Supporting information

Glyoxalase-Based Toolbox for the Enantioselective Synthesis of α-Hydroxy Carboxylic Acids

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General Remarks

Materials and solvents. Unless otherwise specified, all reagents and solvents were purchased from commercial sources and used without further purification. Aryl glyoxal hydrates **1** were synthesized following literature procedure.[1]

NMR spectroscopy. NMR spectroscopic data were recorded with a Bruker Avance III 300 MHz spectrometer (300.13 MHz for ^{1}H, 75.47 MHz for $^{13}C\{^{1}H\}$ and 282.40 MHz for $^{19}F\{^{1}H\}$) and a Bruker Avance III 500 MHz spectrometer (500.13 MHz for ^{1}H and 125.76 MHz for $^{13}C\{^{1}H\}$), in DMSO-*d*6 and were referenced to solvent residual proton signal δH = 2.50 ppm, solvent carbon signal δC = 39.52 ppm and hexafluorobenzene fluorine signal δF = -164.9 ppm.

Mass spectrometry. Mass spectra were recorded on Agilent 6560 ESI-IM-QTOF mass spectrometer equipped with AJS ESI ion source. Samples were injected using direct infusion from syringe pump with flow rate of 5 µl/min. Stock solutions (10 mM) of samples were prepared with MeOH and filtered if needed. Samples were diluted with MeOH to 5, 10 or 15 µM prior to measurements.

Melting points. Melting points were determined in open capillary tubes on Stuart Scientific SMP3 melting point apparatus.

Optical rotations. Optical rotations were obtained with a Perkin-Elmer 343 polarimeter.

Enzymatic reaction progress monitoring. The maximum absorption wavelength (λ_{max}) and molar extinction coefficient at λ_{max} of each aryl glyoxal were acquired using a NanoDrop 2000c spectrophotometer. Enzymatic conversion was continuously monitored by either a decrease in the absorbance at the respective λ_{max} or a shift of maximum absorbance to a new λ_{max} due to the strong absorption by some α-hydroxy acids being produced.

Chiral UHPLC. The methods for the detection and separation of the α-hydroxy acid enantiomers were developed using Astec CLC-D and Astec Chirobiotic T chiral columns on HPLC Accela 600 and Dionex Ultimate 3000 UHPLC respectively. The method for the separation of lactate enantiomers on CLC-D column comprised aqueous solution of 5 mM $CuSO_4$ as a mobile phase, 1.0 mL/min flow rate, 25°C column oven temperature, 10 µL injection volume and UV-Vis detector wavelength of 254 nm. For Astec CLC-D column, L enantiomer generally elutes before D. Separation of aromatic α-hydroxy acid enantiomers on Chirobiotic T column was achieved using 60% H_2O acidified with either 0.1% or 0.05% AcOH and 40% MeOH as a mobile phase, 1 µL injection volume and varying UV-Vis detector wavelengths (220, 228, 234, 260, 280, 224 nm). The specific flow rate, column oven and sample holder temperature are given below for each provided chiral UHPLC chromatogram. Roughly equimolar mixtures of purified **(*R*)-2** and **(*S*)-2** or unpurified αHCAs **2** obtained with *E. coli* Hsp31 were used as racemic or nearly racemic references.

[1] A. A. Peshkov, D. Gapanenok, A. Puzyk, N. Amire, A. S. Novikov, S. D. Martynova, S. Kalinin, D. Dar'in, V. A. Peshkov and M. Krasavin, *The Journal of Organic Chemistry,* 2023, **88**, 10508–10524.

Protein expression and purification

Expression and purification of human DJ-1, *A. thaliana* DJ-1d, *C. albicans* Glx3, *E. coli* Hsp31 proteins was performed as described.[2,3]

Full length *E. coli* Glyoxalase I and Glyoxalase II were amplified from genomic DNA by Phusion polymerase and cloned into pHis-Parallel2 vector using BamHI and SalI restriction sites. BL21(DE3)-RIPL cells were transformed with Glyoxalase I expression construct and plated on agar plates containing 100 μg/ml ampicillin and 25 μg/ml chloramphenicol. The colonies were picked and grown overnight in 15 mL terrific broth with the same antibiotics at 37°C with shaking at 230 rpm. The cells were then transferred to a 400 mL terrific broth medium with antibiotics and 5 μM $NiCl_2 \times 6H_2O$ and grown at 37°C with shaking at 150 rpm. Expression was induced by the addition of isopropyl β-d-1-thiogalactopyranoside to 0.25 mM and the cells were grown for further 3 hours at 120 rpm. Thereafter, the cells were collected by centrifugation at 6,700 × g, resuspended in 20 mM Tris, 0.5 M NaCl, pH 8.0 (Buffer A) and homogenized. For purification, the homogenate was incubated with lysozyme in the presence of PMSF for 10 minutes, sonicated and centrifuged for 30 min at 55,900 × g and 4°C. The supernatant was applied to a freshly prepared 2 mL Ni-NTA gravity column and unbound proteins were washed off with 30 mL Buffer A containing 10 mM imidazole. Glyoxalase I was eluted with Buffer A containing 150 mM imidazole and desalted into PBS using PD-10 columns. Expression and purification of *E. coli* Glyoxalase II was performed following the same procedure except for the addition of $ZnCl_2 \times 2H_2O$ instead of $NiCl_2 \times 6H_2O$ to 5 μM in a 400 mL terrific broth medium.

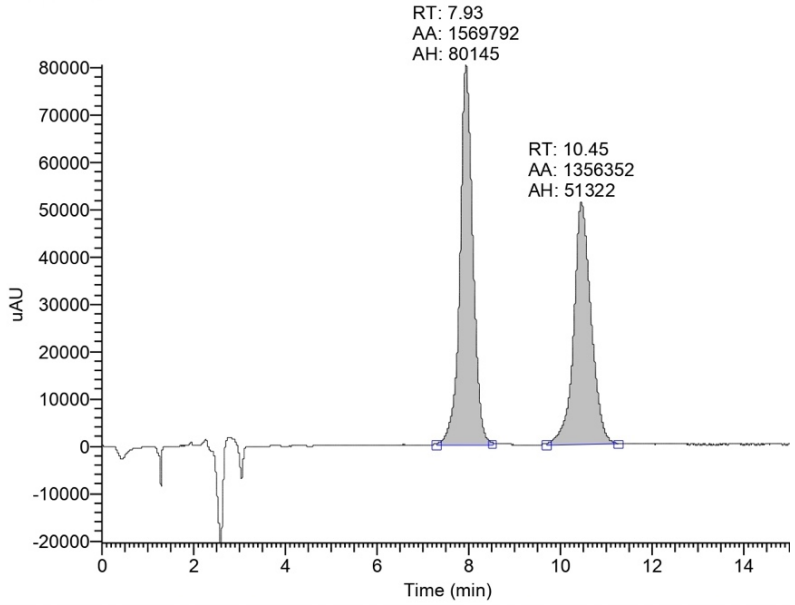
[2] T. Mulikova, Z. Bekkhozhin, A. Abdirassil and D. Utepbergenov, *Analytical Biochemistry*, 2021, **630**, 114317.

[3] A. Andreeva, Z. Bekkhozhin, N. Omertassova, T. Baizhumanov, G. Yeltay, M. Akhmetali, D. Toibazar and D. Utepbergenov, *Journal of Biological Chemistry*, 2019, **294**, 18863–18872.

Screening of type III glyoxalases

For the screening of enzymes and substrate scope, 1 mL reactions were set up with 5 mM aryl glyoxal and respective enzyme in Buffer B. When Glyoxalase I and Glyoxalase II were used, the initial concentration of GSH was kept at 250 µM in the reaction mixture. Upon completion of the reaction as was informed by absorbance measurements, the samples were centrifuged in Amicon Ultra-0.5 mL centrifugal filters (NMWL = 10K) twice at 21,130 × g for 6 minutes to remove protein and any aggregates. The flow-through was transferred to clean HPLC vials. For the analysis of the purified α-hydroxy acids, filtered 1 mL 5 mM aqueous solutions of each product were prepared.

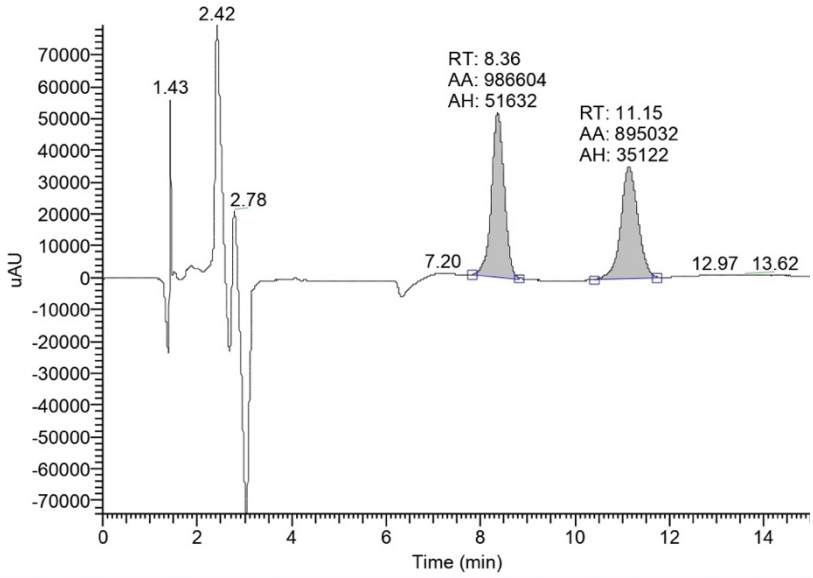
Chiral UHPLC chromatogram of lactic acid (**2a**) racemate:



Peak No.	Retention Time (RT), min	Area (AA), µAU*min	Relative Area, %
1	7.93	1569792	53.65
2	10.45	1356352	46.35
Total:		**2926144**	**100.00**

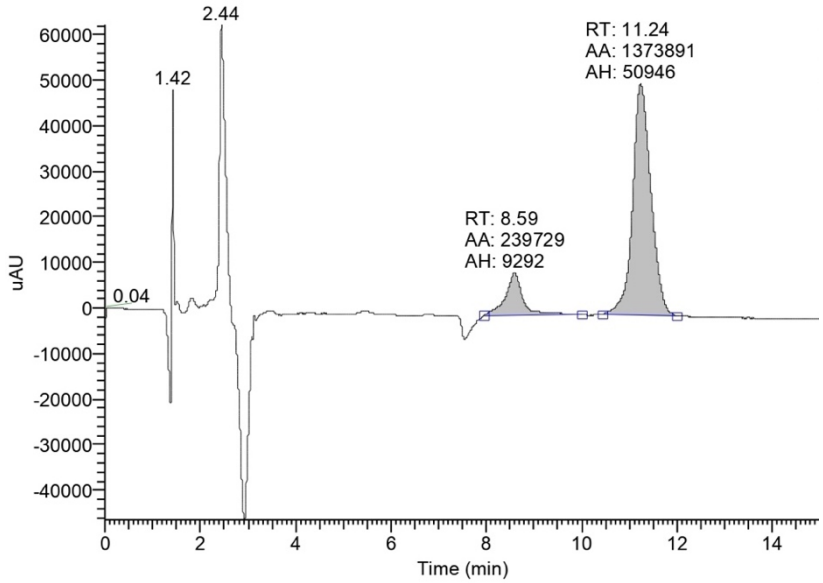
Chiral UHPLC chromatogram of lactic acid (**2a**) formed by the enzymatic conversion of methyl glyoxal hydrate (**1a·H_2O)** by:

a) *E. coli* Hsp31



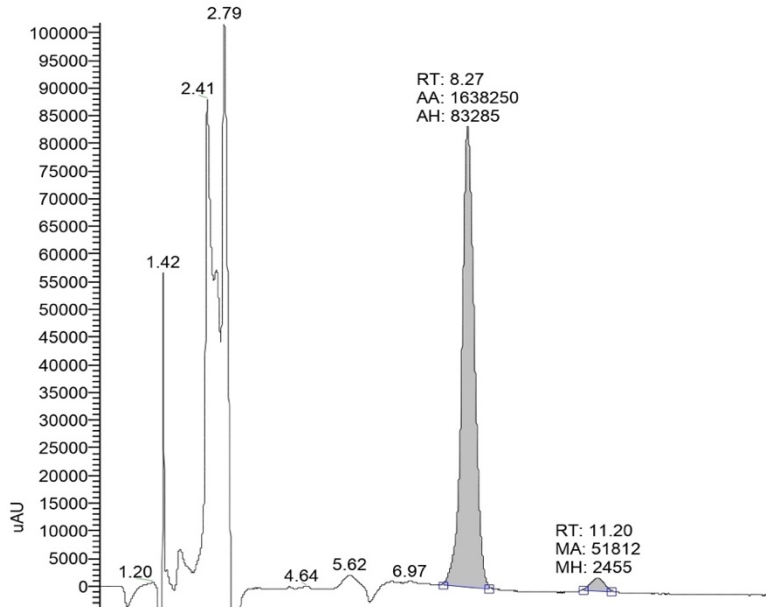
Peak No.	Retention Time (RT), min	Area (AA), µAU*min	Relative Area, %
1	8.36	986604	52.43
2	11.15	895032	47.57
Total:		**1881636**	**100.00**

b) *A. thaliana* DJ-1d



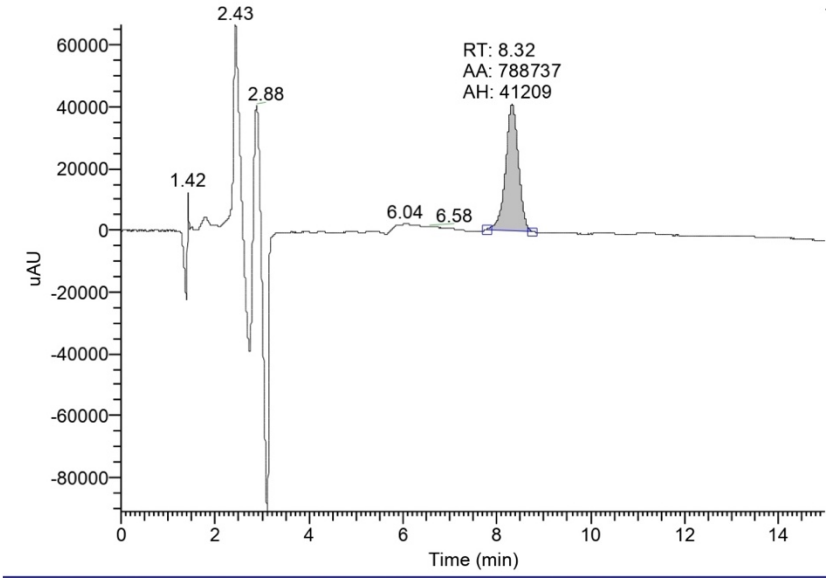
Peak No.	Retention Time (RT), min	Area (AA), µAU*min	Relative Area, %
1	8.59	239729	14.86
2	11.24	1373891	85.14
Total:		**1613620**	**100.00**

c) *C. albicans* Glx3



Peak No.	Retention Time (RT), min	Area (AA), µAU*min	Relative Area, %
1	8.27	1638250	96.93
2	11.20	51812	3.07
Total:		**1690062**	**100.00**

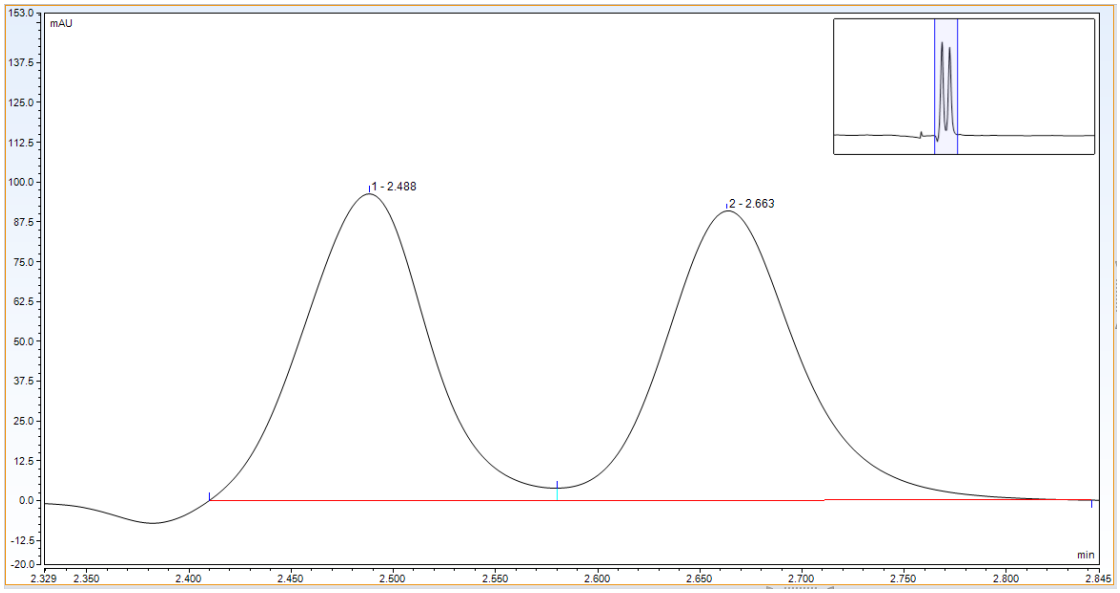
d) Human DJ-1



Peak No.	Retention Time (RT), min	Area (AA), µAU*min	Relative Area, %
1	8.32	788737	100.00
Total:		**788737**	**100.00**

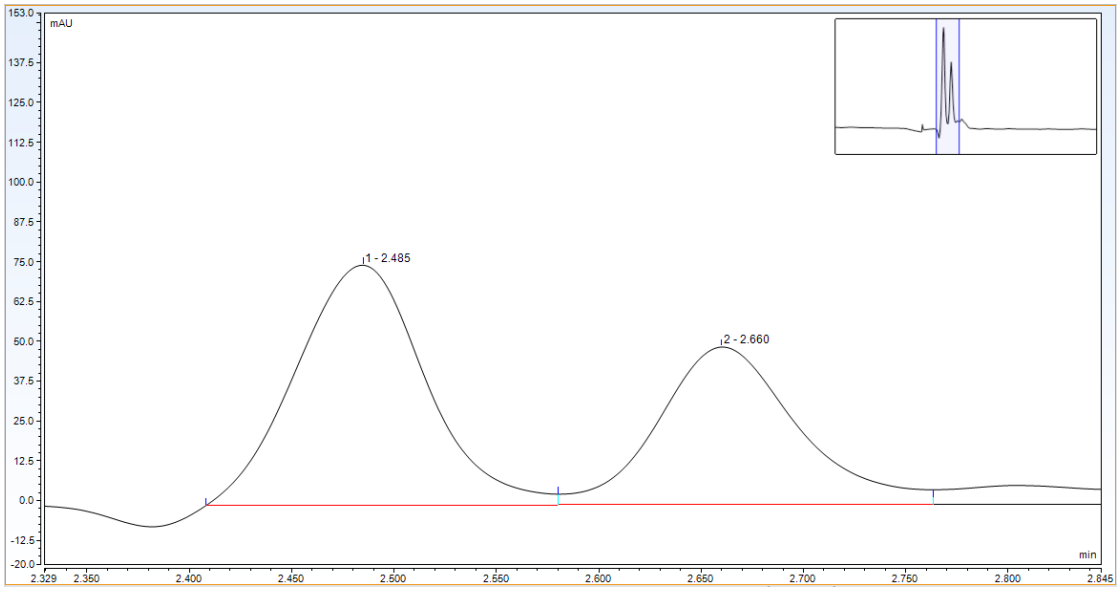
Chiral UHPLC chromatograms obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature of 2-hydroxy-2-phenylacetic acid (**2b**) formed by the enzymatic conversion of phenyl glyoxal hydrate (**1b·H_2O)** by:

e) *E. coli* Hsp31



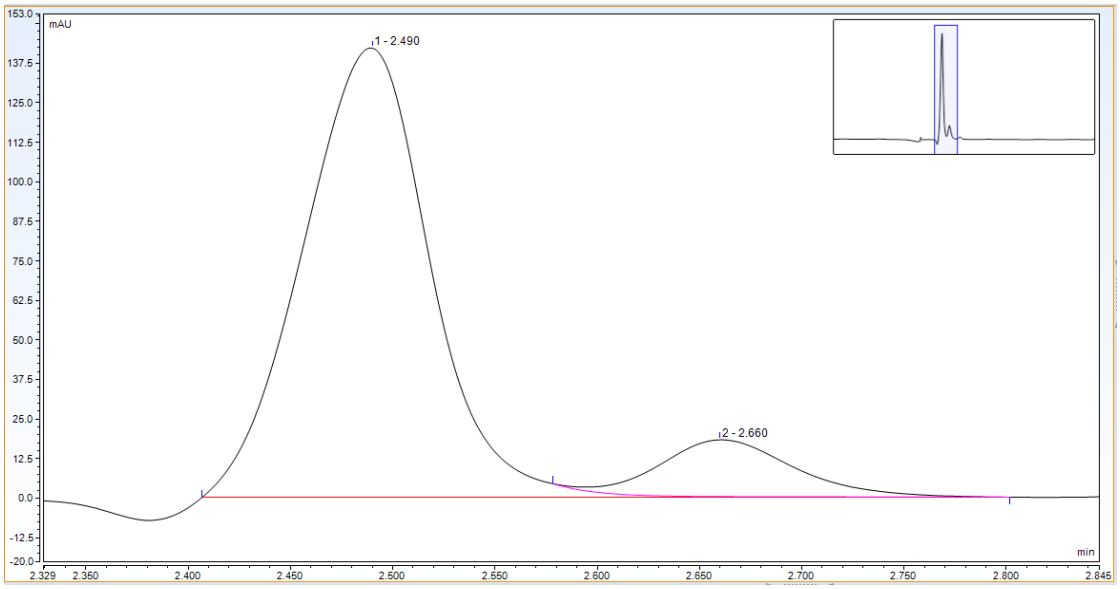
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.488	6.846	49.79
2	2.663	6.904	50.21
Total:		**13.751**	**100.00**

f) *A. thaliana* DJ-1d



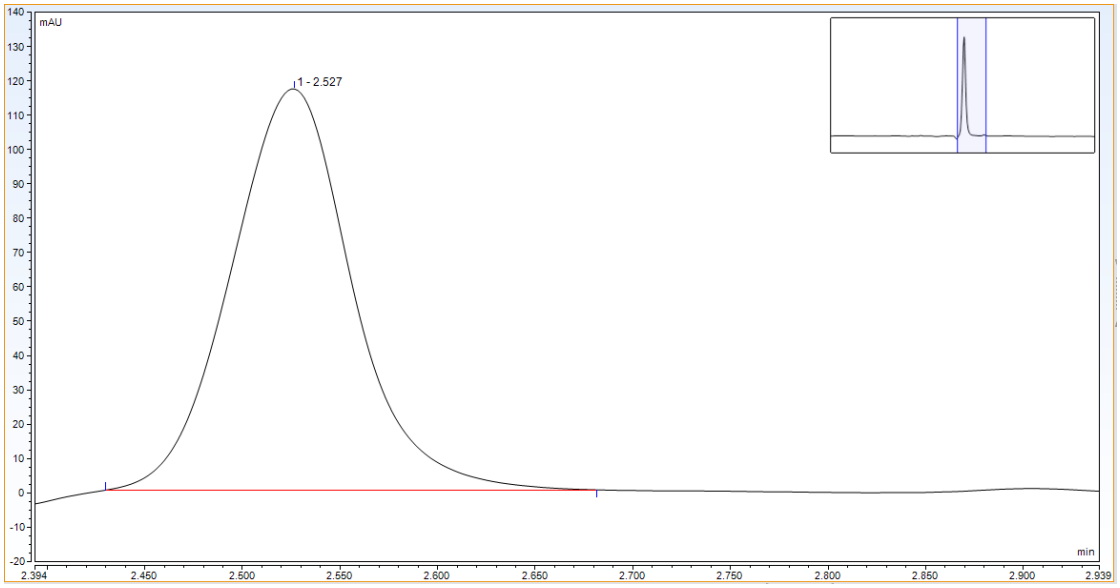
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.485	5.518	58.59
2	2.660	3.900	41.41
Total:		**9.419**	**100.00**

g) *C. albicans* Glx3



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.490	10.264	88.26
2	2.660	1.365	11.74
Total:		**11.629**	**100.00**

h) Human DJ-1



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.527	8.402	100.00
Total:		**8.402**	**100.00**

Optimization of Glyoxalase I/II ratio

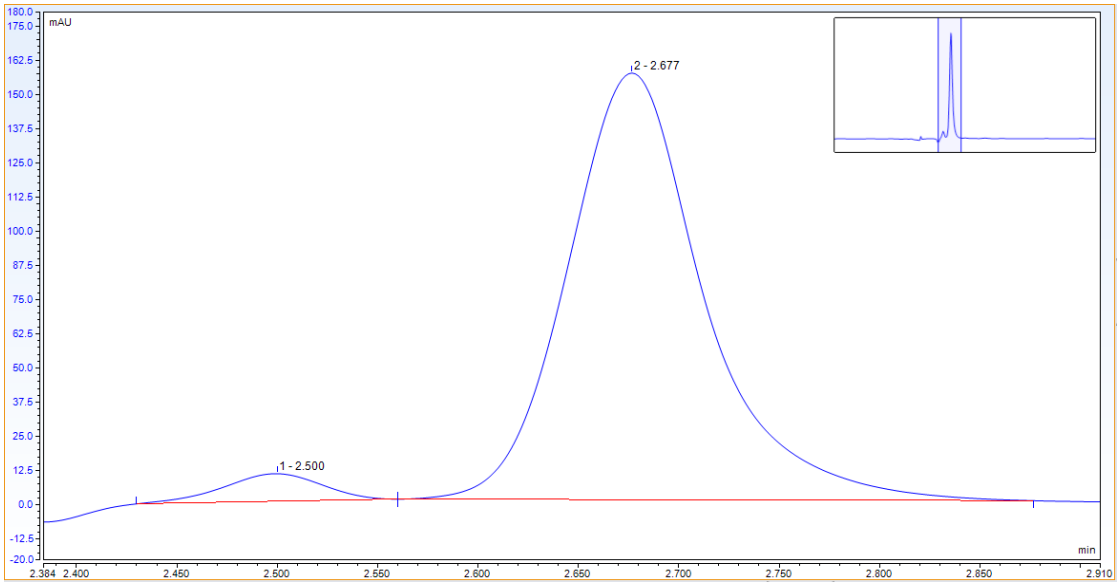
Table 1. Optimization of Glyoxalase I and Glyoxalase II concentration ratio

Glyoxal **1**, R	ee of D-αHCA at the given [GlxI]/[GlxII] ratios, %			
	1:3	3:1	30:1	Delay[4]
1b, Ph	91	89	79	68
1g, 4-BrC_6H_4	91	90	87	81
1h, 4-MeOC_6H_4	97	97	89	92
1n, 5-bromothiophen-2-yl	91	88	72	24

The ee values above were obtained from samples of corresponding 1 mL reaction mixtures, not from extracted and purified αHCA products. Similar or identical preliminary ee values for 1:3 and 3:1 ratios of enzymes, combined with the greater catalytic activity of *E. coli* GlxII compared to GlxI (data not shown), as well as greater ease of extraction and lower enzyme expenditure, prompted us to proceed with around 3:1 ratio of Glyoxalase I and Glyoxalase II for the preparative chemoenzymatic synthesis. Noteworthy, scaling up the protocol resulted only in minor drop of ee for the substrate **1h**, while for the substrates **1b** and **1n**, an increase in the obtained ee values was observed.

Chiral UHPLC chromatograms obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature of 2-hydroxy-2-phenylacetic acid (**2b**) formed by the glutathione-mediated conversion of phenyl glyoxal hydrate (**1b·H_2O)** by *E. coli* Glyoxalase I and Glyoxalase II in the respective concentration ratios:

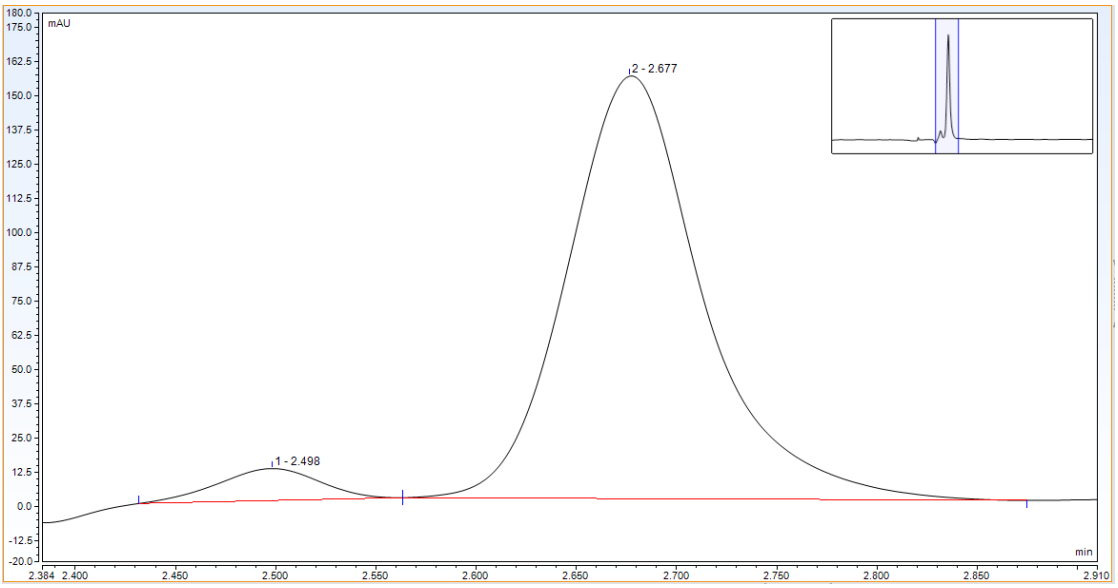
a) 1:3



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.500	0.573	4.45
2	2.677	12.295	95.55
Total:		**12.868**	**100.00**

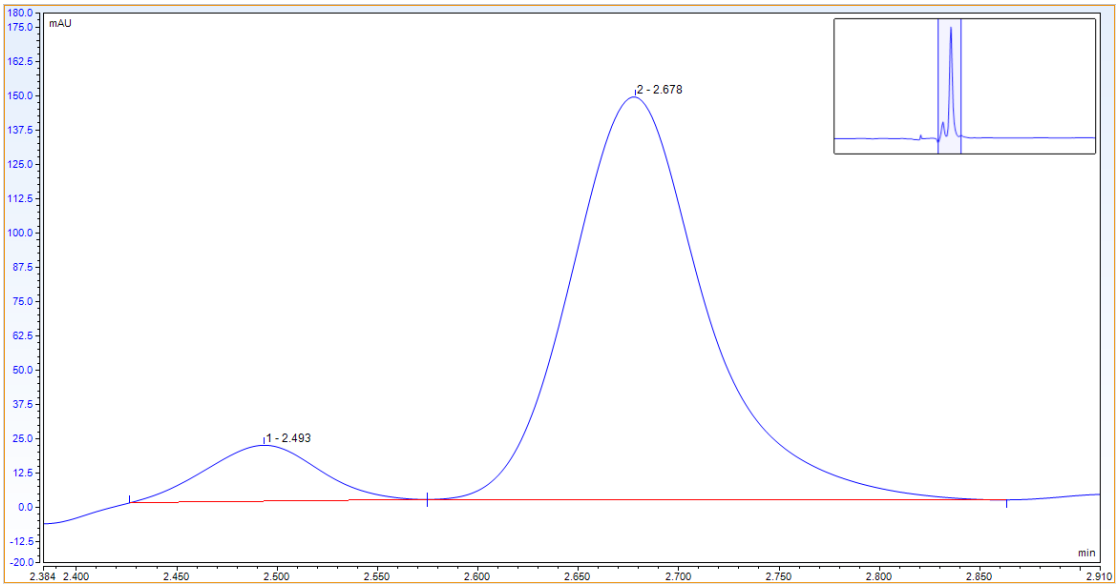
[4] The substrate was incubated with Glyoxalase I and glutathione for 13 hours, followed by the addition of Glyoxalase II to the resulting mixture and further incubation. The final ratio of enzymes was 3:1 respectively.

b) 3:1



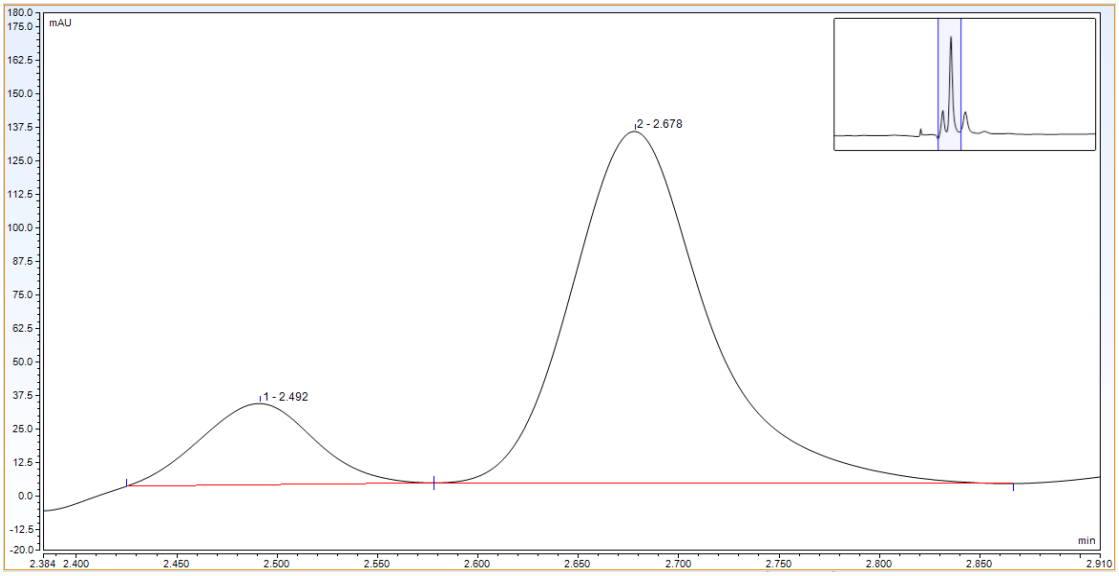
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.498	0.687	5.38
2	2.677	12.093	94.62
Total:		**12.780**	**100.00**

c) 30: 1



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.493	1.334	10.43
2	2.678	11.461	89.57
Total:		**12.795**	**100.00**

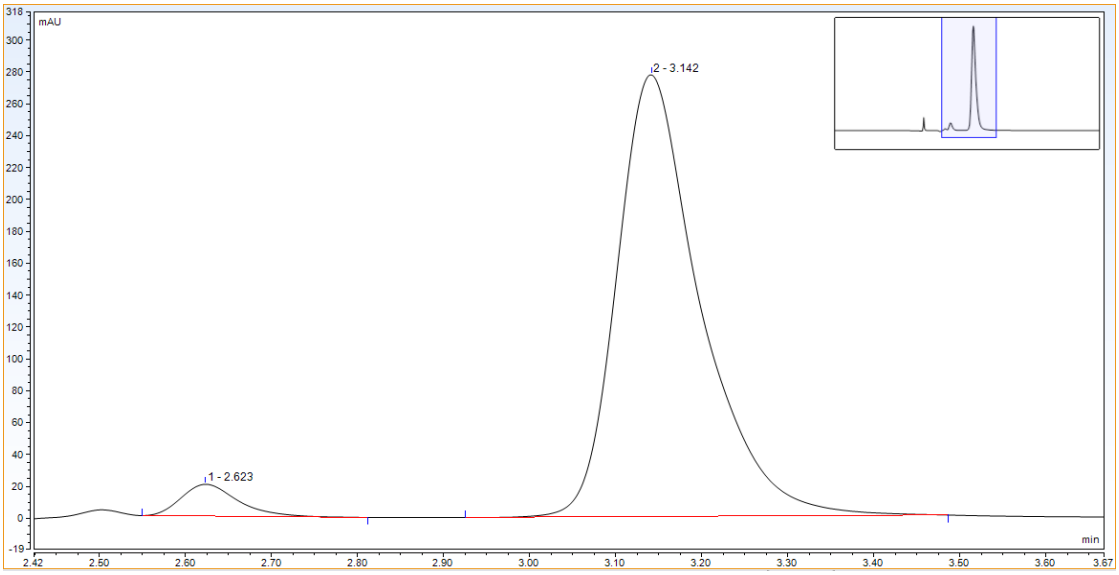
d) Delay



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.492	1.982	16.16
2	2.678	10.281	83.84
Total:		**12.263**	**100.00**

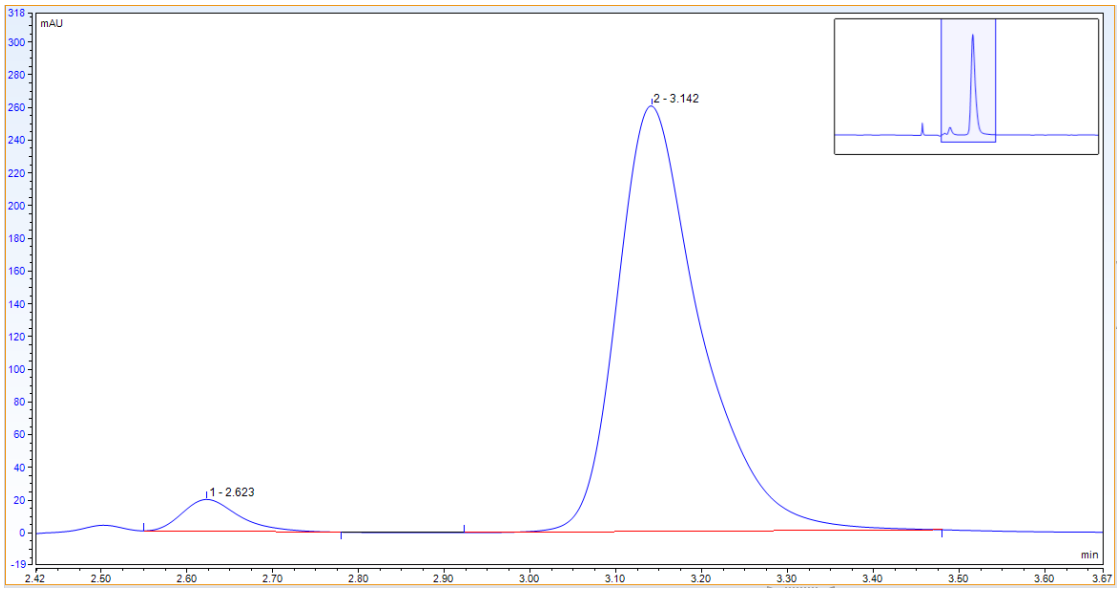
Chiral UHPLC chromatograms obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature of 2-(4-bromophenyl)-2-hydroxyacetic acid **(2g)** formed by the glutathione-mediated conversion of 4-Bromophenyl glyoxal hydrate (**1g·H_2O)** by *E. coli* Glyoxalase I and Glyoxalase II in the respective concentration ratios:

a) 1:3



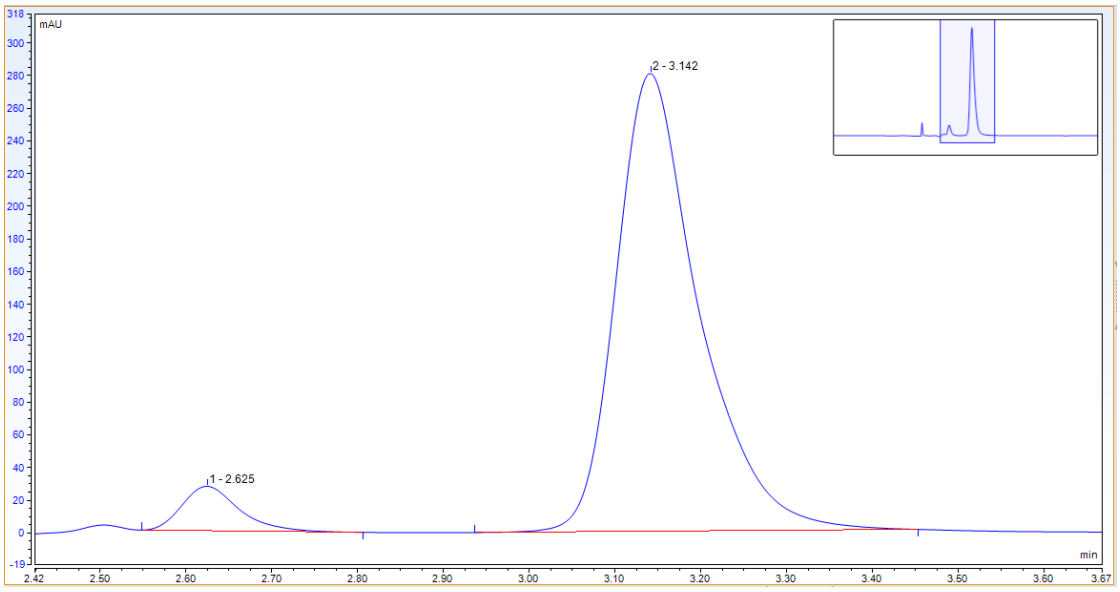
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.623	1.529	4.69
2	3.142	31.075	95.31
Total:		**32.604**	**100.00**

b) 3:1



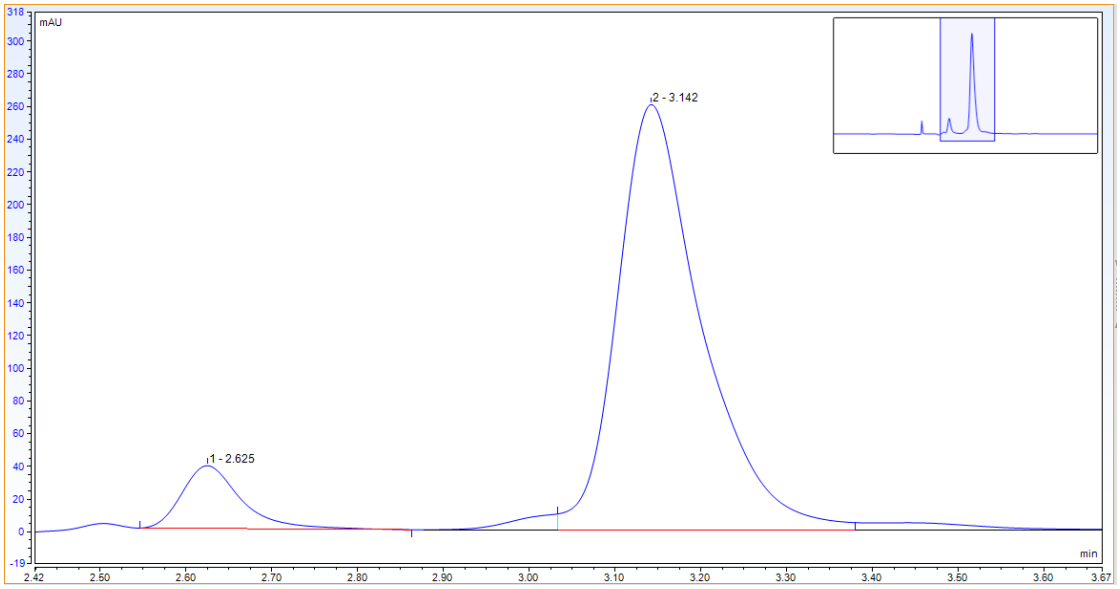
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.623	1.480	4.82
2	3.142	29.213	95.18
Total:		**30.693**	**100.00**

c) 30:1



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.625	2.097	6.26
2	3.142	31.376	93.74
Total:		**33.472**	**100.00**

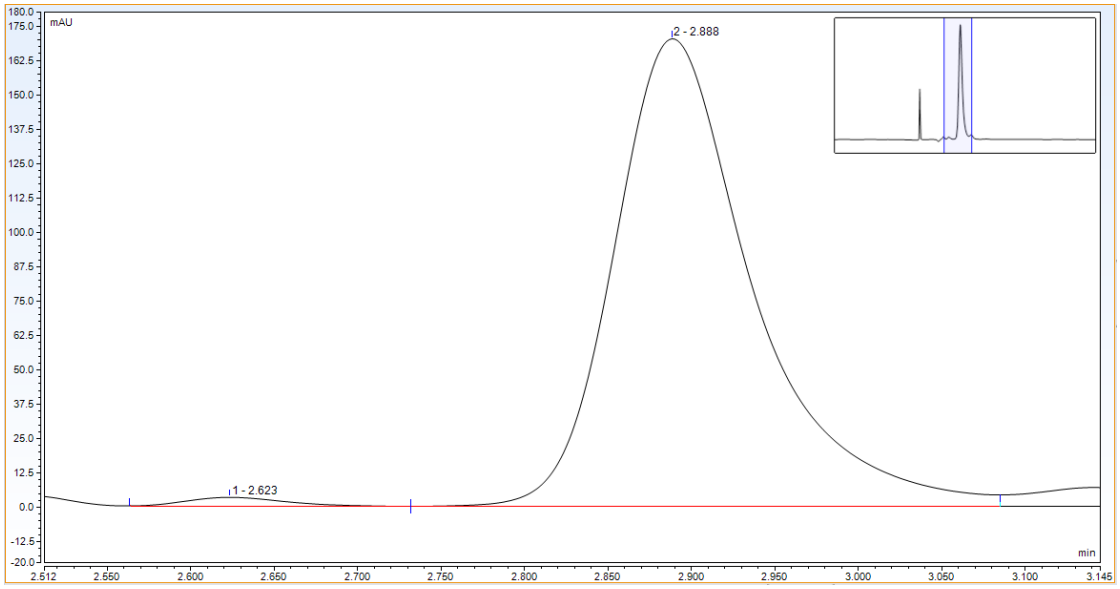
d) Delay



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.625	3.055	9.38
2	3.142	29.517	90.62
Total:		**32.572**	**100.00**

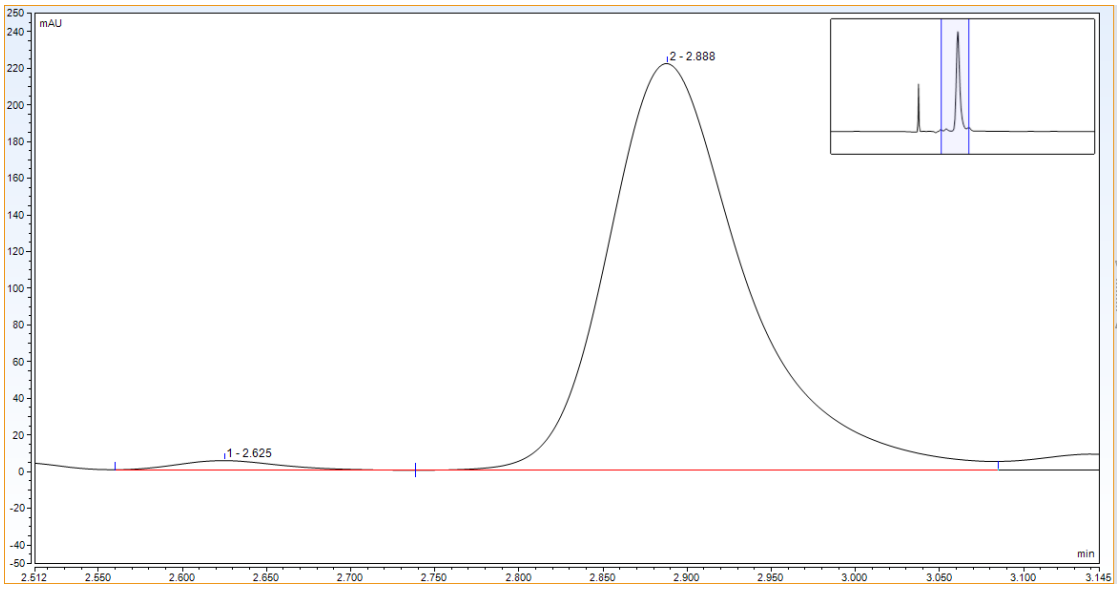
Chiral UHPLC chromatogram obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature of 2-hydroxy-2-(4-methoxyphenyl)acetic acid **(2h)** formed by the glutathione-mediated conversion of 4-Methoxyphenyl glyoxal hydrate (**1h·H_2O)** by *E. coli* Glyoxalase I and Glyoxalase II in the respective concentration ratios:

a) 1:3



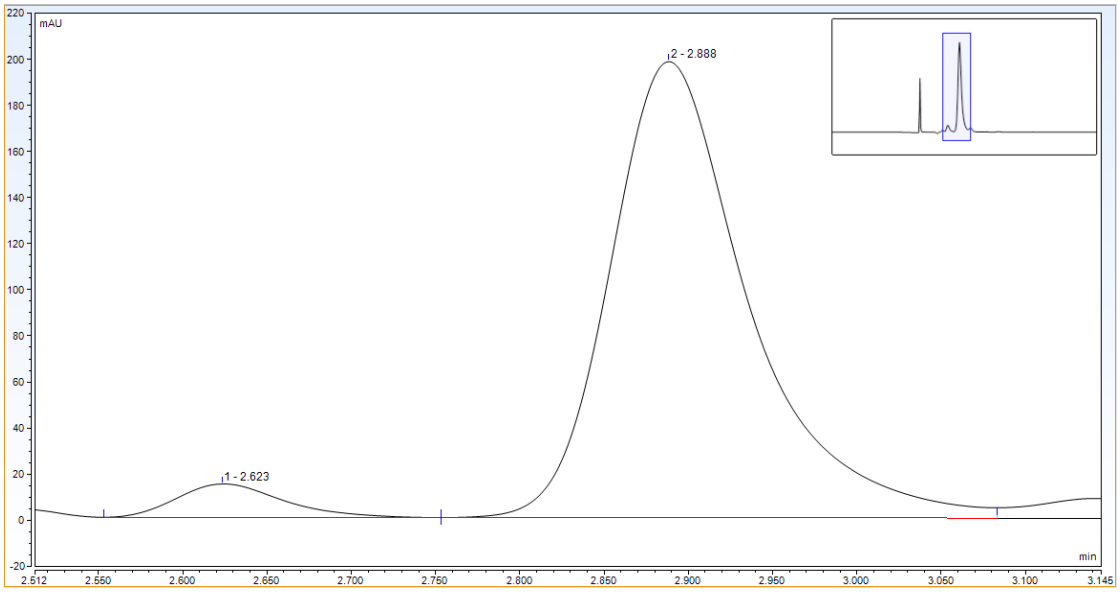
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.623	0.212	1.28
2	2.888	16.367	98.72
Total:		**16.579**	**100.00**

b) 3:1



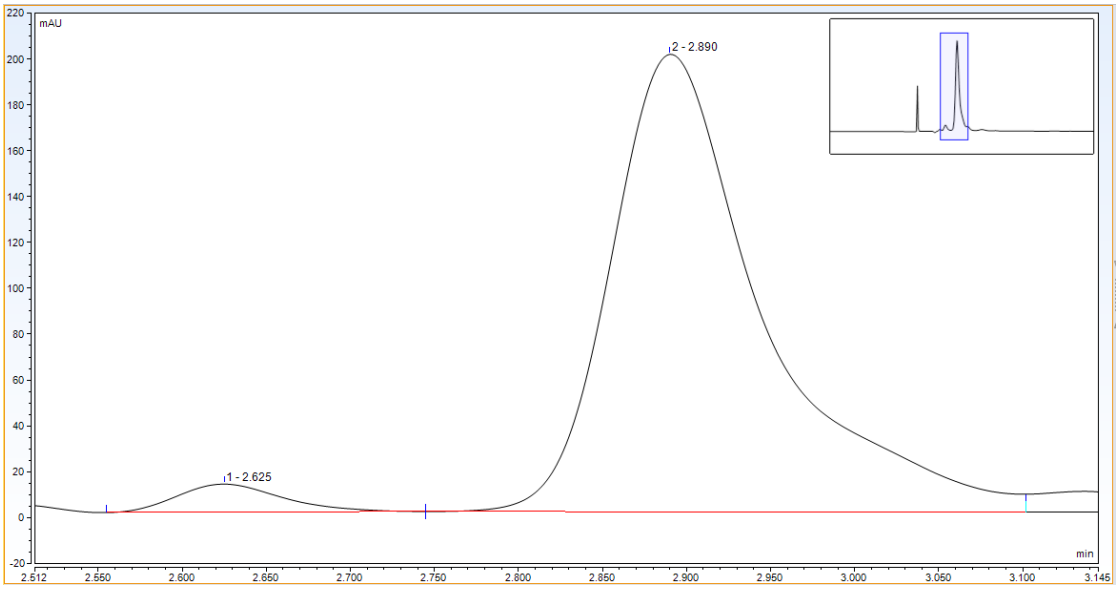
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.625	0.351	1.64
2	2.888	21.057	98.36
Total:		**21.408**	**100.00**

c) 30: 1



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.623	1.055	5.30
2	2.888	18.872	94.70
Total:		**19.927**	**100.00**

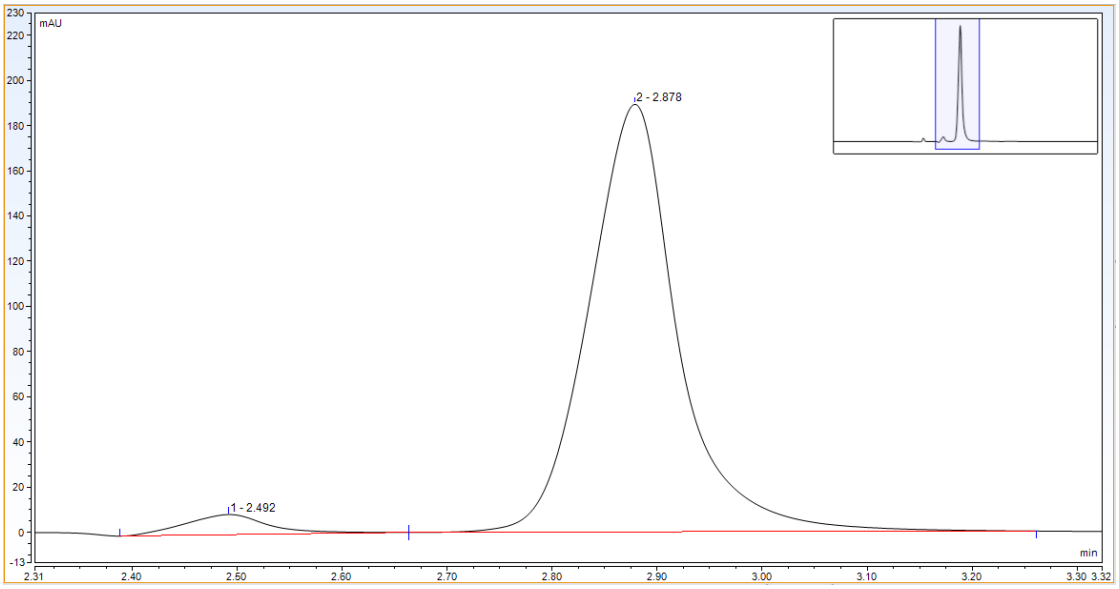
d) Delay



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.625	0.884	4.06
2	2.890	20.893	95.94
Total:		**21.778**	**100.00**

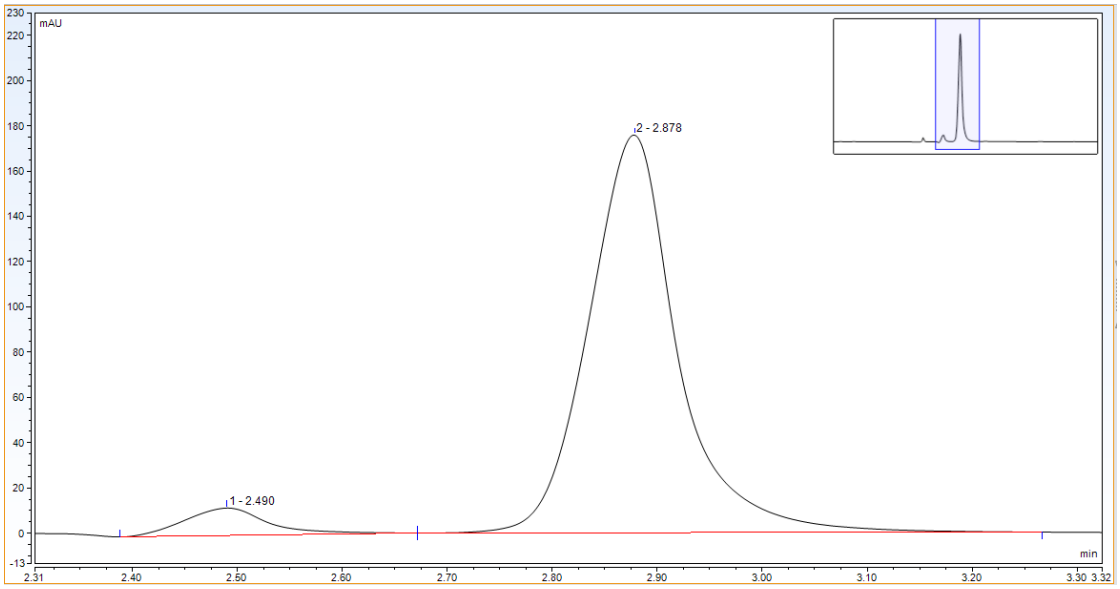
Chiral UHPLC chromatogram obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature of 2-(5-bromothiophen-2-yl)-2-hydroxyacetic acid **(2n)** formed by the glutathione-mediated conversion of 5-Bromothiophen-2-yl glyoxal hydrate **(1n·H_2O)** by *E. coli* Glyoxalase I and Glyoxalase II in the respective concentration ratios:

a) 1:3



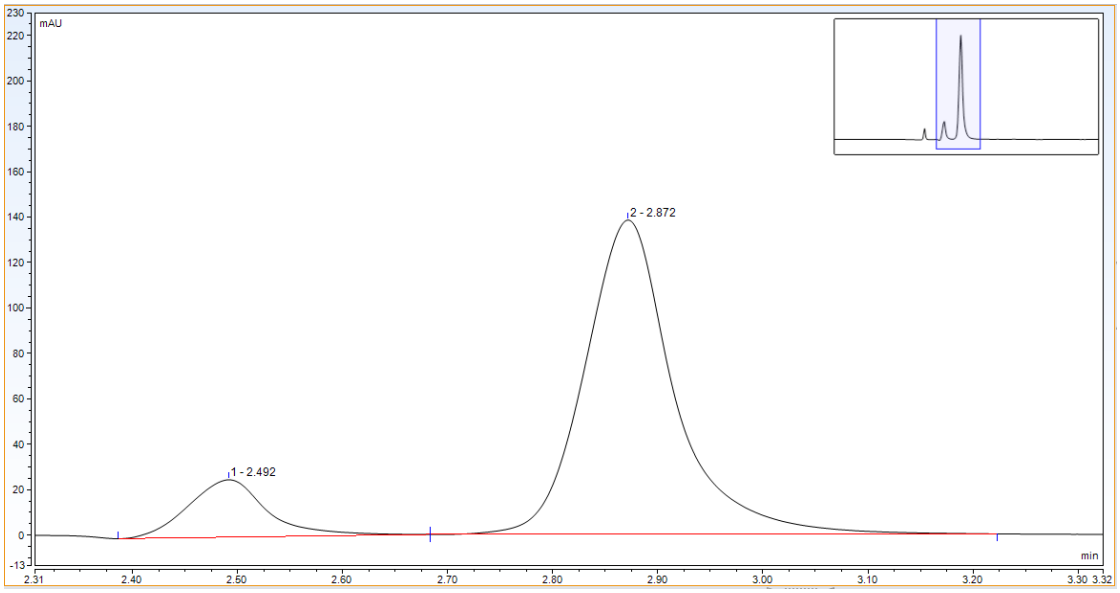
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.492	0.832	4.28
2	2.878	18.599	95.72
Total:		**19.431**	**100.00**

b) 3:1



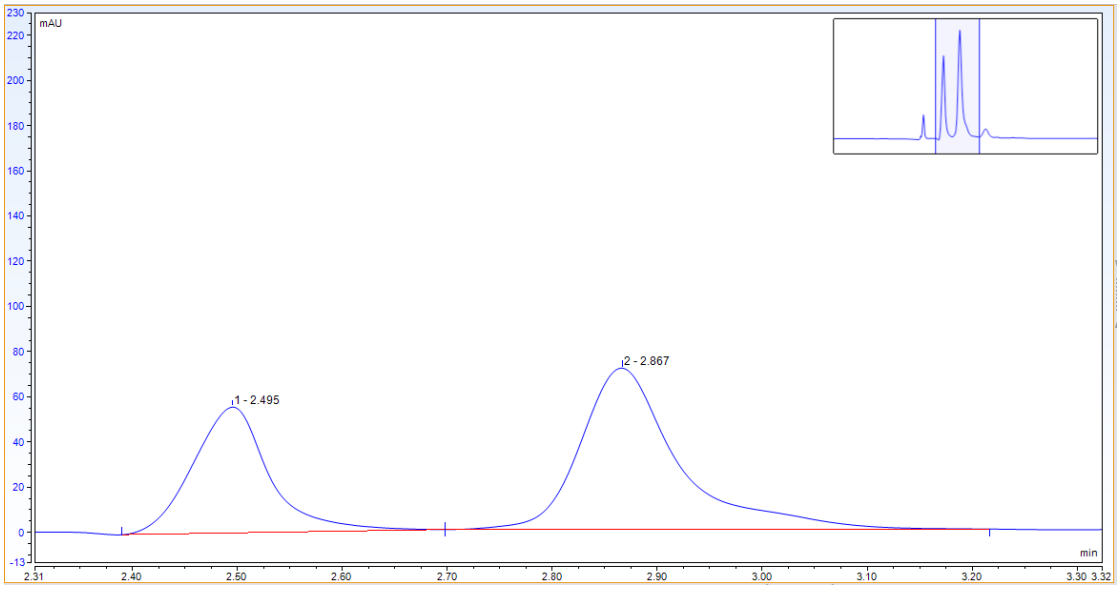
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.490	1.128	6.13
2	2.878	17.266	93.87
Total:		**18.395**	**100.00**

c) 30: 1



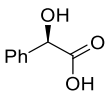
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.492	2.201	13.90
2	2.872	13.631	86.10
Total:		**15.832**	**100.00**

d) Delay



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.495	4.696	37.84
2	2.867	7.712	62.16
Total:		**12.408**	**100.00**

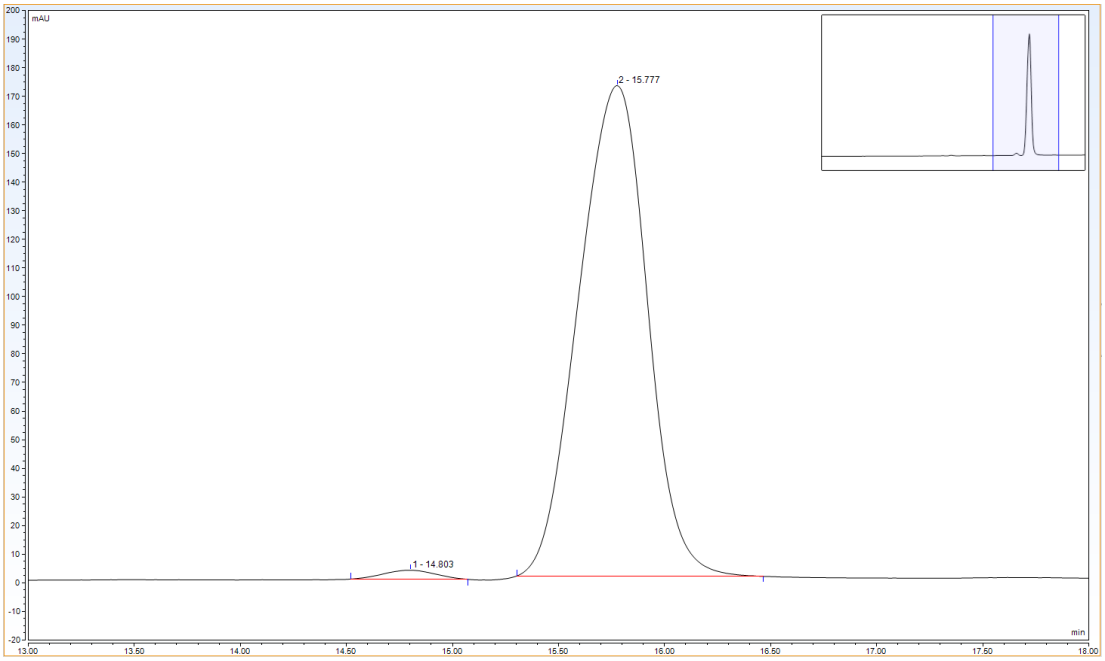
Synthesis and characterization



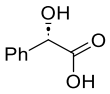
(*R*)-2b

(*R*)-(-)-2-Hydroxy-2-phenylacetic acid ((*R*)-2b). Phenyl glyoxal hydrate (**1b·H₂O**, 100 mg, 0.66 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 760 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (33 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction (determined by monitoring optical absorbance at 250 nm) the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*R*)-2b** (94 mg, 0.62 mmol, 94%) as white powder. The large-scale reaction was set up by dissolving **1b** (1.0 g, 6.6 mmol) and glutathione (0.33 mmol) in 100 mL sodium phosphate buffer (50 mM, pH = 7), and the resulting solution was added dropwise to a 50 mL solution of glyoxalase I and glyoxalase II (157 nmol and 49 nmol respectively) in the same buffer. The large-scale reaction using **1b** (1.0 g, 6.6 mmol) delivered **(*R*)-2b** (0.94 g, 6.2 mmol, 94%) as white powder. ***ee*** = 97 % [UHPLC]. $[\alpha]^{27}_D$ = -150 (c = 0.5, MeOH, EtOH or EtOAc). ***mp:*** 133-135 °C. 0.07-0.8 mL/min flow rate, 5-30°C column oven and 5°C sample holder temperature

Chiral UHPLC chromatogram of (*R*)-(-)-2-hydroxy-2-phenylacetic acid (**(*R*)-2b**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



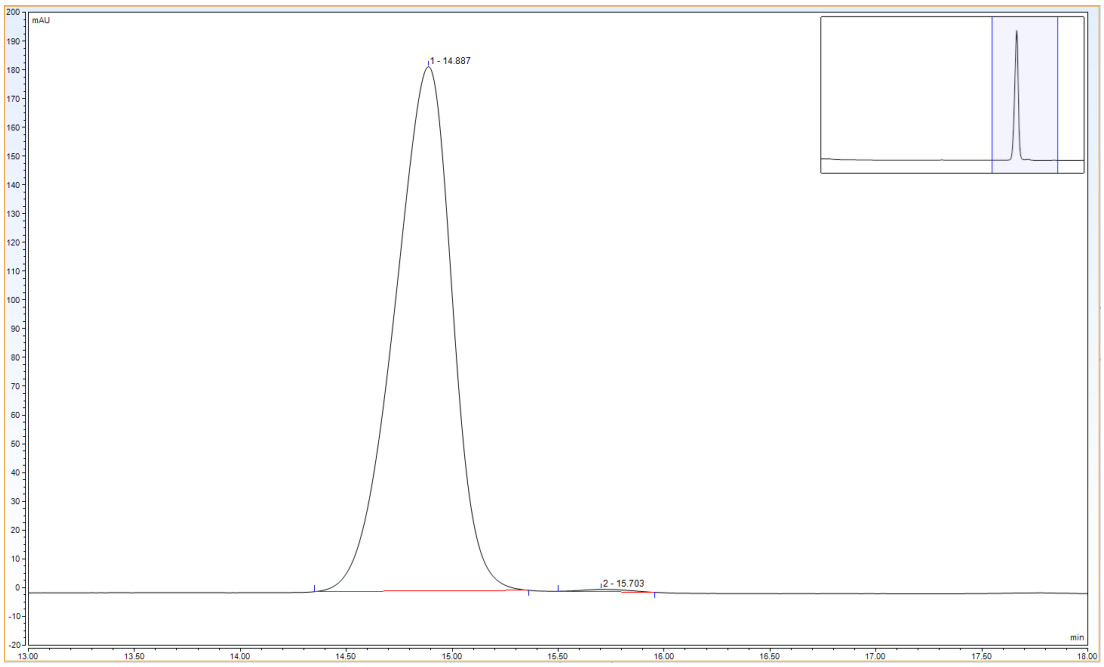
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	14.803	0.8819	1.36
2	15.777	64.187	98.64



(*S*)-2b

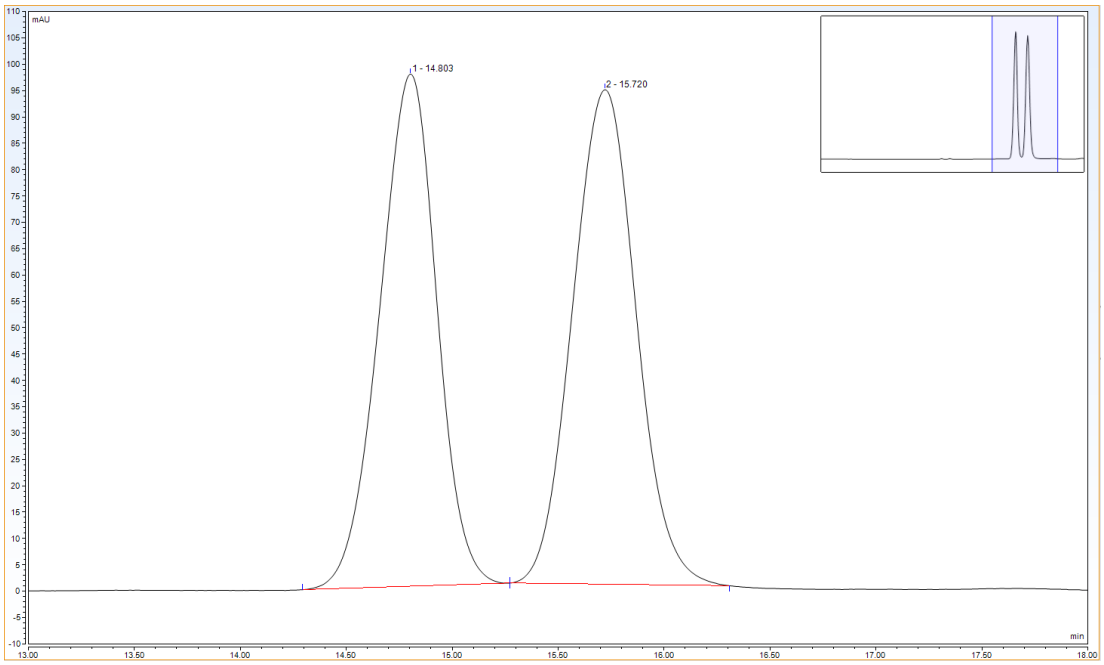
(*S*)-(+)-2-Hydroxy-2-phenylacetic acid ((*S*)-2b). Phenyl glyoxal hydrate (**1b·H₂O**, 100 mg, 0.66 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 0.5 ml phosphate buffer solution containing 280 nmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 250 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*S*)-2b** (98 mg, 0.64 mmol, 98%) as white powder. The large-scale reaction was set up by dissolving **1b** (1.0 g, 6.6 mmol) in 100 mL sodium phosphate buffer (50 mM, pH = 7), and the resulting solution was added dropwise to a 50 mL solution containing 9 µmol DJ-1 in the same buffer. The large-scale reaction using **1b** (1.0 g, 6.6 mmol) delivered **(*S*)-2b** (0.92 g, 6.0 mmol, 92%) as white powder. ***ee*** = >99 % [UHPLC]. $[\alpha]^{27}_{D}$ = +153 (c = 0.5, EtOH or EtOAc). ***mp:*** 134-136 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-hydroxy-2-phenylacetic acid (**(*S*)-2b**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



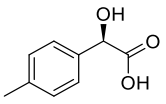
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	14.887	57.5296	99.64
2	15.703	0.2083	0.36

Chiral UHPLC chromatogram of racemic 2-hydroxy-2-phenylacetic acid (**2b**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	14.803	30.3829	48.24
2	15.72	32.5939	51.76

^{1}H NMR (300 MHz, DMSO-D6): δ 7.45 – 7.38 (m, 2H), 7.38 – 7.23 (m, 3H), 5.83 (bs, 1H), 5.02 (s, 1H). ***$^{13}C\{^{1}H\}$ NMR (125 MHz DMSO-D6):*** δ 174.1, 140.3, 128.1, 127.7, 126.7, 72.4; ***HRMS (ESI, [M-H]$^{-}$)*** for $C_8H_7O_3^-$ calcd 151.0401, found 151.0399.

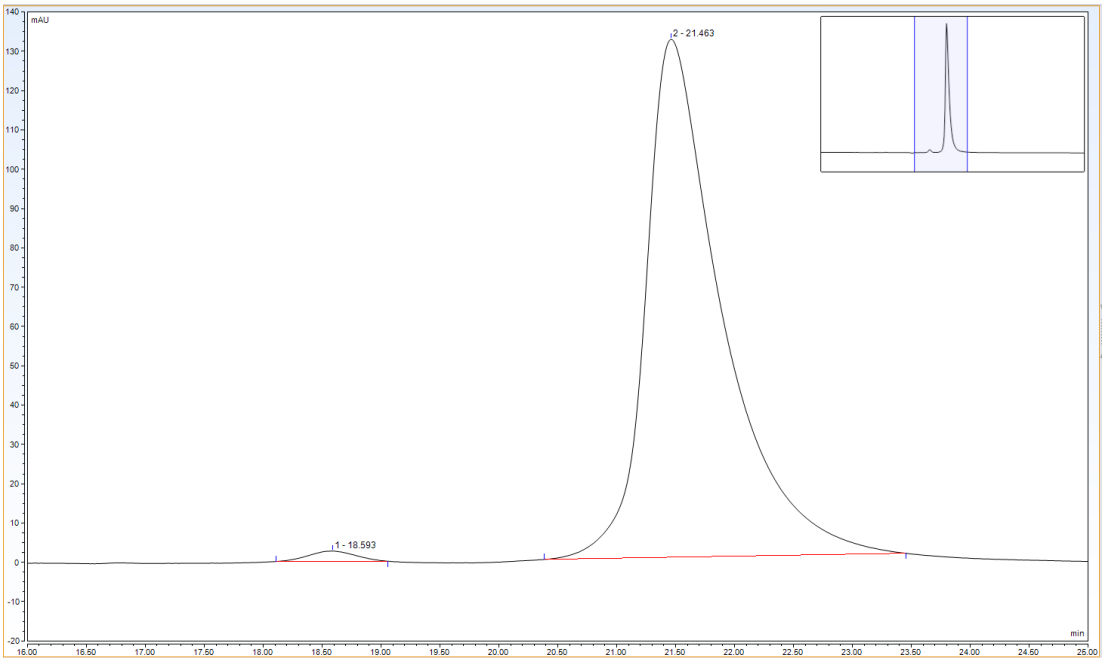


(*R*)-2c

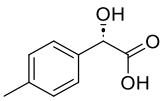
(*R*)-(-)-2-Hydroxy-2-(*p*-tolyl)acetic acid ((*R*)-2c).[5] *p*-Tolyl glyoxal hydrate (**1c·H$_2$O**, 100 mg, 0.60 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 700 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (30 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 263 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*R*)-2c** (99 mg, 0.60 mmol, 99%) as beige solid. ***ee*** = 98 % [UHPLC]. ***[α]$^{27}_D$*** = -146 (c = 0.5, EtOAc). ***mp:*** 118-121 °C.

[5] J. R. E. Hoover, G. L. Dunn, D. R. Jakas, L. L. Lam, J. J. Taggart, J. R. Guarini, and L. Phillips, *Journal of Medicinal Chemistry*, 1974, **17**, 34–41.

Chiral UHPLC chromatogram of (*R*)-(-)-2-hydroxy-2-(*p*-tolyl)acetic acid (**(*R*)-2c**) obtained with 0.125 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



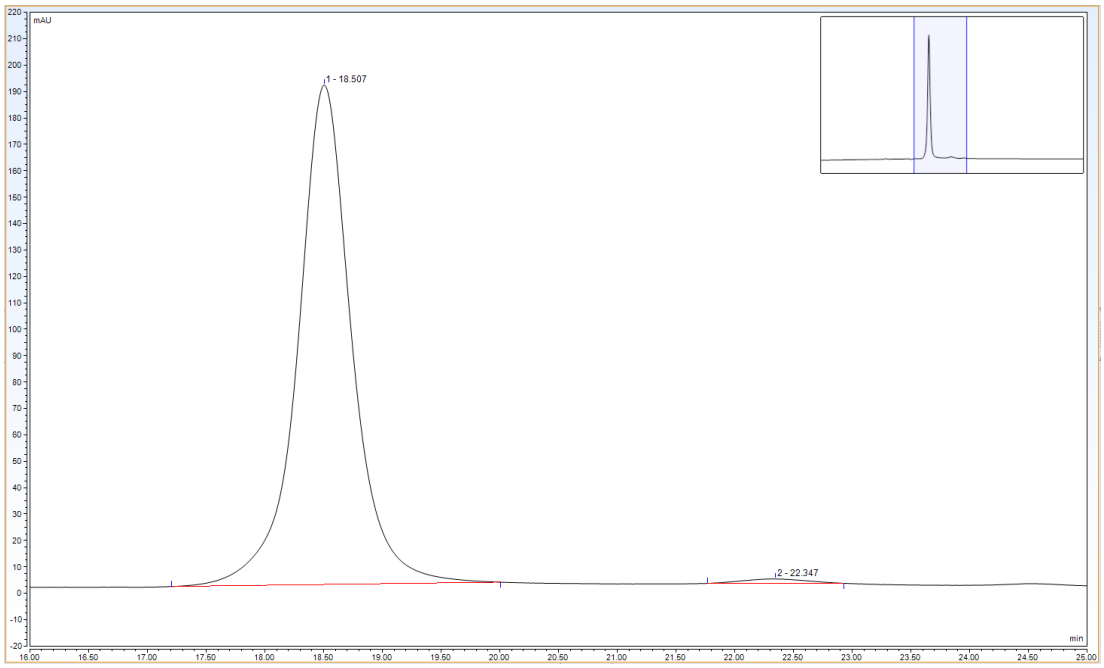
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	18.593	1.2079	1.20
2	21.463	99.1562	98.80



(*S*)-2c

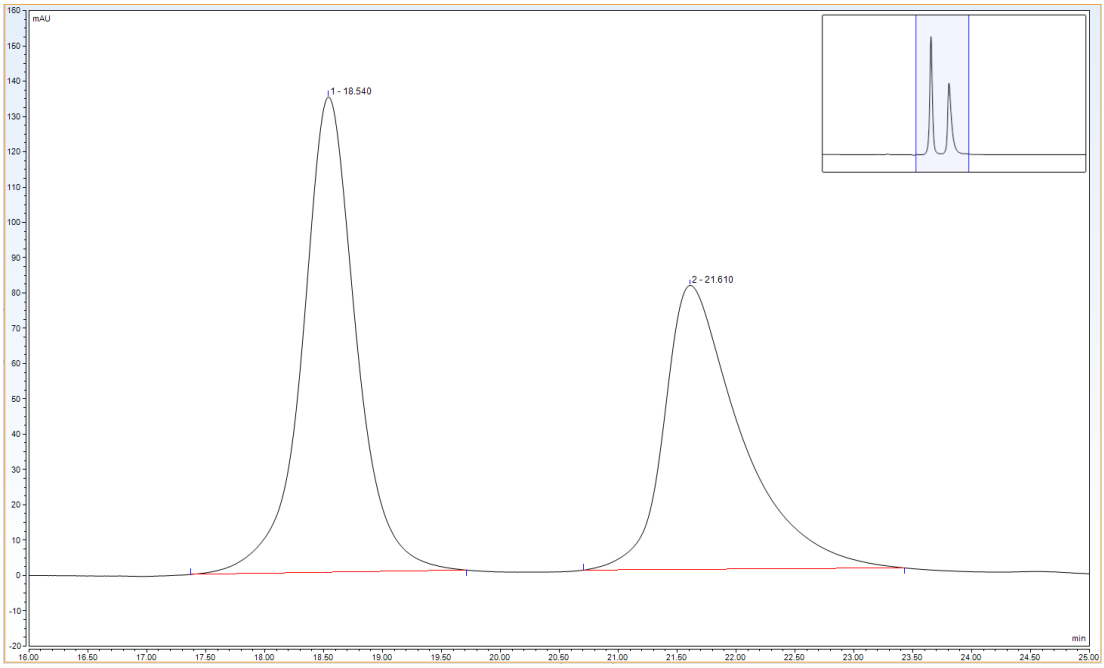
(*S*)-(+)-2-Hydroxy-2-(*p*-tolyl)acetic acid ((*S*)-2c). *p*-Tolyl glyoxal hydrate (**1c·H₂O**, 100 mg, 0.60 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 1.4 ml phosphate buffer solution containing 784 nmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 263 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*S*)-2c** (97 mg, 0.58 mmol, 97%) as beige solid. ***ee*** = 98 % [UHPLC]. $[\alpha]^{27}_D$ = +149 (c = 0.6, EtOAc). ***mp:*** 123-126 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-hydroxy-2-(*p*-tolyl)acetic acid (**(*S*)-2c**) obtained with 0.125 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



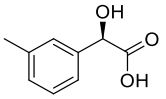
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	18.507	100.9578	99.00
2	22.347	1.0187	1.00

Chiral UHPLC chromatogram of nearly racemic 2-hydroxy-2-(*p*-tolyl)acetic acid (**2c)** obtained with 0.125 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	18.54	71.309	53.93
2	21.61	60.9103	46.07

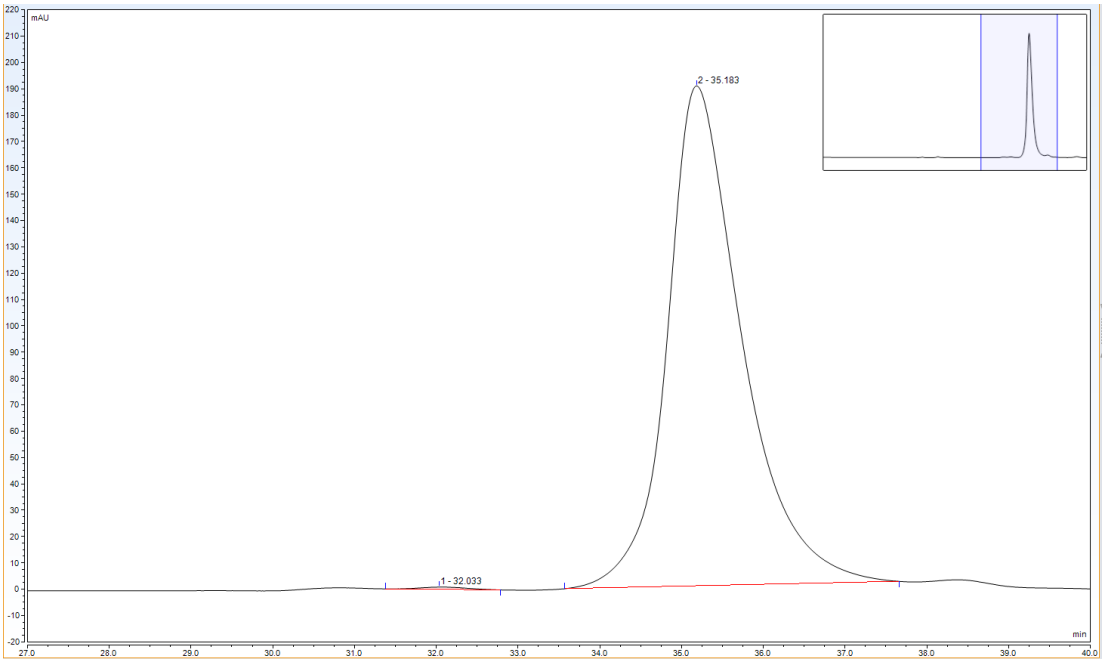
^{1}H NMR (300 MHz, DMSO-D6): δ 7.29 (d, *J* = 8.1 Hz, 2H), 7.14 (d, *J* = 7.9 Hz, 2H), 5.72 (bs, 1H), 4.97 (s, 1H), 2.28 (s, 3H). ***^{13}C{^{1}H} NMR (75 MHz, DMSO-D6):*** δ 174.2, 137.3, 136.7, 128.6, 126.5, 72.2, 20.7; ***HRMS (ESI, [M-H]$^-$)*** for $C_9H_9O_3^-$ calcd 165.0557, found 165.0554.



(*R*)-2d

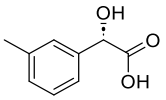
(*R*)-(-)-2-Hydroxy-2-(*m*-tolyl)acetic acid ((*R*)-2d).[5] *m*-Tolyl glyoxal hydrate (**1d·H$_2$O**, 100 mg, 0.60 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 700 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (30 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 255 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate followed by addition of silica gel and concentration in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0→9:1:0.02) as eluent followed by trituration with pentane afforded pure **(*R*)-2d** (67 mg, 0.40 mmol, 67%) as white solid. ***ee*** = >99 % [UHPLC]. ***[α]$^{27}_D$*** = -143 (c = 0.36, EtOAc). ***mp:*** 105-108 °C.

Chiral UHPLC chromatogram of (*R*)-(-)-2-hydroxy-2-(*m*-tolyl)acetic acid (**(*R*)-2d**) obtained with 0.07 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	32.033	0.5797	0.29
2	35.183	200.6875	99.71

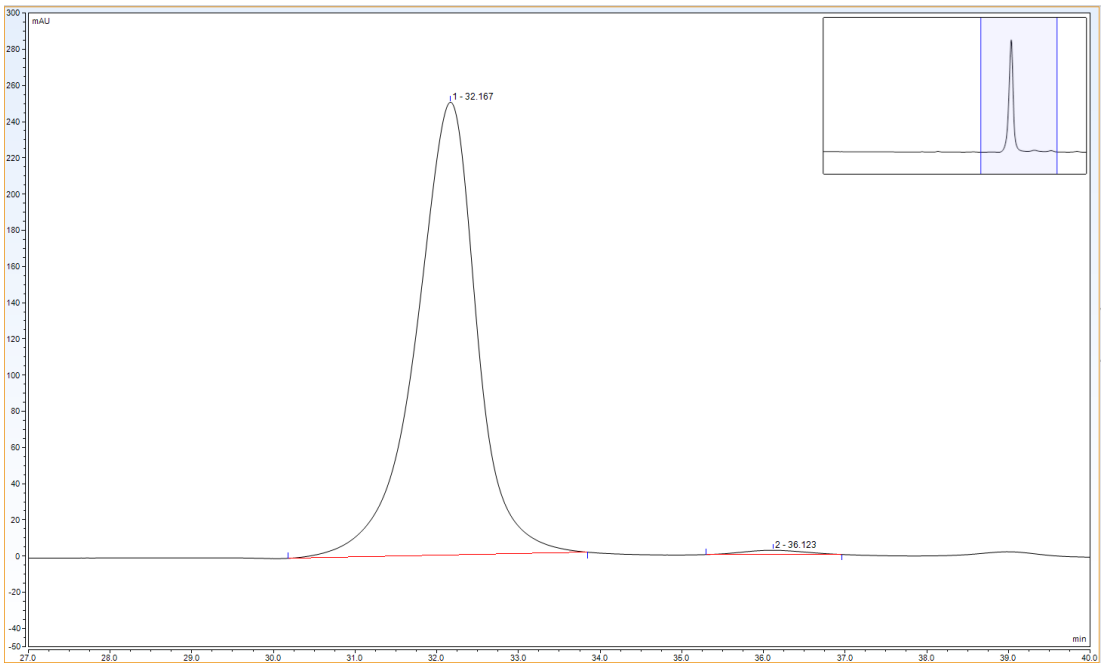
[6] All columns were initially packed with silicagel and DCM. Crude product was applied via solid load, then the elution with the eluent mixture was started.



(*S*)-2d

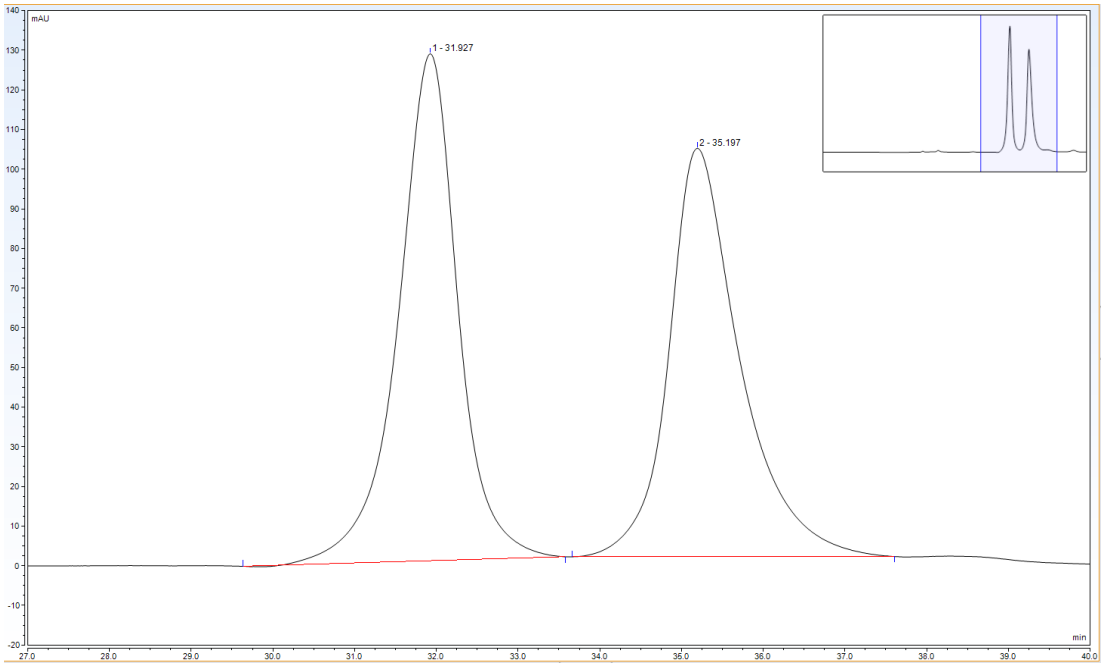
(*S*)-(+)-2-Hydroxy-2-(*m*-tolyl)acetic acid ((*S*)-2d). *m*-Tolyl glyoxal hydrate (**1d·H₂O**, 100 mg, 0.60 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 2.6 ml phosphate buffer solution containing 1.57 µmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 255 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate followed by addition of silica gel and concentration in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0→9:1:0.02) as eluent followed by trituration with pentane afforded pure **(*S*)-2d** (59 mg, 0.36 mmol, 59%) as white solid. ***ee*** = 98 % [UHPLC]. $[\alpha]^{27}_{D}$ = +138 (c = 0.36, EtOAc). ***mp:*** 109-111 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-hydroxy-2-(*m*-tolyl)acetic acid (**(*S*)-2d**) obtained with 0.07 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



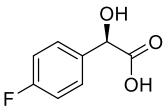
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	32.167	215.9292	99.05
2	36.123	2.0627	0.95

Chiral UHPLC chromatogram of racemic 2-hydroxy-2-(*m*-tolyl)acetic acid (**2d**) obtained with 0.07 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	31.927	108.3441	51.06
2	35.197	103.8379	48.94

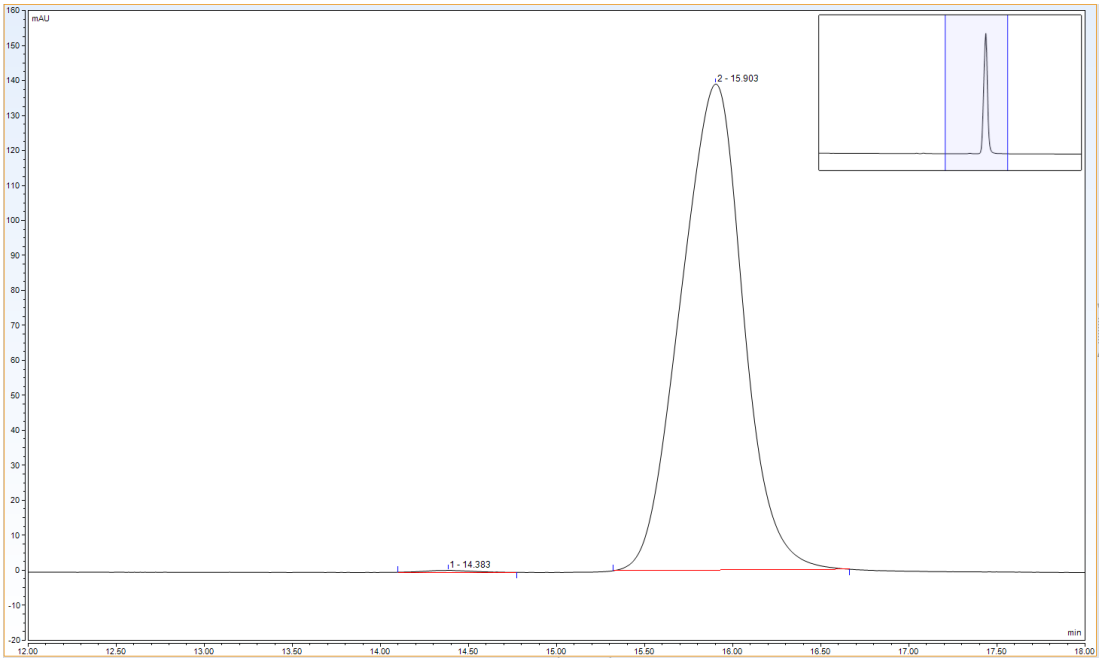
^{1}H NMR (300 MHz, DMSO-D6): δ 7.28 – 7.14 (m, 3H), 7.14 – 7.04 (m, 1H), 4.95 (s, 1H), 2.30 (s, 3H). ***$^{13}C\{^{1}H\}$ NMR (75 MHz, DMSO-D6):*** δ 174.1, 140.1, 137.1, 128.2, 128.0, 127.2, 123.8, 72.4, 21.0; ***HRMS (ESI, [M-H]$^{-}$)*** for $C_9H_9O_3^-$ calcd 165.0557, found 165.0552.



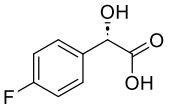
(*R*)-2e

(*R*)-(-)-2-(4-Fluorophenyl)-2-hydroxyacetic acid ((*R*)-2e).[5] 4-Fluorophenyl glyoxal hydrate (**1e·H$_2$O**, 100 mg, 0.59 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 690 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (29.5 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 253 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*R*)-2e** (100 mg, 0.59 mmol, 100%) as beige solid. ***ee*** = >99 % [UHPLC]. ***[α]$^{27}_{D}$*** = -139 (c = 0.46, EtOAc). ***mp:*** 149-152 °C.

Chiral UHPLC chromatogram of (*R*)-(-)-2-(4-fluorophenyl)-2-hydroxyacetic acid (**(*R*)-2e**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



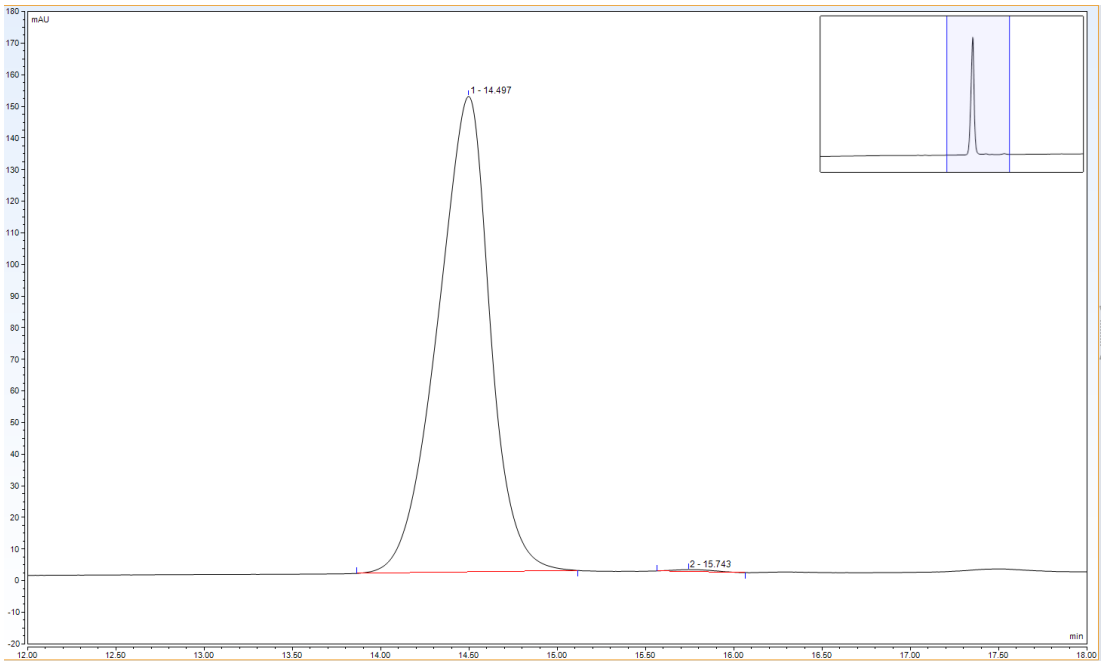
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	14.383	0.1725	0.30
2	15.903	57.1343	99.70



(*S*)-2e

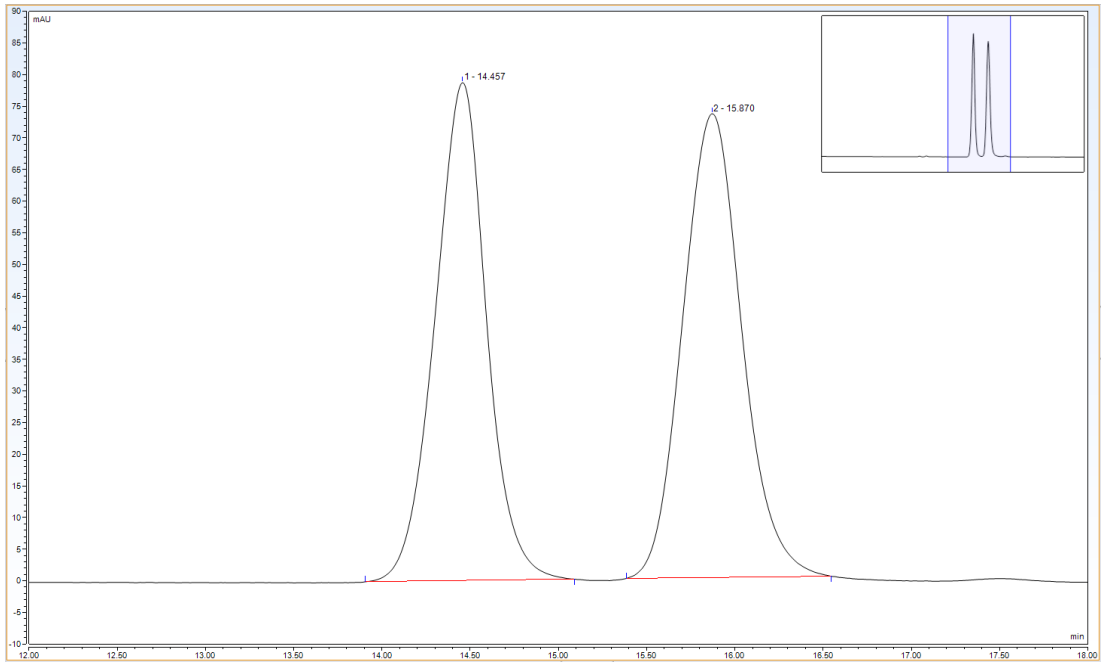
(*S*)-(+)-2-(4-Fluorophenyl)-2-hydroxyacetic acid ((*S*)-2e). 4-Fluorophenyl glyoxal hydrate (**1e·H₂O**, 100 mg, 0.59 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 0.3 ml phosphate buffer solution containing 90 nmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 253 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*S*)-2e** (83 mg, 0.49 mmol, 83%) as white solid. ***ee*** = 99 % [UHPLC]. $[\alpha]^{27}_{D}$ = +146 (c = 0.47, EtOAc). ***mp:*** 151-154 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-(4-fluorophenyl)-2-hydroxyacetic acid (**(*S*)-2e**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



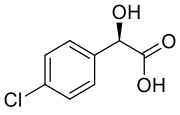
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	14.497	51.7746	99.69
2	15.743	0.1626	0.31

Chiral UHPLC chromatogram of racemic 2-(4-fluorophenyl)-2-hydroxyacetic acid (**2e**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	14.457	26.5929	48.32
2	15.87	28.4396	51.68

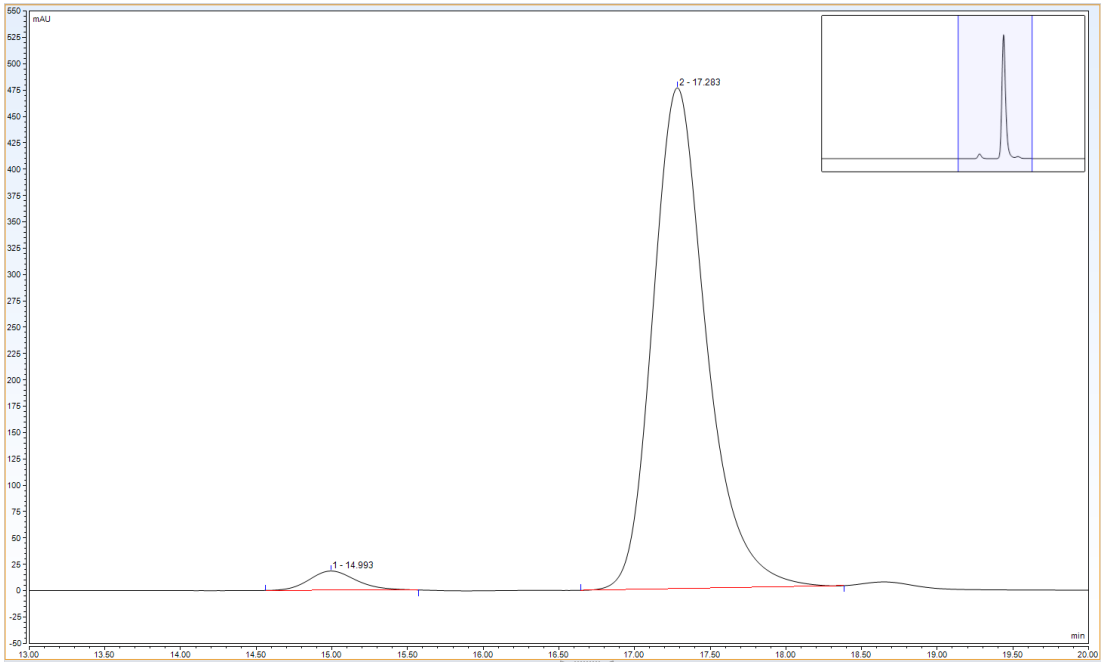
^{1}H NMR (500 MHz, DMSO-D6): δ 7.50 – 7.39 (m, 2H), 7.22 – 7.11 (m, 2H), 5.03 (s, 1H). ***^{13}C{^{1}H} NMR (125 MHz, DMSO-D6):*** δ 173.9, 161.6 (d, *J* = 243.2 Hz), 136.5 (d, *J* = 2.9 Hz), 128.6 (d, *J* = 8.2 Hz), 114.9 (d, *J* = 21.3 Hz), 71.8; ***^{19}F{^{1}H} NMR (282 MHz, DMSO-D6):*** δ -117.1 – (-117.5) (m). ***HRMS (ESI, [M-H]$^{-}$)*** for $C_8H_6FO_3^-$ calcd 169.0306, found 169.0303.



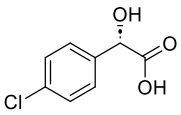
(*R*)-2f

(*R*)-(-)-2-(4-Chlorophenyl)-2-hydroxyacetic acid ((*R*)-2f).[5] 4-Chlorophenyl glyoxal hydrate (**1f·H$_2$O**, 100 mg, 0.54 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 640 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (27 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 261 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0→9:1:0.02) as eluent followed by trituration with pentane afforded pure **(*R*)-2f** (88 mg, 0.47 mmol, 88%) as white sold. ***ee*** = 93 % [UHPLC]. ***[α]$^{27}_{D}$*** = -130 (c = 0.35, EtOAc). ***mp:*** 117-120 °C.

Chiral UHPLC chromatogram of (*R*)-(-)-2-(4-chlorophenyl)-2-hydroxyacetic acid (**(*R*)-2f**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



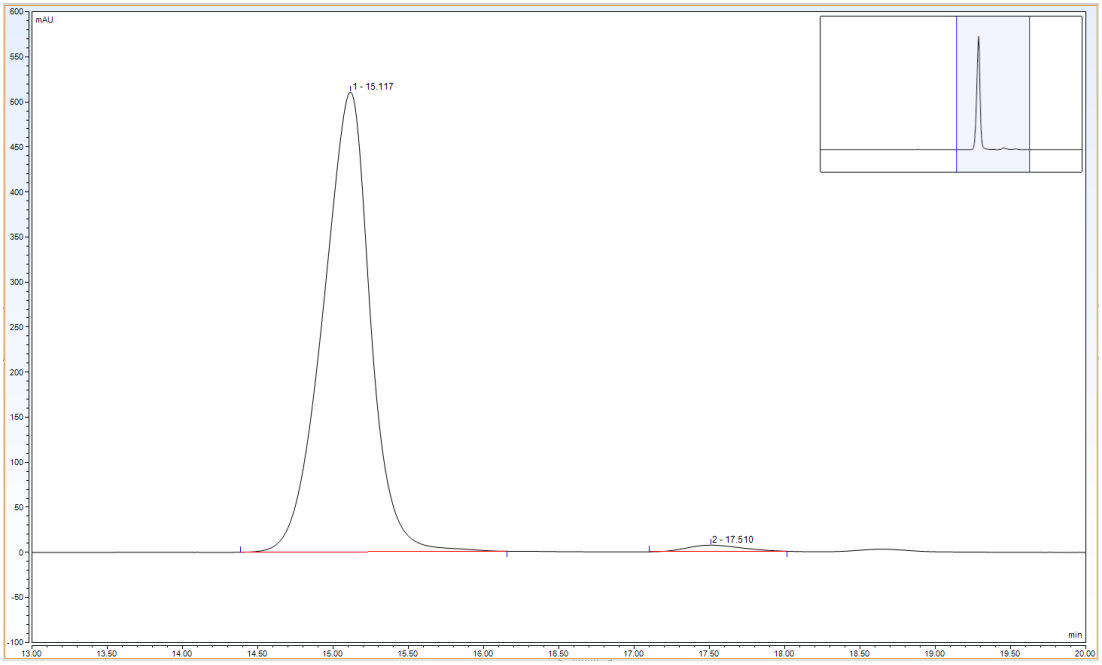
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	14.993	6.5459	3.24
2	17.283	195.335	96.76



(*S*)-2f

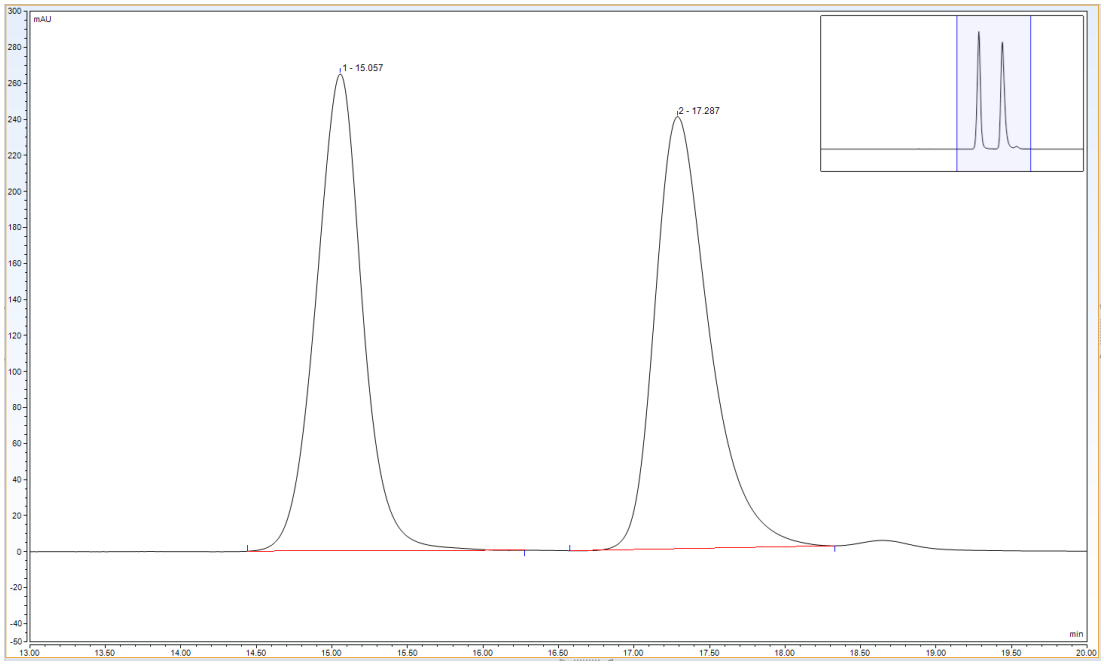
(*S*)-(+)-2-(4-Chlorophenyl)-2-hydroxyacetic acid ((*S*)-2f). 4-Chlorophenyl glyoxal hydrate (**1f·H₂O**, 100 mg, 0.54 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 0.184 ml phosphate buffer solution containing 200 nmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 261 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0→9:1:0.02) as eluent followed by trituration with pentane afforded pure **(*S*)-2f** (92 mg, 0.49 mmol, 92%) as white sold. ***ee*** = 97 % [UHPLC]. $[\alpha]^{27}_{D}$ = +134 (c = 0.38, EtOAc). ***mp:*** 112-115 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-(4-chlorophenyl)-2-hydroxyacetic acid (**(*S*)-2f**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



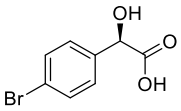
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	15.117	185.5444	98.43
2	17.51	2.9609	1.57

Chiral UHPLC chromatogram of racemic 2-(4-chlorophenyl)-2-hydroxyacetic acid (**2f**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	15.057	94.8104	48.50
2	17.287	100.6816	51.50

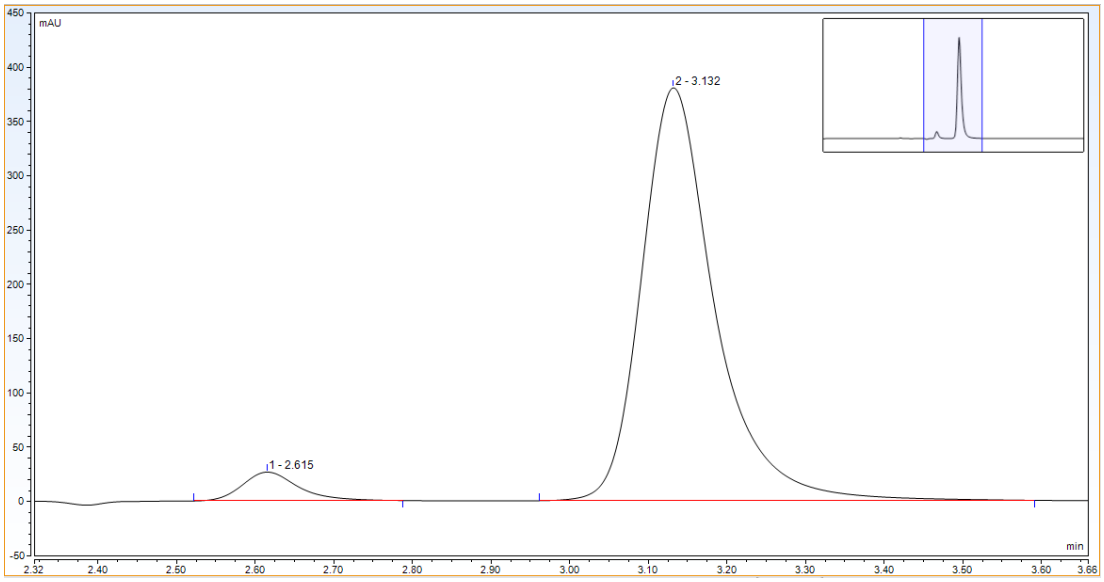
^{1}H NMR (300 MHz, DMSO-D6): δ 7.47 – 7.37 (m, 4H), 5.04 (s, 1H). ***^{13}C{^{1}H} NMR (75 MHz, DMSO-D6):*** δ 173.6, 139.2, 132.1, 128.4, 128.1, 71.6; ***HRMS (ESI, [M-H]$^{-}$)*** for $C_8H_6ClO_3^-$ calcd 185.0011, found 185.0012.



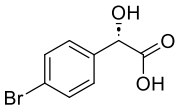
(*R*)-2g

(*R*)-(-)-2-(4-Bromophenyl)-2-hydroxyacetic acid ((*R*)-2g).[5] 4-Bromophenyl glyoxal hydrate (**1g·H$_2$O**, 100 mg, 0.43 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 530 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (21.5 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 265 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*R*)-2g** (95 mg, 0.41 mmol, 95%) as beige solid. ***ee*** = 90 % [UHPLC]. ***[α]$^{27}_{D}$*** = -101 (c = 0.45, EtOAc). ***mp:*** 124-127 °C.

Chiral UHPLC chromatogram of (*R*)-(-)-2-(4-bromophenyl)-2-hydroxyacetic acid (**(*R*)-2g**) obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature:



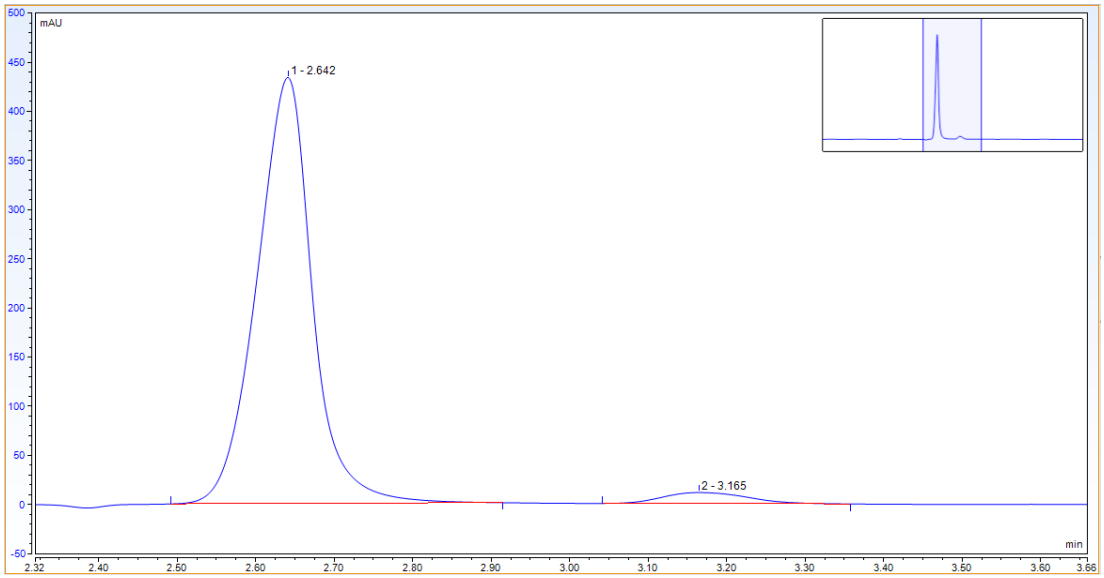
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.615	2.177	5.04
2	3.132	41.050	94.96



(*S*)-2g

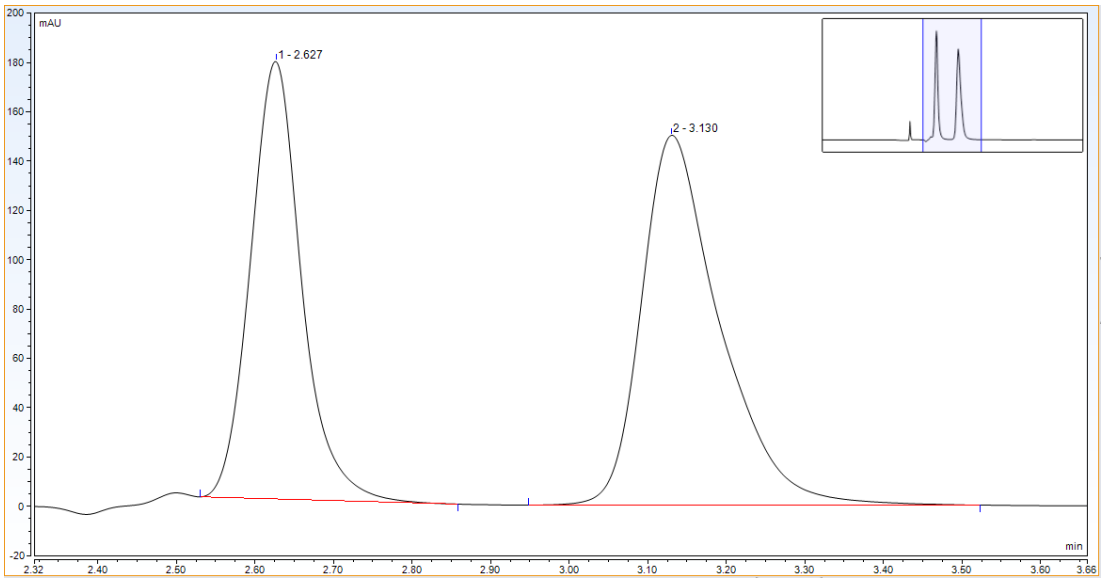
(*S*)-(+)-2-(4-Bromophenyl)-2-hydroxyacetic acid ((*S*)-2g). 4-Bromophenyl glyoxal hydrate (**1g·H₂O**, 100 mg, 0.43 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7)with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 0.105 ml phosphate buffer solution containing 84 nmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 265 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*S*)-2g** (94 mg, 0.41 mmol, 94%) as beige solid. ***ee*** = 92 % [UHPLC]. $[\alpha]^{27}_D$ = +110 (c = 0.4, EtOAc). ***mp:*** 126-129 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-(4-bromophenyl)-2-hydroxyacetic acid (**(*S*)-2g**) obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature:



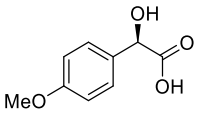
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.642	35.500	96.17
2	3.165	1.414	3.83

Chiral UHPLC chromatogram of scalemic 2-(4-bromophenyl)-2-hydroxyacetic acid (**2g**) obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.627	13.384	43.59
2	3.130	17.318	56.41

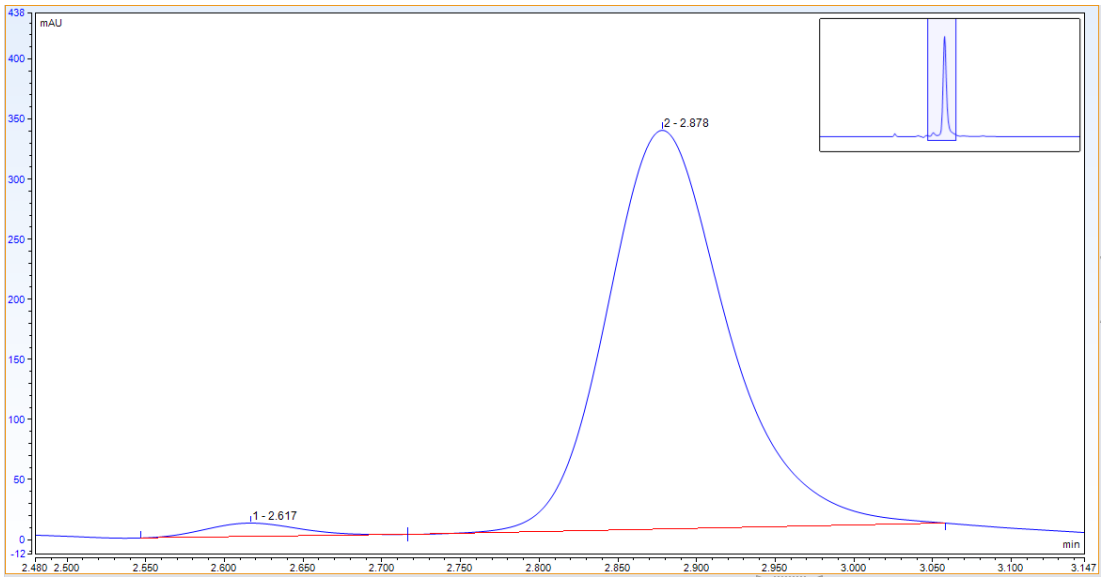
^{1}H NMR (300 MHz, DMSO-D6): δ 7.54 (d, *J* = 8.5 Hz, 2H), 7.37 (d, *J* = 8.3 Hz, 2H), 5.93 (bs, 1H), 5.03 (s, 1H). ***^{13}C{^{1}H} NMR (75 MHz, DMSO-D6):*** δ 173.6, 139.6, 131.0, 128.8, 120.7, 71.7; ***HRMS (ESI, [M-H]$^-$)*** for $C_8H_6BrO_3^-$ calcd 228.9506, found 228.9504.



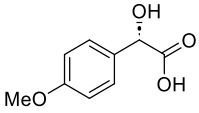
(*R*)-2h

(*R*)-(-)-2-Hydroxy-2-(4-methoxyphenyl)acetic acid ((*R*)-2h).[5] 4-Methoxyphenyl glyoxal hydrate (**1h·H₂O**, 100 mg, 0.55 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 650 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (27.5 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 286 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0.02) as eluent followed by trituration with pentane afforded pure **(*R*)-2h** (80 mg, 0.44 mmol, 80%) as white solid. ***ee*** = 95 % [HPLC]. $[\alpha]^{27}_D$ = -147 (c = 0.5, EtOAc). ***mp:*** 101-103 °C.

Chiral UHPLC chromatogram of (*R*)-(-)-2-hydroxy-2-(4-methoxyphenyl)acetic acid (**(*R*)-2h**) obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature:



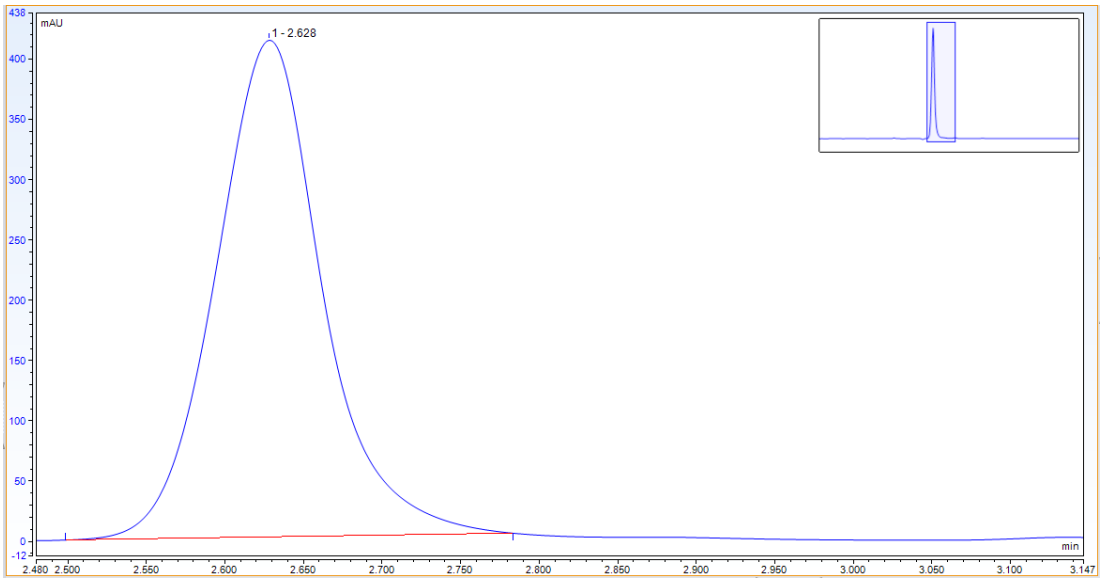
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.617	0.767	2.53
2	2.878	29.533	97.47



(*S*)-2h

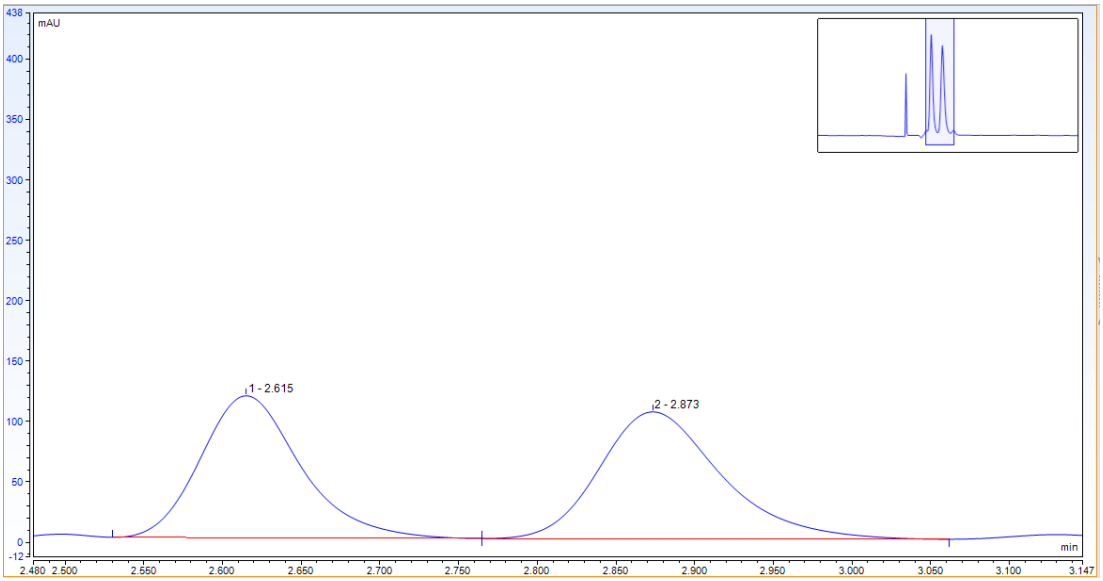
(*S*)-(+)-2-Hydroxy-2-(4-methoxyphenyl)acetic acid ((*S*)-2h). 4-Methoxyphenyl glyoxal hydrate (**1h·H₂O**, 100 mg, 0.55 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7)with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 1.84 ml phosphate buffer solution containing 552 nmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 286 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*S*)-2h** (95 mg, 0.52 mmol, 95%) as white solid. ***ee*** = >99 % [UHPLC]. $[\alpha]^{27}_D$ = +147 (c = 0.43, EtOAc). ***mp:*** 102-104 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-hydroxy-2-(4-methoxyphenyl)acetic acid (**(*S*)-2h**) obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature:



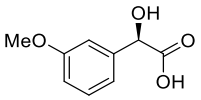
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.628	32.403	100.00

Chiral UHPLC chromatogram of nearly racemic 2-hydroxy-2-(4-methoxyphenyl)acetic acid (**2h**) obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.615	8.658	47.96
2	2.873	9.394	52.04

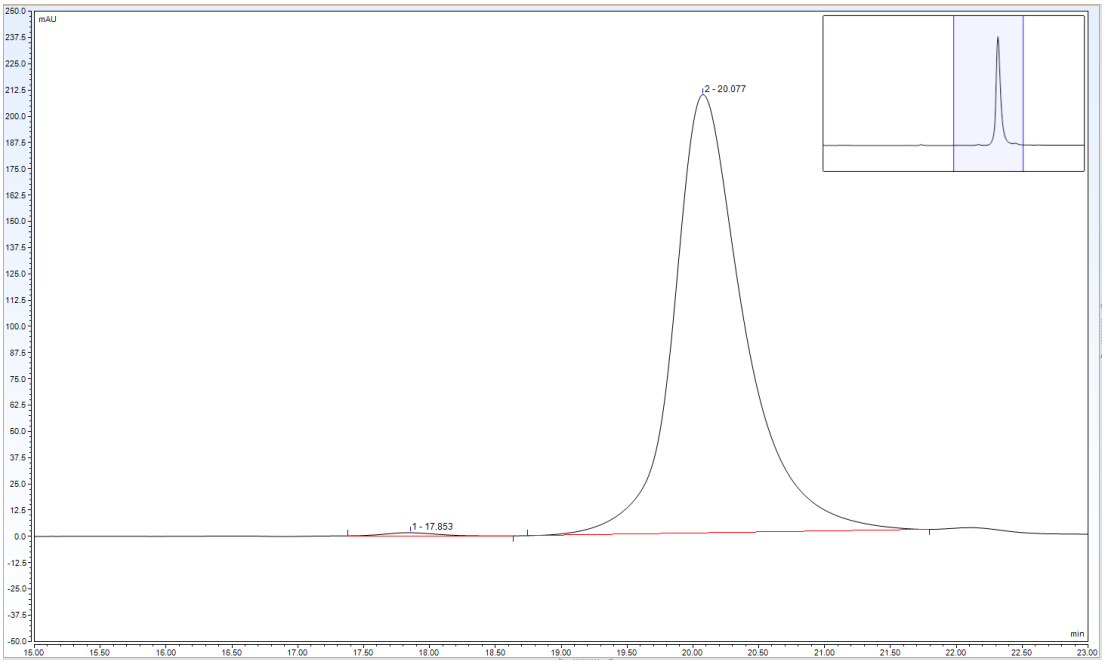
^{1}H NMR (300 MHz, DMSO-D6): δ 7.32 (d, *J* = 8.6 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 4.94 (s, 1H), 3.74 (s, 3H). ***^{13}C{^{1}H} NMR (75 MHz, DMSO-D6):*** δ 174.3, 158.8, 132.3, 127.8, 113.5, 71.9, 55.1; ***HRMS (ESI, [M-H]$^{-}$)*** for $C_9H_9O_4^-$ calcd 181.0506, found 181.0504.



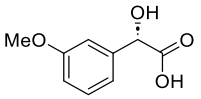
(*R*)-2i

(*R*)-(-)-2-Hydroxy-2-(3-methoxyphenyl)acetic acid ((*R*)-2i).[5] 3-Methoxyphenyl glyoxal hydrate (**1i·H₂O**, 100 mg, 0.55 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 650 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (27.5 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 257 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*R*)-2i** (96 mg, 0.53 mmol, 96%) as beige solid. ***ee*** = 99 % [UHPLC]. $[\alpha]^{27}_D$ = -117 (c = 0.5, EtOAc). ***mp:*** 77-80 °C.

Chiral UHPLC chromatogram of (*R*)-(-)-2-hydroxy-2-(3-methoxyphenyl)acetic acid (**(*R*)-2i**) obtained with 0.125 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



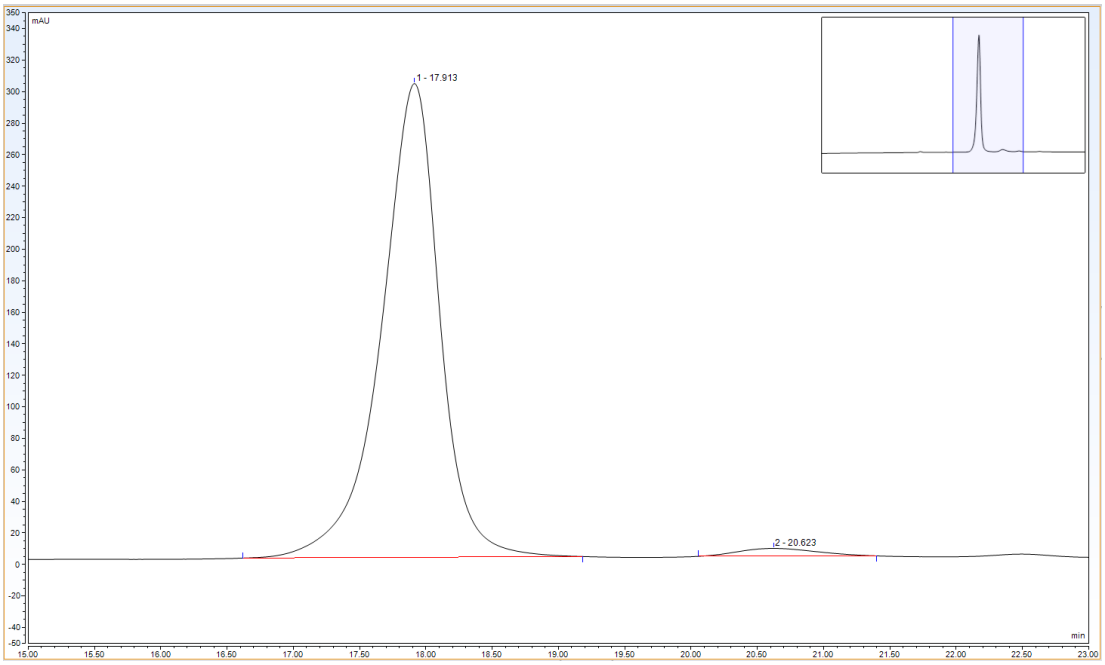
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	17.853	0.6533	0.50
2	20.077	130.7776	99.50



(*S*)-2i

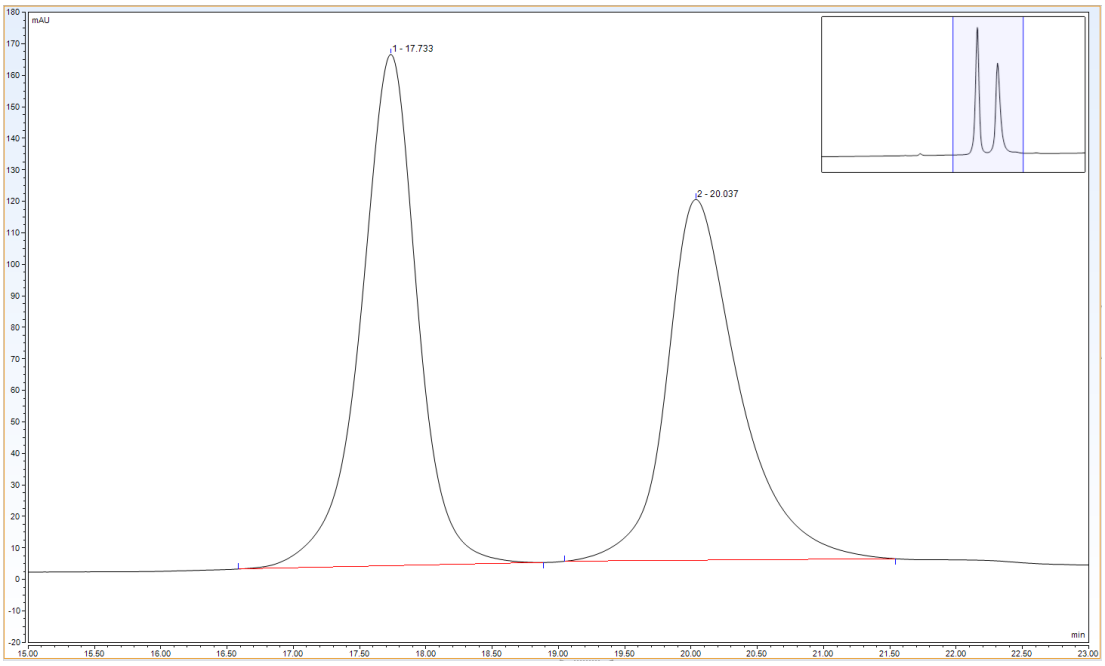
(*S*)-(+)-2-Hydroxy-2-(3-methoxyphenyl)acetic acid ((*S*)-2i). 3-Methoxyphenyl glyoxal hydrate (**1i·H₂O**, 100 mg, 0.55 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 3 ml phosphate buffer solution containing 2.55 µmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 257 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0.02) as eluent followed by trituration with pentane afforded pure **(*S*)-2i** (71 mg, 0.39 mmol, 71%) as beige solid. ***ee*** = 96 % [HPLC]. $[\alpha]^{27}_D$ = +114 (c = 0.34, EtOAc). ***mp:*** 76-79 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-hydroxy-2-(3-methoxyphenyl)acetic acid (**(*S*)-2i**) obtained with 0.125 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



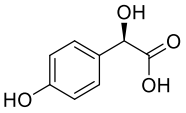
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	17.913	153.6581	97.87
2	20.623	3.3425	2.13

Chiral UHPLC chromatogram of nearly racemic 2-hydroxy-2-(3-methoxyphenyl)acetic acid (**2i**) obtained with 0.125 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	17.733	80.6437	52.63
2	20.037	72.5983	47.37

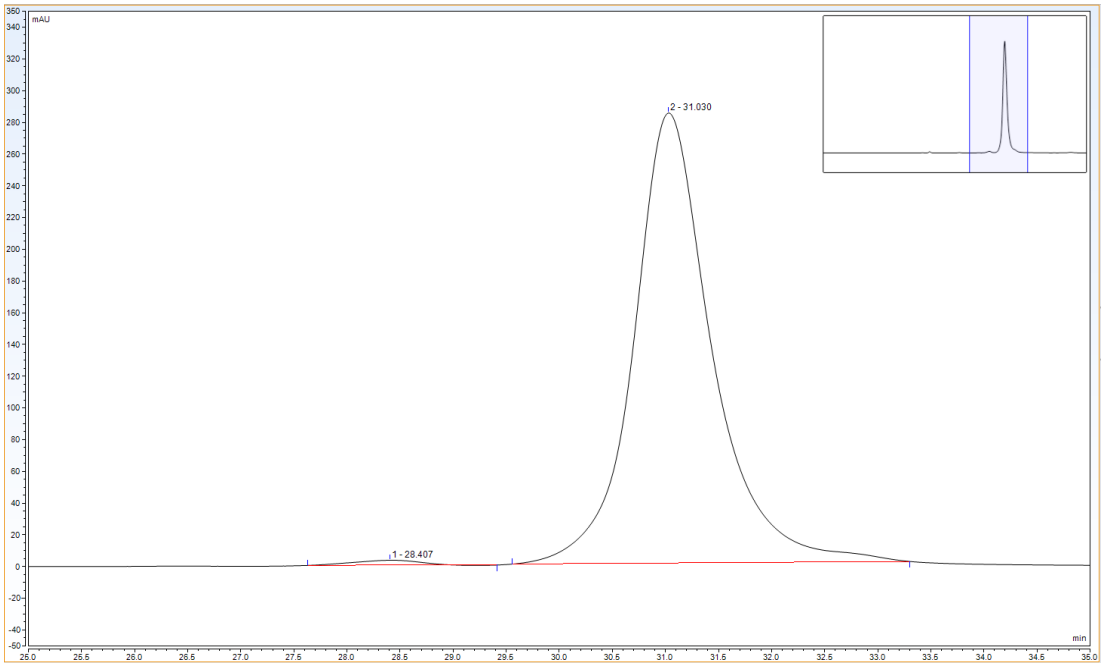
^{1}H NMR (300 MHz, DMSO-D6): δ 7.30 – 7.18 (m, 1H), 7.05 – 6.90 (m, 2H), 6.89 – 6.78 (m, 1H), 5.82 (bs, 1H), 4.99 (s, 1H), 3.74 (s, 3H). ***$^{13}C\{^{1}H\}$ NMR (75 MHz, DMSO-D6):*** δ 173.9, 159.1, 141.7, 129.1, 118.9, 113.0, 112.2, 72.3, 55.0; ***HRMS (ESI, [M-H]$^{-}$)*** for $C_9H_9O_4^-$ calcd 181.0506, found 181.0505.



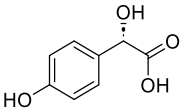
(*R*)-2j

(*R*)-(-)-2-Hydroxy-2-(4-hydroxyphenyl)acetic acid ((*R*)-2j). 4-Hydroxyphenyl glyoxal hydrate (**1j·H₂O**, 100 mg, 0.59 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 690 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (29.5 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 286 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0.02) as eluent followed by trituration with pentane afforded pure **(*R*)-2j** (55 mg, 0.33 mmol, 55%) as white solid. ***ee*** = 98 % [HPLC]. ***[α]$^{27}_{D}$*** = -139 (c = 0.33, MeOH). ***mp:*** 103-106 °C.

Chiral UHPLC chromatogram of (*R*)-(-)-2-hydroxy-2-(4-hydroxyphenyl)acetic acid (**(*R*)-2j**) obtained with 0.07 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



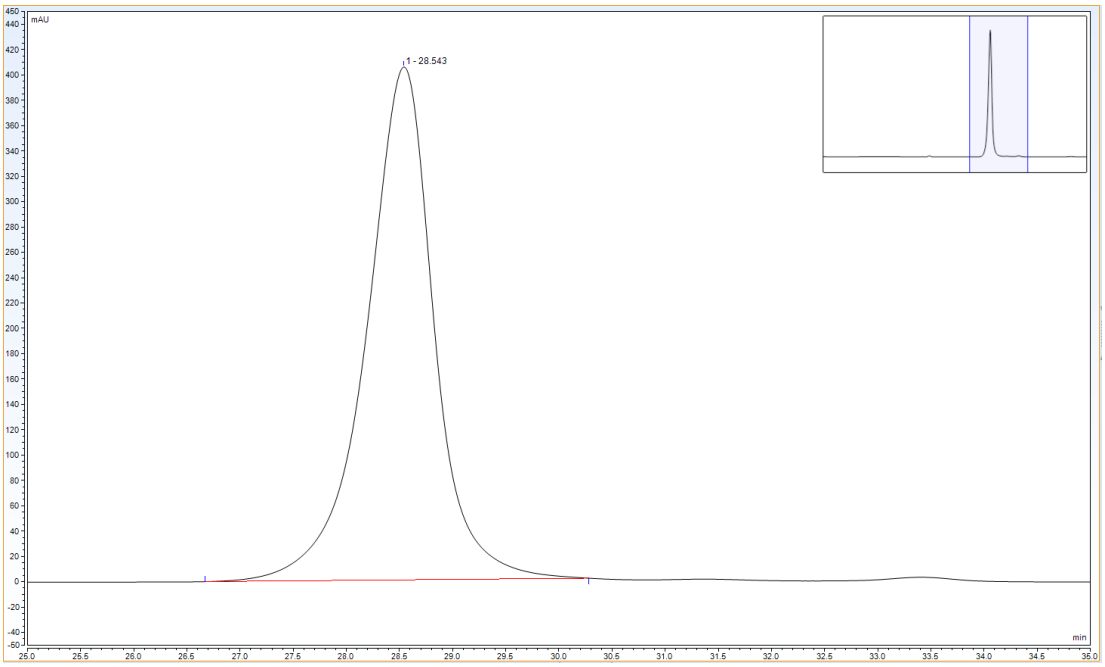
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	28.407	2.2138	0.90
2	31.03	242.7675	99.10



(*S*)-2j

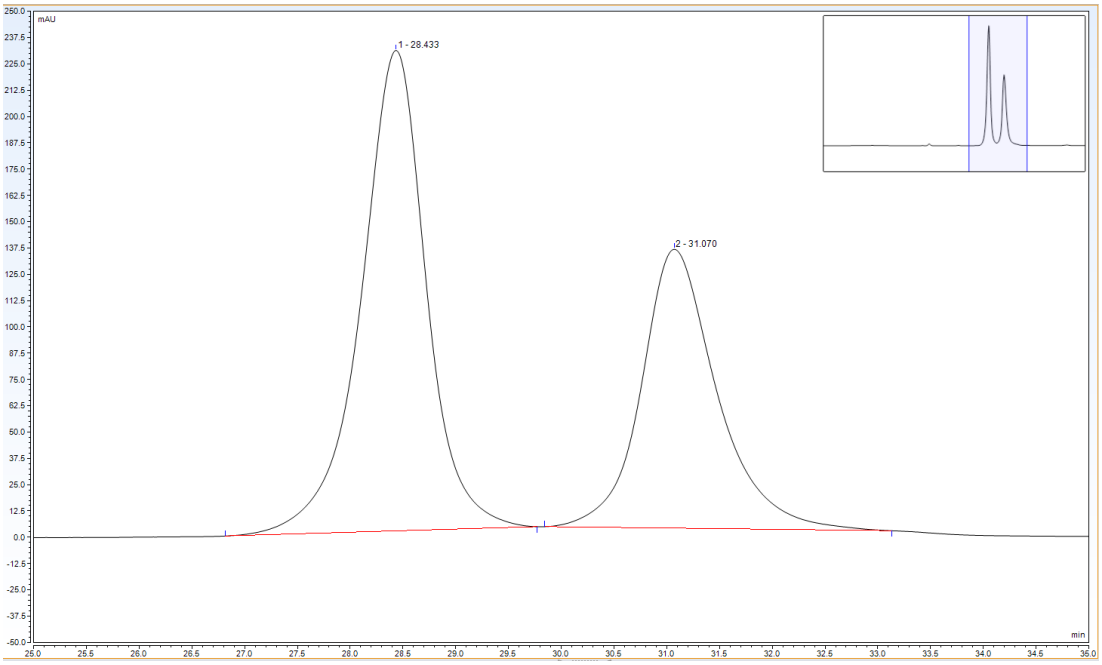
(*S*)-(+)-2-Hydroxy-2-(4-hydroxyphenyl)acetic acid ((*S*)-2j). 4-Hydroxyphenyl glyoxal hydrate (**1j·H₂O**, 100 mg, 0.59 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 5 ml phosphate buffer solution containing 2.8 µmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 286 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0.02) as eluent followed by trituration with pentane afforded pure **(*S*)-2j** (56 mg, 0.33 mmol, 56%) as beige solid. ***ee*** = >99 % [HPLC]. $[\alpha]^{27}_{D}$ = +143 (c = 0.34, MeOH). ***mp:*** 103-106 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-hydroxy-2-(4-hydroxyphenyl)acetic acid (**(*S*)-2j**) obtained with 0.07 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



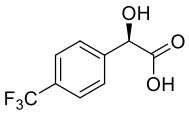
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	28.543	298.6673	100.00

Chiral UHPLC chromatogram of scalemic 2-hydroxy-2-(4-hydroxyphenyl)acetic acid (**2j**) obtained with 0.07 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	28.433	163.3574	59.69
2	31.07	110.3318	40.31

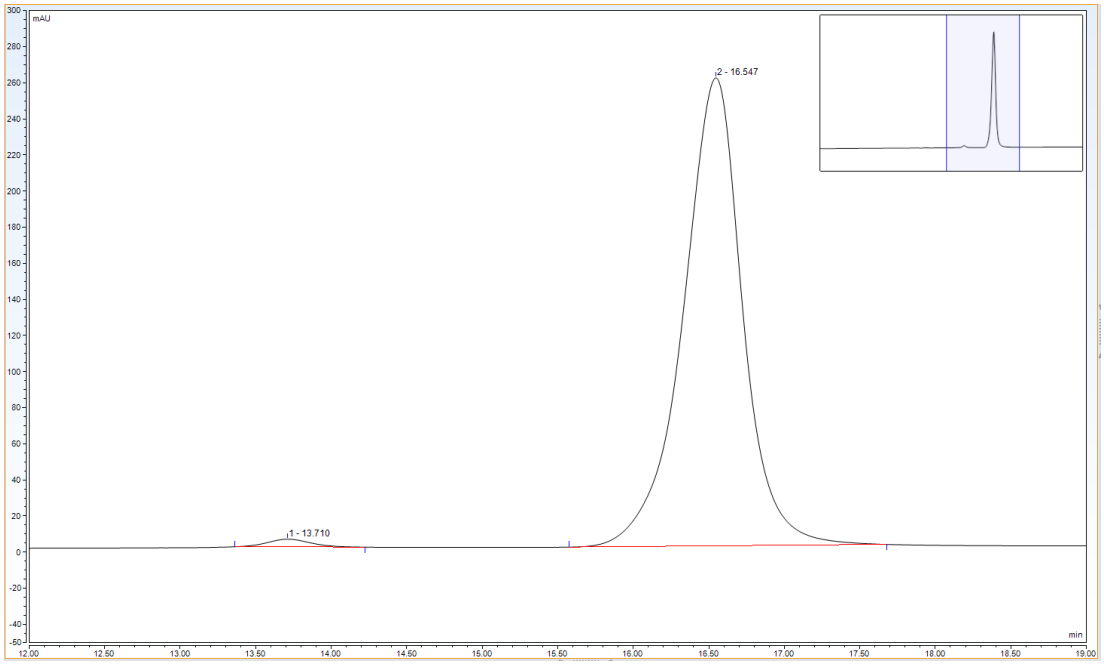
^{1}H NMR (300 MHz, DMSO-D6): δ 9.32 (bs, 1H), 7.18 (d, *J* = 8.4 Hz, 2H), 6.70 (d, *J* = 8.4 Hz, 2H), 4.83 (s, 1H). ***$^{13}C\{^{1}H\}$ NMR (75 MHz, DMSO-D6):*** δ 174.4, 156.9, 130.6, 127.9, 114.8, 72.0; ***HRMS (ESI, [M-H]$^-$)*** for $C_8H_7O_4^-$ calcd 167.0350, found 167.0348.



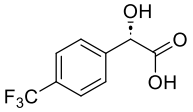
(*R*)-2k

(*R*)-(-)-2-Hydroxy-2-(4-(trifluoromethyl)phenyl)acetic acid ((*R*)-2k).[5] 4-(Trifluoromethyl)phenyl glyoxal hydrate (**1k·H₂O**, 100 mg, 0.45 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 550 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (22.5 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 243 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with cold pentane to afford pure **(*R*)-2k** (91 mg, 0.41 mmol, 91%) as beige solid. ***ee*** = 98 % [UHPLC]. ***[α]$^{27}_D$*** = -99 (c = 0.36, EtOAc). ***mp:*** 135-138 °C.

Chiral UHPLC chromatogram of (*R*)-(-)-2-hydroxy-2-(4-(trifluoromethyl)phenyl)acetic acid (**(*R*)-2k**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



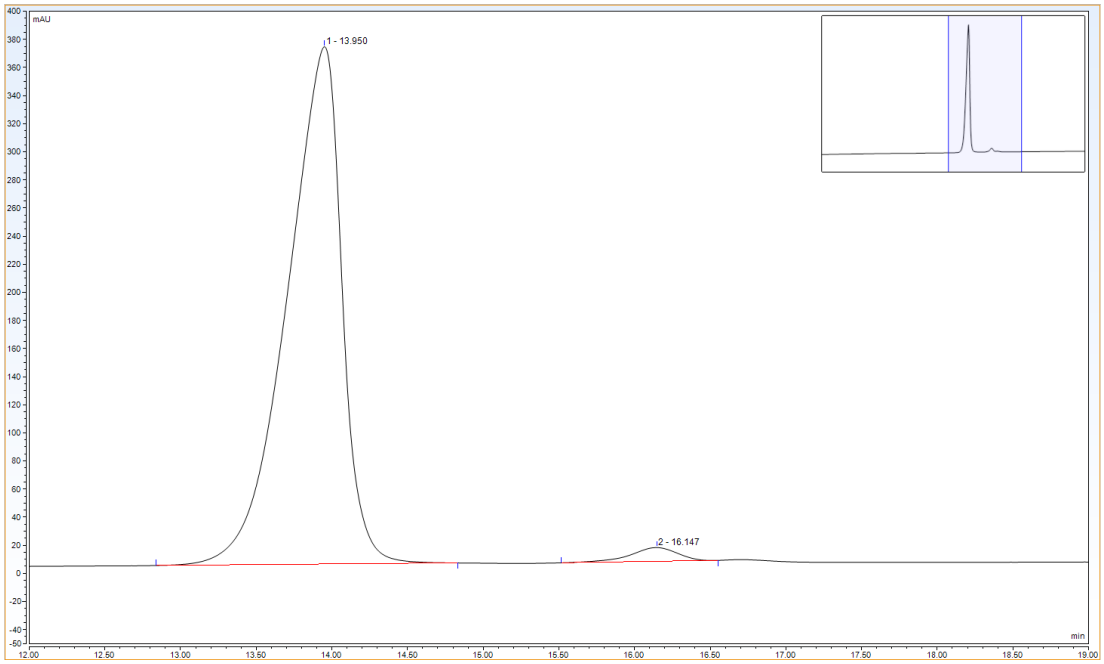
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	13.71	1.424	1.19
2	16.547	118.3575	98.81



(*S*)-2k

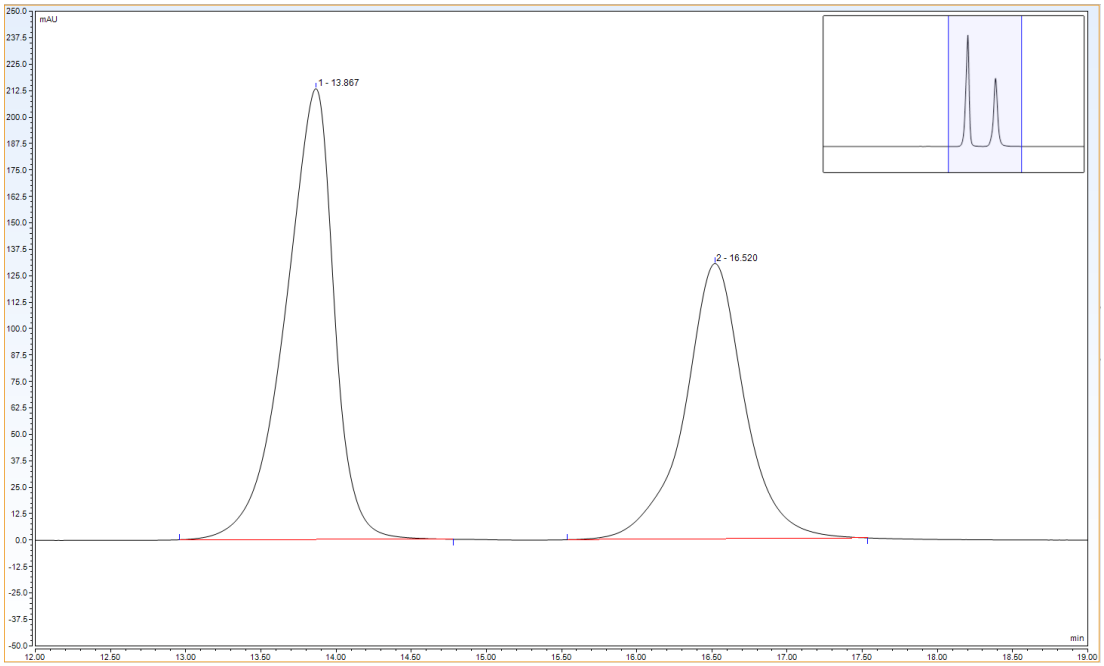
(*S*)-(+)-2-Hydroxy-2-(4-(trifluoromethyl)phenyl)acetic acid ((*S*)-2k). 4-(Trifluoromethyl)phenyl glyoxal hydrate (**1k·H₂O**, 100 mg, 0.45 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7)with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 0.553 ml phosphate buffer solution containing 602 nmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 243 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0→9:1:0.02) as eluent followed by trituration with cold pentane afforded pure **(*S*)-2k** (83 mg, 0.38 mmol, 83%) as white solid. ***ee*** = 96 % [UHPLC]. $[\alpha]^{27}_D$ = +105 (c = 0.38, EtOAc). ***mp:*** 140-143 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-hydroxy-2-(4-(trifluoromethyl)phenyl)acetic acid (**(*S*)-2k**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



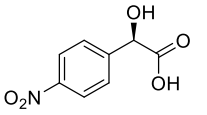
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	13.95	154.1043	97.76
2	16.147	3.5251	2.24

Chiral UHPLC chromatogram of nearly racemic 2-hydroxy-2-(4-(trifluoromethyl)phenyl)acetic acid (**2k**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	13.867	80.3479	57.16
2	16.52	60.2265	42.84

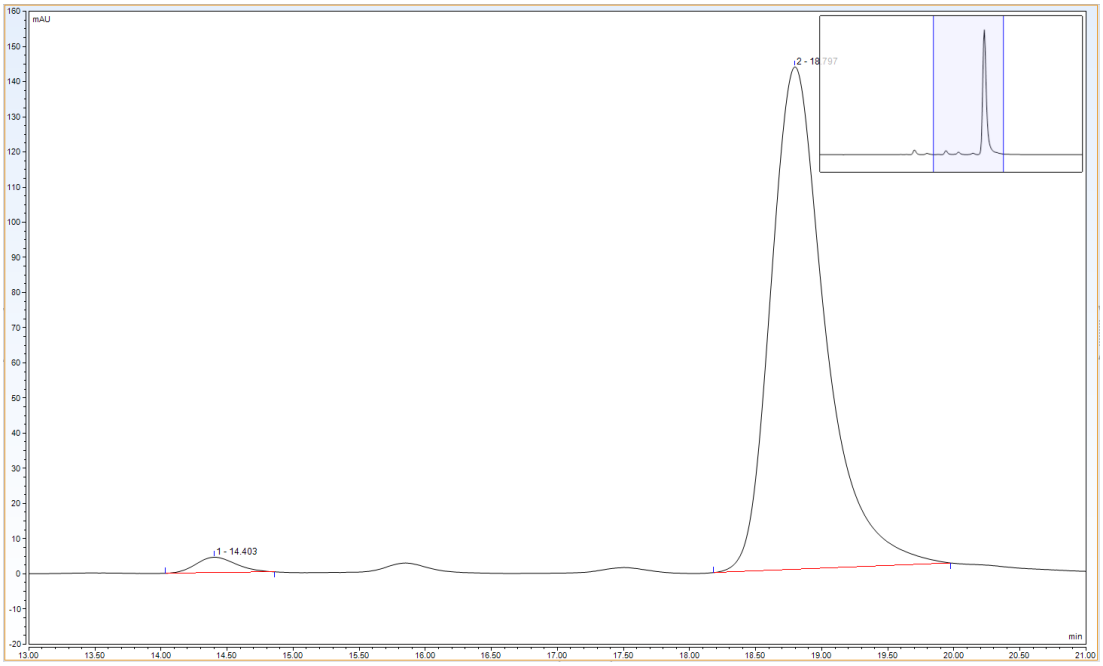
^{1}H NMR (300 MHz, DMSO-D6): δ 7.71 (d, *J* = 8.3 Hz, 2H), 7.64 (d, *J* = 8.3 Hz, 2H), 5.14 (s, 1H). ***^{13}C{^{1}H} NMR (75 MHz, DMSO-D6):*** δ 173.4, 144.9, 128.2 (q, *J* = 31.7 Hz), 127.3, 125.0 (q, *J* = 3.8 Hz), 124.2 (q, *J* = 271.9 Hz), 71.8; ***^{19}F{^{1}H} NMR (282 MHz, DMSO-D6):*** δ -63.2 (s). ***HRMS (ESI, [M-H]$^{-}$)*** for $C_9H_6F_3O_3^-$ calcd 219.0275, found 219.0273.



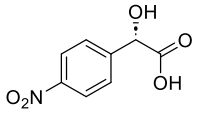
(*R*)-2l

(*R*)-(-)-2-Hydroxy-2-(4-nitrophenyl)acetic acid ((*R*)-2l).[5] 4-Nitrophenyl glyoxal hydrate (**1l·H$_2$O**, 100 mg, 0.51 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 610 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (25.5 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the shift in the UV/vis absorbance maximum to $\lambda_{max,\ product}$ = 280 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0.02) as eluent followed by trituration with pentane afforded pure **(*R*)-2l** (82 mg, 0.42 mmol, 82%) as orange solid. ***ee*** = 96 % [UHPLC]. ***[α]$^{27}_{D}$*** = -117 (c = 0.26, MeOH). ***mp:*** 74-78 °C.

Chiral UHPLC chromatogram of (*R*)-(-)-2-hydroxy-2-(4-nitrophenyl)acetic acid (**(*R*)-2l**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



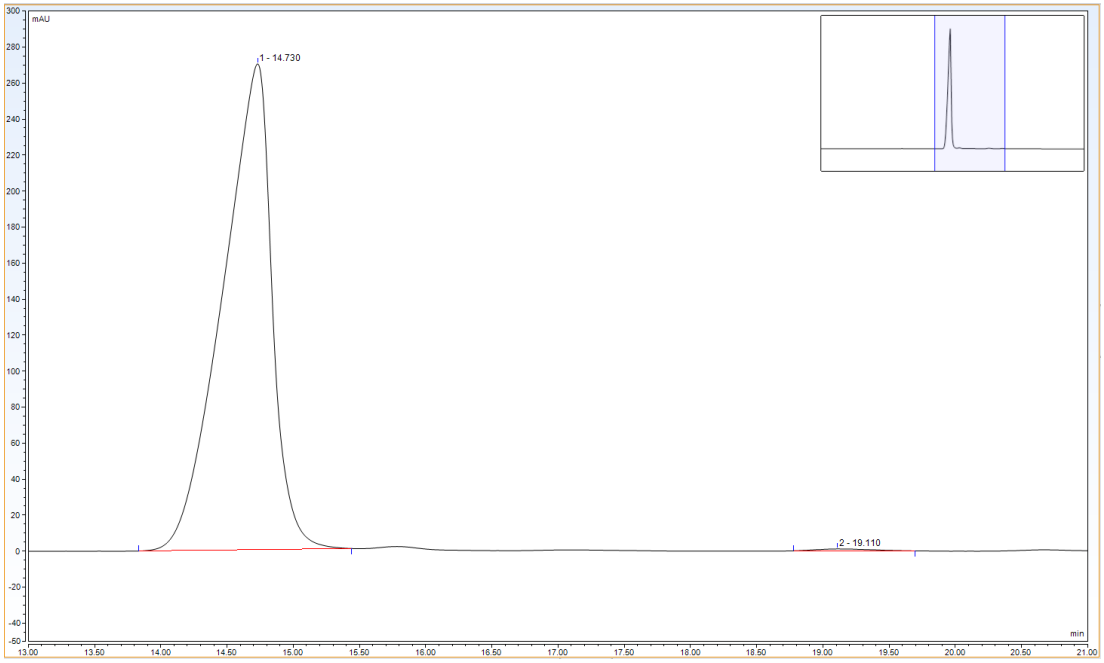
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	14.403	1.5313	2.19
2	18.797	68.4355	97.81



(S)-2l

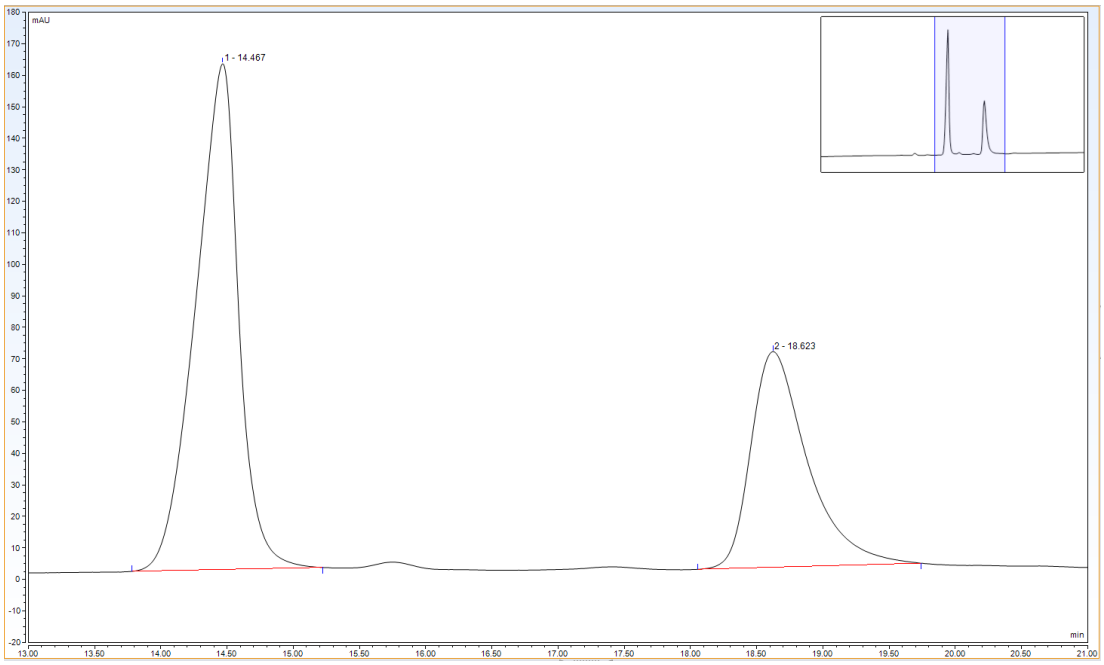
(*S*)-(+)-2-Hydroxy-2-(4-nitrophenyl)acetic acid ((*S*)-2l). 4-Nitrophenyl glyoxal hydrate (**1l·H₂O**, 100 mg, 0.51 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool, mixed with 51 µmol β-mercaptoethanol solution in sodium phosphate buffer (50 mM, pH = 7) and after 5 minutes was mixed with 1.5 ml phosphate buffer solution containing 654 nmol DJ-1. Upon completion of the reaction as was determined by monitoring the shift in the UV/vis absorbance maximum to $\lambda_{max,\ product}$ = 280 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0.02) as eluent followed by trituration with pentane afforded pure **(*S*)-2l** (74 mg, 0.38 mmol, 74%) as yellow solid. ***ee*** = 99 % [UHPLC]. $[\alpha]^{27}_{D}$ = +140 (c = 0.35, MeOH). ***mp:*** 92-95 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-hydroxy-2-(4-nitrophenyl)acetic acid (**(*S*)-2l**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



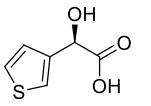
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	14.73	116.6019	99.57
2	19.11	0.5045	0.43

Chiral UHPLC chromatogram of scalemic 2-hydroxy-2-(4-nitrophenyl)acetic acid (**2l**) obtained with 0.125 ml/min flow rate, 20°C column oven and sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	14.467	60.3685	64.02
2	18.623	33.932	35.98

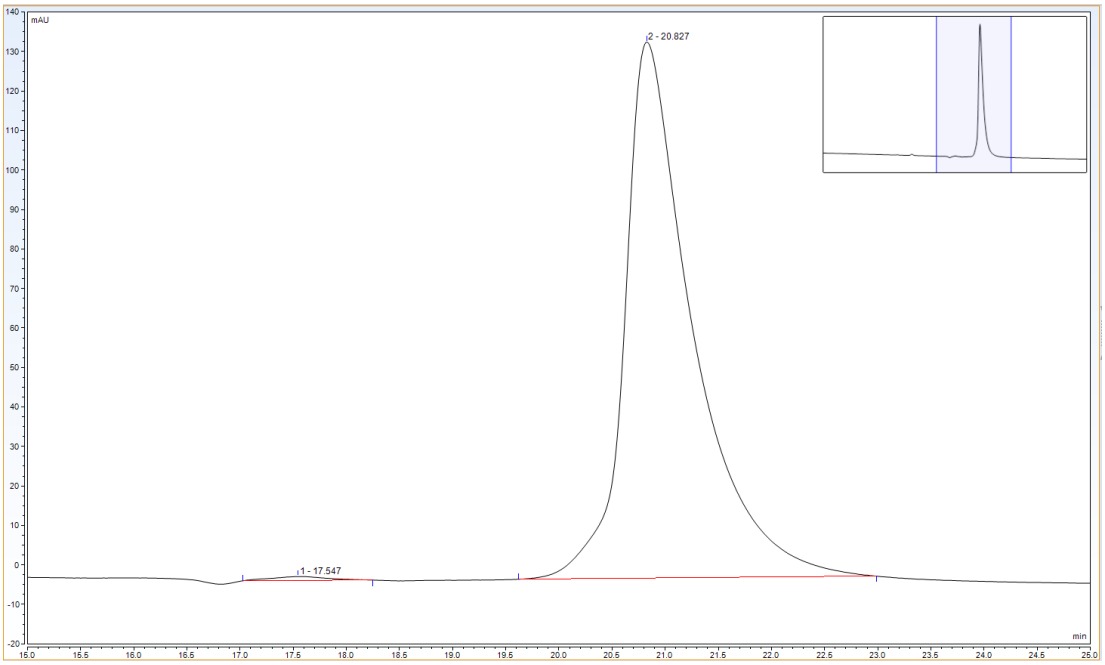
^{1}H NMR (300 MHz, DMSO-D6): δ 8.19 (d, *J* = 8.5 Hz, 2H), 7.70 (d, *J* = 8.6 Hz, 2H), 5.08 (s, 1H). ***$^{13}C\{^{1}H\}$ NMR (75 MHz, DMSO-D6):*** δ 173.0, 147.8, 146.9, 127.7, 123.3, 71.7; ***HRMS (ESI, [M-H]$^{-}$)*** for $C_8H_6NO_5^-$ calcd 196.0251, found 196.0245.



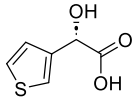
(*R*)-2m

(*R*)-(-)-2-Hydroxy-2-(thiophen-3-yl)acetic acid ((*R*)-2m). Thiophen-3-yl glyoxal hydrate (**1m·H$_2$O**, 100 mg, 0.63 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 730 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (31.5 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 260 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. Column chromatography[6] with DCM/MeOH/HCOOH (9:1:0.02) as eluent followed by trituration with pentane afforded pure **(*R*)-2m** (90 mg, 0.57 mmol, 90%) as brownish solid. ***ee*** = 99 % [UHPLC]. ***[α]$^{27}_{D}$*** = -102 (c = 0.36, EtOAc). ***mp:*** 119-122 °C.

Chiral UHPLC chromatogram of (*R*)-(-)-2-hydroxy-2-(thiophen-3-yl)acetic acid ((***R*)-2m**) obtained with 0.125 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



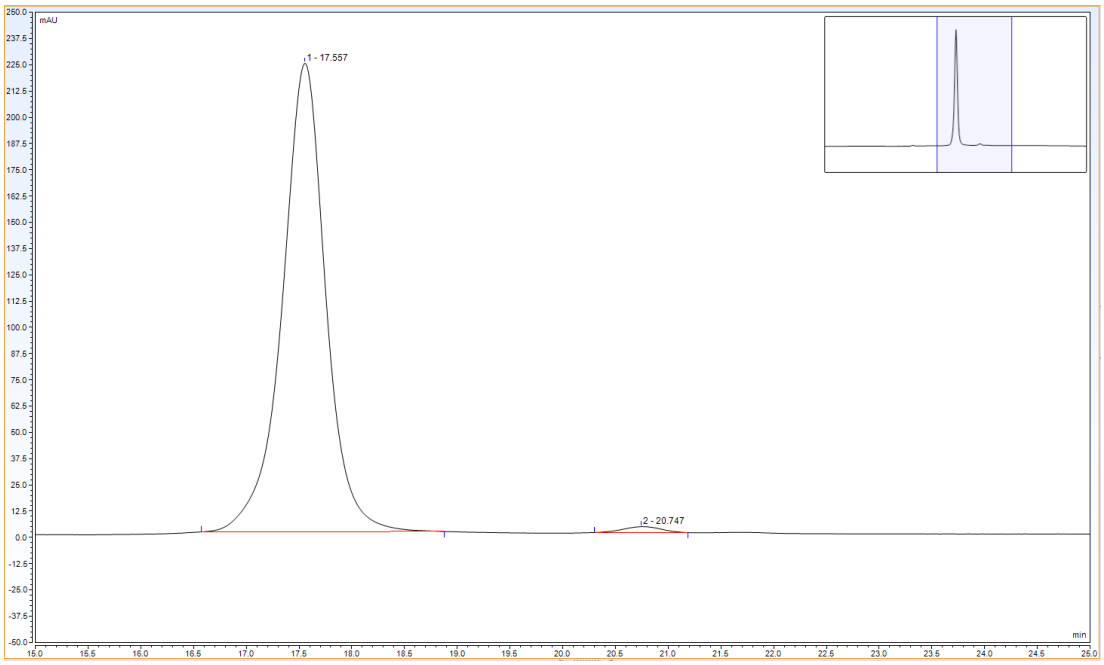
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	17.547	0.6268	0.62
2	20.827	101.0607	99.38



(*S*)-2m

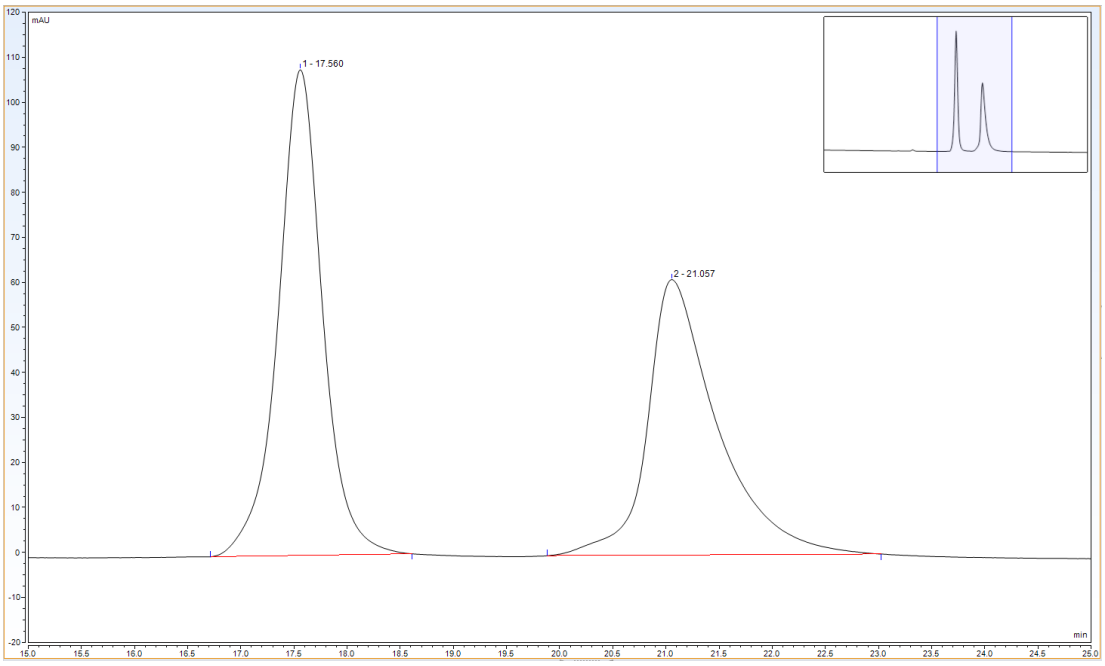
(*S*)-(+)-2-Hydroxy-2-(thiophen-3-yl)acetic acid ((*S*)-2m). Thiophen-3-yl glyoxal hydrate (**1m·H₂O**, 100 mg, 0.63 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 0.855 ml phosphate buffer solution containing 479 nmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 260 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*S*)-2m** (97 mg, 0.61 mmol, 97%) as brownish solid. ***ee*** = 98 % [UHPLC]. $[\alpha]^{27}_{D}$ = +104 (c = 0.34, EtOAc). ***mp:*** 116-119 °C.

Chiral UHPLC chromatogram of (*S*)-(+)-2-hydroxy-2-(thiophen-3-yl)acetic acid (**(*S*)-2m**) obtained with 0.125 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



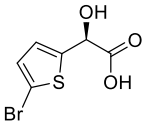
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	17.557	106.5547	98.91
2	20.747	1.178	1.09

Chiral UHPLC chromatogram of nearly racemic 2-hydroxy-2-(thiophen-3-yl)acetic acid (**2m**) obtained with 0.125 ml/min flow rate, 5°C column oven and 20°C sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	17.56	52.207	52.81
2	21.057	46.6434	47.19

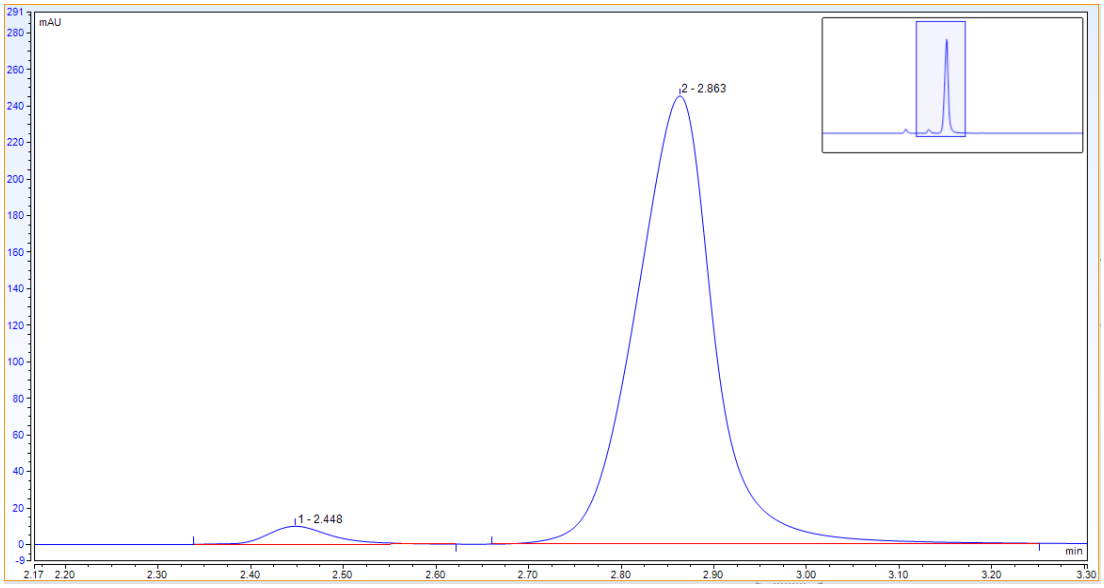
^{1}H NMR (300 MHz, DMSO-D6): δ 7.48 (dd, *J* = 5.0, 3.0 Hz, 1H), 7.44 – 7.39 (m, 1H), 7.10 (dd, *J* = 5.0, 1.1 Hz, 1H), 5.81 (bs, 1H), 5.09 (s, 1H). ***^{13}C{^{1}H} NMR (75 MHz, DMSO-D6):*** δ 173.7, 141.2, 126.5, 126.0, 122.2, 69.0; ***HRMS (ESI, [M-H]$^-$)*** for $C_6H_5O_3S^-$ calcd 156.9965, found 156.9963.



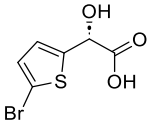
(*S*)-2n

(*S*)-(-)-2-(5-Bromothiophen-2-yl)-2-hydroxyacetic acid ((*S*)-2n). 5-Bromothiophen-2-yl glyoxal hydrate (**1n·H$_2$O**, 100 mg, 0.42 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 520 µl phosphate buffer solution containing glutathione, *E. coli* glyoxalase I and glyoxalase II (21 µmol, 7.85 nmol and 2.45 nmol respectively). Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 305 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*S*)-2n** (86 mg, 0.36 mmol, 86%) as brownish solid. ***ee*** = 94 % [UHPLC]. ***[α]$^{27}_D$*** = -31 (c = 0.36, EtOAc). ***mp:*** 104-107 °C.

Chiral UHPLC chromatogram of (*S*)-(-)-2-(5-bromothiophen-2-yl)-2-hydroxyacetic acid (**(*S*)-2n**) obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature:



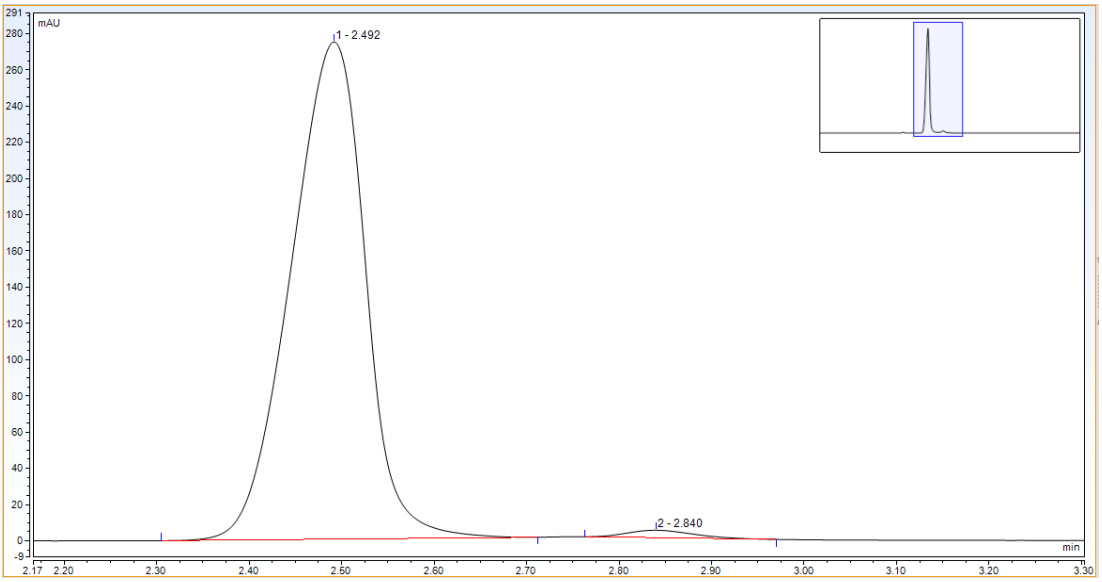
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.448	0.781	3.16
2	2.863	23.955	96.84



(*R*)-2n

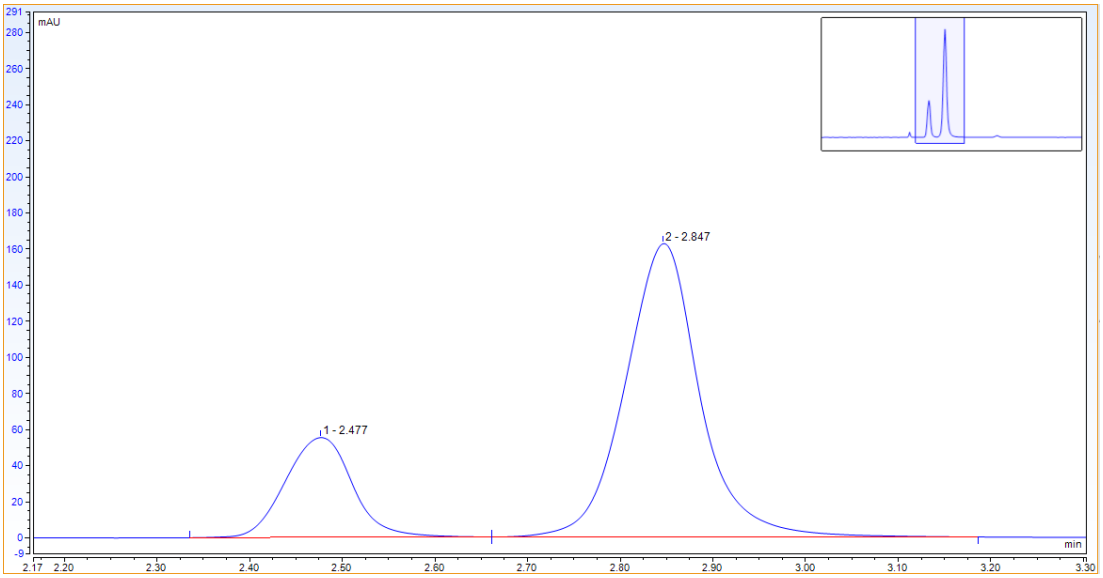
(*R*)-(+)-2-(5-Bromothiophen-2-yl)-2-hydroxyacetic acid ((*R*)-2n). 5-Bromothiophen-2-yl glyoxal hydrate (**1n·H$_2$O**, 100 mg, 0.42 mmol) was placed into a 250 mL glass flask and dissolved in sodium phosphate buffer (50 mM, pH = 7) with stirring and heating to obtain a 5 mM solution. The resulting solution was allowed to cool and was mixed with 0.105 ml phosphate buffer solution containing 59 nmol DJ-1. Upon completion of the reaction as was determined by monitoring the drop in the UV/vis absorbance at λ_{max} = 305 nm the reaction mixture was acidified with a few drops of 12M HCl to pH = 2. The resulting mixture was extracted with ethyl acetate and the organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo. The crude product was triturated with pentane to afford pure **(*R*)-2n** (94 mg, 0.40 mmol, 94%) as brownish solid. ***ee*** = 97 % [UHPLC]. ***[α]$^{27}_D$*** = +39 (c = 0.54, EtOAc). ***mp:*** 106-109 °C.

Chiral UHPLC chromatogram of (*R*)-(+)-2-(5-bromothiophen-2-yl)-2-hydroxyacetic acid (**(*R*)-2n**) obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature:



Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.492	25.502	98.67
2	2.840	0.343	1.33

Chiral UHPLC chromatogram of scalemic 2-(5-bromothiophen-2-yl)-2-hydroxyacetic acid (**2n**) obtained with 0.8 ml/min flow rate, 30°C column oven and 5°C sample holder temperature:

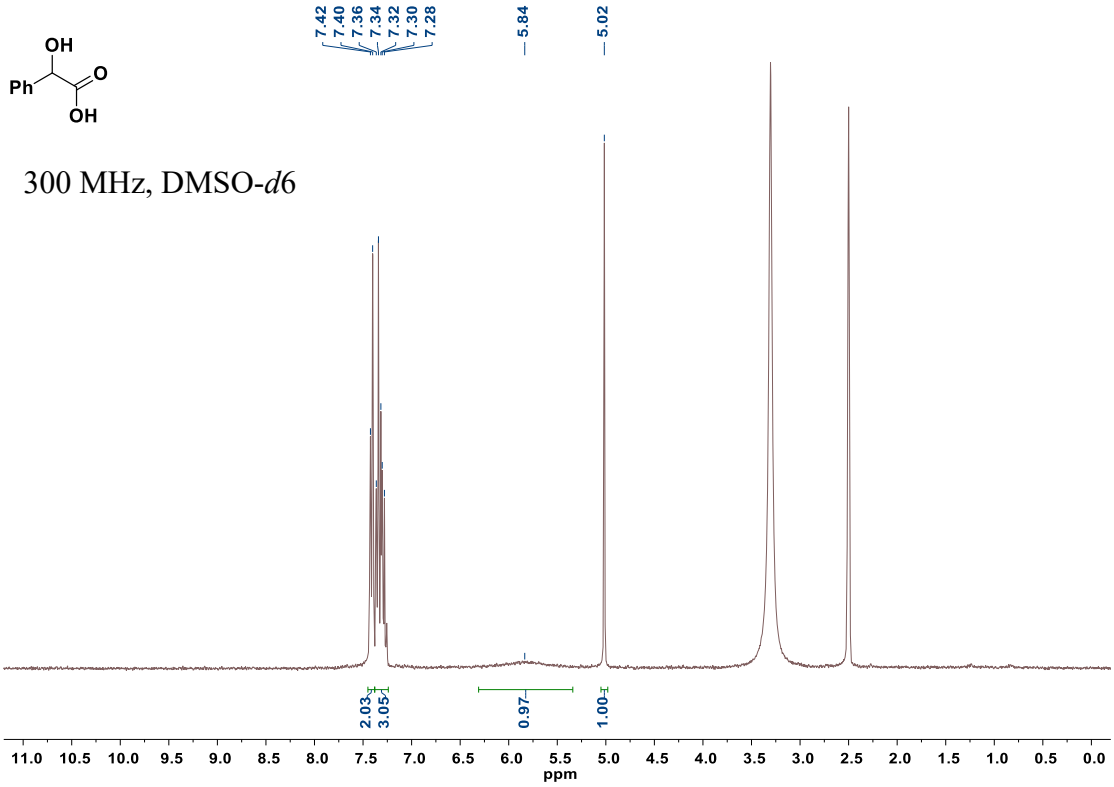


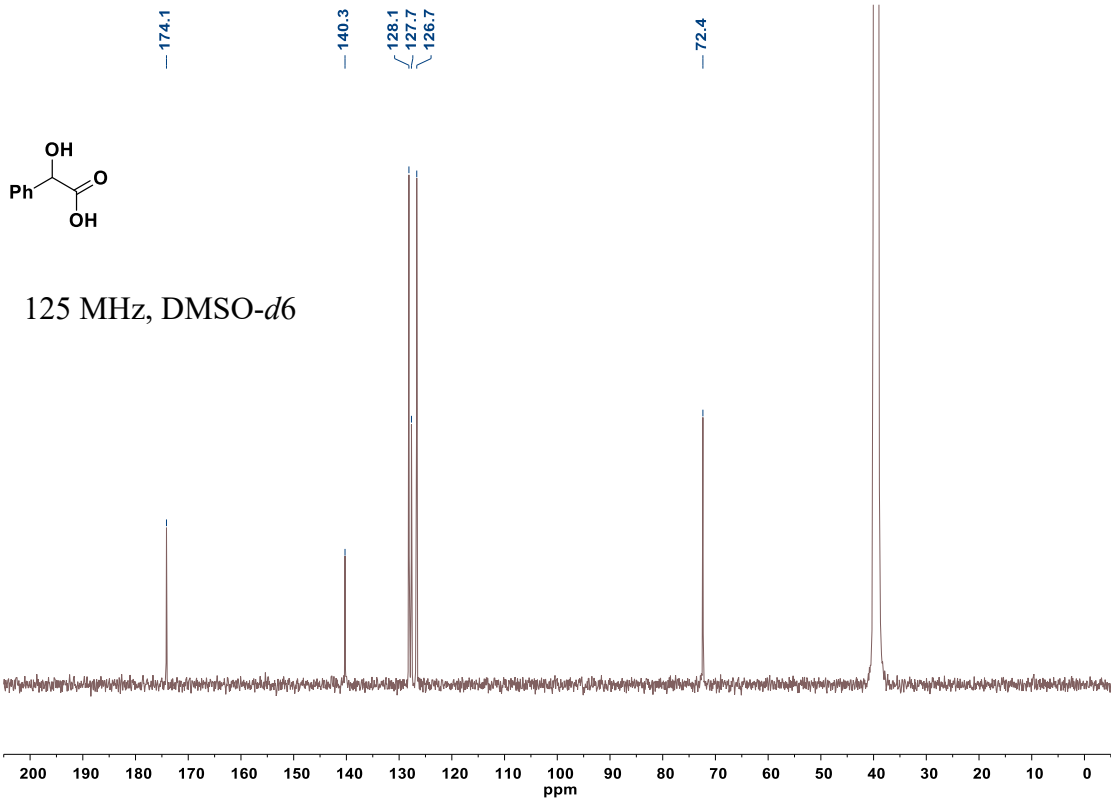
Peak No.	Retention Time (RT), min	Area (AA), mAU*min	Relative Area, %
1	2.477	4.637	23.45
2	2.847	15.134	76.55

^{1}H NMR (300 MHz, DMSO-D6): δ 7.08 (d, *J* = 3.8 Hz, 1H), 6.90 (dd, *J* = 3.8, 1.0 Hz, 1H), 6.29 (bs, 1H), 5.24 (d, *J* = 0.8 Hz, 1H). ***^{13}C{^{1}H} NMR (75 MHz, DMSO-D6):*** δ 172.5, 145.4, 129.8, 125.6, 110.6, 68.7; ***HRMS (ESI, [M-H]$^{-}$)*** for $C_6H_4BrO_3S^-$ calcd 234.9070, found 234.9062.

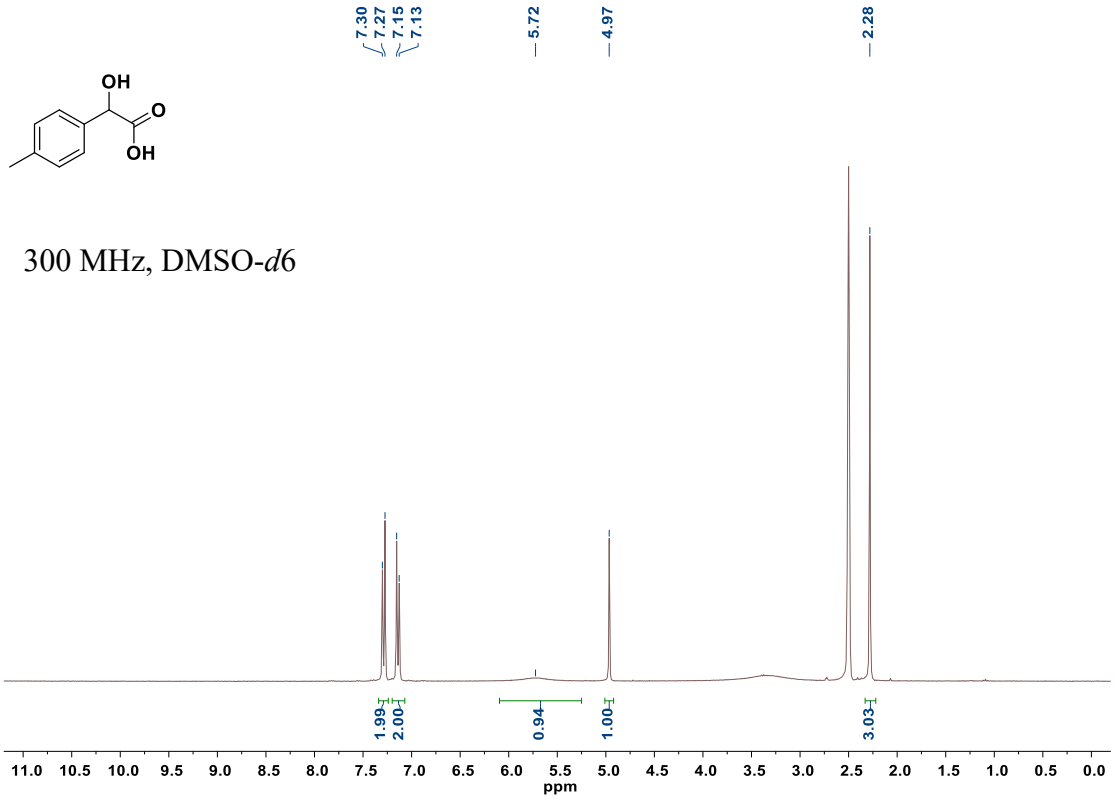
Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compounds 2

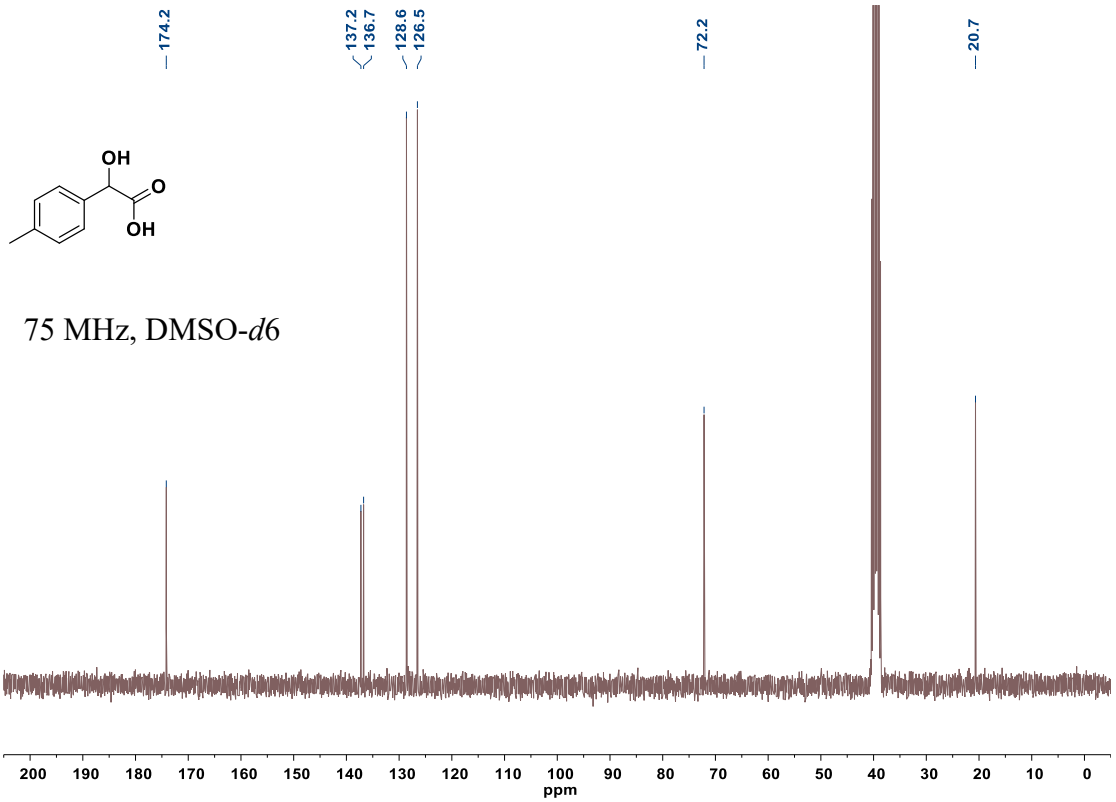
Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compound **2b**



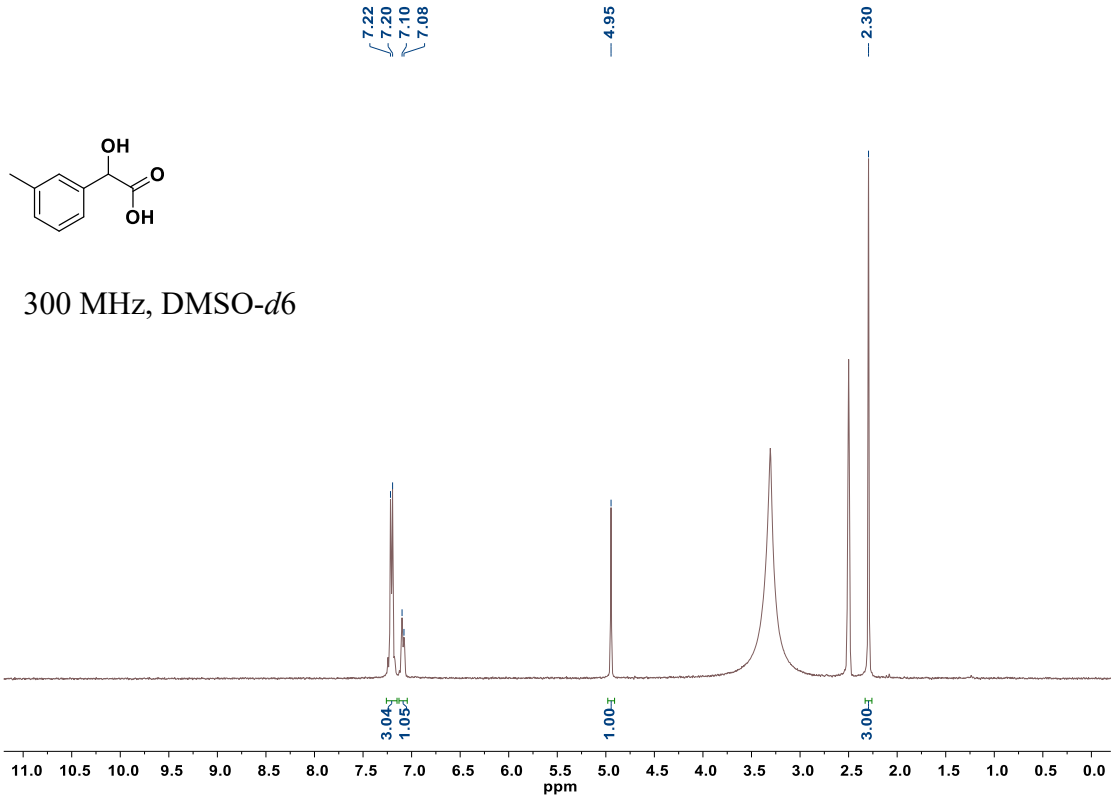


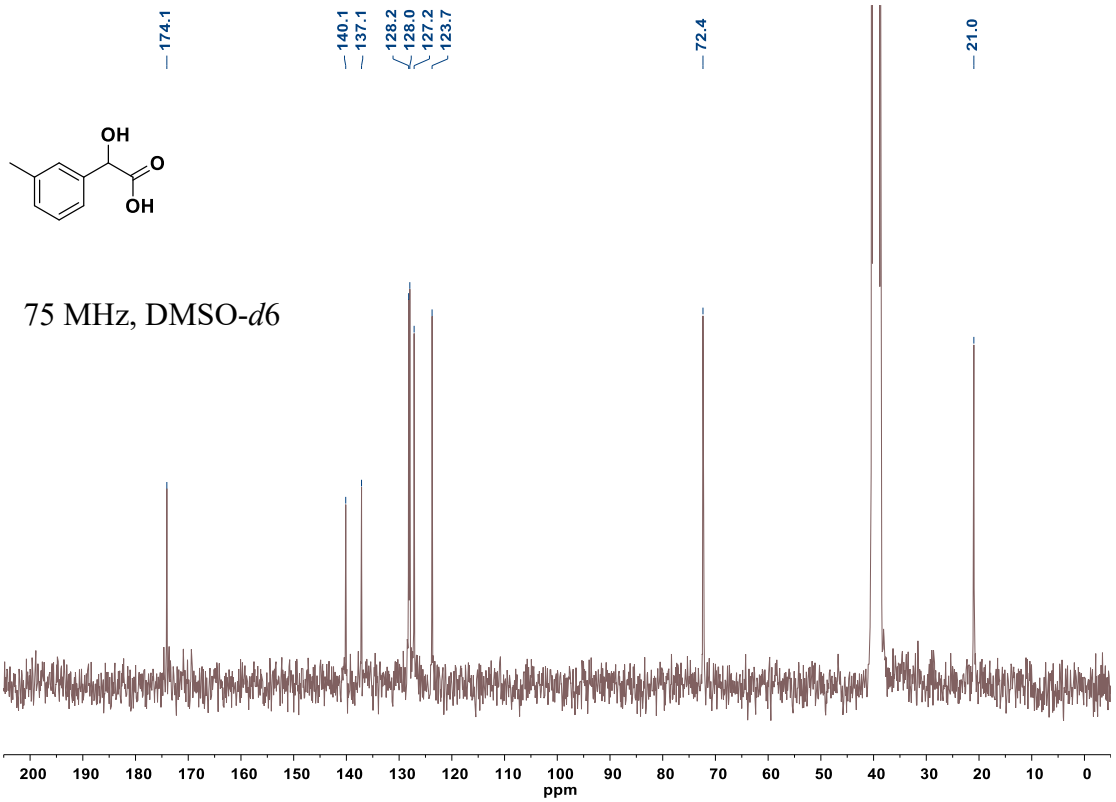
Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compound **2c**



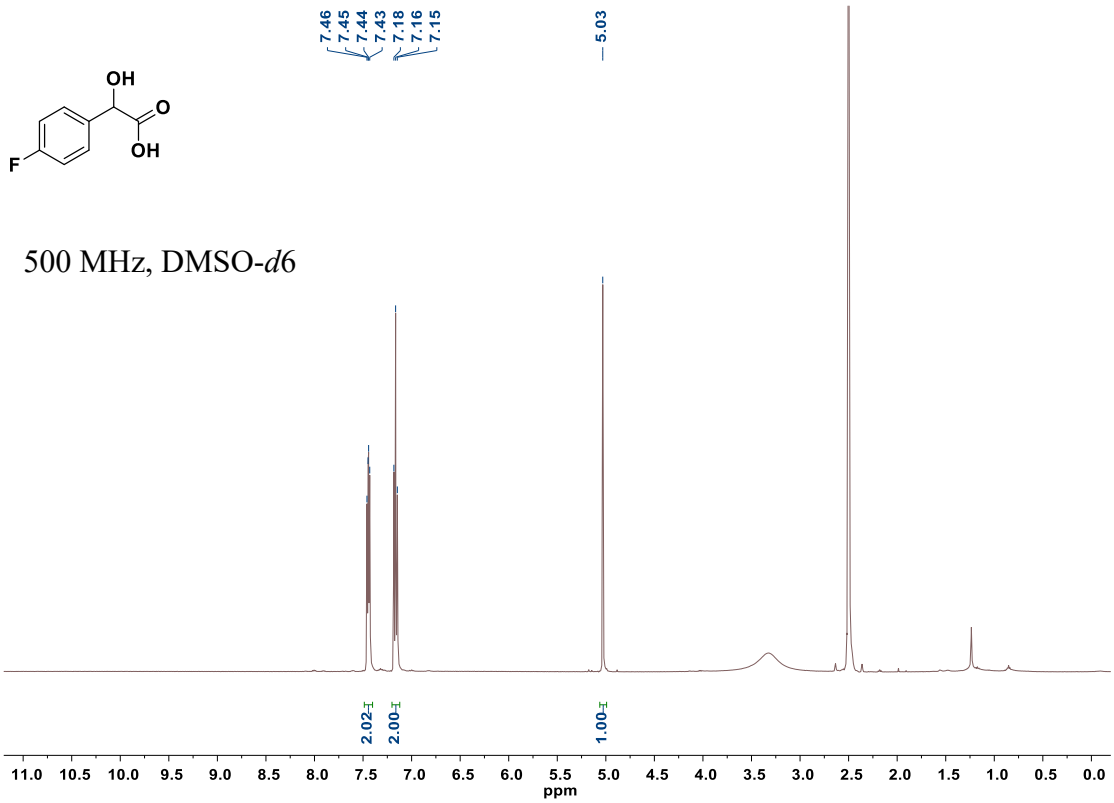


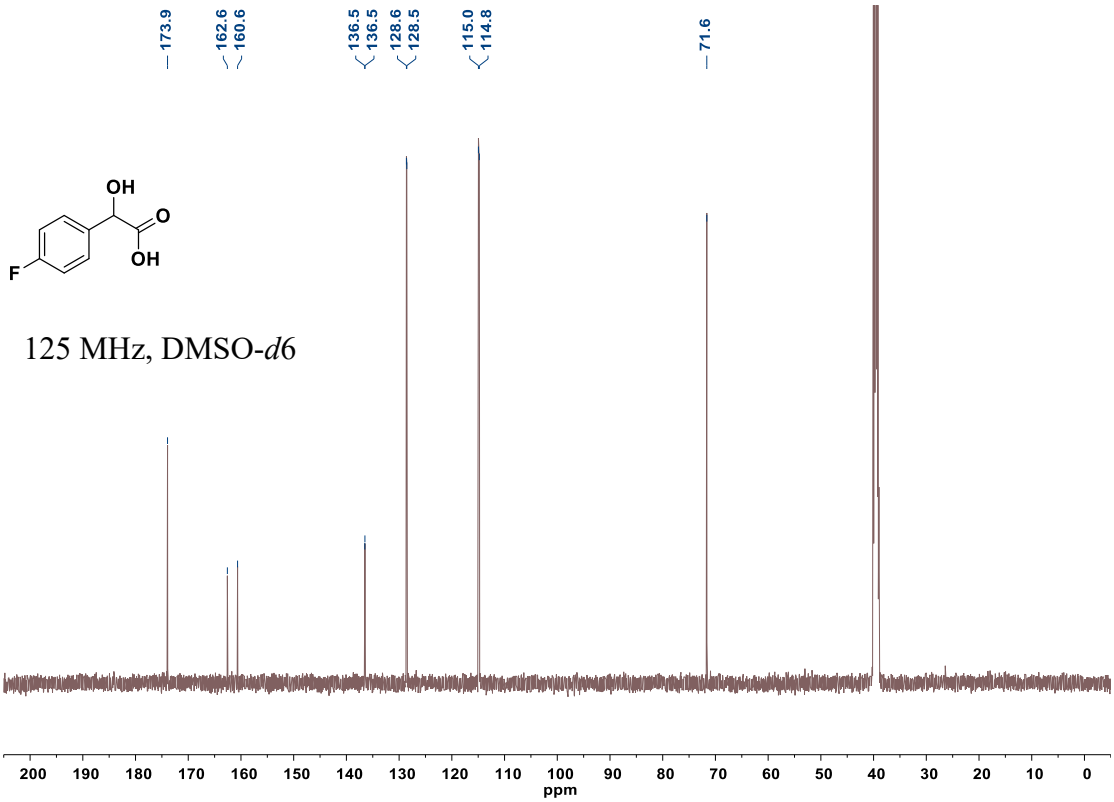
Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compound **2d**

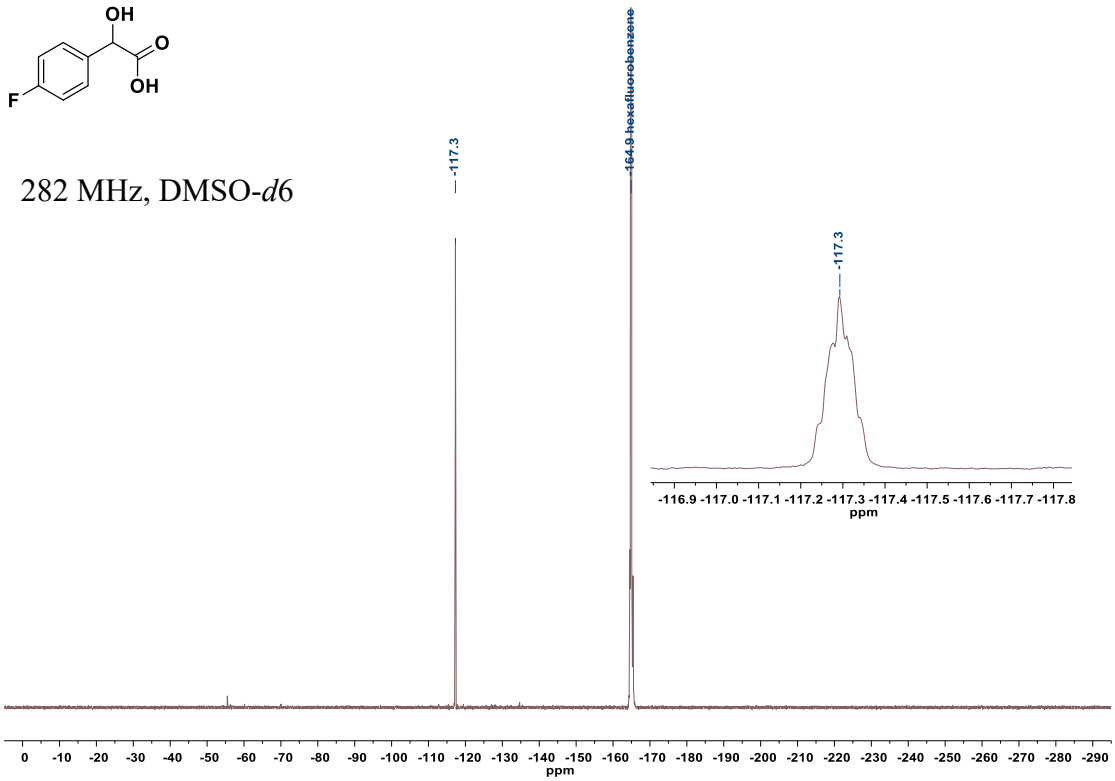




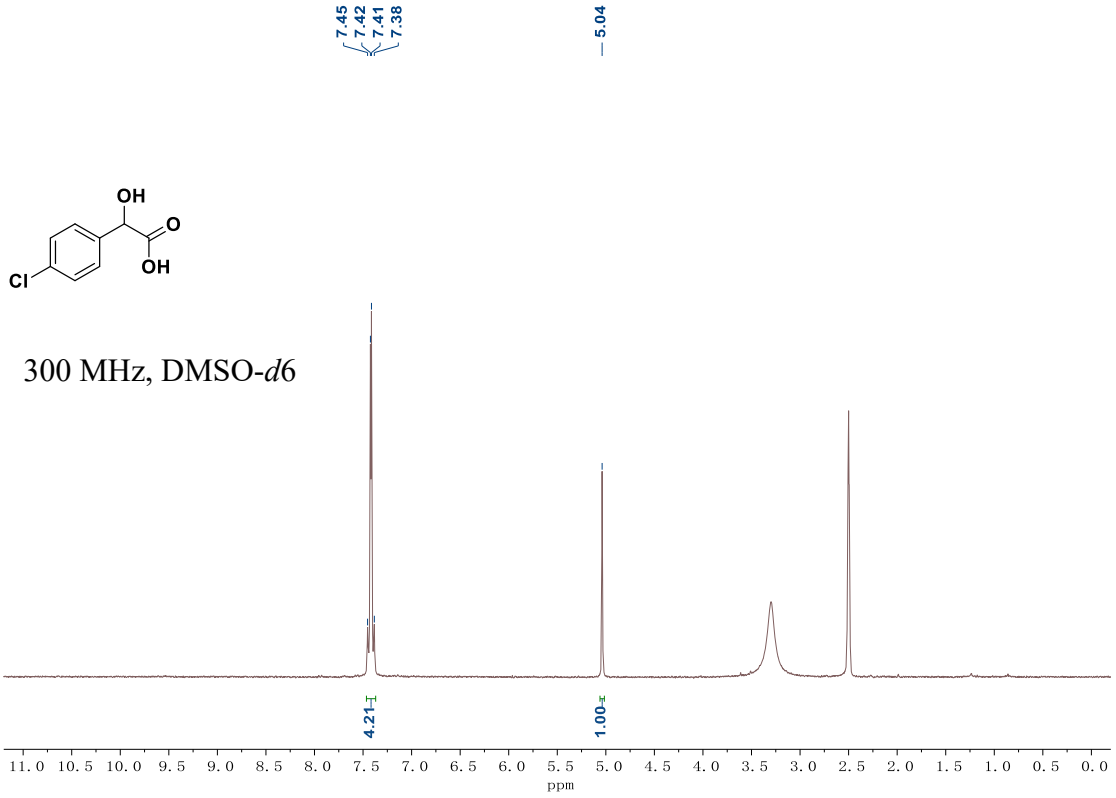
Copies of ^{1}H, $^{13}C\{^{1}H\}$ and $^{19}F\{^{1}H\}$ spectra of compound **2e**

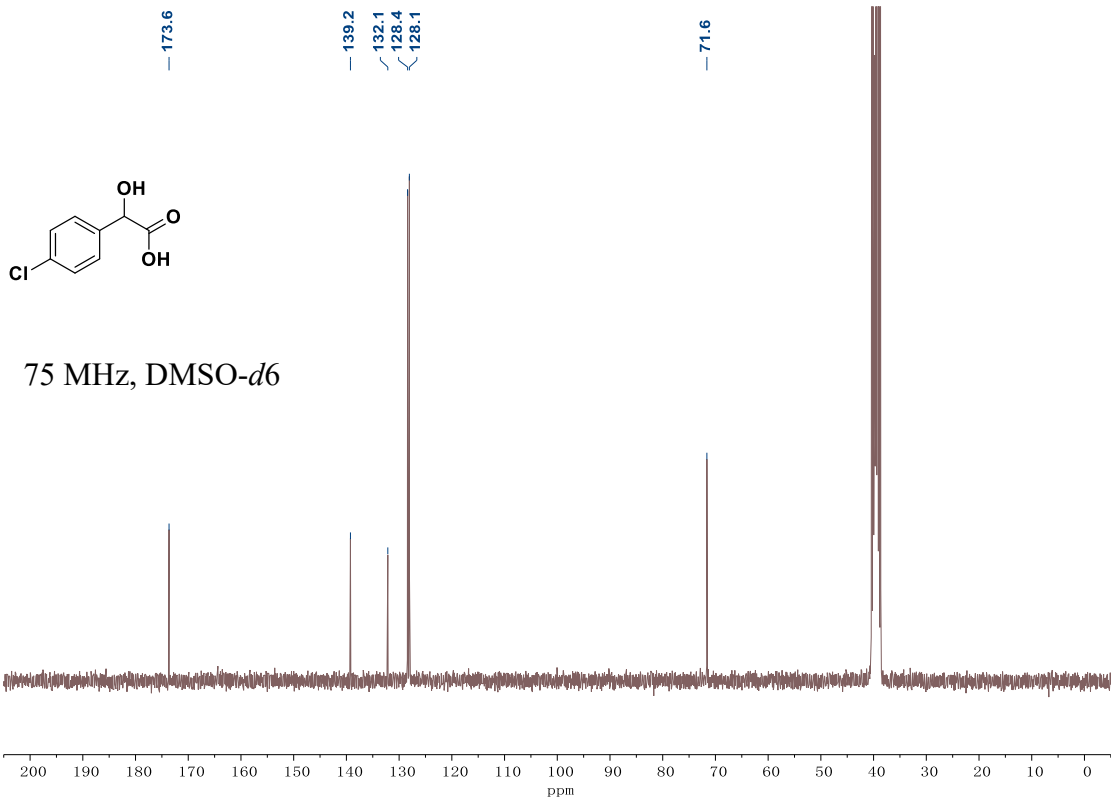




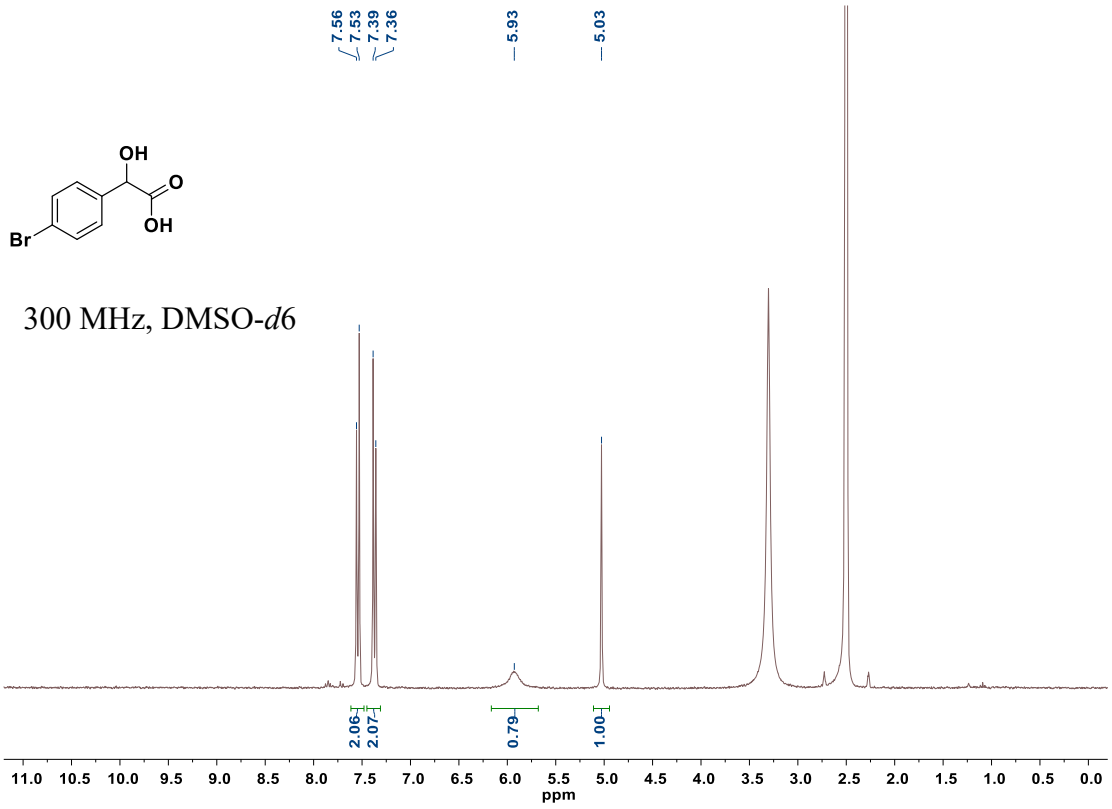

OH
O
OH
F
282 MHz, DMSO-d6
-117.3
-164.9 hexafluorobenzene
-117.3
-116.9 -117.0 -117.1 -117.2 -117.3 -117.4 -117.5 -117.6 -117.7 -117.8
ppm
0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240 -250 -260 -270 -280 -290
ppm

Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compound **2f**

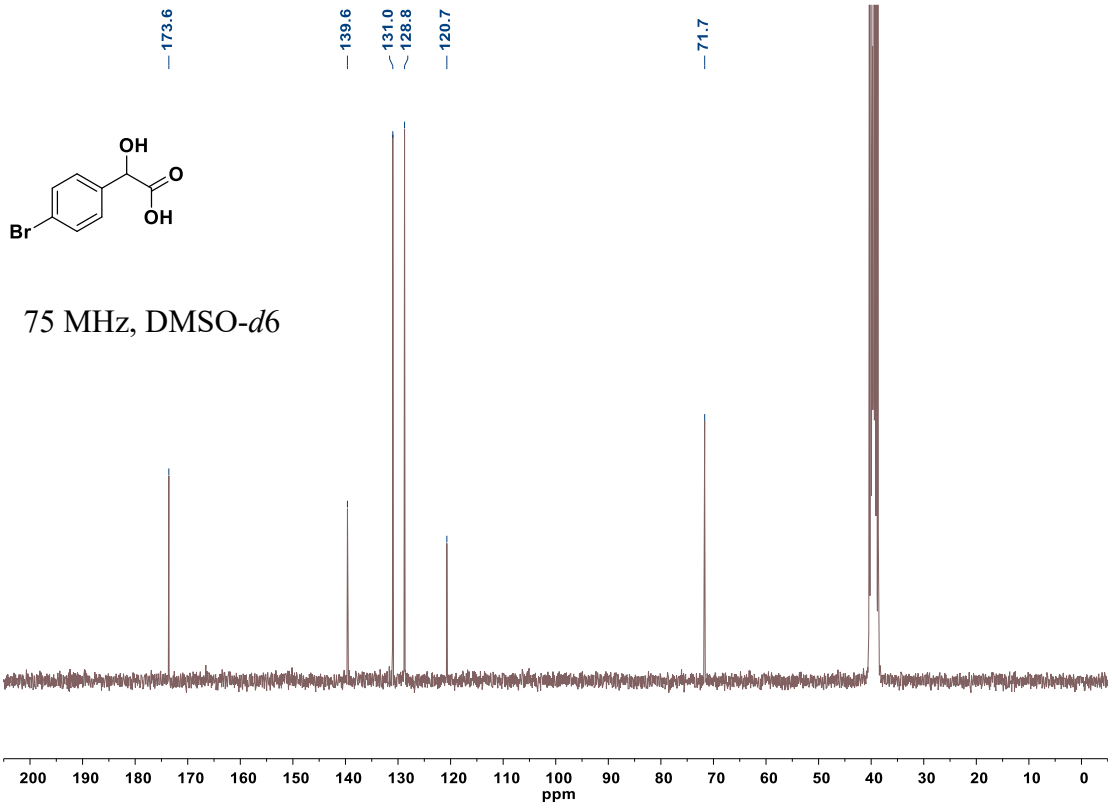




Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compound **2g**

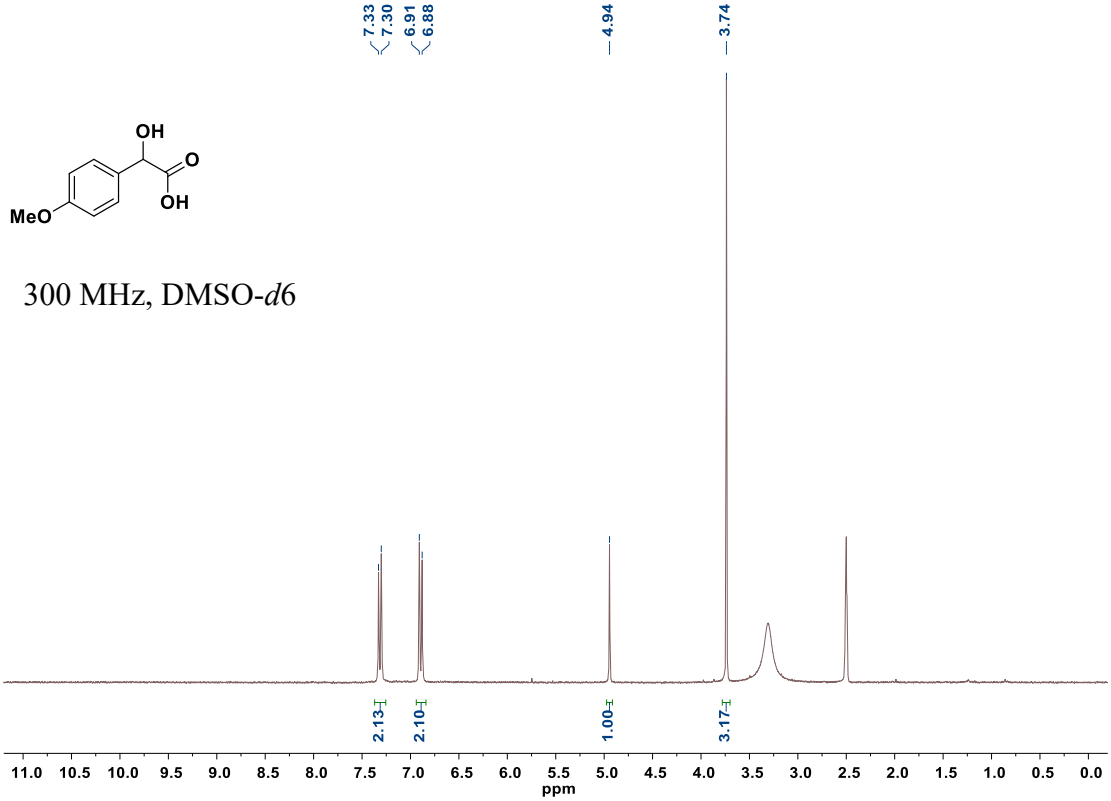


300 MHz, DMSO-*d*6

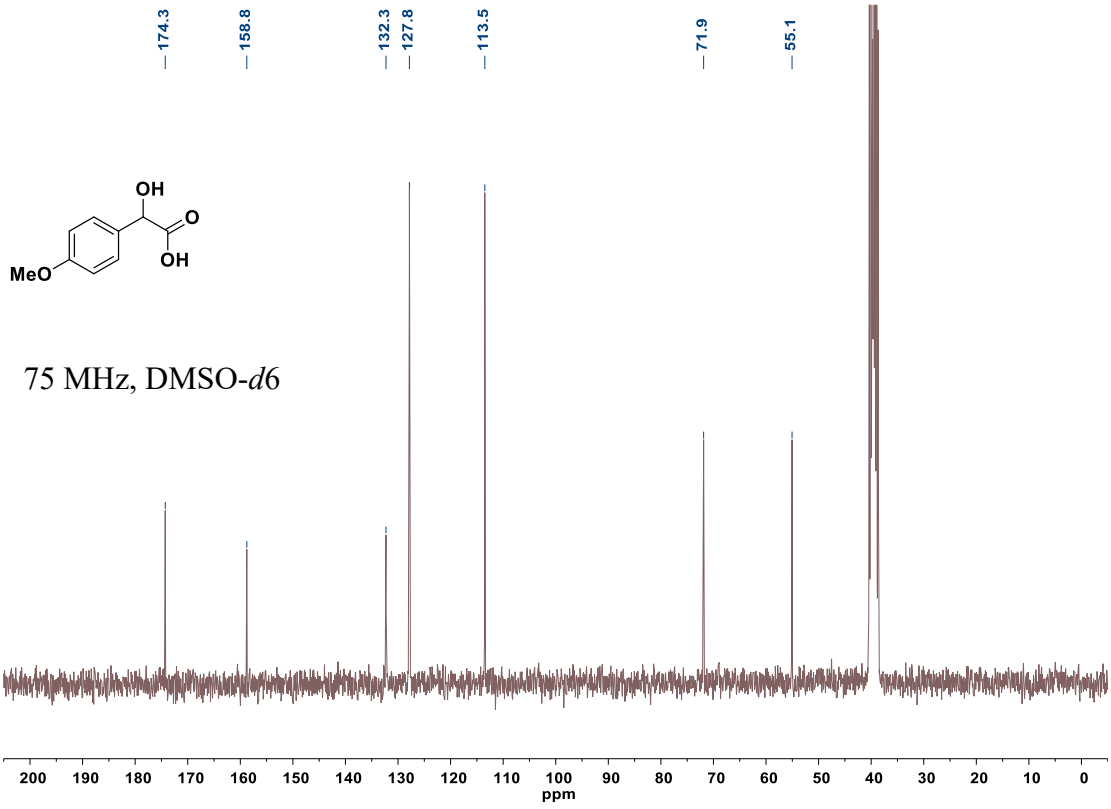


75 MHz, DMSO-*d*6

Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compound **2h**

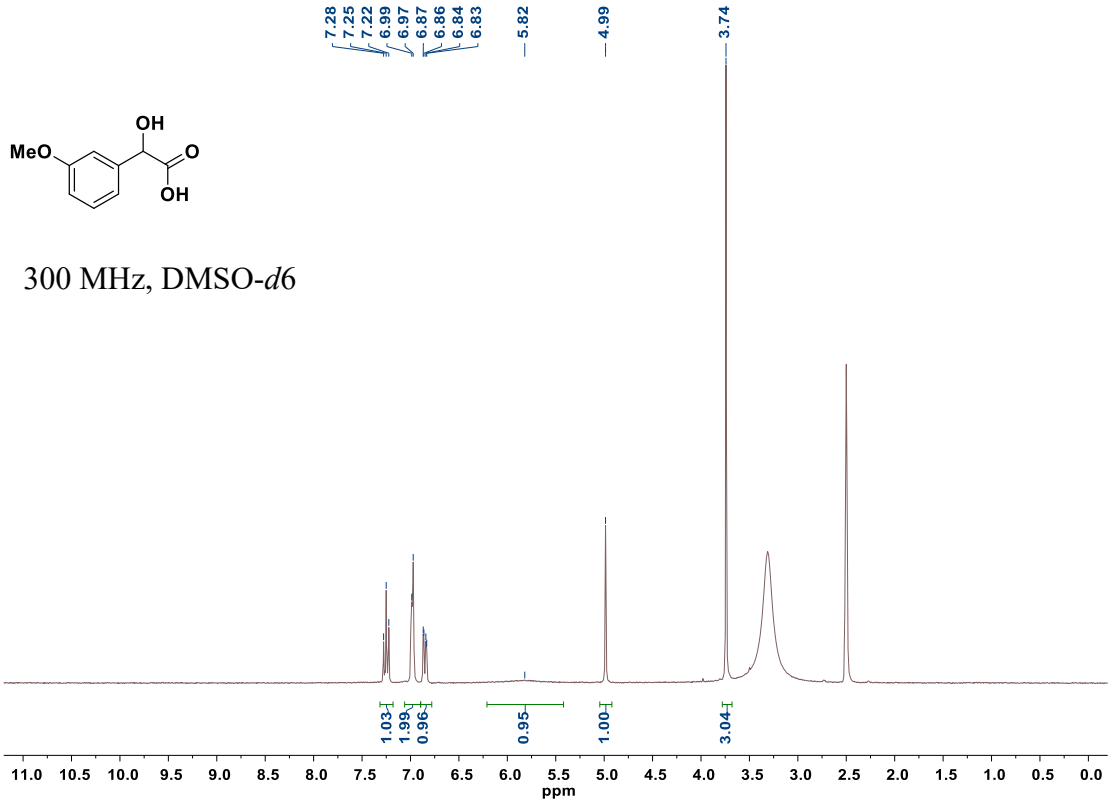


300 MHz, DMSO-*d*6

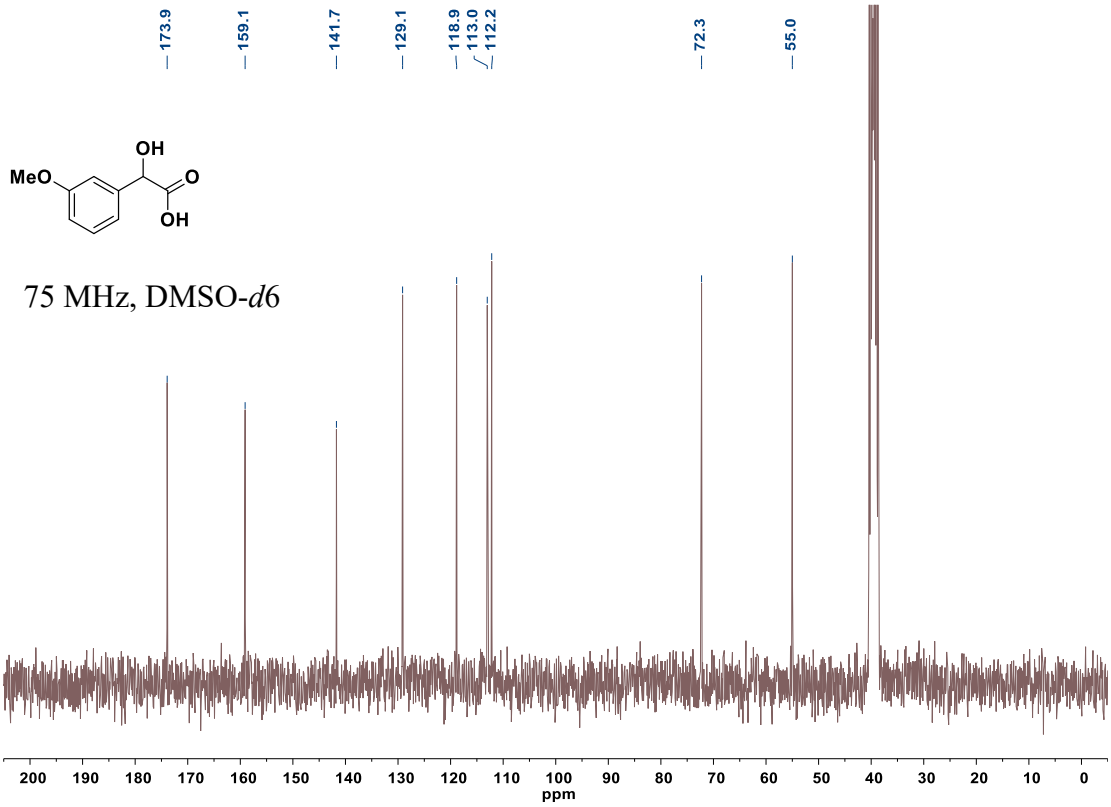


75 MHz, DMSO-*d*6

Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compound **2i**

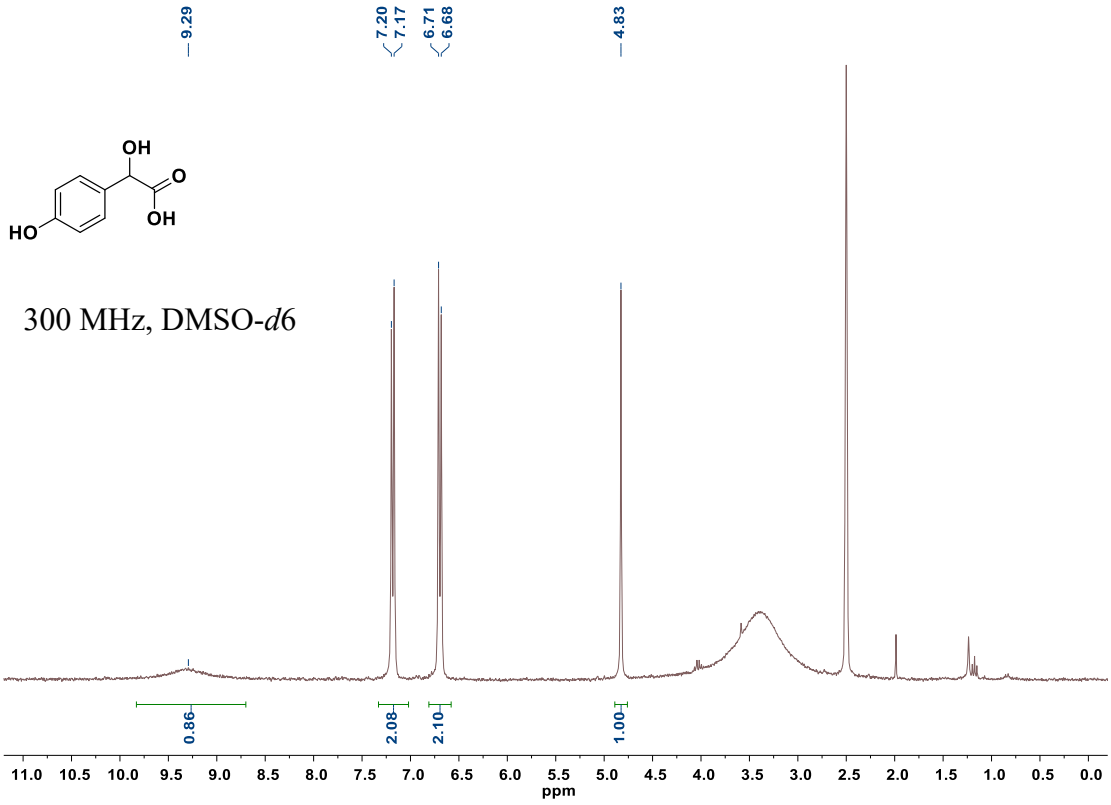


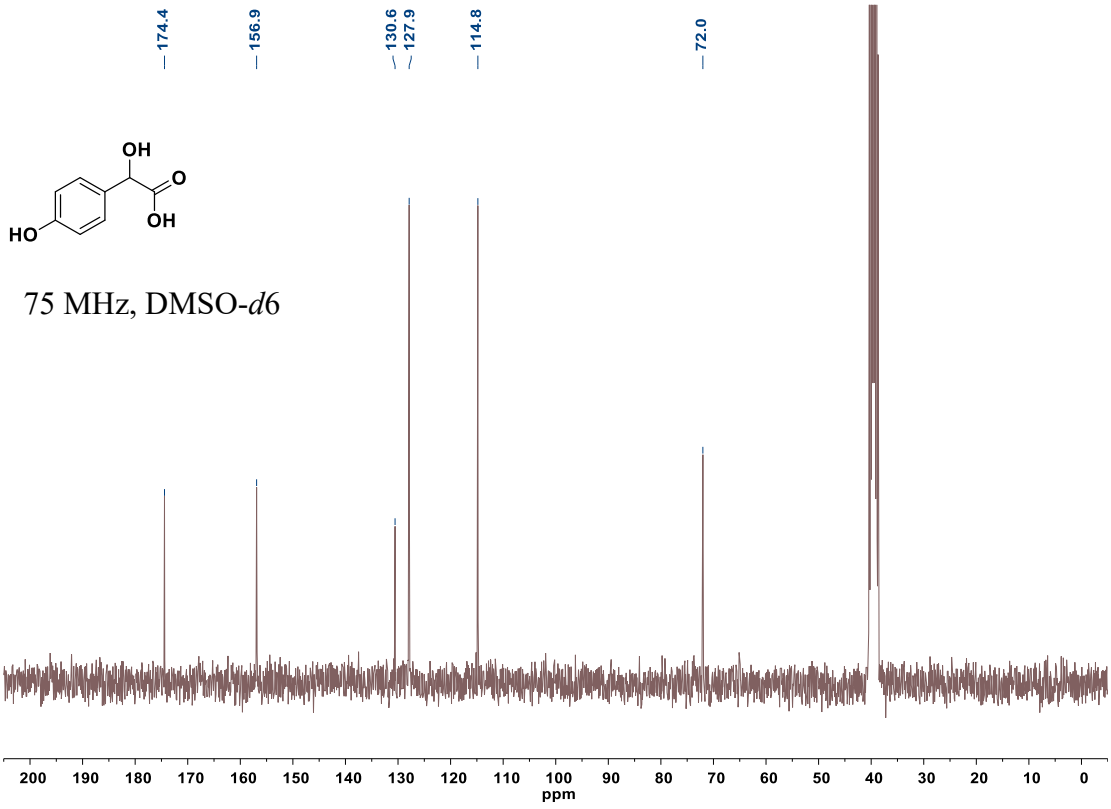
300 MHz, DMSO-*d*6



75 MHz, DMSO-*d*6

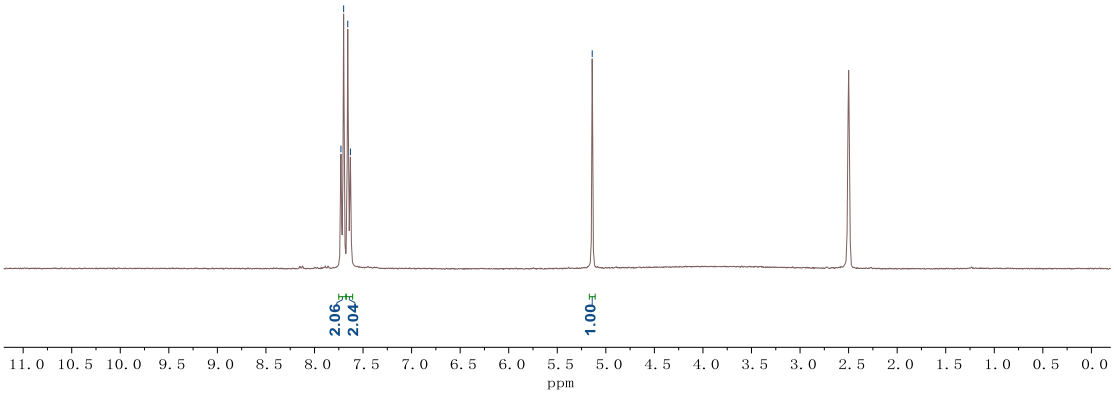
Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compound **2j**

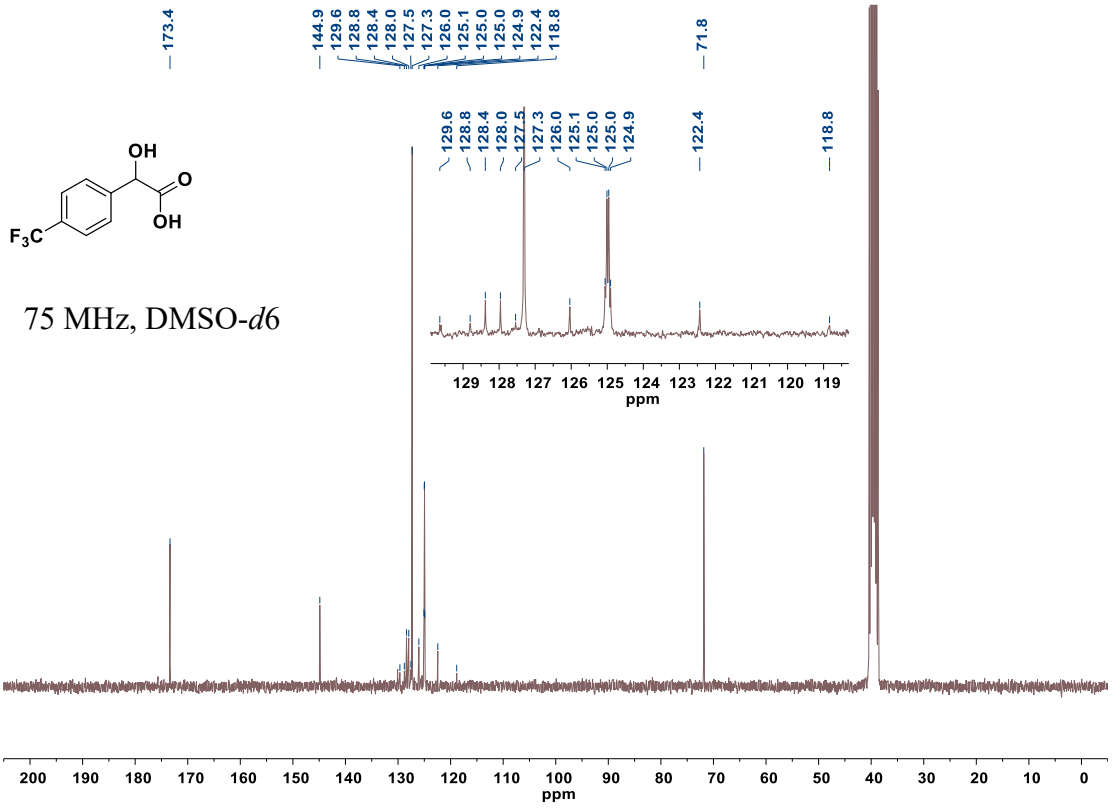




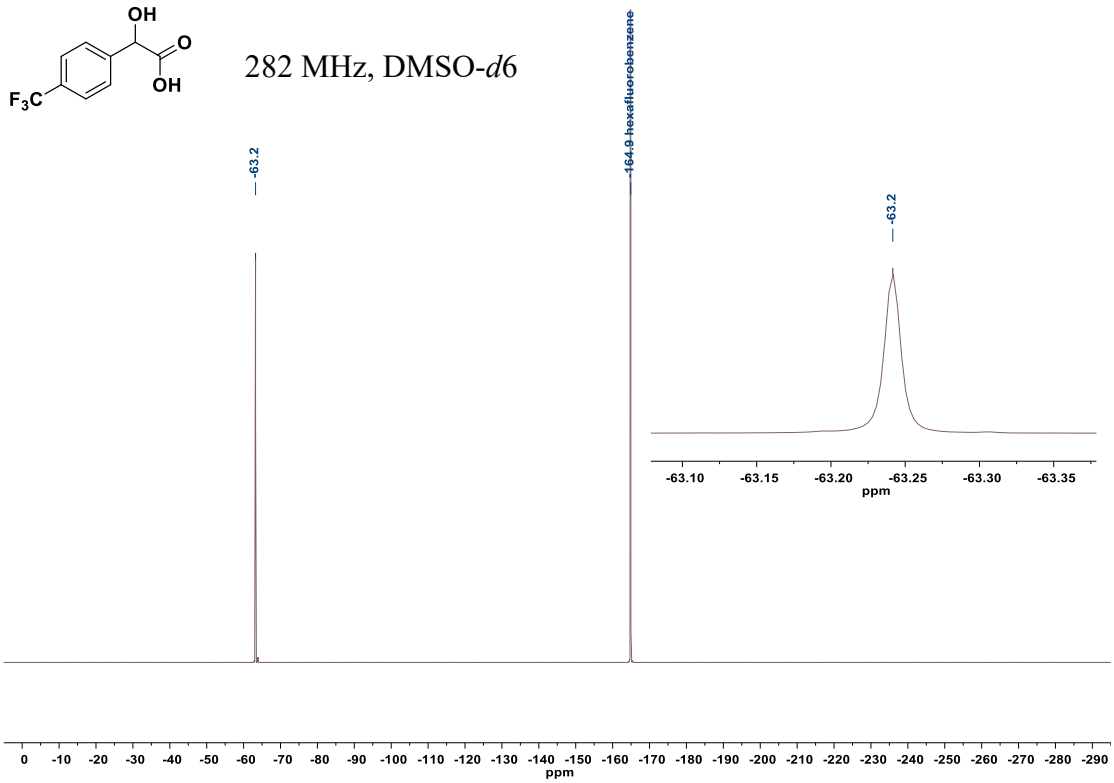
Copies of ^{1}H, $^{13}C\{^{1}H\}$ and $^{19}F\{^{1}H\}$ spectra of compound **2k**

300 MHz, DMSO-*d*6

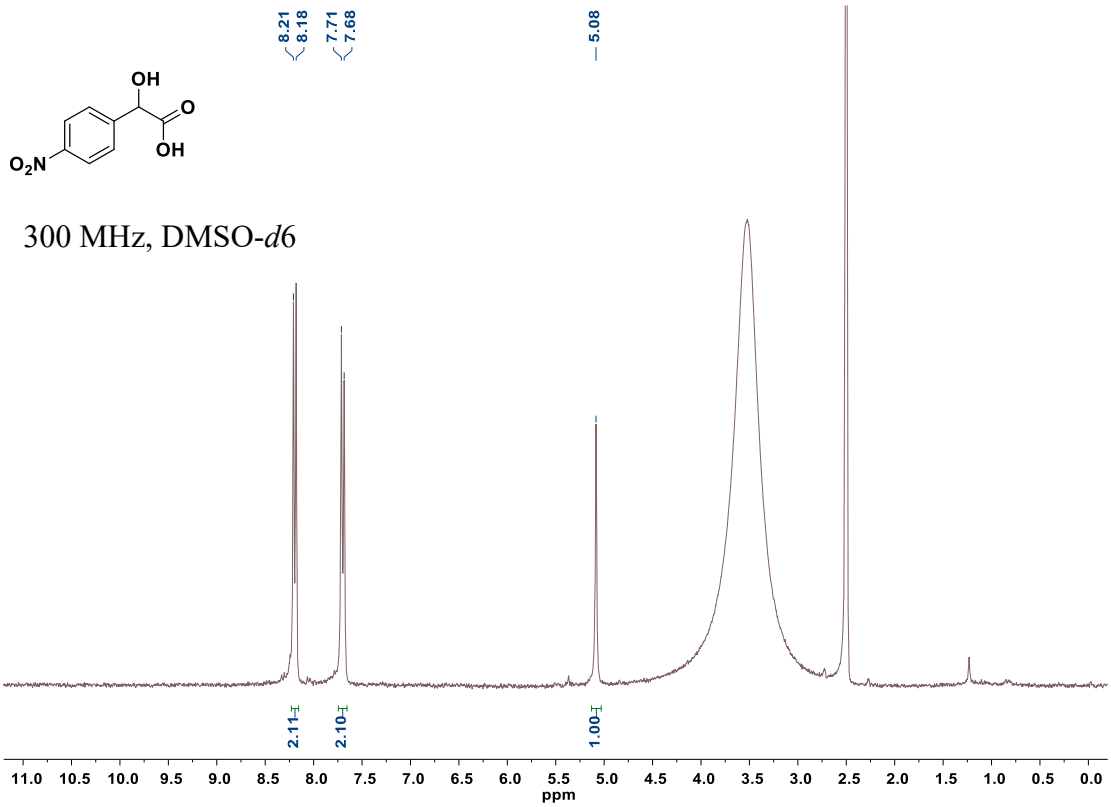


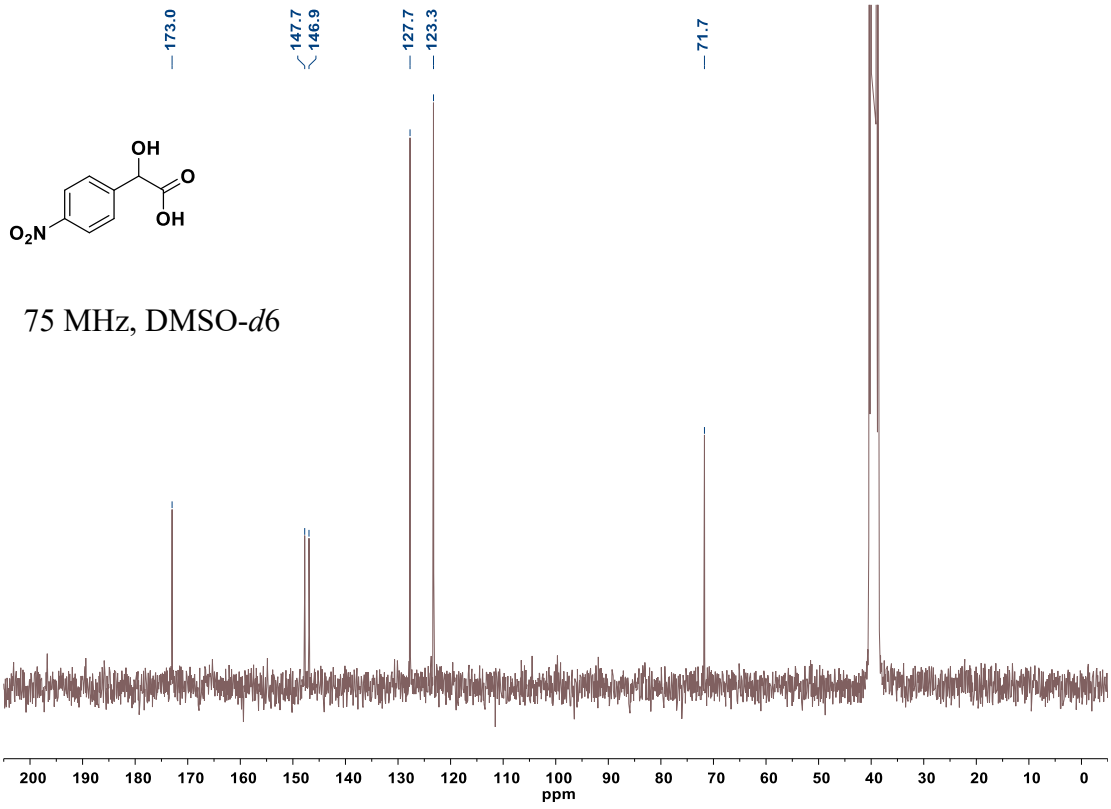


75 MHz, DMSO-*d*6

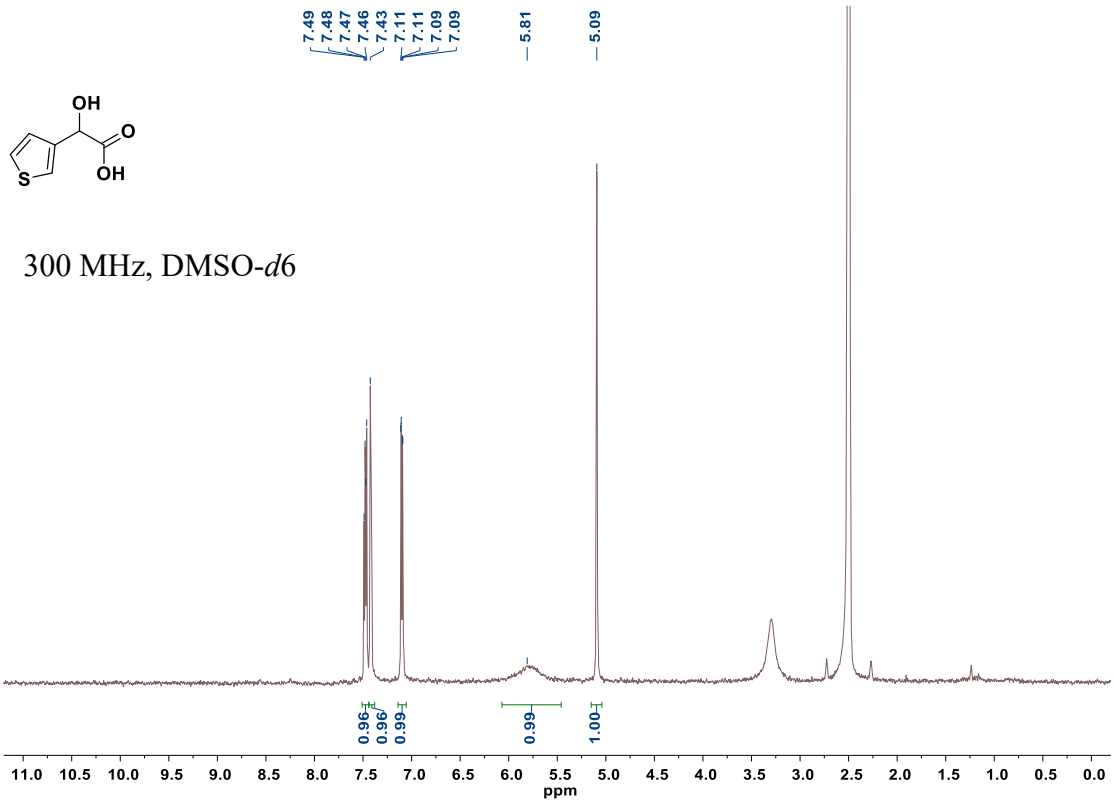

OH
O
OH
F3C
282 MHz, DMSO-d6
-63.2
-164.9 hexafluorobenzene
-63.2
-63.10
-63.15
-63.20
-63.25
-63.30
-63.35
ppm
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
-240
-250
-260
-270
-280
-290
ppm

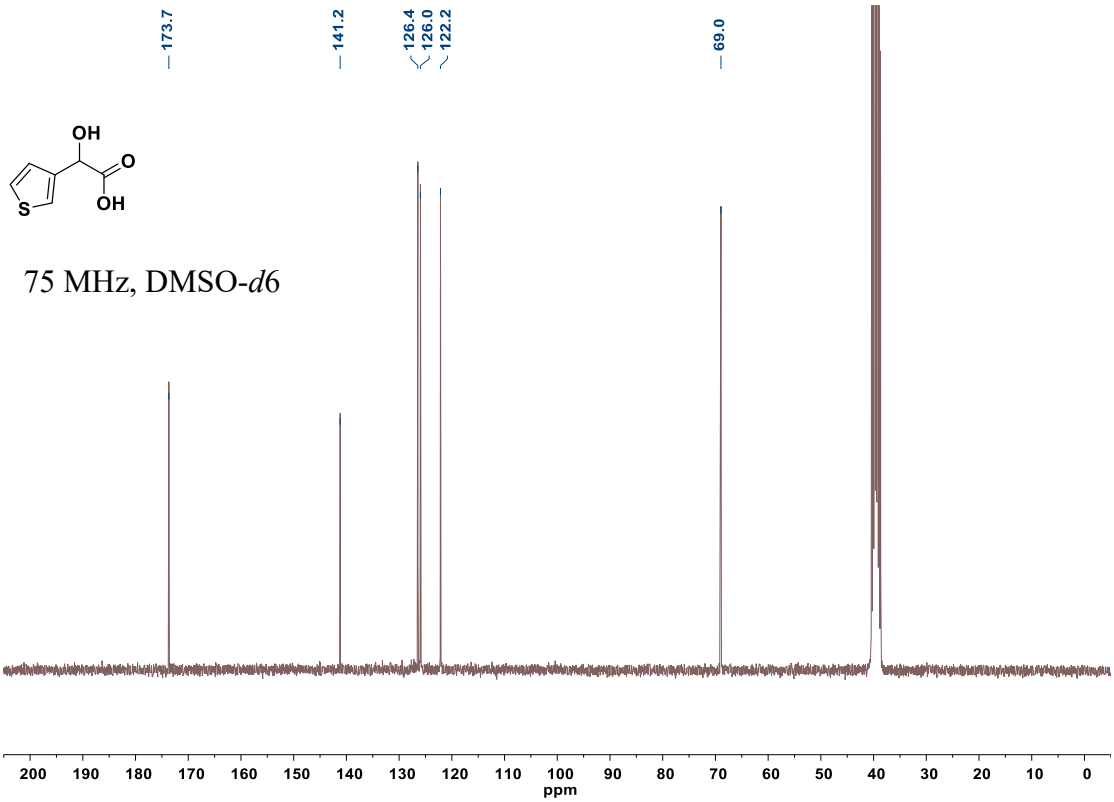
Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compound **2l**



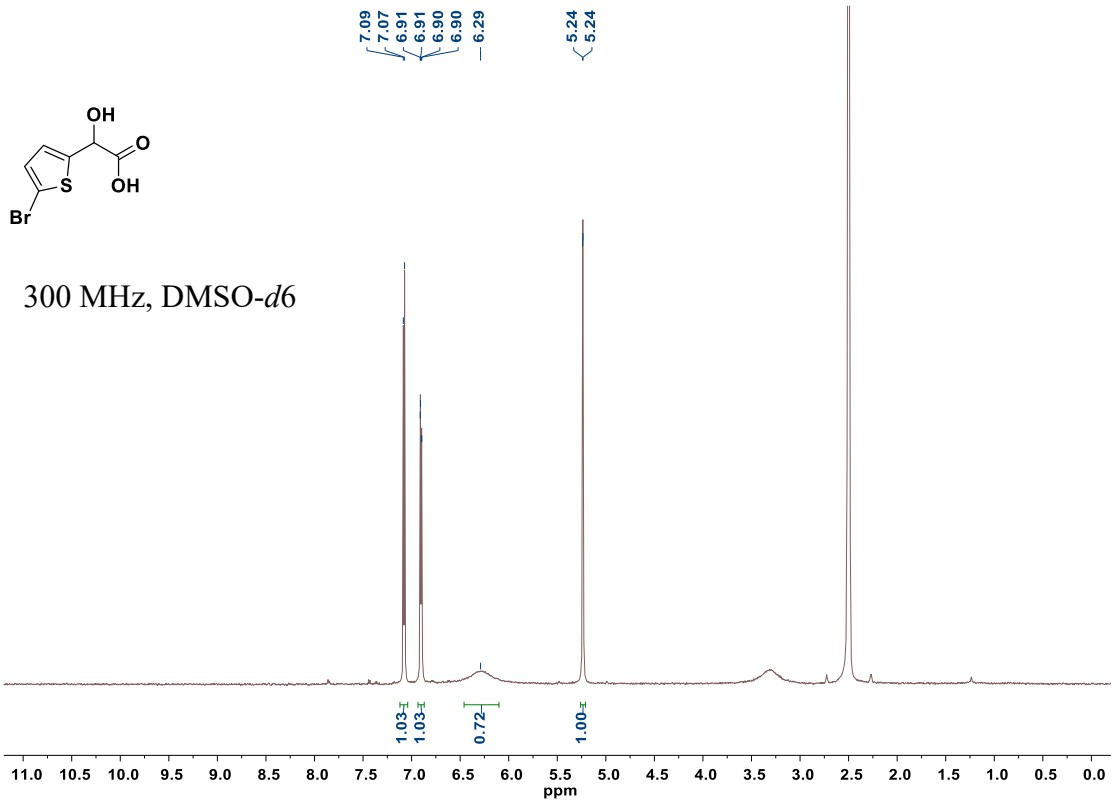


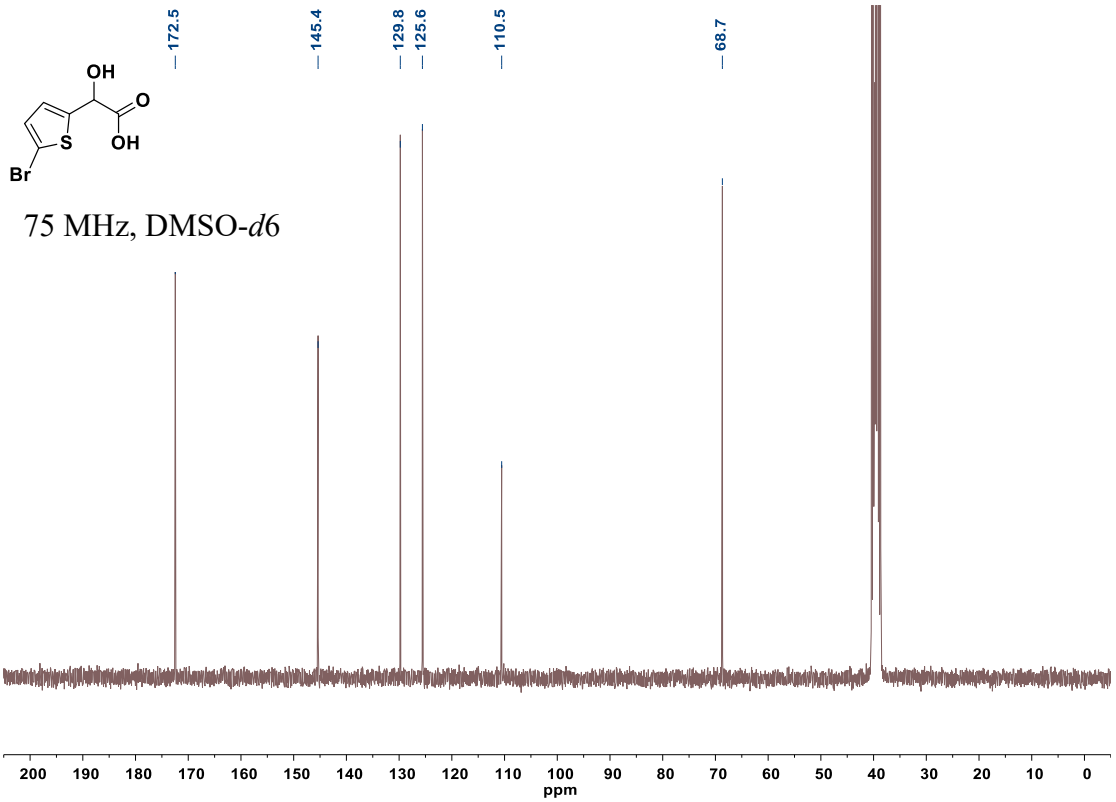
Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compound **2m**





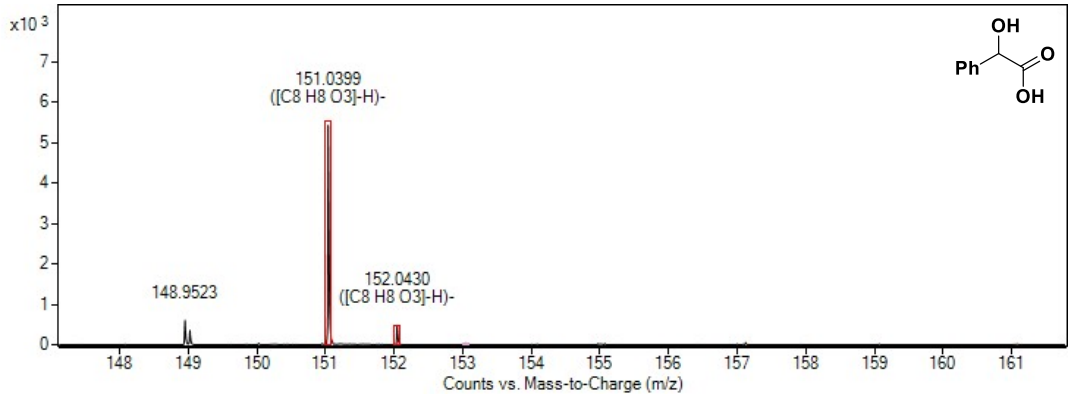
Copies of ^{1}H and $^{13}C\{^{1}H\}$ spectra of compound **2n**



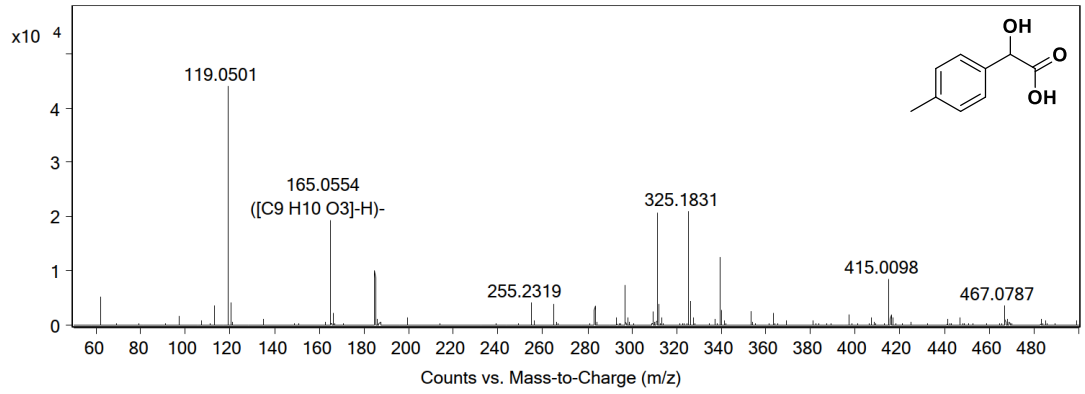


Copies of HRMS spectra of compounds 2

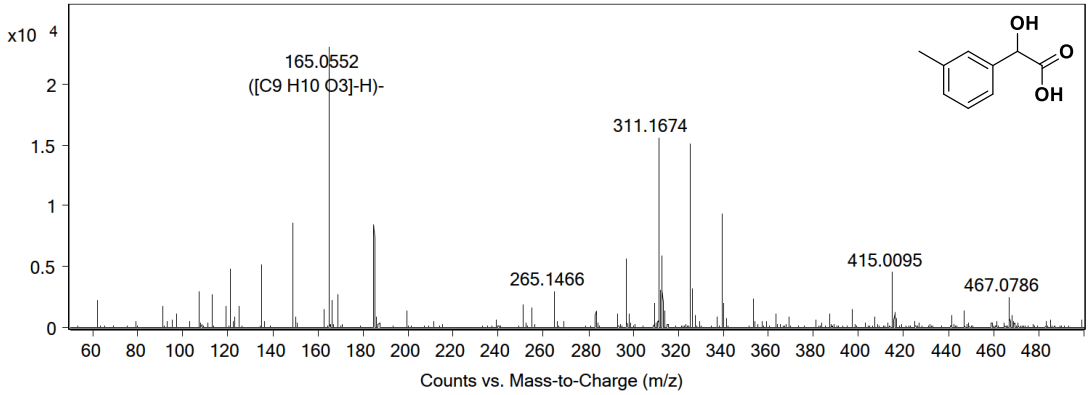
Copy of HRMS spectrum of compound **2b**



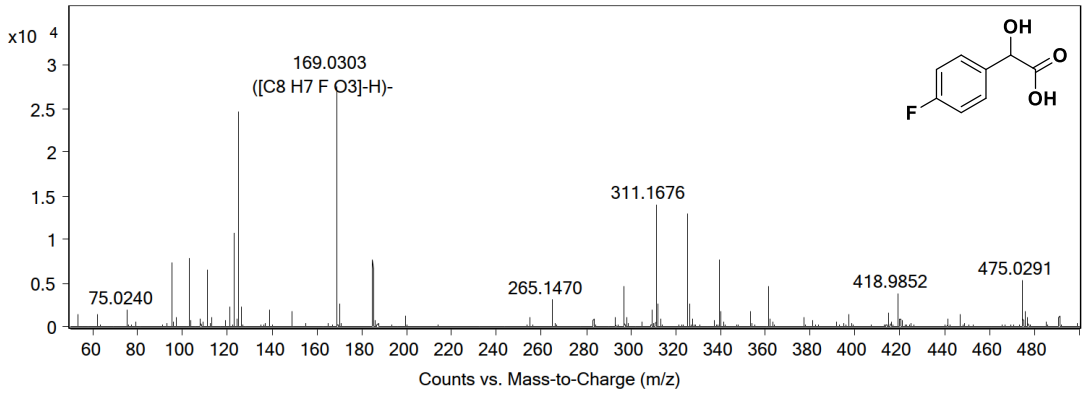
Copy of HRMS spectrum of compound **2c**



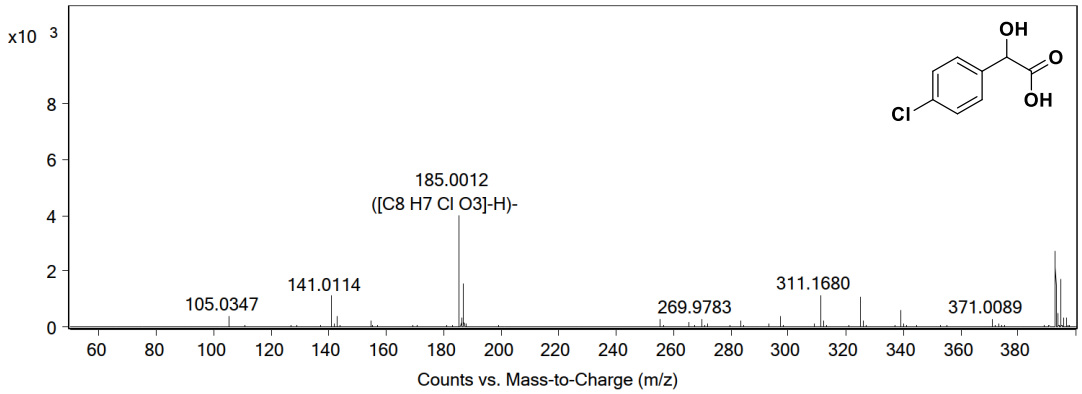
Copy of HRMS spectrum of compound **2d**



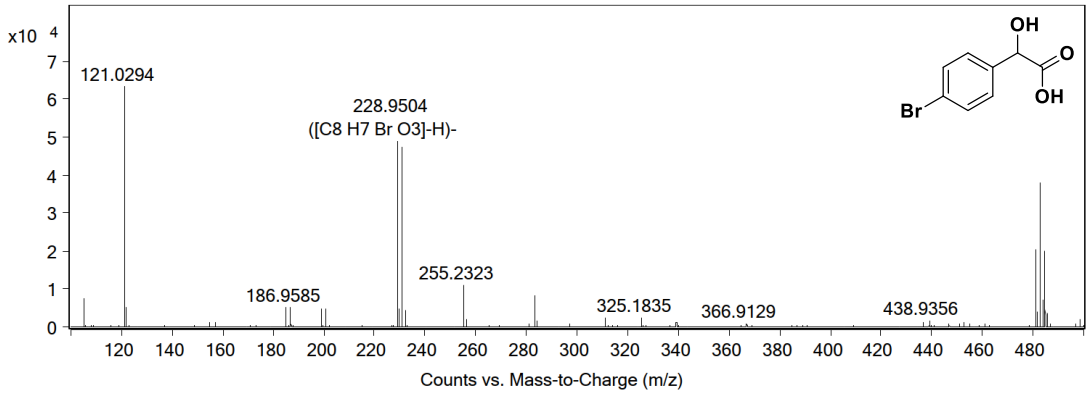
Copy of HRMS spectrum of compound **2e**



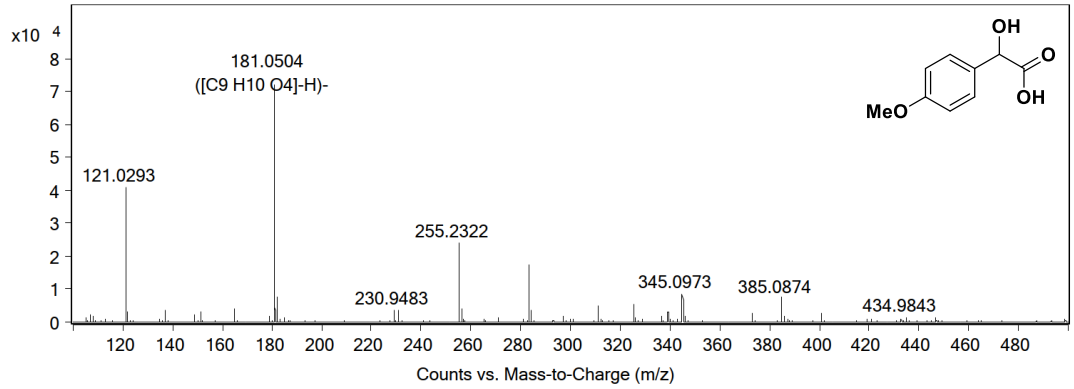
Copy of HRMS spectrum of compound **2f**



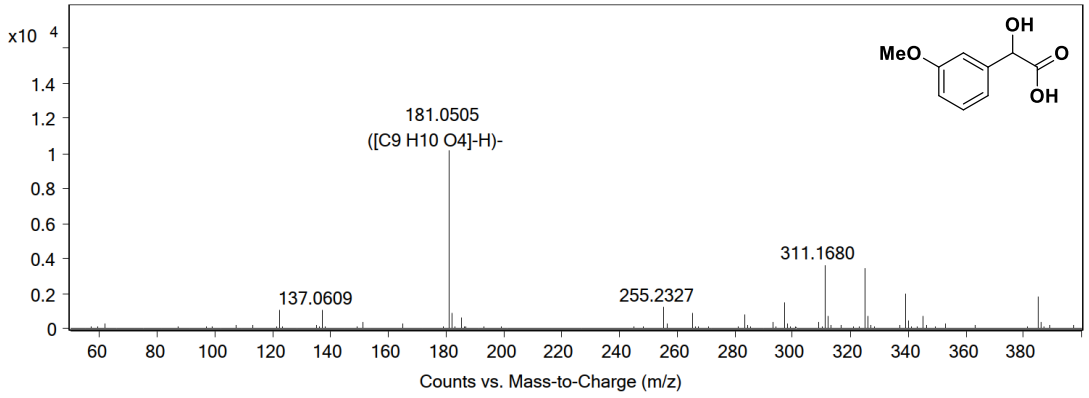
Copy of HRMS spectrum of compound **2g**



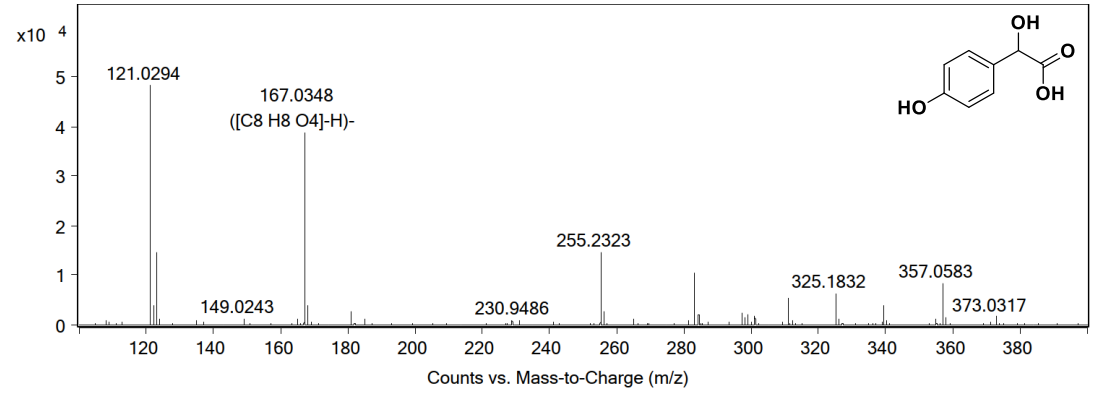
Copy of HRMS spectrum of compound **2h**



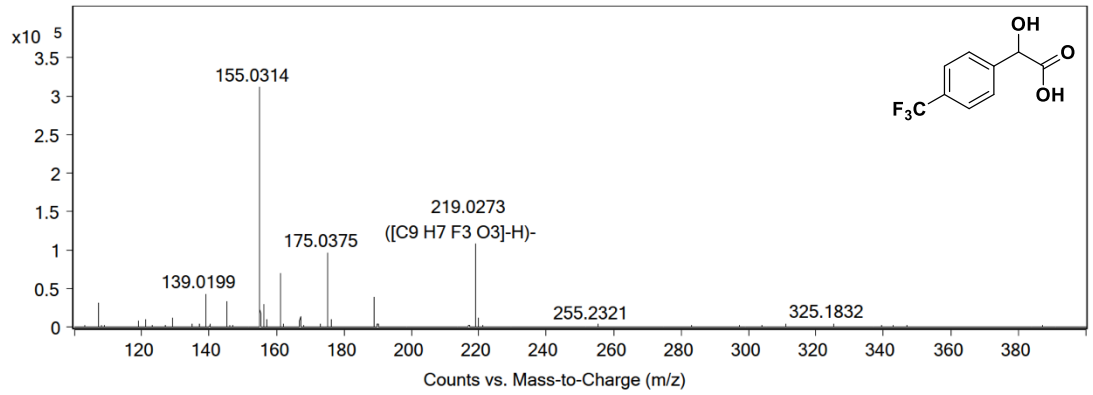
Copy of HRMS spectrum of compound **2i**



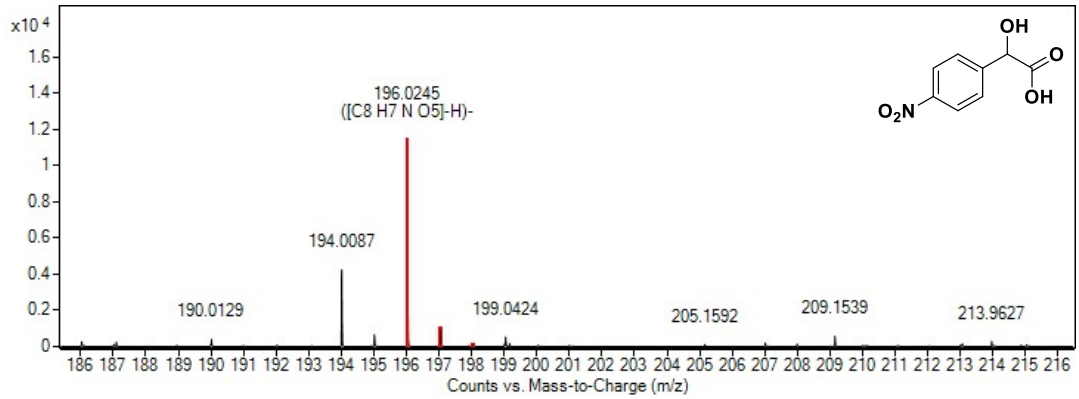
Copy of HRMS spectrum of compound **2j**



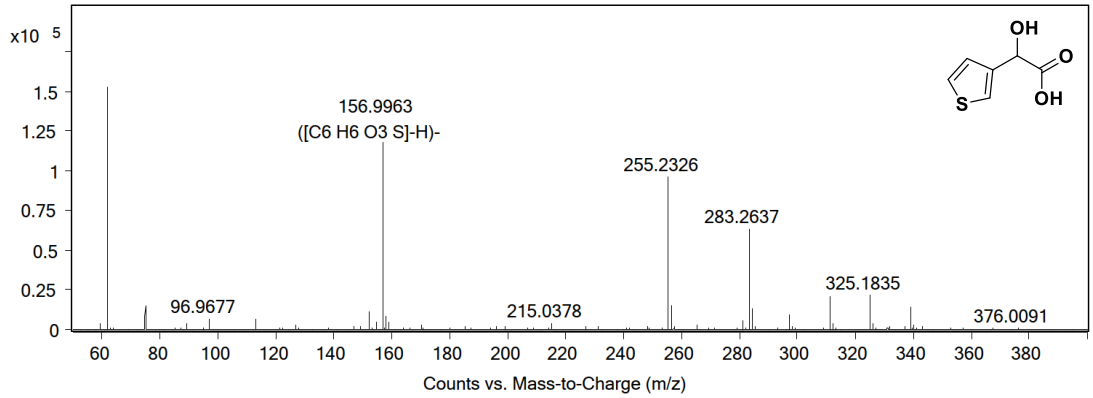
Copy of HRMS spectrum of compound **2k**



Copy of HRMS spectrum of compound **2l**



Copy of HRMS spectrum of compound **2m**



Copy of HRMS spectrum of compound **2n**