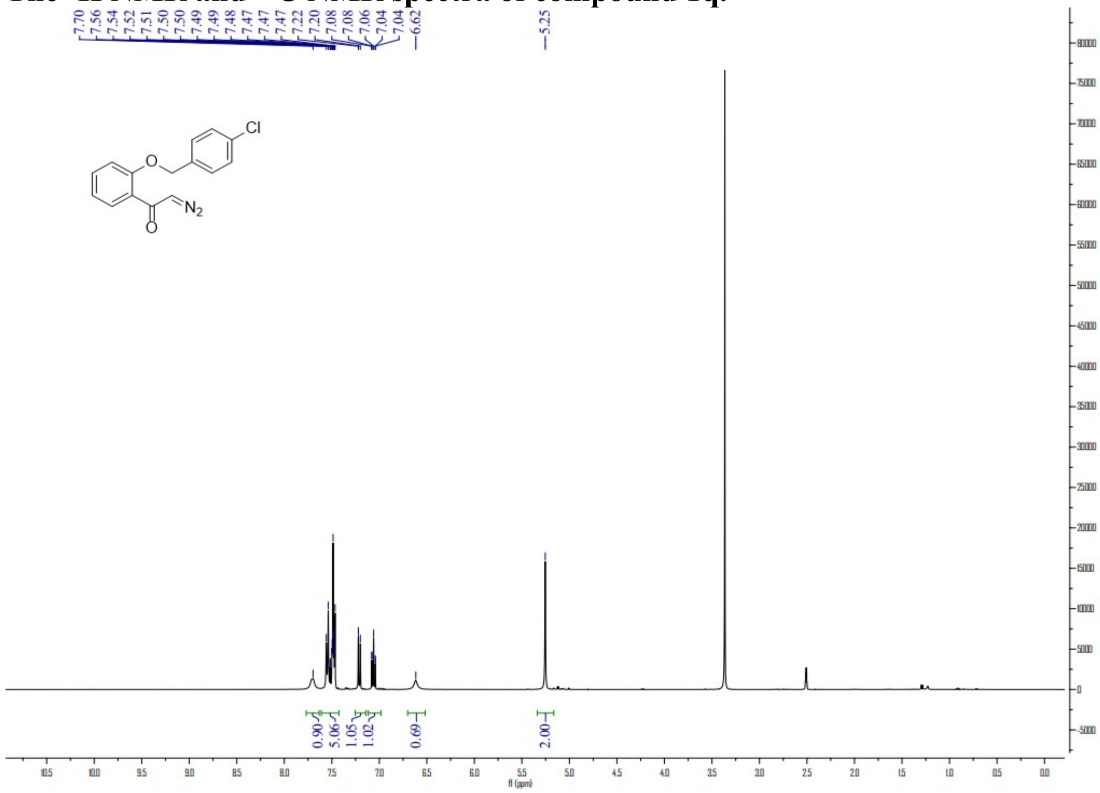


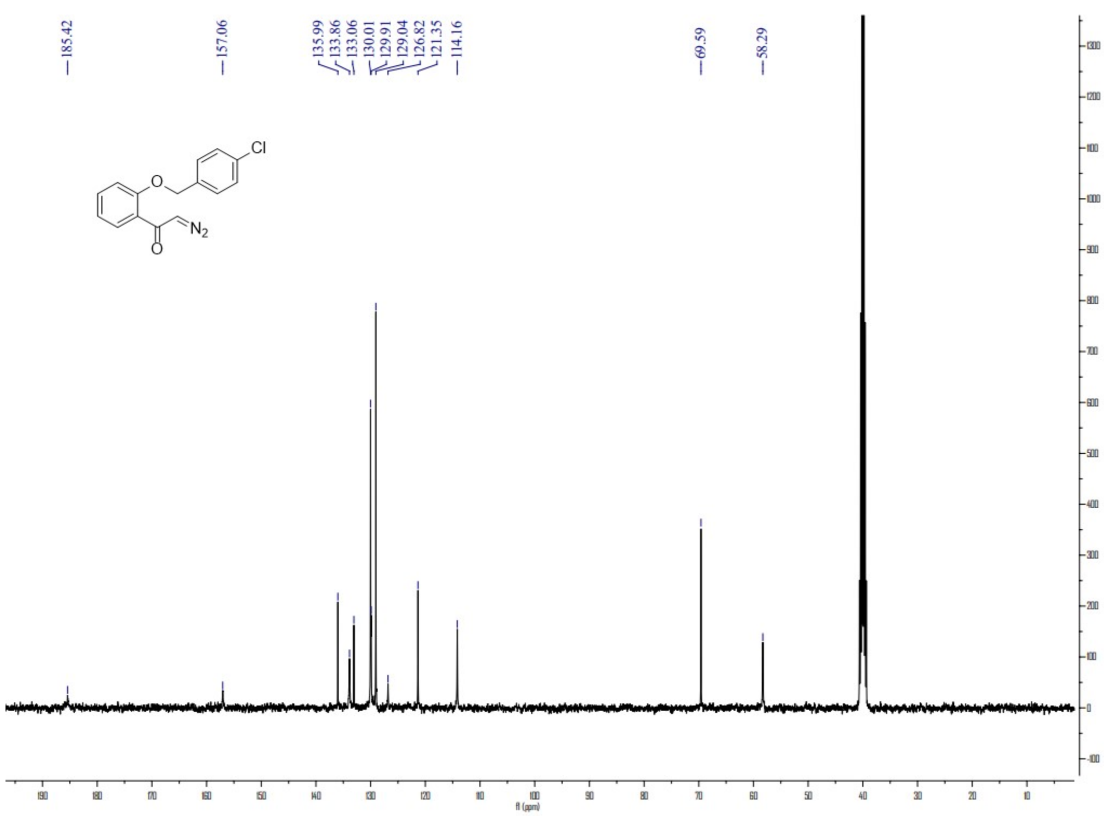
The ^{1}H NMR and ^{13}C NMR spectra of compound 1p.



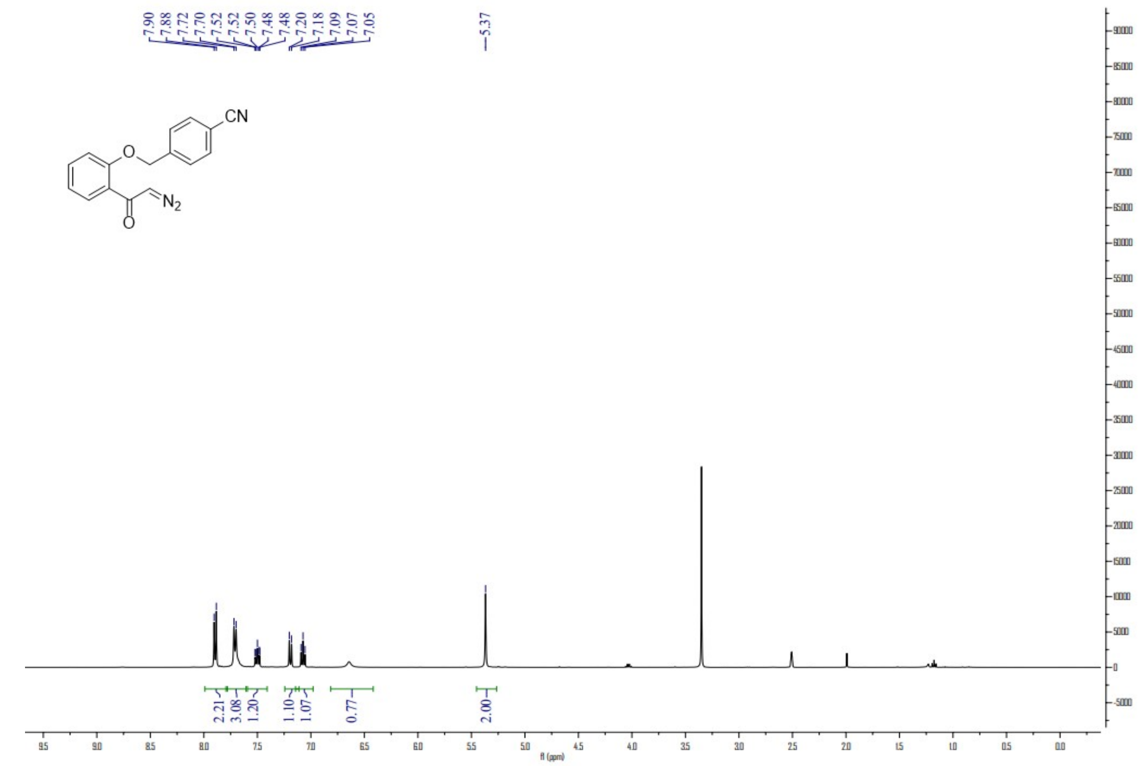


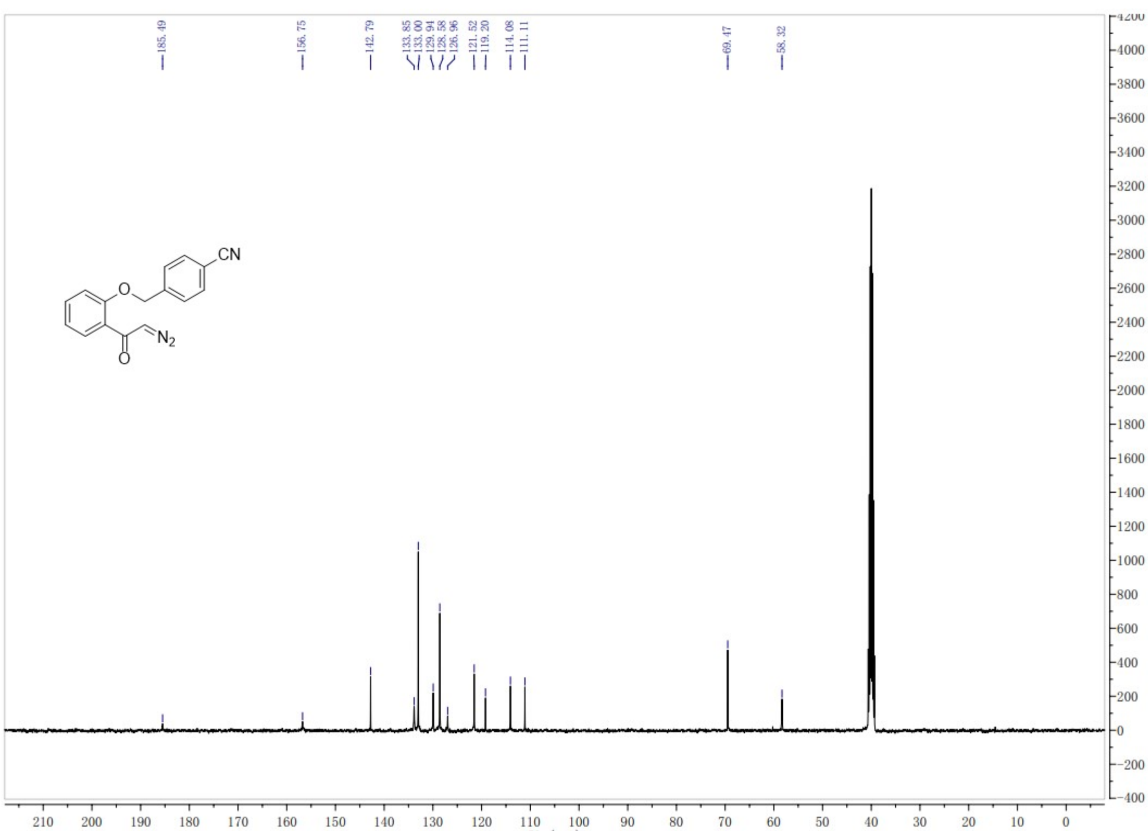
The ^{1}H NMR and ^{13}C NMR spectra of compound 1q.



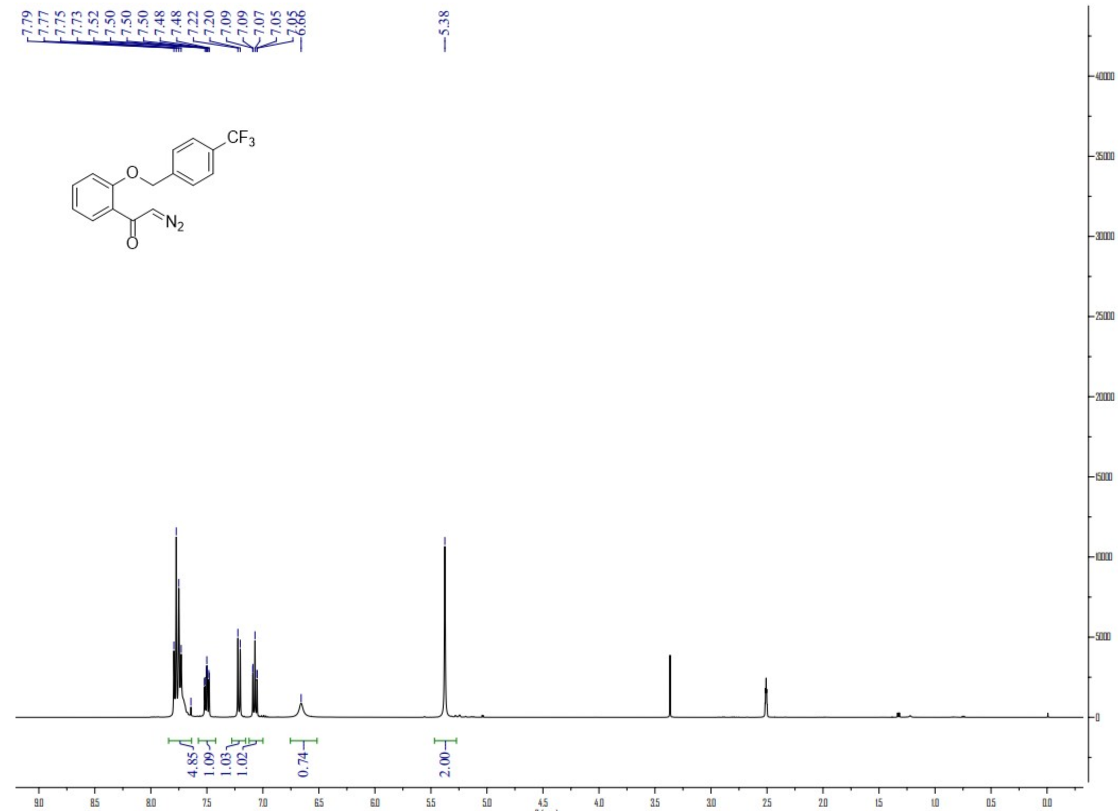


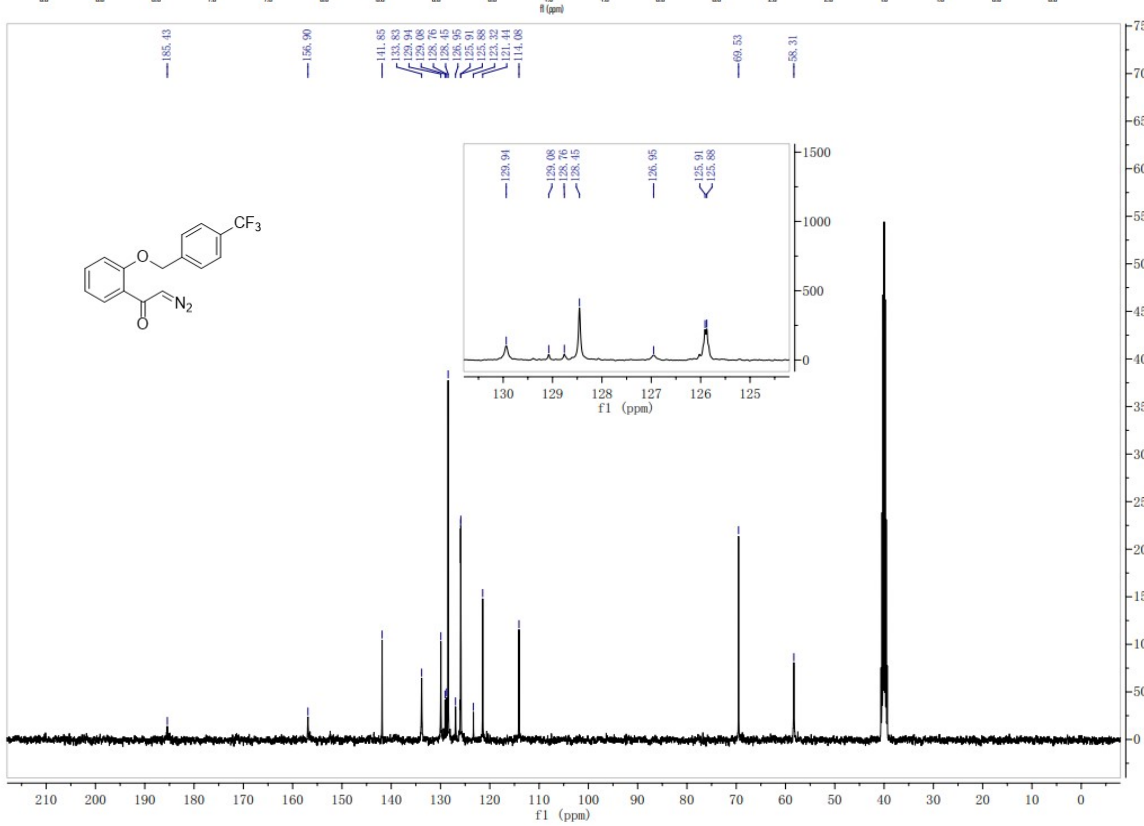
The ^{1}H NMR and ^{13}C NMR spectra of compound 1r.

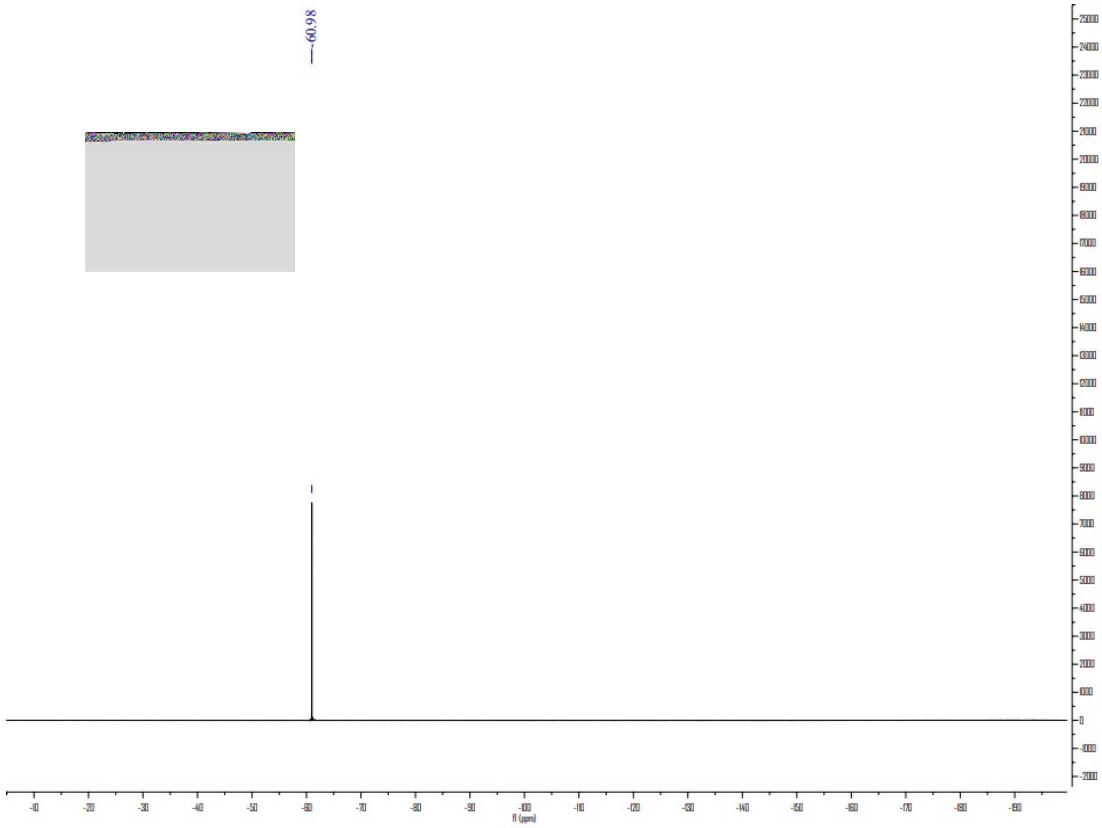




The ^{1}H NMR and ^{13}C NMR spectra of compound 1s.

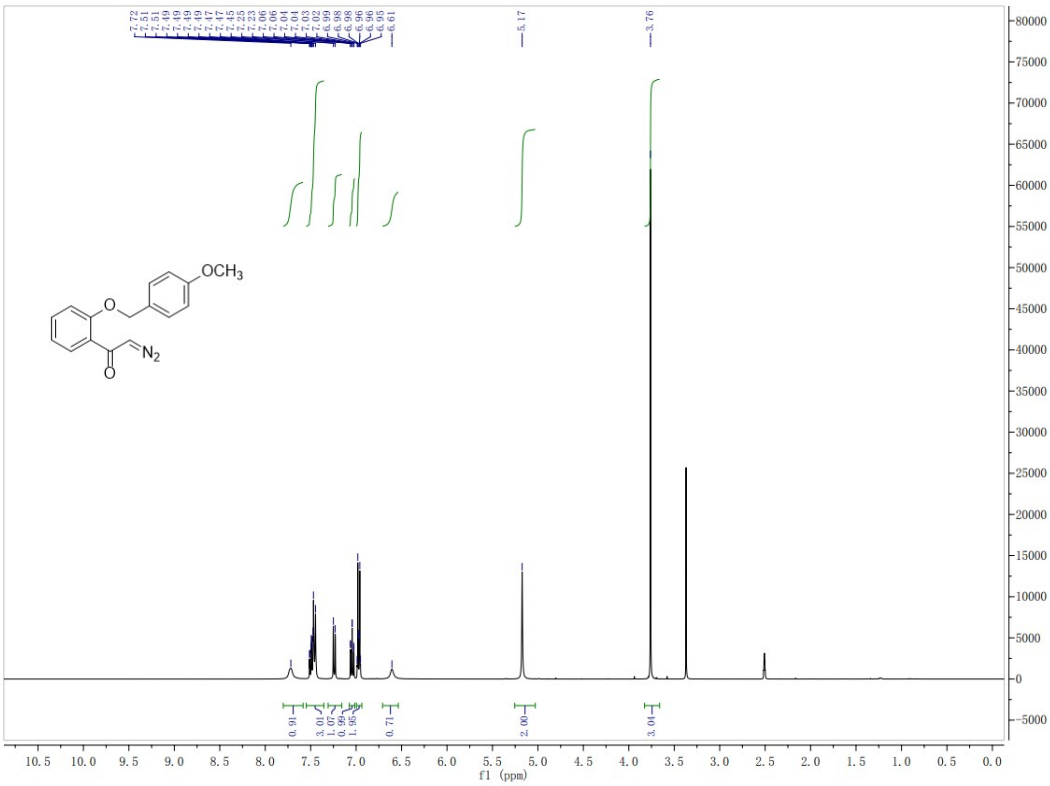


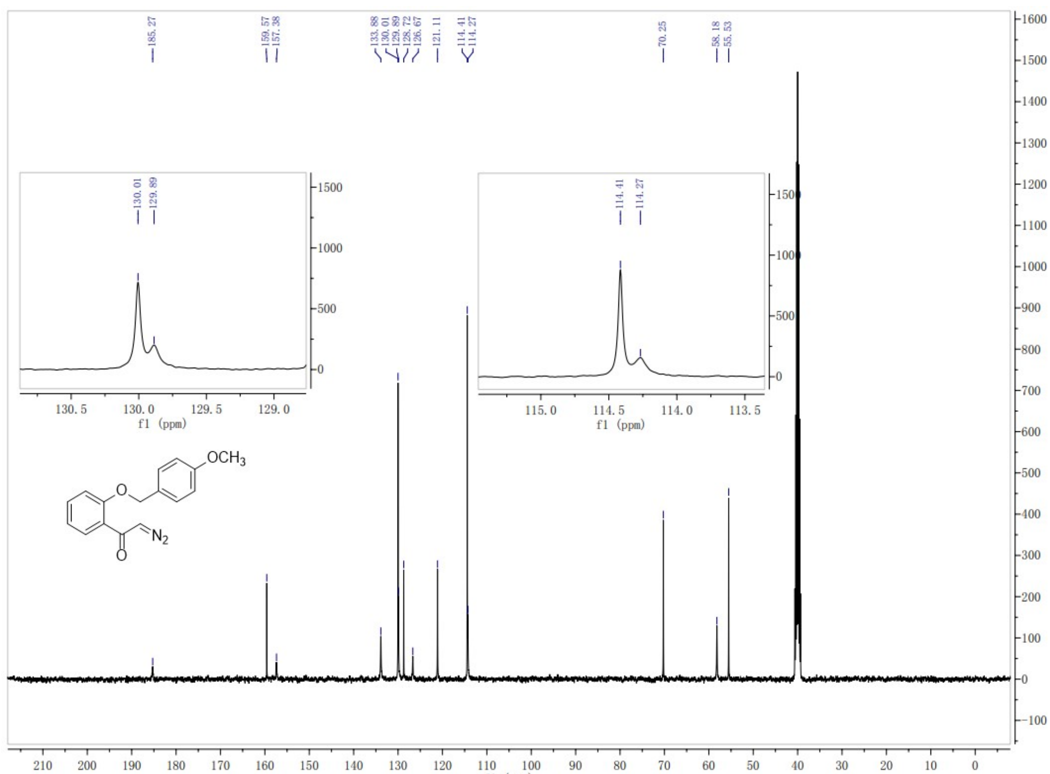




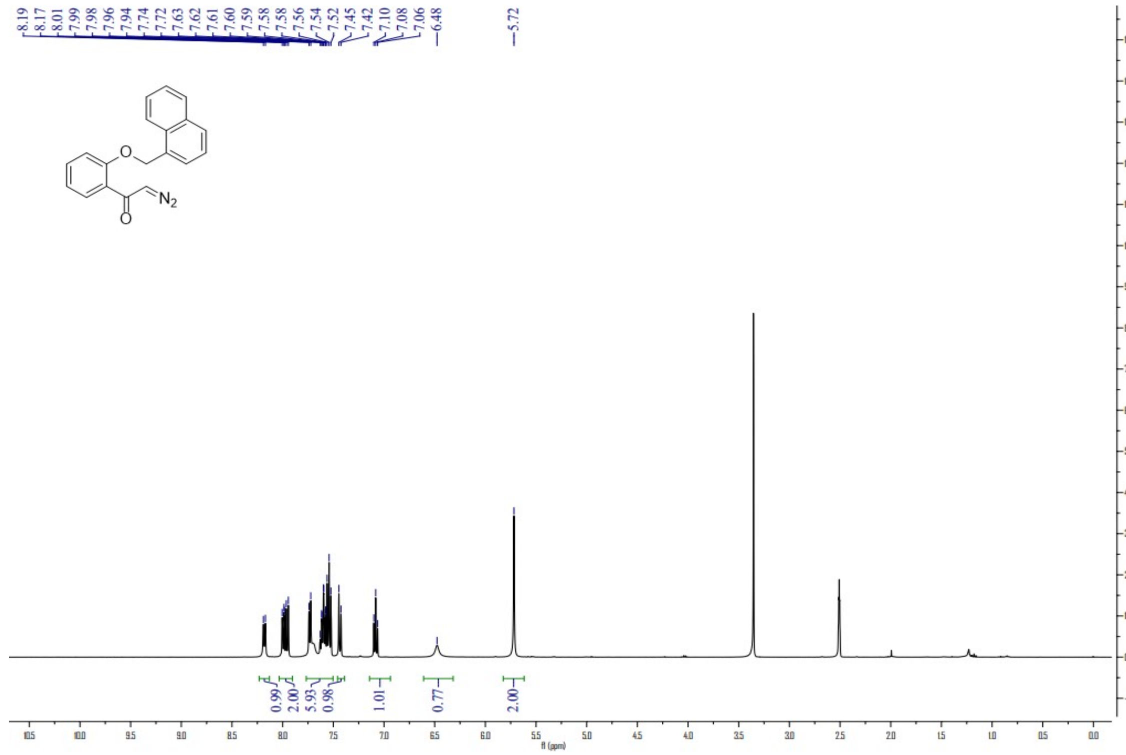
—-60.98
f1 (ppm)

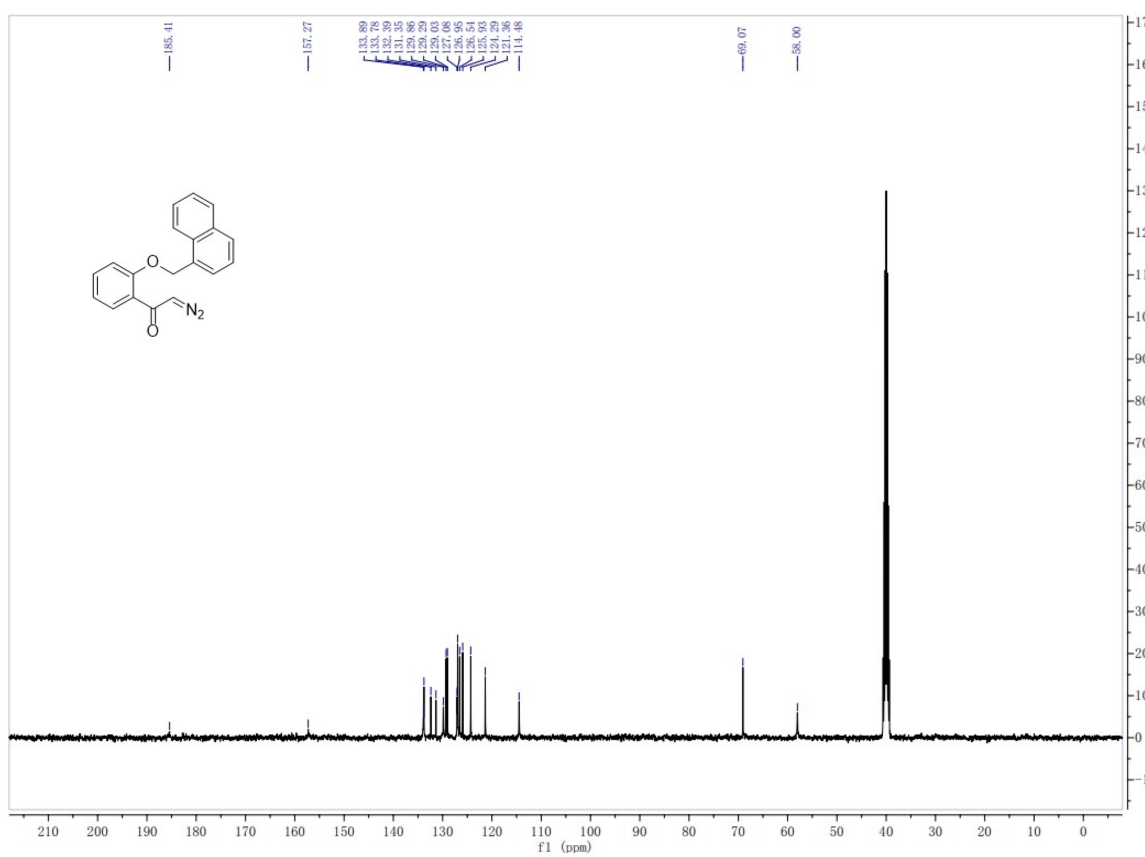
The ^{1}H NMR and ^{13}C NMR spectra of compound 1t.



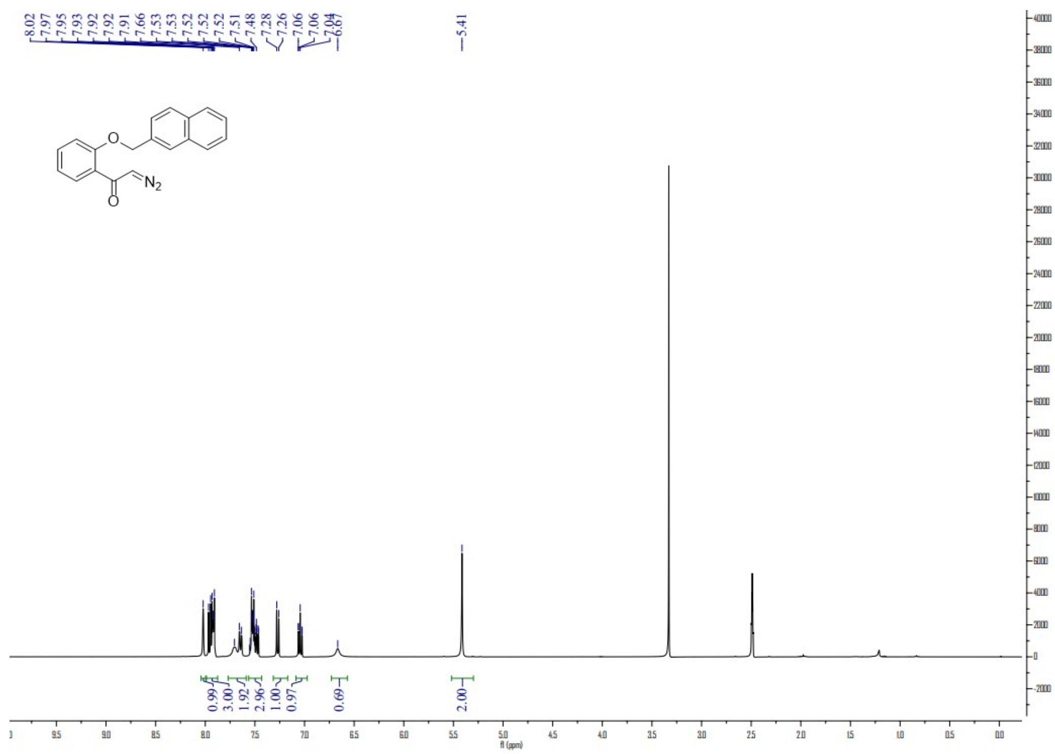


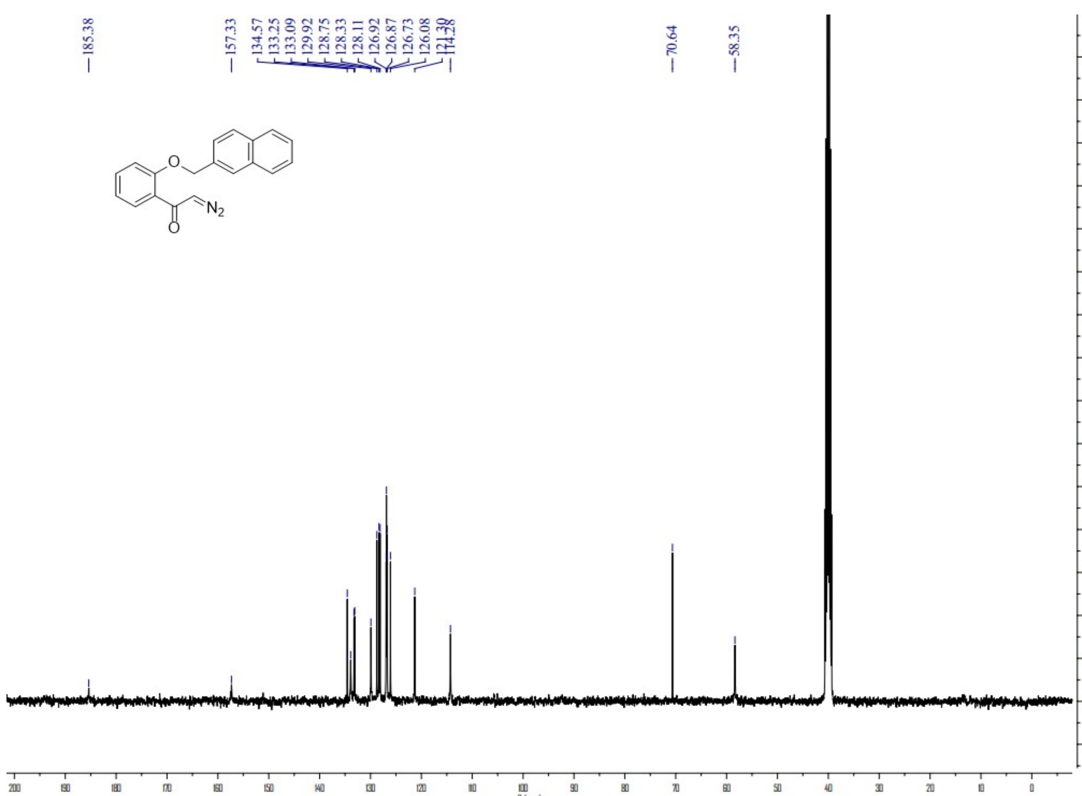
The ^{1}H NMR and ^{13}C NMR spectra of compound 1u.



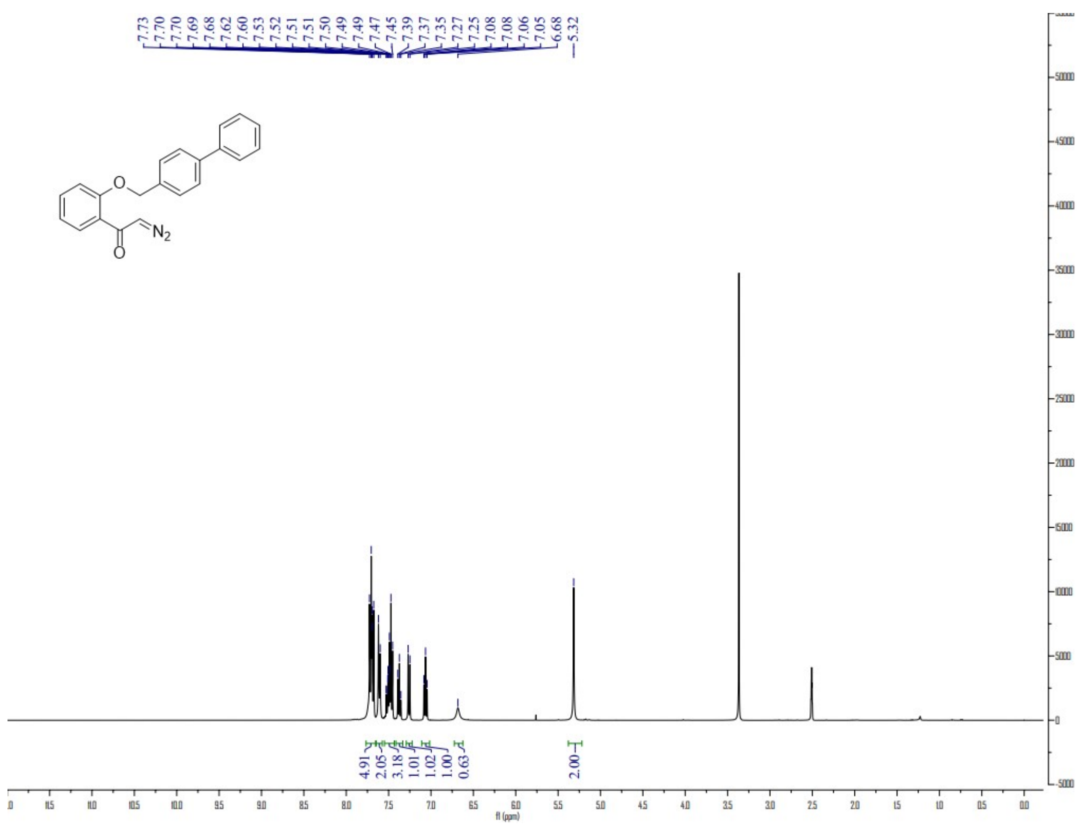


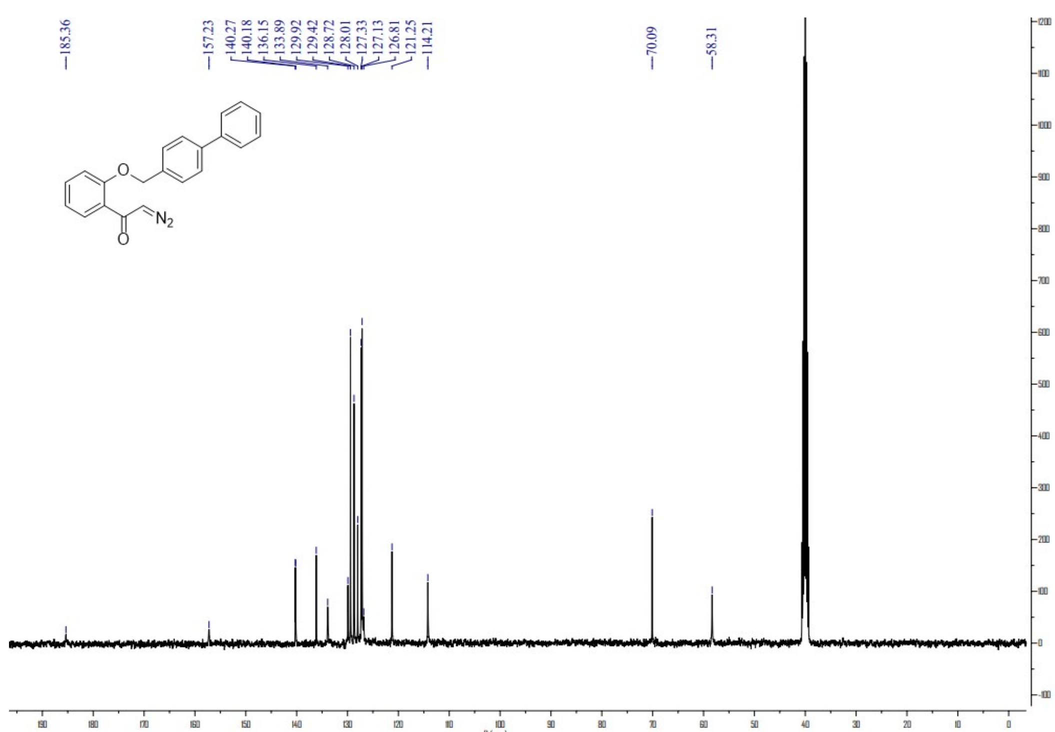
The ^{1}H NMR and ^{13}C NMR spectra of compound 1v.



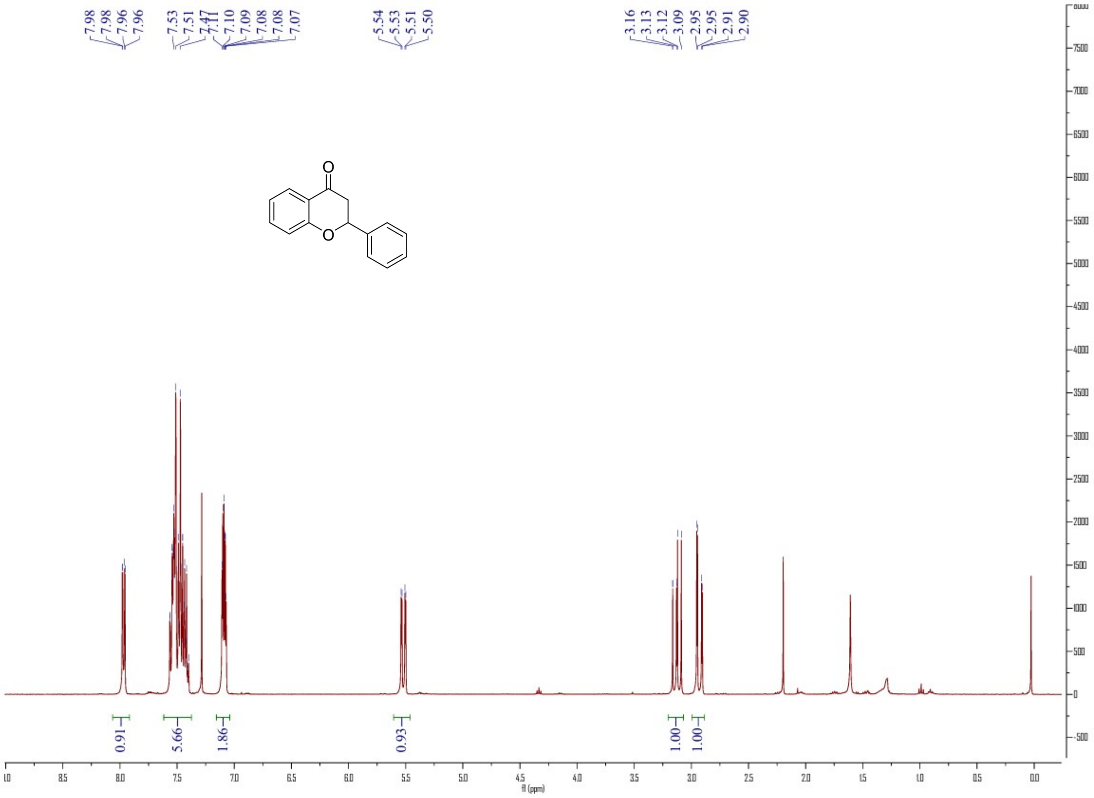


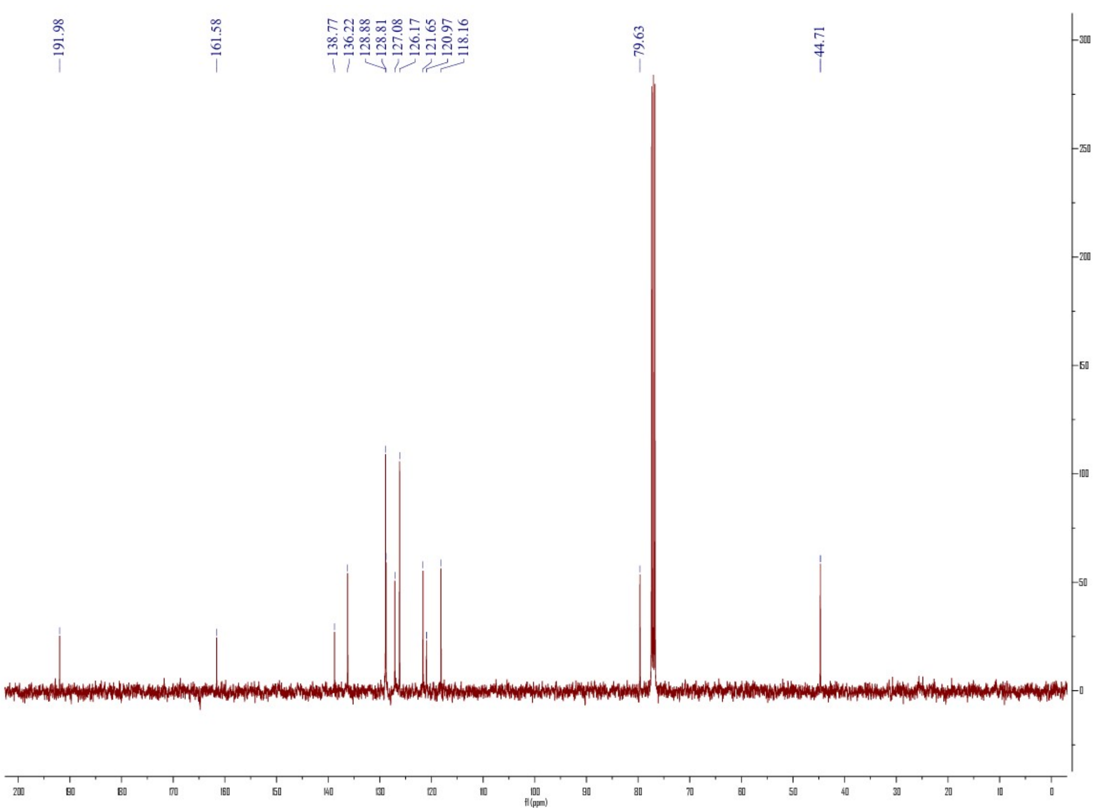
The ^{1}H NMR and ^{13}C NMR spectra of compound 1w.



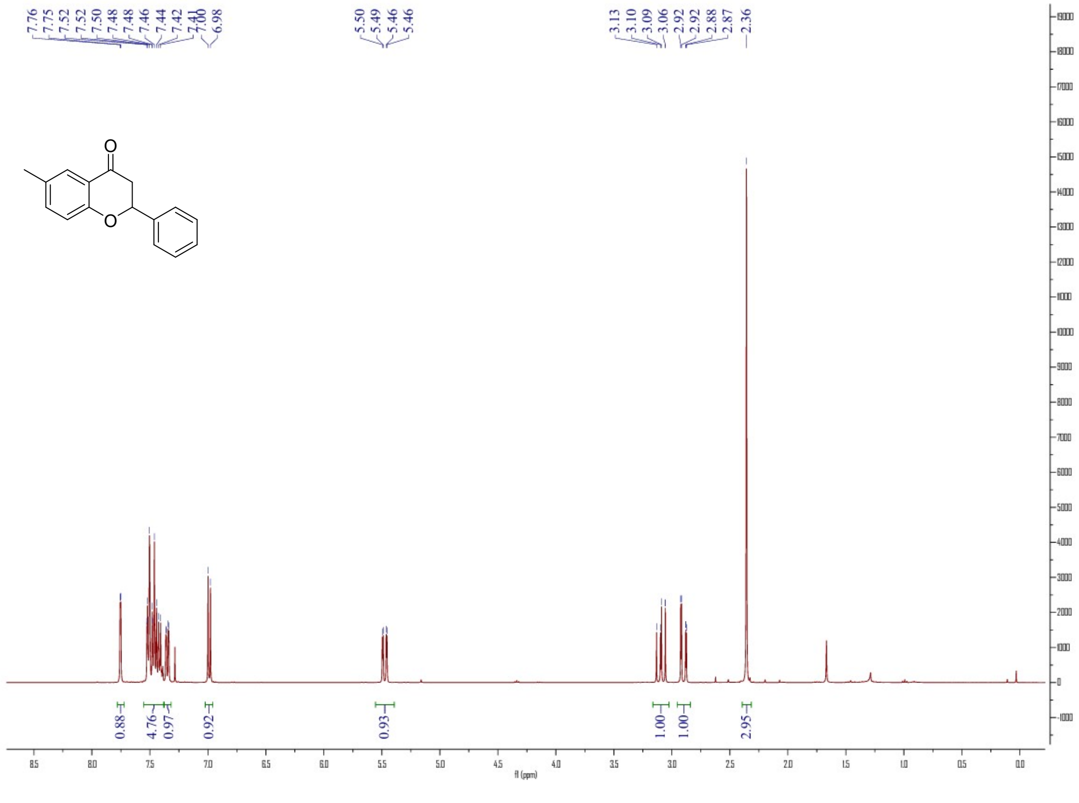


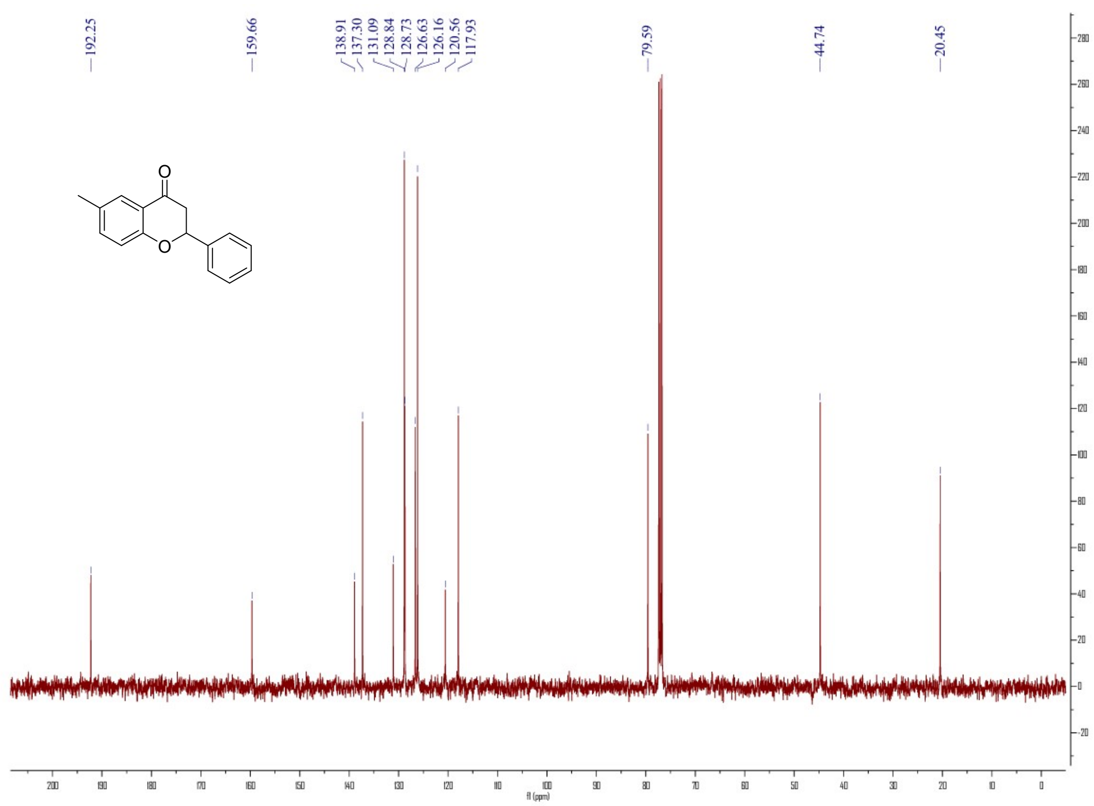
The ^{1}H NMR and ^{13}C NMR spectra of compound 2a.



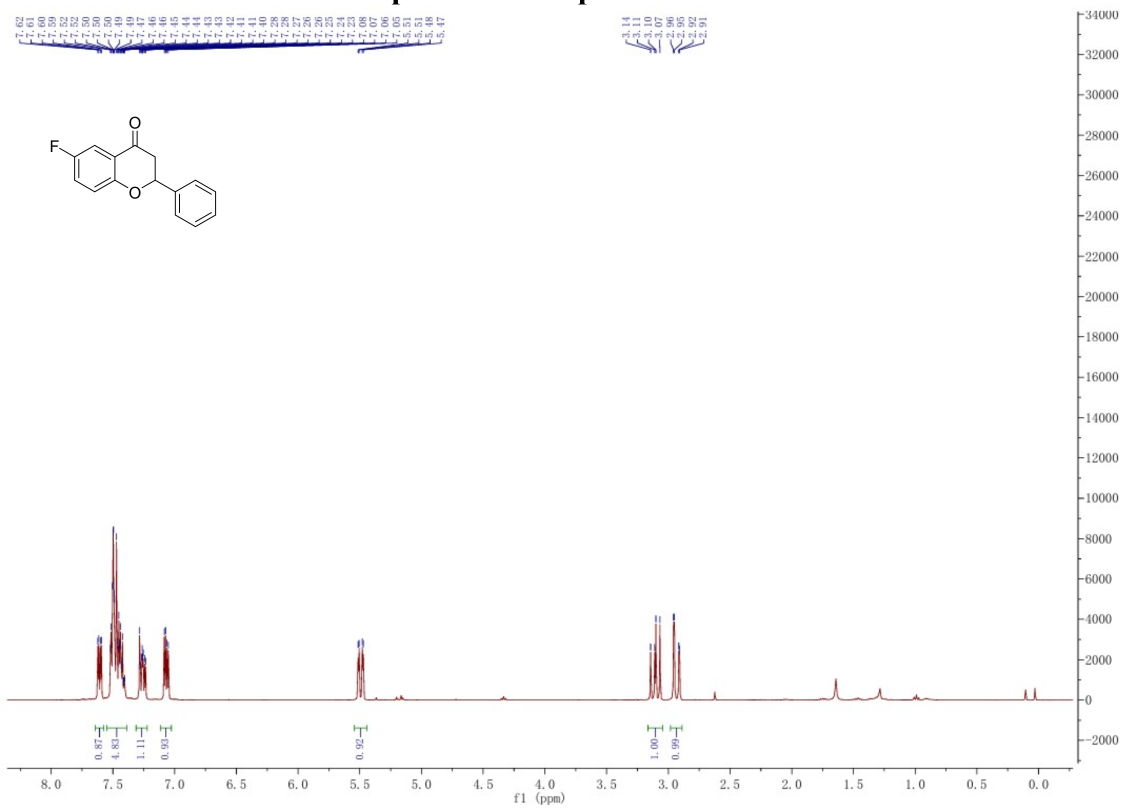


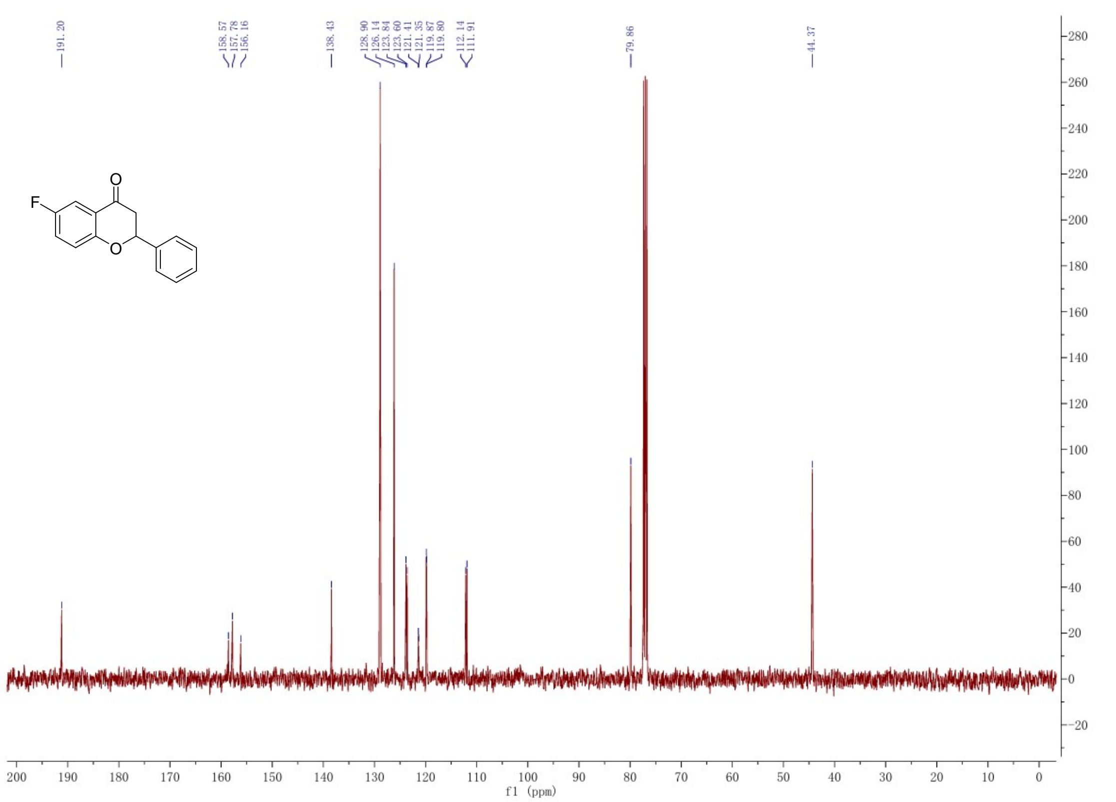
The ^{1}H NMR and ^{13}C NMR spectra of compound 2b.



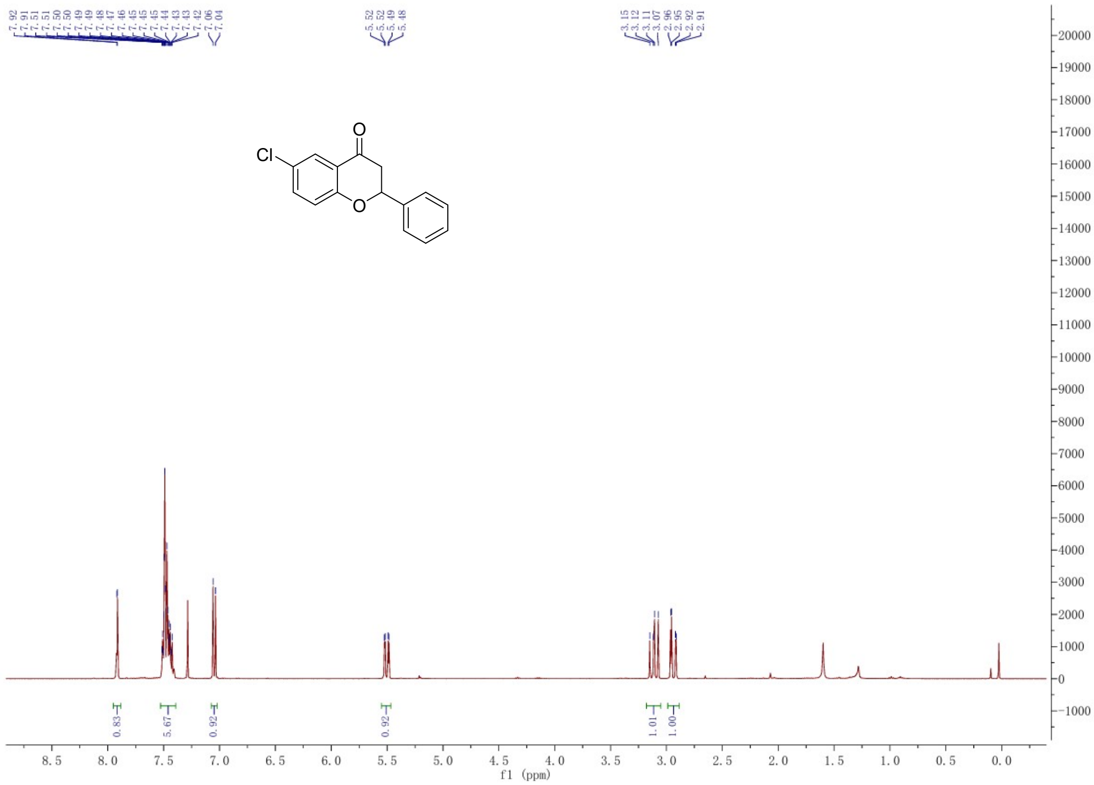


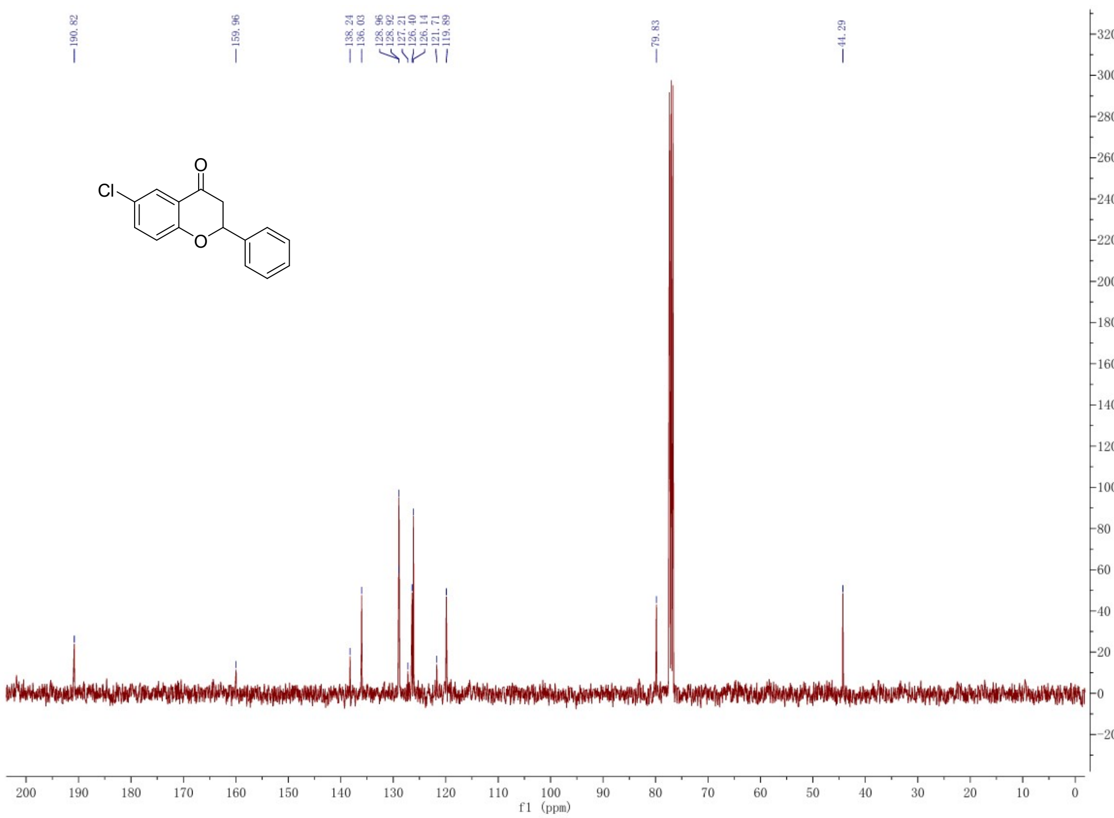
The ^{1}H NMR and ^{13}C NMR spectra of compound 2c.



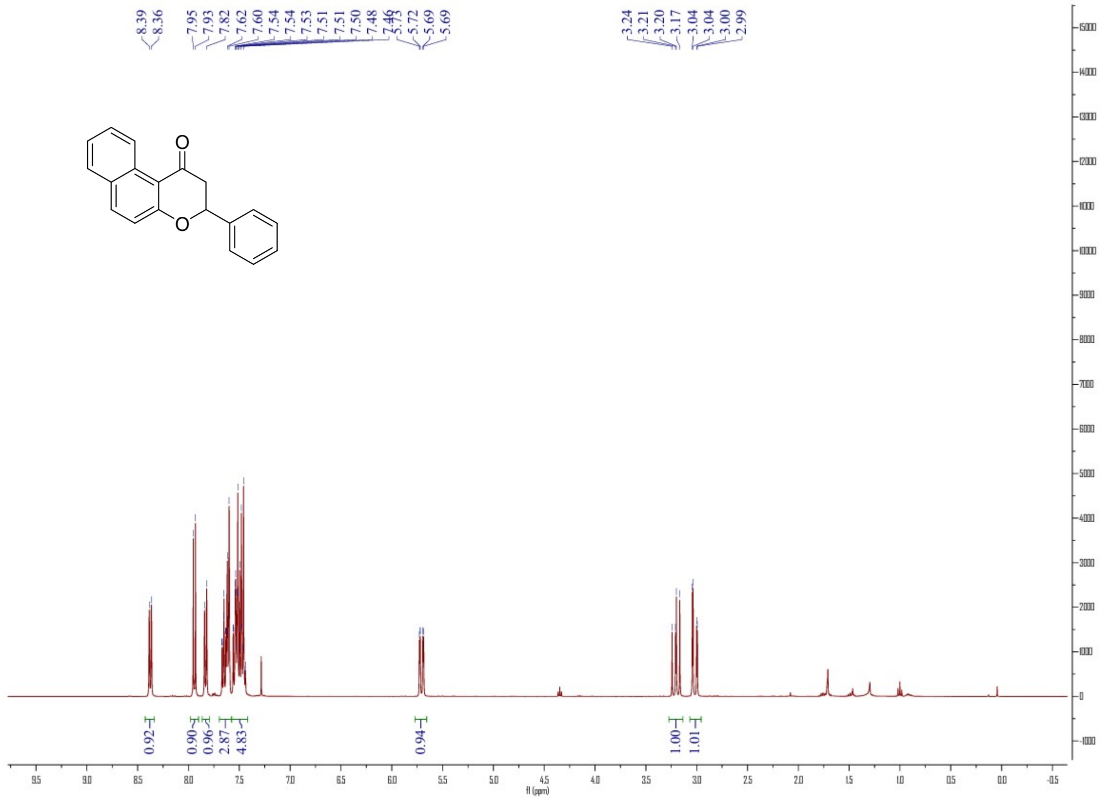


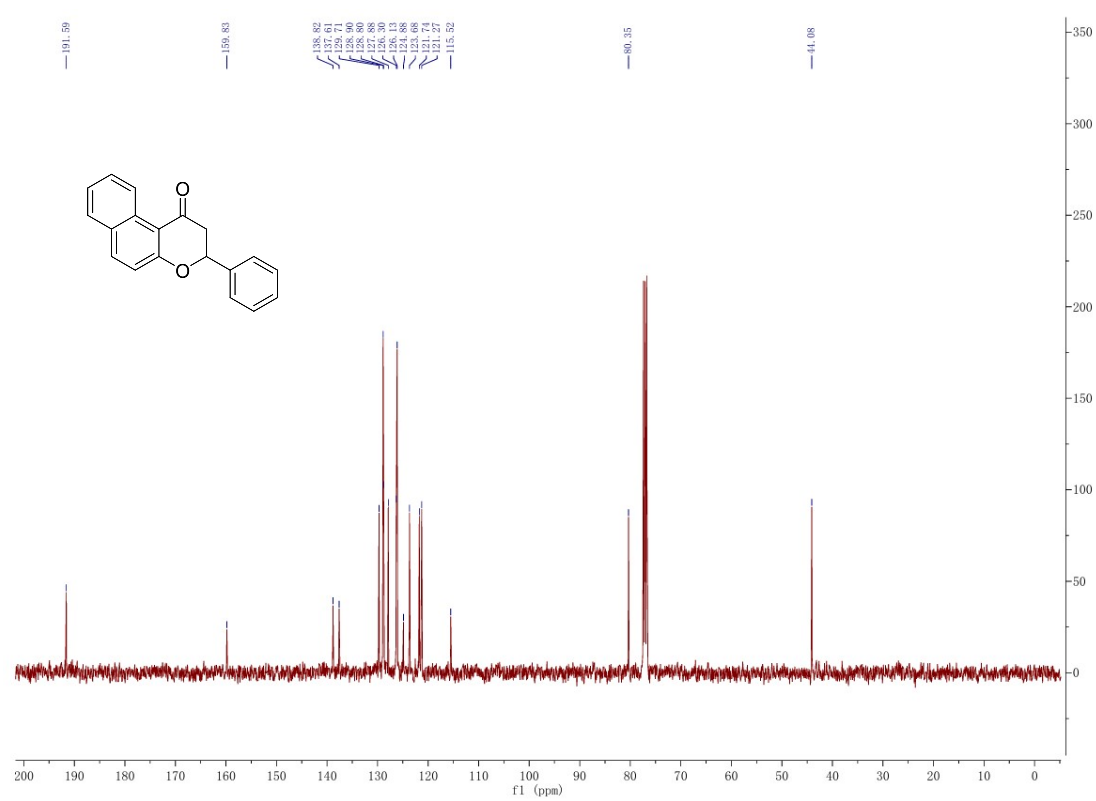
The ^{1}H NMR and ^{13}C NMR spectra of compound 2d.



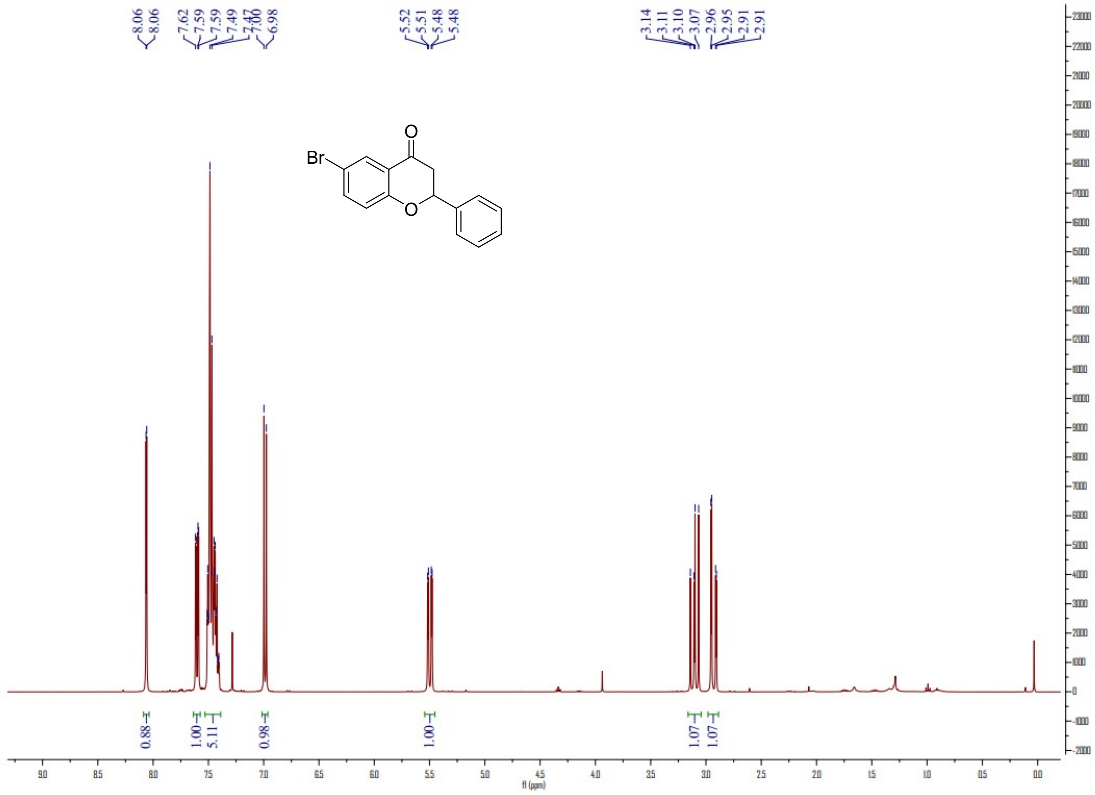


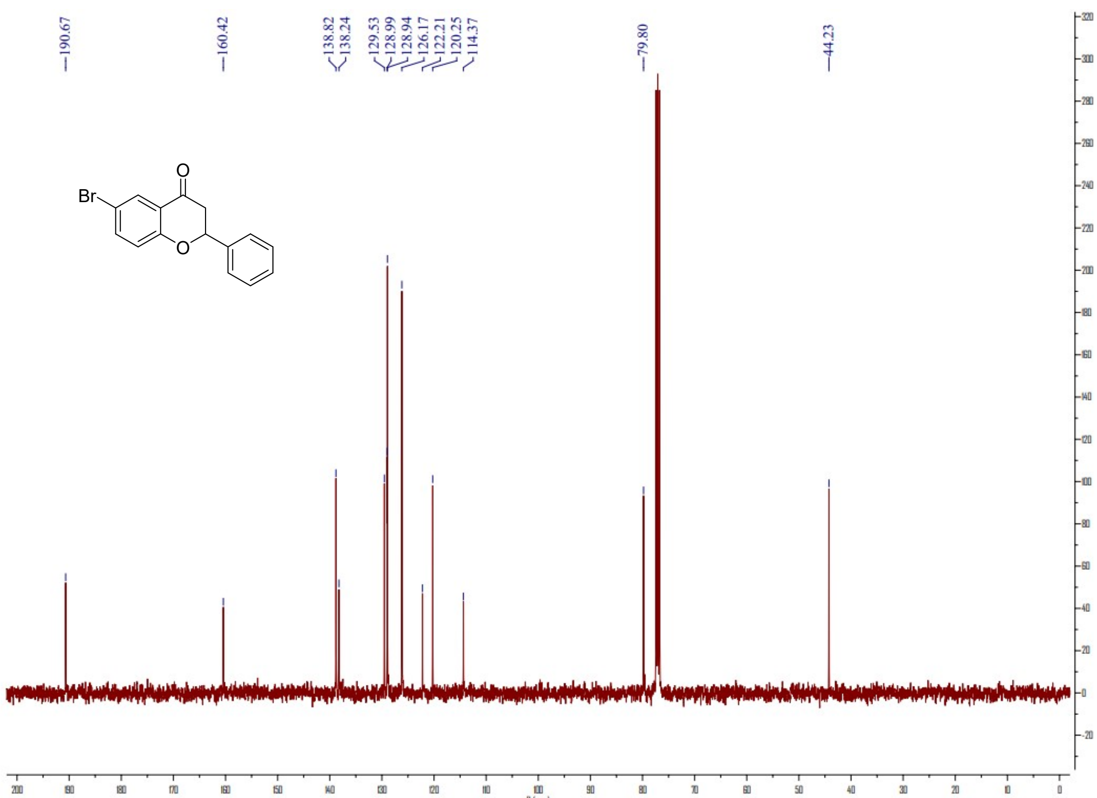
The ^{1}H NMR and ^{13}C NMR spectra of compound 2e.



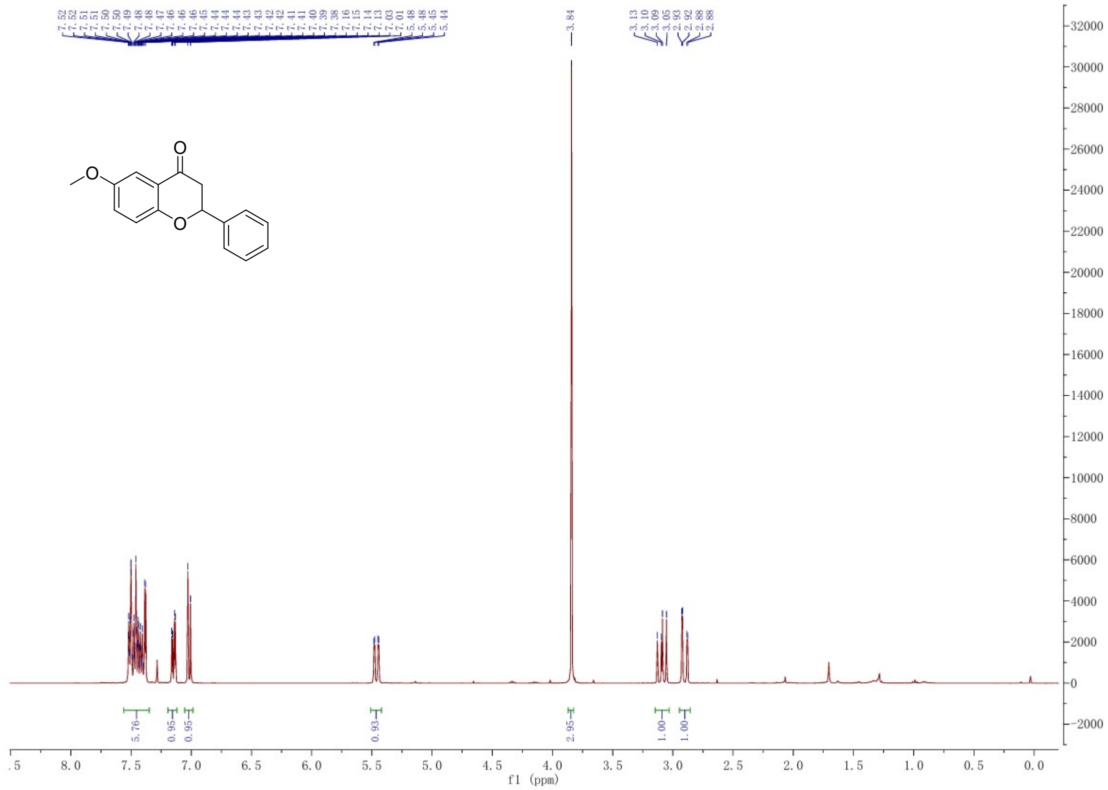


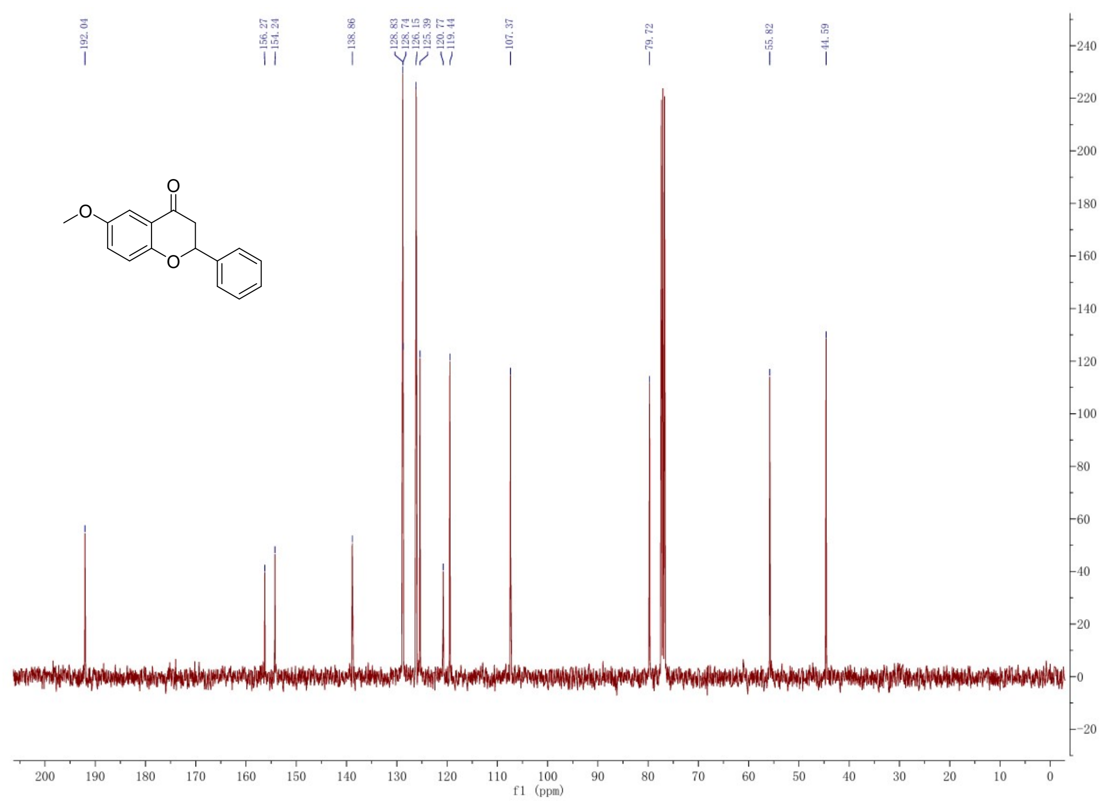
The ^{1}H NMR and ^{13}C NMR spectra of compound 2f.



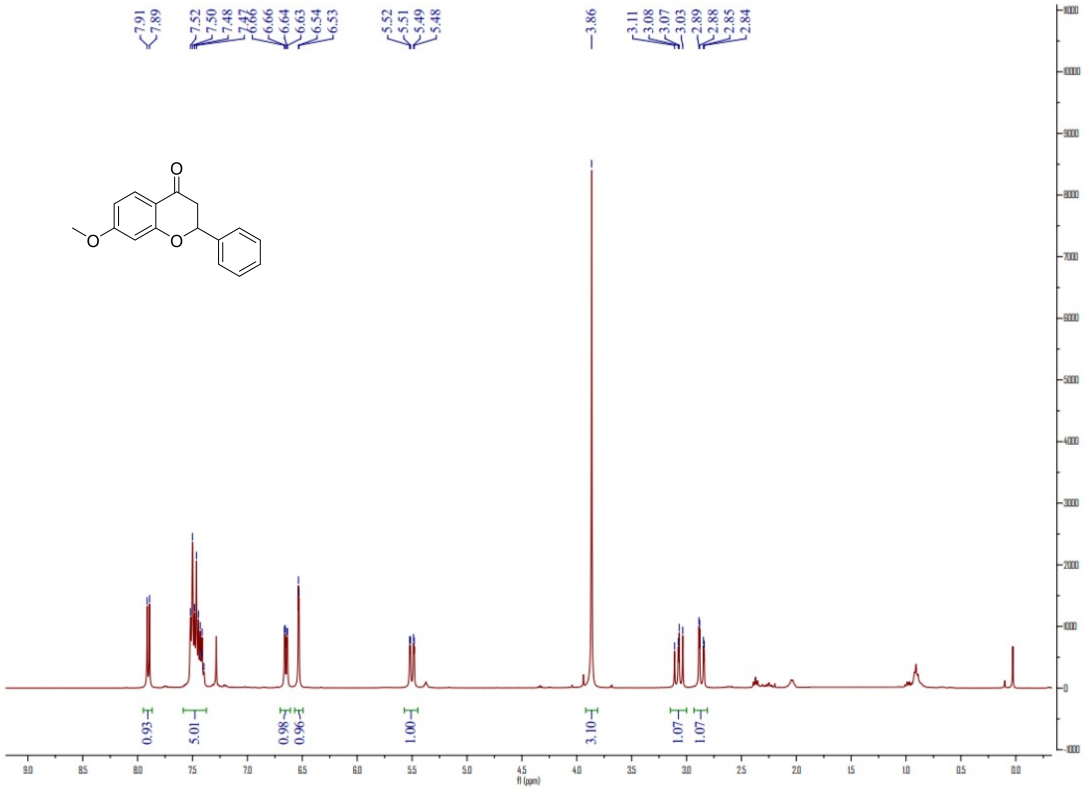


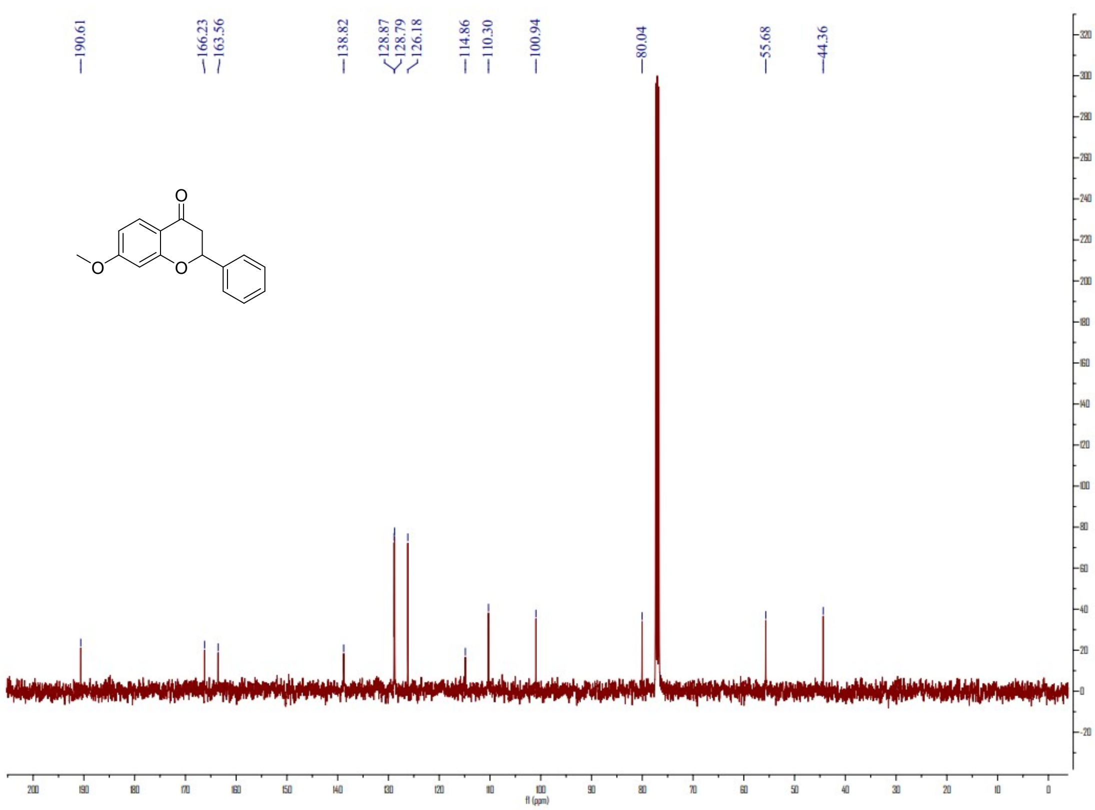
The ^{1}H NMR and ^{13}C NMR spectra of compound 2g.



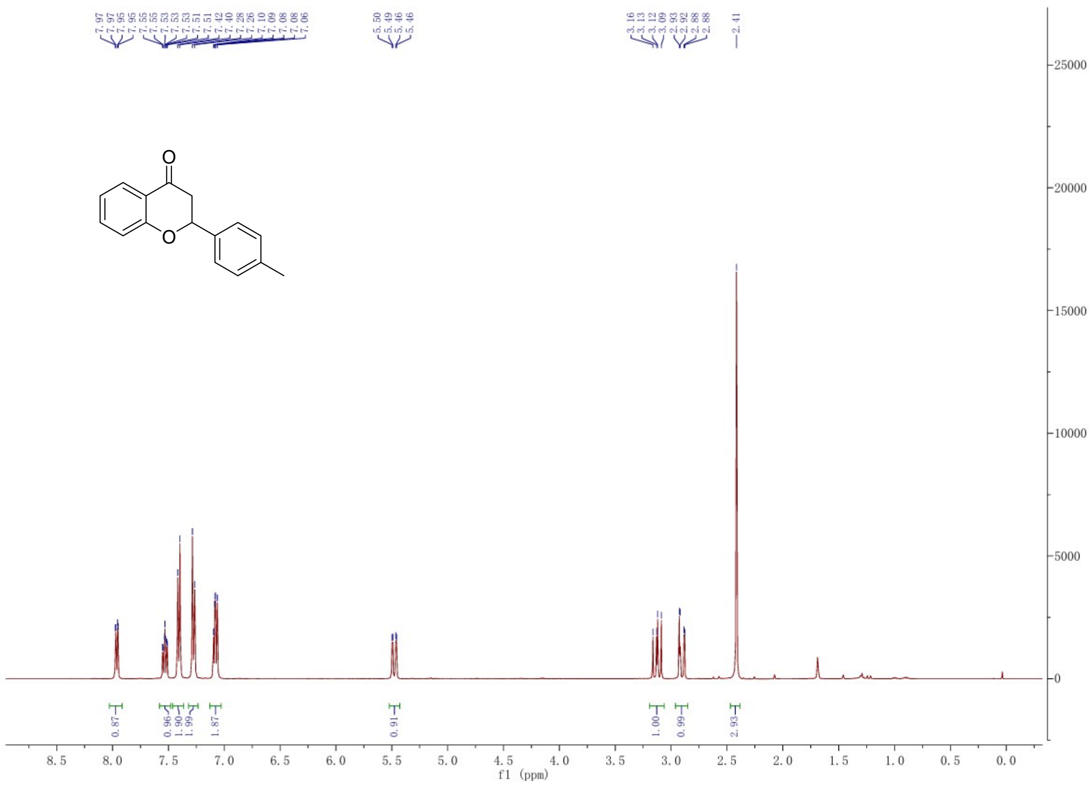


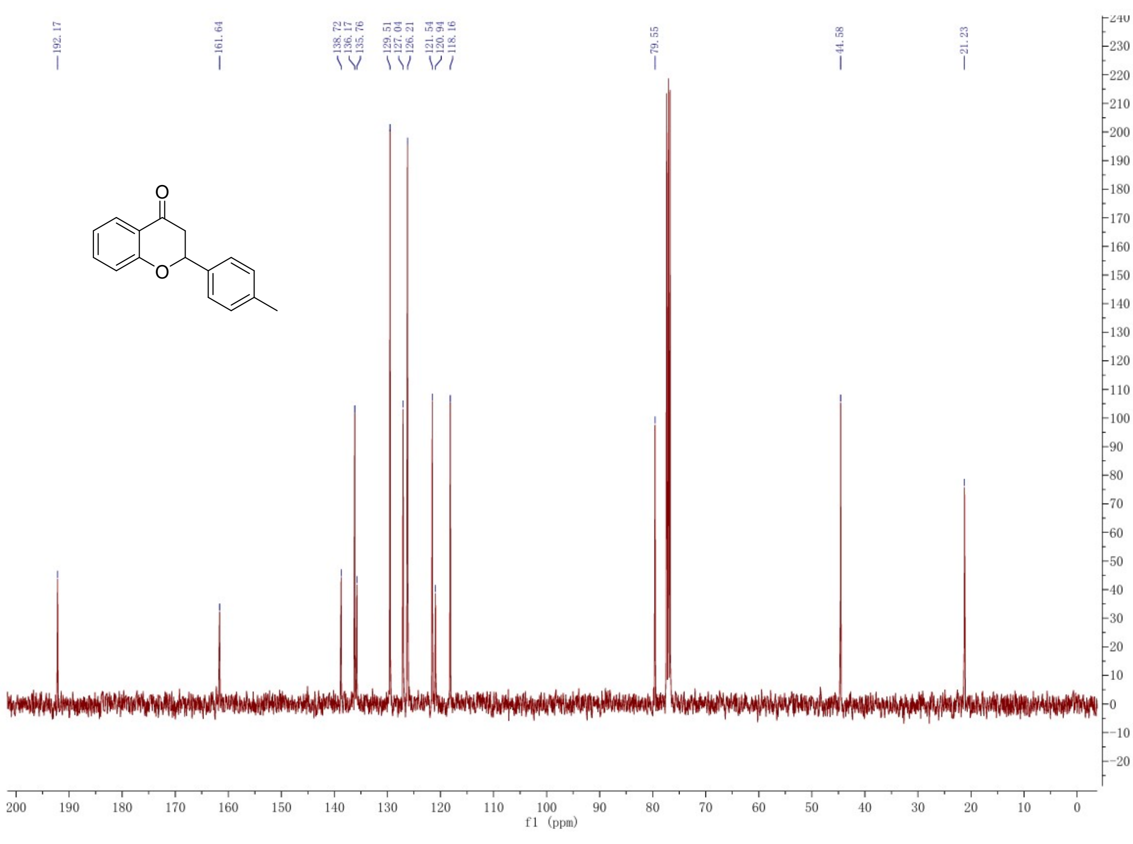
The ^{1}H NMR and ^{13}C NMR spectra of compound 2h.



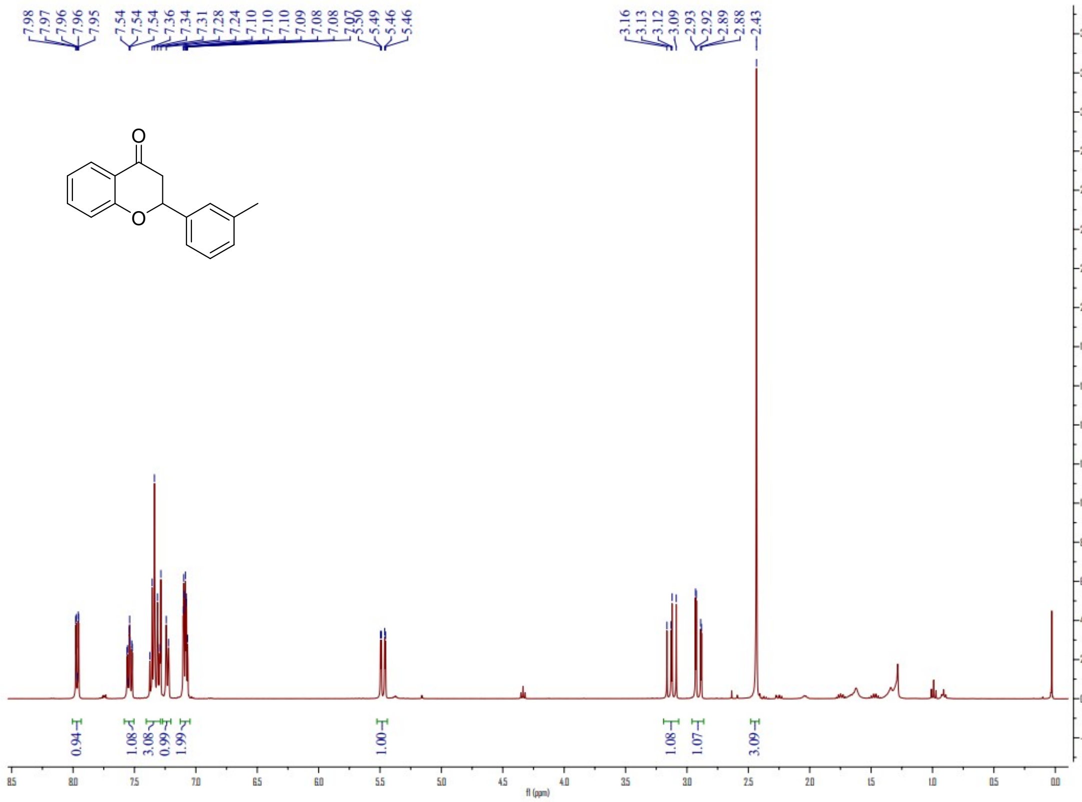


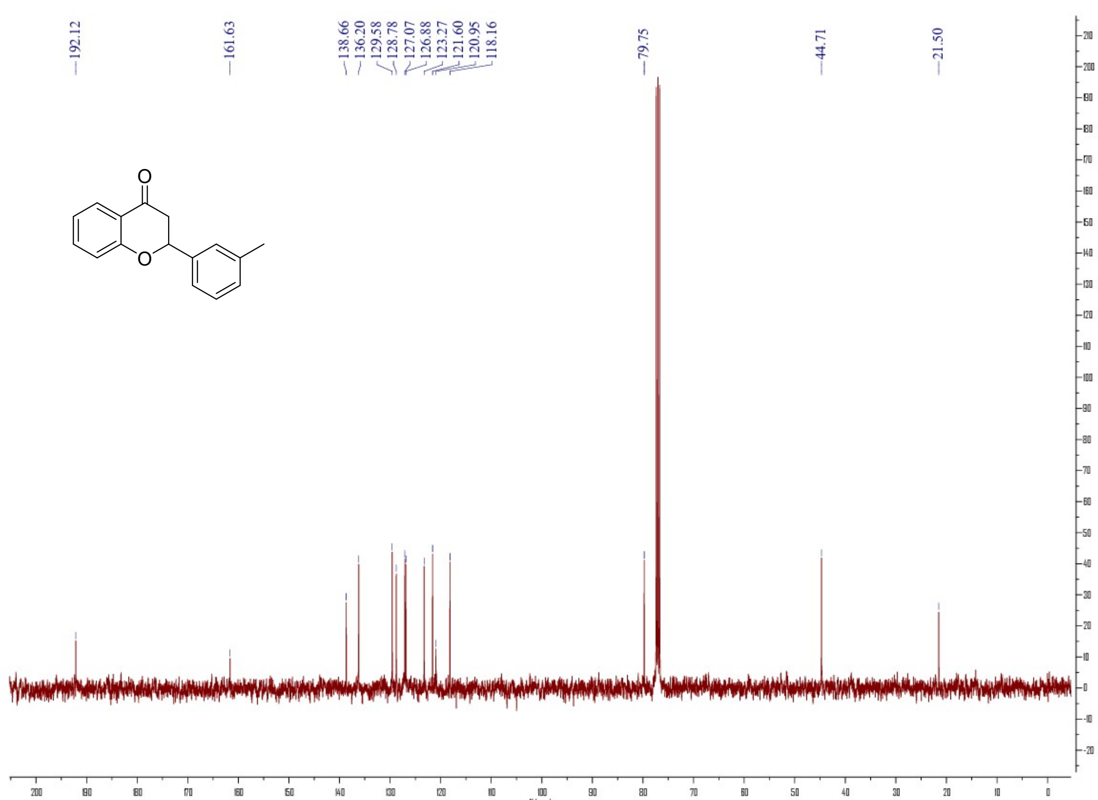
The ^{1}H NMR and ^{13}C NMR spectra of compound 2i.



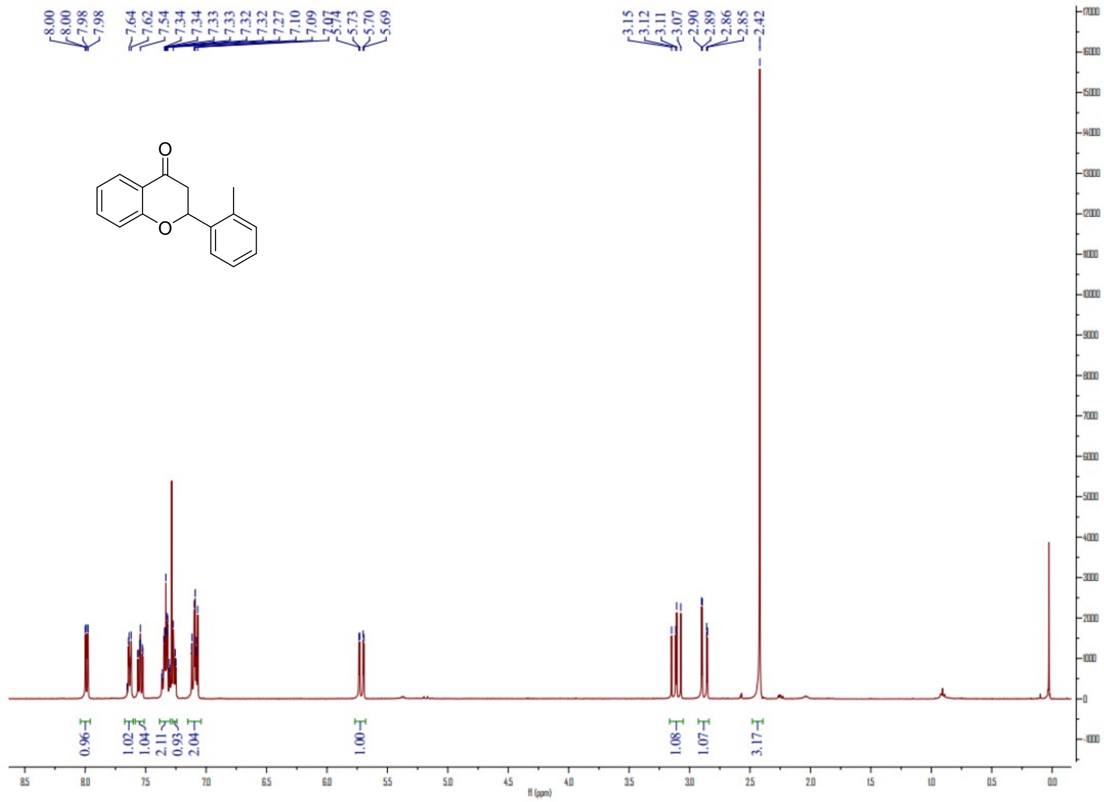


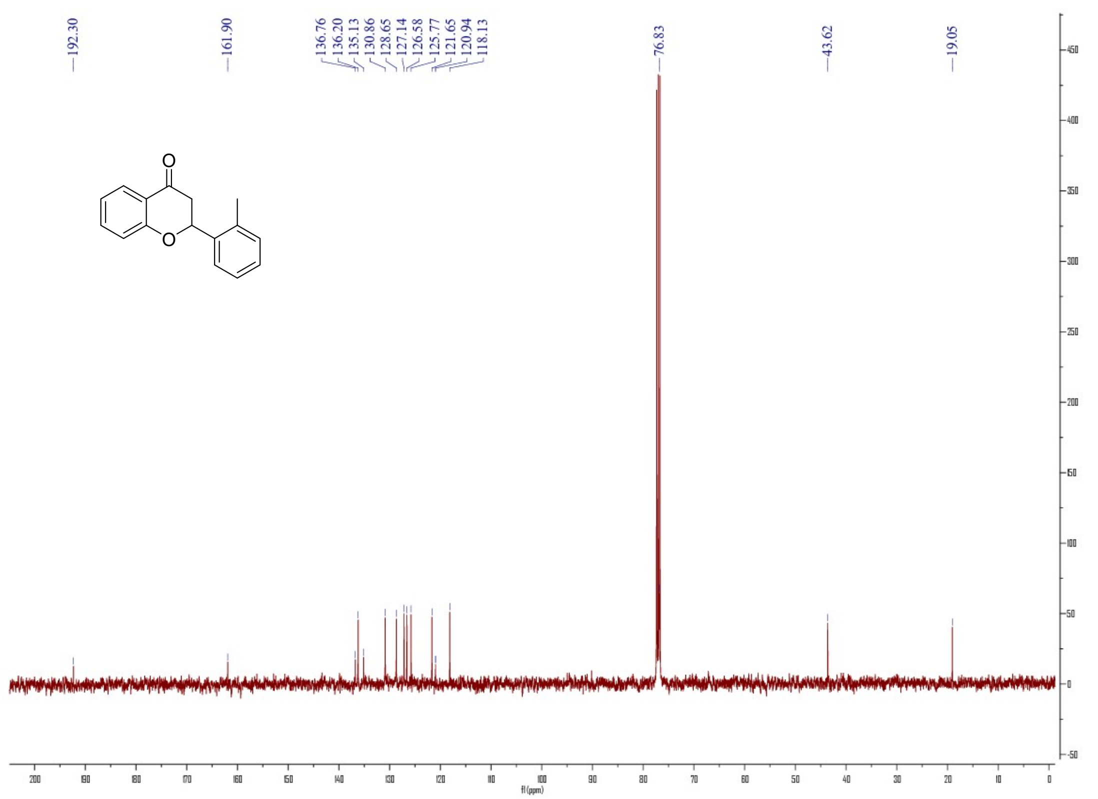
The ^{1}H NMR and ^{13}C NMR spectra of compound 2j.



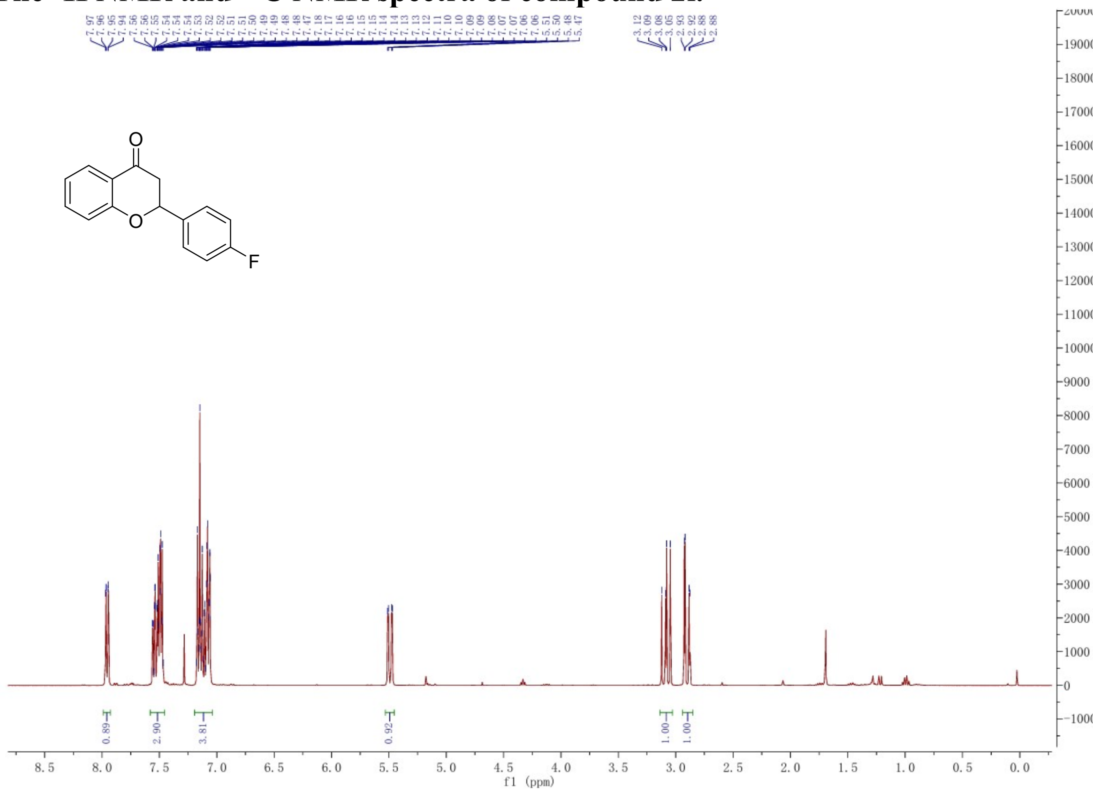


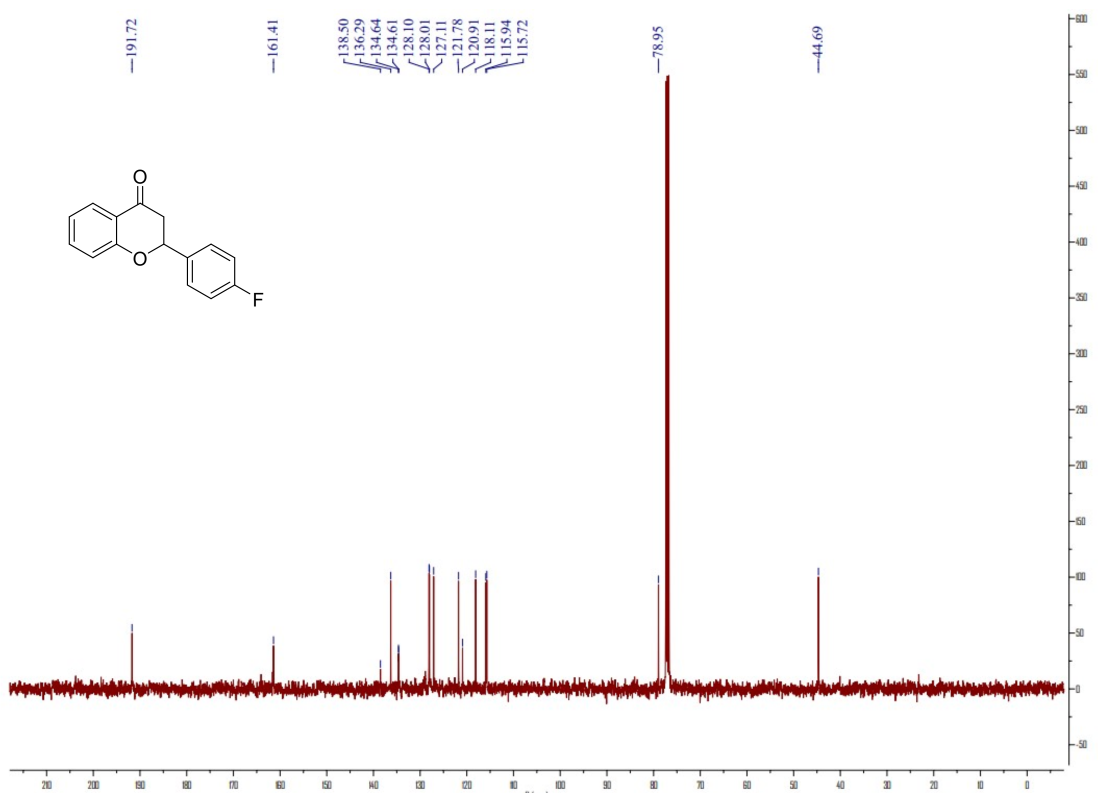
The ^{1}H NMR and ^{13}C NMR spectra of compound 2k.



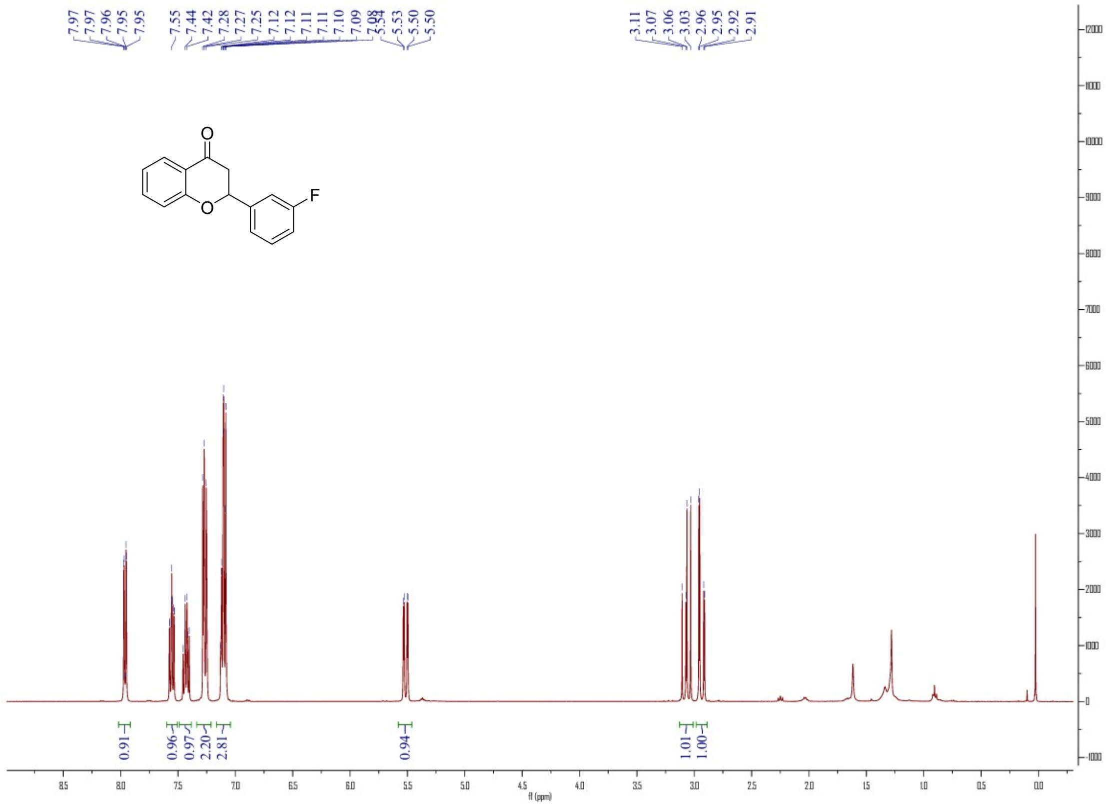


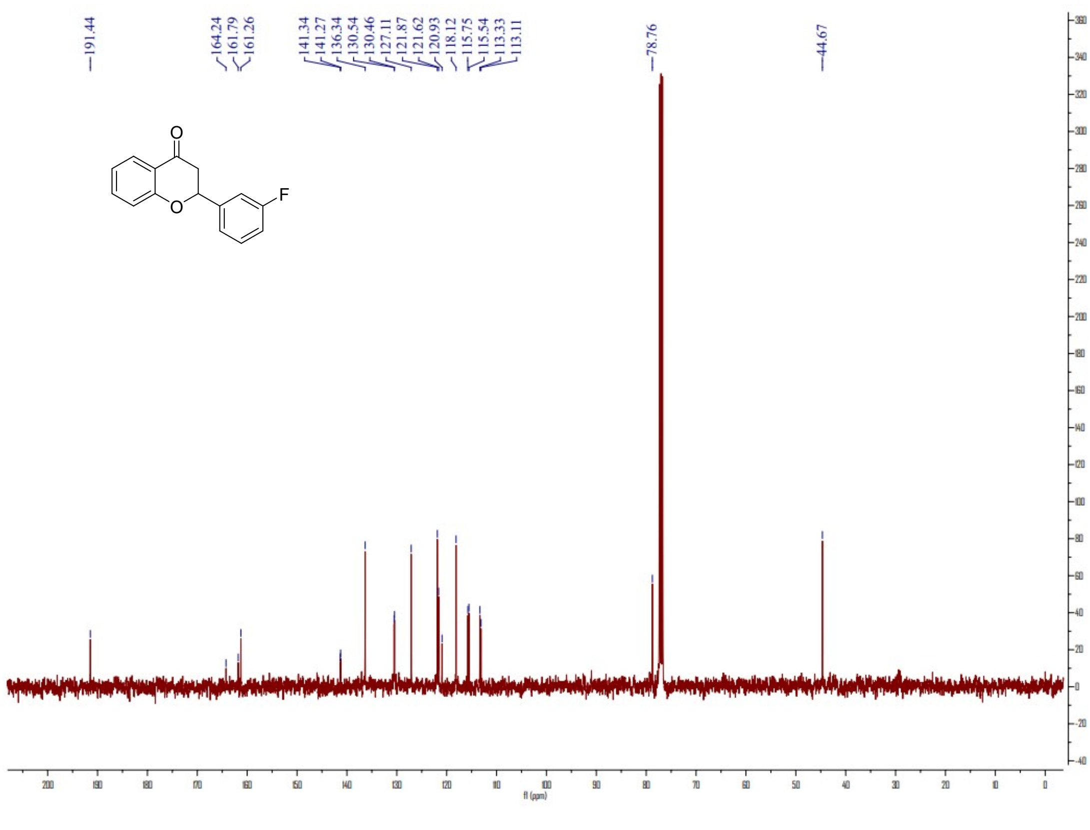
The ^{1}H NMR and ^{13}C NMR spectra of compound 2l.



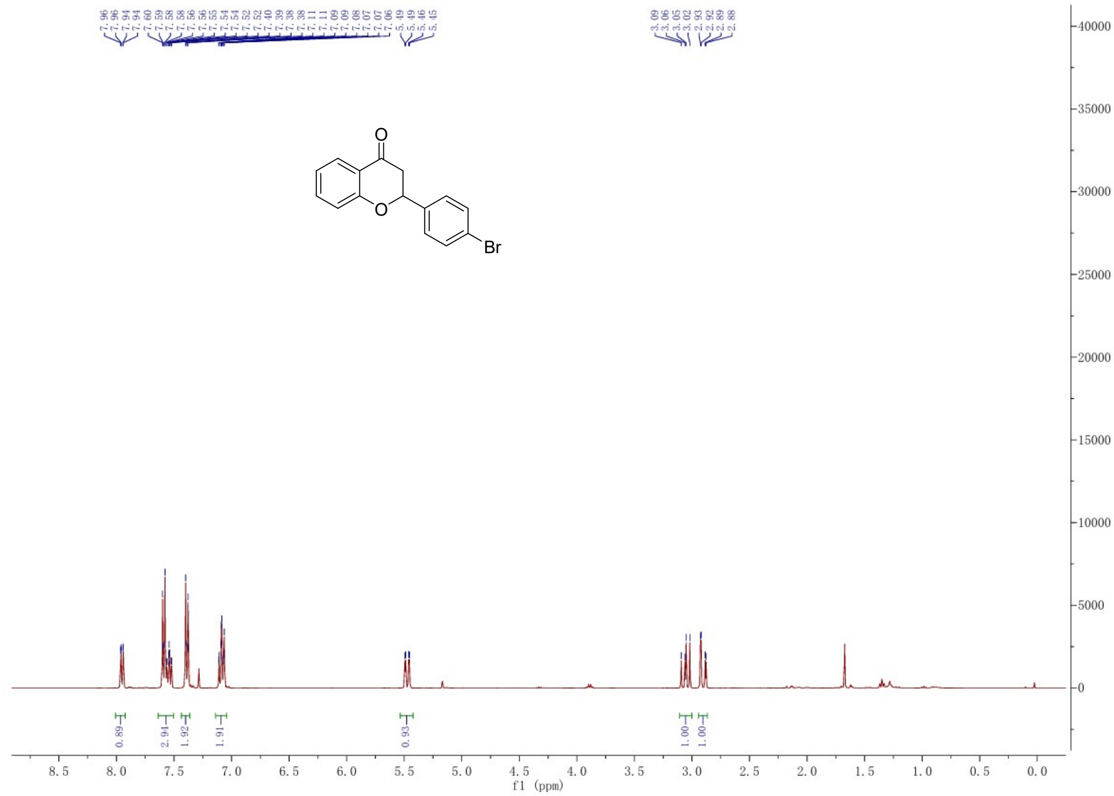


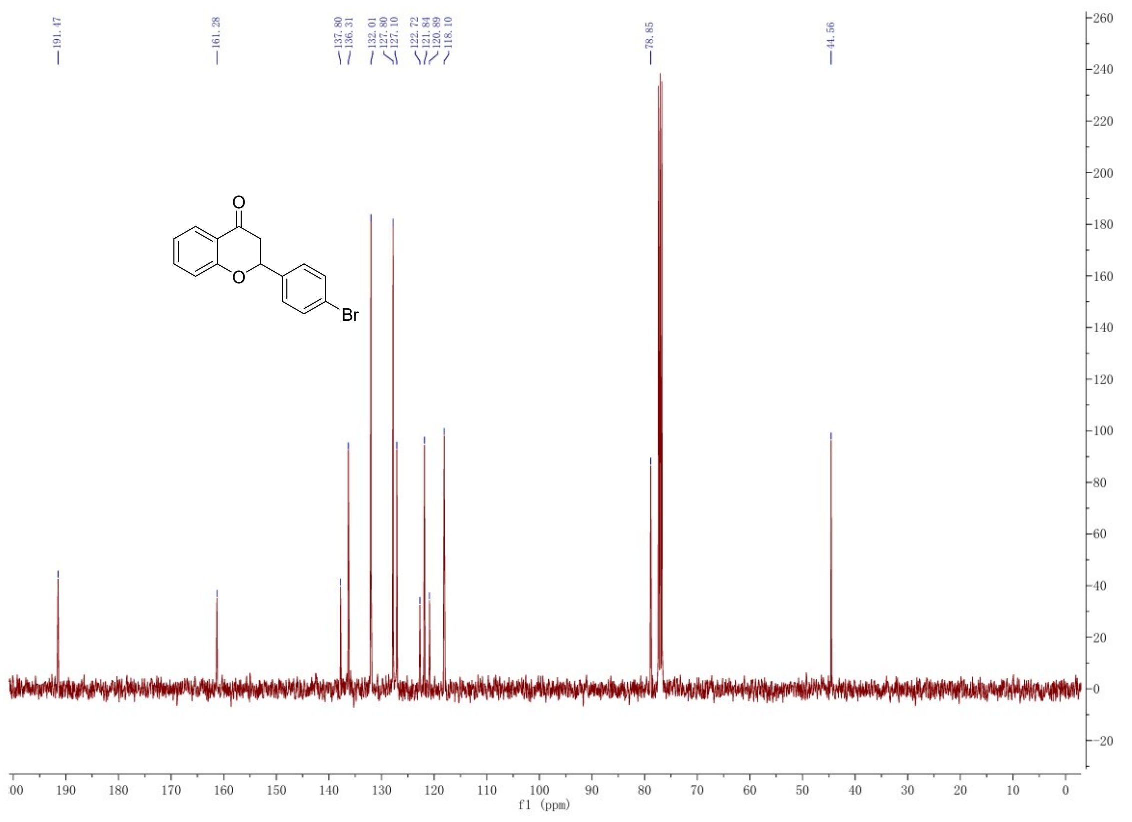
The ^{1}H NMR and ^{13}C NMR spectra of compound 2m.



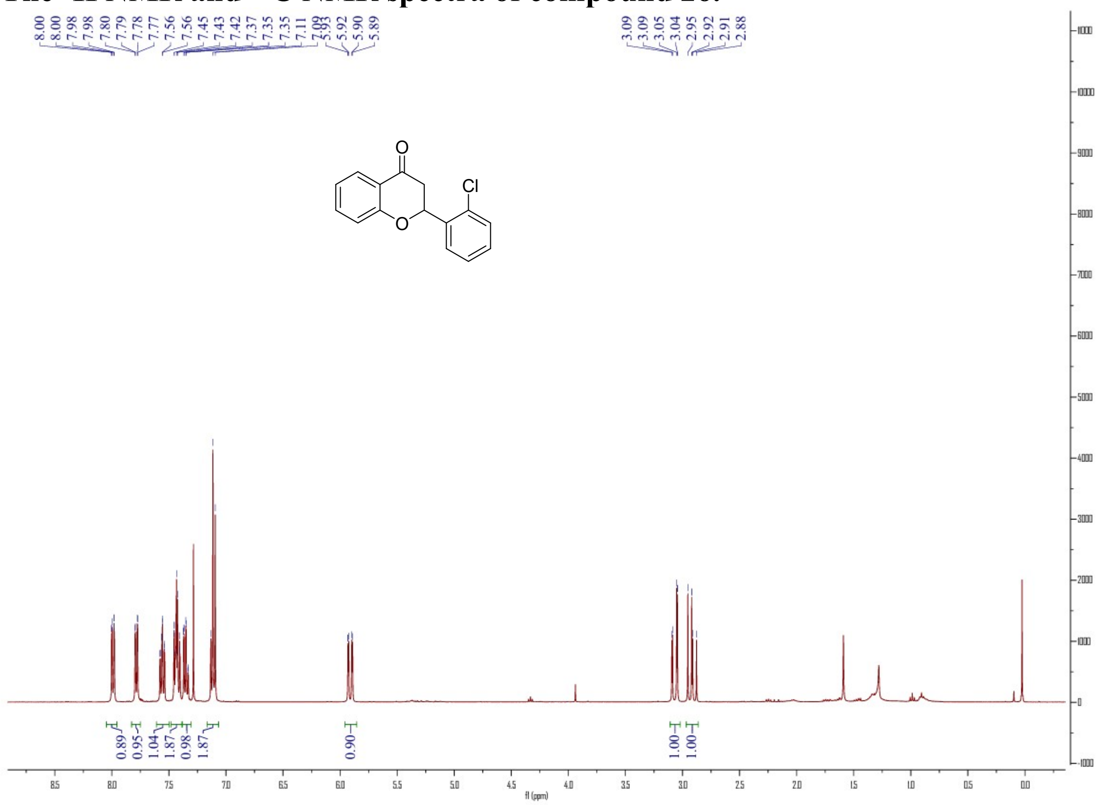


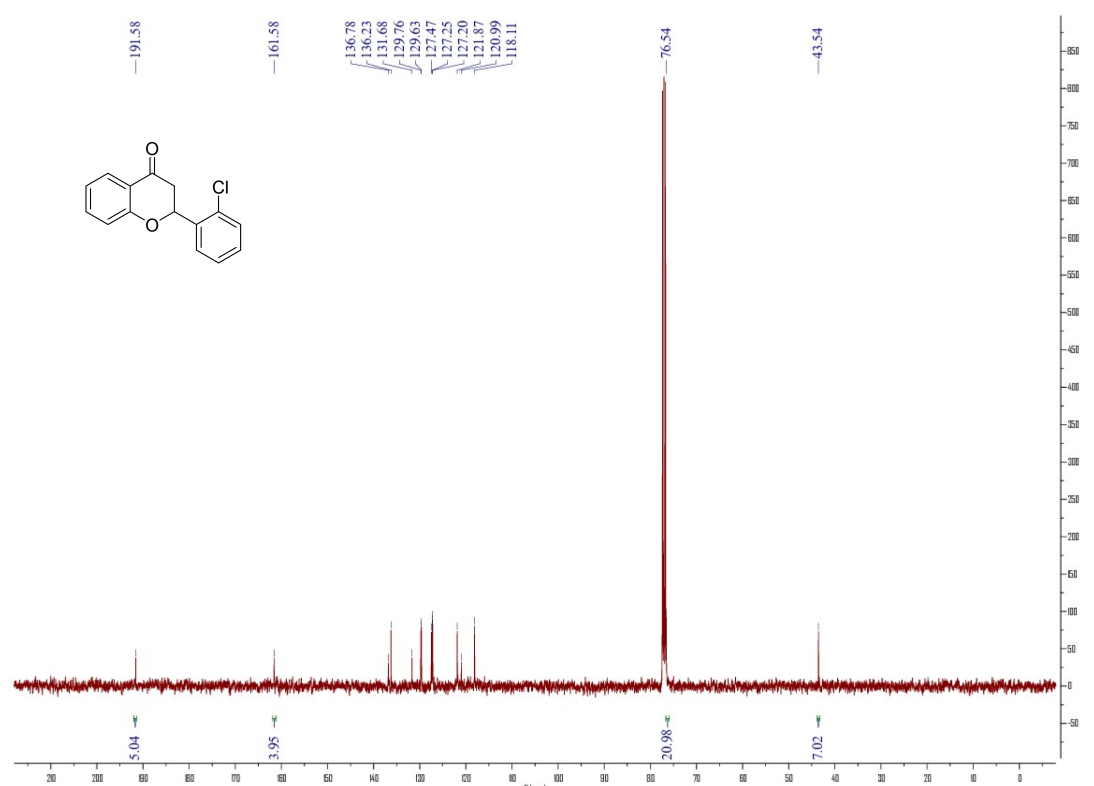
The ^{1}H NMR and ^{13}C NMR spectra of compound 2n.



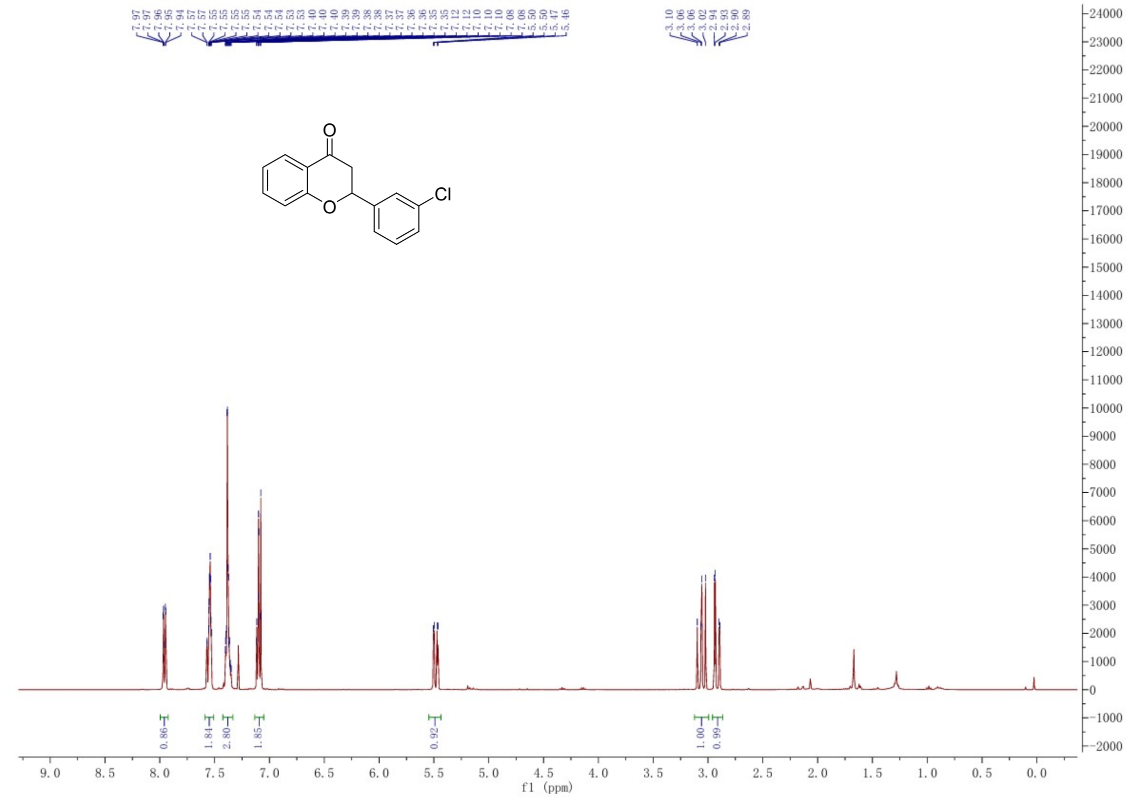


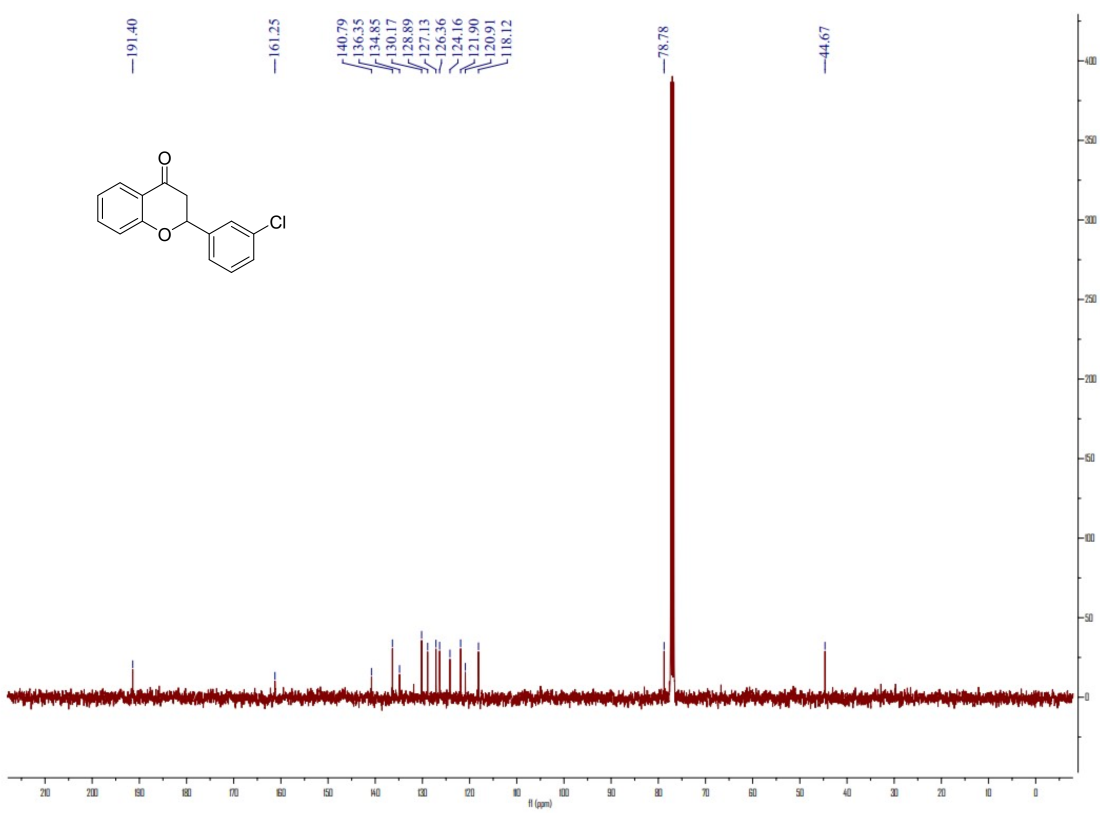
The [1]H NMR and [13]C NMR spectra of compound 2o.



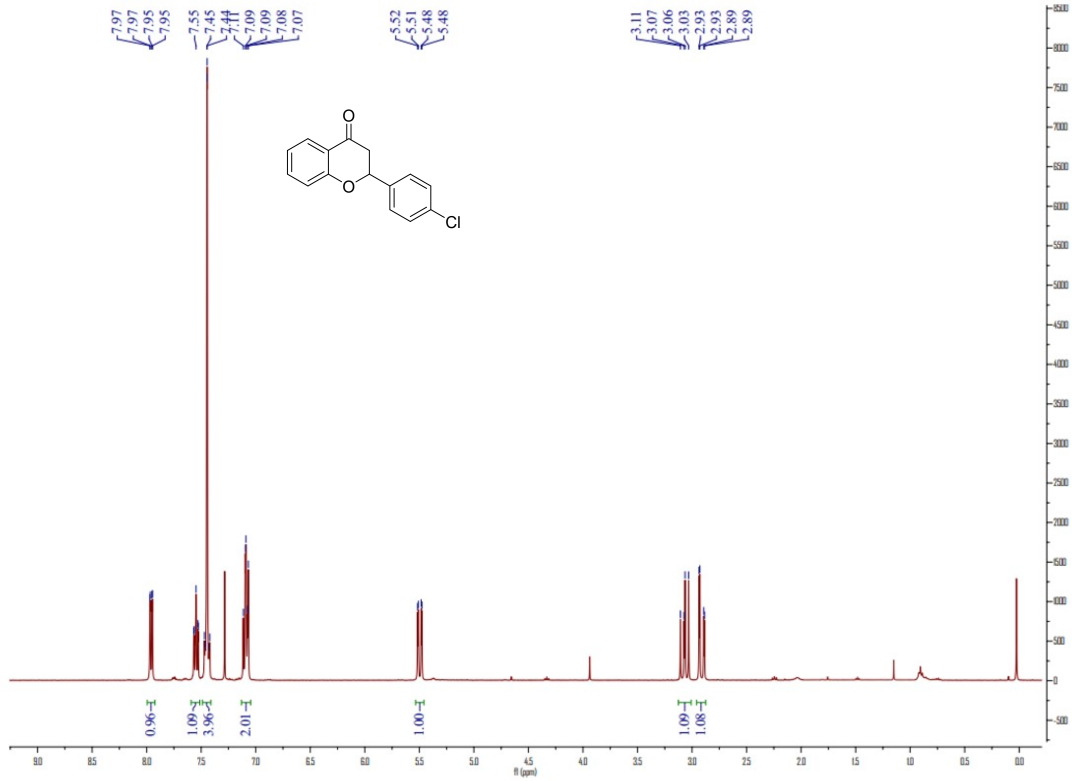


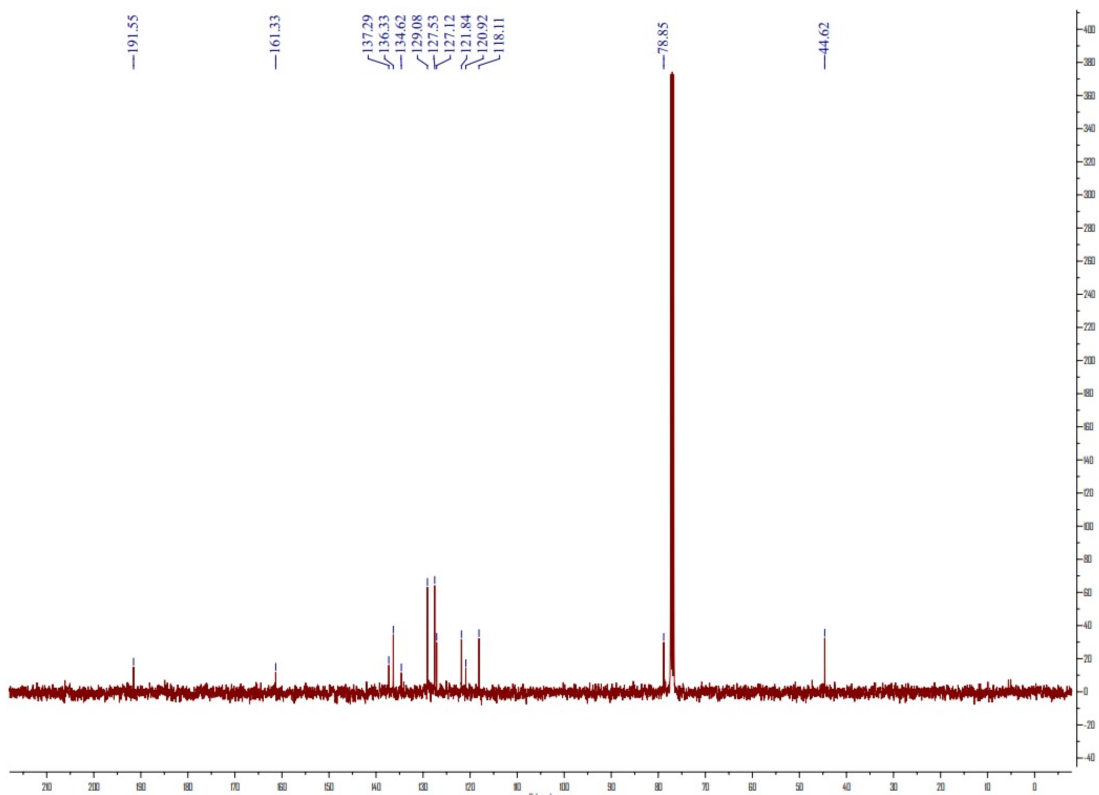
The ^{1}H NMR and ^{13}C NMR spectra of compound 2p.



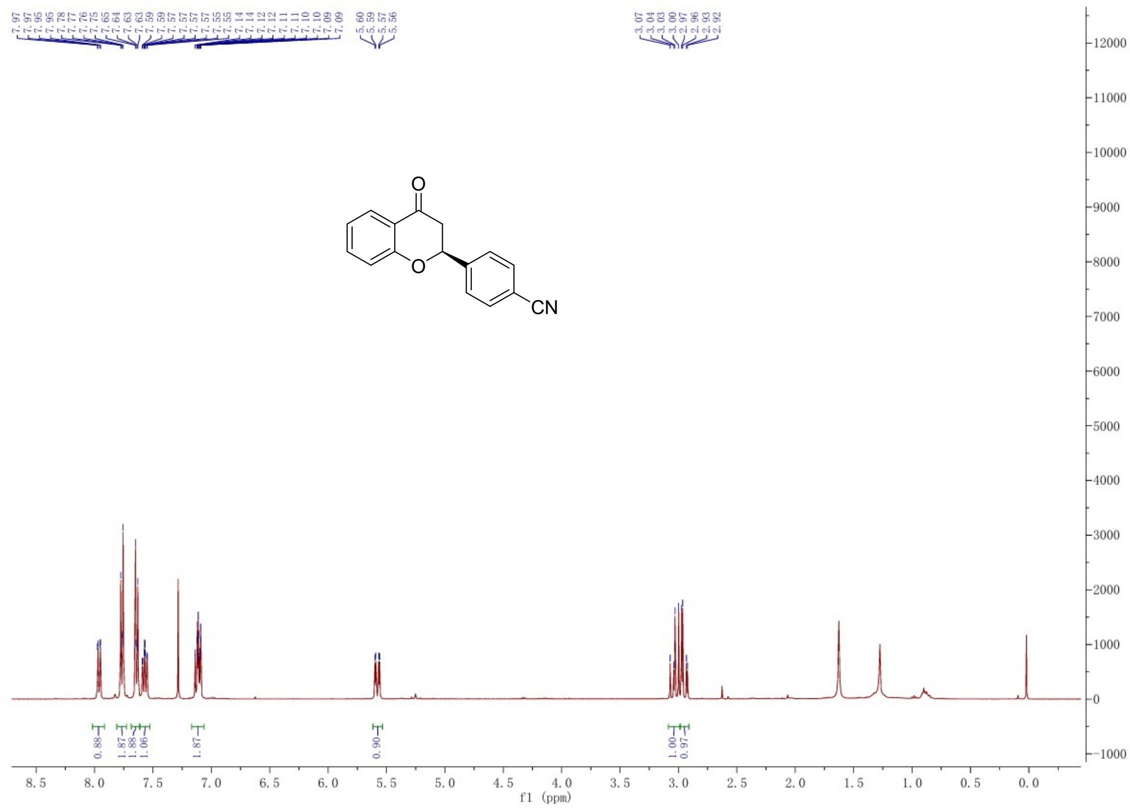


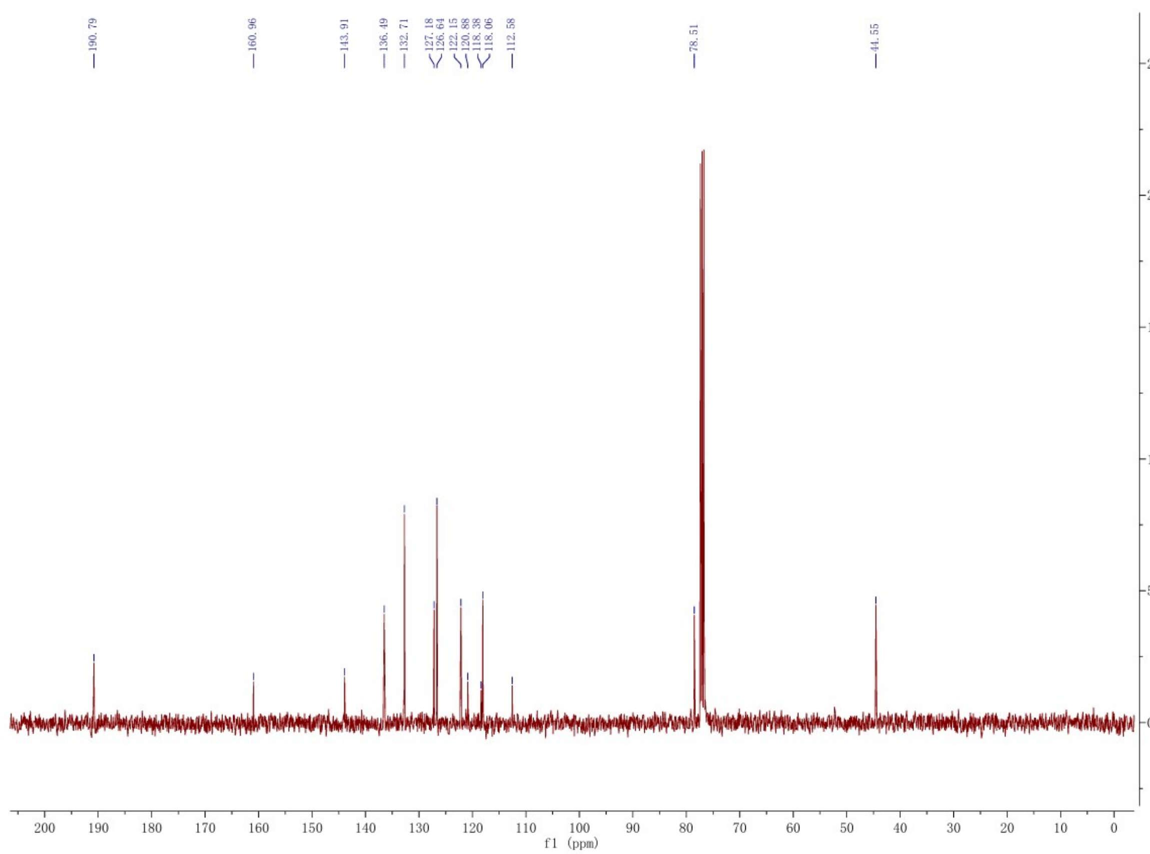
The ^{1}H NMR and ^{13}C NMR spectra of compound 2q.



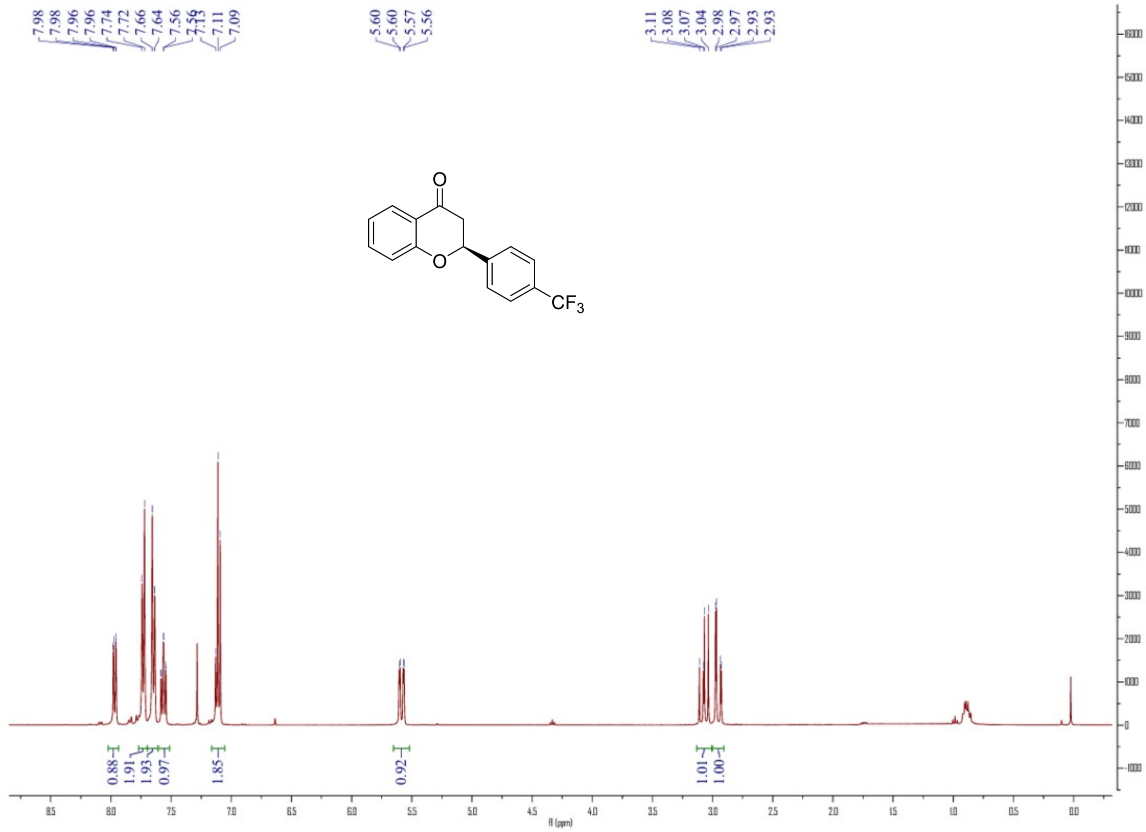


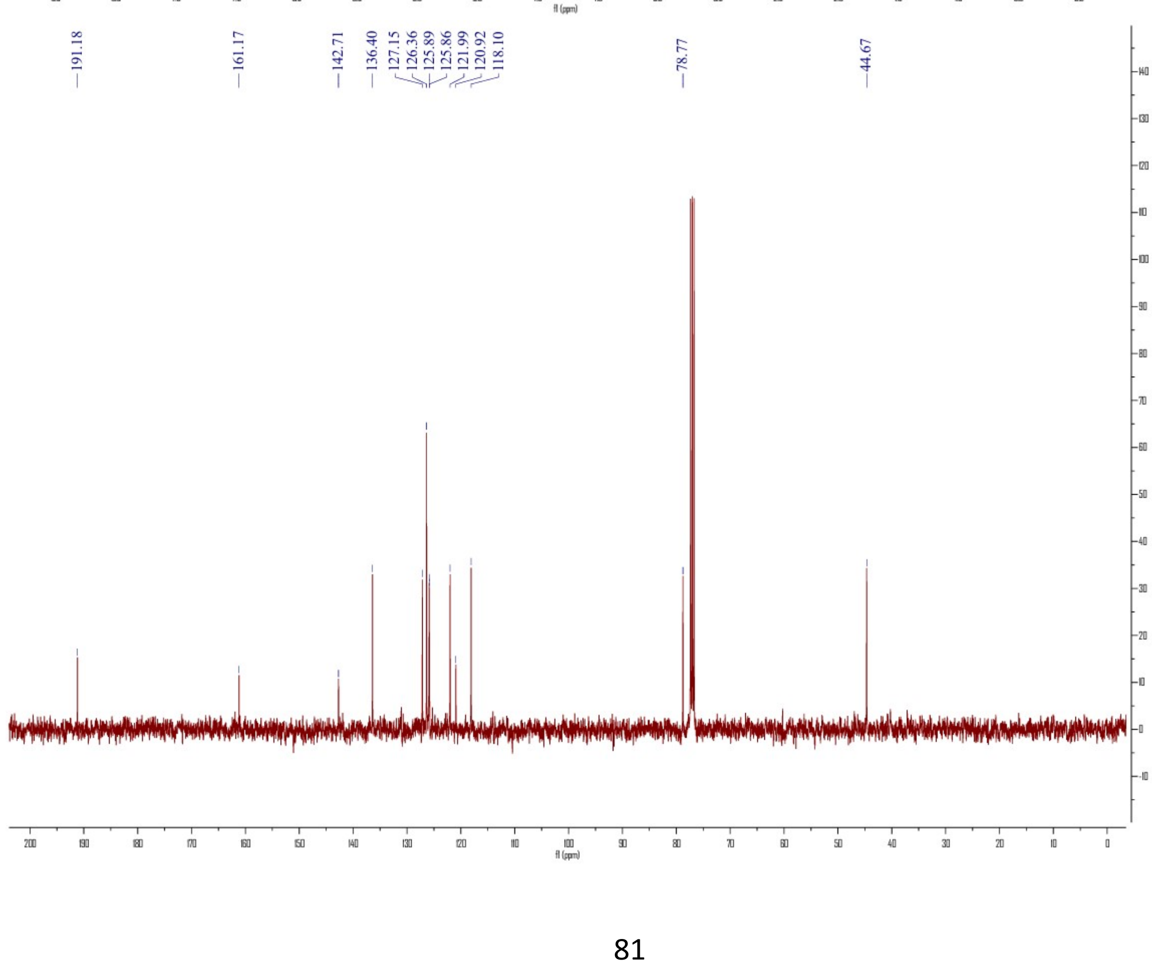
The ^{1}H NMR and ^{13}C NMR spectra of compound 2r.



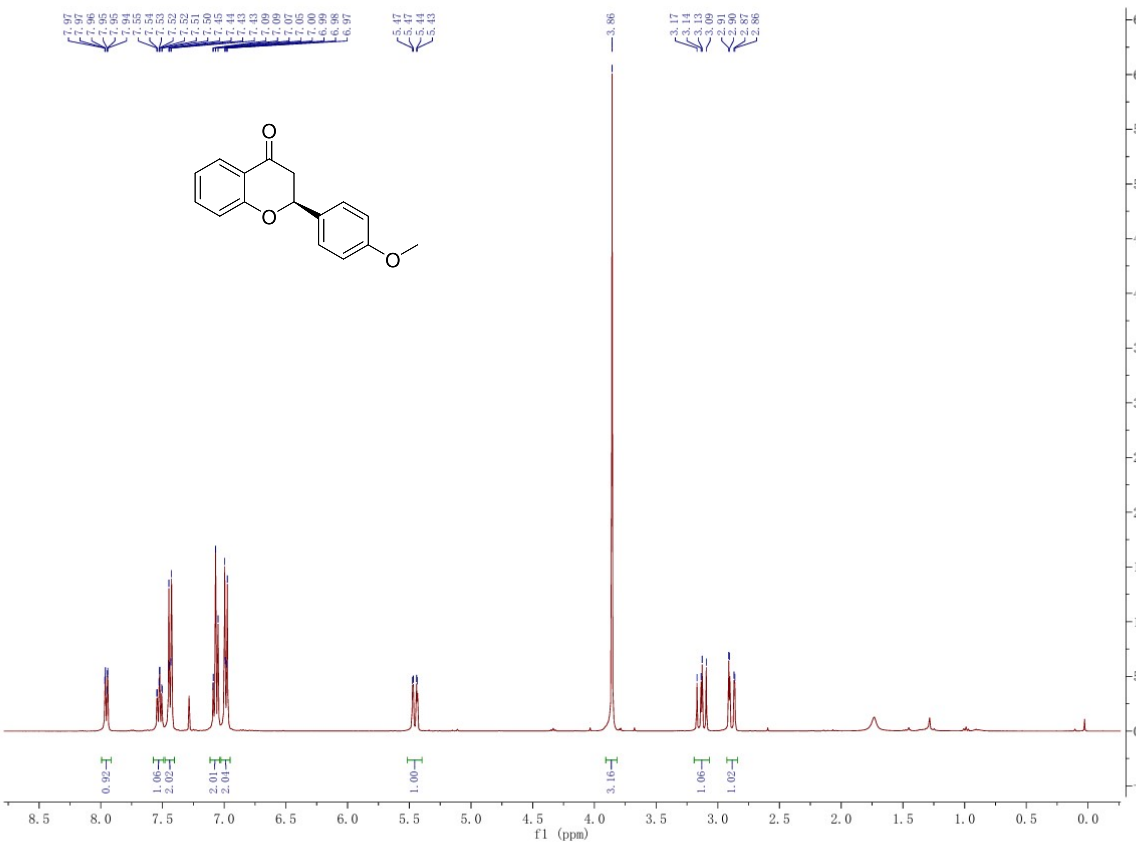


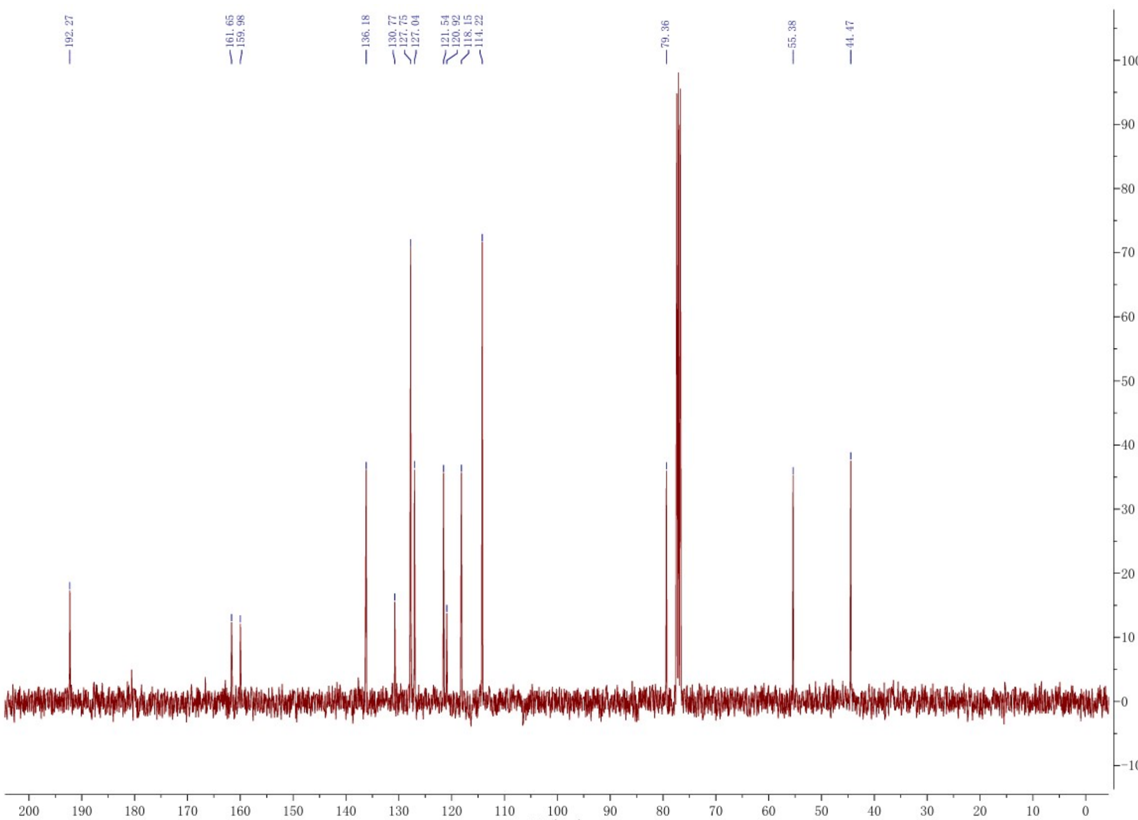
The ^{1}H NMR and ^{13}C NMR spectra of compound 2s.



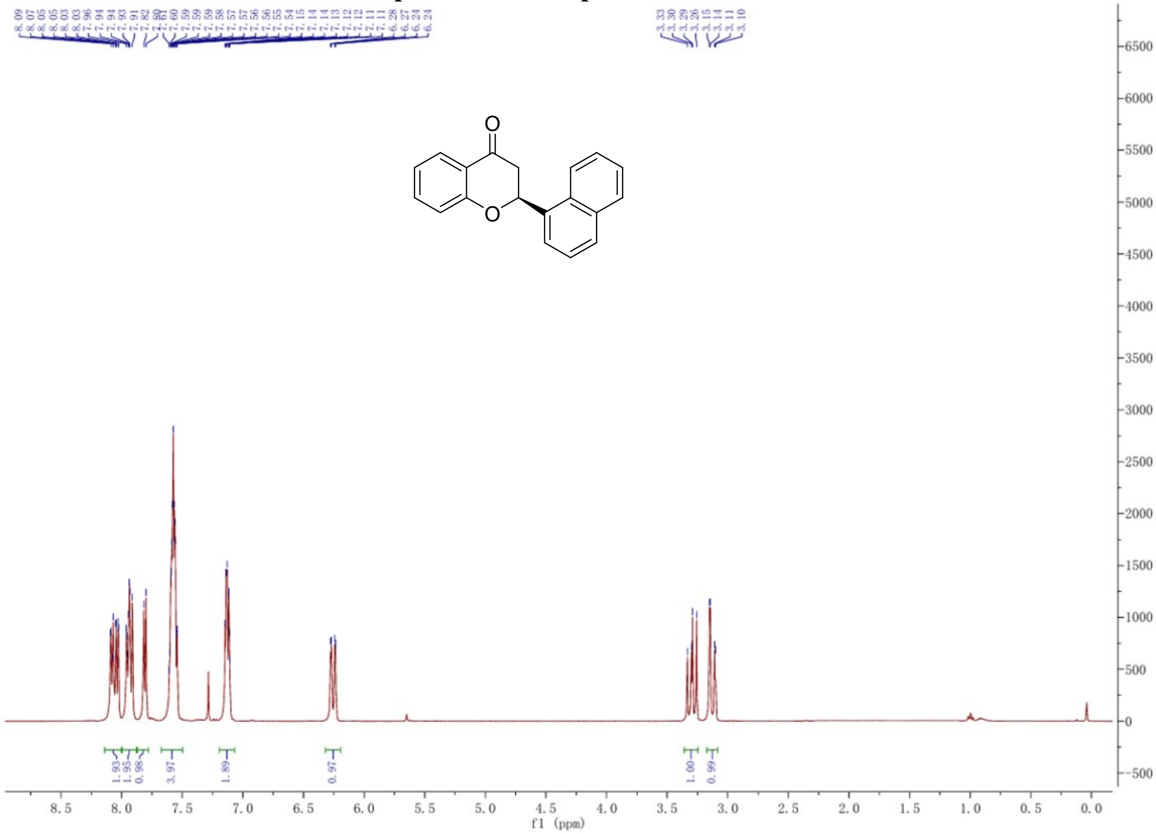


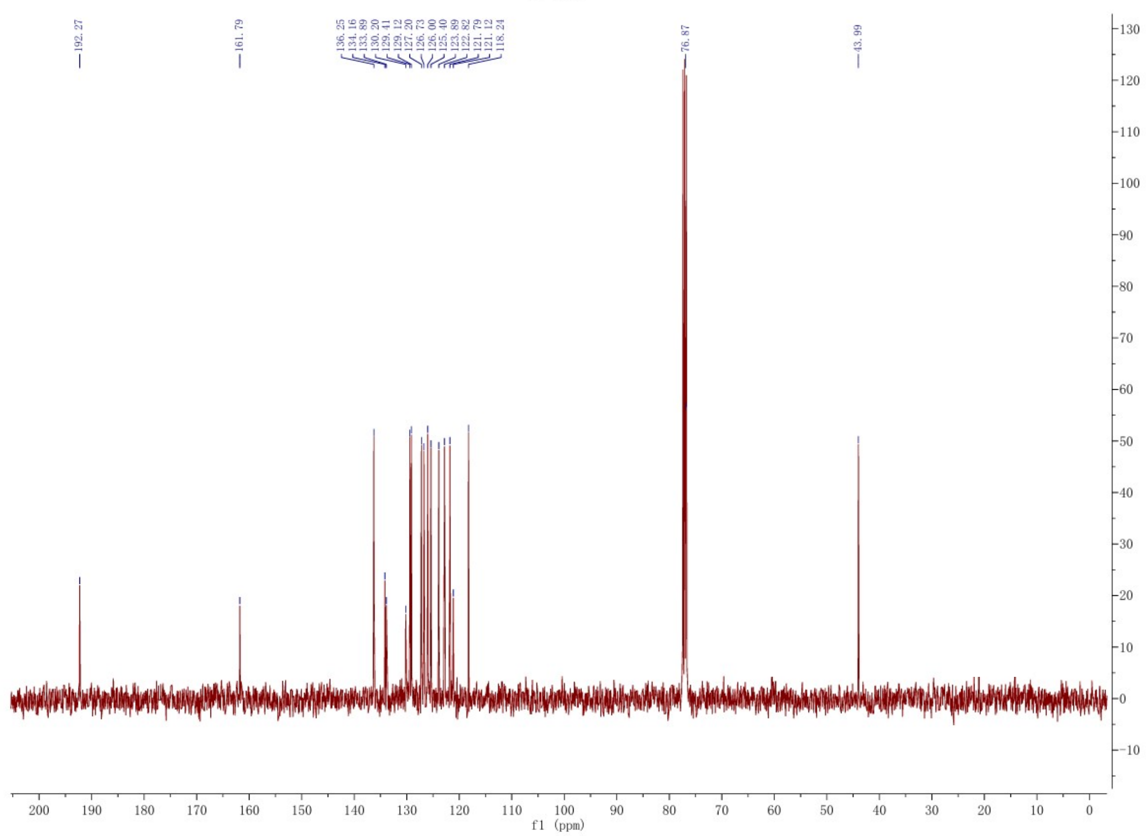
The ^{1}H NMR and ^{13}C NMR spectra of compound 2t.



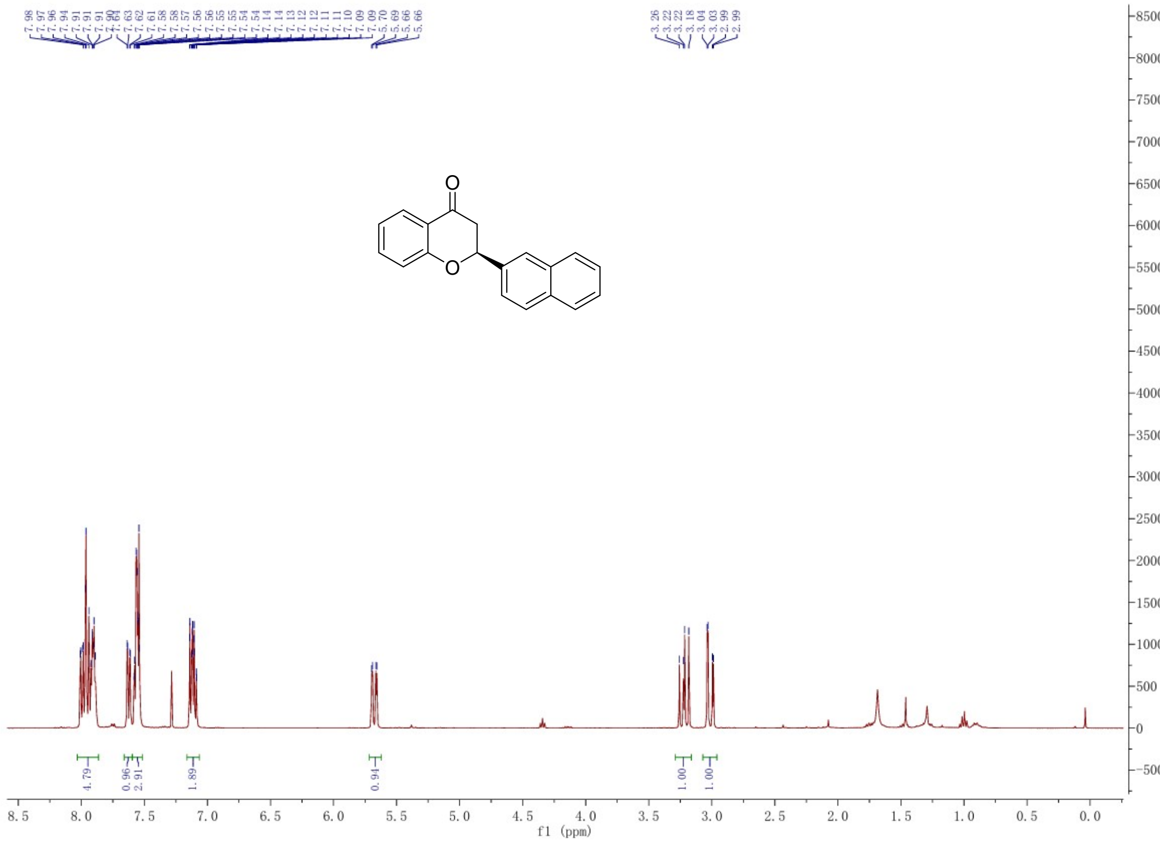


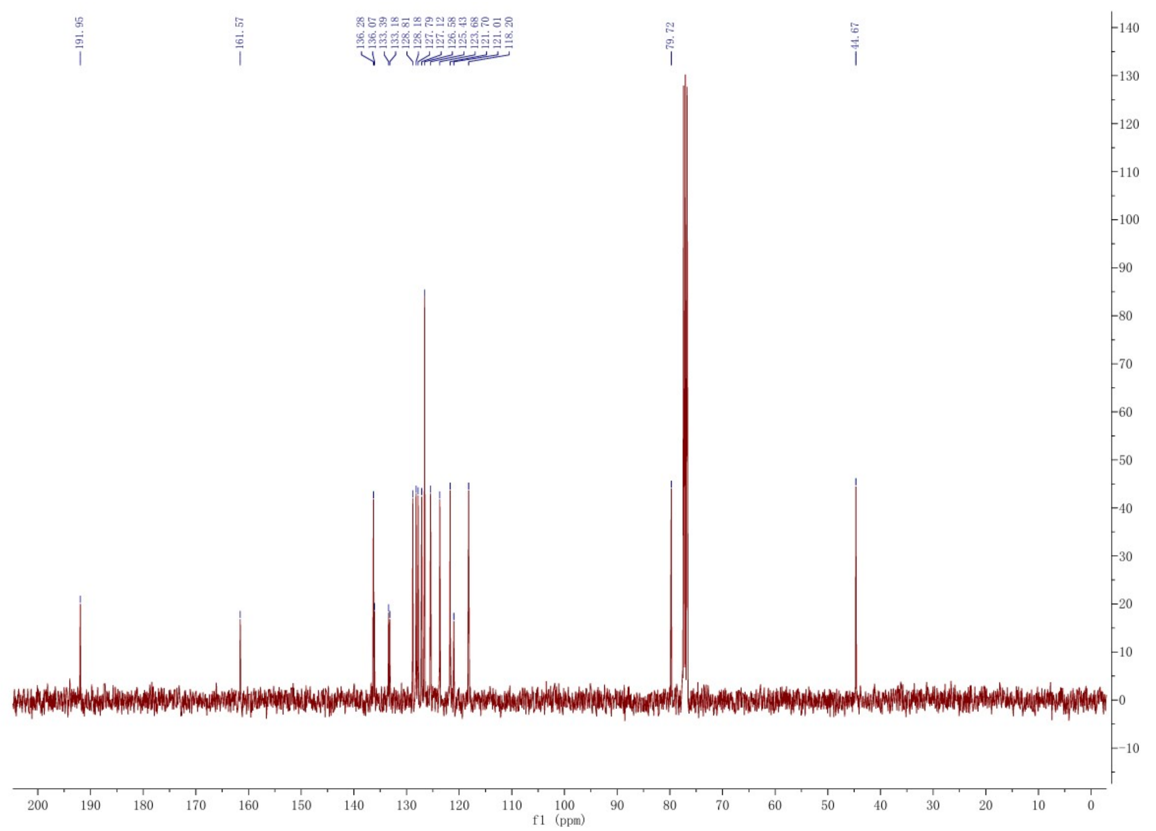
The ^{1}H NMR and ^{13}C NMR spectra of compound 2u.



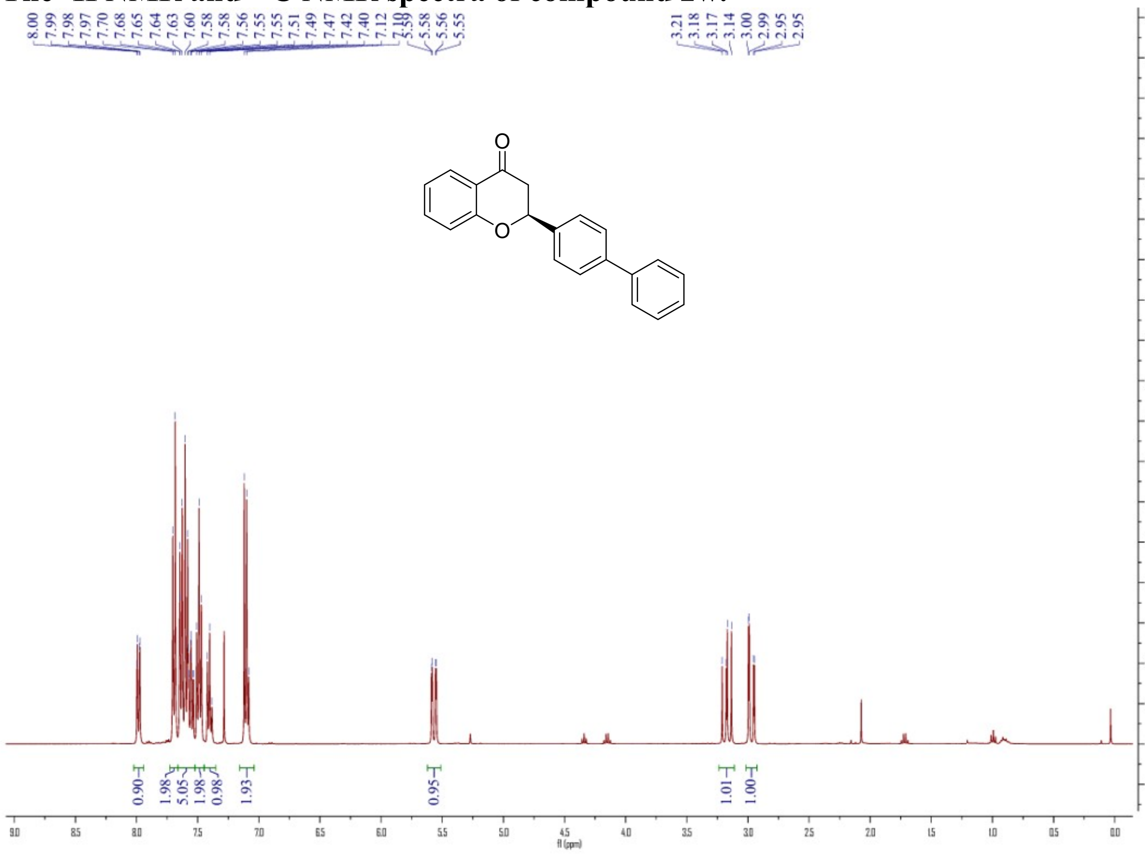


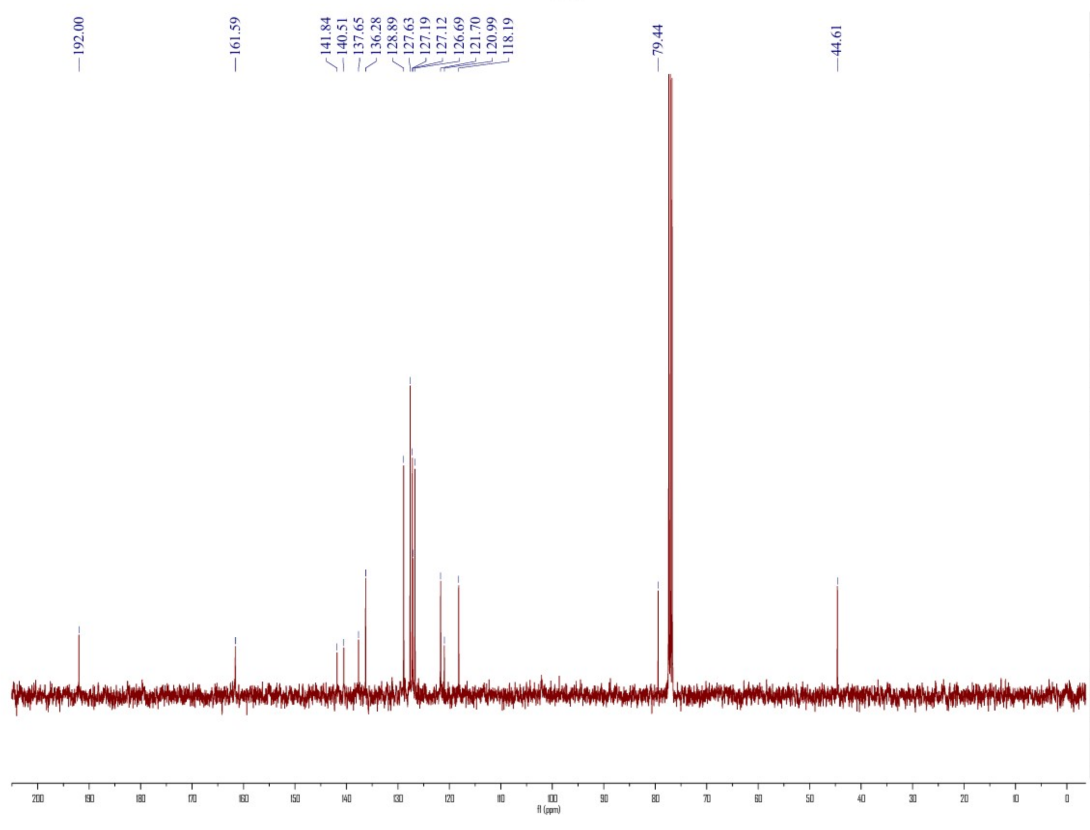
The ^{1}H NMR and ^{13}C NMR spectra of compound 2v.





The ^{1}H NMR and ^{13}C NMR spectra of compound 2w.





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