Supplementary Information, Data S1

Materials and Methods

Protein preparation

The preparation of full-length AtD14, MAX2 and ShHTL7 proteins are described previously [2, 3].

YLG hydrolysis assay

The preparation of YLG and *in vitro* YLG hydrolysis assays were performed by following reported procedures [6]. To determine the K_m and k_{cat} values, the YLG hydrolysis assays were performed by incubating 1 μg of recombinant proteins (AtD14 or ShHTL7) with YLG (0.1~10 μM). The fluorescent intensity was measured by EnSpire Multimode Plate Reader (PerkinElmer) at excitation by 480 nm and detection by 520 nm. The K_m and V_{max} values were calculated by fitting assay values (fluorescence intensity) with a Michaelis-Menten plot, while the k_{cat} values were calculated from formula $k_{cat} = V_{max} / [E]$. For inhibition assays, β-lactones at the range between 0.004 and 640 μM were co-incubated with 1 μM or 5 μM of YLG and 1 μg of recombinant proteins (AtD14 or ShHTL7). IC_{50} values were calculated by fitting competition assay values with a Dose-Response-Inhibition plot on GraphPad Prism (version 5.0).

LC-MS/MS analysis

Purified ShHTL7 (2 μM) was co-incubated with 10 μM TFQ0022 or TFQ0023 at 25˚C for 20 min, then the reaction mixture was subjected to SDS-PAGE. The gel bands of ShHTL7 were excised for trypsin digestion and applied to LC-MS/MS analysis as described previously [2]. Similar approach was performed using AtD14 except that AspN and trypsin double digestion was used for this protein.

Pull-down assay

In vitro pull-down assay was performed using 20 μg His_6-MAX2 as the bait and 12 μg GST-AtD14 as the prey in the absence or presence of indicated chemicals. The pull-down procedure is described previously [2].

Hypocotyl elongation analysis

The hypocotyl length of wild-type *Arabidopsis* Col-0 was measured for seedlings grown on Murashige and Skoog (MS) medium supplemented with indicated chemicals, under continuous low light at 22˚C for 6 days.

SL receptor labelling assay

The CaMV 35S promoter-driven *AtD14-Flag* overexpression transgenic plants were grinded in liquid nitrogen, followed by total protein extraction with extraction buffer (10 mM HEPES, 150 mM NaCl, pH 7.0). The receptor labelling and enrichment procedure was similar to that described by Weerapana et al. [16]. Briefly, the proteomes were incubated with TFQ0023 (10 μM or 20 μM) at room temperature for 1 h, after which proteins were denatured by heating at 95˚C for 5 min. The labeled proteomes were tagged by the click reaction of the terminal alkyne in TFQ0023 with an azide-biotin, which was then subjected to enrichment on magnetic streptavidin beads. The bound proteins were released by heating and subjected to separation by SDS-PAGE. The western blot was performed using anti-Flag as the first antibody. In competitive assays, 20 μM TFQ0022 and TFQ0023 were both added to the proteomes.

Chemical synthesis and characterization

1) General information

Reactions were monitored by Thin Layer Chromatography on plates (HSGF254) supplied by Yantai Chemicals (China). If not specially mentioned, flash column chromatography uses silica gel (200-300 mesh) supplied by Tsingtao Haiyang Chemicals (China).

NMR spectra were recorded on Brüker Advance 400 (^{1}H 400 MHz, ^{13}C 100 MHz). TMS was used as internal standard for ^{1}H NMR (0.00 ppm), and solvent signal was used as reference for ^{13}C NMR ($CDCl_3$, 77.0 ppm). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

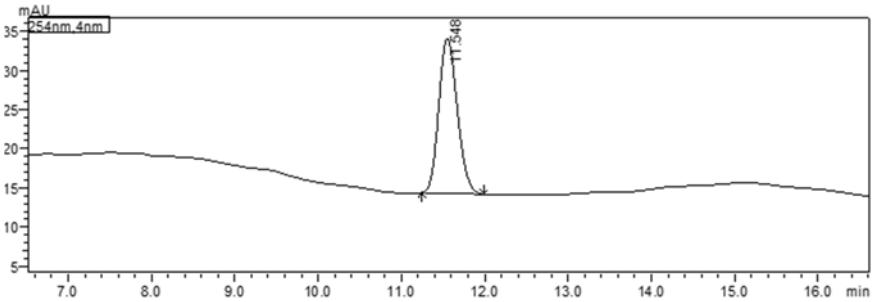
Mass spectrometric data were obtained using Brüker Apex IV FTMS using ESI (electrospray ionization) and Waters GCT (GC-MS) using EI (electron impact ionization). Infrared spectra were recorded on a Thermo Nicolet iS5 spectrometer. Optical rotations were measured on a Perkin-Elmer 341LC digital polarimeter with a sodium lamp at ambient temperature and are reported as follows: $[\alpha]_{\lambda}$ (c g/100 mL).

Analytical high performance liquid chromatography (HPLC) was performed on a Shimadzu@ Modular HPLC equipped with a variable wavelength UV detector (deuterium lamp, 190-700 nm) using a Daicel ChiracelTM OD-H column (4.6 mm×250 mm) or Daicel ChiralpakTM AD column (4.6 mm×250 mm) and HPLC-grade isopropanol and hexanes as the eluting solvents.

Unless otherwise mentioned, all reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions. All the chemicals were purchased commercially and used without further purification. O-trimethylsilylquinidine (TMSQD), O-trimethylsilylquinine (TMSQN) and O-methylquinine (MeQN) were prepared according to the literature procedure [17, 18].

2) General procedure A for asymmetric [2+2] cycloadditions: To a solution of TMSQD or TMSQN (0.1 mmol) and $LiClO_4$ (0.5 mmol) in 1.0 mL of diethyl ether was added 2.0 mL of CH_2Cl_2 and the reaction mixture was cooled to −78˚C. To the resulting mixture was added 0.41 mL of *N*, *N*-diisopropylethylamine (2.5 mmol) followed by the aldehyde (1.0 mmol). A solution of 2.0 mmol of acyl chloride in 0.5 mL of CH_2Cl_2 was then added within 2 h by syringe pump. The reaction mixture was stirred for 7 h then was quenched at the reaction temperature by adding 10 mL of Et_2O and the resulting mixture was filtered through silica gel eluting with Et_2O (3×20 mL). The filtrate was concentrated *in vacuo* and the crude product mixture was purified by flash chromatography [11].

(3R, 4S)-3-Methyl-4-phenethyloxetan-2-one (TFQ0010):
The general procedure A was followed employing 40 mg of TMS-QN (0.1 mmol), 53 mg $LiClO_4$ (0.5 mmol) and 132 µL of phenylpropyl aldehyde (1.0 mmol). Purification by flash chromatography (EtOAc/PE = 1/20) gave 92 mg (48%) of the title compound as a colorless oil. $[\alpha]_D^{25}$ = –47.9 (c = 0.87, $CHCl_3$); ^{1}H NMR (400 MHz, Chloroform-d) δ 7.31 (t, J = 7.4 Hz, 2H), 7.26 – 7.16 (m, 3H), 4.56 (ddd, J = 10.0, 6.4, 4.0 Hz, 1H), 3.74 (p, J = 7.7 Hz, 1H), 2.87 (ddd, J = 14.2, 9.4, 5.2 Hz, 1H), 2.70 (dt, J = 13.8, 8.2 Hz, 1H), 2.08 (dtd, J = 14.4, 9.4, 5.2 Hz, 1H), 1.96 (dddd, J = 13.9, 9.4, 7.4, 4.0 Hz, 1H), 1.26 (d, J = 7.8 Hz, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ 172.53, 140.42, 128.66, 128.50, 126.40, 74.65, 47.24, 31.99, 31.56, 8.15; IR (neat, cm^{-1}): 3027, 2940, 1812, 1454, 839, 698; HRMS-ESI calc. for $C_{12}H_{14}O_2Na$ [M + Na^+]: 213.088600; Found: 213.088172.
Separation of enantiomers by Chiral HPLC [Daicel Chiracel™ OD-H column, 4.6mm× 250 mm, flow rate 1.0 ml/min, 5% ^{i}PrOH, 95% hexane T_r: 11.548 min (3R, 4S) provided only one enantiomer (≥ 99% ee).



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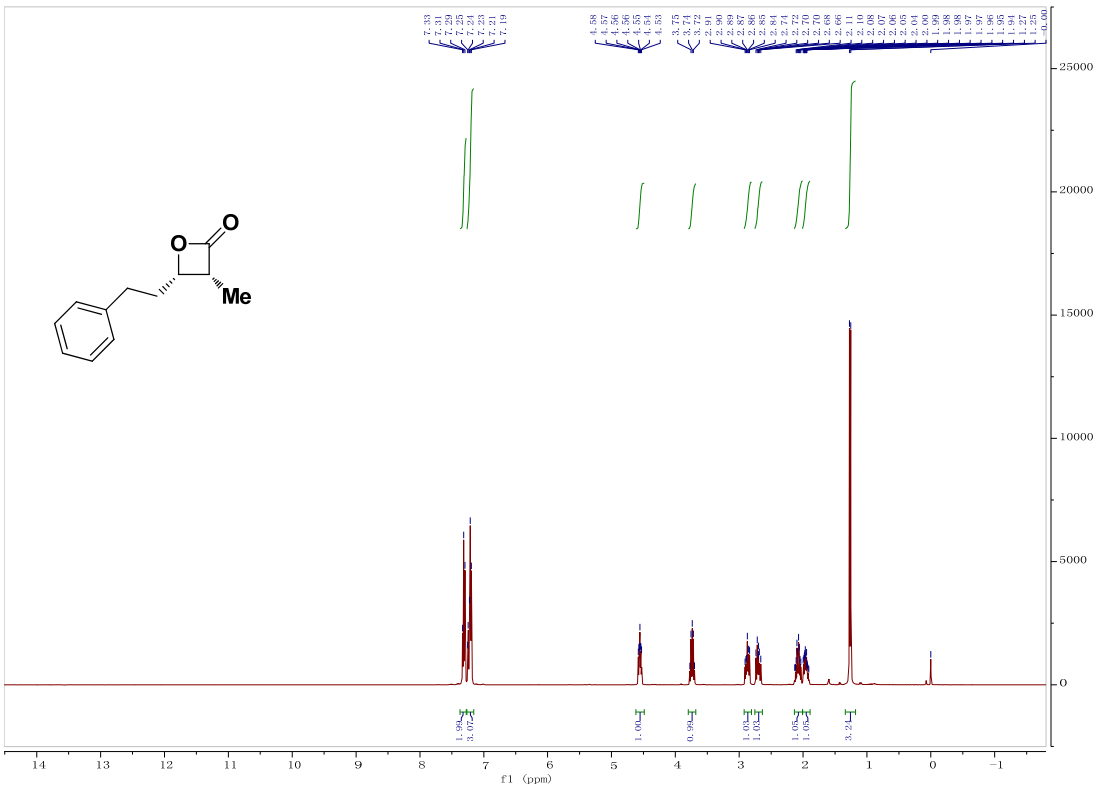
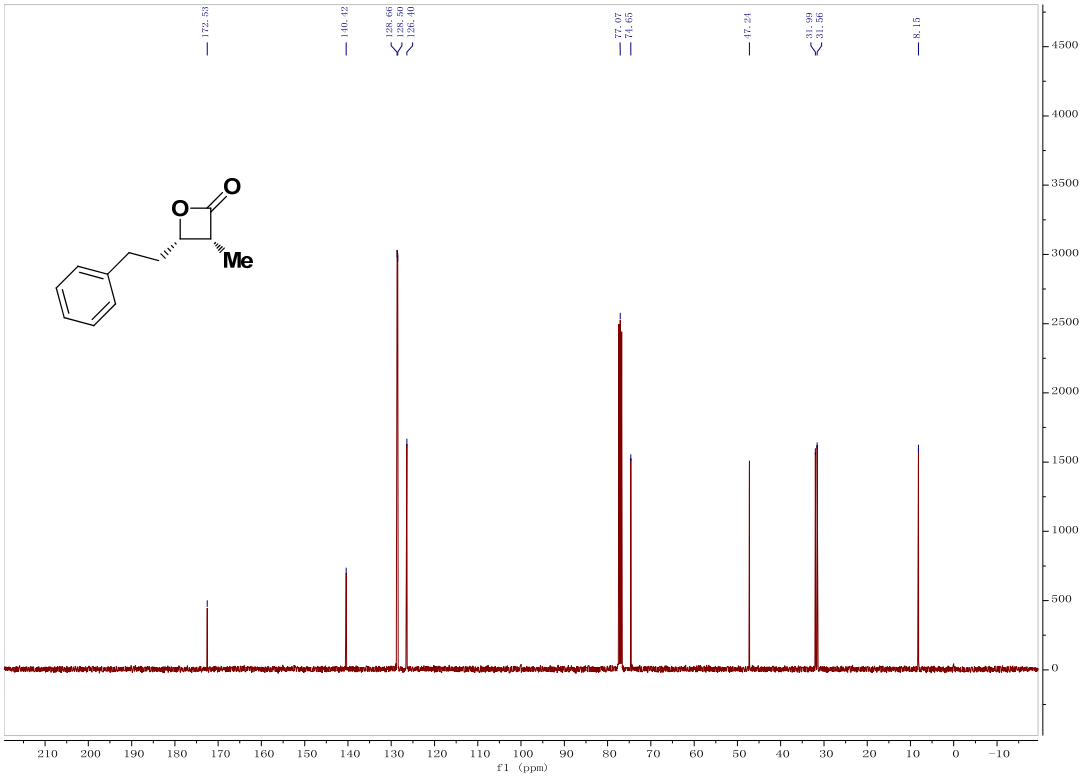


^{1}H NMR of compound TFQ0010

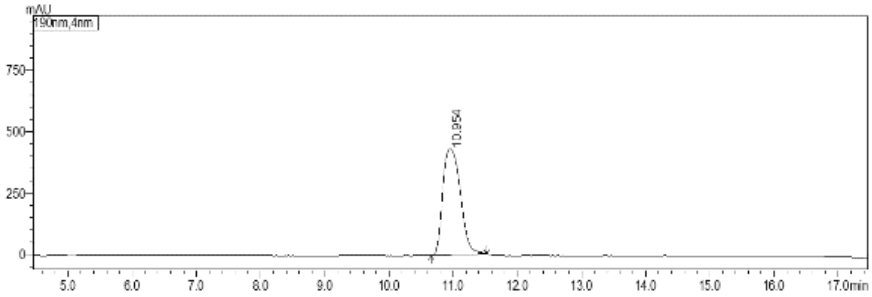


^{13}C NMR of compound TFQ0010

(3S, 4R)-3-Methyl-4-phenethyloxetan-2-one (TFQ0011):

The general procedure A was followed employing 40 mg of TMS-QD (0.1 mmol), 53 mg $LiClO_4$ (0.5 mmol) and 132 μL of phenylpropyl aldehyde (1.0 mmol). Purification by flash chromatography (EtOAc/PE = 1/20) gave 107 mg (56%) of the title compound as a colorless oil. $[\alpha]_D^{25}$ = +43.7 (c = 0.90, $CHCl_3$); ^1H NMR (400 MHz, Chloroform-d) δ 7.31 (t, J = 7.3 Hz, 2H), 7.23 (dt, J = 12.5, 5.4 Hz, 3H), 4.56 (ddd, J = 10.0, 6.4, 4.0 Hz, 1H), 3.74 (p, J = 7.7 Hz, 1H), 2.87 (ddd, J = 14.2, 9.4, 5.2 Hz, 1H), 2.70 (dt, J = 13.8, 8.2 Hz, 1H), 2.08 (dtd, J = 14.4, 9.4, 5.2 Hz, 1H), 1.96 (dddd, J = 13.9, 9.5, 7.4, 4.0 Hz, 1H), 1.26 (d, J = 7.8 Hz, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ 172.53, 140.42, 128.66, 128.50, 126.40, 74.65, 47.24, 31.99, 31.56, 8.15; IR (neat, cm^{-1}): 3027, 2940, 1812, 1454, 840, 700; HRMS-ESI calc. for $C_{12}H_{14}O_2Na$ [M + Na^+]: 213.088600; Found: 213.088203.

Separation of enantiomers by Chiral HPLC [Daicel Chiracel™ OD-H column, 4.6 mm×250 mm, flow rate 1.0 ml/min, 5% ^{i}PrOH, 95% hexane T_r: 10.954 min (3S, 4R) provided only one enantiomer (≥ 99% ee).



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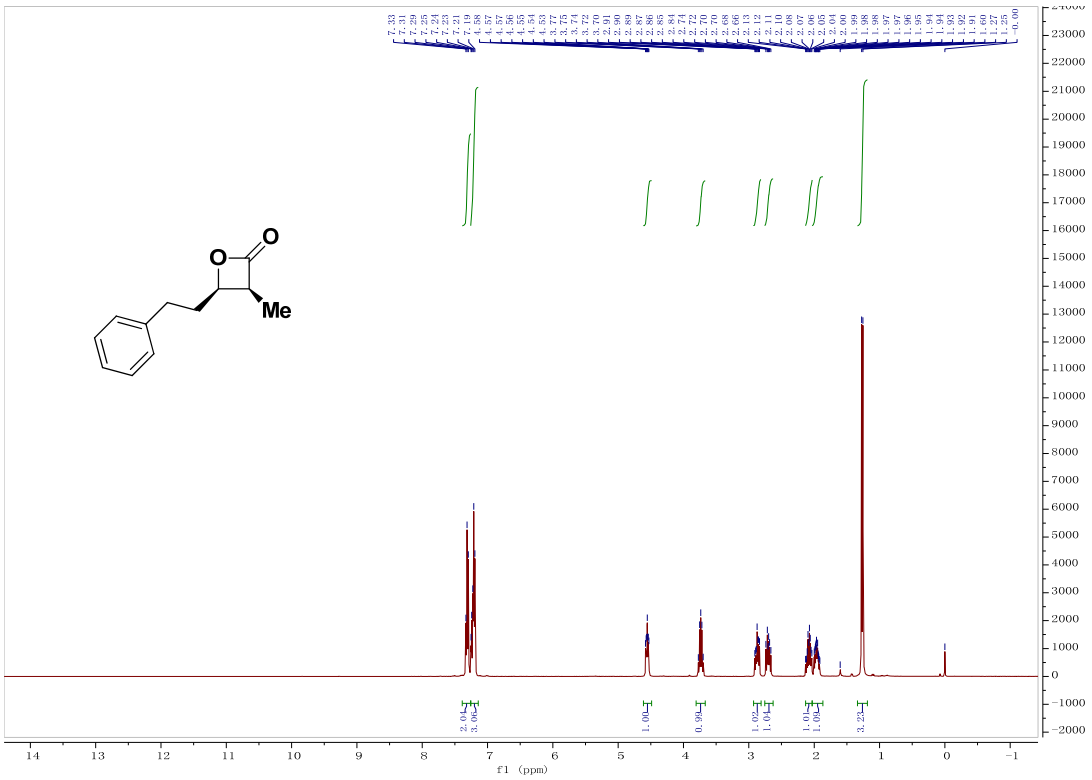
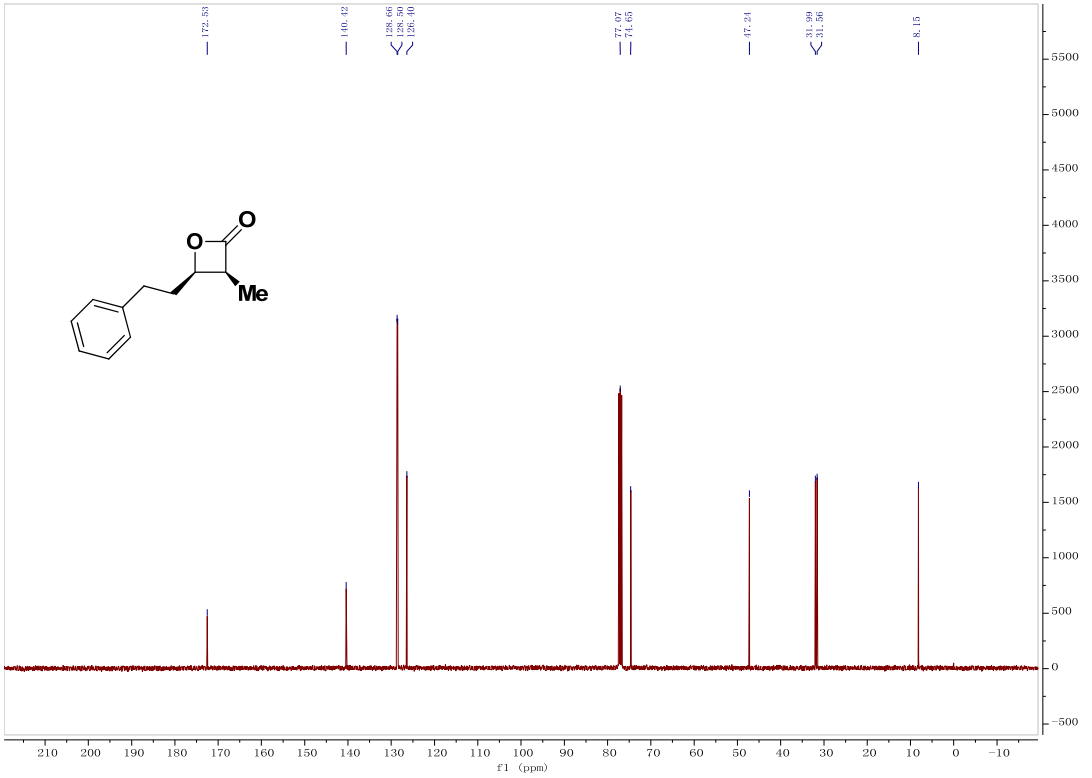


^{1}H NMR of compound TFQ0011

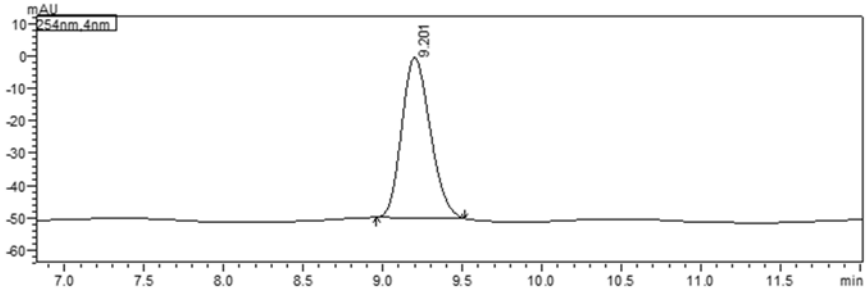


^{13}C NMR of compound TFQ0011

(3R, 4R)-3-Methyl-4-phenyloxetan-2-one (TFQ0020):

The general procedure A was followed employing 40 mg of TMS-QN (0.1 mmol), 53 mg $LiClO_4$ (0.5 mmol) and 102 μL of benzaldehyde (1.0 mmol). Purification by flash chromatography (EtOAc/PE = 1/20) gave 72 mg (44%) of the title compound as a colorless oil. $[\alpha]_D^{25}$ = +145.9 (c = 0.63, $CHCl_3$); 1H NMR (400 MHz, Chloroform-d) δ 7.40 (dq, J = 14.3, 7.1 Hz, 3H), 7.30 (d, J = 7.2 Hz, 2H), 5.65 (d, J = 6.5 Hz, 1H), 4.05 (p, J = 7.6 Hz, 1H), 0.93 (d, J = 7.8 Hz, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ 172.20, 134.64, 128.67, 128.64, 125.70, 75.30, 50.22, 9.61; IR (neat, cm^{-1}):2979, 1820, 1455, 1142, 940, 731; HRMS-EI calc. for $C_{10}H_{10}O_2$ [M^+]: 162.06753; Found: 162.06722.

Separation of the enantiomers by chiral HPLC [Daicel Chiracel™ OD-H column, 4.6 mm×250 mm, flow rate 1.0 ml/min, 3% ^iPrOH, 97% hexane, T_r 9.201 min (3R, 4R) provided only one enantiomer (≥ 99% ee).



Peak	Ret. Time	Area	Area%
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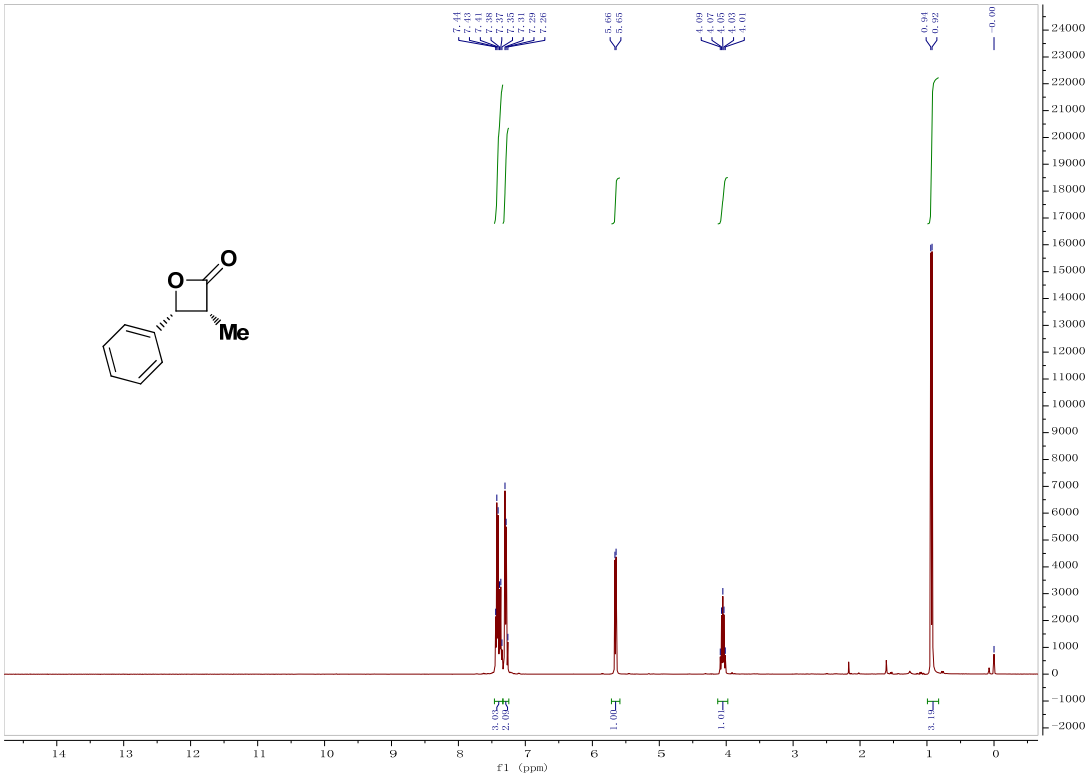
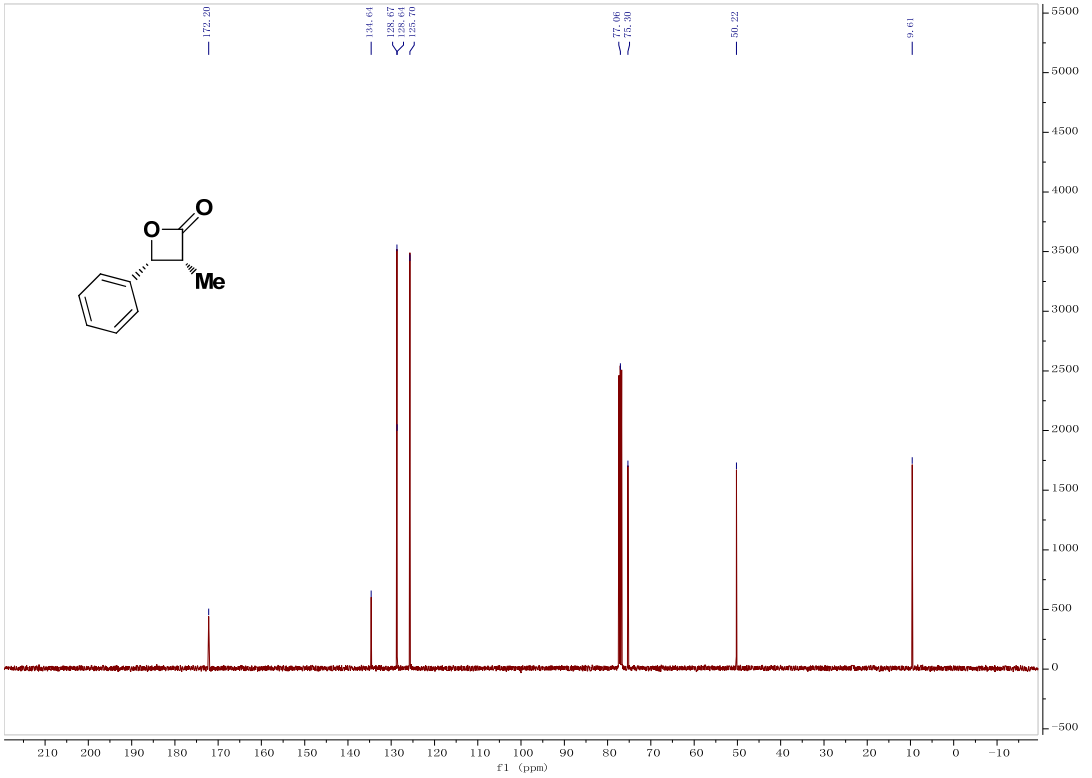
total		616481	100



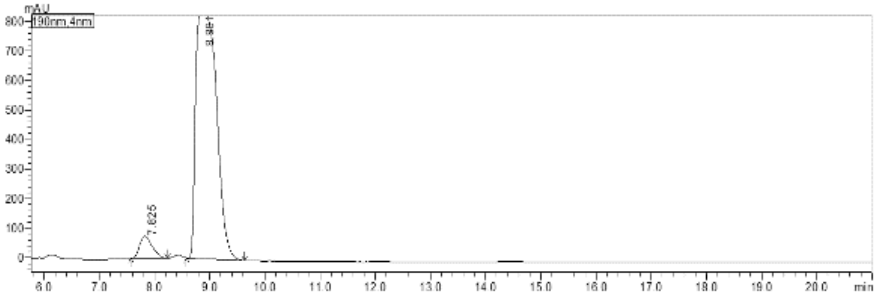
^{1}H NMR of compound TFQ0020



^{13}C NMR of compound TFQ0020

3) General procedure B for asymmetric [2+2] cycloadditions: To a solution of Me-QN (0.1 mmol) and LiI (1.0 mmol) in 0.25 mL of diethyl ether at ambient temperature was slowly added 2.0 mL of CH_2Cl_2. The resulting mixture was cooled to −78˚C and 0.41 mL of *N*, *N*-diisopropylethylamine (2.5 mmol) was added, and then aldehyde (1.0 mmol) was added dropwise. Afterwards a solution of acyl chloride (2.0 mmol) in 0.5 mL CH_2Cl_2 was added over 2 h by syringe pump (the tip of syringe was put into the reaction mixture). The reaction mixture was stirred for 7 h at −78˚C. The reaction was quenched at −78˚C by adding 10 mL of Et_2O and the resulting mixture was filtered through silica gel eluting with 3×20 mL of Et_2O. The filtrate was concentrated in vacuo and the crude product mixture was purified by flash chromatography [19].

(3R, 4S)-3-Ethyl-4-phenethyloxetan-2-one (TFQ0021):
The general procedure B was followed employing 34 mg of Me-QN (0.1 mmol), 134 mg LiI (1.0 mmol) and 132 μL of phenylpropyl aldehyde (1.0 mmol). Purification by flash chromatography (EtOAc/PE = 1/25) gave 16 mg (10%) of the title compound as a colorless oil. $[\alpha]_D^{25}$ = −52.5 (c = 0.33, $CHCl_3$); ^{1}H NMR (400 MHz, Chloroform-d) δ 7.32 (t, J = 7.4 Hz, 2H), 7.22 (dd, J = 11.7, 7.6 Hz, 2H), 4.55 (ddd, J = 10.1, 6.4, 3.4 Hz, 1H), 3.60 – 3.49 (m, 1H), 2.90 (ddd, J = 14.2, 9.5, 5.0 Hz, 1H), 2.77 – 2.65 (m, 1H), 2.09 (ddt, J = 14.3, 9.6, 4.8 Hz, 1H), 1.97 (dddd, J = 13.9, 10.3, 7.5, 3.4 Hz, 1H), 1.84 (ddd, J = 13.7, 8.7, 7.2 Hz, 1H), 1.67 (dq, J = 14.3, 7.4 Hz, 1H), 1.06 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ 171.93, 140.47, 128.63, 128.51, 126.35, 74.57, 54.18, 32.08, 31.62, 17.48, 12.21; IR (neat, cm^{-1}): 2969, 1816, 1454, 1133, 909, 731; HRMS-ESI calc. for $C_{13}H_{16}O_2Na$ [M + Na^+]: 227.104250; Found: 227.103855.
Separation of the enantiomers by chiral HPLC (Daicel Chirapak™ AD-H column, 4.6mm×250mm, flow rate 0.7 ml/min, 10% ^{i}PrOH, 90% hexane, T_r 7.825 (3S, 4R) and 8.881 min (3R, 4S) provided the enantiomer ratio: (3S, 4R):(3R, 4S) = 5.2:94.8 (90% ee).



Peak	Ret. Time	Area	Area%
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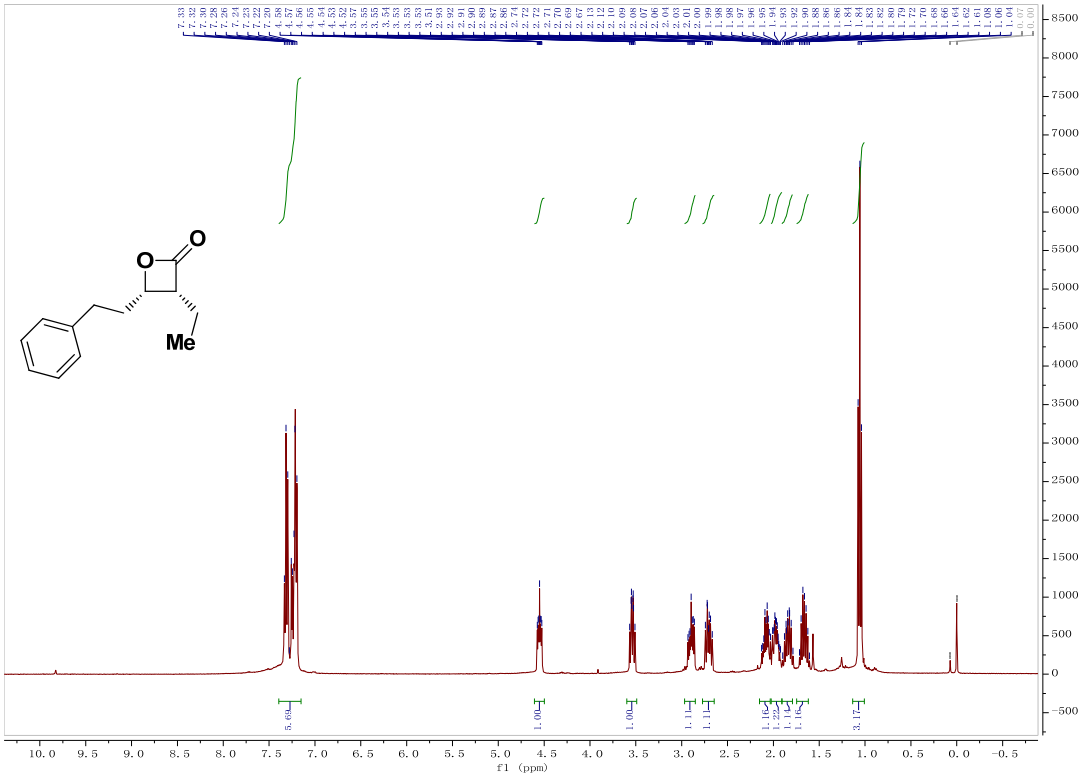
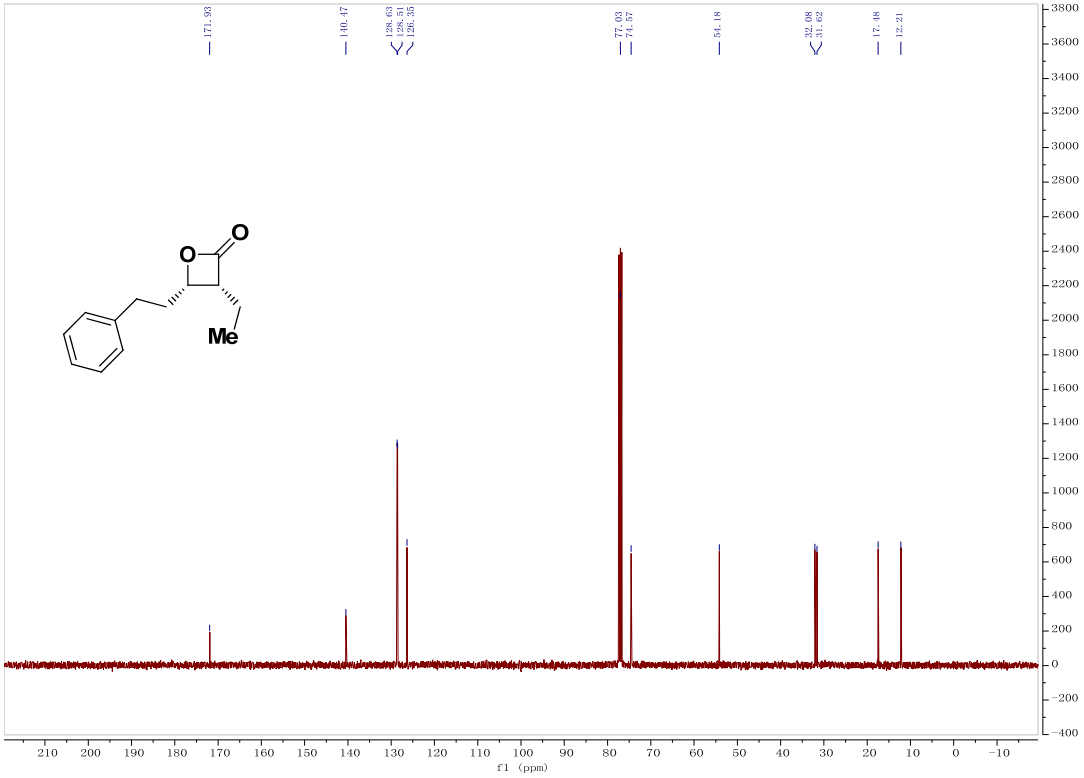
2	8.881 min	21931933	94.8
total		23127233	100



^{1}H NMR of compound TFQ0021

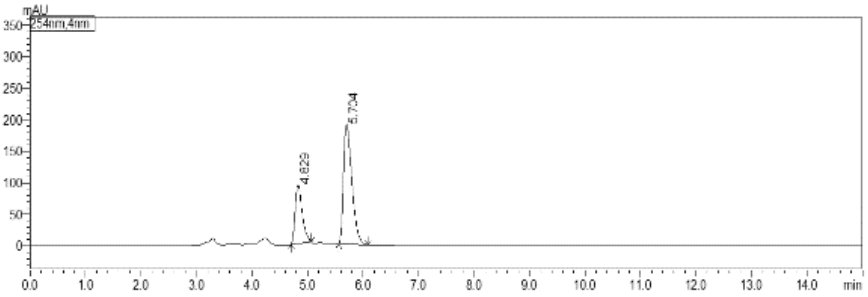


^{13}C NMR of compound TFQ0021

(3R, 4S)- 4-Phenethyl-3-propyloxetan-2-one (TFQ0022):

The general procedure B was followed employing 34 mg of Me-QN (0.1 mmol), 134 mg LiI (1.0 mmol) and 132 μL of phenylpropyl aldehyde (1.0 mmol). Purification by flash chromatography (EtOAc/PE = 1/25) gave 41 mg (17%) of the title compound as a colorless oil. $[\alpha]_D^{25} = -37.7$ (c = 0.93, $CHCl_3$); ^1H NMR (400 MHz, Chloroform-d) δ 7.30 (q, J = 7.3 Hz, 2H), 7.23 (dt, J = 11.7, 5.3 Hz, 3H), 4.54 (ddd, J = 10.1, 6.4, 3.4 Hz, 1H), 3.68 – 3.54 (m, 1H), 2.89 (ddd, J = 14.1, 9.5, 5.0 Hz, 1H), 2.70 (dt, J = 13.8, 8.2 Hz, 1H), 2.07 (dtd, J = 14.3, 9.6, 5.0 Hz, 1H), 1.95 (dddd, J = 14.0, 10.6, 7.5, 3.4 Hz, 1H), 1.76 (ddt, J = 11.5, 7.7, 3.5 Hz, 1H), 1.64 – 1.46 (m, 2H), 1.46 – 1.31 (m, 1H), 0.93 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, Chloroform-d) δ 172.10, 140.52, 128.65, 128.53, 126.37, 74.56, 52.41, 32.24, 31.65, 26.01, 20.90, 13.86; IR (neat, cm^{-1}): 2960, 1813, 1454, 1134, 907, 729; HRMS-ESI calc. for $C_{14}H_{18}O_2Na$ [M + Na^+]: 241.119901; Found: 241.119937.

Separation of the enantiomers by chiral HPLC (Daicel Chirapak™ AD-H column, 4.6 mm×250 mm, flow rate 0.7 ml/min, 10% ^{i}PrOH, 90% hexane, T_r 4.829 (3S, 4R) and 5.704 min (3R, 4S) provided the enantiomer ratio: (3S, 4R):(3R, 4S) = 29.2:70.8 (42% ee).



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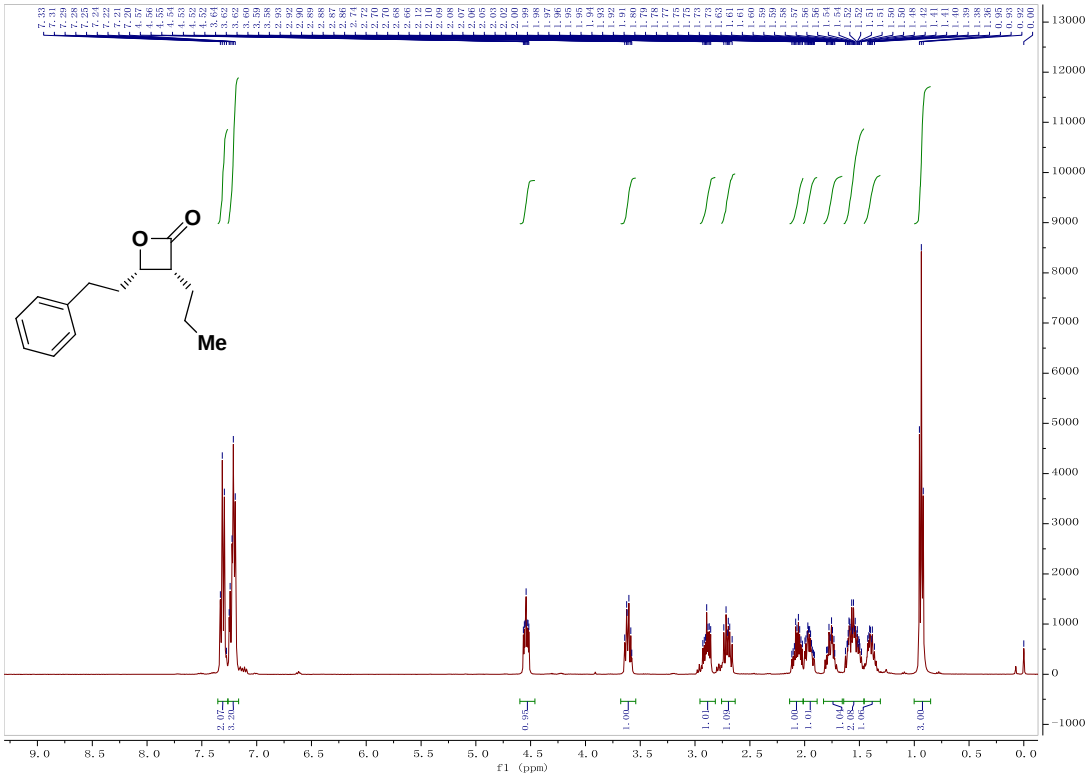
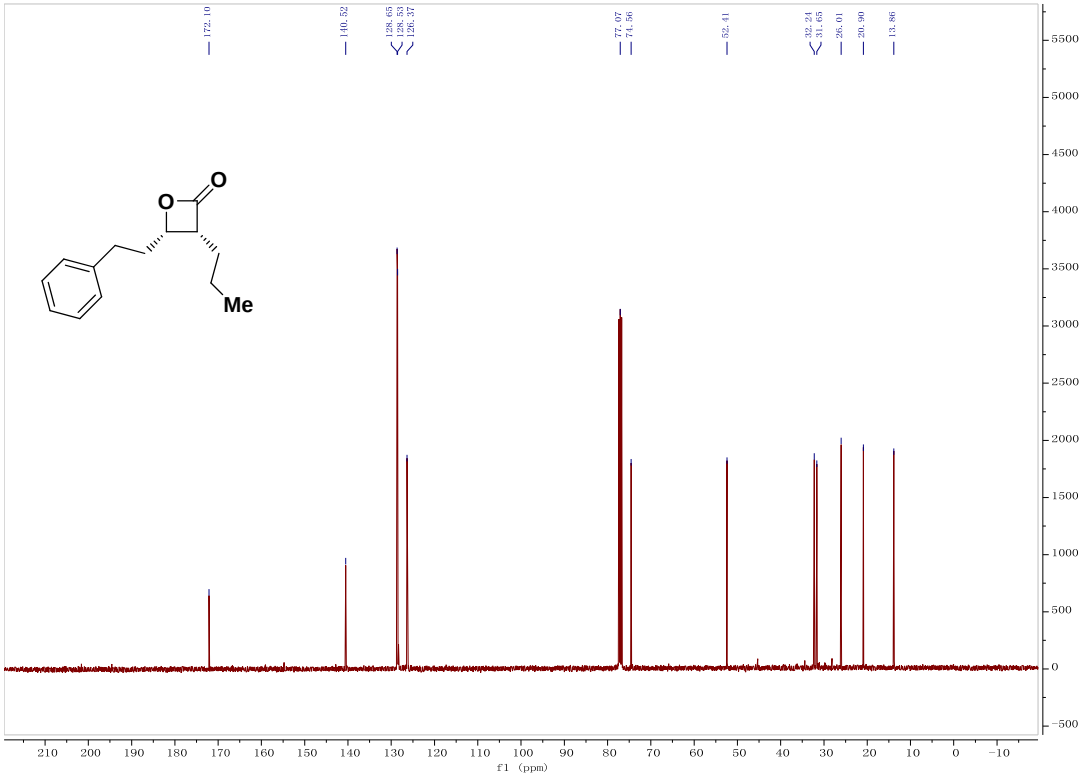
total		2814945	100



^{1}H NMR of compound TFQ0022



^{13}C NMR of compound TFQ0022

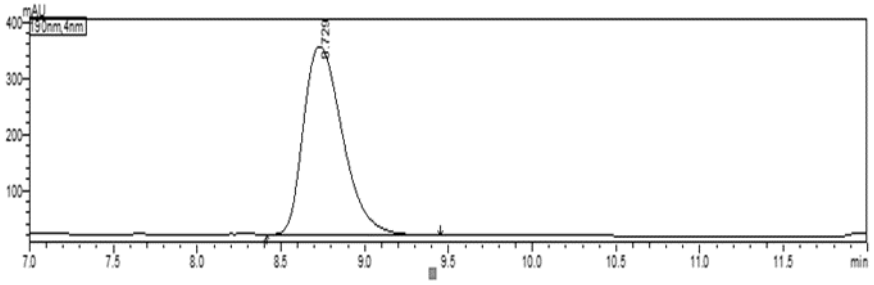
4) Synthesis of 4-pentynoic chloride

To a dry vial with a stirring bar was added 250 mg (2.55 mmol, 1.0 equiv.) 4-pentynoic acid and 8 mL of CH_2Cl_2, followed by 254 uL (3.06 mmol, 1.2 equiv) oxalyl chloride. The solution was allowed to stir for 4 hours at room temperature and then was refluxed for 3 h. The solution was directly evaporated in vacuo and revealed a pungent clear, faintly yellow oil as the desired product (110 mg, 37% yield), which was put into next step immediately without further purification.

(3R, 4S)-4-phenethyl-3-(prop-2-yn-1-yl) oxetan-2-one (TFQ0023):

The general procedure B was followed employing 17 mg of Me-QN (0.05 mmol), 67 mg LiI (0.5 mmol), 66 μL of phenylpropyl aldehyde (0.5 mmol) and 110 mg of 4-pentynoic chloride. Purification by flash chromatography (EtOAc/PE = 1/25) gave 35 mg (33%) of the title compound as a colorless oil. $[\alpha]_D^{25}$ = −103.9 (c = 0.37, $CHCl_3$); ^{1}H NMR (400 MHz, Chloroform-d) δ 7.32 (t, J = 7.4 Hz, 2H), 7.23 (t, J = 8.9 Hz, 3H), 4.63 (ddd, J = 10.0, 6.3, 3.9 Hz, 1H), 3.88 (dt, J = 11.9, 6.0 Hz, 1H), 2.93 (ddd, J = 14.1, 9.0, 5.4 Hz, 1H), 2.82 – 2.71 (m, 1H), 2.71 – 2.52 (m, 2H), 2.17 (dddt, J = 18.7, 14.3, 9.3, 5.2 Hz, 2H), 2.06 (t, J = 2.5 Hz, 1H); ^{13}C NMR (125 MHz, Chloroform-d) δ 168.43, 139.18, 127.63, 127.49, 125.39, 73.45, 69.87, 50.25, 30.45, 12.75; IR (neat, cm^{-1}): 3304, 3028, 2947, 1820, 1454, 1135, 908, 730; HRMS-ESI calc. for $C_{14}H_{15}O_2$ [M + H^+]: 215.10666; Found: 215.10670.

Separation of the enantiomers by chiral HPLC (Daicel Chirapak™ AD-H column, flow rate 0.7 ml/min, 10% ^{i}PrOH, 90% hexane, T_r 8.7 min (3R, 4S) provided only one enantiomer (≥ 99% ee).



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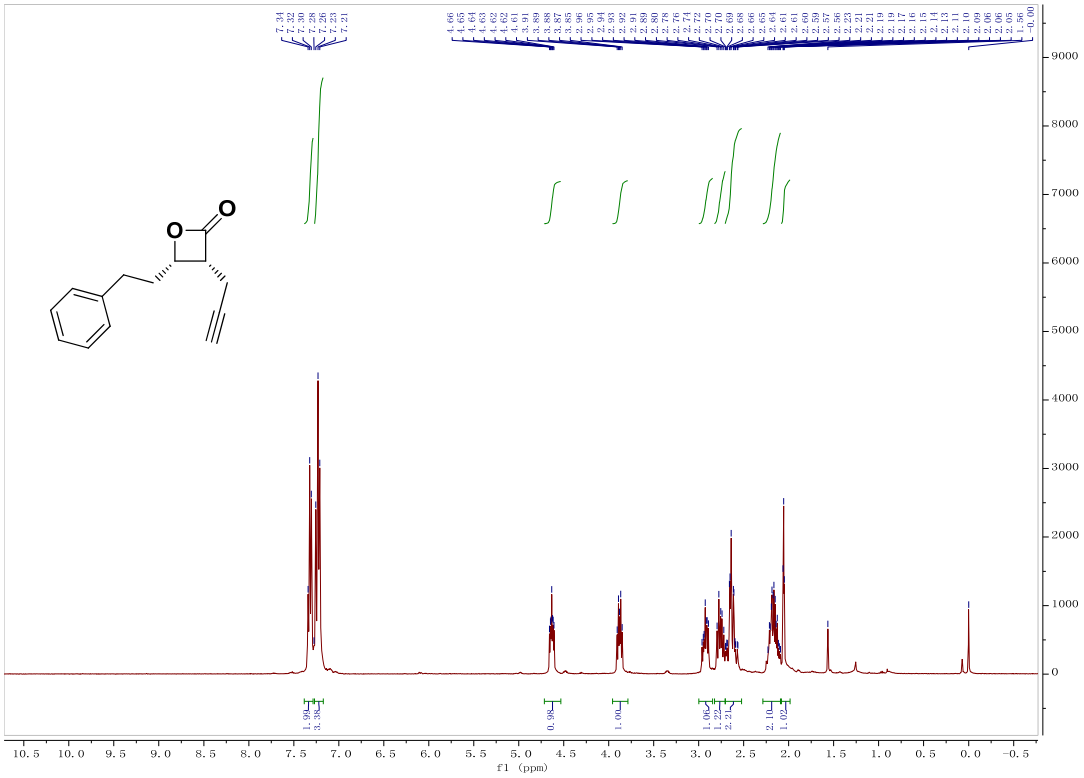
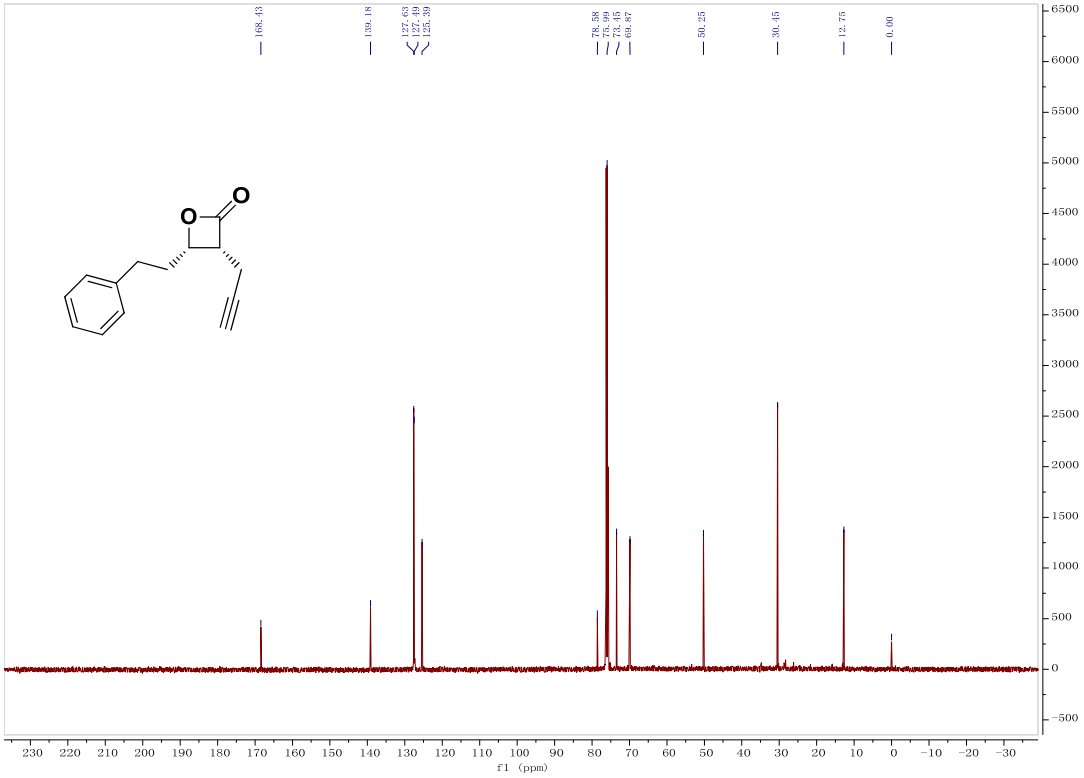


^{1}H NMR of compound TFQ0023



^{13}C NMR of compound TFQ0023

Supplementary References

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