Supporting Information

Biocatalytic Asymmetric Ring-opening of Dihydroisoxazoles: A Cyanide-free Route to Complementary Enantiomers of β-Hydroxy nitriles from Olefins

Daijun Zheng[1,2], Yasuhisa Asano[1,2]

[1] Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180, Imizu, Toyama 939-0398, Japan

[2] Asano Active Enzyme Molecular Project, ERATO, JST, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan

Correspondence and requests for materials should be addressed to Y. A. (asano@pu-toyama.ac.jp) (Tel: +81-766-56-7500

Table of Contents

1. General remarks

Reagents were purchased from commercial sources and were directly used unless otherwise noted. The reaction process was monitored by TLC. ^{1}H NMR and ^{13}C NMR (400 and 100 MHz, respectively) spectra were recorded in $CDCl_3$ and DMSO-d_6. ^{1}H NMR chemical shifts are reported in ppm relative to tetramethylsilane (TMS) with the solvent resonance employed as the internal standard ($CDCl_3$ at 7.26 ppm, DMSO-d_6 at 2.50 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet and m = multiplet), coupling constants (Hz) and integration. ^{13}C NMR chemical shifts are reported in ppm relative to tetramethylsilane (TMS) with the solvent resonance as the internal standard ($CDCl_3$ at 77.16 ppm, DMSO-d_6 at 39.51 ppm). Column chromatography was performed with Merck silica gel 60 (200-300 mesh) as column material. TLC analysis was performed with precoated Silica Gel 60 F_{254} TLC-plate and the compounds were visualized with UV light.

2. Aldoxime dehydratases recombinant construction and enzyme purification

2.1 Construction of Oxd A recombinant and its expression, purfication

The Oxd A gene was amplified from previous recombinant plasmid [1] using forward primer 5′-GTGCCGCGCGGCAGC**CATATG**GAAAGCGCAATTGATAC-3′ with restriction site of *Nde* I (bold font) and reverse primer 5′-TCGACGGAGCTC**GAATTC**TCAGGTCGGTGCAACAAC-3′ with restriction site of *EcoR* I (bold font). The resulting PCR prodcut was purified and ligated with *Nde* I/*EcoR* I-digested pET-28b vector. The ligating mixture was transformed into *E.coli* JM109(DE3) competent cell via thermal shock. Positive transformants were selected on LB agar plates containing 50 μg/mL kanamycin by incubating at 37 °C overnight. New plasmid DNA (pET-28-N-His-OxdA) was isolated and sequenced to verify that no mutation had been introduced during gene amplification. For enzyme production, the positive colony was picked up and inoculated into 5 mL LB medium containing 50 μg/mL kanamycin, which was incubated at 37 °C overnight. Then 5 mL pre-culture was transferred into 1.4 L TB medium containing 50 μg/mL kanamycin, the resulting mixture was incubated at 30 °C for 12 h, then final concentration of 1 mM IPTG was added to induce the expression of target protein at 26 °C for 24 h. The recombiant cells were harvested by centrifugation

(6000 ×g, 15 min, 4 °C) and the pellet was resuspended into lysis buffer (20 mM Tris-HCl, 300 mM NaCl, 20 mM imidazole, pH 8.0), which was applied to sonication (ice-bath, 15 min) for cell disruption. The debris was removed by centrifugation (20,000 × g, 30 min, 4 °C) and the supernatant was loaded onto Ni Sepharose™ 6 Fast flow column equilibrated by lysis buffer (20 bed volumes). The unbinded protein was washed with lysis buffer (30 bed volumes) and the absorbed protein was eluted by elution buffer (20 mM Tris-HCl, 300 mM NaCl, 300 mM imidazole, pH 8.0), the fractions were analyzed by SDS-PAGE containing 12% acrylamide and those fractions containing Oxd A were pooled. The combined fractions were dialyzed against KPB (10 mM, pH 7.0, 4 °C, 5 L×2). The partially purified enzyme was used for reaction.

2.2 Oxd B and Oxd RE cultivation and purfication

The recombinant of $BL_{21}(DE_3)$-PET-22b-His-Oxd-B and $BL_{21}(DE_3)$-PET-22b-His-Oxd RE constructed previously [1] were used directly for enzyme production. The positive colony was picked up and inoculated into 5 mL LB medium containing 100 μg/mL ampicillin, which was incubated at 37 °C overnight. Then 5 mL pre-culture was transferred into 1.4 L TB medium containing 100 μg/mL ampicillin, the resulting mixture was incubated at 30 °C for 12 h, then final concentration of 1 mM IPTG was added to induce the expression of target protein at 26 °C for 24 h. The recombiant cells were harvested by centrifugation (6000 ×g, 15 min, 4 °C) and the pellet was resuspended into lysis buffer (20 mM Tris-HCl, 300 mM NaCl, 20 mM imidazole, pH 8.0), which was applied to sonication (ice-bath, 15 min) for cell disruption. The debris was removed by centrifugation (20,000 × g, 30 min, 4 °C) and the supernatant was loaded onto Ni Sepharose™ 6 Fast flow column equilibrated by lysis buffer (20 bed volumes). The unbinded protein was washed with lysis buffer (30 bed volumes) and the absorbed protein was eluted by elution buffer (20 mM Tris-HCl, 300 mM NaCl, 300 mM imidazole, pH 8.0), the fractions were analyzed by SDS-PAGE containing 12% acrylamide and those fractions containing Oxds were pooled. The combined fractions were dialyzed against KPB (10 mM, pH 7.0, 4 °C, 5 L×2). The partially purified enzyme was used for reaction. For kinetic parameter assay, a further purification of the partially purified enzyme from Ni Sepharose™ 6 Fast flow column was performed by loading it onto the ÄKTA Protein Purification System equipped with Mono-Q 5/50 GL column, which was equilibrated by low-salt buffer (20 mM KPB, pH 7.0, 20 bed volumes). After loading the sample, the unbounded protein was washed by low-salt buffer (20 bed volumes), followed to elute the targe protein by inreasing the ratio of high-salt buffer (20 mM KPB, pH 7.0, 500 mM NaCl) to 20% in 20 bed volumes and kept at 20% high-salt buffer to elute for additional 20 bed volumes. The purity of the fractions were checked by SDS-PAGE containing 12% acrylamide, and pure fractions were pooled to dialyzed against KPB (20 mM, pH 7.0, 5L ×2).

3. Enzyme activity assay

Oxd A activity assay [2]**:** the aldoxime dehydration activity was measured with appropriate amount of enzyme in 500 μL mixture of 5 mM butyraldoxime, 5 mM $Na_2S_2O_4$ in 100 mM KPB (pH 7.0), which was incubated at 30 °C for 5 min. Then 1 mL methanol containing 3 mM isobutyrnitrile (internal standard substance) was added to stop the reaction, the resulting mixture was centrifuged at 20000×g for 10 min, 1 mL supernatant was used for Gas Chromatograph analysis (Shimadazu Gas Chromatograph equipped with Agilent J&W DB-WAX column, carrier gas: H_2/He = 1/1, pressure: 3 kg/cm^2, temperature program: 80 °C-5 min, 10 °C/min to 150 °C, 150 °C-2 min). 1 U enzyme was defined as the amount of enzyme to produce 1 μmol product in 1 min.

Oxd B activity assay [3]**:** the aldoxime dehydration activity was measured with appropriate amount of enzyme in 500 μL mixture of 10 mM *Z*-phenylacetoxime, 1 mM $Na_2S_2O_5$, 0.25 mM FMN, in 100 mM KPB (pH 7.0), which was incubated at 30 °C for 5 min. Then 1 mL ethyl acetate containing 2 mM benzonitrile (internal standard substance) was added to stop the reaction, the resulting mixture was centrifuged at 13,000×g for 1 min, 500 μL upper organic solvent was mixed with 500 μL *iso*-propanol and applied for High Performance Liquid Chromatography analysis (Shimadazu-HPLC equipped with Daicel AD-H chiral column, *n*-hexane/*iso*-propanol=90/10, flow rate 1mL/min, column temperature 30 ºC, injection volume 5 μL, detection wavelength 220 nm). 1 U enzyme was defined as the amount of enzyme to produce 1 μmol product in 1 min.

Oxd RE activity assay [4]**:** the aldoxime dehydration activity was measured with appropriate amount of enzyme in 500 μL mixture of 5 mM *Z*-phenylacetoxime, 1 mM Na_2S, 10% DMSO, in 100 mM KPB (pH 7.0), which was incubated at 30 °C for 10 min. Then 1 mL ethyl acetate containing 2 mM benzonitrile (internal standard substance) was added to stop the reaction, the resulting mixture was centrifuged at 13,000×g for 1 min, 500 μL upper organic solvent was mixed with 500 μL *iso*-propanol and applied for High Performance Liquid Chromatography analysis (Shimadazu-HPLC equipped with Daicel AD-H chiral column, *n*-hexane/*iso*-

propanol=90/10, flow rate 1mL/min, column temperature 30 ºC, injection volume 5 μL, detection wavelength 220 nm). 1 U enzyme was defined as the amount of enzyme to produce 1 μmol product in 1 min.

4. Optimization of the reaction conditions

4.1 Reductants effect to the activity of Oxd B in the catalysis of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole

The partially purified Oxd B from Ni Sepharose™ 6 Fast flow column was used. Initially, different reductants were used for screening on the activity of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole catalyzed by Oxd B, The reactions were performed in 500 μL scale with 100 mM KPB (pH 7.0), 1 U Oxd-B, 20 mM 5-phenyl-4,5-dihydroisoxazole, 0.25 mM FMN and 1 mM reducing reagent at 30 ºC for 10 min. The yield and *ee* were detected by chiral HPLC as showed above. The result was showed in **Fig. S1**.

4.2 Substrate concentration of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole catalzyed by Oxd B and scale up reaction investigation

For substrate concentration investigation, the partially purfied OxdB from Ni Sepharose™ 6 Fast flow column was used, the reactions were performed in 500 μL scale with 100 mM KPB buffer (pH 6.0), 1 U Oxd-B, 5 mM-40 mM 5-phenyl-4,5-dihydroisoxazole, 0.05 mM FMN and 1 mM $Na_2S_2O_5$ at 30 ºC for 10 min. When finished, 1 mL ethyl acetate containing 2 mM benzonitrile was added to stop the reaction and followed with centrifugation (13,000×g, 1 min), 500 μL upper organic solvent was mixed with 500 μL *iso*-propanol. The yield and *ee* were detected by chiral HPLC as showed above, the result was showed in **Fig. S2**.

To explore the practicability of this novel enzymatic reaction in synthesis, the scale-up reactions were performed based on optimized reaction conditions by increasing the enzyme and substrate amount proportionally. In details, the reactions were performed in 500 μL scale with equally proportional amplification of optimal reaction conditions (100 mM KPB buffer (pH 6.0), 1 U Oxd-B, 20 mM 5-phenyl-4,5-dihydroisoxazole, 0.05 mM FMN and 1 mM $Na_2S_2O_5$) at 30 ºC for 2 h. The conversion was calculated by the enantiomer excess of substrate and product, as conversion = $ee_s/(ee_s+ee_p)$ [5]. The yield and *ee* were detected by chiral HPLC as showed above, the result was showed in **Table. S1**.

4.3 Reductants effect to the activity of Oxd RE in the catalysis of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole

The partially purified Oxd RE from Ni Sepharose™ 6 Fast flow column was used. Initially, different reductants were used for screening on the activity of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole catalyzed by Oxd RE, the reactions were performed in 500 μL scale with 100 mM KPB (pH 7.0), 1 U Oxd RE, 20 mM 5-phenyl-4,5-dihydroisoxazole and 1 mM reducing reagent at 30 ºC for 10 min. The yield and *ee* were detected by chiral HPLC as showed above. The result was showed in **Fig. S3**.

Subsequently, the best concentration of Na_2S was investigated by performing the reaction in 500 μL scale with 100 mM KPB (pH 7.0), 1 U Oxd RE, 20 mM 5-phenyl-4,5-dihydroisoxazole and 0 mM-4 mM Na_2S at 30 ºC for 30 min. The yield and *ee* were detected by chiral HPLC as showed above. The result was showed in **Fig. S4**.

4.4 Reaction time curve of ring-opening of 5-phenyl-4,5-dihydroisoxazole with the catalysis of Oxd RE

The partially purified Oxd RE from Ni Sepharose™ 6 Fast flow column was used. The reaction was monitored in time of 10 min to 180 min with the reaction condition of 500 μL reaction scale containing 100 mM KPB (pH 6.0 or pH 7.0), 1 U Oxd RE, 20 mM 5-phenyl-4,5-dihydroisoxazole and 1 mM Na_2S at 30 ºC. The yield and *ee* were detected by chiral HPLC as showed above. And the different reaction modes of resting and shaking were compared in 60 min. The result was showed in **Fig. S5**.

4.5 Substrate concentration of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole catalzyed by Oxd RE

The reactions were performed in 500 μL scale with 100 mM KPB buffer (pH 7.0), 12.5 μg pure Oxd RE, 2 mM-16 mM 5-phenyl-4,5-dihydroisoxazole, 1 mM Na_2S at 30 ºC for 20 min. When finished, 1 mL ethyl acetate containing 2 mM benzonitrile was added to stop the reaction and followed with centrifugation (13,000×g, 1 min), 500 μL upper organic solvent was mixed with 500 μL *iso*-propanol. The yield and *ee* were detected by chiral HPLC as showed above, the result was showed in **Fig. S6**.

4.6 Reductants effect to the activity of Oxd A in the catalysis of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole

The partially purified Oxd A from Ni Sepharose™ 6 Fast flow column was used. Initially, different reductants were used for screening on the activity of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole catalyzed by Oxd A, the reactions were performed in 500 μL scale with 100 mM KPB (pH 7.0), 10 U Oxd-A, 20 mM 5-phenyl-4,5-dihydroisoxazole and 1 mM reducing reagent at 30 ºC for 10 min. The yield and *ee* were detected by chiral HPLC as showed above. The result was showed in **Fig. S7**.

Subsequently, the best concentration of $Na_2S_2O_4$ was investigated by performing the reaction in 500 μL scale with 100 mM KPB (pH 7.0), 10 U Oxd A, 20 mM 5-phenyl-4,5-dihydroisoxazole and 0 mM-5 mM $Na_2S_2O_4$ at 30 ºC for 10 min. The yield and *ee* were detected by chiral HPLC as showed above. The result was showed in **Fig. S8**.

4.7 Reaction time curve of ring-opening of 5-phenyl-4,5-dihydroisoxazole with the catalysis of Oxd-A

The partially purified Oxd A from Ni Sepharose™ 6 Fast flow column was used. The reaction was monitored in time of 10 min to 180 min with the reaction condition of 500 μL reaction scale containing 100 mM KPB (pH 7.0), 10 U Oxd A, 20 mM 5-phenyl-4,5-dihydroisoxazole and 4 mM $Na_2S_2O_4$ at 30 ºC. The yield and *ee* were detected by chiral HPLC as showed above. And the different reaction modes of resting and shaking were compared in 60 min. The result was showed in **Fig. S9**.

4.8 Substrate concentration of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole catalzyed by Oxd A

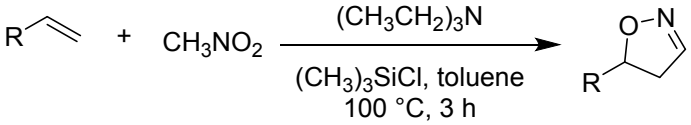
The reactions were performed in 500 μL scale with 100 mM KPB buffer (pH 7.0), 10 U partially purified Oxd A, 2 mM-20 mM 5-phenyl-4,5-dihydroisoxazole, 4 mM $Na_2S_2O_4$ at 30 ºC for 10 min. When finished, 1 mL ethyl acetate containing 2 mM benzonitrile was added to stop the reaction and followed with centrifugation (13,000×g, 1 min), 500 μL upper organic solvent was mixed with 500 μL *iso*-propanol. The yield and *ee* were detected by chiral HPLC as showed above, the result was showed in **Fig. S10**.

4.9 Assay procedure for determination of kinetic parameters of Oxd B and Oxd RE

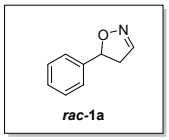
Kinetic assays for Oxd B were performed with 10 μg pure Oxd B and varying substrate concentritions (0.2 mM-50 mM) of **1a** in 100 mM KPB (100 mM, pH 6.0) containing 0.05 mM FMN, 1 mM fresh $Na_2S_2O_5$ at 30 °C and kinetic assays for Oxd RE were performed with 20 μg pure Oxd RE and varying substrate concentritions (0.2 mM-20 mM) of **1a** in 100 mM KPB (100 mM, pH 7.0) containing 1 mM fresh Na_2S at 30 °C, respectively. The reactions were initiated by addition of substrate and each sample was measured at different time points of 2 min, 4 min, 6 min for OxdB, 5 min, 10 min, 20 min for OxdRe, respectively, by taking 200 μL reaction mixture, which was extracted by addition of 400 μL ethyl acetate containing 2 mM benzonitrile. Initial rates (mM/min) determined by subtracting the slope of the control were plotted versus the concentration of **1a**. The data was fitted to the Michaelis-Menten equation and determine the kinetic parameters (**Table. S2**).

5. Substrates and racemic products synthesis

5.1 Synthesis of aromatic dihydroisoxazoles [6]

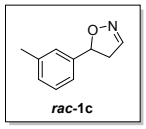


To the 50 mL toluene solution containing styrene derivatives (20 mmol), nitromethane (30 mmol), triethylamine (30 mmol) was added with 30 mmol chlorotrimethylsilane in room temperature. Then the resulting mixture was stirred at 100 °C for 24 h. Then filtered and removed the solvent in reduced pressure, the residual was applied to silica-column for purification (EA/hexane=1/5).



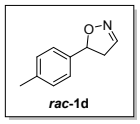
rac-1a

5-phenyl-4,5-dihydroisoxazole: pale yellow Oil, yield 54%. ^{1}H NMR ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.28 (m, 5H), 7.20 (td, *J* = 1.8, 0.6 Hz, 1H), 5.52 (dd, *J* = 11.2, 8.1 Hz, 1H), 3.44 (ddd, *J* = 17.6, 11.2, 1.8 Hz, 1H), 2.98 (ddd, *J* = 17.6, 8.1, 1.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.62, 140.98, 128.75, 128.16, 125.79, 79.89, 43.75. HRMS (ESI): calcd for $C_9H_{10}NO$ $[M + H]^+$, 148.0757; found: 148.0755; calcd for C_9H_9NNaO $[M + Na]^+$, 170.0576; found: 170.0577.



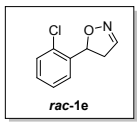
rac-1c

5-(m-tolyl)-4,5-dihydroisoxazole: colourless oil, yield 23%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (dd, *J* = 8.5, 6.6 Hz, 1H), 7.13 (td, *J* = 1.8, 0.6 Hz, 1H), 7.10-7.01 (m, 3H), 5.42 (dd, *J* = 11.2, 8.1 Hz, 1H), 3.35 (ddd, *J* = 17.6, 11.2, 1.8 Hz, 1H), 2.90 (ddd, *J* = 17.6, 8.1, 1.9 Hz, 1H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.53, 140.80, 138.41, 128.81, 128.56, 126.32, 122.77, 79.86, 43.64, 21.35. HRMS (ESI): calcd for $C_{10}H_{12}NO$ $[M + H]^+$, 162.0913; found: 162.0911; calcd for $C_{10}H_{11}NNaO$ $[M + Na]^+$, 184.0733; found: 184.0734.



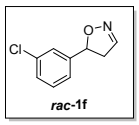
rac-1d

5-(p-tolyl)-4,5-dihydroisoxazole: yellow oil, yield 32%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.04 (m, 5H), 5.47 (dd, *J* = 11.1, 8.2 Hz, 1H), 3.39 (ddd, *J* = 17.6, 11.1, 1.8 Hz, 1H), 2.95 (ddd, 17.6, 8.2, 1.8 Hz, 1H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.64, 137.95, 137.90, 129.40, 125.81, 79.91, 43.66, 21.16. HRMS (ESI): calcd for $C_{10}H_{12}NO$ $[M + H]^+$, 162.0913; found: 162.0910; calcd for $C_{10}H_{11}NNaO$ $[M + Na]^+$, 184.0733; found: 184.0744.



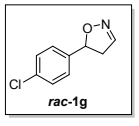
rac-1e

5-(2-chlorophenyl)-4,5-dihydroisoxazole: colorless oil, yield 17%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.40 (m, 1H), 7.33-7.27 (m, 1H), 7.25-7.13 (m, 2H), 7.13-7.08 (m, 1H), 5.75 (dd, *J* = 11.3, 6.9 Hz, 1H), 3.52(ddd, *J* = 17.8, 11.3, 1.8 Hz, 1H), 2.79 (ddd, *J* = 17.8, 6.9, 1.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.78, 139.01, 131.26, 129.65, 129.15, 127.28, 126.61, 76.97, 43.46. HRMS (ESI): calcd for $C_9H_8ClNNaO$ $[M + Na]^+$, 204.0187; found: 204.0186.



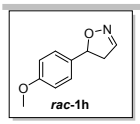
rac-1f

5-(3-chlorophenyl)-4,5-dihydroisoxazole: yellow oil, yield 5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.26 (m, 3H), 7.20-7.18 (m, 2H), 5.49 (dd, *J* = 11.2, 7.7 Hz, 1H), 3.45 (ddd, *J* = 17.6, 11.3, 1.7 Hz, 1H), 2.95 (ddd, *J* = 17.6, 7.7, 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.49, 143.10, 134.64, 130.10, 128.27, 125.86, 123.87, 79.00, 43.82. HRMS (ESI): calcd for $C_9H_8ClNNaO$ $[M + Na]^+$, 204.0187; found: 204.0182.

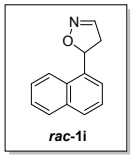


rac-1g

5-(4-chlorophenyl)-4,5-dihydroisoxazole: yellow oil, yield 18%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33-7.20 (m, 5H), 5.51-5.46 (m, 1H), 3.58-3.21 (m, 1H), 2.96-2.89 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.51, 139.51, 133.91, 128.90, 127.16, 79.13, 43.78. HRMS (ESI): calcd for $C_9H_8ClNNaO$ $[M + Na]^+$, 204.0187; found: 204.0185.

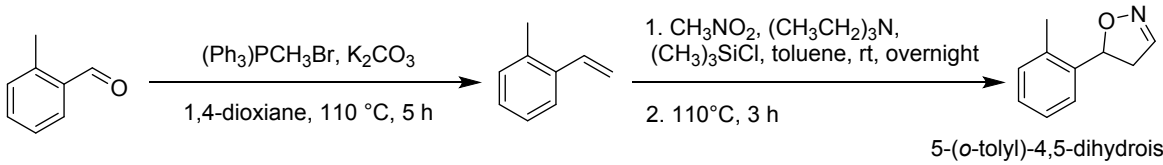


5-(4-methoxyphenyl)-4,5-dihydroisoxazole: colorless oil, yield 25%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26-7.19 (m, 3H), 6.90-6.86 (m, 2H), 5.46 (dd, *J* = 10.9, 8.6 Hz, 1H), 3.79 (s, 3H), 3.37 (ddd, *J* = 17.6, 11.1, 1.7 Hz, 1H), 2.95 (ddd, *J* = 17.6, 8.4, 1.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.55, 145.71, 132.80, 127.26, 114.11, 79.81, 55.33, 43.48. HRMS (ESI): calcd for $C_{10}H_{11}NNaO_2$ $[M + Na]^+$, 200.0682; found: 200.0696.



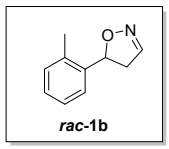
5-(naphthalen-1-yl)-4,5-dihydroisoxazole: yellow oil, yield 18%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90-7.88 (m, 1H), 7.85-7.81 (m, 2H), 7.63-7.62 (d, *J* = 7.1 Hz, 1H), 7.58-7.43 (m, 3H), 7.25 (s, 1H), 6.21 (dd, *J* = 11.3, 7.7 Hz, 1H), 3.65 (dd, *J* = 17.4, 11.4 Hz, 1H), 3.02 (dd, *J* = 17.4, 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.85, 136.06, 133.97, 129.67, 129.14, 128.50, 126.36, 125.76 , 125.51, 122.85, 122.74, 77.62, 43.51. HRMS (ESI): calcd for $C_{13}H_{11}NNaO$ $[M + Na]^+$, 220.0733; found: 220.0748.

5.2 Synthesis of 5-(o-tolyl)-4,5-dihydroisoxazole



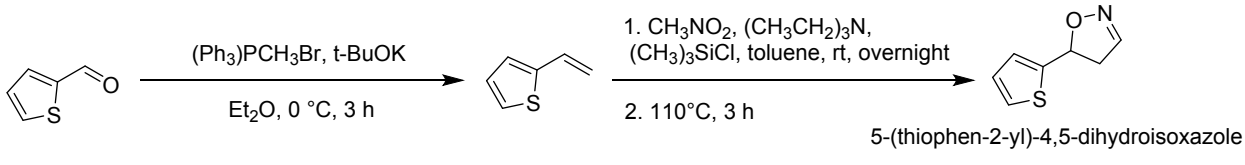
5-(*o*-tolyl)-4,5-dihydroisoxazole

To 30 mL 1,4-dioxane solution containing 2-methylbenzaldehyde (2.65 g, 22 mmol) and K_2CO_3 (3.03 g), 7.86 g methyltriphenylphosphonium was added. The resulting solution was refluxed at 110 °C for 5 h. Then the solution was cooled down to room temperature and filtered, the solvent in filtrate was removed in reduced pressure. The residual was dissolved in 50 mL toluene, then 1.32 g CH_3NO_2, 2.22 g $(CH_3CH_2)_3N$ and 2.2 g $(CH_3)_3SiCl$ were added. The resulting solution was stirred at room temperature for 48 h, followed with refluxing at 110 °C for 3 h. After cooling down, the solvent was removed in vacuum and the residual was purified by silica-column, (Eluent: EA/hexane=1/5).



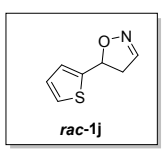
5-(o-tolyl)-4,5-dihydroisoxazole: colorless oil, yield 21%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.27 (m, 1H), 7.23 – 7.04 (m, 4H), 5.62 (dd, *J* = 11.2, 8.1 Hz, 1H), 3.39 (ddd, *J* = 17.4, 11.3, 1.4 Hz, 1H), 2.77 (ddd, *J* = 17.4, 8.0, 0.9 Hz, 1H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.48, 139.08, 134.21, 130.59, 127.84, 126.38, 124.97, 77.45, 42.84, 19.30. HRMS (ESI): calcd for $C_{10}H_{12}NO$ $[M + H]^+$, 162.0913; found: 162.0914; calcd for $C_{10}H_{11}NNaO$ $[M + Na]^+$, 184.0733; found: 184.0733.

5.3 Synthesis of 5-(thiophen-2-yl)-4,5-dihydroisoxazole



5-(thiophen-2-yl)-4,5-dihydroisoxazole

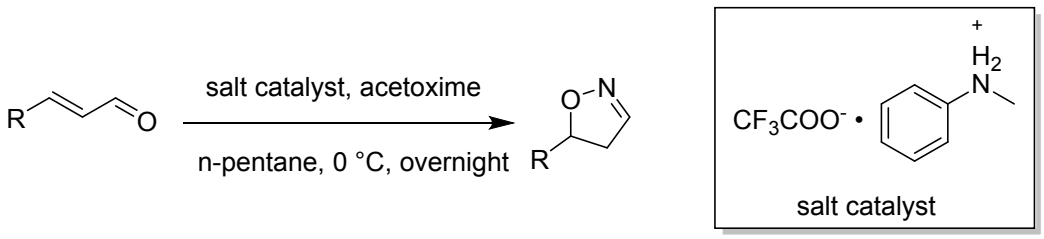
To 50 mL Et_2O solution containing thiophene-2-carbaldehyde (3.36 g, 30 mmol) and t-BuOK (6.6 g, 48 mmol), 12.86 g methyltriphenylphosphonium was added. The resulting solution was stirred at 0 °C for 3 h. Then the solution was cooled down to room temperature and filtered, the solvent in filtrate was removed in reduced pressure. The residual was dissolved in 50 mL toluene, then 1.80 g CH_3NO_2, 3.03 g $(CH_3CH_2)_3N$ and 3.30 g $(CH_3)_3SiCl$ were added. The resulting solution was stirred at room temperature overnight, followed with refluxing at 110 °C for 3 h. After cooling down, the solvent was removed in vacuum and the residual was purified by silica-column (Eluent: EA/hexane=1/5).



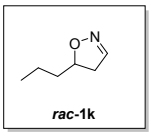
5-(thiophen-2-yl)-4,5-dihydroisoxazole: brown oil, yield 7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 – 7.23 (m, 1H), 7.22 (t, *J* = 1.5 Hz, 1H), 7.06 – 7.00 (m, 1H), 7.00 – 6.93 (m, 1H), 5.72 (dd, *J* = 10.8, 7.8 Hz, 1H), 3.39 (ddd, *J* =

17.6, 10.8, 1.8 Hz, 1H), 3.10 (ddd, *J* = 17.6, 7.8, 1.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.84, 143.46, 126.96, 125.71, 125.38, 75.89, 43.49. HRMS (ESI): calcd for C_7H_7NNaOS [M + Na]$^+$, 176.0141; found: 176.0139.

5.4 Synthesis of aliphatic dihydroisoxazoles [7]

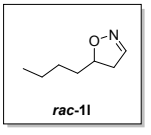


To 50 mL n-pentane solution containing 3.65 g (50 mmol) acetoxime and 2.65 g (20 mol%) salt catalyst was slowly added with 60 mmol aliphatic aldehyde at 0 °C. The resulting solution was stirred at 0°C overnight, then the upper *n*-pentane layer was separated out and the lower layer was washed with *n*-pentane (50 mL ×2). The combined *n*-pentane was concentrated in vacuum and the residual was applied to silica-column for purification (eluent: *n*-pentane/Et_2O=5/1).



rac-1k

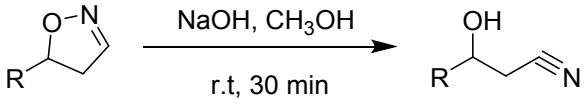
5-propyl-4,5-dihydroisoxazole: light yellow oil, yield 10%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (t, *J* = 1.7 Hz, 1H), 4.58 – 4.33 (m, 1H), 3.02 (ddd, *J* = 17.3, 10.5, 1.8 Hz, 1H), 2.59 (ddd, *J* = 17.3, 8.1, 1.9 Hz, 1H), 1.78 – 1.55 (m, 1H), 1.56 – 1.23 (m, 3H), 1.03 – 0.79 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.90, 78.52, 40.42, 37.18, 18.79, 13.86. HRMS (ESI): calcd for $C_6H_{11}NNaO$ [M + Na]$^+$, 136.0733; found: 136.0708.



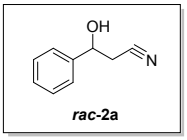
rac-1l

5-butyl-4,5-dihydroisoxazole: light yellow oil, yield 32%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (s, 1H), 4.43 (ddt, *J* = 14.3, 10.4, 7.0 Hz, 1H), 2.97 (ddd, *J* = 17.3, 10.5, 1.8 Hz, 1H), 2.54 (ddd, *J* = 17.4, 8.1, 1.8 Hz, 1H), 1.64-1.58 (m, 1H), 1.49-1.41 (m, 1H), 1.38 – 1.22 (m, 4H), 0.84 (t, *J* = 8.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.90, 78.77, 40.41, 34.77, 27.64, 22.49, 13.94. HRMS (ESI): calcd for $C_7H_{13}NNaO$ [M + Na]$^+$, 150.0889; found: 150.0900.

5.5 Synthesis of racemic β-alcohol nitriles

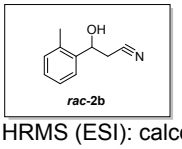


To 10 mL methanol solution containing 200 mg NaOH was added with dihydroisoxazole derivatives (1.5 mmol), then stirred at room temperature for additional 30 min. The solvent was removed in reduced pressure and the residual was dissolve in water following with extraction using ethyl acetate, the ethyl acetate layer was dried against anhydrous $MgSO_4$ and concentrated, then applied to silica-column for purification (EA/hexane=1/5).



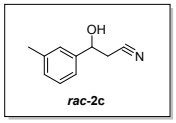
rac-2a

3-hydroxy-3-phenylpropanenitrile: pale yellow oil, 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47-7.30 (m, 5H), 5.05 (m, 1H), 2.81-2.74 (m, 2H), 2.40 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.02, 128.97, 128.88, 125.54, 117.31, 70.15, 27.95. HRMS (ESI): calcd for C_9H_9NNaO [M + Na]$^+$, 170.0576; found: 170.0575.

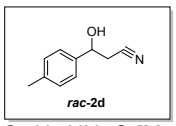


rac-2b

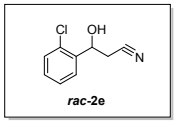
3-hydroxy-3-(o-tolyl)propanenitrile: colorless oil, yield 77%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48-7.46 (m, 1H), 7.26-7.17 (m, 2H), 7.16-7.10 (m, 1H), 5.25-5.13 (m, 1H), 3.16 (s, 1H), 2.64 (dd, *J* = 6.1, 1.3 Hz, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.24, 134.36, 130.75, 128.43, 126.72, 125.04, 117.71, 66.31, 26.66, 18.96. HRMS (ESI): calcd for $C_{10}H_{11}NNaO$ [M + Na]$^+$, 184.0733; found: 184.0733.



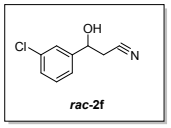
3-hydroxy-3-(m-tolyl)propanenitrile: colorless oil, yield 41%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-7.26 (m, 1H), 7.21-7.15 (m, 3H), 5.00 (td, *J* = 6.2, 3.7 Hz, 1H), 2.76 (dd, *J* = 6.2, 1.8 Hz, 2H), 2.43 (d, *J* = 3.6 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.98, 138.79, 129.63, 128.87, 126.16, 122.57, 117.33, 70.23, 27.90, 21.46. HRMS (ESI): calcd for $C_{10}H_{11}NNaO$ [M + Na]$^+$, 184.0733; found: 184.0735.



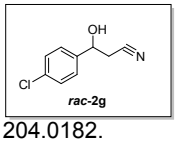
3-hydroxy-3-(p-tolyl)propanenitrile: colorless oil, yield 54%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-7.22 (m, 2H), 7.19 (d, *J* = 7.9 Hz, 2H), 4.96 (t, *J* = 5.9 Hz, 1H), 2.79 (s, 1H), 2.71 (dd, *J* = 12.5, 6.6 Hz, 2H), 2.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.66, 138.15, 129.57, 125.50, 117.50, 69.90, 27.89, 21.18. HRMS (ESI): calcd for $C_{10}H_{11}NNaO$ [M + Na]$^+$, 184.0733; found: 184.0733.



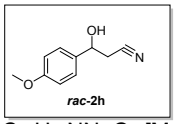
3-(2-chlorophenyl)-3-hydroxypropanenitrile: colorless oil, yield 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74-7.60 (m, 1H), 7.38-7.30 (m, 2H), 7.27 (m, 1H), 5.40 (dt, *J* = 7.3, 3.8 Hz, 1H), 3.28 (d, *J* = 3.8 Hz, 1H), 2.88 (dd, *J* = 16.8, 4.0 Hz, 1H), 2.70 (dd, *J* = 16.7, 7.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.41, 131.29, 129.67, 129.57, 127.56, 127.05, 117.31, 66.31, 26.32. HRMS (ESI): calcd for $C_9H_8ClNNaO$ [M + Na]$^+$, 204.0187; found: 204.0182.



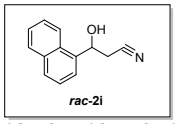
3-(3-chlorophenyl)-3-hydroxypropanenitrile: colorless oil, yield 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.41 (m, 1H), 7.37-7.31 (m, 2H), 7.31-7.26 (m, 1H), 5.03 (td, *J* = 6.2, 3.7 Hz, 1H), 2.76 (d, *J* = 6.2 Hz, 2H), 2.65 (d, *J* = 3.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.97, 134.92, 130.27, 129.00, 125.79, 123.74, 116.93, 69.49, 27.98. HRMS (ESI): calcd for $C_9H_8ClNNaO$ [M + Na]$^+$, 204.0187; found: 204.0184.



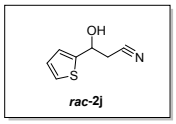
3-(4-chlorophenyl)-3-hydroxypropanenitrile: light yellow oil, yield 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.25 (m, 4H), 4.98 (t, *J* = 6.1 Hz, 1H), 3.23 (s, 1H), 2.71 (dd, *J* = 6.2, 1.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.54, 134.49, 129.05, 127.01, 117.27, 69.19, 27.98. HRMS (ESI): calcd for $C_9H_8ClNNaO$ [M + Na]$^+$, 204.0187; found: 204.0182.



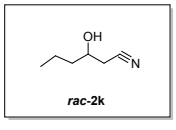
3-hydroxy-3-(4-methoxyphenyl)propanenitrile: colorless oil, yield 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-7.26 (m, 2H), 6.93-6.86 (m, 2H), 4.94 (t, *J* = 5.3 Hz, 1H), 3.79 (s, 3H), 2.96 (s, 1H), 2.71 (dd, *J* = 6.2, 2.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.82, 133.27, 126.91, 117.56, 114.24, 69.62, 55.36, 27.90. HRMS (ESI): calcd for $C_{10}H_{11}NNaO_2$ [M + Na]$^+$, 200.0682; found: 200.0688.



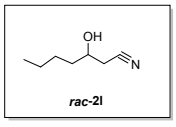
3-hydroxy-3-(naphthalen-1-yl)propanenitrile: colorless oil, yield 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97-7.81 (m, 2H), 7.77 (d, *J* = 8.2 Hz, 1H), 7.64 (d, *J* = 7.2 Hz, 1H), 7.58-7.35 (m, 3H), 5.67 (s, 1H), 3.24 (s, 1H), 2.81 (ddd, *J* = 24.4, 16.8, 6.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 136.46, 133.78, 129.64, 129.28, 129.16, 126.76, 125.97, 125.53, 123.25, 122.16, 117.76, 66.75, 27.10. HRMS (ESI): calcd for $C_{13}H_{11}NNaO$ [M + Na]$^+$, 220.0733; found: 220.0733.



3-hydroxy-3-(thiophen-2-yl)propanenitrile: yellow oil, yield 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.11-7.03 (m, 1H), 7.00 (dd, *J* = 5.0, 3.6 Hz, 1H), 5.26 (t, *J* = 6.2 Hz, 1H), 3.23 (s, 1H), 2.85 (dd, *J* = 6.2, 0.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.51, 127.12, 125.75, 124.72, 117.07, 66.19, 28.21. HRMS (ESI): calcd for C_7H_7NNaOS [M + Na]$^+$, 176.0141; found: 176.0139.



3-hydroxyhexanenitrile: yellow oil, yield 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98-3.85 (m, 1H), 2.68 – 2.38 (m, 3H), 1.65 – 1.25 (m, 4H), 0.98 – 0.87 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 117.87, 67.44, 38.57, 26.10, 18.62, 13.75. HRMS (ESI): calcd for $C_6H_{11}NNaO$ [M + Na]$^+$, 136.0733; found: 136.0729.



3-hydroxyheptanenitrile: yellow oil, yield 89%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.93 – 3.79 (m, 1H), 2.98 (s, 1H), 2.47 (qd, *J* = 16.6 Hz, *J* = 6.4 Hz, 2H), 1.60 – 1.44 (m, 2H), 1.43 – 1.13 (m, 4H), 0.91 – 0.76 (m, 3H); ^{13}C NMR

(100 MHz, $CDCl_3$) δ 118.01, 67.57, 36.15, 27.49, 26.03, 22.39, 13.90. HRMS (ESI): calcd for $C_7H_{13}NNaO$ $[M + Na]^+$, 150.0889; found: 150.0886.

5.6 HPLC/GC analysis methods [8]

The substrates (**1a**-**1l**) and corresponding products (**2a**-**2l**) were analyzed by Shimadazu-HPLC equipped with Daicel chiral columns and Shimadazu Gas Chromatograph equipped with chiral GC columns as showed in **Table S3**.

6. Large scale reaction and absolute conformation measurement by Mosher reagents [9]

For the assay of conformation of **2e**, **2g**, **2k** and **2l**, 50 mL large scale reactions were performed with 5 mmol corresponding substrates, 0.25 mM FMN, 5 mM fresh $Na_2S_2O_5$ and 500 U Oxd B in 100 mM KPB (pH 6.0) at 30 °C for 2 h (slowly stirred by magnetic bar). The products were extracted by ethyl acetate, following with solvent removing in vacuum and the residue was applied for silica chromatography purification, the yield and *ee* were showed in **Table S4**.

The isolated β-alcohol nitriles were derived with (*R*)-MTPA-Cl and (*S*)-MTPA-Cl using pyridine as base in dichloromethane to produce corresponding (*S*)-MTPA-ester and (*R*)-MTPA-ester. The products were purified by silica-chromatography and applied for H NMR analysis. The compound conformation was deduced by the proton relative chemical shift in H NMR data as the model established in published literature. When the substrate group locates at the same side with phenyl group of MTPA, a smaller chemical shift was observed because of the shielding effect. And same shielding effect of phenyl on the substrate to the -OMe group in MTPA was also observed. The NMR spectra results were showed in **Section 9.**

7. Oxd B homology model and mutants activity assay to aldoxime dehydration

The Oxd B homology model was constructed based on the structure of Oxd RE using SWISS-MODEL online. And the docking experiments between homology model and substrates were performed by the software of Molecular Operating Environment (MOE). The Alanine mutants of Oxd B were constructed by QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent) with standard PCR. The Oxd B mutant enzymes were produced and purified as the protocol in **section 2.2**. Then the activity of the mutants to substrate Z-phenylacetoxime were assayed with the reaction mixture of 10 mM substrate, 0.25 mM FMN, 1 mM $Na_2S_2O_5$, 2 μg pure enzymes in 100 mM KPB (pH 7.0) at 30 °C for 5 min. The results showed in **Fig. S11**.

8. Figures, Tables, and NMR spectra

8.1 Figures and Tables

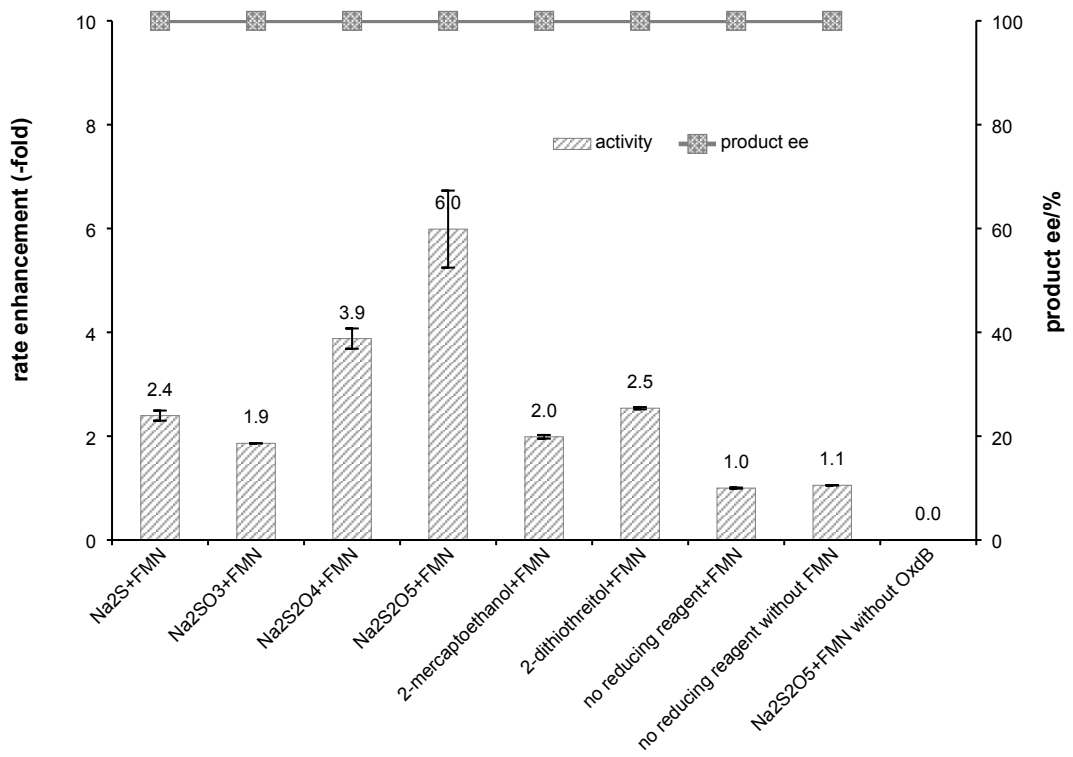


Fig S1: Reductants effect to the activity of Oxd B in the catalysis of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole.

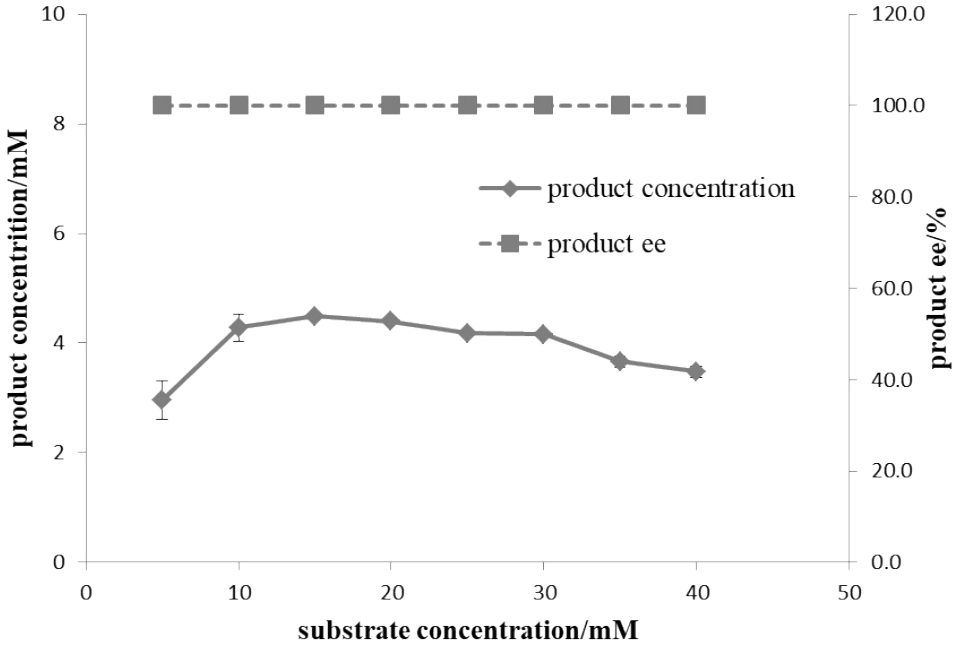


Fig S2: Substrate effect on the asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole with catalysis of Oxd B.

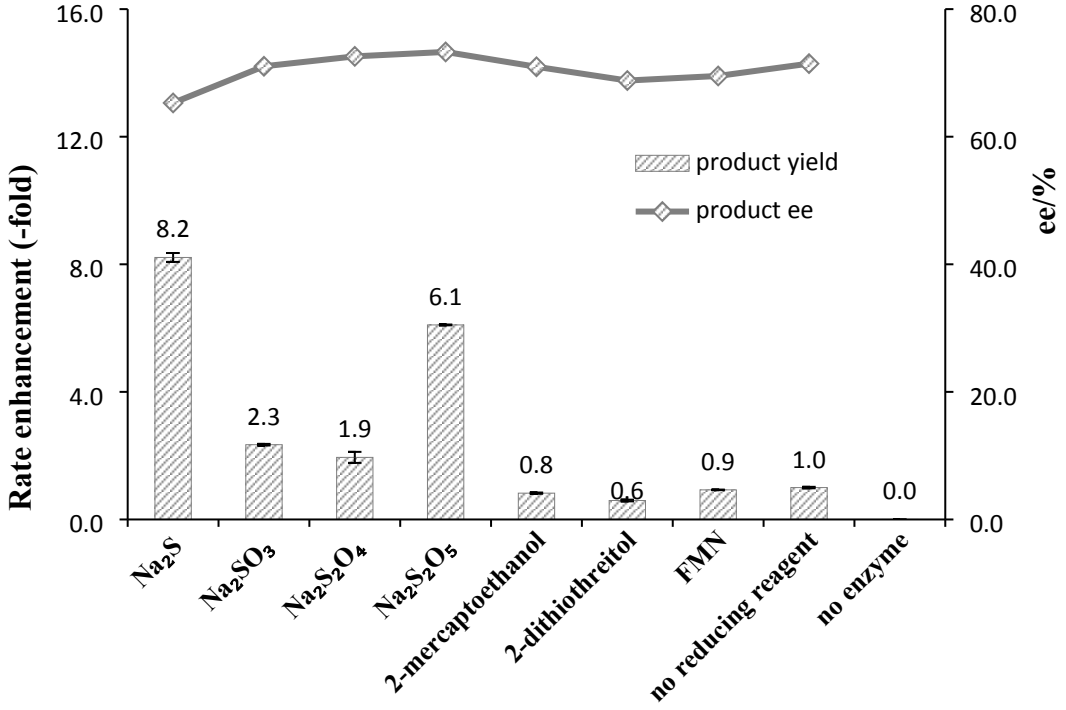


Fig S3: Reductants effect on the activity of Oxd RE in the catalysis of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole. The activity without reductant was taken as 1.0.

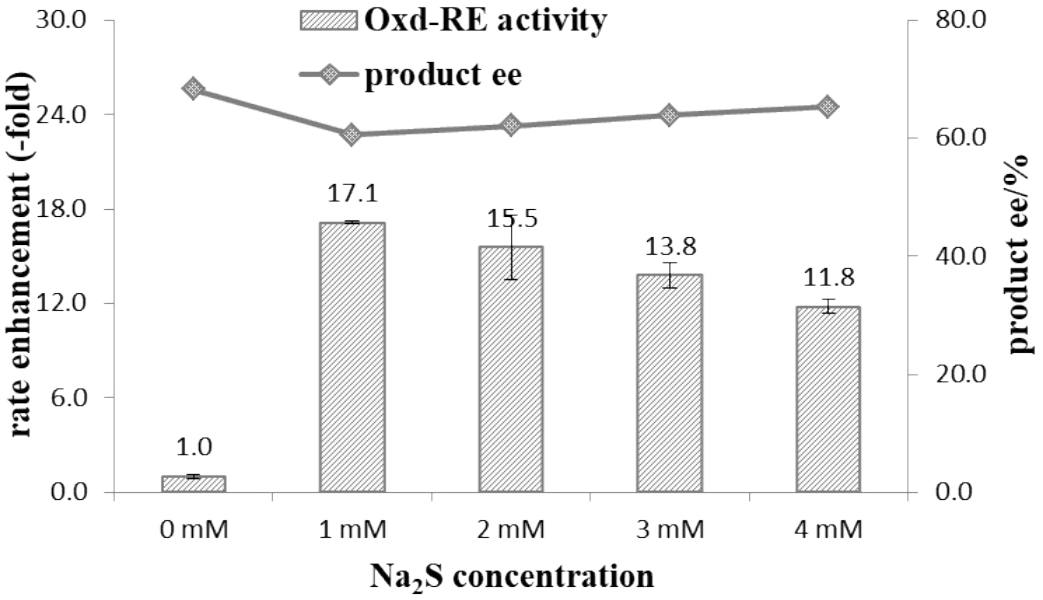


Fig S4: Na_2S effect to the activity of Oxd RE in the catalysis of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole. The activity without reductant was taken as 1.0.

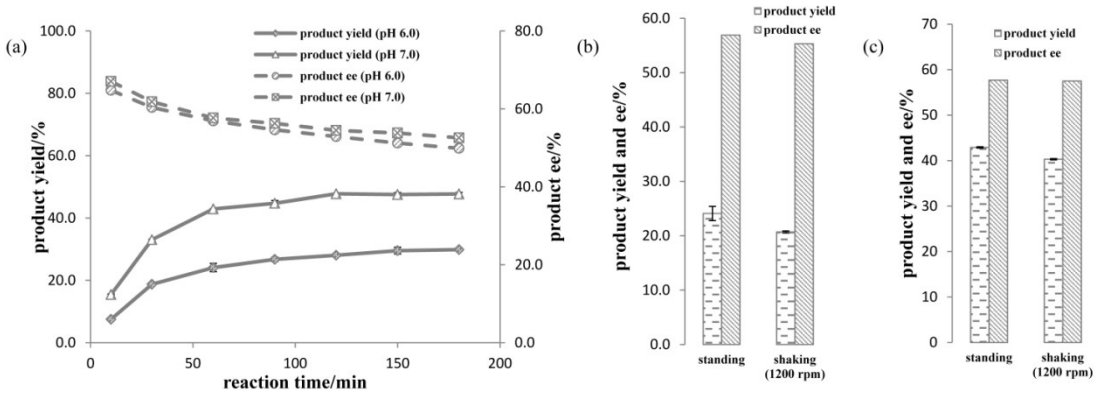


Fig S5: Reaction time curve for the asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole with catalysis of Oxd RE.

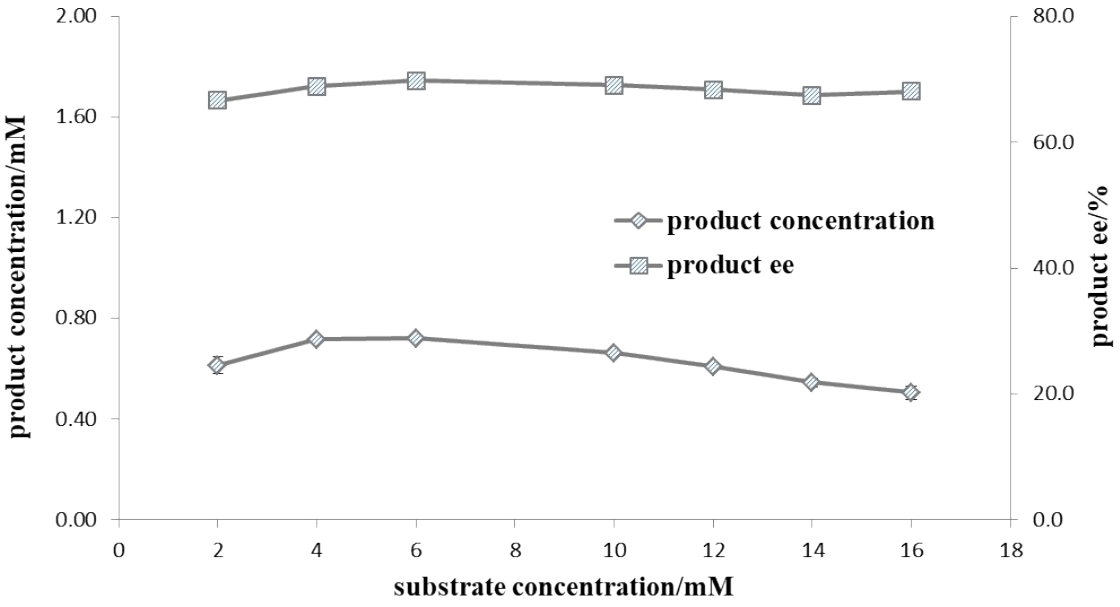


Fig S6: Substrate concentration effect on the activity of Oxd RE to 5-phenyl-4,5-dihydroisoxazole

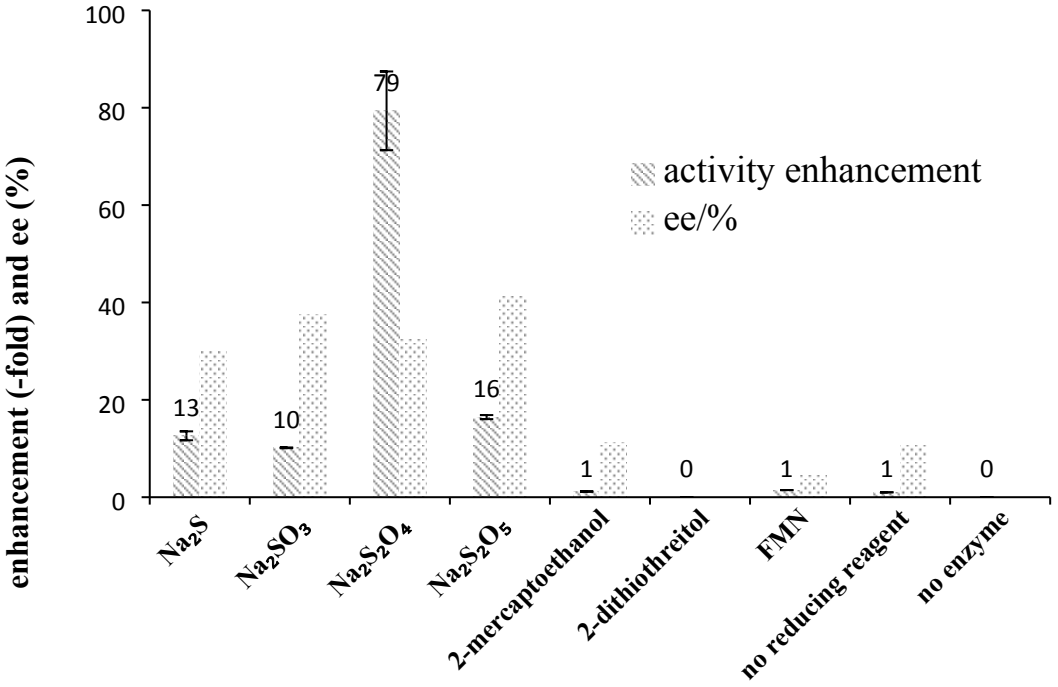


Fig S7: Reductants effect on the activity of Oxd A in the catalysis of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole. The activity without reductant was taken as 1.0.

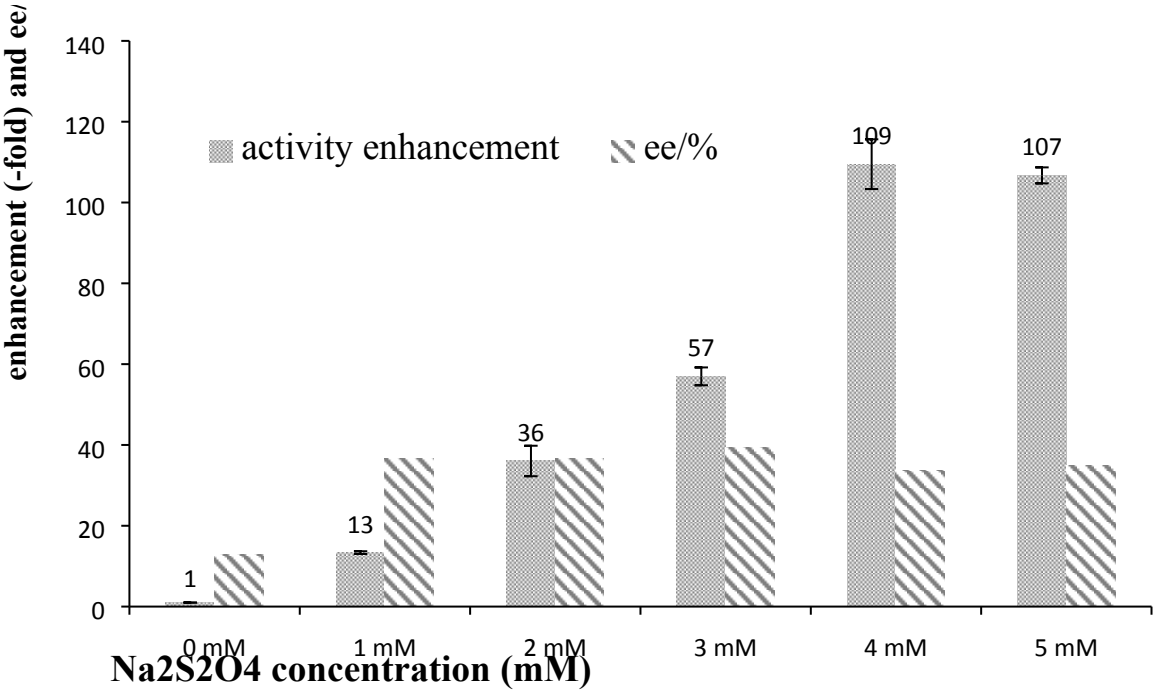


Fig S8: $Na_2S_2O_4$ effect to the activity of Oxd A in the catalysis of asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole. The activity without reductant was taken as 1.0.

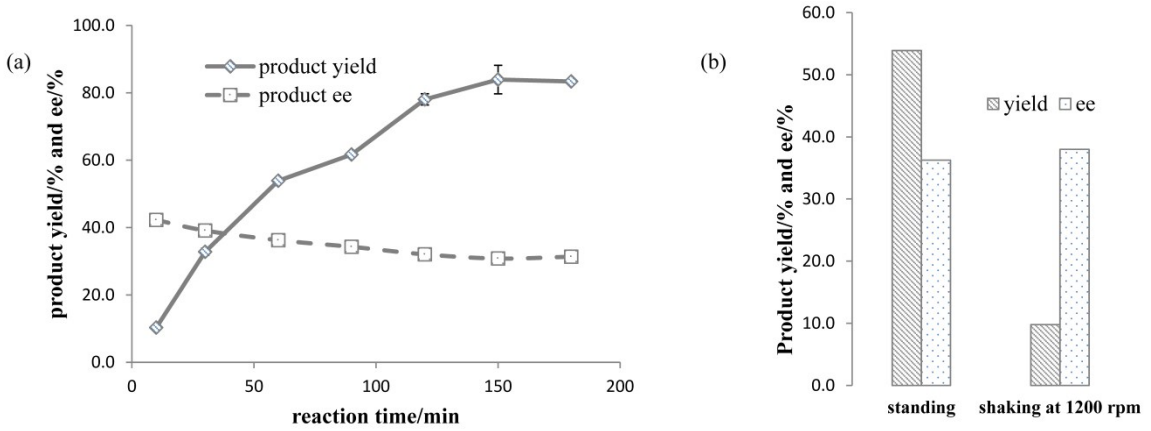


Fig S9: Reaction time curve for the asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole with catalysis of Oxd A

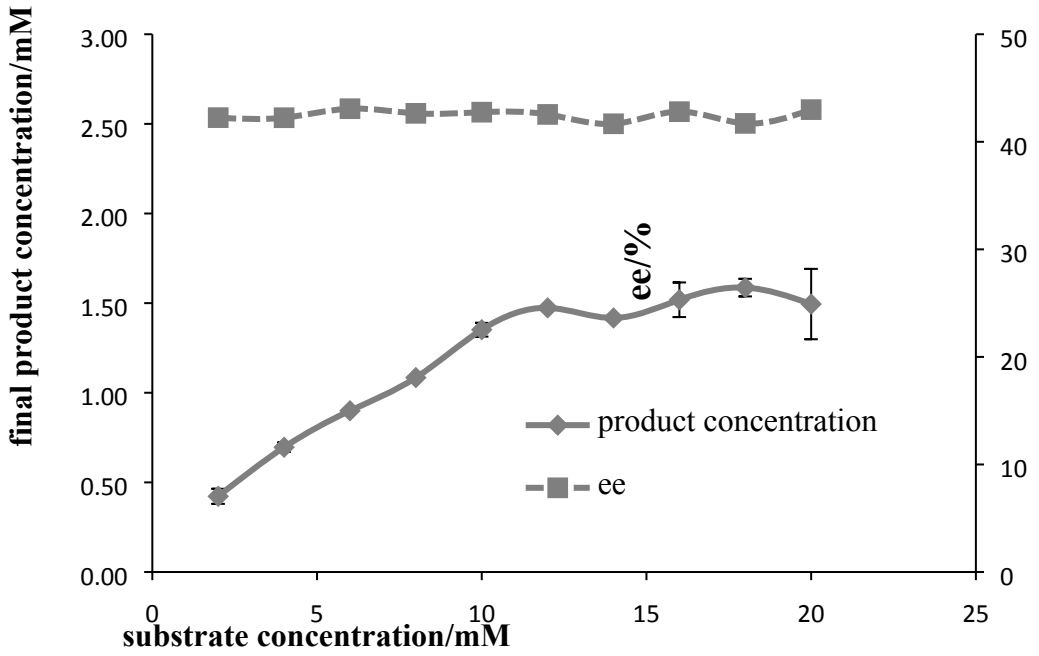


Fig S10: Substrate concentration effect on the activity of Oxd A to 5-phenyl-4,5-dihydroisoxazole

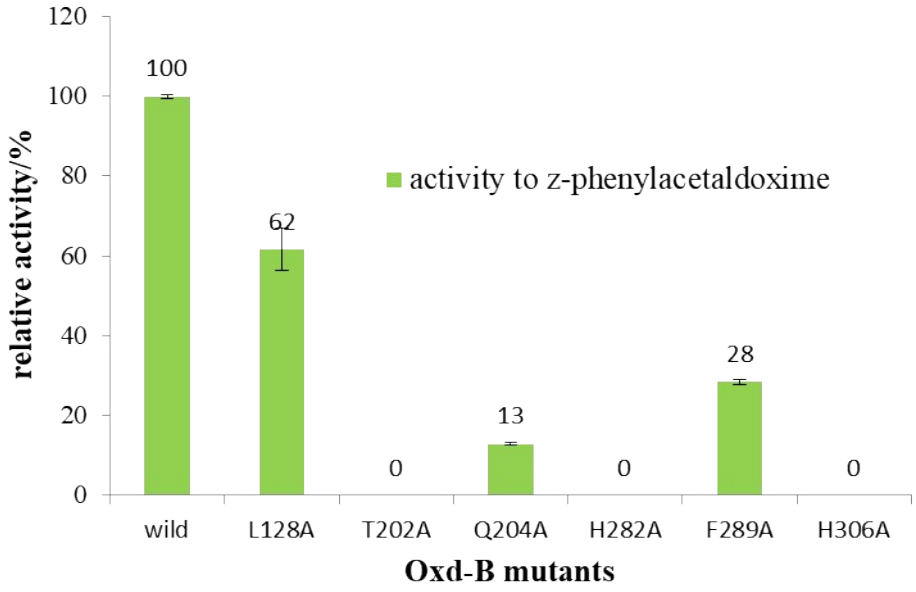


Fig. S11: The activity of Oxd B on the substrate of Z-phenylacetaldoxime. The activity of wild type was taken as 100%.

Table S1: Scale up for asymmetric ring-opening of 5-phenyl-4,5-dihydroisoxazole with catalysis of Oxd B

entry	substrate concentration (mM)	Oxd B (U)	$Na_2S_2O_5$ concentration (mM)	FMN concentration (mM)	substrate ee (%)	product ee (%)	conversion (%)
1	20	1	1	0.05	99	98.7	50.1
2	40	2	2	0.1	99	98.6	50.1
3	60	3	3	0.15	99	98.8	50.1
4	80	4	4	0.2	99	98.6	50.1
5	100	5	5	0.25	99	98.7	50.1
6	120	6	6	0.3	99	98.7	50.1
7	150	7.5	7.5	0.375	99	99.0	50.0
8	200	10	10	0.5	49.4	98.9	33.3

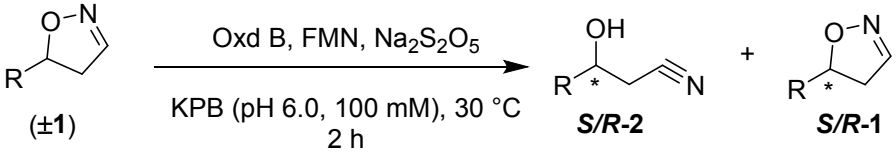
Table S2: Kinetic parameters of Oxd B and Oxd RE for ring-opening of 5-phenyl-4,5-dihydroisoxazole

Enzyme	k_{cat} (s^{-1})	K_m (mM)	K_{cat}/K_m (M^{-1} S^{-1})
Oxd B	11	0.3	36667
Oxd RE	3.0	0.7	4286

Table S3: Analysis methods for substrates and products

analysis method	Substrate			product		
	structure	retention time		structure	retention time	
Daicel Chiral OJ-H, 1 mL/min, Hexane/iso-propanol=9/1, 30 °C	5-phenyl-4,5-dihydroisoxazole	t1=19.027 min	t2=19.696 min	3-hydroxy-3-phenylpropanenitrile	t1=21.838 min	t2=27.085 min
Daicel Chiral OD-H, 1 mL/min, Hexane/iso-propanol=9/1, 30 °C	5-(o-tolyl)-4,5-dihydroisoxazole	t1=10.527 min	t2=11.268 min	3-hydroxy-3-(o-tolyl)propanenitrile	t1=13.419 min	t2=17.597 min
Daicel Chiral OJ-H, 1 mL/min, Hexane/iso-propanol=9/1, 30 °C	5-(m-tolyl)-4,5-dihydroisoxazole	t1=14.420 min	t2=15.420 min	3-hydroxy-3-(m-tolyl)propanenitrile	t1=17.248 min	t2=20.816 min
Daicel Chiral OD-H, 1 mL/min, Hexane/iso-propanol=9/1, 30 °C	5-(p-tolyl)-4,5-dihydroisoxazole	t1=9.067 min	t2=10.731 min	3-hydroxy-3-(p-tolyl)propanenitrile	t1=16.307 min	t2=17.437 min
Daicel Chiral OD-H, 1 mL/min, Hexane/iso-propanol=95/5, 30 °C	5-(2-chlorophenyl)-4,5-dihydroisoxazole	t1=9.025 min	t2=9.908 min	3-(2-chlorophenyl)-3-hydroxypropanenitrile	t1=18.919 min	t2=19.710 min
Daicel Chiral OJ-H, 1 mL/min, Hexane/iso-propanol=9/1, 30 °C	5-(3-chlorophenyl)-4,5-dihydroisoxazole	t1=15.971 min	t2=16.419 min	3-(3-chlorophenyl)-3-hydroxypropanenitrile	t1=21.532 min	t2=25.850 min
Daicel Chiral OD-H, 1 mL/min, Hexane/iso-propanol=95/5, 30 °C	5-(4-chlorophenyl)-4,5-dihydroisoxazole	t1=14.083 min	t2=14.403 min	3-(4-chlorophenyl)-3-hydroxypropanenitrile	t1=34.262 min	t2=39.309 min
Daicel Chiral OJ-H, 1 mL/min, Hexane/iso-propanol=9/1, 30 °C	5-(4-methoxyphenyl)-4,5-dihydroisoxazole	t1=39.105 min	t2=40.344 min	3-hydroxy-3-(4-methoxyphenyl)propanenitrile	t1=42.091 min	t2=43.587 min
Daicel Chiral OJ-H, 1 mL/min, Hexane/iso-propanol=9/1, 30 °C	5-(naphthalen-1-yl)-4,5-dihydroisoxazole	t1=22.175 min	t2=35.406 min	3-hydroxy-3-(naphthalen-1-yl)propanenitrile	t1=39.632 min	t2=47.051 min
Daicel Chiral OD-H, 1 mL/min, Hexane/iso-propanol=95/5, 30 °C	5-(thiophen-2-yl)-4,5-dihydroisoxazole	t1=19.657 min	t2=20.803 min	3-hydroxy-3-(thiophen-2-yl)propanenitrile	t1=39.119 min	t2=41.094 min
BGB-174，40 °C-2 min, 1 °C/min to 100 °C, 100 °C-10 min, 2 °C/min to 150 °C, 150 °C-30 min	5-propyl-4,5-dihydroisoxazole	t1=68.616 min	t2=72.214 min	3-hydroxyhexanenitrile	t1=103.731 min	t2=104.495 min
BGB-174，40 °C-2 min, 1 °C/min to 100 °C, 100 °C-10 min, 2 °C/min to 150 °C, 150 °C-30 min	5-butyl-4,5-dihydroisoxazole	t1=78.849 min	t2=80.736 min	3-hydroxyheptanenitrile	t1=110.752 min	t2=111.740 min

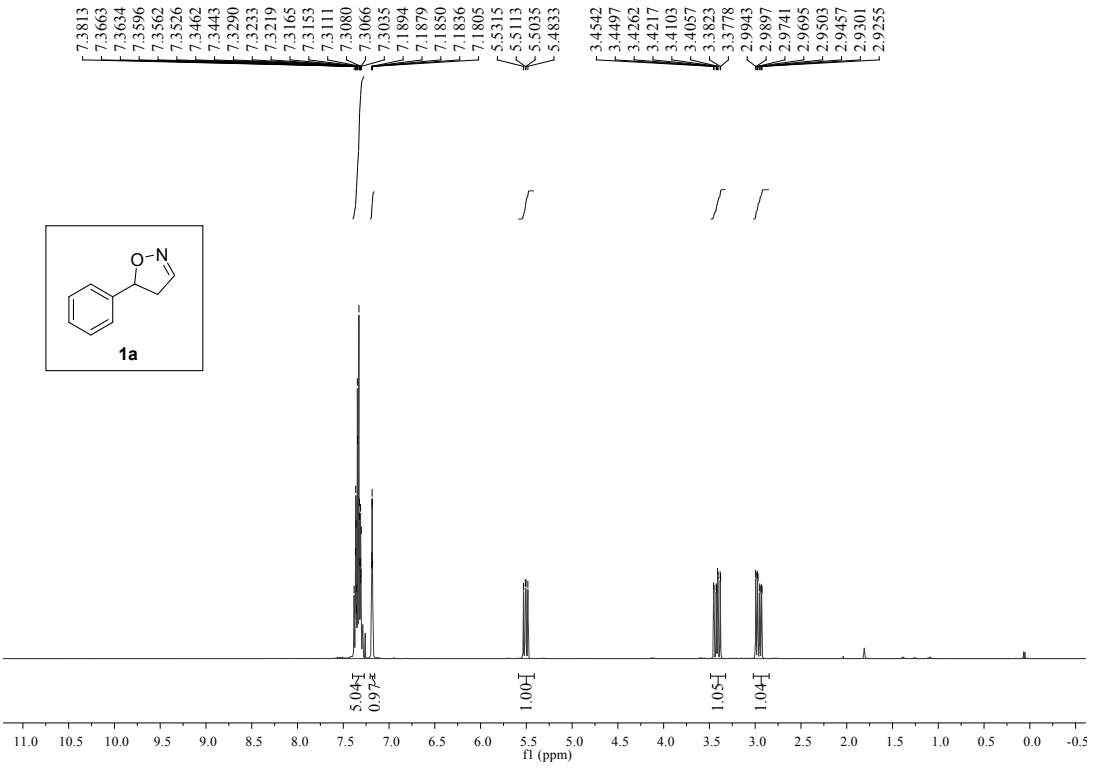
Table S4: Large scale reaction with the catalysis of Oxd B

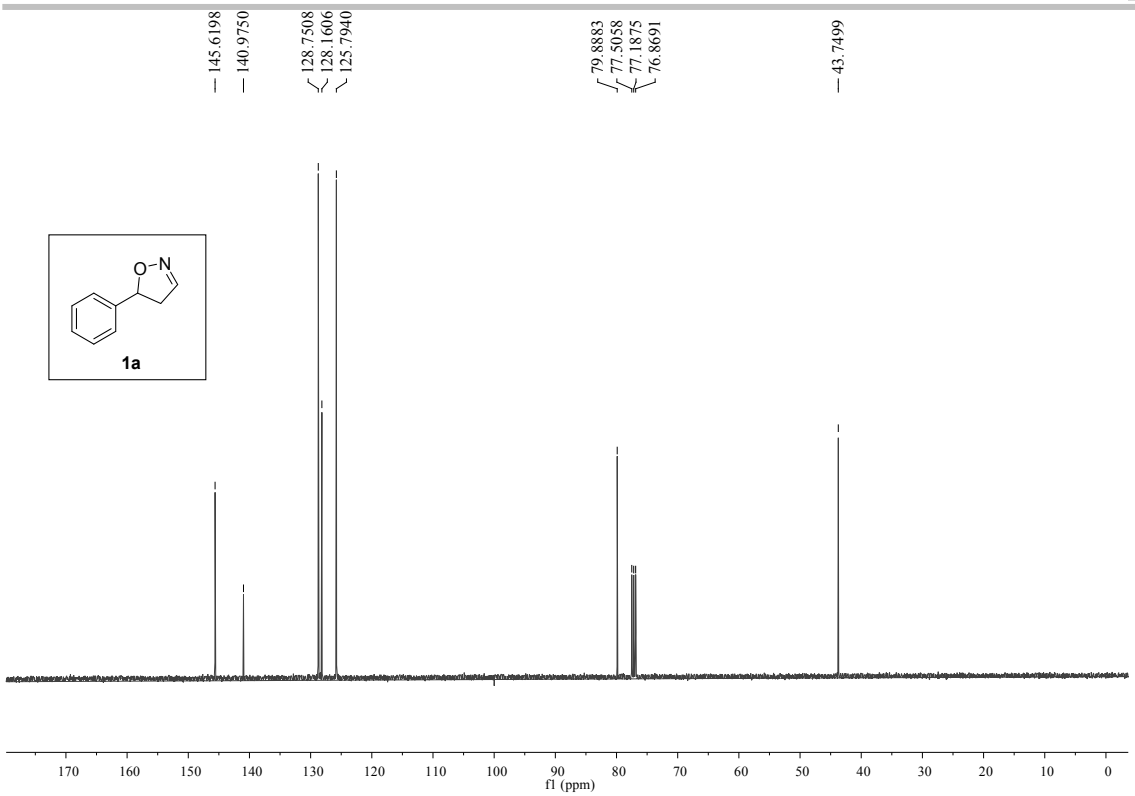


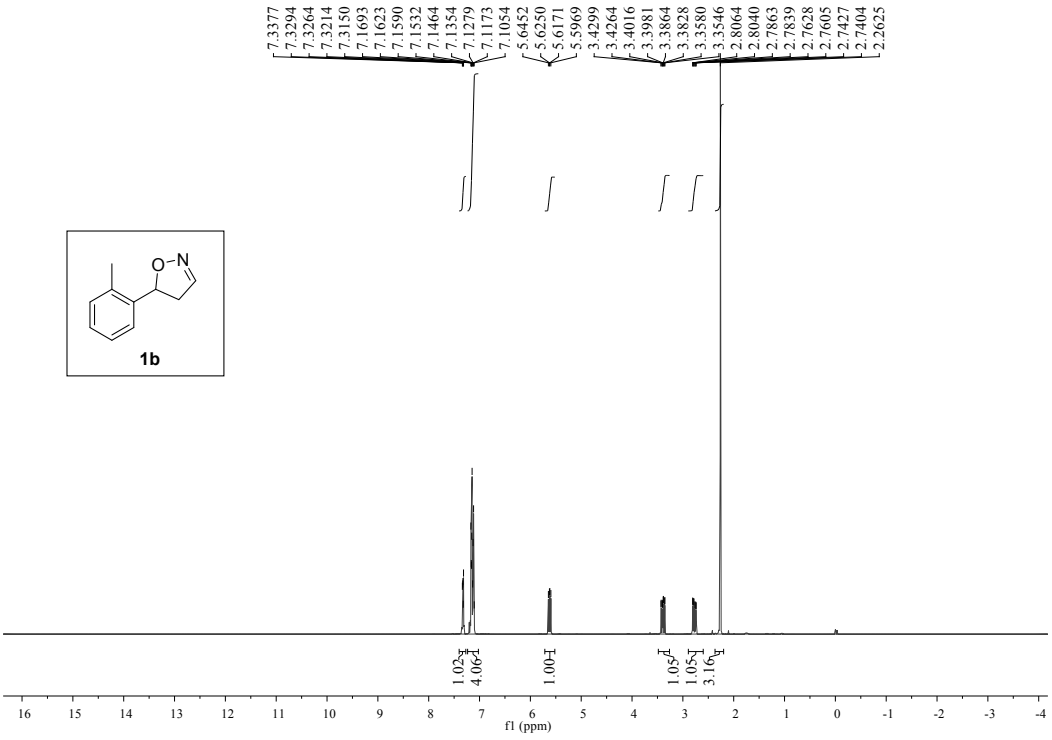
(±**1**) ***S/R*-2** ***S/R*-1**

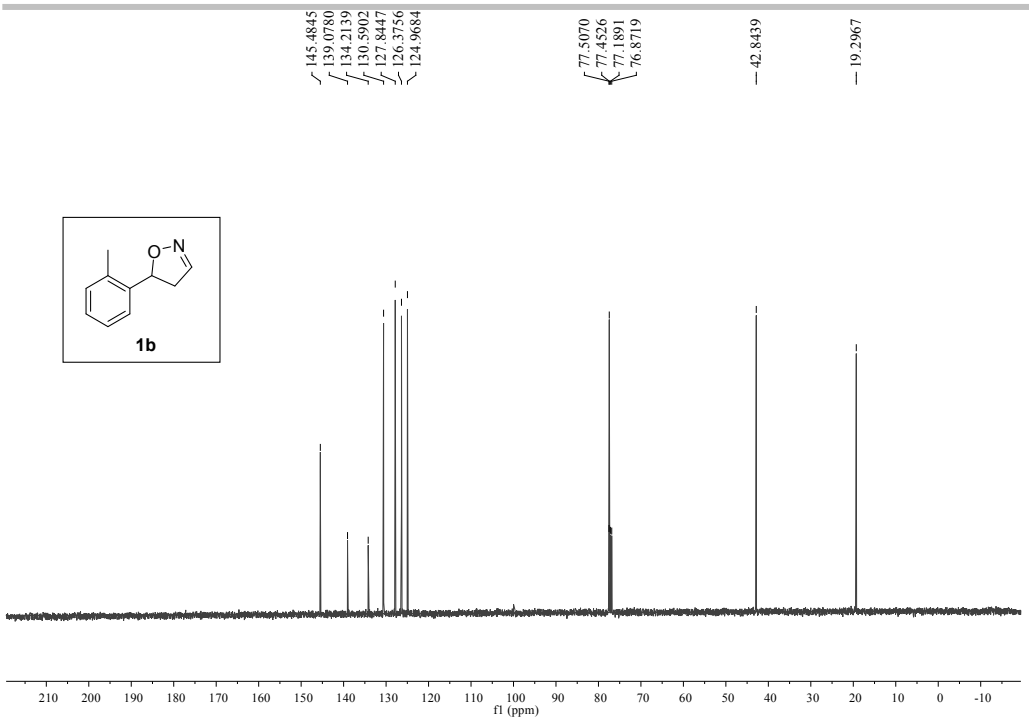
substrate	***S/R*-2**	***S/R*-1**
R = 2-Cl-phenyl (**1e**),	yield 13%, ee 96%	yield 61%, ee 32%
4-Cl-phenyl (**1g**),	yield 13%, ee 99%	yield 64%, ee 31%
n-propyl (**1k**),	yield 21%, ee 98%	yield 32%, ee58%
n-butyl (**1l**)	yield 35%, ee 98%	yield 39%, ee 77%

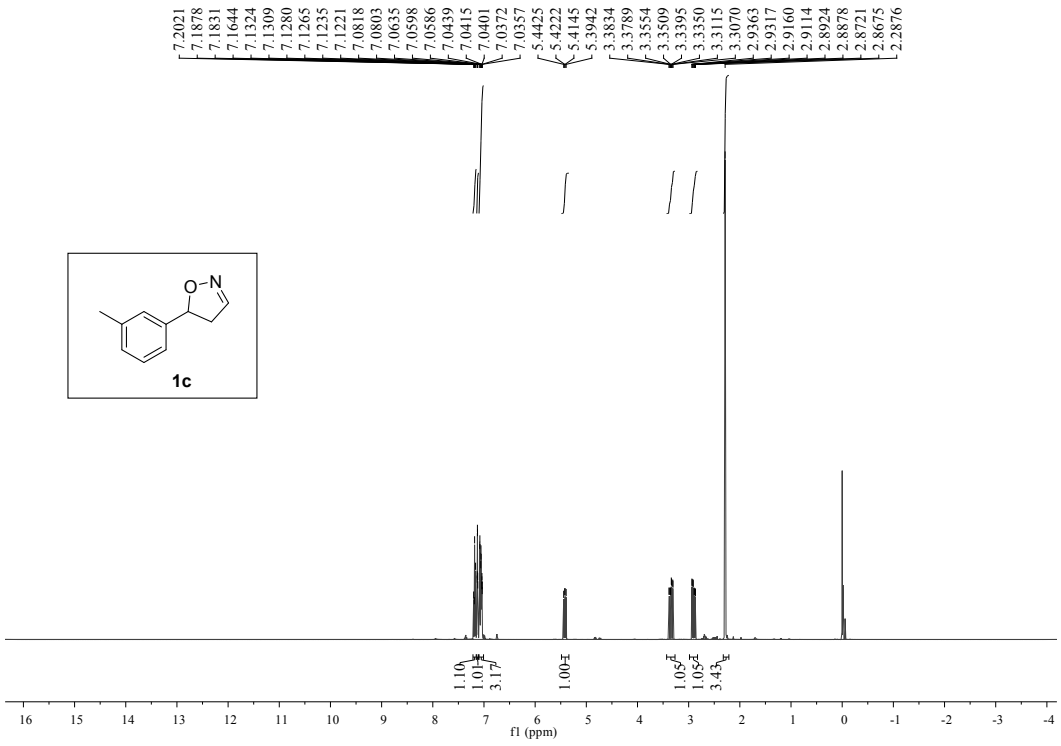
8.2 NMR spectra

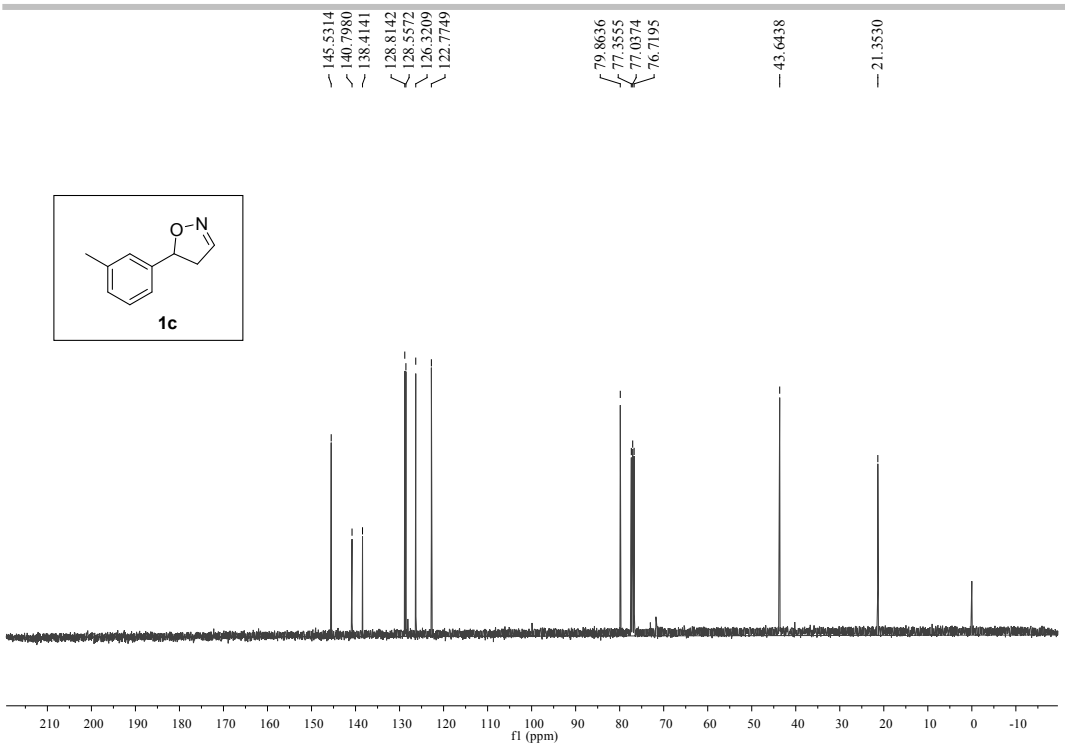


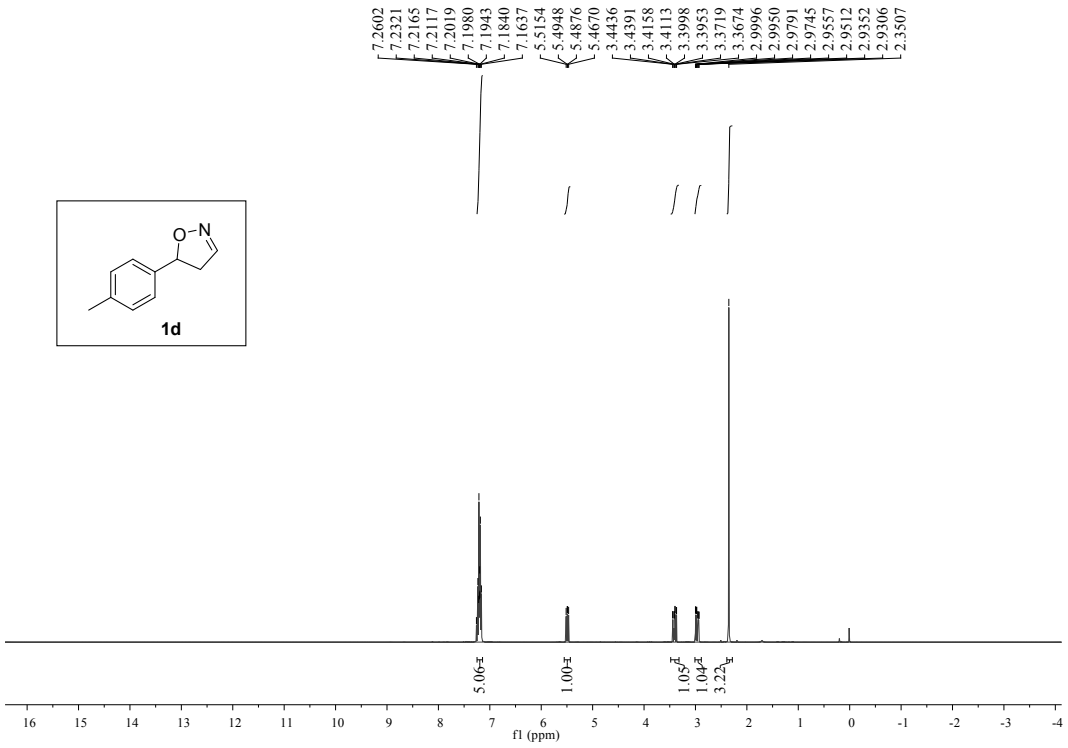

145.6198
140.9750
128.7508
128.1606
125.7940
79.8883
77.5058
77.1875
76.8691
43.7499
1a
f1 (ppm)

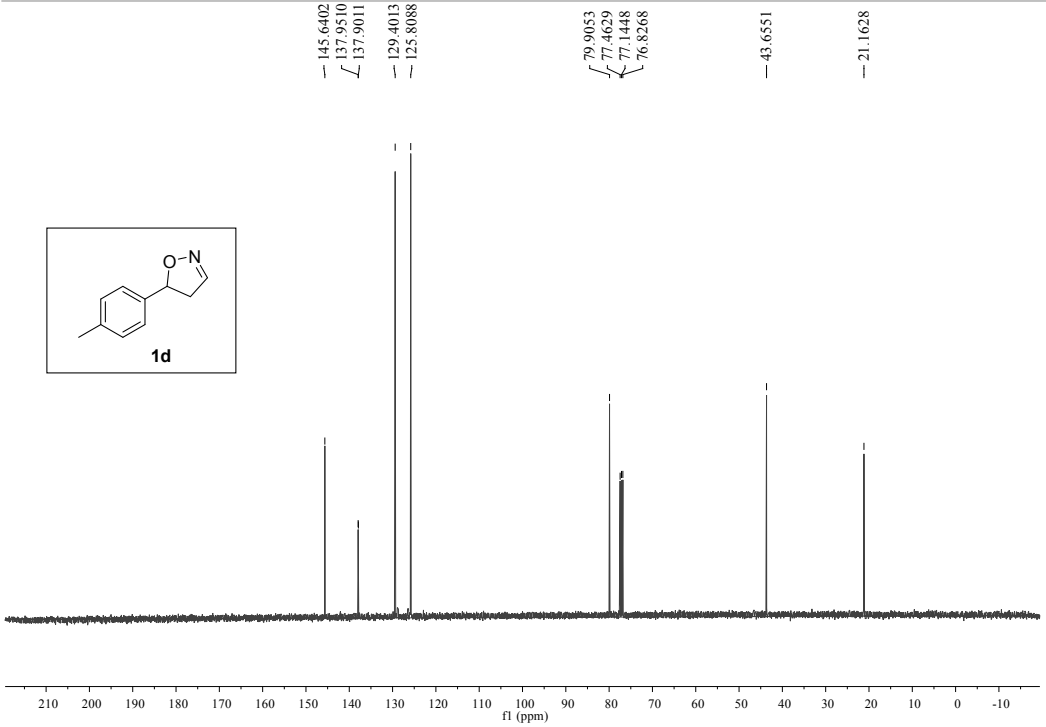

7.3377
7.3294
7.3264
7.3214
7.3150
7.1693
7.1623
7.1590
7.1532
7.1464
7.1354
7.1279
7.1173
7.1054
5.6452
5.6250
5.6171
5.5969
3.4299
3.4264
3.4016
3.3981
3.3864
3.3828
3.3580
3.3546
2.8064
2.8040
2.7863
2.7839
2.7628
2.7605
2.7427
2.7404
2.2625
1.02
4.06
1.00
1.05
1.05
3.16
1b
f1 (ppm)

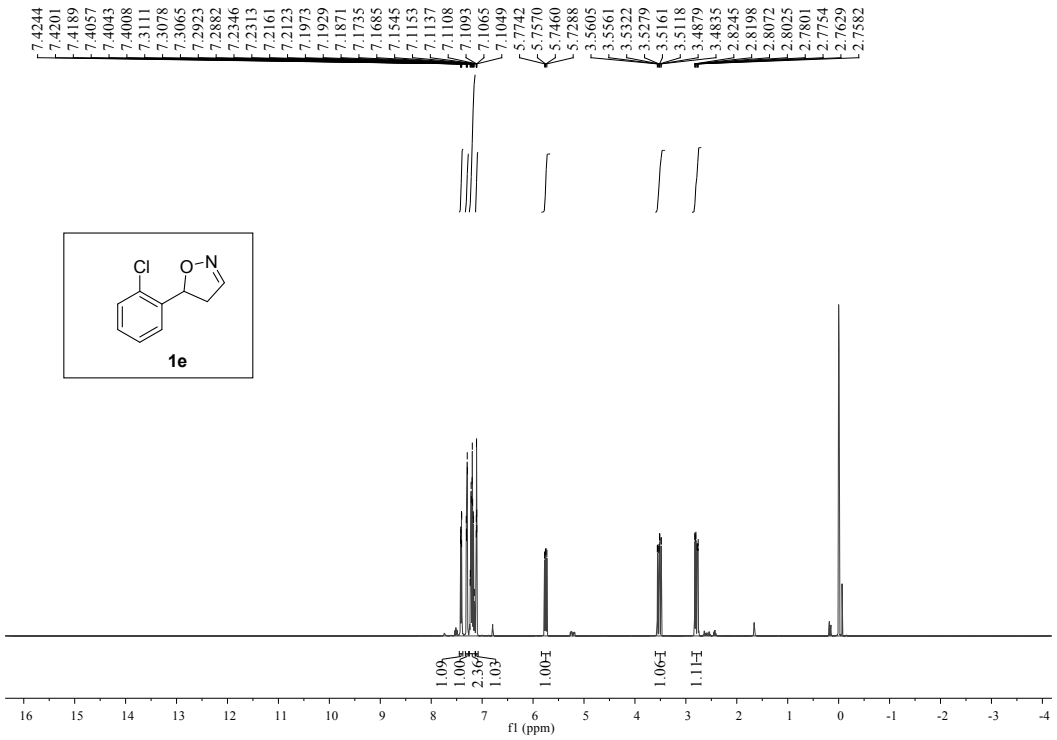

145.4845
139.0780
134.2139
130.5902
127.8447
126.3756
124.9684
77.5070
77.4526
77.1891
76.8719
42.8439
19.2967
1b
f1 (ppm)

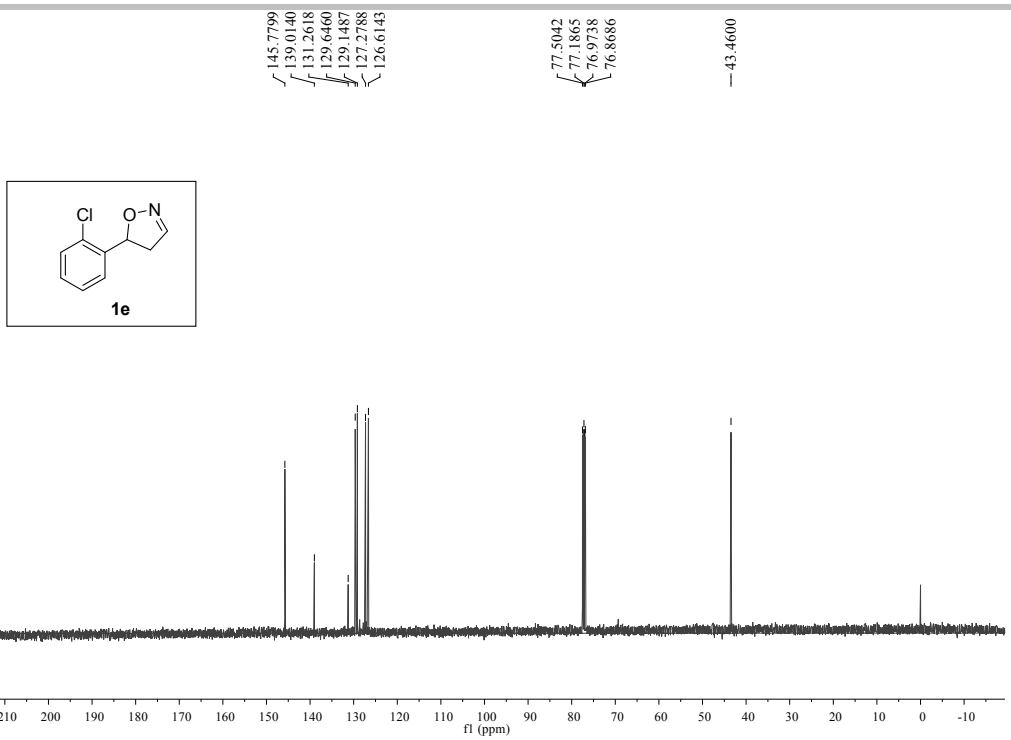

7.2021
7.1878
7.1831
7.1644
7.1324
7.1309
7.1280
7.1265
7.1235
7.1221
7.0818
7.0803
7.0635
7.0598
7.0586
7.0439
7.0415
7.0401
7.0372
7.0357
5.4425
5.4222
5.4145
5.3942
3.3834
3.3789
3.3554
3.3509
3.3395
3.3350
3.3115
3.3070
2.9363
2.9317
2.9160
2.9114
2.8924
2.8878
2.8721
2.8675
2.2876
1.10
1.01
3.17
1.00
1.05
1.05
3.43
1c
f1 (ppm)

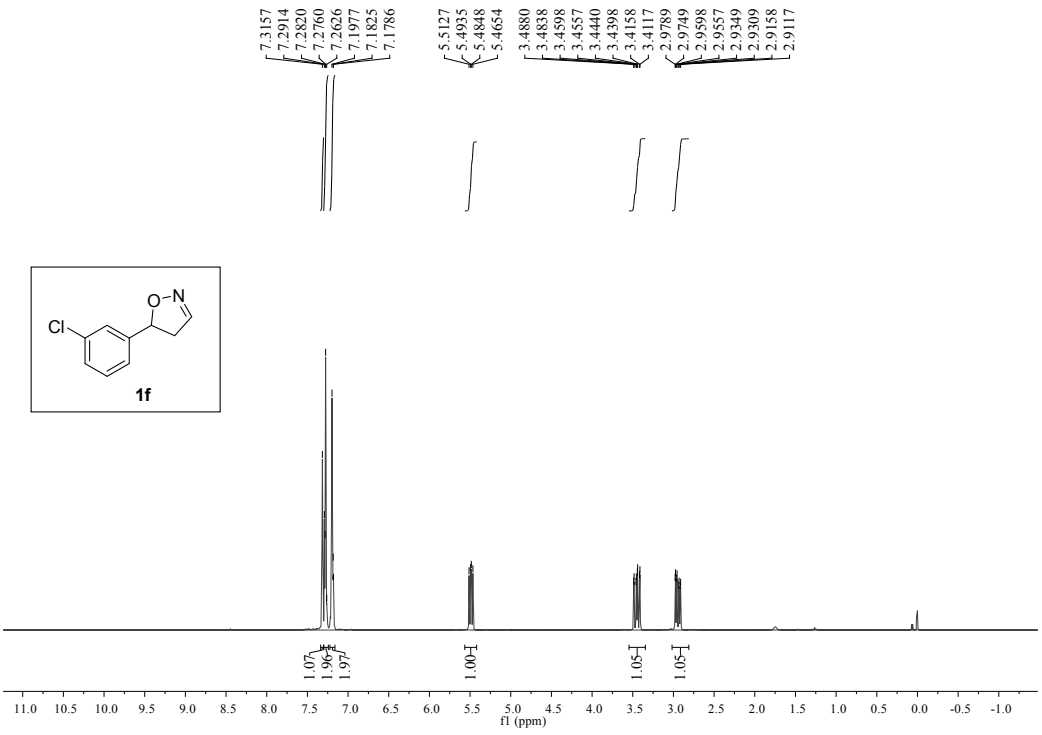

145.5314
140.7980
138.4141
128.8142
128.5572
126.3209
122.7749
79.8636
77.3555
77.0374
76.7195
43.6438
21.3530
1c
f1 (ppm)

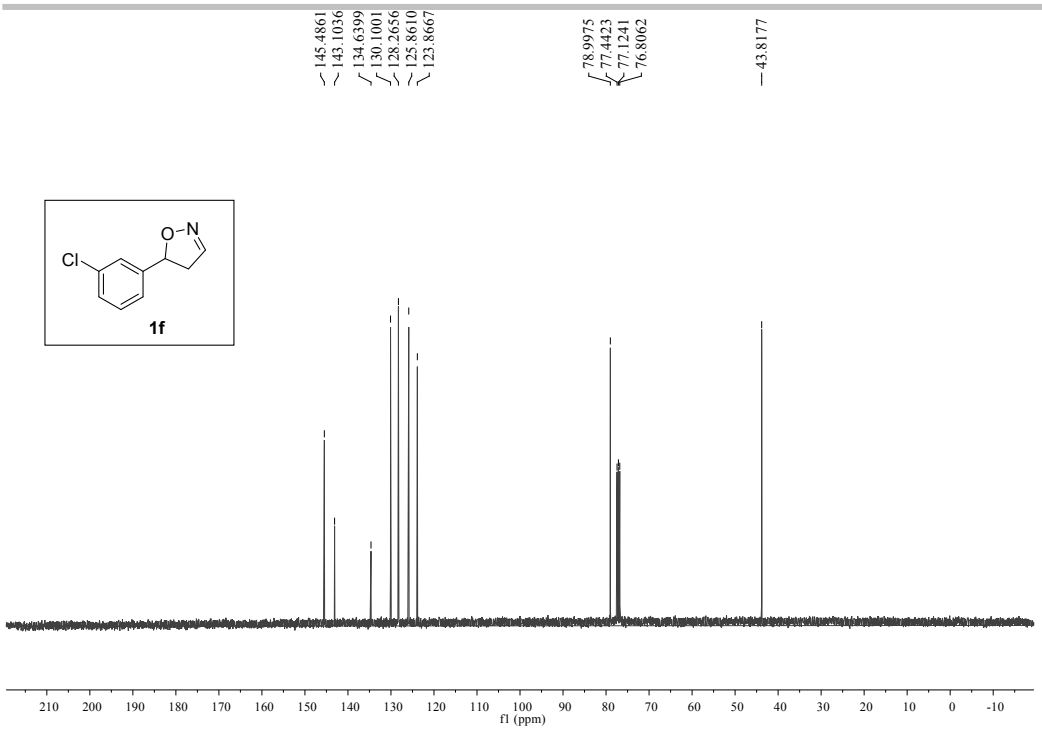

7.2602
7.2321
7.2165
7.2117
7.2019
7.1980
7.1943
7.1840
7.1637
5.5154
5.4948
5.4876
5.4670
3.4436
3.4391
3.4158
3.4113
3.3998
3.3953
3.3719
3.3674
2.9996
2.9950
2.9791
2.9745
2.9557
2.9512
2.9352
2.9306
2.3507
5.06
1.00
1.05
1.04
3.22
1d
f1 (ppm)

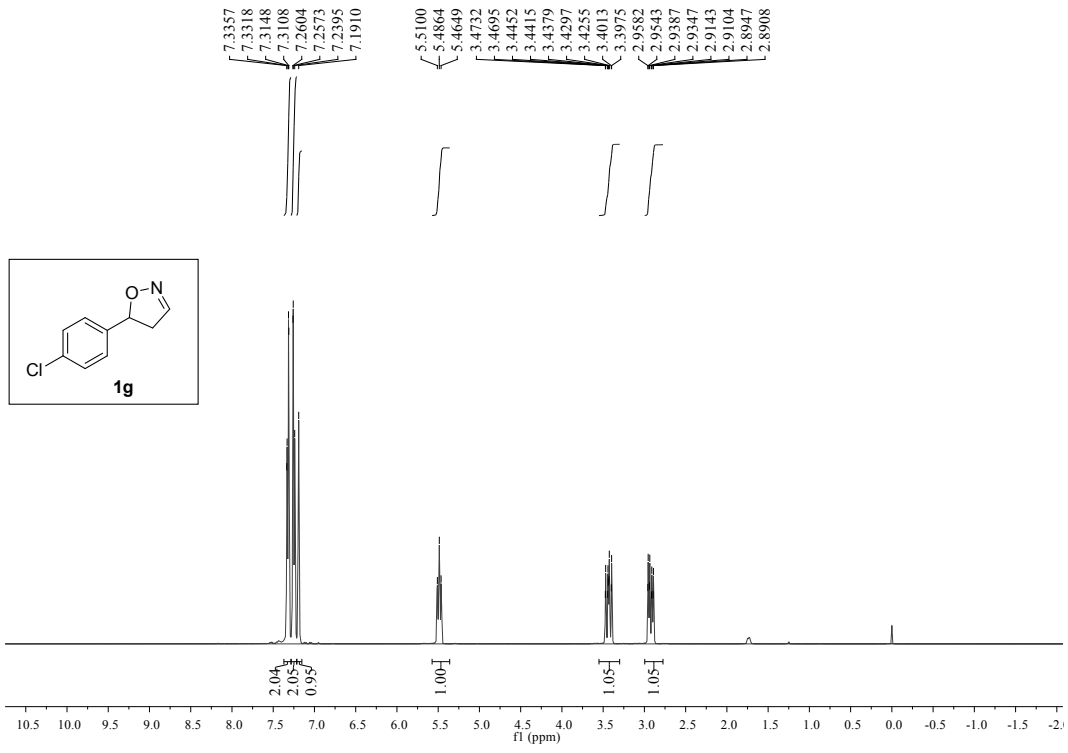

145.6402
137.9510
137.9011
129.4013
125.8088
79.9053
77.4629
77.1448
76.8268
43.6551
21.1628
1d
f1 (ppm)

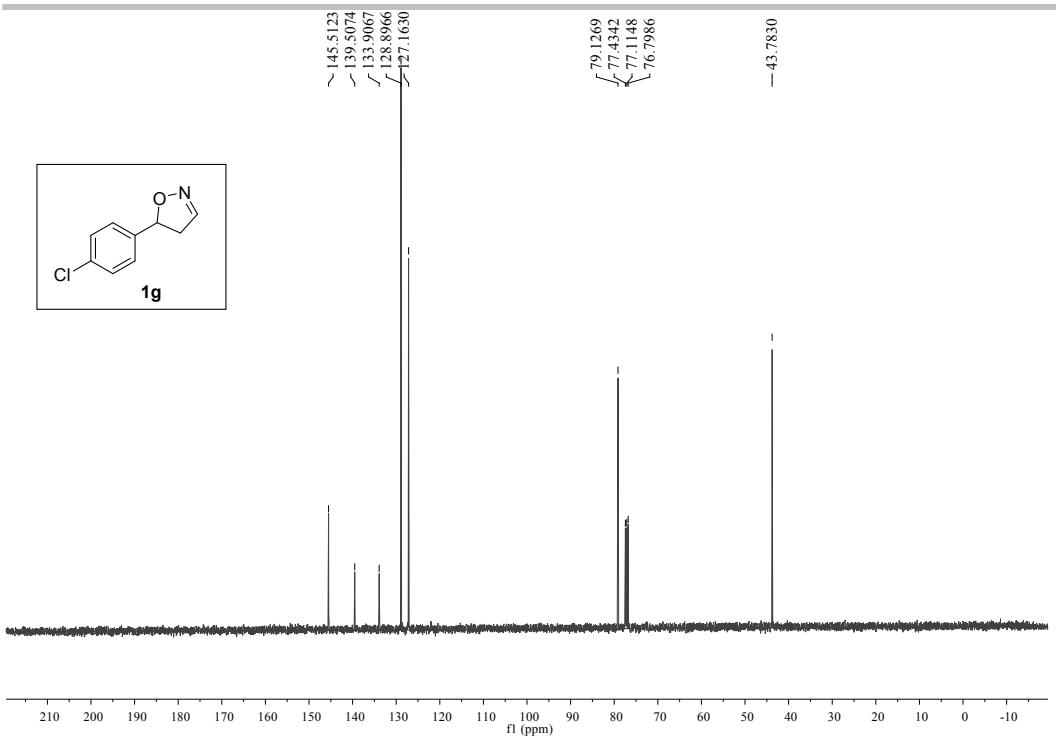

7.4244
7.4201
7.4189
7.4057
7.4043
7.4008
7.3111
7.3078
7.3065
7.2923
7.2882
7.2346
7.2313
7.2161
7.2123
7.1973
7.1929
7.1871
7.1735
7.1685
7.1545
7.1153
7.1137
7.1108
7.1093
7.1065
7.1049
5.7742
5.7570
5.7460
5.7288
3.5605
3.5561
3.5322
3.5279
3.5161
3.5118
3.4879
3.4835
2.8245
2.8198
2.8072
2.8025
2.7801
2.7754
2.7629
2.7582
Cl
1e
1.09
1.00
2.36
1.03
1.00
1.06
1.11
f1 (ppm)

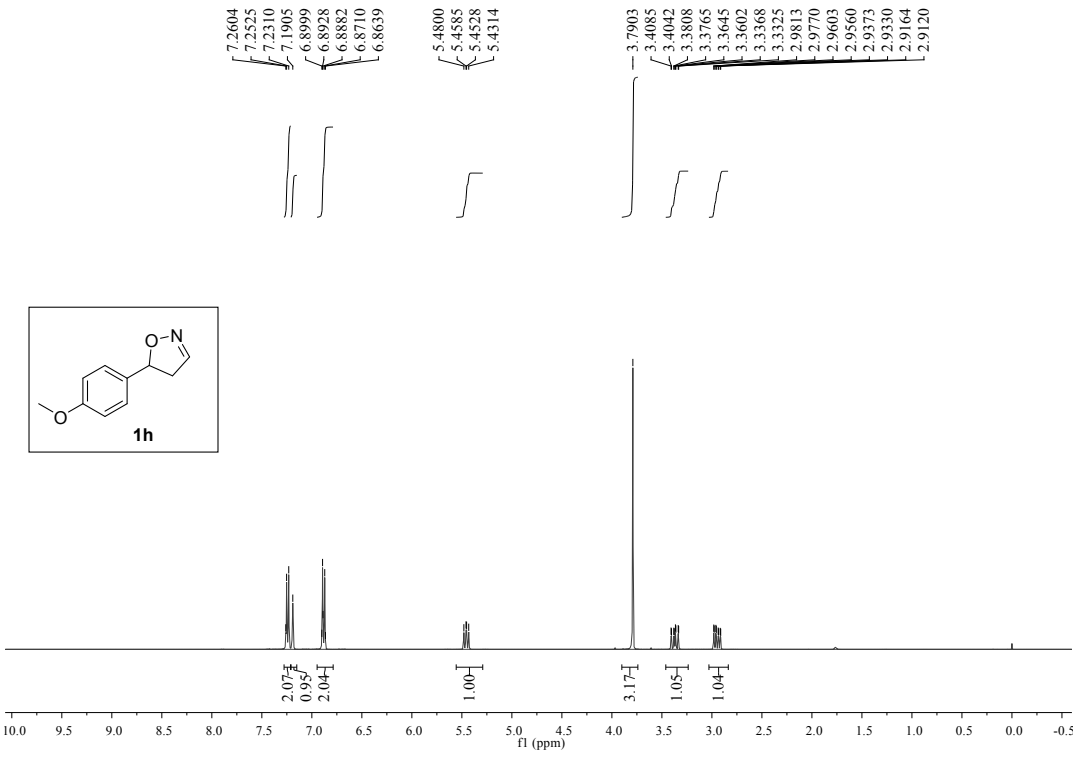

145.7799
139.0140
131.2618
129.6460
129.1487
127.2788
126.6143
77.5042
77.1865
76.9738
76.8686
43.4600
Cl
O
N
1e
f1 (ppm)

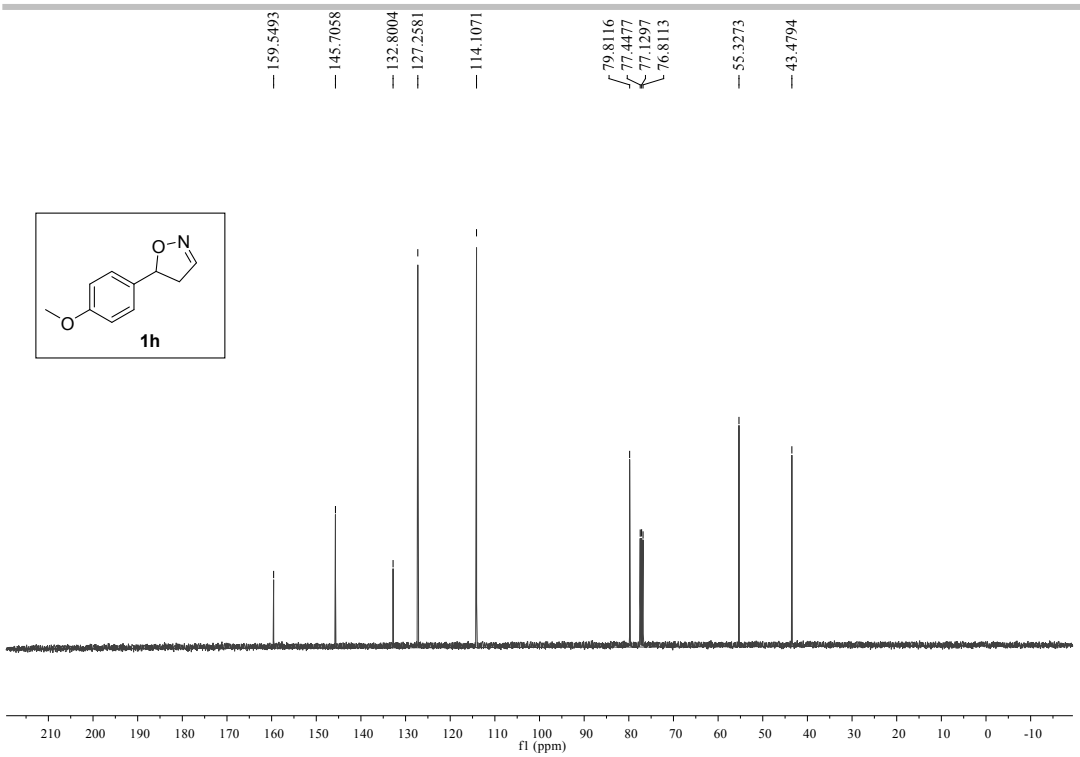

7.3157
7.2914
7.2820
7.2760
7.2626
7.1977
7.1825
7.1786
5.5127
5.4935
5.4848
5.4654
3.4880
3.4838
3.4598
3.4557
3.4440
3.4398
3.4158
3.4117
2.9789
2.9749
2.9598
2.9557
2.9349
2.9309
2.9158
2.9117
Cl
O
N
1f
1.07
1.96
1.97
1.00
1.05
1.05
f1 (ppm)

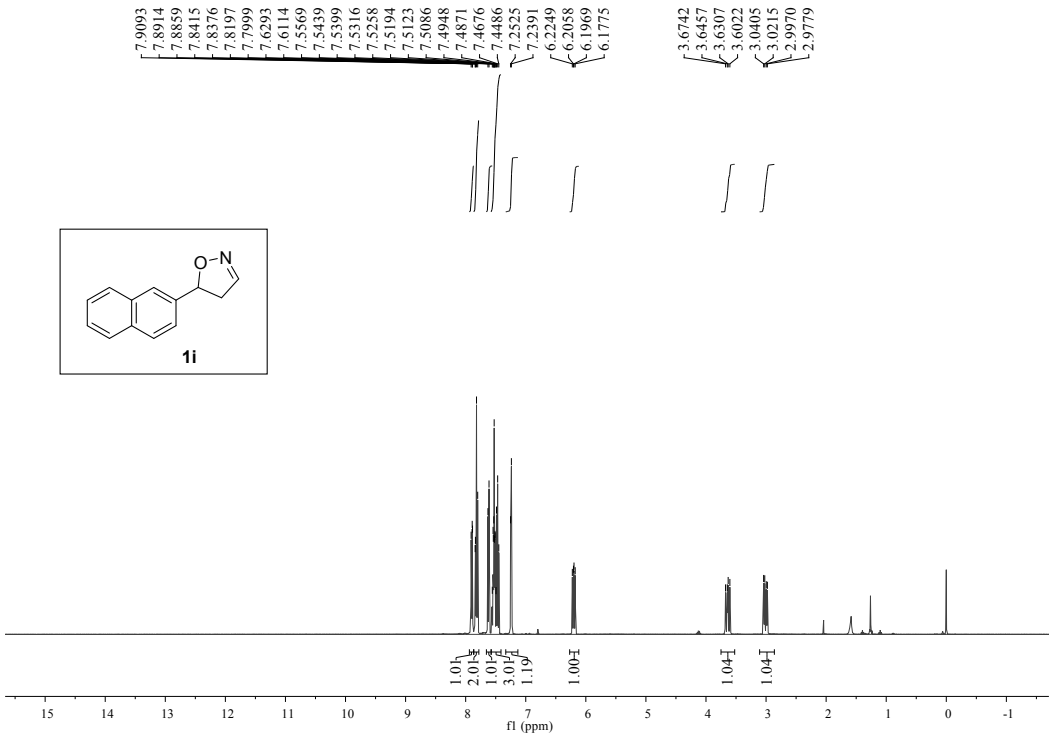

145.4861
143.1036
134.6399
130.1001
128.2656
125.8610
123.8667
78.9975
77.4423
77.1241
76.8062
43.8177
Cl
O
N
1f
f1 (ppm)

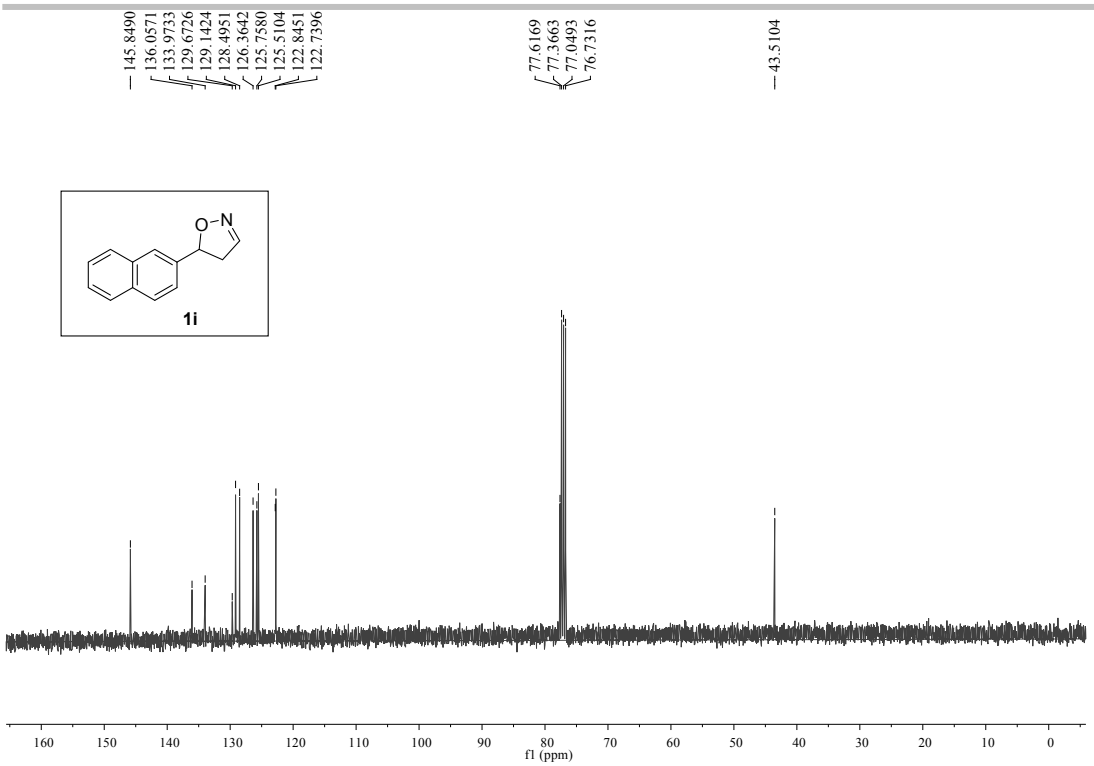

7.3357
7.3318
7.3148
7.3108
7.2604
7.2573
7.2395
7.1910
5.5100
5.4864
5.4649
3.4732
3.4695
3.4452
3.4415
3.4379
3.4297
3.4255
3.4013
3.3975
2.9582
2.9543
2.9387
2.9347
2.9143
2.9104
2.8947
2.8908
Cl
O
N
1g
2.04
2.05
0.95
1.00
1.05
1.05
f1 (ppm)

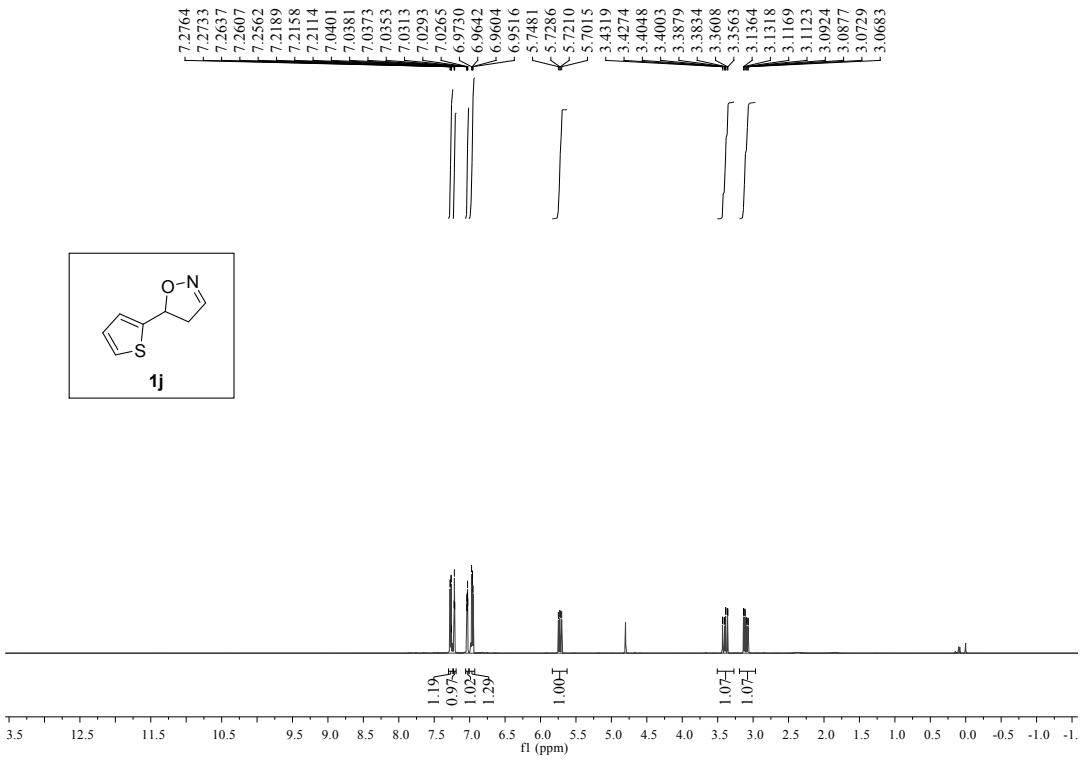

145.5123
139.5074
133.9067
128.8966
127.1630
79.1269
77.4342
77.1148
76.7986
43.7830
O
N
Cl
1g
f1 (ppm)


7.2604
7.2525
7.2310
7.1905
6.8999
6.8928
6.8882
6.8710
6.8639
5.4800
5.4585
5.4528
5.4314
3.7903
3.4085
3.4042
3.3808
3.3765
3.3645
3.3602
3.3368
3.3325
2.9813
2.9770
2.9603
2.9560
2.9373
2.9330
2.9164
2.9120
O
N
O
1h
2.07
0.95
2.04
1.00
3.17
1.05
1.04
f1 (ppm)

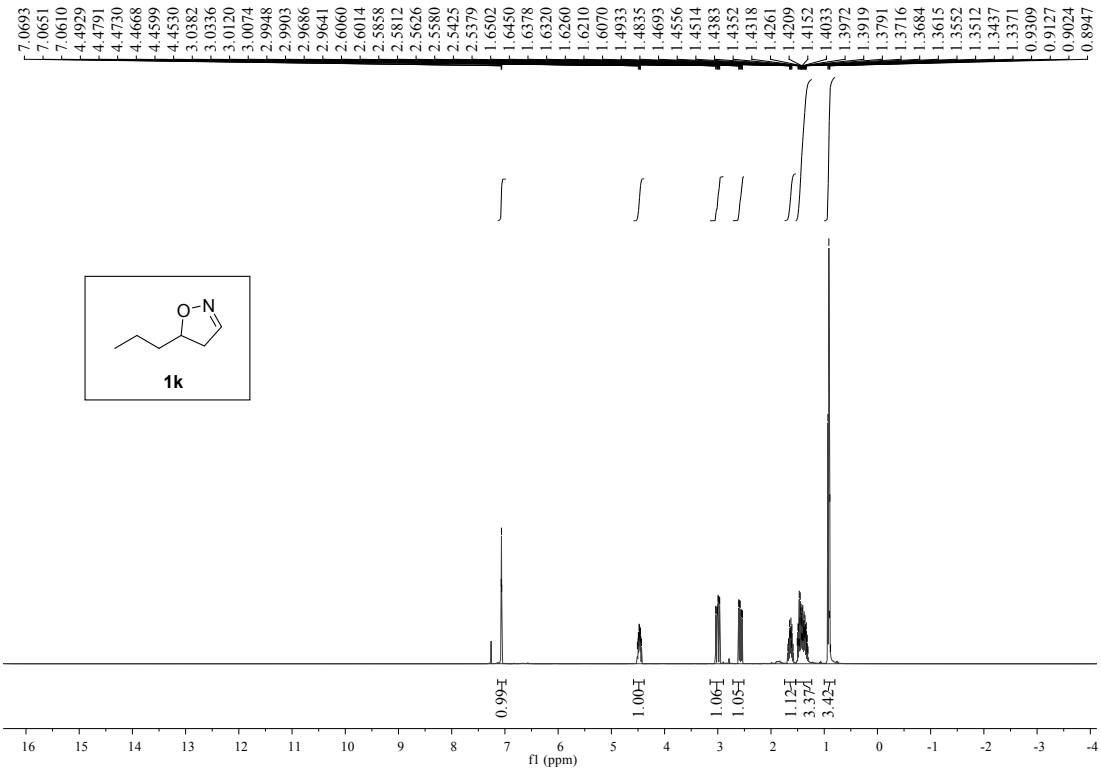

159.5493
145.7058
132.8004
127.2581
114.1071
79.8116
77.4477
77.1297
76.8113
55.3273
43.4794
1h
f1 (ppm)

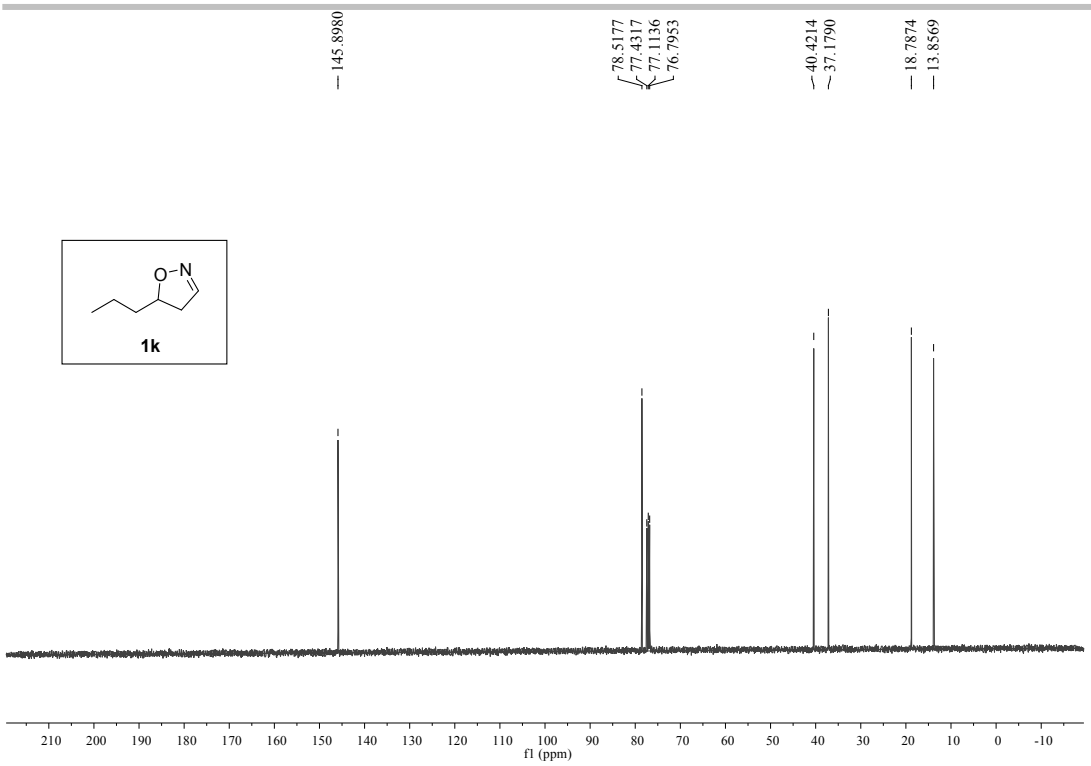

7.9093
7.8914
7.8859
7.8415
7.8376
7.8197
7.7999
7.6293
7.6114
7.5569
7.5439
7.5399
7.5316
7.5258
7.5194
7.5123
7.5086
7.4948
7.4871
7.4676
7.4486
7.2525
7.2391
6.2249
6.2058
6.1969
6.1775
3.6742
3.6457
3.6307
3.6022
3.0405
3.0215
2.9970
2.9779
1i
1.01
2.01
1.01
3.01
1.19
1.00
1.04
1.04
f1 (ppm)

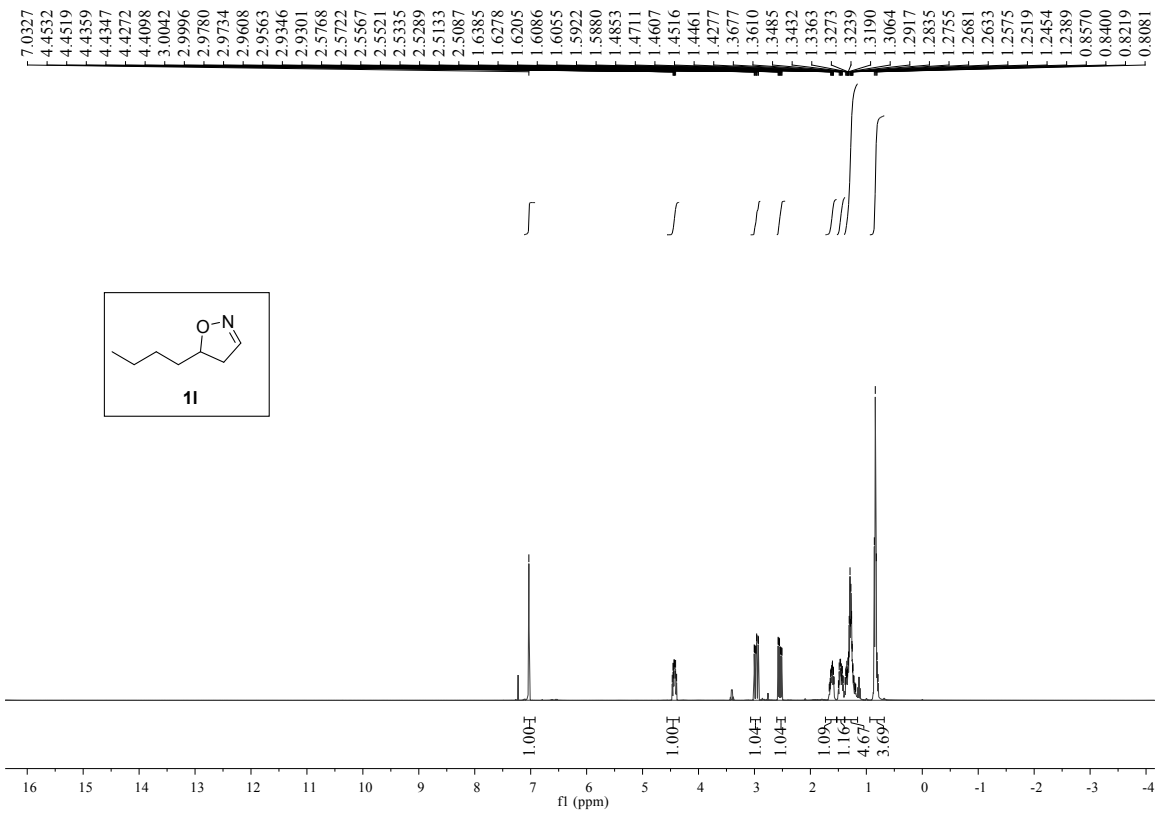

145.8490
136.0571
133.9733
129.6726
129.1424
128.4951
126.3642
125.7580
125.5104
122.8451
122.7396
77.6169
77.3663
77.0493
76.7316
43.5104
O
N
1i
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

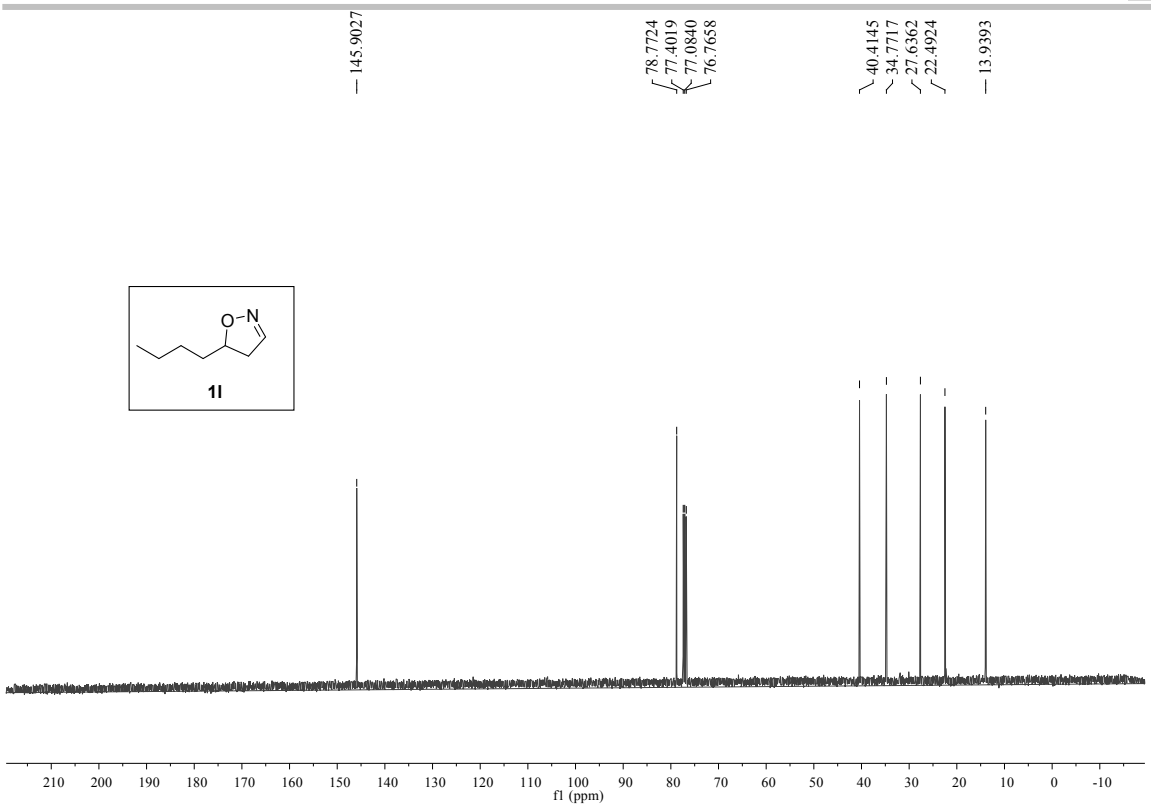

7.2764
7.2733
7.2637
7.2607
7.2562
7.2189
7.2158
7.2114
7.0401
7.0381
7.0373
7.0353
7.0313
7.0293
7.0265
6.9730
6.9642
6.9604
6.9516
5.7481
5.7286
5.7210
5.7015
3.4319
3.4274
3.4048
3.4003
3.3879
3.3834
3.3608
3.3563
3.1364
3.1318
3.1169
3.1123
3.0924
3.0877
3.0729
3.0683
O
N
S
1j
1.19
0.97
1.02
1.29
1.00
1.07
1.07
3.5
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

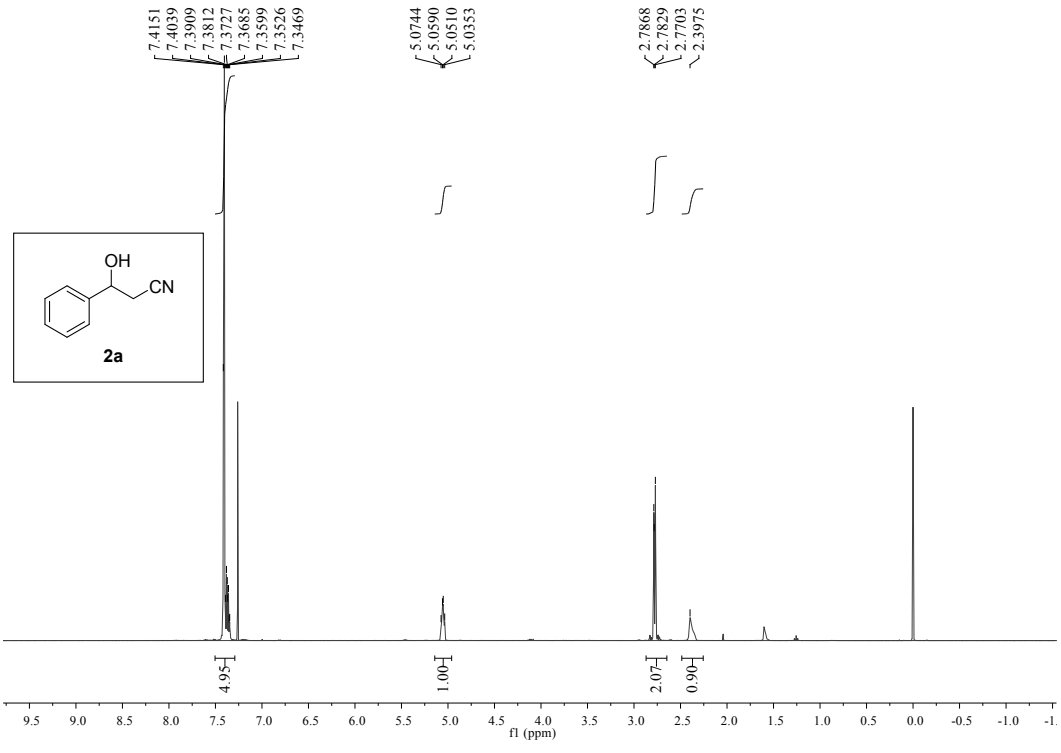

145.8422
143.4638
126.9578
125.7056
125.3820
75.8869
43.4853
O
N
S
1j
fl (ppm)

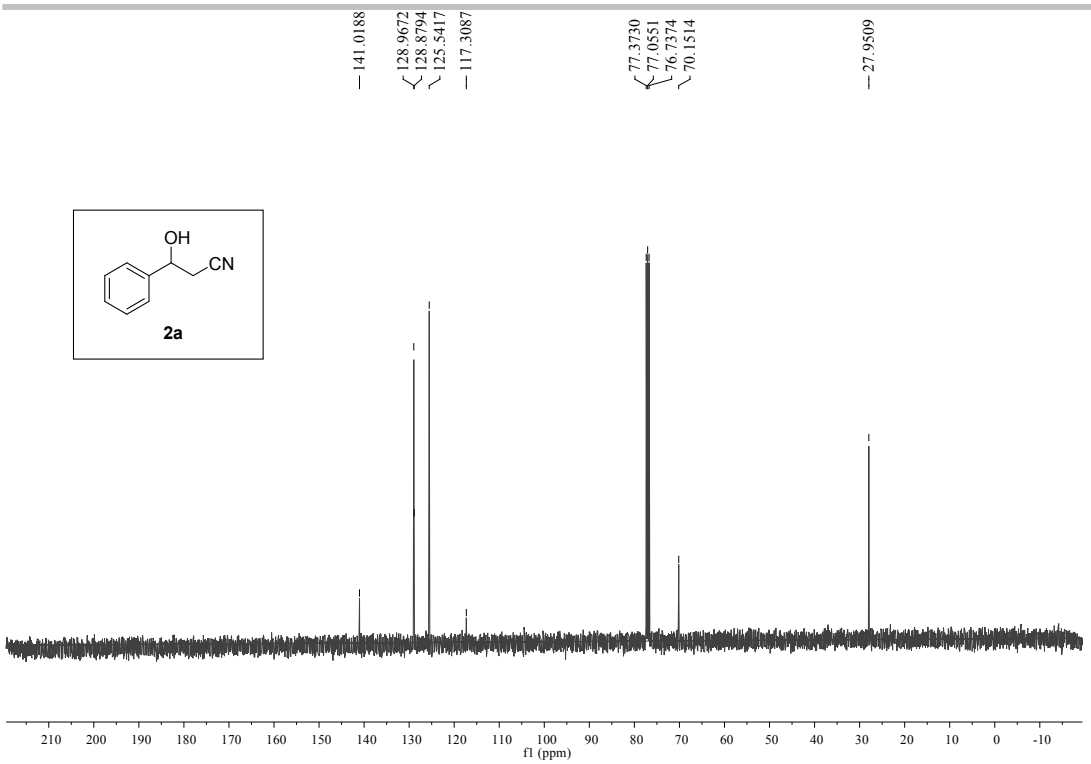

7.0693
7.0651
7.0610
4.4929
4.4791
4.4730
4.4668
4.4599
4.4530
3.0382
3.0336
3.0120
3.0074
2.9948
2.9903
2.9686
2.9641
2.6060
2.6014
2.5858
2.5812
2.5626
2.5580
2.5425
2.5379
1.6502
1.6450
1.6378
1.6320
1.6260
1.6210
1.6070
1.4933
1.4835
1.4693
1.4556
1.4514
1.4383
1.4352
1.4318
1.4261
1.4209
1.4152
1.4033
1.3972
1.3919
1.3791
1.3716
1.3684
1.3615
1.3552
1.3512
1.3437
1.3371
0.9309
0.9127
0.9024
0.8947
O
N
1k
0.99
1.00
1.06
1.05
1.12
3.37
3.42
fl (ppm)

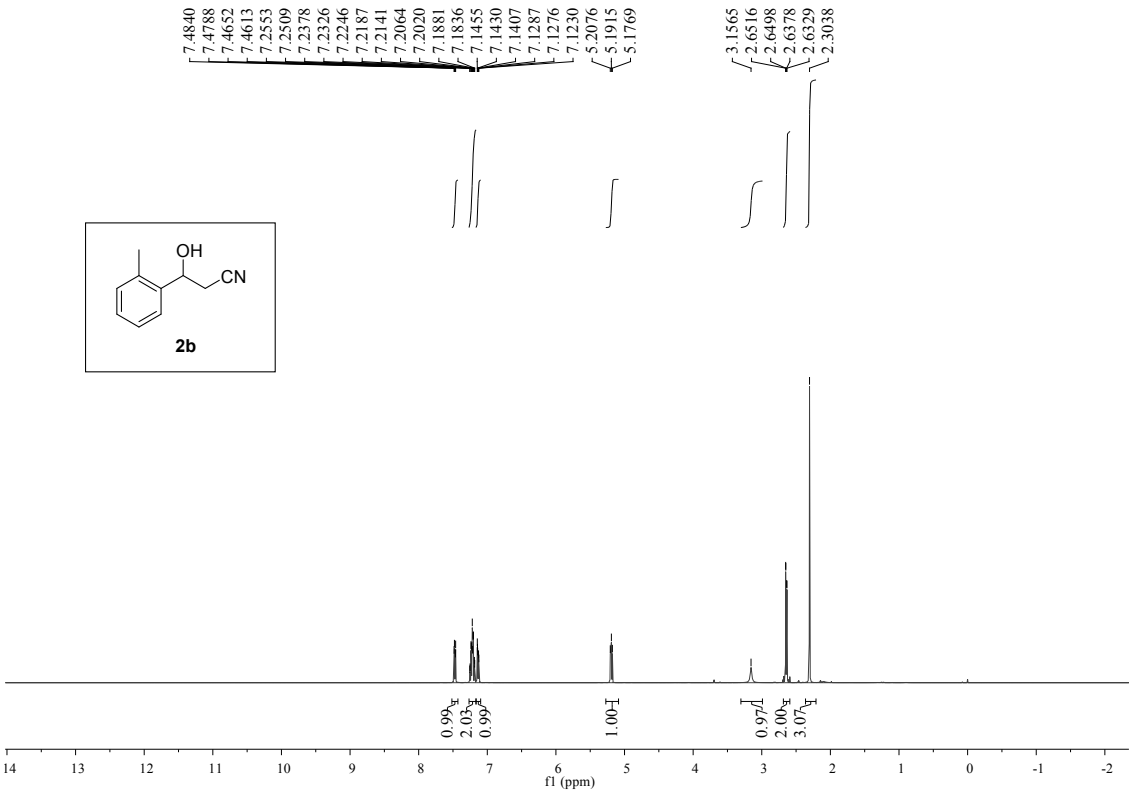

145.8980
78.5177
77.4317
77.1136
76.7953
40.4214
37.1790
18.7874
13.8569
1k
fl (ppm)

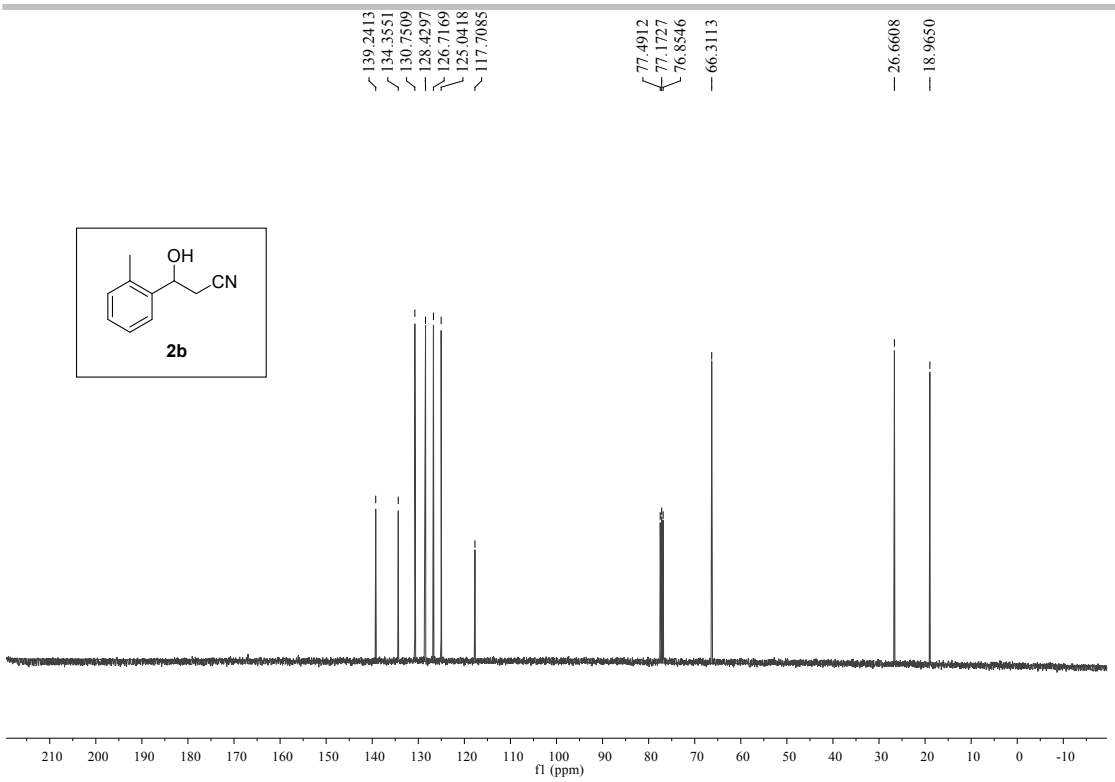

7.0327
4.4532
4.4519
4.4359
4.4347
4.4272
4.4098
3.0042
2.9996
2.9780
2.9734
2.9608
2.9563
2.9346
2.9301
2.5768
2.5722
2.5567
2.5521
2.5335
2.5289
2.5133
2.5087
1.6385
1.6278
1.6205
1.6086
1.6055
1.5922
1.5880
1.4853
1.4711
1.4607
1.4516
1.4461
1.4277
1.3677
1.3610
1.3485
1.3432
1.3363
1.3273
1.3239
1.3190
1.3064
1.2917
1.2835
1.2755
1.2681
1.2633
1.2575
1.2519
1.2454
1.2389
0.8570
0.8400
0.8219
0.8081
1l
1.00
1.00
1.04
1.04
1.09
1.16
4.67
3.69
fl (ppm)

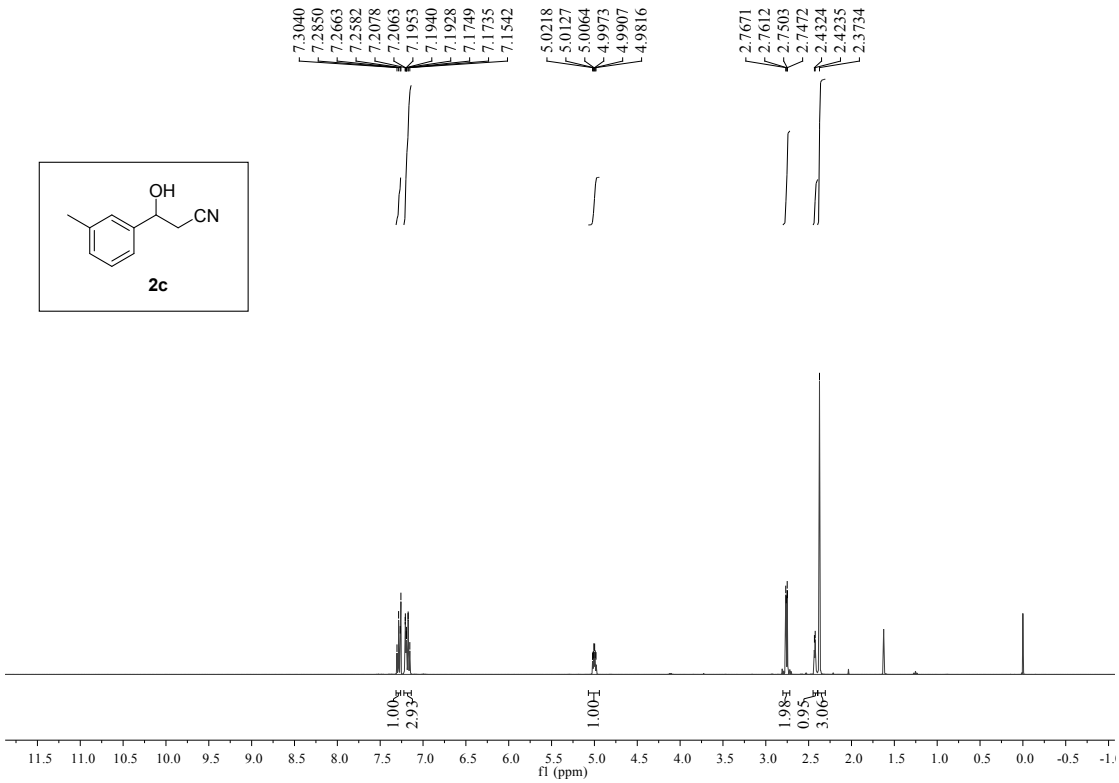

145.9027
78.7724
77.4019
77.0840
76.7658
40.4145
34.7717
27.6362
22.4924
13.9393
1l
f1 (ppm)

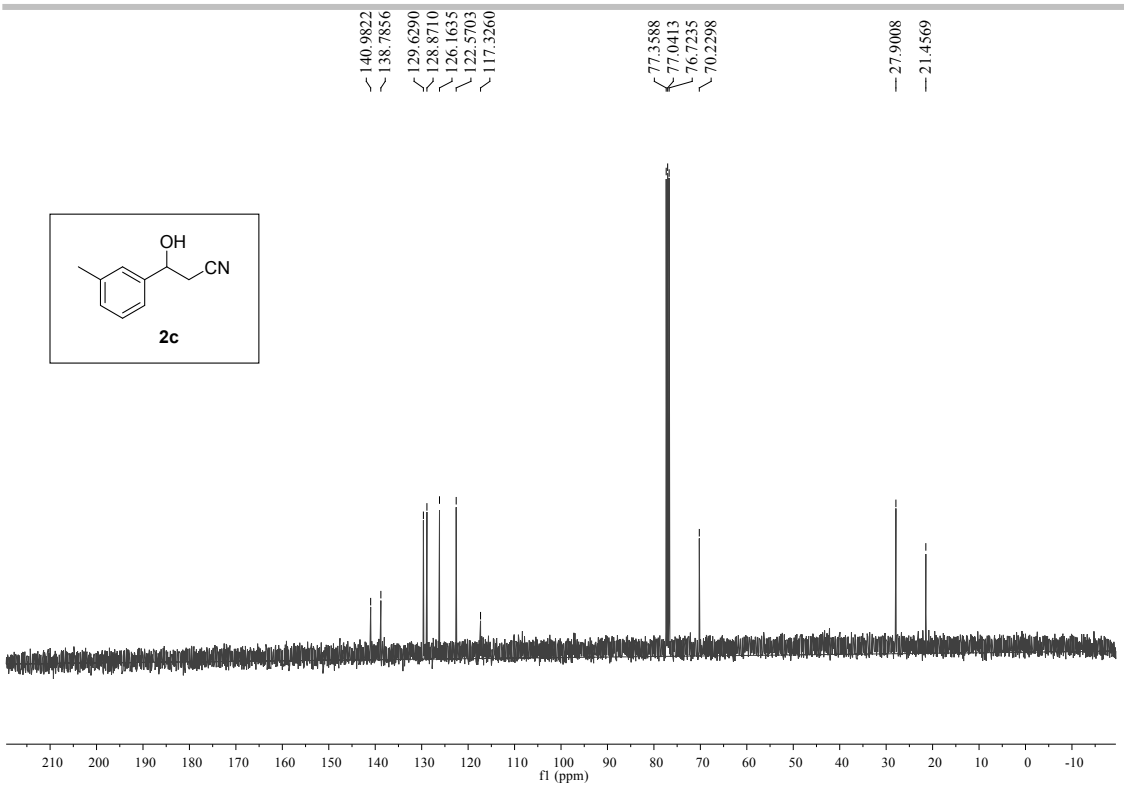

7.4151
7.4039
7.3909
7.3812
7.3727
7.3685
7.3599
7.3526
7.3469
5.0744
5.0590
5.0510
5.0353
2.7868
2.7829
2.7703
2.3975
OH
CN
2a
4.95
1.00
2.07
0.90
f1 (ppm)

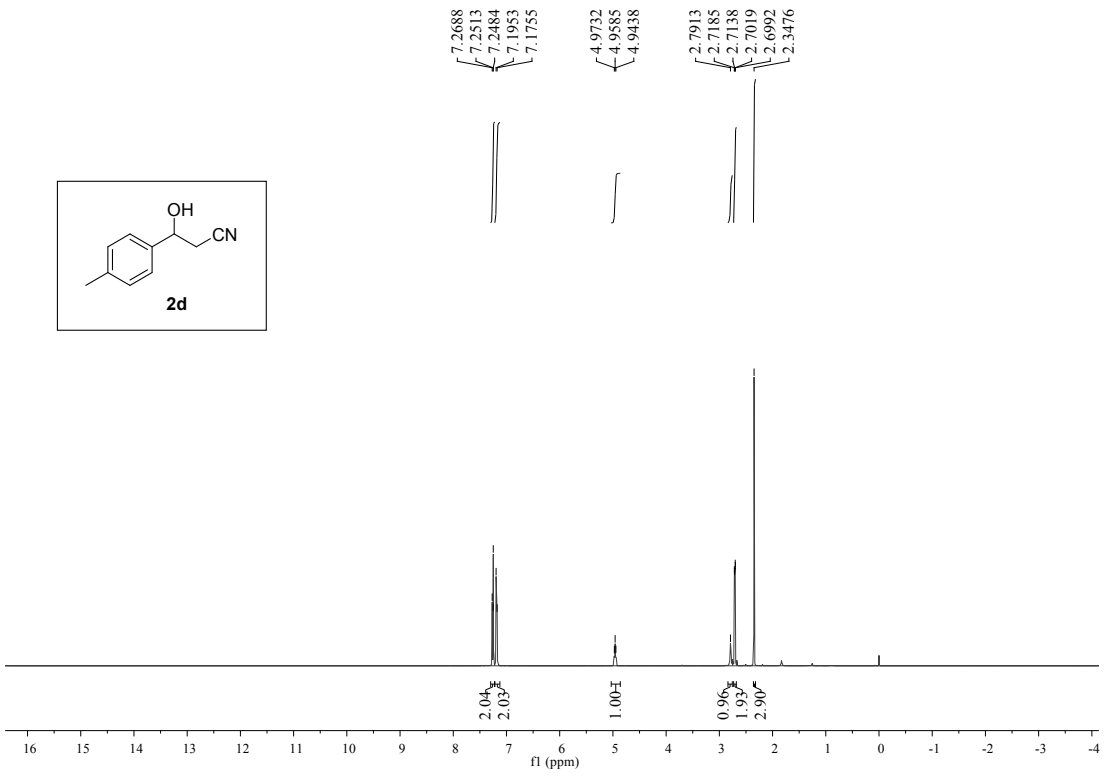

141.0188
128.9672
128.8794
125.5417
117.3087
77.3730
77.0551
76.7374
70.1514
27.9509
OH
CN
2a
f1 (ppm)

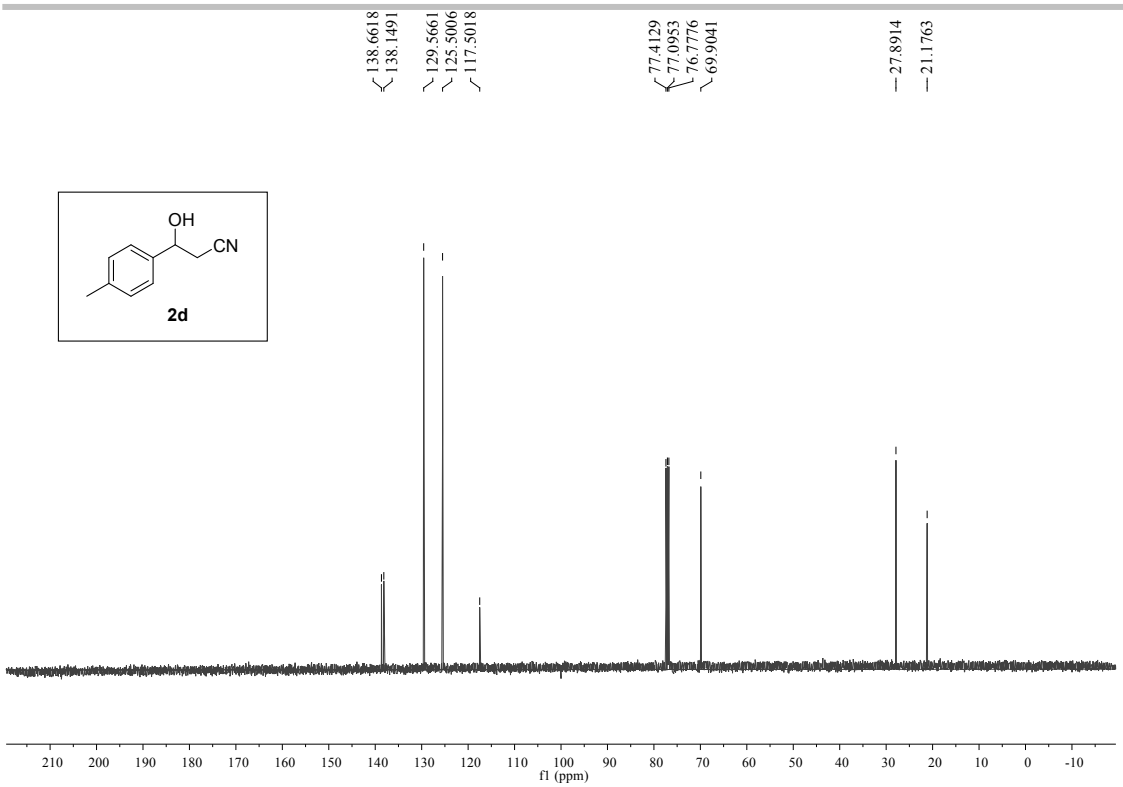

7.4840
7.4788
7.4652
7.4613
7.2553
7.2509
7.2378
7.2326
7.2246
7.2187
7.2141
7.2064
7.2020
7.1881
7.1836
7.1455
7.1430
7.1407
7.1287
7.1276
7.1230
5.2076
5.1915
5.1769
3.1565
2.6516
2.6498
2.6378
2.6329
2.3038
OH
CN
2b
0.99
2.03
0.99
1.00
0.97
2.00
3.07
f1 (ppm)

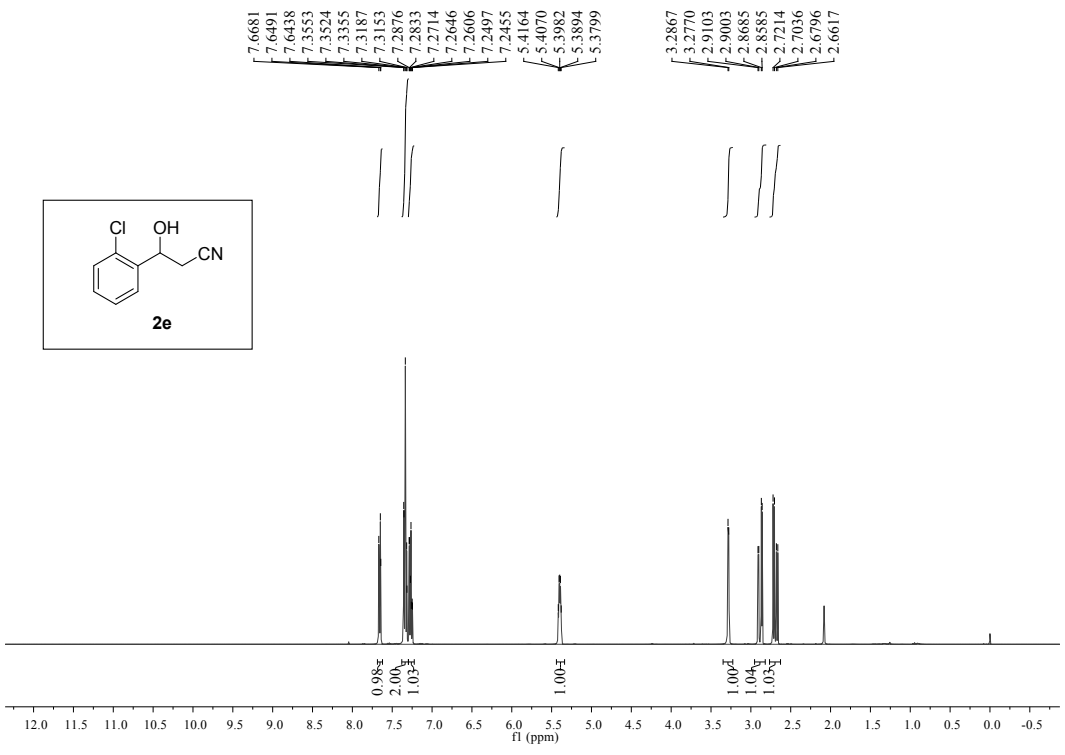

139.2413
134.3551
130.7509
128.4297
126.7169
125.0418
117.7085
77.4912
77.1727
76.8546
66.3113
26.6608
18.9650
OH
CN
2b
f1 (ppm)

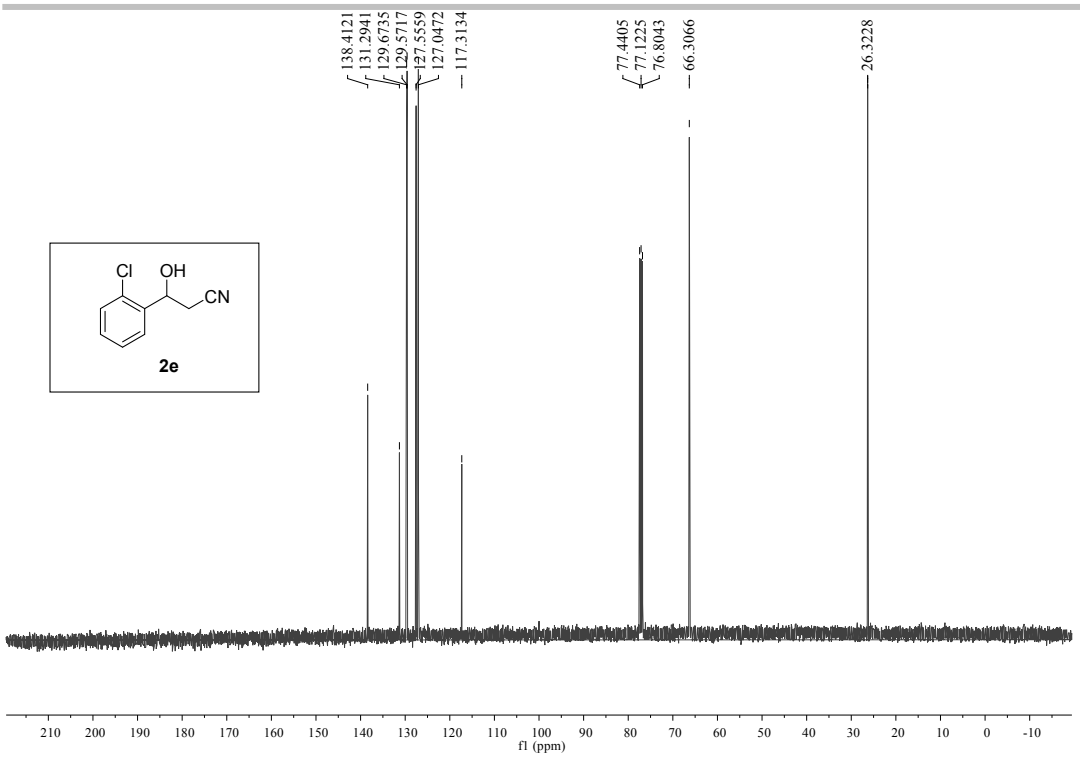

7.3040
7.2850
7.2663
7.2582
7.2078
7.2063
7.1953
7.1940
7.1928
7.1749
7.1735
7.1542
5.0218
5.0127
5.0064
4.9973
4.9907
4.9816
2.7671
2.7612
2.7503
2.7472
2.4324
2.4235
2.3734
OH
CN
2c
1.00
2.93
1.00
1.98
0.95
3.06
f1 (ppm)

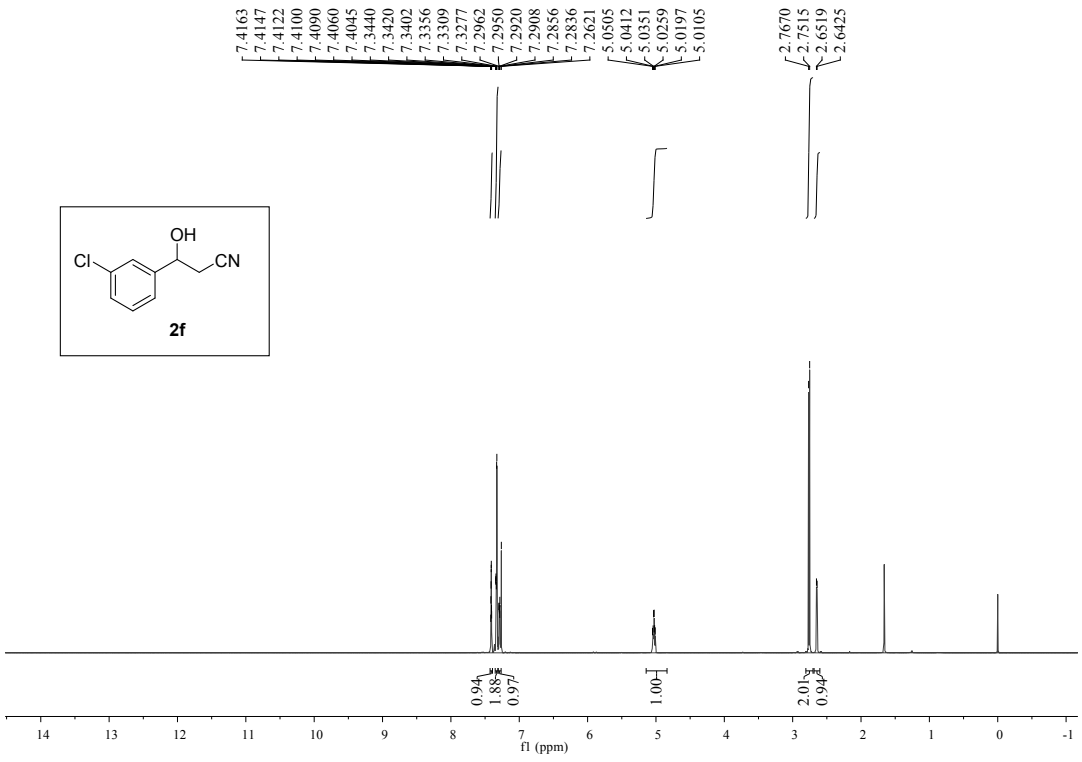

140.9822
138.7856
129.6290
128.8710
126.1635
122.5703
117.3260
77.3588
77.0413
76.7235
70.2298
27.9008
21.4569
OH
CN
2c
f1 (ppm)

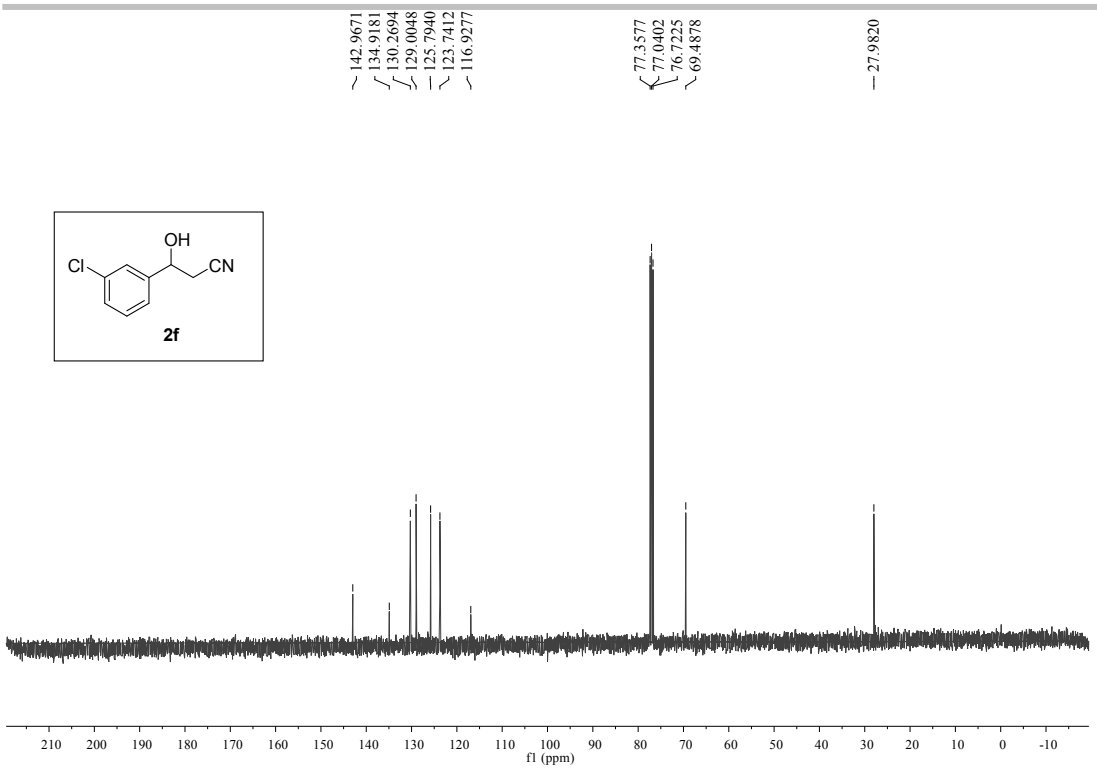

7.2688
7.2513
7.2484
7.1953
7.1755
4.9732
4.9585
4.9438
2.7913
2.7185
2.7138
2.7019
2.6992
2.3476
OH
CN
2d
2.04
2.03
1.00
0.96
1.93
2.90
f1 (ppm)

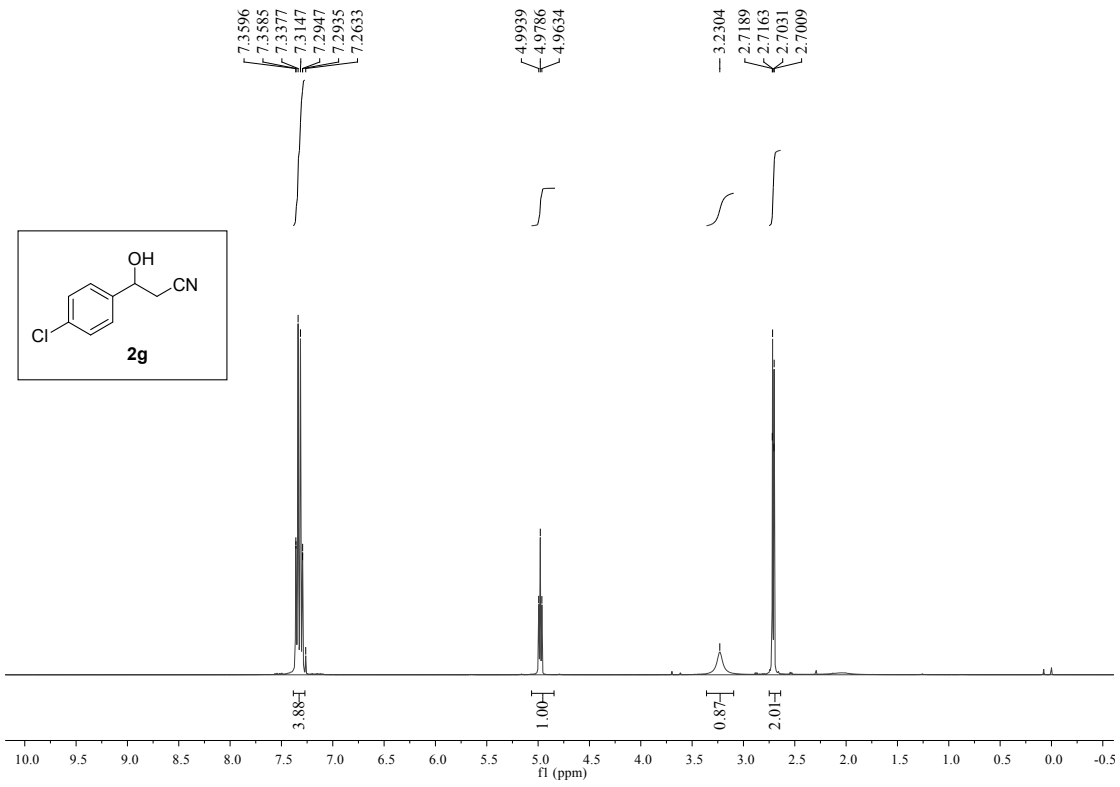

138.6618
138.1491
129.5661
125.5006
117.5018
77.4129
77.0953
76.7776
69.9041
27.8914
21.1763
OH
CN
2d
f1 (ppm)

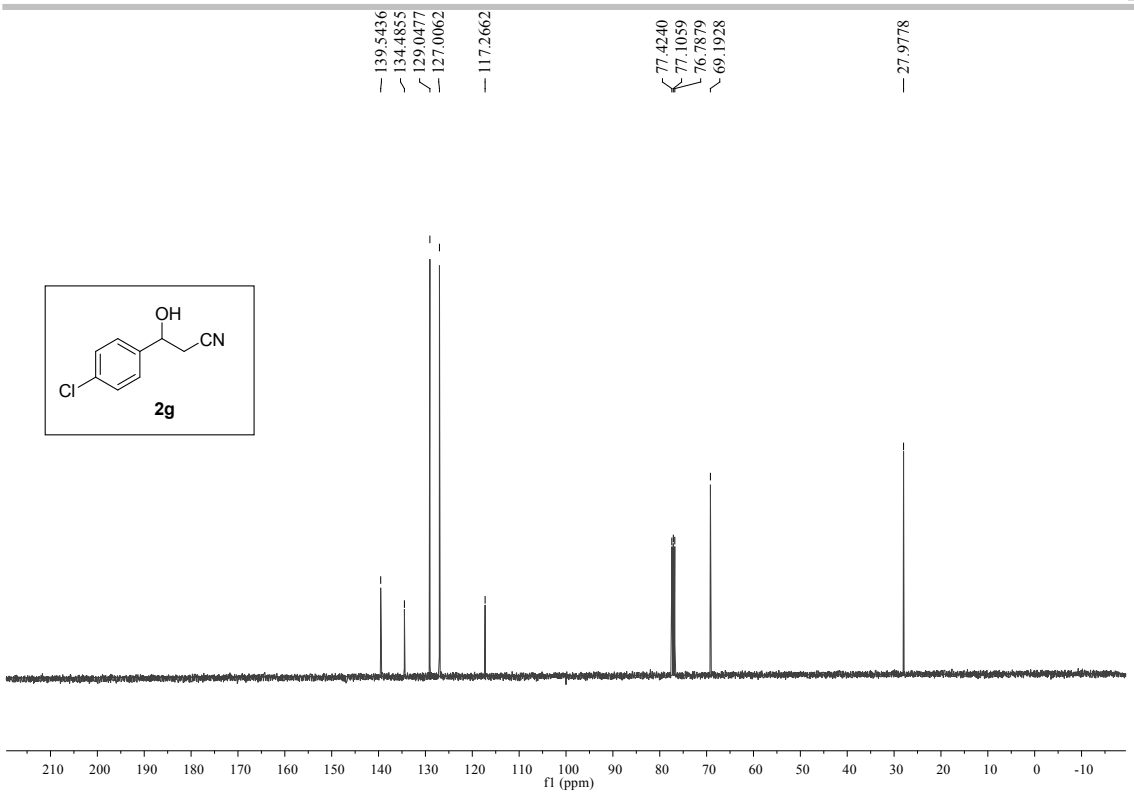

7.6681
7.6491
7.6438
7.3553
7.3524
7.3355
7.3187
7.3153
7.2876
7.2833
7.2714
7.2646
7.2606
7.2497
7.2455
5.4164
5.4070
5.3982
5.3894
5.3799
3.2867
3.2770
2.9103
2.9003
2.8685
2.8585
2.7214
2.7036
2.6796
2.6617
Cl
OH
CN
2e
0.98
2.00
1.03
1.00
1.00
1.04
1.03
f1 (ppm)

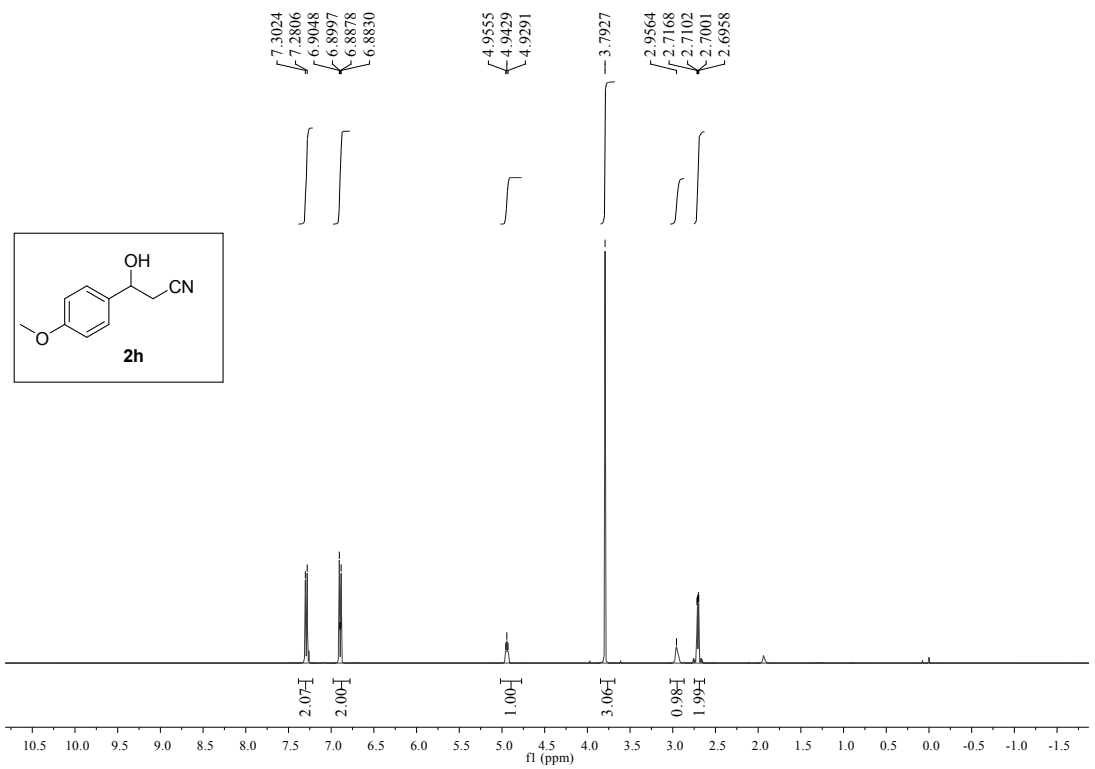

138.4121
131.2941
129.6735
129.5717
127.5559
127.0472
117.3134
77.4405
77.1225
76.8043
66.3066
26.3228
Cl
OH
CN
2e
f1 (ppm)

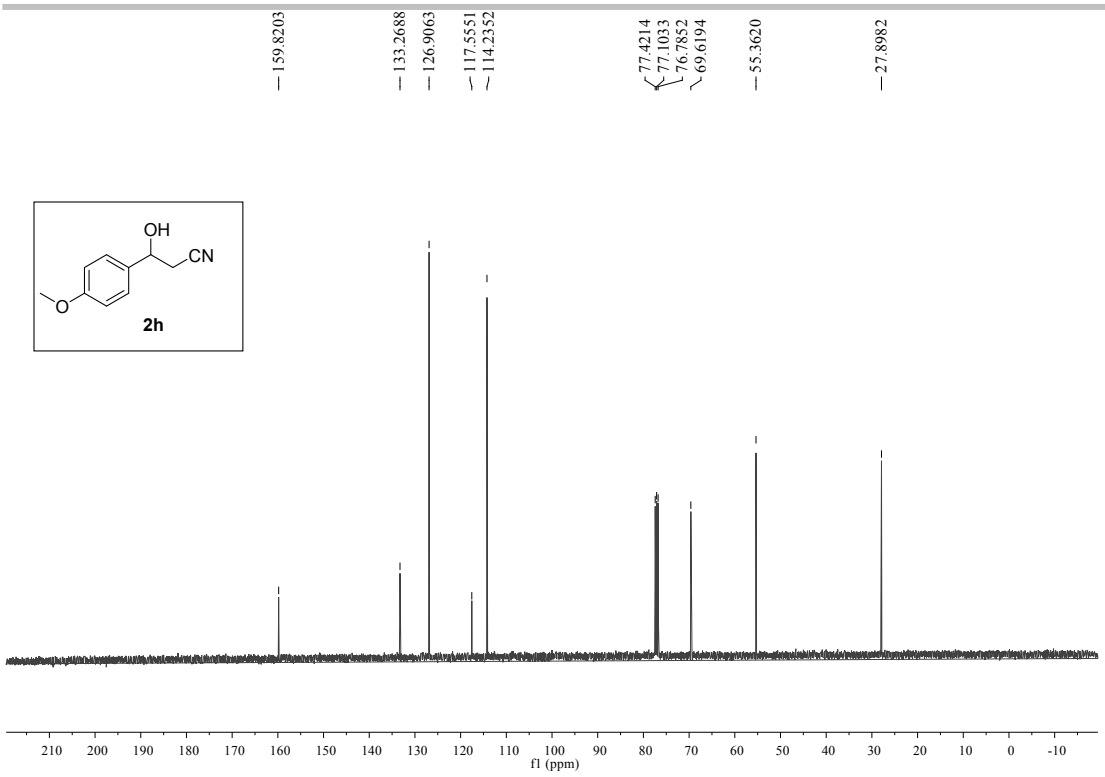

7.4163
7.4147
7.4122
7.4100
7.4090
7.4060
7.4045
7.3440
7.3420
7.3402
7.3356
7.3309
7.3277
7.2962
7.2950
7.2920
7.2908
7.2856
7.2836
7.2621
5.0505
5.0412
5.0351
5.0259
5.0197
5.0105
2.7670
2.7515
2.6519
2.6425
OH
Cl
CN
2f
0.94
1.88
0.97
1.00
2.01
0.94
f1 (ppm)

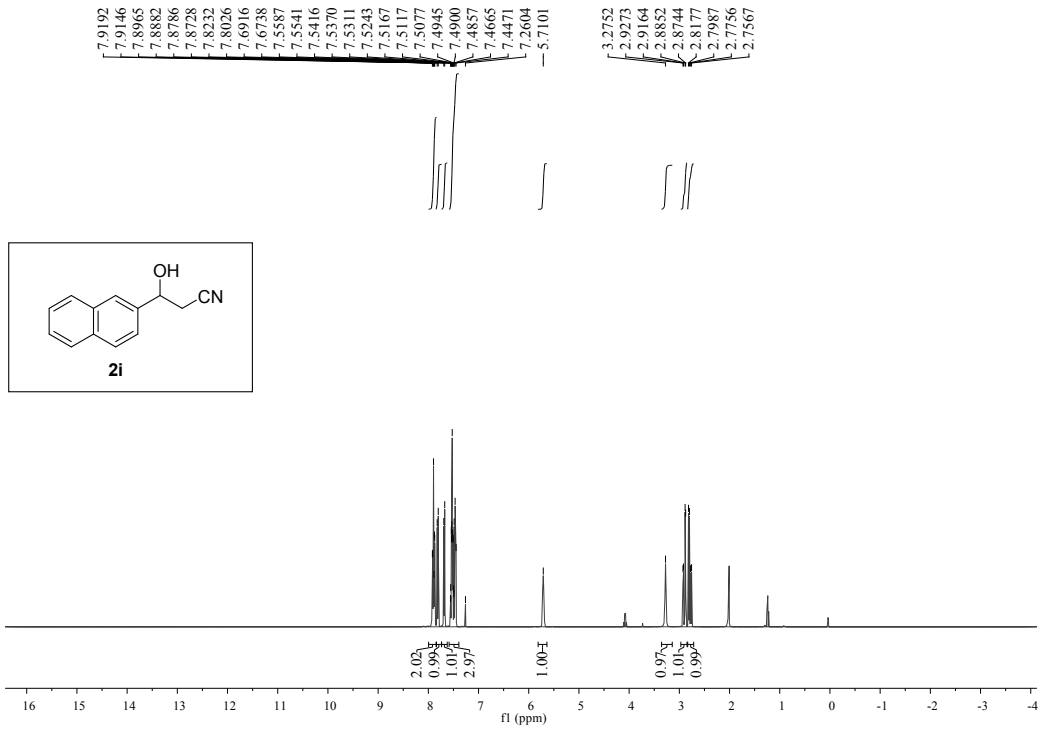

142.9671
134.9181
130.2694
129.0048
125.7940
123.7412
116.9277
77.3577
77.0402
76.7225
69.4878
27.9820
OH
Cl
CN
2f
f1 (ppm)

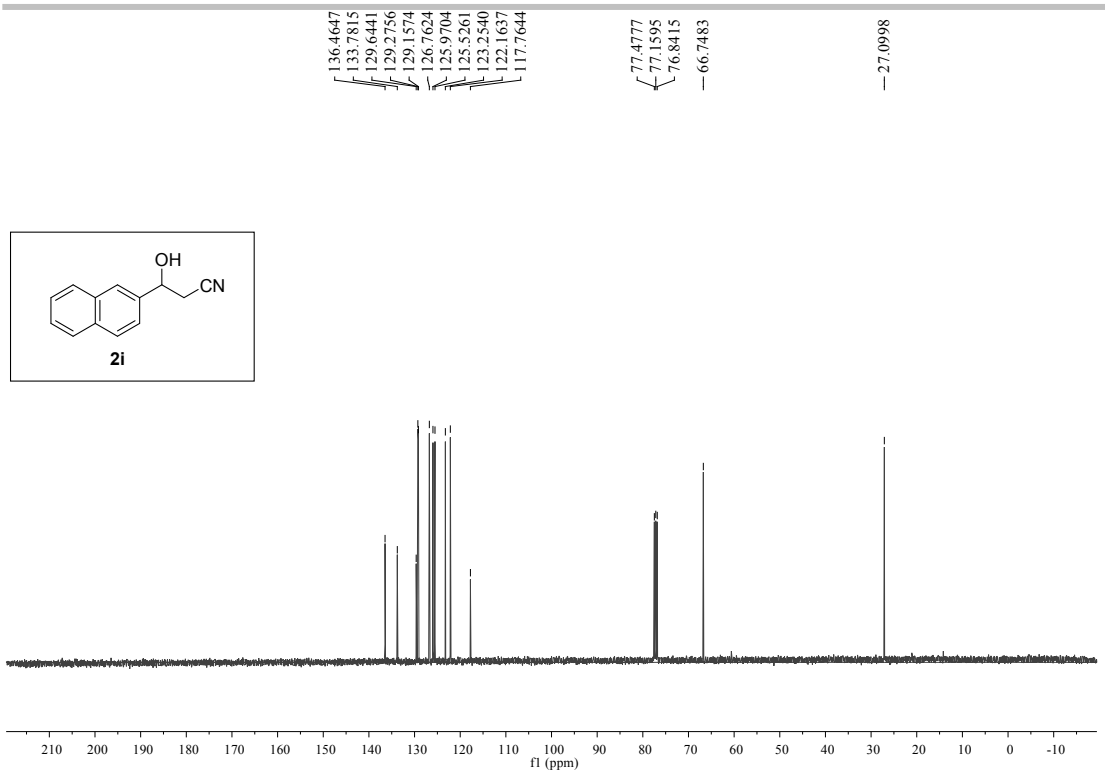

7.3596
7.3585
7.3377
7.3147
7.2947
7.2935
7.2633
4.9939
4.9786
4.9634
3.2304
2.7189
2.7163
2.7031
2.7009
OH
CN
Cl
2g
3.88
1.00
0.87
2.01
f1 (ppm)

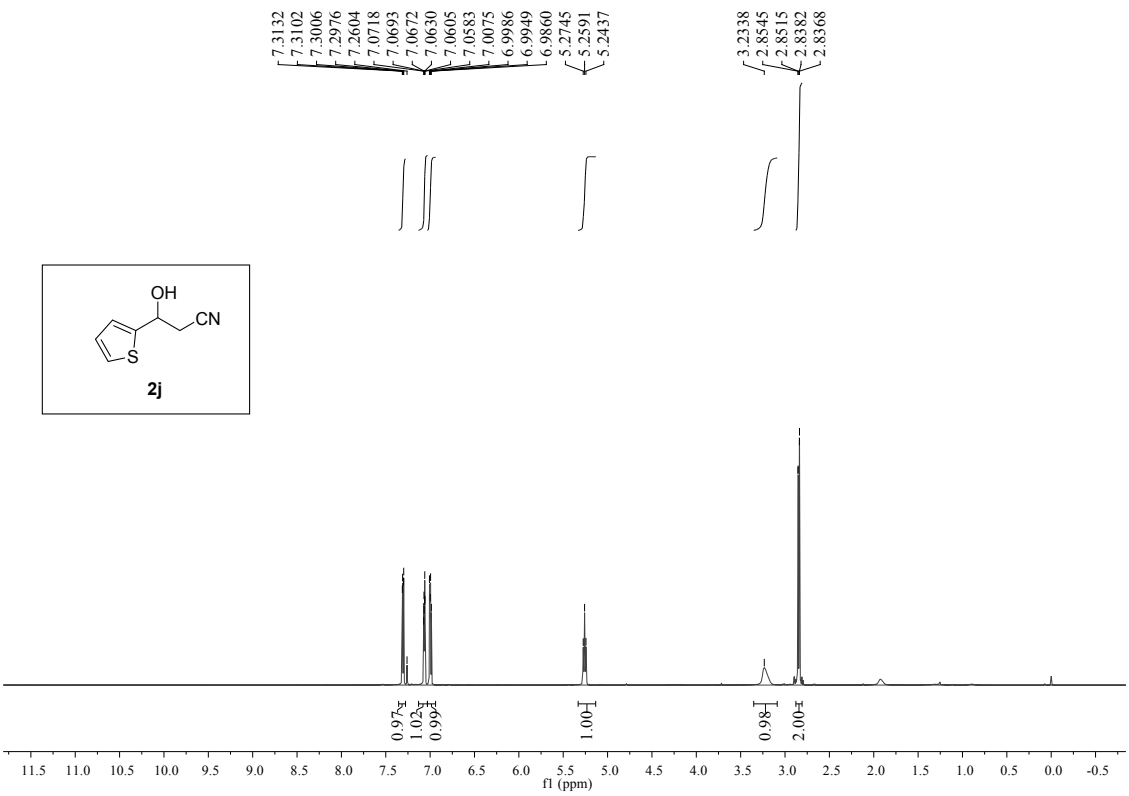

139.5436
134.4855
129.0477
127.0062
117.2662
77.4240
77.1059
76.7879
69.1928
27.9778
OH
CN
Cl
2g
f1 (ppm)

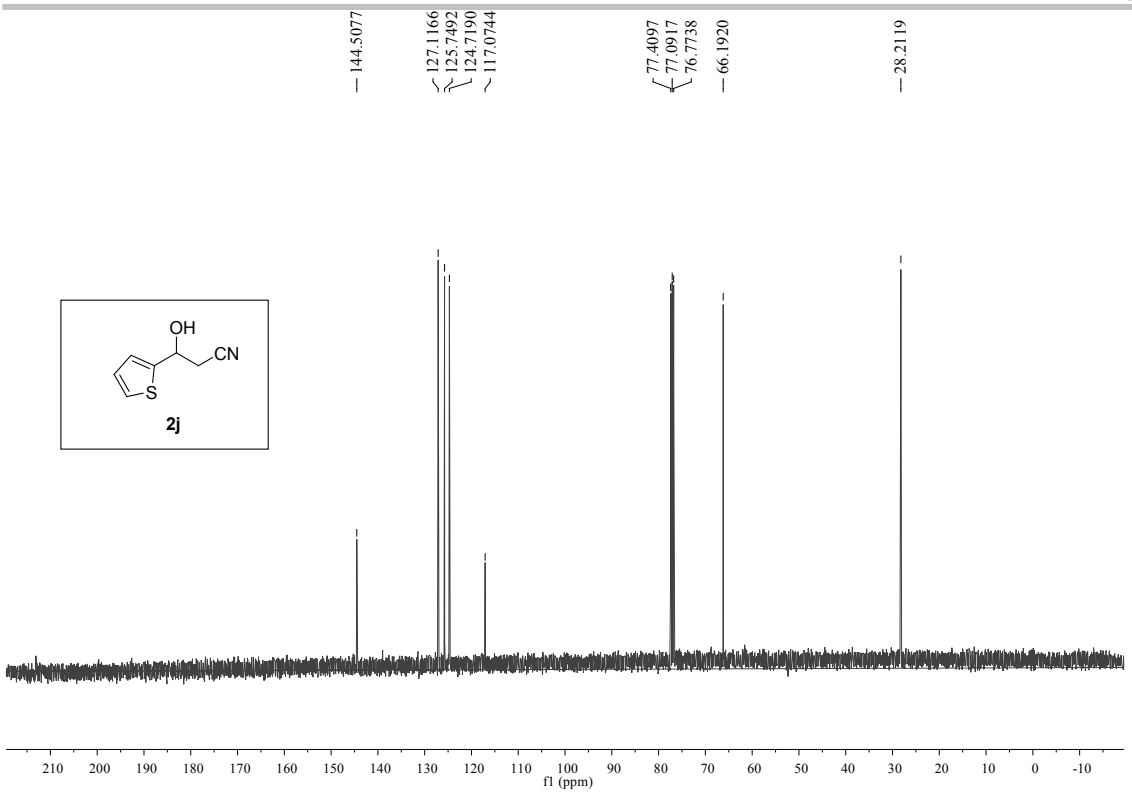

7.3024
7.2806
6.9048
6.8997
6.8878
6.8830
4.9555
4.9429
4.9291
3.7927
2.9564
2.7168
2.7102
2.7001
2.6958
OH
CN
O
2h
2.07
2.00
1.00
3.06
0.98
1.99
f1 (ppm)

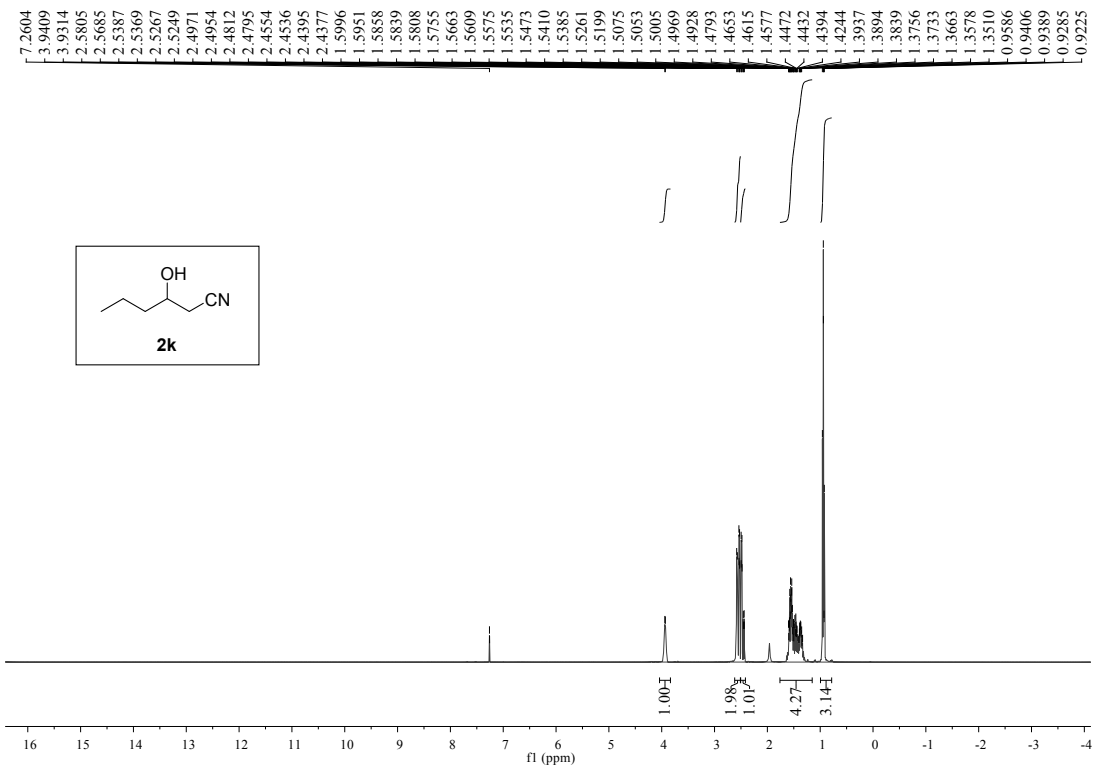

159.8203
133.2688
126.9063
117.5551
114.2352
77.4214
77.1033
76.7852
69.6194
55.3620
27.8982
OH
CN
O
2h
f1 (ppm)

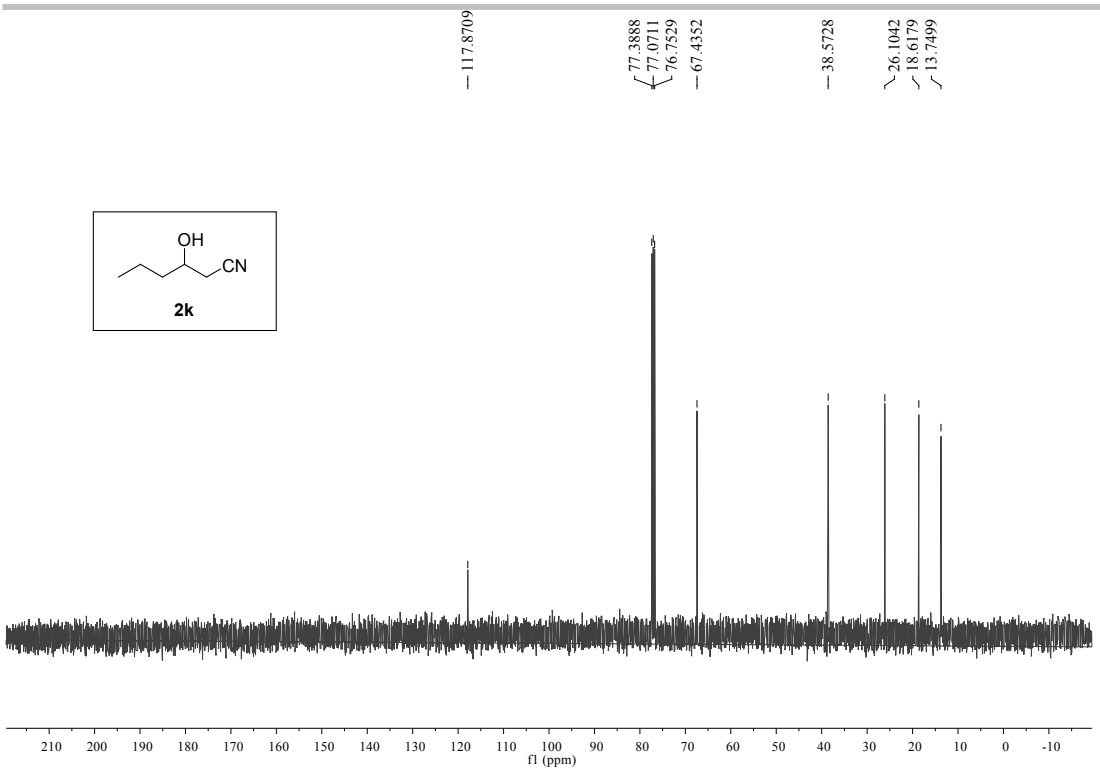

7.9192
7.9146
7.8965
7.8882
7.8786
7.8728
7.8232
7.8026
7.6916
7.6738
7.5587
7.5541
7.5416
7.5370
7.5311
7.5243
7.5167
7.5117
7.5077
7.4945
7.4900
7.4857
7.4665
7.4471
7.2604
5.7101
3.2752
2.9273
2.9164
2.8852
2.8744
2.8177
2.7987
2.7756
2.7567
OH
CN
2i
2.02
0.99
1.01
2.97
1.00
0.97
1.01
0.99
f1 (ppm)

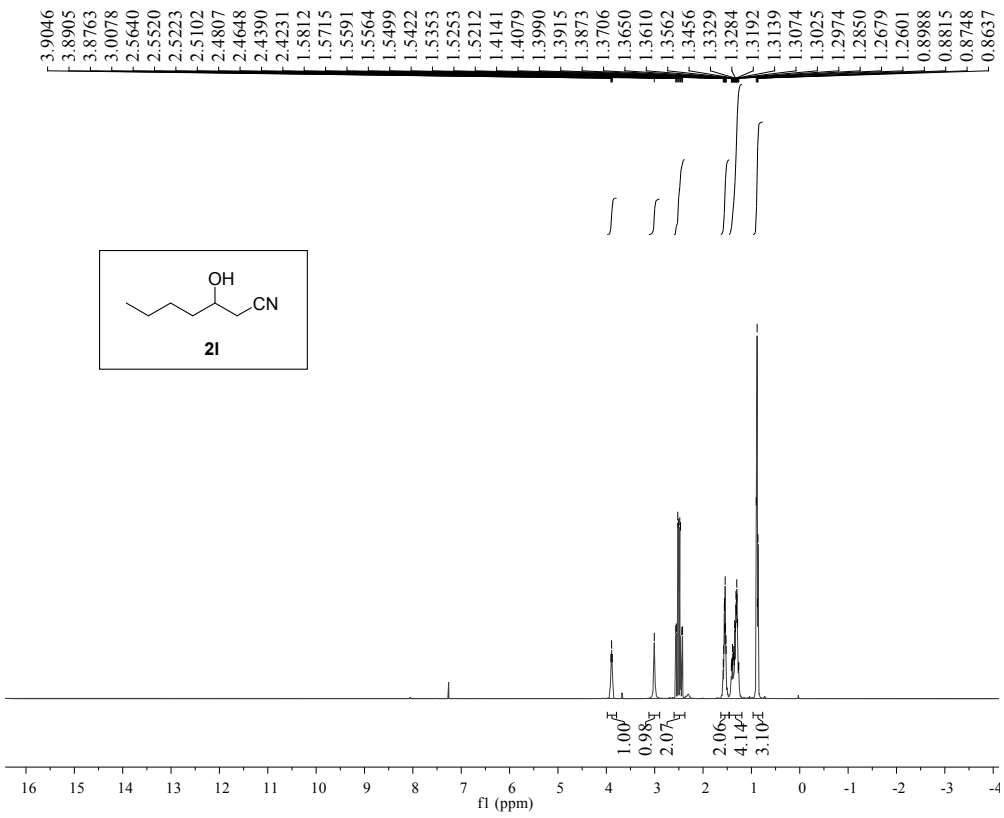

136.4647
133.7815
129.6441
129.2756
129.1574
126.7624
125.9704
125.5261
123.2540
122.1637
117.7644
77.4777
77.1595
76.8415
66.7483
27.0998
OH
CN
2i
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

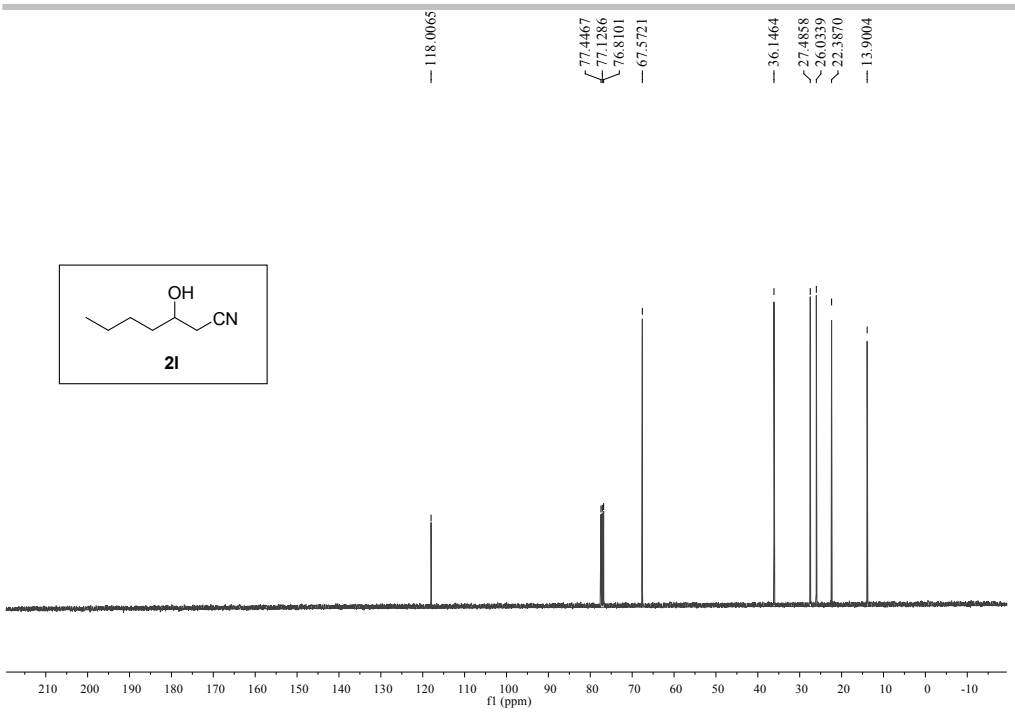

7.3132
7.3102
7.3006
7.2976
7.2604
7.0718
7.0693
7.0672
7.0630
7.0605
7.0583
7.0075
6.9986
6.9949
6.9860
5.2745
5.2591
5.2437
3.2338
2.8545
2.8515
2.8382
2.8368
OH
CN
S
2j
0.97
1.02
0.99
1.00
0.98
2.00
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

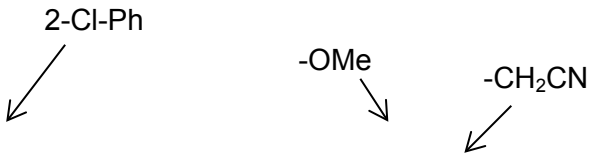

144.5077
127.1166
125.7492
124.7190
117.0744
77.4097
77.0917
76.7738
66.1920
28.2119
OH
CN
S
2j
f1 (ppm)

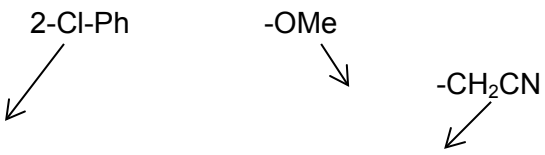

7.2604
3.9409
3.9314
2.5805
2.5685
2.5387
2.5369
2.5267
2.5249
2.4971
2.4954
2.4812
2.4795
2.4554
2.4536
2.4395
2.4377
1.5996
1.5951
1.5858
1.5839
1.5808
1.5755
1.5663
1.5609
1.5575
1.5535
1.5473
1.5410
1.5385
1.5261
1.5199
1.5075
1.5053
1.5005
1.4969
1.4928
1.4793
1.4653
1.4615
1.4577
1.4472
1.4432
1.4394
1.4244
1.3937
1.3894
1.3839
1.3756
1.3733
1.3663
1.3578
1.3510
0.9586
0.9406
0.9389
0.9285
0.9225
OH
CN
2k
1.00
1.98
1.01
4.27
3.14
f1 (ppm)

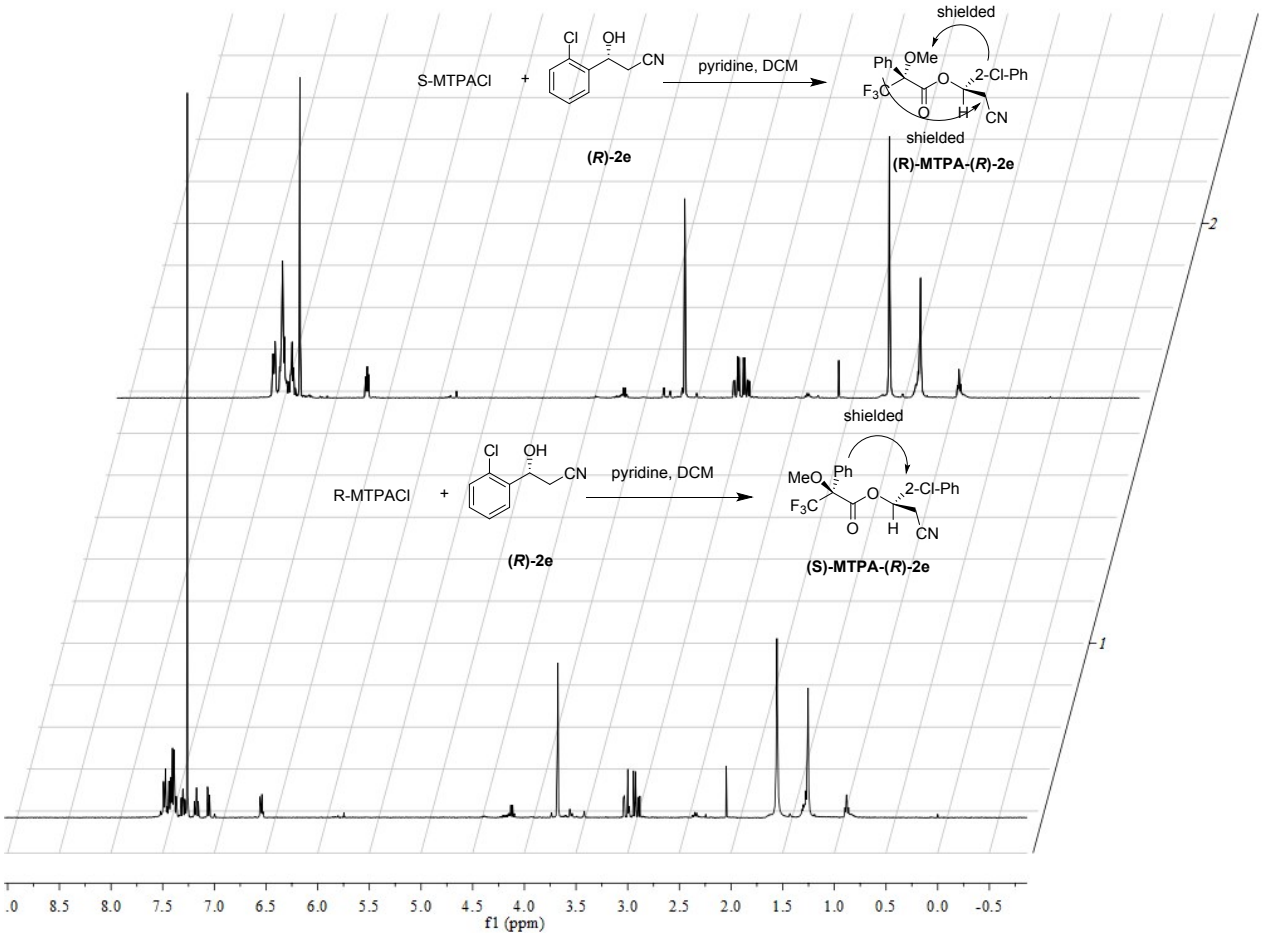

117.8709
77.3888
77.0711
76.7529
67.4352
38.5728
26.1042
18.6179
13.7499
OH
CN
2k
f1 (ppm)

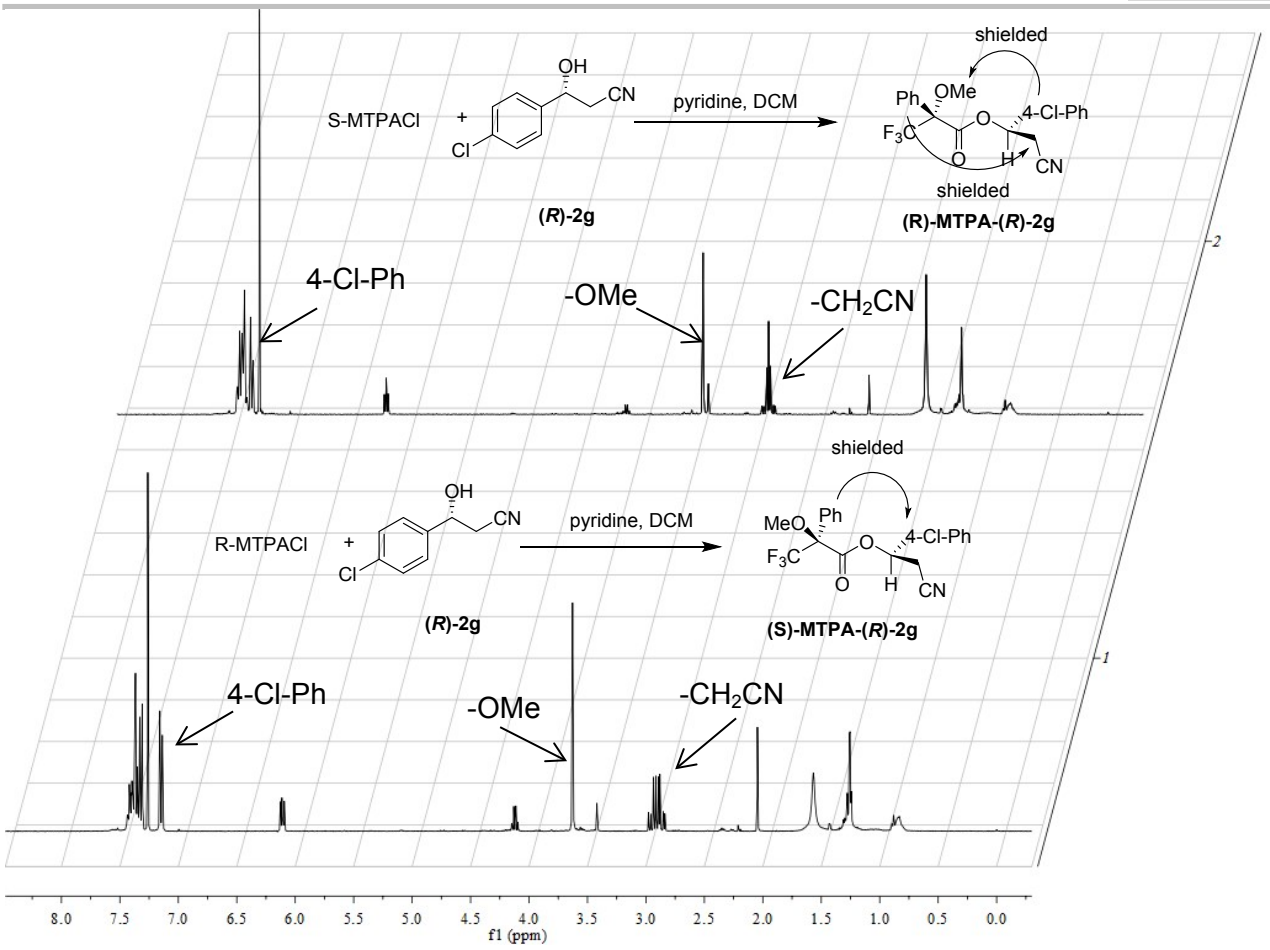

3.9046
3.8905
3.8763
3.0078
2.5640
2.5520
2.5223
2.5102
2.4807
2.4648
2.4390
2.4231
1.5812
1.5715
1.5591
1.5564
1.5499
1.5422
1.5353
1.5253
1.5212
1.4141
1.4079
1.3990
1.3915
1.3873
1.3706
1.3650
1.3610
1.3562
1.3456
1.3329
1.3284
1.3192
1.3139
1.3074
1.3025
1.2974
1.2850
1.2679
1.2601
0.8988
0.8815
0.8748
0.8637
OH
CN
2l
1.00
0.98
2.07
2.06
4.14
3.10
f1 (ppm)

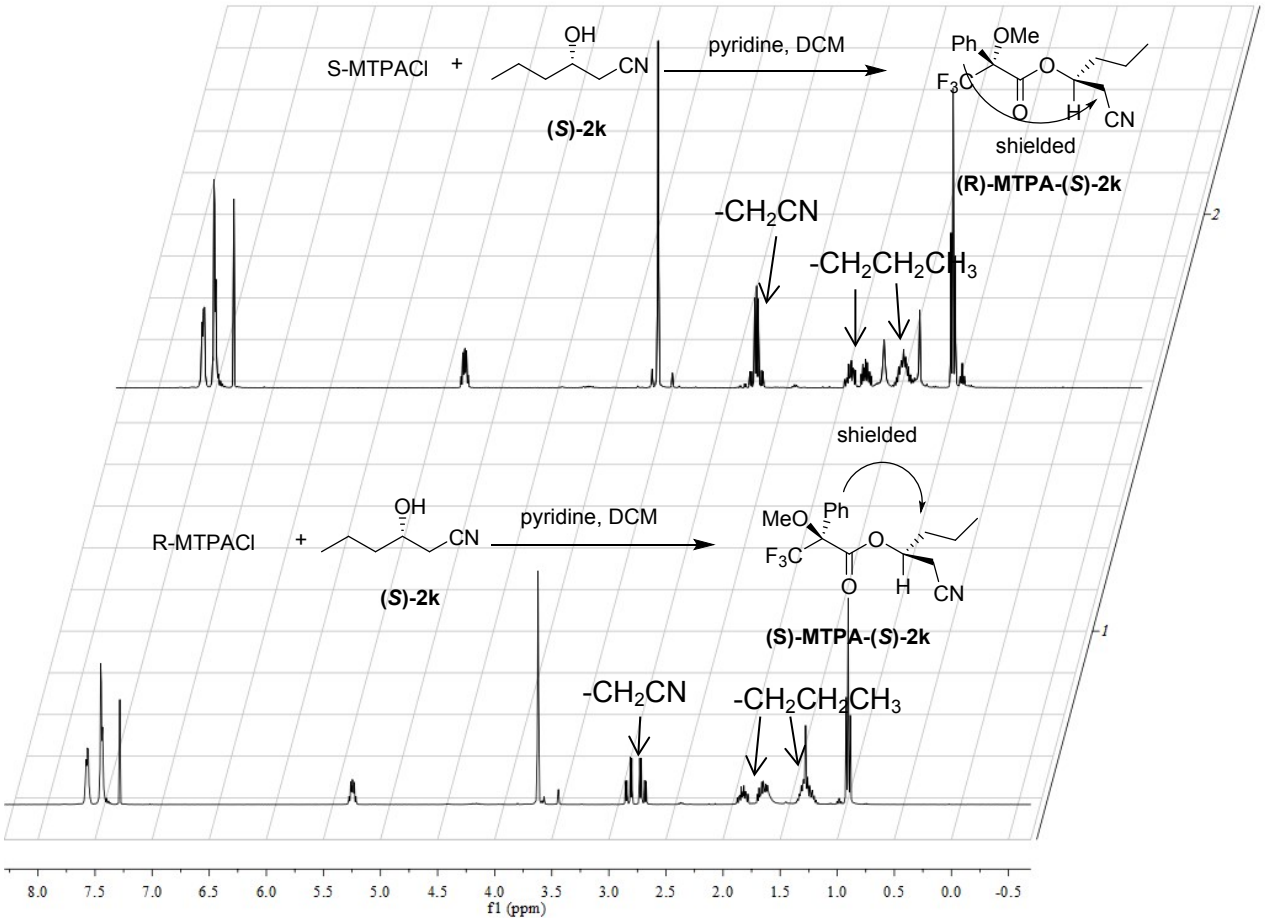

118.0065
77.4467
77.1286
76.8101
67.5721
36.1464
27.4858
26.0339
22.3870
13.9004
OH
CN
2l
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

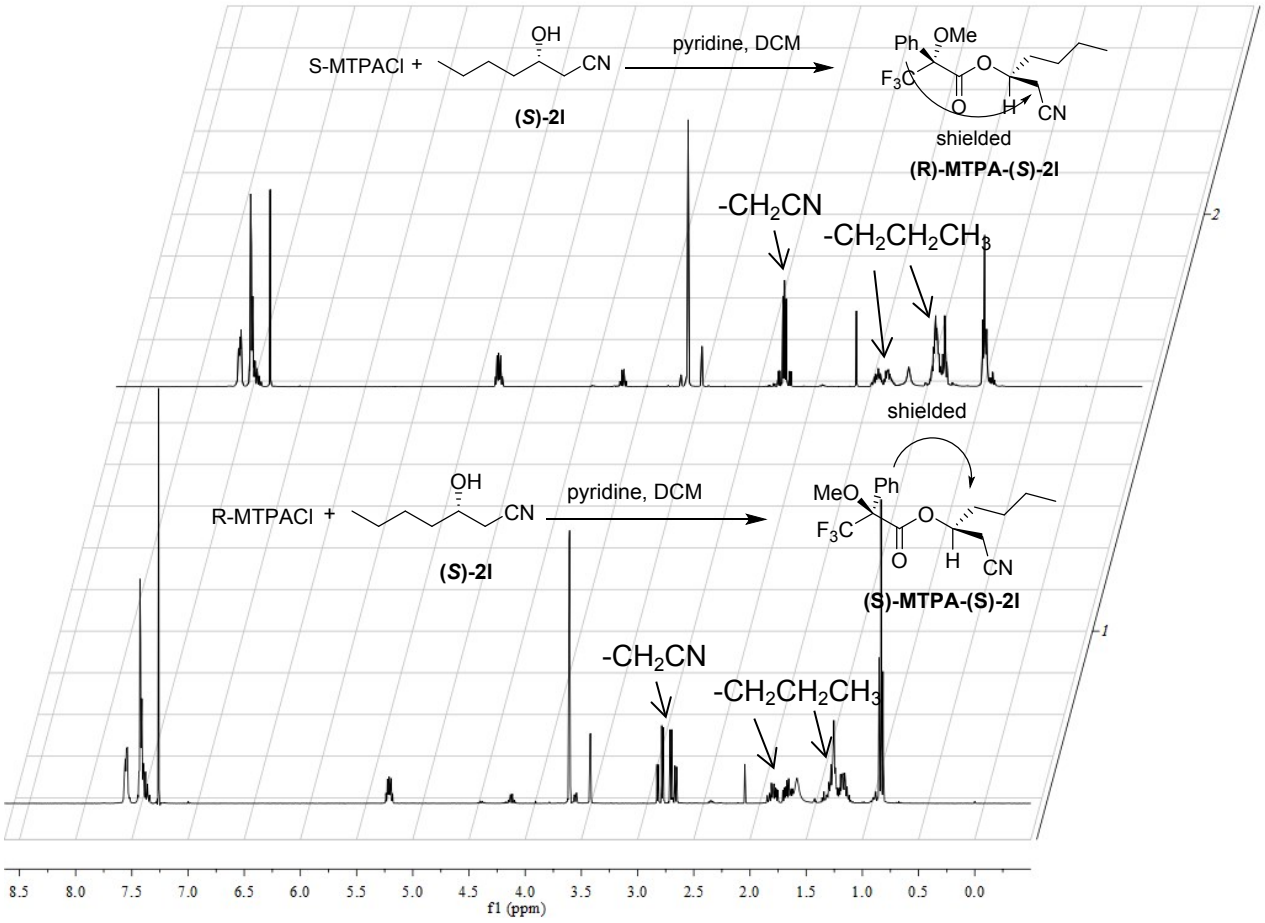

2-Cl-Ph
-OMe
-CH2CN


2-Cl-Ph
-OMe
-CH2CN


shielded
S-MTPACl
+
Cl
OH
CN
pyridine, DCM
Ph
OMe
2-Cl-Ph
F3C
O
O
H
CN
shielded
(R)-2e
(R)-MTPA-(R)-2e
2
shielded
R-MTPACl
+
Cl
OH
CN
pyridine, DCM
MeO
Ph
2-Cl-Ph
F3C
O
O
H
CN
(R)-2e
(S)-MTPA-(R)-2e
1
.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


S-MTPACl
OH
CN
Cl
pyridine, DCM
shielded
Ph
OMe
O
4-Cl-Ph
F3C
O
H
CN
shielded
(R)-2g
(R)-MTPA-(R)-2g
4-Cl-Ph
-OMe
-CH2CN
R-MTPACl
OH
CN
Cl
pyridine, DCM
shielded
MeO
Ph
F3C
O
4-Cl-Ph
O
H
CN
(R)-2g
(S)-MTPA-(R)-2g
4-Cl-Ph
-OMe
-CH2CN
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


S-MTPACl
OH
CN
(S)-2k
pyridine, DCM
Ph
OMe
F3C
O
O
H
CN
shielded
(R)-MTPA-(S)-2k
-CH2CN
-CH2CH2CH3
shielded
R-MTPACl
OH
CN
(S)-2k
pyridine, DCM
MeO
Ph
F3C
O
O
H
CN
(S)-MTPA-(S)-2k
-CH2CN
-CH2CH2CH3
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)



References

[1] Y. Miao, R. Metzner, Y. Asano, *ChemBioChem* **2017**, *18*, 451-454.
[2] K. Oinuma, Y. Hashimoto, K. Konishi, M. Goda, T. Noguchi, H. Higashibata, M. Kobayashi, *J. Biol. Chem.* **2003**, *278*, 29600-29608.
[3] Y. Kato, K. Nakamura, H. Sakiyama, S. G. Mayhew, Y. Asano, *Biochemistry* **2000**, *39*, 800-809.
[4] Y. Kato, S. Yoshida, S.-X. Xie, Y. Asano, *J. Biosci. Bioeng.* **2004**, *97*, 250-259.
[5] C. S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294-7299.
[6] a) F. J. F. Castro, M. M. Vila, P. R. Jenkins, M. L. Sharma, G. Tustin, J. Fawcett, D. R. Russell, *Synlett* **1999**, 798-800; b) O. B. Bondarenko, A. Y. Gavrilova, M. A. Kazantseva, V. N. Tikhanushkina, E. E. Nifant'ev, L. G. Saginova, N. V. Zyk, *Russ. J. Org. Chem.* **2007**, *43*, 564-570.
[7] A. Pohjakallio, P. M. Pihko, U. M. Laitinen, *Chem. - Eur. J.* **2010**, *16*, 11325-11339.
[8] a) O. Soltani, M. A. Ariger, H. Vázquez-Villa, E. M. Carreira, *Org. Lett.* **2010**, *12*, 2893-2895; b) H. Vázquez-Villa, S. Reber, M. A. Ariger, E. M. Carreira, *Angew. Chem. Int. Ed.* **2011**, *50*, 8979-8981.
[9] T. R. Hoye, C. S. Jeffrey, F. Shao, *Nat. Protoc.* **2007**, *2*, 2451.