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

Synthesis of Substrates **1**-**3**

Substrates **1–3** were synthesised using standard methods (Fig. 1) and purified via flash chromatography Cyclohexyloxymethyl benzene (**1**) was synthesised by adding benzylbromide and diisopropylethylamine to a solution of cyclohexanol and heating under reflux for 2 hours at 150 °C. Cyclohexyl benzoate (**2**) and 1-cyclohex-2-enyl benzoate (**3**) were synthesised by adding triethylamine and benzoyl chloride to cyclohexanol and cyclohex-2-enol in dichloromethane and stirring for 16 hours at room temperature. The substrates were purified as described in the experimental section and characterised via NMR spectroscopy.

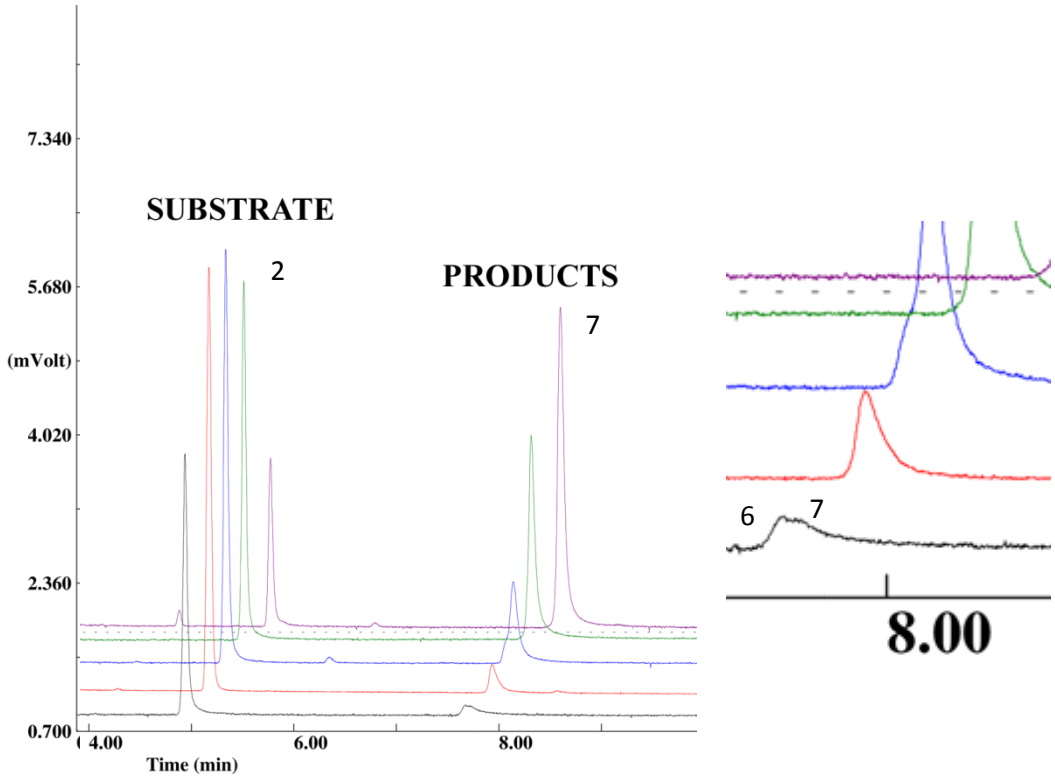
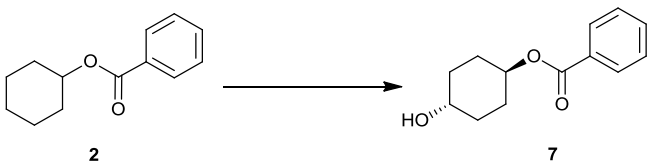


Figure S1 GC analysis of the turnover of cyclohexyl benzoate (**2,** RT 5.0 min) by different P450cam mutants. The amount of product (RT 7.8 min) is greatest for the Y96A mutant (purple). Lower amounts of product are observed for the F87A/Y96F mutant (green), the Y96F mutant (blue) and the WT enzyme (black). The red chromatogram is from the V247A mutant which alters the selectivity towards the minor product, **6** (85%, Table 2 and Fig. S3). For clarity each GC trace has been offset along the x and y axes. The inset shows an expanded view of the product peak in the chromatogram. Two overlapping peaks can be seen for the WT enzyme (black) and a shoulder can be seen in the Y96F mutant (blue). The Y96A mutant (purple) resulted in the formation of >95% of a single product (**7**, Table 2 and Fig. S3).



2 **7**

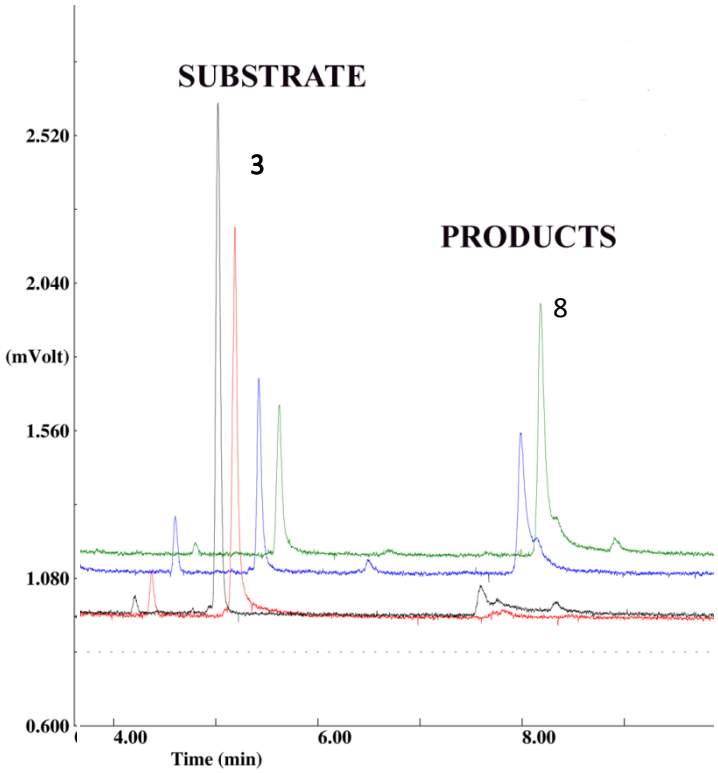
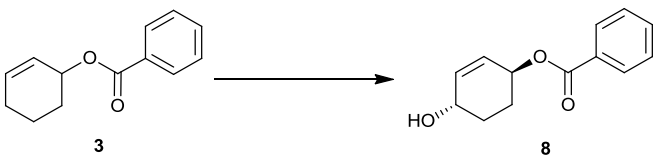


Figure S2 GC analysis of the turnover of 1-cyclohex-2-enyl benzoate (**3**, RT 4.5 min) by different P450cam mutants. The amount of product (RT 7.7 min) is greatest for the F87A/Y96F mutant (green). Significantly lower amounts of product are observed for the Y96A mutant (black) and the WT enzyme (red) while the F87L/Y96F mutant (blue) generated an intermediate level of product. The F87A/Y96F mutant generates predominantly one product, **8** (>90%). The minor products can be seen in the chromatogram as a shoulder on the peak of the majority product. For clarity each GC trace has been offset along the x and y axes.



3 8

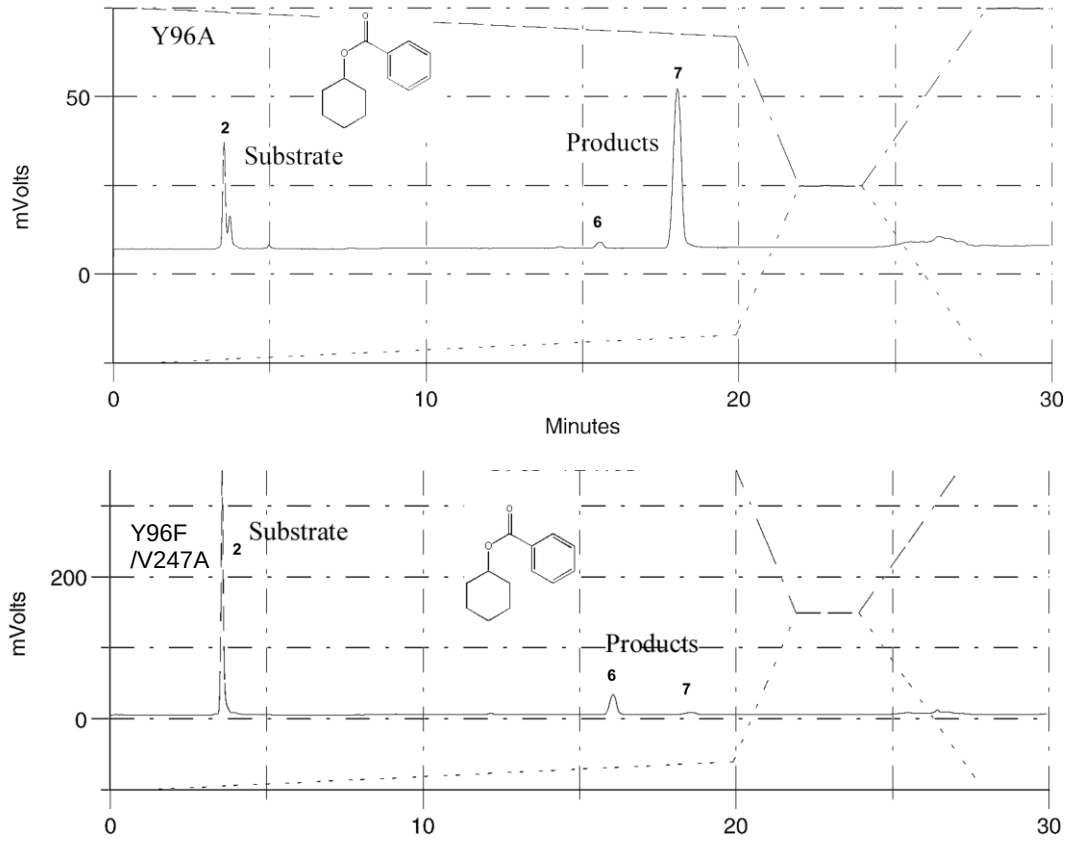


Figure S3 Normal phase HPLC analysis of the turnover of cyclohexyl benzoate (**2,** RT 4.0 min) by Y96A (top chromatogram) and Y96F/V247A (bottom chromatogram). Two products can be seen in both chromatograms. The major product, **7**, with Y96A (RT 18 min) is formed with >95% selectivity and the minor product (RT 16 min) can be seen. The Y96F/V247A mutant forms an excess of product, **6**, at 16 min (85%) with a smaller amount of the product at 18 min (15%).

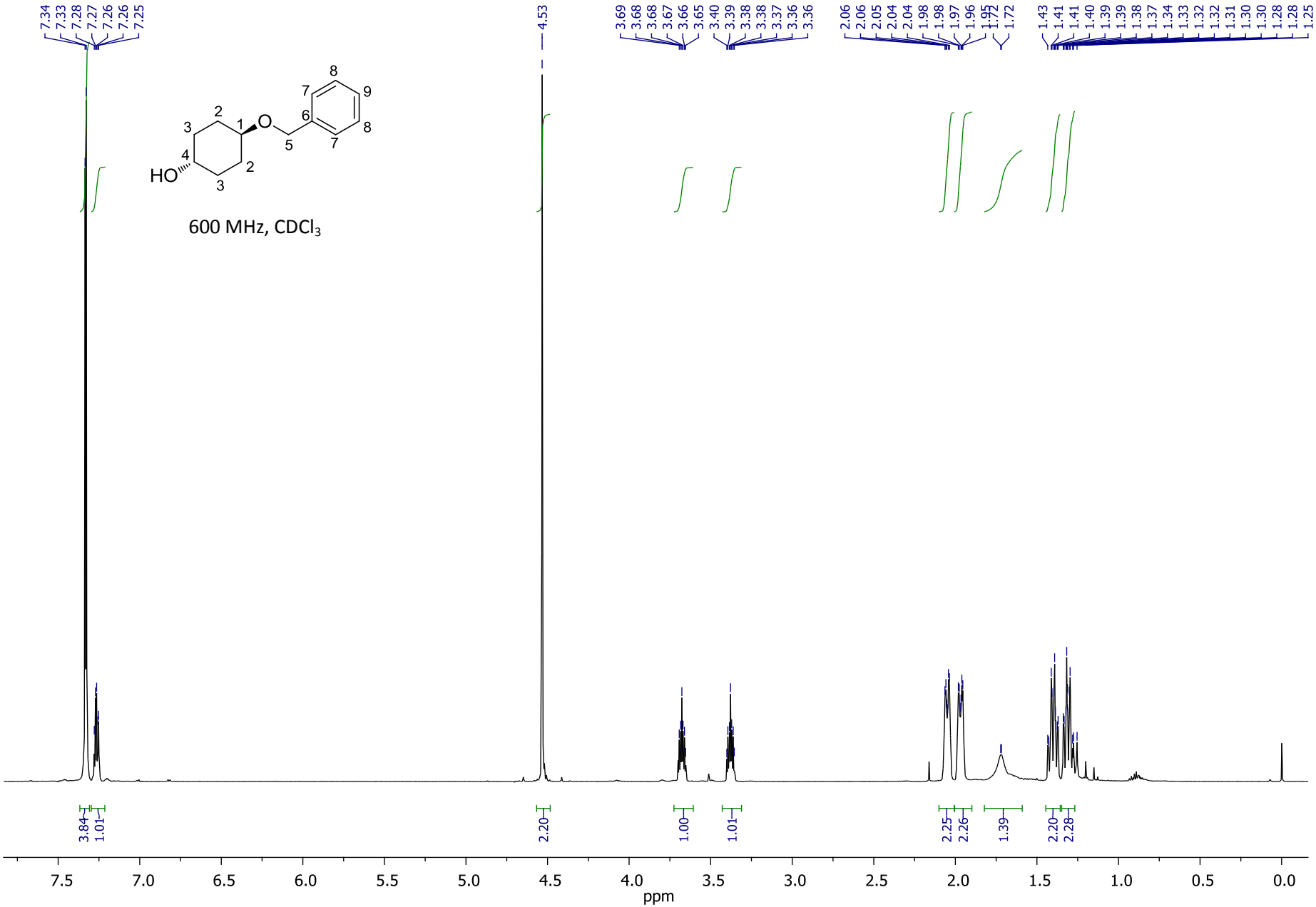


Figure S4a ^{1}H NMR spectrum of 4-(benzyloxy)cyclohexanol (**5**)

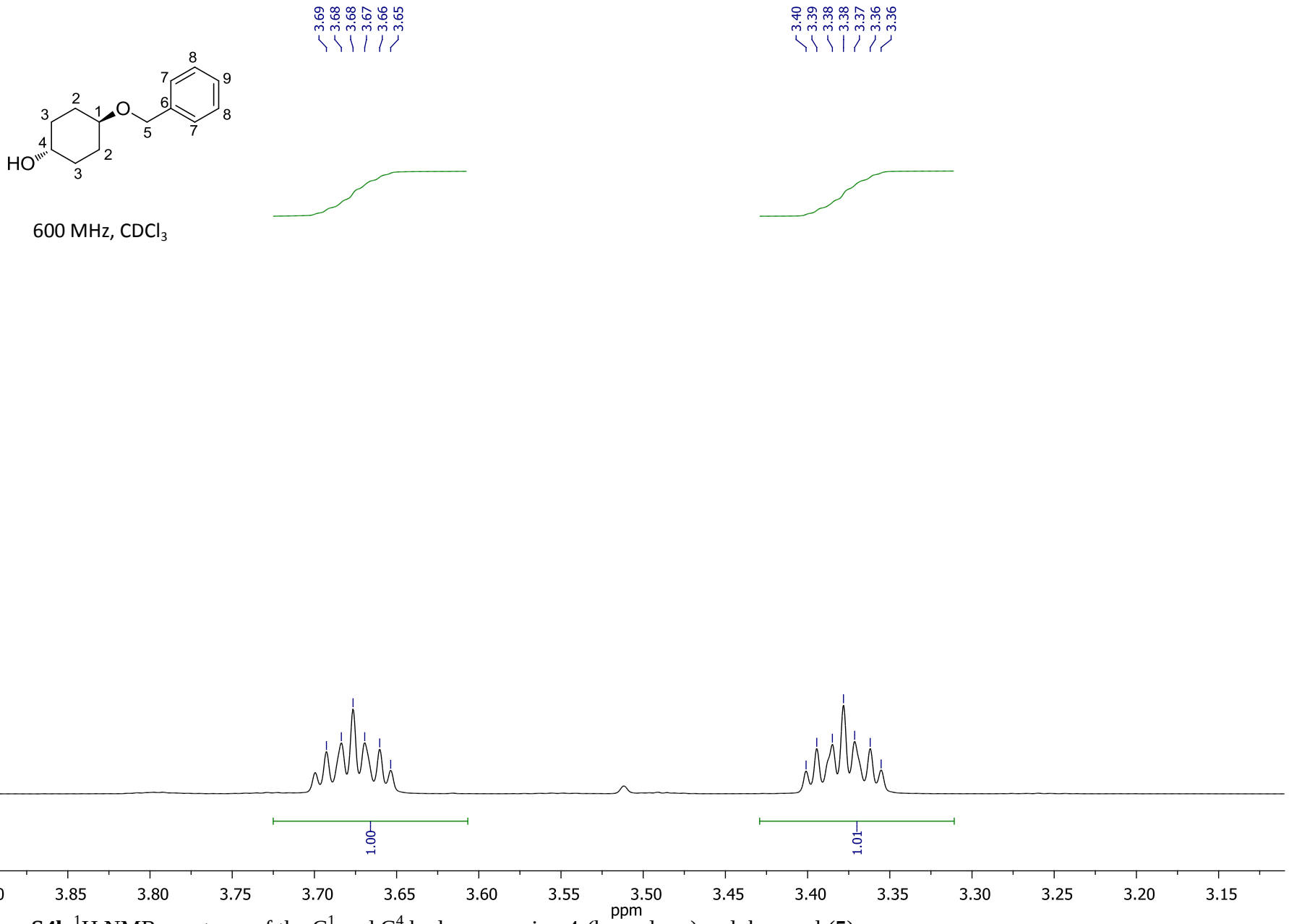


Figure S4b ^{1}H NMR spectrum of the C^{1} and C^{4} hydrogen region 4-(benzyloxy)cyclohexanol (**5**)

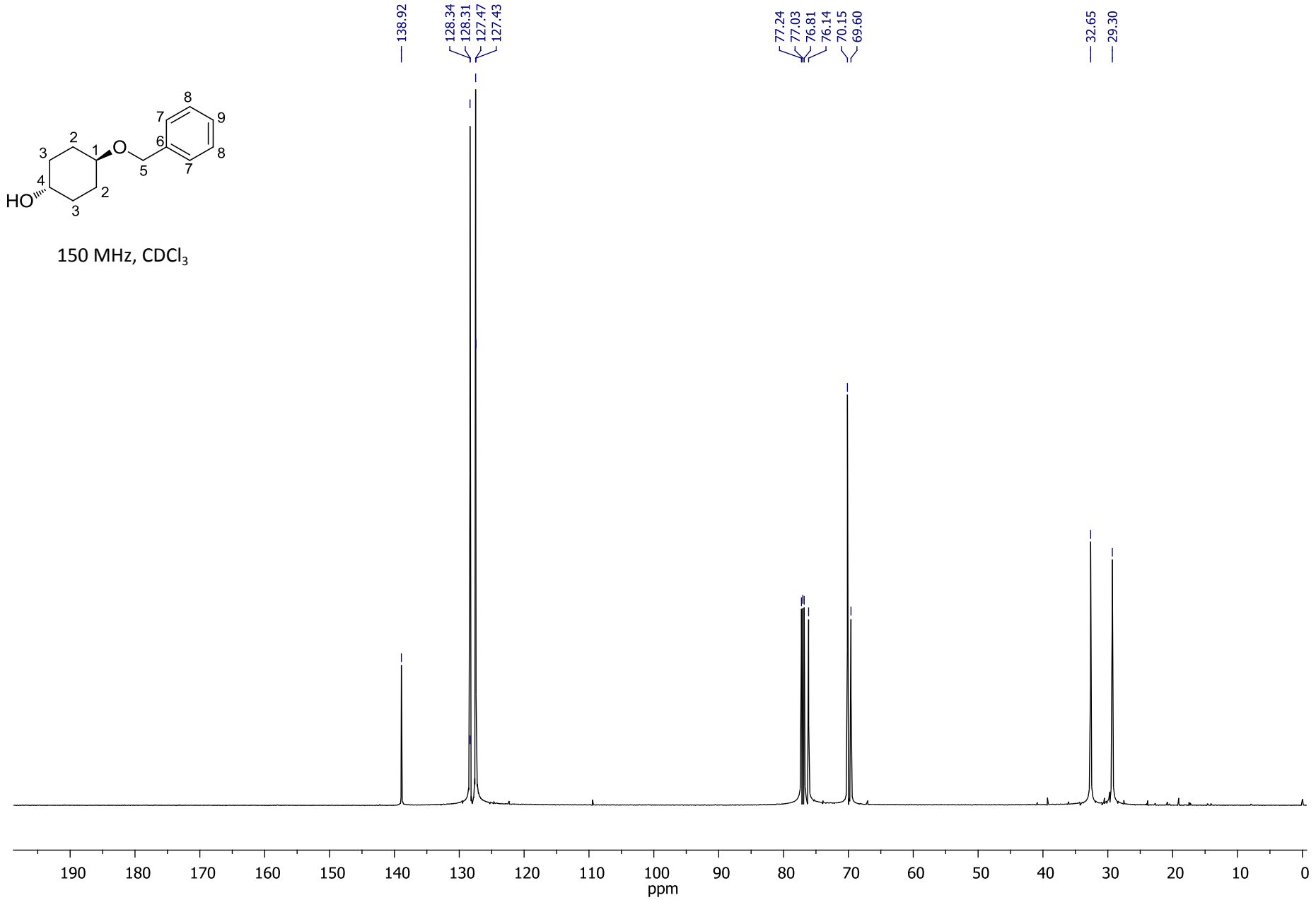


Figure S4c ^{13}C NMR spectrum of 4-(benzyloxy)cyclohexanol (**5**)

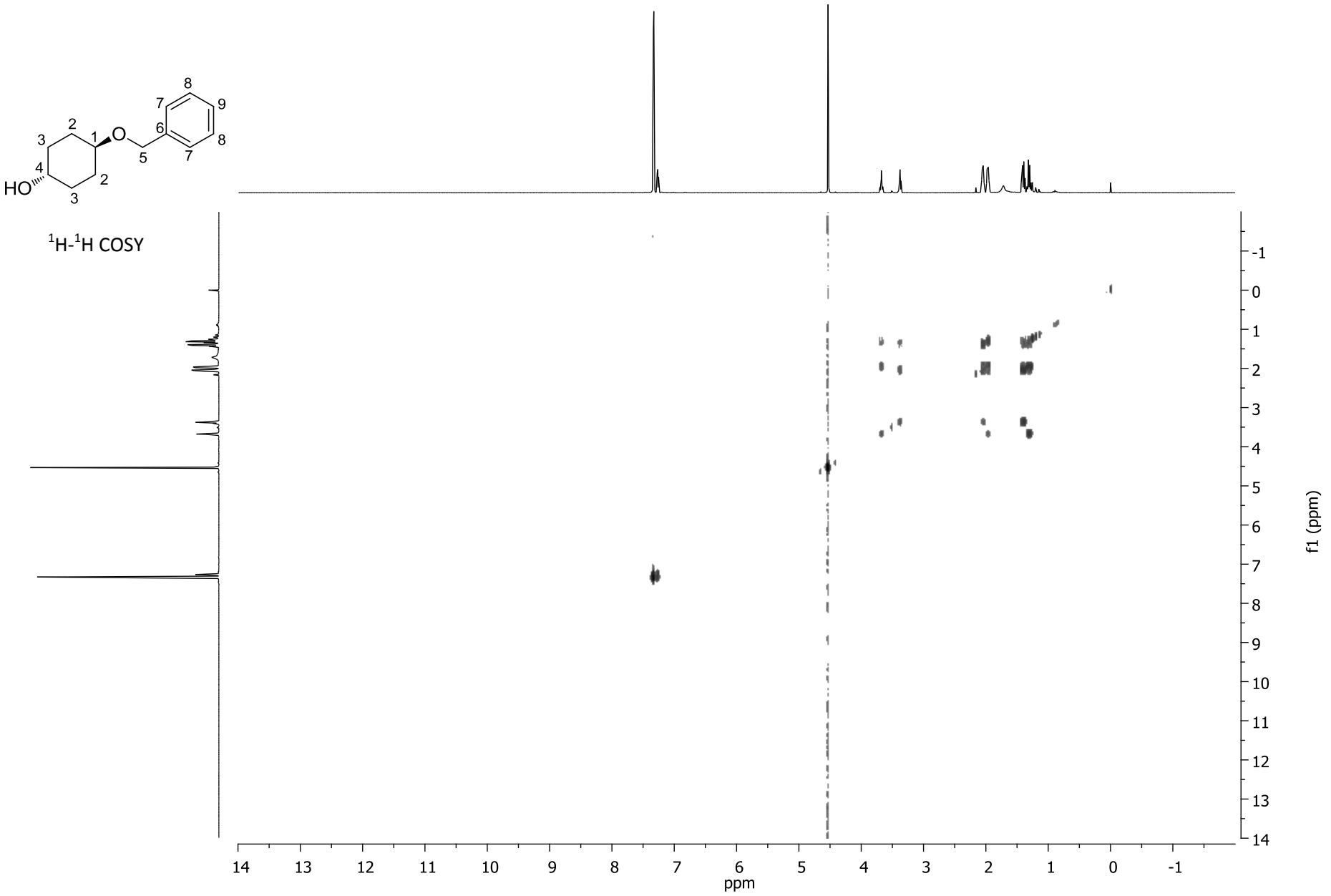


Figure S4d ^{1}H-^{1}H COSY spectrum of 4-(benzyloxy)cyclohexanol (**5**)

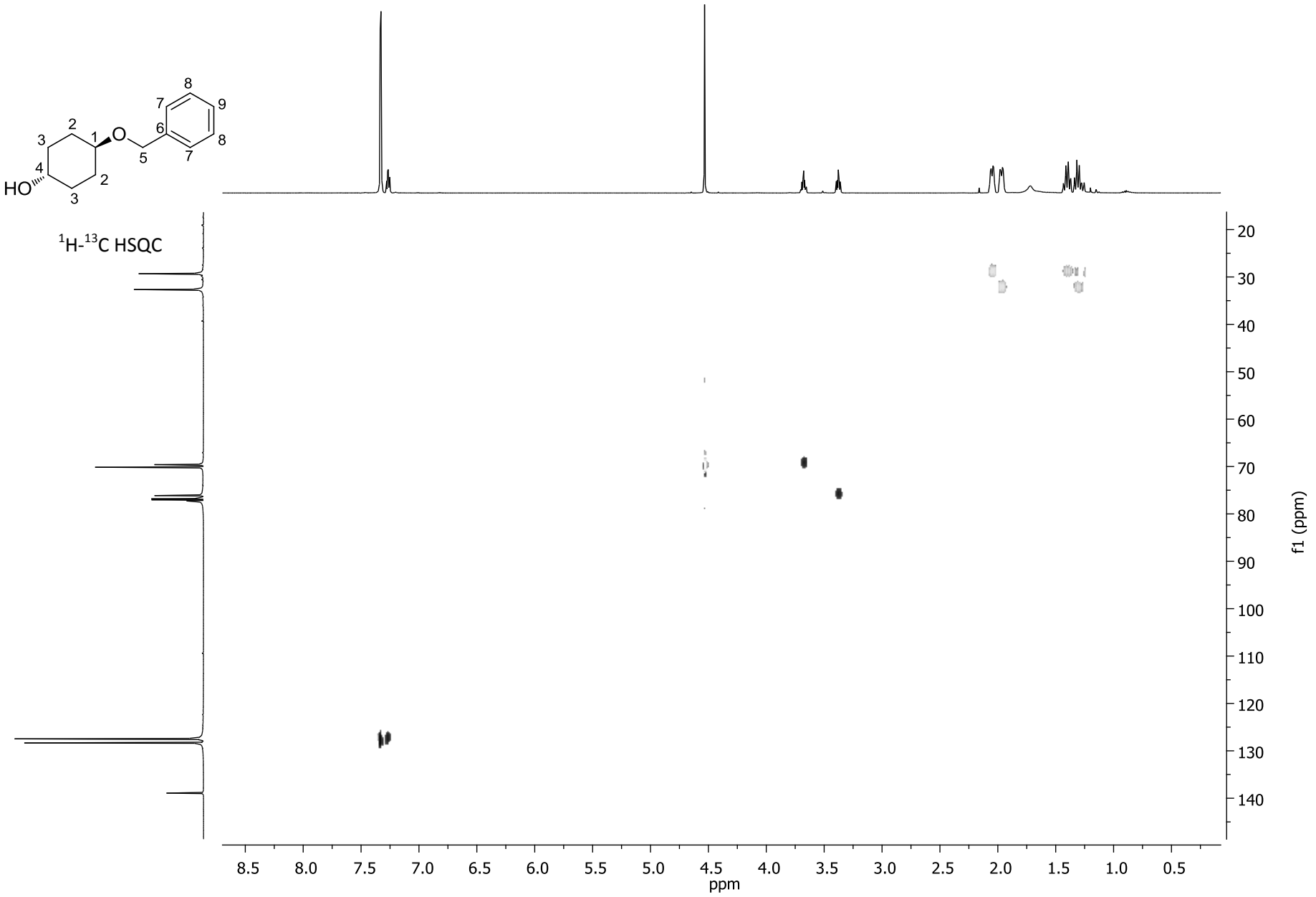


Figure S4e ^{1}H-^{13}C HSQC spectrum of 4-(benzyloxy)cyclohexanol (**5**)

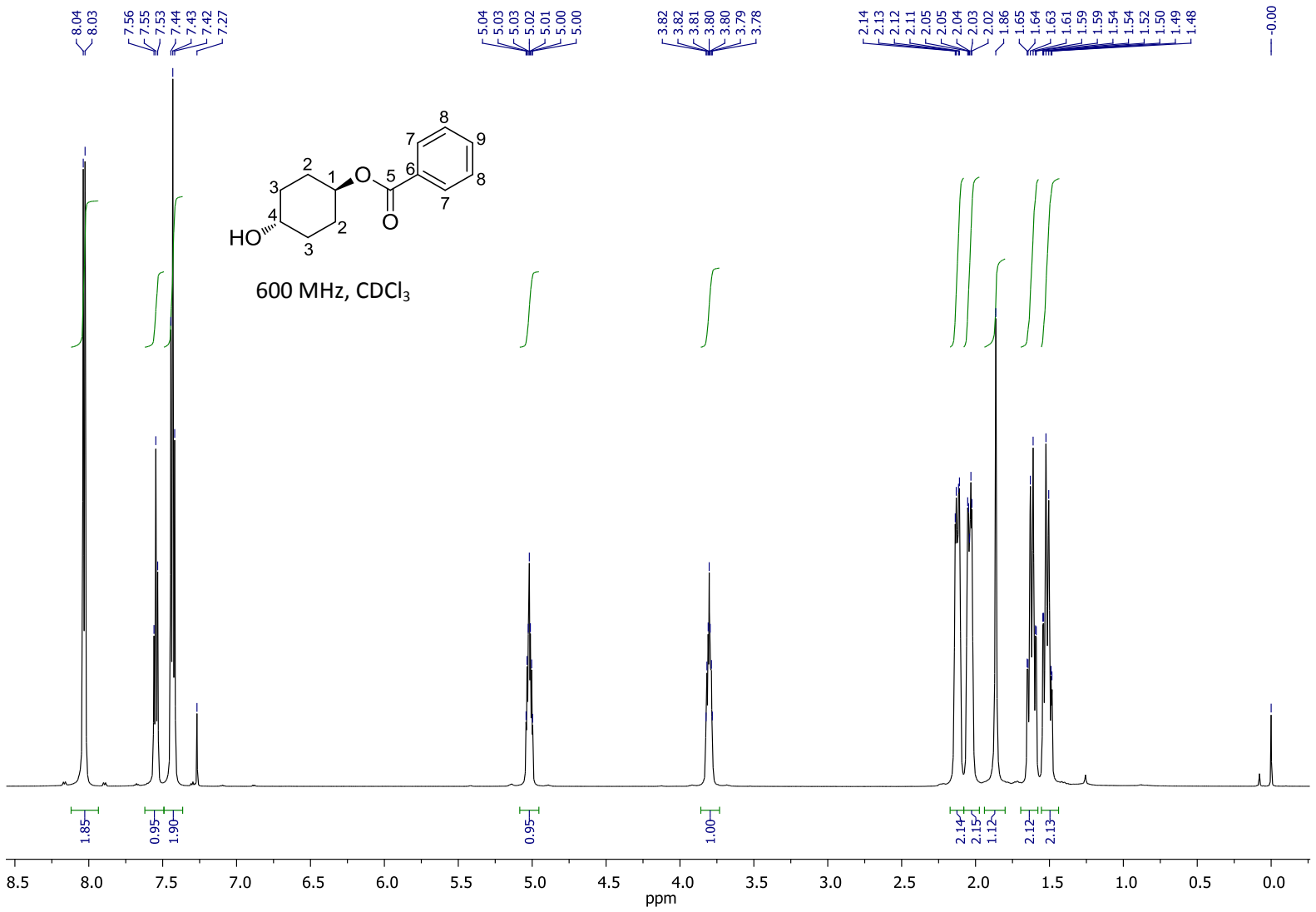


Figure S5a 1H NMR spectrum of benzoic acid 4-hydroxy-cyclohexyl ester (**7**)

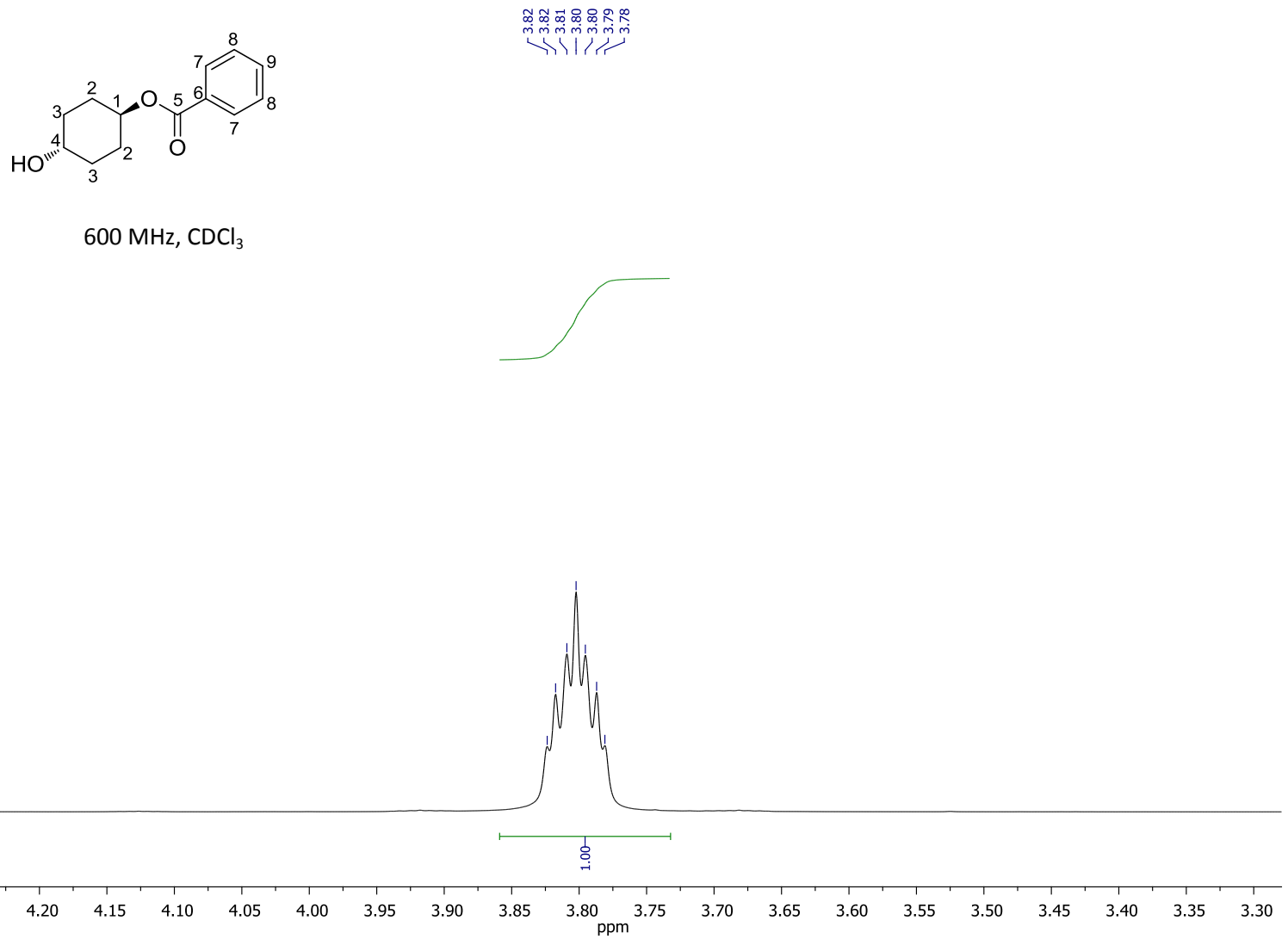


Figure S5b ^{1}H NMR spectrum of the C^{1} hydrogen region of benzoic acid 4-hydroxy-cyclohexyl ester (**7**)

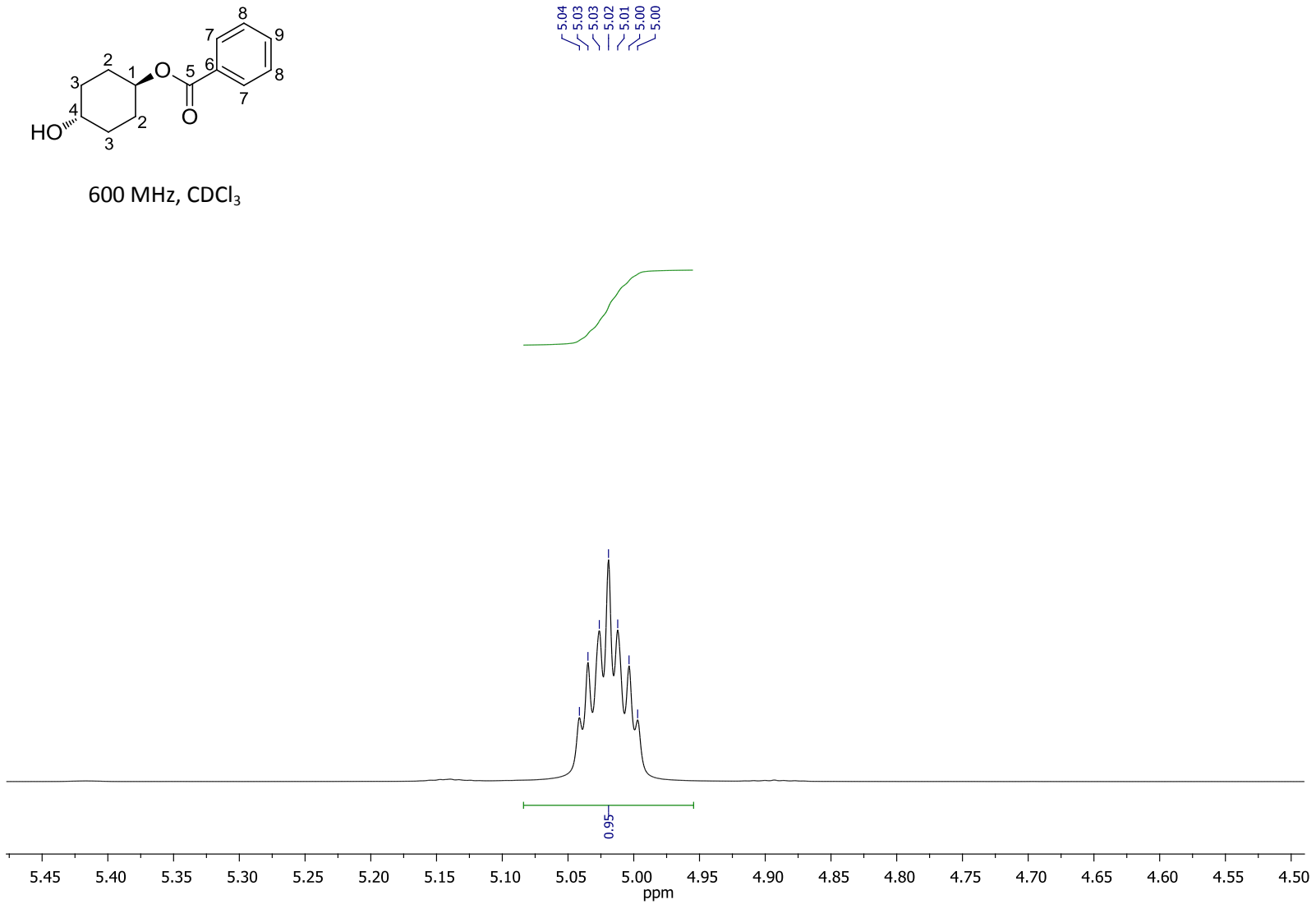


Figure S5c ^{1}H NMR spectrum of the C^{4} hydrogen region of benzoic acid 4-hydroxy-cyclohexyl ester (**7**)

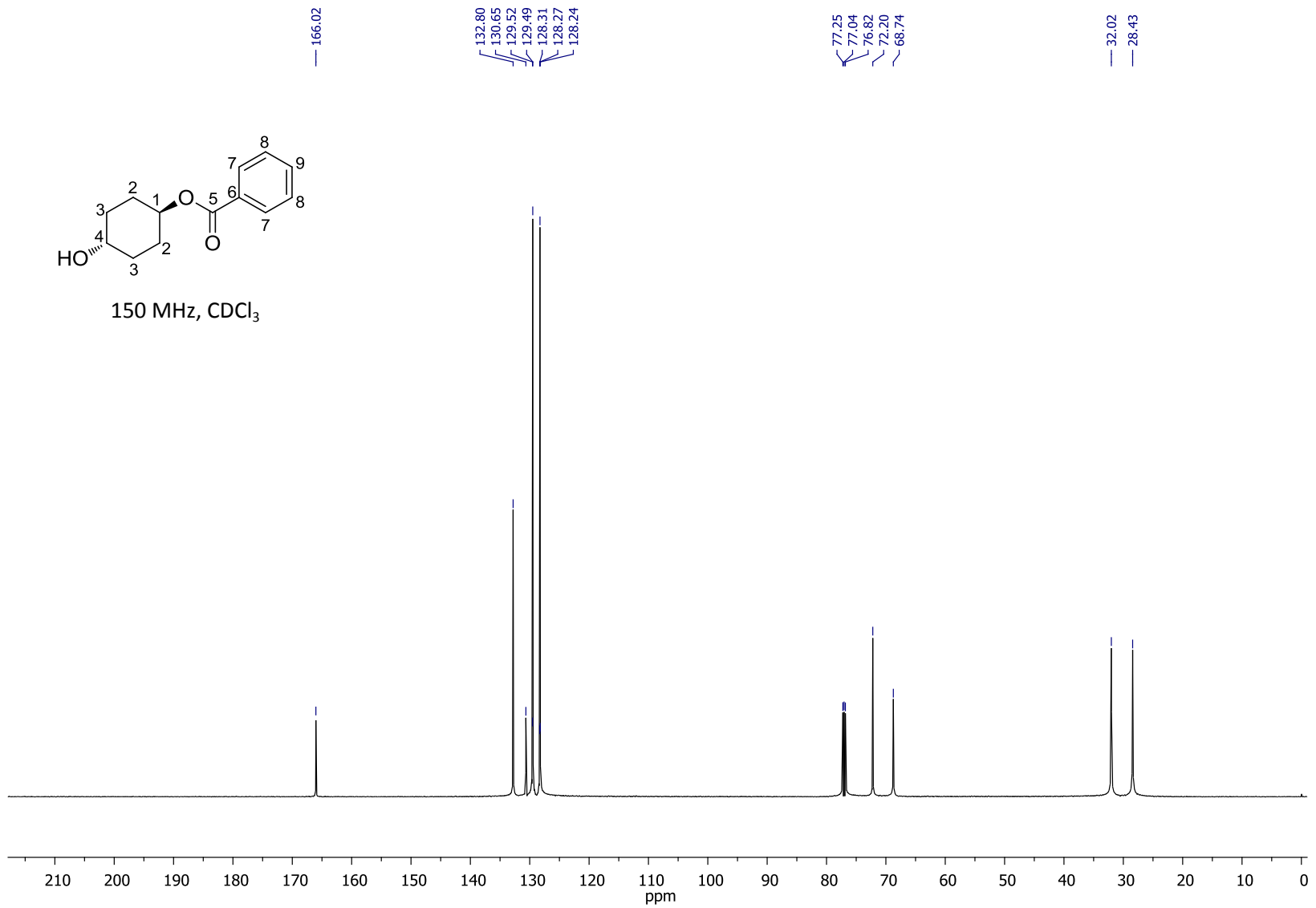


Figure S5d ^{13}C NMR spectrum of benzoic acid 4-hydroxy-cyclohexyl ester (**7**)

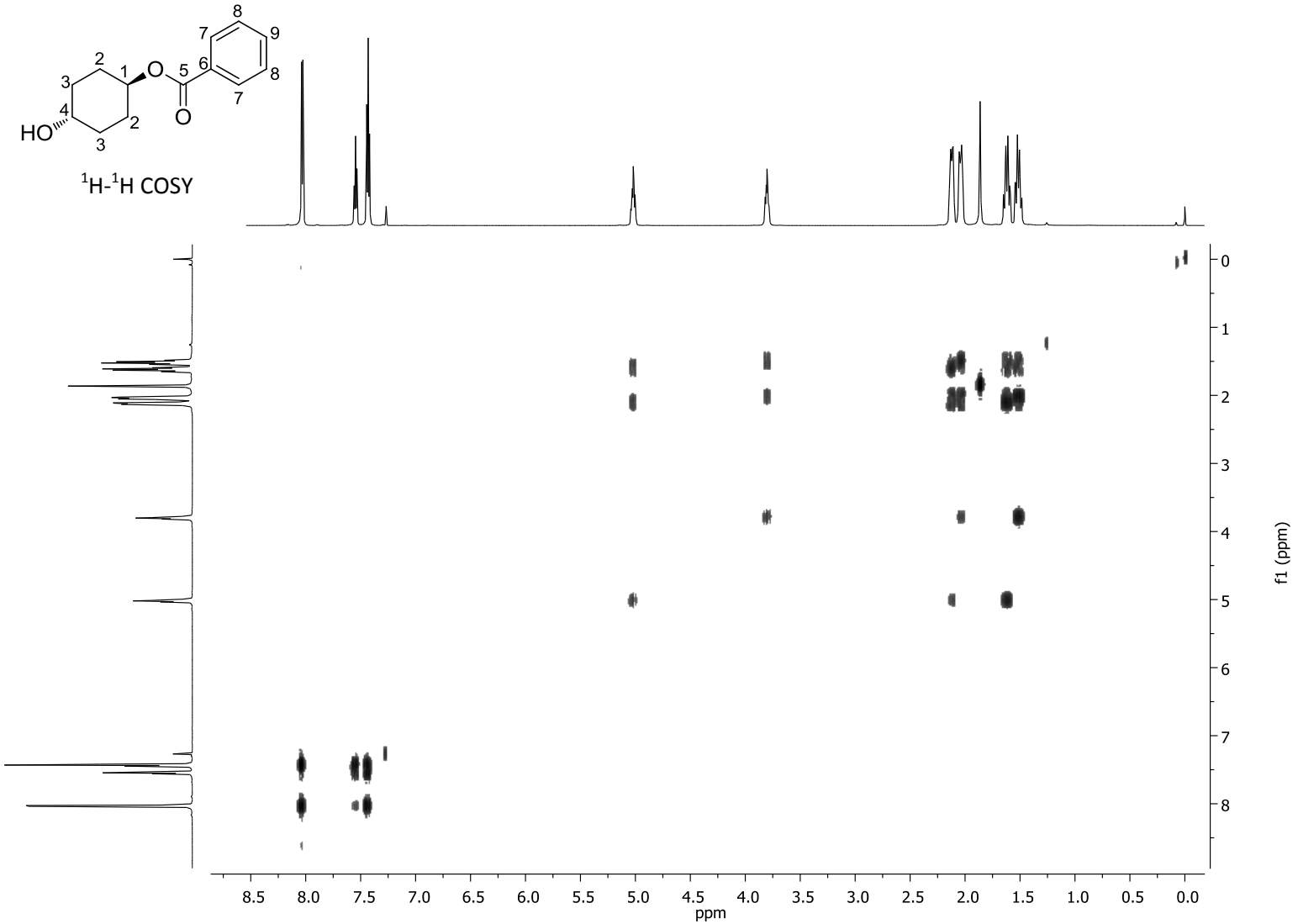


Figure S5e ^{1}H-^{1}H COSY spectrum of benzoic acid 4-hydroxy-cyclohexyl ester (**7**)

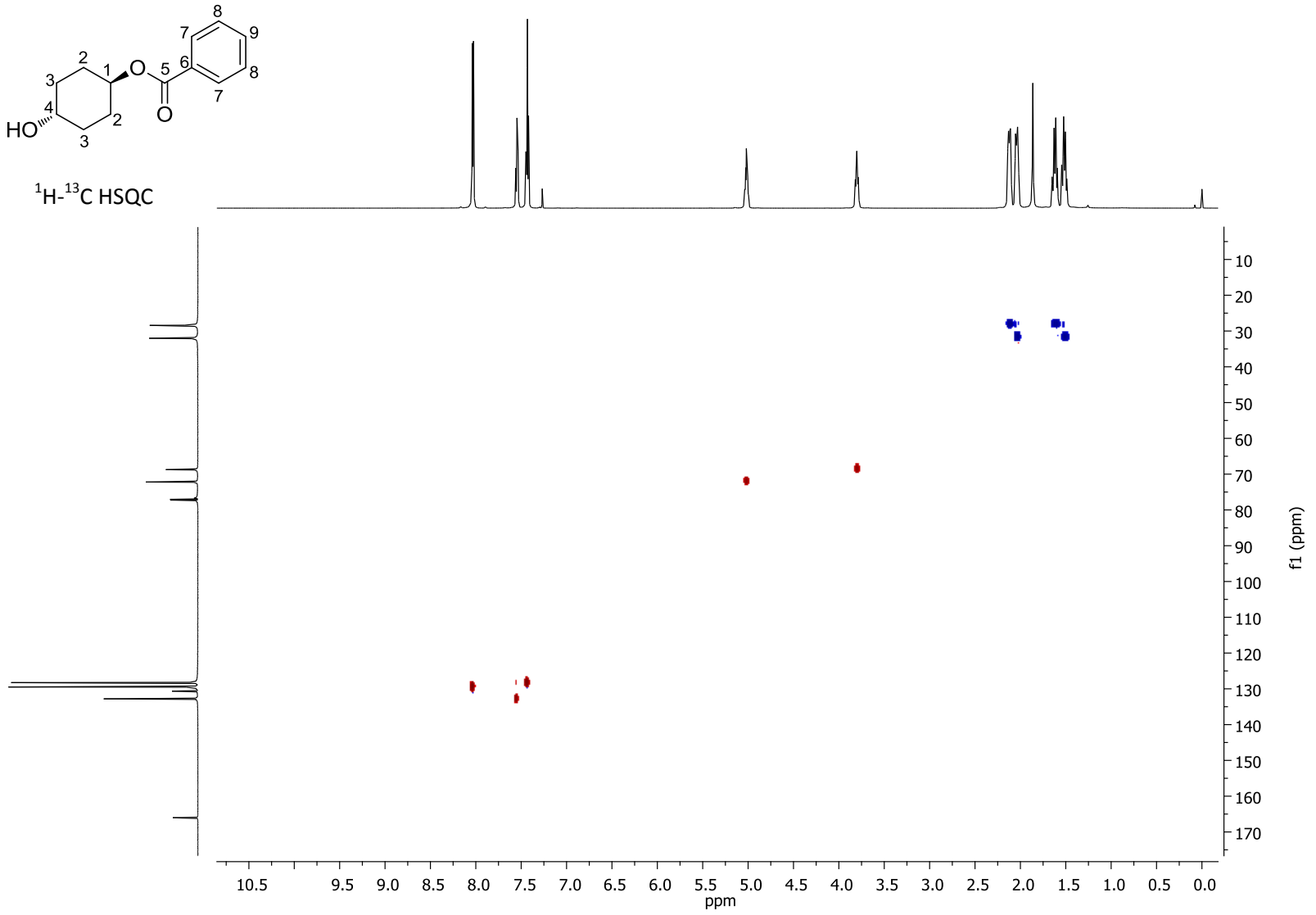


Figure S5f ^{1}H-^{13}C HSQC spectrum of benzoic acid 4-hydroxy-cyclohexyl ester (**7**)

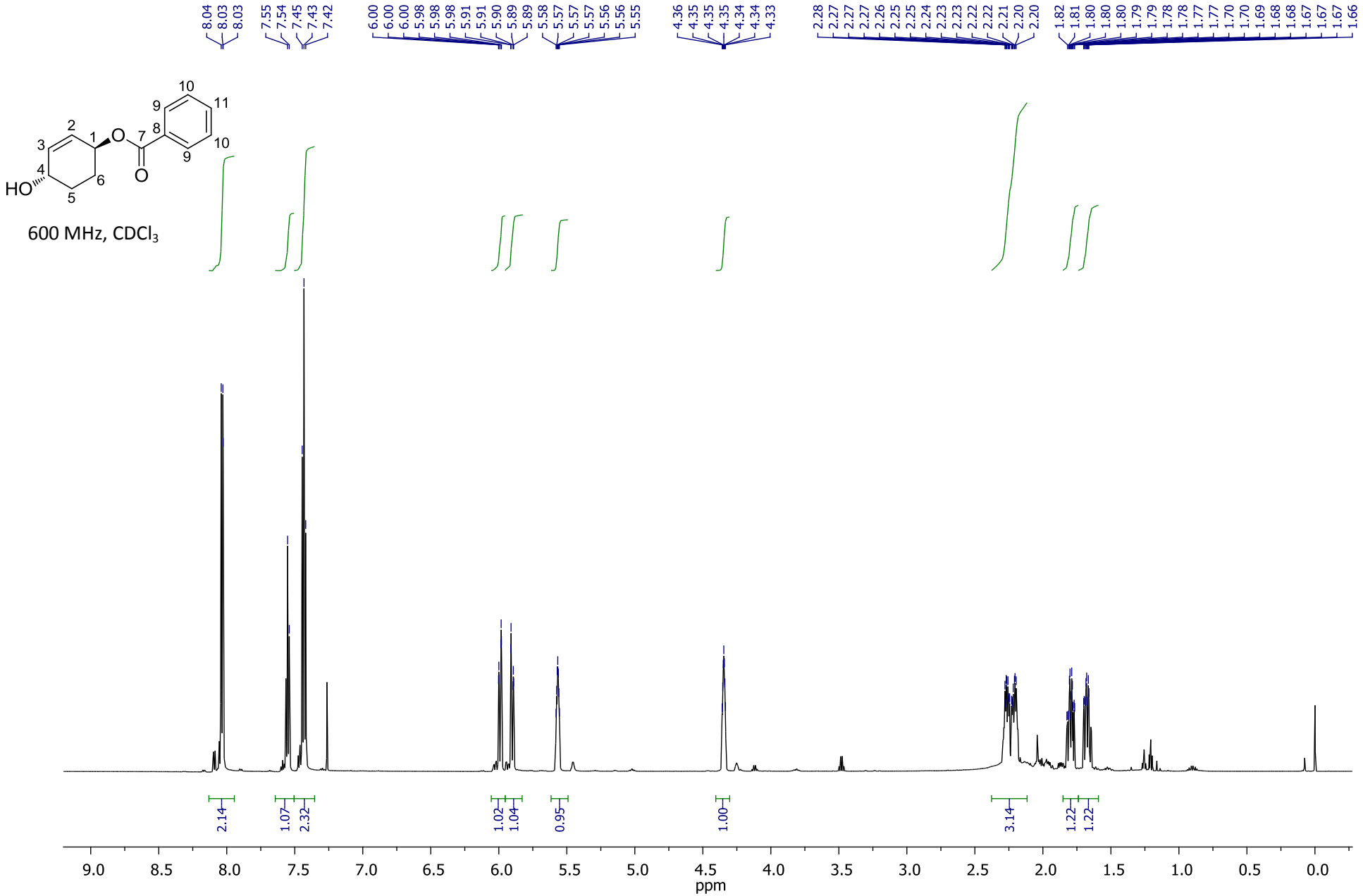


Figure S6a ^{1}H NMR spectrum of benzoic acid 4-hydroxy-cyclohex-2-enyl ester (**8**)

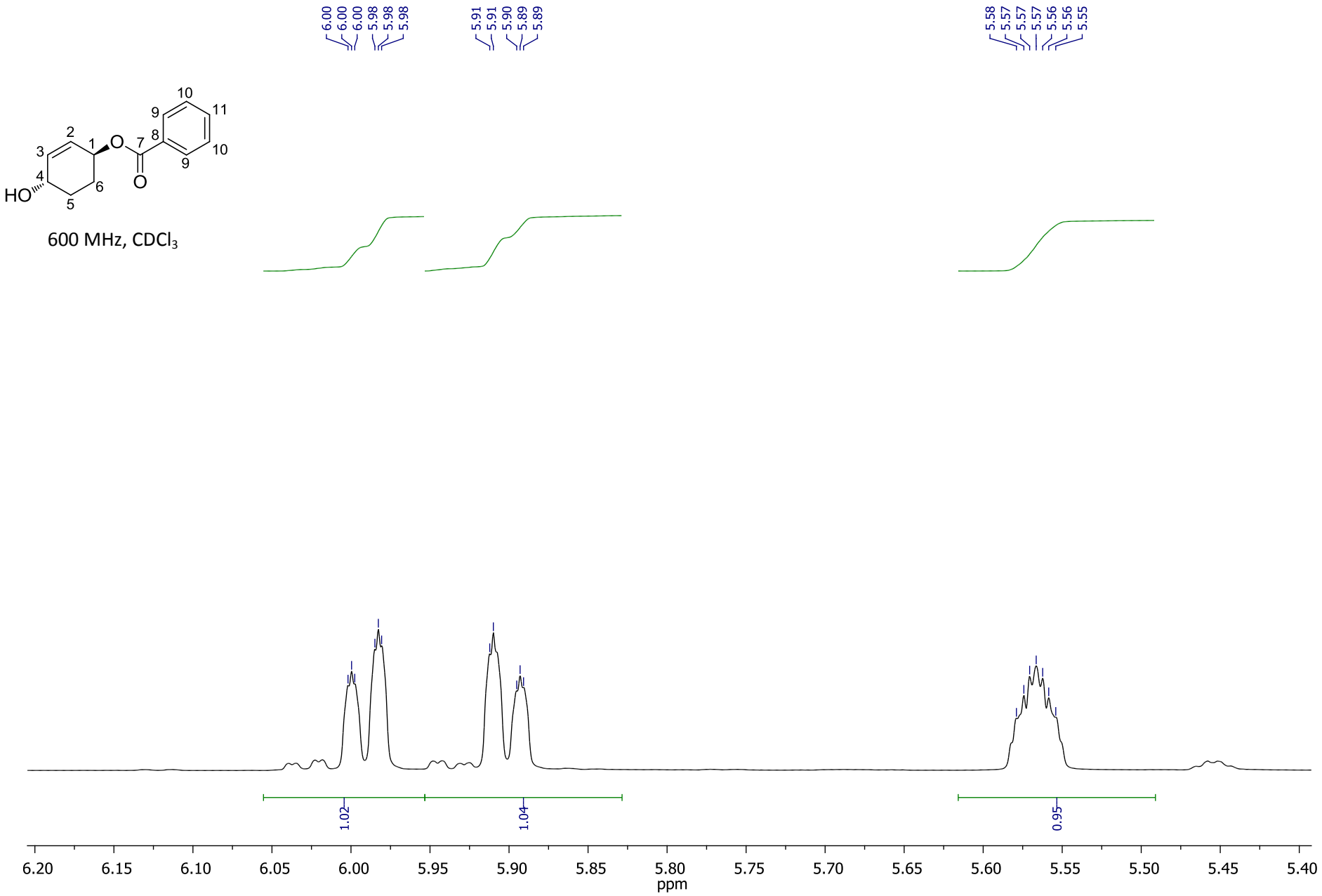


Figure S6b ^{1}H NMR spectrum of the C^{1}, C^{2} and C^{3} hydrogen region of benzoic acid 4-hydroxy-cyclohex-2-enyl ester (**8**)

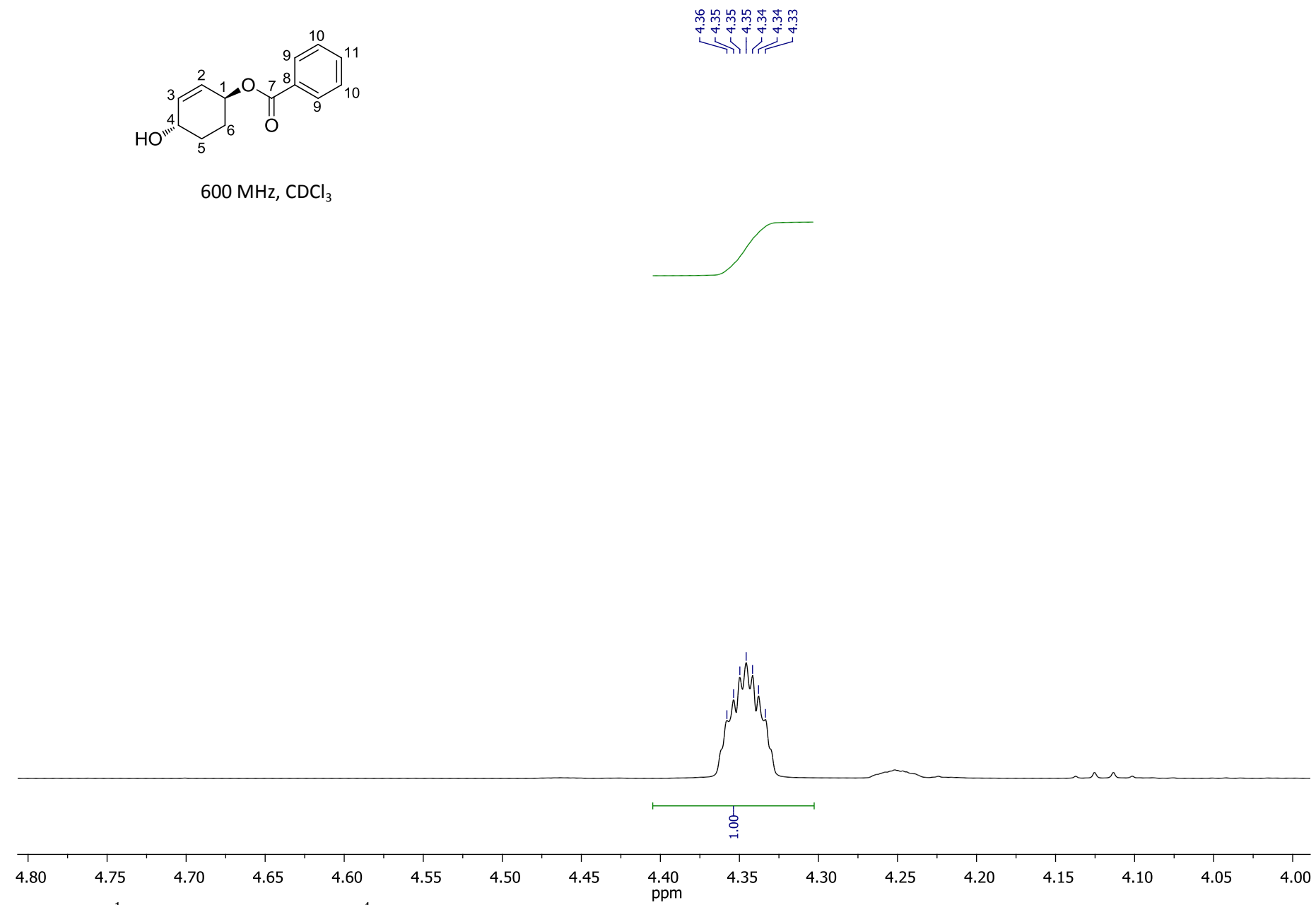


Figure S6c ^{1}H NMR spectrum of the C^{4} hydrogen region of benzoic acid 4-hydroxy-cyclohex-2-enyl ester (**8**)

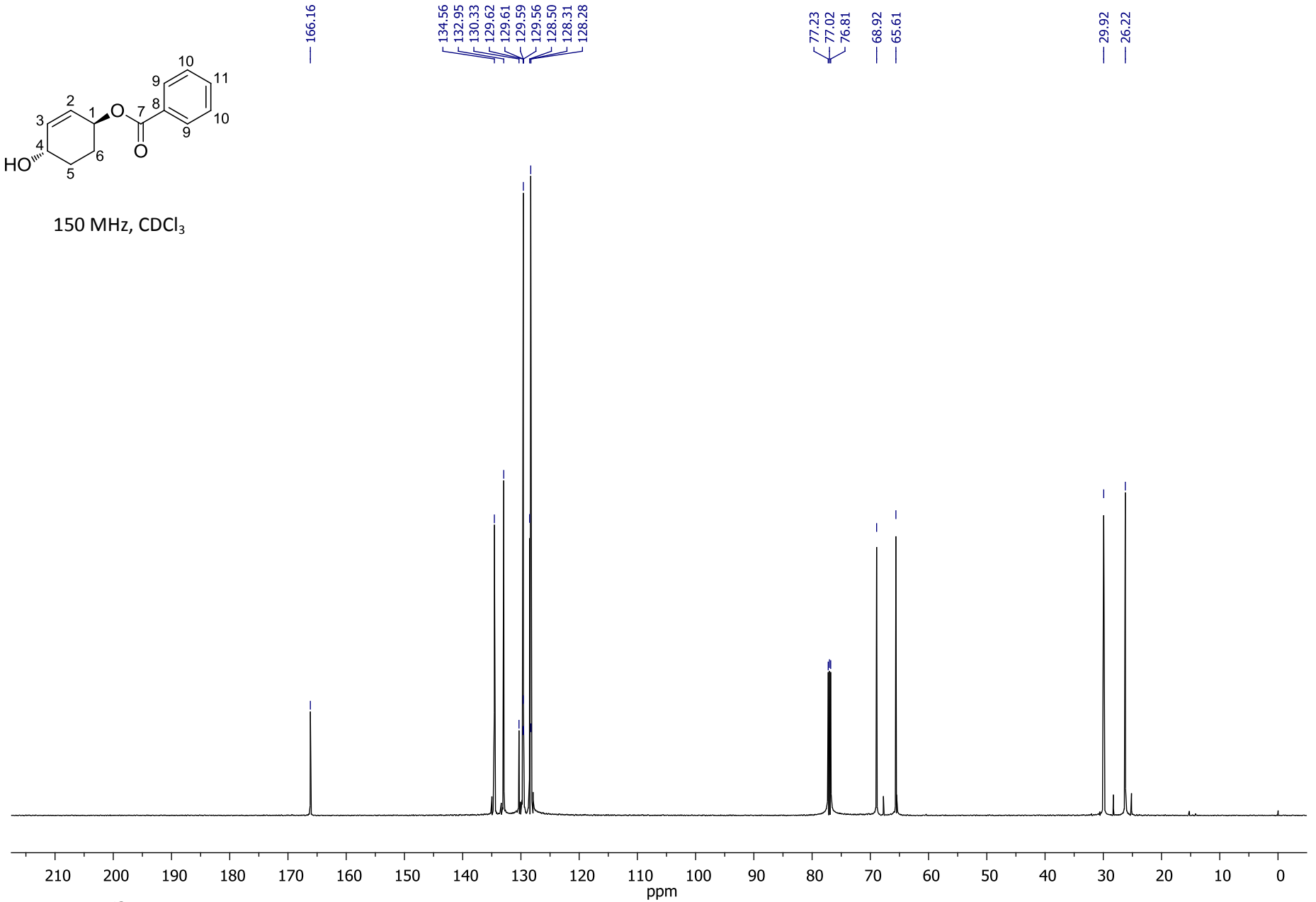


Figure S6d ^{13}C NMR spectrum of benzoic acid 4-hydroxy-cyclohex-2-enyl ester (**8**)

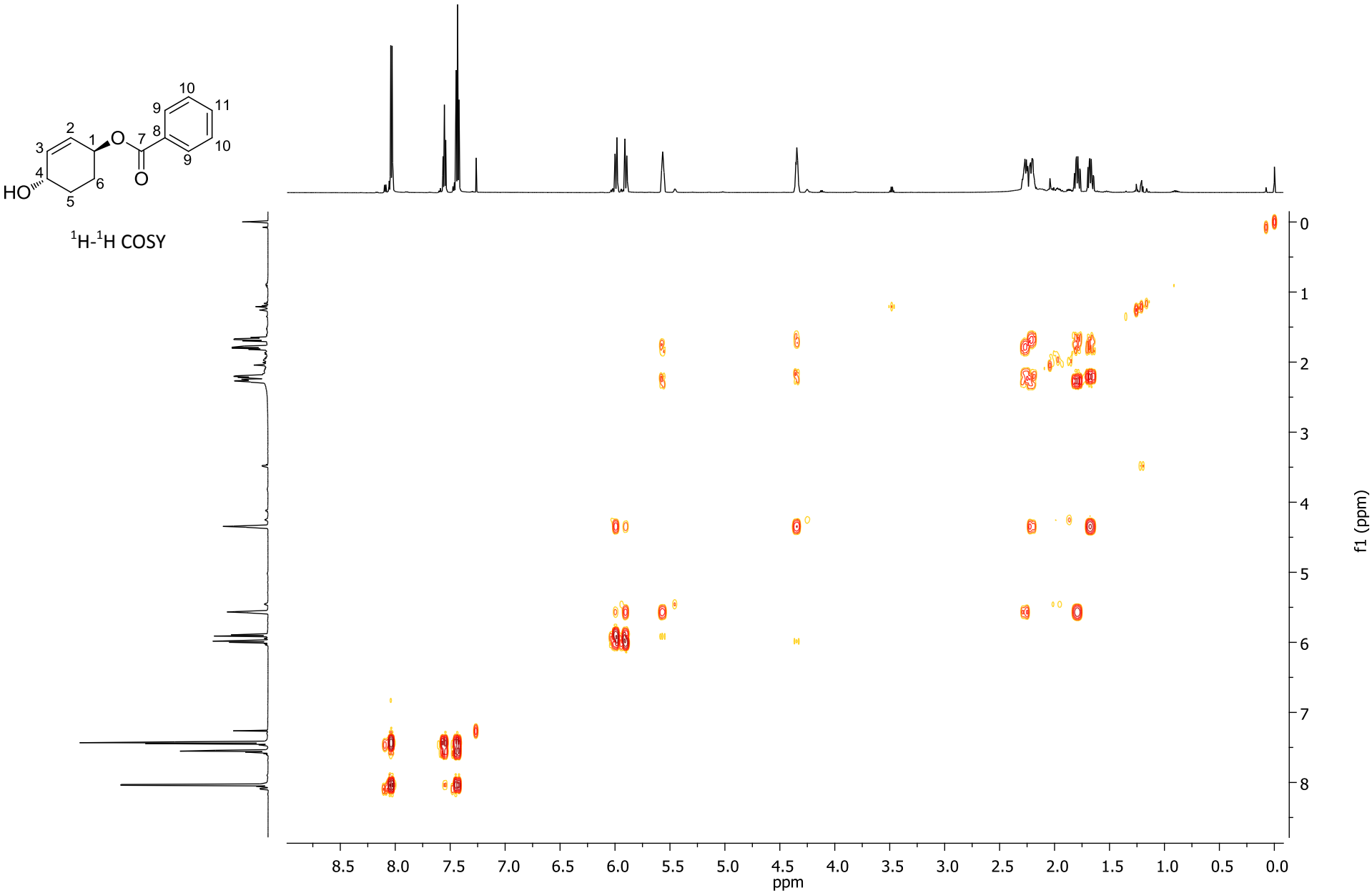


Figure S6e ^{1}H-^{1}H COSY spectrum of benzoic acid 4-hydroxy-cyclohex-2-enyl ester (**8**)

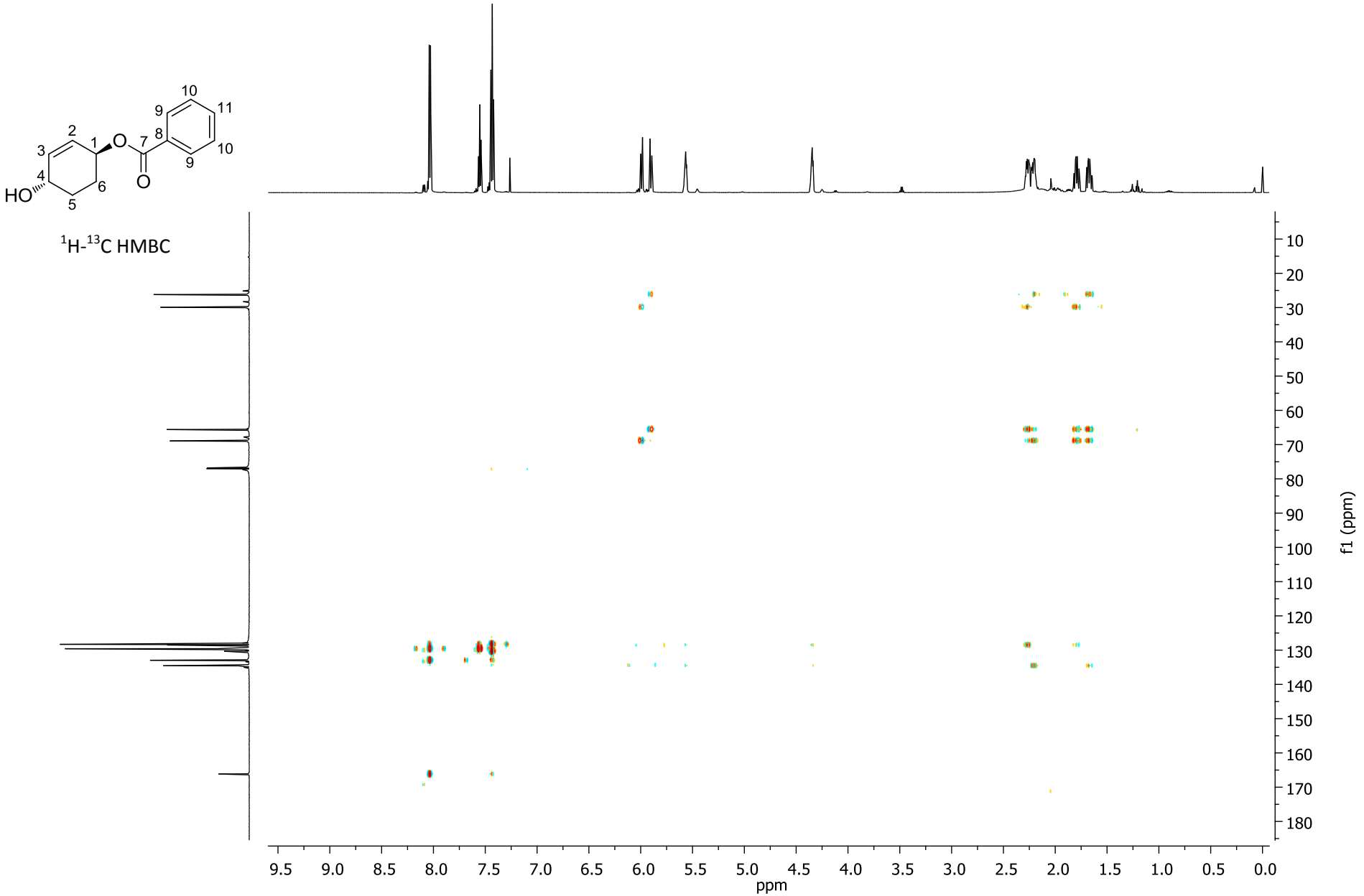


Figure S6f ^{1}H-^{13}C HMBC spectrum of benzoic acid 4-hydroxy-cyclohex-2-enyl ester (**8**)

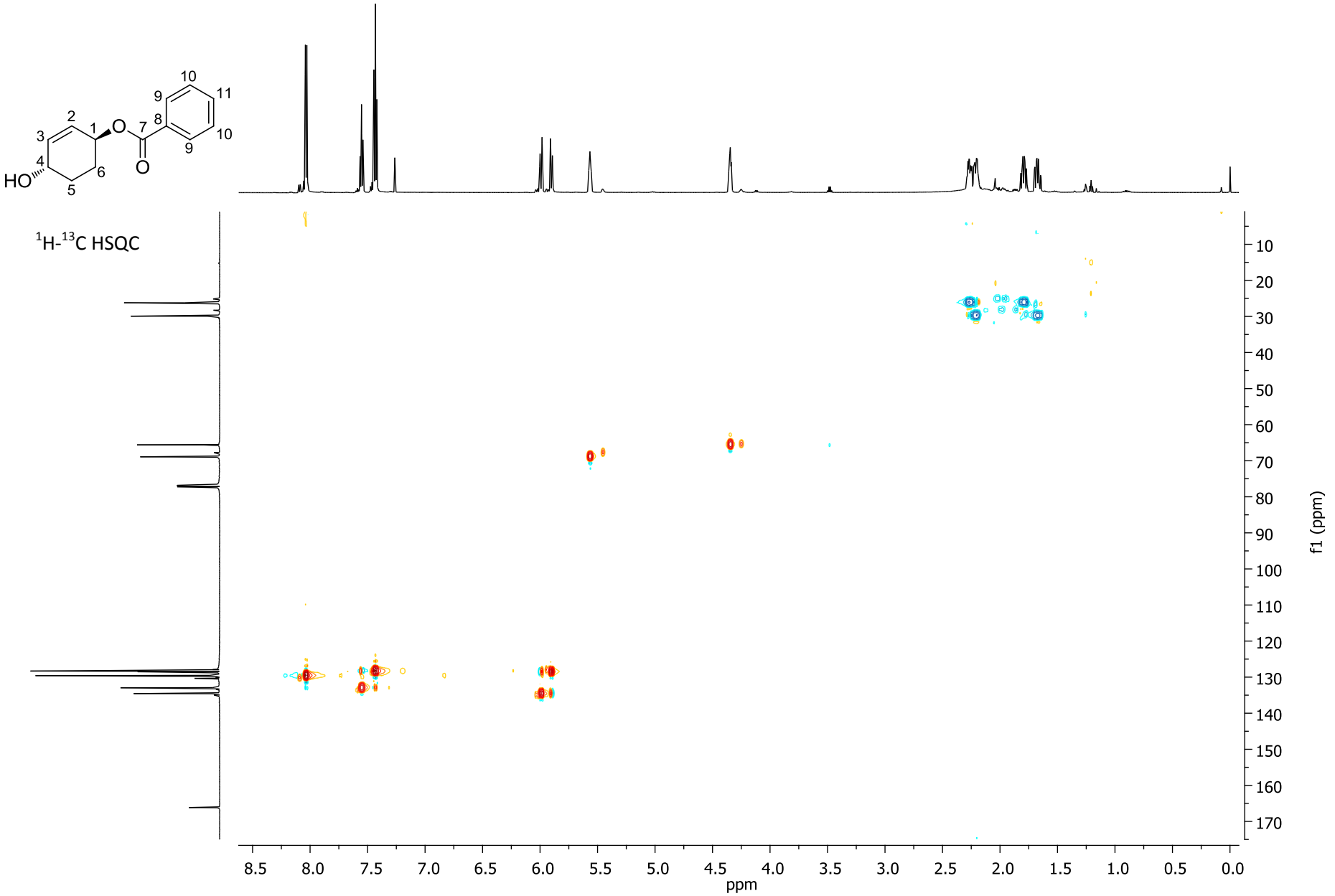


Figure S6g ^{1}H-^{13}C HSQC spectrum of benzoic acid 4-hydroxy-cyclohex-2-enyl ester (**8**)

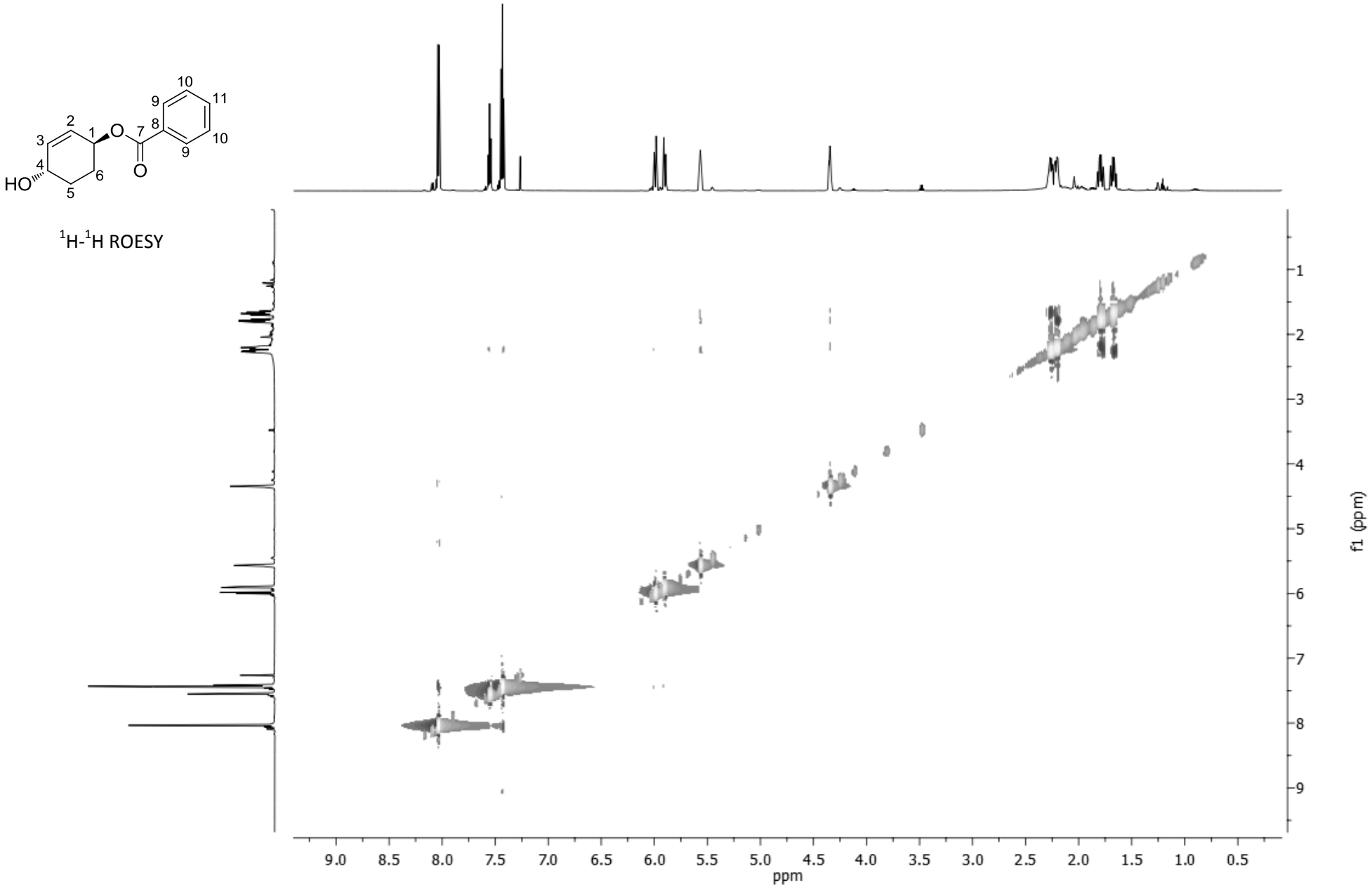


Figure S6h ^{1}H-^{1}H ROESY spectrum of benzoic acid 4-hydroxy-cyclohex-2-enyl ester (**8**)

Figure S7 Chiral chromatogram of Benzoic acid 4-hydroxy-cyclohex-2-enyl ester, **8**.