Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Efficient single-enzymatic cascade for asymmetric synthesis of chiral amines catalyzed by ω-transaminase

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1. General information

All pro-chiral ketones were commercially available and purchased from Sigma-Aldrich (Ludwigshafen, Germany) and were used as received without any purification. All chemicals used were of analytical grade. ^{1}H- and ^{13}C-NMR were recorded on a Bruker 500 MHz spectrometer at 500 MHz and 125.8 MHz, respectively. All chemical shifts (δ) were quoted in parts per million (ppm) and reported relative to an internal tetramethylsilane (TMS, δ 0.00) standard. The following abbreviations were used to define the multiplicities: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; p, pentet and m, multiplet. The conversion of prochiral ketones were measured by gas chromatography using a Hewlett Packard 5890 that was equipped with a CP Chiral-DEX CB DF 0.25 column (25 m x 0.32 mm). All product *ee* values were analyzed by using an Agilent HPLC 1100 that was equipped with a Silica-gel Crownpak CR(+) (0.4 cm x 15 cm) column. Absolute configuration of product was determined by comparing with the standard pure products. Kinetic constants were recorded on a Varian Cary 300 UV-Vis dual beam spectrophotometer with appropriate blank correction.

2. General procedure for cloning, protein expression and purification of transaminase

The gene for *Chromobacterium violaceum* ω-transaminase was inserted in the plasmid pET28a(+) with an N-terminal His_6-tag. After digestion (NheI, HindIII) and sequence verification the construct was transformed into *E. coli* BL21 (DE). Expression was performed by mixing a 20 ml overnight culture with 180 ml of Luria-Bertoni (LB) medium with 50 mg/l Kanamycin and 0.4 mM IPTG, and incubating for 24 h at 25 °C (150 rpm, baffled flask). The cells were thereafter separated from the medium by centrifugation and resuspended in Immobilized Metal ion Affinity Chromatography (IMAC) binding buffer (20 mM sodium phosphate, 500 mM sodium chloride, pH 7.4), disrupted by addition of BugBuster® 10X (Merck) and applied to a column with Chelating Sepharose FastFlow (GE Healthcare) resin treated with a saturated water solution of Cobalt(II) chloride. After washing with the binding buffer, the His_6-tagged enzyme was eluted with IMAC elution buffer. An excess of cofactor (PLP) was added before desalting on a PD10 column (GE Healthcare). The simple procedure of adding PLP before buffer change ensures that the amount of cofactor is balanced with the enzyme concentration, assuming that a negligible amount of enzyme is in apo form during the desalting. The PD10 column also served to change the buffer into 50 mM HEPES.

3. Enzyme reactions

The reactions were performed in 50 mM HEPES buffer solution on a 1 ml scale, the pH of the buffer was 7.0 for *Chromobacterium violaceum* ω-transaminase W60C and 8.2 for other ω-transaminases. All components were dissolved separately in the buffer and the pH was corrected. These solutions were then combined to final concentration of 5 mM acetophenone or other substituted-acetophenones (4-methylacetophenone, 4-methoxylacetophenone, 4-nitroacetophenone, 4-chloroacetophenone), 3-aminocyclohexa-1,5-dienecarboxylic acid (ACHDA) (SigmaAldrich), 5.25 mM, and transaminase (purified ω-transaminases, $[E]_{Cv\text{-}\omega\text{-}TA\ W60C}$ = 25.0 µM, ATA-113, ATA-117, and ω-TA-001 from Codexis, 0.8 U).

4. Determination of kinetics

The initial reaction rates were measured spectrophotometrically at various concentrations of the mixture of 3-aminocyclohexa-1,5-dienecarboxylic acid and 3-oxocyclohexa-1,5-dienecarboxylic acid (**3**+**5**), and the concentration of acetophenone was set at 5 mM for Entry 1-6 and Entry 11-14, and for Entry 7-10., the concentration was 2 mM. By detecting the formation of (*R*)- or (*S*)-1-phenylethylamine at the wavelength of 270 nm. All continuous measurements were done on a dual beam spectrophotometer (Cary 300 UV-Vis, Varian Inc.) with appropriate blank correction.

5. Determination of substrate conversion

When reactions were completed, 10 μl perchloric acid at the concentration of 70% was added to the reaction solution, and the mixed solution was centrifuged at 3000 rpm for 5 min. After that, 300 μl of the supernatant was taken and added into with 0.5 ml ethyl acetate. And then 300 μl organic solution was taken and dried over molecular sieves.

The prepared samples were subjected to gas chromatography (GC). Helium was used as the carrier gas. Substrate conversions were determined by measuring the left prochiral ketones and calculated by referring an internal standard.

6. Determination of enantiomeric excess of chiral amines

The samples were subjected to high performance liquid chromatography (HPLC) with a CrownpakCR(+) column (Daicel), after filtration (0.22 μm) and acidification by perchloric acid. Ultrapure water with addition of perchloric acid to pH 1.6 was used as mobile phase. Retention times:

1-phenylethylamine at flow rate 0.5 ml/min: 31.7 min (S), 42.0 min (R);

1-(4-methylphenyl)ethylamine at flow rate 0.5 ml/min: 35.4 min (S), 47.3 min (R);

1-(4-methoxyphenyl)ethylamine at flow rate 0.5 ml/min: 40.8 min (S), 62.2 min (R);

1-(4-nitrophenyl)ethylamine at flow rate 0.5 ml/min: 46.8 min (S), 54.9 min (R);

1-(4-chlorophenyl)ethylamine at flow rate 0.3 ml/min: 32.0 min (S), 44.7 min (R).

All amines were detected at 254 nm, and ee values were calculated with the equation below (Eq.1):

$$ee_P = \frac{[S]-[R]}{[S]+[R]} x100\% \qquad \text{(Eq.1)}$$

7. General procedure for the preparation of chiral amines in preparation scale

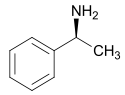
To obtain the desired pure chiral amines, reactions were performed on a 10 ml scale, reaction parameters were identical with which was described in the footnote of Table 1 and Table 2 in the main text of the manuscript. When reactions were completed, 100 μl perchloric acid (70%) was added into the reaction solution to acidify the solution, and the reaction solution was centrifuged for 5 min to remove the denatured transaminase.

The 5 ml ethyl acetate was added to the supernatant, mixed and extracted three times. The organic solution was isolated and combined together. The combined organic solution was then distilled under

evacuation to remove the organic solvent, and the residues were chromatographed to afford the desired pure amines.

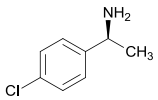
8. ^{1}H NMR and ^{13}C NMR spectrum of chiral amines

8.1 (*S*)-1-phenylethylamine



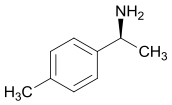
Colorless liquid, ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25 (d, *J* = 2.5 Hz, 3H), 4.12 (q, *J* = 10.0 Hz, 1H), 7.22-7.25 (m, 2H), 7.32-7.36 (m, 3H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 25.7, 51.3, 75.8, 125.7, 126.8, 128.5.

8.2 (*S*)-1-(4-chlorophenyl)ethylamine



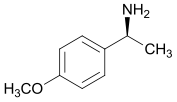
Colorless oil, ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.36 (d, *J* = 2.5 Hz, 3H), 4.11 (q, *J* = 7.5 Hz, 1H), 7.29-7.38 (m, 4H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 25.8, 50.7, 127.1, 128.5, 132.3, 146.2.

8.3 (*R*)-1-(*p*-tolyl)ethylamine



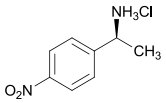
Colorless oil, ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37 (d, *J* = 2.5 Hz, 3H), 2.34 (s, 3H), 4.08 (q, *J* = 7.5 Hz, 1H), 7.14-7.15 (m, 2H), 7.23-7.25 (m, 2H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 21.0, 25.7, 51.0, 76.8, 125.6, 129.1, 136.4, 144.9.

8.4 (*S*)-1-(4-methoxyphenyl)ethylamine



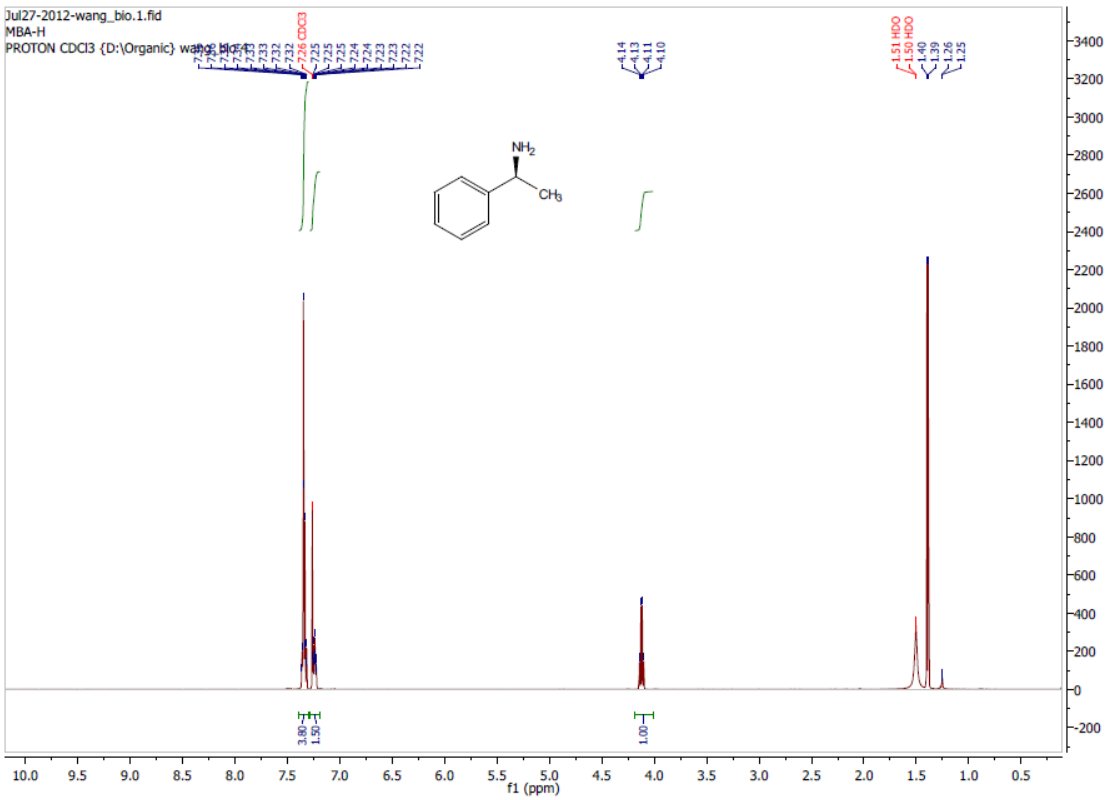
Colorless oil, ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.13 (d, *J* = 2.5 Hz, 3H), 2.29 (s, 3H), 2.58 (q, *J* = 12.5 Hz, 1H), 5.35-5.38 (m, 2H), 5.75-5.78 (m, 2H). ^{13}C NMR (125.8 MHz, $CDCl_3$) δ 25.8, 50.7, 55.3, 113.8, 126.7, 140.0, 158.4.

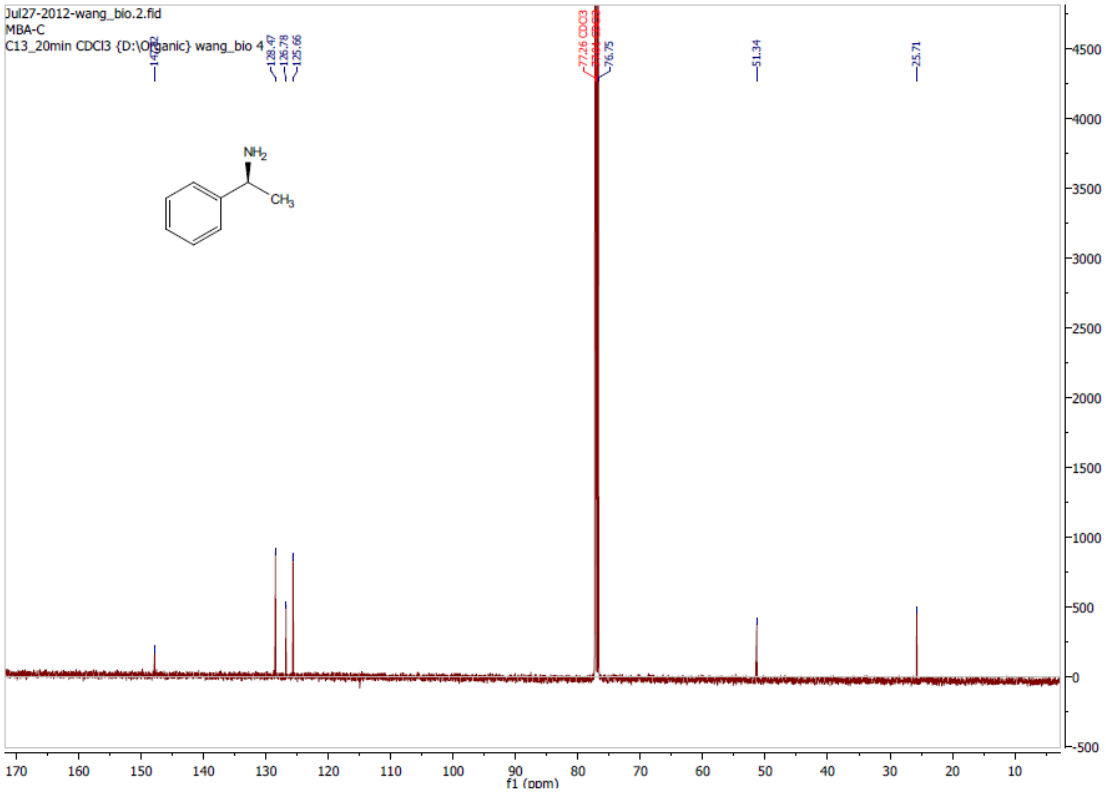
8.5 (*S*)-1-(4-nitrophenyl)ethylamine

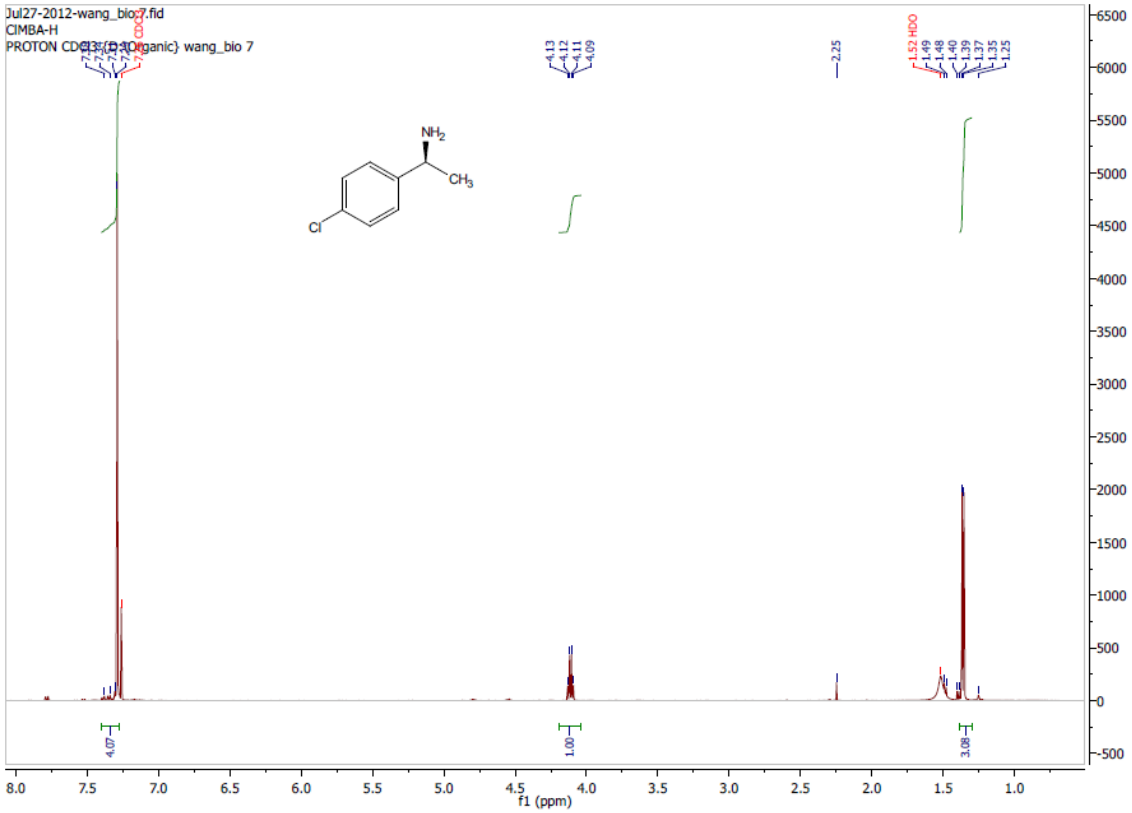


Light yellow oil, ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.53 (d, *J* = 2.5 Hz, 3H), 4.59 (q, *J* = 10.0 Hz, 1H), 7.80-7.82 (m, 2H), 8.29-8.31 (m, 2H), 8.69 (s, 3H). ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 20.5, 38.9, 49.2, 123.7, 128.2, 146.5.

9. ^{1}H NMR and ^{13}C NMR spectra of chiral amines



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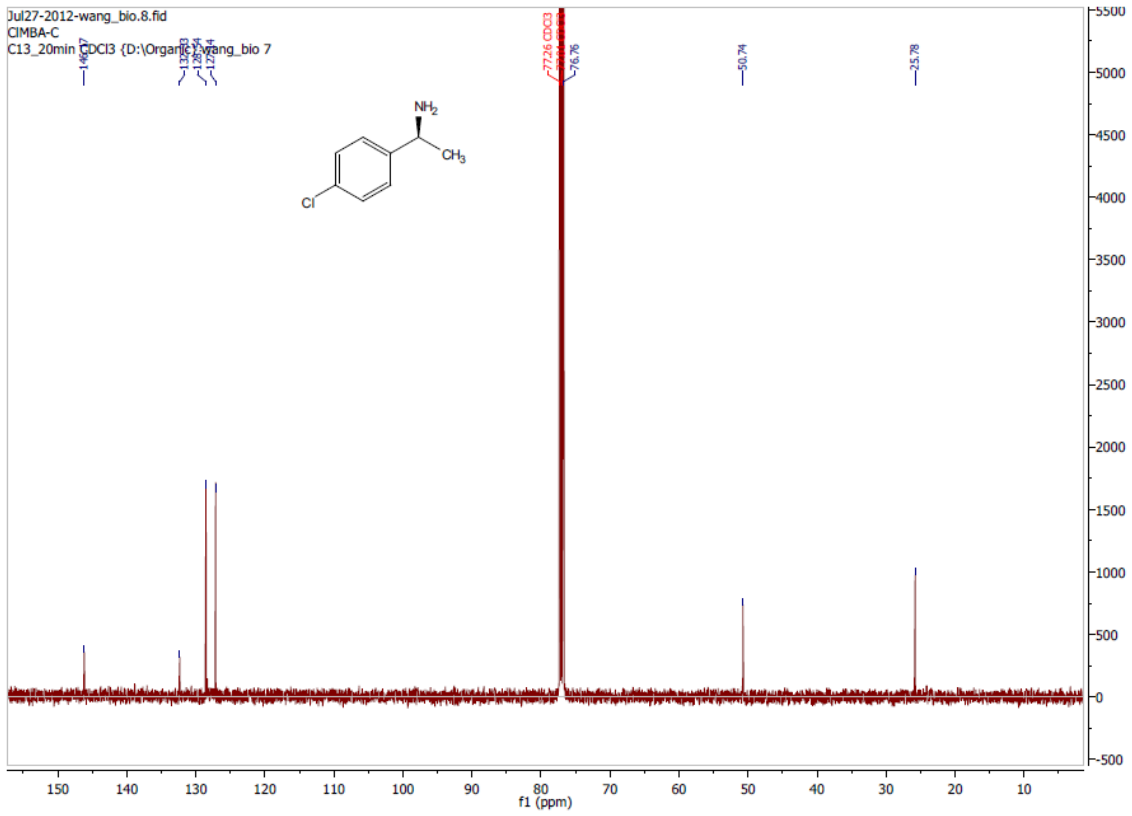
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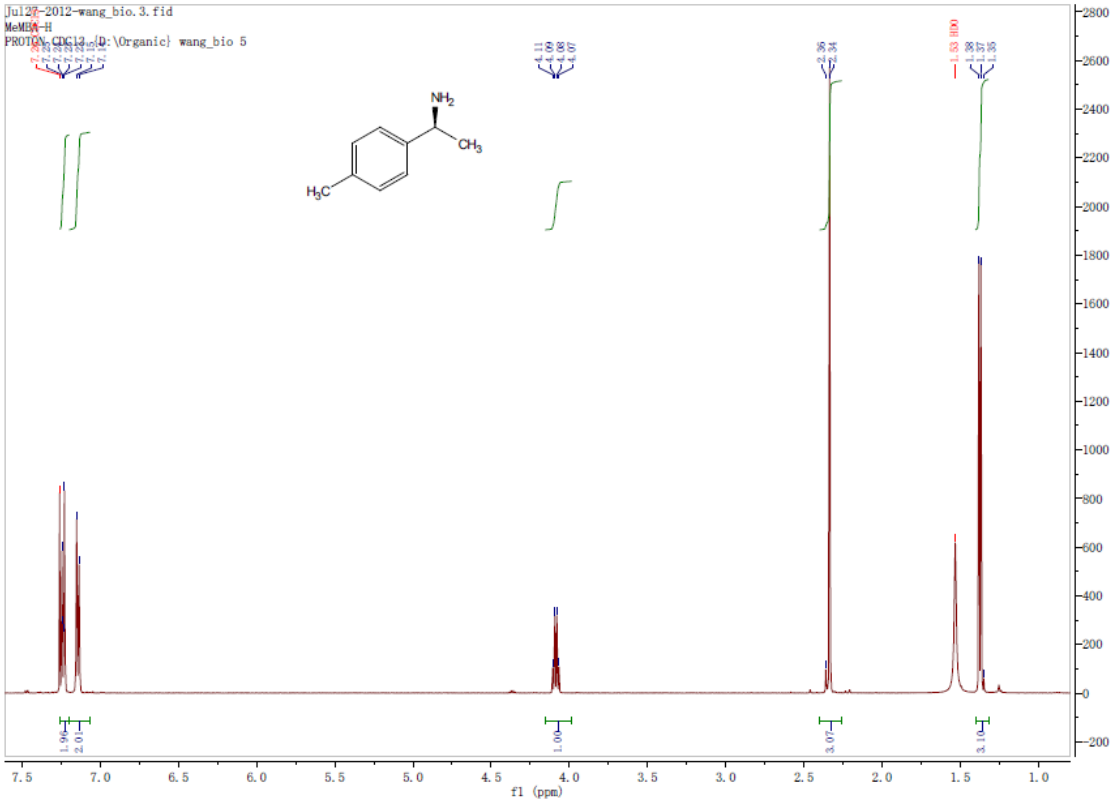
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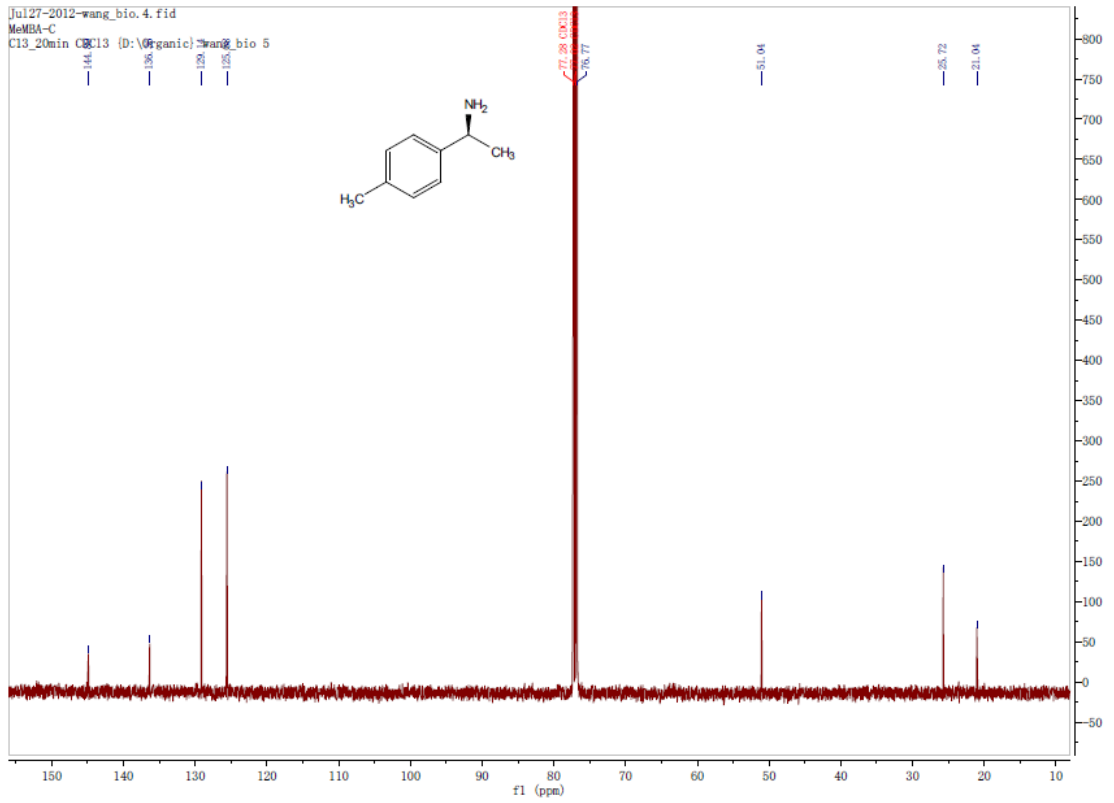
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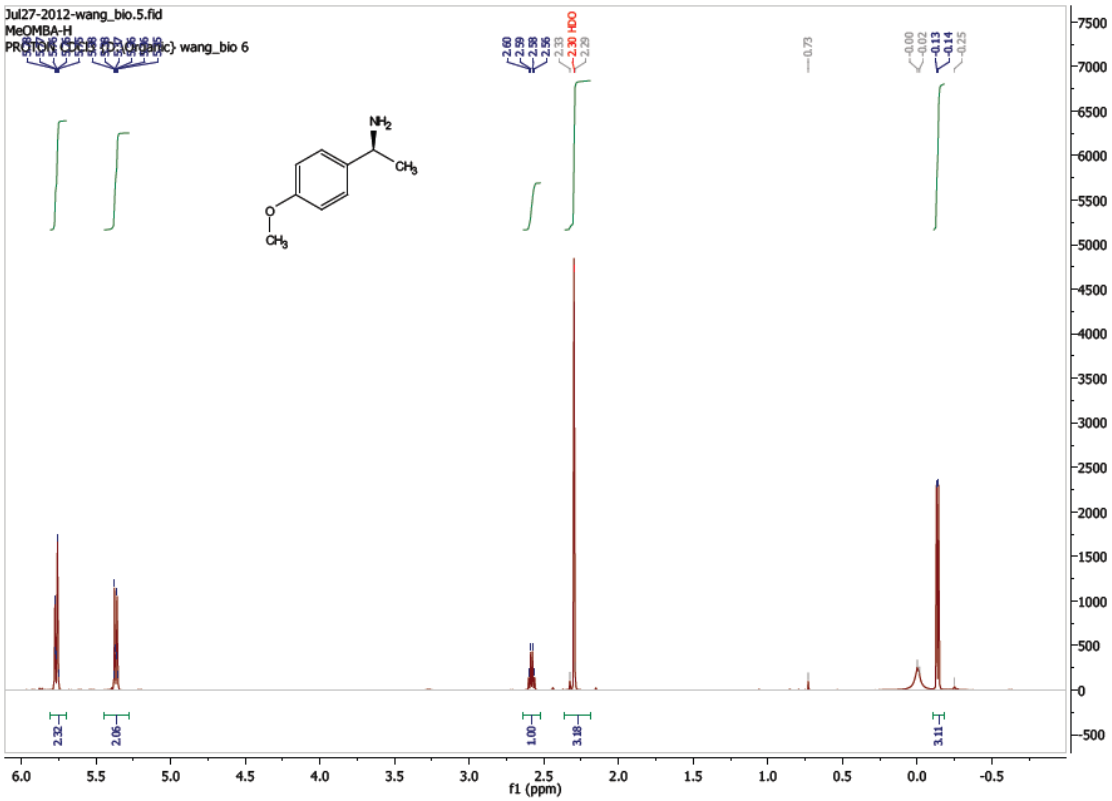
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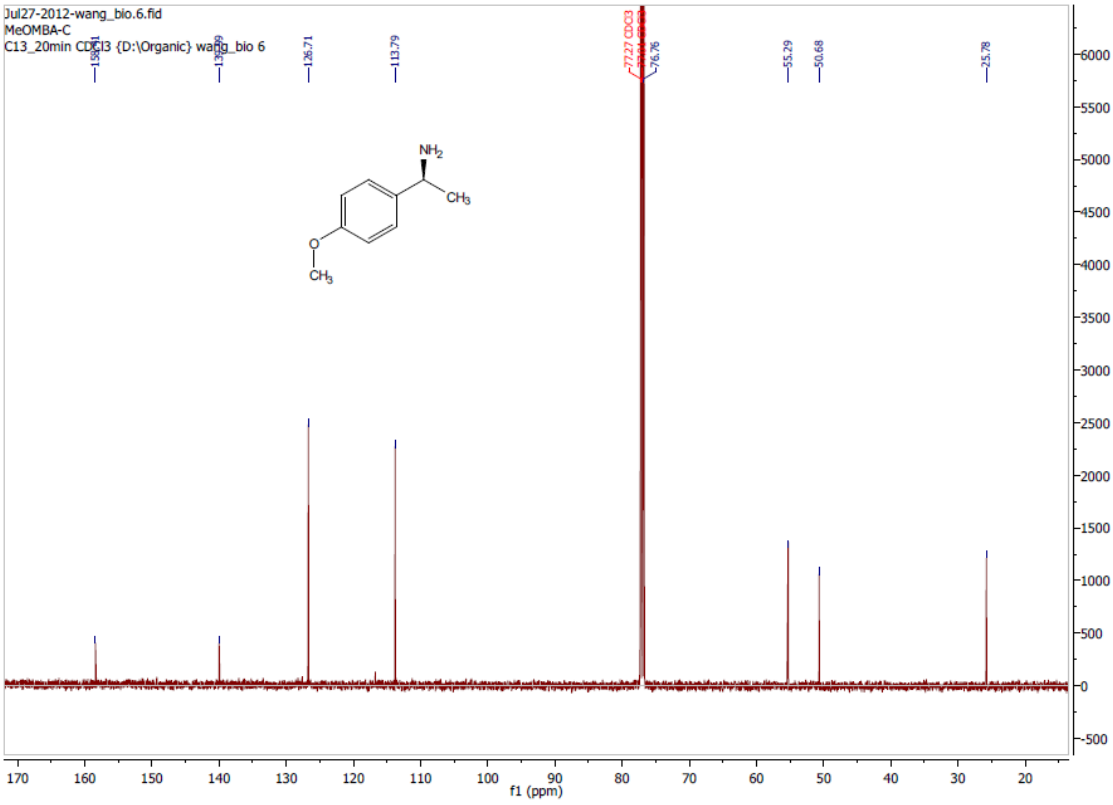
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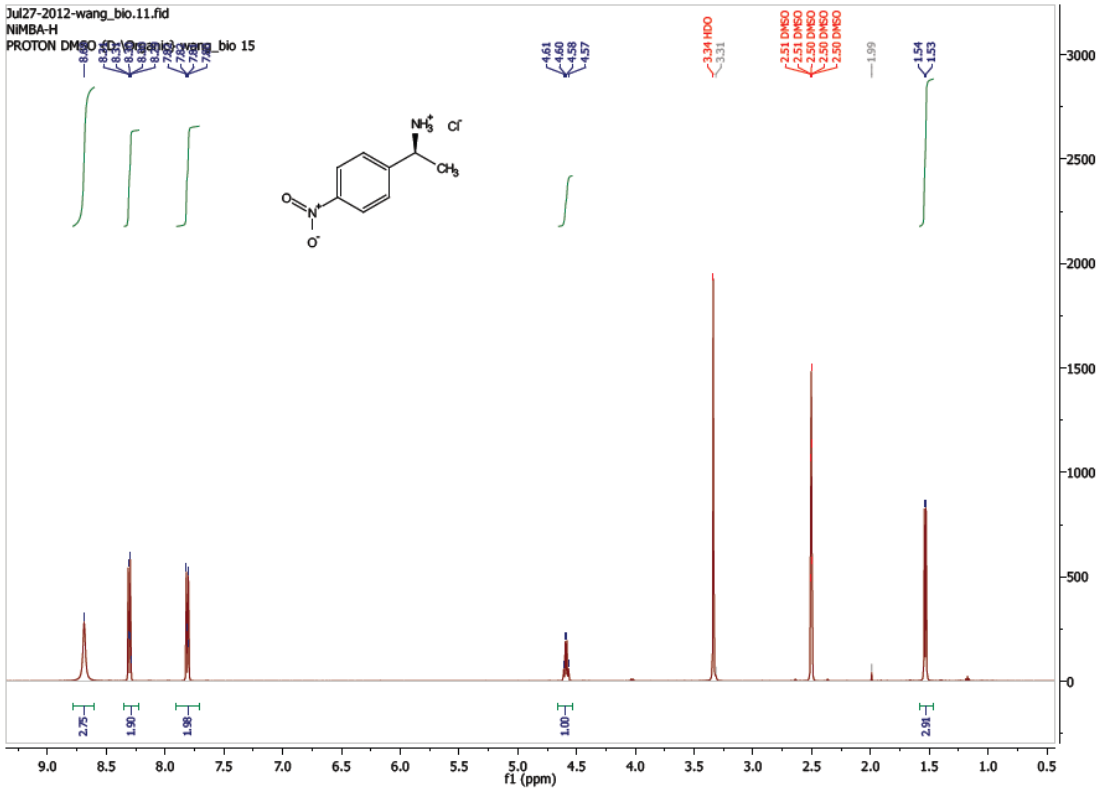
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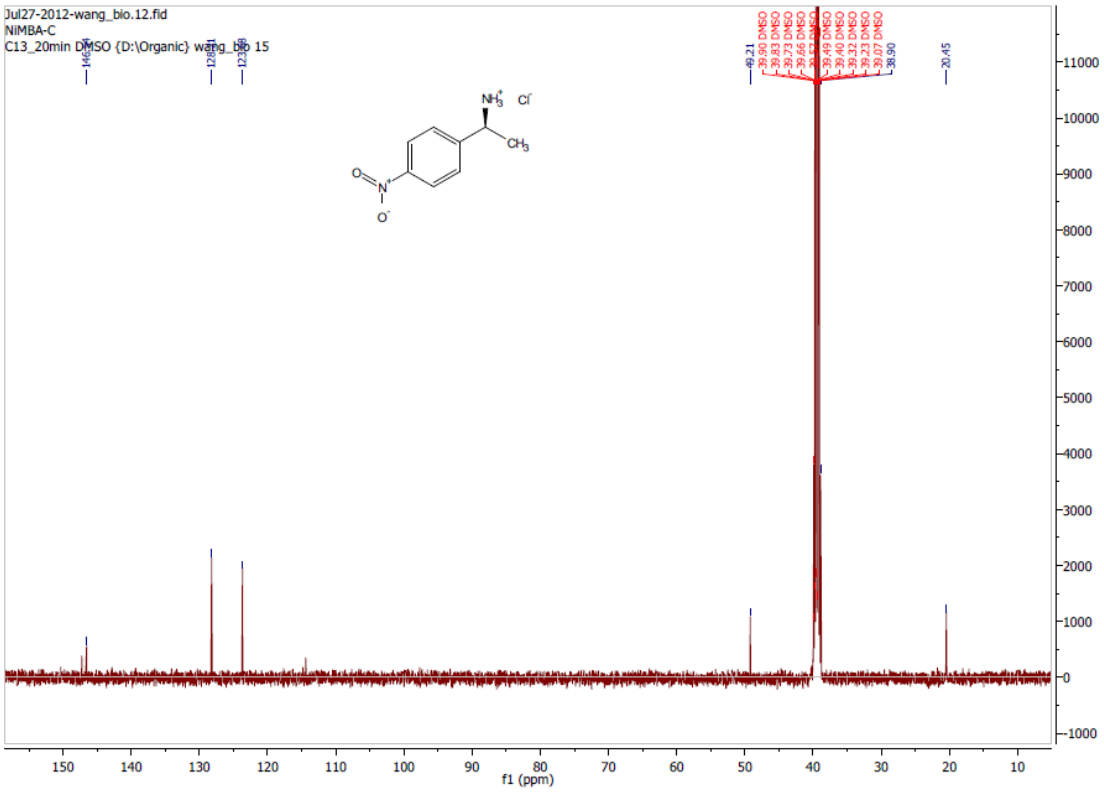
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