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Supporting Information:**An Enzyme Library Approach to Biocatalysis: Development of Nitrilases for Enantioselective Production of Carboxylic Acid Derivatives**

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Hydroxyglutaronitrile was purchased from TCI America and used as received. Amino acids used for the preparation of aryl lactic acid standards were purchased from PepTech (Cambridge, MA). (*R*)-3-hydroxy-4-cyanobutyric acid was obtained from Gateway Chemical Technology (St. Louis, MO). Both (*R*)- and (*S*)- mandelic acid and (*R*)- and (*S*)- phenyl lactic acid standards were purchased from Sigma Aldrich. All other reagents were purchased from Sigma Aldrich and utilized without further purification. Silica Gel, 70-230 mesh, 60 Å, purchased from Aldrich, was used for chromatographic purifications. All ¹H NMRs and ¹³C NMRs were run on Bruker model AM-500 machines, set at room temperature, 500 MHz and 125MHz respectively for ¹H and ¹³C. Mass analyses and unit mass resolution was achieved by flow injection analysis (FIA) using a Perkin-Elmer Sciex API-4000 TurboIon Spray LC/MS/MS system. The LC flow was provided by Shimadzu LC-10Advp pumps, with 0.05% acetic acid and MeOH. Injections were accomplished via a Valco injector valve. The HPLC analysis was done on an Agilent 1100 HPLC with Astec's Chirobiotic R column (100 x 4.6 mm, cat no. 13022 or 150 x 4.6 mm, cat no. 13023) or Daicel's Chiralcel OD column (50 x 4.6 mm, cat no. 14022) and the DAD detector set at 210, 220, 230, and 250 nm. For specific rotations, a Perkin Elmer Model 341 Polarimeter was used, set at 589 nm, Na lamp, at room temperature, with a 100 mm path length cell. Concentrations for specific rotation are reported in grams per 100 mL of solvent. Microbiology techniques were executed in accordance to published protocols.¹ Glycolic acid products were isolated and absolute configurations were determined to be (*R*) in all cases by comparison with literature optical rotation data on configurationally defined compounds except for (-)-3-pyridylglycolic acid, which to our knowledge is not known as a single enantiomer.²

For the aryl lactic acid products, absolute configuration was established to be (*S*) for phenyl lactic acid by comparison with literature optical rotation and for all other phenyl lactic acid products, absolute configurations were predicted based upon elution order using chiral HPLC. Absolute configuration for 3-hydroxy-4-cyano-butanoic acid was established by derivatization to (*R*)-(-)-Methyl-(3-O-[benzoyl]-4-cyano)-butanoate and comparison to literature optical rotation data on configurationally defined compound.³

Nitrilase Discovery and Characterization Methods:**1. Nitrilase Selection.**

An *Escherichia coli* screening host strain, SEL700, was optimized for nitrilase selections on a nitrile substrate. An $Abs_{600nm} = 1$, resuspension of SEL700 screening host in 10 mM MgSO₄ was infected with kanamycin-resistant environmental DNA library for 45 minutes at

37°C, such that complete screening coverage of the library was achieved. Infected cells, now denoted by kanamycin resistance, were plated on kanamycin LB plates and allowed to grow overnight at 30°C. Titer plates were also made to determine infection efficiency. Cells were pooled, washed, and resuspended the next morning with 10 mM MgSO₄. Transformed clones were inoculated into M9 media (without nitrogen) with 10 mM of nitrile substrate. M9 media consisted of 1X M9 salts (NH₄Cl omitted), 0.1mM CaCl₂, 1 mM MgSO₄, 0.2 % glucose, and approximately 10 mM of adiponitrile. The selection cultures were then incubated at 30°C, shaking at 200 rpm, for up to five weeks. Positive nitrilase cultures were identified by growth, due to positive clone's ability to hydrolyze nitrile substrate. Positive clones were isolated by streaking out a selection culture with growth and subsequent secondary culturing of isolated colonies in the same defined media. The DNA from any positive secondary cultures exhibiting re-growth was then isolated and sequenced to confirm discovery of a nitrilase gene and to establish the unique nature of that gene.

2. Nitrilase Biopanning.

Traditional filter lift hybridization screening protocols are limited to libraries with approximately 10⁶ to 10⁷ members. Attempting to screen one library would require approximately 5,000 filter lifts. Therefore, Diversa has developed solution phase and other biopanning formats for ultra high throughput sequence based screening permitting rapid screening of up to 10⁸ member environmental libraries. In the solution format, the DNA from a large number of library clones is mixed with tagged molecules of interest under conditions which promote hybridization. The tagged clones and hybridized DNA are then removed from solution and washed at some level of stringency to remove clones which do not have sequence identity with the probe. The hybridized DNA is then eluted and recovered. Clones of interest are sequenced and cloned to provide enzyme activities of interest. This method has been demonstrated to achieve up to 1,000-fold enrichment per round for sequences of interest.

3. High Throughput Nitrilase Activity Assay.

Activity assays were conducted using 25 mM (~3 mg/mL) substrate, 0.1 mg/mL nitrilase in 0.25 mL of assay solution. Assay solutions consisted of 0-10% (v/v) MeOH in 0.1 M sodium phosphate buffer solution at pH 7 to 9 and temperatures 37°C or 22°C. Specific activities were measured at 5 min transformation time point, unless otherwise noted, and are expressed in units $\mu\text{mol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$. Enantiomeric excess and conversion rates were determined by high throughput HPLC analysis comparing enzyme product concentration to standard curves of racemic acid products. Analytical conditions for the products are tabulated below.

Analytical Methods:

	Acid Product	Column	Liquid Chromatography Method	Retention Times of enantiomers (min)
1.1	mandelic acid	Chirabiotic R 100 x 4.6 mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.4 (<i>S</i>); 2.9 (<i>R</i>)
1.2	2-Cl-mandelic acid	Chirabiotic R 100 x 4.6 mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.3 (<i>S</i>); 2.9 (<i>R</i>)
1.3	2-Br-mandelic acid	Chirabiotic R 100 x 4.6 mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.8; 4.0
1.4	2-CH ₃ -mandelic acid	Chirabiotic R 100 x 4.6 mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	3.1; 3.8
1.5	3-Cl-mandelic	Chirabiotic R 100 x 4.6 mm	10%[0.5% AcOH], 90% CH ₃ CN 1 ml/min	3.1; 3.8
1.6	3-Br-mandelic	Chirabiotic R 100 x 4.6 mm	10%[0.5% AcOH], 90% CH ₃ CN 1 ml/min	3.3; 3.9
1.7	4-F-mandelic	Chirabiotic R 150 x 4.6 mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	3.7; 4.8
1.8	1-naphthylglycolic acid	Chirabiotic R 100 x 4.6 mm	4%[0.5% AcOH], 96% CH ₃ CN 1 ml/min	3.1; 3.7
1.9	2-naphthylglycolic acid	Chirabiotic R 100 x 4.6 mm	4%[0.5% AcOH], 96% CH ₃ CN 1 ml/min	3.7; 4.7
1.10	3-pyridylglycolic acid	Chirabiotic R 100 x 4.6 mm	5% [0.5% AcOH], 65% H ₂ O, 30% CH ₃ CN, 2 ml/min	4.4; 5.5
1.11	3-thienylglycolic acid	Chirabiotic R 100 x 4.6 mm	20%[0.5% AcOH], 80% CH ₃ CN 2 ml/min	1.4; 2.5
2.1	phenyl lactic acid	Chirabiotic R 150 x 4.6 mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.8 (<i>S</i>); 4.0 (<i>R</i>)
2.2	2-methylphenyl lactic acid	Chirabiotic R 150 x 4.6 mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.5; 2.8
2.3	2-bromophenyl lactic acid	Chirabiotic R 150 x 4.6 mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.8; 3.2
2.4	2-fluorophenyl lactic acid	Chirabiotic R 150 x 4.6 mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.6; 2.9
2.5	3-methylphenyl lactic acid	Chirabiotic R 150 x 4.6 mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.4; 3.2
2.6	3-fluorophenyl lactic acid	Chirabiotic R 150 x 4.6mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.8; 3.6
2.7	1-naphthyl lactic acid	Chirabiotic R 150 x 4.6mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.7; 3.1
2.8	2-pyridyl lactic acid	Chirabiotic R 150 x 4.6mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.5; 2.9
2.9	3-pyridyl lactic acid	Chirabiotic R 150 x 4.6mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	2.9; 3.6
2.10	2-thienyl lactic acid	Chirabiotic R 150 x 4.6mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	3.6; 4.6
2.11	3-thienyl lactic acid	Chirabiotic R 150 x 4.6mm	20%[0.5% AcOH], 80% CH ₃ CN 1 ml/min	3.5; 4.6
	Methyl(3-O [benzoyl]-4-cyano)-butanoate	Daicel OD 50 x 4.6 mm	5% isopropanol, 95% hexane 1 ml/min	4.5 (<i>R</i>); 5.4(<i>S</i>)
GC	Methy-3-hydroxy-4-cyano-butanoate	Lipodex A 25 m Macherey-Nagel	95 °C, 1ml/min	51.6 (<i>R</i>); 52.7(<i>S</i>)

Cyanohydrin (Substrate) Synthesis:

Mandelonitrile Synthesis Method A: Acetone cyanohydrin (685 μL , 7.5 mmol), aldehyde (5 mmol), and catalytic DIEA (13 μL , 0.075 mmol) were mixed at 0 $^{\circ}\text{C}$. The reactions were stirred on ice for 45 minutes. To drive the equilibrium toward the product, acetone was removed *in vacuo*. Subsequently, crude reactions were acidified with H_2SO_4 (3 μL) and stored at -20 $^{\circ}\text{C}$. TLC was used to monitor reaction progress (3:1 hexane/ethylacetate (EtOAc)).

Mandelonitrile Synthesis Method B: To a solution of KCN (358 mg, 5.5 mmol) in MeOH (1 mL) at 0 $^{\circ}\text{C}$ was added aldehyde (5 mmol) and acetic acid (315 μL , 5.5 mmol). After stirring for one hour on ice, MeOH was removed *in vacuo*, and the crude mixture was partitioned using EtOAc and H_2O . The organic fraction was retained and concentrated *in vacuo*. TLC analysis was used to monitor reaction progress (3:1 Hexanes/EtOAc).

Aryl Acetaldehyde Cyanohydrin: Arylacetic acid (50 mmol) was dissolved in 50 ml anhydrous tetrahydrofuran (THF) in a two-neck 500 ml round-bottom flask under $\text{N}_2(\text{g})$ atmosphere. To this solution cooled to 0 $^{\circ}\text{C}$, under vigorous mixing, was added slowly 105 mmol of tetrabutylammonium hexafluoroantimonate (2.55 M in methylene chloride). The reaction was allowed to proceed overnight. Excess acetic acid (10 ml) was added to quench and acidify the reaction followed by the addition 10 ml water. After stirring at room temperature for 1 hour, solvent was removed *in vacuo* and the residue was dissolved in 100 ml water and extracted with 200 ml EtOAc. The EtOAc layer was dried over sodium sulfate, filtered and then concentrated *in vacuo*. Subsequently, 60 mmol of KCN, followed by 100 ml methanol was added to the residue. The solution was then cooled to 0 $^{\circ}\text{C}$ and acetic acid (60 mmol) added. The reaction was stirred for 1-2 hours after all KCN dissolved. Solvents were removed *in vacuo* and residue was dissolved in 100 ml water and 200 ml EtOAc. The aqueous layer was extracted with EtOAc one more time. Combined EtOAc extracts were washed with saturated brine and dried over sodium sulfate, filtered and then concentrated *in vacuo* to obtain crude cyanohydrin product. The cyanohydrin was purified by silica-gel column (hexane/EtOAc), as necessary.

2-chloro mandelonitrile: ^1H NMR (CDCl_3 , 500 MHz) δ 7.69 (m, 1H), 7.41 (m, 1H), 7.36 (m, 2H), 5.84 (s, 1H), 3.07 (br, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 132.89, 132.73, 131.22, 130.19, 128.48, 127.84, 118.24, 60.87. MS calc'd for [$\text{C}_8\text{H}_6\text{ClNO}$] 167.01 found 167.9 (LC-MS +).

2-bromomandelonitrile: ^1H NMR (CDCl_3 , 500 MHz) δ 7.72 (d, 1H, $J=6.58$), 7.62 (d, 1H, $J=8.35$), 7.43 (t, 1H, $J=8.42$), 7.30 (t, 1H, $J=7.00$), 5.85 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 134.55, 133.58, 131.56, 128.82, 128.54, 122.57, 118.15, 63.38.

2-methylmandelonitrile: ^1H NMR (CDCl_3 , 500 MHz) δ : 7.60 (d, 1H, $J=7.4$), 7.23-7.35 (m, 3H), 5.66 (s, 1H), 2.44 (s, 3H). ^{13}C NMR (CDCl_3 , 298 K, 125 MHz) δ : 136.43, 133.42, 131.45, 130.15, 127.20, 126.90, 118.95, 18.92. MS calc'd for [$\text{C}_9\text{H}_9\text{NO}$] 147.07, found 147.20 (ESI +).

3-chloromandelonitrile: ^1H NMR (CDCl_3 , 500 MHz) δ 7.55 (s, 1H), 7.43-7.37 (m, 3H), 5.54 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 137.19, 135.48, 130.72, 130.30, 127.05, 124.89, 118.40, 63.16. MS calc'd for $[\text{C}_8\text{H}_6\text{ClNO}]$ 167.01 found 167.9 (LC-MS +).

3-bromomandelonitrile: ^1H NMR (CDCl_3 , 500 MHz) δ 7.69 (s, 1H), 7.56 (d, $J = 6.2$ Hz, 1H), 7.45 (d, $J = 5.5$ Hz, 1H), 7.32 (t, $J = 6.4$ Hz, 1H), 5.53 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 137.38, 133.20, 130.93, 129.21, 125.36, 123.38, 118.46, 63.01. MS calc'd for $[\text{C}_8\text{H}_6\text{BrNO}]$ 212.0 found 211.9 (LC-MS +).

4-fluoromandelonitrile: ^1H NMR (CDCl_3 , 500 MHz) δ 5.54 (s, 1H), 7.13 (m, 2H), 7.51-7.53 (m, 2H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 63.02, 116.44, 118.97, 128.90, 131.54, 132.51, 162.58.

4-chloromandelonitrile: ^1H NMR (CDCl_3 , 500 MHz) δ 7.47 (d, $J = 7.0$ Hz, 2H), 7.42 (d, $J = 7.0$ Hz, 2H), 5.53 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 136.21, 133.85, 129.65, 128.23, 118.63, 63.15. MS calc'd for $[\text{C}_8\text{H}_6\text{ClNO}]$ 167.01 found 167.9 (LC-MS +)

1-naphthyl cyanohydrin : ^1H NMR (CDCl_3 , 500 MHz) δ 8.14 (d, 1H, $J = 8.5$), 7.92 (t, 2H, $J = 6.1$), 7.82 (d, 1H, $J = 5.7$), 7.62 (t, 1H, $J = 6.1$), 7.56 (t, 1H, $J = 6.1$), 7.50 (t, 1H, $J = 6.1$), 6.18 (s, 1H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 137.0, 135.7, 134.2, 131.1, 129.2, 127.5, 126.7, 125.8, 125.3, 123.1, 119.0, 62.4; MS calc'd for $[\text{C}_{12}\text{H}_9\text{O}]$ 183.21, found 183.2 (ESI +).

2-naphthyl cyanohydrin: ^1H NMR (CDCl_3 , 500 MHz) δ 8.03 (s, 1H), 7.92 (d, 1H, $J = 8.6$), 7.87-7.91 (m, 2H), 7.61 (dd, 1H, $J = 6.7, 1.2$), 7.55-7.60 (m, 2H), 5.72 (s, 1H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 134.9, 133.9, 132.7, 129.6, 128.6, 128.0, 127.4, 127.2, 126.4, 123.9, 118.9, 64.1; MS calc'd for $[\text{C}_{12}\text{H}_9\text{O}]$ 183.21, found 183.2 (ESI +).

3-pyridyl cyanohydrin: ^1H NMR (CDCl_3 , 500 MHz) δ : 8.62 (d, 1H, $J = 1.8$), 8.57 (d, 1H, $J = 5.1$), 7.94 (d, 1H, $J = 8.1$), 7.41 (dd, 1H, $J = 8.1, 5.1$), 5.64 (s, 1H); ^{13}C NMR (CDCl_3 , 125 MHz) δ 149.92, 147.36, 135.41, 133.04, 124.44, 118.98, 61.09. MS calc'd for $[\text{C}_7\text{H}_6\text{N}_2\text{O}]$ 134.05, found 135.2 (ESI +).

3-thienyl cyanohydrin: ^1H NMR (CDCl_3 , 500 MHz) δ 7.45 (d, $J = 2.2$ Hz, 1H), 7.56 (dd, $J = 6.2$ Hz, 1H), 7.45 (d, $J = 5.5$ Hz, 1H), 7.32 (t, $J = 6.4$ Hz, 1H), 5.53 (s, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 137.38, 133.20, 130.93, 129.21, 125.36, 123.38, 118.46, 63.006. MS calc'd for $[\text{C}_6\text{H}_5\text{NOS}]$ 139.01 found 139.9 (LC-MS +).

phenyl acetaldehyde cyanohydrin: ^1H NMR (CDCl_3 , 500 MHz) δ 7.34 (m, 5H), 4.64 (t, $J = 6.75$ Hz, 1H), 3.11 (d, $J = 6.75$ Hz, 2H), 2.75 (br, 1H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 133.96, 129.91, 129.16, 128.08, 119.47, 62.33, 41.55.

2-methylphenyl acetaldehyde cyanohydrin: ^1H NMR (CDCl_3 , 500 MHz) δ 7.11 (m, 4H), 4.61 (t, $J = 6.62$ Hz, 1H), 3.12 (d, $J = 6.62$ Hz, 2H), 2.14 (s, 3H). ^{13}C NMR (CDCl_3 , 125

MHz) δ 136.94, 136.47, 132.57, 130.48, 127.61, 125.75, 120.11, 62.95, 44.73 MS calc'd for [C₁₀H₁₁NO]: 161.08, found 162.2 (M+Na, ESI +)

2-bromophenyl acetaldehyde cyanohydrin: ¹H NMR (CDCl₃, 500 MHz) δ 7.20 (m, 4H), 4.78 (t, *J* = 6.5 Hz, 1H), 3.26 (d, *J* = 6.5 Hz, 2H). ¹³C NMR (CDCl₃, 100 MHz) δ 133.93, 132.82, 131.72, 129.21, 128.12, 124.86, 119.41, 63.02, 44.89.

2-fluorophenyl acetaldehyde cyanohydrin: ¹H NMR (CDCl₃, 500 MHz) δ 7.2 (m, 2H), 7.02 (m, 2H), 4.50 (dd, *J* = 4.62 Hz, *J* = 7.88 Hz, 1H), 3.23 (dd, *J* = 4.62 Hz, 1 *J* = 14.12 Hz, 1H), 2.97 (dd, 7.88 Hz, 14.12 Hz, 1H). ¹³C NMR (CDCl₃, 125 MHz) δ 132.18, 131.52, 129.66, 129.03, 128.07, 124.05, 115.8, 63.02, 44.79 MS calc'd for [C₉H₈FNO] 165.06, found 164.2 (ESI +).

3-methylphenyl acetaldehyde cyanohydrin: ¹H NMR (CDCl₃, 500 MHz) δ 7.18 (m, 1H), 7.02 (m, 3H), 4.54 (dd, *J* = 4.62 Hz, *J* = 8 Hz, 1H), 3.06 (dd, *J* = 4.62 Hz, *J* = 14.38 Hz, 1H), 2.83 (dd, *J* = 8 Hz, *J* = 14.38 Hz, 1H), 2.36 (s, 3H) ¹³C NMR (CDCl₃, 125 MHz) δ 176.25, 138.18, 136.0, 130.97, 128.93, 127.68, 126.58, 76.42, 34.29, 37.69 MS calc'd for [C₁₀H₁₂O₃] 180.08, found 180.0 (ESI +).

3-fluorophenyl acetaldehyde cyanohydrin: ¹H NMR (CDCl₃, 500 MHz) δ 7.18 (m, 2H), 6.95 (m, 2H), 4.44 (dd, 1H), 3.11 (dd, 1H). ¹³C NMR (CDCl₃, 125 MHz) δ 130.40, 125.53, 124.85, 116.92, 114.87, 114.50, 119.77, 61.97, 41.27.

1-naphthyl acetaldehyde cyanohydrin: ¹H NMR (CDCl₃, 500 MHz) δ 8.07 (m, 1H), 7.86 (m, 1H), 7.74 (m, 1H), 7.41 (m, 4H), 4.20 (t, *J* = 7 Hz, 1H), 3.33 (d, *J* = 6.8 Hz, 2H) ¹³C NMR (CDCl₃, 125 MHz) δ 177.7, 140.31, 129.74, 129.24, 128.92, 128.26, 127.84, 125.63, 124.53, 124.05, 123.42, 70.58, 38.0 MS calc'd for [C₁₃H₁₁NO] 197.08, found 197.1 (ESI +).

3-pyridyl acetaldehyde cyanohydrin: ¹H NMR (CDCl₃, 500 MHz) δ 8.62 (d, 1H, *J* = 1.8), 8.57 (d, 1H, *J* = 5.1), 7.94 (d, 1H, *J* = 8.1), 7.41 (dd, 1H, *J* = 8.1, 5.1), 5.64 (s, 1H). ¹³C NMR (CDCl₃, 125 MHz) δ : 149.92, 147.36, 135.41, 133.04, 124.44, 118.98, 61.09. Exact Mass calculated for [C₇H₆N₂O]: 134.05, found: 135.2 (ESI +).

2-thienyl acetaldehyde cyanohydrin:

¹H NMR (CDCl₃, 500 MHz) δ 7.1 (m, 1H), 6.9 (m, 1H), 6.8 (m, 1H), 4.11 (t, *J* = 7.0 Hz, 1H), 2.86 (d, *J* = 7.0 Hz, 2H). ¹³C NMR (CDCl₃, 125 MHz) δ 127.68, 127.41, 125.58, 124.60, 118.70, 63.25, 44.84.

3-thienyl acetaldehyde cyanohydrin: ¹H NMR (CDCl₃, 500 MHz) δ 7.09 (m, 3H), 4.60 (t, *J* = 6.25 Hz, 1H), 3.12 (d, *J* = 6.25 Hz, 2H). ¹³C NMR (CDCl₃, 125 MHz) δ 129.05, 127.16, 125.27, 122.65, 119.87, 61.58, 44.90.

Preparation of racemic mandelic acids standards from corresponding cyanohydrins:⁴ 2-bromomandelonitrile (230 mg, 1.08 mmol) was dissolved in conc. HCl (1 mL) and stirred at room temperature for 18 h and then at 70 °C for 24 h. After cooling, the reaction mixture

was extracted with diethyl ether (4 x 2 mL). Organic extracts were, combined, dried over MgSO₄, filtered and concentrated *in vacuo*. 2-bromomandelic acid was isolated as a colorless powder (180 mg, 0.78 mmol, 70 % yield).

Preparation of racemic arylactic acids standards from corresponding amino acids: Phenylalanine (10 mmol, 1.65g) was dissolved in 30 ml 2N H₂SO₄ at room temperature under N₂ (g) atmosphere. Sodium nitrite (1.4 g in 3 ml aqueous solution, 2 eq) solution was added slowly to the reaction mixture over a period of 3-4 hours with vigorous stirring at room temperature under N₂ (g) atmosphere. The reaction mixture was stirred overnight and the phenyllactic acid product was then extracted into diethylether (3 x 30 ml). Combined ether extracts were dried over MgSO₄ and then filtered and concentrated *in vacuo*.⁵

General Method for Enzymatic Preparation of α -hydroxy acids:

(R)-(-)-Mandelic Acid To a solution of mandelonitrile (1.005 g, 7.56 mmol) in 150 mL of sodium phosphate (100 mM) buffer at pH 8 with 10% v/v methanol, that had been N₂ (g) sparged, at 37 °C, was added 9 mg of nitrilase 1 (nitrilase content estimated by PAGE and protein assay). The reaction was conducted under N₂ (g) atmosphere on a rotating platform shaker. Reaction progress was monitored by withdrawing aliquots for HPLC analysis. After 3 h incubation, the reaction mixture was acidified to pH 2 with 1 N HCl and extracted with diethyl ether (4 x 50 ml). Organic fractions were concentrated *in vacuo* and then the residue was taken up in 10% sodium bicarbonate solution. This aqueous solutions was then washed with diethyl ether (3 x 50 ml) and then acidified to pH 2 with 1 N HCl and extracted with diethyl ether (3 x 50 ml). Organic fractions were combined, washed with brine, dried over MgSO₄, filtered and then concentrated *in vacuo*. (R)-(-)-Mandelic acid (933 mg, 6.22 mmol) was isolated as a colorless powder in 86 % yield. ¹H NMR (DMSO-d₆, 500 MHz) δ 12.6 (br, s, 1H) 7.41 (m, 2H), 7.34 (m, 2H), 7.28 (m, 1H), 5.015 (s, 1H). ¹³C NMR DMSO-d₆, 125 MHz) δ 174.083, 140.216, 128.113, 127.628, 126.628, 72.359. MS calc'd for [C₈H₈O₃] 150.07, found 150.9 (ESI +); *ee* = 98 % [HPLC]. [α]₅₉₈²⁰ = -134.6 (*c* = 0.5, methanol).

(-)-2-chloromandelic acid ¹H NMR (DMSO-d₆, 500 MHz) δ 7.75 (m, 1H), 7.44 (m, 1H), 7.34 (m, 2H), 5.34 (s, 1H). ¹³C NMR (DMSO, 298K, 125MHz) δ 173.07, 137.99, 132.10, 129.40, 129.16, 128.71, 127.23. MS calc'd for [C₈H₇ClO₃] 186.0, found 185.0 (LC-MS -). *ee* = 96 % [HPLC]. 92 % yield. [α]₅₉₈²⁰ = -137.6 (*c* = 0.5, ethanol).

(-)-2-bromomandelic acid ¹H NMR (DMSO-d₆, 500 MHz) δ 7.60 (d, *J* = 7.93, 1H), 7.48 (m, 1H), 7.40 (m, 1H), 7.25 (m, 1H), 5.30 (s, 1H). ¹³C NMR DMSO-d₆, 125 MHz) δ 172.994, 139.61, 132.36, 129.65, 128.75, 127.75, 122.68, 71.64. MS calc'd for [C₈H₇BrO₃] 230.0, found 230.9. *ee* = 96% [HPLC]. 92% yield. [α]₅₉₈²⁰ = -116.4 (*c* = 0.5, ethanol).

(-)-2-methylmandelic acid ¹H NMR (DMSO-d₆, 500 MHz) δ 11.78 (bs, 1H) 7.38 (m, 1H), 7.16-7.38 (m, 3H), 5.18 (s, 1H), 2.35 (s, 3H). ¹³C NMR DMSO-d₆, 125 MHz) δ 174.230, 138.62, 135.650, 130.130, 127.49, 126.99, 125.70, 125.70, 69.73, 18.90. MS calc'd for [C₉H₁₀O₃] 166.1, found 165.2. *ee* = 91 % [HPLC]. 86 % yield. [α]₅₉₈²⁰ = -164.4 (*c* = 0.5, ethanol).

(-)-3-chloromandelic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.46 (s, 1H), 7.36 (m, 3H), 5.07 (s, 1H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 173.55, 142.69, 132.81, 130.07, 127.57, 126.36, 125.289, 71.659. MS calc'd for $[\text{C}_8\text{H}_7\text{ClO}_3]$ 186.0, found 185.34 (MALDI TOF -). *ee* = 98 % [HPLC]. 70 % yield. $[\alpha]^{20}_{598} = -120.48$ ($c = 0.5$, methanol).

(-)-3-bromomandelic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.60 (s, 1H), 7.49 (m, 1H), 7.42 (m, 1H), 7.31 (m, 1H), 5.06 (s, 1H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 173.55, 142.92, 130.47, 130.38, 129.28, 125.69, 121.40, 71.60. MS calc'd for $[\text{C}_8\text{H}_7\text{BrO}_3]$ 229.98, found 229.1 (LC-MS). *ee* = 98 % [HPLC]. 82 % yield. $[\alpha]^{20}_{598} = -84.8$ ($c = 0.5$, ethanol).

(-)-4-fluoromandelic acid ^1H NMR (DMSO, 298K, 500MHz) δ 12.65 (s, 1H), 7.44 (m, 2H), 7.17 (m, 2H), 5.91 (s, 1H), 5.03 (s, 1H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 173.93, 162.57, 136.47, 128.61, 128.55, 114.96, 114.80, 71.61. MS calc'd for $[\text{C}_8\text{H}_7\text{FO}_3]$ 170.0, found 168.8. *ee* = 99% [HPLC]. 81% yield. $[\alpha]^{20}_{598} = -152.8$ ($c = 0.5$, methanol).

(-)-1-naphthylglycolic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.28-8.26 (m, 1H), 7.87-7.93 (m, 2H), 7.47-7.58 (m, 4H), 5.66 (s, 1H). ^{13}C NMR DMSO- d_6 , 125 MHz) δ 174.29, 136.28, 133.42, 130.65, 128.35, 128.19; 125.93, 125.69, 125.61, 125.27, 124.56, 70.94. MS calc'd for $[\text{C}_{12}\text{H}_{10}\text{O}_3]$: 202.21 found 201.37 (MALDI TOF -). *ee* = 95% [HPLC]. 90 % yield $[\alpha]^{20}_{598} = -115.4$ ($c = 0.5$, ethanol).

(-)-2-naphthylglycolic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 12.6 (bm, 1H), 7.88-7.93 (m, 4H), 7.48-7.56 (m, 3H), 5.20 (s, 1H). ^{13}C NMR DMSO- d_6 , 125 MHz) δ 174.00, 137.76, 132.64, 132.50, 127.81, 127.66, 127.51, 127.21, 125.99, 125.33, 124.76, 72.47. MS calc'd for $[\text{C}_{12}\text{H}_{10}\text{O}_3]$ 202.21, found 201.37 (MALDI TOF). *ee* = 98% [HPLC]. 68% yield. $[\alpha]^{20}_{598} = -115.4$ ($c = 0.5$, ethanol).

(-)-3-pyridylglycolic acid This Reaction was performed in 100 mM ammonium formate buffer at pH 8. To isolate the product, the reaction mixture was filtered through a 10,000 MWCO membrane to remove enzyme and then concentrated *in vacuo*. ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.56 (s, 1H), 8.36 (d, $J = 4.57$ Hz, 1H), 8.25 (s, 1H), 7.71 (m, 1H), 7.25 (dd, $J = 4.98$, 4.80 Hz 1H), 5.45 (s, 1H). ^{13}C NMR DMSO- d_6 , 125 MHz) δ 165.911, 147.862, 147.251, 139.118, 133.381, 122.746, 71.508. MS calc'd for $[\text{C}_7\text{H}_7\text{NO}_3]$ 153.04, found 154.0 ((MALDI TOF). *ee* = 92% [HPLC], 84% yield, $[\alpha]^{20}_{598} = -65.2$ ($c = 0.5$, H_2O).

(-)-3-thienylglycolic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.48 (m, 1H), 7.45 (d, $J = 2.81$, 1H), 7.10 (m, 1H), 5.09 (s, 1H), 3.33 (s, 1H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 173.70, 141.11, 126.45, 126.04, 122.25, 68.92 MS calc'd for $[\text{C}_6\text{H}_6\text{O}_3\text{S}]$ 158.00, found 157.224 (MALDI TOF). *ee* = 95 % [HPLC]. 70 % yield. $[\alpha]^{20}_{598} = -123.28$ ($c = 0.5$, methanol).

(S)-(-)-phenyllactic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.28(m, 5H), 4.17(dd, $J = 4.5$ Hz, $J = 8.3$ Hz, 1H), 2.98(dd, $J = 4.5$ Hz, $J = 13.7$ Hz, 1H), 2.79 (dd, $J = 8.3$ Hz, $J = 13.7$ Hz, 1H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 178.16, 133.4, 129.27, 128.6, 127.3, 70.45, 44.12. *ee* = 97 % [HPLC], 84 % yield. $[\alpha]^{20}_{598} = -17.8$ ($c = 0.5$, methanol).

(-)-2-methylphenyllactic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.16 (m, 4H), 4.47 (dd, $J = 3.9$ Hz, $J = 8.8$ Hz, 1H), 3.25 (dd, $J = 3.9$ Hz, $J = 14.3$ Hz, 1H), 2.94 (dd, $J = 8.8$ Hz, $J = 14.3$ Hz), 2.35 (s, 3H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 178.61, 137.08, 134.74, 130.80, 130.25, 127.44, 126.34, 70.93, 37.67, 19.79. MS calc'd [$\text{C}_{10}\text{H}_{12}\text{O}_3$] 180.08, found 180.0 (ESI +). 86 % yield. $ee = 95$ % [HPLC]. $[\alpha]^{20}_{598} = -13.2$ ($c = 0.5$, methanol).

(-)-2-bromophenyllactic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.28 (m, 4H), 4.60 (dd, $J = 4.0$ Hz, $J = 9.1$ Hz, 1H), 3.45 (dd, $J = 4.0$ Hz, $J = 14.1$ Hz, 1H), 3.04 (dd, $J = 8.0$ Hz, $J = 14.1$ Hz, 1H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 178.70, 136.05, 133.21, 132.10, 128.99, 127.72, 125.0, 70.04, 40.76. MS calc'd for [$\text{C}_9\text{H}_9\text{BrO}_3$] 243.9, found 243.3 (ESI +). 91 % yield. $ee = 93$ % [HPLC], $[\alpha]^{20}_{598} = -17.6$ ($c = 0.5$, methanol)

(-)-2-fluorophenyllactic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.10 (m, 4H), 4.64 (t, $J = 6.8$ Hz, 1H), 3.11 (d, $J = 6.8$ Hz, 2H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 132.18, 131.52, 129.66, 129.03, 128.07, 124.05, 115.8, 63.02, 44.79. MS calc'd for [$\text{C}_9\text{H}_8\text{FNO}$]: 165.06, found 164.2 (ESI +). 91 % yield. $ee = 88$ % [HPLC]. $[\alpha]^{20}_{598} = -14.0$ ($c = 0.5$, methanol).

(-)-3-methylphenyllactic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.18 (m, 1H), 7.02 (m, 3H), 4.54 (dd, $J = 4.6$ Hz, $J = 8.0$ Hz, 1H), 3.06 (dd, $J = 4.54$ Hz, $J = 14.4$ Hz, 1H), 2.83 (dd, $J = 8.0$ Hz, $J = 14.4$ Hz, 1H), 2.36 (s, 3H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 175.88, 163.80, 130.33, 130.09, 125.7, 116.68, 113.75, 71.31, 34.28. MS calc'd for [$\text{C}_{10}\text{H}_{11}\text{NO}$] 161.08, found 162.2 (ESI +). 80 % yield. $ee = 98$ % [HPLC]. $[\alpha]^{20}_{598} = -2.4$ ($c = 0.5$, methanol).

(-)-3-fluorophenyllactic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 7.2 (m, 1H), 6.9 (m, 3H), 4.56 (dd, 4.5 Hz, $J = 7.9$ Hz, 1H), 3.09 (dd, $J = 4.5$ Hz, $J = 14.1$ Hz, 1H), 2.86 (dd, $J = 7.9$ Hz, $J = 14.1$ Hz, 1H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 175.88, 163.80, 130.33, 130.09, 125.7, 116.68, 113.75, 71.31, 34.28. MS calc'd for [$\text{C}_9\text{H}_9\text{O}_3\text{F}$] 184.05, found 184.1 (ESI +). 82 % yield. $ee = 97$ % [HPLC]. $[\alpha]^{20}_{598} = -5.2$ ($c = 0.5$, methanol).

(-)-1-naphthyllactic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.57 (m, 1H), 8.21 (m, 1H), 8.08 (m, 1H), 7.61 (m, 4H), 4.64 (dd, 3.5 Hz, 8.5 Hz, 1H), 3.84 (dd, $J = 3.5$ Hz, $J = 14.5$ Hz, 1H), 3.38 (dd, $J = 8.5$ Hz, $J = 14.5$ Hz, 1H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 177.7, 140.31, 129.74, 129.24, 128.92, 128.26, 127.84, 125.63, 124.53, 124.05, 123.42, 70.58, 38.0. MS calc'd for [$\text{C}_{13}\text{H}_{11}\text{NO}$] 197.08, found 197.1 (ESI +). 87 % yield. $ee = 94$ % [HPLC]. $[\alpha]^{20}_{598} = -16.2$ ($c = 0.5$, methanol).

(-)-2-pyridyllactic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.49 (m, 1H), 7.62 (m, 1H), 7.21 (m, 2H), 4.50 (t, $J = 5.0$ Hz, 1H), 3.01 (d, $J = 5.0$ Hz, 2H). ^{13}C NMR (DMSO, 298K, 125 MHz) δ 178.8, 159.79, 148.84, 136.89, 124.35, 121.75, 71.14, 44.09. MS calc'd for [$\text{C}_8\text{H}_9\text{NO}_3$]: 167.06, found 167.0. (ESI +). 62 % yield. $ee = 94$ % [HPLC], $[\alpha]^{20}_{598} = -3.6$ ($c = 0.5$, methanol).

(-)-3-pyridyllactic acid ^1H NMR (DMSO- d_6 , 500 MHz) δ 8.43 (m, 2H), 7.62 (m, 1H), 7.28 (m, 1H), 4.57 (t, 5.37 Hz, 1H), 2.85 (d, 5.37 Hz, 2H). ^{13}C NMR (DMSO, 298K, 125 MHz)

δ 176.6, 150.03, 147.12, 136.41, 129.45, 123.26, 61.56, 31.46 MS calc'd for $[C_8H_9NO_3]$ 167.06, found 167.0 (ESI +). 59 % yield. *ee* = 94 % [HPLC]. $[\alpha]^{20}_{598} = -4.0$ (*c* = 0.5, methanol).

(-)-2-thienyllactic acid 1H NMR (DMSO- d_6 , 500 MHz) δ 7.18(m, 1H), 6.94(m, 1H), 6.90(m, 1H), 4.49 (dd, *J* = 4.1 Hz, *J* = 6.25 Hz, 1H), 3.36 (dd, *J* = 4.1 Hz, *J* = 15.0 Hz, 1H), 3.26(dd, *J* = 6.25 Hz, *J* = 15.0 Hz, 1H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 127.68, 127.41, 125.58, 124.60, 118.70, 63.25, 44.84. MS calc'd for $[C_7H_7NOS]$ 153.02, found 153.0 (ESI +). 85 % yield. *ee* = 95 % [HPLC]. $[\alpha]^{20}_{598} = -13.0$ (*c* = 0.5, methanol).

(-)-3-thienyllactic acid 1H NMR (DMSO- d_6 , 500 MHz) δ 7.30(m, 1H), 7.13(m, 1H), 7.01(m, 1H), 4.50 (dd, *J* = 4.25 Hz, *J* = 6.5 Hz, 1H), 3.21(dd, *J* = 4.25 Hz, *J* = 15.0 Hz, 1H), 3.10 (dd, *J* = 6.5 Hz, *J* = 15.0 Hz, 1H). ^{13}C NMR (DMSO, 298K, 125MHz) δ 127.50, 136.09, 128.83, 126.24, 123.32, 70.65, 34.84. MS calc'd for $[C_7H_8O_3S]$ 172.02, found 172.1 (ESI +). 81 % yield. *ee* = 96 % [HPLC]. $[\alpha]^{20}_{598} = -18.8$ (*c* = 0.5, methanol).

Enzymic Hydrolysis of 3-Hydroxyglutaronitrile:

3-Hydroxyglutaronitrile (1.0 g, 9.0 mmol, 240 mM) was suspended in N_2 (g) sparged sodium phosphate buffer (37.5 mL, pH 7, 100 mM) at room temperature. Cell lysate (30 mg, normalized for nitrilase content) was added to bring the concentration to 0.8 mg/ml enzyme and the reaction was at shaken at 100 rpm, room temperature. Reaction progress was monitored by TLC (1:1 EtOAc:Hexanes, $R_f=0.32$, nitrile; $R_f=0.0$, acid) After 22 h, the reaction was acidified with 1M HCl. The reaction mixture was continuously extracted with diethyl ether. The acid product was isolated as a yellow oil (1.15 g, 98 % yield). 1H NMR (DMSO, 298K, 500MHz) δ 12.32 (s, 1H), 5.52 (s, 1H), 4.10 (m, 1H), 2.70 (dd, 1H, *J* = 16.8, 4.1 Hz), 2.61 (dd, 1H, *J* = 16.9, 6.3 Hz), 2.44 (dd, 1H, *J* = 15.4, 5.3 Hz), 2.37 (dd, 1H, *J* = 15.6, 7.8 Hz). ^{13}C NMR (DMSO, 298K, 125 MHz) δ 171.9, 118.7, 63.4, 41.2, 25.2 MS calc'd for $[C_5H_7NO_3]$: 129.0, found 130.0 $[M+H^+]$, (ESI +). For *ee* determination, acid product was derivatized to (*R*)-(-)-Methyl (3-*O*-[benzoyl]-4-cyano)-butanoate as described below.

Preparation of (*R*)-(-)-Methyl-4-cyano-butanoate

(*R*)-3-hydroxy-4-cyano butyric acid (658 mg, 5.1 mmol) was dissolved in methanol (20 ml) and the solution was treated with dropwise addition of TMS-diazomethane (2 M solution in hexane, 150 mmol) at 22 °C for 1 hr to yield the methyl ester product. The mixture was concentrated *in vacuo*, extracted with ethyl acetate (3 x 40 ml) and then the combined organic extracts were dried over $MgSO_4$, filtered and concentrated *in vacuo* giving a yellow oil (549 mg, 3.84 mmol, 74 % yield). 1H NMR (DMSO, 298K, 500MHz) δ 5.60 (d, 1H, *J* = 5.26 Hz), 4.13 (m, 1H), 3.60 (s 3H), 2.71 (dd, 1H, *J* = 16.9, 4.2 Hz), 2.63 (dd, 1H, *J* = 16.9, 6.7), 2.54 (dd, 1H, *J* = 14.9, 4.9), 2.44 (dd, 1H, *J* = 15.7, 7.9) ^{13}C NMR (DMSO, 298K, 125MHz) 170.7, 118.6, 63.4, 51.4, 40.9, 25.1 Exact Mass Calculated for $[C_6H_9NO_3]$: 143.1, found: 144.0 $[M+H^+]$. *ee* = 95% [GC].

Preparation of (*R*)-(-)-Methyl (3-*O*-[benzoyl]-4-cyano)-butanoate

Benzoyl chloride (0.068 ml, 0.752 mmol) was added to a stirred solution of (*R*)-methyl-(3-hydroxy-4-cyano)-butanoate (71.7 mg, 0.501mmol) in pyridine (2.0 ml), at room

temperature. After 19 hours, add an additional 0.5 equivalent of benzoyl chloride (0.023ml, 0.251mmol). Reaction was complete at 23 h, as determined by TLC. Add 1ml H₂O, extract with ether (3 x 10ml). Wash with brine (2 x 10ml). Dry combined aqueous extracts with MgSO₄. Filter off drying agent and remove solvent by rotary evaporation. Purify by column chromatography (hexane:ethyl acetate [2:1]). Rotary evaporation of fractions yielded the product as a yellow oil (46 mg, 0.186 mmol, 37%). ¹H NMR (DMSO, 298K, 500MHz) δ 7.96 (d, 2H, J = 7.8), 7.70 (t, 1H, J = 7.25), 7.56 (t, 2H, J = 7.8), 5.55 (m, 1H), 3.59 (s, 3H), 3.13 (m, 2H), 2.90 (m, 2H). ¹³C NMR (DMSO, 298K, 125MHz) δ 169.6, 164.5, 133.8, 129.3, 128.9, 128.5, 117.3, 66.0, 51.8, 37.5, 22.2 MS calc'd for [C₁₃H₁₃NO₄]: 247.25, found 270.3 [M+Na⁺] *ee* = 95% [HPLC]. [α]_D²⁰₅₉₈ -32.4 (c = 0.5, CHCl₃).

Synthesis of (R)-Ethyl-(3-hydroxy-4-cyano)-butanoate

A 0.2 M solution of (R)-3-hydroxy-4-cyano-butanoic acid (50 mg, 0.387 mmol) in anhydrous ethanol (1.94 mL) was prepared. The ethanol solution was added dropwise to 1.0 ml of a 50:50 (v/v) mixture of anhydrous 1 M HCl ethereal solution and anhydrous ethanol over sieves. The reaction was stirred overnight at room temperature under N₂ (g) atmosphere. The reaction was monitored by TLC, (1:1 EtOAc:Hexanes, R_f = 0.45, ester; R_f = 0.0, acid, stained with p-anisaldehyde). After 30 hrs, solvent was removed by rotary evaporation. The crude product was taken up in 25 mL ether, washed with 5 mL saturated bicarbonate and then 5 mL brine. The organic extract was dried over MgSO₄, filtered and then concentrated *in vacuo*, yielding the product as a clear oil. ¹H NMR (DMSO, 298K, 500MHz) δ 5.60 (d, 1H, J = 5.58 Hz), 4.12 (m, 1H), 4.07 (q, 2H, J = 7.1), 2.66 (m, 2H), 2.47 (m, 2H), 1.87 (t, 3H, J = 7.0). ¹³C NMR (DMSO, 298K, 125 MHz) δ 170.21, 118.60, 63.40, 59.98, 41.10, 25.14, 14.02. MS calc'd for [C₇H₁₁NO₃]: 157.1, found 158.2. [M+H⁺] *ee* = 95% [GC].

Amino Acid Sequences of Nitrilase Enzymes (standard one letter amino acid code used)

NITRILASE I

MKEAIKVACVQAAPYMDLEATVVDKTIELMEEAARNNARLIAFPETWIPG
 YPWFLWLDSPA WAMQFVRQYHENSLELDGPQAKRISDAAKRLGIMVTLGM
 SERVGGTL YISQWFIGDNGDTIGARRKLPKPTFVERTLFGEGDGSSLA VFE
 TSVGRLGGLCCWEHLQPLTKYALYAQNEEIHCAA WPSFSLYPNAAKALGP
 DVNVAASRIYAVEGQCFVLASCALVSQSMIDMLCTDDEKHALLAGGGHS
 RIIGPDGGDLVAPLAENEEGILYANLDPGVRLAKMAADPAGHYSRPDIT
 RLLIDRSPKLPVVEIEGDLRPYALGKASETGAQLEEI

NITRILASE II

MGEFGEVTLGVAQAAPVYFDREASTEKARGLIREAGEKGVDLLAFGETWL
 TGYPYWKDAPWSREYNDLRARYVANGVMIPGPETDALCQAAAEAGVDVAI
 GVVELEPGSLSSVYCTLLFISREGEILGRHRKLPKPTDSERRYWSEGDATG
 LRVYERPYGRLSGLNCWEHLMMLPGYALAAQGTQFHVAA WPNMASSASEL
 LSRAYAYQAGCYVLCAGGLGPAPGELPDGIAAESLDHLTGESCIIDPWGK
 VIAGPVSCEETLITARVSTASIYRRKSLTDVGGHYSRPDVFRFEVDRSER
 PRVFRDGDVDDRG

NITRILASE III

MKVVKAAAVQISPVLYSREATVEKVVKKIHELGLGVQFATFPETVVPYY
PYFSAVQTGIELLSGTEHLRLLDQAVTVPSPATDAIGEAAARKAGMVVSIG
VNERDGGTLYNTQLLFDADGTLIQRRRKITPTHFERMIWGQGDGSGLRV
DSKVGRIGQLACFEHNNPLARYALIADGEQIHSAMYPGSAFGEGFAQRME
INIRQHALES GAFVFNATAWLDADQQAQIIKDTGCGIGPISGGCFTTIVA
PDGMLMAEPLRSGEGEVIVDLDFTLIDRRKMLMDSAGHYNRPELLSLMID
RTATAHVHERAAHPVSGAEQGPEDLRTPAA

References:

1. Sambrook, J. Fritsch, EF, Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual (2nd ed.), Cold Spring Harbor Laboratory Press, Plainview NY.
2. (a) For mandelic, 2-chloromandelic, 2-methyl mandelic, 3-chloromandelic, 3-bromomandelic and 4-fluoromandelic acid see Hoover, J.R. E.; Dunn, G. L.; Jakas, D.R.; Lam, L.L.; Taggart, J. J.; Guarini, J.R.; Phillips, L. *J. Med. Chem.* **1974**, *17*(1), 34-41. (b) For 2-bromo mandelic acid see Collet, A.; Jacques, J.; *Bull. Soc. Chem. Fr.* **1973**, *12*, 3330-3331. (c) For 1- and 2-naphthylglycolic acid see Takahashi, I; Y. Aoyagi, I. Nakamura, Kitagawa, A., Matsumoto, K., Kitajima, H. Isa, K. Odashima, K. Koga, K. *Heterocycles* **1999**, *51*(6), 1371-88. (d) For 3-thienylglycolic acid Gronowitz, S. Ark. Kemi, **1957**, *11*, 519-525.
3. Beard, T. Cohen, M. A. Parratt, J.S. Turner, N. J. *Tetrahedron:Asymm.* **4**(6), **1993**, 1085-1104.
4. Stoughton, R.W. *J. Am. Chem. Soc.* **1941**, *63*, 2376.
5. Kenji, I.; Susumu, A.; Masaru, M.; Yasuyoshi, U.; Koki, Y.; Koichi, K. Patent Number, WO0155074, Publication date: 2001-08-02.