Supplementary Information

A study on the chiral inversion of mandelic acid in humans

Maksims Yevglevskis, Catherine R. Bowskill, Chloe C. Y. Chan, Justin H.-J. Heng, Michael D. Threadgill, Timothy J. Woodman, and Matthew D. Lloyd*
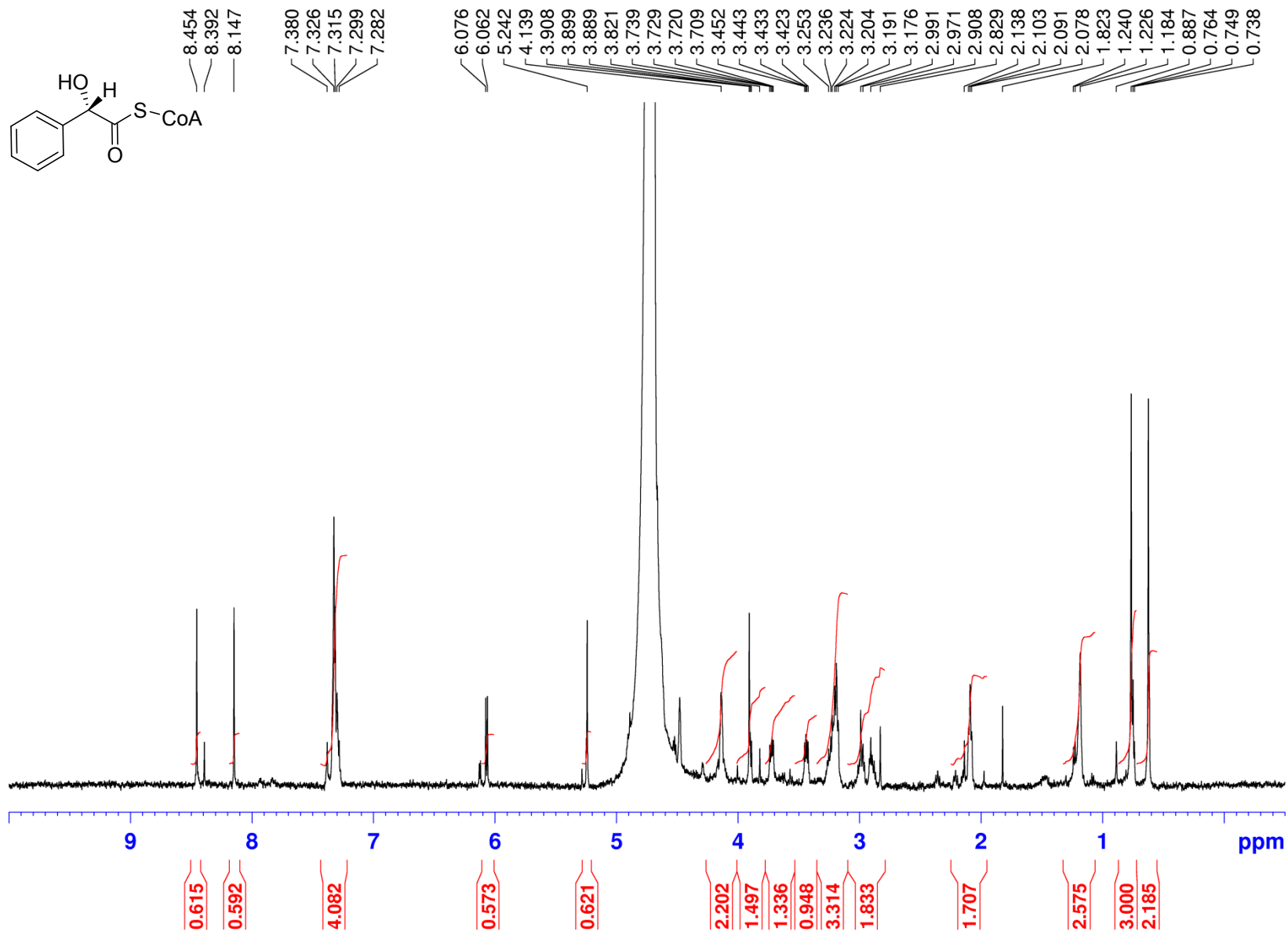
Medicinal Chemistry, Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, U. K.
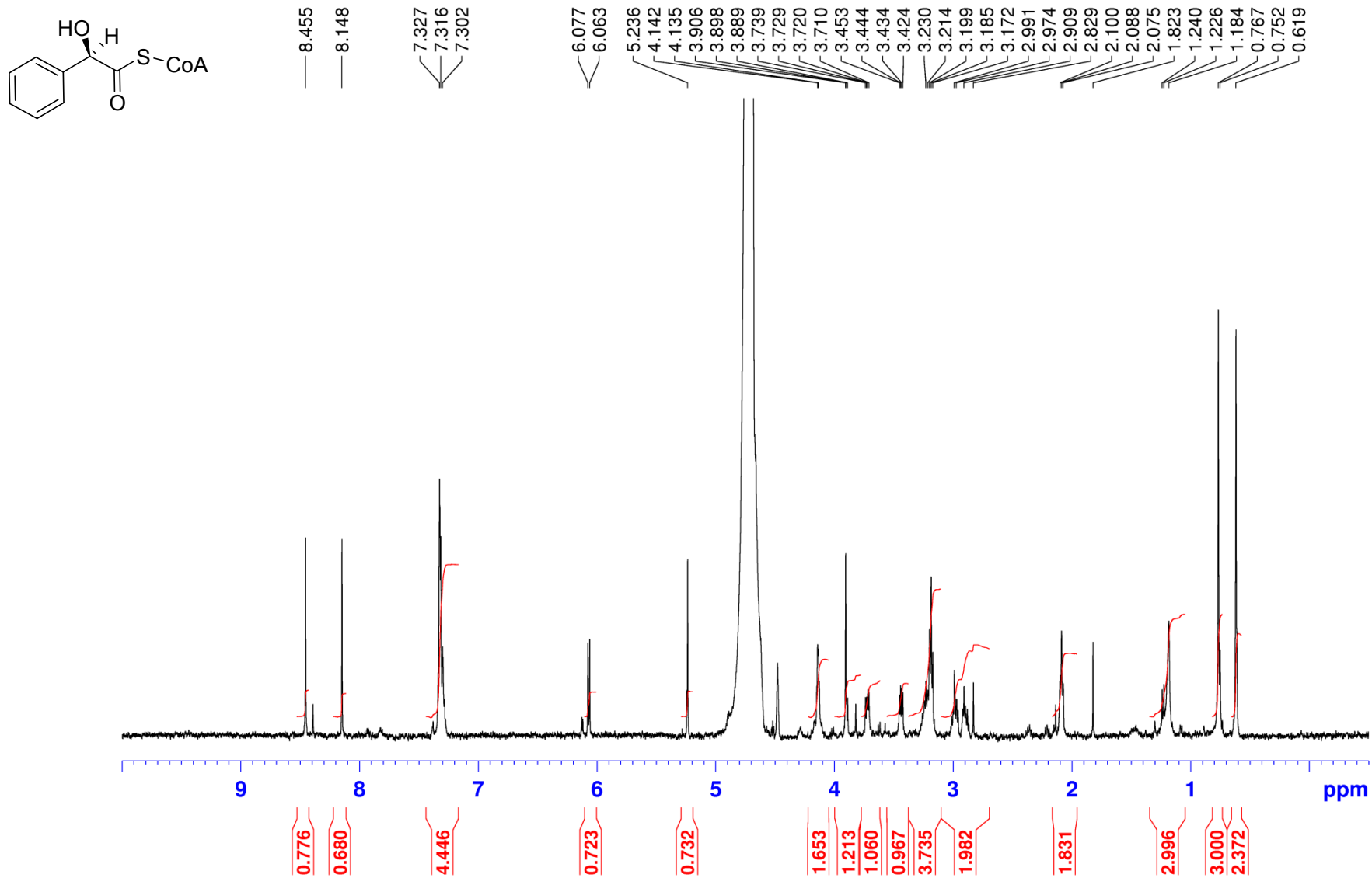
Email: M.D.Lloyd@bath.ac.uk
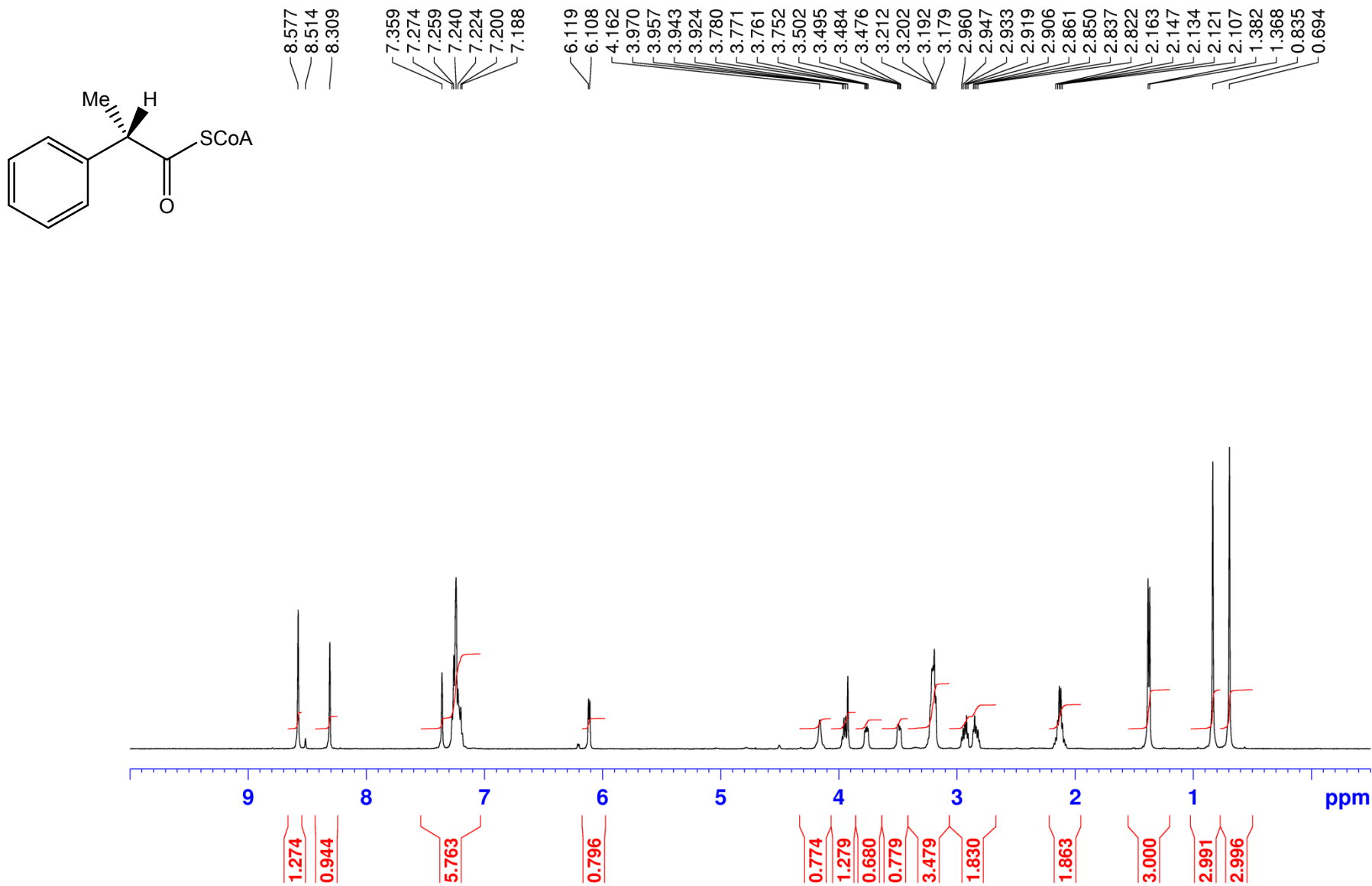
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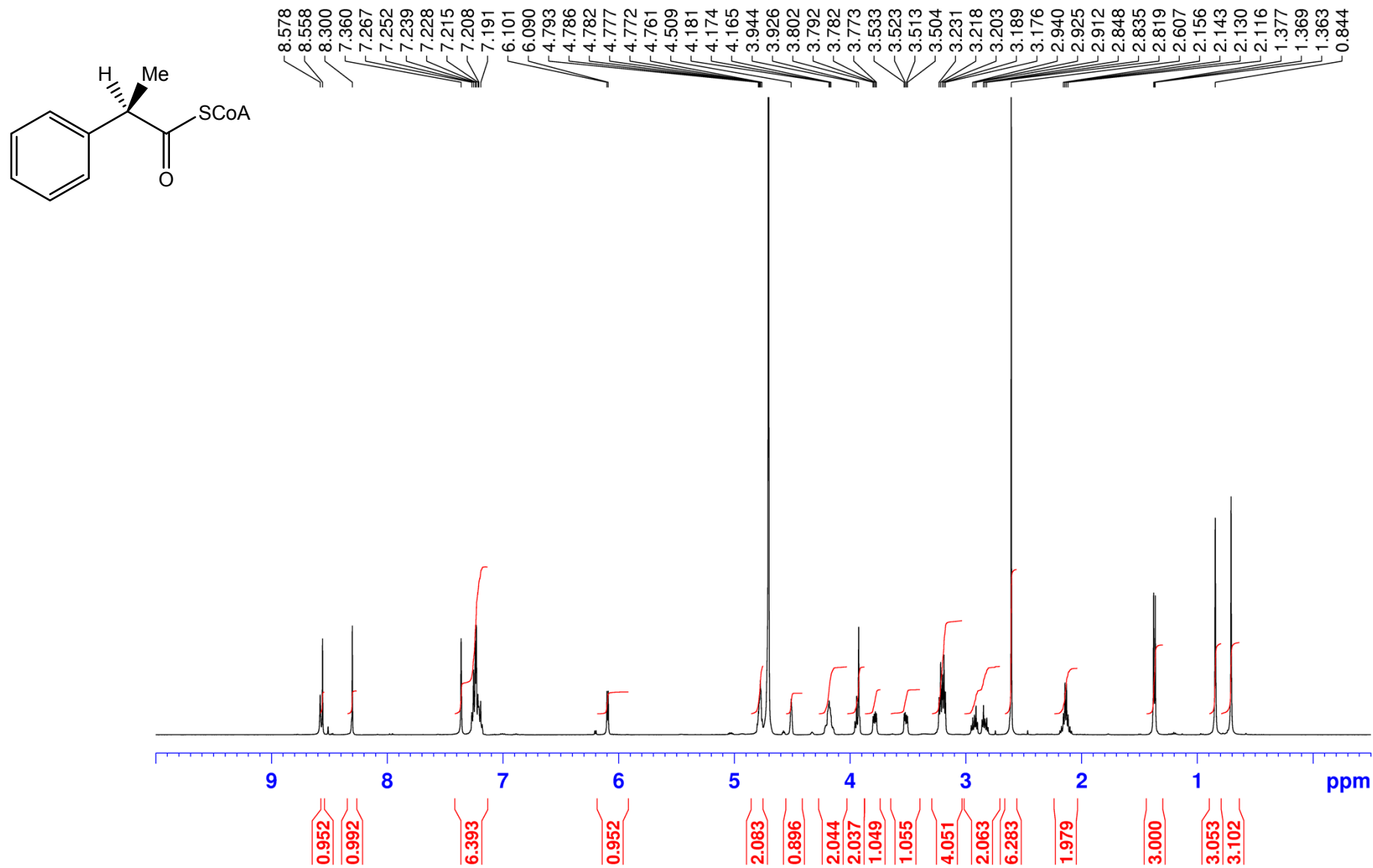*S*-2-Hydroxyphenylacetyl-CoA **2*S*** (¹H NMR, 500.13 MHz)

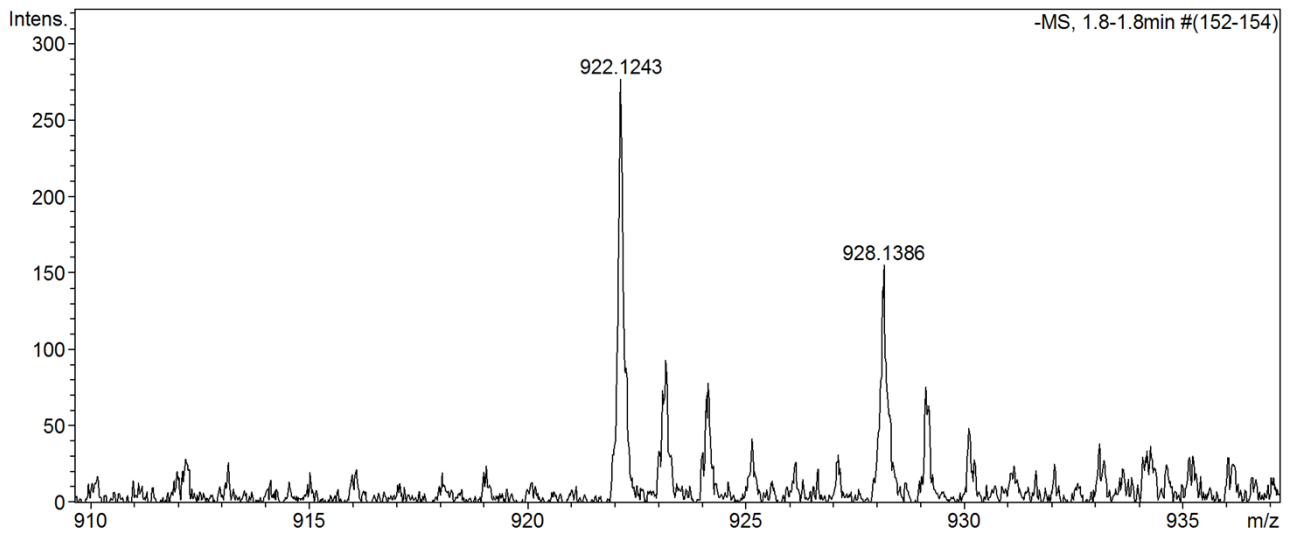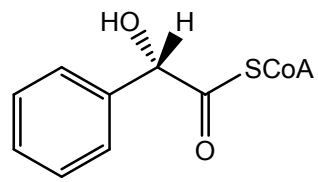*R*-2-Hydroxy-2-phenylacetyl-CoA **2R** (^{1}H NMR, 500.13 MHz)

S-2-Methyl-2-phenylacetyl-CoA **8S** (¹H NMR, 500.13 MHz)

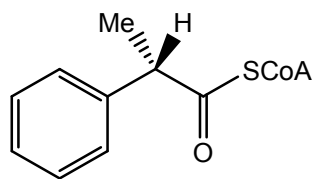*R*-2-Methyl-2-phenylacetyl-CoA **8R** (^{1}H NMR, 500.13 MHz)

8.578, 8.558, 8.300, 7.360, 7.267, 7.252, 7.239, 7.228, 7.215, 7.208, 7.191, 6.101, 6.090, 4.793, 4.786, 4.782, 4.777, 4.772, 4.761, 4.509, 4.181, 4.174, 4.165, 3.944, 3.926, 3.802, 3.792, 3.782, 3.773, 3.533, 3.523, 3.513, 3.504, 3.231, 3.218, 3.203, 3.189, 3.176, 2.940, 2.925, 2.912, 2.848, 2.835, 2.819, 2.607, 2.156, 2.143, 2.130, 2.116, 1.377, 1.369, 1.363, 0.844

0.952, 0.992, 6.393, 0.952, 2.083, 0.896, 2.044, 2.037, 1.049, 1.055, 4.051, 2.063, 6.283, 1.979, 3.000, 3.053, 3.102
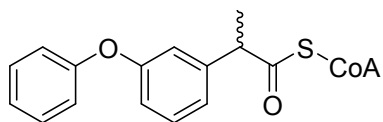
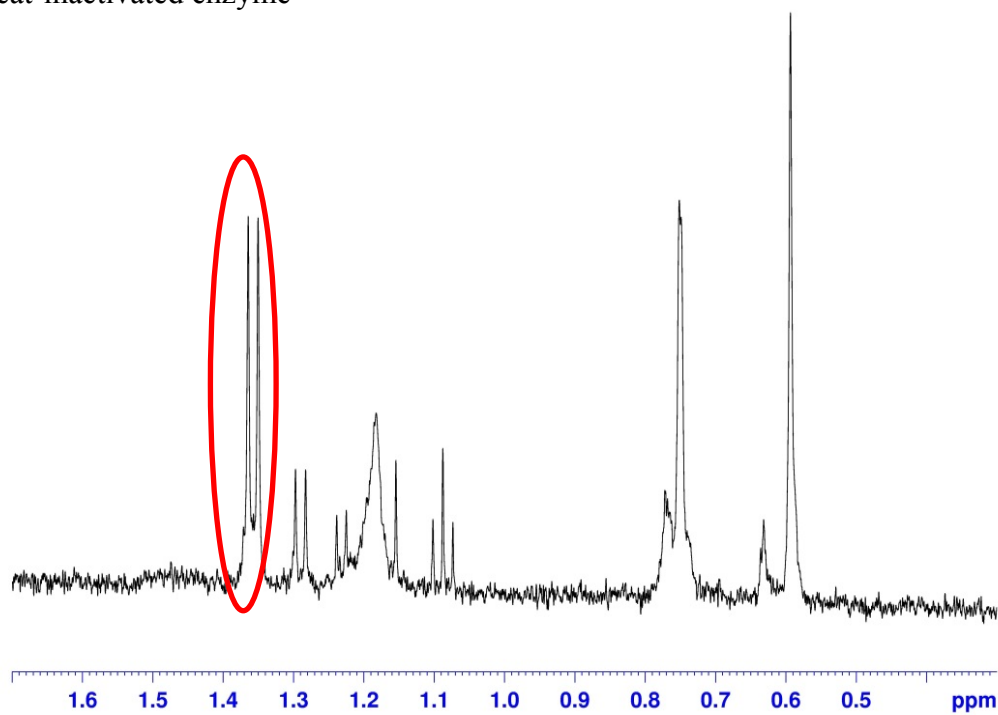## *S*-2-Hydroxy-2-phenylacetyl-CoA 2*S* (as Na adduct)
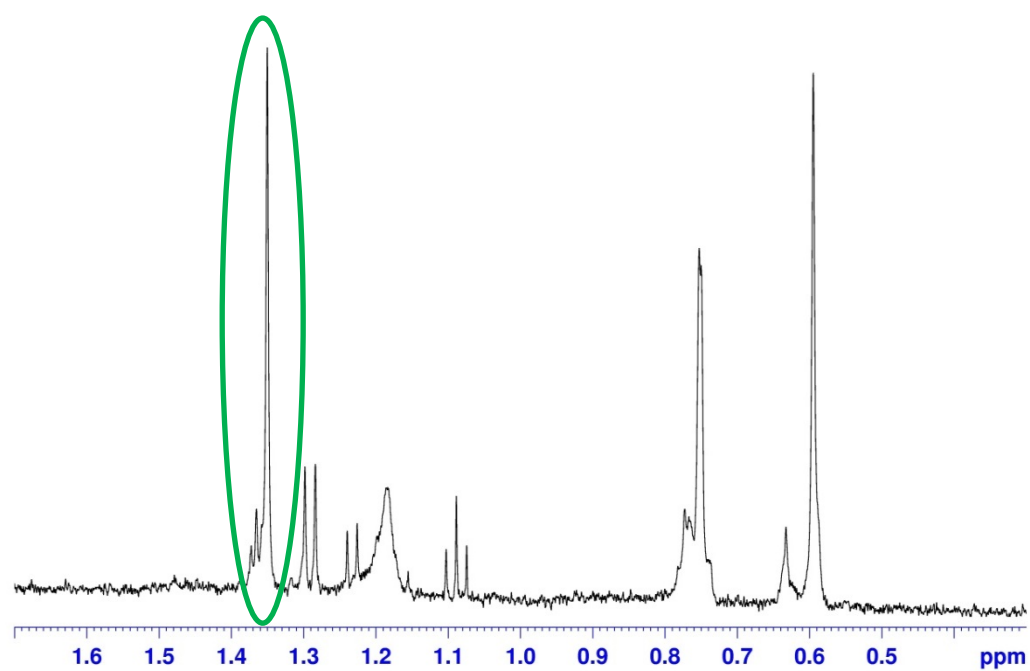
S-2-Phenylpropanoyl-CoA **8*S***
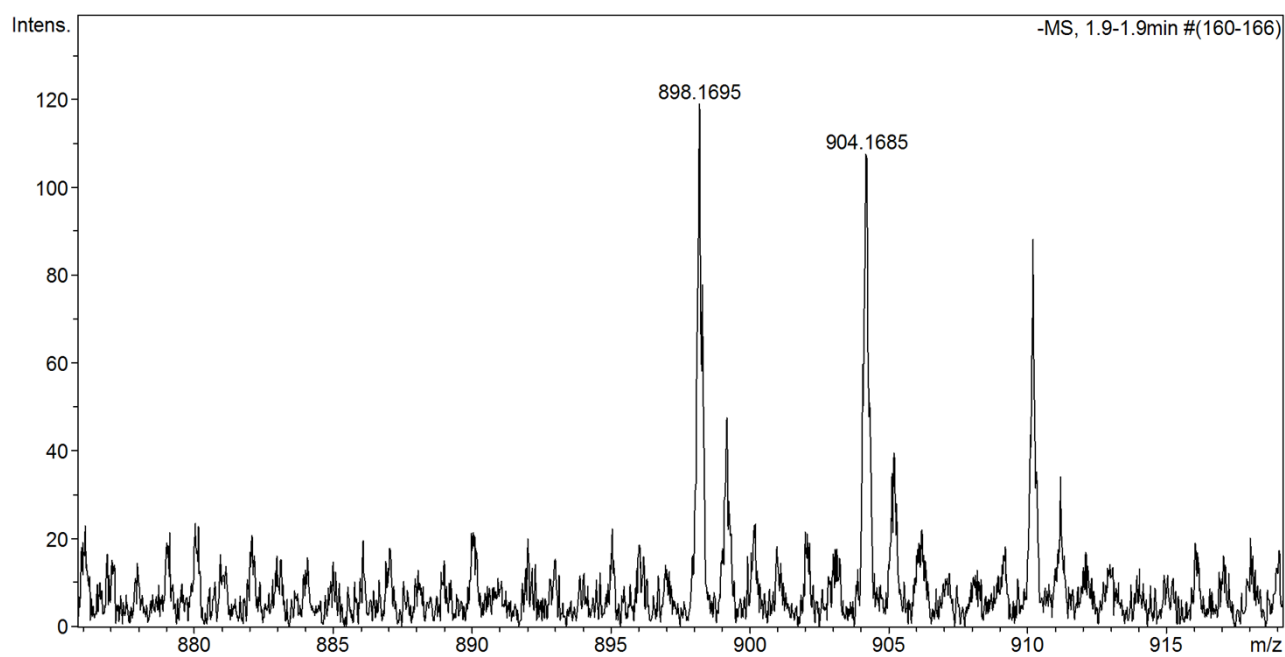
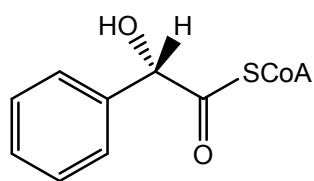Incubation of ±-Fenoprofenoyl-CoA with human recombinant AMACR 1A
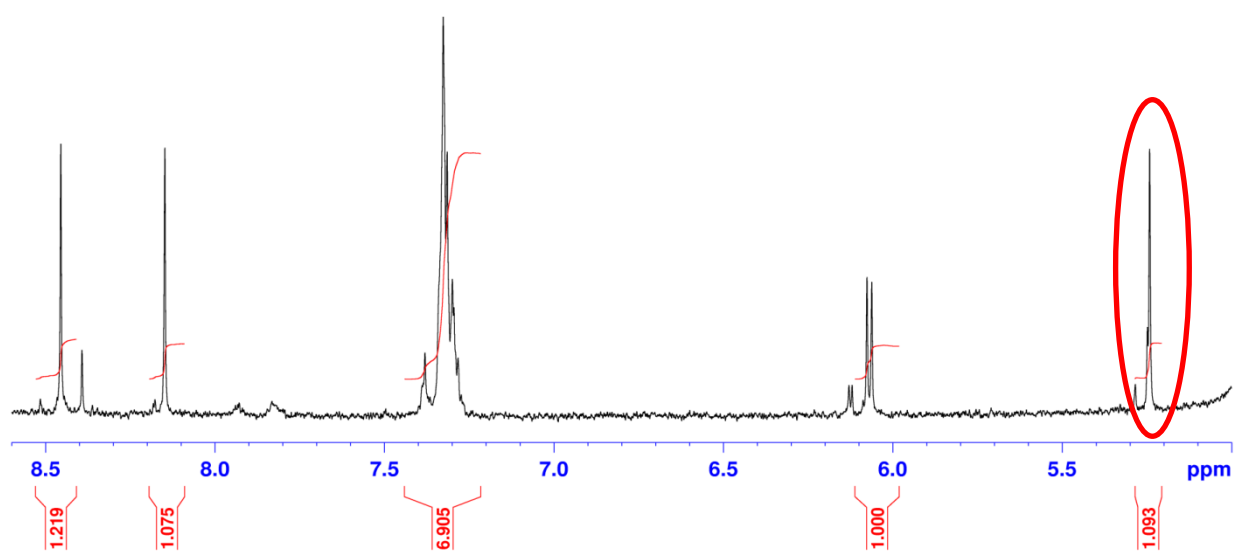

Heat-inactivated enzyme

Live enzyme

Incubation of *S*-2-hydroxy-2-phenylacetyl-CoA **2*S*** with human recombinant AMACR 1A

Heat-inactivated enzyme

Live enzyme

S-2-Hydroxy-2-phenylacetyl-CoA **2S** incubated with heat inactivated AMACR (full spectrum)

S-2-Hydroxy-2-phenylacetyl-CoA **2S** incubated with active AMACR (full spectrum)
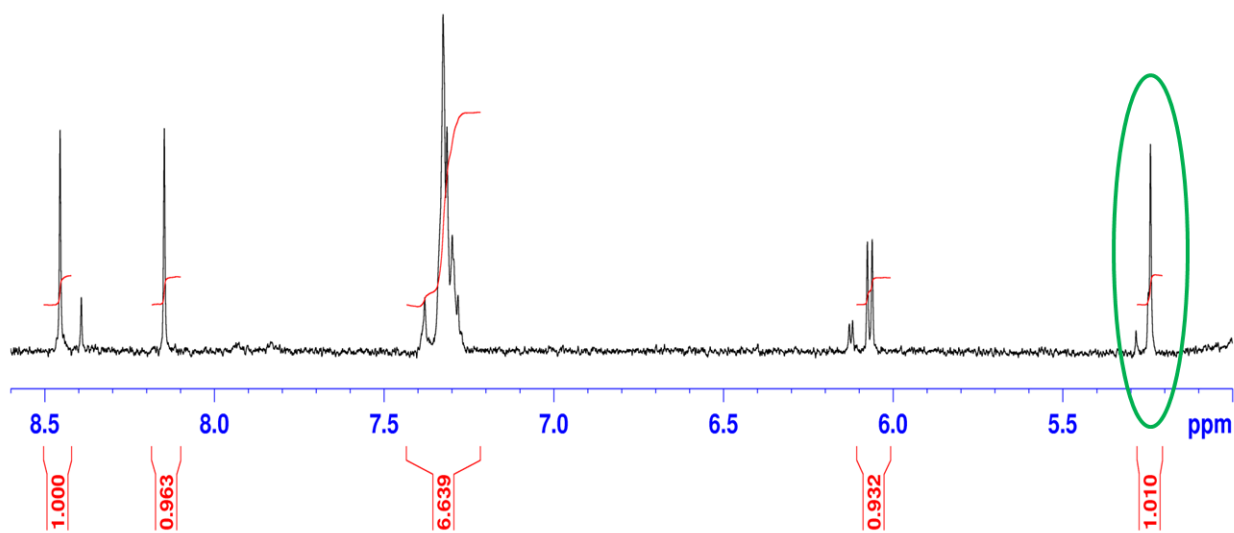
Incubation of *R*-2-hydroxy-2-phenylacetyl-CoA **2R** with human recombinant AMACR 1A
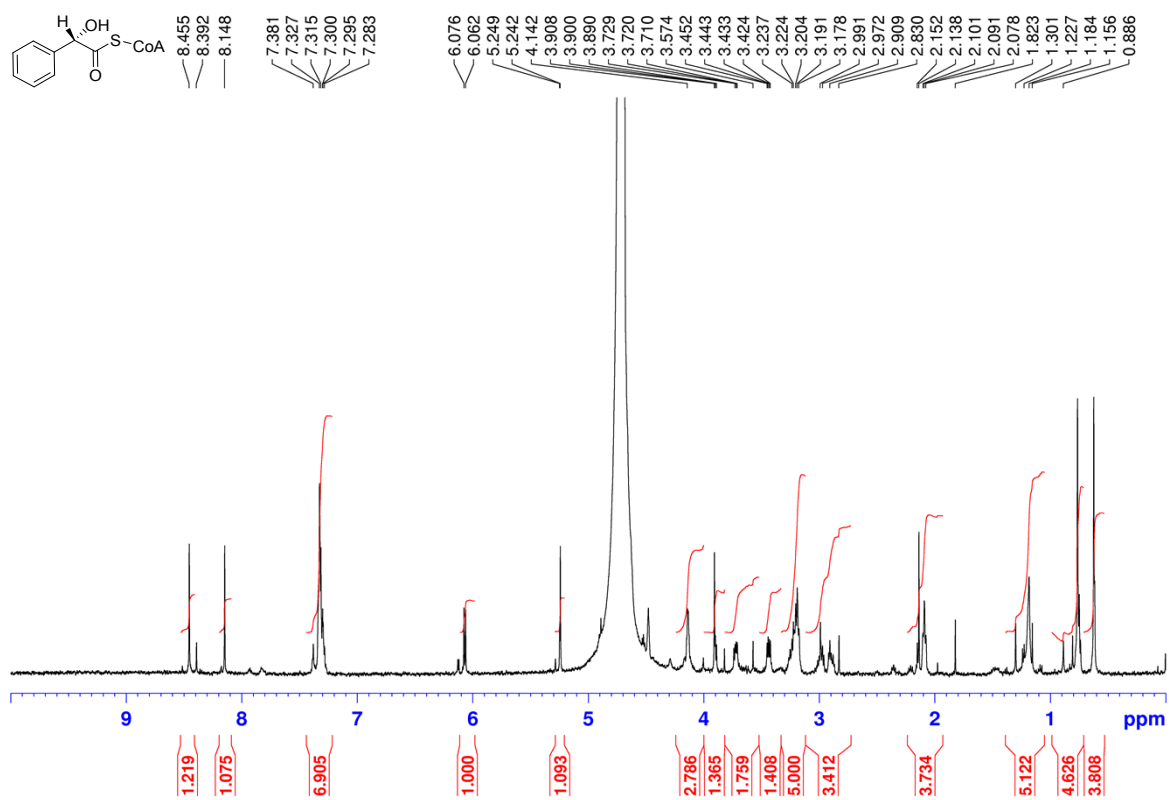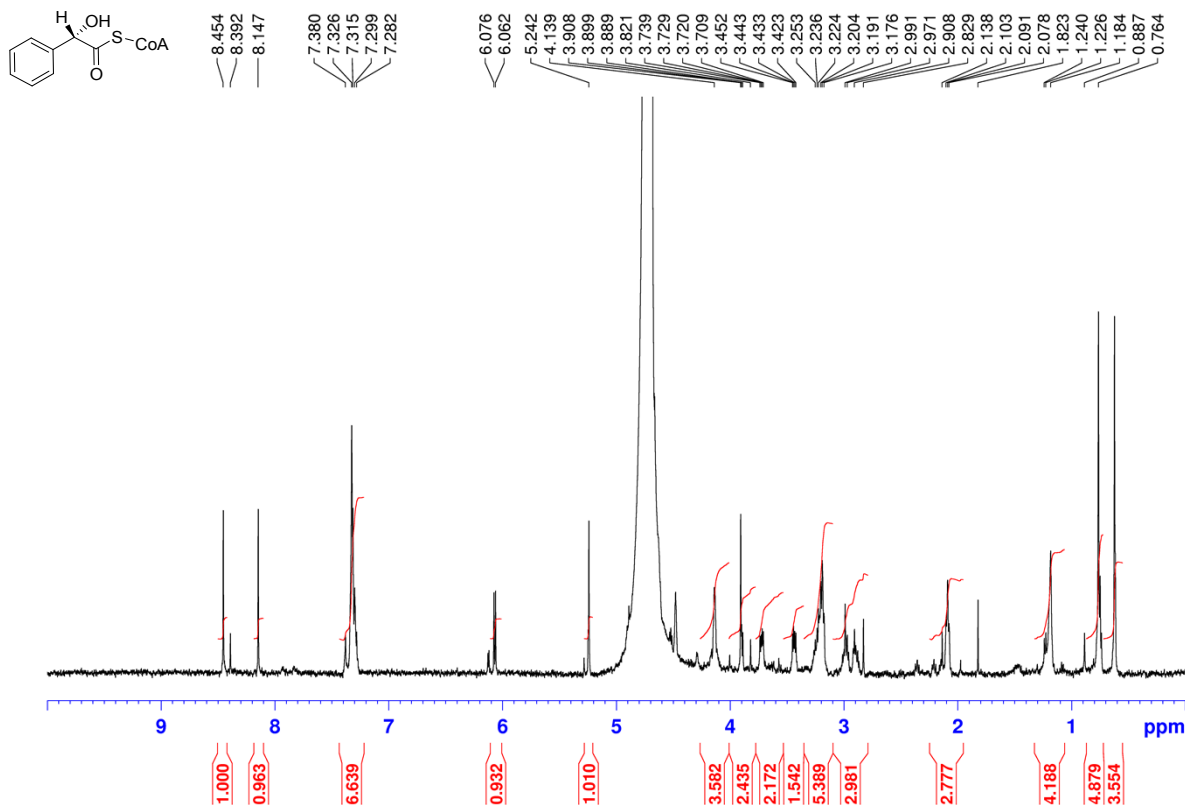

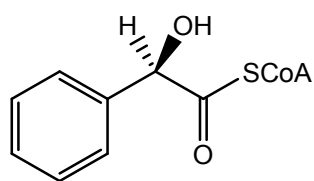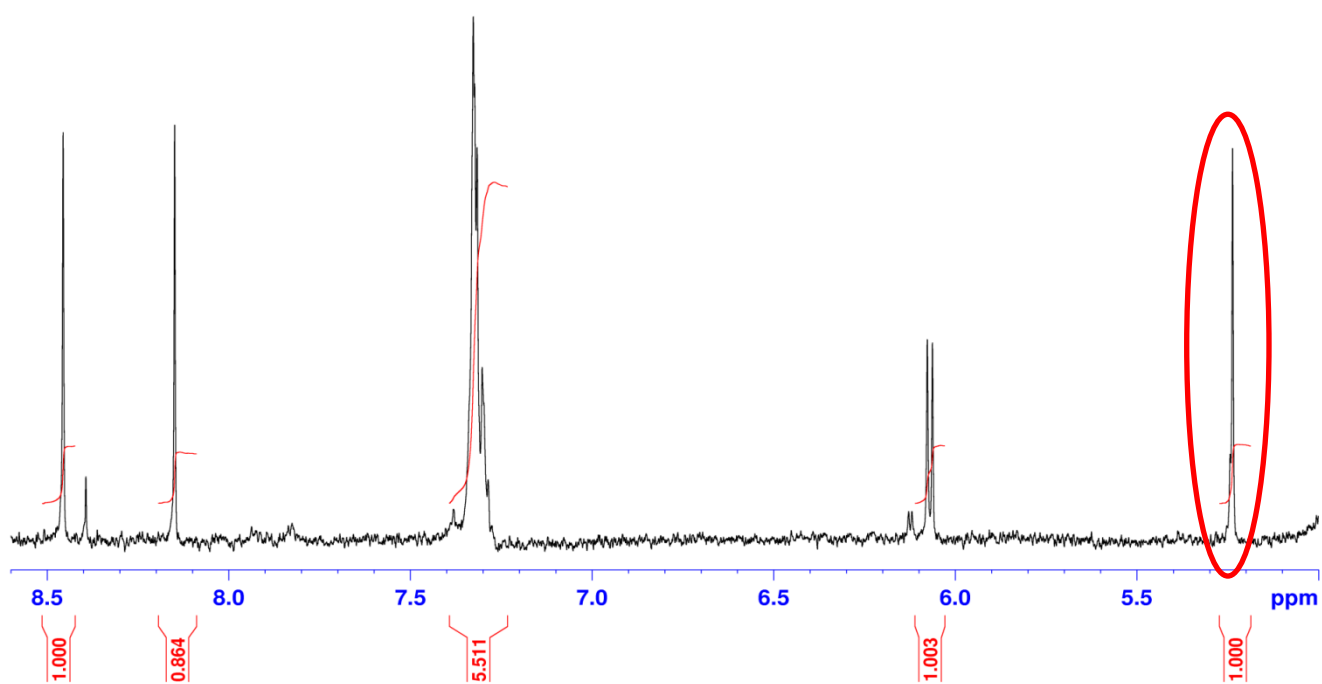Heat-inactivated enzyme

Live enzyme
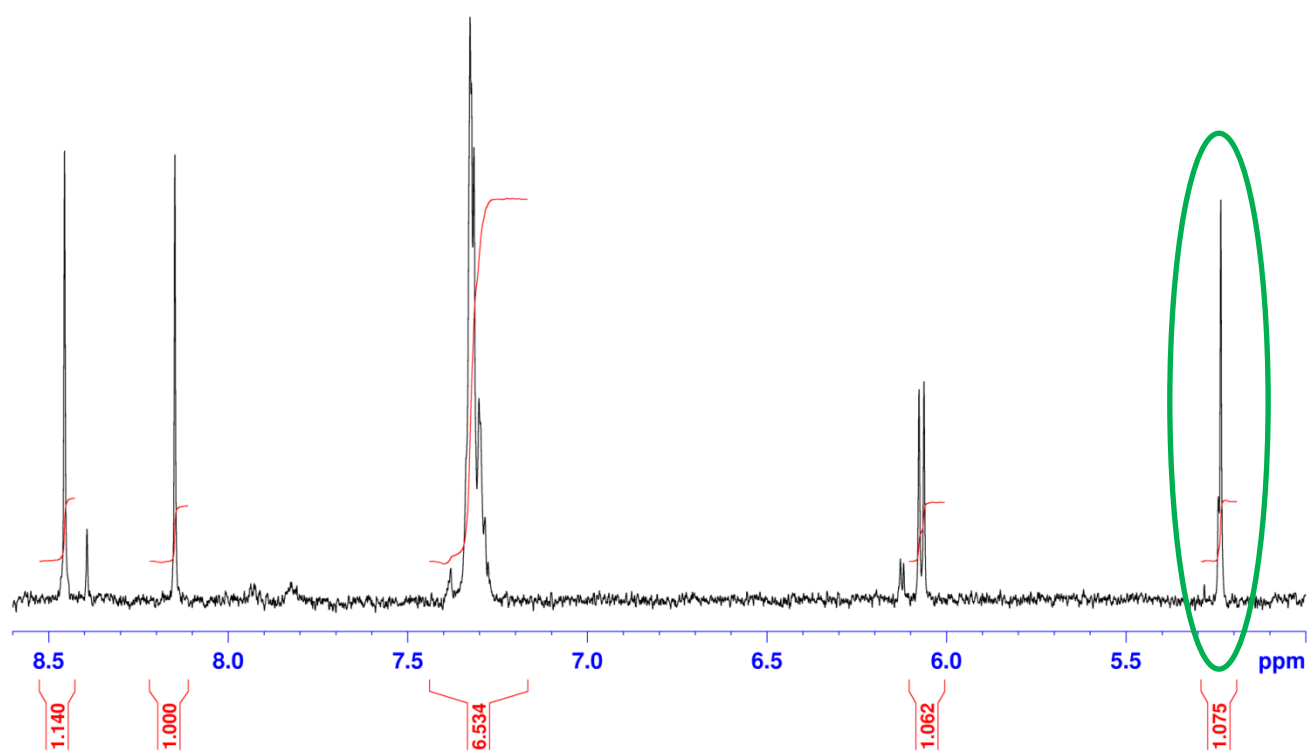
R-2-Hydroxy-2-phenylacetyl-CoA **2R** incubated with heat inactivated AMACR (full spectrum)
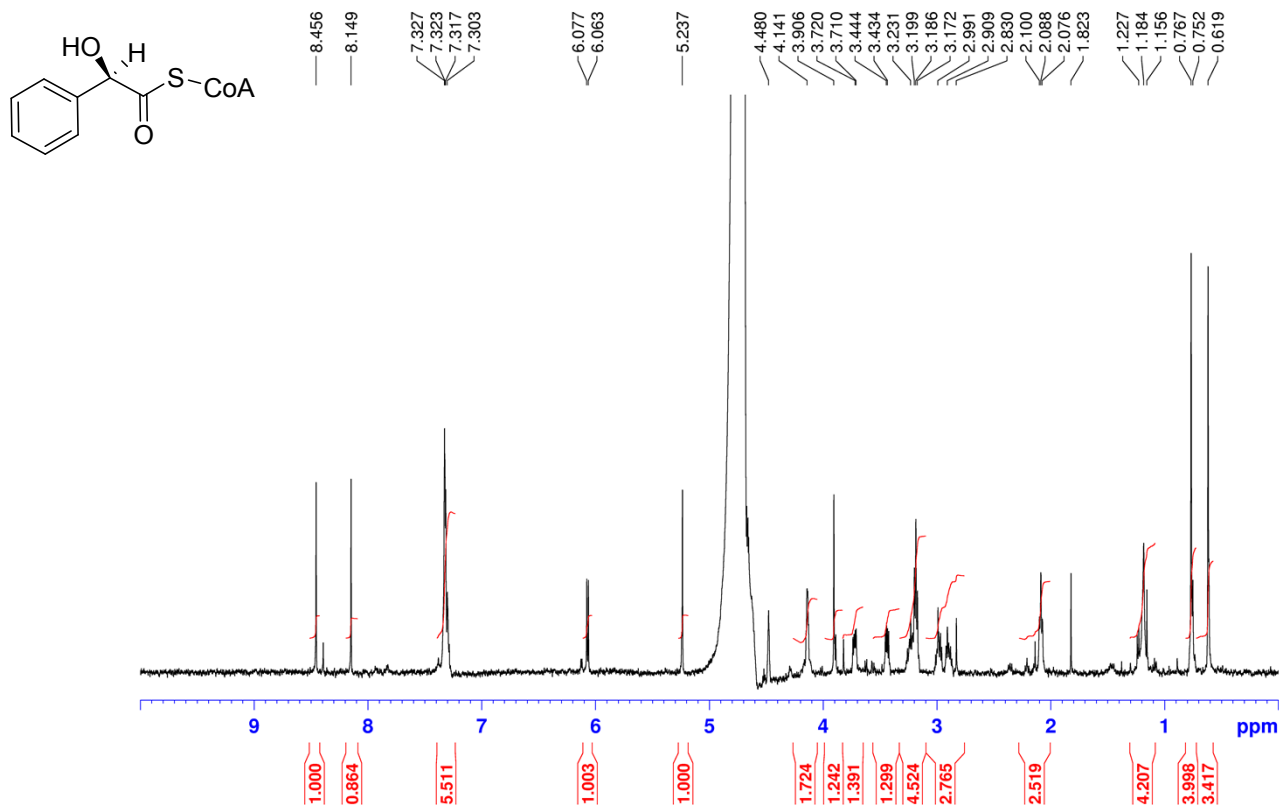
R-2-Hydroxy-2-phenylacetyl-CoA **2R** incubated with active AMACR (full spectrum)
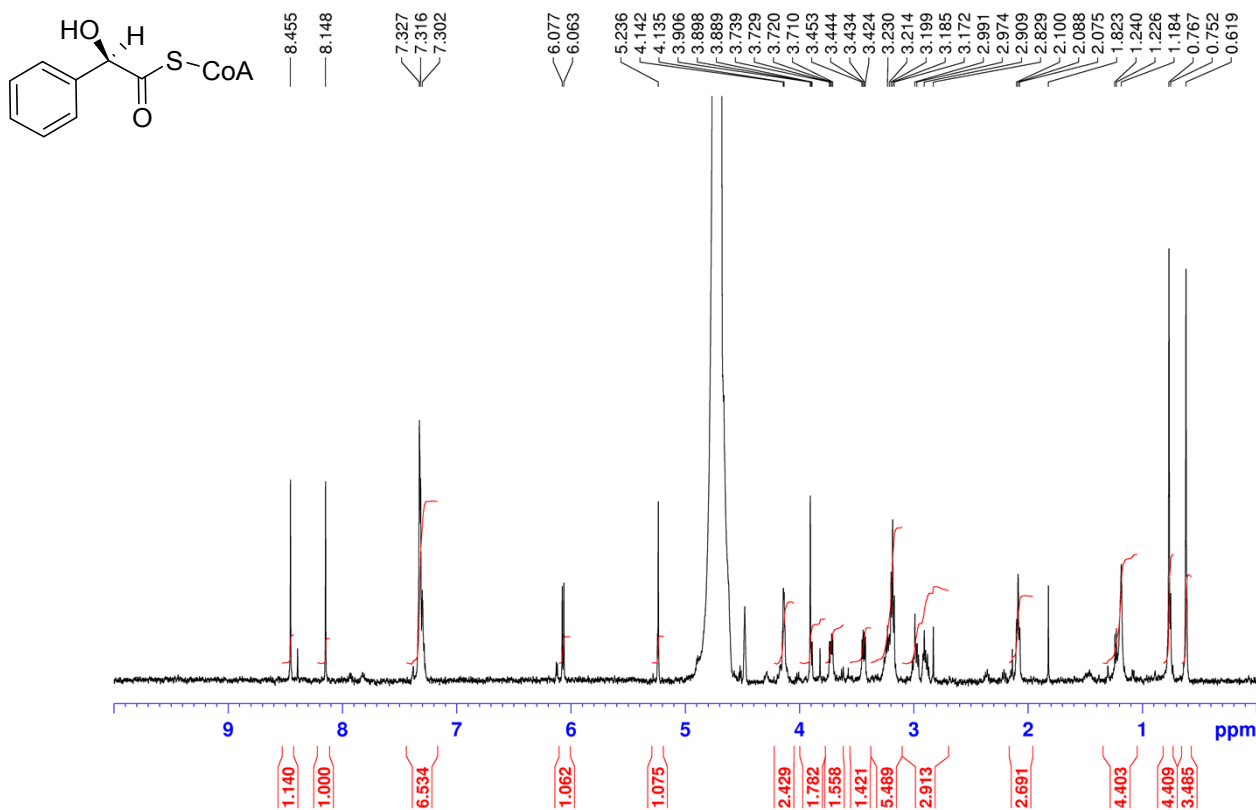
Stack plot showing incubation of **2R** and **2S** with live and heat-inactivated AMACR

2R, Live enzyme
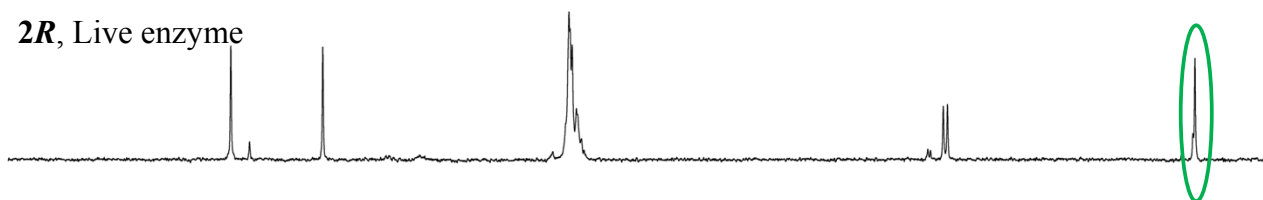
2R, heat-inactivated enzyme
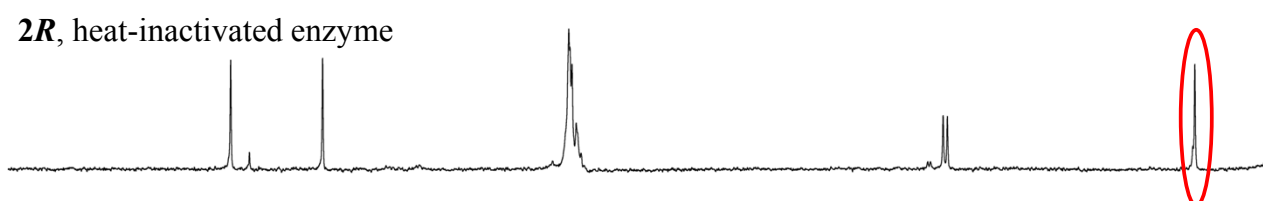
2S, Live enzyme
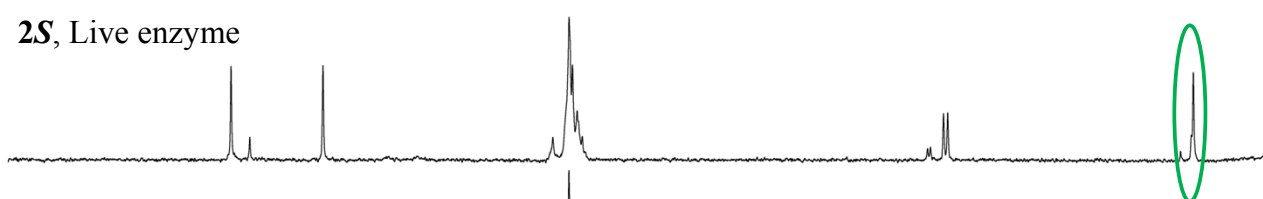
2S, heat-inactivated enzyme
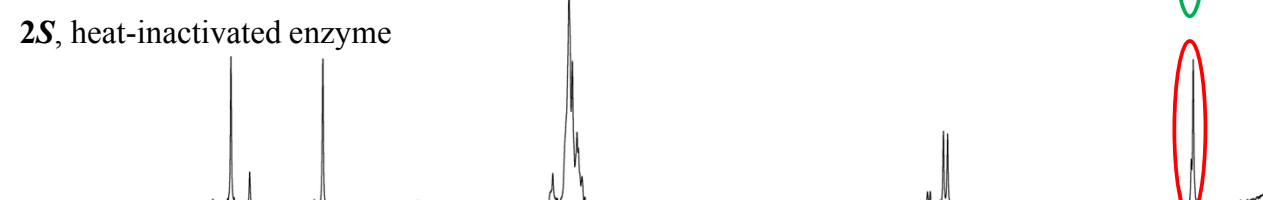
9.0   8.5   8.0   7.5   7.0   6.5   6.0   5.5   ppm

Incubation of *S*-2-phenylpropanoyl-CoA **8S** with human recombinant AMACR 1A
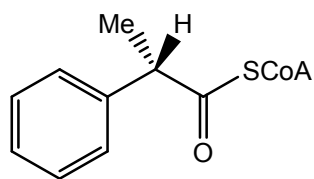
Heat-inactivated enzyme
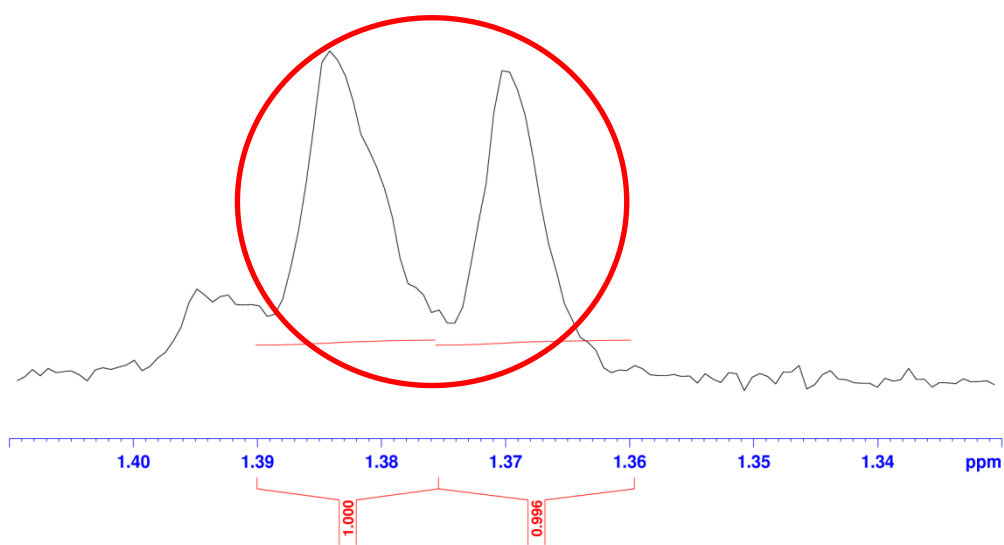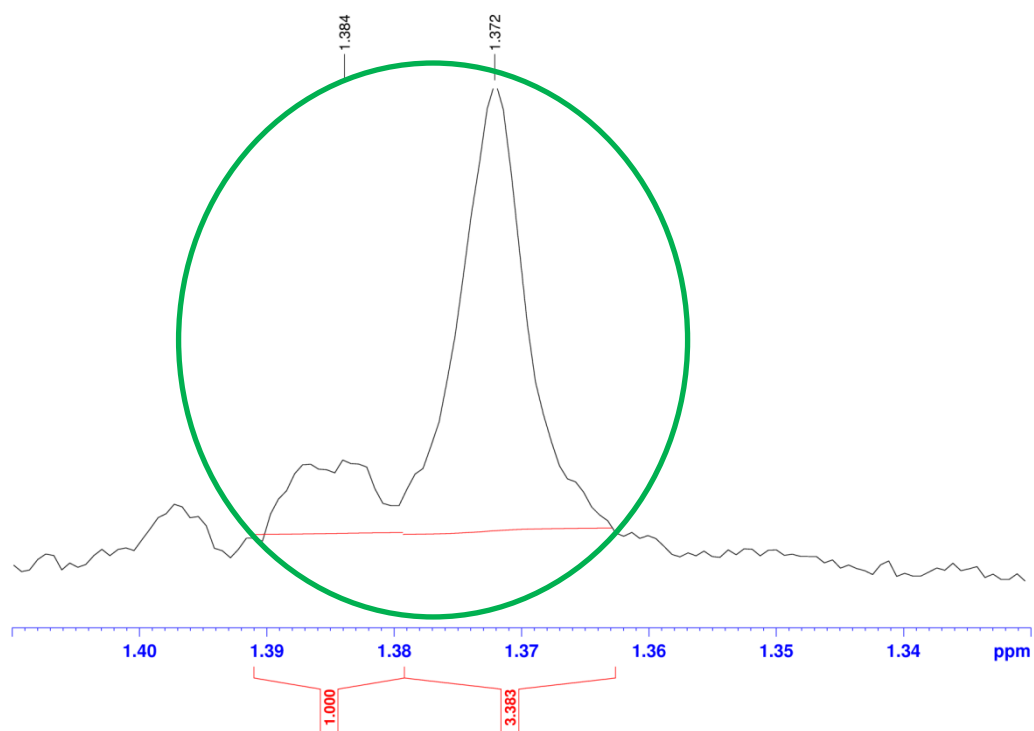
Live enzyme

Incubation of *R*-2-phenylpropanoyl-CoA **8R** with human recombinant AMACR 1A
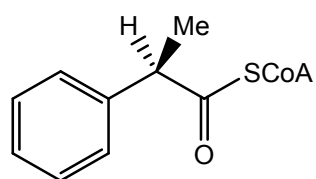
Heat-inactivate enzyme
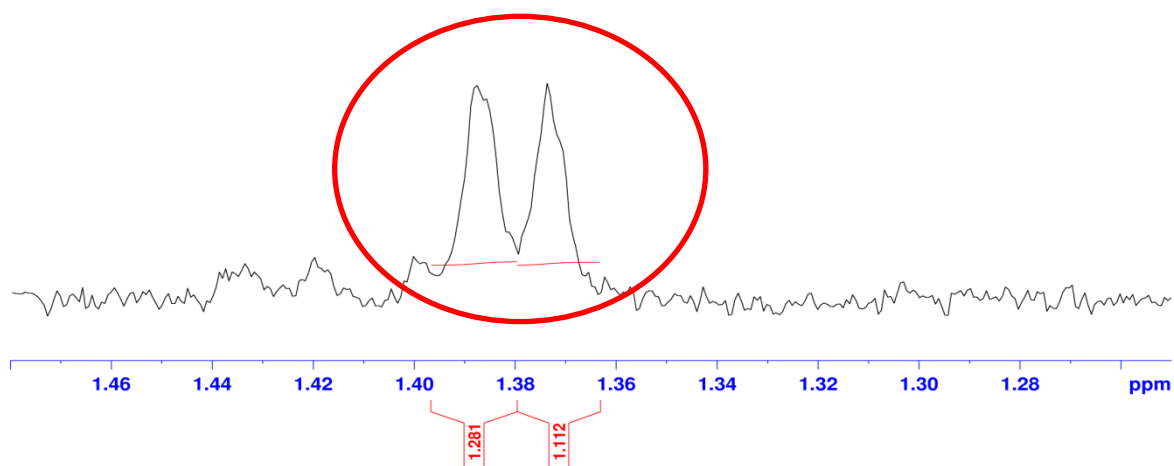
Live enzyme
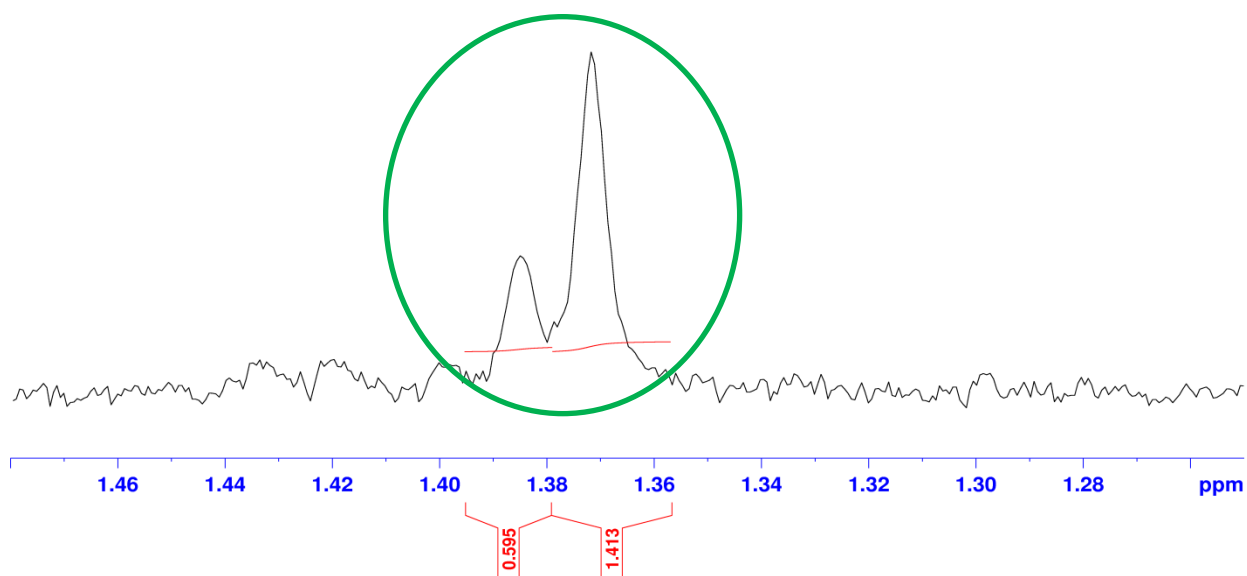
ACOT1, Myristoyl-CoA 11
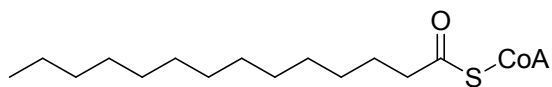
Parameters

	Value	±Std. Error	95% Conf. Interval		
Vmax	68.1711	118.7410	-178.7692	to	315.1114
Km	84.3676	170.3888	-269.9825	to	438.7178
Ki	28.1262	69.3833	-116.1673	to	172.4196

Goodness of Fit

|	
Degrees of Freedom	21
AICc	48.022
R²	0.832
Sum of Squares	116.501
Sy.x	2.355
Runs Test p Value	0.501

Data

Number of x values	8
Number of replicates	3
Total number of values	24
Number of missing values	0

Michaelis-Menten
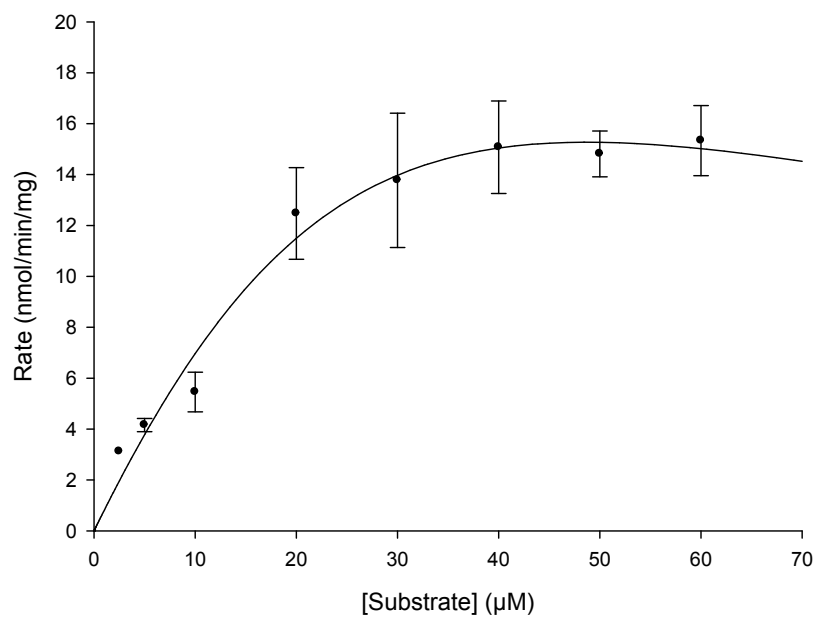
Lineweaver-Burk
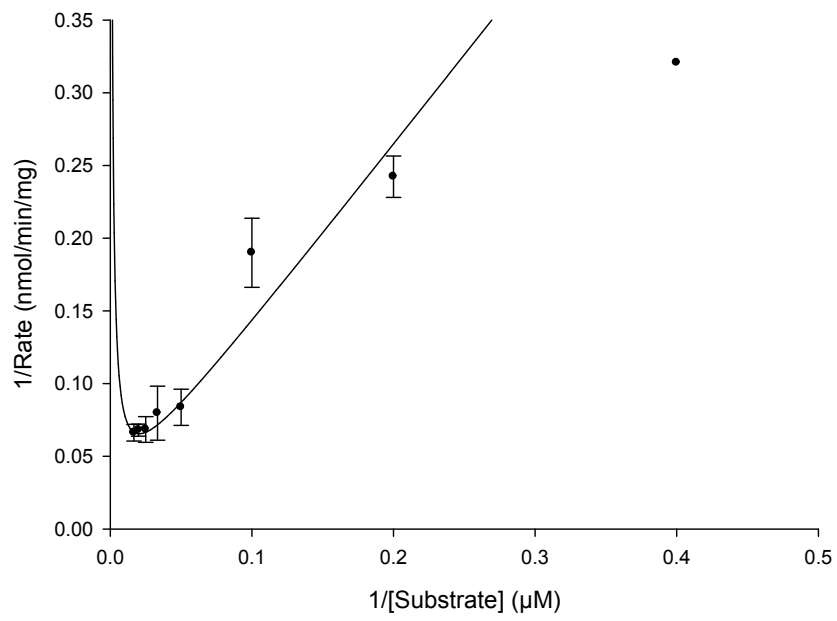
Residuals
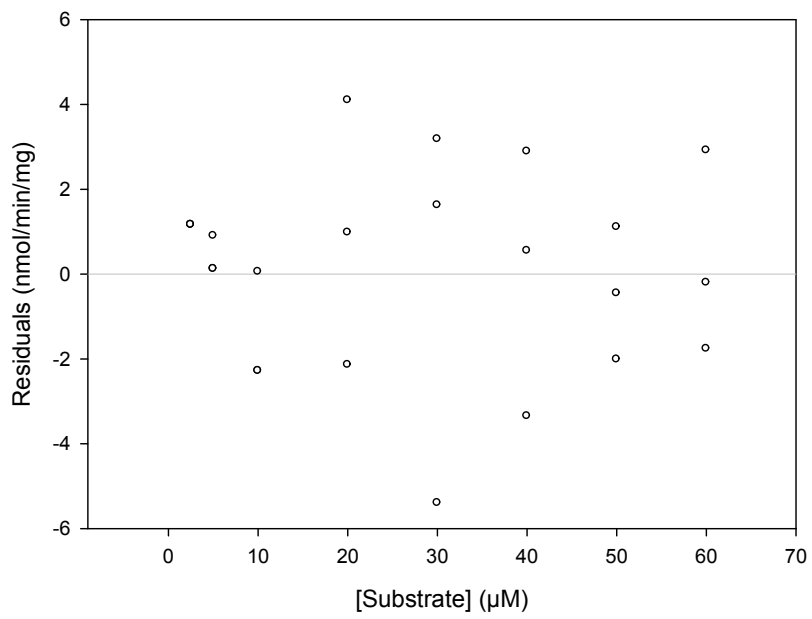
ACOT-1, *S*-2-hydroxy-2-phenylacetyl-CoA **2S**
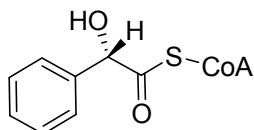
Parameters

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Vmax	75.4081	56.1754	-41.4173	to	192.2335
Km	156.4288	138.3438	-131.2787	to	444.1362
Ki	40.4438	38.0679	-38.7243	to	119.6120

Goodness of Fit

Degrees of Freedom	21
AICc	28.657
R²	0.913
Sum of Squares	51.989
Sy.x	1.573
Runs Test p Value	0.486

Data

Number of x values	8
Number of replicates	3
Total number of values	24
Number of missing values	0

Michaelis-Menten
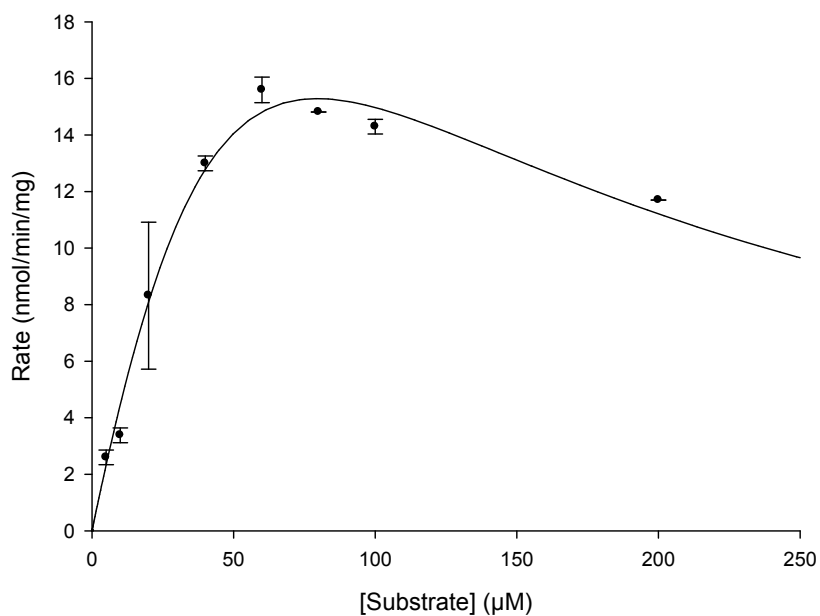
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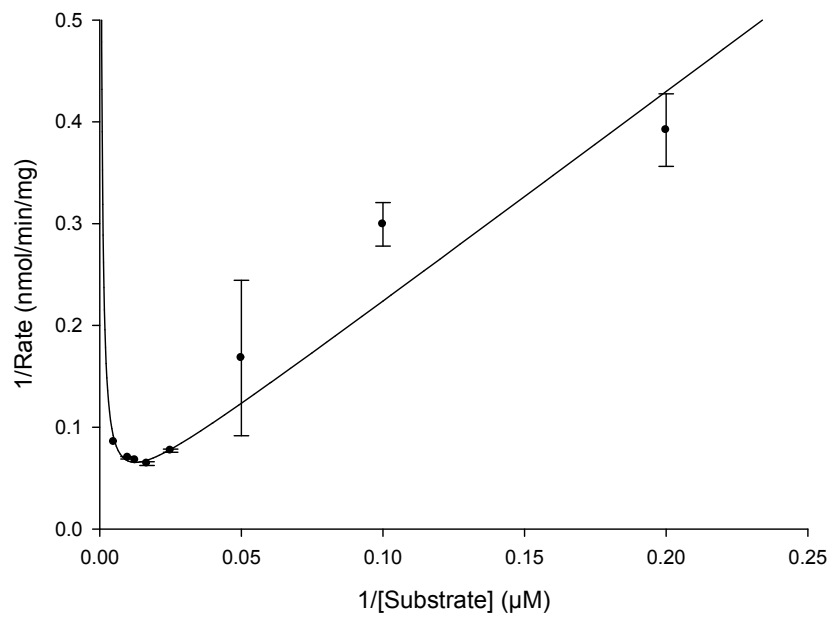
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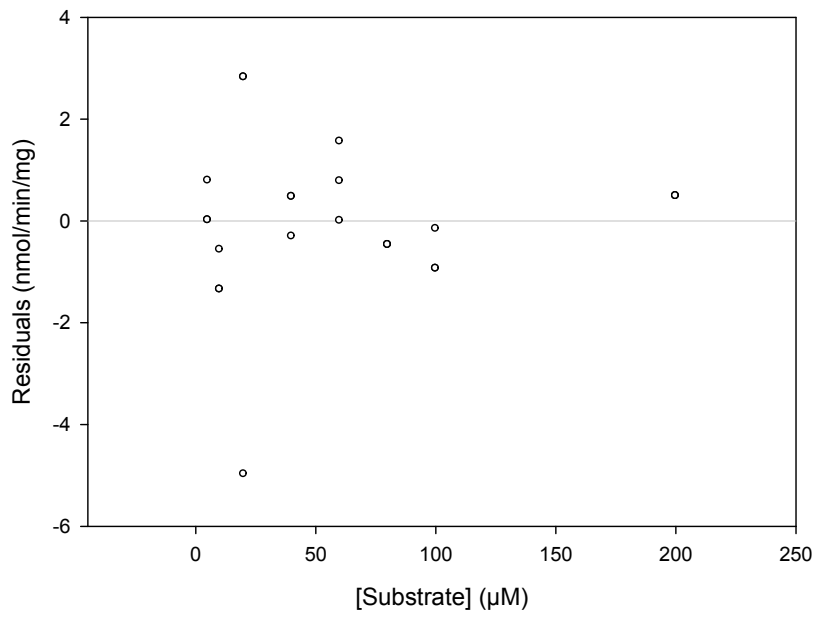
ACOT-1, *R*-2-hydroxy-2-phenylacetyl-CoA **2R**
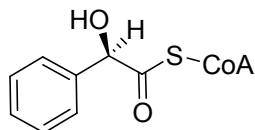
Parameters

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Vmax	12.2541	1.5178	9.0975	to	15.4106
Km	61.6170	18.1924	23.7830	to	99.4509

Goodness of Fit

Degrees of Freedom	21
AICc	13.563
R²	0.837
Sum of Squares	30.247
Sy.x	1.200
Runs Test p Value	0.334

Data

Number of x values	8
Number of replicates	3
Total number of values	23
Number of missing values	1

Michaelis-Menten
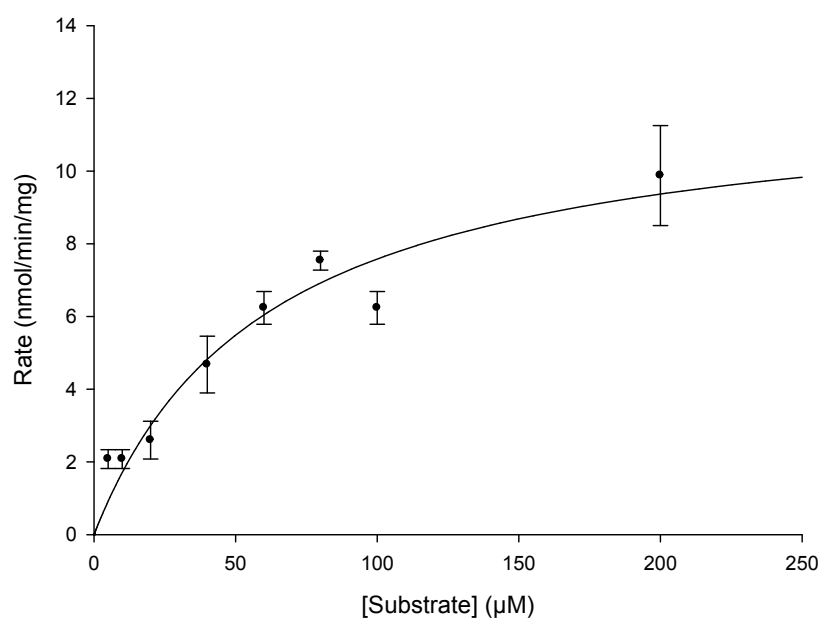
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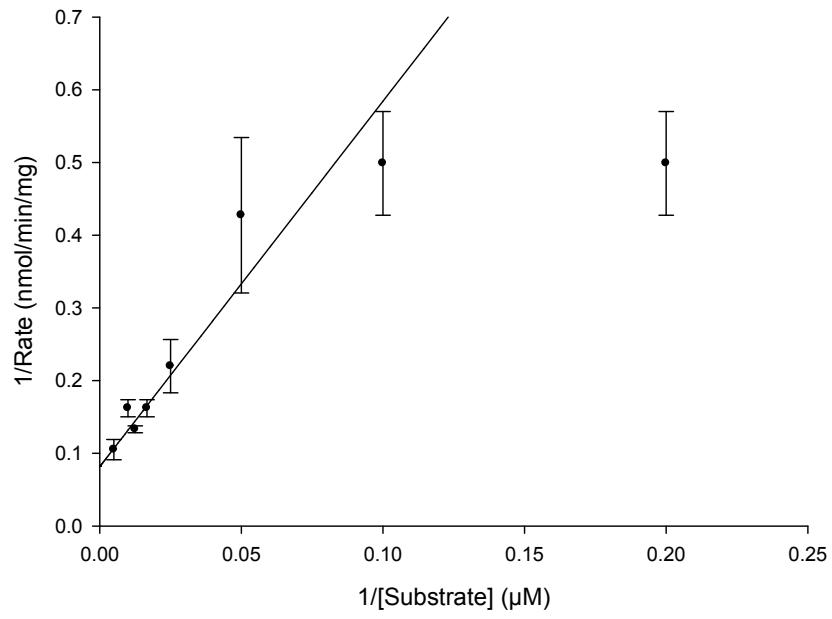
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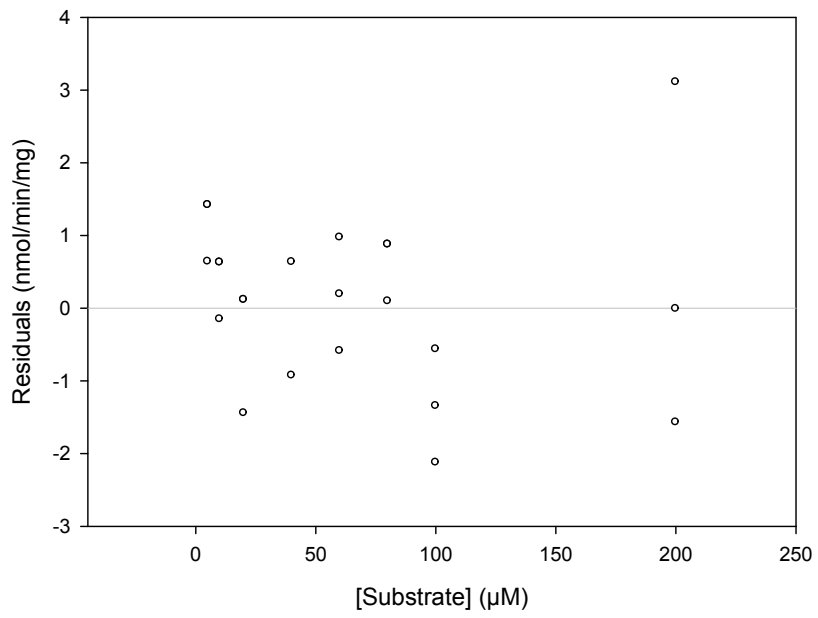
ACOT-1, *S*-2-Phenylpropanoyl-CoA **8S**
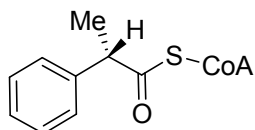
Parameters

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Vmax	26,802.5216	6.645e+6	-1.379e+7	to	1.385e+7
Km	35,967.0081	8.924e+6	-1.852e+7	to	1.859e+7
Ki	0.1140	28.2948	-58.7294	to	58.9574

Goodness of Fit

Degrees of Freedom	21
AICc	62.143
R²	0.892
Sum of Squares	209.830
Sy.x	3.161
Runs Test p Value	0.126

Data

Number of x values	8
Number of replicates	3
Total number of values	24
Number of missing values	0

Michaelis-Menten
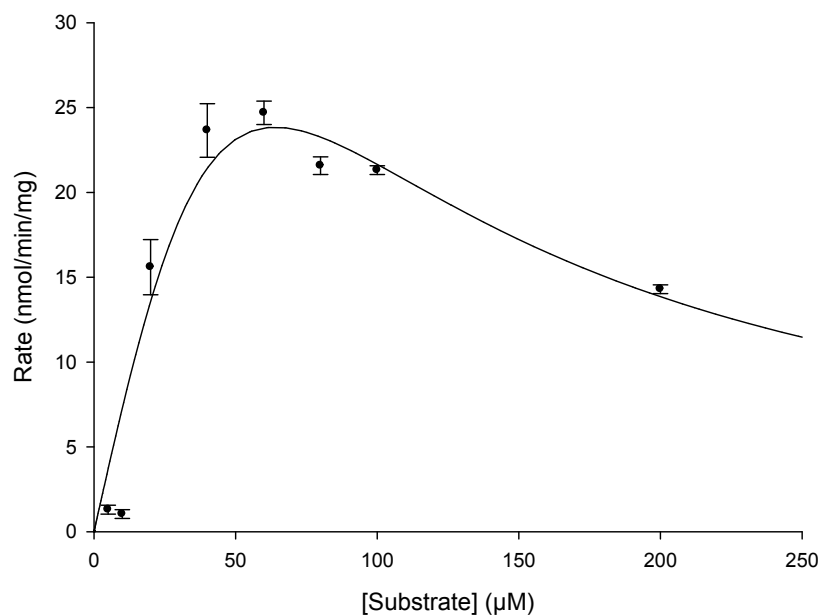
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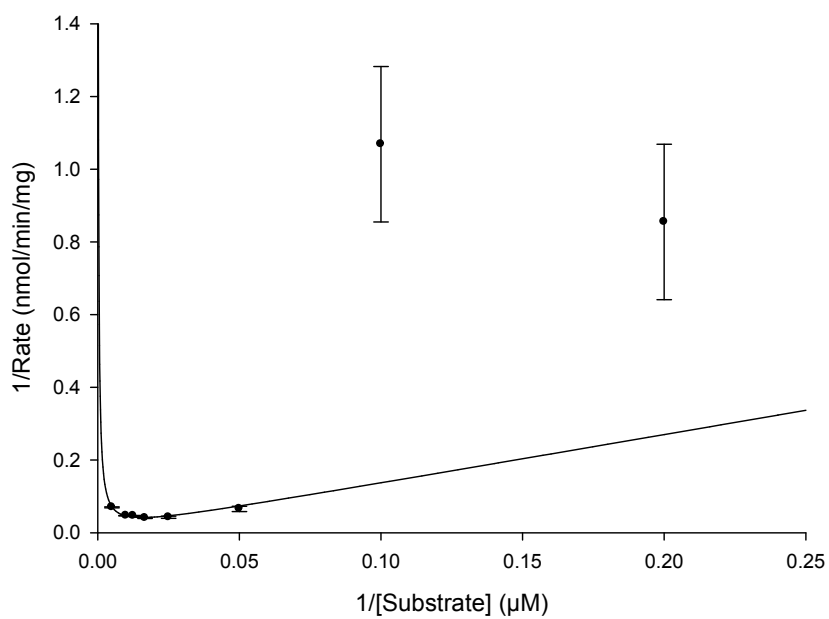
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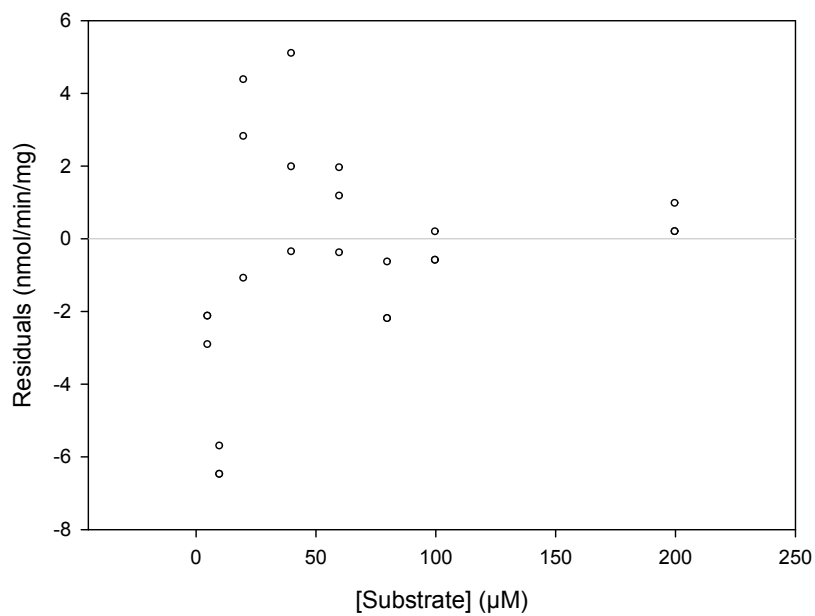
ACOT-1, *R*-2-phenylpropanoyl-CoA **8R**
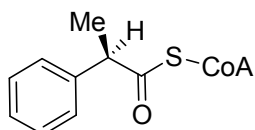
Parameters

	Value	±Std. Error	95% Conf. Interval		
Vmax	4.9646	0.3707	4.1959	to	5.7334
Km	22.1012	5.7426	10.1914	to	34.0109

Goodness of Fit

Degrees of Freedom	22
AICc	-18.897
R²	0.800
Sum of Squares	8.090
Sy.x	0.606
Runs Test p Value	0.501

**Data**

Number of x values	8
Number of replicates	3
Total number of values	24
Number of missing values	0

Michaelis-Menten
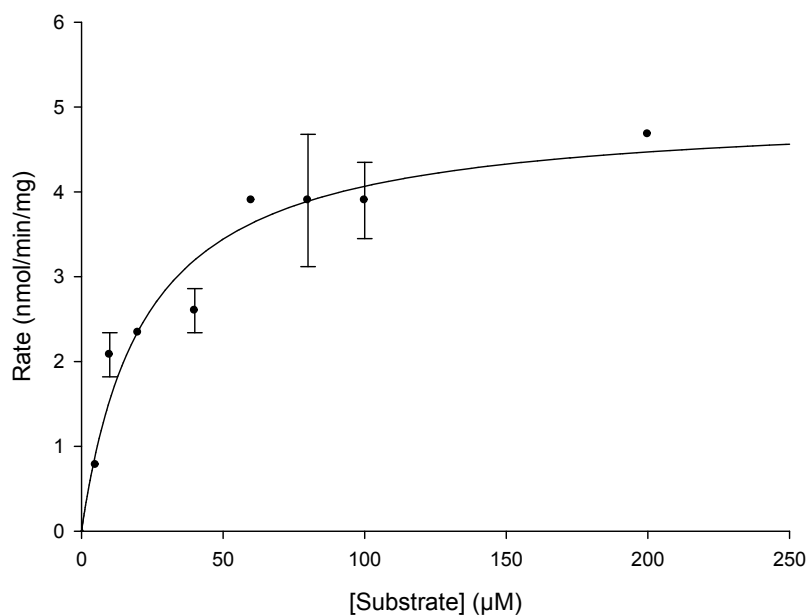
Lineweaver-Burk
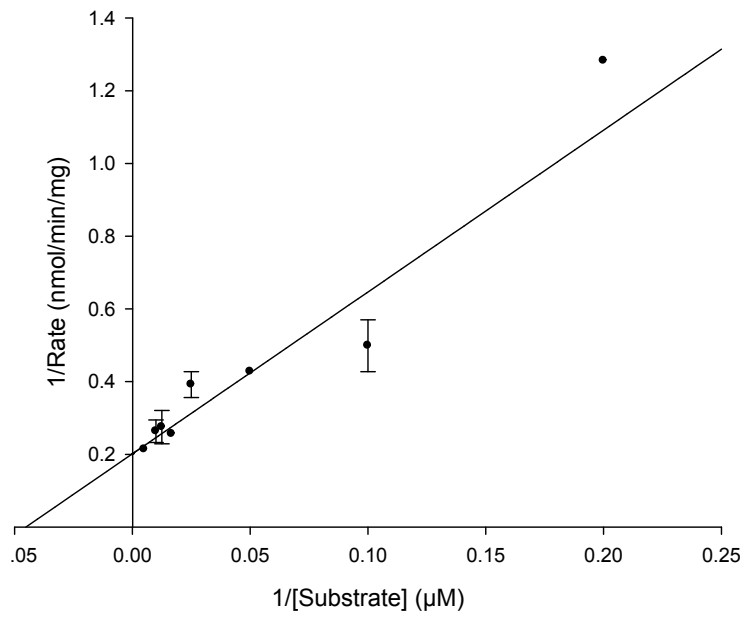
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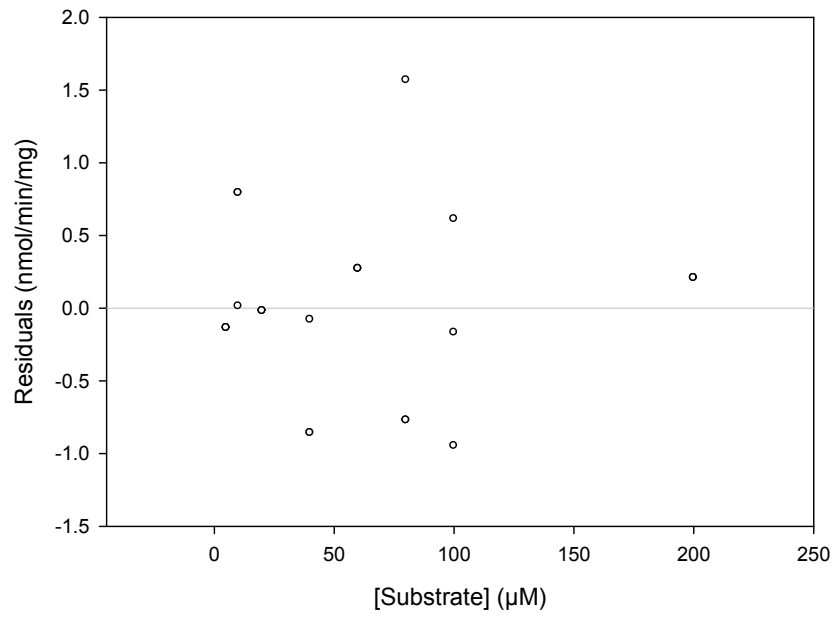
ACOT-2, Myristoyl-CoA 11
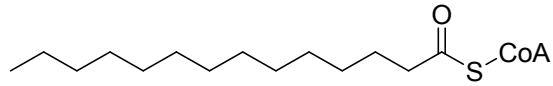
Parameters

	Value	±Std. Error	95% Conf. Interval		
Vmax	29.3472	11.0834	6.2271	to	52.4672
Km	15.7935	9.9807	-5.0262	to	36.6132
Ki	152.5699	205.6633	-276.4448	to	581.5847

Goodness of Fit

Degrees of Freedom	20
AICc	48.032
R²	0.826
Sum of Squares	119.032
Sy.x	2.440
Runs Test p Value	0.500

Data

Number of x values	8
Number of replicates	3
Total number of values	23
Number of missing values	1

Michaelis-Menten
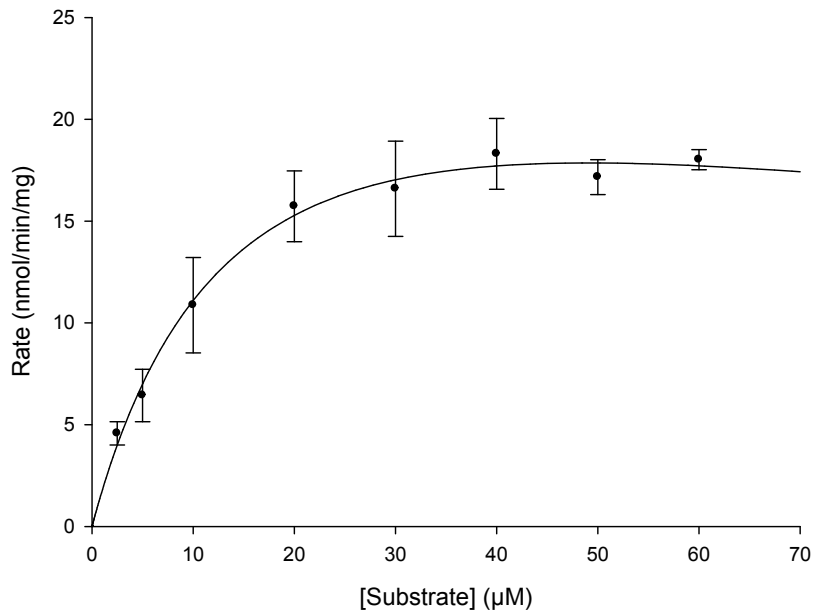
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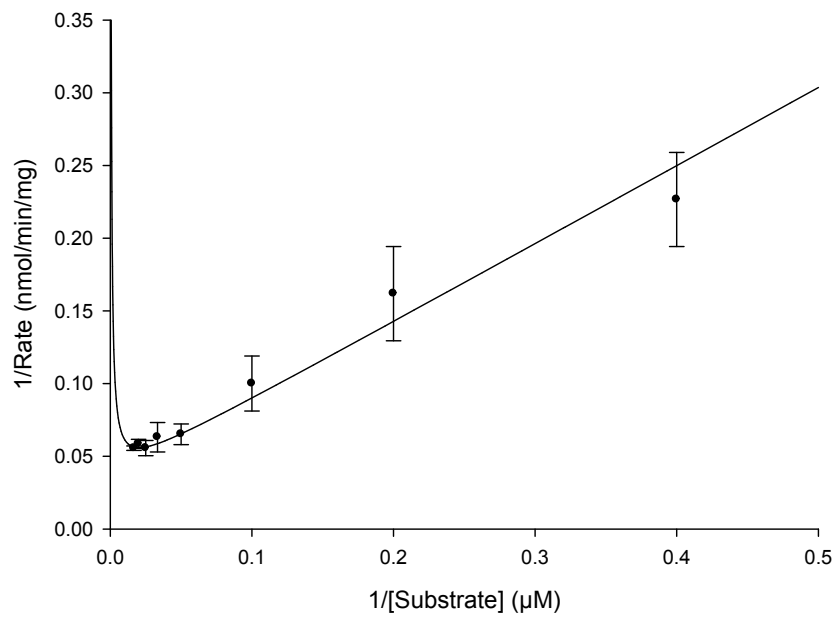
Residuals
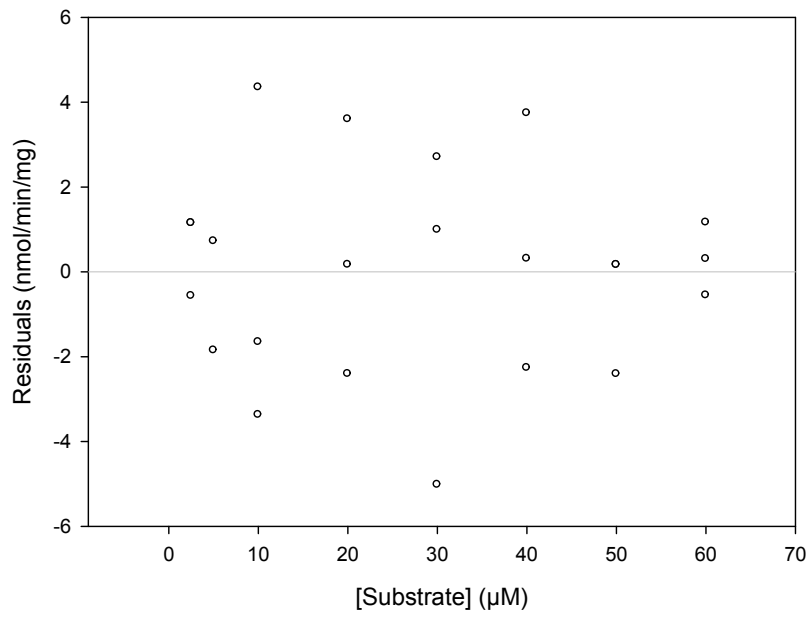
ACOT-2, *S*-2-hydroxy-2-phenylacetyl-CoA **2S**
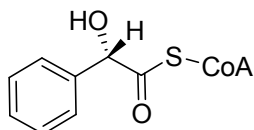
Parameters

	Value	±Std. Error	95% Conf. Interval		
Vmax	102.8182	103.7608	-112.9686	to	318.6050
Km	171.6409	198.9373	-242.0801	to	585.3619
Ki	26.8023	32.3655	-40.5068	to	94.1115

Goodness of Fit

Degrees of Freedom	21
AICc	38.715
R²	0.902
Sum of Squares	79.053
Sy.x	1.940
Runs Test p Value	0.338

Data

Number of x values	8
Number of replicates	3
Total number of values	24
Number of missing values	0

Michaelis-Menten
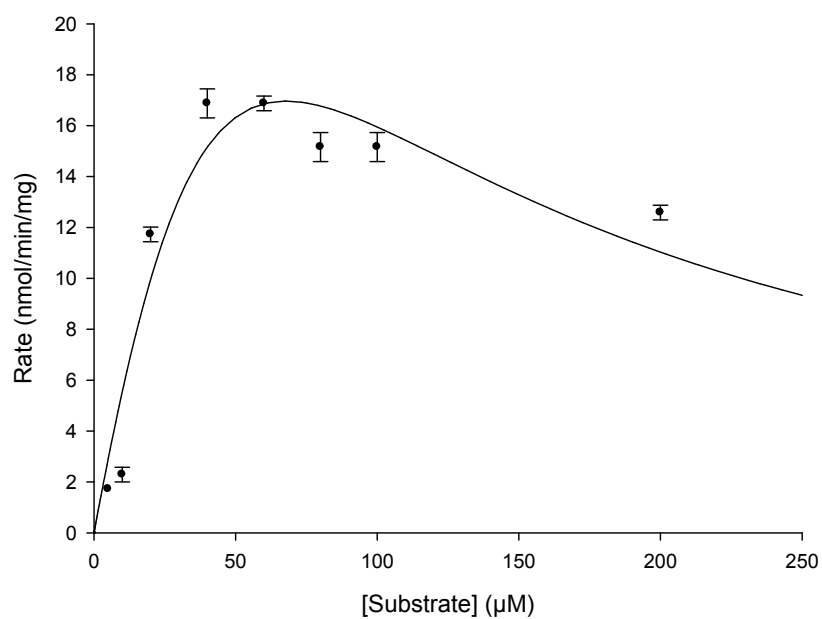
Lineweaver-Burk
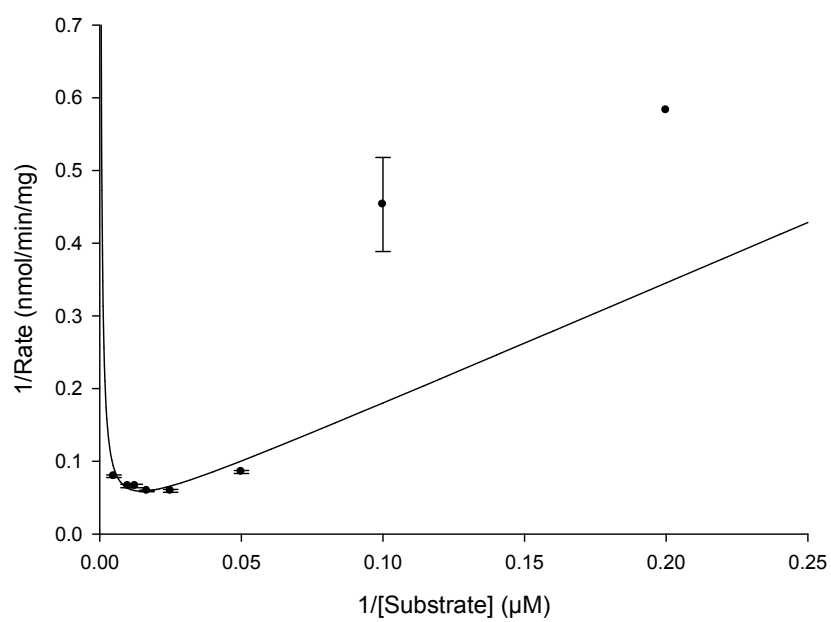
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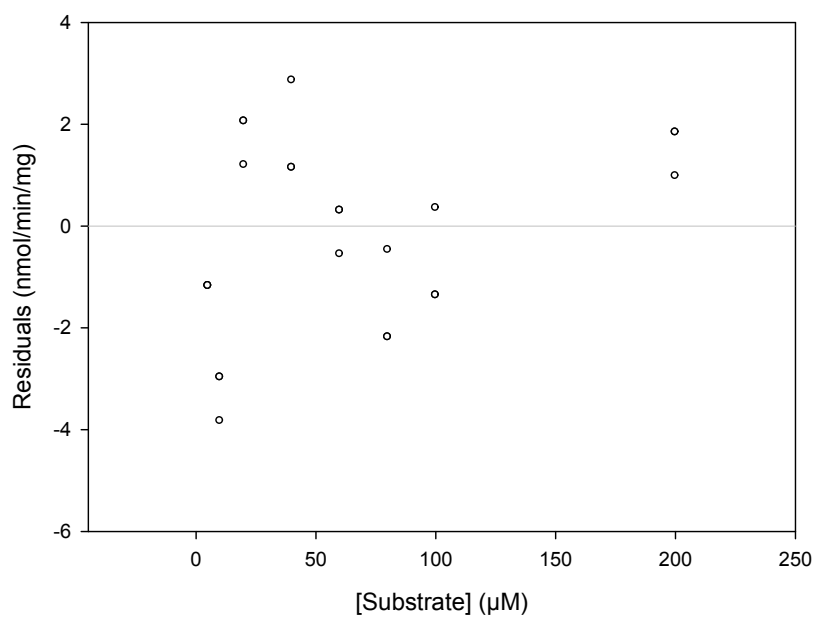
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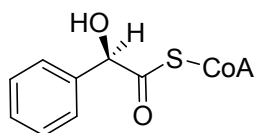
Parameters

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Vmax	20.3377	3.1959	13.6231	to	27.0522
Km	130.7584	37.3894	52.2047	to	209.3122

Goodness of Fit

Degrees of Freedom	18
AICc	14.166
R²	0.893
Sum of Squares	27.911
Sy.x	1.245
Runs Test p Value	0.179

Data

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Number of replicates	3
Total number of values	20
Number of missing values	1

Michaelis-Menten
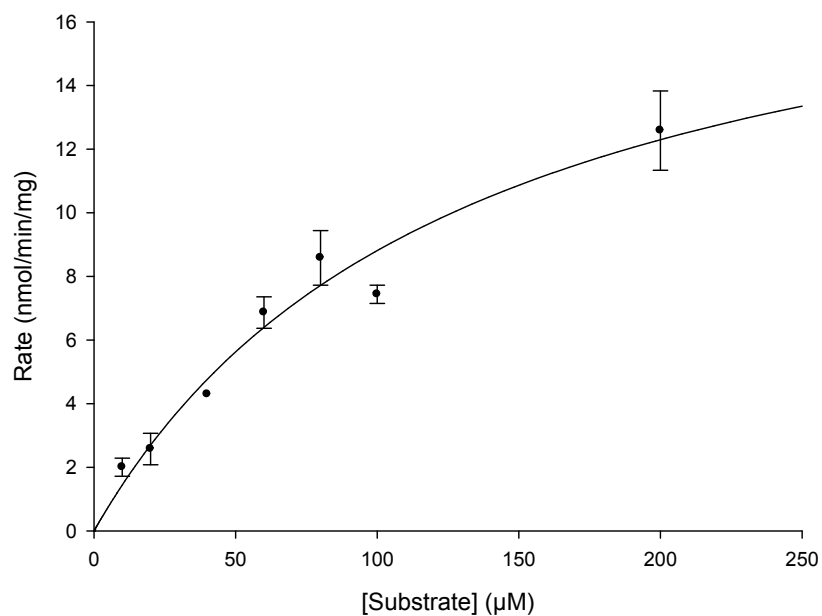
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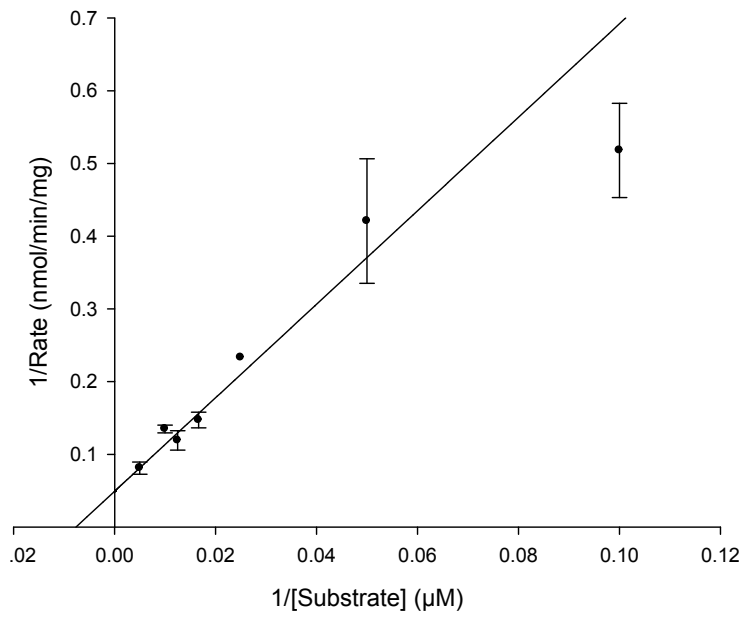
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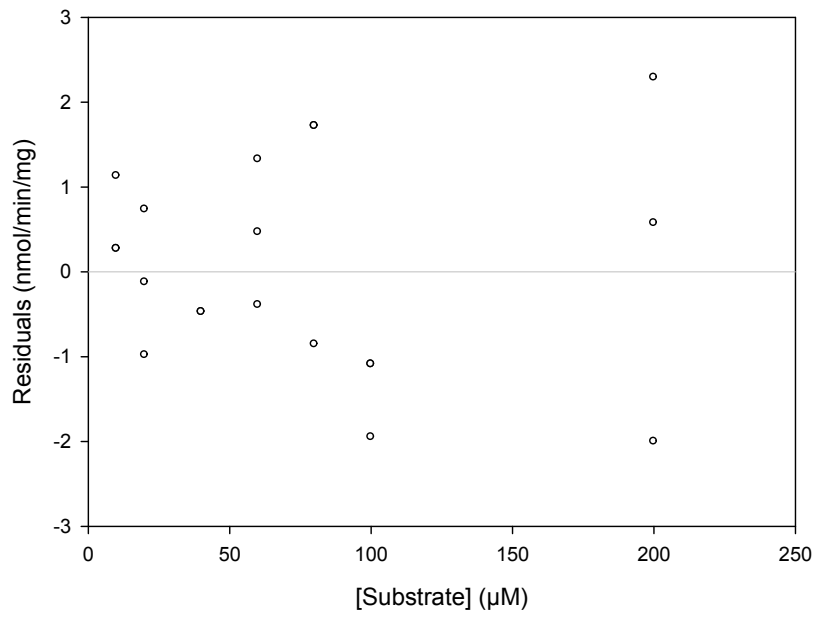
ACOT-2, *S*-2-Phenylpropanoyl-CoA **8*S***
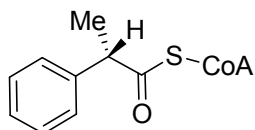
Parameters

	Value	±Std. Error	95% Conf. Interval		
Vmax	33,339.3892	7.917e+6	-1.643e+7	to	1.650e+7
Km	46,916.6408	1.115e+7	-2.314e+7	to	2.323e+7
Ki	0.1081	25.6837	-53.3051	to	53.5212

Goodness of Fit

Degrees of Freedom	21
AICc	54.443
R²	0.930
Sum of Squares	152.241
Sy.x	2.693
Runs Test p Value	0.501

Data

Number of x values	8
Number of replicates	3
Total number of values	24
Number of missing values	0

Michaelis-Menten
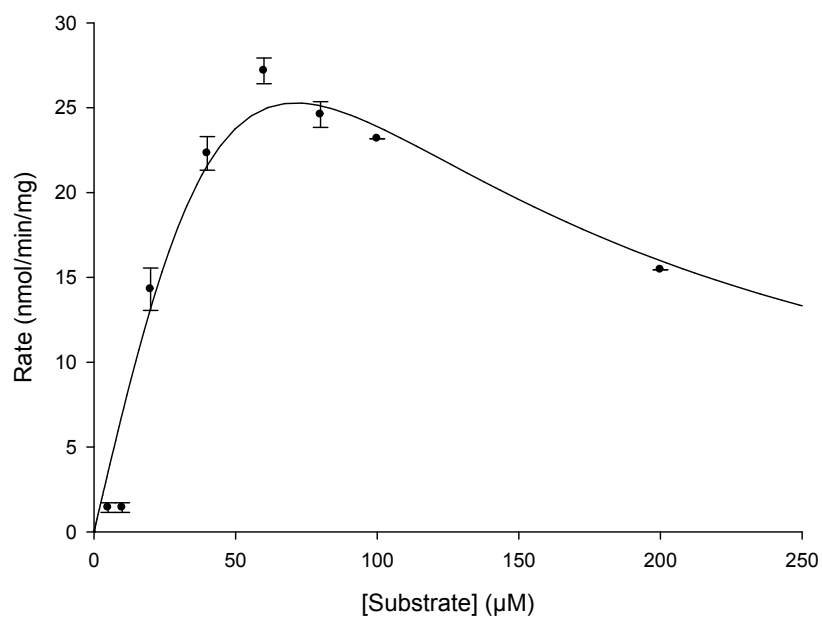
Lineweaver-Burk

Residuals

ACOT-2, *R*-2-Phenylpropanoyl-CoA **8R**

$$\text{Me} \quad \text{H}$$

(structure: phenyl group attached to a chiral carbon bearing Me and H, connected to C(=O)–S–CoA)
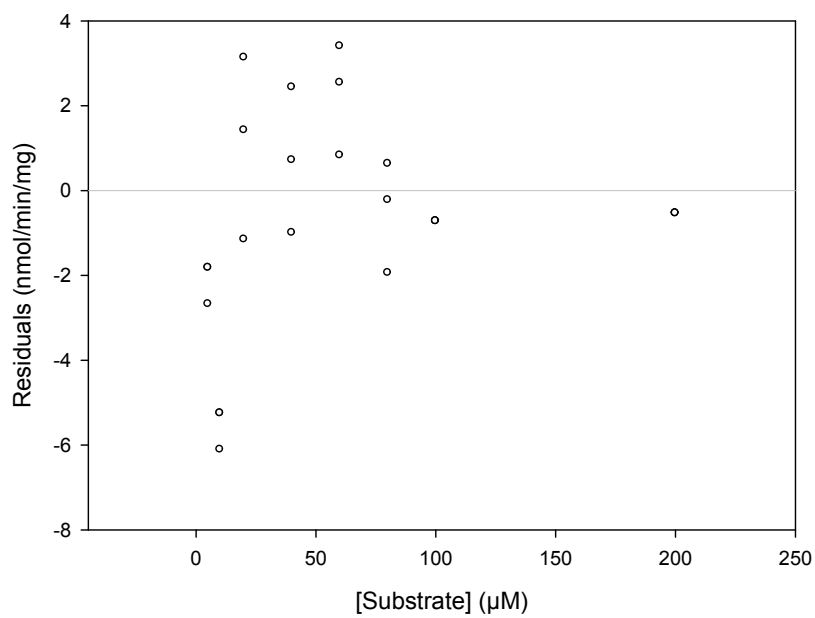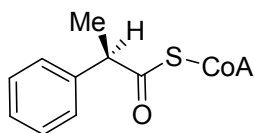
Parameters

	Value	±Std. Error	95% Conf. Interval		
Vmax	5.3167	0.3275	4.6375	to	5.9960
Km	27.3880	5.4159	16.1558	to	38.6202

Goodness of Fit

Degrees of Freedom	22
AICc	-31.069
R²	0.882
Sum of Squares	4.872
Sy.x	0.471
Runs Test p Value	0.023

Data

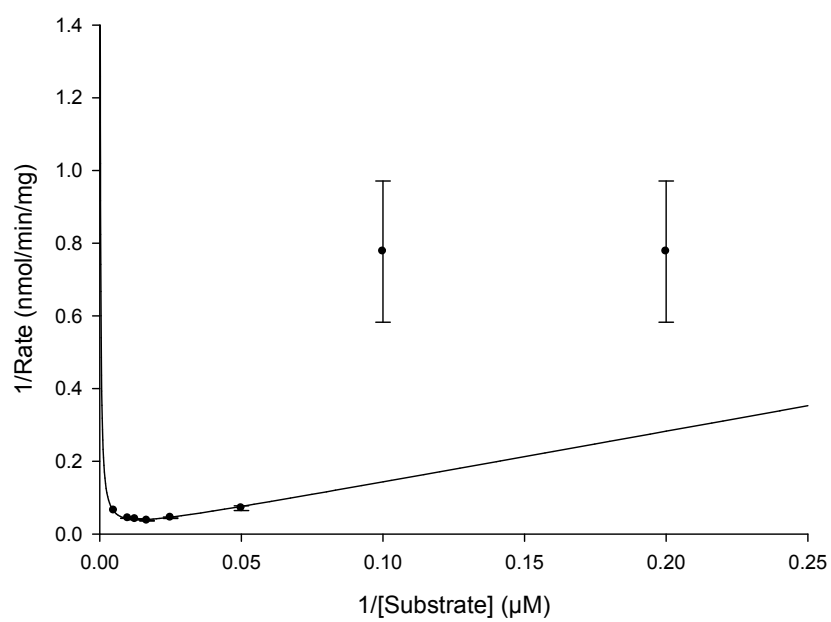|	
Number of x values	8
Number of replicates	3
Total number of values	24
Number of missing values	0

Michaelis-Menten
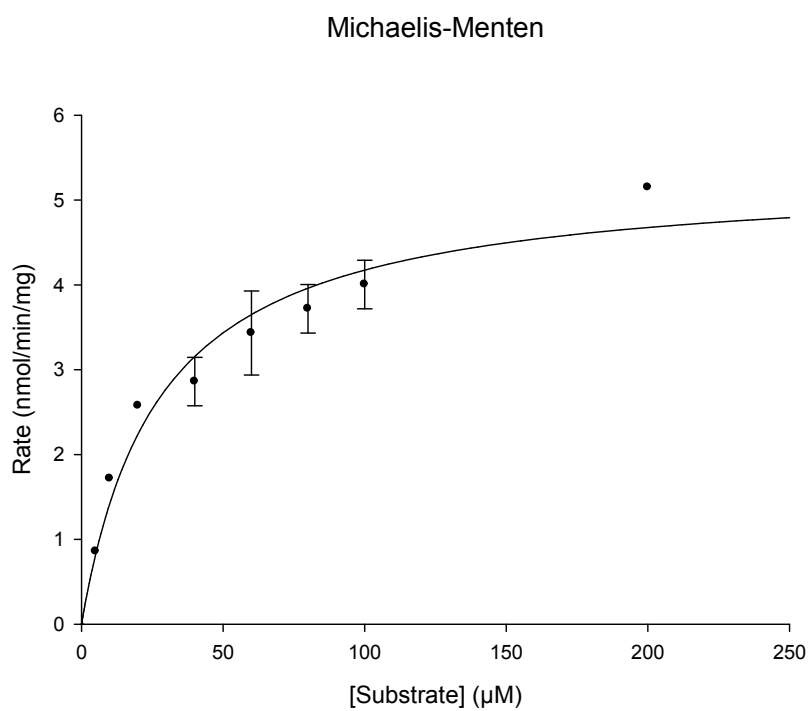
Lineweaver-Burk
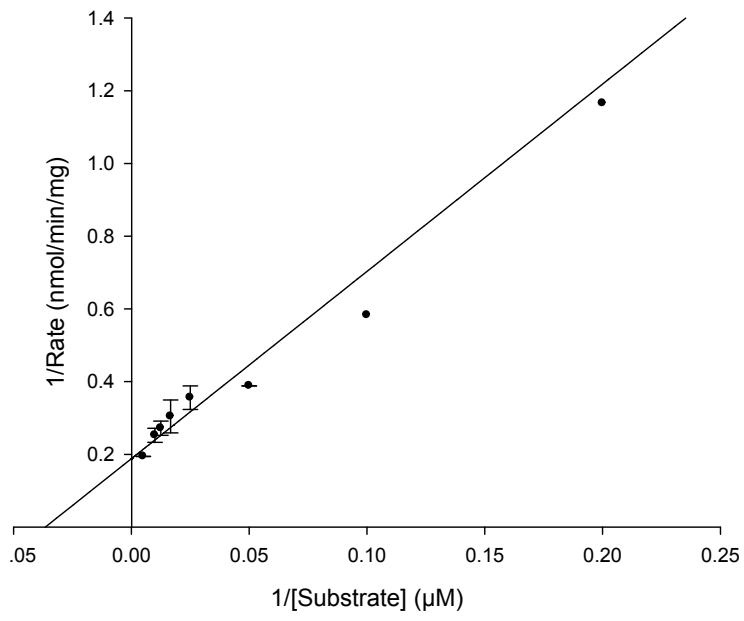
Residuals
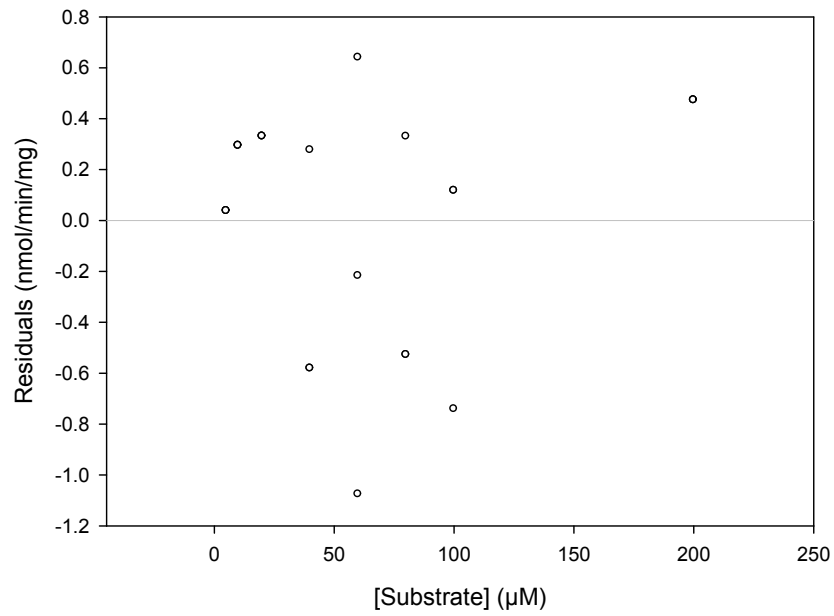
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MCR        MAGPLSGLRVVELAGIGPGPHAAMILGDLGADVVRIDRPSSVDGISRDAMLRNRRIVTAD
AMACR      --MALQGISVVELSGLAPGPFCAMVLADFGARVVRVDRPGSRYDVSR--LGRGKRSLVLD

MCR        LKSDQGLELALKLIAKADVLIEGYRPGVTERLGLGPEECAKVNDRLIYARMTGWGQTGPR
AMACR      LKQPRGAAVLRRLCKRSDVLLEPFRRGVMEKLQLGPEILQRENPRLIYARLSGFGQSGSF

MCR        SQQAG**HD**INYISLNGILHAIGRGDERPVPPL**N**LVG**D**FGGGSMFLLVGILAALWERQSSGK
AMACR      CRLAG**HD**INYLALSGVLSKIGRSGENPYAPL**N**LLA**D**FAGGGLMCALGIIMALFDRTRTGK

MCR        GQVVDAAMVDGSSVLIQMMWAMRATGMWTDTRGANM**L**DGGAPY**Y**DTYECADGRYVAVGA**I**
AMACR      GQVIDANMVEGTAYLSSFLWKTQKSSLWEAPRGQNM**L**DGGAPF**Y**TTYRTADGEFMAVGA**I**

MCR        EPQFYAAMLAGLGLDAAELPPQNDRARWPELRALLTEAFASHDRDHWGAVFANSDACVTP
AMACR      EPQFYELLIKGLGLKSDELPNQMSMDDWPEMKKKFADVFAKKTKAEWCQIFDGTDACVTP

MCR        VLAFGEVHNEPHIIERNTFYEANG---GWQPMPAPRFSRTASSQPRPPAATIDIEAVLTD
AMACR      VLTFEEVVHHDHNKERGSFITSEEQDVSPRPAPLLLNTPAIPSFKRDPFIGEHTEEILEE

MCR        WDG----------------------
AMACR      FGFSREEIYQLNSDKIIESNKVKASL
```

Figure S1: Sequence alignment of MCR from *M. tuberculosis* (O06543) and human AMACR 1A (Q9UHK6), showing residues involved in methyl group binding pocket (Bold).