Asymmetric Synthesis of Propargylic Alcohols Using Bifunctional Glucose Dehydrogenases

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1. General Information

All catalytic reactions were performed in centrifuge tubes (1.5 mL or 50 mL) under rotation or in conical flasks (60 mL, 500 mL or 2 L) with shaking. Substrates **1a-j** and other reagents were purchased from commercial suppliers, such as Sigma-Aldrich, Oakwood Chemical, Enamine, Ambeed, Combi-Blocks, AA Blocks, and Cambridge Isotope Laboratories, and were used without further purification. The racemic compounds **2** were synthesized according to previously reported procedures using sodium borohydride ($NaBH_4$). Column chromatography was performed with ultrapure silica gel SiliaFlash® P60 from SILICYCLE Inc. (irregular shaped, 230-400 mesh). Nuclear magnetic resonance was performed on Bruker-Avance 400 MHz instrument. All ^1H NMR experiments are reported in units, parts per million (ppm) and were measured relative to the deuterated chloroform signal (7.26). All proton decoupled-^{13}C NMR spectra are reported in ppm relative to deuterated chloroform (77.16). Chiral separation and enantiomeric purity check of the reaction products were determined with chiral columns on an Agilent 1260 Infinity II system using hexanes/isopropanol as the mobile phases. Molecular biology reagents were purchased from Fisher Scientific, Sigma-Aldrich, or New England Biolabs unless specified otherwise. *Escherichia coli* BL21 (DE3) was purchased from New England Biolab which was cultured in Luria–Bertani broth. The plasmids containing the DNA sequences of the enzymes were ordered from Twist Bioscience (San Francisco, CA).

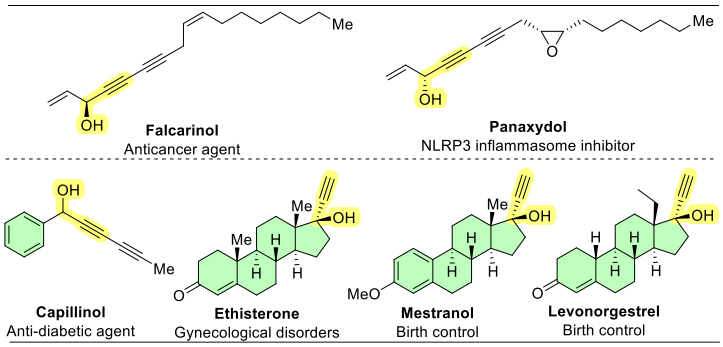


Figure S1. Representative bioactive compounds containing a propargyl alcohol moiety.

2. Expression and Purification of Enzymes

The following procedures for protein expression and purification related to RasADH,[1] PTDH,[2] GDHs and their mutants were described elsewhere with slight modifications. The volume of 20 μL stocked *E. Coli* BL21 cells harboring plasmid of the target enzyme was inoculated in 5 mL LB medium containing 100 μg/mL kanamycin and grown overnight at 37°C. This overnight culture was utilized to inoculate 500 mL of TB medium containing 100 μg/mL kanamycin, which was grown at 37 °C at 250 rpm until an OD_{600} reached to 0.4-0.6, and subsequently induced with the addition of 0.2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The induced culture was placed at 18 °C, 200 rpm, and cultured for 22 h

for protein expression. Following the above-mentioned expression procedures, the *E. Coli* BL21 cells containing the target enzyme were collected by centrifugation (3,550 × g for 15 min) and resuspended with sodium phosphate buffer (50 mM, pH 8.5). The mixture was lysed by sonication and clarified by centrifugation (4 °C, 3,550 × g for 30 min). The protein was subsequently purified by affinity chromatography using HisPur™ Ni-NTA Resin (88222, Thermo Scientific™) according to the manufacturer's instructions. The purified protein was buffer exchanged against 50 mM sodium phosphate buffer, pH 8.5 using an Amicon Ultra concentration tube with 10 kDa cut-off. After that, the proteins were stored in 16% glycerol as 100-200 µL aliquots at −80 °C. Enzyme concentrations were determined spectrophotometrically using a NanoDrop instrument at 280 nm, based on calculated extinction coefficients from the amino acid sequence. The purified OsGDH protein was qualitatively analysed using 12% SDS-PAGE and gel was stained using Coomassie Brilliant Blue R-250. The PageRuler™ Prestained Protein Ladder (26616, Thermo Scientific™) was utilized to determine the molecular weights of key enzymes.

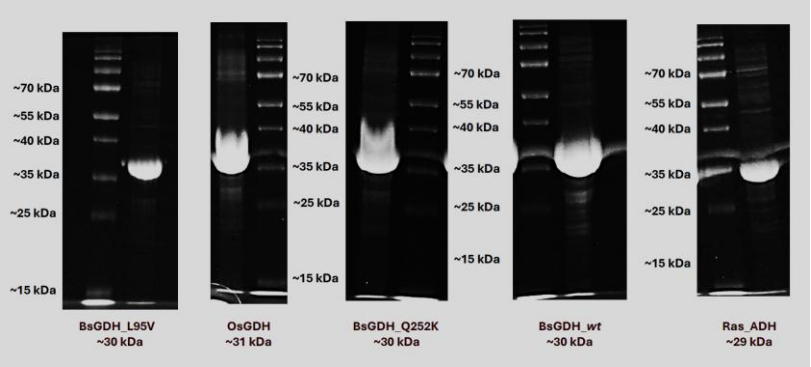


Figure S2: SDS-PAGE gels of key enzymes

Enzyme sequence

The enzyme sequences are underlined.

RasADH:
MGSSHHHHHHSSGLVPRGSHMYRLLNKTAVITGGNSGIGLATAKRFVAEGAYVFIVGRRRKE
LEQAAAEIGRNVTAVKADVTKLEDLDRLYAIVREQRGSIDVLFANSGAIEQKTLEEITPEHYDR
TFDVNVRGLIFTVQKALPLLRDGGSVILTSSVAGVLGLQAHDTYSAAKAAVRSLARTWTTELK
GRSIRVNAVSPGAIDTPIIENQVSTQEEADELRAKFAAATPLGRVGRPEELAAAVLFLASDDSSY
VAGIELFVDGGLTQV

OsGDH:
MGSSHHHHHHSSGLVPRGSHMSVSPYLLKGQKALVTGGSSGIGEAVARYLAKAGASVAINYH
SHPEEADKIVSDIQAGNGEAIAIQANVSKEDEVKAMFAKMFQEFGTIDILVNNAGMQKDAAFL
DMTLEQWNLVINVNLTGQFLCAREAAKEFLRRGVQPHISSAAGKIICMSSVHEVIPWAGHVNY
AASKGGINMMMRSMAQELAPHKIRVNSIAPGAIKTPINKSAWETPEAEAKLLTLIPAGRVGQV
EDIAKAAVWLASDDSDYVSGATLFVDGGMTLYPAFARGG

BsGDH:

MGSSHHHHHHSSGLVPRGSHMMYPDLKGKVVAITGAASGLGKAMAIRFGKEQAKVVINYYS
NKQDPNEVKEEVIKAGGEAVVVQGDVTKEEDVKNIVQTAIKEFGTLDIMINNAGLENPVPSHE
MPLKDWDKVIGTNLTGAFLGSREAIKYFVENDIKGNVINMSSVHEVIPWPLFVHYAASKGGIK
LMTETLALEYAPKGIRVNNIGPGAINTPINAEKFADPKQKADVESMIPMGYIGEPEEIAAVAAW
LASKEASYVTGITLFADGGMTQYPSFQAGRG

BsGDH_Q252K:
MGSSHHHHHHSSGLVPRGSHMMYPDLKGKVVAITGAASGLGKAMAIRFGKEQAKVVINYYS
NKQDPNEVKEEVIKAGGEAVVVQGDVTKEEDVKNIVQTAIKEFGTLDIMINNAGLENPVPSHE
MPLKDWDKVIGTNLTGAFLGSREAIKYFVENDIKGNVINMSSVHEVIPWPLFVHYAASKGGIK
LMTETLALEYAPKGIRVNNIGPGAINTPINAEKFADPKQKADVESMIPMGYIGEPEEIAAVAAW
LASKEASYVTGITLFADGGMTKYPSFQAGRG

BsGDH_L95V:
MGSSHHHHHHSSGLVPRGSHMMYPDLKGKVVAITGAASGLGKAMAIRFGKEQAKVVINYYS
NKQDPNEVKEEVIKAGGEAVVVQGDVTKEEDVKNIVQTAIKEFGTLDIMINNAGVENPVPSHE
MPLKDWDKVIGTNLTGAFLGSREAIKYFVENDIKGNVINMSSVHEVIPWPLFVHYAASKGGIK
LMTETLALEYAPKGIRVNNIGPGAINTPINAEKFADPKQKADVESMIPMGYIGEPEEIAAVAAW
LASKEASYVTGITLFADGGMTQYPSFQAGRG

3. Standard reaction conditions with 1a as substrate in 0.0025 mmol scale

To a 1.5 mL centrifuge tube, $NADP^+$ (1 mM), D-glucose (100 mM), and BsGDH or its mutant or OsGDH (1 mol%) in sodium phosphate buffer (50 mM, pH 7.0), and compound **1a** (10 μL of a 250 mM DMSO stock, 0.0025 mmol) were added sequentially. The total volume of the reaction mixture was 0.5 mL and the reaction was then allowed to rotate at 30 °C in a rotator (BS-RTTT-2, Stellar Scientific) for 24 hours. After the completion of reaction, 1,1,2,2-tetrachloroethane (10 μL of 250mM $CDCl_3$ stock, 0.0025 mmol) was added to the mixture. The mixture was extracted by adding 500 μL $CDCl_3$ and cenrifuged with dried sodium sulphate. The contents were then transferred to a NMR tube and the product yields were then determined by ^1H NMR analysis.

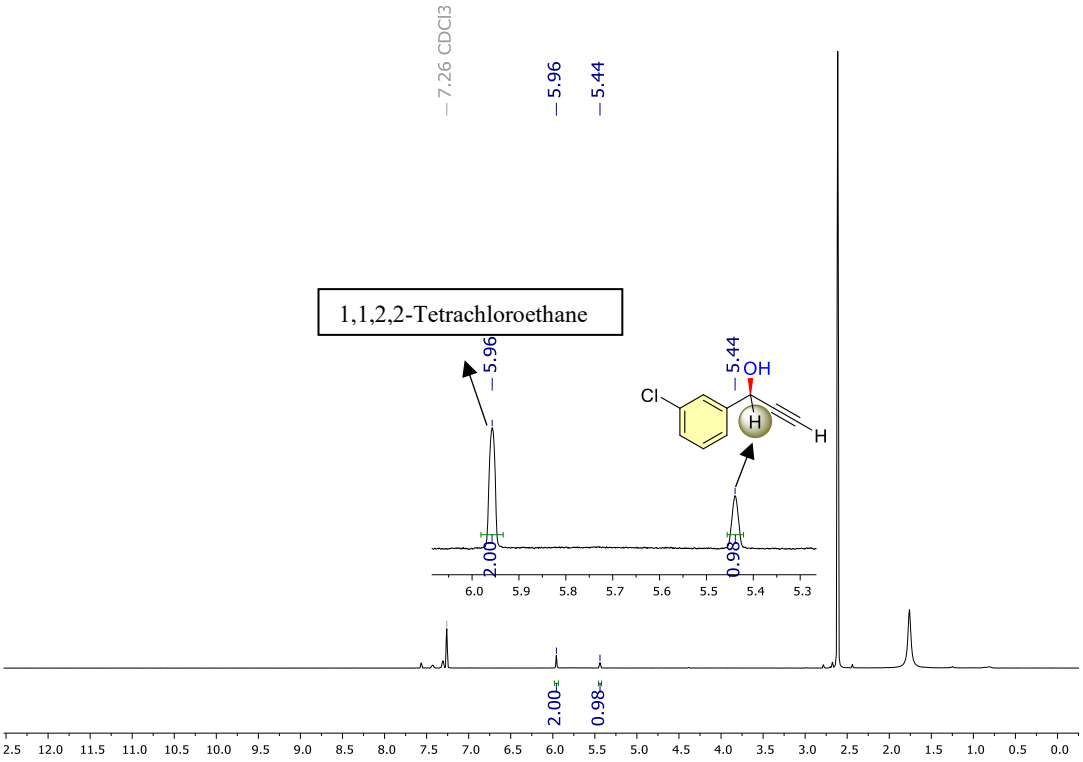
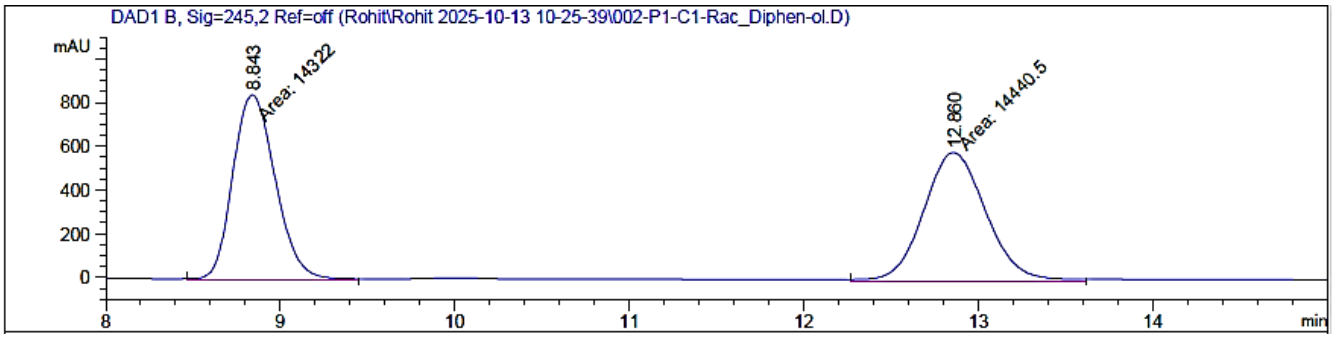


Figure S3: Representative example of NMR yield calculation of product **2d**

4. Reactions of *Ras*-ADH with substrate 1a

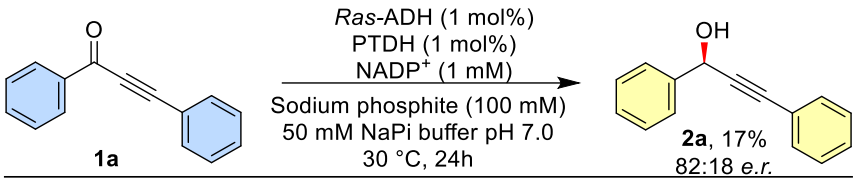
To a 1.5 mL centrifuge tube, $NADP^+$ (1 mM), sodium phosphite (100 mM), *Ras*-ADH (1 mol%) and PTDH (1 mol%) in sodium phosphate buffer (50 mM, pH 7.0), and compound **1a** (10 µL of a 250 mM DMSO stock, 0.0025 mmol) were added sequentially. The total volume of the reaction mixture was 0.5 mL and the reaction was then allowed to rotate at 30 °C in a rotator (BS-RTTT-2, Stellar Scientific) for 24 hours. After the completion of reaction, 1,1,2,2-tetrachloroethane (10 µL of 250 mM $CDCl_3$ stock, 0.0025 mmol) was added to the mixture. The mixture was extracted by adding 500 µL $CDCl_3$ and cenrifuged with dried sodium sulfate. The contents were then transferred to a NMR tube and the product yields were then determined by ^1H NMR analysis. The reactions were done in duplicates.

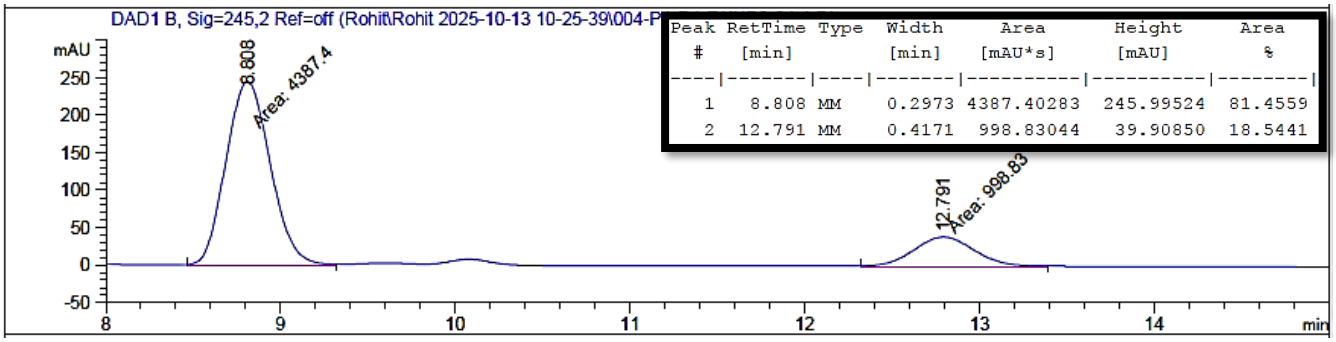
After the determination of NMR yields, the compounds were transferred to HPLC vials fitted with inserts and were dried using nitrogen gas, after drying the mixture was redissolved in hexane:isopropanol (19:1). The enantiomeric ratio of the product was determined using an Agilent system equipped with a chiral HPLC column (Chiralcel® - OJ-H, 250 mm; *n*-hexane/*i*-PrOH = 70:30; 1 mL/min; detection at 245 nM). HPLC trace of racemic **2a**:



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.843	MM	0.2826	1.43220e4	844.55737	49.7941
2	12.860	MM	0.4116	1.44405e4	584.79803	50.2059

Reaction: *Ras*-ADH and PTDH as cofactor regeneration system





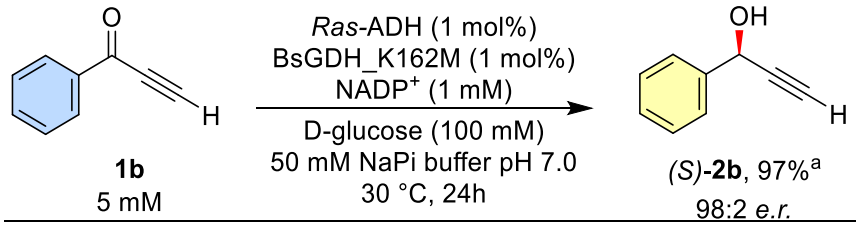
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.808	MM	0.2973	4387.40283	245.99524	81.4559
2	12.791	MM	0.4171	998.83044	39.90850	18.5441

5. Determination of enantiomeric ratio

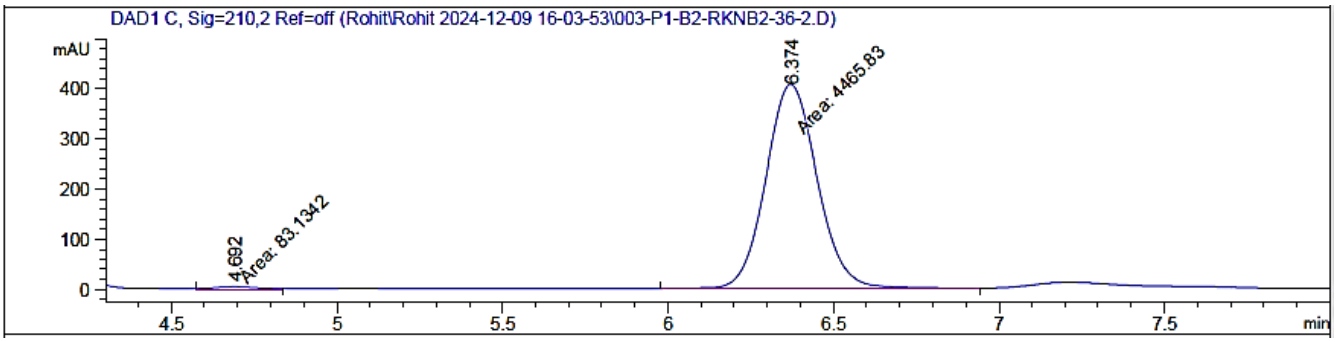
After the isolation, compounds were dissolved in hexane:isopropanol (19:1) and transferred to HPLC vials. The enantiomeric ratios were determined by the agilent 1260 Infinity II system with Chiralcel® - OD-H column.

5.1 Determination of stereochemistry of compounds

The stereochemistry of the compounds was confirmed using a previously known *Ras*-ADH which provides same products in *(S)*-configuration. The reaction of substrate **1b** was performed in the presence of *Ras*-ADH using BsGDH_K162M[3] mutant for cofactor regeneration. The yield of product *(S)*-**2b** was confirmed using NMR (97%) and enantiomeric ratio was determined using chiral HPLC. HPLC separation (Chiralcel® - OD-H, *n*-hexane/*i*-PrOH = 90:10, 1 mL/min, detection at 210 nM).



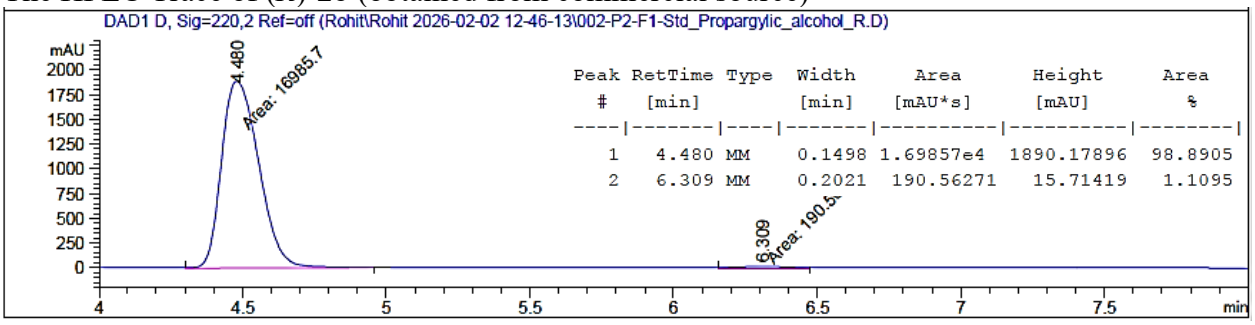
HPLC trace of enantioenriched (*S*)-**2b** obtained from the catalytic reduction by *Ras*-ADH:



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.692	MM	0.1900	83.13416	7.29342	1.8275
2	6.374	MM	0.1821	4465.83105	408.80106	98.1725

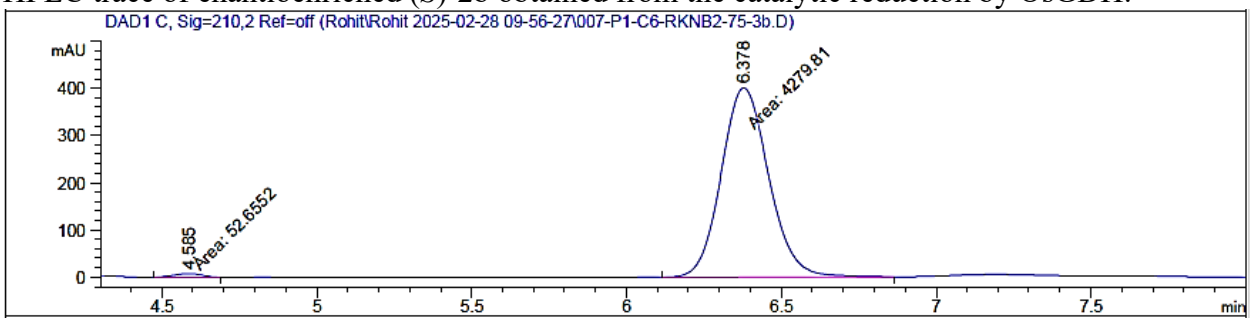
5.2 Dterermining stereochemistry using commercially available (*R*)-2b ((R)-1-Phenyl-2-propyn-1-ol CAS No.: 61317-73-5

The HPLC Trace of (*R*)-2b (obtained from commercial source)

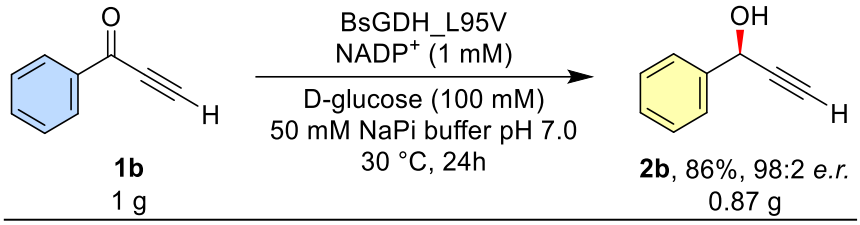


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.480	MM	0.1498	1.69857e4	1890.17896	98.8905
2	6.309	MM	0.2021	190.56271	15.71419	1.1095

HPLC trace of enantioenriched (*S*)-2b obtained from the catalytic reduction by OsGDH:



6. Gram-scale synthesis of product 2b using BsGDH_L95V

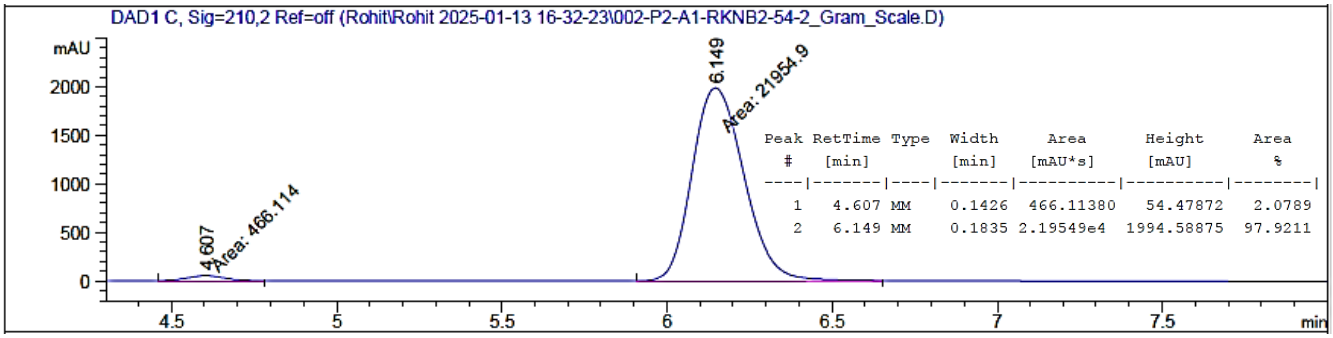


1b
1 g

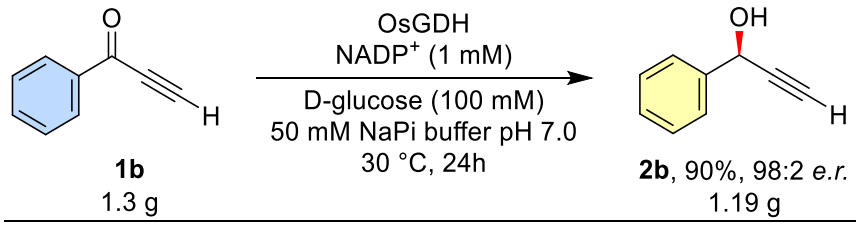
2b, 86%, 98:2 *e.r.*
0.87 g

To a 2 L glass flask, $NADP^+$ (1 mM), D-glucose (100 mM), and BsGDH_L95V wet cells (23.30 g, 38.83 g/L) in sodium phosphate buffer (50 mM, pH 7.0), DMSO and compound **1b** (1g dissolved in 30 mL DMSO) were added sequentially. The total volume of the reaction mixture was 600 mL, the reaction mixture was then kept in a shaker at 30°C at 220 rpm for 24 hours. Then the reaction mixture was diluted with 300 mL water which was extracted by 150 mL ethyl acetate. After two additional extractions, the combined organic layers were washed with brine (300 mL). The organic layer was dried over sodium sulfate and concentrated under vacuum to give the crude extract. The residue was then purified by coloumn chromatography (90:10 hexane:ethyl acetate) on silica gel to give the target product, yield = 86% (870 mg).

HPLC chromatogram



7. Gram scale synthesis of product 2b using OsGDH



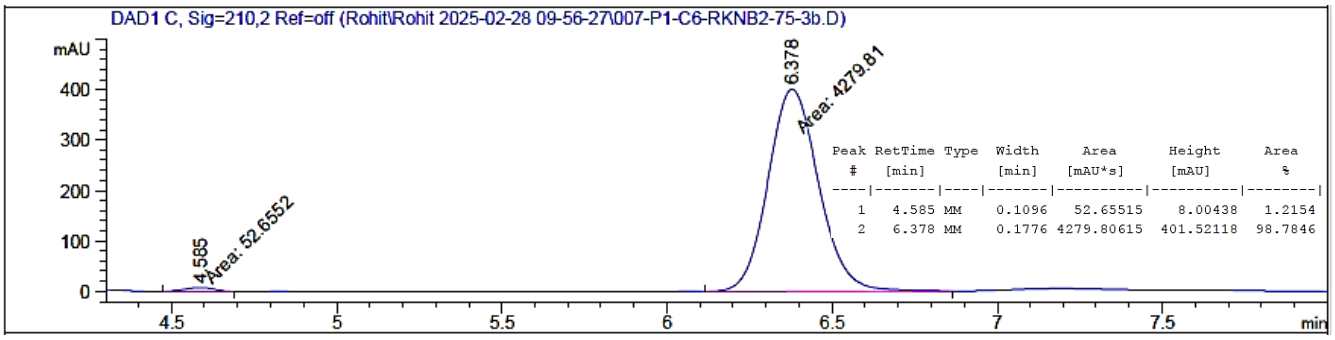
1b
1.3 g

2b, 90%, 98:2 *e.r.*
1.19 g

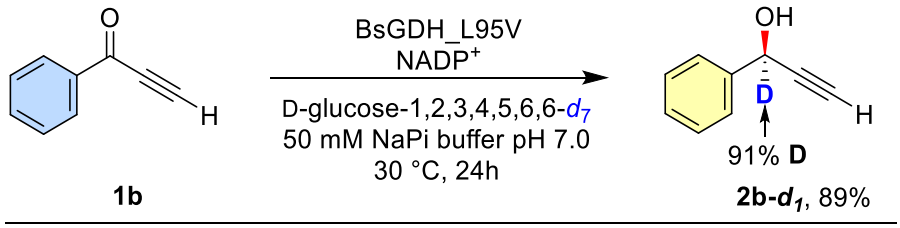
To a 2 L glass bottle, $NADP^+$ (1 mM), D-glucose (100 mM), and OsGDH wet cells (26.62 g, 44.36 g/L) in sodium phosphate buffer (50 mM, pH 7.0), DMSO and compound **1b** (1.3 g dissolved in 30 mL DMSO) were added sequentially. The total volume of the reaction mixture was 600 mL, the reaction mixture was then kept in a shaker at 30°C at 220 rpm for 24 hours. Then the reaction mixture was diluted with 300 mL water which was extracted by 150 mL ethyl acetate. After two additional extractions, the combined organic layers were washed with brine (300 mL). The organic layer was dried over sodium sulfate and concentrated under vacuum to give the crude extract. The residue was then purified by

coloumn chromatography (90:10 hexane:ethyl acetate) on silica gel to give the target product, yield = 90% (1.194 g) *e.r.* = 98:2.

HPLC chromatogram



8.1 Deuterium labeling experiment with 1b as substrate in 0.1 mmol scale



To a 50 mL falcon tube, $NADP^+$ (1 mM), D-glucose-1,2,3,4,5,6,6-d_7 (100 mM), and BsGDH_L95V (1 mol%) in sodium phosphate buffer (50 mM, pH 7.0), and compound **1a** (400 µL of a 250 mM DMSO stock, 0.1 mmol) were added sequentially. The total volume of the reaction mixture was 15 mL and the reaction was then allowed to rotate at 30 °C in a rotator for 24 hours. After the completion of reaction, the reaction mixture was transferred to separating funnel and was extracted by ethyl acetate (2 × 15 mL). The combined organic layers were dried over Na_2SO_4 and the contents were concentrated under reduced pressure. The residue was then purified by using preparative TLC to give the target product (**2b-*d*$_1$**), yield = 89% (11.8 mg). 91% Deuterium incorporation was observed at the α-position of the isolated product.

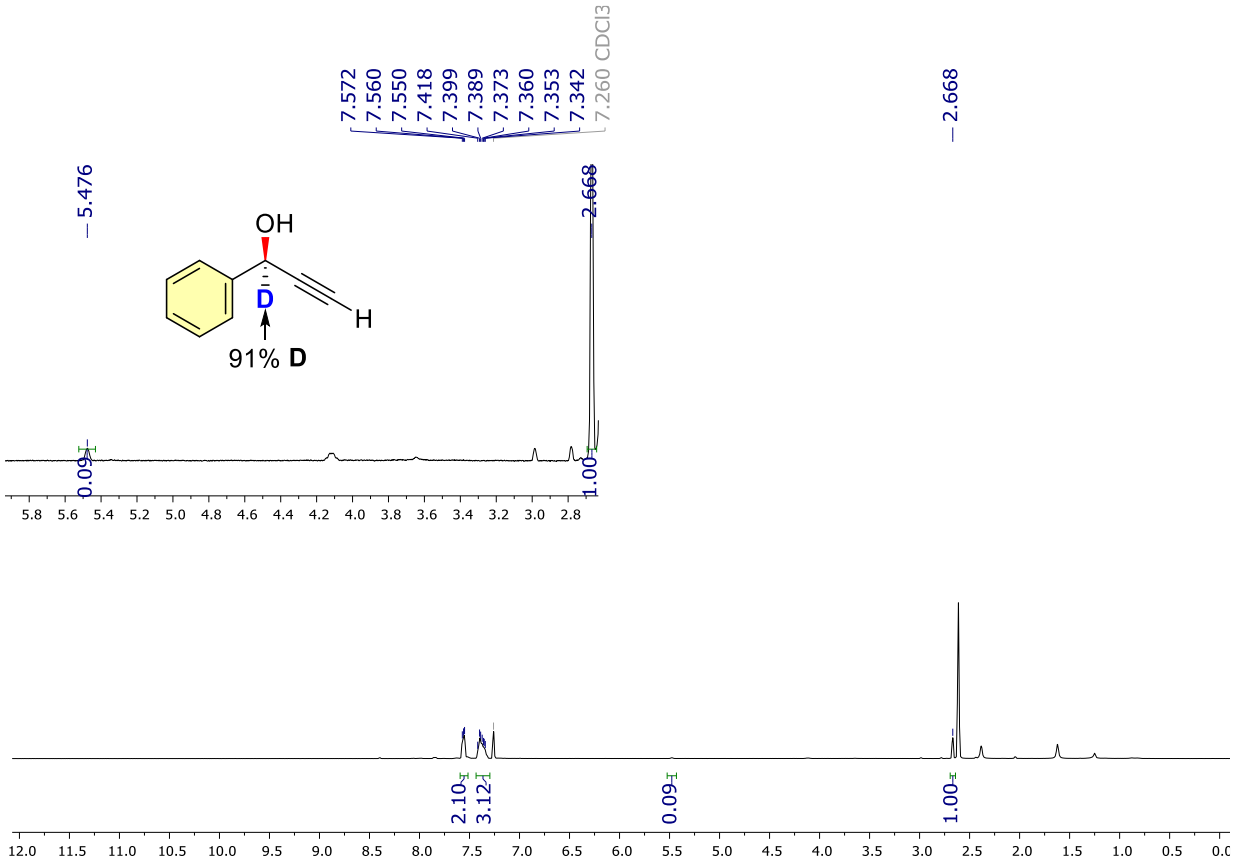


Figure S4: NMR spectra of **2b-*d*$_1$**

8.2 Proposed reaction mechanism

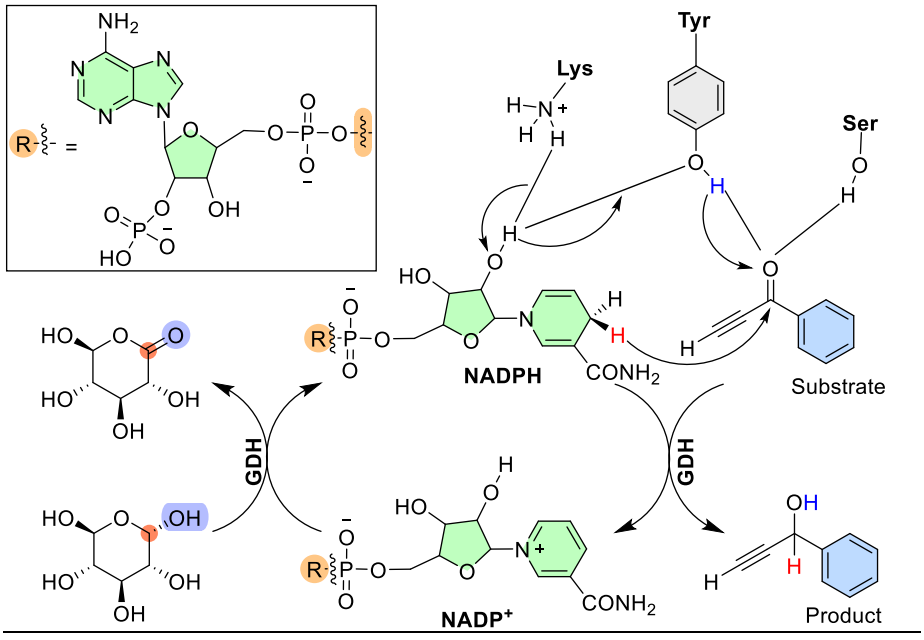


Figure S5: GDH catalytic mechanism

9. Procedure a: General procedure for the chemical reduction of ketones (1a-j):

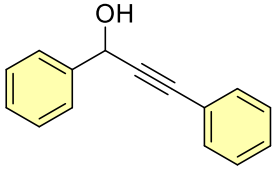
The ketone (0.1mmol) was dissolved in methanol (2 mL) and cooled to 0 °C with stirring. An excess of sodium borohydride (21.7 mg) was added, and the mixture was stirred for 15 min at 0 °C and then stirred additional four hours at room temperature. The reaction was quenched by dropwise addition of 1 M HCl until bubbling ceased. The volume of the mixture was reduced to 2 mL under reduced pressure, and ethyl acetate (10 mL) was added and the combined organic layers were washed by brine and then evaporated under reduced pressure to afford an oil. The resulting residue was purified by preparative TLC to afford corresponding alcohols.

10. Procedure b: General procedure for the biocatalytic reduction of ketones using BsGDH_L95V and OsGDH (1a-j):

To a 50 mL falcon tube, ketone (**1a-j**) (0.1mmol in 400 µL DMSO) along with $NADP^+$ (1 mM) (400 µL from 50 mM stock), D-glucose (100 mM) (2 mL from 1M stock), and BsGDH_L95V or OsGDH (1 mol%) was added to 50 mM NaPi buffer (10 mL) and total reaction volume was adjusted to 20 mL using sodium phosphate buffer (50 mM, pH 7.0). The reaction was then allowed to rotate at 30 °C in a rotator for 24 hours. After completion of the reaction, the reaction mixture was transferred to a separatory funnel and extracted with ethyl acetate (2 × 15 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by preparative TLC to afford the target compound.

11. Characterization data

*1,3-diphenylprop-2-yn-1-ol (Table 2, Entry **2a**)*

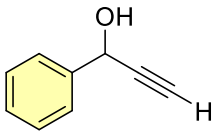


Following the general procedure a, 1,3-diphenylprop-2-yn-1-one **(1a)** (20.6 mg, 0.1 mmol), sodium borohydride (21.7 mg). Title compound was isolated by preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, Yield = 87% (18 mg). HPLC separation (racemic) (Chiralcel® - OD-H), *n*-hexane/*i*-PrOH 90:10, 1 mL/min, detection at 254 nM): = t_{r1} = 7.28 min, t_{r2} = 13.23 min.

Following the general procedure b, the title compound was isolated using preprative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 44% (9.2 mg), *e.r.* = 99.9:0.1 (*S*) using BsGDH_L95V and yield = 57% (11.9 mg) *e.r.* = 99.9:0.1 (*S*) using OsGDH. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.63 (d, J = 7.6 Hz, 2H), 7.49 (d, J = 5.6 Hz, 2H), 7.42 (t, J = 7.2 Hz, 2H), 7.38 – 7.30 (m, 3H), 5.70 (s, 1H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, δ): 140.7, 131.9, 128.8, 128.8, 128.6, 128.4, 126.9, 122.5, 88.8, 86.8, 65.3. Spectroscopic data are in agreement with the reported values in the literature.[4]

*1-phenylprop-2-yn-1-ol (Table 2, Entry **2b**)*

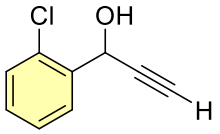
Following the general procedure for reduction, 1-phenylprop-2-yn-1-one **(1b)** (13 mg, 0.1 mmol), sodium borohydride (21.7 mg). Title compound was isolated by preparative TLC (30% EtOAc/*n*-hexane) as yellow oil, yield = 91% (12 mg). HPLC separation (racemic) (Chiralcel® - OD-H), *n*-hexane/*i*-PrOH 90:10, 1 mL/min, detection at 210 nM): = t_{r1} = 4.73 min, t_{r2} = 6.29 min.



Following the general procedure b, the title compound was isolated using preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 89% (11.8 mg) *e.r.* = 1:99 (*S*) using BsGDH_L95V and yield = 92% (12.2 mg) *e.r.* = 4:96 (*S*) using OsGDH. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.56 (d, J = 7.2 Hz, 2H), 7.42 – 7.33 (m, 3H), 5.48 (s, 1H), 2.68 (s, 1H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$, δ): 140.1, 128.8, 128.7, 126.7, 83.6, 75.0, 64.6. Spectroscopic data are in agreement with the reported values in the literature.[1]

*1-(2-chlorophenyl)prop-2-yn-1-ol (Table 2, Entry **2c**)*

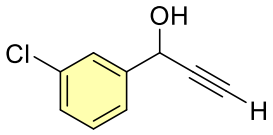
Following the general procedure for reduction, 1-(2-chlorophenyl)prop-2-yn-1-one **(1c)** (16.4 mg, 0.1 mmol), sodium borohydride (21.7 mg). Title compound was isolated by preparative TLC (30% EtOAc/*n*-hexane) as yellow oil, yield = 84% (14 mg). HPLC separation (racemic) (Chiralcel® - OD-H), *n*-hexane/*i*-PrOH 90:10, 1 mL/min, detection at 220 nM): = t_{r1} = 4.38 min, t_{r2} = 4.93 min.



Following the general procedure b, the title compound was isolated using preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 93% (15.5 mg), *e.r.* = 1:99 (*S*) using BsGDH_L95V and yield = 96% (16 mg), *e.r.* = 1:99 (*S*) using OsGDH. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.80 (d, J = 7.2 Hz, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.33 (p, J = 8.0, 7.2 Hz, 2H), 5.85 (s, 1H), 2.68 (s, 1H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$, δ): 137.4, 132.8, 129.9, 129.8, 128.4, 127.4, 82.4, 75.0, 61.8. Spectroscopic data are in agreement with the reported values in the literature.[5]

*1-(3-chlorophenyl)prop-2-yn-1-ol (Table 2, Entry **2d**)*

Following the general procedure for reduction, 1-(3-chlorophenyl)prop-2-yn-1-one **(1d)** (16.4 mg, 0.1 mmol), sodium borohydride (21.7 mg). Title compound was isolated by preparative TLC (30% EtOAc/*n*-hexane) as yellow oil, yield = 87% (14.4 mg). HPLC separation (racemic) (Chiralcel® - OD-H), *n*-hexane/*i*-PrOH 90:10, 1 mL/min, detection at 220 nM): = t_{r1} = 4.50 min, t_{r2} = 4.88 min.

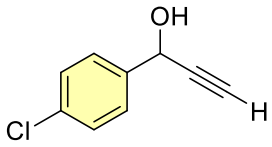


Following the general procedure b, the title compound was isolated using preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 91% (15.2 mg), *e.r.* = 1:99 (*S*) using BsGDH_L95V and yield = 92% (15.3 mg), *e.r.* = 1:99 (*S*) using OsGDH. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.56 (s, 1H), 7.46 – 7.40 (m, 1H), 7.34 – 7.29 (m, 2H), 5.44 (s, 1H), 2.69 (s, 1H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$, δ): 141.9,

134.6, 130.1, 128.8, 126.9, 124.9, 82.9, 75.5, 63.8. Spectroscopic data are in agreement with the reported values in the literature.[6]

*1-(4-chlorophenyl)prop-2-yn-1-ol (Table 2, Entry **2e**)*

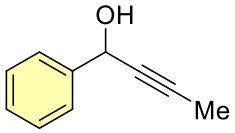
Following the general procedure for reduction, 1-(4-chlorophenyl)prop-2-yn-1-one **(1e)** (16.4 mg, 0.1 mmol), sodium borohydride (21.7 mg). Title compound was isolated by preparative TLC (30% EtOAc/*n*-hexane) as yellow oil, yield = 81% (13.4 mg). HPLC separation (racemic) (Chiralcel® - OD-H), *n*-hexane/*i*-PrOH 98:02, 1 mL/min, detection at 220 nM): = t_{r1} = 11.16 min, t_{r2} = 11.74 min.



Following the general procedure b, the title compound was isolated using preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 95% (15.8 mg), 1:99 (*S*) using BsGDH_L95V and yield = 91% (15.1 mg), 6:94 (*S*) using OsGDH. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.43 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 5.39 (s, 1H), 2.63 (s, 1H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$, δ): 138.5, 134.5, 128.9, 128.1, 83.2, 75.3, 63.8. Spectroscopic data are in agreement with the reported values in the literature.[1]

*1-phenylbut-2-yn-1-ol (Table 2, Entry **2f**)*

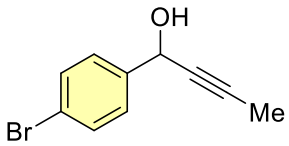
Following the general procedure for reduction, 1-phenylbut-2-yn-1-one **(1f)** (14.4 mg, 0.1 mmol), sodium borohydride (21.7 mg). Title compound was isolated by preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 88% (12.8 mg). HPLC separation (racemic) (Chiralcel® - OD-H), *n*-hexane/*i*-PrOH 90:10, 1 mL/min, detection at 210 nM): = t_{r1} = 4.32 min, t_{r2} = 6.25 min.



Following the general procedure b, the title compound was isolated using preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 53% (7.8 mg), *e.r.* = 13:87 (*S*) using BsGDH_L95V and yield = 69% (10.1 mg), *e.r.* = 57:43 (*R*) using OsGDH. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.54 (d, J = 7.6 Hz, 2H), 7.38 (t, J = 7.2 Hz, 2H), 7.35 – 7.30 (m, 1H), 5.44 (s, 1H), 1.92 (s, 3H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$, δ): 141.3, 128.7, 128.4, 126.7, 83.3, 79.2, 64.9, 3.9. Spectroscopic data are in agreement with the reported values in the literature.[1]

*1-(4-bromophenyl)but-2-yn-1-ol (Table 2, Entry **2g**)*

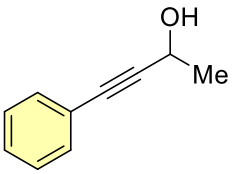
Following the general procedure for reduction, 1-(4-bromophenyl)but-2-yn-1-one **(1g)** (22.3 mg, 0.1 mmol), sodium borohydride (21.7 mg). Title compound was isolated by preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 87% (19.6 mg). HPLC separation (racemic) (Chiralcel® - OD-H), *n*-hexane/*i*-PrOH 90:10, 1 mL/min, detection at 220 nM): = t_{r1} = 4.35 min, t_{r2} = 5.084 min.



Following the general procedure b, the title compound was isolated using preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 42% (9.5 mg), *e.r.* = 11:89 (*S*) using BsGDH_L95V and yield = 61% (13.8 mg), *e.r.* = 73:27 (*R*) using OsGDH. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.49 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 8.0 Hz, 2H), 5.38 (s, 1H), 1.90 (s, 3H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$, δ): 140.3, 131.7, 128.4, 122.3, 83.7, 78.8, 64.3, 3.8. Spectroscopic data are in agreement with the reported values in the literature.[7]

*4-phenylbut-3-yn-2-ol (Table 2, Entry **2h**)*

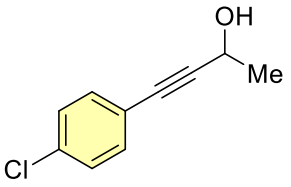
Purchased from Combi Blocks and used as such. HPLC separation (racemic) (Chiralcel® - OD-H), *n*-hexane/*i*-PrOH 90:10, 1 mL/min, detection at 220 nM): = t_{r1} = 4.06 min, t_{r2} = 9.40 min.



Following the general procedure b, the title compound was isolated using preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 58% (8.3 mg) using BsGDH_L95V and yield = 93% (13.6 mg) using OsGDH. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.46 – 7.39 (m, 2H), 7.30 (dd, J = 5.2, 2.0 Hz, 3H), 4.76 (q, J = 6.4 Hz, 1H), 1.56 (d, J = 6.4 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$, δ): 131.8, 128.5, 128.4, 122.7, 91.1, 84.1, 58.9, 24.5. Spectroscopic data are in agreement with the reported values in the literature.[7]

*4-(4-chlorophenyl)but-3-yn-2-ol (Table 2, Entry **2i**)*

Following the general procedure for reduction, 4-(4-chlorophenyl)but-3-yn-2-one **(1i)** (17.8 mg, 0.1 mmol), sodium borohydride (21.7 mg). Title compound was isolated by preparative TLC (30% EtOAc/*n*-hexane) as light yellow oil, yield = 83% (15 mg). HPLC separation (racemic) (Chiralcel® - OD-H), *n*-hexane/*i*-PrOH 90:10, 1 mL/min, detection at 254 nM): = t_{r1} = 3.32 min, t_{r2} = 3.92 min.

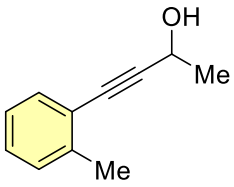


Following the general procedure b, the title compound was isolated by preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 54% (9.8 mg), *e.r.* = 24:76 (*S*) using BsGDH_L95V and yield = 94% (17 mg), *e.r.* = 84:16 (*R*) using OsGDH. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.36 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 4.76 (q, J = 6.4 Hz, 1H), 1.56 (d, J = 6.4 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$, δ): 134.4, 132.9, 128.6, 121.1, 91.9, 82.9, 58.8, 24.3. Spectroscopic data are in agreement with the reported values in the literature.[8]

*4-(o-tolyl)but-3-yn-2-ol (Table 2, Entry **2j**)*

Following the general procedure for reduction, 4-(*o*-tolyl)but-3-yn-2-one **(1j)** (15.8 mg, 0.1 mmol), sodium borohydride (21.7 mg). Title compound was isolated by preparative TLC (30% EtOAc/*n*-hexane)

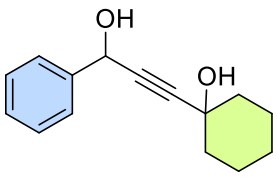
as light yellow oil, yield = 79% (12.6 mg). HPLC separation (racemic) (Chiralcel® - OD-H), *n*-hexane/*i*-PrOH 90:10, 1 mL/min, detection at 210 nM): = t_{r1} = 3.84 min, t_{r2} = 6.09 min.



Following the general procedure b, the title compound was isolated using preparative TLC (30% EtOAc/*n*-hexane) as colorless oil, yield = 77% (12.3 mg), *e.r.* = 13:87 (*S*) using BsGDH_L95V and yield = 93% (14.9 mg) *e.r.* = 72:28 (*R*) using OsGDH. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.39 (d, J = 7.6 Hz, 1H), 7.24 – 7.18 (m, 2H), 7.13 (t, J = 6.8 Hz, 1H), 4.80 (q, J = 6.4 Hz, 1H), 2.43 (s, 3H), 1.58 (d, J = 6.4 Hz, 3H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, δ): 140.3, 132.1, 129.5, 128.5, 125.6, 122.4, 95.1, 82.9, 59.1, 24.7, 20.7. Spectroscopic data are in agreement with the reported values in the literature. [9]

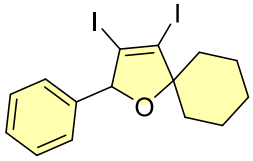
*1-(3-hydroxy-3-phenylprop-1-yn-1-yl)cyclohexan-1-ol (Scheme 3, Entry **3a**)*

To a stirred solution of the **2b** (2 mmol, 264 mg) in THF, was added ethylmagnesium bromide (Et-Mg-Br) (1.0M in THF, 2.0 equivs.) at room temperature. The resulting solution was reflux for 1 h at 80 °C. Then cyclohexanone (2 mmol, 1.0 equiv.) in THF (0.35M) was added slowly by syringe to the resulting solution at room temperature and stirred for 3 h. The reaction mixture was quenched by addition of saturated aqueous ammonium chloride (40 mL) and extracted with ethyl ether (2 x 40 mL). The combined organic layers were washed with brine, dried over Na2SO4, and concentrated under reduced pressure. The crude material was purified by column chromatography. Isolated as light yellow oil, yield = 79% (375 mg). HPLC separation (racemic) (Chiralcel® - OD-H 250 mm then OJ-H 250 mm), *n*-hexane/*i*-PrOH 80:20, 1 mL/min, detection at 210 nM): = t_{r1} = 8.94 min, t_{r2} = 9.93 min. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.53 (d, J = 7.2 Hz, 2H), 7.39 – 7.29 (m, 3H), 5.48 (s, 1H), 2.83 (s, 1H), 2.53 (s, 1H), 1.95 – 1.91 (m, 2H), 1.72 – 1.65 (m, 2H), 1.623 – 1.48 (m, 5H), 1.25 (t, J = 7.2 Hz, 1H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, δ): 140.8, 128.7, 128.4, 126.8, 90.5, 84.1, 68.8, 64.6, 39.9, 39.9, 25.2, 23.4. Spectroscopic data are in agreement with the reported values in the literature.[10]



*3,4-diiodo-2-phenyl-1-oxaspiro[4.5]dec-3-ene (Scheme 3, Entry **4a**)*

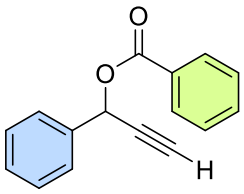
To a solution of **3a** (0.30 mmol, 70 mg) in wet CH_2Cl_2 (3.0 mL) was added 152.4 mg (0.6 mmol, 2equiv) of I_2 at room temperature for 4 hours. After 4 h, the reaction mixture was diluted with ethyl ether (40 mL), washed with water, saturated brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by chromatography on silica gel to afford corresponding product **4a**. Isolated as white solid, yield = 92% (129 mg). HPLC separation (racemic) (Chiralcel® - OD-H 250 mm then OJ-H 250 mm), gradient method *n*-hexane/*i*-PrOH 80:20, 0.5 mL/min from 0 min to 20 min, detection at 210 nM): = t_{r1} = 15.80 min, t_{r2} =



17.12 min. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.39 – 7.31 (m, 5H), 5.56 (s, 1H), 2.01 - 1.93 (m, 1H), 1.79 - 1.64 (m, 8H), 1.26 – 1.14 (m, 1H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$, δ): 139.6, 128.8, 128.5, 128.0, 115.8, 107.3, 93.3, 92.1, 37.0, 34.3, 24.9, 22.3, 21.9. Spectroscopic data are in agreement with the reported values in the literature.[10]

*1-phenylprop-2-yn-1-yl benzoate (Scheme 3, Entry **4a**)*

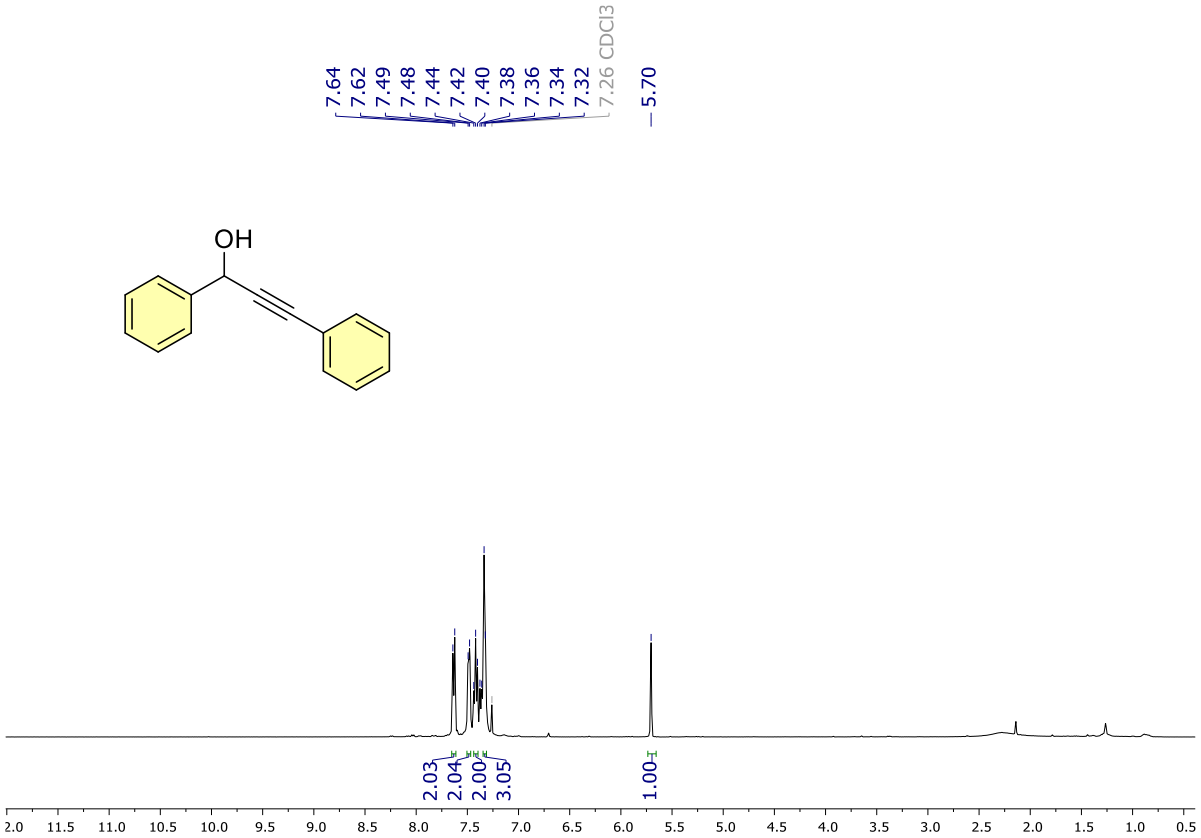
Phenyl propargyl alcohol, **2b** (264 mg, 2 mmol) was dissolved in CH_2Cl_2 (4 mL). DMAP (12 mg, 0.10 mmol), Et_3N (0.60 mL, 4.0 mmol) and benzoyl chloride (0.36 mL, 2.2 mmol) were sequentially added. The resulting mixture was stirred for 2 h. The solvent was evaporated and the residue was purified by column chromatography on silica gel affording the desired product **4a** (420 mg, 89%). HPLC separation (racemic) (Chiralcel® - OD-H 250 mm then OJ-H 250 mm), *n*-hexane/*i*-PrOH 80:20, 1 mL/min, detection at 210 nM): = t_{r1} = 14.05 min, t_{r2} = 16.92 min. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.10 – 8.08 (m, 2H), 7.65 – 7.62 (m, 2H), 7.59 – 7.55 (m, 1H), 7.46 – 7.39 (m, 5H), 6.72 (d, J = 2.0 Hz, 1H), 2.71 (d, J = 2.4 Hz, 1H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$, δ): 165.5, 136.7, 133.5, 130.0, 129.7, 128.9, 128.5, 127.8, 80.4, 75.8, 65.9. Spectroscopic data are in agreement with the reported values in the literature.[11]



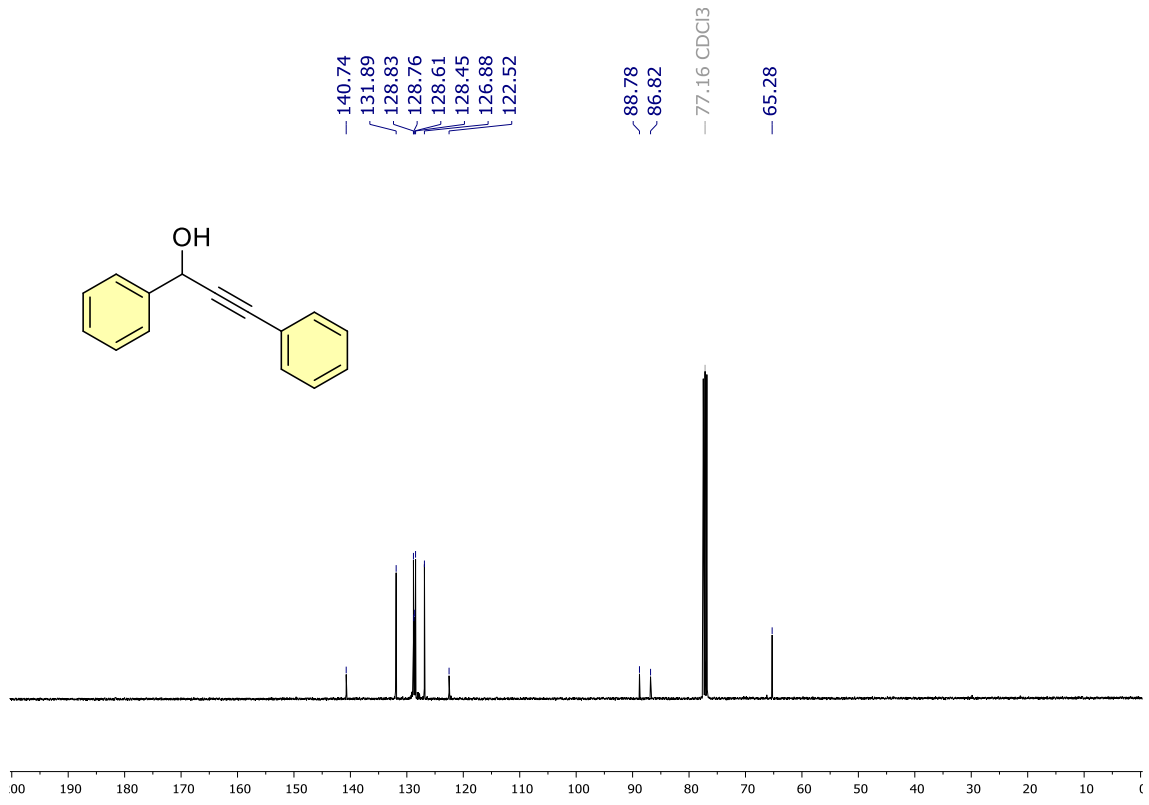
13. ^{1}H and ^{13}C Spectral data

*1,3-diphenylprop-2-yn-1-ol (Table 3, Entry **2a**)*

^{1}H NMR (400 MHz)

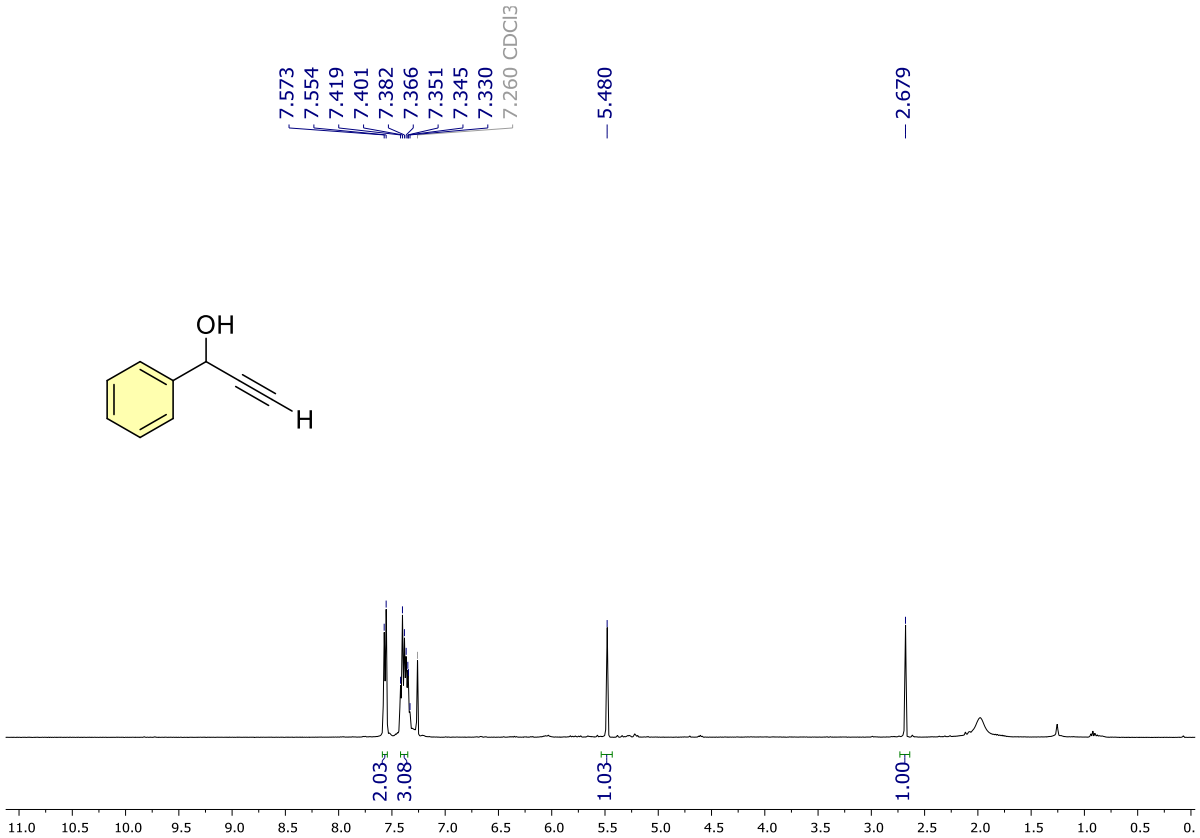


^{13}C (100 MHz)

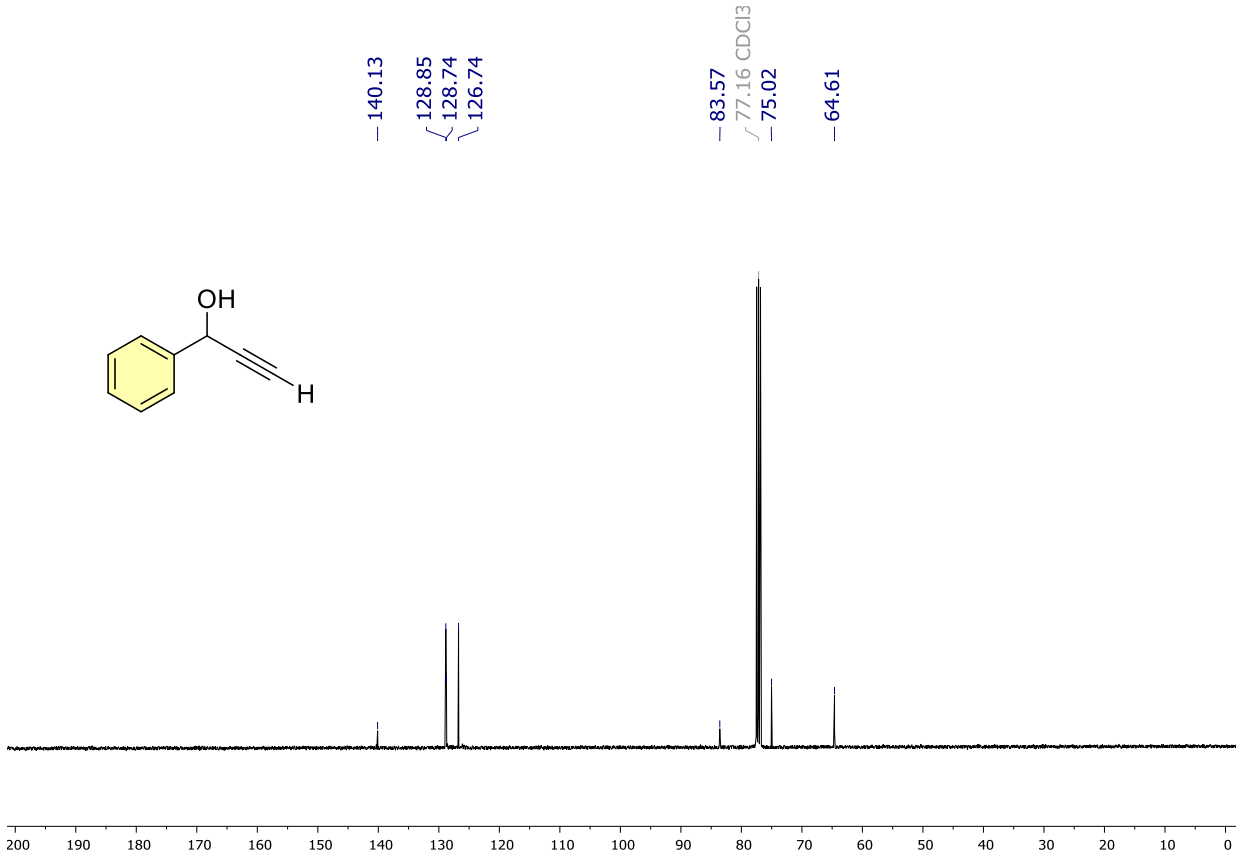


*1-phenylprop-2-yn-1-ol (Table 3, Entry **2b**)*

^{1}H NMR (400 MHz)

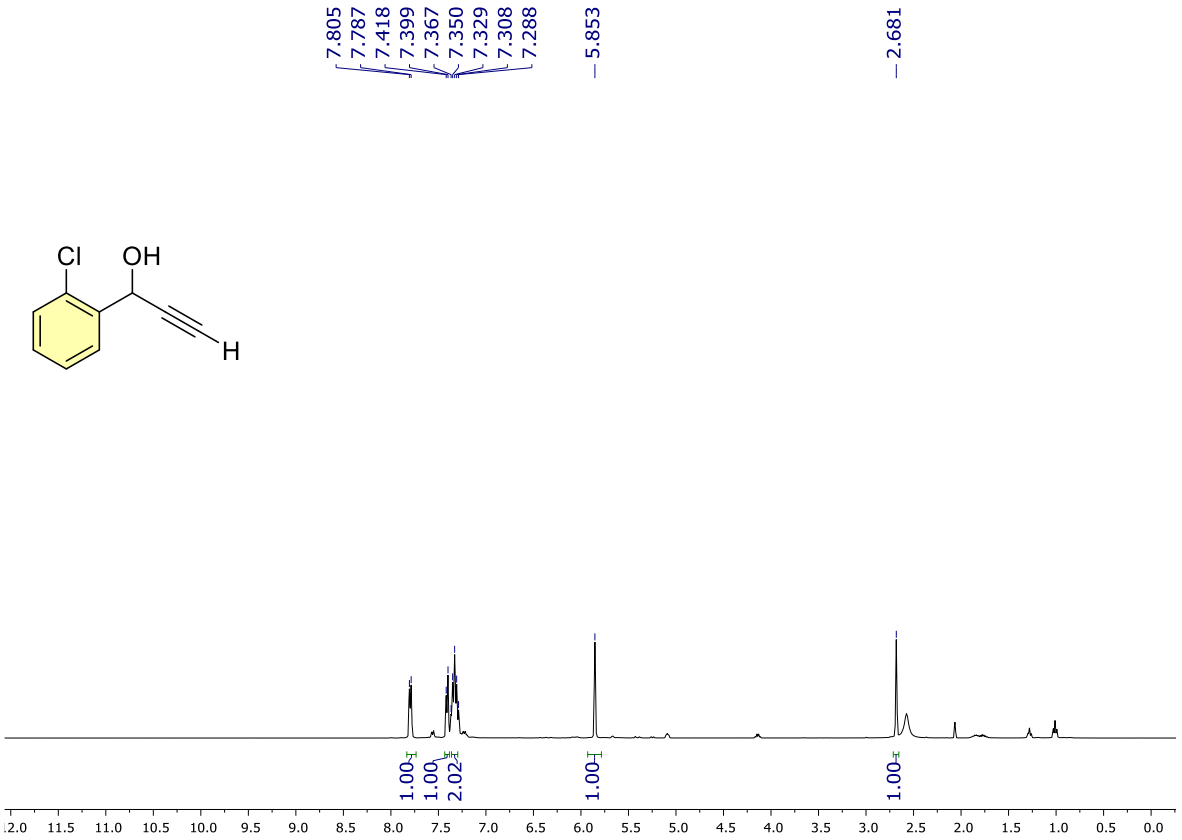


^{13}C (100 MHz)

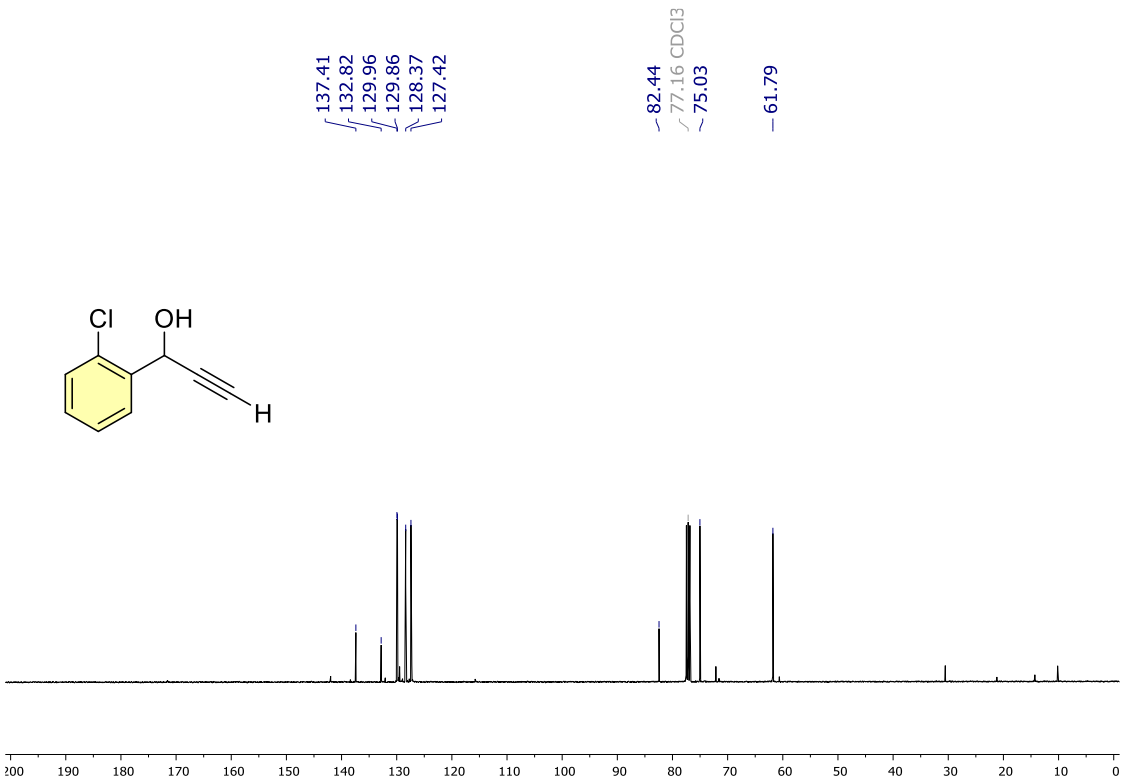


*1-(2-chlorophenyl)prop-2-yn-1-ol (Table 3, Entry **2c**)*

^{1}H NMR (400 MHz)

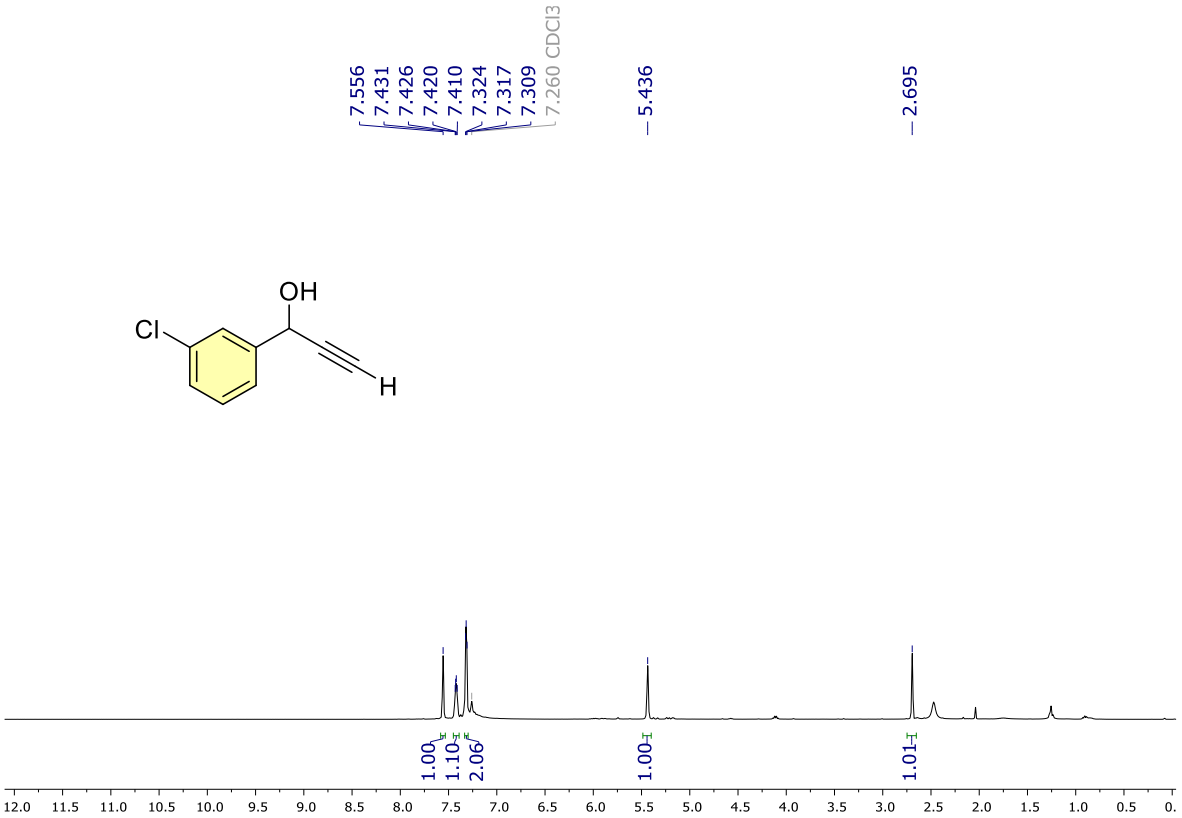


^{13}C (100 MHz)

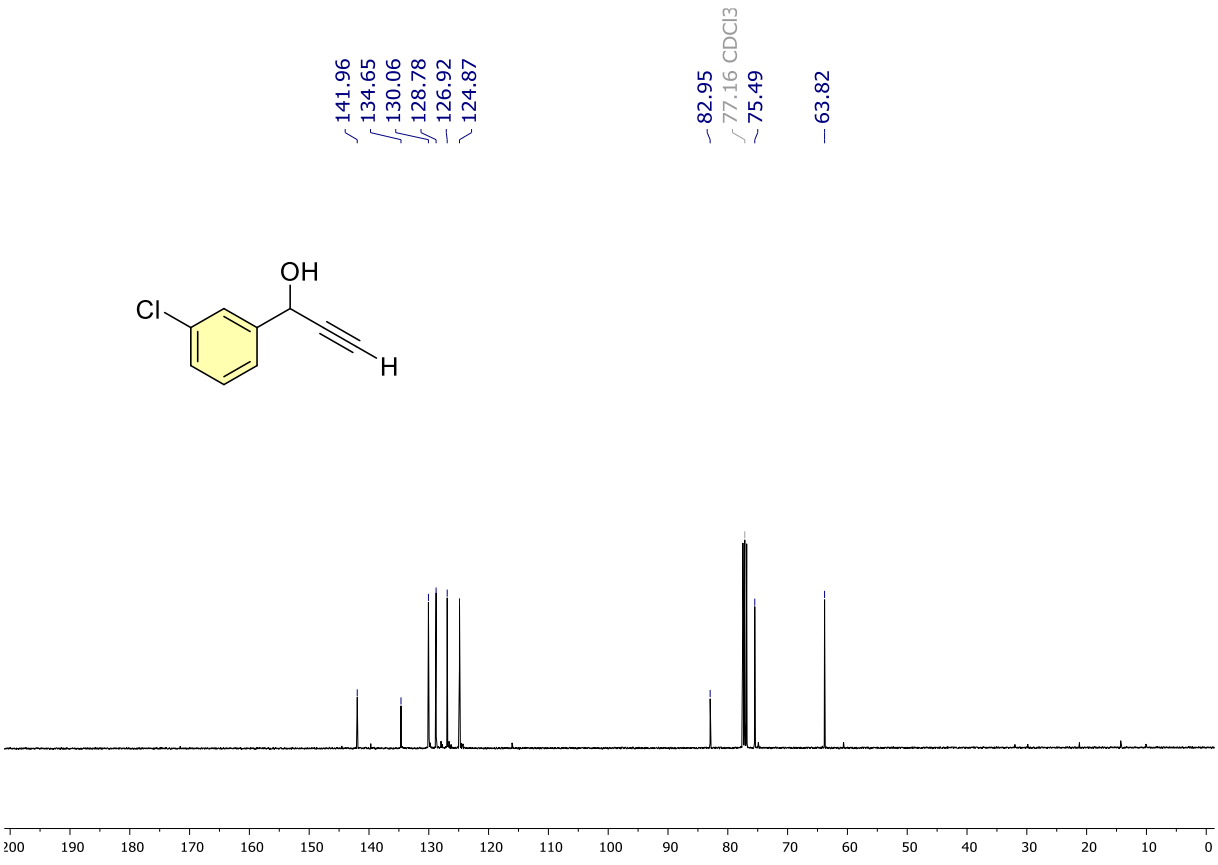


*1-(3-chlorophenyl)prop-2-yn-1-ol (Table 3, Entry **2d**)*

^{1}H NMR (400 MHz)

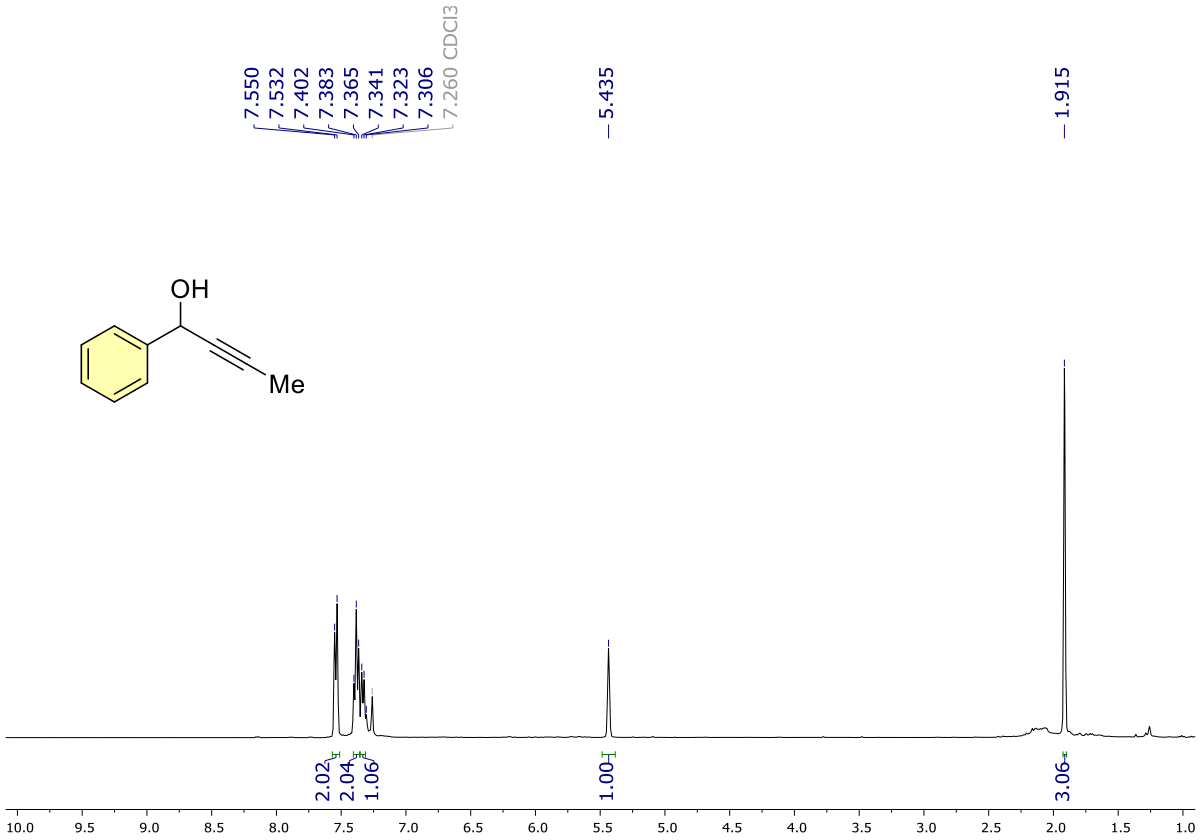


^{13}C (100 MHz)

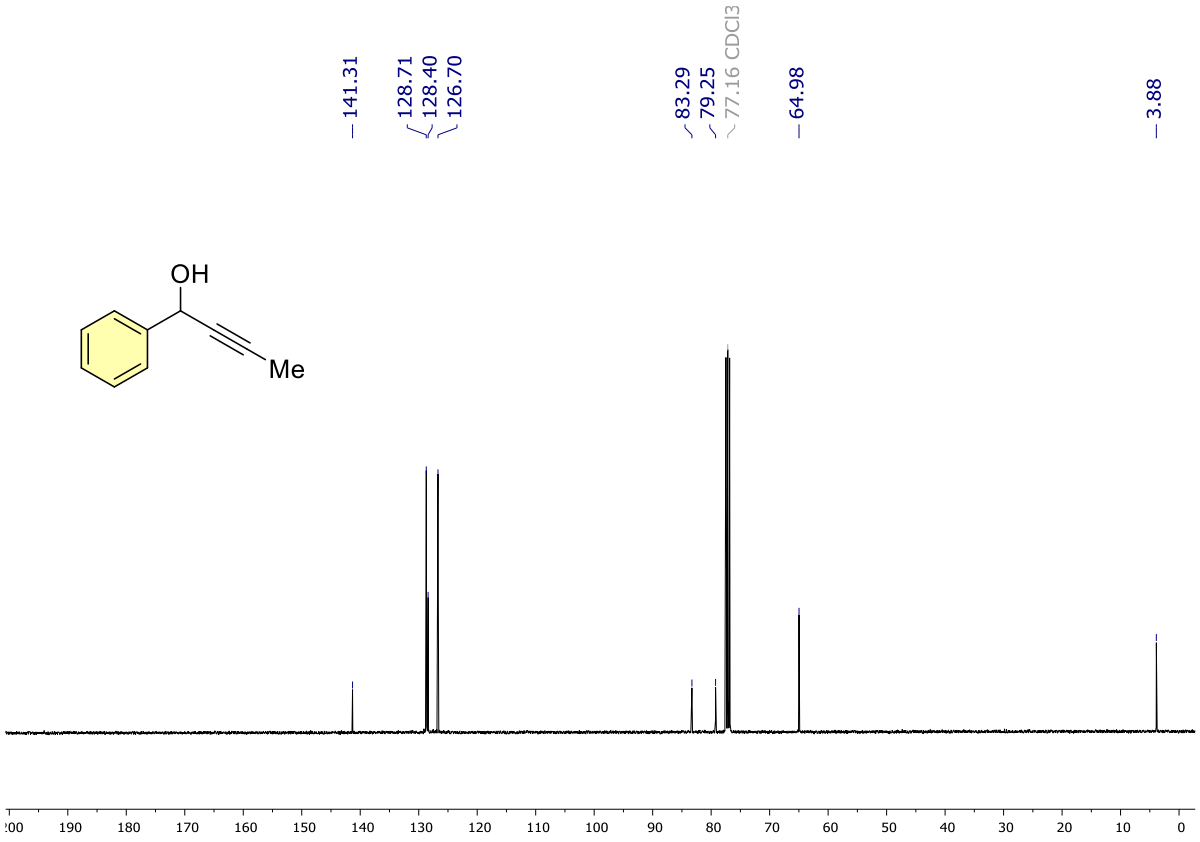


*1-phenylbut-2-yn-1-ol (Table 2, Entry **2f**)*

^{1}H NMR (400 MHz)

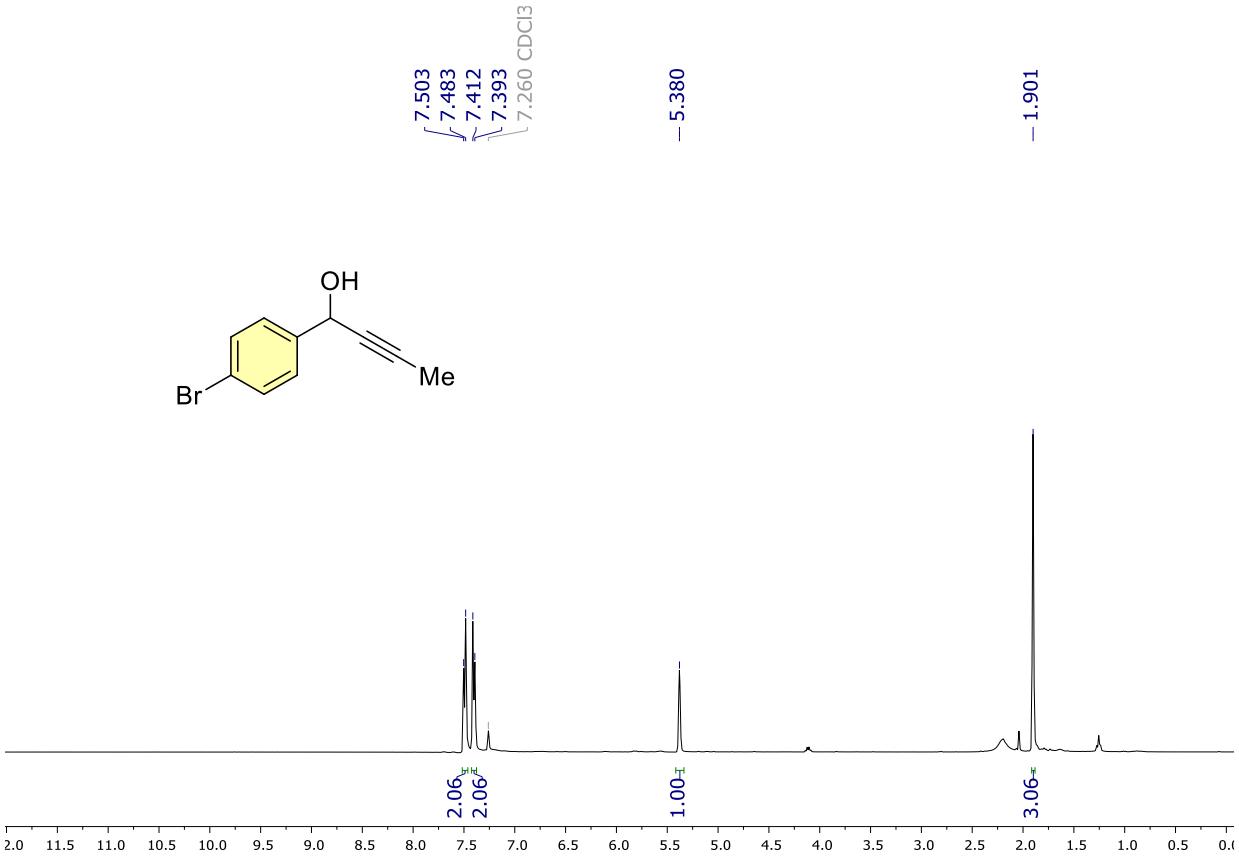


^{13}C (100 MHz)

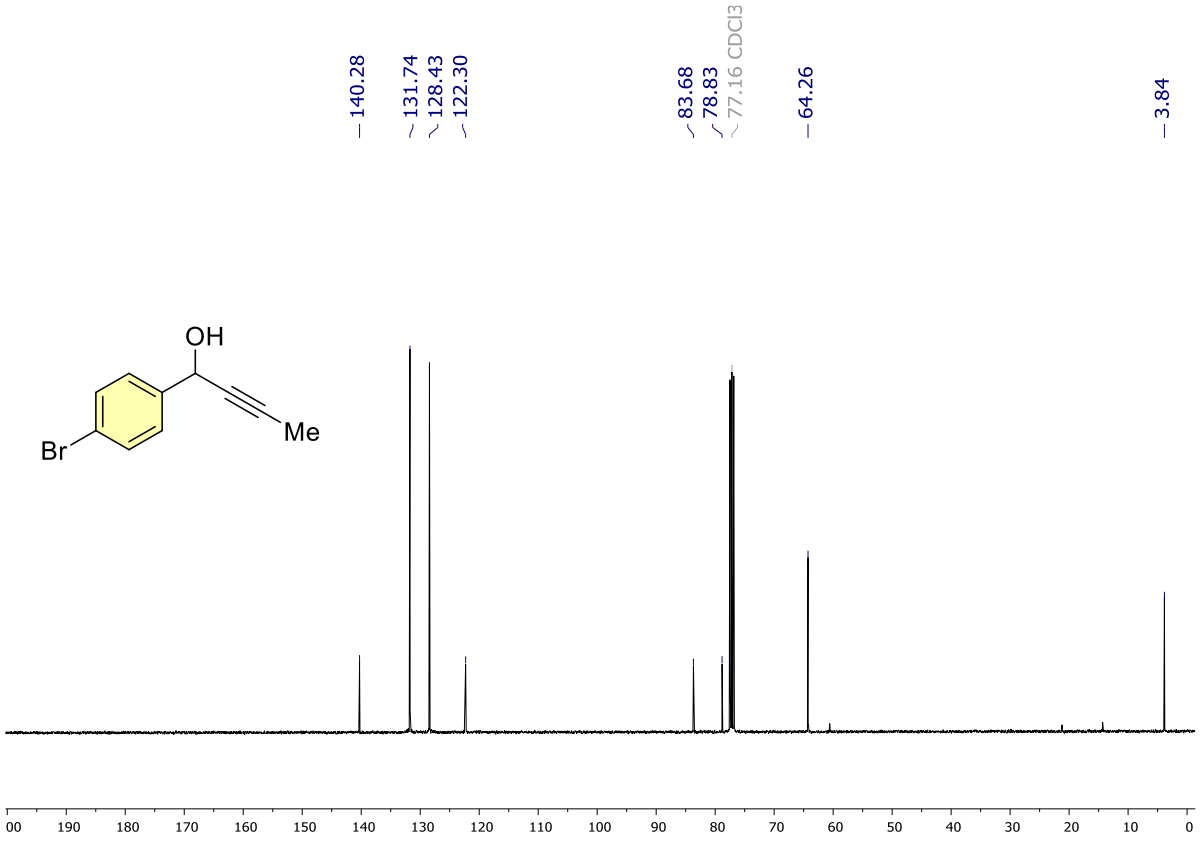


*1-(4-bromophenyl)but-2-yn-1-ol (Table 2, Entry **2g**)*

^{1}H NMR (400 MHz)

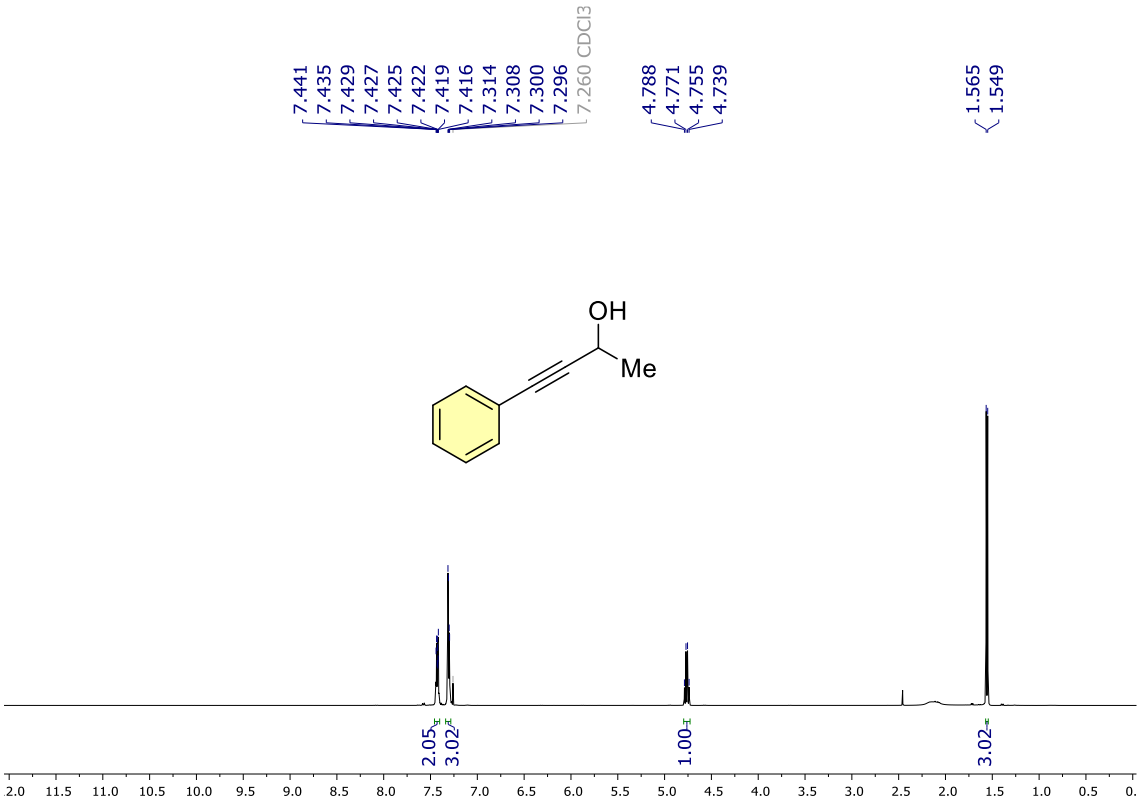


^{13}C (100 MHz)

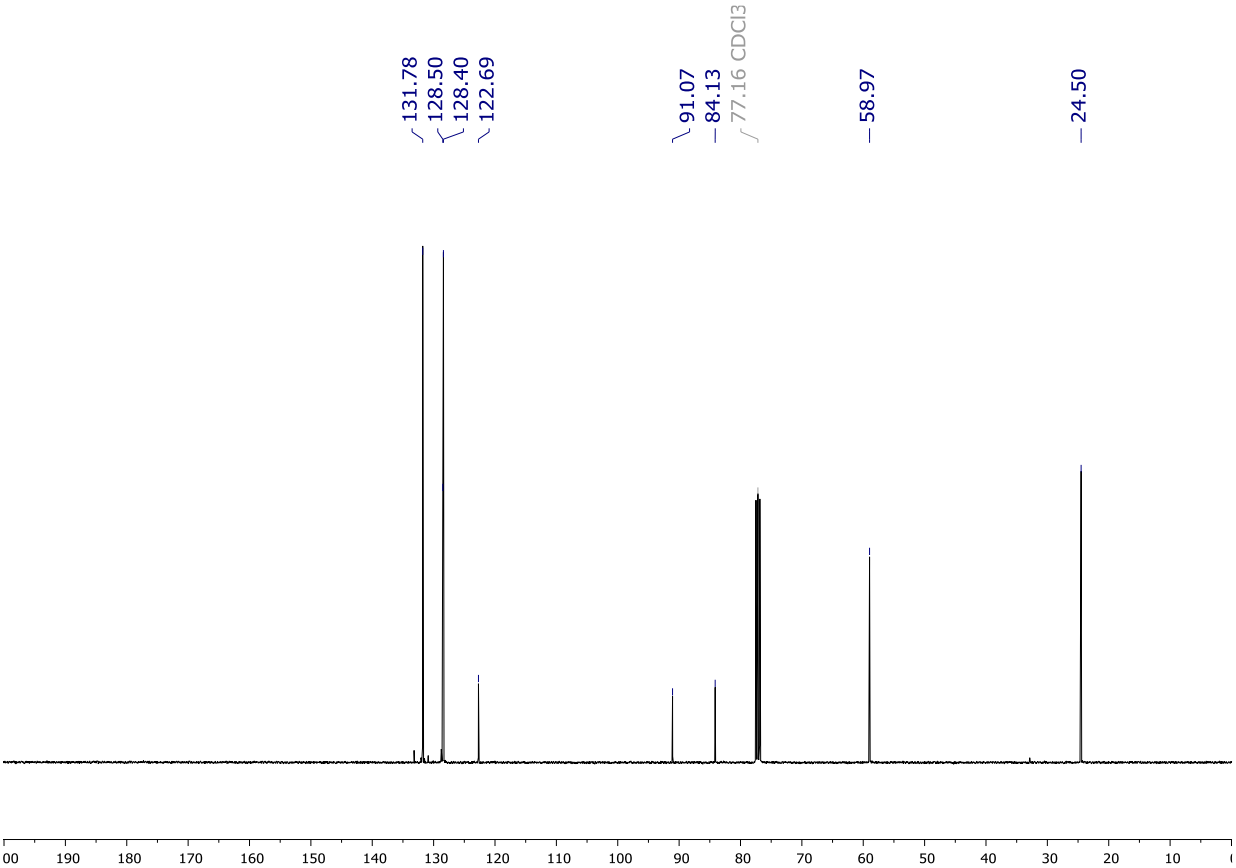


*4-phenylbut-3-yn-2-ol (Table 2, Entry **2h**)*

^{1}H NMR (400 MHz)

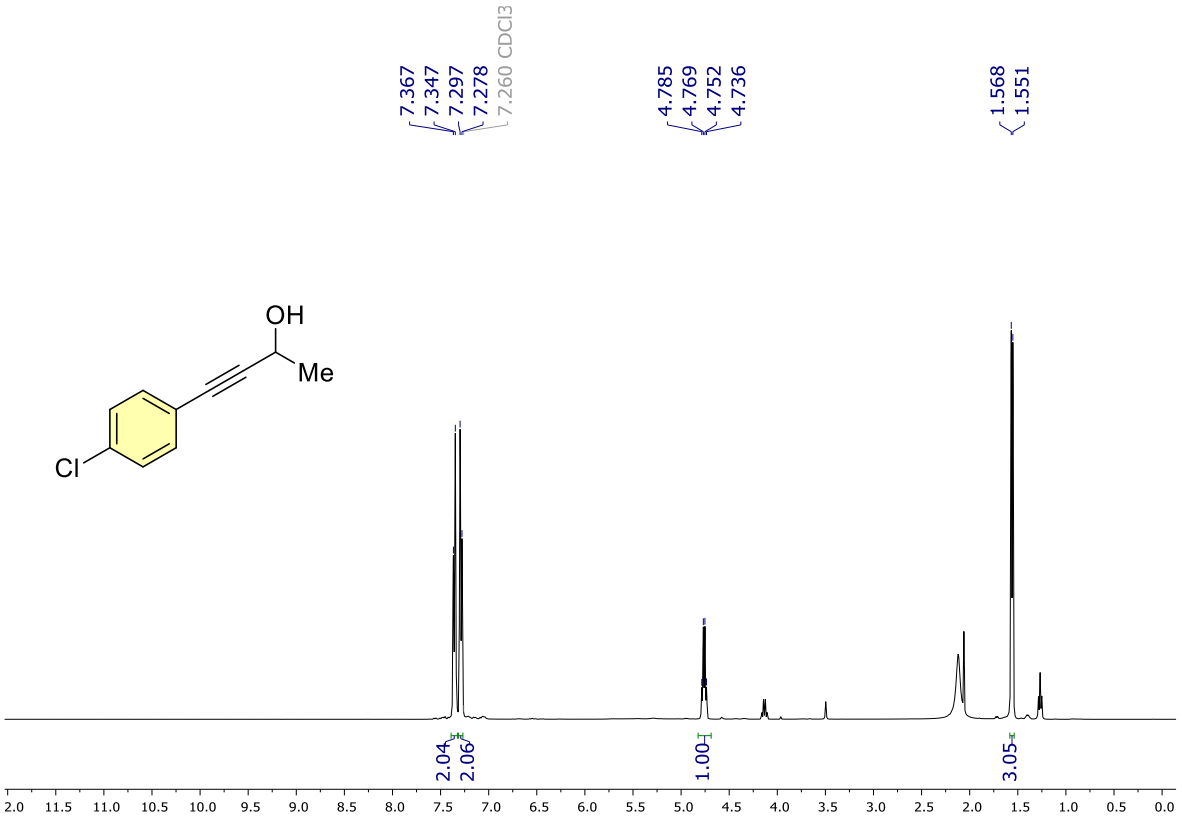


^{13}C (100 MHz)

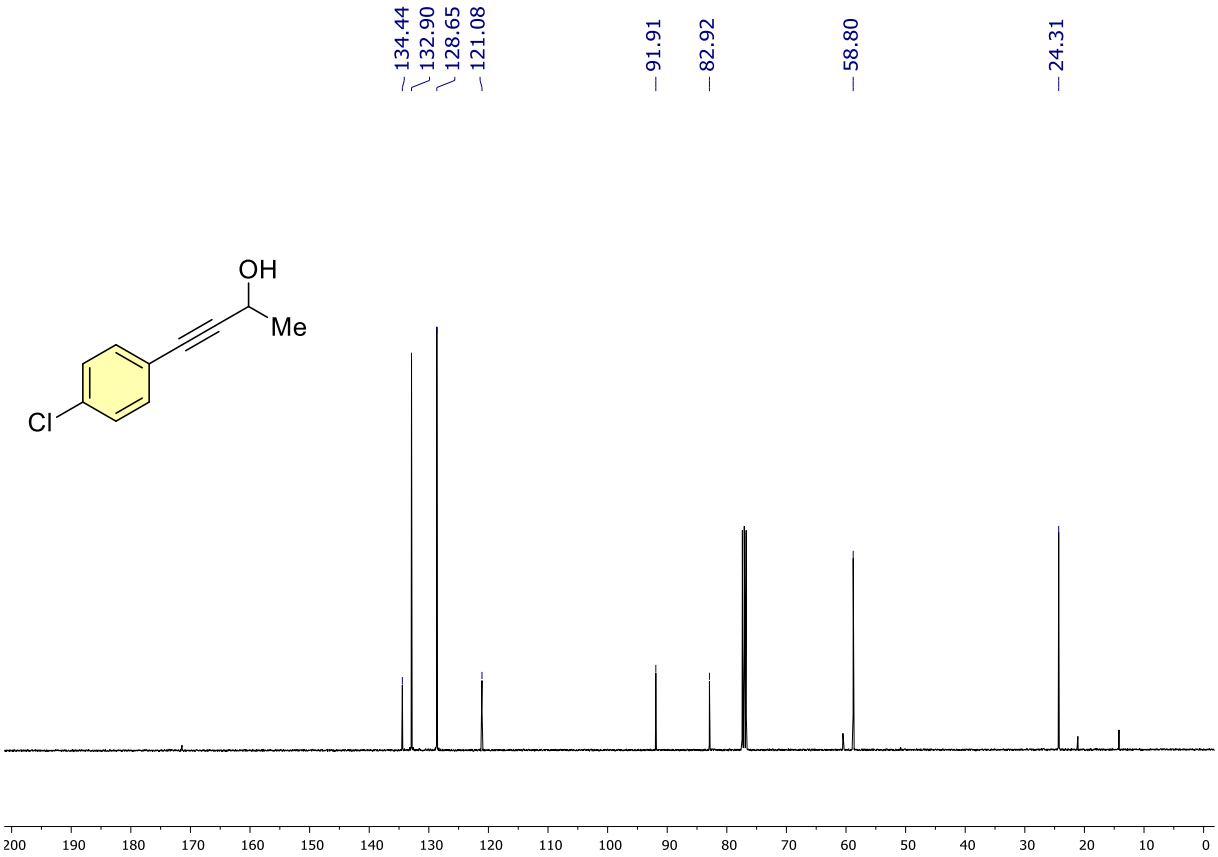


*4-(4-chlorophenyl)but-3-yn-2-ol (Table 2, Entry **2i**)*

^{1}H NMR (400 MHz)

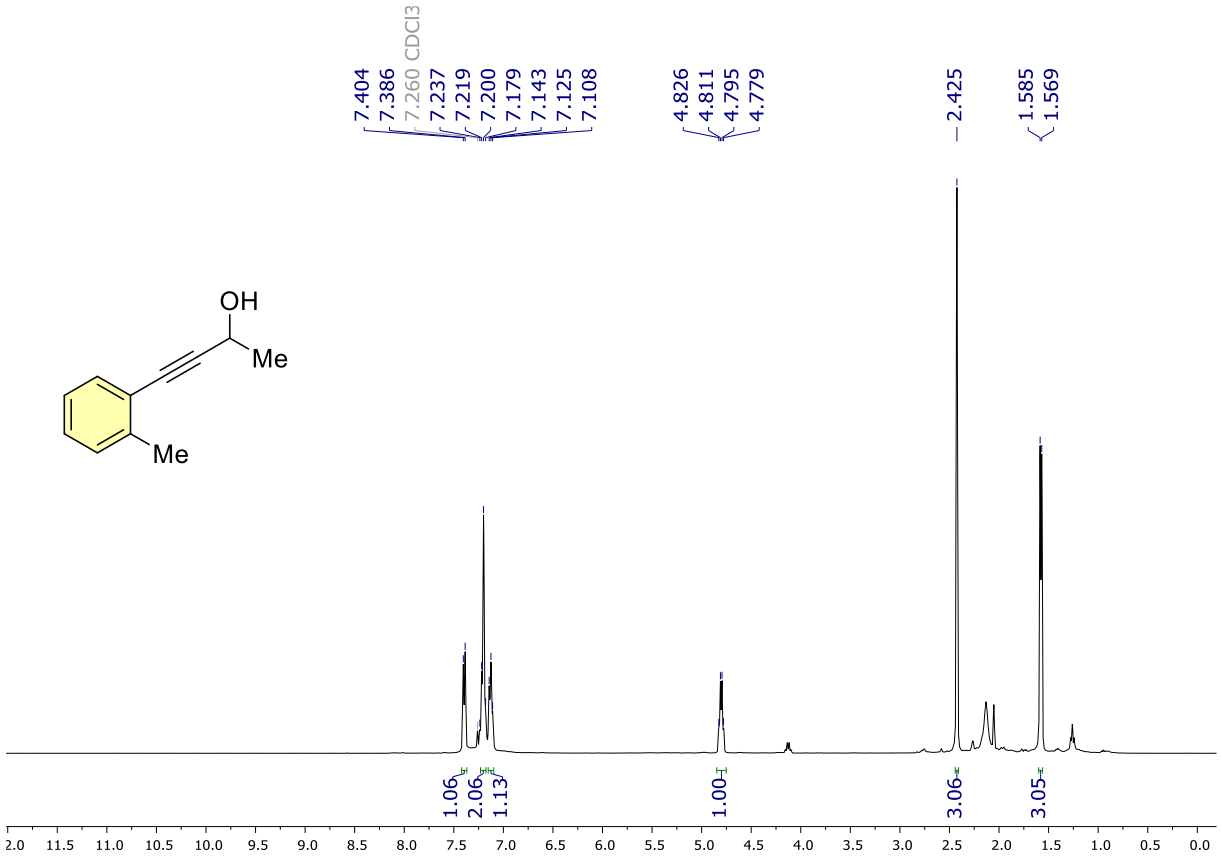


^{13}C (100 MHz)

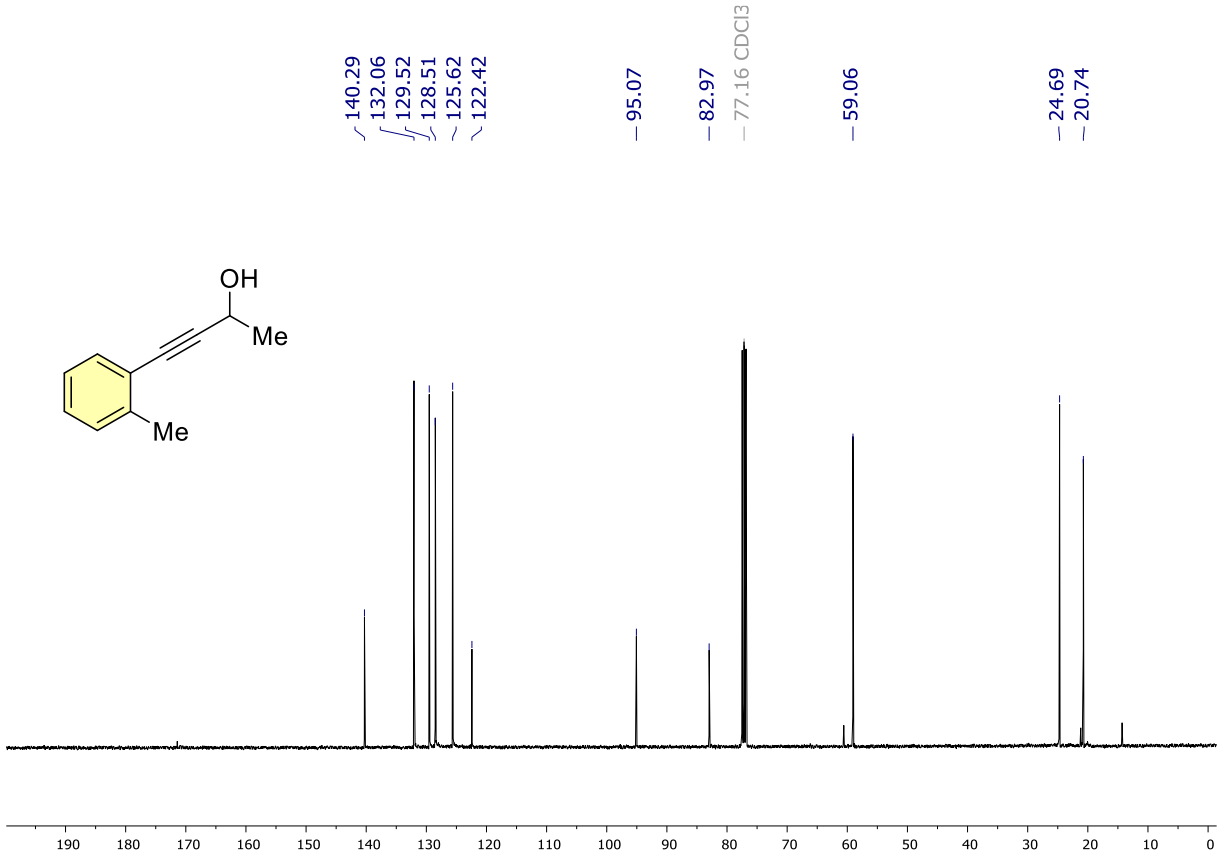


*4-(o-tolyl)but-3-yn-2-ol (Table 2, Entry **2j**)*

^{1}H NMR (400 MHz)

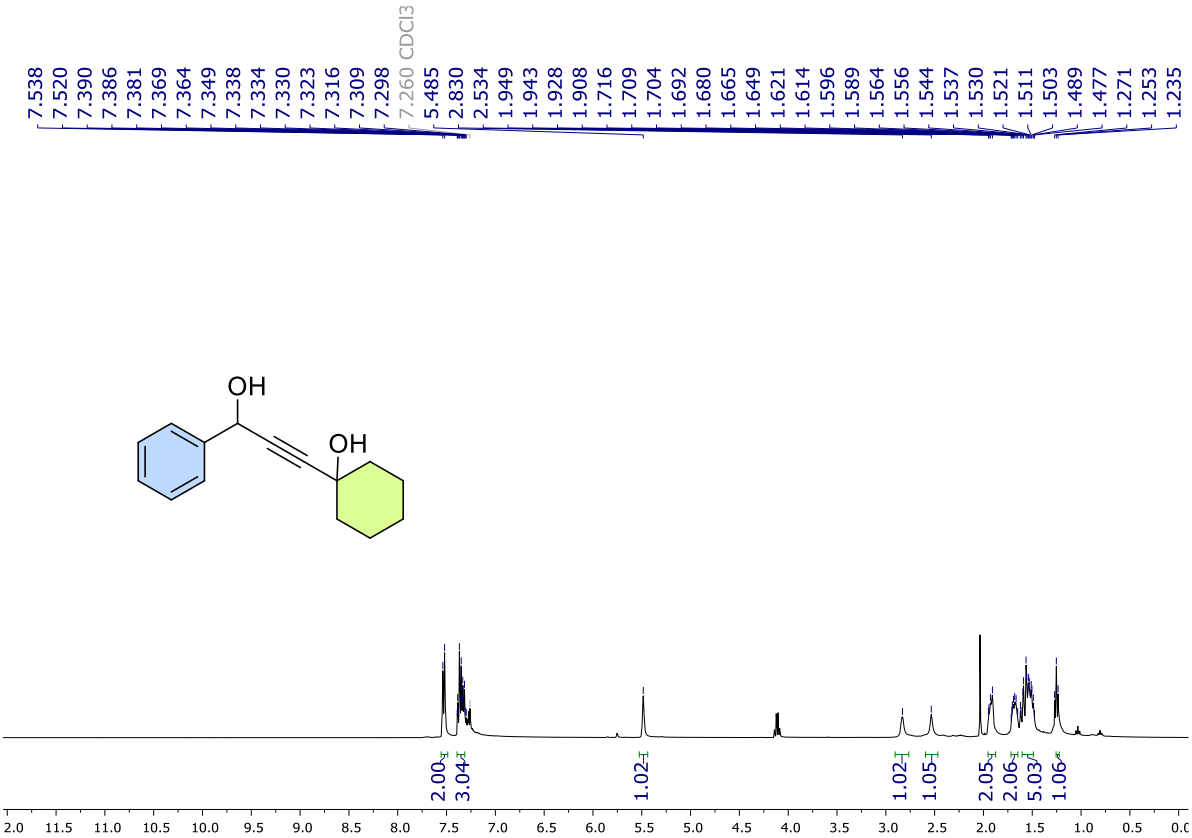


^{13}C (100 MHz)

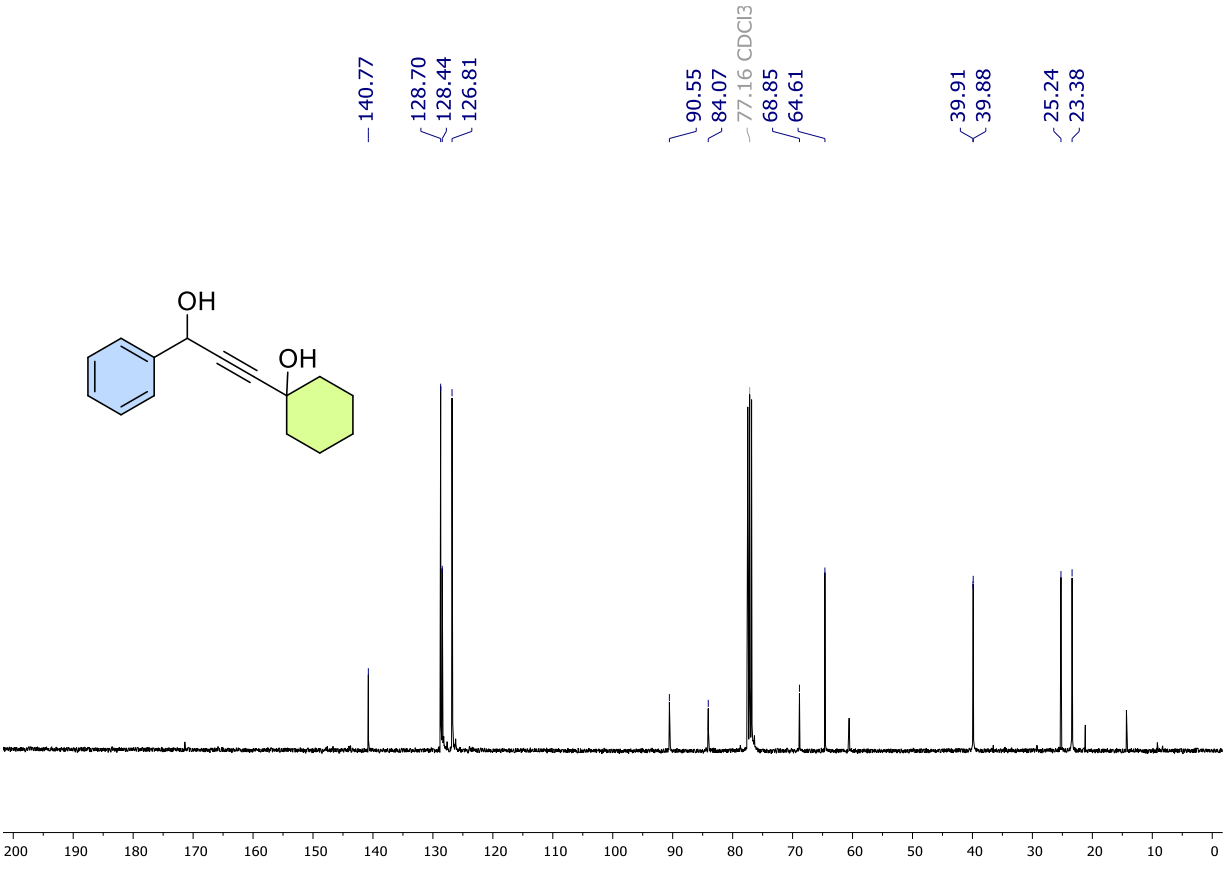


*1-(3-hydroxy-3-phenylprop-1-yn-1-yl)cyclohexan-1-ol (Scheme 3, Entry **3a**)*

^{1}H NMR (400 MHz)

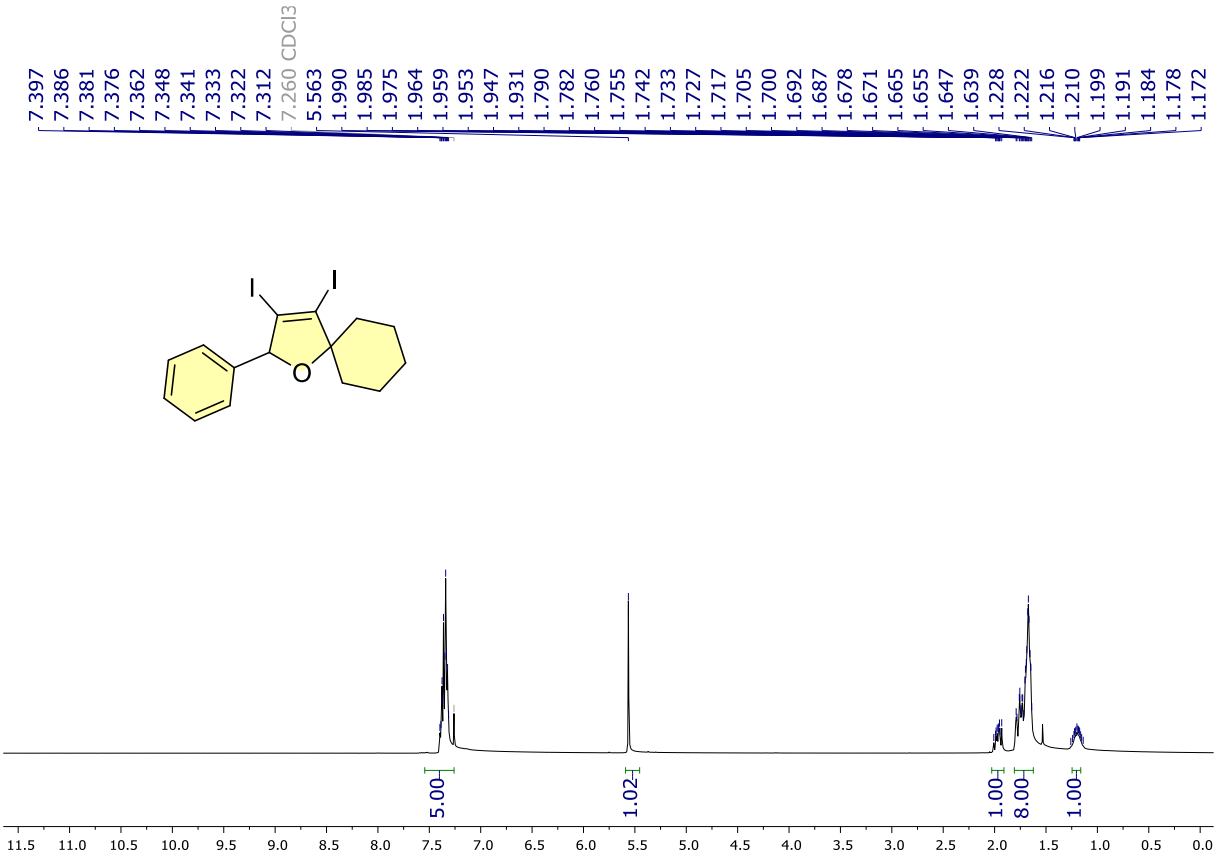


^{13}C (100 MHz)

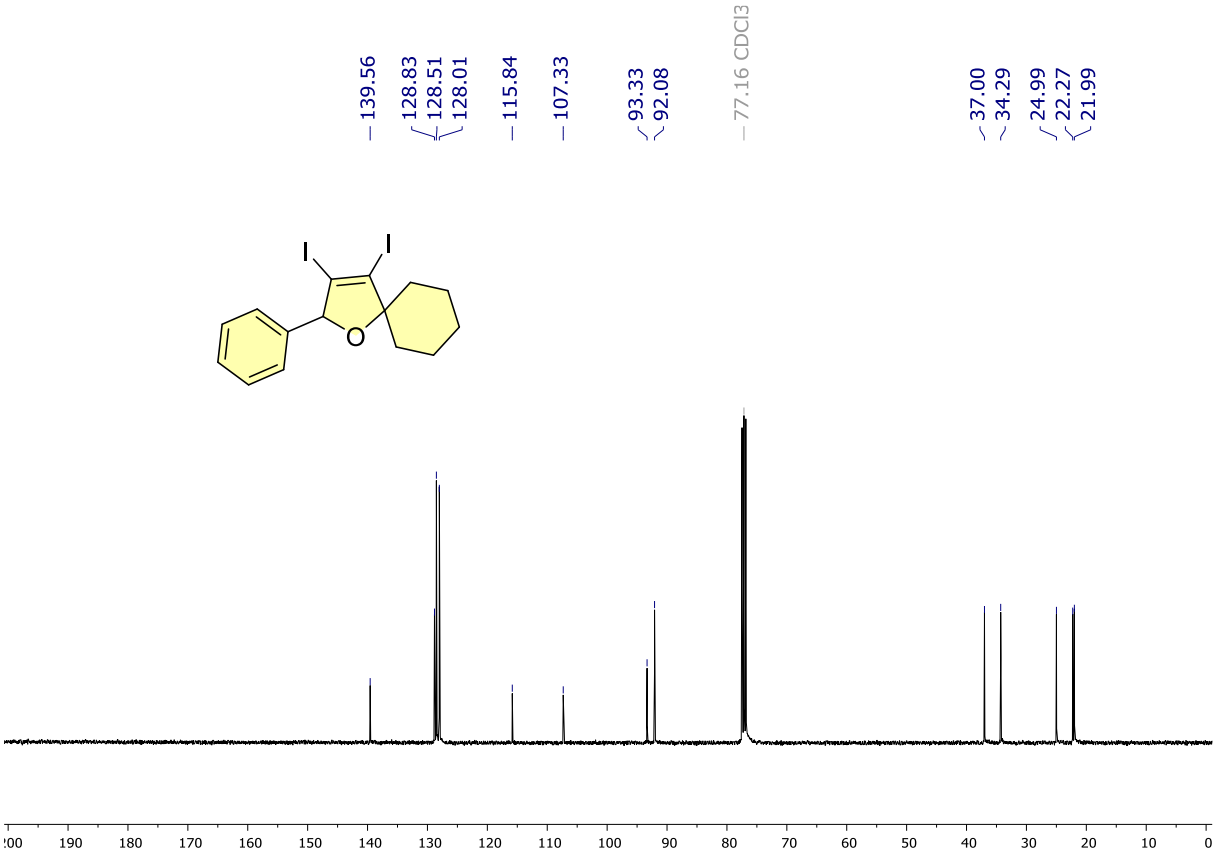


*3,4-diiodo-2-phenyl-1-oxaspiro[4.5]dec-3-ene (Scheme 3, Entry **4a**)*

^{1}H NMR (400 MHz)

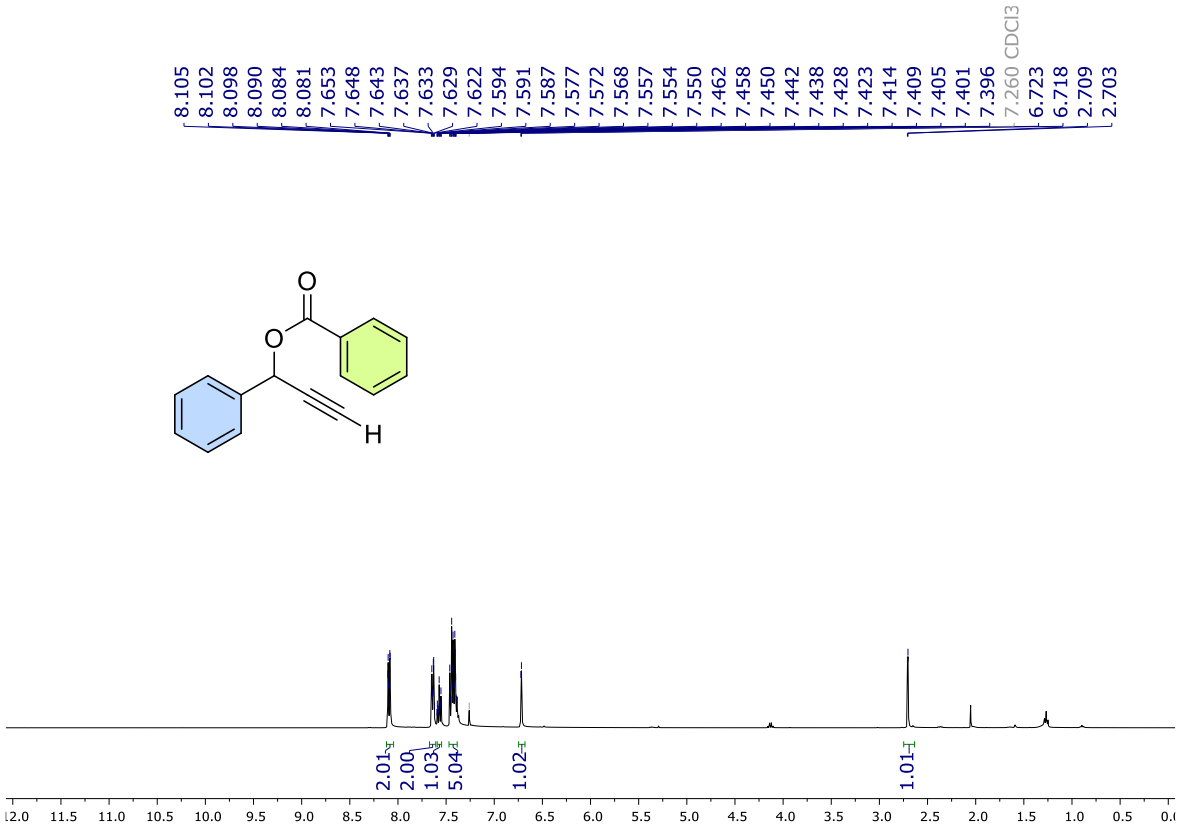


^{13}C (100 MHz)

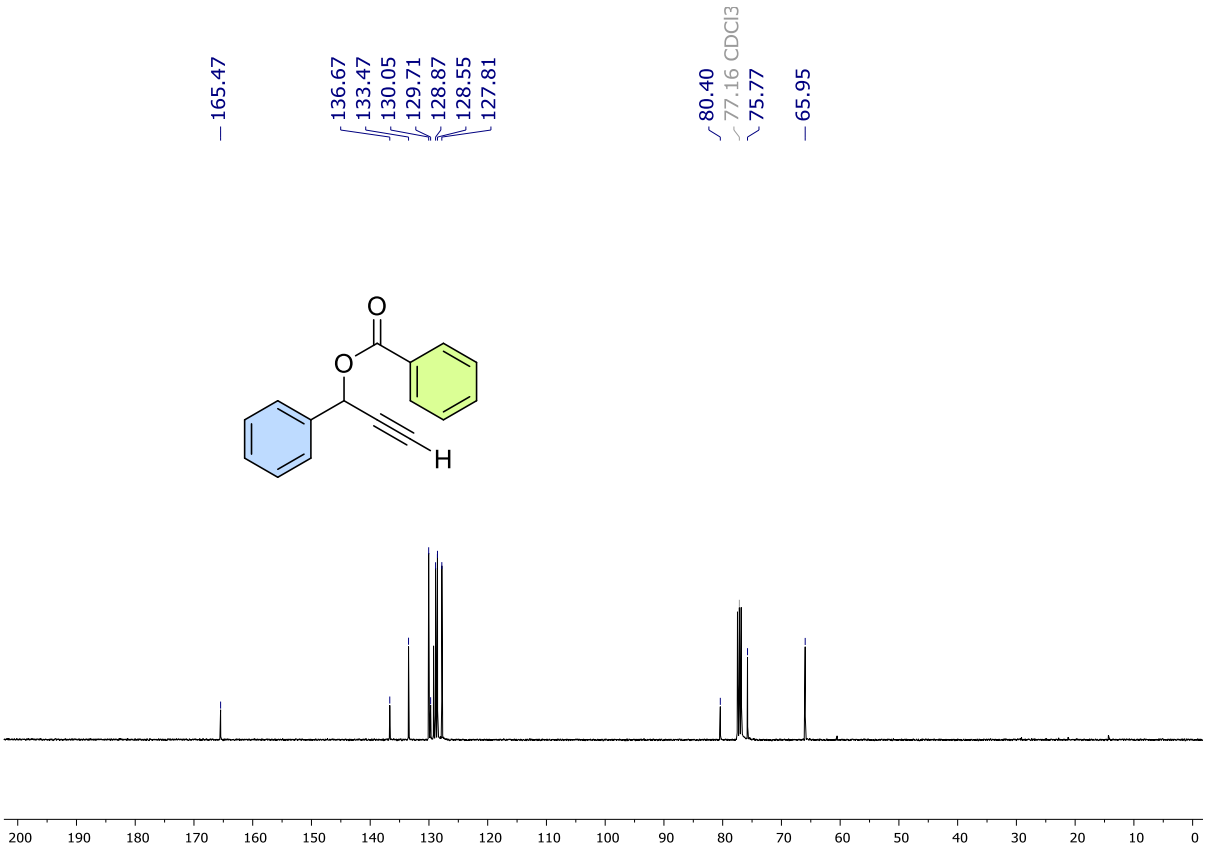


*1-phenylprop-2-yn-1-yl benzoate (Scheme 3, Entry **4b**)*

^{1}H NMR (400 MHz)

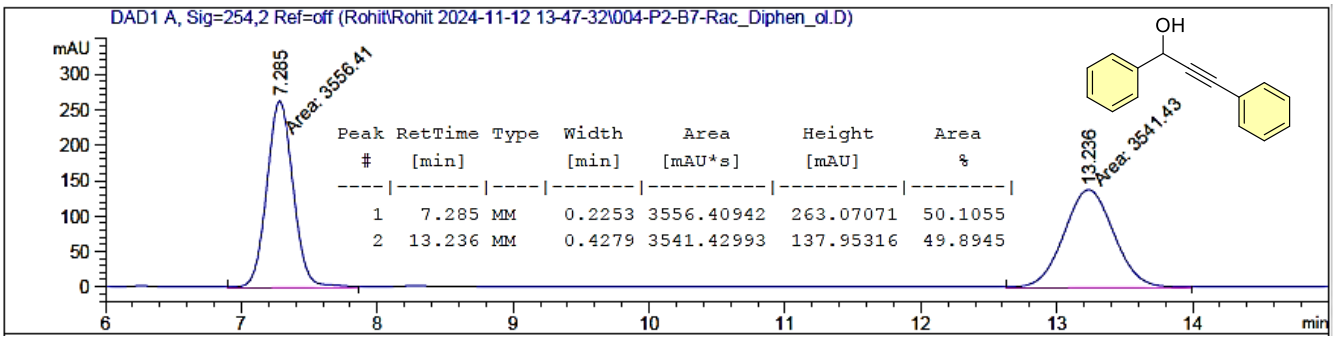


^{13}C (100 MHz)

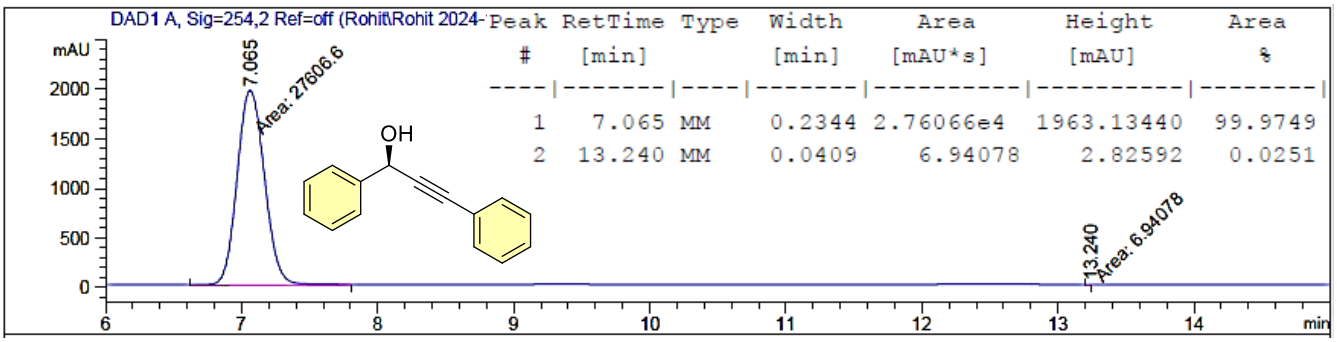


14. HPLC spectra

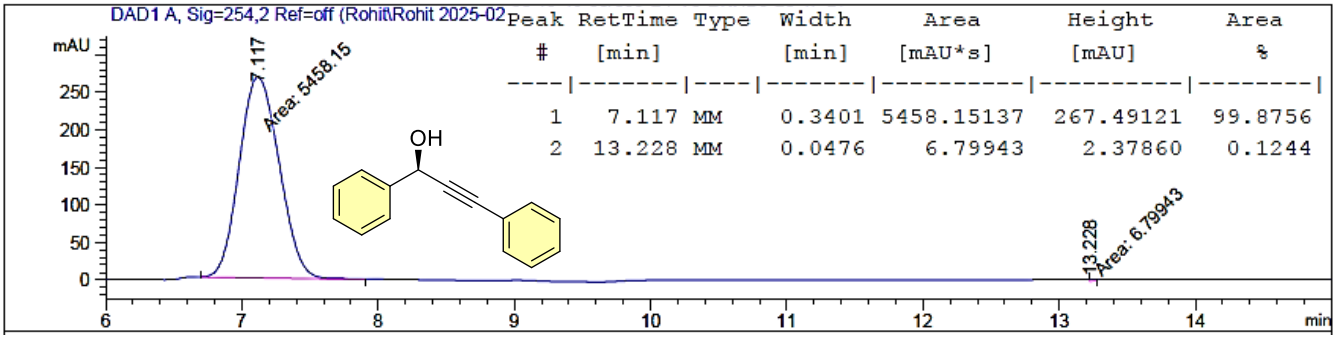
HPLC trace of **rac-2a**:



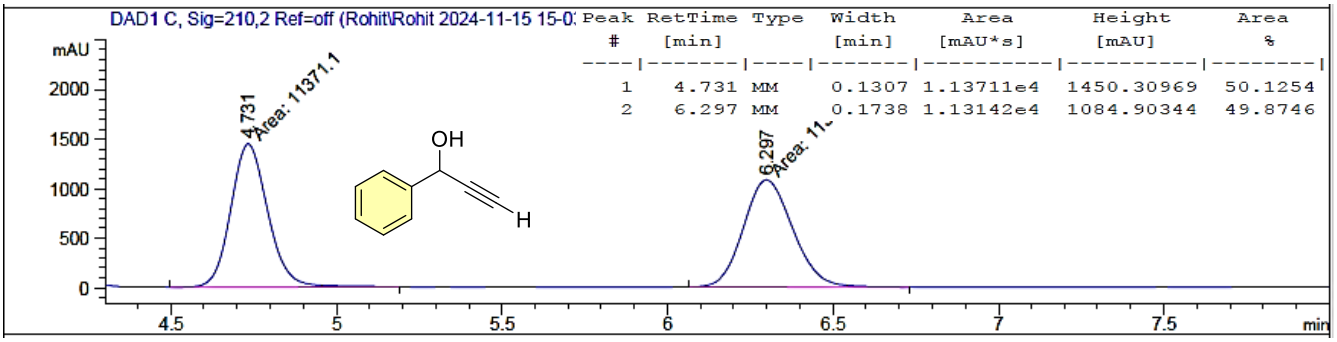
HPLC trace of enantioenriched (*R*)-**2a** obtained from the catalytic reduction by BsGDH_L95V:



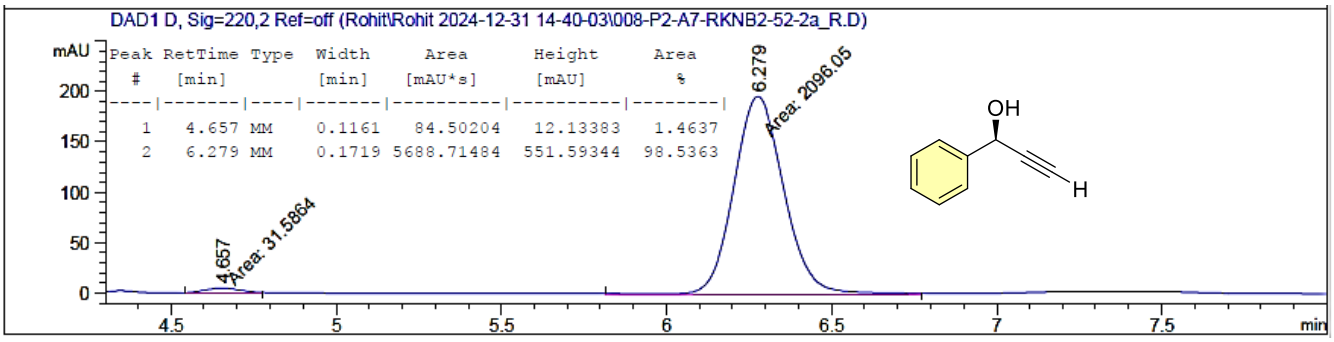
HPLC trace of enantioenriched (*R*)-**2a** obtained from the catalytic reduction by OsGDH:



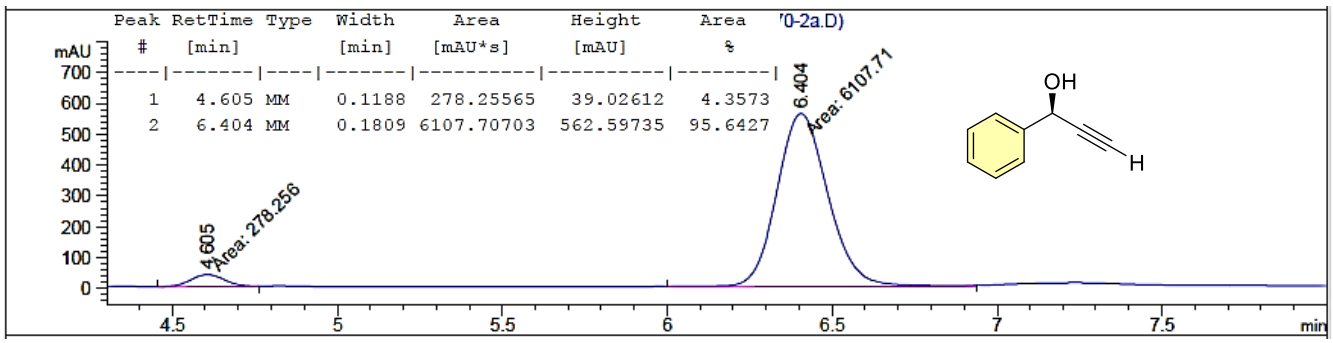
HPLC trace of **rac-2b**:



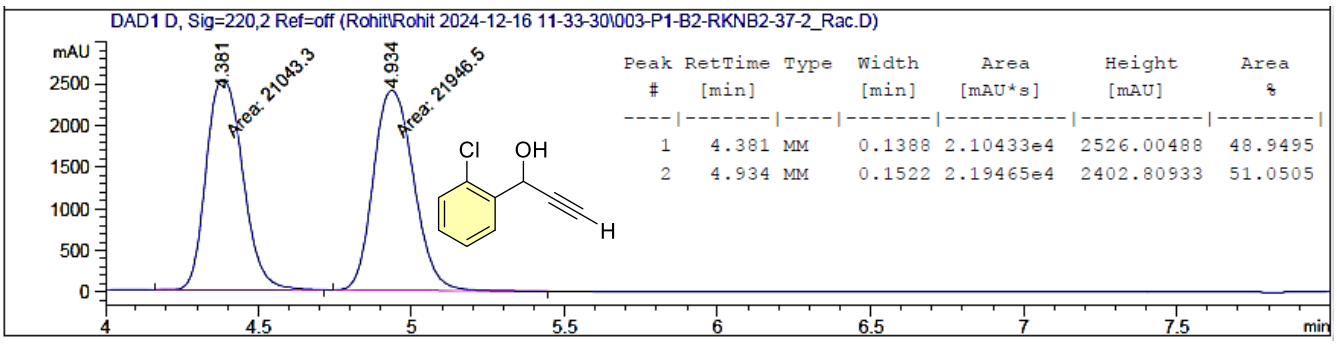
HPLC trace of enantioenriched (*S*)-**2b** obtained from the catalytic reduction by BsGDH_L95V:



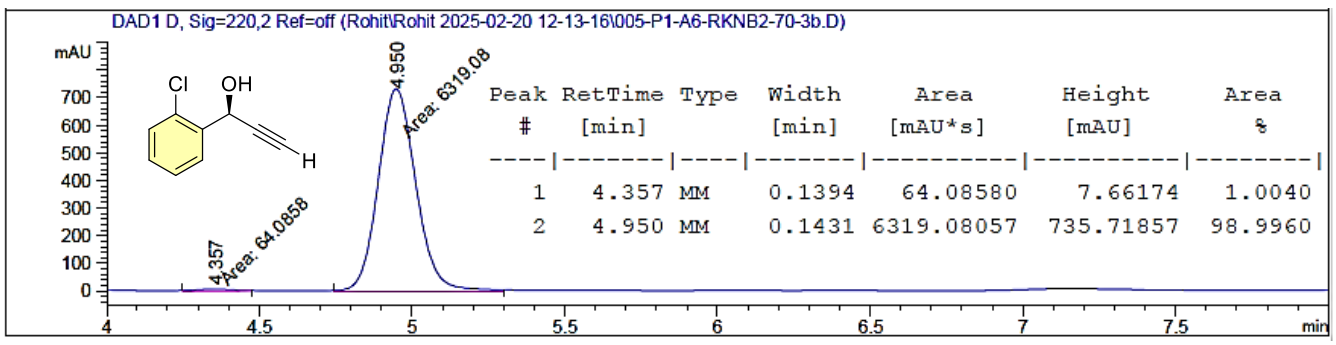
HPLC trace of enantioenriched (*S*)-**2b** obtained from the catalytic reduction by OsGDH:



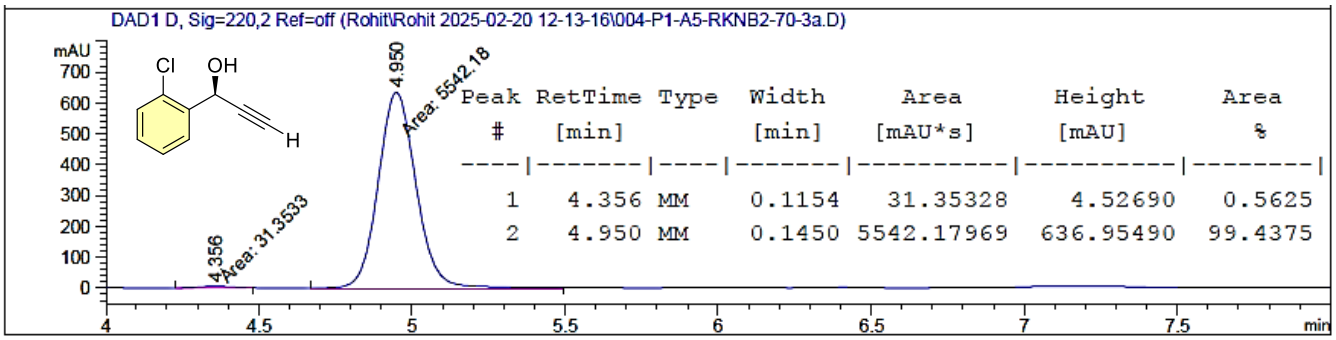
HPLC trace of **rac-2c**:



HPLC trace of enantioenriched (*S*)-**2c** obtained from the catalytic reduction by BsGDH_L95V:

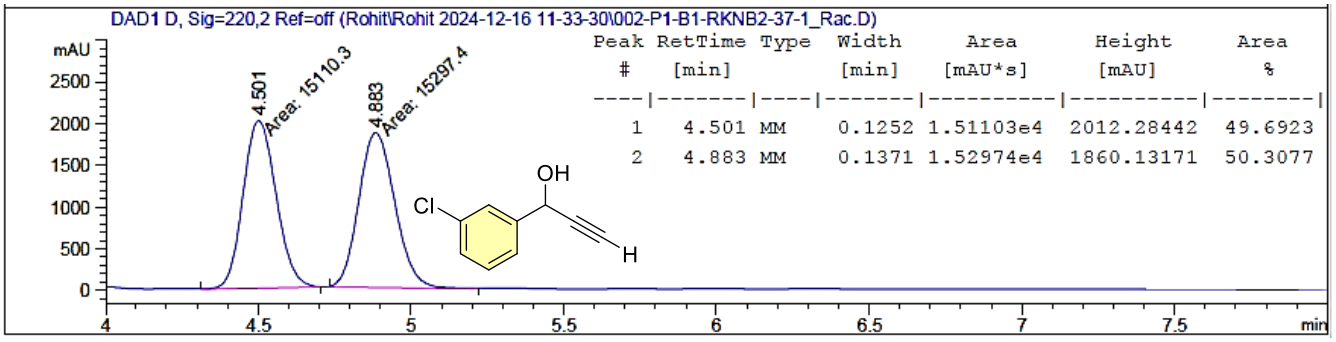


HPLC trace of enantioenriched (*S*)-**2c** obtained from the catalytic reduction by OsGDH:



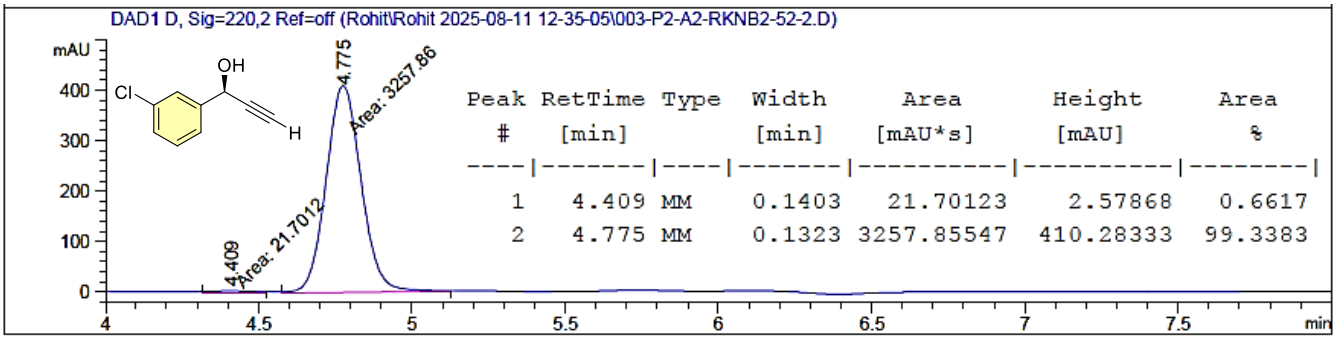
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.356	MM	0.1154	31.35328	4.52690	0.5625
2	4.950	MM	0.1450	5542.17969	636.95490	99.4375

HPLC trace of **rac-2d**:



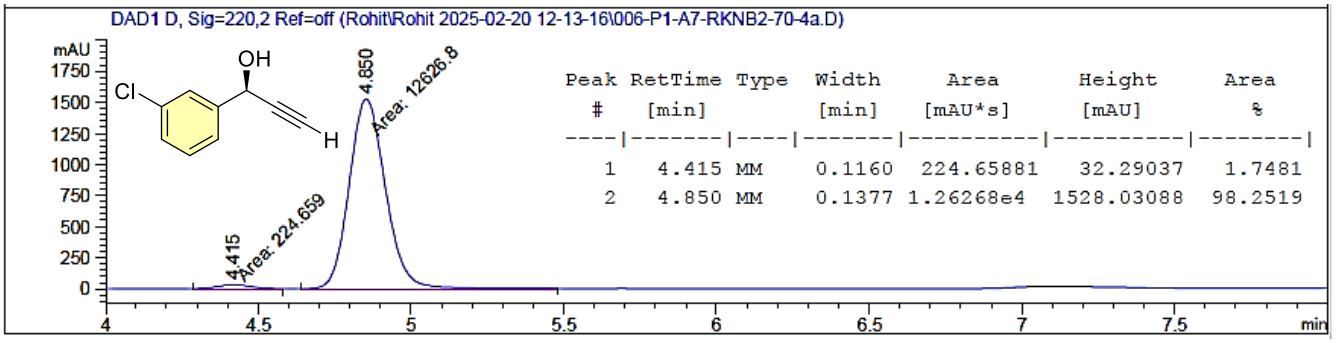
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.501	MM	0.1252	1.51103e4	2012.28442	49.6923
2	4.883	MM	0.1371	1.52974e4	1860.13171	50.3077

HPLC trace of enantioenriched (*S*)-**2d** obtained from the catalytic reduction by BsGDH_L95V:



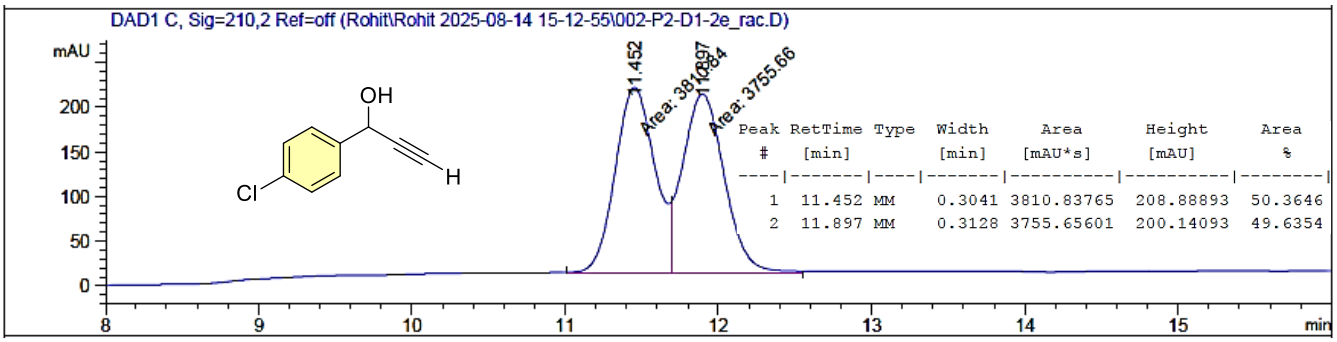
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.409	MM	0.1403	21.70123	2.57868	0.6617
2	4.775	MM	0.1323	3257.85547	410.28333	99.3383

HPLC trace of enantioenriched (*S*)-**2d** obtained from the catalytic reduction by OsGDH:

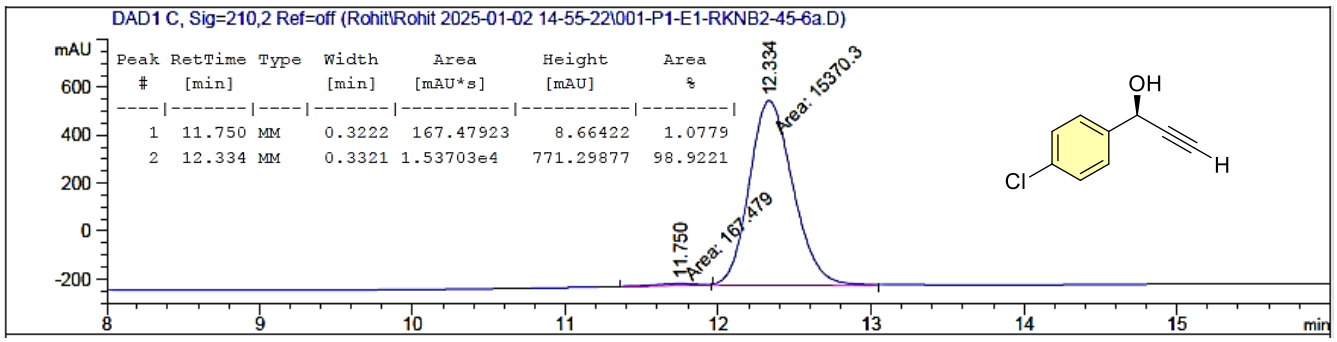


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.415	MM	0.1160	224.65881	32.29037	1.7481
2	4.850	MM	0.1377	1.26268e4	1528.03088	98.2519

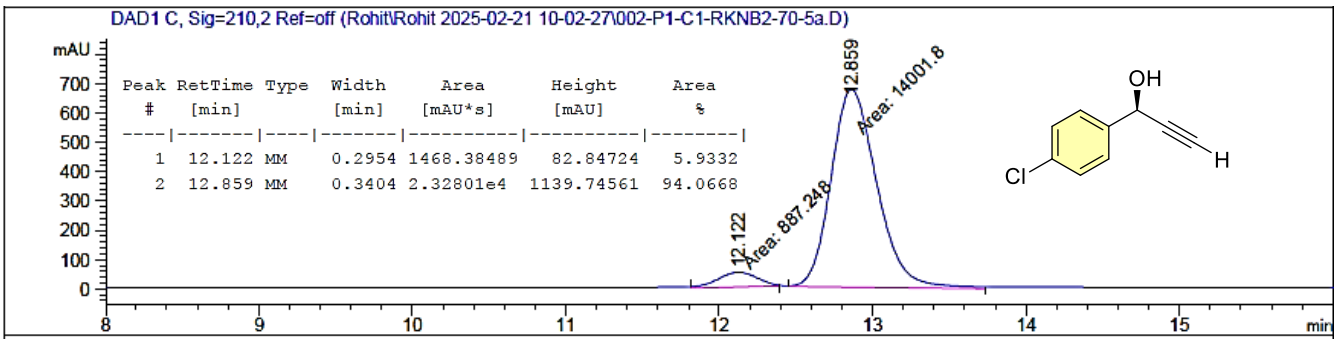
HPLC trace of **rac-2e**:



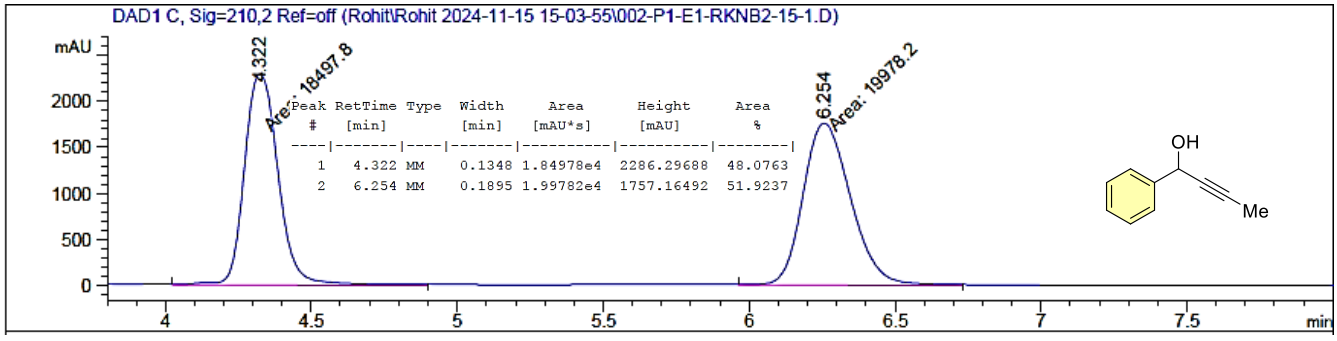
HPLC trace of enantioenriched (*S*)-**2e** obtained from the catalytic reduction by BsGDH_L95V:



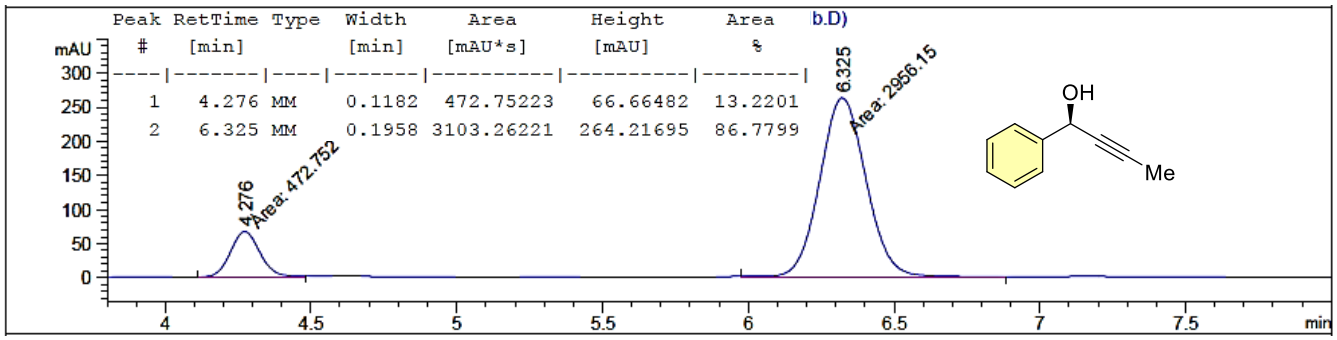
HPLC trace of enantioenriched (*S*)-**2e** obtained from the catalytic reduction by OsGDH:



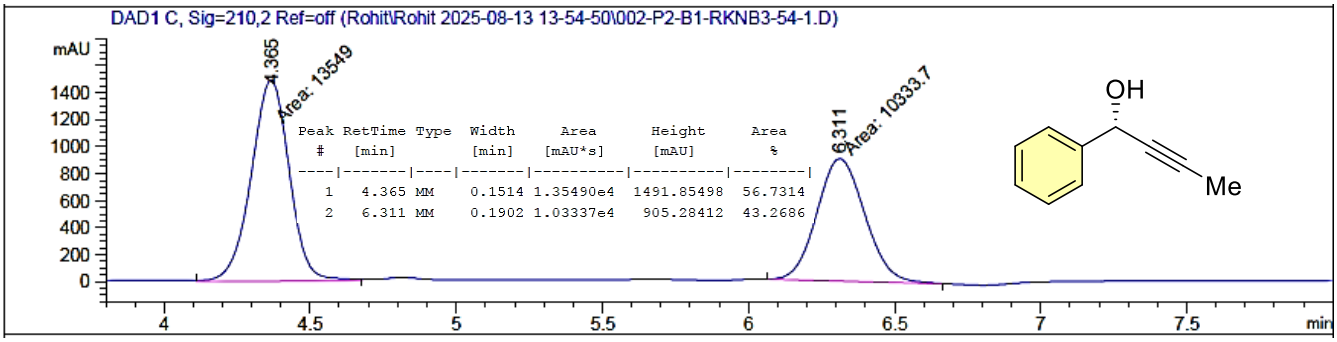
HPLC trace of **rac-2f**:



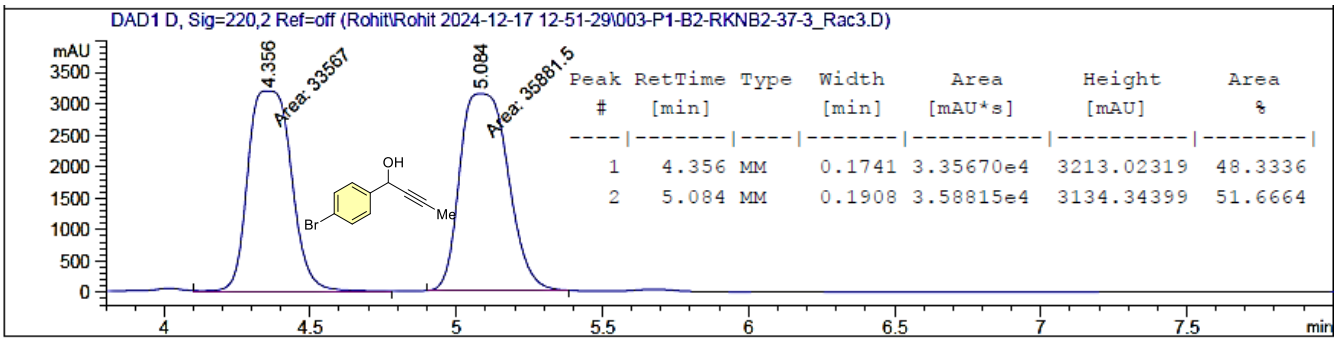
HPLC trace of enantioenriched (*R*)-**2f** obtained from the catalytic reduction by BsGDH_L95V:



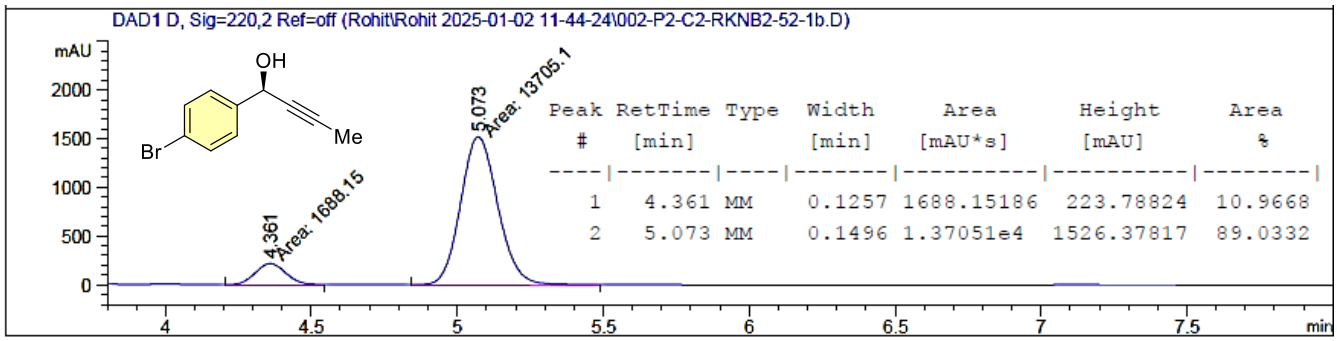
HPLC trace of enantioenriched (*S*)-**2f** obtained from the catalytic reduction by OsGDH:



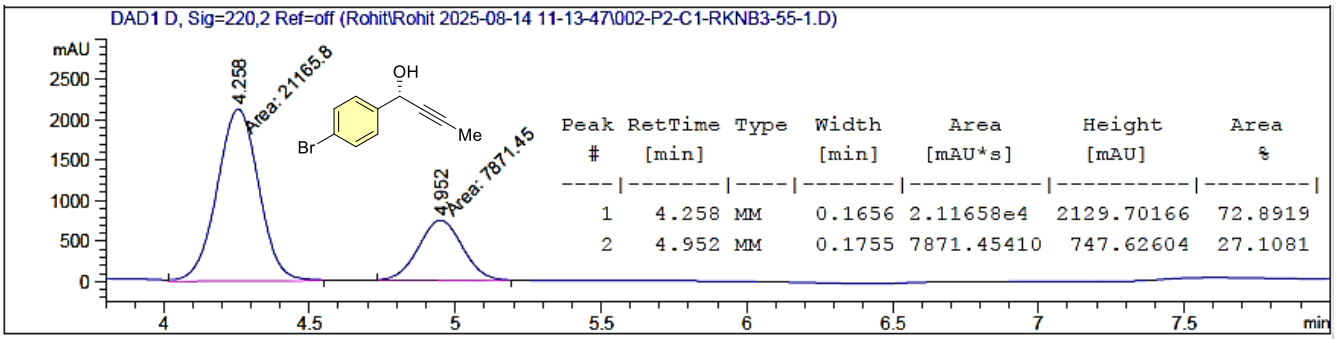
HPLC trace of **rac-2g**:



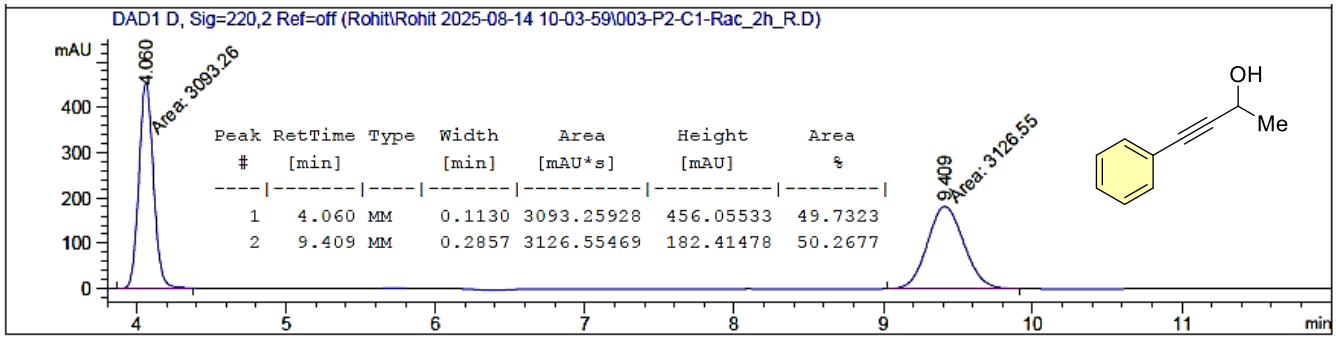
HPLC trace of enantioenriched (*R*)-**2g** obtained from the catalytic reduction by BsGDH_L95V:



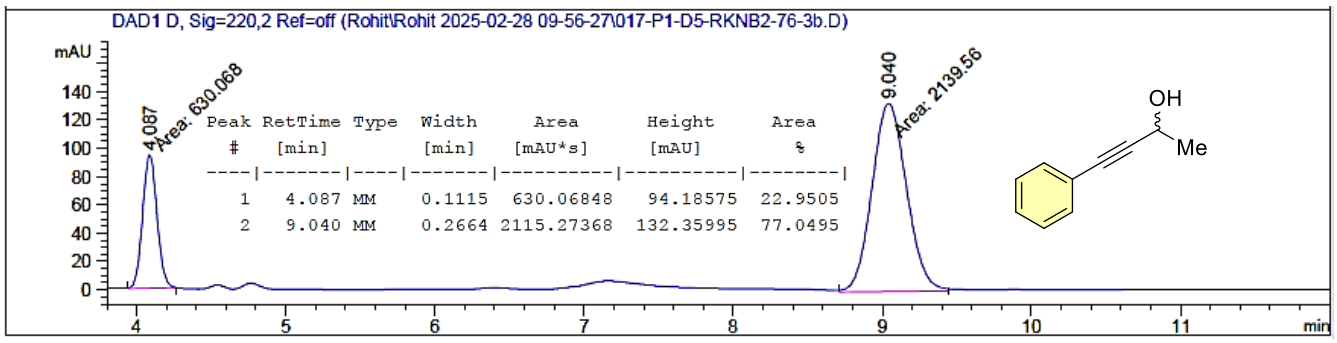
HPLC trace of enantioenriched (*S*)-**2g** obtained from the catalytic reduction by OsGDH:



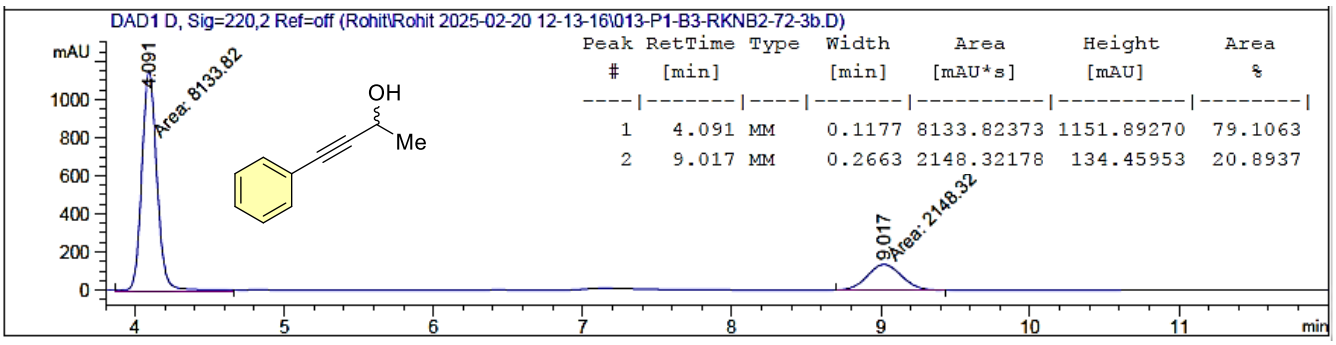
HPLC trace of **rac-2h**:



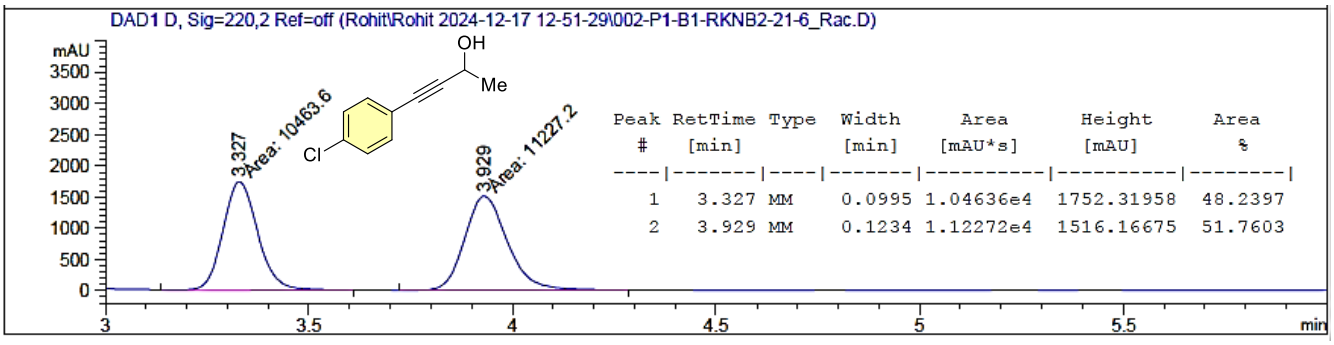
HPLC trace of enantioenriched (*R*)-**2h** obtained from the catalytic reduction by BsGDH_L95V:



HPLC trace of enantioenriched (*S*)-**2h** obtained from the catalytic reduction by OsGDH:

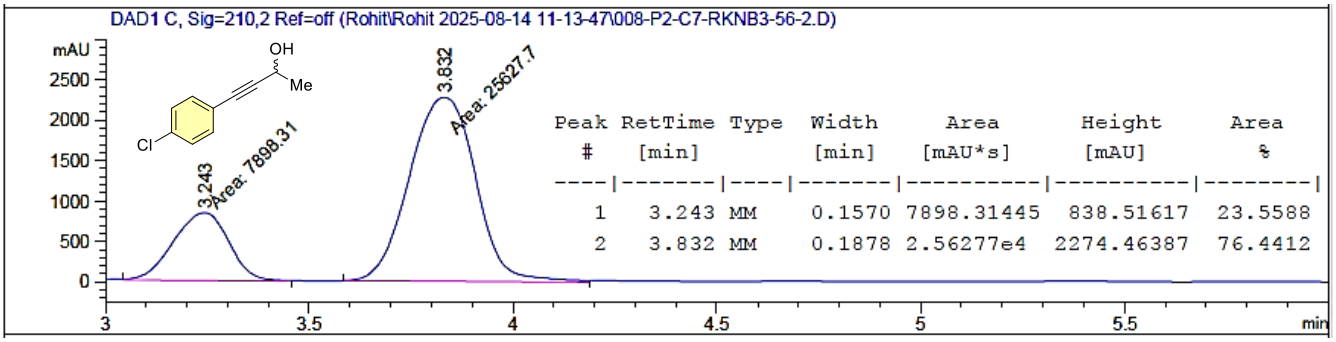


HPLC trace of **rac-2i**:



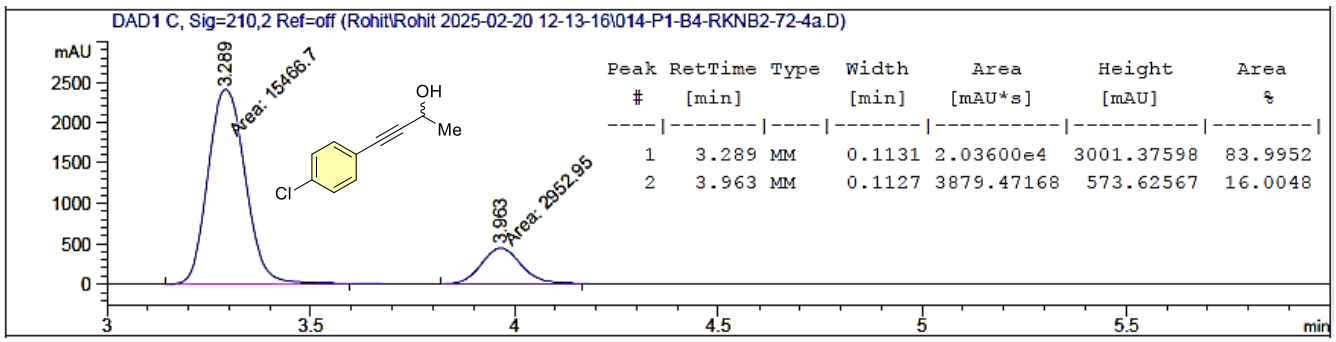
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.327	MM	0.0995	1.04636e4	1752.31958	48.2397
2	3.929	MM	0.1234	1.12272e4	1516.16675	51.7603

HPLC trace of enantioenriched (*R*)-**2i** obtained from the catalytic reduction by BsGDH_L95V:



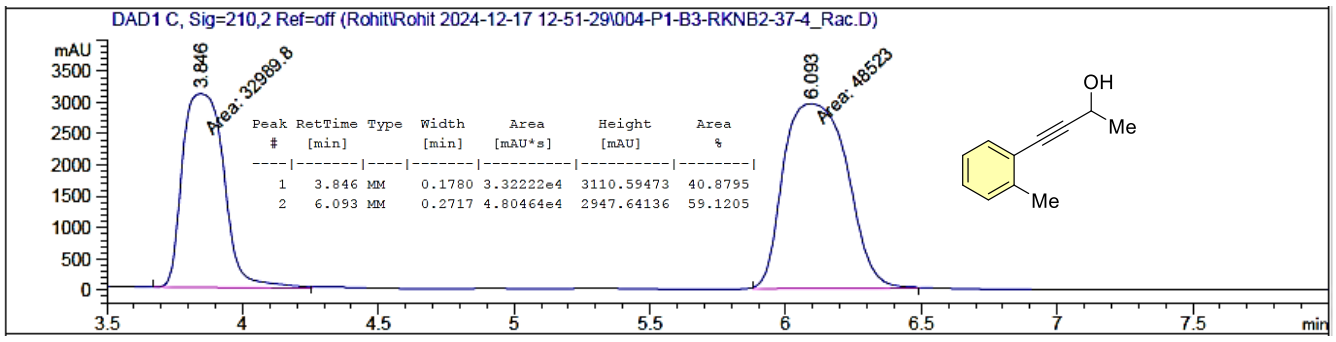
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.243	MM	0.1570	7898.31445	838.51617	23.5588
2	3.832	MM	0.1878	2.56277e4	2274.46387	76.4412

HPLC trace of enantioenriched (*S*)-**2i** obtained from the catalytic reduction by OsGDH:



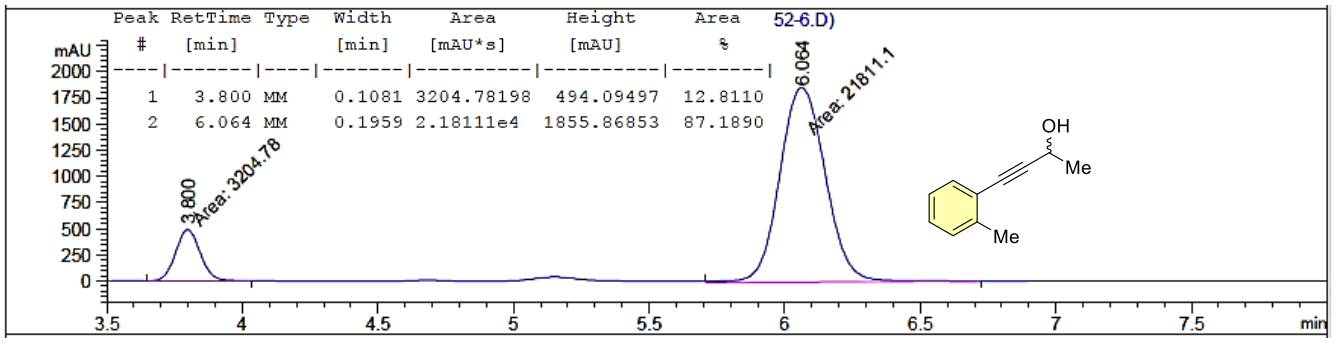
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.289	MM	0.1131	2.03600e4	3001.37598	83.9952
2	3.963	MM	0.1127	3879.47168	573.62567	16.0048

HPLC trace of **rac-2j**:

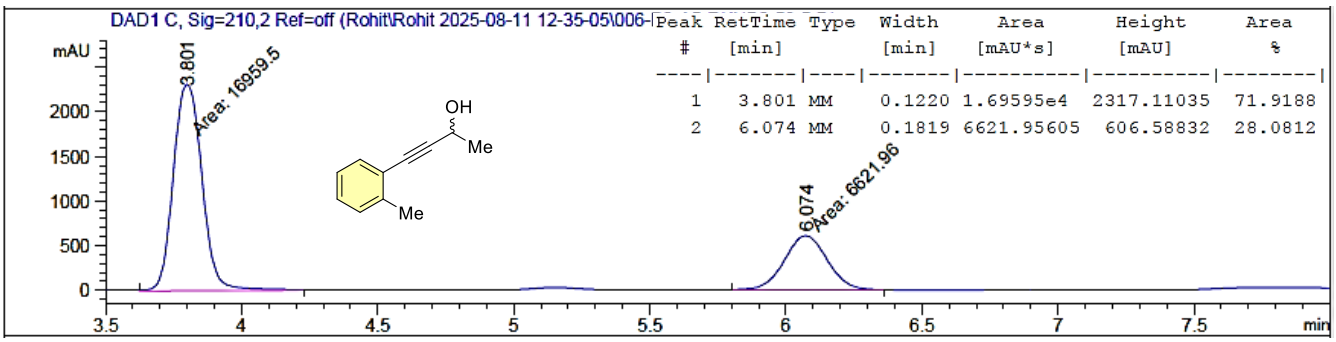


Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.846	MM	0.1780	3.32222e4	3110.59473	40.8795
2	6.093	MM	0.2717	4.80464e4	2947.64136	59.1205

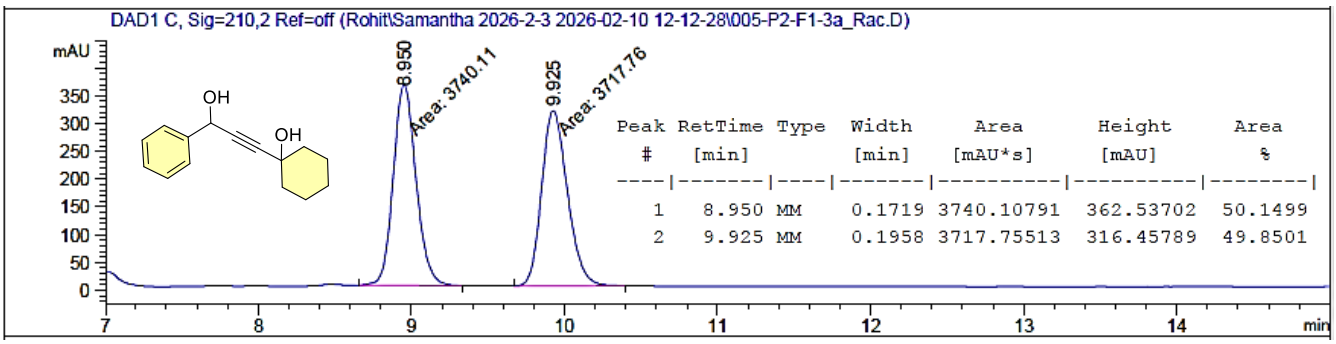
HPLC trace of enantioenriched (*R*)-**2j** obtained from the catalytic reduction by BsGDH_L95V:



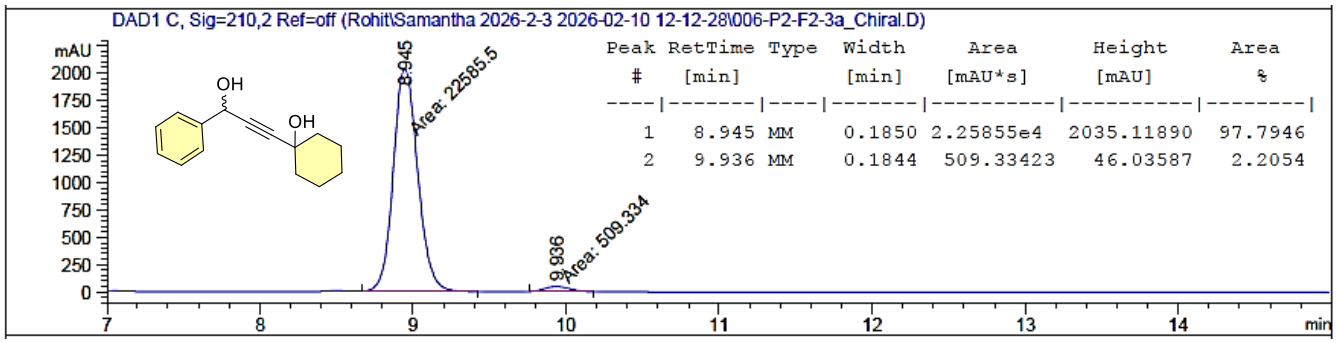
HPLC trace of enantioenriched (*S*)-**2j** obtained from the catalytic reduction by OsGDH:



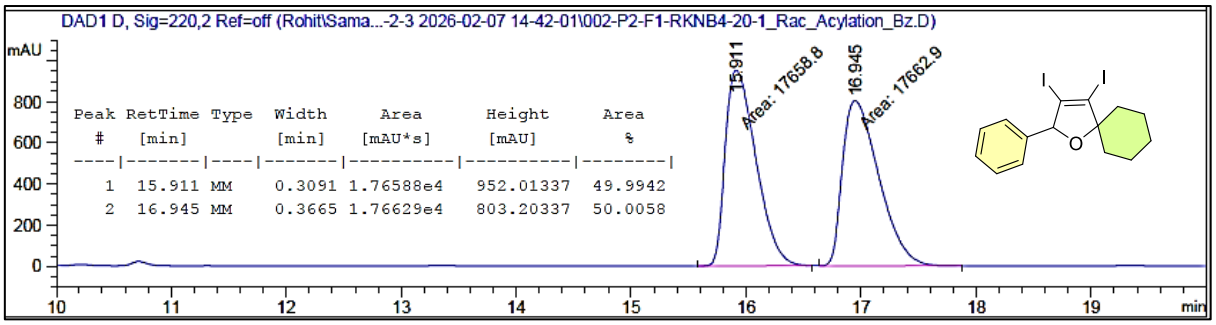
HPLC trace of **rac-3a**:



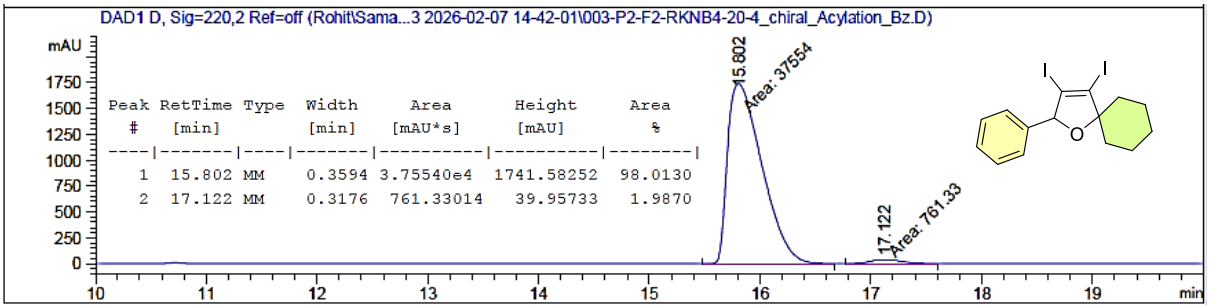
HPLC trace of enantioenriched **3a**:



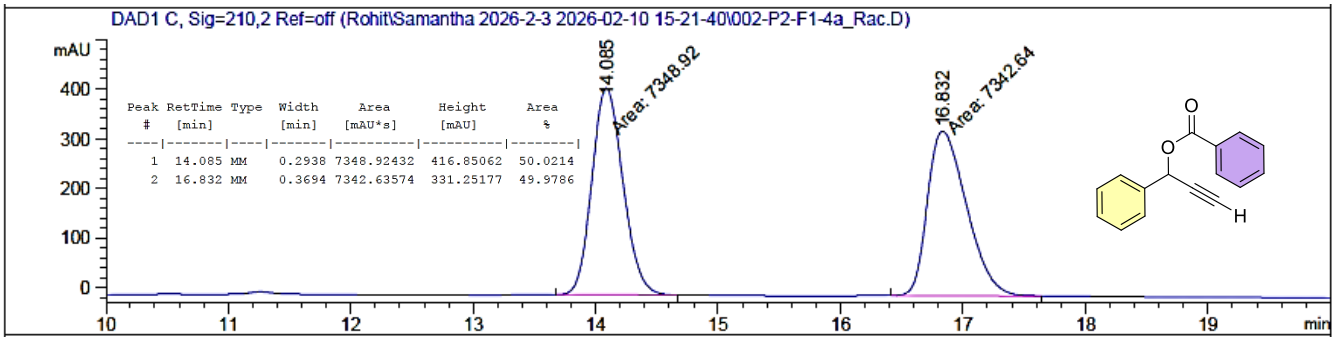
HPLC trace of **rac-4a**:



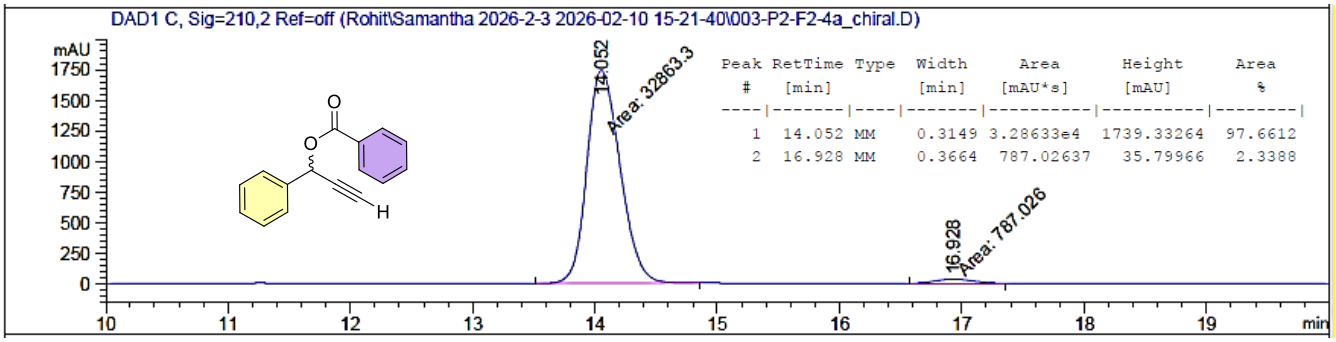
HPLC trace of enantioenriched **4a**:



HPLC trace of **rac-4b**:



HPLC trace of enantioenriched **4b**:



12. References

1. S. González Granda, D. Méndez Sánchez, I. Lavandera and V. Gotor Fernández, *ChemCatChem*, 2020, **12**, 520-527.
2. T. W. Johannes, R. D. Woodyer and H. Zhao, *Biotechnol. Bioeng.*, 2007, **96**, 18-26.
3. G. Jiang, J. Lu, M. Zhou, W. Harrison and H. Zhao, *ACS Catal.*, 2024, **14**, 7074-7079.
4. C. Kamilya, S. S. Gorad and P. Ghorai, *Chem. Eur. J.*, 2024, **30**, e202303980.
5. G. Ieronimo, G. Palmisano, A. Maspero, A. Marzorati, L. Scapinello, N. Masciocchi, G. Cravotto, A. Barge, M. Simonetti and K. L. Ameta, *Org. Biomol. Chem.*, 2018, **16**, 6853-6859.
6. Y. Horino, J. Sakamoto, M. Murakami and M. Sugata, *Synlett*, 2020, **31**, 1323-1327.
7. Z. Zhang, J. T. Deng, J. Y. Feng, J. Y. Liang, X. T. Xu and J. B. Peng, *J. Org. Chem.*, 2023, **88**, 12054-12063.
8. X. Sang, F. Tong, Z. Zeng, M. Wu, B. Yuan, Z. Sun, X. Sheng, G. Qu, M. Alcalde and F. Hollmann, *Org. Lett.*, 2022, **24**, 4252-4257.
9. A. Falconnet, M. Magre, B. Maity, L. Cavallo and M. Rueping, *Angew. Chem. Int. Ed.*, 2019, **58**, 17567-17571.
10. K. G. Ji, H. T. Zhu, F. Yang, X. Z. Shu, S. C. Zhao, X. Y. Liu, A. Shaukat and Y. M. Liang, *Chem. Euro. J.*, 2010, **16**, 6151-6154.
11. X. Huang, T. de Haro and C. Nevado, *Chem. Euro. J.*, 2009, **15**, 5904-5908.