Supporting Information for:

**Enantioselective Desymmetrization of Methylenedianilines via Enzyme-Catalyzed Remote Halogenation**

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*Materials:*

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Deuterated solvents were obtained from Cambridge Isotope labs. Silicycle silica gel plates (250 mm, 60 F254) were used for analytical TLC, and preparative chromatography was performed using SiliCycle SiliaFlash silica gel (230-400 mesh). Oligonucleotides were purchased from Integrated DNA Technologies (San Diego, CA). BL21(DE3) *E. coli* cells were purchased from Invitrogen (Carlsbad, CA). T7 DNA ligase, Taq DNA polymerase, and Phusion HF polymerase were purchased from New England Biolabs (Ipswitch, MA). Luria Broth (LB) and Terrific Broth (TB) media were purchased from Research Products International (Mt. Prospect, IL). Qiagen Miniprep Kits were purchased from QIAGEN

Inc. (Valencia, CA) and used according to the manufacturer's instructions. All genes were confirmed by sequencing at the University of Chicago Comprehensive Cancer Center DNA Sequencing & Genotyping Facility (900 E. 57th Street, Room 1230H, Chicago, IL 60637). Electroporation was carried out on a Bio-Rad MicroPulser using method Ec2. Ninitrilotriacetic acid (Ni-NTA) resin and Pierce® BCA Protein Assay Kits were purchased from Fisher Scientific International, Inc. (Hampton, NH), and the manufacturer's instructions were following when using both products (for Ni-NTA resin, 5 mL resin were used, with buffers delivered by a peristaltic pump at a rate of 1 mL/min, in a 4 °C cold cabinet). Amicon® 30 kD spin filters for centrifugal concentration were purchased from EMD Millipore (Billerica, MA) and used at 4,000 g at 4 °C. Biotage reverse phase columns (SNAP KP-C18-HS) were purchased from Biotage. HPLC analyses were performed using HPLC grade acetonitrile (Fisher Scientific), 18 MΩ water from a Milli-Q purification system (model No. QGARD00D2), and trifluoroacetic acid (Oakwood Chemicals). SFC analyses were performed using bone-dry grade $CO_2$ (Cylinder Gas Operations, University of Chicago, Chicago, IL), HPLC grade methanol (Fisher Scientific), and isobutanol (Sigma-Aldrich). Glucose dehydrogenase (GDH, product No. GDH-105), and NAD (product No. NAD-004626) were purchased from Codexis (Redwood City, CA). FAD (product No. 00151) was purchased from Chem-Impex International (Wood Dale, IL). AeraSeal film was purchased from Research Products International (product No. 202504).

*General Procedures:*

Standard molecular cloning procedures were followed, and the same PCR conditions were used as previously reported.[1] Point mutations in RebH variants DA, AA, YD, and ND were introduced via sequence-overlap extension PCR using primers with sequences shown in Table S1.[2]

Reactions were monitored using UPLC (Agilent 1200 UPLC with a 1290 DAD detector (G4212A) with an Agilent Eclipse Plus C18 4.6 x 150 mm column, 3.5 μM particle size; C18 4.6 x 50 mm column, 3.5 μM particle size; and C18 2.1 x 50 mm column, 1.8 μM particle size; solvent A = $H_2O$/0.1% TFA, solvent B = $CH_3CN$). Product enantioselectivity was determined using an Agilent 1200 UPLC with a 1260 Infinity SFC Control Module (G4301A) supplying supercritical $CO_2$ to channel A with a 1260 DAD VL+ detector (G1315C) (columns and conditions specified below). Reverse phase preparative chromatography was carried out using a Biotage Isolera One. $^{1}H$ and $^{13}C$ NMR spectra were acquired on a Bruker DMX-500 or DRX-500 spectrometer at room temperature, and chemical shifts are reported relative to residual solvent peaks with coupling constants reported in Hz. Mass spectra were obtained from the University of Chicago mass spectrometry facility using an Agilent Technologies 6224 TOF LC/MS.

**Table S1.** Sequences of primers used for RebH mutagenesis.

| Primer Name (Mutations) | Primer Sequence – mutations in bold |
|---|---|
| RebH_YE-EE_F (Y455E E461E) | 5’ - CTCTAC**GAA**GGCAACTTCGAGGAG**GAG**TTCCGC - 3’ |
| RebH_YE-EE_R (Y455E E461E) | 5’ - GCGGAA**CTC**CTCCTCGAAGTTGCC**TTC**GTAGAG - 3’ |
| RebH_YE-DD_F (Y455D E461D) | 5’ - CTCTAC**GAC**GGCAACTTCGAGGAG**GAC**TTCCGC - 3’ |
| RebH_YE-DD_R (Y455D E461D) | 5’ - GCGGAA**GTC**CTCCTCGAAGTTGCC**GTC**GTAGAG - 3’ |
| RebH_N-E_F (N54E) | 5’ - ACGATCCCC**GAA**CTGCAGACGGCG - 3’ |
| RebH_N-E_R (N54E) | 5’ - CGCCGTCTGCAG**TTC**GGGGATCGT - 3’ |
| RebH_N-D_F (N54D) | 5’ - ACGATCCCC**GAT**CTGCAGACGGCG - 3’ |
| RebH_N-D_R (N54D) | 5’ - CGCCGTCTGCAG**ATC**GGGGATCGT - 3’ |
| RebH_YE-DA_F (Y455D E461A) | 5’ - CTCTAC**GAC**GGCAACTTCGAGGAG**GCG**TTCCGC - 3’ |
| RebH_YE-DA_R (Y455D E461A) | 5’ - GCGGAA**CGC**CTCCTCGAAGTTGCC**GTC**GTAGAG - 3’ |
| RebH_YE-AA_F (Y455A E461A) | 5’ - CTCTAC**GCC**GGCAACTTCGAGGAG**GCG**TTCCGC - 3’ |
| RebH_YE-AA_R (Y455A E461A) | 5’ - GCGGAA**CGC**CTCCTCGAAGTTGCC**GGC**GTAGAG - 3’ |
| RebH_YE-YD_F (Y455Y E461D) | 5’ - CTCTAC**TAC**GGCAACTTCGAGGAG**GAC**TTCCGC - 3’ |
| RebH_YE-YD_R (Y455Y E461D) | 5’ - GCGGAA**GTC**CTCCTCGAAGTTGCC**GTA**GTAGAG - 3’ |

*Experimental Procedures*

**Enzyme purification:** The MBP-RebF and RebH variants used for analytical and 10 mg bioconversions were produced and purified according to a previous report.[3] For all halogenase variants, an overnight starter culture was used to inoculate either 50 mL or 750 mL TB (with 50 μg/mL kanamycin and 20 μg/mL chloramphenicol for the pGro7 plasmid) in a 250 mL Erlenmeyer flask or a 2.8 L Fernbach flask, respectively. Following growth at 37 °C, 250 rpm, until $OD_{600}$ = 0.6-0.8, gene expression was induced with IPTG and arabinose to final concentrations of 100 μM and 2 mg/mL, respectively. Gene expression continued for ~20 h at 30 °C, 250 rpm, after which time cells were harvested by centrifugation and stored at -80 °C. Cell pellets were thawed, suspended in 10 mL (for 50 mL cultures) or 30 mL (for 750 mL cultures) 25 mM HEPES (pH 7.4) and lysed by sonication while kept on ice (Qsonica S-4000 with a 0.5" horn; 5 x 1 min with 1 min rests, 20 % duty cycle delivering 40-50 W). The resulting suspensions were clarified by centrifugation, and the MBP-RebF and RebH variants were purified by Ni-NTA affinity chromatography and exchanged into a buffer of 25 mM HEPES (pH 7.4) and 10 % glycerol. Protein concentrations were measured using the Pierce® BCA Protein Assay Kit and protein solutions were then stored at -20 °C until use.

**Chlorination to Produce Racemic Standards:**[4] To the starting material (**1-7**, 0.05-0.1 mmol) in 10 mL acetonitrile in a 25 mL round-bottomed flask equipped with a reflux condenser was added 1 equivalent of *N*-chlorosuccinimide. The reaction was then stirred at 80 °C for 48 hours. The solvent was then removed and the crude material was purified by reverse-phase chromatography using a Biotage chromatography system to yield the TFA salts of the racemic chlorinated compounds. All of these except **1a**•TFA were then dissolved in saturated $Na_2CO_3$, the mixture

was extracted three times with methylene chloride, and the combined organic extracts were dried over $Na_2SO_4$ and concentrated to yield the corresponding free bases.

**General Procedure for Analytical Bioconversions:** Substrate (37.5 nmol) was added to a 1.5 mL Eppendorf tube as a 10 mM solution in MeOH. Individual solutions of NAD (0.2 equiv., 100 μM final concentration), FAD (0.2 equiv., 100 μM final concentration), NaCl (20 equiv., 10 mM final concentration), and glucose dehydrogenase (9 U/mL final concentration GDH) were added to the reaction. This was diluted such that the final reaction volume was 75 μL with HEPES buffer, and halogenase variant (0.005-0.05 equiv., 2.5-25 μM final concentration) and MBP-RebF (0.005 equiv., 2.5 μM final concentration) were added as solutions of HEPES/glycerol buffer (25 mM HEPES, pH 7.4, 10% glycerol v/v). The reaction was initiated with a solution of 1 M glucose (40 equiv., 20 mM final concentration), the tube was closed, and incubated at 25 °C at 600 rpm. Reactions were quenched by addition of a reaction volume of MeOH after 16 hours. These reactions were analyzed by UPLC (Agilent 1200 UPLC with an Agilent Eclipse Plus C18 4.6 x 150 mm column, 3.5 μM particle size; solvent A = $H_2O$/0.1% TFA, solvent B = $CH_3CN$). The following method was used for all substrates: 0-10 min, B = 15%; 10-20 min, B = 15-100%; 20-24 min, B = 100%.

**General Procedure for Large Scale Bioconversions (without subsequent cross-coupling)**: Substrate (0.1 mg-10.0 mg) was added to a 20 mL scintillation vial (for 0.1-1 mg bioconversions) or a crystallization dish (100 x 50 mm) (for 10 mg bioconversions) as a solution in MeOH. Solutions of NAD (0.2 equiv., 100 μM final concentration), FAD (0.2 equiv., 100 μM final concentration), NaCl (20 equiv., 10 mM final concentration), and a glucose dehydrogenase

(9 U/mL final concentration GDH) were added to the vial. The resulting solution was diluted to the appropriate volume with HEPES buffer, and halogenase variant (0.01-0.05 equiv., 5-25 μM final concentration) and MBP-RebF (0.005 equiv., 2.5 μM final concentration) were added as solutions of HEPES/glycerol buffer (25 mM HEPES, pH 7.4, 10% glycerol v/v). The reaction was initiated with a solution of 1 M glucose (40 equiv., 20 mM final concentration), sealed with an AeraSeal film, and left on the benchtop at room temperature without shaking. These reactions were analyzed by UPLC (Agilent 1200 UPLC with an Agilent Eclipse Plus C18 4.6 x 150 mm column, 3.5 μM particle size; solvent A = $H_2O$/0.1% TFA, solvent B = $CH_3CN$). The following method was used for all substrates: 0-10 min, B = 15%; 10-20 min, B = 15-100%; 20-24 min, B = 100%. Reactions were monitored by UPLC and in most cases stopped after 16 hours. The bioconversions were quenched with HCl (5 M, until pH<2) and saturated with NaCl. For 0.1-1 mg bioconversions, precipitated protein was spun down by centrifugation (15,000 g for 10 min.), and the supernatant was decanted and brought to pH>12 through addition of NaOH (5M). For 10 mg bioconversions, precipitated protein was filtered out through a pad of Celite, and the filtrate was brought to pH>12 through addition of NaOH (5M). The filtrate was extracted into $CH_2Cl_2$. The crude material was either analyzed directly or purified by reverse phase chromatography (Biotage SNAP-KP-C18-HS, gradient from pure $H_2O$ to 40% $CH_3CN/H_2O$). See traces below.

**SFC Analysis:** To determine the e.r. values of the products, analyses were carried out using an Agilent 1200 UPLC with a 1260 Infinity SFC Control Module (G4301A) supplying supercritical $CO_2$ to channel A with a 1260 DAD VL+ detector (G1315C). The crude product of a typically 0.5 mg bioconversion was resuspended in 200 μL of MeOH, of which 15 μL were injected into a

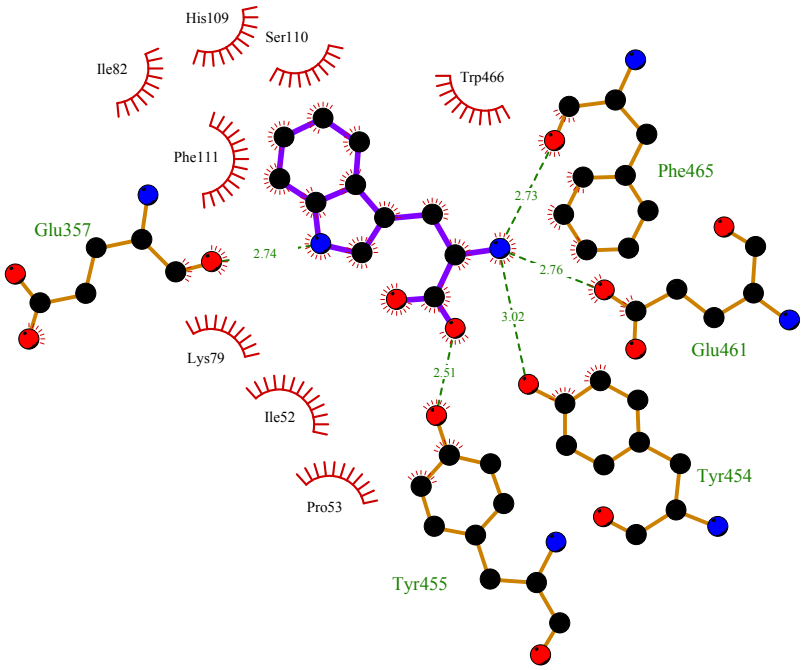
5 µL loop. A Daicel Chiral Technologies (West Chester, PA, USA) IC-3 column was used with an isocratic method of 65% $CO_2$, 35% MeOH/25 mM isobutanol, 3.0 mL/min, for 10 minutes for all substrates except the *t*-Bu substrate, **1**, which was analyzed using an Agilent 1200 UPLC with a 1290 DAD detector (G4212A) with a Phenomenex Lux 3u Cellulose-1 250 x 4.6 mm column with an isocratic method of 70% $H_2O$/0.1% triethylamine, 30% ACN/0.1% triethylamine, 1.0 mL/min, for 40 minutes. E.r. values were determined by taking the ratios of the areas of the UV peaks (at 254 nm) that match the retention times observed for identical analyses of the racemic products.

**Docking Simulations**

A crystal structure of RebH at a resolution of 2.15Å (PDB ID: 2OA1) was used for molecular simulations[5]. The FAD, CL and TRP ligands were removed, the 4V and YE-DA variants were modeled, and geometry optimization of the resulting variants was conducted using Swiss-PDBViewer [6]. Swiss-PDBViewer uses GROMOS 43B1 force field for geometry optimization. Molecular docking simulations were carried out with AutoDock Vina, which has been widely used for detecting binding poses of ligands[7]. All protein structures including ligands (halogenation substrates) were converted into PDBQT format by AutoDock tools prior to docking simulations. Polar hydrogen bonds were added to the receptors (protein structures) and Gasteiger charges were typed to the structures. A cubic grid box (grid spacing = 1.000 Å; 24 × 20 × 20 grid points) was centered on the coordinates of the tryptophan in original PDB (PDB ID: 2OA1). Exhaustiveness and number of binding modes were set to 15. The resulting docking poses were analyzed based on binding energies and the mechanism of halogenase which could ensure feasibility each reactive position as previously reported[1]. LigPlot[8] structures for each pose

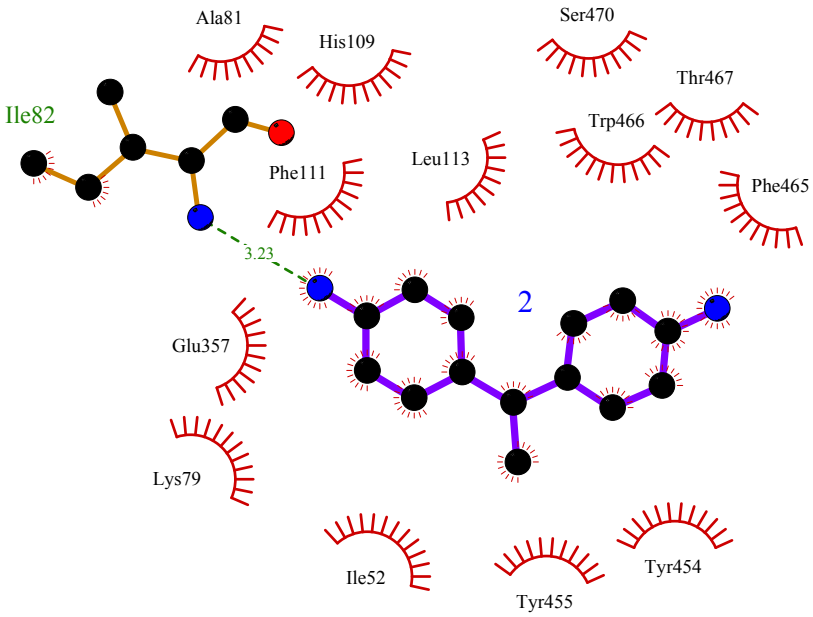
were also generated to visualize interactions between the docked ligand and the enzyme (Fig. S1a-d). These correspond to Fig. 1a-d in the manuscript.

**Figure S1a.** Ligplot diagram for Fig. 1a.




4V_Trp

**Figure S1b.** LigPlot diagram for Fig. 1b.




4V_0001E (cyan)

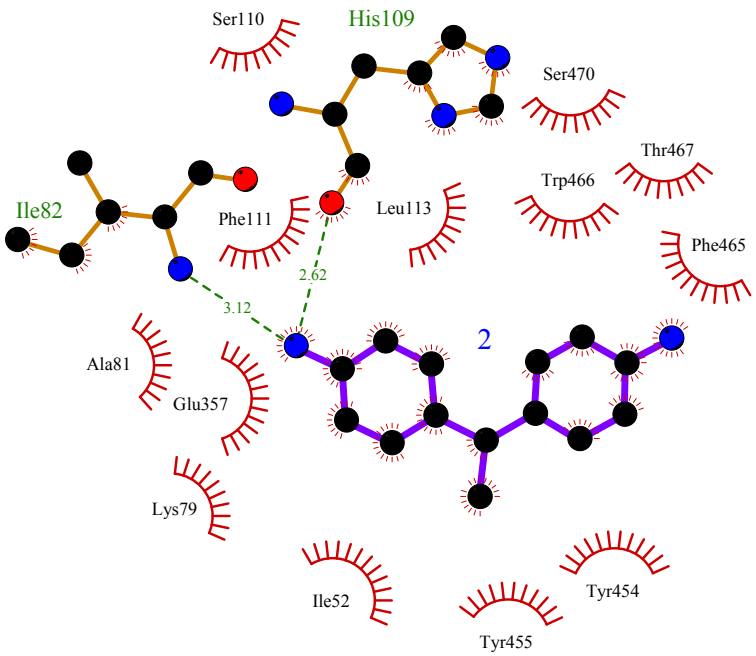
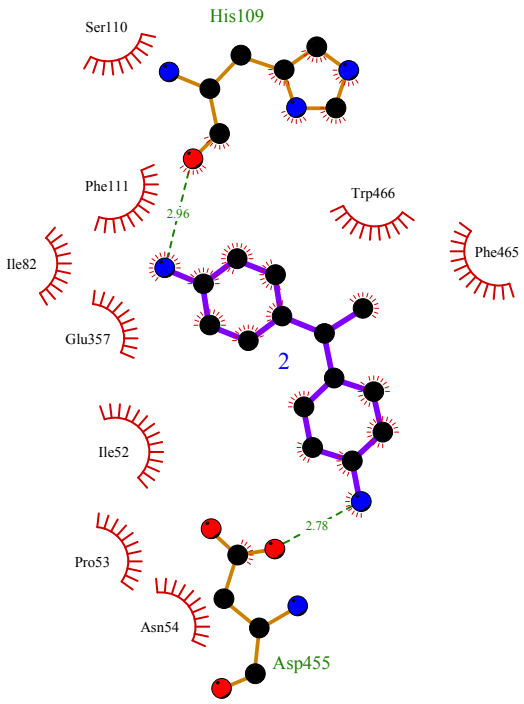
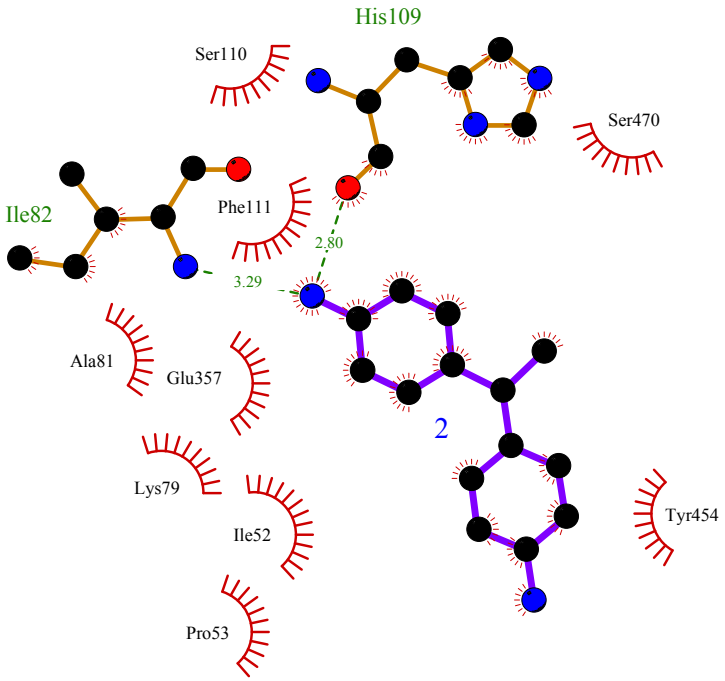



**Figure S1c.** LigPlot diagram for Fig. 1c.

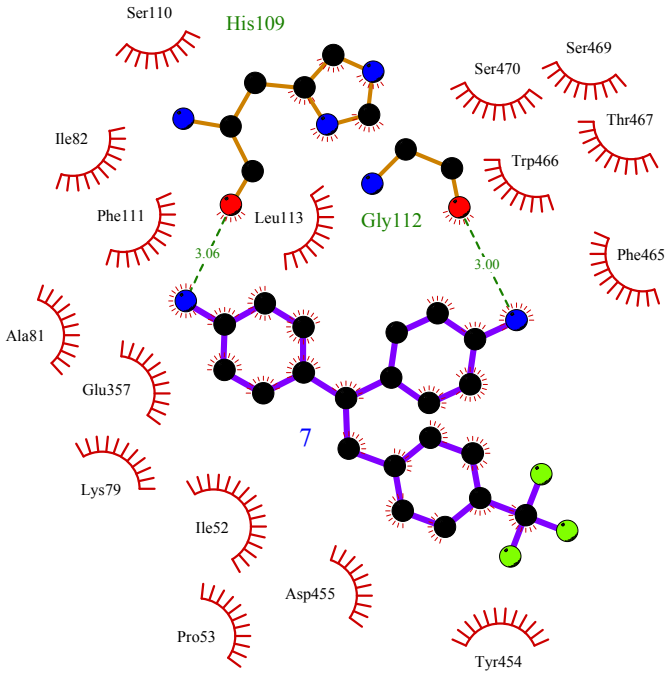



YE-DA_0005E(cyan)

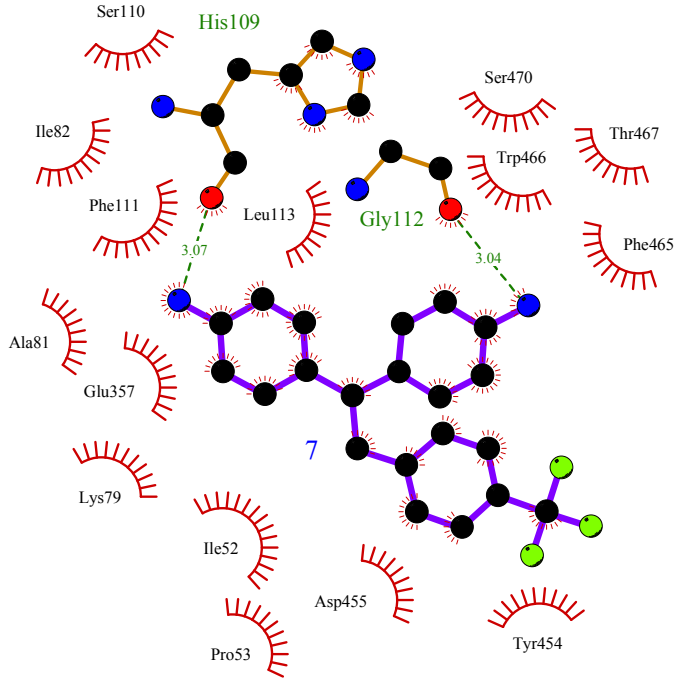



YE-DA_0007E(green)

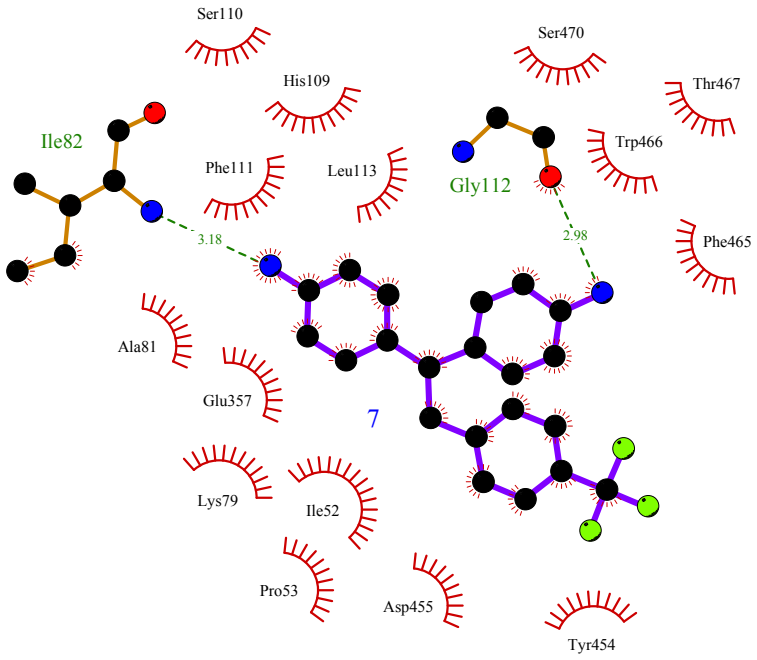
**Figure S1d.** LigPlot diagram for Fig. S1d.




YE-DA_ye-da_3_0001




YE-DA_ye-da_3_0002




YE-DA_ye-da_3_0003

*Detailed Synthesis and Characterization*

***t*-Bu dianilinemethane (1):** This compound was synthesized as described in a previous report.[9] $^{1}$H NMR (500 MHz, MeOD) δ 7.19 (d, $J$ = 8.4 Hz, 4H), 6.66 (d, $J$ = 8.5 Hz, 4H), 3.51 (s, 1H), 0.98 (s, 9H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 144.07, 134.13, 130.51, 114.80, 62.69, 31.14, 29.26. HRMS (ESI-TOF) calc'd for $C_{17}H_{23}N_2$ $[M + H]^+$: 255.1861, found: 255.1182.

***n*-Pr dianilinemethane (2):** This compound was synthesized as described in a previous report.[10] $^{1}$H NMR (500 MHz, $CDCl_3$) δ 6.98 (d, $J$ = 8.4 Hz, 4H), 6.59 (d, $J$ = 8.4 Hz, 4H), 3.68 (t, $J$ = 7.8 Hz, 1H), 1.89 (q, $J$ = 7.7 Hz, 2H), 1.24 (sextet, $J$ = 7.7 Hz, 2H), 0.88 (t, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 144.14, 136.33, 128.56, 115.19, 49.28, 38.31, 21.18, 14.08. HRMS (ESI-TOF) calc'd for $C_{16}H_{21}N_2$ $[M + H]^+$: 241.1705, found: 241.1023.

**Methyl dianilinemethane (3):** This compound was synthesized as described in a previous report.[11] $^{1}$H NMR (500 MHz, $CDCl_3$) δ 6.99 (d, $J$ = 8.1 Hz, 4H), 6.61 (d, $J$ = 8.5 Hz, 5H), 3.94 (q, $J$ = 7.3 Hz, 1H), 1.53 (d, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 144.30, 137.34, 128.37, 115.22, 43.10, 22.36. HRMS (ESI-TOF) calc'd for $C_{14}H_{17}N_2$ $[M + H]^+$: 213.1392, found: 213.0716.

**Ethyl ester dianilinemethane (4):** To a stirred solution of 0.5 mmol (129.1 mg) of bis(4-nitrophenyl)methane in 4 mL THF at 0 °C were added 1 equiv. (0.5 mmol, 56.1 mg) KO*t*Bu and 1.1 equiv. (0.55 mmol, 145.4 mg) 18-C-6. This solution was stirred for 10 minutes and cooled to -78 °C. 1 equiv. (0.5 mmol, 83.5 mg) of ethyl 2-bromoacetate in 1 mL THF was then added dropwise via syringe and the reaction was allowed to warm to room temperature. The reaction mixture was quenched with saturated ammonium chloride and extracted with ethyl acetate. The combined organic extracts were dried with $Na_2SO_4$, filtered, and concentrated via rotary evaporation. The resulting residue was purified via normal phase silica flash chromatography (10% ethyl acetate in hexanes) to provide the alkylated product as a yellow liquid. The entirety of this material was dissolved in 4 mL MeOH, added to a Parr apparatus, and stirred at room temperature with 14 mg of Pd/C (10%) under a $H_2$ atmosphere. The reaction mixture was filtered, the filtrate was concentrated, and the residue was purified by normal phase silica flash chromatography (30% ethyl acetate in hexanes) to afford 116 mg (82%) of **4**. $^{1}$H NMR (500 MHz, MeOD) δ 7.01 – 6.94 (d, 4H), 6.70 – 6.63 (d, 4H), 4.26 (t, $J$ = 8.2 Hz, 1H), 4.00 (q, $J$ = 7.1 Hz, 2H), 2.93 (d, $J$ = 8.2 Hz, 2H), 1.11 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.22, 144.61, 134.33, 128.44, 115.24, 60.29, 45.54, 41.38, 14.14. HRMS (ESI-TOF) calc'd for

$C_{11}H_{14}NO_2$ [M – $C_6H_7N$ (elimination to lose aniline to form α,β-unsaturated system) + H]$^+$: 192.l025, found: 192.0402.

**Benzyl dianilinemethane (5):** This compound was synthesized as described in a previous report.[12] $^1$H NMR (500 MHz, $CDCl_3$) δ 7.16 (t, *J* = 7.1 Hz, 2H), 7.10 (t, *J* = 7.5 Hz, 1H), 6.99 (d, *J* = 6.8 Hz, 4H), 6.57 (d, *J* = 8.3 Hz, 4H), 4.02 (t, *J* = 7.7 Hz, 1H), 3.24 (d, *J* = 7.8 Hz, 2H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 144.29, 140.89, 135.43, 129.18, 128.78, 127.97, 125.68, 115.18, 51.41, 42.55. HRMS (ESI-TOF) calc'd for $C_{20}H_{21}N_2$ [M + H]$^+$: 289.1705, found: 289.0049.

***m*-Methoxybenzyl dianilinemethane (6):** To a stirred solution of 0.5 mmol (129.1 mg) of bis(4-nitrophenyl)methane in 4 mL THF at 0 °C were added 1 equiv. (0.5 mmol, 56.1 mg) KO*t*Bu and 1.1 equiv. (0.55 mmol, 145.4 mg) 18-C-6. This solution was stirred for 10 minutes and cooled to -78 °C. 1 equiv. (0.5 mmol, 100.5 mg) of *m*-MeO-benzyl bromide in 1 mL THF was then added dropwise via syringe and the reaction was allowed to warm to room temperature. The reaction mixture was quenched with saturated ammonium chloride and extracted with ethyl acetate. The combined organic extracts were dried with $Na_2SO_4$, filtered, and concentrated via rotary evaporation. The resulting residue was purified using silica gel chromatography using ethyl acetate/hexanes to provide the alkylated product. The entirety of this material was dissolved in 2 mL MeOH, added to a Parr apparatus under a $H_2$ atmosphere (200 psi). The reaction mixture was filtered through a pad of celite, the filtrate was concentrated, and the residue was purified by silica gel chromatography using ethyl acetate/hexanes containing triethylamine to afford 128 mg (80% yield) of **6**.$^1$H NMR (500 MHz, MeOD) δ 7.04 (t, *J* = 7.9 Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 4H), 6.63 (m, 6H), 6.54 (s, 1H), 3.99 (t, *J* = 7.8 Hz, 1H), 3.63 (s, 3H), 3.20 (d, *J* = 7.9 Hz, 2H).

$^{13}$C NMR (126 MHz, $CDCl_3$) δ 159.23, 144.3, 142.52, 135.40, 128.89, 128.77, 121.66, 115.19, 114.77, 111.26, 55.06, 51.27, 42.57. HRMS (ESI-TOF) calc'd for $C_{21}H_{23}N_2O$ $[M + H]^+$: 319.1811, found: 319.0985.

***p*-Trifluoromethylbenzyl dianilinemethane (7):** To a stirred solution of 0.5 mmol (129.1 mg) of bis(4-nitrophenyl)methane in 4 mL THF at 0 °C were added 1 equiv. (0.5 mmol, 56.1 mg) KO*t*Bu and 1.1 equiv. (0.55 mmol, 145.4 mg) 18-C-6. This solution was stirred for 10 minutes and cooled to -78 °C. 1 equiv. (0.5 mmol, 119.5 mg) of *p*-$CF_3$-benzyl bromide in 1 mL THF was then added dropwise via syringe and the reaction was allowed to warm to room temperature. The reaction mixture was quenched with saturated ammonium chloride and extracted with ethyl acetate. The combined organic extracts were dried with $Na_2SO_4$, filtered, and concentrated via rotary evaporation. The resulting residue was purified by normal phase silica flash chromatography (5% ethyl acetate in hexanes) to provide the alkylated product. The entirety of this material was dissolved in 2 mL MeOH, added to a Parr apparatus, and stirred at room temperature with 20 mg of Pd/C (10%) under a $H_2$ atmosphere (200 psi). The reaction mixture was filtered through a pad of celite, and the filtrate was concentrated to afford 137 mg (77% yield) of **7**. $^1$H NMR (500 MHz, MeOD) δ 7.40 (d, $J$ = 8.0 Hz, 2H), 7.17 (d, $J$ = 7.9 Hz, 2H), 6.97 (d, $J$ = 8.3 Hz, 4H), 6.64 (d, $J$ = 8.3 Hz, 4H), 4.03 (t, $J$ = 7.9 Hz, 1H), 3.29 (d, $J$ = 8.0 Hz, 2H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 145.03, 144.50, 134.76, 129.41, 128.68, 124.87 (q, $J$= 3.8 Hz), 115.21, 51.18, 42.34. HRMS (ESI-TOF) calc'd for $C_{21}H_{20}F_3N_2$ $[M + H]^+$: 357.1579, found: 357.0754.

**Racemic monochlorinated *t*-Bu dianilinemethane (1a):** This compound was prepared from 0.2 mmol (51 mg) of **1** as described in the general procedures. After reaction completion, a significant amount of dichlorinated material was observed, which was removed by normal phase flash chromatography (eluting with 20:1 DCM:MeOH) before the reaction mixture was subsequently purified by reverse phase chromatography as described in the general procedures to afford **1a** in 14% yield (11.1 mg of **1a**•TFA, 0.028 mmol). $^{1}$H NMR (500 MHz, MeOD) δ 7.59 (d, $J$ = 8.5 Hz, 2H), 7.28 (m, 3H), 7.18 (dd, $J$ = 8.3, 2.0 Hz, 1H), 6.80 (d, $J$ = 8.3 Hz, 1H), 3.72 (s, 1H), 1.01 (s, 9H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 144.36, 140.68, 135.03, 133.40, 130.45, 130.3, 128.96, 118.84, 115.48, 114.87, 62.46, 35.16, 29.21. HRMS (ESI-TOF) calc'd for $C_{17}H_{22}N_2Cl$ $[M + H]^+$: 289.1472 and 291.1442, found: 289.0621 and 291.0592.

**Racemic monochlorinated *n*-Pr dianilinemethane (3a):** This compound was prepared from 0.473 mmol (113.6 mg) of **2** as described in the general procedures. After reaction completion, the reaction mixture was purified as described in the general procedures to afford **3a** in 63% yield (81.8 mg, 0.298 mmol). $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.11 (d, $J$ = 1.8 Hz, 1H), 7.01 (d, $J$ = 8.2 Hz, 2H), 6.93 (dd, $J$ = 8.2, 1.7 Hz, 1H), 6.69 (d, $J$ = 8.2 Hz, 1H), 6.64 (d, $J$ = 8.5 Hz, 2H), 3.69 (t, $J$ = 8.0 Hz, 1H), 1.91 (q, $J$ = 7.3 Hz, 2H), 1.28 (sextet, $J$ = 7.7 Hz, 2H), 0.93 (t, $J$ = 7.3 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 144.37, 140.66, 137.35, 135.56, 128.53, 128.43, 127.07, 119.24, 115.93, 115.25, 49.09, 38.13, 21.12, 14.06. HRMS (ESI-TOF) calc'd for $C_{16}H_{20}N_2Cl$ $[M + H]^+$: 275.1315 and 277.1286, found: 275.0503 and 277.0475.

**Racemic monochlorinated methyl dianilinemethane (2a):** This compound was prepared from 0.309 mmol (65.8 mg) of **3** as described in the general procedures. After reaction completion, the

reaction mixture was purified as described in the general procedures to afford **2a** in 36% yield (27.3 mg, 0.111 mmol). $^{1}$H NMR (500 MHz, $CHCl_3$) δ 7.11 (d, $J$ = 1.7 Hz, 1H), 7.00 (d, $J$ = 8.2 Hz, 2H), 6.92 (dd, $J$ = 8.2, 1.7 Hz, 1H), 6.70 (d, $J$ = 8.2 Hz, 1H), 6.65 (d, $J$ = 8.5 Hz, 2H), 3.95 (m, 3H), 1.55 (d, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 144.41, 140.69, 138.35, 136.58, 128.30, 128.21, 126.87, 115.88, 115.21, 115.16, 42.89, 22.12. HRMS (ESI-TOF) calc'd for $C_{14}H_{16}N_2Cl$ $[M + H]^+$: 247.1002 and 249.0973, found: 247.0222 and 249.0189.

**Racemic monochlorinated ethyl ester dianilinemethane (4a):** This compound was prepared from 0.530 mmol (150.5 mg) of **4** as described in the general procedures. After reaction completion, the reaction mixture was purified as described in the general procedures to afford **4a** in 33% yield (54.9 mg 0.173 mmol). $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.03 (d, $J$ = 8.3 Hz, 2H), 6.98 (d, $J$ = 1.8 Hz, 1H), 6.88 (dd, $J$ = 8.0, 2.1 Hz, 1H), 6.62 (d, $J$ = 8.4 Hz, 2H), 6.42 (d, $J$ = 8.1 Hz, 1H), 4.35 (t, $J$ = 8.1 Hz, 1H), 4.05 (q, $J$ = 7.1 Hz, 2H), 2.95 (d, $J$ = 8.1 Hz, 2H), 1.14 (t, $J$ = 7.2 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.15, 144.62, 140.09, 134.29, 133.67, 128.43, 127.50, 126.32, 123.23, 115.22, 113.34, 60.26, 45.83, 41.48, 14.12. HRMS (ESI-TOF) calc'd for $C_{11}H_{13}NO_2Cl$ [M – $C_6H_7N$ (elimination to lose aniline to form α,β-unsaturated system) + H]$^+$: 226.0635 and 228.0605, found: 225.9876 and 227.9844.

**Racemic monochlorinated benzyl dianilinemethane (5a):** This compound was prepared from 0.235 mmol (67.8 mg) of **5** as described in the general procedures. After reaction completion, the reaction mixture was purified as described in the general procedures to afford **5a** in 37% yield (36.2 mg, 0.112 mmol). $^{1}$H NMR (500 MHz, $CDCl_3$) δ 7.20 (t, $J$ = 7 Hz, 2H), 7.15 (t, $J$ = 7.3 Hz, 2H), 7.09 (d, $J$ = 1.8 Hz, 1H), 7.03 (d, $J$ = 7.1, 2H), 6.98 (d, $J$ = 8.3 Hz, 2H), 6.89 (dd, $J$ = 8.2,

1.8 Hz, 1H), 6.66 (d, $J$ = 8.2 Hz, 1H), 6.61 (d, $J$ = 8.5 Hz, 2H), 4.03 (t, $J$ = 7.8 Hz, 1H), 3.26 (dd, $J$ = 7.9 Hz, 3.3 Hz, 2H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 144.50, 140.80, 140.46, 136.38, 134.69, 129.13, 128.73, 128.65, 128.04, 127.27, 125.82, 119.22, 115.83, 115.21, 51.20, 42.37. HRMS (ESI-TOF) calc'd for $C_{20}H_{20}N_2Cl$ $[M + H]^+$: 323.1315 and 325.1286, found: 323.0427 and 325.0394.

**Racemic monochlorinated *m*-methoxybenzyl dianilinemethane (6a):** This compound was prepared from 0.111 mmol (35.2 mg) of **6** as described in the general procedures. After reaction completion, the reaction mixture was purified as described in the general procedures to afford **6a** in 35% yield (13.6 mg, 0.039 mmol). $^1$H NMR (500 MHz, MeOD) δ 7.09 (d, $J$ = 8.2 Hz, 2H), 7.07 – 7.04 (m, 2H), 6.93 (dd, $J$ = 8.3, 2.0 Hz, 1H), 6.82 (d, $J$ = 8.3 Hz, 2H), 6.74 (d, $J$ = 8.3 Hz, 1H), 6.68-6.63 (m, 2H), 6.57 (s, 1H), 4.06 (t, $J$ = 7.8 Hz, 1H), 3.68 (s, 3H), 3.23 (d, $J$ = 8.1 Hz, 2H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 159.30 144.51, 142.04, 140.81, 136.35, 134.68, 128.94, 128.71, 128.65, 127.25, 121.58, 115.81, 115.20, 115.15, 114.74, 111.39, 55.05, 51.04, 42.39. HRMS (ESI-TOF) calc'd for $C_{21}H_{22}N_2OCl$ $[M + H]^+$: 353.1421 and 325.1391, found: 353.0489 and 355.0458.

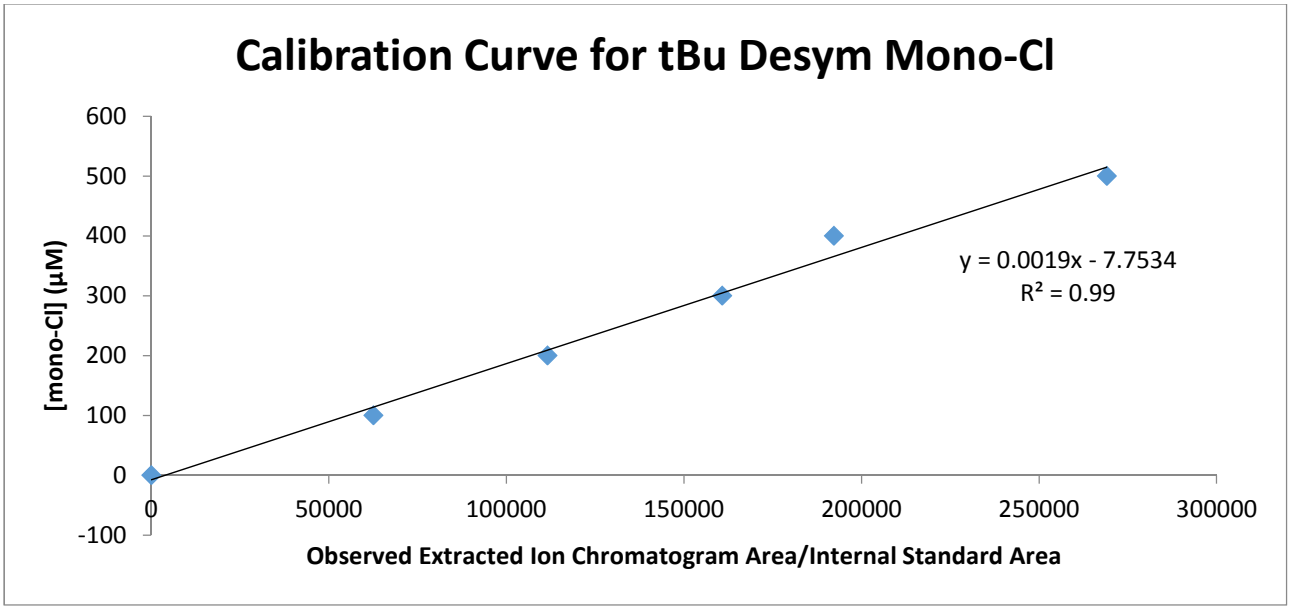
**Racemic monochlorinated *p*-trifluoromethylbenzyl dianilinemethane (7a):** This compound was prepared from 0.315 mmol (112.3 mg) of **7** as described in the general procedures. After reaction completion, the reaction mixture was purified as described in the general procedures to afford **7a** in 47% yield (58.3 mg, 0.149 mmol). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.45 (d, $J$ = 8 Hz, 2H), 7.12 (d, $J$ = 8 Hz, 2H), 7.08 (d, $J$ = 1.9 Hz, 1H), 6.96 (d, $J$ = 8.2 Hz, 2H), 6.87 (dd, $J$ = 8.2, 1.9 Hz, 1H), 6.67 (d, $J$ = 8.2 Hz, 1H), 6.61 (d, $J$ = 8.4 Hz, 2H), 4.01 (t, $J$ = 7.8 Hz, 1H), 3.30

(d, $J$ = 7.9 Hz, 2H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 144.72, 144.58, 141.03, 135.74, 133.98, 129.38, 128.65, 128.53, 128.15 (q, $J$ = 31.5 Hz), 127.16, 124.96 (q, $J$ = 3.8 Hz), 124.35 (q, $J$ = 271.8 Hz), 119.28, 115.87, 115.25, 50.95, 42.15. HRMS (ESI-TOF) calc'd for $C_{21}H_{18}F_3N_2Cl$ [M + H]$^+$: 391.1189 and 393.1159, found: 391.1401 and 393.1960.

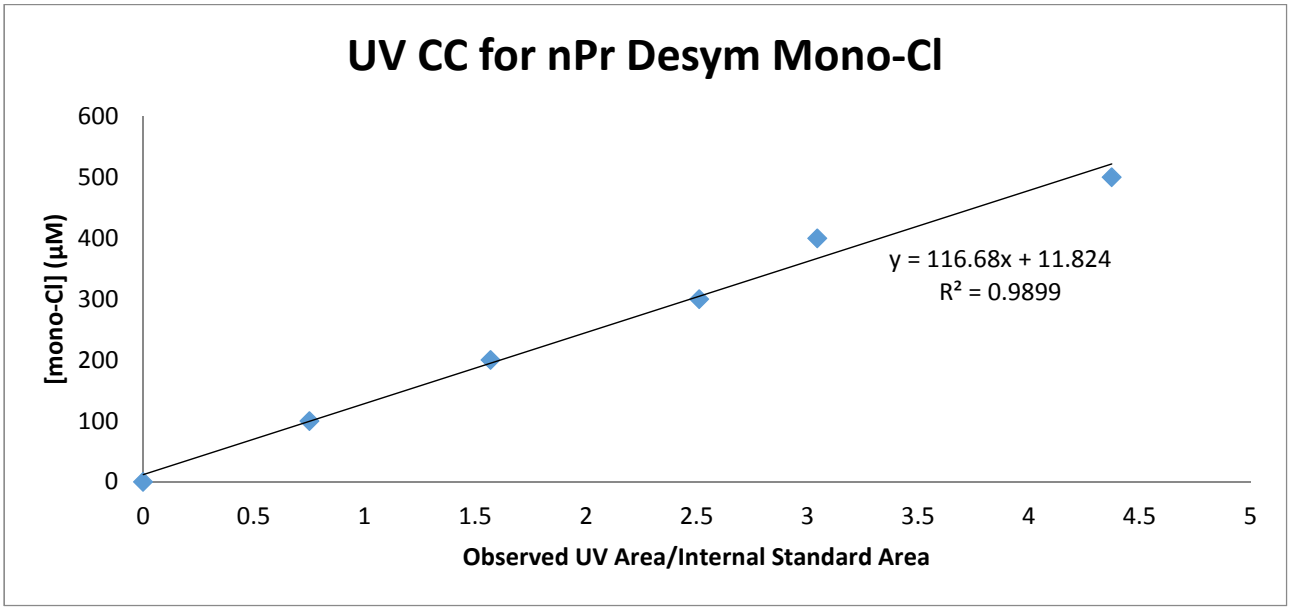
**aniline 1b:** This compound was prepared following a previously reported procedure.[13] In a glovebox, palladium (II) acetate (1.2 mg, 0.0058 mmol, 0.05 equiv.), 1,1'bis(di-*tert*-butylphosphino)ferrocene (3.5 mg, 0.0075 mmol, 0.065 equiv.), monochlorinated methylene dianiline **1a** (33.7 mg, 0.117 mmol, 1 equiv.), and potassium carbonate (32.4 mg, 0.230 mmol, 2 equiv.), were added to an oven-dried micro reaction vial containing a stir vane. NMP (0.25 mL, to give 0.5 M substrate concentration) and 4-octyne (21 μL, 0.14 mmol, 1.2 equiv.) were added via syringe. The vial was sealed with a Mininert valve, removed from the glovebox, and heated with stirring at 130 °C for 4 h. The reaction was then concentrated onto silica gel and dry loaded onto a silica gel column packed using 20% ethyl acetate in hexanes. 20% ethyl acetate in hexanes was passed through the column to provide the product 42.3 mg (73%) 1b as an off white solid. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.51 (s, 1H), 7.30-7.27 (m, 2H), 7.20-7.14 (m, 2H), 6.62 (d, $J$ = 8.4 Hz, 2H), 3.72 (s, 1H), 2.68 (q, $J$ = 7.8 Hz, 4H), 1.67 (septet, $J$ = 7.1 Hz, 4H), 1.05 (s, 9H), 0.99 (dt, $J$ = 7.4, 2.3 Hz, 6H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 144.40, 135.42, 134.37, 133.84, 130.55, 128.64, 122.89, 119.02, 114.42, 111.82, 109.2, 63.55, 35.04, 29.67, 29.20, 28.08, 26.11, 24.21, 23.17, 13.95, 13.69. HRMS (ESI-TOF) calc'd for $C_{25}H_{35}N_2$ [M + H]$^+$: 364.2878, found: 364.2792.

*Calibration Curves for Determining Halogenated Product Yields*

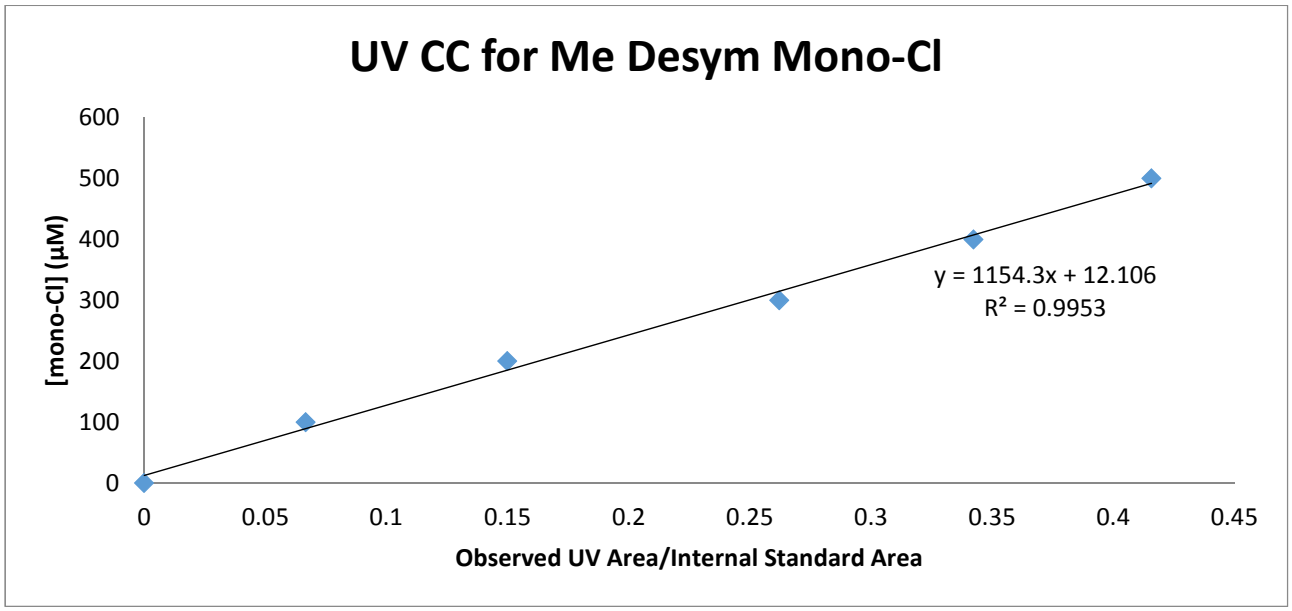
**Calibration Curve for Determining Yield of 1a**



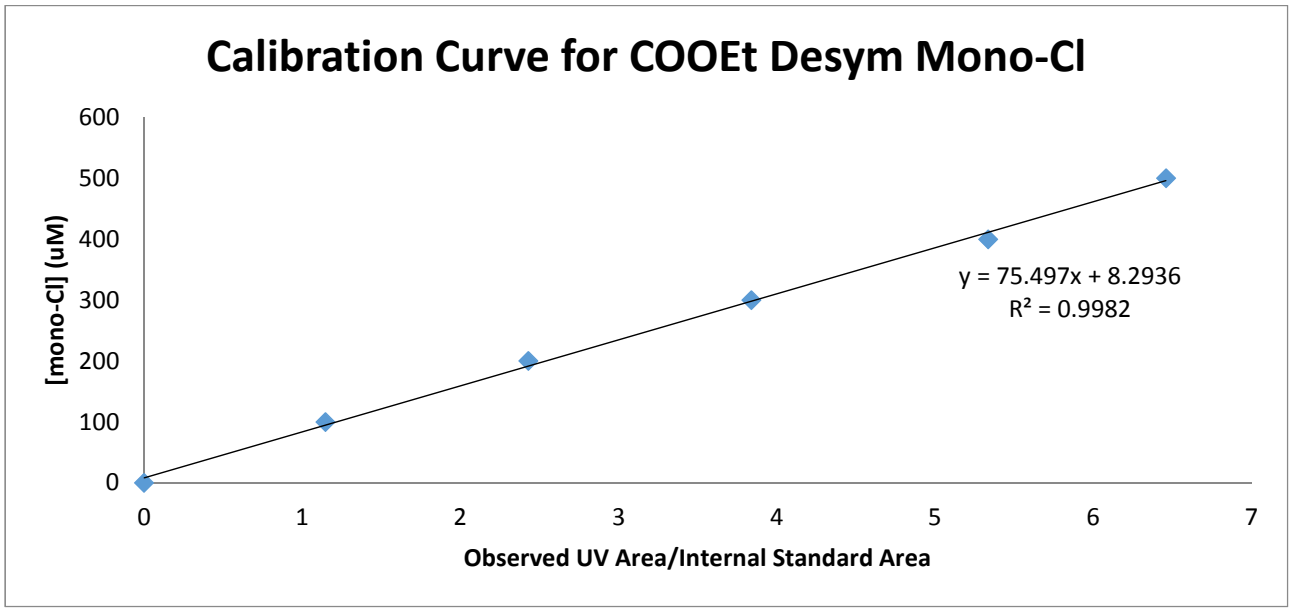

**Calibration Curve for Determining Yield of 2a**



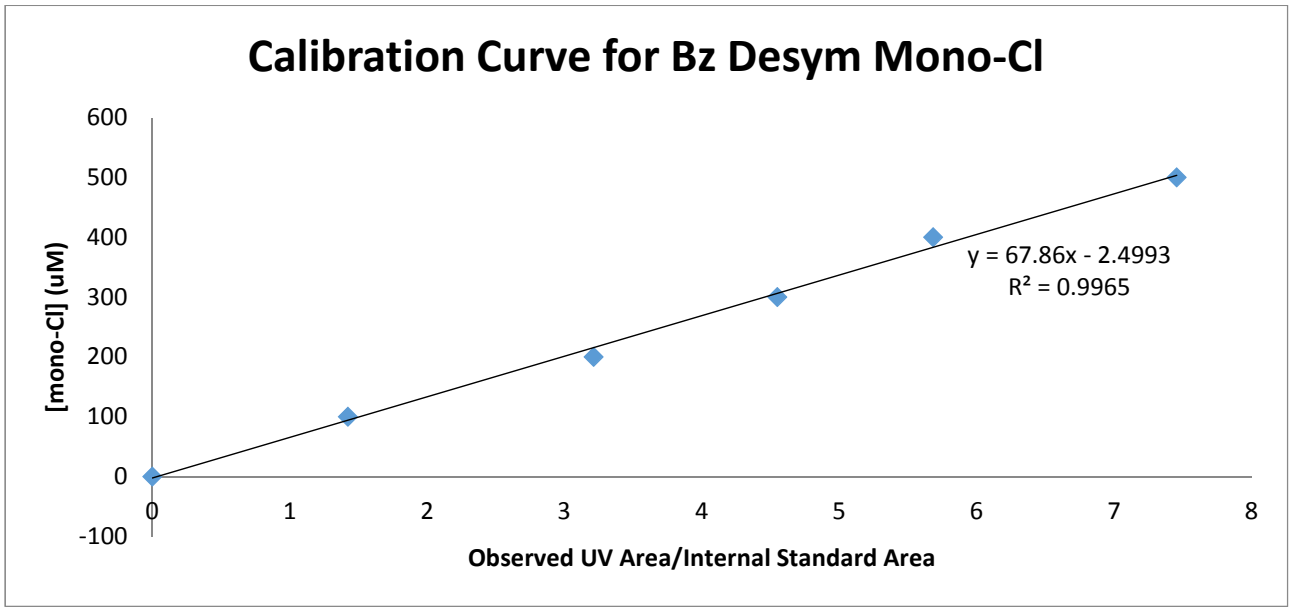
**Calibration Curve for Determining Yield of 3a**



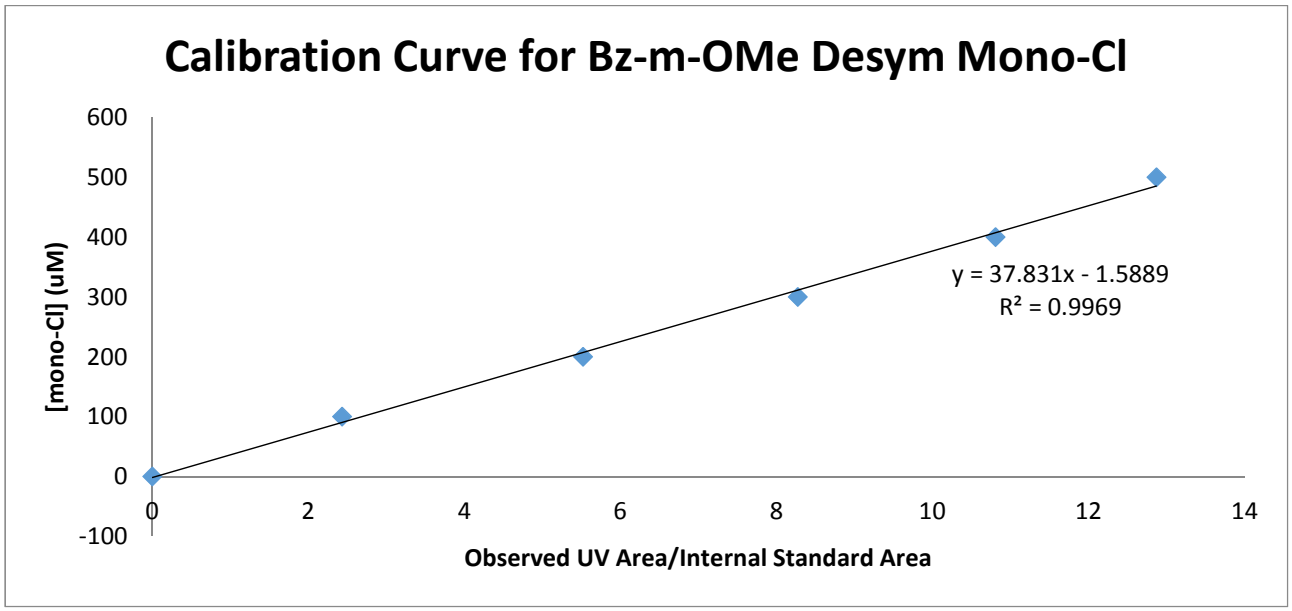

**Calibration Curve for Determining Yield of 4a**



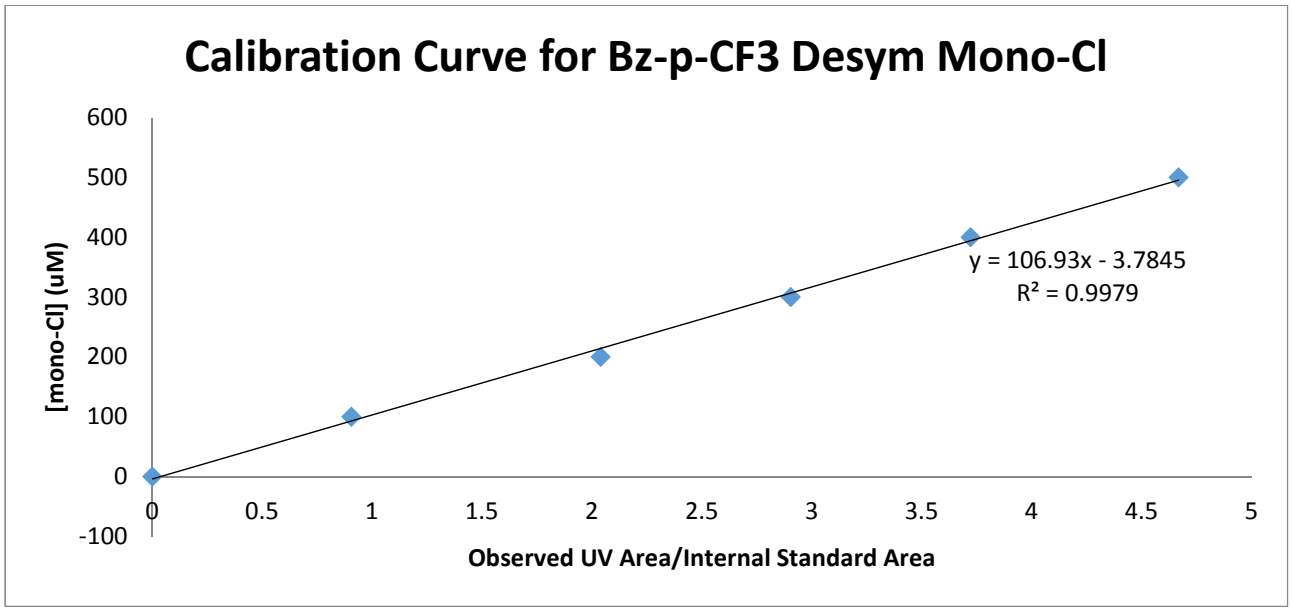
**Calibration Curve for Determining Yield of 5a**




**Calibration Curve for Determining the Yield of 6a**

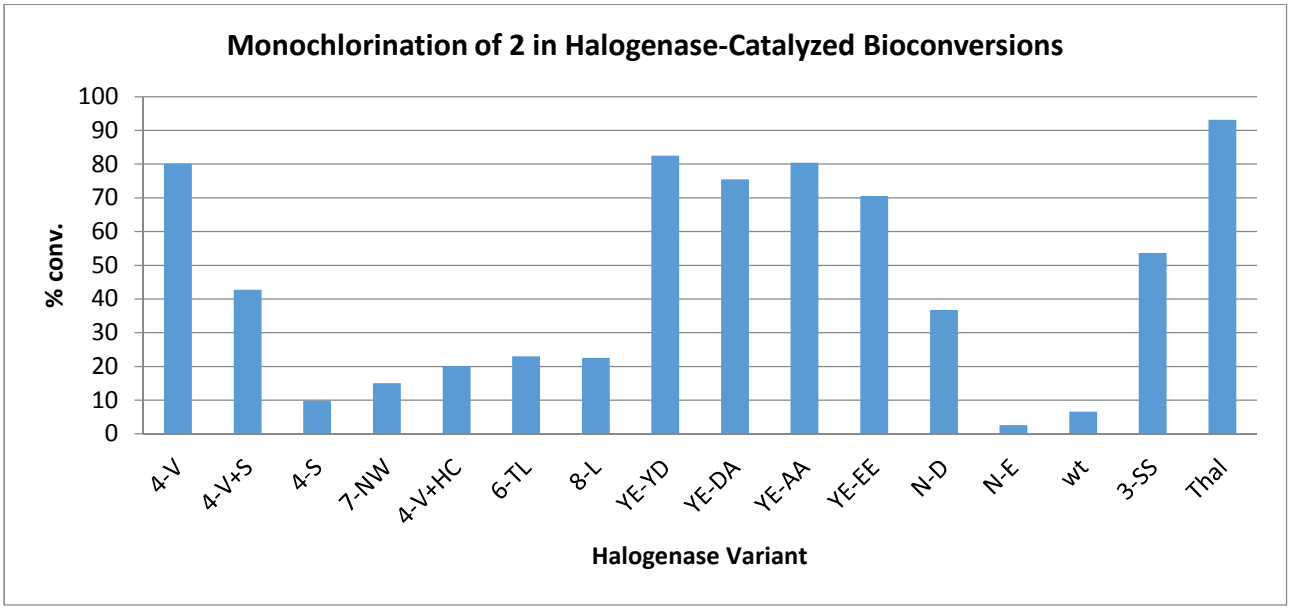


**Calibration Curve for Determining the Yield of 7a**

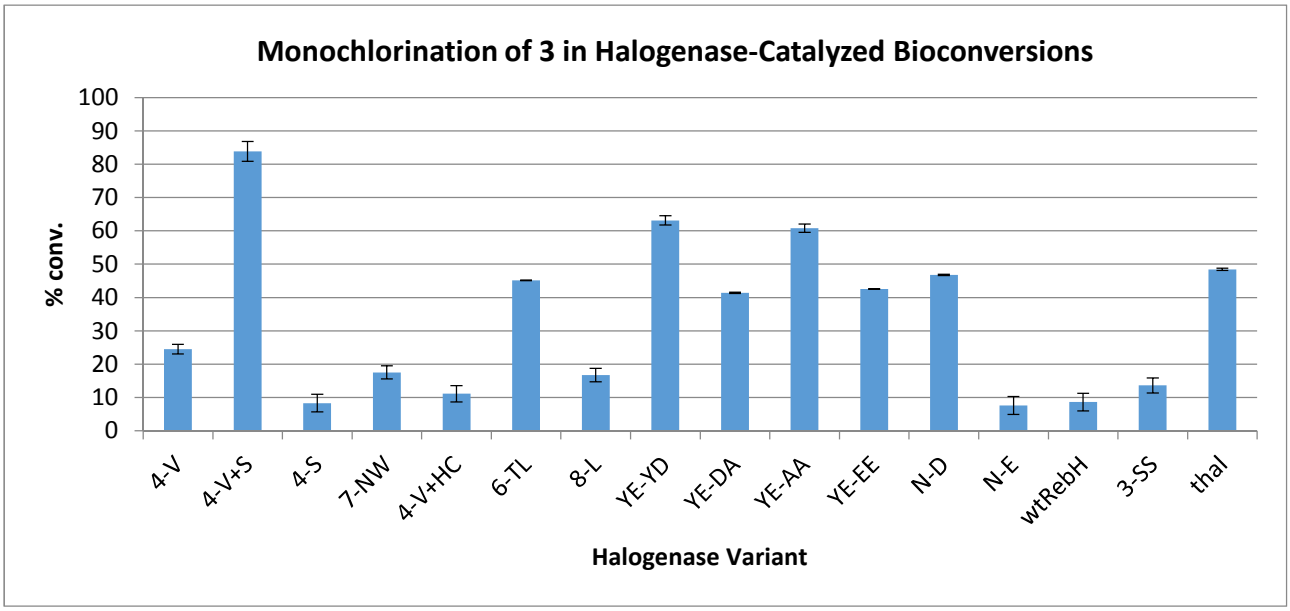



*Halogenase Variant Conversions*

**Halogenase variant conversions of *t*-Bu substrate 1.**

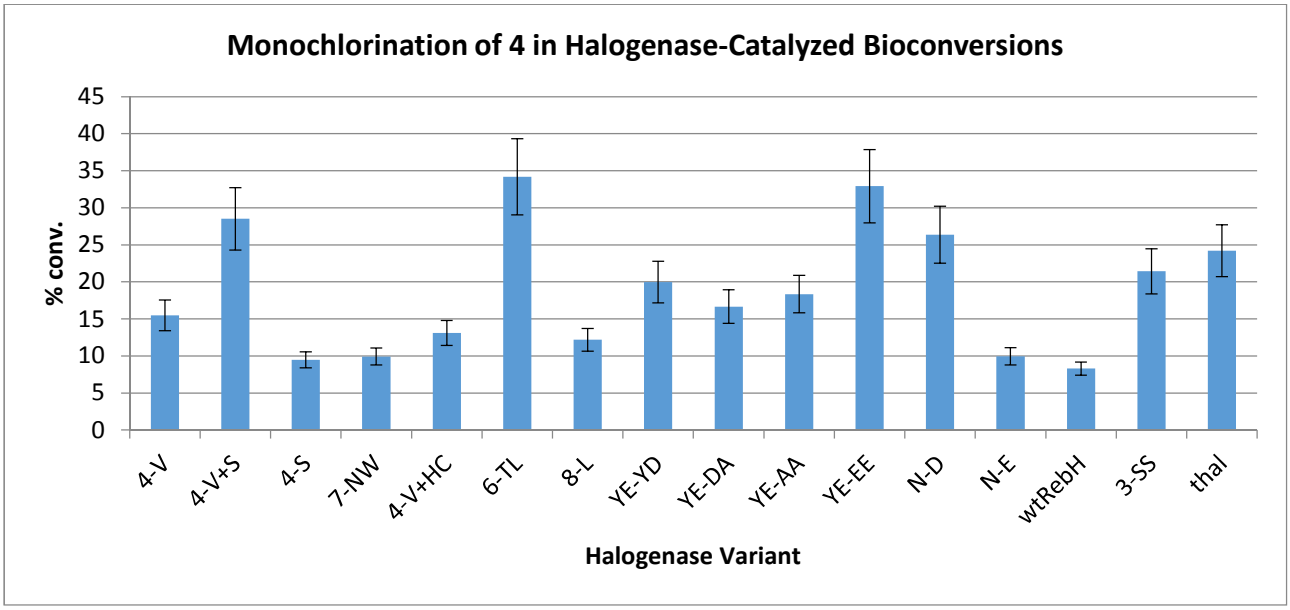



**Halogenase variant conversions of *n*-Pr substrate 2.**



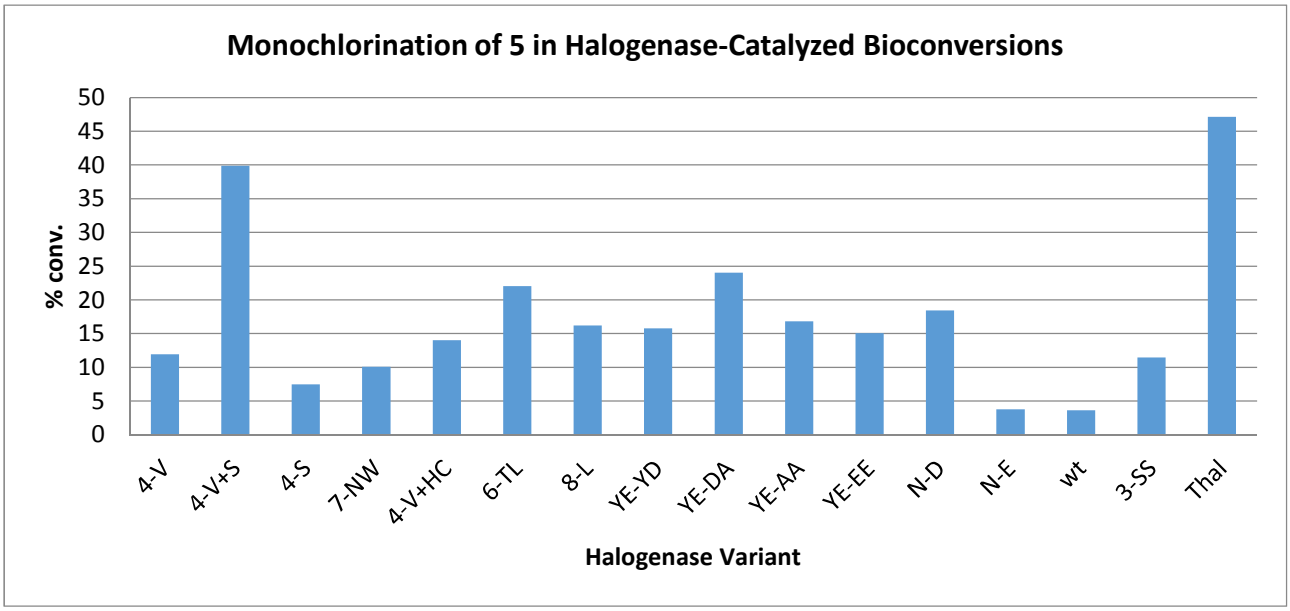

Error bars represent the standard deviation from the average of two independent measurements.

**Halogenase variant conversions of methyl substrate 3.**

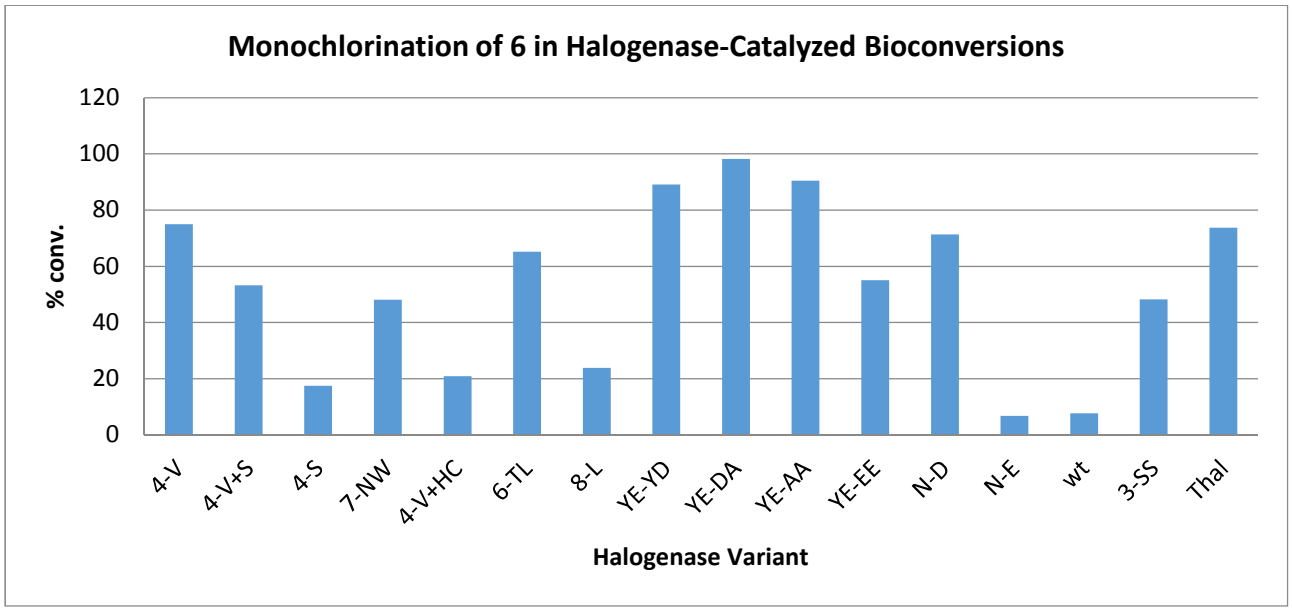



Error bars represent the standard deviation from the average of two independent measurements.

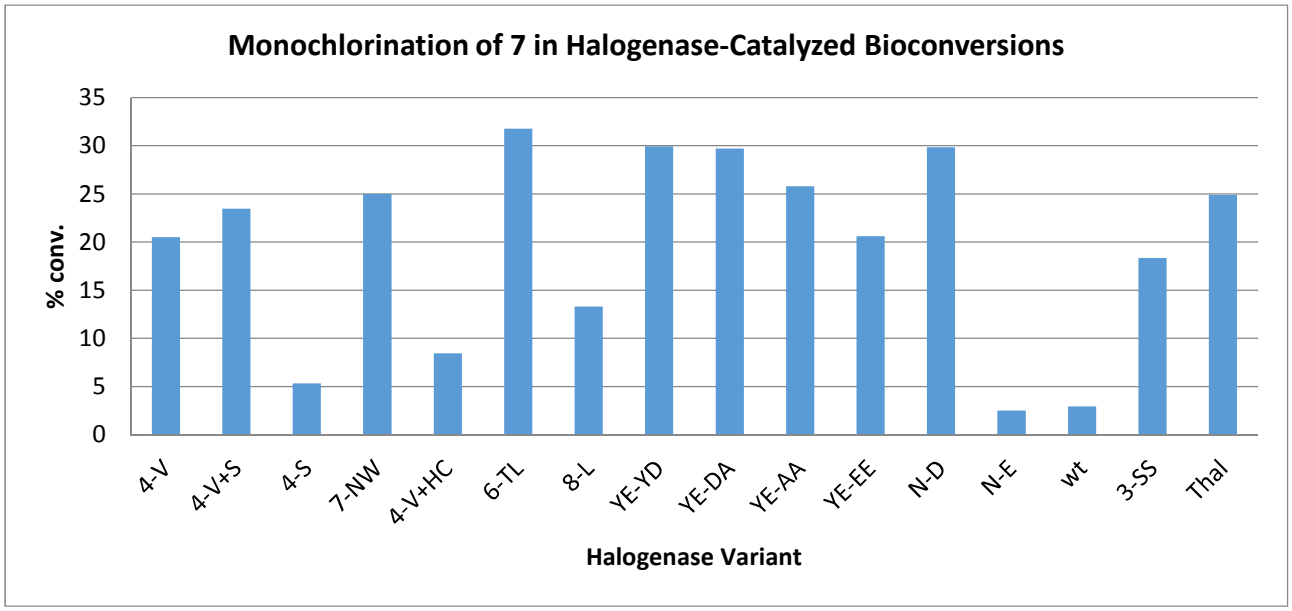
**Halogenase variant conversions of ethyl ester substrate 4.**



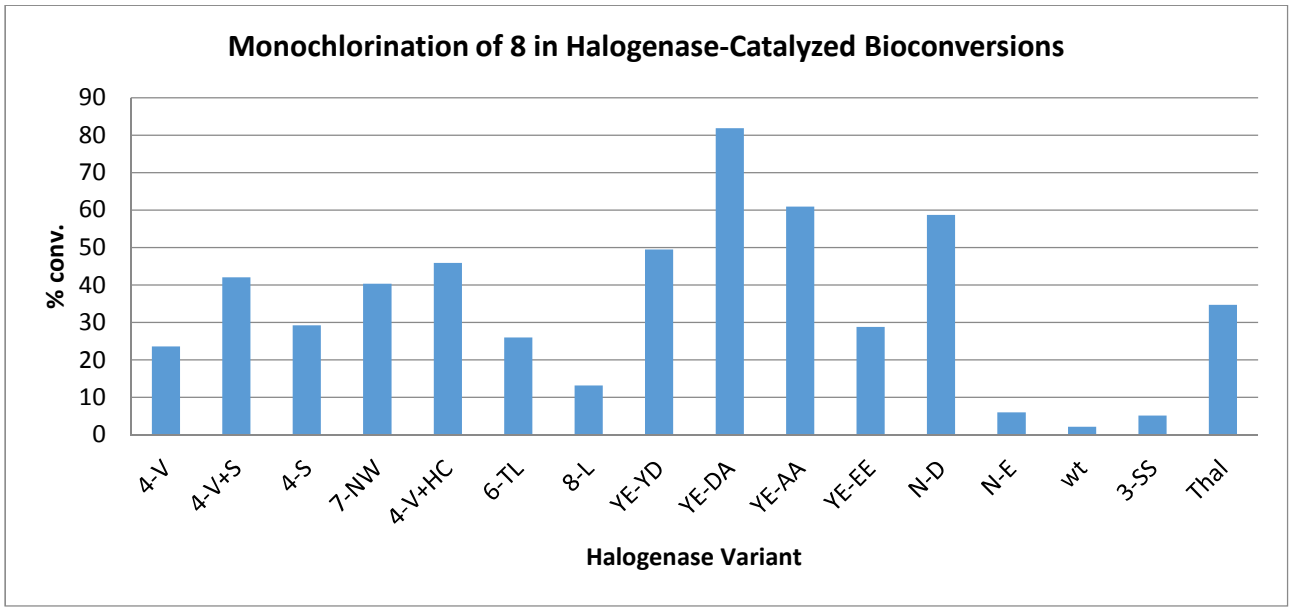

**Halogenase variant conversions of benzyl substrate 5.**




**Halogenase variant conversions of *m*-MeO-benzyl substrate 6.**

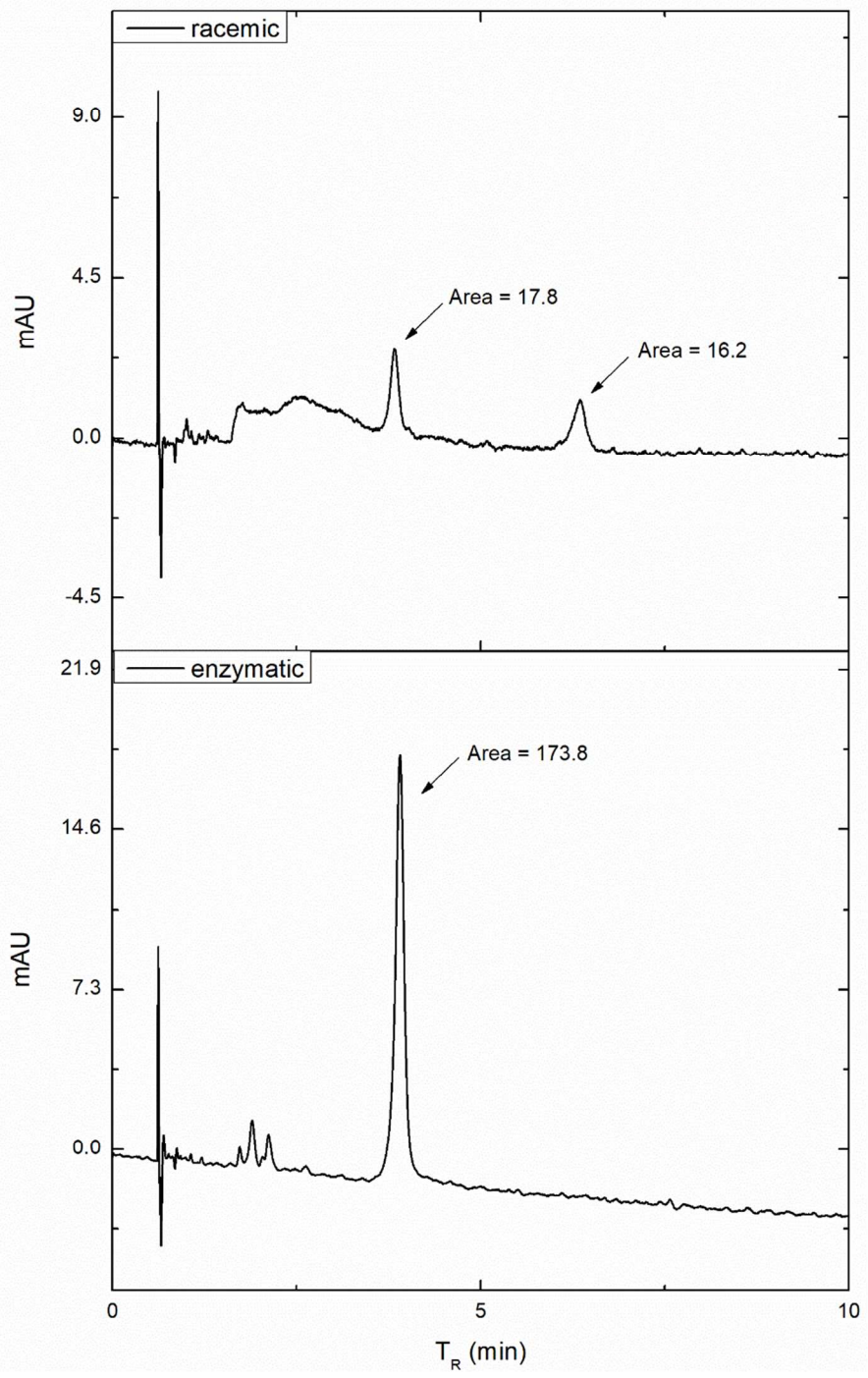



**Halogenase variant conversions of *p*-$CF_3$-benzyl substrate 7.**

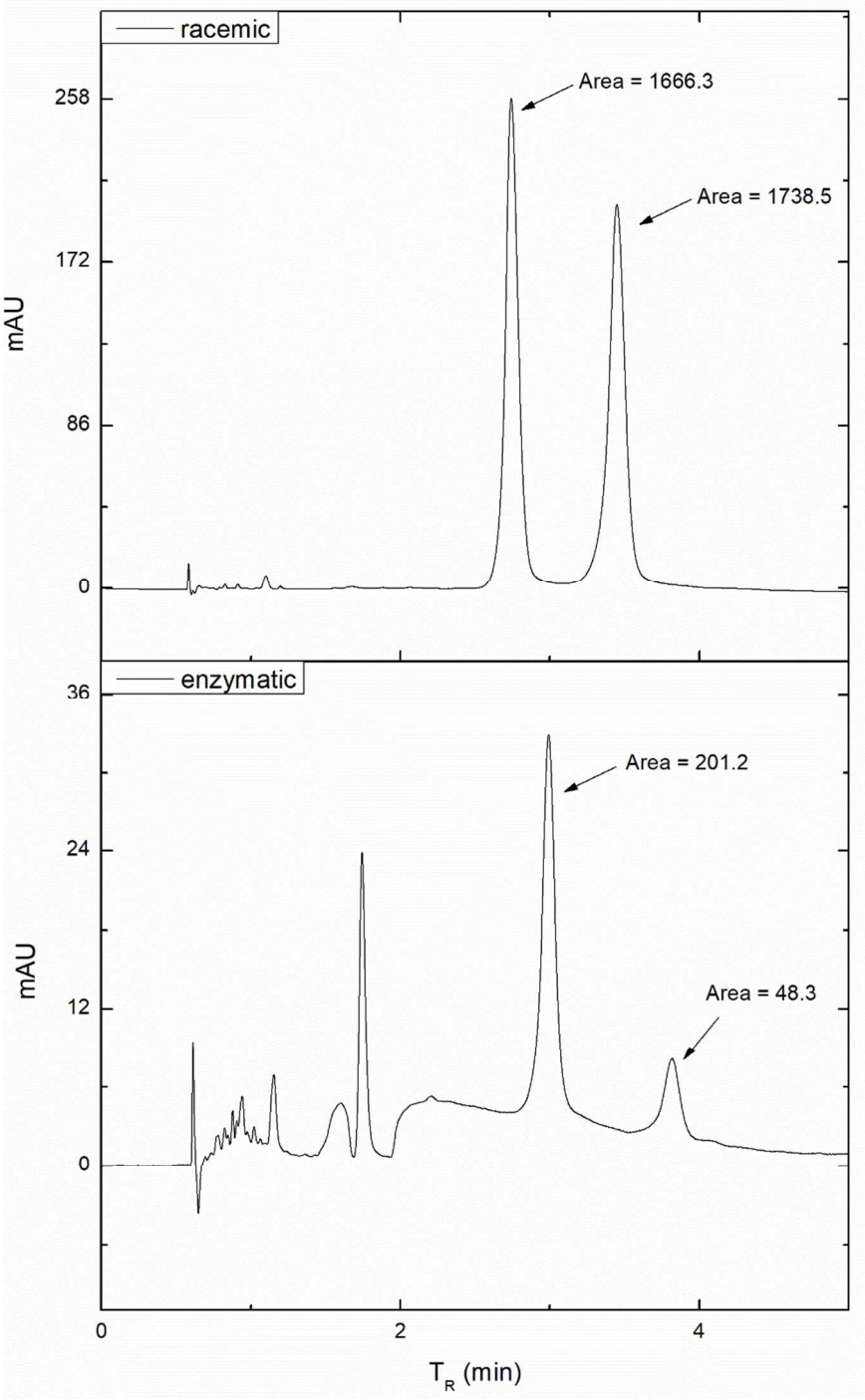

Monochlorination of 8 in Halogenase-Catalyzed Bioconversions
% conv.
90
80
70
60
50
40
30
20
10
0
4-V
4-V+S
4-S
7-NW
4-V+HC
6-TL
8-L
YE-YD
YE-DA
YE-AA
YE-EE
N-D
N-E
wt
3-SS
Thal
Halogenase Variant

*Chiral SFC Traces of Bolded Entries in Table 2 and Their Racemic Forms*

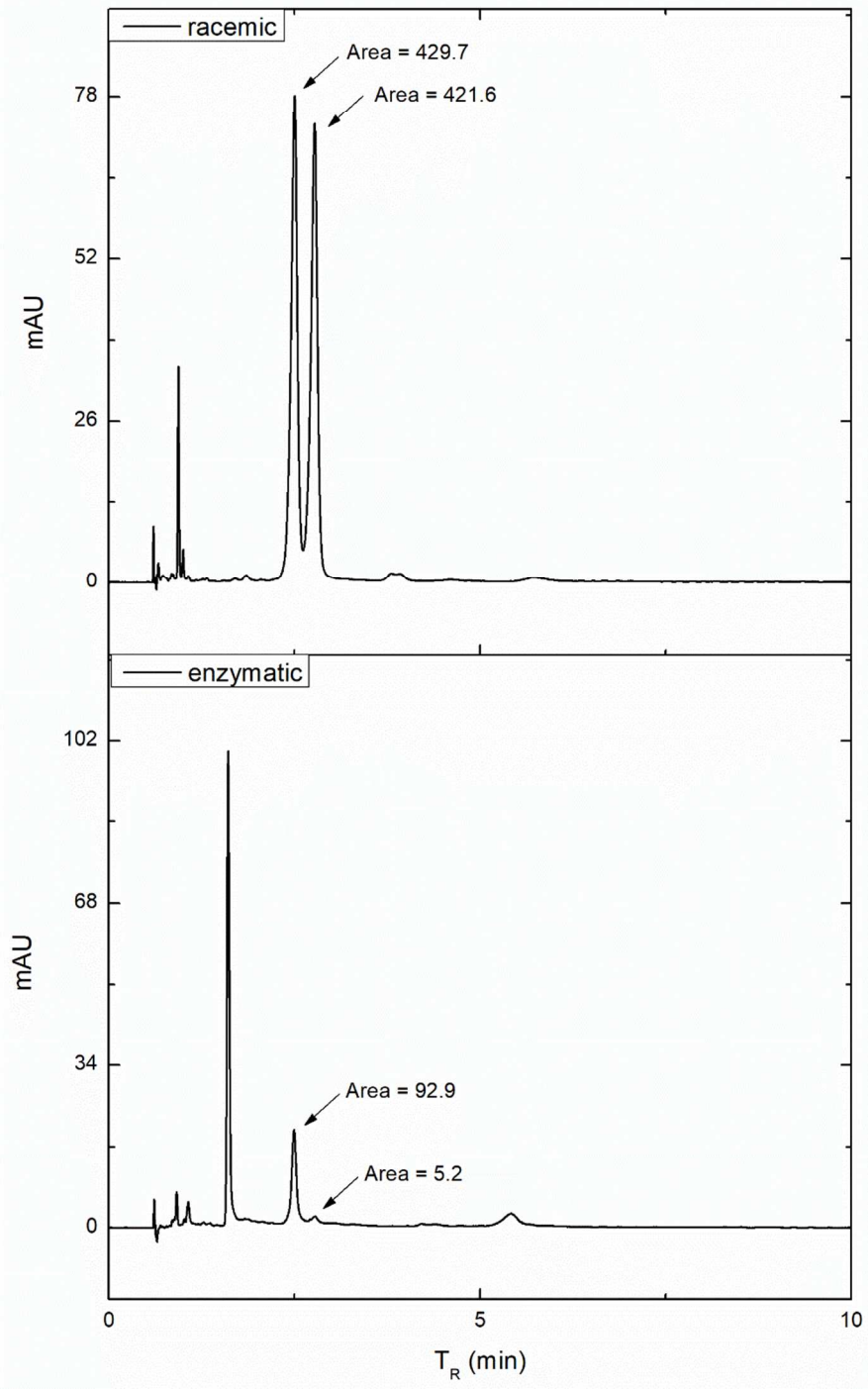
**Trace of 1a produced with 4-V**



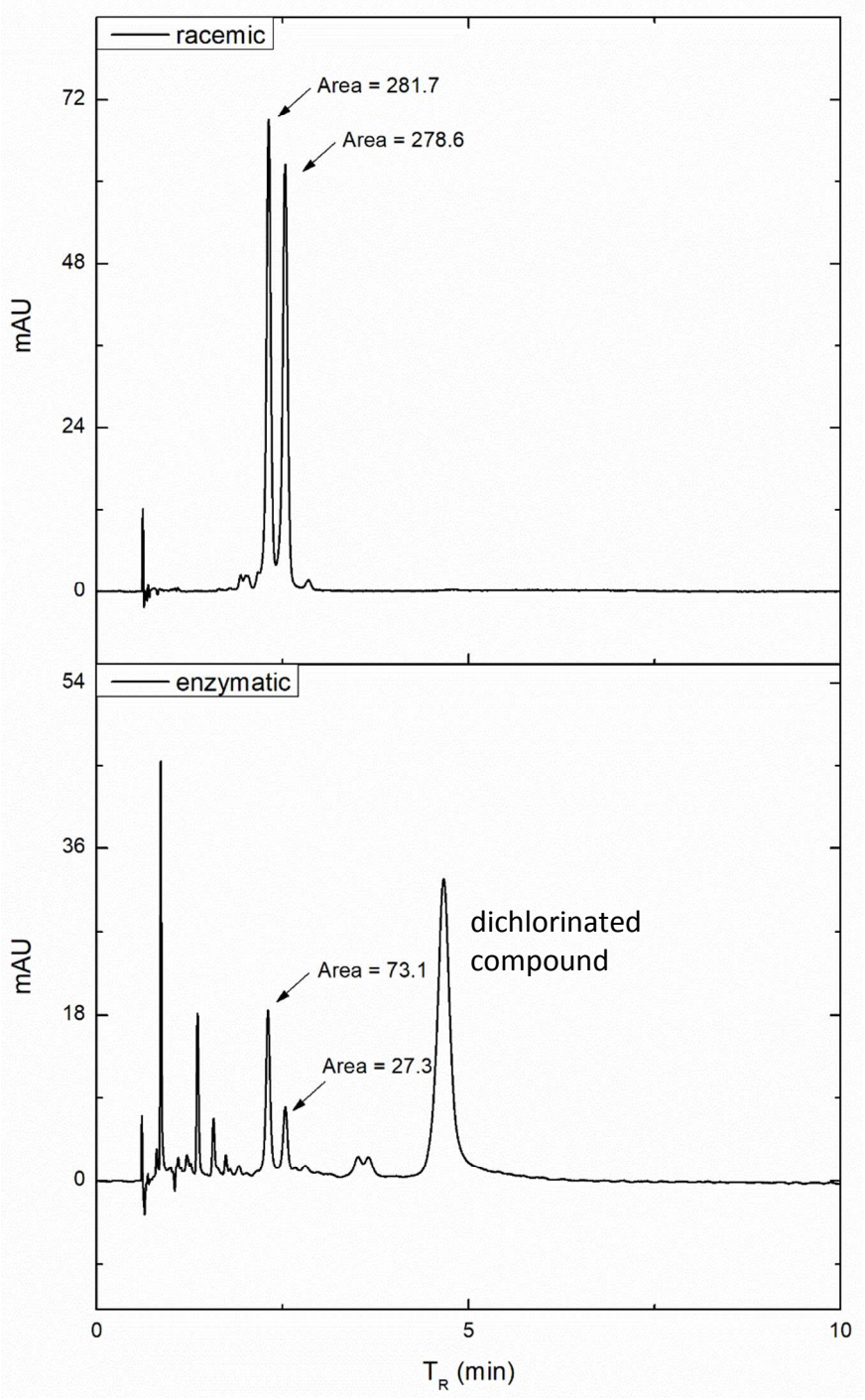
**Trace for 2a produced with 4-V**



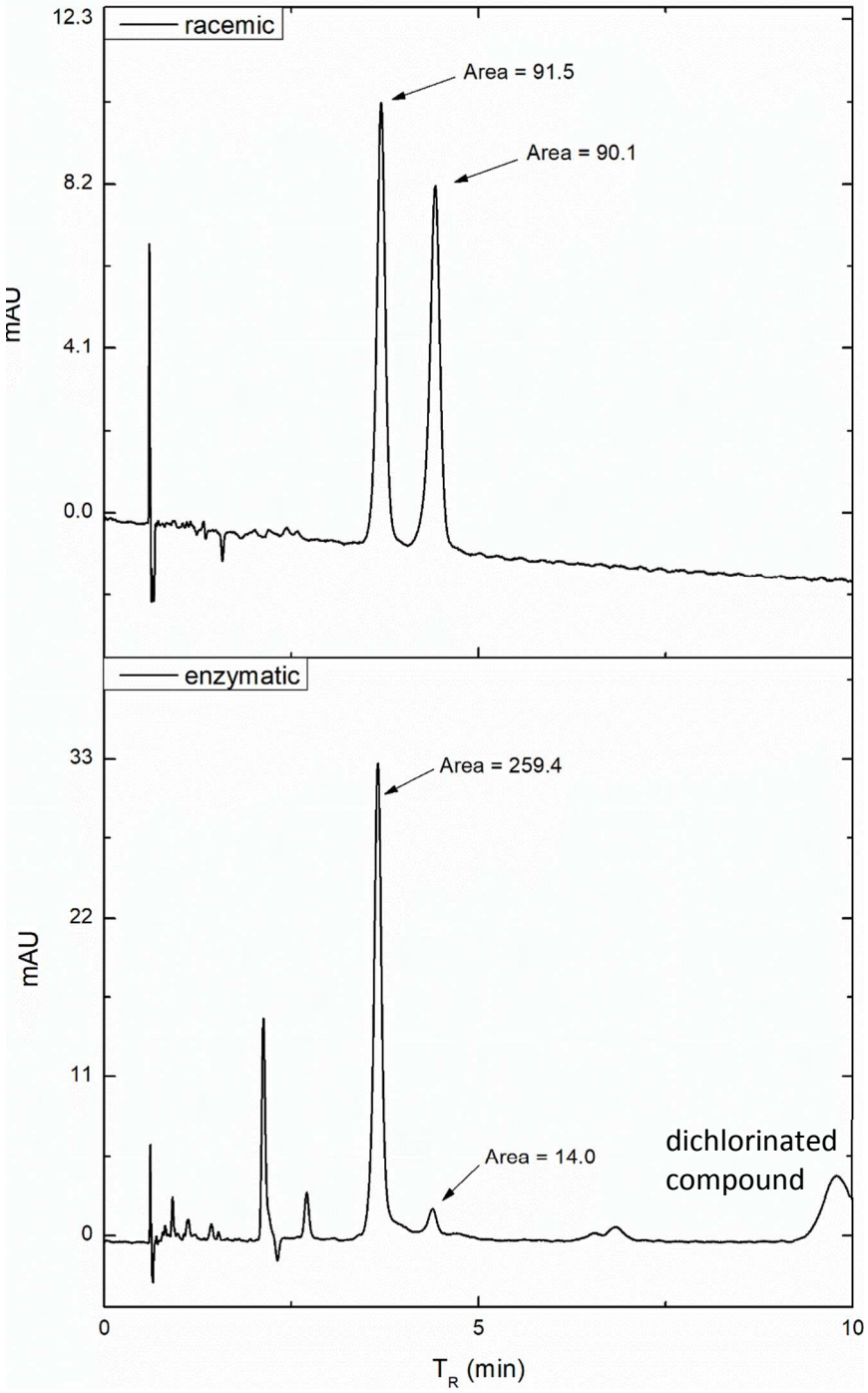
**Trace for 3a produced with 3-SS**



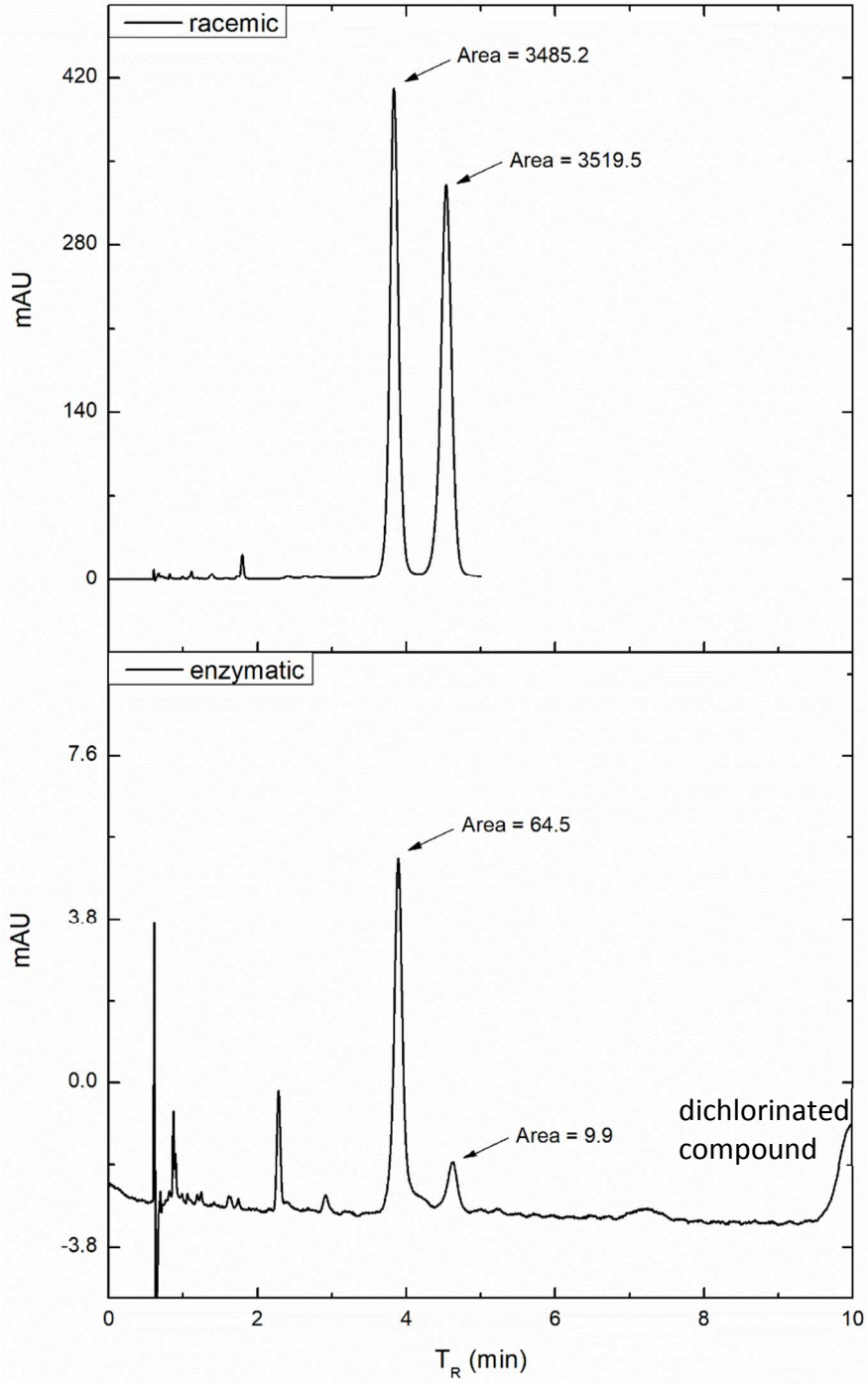
**Trace for 4a produced with 6-TL**



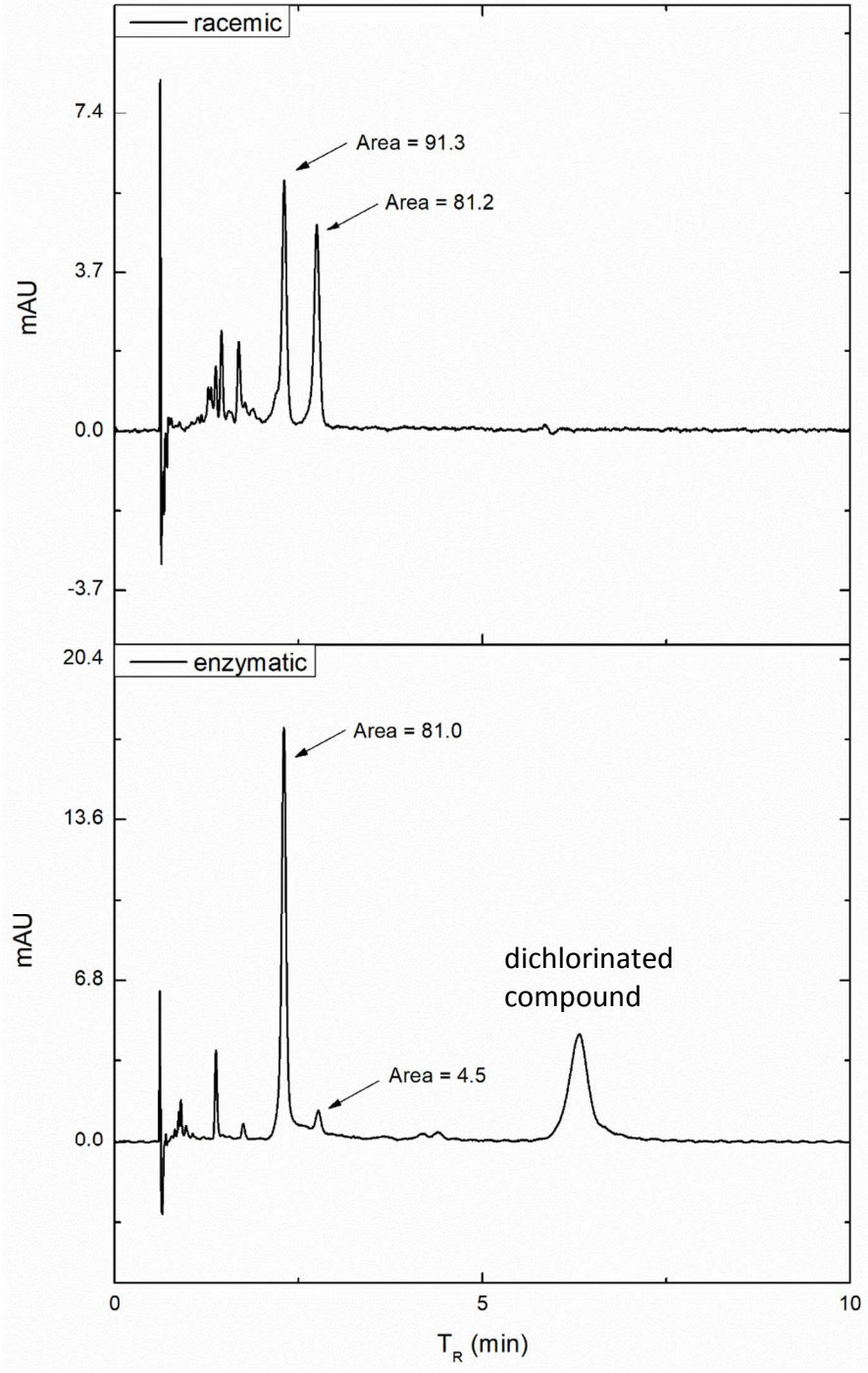
**Trace for 5a produced with YE-AA**



**Trace for 6a produced with YE-YD**



**Trace for 7a produced with YE-DA**

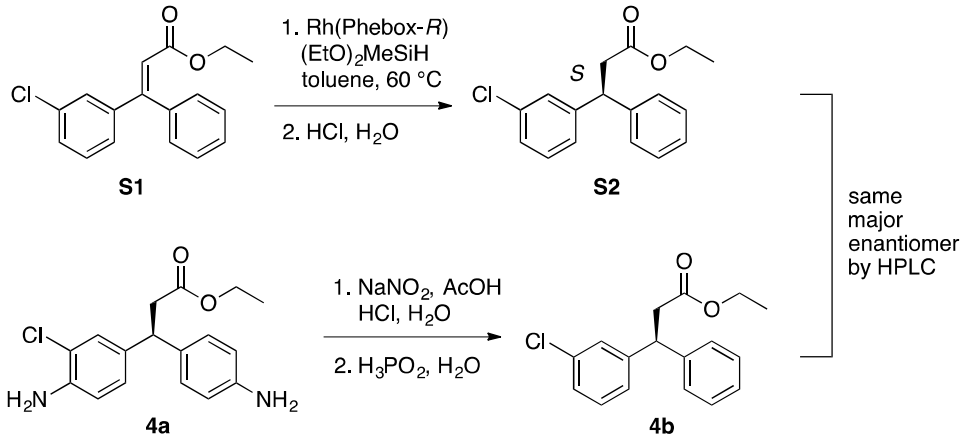


**Establishing Absolute Chirality**

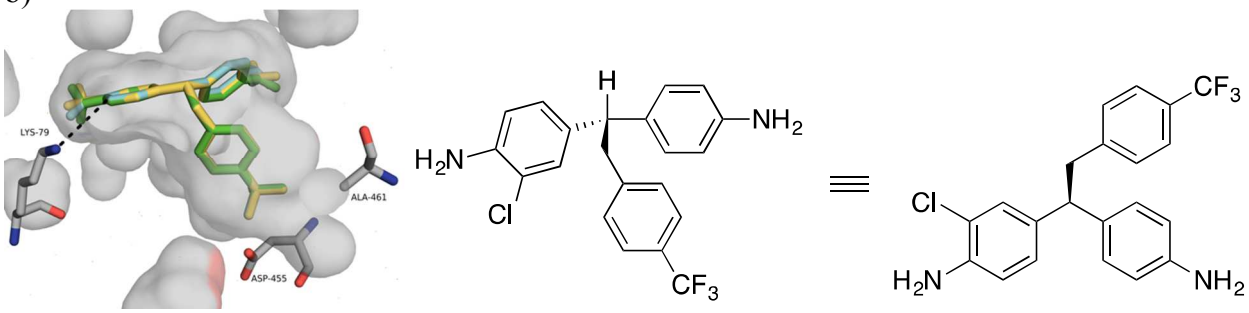
While the absolute chirality of the different desymmetrization products could vary due to subtle aspects of substrate binding (as seen in our docking simulations), evidence supporting the *S* absolute configuration for one of the products, **4a**, was obtained by comparison to a known compound. Specifically, hydrosilylation/hydrolysis of compound **S1** was carried out as reported in the literature using *R*-Rh(Phebox) as a catalyst.[14] This procedure was reported to provide the *S*-enantiomer of a range of different 3,3-diarylpropanoates, including **S2**. Diazotization/reduction of compound **4a** led to formation of **4b** as a mixture of enantiomers favoring the same (*S*) enantiomer of **S2** formed using the *R*-Rh(Phebox) catalyst. Notably, this enantiomer corresponds to the enantiomer expected based on docking simulations of substrate **7** (manuscript Fig. 1D, reproduced below).

**Figure S2.** a) Synthetic sequence used to generate **S2** and **4b** for comparison by chiral HPLC (see below). b) Docking of substrate **7** and structure of **7a** expected from the pose observed.

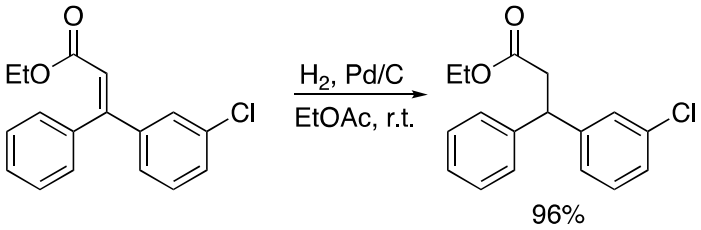
a)




b)



Synthesis of racemic product:

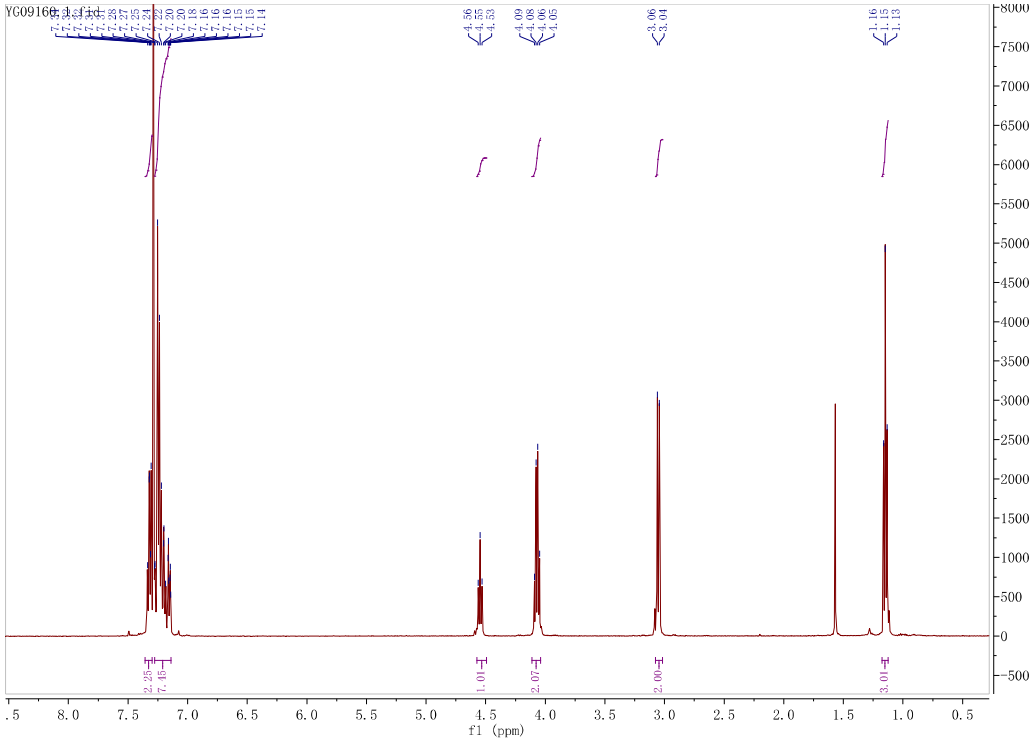



96%

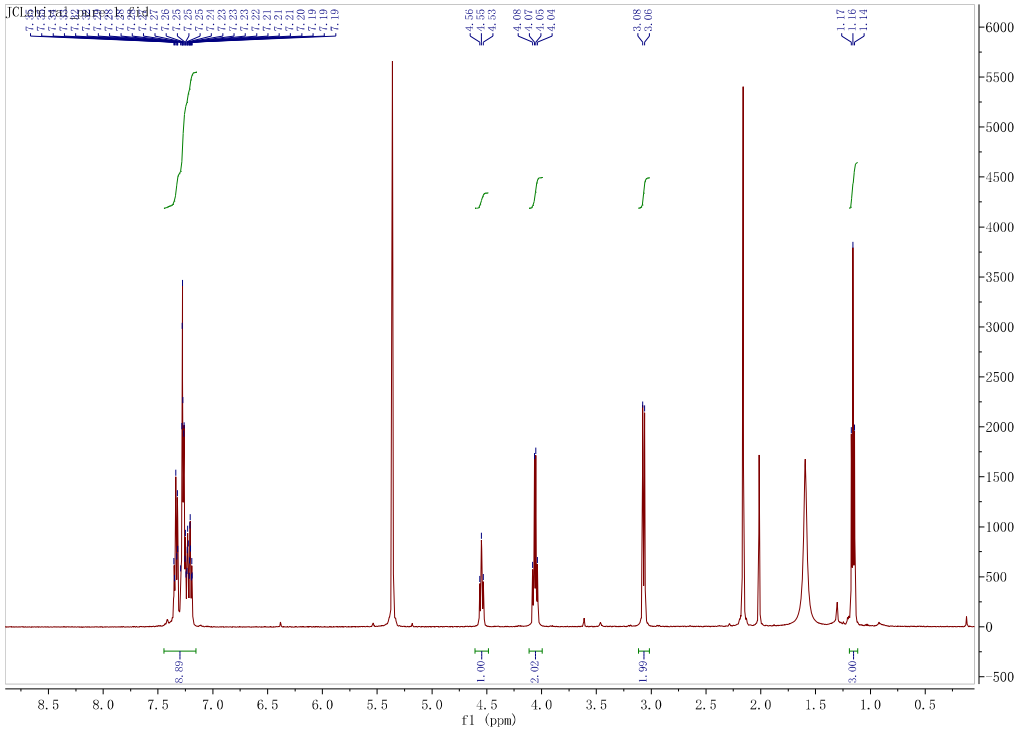
A mixture of ethyl 3-phenyl-3'-(3-chlorophenyl)acrylate (30 mg, 0.1 mmol) and Pd on carbon (5%, 5.6 mg, 2.5 mol%) in ethyl acetate (5 mL) was bubbled with hydrogen balloon for 30 min. The reaction was further stirred under hydrogen balloon at r.t. for 2 h. After completion, the reaction was filtered through a pad of celite, the filtrate was concentrated and further purified by silica column. The product was a colorless oil, 29 mg, 96% yield. $^1$H NMR (500 MHz, Chloroform-*d*) δ 7.32 – 7.14 (m, 9H), 4.55 (t, *J* = 8.0 Hz, 1H), 4.07 (q, *J* = 7.2 Hz, 2H), 3.05 (d, *J* = 7.9 Hz, 2H), 1.15 (t, *J* = 7.2 Hz, 3H). LCMS (M+H$^+$): 289.1 (cal. 289.1).

Synthesis of chiral product (**S2**):
Prepared according to a literature procedure.[14] $^1$H NMR (500 MHz, Methylene Chloride-$d_2$) δ 7.38 – 7.15 (m, 9H), 4.55 (t, *J* = 8.0 Hz, 1H), 4.06 (q, *J* = 7.1 Hz, 2H), 3.07 (d, *J* = 8.0 Hz, 2H), 1.16 (t, *J* = 7.1 Hz, 3H). LCMS (M+H$^+$): 289.1 (cal. 289.1).

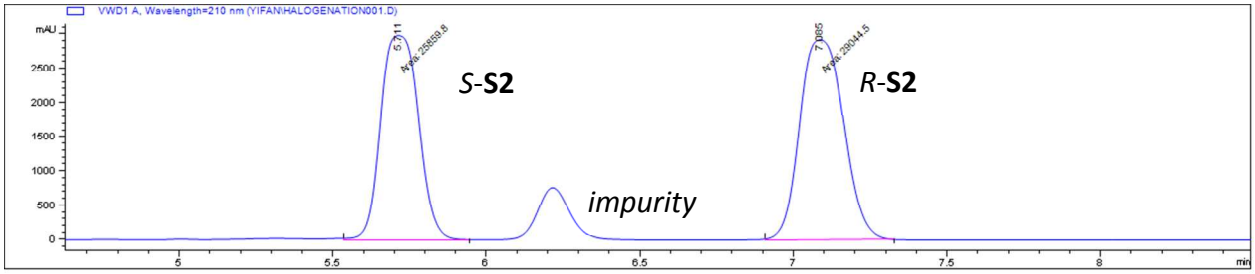
Racemic compound:



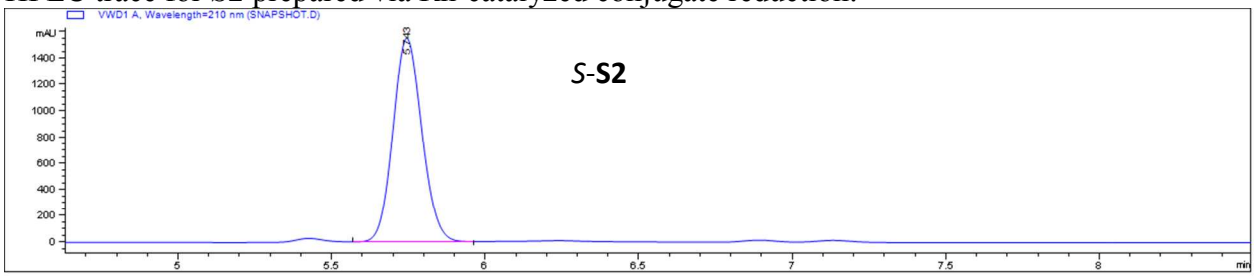
Chiral compound (**S2**):



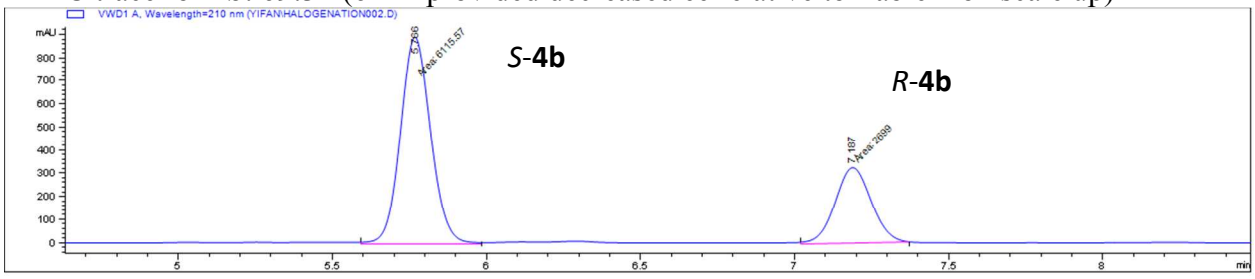
HPLC trace for racemic **S2**: 47:53




HPLC trace for **S2** prepared via Rh-catalyzed conjugate reduction:

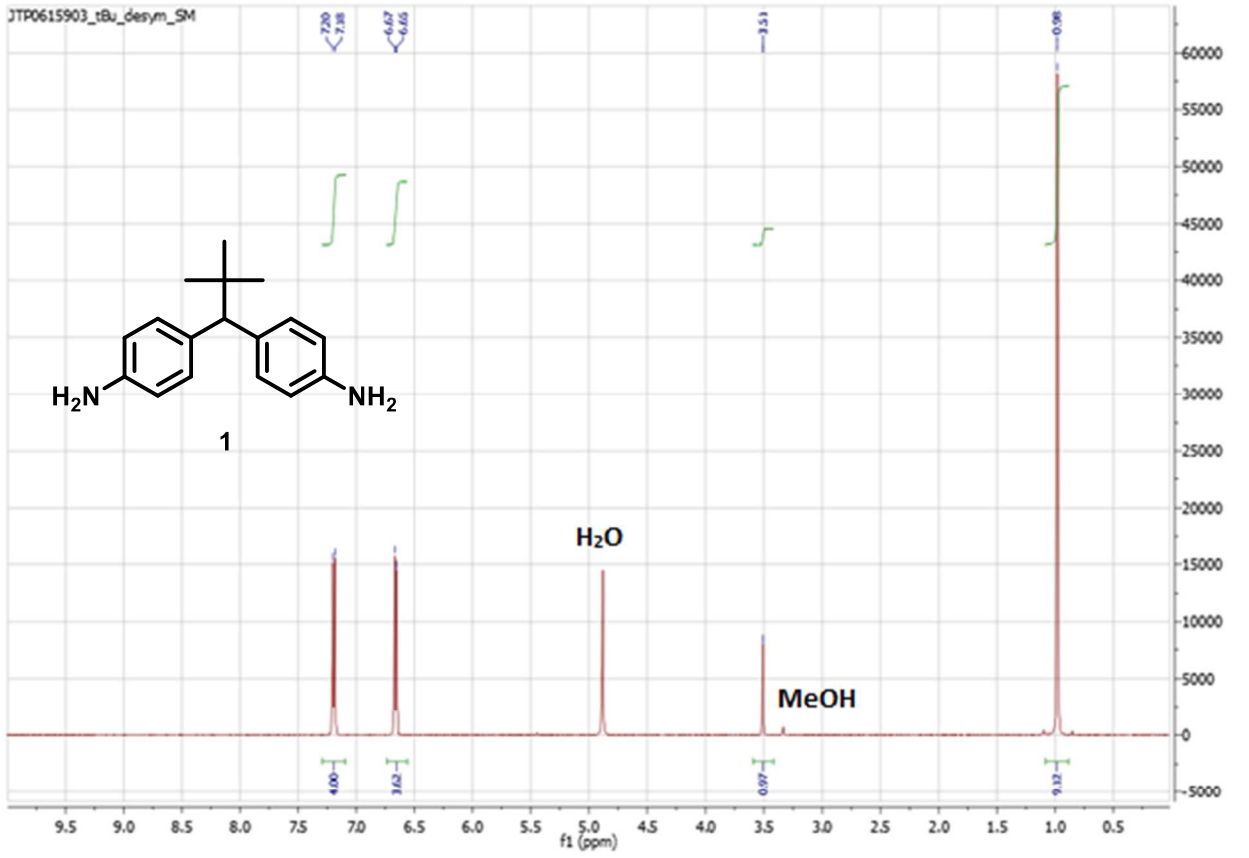



HPLC trace for **4b**: 69:31 (6-TL provided decreased ee relative to Table 2 on scale up)

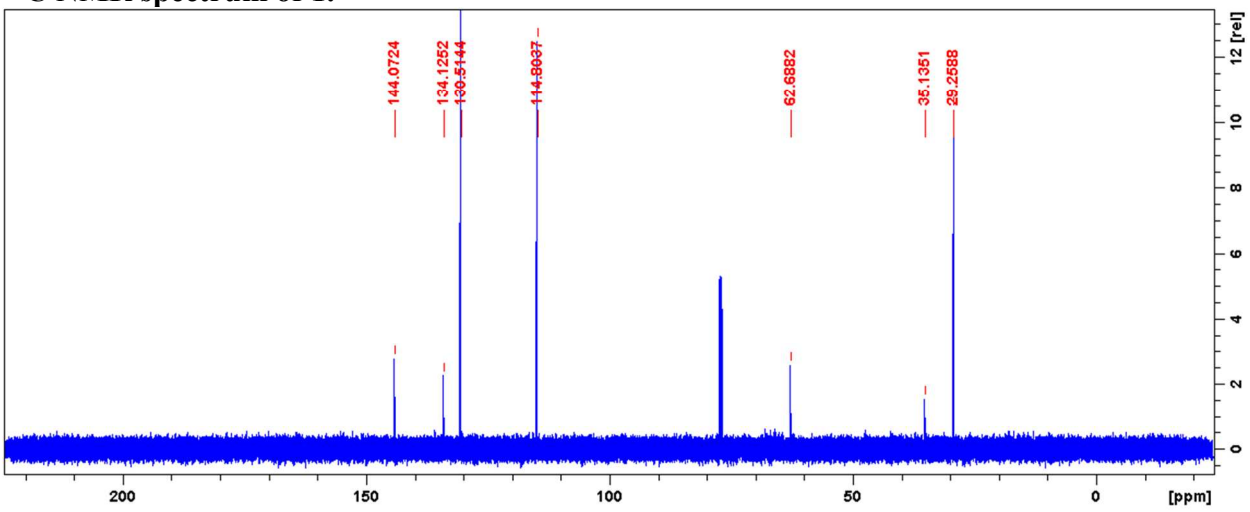


*NMR Spectra:*

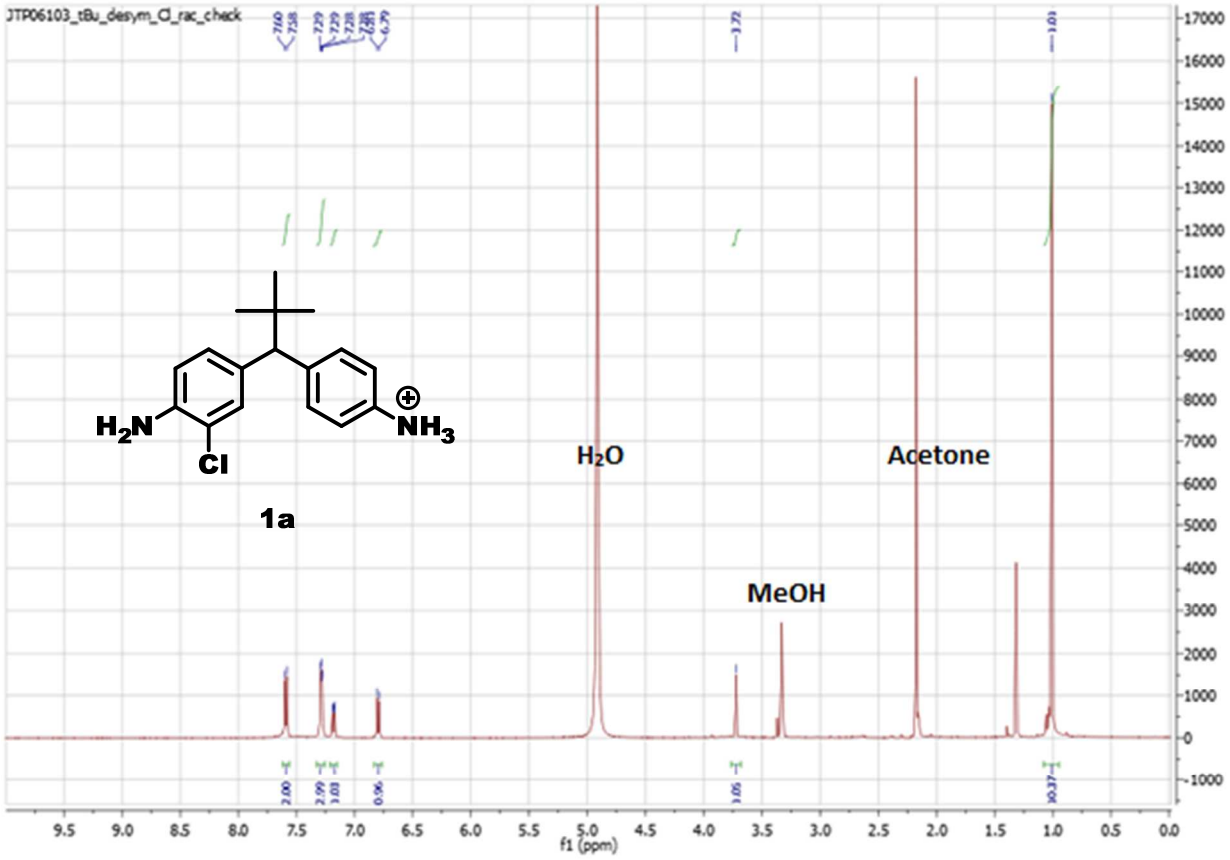
**$^{1}$H NMR spectrum of 1.**



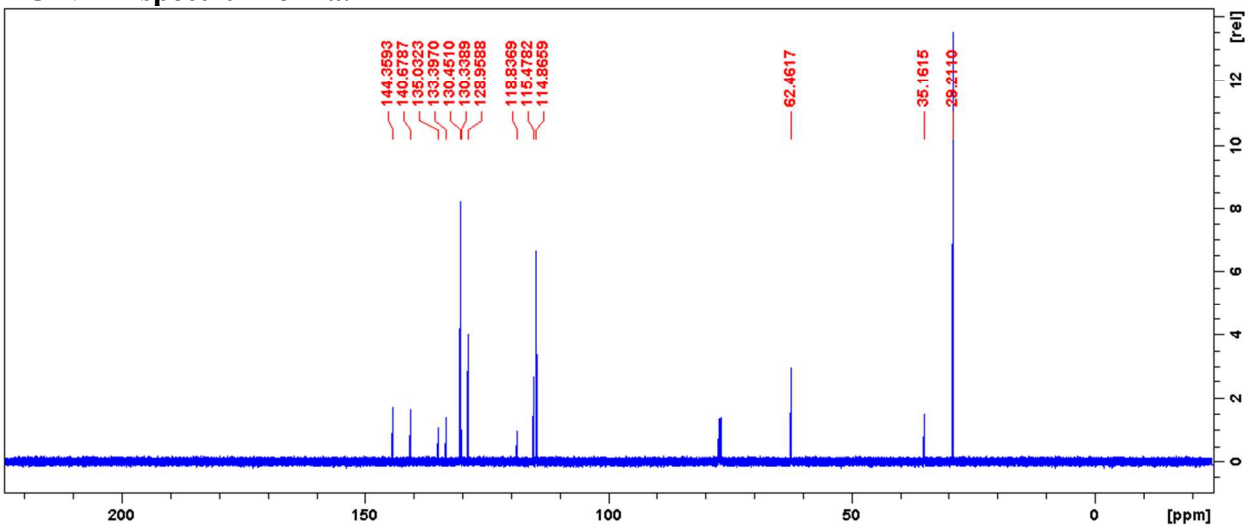

**$^{13}$C NMR spectrum of 1.**



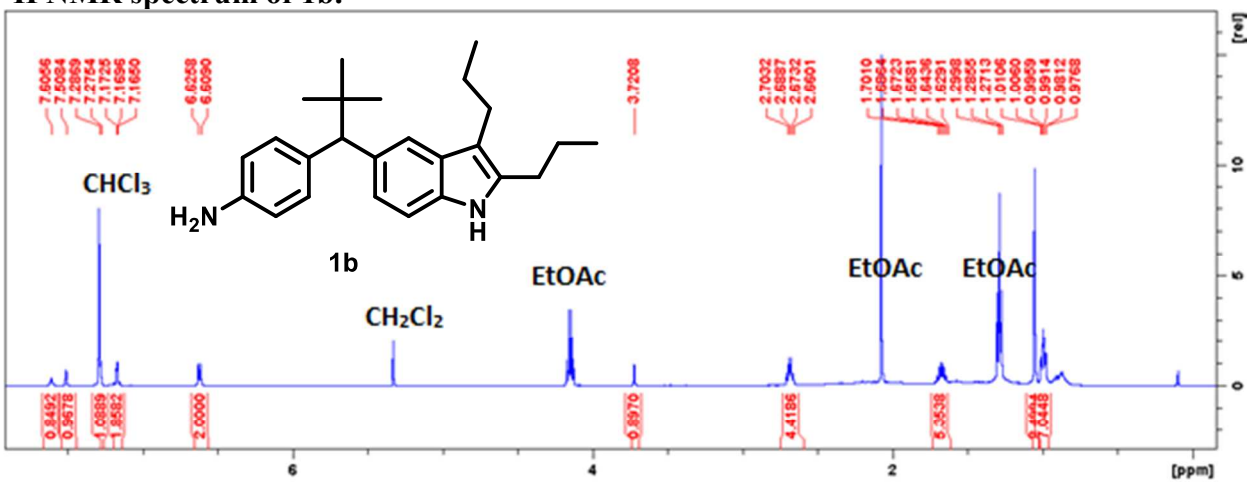
**$^{1}$H NMR spectrum of 1a.**



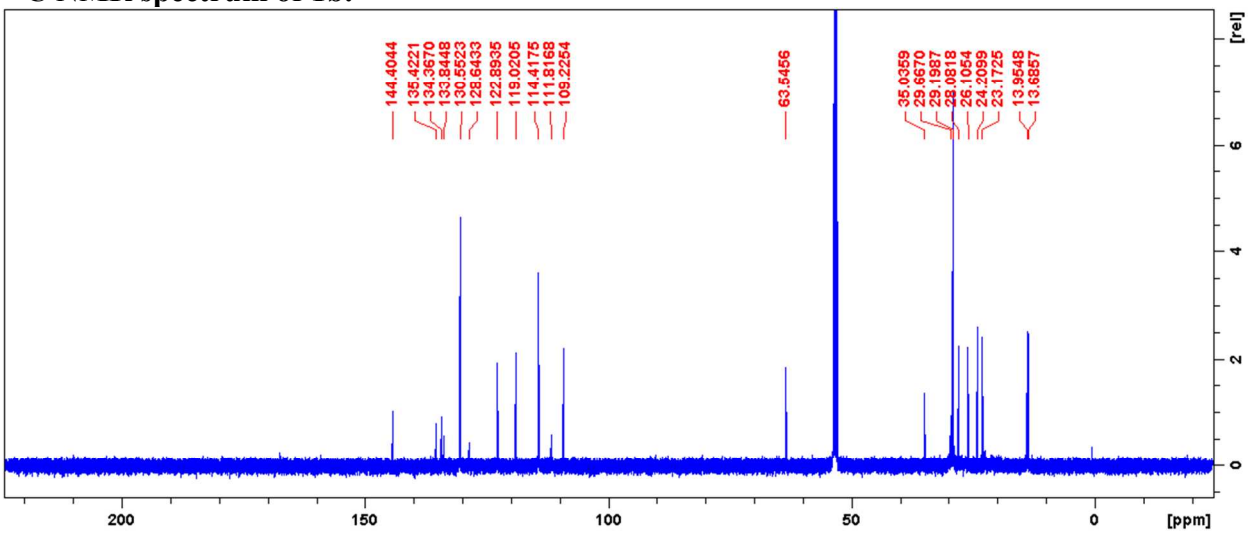

**$^{13}$C NMR spectrum of 1a.**



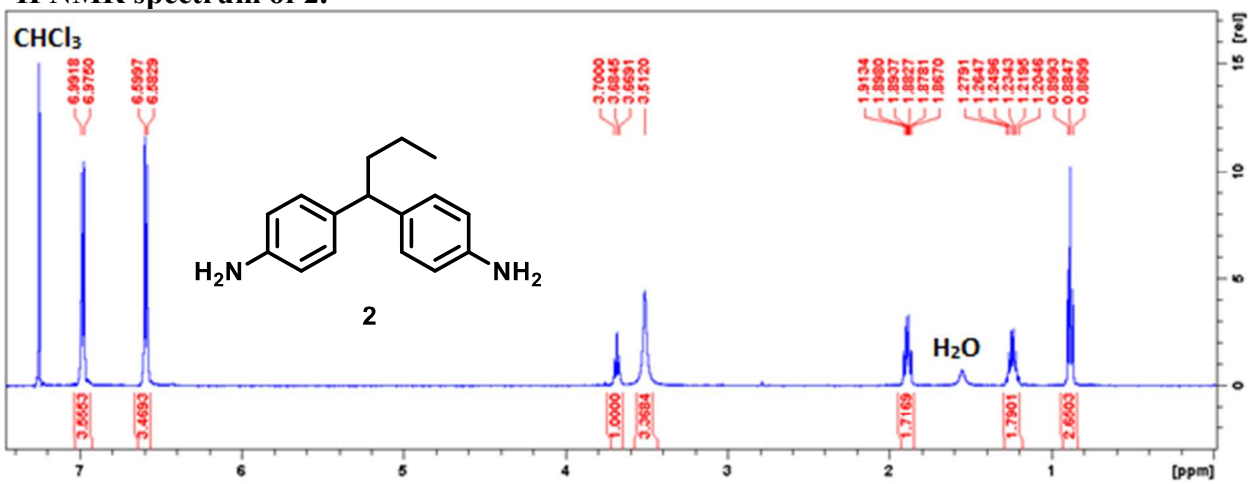
**$^{1}$H NMR spectrum of 1b.**



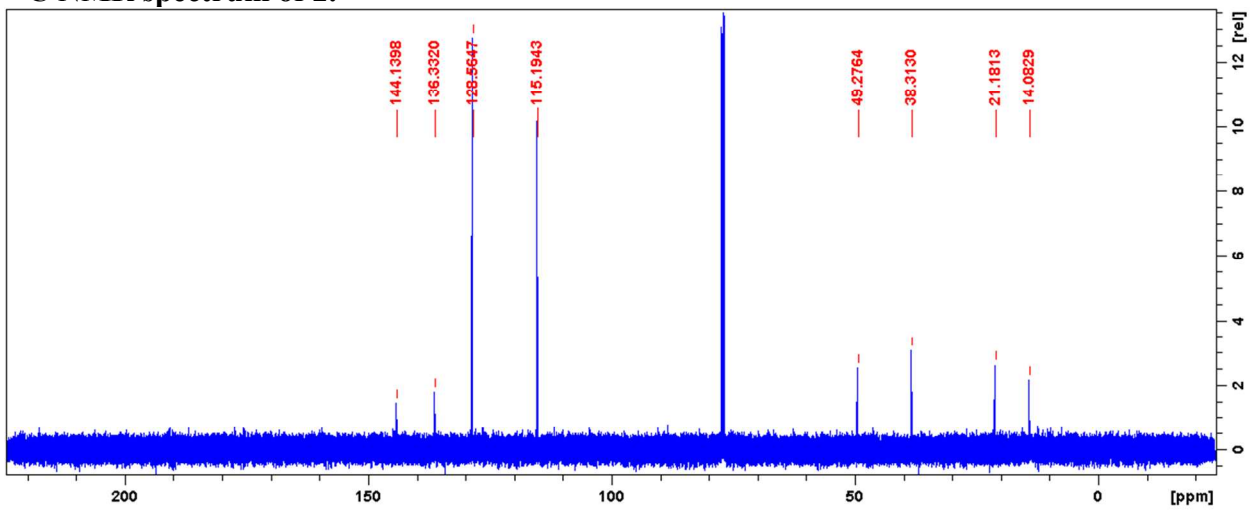

**$^{13}$C NMR spectrum of 1b.**



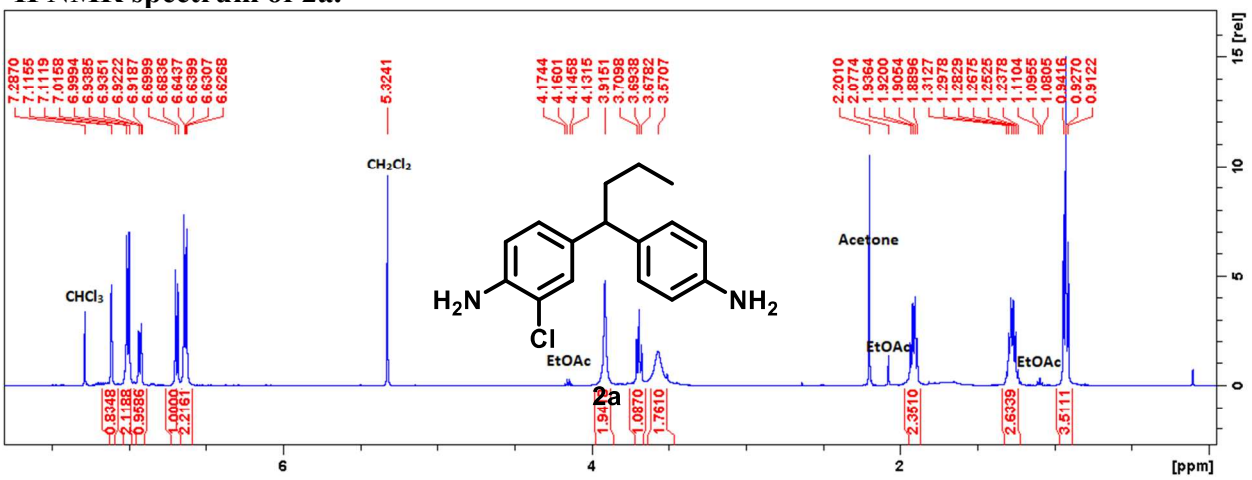
**$^{1}$H NMR spectrum of 2.**



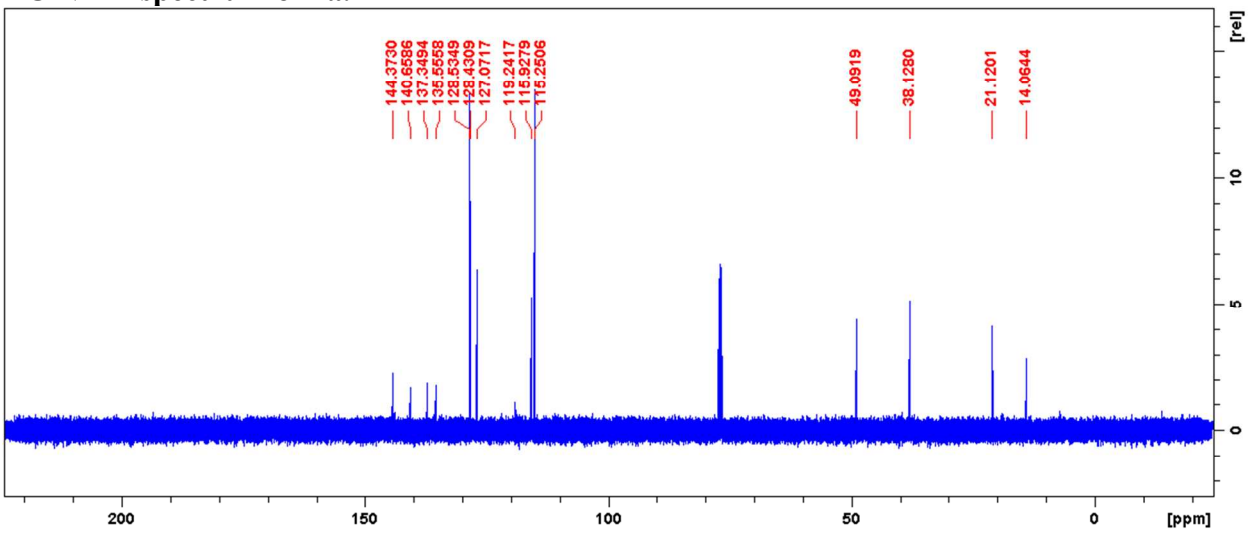

**$^{13}$C NMR spectrum of 2.**



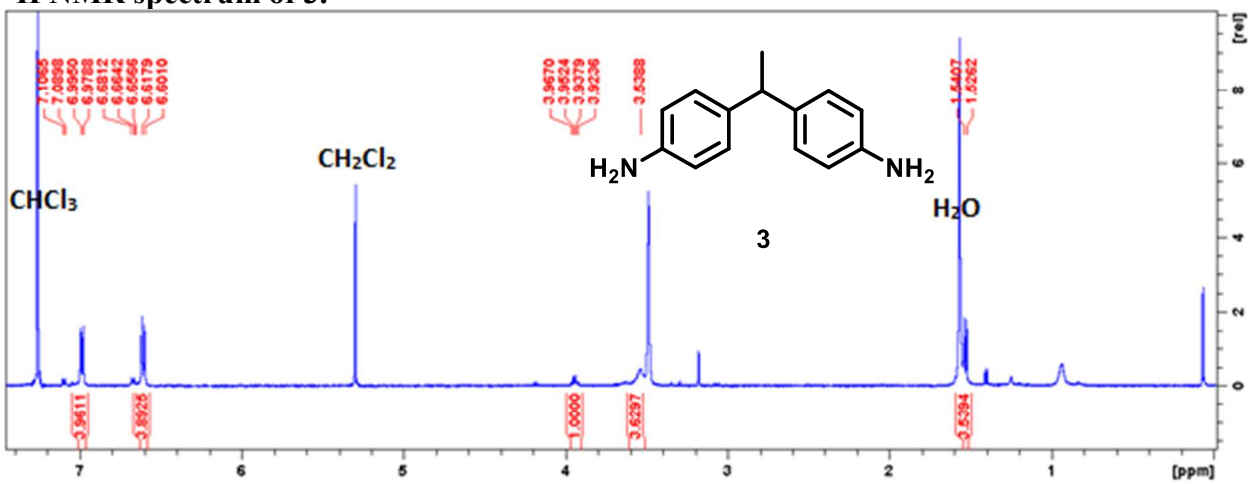
**$^{1}$H NMR spectrum of 2a.**



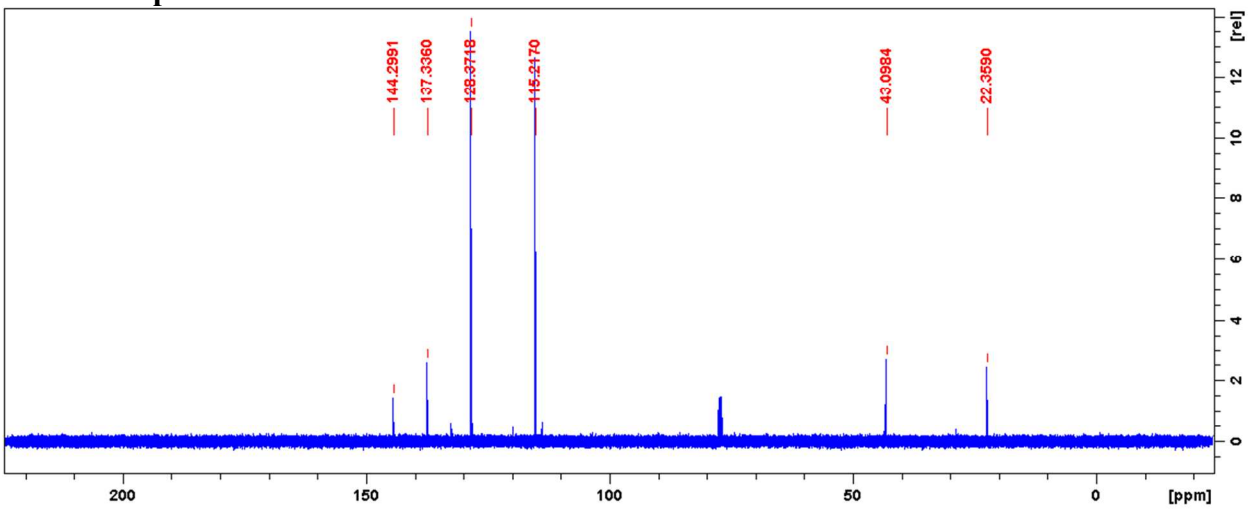

**$^{13}$C NMR spectrum of 2a.**



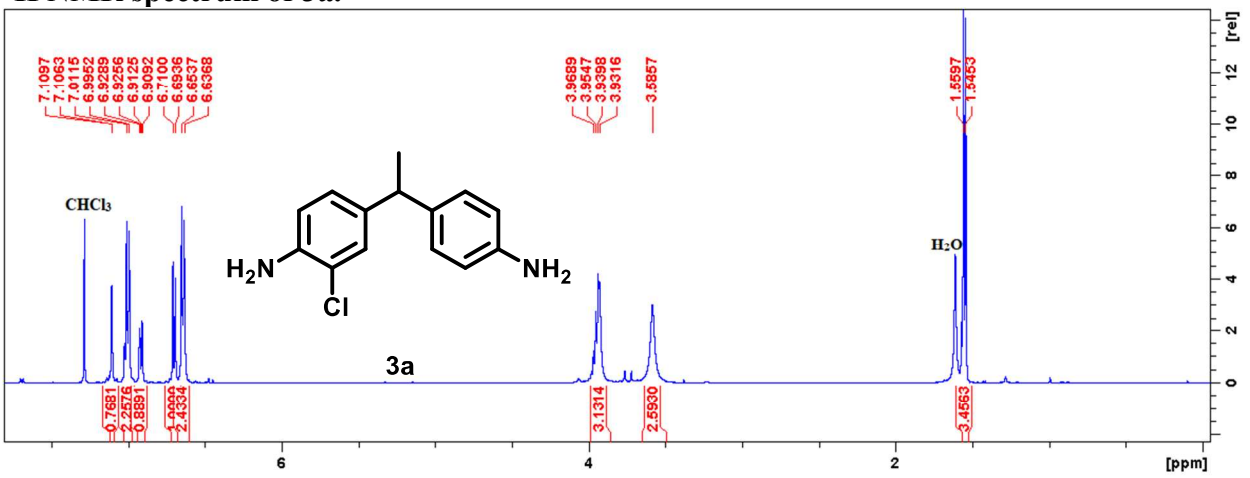
**$^{1}$H NMR spectrum of 3.**



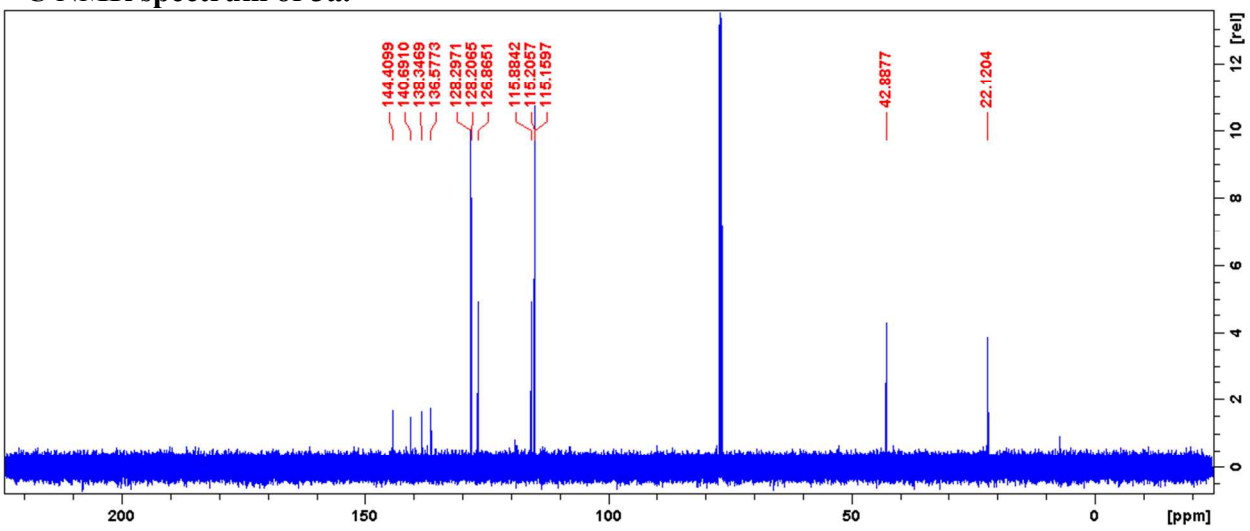

**$^{13}$C NMR spectrum of 3.**



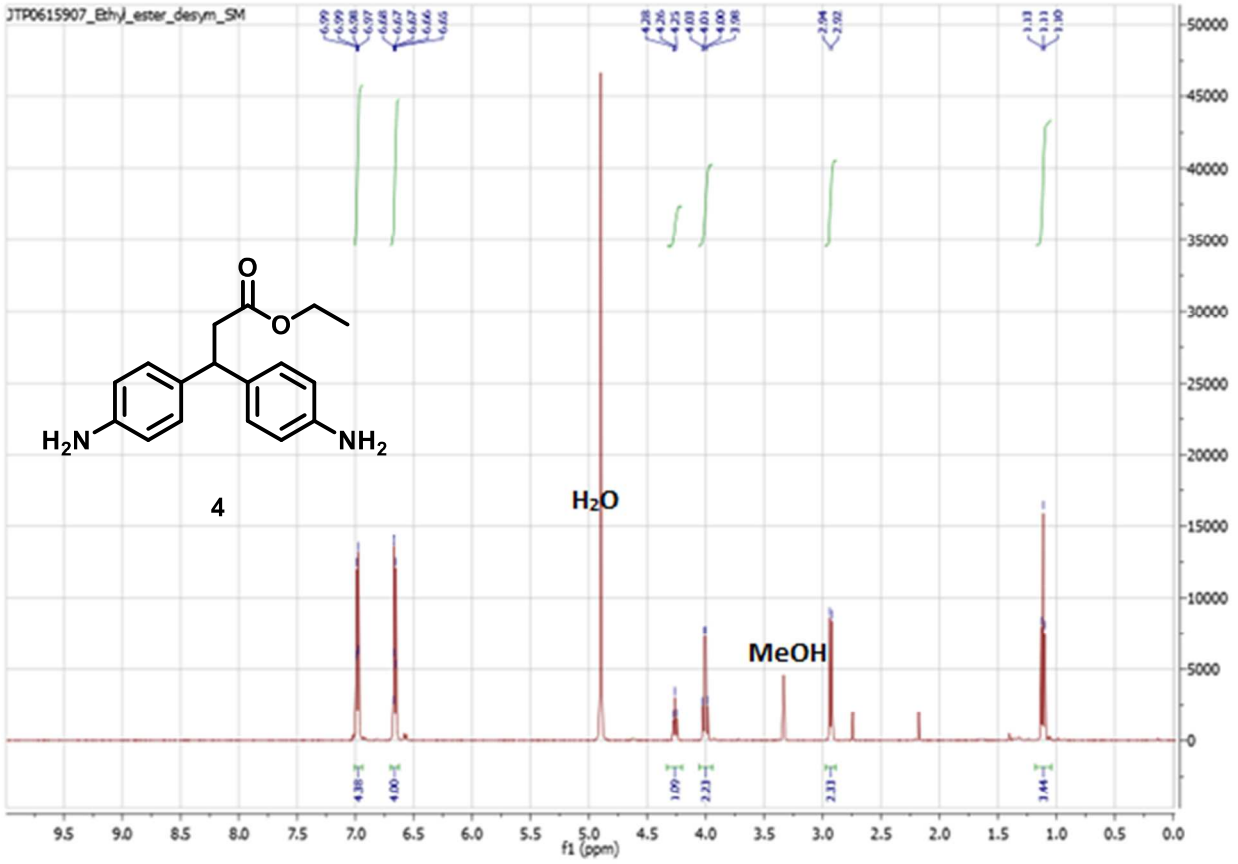
**$^{1}$H NMR spectrum of 3a.**



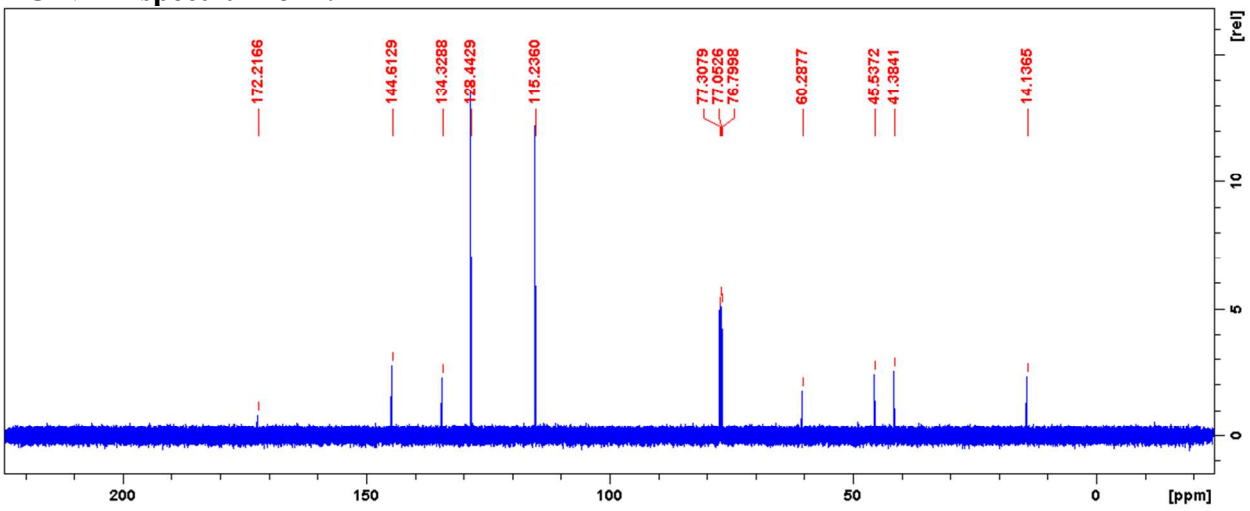

**$^{13}$C NMR spectrum of 3a.**



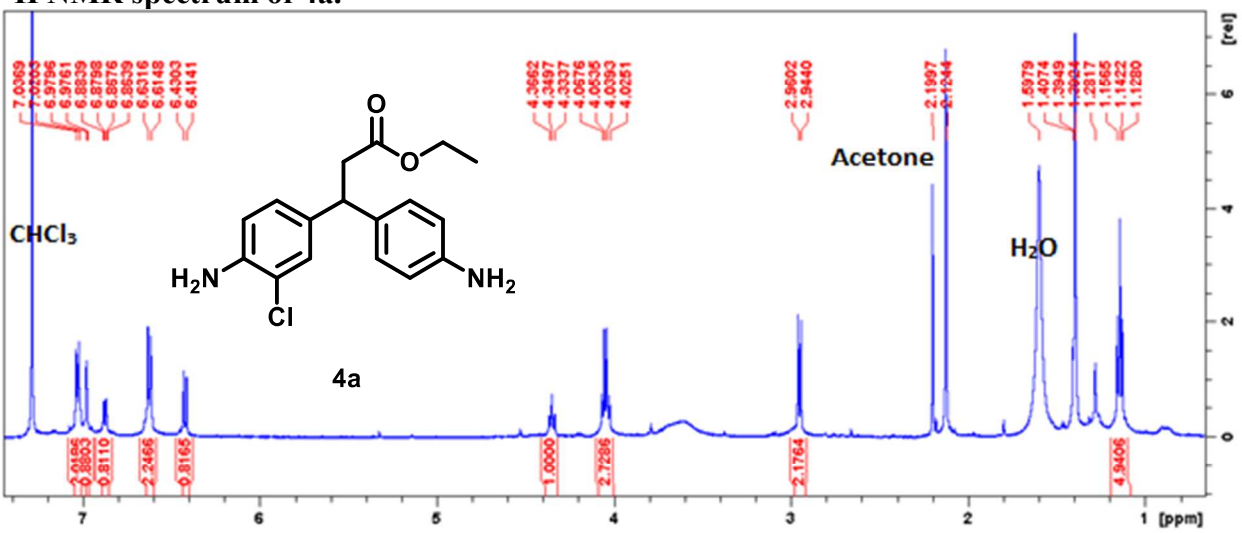
**$^{1}$H NMR spectrum of 4.**



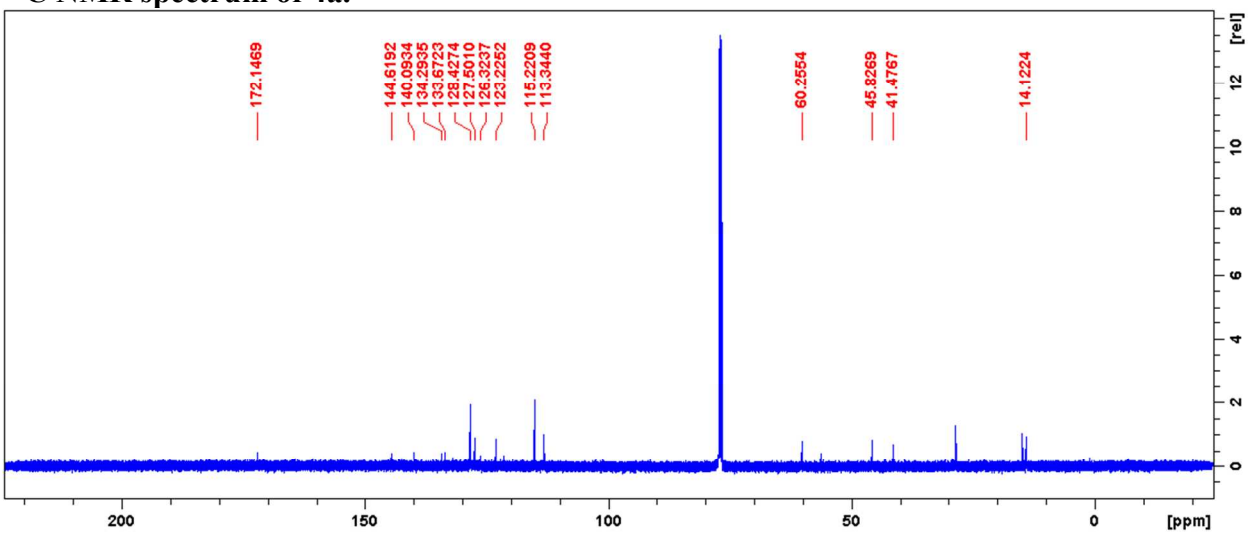

**$^{13}$C NMR spectrum of 4.**



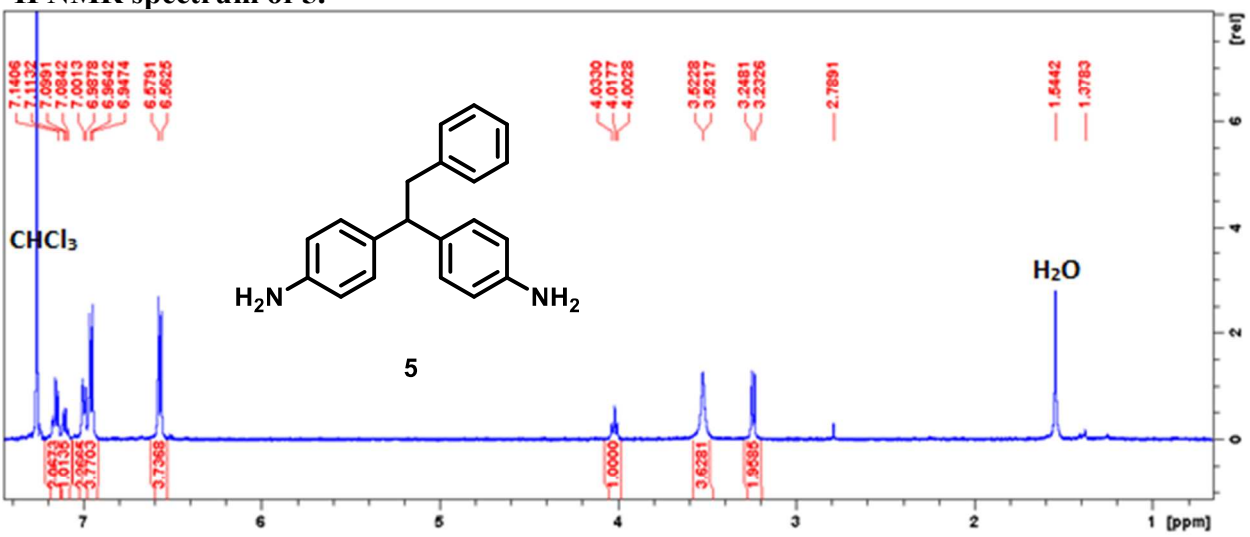
**$^{1}$H NMR spectrum of 4a.**



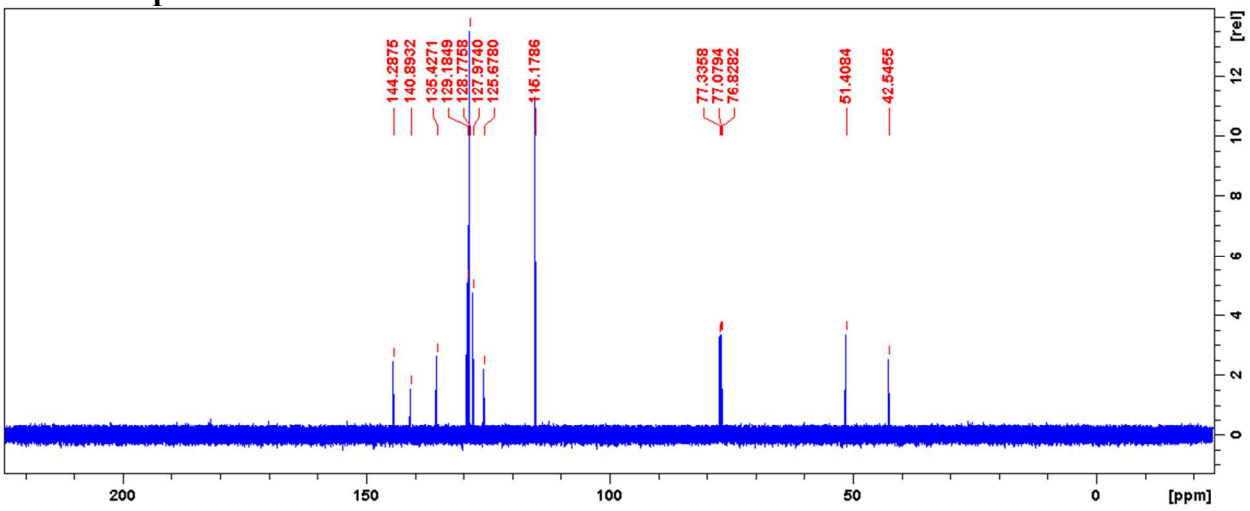

**$^{13}$C NMR spectrum of 4a.**



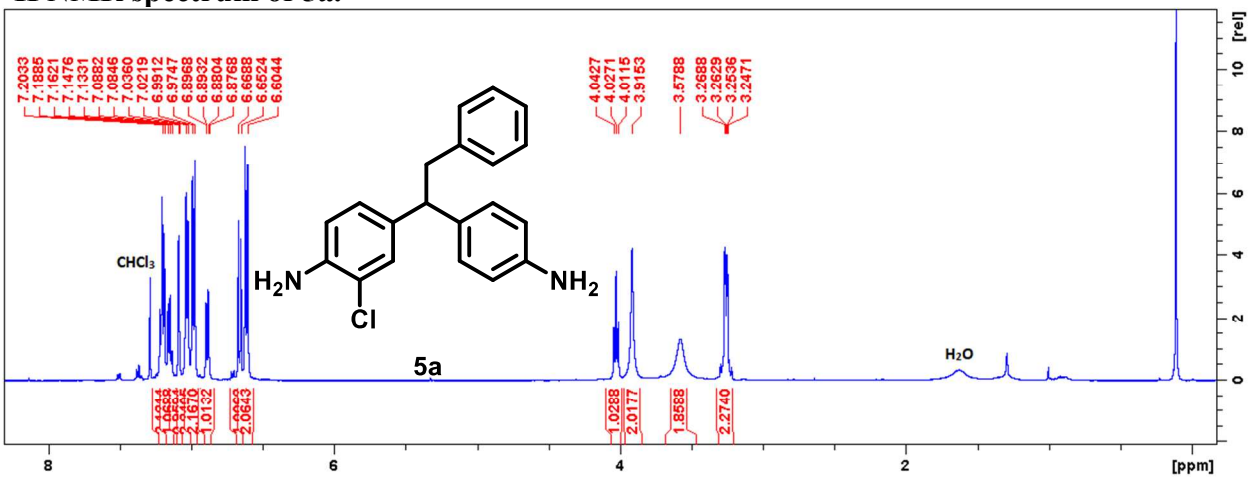
**$^{1}$H NMR spectrum of 5.**



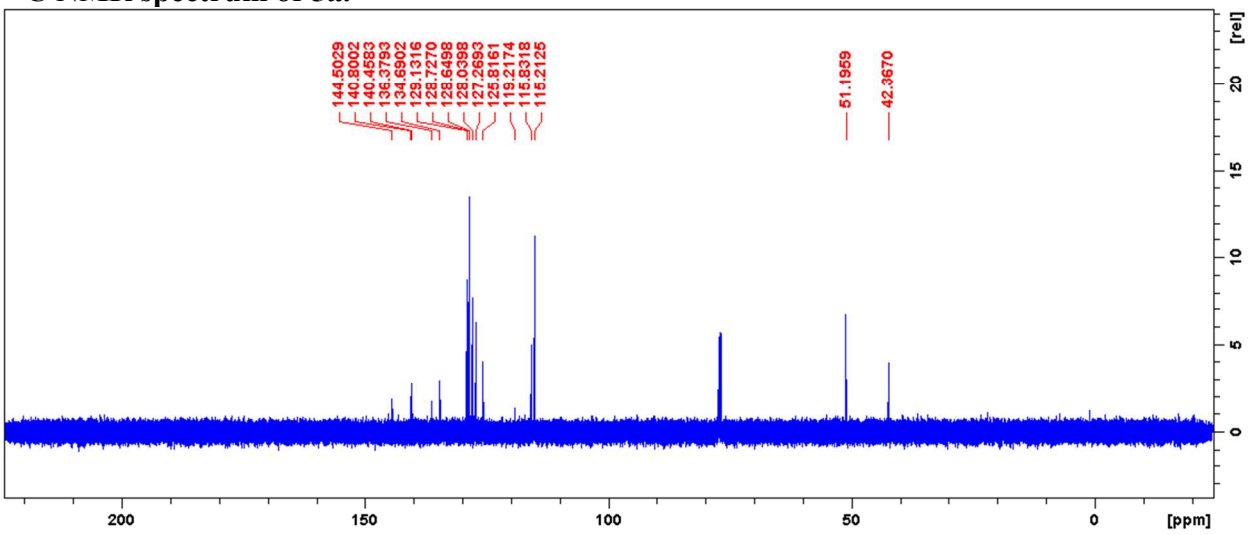

**$^{13}$C NMR spectrum of 5.**



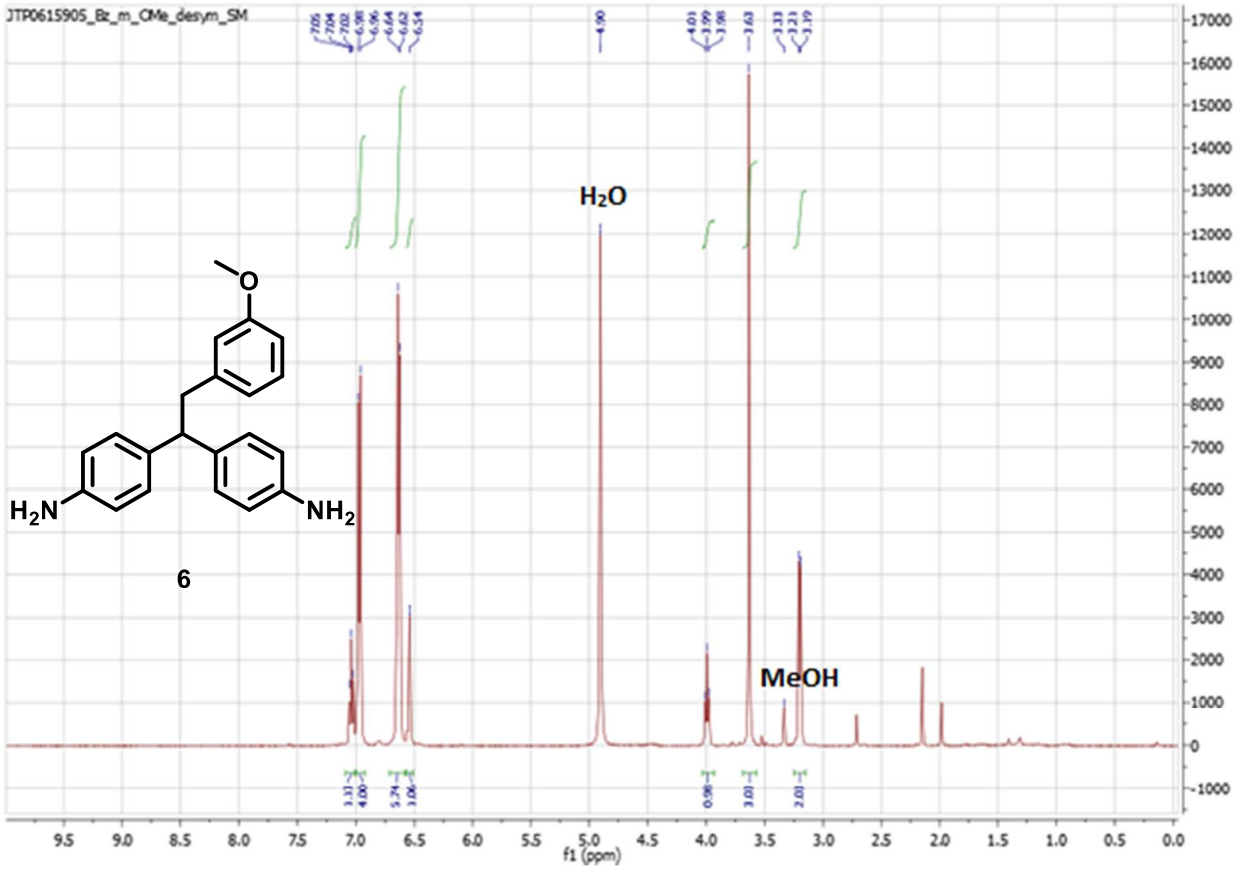
**$^{1}$H NMR spectrum of 5a.**



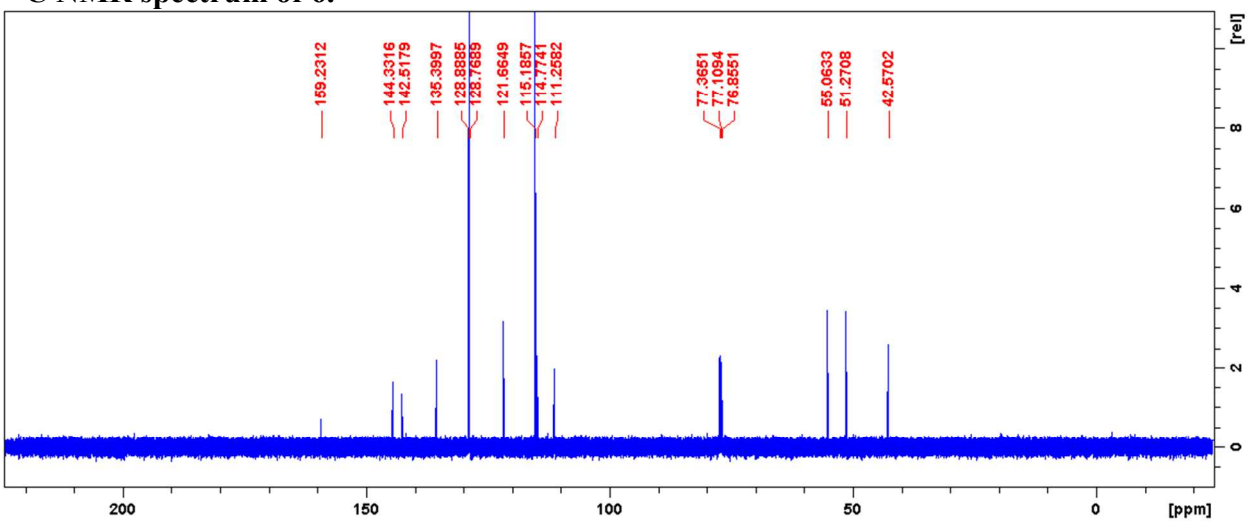

**$^{13}$C NMR spectrum of 5a.**



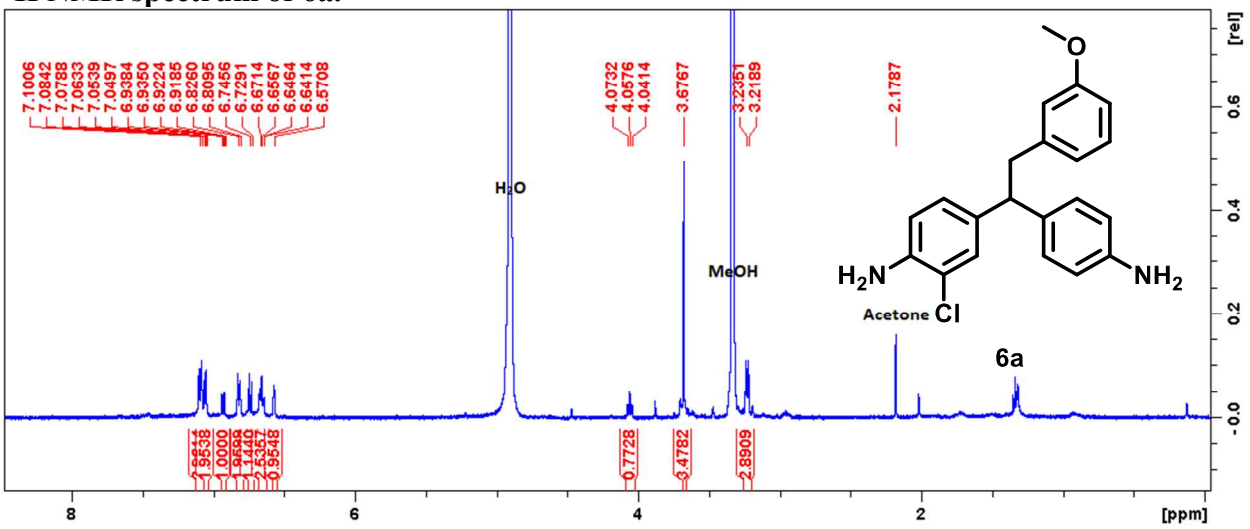
**$^{1}$H NMR spectrum of 6.**



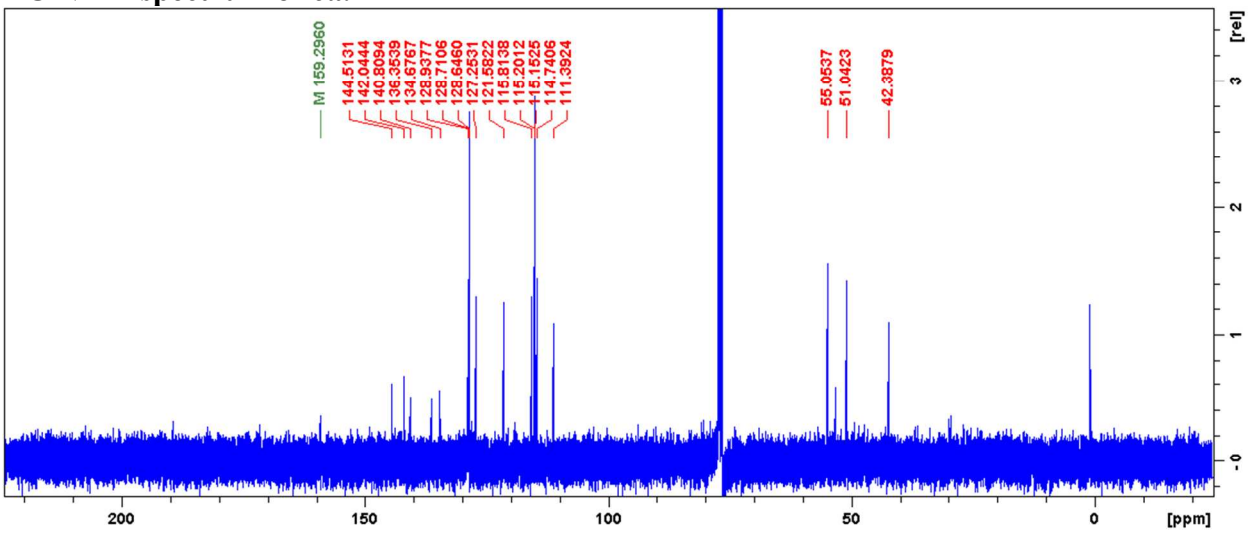

**$^{13}$C NMR spectrum of 6.**



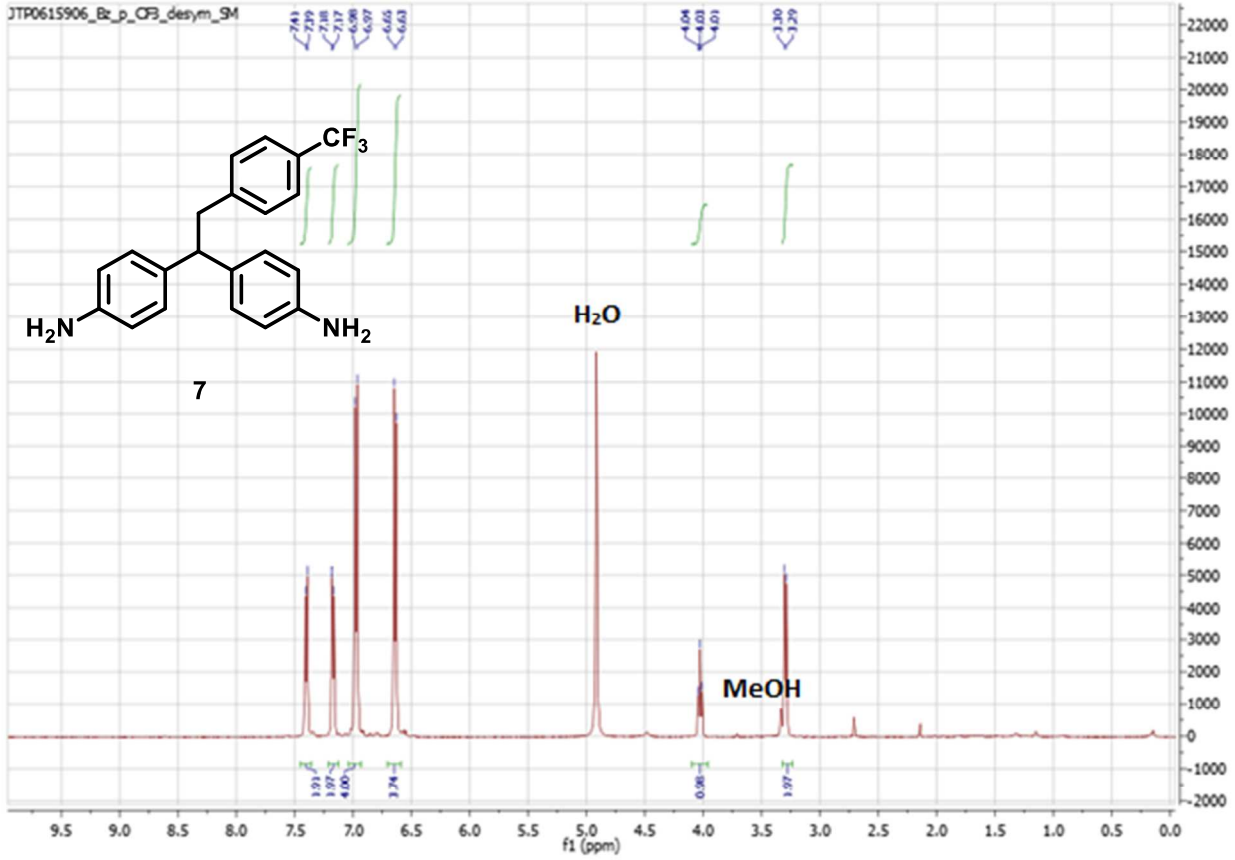
**$^{1}$H NMR spectrum of 6a.**



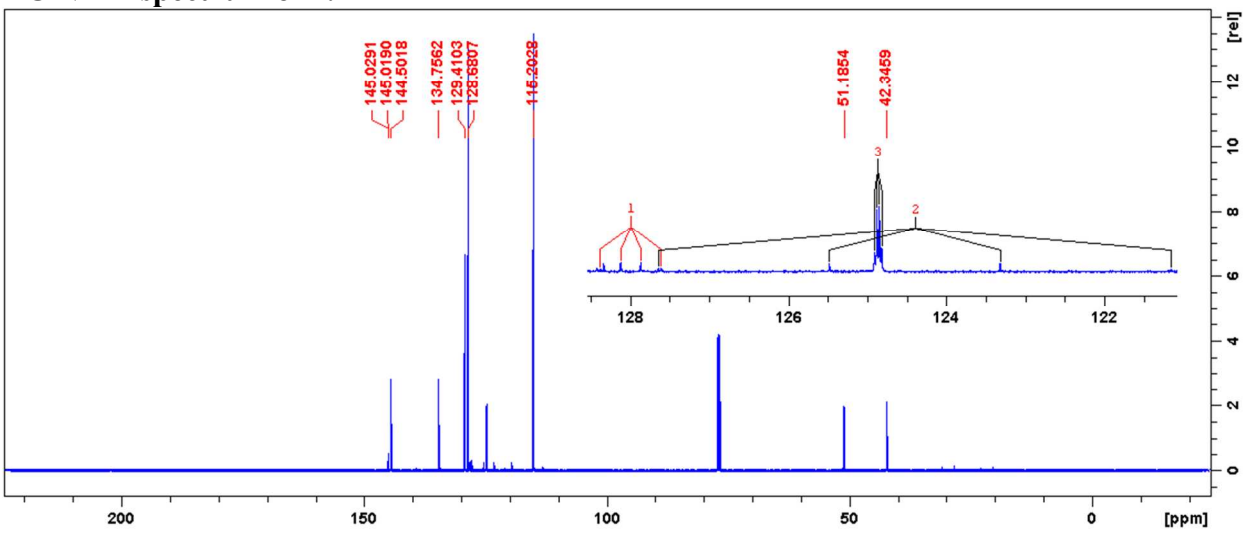

**$^{13}$C NMR spectrum of 6a.**



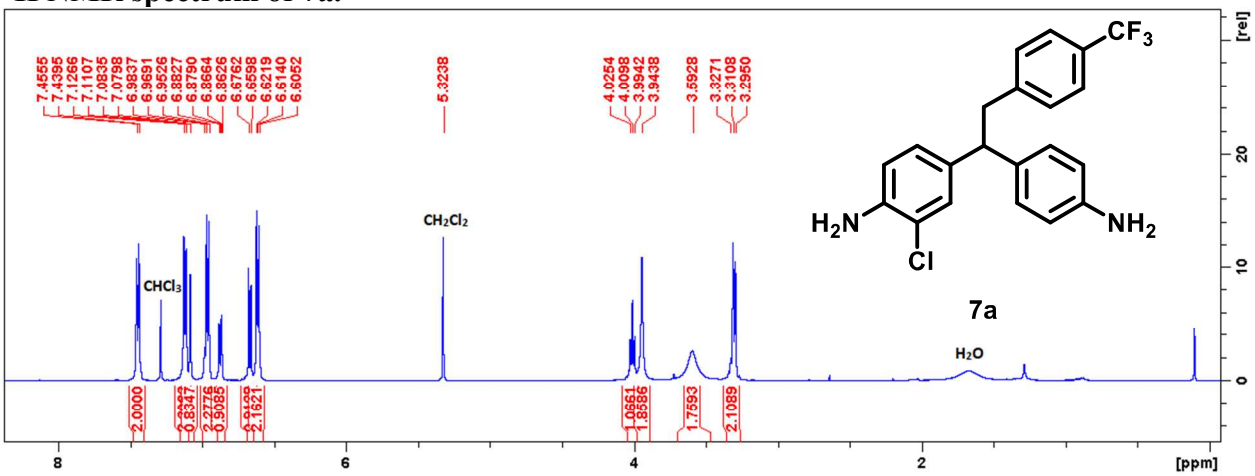
**$^{1}$H NMR spectrum of 7.**



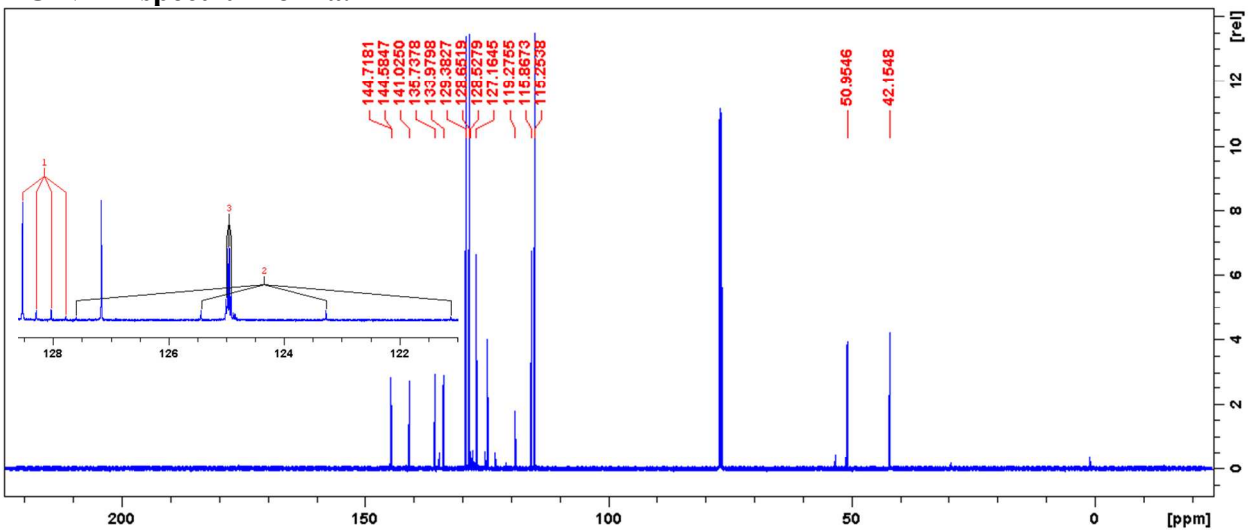

**$^{13}$C NMR spectrum of 7.**



**$^{1}$H NMR spectrum of 7a.**




**$^{13}$C NMR spectrum of 7a.**



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