Supplementary Information

A Colorimetric Method for Quantifying Cis- and Trans-Alkenes Using an Indicator Displacement Assay

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Contents

^{11}B NMR Procedure with d,l- and meso-butanediol:

Methanol-d_4 (Cambridge Isotopes, 99.8%) was used as the solvent and quartz NMR tubes (Wilmad Precision) were used for the NMR experiment. Four [40 mM] NMR solutions were prepared with the following procedure(s):

(1) [2-(pyrrolidine-1-ylmethyl)phenyl] boronic acid (Combi-Blocks, >95%)

(2) [2-(pyrrolidine-1-ylmethyl)phenyl] boronic acid, and (2R, 3R)-(-)-2,3-Butanediol (Sigma-Aldrich, 97%) / with an equivalency of 1:2 (boronic acid/d,l-butanediol)

(3) [2-(pyrrolidine-1-ylmethyl)phenyl] boronic acid, and meso-2,3-Butanediol (Sigma-Aldrich, 99%) / with an equivalency of 1:2 (boronic acid/meso-butanediol)

(4) [2-(pyrrolidine-1-ylmethyl)phenyl] boronic acid, (2R, 3R)-(-)-2,3-Butanediol, and meso-2,3-Butanediol / with an equivalencies of 1:2:2 (boronic acid/ d,l-butanediol/ meso-butanediol)

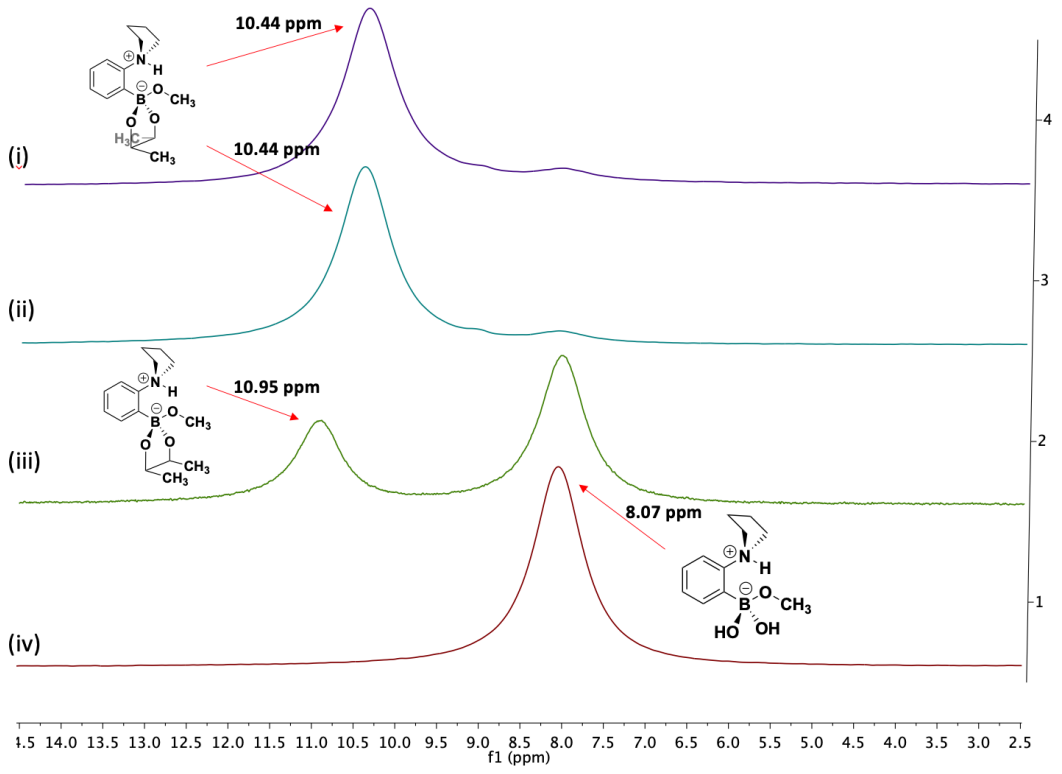


Figure S1: The ^{11}B NMR of meso- and d,l-butanediol with the host, **H**. The conditions used are described above. Where: (i) follows procedure (4), (ii) follows procedure (2), (iii) follows procedure (3), and (iv) follows procedure (1). **H** has a chemical shift of 8.07 ppm. While the boronate ester with meso-butanediol has a chemical shift of 10.95 ppm. In contrast, the boronate ester with d,l-butanediol has a chemical shift of 10.44 ppm. When both meso- and d,l-butanediol are able to compete with the host the chemical shift of the boronate ester is 10.44 ppm. This strongly suggests **H** prefers to bind with the d,l-butanediol.

^{11}B NMR Procedure with G3ER & G3TH:

A binary solvent mixture (1:1) of acetonitrile-d_3 and methanol-d_4 (Cambridge Isotopes, 99.8%) was used and quartz NMR tubes (Wilmad Precision) were used for the experiment. Four [40 mM] NMR solutions were prepared under the following conditions:

(1) [2-(pyrrolidine-1-ylmethyl)phenyl] boronic acid (Combi-Blocks, >95%)

(2) [2-(pyrrolidine-1-ylmethyl)phenyl] boronic acid, and **G3TH** / equivalencies of 1:1

(3) [2-(pyrrolidine-1-ylmethyl)phenyl] boronic acid, and **G3ER** / equivalencies of 1:1

(4) [2-(pyrrolidine-1-ylmethyl)phenyl] boronic acid, **G3ER** and **G3TH** / equivalencies of 1:1:1

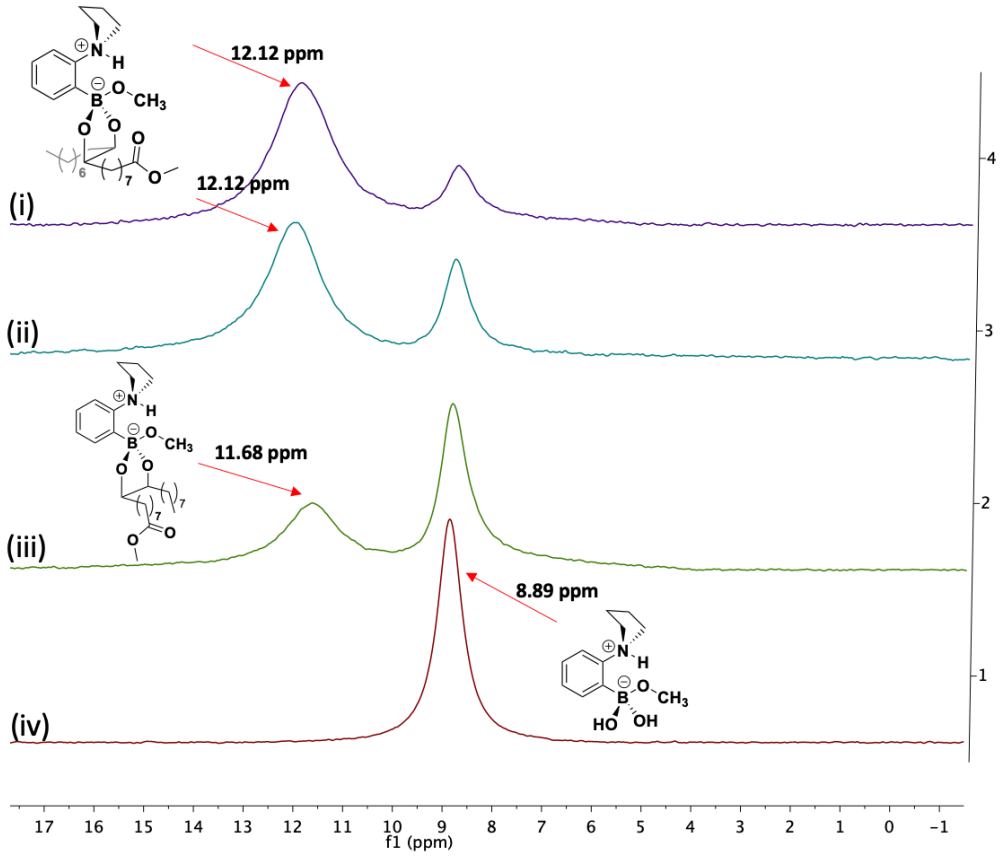


Figure S2: The ^{11}B NMR study using [2-(pyrrolidine-1-ylmethyl)phenyl] boronic acid as the host, **H**, and **G3TH** and **G3ER** as the guest. Where the chemical shift of the host is 8.89 ppm shown with (iv). Upon the addition of **G3ER**, the chemical shift of the boronate species shifts to 11.68 ppm, shown with (iii). However, there is a strong presence of original host shown at 8.89 ppm in (iii). In comparison, the addition of **G3TH** changes the chemical shift to 12.12 ppm with less presence of the uncomplexed **H** in part (ii). The competition between **G3TH** and **G3ER** with the **H** shows the chemical shift of the boronate species at 12.12 ppm which strongly suggests **G3TH** will preferably bind to the host compared to **G3ER**.

General Procedure for UV-Vis titrations:

UV-vis measurements were performed on a Beckman DU-640 UV-vis spectrophotometer. pH measurements were performed on an Orion 720ApH meter with a glass electrode. All the selected chemicals were commercially sourced unless otherwise stated. All titrations were carried out using a 1 cm light path UV-vis quartz semi-micro cell, equipped with a silicon septum, and Hamilton gas-tight microliter syringes were used to transfer solutions. Stock solutions of host, indicator and analytes were made in 10 mM solutions of *para*-toluenesulfonic acid and Hünig's base (N,N-diisopropylethylamine) in 1:1 spectral grade degassed methanol/acetonitrile, and the pH of all the solutions was adjusted in the range 8.1 - 8.4. All measurements were taken at 25 °C.

Solvent System titrations of H with PV:

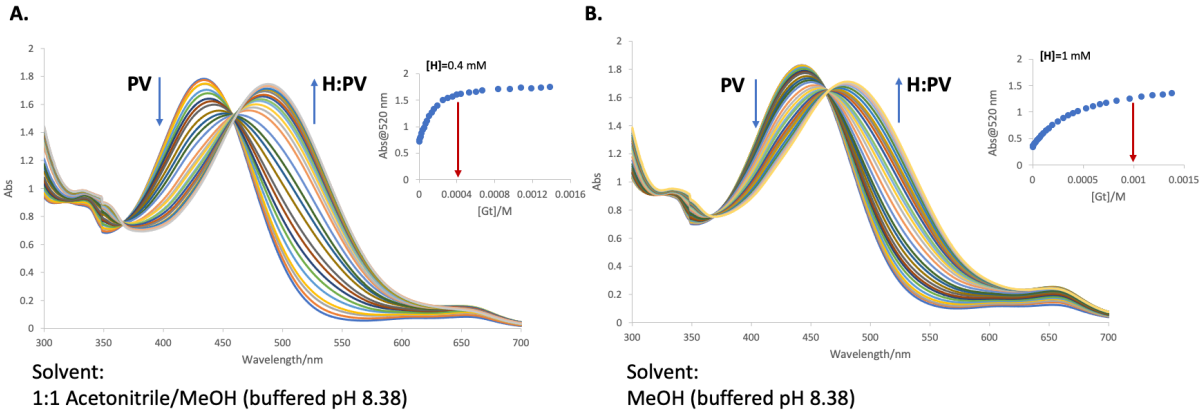


Figure S3: A. Solvent system, 1:1 acetonitrile/MeOH (buffered pH=8.38) using **H** and **PV**. Inset shows the [**H**] saturation at 0.4 mM and the UV-Vis titration shows a large dynamic shift between the **H** and **H:PV** complex. B. Solvent system, MeOH (buffered pH=8.38) using **H** and **PV**. Inset shows the [**H**] saturation at 1 mM and the UV-Vis titration shows dynamic shift between the **H** and **H:PV** complex. Due to the dynamic shift between **H** and **H:PV** complex being greater for (A), the 1:1 acetonitrile/MeOH (buffered pH=8.38) was chosen as the solvent system.

Determination of $K_{H:PV}$ between H and pyrocatechol violet (PV):

The binding constant, $K_{H:PV}$, of **H** to **PV** was calculated by measuring the change in the absorbance of the pyrocatechol violet indicator with the addition of host.

Two solutions **A** and **B** were prepared as follows: solution **A**, 150 µM **PV**; solution B, 150 µM **PV**; and 1.6 mM **H** (10 equiv of boronic acid host to the indicator). Initially 700 µL of solution **A** was added to a UV-vis cuvette, and the change in absorbance was monitored with the continual addition of solution **B** to solution **A** in the cuvette. With each addition of **B** to **A**, the solution was allowed to equilibrate for

three minutes before taking the measurement. The addition was continued until the change in the absorbance from subsequent readings was negligible. The change in absorbance at 520 nm was plotted against the total host concentration and the isotherm was used to solve for the 1:1 binding constant, $K_{\mathbf{H:PV}}$, using BindFit v 0.5 (**Figure S4**).[1]

All titrations were carried out in 1:1 methanol/acetonitrile, 10 mM *para*-toluenesulfonic acid and Hünig's base buffer, pH=8.1-8.4. All measurements were taken at 25°C.

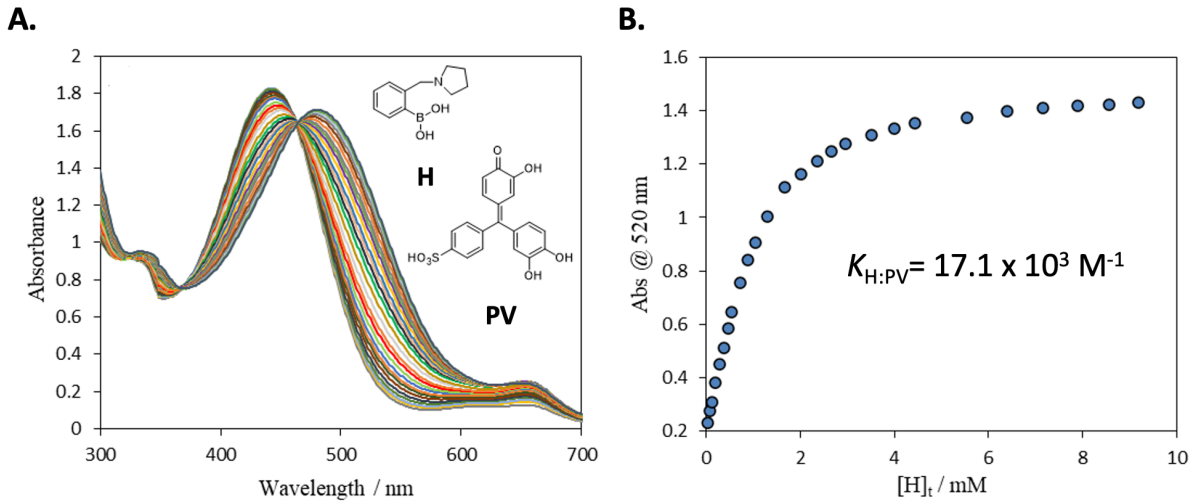


Figure S4: A. UV-visible titration of host **H** with **PV** (150μM) B. 1:1 binding isotherm (plot of the difference in absorbance at 520 nm with the addition of the host).

Binding Constants, $K_{\mathbf{H:G}}$, between H & specified threo and erythro diol guests (G):

The binding constant between **H** and the analyte, $K_{H:G}$, was determined by measuring the change in absorbance of the host indicator solution with the addition of the analyte. The procedure described here is the titration of **G2TH** into a solution of **H** and **PV** to determine $K_{\mathbf{H:G2TH}}$. The optimum ratio of **H** to **PV** which was used in the displacement assay (~90% saturation) was determined by using the 1:1 binding isotherm of **H** and **PV**. Two solutions, **C** and **D** were made, whose compositions were: solution **C**, 150 μM **PV** and 400 μM **H**; solution **D** 150 μM **PV**, 400 μM **H,** and 20 mM **G2TH**. The UV-Vis titration was carried out with these two solutions in a similar manner as described above. To determine, $K_{\mathbf{H:G2ER}}$ of **H** with **G2ER**, another titration in this manner was performed. Similarly, the binding

constant of all selected sets of the threo and erythro diol guests were determined in this way using the sensing ensemble **H** and **PV,** except the concentration of guest in solution **D** was different for each case – depending on the concentration required to fully displace **PV**, **Figures S5-S10**. All titrations were carried out in 1:1 methanol/acetonitrile, 10 mM *para*-toluenesulfonic acid and Hünig's base buffer, pH: 8.1-8.4. All measurements were taken at 25°C.

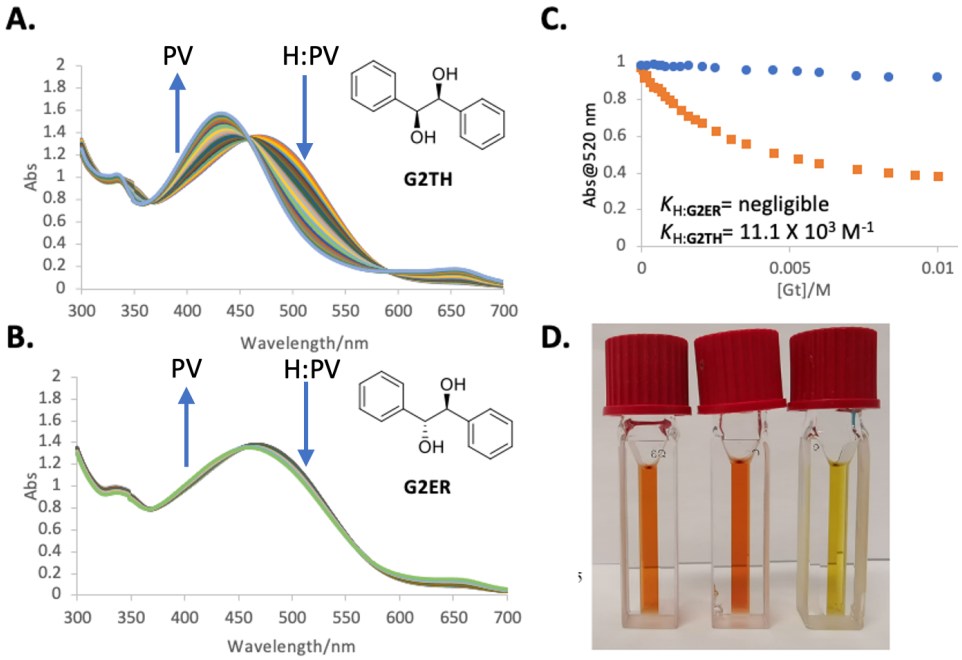


Figure S5: A. UV-Vis titration of **H** with **G2TH** using **PV**. B. UV-Vis titration of **H** with **G2ER** using **PV**. C. Monitoring the absorbance with the increase of guest concentration [Gt]/M. D. Qualitative analysis of the IDA of with **G2TH** and **G2ER** with: **H** and **PV** only (left), **H** and **PV** with 10mM **G2ER** (middle), and **H** & **PV** with 10mM **G2TH** (right). The **H:PV** complex has an orange color, while in contrast the **H:G2TH** complex has a yellow color. Lastly, the **H:G2ER** complex has a red color.

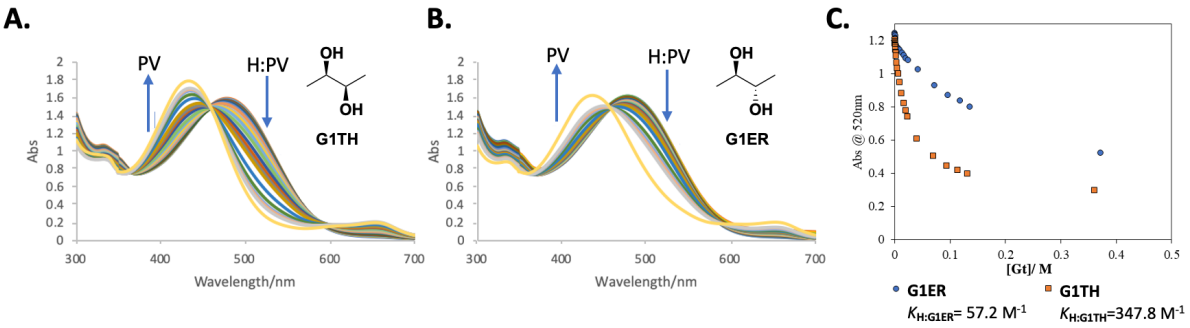


Figure S6: Quantitative analysis of an IDA of **H:PV** with **G1TH** and **G2ER**. A. UV-Vis titration of **H:PV** with **G1TH**. B. UV-Vis titration of **H:PV** with **G1ER**. C. Monitoring the absorbance with the increase of guest concentration [Gt]/M and the associated binding constants.

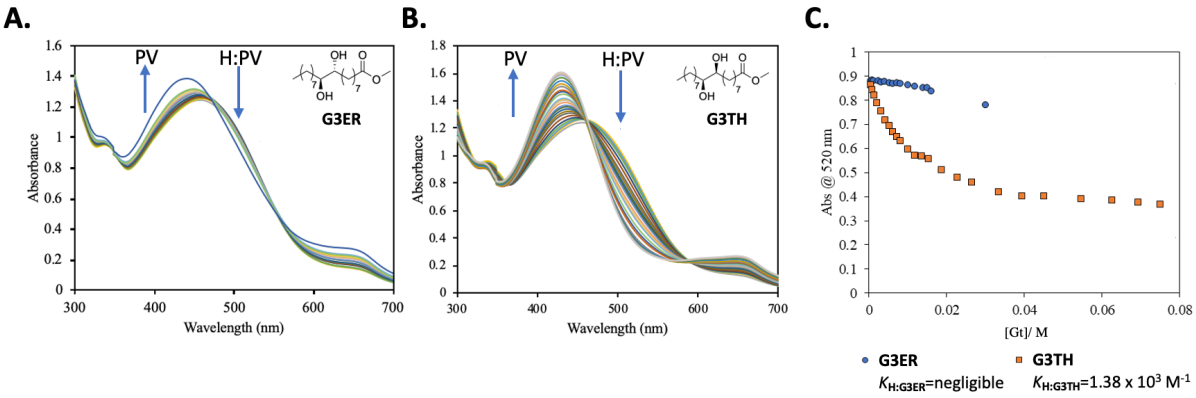


Figure S7: Quantitative analysis of an IDA of **H:PV** with **G3TH** and **G3ER**. A. UV-Vis titration of **H:PV** with **G3ER**. B. UV-Vis titration of **H:PV** with **G3TH**. C. Monitoring the absorbance with the increase of guest concentration [Gt]/M and the associated binding constants.

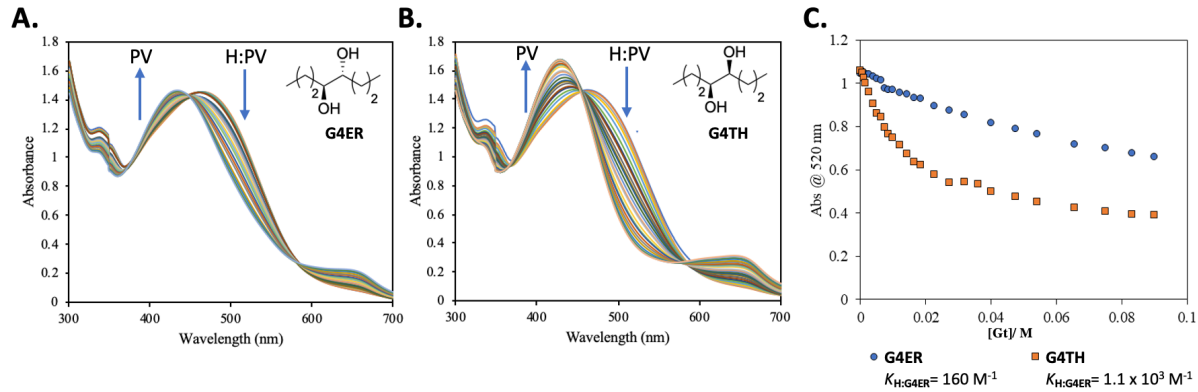


Figure S8: Quantitative analysis of an IDA of **H:PV** with **G4TH** and **G4ER**. A. UV-Vis titration of **H:PV** with **G4ER**. B. UV-Vis titration of **H:PV** with **G4TH**. C. Monitoring the absorbance with the increase of guest concentration [Gt]/M and the associated binding constants

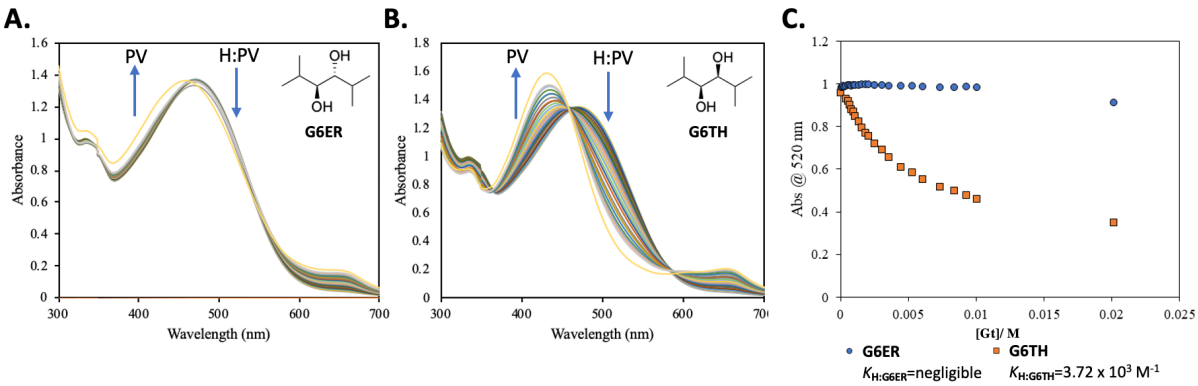


Figure S9: Quantitative analysis of an IDA of **H:PV** with **G6TH** and **G6ER**. A. UV-Vis titration of **H:PV** with **G6ER**. B. UV-Vis titration of **H:PV** with **G6TH**. C. Monitoring the absorbance with the increase of guest concentration [Gt]/M and the associated binding constants

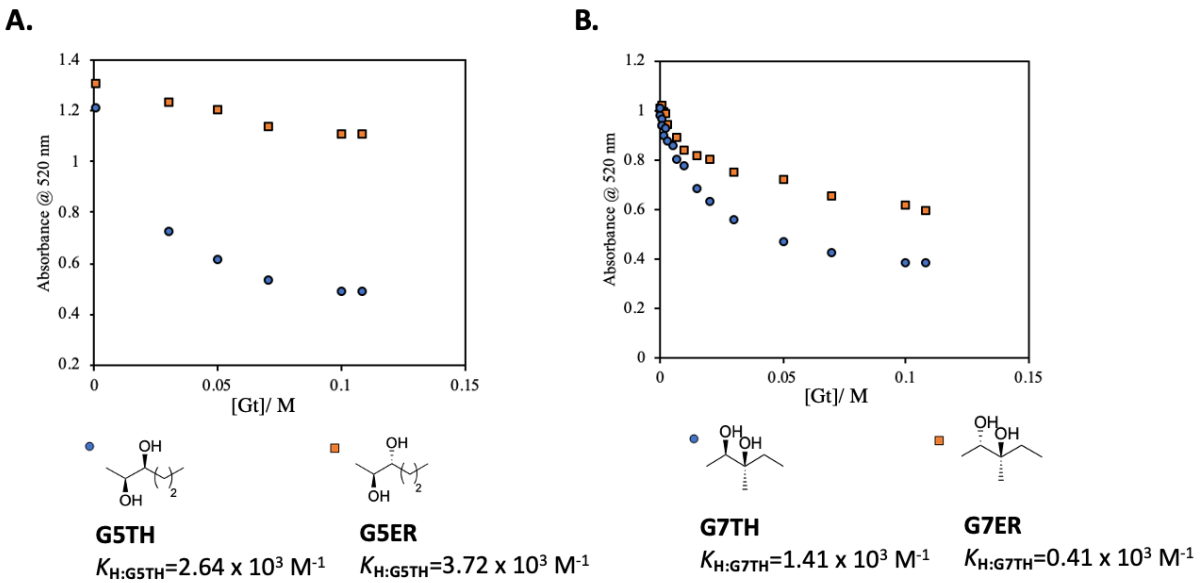


Figure S10: The 96-well plate analysis of the binding constant for **G5TH/G5ER** and **G7TH/G7ER**. The same experimental analysis was undertaken except the implimented using a UV-Vis plate reader. A. The absorbance monitored with the increase of guest concentration [Gt]/M and the associated binding constants for **G5TH** and **G5ER**. B. The absorbance monitored with the increase of guest concentration [Gt]/M and the associated binding constants for **G7TH** and **G7ER**.

Wavelength Analysis and Guest Concentration:

The optimal wavelength for analysis and guest concentration was discerned by both qualitative and quantitative means by assessing the change in color/absorbance of the host indicator solution with the addition of the analyte. Solutions were made with sensing ensemble **H** (400 μM) and **PV** (150 μM), with the addition of varying [guest]. **Figure S11** provides detail on the assessment of hydrobenzoin guest 10mM – 360 mM. It was concluded according to this data, that a wavelength of 520 nm at hydrobenzoin concentration of 10 mM gave the best dynamic range for study. All titrations were carried out in 1:1 methanol/acetonitrile, 10 mM *para*-toluenesulfonic acid, and Hünig's base buffer, pH: 8.1-8.4. All measurements were taken at 25°C.

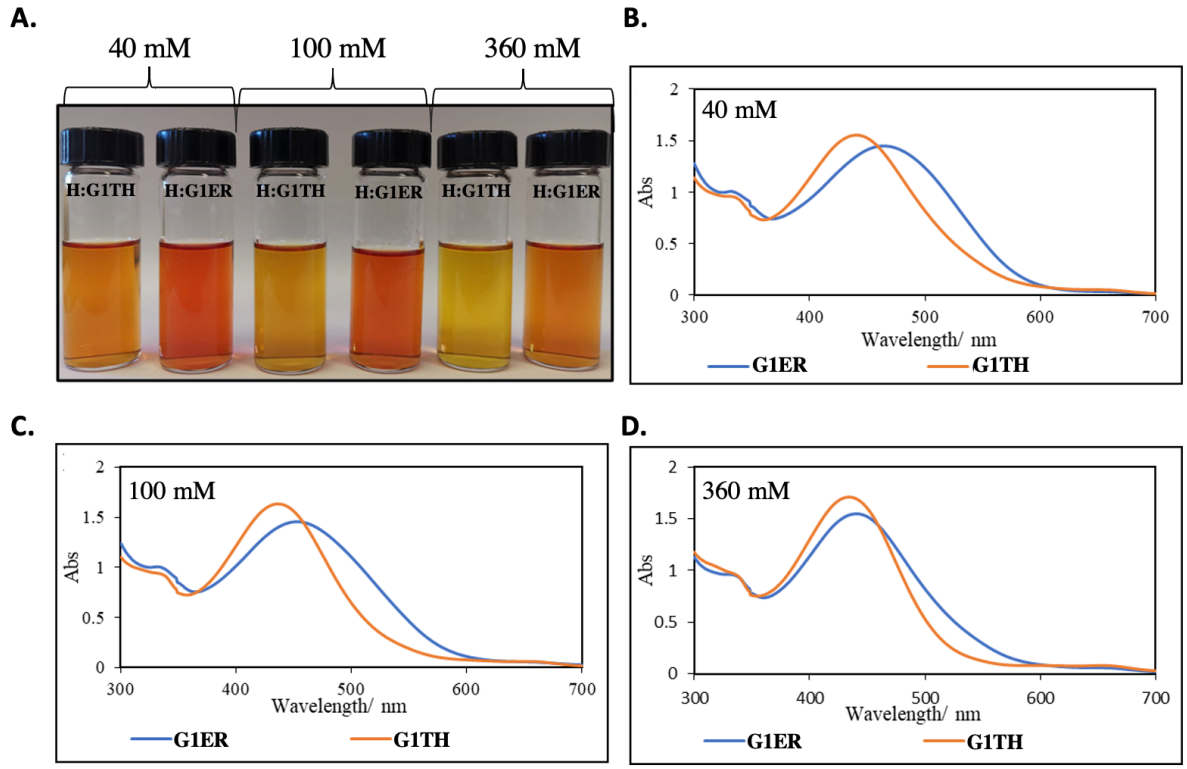


Figure S11: Qualitative and quantitative assessment of varying guest concentration. For all studies: solvent 1:1 methanol/acetonitrile, pH 8.1-8.4, 10mM buffer (*para*-toluenesulfonic acid and Hünig's base), **H** (400µM) and **PV** (150µM). A. qualitative assessment of the **H:G1TH** and **H:G1ER** complex at the concentrations of 40, 100, and 360 mM. B. quantitative assessment at 40 mM for **H:G1TH** and **H:G1ER** complexes. C. quantitative assessment at 100 mM for **H:G1TH** and **H:G1ER** complexes. D. quantitative assessment at 360 mM for **H:G1TH** and **H:G1ER** complexes.

Calibration Curves for G2T/G2C and G3T/G3C:

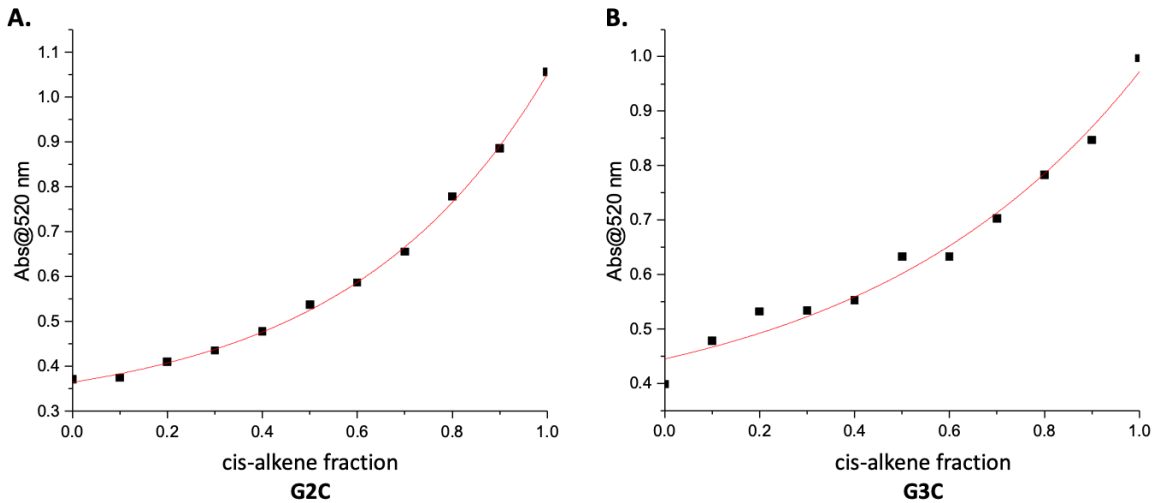
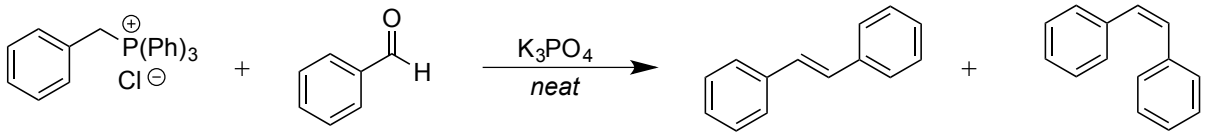


Figure S12: The calibration curves used for the three different unknown mixtures for **G2TH/G2ER** and **G3TH/G3ER** from their respective alkenes of **G2T/G2C or G3T/G3C**. All calibration curves underwent the procedures described under the "96-well plate analysis" in the supplementary information.

Wittig Reaction Materials:

All materials were used upon arrival unless otherwise stated. The ylide, benzyltriphenylphosphonium chloride (99%) was purchased from Janssen Chimica, benzaldehyde (>99%) was purchased from Sigma-Aldrich and potassium phosphate tribasic monohydrate (96%) was purchased from Acros Organics.

Wittig Reaction Procedure:



Benzyltriphenylphosphonium chloride (1.3 g, 3.34 mmol), benzaldehyde (338 μL, 3.34 mmol), and K_3PO_4 (2.13 g, 10.03 mmol) were combined into a porcelain mortar. The mixture was ground by hand with a pestle for 30 minutes and monitored by TLC (Ethyl acetate/Hexane 20:80, $R_{f\ product}$=0.75). Upon completion, the reaction mixture purified by passing the crude reaction mixture through a silica plug using ethyl acetate as the solvent. The ethyl acetate was removed using rotary evaporation resulting in the desired product. Yielding a white solid: 536.59 mg (89.1%)

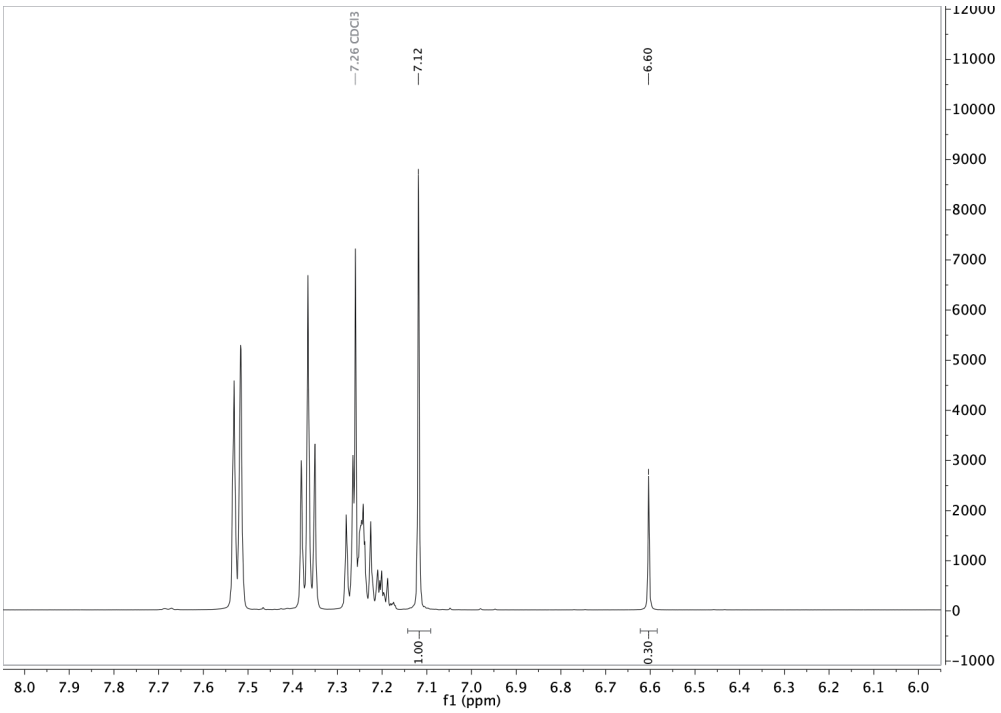
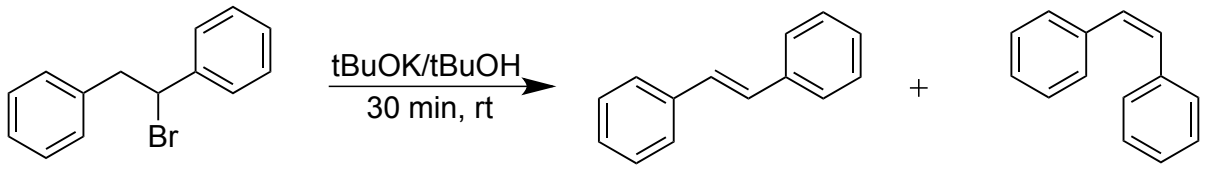


Figure S13: The ^{1}H NMR (600 MHz, $CDCl_3$) of the Wittig reaction mixture to make a random percentage of cis- and trans-stilbene. Where cis- and trans-stilbene are known to have the chemical shift for the olefin's proton to be at 6.57 ppm and 7.15 ppm respectively.[2] Upon integration of the olefin's protons the percent conversion between cis- and trans-stilbene is 23% and 77% respectively.

Second Order Elimination (E_2) Reaction Materials:

All materials were used as received unless otherwise stated. Commercially available, (1-bromo-2-phenylethyl)benzene (95%) from Enamine Building Blocks and potassium tert-butoxide (98+%) from Acros Organics was used. Certified tert-butanol from Fisher Chemical was used as the solvent.

E_2 Reaction:



A round bottom flask was charged with: (1-bromo-2-phenylethyl)benzene (0.88 mmol, 0.23 g), potassium tert-butoxide (0.86 mmol, 97.0 mg) and dissolved in tert-butanol (7 mL). The reaction was allowed to stir at room temperature (22-23 ^{o}C) for 30 minutes. Upon completion the reaction was extracted with DCM (30 mL total) and water saturated with Na_2CO_3. The organic layer was dried over

sodium sulphate prior to concentrating the resulting reaction using rotatory evaporation. Yielding a white solid: 0.1574 g (98.9% yield).

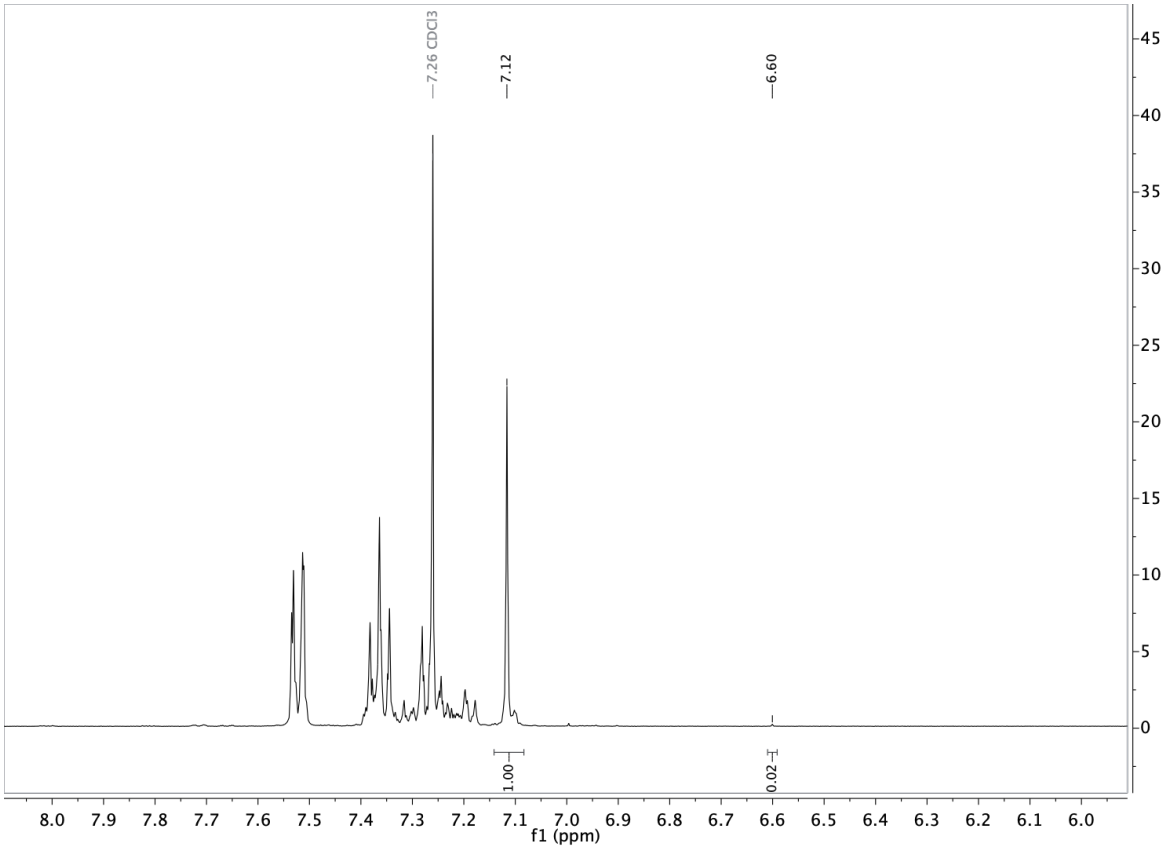
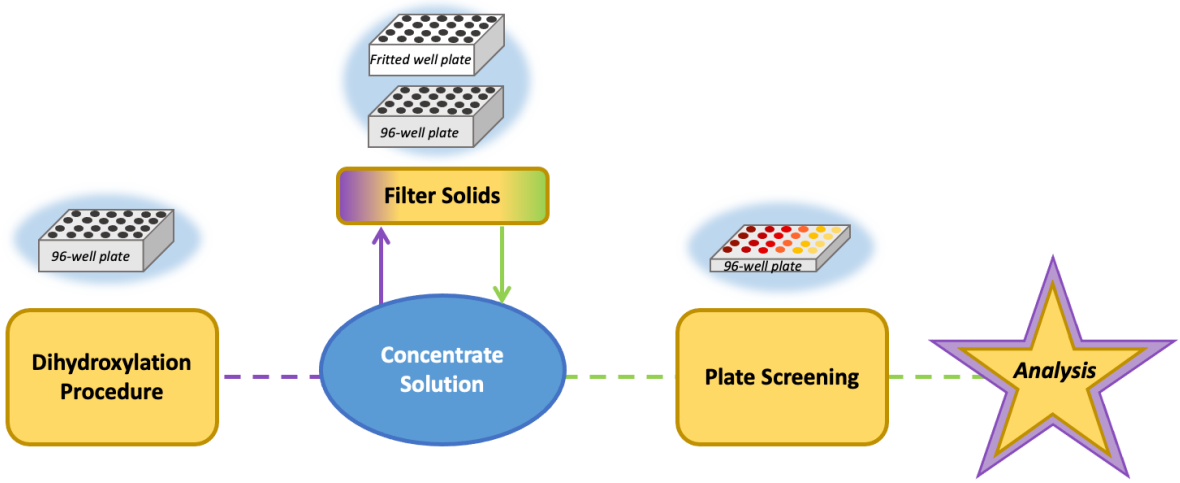


Figure S14: The ^{1}H NMR (600 MHz, $CDCl_3$) of the E_2 reaction mixture. Where cis- and trans-stilbene are known to have the chemical shift for the olefin's proton to be at 6.57 ppm and 7.15 ppm respectively.[2] Upon integration of the olefin's protons the percent conversion between cis- and trans-stilbene is 2% and 98% respectively.

General Work-Flow:



96-well Plate Analysis:

Arrays were made by mixing stock solutions of **H** and **PV**, with analyte stock solutions within a Costar EIA/RIA polystyrene 96-well flat bottom plates. Absorbance spectra were recorded at ambient temperature on a BioTek SynergyTM 4 multi-detection microplate reader. Eppendorf Repeater Stream electronic micropipettes were used to add stock solutions to the 96-well plate. Each well contained a total solution volume of 300 µl, and methanol buffer and acetonitrile solvents in 1:1 ratio. After making the plate, it was sealed with a UC-500 sealing film to prevent solvent evaporation and allowed to equilibrate for 3 minutes before reading.

Plate Screening:

Screening plates were designed for each diol to discern the response curve as the ratio of guest was varied from 100% erythro through to 100% threo diol. The architecture of the screening plate for **G5ER** and **G5TH** shown in **Figure S15**. Various concentrations of guest were analysed to determine the optimal concentration for both qualitative and quantitative analysis, in the case of **G5ER** and **G5TH** the concentrations used were: 2 mM, 5 mM, 10 mM, 25 mM, 50 mM and 100 mM. The plate was sealed with the sealing film, allowed to equilibrate for three minutes before the absorbance spectra was recorded and analysed.

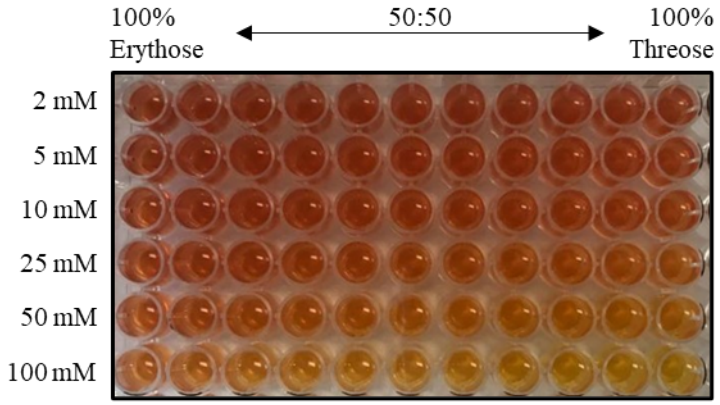


Figure S15: Layout of the screening plate shown, solvent 1:1 methanol/acetonitrile, pH 8.1-8.4, 10mM buffer (*para*-toluenesulfonic acid and Hünig's base), **H** (400µM) and **PV** (150µM) with analyte **G5ER** and **G5TH** in the concentrations 2mM – 100 mM.

Similarly, the optimal concentration of guest for selected threo and erythro diol guests were determined in this way using the sensing ensemble **H** (400µM) and **PV** (150µM), allowing response

curves at each concentration to be plotted (**Figure S16 – S22**). All screening plate analyses were carried out in 1:1 methanol/acetonitrile, 10 mM *para*-toluenesulfonic acid and Hünig's base buffer, pH: 8.1-8.4. All measurements were taken at 25°C, and curves analysed using Origin (Microcal Software, Inc.).

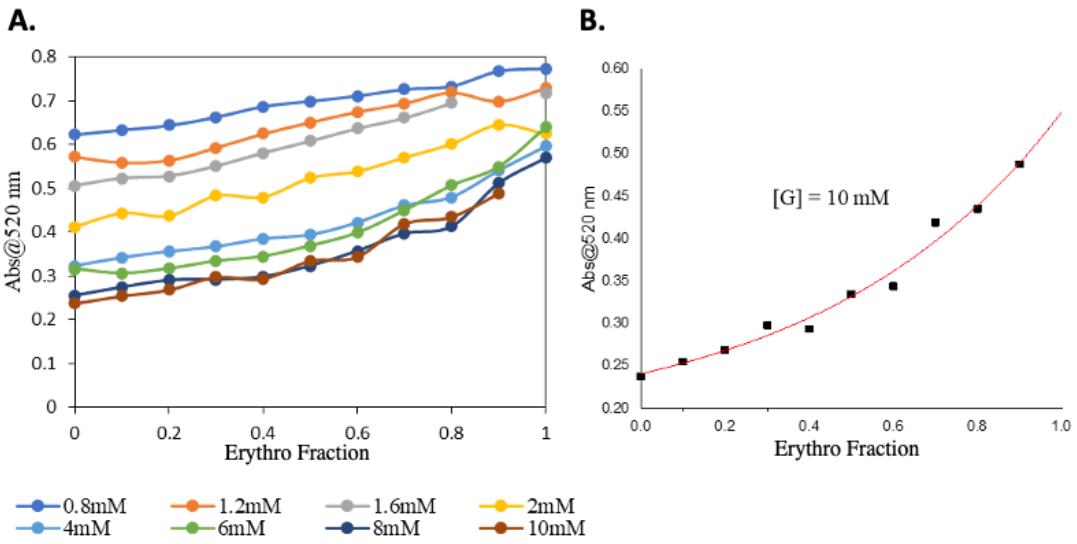


Figure S16: Screening plate response curves for **G2TH** and **G2ER**, solvent 1:1 methanol/acetonitrile, pH 8.1-8.4, 10mM buffer (*para*-toluenesulfonic acid and Hünig's base), **H** (400µM) and **PV** (150µM). A. Response curves for **G2TH** and **G2ER** concentrations 0.8 mM – 10 mM. B. Optimal concentration for **G2TH** and **G2ER** chosen to be 10 mM.

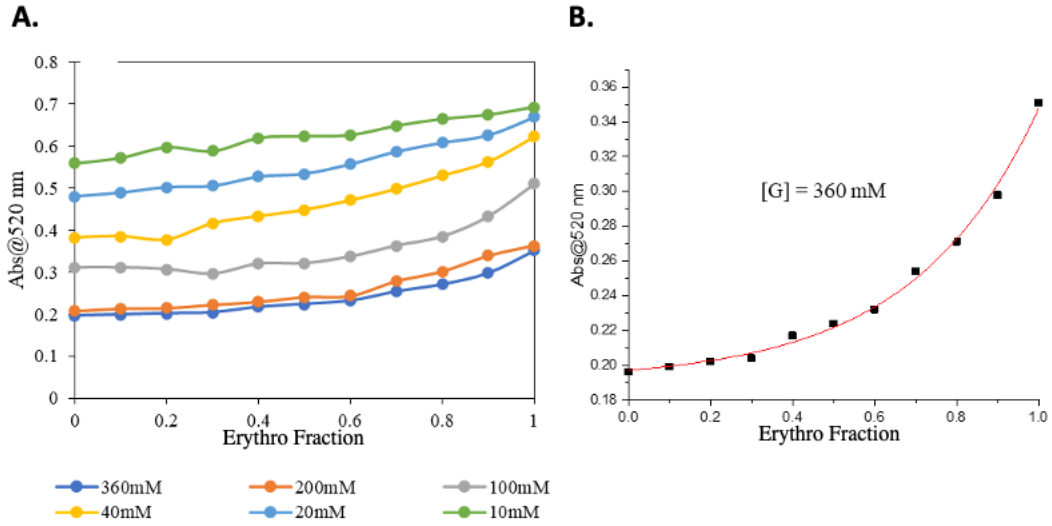


Figure S17: Screening plate response curves for **G1ER** and **G1TH**, solvent 1:1 methanol/acetonitrile, pH 8.1-8.4, 10mM buffer (*para*-toluenesulfonic acid and Hünig's base), **H** (400µM) and **PV** (150µM). A. Response curves for **G1ER** and **G1TH** concentrations 10 mM – 360 mM. B. Optimal concentration for **G1ER** and **G1TH** was chosen to be 360 mM.

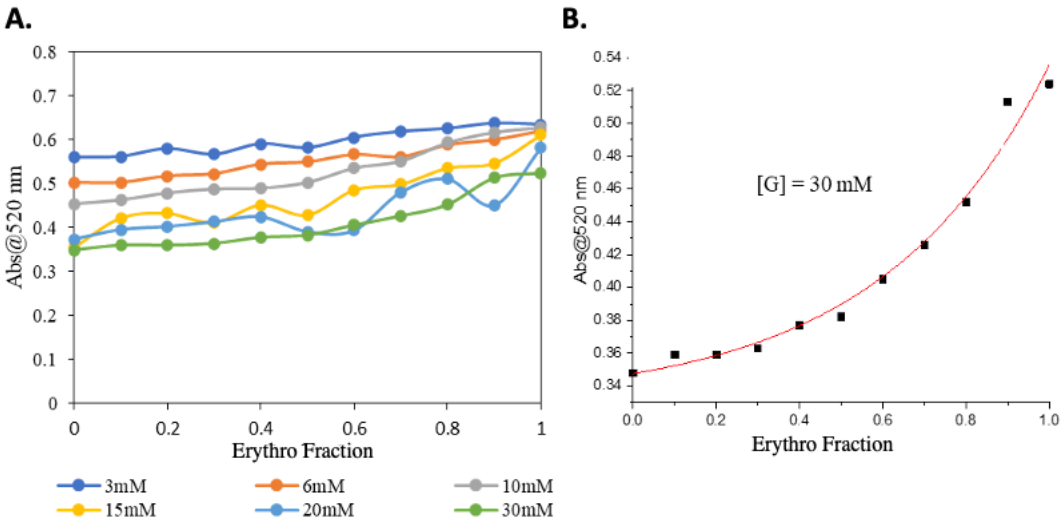


Figure S18: Screening plate response curves for **G3TH** and **G3ER**, solvent 1:1 methanol/acetonitrile, pH 8.1-8.4, 10mM buffer (*para*-toluenesulfonic acid and Hünig's base), **H** (400µM) and **PV** (150µM). A. Response curves for **G3TH** and **G3ER** concentrations 3 mM – 30 mM. B. Optimal concentration for **G3TH** and **G3ER** set was chosen to be 30 mM.

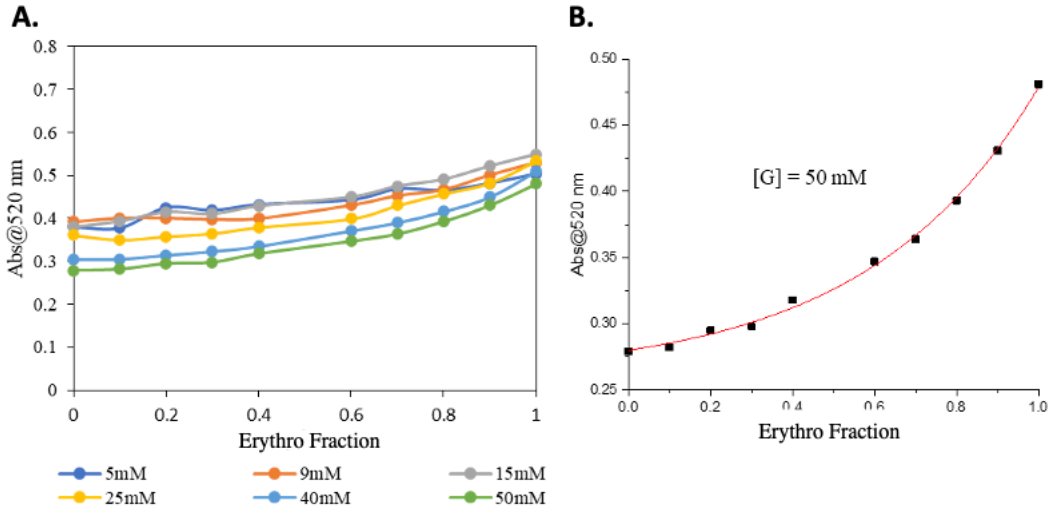


Figure S19: Screening plate response curves for **G3TH** and **G4ER**, solvent 1:1 methanol/acetonitrile, pH 8.1-8.4, 10mM buffer (*para*-toluenesulfonic acid and Hünig's base), **H** (400µM) and **PV** (150µM). A. Response curves for **G3TH** and **G4ER** 5 mM – 50 mM. B. Optimal concentration for **G3TH** and **G4ER** was chosen to be 50 mM.

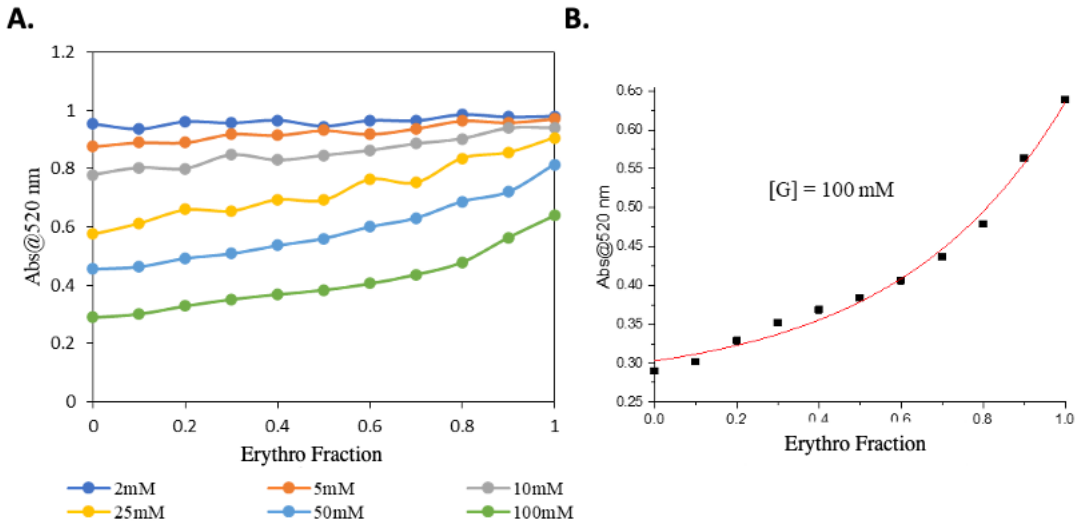


Figure S20: Screening plate response curves for **G5TH** and **G5ER**, solvent 1:1 methanol/acetonitrile, pH 8.1-8.4, 10mM buffer (*para*-toluenesulfonic acid and Hünig's base), **H** (400µM) and **PV** (150µM). A. Response curves for **G5TH** and **G5ER** concentrations 2 mM – 100 mM. B. Optimal concentration for **G5TH** and **G5ER** was chosen to be 100 mM.

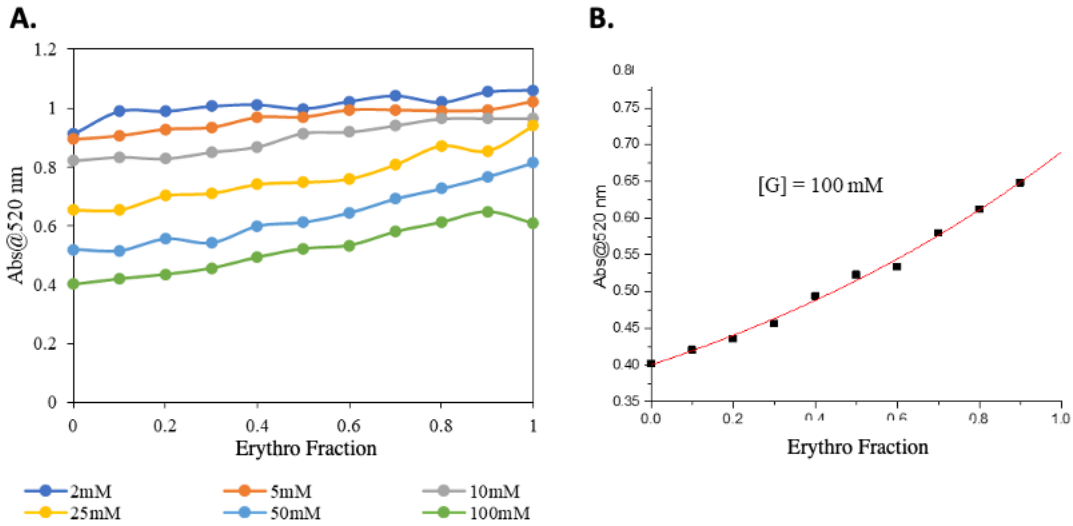


Figure S21: Screening plate response curves for **G7ER** and **G7TH**, solvent 1:1 methanol/acetonitrile, pH 8.1-8.4, 10mM buffer (*para*-toluenesulfonic acid and Hünig's base), **H** (400µM) and **PV** (150µM). A. Response curves for **G7ER** and **G7TH** concentrations 2 mM – 100 mM. B. Optimal concentration for **G7ER** and **G7TH** was chosen to be 100 mM.

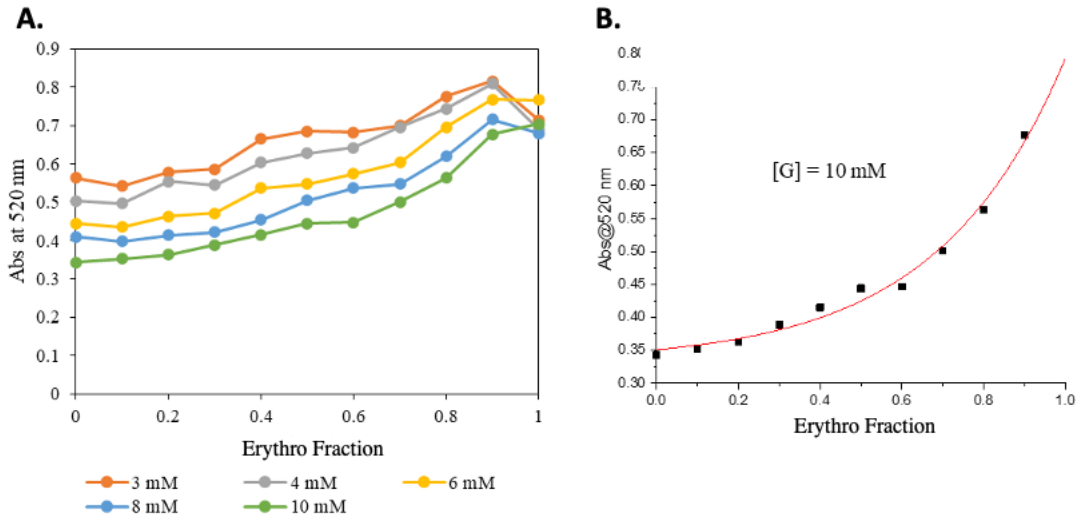


Figure S22: Screening plate response curves for **G6TH** and **G6ER**, solvent 1:1 methanol/acetonitrile, pH 8.1-8.4, 10mM buffer (*para*-toluenesulfonic acid and Hünig's base), **H** (400µM) and **PV** (150µM). A. Response curves **G6TH** and **G6ER** concentrations 3 mM – 10 mM. B. Optimal concentration for **G6TH** and **G6ER** was chosen to be 10 mM.

Plate Processing:

1) **OsO_4 reaction:**

Arrays were made by mixing the desired alkene, NMO and OsO_4 stock solutions within a 96-deep-well Glass coated PP Microplate, 2.4 mL. Eppendorf Repeater Streamer electronic micropipettes were used to add stock solutions to the plate. In the case of the **G3T**/**G3C** set, 60 mM of the alkene was added with 1.1 eq. NMO and 0.04 eq., OsO_4, acetone/water (10:1), into each well. After making the plate, it was sealed with a UC-500 sealing film to prevent solvent evaporation and placed on nutating mixer (VWR International) shaker for 24 hours. Similarly, with the **G2T**/**G2C** set, 20 mM of the alkene was added together with 1.1 eq. NMO, 0.5 eq. OsO_4, plus 1 eq. DABCO, acetone/water (10:1), before sealing and placing on the shaker for 24 hours.

After this period 2.5 eq. of $NaHSO_3$ to OsO_4, was added to quench the reaction before being transferred to DD-4X Genevac to remove solvent. Then the remaining crude reaction mixture is re-dissolved in ethyl acetate (1.5 mL) and the impurities are removed by filtering through a 96-deep-well SiliaPrep (Silicycle, 40-60 µm, 100 mg) using a SUPELCO 96-well plate PlatePrep Vacuum Manifold before removing residual solvent using the Genevac.

2) Analysis:

The solid diol left remaining in the plate is dissolved in degassed 240µl methanol, 10mM buffer (*para*-toluenesulfonic acid and Hünig's base, pH 8.1-8.4), sealed with a sealing film, and sonicated using 2510 Branson (Bransonic Sonicator) until fully dissolved. 120 µl of the analyte solution is transferred to a Costar EIA/RIA polystyrene 96-well plate into which the sensing ensemble, **H** (400µM) and **PV** (150µM) is added. Each well contains a total solution volume of 300 µL, and methanol buffer and acetonitrile solvents in 1:1 ratio. After making the plate, it was sealed with a UC-500 sealing film to prevent solvent evaporation and allowed to equilibrate for 3 minutes before reading. A Cytation|3 Imaging Reader (BioTek) was used and read the absorbance at 520 nm for each well a total of three times for the 3-, 6-, and 9-minute time mark.

The blank sample consisted of a 1:1 methanol and acetonitrile and was placed in each row of the 96-well plate as a reference. While a control for each plate analysis was prepared under the same conditions as (1) and (2), however, had the absence of any alkene. Additionally, we made calibration standards with commercially available diols for our calibration curves.

Colorimetric High-Throughput Screening of Alkenes:

Following the method described in (1)-Osmium Reaction and (2)-Analysis (see above), two different reactions, Wittig and E_2, were conducted to create a mixture of cis- and trans- stilbene. Additionally, unknown mixtures were prepared to further assess the procedural error. The high-throughput screening was conducted to determine the corresponding erythro and threo diols.

Wittig: Material used from, supplementary information Wittig Reaction, was used for the Wittig reaction and compared to the ^{1}H NMR results shown in **Figure S13**. The Wittig reaction mixture of cis- and trans-stilbene was used for the high-throughput processing. **Figure S23** is a visual representation of the 96-well plate used for the analysis. The calibration curve (**Figure S24**) and analysis previously described, determined the reaction to have 19% cis-stilbene.

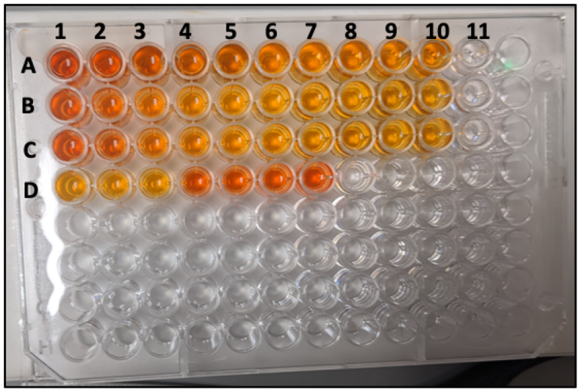


Plate Layout:
A1:C1 = 100 % cis-stilbene
A2:C2 = 90:10 cis-stilbene/trans-stilbene
A3:C3 = 80:20 cis-stilbene/trans-stilbene
A4:C4 = 70:30 cis-stilbene/trans-stilbene
A5:C5 = 60:40 cis-stilbene/trans-stilbene
A6:C6 = 50:50 cis-stilbene/trans-stilbene
A7:C7 = 60:40 cis-stilbene/trans-stilbene
A8:C8 = 70:30 cis-stilbene/trans-stilbene
A9:C9 = 80:20 cis-stilbene/trans-stilbene
A10:C10 = 90:10 cis-stilbene/trans-stilbene
A11:C11 = blank
D1:D3 = 100% trans-stilbene
D4:D6 = Wittig
D7 = control
D8 = blank
A11:C11= blank

Figure S23: Representative plate of the cis- and trans-stilbene high-through put screening from the Wittig reaction mixture.

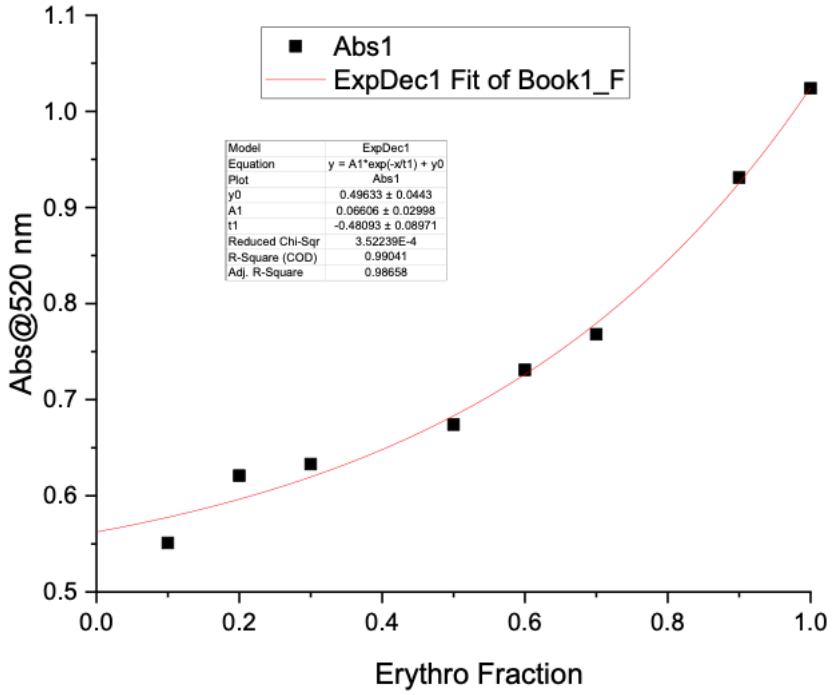


Figure S24: The calibration plot from the plate analysis of the Wittig reaction mixture.

E_2 Reaction: Material used from the supplementary information section, Second Order Elimination Reaction, was used for the E_2 reaction and compared to the ^{1}H NMR results shown in **Figure S14**.

Preparations of an Unknown Mixutres for G2T/G2C and G3T/G3C: Each unknown was prepared by author C. Y. Yao and author H. S. N. Crory blindly analysed the data. Then author C. Y. Yao compared the unknown she prepared to was H. S. N. Crory determined through the analysis.

Color Wheel Experiment:

The color wheel experiment was designed to alter the color change of the IDA to different regions of the color wheel, in order to discern the most distinguishable visual difference between erythro and threo samples. Food coloring dyes were diluted by a factor of 25 in methanol, 10 mM buffer (*para*-toluenesulfonic acid and Hünig's base). Three solutions, **E**, **F** and **G** were made, whose compositions were: solution **E**, 150 μM **PV** and 400 μM **H**; solution **F** 150 μM **PV**, 400 μM **H** and 10 mM **G2ER** and solution **G** 150 μM **PV**, 400 μM **H** and 10 mM **G2TH**. The total volume of the solution contained methanol buffer and acetonitrile solvents in 1:1 ratio. Into solutions **E**, **F** and **G** was placed 50 μl or 25 μl of each dye, shown in **Figure S25-S26**.

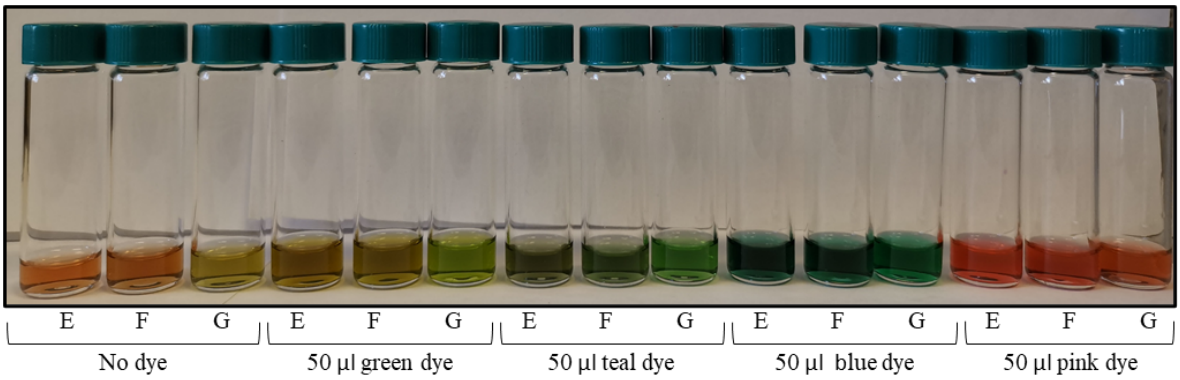


Figure S25: Color wheel experiment: solution **E**, 150 μM **PV** and 400 μM **H**; solution **F** 150 μM **PV**, 400 μM **H**, 10 mM **G2ER** and solution **G** 150 μM **PV**, 400 μM **H** and 10 mM **G2TH**. 1:1 methanol buffer and acetonitrile. 50μl of each food coloring solution was added as indicated.

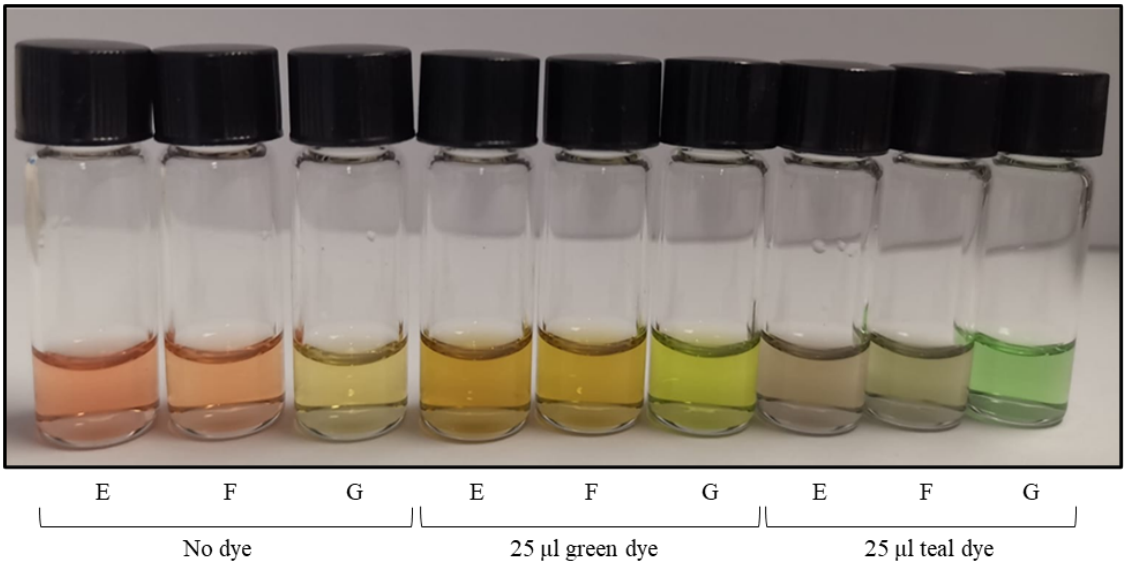


Figure S26: Colour wheel experiment: solution E, 150 µM **PV** and 400 µM **1**; solution F 150 µM **PV**, 400 µM **H**, 10 mM **G2ER** and solution G 150 µM **PV**, 400 µM **H** and 10 mM **G2TH**. 1:1 methanol buffer and acetonitrile. 25µl of each food coloring solution was added as indicated.

General Procedure for Osmium Tetroxide Dihydroxylation of Alkenes:

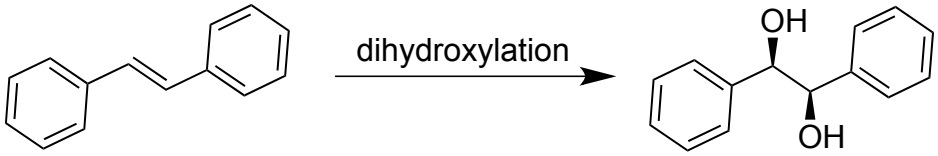
A 206.15 mM solution containing the desired alkene and a binary solvent system of acetone/water (1:3) was combined in a round bottom flask. Osmium tetroxide (0.25 mmol), and 4-methylmorpholine N-oxide (14.83 mmol) were subsequently added to the round bottom flask. The reaction was able to stir at room temperature (22-23 ºC) for 12-72 hours. Upon completion, sodium bisulfite (14.83 mmol) was added directly to the reaction vessel prior to handling. The alkene was then extracted with DCM (100 mL total) and water (50 mL total). The organic layers were combined and dried over magnesium sulfate. Prior to concentrating the organic layer using rotary evaporation, the organic layer was passed through a silica gel plug.

Osmium Tetroxide Dihydroxylation Materials:

Unless otherwise state all materials for the dihydroxylation were used as received. Laboratory grade sodium hydrosulfite (Fisher Chemical) was used to quench the reaction. Certified ACS Grade Acetone (Fisher Chemical) was used for the solvent and 4-menthylmorpholine N-oxide (>95%, TCI) was used. Osmium tetroxide (Sigma-Aldrich) was stored as a 0.1277 M solution in tert-butanol (Fisher Chemical).

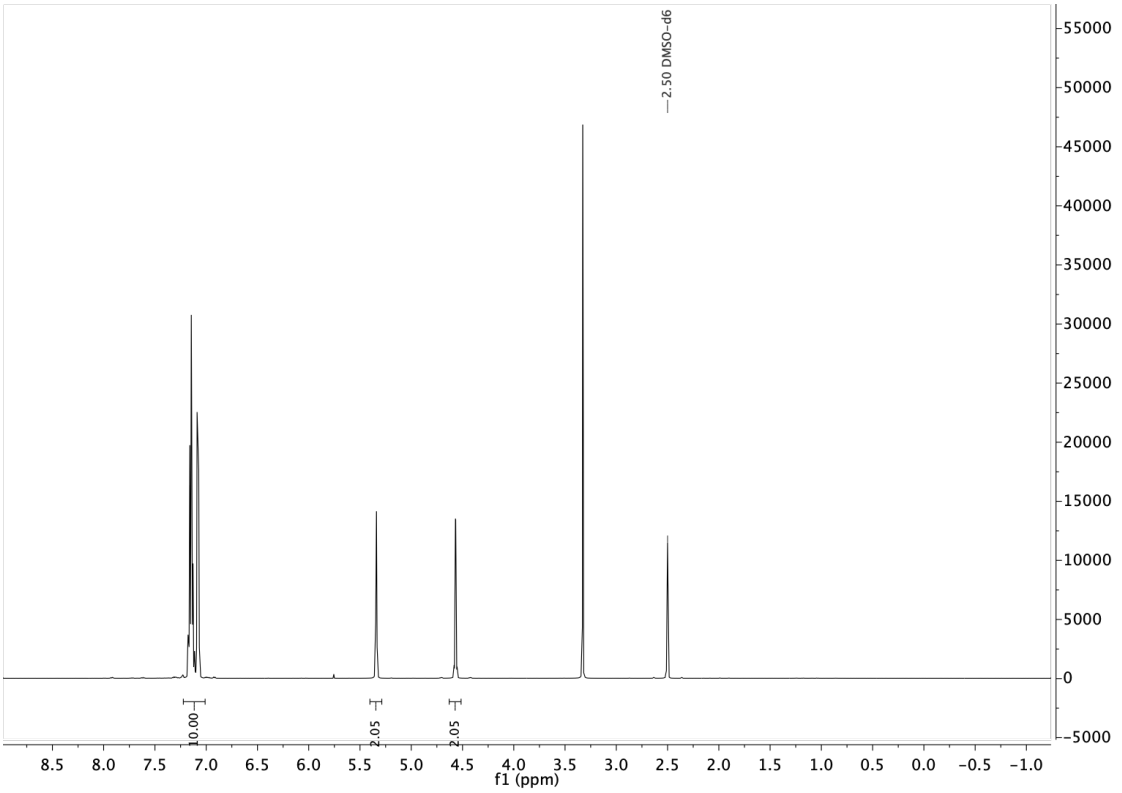
Magnesium sulfate (Fisher Chemical) was used as a drying reagent. Premium grade silica gel (Sorbtech, 60 Å, 230x400 mesh) was used for the silica gel plug. Certified ACS grade DCM was used for the extraction.

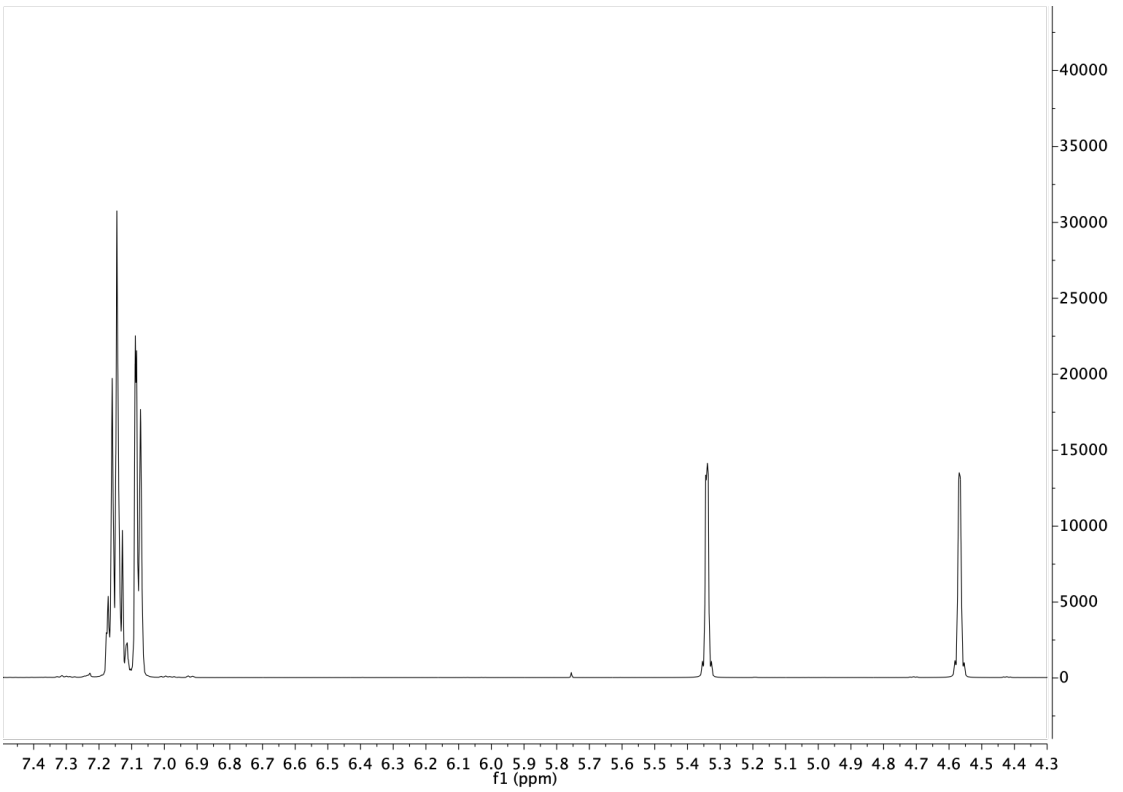
Osmium Tetroxide Dihydroxylation Reactions:



The alkene, trans-stilbene (1.15 g, 6.38 mmol) manufactured by Sigma-Aldrich 96%, was subjected to the previously described dihydroxylation procedures. Once purified, 173.58 mg of the dehydroxylated product was isolated as a white solid. Yield: 12.7%

^{1}H NMR (600 MHz, DMSO-d_6): δ 7.11 (10H, m), 5.34 (2H, m), 4.57 (2H, m). ^{13}C NMR (150 MHz, DMSO-d_6): δ 142.82, 127.73, 127.63, 127.15, 78.12.

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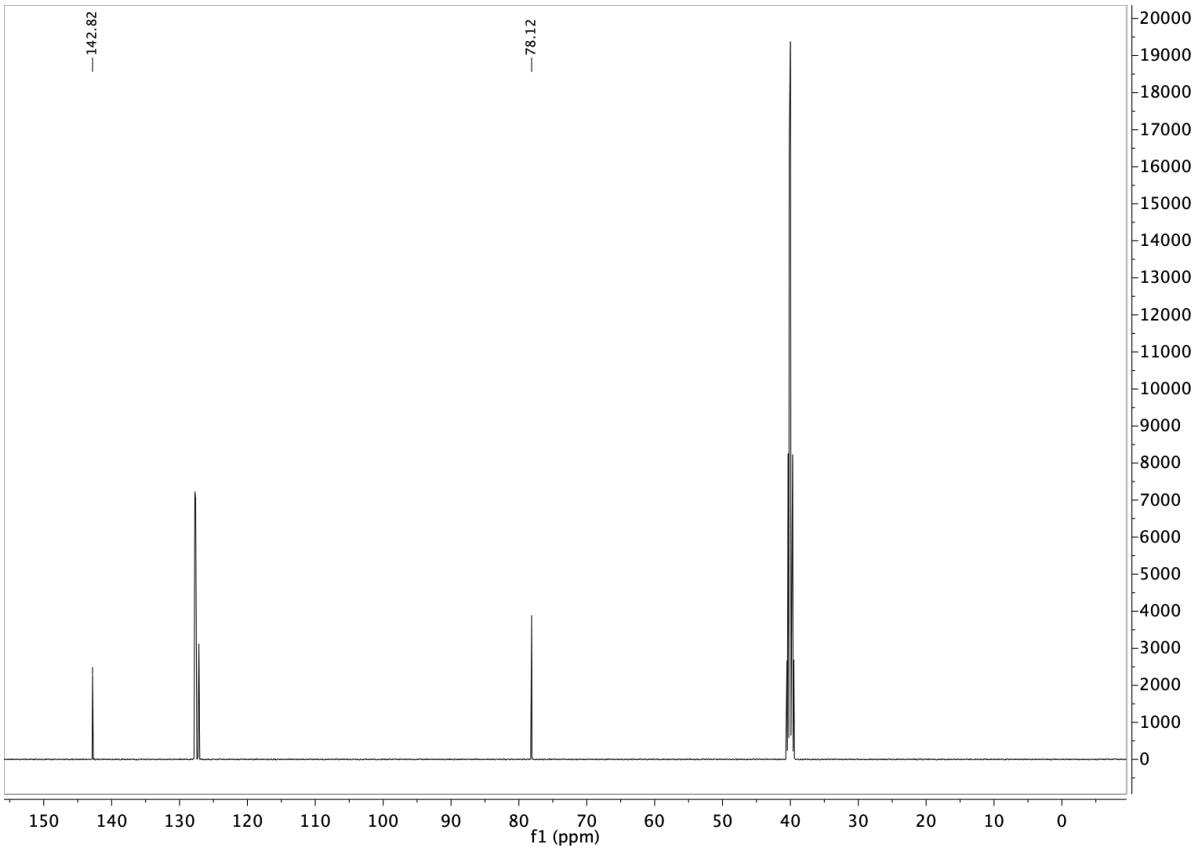
30000
25000
20000
15000
10000
5000
0
7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3
f1 (ppm)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78.12
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19000
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
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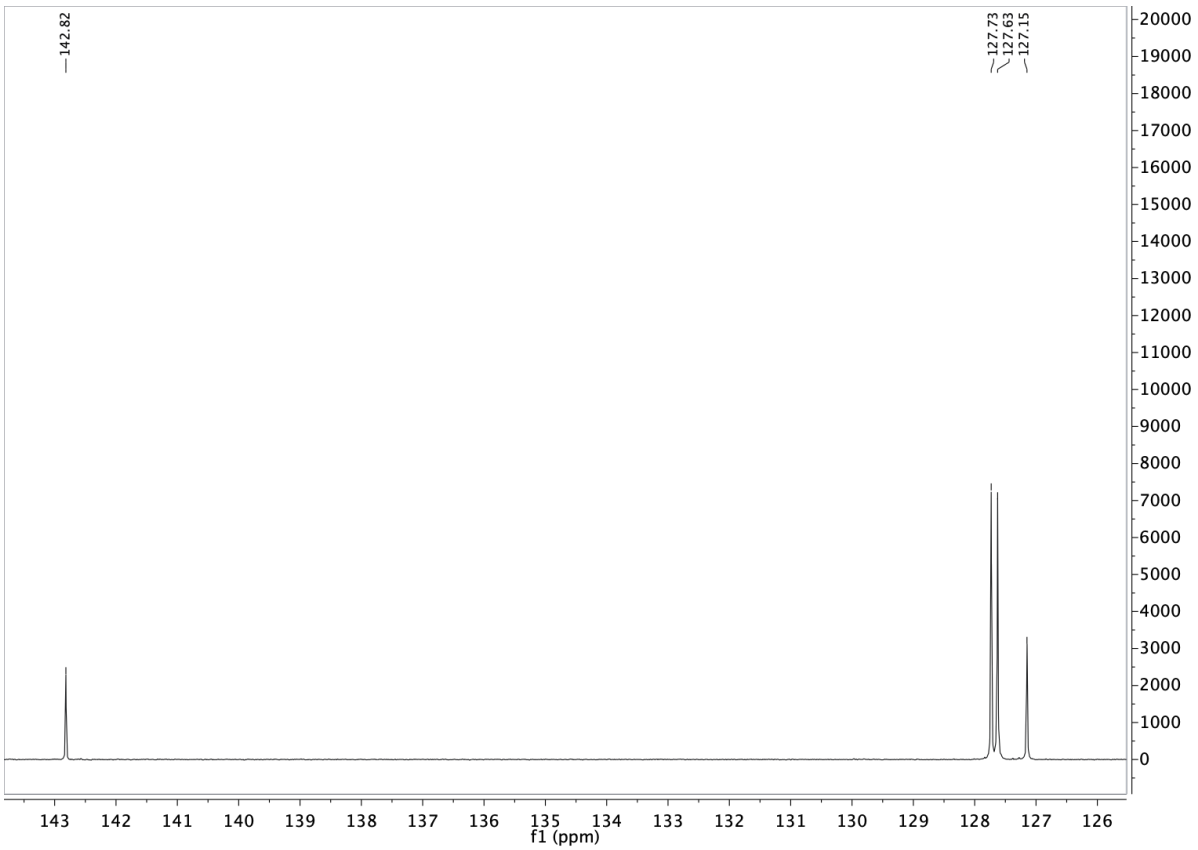
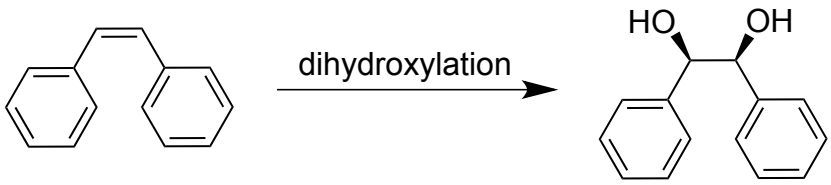
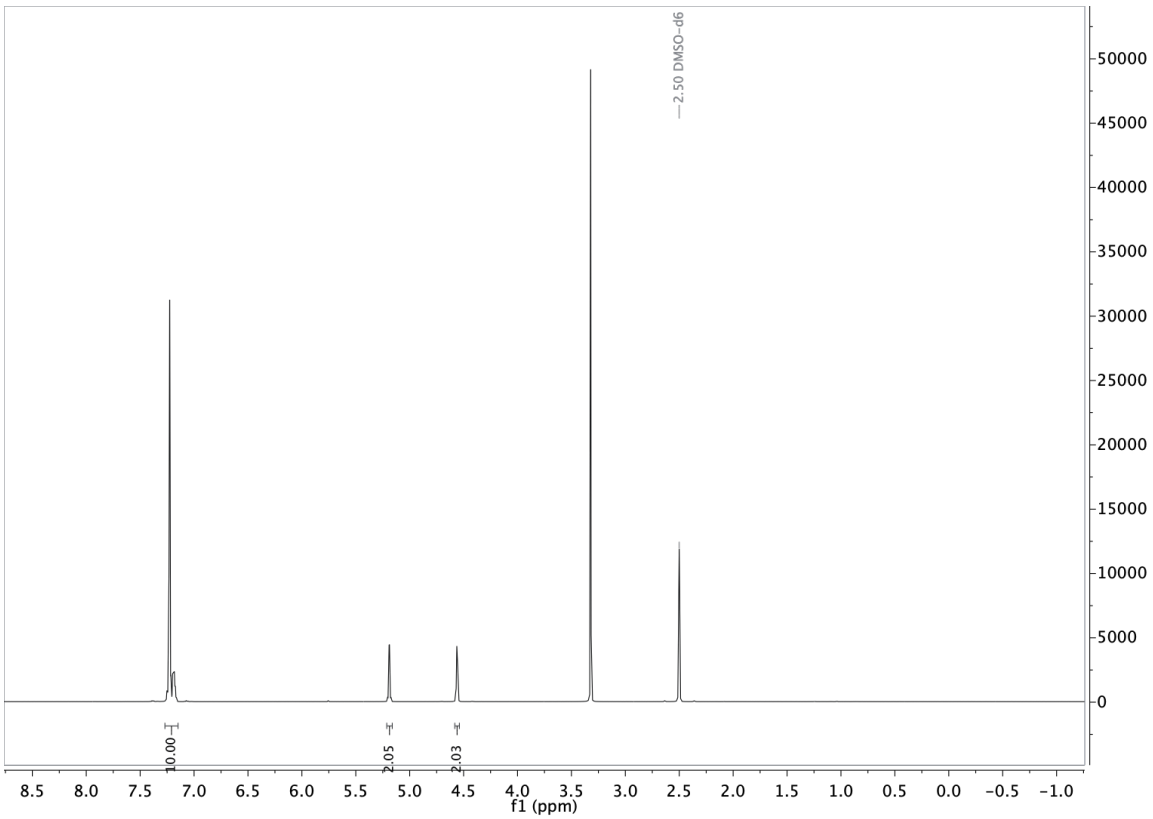
f1 (ppm)





The alkene, cis-stilbene (1.0 mL, 5.61 mmol) manufactured by Aldrich 96% was subjected to the dihydroxylation procedures previously described. Upon purification, 318.66 mg of the dehydroxylated product was isolated as a white solid. Yield: 26.5%

^{1}H NMR (600 MHz, DMSO-d_6): δ 7.21 (10H, m), 5.19 (2H, m), 4.56 (2H, m). ^{13}C NMR (150 MHz, DMSO-d_6): δ 143.75, 127.84, 127.71, 127.07, 77.45.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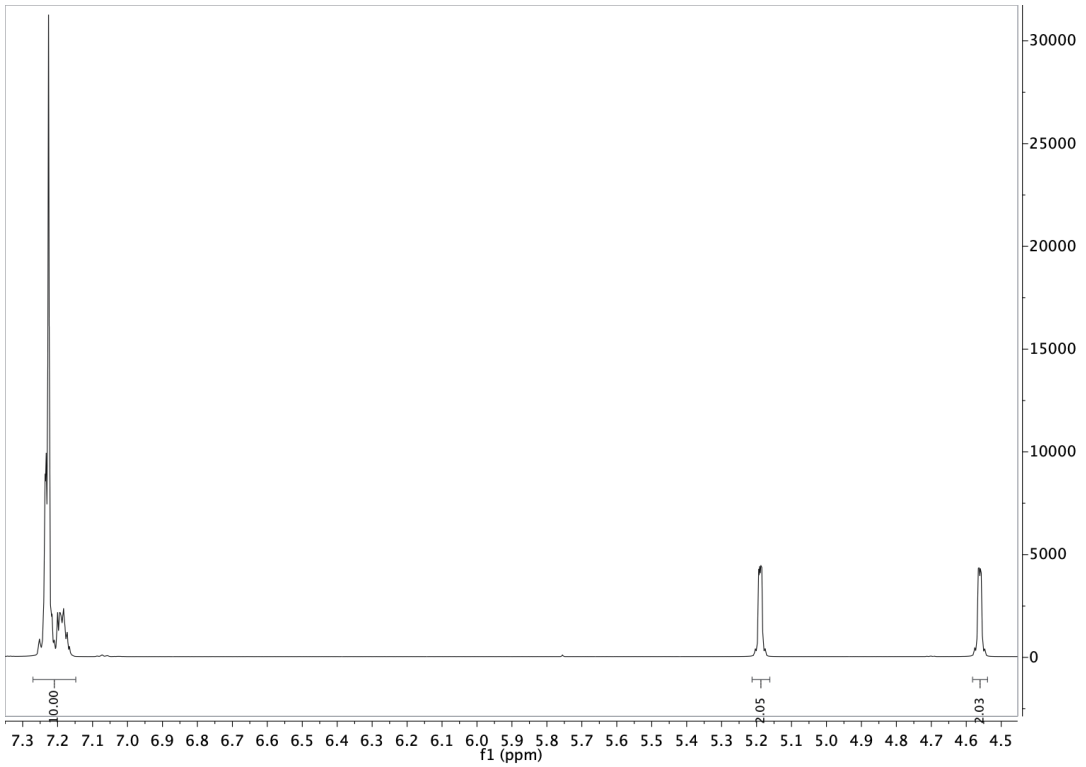
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
10.00
2.05
2.03
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)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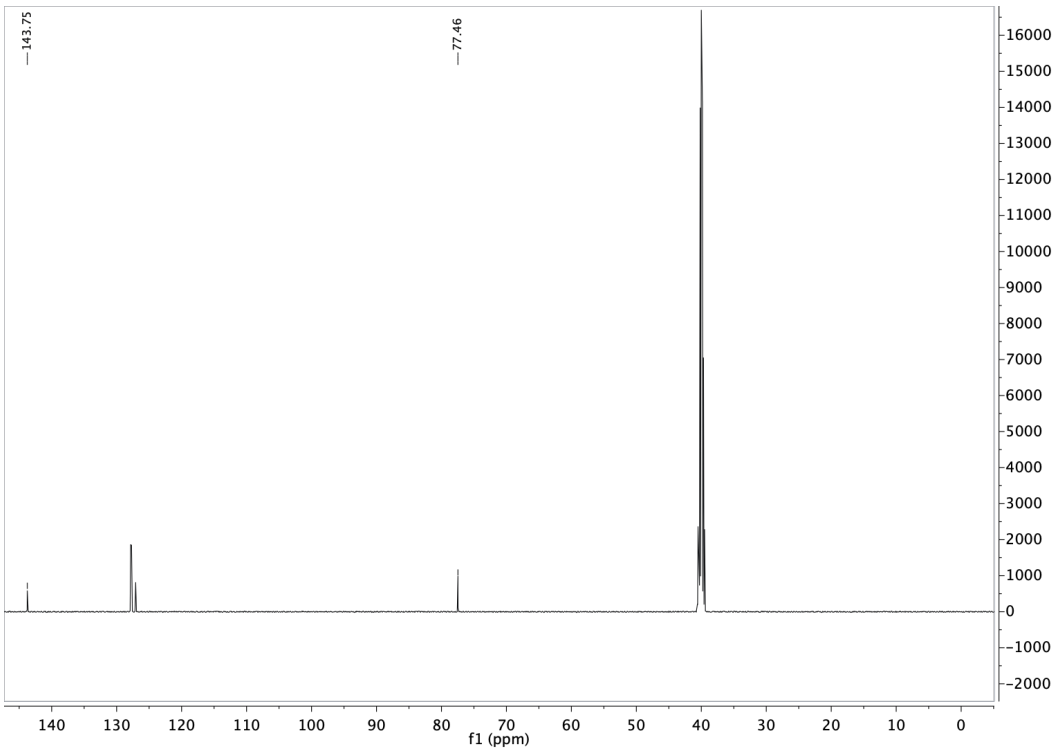
20000
15000
10000
5000
0
10.00
2.05
2.03
7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5
f1 (ppm)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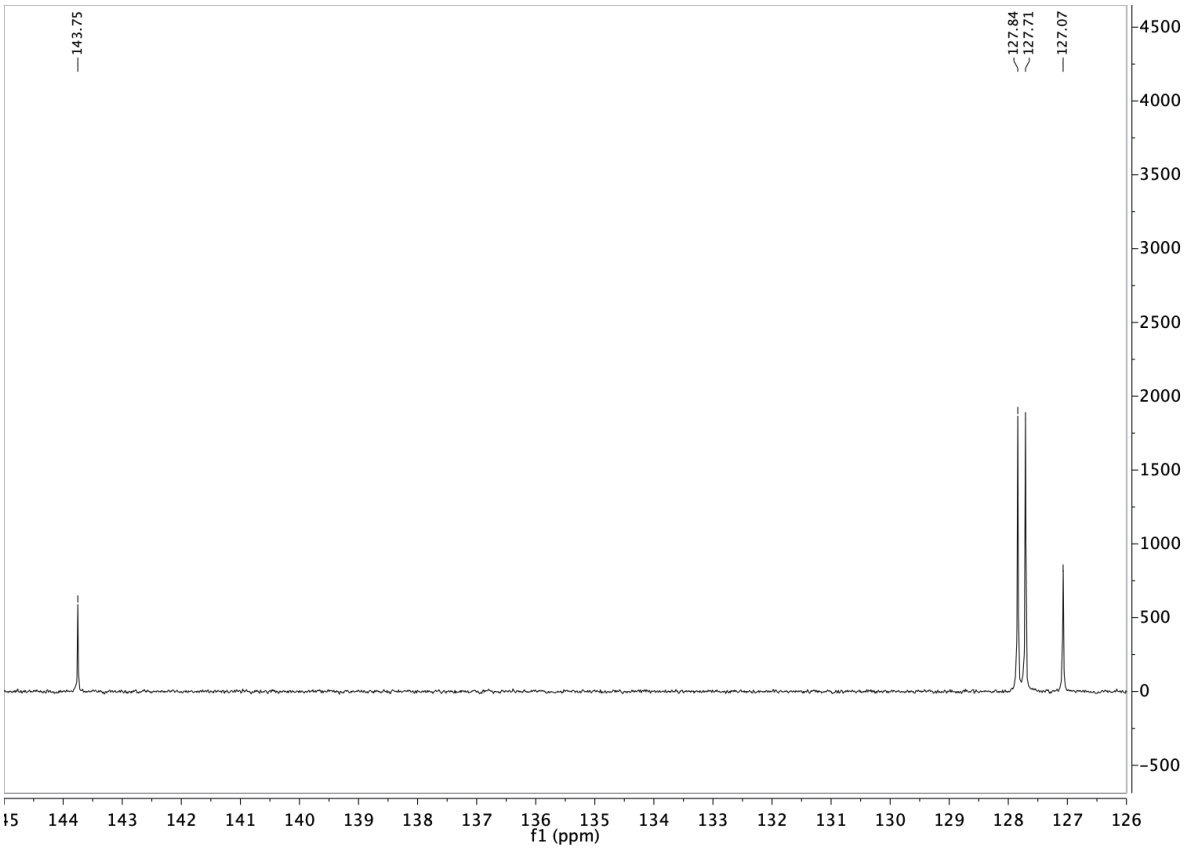
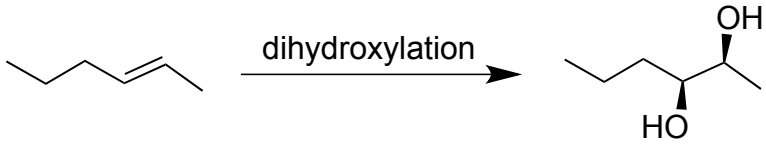
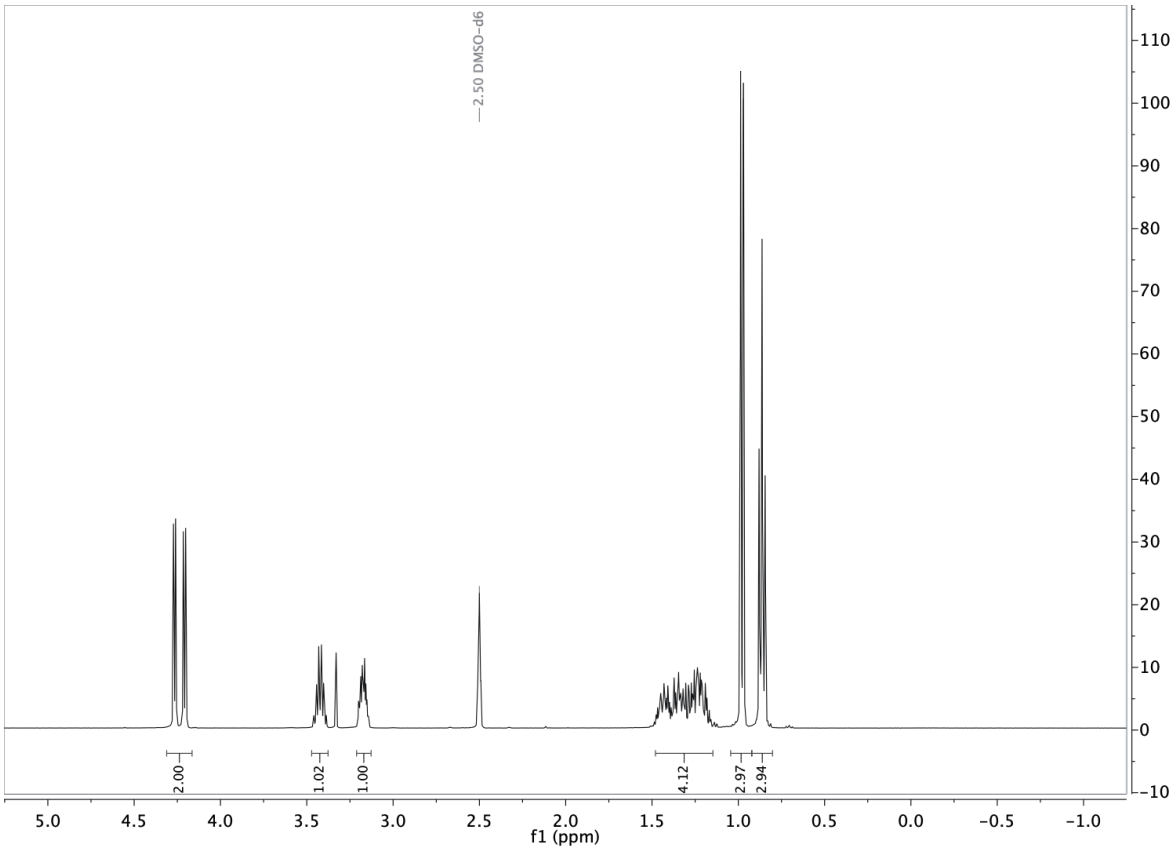
77.46
f1 (ppm)

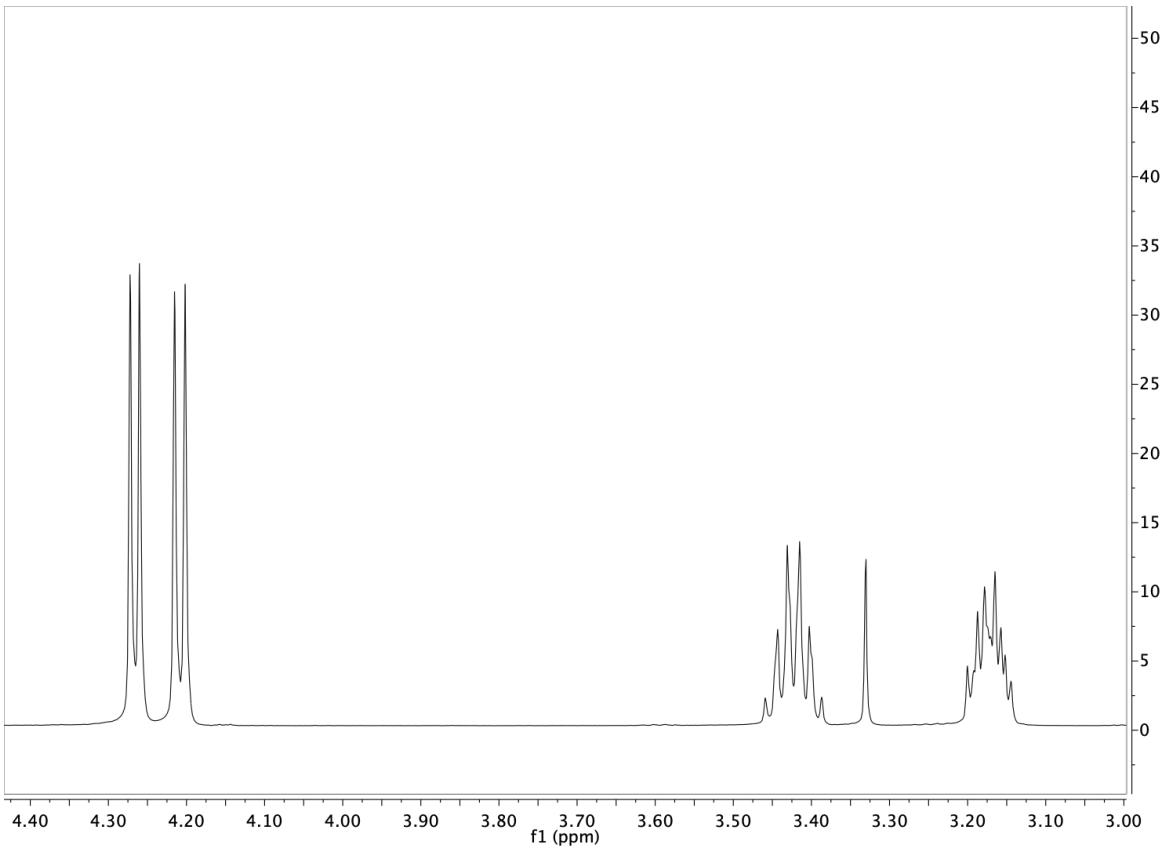

143.75
127.84
127.71
127.07
f1 (ppm)


dihydroxylation
OH
HO

Trans-2-hexene (1.5 mL, 11.92 mmol) purchased from Sigma-Aldrich 95%, underwent the dihydroxylation conditions previously described. Upon purification, 798.39 mg of the desired product resulted in a clear oil. Yield: 56.7%

^{1}H NMR (600 MHz, DMSO-d_6): δ 4.24 (2H, dd, J=5.2 Hz), 3.42 (1H, sx, J=5.7 Hz), 3.17 (1H, m), 1.31 (4H, m), 0.98 (1H, d, J=6.2 Hz), 0.86 (1H, t, J=7.0 Hz). ^{13}C NMR (150 MHz, DMSO-d_6): δ 74.38, 69.78, 34.42, 19.21, 19.05, 14.62.

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40
35
30
25
20
15
10
5
0
4.40
4.30
4.20
4.10
4.00
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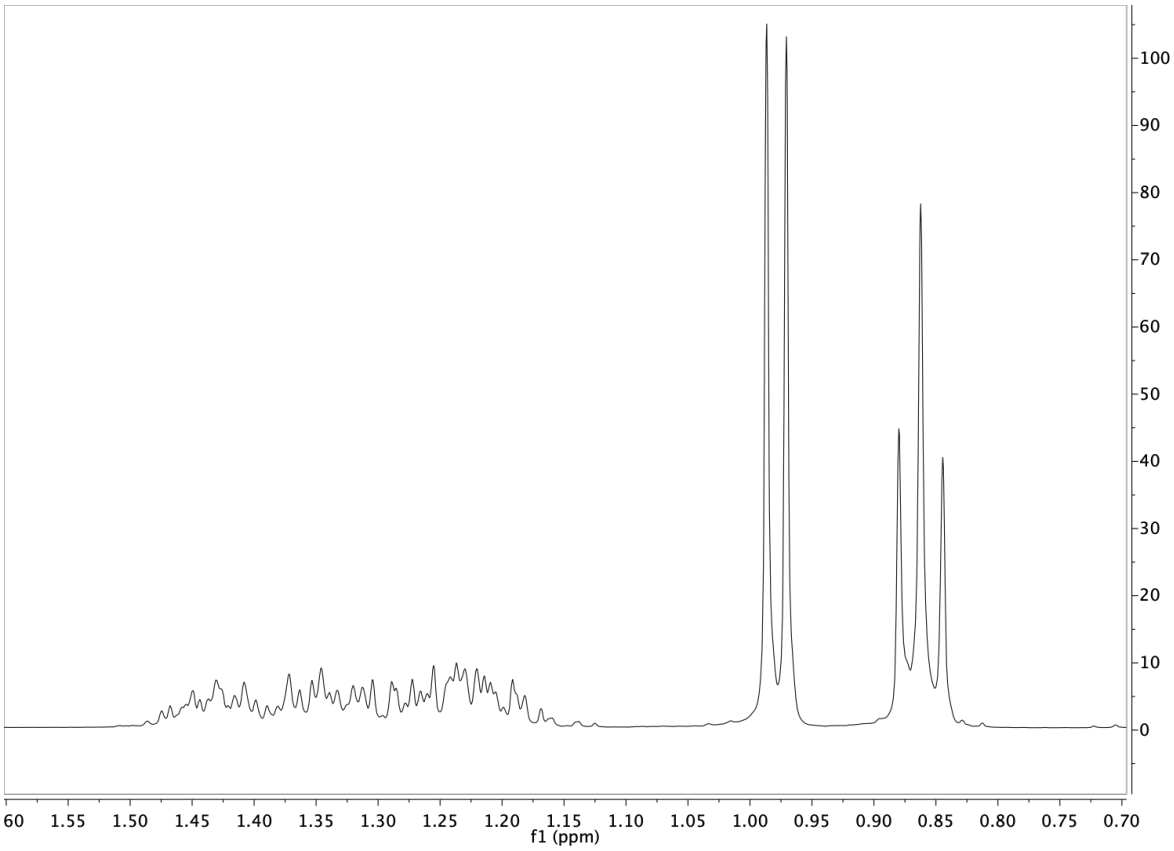
3.30
3.20
3.10
3.00
f1 (ppm)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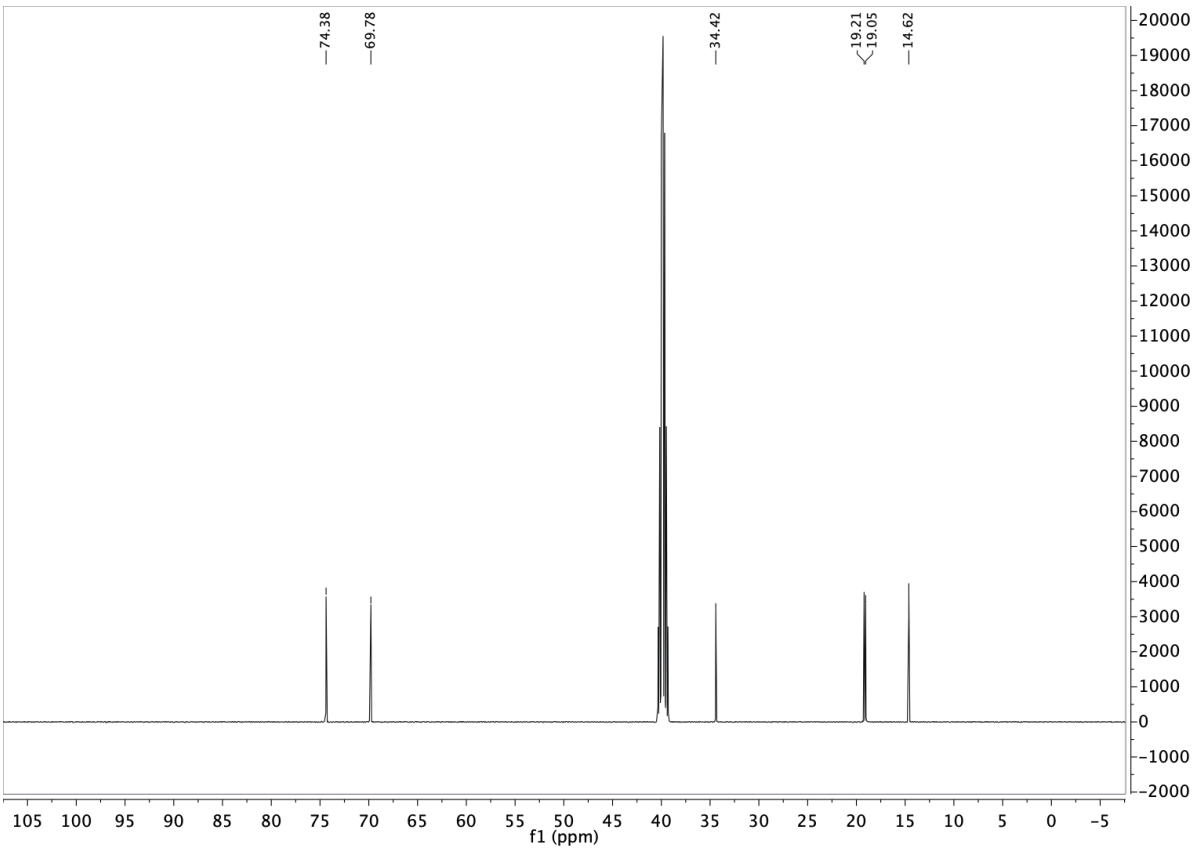
90
80
70
60
50
40
30
20
10
0
60
1.55
1.50
1.45
1.40
1.35
1.30
1.25
1.20
1.15
1.10
1.05
1.00
0.95
0.90
0.85
0.80
0.75
0.70
f1 (ppm)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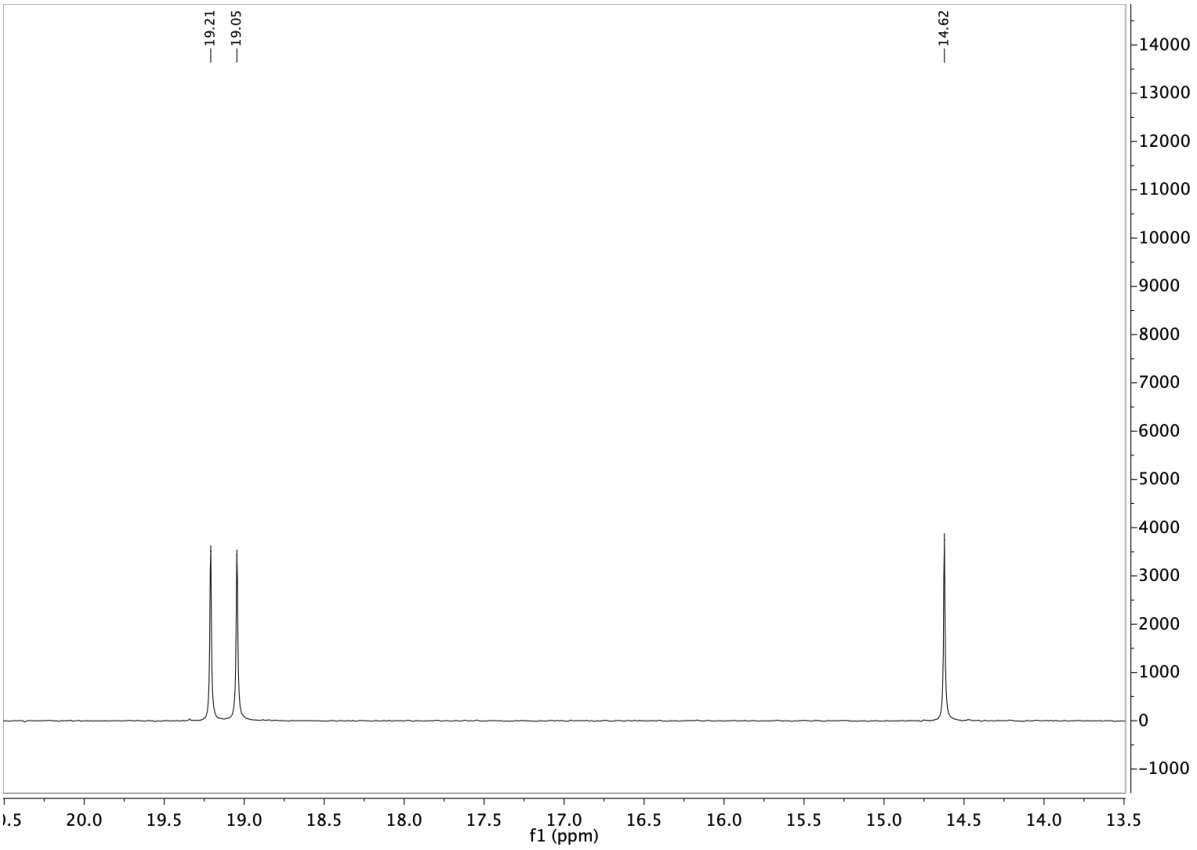
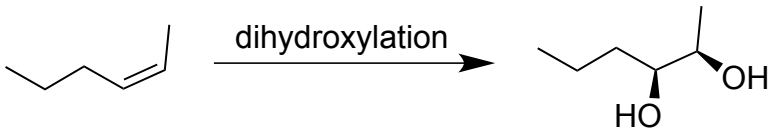
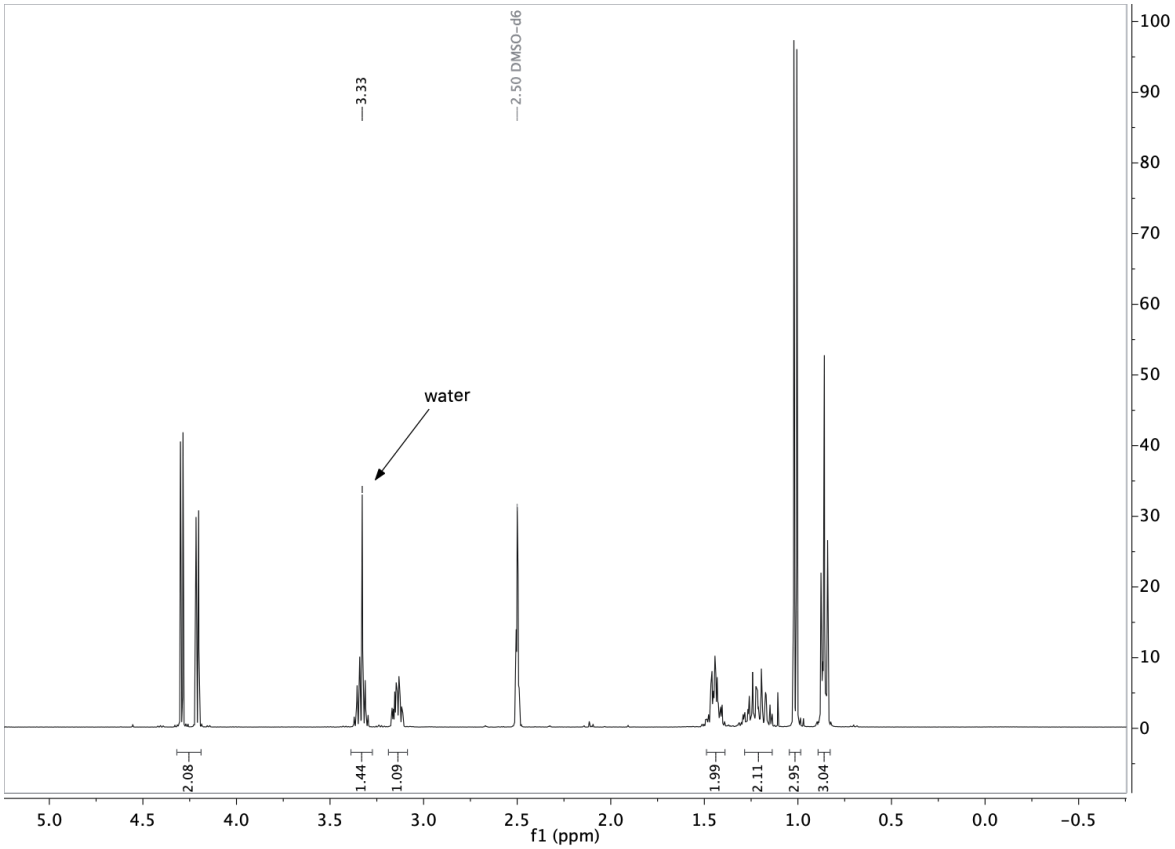
69.78
34.42
19.21
19.05
14.62
f1 (ppm)

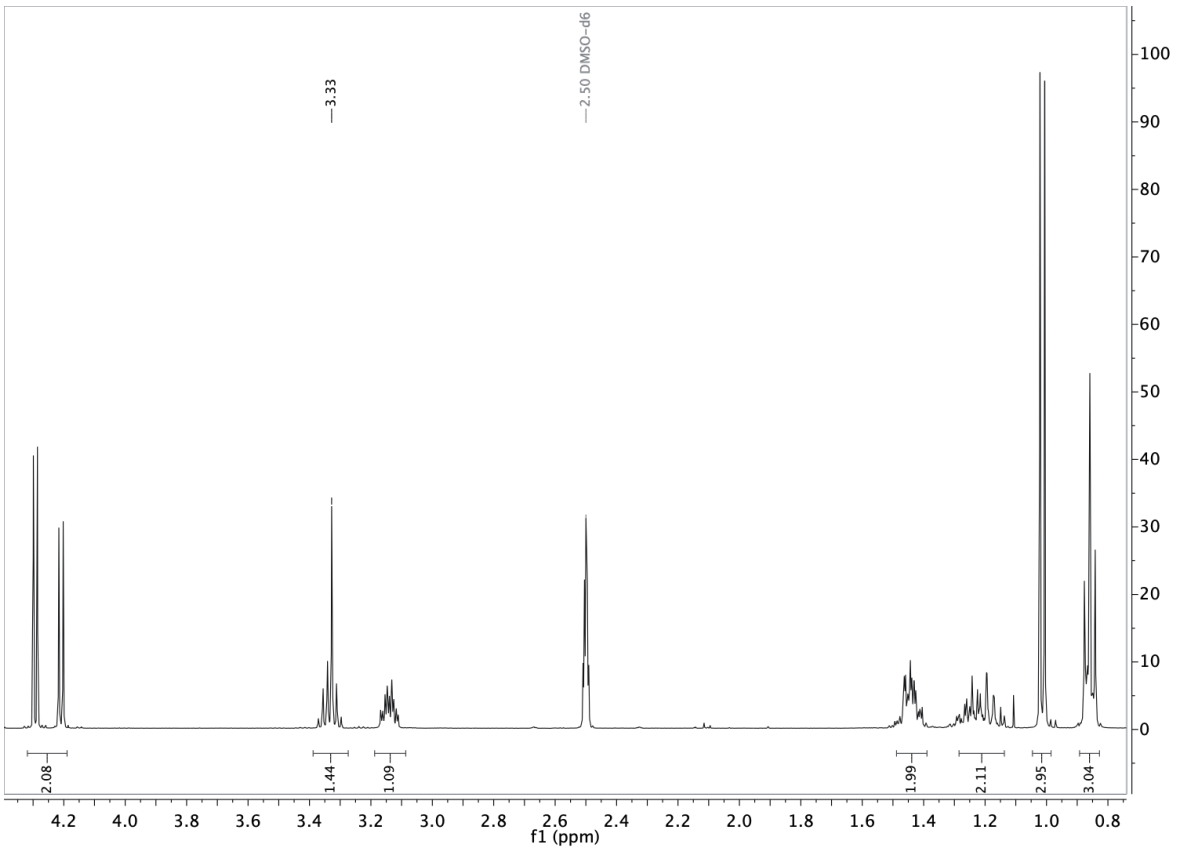

19.21
19.05
14.62
f1 (ppm)

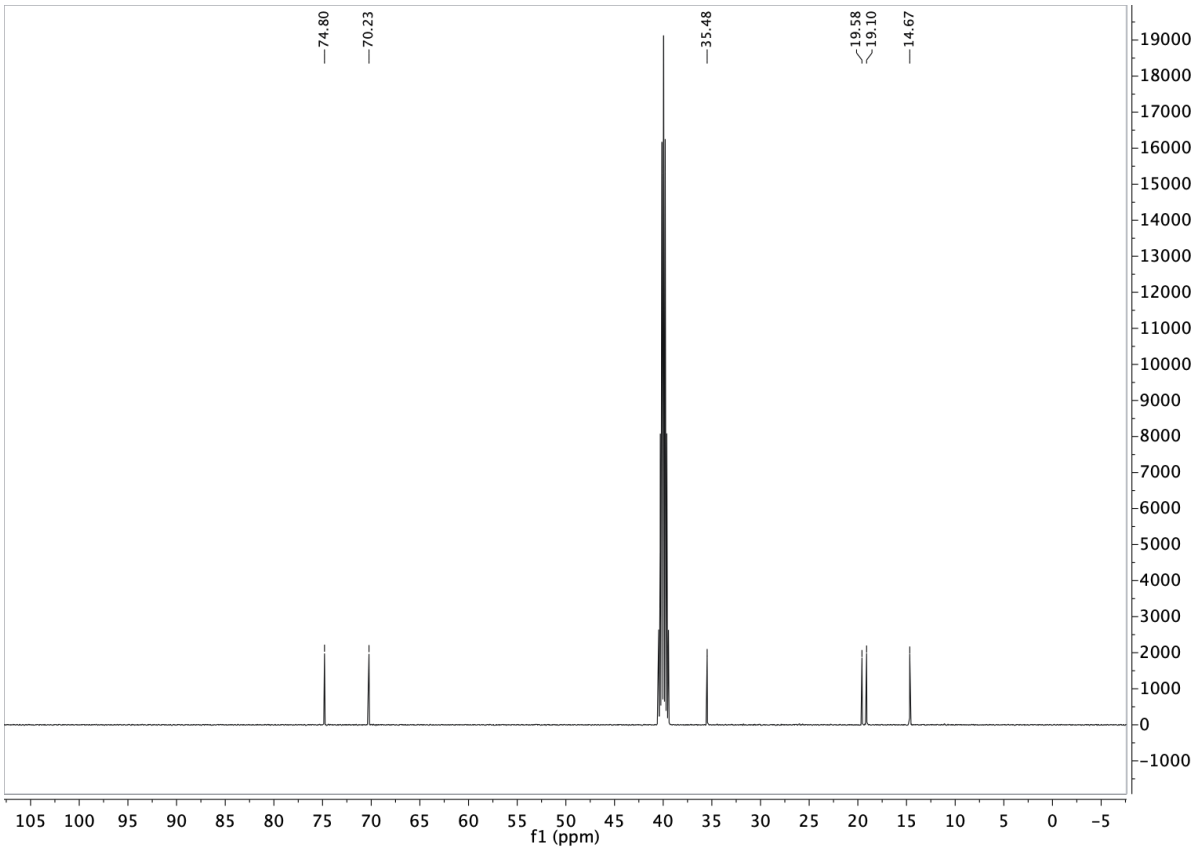


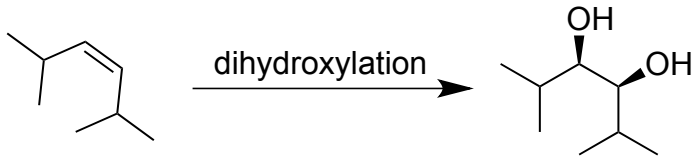
The alkene, cis-2-hexene (1.5 mL, 11.92 mmol) purchased from Sigma-Aldrich 95%, was subjected to the described dihydroxylation procedures shown above. Upon purification, 150.34 mg of the desired clear oil was collected. Yield: 10.7%

^{1}H NMR (600 MHz, DMSO-d_6): δ 4.25 (2H, dd, J=5.5 Hz), 3.33 (1H, m), 3.14 (1H, m), 1.44 (2H, m), 1.21 (2H, m), 1.01 (3H, d, J=6.3 Hz), 0.86, (3H, t, J=7.1 Hz). ^{13}C NMR (150 MHz, DMSO-d_6): δ 74.80, 70.23, 35.48, 19.58, 19.10, 14.67.



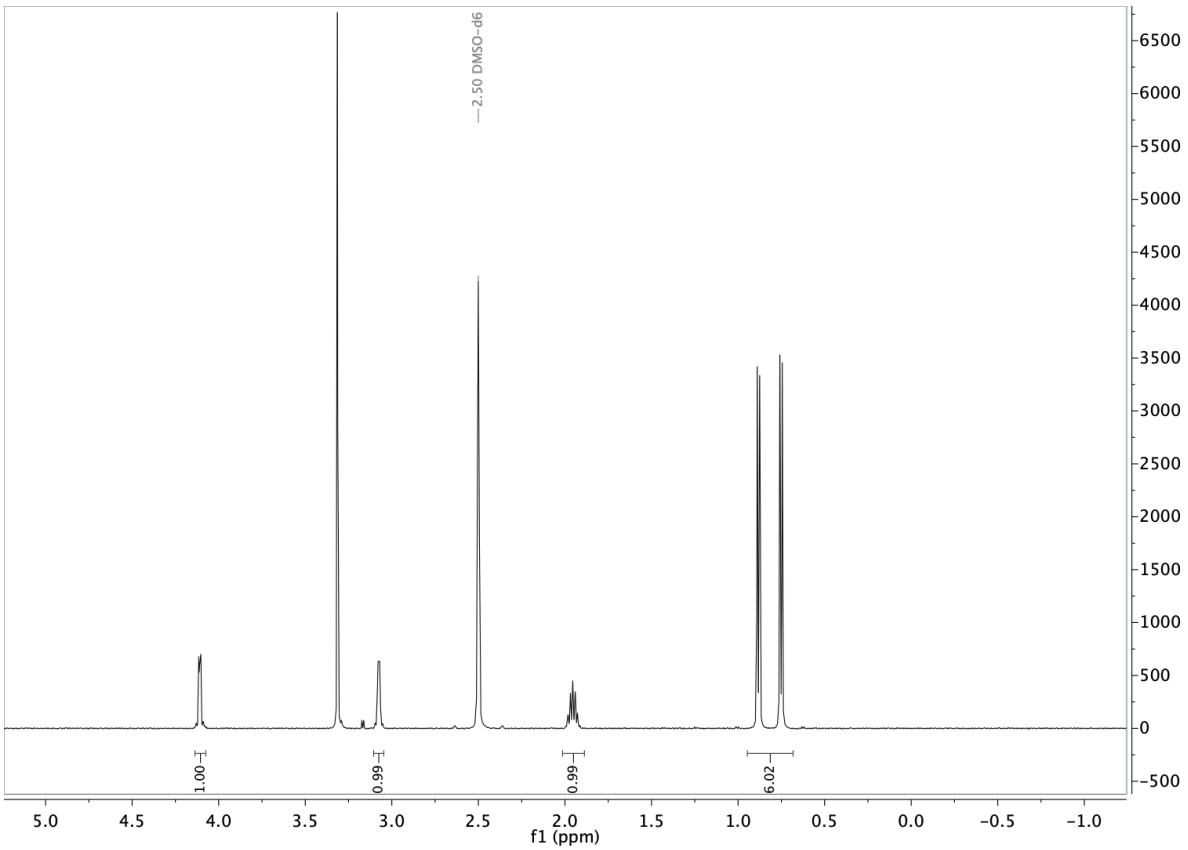

3.33
2.50 DMSO-d6
2.08
1.44
1.09
1.99
2.11
2.95
3.04
f1 (ppm)

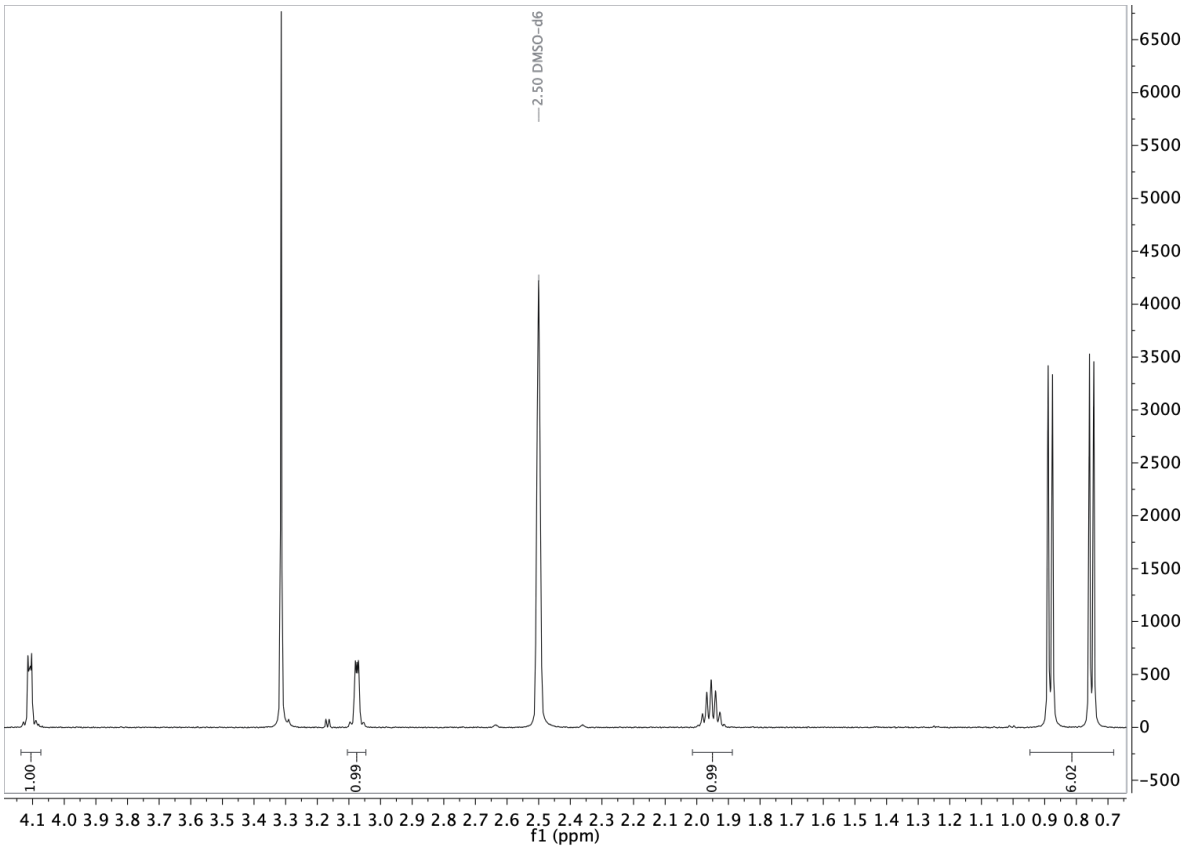

74.80
70.23
35.48
19.58
19.10
14.67
f1 (ppm)

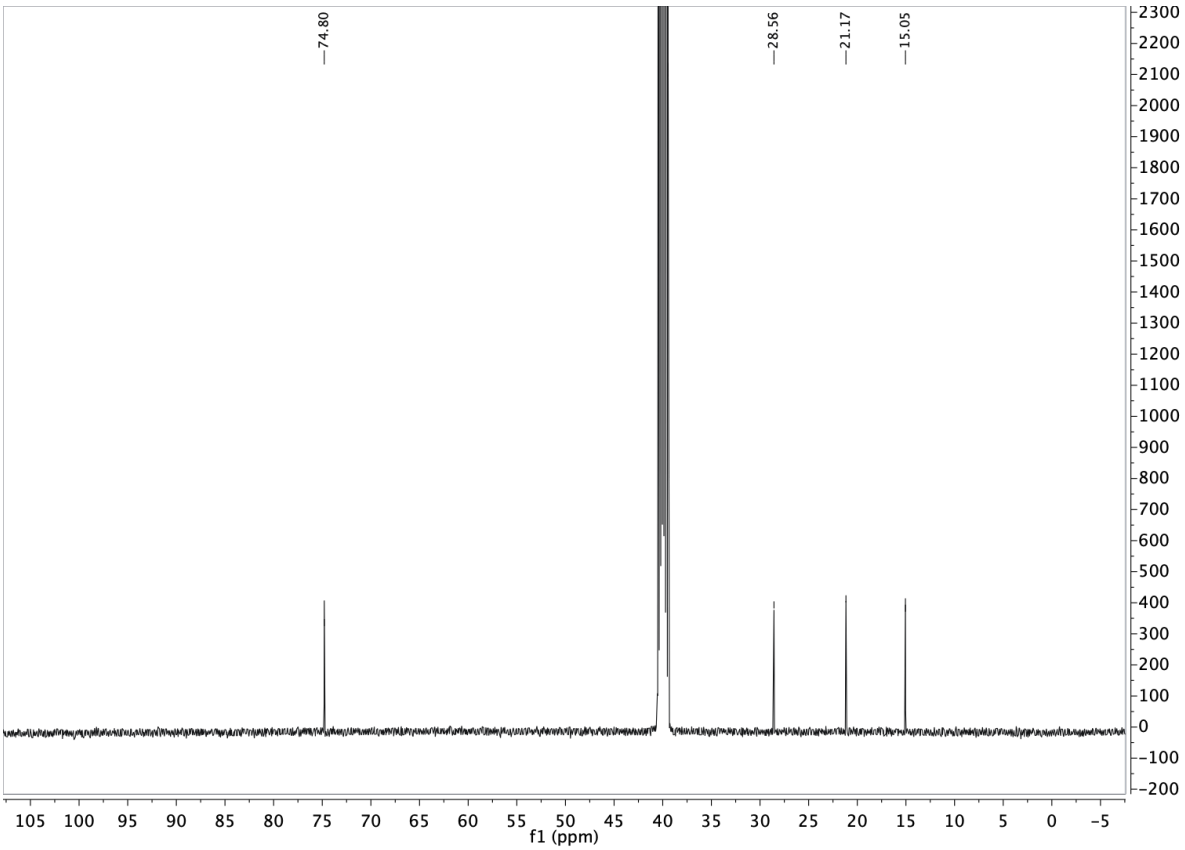


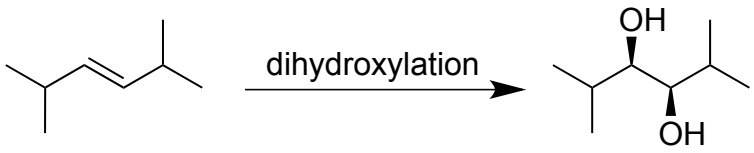
The alkene, cis-2,5-dimethyl-3-hexene (1.5 mL, 9.68 mmol) purchased from Pfaltz & Bauer 99%, was subjected to the described dihydroxylation procedures shown above. Upon purification, 69.61 mg of the desired product was collected as a white solid. Yield: 5.0%

^{1}H NMR (600 MHz, DMSO-d_6): δ 4.11 (1H, d, J=6.0 Hz), 3.07 (1H, m), 1.96 (1H, m), 0.81 (6H, dd, J=7.0 Hz). ^{13}C NMR (150 MHz, DMSO-d_6): δ 74.80, 28.56, 21.17, 15.05.


2.50 DMSO-d6
1.00
0.99
0.99
6.02
f1 (ppm)

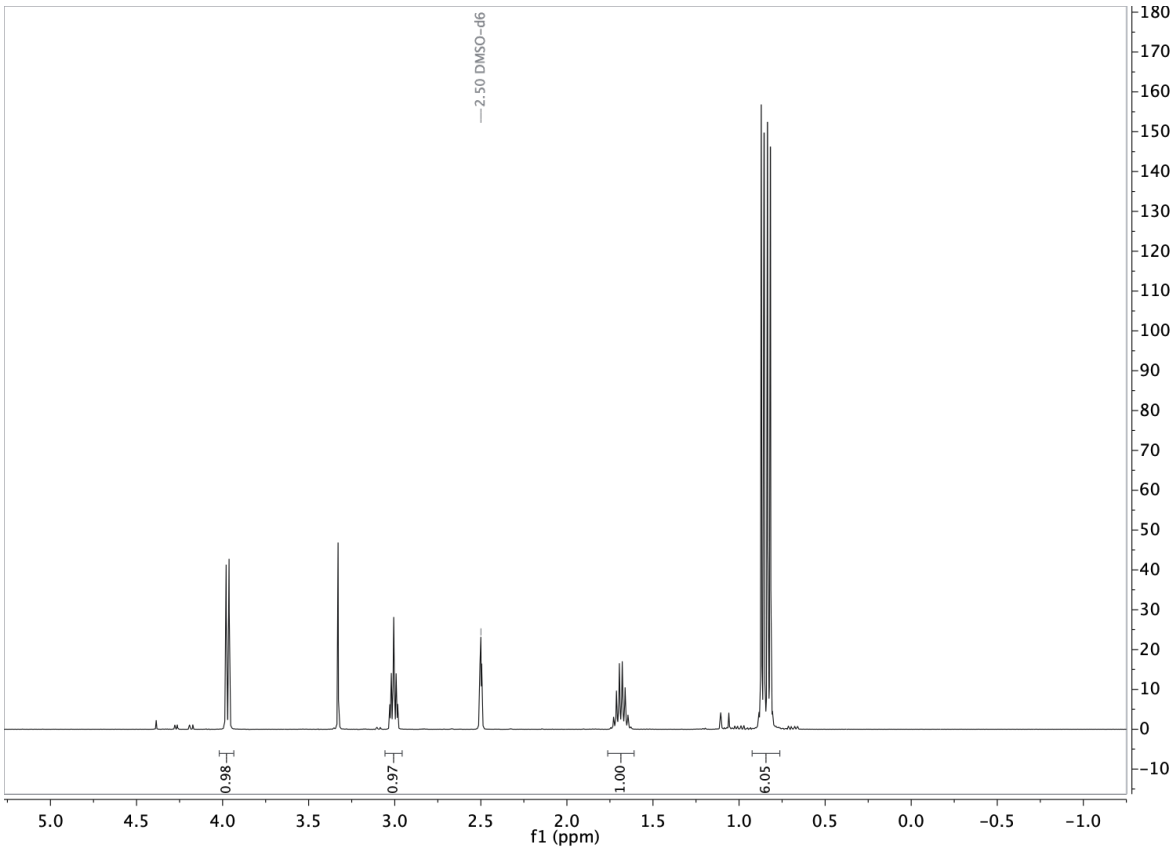

2.50 DMSO-d6
1.00
0.99
0.99
6.02
f1 (ppm)





The alkene, trans-2,5-dimethyl-3-hexene (1.5 mL, 9.68 mmol) purchased from Pfaltz & Bauer 99%, was subjected to the previously described dihydroxylation procedure. Upon purification, the product yielded 134.1 mg of a white solid. Yield: 9.5%

^{1}H NMR (600 MHz, DMSO-d_6): δ 3.97 (1H, d, J=6.2 Hz), 3.01 (1H, m), 1.69 (1H, m), 0.85 (6H, dd, J=6.9 Hz). ^{13}C NMR (150 MHz, DMSO-d_6): δ 75.84, 30.54, 20.11, 18.80.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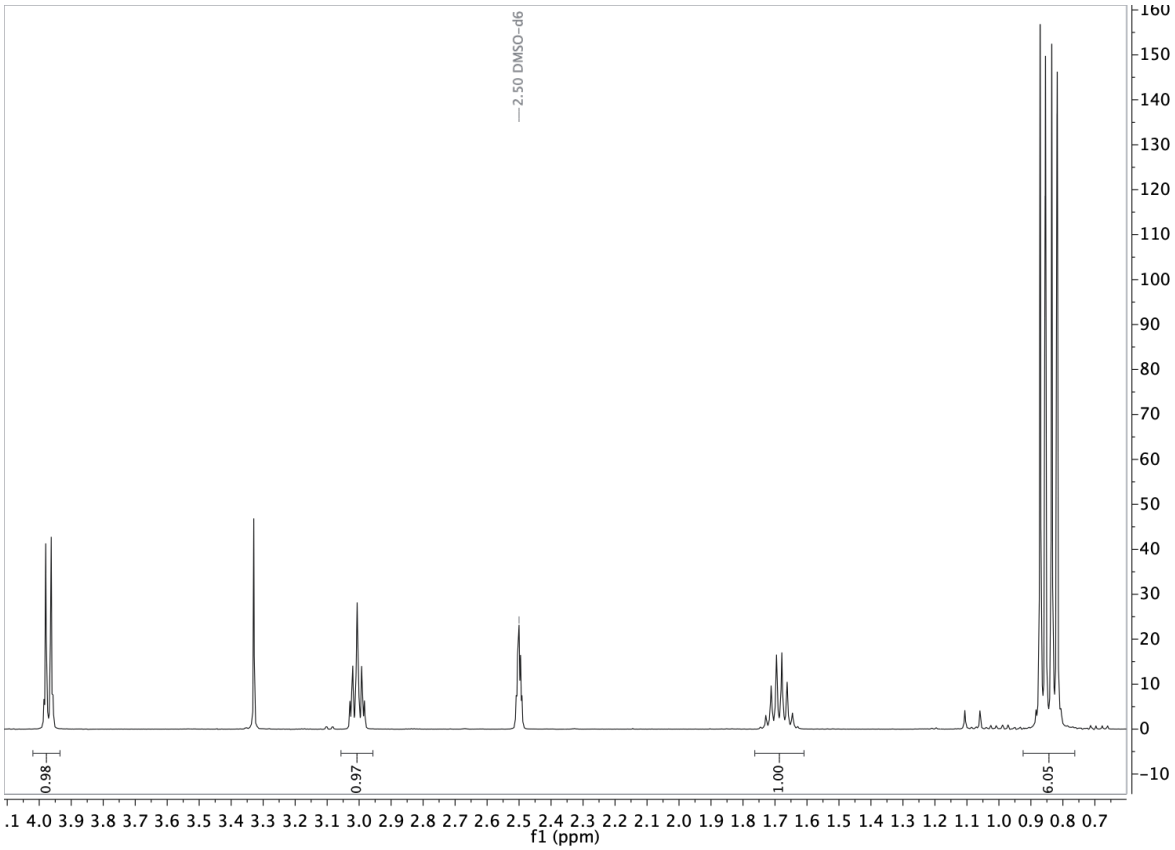
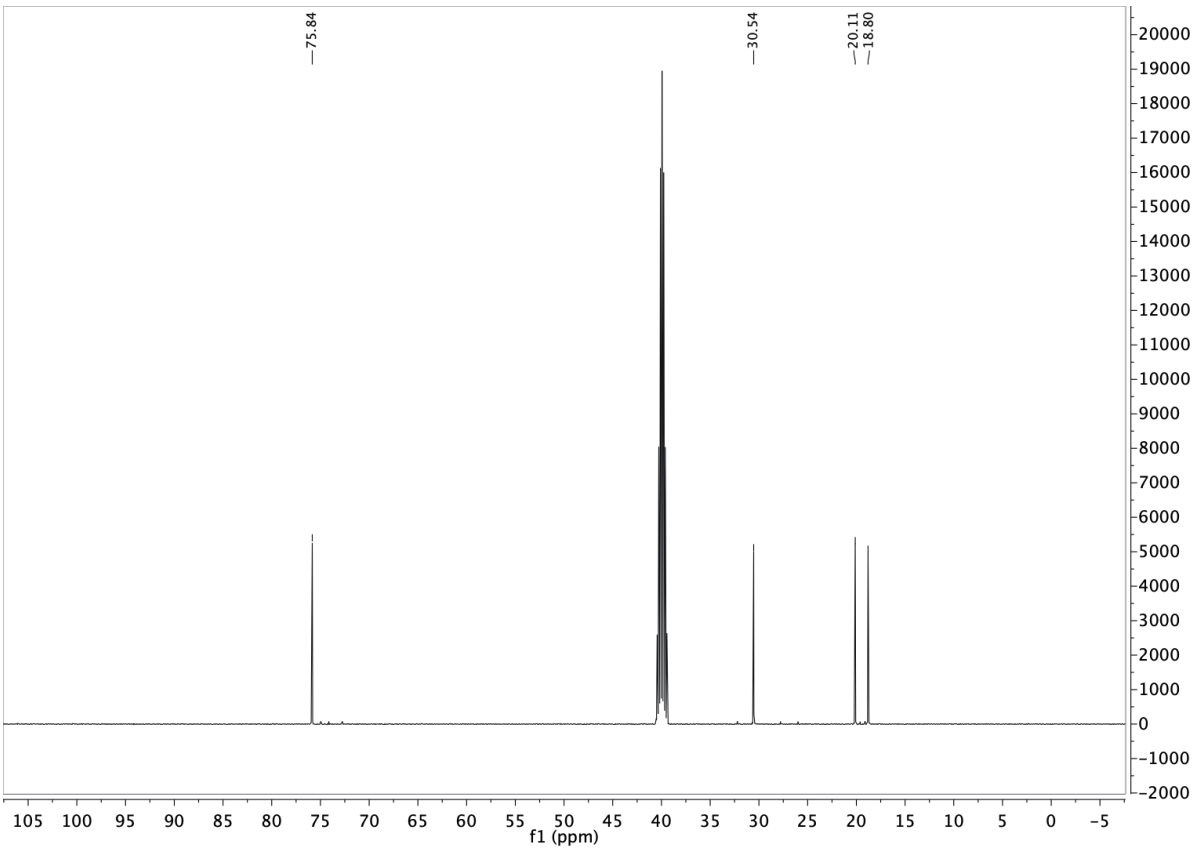
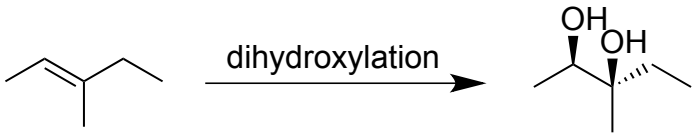
0.98
0.97
1.00
6.05
f1 (ppm)

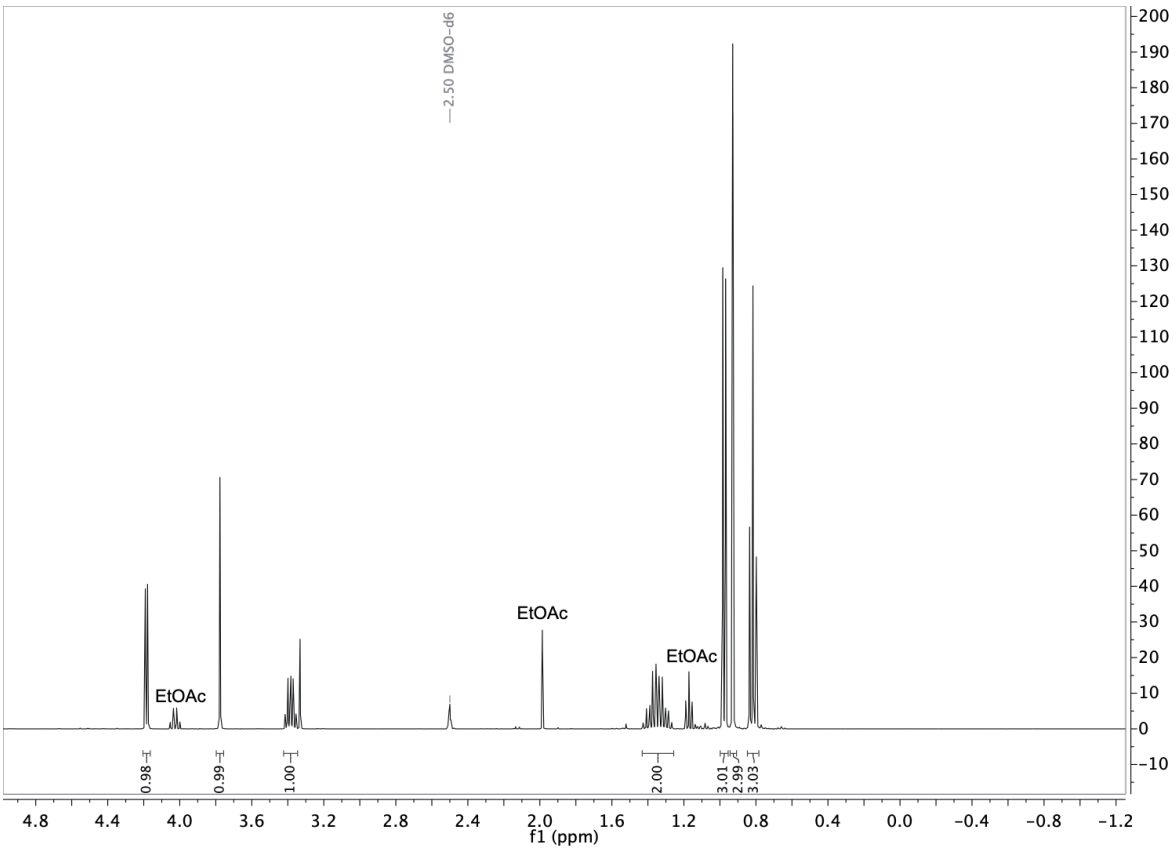

2.50 DMSO-d6
0.98
0.97
1.00
6.05
f1 (ppm)

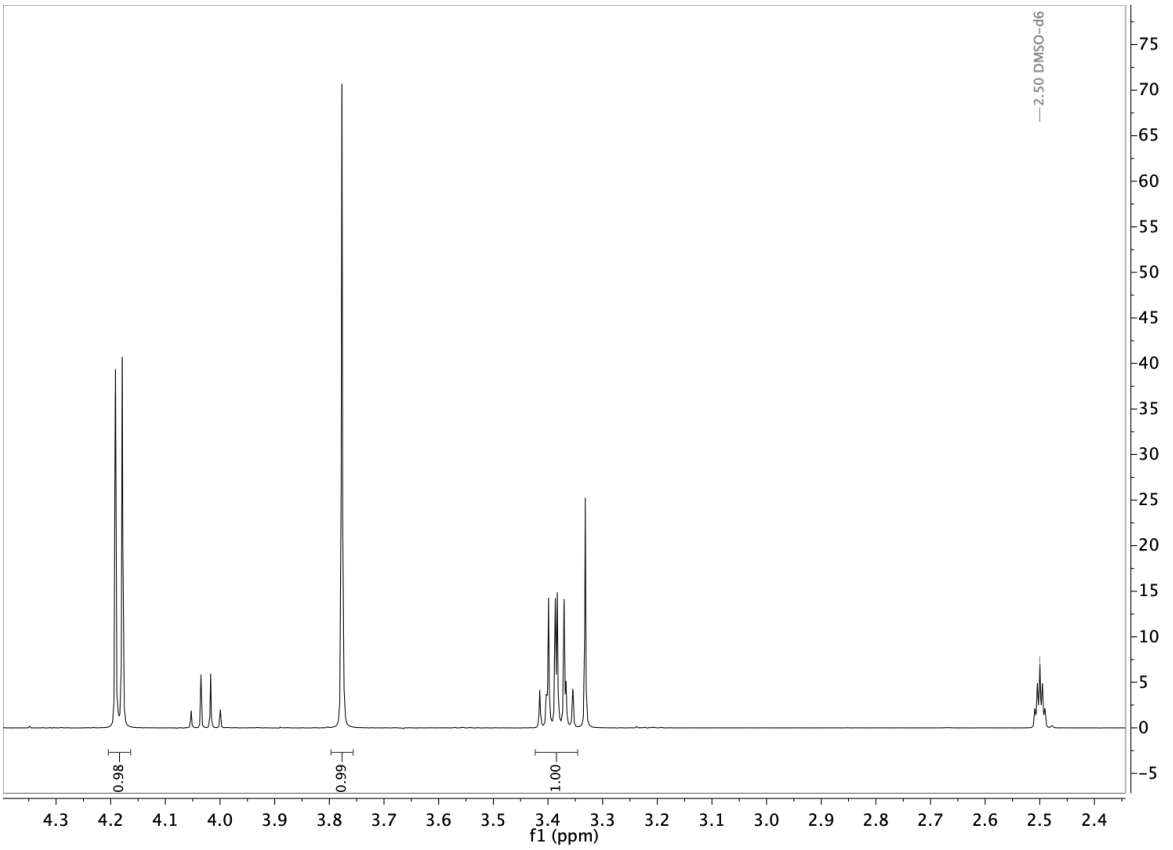


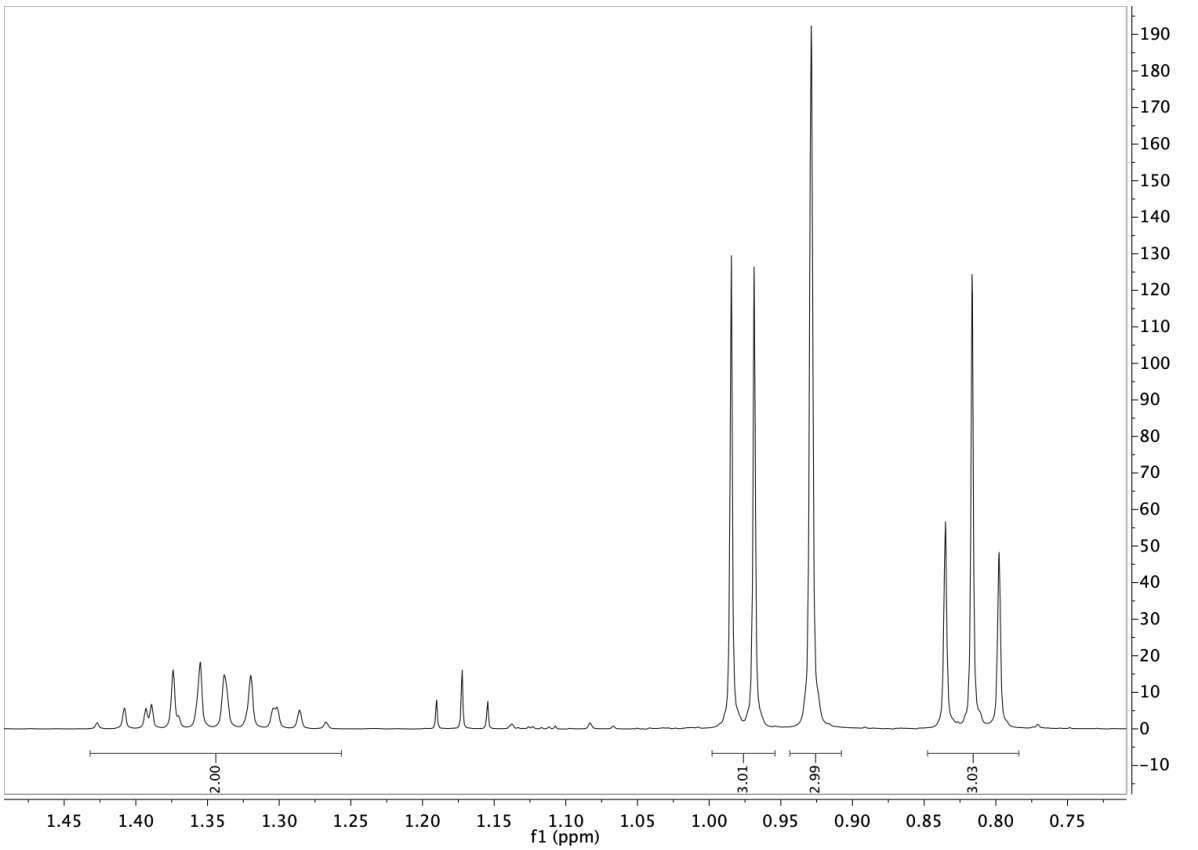


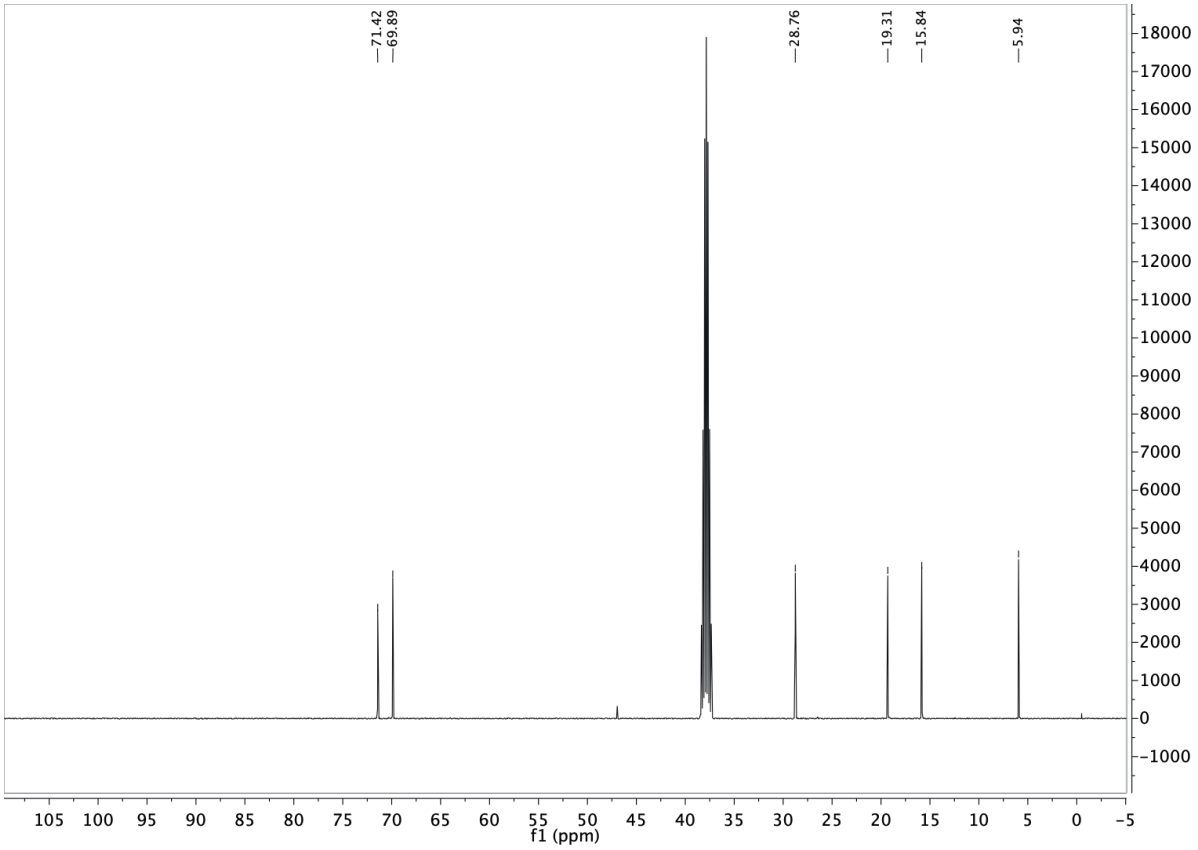
The alkene, trans-3-methyl-2-pentene (1.6 mL, 13.12 mmol) purchased from TCI >99%, underwent the dihydroxylation procedures. Upon purification, 724.85 mg of a clear oil was collected. Yield: 46.8%

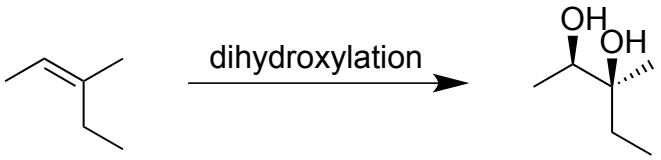
^{1}H NMR (600 MHz, DMSO-d_6): δ 4.19 (1H, d, J=4.7 Hz), 3.78 (1H, s), 3.37 (1H, m), 1.35 (2H, m), 0.98 (3H, d, J=6.9 Hz), 0.93 (3H, s), 0.82 (3H, t, J=7.4 Hz). ^{13}C NMR (150 MHz, DMSO-d_6): δ 71.42, 69.89, 28.76, 19.31, 15.84, 5.94.


2.50 DMSO-d6
EtOAc
EtOAc
EtOAc
0.98
0.99
1.00
2.00
3.01
2.99
3.03
f1 (ppm)


2.50 DMSO-d6
0.98
0.99
1.00
f1 (ppm)

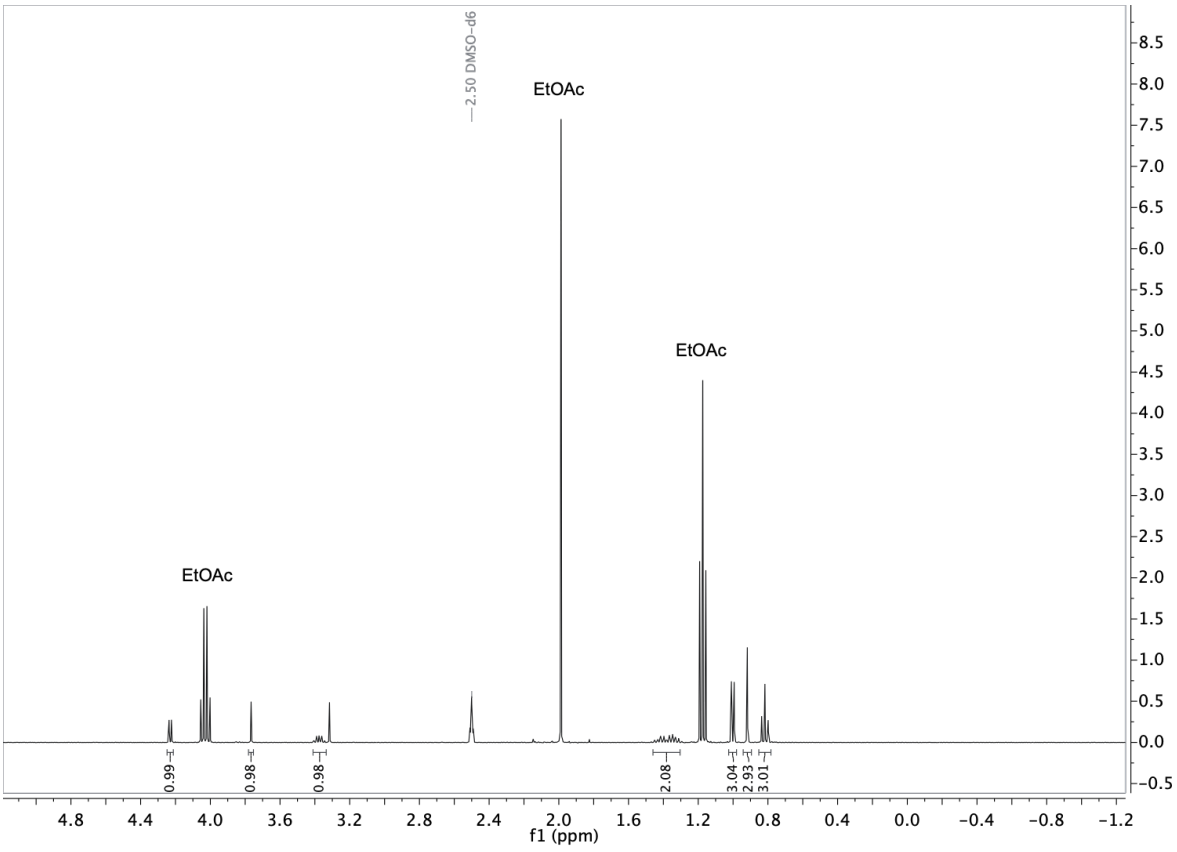

2.00
3.01
2.99
3.03
f1 (ppm)

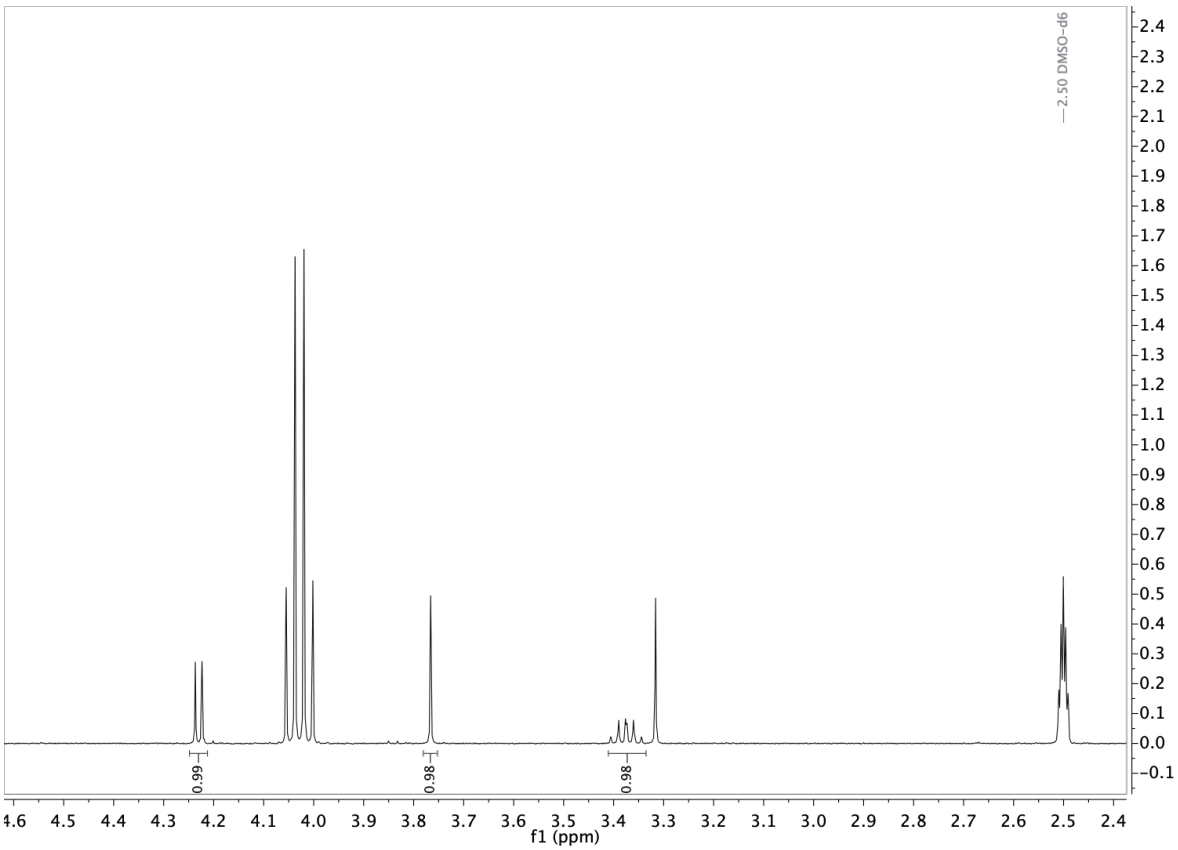

71.42
69.89
28.76
19.31
15.84
5.94
f1 (ppm)

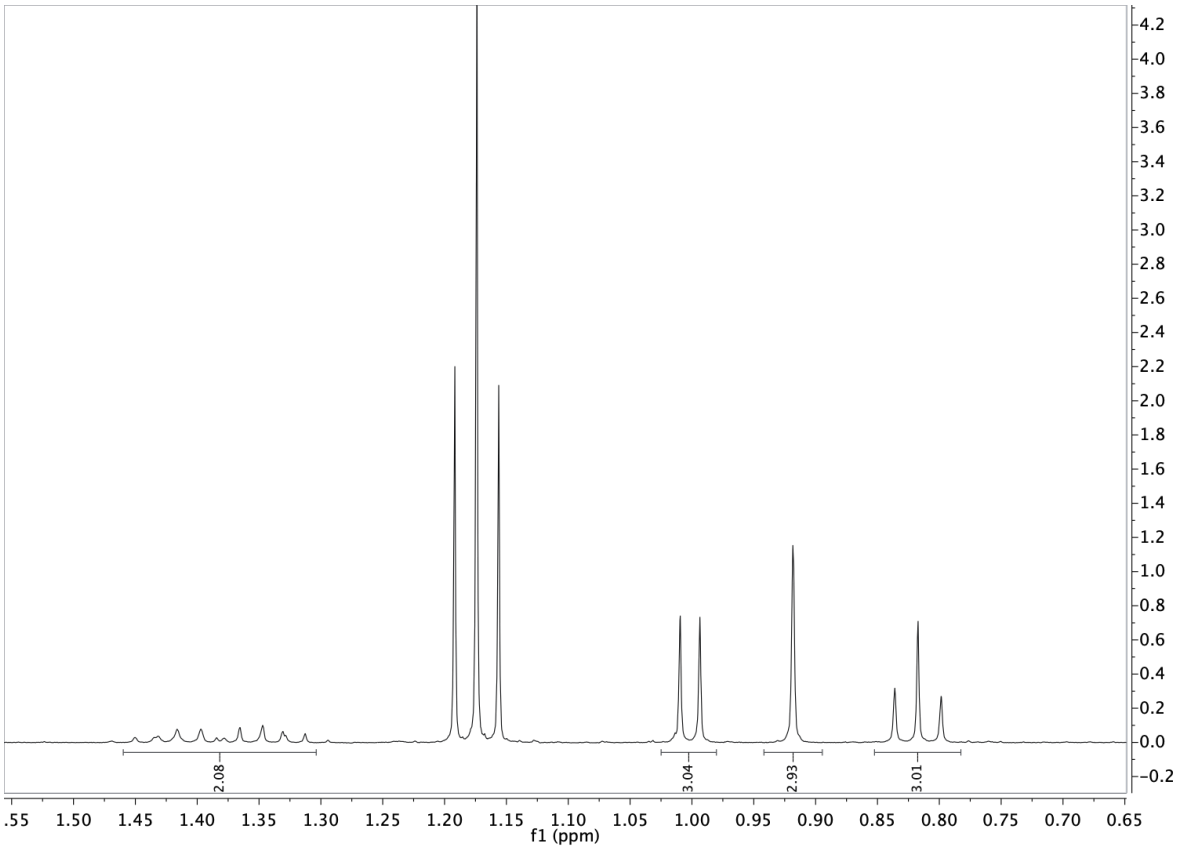


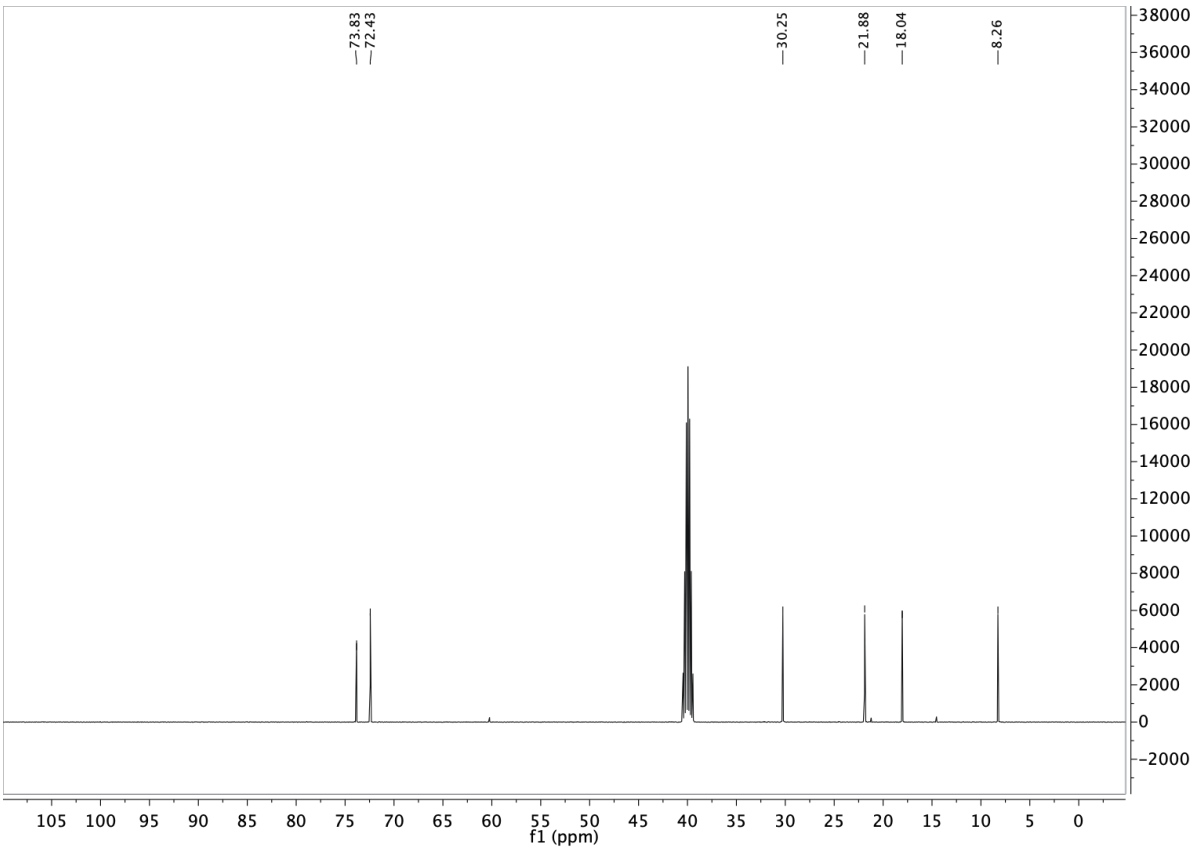
The alkene, cis-3-methyl-2-pentene (1.8 mL, 14.75 mmol) purchased from TCI >95%, underwent the dihydroxylation procedures. Upon purification, 668.08 mg of a clear oil was collected. Yield: 38.3%

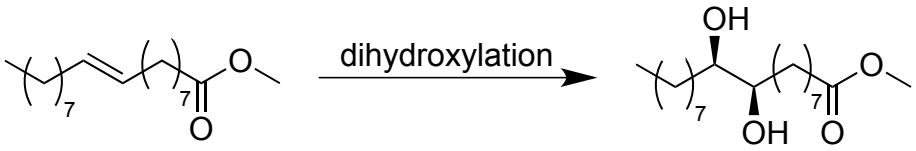
^{1}H NMR (600 MHz, DMSO-d_6): δ 4.23 (1H, d, J=5.4 Hz), 3.77 (1H, s), 3.37 (1H, m), 1.38 (2H, m), 1.00 (3H, d, J=6.6 Hz), 0.92 (3H, s), 0.82 (3H, t, J=7.5 Hz). ^{13}C NMR (150 MHz, DMSO-d_6): δ 73.83, 72.43, 30.25, 21.88, 18.04, 8.26.



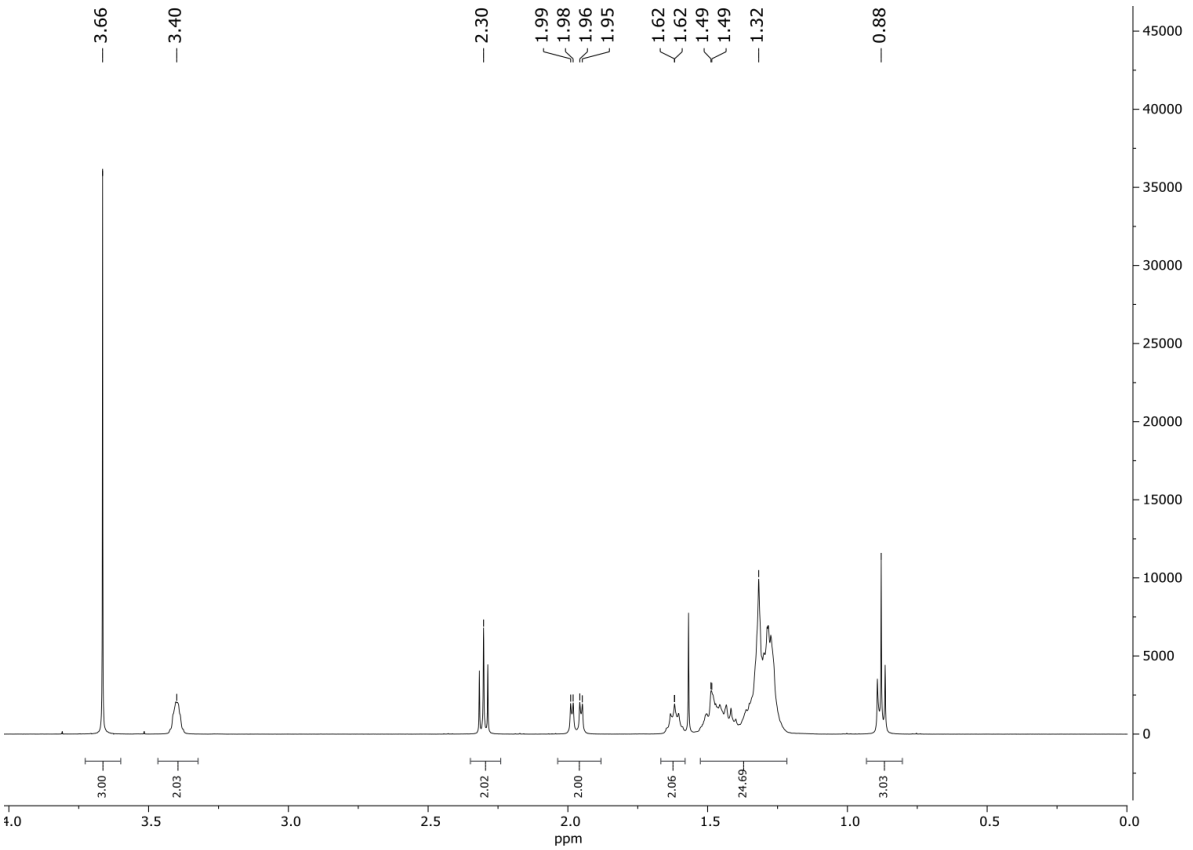

2.50 DMSO-d6
0.99
0.98
0.98
f1 (ppm)

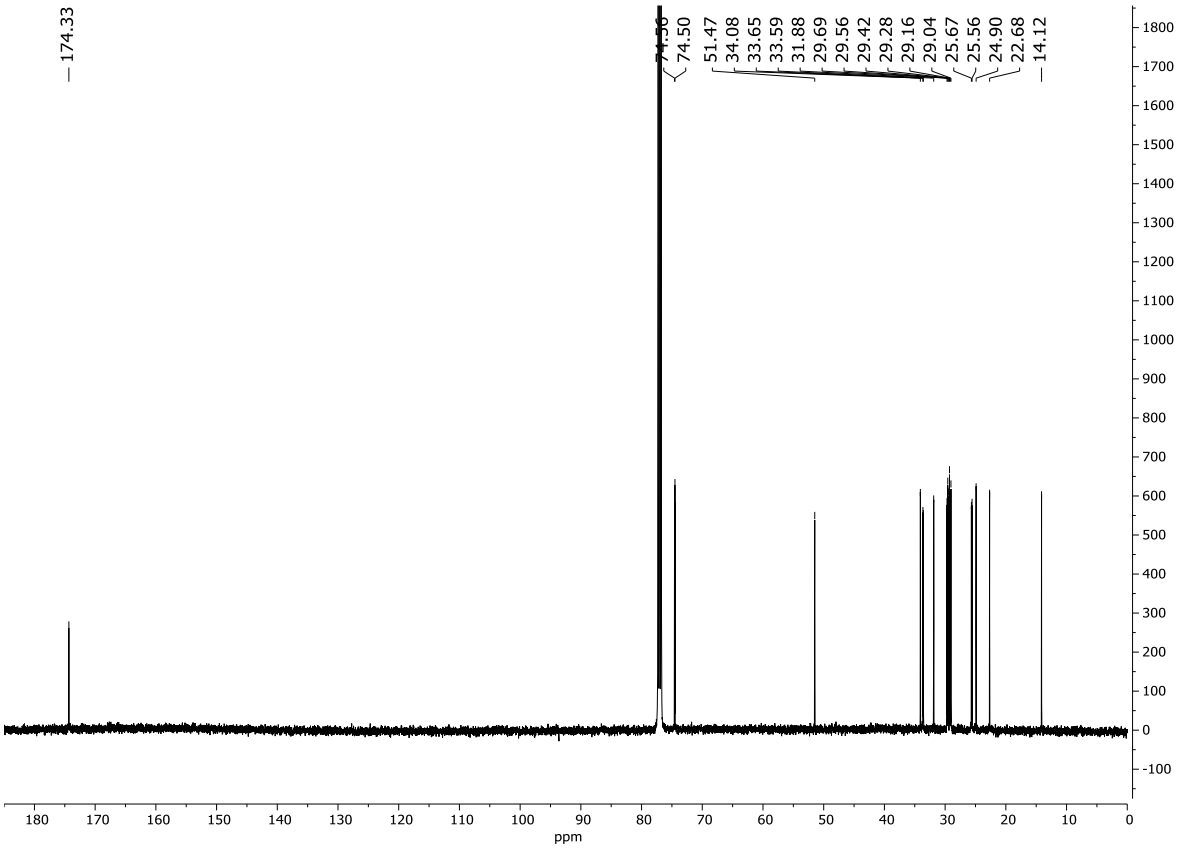

2.08
3.04
2.93
3.01
f1 (ppm)





General procedure dihydroxylation procedure afforded the title compound as a white solid (666.7 mg, 67% yield). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 0.88 (t, 3H), 1.40 (m, 24H), 1.58 – 1.67 (t, 2H), 1.97 (dd, J = 16.4, 4.4 Hz, 2H), 2.30 (t, 2H), 3.40 (s, 2H), 3.66 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.33, 74.56, 74.50, 51.47, 34.08, 33.65, 33.59, 31.88, 29.69, 29.56, 29.42, 29.28, 29.16, 29.04, 25.67, 25.56, 24.90, 22.68, 14.12.


3.66
3.40
2.30
1.99
1.98
1.96
1.95
1.62
1.62
1.49
1.49
1.32
0.88
3.00
2.03
2.02
2.00
2.06
24.69
3.03
ppm


174.33
74.56
74.50
51.47
34.08
33.65
33.59
31.88
29.69
29.56
29.42
29.28
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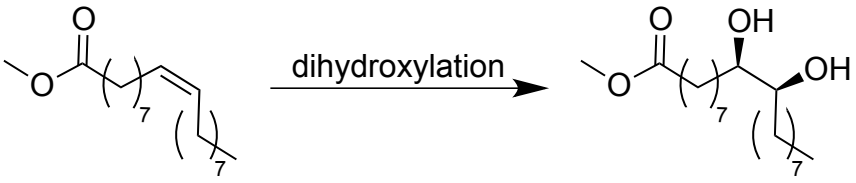
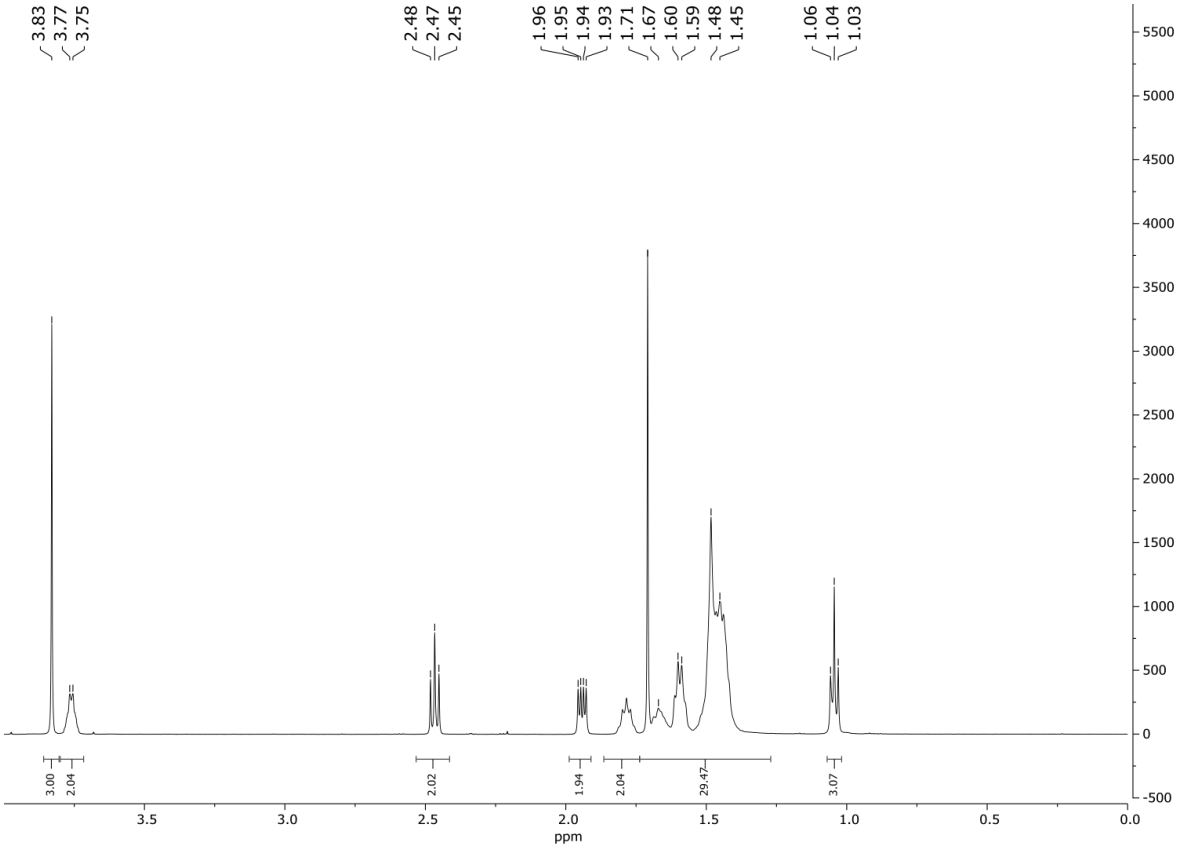
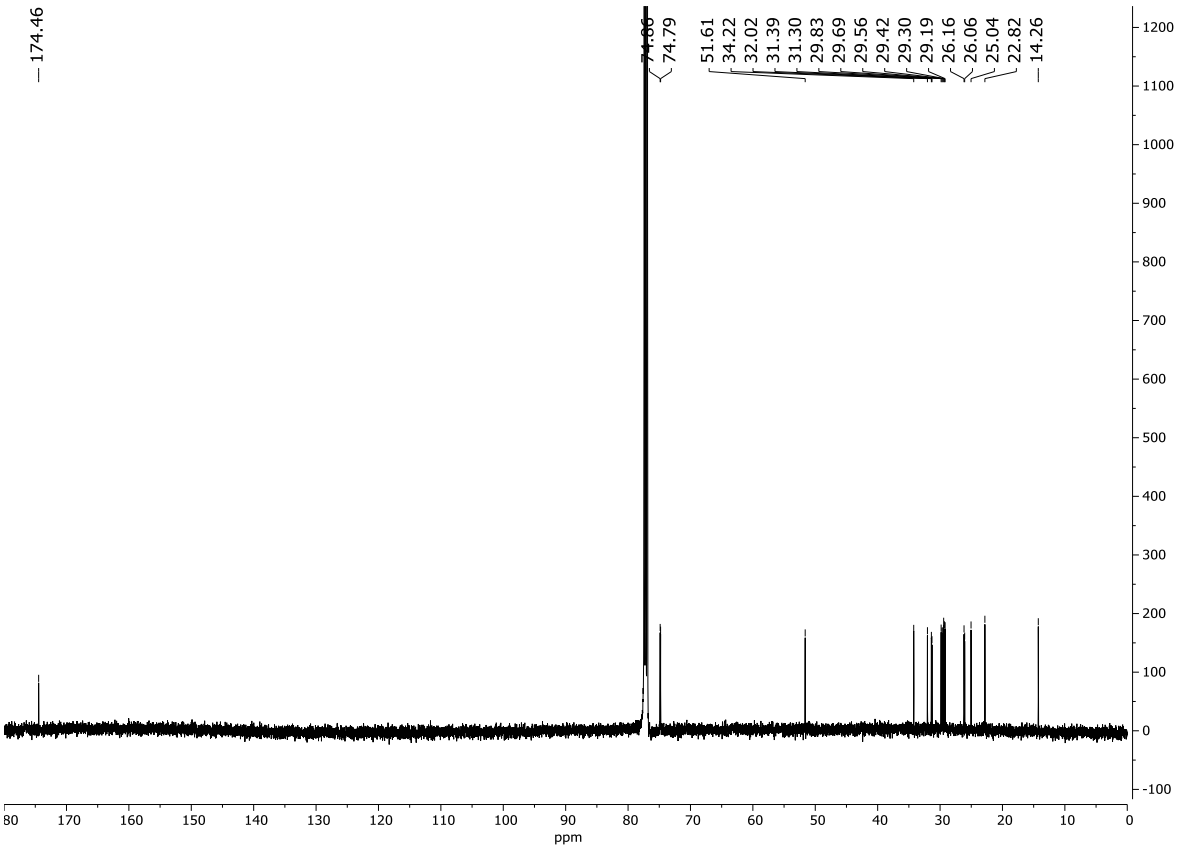
25.67
25.56
24.90
22.68
14.12
ppm



General dihydroxylation procedure afforded the title compound as a white solid (951.1 mg, 95% yield). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 1.04 (t, J = 6.8 Hz, 3H), 1.32 – 1.68 (m, 24H), 1.78 (t, J = 7.2 Hz, 2H), 1.94 (dd, J = 9.6, 4.8 Hz, 2H), 2.47 (t, J = 7.5 Hz, 2H), 3.76 (d, J = 5.7 Hz, 2H), 3.83 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.46, 74.86, 74.79, 51.61, 34.22, 32.02, 31.39, 31.30, 29.83, 29.69, 29.56, 29.42, 29.30, 29.19, 26.16, 26.06, 25.04, 22.82, 14.26.




174.46
74.86
74.79
51.61
34.22
32.02
31.39
31.30
29.83
29.69
29.56
29.42
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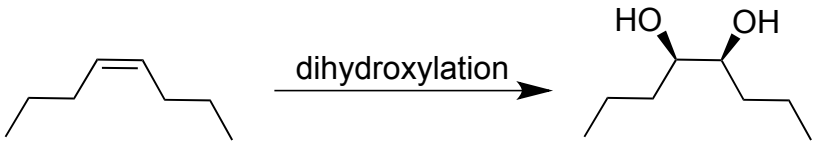
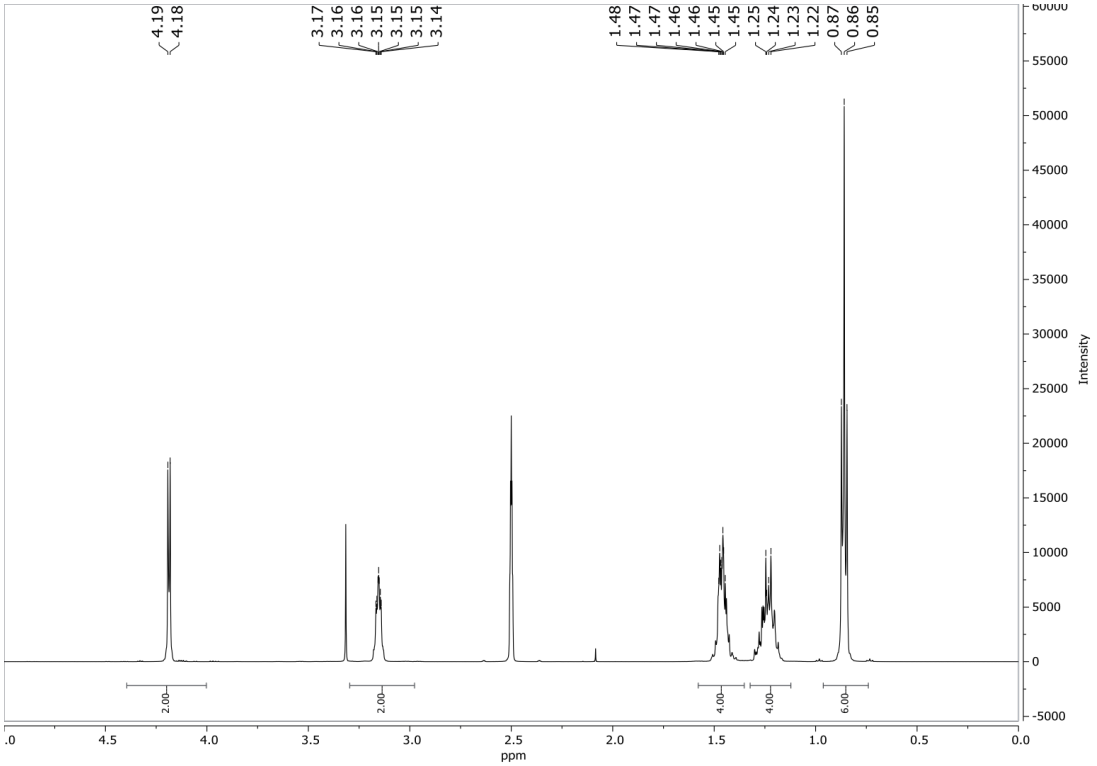
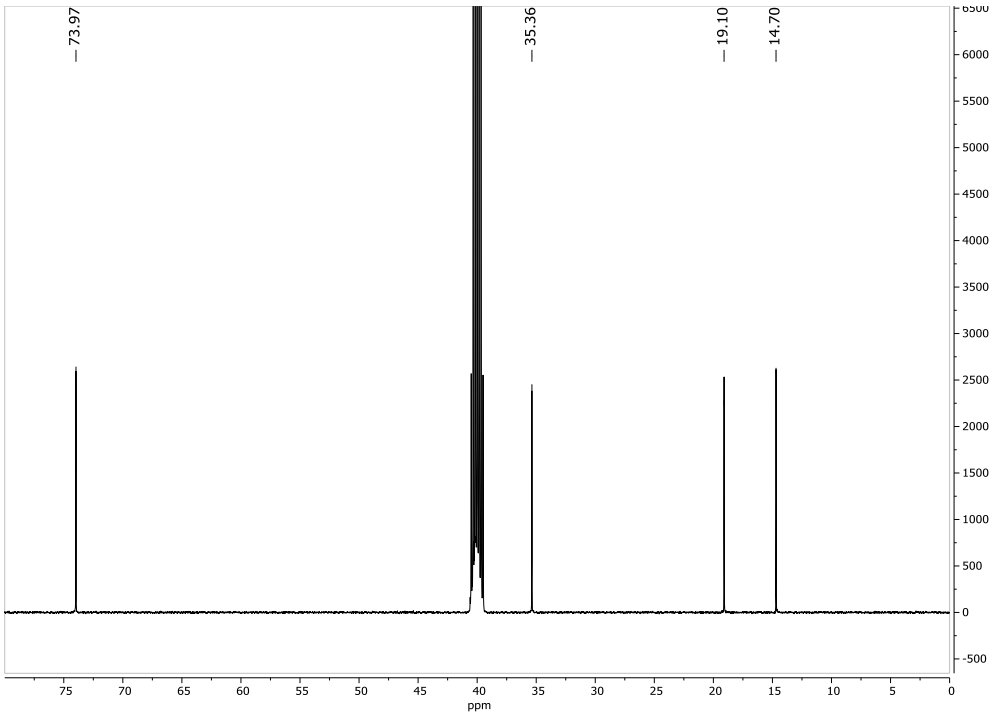
26.16
26.06
25.04
22.82
14.26
ppm



General procedure for the dihydroxylation afforded the title compound after recrystallization in hexanes as an off-white solid (928 mg, 93% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.86 (t, J = 6.9 Hz, 6H), 1.17 – 1.29 (m, 4H), 1.38 – 1.59 (m, 4H), 3.11 – 3.19 (m, 1H), 4.19 (d, J = 5.6 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 73.97, 35.36, 19.10, 14.70.




73.97
35.36
19.10
14.70
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
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70
65
60
55
50
45
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35
30
25
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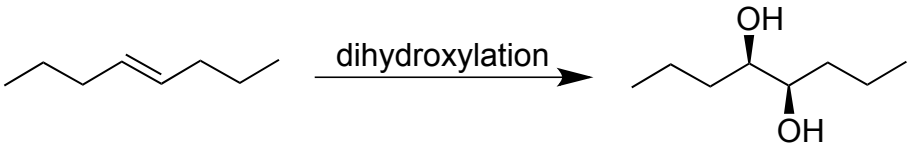
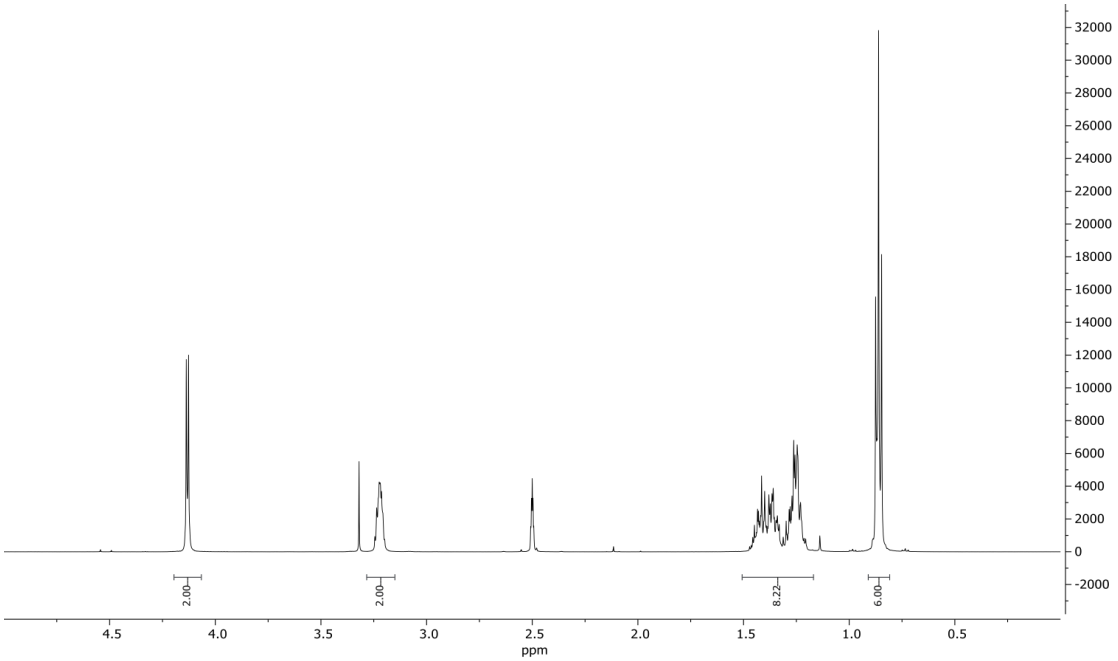
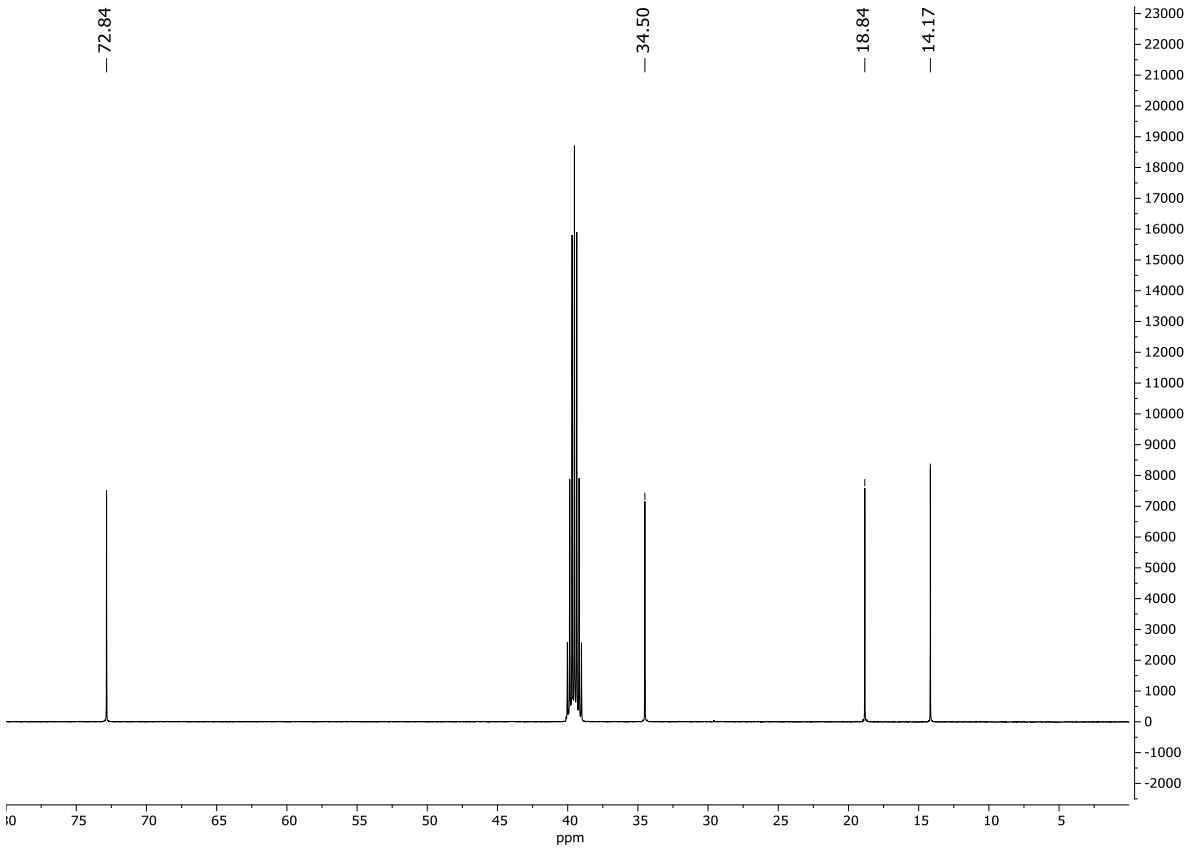
15
10
5
0
ppm



General procedure for the dihydroxylation afforded the title compound after recrystallization in hexanes as an off-white solid (729mg, 73% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.86 (t, J = 7.1 Hz, 6H), 1.18 – 1.59 (m, 8H), 3.17 – 3.27 (m, 2H), 4.13 (d, J = 5.4 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 72.84, 34.50, 18.84, 14.17.

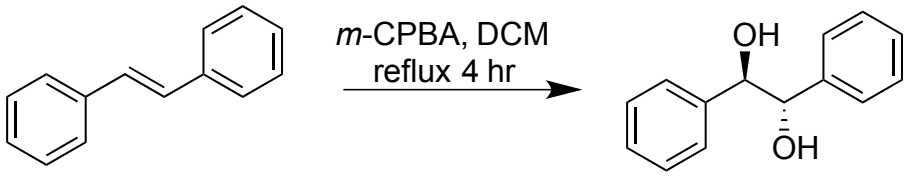




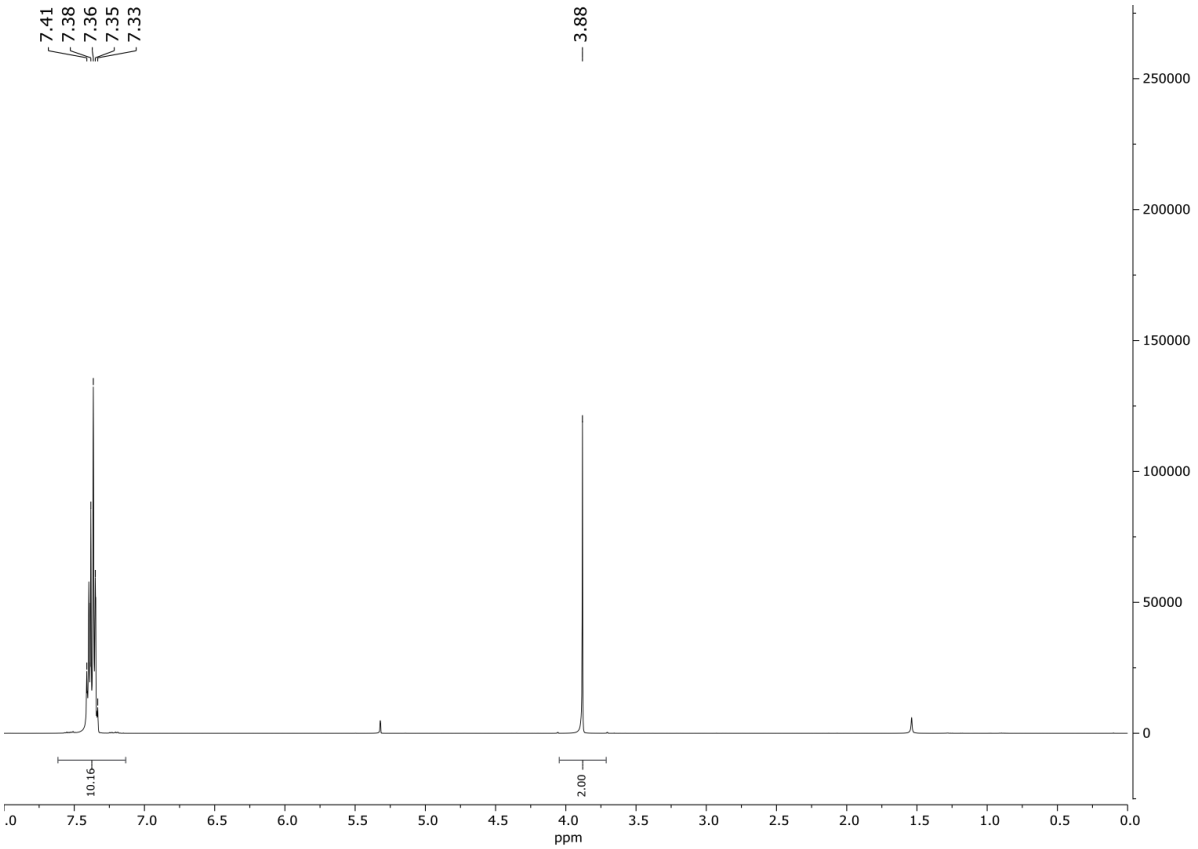
General Procedure for the *m*-CPBA Reaction:

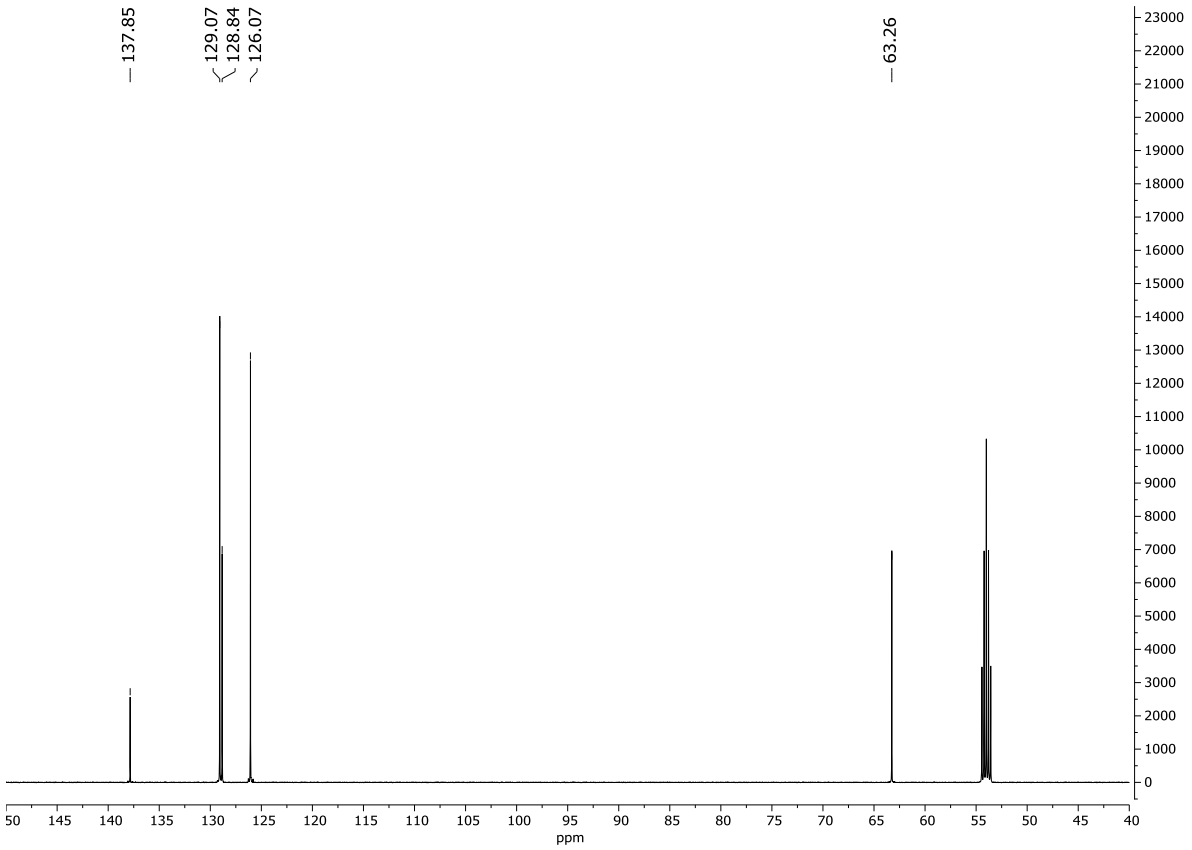
To a clean 100 mL round-bottom flask was added alkene (1 eq., 6.0 mmol),15 mL dichloromethane, and a stir bar. At room temperature, 25 mL of a 0.26 mM (1.1 eq., 6.6 mmol) solution of *m*-CPBA (Pfaltz & Bauer, 65-75% with >17% H_2O) in dichloromethane was added slowly to the flask. The flask was then equipped with a condenser and brought to reflux for 4 hours and monitored by TLC. Once the starting alkene had been consumed, the reaction was allowed to cool to room temperature and washed with 5 mL sat. $NaHCO_3$ followed by extraction with DCM (3 x 20 mL) then concentrated in vacuo. The crude epoxide was used without further purification.

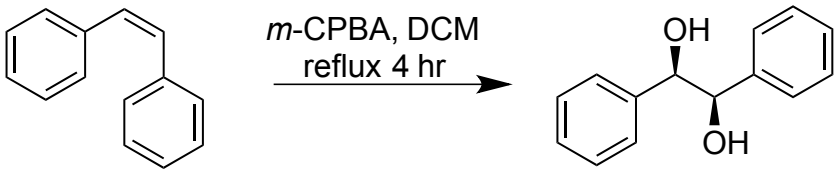
To the crude epoxide was added 30 mL DMSO and 22.5 mL of a 0.4 M KOH solution in water (1.5 eq., 9 mmol). The flask was sealed with septum and heated at 80 °C for 18 hours. The reaction was allowed to cool to room temperature and diluted with deionized water. The product was extracted with EtOAc (3 x 20 mL), concentrated in vacuo, and purified by column chromatography (1:3 ethylacetate:hexanes).



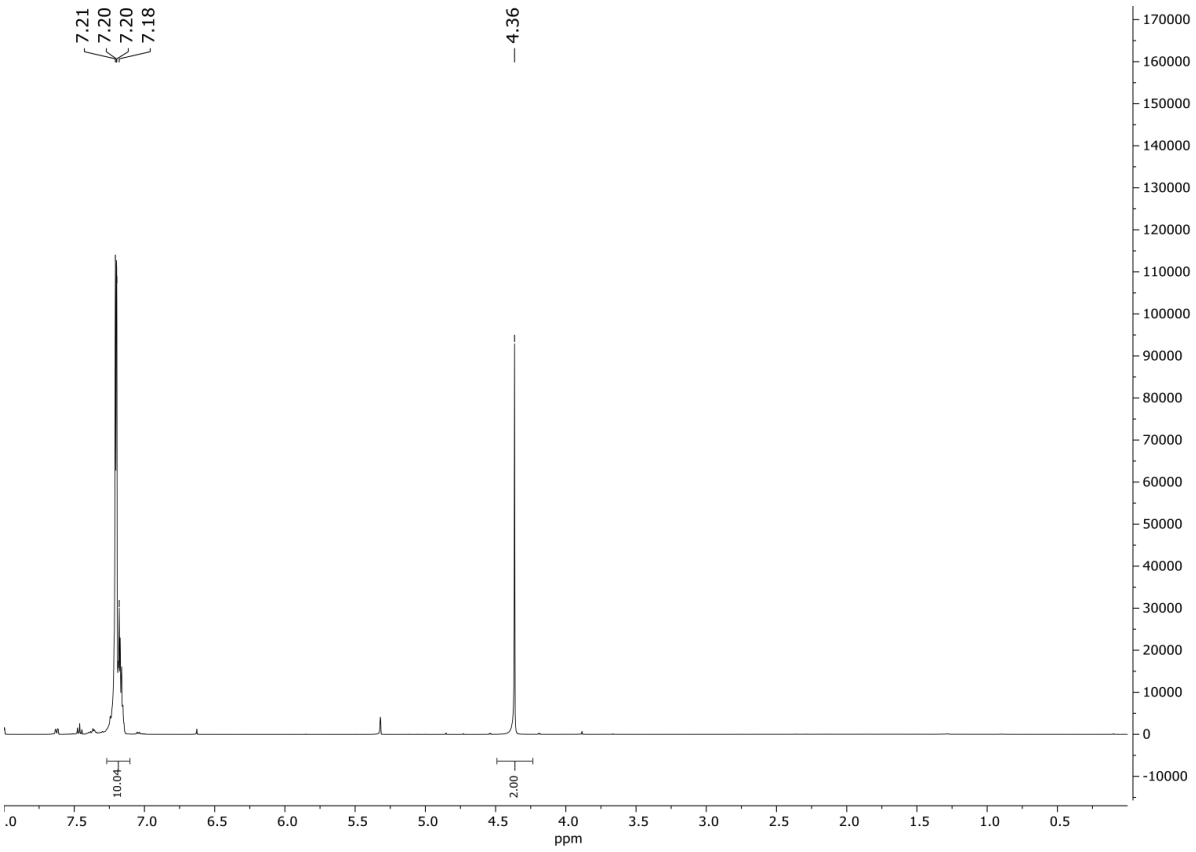
General procedure for the *m*-CPBA afforded the titled compound as a white solid (121 mg, 18% yield). ^{1}H NMR (500 MHz, Methylene Chloride-d_2) δ 3.88 (s, 2H), 7.37 (m, 10H). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 137.85, 129.07, 128.84, 126.07, 63.26.

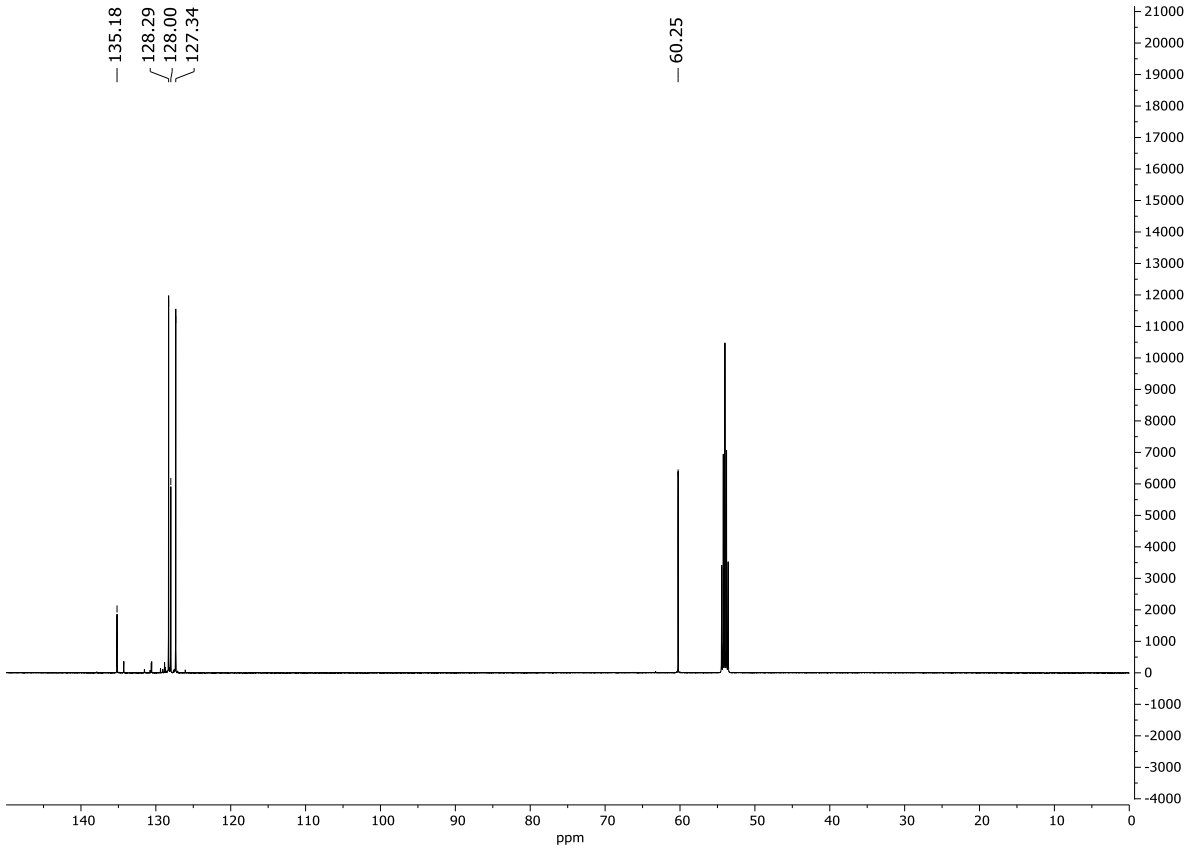



137.85
129.07
128.84
126.07
63.26
ppm



General procedure afforded the title compound as a white solid (29 mg, 5% yield). ^{1}H NMR (500 MHz, Methylene Chloride-d_2) δ 4.36 (s, 2H), 7.05 – 7.35 (m, 10H). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 135.18, 128.29, 128.00, 127.34, 60.25.





Reference:

(1) Supramolecular Bindfit. www.supramolecular.org

(2) a) ChemicalBook. (September 1, 2016) "Cis-Stilbene(645-49-8) ^{1}H NMR". https://www.chemicalbook.com/SpectrumEN_645-49-8_1HNMR.htm. b) ChemicalBook. (September 1, 2016) "Trans-Stilbene(103-30-0) ^{1}H NMR". https://www.chemicalbook.com/SpectrumEN_103-30-0_1hnmr.htm.