Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Chemoenzymatic Total Synthesis of Rotigotine via IRED-Catalyzed Reductive Amination

Dongyu Tang[a,b], Yaqing Ma[b], Jinping Bao[b], Shushan Gao[b,c], Shuli Man[*a], Chengsen Cui[*b,c]

[a] College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, China
[b]Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China
[c]National Technology Innovation Center of Synthetic Biology, Tianjin 300308, China

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1. General

^{1}H NMR spectra were recorded in $CDCl_3$ (400MHz). Residual solvent peaks are used as the internal reference; the signals at 7.26 ppm are set for ^{1}H NMR spectra, taken in $CDCl_3$. Silica gel plates pre-coated on glass were used for thin-layer chromatography using UV light, or 7% ethanolic phosphomolybdic acid or potassium permanganate solution and heating as the visualizing methods. Silica gel was used for flash column chromatography with mixed CH_2Cl_2 and MeOH or ethyl acetate (EtOAc) and hexane as the eluting solvents. HPLC analysis was performed on Waters 2695 using C18 analytical column (Agilent Eclipse XDB-C_{18}, 4.6×250 mm, 5 μm). LC-MS analysis was performed on Agilent 1100 with a mass spectrum detector (MSD) using an analytical column (Ultimate XB-C_{18}, 2.1×100 mm, 3.0 μm). Chiral HPLC analysis was performed on Waters 2695 using chiral analytical columns (CHIRALPAK AD/AY-H, etc., 4.6×250 mm, 5 μm). NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer.

2. Materials

Commercially available chemicals and reagents, including ketones **1**, amines **a**, and $NADP^+$, were purchased from Meryer (Shanghai, China), Macklin (Shanghai, China), J&K Scientific (Beijing, China), or Sigma-Aldrich (Poole, Dorset, UK) unless stated otherwise. All HPLC or LC-MS grade solvents, including acetonitrile, n-hexane, and ethanol, were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Sodium phosphate buffer (0.1 M, pH 7.0) was prepared in-house.

3. Preparation of standard amines

3.1. Reductive amination procedure for the preparation of racemic amine (**1a**) standards

To a stirred solution of 5-methoxy-3,4-dihydronaphthalen-2(1H)-one **1** (0.57 mmol) and the 2-(thiophen-2-yl)ethan-1-amine **a** (0.85 mmol) in dry MeOH (10 mL) was added acetic acid (300 μL) under an N_2 atmosphere. The reaction mixture was stirred for 1.5 h at room temperature and then cooled to 0 °C (ice/water bath) for 5 min and then $NaCNBH_3$ (0.107 g, 1.7 mmol) was added. The reaction mixture was gradually warmed to room temperature and stirred overnight. The reaction progress was monitored by TLC and following completion was quenched with sat. $NaHCO_3$ solution (10 mL) and stirred for an additional 30 minutes. The mixture was extracted with EtOAc (3 × 10 mL) and the combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to give the crude product. The crude product was purified by flash column chromatography (silica gel, gradient elute from pure PE-PE/EA, 85:15) affords the corresponding racemic **1a**.

***rac*-1a:** dark brown oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.17 (dd, J = 5.1, 1.2 Hz, 1H), 7.11 (t, J = 7.9 Hz, 1H), 6.96 (dd, J = 5.1, 3.4 Hz, 1H), 6.89-6.86 (m, 1H), 6.70 (dd, J = 16.5, 7.9 Hz, 2H), 3.82 (s, 3H), 3.09-2.88 (m, 7H), 2.60 (qd, J = 11.0, 10.2, 4.6 Hz, 2H), 2.14-2.05 (m, 1H), 1.58 (dtd, J = 12.4, 10.5, 5.8 Hz, 1H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 157.23 , 142.61 , 136.63 , 126.92 , 126.27 , 125.11 , 125.11 , 123.65 , 121.58 , 107.13 , 55.28 , 53.03 , 48.42 , 36.79 , 30.73 , 29.23 , 22.25 .

3.2. Reductive amination procedure for the preparation of chiral amine standards

Chiral amine standards were obtained from commercially available standards and were purchased from Leyan (Shanghai, China)

3.3. Enzymatic synthesis compound **1a**

Milligram scale synthesis:

At room temperature, a 10 ml enzymatic reaction is performed which consists of 1 mM $NADP^+$ (7.43 mg), cell-free extract (10 g/L wet cells weight, M5-F260W-M147Y), 5-methoxy-3,4-dihydronaphthalen-2(1H)-one **1** (52.8 mg, 30 mM), 2-(thiophen-2-yl)ethan-1-amine **a** (76.3 mg, 60 mM, stock amine solution was adjusted to pH 7.0 with 1 M HCl), D-glucose (108 mg, 60 mM), GDH (1 mL, 1 mg/mL), 10% DMSO (1 mL) in sodium phosphate buffer (6.6 mL, 100 mM, pH 7.0). After being shaken for 24 h at 30 °C with 200 rpm, the reaction mixture was quenched with acetic acid to give a solution with pH 3.0, and celite was added. The mixture was filtered and rinsed with water. The filtrate was extracted with ethyl acetate (3 × 10 mL) to remove the neutral materials. The aqueous phase was alkalified to pH 10 with saturated sodium carbonate, and then extracted with ethyl acetate (3 × 10 mL). The solvent of the combined organic extract was removed under reduced pressure to afford the crude products. The crude products were purified by silica gel column chromatography (gradient elute from pure PE-PE/ EA85:15) to give product **1a** as dark brown oil (73.2 mg, 85%). HRMS calcd. for $C_{17}H_{22}NOS^+$ 288.1422 $[M+H]^+$, found 288.1422.

Gram scale synthesis:

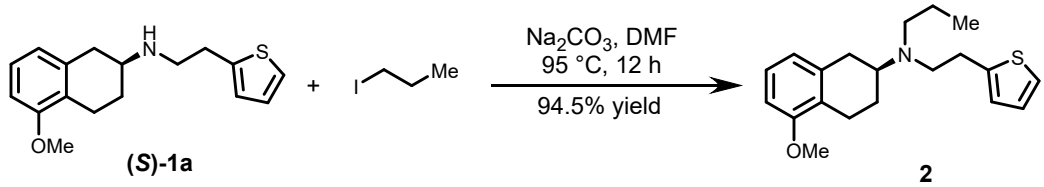
A typical 300 mL reaction mixture contained 1 mM $NADP^+$(223 mg), cell-free extract (10 g/L wet cells weight, M5-F260W-M147Y), 30 mM (1.5 g, 30 mM) of 5-methoxy-3,4-dihydronaphthalen-2(1H)-one **1**, 45 mM (1.7 g, stock amine solution was adjusted to pH 7.0 with 1 M HCl) of 2-(thiophen-2-yl)ethan-1-amine **a**, D-glucose (3.24 g, 60 mM), GDH (30 ml, 1 mg/mL), 10% DMSO (30 ml) in sodium phosphate buffer (203 mL, 100 mM, pH 7.0). After being shaken for 24 h at 30 °C with 200 rpm, the reactions were quenched with acetic acid to give a solution with pH 3.0, and celite was added. The mixtures were filtered and rinsed with water. The filtrate was extracted with ethyl acetate (3 × 300 mL) to remove the neutral materials. The aqueous phase was alkalified to pH 10 with saturated sodium carbonate, and then extracted with ethyl acetate (3 × 300 mL). The solvent of the combined organic extract was removed under reduced pressure to afford the crude products. The crude products were purified by silica gel column chromatography (gradient elute from pure PE-PE/ EA, 85:15) to give product **1a** as dark brown oil (1.86 g, 72%).

1: Shake Flask Fermentation M5-F260W-M147Y.

2: Enzymatic Catalytic Reaction Process.

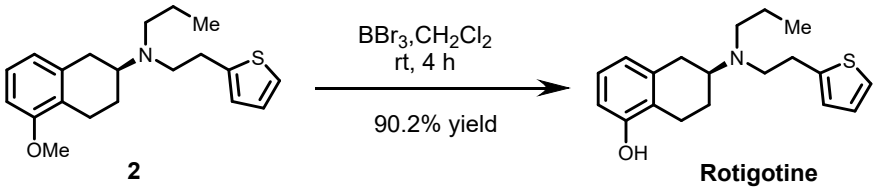
3.4. Total synthesis of rotigotine

(*S*)-5-methoxy-*N*-propyl-*N*-(2-(thiophen-2-yl)ethyl)-1,2,3,4-tetrahydronaphthalen-2-amine, **2**



To a stirred solution of compound **(*S*)-1a** (1.0 g, 3.48 mmol), iodopropane (3.53 g, 21 mmol), Na_2CO_3 (1.5 g, 14 mmol) and DMF (15 ml) were added to a three-mouth bottle with a condensing tube at room temperature and heated to 95 ℃ for 12 h under nitrogen protection. When the reaction was completed, the mixture was cooled to room temperature and diluted with EtOAc (15 ml) and H_2O (15 ml). The aqueous phase was extracted with EtOAc (3×15 ml), and the combined organic phase was washed with brine, filtered, dried over $MgSO_4$, and then concentrated under reduced pressure to obtain crude product. The crude product was purified by silica gel column chromatography (gradient elute from pure PE-PE/ EA, 90:10), to give product **2** as yellow oil (1.10 g, 95%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.16-7.08 (m, 2H), 6.94 (dd, J = 5.2, 3.6 Hz, 1H), 6.84 (d, J = 3.3 Hz, 1H), 6.73 (d, J = 7.7 Hz, 1H), 6.67 (d, J = 8.1 Hz, 1H), 3.83 (s, 3H), 3.06-2.97 (m, 4H), 2.93-2.84 (m, 3H), 2.77 (dd, J = 16.1, 11.4 Hz, 1H), 2.62-2.50 (m, 3H), 2.14-2.04 (m, 1H), 1.57 (ddt, J = 28.9, 15.0, 7.0 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 157.6, 143.7, 138.5, 127.0, 126.6, 125.7, 124.9, 123.6, 122.1, 107.3, 57.2, 55.7, 53.2, 53.2, 32.8, 30.6, 26.2, 24.3, 22.8, 12.4; HRMS calcd. for $C_{20}H_{28}NOS^+$ 330.1892 $[M+H]^+$, found 330.1893.

Rotigotine



At room temperature, compound **2** (1.5 g, 3.03 mmol) and CH_2Cl_2 (15 ml) were added to the sealed tube, and the temperature was lowered to 0 ℃. BBr_3(1.9 g, 5.22 mmol, 1.0 M) dichloromethane solution was added to the reaction solution, and the reaction mixture was raised to room temperature after addition. After the reaction stirred for 4 h, saturated $NaHCO_3$ aqueous solution was added for neutralization, and the mixed solution was transferred to the separation funnel and extracted with CH_2Cl_2 (3×15 ml). The organic layer was combined, dried with $MgSO_4$, and then vacuum concentrated to obtain the crude product. The crude product was purified by silica gel column (gradient elute from pure PE-PE/ EA, 90:10), and the pure product was yellow oil (1.35 g, 90.2%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.13 (dd, J = 5.1, 1.1 Hz, 1H), 7.00 (t, J = 7.8 Hz, 1H), 6.94 (dd, J = 5.1, 3.5 Hz, 1H), 6.83 (d, J = 3.3 Hz, 1H), 6.69 (d, J = 7.6 Hz, 1H), 6.58 (d, J = 7.9 Hz, 1H), 3.04-2.94 (m, 4H), 2.92-2.83 (m, 3H), 2.77 (dd, J = 16.1, 11.4 Hz, 1H), 2.66-2.51 (m, 3H), 2.12 (ddt, J = 10.4, 4.4, 2.2 Hz, 1H), 1.71-1.47 (m, 3H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 153.9, 143.5, 138.8, 127.0, 126.8, 125.0, 123.7, 123.5, 122.1, 112.4, 57.2, 53.2, 53.1, 32.7, 30.3, 26.1, 24.1, 22.5, 12.4; HRMS calcd. for $C_{19}H_{26}NOS^+$ 316.1735 $[M+H]^+$, found 316.1736.

4. Site-saturation mutagenesis library construction and enantioselectivity analysis

Site-saturation mutagenesis libraries were constructed using Fast Mutagenesis System (TransGen Biotech). The codon of the mutation site was replaced by the NNK degeneracy codon (Supplementary Table 1). *E. coli* BL21 (DE3) was transformed with pET28a-IR-36-M5 generated by saturation mutagenesis. Colonies were picked up in deep-well plates containing 300 μL LB medium with 50 μg/mL kanamycin and cultured overnight at 37 ℃ with 800 rpm. The 19 diverse variants with the mutation site respectively replaced by the remaining AA residues were obtained through DNA sequencing. For proteins expression, pre-cultures of the 19 variants were grown in LB-medium (5 mL) containing 50 μg/mL kanamycin overnight at 37 °C with shaking at 220 rpm. An aliquot of 500 μL was transferred to a new culture flask containing 50 mL LB medium with 50 μg/mL kanamycin and cultured at 37 ℃ with shaking at 220 rpm to an OD_{600} of 0.6~0.8. Gene expression was induced by the addition of IPTG (0.1 mM) for 24 h at 20 °C, 220 rpm. The cells were harvested and washed twice with 100 mM pH 7.0 potassium phosphate buffer and centrifuged for 30 min with 4000 rpm. Then, the pellets were resuspended in 5 mL of the same buffer containing 6 U DNase I and 1 mg/mL lysozyme for breaking the cell at 30 °C, 200 rpm for 2 h. Then, 500 μL stock solution containing $NADP^+$ (1 mM), ketone 1 (30 mM, 1eq), amine a (60 mM, 2eq), *D*-glucose (60 mM), 1 mg/mL glucose dehydrogenase (GDH) and 10% v/v DMSO was added. After being shaken for 24 h at 30 °C with 200 rpm, reactions were quenched by the addition of 1 mL saturated

sodium carbonate and extracted with ethyl acetate (3×10 mL). The combined organic extracts were finally dried using anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The extracts were dissolved in ethanol and subjected to HPLC analysis for measurement of the *ee* values.

5. Triple code saturation mutagenesis (A/L/F) library construction and enantioselectivity analysis

Site-saturation mutagenesis libraries were constructed using Fast Mutagenesis System (TransGen Biotech). The codon of the mutation site was replaced by the NNK degeneracy codon (Supplementary Table 1). Subsequent procedure of mutagenesis (A/L/F) library construction and enantioselectivity analysis is the same as the above method.

6. Expression and purification of IR-36-M5

The plasmids containing the genes for target enzymes were used to transform *E. coli* BL21(DE3) competent cells for gene expression. Pre-cultures were grown in LB-medium (10 mL) containing 50 μg/mL kanamycin overnight at 37 °C with shaking at 220 rpm. 1L volume cultures were inoculated with the pre-culture (10 mL) and incubated at 37°C, with shaking at 220 rpm to an OD_{600} of 0.6-0.8. Gene expression was induced by the addition of 0.1 mM isopropyl-*β*-*D*-thiogalactopyranoside (IPTG) and shaking was continued for 16 h at 18 °C, 180 rpm. The cells were then harvested by centrifugation at 5000 rpm for 30 min and resuspended in binding buffer (50 mM Tris-HCl buffer pH 8.0, 300 mM NaCl, containing 20 mM imidazole). Cells were disrupted by ultrasonication for 30 min, 5 s on, 9 s off cycles, and the suspension was centrifuged at 1,2000 rpm for 25 min to yield a clear lysate. The *N*-terminal His-tagged proteins were purified using the Ni-NTA column. In each case, the lysate was loaded onto a pre-equilibrated Ni-NTA column, followed by washing with 40~60 mL a washing buffer (50 mM Tris-HCl buffer pH 8.0, 300 mM NaCl, containing 40 mM imidazole). The bound protein was eluted with elution buffer containing 250 mM imidazole. Proteins were concentrated, and used for biotransformation reactions.

7. Enzymic reductive aminations

Purified proteins of the variants of IR-36-M5 were used to measuring the substrate loading. A typical 5 mL reaction mixture contained 1 mM $NADP^+$, 10 mg/mL purified enzymes, 30-100 mM ketone **1**, amine **a** (2 eq), *D*-glucose (2 eq), 1 mg/mL GDH, 10% DMSO in sodium phosphate buffer (100 mM, pH 7.0). All reactions were incubated at 30 °C with shaking at 220 rpm for 24 h, after which they were quenched by the addition of 5 mL acetonitrile containing 1M acetic acid. Then, the

mixtures were centrifuged at 12000 rpm for 10 minutes, and the supernatant was subjected to HPLC analysis.

8. Biotransformation using cell-free extract

Cell-free extract of M5-F260W-M147Y were used to measuring the conversion and enantioselectivity for ketone **1** and amine **a**. A typical 10 mL reaction mixture contained 1 mM NADP+, cell-free extract (10 g/L wet cells weight), 30 mM (for M5-F260W-M147Y catalyzed reactions) 5-methoxy-3,4-dihydronaphthalen-2(1H)-one 1, 2-(thiophen-2-yl)ethan-1-amine a (2 eq, stock amine solution was adjusted to pH 7.0 with 1 M HCl), D-glucose (2 eq), 1 mg/mL GDH, 10% DMSO in sodium phosphate buffer (100 mM, pH 7.0). After being shaken for 24 h at 30 °C with 200 rpm, the reactions were quenched with acetic acid to give a solution with pH 3.0, and celite was added. The mixtures were filtered and rinsed with water. The filtrate was extracted with dichloromethane (10 mL × 3) to remove the neutral materials. The aqueous phase was alkalified to pH 10 with saturated sodium carbonate, and then extracted with dichloromethane (10 mL × 3). The solvent of the combined organic extract was removed under reduced pressure to afford the corresponding amine products.

9. HPLC and chiral HPLC analysis

All biotransformation products were analyzed by HPLC and the products were confirmed by UV spectrum comparison with standards. Substrate loading and conversions were calculated according to the standard curves. Standard curves were plotted for varying concentrations of standards using the UV detection wavelength of 210 nm (**1a**). The samples above were analyzed by Waters 2695 Separation Module and Wates 2996 Photodiode Array Detector with gradient method (1.0 mL/min, 20 min, H_2O/MeCN, 90/10→10/90, v/v) using C-18 analytic column (Phenomenex Gemini, 4.6×250 mm, 5 μm).

The ee values of racemic amine standards, chiral amine standards and biotransformation products for **1a** were analyzed by chiral HPLC with chiral columns and different solvent ratios of *n*-hexane and ethanol containing 0.02% diethylamine.

10. Molecular docking

The previously reported crystal structure of M5 (7WNW) which is a complex with $NADP^+$, is used as the receptor target in this study. The structures of M5-F20W-M147Y are generated by using the standard mutation protocol of Discovery Studio 2019 Client. Sites 147 and 260 were set as flexible residues using Discovery Studio 2019 Client. Flexible dockings of imine intermediate of **1** and **a** into M5, M5-F260W-M147Y were performed using a flexible docking protocol. Docking runs were

carried out using the standard parameters of the program. The conformations with the lowest energy were chosen for the analysis of substrate-enzyme interactions and distance between carbonyl group of the substrate and C4-H of NADPH.

11. Sequence of IR-36-M5 and the virants

DNA sequence of IR-36-M5

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGCCGG
AATCTACCACCCCGAGTACCGCCACCCCGGTGACCATCATCGGTCTTGGTGCAATGGGCACCGCCCT
GGCAAACGCATTCCTCGATGCAGGTCATAGTACCACCGTTTGGAATCGTACCGCAGCACGCGCCACC
GCATTAGCCGCACGCGGCGCACATCATGCAGAAACCGTGACCGAAGCCATTGCAGCCTCTCCGTTAG
TGATTGCCTGTGTGCTGGATTATGATGCCTTTCATGAAACCTTAGCCCCGGCTACAGACGCGCTGGCA
GGTCGCGCCCTGGTTAATCTGACCACAGGTACCCCGAAACAGGCACGCGAAACCGCCTCTTGGGCAG
CCGATCATCGTATTGATTATCTGGATGGCAAAATTATGGCCATTCCGCCGGGTATTGCAACCCCGGAT
AGTTTTATTCTGTATAGCGGTCCGTTAGGTACCTTTGAAGCACATCGCTCAACCTTAGAAGTGCTGGG
CGCAGCAAATCATGTGGGTACCGATGCAGGTTTGGCGAGCTTACATGATATTGCACTGCTGACCGGT
ATGTATGGCATGATTGCAGGCATTTTACAGGCCTTTGCCTTAATTGATAGTGAAGGTATTCCGGCAGG
CGATCTGGCCCCGATGTTAACCAATTGGTTAACCGGCGCAGCACATAGCGTGGCCCATTATGCCCAG
CAGATTGATACCGGCGATTATGAAACCGGTGTTGTGTTTAATTTAGCACATCAGAGCCATGGCTTTGC
AAAATTAGTTCAGGCCGGTGAAGATCAGGGTGTGGATGTGGGCTTACTGCGTCCGCTGTTTGAACTG
ATGCGTCATCAGGTTGCCGCAGGCTATGGTAATGGTGATGTTGCCTCAGTTATTGAACTGATTCGTCG
CGAAGAACGTCGTCAGCCGGCCAAAAGTCCGGGCGCAGATAAAATTACCCGTGCACGTCGTCCGTA
A

Amino acid sequence IR-36-M5

MGSSHHHHHHSSGLVPRGSHMPESTTPSTATPVTIIGLGAMGTALANAFLDAGHSTTVWNRTAARATAL
AARGAHHAETVTEAIAASPLVIACVLDYDAFHETLAPATDALAGRALVNLTTGTPKQARETASWAADHR
IDYLDGKIMAIPPGIATPDSFILYSGPLGTFEAHRSTLEVLGAANHVGTDAGLASLHDIALLTGMYGMIAGI
LQAFALIDSEGIPAGDLAPMLTNWLTGAAHSVAHYAQQIDTGDYETGVVFNLAHQSHGFAKLVQAGED
QGVDVGLLRPLFELMRHQVAAGYGNGDVASVIELIRREERRQPAKSPGADKITRARRP*

DNA sequence of IR-36-M5-F260W-M147Y

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGCCGG
AATCTACCACCCCGAGTACCGCCACCCCGGTGACCATCATCGGTCTTGGTGCAATGGGCACCGCCCT
GGCAAACGCATTCCTCGATGCAGGTCATAGTACCACCGTTTGGAATCGTACCGCAGCACGCGCCACC
GCATTAGCCGCACGCGGCGCACATCATGCAGAAACCGTGACCGAAGCCATTGCAGCCTCTCCGTTAG
TGATTGCCTGTGTGCTGGATTATGATGCCTTTCATGAAACCTTAGCCCCGGCTACAGACGCGCTGGCA

GGTCGCGCCCTGGTTAATCTGACCACAGGTACCCCGAAACAGGCACGCGAAACCGCCTCTTGGGCAG
CCGATCATCGTATTGATTATCTGGATGGCAAAATTTATGCCATTCCGCCGGGTATTGCAACCCCGGAT
AGTTTTATTCTGTATAGCGGTCCGTTAGGTACCTTTGAAGCACATCGCTCAACCTTAGAAGTGCTGGG
CGCAGCAAATCATGTGGGTACCGATGCAGGTTTGGCGAGCTTACATGATATTGCACTGCTGACCGGT
ATGTATGGCATGATTGCAGGCATTTTACAGGCCTTTGCCTTAATTGATAGTGAAGGTATTCCGGCAGG
CGATCTGGCCCCGATGTTAACCAATTGGTTAACCGGCGCAGCACATAGCGTGGCCCATTATGCCCAG
CAGATTGATACCGGCGATTATGAAACCGGTGTTGTGTGGAATTTAGCACATCAGAGCCATGGCTTTG
CAAAATTAGTTCAGGCCGGTGAAGATCAGGGTGTGGATGTGGGCTTACTGCGTCCGCTGTTTGAACT
GATGCGTCATCAGGTTGCCGCAGGCTATGGTAATGGTGATGTTGCCTCAGTTATTGAACTGATTCGTC
GCGAAGAACGTCGTCAGCCGGCCAAAAGTCCGGGCGCAGATAAAATTACCCGTGCACGTCGTCCGT
AA

Amino acid sequence IR-36-M5-F260W-M147Y

MGSSHHHHHHSSGLVPRGSHMPESTTPSTATPVTIIGLGAMGTALANAFLDAGHSTTVWNRTAARATAL
AARGAHHAETVTEAIAASPLVIACVLDYDAFHETLAPATDALAGRALVNLTTGTPKQARETASWAADHR
IDYLDGKIYAIPPGIATPDSFILYSGPLGTFEAHRSTLEVLGAANHVGTDAGLASLHDIALLTGMYGMIAGI
LQAFALIDSEGIPAGDLAPMLTNWLTGAAHSVAHYAQQIDTGDYETGVVWNLAHQSHGFAKLVQAGED
QGVDVGLLRPLFELMRHQVAAGYGNGDVASVIELIRREERRQPAKSPGADKITRARRP*

Table 1. List of primers in this study

Primer	mutant	Sequences (5′→3′)
F	F260A	GTTGTGGCCAATTTAGCACATCAGAG
R	F260A	CTAAATTGGCCACAACACCGGTTTCA
F	F260L	GTTGTGTTAAATTTAGCACATCAGAG
R	F260L	CTAAATTTAACACAACACCGGTTTCA
F	F260NNK	GTTGTGNNKAATTTAGCACATCAGAG
R	F260NNK	CTAAATTMNNCACAACACCGGTTTCA
F	W234A	ACCAATGCCTTAACCGGCGCAGCACATAGC
R	W234A	GCCGGTTAAGGCATTGGTTAACATCGGGGC
F	W234L	ACCAATTTATTAACCGGCGCAGCACATAGC
R	W234L	GCCGGTTAATAAATTGGTTAACATCGGGGC
F	W234F	ACCAATTTTTTAACCGGCGCAGCACATAGC
R	W234F	GCCGGTTAAAAAATTGGTTAACATCGGGGC
F	W234NNK	ACCAATNNKTTAACCGGCGCAGCACATAGC
R	W234NNK	GCCGGTTAAMNNATTGGTTAACATCGGGGC

F	M147A	GCAAAATTGCAGCCATTCCGCCGGGTATTGCAACC
R	M147A	GCGGAATGGCTGCAATTTTGCCATCCAGATAATCA
F	M147L	GCAAAATTGCATTAATTCCGCCGGGTATTGCAACC
R	M147L	GCGGAATTAATGCAATTTTGCCATCCAGATAATCA
F	M147F	GCAAAATTGCATTTATTCCGCCGGGTATTGCAACC
R	M147F	GCGGAATAAATGCAATTTTGCCATCCAGATAATCA
F	M147NNK	GCAAAATTGCANNKATTCCGCCGGGTATTGCAACC
R	M147NNK	GCGGAATMNNTGCAATTTTGCCATCCAGATAATCA
F	L200A	ACCGGTTGCCAGTGCAATATCATGTAAGCTCGCCA
R	L200A	TATTGCACTGGCAACCGGTATGTATGGCATGATTG
F	L200F	ACCGGTTTTTAGTGCAATATCATGTAAGCTCGCCA
R	L200F	TATTGCACTAAAAACCGGTATGTATGGCATGATTG
F	L200NNK	ACCGGTTNNKAGTGCAATATCATGTAAGCTCGCCA
R	L200NNK	TATTGCACTMNNAACCGGTATGTATGGCATGATTG
F	S241A	AGCACATGCCGTGGCCCATTATGCCCA
R	S241A	GGGCCACGGCATGTGCTGCGCCGGTTAA
F	S241L	AGCACATTTAGTGGCCCATTATGCCCAG
R	S241L	GGGCCACTAAATGTGCTGCGCCGGTTAA
F	S241F	GCGCAGCACATTTTGTGGCCCATTATGCCCAGCAGATT
R	S241F	ATAATGGGCCACAAAATGTGCTGCGCCGGTTAACCAATT
F	S241NNK	AGCACATNNKGTGGCCCATTATGCCCAG
R	S241NNK	GGGCCACMNNATGTGCTGCGCCGGTTAA
F	M203A	ACCGGTGCATATGGCATGATTGCAGGCATT
R	M203A	GCCATATGCACCGGTCAGCAGTGCAATAT
F	M203L	ACCGGTTTATATGGCATGATTGCAGGCATT
R	M203L	GCCATATAAACCGGTCAGCAGTGCAATATC
F	M203F	ACCGGTTTTTATGGCATGATTGCAGGCATT
R	M203F	GCCATAAAAACCGGTCAGCAGTGCAATATC
F	M203NNK	ACCGGTNNKTATGGCATGATTGCAGGCATT
R	M203NNK	GCCATAMNNACCGGTCAGCAGTGCAATATC
F	I149A	TATGGCCGCACCGCCGGGTATTGCAACCC
R	I149A	CCCGGCGGTGCGGCCATAATTTTGCCATC
F	I149L	TATGGCCTTACCGCCGGGTATTGCAACCC
R	I149L	CCCGGCGGTAAGGCCATAATTTTGCCATCC
F	I149F	TATGGCCTTTCCGCCGGGTATTGCAACCC

R	I149F	CCCGGCGGAAAGGCCATAATTTTGCCATCC
F	I149NNK	TATGGCCNNKCCGCCGGGTATTGCAACCC
R	I149NNK	CCCGGCGGMNNGGCCATAATTTTGCCATCC

Table 2. Enantioselectivity of the mutants generated by site-saturation mutagenesis of residue M147 over M5

Mutants	ee value	Mutants	ee value	Mutants	ee value
M147A	80.36%, *S*	M147V	84.46%, *S*	M147N	82.54%, *S*
M147L	84.44%, *S*	M147P	82.74%, *S*	M147Q	81.7%, *S*
M147F	83.6%, *S*	M147W	86.58%, *S*	M147Y	93.56%, *S*
M147H	89.96%, *S*	M147G	83.26%, *S*	M147D	80.46%, *S*
M147R	81.56%, *S*	M147C	73.38%, *S*	M147E	87.74%, *S*
M147K	N.D.	M147S	84.02%, *S*		
M147I	75.88%, *S*	M147T	75.78%, *S*		

Table 3. Enantioselectivity of the mutants generated by site-saturation mutagenesis of residue M203 over M5

Mutants	ee value	Mutants	ee value	Mutants	ee value
M203A	16.8%, *S*	M203V	69.28%, *S*	M203N	62.04%, *S*
M203L	76.74%, *S*	M203P	57.82%, *S*	M203Q	86.7%, *S*
M203F	22.94%, *S*	M203W	4.64%, *R*	M203Y	3.46%, *R*
M203H	0.9%, *R*	M203G	N.D.	M203D	N.D.
M203R	60%, *S*	M203C	68%, *S*	M203E	75.86%, *S*
M203K	92.5%, *S*	M203S	45.5%, *S*		
M203I	N.D.	M203T	70.34%, *S*		

Table 4. Enantioselectivity of the mutants generated by site-saturation mutagenesis of residue W234 over M5

Mutants	ee value	Mutants	ee value	Mutants	ee value
W234A	78.02%, *S*	W234V	50.26%, *S*	W234N	65.1%, *S*
W234L	61.26%, *S*	W234M	76.44%, *S*	W234Q	76.13%, *S*
W234F	83.5%, *S*	W234P	69.64%, *S*	W234Y	81.02%, *S*
W234H	73.42%, *S*	W234G	80.02%, *S*	W234D	63.76%, *S*
W234R	76.18%, *S*	W234C	76.88%, *S*	W234E	65.4%, *S*
W234K	76.11%, *S*	W234S	77.14%, *S*		
W234I	41.68%, *S*	W234T	55.54%, *S*		

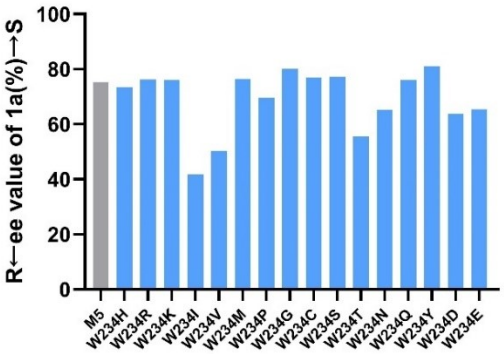


Figure 1. Enantioselectivity of IR-36-M5 and its mutants at sites W234

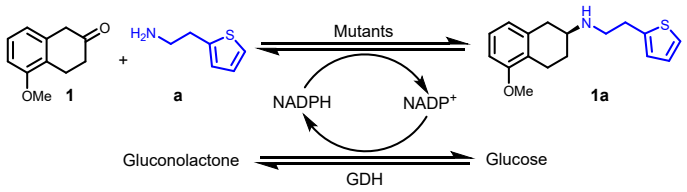
Table 5. Enantioselectivity of the mutants generated by site-saturation mutagenesis of residue F260 over M5

Mutants	ee value	Mutants	ee value	Mutants	ee value
F260A	63.84%, *S*	F260W	92.5%, *S*	F260E	N.D.
F260L	68.5%, *S*	F260G	N.D.		
F260H	N.D.	F260C	50.36%, *S*		
F260R	N.D.	F260S	N.D.		
F260I	35.62%, *S*	F260T	N.D.		
F260V	75.64%, *S*	F260N	64.9%, *S*		
F260P	N.D.	F260Y	N.D.		

Table 6. Enantioselectivity of the mutants generated by Triple code saturation mutagenesis (A/L/F) of residues I149, L200, and S241 over M5

Mutants	ee value	Mutants	ee value	Mutants	ee value
I149A	59.22%, *S*	I149L	21.86%, *S*	I149F	29.14%, *S*
L200A	75.42%, *S*	L200F	86.28%, *S*		
S241A	57.44%, *S*	S241L	71.26%, *S*	S241F	76.7%, *S*

Table7. Conversion and stereoselectivities of IRED mutants towards **1a**



Entry	Mutants	Substrate loading (mM)	Enzyme loading (mg mL^{-1})	*ee* (%)
1	M203K/W234F	30	10	87, S
2	M203K/M147Y	30	10	97, S
3	M203K/F260W	30	10	71, S
4	W234F/F260W	30	10	92, S
5	W234F/M147Y	30	10	93, S
6	F260W/M147Y	30	10	＞99, S

Chiral HPLC Chromatograms

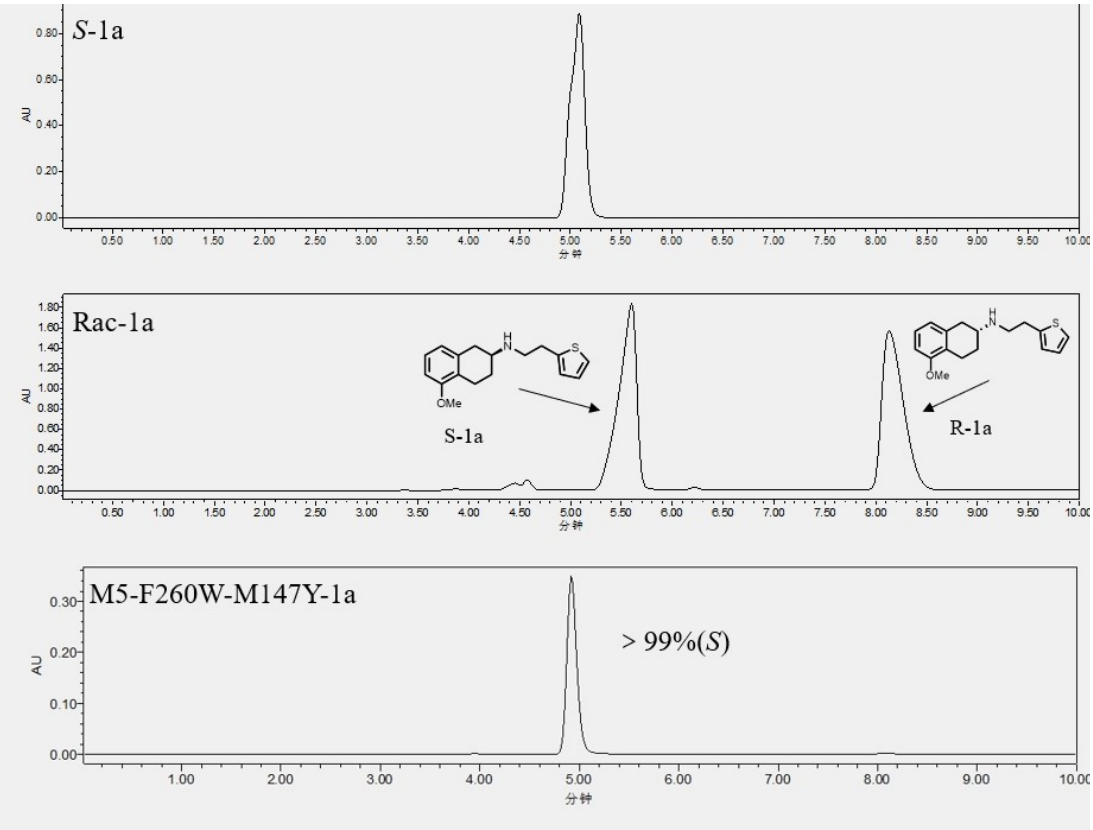


Figure 2. Chiral HPLC analysis of racemic standard of **1a**, and chiral amine standards of **1a**, M5-F260W-M147Y catalytic product **1a**. HPLC conditions: CHIRALPAK AY-H column with a mobile phase of *n*-hexane/ethanol (80:20, v/v, 0.2‰ diethylamine), flow rate 1.0 mL/min, 30 °C, UV detection at 277 nm

NMR spectra of amine products

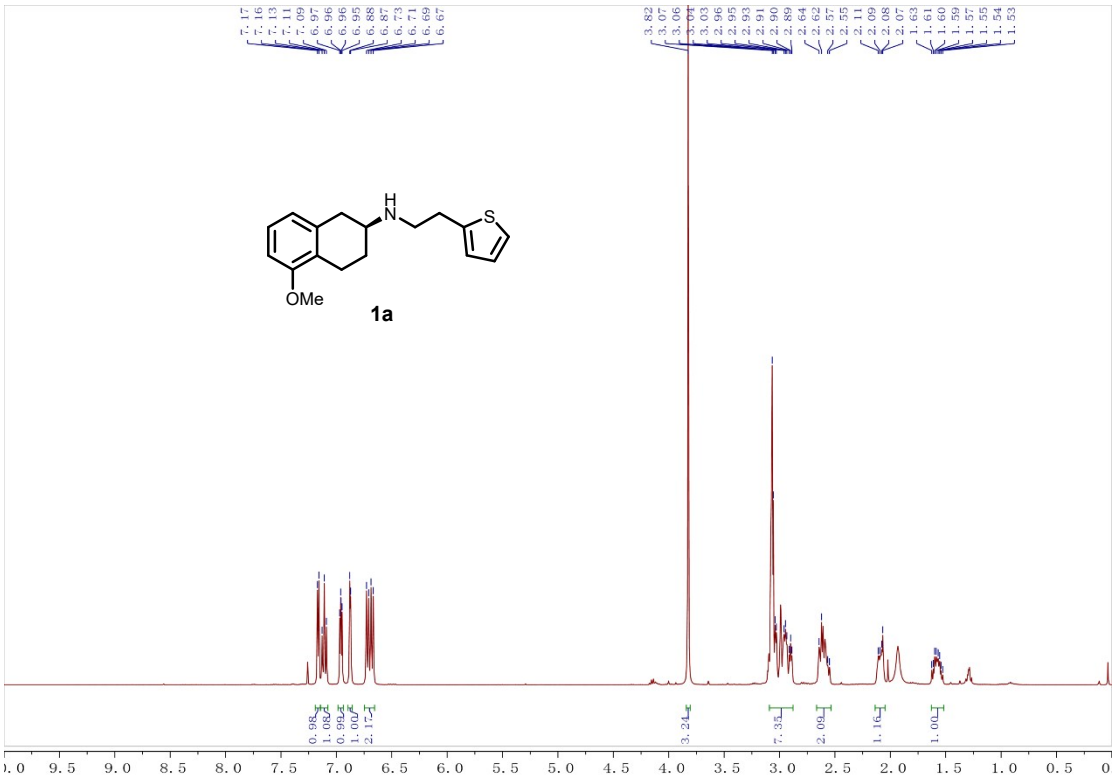


Figure 3. The ^{1}H NMR spectrum of **1a** in chloroform-*d* (400 MHz)

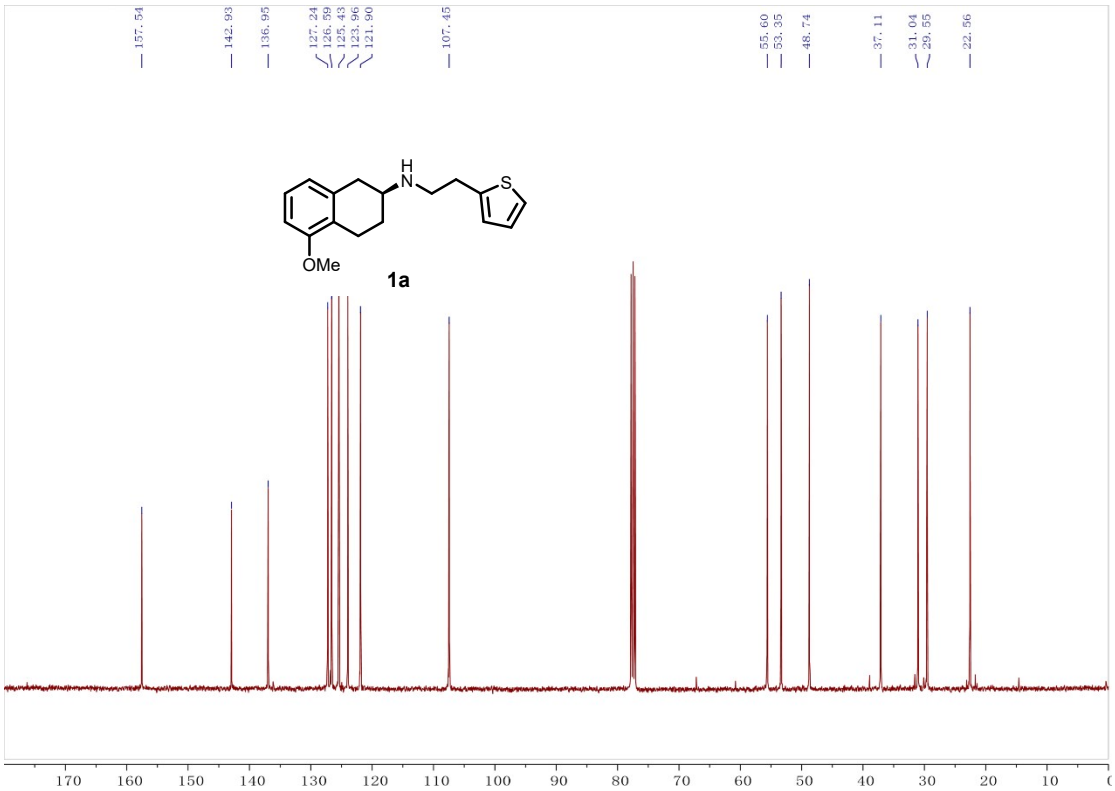


Figure 4. The ^{13}C NMR spectrum of **1a** in chloroform-*d* (100 MHz)

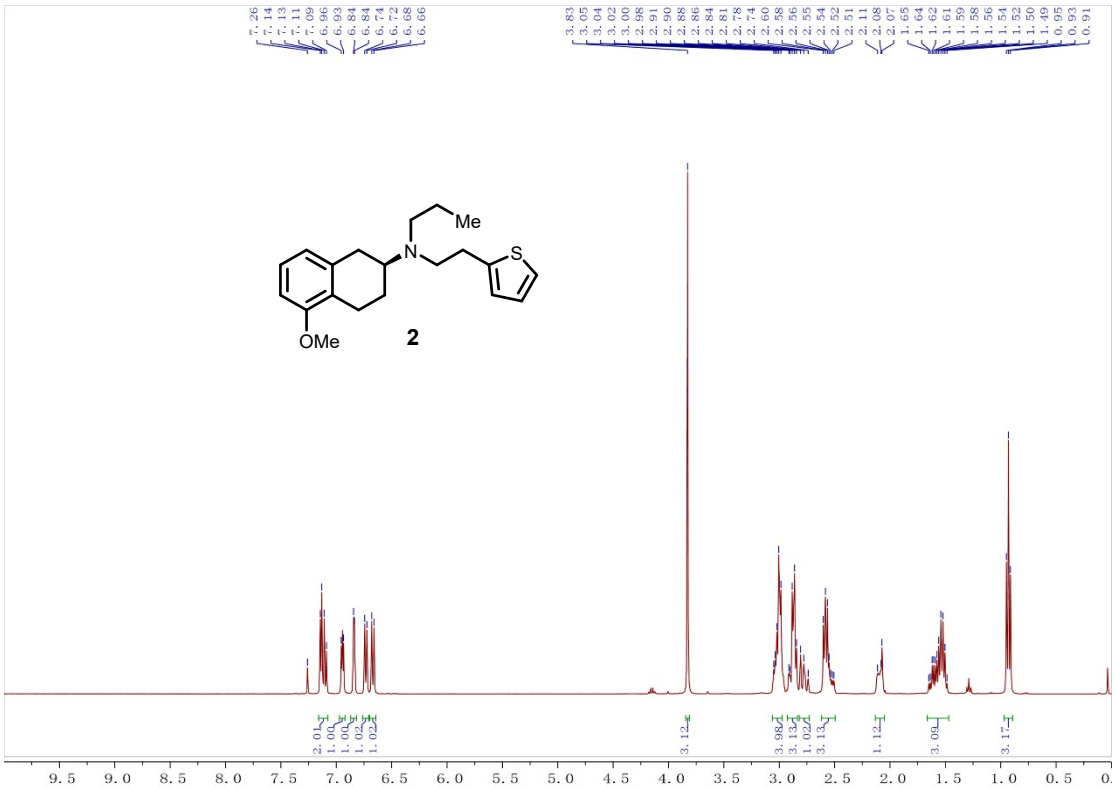


Figure 5. The ^{1}H NMR spectrum of **2** in chloroform-*d* (400 MHz)

Figure 6. The ^{13}C NMR spectrum of **2** in chloroform-*d* (100 MHz)

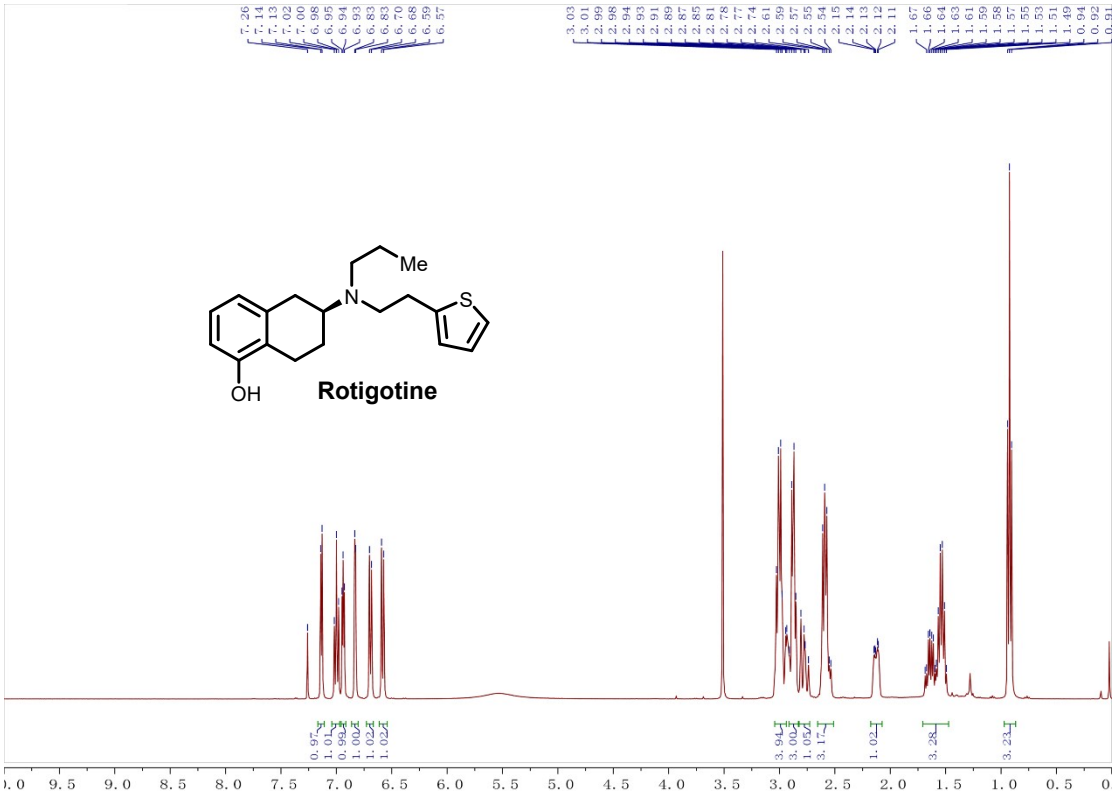


Figure 7. The ^{1}H NMR spectrum of **Rotigotine** in methanol-d_4 (400 MHz)

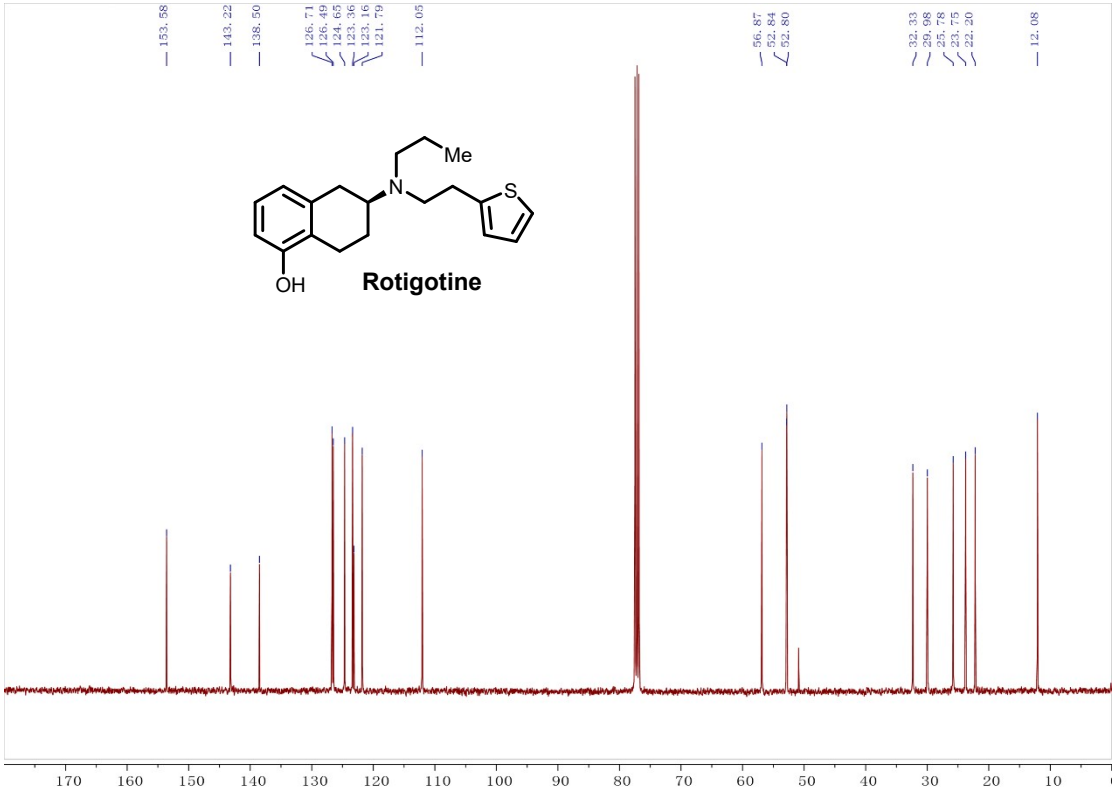


Figure 8. The ^{13}C NMR spectrum of **Rotigotine** in methanol-d_4 (100 MHz)

HRMS spectra of products

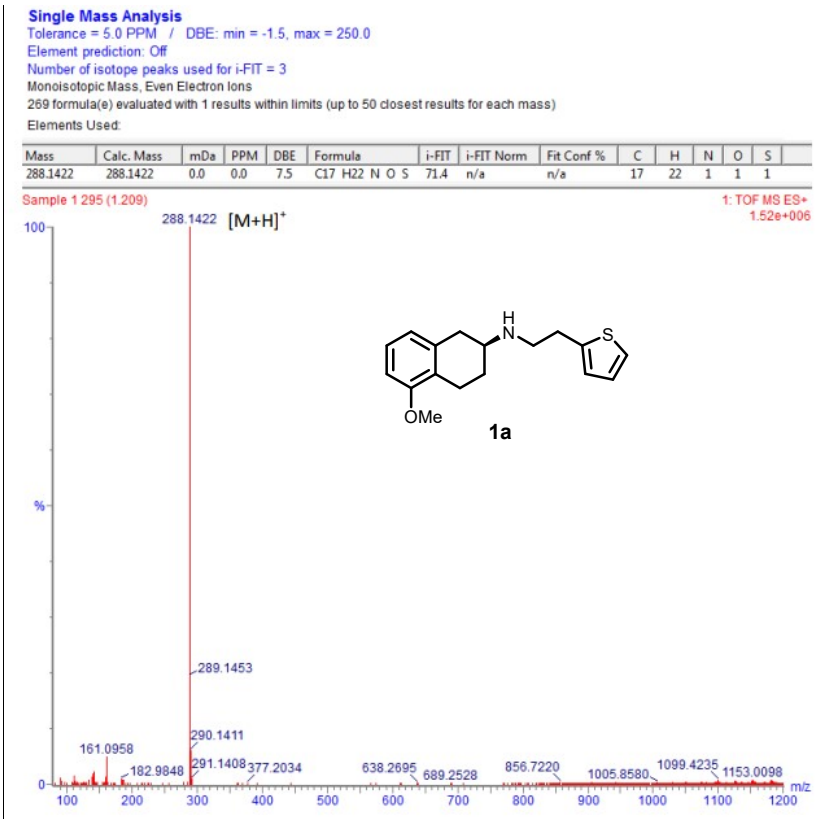


Figure 9. The HRMS spectrum of **1a**.

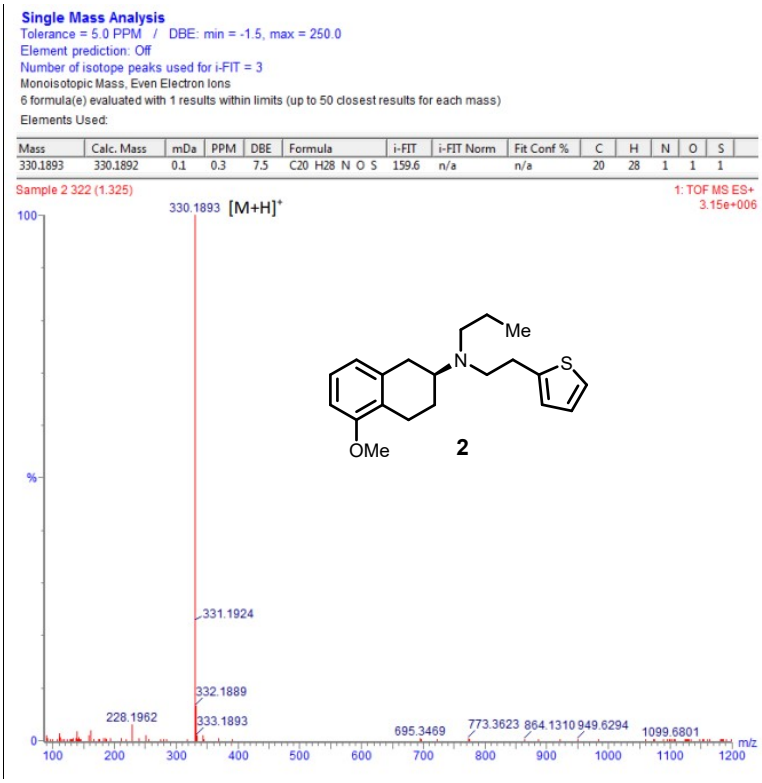


Figure 10. The HRMS spectrum of **2**.

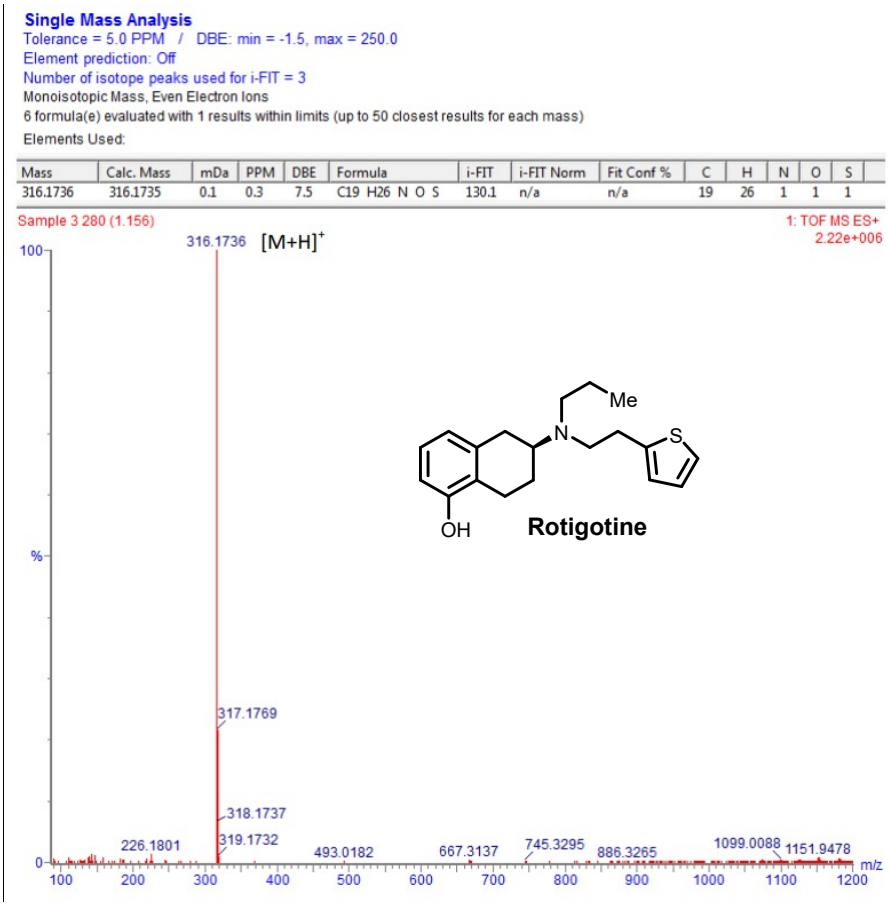


Figure 11. The HRMS spectrum of **Rotigotine**.