Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary information for '**Synthesis and use of isotope-labelled substrates for a mechanistic study on human α-methylacyl-CoA racemase 1A (AMACR; P504S)**'

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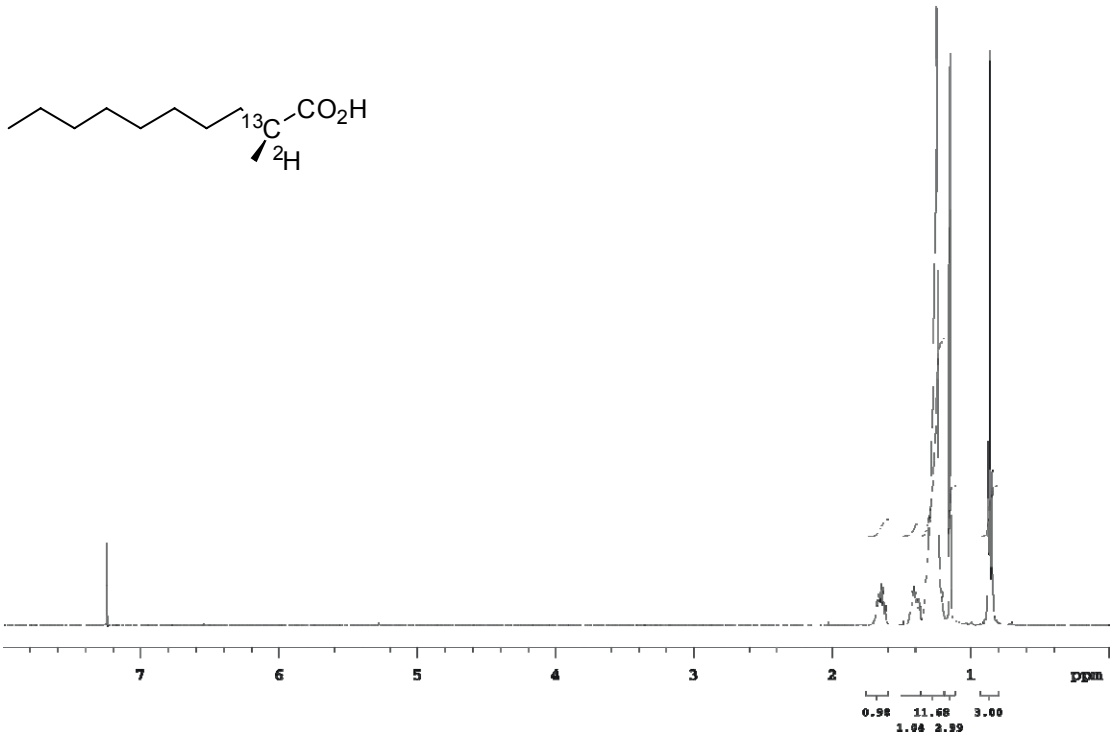
Contents

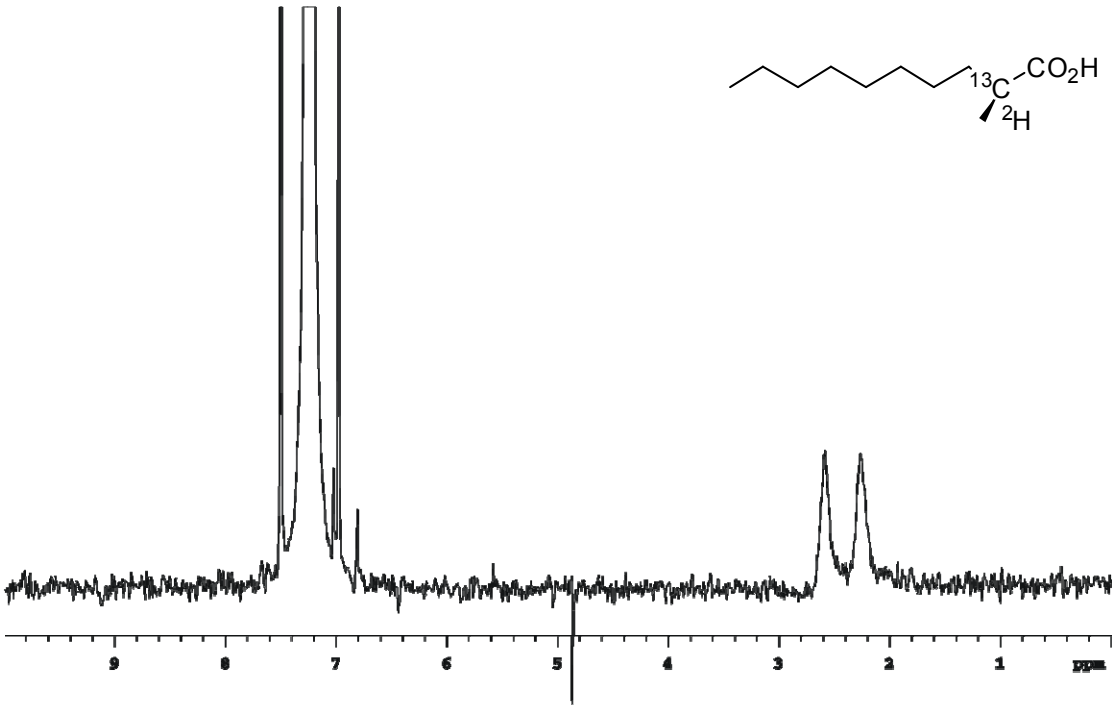
NMR spectra

S-2-[^{13}C]-2-[^{2}H]-Methyldecanoic Acid 16*S*

^{1}H NMR spectrum (400 MHz, $CDCl_3$)

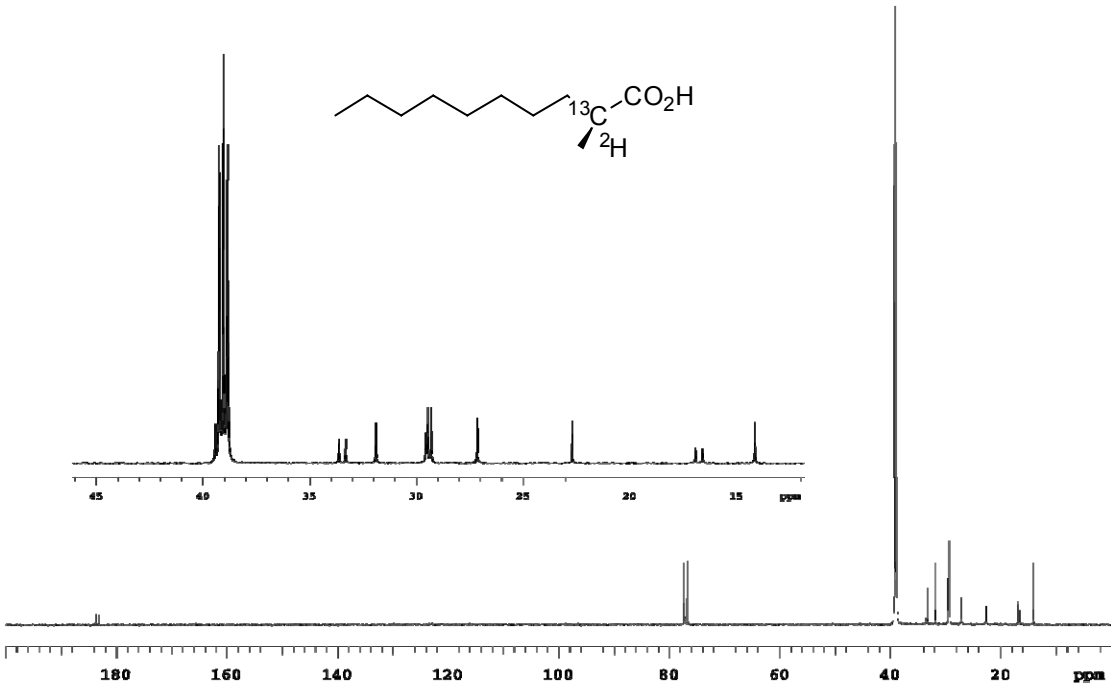


^{2}H NMR spectrum (61.41 MHz, $CDCl_3$)



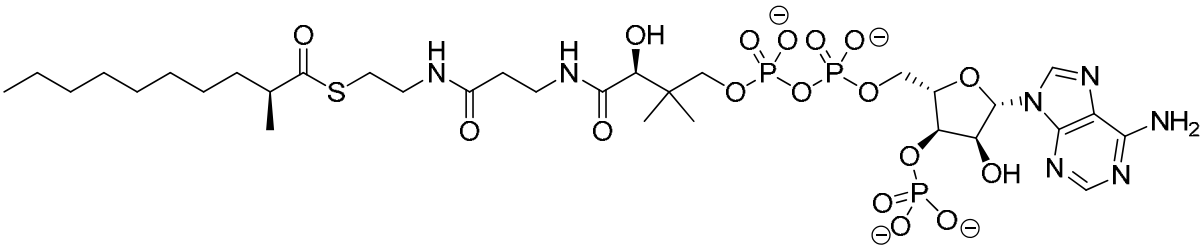
S-2-[^{13}C]-2-[^{2}H]-Methyldecanoic Acid 16*S*

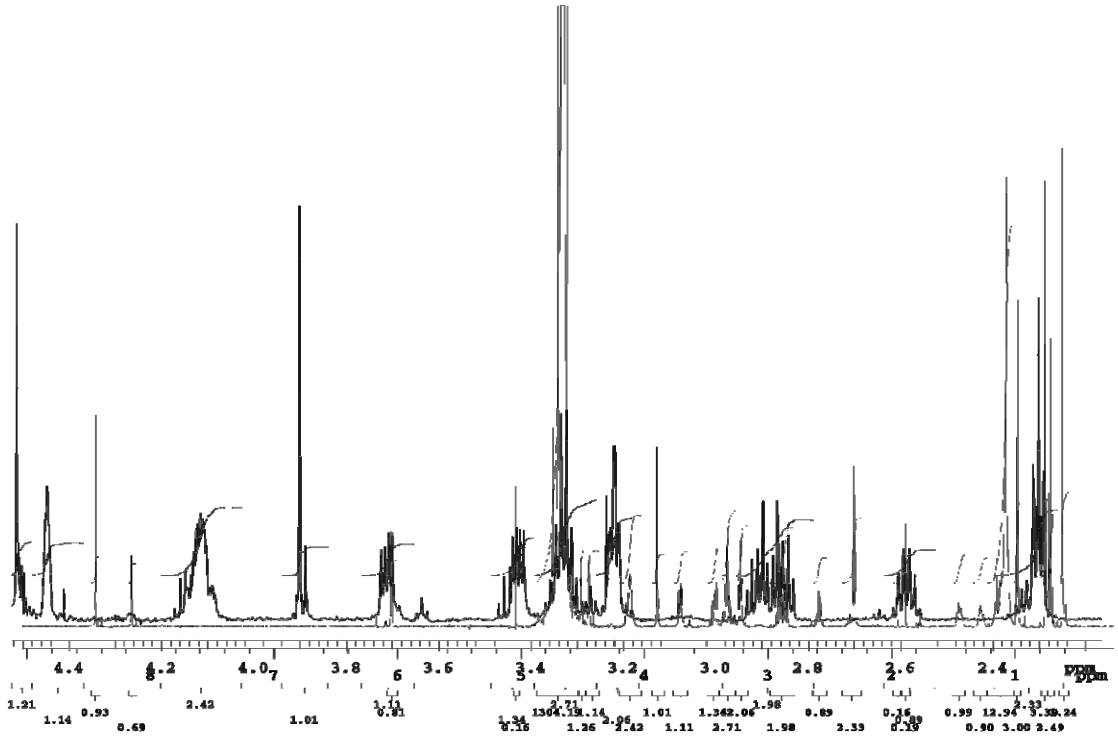
^{13}C NMR spectrum (100 MHz, $CDCl_3$)

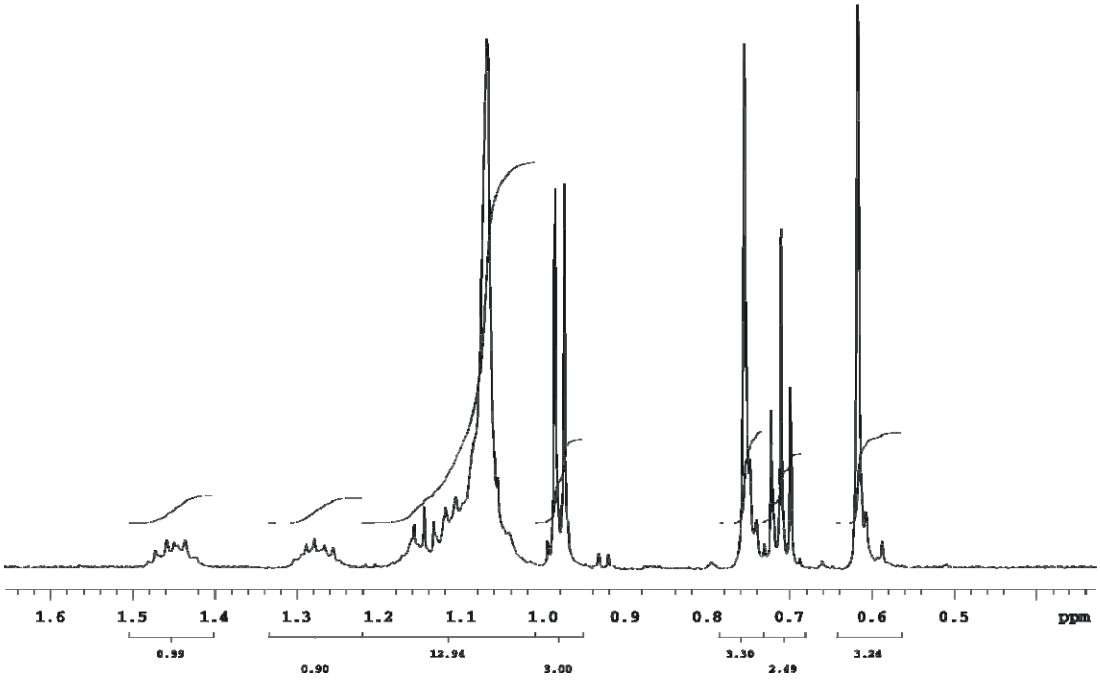


S-2-Methyldecanoyl-CoA **10*S***

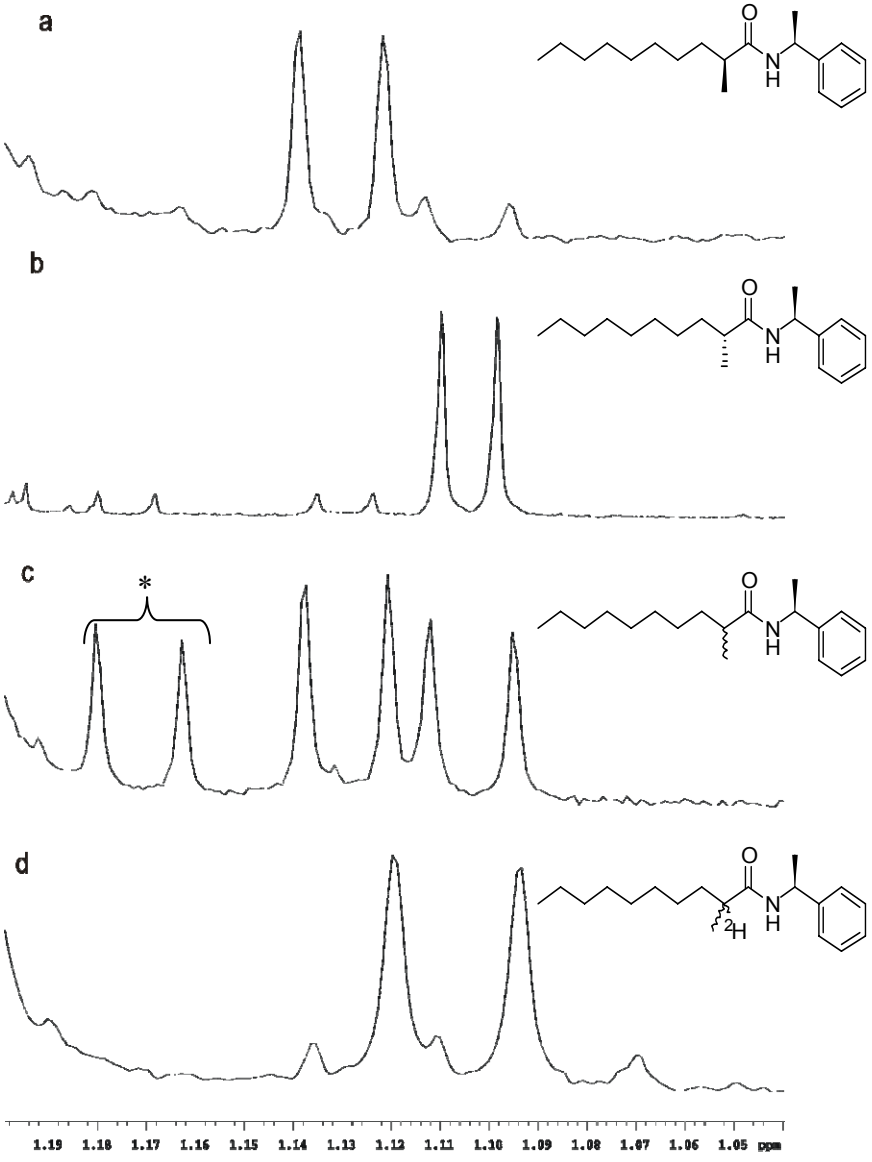
^{1}H NMR spectrum (400 MHz, D_2O)






1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
ppm
0.99
0.90
12.94
3.00
3.30
2.49
3.24

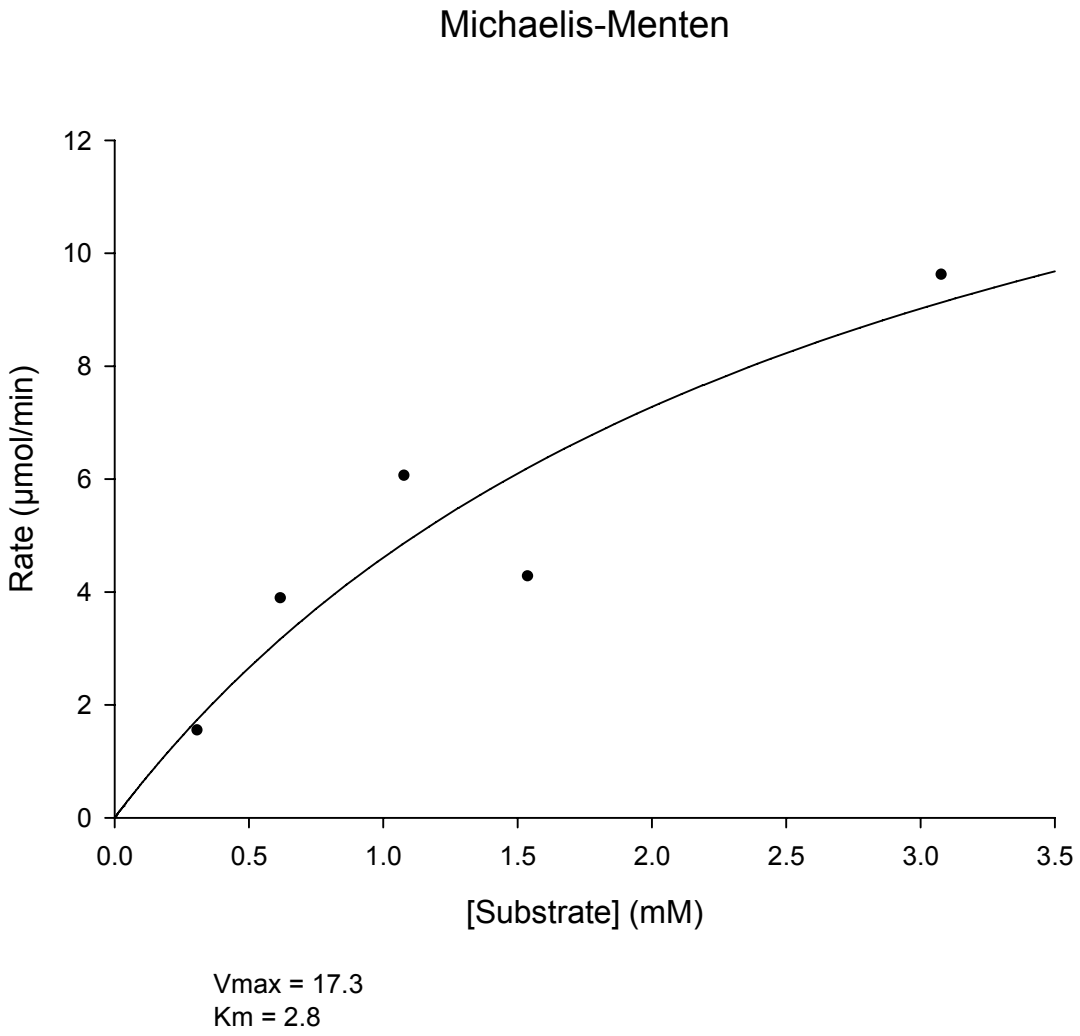
^{1}H NMR part spectra of *S*-1-phenylethylamides 18 (400 MHz, $CDCl_3$)



^{1}H NMR spectra of 2-methyl-N-(*S*-1-phenylethyl)decanamides **18**: (a) *S*-2-methyl-N-(*S*-1-phenylethyl)decanamide standard **18*S***; (b) *R*-2-methyl-N-(*S*-1-phenylethyl)decanamide standard **18*R***; (c) *S*-1-phenylethyl amides derived from *S*-2-methyldecanoyl-CoA **10*S*** with AMACR 1A in H_2O and hydrolysis of the CoA ester. The doublet indicated by an asterisk (*) arises from underivatised acid; (d) *S*-1-phenylethyl amides derived from equilibration of *S*-2-methyldecanoyl-CoA with AMACR 1A in ^2H_2O and hydrolysis of the CoA ester.

Kinetic analysis of racemisation of *S*-2-methyldecanoyl-CoA 10*S* using NMR assay

	Value	±Std. Error	95% Conf. Interval		
V_{max}	17.3040	9.2036	-11.9794	to	46.5875
K_m	2.7567	2.4711	-5.1057	to	10.6191



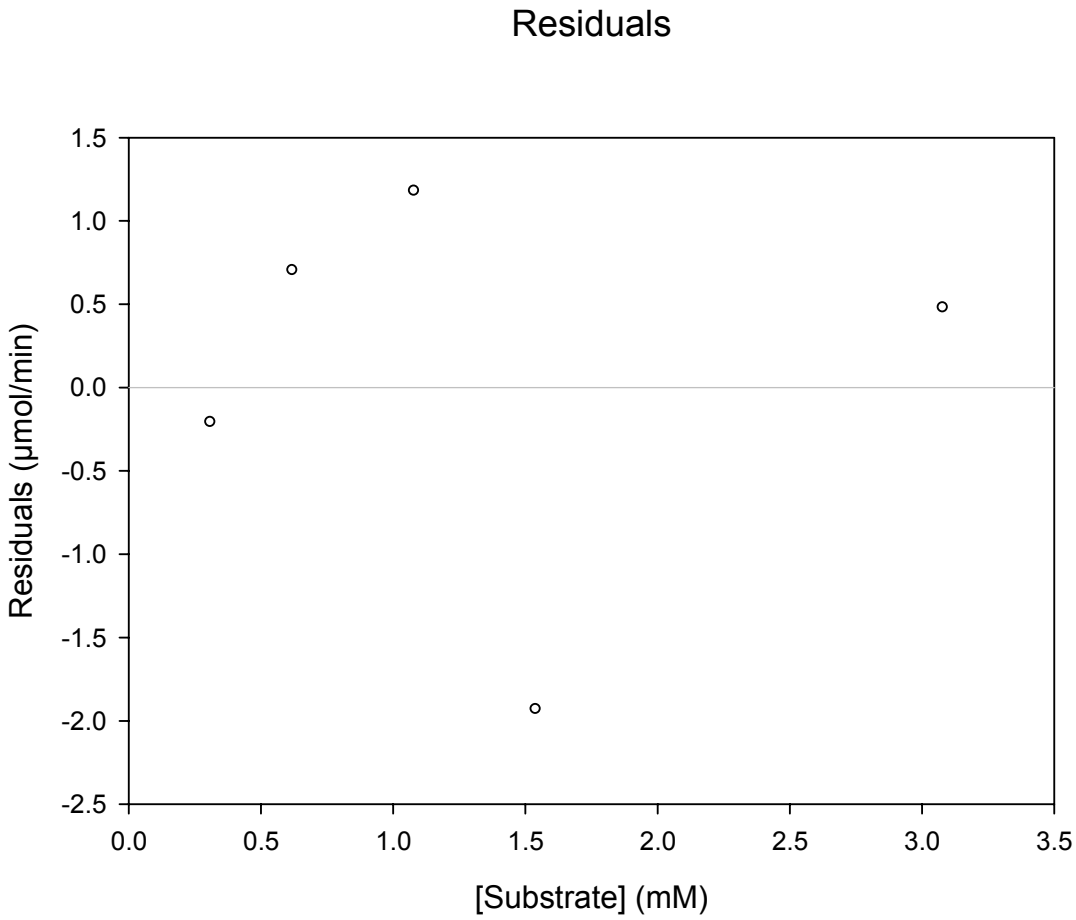
Vmax = 98.6 ± 52.4 µmol.min^{-1}.mg^{-1}

K_m = 2.8 ± 2.4 mM

k_{cat} = 77.5 ± 41 s^{-1}

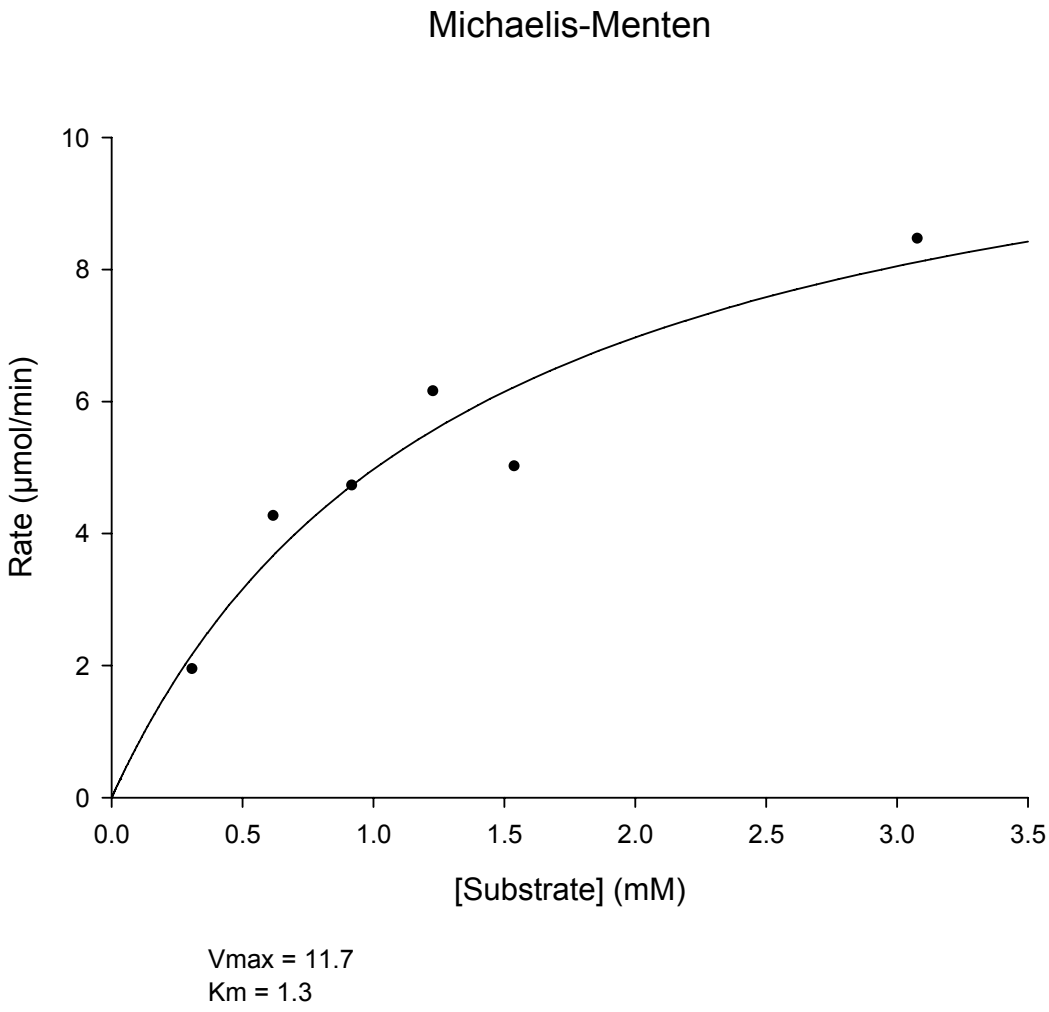
k_{cat}/K_m = 27679 s^{-1} M^{-1}.

Enzyme = 0.1755 mg per assay (3.72 nmoles)


Residuals
Residuals (µmol/min)
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
[Substrate] (mM)

Kinetic analysis of racemisation of *R*-2-methyldecanoyl-CoA 10*R* using NMR assay

	Value	±Std. Error	95% Conf. Interval		
Vmax	11.6599	2.3066	5.2558	to	18.0640
Km	1.3450	0.5561	-0.1991	to	2.8891



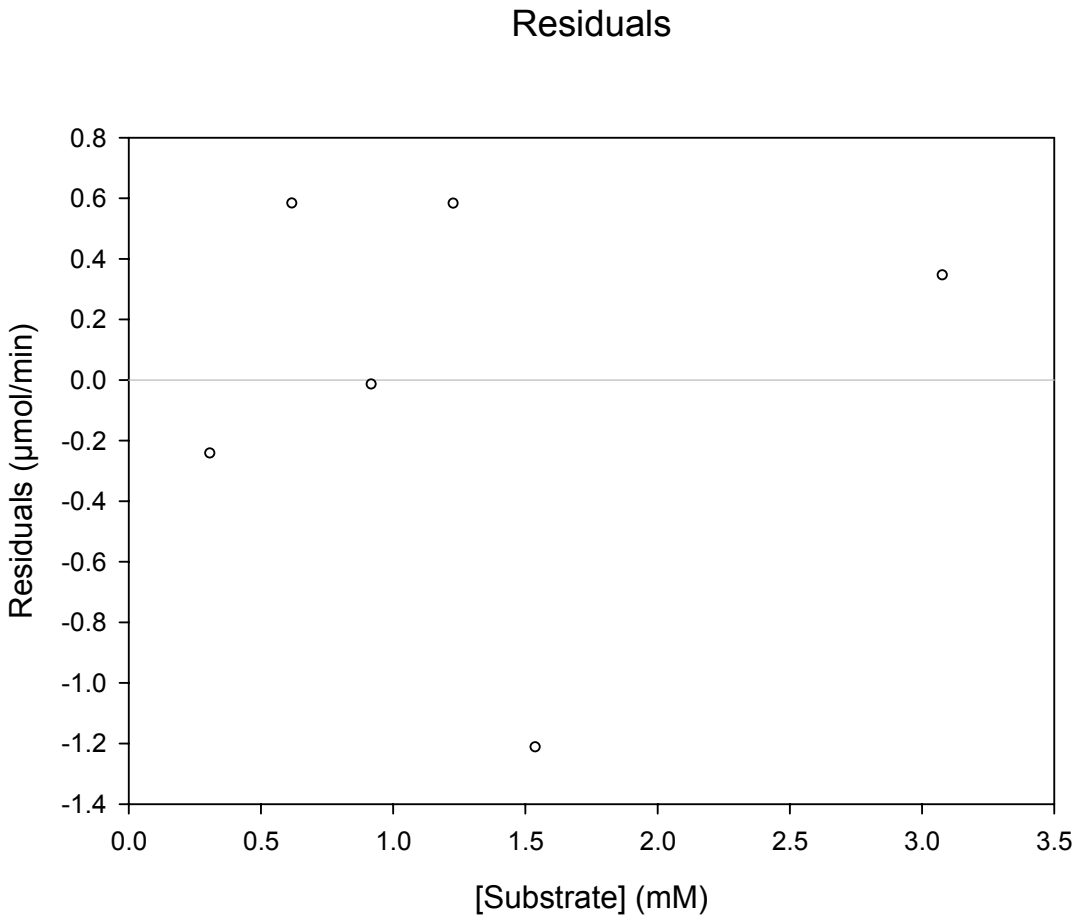
Vmax = 66.6 ± 13 µmol.min^{-1}.mg^{-1}

K_m = 1.3 ± 0.55 mM

k_{cat} = 52.4 ± 10.2 s^{-1}

k_{cat}/K_m = 40307 s^{-1} M^{-1}

Enzyme = 0.1755 mg per assay (3.72 nmoles)


Residuals
Residuals (µmol/min)
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
[Substrate] (mM)

Analysis of Figure 3b
Equation: Hyperbola, Single Rectangular, 2 Parameter
f=a*x/(b+x)

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9971	0.9942	0.9941	1.1224

	Coefficient	Std. Error	t	P	VIF
a	52.0249	0.2889	180.0799	<0.0001	2.8939
b	311.4455	8.8749	35.0928	<0.0001	2.8939

Analysis of Variance:

Uncorrected for the mean of the observations:

	DF	SS	MS
Regression	2	118234.2077	59117.1039
Residual	76	95.7517	1.2599
Total	78	118329.9594	1517.0508

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	1	16441.9139	16441.9139	13050.2719	<0.0001
Residual	76	95.7517	1.2599		
Total	77	16537.6656	214.7749		