A Chemoenzymatic Process to Achieve Optically Active Mandelic Acid Employing Continuous-Flow Resolution of Cyanohydrin as a Key Step

Síntese Químio-Enzimática do Ácido Mandélico Opticamente Ativo Empregando a Resolução em Fluxo Contínuo do Precursor Cianoidrina como Etapa Chave

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Supporting Information

General Information

^{1}H NMR spectra were recorded on a Bruker DPX 200 MHz spectrometer. ^{13}C NMR spectra were recorded on the same instrument at 50 MHz. The chemical shifts of ^{1}H and ^{13}C NMR were quoted relative to internal tetramethylsilane ($\delta_{TMS} = 0.00$). ^{1}H NMR data are reported as follows: chemical shift in ppm (δ), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz) and relative intensity (integral). ^{13}C NMR data are reported as chemical shift in ppm (δ).

Infrared spectra were recorded from KBr discs on a Bomem MB100 spectrometer coupled with FTIR. Maximum absorption (ν_{max}) is reported in wavenumber (cm^{-1}).

Optical rotations were measured by Jasco P2000 polarimeter with a 100 mm optical path cell.

Chiral GC analyses were performed on a Shimadzu model GC-17A chromatograph (FID) equipped with Beta Dex 325 column (30 m × 0.25 mm diameter, 0.25 µm film thickness). The split ratio was 1:50 and N_2 was used as carrier gas. The injector and detector temperatures were set at 220°C. Mandelonitrile (**3**) was derivatized to corresponding propionate **3a** and mandelic acid (**1**) was derivatized to corresponding acetal (**1a**) before analysis.

Temperature programming: 100 ^{o}C, increasing in 10 ^{o}C min^{-1} up to 175 ^{o}C, kept at 175 ^{o}C for 2 min, increasing in 5 ^{o}C min^{-1} up to 180 ^{o}C, kept at 180 ^{o}C for 7 min.

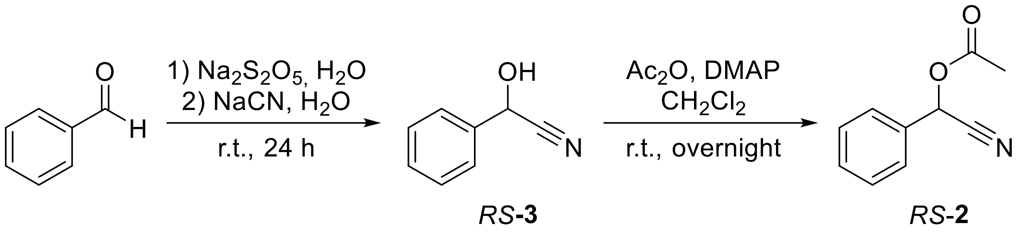
Retention times (min): (*R*)-**2**: 11.1, (*S*)-**2**: 11.5, (*R*)-**3b**: 12.9, (*S*)-**3b**: 13.1.

Temperature programming: 100 °C, increasing in 10 °C min^{-1} up to 180 °C, kept at 180 °C for 30 min.

Retention times (min): (*R*)-**1a**: 28.7, (*S*)-**1a**: 29.1.

Experimental Procedures

Synthesis of Mandelonitrile Acetate (*RS*-2) and Mandelonitrile (*RS*-3)



Benzaldehyde (2.122 g, 20 mmol) was added dropwise to a solution of $Na_2S_2O_5$ (2.282 g, 12 mmol) in water (10 mL), which was previous cooled in an ice bath and maintained under continuous stirring. After 10 min, a solution of NaCN (0.490 g, 20 mmol) in cold water (10 mL) was added dropwise and the mixture was stirred at room temperature for 24 h. The reaction media was extracted with CH_2Cl_2 (3 x 15 mL), dried with $MgSO_4$, filtered and the solvent was evaporated under reduced pression. The crude cyanohydrin was dissolved in CH_2Cl_2 (20 mL), and acetic anhydride (2.84 mL, 30 mmol) and DMAP (one crystal) were added. The mixture was stirred at room temperature overnight. Then, the reaction media was washed with a solution of $NaHCO_3$, dried with $MgSO_4$, filtered and solvent was evaporated under reduced pressure. Remaining aldehyde was removed via crystallization as its bisulfide salt.[1] Mandelonitrile (*RS*-**3**) and mandelonitrile acetate (*RS*-**2**) were obtained in 86% and 62% yield, respectively.

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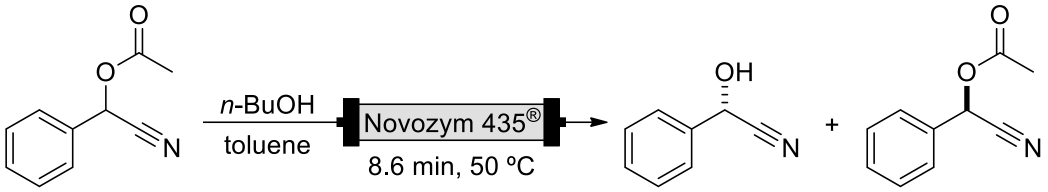
[1] a) Corson, B. B.; Dodge, R. A.; Harris, S. A.; Yeaw, J. S.; Mandelic acid. *Organic Synthesis* **1926**, *6*, 58. [Crossref] b) Thomas, J. C.; Aggio, B. B.; Oliveira, A. R. M.; Piovan, L.; High-throughput preparation of optically active cyanohydrins mediated by lipases. *European Journal of Organic Chemistry* **2016**, *2016*, 5964. [Crossref]

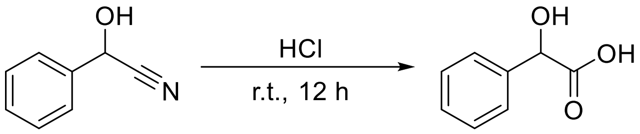
Enzymatic Kinetic Resolution in Continuous-Flow Mode



(*RS*)-**2** (*S*)-**3** (*R*)-**2**

Mandelonitrile acetate (*RS*-**2**) (2.628 g, 15 mmol) and *n*-butanol (5.50 mL, 60 mmol) were dissolved in toluene (150 mL), and the solution was eluted through a reactor filled with Novozym 435® (200 mg) with a flow rate of 0.1 mL min^{-1} for two cycles (residence time = 8.6 min), resulting in (*R*)-**2** (94% e.e.) and (*S*)-**3** (98% e.e.). Then, solvent was removed under reduced pressure and the mixture was purified by chromatography (hexanes/ethyl acetate 10:1). The solvent was removed and (*R*)-**2** and (*S*)-**3** were obtained in 34% and 37% yield, respectively.[2]

Synthesis of Mandelic Acid (*RS*-1)



RS-**3** *RS*-**1**

Mandelonitrile (0.666 g, 5 mmol) and HCl (36%, 5.8 mL) were added to a mortar. The reaction media was left without stirring at room temperature for 12 h. Then, solvent was removed via evaporation with water steam bath and left drying overnight at room temperature. The crude residue was transferred to a becker and dissolved in ethyl acetate (35 mL), under magnetic stirring for 5 min. This solution was filtered to remove NH_4Cl (remaining as a solid) and the solvent was removed under reduced pressure. After that, the solid residue was powdered, washed twice with cold benzene (2 x 4 mL, 5-10 °C) and then recrystallized in benzene (22 mL), filtered off in a sintered

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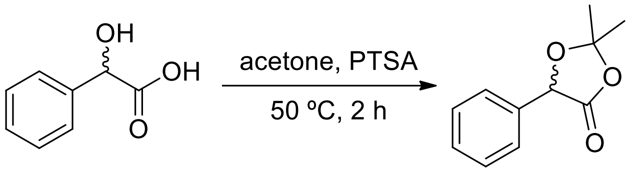
[2] Thomas, J. C.; Aggio, B. B.; Oliveira, A. R. M.; Piovan, L.; High-throughput preparation of optically active cyanohydrins mediated by lipases. *European Journal of Organic Chemistry* **2016**, *2016*, 5964. [Crossref]

glass funnel (5-10 °C) and washed with cold benzene (8 mL, 5-10 °C) to give (*RS*)-**1**, (*R*)-**1** and (*S*)-**1** in 74%, 70% and 80% yield, respectively.[3]

Determination of enantiomeric excess of optically active mandelic acid (1)



(*R*) or (*S*)-**1** (*R*) or (*S*)-**1a**

In a 4 mL sealed vial, optically active mandelic acid (0.045 g, 0.25 mmol) was dissolved in acetone (2 mL) and PTSA was added (10 mg). The reaction media was left stirring at 50 °C for 3 h.[4] After that, one aliquot (200 µL) was taken from reaction media, washed three times with a solution of $NaHCO_3$ (3 x 500 µL), dried over $MgSO_4$, filtered and analyzed via gas chromatography. Compounds (*R*)-**1a** and (*S*)-**1a** were obtained in 94% and 98% enantiomeric excess, respectively.

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[3] Corson, B. B.; Dodge, R. A.; Harris, S. A.; Yeaw, J. S.; Mandelic acid. *Organic Synthesis* **1926**, *6*, 58. [Crossref].

[4] Prousis, K. C.; Markopoulos, J.; Mckee, V.; Markopoulou, O. I.; An efficient synthetic approach towards fully functionalized tetronic acids: the use of 1, 3-dioxolane-2, 4-diones as novel protected-activated synthons of α-hydroxy acids. *Tetrahedron* **2015**, *71*, 8637. [Crossref]

Chromatograms

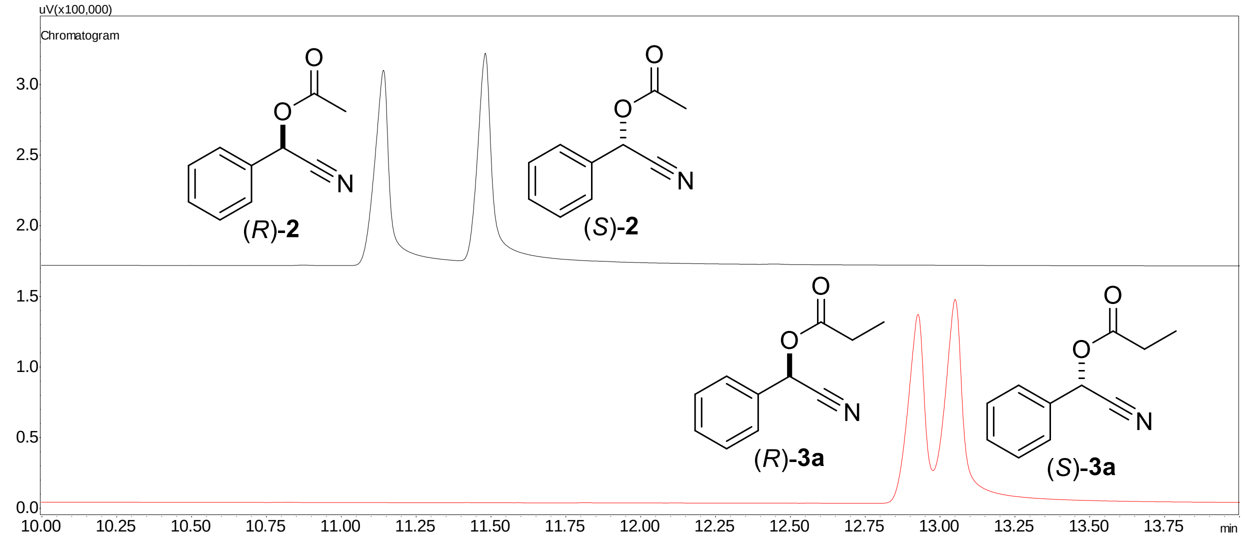

Figure S1. Chromatograms of racemic mandelonitrile acetate (**2**) and racemic mandelonitrile propionate (**3a**), obtained via derivatization of racemic mandelonitrile (**3**)

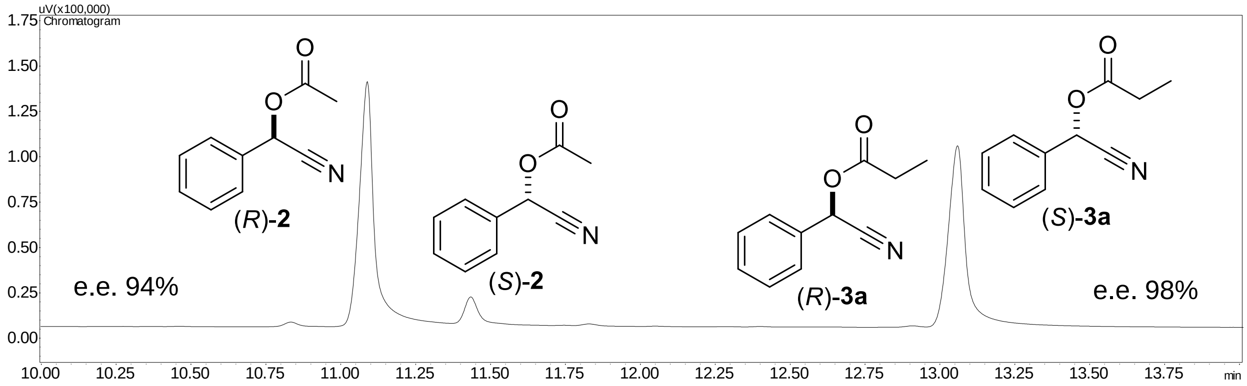

Figure S2. Chromatograms of optically active mandelonitrile acetate (**2**) and mandelonitrile propionate (**3a**), obtained via derivatization of mandelonitrile (**3**)[5]

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[5] Absolute configuration is assigned based on enzyme's known selectivity

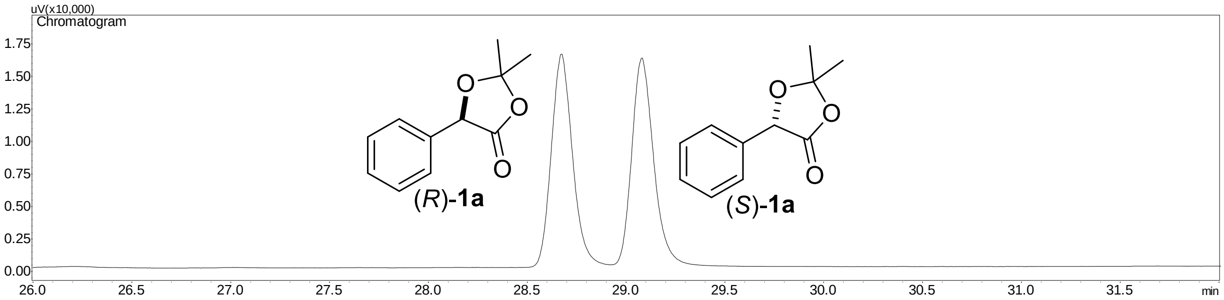


Figure S3. Chromatograms of racemic **1a**, obtained via derivatization of mandelic acid (**1**)

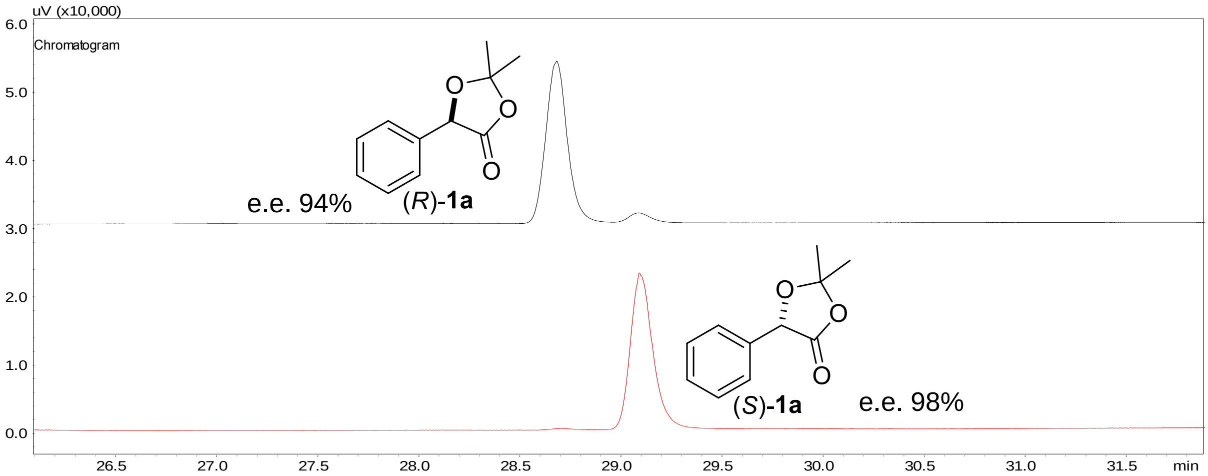


Figure S4. Chromatograms of optically active compounds (*R*)-**1a** and (*S*)-**1a**, obtained via derivatization of mandelic acid (**1**)

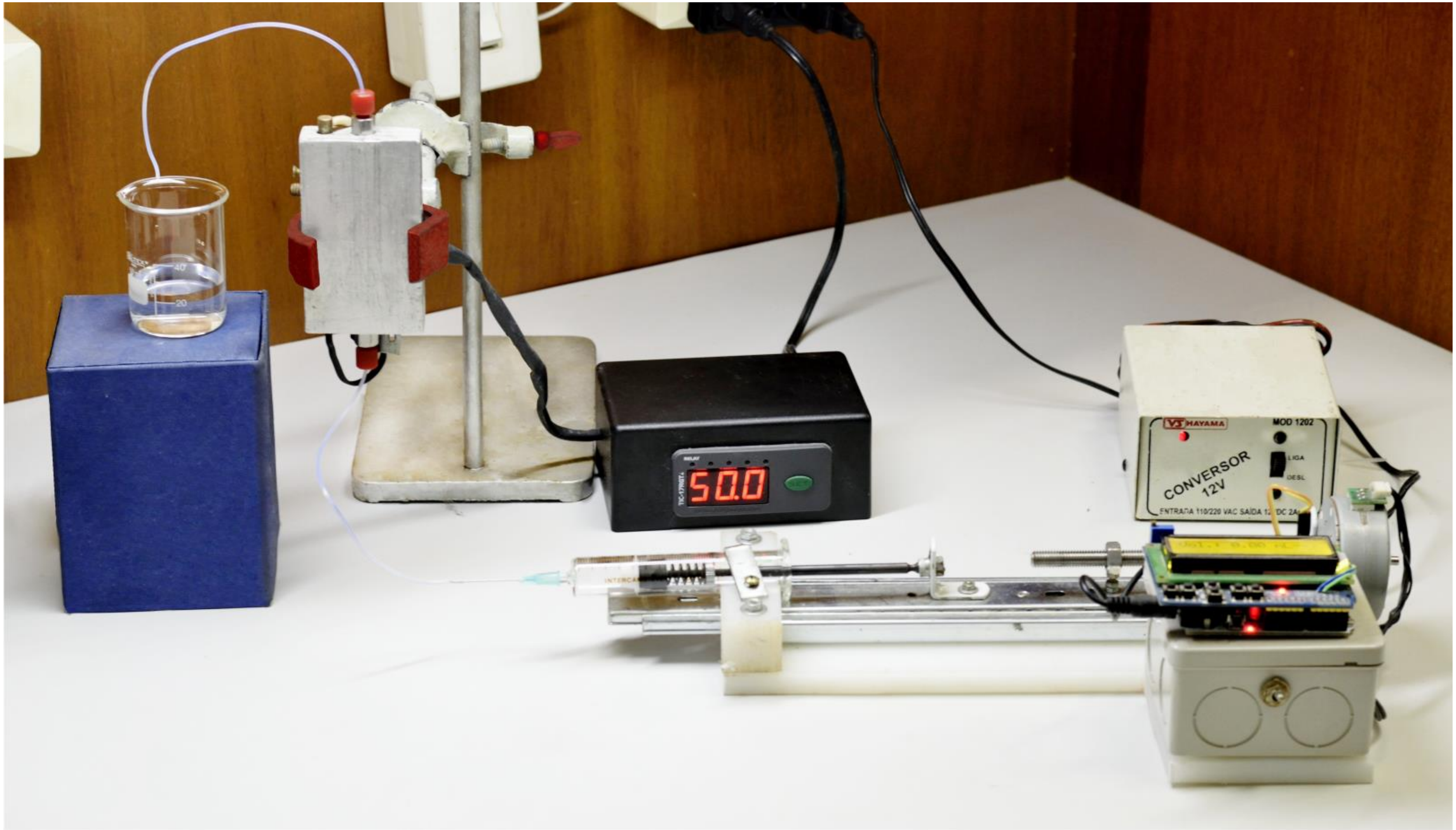
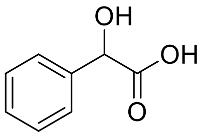


Figure S5. Continuous-flow system



$C_8H_8O_3$
152,147

Mandelic Acid (*RS*-**1**)

Yield: 74%.

^{1}H NMR (200 MHz, MeOD, TMS), δ 5.14 (s, 1H); 7.30–7.48 (m, 5H).

^{13}C NMR (50 MHz, MeOD), δ 72.8; 126.5; 127.8; 128.0; 139.4; 174.8.

IR (KBr) ν/cm^{-1} 3400, 3029, 2967, 2716, 2628, 1717, 1452, 1299, 1190, 1059, 938, 888, 732, 696.

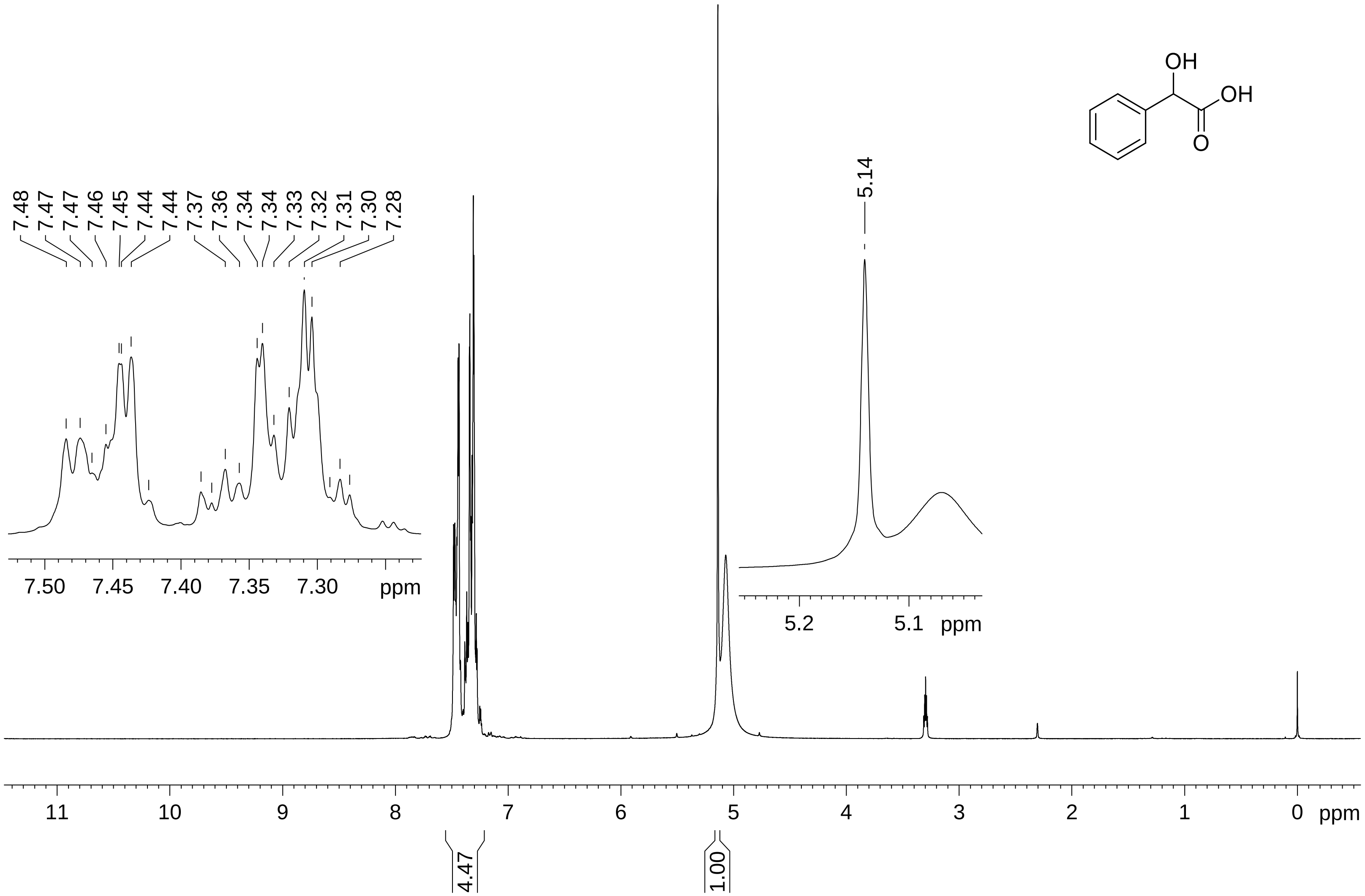


Figure S6. ^{1}H NMR spectrum (200 MHz, MeOD, TMS) of compound *RS*-**1**

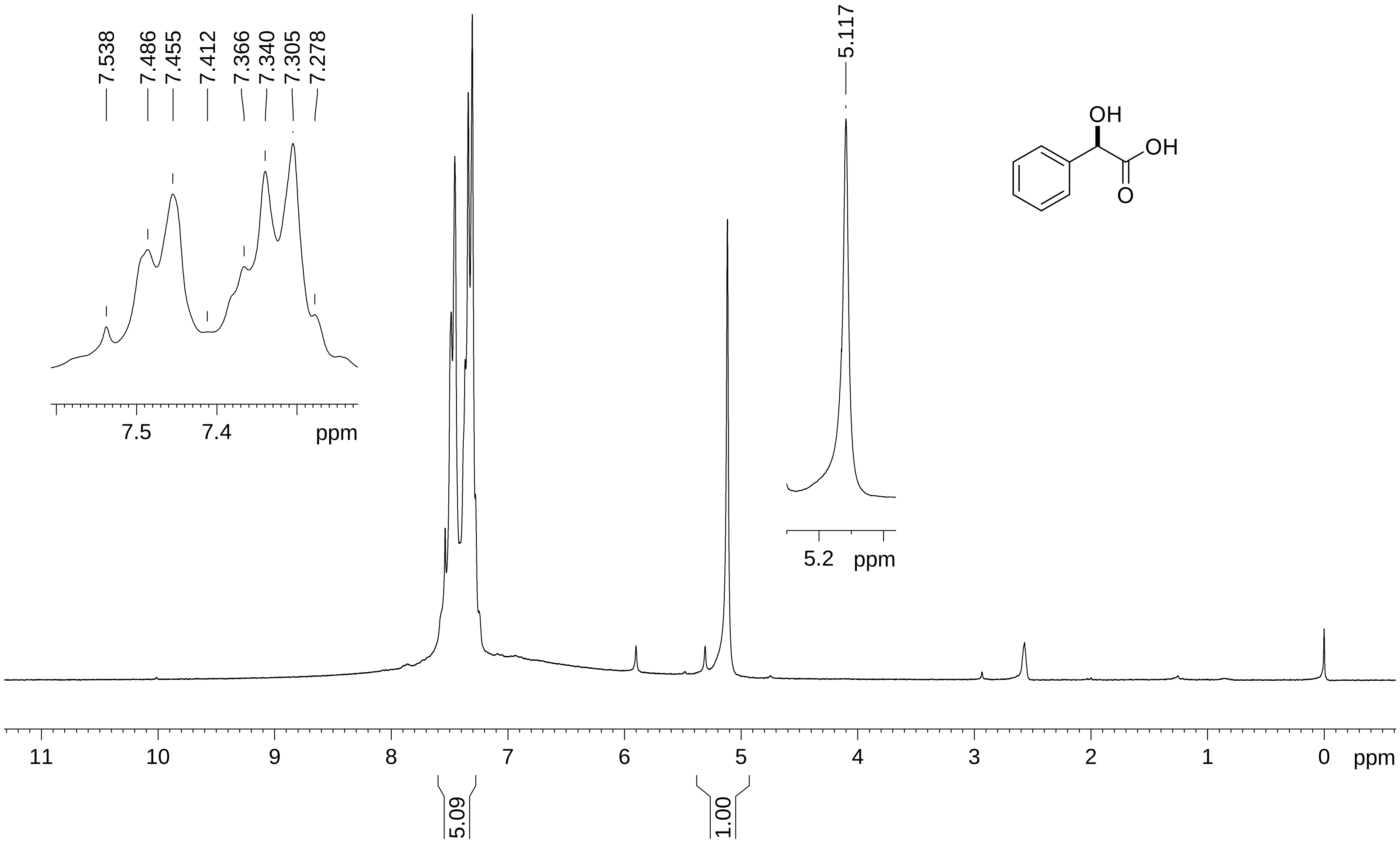


Figure S7. ^{1}H NMR spectrum (200 MHz, $CDCl_3$/DMSO-d6, TMS) of compound *R*-**1**

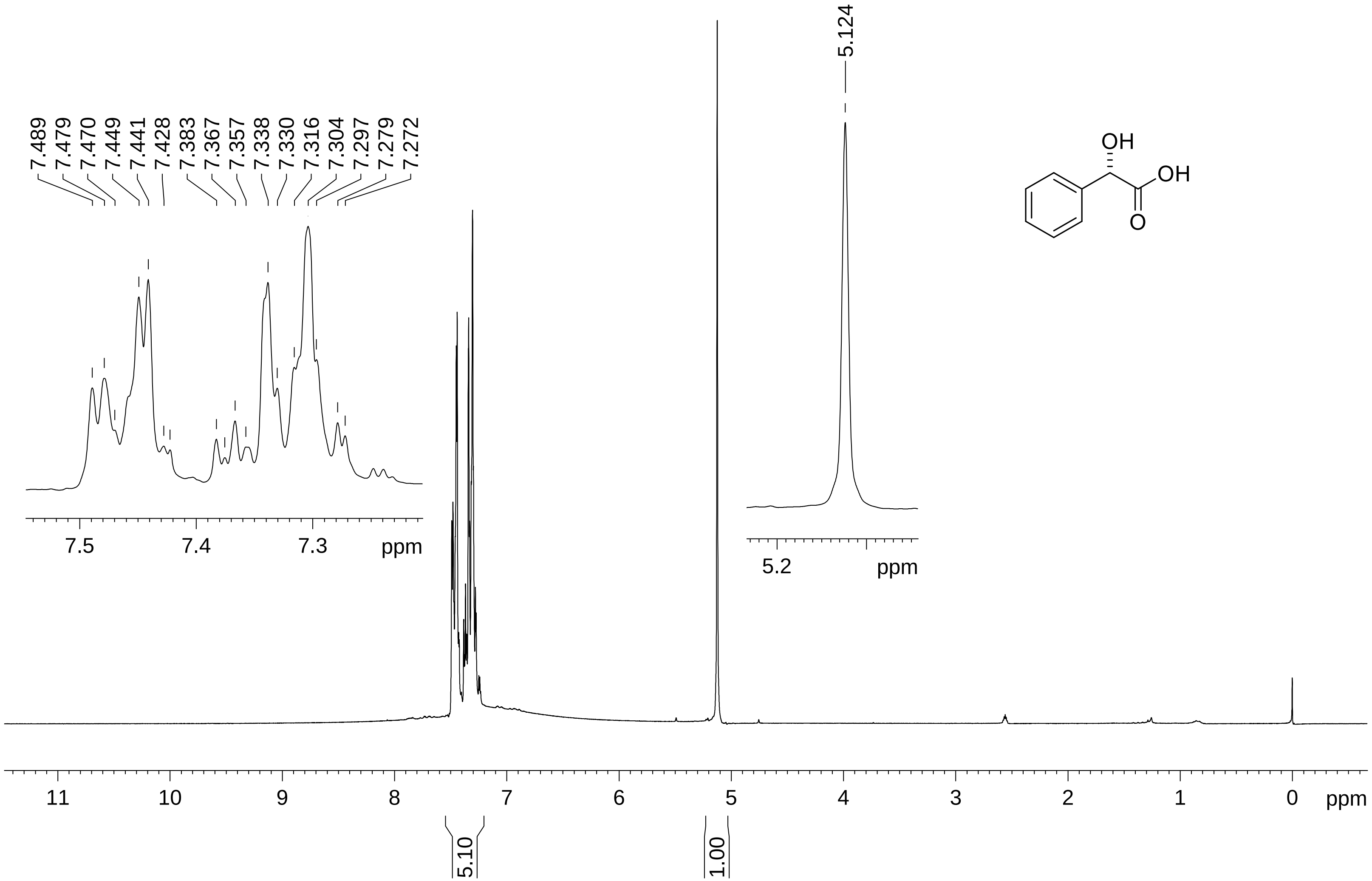


Figure S8. ^{1}H NMR spectrum (200 MHz, $CDCl_3$/DMSO-d6, TMS) of compound *S*-**1**

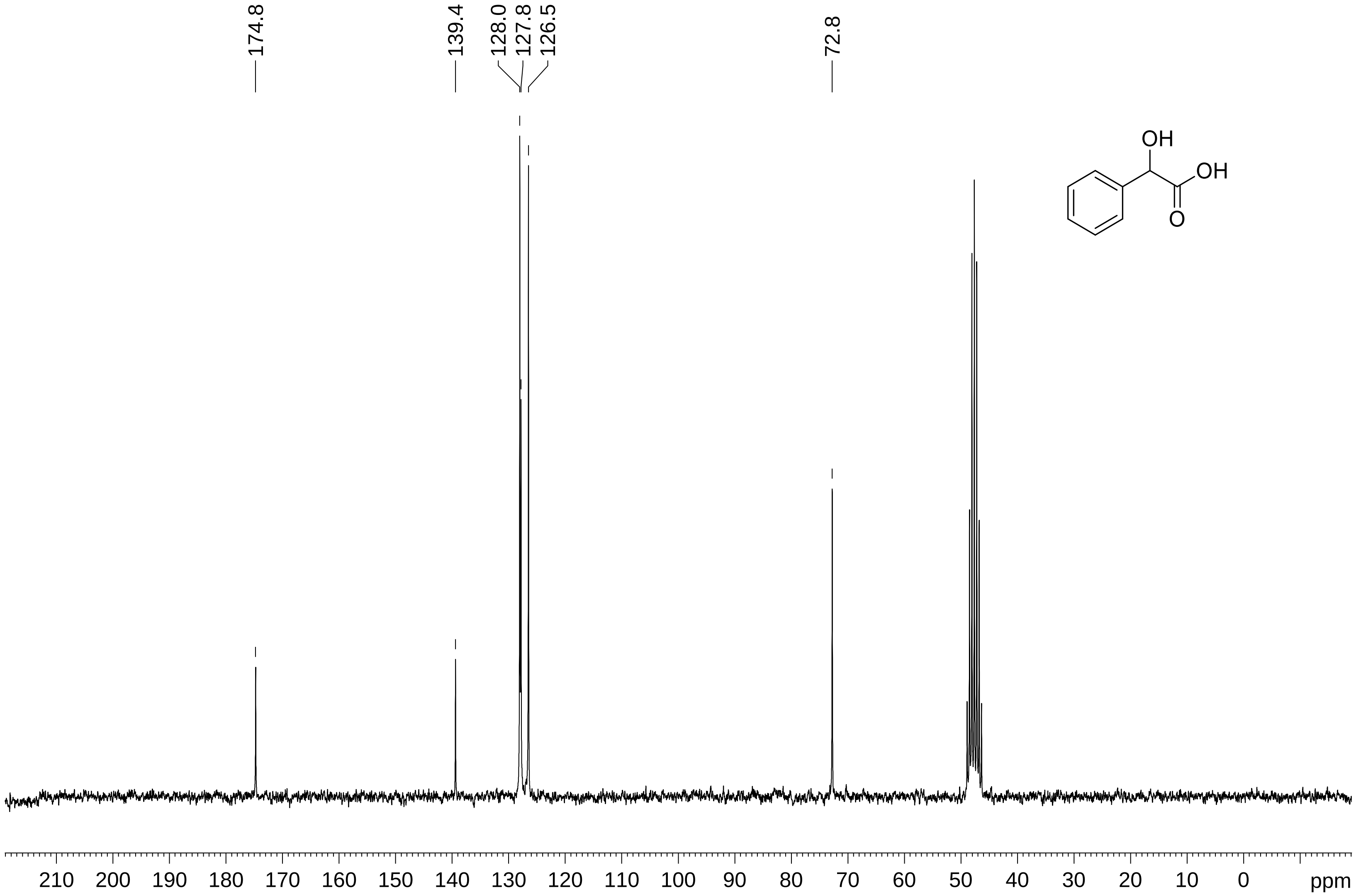


Figure S9. ^{13}C NMR spectrum (50 MHz, MeOD) of compound **1**

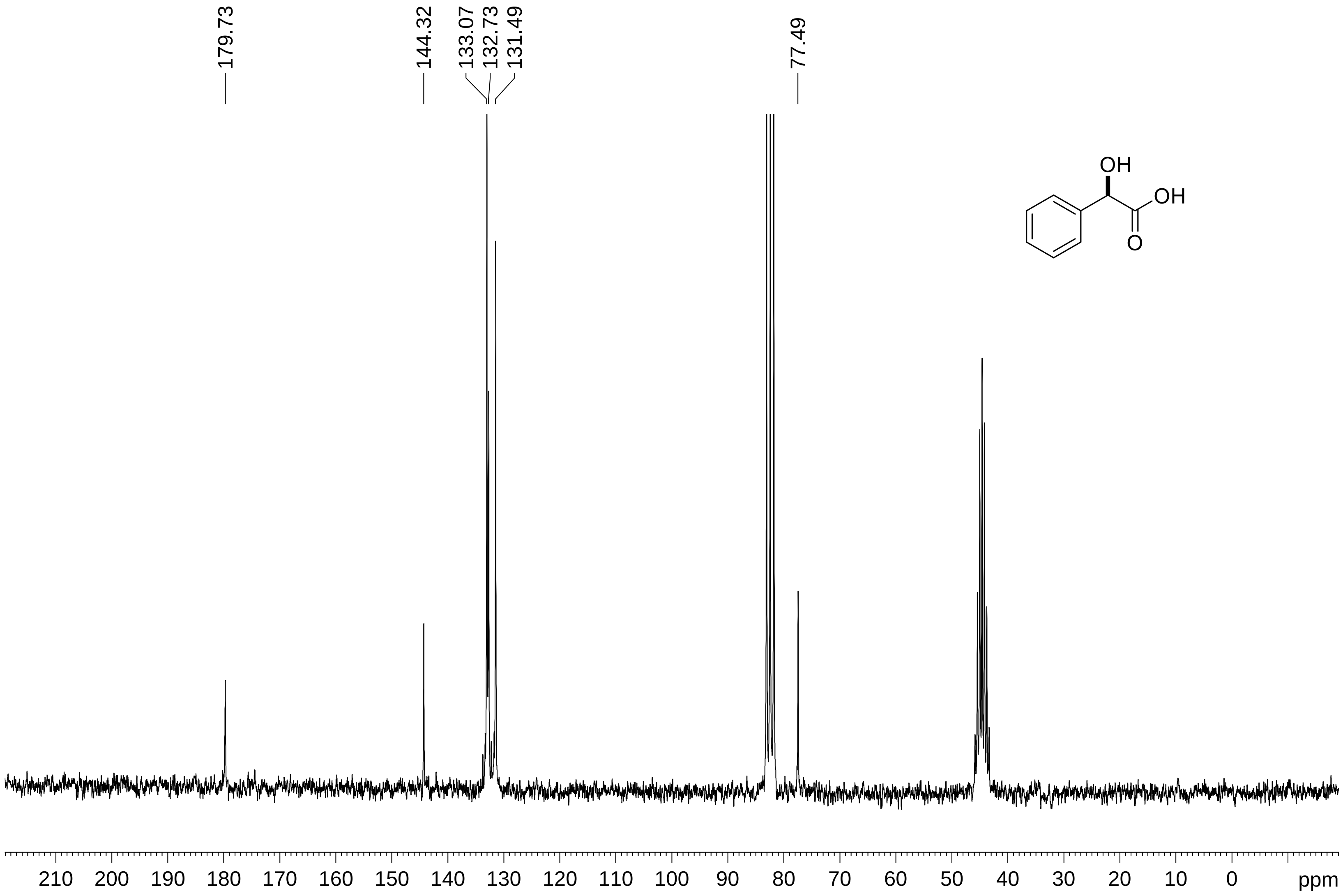


Figure S10. ^{13}C NMR spectrum (50 MHz, $CDCl_3$/DMSO-d6) of compound *R*-**1**

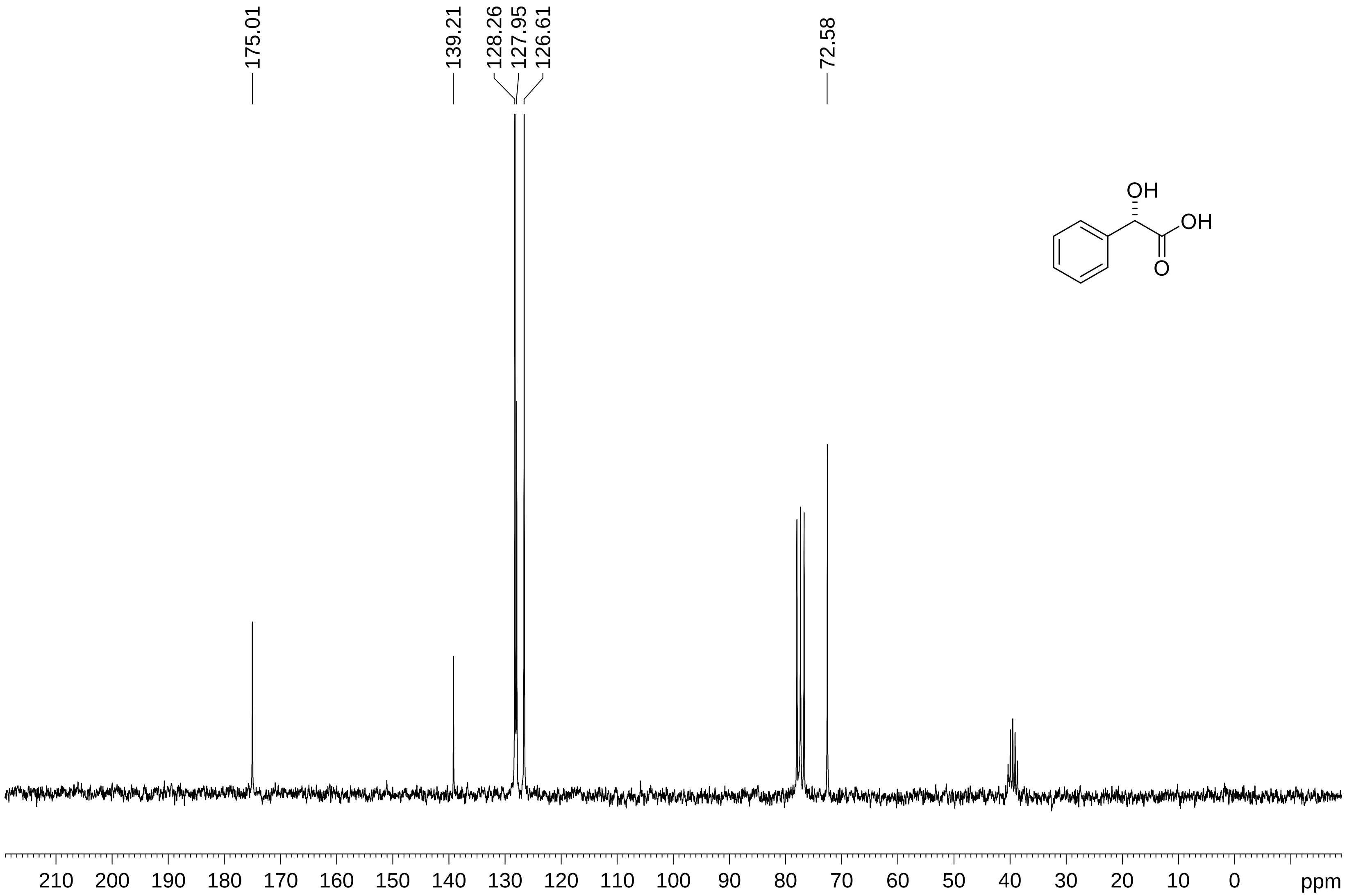


Figure S11. ^{13}C NMR spectrum (50 MHz, $CDCl_3$/DMSO-d6) of compound *S*-**1**

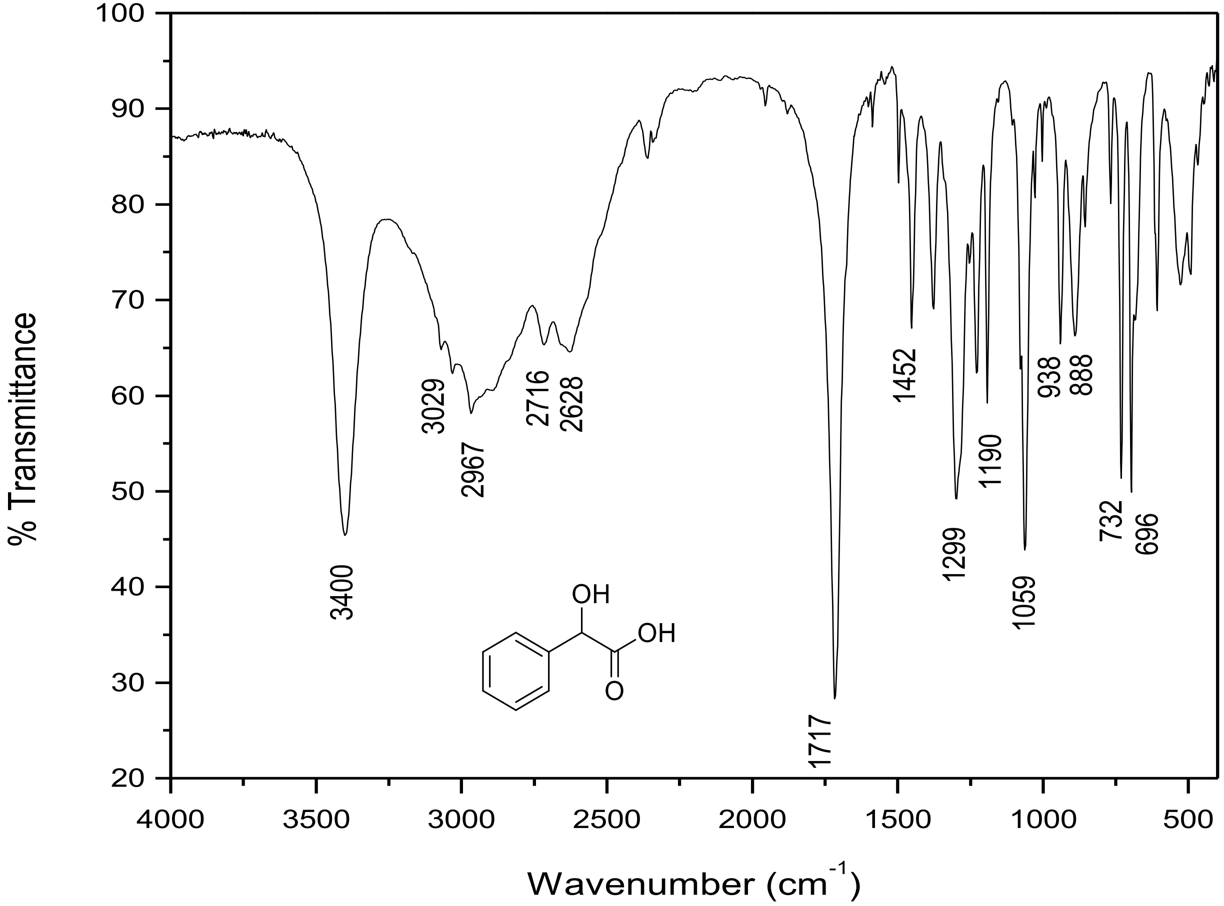
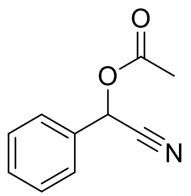


Figure S12. IR spectrum of compound *RS*-**1**



$C_{10}H_9NO_2$
175.18 g mol^{-1}

Mandelonitrile acetate (*RS*-**2**)

Yield: 62%.

GC-MS (70 eV), *m/z* (relative intensity) 175 ($M^{\bullet+}$, 16%), 133 (76%), 115 (74%), 105 (35%), 89 (30%), 77 (21%), 63 (16%), 51 (19%), 43 (100%).

^{1}H NMR (200 MHz, $CDCl_3$, TMS), δ 2.17 (s, 3H); 6.41 (s, 1H); 7.42-7.54 (m, 5H).

^{13}C NMR (50 MHz, $CDCl_3$), δ 20.4; 62.9; 116.1; 127.9; 129.2; 130.4; 131.8; 168.9.

IR (KBr) ν/cm^{-1} 3067, 3037, 2944, 1754, 1496, 1458, 1369, 1216, 1024, 756, 697.

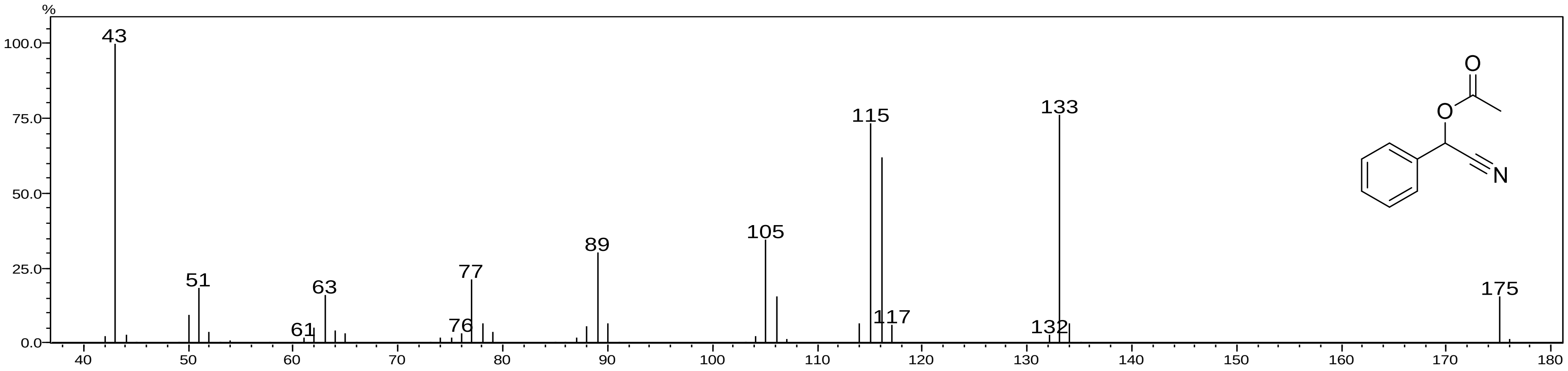


Figure S13. Mass spectrum of compound *RS*-**2**

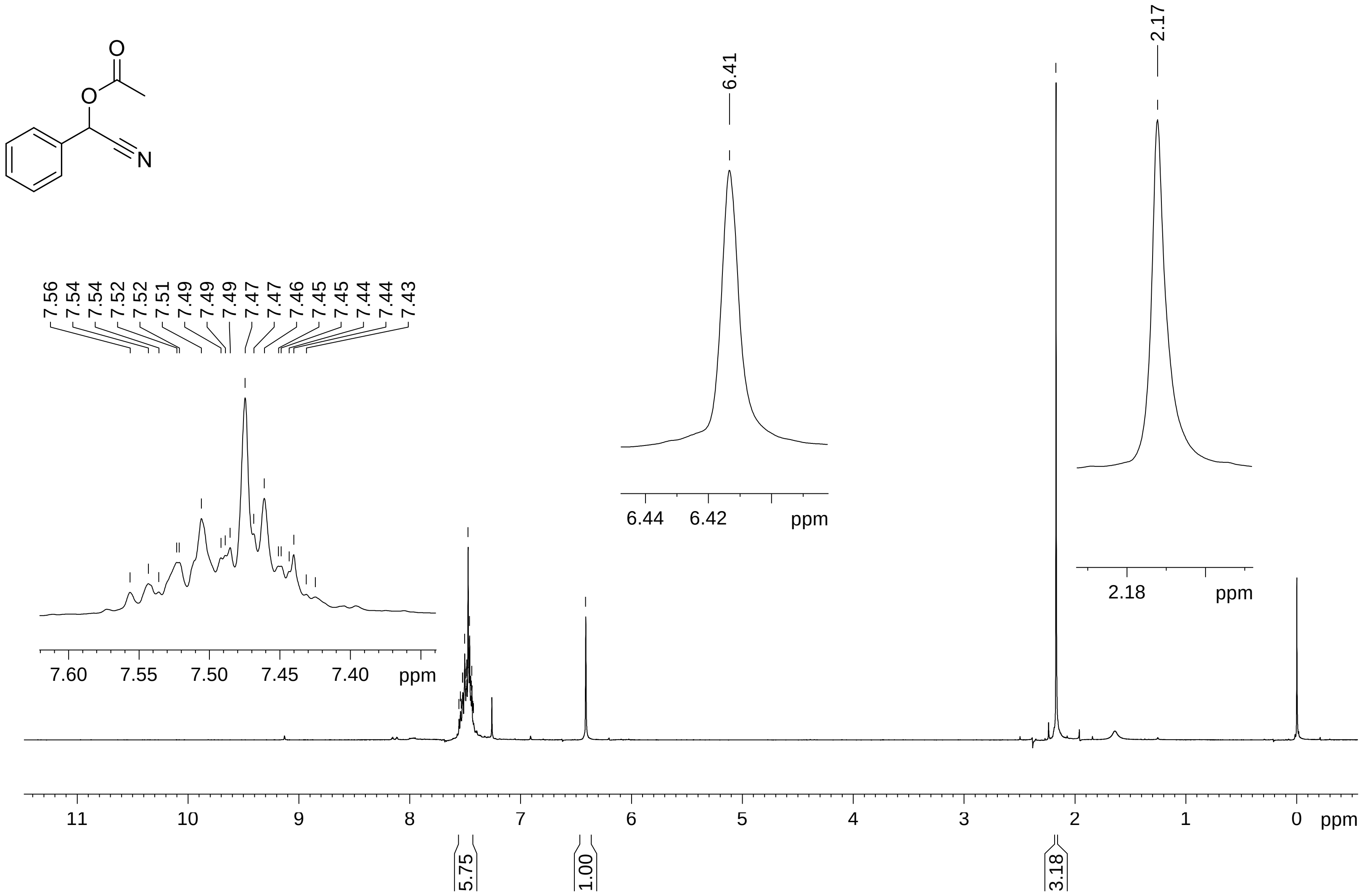


Figure S14. ^{1}H NMR spectrum (200 MHz, $CDCl_3$, TMS) of compound *RS*-**2**

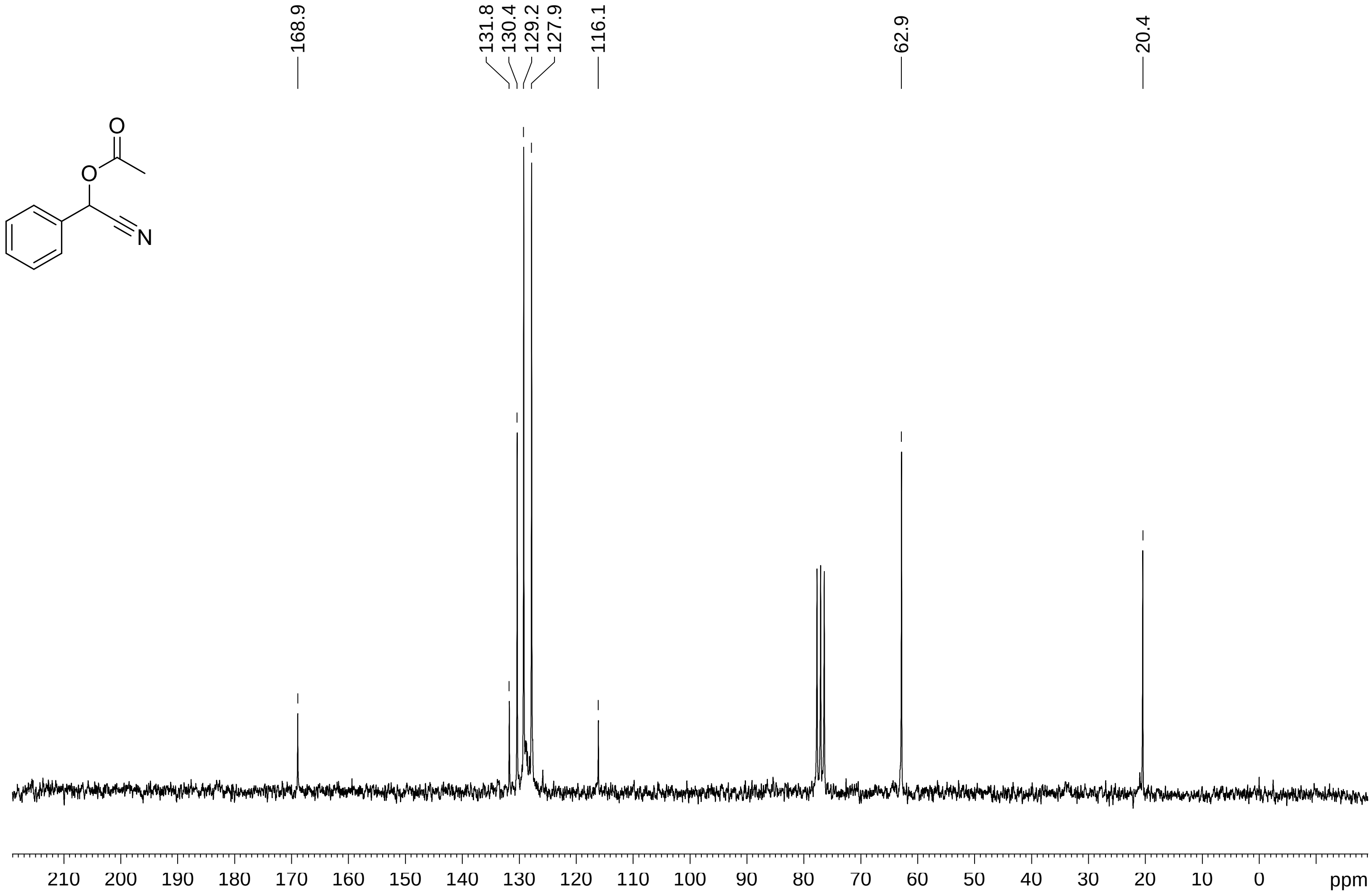


Figure S15. ^{13}C NMR spectrum (50 MHz, $CDCl_3$) of compound *RS*-**2**

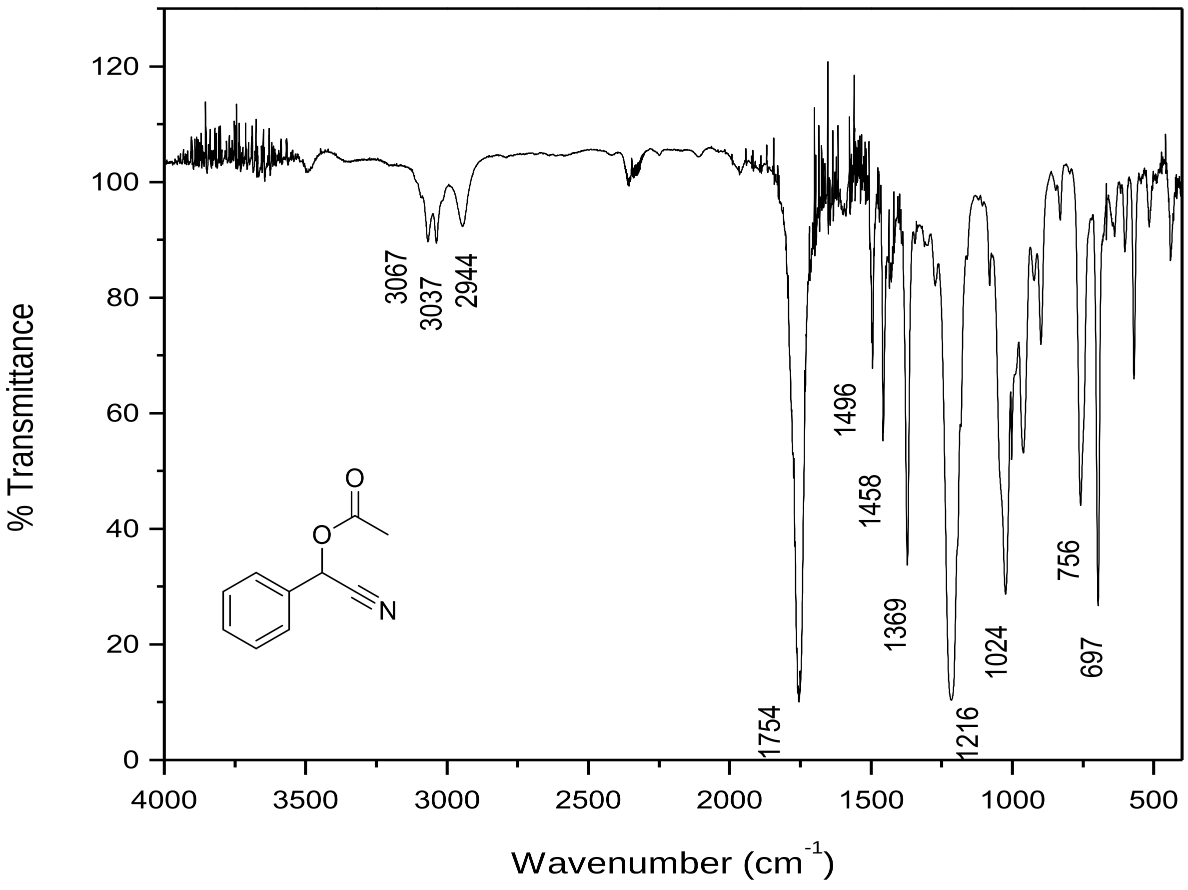
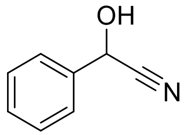


Figure S16. IR spectrum of compound *RS*-**2**



C_8H_7NO
133,147

Mandelonitrile (*RS*-**3**)

Yield: 86%.

^1H NMR (200 MHz, $CDCl_3$, TMS), δ 5.55 (s, 1H); 7.42–7.57 (m, 5H).

^{13}C NMR (50 MHz, $CDCl_3$), δ 63.5; 118.9; 126.7; 129.2; 129.8; 135.2.

IR (KBr) ν/cm^{-1} 3400, 3029, 2967, 2716, 2628, 1717, 1452, 1299, 1190, 1059, 938, 888, 732, 696.

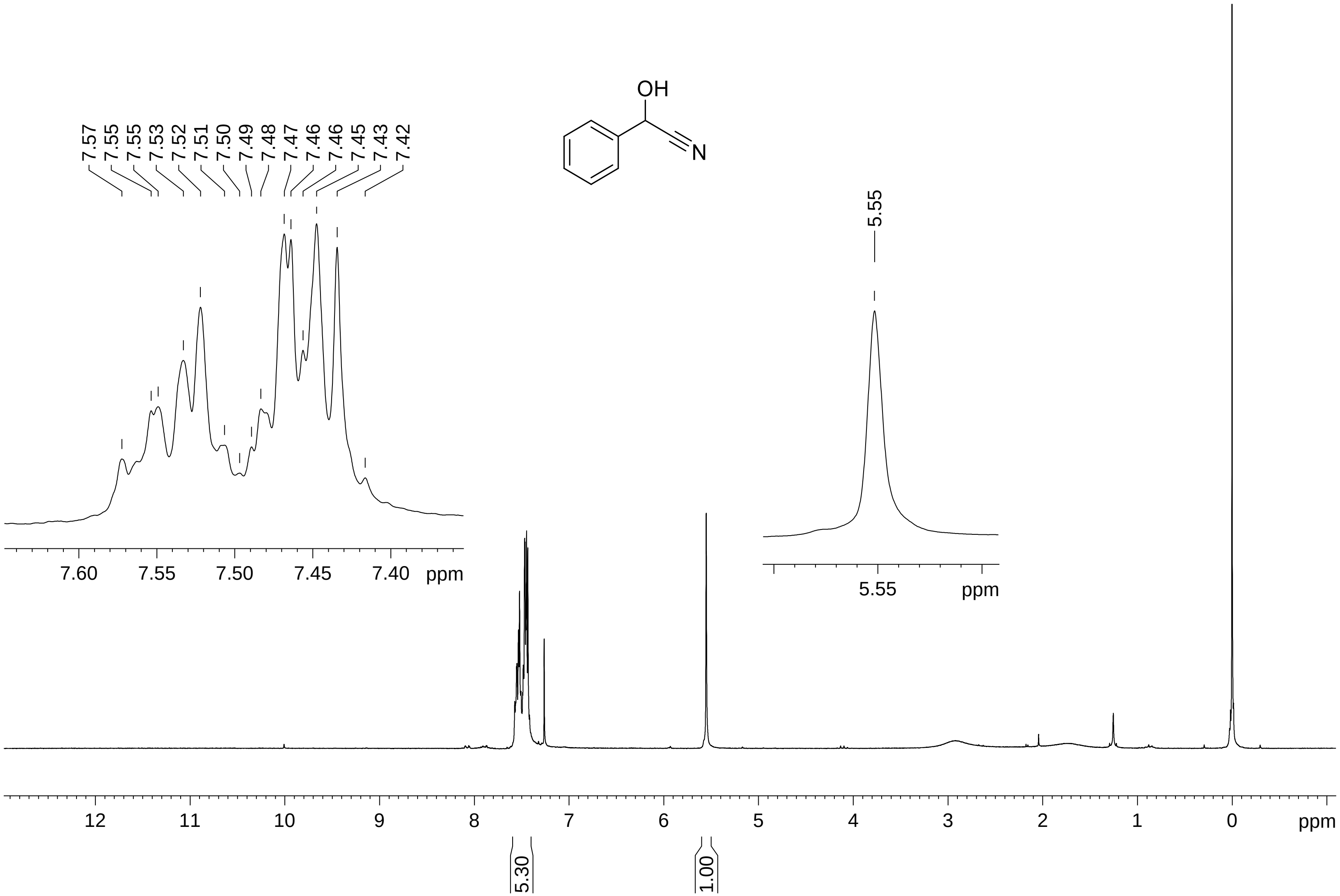


Figure S17. ^{1}H NMR spectrum (200 MHz, $CDCl_3$, TMS) of compound *RS*-**3**

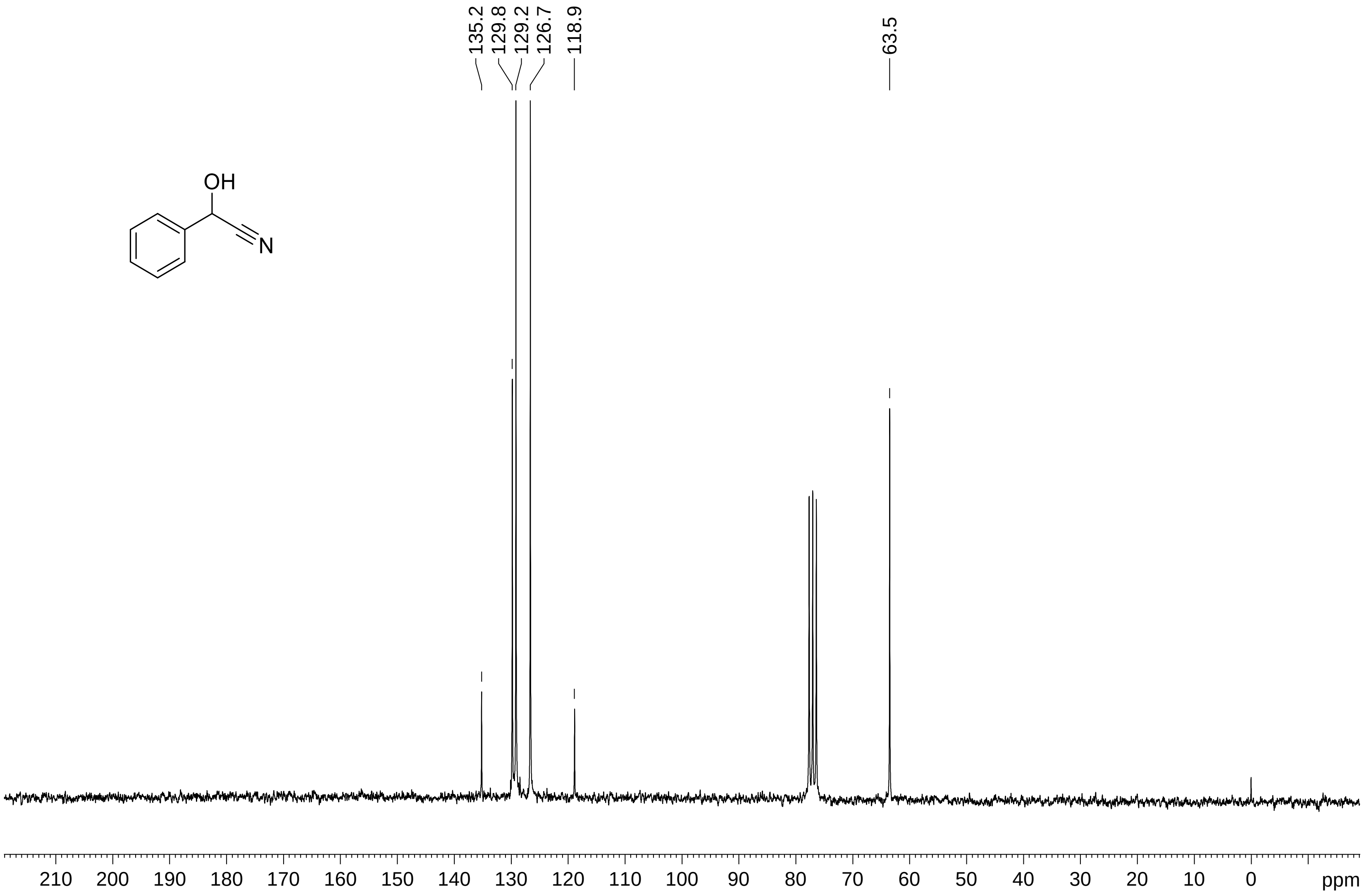


Figure S18. ^{13}C NMR spectrum (50 MHz, $CDCl_3$) of compound *RS*-**3**

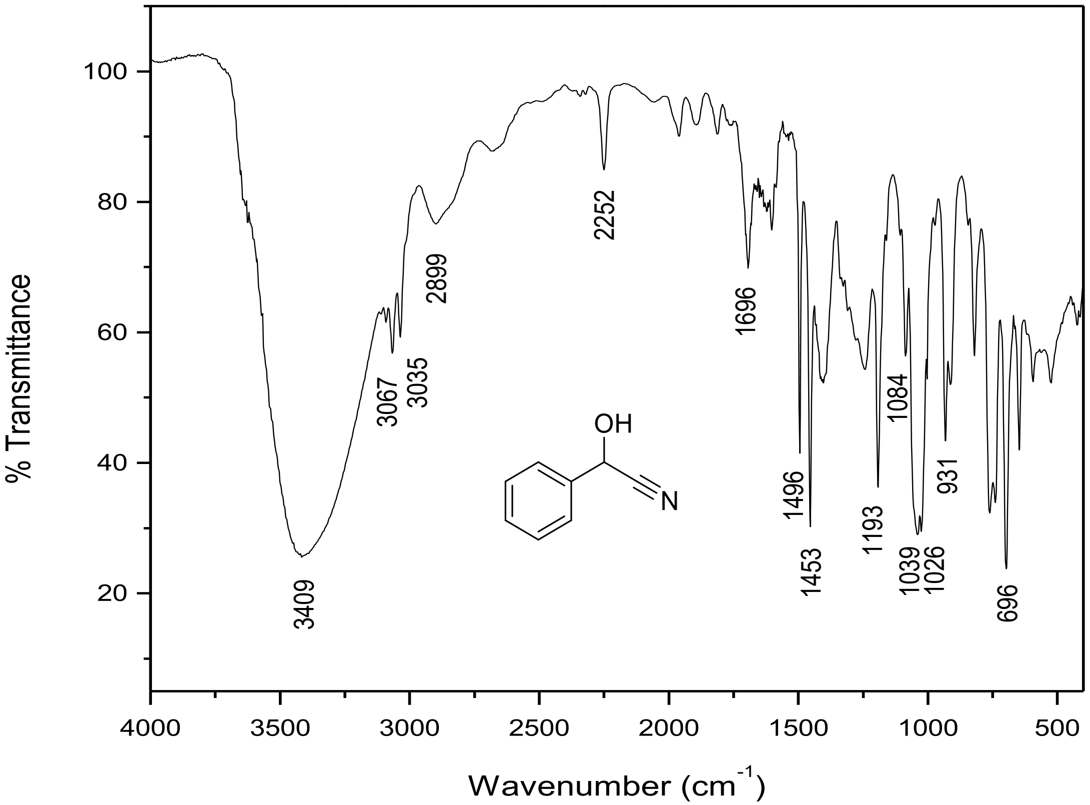


Figure S19. IR spectrum of compound *RS*-**3**