

Supplementary Information

Engineered halohydrin dehalogenase mediates remote enantiocontrolled dehalogenative hydroxylation via an unconventional mechanism

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1. Materials and methods

General.

^1H -NMR (400 MHz) and ^{13}C -NMR (100 MHz) spectra were recorded on an Agilent Technologies 400 MR spectrometer at ambient temperature. Chemical shifts (δ) are reported in parts per million (ppm) using residual solvent signals as internal references, coupling constants (J) are given in Hertz (Hz), and peak integrals are expressed as relative ratios. Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets. High-resolution mass spectra (HRMS) were acquired using electrospray ionization time-of-flight (ESI-TOF) or field ionization time-of-flight (FI-TOF) detector. Flash column chromatography was carried out using 300–400 mesh silica gel.

Materials

Racemic ε -haloalcohols and racemic ε -diols used in this study were synthesized following established chemical procedures. The following compounds were obtained from commercial suppliers: 2-chloro-1-phenylethan-1-ol (**1a1**), (S)-**1c1**, 3-chloro-1-phenylpropan-1-ol (**1a2**), 5-chloro-1-phenylpentan-1-one (**1a4**), 2-phenyloxirane (**1c1**), and (S)-**1c2**. Compounds 2-phenyloxetane (**1c2**), 4-chloro-1-phenylbutan-1-ol (**1a3**) and 2-phenyltetrahydrofuran (**1c3**) and their corresponding enantiomers were prepared in our earlier work and used directly in this study. PrimeSTAR DNA polymerase and *Dpn* I endonuclease were purchased from Takara. Cells were cultured in Terrific Broth (TB) medium (containing 12 g/L peptone, 24 g/L yeast extract, and 4 mL/L glycerol). Isopropyl- β -D-thiogalactopyranoside (IPTG), kanamycin sulfate, and ampicillin (Amp) were obtained from Solarbio (Beijing, China). Reaction buffers, including phosphate buffer (PB, $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$) and glycine-NaOH (Gly-NaOH), served as the buffering systems for whole-cell biotransformations. Unless otherwise specified, all other reagents and solvents were obtained from commercial sources and used as received.

Chromatography analysis.

The enantiomeric composition of all biotransformation samples, isolated enzymatic products, and derivatization products was analyzed by chiral high-performance liquid chromatography (HPLC) using a Shimadzu LC-20A system. The following chiral columns were employed as appropriate for each compound: Chiralpak AY-H (4.6 mm × 250 mm, 5 μm), Chiralcel OD-H (4.6 mm × 250 mm, 5 μm), Chiralpak AD-H (4.6 mm × 250 mm, 5 μm), Chiralcel OJ-H (4.6 mm × 250 mm, 5 μm), Chiralcel OB-H (4.6 mm × 250 mm, 5 μm), Chiralcel OX-3 (4.6 mm × 250 mm, 3 μm), Chiralcel OD-3 (4.6 mm × 250 mm, 3 μm), Chiralpak IB (4.6 mm × 250 mm, 5 μm), and Chiralpak IF (4.6 mm × 250 mm, 5 μm). For ε-haloalcohol **11a**, enantiomeric excess (*ee*) was determined by chiral gas chromatography (GC) on an Agilent 7890B instrument equipped with a flame ionization detector (FID) and an Rt-βDEXcst column, using nitrogen as the carrier gas. Detailed chiral HPLC and GC methods are provided in Section 9 and Section 10. The (*R*)- and (*S*)-enantiomers of the different substrates and products in chiral HPLC were assigned by analogy based on the *S*-enantioselectivity of biotransformation. Specifically, by comparing the consumption of racemic substrate standards in chiral HPLC analysis, the more rapidly depleted substrate peak was assigned as the *S*-enantiomer, while the less consumed peak corresponded to the *R*-enantiomer. Correspondingly, the major product peak formed was assigned as the *S*-enantiomer, and the minor product peak as the *R*-enantiomer.

Preparation and screening of *E. coli* (HHDH).

Recombinant *E. coli* strains for halohydrin dehalogenase (HHDH) expression were constructed and maintained in our laboratory, including *E. coli* (HHDH) variants¹⁻³ (Supplementary Table 1), *E. coli* (HheG) variants^{4,5} (Supplementary Table 2), and *E. coli* (HheC) variants^{6,7} (Supplementary Table 3). Each variant was expressed using plasmid pET-28b(+) (Novagen, Kit Lot No. 69865) or pET-32a(+) (Novagen, Kit Lot No. 69015) in *E. coli* BL21(DE3) as the host strain.¹⁻³ Cells were grown in TB medium supplemented with 50 μg/mL kanamycin or 100 μg/mL ampicillin at 37 °C until OD₆₀₀ reached 0.6–0.8. Subsequently, IPTG was added to a final concentration of 0.2 mM to

induce enzyme expression, and the culture was incubated at 28 °C for an additional 12 h. Cells expressing the target HHDH were harvested by centrifugation at $8800 \times g$ for 3 min at 4 °C. The pellet was resuspended in the appropriate reaction buffer to the desired cell density for subsequent biotransformation assays. Analytical-scale reactions were typically performed in 5 mL of PB buffer (pH 8.5, 50 mM) containing 5-10 mM substrate (with 1% v/v DMSO as co-solvent) and 10 g dcw/L of *E. coli* cells at 30 °C for 24 h. The analytical yields of ϵ -diol **1b** were determined by HPLC and quantified using the corresponding calibration curve. Conversions and enantioselectivity (*E* values) were calculated from the enantiomeric excess (ee) of the substrate (*ee_s*) and the product (*ee_p*), using the following equations:

$$\text{Conversion (\%)} = ee_s / (ee_s + ee_p) \times 100.$$

$$\text{Enantioselectivity (E): } E = \ln[(1-ee_s)/(1+ee_s/ee_p)] / \ln[(1+ee_s)/(1+ee_s/ee_p)].$$

Molecular dynamics simulations

The X-ray crystal structure of wild-type HheC (HheC-WT) has been previously determined (PDB ID: 1PWZ).⁸ The initial HheC-SM9 model was obtained by introducing the L142A mutation into the structure of HheC-WT (chain A) using the mutagenesis toolkit in PyMOL software. Molecular dynamics (MD) simulations of HheC-SM9 model were performed using the GPU-accelerated pmemd code in AMBER 22.⁹ Protonation states of ionizable residues were assigned with the H++ server (<http://biophysics.cs.vt.edu/H++>).¹⁰ The ff14SB force field¹¹ was applied to the enzyme, and TIP3P¹² water molecules were used for solvation. The system was placed in a truncated octahedral box with a 10 Å buffer of explicit water, resulting in approximately 12,000 solvent molecules. The system was neutralized with Na⁺ and Cl⁻ ions. Prior to production simulations, the system underwent two-stage energy minimization: first with positional restraints on the protein (500 kcal/mol·Å²) applied to water and ions, followed by minimization of the entire system. The system was gradually heated from 0 K to 300 K in six 50-ps steps under the NVT ensemble using a Langevin thermostat, with a weak restraint (10 kcal/mol·Å²) and an 8 Å nonbonded

cutoff. A time step of 1 fs was used during heating. Equilibration was then performed for 2 ns under the NPT ensemble using a Berendsen barostat and Langevin thermostat, with a time step of 2 fs. Finally, a 600 ns production simulation was carried out under the NVT ensemble with a Langevin thermostat.

Enzyme expression and purification of HheC-SM9.

To purify the HheC-SM9 (L142A) mutant, we introduced an N-terminal 6×His-tag, generating the recombinant strain *E. coli* (His+HheC-SM9). A single colony was used to inoculate 50 mL of TB medium supplemented with 50 µg/mL kanamycin, followed by overnight incubation at 37 °C and 250 rpm. The overnight culture was diluted 1:50 into 50 mL (×10) of fresh TB medium containing the same antibiotic and grown under identical conditions until OD₆₀₀ reached ~0.8 (2–3 h). Protein expression was induced by adding IPTG to a final concentration of 0.2 mM, after which the culture was incubated at 28 °C and 250 rpm overnight. Cells were harvested by centrifugation (8,800 × *g*, 4 °C, 5 min), resuspended in lysis buffer A (50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl), and disrupted by sonication. Following clarification by centrifugation (11,000 × *g*, 4 °C, 20 min), the supernatant containing the His-tagged HheC-SM9 was loaded onto a Ni-NTA affinity column equipped with BeyoGold™ His-tag purification resin (Beyotime Biotechnology, China) and pre-equilibrated with buffer A. Non-specifically bound proteins were removed by washing with 5 column volumes of buffer B (50 mM NaH₂PO₄, 300 mM NaCl, 2 mM imidazole, pH 8.0). Bound target protein was then eluted with 10 column volumes of elution buffer C (50 mM NaH₂PO₄, 300 mM NaCl, 50 mM imidazole, pH 8.0). Eluted fractions were pooled, concentrated using a 10 kDa ultrafiltration device (Amicon® Ultra-15, Millipore), buffer-exchanged into sterile water, and finally lyophilized.

Isotope-Labeling experiments

Reaction in H₂¹⁶O: Lyophilized HheC-SM9 powder (500 nmol) was dissolved in 50 µL of H₂¹⁶O in a 1.5 mL microcentrifuge tube. Substrate (*rac*)-**1a** (500 nmol) was added with 1% v/v DMSO as co-solvent, and the mixture was shaken vigorously (1000 rpm)

at 30 °C for 12 h. The reaction was quenched by addition of 800 μ L ethyl acetate, followed by centrifugation (12000 rpm, 3 min). The organic layer was separated, dried over anhydrous Na_2SO_4 , and analyzed by GC/TOF-MS (FI positive ion mode).

Reaction in H_2^{18}O : The procedure described above was repeated using H_2^{18}O (50 μ L) in place of H_2^{16}O .

Reaction in a 1:1 $\text{H}_2^{16}\text{O}/\text{H}_2^{18}\text{O}$ mixture: The same protocol was followed, with HheC-SM9 (500 nmol) dissolved in a 1:1 mixture of H_2^{16}O (25 μ L) and H_2^{18}O (25 μ L). All other steps were identical.

Docking study.

A representative snapshot of HheC-SM9 was selected for docking with the preferred substrate (S)-**1a**. The snapshot was taken after the system reached equilibrium, as indicated by stabilization of the RMSD profile at approximately 30 ns of the molecular dynamics simulation. Molecular docking was performed using AutoDock 4.0,¹³ with search parameters set to the “Genetic Algorithm” and default docking settings. The docking pose was selected based on the proposed catalytic mechanism, which requires hydrogen bonds between the hydroxyl group of (S)-**1a** and the hydroxyl groups of Ser132 and Tyr145. The selected pose was then visually analyzed with PyMOL.¹⁴

Directed evolution of HheC-SM9 (HheC- ϵ M1).

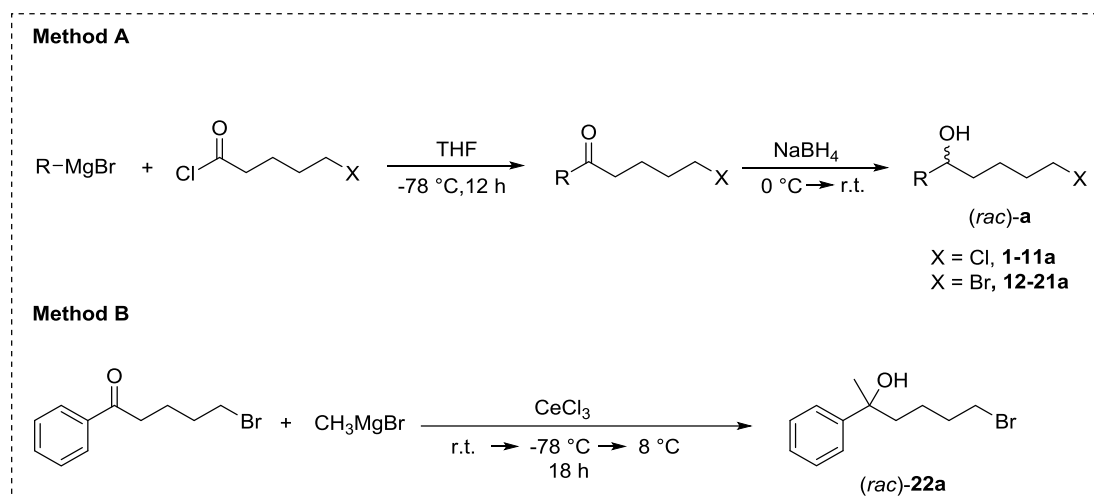
Site-saturation mutagenesis of HheC- ϵ M1 was performed via QuickChange PCR using degenerate codons. The amplified products were digested with *Dpn* I, purified, and transformed into *E. coli* BL21(DE3) competent cells by heat shock. Single colonies were picked and grown in 2-mL 96-deep-well plates containing 300 μ L TB-Kan medium (50 μ g/mL kanamycin) at 37 °C for 6 h. Subsequently, 900 μ L of fresh TB-Kan medium supplemented with 0.2 mM IPTG was added to each well, and the plates were incubated at 28 °C and 900 rpm for 12 h. Cells were harvested by centrifugation ($1600 \times g$, 15 min, 4 °C) and used directly for biotransformations.

For dehalogenation performance screening, harvested cells were resuspended in 500 μ L of PB buffer (100 mM, pH 8.5) containing 10 mM (*rac*)-**1a** (with 1% v/v DMSO

as co-solvent). The plates were shaken at 30 °C and 900 rpm for 10 h. Reactions were extracted with 700 μ L ethyl acetate, and the organic phases were separated, dried over anhydrous Na₂SO₄, and analyzed by chiral HPLC. Mutants showing improved catalytic activity and/or enantioselectivity were further validated in 5-mL biotransformation reactions (PB buffer, pH 8.5, 100 mM; 10 mM (*rac*)-**1a**; 10 g dcw/L *E. coli* cells) at 30 °C for 10 h. Conversions and *ee* values (**1a** and **1b**) were determined by chiral HPLC. Positive variants were preserved for sequencing by Sangon Biotechnology Co. Ltd. (Shanghai, China). The site-directed mutagenesis was performed following the same protocol as used for site-saturation mutagenesis. Three colonies were selected and sequenced to confirm the presence of the desired mutation.

2. Chemical synthesis and characterization of racemic compounds

Synthesis of racemic ϵ -haloalcohols:

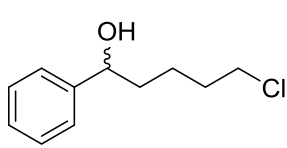


The synthesis of racemic ϵ -haloalcohols **1-21a** was achieved by treating Grignard reagents with 5-chloropentanoyl chloride or 5-bromopentanoyl chloride. (Method A).¹⁵ General procedure: Under anhydrous and anaerobic conditions, 2.6 mL of 5-chloropentanoyl chloride was added to a round-bottom flask, followed by 60 mL of anhydrous tetrahydrofuran. The mixture was cooled to $-78\text{ }^\circ\text{C}$ with stirring, and 20 mL of phenylmagnesium bromide (1.0 M in THF) was added dropwise over a period of 20 minutes. After complete addition, the reaction was maintained at $-78\text{ }^\circ\text{C}$ for 30 min and then allowed to warm to room temperature and stirred for an additional 12 h. The reaction was quenched with water and extracted with ethyl acetate ($3 \times 30\text{ mL}$). The combined organic layers were washed with saturated aqueous NaCl ($3 \times 30\text{ mL}$), dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 80:1 to 20:1) to give the corresponding haloketone intermediate. The obtained haloketone was then dissolved in methanol in a round-bottom flask equipped with a magnetic stirrer. After cooling to $0\text{ }^\circ\text{C}$, sodium borohydride (2.0 equiv) was added portionwise. The mixture was then warmed to room temperature and stirred for 1 h (reaction progress was monitored by TLC). The solvent was removed under reduced pressure, and the residue was taken up in water and extracted with ethyl acetate ($3 \times 30\text{ mL}$). The organic extracts were washed with saturated aqueous NaCl ($3 \times 30\text{ mL}$), dried over anhydrous Na_2SO_4 ,

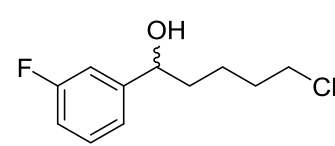
and concentrated in vacuo. The crude product was further purified by flash chromatography (petroleum ether: ethyl acetate = 20:1 ~ 5:1) to afford the racemic ϵ -haloalcohol (*rac*)-**a**.

Racemic ϵ -haloalcohol **22a** was synthesized from 5-bromo-1-phenylpentan-1-one via reaction with methylmagnesium bromide in the presence of cerium(III) chloride.¹⁶ In a nitrogen-filled glovebox, a 200-mL round-bottom flask was charged with anhydrous CeCl₃ (5.6 g, 22.6 mmol) and dry THF (40 mL). The mixture was stirred at room temperature for 3.5 h and then cooled to -78 °C. Methylmagnesium bromide (1 M in THF, 40 mL) was added, and the reaction was maintained at -78 °C for 2 h. A solution of 5-bromo-1-phenylpentan-1-one (3.4 g, 20 mmol) in dry THF (30 mL) was added dropwise. The reaction temperature was then allowed to rise gradually to 8 °C and stirred for 18 h. The mixture was quenched with saturated NH₄Cl solution (15 mL), extracted with ethyl acetate (3 × 20 mL), and washed with saturated brine. The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate = 30:1 ~ 10:1) to afford racemic (*rac*)-**22a**.

(*rac*)-5-chloro-1-phenylpentan-1-ol [(*rac*)-1a]¹⁷

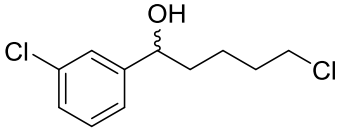
 ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.32 – 7.29 (m, 4H), 7.23 – 7.19 (m, 1H), 5.17 (d, *J* = 4.4 Hz, 1H), 4.53 – 4.49 (m, 1H), 3.60 (t, *J* = 6.5 Hz, 2H), 1.74 – 1.53 (m, 4H), 1.50 – 1.31 (m, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 146.4, 128.0, 126.6, 125.8, 72.1, 45.5, 38.6, 32.1, 22.9. HRMS (ESI) *m/z*: calculated for C₁₁H₁₅ClNaO [M+Na]⁺: 221.0704, found: 221.0709.

(*rac*)-5-chloro-1-(3-fluorophenyl)pentan-1-ol [(*rac*)-2a]

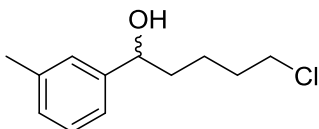
 ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.34 (q, *J* = 7.8 Hz, 1H), 7.14 (t, *J* = 7.2 Hz, 2H), 7.05 – 7.00 (m, 1H), 5.32 (d, *J* = 4.5 Hz, 1H), 4.55 (q, *J* = 5.6 Hz, 1H), 3.60 (t, *J* = 6.5 Hz, 2H), 1.76 – 1.67 (m, 2H), 1.62 – 1.55 (m, 2H), 1.51 – 1.32 (m, 2H). ¹³C NMR (100

MHz, DMSO-*d*₆) δ 162.2 (d, J = 241.0 Hz, 1C), 149.6 (d, J = 6.0 Hz, 1C), 129.9 (d, J = 8.0 Hz, 1C), 121.8 (d, J = 3.0 Hz, 1C), 113.3 (d, J = 21.0 Hz, 1C), 112.4 (d, J = 21.0 Hz, 1C), 71.4, 45.5, 38.3, 32.1, 22.7. **HRMS (ESI)** m/z : calculated for C₁₁H₁₄ClFNaO [M+Na]⁺: 239.0609, found: 239.0615.

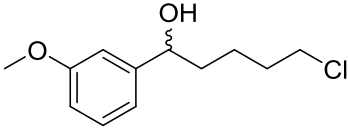
(*rac*)-5-chloro-1-(3-chlorophenyl)pentan-1-ol [(*rac*)-3a]

 **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.38 (t, J = 2.0 Hz, 1H), 7.35 – 7.31 (m, 1H), 7.28 – 7.24 (m, 2H), 5.33 (d, J = 3.4 Hz, 1H), 4.55 (t, J = 6.6 Hz, 1H), 3.60 (t, J = 6.5 Hz, 2H), 1.77 – 1.67 (m, 2H), 1.63 – 1.57 (m, 2H), 1.52 – 1.31 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 149.0, 132.9, 129.9, 126.5, 125.6, 124.5, 71.5, 45.4, 38.3, 32.0, 22.7. **HRMS (ESI)** m/z : calculated for C₁₁H₁₄Cl₂NaO [M+Na]⁺: 255.0314, found: 255.0319.

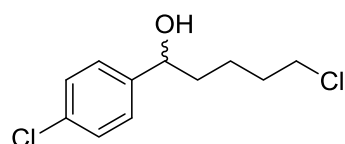
(*rac*)-5-chloro-1-(*m*-tolyl)pentan-1-ol [(*rac*)-4a]

 **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.22 – 7.11 (m, 3H), 7.03 (d, J = 7.5 Hz, 1H), 5.15 (d, J = 3.6 Hz, 1H), 4.50 (t, J = 6.8 Hz, 1H), 3.59 (t, J = 6.6 Hz, 2H), 2.31 (s, 3H), 1.76 – 1.68 (m, 2H), 1.65 – 1.54 (m, 2H), 1.53 – 1.33 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 146.4, 136.9, 127.9, 127.2, 126.5, 123.0, 72.2, 45.4, 38.6, 32.2, 23.0, 21.2. **HRMS (ESI)** m/z : calculated for C₁₂H₁₈ClO [M+H]⁺: 213.1041, found: 213.1046.

(*rac*)-5-chloro-1-(3-methoxyphenyl)pentan-1-ol [(*rac*)-5a]

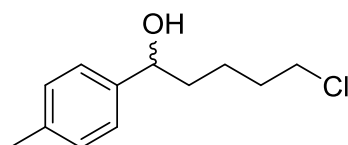
 **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.22 (t, J = 7.8 Hz, 1H), 6.91 – 6.88 (m, 2H), 6.79 – 6.76 (m, 1H), 5.17 (s, 1H), 4.50 (t, J = 6.2 Hz, 1H), 3.74 (s, 3H), 3.60 (t, J = 6.7 Hz, 2H), 1.77 – 1.67 (m, 2H), 1.64 – 1.57 (m, 2H), 1.52 – 1.33 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 159.2, 148.2, 129.0, 118.1, 112.0, 111.4, 72.1, 54.9, 45.5, 38.5, 32.1, 22.9. **HRMS (ESI)** m/z : calculated for C₁₂H₁₈ClO₂ [M+H]⁺: 229.0990, found: 229.0995.

(rac)-5-chloro-1-(4-chlorophenyl)pentan-1-ol [(rac)-6a]¹⁸



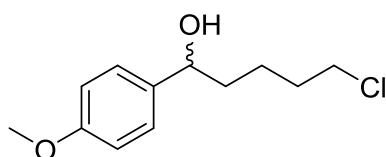
¹H NMR (400 MHz, DMSO-*d*₆) δ 7.37 – 7.32 (m, 4H), 5.26 (d, *J* = 4.3 Hz, 1H), 4.55 – 4.51 (m, 1H), 3.60 (t, *J* = 6.6 Hz, 2H), 1.76 – 1.66 (m, 2H), 1.63 – 1.51 (m, 2H), 1.49 – 1.29 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 145.3, 131.0, 127.9, 127.7, 71.4, 45.4, 38.4, 32.0, 22.7. **HRMS (ESI)** *m/z*: calculated for C₁₁H₁₄Cl₂NaO [M+Na]⁺: 255.0314, found: 255.0319.

(rac)- 5-chloro-1-(*p*-tolyl)pentan-1-ol [(rac)-7a]



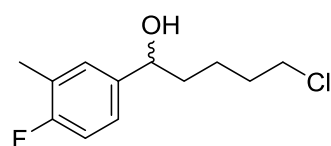
¹H NMR (400 MHz, DMSO-*d*₆) δ 7.21 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 5.11 (d, *J* = 4.0 Hz, 1H), 4.51 – 4.47 (m, 1H), 3.59 (t, *J* = 6.6 Hz, 2H), 2.28 (s, 3H), 1.75 – 1.67 (m, 2H), 1.64 – 1.54 (m, 2H), 1.50 – 1.30 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 143.3, 135.5, 128.6, 125.9, 72.0, 45.5, 38.6, 32.2, 22.9, 20.8. **HRMS (ESI)** *m/z*: calculated for C₁₂H₁₈ClO [M+H]⁺: 213.1041, found: 213.1046.

(rac)-5-chloro-1-(4-methoxyphenyl)pentan-1-ol [(rac)-8a]



¹H NMR (400 MHz, DMSO-*d*₆) δ 7.26 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 6.9 Hz, 2H), 5.10 (s, 1H), 4.49 (t, *J* = 5.4 Hz, 1H), 3.74 (s, 3H), 3.59 (t, *J* = 6.6 Hz, 2H), 1.75 – 1.68 (m, 2H), 1.66 – 1.54 (m, 2H), 1.49 – 1.30 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 158.1, 138.4, 127.0, 113.3, 71.9, 55.0, 45.5, 38.7, 32.2, 23.0. **HRMS (ESI)** *m/z*: calculated for C₁₂H₁₈ClO₂ [M+H]⁺: 229.0990, found: 229.0995.

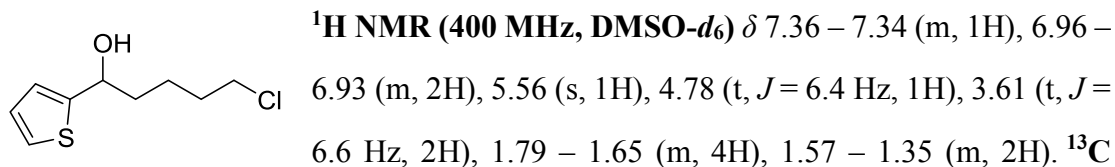
(rac)-5-chloro-1-(4-fluoro-3-methylphenyl)pentan-1-ol [(rac)-9a]



¹H NMR (400 MHz, DMSO-*d*₆) δ 7.23 – 7.20 (m, 1H), 7.17 – 7.13 (m, 1H), 7.06 – 7.01 (m, 1H), 5.19 (d, *J* = 4.4 Hz, 1H), 4.50 – 4.46 (m, 1H), 3.60 (t, *J* = 6.6 Hz, 2H), 2.2 (s, 3H), 1.76 – 1.67 (m, 2H), 1.62 – 1.51 (m, 2H), 1.49 – 1.29 (m, 2H). **¹³C NMR (100**

MHz, DMSO-*d*₆) δ 159.6 (d, J = 239.0 Hz, 1C), 142.2 (d, J = 4.0 Hz, 1C), 129.0, 124.9 (d, J = 8.0 Hz, 1C), 123.5 (d, J = 17.0 Hz, 1C), 114.3 (d, J = 22.0 Hz, 1C), 71.6, 45.4, 38.6, 32.1, 22.9, 14.3 (d, J = 4.0 Hz, 1C). **HRMS (ESI)** m/z : calculated for C₁₂H₁₇ClFO [M+H]⁺: 231.0946, found: 231.0952.

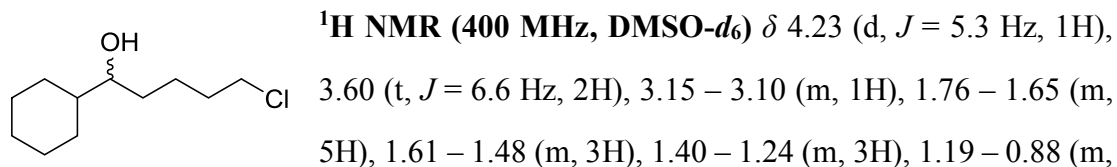
5-chloro-1-(thiophen-2-yl)pentan-1-ol [(*rac*)-10a]



NMR (100 MHz, DMSO-*d*₆) δ 150.9, 126.5, 123.9, 122.8, 68.3, 45.4, 38.7, 32.0, 22.8.

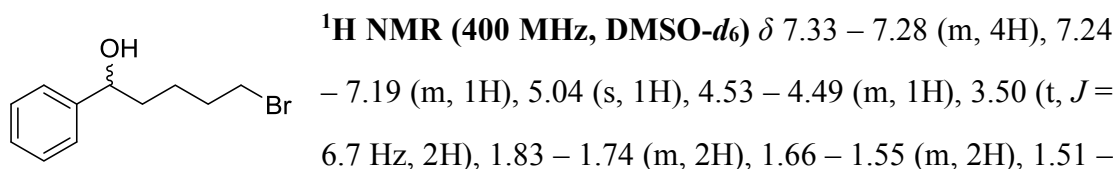
HRMS (ESI) m/z : calculated for C₉H₁₃ClNaOS [M+Na]⁺: 227.0268, found: 227.0273.

(*rac*)-5-chloro-1-cyclohexylpentan-1-ol [(*rac*)-11a]



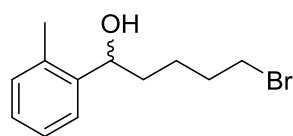
26.1, 26.0, 23.1. **HRMS (ESI)** m/z : calculated for C₁₁H₂₂ClO [M+H]⁺: 205.1354, found: 205.1359.

(*rac*)-5-bromo-1-phenylpentan-1-ol [(*rac*)-12a]¹⁹



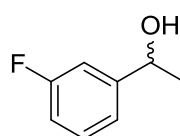
1.34 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 146.3, 127.9, 126.6, 125.8, 72.1, 38.4, 35.2, 32.3, 24.1. **HRMS (ESI)** m/z : calculated for C₁₁H₁₅BrNaO [M+Na]⁺: 265.0198, found: 265.0204.

(rac)-5-bromo-1-(o-tolyl)pentan-1-ol [(rac)-13a]



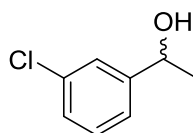
¹H NMR (400 MHz, DMSO-*d*₆) δ 7.49 – 7.46 (m, 1H), 7.21 – 7.19 (m, 1H), 7.13 – 7.11 (m, 2H), 5.09 (s, 1H), 4.79 – 4.75 (m, 1H), 3.53 – 3.48 (m, 2H), 2.31 (s, 3H), 1.89 – 1.79 (m, 2H), 1.61 – 1.56 (m, 3H), 1.52 – 1.46 (m, 1H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 144.4, 133.6, 129.9, 126.3, 125.7, 125.4, 68.5, 37.1, 35.3, 32.3, 24.4, 18.7. **HRMS (ESI)** *m/z*: calculated for C₁₂H₁₇BrNaO [M+Na]⁺: 279.0355, found: 279.0360.

(rac)-5-bromo-1-(3-fluorophenyl)pentan-1-ol [(rac)-14a]



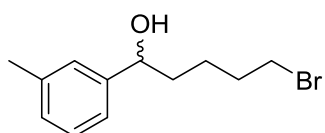
¹H NMR (400 MHz, DMSO-*d*₆) δ 7.34 (q, *J* = 7.6 Hz, 1H), 7.16 – 7.12 (m, 2H), 7.04 – 6.99 (m, 1H), 5.17 (s, 1H), 4.55 (t, *J* = 6.3 Hz, 1H), 3.50 (t, *J* = 6.8 Hz, 2H), 1.84 – 1.76 (m, 2H), 1.63 – 1.57 (m, 2H), 1.49 – 1.33 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 162.2 (d, *J* = 242.0 Hz, 1C), 149.6 (d, *J* = 6.0 Hz, 1C), 129.8 (d, *J* = 8.0 Hz, 1C), 121.8 (d, *J* = 3.0 Hz, 1C), 113.2 (d, *J* = 21.0 Hz, 1C), 112.4 (d, *J* = 21.0 Hz, 1C), 71.4, 38.2, 35.1, 32.2, 24.0. **HRMS (ESI)** *m/z*: calculated for C₁₁H₁₅BrFO [M+H]⁺: 261.0285, found: 261.0290.

(rac)-5-bromo-1-(3-chlorophenyl)pentan-1-ol [(rac)-15a]



¹H NMR (400 MHz, DMSO-*d*₆) δ 7.40 (s, 1H), 7.35 – 7.24 (m, 3H), 5.34 (d, *J* = 5.9 Hz, 1H), 4.58 – 4.54 (m, 1H), 3.50 – 3.46 (m, 2H), 1.85 – 1.78 (m, 2H), 1.62 – 1.58 (m, 2H), 1.52 – 1.39 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 149.0, 132.9, 129.8, 126.5, 125.7, 124.4, 71.5, 38.2, 35.0, 32.3, 24.1. **HRMS (ESI)** *m/z*: calculated for C₁₁H₁₅BrClO [M+H]⁺: 276.9989, found: 276.9995.

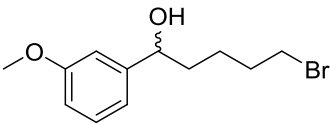
(rac)-5-bromo-1-(m-tolyl)pentan-1-ol [(rac)-16a]



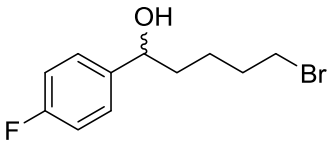
¹H NMR (400 MHz, DMSO-*d*₆) δ 7.21 – 7.16 (m, 1H), 7.14 – 7.09 (m, 2H), 7.04 – 7.01 (m, 1H), 5.04 (s, 1H), 4.50 – 4.45 (m, 1H), 3.52 – 3.47 (m, 2H), 2.30 (d, *J* = 4.3 Hz, 3H),

1.82 – 1.77 (m, 2H), 1.64 – 1.53 (m, 2H), 1.49 – 1.34 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 146.3, 136.9, 127.9, 127.2, 126.4, 123.0, 72.2, 38.4, 35.2, 32.4, 24.2, 21.2. **HRMS (ESI) *m/z***: calculated for C₁₂H₁₇BrKO [M+K]⁺: 295.0094, found: 295.0100.

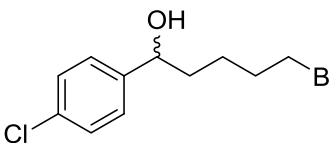
(*rac*)-5-bromo-1-(3-methoxyphenyl)pentan-1-ol [(*rac*)-17a]

 **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.22 (t, *J* = 7.8 Hz, 1H), 6.93 – 6.89 (m, 2H), 6.80 – 6.77 (m, 1H), 5.09 (s, 1H), 4.52 (t, *J* = 6.0 Hz, 1H), 3.74 (s, 3H), 3.49 (t, *J* = 6.7 Hz, 2H), 1.85 – 1.77 (m, 2H), 1.65 – 1.57 (m, 2H), 1.54 – 1.34 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 159.2, 148.1, 129.0, 118.1, 111.9, 111.3, 72.1, 54.9, 38.4, 35.1, 32.4, 24.2. **HRMS (ESI) *m/z***: calculated for C₁₂H₁₇BrNaO₂ [M+Na]⁺: 295.0304, found: 295.0310.

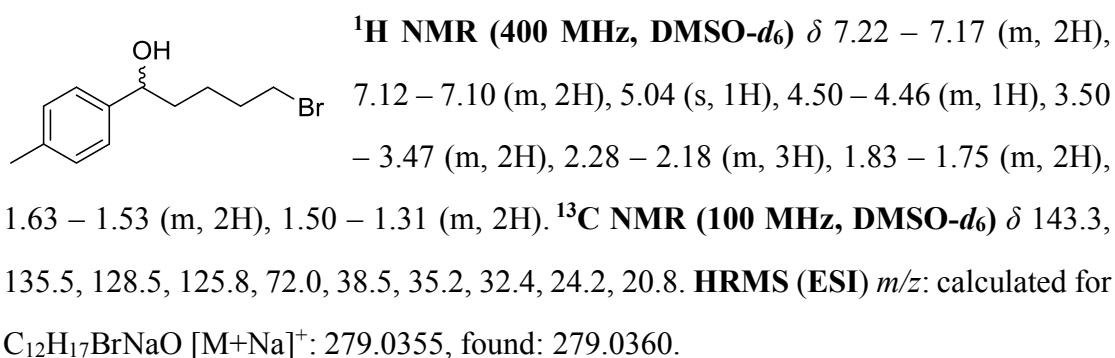
(*rac*)-5-bromo-1-(4-fluorophenyl)pentan-1-ol [(*rac*)-18a]

 **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.39 – 7.34 (m, 2H), 7.13 – 7.08 (m, 2H), 4.92 (d, *J* = 16.1 Hz, 1H), 4.54 (d, *J* = 7.7 Hz, 1H), 3.50 – 3.45 (m, 2H), 1.82 – 1.77 (m, 2H), 1.64 – 1.58 (m, 2H), 1.49 – 1.32 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 161.1 (d, *J* = 240.0 Hz, 1C), 142.5 (d, *J* = 3.0 Hz, 1C), 127.6 (d, *J* = 8.0 Hz, 2C), 114.6 (d, *J* = 21.0 Hz, 2C), 71.5, 38.5, 35.0, 32.3, 24.1. **HRMS (ESI) *m/z***: calculated for C₁₁H₁₄BrFNaO [M+Na]⁺: 283.0104, found: 283.0110.

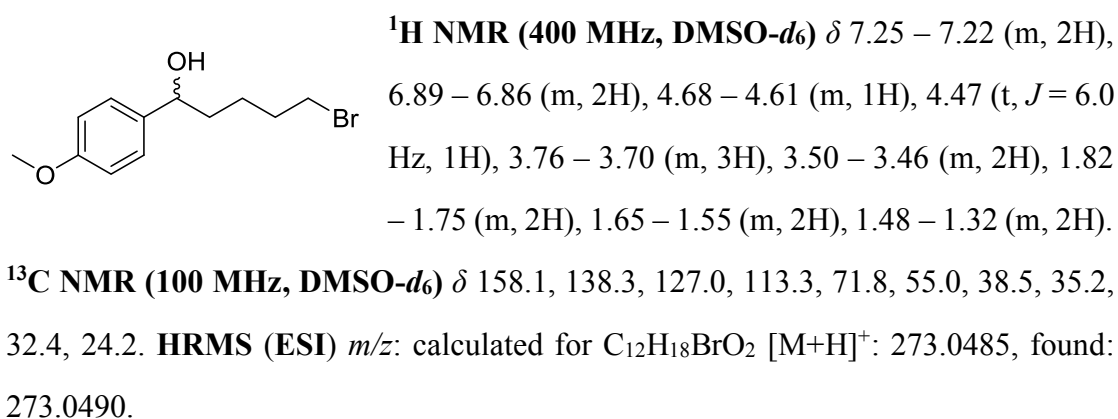
(*rac*)-5-bromo-1-(4-chlorophenyl)pentan-1-ol [(*rac*)-19a]

 **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.37 – 7.32 (m, 4H), 5.27 (s, 1H), 4.55 – 4.52 (m, 1H), 3.49 (t, *J* = 6.8 Hz, 2H), 1.83 – 1.73 (m, 2H), 1.62 – 1.53 (m, 2H), 1.49 – 1.30 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 145.3, 131.0, 127.9, 127.7, 71.4, 38.2, 35.1, 32.3, 24.0. **HRMS (ESI) *m/z***: calculated for C₁₁H₁₄BrClKO [M+K]⁺: 314.9548, found: 314.9554.

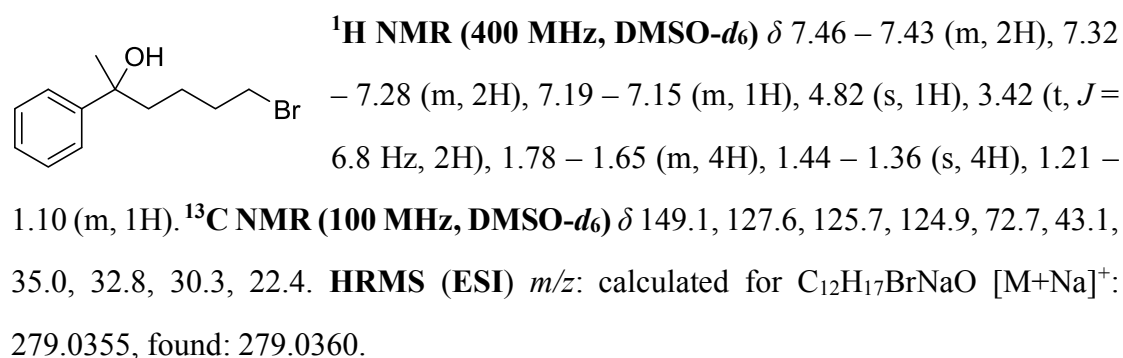
(rac)-5-bromo-1-(p-tolyl)pentan-1-ol [(rac)-20a]



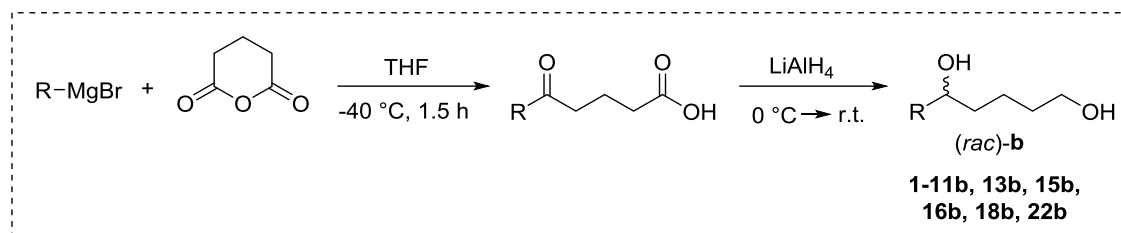
(rac)-5-bromo-1-(4-methoxyphenyl)pentan-1-ol [(rac)-21a]



(rac)-6-bromo-2-phenylhexan-2-ol [(rac)-22a]



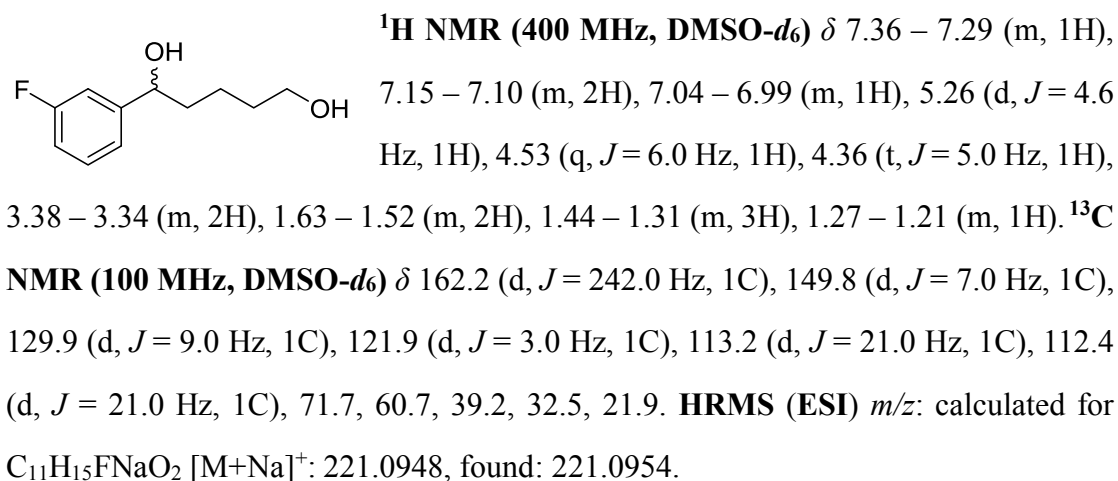
Synthesis of racemic ϵ -diols:



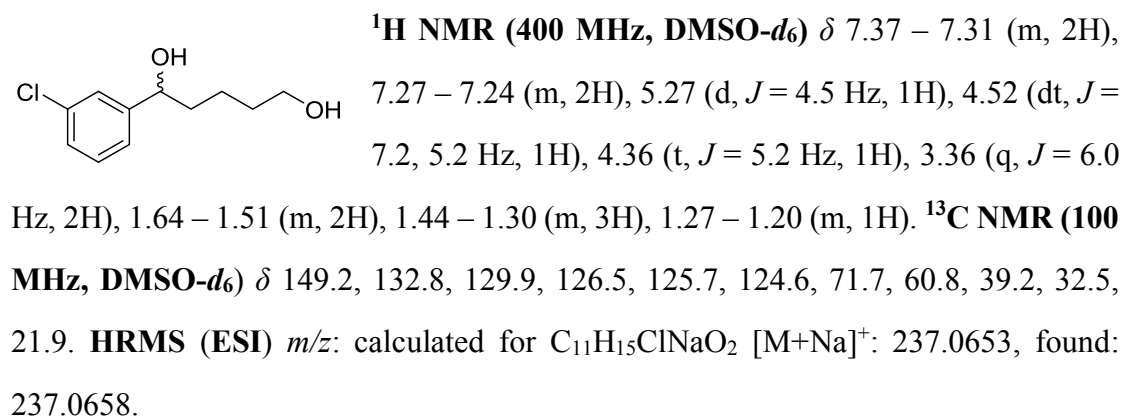
The racemic ϵ -diols are synthesized by the reaction of Grignard reagents with dihydro-2H-pyran-2,6(3H)-dione.^{20,21} General procedure: Under anhydrous and oxygen-free conditions, glutaric anhydride (5.0 mmol) was placed in a 50 mL round-bottom flask and dissolved in anhydrous tetrahydrofuran (10 mL). The solution was cooled to $-40\text{ }^{\circ}\text{C}$, and phenylmagnesium bromide (5.0 mmol) was added dropwise. The mixture was stirred at this temperature for 1.5 h, then quenched with 1 M HCl (5 mL). The aqueous layer was extracted with ethyl acetate ($3 \times 15\text{ mL}$), and the combined organic phases were washed with saturated NaCl solution. After removal of the solvent under reduced pressure, a white solid crude product was obtained. Purification by column chromatography (petroleum ether/ethyl acetate = 5:1 to 1:1) afforded benzenepentanoic acid intermediate. Benzenepentanoic acid (1.0 equiv) was then dissolved in dry THF in a 50 mL round-bottom flask. The solution was cooled to $0\text{ }^{\circ}\text{C}$, and LiAlH_4 (4.0 equiv) was added portionwise. The reaction was stirred at room temperature for 1 h, then carefully quenched by sequential addition of water (1 mL), 15 % NaOH (1 mL), and additional water (3 mL per 1 g of LiAlH_4 used). Diethyl ether and anhydrous MgSO_4 were added, and the mixture was stirred for 30 min. After filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 3:1 ~ 1:1) to yield racemic (*rac*)-**1b**. (*rac*)-1-phenylpentane-1,5-diol [(*rac*)-**1b**]²²

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.30 (d, $J = 4.4\text{ Hz}$, 4H), 7.23 – 7.18 (m, 1H), 5.11 (d, $J = 4.4\text{ Hz}$, 1H), 4.51 – 4.46 (m, 1H), 4.34 (t, $J = 5.2\text{ Hz}$, 1H), 3.35 – 3.32 (m, 2H), 1.65 – 1.49 (m, 2H), 1.44 – 1.30 (m, 3H), 1.26 – 1.15 (m, 1H). **^{13}C NMR (100 MHz, $\text{DMSO}-d_6$)** δ 146.6, 128.0, 126.6, 125.9, 72.4, 60.8, 39.4, 32.6 22.0. **HRMS (ESI)** m/z : calculated for $\text{C}_{11}\text{H}_{16}\text{NaO}_2$ $[\text{M}+\text{Na}]^+$: 203.1043, found: 203.1048.

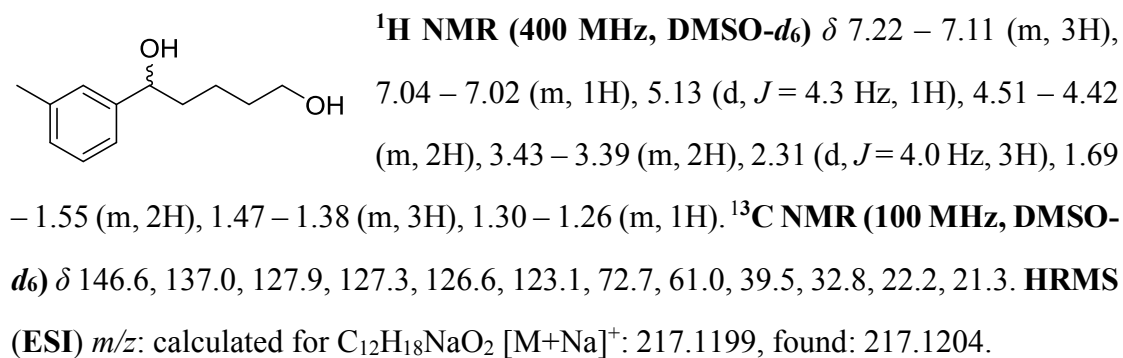
(rac)-1-(3-fluorophenyl)pentane-1,5-diol [(rac)-2b]



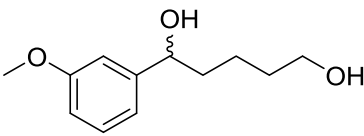
(rac)-1-(3-chlorophenyl)pentane-1,5-diol [(rac)-3b]



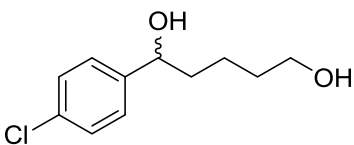
(rac)-1-(*m*-tolyl)pentane-1,5-diol [(rac)-4b]



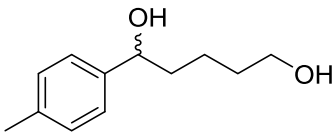
(rac)-1-(3-methoxyphenyl)pentane-1,5-diol [(rac)-5b]

**¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.21 (t, *J* = 7.6 Hz, 1H), 6.90 – 6.87 (m, 2H), 6.79 – 6.76 (m, 1H), 5.13 (d, *J* = 4.7 Hz, 1H), 4.51 – 4.47 (m, 1H), 4.39 – 4.36 (m, 1H), 3.75 (t, *J* = 3.2 Hz, 3H), 4.39 – 4.35 (m, 2H), 1.66 – 1.53 (m, 2H), 1.46 – 1.35 (m, 3H), 1.28 – 1.21 (m, 1H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 159.2, 148.4, 129.0, 118.2, 111.9, 111.5, 72.4, 60.9, 54.9, 39.4, 32.7, 22.1. **HRMS (ESI)** *m/z*: calculated for C₁₂H₁₈NaO₃ [M+Na]⁺: 233.1148, found: 233.1154.

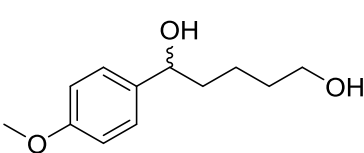
(rac)-1-(4-chlorophenyl)pentane-1,5-diol [(rac)-6b]

**¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.36 – 7.29 (m, 4H), 5.20 (d, *J* = 4.4 Hz, 1H), 4.50 (dt, *J* = 7.1, 5.2 Hz, 1H), 4.33 (t, *J* = 5.2 Hz, 1H), 3.37 – 3.33 (m, 2H), 1.64 – 1.49 (m, 2H), 1.43 – 1.30 (m, 3H), 1.27 – 1.18 (m, 1H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 145.5, 131.0, 127.9, 127.7, 71.7, 60.8, 39.2, 32.5, 21.9. **HRMS (ESI)** *m/z*: calculated for C₁₁H₁₆ClO₂ [M+H]⁺: 215.0833, found: 215.0839.

(rac)-1-(p-tolyl)pentane-1,5-diol [(rac)-7b]

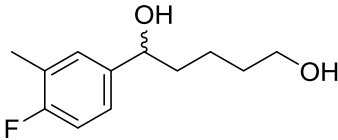
**¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.22 – 7.17 (m, 2H), 7.12 – 7.09 (m, 2H), 5.07 – 5.02 (m, 1H), 4.47 – 4.43 (m, 1H), 4.39 – 4.33 (m, 1H), 3.39 – 3.33 (m, 2H), 2.27 (s, 3H), 1.63 – 1.50 (m, 2H), 1.44 – 1.19 (m, 4H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 143.5, 135.5, 128.5, 125.8, 72.3, 60.8, 39.4, 32.6, 22.0, 20.7. **HRMS (ESI)** *m/z*: calculated for C₁₂H₁₈NaO₂ [M+Na]⁺: 217.1199, found: 217.1204.

(rac)-1-(4-methoxyphenyl)pentane-1,5-diol [(rac)-8b]

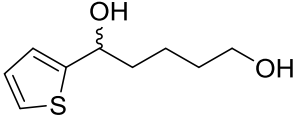
**¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.23 – 7.20 (m, 2H), 6.88 – 6.85 (m, 2H), 4.98 (d, *J* = 4.3 Hz, 1H), 4.45 – 4.41 (m, 1H), 4.32 (t, *J* = 5.1 Hz, 1H), 3.72 (s, 3H), 3.36 – 3.32 (m, 2H), 1.62 – 1.48 (m, 2H), 1.43 – 1.35 (m, 2H),

1.34 – 1.15 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 158.0, 138.5, 127.0, 113.3, 72.0, 60.8, 55.0, 39.4, 32.6, 22.0. **HRMS (ESI)** m/z : calculated for C₁₂H₁₈NaO₃ [M+Na]⁺: 233.1148, found: 233.1154.

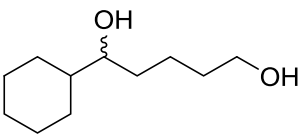
(*rac*)-1-(4-fluoro-3-methylphenyl)pentane-1,5-diol [(*rac*)-9b]

 **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.20 (dd, J = 7.8, 2.2 Hz, 1H), 7.15 – 7.11 (m, 1H), 7.05 – 7.00 (m, 1H), 5.12 (d, J = 4.4 Hz, 1H), 4.49 – 4.44 (m, 2H), 4.36 (t, J = 5.1 Hz, 1H), 3.39 – 3.34 (m, 2H), 2.22 (d, J = 2.0 Hz, 3H), 1.63 – 1.49 (m, 2H), 1.45 – 1.31 (m, 3H), 1.27 – 1.20 (m, 1H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 159.9 (d, J = 239.0 Hz, 1C), 142.5 (d, J = 4.0 Hz, 1C), 129.0 (d, J = 13.0, 5.0 Hz, 1C), 125.0 (d, J = 13.0 Hz, 1C), 123.4 (d, J = 17.0 Hz, 1C), 114.4 (d, J = 22.0 Hz, 1C), 71.9, 60.9, 39.4, 32.6, 22.1, 14.3 (d, J = 3.0 Hz, 1C). **HRMS (ESI)** m/z : calculated for C₁₂H₁₇FNaO₂ [M+Na]⁺: 235.1105, found: 235.1110.

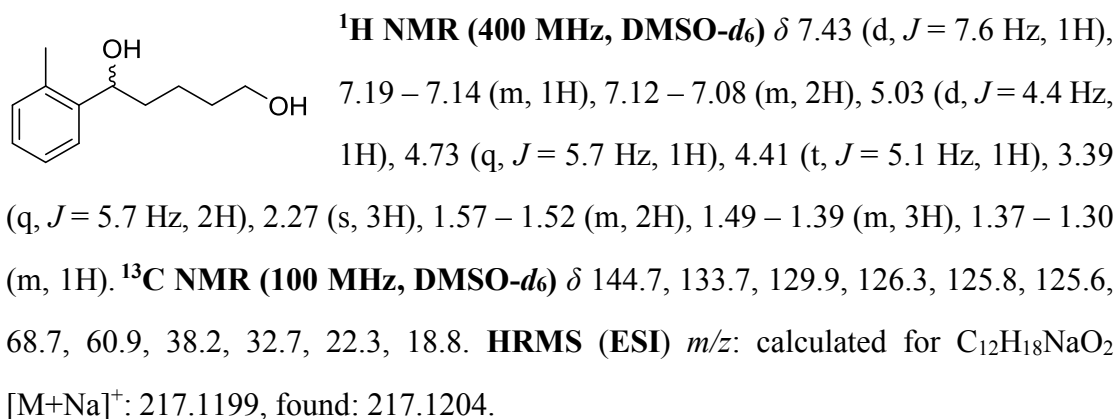
(*rac*)-1-(thiophen-2-yl)pentane-1,5-diol [(*rac*)-10b]

 **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.36 – 7.34 (m, 1H), 6.96 – 6.90 (m, 2H), 5.48 – 5.46 (m, 1H), 4.76 – 4.71 (m, 1H), 4.36 – 4.32 (m, 1H), 3.39 – 3.34 (m, 2H), 1.72 – 1.60 (m, 2H), 1.45 – 1.34 (m, 3H), 1.30 – 1.23 (m, 1H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 151.1, 126.5, 123.9, 122.8, 68.6, 60.9, 39.6, 32.5, 22.0. **HRMS (ESI)** m/z : calculated for C₉H₁₄NaO₂S [M+Na]⁺: 209.0607, found: 209.0612.

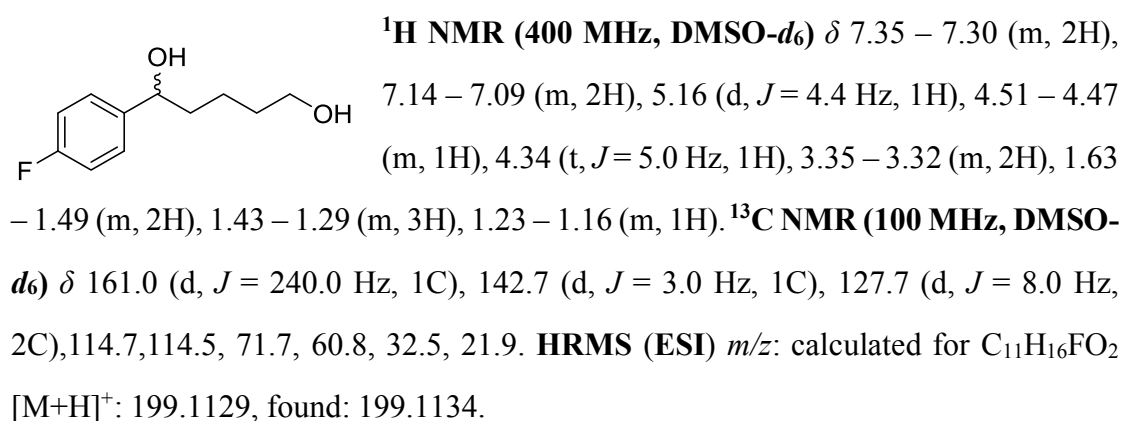
(*rac*)-1-cyclohexylpentane-1,5-diol [(*rac*)-11b]

 **¹H NMR (400 MHz, DMSO-*d*₆)** δ 4.34 (dd, J = 5.3, 10.5 Hz, 1H), 4.16 (t, J = 5.5, 1H), 3.37 (t, J = 5.9, 2H), 3.11 (s, 1H), 1.74 – 0.83 (m, 17H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 73.9, 60.9, 43.4, 33.7, 32.9, 29.1, 27.7, 26.4, 26.2, 26.0, 22.2. **HRMS (ESI)** m/z : calculated for C₁₁H₂₂NaO₂ [M+Na]⁺: 209.1512, found: 209.1517.

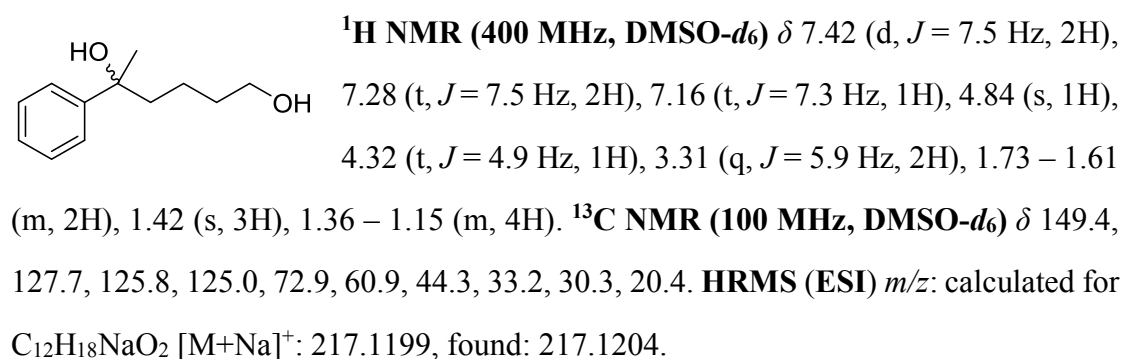
(rac)-1-(o-tolyl)pentane-1,5-diol [(rac)-13b]



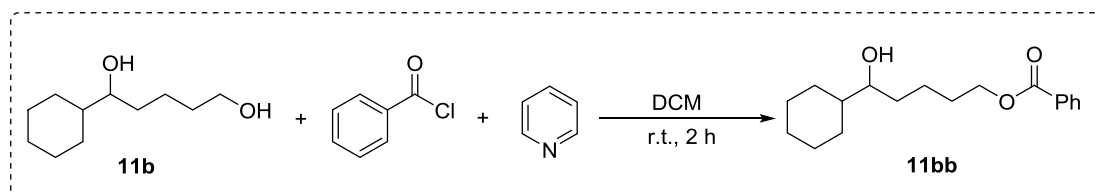
(rac)-1-(4-fluorophenyl)pentane-1,5-diol [(rac)-18b]



(rac)-5-phenylhexane-1,5-diol [(rac)-22b]²³

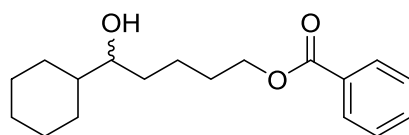


Synthesis of 11bb from 11b:²⁴



The chiral ϵ -doil **11b** (0.2 mmol) was reacted with benzoyl chloride (70 mg, 0.5 mmol) in the presence of pyridine (47 mg, 0.6 mmol) in DCM (5 mL) for 2 h. The reaction mixture was then quenched with saturated NH_4Cl (5 mL) and extracted with DCM (5 mL $\times$ 3). The combined extracts were washed with brine, dried over anhydrous MgSO_4 and concentrated in vacuo. After a flash chromatography on silica gel (petroleum ether/ethyl acetate = 10:1), the desired benzoyl esters were obtained in nearly quantitative yields.

(rac)- 5-cyclohexyl-5-hydroxypentyl benzoate [(rac)-11bb]



^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.98 – 7.95 (m, 2H), 7.66 – 7.59 (m, 1H), 7.55 – 7.47 (m, 2H), 4.29 – 4.20 (m, 2H), 3.36 (s, 1H), 3.17 – 3.12 (m, 1H), 1.74 – 1.54 (m, 7H), 1.43 – 1.29 (m, 3H), 1.24 – 0.90 (m, 7H). **^{13}C NMR (100 MHz, $\text{DMSO}-d_6$)** δ 165.7, 133.2, 129.9, 129.1, 128.7, 73.7, 64.7, 43.4, 33.3, 29.0, 28.4, 27.7, 26.3, 26.1, 25.9, 22.2. **HRMS (ESI)** m/z : calculated for $\text{C}_{18}\text{H}_{26}\text{NaO}_3$ $[\text{M}+\text{Na}]^+$: 313.1774, found: 313.1780.

3. Supplementary Tables 1-35

Supplementary Table 1. Wild-type HHDHs tested in this study that showed no detectable activity toward ϵ -haloalcohol substrate **1a**.^a

OCC(Cc1ccccc1)CCCl $\xrightarrow[\text{30 } ^\circ\text{C, 12 h}]{\text{E. coli (HHDH) cells, PB buffer (pH 8.5, 50 mM)}}$ CC1(C)OCC(Cc2ccccc2)CC1 + OCC(Cc1ccccc1)CCO

(rac)-**1a** **1c** **1b**

entry	HHDHs	Source/ Accession	Conversion 1a (%) ^b
1	-	-	NR
2	HheA2	<i>Arthrobacter</i> sp. AD2/ AAK92100	NR
3	HheA5	<i>Tistrella mobilis</i> KA081020-065/ WP_014743557	NR
4	HheA8	<i>Sneathiella glossodoripedis</i> / WP_025899379.1	NR
5	HheA10	<i>Tsukamurella</i> sp. 1534/ WP_019201195	NR
6	HheA11	<i>Reyranella massiliensis</i> 521/ WP_020698933	NR
7	HheA13	<i>Pseudomonas</i> sp. G5(2012)/ WP_042955870	NR
8	HheB3	marine metagenome/ EBL02020	NR
9	HheB4	marine metagenome/ EBP61646	NR
10	HheB6	marine metagenome/ EDB56284	NR
11	HheC	<i>Agrobacterium tumefaciens</i> AD1/ AAK92099	NR
12	HheD	<i>Dechloromonas aromatica</i> RCB/ WP_011285856	NR
13	HheD2	<i>gamma proteobacterium</i> HTCC2207/ WP_007233072	NR
14	HheD6	<i>Marinobacter nanhaiticus</i> D15-8W/ WP_004579485	NR
15	HheD7	<i>Thauera</i> sp. 27/ WP_002926105	NR
16	HheD8	<i>Thauera aminoaromatica</i> S2/ WP_004302136	NR
17	HheD9	<i>Thauera phenylacetica</i> B4P/ WP_004355811	NR
18	HheD14	<i>Gammaproteobacteria</i> bacterium MOLA455/ WP_035490777	NR
19	HheD15	<i>Candidatus Competibacter denitrificans</i> Run_A_D11/ WP_048670059	NR
20	HheD16	<i>Methylibium</i> sp. T29/ WP_036236554	NR
21	HheD17	<i>Curvibacter gracilis</i> ATCC BAA-807/ WP_051443321	NR
22	HheD20	<i>Rhodocyclaceae</i> bacterium PG1-Ca6/ AJP48347	NR
23	HheE	marine metagenome/ EBP63112	NR
24	HheE5	marine metagenome/ WP_009577001	NR
25	HheF	uncultured bacterium/ BAH89601	NR
26	HheG	<i>Ilumatobacter coccineus</i> YM16-304/ WP_015443096	NR
27	HheG2	<i>Ilumatobacter nonamiensis</i> YM16-303/ WP_040495182	NR
28	HHDHn1	<i>Rhodobiaceae</i> bacterium/ PCJ71437.1	NR
29	HHDHn2	<i>Alphaproteobacteria</i> bacterium HGW Alphaproteobacteria-3/ PKP78298.1	NR
30	HHDHn3	<i>Alphaproteobacteria</i> bacterium 46_93_T64/ OUR79898	NR
31	HHDHn4	<i>Reyranella massiliensis</i> / WP_020698933.1	NR

32	HHDHn5	<i>Alphaproteobacteria</i> bacterium 65-37/ OJU34764.1	NR
33	HHDHn6	<i>Rhodospirillales</i> bacterium URHD0017/ WP_092824593.1	NR
34	HHDHn7	<i>Alphaproteobacteria</i> bacterium RIFCSPHIGHO2_12_FULL_66_14/ OFW98094.1	NR
35	HHDHn8	<i>Enhydrobacter aerosaccus</i> / WP_085934632.1	NR
36	HHDHn9	<i>Rhodospirillaceae</i> bacterium TMED8/ OUT52242.1	NR
37	HHDHn10	<i>Rhodomicrobium</i> / WP_088343515.1	NR
38	HHDHn11	<i>Aurantiochytrium</i> sp. FCC1311/ GBG24028.1	NR
39	HHDHamb	<i>Acidimicrobiia</i> bacterium/ MSO17354.1	NR
40	HHDHapb	<i>Alphaproteobacteria</i> bacterium 32-64-14/ OYX46376.1	NR
41	HHDHabb	<i>Actinobacteria</i> bacterium IMCC26256/ AKL74579.1	NR
42	HHDHnsr	<i>Novosphingobium resinovorum</i> / WP_069709913.1	NR

^aThe reactions were carried out in 5 mL PB buffer (pH 8.5, 50 mM) containing 5 mM (*rac*)-**1a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HHDH) cells at 30 °C for 12 h. ^bThe conversions were determined by chiral HPLC. NR= no reaction.

Supplementary Table 2. Screening of HheG mutant library for dehalogenation activity toward ϵ -haloalcohol substrate **1a**.^a

entry	<i>E. coli</i> (HheG)	Mutation	Conversion 1a (%) ^b
1	RM1	I104W	NR
2	RM2	I104F	NR
3	RM3	I104W/N196S	NR
4	RM4	I104F/N196S	NR
5	RM5	I104W/N196S/F200W	NR
6	RM6	I104F/N196S/F200W	NR
7	<i>rac</i> -M4	I104F/N196G	NR
8	M3	N196G/Y197W	NR
9	M4	V162W/N196G/Y197W	NR
10	M5	V162W/N196G/Y197W/A206M	NR
11	M6	V162W/T195A/N196G/Y197W/A206M	NR
12	M7	T106V/V162W/T195A/N196G/Y197W/A206M	NR
13	M8	T106V/V162W/T195A/N196G/Y197W/F203L/A206M	NR

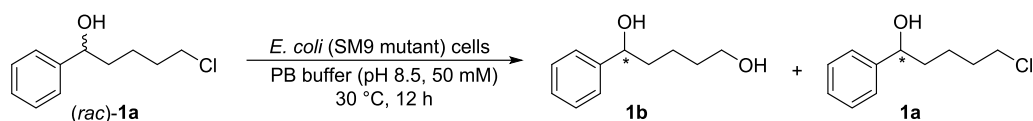
^aThe reactions were carried out in 5 mL PB buffer (pH 8.5, 50 mM) containing 5 mM (*rac*)-**1a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheG mutant) cells at 30 °C for 12 h. ^bThe conversions were determined by chiral HPLC. NR= no reaction.

Supplementary Table 3. Screening of HheC mutant library for dehalogenation activity toward ϵ -haloalcohol substrate **1a**.^a

entry	<i>E. coli</i> (HheC)	Mutation	Yield 1c (%) ^b	Yield 1b (%) ^b	<i>ee</i> 1b (%) ^b	<i>ee</i> 1a (%) ^b
1	SM1	N176A	0	NR	ND	0
2	SM2	N176G	0	NR	ND	0
3	SM3	T134A	0	NR	ND	0
4	SM4	T134L	0	NR	ND	0
5	SM5	P84T	0	3±0.3	33±0.8(<i>R</i>)	<5
6	SM6	P84D	0	NR	ND	0
7	SM7	W249F	0	4±0.2	8±0.2(<i>R</i>)	<5
8	SM8	T134S	0	NR	ND	0
9	SM9	L142A	0	19±0.2	59±0.3(<i>S</i>)	15±0.5(<i>R</i>)
10	DM1	T134A/N176G	0	NR	ND	0
11	DM2	T134I/N176A	0	NR	ND	0
12	DM3	T134L/N176A	0	NR	ND	0
13	DM4	T134S/N176A	0	NR	ND	0
14	DM5	T134S/N176G	0	NR	ND	0
15	DM6	T134V/N176G	0	NR	ND	0
16	DM7	P84D/W249Y	0	NR	ND	0
17	TM1	F86H/T134V/N176G	0	NR	ND	0
18	TM2	F86V/T134V/N176G	0	NR	ND	0
19	QM1	P84V/F86P/T134A/N176A	0	NR	ND	0

^aThe reactions were carried out in 5 mL PB buffer (pH 8.5, 50 mM) containing 5 mM (*rac*)-**1a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC mutant) cells at 30 °C for 12 h. ^bThe yields and *ee* values were determined by chiral HPLC. NR= no reaction. ND= not detected. Reactions were carried out in triplicate.

Supplementary Table 4. Dehalogenative hydroxylation activity of SM9-derived mutants toward ϵ -haloalcohol substrate **1a**.^a



entry	<i>E. coli</i> (HheC)	Mutation	Yield 1b (%) ^b	<i>ee</i> 1b (%) ^b	<i>ee</i> 1a (%) ^b
1	SM9	L142A	21.4 ± 1.1	60 ± 0.3(<i>S</i>)	18 ± 0.2(<i>R</i>)
2	SM9+D80A	L142A/D80A	NR	ND	<5
3	SM9+D80E	L142A/D80E	NR	ND	<5
4	SM9+D182A	L142A/D182A	5.0 ± 0.2	60 ± 0.3(<i>S</i>)	<5
5	SM9+D182E	L142A/D182E	14.8 ± 0.1	62 ± 0.9(<i>S</i>)	12 ± 0.1(<i>R</i>)

^aThe reactions were carried out in 5 mL PB buffer (pH 8.5, 50 mM) containing 5 mM (*rac*)-**1a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-SM9) cells at 30 °C for 12 h. ^bThe yields and *ee* values were determined by chiral HPLC. NR= no reaction. ND= not detected. Reactions were carried out in triplicate.

Supplementary Table 5. Dehalogenative cyclization activity of SM9-derived mutants toward β -haloalcohol substrate **1a1**.^a

ClCC(O)c1ccccc1 $\xrightarrow[\text{30 } ^\circ\text{C, 1 h}]{\text{E. coli (SM9) cells, PB buffer (pH 8.5, 50 mM)}}$ C1CC1(O)c2ccccc2 + ClCC(O)c3ccccc3
 $(rac)\text{-1a1}$ **1c1** **1a1**

entry	<i>E. coli</i> (HheC)	Mutation	Yield 1c1 (%) ^b	<i>ee</i> 1c1 (%) ^b	<i>ee</i> 1a1 (%) ^b
1 ^c	-	-	0	ND	<5
2	WT	no	57.6 $\pm$ 2.7	<5	7 $\pm$ 0.7(<i>R</i>)
3	SM9	L142A	58.2 $\pm$ 0.5	<5	<5
4	SM9+D80A	L142A/D80A	0	ND	6 $\pm$ 1.0(<i>R</i>)
5	SM9+D182A	L142A/D182A	60.4 $\pm$ 0.8	<5	<5

^aThe reactions were carried out in 5 mL PB buffer (pH 8.5, 50 mM) containing 5 mM *(rac)*-**1a1** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC) cells at 30 °C for 1 h. ^bThe yields and *ee* values were determined by chiral HPLC. ^cThe reaction was carried out using *E. coli* cells in the absence of the HheC enzyme. ND= not detected. Reactions were carried out in triplicate.

Supplementary Table 6. Dehalogenative cyclization activity of SM9-derived mutants toward γ -haloalcohol substrate **1a2**.^a

Reaction scheme: (rac)-**1a2** $\xrightarrow[\text{PB buffer (pH 8.5, 50 mM), 30 °C, 3 h}]{E. coli \text{ (SM9) cells}}$ **1c2** + **1a2**

entry	<i>E. coli</i> (HheC)	Mutation	Yield 1c2 (%) ^b	<i>ee</i> 1c2 (%) ^b	<i>ee</i> 1a2 (%) ^b
1 ^c	-	-	NR	ND	<5
2	WT	no	22.3 ± 0.2	85 ± 0.4(<i>S</i>)	28 ± 0.3(<i>R</i>)
3	SM9	L142A	3.3 ± 0.1	9 ± 0.4(<i>S</i>)	<5
4	SM9+D80A	L142A/D80A	NR	ND	<5
5	SM9+D182A	L142A/D182A	0	ND	<5

^aThe reactions were carried out in 5 mL PB buffer (pH 8.5, 50 mM) containing 5 mM (*rac*)-**1a2** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC) cells at 30 °C for 3 h. ^bThe yields and *ee* values were determined by chiral HPLC. ^cThe reaction was carried out using *E. coli* cells in the absence of the HheC enzyme. NR= no reaction. ND= not detected. Reactions were carried out in triplicate.

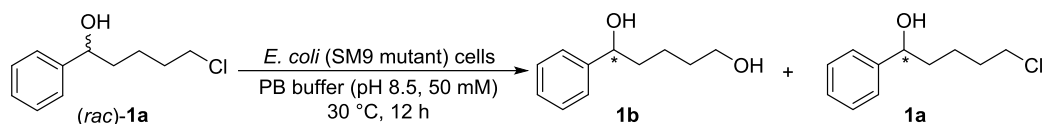
Supplementary Table 7. Dehalogenative cyclization activity of SM9-derived mutants toward δ -haloalcohol substrate **1a3**.^a

c1ccccc1[C@H](O)CCCCl $\xrightarrow[\text{PB buffer (pH 8.5, 50 mM), 30 }^\circ\text{C, 3 h}]{E. coli \text{ (SM9) cells}}$ c1ccccc1[C@H]2OCCCC2 + c1ccccc1[C@H](O)CCCCl
(rac)-1a3 **1c3** **1a3**

entry	<i>E. coli</i> (HheC)	Mutation	Yield 1c3 (%) ^b	<i>ee</i> 1c3 (%) ^b	<i>ee</i> 1a3 (%) ^b
1 ^c	-	-	1	<5	<5
2	WT	no	43.0 $\pm$ 2.7	68 $\pm$ 1.7(<i>S</i>)	99 $\pm$ 0.1(<i>R</i>)
3	SM9	L142A	48.8 $\pm$ 2.2	38 $\pm$ 0.2(<i>S</i>)	56 $\pm$ 0.4(<i>R</i>)
4	SM9+D80A	L142A/D80A	1	<5	<5
5	SM9+D182A	L142A/D182A	23.9 $\pm$ 0.6	33 $\pm$ 0.0(<i>S</i>)	13 $\pm$ 0.4(<i>R</i>)

^aThe reactions were carried out in 5 mL PB buffer (pH 8.5, 50 mM) containing 5 mM **(rac)-1a3** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC) cells at 30 °C for 3 h. ^bThe yields and *ee* values were determined by chiral HPLC. ^cThe reaction was carried out using *E. coli* cells in the absence of the HheC enzyme. Reactions were carried out in triplicate.

Supplementary Table 8. Functional analysis of NCT residues (S132, Y145, R149) on dehalogenative hydroxylation activity by site-directed mutagenesis on SM9.^a



entry	<i>E. coli</i> (HheC)	Mutation	Yield 1b (%) ^b	<i>ee</i> 1b (%) ^b	<i>ee</i> 1a (%) ^b
1	SM9+S132A	L142A/S132A	NR	ND	<5
2	SM9+S132T	L142A/S132T	NR	ND	<5
3	SM9+S132C	L142A/S132C	NR	ND	<5
4	SM9+Y145A	L142A/Y145A	NR	ND	<5
5	SM9+Y145F	L142A/Y145F	NR	ND	<5
6	SM9+Y145W	L142A/Y145W	NR	ND	<5
7	SM9+R149A	L142A/R149A	NR	ND	<5
8	SM9+R149K	L142A/R149K	NR	ND	<5
9	SM9+R149D	L142A/R149D	NR	ND	<5
10	SM9+R149E	L142A/R149E	NR	ND	<5

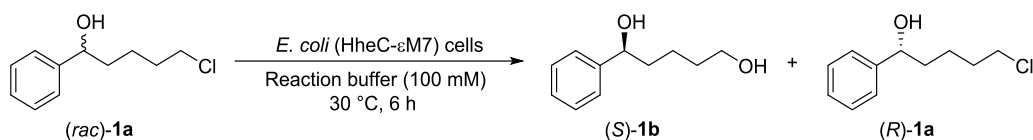
^aThe reactions were carried out in 5 mL PB buffer (pH 8.5, 50 mM) containing 5 mM *(rac)*-**1a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (SM9 mutant) cells at 30 °C for 12 h. ^bThe yields and *ee* values were determined by chiral HPLC. NR= no reaction. ND= not detected.

Supplementary Table 10. Effect of buffer concentration on the dehalogenative hydroxylation activity of SM9 toward ϵ -haloalcohol substrate **1a**.^a

entry	PB buffer (mM)	<i>ee</i> 1b (%) ^b	<i>ee</i> 1a (%) ^b	Conversion 1a (%) ^b
1	25	63 ± 2.1	5 ± 0.6	7 ± 1.0
2	50	59 ± 0.3	15 ± 0.5	21 ± 0.6
3	100	55 ± 0.4	38 ± 1.0	41 ± 0.8

^aThe reactions were carried out in 5 mL PB buffer (pH 8.5) containing 5 mM (*rac*)-**1a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (SM9) cells at 30 °C for 12 h. ^bThe conversions and *ee* values were determined by chiral HPLC. Reactions were carried out in triplicate.

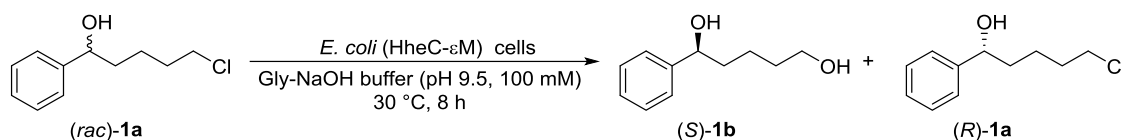
Supplementary Table 11. Optimization of reaction buffer pH for the enantioselective dehalogenation of (*rac*)-**1a**.^a



entry	Buffer	pH	<i>ee</i> 1b (%) ^b	<i>ee</i> 1a (%) ^b	Conversion 1a (%) ^b
1	PB	6.5	>99	<5	3 ± 0.0
2	PB	7.5	98 ± 0.3	46 ± 0.9	32 ± 1.3
3	PB	8.5	97 ± 0.1	62 ± 0.9	39 ± 0.3
4	Gly-NaOH	9.5	96 ± 0.1	89 ± 0.7	48 ± 0.2
5	Gly-NaOH	10.5	94 ± 0.1	92 ± 1.7	49 ± 0.5

^aThe reactions were carried out in 5 mL buffer (100 mM) containing 10 mM (*rac*)-**1a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 6 h. ^bThe conversion and *ee* values were determined by chiral HPLC. Reactions were carried out in triplicate.

Supplementary Table 12. Evaluation of HheC-εM mutants for the enantioselective dehalogenation of (*rac*)-**1a** under optimized conditions. ^a

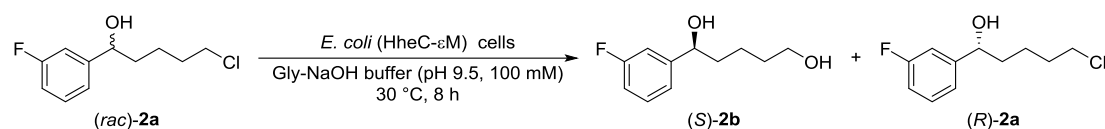


entry	HheC-εM	Mutation	<i>ee</i> 1b (%) ^b	<i>ee</i> 1a (%) ^b	Conversion 1a (%) ^c	<i>E</i> ^c
1	εM1	L142A	54±0.3	42±1.3	44±0.6	5
2	εM2	L142A/W249P	78±3.2	98±0.1	56±1.1	36
3	εM3	L142A/W249P/P84G	92±2.5	94±2.4	50±1.3	85
4	εM4	L142A/W249P/T134L	95±0.3	74±1.3	44±0.3	87
5	εM5	L142A/W249P/P84G/T134L	96±0.1	45±2.0	32±1.0	76
6	εM6	L142A/W249P/P84G/N176Q	94±0.9	94±3.3	50±1.1	115
7	εM7	L142A/W249P/P84G/F186Y	96±0.4	93±1.3	49±0.4	168
8	εM8	L142A/W249P/P84G/F186Y/N176Q	95±0.1	91±0.8	49±0.2	124

^aThe reactions were carried out in 5 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 10 mM (*rac*)-**1a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM7) cells at 30 °C for 8 h. ^bThe *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{1a}/(ee_{1a}+ee_{1b})$.

^dCalculated enantioselectivity: $E = \ln[(1-ee_{1a})/(1+ee_{1a}/ee_{1b})]/\ln[(1+ee_{1a})/(1+ee_{1a}/ee_{1b})]$. Reactions were carried out in triplicate.

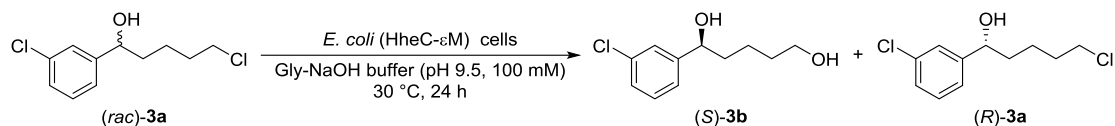
Supplementary Table 13. Enantioselective dehalogenation of (*rac*)-**2a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 2b (%) ^b	<i>ee</i> 2a (%) ^b	Conversion 2a (%) ^c	<i>E</i> ^c
1	εM1	45±0.6	30±0.4	41±0.2	3
2	εM2	81±1.0	>99±0.0	55±0.3	49
3	εM3	89±0.8	93±0.8	51±0.1	58
4	εM4	97±0.8	62±0.8	39±0.4	124
5	εM5	96±0.6	48±0.7	33±0.2	79
6	εM6	90±1.1	94±1.2	51±0.1	67
7	εM7	90±0.6	95±0.2	51±0.2	71
8	εM8	90±1.4	86±0.1	49±0.4	53

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 10 mM (*rac*)-**2a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 8 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{2a}/(ee_{2a}+ee_{2b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{2a})/(1+ee_{2a}/ee_{2b})]/\ln[(1+ee_{2a})/(1+ee_{2a}/ee_{2b})]$. Reactions were carried out in triplicate.

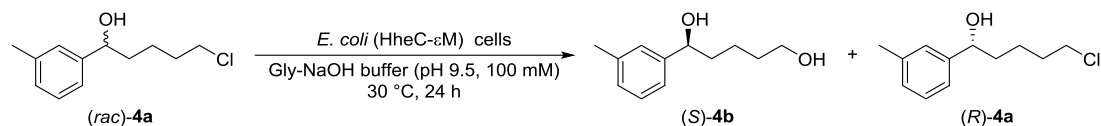
Supplementary Table 14. Enantioselective dehalogenation of (*rac*)-**3a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 3b (%) ^b	<i>ee</i> 3a (%) ^b	Conversion 3a (%) ^c	<i>E</i> ^c
1	εM1	>99±0.0	9±0.5	9±0.4	218
2	εM2	79±0.2	97±0.6	55±0.2	35
3	εM3	78±0.7	85±0.8	52±0.1	22
4	εM4	>99±0.0	30±0.6	23±0.3	267
5	εM5	>99±0.0	28±0.6	22±0.4	262
6	εM6	80±0.8	67±1.0	46±0.1	18
7	εM7	82±0.8	73±0.7	47±0.0	22
8	εM8	87±1.2	51±1.1	37±0.6	24

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 10 mM (*rac*)-**3a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 24 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{3a}/(ee_{3a}+ee_{3b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{3a})/(1+ee_{3a}/ee_{3b})]/\ln[(1+ee_{3a})/(1+ee_{3a}/ee_{3b})]$. Reactions were carried out in triplicate.

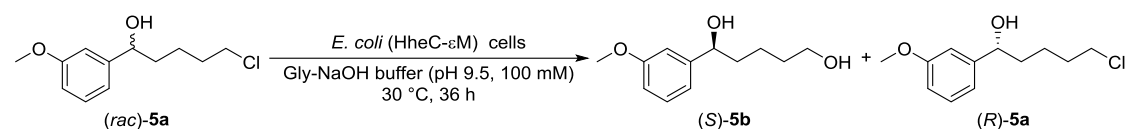
Supplementary Table 15. Enantioselective dehalogenation of (*rac*)-**4a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 4b (%) ^b	<i>ee</i> 4a (%) ^b	Conversion 4a (%) ^c	<i>E</i> ^c
1	εM1	73±0.9	10±0.2	13±0.2	7
2	εM2	64±1.4	96±1.5	60±0.9	17
3	εM3	84±0.2	73±1.7	47±0.6	25
4	εM4	87±0.8	28±0.3	24±0.3	19
5	εM5	90±1.4	29±0.4	24±0.3	23
6	εM6	80±0.5	80±0.5	50±0.0	22
7	εM7	84±0.9	74±0.2	47±0.3	25
8	εM8	88±0.5	50±0.1	36±0.1	26

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 10 mM (*rac*)-**4a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 24 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{4a}/(ee_{4a}+ee_{4b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{4a})/(1+ee_{4a}/ee_{4b})]/\ln[(1+ee_{4a})/(1+ee_{4a}/ee_{4b})]$. Reactions were carried out in triplicate.

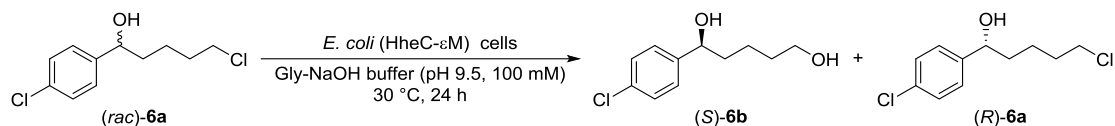
Supplementary Table 16. Enantioselective dehalogenation of (*rac*)-**5a** using the engineered HheC-εM variants.^a



entry	HheC-εM	<i>ee</i> 5b (%) ^b	<i>ee</i> 5a (%) ^b	Conversion 5a (%) ^c	<i>E</i> ^c
1	εM1	75±0.2	22±1.7	23±1.4	9
2	εM2	50±0.8	>99±0.0	67±0.4	14
3	εM3	83±0.4	98±0.9	54±0.2	49
4	εM4	83±0.9	28±1.8	25±1.1	14
5	εM5	90±0.8	38±1.9	30±1.2	28
6	εM6	79±1.1	99±1.0	56±0.4	44
7	εM7	85±1.6	93±4.7	52±1.7	42
8	εM8	87±1.5	68±6.7	44±2.8	29

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 10 mM (*rac*)-**5a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 36 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{5a}/(ee_{5a}+ee_{5b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{5a})/(1+ee_{5a}/ee_{5b})]/\ln[(1+ee_{5a})/(1+ee_{5a}/ee_{5b})]$. Reactions were carried out in triplicate.

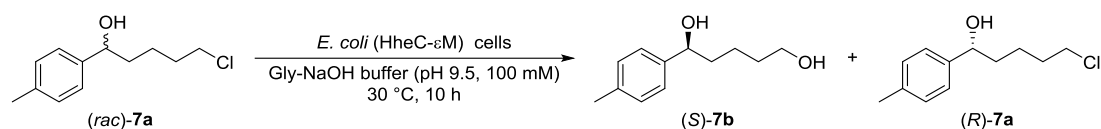
Supplementary Table 17. Enantioselective dehalogenation of (*rac*)-**6a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 6b (%) ^b	<i>ee</i> 6a (%) ^b	Conversion 6a (%) ^c	<i>E</i> ^c
1	εM1	33±1.4	52±1.8	61±1.7	3
2	εM2	53±2.1	>99±0.0	65±0.9	15
3	εM3	91±0.9	68±1.4	43±0.6	43
4	εM4	91±0.5	91±0.8	50±0.4	67
5	εM5	94±0.4	30±0.2	24±0.1	43
6	εM6	92±0.1	64±0.6	41±0.2	46
7	εM7	90±1.6	76±7.2	46±2.8	44
8	εM8	93±0.2	57±1.8	38±0.8	49

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 10 mM (*rac*)-**6a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 24 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: *conv.* = $ee_{6a}/(ee_{6a}+ee_{6b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{6a})/(1+ee_{6a}/ee_{6b})]/\ln[(1+ee_{6a})/(1+ee_{6a}/ee_{6b})]$. Reactions were carried out in triplicate.

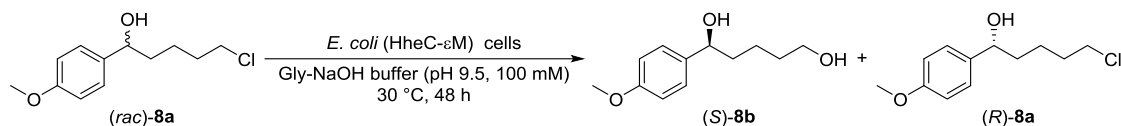
Supplementary Table 18. Enantioselective dehalogenation of (*rac*)-**7a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 7b (%) ^b	<i>ee</i> 7a (%) ^b	Conversion 7a (%) ^c	<i>E</i> ^c
1	εM1	34±0.5	14±0.7	29±1.3	2
2	εM2	63±1.3	>99±0.0	61±0.5	22
3	εM3	91±0.2	35±2.9	28±1.6	30
4	εM4	91±0.1	59±1.8	39±0.7	39
5	εM5	91±0.1	18±0.7	17±0.5	25
6	εM6	89±0.4	74±2.0	45±0.8	38
7	εM7	89±0.1	55±1.7	38±0.7	30
8	εM8	90±0.3	60±1.7	40±0.7	35

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 10 mM (*rac*)-**7a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 10 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{7a}/(ee_{7a}+ee_{7b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{7a})/(1+ee_{7a}/ee_{7b})]/\ln[(1+ee_{7a})/(1+ee_{7a}/ee_{7b})]$. Reactions were carried out in triplicate.

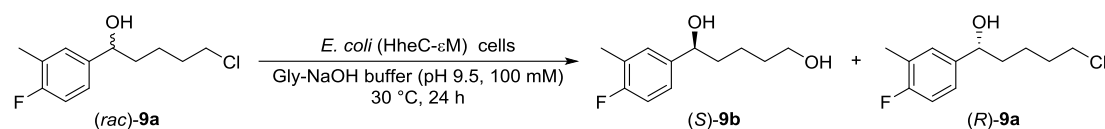
Supplementary Table 19. Enantioselective dehalogenation of (*rac*)-**8a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 8b (%) ^b	<i>ee</i> 8a (%) ^b	Conversion 8a (%) ^c	<i>E</i> ^c
1	εM1	6±1.0	6±0.8	50±3.0	1
2	εM2	51±1.4	53±1.2	51±1.3	5
3	εM3	65±0.6	33±0.7	34±0.3	6
4	εM4	72±0.6	12±0.3	14±0.3	7
5	εM5	59±1.4	8±1.2	12±1.8	4
6	εM6	64±0.2	30±3.3	32±2.4	6
7	εM7	59±0.4	62±0.8	51±0.4	7
8	εM8	69±1.0	26±3.5	27±2.3	7

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 10 mM (*rac*)-**8a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 48 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{8a}/(ee_{8a}+ee_{8b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{8a})/(1+ee_{8a}/ee_{8b})]/\ln[(1+ee_{8a})/(1+ee_{8a}/ee_{8b})]$. Reactions were carried out in triplicate.

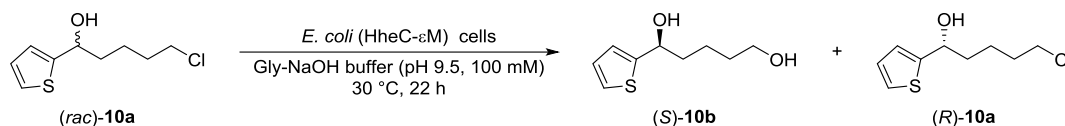
Supplementary Table 20. Enantioselective dehalogenation of (*rac*)-**9a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 9b (%) ^b	<i>ee</i> 9a (%) ^b	Conversion 9a (%) ^c	<i>E</i> ^c
1	εM1	52±0.2	19±0.0	27±0.1	4
2	εM2	54±0.6	86±0.7	61±0.4	9
3	εM3	64±0.5	40±0.1	38±0.1	7
4	εM4	79±0.8	23±0.2	22±0.2	11
5	εM5	79±0.7	16±0.2	17±0.1	10
6	εM6	74±0.2	27±0.5	27±0.4	9
7	εM7	56±0.2	45±0.3	44±0.1	5
8	εM8	70±0.3	30±1.0	30±0.8	8

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 10 mM (*rac*)-**9a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 24 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{9a}/(ee_{9a}+ee_{9b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{9a})/(1+ee_{9a}/ee_{9b})]/\ln[(1+ee_{9a})/(1+ee_{9a}/ee_{9b})]$. Reactions were carried out in triplicate.

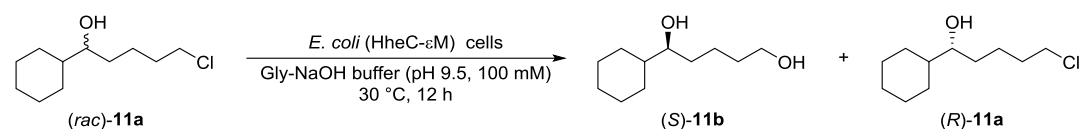
Supplementary Table 21. Enantioselective dehalogenation of (*rac*)-**10a** using the engineered HheC-εM variants.^a



entry	HheC-εM	<i>ee</i> 10b (%) ^b	<i>ee</i> 10a (%) ^b	Conversion 10a (%) ^c	<i>E</i> ^c
1	εM1	65±0.3	45±0.3	41±0.2	7
2	εM2	83±0.8	>99±0.0	55±0.3	56
3	εM3	96±0.4	65±1.4	40±0.4	96
4	εM4	93±0.5	68±0.9	42±0.4	56
5	εM5	98±0.5	23±0.7	19±0.5	124
6	εM6	95±0.5	72±0.6	43±0.3	84
7	εM7	96±0.2	83±1.3	46±0.4	128
8	εM8	96±0.2	66±0.5	41±0.2	98

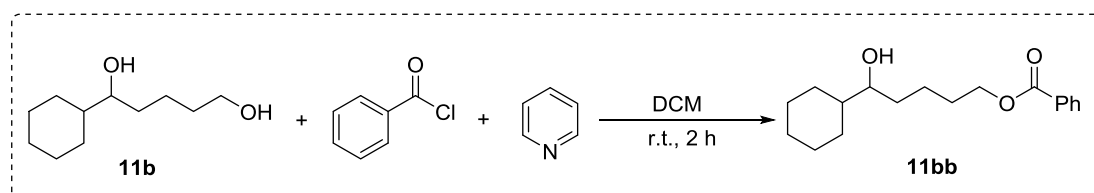
^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**10a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 22 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{10a}/(ee_{10a}+ee_{10b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{10a})/(1+ee_{10a}/ee_{10b})]/\ln[(1+ee_{10a})/(1+ee_{10a}/ee_{10b})]$. Reactions were carried out in triplicate.

Supplementary Table 22. Enantioselective dehalogenation of (*rac*)-**11a** using the engineered HheC-εM variants.^a



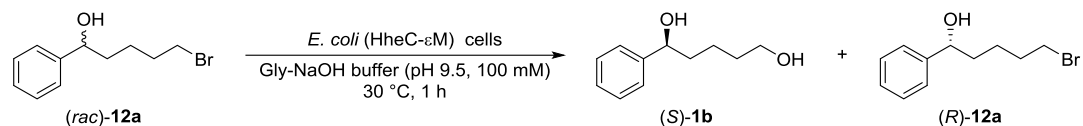
entry	HheC-εM	<i>ee</i> 11b (%) ^b	<i>ee</i> 11a (%) ^b	Conversion 11a (%) ^c	<i>E</i> ^c
1	εM1	58	6	9	4
2	εM2	70	73	51	12
3	εM3	91	51	36	35
4	εM4	84	16	16	13
5	εM5	86	13	13	15
6	εM6	91	68	43	43
7	εM7	77	99	56	39
8	εM8	84	29	26	15

^aThe reactions were carried out in 5 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 10 mM (*rac*)-**11a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 12 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{11a}/(ee_{11a}+ee_{11b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{11a})/(1+ee_{11a}/ee_{11b})]/\ln[(1+ee_{11a})/(1+ee_{11a}/ee_{11b})]$. The *ee* of **11b** was determined after derivatization with benzoyl chloride.



The *ee* values of the enzymatically generated **11b** was determined by derivatizing **11b** to yield **11bb**. The biotransformation mixture was extracted with ethyl acetate (5 mL). The organic layer was separated by centrifugation (8800 × *g*, 3 min), dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. To the resulting residue, benzoyl chloride (7 mg, 0.05 mmol) and pyridine (5 mg) in DCM (2 mL) were added, and the reaction was stirred at room temperature for 2 h. The mixture was then quenched with saturated NH₄Cl (2 mL) and extracted with DCM (2 mL). The organic extract was washed with brine, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. A 5 μL aliquot of the resulting mixture was diluted with 500 μL ethyl acetate and analyzed by chiral HPLC.

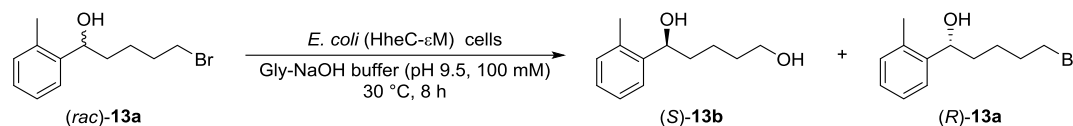
Supplementary Table 23. Enantioselective dehalogenation of (*rac*)-**12a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 1b (%) ^b	<i>ee</i> 12a (%) ^b	Conversion 12a (%) ^c	<i>E</i> ^c
1	εM1	51±0.9	47±2.7	48±1.6	5
2	εM2	84±0.7	98±0.4	54±0.3	52
3	εM3	95±0.5	83±0.7	47±0.3	102
4	εM4	96±0.2	65±1.7	40±0.7	96
5	εM5	97±0.4	40±1.2	29±0.7	97
6	εM6	91±0.3	96±0.3	51±0.1	83
7	εM7	94±0.4	93±0.4	50±0.2	110
8	εM8	93±0.2	96±0.5	51±0.2	109

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**12a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 1 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{12a}/(ee_{12a}+ee_{1b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{12a})/(1+ee_{12a}/ee_{1b})]/\ln[(1+ee_{12a})/(1+ee_{12a}/ee_{1b})]$. Reactions were carried out in triplicate.

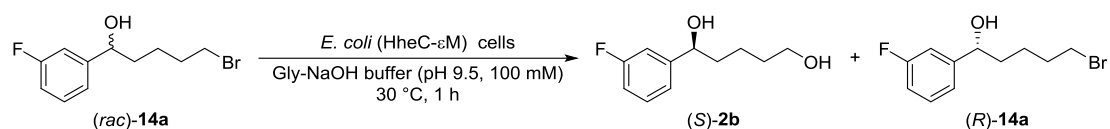
Supplementary Table 24. Enantioselective dehalogenation of (*rac*)-**13a** using the engineered HheC-εM variants.^a



entry	HheC-εM	<i>ee</i> 13b (%) ^b	<i>ee</i> 13a (%) ^b	Conversion 13a (%) ^c	<i>E</i> ^c
1	εM1	57±0.7	59±1.0	51±0.7	6
2	εM2	14±0.5	11±0.1	43±0.9	1
3	εM3	15±0.4	12±0.2	46±0.9	2
4	εM4	13±0.4	<5	25±0.5	1
5	εM5	14±0.5	5±0.1	25±1.2	1
6	εM6	15±0.1	20±0.3	58±0.6	2
7	εM7	21±0.4	18±0.5	47±0.6	2
8	εM8	19±0.3	15±0.3	44±0.9	2

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**13a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 8 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{13a}/(ee_{13a}+ee_{13b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{13a})/(1+ee_{13a}/ee_{13b})]/\ln[(1+ee_{13a})/(1+ee_{13a}/ee_{13b})]$. Reactions were carried out in triplicate.

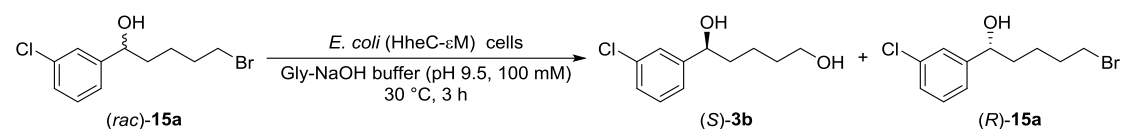
Supplementary Table 25. Enantioselective dehalogenation of (*rac*)-**14a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 2b (%) ^b	<i>ee</i> 14a (%) ^b	Conversion 14a (%) ^c	<i>E</i> ^c
1	εM1	42±0.8	24±0.4	36±0.3	3
2	εM2	85±1.0	98±1.5	54±0.7	56
3	εM3	90±2.5	77±2.2	46±1.4	44
4	εM4	96±0.3	69±1.1	42±0.3	102
5	εM5	94±1.3	35±0.5	27±0.2	46
6	εM6	87±0.5	95±1.4	52±0.5	53
7	εM7	90±1.8	92±2.7	51±1.2	62
8	εM8	87±1.4	98±1.6	53±0.8	65

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**14a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 1 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{14a}/(ee_{14a}+ee_{2b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{14a})/(1+ee_{14a}/ee_{2b})]/\ln[(1+ee_{14a})/(1+ee_{14a}/ee_{2b})]$. Reactions were carried out in triplicate.

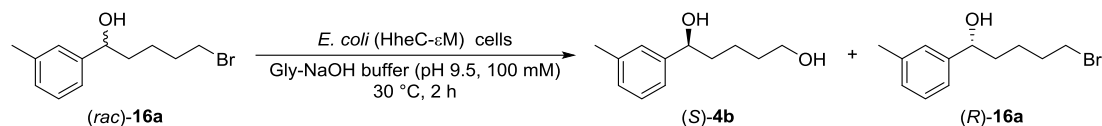
Supplementary Table 26. Enantioselective dehalogenation of (*rac*)-**15a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 3b (%) ^b	<i>ee</i> 15a (%) ^b	Conversion 15a (%) ^c	<i>E</i> ^c
1	εM1	59±1.7	11±1.3	16±1.6	4
2	εM2	76±0.9	93±1.5	55±0.2	24
3	εM3	93±1.4	38±1.1	29±0.9	40
4	εM4	79±1.4	54±2.9	40±1.0	15
5	εM5	83±0.5	21±0.4	20±0.4	13
6	εM6	72±0.9	77±0.5	52±0.3	14
7	εM7	80±0.9	73±1.2	48±0.7	20
8	εM8	76±0.1	75±0.6	50±0.2	16

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**15a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 3 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{15a}/(ee_{15a}+ee_{3b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{15a})/(1+ee_{15a}/ee_{3b})]/\ln[(1+ee_{15a})/(1+ee_{15a}/ee_{3b})]$. Reactions were carried out in triplicate.

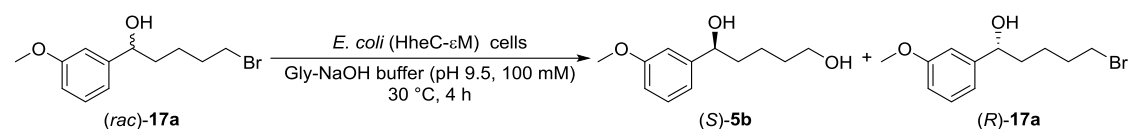
Supplementary Table 27. Enantioselective dehalogenation of (*rac*)-**16a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 4b (%) ^b	<i>ee</i> 16a (%) ^b	Conversion 16a (%) ^c	<i>E</i> ^c
1	εM1	75±1.5	13±0.2	15±0.4	8
2	εM2	78±1.3	73±1.1	49±0.5	18
3	εM3	89±1.1	50±1.4	36±0.9	28
4	εM4	92±1.7	27±3.5	23±2.5	31
5	εM5	92±1.3	23±0.9	20±0.6	30
6	εM6	85±1.0	65±3.5	44±1.1	24
7	εM7	85±0.7	69±1.3	45±0.6	25
8	εM8	86±0.9	62±2.8	42±0.9	25

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**16a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 2 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{16a}/(ee_{16a}+ee_{4b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{16a})/(1+ee_{16a}/ee_{4b})]/\ln[(1+ee_{16a})/(1+ee_{16a}/ee_{4b})]$. Reactions were carried out in triplicate.

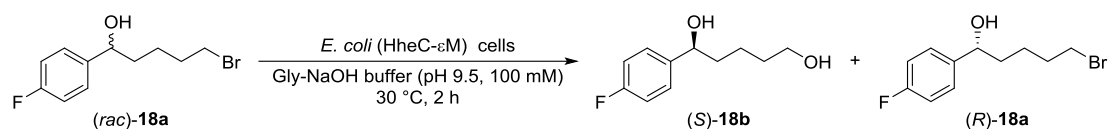
Supplementary Table 28. Enantioselective dehalogenation of (*rac*)-**17a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 5b (%) ^b	<i>ee</i> 17a (%) ^b	Conversion 17a (%) ^c	<i>E</i> ^c
1	εM1	75±1.1	29±3.1	28±2.5	9
2	εM2	69±2.4	87±2.7	56±0.1	15
3	εM3	90±0.1	75±3.6	45±1.2	43
4	εM4	82±0.8	25±4.2	23±2.7	13
5	εM5	93±0.7	25±3.2	21±2.2	35
6	εM6	82±1.2	91±2.1	53±0.9	32
7	εM7	88±0.4	79±2.1	47±0.7	38
8	εM8	85±2.0	74±3.3	47±1.2	27

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**17a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 4 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{17a}/(ee_{17a} + ee_{5b})$. ^dCalculated enantioselectivity: $E = \ln[(1 - ee_{17a})/(1 + ee_{17a}/ee_{5b})]/\ln[(1 + ee_{17a})/(1 + ee_{17a}/ee_{5b})]$. Reactions were carried out in triplicate.

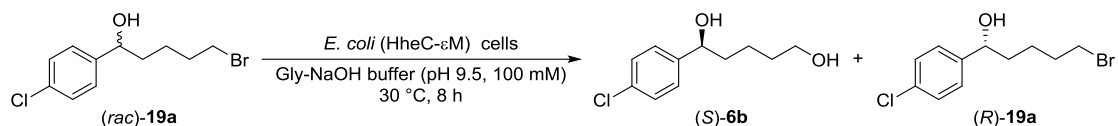
Supplementary Table 29. Enantioselective dehalogenation of (*rac*)-**18a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 18b (%) ^b	<i>ee</i> 18a (%) ^b	Conversion 18a (%) ^c	<i>E</i> ^c
1	εM1	48±1.2	49±1.0	51±1.1	5
2	εM2	85±0.7	97±1.1	54±0.5	51
3	εM3	95±0.9	76±1.1	44±0.2	90
4	εM4	94±1.3	73±2.5	44±1.0	71
5	εM5	97±0.5	33±2.0	25±1.2	91
6	εM6	94±0.1	88±1.0	49±0.3	94
7	εM7	94±0.3	90±0.6	49±0.2	100
8	εM8	93±0.3	94±0.5	50±0.2	98

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**18a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 2 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{18a}/(ee_{18a}+ee_{18b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{18a})/(1+ee_{18a}/ee_{18b})]/\ln[(1+ee_{18a})/(1+ee_{18a}/ee_{18b})]$. Reactions were carried out in triplicate.

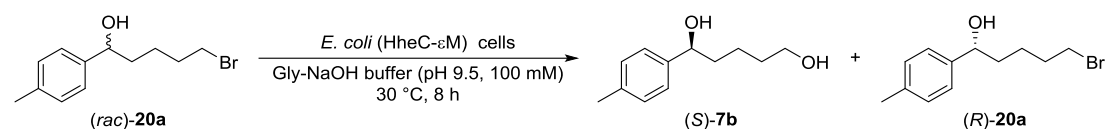
Supplementary Table 30. Enantioselective dehalogenation of (*rac*)-**19a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 6b (%) ^b	<i>ee</i> 19a (%) ^b	Conversion 19a (%) ^c	<i>E</i> ^c
1	εM1	22±0.2	50±1.0	70±0.2	2
2	εM2	50±0.5	93±1.9	65±0.2	9
3	εM3	87±0.1	86±2.7	50±0.8	40
4	εM4	91±0.4	55±1.0	38±0.3	37
5	εM5	90±2.5	20±0.4	18±0.7	23
6	εM6	87±0.5	82±1.4	49±0.3	37
7	εM7	89±1.2	68±0.8	44±0.1	35
8	εM8	87±0.4	81±1.3	48±0.3	36

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**19a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 8 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{19a}/(ee_{19a}+ee_{6b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{19a})/(1+ee_{19a}/ee_{6b})]/\ln[(1+ee_{19a})/(1+ee_{19a}/ee_{6b})]$. Reactions were carried out in triplicate.

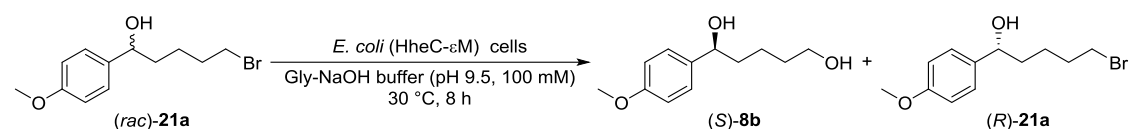
Supplementary Table 31. Enantioselective dehalogenation of (*rac*)-**20a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 7b (%) ^b	<i>ee</i> 20a (%) ^b	Conversion 20a (%) ^c	<i>E</i> ^c
1	εM1	25±0.2	11±1.3	30±2.8	2
2	εM2	72±0.2	>99±0.0	58±0.1	31
3	εM3	93±0.3	14±1.9	13±0.5	32
4	εM4	92±0.7	77±3.0	46±0.9	56
5	εM5	88±1.1	6±0.5	7±0.5	17
6	εM6	90±0.3	95±0.1	51±0.1	71
7	εM7	91±0.5	77±0.6	46±0.1	49
8	εM8	92±0.2	56±6.0	38±2.5	42

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**20a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 8 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{20a}/(ee_{20a}+ee_{7b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{20a})/(1+ee_{20a}/ee_{7b})]/\ln[(1+ee_{20a})/(1+ee_{20a}/ee_{7b})]$. Reactions were carried out in triplicate.

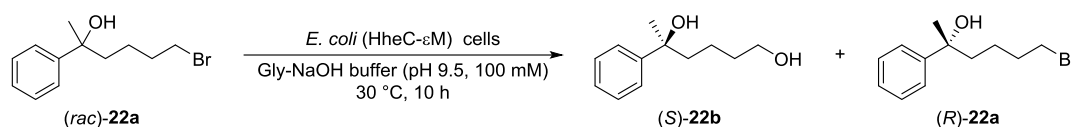
Supplementary Table 32. Enantioselective dehalogenation of (*rac*)-**21a** using the engineered HheC-εM variants. ^a



entry	HheC-εM	<i>ee</i> 8b (%) ^b	<i>ee</i> 21a (%) ^b	Conversion 21a (%) ^c	<i>E</i> ^c
1	εM1	6±0.1	6±0.6	50±2.6	1
2	εM2	50±0.7	56±0.6	53±0.6	5
3	εM3	64±0.3	21±1.1	25±0.9	6
4	εM4	73±0.8	17±0.6	19±0.4	8
5	εM5	67±1.1	<5	5±0.7	5
6	εM6	60±0.6	38±1.2	39±1.0	6
7	εM7	61±1.2	58±1.2	49±1.0	7
8	εM8	60±0.4	46±0.3	43±0.1	6

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**21a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 8 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{21a}/(ee_{21a}+ee_{8b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{21a})/(1+ee_{21a}/ee_{8b})]/\ln[(1+ee_{21a})/(1+ee_{21a}/ee_{8b})]$. Reactions were carried out in triplicate.

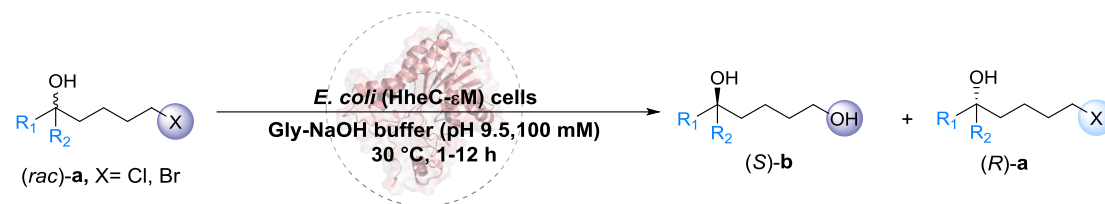
Supplementary Table 33. Enantioselective dehalogenation of (*rac*)-**22a** using the engineered HheC-εM variants.^a



entry	HheC-εM	<i>ee</i> 22b (%) ^b	<i>ee</i> 22a (%) ^b	Conversion 22a (%) ^c	<i>E</i> ^c
1	εM1	10±0.4	86±1.2	90±0.5	3
2	εM2	57±0.6	65±0.9	53±0.6	7
3	εM3	61±0.2	81±0.3	57±0.2	10
4	εM4	64±0.4	16±0.3	20±0.3	5
5	εM5	79±0.6	25±0.3	24±0.4	11
6	εM6	64±0.2	91±0.4	59±0.1	14
7	εM7	52±0.4	44±0.4	46±0.3	5
8	εM8	69±0.4	29±0.9	30±0.8	7

^aThe reactions were carried out in 2 mL Gly-NaOH buffer (pH 9.5, 100 mM) containing 20 mM (*rac*)-**22a** (with 1% v/v DMSO as co-solvent) and 10 g dcw/L *E. coli* (HheC-εM) cells at 30 °C for 10 h. ^bThe conversion and *ee* values were determined by chiral HPLC. ^cThe conversions and enantioselectivity (*E* value) were calculated from *ee* values of substrates and products. Calculated conversion: $conv. = ee_{22a}/(ee_{22a}+ee_{22b})$. ^dCalculated enantioselectivity: $E = \ln[(1-ee_{22a})/(1+ee_{22a}/ee_{22b})]/\ln[(1+ee_{22a})/(1+ee_{22a}/ee_{22b})]$. Reactions were carried out in triplicate.

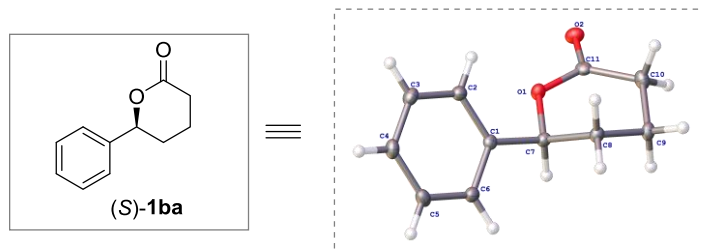
Supplementary Table 34. Preparative-scale biocatalytic enantioselective dehalogenation of ϵ -haloalcohols.^a



Substrate	<i>E. coli</i> (HheC- ϵ M)	Product	T (h)	yield b (%) ^b	<i>ee</i> (<i>S</i>)- b (%) ^c	yield a (%) ^b	<i>ee</i> (<i>R</i>)- a (%) ^c	<i>E</i> ^d
1a	ϵ M7	1b	10	48	96	45	96	194
10a	ϵ M7	10b	12	49	96	49	91	156
12a	ϵ M7	1b	1	42	90	47	98	87
18a	ϵ M8	18b	2	48	89	46	90	53
20a	ϵ M6	7b	6	43	88	47	90	48

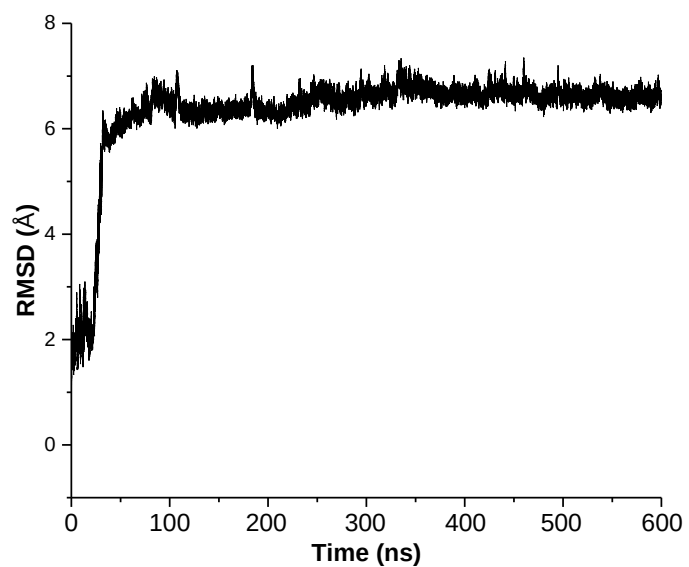
^aReactions were performed in 100 mL of Gly-NaOH buffer (100 mM, pH 9.5) using 10 g dcw/L of *E. coli* (HheC- ϵ M) cells, with 1–2 mmol of ϵ -haloalcohol substrates **a** (with 1% v/v DMSO as co-solvent), at 30 °C for 1–12 h. ^bThe isolated yields were obtained by silica gel chromatography. ^cThe *ee* values of the isolated products (**b**) were determined by chiral HPLC. ^dCalculated enantioselectivity: $E = \ln[(1-ee_a)/(1+ee_a/ee_b)]/\ln[(1+ee_a)/(1+ee_a/ee_b)]$.

Supplementary Table 35. Crystal data and structure refinement for (*R*)-**1ba**.

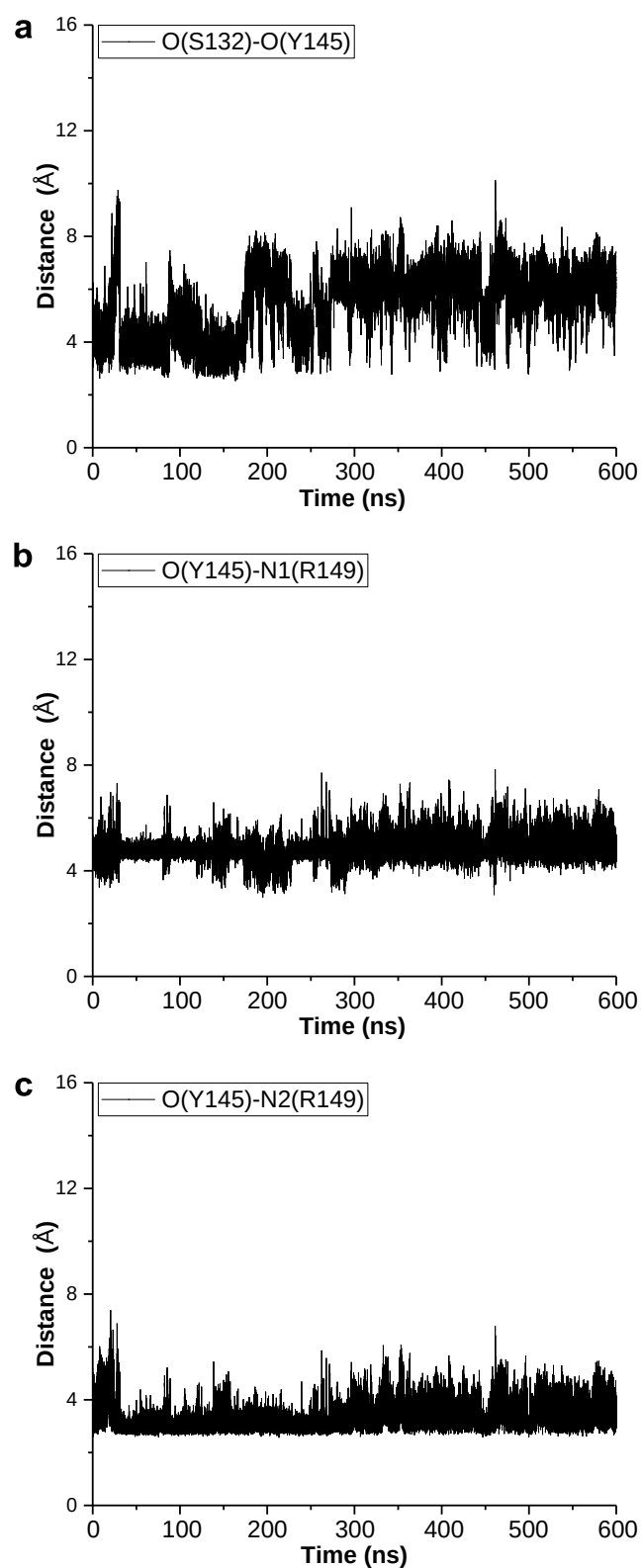


CCDC number	2519677
Empirical formula	C ₁₁ H ₁₂ O ₂
Formula weight	176.21
Temperature/K	100.00(10)
Crystal system	orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁
a/Å	6.15020(10)
b/Å	8.47550(10)
c/Å	17.2230(2)
α /°	90
β /°	90
γ /°	90
Volume/Å ³	897.77(2)
Z	4
$\rho_{\text{calc}}/\text{cm}^3$	1.304
μ/mm^{-1}	0.715
F(000)	376.0
Crystal size/mm ³	0.14 × 0.12 × 0.1
Radiation	Cu K α (λ = 1.54184)
2 Θ range for data collection/°	11.636 to 149.854
Index ranges	-6 ≤ h ≤ 7, -10 ≤ k ≤ 10, -21 ≤ l ≤ 21
Reflections collected	10185
Independent reflections	1792 [R _{int} = 0.0182, R _{sigma} = 0.0087]
Data/restraints/parameters	1792/0/118
Goodness-of-fit on F ²	1.154
Final R indexes [I ≥ 2 σ (I)]	R ₁ = 0.0319, wR ₂ = 0.0871
Final R indexes [all data]	R ₁ = 0.0319, wR ₂ = 0.0871
Largest diff. peak/hole / e Å ⁻³	0.20/-0.17
Flack/Hooft parameter	-0.08(5)/-0.02(2)

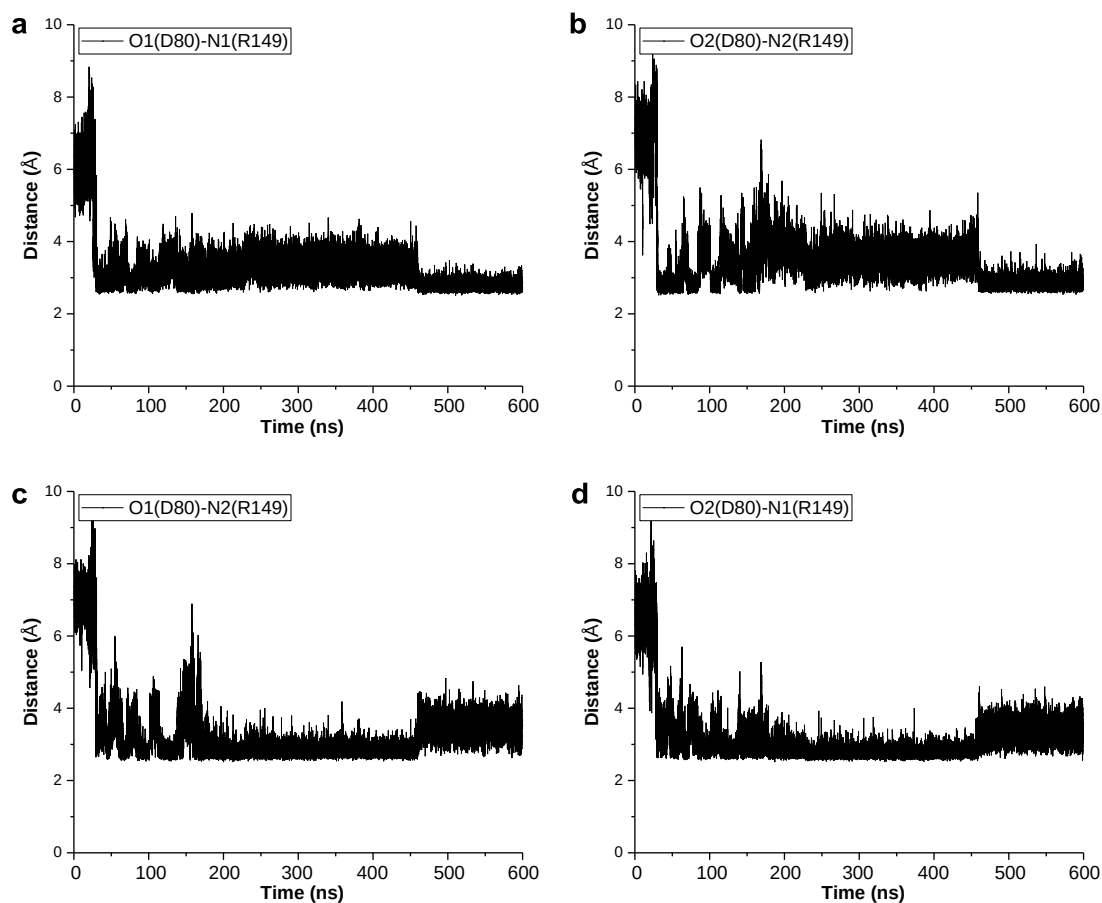
4. Supplementary Figures 1-9



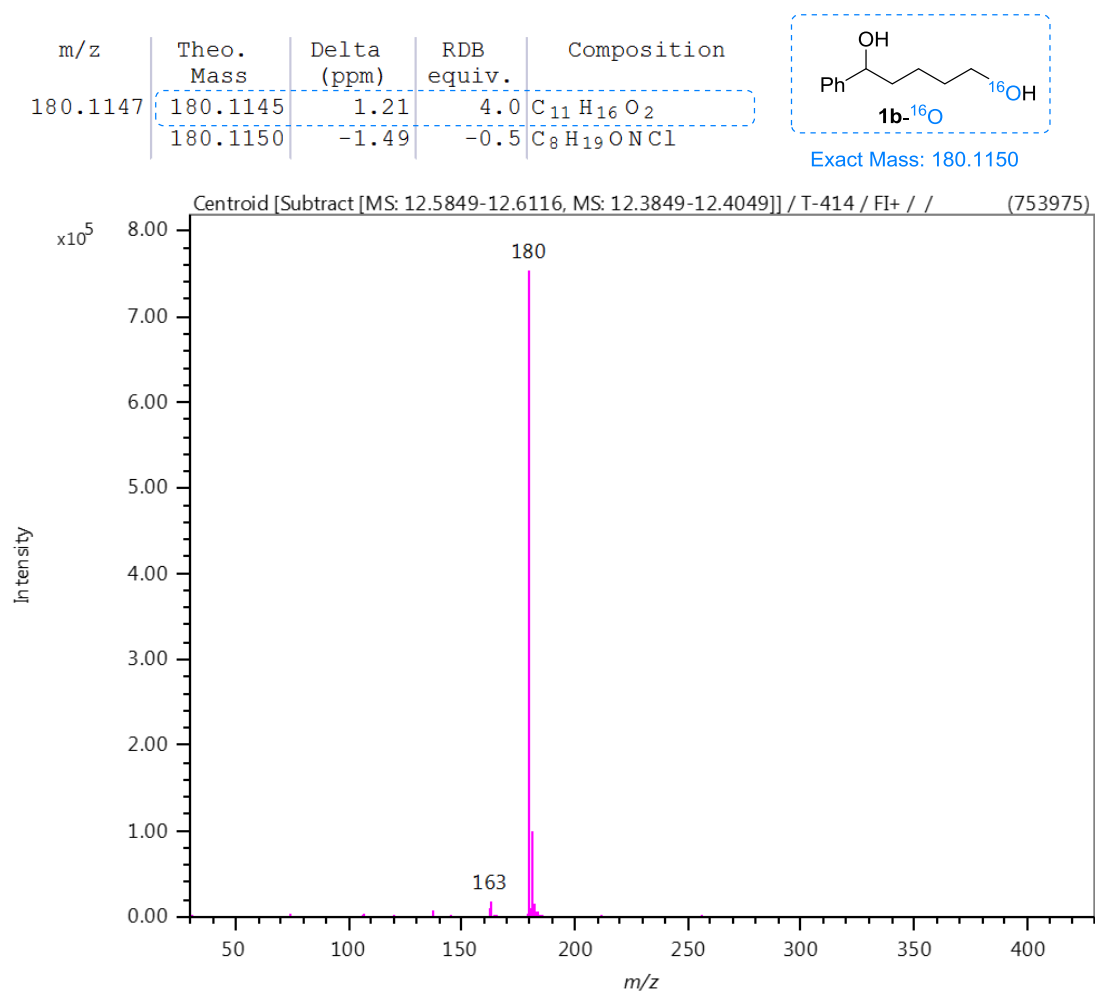
Supplementary Fig. 1 RMSD values of Ca atoms of the whole system with respect to the model structure of SM9 during the MD simulation.



Supplementary Fig. 2 The changes of distances of the NCT (S132-Y145-R149) residues during the MD simulation. (a) The changes of distances between S132-O and Y145-O. (b) The changes of distances between Y145-O and R149-N1. (c) The changes of distances between Y145-O and R149-N2.



Supplementary Fig. 3 The changes of distances of between D80 and R149 residues during the MD simulation. (a) The changes of distances between D80-O1 and R149-N1. (b) The changes of distances between D80-O2 and R149-N2. (c) The changes of distances between D80-O1 and R149-N2. (d) The changes of distances between D80-O2 and R149-N1.

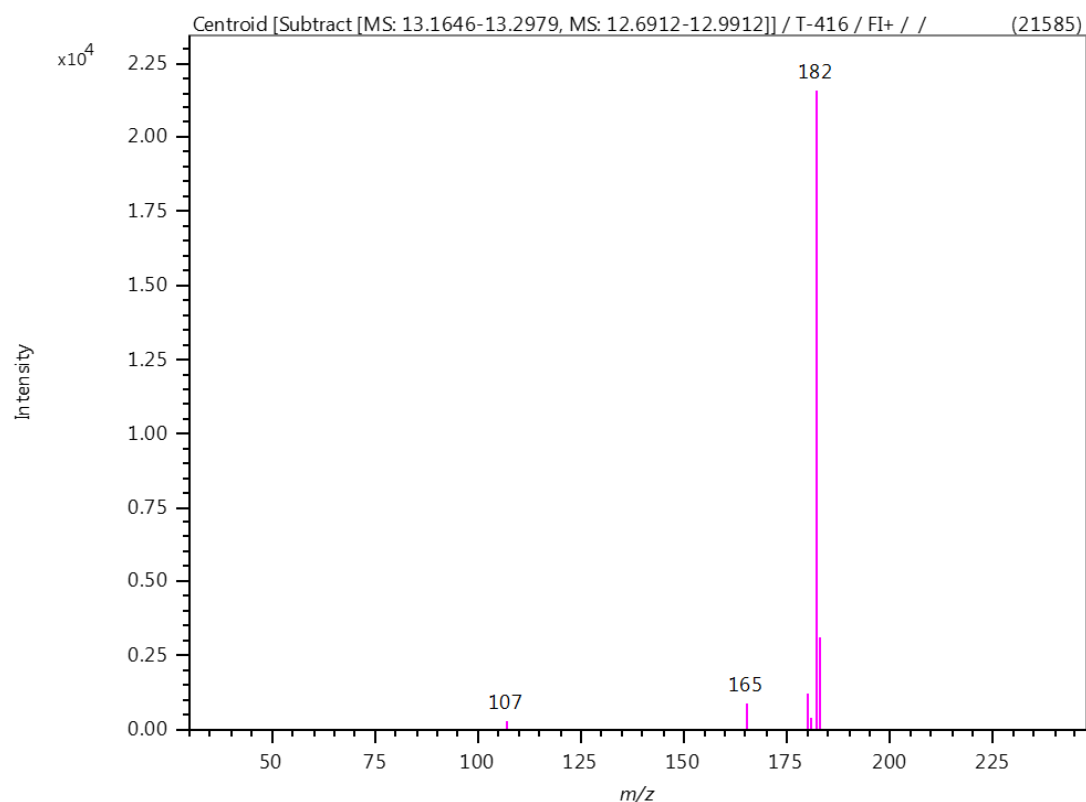


Supplementary Fig. 4 HRMS analysis of product **1b** from the SM9-catalyzed dehalogenative hydroxylation of **1a** in H₂¹⁶O solvent.

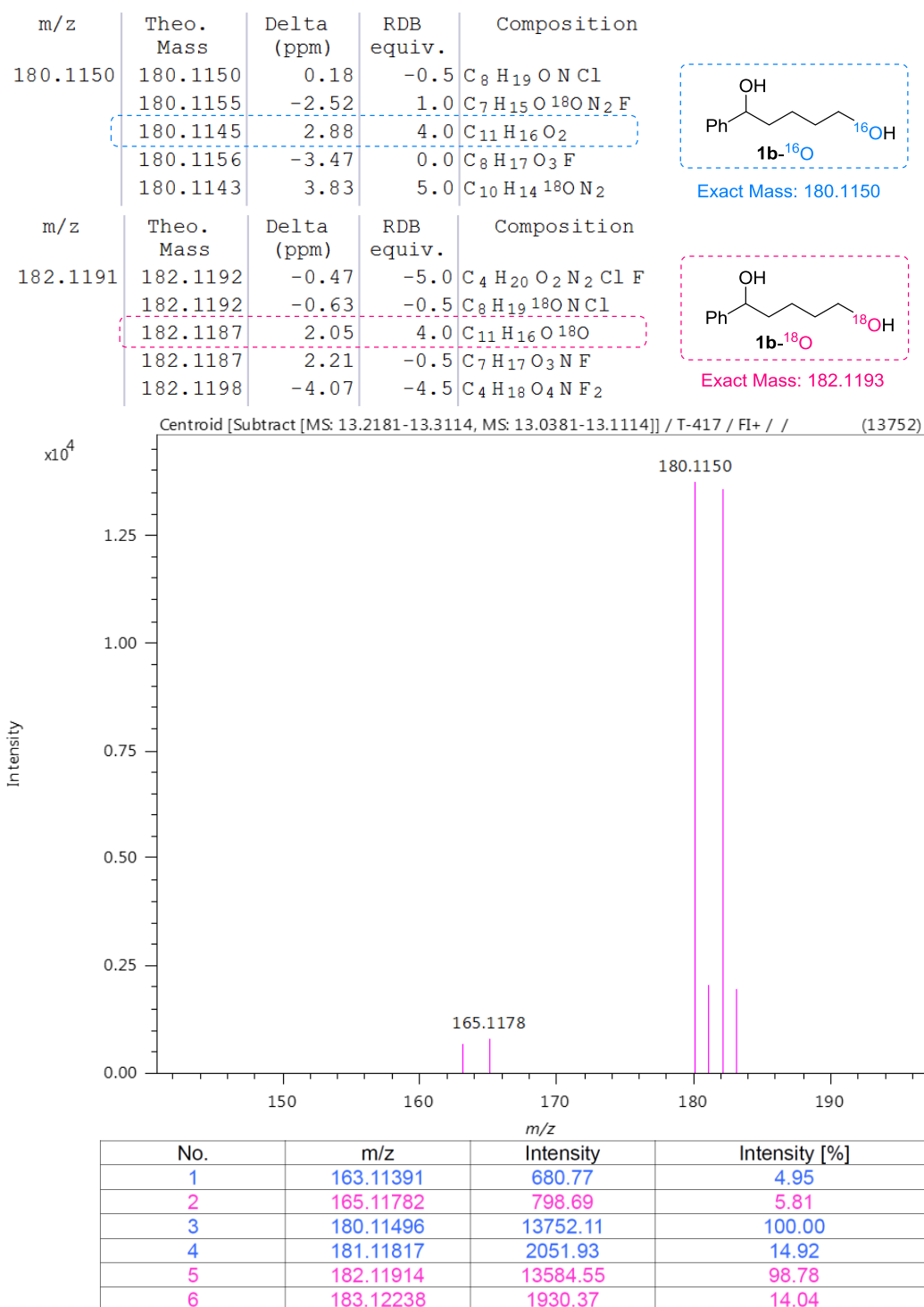
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
182.1191	182.1192	-0.47	-5.0	C ₄ H ₂₀ O ₂ N ₂ Cl F
	182.1192	-0.63	-0.5	C ₈ H ₁₉ ¹⁸ O N Cl
	182.1187	2.05	4.0	C ₁₁ H ₁₆ O ¹⁸ O
	182.1187	2.21	-0.5	C ₇ H ₁₇ O ₃ N F
	182.1198	-4.07	-4.5	C ₄ H ₁₈ O ₄ N F ₂

Oc1ccccc1C(O)CCCC[18O]
1b-¹⁸O

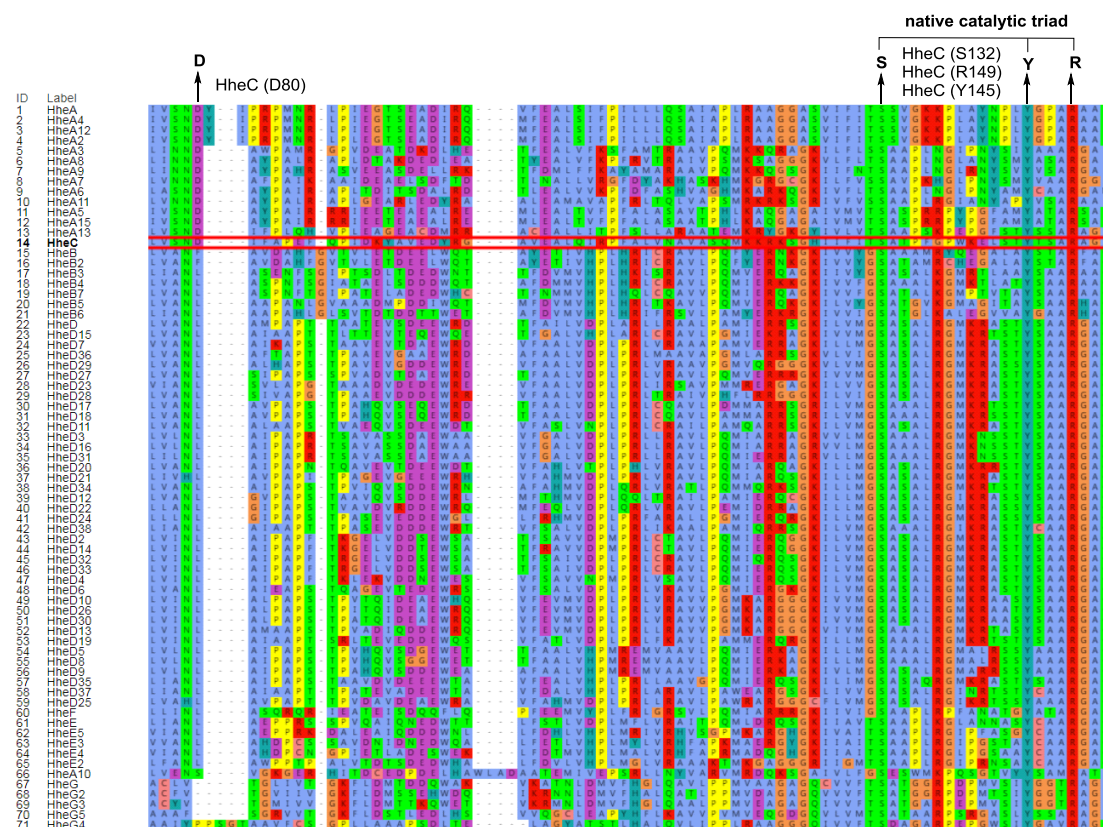
Exact Mass: 182.1193



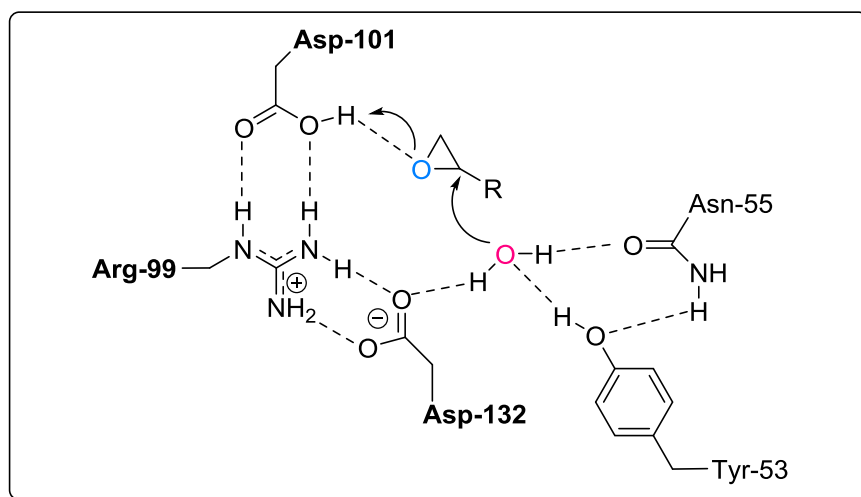
Supplementary Fig. 5 HRMS analysis of product **1b** from the SM9-catalyzed dehalogenative hydroxylation of **1a** in H₂¹⁸O solvent.



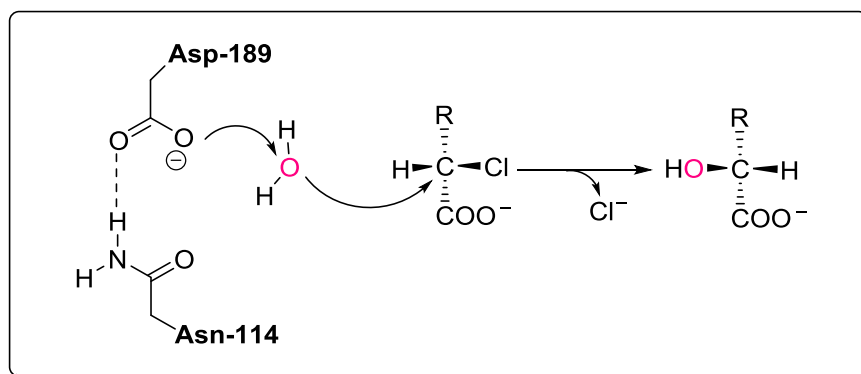
Supplementary Fig. 6 HRMS analysis of product **1b** from the SM9-catalyzed dehalogenative hydroxylation of **1a** in mixed H₂¹⁶O: H₂¹⁸O (1:1) solvent.



Supplementary Fig. 7 Sequence alignment of classified HHDHs across subtypes A–G. Detailed sequences of the classified HHDHs were obtained from previous reports.^{25,26} The sequence alignment was performed using the MAFFT online service^{27,28} and visualized with MSASviewer.²⁹

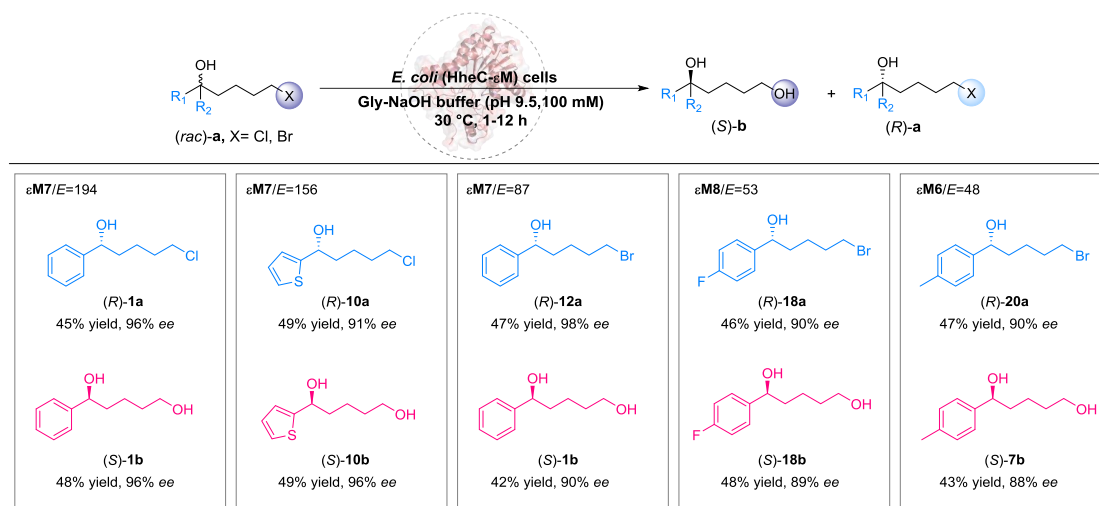


Supplementary Fig. 8. Water activation by the Arg–Asp pair in limonene-1,2-epoxide hydrolase from *Rhodococcus erythropolis*. The mechanism is adapted from Arand et al.,³⁰ and the reaction was performed in 100 mM sodium phosphate buffer (pH 7.4).



Supplementary Fig. 9 Water activation by the Asn–Asp pair in the DL-2-haloacid dehalogenase from *Pseudomonas putida* PP3. The mechanism is adapted from Schmidberger et al.,³¹ and the reaction was performed in 150 mM Tris-SO₄ buffer (pH 7.9).³²

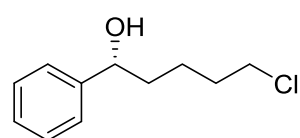
5. Preparative-scale biocatalytic enantioselective dehalogenation



General procedure:

A resting cell suspension of *E. coli* (HheC- ϵ M) was prepared at a concentration of 10 g dcw/L in PB buffer (100 mM, pH 9.5, 100 mL) in a 200 mL round-bottom flask. To this suspension was added ϵ -haloalcohol $(rac)\text{-a}$ (1–2 mmol, with 1% v/v DMSO as co-solvent), yielding a final substrate concentration of 10–20 mM. The reaction mixture was stirred at 30 °C for 1–12 h. Upon completion, the mixture was extracted with ethyl acetate (3×100 mL), and the organic layers were separated by centrifugation ($8800 \times g$, 3 min), combined, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. Flash chromatography on silica gel (petroleum ether/ethyl acetate = 10:1 to 1:1) afforded chiral ϵ -diols $(S)\text{-b}$ and ϵ -haloalcohols $(R)\text{-a}$.

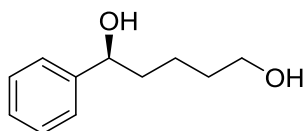
$(R)\text{-5-chloro-1-phenylpentan-1-ol}$ [$(R)\text{-1a}$]



Prepared according to the general procedure: 100 mL Gly-NaOH buffer (100 mM, pH 9.5) containing 10 mM **1a** (1 mmol), 10 g dcw/L *E. coli* (ϵ M7) cells, 30 °C, reaction for 10 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 10:1) to provide $(R)\text{-1a}$ as a light white liquid in 45% yield (92.0 mg). ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.33 – 7.29 (m, 4H), 7.23 – 7.19 (m, 1H), 5.22 – 5.17 (m, 1H), 4.54 – 4.48 (m, 1H), 3.62 – 3.58 (m, 2H), 1.76 – 1.54 (m, 4H), 1.52 – 1.30 (m, 2H). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 146.4, 128.0, 126.6, 125.8,

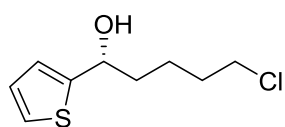
72.1, 45.5, 38.6, 32.1, 22.9. $[\alpha]_D^{25} = +32.00$ ($c = 1.00$, CH_2Cl_2). **HPLC analysis** (AY-H, 12 % IPA in *n*-hexane, 1.0 ml/min, $\lambda = 210$ nm) indicated 96% *ee*: $t_{(R)}$ (major) = 5.8 min, $t_{(S)}$ (minor) = 7.0 min. **HRMS (ESI)** m/z : calculated for $\text{C}_{11}\text{H}_{15}\text{ClNaO}$ $[\text{M}+\text{Na}]^+$: 221.0704, found: 221.0713.

(*S*)-1-phenylpentane-1,5-diol [(*S*)-1b]



Prepared according to the general procedure: 100 mL Gly-NaOH buffer (100 mM, pH 9.5) containing 10 mM **1a** (1 mmol), 10 g dcw/L *E. coli* (ϵM7) cells, 30 °C, reaction for 10 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 10:1 ~ 1:1) to provide (*S*)-**1b** as a light yellow liquid in 48% yield (88.5 mg). **^1H NMR (400 MHz, DMSO- d_6)** δ 7.31 – 7.27 (m, 4H), 7.22 – 7.18 (m, 1H), 5.11 – 5.09 (m, 1H), 4.50 – 4.46 (m, 1H), 4.34 – 4.31 (m, 1H), 3.37 – 3.34 (m, 2H), 1.65 – 1.49 (m, 2H), 1.44 – 1.30 (m, 3H), 1.25 – 1.18 (m, 1H). **^{13}C NMR (100 MHz, DMSO- d_6)** δ 146.5, 127.9, 126.6, 125.8, 72.4, 60.8, 39.4, 32.6, 22.0. $[\alpha]_D^{25} = -34.40$ ($c = 1.00$, CH_2Cl_2). **HPLC analysis** (AY-H, 12 % IPA in *n*-hexane, 1.0 ml/min, $\lambda = 210$ nm) indicated 96% *ee*: $t_{(R)}$ (minor) = 11.4 min, $t_{(S)}$ (major) = 15.7 min. **HRMS (ESI)** m/z : calculated for $\text{C}_{11}\text{H}_{16}\text{NaO}$ $[\text{M}+\text{Na}]^+$: 203.1043, found: 203.1053. **m.p.** : 34.7 – 35.6 °C .

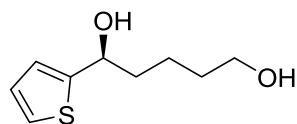
(*R*)-5-chloro-1-(thiophen-2-yl)pentan-1-ol [(*R*)-10a]



Prepared according to the general procedure: 100 mL Gly-NaOH buffer (100 mM, pH 9.5) containing 10 mM **10a** (1 mmol), 10 g dcw/L *E. coli* (ϵM7) cells, 30 °C, reaction for 12 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 10:1) to provide (*R*)-**10a** as a light yellow liquid in 49% yield (202.6 mg). **^1H NMR (400 MHz, DMSO- d_6)** δ 7.37 – 7.35 (m, 1H), 6.96 – 6.91 (m, 2H), 5.55 (s, 1H), 4.77 (q, $J = 6.4$ Hz, 1H), 3.63 – 3.59 (m, 2H), 1.76 – 1.63 (m, 4H), 1.55 – 1.46 (m, 1H), 1.44 – 1.35 (m, 1H). **^{13}C NMR (100 MHz, DMSO- d_6)** δ

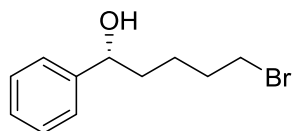
150.9, 126.5, 123.9, 122.8, 68.3, 45.4, 38.7, 32.0, 22.8. $[\alpha]_D^{25} = +13.40$ ($c = 1.00$, CH_2Cl_2). **HPLC analysis** (AY-H, 12 % IPA in *n*-hexane, 1.0 ml/min, $\lambda = 210$ nm) indicated 91% *ee*: $t_{(R)}$ (major) = 6.9 min, $t_{(S)}$ (minor) = 7.5 min. **HRMS (ESI)** m/z : calculated for $\text{C}_9\text{H}_{13}\text{ClNaOS}$ $[\text{M}+\text{Na}]^+$: 227.0266, found: 227.0268.

(*S*)-1-(thiophen-2-yl)pentane-1,5-diol [(*S*)-10b]



Prepared according to the general procedure: 100 mL Gly-NaOH buffer (100 mM, pH 9.5) containing 10 mM **10a** (1 mmol), 10 g dcw/L *E. coli* (ϵM7) cells, 30 °C, reaction for 12 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 2:1) to provide (*S*)-**10b** as a light white liquid in 45% yield (168.86 mg). **^1H NMR (400 MHz, DMSO- d_6)** δ 7.35 (dd, $J = 1.3, 5.0$ Hz, 1H), 6.95 – 6.91 (m, 2H), 4.48 (d, $J = 4.7$ Hz, 1H), 4.76 – 4.71 (m, 1H), 4.35 (t, $J = 5.1$ Hz, 1H), 3.38 – 3.37 (m, 1H), 3.35 – 3.34 (m, 1H), 1.73 – 1.59 (m, 2H), 1.45 – 1.22 (m, 4H). **^{13}C NMR (100 MHz, DMSO- d_6)** δ 151.0, 126.4, 123.9, 122.8, 68.4, 60.8, 39.5, 32.4, 21.9. $[\alpha]_D^{25} = -13.40$ ($c = 1.00$, CH_2Cl_2). **HPLC analysis** (AY-H, 12 % IPA in *n*-hexane, 1.0 ml/min, $\lambda = 210$ nm) indicated 96% *ee*: $t_{(R)}$ (major) = 11.3 min, $t_{(S)}$ (minor) = 12.5 min. **HRMS (ESI)** m/z : calculated for $\text{C}_9\text{H}_{13}\text{NaClOS}$ $[\text{M}+\text{Na}]^+$: 209.0607, found: 209.0613.

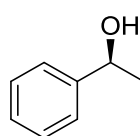
(*R*)-5-bromo-1-phenylpentan-1-ol [(*R*)-12a]



Prepared according to the general procedure: 100 mL Gly-NaOH buffer (100 mM, pH 9.5) containing 20 mM **12a** (2 mmol), 10 g dcw/L *E. coli* (ϵM7) cells, 30 °C, reaction for 1 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 10:1) to provide (*R*)-**12a** as a light yellow liquid in 47% yield (228.5 mg). **^1H NMR (400 MHz, DMSO- d_6)** δ 7.33 – 7.28 (m, 4H), 7.24 – 7.19 (m, 1H), 4.68 – 4.49 (m, 2H), 3.52 – 3.48 (m, 2H), 1.83 – 1.75 (m, 2H), 1.66 – 1.54 (m, 2H), 1.49 – 1.32 (m, 2H). **^{13}C NMR (100 MHz, DMSO- d_6)** δ 146.3, 127.9, 126.6, 125.8, 72.1, 38.4, 35.2, 32.3, 24.1. $[\alpha]_D^{25} = +20.67$ ($c = 1.00$, CH_2Cl_2). **HPLC**

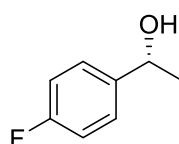
analysis (AY-H, 12 % IPA in *n*-hexane, 1.0 ml/min, λ =210 nm) indicated 98% *ee*: $t_{(R)}$ (major) = 5.9 min, $t_{(S)}$ (minor) = 7.4 min. **HRMS (ESI)** m/z : calculated for $C_{11}H_{16}BrO$ $[M+H]^+$: 243.0379, found: 243.0379.

(S)-1-phenylpentane-1,5-diol [(S)-1b] from (rac)-12a



Prepared according to the general procedure: 100 mL Gly-NaOH buffer (100 mM, pH 9.5) containing 10 mM **12a** (1 mmol), 10 g dcw/L *E. coli* (ϵ M7) cells, 30 °C, reaction for 1 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 10:1 ~ 1:1) to provide (*S*)-**1b** as a light yellow liquid in 42% yield (152.5 mg). **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.31 – 7.29 (m, 4H), 7.23 – 7.18 (m, 1H), 5.19 – 5.04 (m, 1H), 4.51 – 4.46 (m, 1H), 4.35 – 4.32 (m, 1H), 3.37 – 3.32 (m, 2H), 1.62 – 1.51 (m, 2H), 1.40 – 1.36 (m, 3H), 1.25 – 1.16 (m, 1H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 146.5, 127.9, 126.5, 125.8, 72.4, 60.8, 39.3, 32.6, 22.0. $[\alpha]_D^{25}$ = -33.40(c = 1.00, CH_2Cl_2). **HPLC analysis** (AY-H, 12 % IPA in *n*-hexane, 1.0 ml/min, λ =210 nm) indicated 90% *ee*: $t_{(R)}$ (minor) = 11.3 min, $t_{(S)}$ (major) = 15.5 min. **HRMS (ESI)** m/z : calculated for $C_{11}H_{16}NaO$ $[M+Na]^+$: 203.1043, found: 203.1048. **m.p.** : 34.7–35.6 °C .

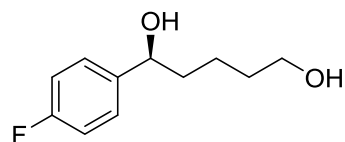
(R)-5-bromo-1-(4-fluorophenyl)pentan-1-ol [(R)-18a]



Prepared according to the general procedure: 100 mL Gly-NaOH buffer (100 mM, pH 9.5) containing 20 mM **18a** (2 mmol), 10 g dcw/L *E. coli* (ϵ M8) cells, 30 °C, reaction for 2 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 10:1 ~ 1:1) to provide (*R*)-**18a** as a light yellow liquid in 46% yield (244.3 mg). **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.37 – 7.33 (m, 2H), 7.15 – 7.09 (m, 2H), 4.96 – 4.50 (m, 2H), 3.49 (t, J = 6.7 Hz, 2H), 1.82 – 1.73 (m, 2H), 1.65 – 1.53 (m, 2H), 1.49 – 1.29 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 161.0 (d, J = 240.0 Hz, 1C), 142.5 (d, J = 3.0 Hz, 1C), 127.6 (d, J = 8.0 Hz, 2C), 114.6 (d, J = 21.0

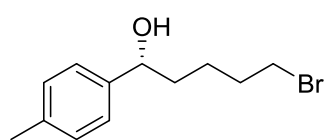
Hz, 2C), 71.4, 38.4, 35.3, 32.3, 24.1. $[\alpha]_D^{25} = +10.30$ ($c = 1.00$, CH_2Cl_2). **HPLC analysis** (AY-H, 12 % IPA in *n*-hexane, 1.0 ml/min, $\lambda = 210$ nm) indicated 90% *ee*: $t_{(R)}$ (major) = 5.5 min, $t_{(S)}$ (minor) = 7.8 min. **HRMS (ESI)** m/z : calculated for $\text{C}_{11}\text{H}_{15}\text{BrFO}$ $[\text{M}+\text{H}]^+$: 261.0285, found: 261.0285.

(S)-1-(4-fluorophenyl)pentane-1,5-diol [(S)-18b]



Prepared according to the general procedure: 100 mL Gly-NaOH buffer (100 mM, pH 9.5) containing 20 mM **18a** (2 mmol), 10 g dcw/L *E. coli* (ϵM8) cells, 30 °C, reaction for 2 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 10:1 ~ 1:1) to provide (*S*)-**18b** as a light yellow liquid in 48% yield (193.7 mg). **^1H NMR (400 MHz, DMSO- d_6)** δ 7.35 – 7.31 (m, 2H), 7.14 – 7.08 (m, 2H), 5.16 (d, $J = 4.4$ Hz, 1H), 4.51 – 4.47 (m, 1H), 4.33 (t, $J = 5.1$ Hz, 1H), 3.36 – 3.32 (m, 2H), 1.63 – 1.47 (m, 2H), 1.43 – 1.36 (m, 2H), 1.34 – 1.14 (m, 2H). **^{13}C NMR (100 MHz, DMSO- d_6)** δ 161.0 (d, $J = 240.0$ Hz, 1C), 142.7 (d, $J = 3.0$ Hz, 1C), 127.7 (d, $J = 7.0$ Hz, 2C), 114.7, 114.5, 71.7, 60.7, 32.5, 21.9. $[\alpha]_D^{25} = -17.63$ ($c = 1.00$, CH_2Cl_2). **HPLC analysis** (AY-H, 12 % IPA in *n*-hexane, 1.0 ml/min, $\lambda = 210$ nm) indicated 89% *ee*: $t_{(R)}$ (major) = 8.9 min, $t_{(S)}$ (minor) = 10.9 min. **HRMS (ESI)** m/z : calculated for $\text{C}_{11}\text{H}_{15}\text{FNaO}_2$ $[\text{M}+\text{Na}]^+$: 221.0948, found: 221.0956. **m.p.** : 56.9 – 61.6 °C.

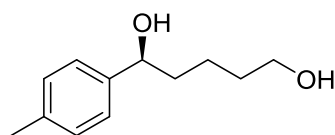
(R)-5-bromo-1-(p-tolyl)pentan-1-ol [(R)-20a]



Prepared according to the general procedure: 100 mL Gly-NaOH buffer (100 mM, pH 9.5) containing 20 mM **20a** (2 mmol), 10 g dcw/L *E. coli* (ϵM6) cells, 30 °C, reaction for 6 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 10:1 ~ 5:1) to provide (*R*)-**20a** as a light yellow liquid in 47% yield (243.2 mg). **^1H NMR (400 MHz, DMSO- d_6)** δ 7.19 (d, $J = 8.0$ Hz, 2H), 7.10 (d, $J = 7.9$ Hz, 2H), 4.88 (s, 1H), 4.48 – 4.45 (m, 1H), 3.49 (t, $J = 6.7$ Hz, 2H),

2.27 (s, 3H), 1.82 – 1.74 (m, 2H), 1.65 – 1.50 (m, 2H), 1.49 – 1.28 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 143.3, 135.5, 128.5, 125.8, 71.9, 38.4, 35.2, 32.3, 24.1, 20.7. **$[\alpha]_D^{25}$** = + 5.30 (*c* = 1.00, CH₂Cl₂). **HPLC analysis** (IF, 10 % IPA in *n*-hexane, 1.0 ml/min, λ = 210 nm) indicated 90% *ee*: *t*_(R) (major) = 7.2 min, *t*_(S) (minor) = 7.7 min. **HRMS (ESI) *m/z***: calculated for C₁₂H₁₇BrNaO [M+Na]⁺: 279.0355, found: 279.0364. **m.p.** : 32.0 - 33.6 °C .

(*S*)-1-(*p*-tolyl)pentane-1,5-diol [(*S*)-7b]



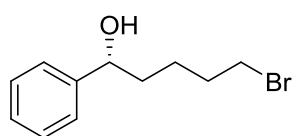
Prepared according to the general procedure: 100 mL Gly-NaOH buffer (100 mM, pH 9.5) containing 20 mM **20a** (2 mmol), 10 g dcw/L *E. coli* (εM6) cells, 30 °C, reaction for 6 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 10:1 ~ 1:1) to provide (*S*)-**7b** as a light yellow liquid in 43% yield (169.7 mg). **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.18 (d, *J* = 7.9 Hz, 2H), 7.10 (d, *J* = 7.8 Hz, 2H), 5.02 (d, *J* = 4.4 Hz, 1H), 4.46 – 4.42 (m, 1H), 4.33 (t, *J* = 5.1 Hz, 1H), 3.37 – 3.32 (m, 2H), 2.27 (s, 3H), 1.62 – 1.47(m, 2H), 1.43 – 1.36 (m, 2H), 1.34 – 1.15 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 143.5, 135.5, 128.5, 125.8, 72.2, 60.8, 39.4, 32.6, 22.0, 20.7. **$[\alpha]_D^{25}$** = – 15.80(*c* = 1.00, CH₂Cl₂). **HPLC analysis** (IF, 10 % IPA in *n*-hexane, 1.0 ml/min, λ = 210 nm) indicated 88% *ee*: *t*_(S) (minor) = 15.9 min, *t*_(R) (major) = 17.9 min. **HRMS (ESI) *m/z***: calculated for C₁₂H₁₈NaO₂ [M+Na]⁺: 217.1199, found: 217.1209.

6. Gram-scale and derivatization reactions

Gram-scale reaction:

A resting cell suspension of *E. coli* (HheC- ϵ M7) was prepared at a concentration of 10 g dcw/L in Gly-NaOH buffer (500 mM, pH 9.5, 100 mL) in a 250 mL three-neck round-bottom flask. To the suspension was added ϵ -haloalcohol (*rac*)-**12a** (2.92 g, 12 mmol) dissolved in DMSO (2 mL), giving a final substrate concentration of 120 mM. The reaction mixture was stirred at 30 °C, and the pH was maintained at 8.5 ± 0.1 with a pH-stat using 5 M NaOH as the alkaline solution. After 57 h, the enzymatic reaction was complete. The mixture was extracted with ethyl acetate (3×90 mL), and the organic layers were separated by centrifugation ($8800 \times g$, 3 min), combined, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate = 10:1 to 1:1) to afford chiral ϵ -haloalcohol (*R*)-**12a** (1.36 g, 47% yield, 93% *ee*) and chiral 1-phenylpentane-1,5-diol (*S*)-**1b** (1.05 g, 47% yield, 95% *ee*).

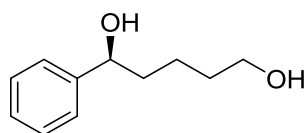
(R)-5-bromo-1-phenylpentan-1-ol [(R)-12a]



Prepared according to the general procedure: 100 mL Gly-NaOH buffer (500 mM, pH 9.5) containing 120 mM (2.92 g) (*rac*)-**12a**, 10 g dcw/L *E. coli* (HheC- ϵ M7) cells, 30 °C, reaction for 57 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 10:1) to provide (*R*)-**12a** as a light yellow liquid in 47% yield (1.36 g). **HPLC analysis** (AY-H, 12 % IPA in *n*-hexane, 1.0 ml/min, $\lambda = 210$ nm) indicated 93% *ee*: $t_{(R)}$ (major) = 5.9 min, $t_{(S)}$ (minor) = 7.4 min.

(S)-1-phenylpentane-1,5-diol [(S)-1b] from 12a

Prepared according to the general procedure: 100 mL Gly-NaOH buffer (500 mM, pH 9.5) containing 120 mM (2.92 g) (*rac*)-**12a**, 10 g dcw/L *E. coli* (HheC- ϵ M7) cells, 30 °C, reaction for 57 h. The crude product was purified by flash column chromatography on silica gel (petroleum ether : ethyl acetate = 1:1) to provide (*S*)-**1b** as a white solid in



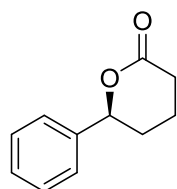
47% yield (1.05 g). **HPLC analysis** (AY-H, 12 % IPA in *n*-hexane, 1.0 ml/min, λ = 210 nm) indicated 95% *ee*: $t_{(R)}$ (minor) = 11.3 min, $t_{(S)}$ (major) = 15.5 min.

Derivatization reactions:

Synthesis of (*S*)-1ba** from (*S*)-**1b**.**³³

To a 100 mL round-bottom flask, (*S*)-**1b** (0.1992 g, 1.0 mmol), TEMPO (0.0173 g, 0.1 mmol), and tetrabutylammonium iodide (0.0412 g, 0.1 mmol) were dissolved in a mixture of 10 mL dichloromethane and 10 mL of water containing NaHCO₃ (0.5 M) and K₂CO₃ (0.05 M). The mixture was stirred vigorously at room temperature, after which N-Chlorosuccinimide (0.4427 g, 3.0 mmol) was added. The reaction was monitored until completion. The organic layer was separated and extracted with dichloromethane (3 × 15 mL). The combined organic extracts were washed with saturated brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The resulting mixture was purified by column chromatography (petroleum ether: ethyl acetate = 8:1 ~ 5:1) to afford the desired chiral product (*S*)-**1ba** as a white solid. The racemic **1ba** was prepared from (*rac*)-**1b** following the same protocol and used for chiral HPLC analysis.

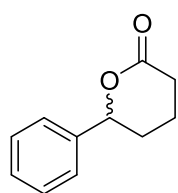
(*S*)-6-phenyltetrahydro-2H-pyran-2-one [(*S*)-1ba**]**



White solid in 88% yield (171.2 mg). **¹H NMR (400 MHz, CDCl₃)** δ 7.41 - 7.29 (m, 5H), 5.35 (dd, J = 10.6, 3.5 Hz, 1H), 2.76 - 2.52 (m, 2H), 2.21 - 2.13 (m, 1H), 2.03 - 1.95 (m, 2H), 1.92 - 1.81 (m, 1H). **¹³C NMR (100 MHz, CDCl₃)** δ 171.5, 139.9, 128.7, 128.4, 125.8, 81.8, 30.6, 29.6,

18.7. $[\alpha]_D^{25}$ = - 10.86 (c = 1.00, CH₂Cl₂). **HPLC analysis** (AY-H, 20% IPA in *n*-hexane, 1.2 ml/min, 210 nm) indicated 95 % *ee*: $t_{(S)}$ (major) = 14.0 min, $t_{(R)}$ (minor) = 18.3 min. **HRMS (ESI)** m/z : calculated for C₁₁H₁₂NaO₂ [M +Na]⁺: 199.0730, found: 199.0737. **m.p.** : 90.6 - 92.7 °C.

(rac)-6-phenyltetrahydro-2H-pyran-2-one [(rac)-1ba]

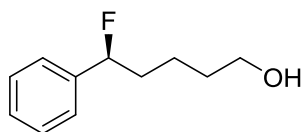


¹H NMR (400 MHz, CDCl₃) δ 7.42 - 7.29 (m, 5H), 5.36 (dd, J = 10.5, 3.5 Hz, 1H), 2.76 - 2.52 (m, 2H), 2.22 - 2.13 (m, 1H), 2.03 - 1.95 (m, 2H), 1.91 - 1.81 (m, 1H). **¹³C NMR (100 MHz, CDCl₃)** δ 171.5, 139.9, 128.7, 128.4, 125.8, 81.8, 30.6, 29.6, 18.7.

Synthesis of (*S*)-1bb from (*S*)-1b.³⁴

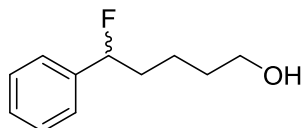
To a 50 mL round-bottom flask, (*S*)-1b (0.0925 g, 0.5 mmol), triphenylphosphine (0.3365 g, 1.25 mmol) and KF (0.0596 g, 1.0 mmol) were dissolved in a mixture of DMF and CCl₄ (4:1, 2.5 mL). The reaction mixture was stirred vigorously at room temperature for 1 h and monitored by TLC. After completion, the mixture was extracted with *n*-hexane (3 $\times$ 15 mL). The combined organic phases were washed with saturated brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by column chromatography (petroleum ether: ethyl acetate = 15:1 ~ 8:1) to afford chiral product (*S*)-1bb as a light yellow liquid. Racemic 1bb was prepared from (*rac*)-1b following the same protocol and used for chiral HPLC analysis.

(*S*)-5-fluoro-5-phenylpentan-1-ol [(*S*)-1bb]



Light yellow liquid in 87% yield (81.6 mg). **¹H NMR (400 MHz, CDCl₃)** δ 7.35 - 7.24 (m, 5H), 4.61 (dd, J = 7.6, 5.7 Hz, 1H), 3.48 (t, J = 6.7 Hz, 2H), 2.22 (s, 1H), 1.82 - 1.63 (m, 4H), 1.59 - 1.48 (m, 1H), 1.44 - 1.33 (m, 1H). **¹³C NMR (100 MHz, CDCl₃)** δ 144.7, 128.6, 127.7, 125.9, 74.4, 45.0, 38.3, 32.5, 23.3. . $[\alpha]_D^{25}$ = - 9.33 (c = 1.00, CH₂Cl₂). **HPLC analysis** (OB-H, 10% IPA in *n*-hexane, 0.7 ml/min, 210 nm) indicated 95 % *ee*: $t_{(S)}$ (major) = 15.6 min, $t_{(R)}$ (minor) = 19.5 min. **HRMS (ESI)** m/z : calculated for C₁₁H₁₆FO $[M+H]^+$: 183.1180, found: 183.1181.

(rac)-5-fluoro-5-phenylpentan-1-ol [(rac)-1bb]



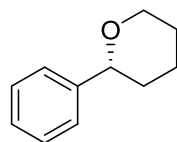
¹H NMR (400 MHz, CDCl₃) δ 7.35 - 7.24 (m, 5H), 4.62 (dd, J = 7.6, 5.7 Hz, 1H), 3.49 (t, J = 6.7 Hz, 2H), 2.17 (s, 1H), 1.80 - 1.64 (m, 4H), 1.60 - 1.33 (m, 2H). **¹³C NMR (100 MHz,**

CDCl_3) δ 144.7, 128.6, 127.7, 125.9, 74.4, 45.0, 38.3, 32.5, 23.3.

Synthesis of (*R*)-**12aa** from (*R*)-**12a**.¹⁵

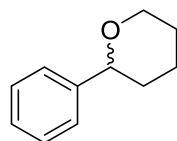
The (*R*)-**12a** (0.2364 g, 1 mmol) was dissolved in THF (20 mL) in a 100 mL round-bottom flask. Potassium *tert*-butoxide (0.3273 g, 3.0 mmol) was added, and the mixture was stirred at room temperature for 4 h. The reaction was quenched with distilled water (10 mL) and extracted with ethyl acetate (3 $\times$ 15 mL). The combined organic layers were washed with saturated brine, dried over anhydrous Na_2SO_4 , and concentrated under reduced pressure. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate = 20:1 $\sim$ 12:1) to afford the desired chiral product (*R*)-**12aa** as a light yellow liquid. Racemic **12aa** was prepared from (*rac*)-**12a** following the same protocol and used for chiral HPLC analysis.

(*R*)-2-phenyltetrahydro-2H-pyran [(*R*)-12aa**]**



Light yellow liquid in 87% yield (137.2 mg). **¹H NMR (400 MHz, CDCl_3)** δ 7.37 - 7.30 (m, 4H), 7.27 - 7.22 (m, 1H), 4.35 (dd, J = 10.8, 2.4 Hz, 1H), 4.17 - 4.10 (m, 1H), 3.66 - 3.57 (m, 1H), 1.98 - 1.79 (m, 2H), 1.74 - 1.54 (m, 4H).. **¹³C NMR (100 MHz, CDCl_3)** δ 143.5, 128.4, 127.4, 126.0, 80.3, 69.1, 34.2, 26.0, 24.1. $[\alpha]_{\text{D}}^{25}$ = + 25.03 (c = 1.00, CH_2Cl_2). **HPLC analysis** (OJ-H, 5% IPA in *n*-hexane, 1.0 ml/min, 210 nm) indicated 93 % *ee*: $t_{(R)}$ (major) = 10.3 min, $t_{(S)}$ (minor) = 9.2 min. **HRMS (ESI)** m/z : calculated for $\text{C}_{11}\text{H}_{15}\text{O}$ $[\text{M}+\text{H}]^+$: 163.1117, found: 163.1121.

(*rac*)-2-phenyltetrahydro-2H-pyran [(*rac*)-12aa**]**

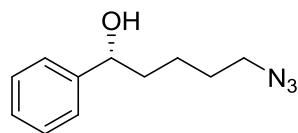


¹H NMR (400 MHz, CDCl_3) δ 7.37 - 7.29 (m, 4H), 7.27 - 7.21 (m, 1H), 4.31 (dd, J = 10.7, 2.3 Hz, 1H), 4.17 - 4.10 (m, 1H), 3.65 - 3.56 (m, 1H), 1.96 - 1.77 (m, 2H), 1.71 - 1.53 (m, 4H). **¹³C NMR (100 MHz, CDCl_3)** δ 143.4, 128.4, 127.4, 125.9, 80.2, 69.1, 34.1, 26.0, 24.1.

Synthesis of (*R*)-**12ab** from (*R*)-**12a**.³⁵

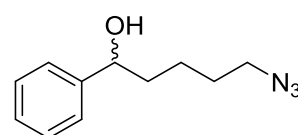
The (*R*)-**12a** (0.1665 g, 1.0 equiv) was placed in a 10 mL round-bottom flask and dissolved in DMF (2 mL). Sodium azide (0.1821 g, 4.0 equiv) was added, and the reaction mixture was heated to 85 °C with stirring for 2 h. Upon completion, the reaction was quenched with saturated aqueous NH₄Cl. The aqueous layer was extracted with ethyl acetate (3 × 10 mL), and the combined organic extracts were washed sequentially with saturated aqueous NH₄Cl (3 × 10 mL) and saturated brine. The organic phase was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (petroleum ether: ethyl acetate = 10:1 ~ 5:1) to afford chiral product (*R*)-**12ab** as a light yellow liquid. The racemic **12ab** was prepared from (*rac*)-**12a** following the same protocol and used for chiral HPLC analysis.

(*R*)-5-azido-1-phenylpentan-1-ol [(*R*)-12ab**]**



Light yellow liquid in 89% yield (125.2 mg). **¹H NMR (400 MHz, DMSO-*d*₆)** δ 7.34 - 7.28 (m, 4H), 7.24 - 7.18 (m, 1H), 5.15 - 5.11 (m, 1H), 4.55 - 4.50 (m, 1H), 3.31 - 3.27 (m, 2H), 1.66 - 1.49 (m, 4H), 1.45 - 1.25 (m, 2H). **¹³C NMR (101 MHz, DMSO-*d*₆)** δ 146.3, 127.9, 126.5, 125.7, 72.1, 50.7, 38.7, 28.2, 22.6. **$[\alpha]_D^{25}$** = + 13.16 (c = 1.00, CH₂Cl₂). **HPLC analysis** (AY-H, 12% IPA in *n*-hexane, 1.0 ml/min, 210 nm) indicated 93 % *ee*: *t*_(*R*) (major) = 6.9 min, *t*_(*S*) (minor) = 10.2 min. **HRMS (ESI)** *m/z*: calculated for C₁₁H₁₅N₃ONa [M+H]⁺: 228.1107, found: 228.1113.

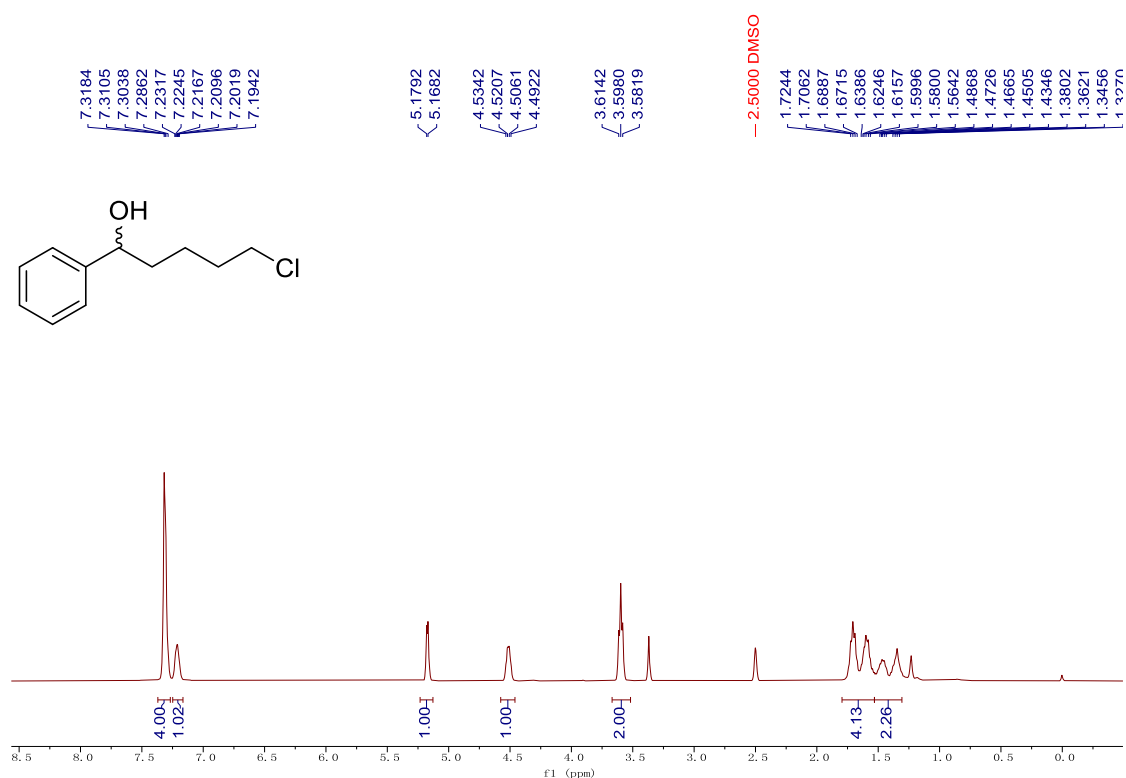
(*rac*)-5-azido-1-phenylpentan-1-ol [(*rac*)-12ab**]**



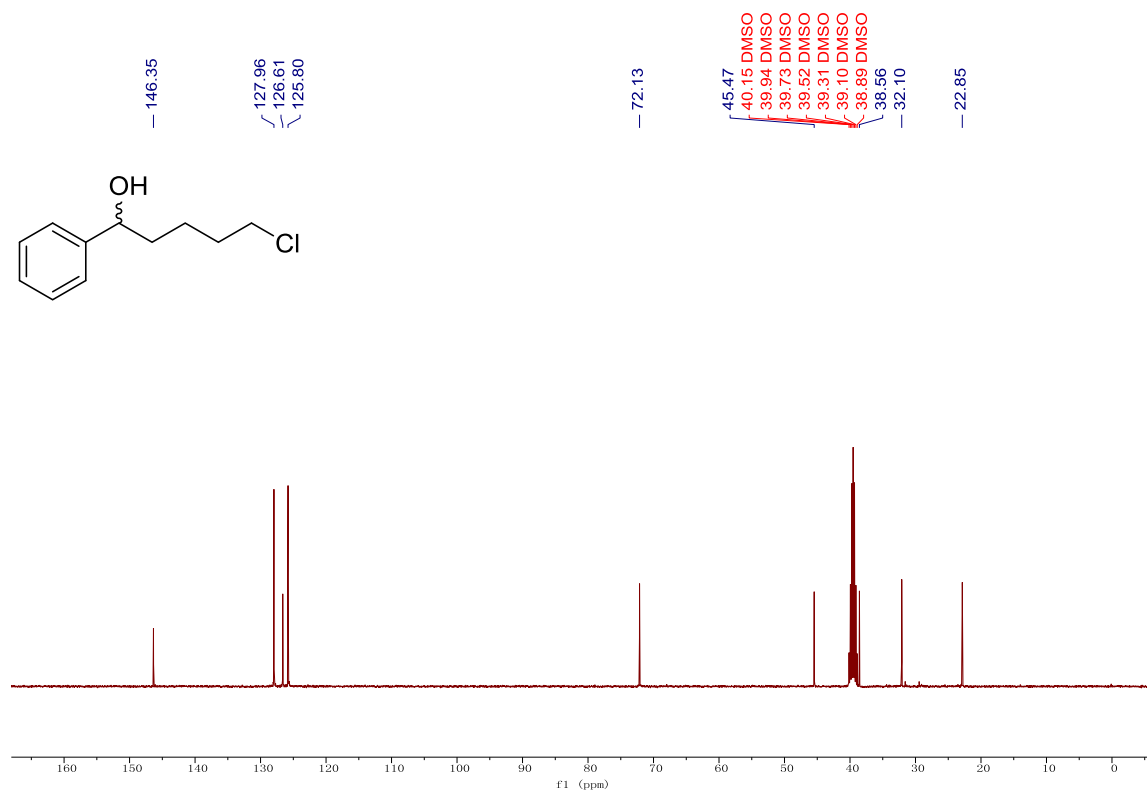
¹H NMR (400 MHz, DMSO-*d*₆) δ 7.33 - 7.38 (m, 4H), 7.24 - 7.19 (m, 1H), 5.14 - 5.11 (m, 1H), 4.55 - 4.50 (m, 1H), 3.32 - 3.27 (m, 2H), 1.68 - 1.49 (m, 4H), 1.45 - 1.24 (m, 2H). **¹³C NMR (100 MHz, DMSO-*d*₆)** δ 146.3, 127.9, 126.5, 125.7, 72.1, 50.7, 38.7, 28.2, 22.6.

7. Copies of NMR spectra for racemic compounds

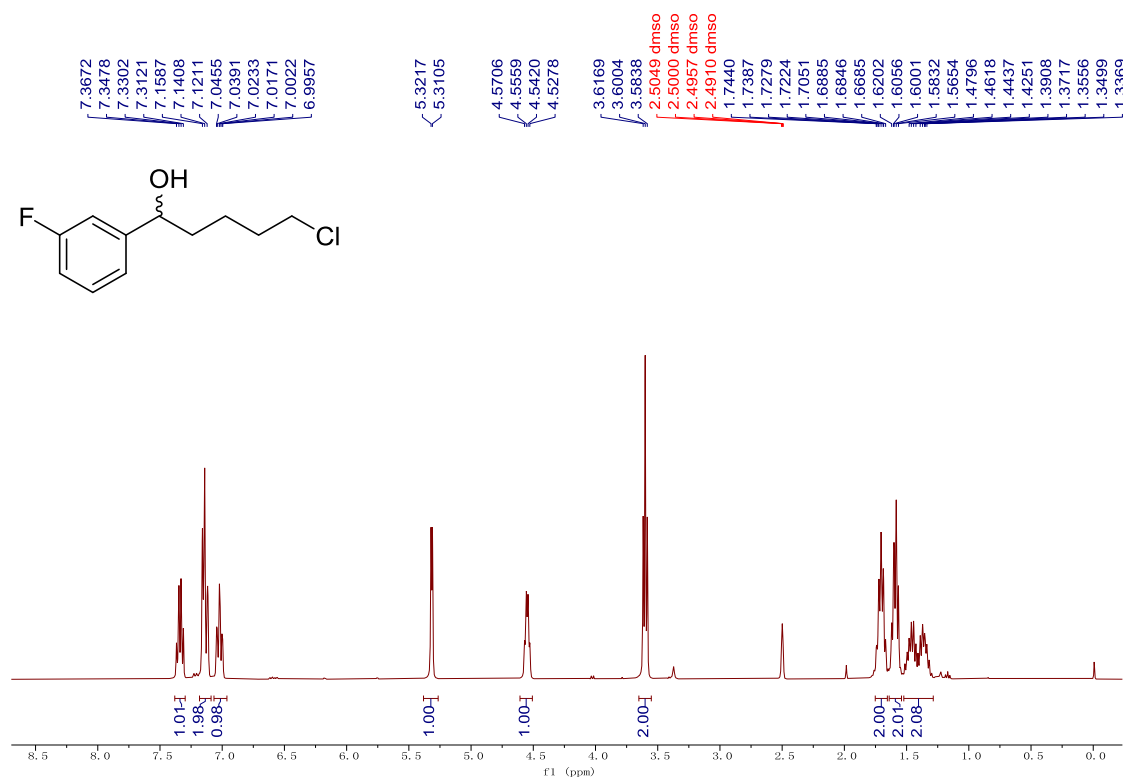
(Supplementary Figures 10–91)



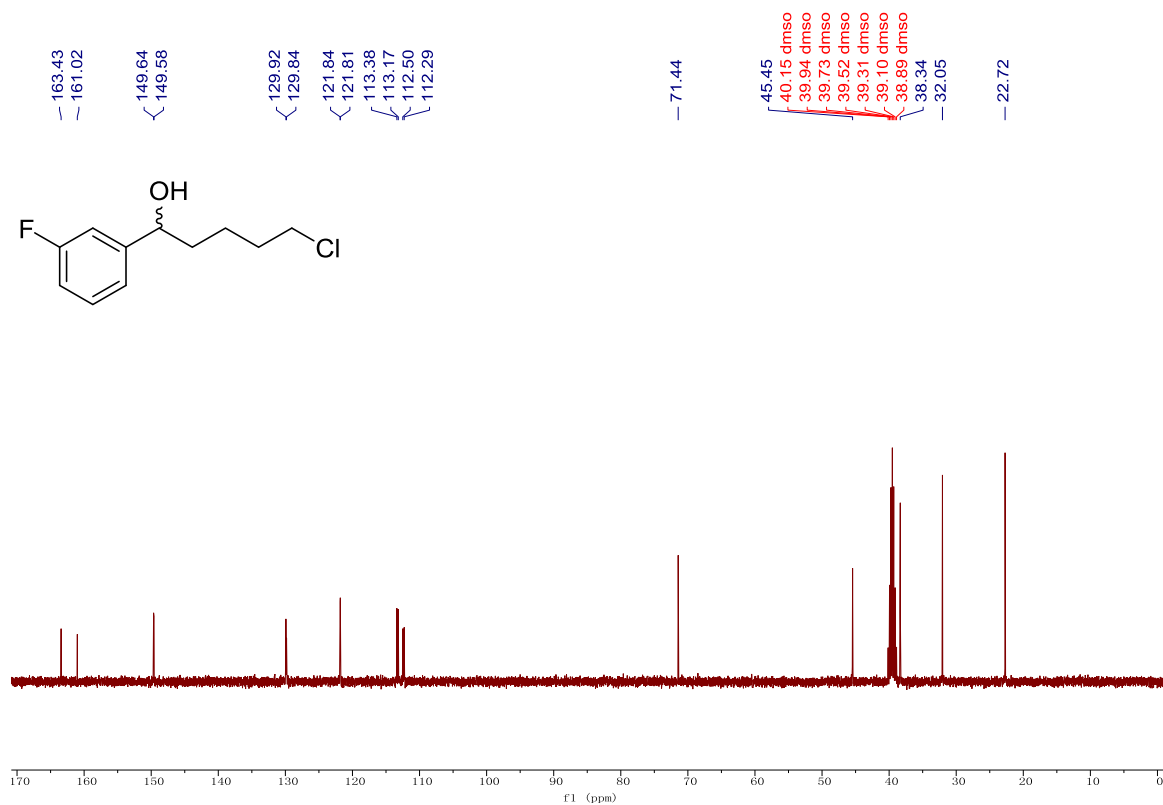
Supplementary Fig. 10 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of *(rac)*-1a.



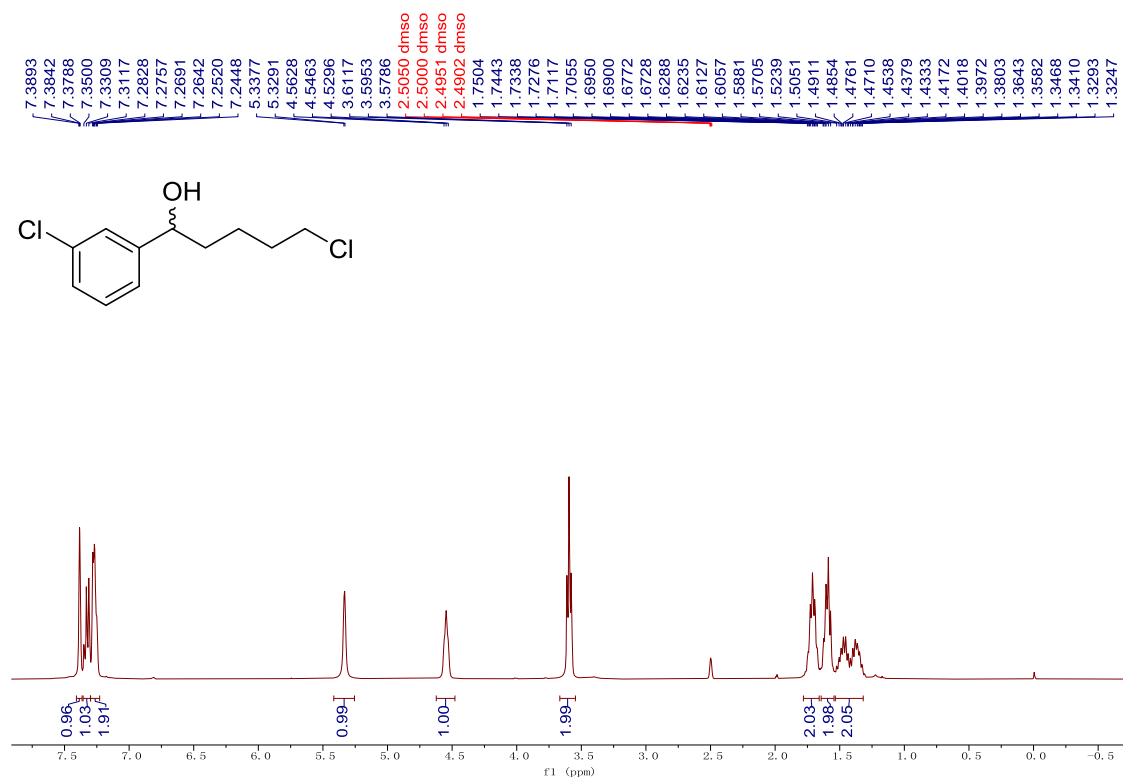
Supplementary Fig. 11 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of *(rac)*-1a.



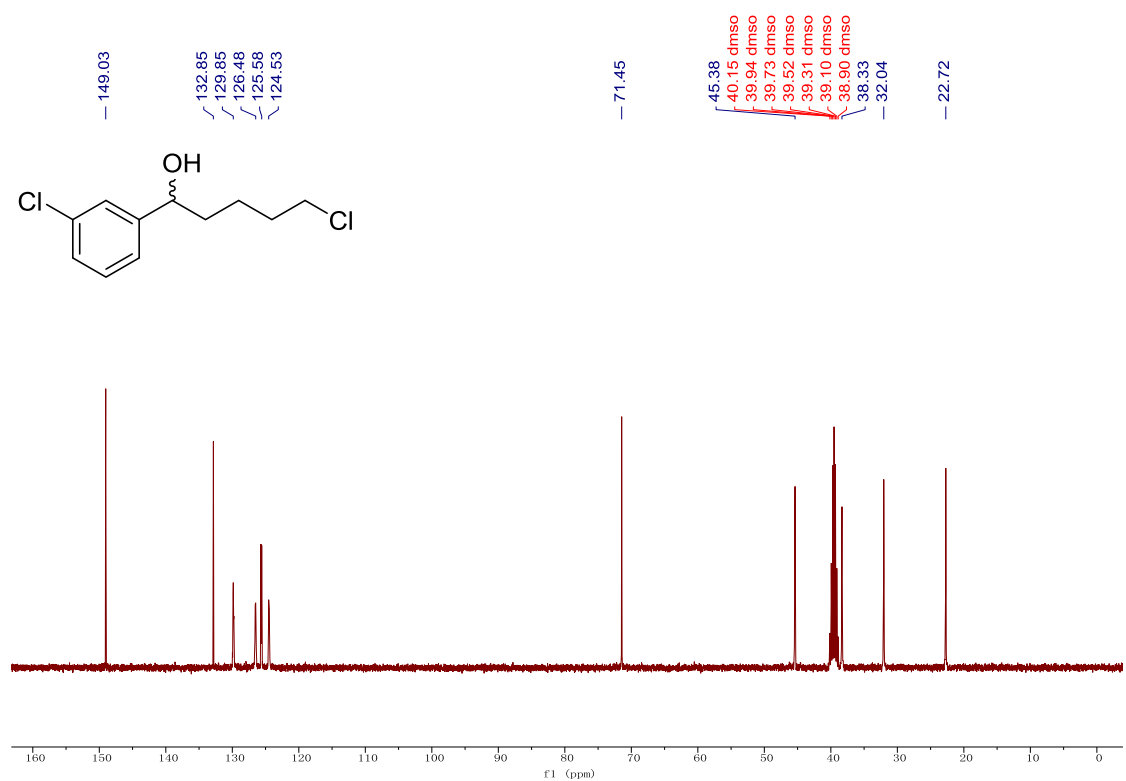
Supplementary Fig. 12 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (*rac*)-**2a**.



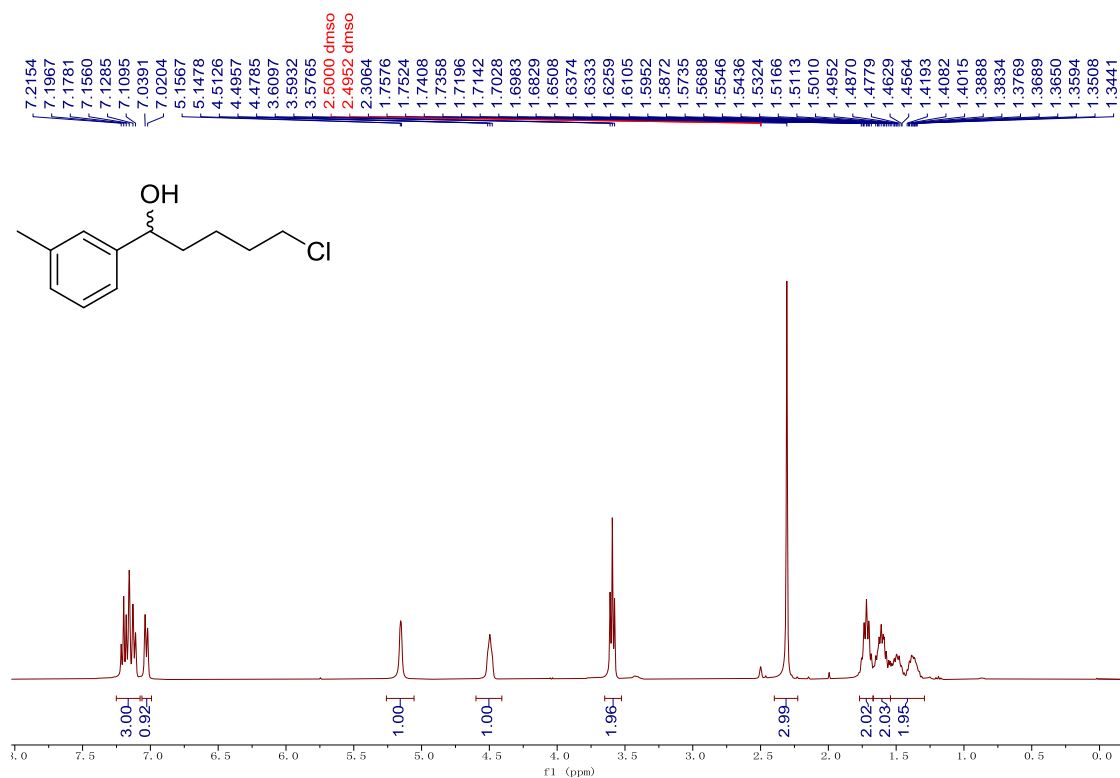
Supplementary Fig. 13 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (*rac*)-**2a**.



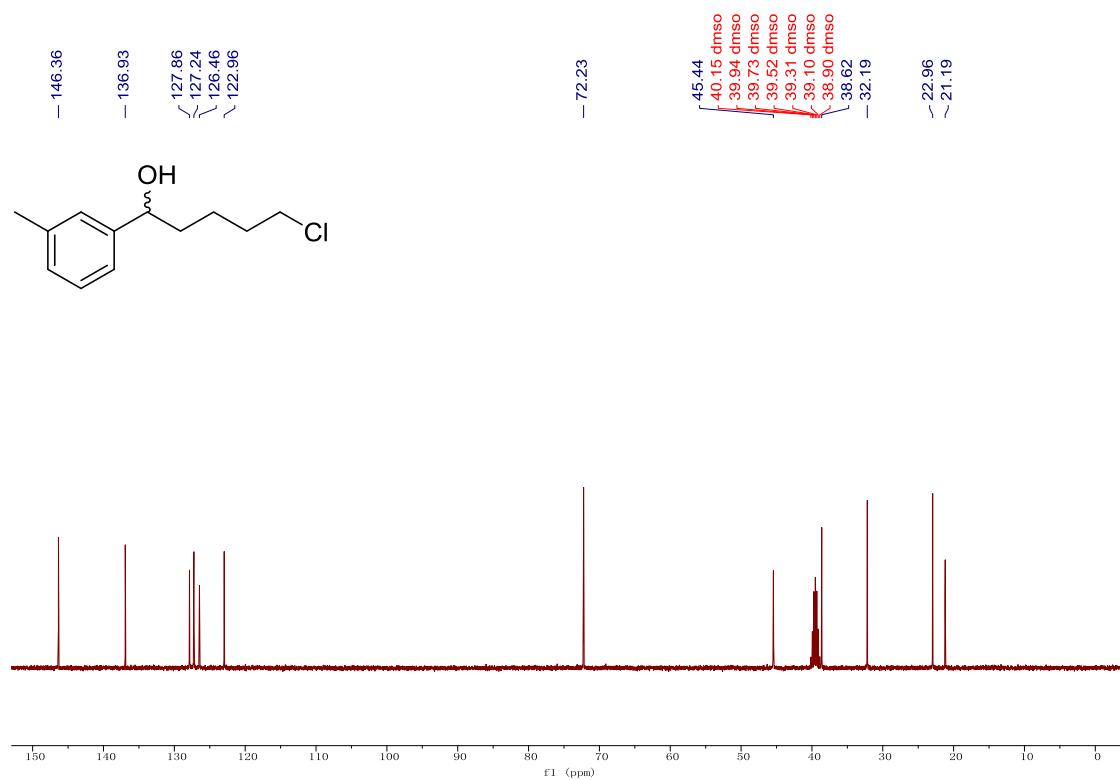
Supplementary Fig. 14 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-3a.



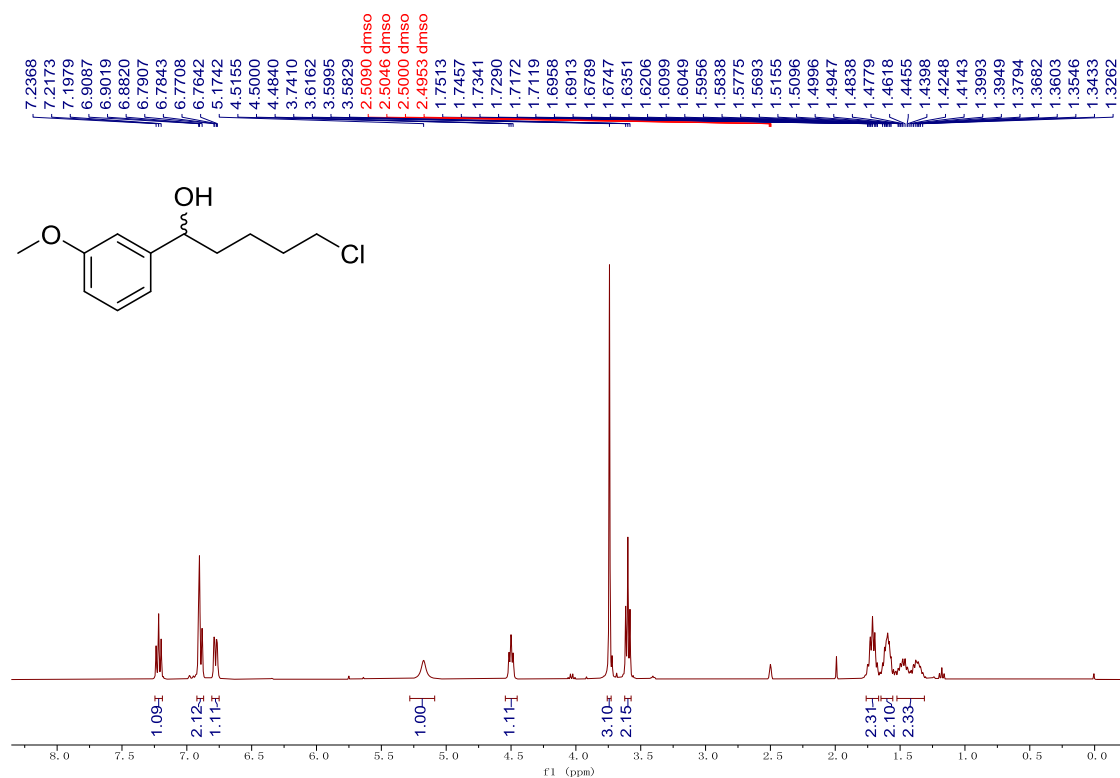
Supplementary Fig. 15 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-3a.



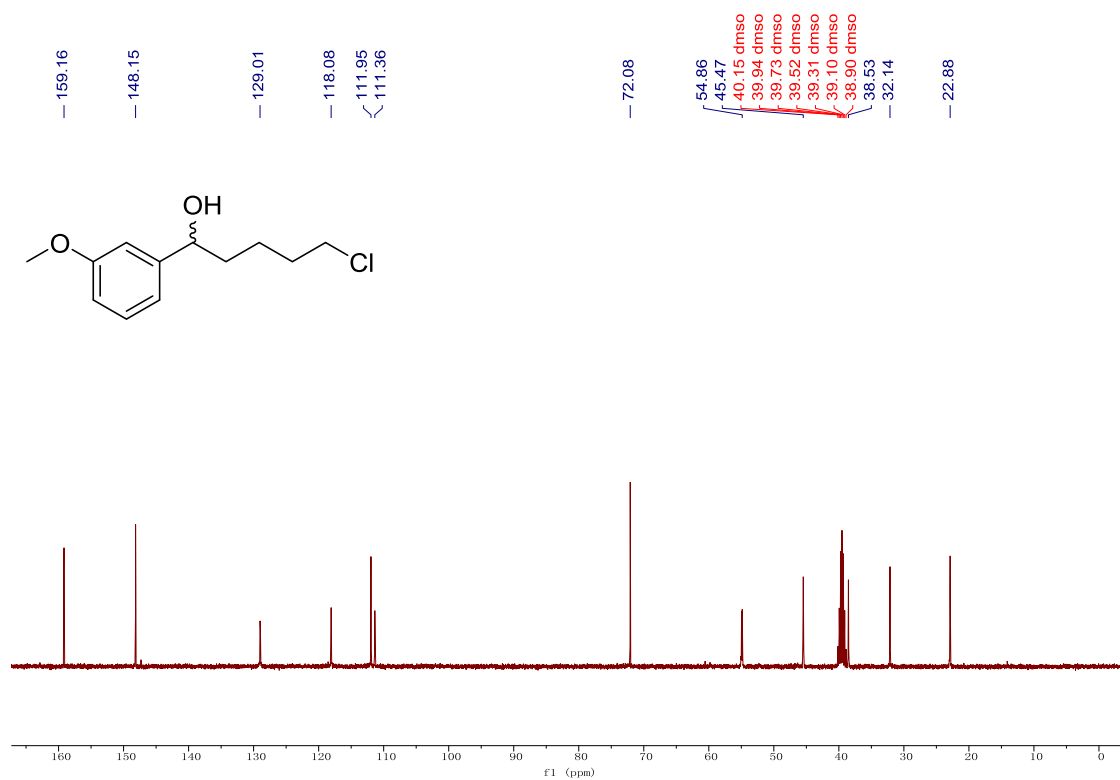
Supplementary Fig. 16 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-4a.



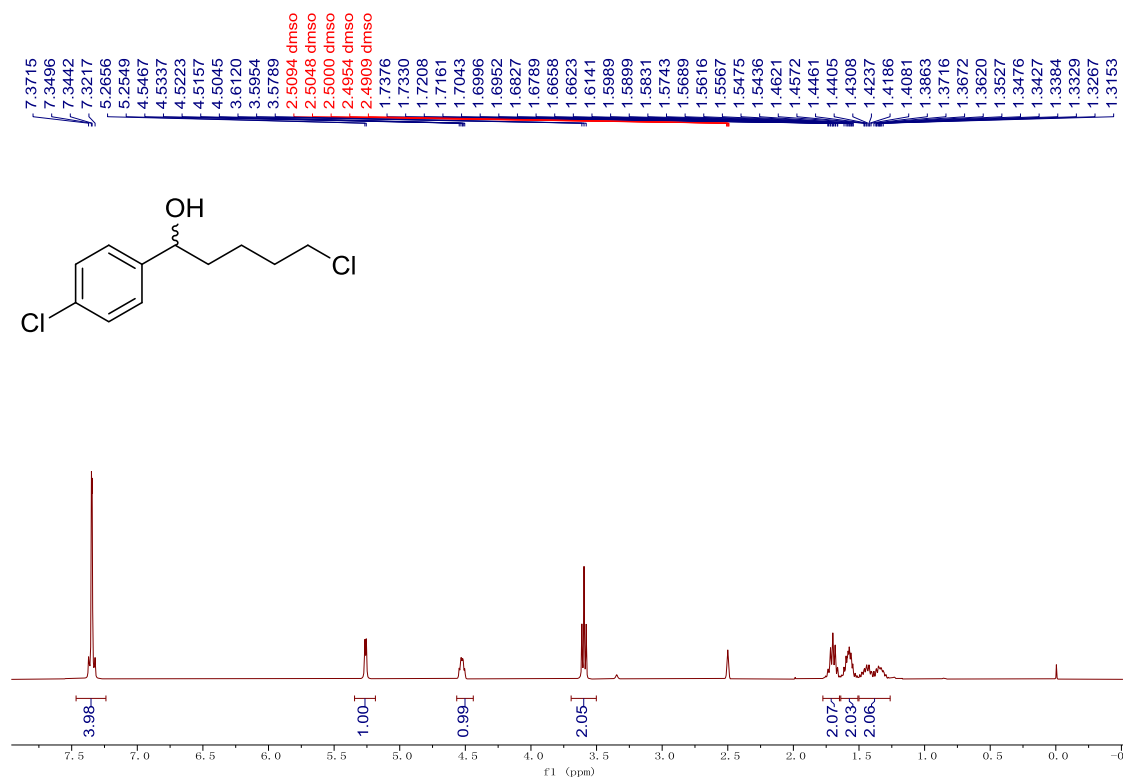
Supplementary Fig. 17 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-4a.



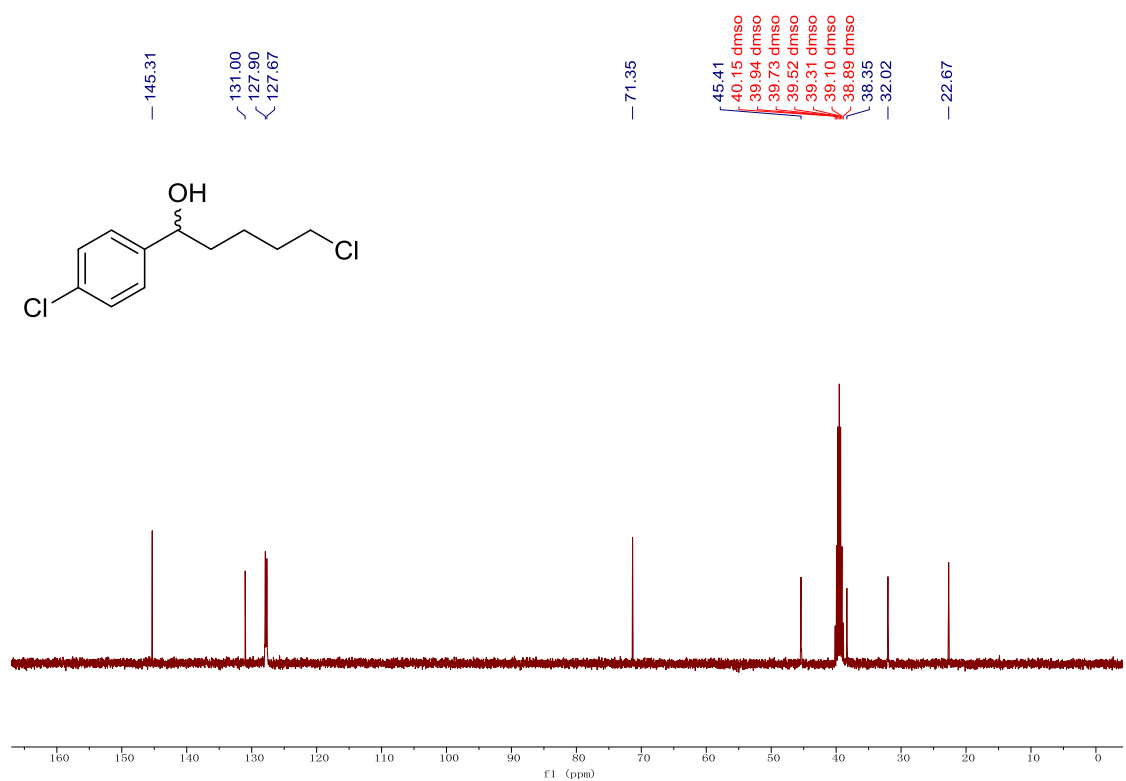
Supplementary Fig. 18 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-5a.



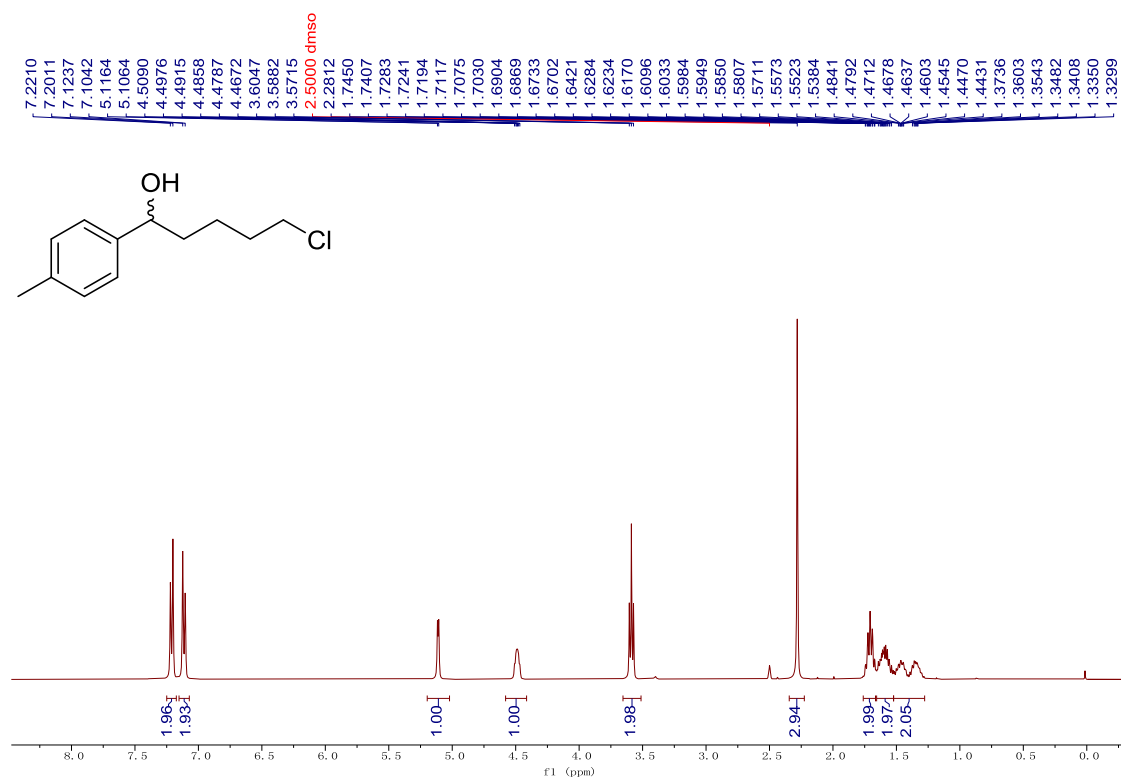
Supplementary Fig. 19 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-5a.



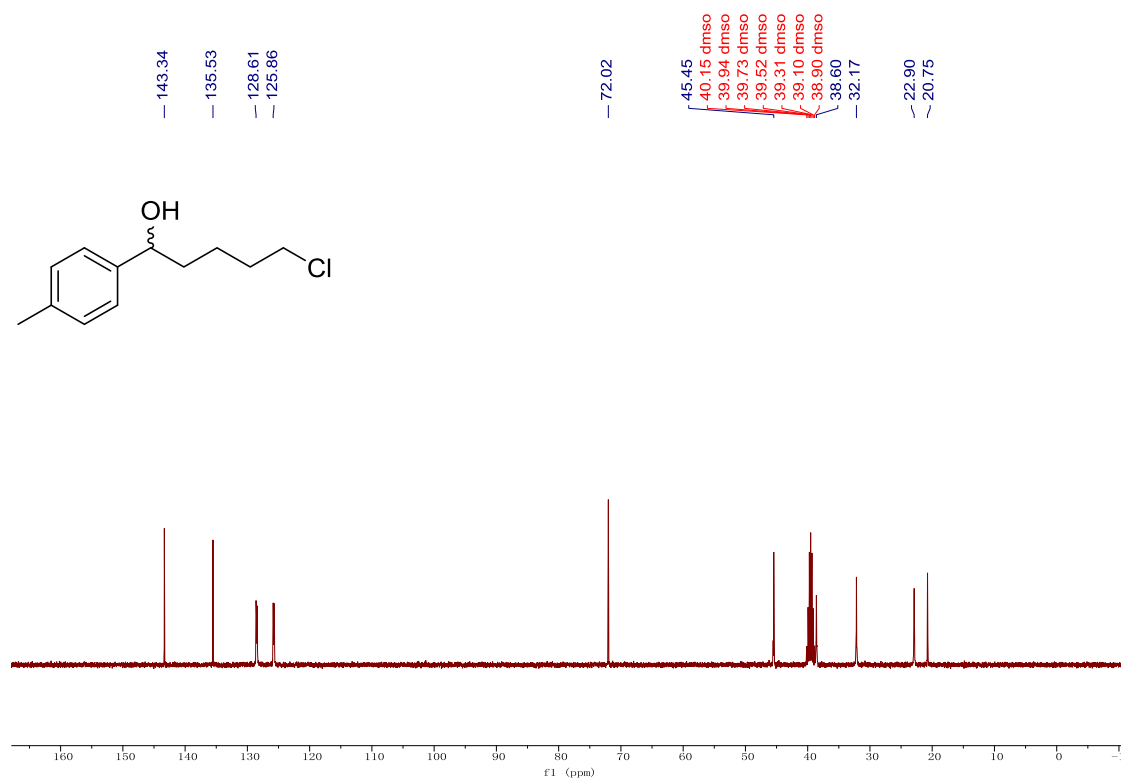
Supplementary Fig. 20 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-6a.



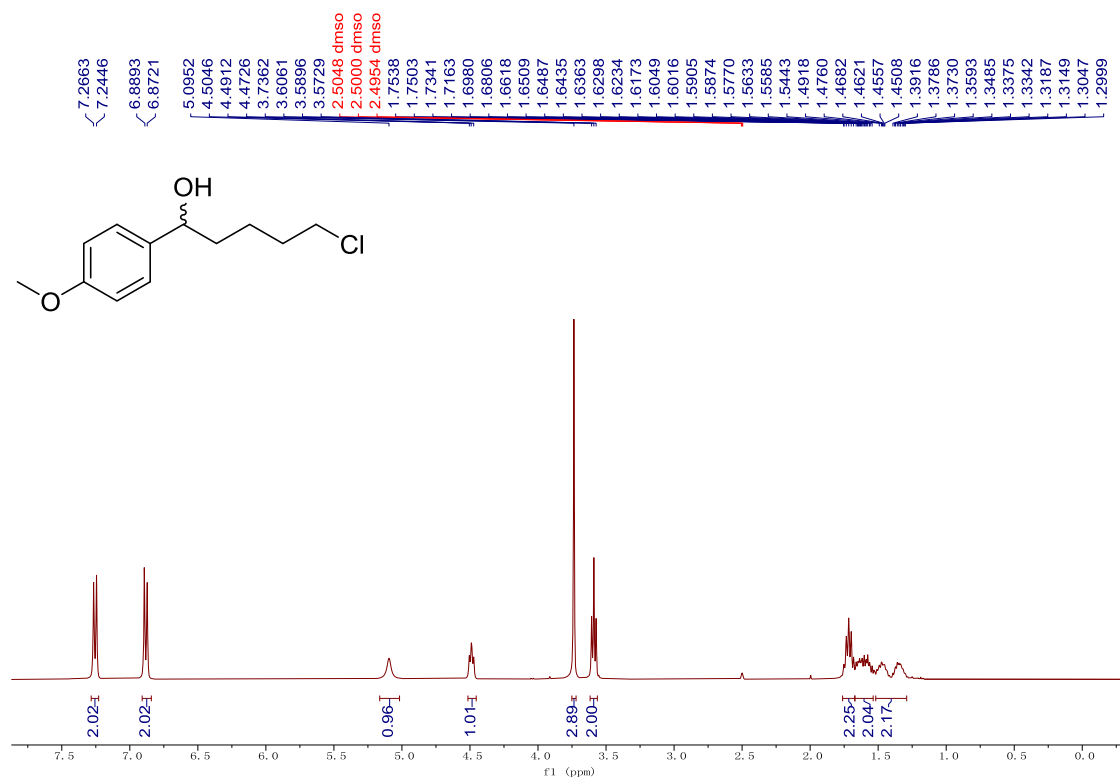
Supplementary Fig. 21 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-6a.



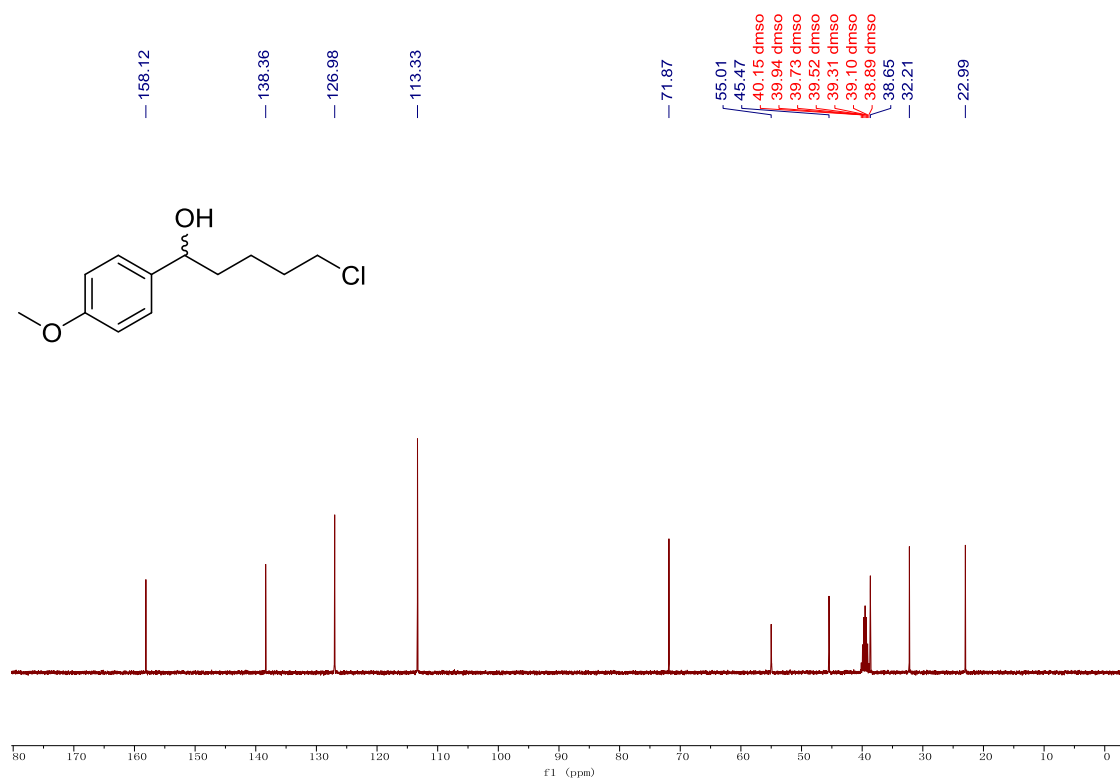
Supplementary Fig. 22 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-7a.



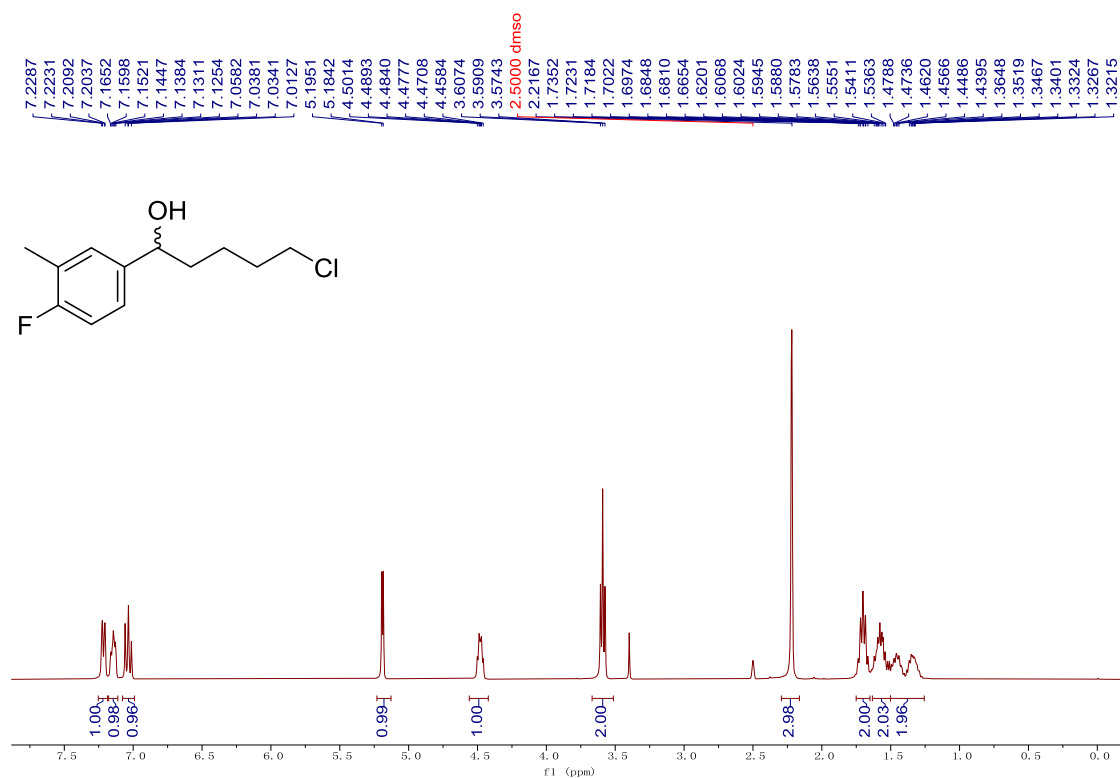
Supplementary Fig. 23 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-7a.



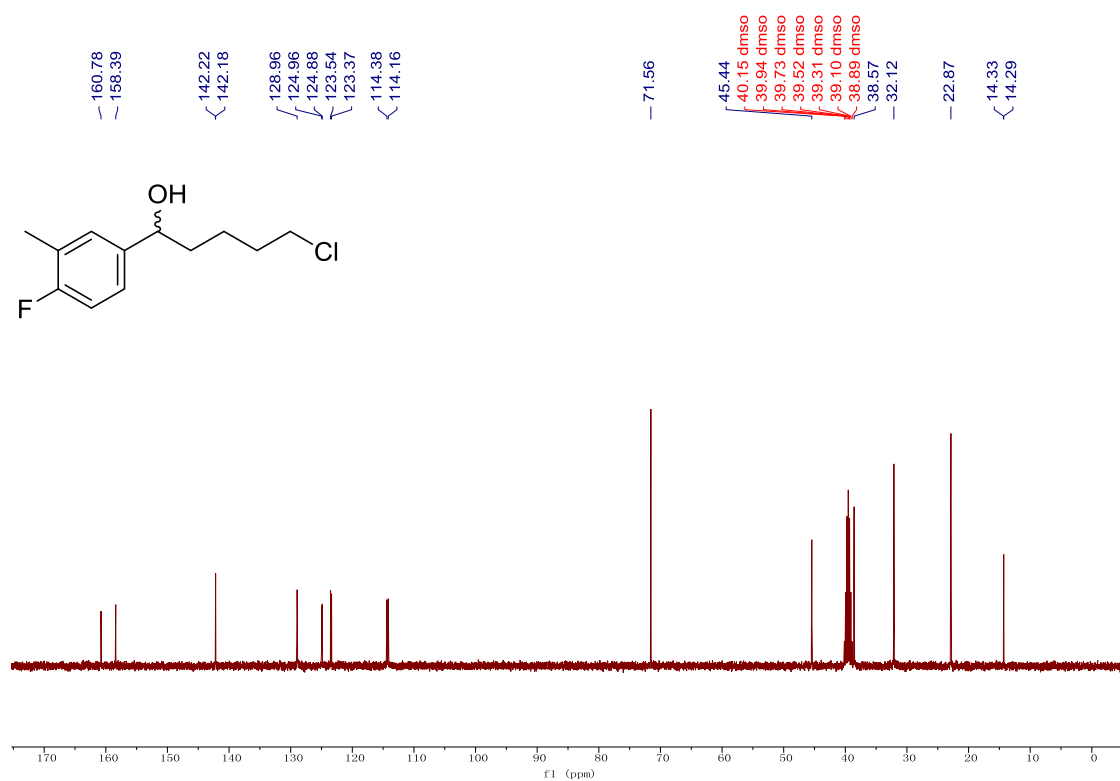
Supplementary Fig. 24 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of *(rac)*-**8a**.



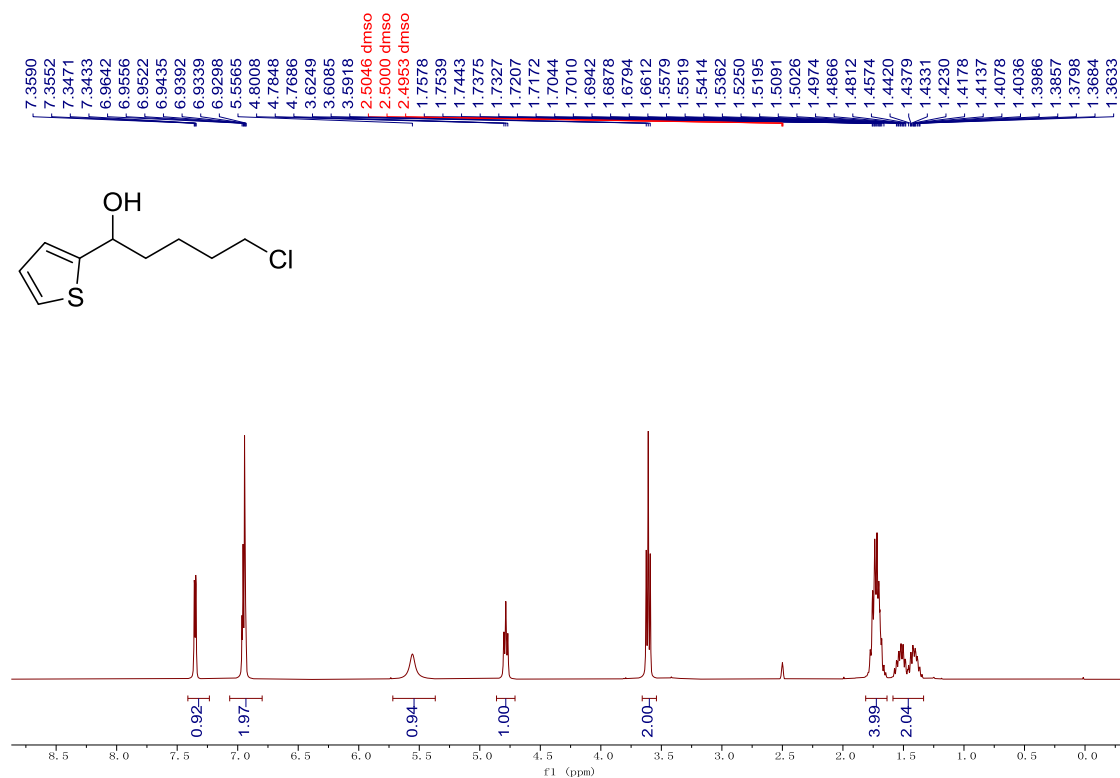
Supplementary Fig. 25 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of *(rac)*-**8a**.



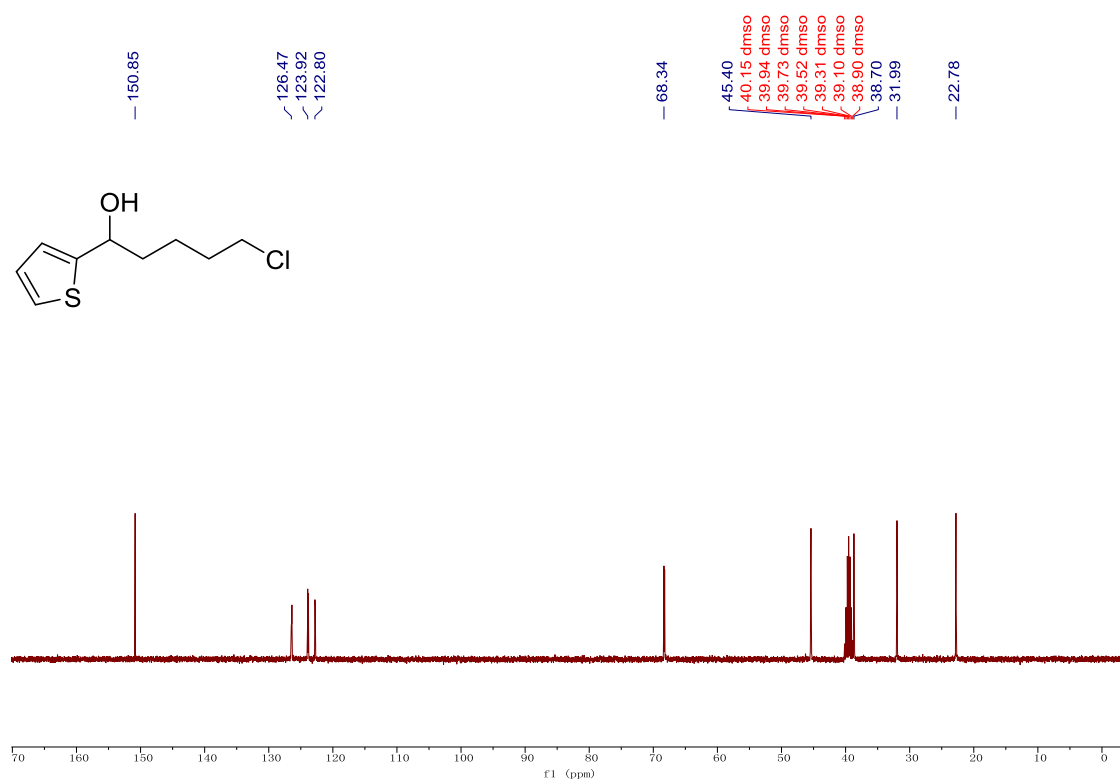
Supplementary Fig. 26 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-9a.



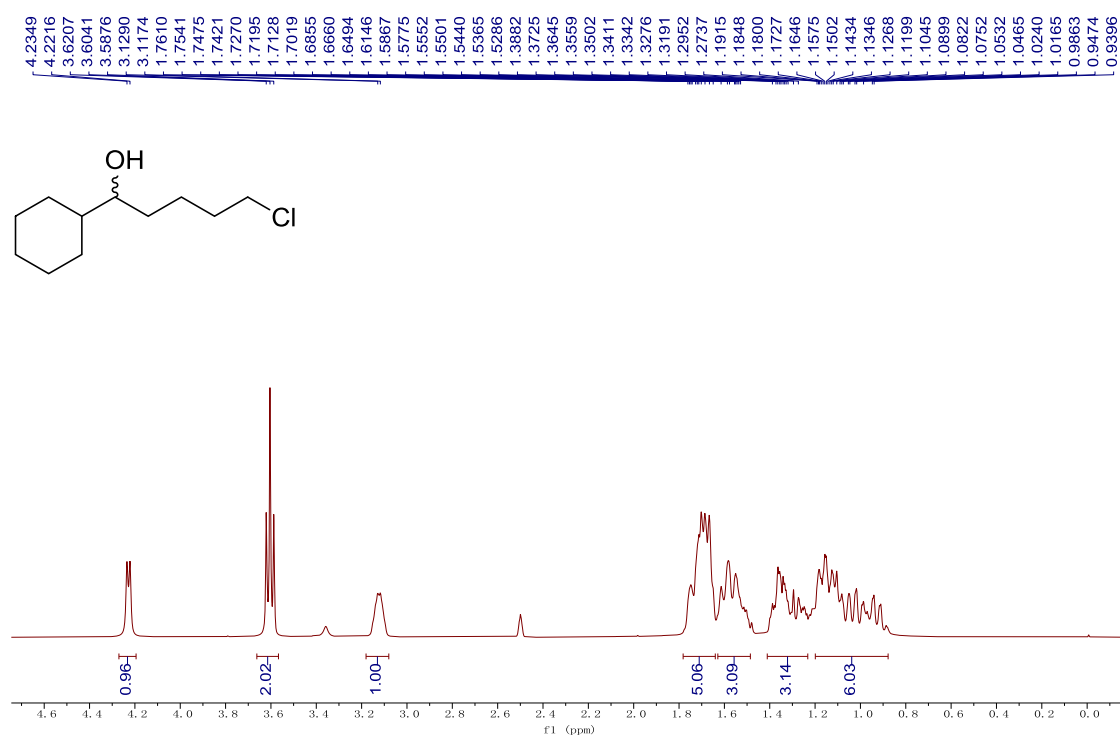
Supplementary Fig. 27 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-9a.



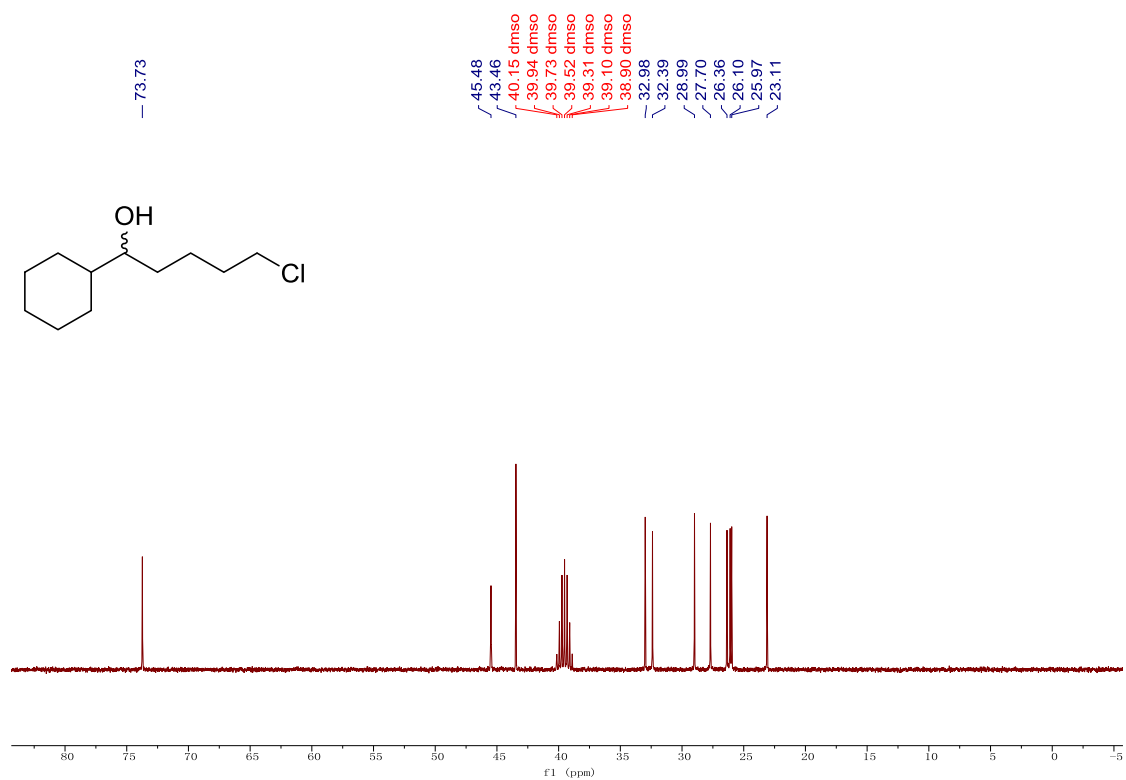
Supplementary Fig. 28 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (*rac*)-**10a**.



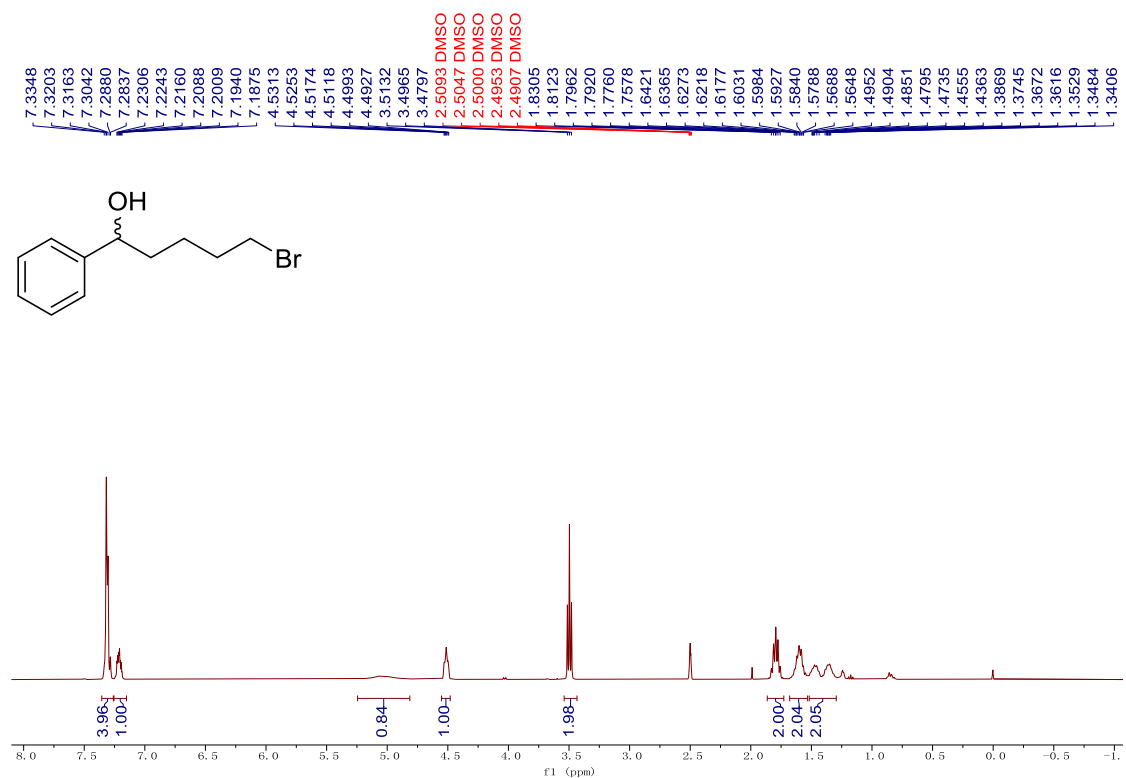
Supplementary Fig. 29 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (*rac*)-**10a**.



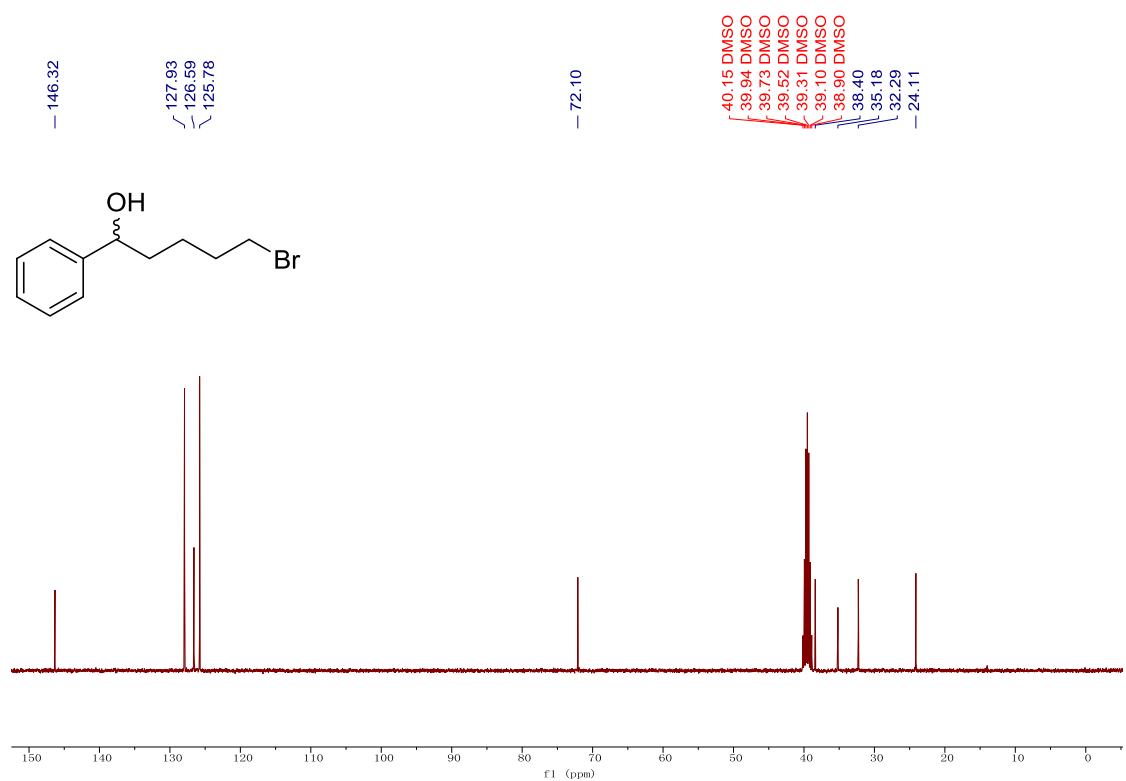
Supplementary Fig. 30 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-11a.



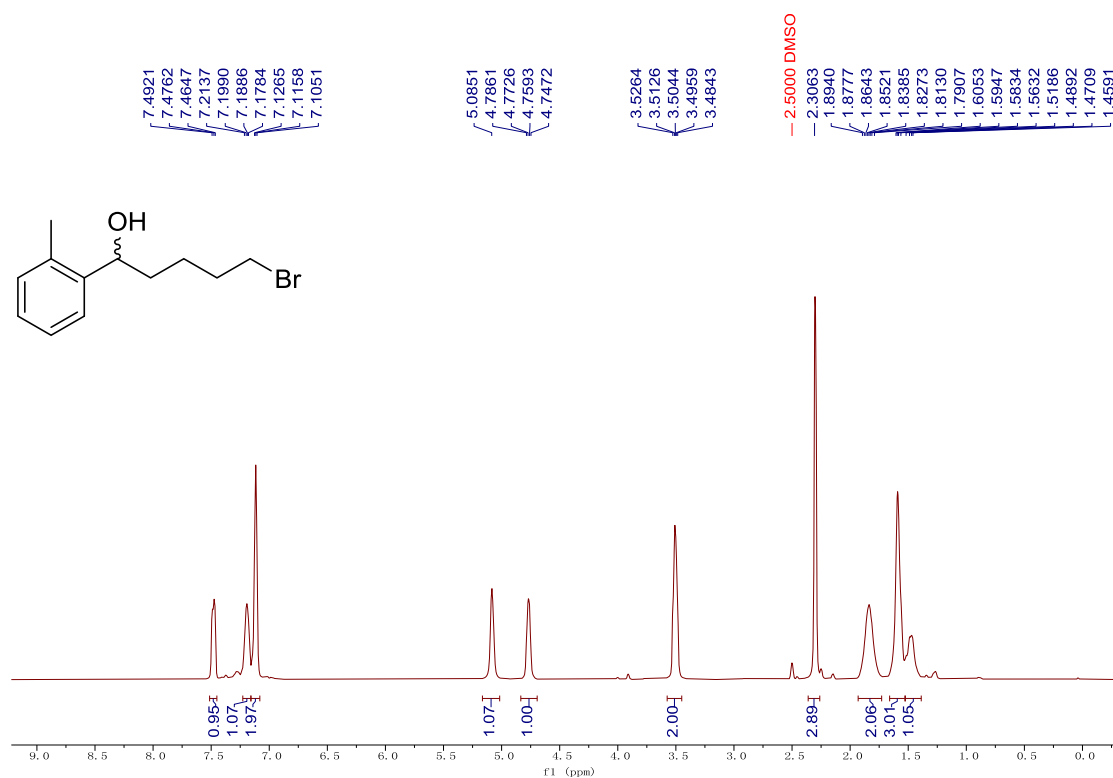
Supplementary Fig. 31 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-11a.



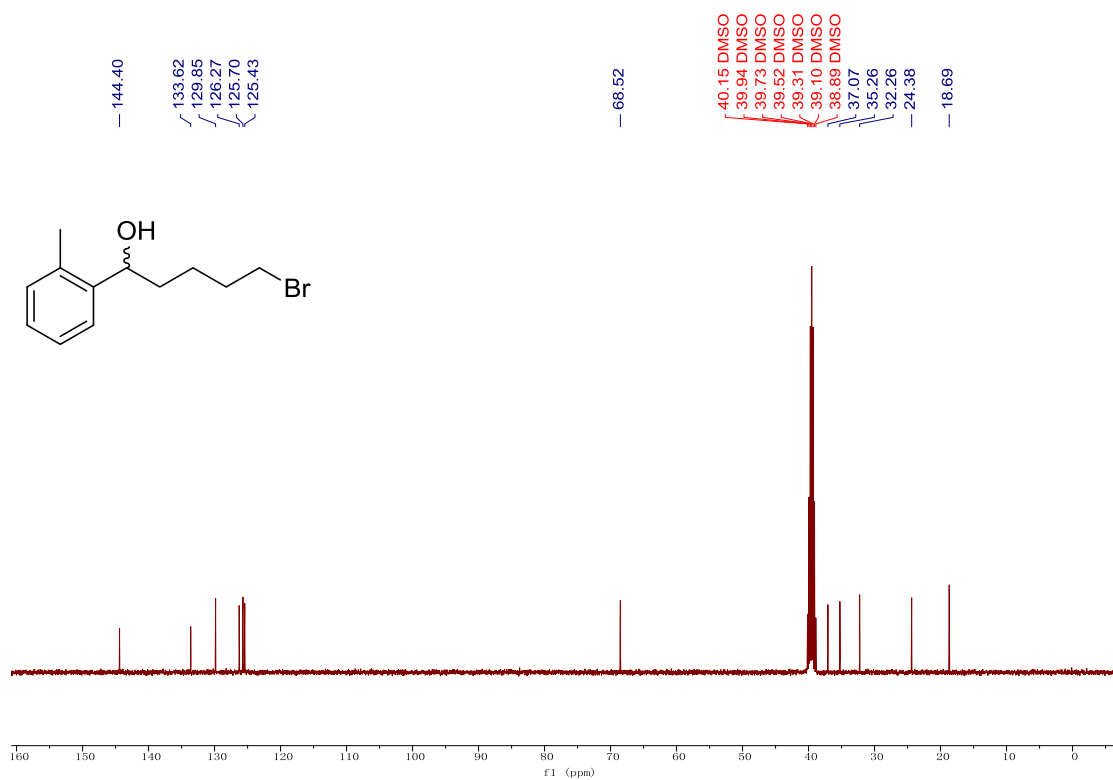
Supplementary Fig. 32 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-12a.



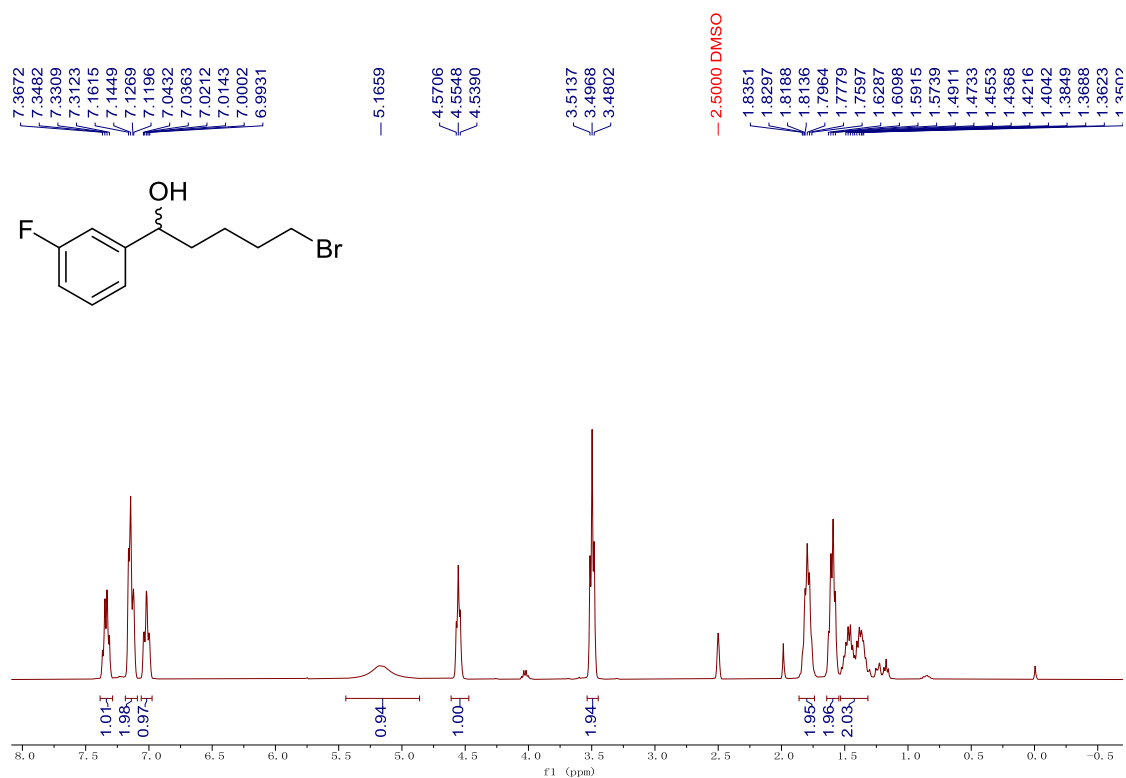
Supplementary Fig. 33 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-12a.



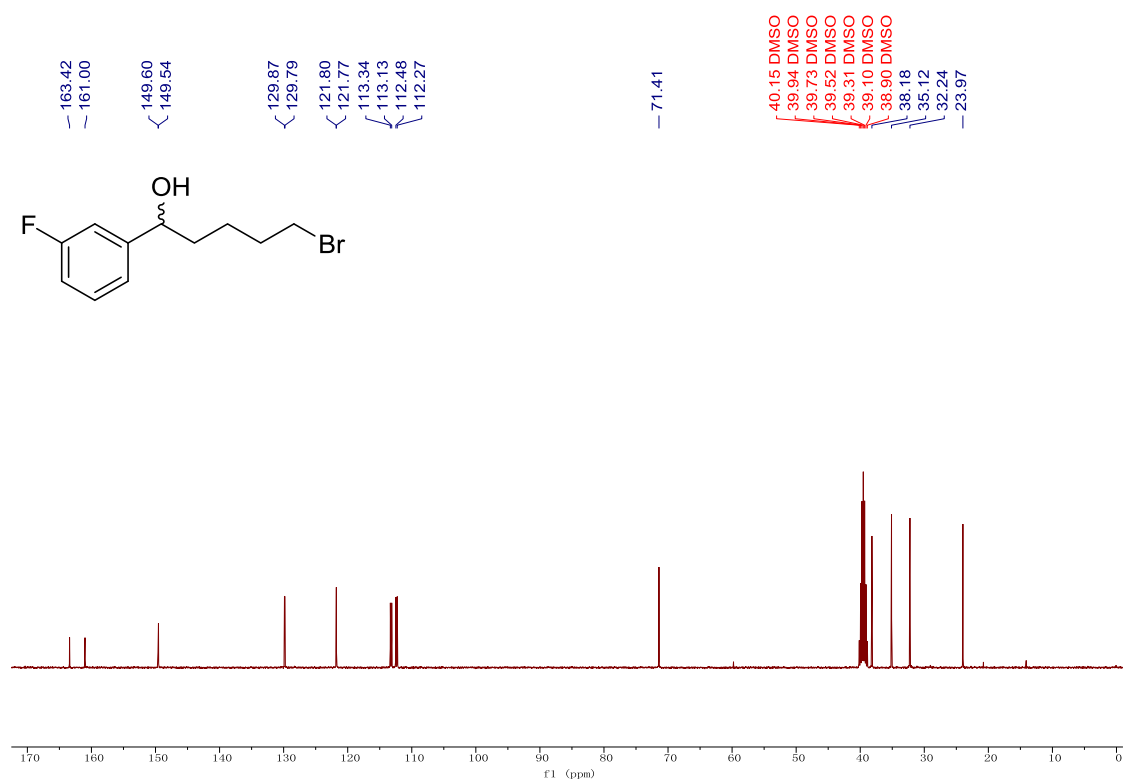
Supplementary Fig. 34 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (*rac*)-13a.



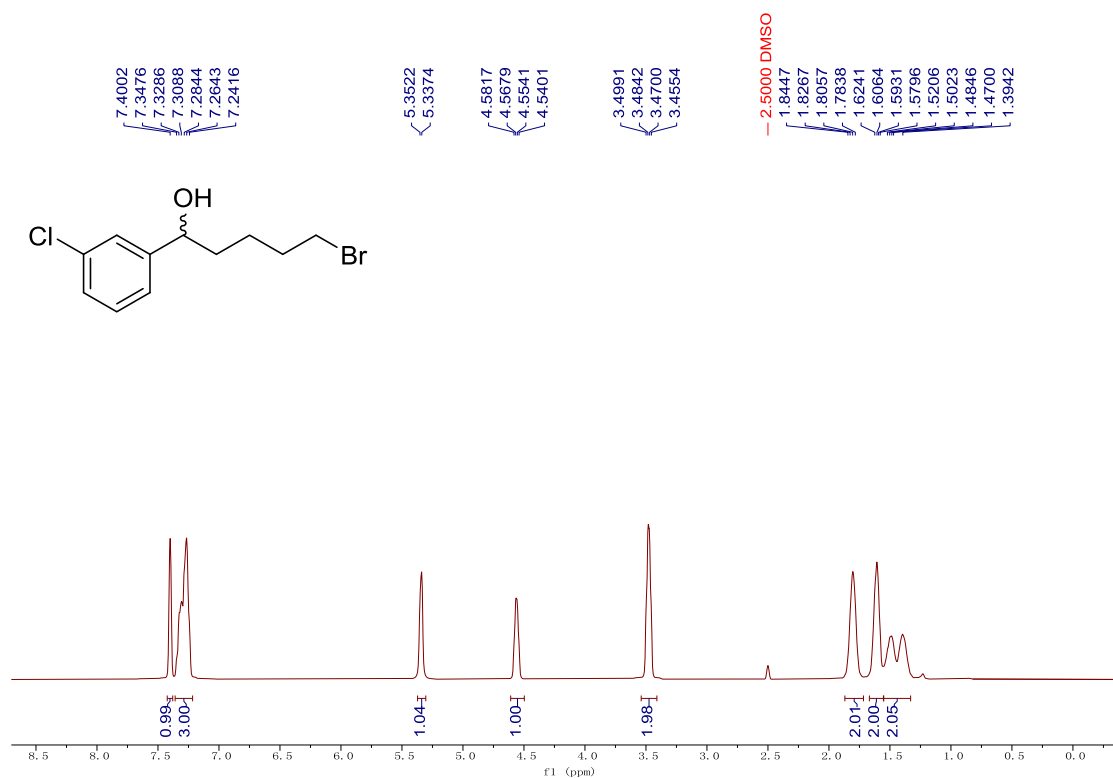
Supplementary Fig. 35 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (*rac*)-13a.



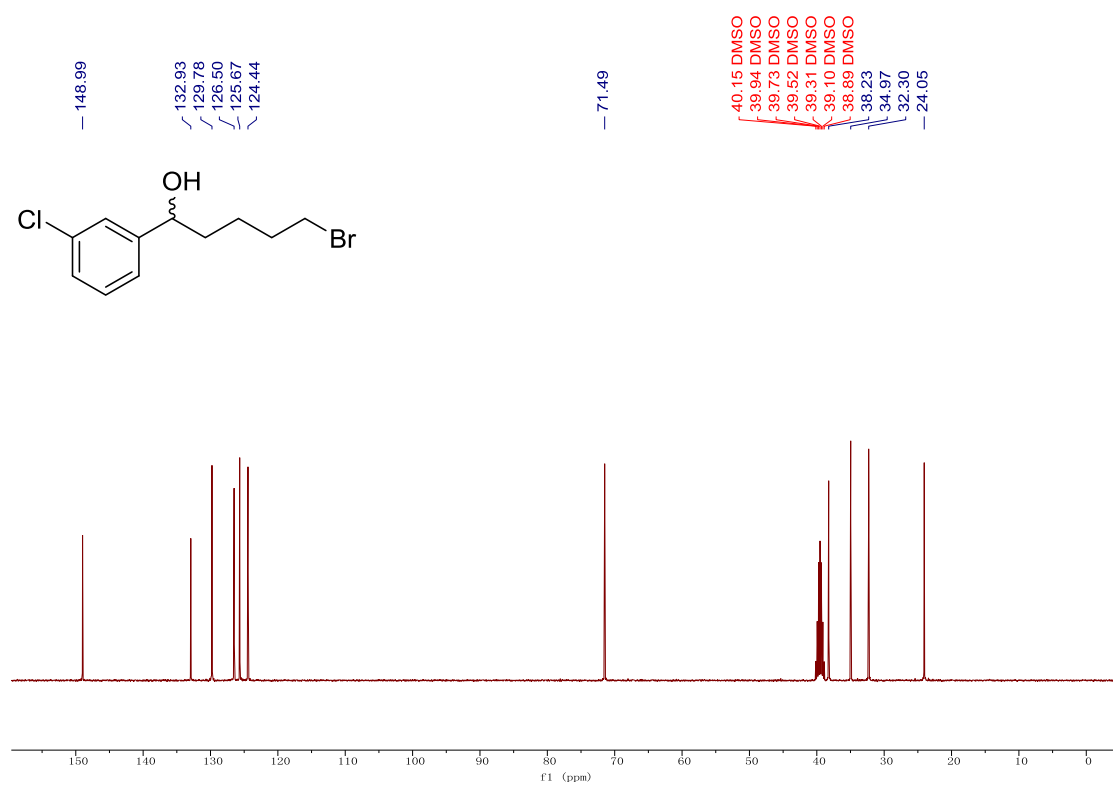
Supplementary Fig. 36 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-14a.



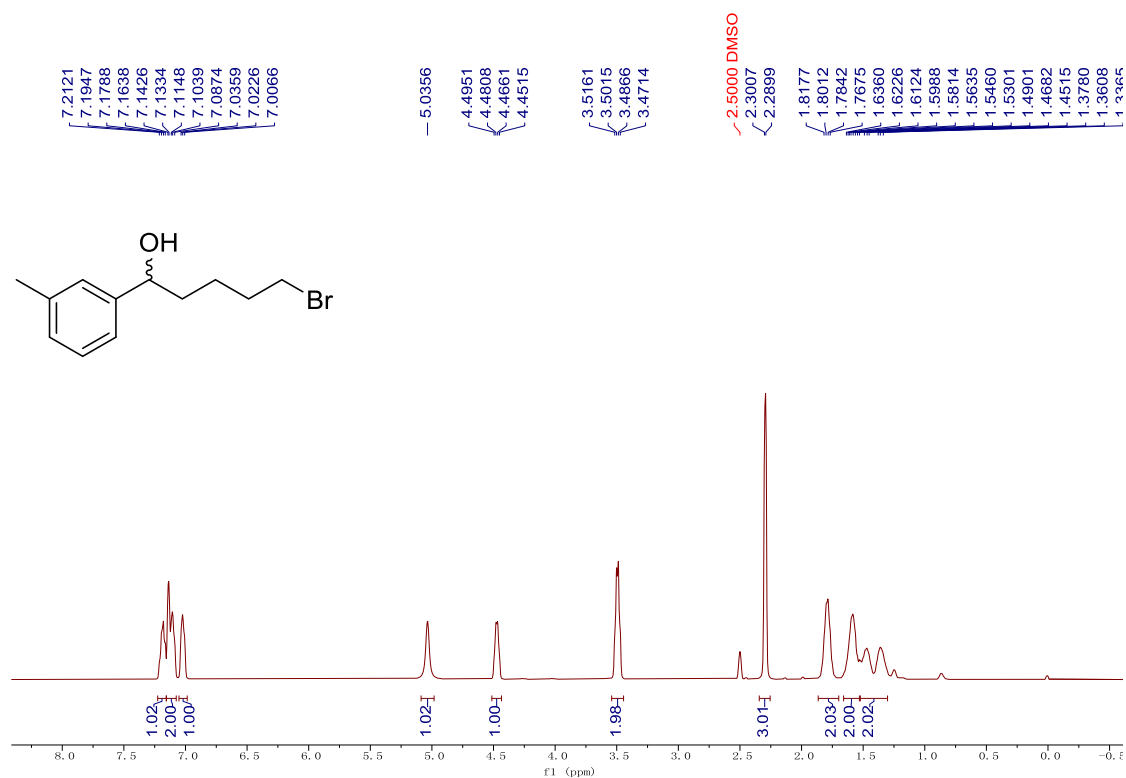
Supplementary Fig. 37 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-14a.



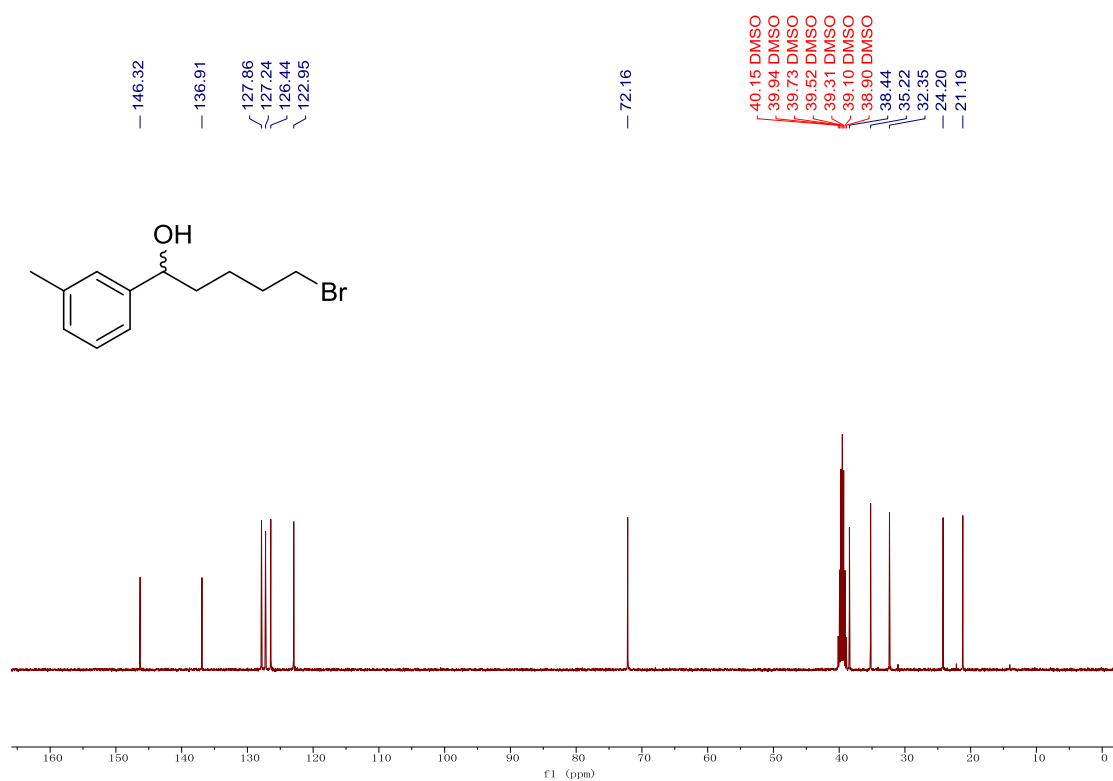
Supplementary Fig. 38 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-15a.



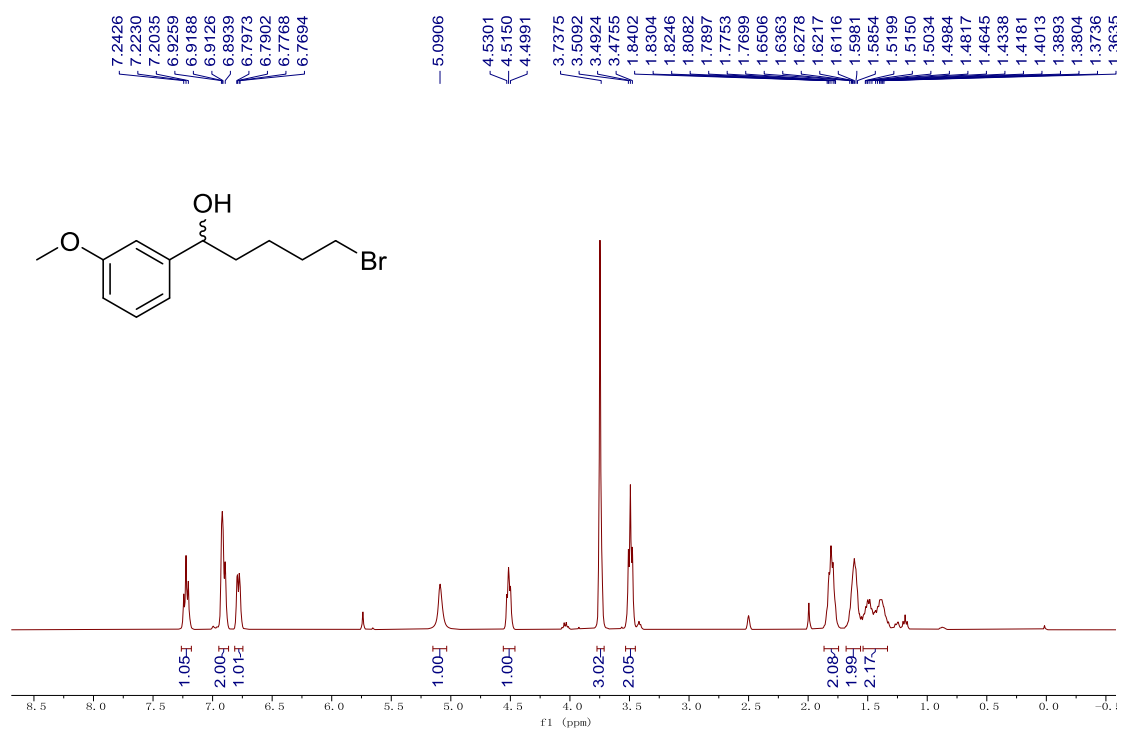
Supplementary Fig. 39 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-15a.



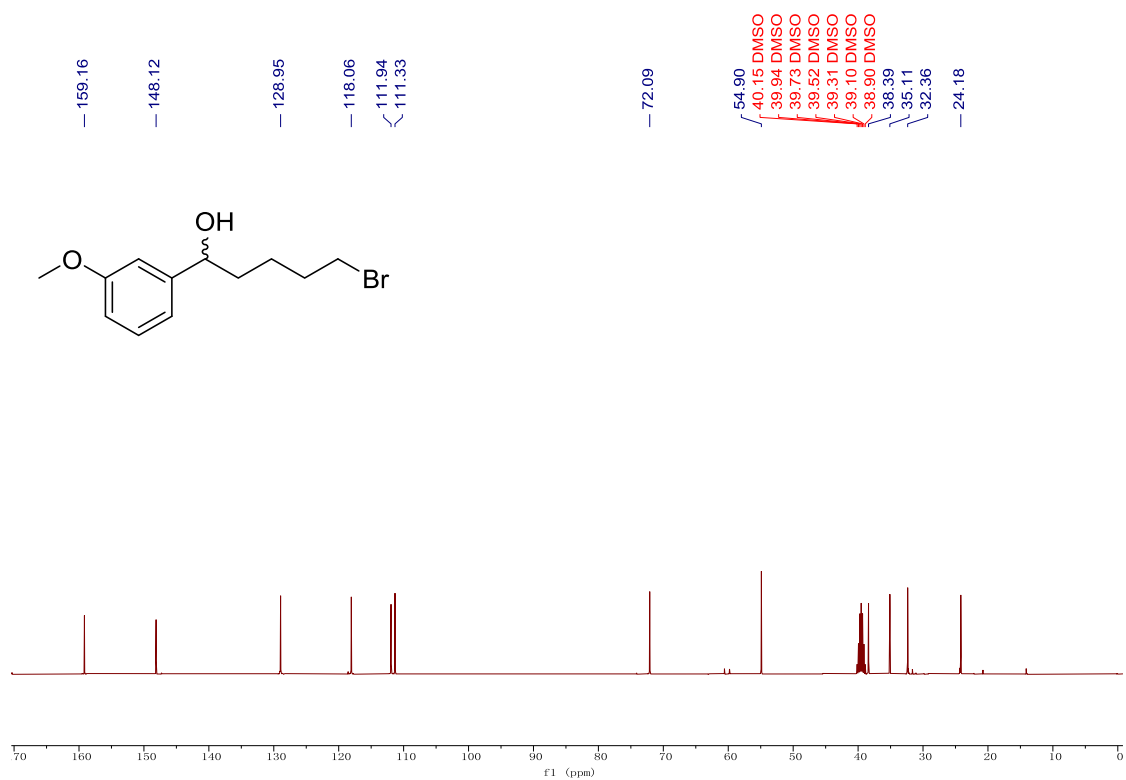
Supplementary Fig. 40 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-16a.



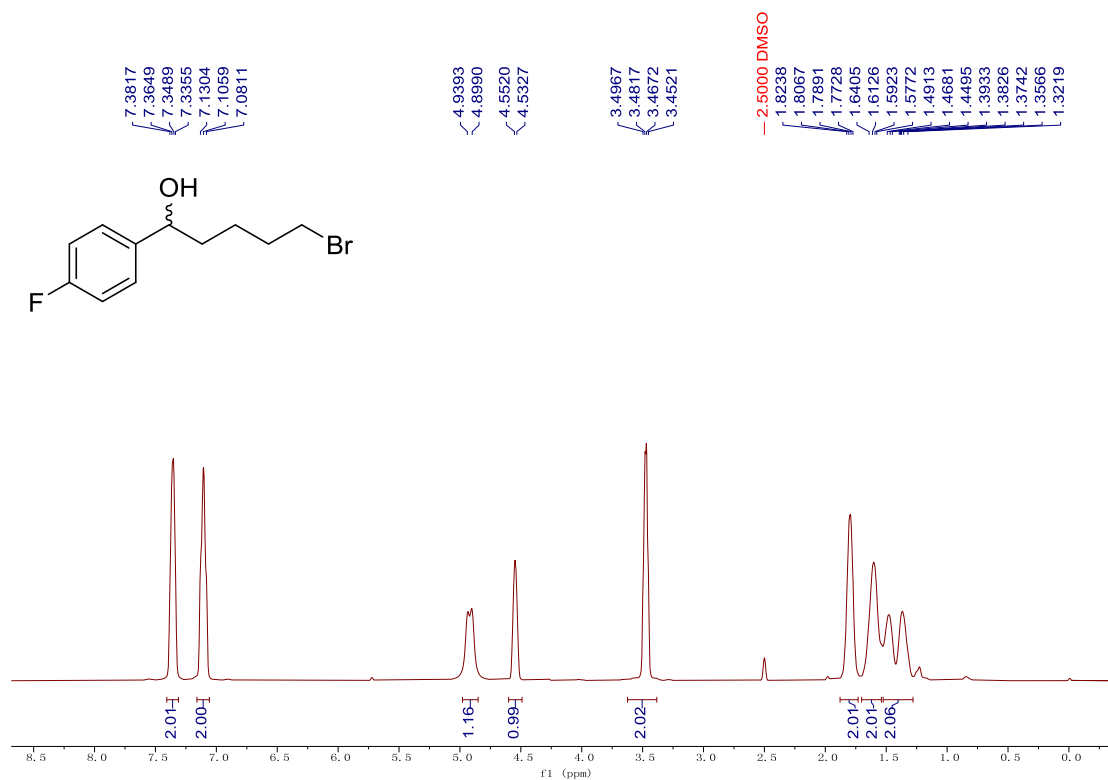
Supplementary Fig. 41 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-16a.



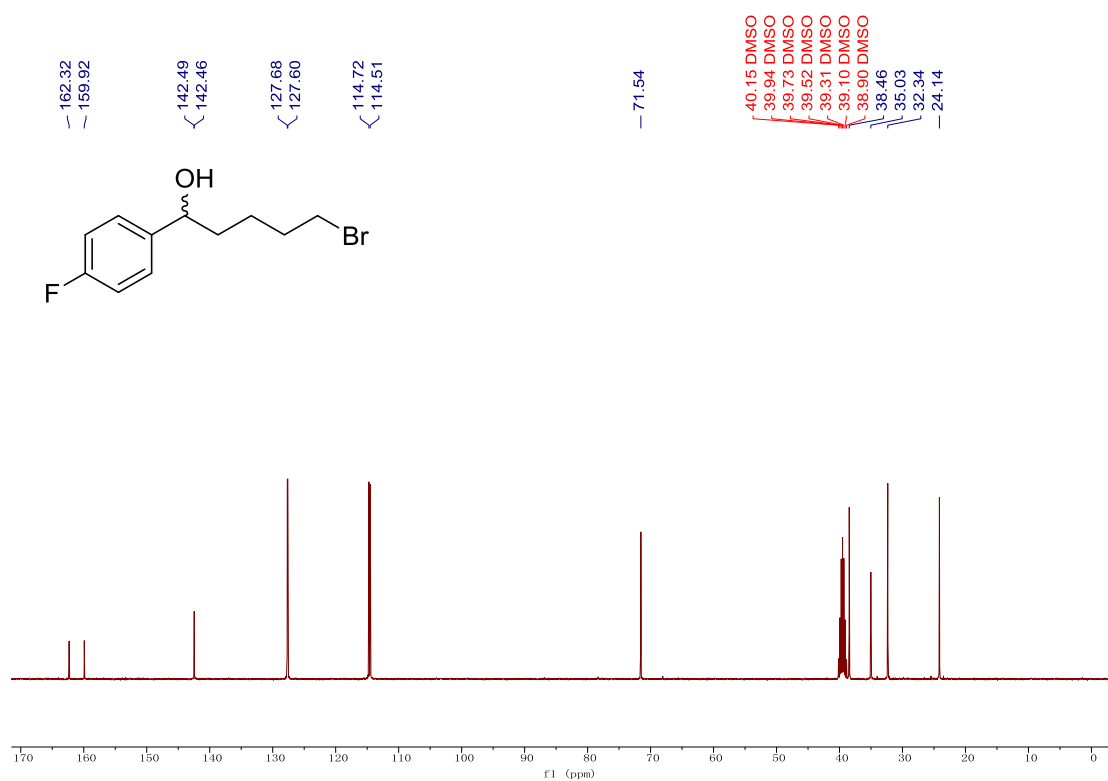
Supplementary Fig. 42 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-17a.



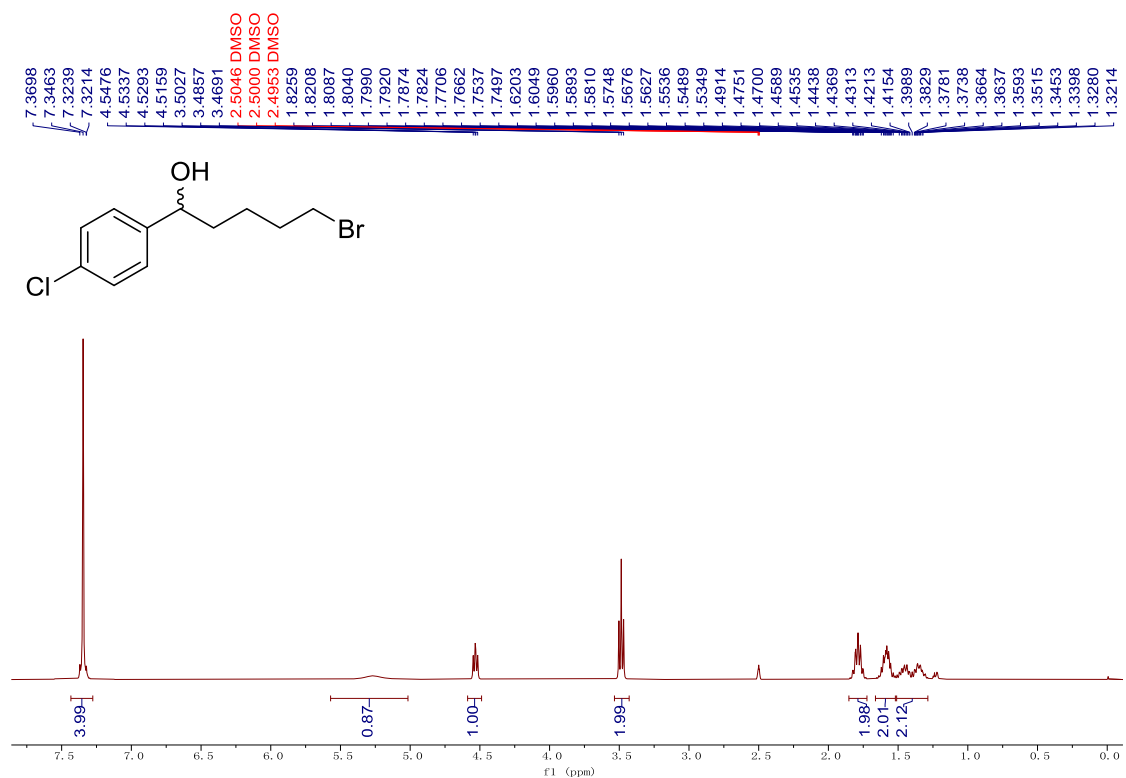
Supplementary Fig. 43 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-17a.



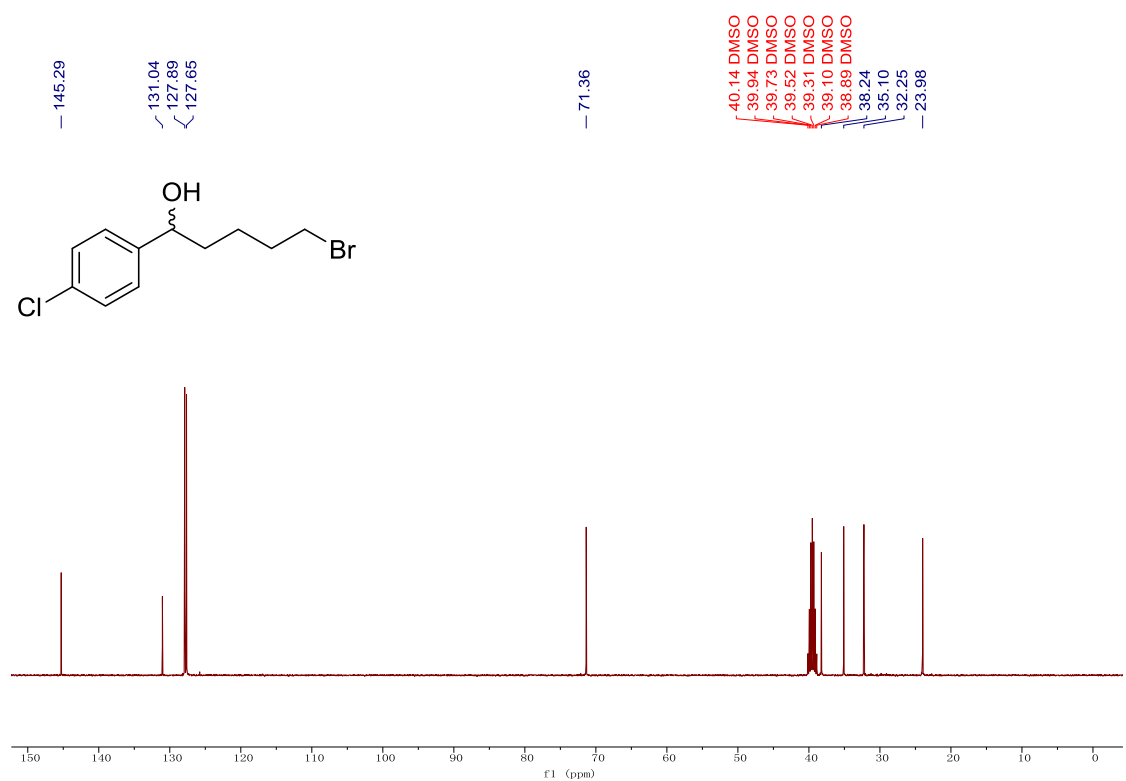
Supplementary Fig. 44 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of *(rac)*-**18a**.



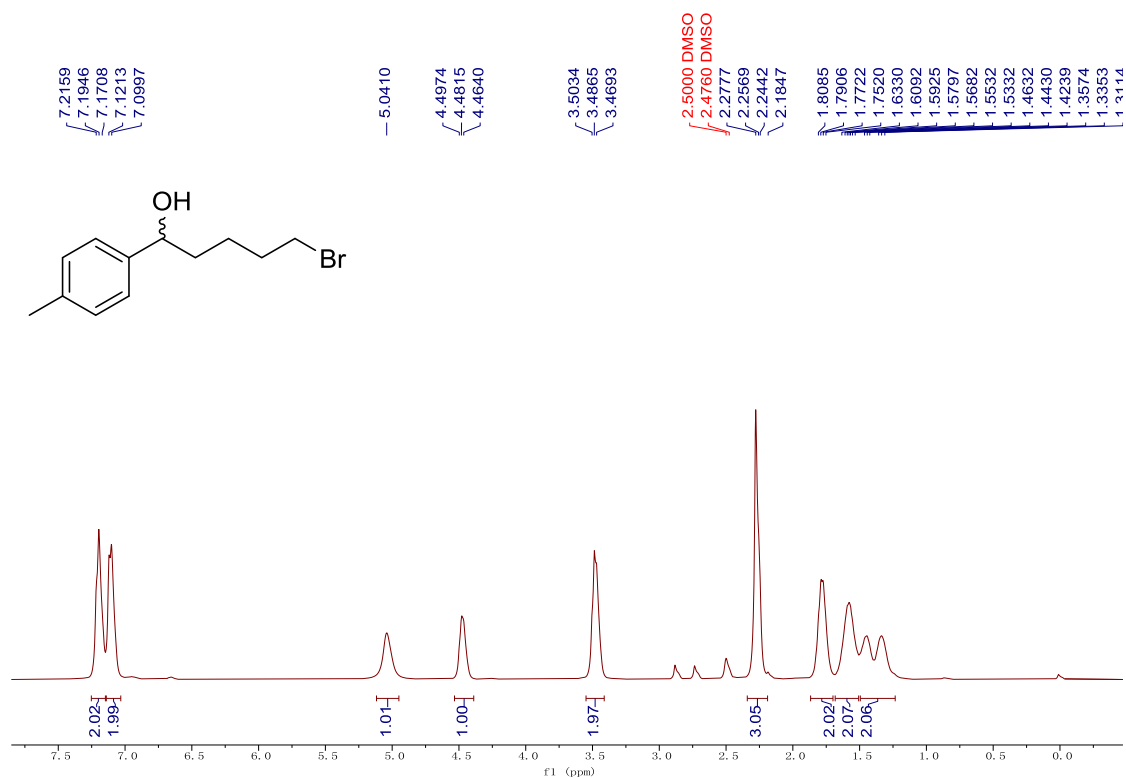
Supplementary Fig. 45 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of *(rac)*-**18a**.



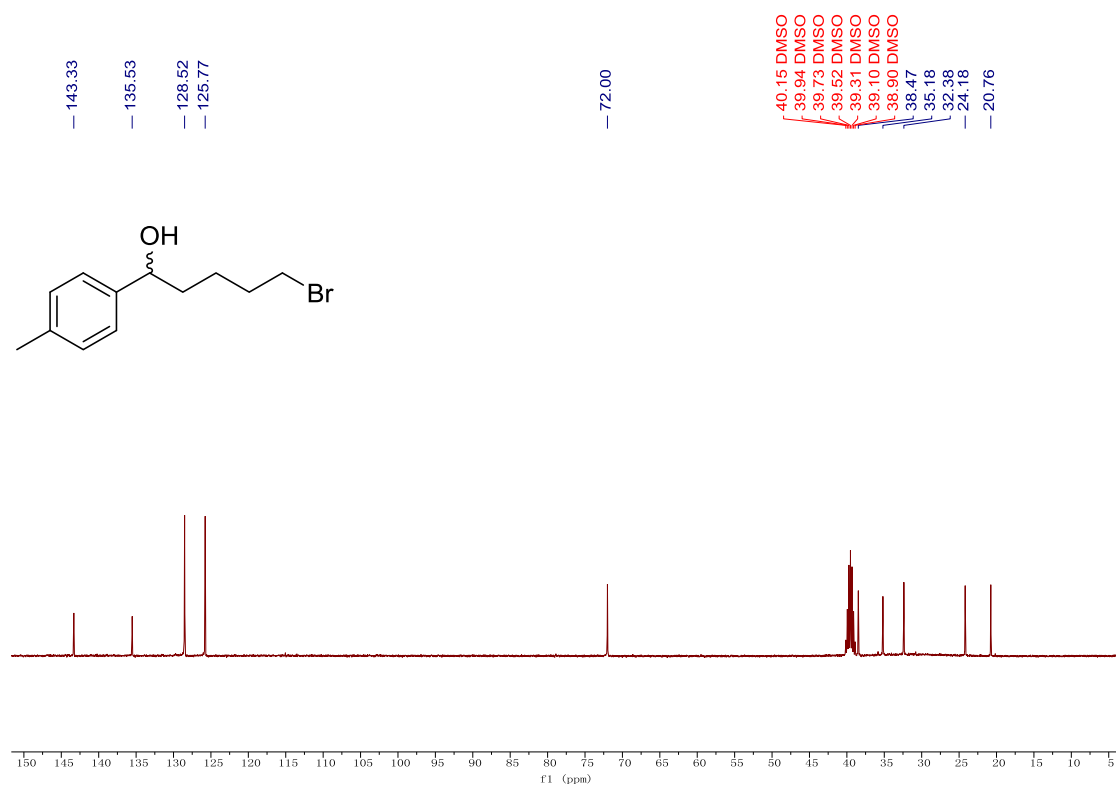
Supplementary Fig. 46 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-19a.



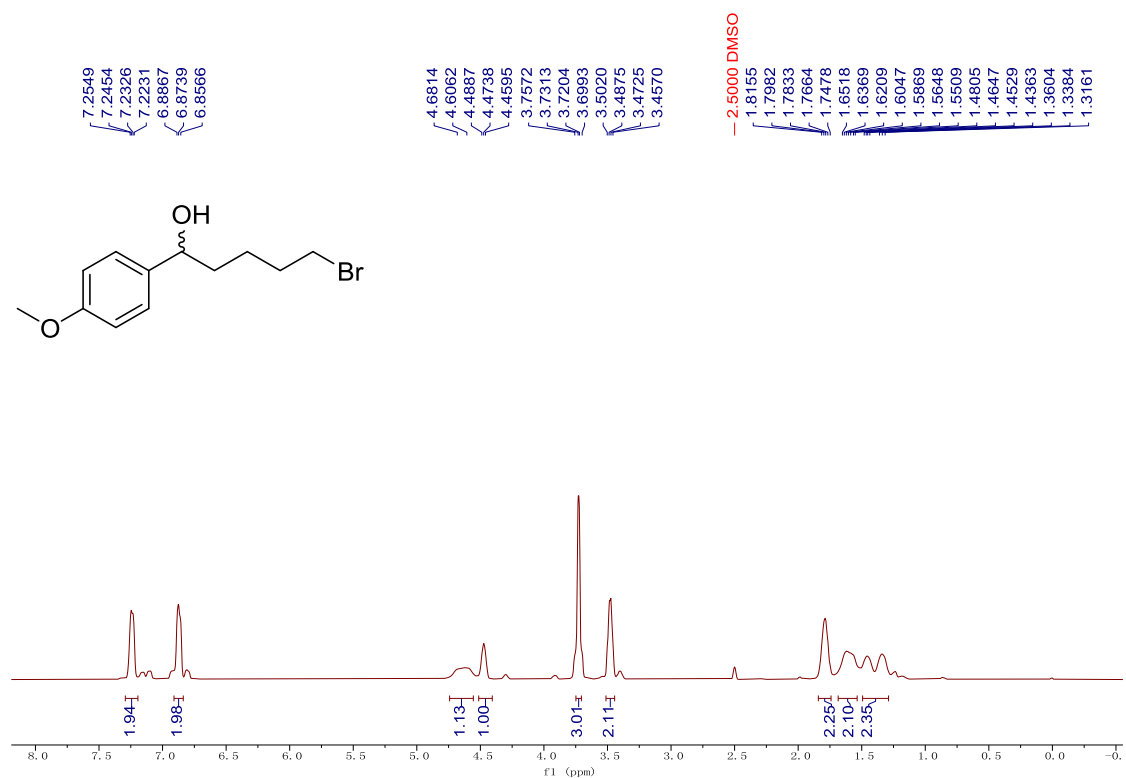
Supplementary Fig. 47 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-19a.



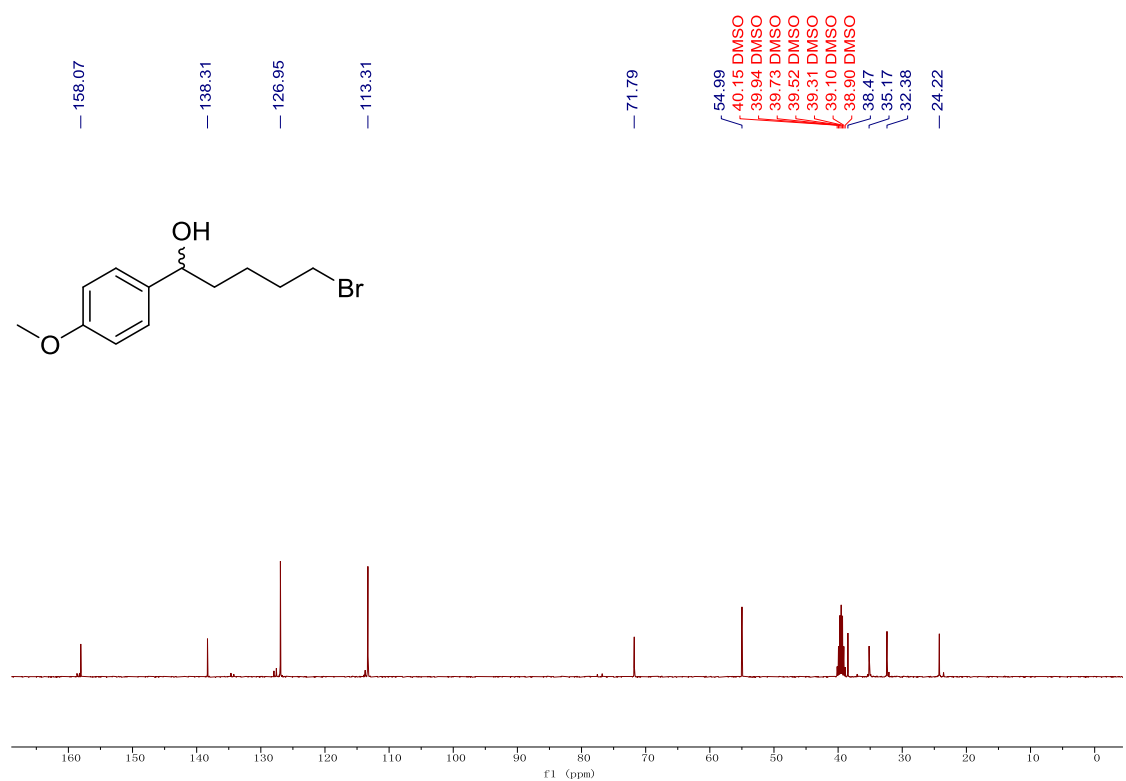
Supplementary Fig. 48 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-20a.



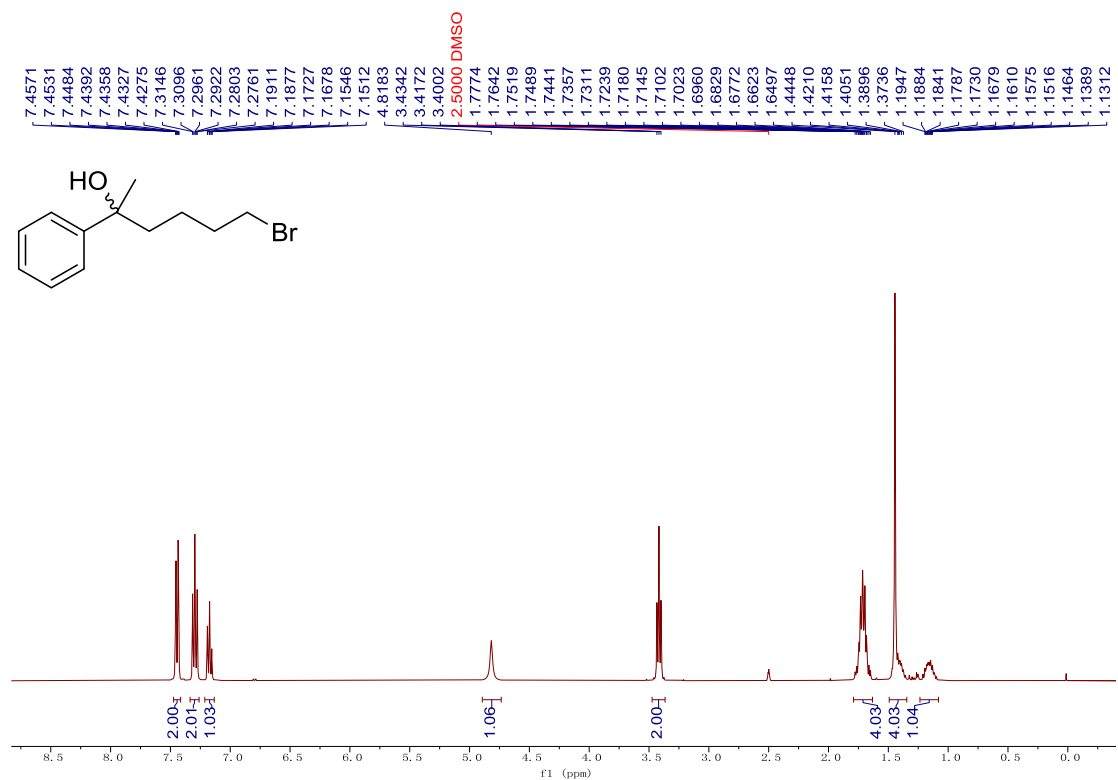
Supplementary Fig. 49 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-20a.



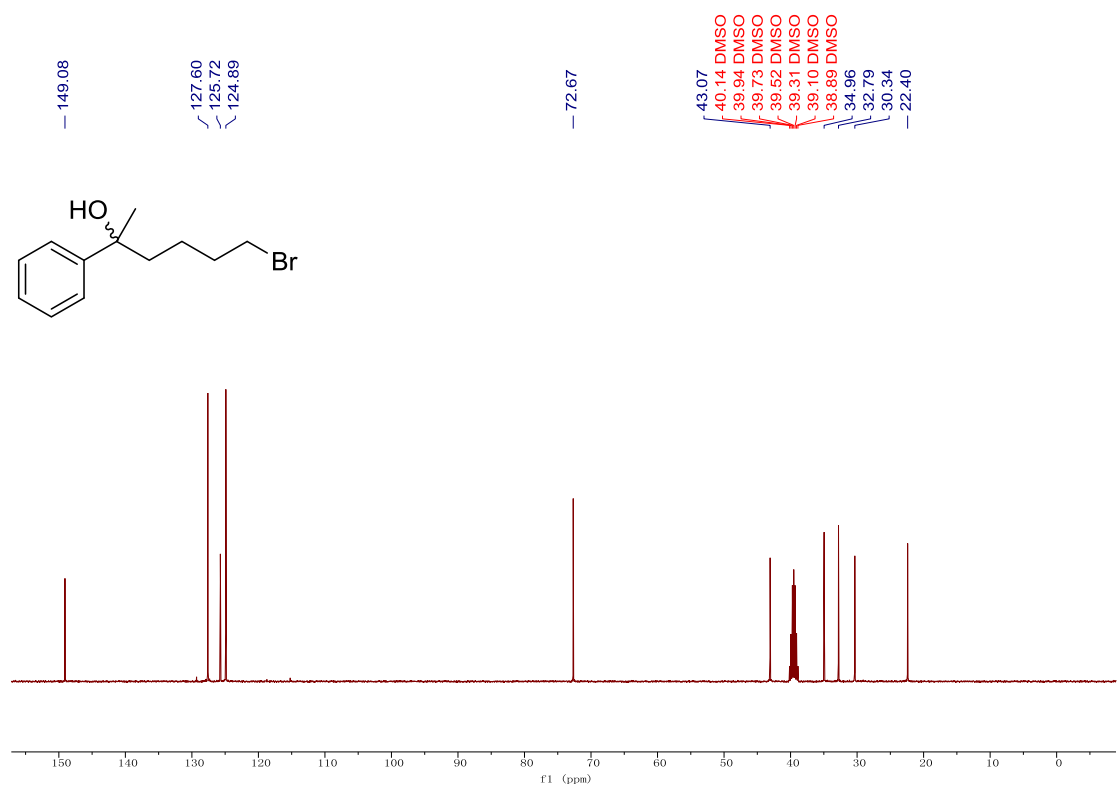
Supplementary Fig. 50 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-21a.



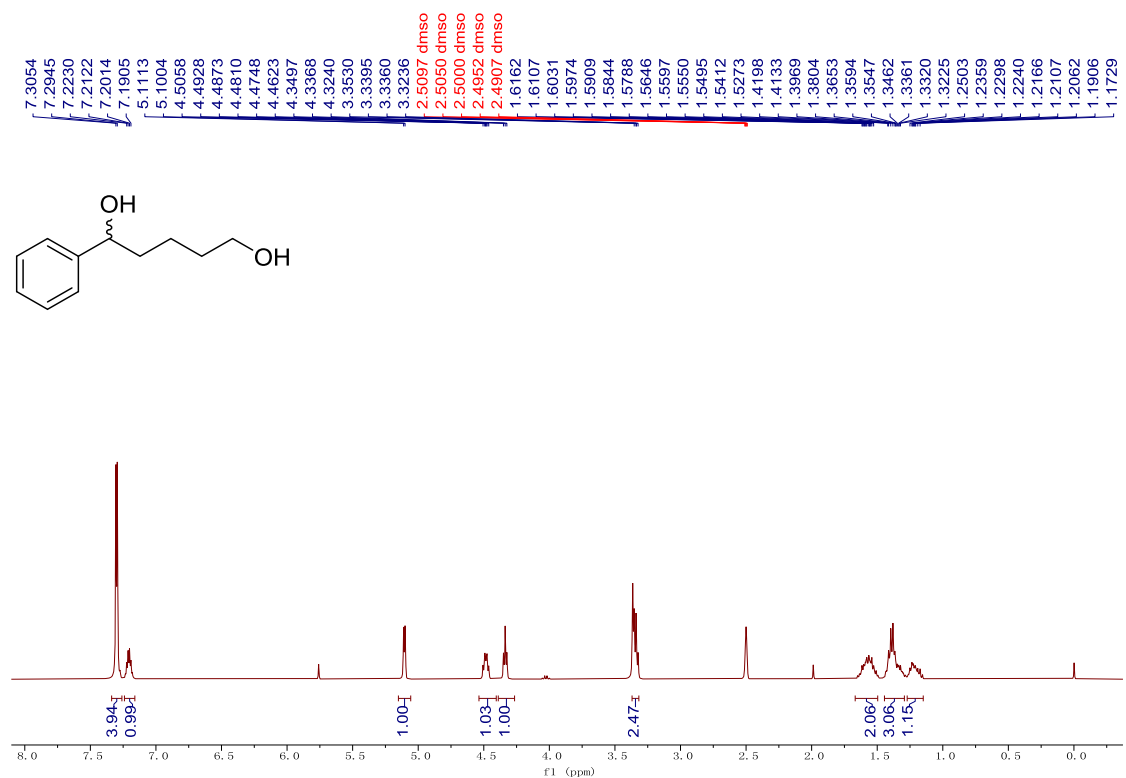
Supplementary Fig. 51 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-21a.



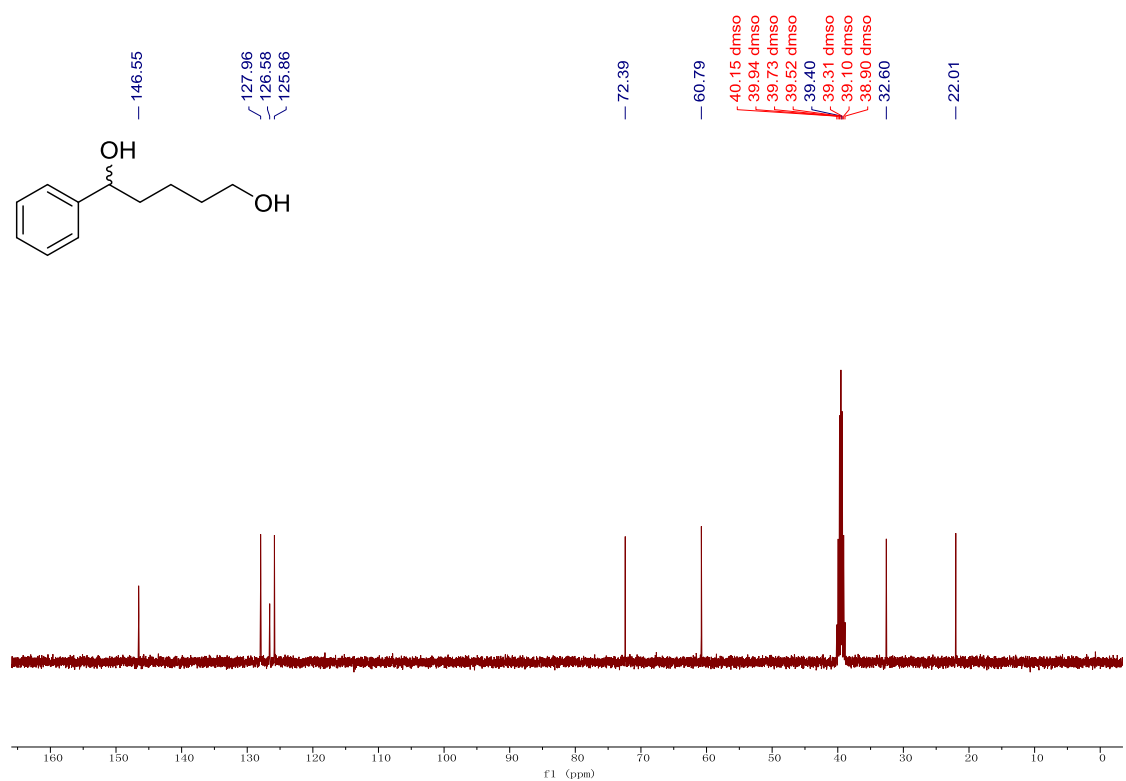
Supplementary Fig. 52 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-22a.



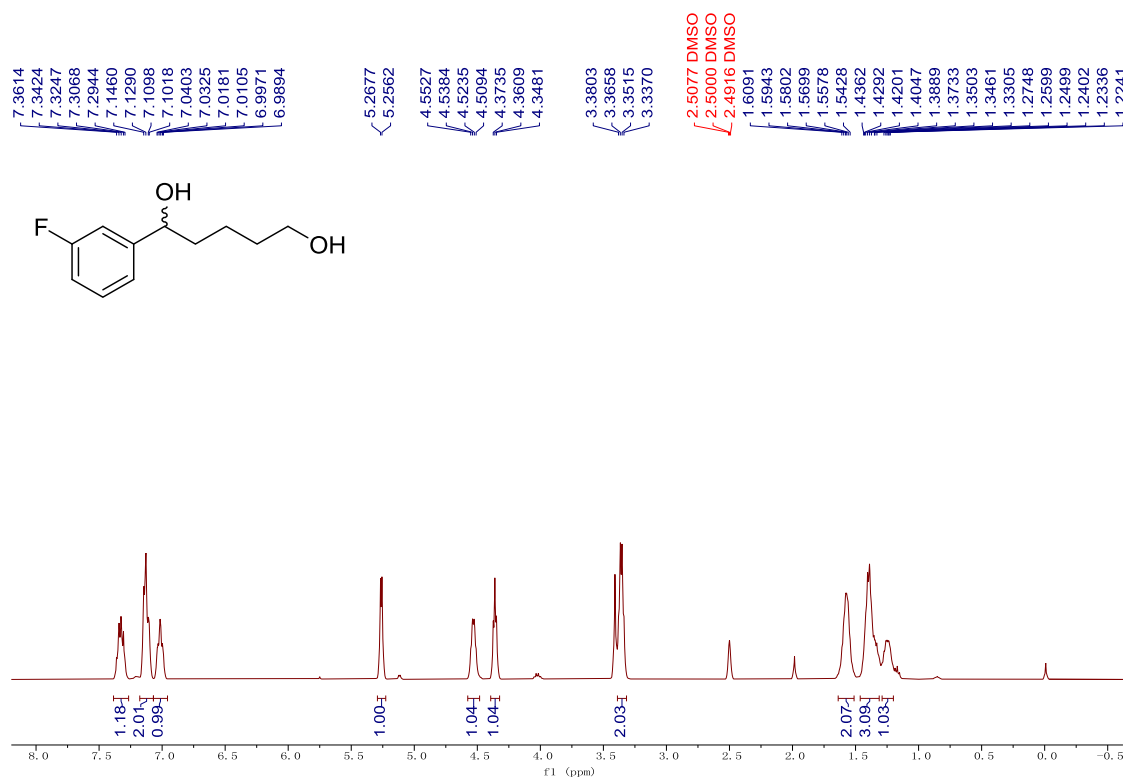
Supplementary Fig. 53 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-22a.



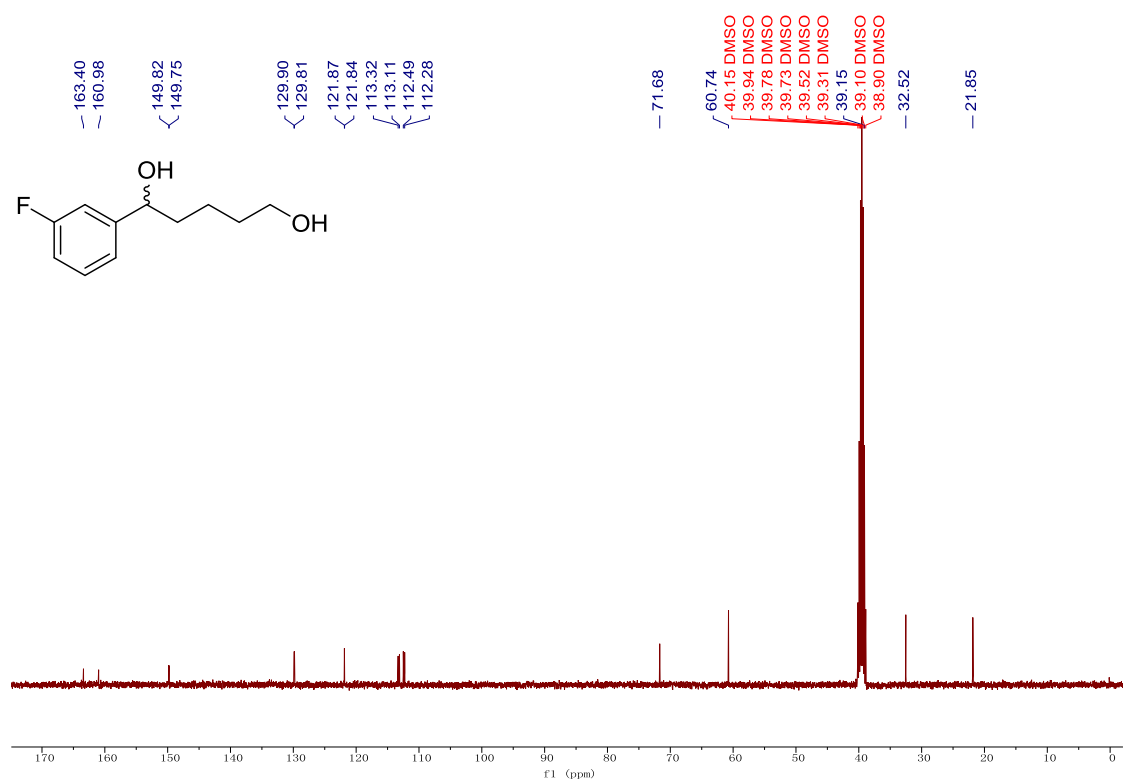
Supplementary Fig. 54 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-1b.



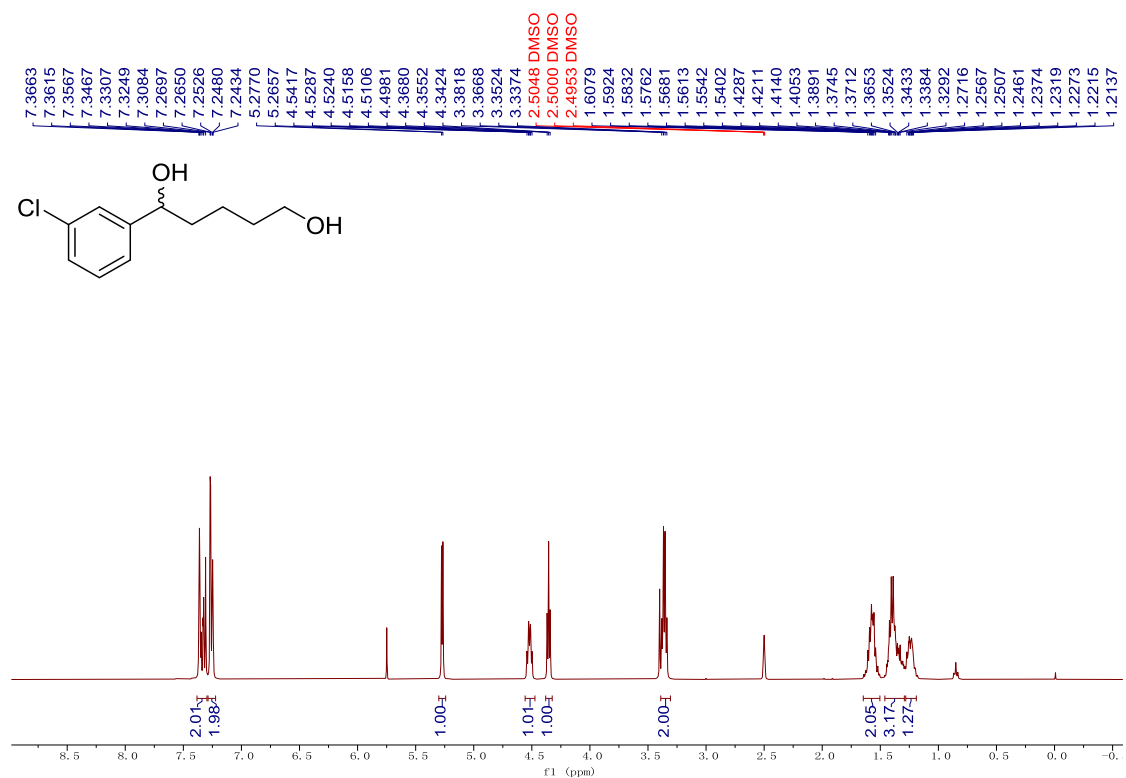
Supplementary Fig. 55 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-1b.



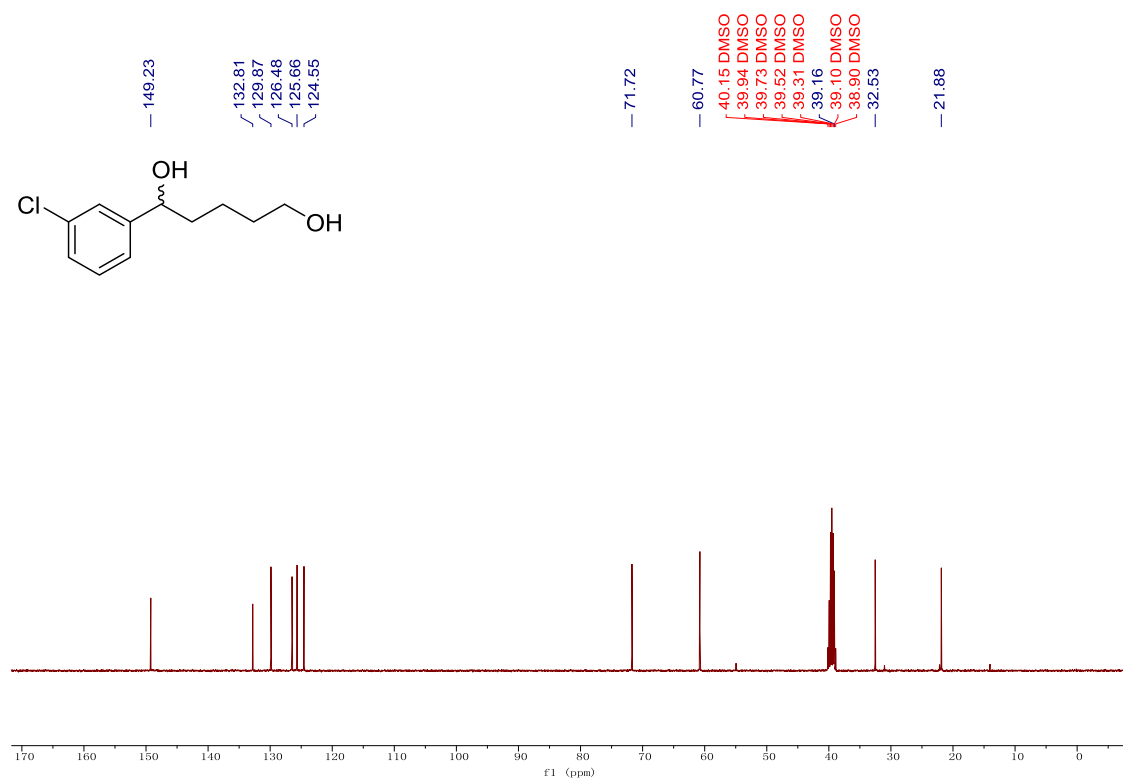
Supplementary Fig. 56 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (*rac*)-**2b**.



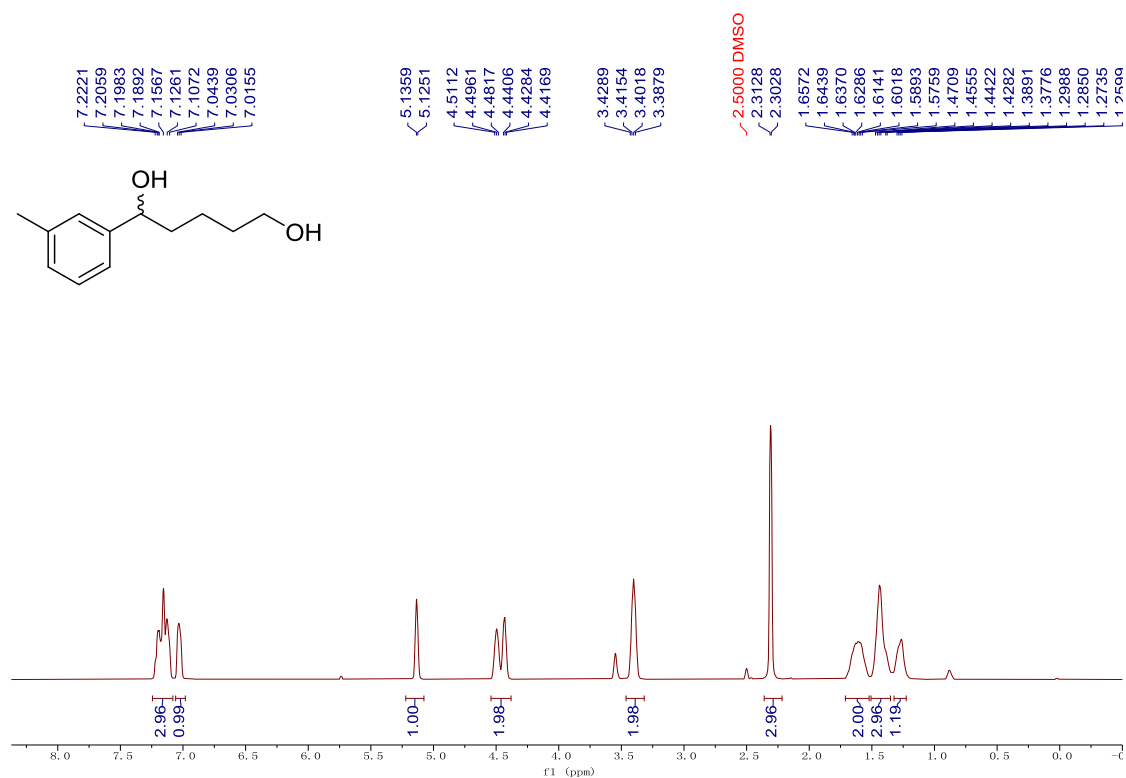
Supplementary Fig. 57 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (*rac*)-**2b**.



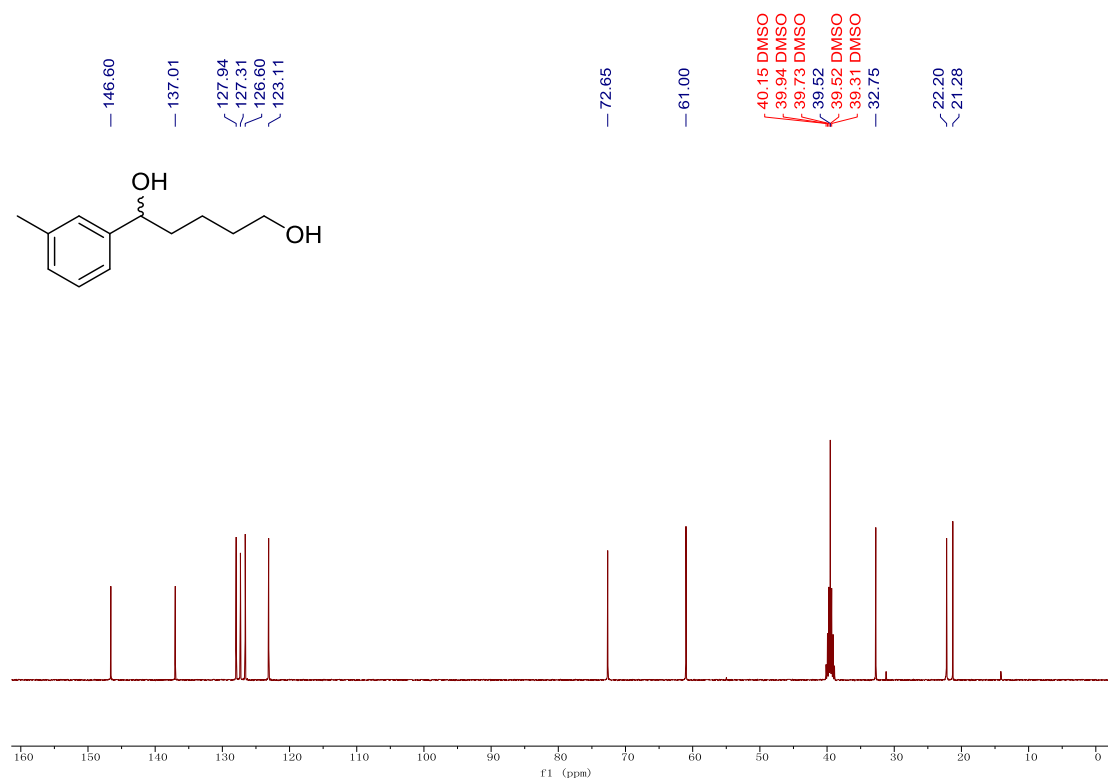
Supplementary Fig. 58 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-3b.



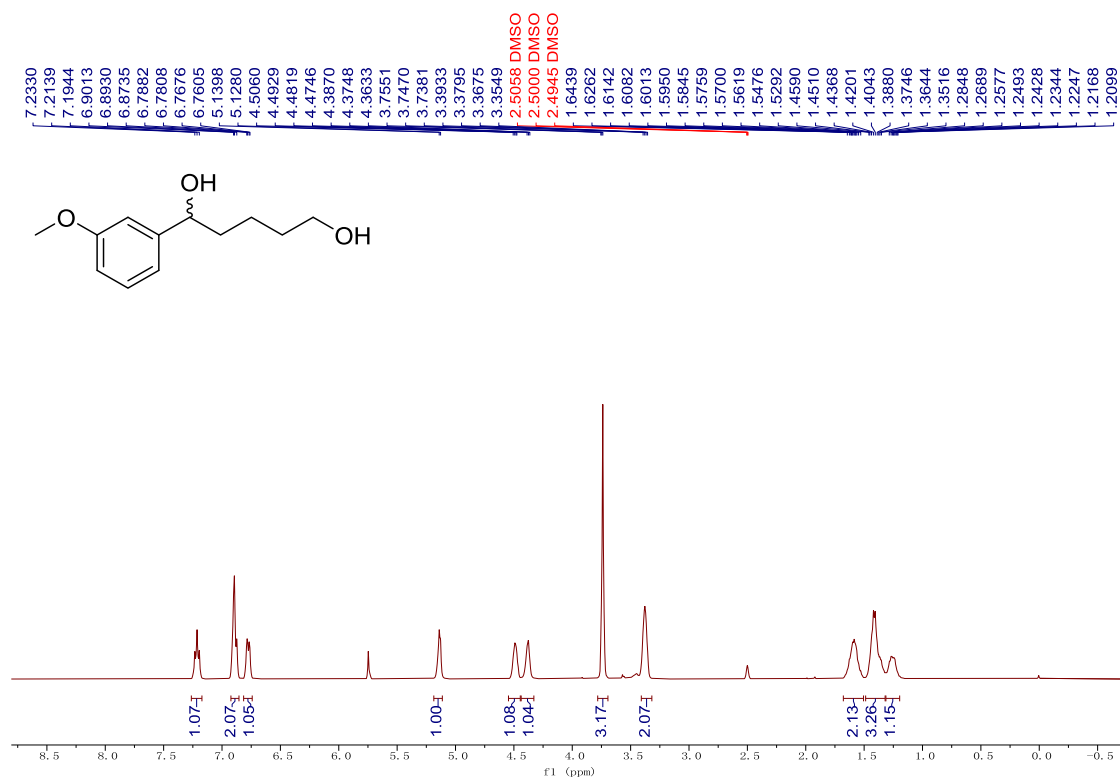
Supplementary Fig. 59 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-3b.



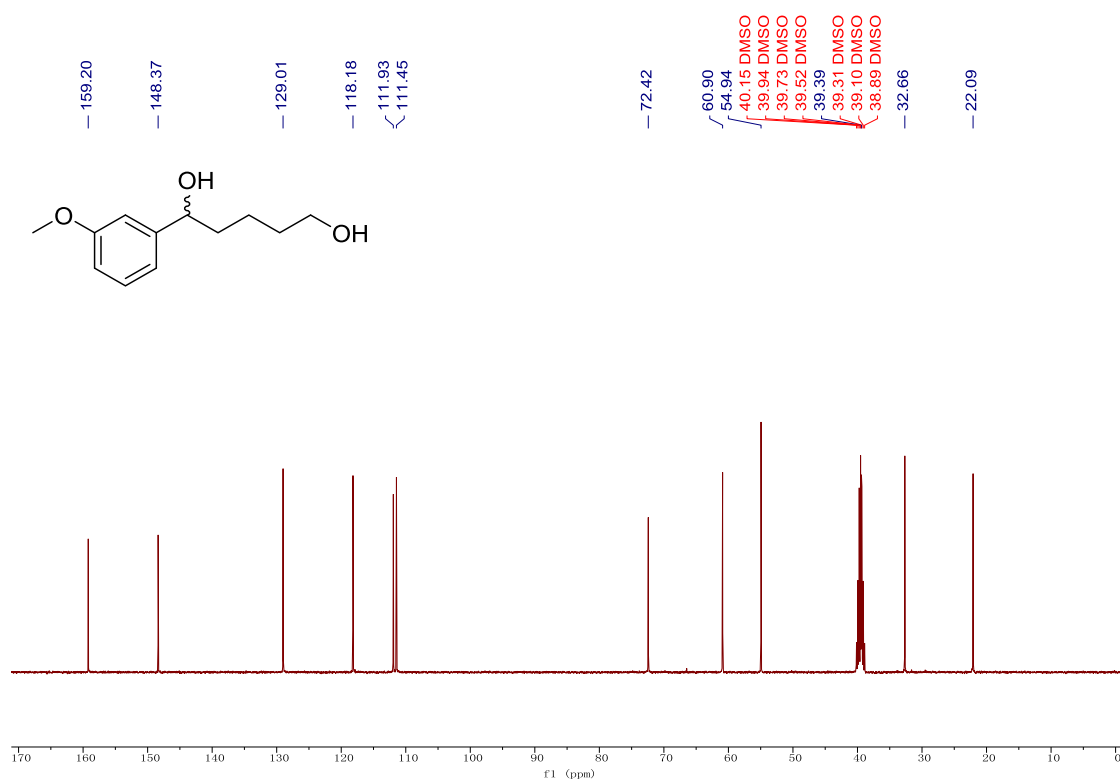
Supplementary Fig. 60 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-4b.



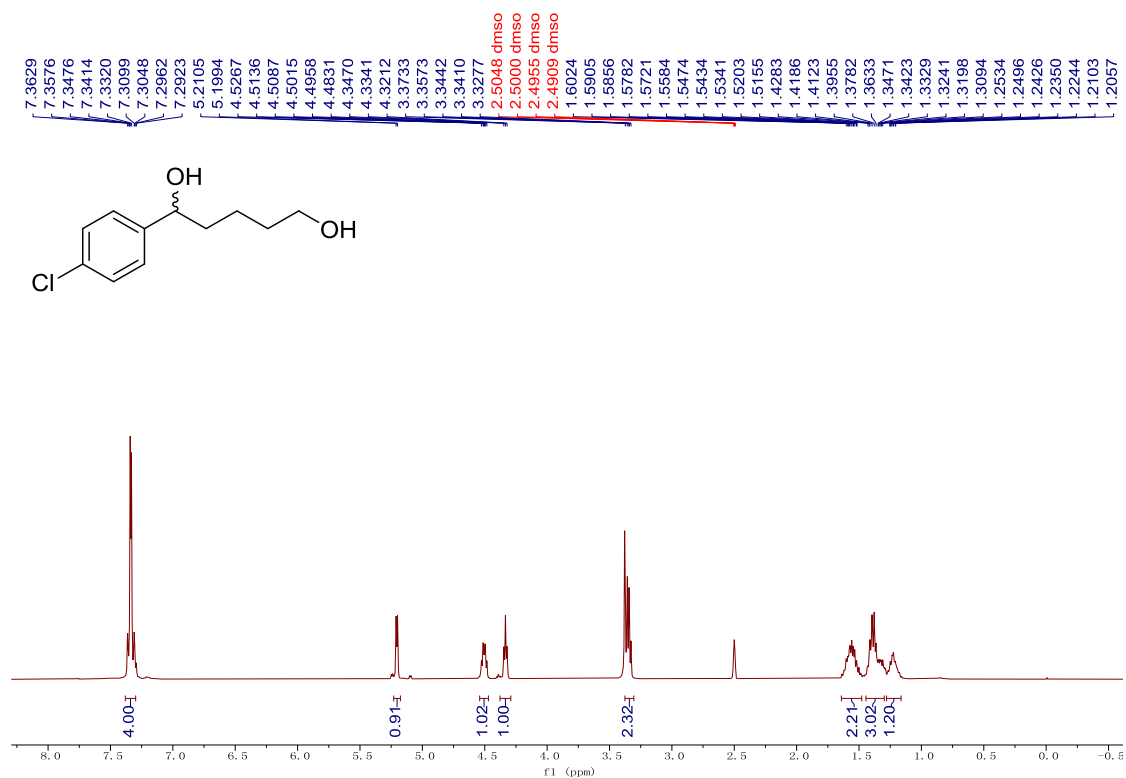
Supplementary Fig. 61 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-4b.



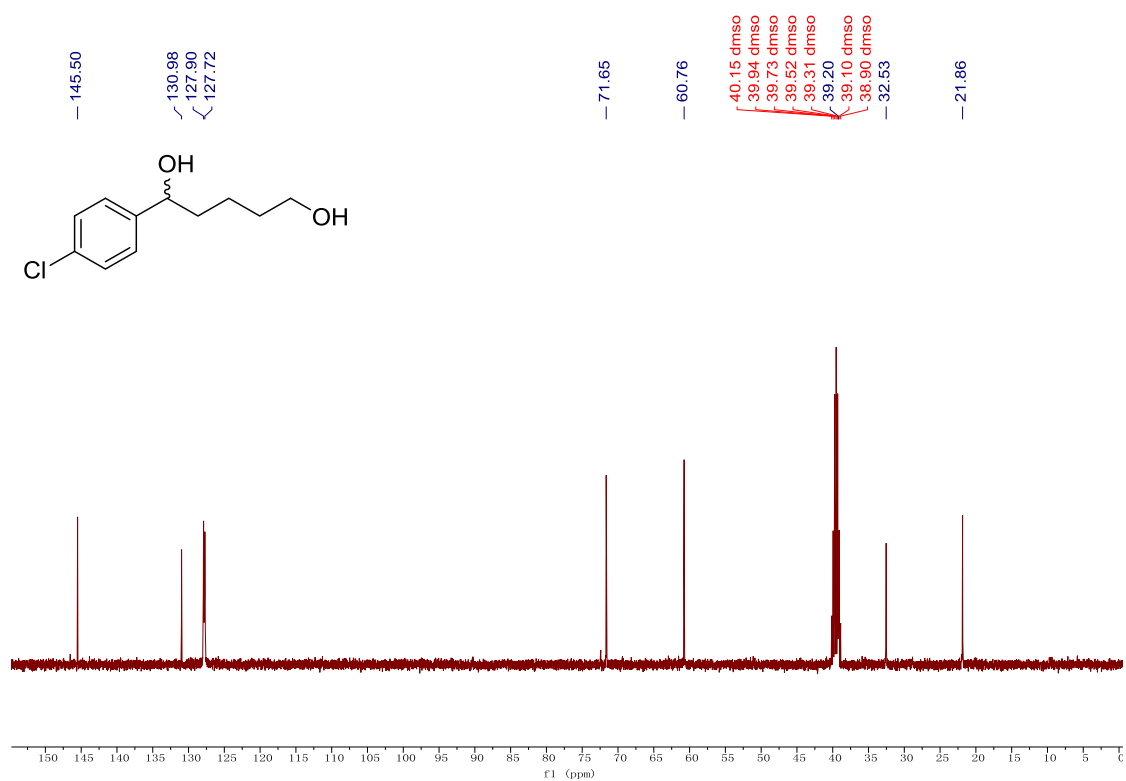
Supplementary Fig. 62 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-5b.



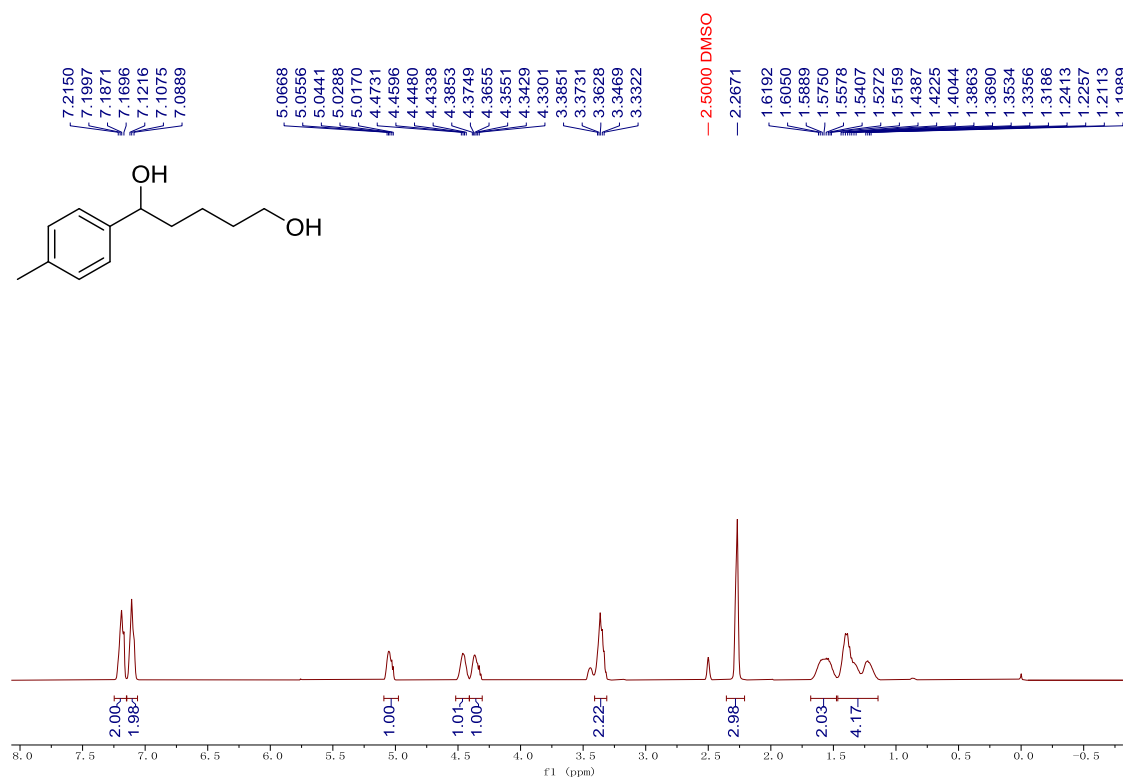
Supplementary Fig. 63 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-5b.



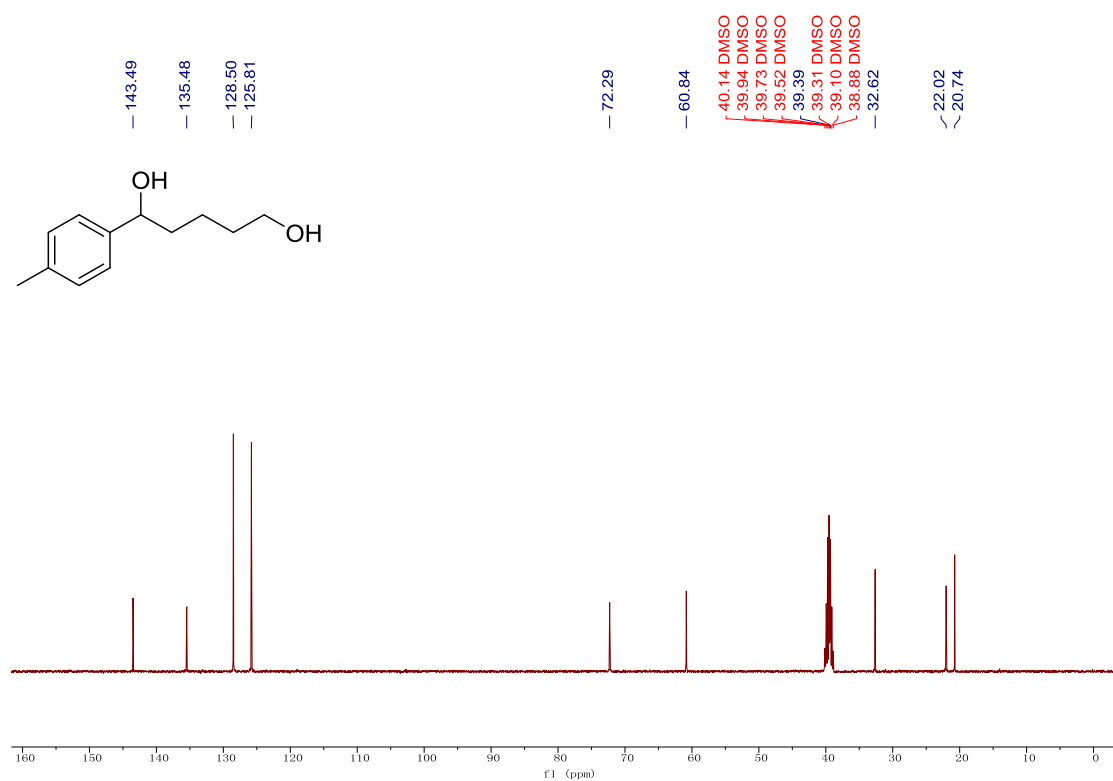
Supplementary Fig. 64 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-6b.



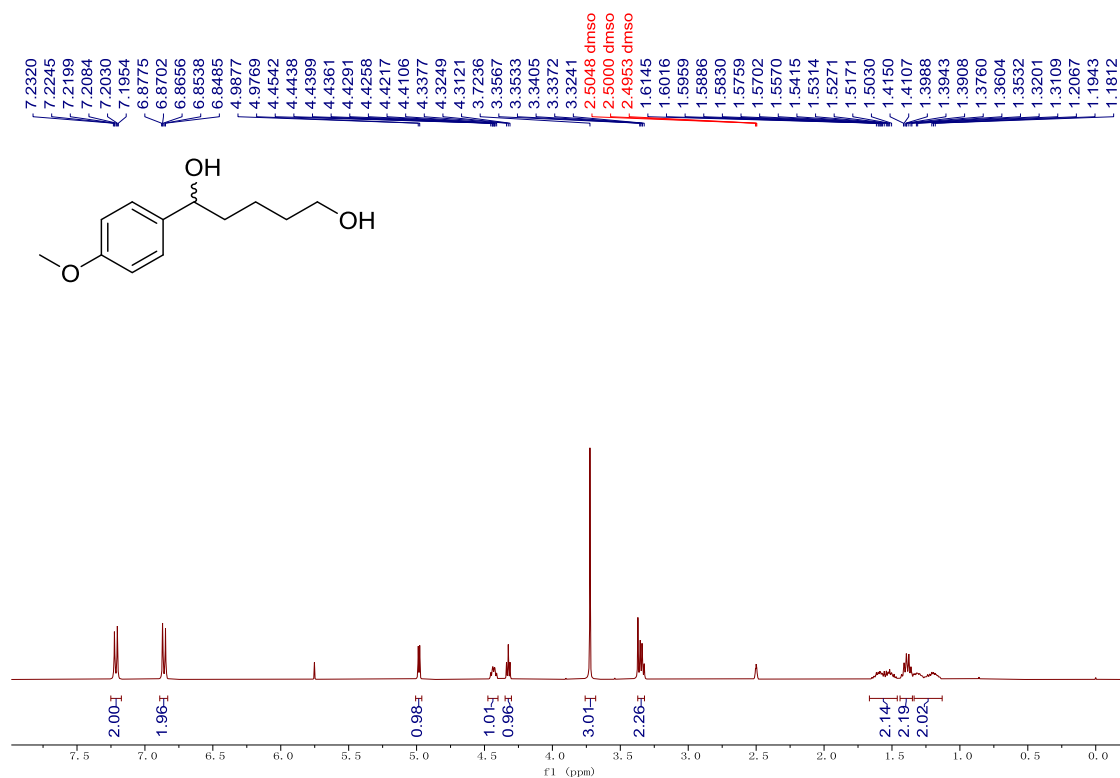
Supplementary Fig. 65 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-6b.



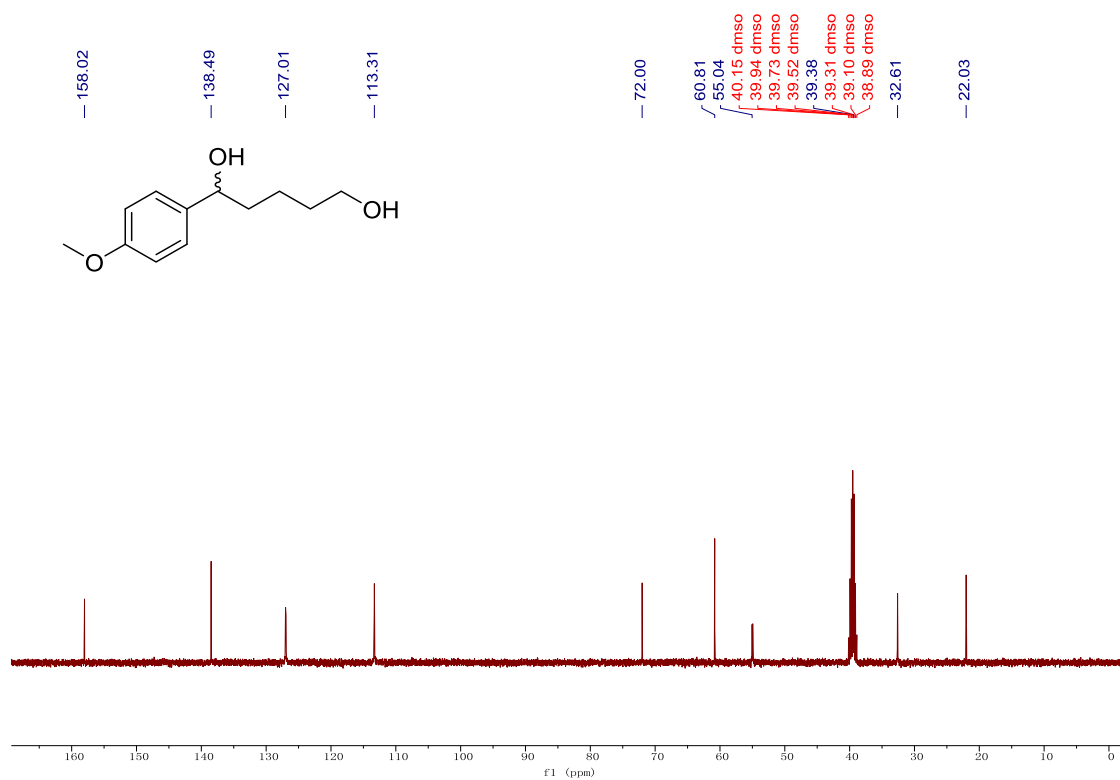
Supplementary Fig. 66 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-7b.



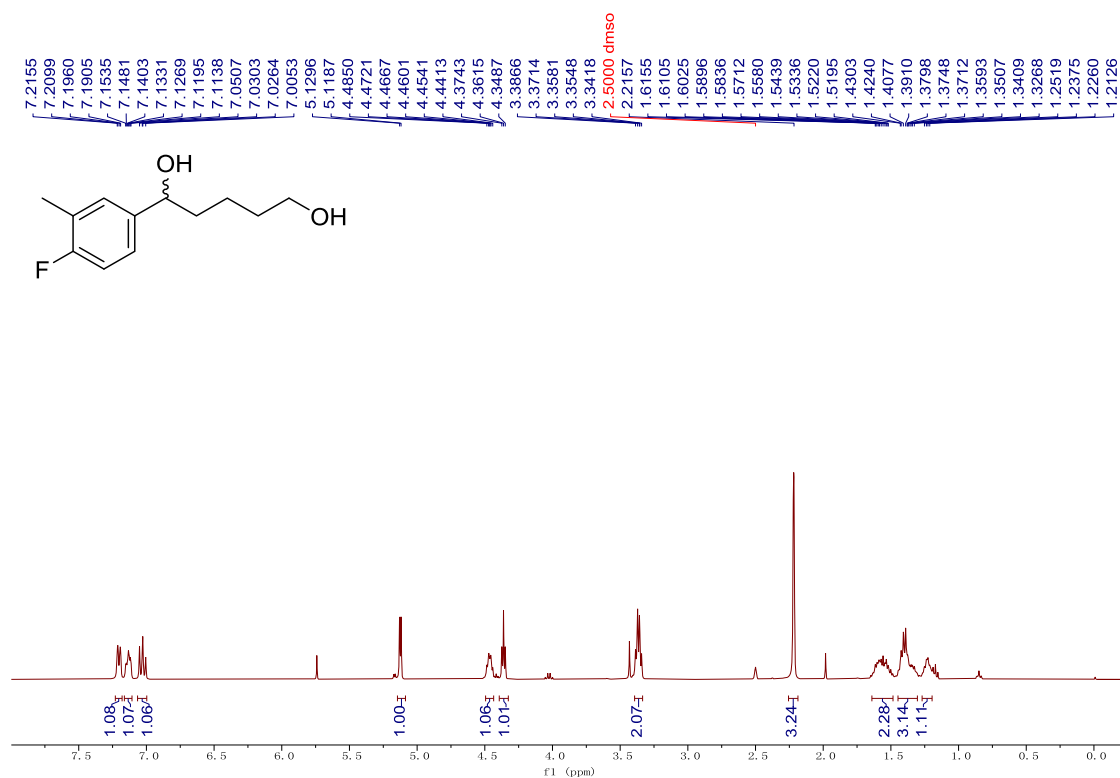
Supplementary Fig. 67 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-7b.



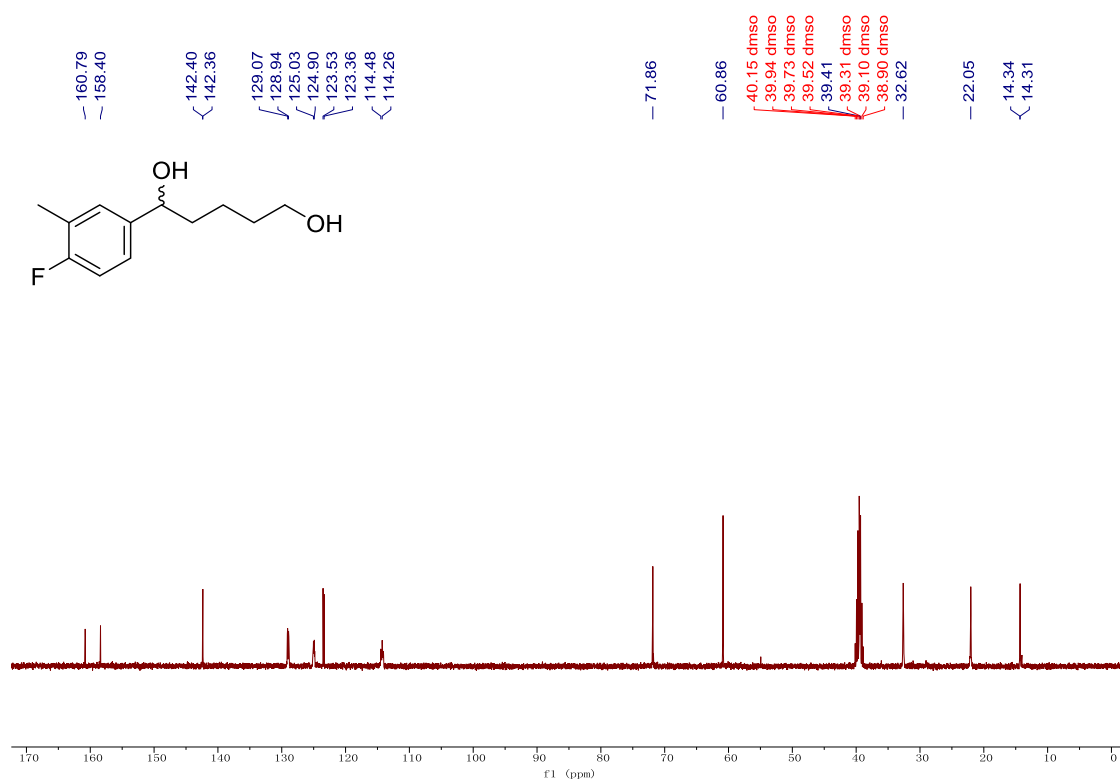
Supplementary Fig. 68 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-8b.



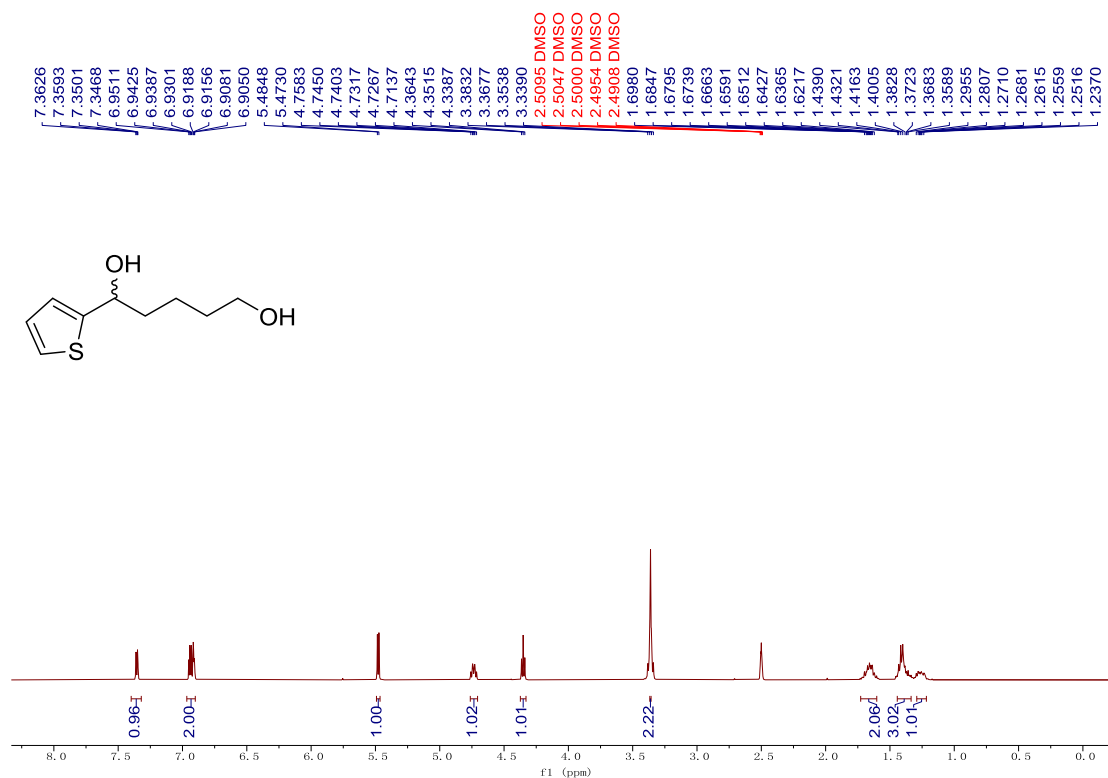
Supplementary Fig. 69 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-8b.



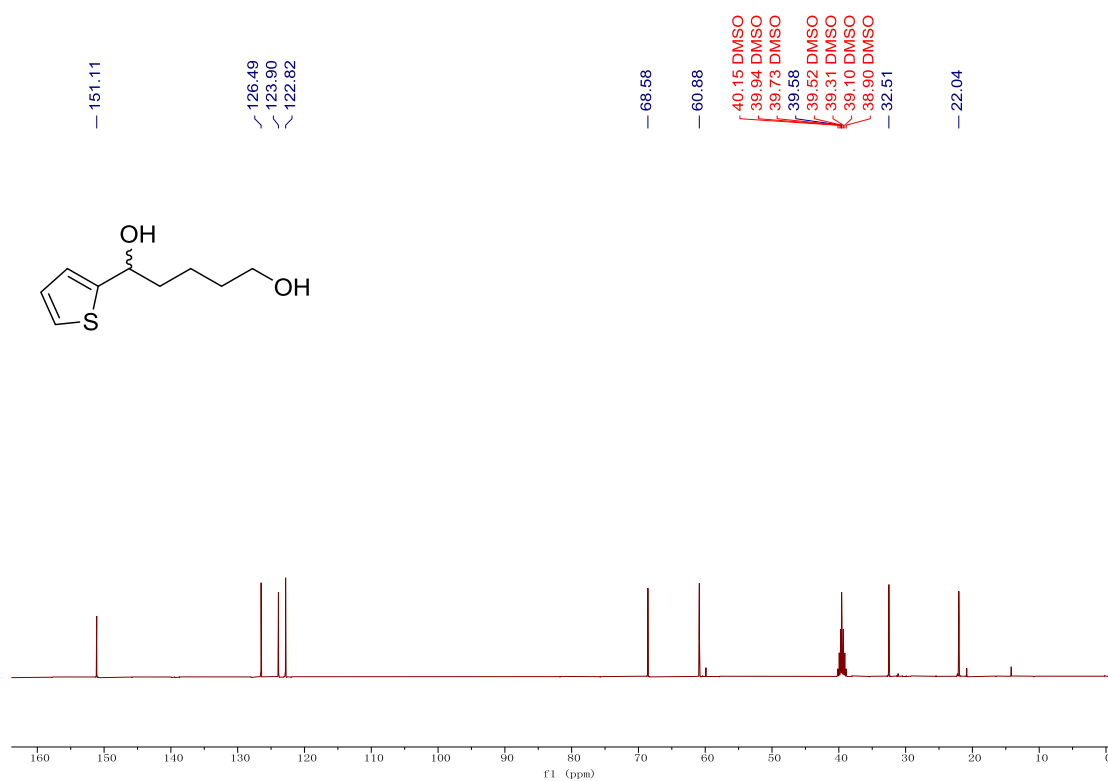
Supplementary Fig. 70 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (*rac*)-**9b**.



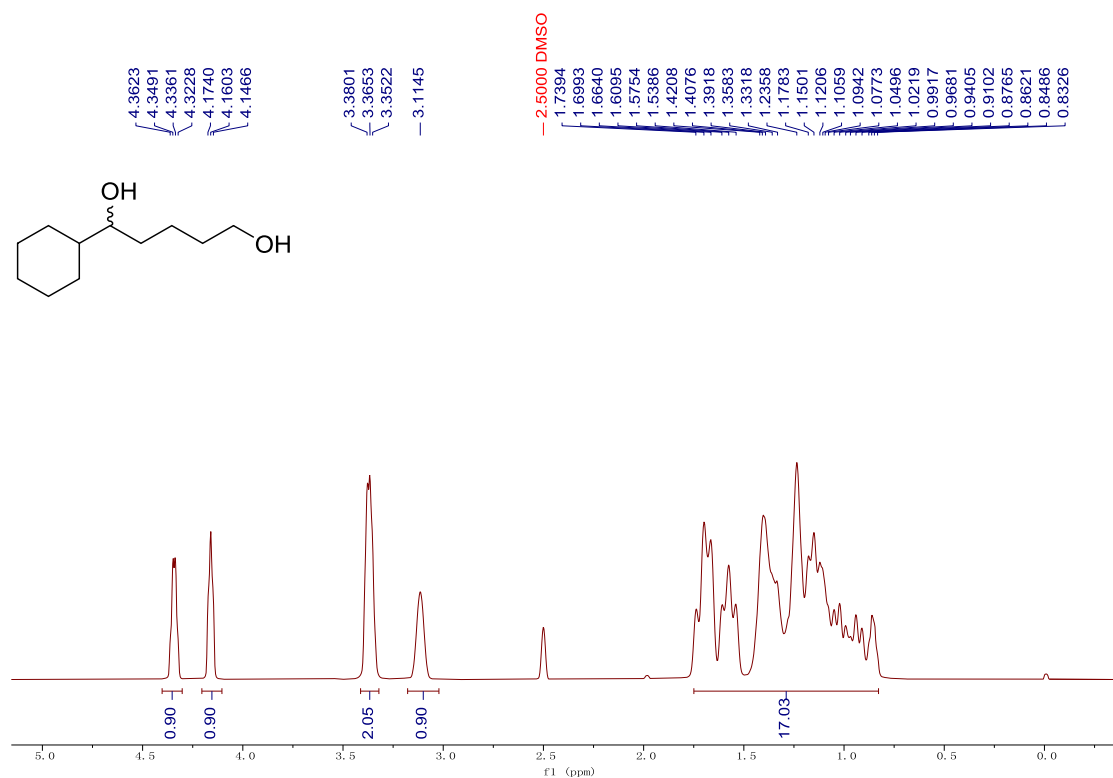
Supplementary Fig. 71 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (*rac*)-**9b**.



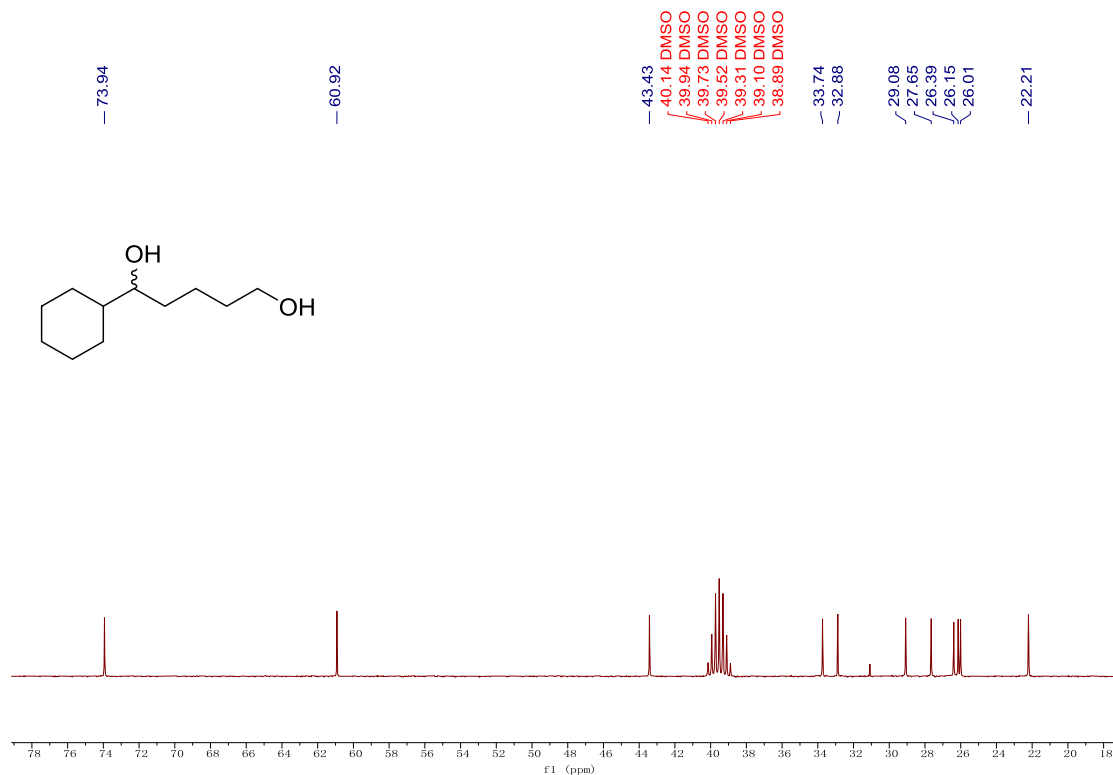
Supplementary Fig. 72 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-10b.



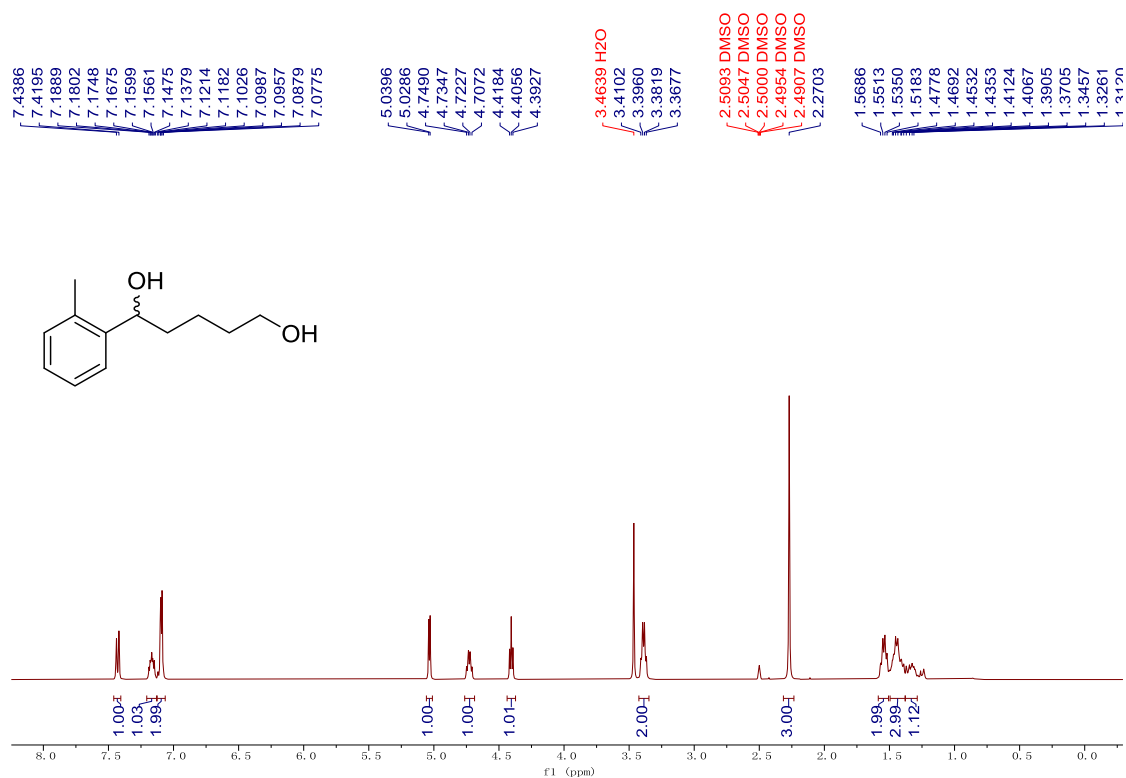
Supplementary Fig. 73 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-10b.



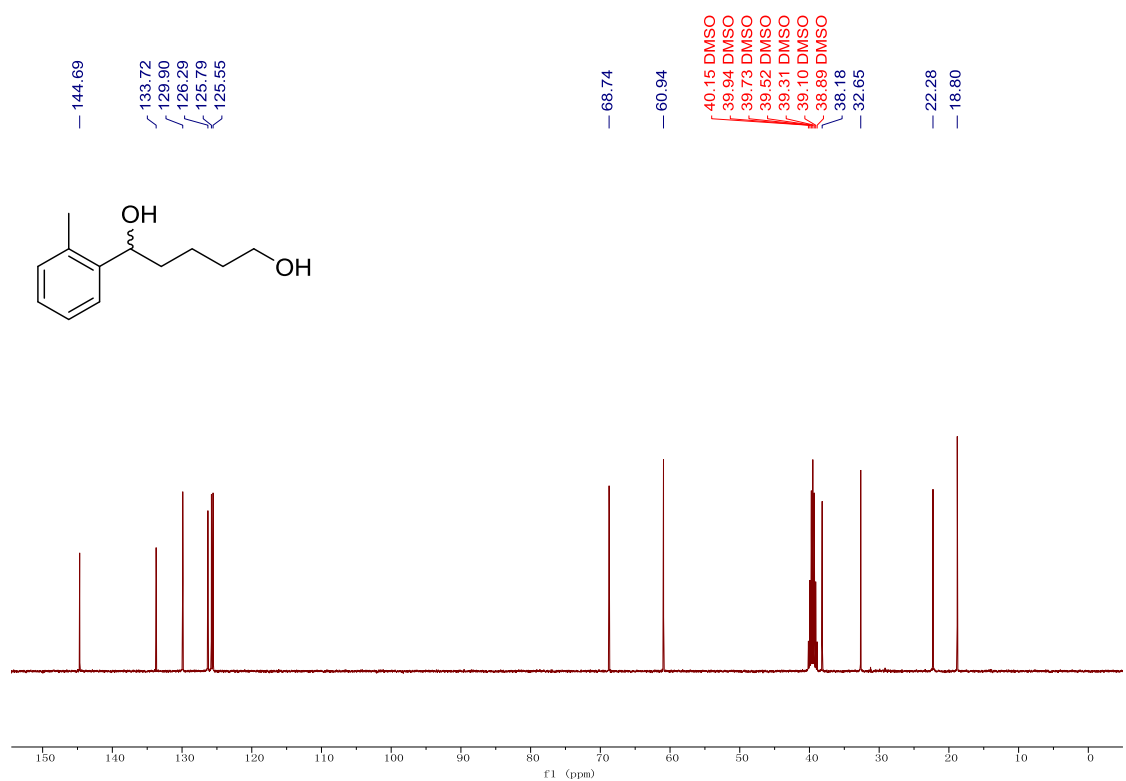
Supplementary Fig. 74 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-11b.



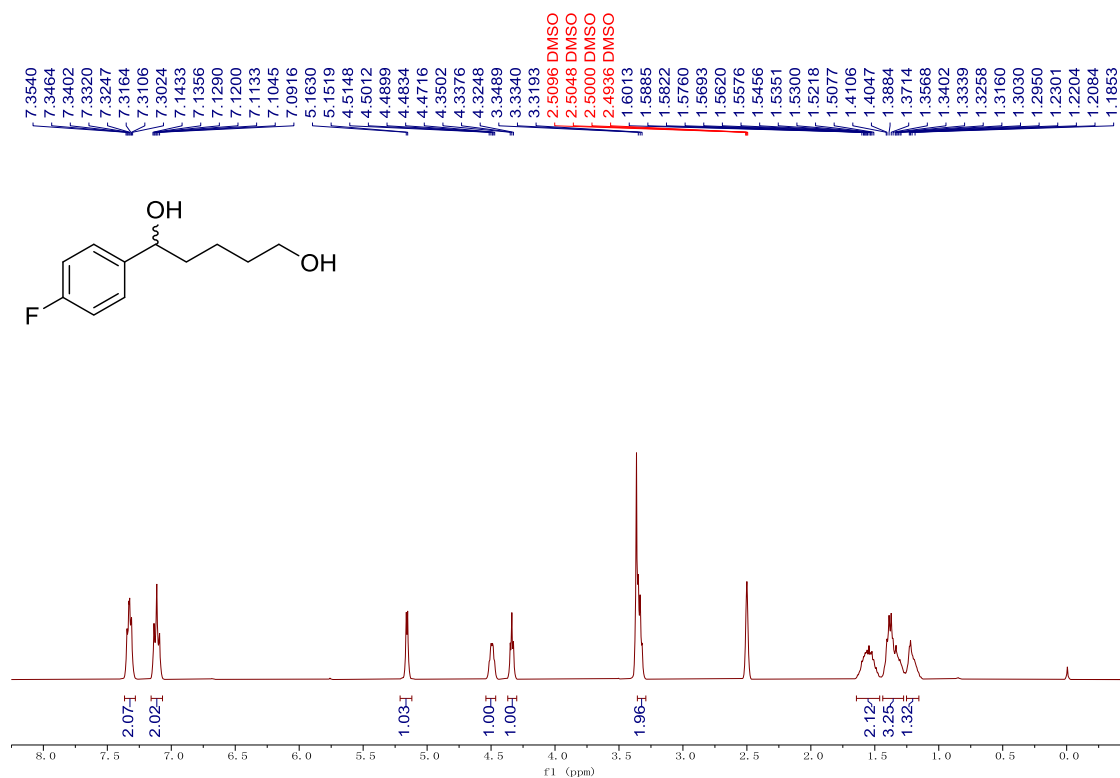
Supplementary Fig. 75 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-11b.



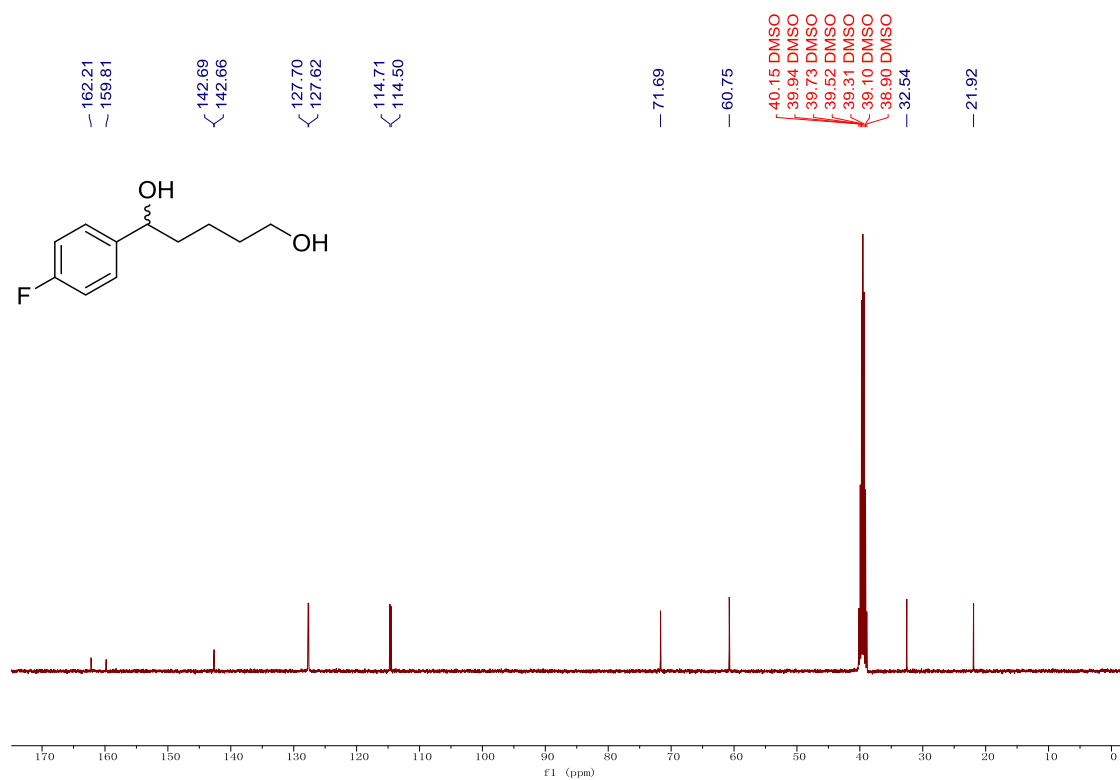
Supplementary Fig. 76 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-13b.



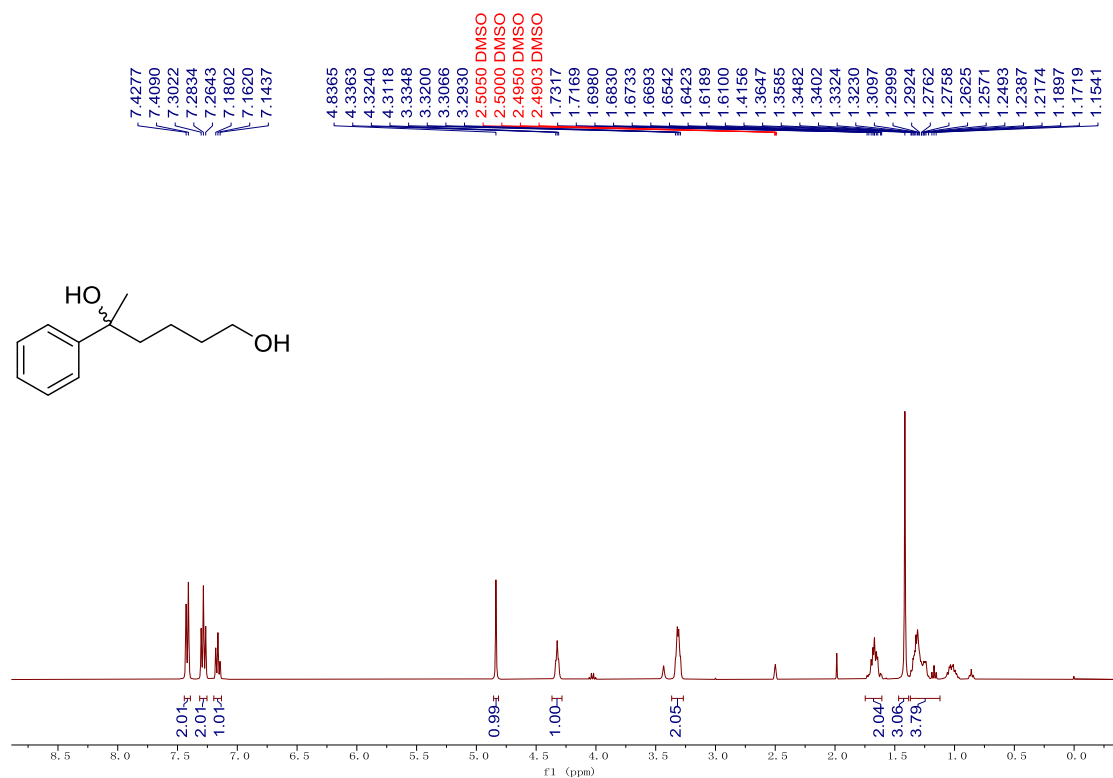
Supplementary Fig. 77 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-13b.



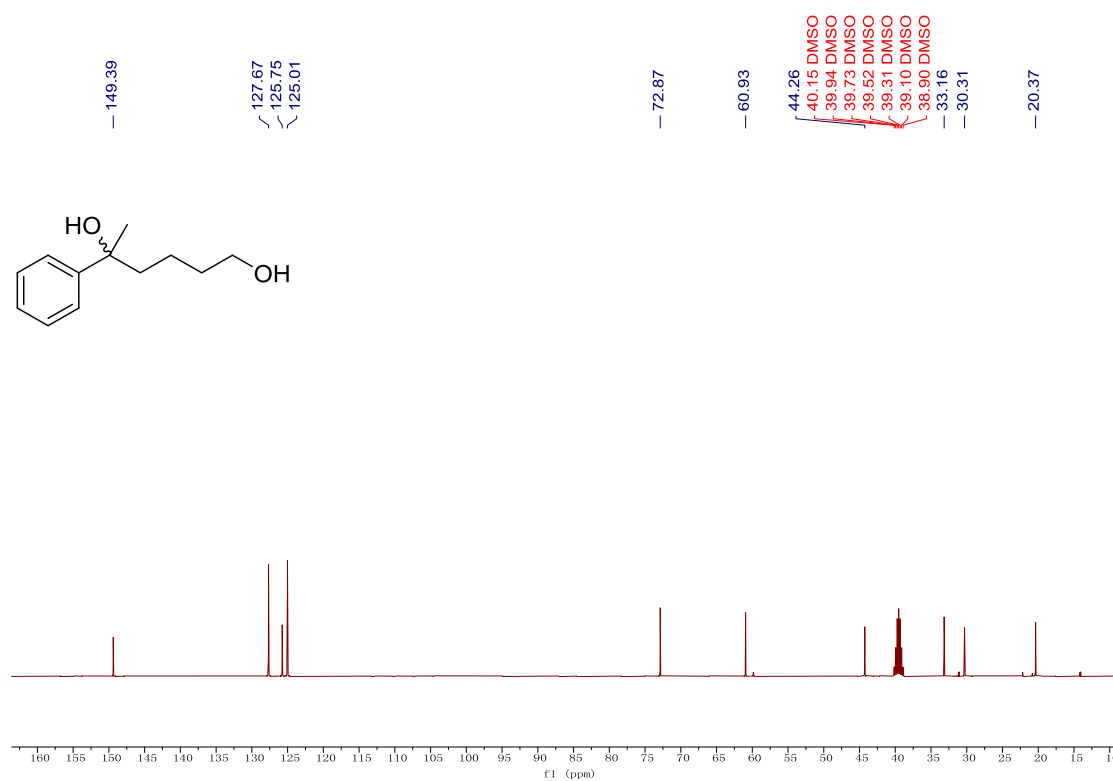
Supplementary Fig. 78 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-18b.



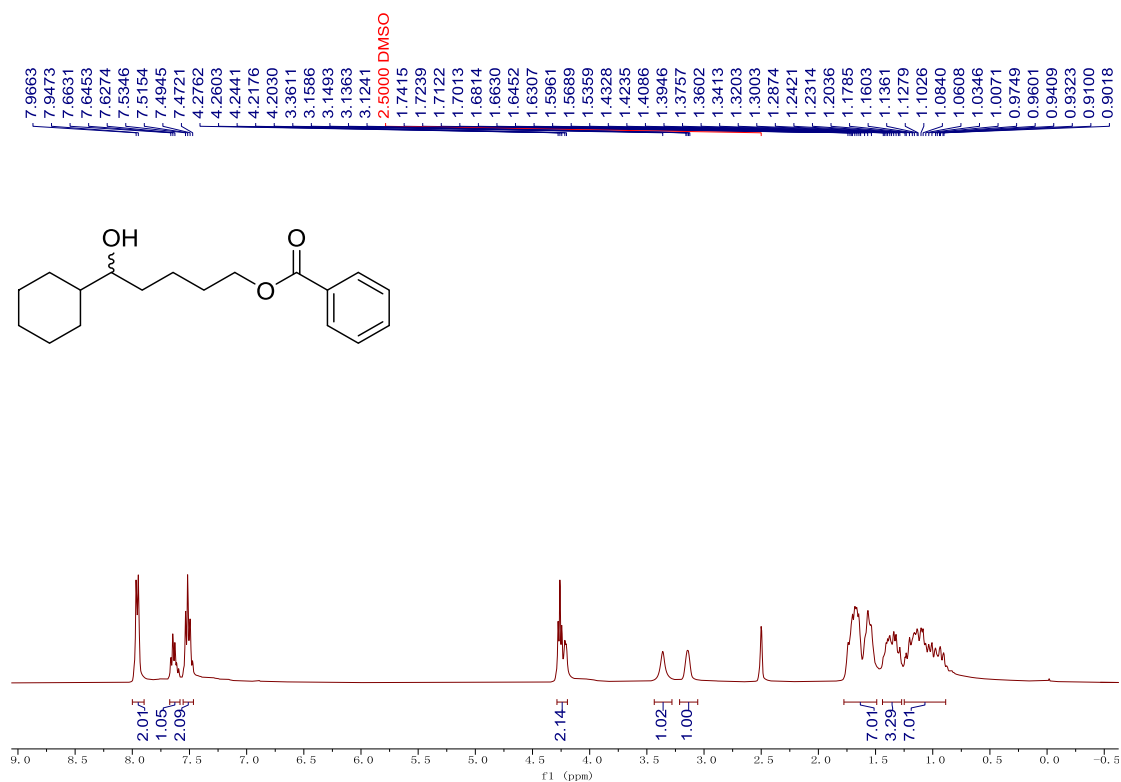
Supplementary Fig. 79 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-18b.



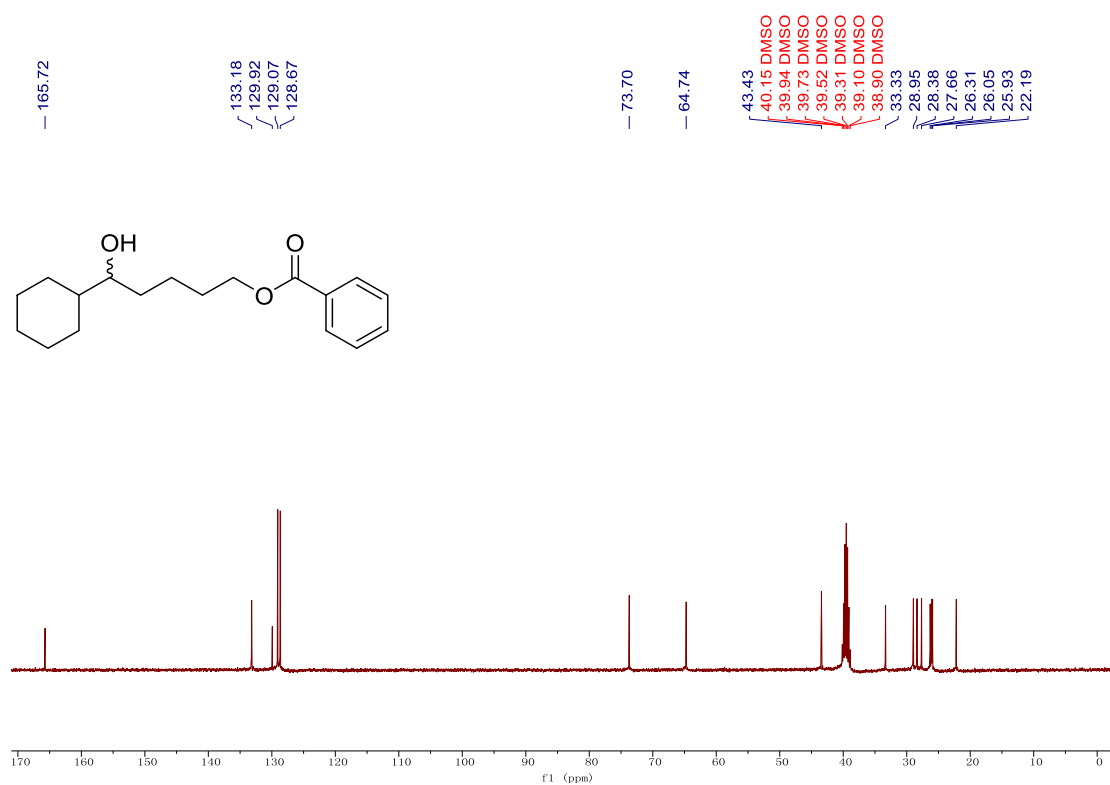
Supplementary Fig. 80 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-22b.



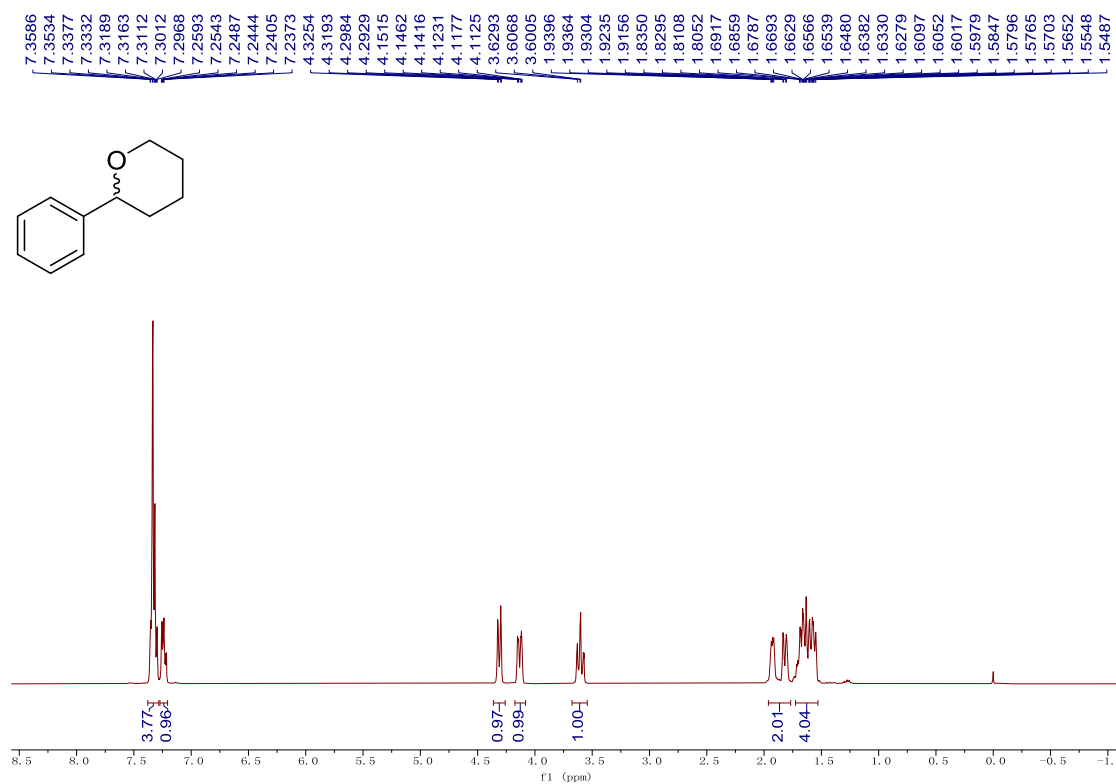
Supplementary Fig. 81 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-22b.



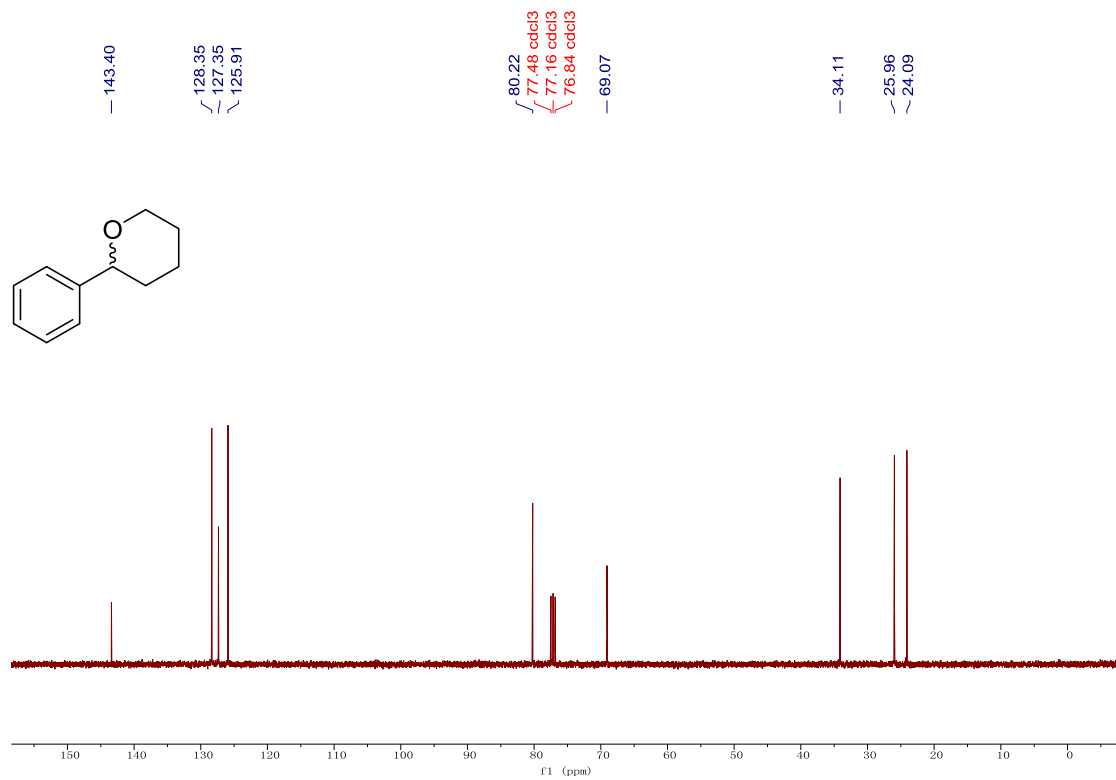
Supplementary Fig. 82 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-11bb.



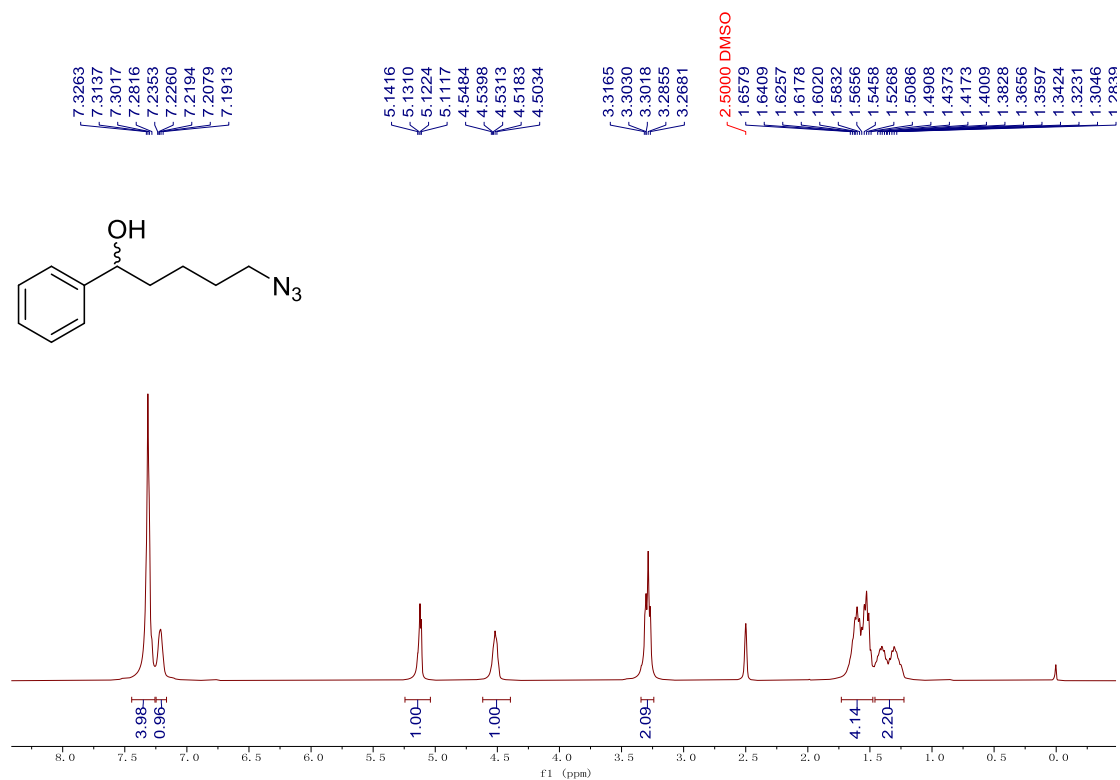
Supplementary Fig. 83 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-11bb.



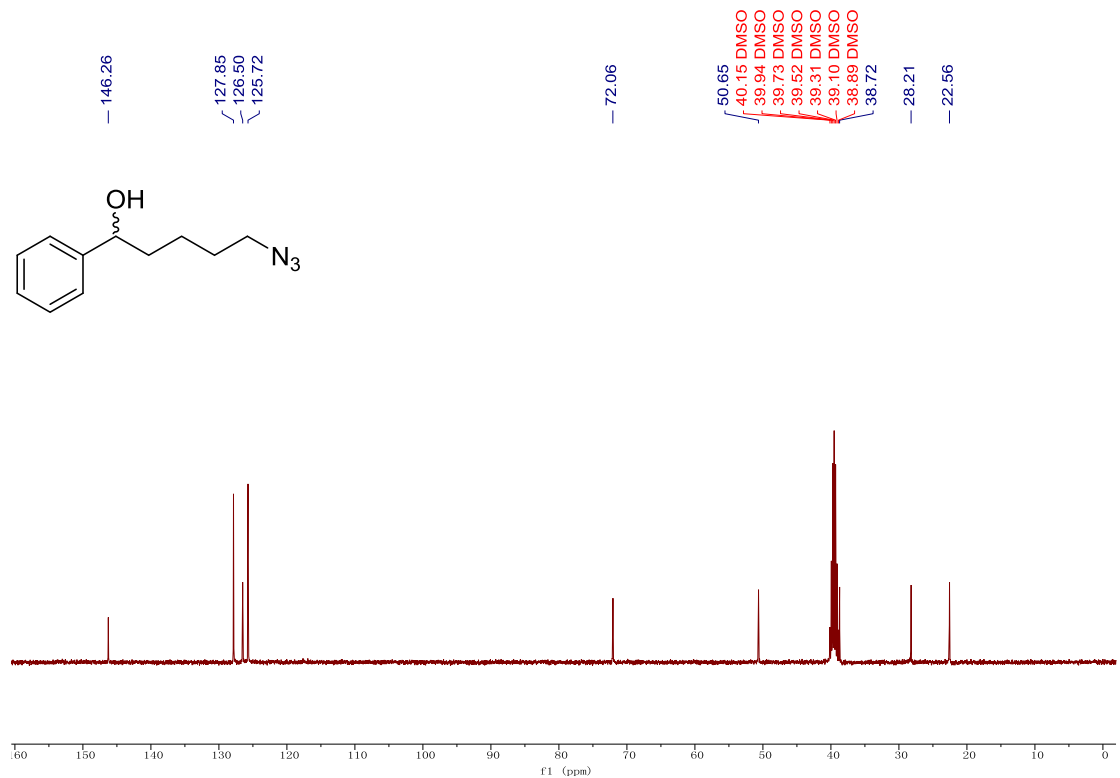
Supplementary Fig. 84 | ¹H NMR (400 MHz, CDCl₃) spectrum of *(rac)*-12aa.



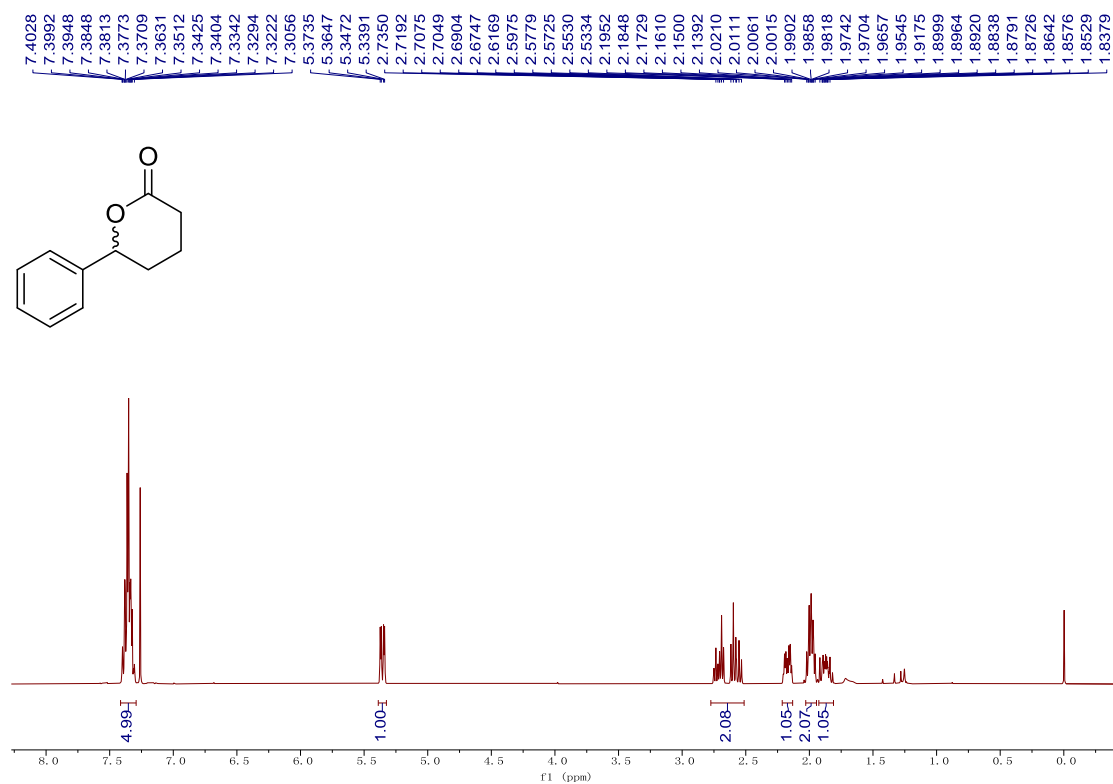
Supplementary Fig. 85 | ¹³C NMR (100 MHz, CDCl₃) spectrum of *(rac)*-12aa.



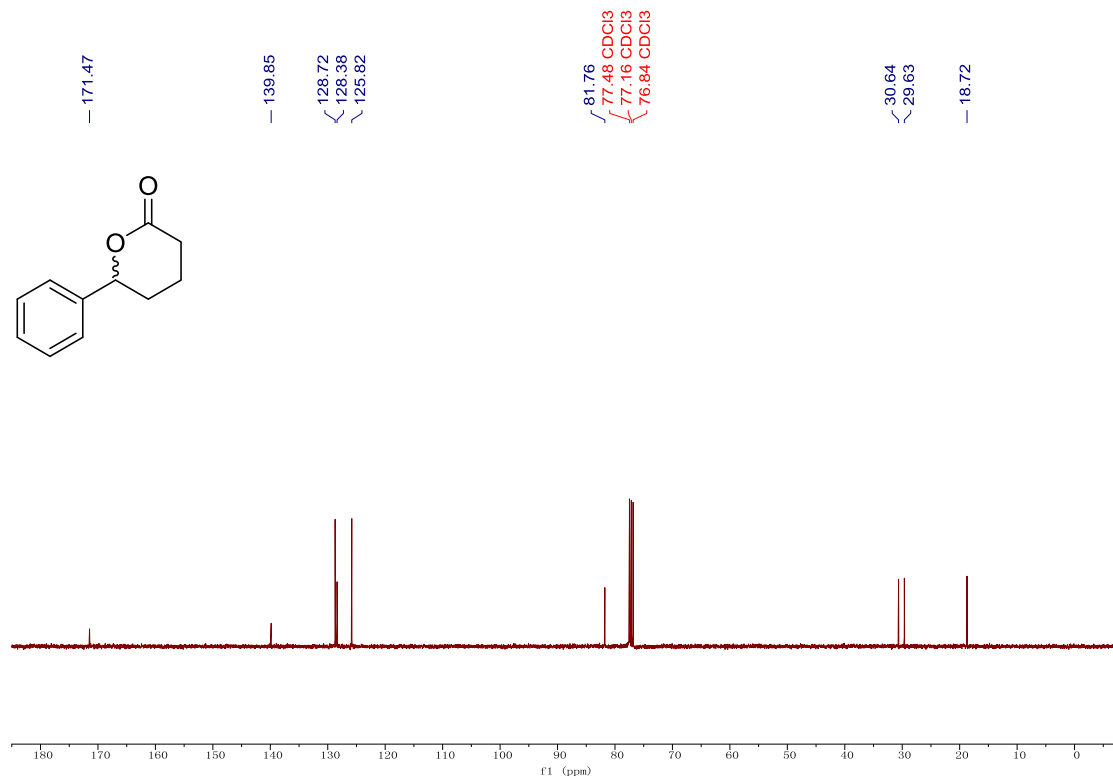
Supplementary Fig. 86 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (rac)-12ab.



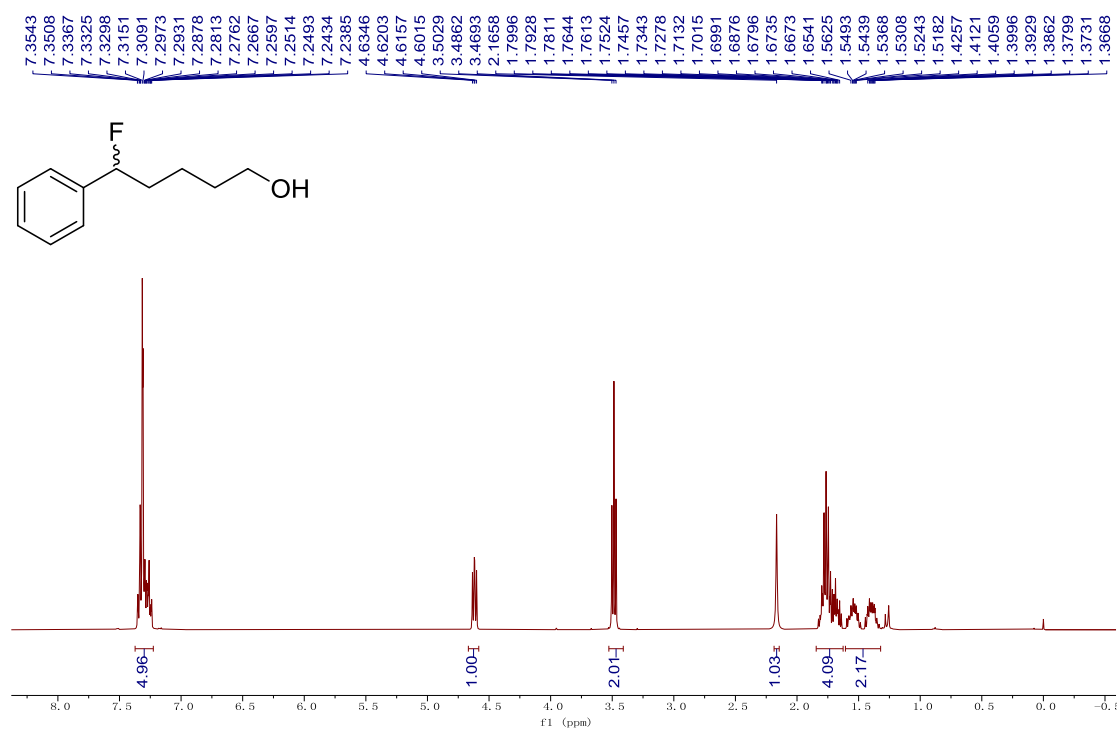
Supplementary Fig. 87 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (rac)-12ab.



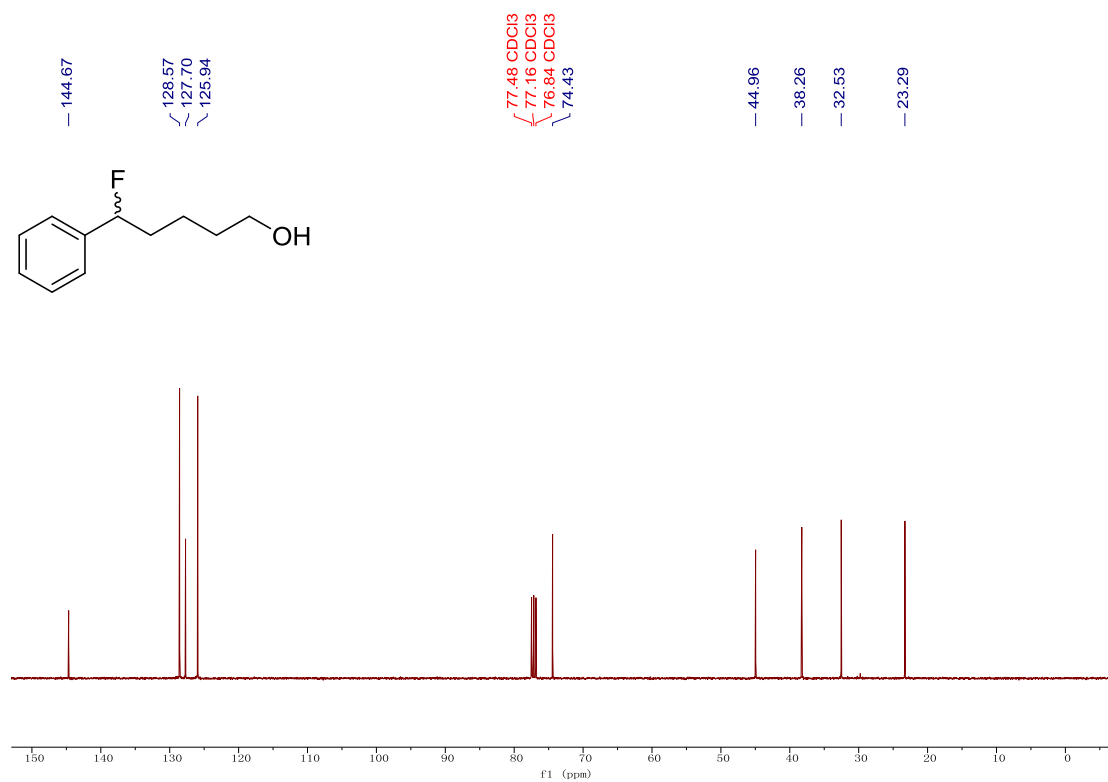
Supplementary Fig. 88 | ¹H NMR (400 MHz, CDCl₃) spectrum of *(rac)*-1ba.



Supplementary Fig. 89 | ¹³C NMR (100 MHz, CDCl₃) spectrum of *(rac)*-1ba.



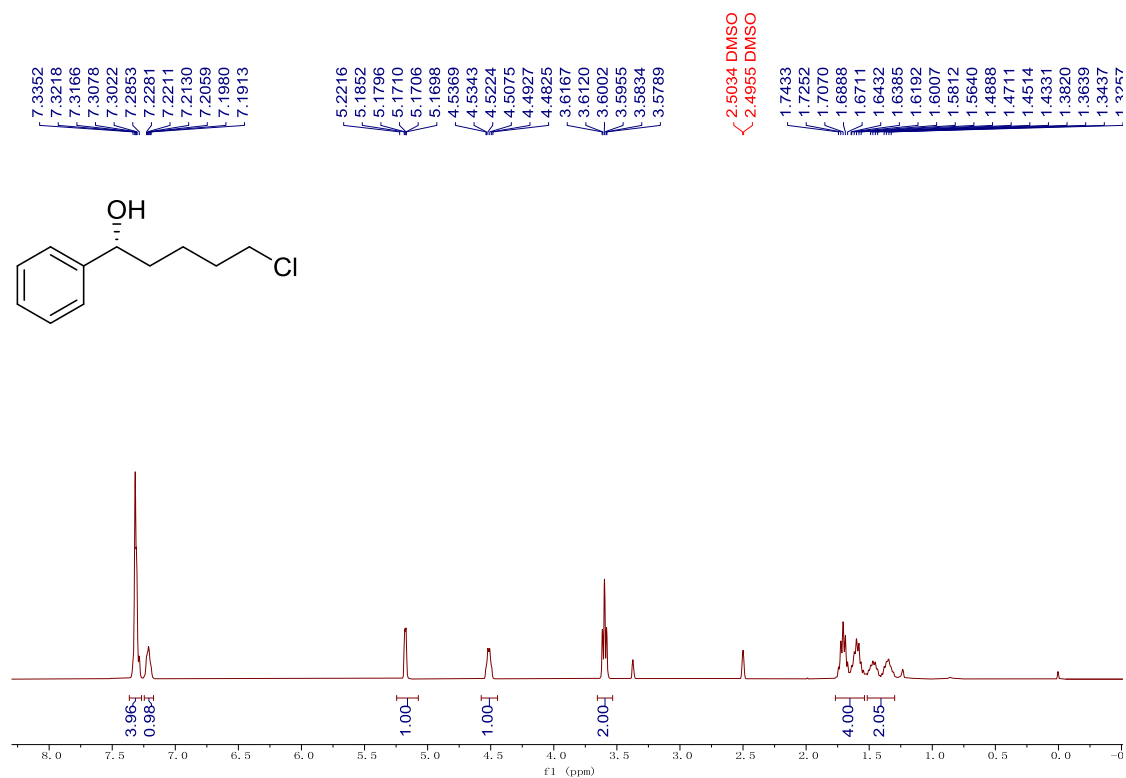
Supplementary Fig. 90 | ¹H NMR (400 MHz, CDCl₃) spectrum of (*rac*)-**1bb**.



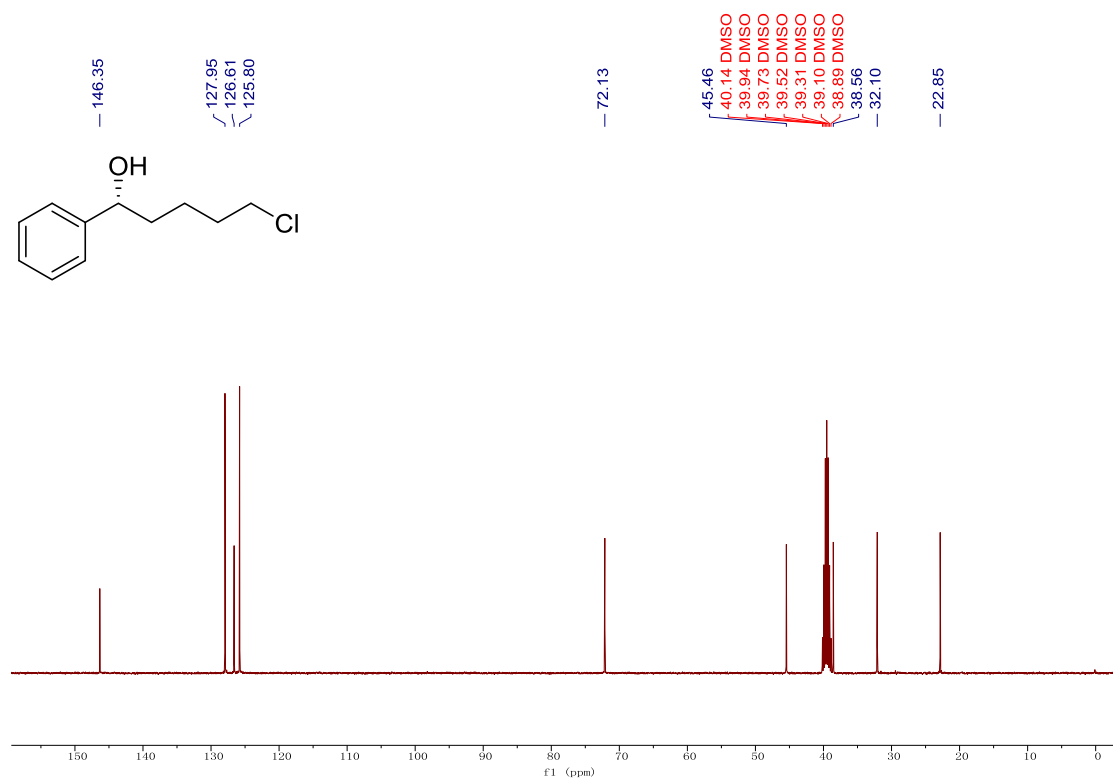
Supplementary Fig. 91 | ¹³C NMR (100 MHz, CDCl₃) spectrum of (*rac*)-**1bb**.

8. Copies of NMR spectra for chiral compounds

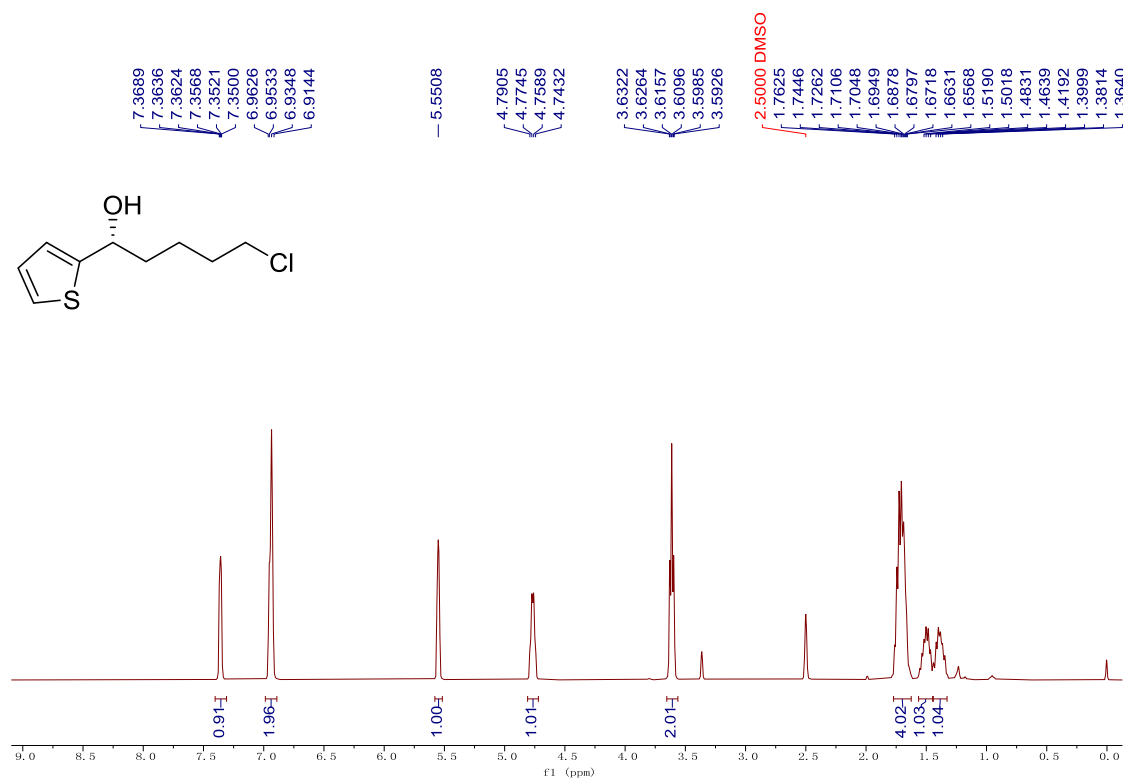
(Supplementary Figures 92–119)



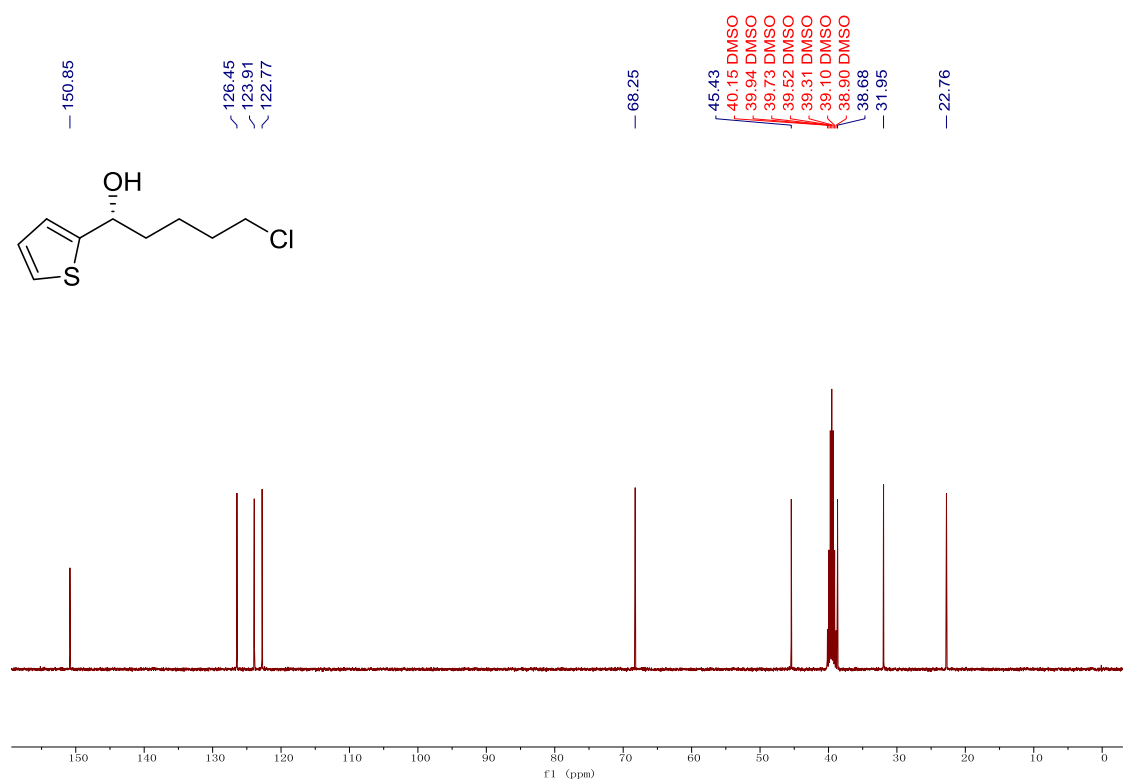
Supplementary Fig. 92 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (R)-1a.



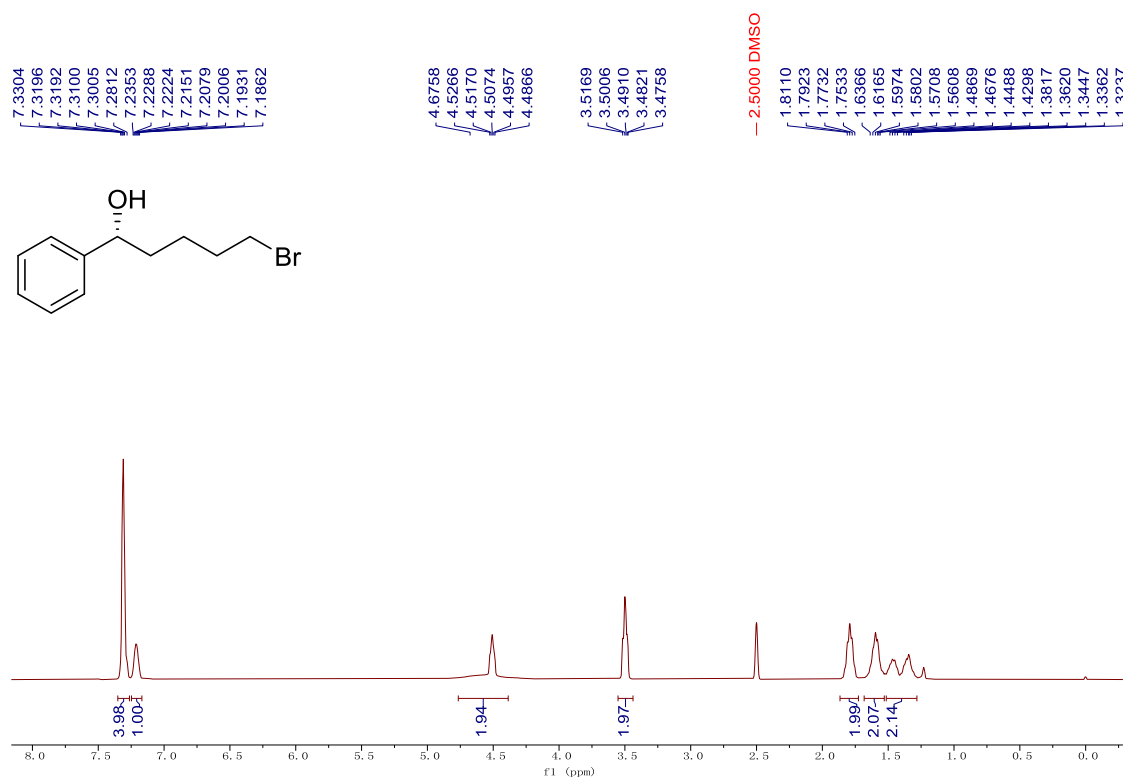
Supplementary Fig. 93 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (R)-1a.



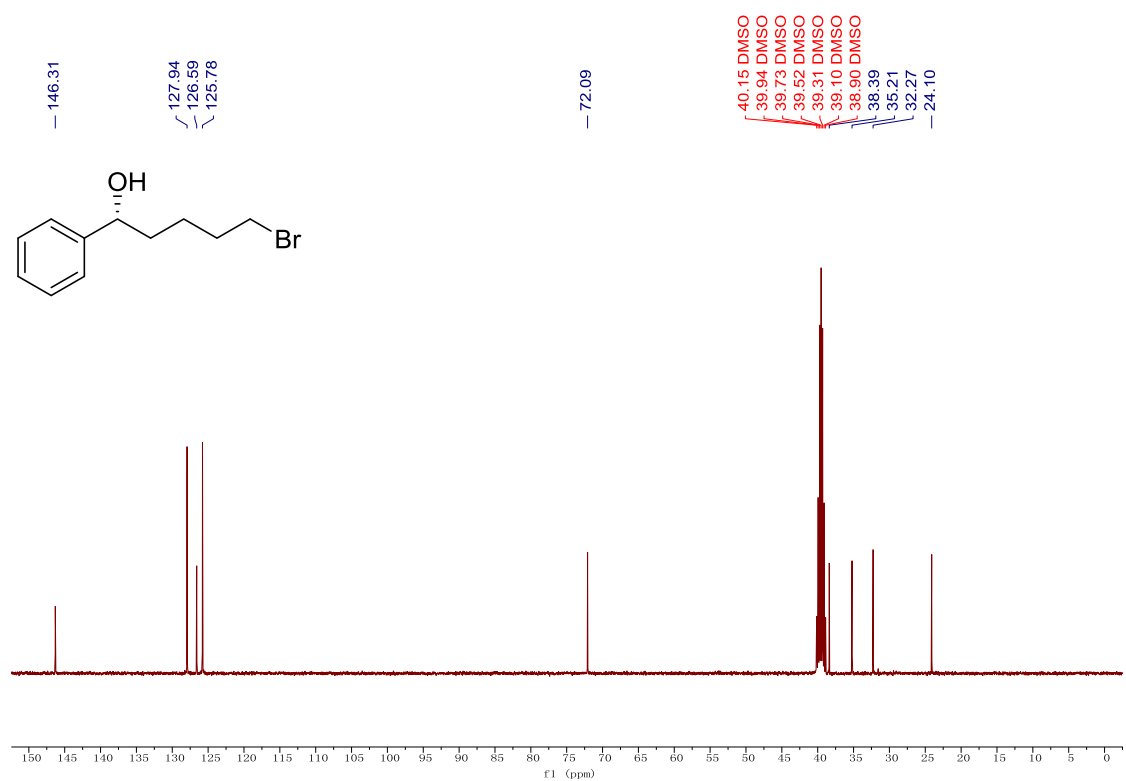
Supplementary Fig. 94 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (R)-10a.



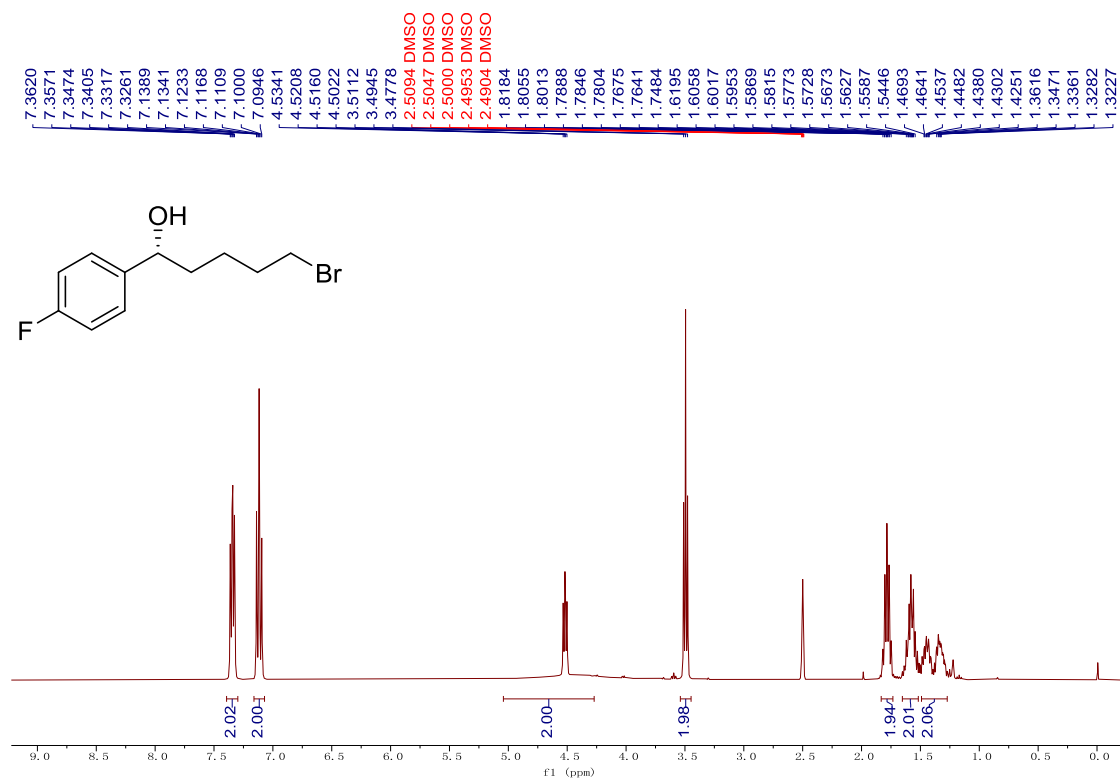
Supplementary Fig. 95 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (R)-10a.



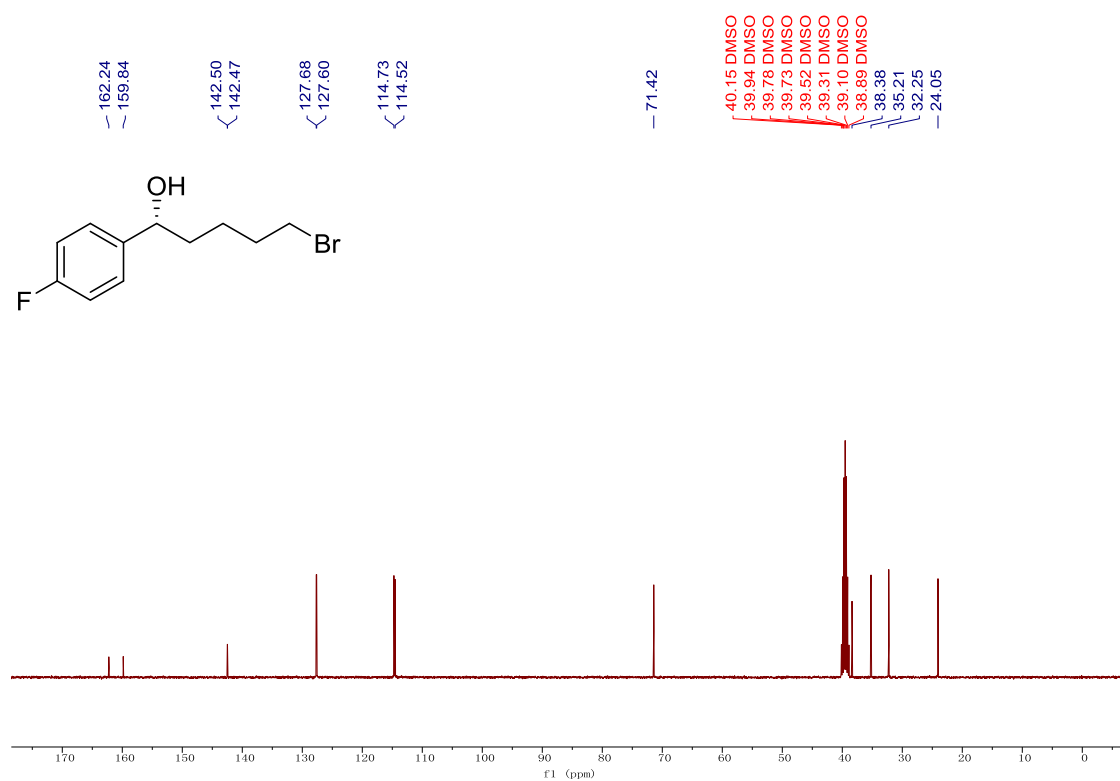
Supplementary Fig. 96 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (*R*)-**12a**.



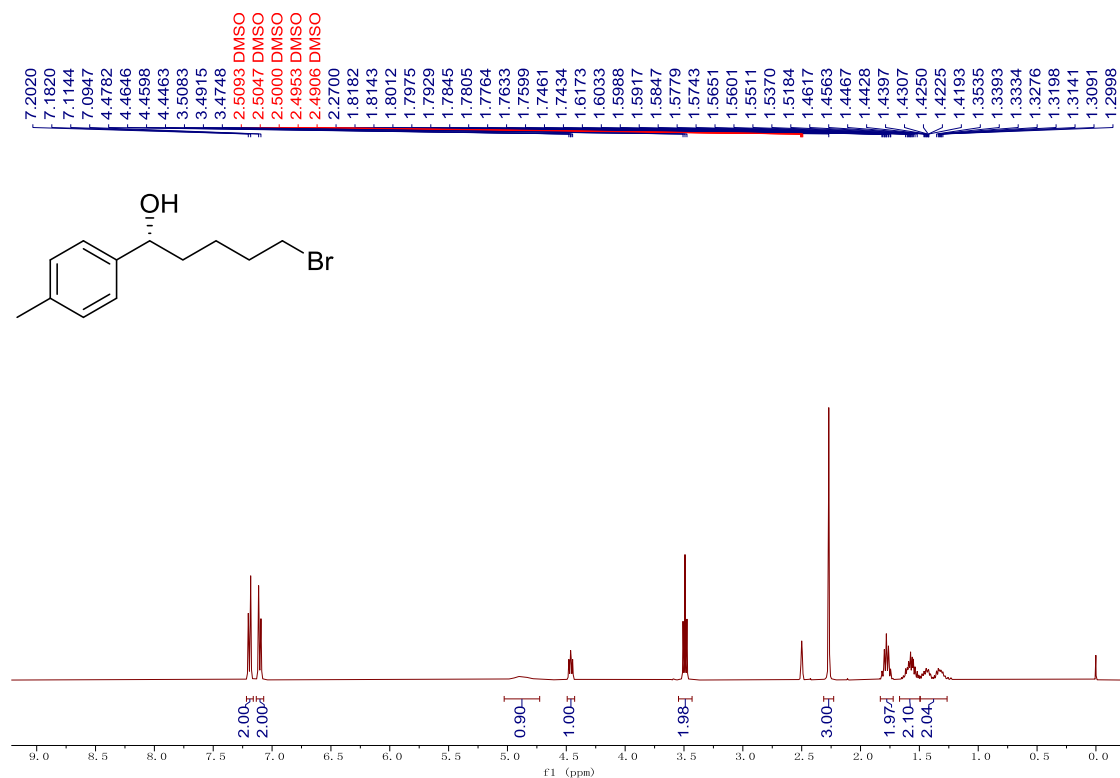
Supplementary Fig. 97 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (*R*)-**12a**.



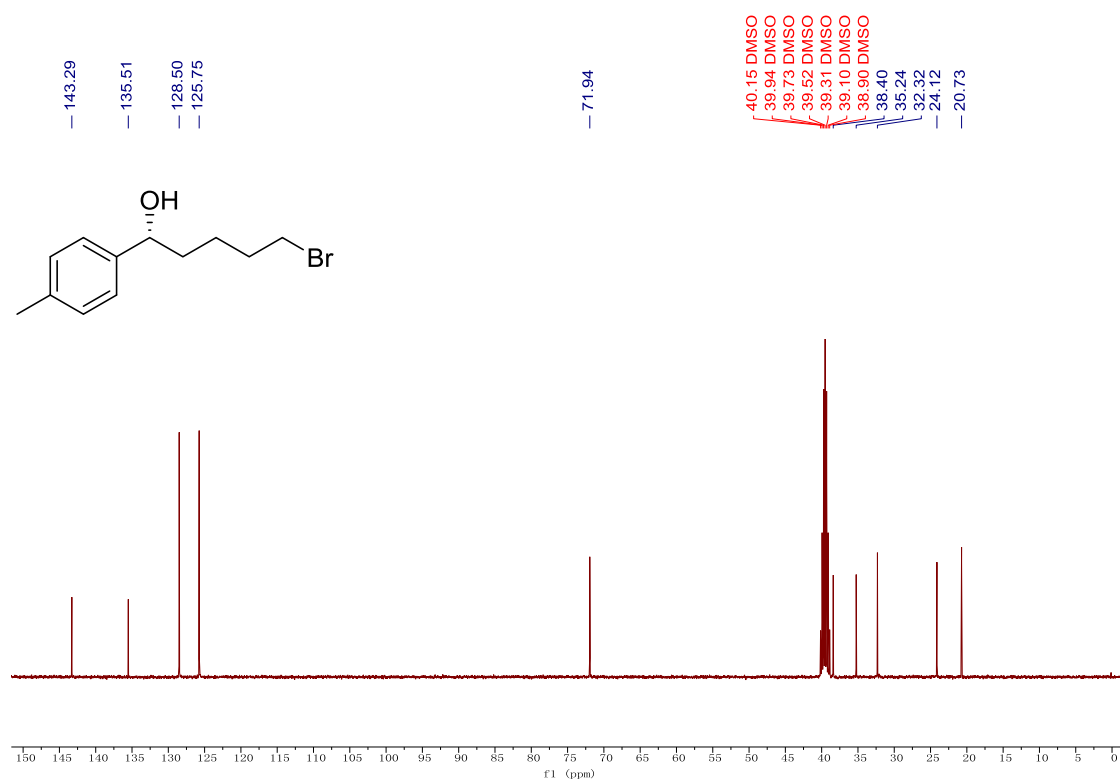
Supplementary Fig. 98 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (R)-18a.



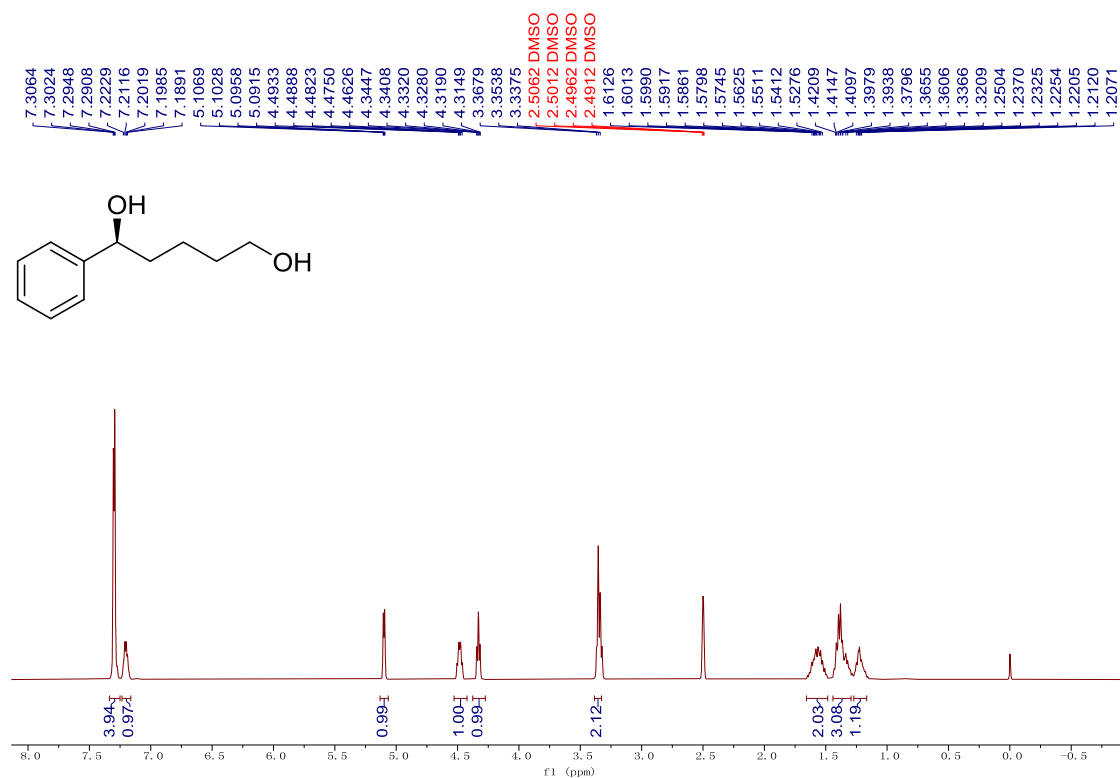
Supplementary Fig. 99 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (R)-18a.



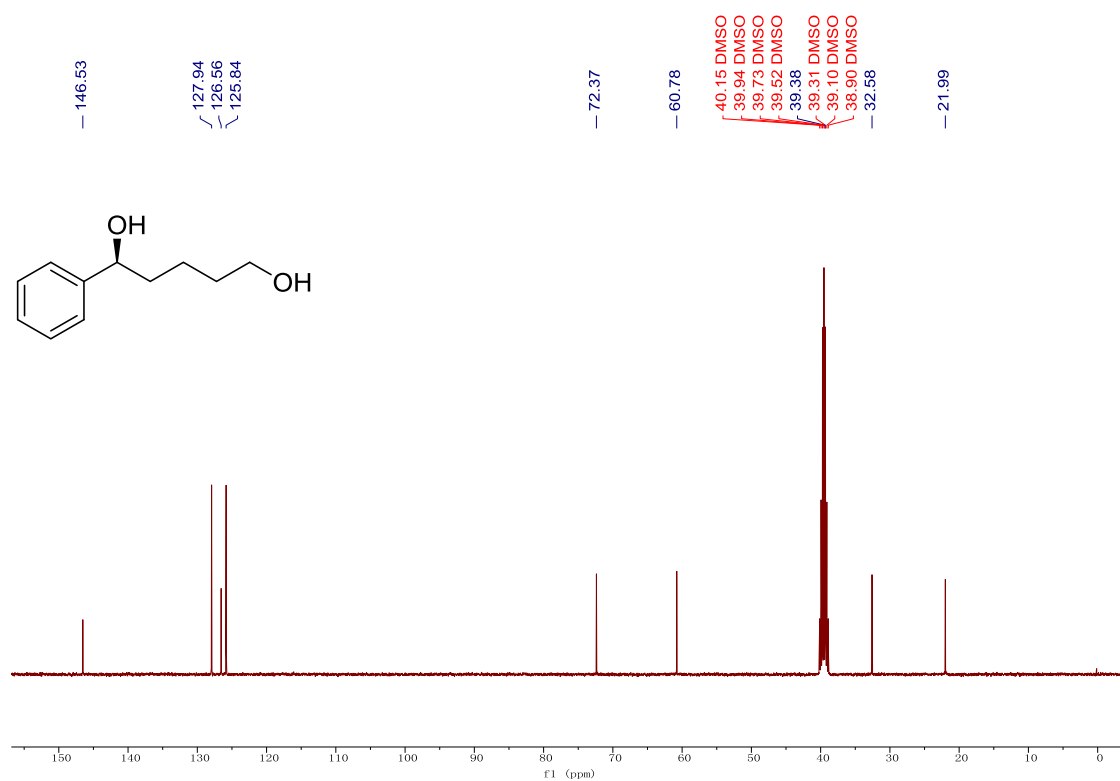
Supplementary Fig. 100 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (R)-20a.



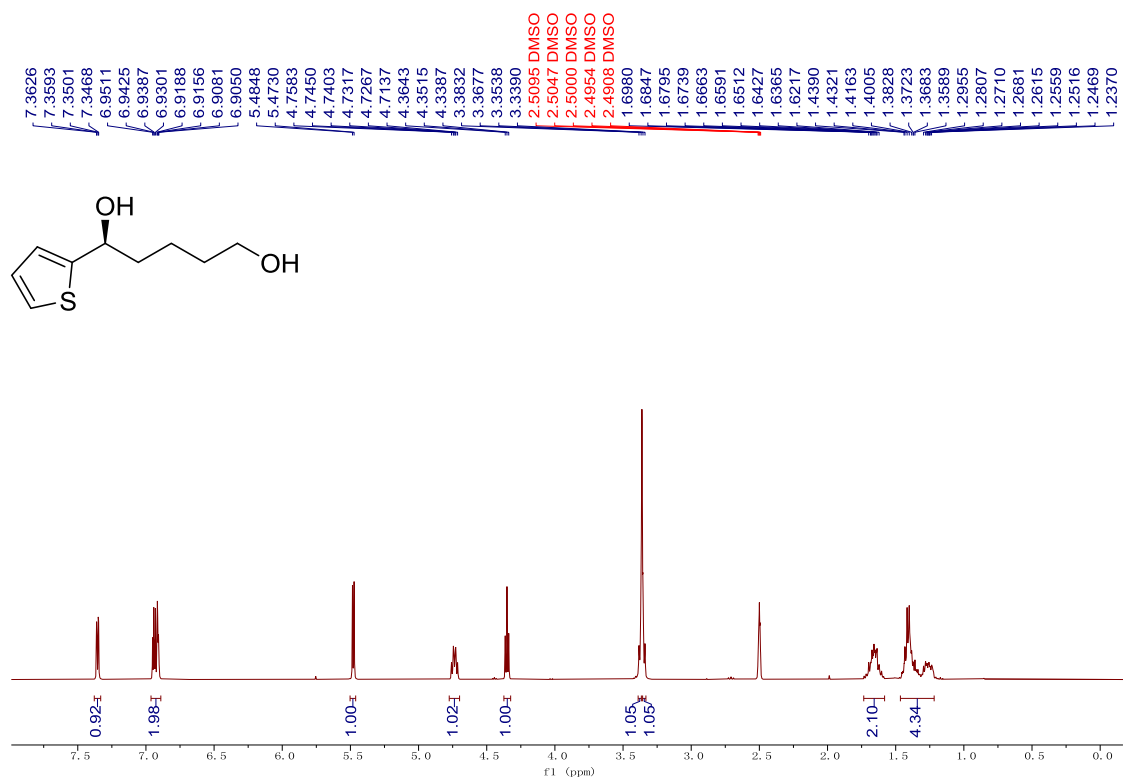
Supplementary Fig. 101 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (R)-20a.



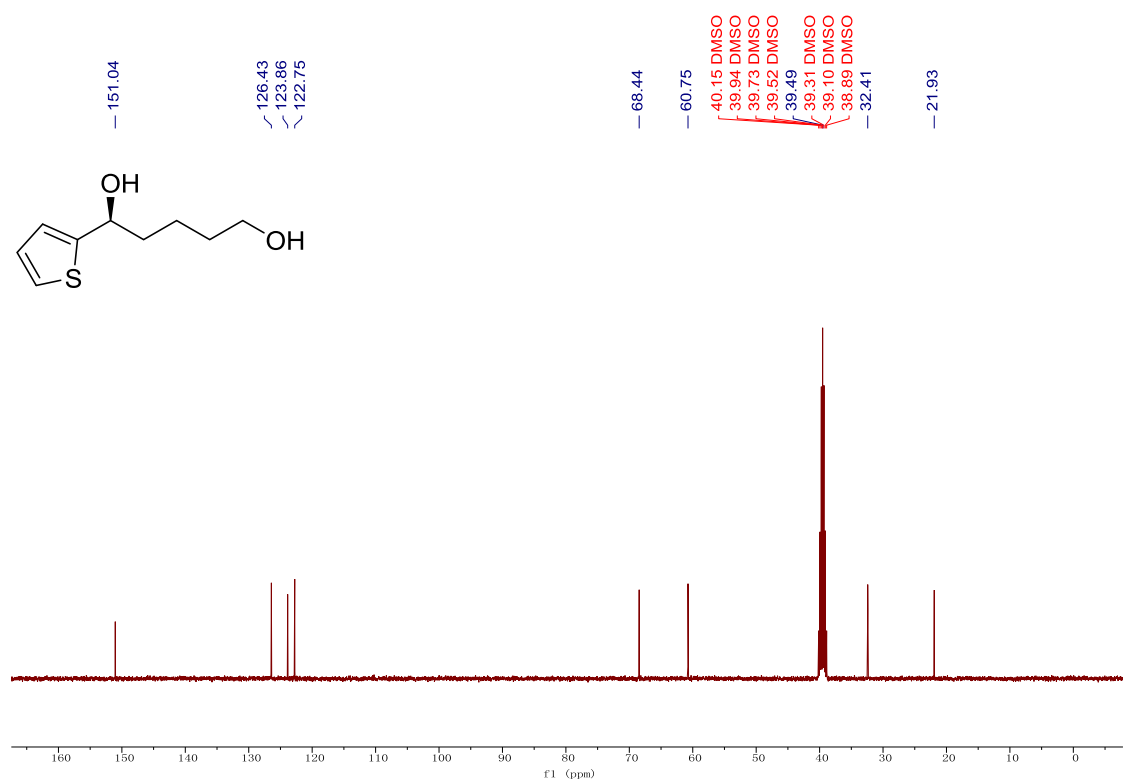
Supplementary Fig. 102 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (*S*)-1b.



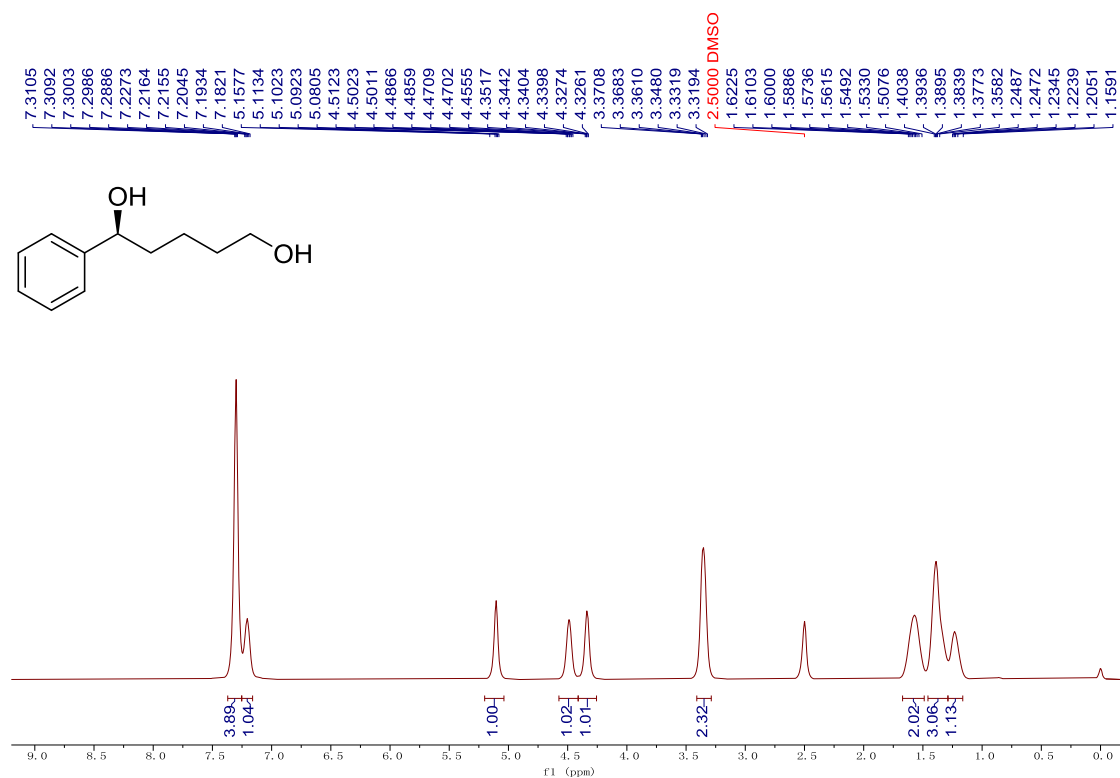
Supplementary Fig. 103 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (*S*)-1b.



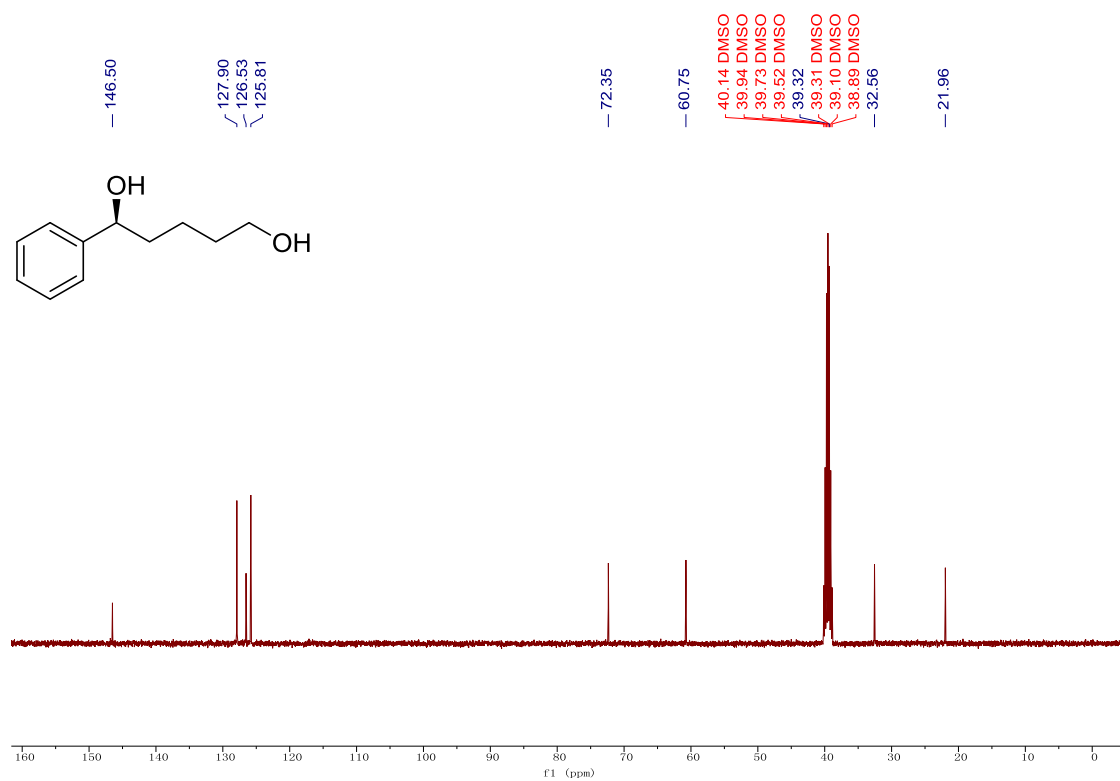
Supplementary Fig. 104 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (S)-10b.



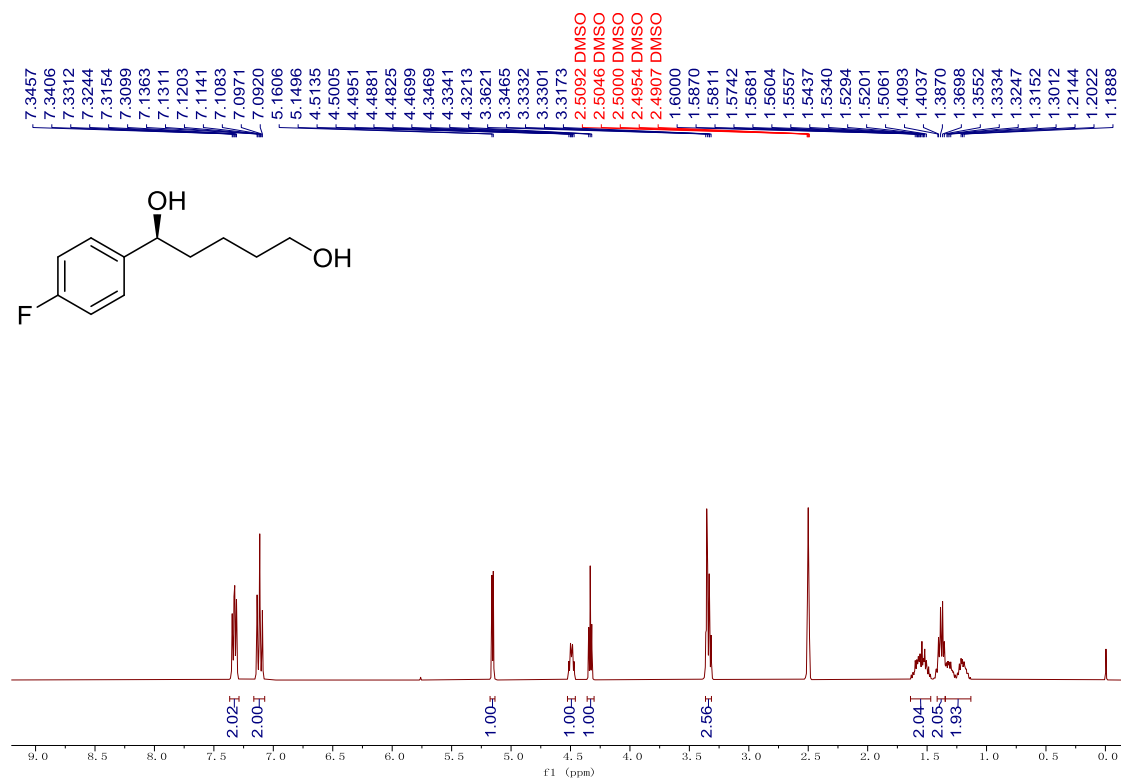
Supplementary Fig. 105 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (S)-10b.



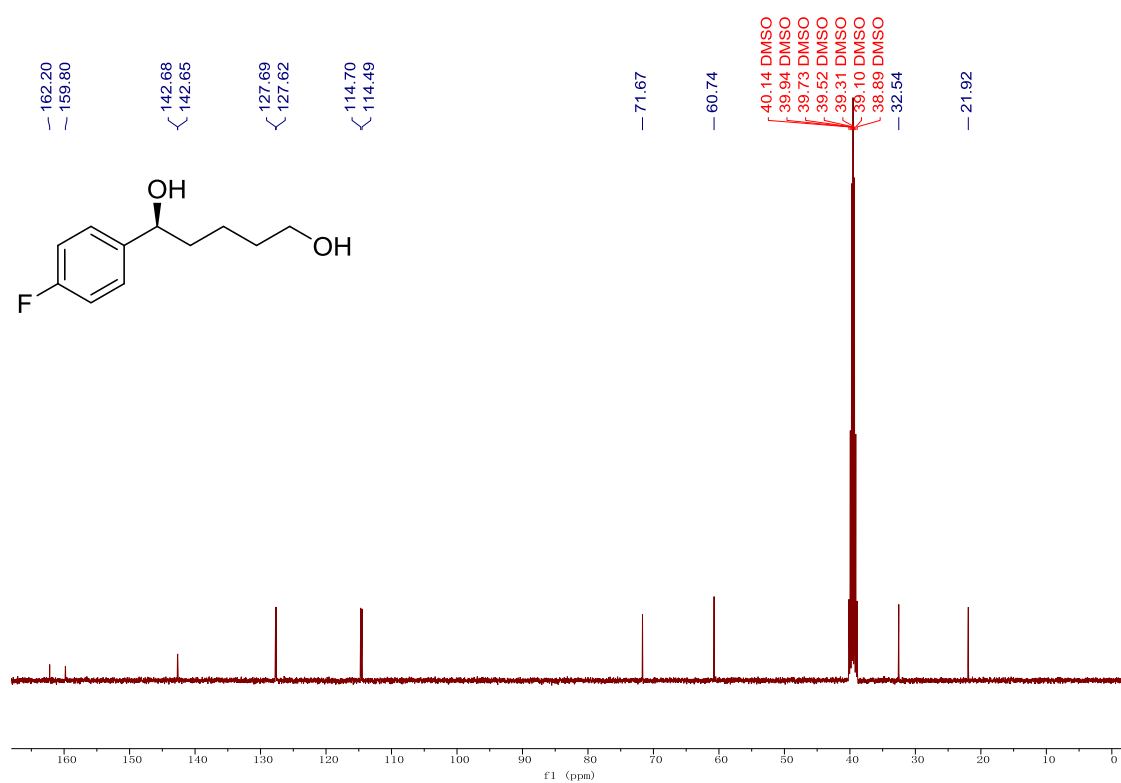
Supplementary Fig. 106 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of *(S)*-1b from 12a.



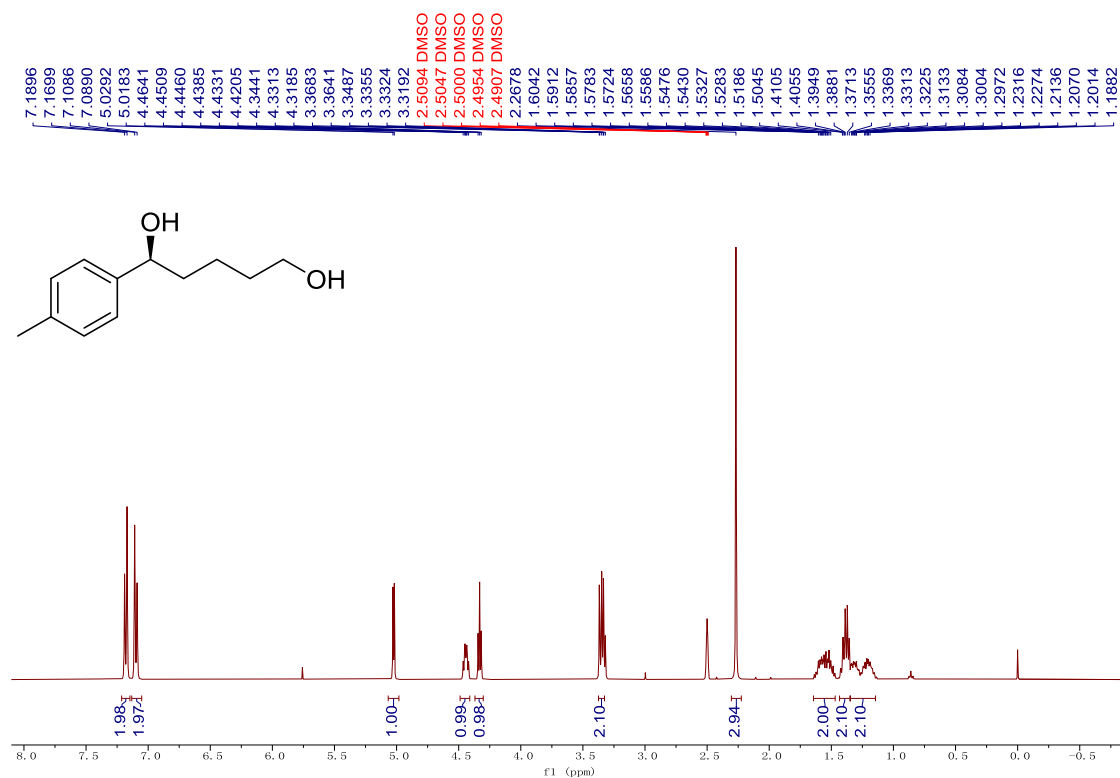
Supplementary Fig. 107 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of *(S)*-1b from 20a.



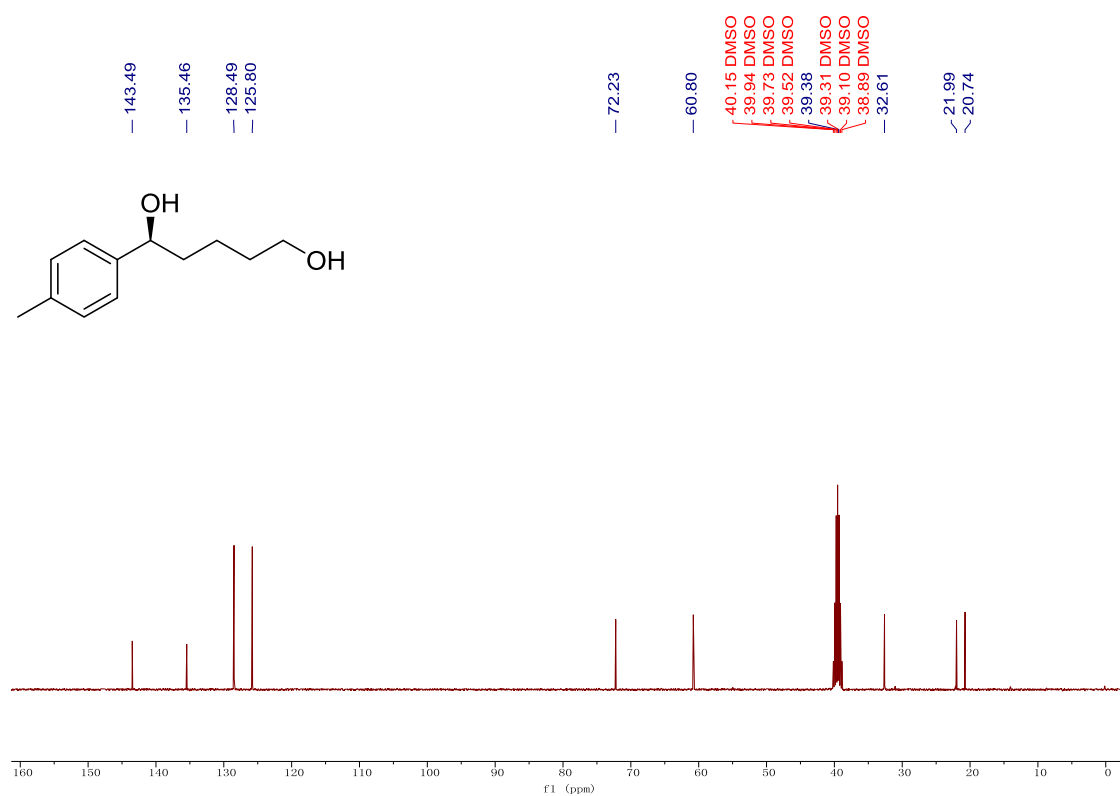
Supplementary Fig. 108 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (S)-18b.



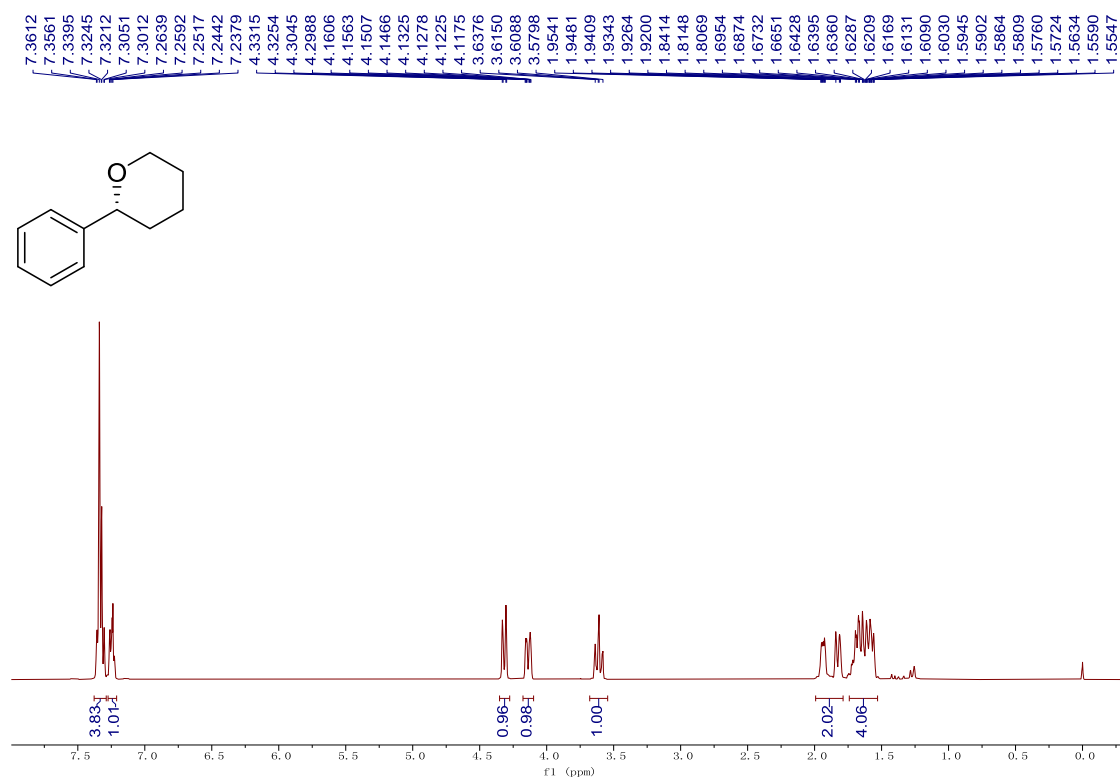
Supplementary Fig. 109 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (S)-18b.



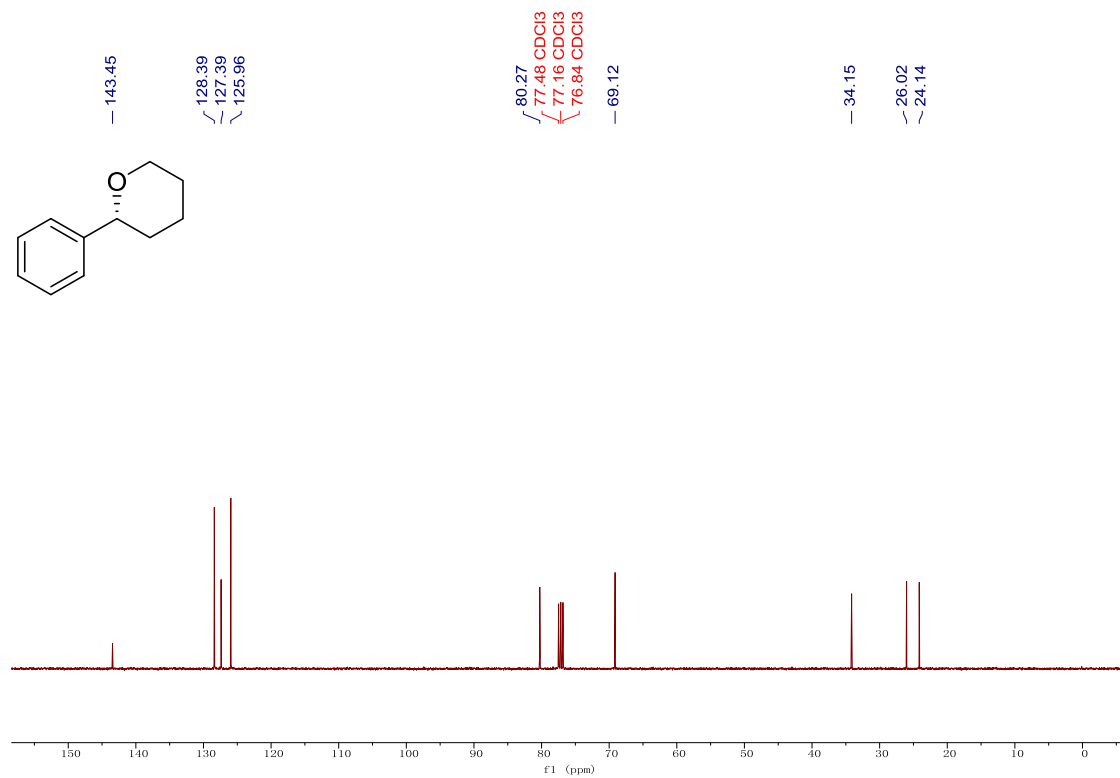
Supplementary Fig. 110 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (S)-7b from 20a.



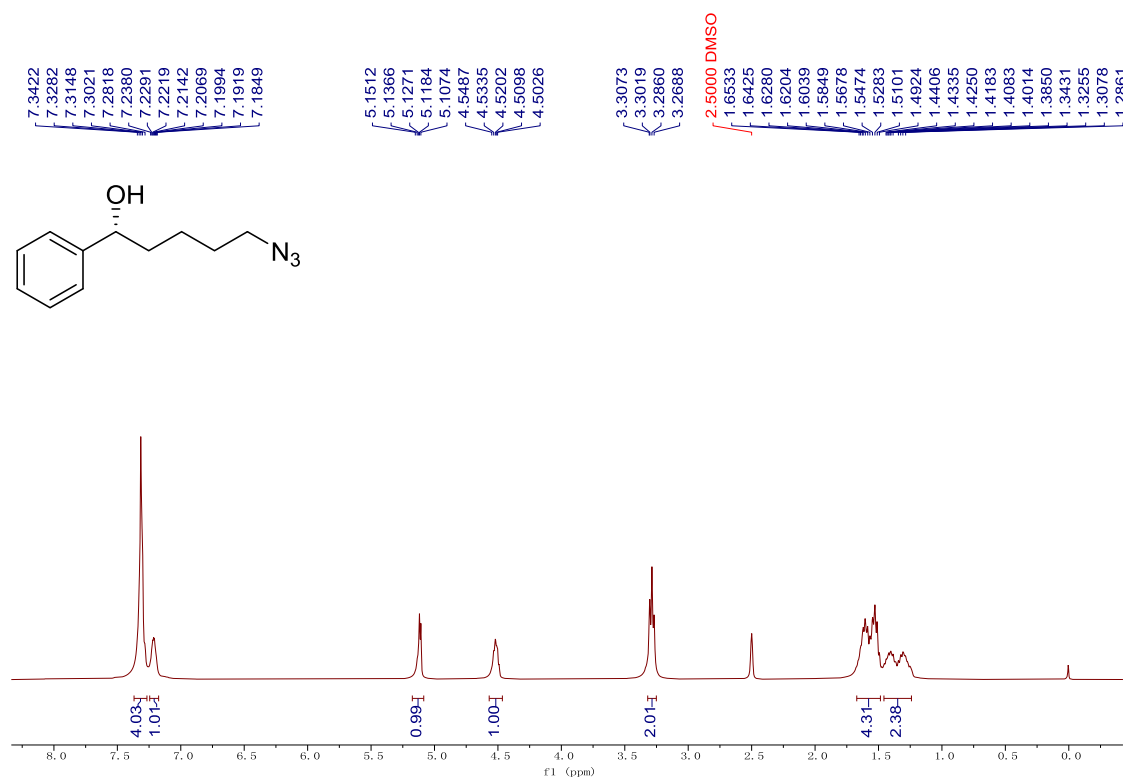
Supplementary Fig. 111 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (S)-7b from 20a.



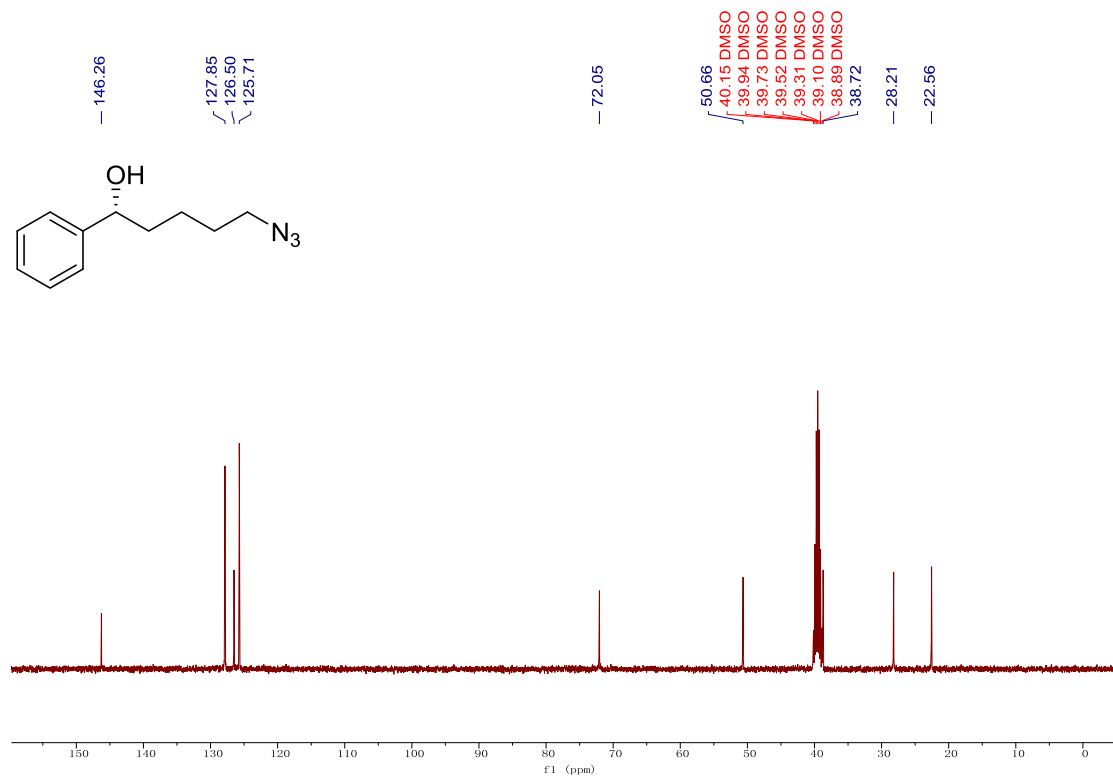
Supplementary Fig. 112 | ¹H NMR (400 MHz, CDCl₃) spectrum of (R)-12aa.



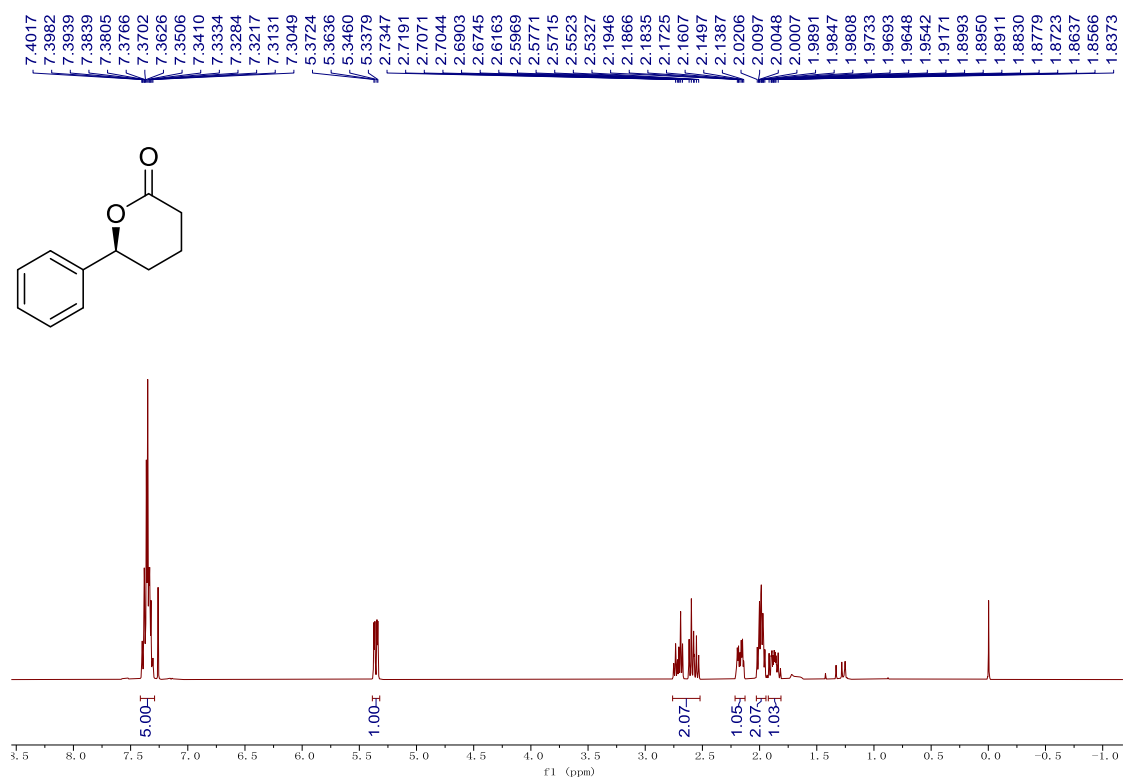
Supplementary Fig. 113 | ¹³C NMR (100 MHz, CDCl₃) spectrum of (R)-12aa.



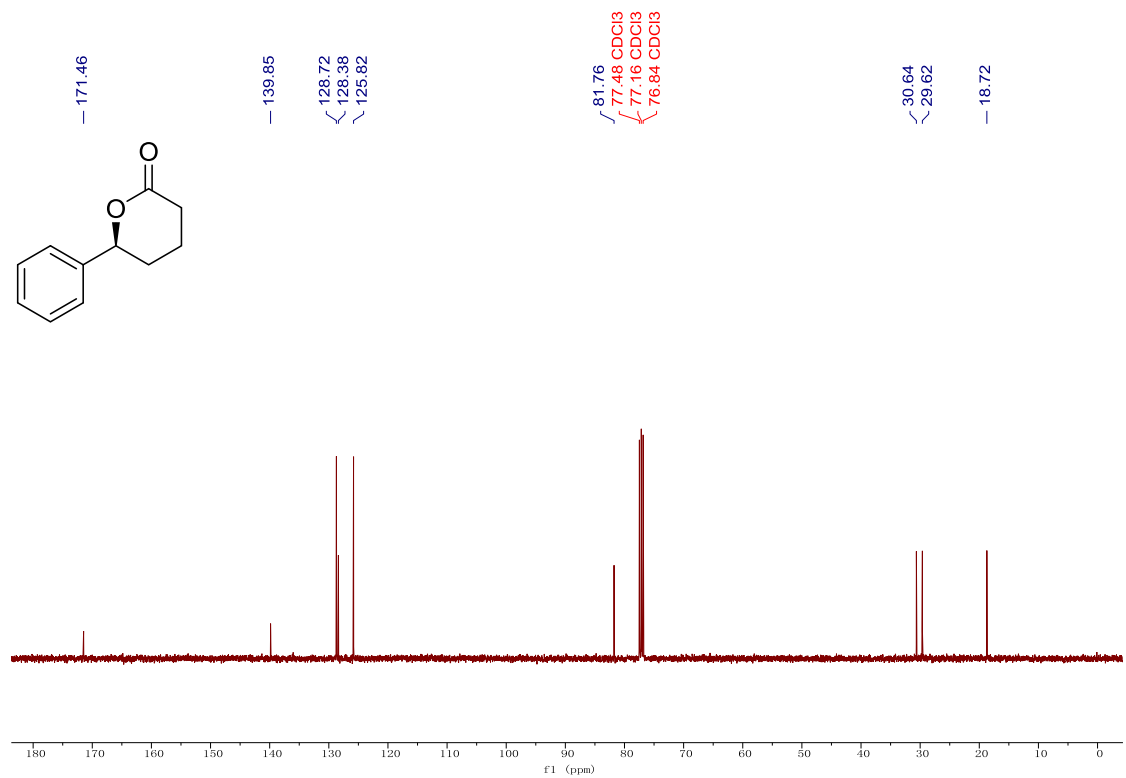
Supplementary Fig. 114 | ¹H NMR (400 MHz, DMSO-*d*₆) spectrum of (R)-12ab.



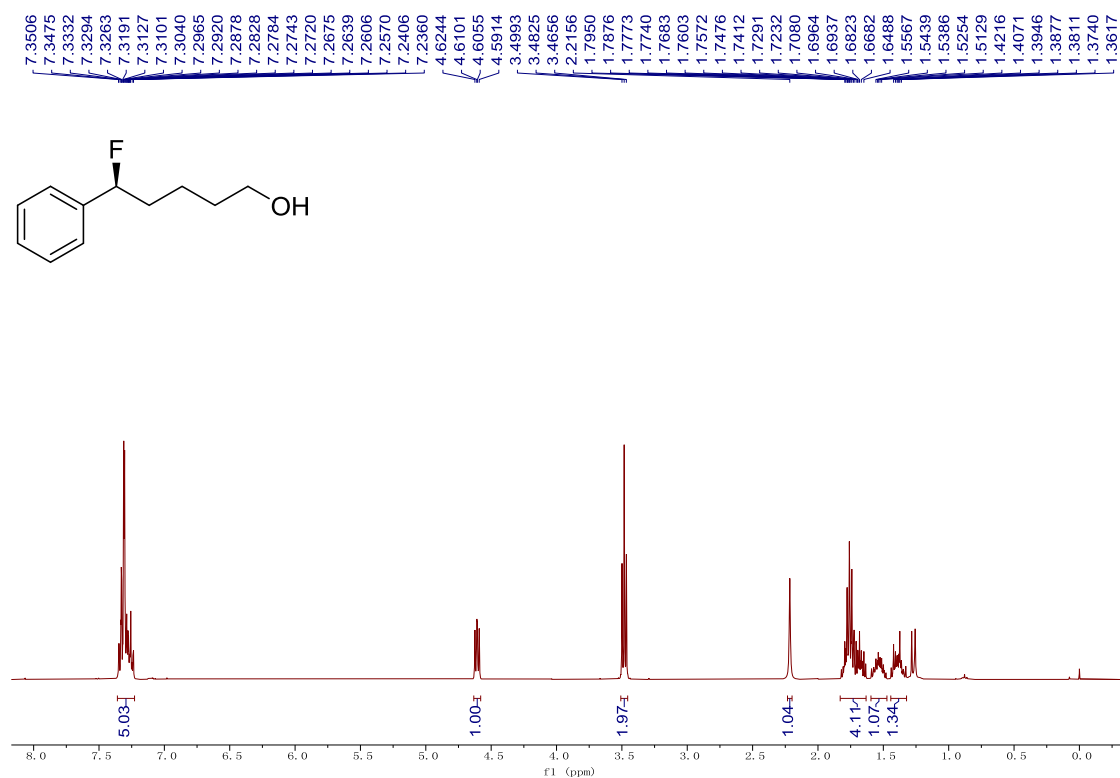
Supplementary Fig. 115 | ¹³C NMR (100 MHz, DMSO-*d*₆) spectrum of (R)-12ab.



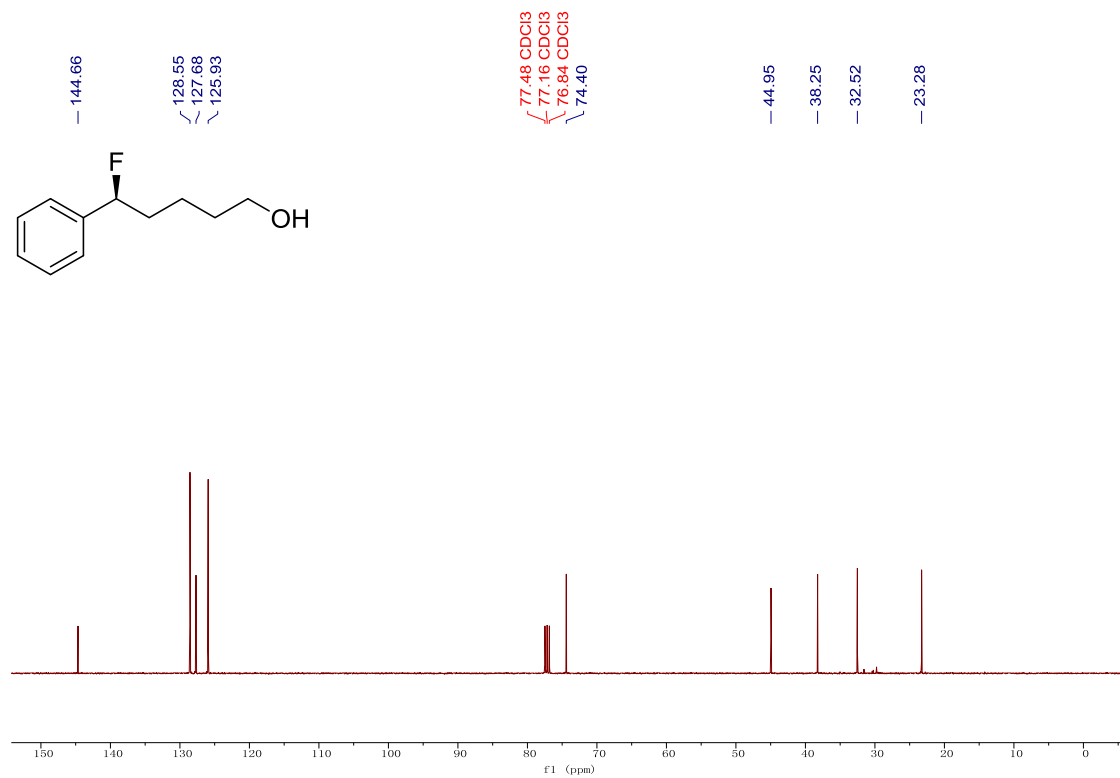
Supplementary Fig. 116 | ¹H NMR (400 MHz, CDCl₃) spectrum of (S)-1ba.



Supplementary Fig. 117 | ¹³C NMR (100 MHz, CDCl₃) spectrum of (S)-1ba.



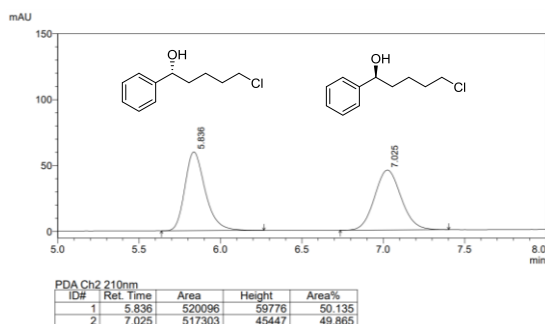
Supplementary Fig. 118 | ¹H NMR (400 MHz, CDCl₃) spectrum of (S)-1bb.



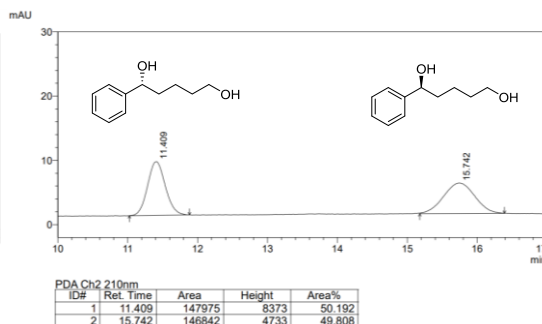
Supplementary Fig. 119 | ¹³C NMR (100 MHz, CDCl₃) spectrum of (S)-1bb.

9. Chromatography analysis for biotransformation

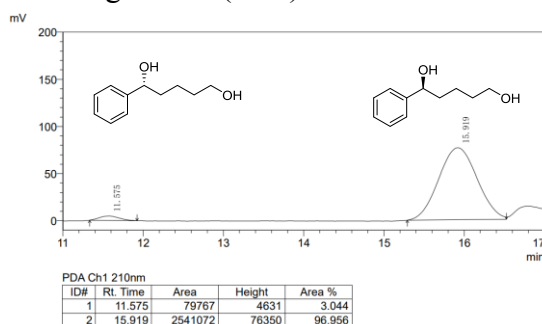
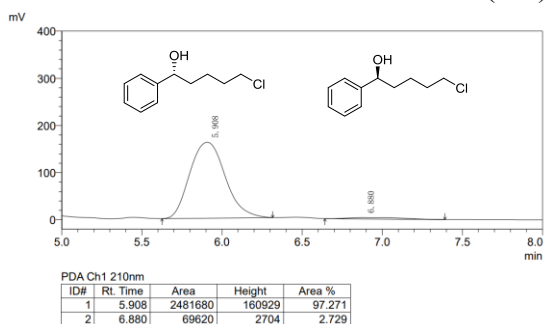
(rac)-1a



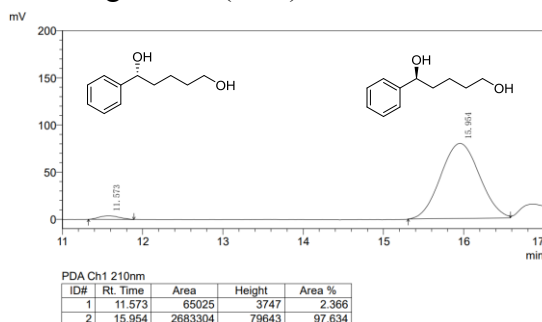
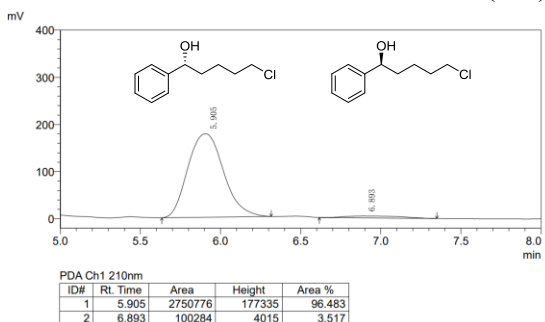
(rac)-1b



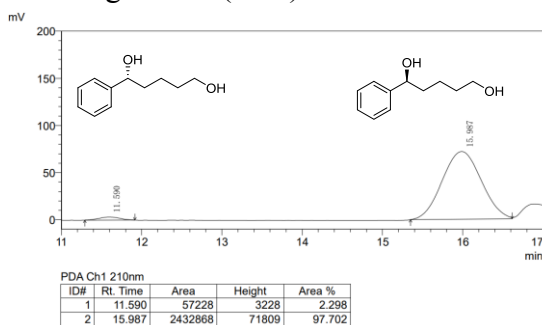
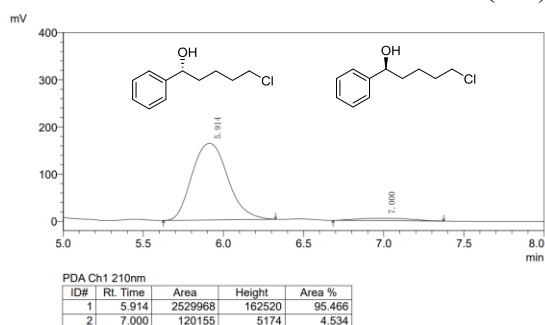
Biotransformation of (rac)-1a using *E. coli* (εM6) cells



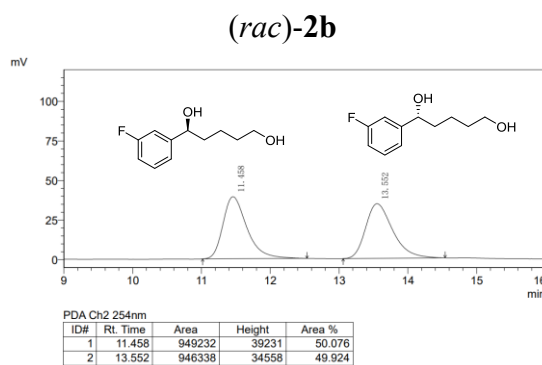
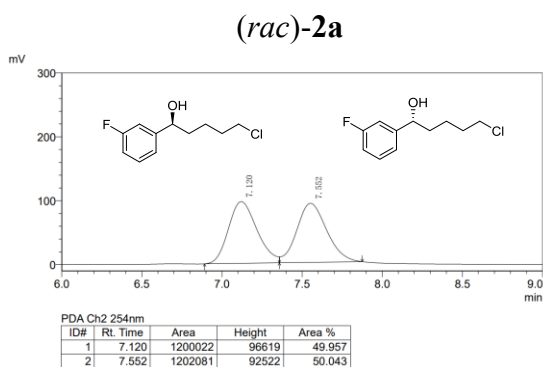
Biotransformation of (rac)-1a using *E. coli* (εM7) cells



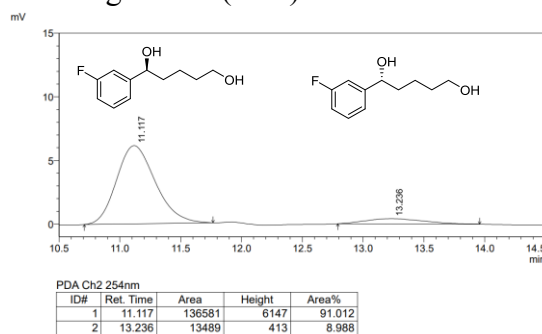
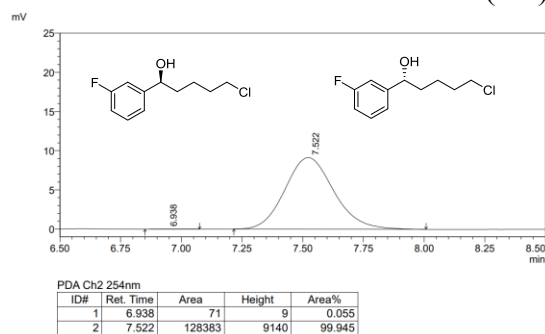
Biotransformation of (rac)-1a using *E. coli* (εM8) cells



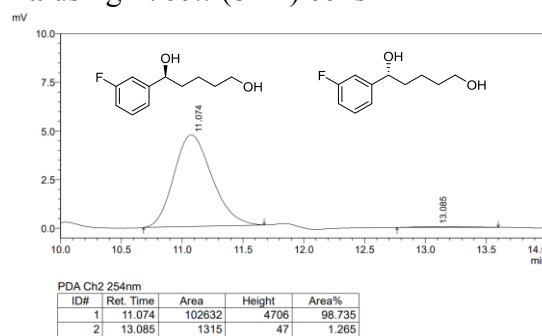
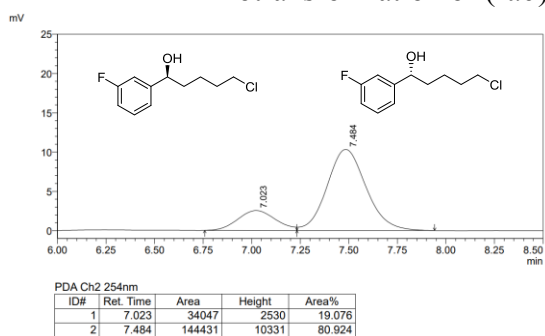
Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 5.8 min, $t_{(S)}$ = 7.0 min; $t_{(R)}$ = 11.4 min, $t_{(S)}$ = 15.7 min.



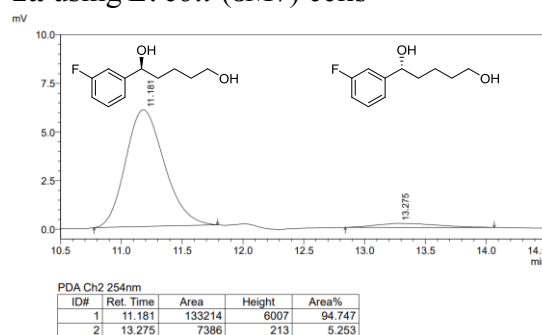
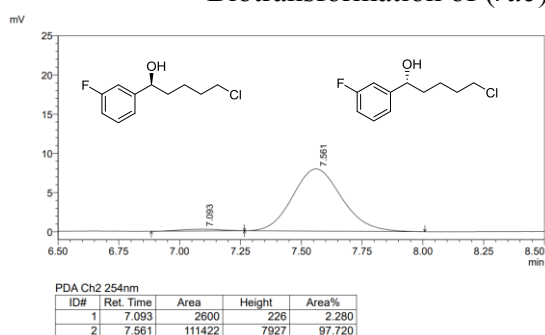
Biotransformation of (rac)-2a using *E. coli* (εM2) cells



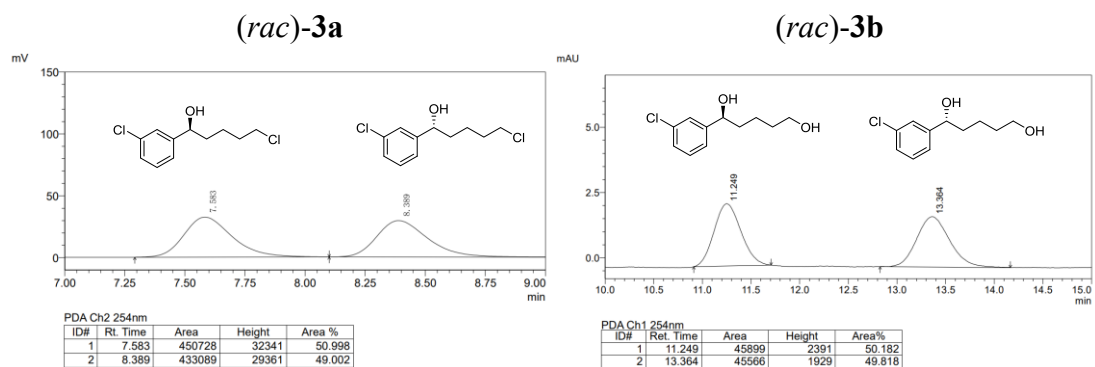
Biotransformation of (rac)-2a using *E. coli* (εM4) cells



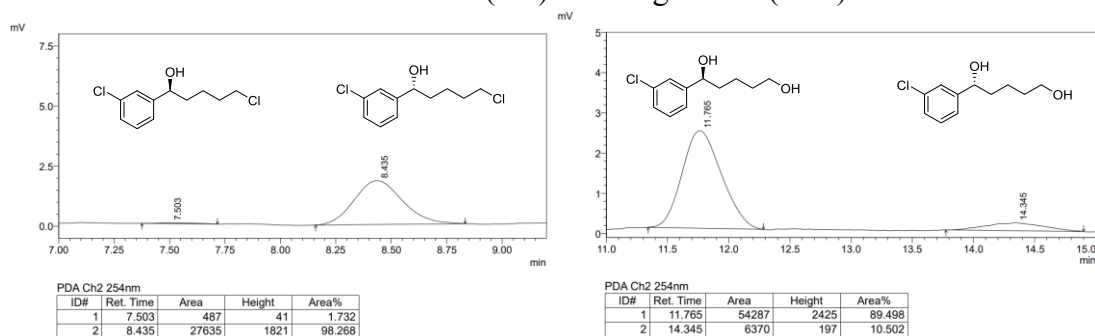
Biotransformation of (rac)-2a using *E. coli* (εM7) cells



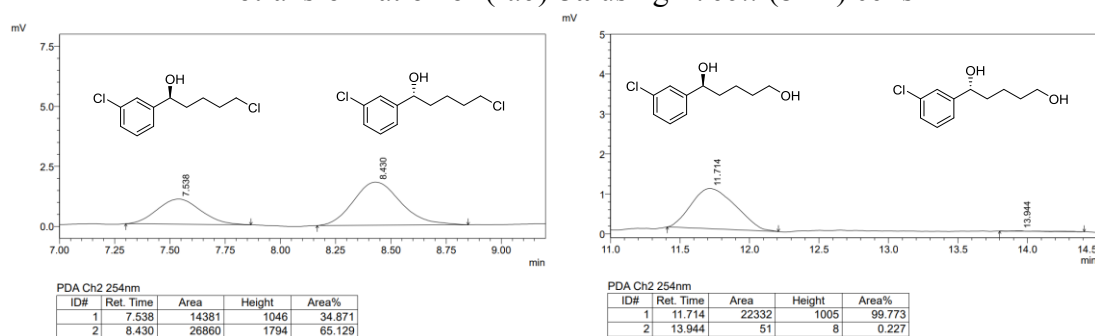
Chiral HPLC analysis: Diacel Chiralpak OD-H, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 254 nm, $t_{(S)}$ = 7.1 min, $t_{(R)}$ = 7.5 min; $t_{(S)}$ = 11.5 min, $t_{(R)}$ = 13.5 min.



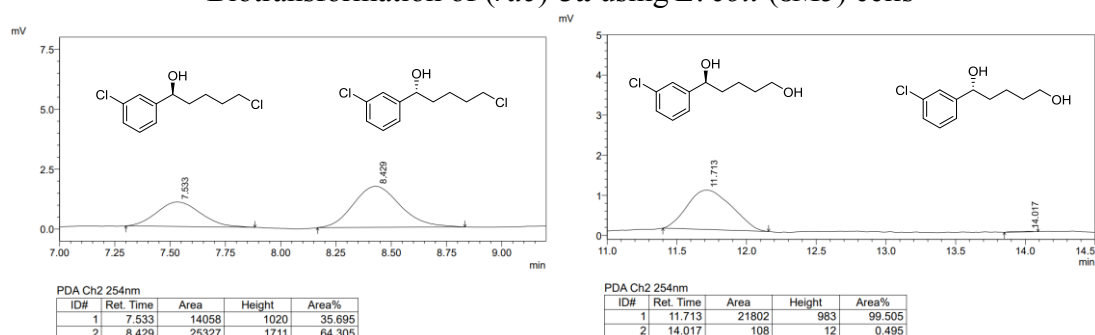
Biotransformation of (rac)-3a using *E. coli* (εM2) cells



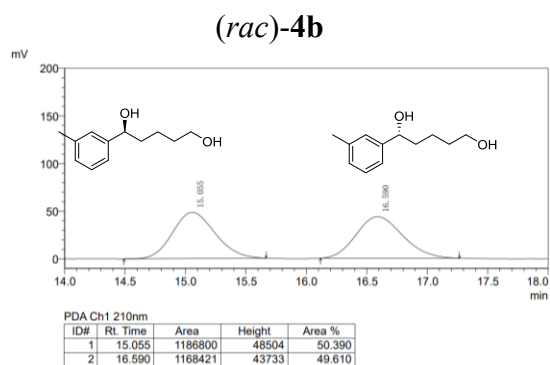
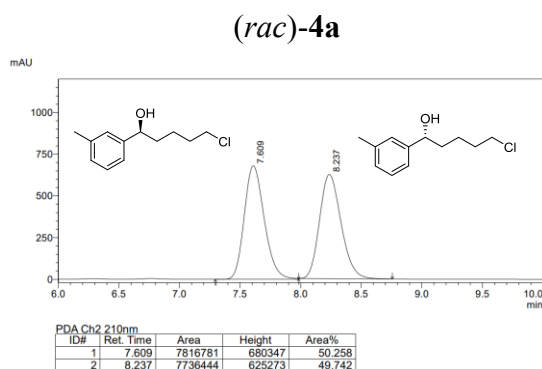
Biotransformation of (rac)-3a using *E. coli* (εM4) cells



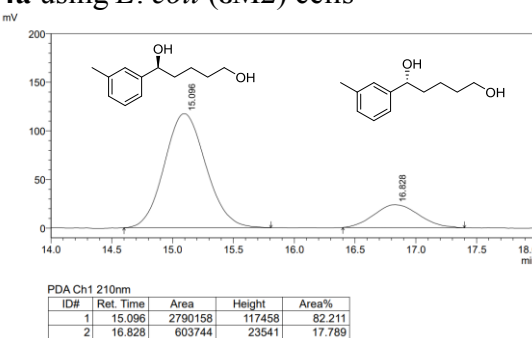
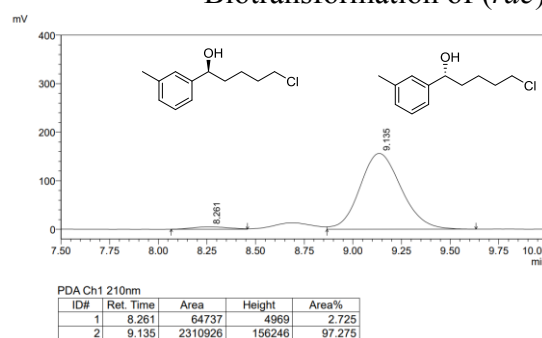
Biotransformation of (rac)-3a using *E. coli* (εM5) cells



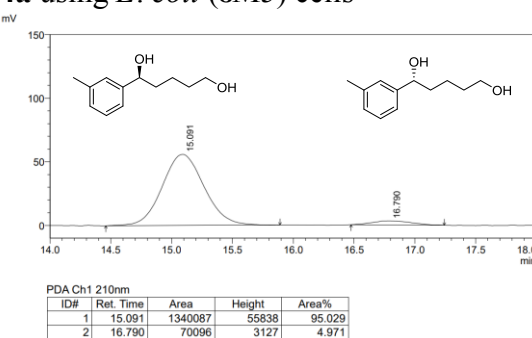
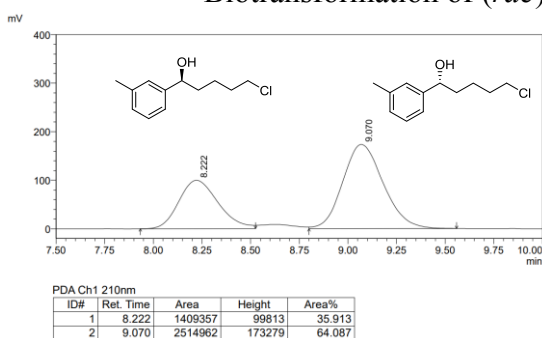
Chiral HPLC analysis: Diacel Chiralpak OD-H, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 254 nm, $t_{(S)}$ = 7.5 min, $t_{(R)}$ = 8.3 min; $t_{(S)}$ = 11.2 min, $t_{(R)}$ = 13.3 min.



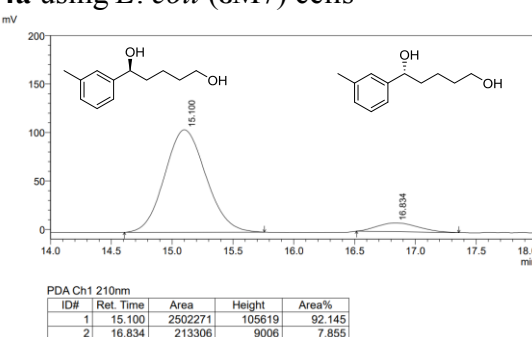
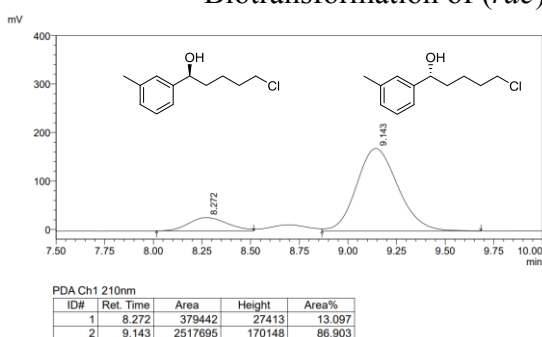
Biotransformation of (rac)-4a using *E. coli* (εM2) cells



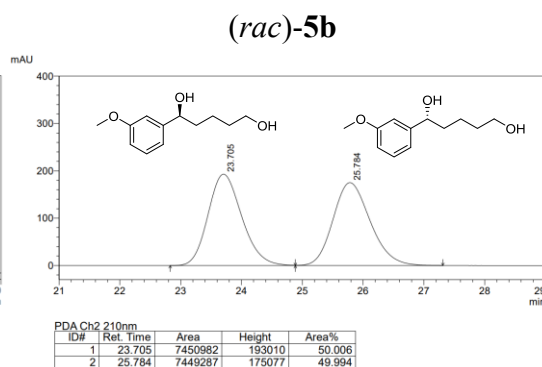
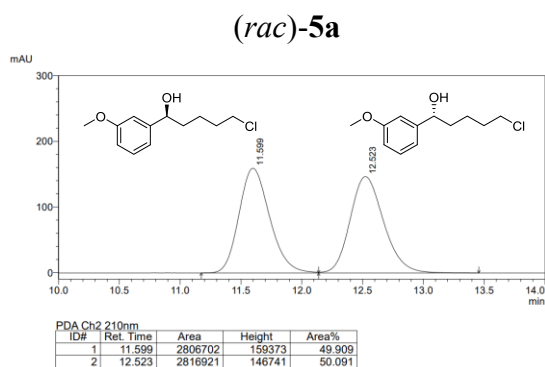
Biotransformation of (rac)-4a using *E. coli* (εM5) cells



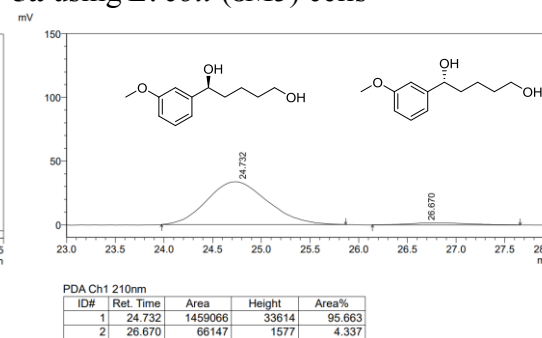
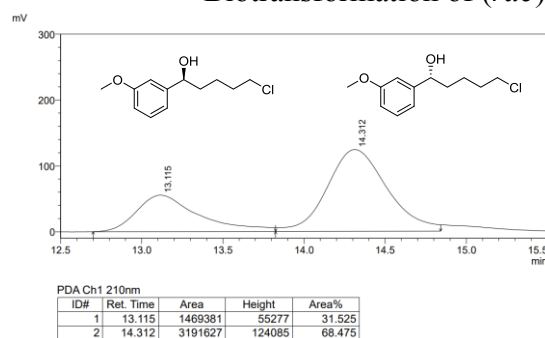
Biotransformation of (rac)-4a using *E. coli* (εM7) cells



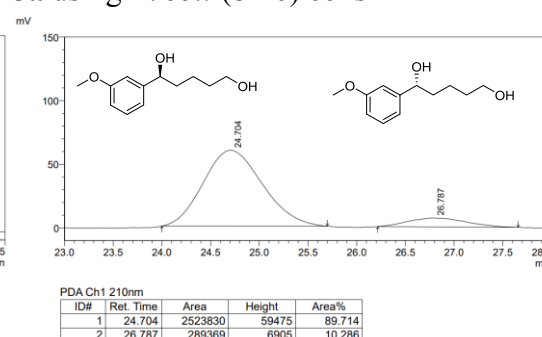
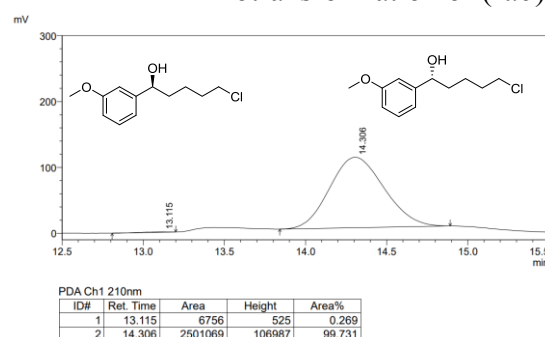
Chiral HPLC analysis: Diacel Chiralpak AD-H, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 210 nm, $t_{(S)}$ = 7.6 min, $t_{(R)}$ = 8.2 min; $t_{(S)}$ = 14.4 min, $t_{(R)}$ = 15.8 min.



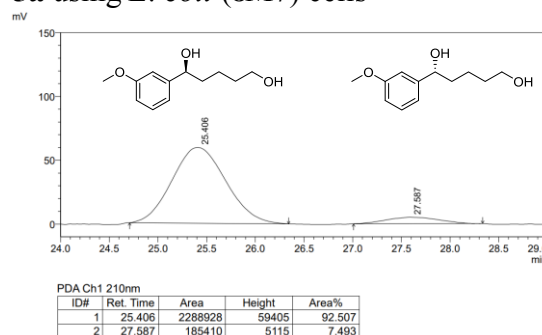
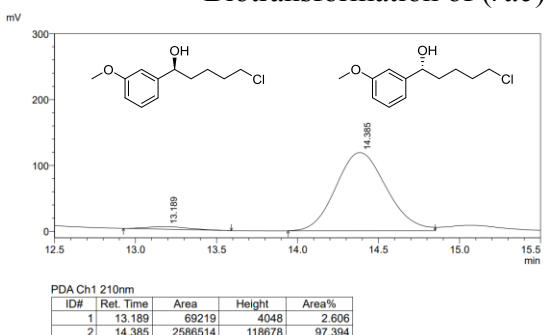
Biotransformation of (rac)-5a using *E. coli* (εM5) cells



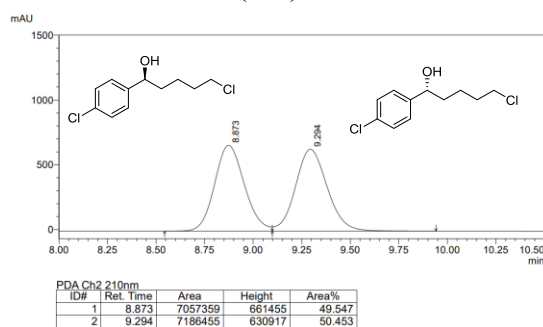
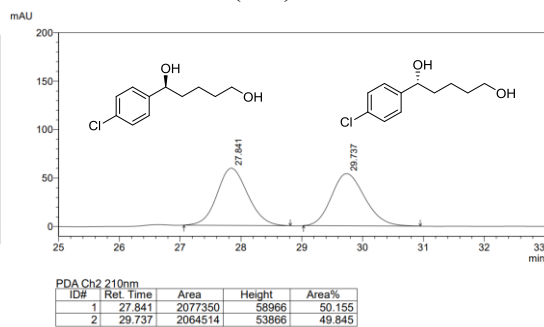
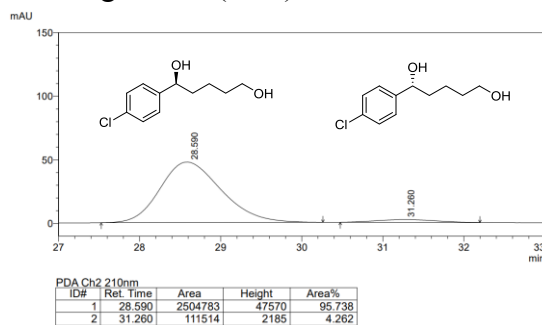
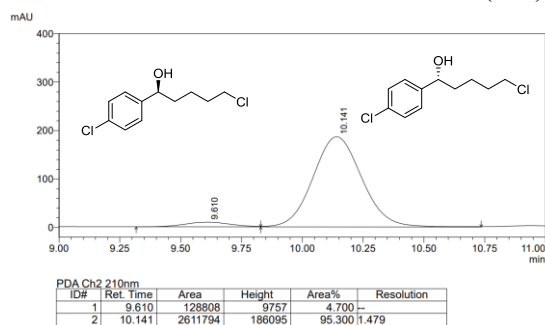
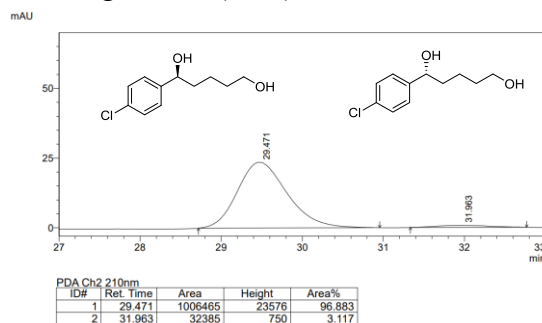
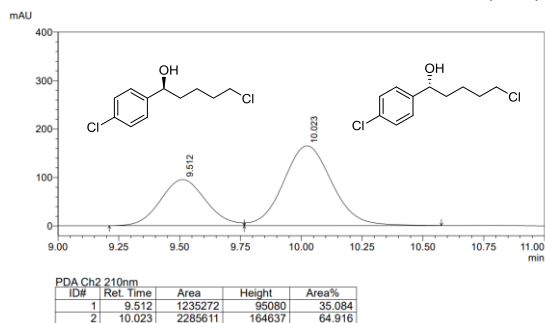
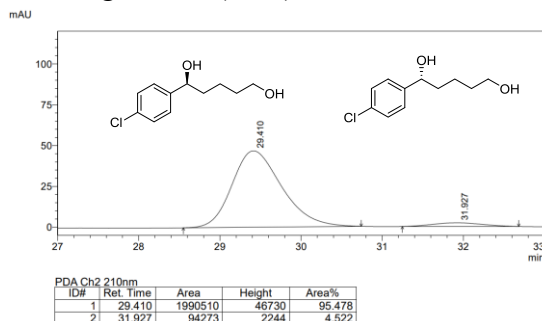
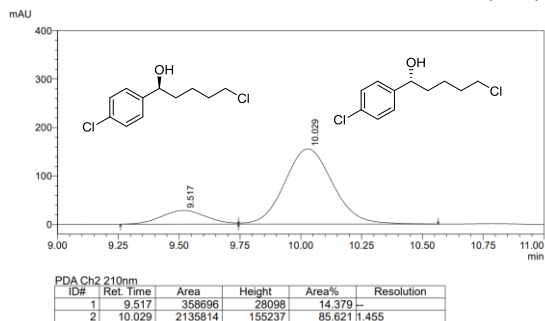
Biotransformation of (rac)-5a using *E. coli* (εM6) cells



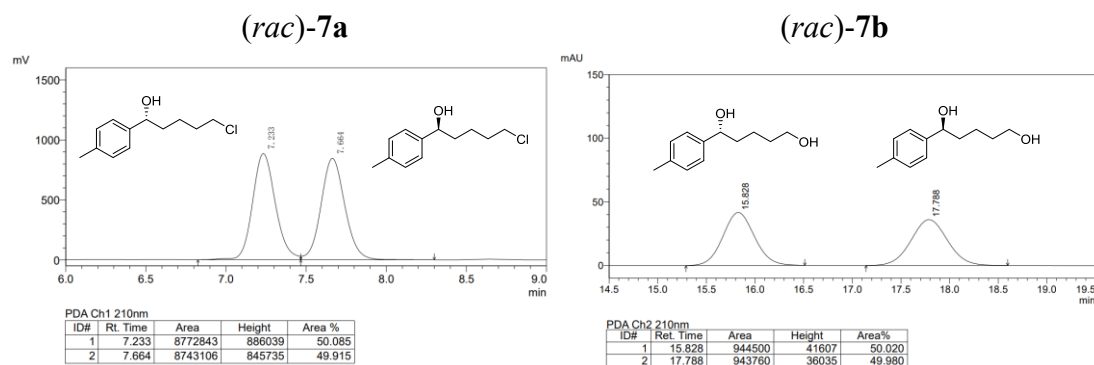
Biotransformation of (rac)-5a using *E. coli* (εM7) cells



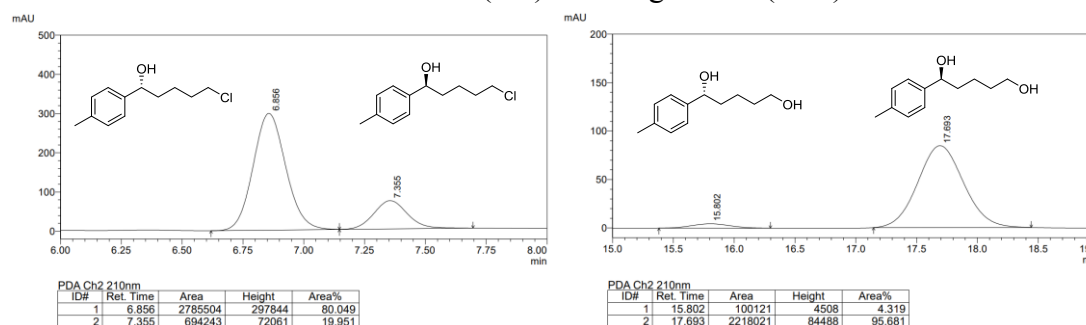
Chiral HPLC analysis: Diacel Chiralpak AD-H, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 210 nm, $t_{(S)}$ = 11.6 min, $t_{(R)}$ = 12.5 min; $t_{(S)}$ = 23.7 min, $t_{(R)}$ = 25.8 min.

(rac)-6a**(rac)-6b****Biotransformation of (rac)-6a using *E. coli* (εM4) cells****Biotransformation of (rac)-6a using *E. coli* (εM5) cells****Biotransformation of (rac)-6a using *E. coli* (εM7) cells**

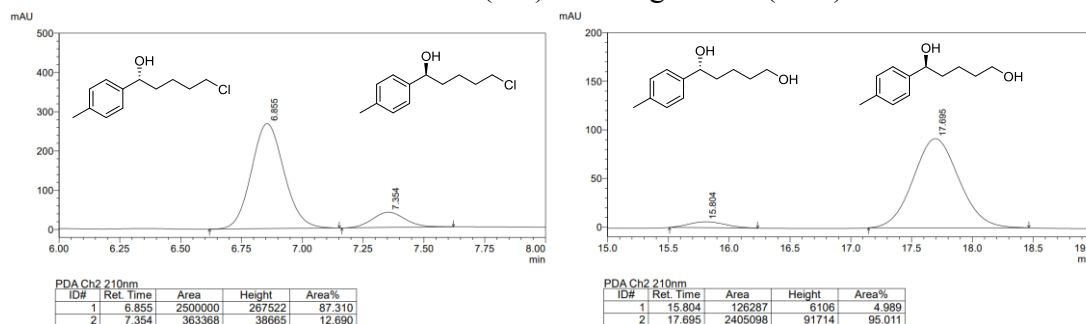
Chiral HPLC analysis: Diacel Chiralpak IB, *n*-hexane/*i*-PrOH = 95/5, flow rate 1.0 mL/min, λ = 210 nm, $t_{(S)}$ = 8.8 min, $t_{(R)}$ = 9.3 min; $t_{(S)}$ = 27.8 min, $t_{(R)}$ = 29.7 min.



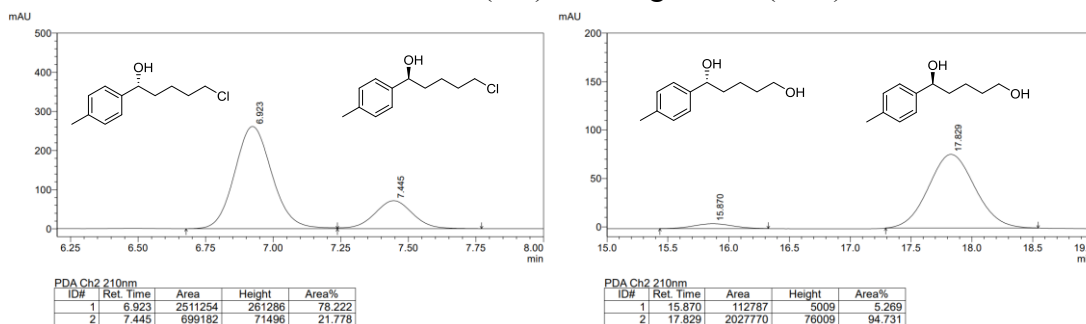
Biotransformation of (rac)-7a using *E. coli* (εM4) cells



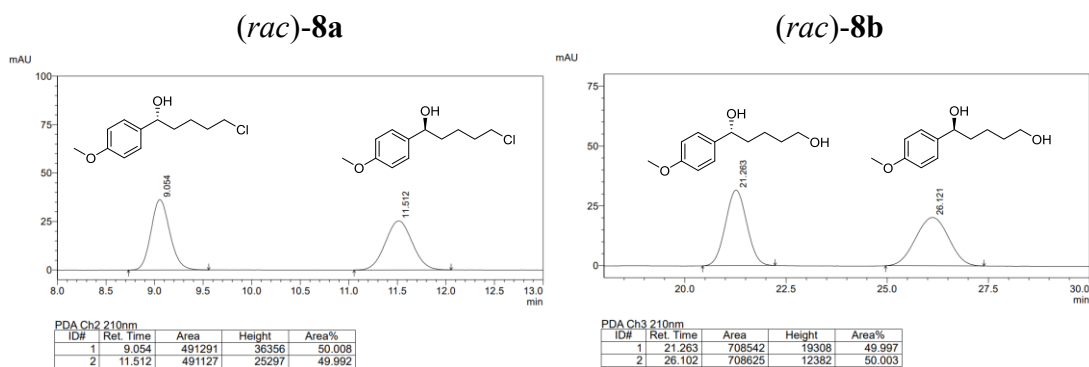
Biotransformation of (rac)-7a using *E. coli* (εM6) cells



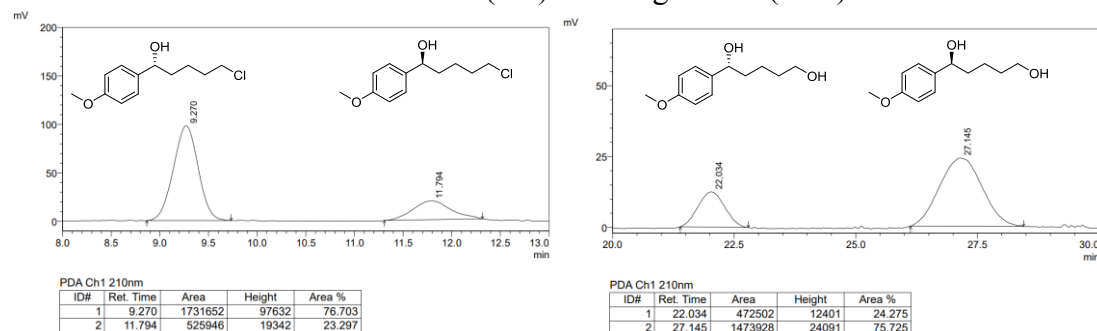
Biotransformation of (rac)-7a using *E. coli* (εM7) cells



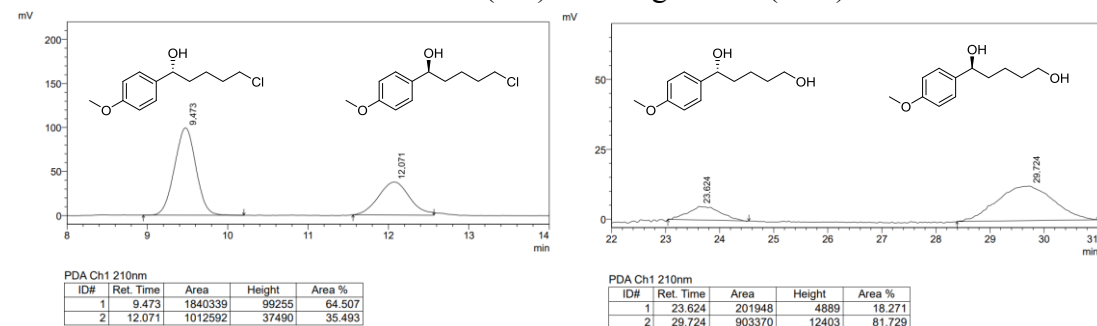
Chiral HPLC analysis: Diacel Chiralpak IF, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 7.2 min, $t_{(S)}$ = 7.6 min; $t_{(R)}$ = 15.8 min, $t_{(S)}$ = 17.8 min.



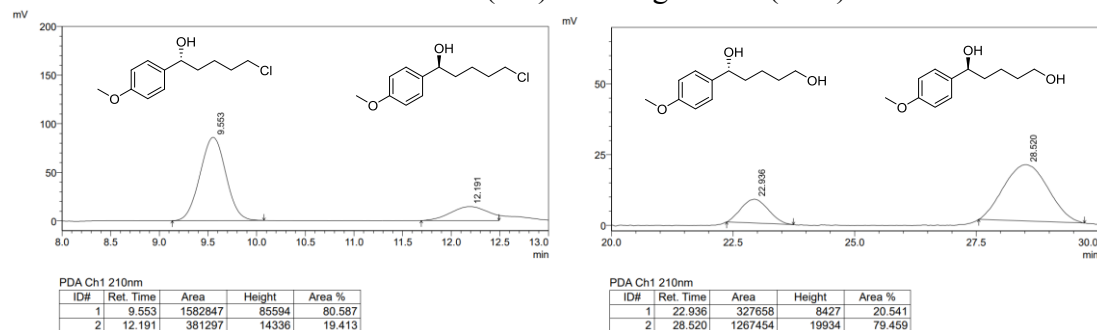
Biotransformation of (rac)-8a using *E. coli* (εM2) cells



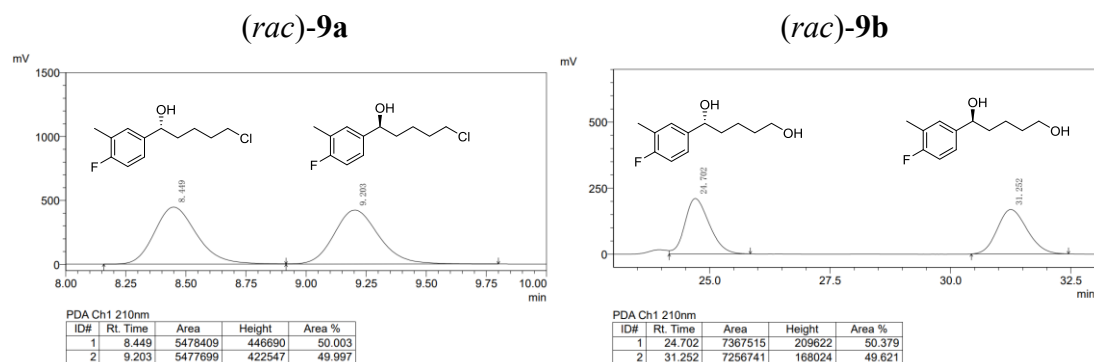
Biotransformation of (rac)-8a using *E. coli* (εM6) cells



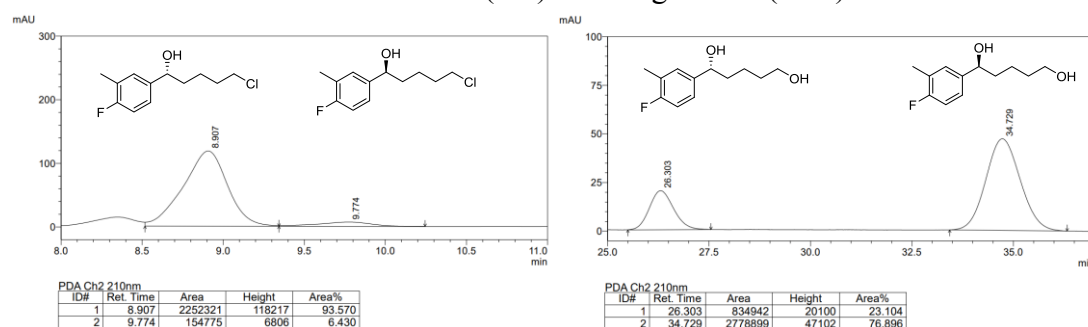
Biotransformation of (rac)-8a using *E. coli* (εM7) cells



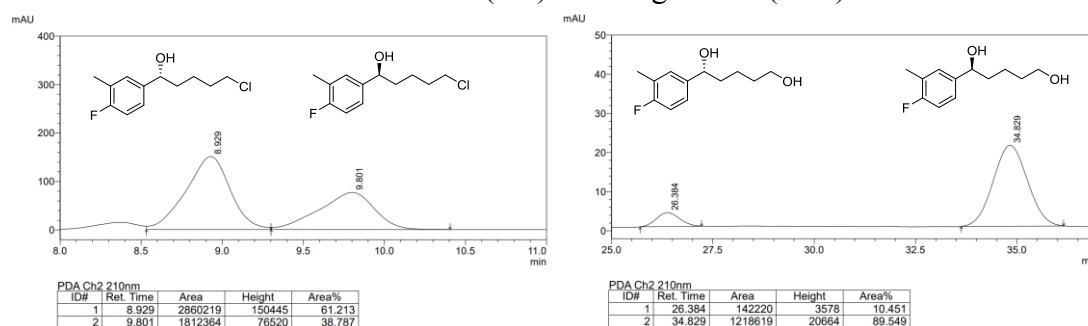
Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 9.0 min, $t_{(S)}$ = 11.5 min; $t_{(R)}$ = 21.2 min, $t_{(S)}$ = 26.1 min.



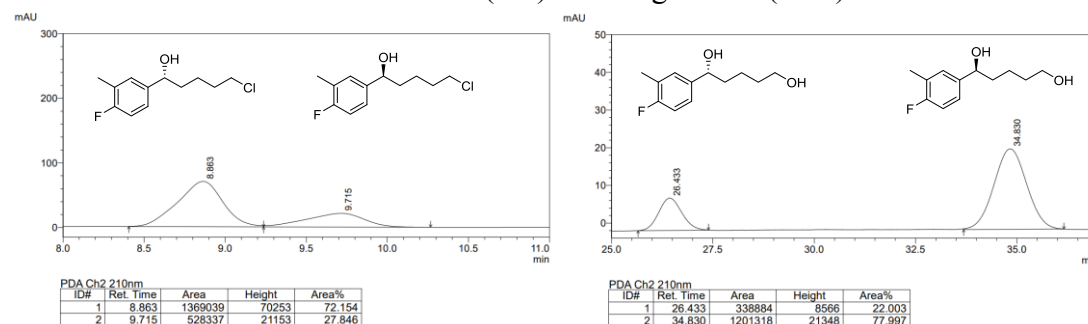
Biotransformation of (rac)-9a using *E. coli* (εM2) cells



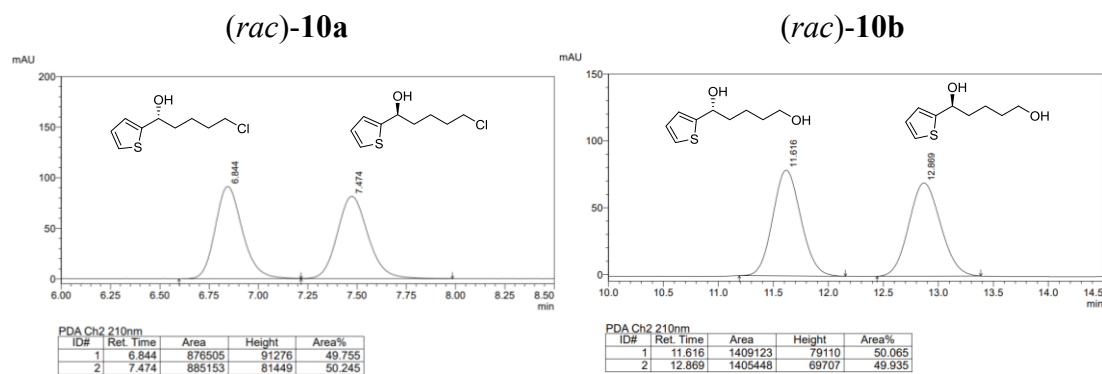
Biotransformation of (rac)-9a using *E. coli* (εM4) cells



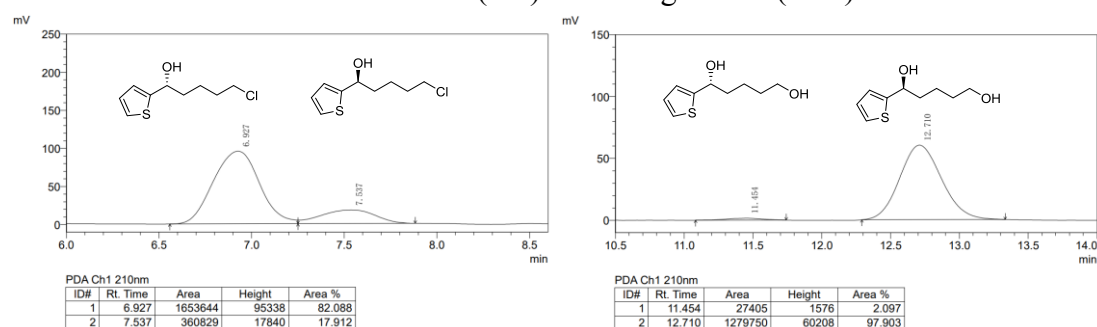
Biotransformation of (rac)-9a using *E. coli* (εM7) cells



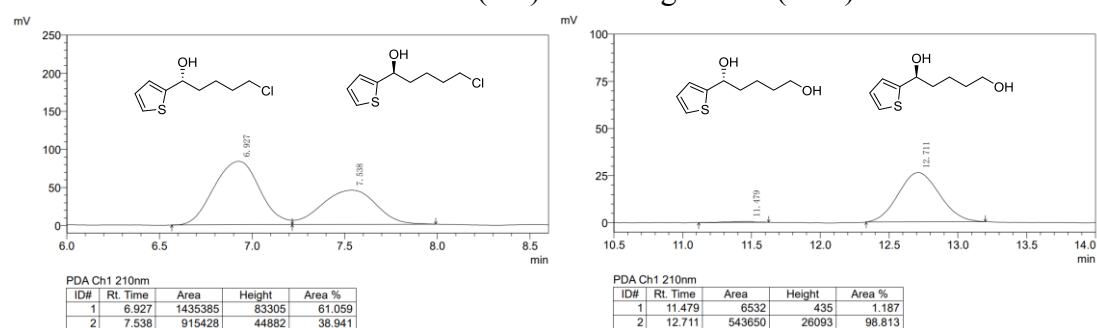
Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 95/5, flow rate 0.8 mL/min, λ = 210 nm, $t_{(R)}$ = 8.4 min, $t_{(S)}$ = 9.2 min; $t_{(R)}$ = 24.7 min, $t_{(S)}$ = 32.3 min.



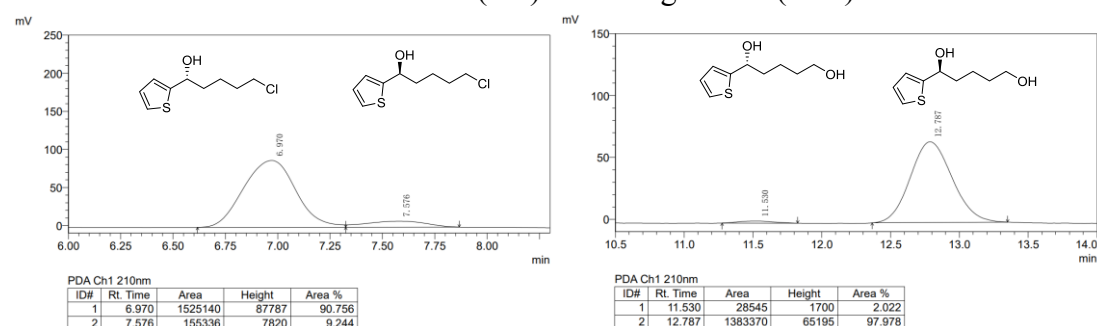
Biotransformation of (rac)-10a using *E. coli* (εM3) cells



Biotransformation of (rac)-10a using *E. coli* (εM5) cells

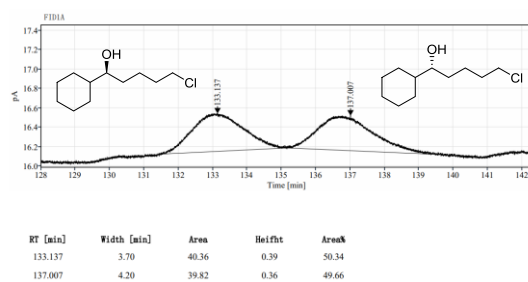


Biotransformation of (rac)-10a using *E. coli* (εM7) cells

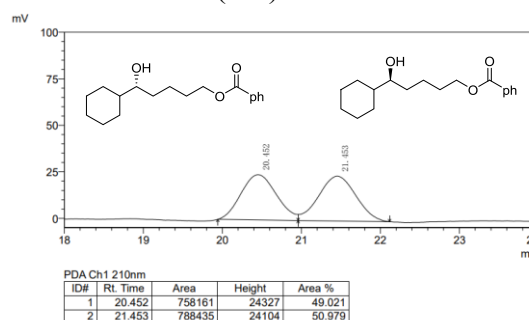


Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 6.8 min, $t_{(S)}$ = 7.5 min; $t_{(R)}$ = 11.6 min, $t_{(S)}$ = 12.8 min.

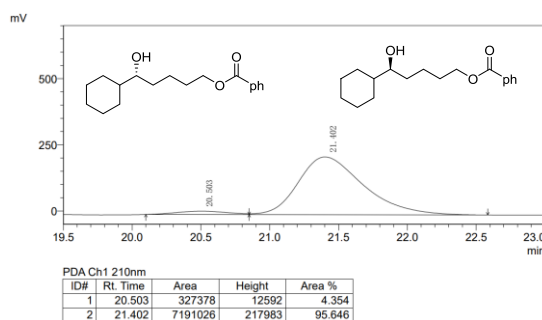
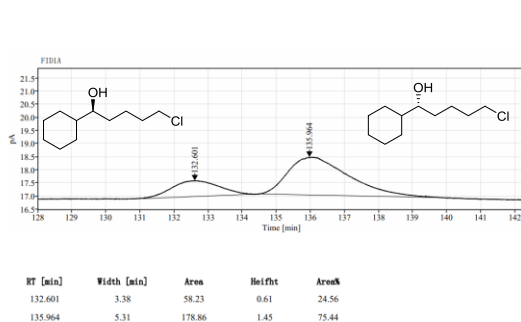
(rac)-11a



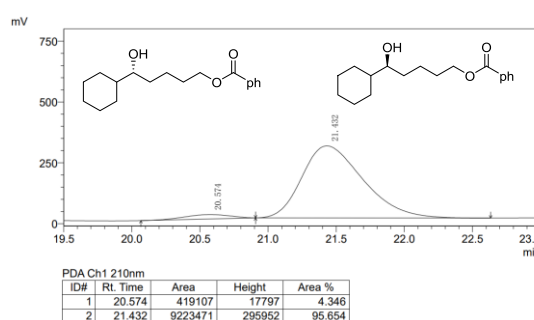
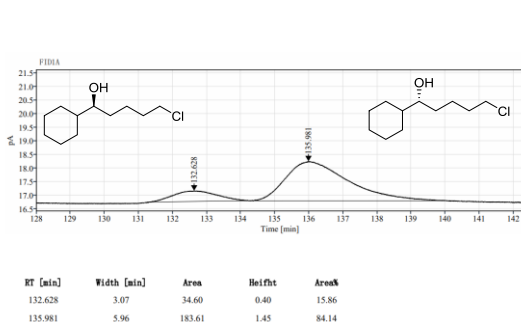
(rac)-11b



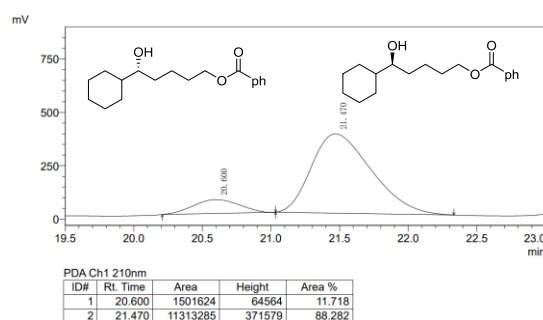
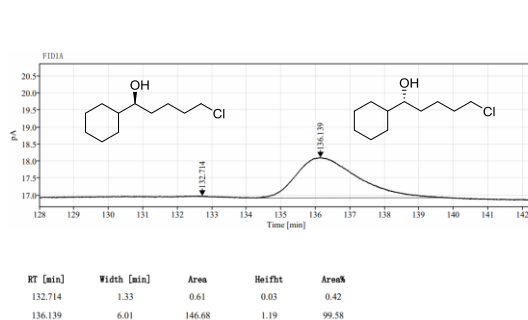
Biotransformation of (rac)-10a using *E. coli* (εM3) cells



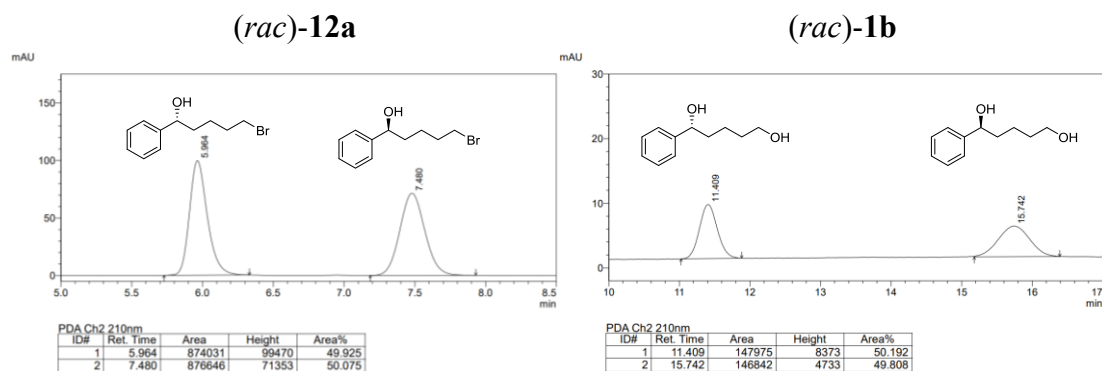
Biotransformation of (rac)-10a using *E. coli* (εM6) cells



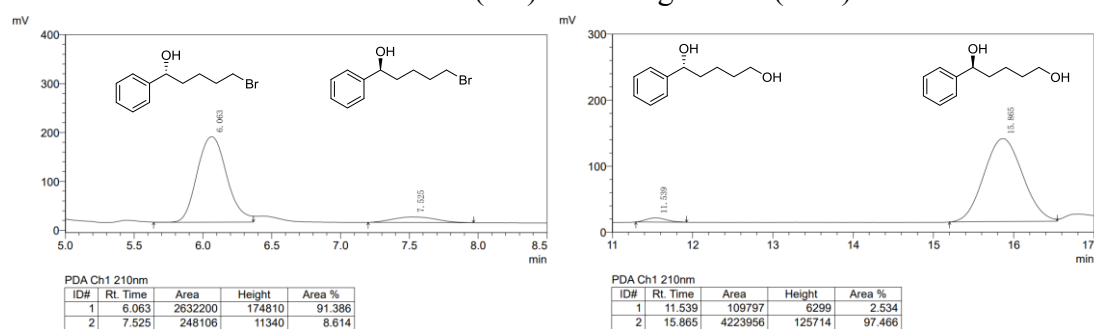
Biotransformation of (rac)-10a using *E. coli* (εM7) cells



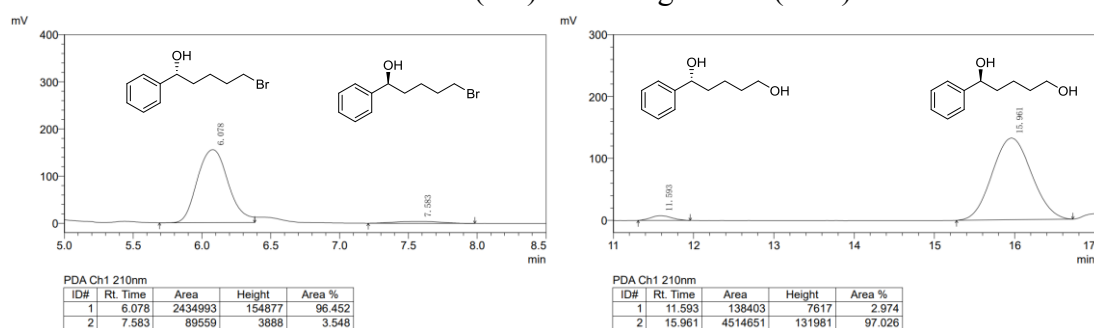
Chiral GC analysis: (Rt-bDEXcst, 140 °C for 150 min) $t_{(S)} = 133.1$ min, $t_{(R)} = 137.0$ min. **Chiral HPLC analysis:** Diacel Chiralpak OD-H, *n*-hexane/*i*-PrOH = 97/3, flow rate 0.7 mL/min, $\lambda = 210$ nm, $t_{(R)} = 20.5$ min, $t_{(S)} = 21.5$ min.



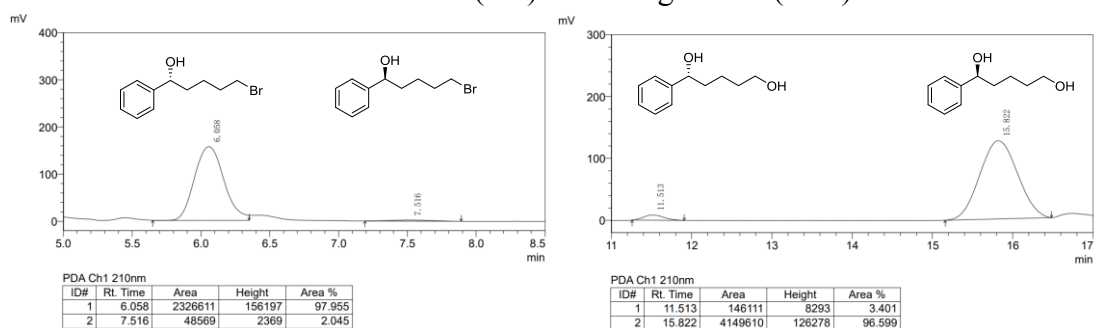
Biotransformation of (rac)-12a using *E. coli* (εM3) cells



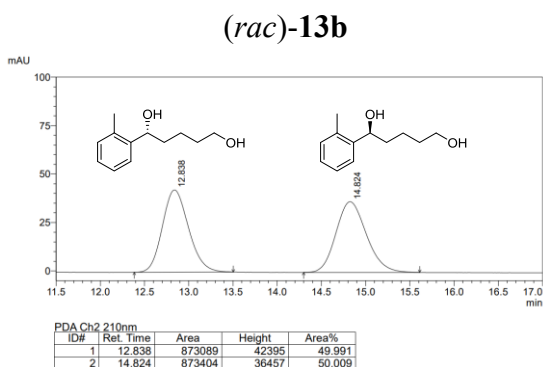
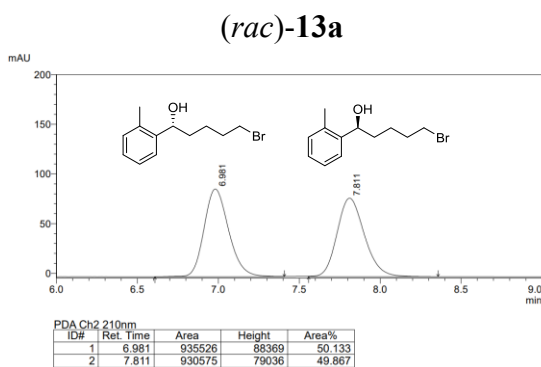
Biotransformation of (rac)-12a using *E. coli* (εM7) cells



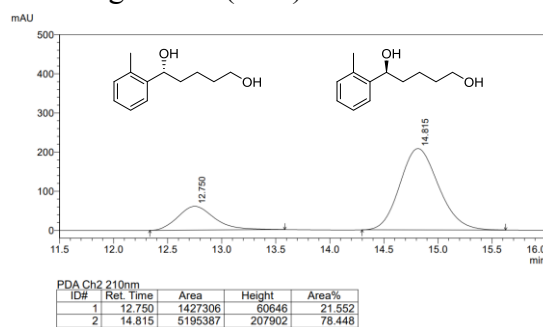
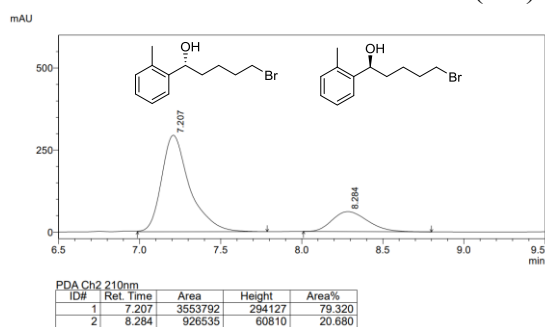
Biotransformation of (rac)-12a using *E. coli* (εM8) cells



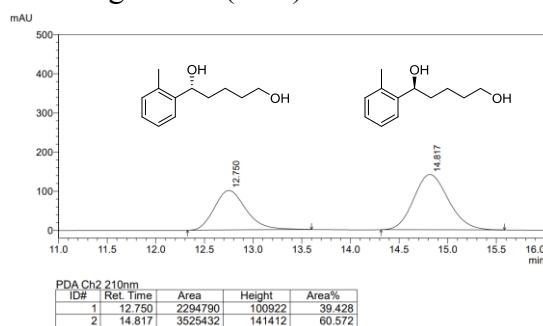
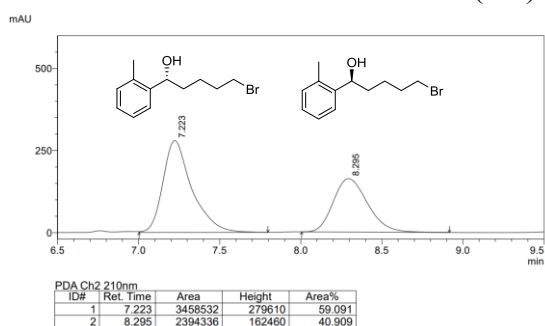
Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, λ = 210 nm, $t_{(S)}$ = 6.0 min, $t_{(R)}$ = 7.5 min; $t_{(S)}$ = 11.4 min, $t_{(R)}$ = 15.7 min.



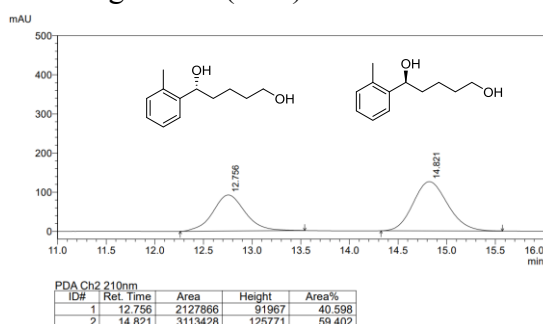
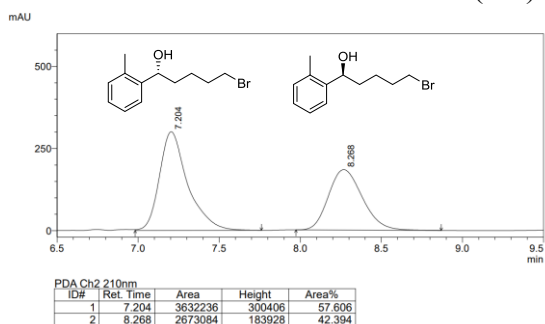
Biotransformation of (rac)-13a using *E. coli* (εM1) cells



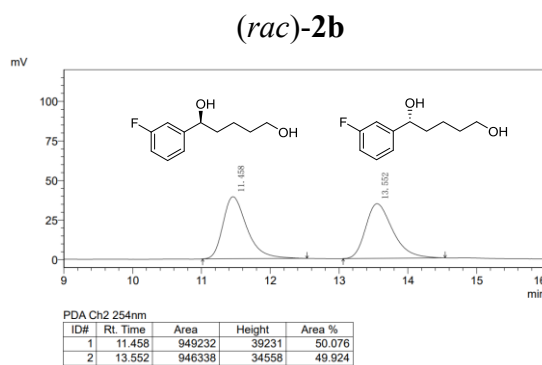
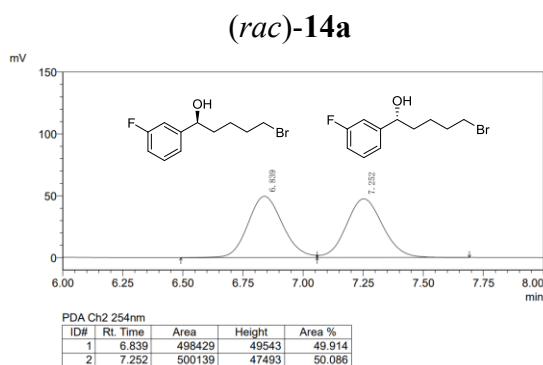
Biotransformation of (rac)-13a using *E. coli* (εM7) cells



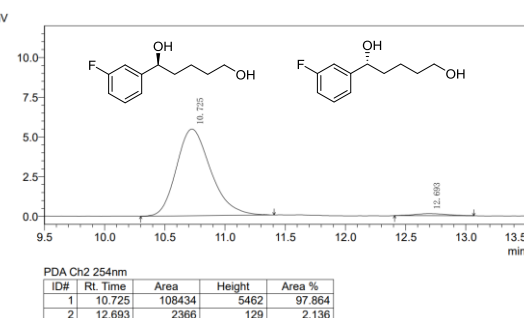
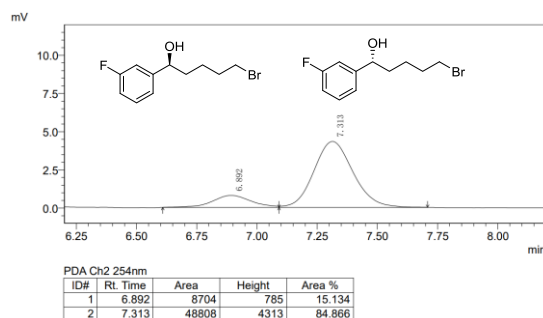
Biotransformation of (rac)-13a using *E. coli* (εM8) cells



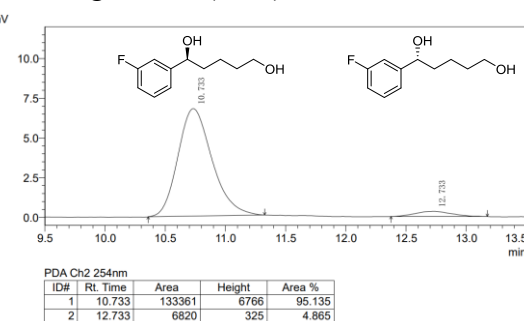
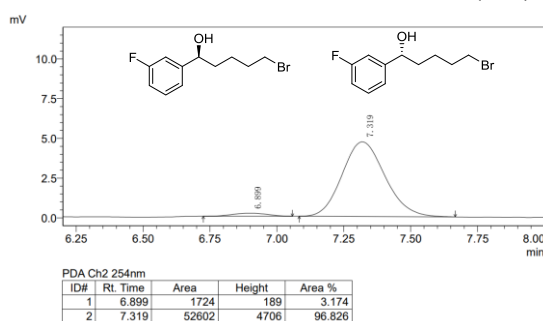
Chiral HPLC analysis: Diacel Chiralpak AD-H, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 7.2 min, $t_{(S)}$ = 8.3 min; $t_{(R)}$ = 12.8 min, $t_{(S)}$ = 14.8 min.



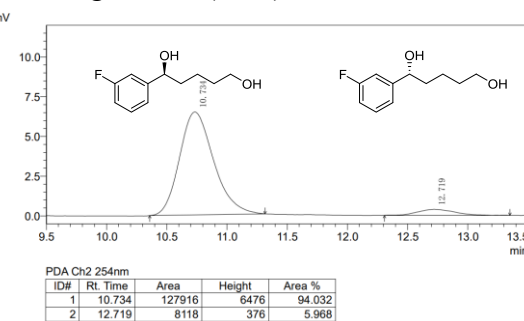
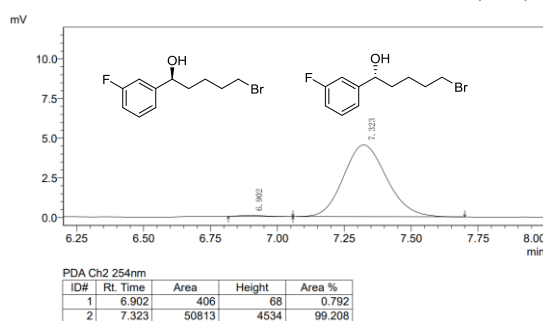
Biotransformation of (rac)-14a using *E. coli* (εM4) cells



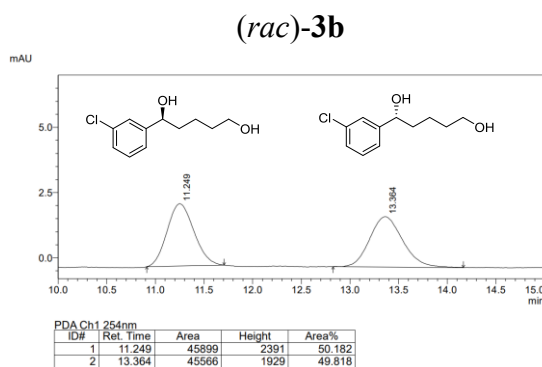
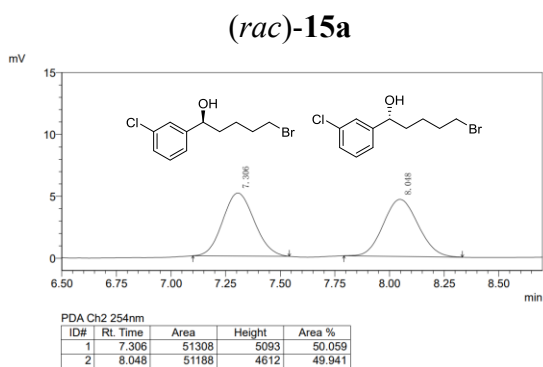
Biotransformation of (rac)-14a using *E. coli* (εM7) cells



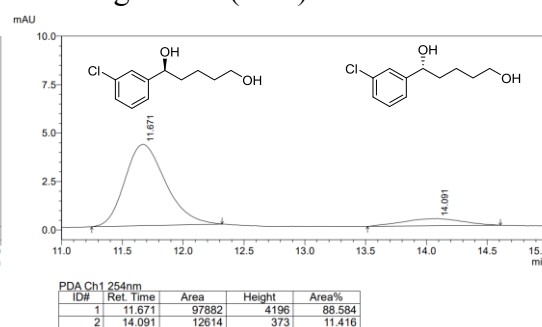
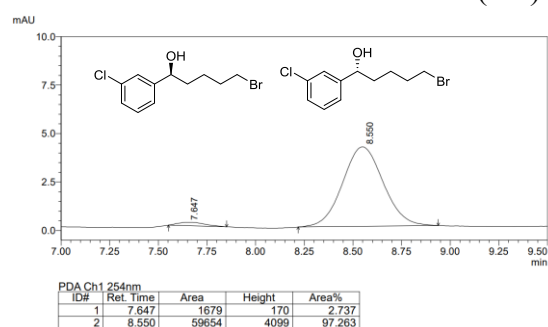
Biotransformation of (rac)-14a using *E. coli* (εM8) cells



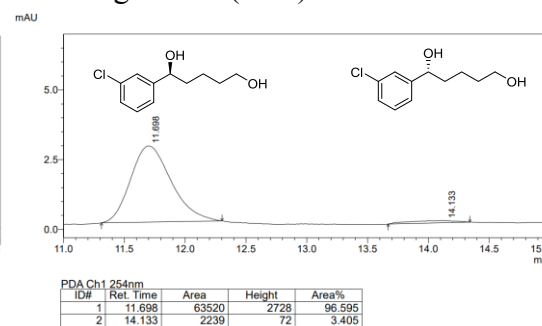
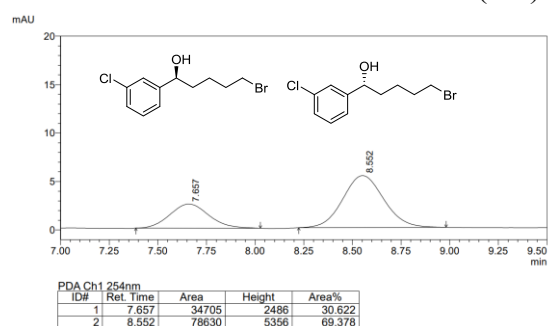
Chiral HPLC analysis: Diacel Chiralpak OD-H, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 254 nm, $t_{(S)}$ = 6.9 min, $t_{(R)}$ = 7.3 min; $t_{(S)}$ = 11.5 min, $t_{(R)}$ = 13.6 min.



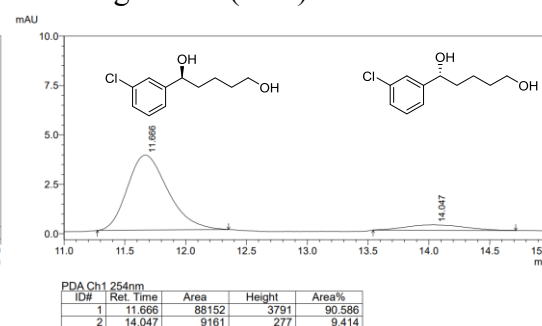
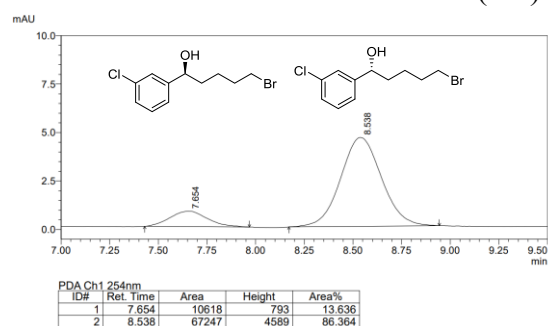
Biotransformation of (rac)-15a using *E. coli* (εM2) cells



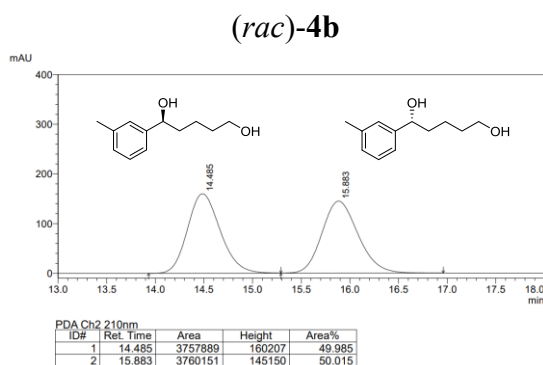
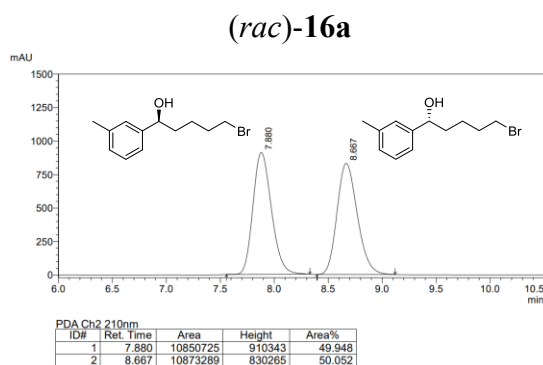
Biotransformation of (rac)-15a using *E. coli* (εM3) cells



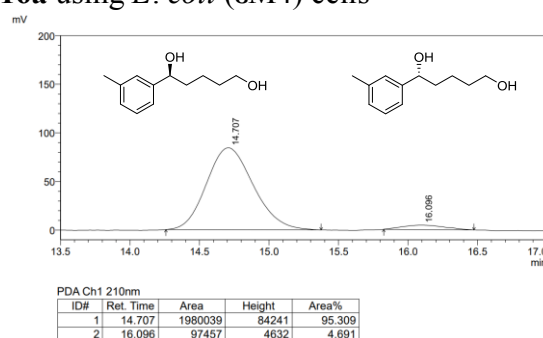
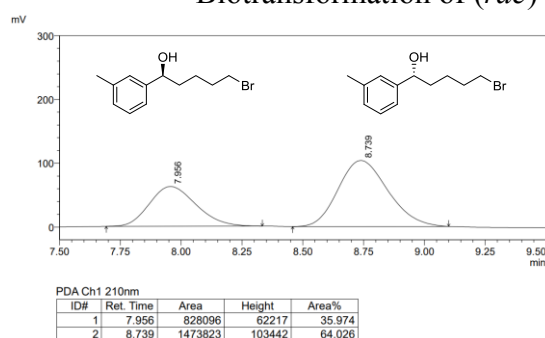
Biotransformation of (rac)-15a using *E. coli* (εM7) cells



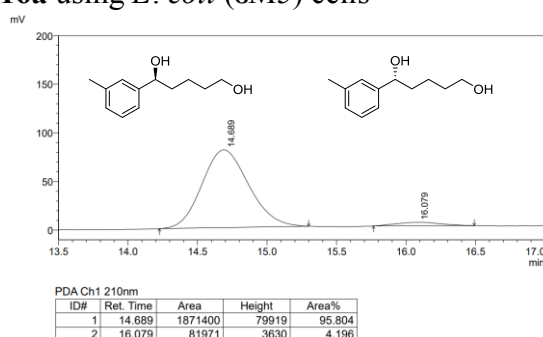
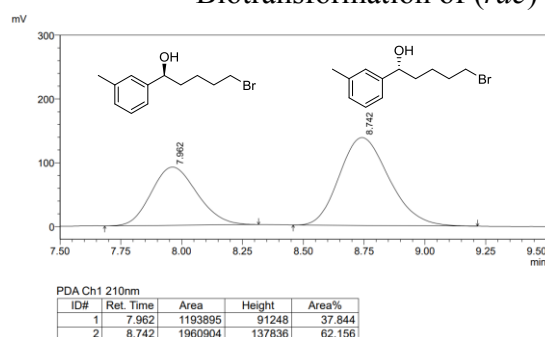
Chiral HPLC analysis: Diacel Chiralpak AD-H, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 210 nm, $t_{(S)}$ = 7.6 min, $t_{(R)}$ = 8.2 min; $t_{(S)}$ = 14.4 min, $t_{(R)}$ = 15.8 min.



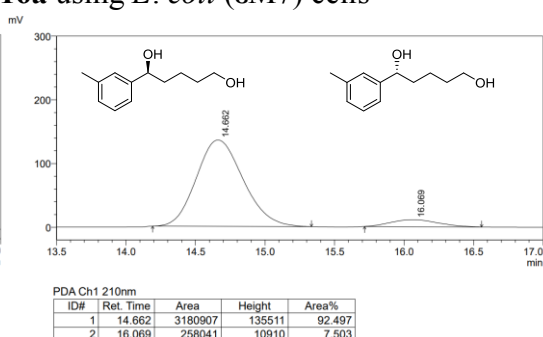
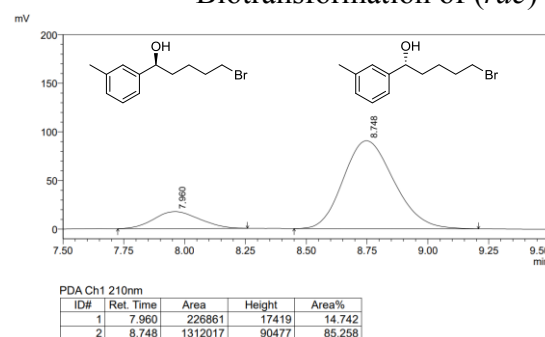
Biotransformation of (rac)-16a using *E. coli* (εM4) cells



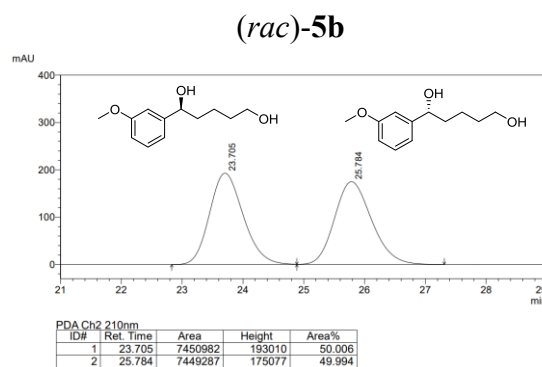
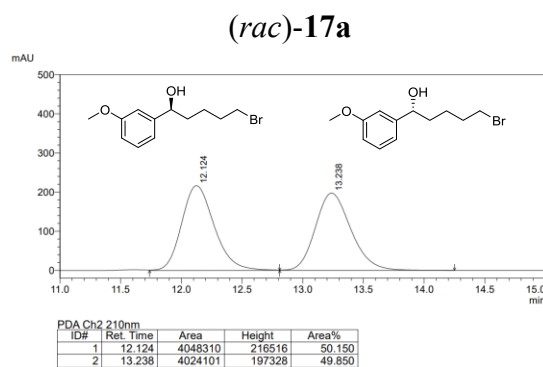
Biotransformation of (rac)-16a using *E. coli* (εM5) cells



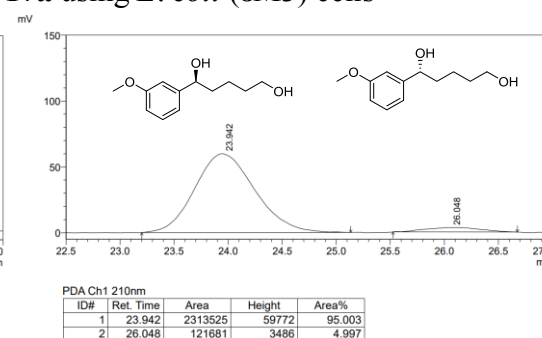
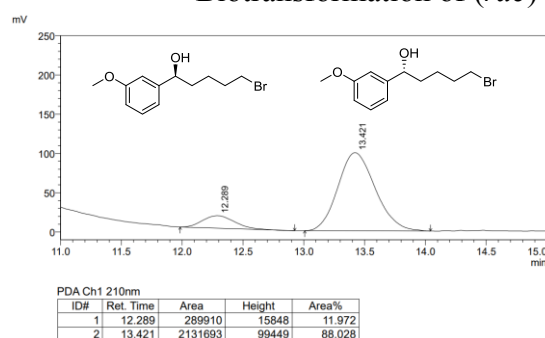
Biotransformation of (rac)-16a using *E. coli* (εM7) cells



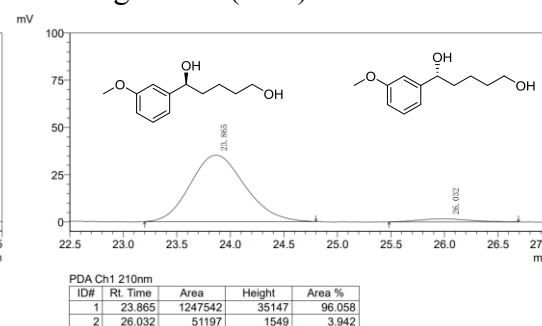
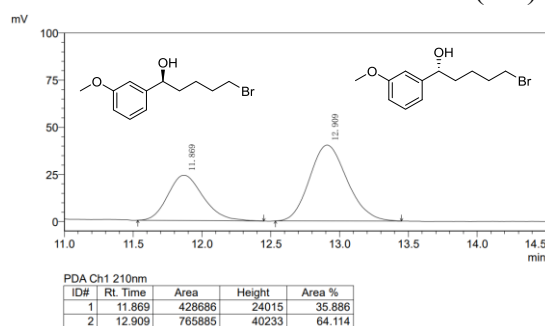
Chiral HPLC analysis: Diacel Chiralpak AD-H, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 210 nm, $t_{(S)}$ = 7.9 min, $t_{(R)}$ = 8.7 min; $t_{(S)}$ = 14.5 min, $t_{(R)}$ = 15.9 min.



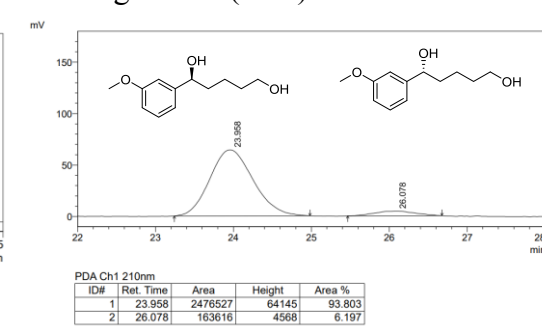
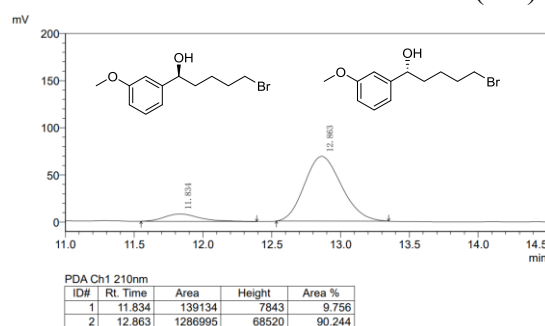
Biotransformation of (rac)-17a using *E. coli* (εM3) cells



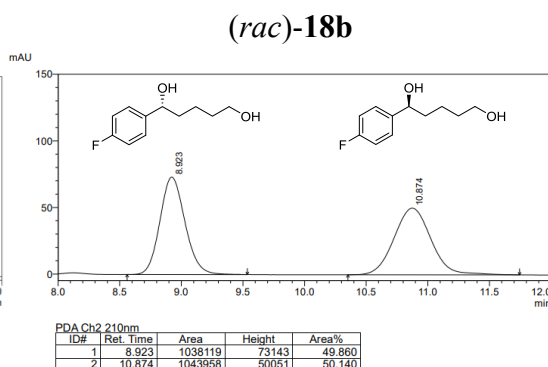
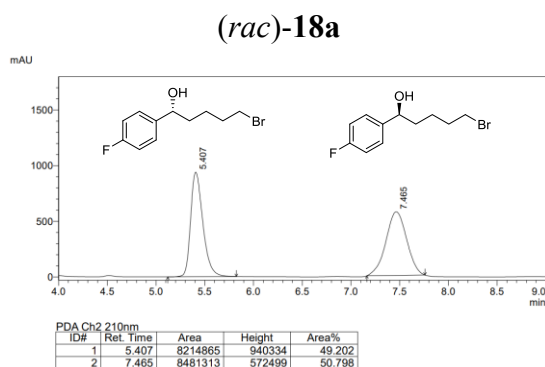
Biotransformation of (rac)-17a using *E. coli* (εM5) cells



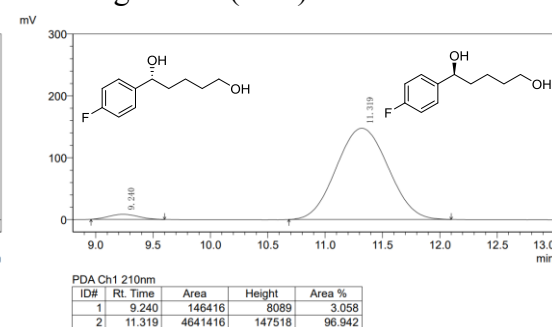
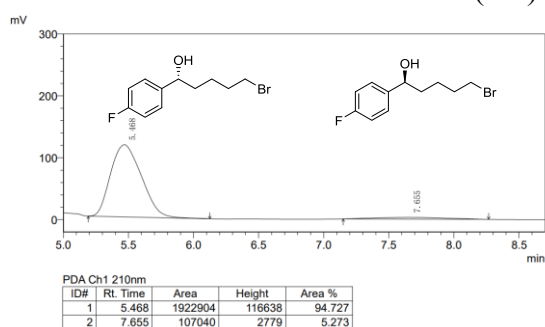
Biotransformation of (rac)-17a using *E. coli* (εM7) cells



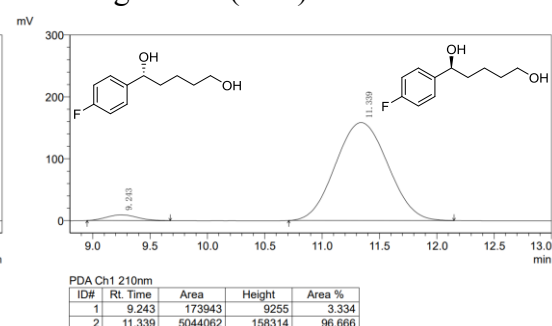
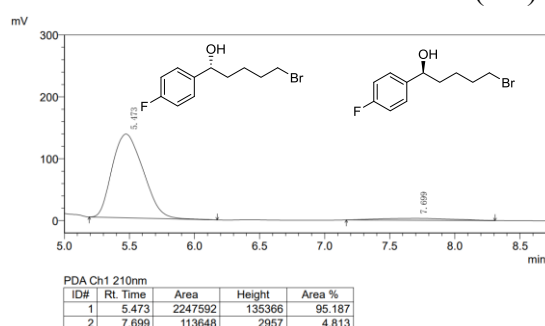
Chiral HPLC analysis: Diacel Chiralpak AD-H, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 210 nm, $t_{(S)}$ = 7.6 min, $t_{(R)}$ = 8.2 min; $t_{(S)}$ = 14.4 min, $t_{(R)}$ = 15.8 min.



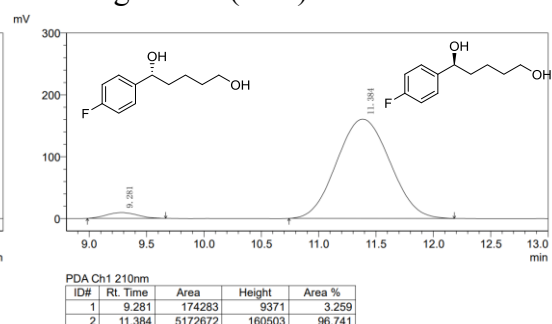
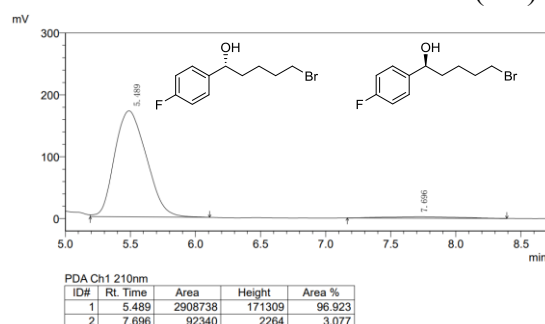
Biotransformation of (rac)-18a using *E. coli* (εM6) cells



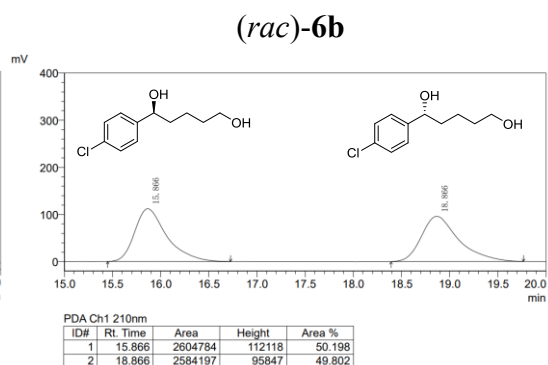
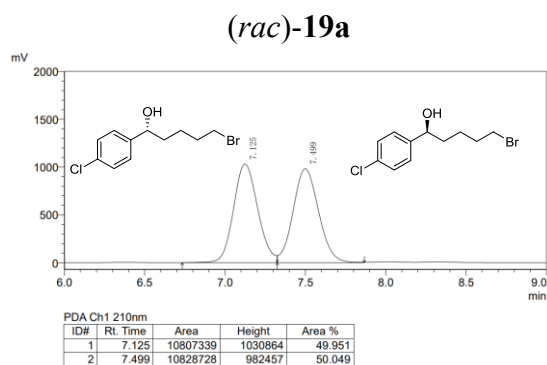
Biotransformation of (rac)-18a using *E. coli* (εM7) cells



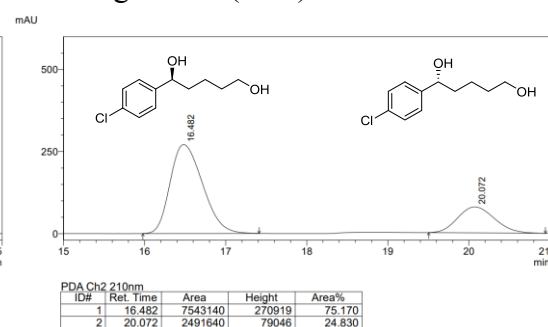
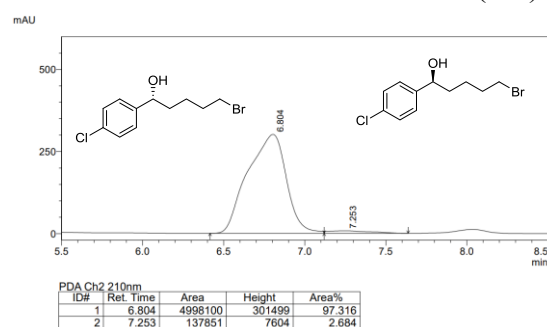
Biotransformation of (rac)-18a using *E. coli* (εM8) cells



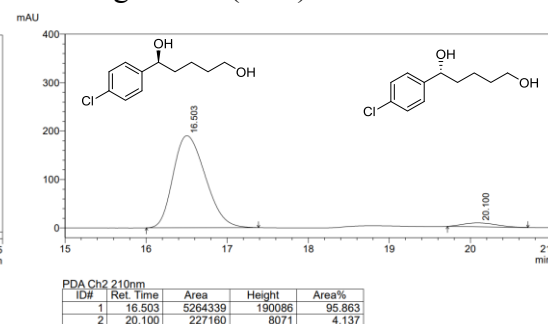
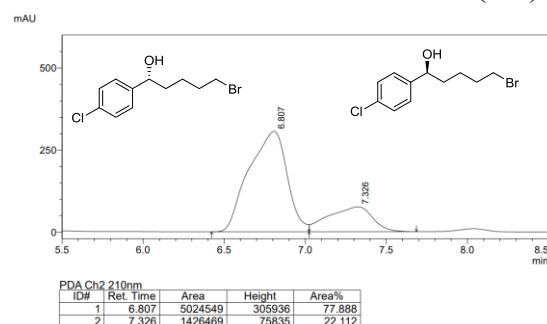
Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 5.4 min, $t_{(S)}$ = 7.5 min; $t_{(R)}$ = 8.9 min, $t_{(S)}$ = 10.9 min.



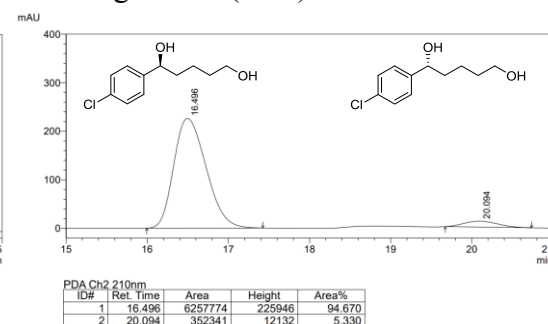
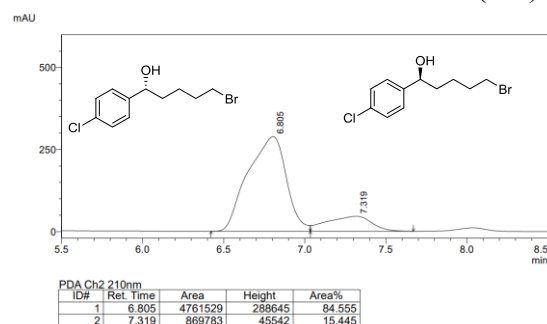
Biotransformation of (rac)-19a using *E. coli* (εM2) cells



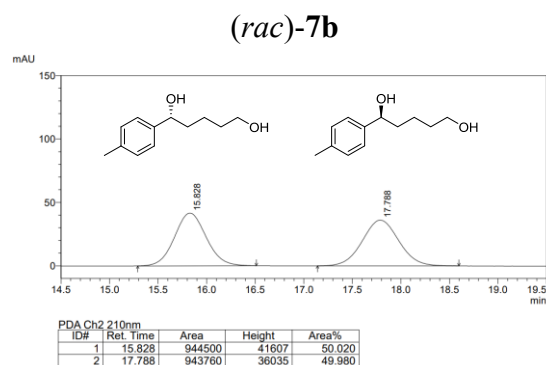
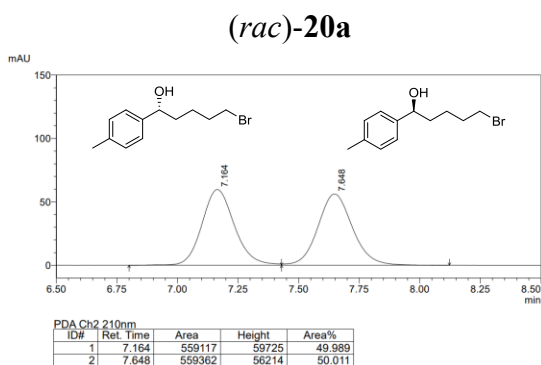
Biotransformation of (rac)-19a using *E. coli* (εM4) cells



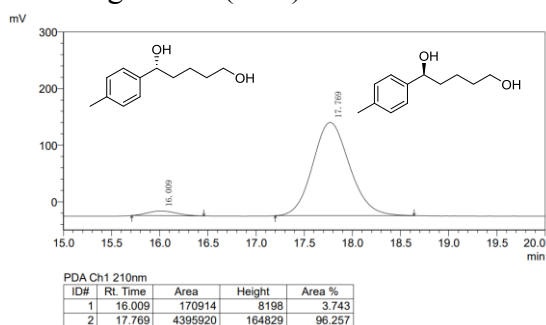
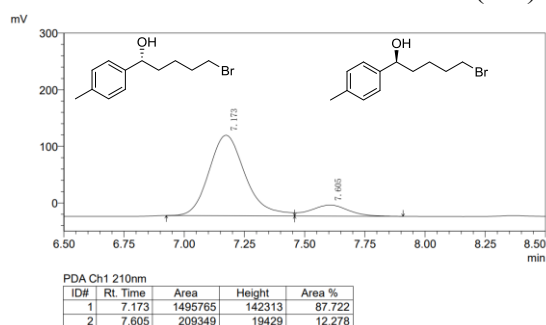
Biotransformation of (rac)-19a using *E. coli* (εM7) cells



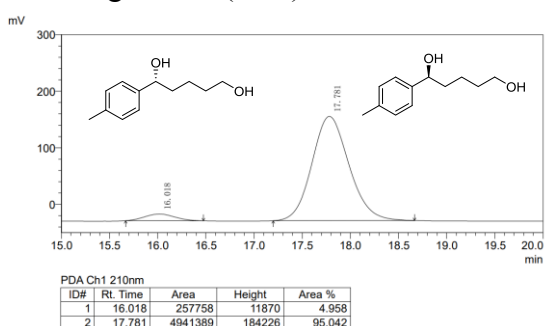
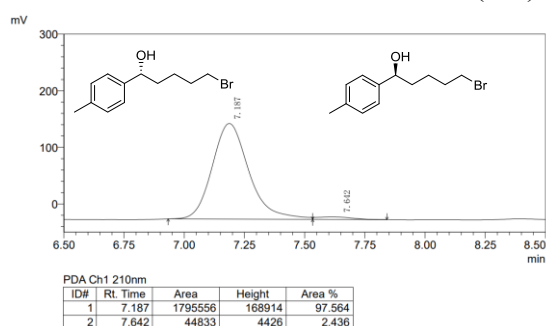
Chiral HPLC analysis: Diacel Chiralpak OX-3, *n*-hexane/*i*-PrOH = 92/8, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 7.1 min, $t_{(S)}$ = 7.5 min; $t_{(S)}$ = 15.9 min, $t_{(R)}$ = 18.9 min.



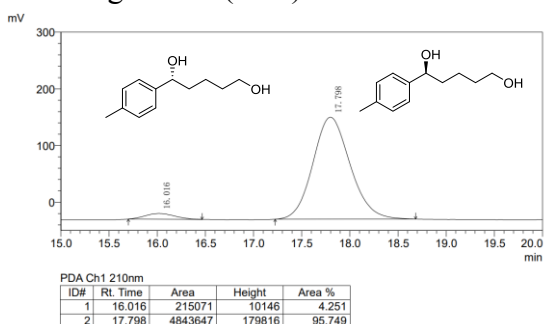
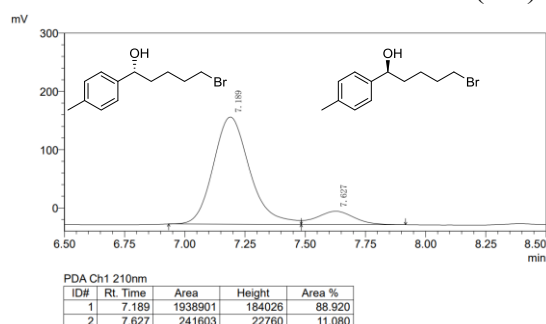
Biotransformation of (rac)-20a using *E. coli* (εM4) cells



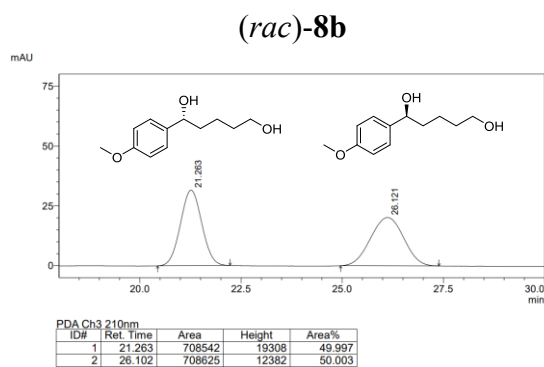
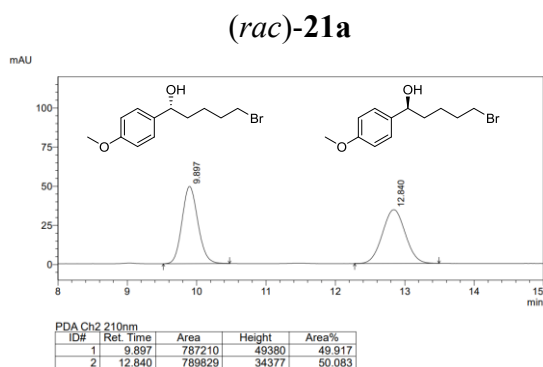
Biotransformation of (rac)-20a using *E. coli* (εM6) cells



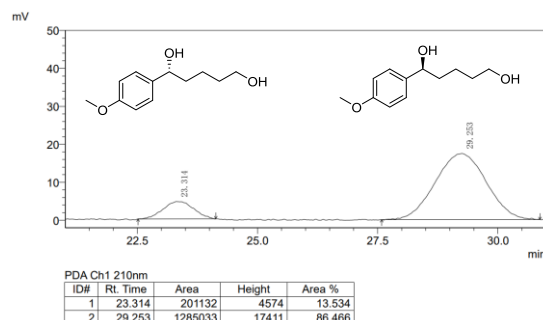
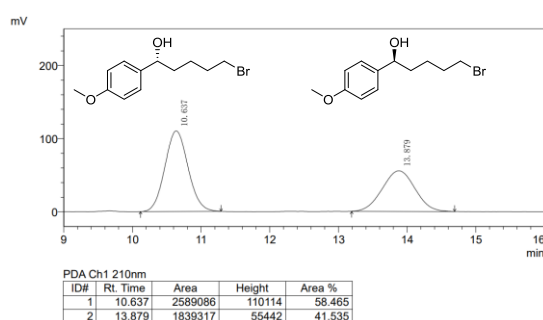
Biotransformation of (rac)-20a using *E. coli* (εM7) cells



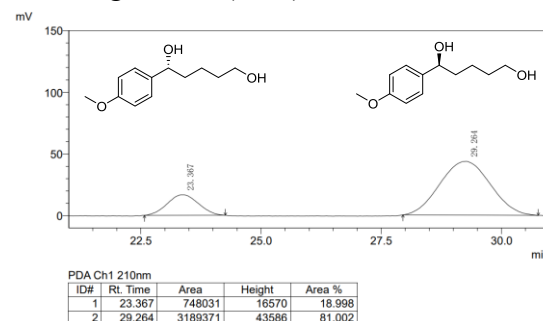
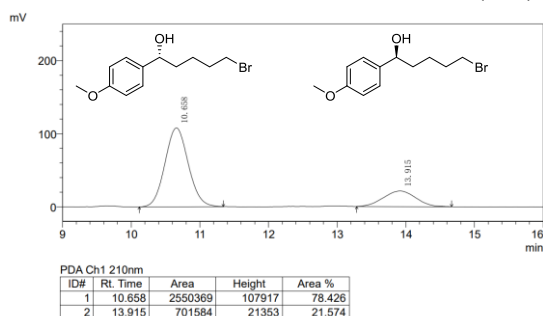
Chiral HPLC analysis: Diacel Chiralpak IF, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 7.2 min, $t_{(S)}$ = 7.6 min; $t_{(R)}$ = 15.8 min, $t_{(S)}$ = 17.8 min.



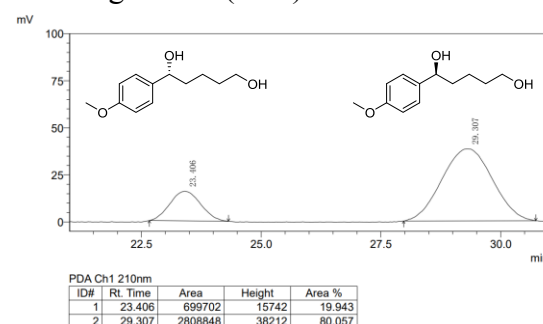
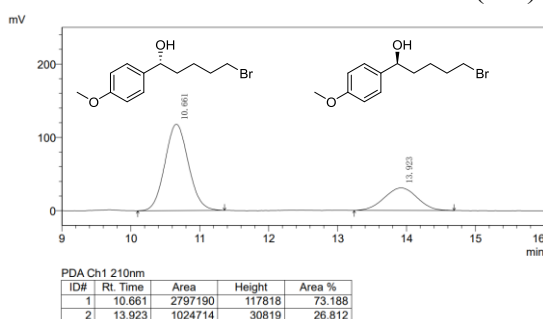
Biotransformation of (rac)-21a using *E. coli* (εM4) cells



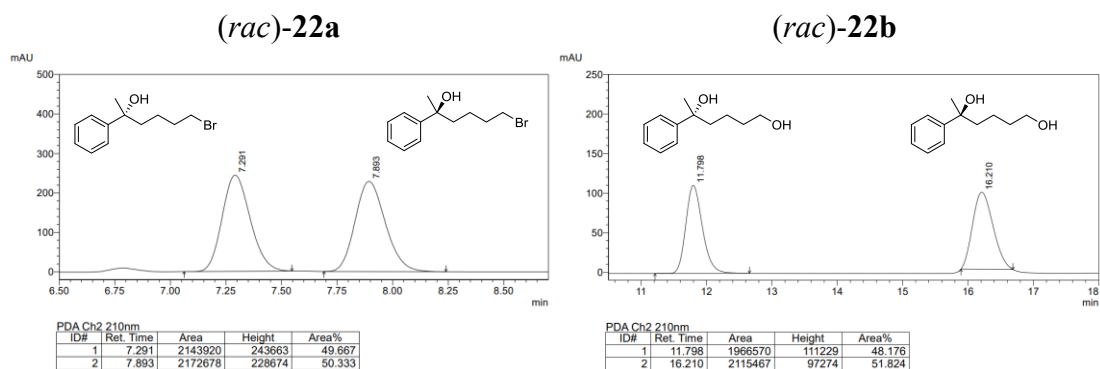
Biotransformation of (rac)-21a using *E. coli* (εM7) cells



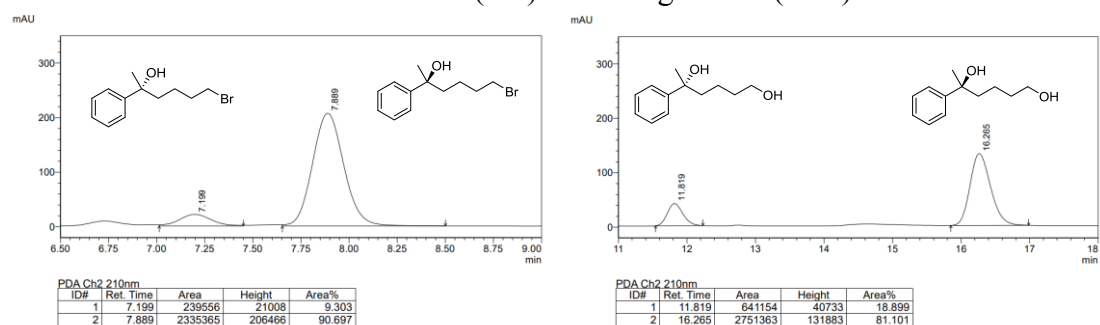
Biotransformation of (rac)-21a using *E. coli* (εM8) cells



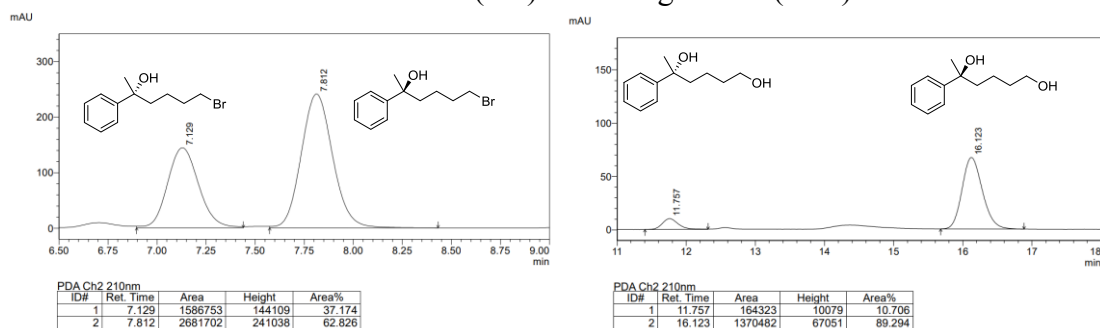
Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 9.9 min, $t_{(S)}$ = 12.8 min; $t_{(R)}$ = 21.3 min, $t_{(S)}$ = 26.1 min.



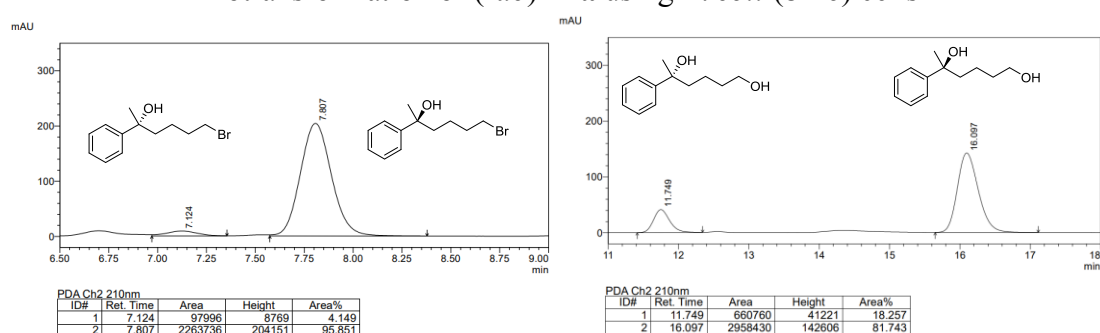
Biotransformation of (rac)-22a using *E. coli* (εM3) cells



Biotransformation of (rac)-22a using *E. coli* (εM5) cells



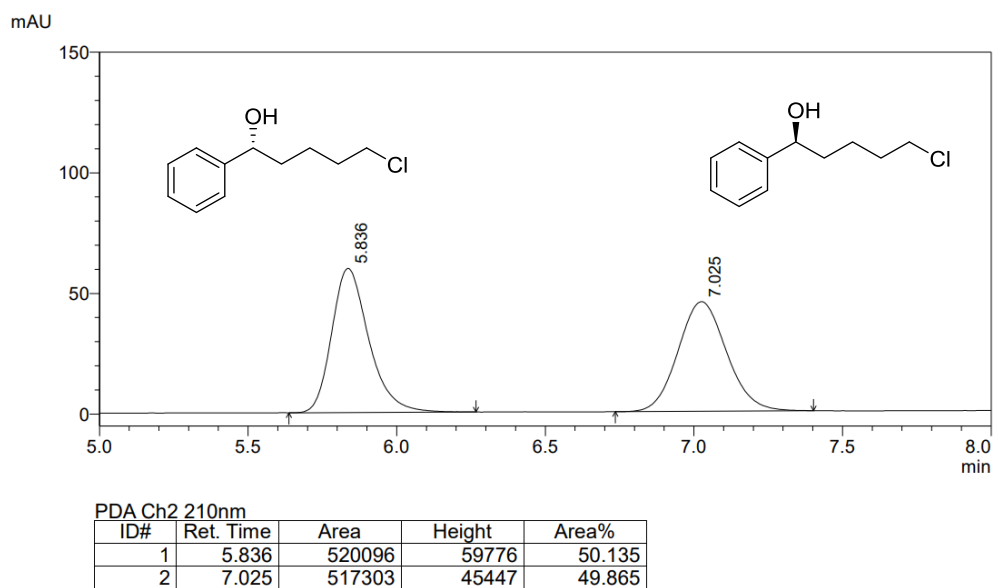
Biotransformation of (rac)-22a using *E. coli* (εM6) cells



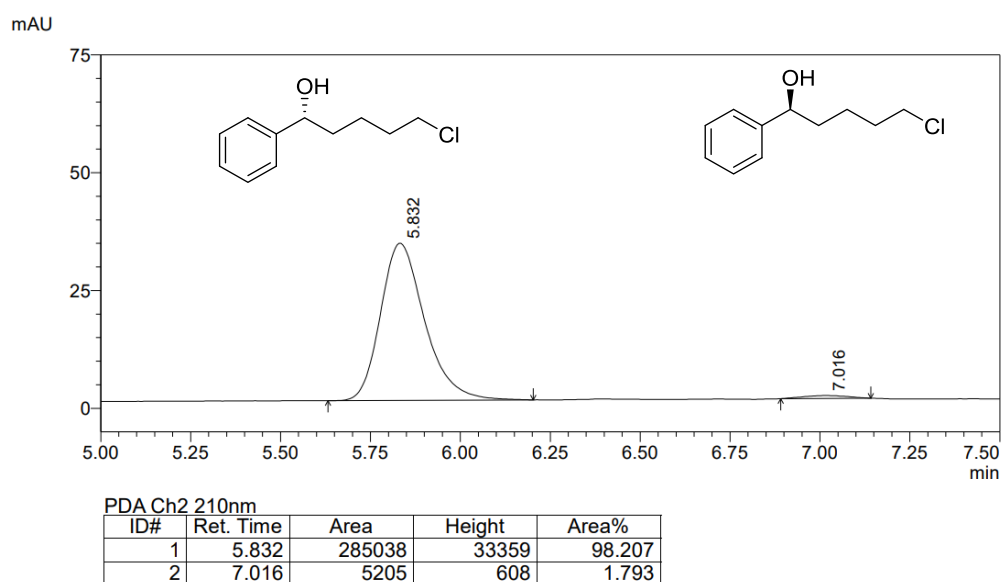
Chiral HPLC analysis: Diacel Chiralpak OD-3, *n*-hexane/*i*-PrOH = 92/8, flow rate 1.0 mL/min, λ = 210 nm, $t_{(S)}$ = 7.3 min, $t_{(R)}$ = 7.9 min; $t_{(R)}$ = 11.8 min, $t_{(S)}$ = 16.2 min.

10. Chromatography analysis for isolated products

Chemical synthesized (*rac*)-1a

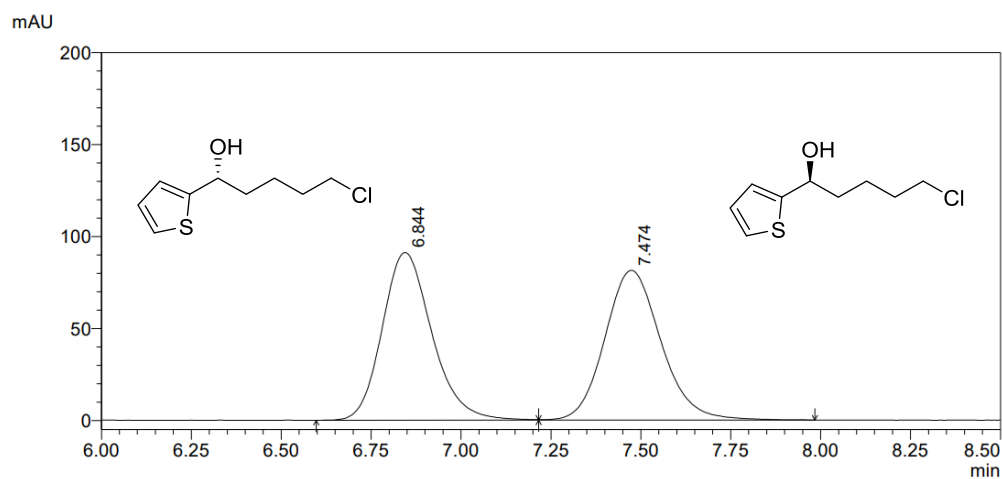


Enzymatic synthesized (*R*)-1a



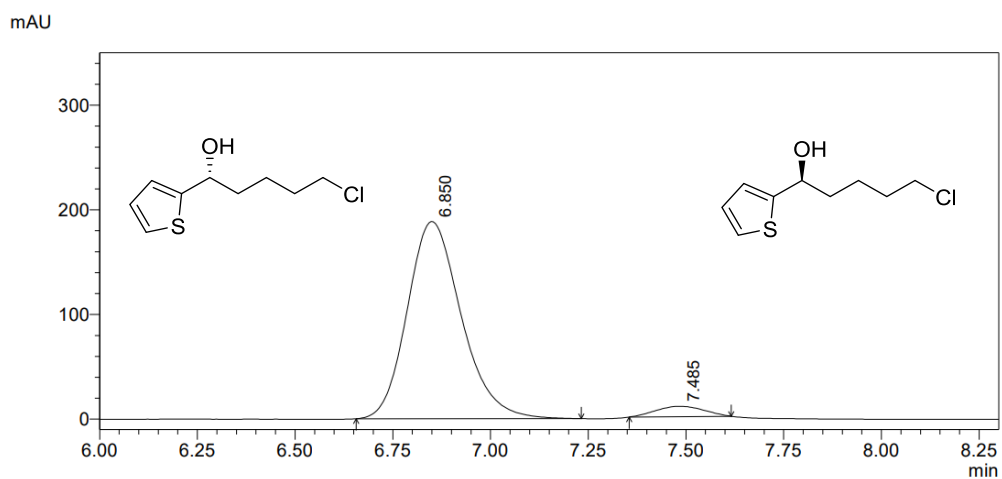
Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, $\lambda = 210$ nm, $t_{(R)} = 5.8$ min, $t_{(S)} = 7.0$ min.

Chemical synthesized (*rac*)-10a



PDA Ch2 210nm				
ID#	Ret. Time	Area	Height	Area%
1	6.844	876505	91276	49.755
2	7.474	885153	81449	50.245

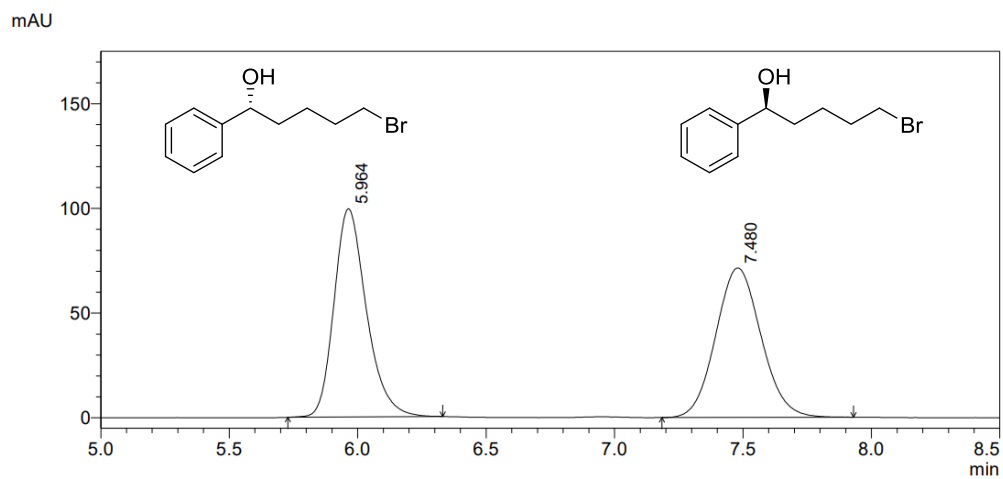
Enzymatic synthesized (*R*)-10a



PDA Ch2 210nm				
ID#	Ret. Time	Area	Height	Area%
1	6.850	1812209	188440	95.535
2	7.485	84702	9880	4.465

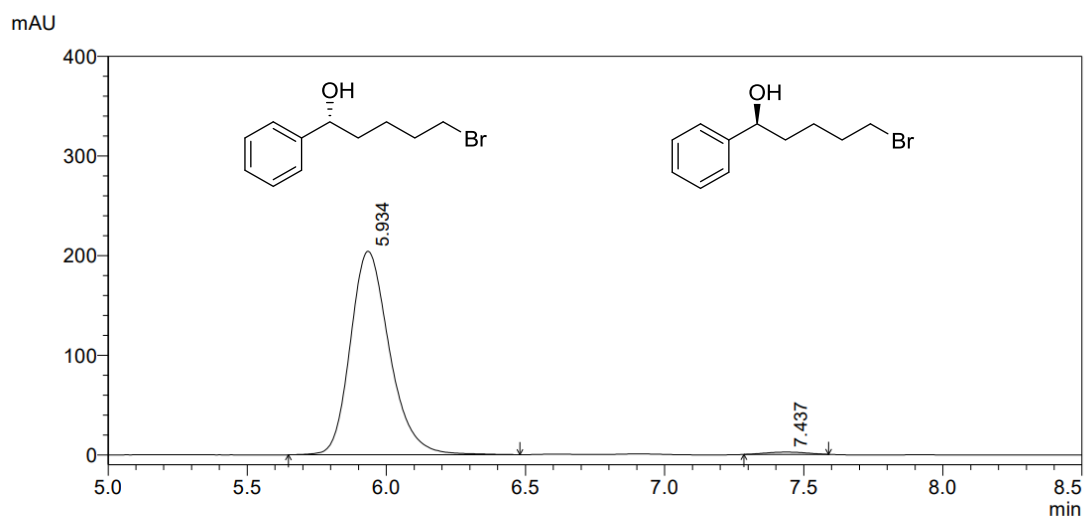
Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 6.9 min, $t_{(S)}$ = 7.5 min.

Chemical synthesized (*rac*)-**12a**



PDA Ch2 210nm				
ID#	Ret. Time	Area	Height	Area%
1	5.964	874031	99470	49.925
2	7.480	876646	71353	50.075

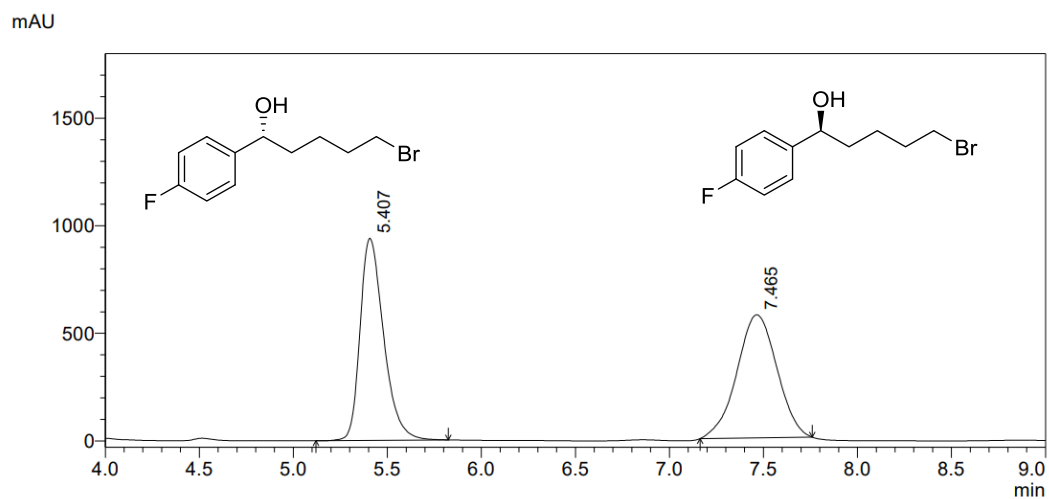
Enzymatic synthesized (*R*)-**12a**



PDA Ch2 210nm				
ID#	Ret. Time	Area	Height	Area%
1	5.934	1999101	204310	98.845
2	7.437	23352	2289	1.155

Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, $\lambda = 210$ nm, $t_{(R)} = 5.9$ min, $t_{(S)} = 7.4$ min.

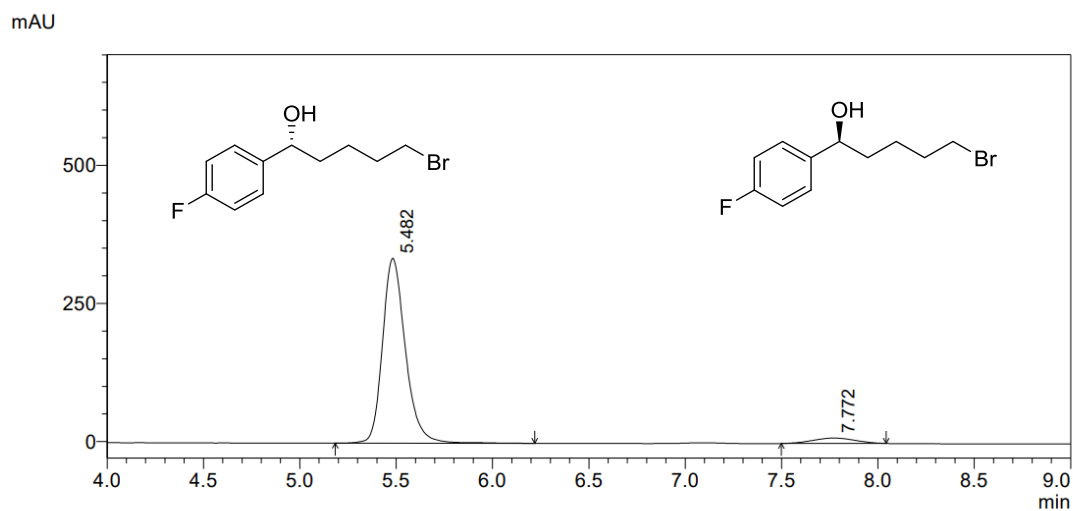
Chemical synthesized (*rac*)-**18a**



PDA Ch2 210nm

ID#	Ret. Time	Area	Height	Area%
1	5.407	8214865	940334	49.202
2	7.465	8481313	572499	50.798

Enzymatic synthesized (*R*)-**18a**

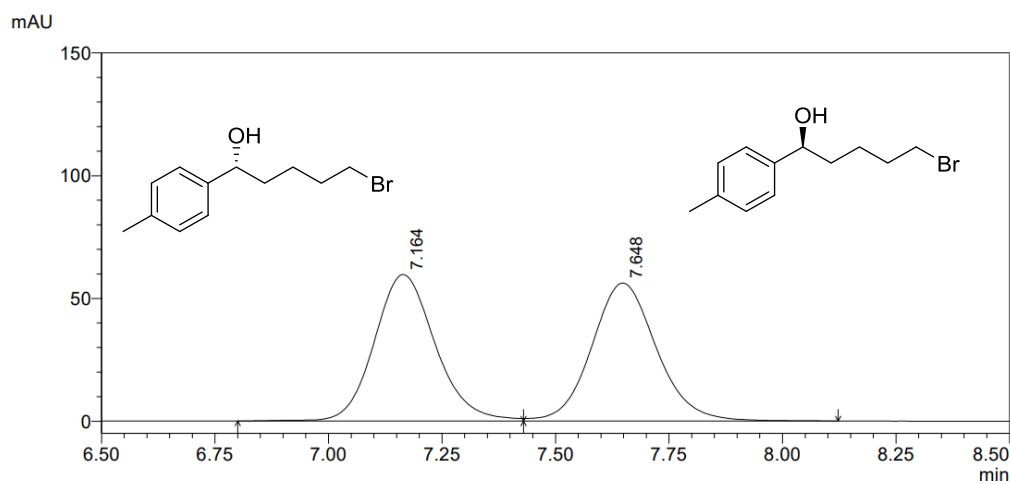


PDA Ch2 210nm

ID#	Ret. Time	Area	Height	Area%
1	5.482	2772407	335022	94.892
2	7.772	149222	9789	5.108

Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 5.5 min, $t_{(S)}$ = 7.8 min.

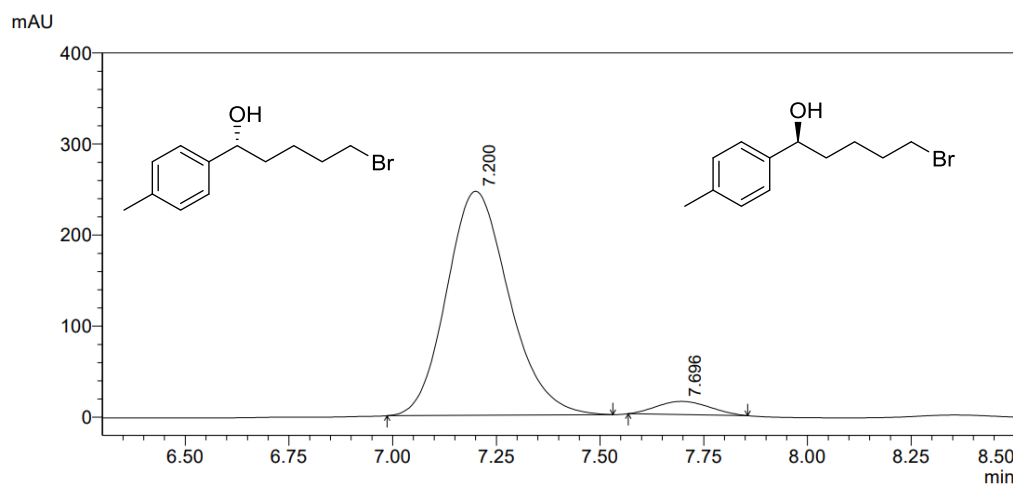
Chemical synthesized (*rac*)-**20a**



PDA Ch2 210nm

ID#	Ret. Time	Area	Height	Area%
1	7.164	559117	59725	49.989
2	7.648	559362	56214	50.011

Enzymatic synthesized (*R*)-**20a**

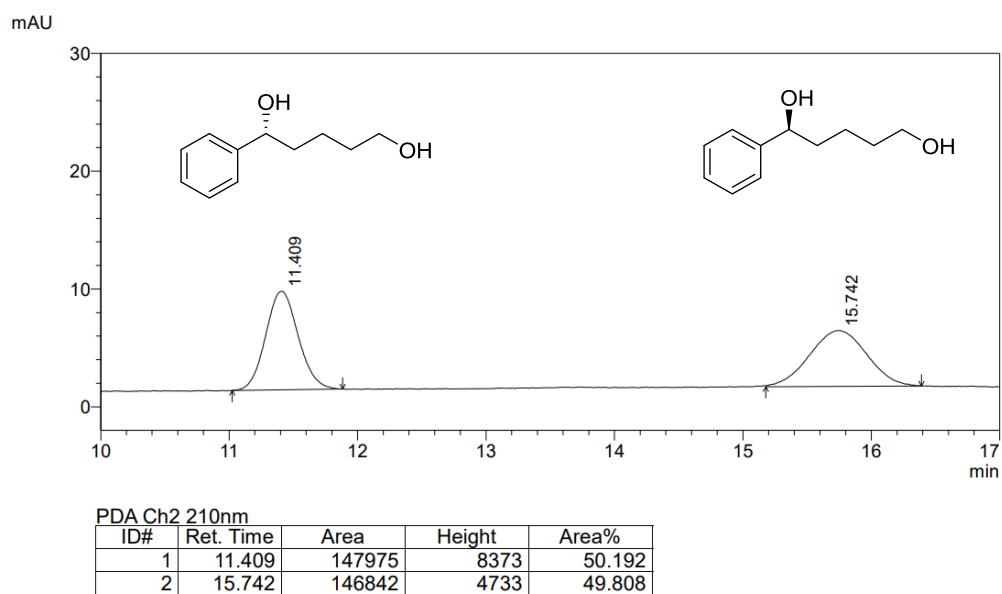


PDA Ch2 210nm

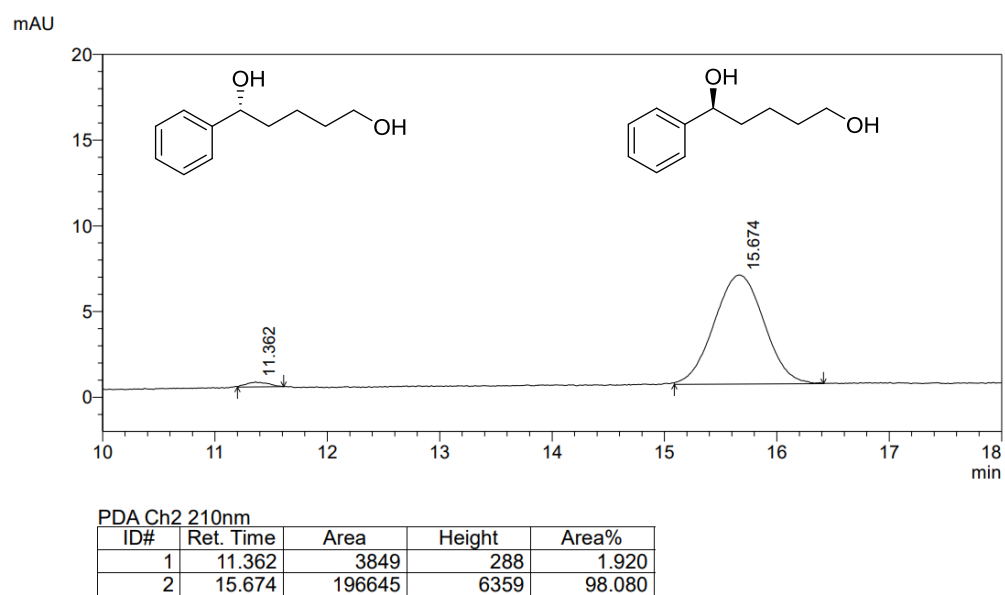
ID#	Ret. Time	Area	Height	Area%
1	7.200	2576571	246107	95.237
2	7.696	128871	14519	4.763

Chiral HPLC analysis: Diacel Chiralpak IF, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 7.2 min, $t_{(S)}$ = 7.7 min.

Chemical synthesized (*rac*)-**1b**

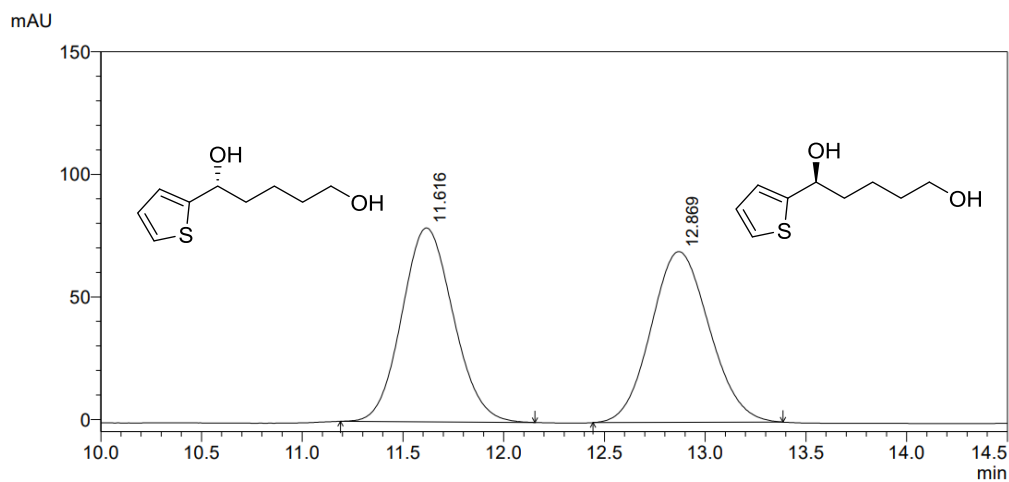


Enzymatic synthesized (*S*)-**1b**



Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 11.4 min, $t_{(S)}$ = 15.7 min.

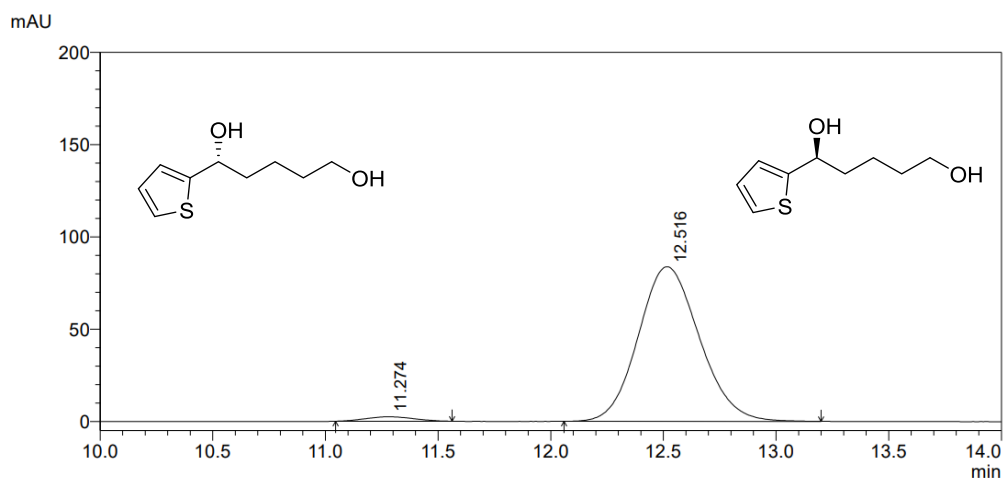
Chemical synthesized (*rac*)-**10b**



PDA Ch2 210nm

ID#	Ret. Time	Area	Height	Area%
1	11.616	1409123	79110	50.065
2	12.869	1405448	69707	49.935

Enzymatic synthesized (*S*)-**10b**



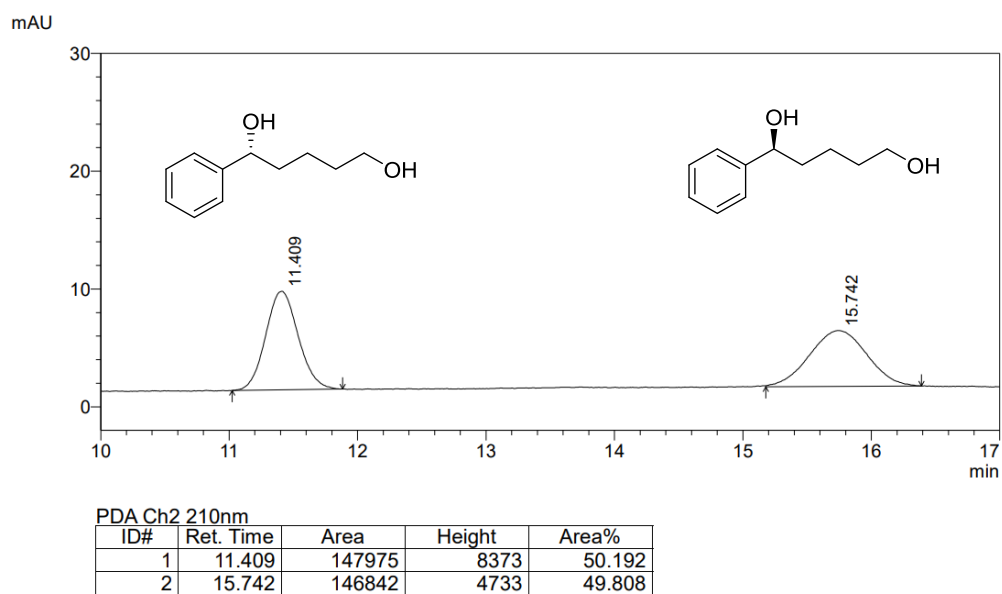
PDA Ch2 210nm

ID#	Ret. Time	Area	Height	Area%
1	11.274	35648	2380	2.199
2	12.516	1585100	83822	97.801

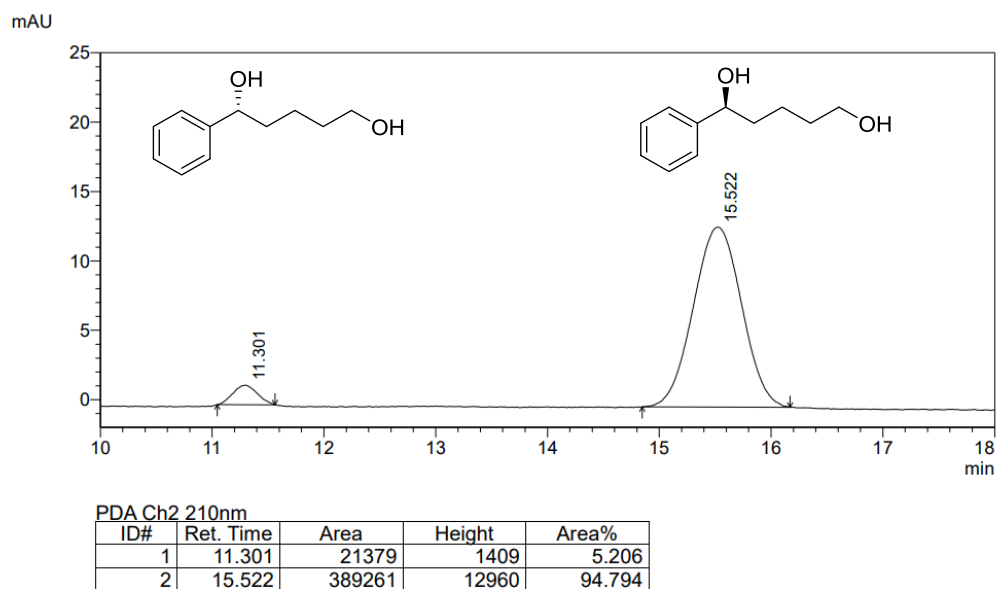
Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate

1.0 mL/min, $\lambda = 210$ nm, $t_{(R)} = 11.3$ min, $t_{(S)} = 12.5$ min.

Chemical synthesized (*rac*)-**1b**

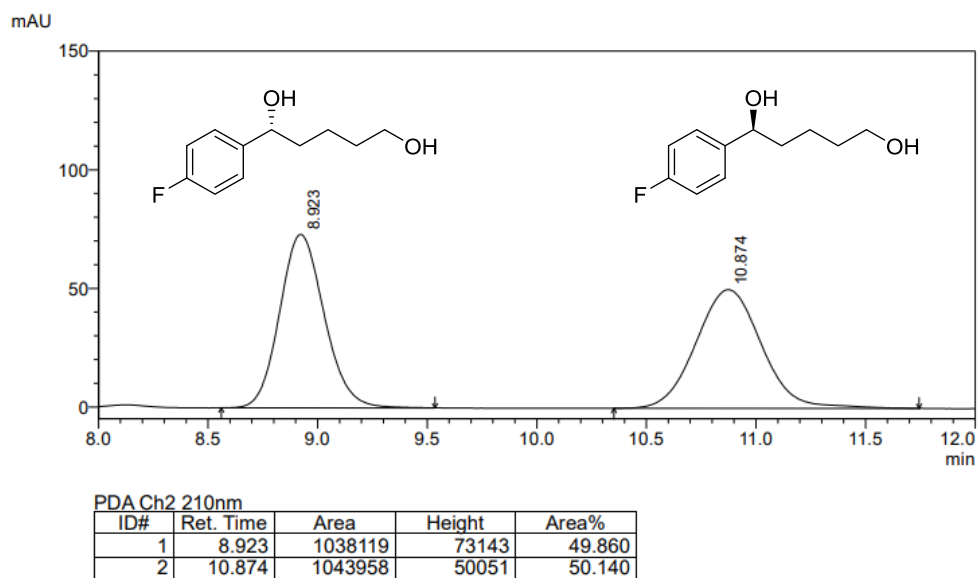


Enzymatic synthesized (*S*)-**1b** form (*rac*)-**12a**

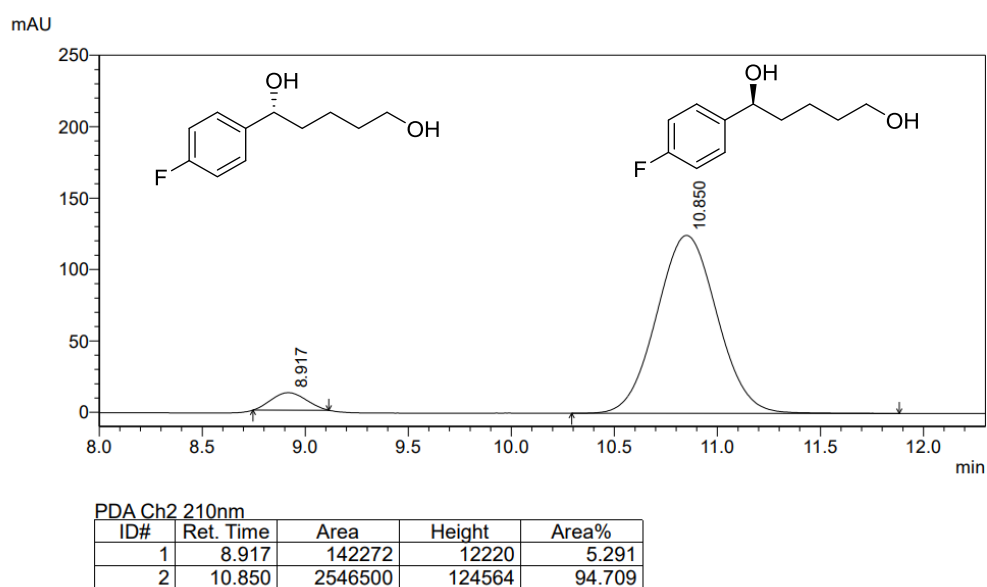


Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 11.3 min, $t_{(S)}$ = 15.5 min.

Chemical synthesized (*rac*)-**18b**

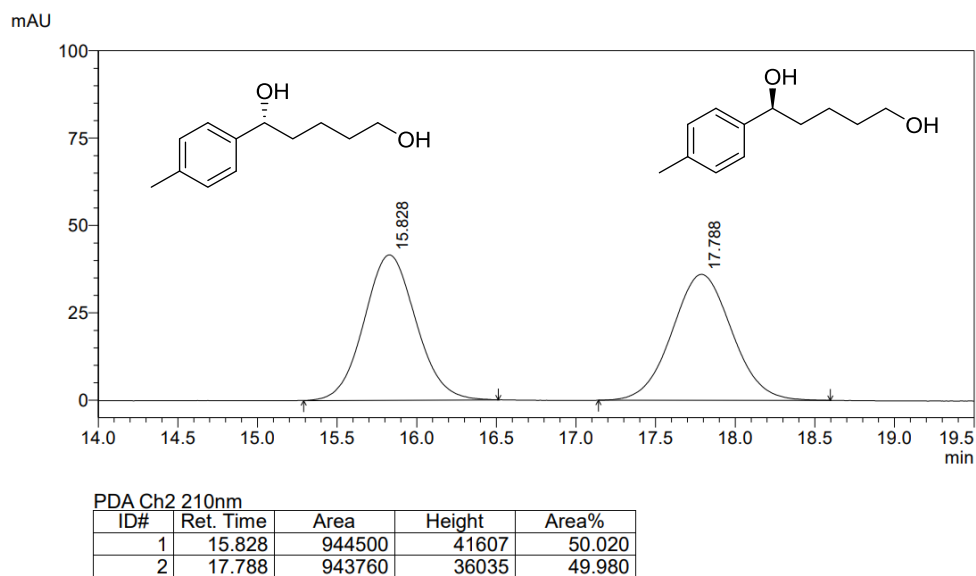


Enzymatic synthesized (*S*)-**18b**

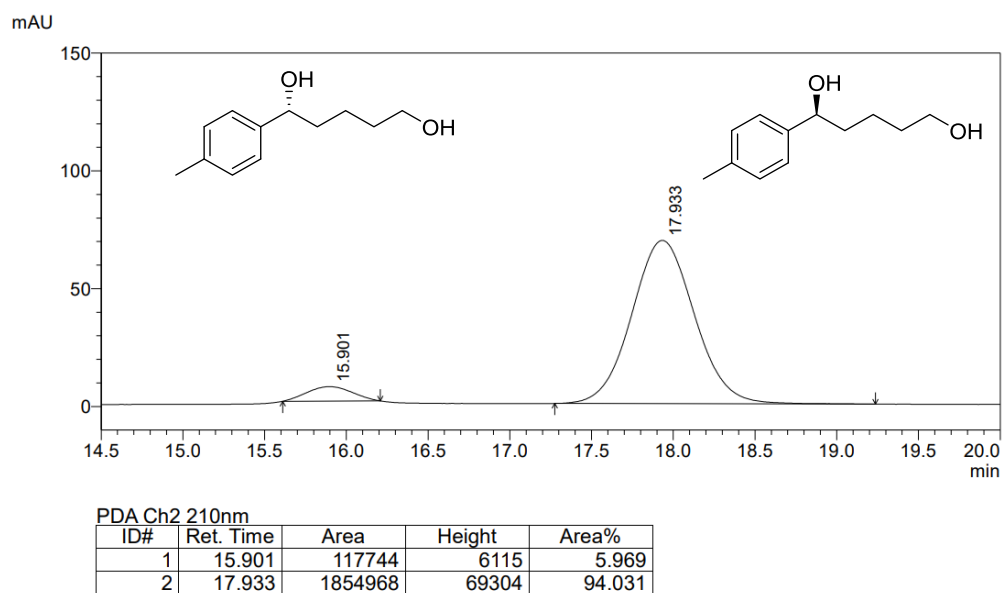


Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 8.9 min, $t_{(S)}$ = 10.9 min.

Chemical synthesized (*rac*)-**7b**



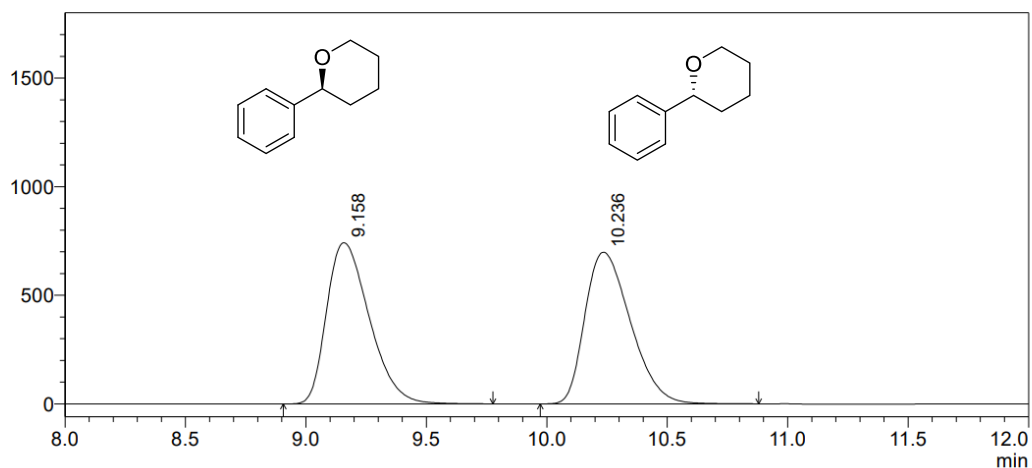
Enzymatic synthesized (*S*)-**7b** form (*rac*)-**20a**



Chiral HPLC analysis: Diacel Chiralpak IF, *n*-hexane/*i*-PrOH = 90/10, flow rate 1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 15.8 min, $t_{(S)}$ = 17.8 min.

Chemical synthesized (*rac*)-**12aa**

mAU

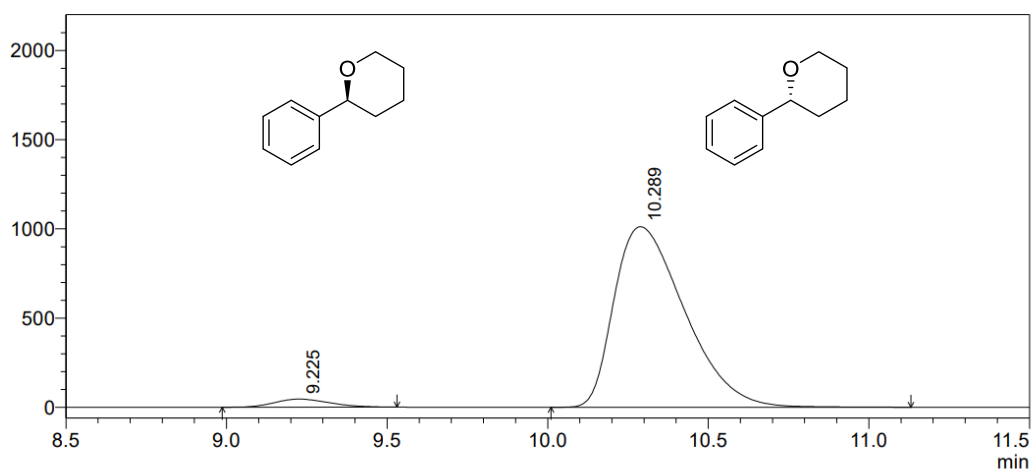


PDA Ch2 210nm

ID#	Ret. Time	Area	Height	Area%
1	9.158	9158029	742807	49.952
2	10.236	9175791	698380	50.048

Chemical synthesized (*R*)-**12aa**

mAU



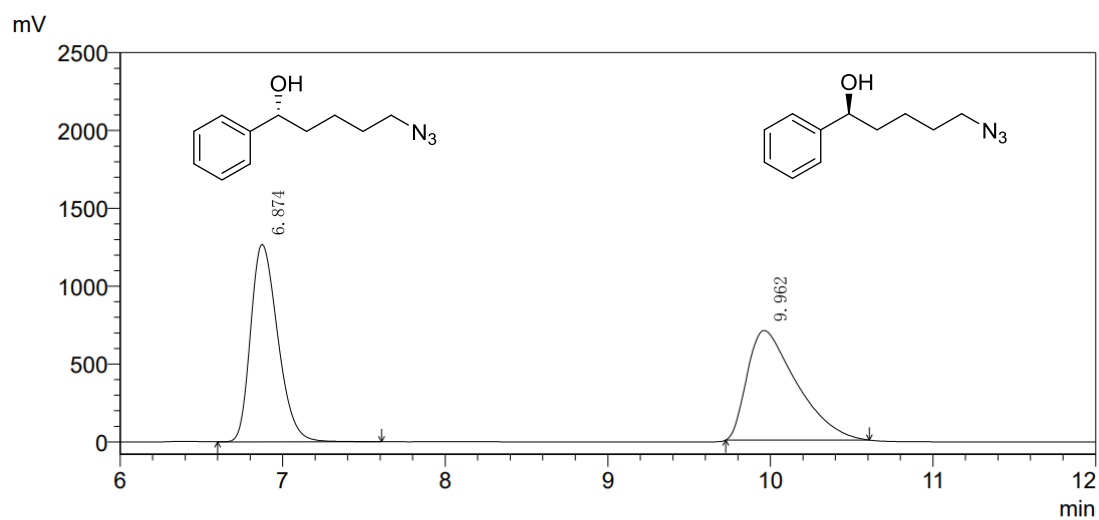
PDA Ch2 210nm

ID#	Ret. Time	Area	Height	Area%
1	9.225	535718	45333	3.349
2	10.289	15459757	1011860	96.651

Chiral HPLC analysis: Diacel Chiralpak OJ-H, *n*-hexane/*i*-PrOH = 95/5, flow rate

1.0 mL/min, λ = 210 nm, $t_{(S)}$ = 9.2 min, $t_{(R)}$ = 10.3 min.

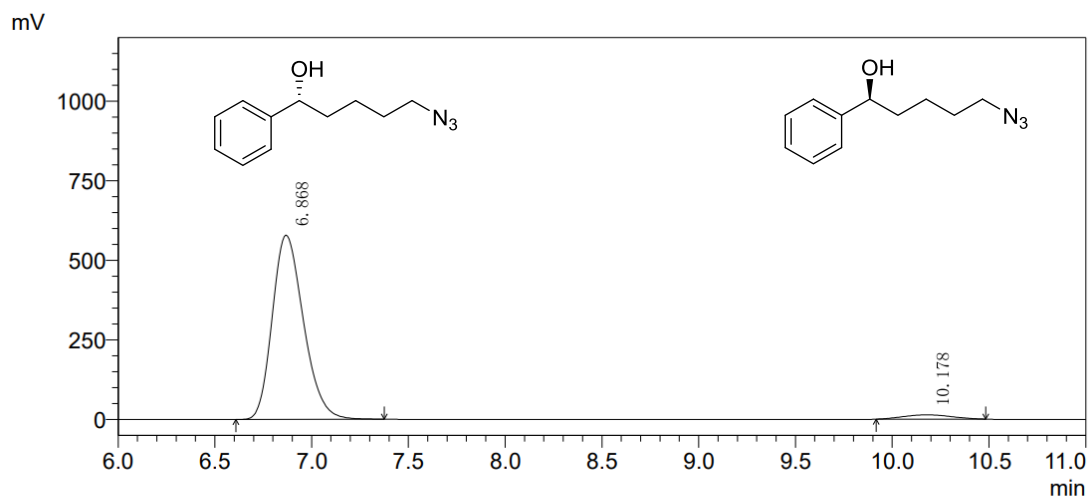
Chemical synthesized (*rac*)-**12ab**



PDA Ch1 210nm

ID#	Rt. Time	Area	Height	Area %
1	6.874	14790681	1268225	49.867
2	9.962	14869747	703754	50.133

Chemical synthesized (*R*)-**12ab**



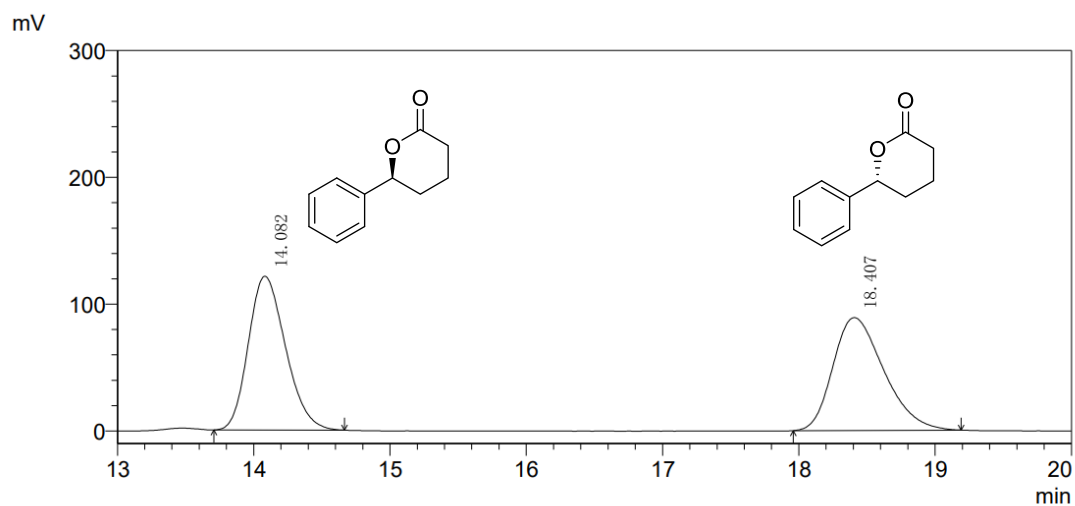
PDA Ch1 210nm

ID#	Rt. Time	Area	Height	Area %
1	6.868	6480974	579279	96.511
2	10.178	234307	13589	3.489

Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 88/12, flow rate

1.0 mL/min, λ = 210 nm, $t_{(R)}$ = 6.9 min, $t_{(S)}$ = 10.2 min.

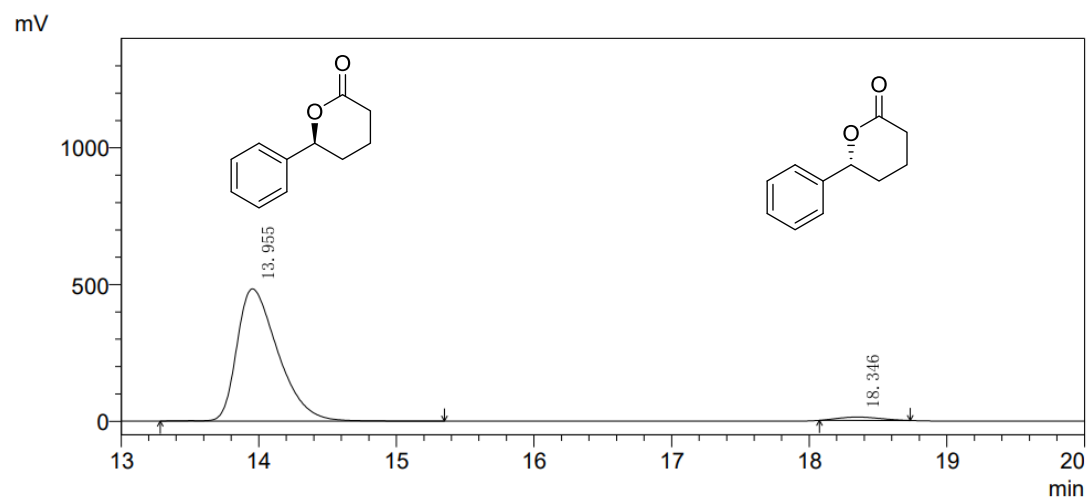
Chemical synthesized (*rac*)-**1ba**



PDA Ch1 210nm

ID#	Rt. Time	Area	Height	Area %
1	14.082	2302969	121338	49.905
2	18.407	2311775	89016	50.095

Chemical synthesized (*S*)-**1ba**

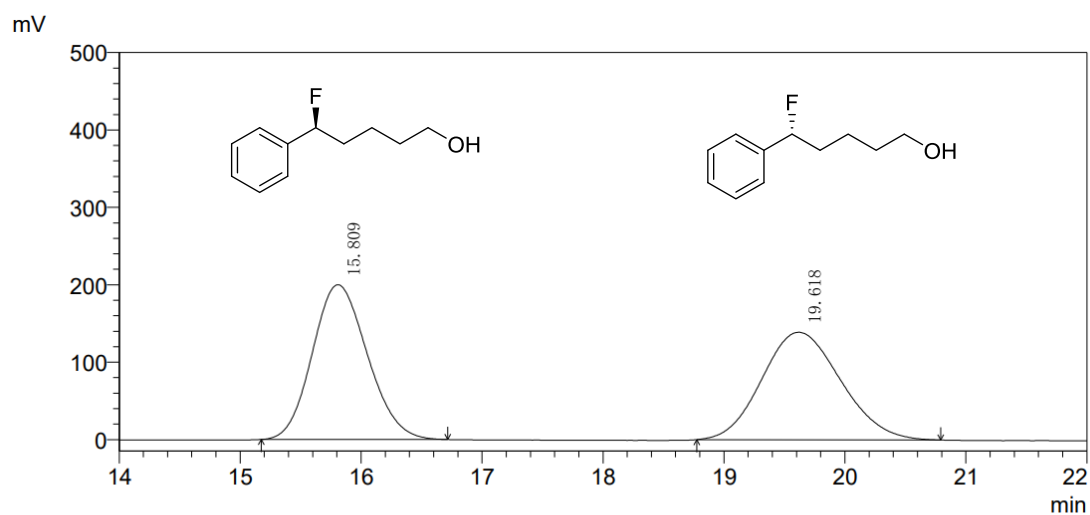


PDA Ch1 210nm

ID#	Rt. Time	Area	Height	Area %
1	13.955	10025988	483456	97.473
2	18.346	259919	12310	2.527

Chiral HPLC analysis: Diacel Chiralpak AY-H, *n*-hexane/*i*-PrOH = 80/20, flow rate 1.2 mL/min, $\lambda = 210$ nm, $t_{(R)}$ = 14.0 min, $t_{(S)}$ = 18.3 min.

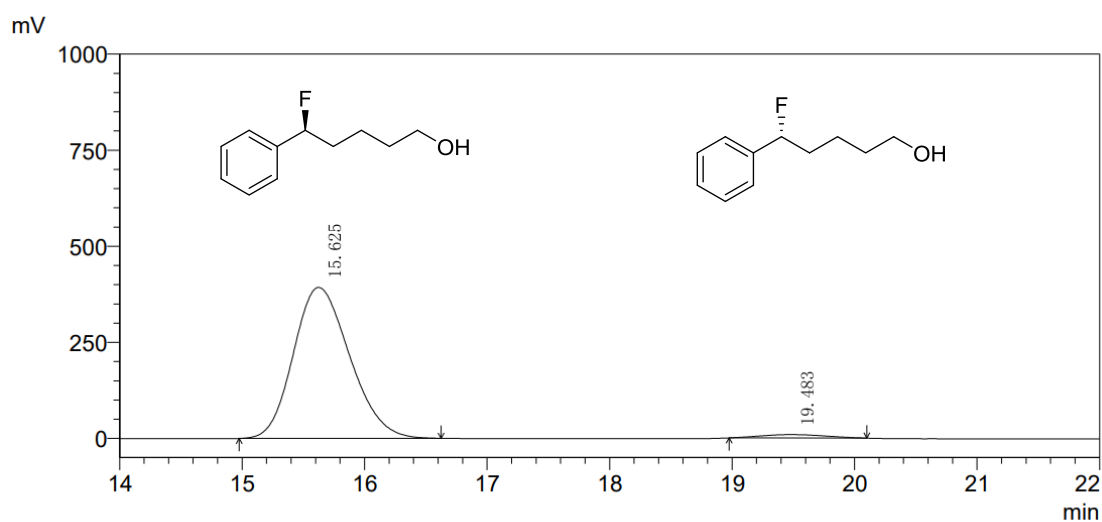
Chemical synthesized (*rac*)-**1bb**



PDA Ch1 210nm

ID#	Rt. Time	Area	Height	Area %
1	15.809	6335167	199943	50.160
2	19.618	6294795	138924	49.840

Chemical synthesized (*S*)-**1bb**



PDA Ch1 210nm

ID#	Rt. Time	Area	Height	Area %
1	15.625	12563708	393049	97.452
2	19.483	328440	8897	2.548

Chiral HPLC analysis: Diacel Chiralpak OB-H, *n*-hexane/*i*-PrOH = 90/10, flow rate 0.7 mL/min, λ = 210 nm, $t_{(S)}$ = 15.6 min, $t_{(R)}$ = 19.5 min.

11. Gene and protein sequences of key variants

>HheC-εM1 (gene sequence)

ATGGCTTCTACCGCTATTGTGACTAACGTAAAGCATTTCGGTGGCATGGGC
TCTGCGCTGCGTCTGTCTGAAGCTGGTCACACTGTTGCTTGCCATGACGAA
AGCTTCAAACAGAAAGATGAACTGGAAGCTTTCGCGGAAACTTATCCTCA
GCTGAAACCGATGTCTGAACAGGAACCGGCTGAACTGATTGAAGCTGTGA
CCTCTGCCTACGGCCAAGTTGACGTCCTGGTGTCCAACGATATTTTCGCGC
CGGAATTCCAGCCGATCGATAAATATGCTGTGGAAGATTACCGTGGTGCT
GTCGAAGCTCTGCAGATCCGCCCATTTCGACTGGTTAACGCGGTGGCTTCC
CAGATGAAGAAACGTAAATCTGGCCACATCATCTTCATTACCTCTGCAAC
TCCATTTCGGTCCGTGGAAAGAA**CGG**TCCACTTATACTTCCGCCCCGTGCTGG
CGCTTGCACTCTGGCAAACGCGCTGTCCAAAGAGCTGGGCGAATAACA
TTCCGGTTTTTCGCGATCGGTCCGAACCTGCACTCTGAAGACAGCCCGT
ACTTCTACCCGACCGAACCGTGGAAAATAACCCGGAACACGTGGCGCAC
GTAAAAAAGGTTACCGCACTGCAGCGTCTGGGTACCCAAAAAGAACTGG
GCCAACTGGTTGCGTTCCTGGCATCTGGTTCCTGTGATTACCTGACCGGTC
AAGTCTTTTGGCTGGCAGGTGGCTTCCCGATGATCGAACGTTGGCCGGGT
ATGCCGGAATAG

>HheC-εM1 (protein sequence)

MASTAIVTNVKHFGGMGSA LRLSEAGHTVACHDESFQKQKDELEAFAETYPQ
LKPMSEQEP AELIEAVTSAYGQVDVLVSNDIFAPEFQPIDKYAVEDYRGAVEA
LQIRPFALVNAVASQMKKRKSGHIIIFITSATPFGPWKE**A**STYTSARAGACTLA
NALSKELGEYNIPVFAIGPNYLHSEDSFYFYPTEPWKTNPEHVAHVKKVTALQ
RLGTQKELGELVAFLASGSCDYLTGQVFWLAGGFPMIERWPGMPE

>HheC-εM2 (gene sequence)

ATGGCTTCTACCGCTATTGTGACTAACGTAAAGCATTTCGGTGGCATGGGC
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AGCTTCAAACAGAAAGATGAACTGGAAGCTTTCGCGGAAACTTATCCTCA
GCTGAAACCGATGTCTGAACAGGAACCGGCTGAACTGATTGAAGCTGTGA
CCTCTGCCTACGGCCAAGTTGACGTCCTGGTGTCCAACGATATTTTCGCGC
CGGAATTCCAGCCGATCGATAAATATGCTGTGGAAGATTACCGTGGTGCT
GTCGAAGCTCTGCAGATCCGCCCATTGCACTGGTTAACGCGGTGGCTTCC
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CGTTGCACTCTGGCAAACGCGCTGTCCAAAGAGCTGGGCGAATACAACA
TTCCGGTTTTTCGCGATCGGTCCGAACCTGCACTCTGAAGACAGCCCGT
ACTTCTACCCGACCGAACCGTGGA³AACTAACCCGGAACACGTGGCGCAC
GTAAAAAAGGTTACCGCACTGCAGCGTCTGGGTACCCAAAAAGAACTGG
GCGAACTGGTTGCGTTCCTGGCATCTGGTTCCTGTGATTACCTGACCGGTC
AAGTCTTTTGGCTGGCAGGTGGCTTCCCGATGATCGAACGTCCT**CCGG**GTA
TGCCGGAATAG

>HheC-εM2 (protein sequence)

MASTAIVTNVKHF¹FGMG²SALRLSEAGHTVACHDE³SKQKDELEAFAETYPQ
LKPMSEQEP⁴AE⁵LIEAVTSAYGQVDVLVSNDIFAPEFQPIDKYAVEDYRGAVEA
LQIRPFALVNAVASQM⁶KKRKSGHII⁷FITSATPF⁸GPWKE⁹A¹⁰STYTSARAGACTLA
NALS¹¹KELGEYNIPVFAIGPNYLHSEDS¹²PFYPT¹³EPWKTNPEHVAHVKKVTALQ
RLGTQKELGELVAFLASGSCDYLTGQVFWLAGGFPMIER¹⁴PPGMPE

>HheC-εM3 (gene sequence)

ATGGCTTCTACCGCTATTGTGACTAACGTAAAGCATTTCGGTGGCATGGGC
TCTGCGCTGCGTCTGTCTGAAGCTGGTCACACTGTTGCTTGCCATGACGAA
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GCTGAAACCGATGTCTGAACAGGAACCGGCTGAACTGATTGAAGCTGTGA
CCTCTGCCTACGGCCAAGTTGACGTCCTGGTGTCCAACGATATTTTCGCGG
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GTCGAAGCTCTGCAGATCCGCCCATTGCACTGGTTAACGCGGTGGCTTCC
CAGATGAAGAAACGTAAATCTGGCCACATCATCTTCATTACCTCTGCAAC
TCCATTTCGGTCCGTGGAAAGAAAGCTCCACTTATACTTCCGCCCCGTGCTGG
CGTTGCACTCTGGCAAACGCGCTGTCCAAAGAGCTGGGCGAATACAACA
TTCCGGTTTTTCGCGATCGGTCCGAACCTGCACTCTGAAGACAGCCCGT
ACTTCTACCCGACCGAACCGTGGAAGAACTAACCCGGAACACGTGGCGCAC
GTAAAAAAGGTTACCGCACTGCAGCGTCTGGGTACCCAAAAAGAACTGG
GCGAACTGGTTGCGTTCCTGGCATCTGGTTCCTGTGATTACCTGACCGGTC
AAGTCTTTTGGCTGGCAGGTGGCTTCCCGATGATCGAACGTCCTCCGGGTA
TGCCGGAATAG

>HheC-εM3 (protein sequence)

MASTAIVTNVKHFGGMGSA LRLSEAGHTVACHDESFKQKDELEAFAETYPQ
LKPMSEQEP AELIEAVTSAYGQVDVLVSNDIFA GEFQPIDKYAVEDYRGAVE
ALQIRPFALVNAVASQMKKRKSGHII FITSATPFGPWKEASTYTSARAGACTL
ANALSKELGEYNIPVFAIGPNYLHSEDSFYPT EPWKTNP EHVAVHVKKVTAL
QRLGTQKELGELVAFLASGSCDYLTGQVFWLAGGFPMIERPPGMPE

>HheC-εM4 (gene sequence)

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GCTGAAACCGATGTCTGAACAGGAACCGGCTGAACTGATTGAAGCTGTGA
CCTCTGCCTACGGCCAAGTTGACGTCCTGGTGTCCAACGATATTTTCGCGC
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GCGAACTGGTTGCGTTCCTGGCATCTGGTTCCTGTGATTACCTGACCGGTC
AAGTCTTTTGGCTGGCAGGTGGCTTCCCGATGATCGAACGTCCTCCGGGTA
TGCCGGAATAG

>HheC-εM4 (protein sequence)

MASTAIVTNVKHFGGMGSA LRLSEAGHTVACHDESFKQKDELEAFAETYPQ
LKPMSEQEPAELIEAVTSAYGQVDVLVSNDIFAPEFQPIDKYAVEDYRGAVEA
LQIRPFALVNAVASQMKKRKSGHIFITSALPFGPWKEASTYTSARAGACTLA
NALSKELG EYNIPVFAIGPNYLHSEDSFYPTPEPWKTNPEHVAHVKKVTALQ
RLGTQKELGELVAFLASGSCDYLTGQVFWLAGGFPMIERPPGMPE

>HheC-εM5 (gene sequence)

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CCGGAATAG

>HheC-εM5 (protein sequence)

MASTAIVTNVKHFGGMGSA LRLSEAGHTVACHDESFQKQKDELEAFAETYPQ
LKPMSEQEP AELIEAVTSAYGQVDVLVSNDIFA GEFQPIDKYAVEDYRGAVE
ALQIRPFALVNAVASQMKKRKSGHIFITSA LPFGPWKEASTYTSARAGACTL
ANALSKELGEYNIPVFAIGPNYLHSEDSFYPTPEPWKTNP EHVAVHKKVTAL
QRLGTQKELGELVAFLASGSCDYL TGQVFWLAGGFPMIERPPGMPE

>HheC-εM6 (gene sequence)

ATGGCTTCTACCGCTATTGTGACTAACGTAAAGCATTTCGGTGGCATGGGC
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GCGAACTGGTTGCGTTCCTGGCATCTGGTTCCTGTGATTACCTGACCGGTC
AAGTCTTTTGGCTGGCAGGAGGCTTCCCGATGATCGAACGTCCTCCGGGT
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>HheC-εM6 (protein sequence)

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LKPMSEQEPAELIEAVTSAYGQVDVLVSNDIFA GEFQPIDKYAVEDYRGAVE
ALQIRPFALVNAVASQMKKRKSGHIFITSATPFGPWKE ASTYTSARAGACTL
ANALSKELGEYNIPVFAIGP QYLHSEDSFYFPTPEWKTNPETHVAHVKKVTA
LQRLGTQKELGELVAFLASGSCDYLTGQVFWLAGGFPMIER PPGMPE

>HheC-εM7 (gene sequence)

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GCGAACTGGTTGCGTTCCTGGCATCTGGTTCCTGTGATTACCTGACCGGTC
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TGCCGGAATAG

>HheC-εM7 (protein sequence)

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ANALSKELGEYNIPVFAIGPNYLHSEDSPI YPTEPWKTNPEHVAHVKKVTA
LQRLGTQKELGELVAFLASGSCDYLTGQVFWLAGGFPMIERPPGMPE

>HheC-εM8 (gene sequence)

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TGCCGGAATAG

>HheC-εM8 (protein sequence)

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LKPMSEQEPAELIEAVTSAYGQVDVLVSNDIFAGEFQPIDKYAVEDYRGAVE
ALQIRPFALVNAVASQMKKRKSGHIFITSATPFGPWKEASTYTSARAGACTL
ANALSKELGEYNIPVFAIGPQYLHSEDSPLYYPTEPWKTNPEHVAHVKKVTA
LQRLGTQKELGELVAFLASGSCDYLTGQVFWLAGGFPMIERPPGMPE

>HheC-εM1+N-His-tag (gene sequence)

ATGGCTCATCATCATCATCACTCTACCGCTATTGTGACTAACGTAAAG
CATTTCGGTGGCATGGGCTCTGCGCTGCGTCTGTCTGAAGCTGGTCACACT
GTTGCTTGCCATGACGAAAGCTTCAAACAGAAAGATGAACTGGAAGCTTT
CGCGGAAACTTATCCTCAGCTGAAACCGATGTCTGAACAGGAACCGGCTG
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CCAACGATATTTTCGCGCCGGAATTCCAGCCGATCGATAAATATGCTGTG
GAAGATTACCGTGGTGCTGTGCAAGCTCTGCAGATCCGCCCATTTCGACT
GGTTAACGCGGTGGCTTCCCAGATGAAGAAACGTAAATCTGGCCACATCA
TCTTCATTACCTCTGCAACTCCATTCGGTCCGTGGAAAGAA^{CGT}TCCACTT
ATACTTCCGCCCCGTGCTGGCGCTTGCACCTCTGGCAAACGCGCTGTCCAAA
GAGCTGGGCGAATACAACATTCCGGTTTTTCGCGATCGGTCCGAACTACCT
GCACTCTGAAGACAGCCCGTACTTCTACCCGACCGAACCGTGGAAAATA
ACCCGGAACACGTGGCGCACGTAAAAAAGGTTACCGCACTGCAGCGTCTG
GGTACCCAAAAAAGAACTGGGCGAACTGGTTGCGTTCCTGGCATCTGGTTC
CTGTGATTACCTGACCGGTCAAGTCTTTTGGCTGGCAGGTGGCTTCCCGAT
GATCGAACGTTGGCCGGGTATGCCGGAATAG

>HheC-εM1+N-His-tag (protein sequence)

MA^{HHHHH}STAIVTNVKHFGGMGSALRLSEAGHTVACHDESFKQKDELEAF
AETYPQLKPMSEQEPAELIEAVTSAYGQVDVLVSNDIFAPEFQPIDKYAVEDY
RGAVEALQIRPFALVNAVASQMKKRKSGHIIFITSATPFGPWKE^ASTYTSARA
GACTLANALSKELGEYNIPVFAIGPNYLHSEDSFYFYPEPWKTNPETHVAHVK
KVTALQRLGTQKELGELVAFLASGSCDYLTGQVFWLAGGFPMIERWPGMPE

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