Supplementary Information

Enantioinduction of triplet state bonding with genetically repurposed photoenzymes

Ningning Sun[#1], Jianjian Huang[#1], Junyi Qian [2], Taiping Zhou[1], Juan Guo[1], Weining Zhao[3], Guojiao Wu[1], Rongzhen Liao[1], Xi Chen[*2], Yuzhou Wu[*1], Fangrui Zhong[*1]

1 Hubei Engineering Research Center for Biomaterials and Medical Protective Materials, Hubei Key Laboratory of Bioinorganic Chemistry & Materia Medica, Key Laboratory of Material Chemistry for Energy Conversion and Storage, Ministry of Education, School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology (HUST), 1037 Luoyu Road, Wuhan 430074, China.

2 Key Laboratory of Synthetic and Natural Functional Molecule of the Ministry of Education, College of Chemistry and Materials Science, Northwest University, Xi'an, 710127, China.

3 College of Pharmacy, Shenzhen Technology University, Shenzhen 518118, China

[#] These authors contributed equally to this work

Table of Contents

1 General information

Unless otherwise noted, all chemicals and reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. Reactions were monitored by TLC. ^{1}H NMR and ^{13}C NMR spectra were recorded on 400 MHz or 600 MHz Bruker spectrometers. Chemical shifts of ^{1}H NMR were reported in part per million relative to the $CDCl_3$ residual peak (δ 7.26). Chemical shifts of ^{13}C NMR were reported relative to $CDCl_3$ (δ 77.16). The used abbreviations are as follows: s (singlet), d (doublet), t (triplet), quart. (quartet), quint. (quintet), m (multiplet), br (broad). Multiplets which arise from accidental equality of coupling constants of magnetically non-equivalent protons are marked as virtual (*virt.*). High resolution mass spectra (HRMS) data were measured on an ESI-microTOF. HPLC analyses were performed using a chiral stationary phase (ChiralPak IA, IC, IB, AS-H, 250×4.6 mm), UV detection; Daicel Chemical Industries) employing n-hexane/i-PrOH. Photoreactions were performed with WATTCAS photoreactor (WP-TEC-1020LC) Reactions were monitored by TLC analysis using silica gel 60 Å F-254 thin layer plates and compounds were visualized with a UV light at 254 nm or 365 nm. Further visualization was achieved by staining with or $KMnO_4$ followed by heating on a hot plate. Flash column chromatography was performed on silica gel 60 Å, 10-40 μm.

Unnatural amino acids (BpA and FBpA) is prepared and purified according to literature reported method [1, 2]. Absorption spectrum of benzophenone (Bp) and fluoro-benzophenone (FBp) was detected on the instrument Varioskan LUX (Thermo).

E.coli DH5α and BL21 (DE3) cells were purchased from the Takara Biomedical Technology. Phenylmethanesulfonyl fluoride (PMSF), dethiobiotin and DNAse Ⅰ was purchased from Sigma-Aldrich. 2×High fidelity PCR Master Mix, Isopropyl-β-D-thiogalactoside (IPTG), L-Arabinose, ampicillin, chloramphenicol and LB medium were obtained from Sangon Biotech. Enzyme DpnI and (4-hydroxyazobenzene-2-carboxylic acid) HABA were purchased from Thermo Scientific. Strep-Tactin column was obtained from IBA Life Sciences.

2 Cloning, expression, purification and mutagenesis

2.1 Cloning and Expression

LmrR

The LmrR was originally derived from L. lactis MG1363. The codon-optimized gene of LmrR referring to the literature [3] was synthesized by Genewiz/Azenta and cloned in pET17b with C-terminal Strep-tag Ⅱ. For the expression of LmrR, an *E. coli* BL21 colony harboring pET17b_LmrR were grown overnight in 10 mL fresh LB medium containing 100 μg/mL of ampicillin. 8 mL of this preculture was used to inoculate 1 L of fresh LB medium containing 100 μg/mL of ampicillin. When the culture reached an optical density at 600 nm (OD600) of 0.8-1.0, the expression culture was supplemented with isopropyl-β-D-1-thiogalactopyranoside (IPTG) (final concentration 1mM). Cells was continued for 20-24 h at 30 ºC and shaken at 200 rpm.

The cultures were centrifuged (8000 rpm, 5 min, and 4 ºC) and the supernatant was removed, the pellets can be freezed at -20 ºC for subsequent protein purification.

TPe variants

The unnatural amino acid 4-benzoylphenylalanine (BpA) or 3'-fluoro-4-benzoylphenylalanine (FBpA) was introduced to LmrR to construct TPes. Amber codon TAG was used to encode BpA/FBpA. The substitution of TAG at the desired position was accomplished by using the site-directed mutagenesis primers (a list primers can be found in the section 11 sequence information) and PCR programs. The PCR reaction solution contains 1μL forward primer, 1μL reverse primer, 1μL template (LmrR), 25μL 2×High fidelity PCR Master Mix and 22 μL ddH_2O. The following PCR cycles were used: initial denaturation at 95 ºC for 1 min, denaturation at 95 ºC for 30 s, annealing at 55-65 ºC for 30s (depending on the Tm of the particular mutant) and extension at 72 ºC for 4.5 min. The thermal cycle was repeated 20 times. The PCR products were digested with DpnI for 2h at 37 ºC and transformed into the *E.Coli* DH5α clonal strains, cultured overnight on ampicillin plate, and monoclones were selected for plasmid extraction and gene sequencing (Sangon Biotech). Each pET17b-LmrR mutant and pEVOL-pBpF plasmid should be co-transformed into *E.Coli* BL21 (DE3) cells. Co-transformed strains were screened by resistant plates (ampicillin and chloramphenicol), then the single colony was selected for subsequent protein expression experiments.

E. Coli BL21 (DE3) cells transformed with pET17b-LmrR mutation and pEVOL-pBpF plasmid were grown overnight in 10 mL fresh LB medium containing 100 μg/mL of ampicillin and 34 μg/ml of chloramphenicol. Then 8 mL of this preculture was used to inoculate 1 L of fresh LB medium containing 100 μg/mL of ampicillin and 34 μg/ml of chloramphenicol. When the culture reached an optical density at 600 nm (OD600) of 0.8-1.0, the expression culture was supplemented with isopropyl β-D-1-thiogalactopyranoside (IPTG) (final concentration 1mM), L-Arabinose (final concentration 0.02%) and BpA or FBpA (final concentration 1mM). Cells were further cultured for 20-24 h at 30 °C and shaken at 200 rpm. The cultures were centrifuged (8000 rpm, 5 min, and 4 °C) and the supernatant was removed, the pellets can be freezed at -20 °C for subsequent protein purification and photocatalytic reactions.

2.2 The preparation of crude TPes (TPes lysate)

The pellets were resuspended in MOPS buffer (20 mM MOPS, 150 NaCl, pH 7.0), followed by adding DNAseI (final concentration 0.1 mg/mL with 10 mM $MgCl_2$) and PMSF solution (final concentration 0.1 mM). Subsequently, the cells were sonicated (60%, 200W) for 40 min (5 sec on, 8 sec off) and centrifuged (8000 rpm, 1h, 4 ºC). The supernatant was collected as crude enzyme solution (10-18 mg/ml) which could be directly used for photocatalytic reactions.

2.3 Protein purification

The pellets were resuspended in washing buffer (100 mM Tris-HCl, 150 mM NaCl, 10% glycerol. pH 8.0), followed by adding DNAseI (final concentration 0.1 mg/mL with 10 mM $MgCl_2$) and PMSF solution (final concentration 0.1 mM). Subsequently, the cells were sonicated (60% (200W) for 40 min (5 sec on, 8 sec off) and centrifuged (8000 rpm, 1 h, 4 ºC), the supernatant was loaded on a Strep-Tactin column and incubated for 1 h at 4 ºC. The column was washed with 5 x 1 CV (column volume) washing buffer, and eluted with 10 x 0.5 CV of washing buffer containing 2.5 mM desthiobiotin. The eluate was collected. About 15-18 mg protein LmrR can be obtained, while photoenzyme containing BpA or FBpA can be purified in the range of 6-10 mg.

2.4 Site directed mutagenesis for directed evolution

TPe1.0 (*i.e.*, LmrR_F93BpA) was taken as a template to carry out saturation mutation of M8, A11, N19, V15, M89, W96, D100 sites. Codon NNN or NNK was used to obtain a large number of mutants and build a photoenzyme library. The mutant TPe1.0_W96L (TPe2.0) was identified to be the best catalyst. The same procedure was conducted using TPe2.0 as template to carry out saturation mutation of M8, V15, N19, D100 sites, and TPe2.0_M8L (TPe3.0) was identified to be the best catalyst. Finally, TPe3.0 was used as a template to carry out site directed mutation of A11 and L18S. All the above primers can be found in section 6 sequence information. The PCR reaction solution contains 1μL forward primer, 1μL reverse primer, 1μL template, 25μL 2×High fidelity PCR Master Mix and 22 μL ddH_2O. The following PCR cycles were used: initial denaturation at 95 ºC for 1 min, denaturation at 95 ºC for 30 s, annealing at 55-65 ºC for 30 s (depending on the T_m of the particular mutant) and extension at 72 ºC for 4.5 min. The thermal cycle was repeated 20 times. After PCR procedure, the PCR products were digested with DpnI for 2h at 37 ºC and transformed into the *E.Coli* DH5α clonal strains, cultured overnight on ampicillin plate, and monoclones were selected for plasmid extraction and gene sequencing (Sangon Biotech). Each pET17b-TPe mutant and pEVOL-pBpF plasmid should be co-transformed into *E.Coli* BL21 (DE3) cells. Co-transformed strains were screened by resistant plates (ampicillin and chloramphenicol), then the single colony was selected for subsequent protein expression experiments.

E. Coli BL21 (DE3) cells transformed with pET17b-TPe mutation and pEVOL-pBpF plasmid were grown overnight in 10 mL fresh LB medium containing 100 μg/mL of ampicillin and 34 μg/ml of chloramphenicol. Then 8 mL of this preculture was used to inoculate 1 L of fresh LB medium containing 100 μg/mL of ampicillin and 34 μg/ml of chloramphenicol. When the culture reached an optical density at 600 nm (OD600) of 0.8-1.0, the expression culture was supplemented with isopropyl β-D-1-thiogalactopyranoside (IPTG) (final concentration 1 mM), L-Arabinose (final concentration 0.02%) and BpA or FBpA (final concentration 1 mM). Cells were further cultured for 20-24 h at 30 °C and shaken at 200 rpm. The cultures were centrifuged

(8000 rpm, 5 min, and 4 °C) and supernatant was removed, and the pellets can be freezed at -20 °C for subsequent protein purification and photocatalytic reaction.

3. General protocol for photocatalytic reactions with TPes

A reaction mixture (1.0 mL) containing the TPe lysates or purified TPes (2.5-5 mol%) and substrate **1** (200 mM) in MOPS buffer (20 mM MOPS, 150 mM NaCl, pH 7.0) with 10% (V/V) DMSO in a glass tube was illuminated using photoreactor (WATTCAS, WP-TEC-1020LC, 365nm, 4 ℃, 10 W, 162384 μW/cm^2). After the reactions were completed, the mixture was extracted with ethyl acetate (3×3 mL). The combined organic phase was concentrated by rotary evaporation. Then, the crude product was dissolved in isopropanol (90 μL) and internal standard thioxanthone in isopropanol (0.01 mg/mL, 60 μL) was added. Yields of photocycloaddition products **2** were measured using normal-phase HPLC based on an average of triplicate runs. The *e.e.* values were determined by normal-phase HPLC on a chiral stationary phase.

The 100-fold scale-up reactions (10 mg-scale) were performed with the reaction mixture (100 mL) containing the TPe lysates and substrate **1** (200 mM) in MOPS buffer (20 mM MOPS, 150 mM NaCl, pH 7.0) with 10% (V/V) DMSO in a 250 mL round-bottle flask was illuminated under LED lamps (365 nm, 6 W, 39300 μW/cm^2) for 20 h in ice bath. the mixture was extracted with ethyl acetate (100×3 mL). The combined organic phase was concentrated by rotary evaporation. The crude products were purified by flash chromatography on silica gel (PE/EtOAc, 20:1 to 10:1) to afford the photocycloaddition products.

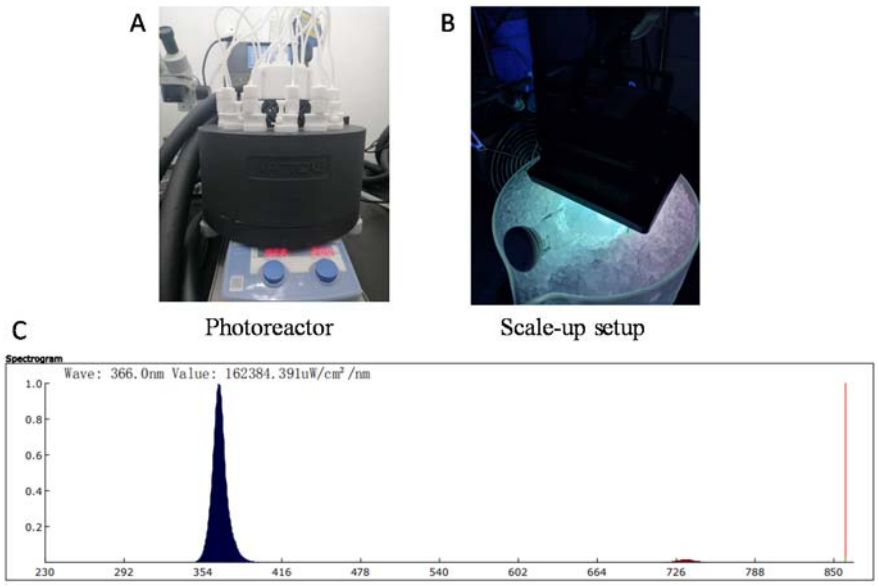


Figure S1. The reaction setup and emission spectrum of the lamp. **A.** The photoreactor used for analytical scale reactions. **B.** The scale-up reaction setup. **C.** The emission spectrum of the lamps.

4. The results of photocycloaddition during TPe evolution

4.1 The 1st Round of evolution: screen the positions for BpA insertion

Table S1. The results of photocycloaddition of substrate 1a with different catalysts.
Reaction conditions: 200 μM substrate **1a**, 2.5 mol% catalyst in 1.0 mL solvent (BpA: CH_3CN, DMF: MOPS (1:10) for enzyme), 365 nm, 12 h, room temperature. (ND: not detected)

Entry	Catalyst	Yield (%)	*e.e.* (%)
1	Benzophenone (10 mol%)	>90	0
2	LmrR	<5	ND
3	TPe_V15BpA	<5	ND
4	TPe_N19BpA	<5	ND
5	TPe_M89BpA	<5	ND
6	TPe_F93BpA (TPe1.0)	50	0

4.2 The 2nd round of evolution

Table S2. The results of photocycloaddition of 1a catalysed by TPe1.0 mutants.
Reaction conditions: 200 μM substrate **1a**, 2.5 mol% catalyst in 1.0 mL solvent (DMF: MOPS (1:10) for enzyme), 365 nm, 12 h, room temperature.

Entry	Catalyst	Yield (%)	*e.e.* (%)
1	TPe1.0_M8H	67	0
2	TPe1.0_M8P	26	6
3	TPe1.0_M8S	14	9
4	TPe1.0_M8F	14	0
5	TPe1.0_M8E	57	0
6	TPe1.0_M8R	67	0
7	TPe1.0_N19V	13	0
8	TPe1.0_N19D	25	7
9	TPe1.0_N19T	25	0
10	TPe1.0_N19S	22	0
11	TPe1.0_N19F	6	0
12	TPe1.0_N19Y	42	9
13	TPe1.0_N19G	16	6
14	TPe1.0_N19L	20	<5
15	TPe1.0_N19I	25	7
16	TPe1.0_M89Y	35	0
17	TPe1.0_M89S	23	0
18	TPe1.0_M89E	52	9
19	TPe1.0_M89F	30	0
20	TPe1.0_M89I	20	0
21	TPe1.0_M89P	29	0
22	TPe1.0_M89A	44	6
23	TPe1.0_M89N	33	0

24	TPe1.0_M89Q	36	10
25	TPe1.0_M89R	10	0
27	TPe1.0_M89D	48	10
28	TPe1.0_W96T	30	0
29	TPe1.0_W96Q	38	0
30	TPe1.0_W96L	30	49
31	TPe1.0_W96V	33	38
32	TPe1.0_W96E	25	0
33	TPe1.0_W96I	22	17
34	TPe1.0_D100G	73	5
35	TPe1.0_D100N	67	5
36	TPe1.0_D100Q	65	9
37	TPe1.0_D100K	70	<5
38	TPe1.0_D100P	66	0
39	TPe1.0_D100D	42	9
40	TPe1.0_D100R	26	9
41	TPe1.0_D100L	18	7
42	TPe1.0_D100A	54	26

4.3 The 3rd round of evolution

Table S3. The results of photocycloaddition of 1a catalysed by TPe2.0 mutants.
Reaction conditions: 200 μM substrate **1a**, 2.5 mol% catalyst in 1.0 mL solvent (DMSO: MOPS (1:10) for enzyme), 365 nm, 12 h, room temperature.

Entry	Catalyst	Yield (%)	*e.e.* (%)
1	TPe2.0_M8A	53	0
2	TPe2.0_M8R	40	0
3	TPe2.0_M8K	53	0
4	TPe2.0_M8S	47	0
5	TPe2.0_M8Y	12	0
6	TPe2.0_M8P	9	0
7	TPe2.0_M8Q	9	0
8	TPe2.0_M8T	18	0
9	TPe2.0_M8G	17	0
10	TPe2.0_M8F	32	34
11	TPe2.0_M8V	38	59
12	TPe2.0_M8L	58	71
13	TPe2.0_V15K	31	0
14	TPe2.0_V15A	34	12
15	TPe2.0_V15G	22	0
16	TPe2.0_V15L	29	0
17	TPe2.0_V15R	27	8
18	TPe2.0_N19T	26	0
19	TPe2.0_N19E	43	0
20	TPe2.0_N19G	59	<5
21	TPe2.0_N19M	34	0
22	TPe2.0_N19L	18	0
23	TPe2.0_N19F	39	0

24	TPe2.0_N19S	39	6
25	TPe2.0_N19V	22	0
26	TPe2.0_N19Q	18	6
27	TPe2.0_N19R	14	0
28	TPe2.0_D100L	36	53
29	TPe2.0_D100Q	39	3
30	TPe2.0_D100P	26	13
31	TPe2.0_D100R	39	3

5. Optimizations of the reaction conditions

5.1 The effect of enzyme dose

Table S4. Effect of enzyme dose. The reaction condition: substrate **1a** (200 μM), TPe2.0 lysate 2.5-5 mol %), MOPS buffer (20 mM MOPS, 150 mM NaCl, pH 7.0), DMSO (10% v/v), stirred at room temperature for 12 h under aerobic condition with irradiation by 365 nm UV light.

Entry	Catalyst (mol%)	Yield (%)	*e.e.* (%)
1	2.5	30	49
2	5	45	65

5.2 The effect of reaction medium

Table S5. Effect of medium on catalytic activity. The reaction condition: substrate **1a** (200 μM), TPe2.0 lysate (5 mol%), MOPS buffer (20 mM MOPS, 150 mM NaCl, pH 7.0) containing different organic solvents, stirred at room temperature for 12 h under aerobic condition with irradiation by 365 nm UV light.

Entry	Solvent (1:10)	Yield (%)	*e.e.* (%)
1	MOPS	12	19
2	DMF/MOPS	43	63
3	MeCN/MOPS	34	63
4	Isopropanol/MOPS	27	63
5	DMSO/MOPS	45	65
6	EtOA/MOPS	14	47
7	Glycerol/MOPS	25	35

6. Sequence information

6.1. Sequence of primers using for LmrR, Tpe1.0, Tpe2.0 and Tpe3.0 mutagenesis

LmrR_V15BpA_fw	GCT CAA ACC AAT **TAG** ATC CTG CTG AAT
LmrR_V15BpA_rv	ATT CAG CAG GAT **CTA** ATT GGT TTG AGC
LmrR_N19BpA_fw	GTC ATC CTG CTG **TAG** GTC CTG AAA CAA G
LmrR_N19BpA_rv	C TTG TTT CAG GAC **CTA** CAG CAG GAT GAC
LmrR_M89BpA_fw	GGC CAT GAA AAC **TAG** CGC CTG GCG TTC
LmrR_M89BpA_rv	GAA CGC CAG GCG **CTA** GTT TTC ATG GCC
LmrR_F93BpA_fw	ATG CGC CTG GCG **TAG** GAA TCC TGG AGT
LmrR_F93BpA_rv	ACT CCA GGA TTC **CTA** CGC CAG GCG CAT
LmrR_W96BpA_fw	GCG TTC GAA TCC **TAG** AGT CGT GTG GAC
LmrR_W96BpA_rv	GTC CAC ACG ACT **CTA** GGA TTC GAA CGC
M8N_fw	ATC CCG AAA GAA **NNK** CTG CGT GCT CAA
M8N_rv	TTG AGC ACG CAG **MNN** TTC TTT CGG GAT
V15N_fw	GCT CAA ACC AAT **NNN** ATC CTG CTG AAT
V15N_rv	ATT CAG CAG GAT **NNN** ATT GGT TTG AGC
N19N_fw	GTC ATC CTG CTG **NNK** GTC CTG AAA CAA G
N19N_rv	C TTG TTT CAG GAC **MNN** CAG CAG GAT GAC
F93BpA_M89N_fw	GGC CAT GAA AAC **NNN** CGC CTG GCG TAG
F93BpA_M89N_rv	CTA CGC CAG GCG **NNN** GTT TTC ATG GCC
F93BpA_W96N_fw	GCG TAG GAA TCC **NNN** AGT CGT GTG GAC
F93BpA_W96N_rv	GTC CAC ACG ACT **NNN** GGA TTC CTA CGC
D100N_fw	TGG AGT CGT GTG **NNK** AAA ATC ATT GAA
D100N_rv	TTC AAT GAT TTT **MNN** CAC ACG ACT CCA
M8L_A11S_fw	GAA TTG CTG CGT TCC CAA ACC AAT GTC
M8L_A11S_rv	GAC ATT GGT TTG GGA ACG CAG CAA TTC
M8L_A11T_fw	GAA TTG CTG CGT ACG CAA ACC AAT GTC
M8L_A11T_rv	GAC ATT GGT TTG CGT ACG CAG CAA TTC
M8L_A11N_fw	GAA TTG CTG CGT AAT CAA ACC AAT GTC
M8L_A11N_rv	GAC ATT GGT TTG ATT ACG CAG CAA TTC
M8L_A11H_fw	GAA TTG CTG CGT CAT CAA ACC AAT GTC
M8L_A11H_rv	GAC ATT GGT TTG ATG ACG CAG CAA TTC
L18S_fw	AAT GTC ATC CTG TCC AAT GTC CTG AAA
L18S_rv	TTT CAG GAC ATT GGA CAG GAT GAC ATT
L18T_fw	AAT GTC ATC CTG ACG AAT GTC CTG AAA
L18T_rv	TTT CAG GAC ATT CGT CAG GAT GAC ATT

L18N_fw	AAT GTC ATC CTG AAT AAT GTC CTG AAA
L18N_rv	TTT CAG GAC ATT ATT CAG GAT GAC ATT
L18H_fw	AAT GTC ATC CTG CAT AAT GTC CTG AAA
L18H_rv	TTT CAG GAC ATT ATG CAG GAT GAC ATT

6.2. Nucleic acid and protein sequences of LmrR and TPes

LmrR

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAAATGCTGCGTGCTCAAACCAATGTCATCCTG
CTGAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGA
AAGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTT
TTGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTTCGAATCCTGGAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKEML RAQTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLAFESWSRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe_V15BpA

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAAATGCTGCGTGCTCAAACCAATTAGATCCTG
CTGAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGA
AAGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTT
TTGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTTCGAATCCTGGAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKEML RAQTN**V(BpA)**ILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLAFESWSRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe_N19BpA

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAAATGCTGCGTGCTCAAACCAATGTCATCCTG
CTGTAGGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGA
AAGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTT
TTGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTTCGAATCCTGGAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKEML RAQTNVILL**N(BpA)** VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLAFESWSRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe_M89BpA

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAAATGCTGCGTGCTCAAACCAATGTCATCCTG
CTGAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGA
AAGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTT
TTGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACTAGCG
CCTGGCGTTCGAATCCTGGAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKEML RAQTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHEN**M(BpA)** RLAFESWSRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe_F93BpA(TPe1.0)

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAAATGCTGCGTGCTCAAACCAATGTCATCCTG
CTGAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGA
AAGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTT
TTGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTAGGAATCCTGGAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKEML RAQTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(BpA)**ESWSRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe2.0

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAAATGCTGCGTGCTCAAACCAATGTCATCCTG
CTGAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGA
AAGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTT
TTGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTAGGAATCCCTTAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKEML RAQTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(BpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe3.0

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAATTGCTGCGTGCTCAAACCAATGTCATCCTG
CTGAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGA
AAGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTT
TTGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTAGGAATCCCTTAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKE**L**LL RAQTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(BpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe3.0_FBpA

Protein Sequence

MGAEIPKE**L**LL RAQTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(FBpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe3.0_A11S

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAATTGCTGCGTTCCCAAACCAATGTCATCCTGC
TGAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGAA
AGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTTT
TGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTAGGAATCCCTTAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKE**L**LL R**S**QTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(BpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe3.0-A11N (TPe 4.0)

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAATTGCTGCGTAATCAAACCAATGTCATCCTGC
TGAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGAA
AGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTTT
TGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTAGGAATCCCTTAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKE**L**LL R**N**QTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(BpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe 4.0_FBpA

Protein Sequence

MGAEIPKE**L**LL R**N**QTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(FBpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe3.0-A11T

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAATTGCTGCGTACGCAAACCAATGTCATCCTG
CTGAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGA
AAGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTT
TTGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTAGGAATCCCTTAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKE**L**LL R**T**QTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(BpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe3.0_A11H

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAATTGCTGCGTCATCAAACCAATGTCATCCTGC
TGAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGAA
AGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTTT
TGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTAGGAATCCCTTAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKE**L**LL R**H**QTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(BpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe3.0_FBpA_A11H

Protein Sequence

MGAEIPKE**L**LL R**H**QTNVILLN VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(FBpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe3.0_L18T

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAATTGCTGCGTGCTCAAACCAATGTCATCCTG
ACGAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGA
AAGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTT
TTGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTAGGAATCCCTTAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKE**L**LL RAQTNVIL**T**N VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(BpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe3.0_L18S

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAATTGCTGCGTGCTCAAACCAATGTCATCCTGT
CCAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGAA
AGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTTT
TGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTAGGAATCCCTTAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGATGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCCA
CCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKE**L**LL RAQTNVIL**S**N VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(BpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe3.0_L18H

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAATTGCTGCGTGCTCAAACCAATGTCATCCTG
CATAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGA
AAGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTT
TTGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTAGGAATCCCTTAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKE**L**LL RAQTNVIL**H**N VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(BpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

TPe3.0_L18N

Nucleic acid sequence

ATGGGTGCCGAAATCCCGAAAGAATTGCTGCGTGCTCAAACCAATGTCATCCTG
AATAATGTCCTGAAACAAGGCGATAACTATGTGTATGGCATTATCAAACAGGTGA
AAGAAGCGAGCAACGGTGAAATGGAACTGAATGAAGCCACCCTGTATACGATTT
TTGATCGTCTGGAACAGGACGGCATTATCAGCTCTTACTGGGGTGATGAAAGTCA
AGGCGGTCGTCGCAAATATTACCGTCTGACCGAAATCGGCCATGAAAACATGCG
CCTGGCGTAGGAATCCCTTAGTCGTGTGGACAAAATCATTGAAAATCTGGAAGC
AAACAAAAAATCTGAAGCGATCAAATCTAGAGGTGGCAGCGGTGGCTGGAGCC
ACCCGCAGTTCGAAAAATAA

Protein Sequence

MGAEIPKE**L**LL RAQTNVIL**N**N VLKQGDNYVY GIIKQVKEAS NGEMELNEAT LYTIFDRLEQ DGIISSYWGD ESQGGRRKYY RLTEIGHENM RLA**F(BpA)**ES**L**SRV DKIIENLEAN KKSEAIKSRG GSGGWSHPQF EK

7. The absorption spectrum of benzophenone and 3'-fluoro-benzophenone

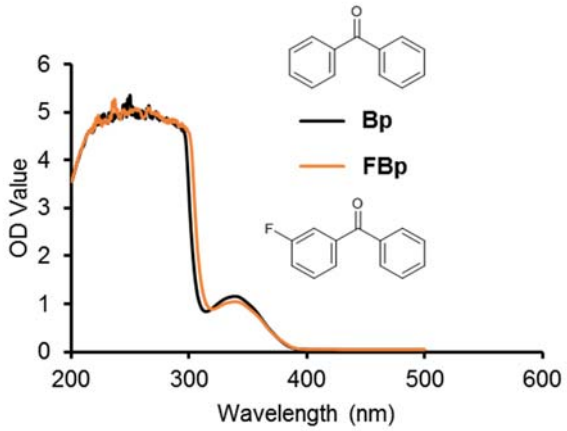


Figure S2. Absorption spectrum of benzophenone (Bp) and 3'-fluorobenzophenone (FBp).

8. Crystallization conditions for photoenzyme-substrate complex

Crystallization of TPe3.0 and the TPe3.0-1b complex: The crystals of TPe3.0 (PDB: 7XUP) were obtained under the condition of 0.2 M NaCl, 0.2 M $MgCl_2$, 0.1 M Tris-HCl, pH 8.5, 25% (w/v) PEG3350. The crystallization condition for TPe3.0-**1b** complex (7XUQ) was 0.2 M $MgCl_2$, 0.1 M Tris-HCl, pH 8.5, 25% (w/v) PEG3350. The crystals were transferred into a cryoprotectant with 25% Paraffin Oil and 75% Parabar and flash-frozen in liquid nitrogen until X-ray data collection on beamline BL18U1 at the Shanghai Synchrotron Radiation Facility (Shanghai, China). Data were processed and scaled by HKL2000 [4], XDS [5] and aimless [6] and the structures were solved by molecular replacement with the program MOLREP [6] using LmrR-Daunomycin (PDB: 3F8F) without the ligand as the template. Refinement was performed with Phenix.refine[7] followed by manual examination and rebuilding of the refined coordinates in the program Coot[8].

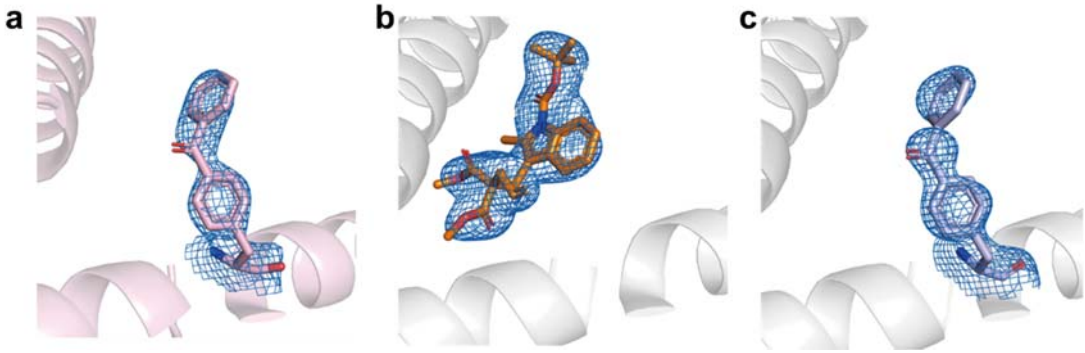


Figure S3. a. Composite omit map for BpA in TPe3.0 (contoured at 1.0 σ blue mesh). b. Composite omit map for substrate **1b** in the TPe3.0-**1b** complex structure (contoured at 1.0 σ, blue mesh). c. Composite omit map for BpA in the TPe3.0-**1b** complex structure (contoured at 1.0 σ, blue mesh).

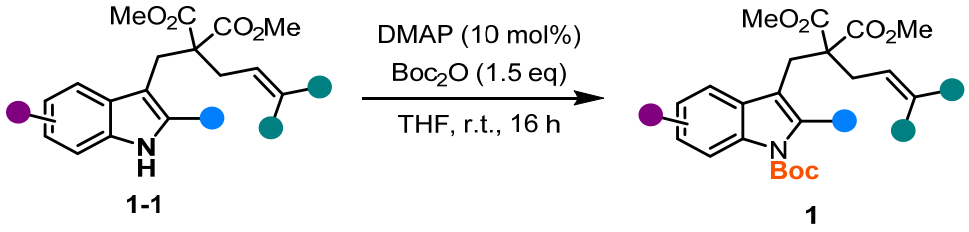
Table S6. Data collection and refinement statistics

data set	TPe3.0	TPe3.0 complexed with N-Boc-3-alkenylindole
Wavelength, Å	0.97915	0.97915
Resolution range, Å	19.13 - 2.602 (2.695 - 2.602)	42.4 - 2.5 (2.589 - 2.5)
Space group	P 32 1 2	P 32 1 2
Unit cell	69.697 69.697 61.787 90 90 120	68.764 68.764 60.386 90 90 120
Total reflections	14571 (974)	115700 (13598)
Unique reflections	5259 (454)	5740 (562)
Multiplicity	2.8(1.7)	20.1 (21.2)
Completeness (%)	97.17 (83.58)	98.96 (98.94)
Mean I/sigma(I)	19.8(4.3)	16.4 (2.0)
Wilson B-factor	31.70	55.29
R-merge	0.045 (0.188)	0.139 (1.955)
R-meas	0.056 (0.265)	0.143 (2.001)
R-pim	0.032 (0.188)	0.032 (0.428)
CC1/2	0.996 (0.887)	0.998 (0.736)
Reflections used in refinement	5258 (453)	5735 (561)
Reflections used for R-free	261 (23)	268 (29)
R-work	0.2130 (0.2617)	0.2088 (0.3378)
R-free	0.2520 (0.2679)	0.2608 (0.3311)
Number of non-hydrogen atoms	893	955
macromolecules	858	901
ligands	19	49
solvent	16	5
Protein residues	108	113
RMS (bonds,Å)	0.004	0.009
RMS (angles,deg)	0.61	1.11
Ramachandran favored (%)	100.00	94.44
Ramachandran allowed (%)	0.00	5.56
Ramachandran outliers (%)	0.00	0.00
Rotamer outliers (%)	0.00	0.00
Clashscore	3.46	5.37
Average B-factor	46.77	67.59
macromolecules	46.79	67.85
ligands	52.21	64.70
solvent	39.03	49.00

Statistics for the highest-resolution shell are shown in parentheses.

9. Synthesis of substrates

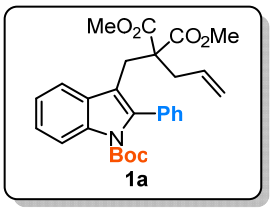
9.1 General procedure for the synthesis of substrates



N-Boc indole derivatives **1** were synthesized in a single step from the NH precursors 1-1 that were reported in literature [9]. A mixture of indole 1-1 (1.0 eq) in anhydrous THF was added $(Boc)_2O$ (1.1 eq), DMAP (5 mol%) at room temperature. The mixture was stirred for 16 h before quenching with saturated aqueous Na_2CO_3 solution. The organic layer was collected and the aqueous layer was extracted with EtOAc (30 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4 and concentrated by rotary evaporation. Purification by flash column chromatography (PE/EtOAc: 100/1 to 50/1) afforded the desired products.

9.2 Characterization data of substrates

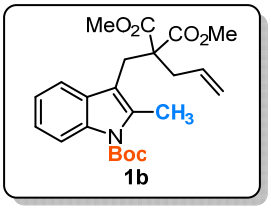
Substrate 1a



White solid. **TLC**: R_f = 0.54 (Hexane/EtOAc = 10:1) [UV].

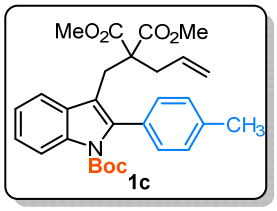
^{1}H NMR (600MHz, $CDCl_3$) δ 8.22 (d, *J* = 8.5 Hz, 1H), 7.52 (d, *J* = 7.8Hz, 1H), 7.48 – 7.38 (m, 5H), 7.35 – 7.30 (m, 1H), 7.27 – 7.22 (m, 1H), 4.87 – 4.76 (m, 1H), 4.76 – 4.68 (m, 2H), 3.50 (s, 7H), 3.46 (s, 2H), 2.25 (d, *J* = 6.5 Hz, 2H), 1.23 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.49, 150.02, 138.37, 136.16, 134.03, 132.49, 130.63, 129.61, 128.28, 127.85, 124.54, 122.38, 119.33, 118.16, 114.89, 114.57, 83.22, 58.98, 52.18, 37.41, 27.46, 26.84.
HRMS (ESI): Calcd for $C_{28}H_{32}NO_6$ $[M+H]^+$: 478.2224; Found: 478.2231.

Substrate 1b



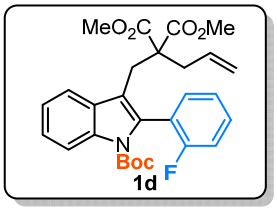
White solid. **TLC**: R_f = 0.52 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.1 Hz, 1H), 7.44 – 7.16 (m, 1H), 7.25 – 7.16 (m, 2H), 5.94 – 5.89 (m, 1H), 5.19 – 5.10 (m, 2H), 3.65 (s, 6H), 3.41 (s, 2H), 2.65 (d, *J* = 6.1 Hz, 2H), 2.54 (s, 3H), 1.70 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.53, 150.59, 135.89, 135.60, 133.07, 130.27, 123.38, 122.20, 118.82, 118.31, 115.27, 112.90, 83.72, 59.09, 52.29, 52.18, 37.98, 28.30, 28.20, 14.94.
HRMS (ESI): Calcd for $C_{23}H_{30}NO_6$ $[M+H]^+$: 416.2068; Found: 416.2075.

Substrate 1c



White solid. **TLC**: R_f = 0.51 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, *J* = 8.4 Hz, 1H), 7.51 (d, *J* = 7.9 Hz, 1H), 7.35 – 7.22 (m, 6H), 4.91 – 4.82 (m, 1H), 4.78 – 4.69 (m, 2H), 3.50 (s, 6H), 3.45 (s, 2H), 2.43 (s, 3H), 2.26 (d, *J* = 6.8 Hz, 2H), 1.27 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.53, 150.07, 138.61, 137.60, 136.08, 132.66, 130.92, 130.46, 129.68, 128.89, 124.38, 122.31, 119.26, 117.96, 114.87, 114.40, 83.20, 58.97, 52.17, 37.41, 27.50, 26.89, 21.29.
HRMS (ESI): Calcd for $C_{29}H_{34}NO_6$ $[M+H]^+$: 492.2381; Found: 492.2389.

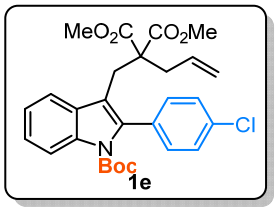
Substrate 1d



Yellow solid. **TLC**: R_f = 0.56 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, *J* = 8.3 Hz, 1H), 7.57 (d, *J* = 7.4 Hz, 1H), 7.47 – 7.32 (m, 3H), 7.30 – 7.23 (m, 1H), 7.17 (t, *J* = 9.6 Hz, 1H), 4.95 – 4.85 (m, 1H), 4.84 – 4.74 (m, 2H), 3.55 (s, 3H), 3.49 (s, 3H), 3.53 – 7.23 (m, 2H), 2.41 (dd, *J* = 14.3, 7.7 Hz, 1H), 2.25 (dd, *J* = 14.3, 6.0 Hz, 1H), 1.32 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.44, 171.37, 160.98 (d, *J* = 247.6 Hz), 149.75, 136.39, 132.20 (d, *J* = 22.7 Hz), 130.31 (d, *J* = 8.1 Hz), 129.50, 124.91, 124.22 (d, *J* = 3.7 Hz), 122.47 (d, *J* = 12.1 Hz), 119.45, 118.26, 115.94, 115.80, 115.59, 115.27, 83.36, 58.85, 52.27, 52.16, 37.03, 27.48, 27.18.

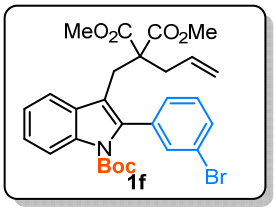
HRMS (ESI): Calcd for $C_{28}H_{31}FNO_6$ $[M+H]^+$: 496.2130; Found: 496.2132.

Substrate 1e



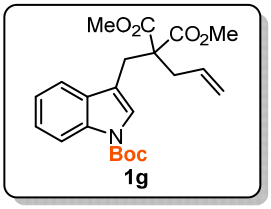
White solid. **TLC**: R_f = 0.52 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.3 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.49 – 7.44 (m, 2H), 7.38 – 7.32 (m, 3H), 7.26 (t, *J* = 7.0 Hz, 1H), 5.06 – 4.92 (m, 1H), 4.89 – 4.74 (m, 2H), 3.51 (s, 6H), 3.45 (s, 2H), 2.29 (d, *J* = 8.3 Hz, 2H), 1.30 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.34, 149.81, 136.93, 136.16, 134.00, 132.56, 132.19, 132.01, 129.55, 128.46, 124.84, 122.58, 119.45, 118.41, 115.23, 115.08, 83.59, 58.81, 52.23, 37.63, 27.56, 26.95.
HRMS (ESI): Calcd for $C_{28}H_{31}ClNO_6$ $[M+H]^+$: 512.1834; Found: 512.1833.

Substrate 1f



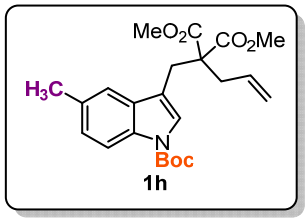
Yellow solid. **TLC**: R_f = 0.52 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.3 Hz, 1H), 7.58 – 7.51 (m, 3H), 7.38 – 7.32 (m, 3H), 7.29 – 7.24 (m, 1H), 5.03 – 4.91 (m, 1H), 4.85 – 4.76 (m, 2H), 3.50 (s, 6H), 3.44 (s,2H), 2.31 (d, *J* = 7.1 Hz, 2H), 1.28 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.36, 149.75, 136.43, 136.20, 136.15, 133.75, 132.06, 130.83, 129.75, 129.45, 129.10, 124.94, 122.59, 122.24, 119.49, 118.47, 115.36, 115.06, 83.57, 58.80, 52.26, 37.56, 27.54, 26.89.
HRMS (ESI): Calcd for $C_{28}H_{31}BrNO_6$ $[M+H]^+$: 556.1329; Found: 556.1336.

Substrate 1g



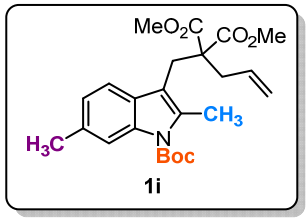
White solid. **TLC**: R_f = 0.58 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 7.1 Hz, 1H), 7.50 (d, *J* = 7.3 Hz, 1H), 7.41 (s, 1H), 7.33 – 7.28 (m, 1H), 7.26– 7.21 (m, 1H), 5.88 – 5.75 (m, 1H), 5.22 – 5.14 (m, 2H), 3.70 (s, 6H), 3.37 (s, 2H), 2.72 (d, *J* = 7.2 Hz, 2H), 1.69 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 1171.27, 149.56, 135.02, 132.61, 130.96, 124.67, 124.34, 122.34, 119.40, 118.95, 115.16, 114.62, 83.63, 58.56, 52.41, 37.31, 28.20, 27.69.
HRMS (ESI): Calcd for $C_{22}H_{28}NO_6$ $[M+H]^+$: 402.1911; Found: 402.1914.

Substrate 1h



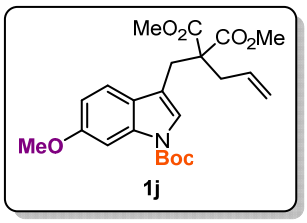
White solid. **TLC**: R_f = 0.51 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.36 (s, 1H), 7.28 (s, 1H), 7.12 (dd, *J* = 8.4, 1.9 Hz, 1H), 5.87 – 5.74 (m, 1H), 5.22 – 5.13 (m, 2H), 3.70 (s, 6H), 3.34 (s, 2H), 2.71 (d, *J* = 7.3 Hz, 2H), 2.45 (s,3H), 1.68 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.29, 149.59, 132.66, 131.69, 131.13, 125.68, 124.70, 119.37, 118.89, 114.79, 114.32, 83.43, 58.47, 52.37, 37.22, 28.21, 27.65, 21.38.
HRMS (ESI): Calcd for $C_{23}H_{30}NO_6$ $[M+H]^+$: 416.2068; Found: 416.2071.

Substrate 1i



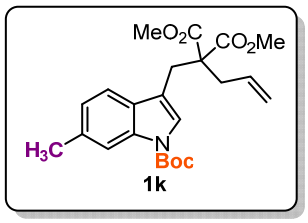
White solid. **TLC**: R_f = 0.54 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.5 Hz, 1H), 7.20 (s, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 5.95 – 5.82 (m, 1H), 5.20 – 5.10 (m, 2H), 3.66 (s, 6H), 3.38 (s, 2H), 2.64 (d, *J* = 7.1 Hz, 2H), 2.52 (s, 3H), 2.43 (s, 3H), 1.69 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.54, 150.62, 135.94, 133.78, 133.12, 131.44, 130.42, 124.64, 118.81, 118.31, 114.98, 112.60, 83.51, 59.02, 52.25, 37.79, 28.31, 28.13, 21.31, 14.99.
HRMS (ESI): Calcd for $C_{24}H_{32}NO_6$ $[M+H]^+$: 430.2224; Found: 430.2230.

Substrate 1j



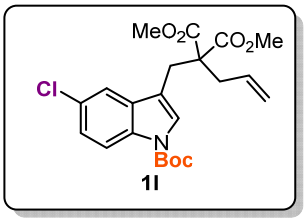
White solid. **TLC**: R_f = 0.59 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.34 (d, *J* = 8.7 Hz, 1H), 7.26 (s, 1H), 6.85 (dd, *J* = 8.7, 2.4 Hz, 1H), 5.85 – 5.72 (m, 1H), 5.20 – 5.09 (m, 2H), 3.85 (s, 3H), 3.67 (s, 6H), 3.30 (s, 2H), 2.69 (d, *J* = 7.2 Hz, 2H), 1.66 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.23, 157.82, 149.57, 136.00, 132.63, 124.68, 123.22, 119.43, 119.32, 114.56, 111.72, 99.20, 83.41, 58.51, 55.49, 52.35, 37.25, 28.14, 27.72.
HRMS (ESI): Calcd for $C_{23}H_{30}NO_7$ $[M+H]^+$: 432.2017; Found: 432.2027.

Substrate 1k



White solid. **TLC**: R_f = 0.52 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98 (br s, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.31 (br s, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 5.85 – 5.76 (m, 1H), 5.22 – 5.10 (m, 2H), 3.70 (s, 6H), 3.34 (s, 2H), 2.71 (d, *J* = 7.2 Hz, 2H), 2.49 (s, 3H), 1.68 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 171.32, 149.64, 134.37, 132.68, 128.72, 123.98, 123.82, 119.36, 118.58, 115.43, 114.58, 83.43, 58.59, 52.41, 37.29, 28.19, 27.75, 21.89.
HRMS (ESI): Calcd for $C_{23}H_{30}NO_6$ $[M+H]^+$: 416.2068; Found: 416.2065.

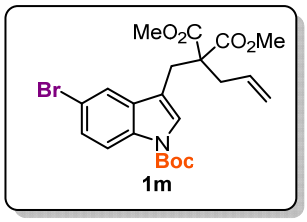
Substrate 1l



White solid. **TLC**: R_f = 0.51 (Hexane/EtOAc = 10:1) [UV].

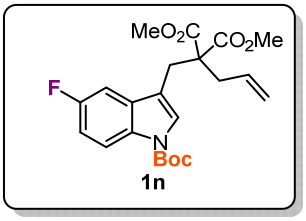
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 6.8 Hz, 1H), 7.42 (d, *J* = 2.1 Hz, 1H), 7.40 (s, 1H), 7.21 (dd, *J* = 8.8, 2.1 Hz, 3H), 5.82 – 5.69 (m, 1H), 5.25 – 5.12 (m, 2H), 3.68 (s, 6H), 3.28 (s, 2H), 2.68 (d, *J* = 7.3 Hz, 2H), 1.65 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.02, 149.09, 133.37, 132.43, 132.05, 128.11, 126.05, 124.40, 119.57, 118.67, 116.20, 113.97, 84.04, 58.19, 52.42, 37.25, 28.09, 27.53.
HRMS (ESI): Calcd for $C_{22}H_{26}ClNO_6$ $[M+H]^+$: 436.1521; Found: 436.1526.

Substrate 1m



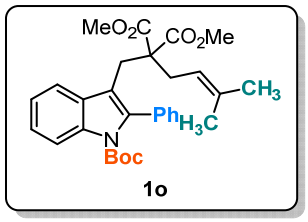
White solid. **TLC**: R_f = 0.53 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 7.4 Hz, 1H), 7.61 (d, *J* = 2.0 Hz, 1H), 7.40 (br s, 1H), 7.38 (dd, *J* = 8.8, 1.9 Hz, 1H), 5.87 – 5.69 (m, 1H), 5.32 – 5.13 (m, 2H), 3.71 (s, 6H), 3.30 (s, 2H), 2.70 (d, *J* = 7.2 Hz, 2H), 1.67 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.07, 149.15, 133.75, 132.57, 132.40, 127.12, 125.93, 121.80, 119.64, 116.65, 115.81, 113.90, 84.15, 58.16, 52.50, 37.22, 28.15, 27.51.
HRMS (ESI): Calcd for $C_{22}H_{27}BrNO_6$ $[M+H]^+$: 480.1016; Found: 480.1014.

Substrate 1n



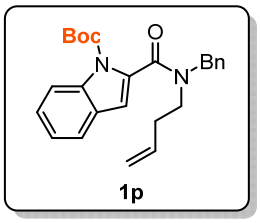
White solid. **TLC**: R_f = 0.51 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.43 (s, 1H), 7.14 (dd, *J* = 9.2, 2.5 Hz, 1H), 7.02 (td, *J* = 9.2, 2.5 Hz, 1H), 5.85 – 5.71 (m, 1H), 5.23 – 5.14 (m, 2H), 3.71 (s, 6H), 3.30 (s, 2H), 2.70 (d, *J* = 7.2 Hz, 2H), 1.67 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.14, 160.38, 158.00, 149.26, 132.44, 131.89 (d, *J* = 9.3 Hz), 126.31, 119.56, 116.13 (d, *J* = 9.0 Hz), 114.37 (d, *J* = 4.0 Hz), 112.10 (d, *J* = 25.0 Hz), 104.58 (d, *J* = 24.1 Hz).83.92, 58.35, 52.45, 37.30, 28.16, 27.70.
HRMS (ESI): Calcd for $C_{22}H_{27}FNO_6$ $[M+H]^+$: 420.1817; Found: 420.1826.

Substrate 1o



White solid. **TLC**: R_f = 0.53 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, *J* = 8.3 Hz, 1H), 7.54 – 7.49 (d, *J* = 8.1 Hz, 1H), 7.47 – 7.36 (m, 5H), 7.35 – 7.21 (m, 2H), 4.17 – 4.10 (m, 1H), 3.50 (s, 6H), 3.46 (s, 2H), 2.23 (d, *J* = 7.2 Hz, 2H), 1.45 (s, 3H), 1.37 (s, 3H), 1.23 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 171.96, 150.08, 138.46, 136.18, 134.35, 133.96, 130.64, 129.73, 128.17, 127.73, 124.49, 122.37, 119.34, 117.71, 114.85, 114.72, 83.17, 58.61, 52.17, 31.15, 27.46, 26.93, 25.82, 17.56.
HRMS (ESI): Calcd for $C_{30}H_{36}NO_6$ $[M+H]^+$: 506.2537; Found: 506.2545.

Substrate 1p



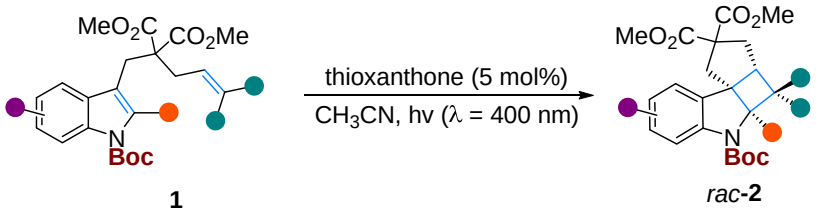
White solid. **TLC**: R_f = 0.47 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.4 Hz, 0.43H), 8.16 (d, *J* = 8.4 Hz, 0.54H), 7.62 (d, *J* = 7.9 Hz, 0.43H), 7.57 (d, *J* = 7.9 Hz, 0.54H), 7.50 (d, *J* = 7.4 Hz, 1H), 7.44 – 7.24 (m, 6H), 6.72 (d, *J* = 4.9 Hz, 1H), 5.94 – 5.84 (m, 0.52H), 5.64 – 5.55 (m, 0.41H), 5.18 (d, *J* = 17.1 Hz, 0.51H), 5.12 (d, *J* = 10.3 Hz, 0.52H), 5.02 – 4.83 (m, 2H), 4.60 (s, 3H), 3.61 (t, *J* = 7.5 Hz, 1H), 3.37 (t, *J* = 7.5 Hz, 1H), 2.49 (q, *J* = 7.2 Hz, 2H), 2.30 (q, *J* = 7.2 Hz, 2H), 1.72 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 164.98, 164.68, 149.28, 149.23, 137.19, 136.43, 135.70, 135.64, 134.40, 133.26, 133.23, 128.73, 128.68, 128.36, 127.87, 127.85, 127.51, 125.26, 123.27, 123.23, 121.40, 117.26, 116.78, 115.54, 108.48, 84.87, 53.03, 48.05, 47.95, 44.03, 32.66, 31.38, 28.27. (This compound contains a pile of rotamers)

10. Synthesis of racemic product standards

10.1 General procedure for the synthesis of racemic product Standards

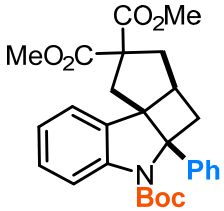


1 *rac*-**2**

In a 10 mL tube containing a stirring bar was added substrate **1** (0.2 mmol) in acetonitrile (5.0 mL). After addition of thioxanthone (5 mol%), the reaction mixture was deoxygenated by bubbling N_2 for 10 min, and was then illuminated by a 6 W blue LED at room temperature for 24-36 h monitored by TLC. The mixture was then concentrated in vacuo and purified by silica gel flash chromatography (PE/EA: 30/1 to 20/1) to afford the corresponding *rac*-**2**.

10.2 Characterization data of racemic product standards

Product *rac*-2a



***rac*-2a**

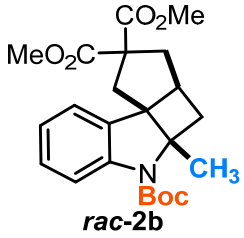
White solid. **TLC**: R_f = 0.50 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, *J* = 8.0 Hz, 1H), 7.33 – 7.28 (m, 2H), 7.27 (m, 2H), 7.19 – 7.07 (m, 3H), 7.05 – 7.00 (m, 1H), 3.75 (s, 3H), 3.50 (s, 3H), 3.08 – 2.99 (m, 1H), 2.97 – 2.83 (m, 2H), 2.81 – 2.70 (m, 1H), 2.61 – 2.52 (m, 1H), 2.40 – 2.29 (m, 2H), 1.18 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 172.54, 172.10, 151.79, 145.20, 140.56, 134.61, 128.20, 127.93, 127.14, 126.66, 122.78, 122.15, 114.55, 80.57, 72.41, 63.52, 53.01, 52.73, 44.75, 40.31, 38.40, 32.91, 28.02.

HRMS (ESI): Calcd for $C_{28}H_{32}NO_6$ $[M+H]^+$: 478.2224; Found 478.2232.

Product *rac*-2b



***rac*-2b**

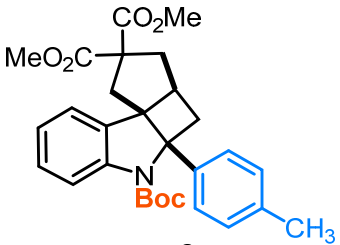
White solid. **TLC**: R_f = 0.46 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (br s, 1H), 7.18 (td, *J* = 7.7, 1.4 Hz, 1H), 7.11 (dd, *J* = 7.5, 1.3 Hz, 1H), 6.97 (td, *J* = 7.4, 1.1 Hz, 1H), 3.84 (s, 3H), 3.83 (s, 3H), 3.04 – 2.96 (m, 1H), 2.81 – 2.75 (m, 2H), 2.71 – 2.60 (m, 1H), 2.55 – 2.46 (m, 1H), 2.43 (dd, *J* = 14.0, 6.1 Hz, 1H), 1.97 (dd, *J* = 13.7, 6.0 Hz, 1H), 1.56 (s, 9H), 1.46 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.63, 172.53, 151.98, 144.16, 135.09, 127.99, 122.69, 122.30, 115.18, 80.77, 68.02, 63.88, 60.37, 53.08, 52.96, 44.54, 40.82, 38.63, 37.47, 28.52, 20.36.

HRMS (ESI): Calcd for $C_{23}H_{30}NO_6$ $[M+H]^+$: 416.2068; Found: 416.2069.

Product *rac*-2c



***rac*-2c**

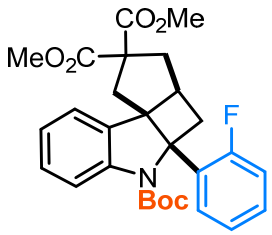
White solid. **TLC**: R_f = 0.45 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, *J* = 8.1 Hz, 1H), 7.23 (t, *J* = 7.7 Hz, 1H), 7.14 (d, *J* = 7.5 Hz, 1H), 7.09 (d, *J* = 7.8 Hz, 2H), 7.06 – 6.90 (m, 3H), 3.73 (s, 3H), 3.50 (s, 3H), 3.02 – 2.96 (m, 1H), 2.95 – 2.80 (m, 2H), 2.77 – 2.80 (m, 1H), 2.54 (dd, *J* = 14.0, 2.9 Hz, 1H), 2.41 – 2.34 (m, 2H), 2.32 (s, 3H), 1.18 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 172.55, 172.08, 151.84, 145.24, 137.56, 136.60, 134.74, 128.58, 128.13, 126.57, 122.72, 122.16, 114.53, 80.44, 72.35, 63.55, 63.33, 52.96, 52.65, 44.71, 40.38, 38.45, 33.10, 28.05, 21.06.

HRMS (ESI): Calcd for $C_{29}H_{34}NO_6$ $[M+H]^+$: 492.2381; Found: 492.2386.

Product *rac*-2d

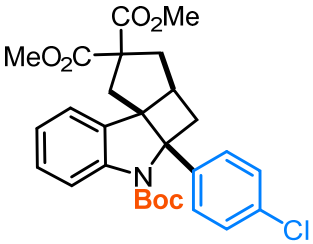


***rac*-2d**

White solid. **TLC**: R_f = 0.52 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (br s, 1H), 7.49 – 7.34 (m, 1H), 7.29 – 7.13 (m, 4H), 7.03 (t, *J* = 7.4 Hz, 1H), 6.98 – 6.90 (t, *J* = 7.3 Hz, 1H), 3.74 (s, 3H), 3.33 (s, 3H), 3.11 – 2.99 (m, 2H), 2.94 – 2.79 (m, 1H), 2.59 – 2.45 (m, 2H), 2.35 – 2.22 (m, 1H), 1.29 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 172.35, 171.94, 162.28, 159.79, 151.56, 144.44, 134.85, 131.49 (d, *J* = 5.0 Hz), 129.33 (d, *J* = 8.0 Hz), 127.95, 126.40 (d, *J* = 13.0 Hz), 122.68, 120.94, 115.87(d, *J* = 20.0 Hz), 114.66, 80.80, 70.06, 63.32, 62.78, 53.03, 52.72, 44.13, 40.05, 38.69, 33.43, 28.22.
HRMS (ESI): Calcd for $C_{28}H_{31}FNO_6$ $[M+H]^+$: 496.2130; Found: 496.2130.

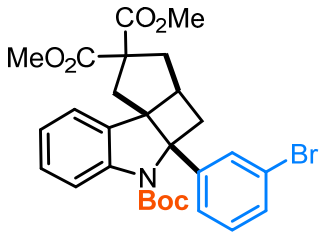
Product *rac*-2e



***rac*-2e**

White solid. **TLC**: R_f = 0.47 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, *J* = 6.9 Hz, 1H), 7.33 – 7.25 (m, 3H), 7.16 (d, *J* = 7.6 Hz, 1H), 7.11 – 6.99 (m, 1H), 3.76 (s, 3H), 3.53 (s, 3H), 3.01 – 2.84 (m, 3H), 2.82 – 2.71 (m, 1H), 2.59 – 2.50 (m, 1H), 2.43 – 2.27 (m, 2H), 1.21 (s, 9H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 172.40, 172.10, 151.55, 144.98, 139.23, 134.24, 132.92, 128.36, 128.20, 122.98, 122.19, 114.67, 80.86, 71.77, 63.45, 53.07, 52.77, 44.67, 40.25, 38.36, 33.09, 28.12.
HRMS (ESI): Calcd for $C_{28}H_{31}ClNO_6$ $[M+H]^+$: 512.1834; Found: 512.1841.

Product *rac*-2f



***rac*-2f**

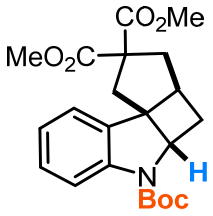
White solid. **TLC**: R_f = 0.47 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, *J* = 8.4 Hz, 1H), 7.39 (d, *J* = 7.7 Hz, 1H), 7.33 – 7.23 (m, 2H), 7.16 (d, *J* = 7.5 Hz, 2H), 7.04 (t, *J* = 6.9 Hz, 2H), 3.75 (s, 3H), 3.56 (s, 3H), 3.00 – 2.94 (m, 1H), 2.94 – 2.89 (m, 2H), 2.79 – 2.71 (m, 1H), 2.58 (d, *J* = 14.3 Hz, 1H), 2.39 (d, *J* = 15.8 Hz, 1H), 2.34 (br s, 1H), 1.22 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 172.40, 172.04, 151.50, 144.95, 143.08, 134.15, 130.29, 129.77, 128.40, 125.22, 123.01, 122.07, 114.70, 80.95, 71.68, 63.55, 63.42, 53.09, 52.98, 44.74, 40.05, 38.19, 32.94, 28.09.

HRMS (ESI): Calcd for $C_{28}H_{31}BrNO_6$ $[M+H]^+$: 556.1329; Found: 556.1327.

Product *rac*-2g



***rac*-2g**

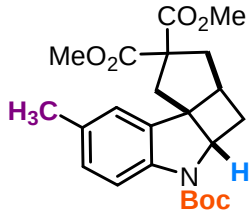
White solid. **TLC**: R_f = 0.51 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, *J* = 8.1 Hz,0.65H), 7.50 (d, *J* = 6.7 Hz, 0.33H), 7.21 (t, *J* = 7.7 Hz, 1H), 7.16 (d, *J* = 7.3 Hz, 1H), 7.02 – 6.95 (m, 1H), 4.55 (s, 0.29H), 4.39 (s, 0.65H), 3.86 (s, 3H), 3.80 (s, 3H), 2.90 – 2.79 (m, 2H), 2.63 – 2.47 (m, 3H), 2.28 – 2.09 (m,2H), 1.60 (s, 2.62H), 1.51 (s, 6.32H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 172.21, 151.72, 144.10, 134.47, 128.35, 122.63, 114.91, 80.30, 63.11, 61.85, 57.34, 53.05, 52.77, 45.65, 43.30, 40.85, 32.96, 28.41.

HRMS (ESI): Calcd for $C_{22}H_{28}NO_6$ $[M+H]^+$: 402.1911; Found: 402.1918 .

Product *rac*-2h



***rac*-2h**

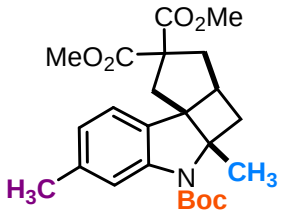
White solid. **TLC**: R_f = 0.45 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.4 Hz, 0.64H), 7.37 (d, *J* = 6.5 Hz, 0.31H), 7.03 (d, *J* = 7.6 Hz, 1H), 6.97 (s, 1H), 4.54 (s, 0.31H), 4.37 (s, 0.67H), 3.86 (s, 3H), 3.81 (s, 3H), 2.91 – 2.79 (m, 2H), 2.62 – 2.48 (m, 3H), 2.32 (s, 3H), 2.26 – 2.08 (m, 2H), 1.60 (s, 2.72H), 1.50 (s, 6.27H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 172.27, 151.71, 141.77, 134.48, 132.14, 128.83, 123.25, 114.65, 80.12, 63.09, 61.92, 57.38, 53.04, 52.74, 45.60, 43.21, 40.79, 32.90, 28.44, 20.92.

HRMS (ESI): Calcd for $C_{23}H_{30}NO_6$ $[M+H]^+$: 416.2068; Found: 416.2072.

Product *rac*-2i



***rac*-2i**

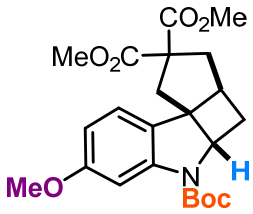
White solid. **TLC**: R_f = 0.49 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 6.98 d, *J* = 8.1 Hz,, 1H), 6.90 (s, 1H), 3.84 (s, 3H), 3.84 (s, 3H), 2.98 (d, *J* = 15.7 Hz, 1H), 2.86 – 2.74 (m, 2H), 2.70 – 2.58 (m, 1H), 2.52 – 2.40 (m, 2H), 2.31 (s, 3H), 1.95 (dd, *J* = 13.7, 6.2 Hz, 1H), 1.55 (s, 9H), 1.43 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ) 172.70, 172.58, 151.98, 141.89, 135.09, 132.13, 128.40, 122.84, 114.96, 80.51, 68.11, 63.84, 60.40, 53.06, 52.96, 44.54, 40.62, 38.39, 37.28, 28.55, 20.95, 20.36.

HRMS (ESI): Calcd for $C_{24}H_{32}NO_6$ $[M+H]^+$: 430.2224; Found: 430.2232.

Product *rac*-2j



***rac*-2j**

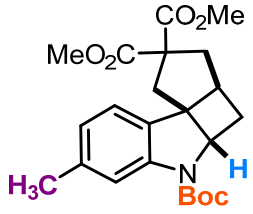
White solid. **TLC**: R_f = 0.52 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (s, 0.62H), 7.14 (s, 0.26H), 7.04 (d, *J* = 8.1 Hz, 1H), 6.54 (dd, *J* = 8.3, 2.5 Hz, 1H), 4.56 (s, 0.28H), 4.40 (s, 0.68H), 3.85 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 2.84 – 2.74 (m, 2H), 2.62– 2.53 (m, 1H), 2.54 – 2.46 (m, 2H), 2.27 – 2.05 (m, 2H), 1.61 (s, 2.52H), 1.51 (s, 6.46H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 172.26, 160.32, 151.68, 145.32, 126.38, 123.04, 109.23, 100.56, 80.35, 62.99, 62.75, 56.82, 55.55, 53.02, 52.73, 45.39, 43.37, 40.82, 32.86, 28.41.

HRMS (ESI): Calcd for $C_{23}H_{30}NO_7$ $[M+H]^+$: 432.2017; Found: 432.2024.

Product *rac*-2k



***rac*-2k**

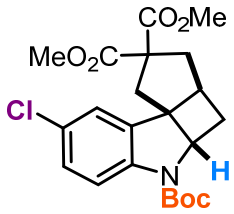
White solid. **TLC**: R_f = 0.46 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76 (s, 0.70H), 7.36 (s, 0.23H), 7.05 (d, *J* = 7.8 Hz, 1H), 6.82 (d, *J* = 7.7 Hz, 1H), 4.55 (s, 0.26H), 4.39 (s, 0.71H), 3.86 (s, 3H), 3.81 (s, 3H), 2.86 – 2.76 (m, 2H), 2.65 – 2.48 (m, 3H), 2.36 (s, 3H), 2.27 – 2.12 (m, 2H), 1.63 (s, 2.96H), 1.52 (s, 5.98H).

^{13}C NMR (151 MHz, $CDCl_3$): δ 172.26, 151.81, 144.19, 138.51, 131.69, 123.38, 122.34, 115.62, 80.31, 63.09, 62.23, 57.14, 53.03, 52.74, 45.55, 43.34, 40.85, 32.93, 28.42, 21.66.

HRMS (ESI): Calcd for $C_{23}H_{30}NO_6$ $[M+H]^+$: 416.2068; Found: 416.2069.

Product *rac*-2l



***rac*-2l**

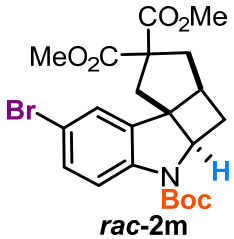
White solid. **TLC**: R_f = 0.45 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, *J* = 8.2 Hz, 0.69H), 7.40 (d, *J* = 16.7 Hz, 0.31H), 7.17 (d, *J* = 8.7 Hz, 1H), 7.12 (s, 1H), 4.56 (s, 0.28H), 4.41 (s, 0.71H), 3.86 (s, 3H), 3.81 (s, 3H), 2.92 – 2.80 (m, 2H), 2.59 (dd, *J* = 14.0, 8.2 Hz, 1H), 2.54 – 2.46 (m, 2H), 2.30 – 2.11 (m, 2H), 1.59 (s, 2.13H), 1.50 (s, 6.83H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 171.98, 151.55, 142.78, 136.43, 132.39, 128.18, 127.47, 122.97, 115.85, 80.70, 63.09, 62.22, 53.12, 52.82, 45.76, 43.16, 40.80, 32.98, 29.70, 28.37.

HRMS (ESI): Calcd for $C_{22}H_{26}ClNO_6$ $[M+H]^+$: 436.1521; Found: 436.1526.

Product *rac*-2m



***rac*-2m**

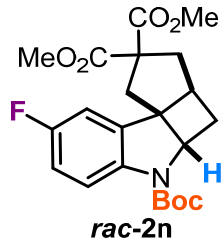
White solid. **TLC**: R_f = 0.48 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 9.2 Hz, 1H), 7.31 (d, *J* = 8.8 Hz,1H), 7.26 (s, 1H), 4.55 (s, 0.26H), 4.40 (s, 0.74H), 3.86 (s, 3H), 3.81 (s, 3H), 2.91 – 2.77 (m, 2H), 2.58 (dd, *J* = 14.1, 8.3 Hz, 1H), 2.54 – 2.46(m, 2H), 2.30 – 2.05 (m, 1H), 1.59 (s, 2.26H), 1.50 (s, 6.77H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 172.17, 171.98, 151.54, 143.33, 136.85, 131.11, 125.85, 116.38, 114.83, 80.74, 63.09, 62.19, 57.13, 53.13, 52.82, 45.82, 43.16, 40.77, 33.03, 28.37.

HRMS (ESI): Calcd for $C_{22}H_{27}BrNO_6$ $[M+H]^+$: 480.1016; Found: 480.1014.

Product *rac*-2n



***rac*-2n**

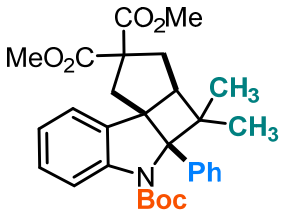
White solid. **TLC**: R_f = 0.45 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (s, 0.68 H), 7.39 (s, 0.29H), 6.93 – 6.85 (m, 2H), 4.58 (s, 0.29H), 4.42 (s, 0.70H), 3.85 (s, 3H), 3.80 (s, 3H), 2.91 – 2.79 (m, 2H), 2.59 (dd, *J* = 14.0, 8.4 Hz, 1H), 2.56 – 2.46 (m, 2H), 2.29 – 2.08 (m,2H), 1.59 (s, 2.36H), 1.50 (s, 6.66H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 172.03, 160.20, 157.81, 151.63, 140.06, 136.39, 115.55 (d, *J* = 7.1 Hz), 114.48 (d, *J* = 22.3 Hz), 110.05 (d, *J* = 22.6 Hz), 80.45, 63.14, 62.30, 57.21, 53.10, 52.80, 45.66, 43.30, 40.90, 32.86, 28.39.

HRMS (ESI): Calcd for $C_{22}H_{27}FNO_6$ $[M+H]^+$: 420.1817; Found: 420.1823.

Product *rac*-2o



***rac*-2o**

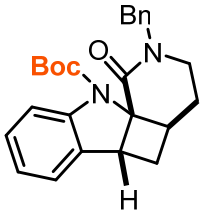
White solid. **TLC**: R_f = 0.49 (Hexane/EtOAc = 10:1) [UV].

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (br s, 1H), 7.73 (br s, 1H), 7.42 – 7.19 (m, 5H), 7.11 (d, *J* = 7.7 Hz, 1H), 7.04 – 6.97 (m, 1H), 3.84 (s, 3H), 3.63 (s, 3H), 2.70 (dd, *J* = 13.8, 8.8 Hz, 1H), 2.57 (dd, *J* = 7.5 Hz, 1H), 2.49 (s, 2H), 2.42 (dd, *J* = 13.8, 7.5 Hz, 1H), 1.53 (s, 3H), 1.29 (s, 3H), 1.28 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 172.82, 171.57, 152.21, 145.25, 141.23, 136.73, 128.29, 128.01, 126.85, 122.92, 122.87, 114.50, 80.95, 78.80, 63.72, 60.08, 56.67, 52.91, 52.75, 44.80, 43.43, 36.29, 29.41, 28.06, 24.42.

HRMS (ESI): Calcd for $C_{30}H_{36}NO_6$ $[M+H]^+$: 506.2537; Found: 506.2538.

Product *rac*-2p



***rac*-2p**

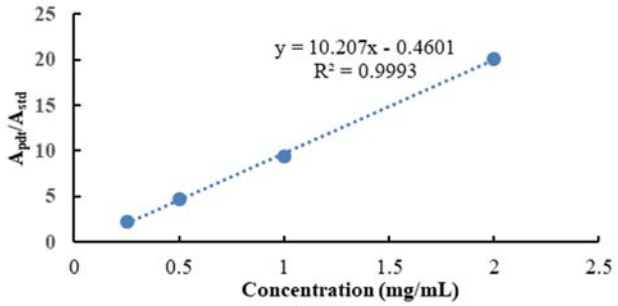
White solid. **TLC**: R_f = 0.12 (Hexane/EtOAc = 10:1) [UV].
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, *J* = 8.2 Hz, 0.69H), 7.56 (d, *J* = 8.0 Hz, 0.29H), 7.41 – 7.30 (m, 5H), 7.26 – 7.17 (m, 1H), 7.05 (d, *J* = 7.5 Hz, 1H), 6.96 (t, *J* = 7.4 Hz, 1H), 4.88 – 4.80 (m, 1H), 4.67 – 4.55 (m, 1H), 3.84 (t, *J* = 7.6 Hz, 1H), 3.68 (t, *J* = 12.7 Hz, 0.73H), 3.56 – 3.48 (t, *J* = 12.7 Hz, 0.27H), 3.57 – 3.40(m, 1H), 3.13 – 3.04 (m, 1H), 2.32 – 2.20 (m, 2H), 2.12 – 2.05 (m, 1H), 1.82 – 1.69 (m, 1H), 1.65 (s, 2.98H), 1.49 (s, 6H).
^{13}C NMR (151 MHz, $CDCl_3$): δ 168.79, 151.24, 144.77, 137.33, 132.56, 128.69, 128.61, 128.19, 128.13, 127.64, 123.69, 122.60, 81.08, 67.16, 51.19, 44.27, 43.81, 39.30, 29.45, 28.53, 28.43, 26.37.

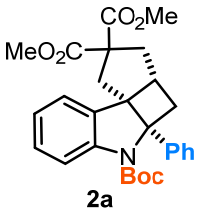
11. Analytical data of enantiomerically enriched photocycloaddition products

The calibration curves of products of intramolecular [2 + 2] photocycloaddition reactions were created for the determination of yield. For each substrate, five different concentrations of product (0.125, 0.25, 0.5, 1, and 2 mg/mL) in isopropanol were mixed each with 30 μL of 0.01 mg/mL internal standard (thioxanthone) solution. The mixtures were vortexed and then analyzed by chiral HPLC. All data were repeated three times and shown in the section 13 Data for photocatalytic reactions.

Product **2a**: Synthesized from substrate **1a** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA**.

Standard Curve:



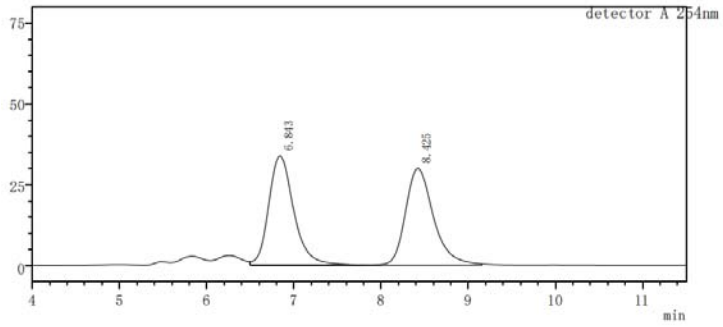


2a

Yield: 90%

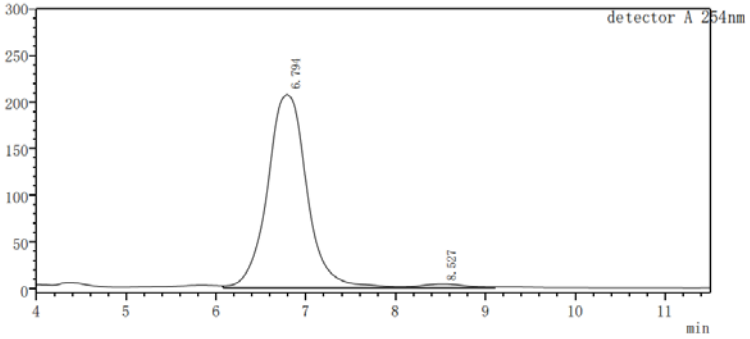
Enantioselectivity: 95% *e.e.* (Daicel, IA-2% IPA/Hexanes, 1.0 mL/min, t_{major} = 6.843 min, t_{minor}=8.425 min)

HPLC trace of racemate **2a:**



Peak	RetTime	Area	Height	Area%
1	6.843	679389	33912	50.351
2	8.425	669916	30013	49.649
Total		1349305	63925	

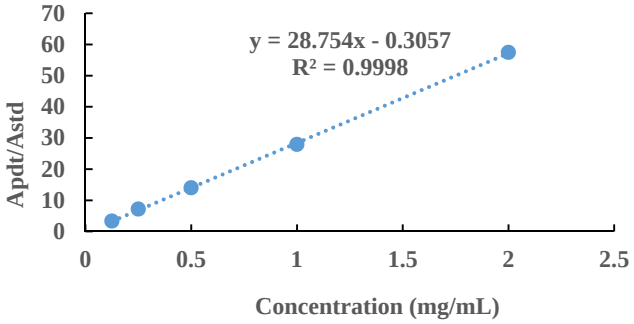
HPLC trace of enantiomerically enriched **2a**:

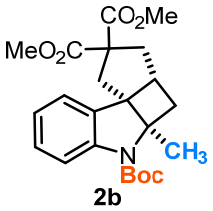


Peak	RetTime	Area	Height	Area%
1	6.794	6366177	208142	97.686
2	8.527	150785	4196	2.314
Total		6516962	212338	

Product 2b: Synthesized from substrate **1b** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA**.

Standard Curve:



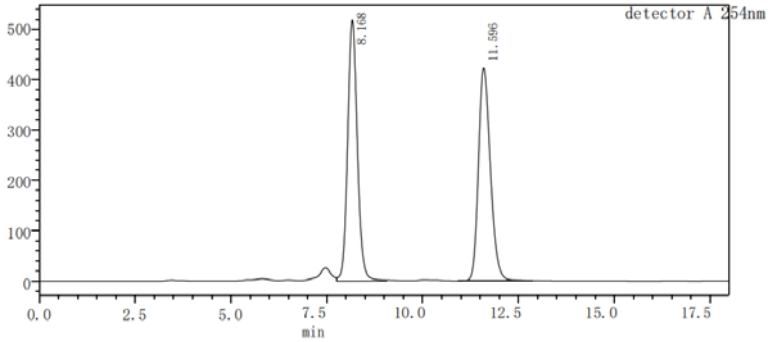


2b

Yield: 97%

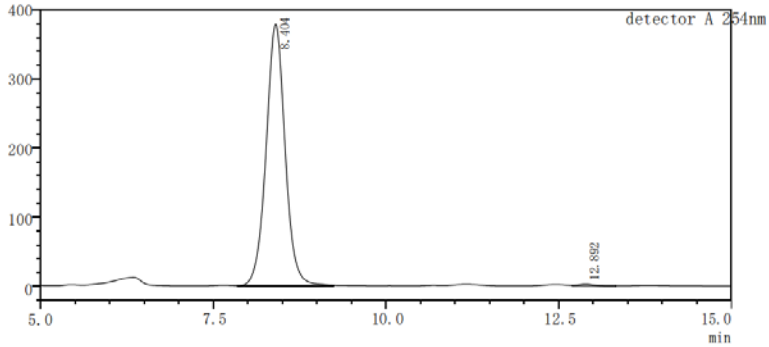
Enantioselectivity: 99% *ee* (Daicel, IC-5% IPA/Hexanes, 1mL/min, t_{major} = 8.168 min, t_{minor}=11.596 min)

HPLC trace of racemate **2b**:



Peak	RetTime	Area	Height	Area%
1	8.168	9173211	518938	49.826
2	11.596	9237103	423986	50.174
Total		18410314	942924	

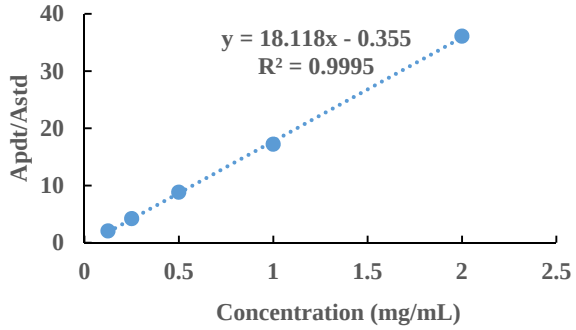
HPLC trace of enantiomerically enriched **2b**:

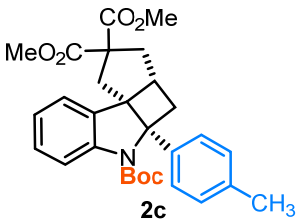


Peak	RetTime	Area	Height	Area%
1	8.404	7325526	378855	99.420
2	12.892	42772	2643	0.580
Total		7368298	381498	

Product 2c: Synthesized from substrate **1c)** according to the general procedure for photocatalytic by enzyme **TPe_FBpA**.

Standard Curve:

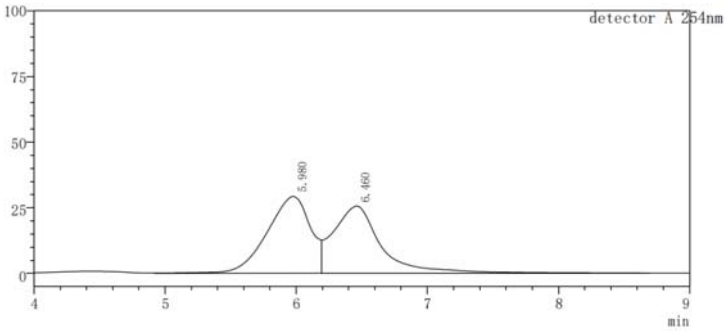




Yield: 84%

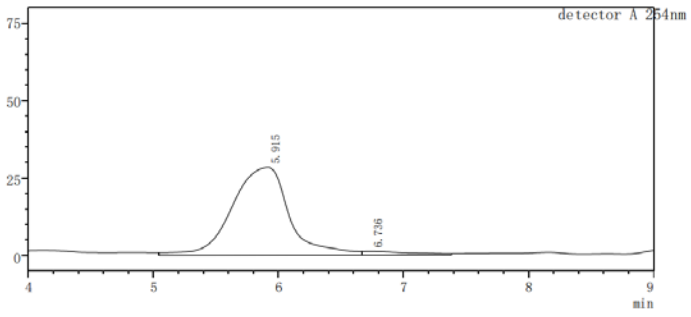
Enantioselectivity: 93% *ee* (Daicel, IB-1.5% IPA/Hexanes, 1mL/min， t_{major} = 5.980 min, t_{minor}=6.460 min)

HPLC trace of racemate **2c**:



Peak	RetTime	Area	Height	Area%
1	5.980	642773	29311	50.057
2	6.460	641310	25713	49.943
Total		1284083	55024	

HPLC trace of enantiomerically enriched **2c**:

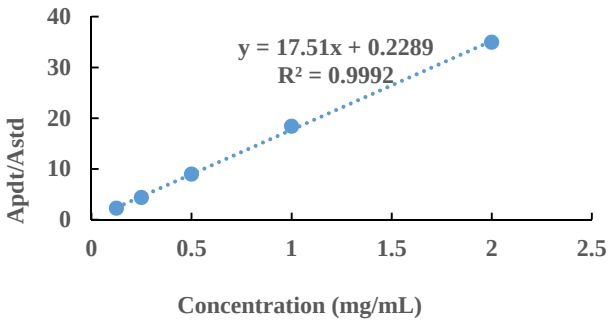


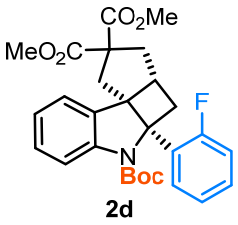
Peak	RetTime	Area	Height	Area%
1	5.915	898821	28394	96.494
2	6.736	32653	1184	3.506
Total		931474	29578	

Product-2d:

synthesized from substrate**1d** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA**.

Standard Curve:



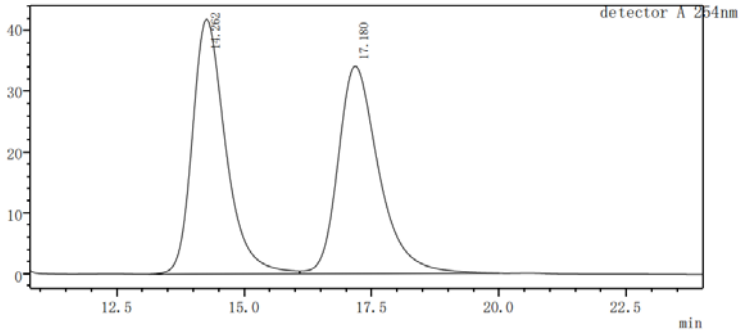


2d

Yield: 94%

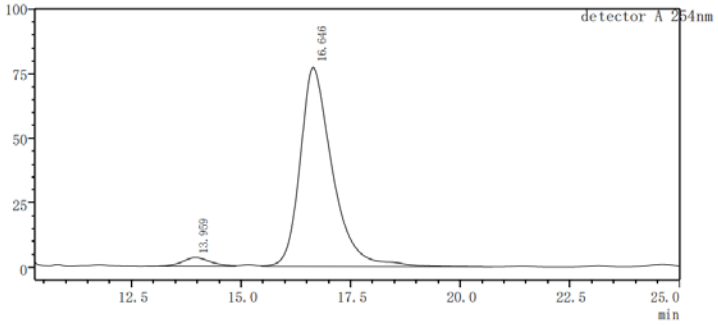
Enantioselectivity: 92% *ee* (Daicel, IC-2% IPA/Hexanes, 1mL/min, t_{major} =17.180 min, t_{minor}=14.262 min)

HPLC trace of racemate **2d**:



Peak	RetTime	Area	Height	Area%
1	14.262	1908764	41819	50.158
2	17.180	1896736	34091	49.842
Total		3805500	75909	

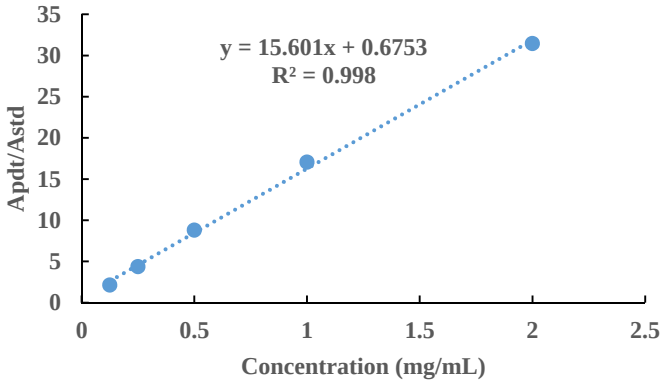
HPLC trace of enantiomerically enriched **2d**:

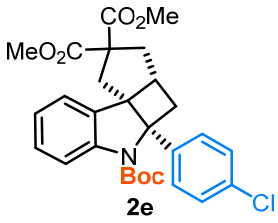


Peak	RetTime	Area	Height	Area%
1	13.959	157071	3410	3.813
2	16.646	3961899	77207	96.187
Total		4118970	80617	

Product 2e: Synthesized from substrate **1e** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA**.

Standard Curve:

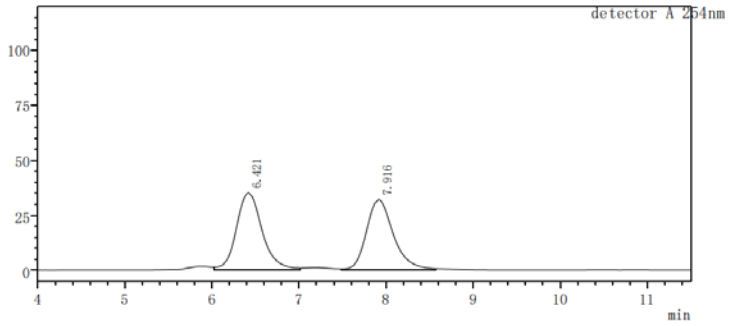




Yield: 89%

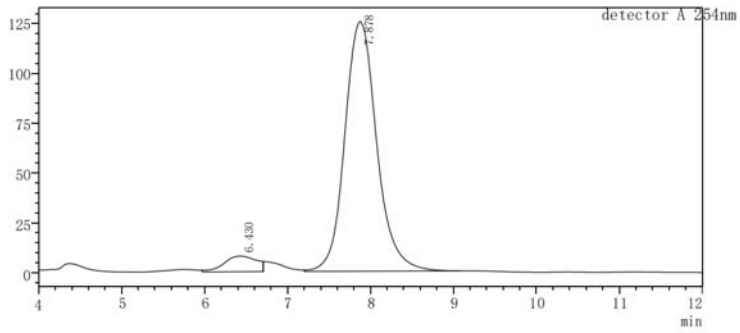
Enantioselectivity: 88% *ee* (Daicel, IA-2% IPA/Hexanes, 1mL/min, t_{major} =7.916 min, t_{minor}=6.421 min)

HPLC trace of racemate **2e**:



Peak	RetTime	Area	Height	Area%
1	6.421	699433	34871	49.740
2	7.916	706755	32049	50.260
Total		1406188	66920	

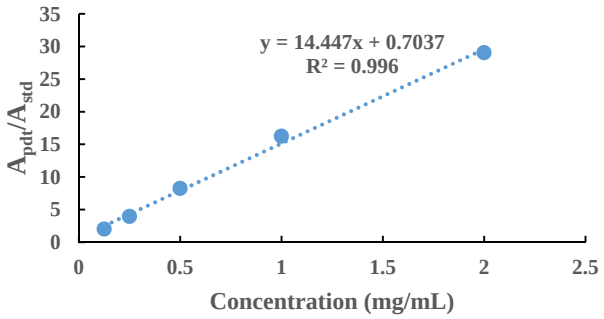
HPLC trace of enantiomerically enriched **2e**:

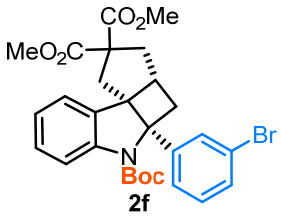


Peak	RetTime	Area	Height	Area%
1	6.430	214216	7805	5.995
2	7.878	3359225	125458	94.005
Total		3573441	133263	

Product 2f: Synthesized from substrate**1f** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA**.

Standard Curve:

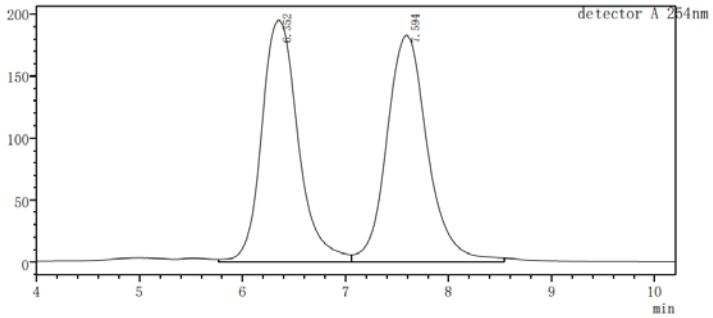




Yield: 82%

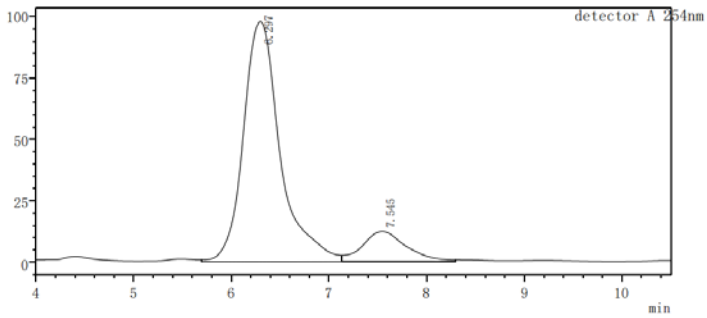
Enantioselectivity: 74% *ee* (Daicel, IA-2% IPA/Hexanes, 1mL/min, t_{major} =6.352 min, t_{minor}=7.594 min)

HPLC trace of racemate **2f**:



Peak	RetTime	Area	Height	Area%
1	6.352	4812391	195301	49.922
2	7.594	4827492	182974	50.078
Total		9639883	378275	

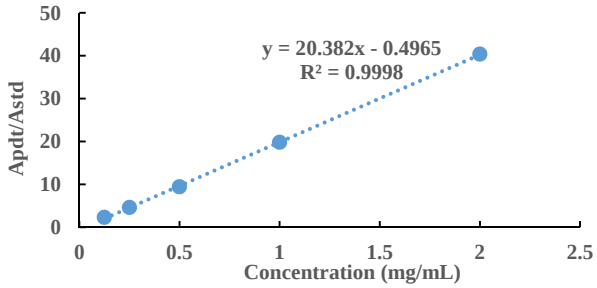
HPLC trace of enantiomerically enriched **2f** :

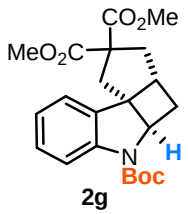


Peak	RetTime	Area	Height	Area%
1	6.297	2566940	97866	86.902
2	7.545	386886	12366	13.098
Total		2953826	110233	

Product 2g : Synthesized from substrate **1g** according to the general procedure for photocatalytic by enzyme **TPe3.0_FBpA_A11H**.

Standard Curve:



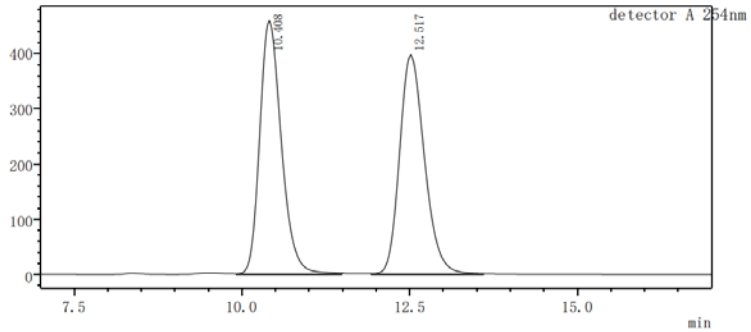


2g

Yield: 91%

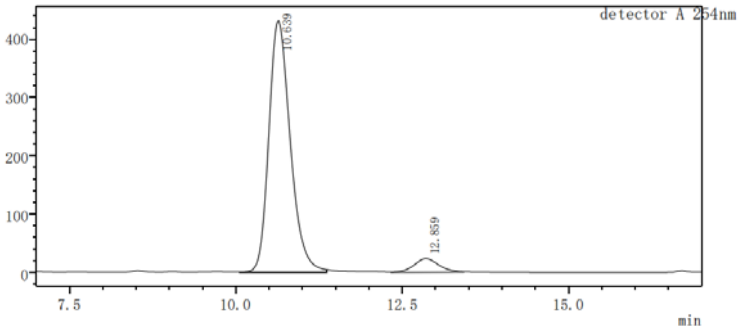
Enantioselectivity: 88% *ee* (Daicel, IC-5% IPA/Hexanes, 1mL/min, t_{major} =10.408 min, t_{minor}=12.517 min)

HPLC trace of racemate **2g:**



Peak	RetTime	Area	Height	Area%
1	10.408	10358350	460059	50.089
2	12.517	10321442	398039	49.911
Total		20679792	858098	

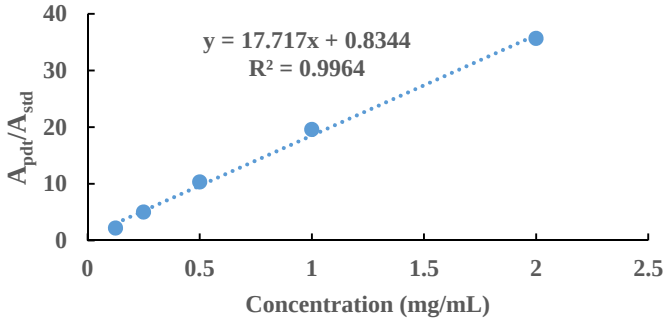
HPLC trace of enantiomerically enriched **2g**:

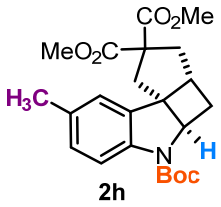


Peak	RetTime	Area	Height	Area%
1	10.639	9689993	431806	94.066
2	12.859	611300	23821	5.934
Total		10301293	455627	

Product 2h: Synthesized from substrate **1h** according to the general procedure for photocatalytic by enzyme **TPe3.0_FBpA_A11H**.

Standard Curve:



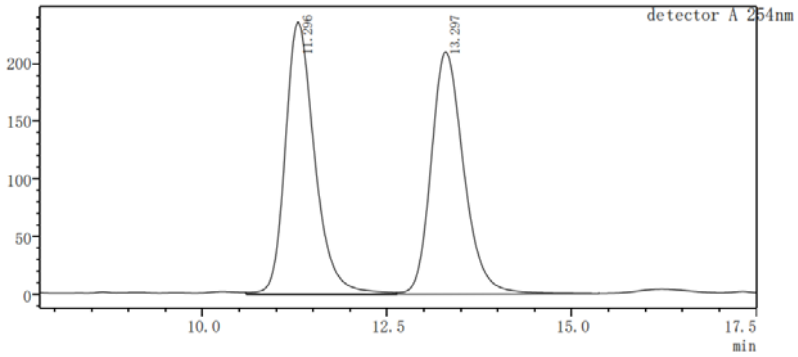


2h

Yield: 85%

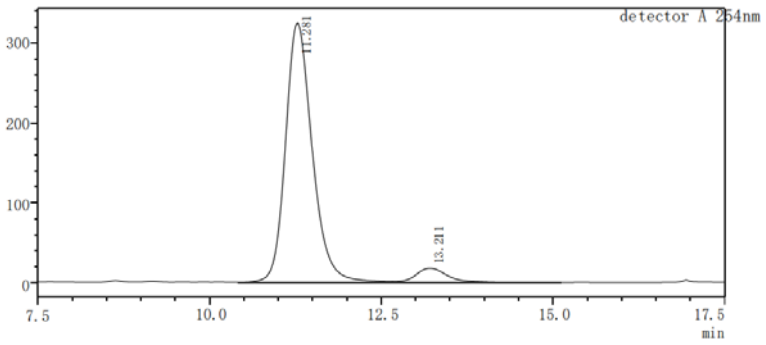
Enantioselectivity: 88% *ee* (Daicel, IC-5% IPA/Hexanes, 1mL/min, t_{major} =11.296 min, t_{minor}=13.297 min)

HPLC trace of racemate 2h:



Peak	RetTime	Area	Height	Area%
1	11.296	6526405	235626	50.286
2	13.297	6452211	210104	49.714
Total		12978617	445731	

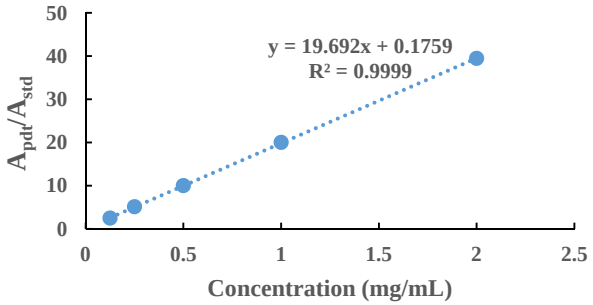
HPLC trace of enantiomerically enriched **2h**:

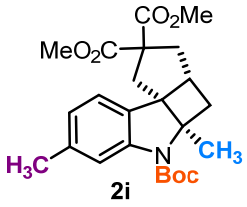


Peak	RetTime	Area	Height	Area%
1	11.281	8802376	324975	93.907
2	13.211	571134	17859	6.093
Total		9373510	342834	

Product 2i: Synthesized from substrate**1i** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA** .

Standard Curve:



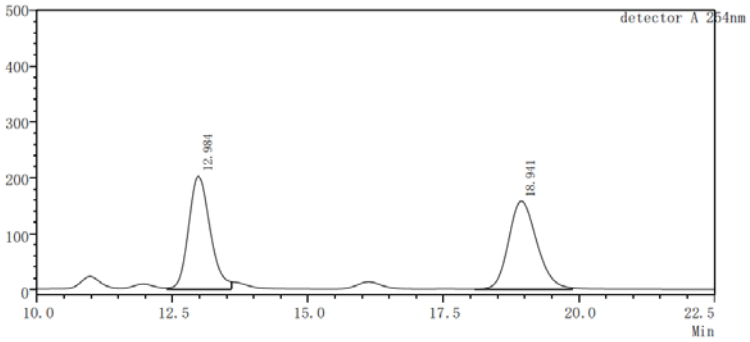


2i

Yield: 93%

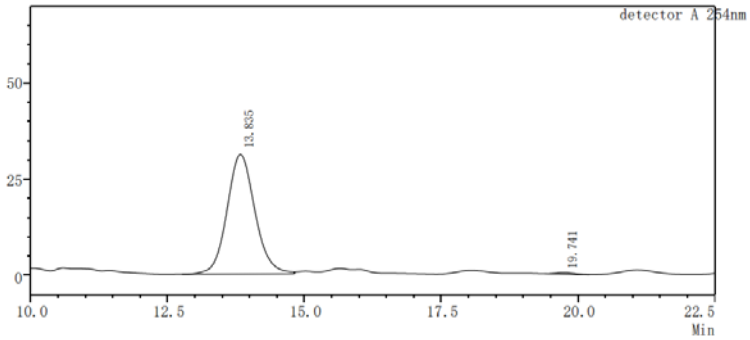
Enantioselectivity: 99% *ee* (Daicel, IC-2% IPA/Hexanes, 1mL/min, t_{major} =12.984 min, t_{minor}=18.941 min)

HPLC trace of racemate **2i**:



Peak	RetTime	Area	Height	Area%
1	12.984	5600326	202311	49.566
2	18.941	5698345	157796	50.434
Total		11298671	360107	

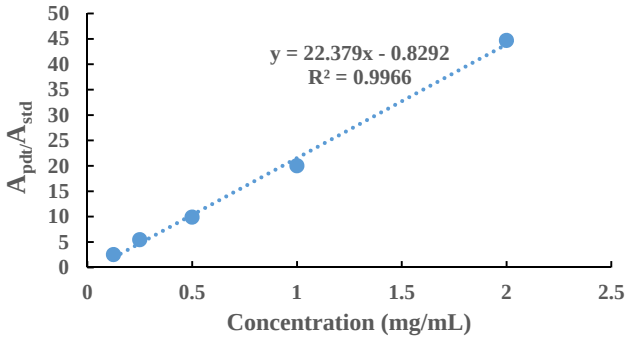
HPLC trace of enantiomerically enriched **2i:**

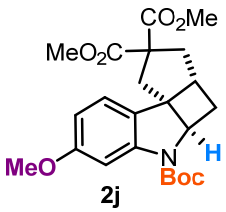


Peak	RetTime	Area	Height	Area%
1	13.835	1102048	31061	99.255
2	19.741	8270	367	0.745
Total		1110318	31428	

Product 2j: Synthesized from substrate **1j** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA**.

Standard Curve:



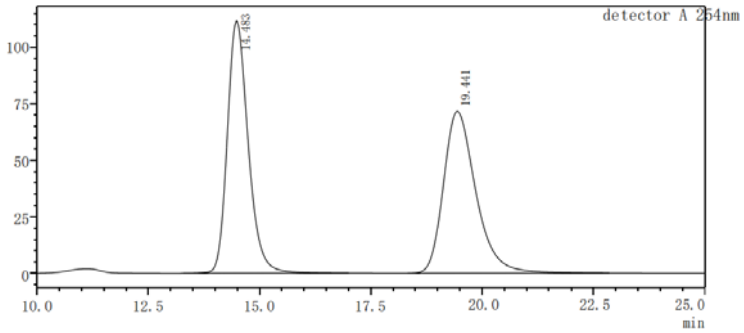


2j

Yield:86%

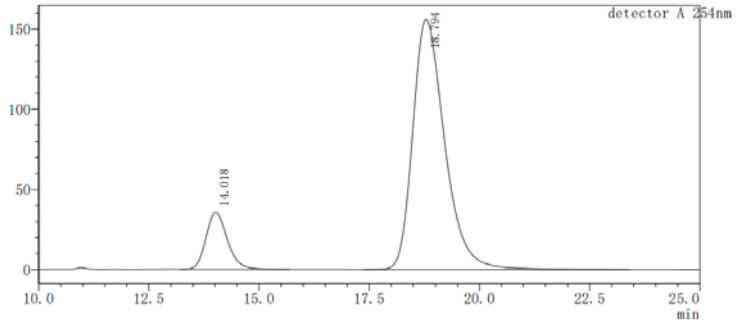
Enantioselectivity: 73% *ee* (Daicel, IC-10% IPA/Hexanes, 1mL/min, t_{major} =19.441 min, t_{minor}= 14.483min)

HPLC trace of racemate **2j:**



Peak	RetTime	Area	Height	Area%
1	14.483	3657954	111828	50.211
2	19.441	3627222	71672	49.789
Total		7285176	183500	

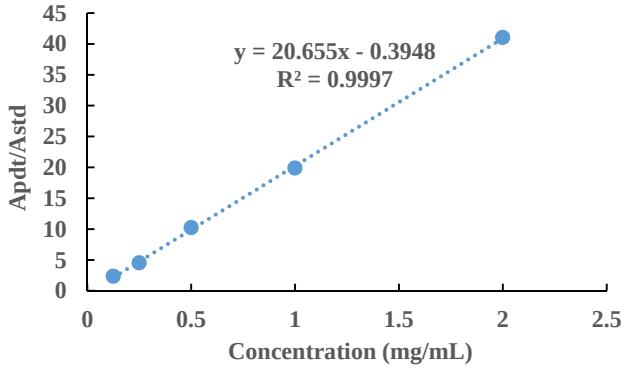
HPLC trace of enantiomerically enriched **2j**:



Peak	RetTime	Area	Height	Area%
1	14.018	1205543	35583	13.339
2	18.794	7832317	155862	86.661
Total		9037860	191445	

Product 2k： Synthesized from substrate**1k** according to the general procedure for photocatalytic by enzyme **TPe3.0_FBpA_A11H**.

Standard Curve:

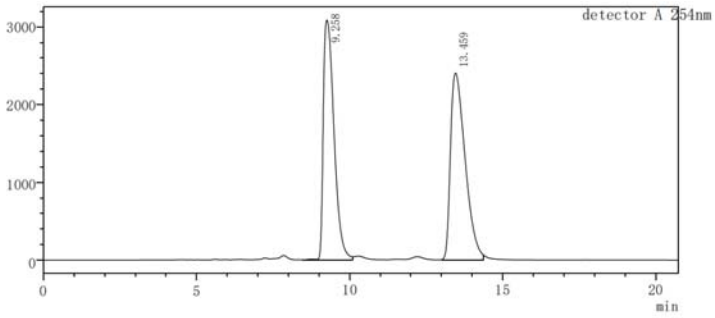


2k

Yield:85%

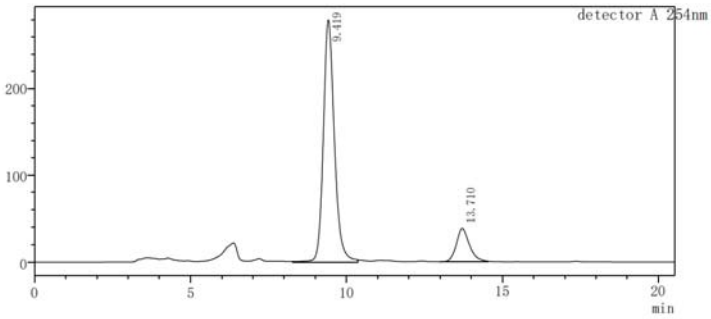
Enantioselectivity: 71% *ee* (Daicel, IC-5% IPA/Hexanes, 1mL/min, t_{major} =9.258 min, t_{minor}=13.459 min)

HPLC trace of racemate **2k**:



Peak	RetTime	Area	Height	Area%
1	9.258	75443563	3086503	48.333
2	13.459	80647125	2406323	51.667
Total		156090689	5492826	

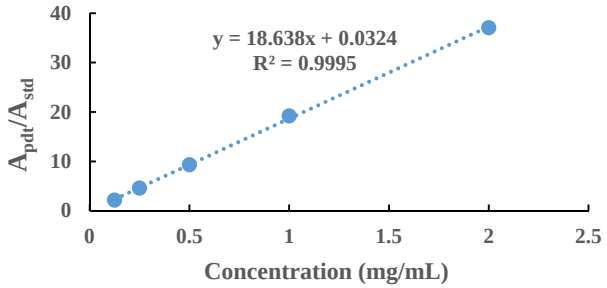
HPLC trace of enantiomerically enriched **2k:**

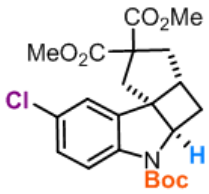


Peak	RetTime	Area	Height	Area%
1	9.419	6873900	279460	85.693
2	13.710	1147621	38782	14.307
Total		8021522	318242	

Product **2l:** Synthesized from substrate**1k** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA**.

Standard Curve:



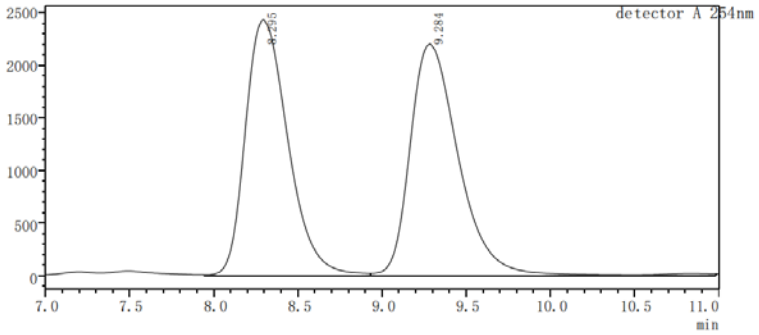


2l

Yield: 92%

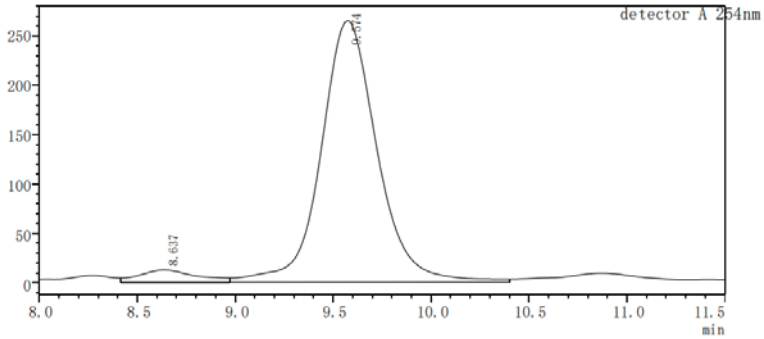
Enantioselectivity: 91% *ee* (Daicel, IC-5% IPA/Hexanes, 1mL/min, t_{major} = 9.284 min, t_{minor}=8.295 min)

HPLC trace of racemate **2l**:



Peak	RetTime	Area	Height	Area%
1	8.295	42787062	2432607	49.178
2	9.284	44217618	2205005	50.822
Total		87004679	4637612	

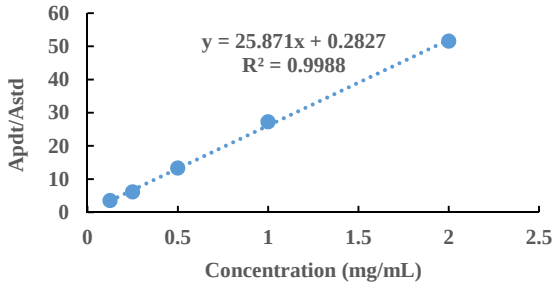
HPLC trace of enantiomerically enriched **2l**:

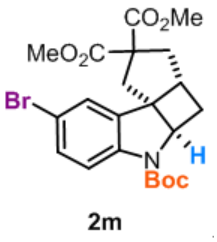


Peak	RetTime	Area	Height	Area%
1	8.637	260874	12499	4.693
2	9.574	5298261	265125	95.307
Total		5559135	277624	

Product 2m： Synthesized from substrate**1l** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA**.

Standard Curve:



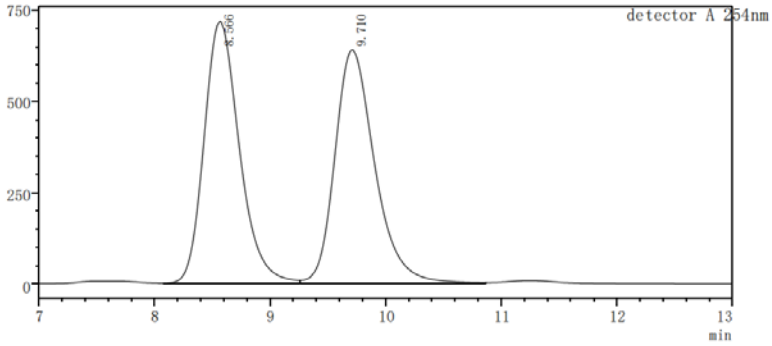


2m

Yield: 93%

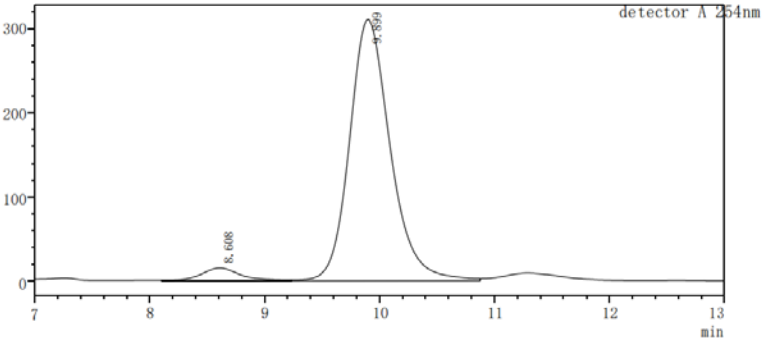
Enantioselectivity: 91% *ee* (Daicel, IC-5% IPA/Hexanes, 1mL/min, t_{major} = 9.710 min, t_{minor} = 8.566 min)

HPLC trace of racemate **2m**:



Peak	RetTime	Area	Height	Area%
1	8.566	15304119	719057	49.761
2	9.710	15450888	641385	50.239
Total		30755007	1360442	

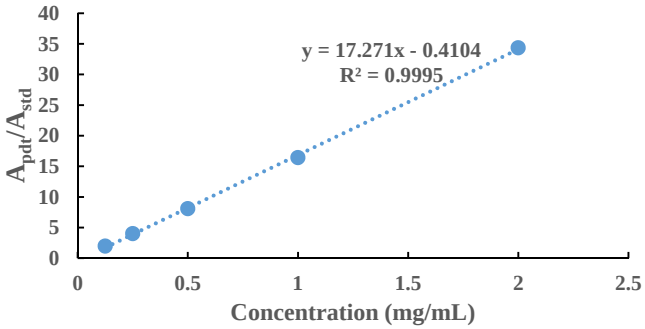
HPLC trace of enantiomerically enriched **2m**:

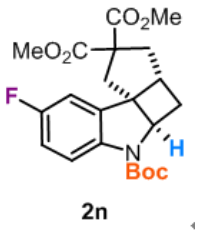


Peak	RetTime	Area	Height	Area%
1	8.608	367061	15281	4.562
2	9.899	7679832	310564	95.438
Total		8046894	325845	

Product 2n: Synthesized from substrate**1n** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA**.

Standard Curve:



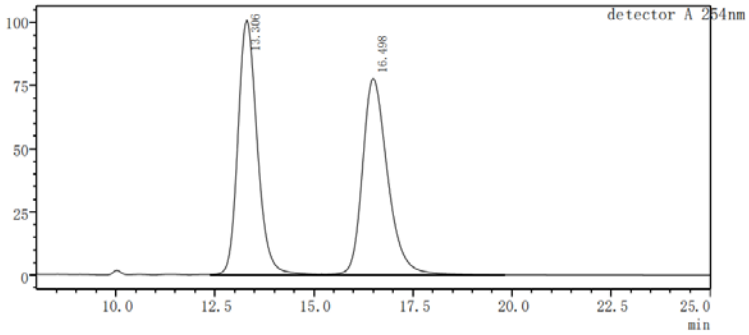


2n

Yield: 91%

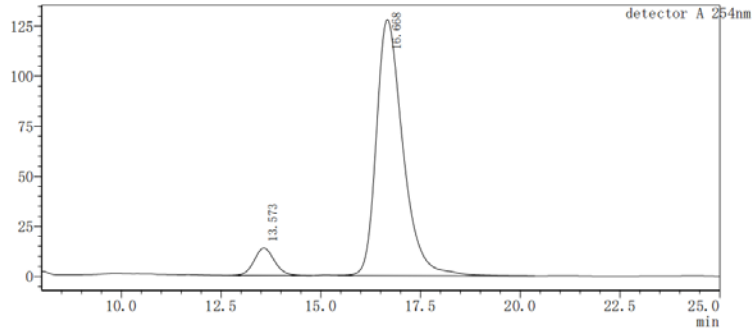
Enantioselectivity: 85% *ee* (Daicel, IC-5% IPA/Hexanes, 1mL/min, t_{major} = 16.498 min, t_{minor}= 13.306 min)

HPLC trace of racemate **2n**:



Peak	RetTime	Area	Height	Area%
1	13.306	3426881	100701	49.932
2	16.498	3436222	77604	50.068
Total		6863103	178305	

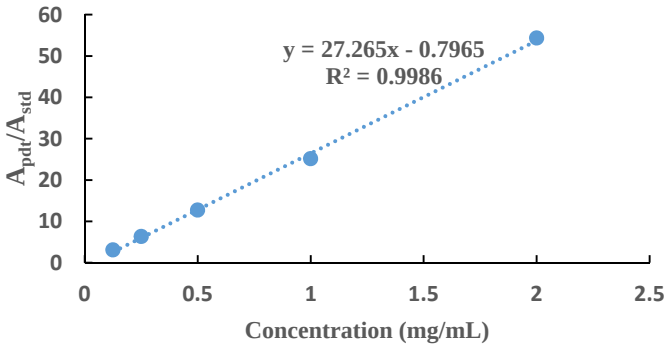
HPLC trace of enantiomerically enriched **2n**:

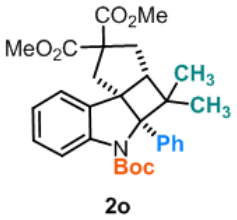


Peak	RetTime	Area	Height	Area%
1	13.573	488170	13626	7.574
2	16.668	5957591	127938	92.426
Total		6445761	141563	

Product 2o: Synthesized from substrate **1o** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA**.

Standard Curve:



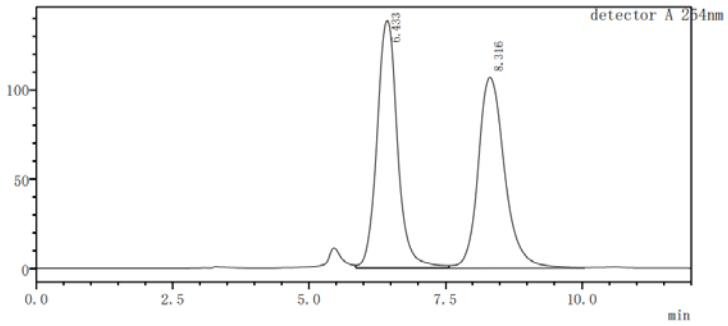


2o

Yield: 81%

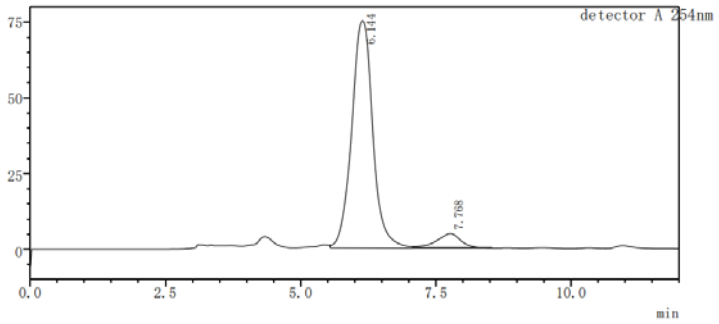
Enantioselectivity: 86%*ee* (Daicel, IA-2% IPA/Hexanes, 1mL/min, t_{major}= 6.433 min, t_{minor}= 8.316 min)

HPLC trace of racemate **2o**:



Peak	RetTime	Area	Height	Area%
1	6.433	3651307	138927	50.253
2	8.316	3614557	106960	49.747
Total		7265864	245888	

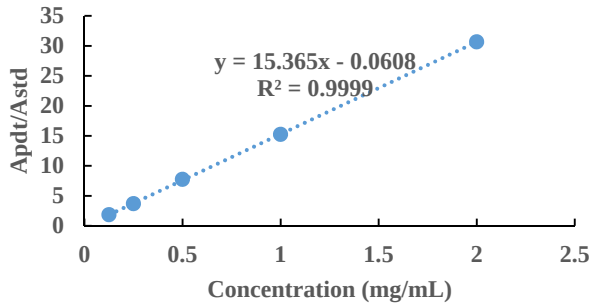
HPLC trace of enantiomerically enriched **2o**:

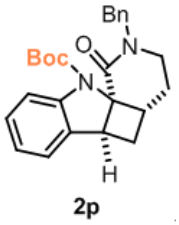


Peak	RetTime	Area	Height	Area%
1	6.144	2034489	74972	93.087
2	7.768	151095	4797	6.913
Total		2185584	79769	

Product 2p: Synthesized from substrate **1p** according to the general procedure for photocatalytic by enzyme **TPe4.0_FBpA**.

Standard Curve:



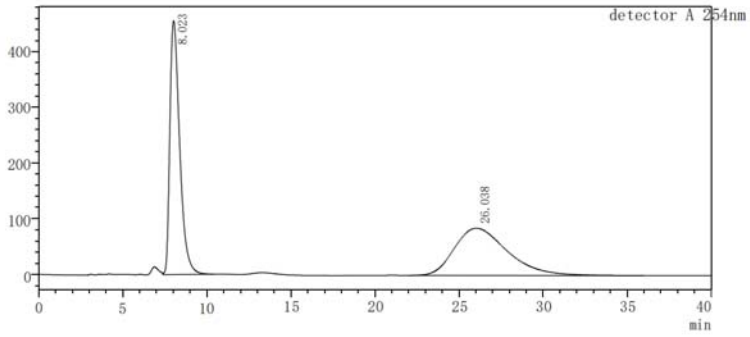


2p

Yield: 92%

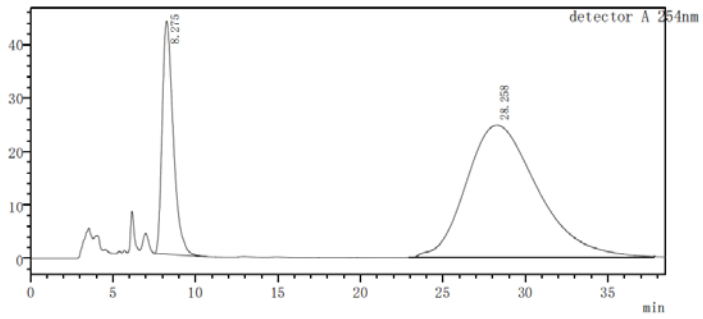
Enantioselectivity: 57% *ee* (Daicel, ASH-10% IPA/Hexanes, 1mL/min, t_{major} =26.038 min, t_{minor}= 8.023min)

HPLC trace of racemate **2p**:



Peak	RetTime	Area	Height	Area%
1	8.023	18503424	455942	49.780
2	26.038	18666621	84869	50.220
Total		37170044	540811	

HPLC trace of enantiomerically enriched **2p**



Peak	RetTime	Area	Height	Area%
1	8.275	2030442	43568	21.433
2	28.258	7442916	24729	78.567
Total		9473358	68297	

12. Determination of the absolute stereochemistry of photocycloaddition products.

The absolute stereochemistry of photoadduct **2m** was assessed by comparison of measured and calculated ECD data. The absolute stereochemistry of products **2a-2l**, **2n-2o** were assigned by analogy to **2m**. The experimental spectrum of **2m** is recorded in Methanol.

Theoretical Methods and Technical Details of the Computations of the ECD spectra: The density functional calculations were completed with the B3LYP-D3 functional (with Grimme's D3 dispersion)[10,11] as implemented in the Gaussian 16 program package.[12] The def2-TZVP basis set was used for geometry optimization and frequency calculations. The UV/Vis and ECD spectra in methanol were calculated using the time-dependent density functional theory (TD-DFT) with the B3LYP-D3 and M06-D3[13] functionals and the SMD continuum solvation model.[14] The absorption data were extracted from the Multiwfn 3.8 program. [15] The cartesian coordinates of optimized structure are given in Table S7.

All calculated UV/Vis spectra are depicted (Figure S4) together with the experimental spectrum in Methanol. The corresponding ECD spectra are given in Figure S5.

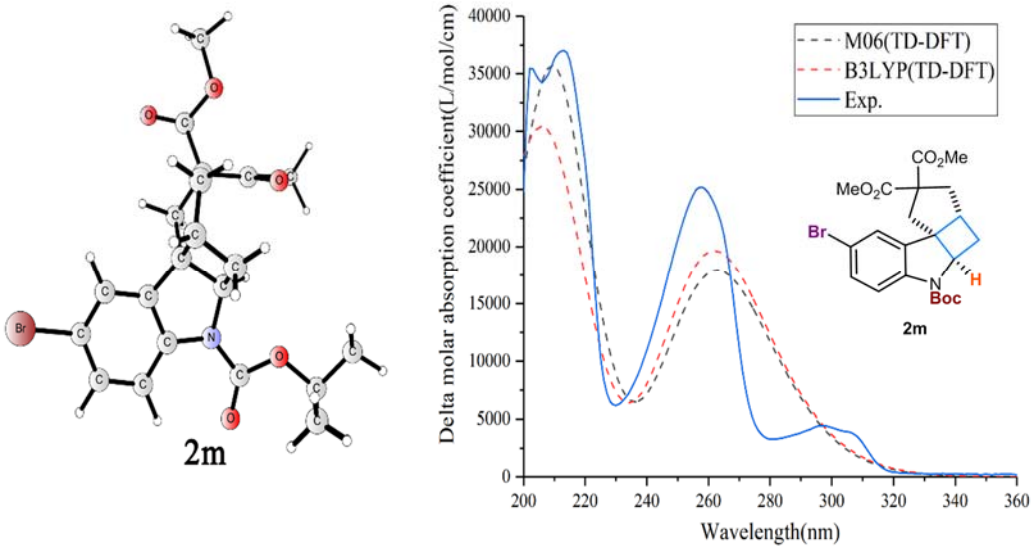


Figure S4. The calculated UV/Vis spectra and experimental UV/Vis spectrum of **2m** in methanol.

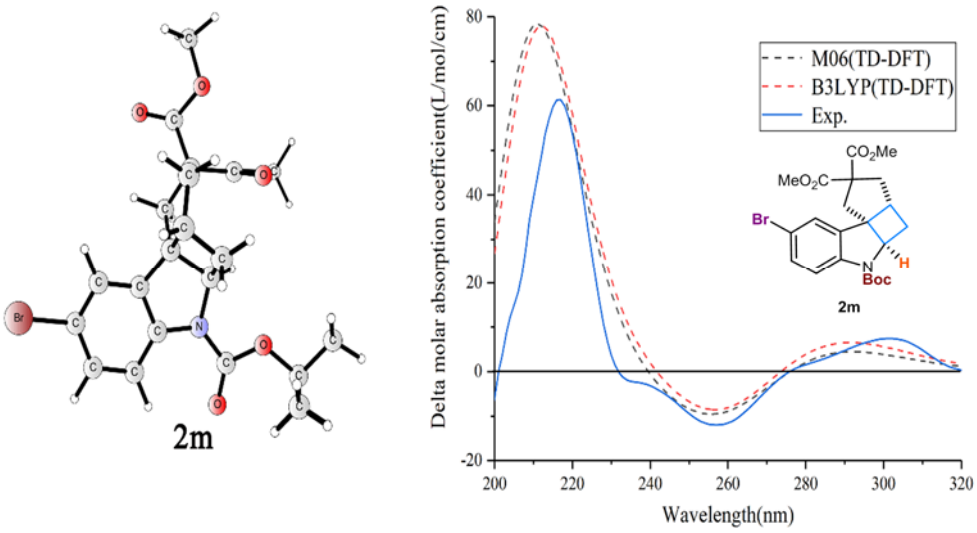


Figure S5. The calculated ECD spectra and experimental ECD spectrum of **2m** in methanol.

Table S7. Cartesian coordinates of optimized structure of **2m**

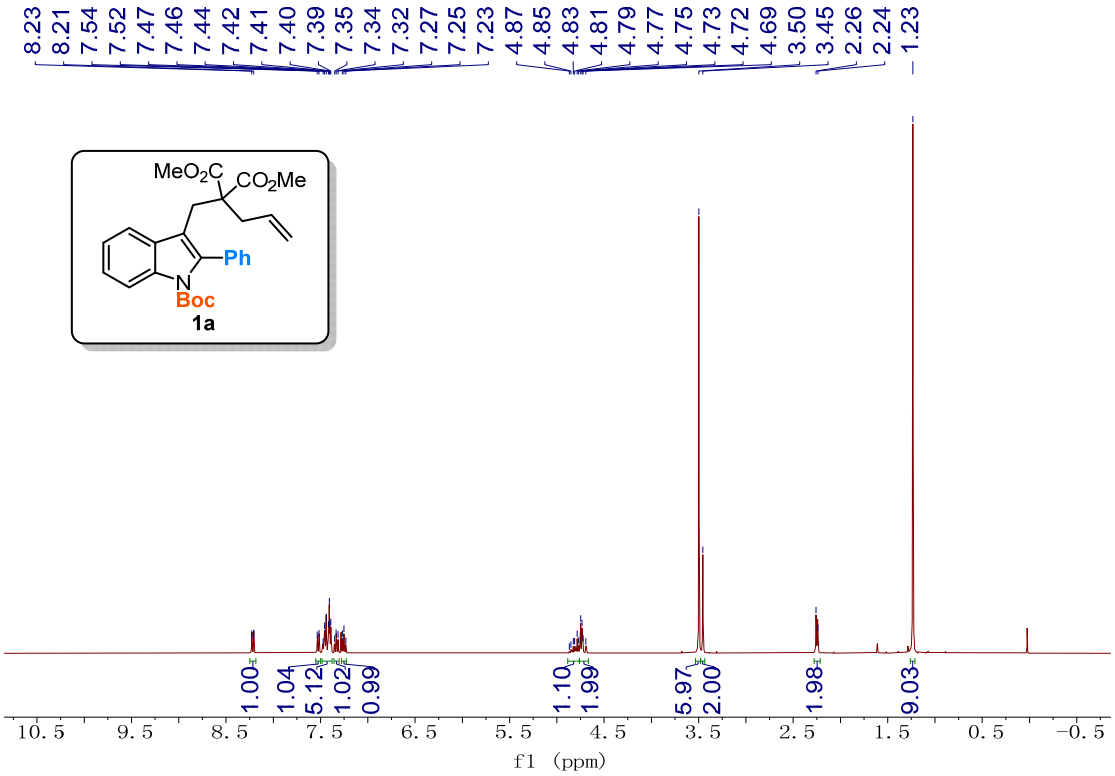
Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	1.520153	-0.029115	0.084861
2	6	0	1.480455	1.359511	-0.088619
3	6	0	2.647619	2.087294	-0.290398
4	6	0	3.859378	1.399014	-0.318125
5	6	0	3.891268	0.021783	-0.147716
6	6	0	2.723699	-0.709134	0.054385
7	6	0	0.139663	-0.570493	0.298060
8	6	0	-0.759714	0.702050	0.264039
9	1	0	2.613399	3.156161	-0.423987
10	1	0	4.779407	1.944262	-0.475094
11	1	0	2.761388	-1.782117	0.184753
12	1	0	-1.591154	0.704605	-0.437033
13	7	0	0.150779	1.815684	-0.022269
14	6	0	-0.257529	3.119590	-0.120038
15	8	0	0.477807	4.062486	-0.323875
16	8	0	-1.594844	3.177365	0.045740
17	6	0	-2.319588	4.455977	0.012945
18	6	0	-2.167297	5.110347	-1.357663
19	1	0	-2.817252	5.985315	-1.414714
20	1	0	-1.140842	5.422260	-1.532388
21	1	0	-2.464263	4.412685	-2.142644
22	6	0	-1.834372	5.358550	1.144322
23	1	0	-0.804749	5.668637	0.985232
24	1	0	-2.466575	6.246735	1.194997
25	1	0	-1.904164	4.834670	2.099104
26	6	0	-3.761456	4.019022	0.248995
27	1	0	-3.853629	3.505664	1.206603
28	1	0	-4.419122	4.889077	0.253750
29	1	0	-4.088765	3.339191	-0.538567
30	35	0	5.571452	-0.895282	-0.189825
31	6	0	-1.148952	0.482959	1.745216
32	1	0	-2.216784	0.384802	1.923694
33	1	0	-0.739691	1.250990	2.400157
34	6	0	-0.344436	-0.847386	1.768573
35	1	0	0.439805	-0.885232	2.522573
36	6	0	-1.135613	-2.159598	1.713107
37	1	0	-0.504569	-2.976954	2.067057
38	1	0	-2.041820	-2.142682	2.316011
39	6	0	-0.271846	-1.777602	-0.535270

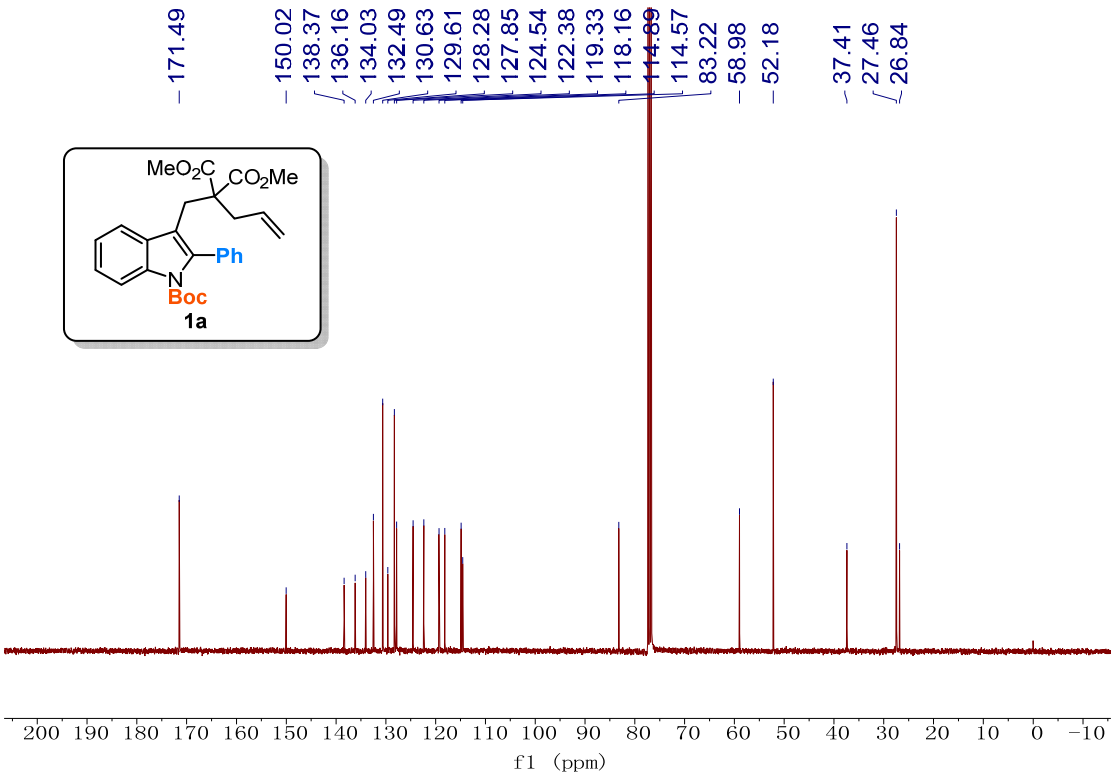
40	1	0	0.537463	-2.508103	-0.555911
41	1	0	-0.512543	-1.524046	-1.566443
42	6	0	-1.463322	-2.416017	0.213972
43	6	0	-2.797520	-1.780160	-0.186429
44	8	0	-3.566863	-1.226226	0.554305
45	8	0	-3.005323	-1.912914	-1.507937
46	6	0	-1.543065	-3.922508	-0.033392
47	8	0	-0.628914	-4.613546	-0.397333
48	8	0	-2.760419	-4.396732	0.278323
49	6	0	-4.245464	-1.382070	-2.005276
50	1	0	-4.302242	-0.309959	-1.817472
51	1	0	-4.243417	-1.585090	-3.072460
52	1	0	-5.088410	-1.873154	-1.519731
53	6	0	-2.940873	-5.817409	0.138511
54	1	0	-2.250901	-6.352901	0.789995
55	1	0	-3.970181	-6.010007	0.427291
56	1	0	-2.765340	-6.120821	-0.893181

---

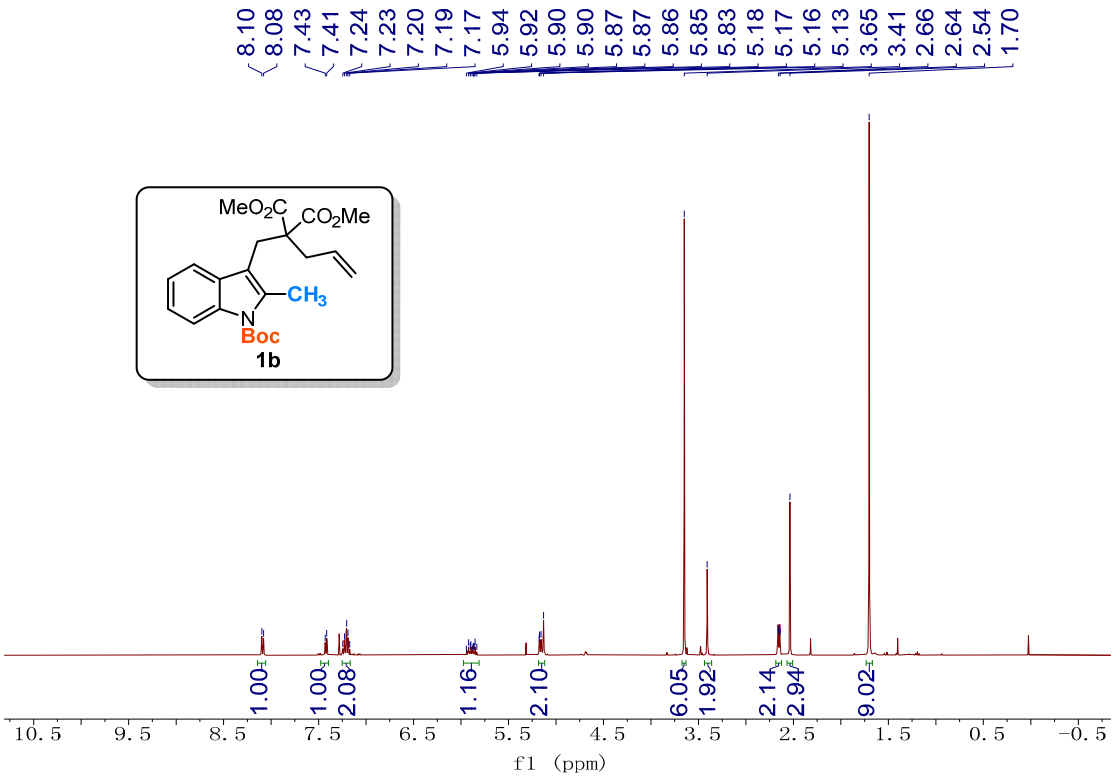
13. NMR spectrum

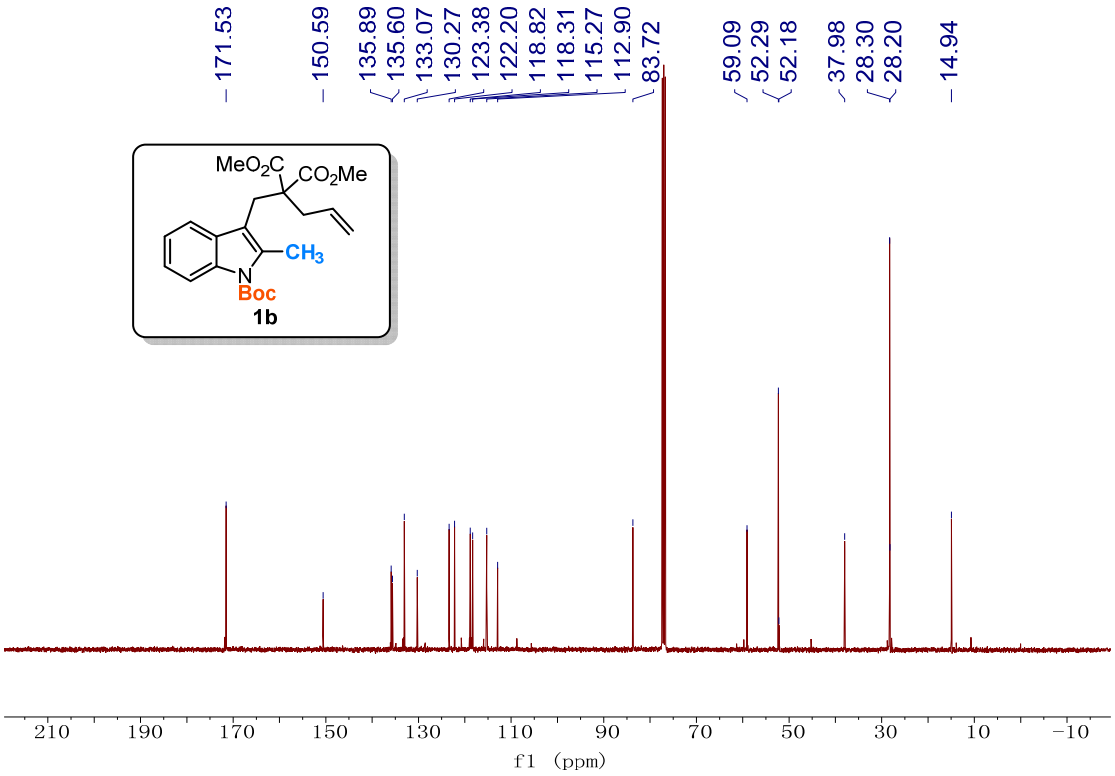
^{1}H & ^{13}C NMR spectra of **1a**



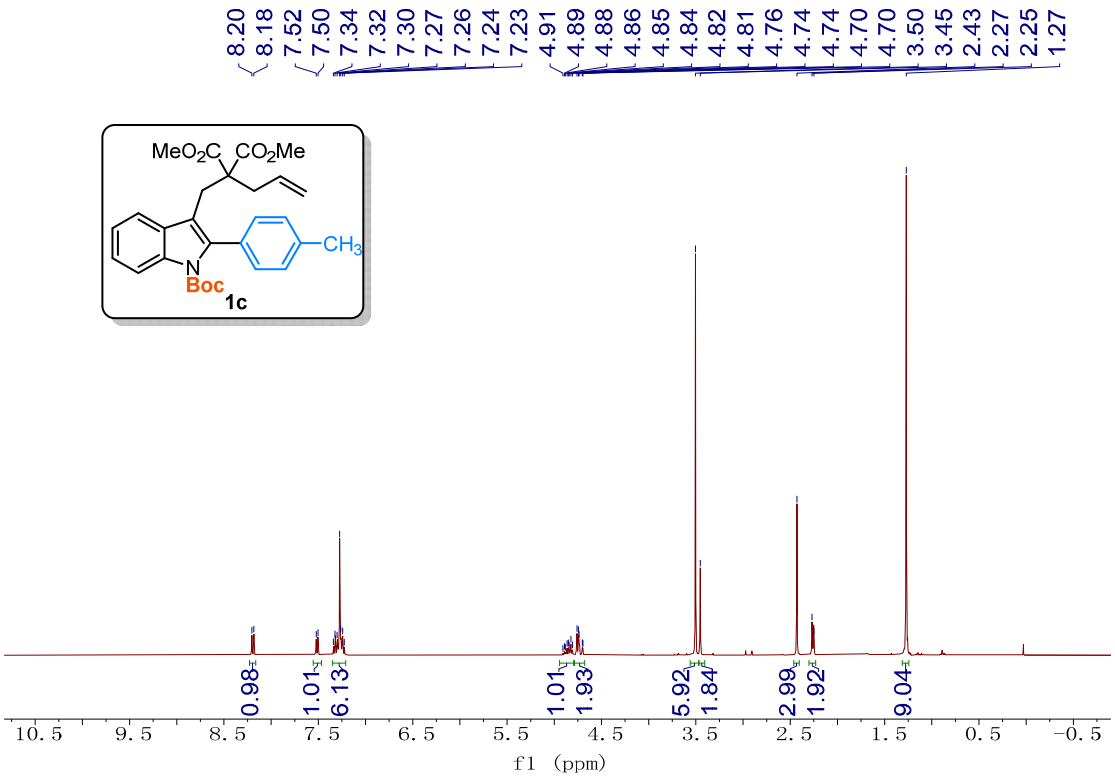


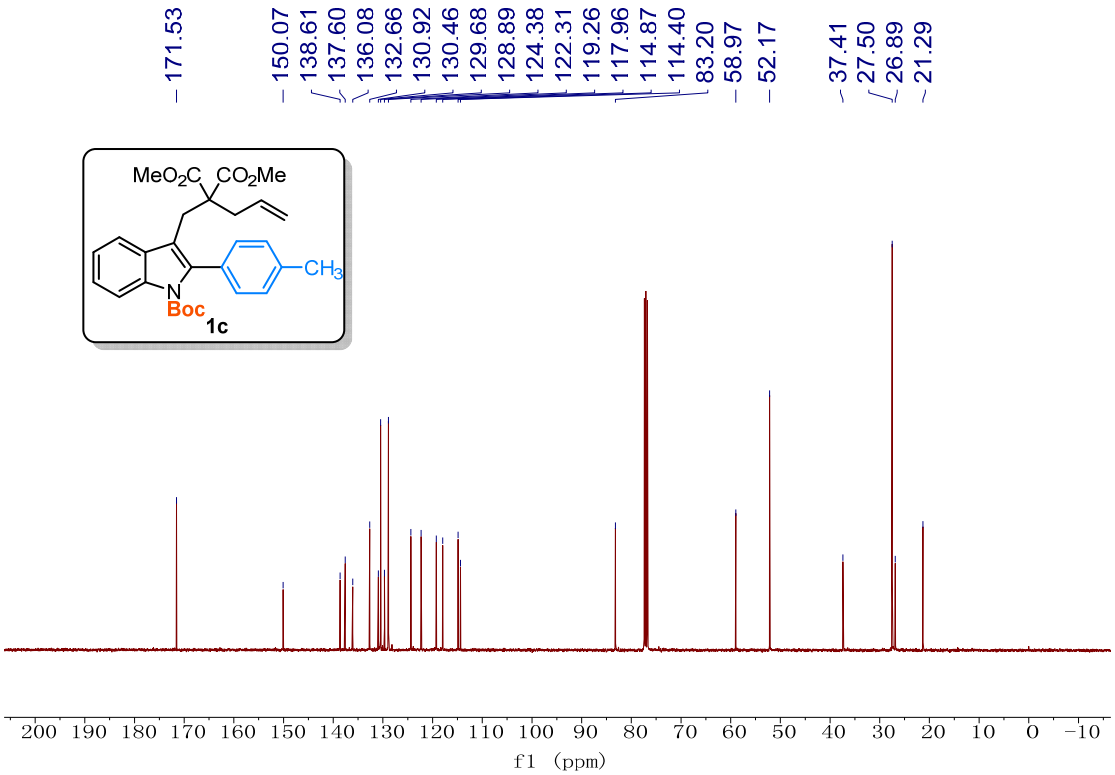
^{1}H & ^{13}C NMR spectra of **1b**



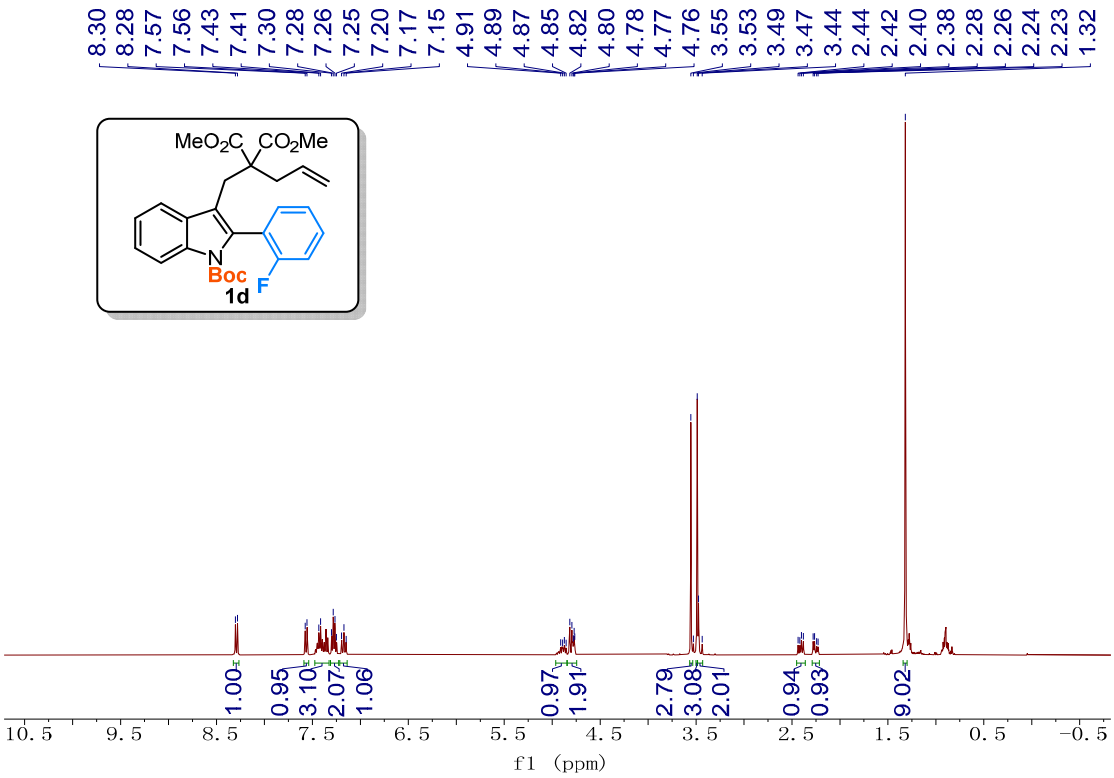


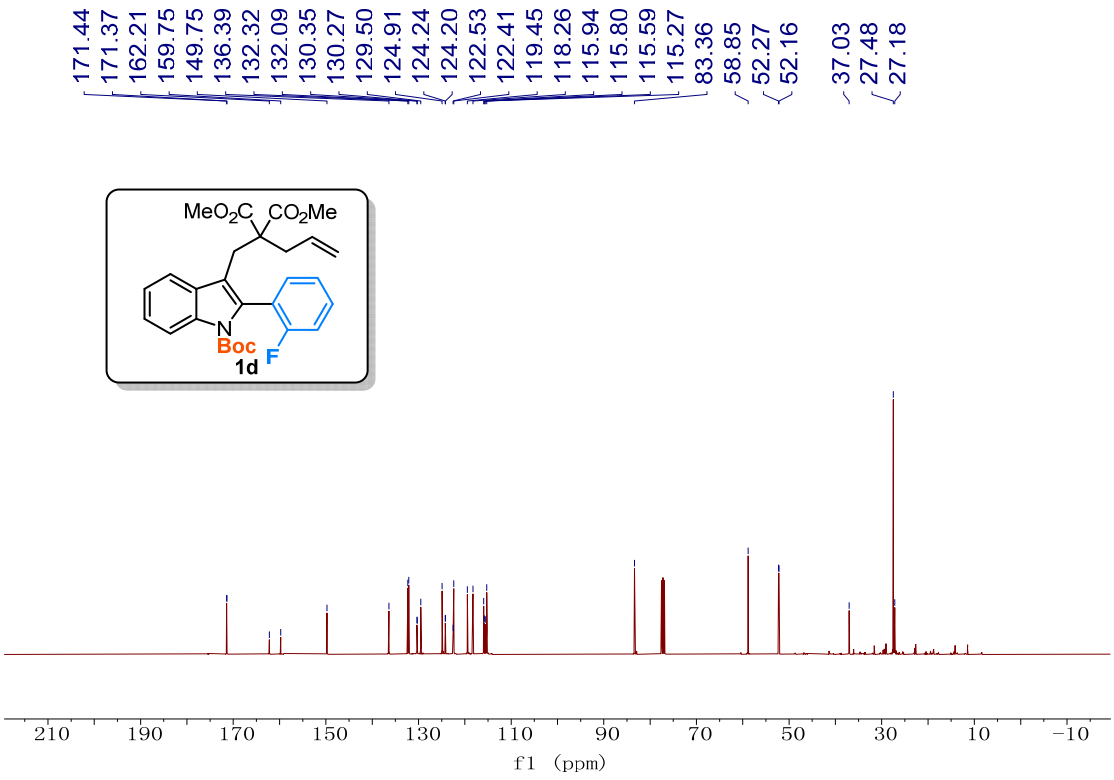
^{1}H & ^{13}C NMR spectra of **1c**



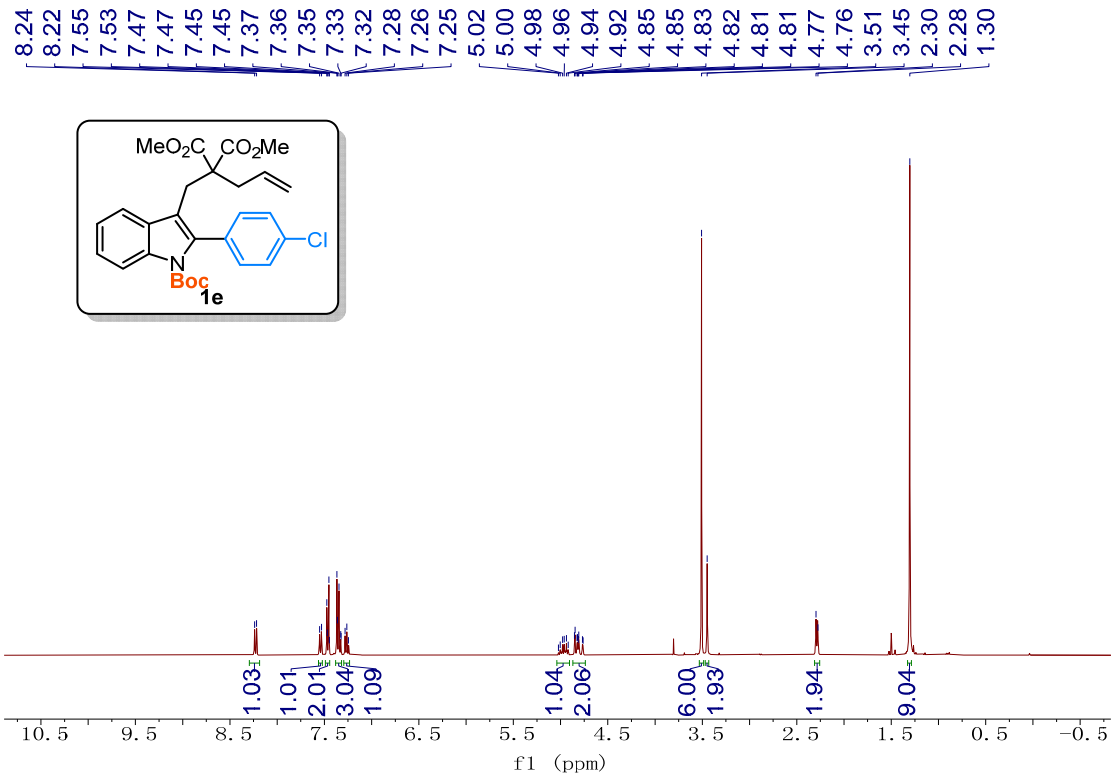


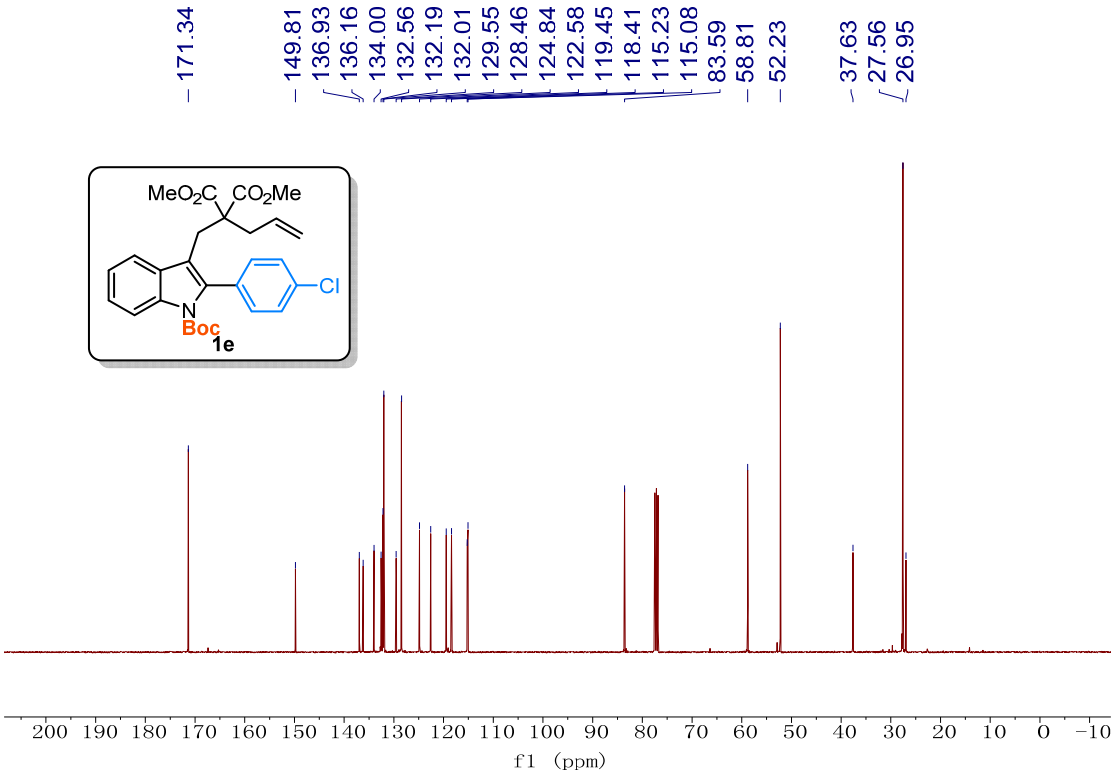
^{1}H & ^{13}C NMR spectra of **1d**



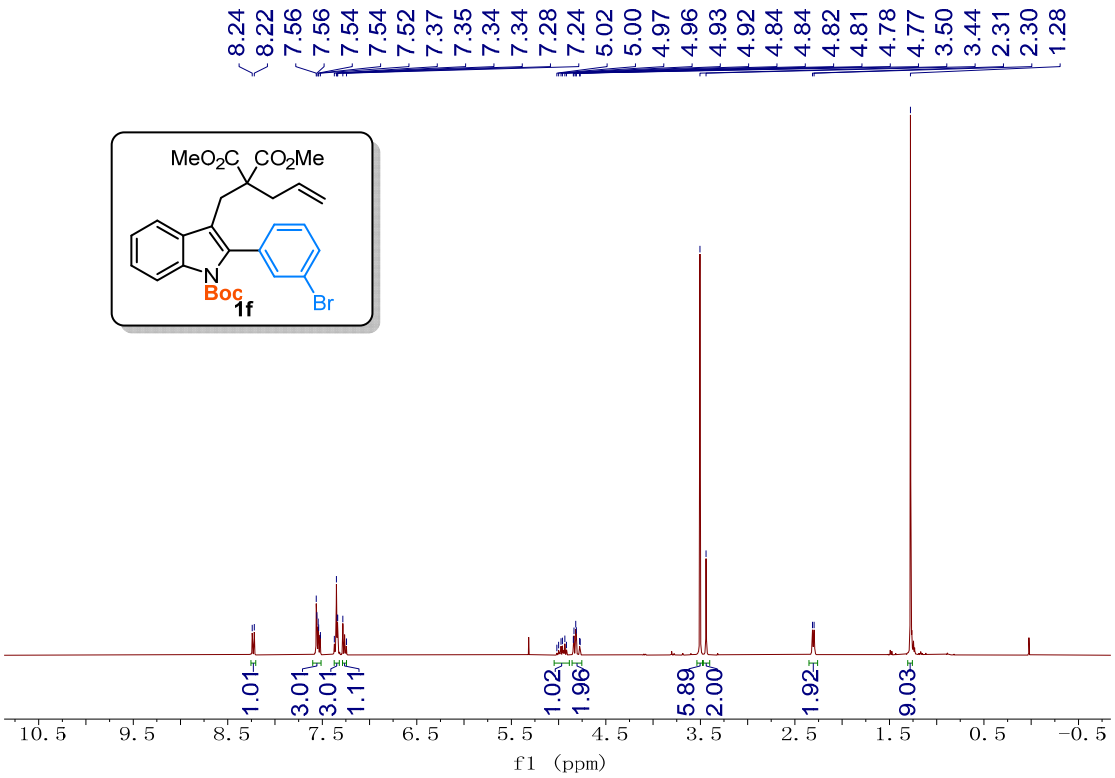


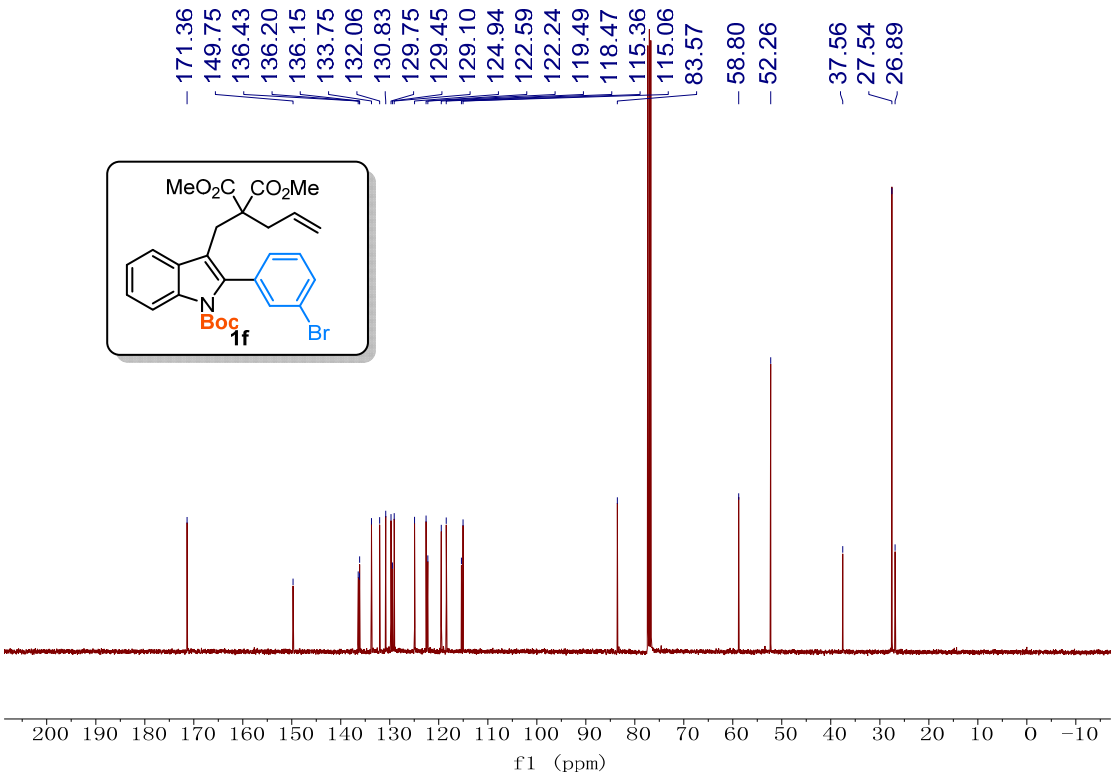
^{1}H & ^{13}C NMR spectra of **1e**



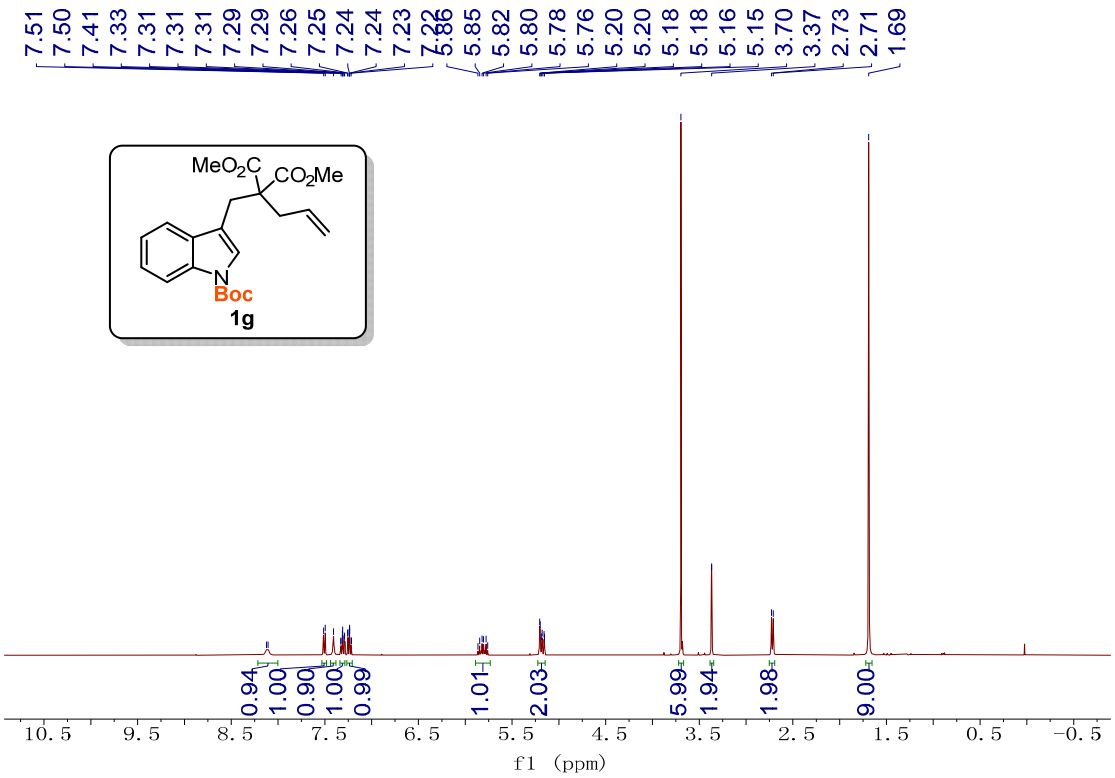


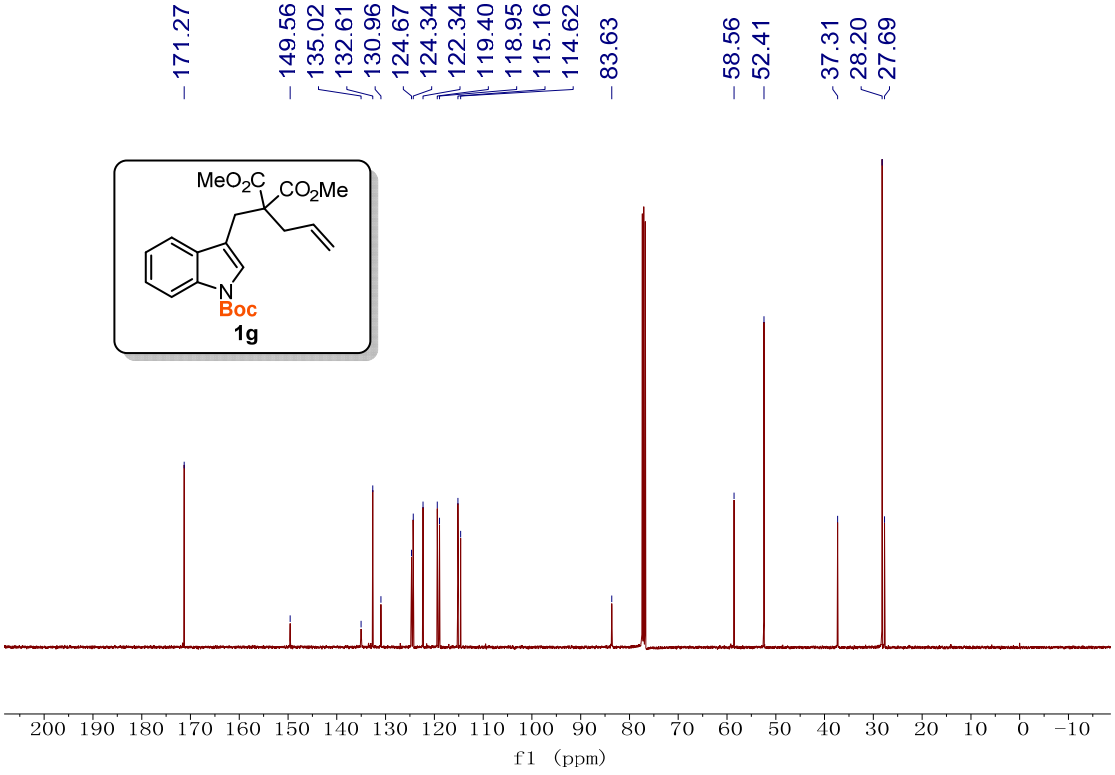
^{1}H & ^{13}C NMR spectra of **1f**



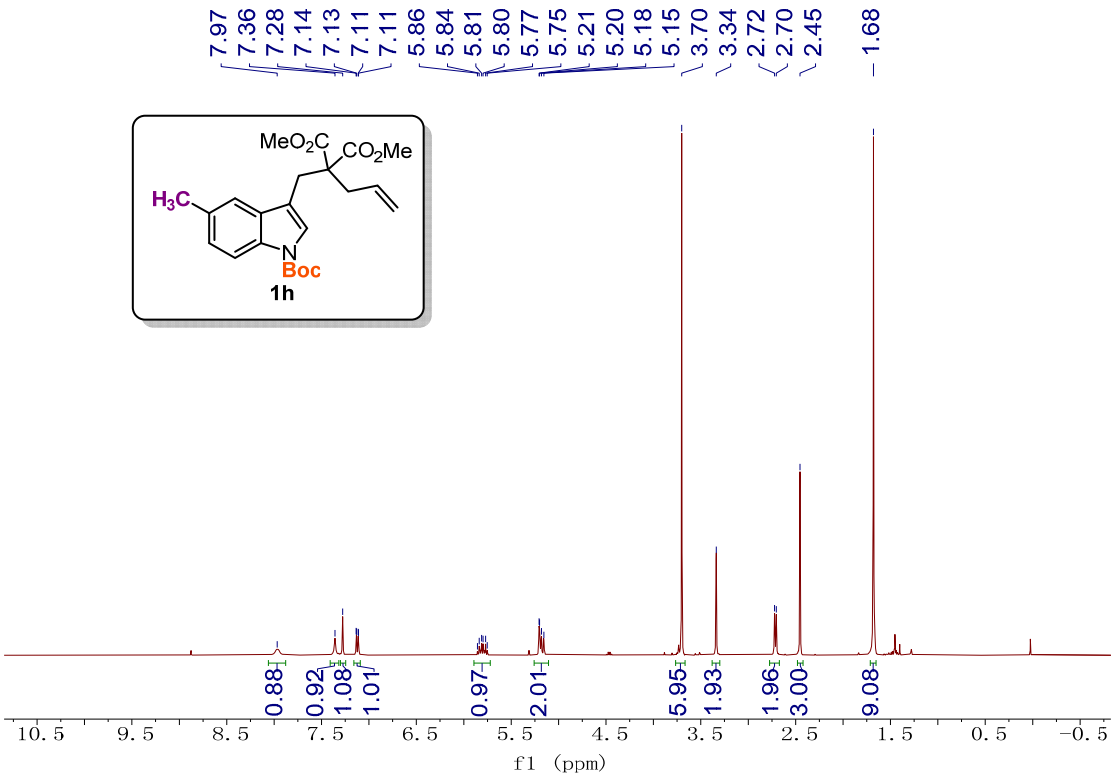


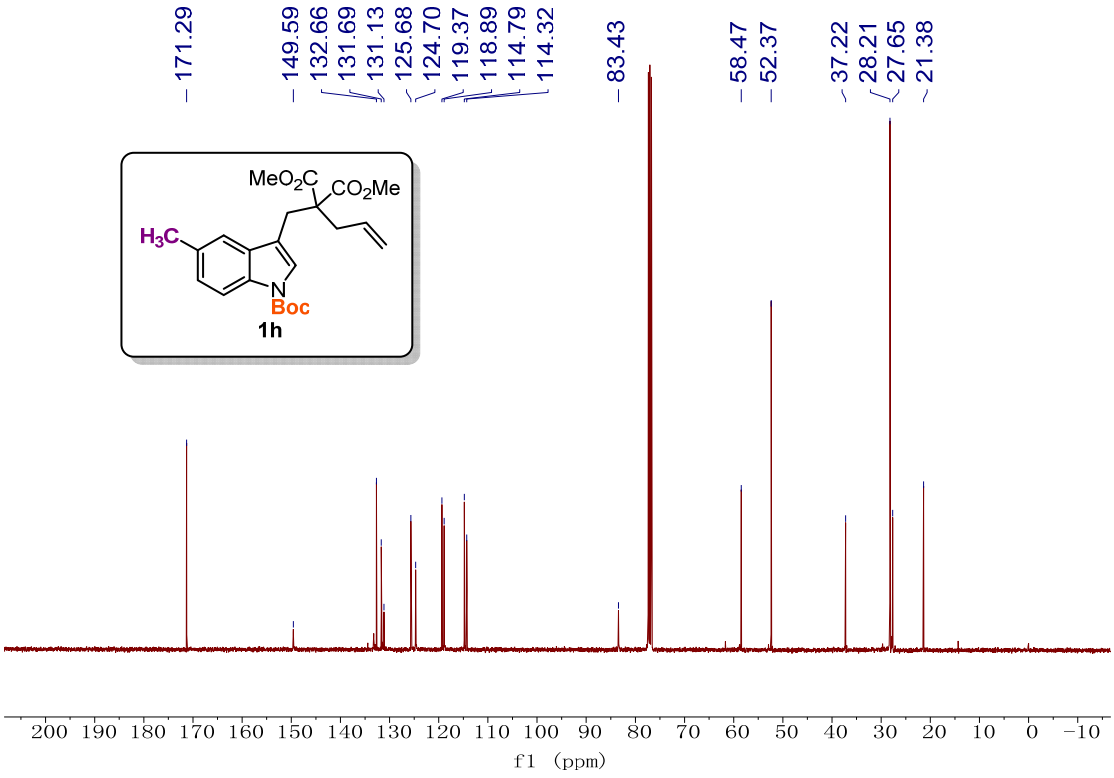
^{1}H & ^{13}C NMR spectra of **1g**



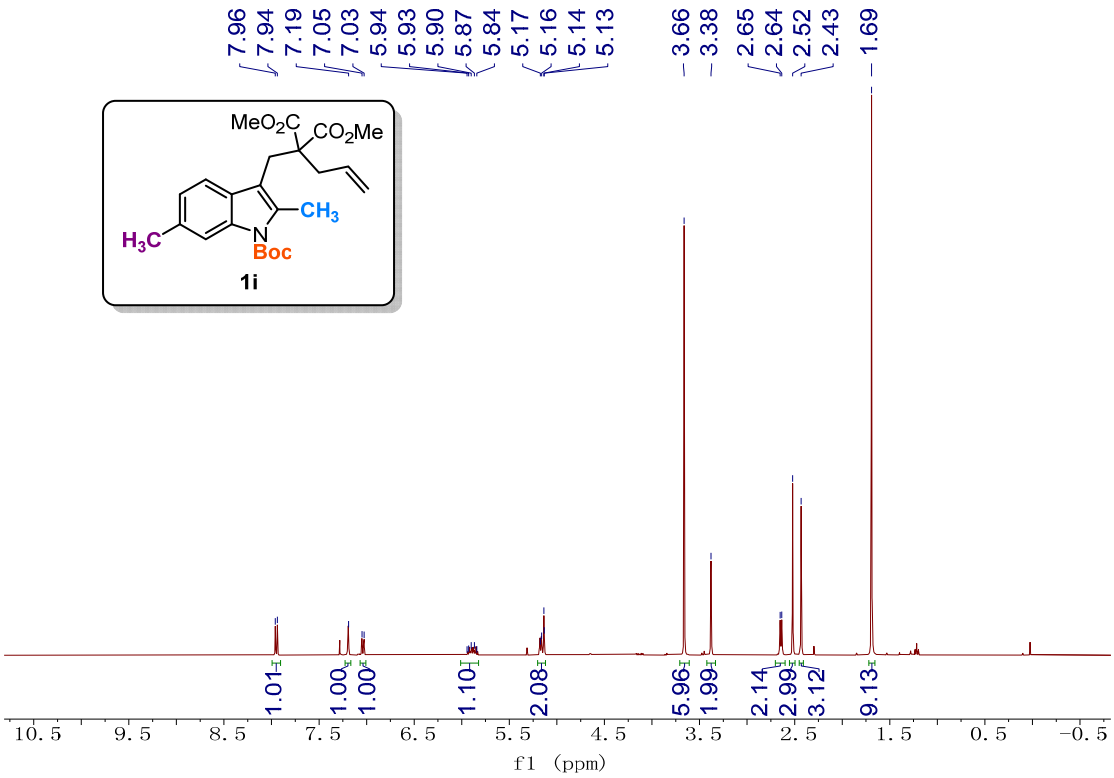


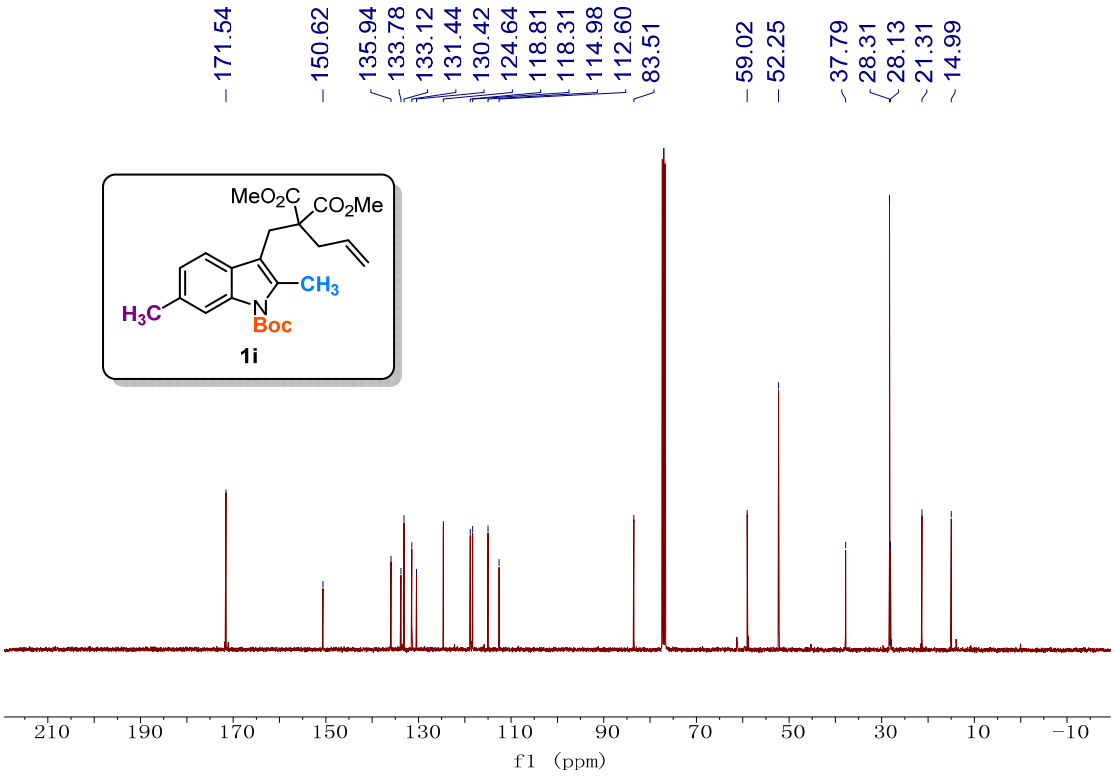
^{1}H & ^{13}C NMR spectra of **1h**



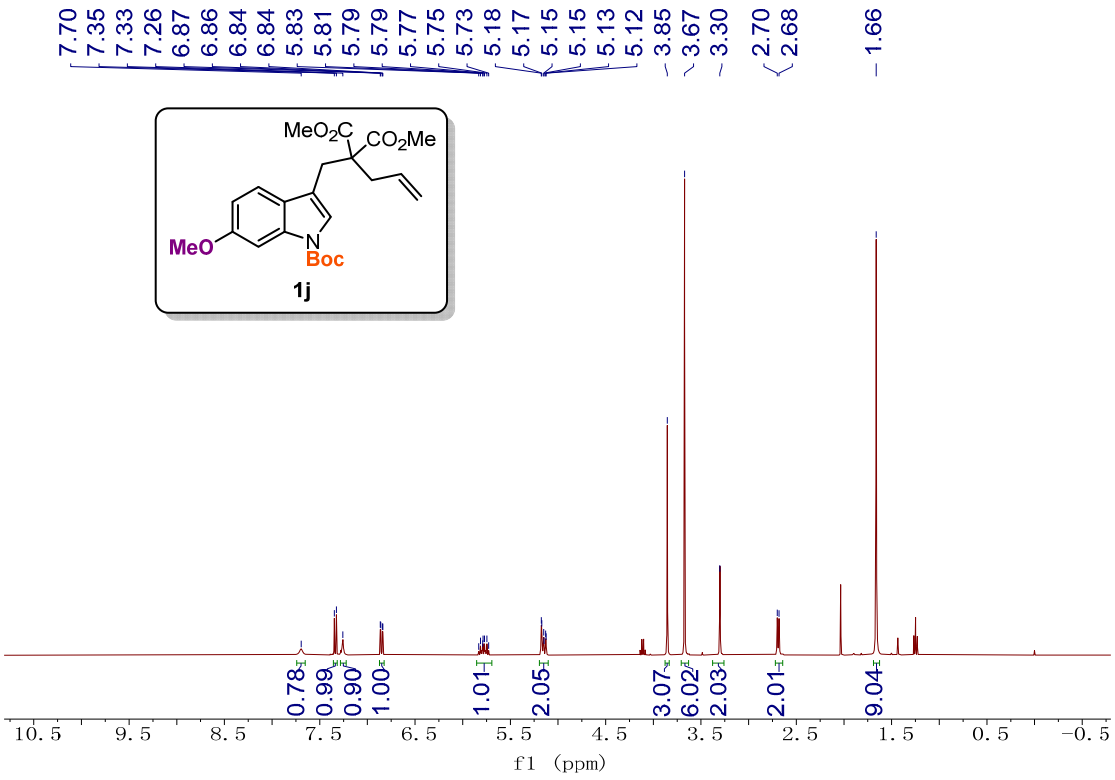


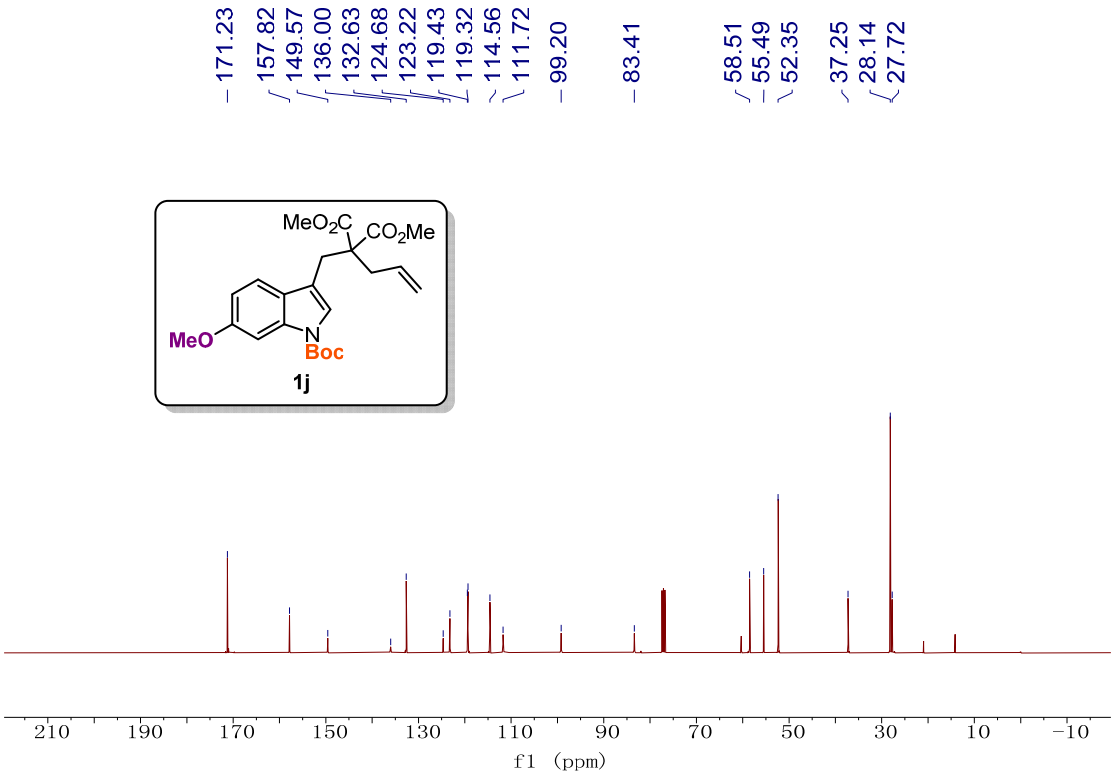
^{1}H & ^{13}C NMR spectra of **1i**



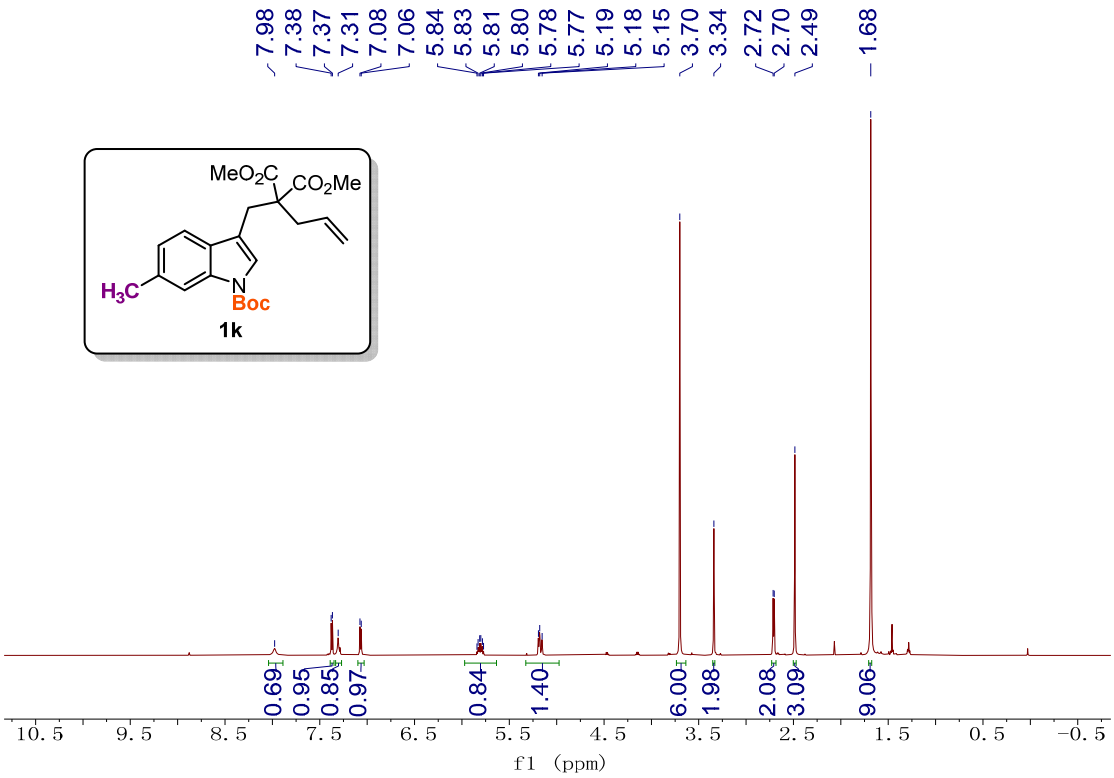


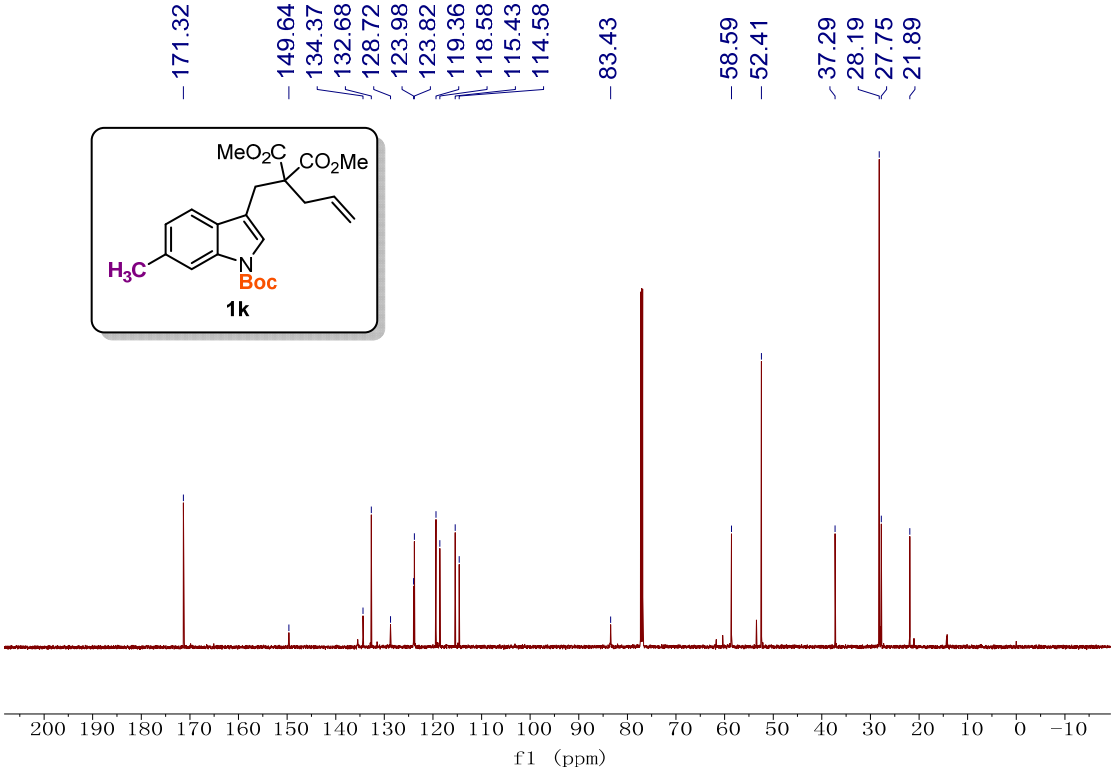
^{1}H & ^{13}C NMR spectra of **1j**



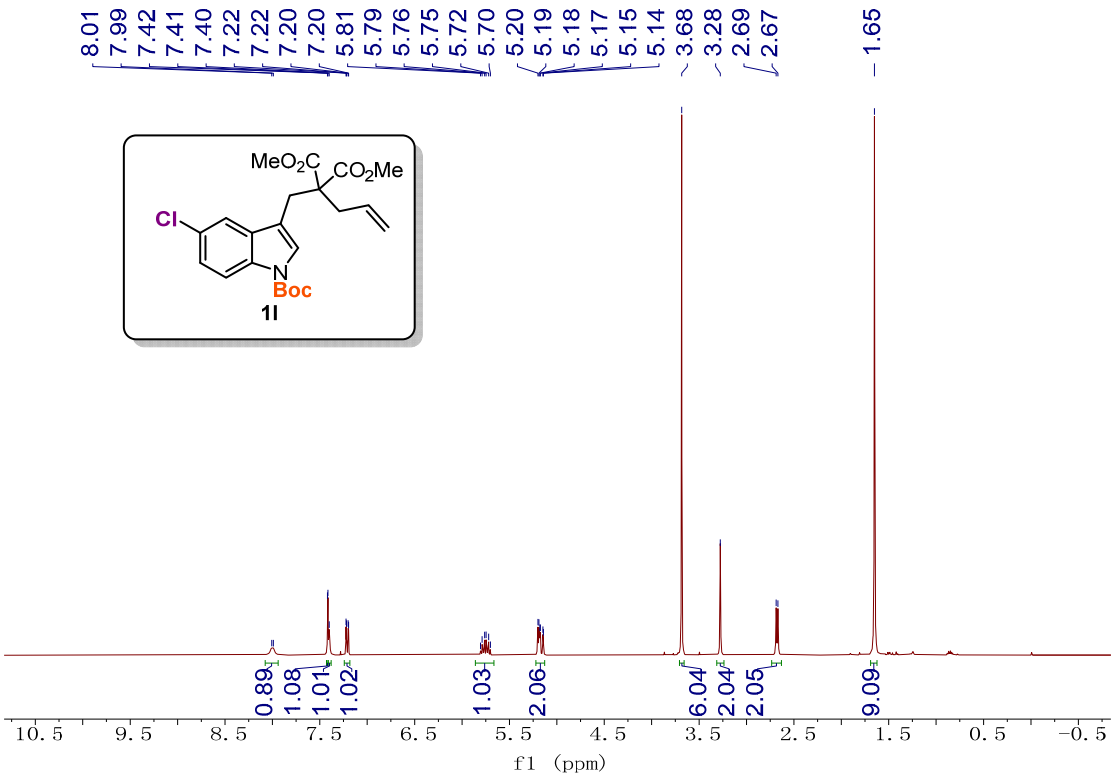


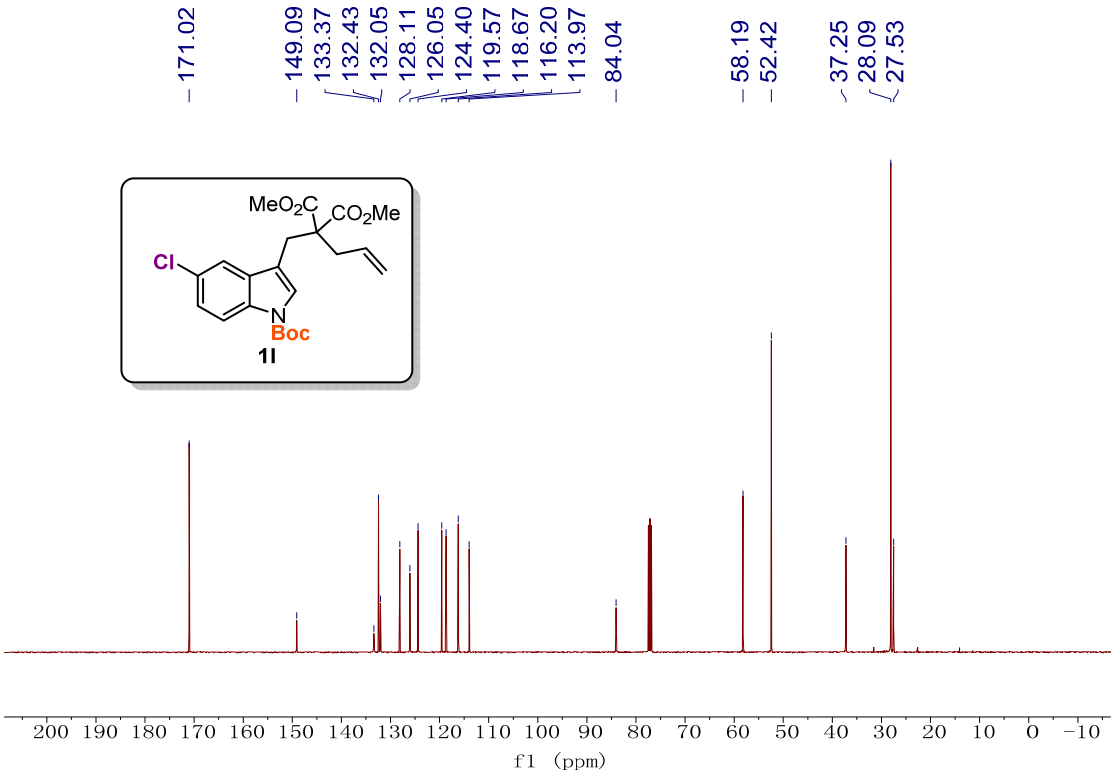
^{1}H & ^{13}C NMR spectra of **1k**



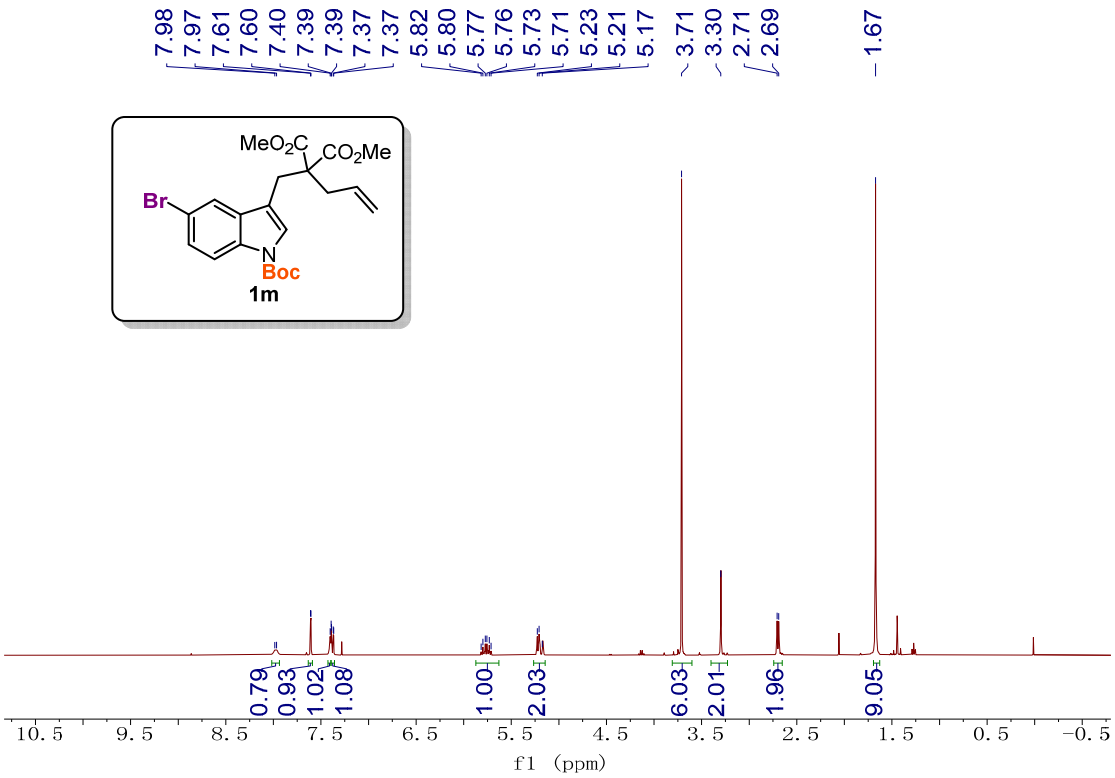


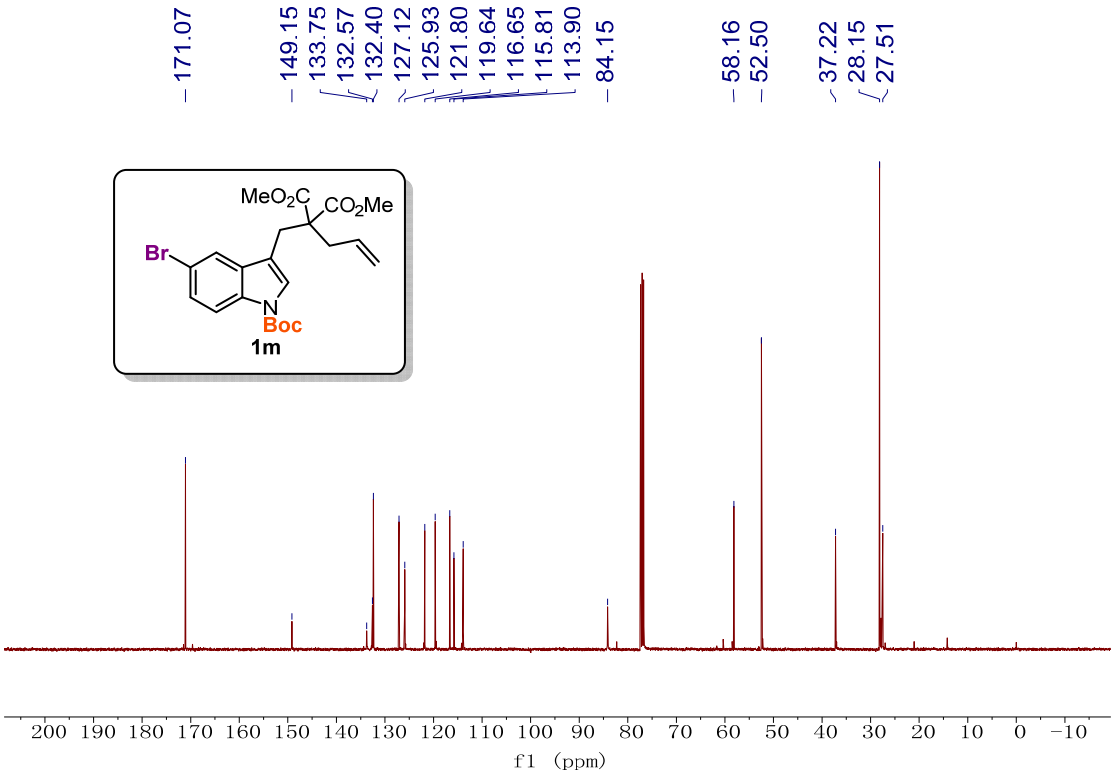
^{1}H & ^{13}C NMR spectra of **1l**



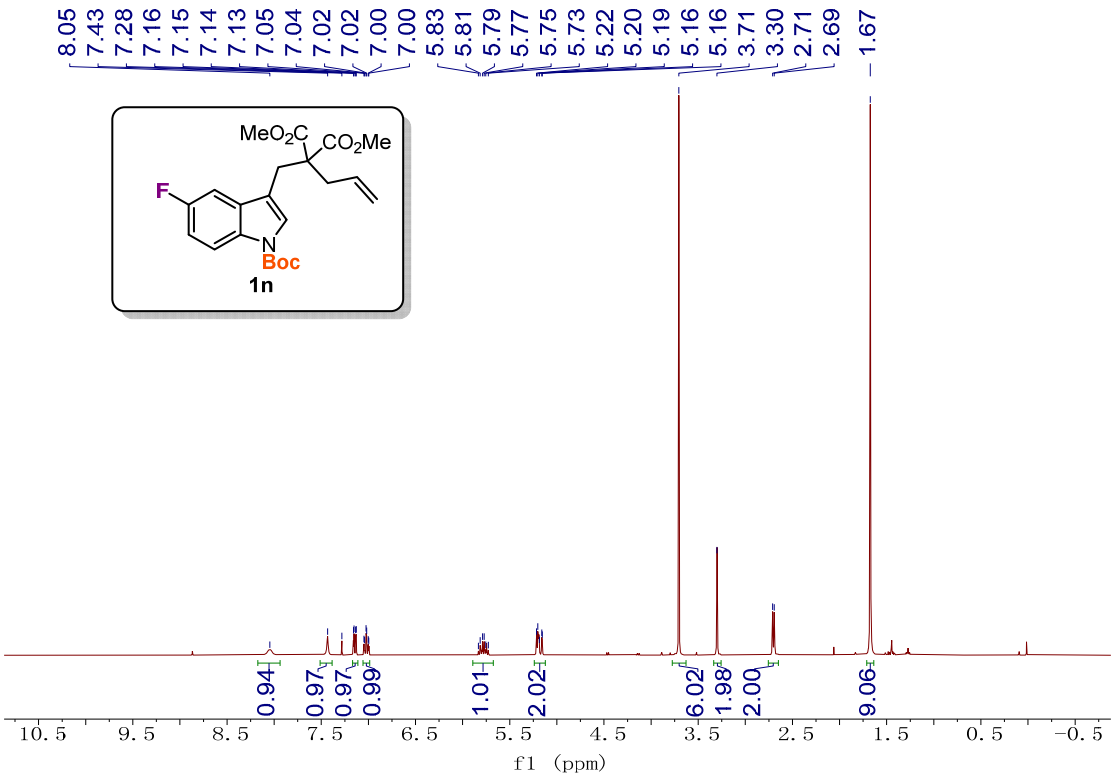


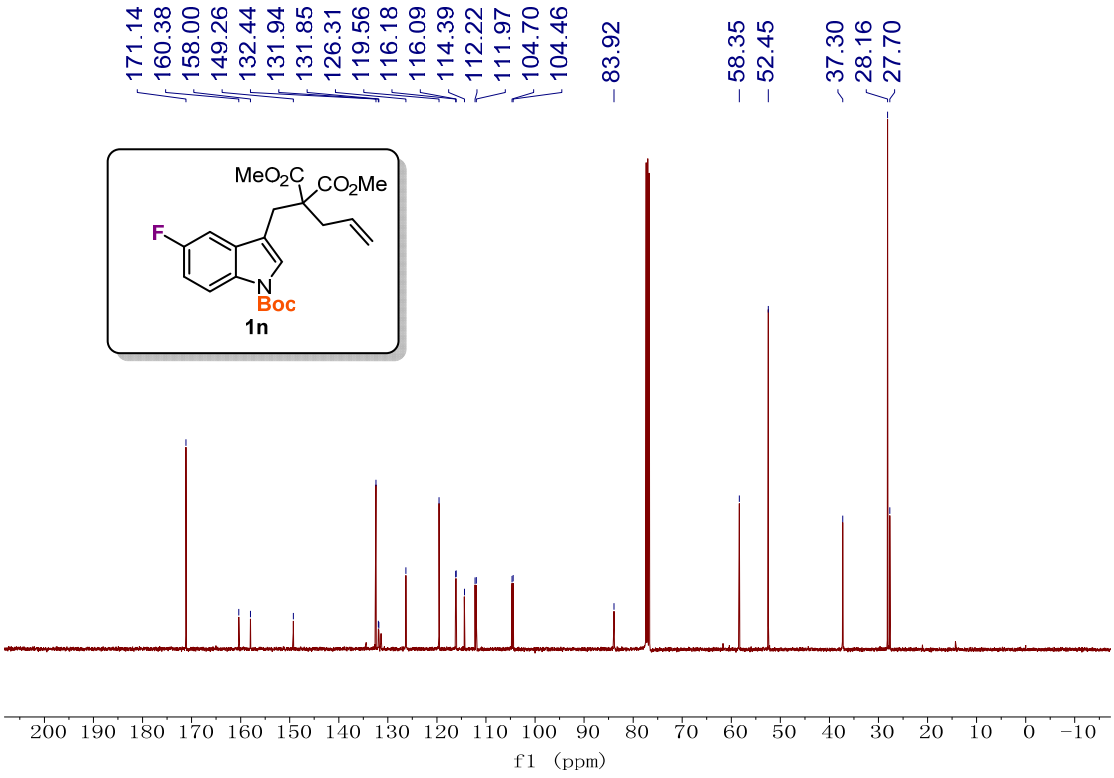
^{1}H & ^{13}C NMR spectra of **1m**



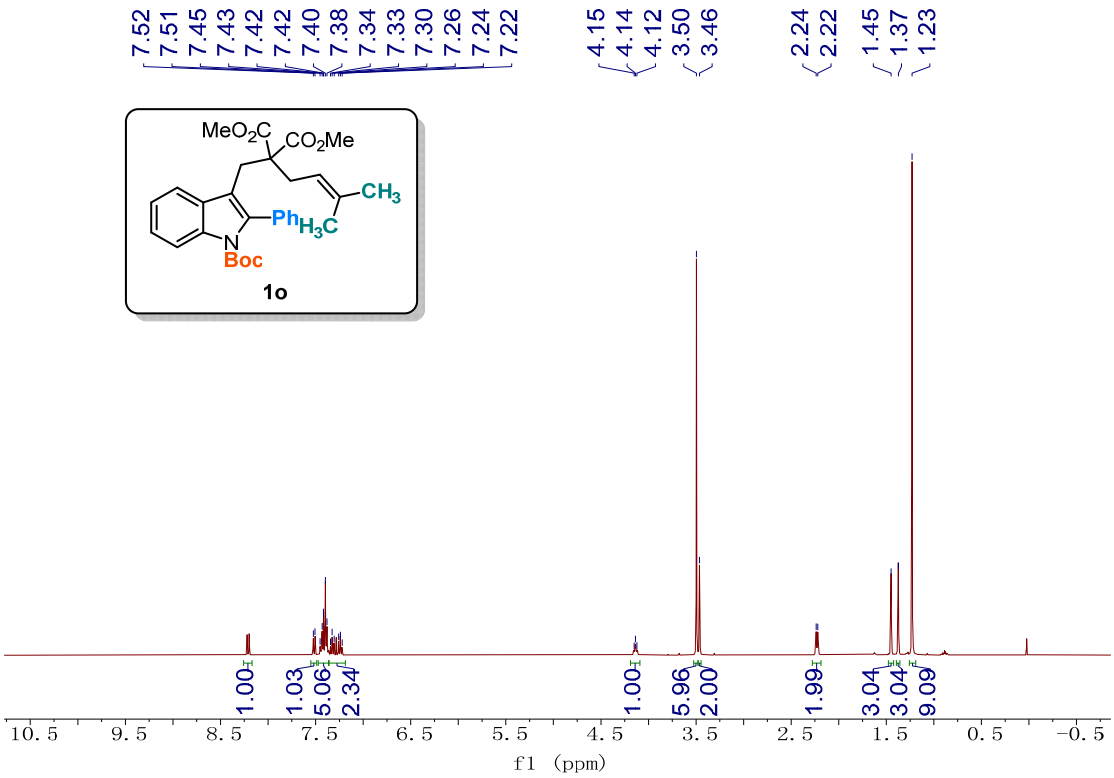


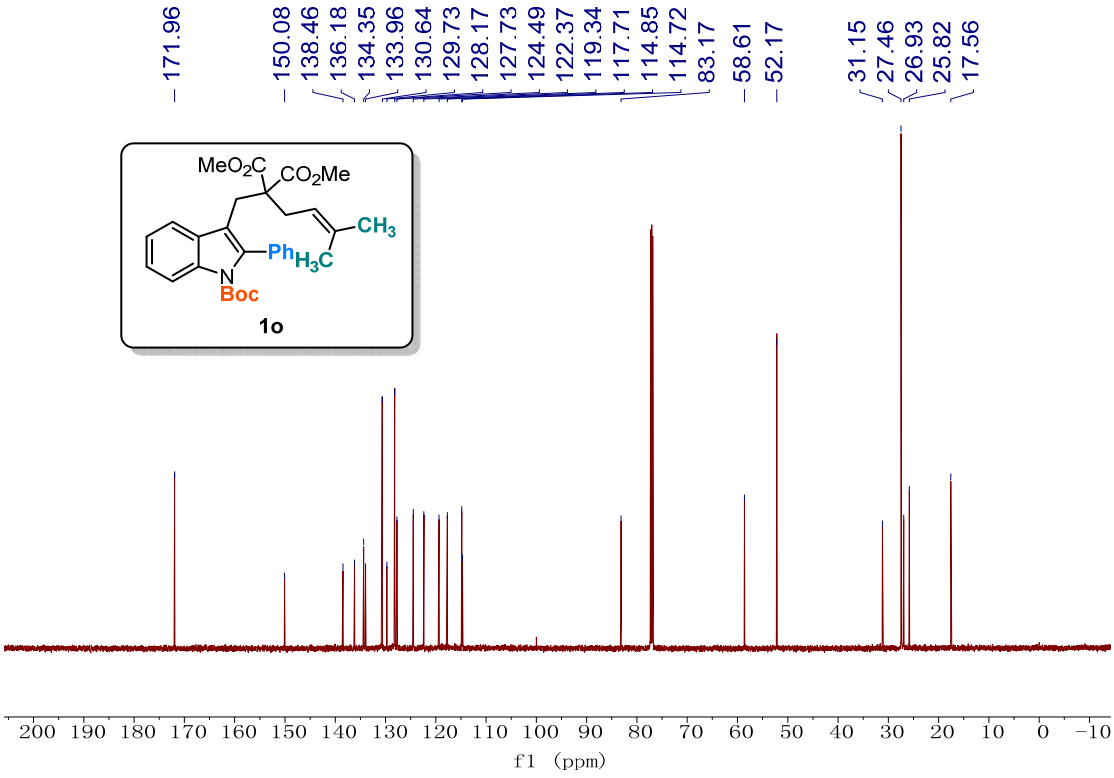
^{1}H & ^{13}C NMR spectra of **1n**



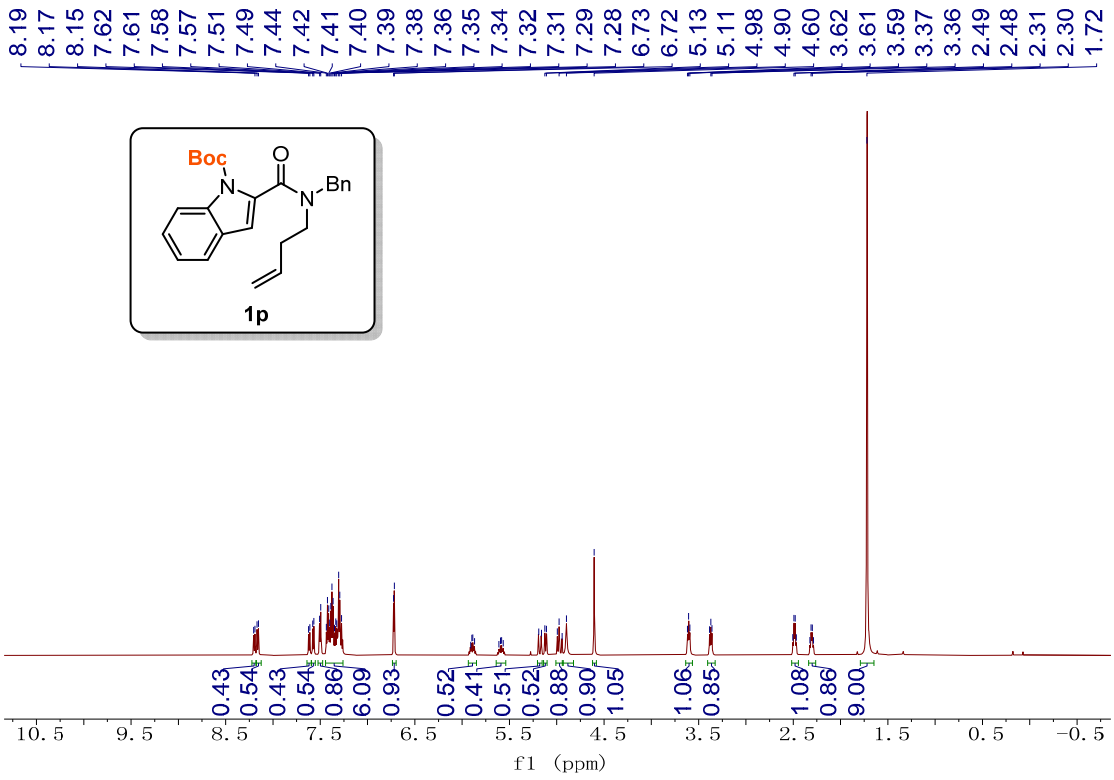


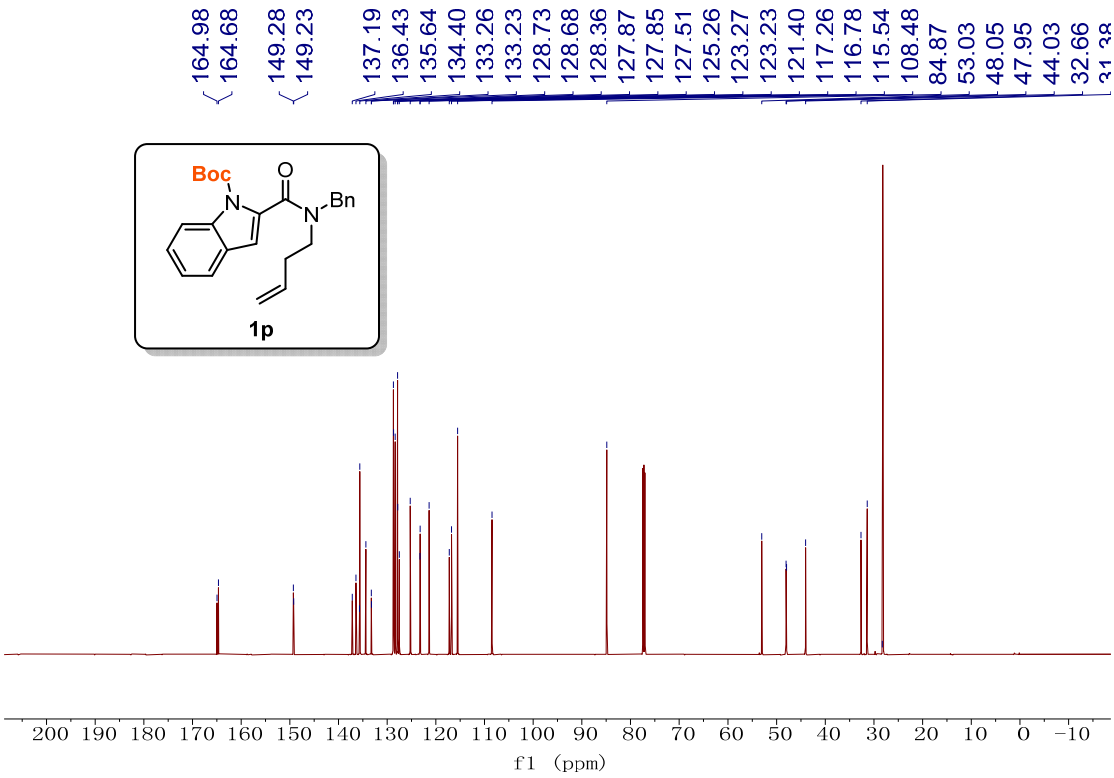
^{1}H & ^{13}C NMR spectra of **1o**





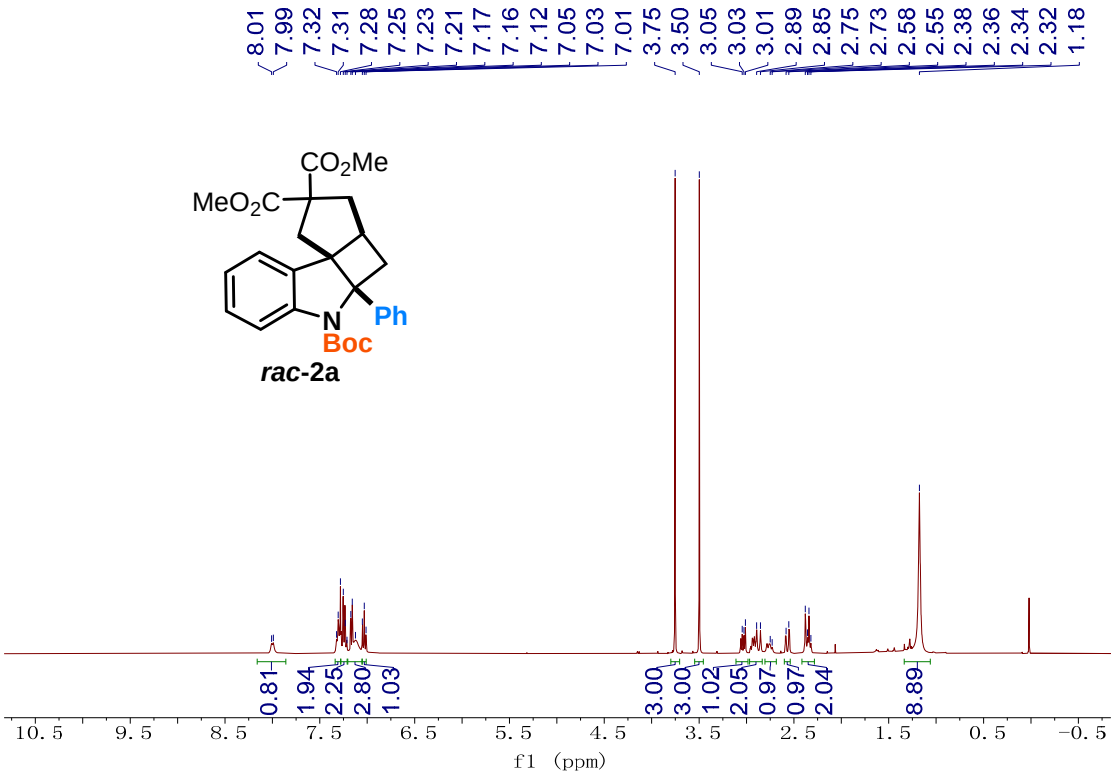
^{1}H & ^{13}C NMR spectra of **1p**

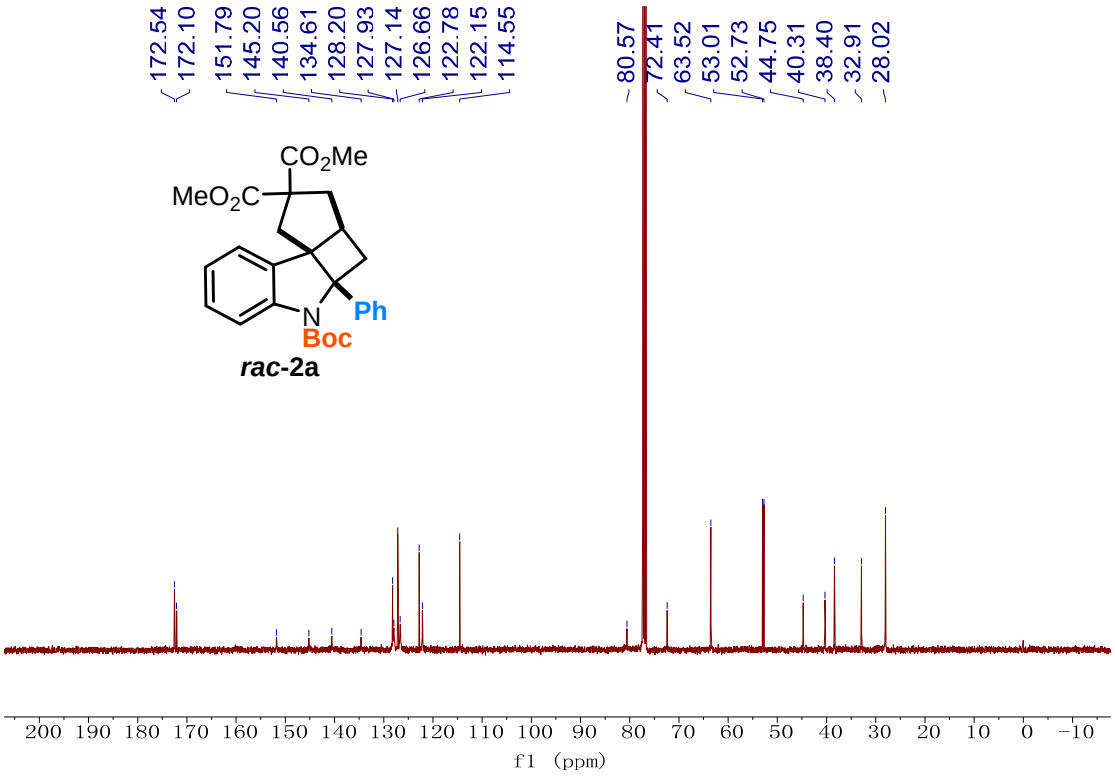




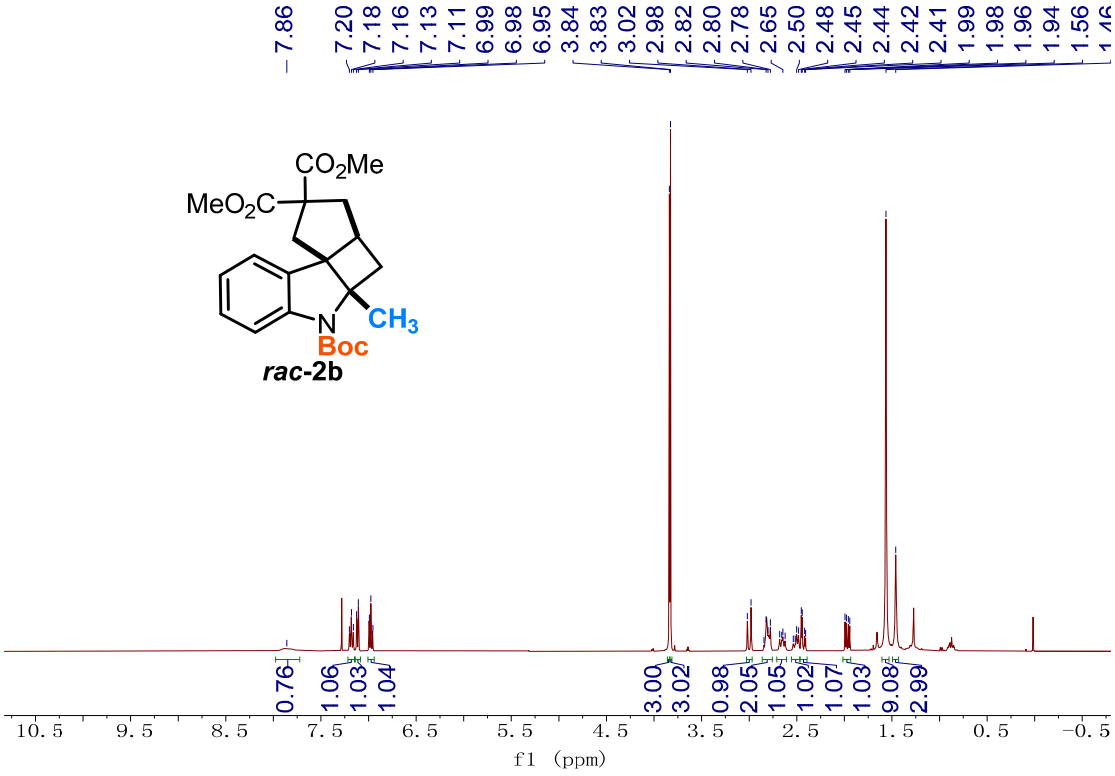
NMR Spectra

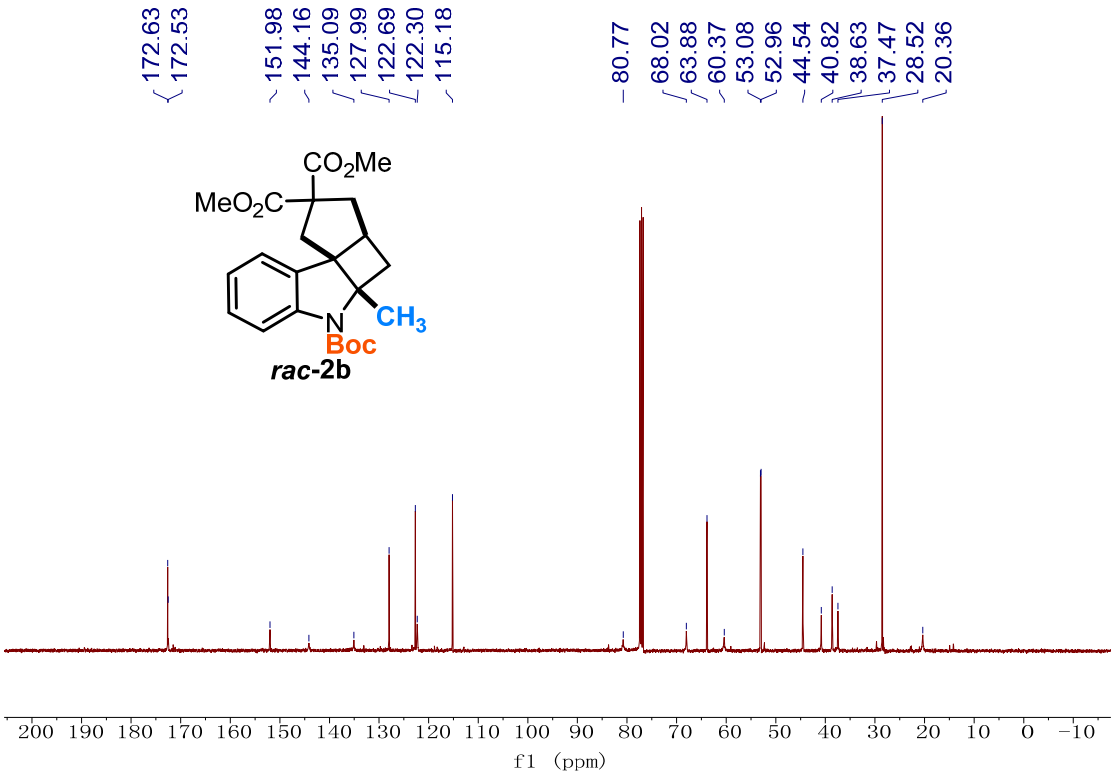
^{1}H & ^{13}C NMR spectra of ***rac*-2a**



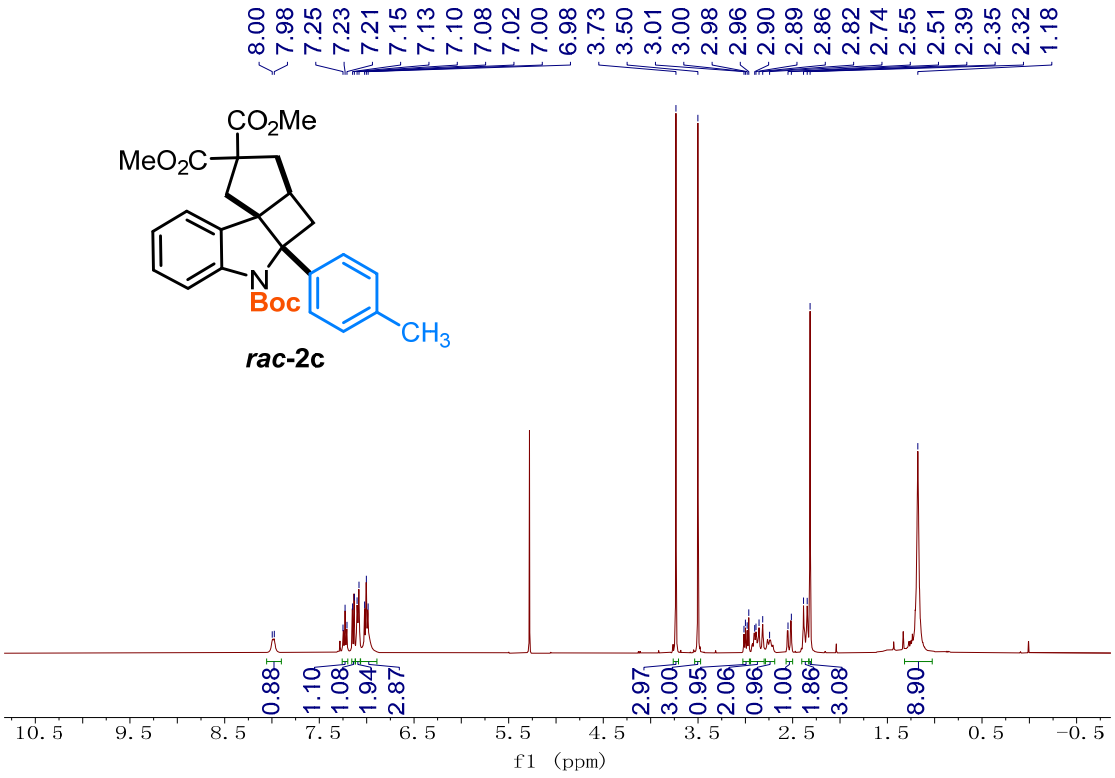


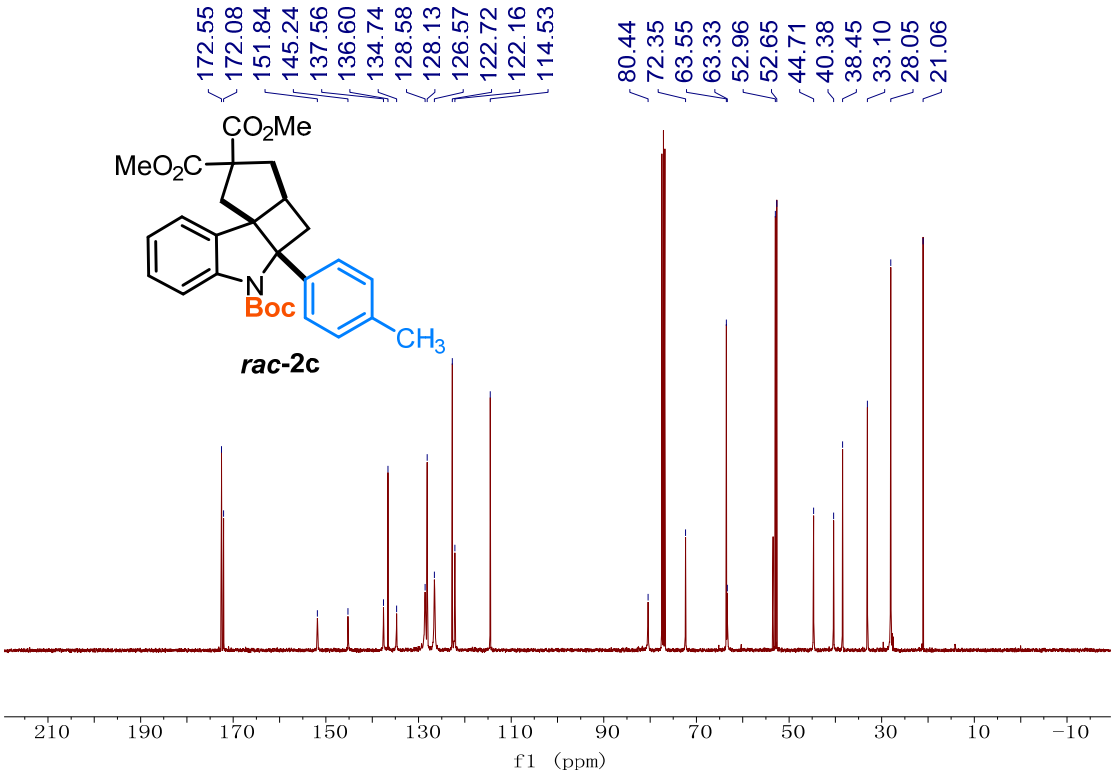
^{1}H & ^{13}C NMR spectra of ***rac*-2b**



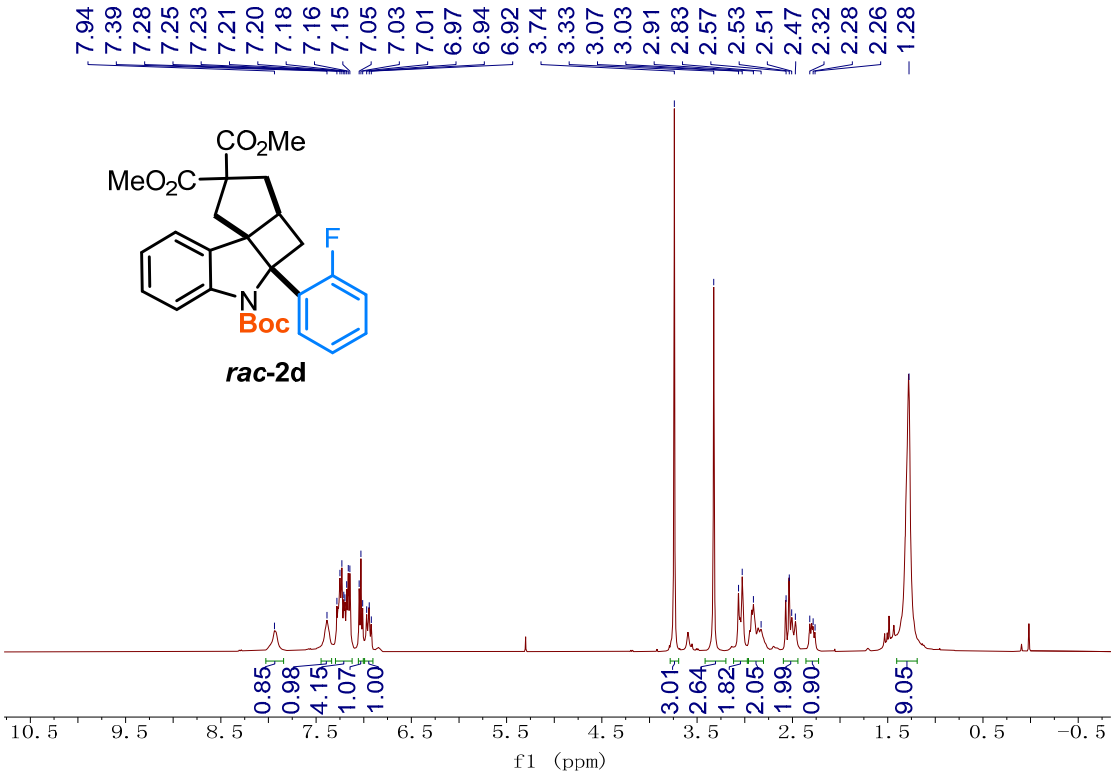


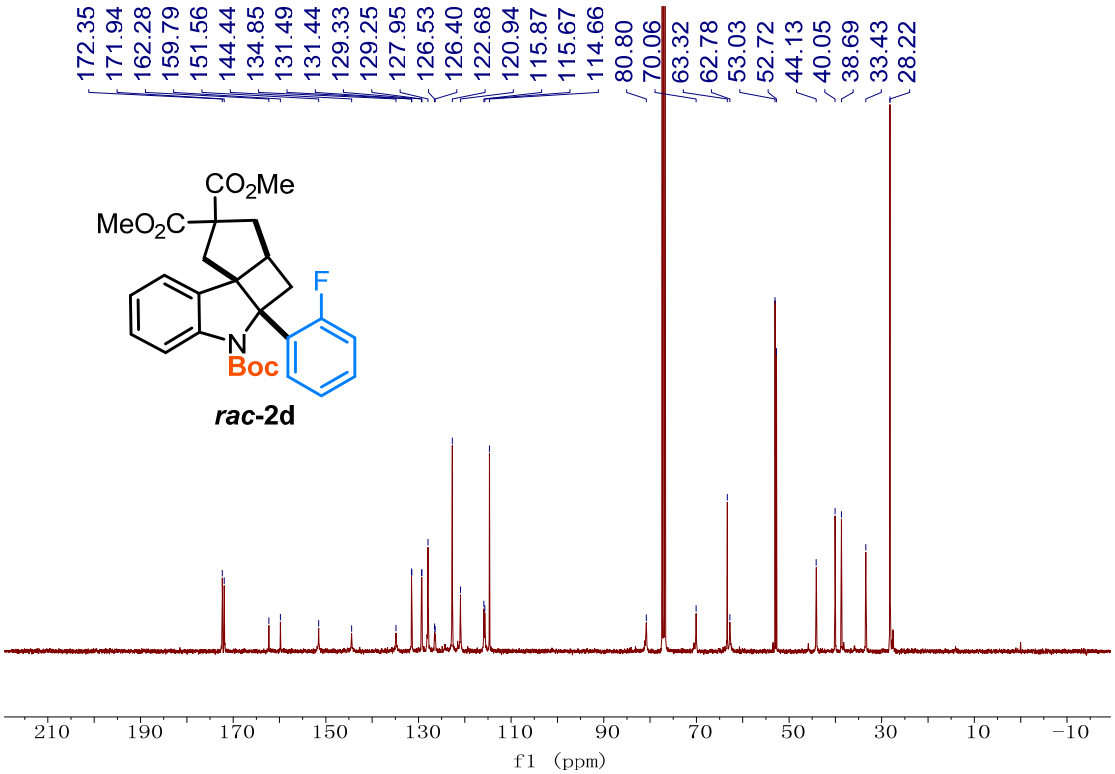
^{1}H & ^{13}C NMR spectra of ***rac*-2c**



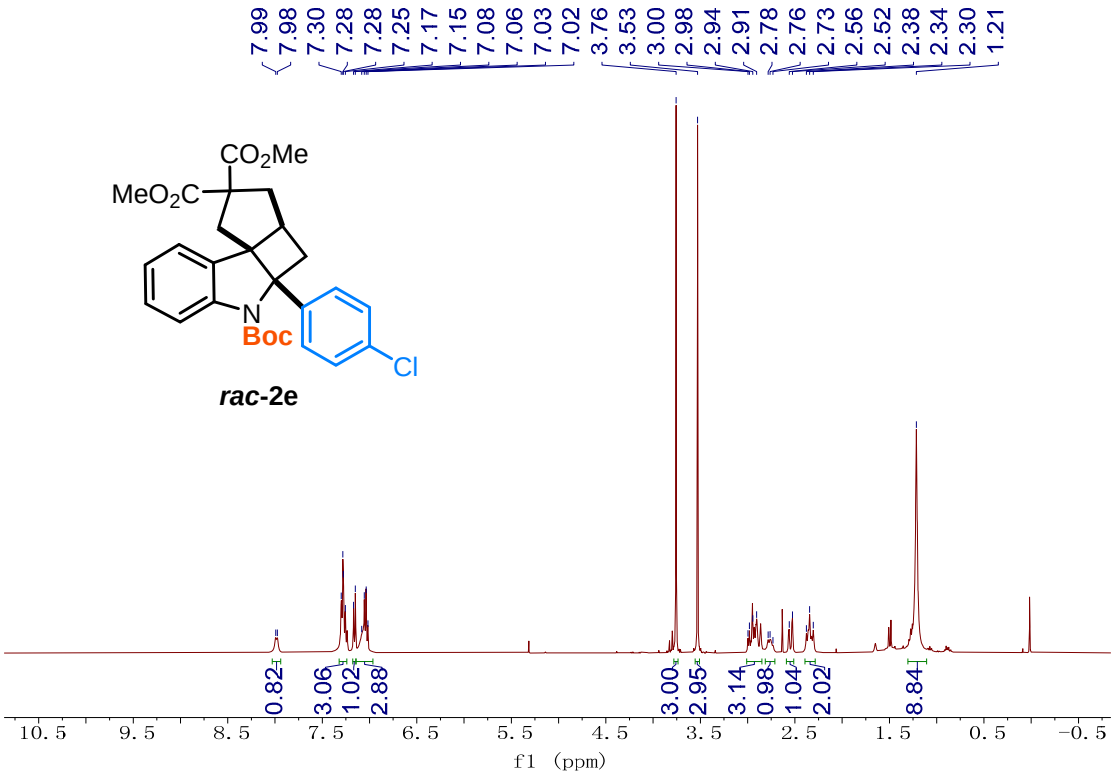


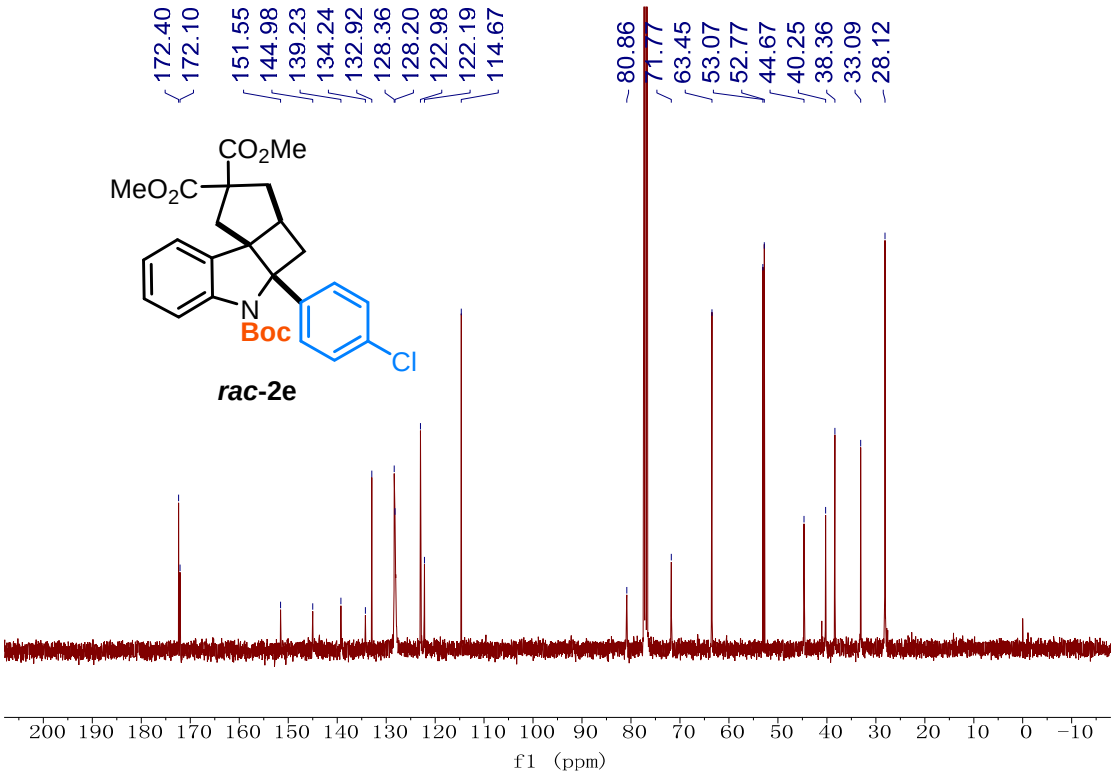
^{1}H & ^{13}C NMR spectra of ***rac*-2d**



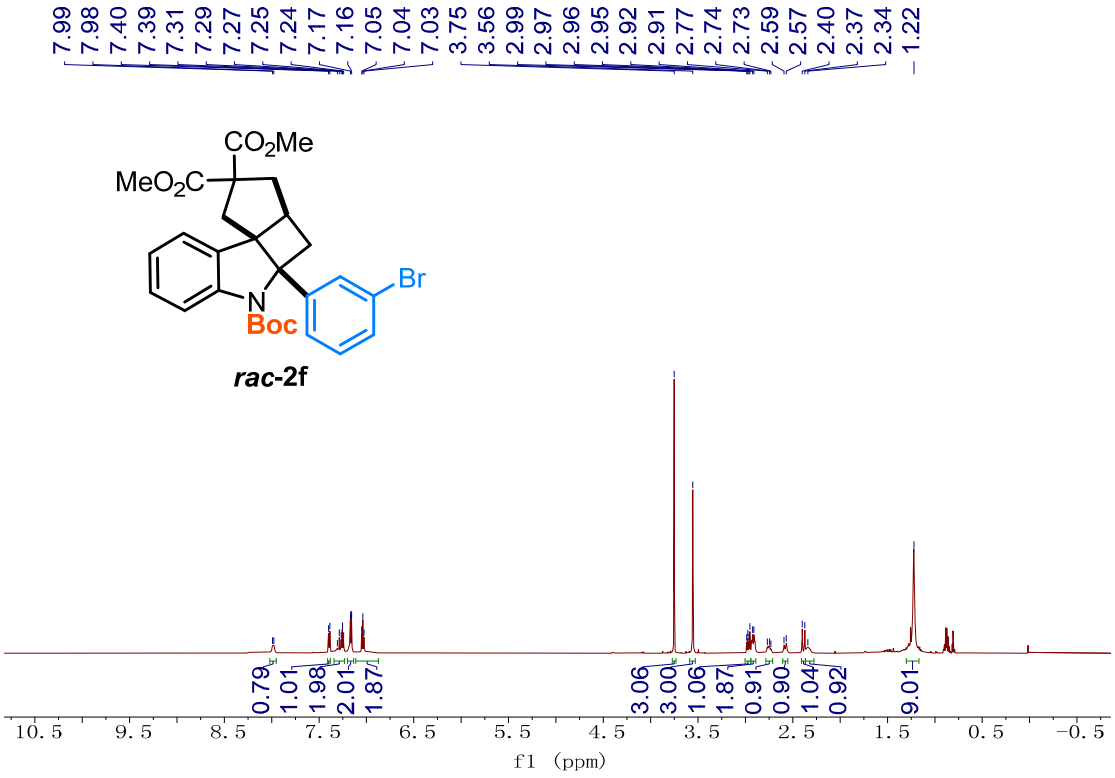


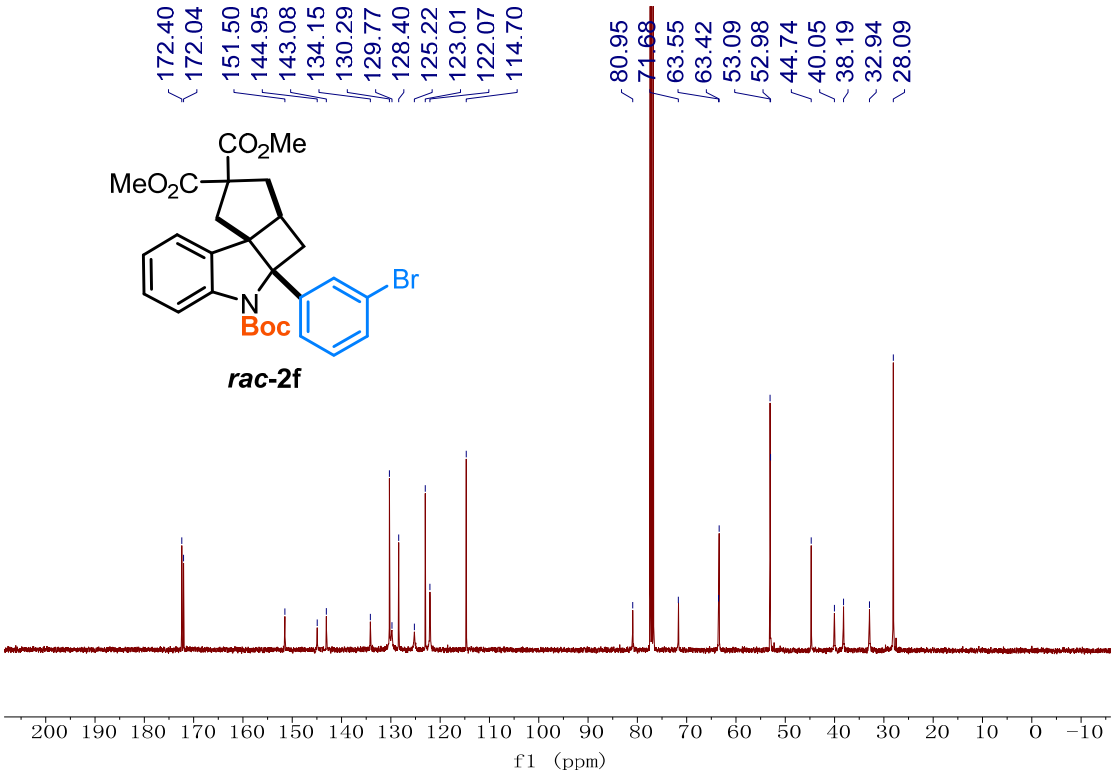
^{1}H & ^{13}C NMR spectra of ***rac*-2e**



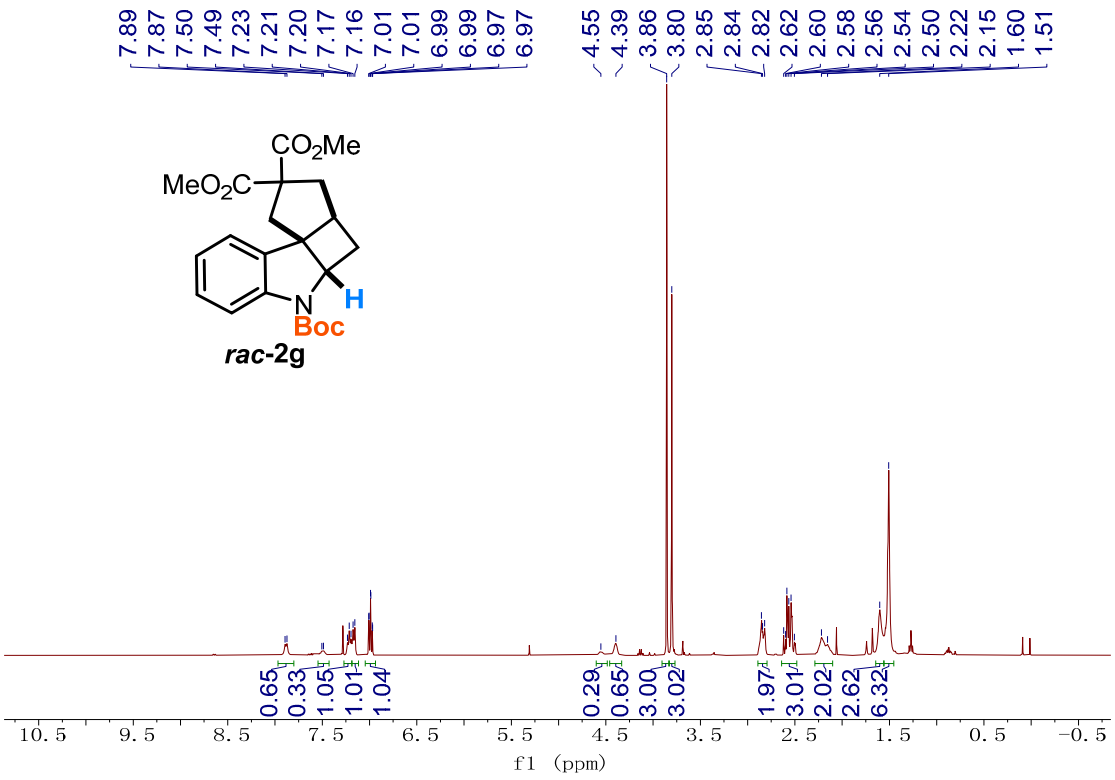


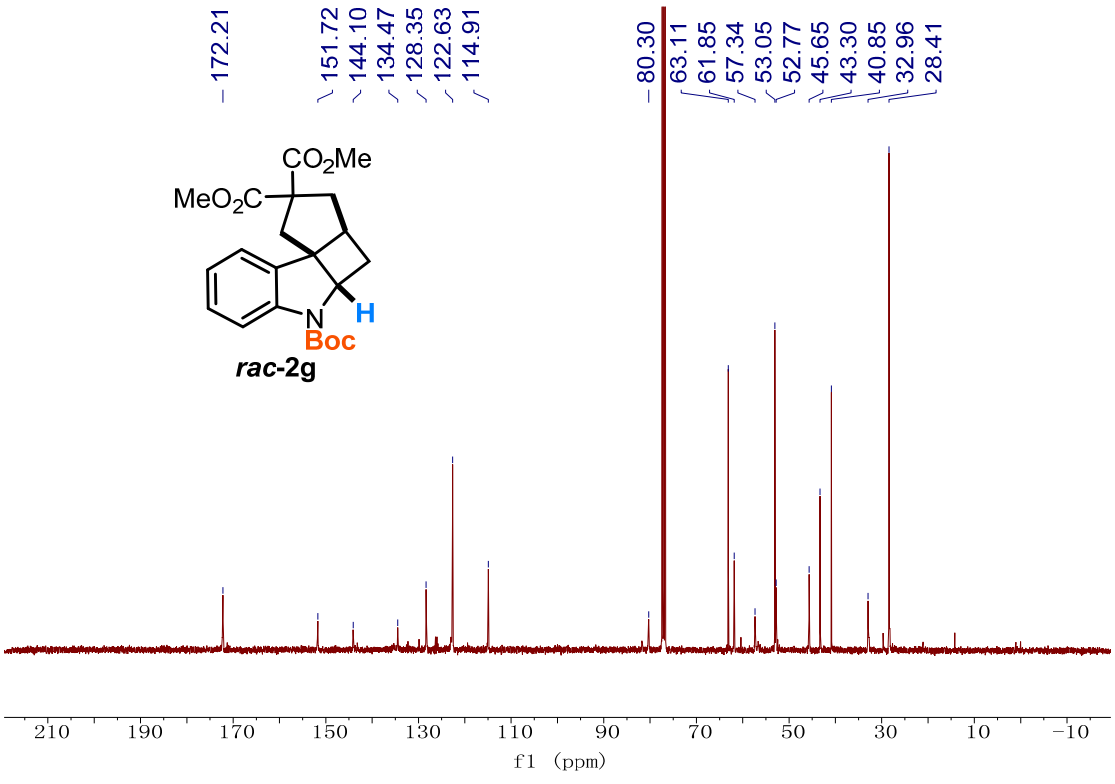
^{1}H & ^{13}C NMR spectra of ***rac*-2f**



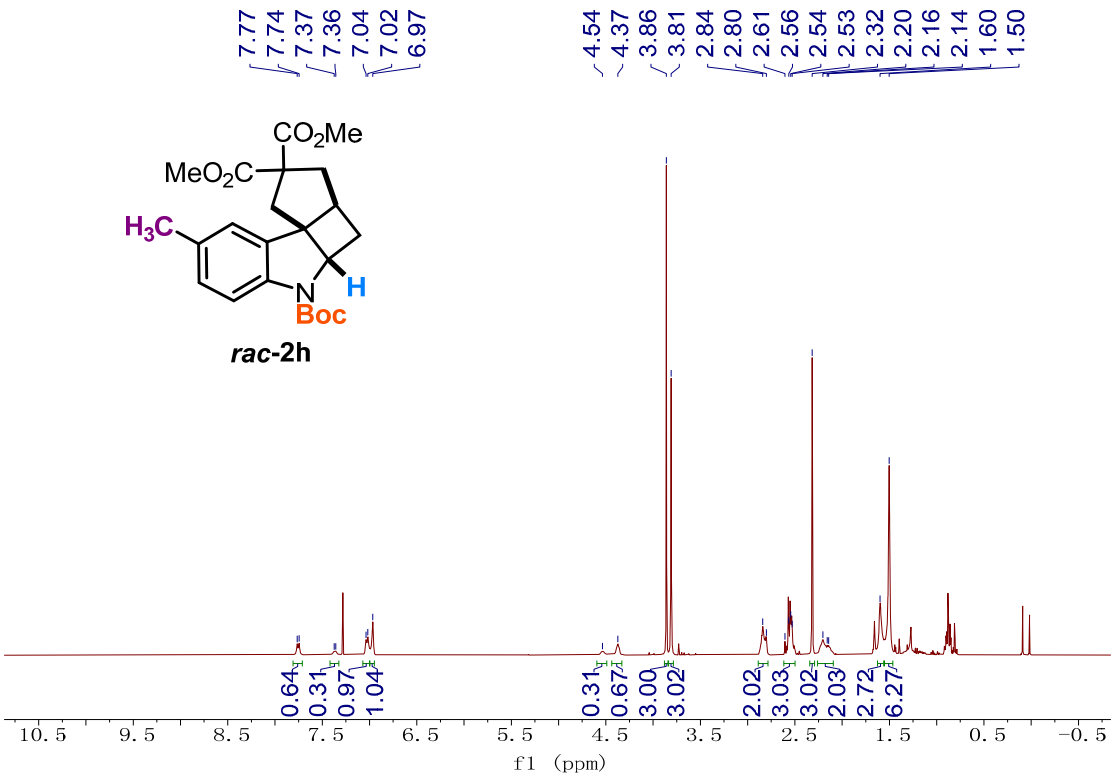


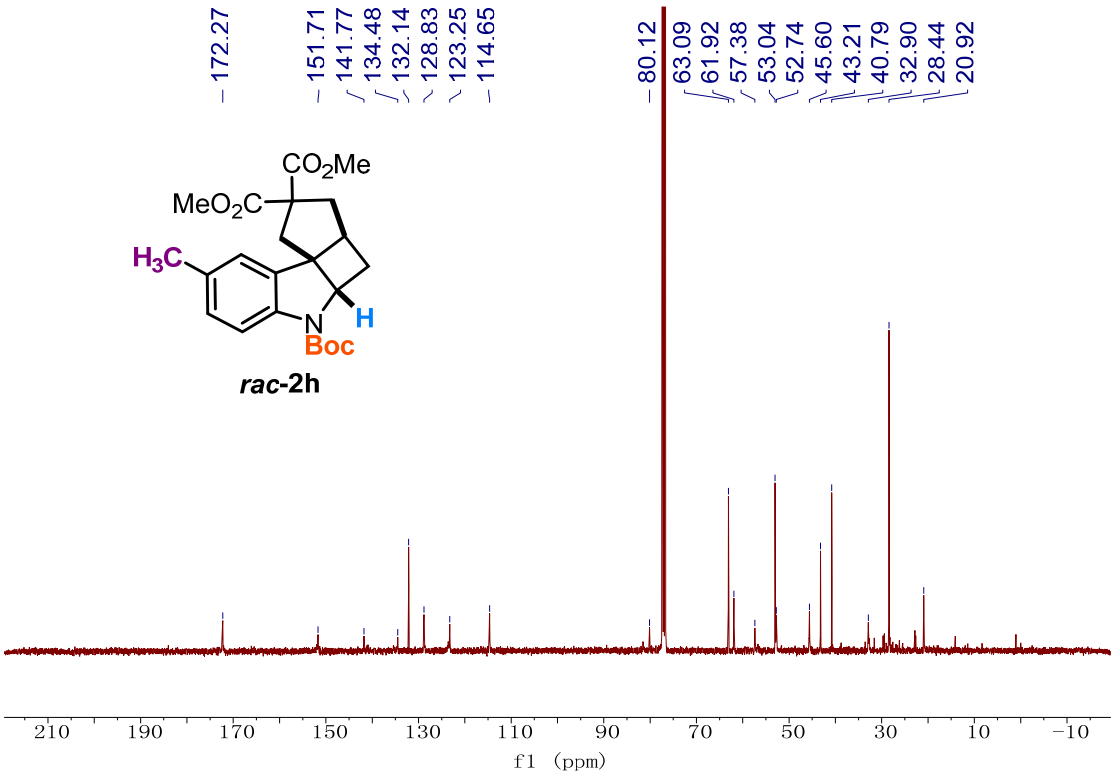
^{1}H & ^{13}C NMR spectra of ***rac*-2g**



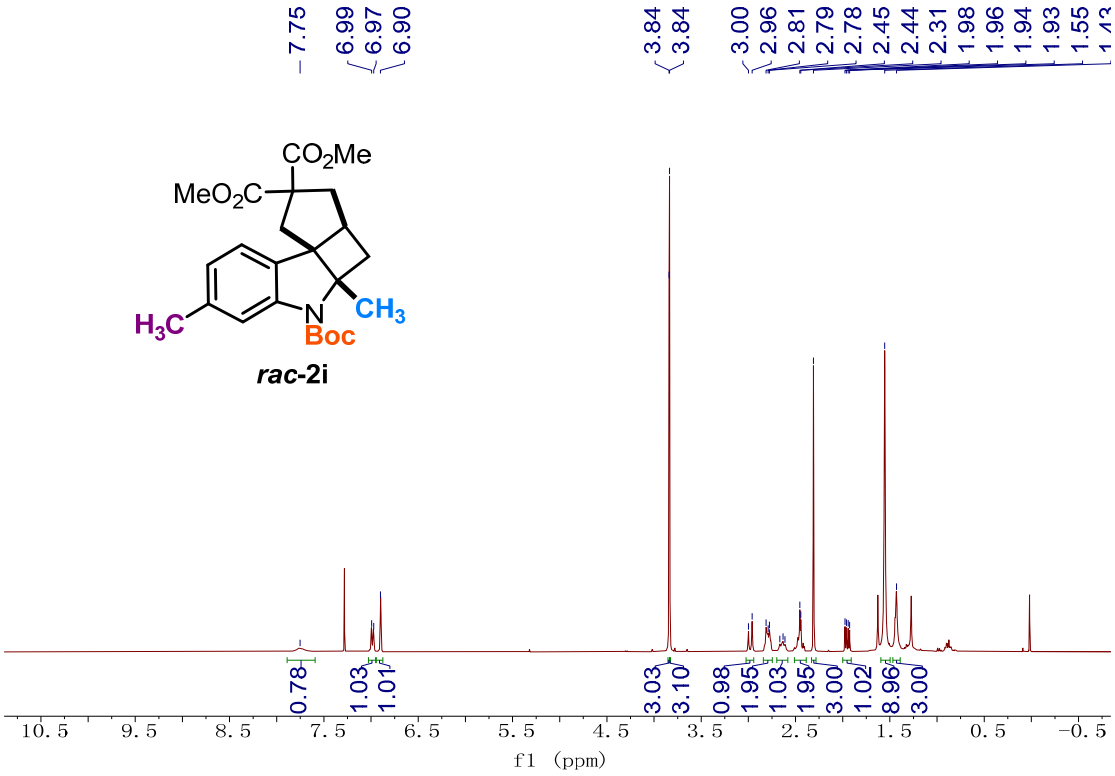


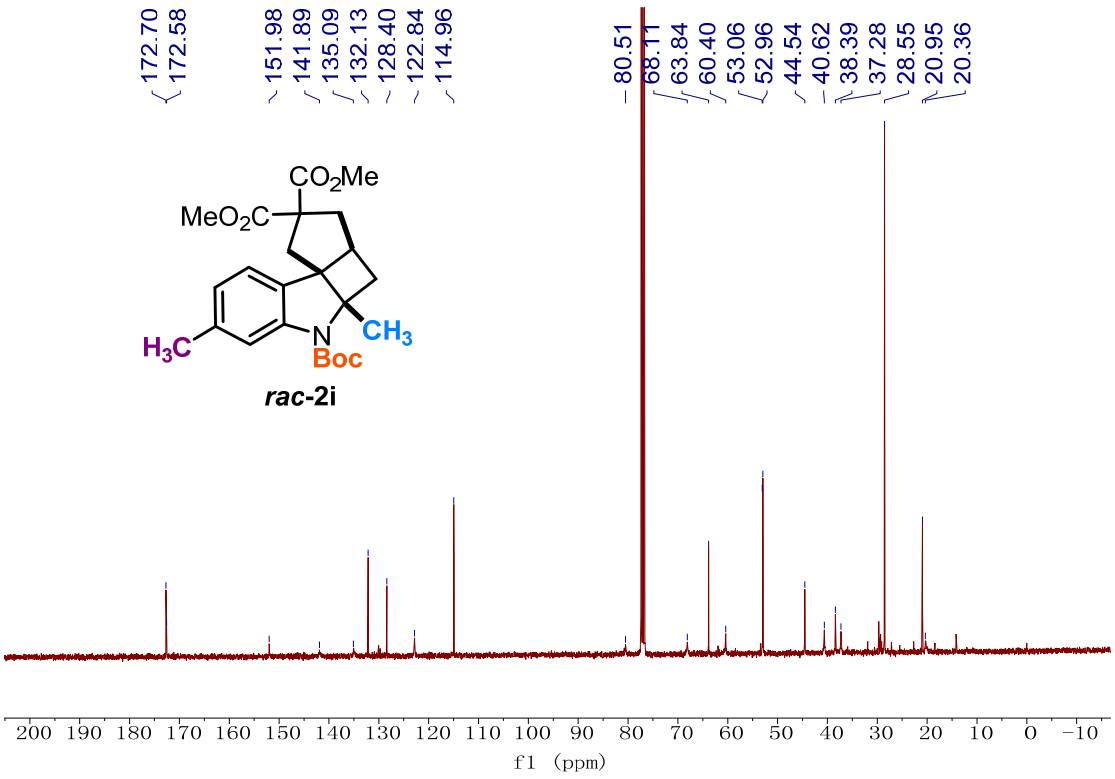
^{1}H & ^{13}C NMR spectra of ***rac*-2h**



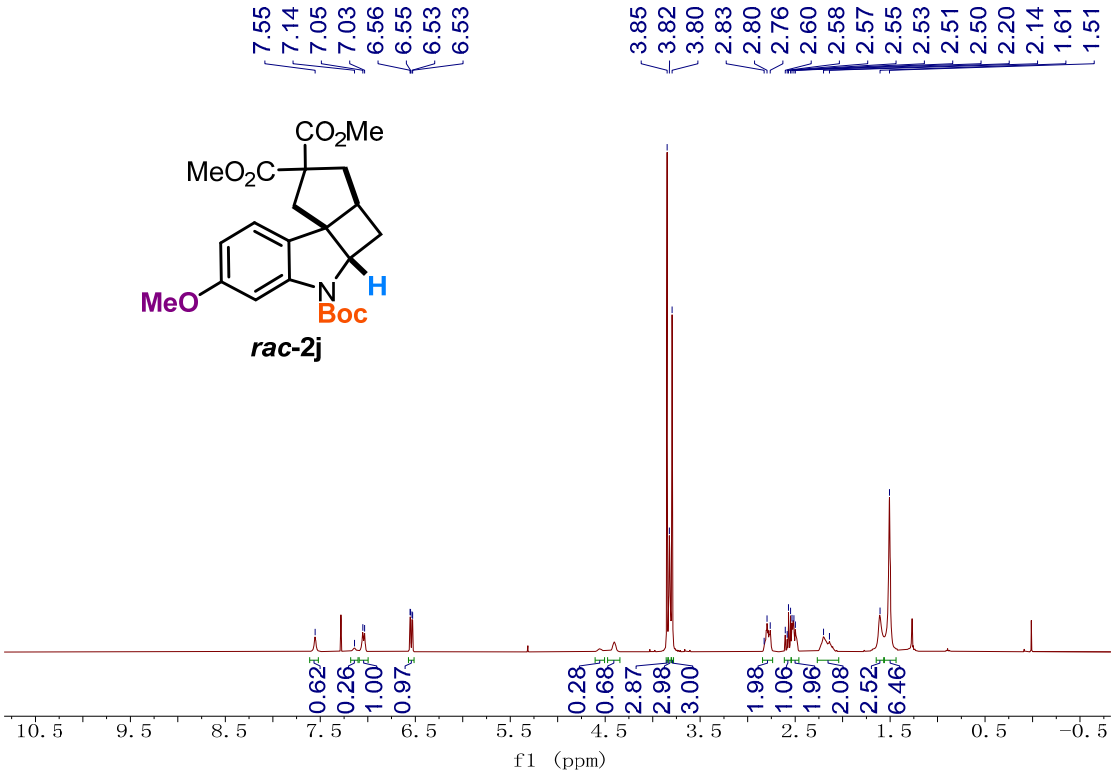


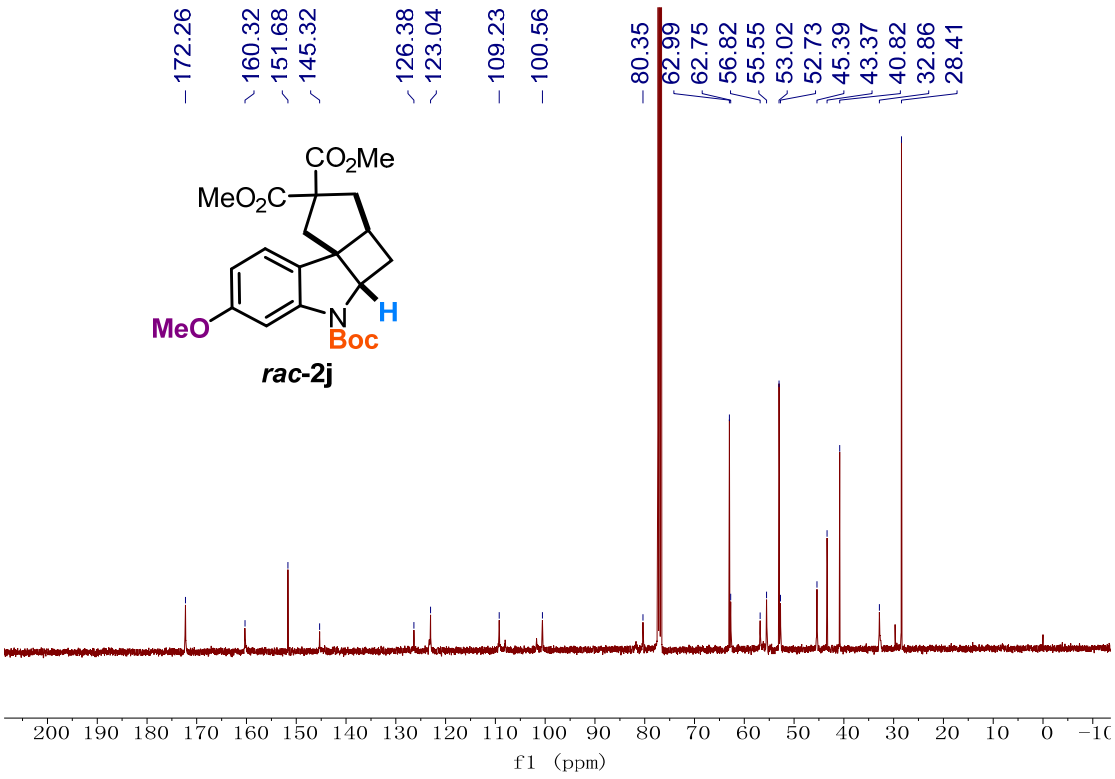
^{1}H & ^{13}C NMR spectra of ***rac*-2i**



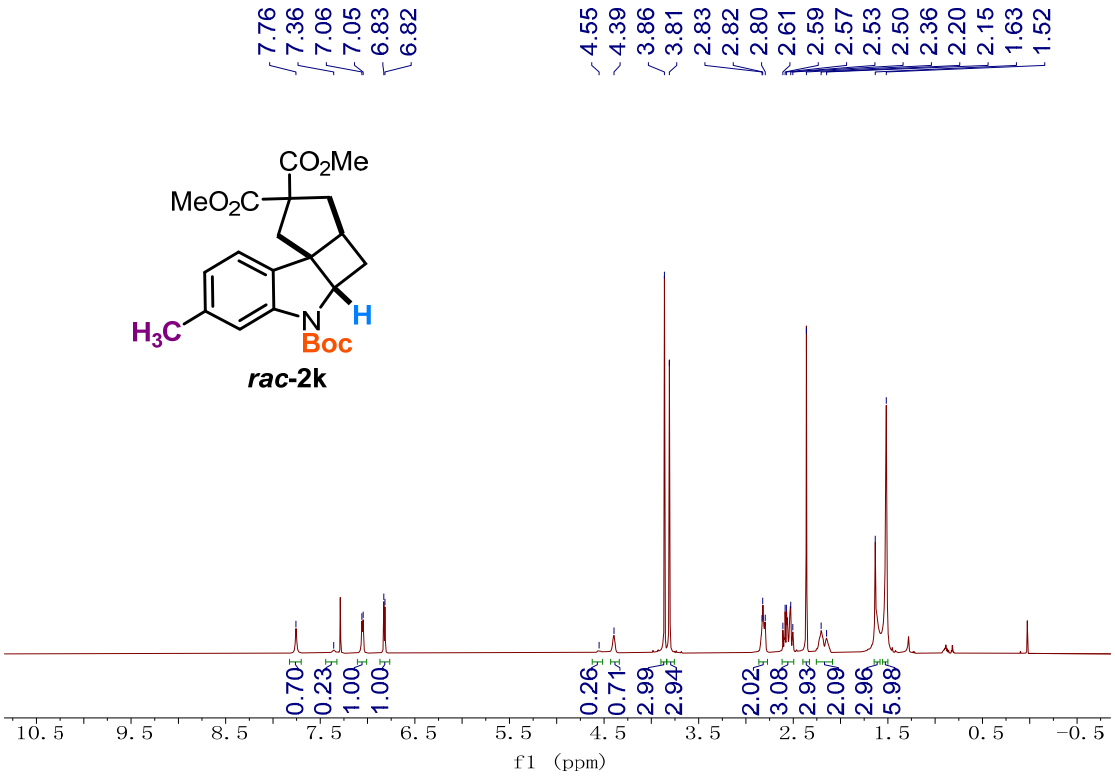


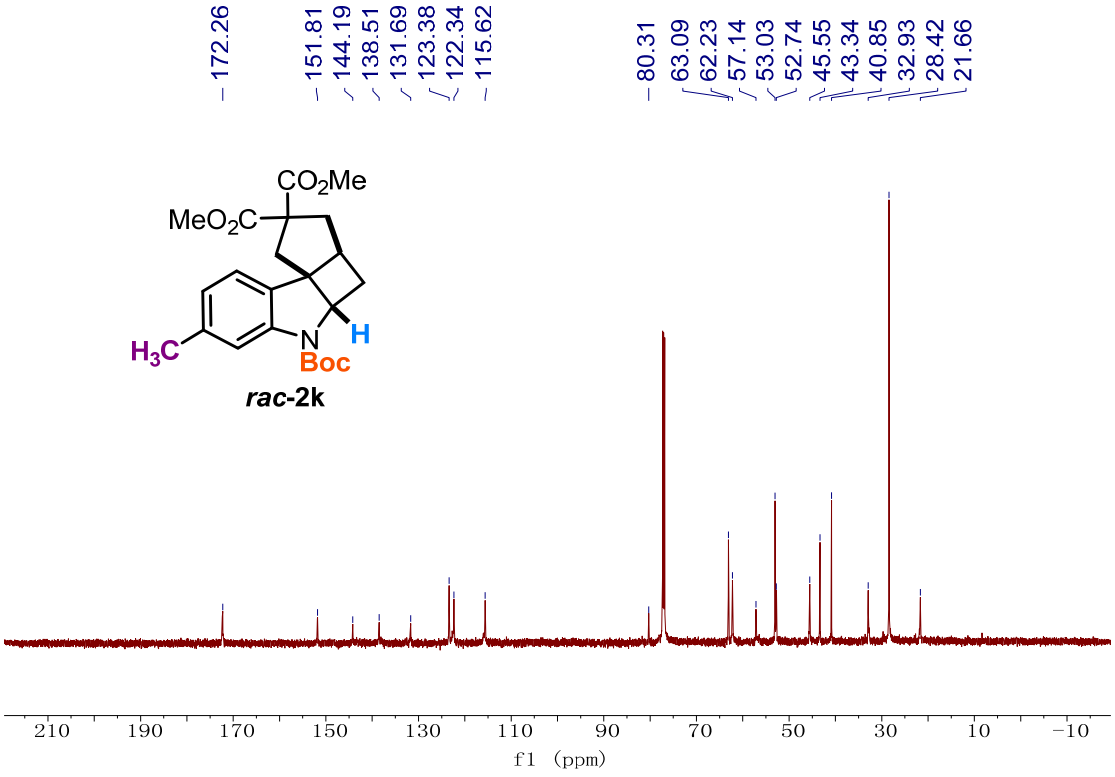
^{1}H & ^{13}C NMR spectra of ***rac*-2j**



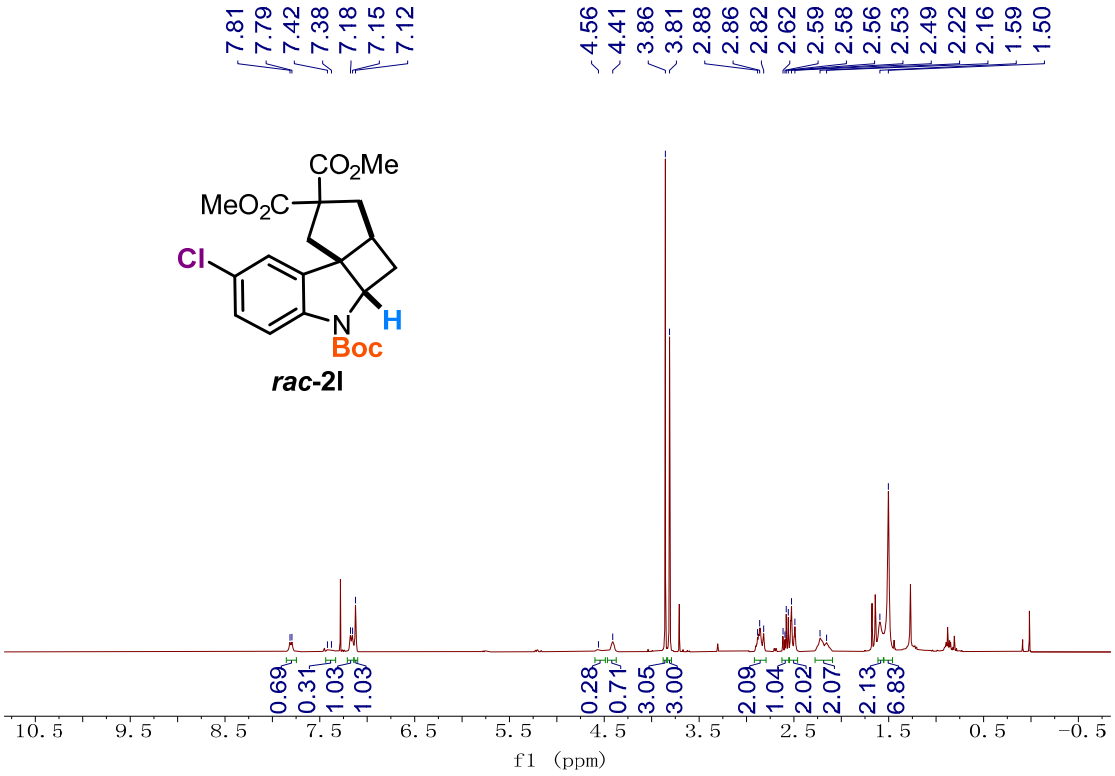


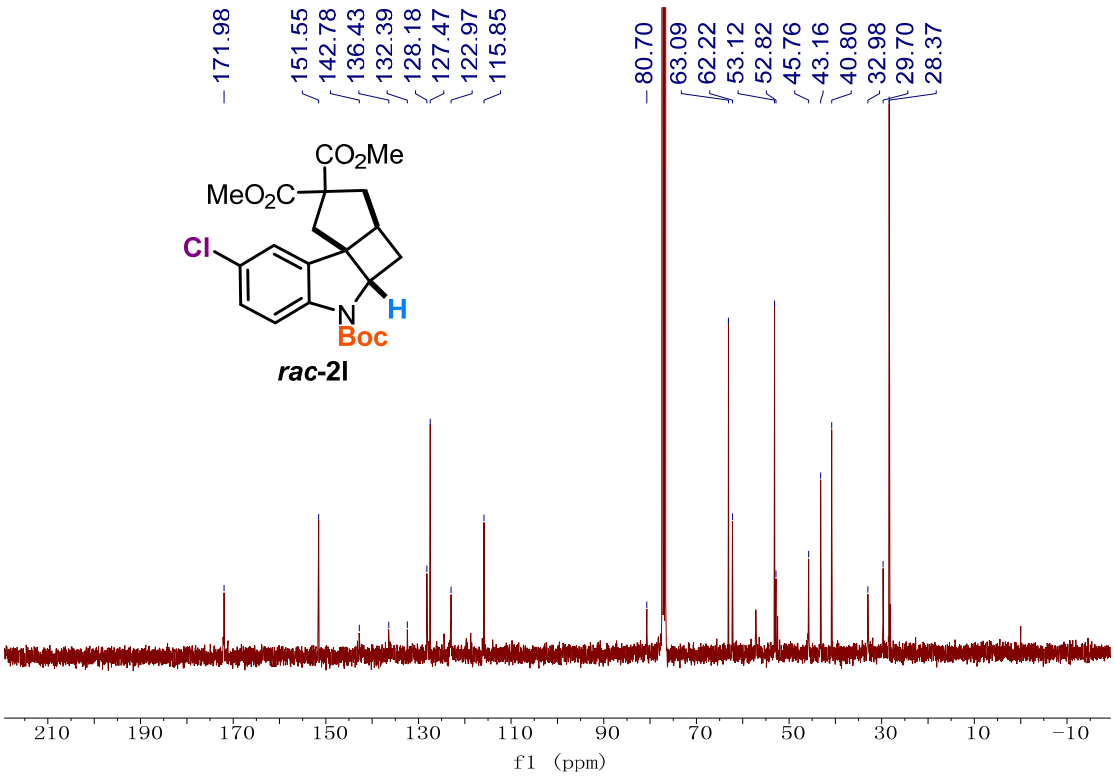
^{1}H & ^{13}C NMR spectra of ***rac*-2k**



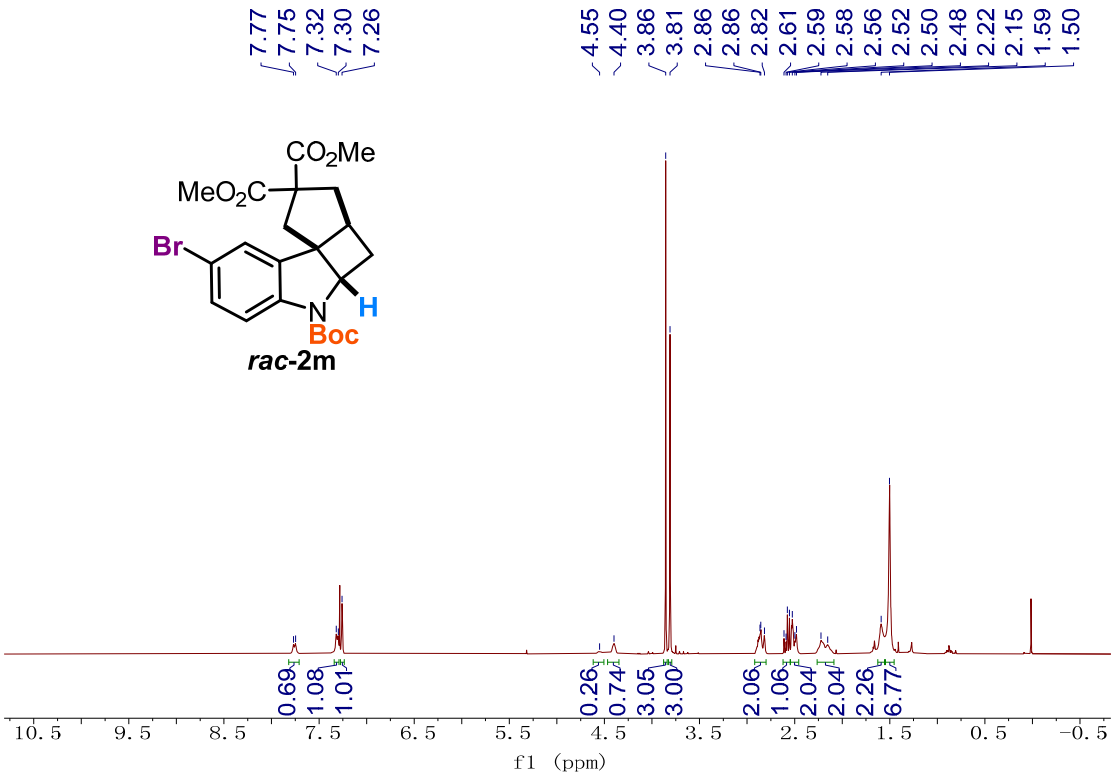


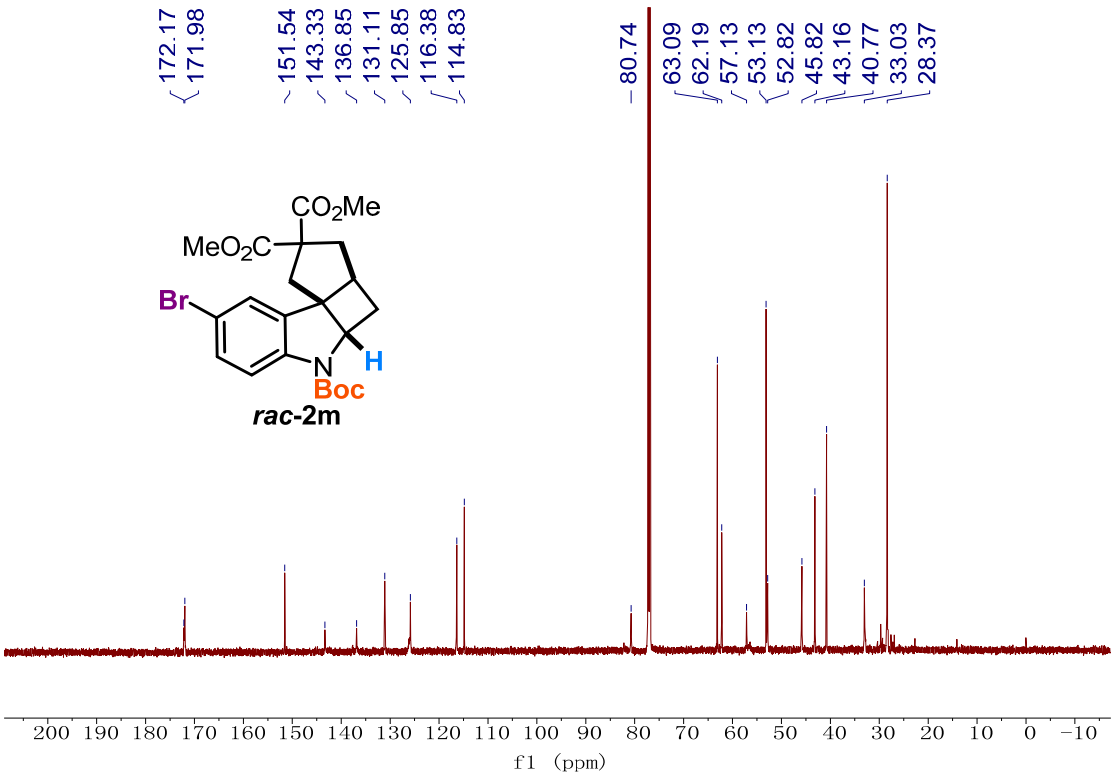
^{1}H & ^{13}C NMR spectra of ***rac*-2l**



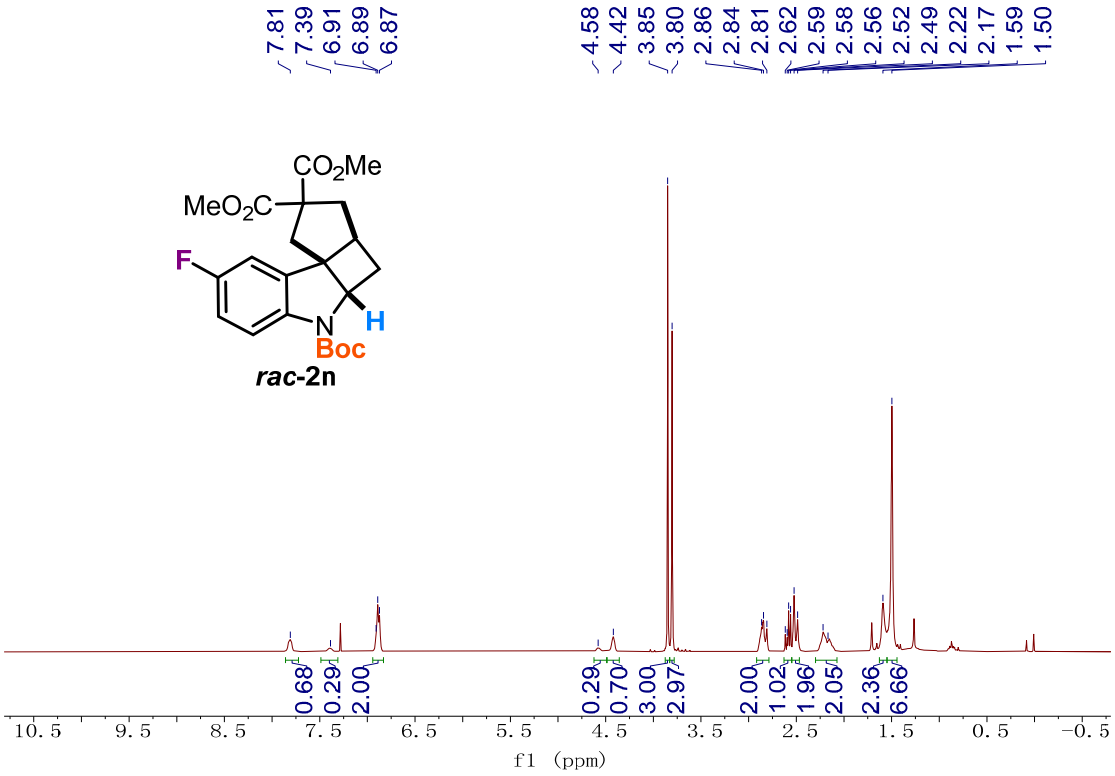


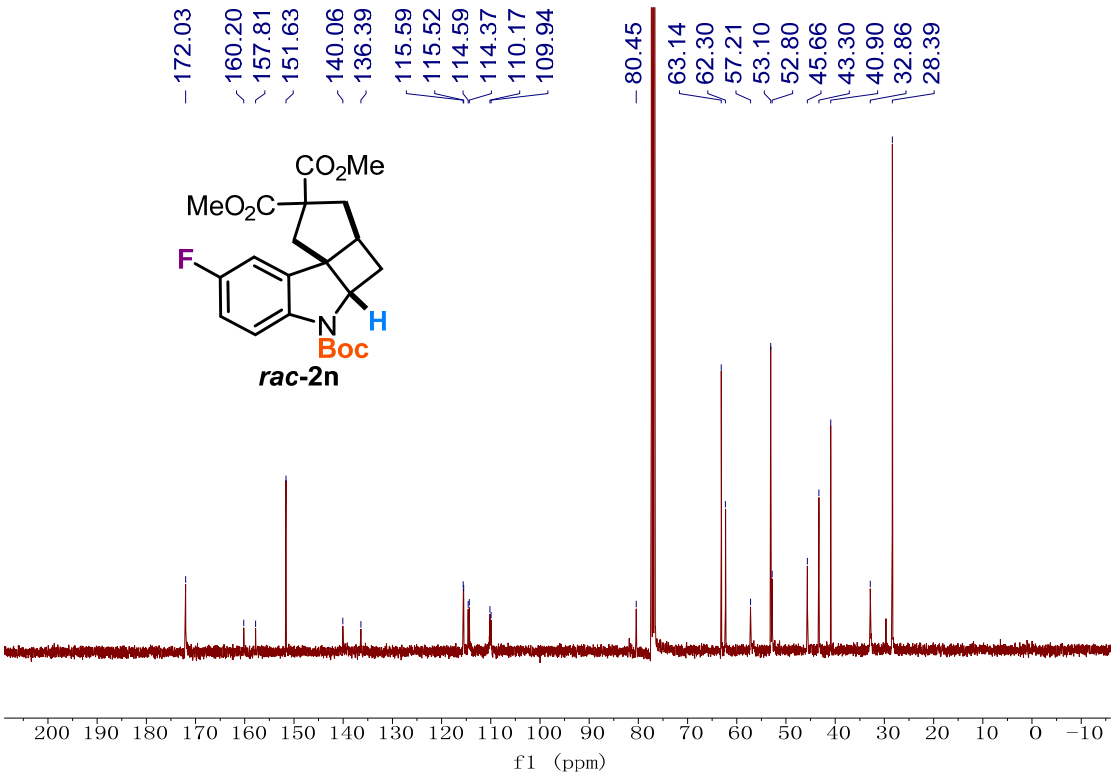
^{1}H & ^{13}C NMR spectra of ***rac*-2m**



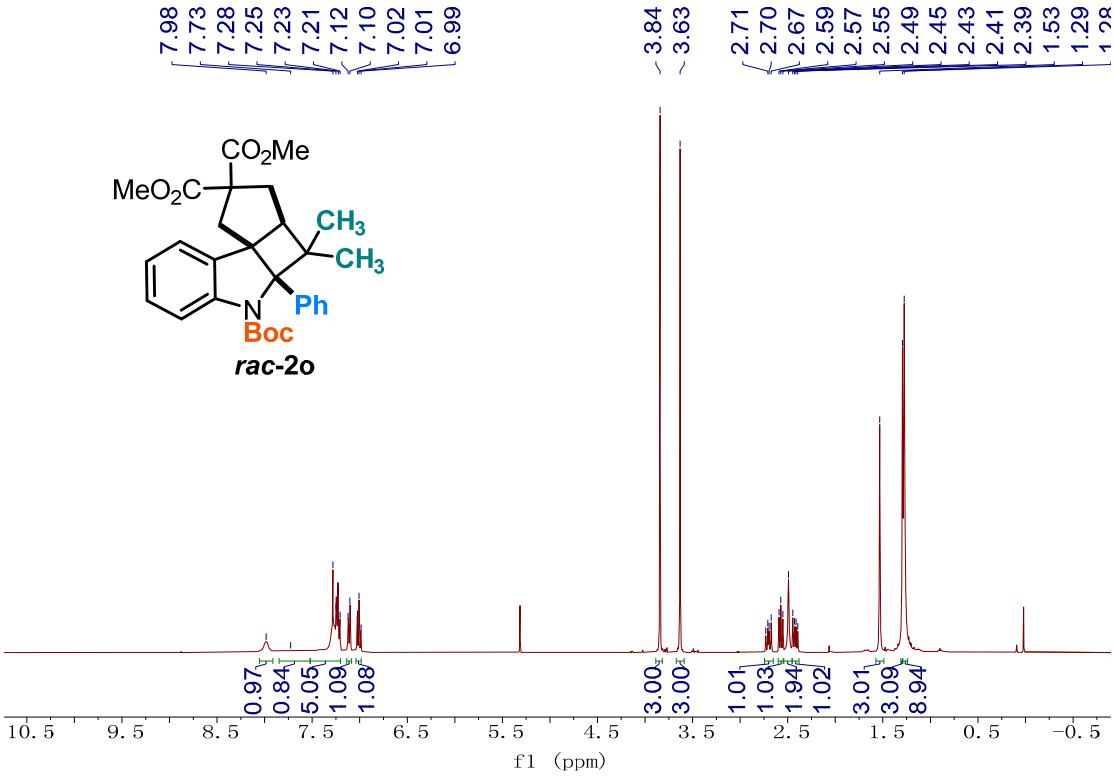


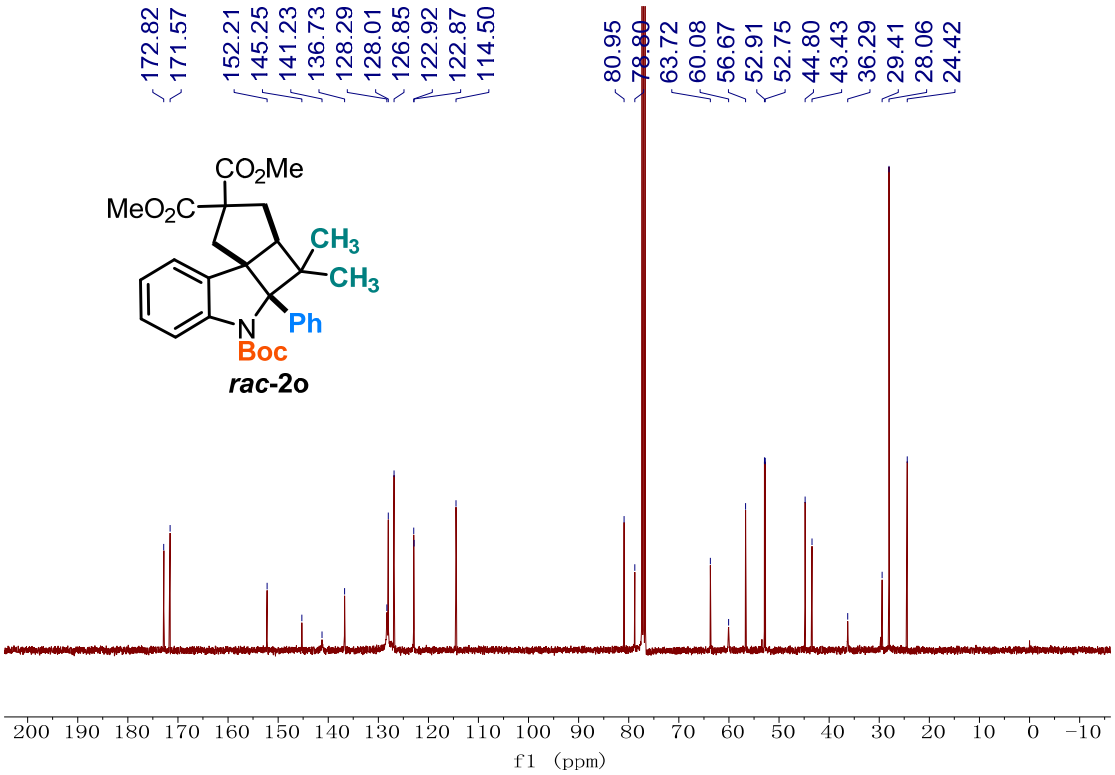
^{1}H & ^{13}C NMR spectra of ***rac*-2n**



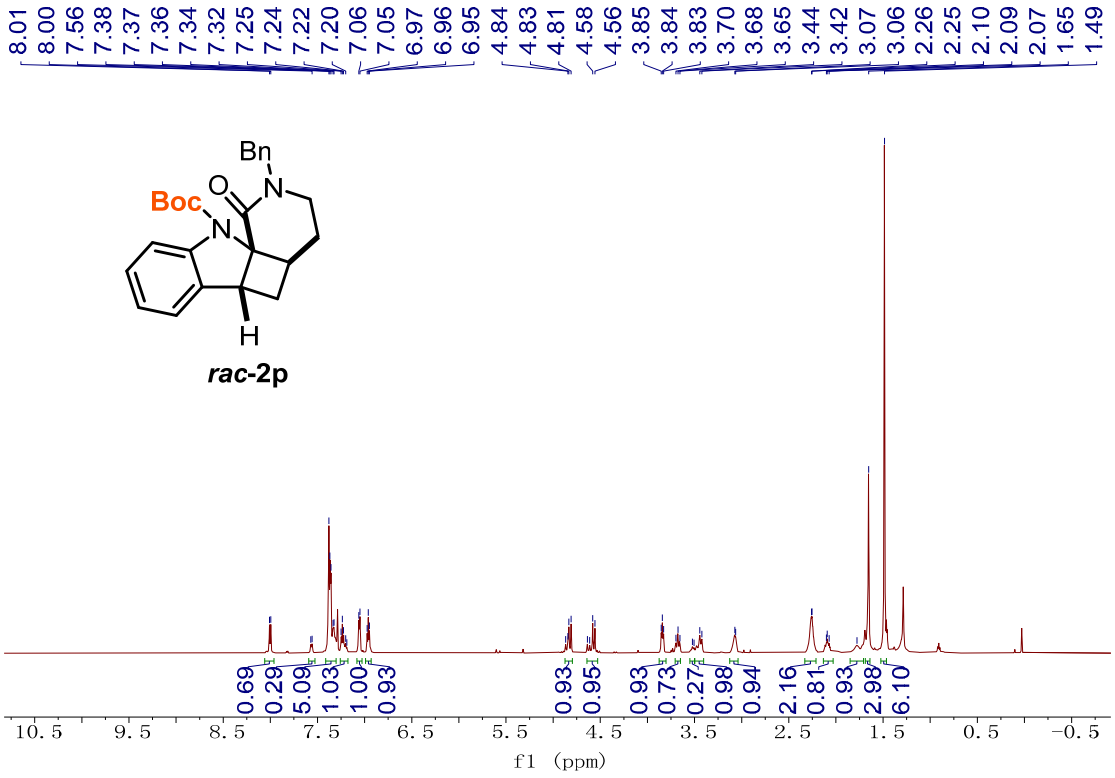


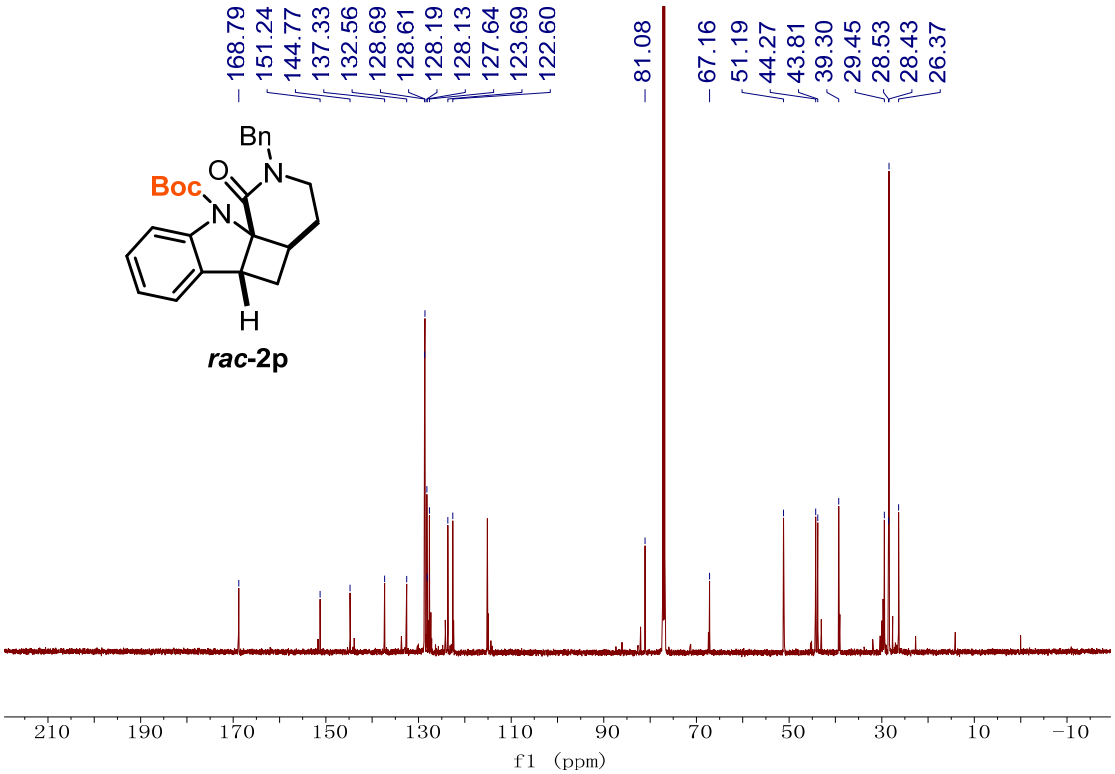
^{1}H & ^{13}C NMR spectra of ***rac*-2o**





^{1}H & ^{13}C NMR spectra of ***rac*-2p**





14. Supplementary references

[1] Nie, L., Lavinder, J. J., Sarka,r M. *et al*. Synthetic approach to stop-codon scanning mutagenesis. *J. Am. Chem. Soc.* **133**, 6177-86(2011).

[2] Joiner, C. M., Breen, M. E., Mapp, A. K. Electron‐deficient *p*‐benzoyl‐L‐phenylalanine derivatives increase covalent chemical capture yields for protein–protein interactions. *Protein Sci.* **28**, 1163-1170(2019).

[3] Bos, J. F., Fusetti, P., Driessen, J. M. *et al*. Enantioselective artificial metalloenzymes by creation of a novel active site at the protein dimer interface. *Angew. Chem. Int. Ed.* **51**, 7590-7593(2012).

[4] Minor, W. *et al*. HKL-3000: the integration of data reduction and structure solution - from diffraction images to an initial model in minutes. *Acta. Crystallogr. D* **62** 859-866(2006).

[5] Kabsch, W. *et al*. Integration, scaling, space-group assignment and post-refinement. *Acta. Crystallogr. D.* **66,** 44-133(2010).

[6] Collaborative Computational Project, N4. The *CCP4* suite: programs for protein crystallography. *Acta. Crystallogr. D Biol. Crystallogr.* **50**, 760-763(1994).

[7] Adams, P. D. *et al*. Phenix: building new software for automated crystallographic structure determination. *Acta. Crystallogr. D Biol. Crystallogr.* **58**, 1948-1954(2002).

[8] Emsley, P. *et al*. Features and development of Coot. *Acta. Crystallogr. D Biol. Crystallogr.* **66**, 486-501(2010).

[9] Zhu, M., Zheng, C., Zhang, X. *et al*. Synthesis of cyclobutane-fused angular tetracyclic spiroindolines via visible-light-promoted intramolecular dearomatization of indole derivatives. *J. Am. Chem. Soc.* **141,** 2636-2644(2019).

[10] Becke, A. D. *et al*. Density functional thermochemistry. III. The role of exact exchange. J. *Chem. Phys.* **98**, 5648-5652(1993).

[11] Grimme, S., Antony, J., Ehrlich, S., Krieg, H. A consistent and accurate *ab* initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. *J. Chem. Phys.* **132**, 104-154(2010).

[12]. Frisch, M. J., Trucks, G. W., Schlegel, H. B., Farkas, O., Foresman, J. B., Fox, D. J. *et al*. *Gaussian 16, Revision A.03*, Wallingford, CT(2016).

[13]. Zhao, Y., Truhlar, D. G. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc.* **120**, 215-241(2008).

[14]. Marenich, A. V., Cramer, C. J., Truhlar, D. G. Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. *J. Phys. Chem. B* **113**, 6378–6396(2009).

[15]. Lu, T., Chen, F. Multiwfn: A multifunctional wavefunction analyzer. *J. Comput. Chem.* **33**, 580-592(2012).