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Electronic Supplementary Information

Fatty Acyl Incorporation in the Biosynthesis of WAP-8294A, a Group of Potent Anti-MRSA Cyclic Lipodepsipeptides

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Table S1. Primers used in this paper

Primers for 7 ACL genes disruption

Name	No.	Fragment Length	Primers
ACL1	LysEGL003270	496bp	Forward: TTACTCGAGTCAATCCCAAGCTCAAGC
			Reverse: TATGGATCCTAGTTGTGCAGCACCAGC
ACL2	LysEGL000201	488bp	Forward: TAACTCGAGATTCCAGCCACGAA
			Reverse: TATGGATCCATGCTTGAGGATGCGTTC
ACL3	LysEGL000048	579bp	Forward: TAACTCGAGAGCTCGAACTCAAGAAGG
			Reverse: AATGGATCCTGACGATGATTTCCTGGC
ACL4	LysEGL000406	555bp	Forward: ATCCTCGAGTTCTTCCTGCTGATGTTC
			Reverse: TATGGATCCTAGCGCATCGTGCAACT
ACL5	LysEGL003038	604bp	Forward: TATCTCGAGTCAACACCAATCCGATGT
			Reverse: TAG GGATCCTTTTCCTGCAGTTCCTTC
ACL6	LysEGL001572	630bp	Forward: TAACTCGAGCACTCCGAAAACCATCTG
			Reverse: AATGGATCCTCGTCGTGGAAGATCG
ACL7	LysEGL003969	700bp	Forward: TAACTCGAGGATCTCGCATCGCAAC
			Reverse: TATGGATCCGGTCGTGAGGTAATAGC

Primers for 7 ACL genes expression

Name	Fragment Length	Protein size (Da)	Primers
ACL1	1677bp	58677.4	Forward: ATAGAATTCAGCCCGACCGTCAGCC
			Reverse: AGGAAGCTTAGCACAACAGGACCC
ACL2	1635bp	58767.3	Forward: AACGAATTCATGCTCTCCCGACTGA
			Reverse: TATCTCGAGCGGAGCGAAAGGGAAG
ACL3	1737bp	60454.5	Forward: TTAGAATTCTGGGCGCTCGTATCC
			Reverse: ATACTCGAGCGCTTTCACCGCAATG
ACL4	1695bp	59778.6	Forward: TATGAATTCCCACCCGCTCGGCTGA
			Reverse: TAAAAGCTTCAGTGCCGCCTCTCAA
ACL5	1677bp	61084.6	Forward: TATGAATTCAGTTTGAACCGTCCGT
			Reverse: TATAAGCTTACCCATGCTCACGCGT
ACL6	1395bp	47157.1	Forward: TAAGAATTCCAACAACGAATCGCTG
			Reverse: ATGAAGCTTATGACCCGGGATTTAT
ACL7	1713bp	58643	Forward: TAAGAATTCTCAAGGACCGATCGAA Reverse: ATTAAGCTTCCATCTGCAGGGTCAT

Primers for construction of ACL-6 deletion mutant

ACL6	upstream	412bp	ATC GTC GAC CTT CTT CCT GCA A
			CTC GGATCC TTC GTT GTT GAG G
ACL6	downstream	280bp	CTA GGATCC CCT GAA GCA GAT C

	GCG TCT AGA TGA GCG GTT CCA T	
Diagnostic Primers		
No.	Forw-diagnostic primer sequence	Expected length*
ACL1	TGTACCACGACAAATCCATCC	632bp
ACL2	TGATGATCGTGCACACCTCC	903bp
ACL3	AGATAGATCCCGACGCGCTG	796bp
ACL4	TGTGCAACATCGCCGC	840bp
ACL5	AGCTCAAGCTCAAGAAGGGC	770bp
ACL6	ATCGACATGCTGGCGTTG	690bp
ACL7	TGTTCGAAGCCAAGGCC	903bp
ACL6 deletion	GAA TAC CTG GCC CTG CAA	730bp
Primer on vector	ACCATGATTACGCCAAGC	

* This length refers to the expected size of diagnostic PCR using diagnostic primer (on the genome, outside the recombination region) and primer on vector

Table S2A. Kinetic parameters for ACL with (*R*)-3-hydroxy-7-methyloctanoic acid as substrate

	K_M (μM)	V_{max} (μM/min)	k_{cat} (min^{-1})	k_{cat}/K_M
ACL1	70.24 ± 12.43	0.2420 ± 0.0065	0.0161 ± 0.0004	2.3×10^{-4}
ACL3	6.12 ± 1.67	0.3148 ± 0.0073	0.0209 ± 0.0004	34.2×10^{-4}
ACL4	511.76 ± 379.39	0.6342 ± 0.0859	0.0422 ± 0.0057	0.8×10^{-4}
ACL5	49.98 ± 11.87	0.9535 ± 0.0397	0.0636 ± 0.0027	12.7×10^{-4}
ACL6	5.84 ± 1.29	4.9006 ± 0.0810	0.3267 ± 0.0054	559.4×10^{-4}
ACL7	N/A	N/A	N/A	N/A

*ACL2 was insoluble when purified; ACL7 did not show clear activity toward this substrate.

Table S2B. Kinetic parameters for ACL with (*R*)-3-hydroxyoctanoic acid as substrate

	K_M (μM)	V_{max} (μM/min)	k_{cat} (min^{-1})	k_{cat}/K_M
ACL1	68.88 ± 20.67	0.1671 ± 0.0075	0.0111 ± 0.0005	1.6×10^{-4}
ACL3	62.65 ± 1.29	0.2248 ± 0.0053	0.0149 ± 0.0003	2.4×10^{-4}
ACL4	3.41 ± 0.99	0.1845 ± 0.0066	0.0123 ± 0.0004	36.1×10^{-4}
ACL5	15.00 ± 1.89	0.2983 ± 0.0044	0.0199 ± 0.0003	13.3×10^{-4}
ACL6	342.07 ± 165.83	0.0405 ± 0.0081	0.0027 ± 0.0005	0.1×10^{-4}
ACL7	15.49 ± 1.05	0.0864 ± 0.0039	0.0057 ± 0.0002	3.7×10^{-4}

*ACL2 was insoluble when purified.

Fig. S1A Alignment of the amino acid sequence of the seven putative ACLs using ClustalW.

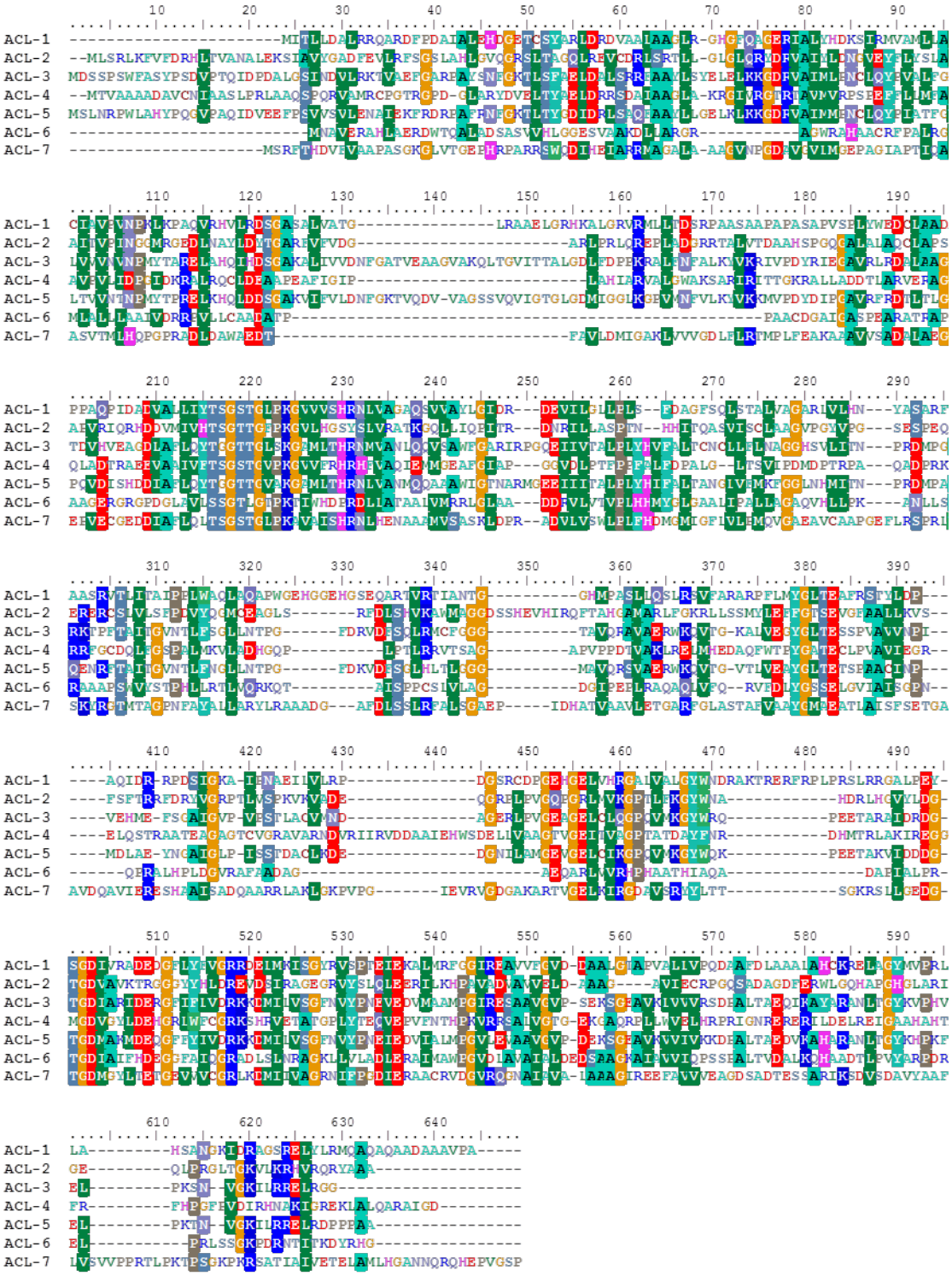
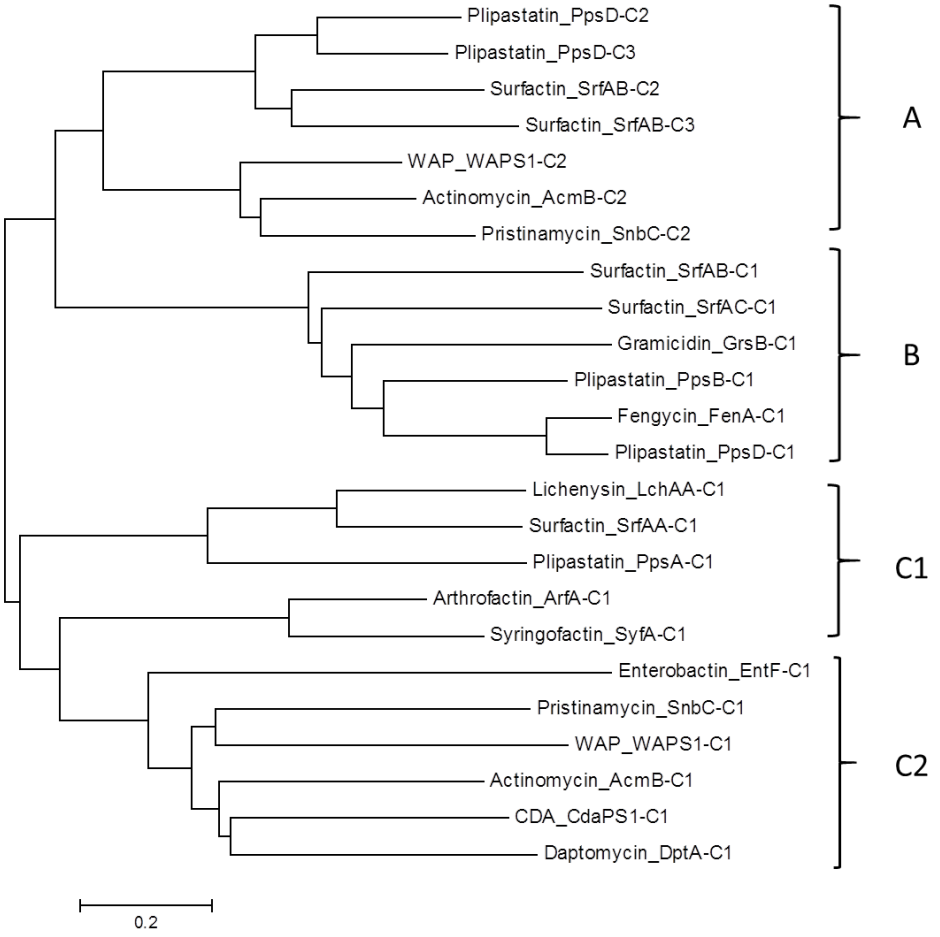
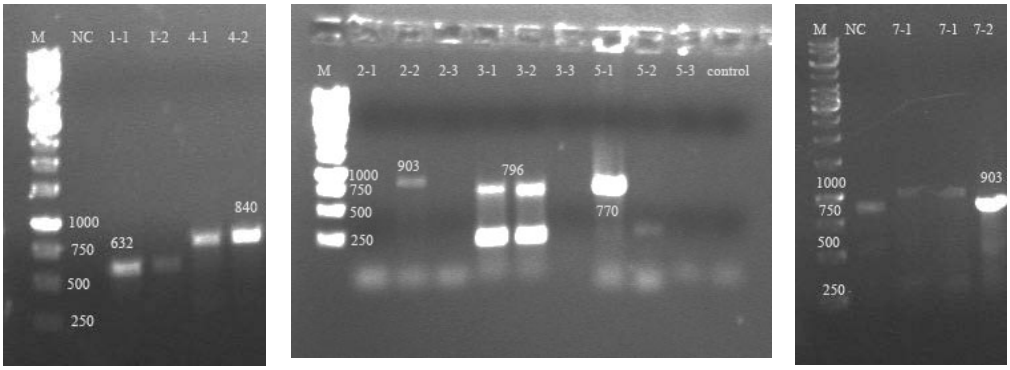


Fig. S1B Phylogenetic analysis of condensation domains of selected NRPS. The C domains in Group-A accept L-amino acids from the upstream donor PCP domain, the C domains in Group-B accept D-amino acids from the upstream donor PCP domain, and the C domains in Group-C accept (fatty)acyl donors, either as free acyl-CoA (Group-C1) or as bound acyl-ACP (Group-C2). Note that the starter C domain of WAP NPRS (WAP_WAPS1_C1) groups with Group-C2, although this C domain can use free acyl-CoA as substrate and there is no free-stand ACP present in the WAP gene cluster. The amino acid sequences of the C domains were retrieved from publicly accessible databases (http://www.ncbi.nlm.nih.gov). The GenBank accession numbers are listed as follows: C domain with L-amino acid donors (7 sequences, including PpsD-C2 and PpsD-C3 for Plipastatin biosynthesis, NP_389713.1; SrfAB-C2 and SrfAB-C3 for Surfactin biosynthesis, NP_388231.1; WAPS1-C2 for WAP-8294A2 biosynthesis, AEP18656.1; AcmB-C2 for Actinomycin biosynthesis, O68487; SnbC-C2 for Pristinamycin biosynthesis, Q54959), C domains with D-amino acid donors (6 sequences, including SrfAB-C1 for Surfactin biosynthesis, NP_388231.1; SrfAC-C1 for Surfactin biosynthesis, NP_388233.2; GrsB-C1 for Gramicidin biosynthesis; X61658.1; PpsB-C1 for Plipastatin biosynthesis, NP_389715.1; FenA-C1 for Fengycin biosynthesis, AF023464.2; PpsD-C1 for Plipasatain biosynthesis, NP_389713.1), and C domains with acyl donors (11 sequences, including ArfA-C1 for Arthrofactin biosynthesis, BAC67534.2; SyfA-C1 for Syringofactin biosynthesis, NP_792633.1; LchAA-C1 for Lichenysin biosynthesis, AAU22002.1; SrfAA-C1 for Surfactin biosynthesis, NP_388230.1; PpsA-C1 for Plipastatin biosynthesis, NP_389716.1; EntF-C1 for Enterobactin biosynthesis, CCN28675.1; SnbC-C1 for Pristinamycin biosynthesis, Q54959; ; WAPS1-C1 for WAP-

8294A2 biosynthesis, AEP18656.1; AcmB-C1 for Actinomycin biosynthesis, O68487; CdaPS1-C1 for Calcium-Dependent Antibiotics (CDA) biosynthesis, CAB38518.1; DptA-C1 for Daptomycin biosynthesis, AAX31557.1). The amino acid sequences of the C domains were predicted using PKS/NRPS analysis website (http://nrps.igs.umaryland.edu). Similar sequences were aligned with ClustalW and the tree shown was generated using the MEGA 5.0.





a b c

Fig. S2 Diagnostic PCR for disruption mutants of six ACL genes (ACL1, 4, 2, 3, 5, and 7)

a: Diagnostic PCR result of ACL1 (632 bp) and ACL4 (840 bp) disruption mutants. M: marker; NC: negative control with water as template; 1-1 and 1-2: ACL1 disruption mutant-1 and mutant-2; 4-1 and 4-2: ACL4 disruption mutants 1 and 2.

b: Diagnostic PCR result of ACL2 (903 bp), ACL-3 (796 bp), and ACL5 (770 bp) mutants. M: marker; 2-1, 2-2, and 2-3: ACL2 disruption mutants 1, 2, and 3 (2-2 is a true mutant); 3-1, 3-2, and 3-3: ACL3 disruption mutants 1, 2, and 3 (3-1 and 3-2 are true mutants); 5-1, 5-2, and 5-3: ACL5 disruption mutant 1, 2, and 3 (5-1 is a true mutant); control: negative control with water as template.

c: Diagnostic PCR result of ACL7 (903 bp) mutant. M: marker; NC: negative control with water as template; 7-1, 7-2, 7-3: ACL7 disruption mutants 1, 2, and 3 (7-2 is a true mutant)

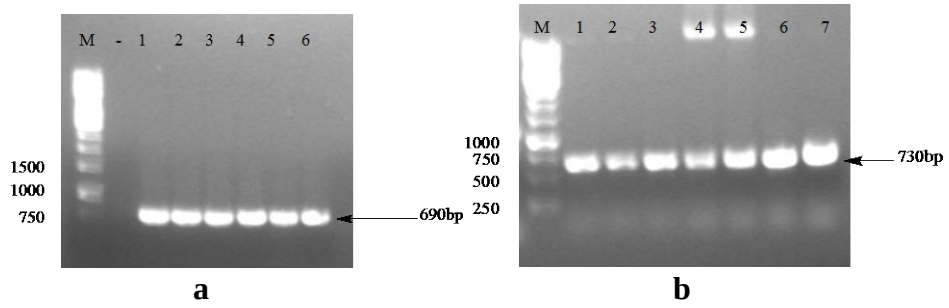


Fig. S3 Diagnostic PCR for disruption mutants and deletion mutants of ACL6.

a: Diagnostic PCR result of ACL6 (690 bp) disruption mutants. M: marker; -: negative control with water as template; 1-6: ACL6 disruption mutants 1-6.

b: Diagnostic PCR result of ACL6 (730 bp) deletion mutants. M: marker; 1-7: ACL6 deletion mutants 1-7.

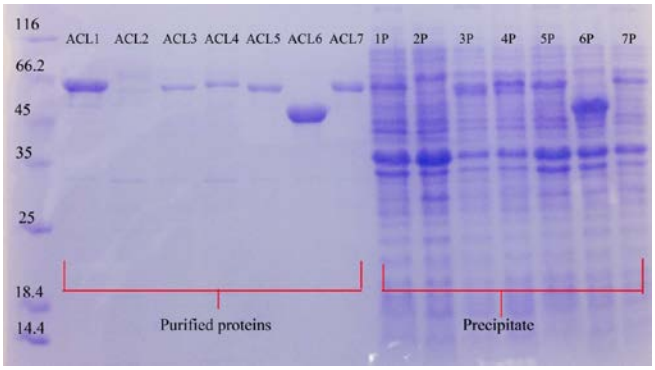


Fig. S4 Expression of seven ACL genes in *E. coli* and purification of the proteins. The first lane: protein markers with sizes indicated; lanes ACL1 through ACL7: purified ACL proteins (except ACL2); lanes 1P through 7P: insoluble fractions of the respective ACL1 through ACL7 protein extracts.

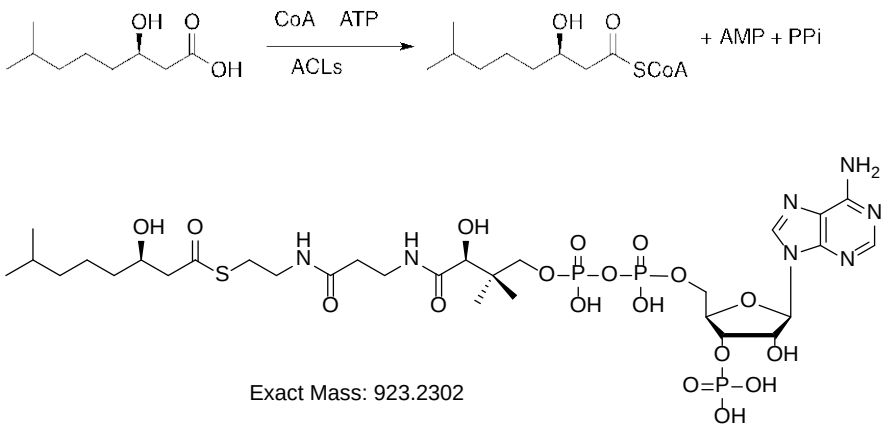


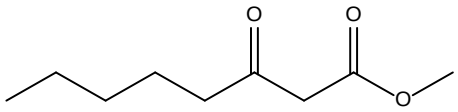
Fig. S5 Reaction scheme of the activation of (*R*)-3-hydroxy-7-methyloctanoic acid by ACL and the structure of (*R*)-3-hydroxy-7-methyloctanoyl-CoA.

Organic synthesis

General Methods

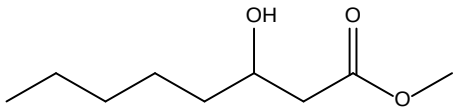
All reactions were conducted under an atmosphere of N_2 in flame-dried glassware. Reagents and solvents were used as supplied commercially, except CH_2Cl_2 (distilled from CaH_2) and THF (distilled from Na/benzophenone). Extracted organic layers were dried using sodium sulfate and filtered through a cotton plug. ^{1}H NMR and ^{13}C NMR spectra were acquired in $CDCl_3$ at the described spectrometer frequency. Chemical shifts are reported relative to residual chloroform (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C); ^{1}H spectra are reported as chemical shift (multiplicity, J couplings in Hz, number of protons). IR spectra were obtained on neat films (ZnSe, ATR mode) with selected absorbances reported in wavenumbers (cm^{-1}). Enantiomeric excess was determined by chiral GC fitted with a CycloSil-B column. Optical rotations were acquired on a digital polarimeter in a 10 cm cell of 2 ml volume. Melting points are uncorrected. Flash column chromatography was performed on 230-400 μM silica gel. Thin-layer chromatography (TLC) was performed on 0.25 mm hard-layer silica G plates containing a fluorescent indicator; developed TLC plates were visualized with a hand-held UV lamp or by heating after staining with a solution of 2.5% ammonium molybdate and 0.5% ceric sulfate in 10% sulfuric acid. Abbreviations throughout: EA = ethyl acetate; Hex = hexane; DCM = dichloromethane

Methyl 3-oxooctanoate (**1a**)



To a 0 °C solution of diisopropylamine (16.82 ml, 120 mmol) in 80 ml of THF was added 76.25 ml of a solution of n-butyl lithium in hexane (nominally 1.6 M in hexane, 122 mmol). The solution was allowed to stir for 15 minutes after which was added 6.9640 g of methyl acetoacetate (60 mmol). The reaction mixture was stirred for one hour at 0 °C and then 8.9050 g of bromobutane (65mmol)**.** The reaction was stirred for an additional hour and then quenched with 30 ml of 6M aq. HCl. The resulting solution was extracted with ether (3 x 60 ml). The combined organic extracts were dried and then concentrated under reduced pressure. The residue was purified by chromatography (8%EA/Hex) to yield 5.1666 g (50%) of **1a** as a yellow oil R_f: 0.43 (10% EA/Hex); ^{1}H NMR (400 MHz): δ 0.915 (t, 3H, J= 7.2), 1.259-1.350 (overlapping peaks, 4H), 1.610 (m, 2H), 2.537 (t, 1.9 H, J= 7.6), 3.456 (s, 1.9H), 3.745 (s, 3H); ^{13}C NMR (100 MHz): δ 14.04 (CH3), 22.56 (CH2), 23.32 (CH2), 31.32(CH2), 43.20 (CH2), 49.19 (CH2), 52.48 (CH3), 167.88 (C), 203.02 (C); HRMS calculated for $C_9H_{16}O_3$ $(M + Na)^+$: 195.0997; found: 195.0993.

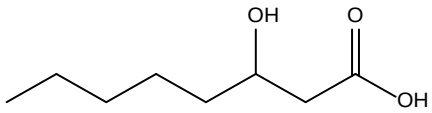
(*R*)-Methyl 3-hydroxyoctanoate (*R*-**2a**)



To a 0 °C solution of 1a (2.79 g, 16.22 mmol) in 20 ml of THF was added 0.62 ml of 1M (*R*)-(+)-2-methyl-CBS-oxazaborolidine (1.62 mmol) in toluene, followed by addition of 12.17 ml of a solution of borane dimethyl sulfide complex (nominally 2M, 24.33 mmol)

in THF over 1 hour. The reaction was stirred for 2 h at 0 °C and then quenched by slow addition of aq. 6M HCl (5 mL). The resulting solution was extracted with ether (3 x 60 ml) and the combined organic layers were dried and then concentrated under reduced pressure. The residue was purified by chromatography (10%EA/Hex) to yield 1.2920 grams (45%) of *R*-**2a** as a yellow oil. R_f: 0.18 (20% EA/Hex); ^{1}H NMR (300 MHz): δ 0.90 (t, 3H, J= 6.6), 1.32-1.53 (overlapping peaks, 8H), 2.53 (dd, 1H, 16.5, 3.3), 2.42 (dd, 1H, J=16.3, 9), 2.89 (d, OH, J= 3.6), 3.73 (s, 2.9H), 4.01 (m, 1H); ^{13}C NMR (75 MHz): δ 13.99 (CH3), 22.56 (CH2), 25.14 (CH2), 31.69 (CH2), 36.49 (CH2), 41.11 (CH2), 51.71 (CH3), 68.02 (CH), 173.49 (C); α_D^{21} -21.9° (*c* 1.00, $CHCl_3$); 87 (±3) % e.e. by chiral GC; HRMS calculated for $C_9H_{18}O_3$ $(M + Na)^+$: 197.1154; found: 197.1154.

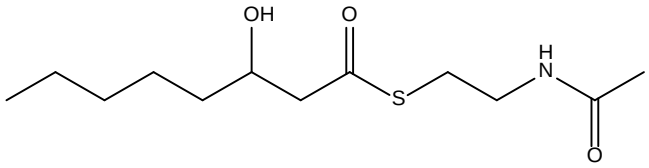
(*R*)-3-Hydroxyoctanoic acid (**3a**)



To a 0 °C solution of **2a** (1.2920 g, 7.42 mmol) in 25 ml of MeOH/THF/H_2O (2:2:1) was added LiOH (5.5 g, 130 mmol) was added. The mixture was allowed to warm to rt and stirred for 3 hours. The solution was then quenched with 1M aq. HCl until the solution reached pH 3. The solution was then extracted with EA (3 x 60 ml). The combined organic extracts were dried and then concentrated under reduced pressure to yield 1.2917 grams of **3a** as a white solid that required no further purification (99%). R_f: 0.29 (10% EA/89% Hex/1% acetic acid); mp 37-39 °C, ^{1}H NMR (400 MHz): δ 0.908 (t, 3H, J= 6.8), 1.29-1.56 (overlapping peaks, 8H), 2.58 (dd, 0.95H, J= 16.4, 3.2), 2.46 (dd, 0.95H, J=

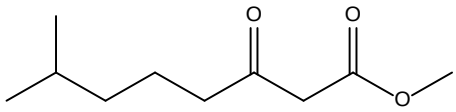
16.4, 8.9), 4.06 (m, 0.9H); ^{13}C NMR (100 MHz): δ 13.99 (CH3), 22.56 (CH2), 25.11 (CH2), 31.65 (CH2), 36.42 (CH2), 41.11 (CH2), 68.12 (CH), 177.72 (C).

(*R*)-3-Hydroxyoctanethoic acid, *S*-[2-(acetylamino)ethyl] ester (**4a**)



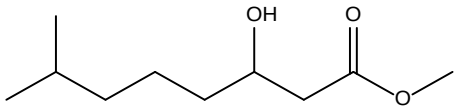
To a 0 °C stirred solution of 3a (0.5145 g, 3.22 mmol) in DCM (10 mL) was added *N*-acetylcysteamine (0.5745 g, 4.82 mmol) and 4-dimethylaminopyridine (0.0785 g, 3.86 mmol), followed by *N*,*N*′-dicyclohexylcarbodiimide (0.7960 g 3.86 mmol). The reaction was allowed to warm to rt and stirred for 4 hours. The reaction was quenched with 10 ml of 1M aq. HCl and then filtered. The combined DCM extracts (3 x 30ml) were washed with aq. 0.5 M HCl (10ml), washed with sat. aq. $NaHCO_3$ (10ml) and finally washed with 20ml sat. aq. NaCl. The combined organic extracts were dried and then concentrated under reduced pressure. The residue is then purified by chromatography (3% MeOH/DCM) to yield 0.6713 grams (80%) of **4a** as a white solid. R_f: 0.13 (2% methanol/DCM); mp 41-43 °C, ^{1}H NMR (300 MHz): δ 0.87 (t, 3H, J= 6.3), 1.28-1.46 (overlapping peaks, 8H), 1.96 (s, 3H), 2.70 (dd, 1H, J= 15.3, 3.9), 2.66 (dd, 1H, J= 15, 8.1), 3.03 (t, 2.3H, J= 3.9), 3.42 (q, 2H, J= 6.3), 4.04 (m, 1H), 6.24 (s, 0.9H); ^{13}C NMR (75 MHz): δ 13.98 (CH3), 22.54 (CH2), 23.13 (CH3), 25.10 (CH2), 28.79 (CH2), 31.64 (CH2), 36.76 (CH2), 39.20 (CH2) 51.15 (CH2), 51.43 (CH2), 68.78 (CH), 170.64 (C), 199.38 (C); IR: 3310, 2923, 1639, 1043; α_D^{20} -7.5 (6.50, $CHCl_3$); HRMS calculated for $C_{12}H_{23}NO_3S$ $(M + Na)^+$: 284.1296; found: 284.1305.

Methyl 7-methyl-3-oxooctanoate (**1b**)



To a 0 °C solution of diisopropylamine (6.306 ml, 45 mmol) in 40 ml of THF was added 28.59 ml of a solution of *n*-butyl lithium in hexane (nominally 1.6 M, 45.75 mmol). The solution is allowed to stir for 15 minutes after which was added 2.6115 g of methyl acetoacetate (22.5 mmol). The reaction mixture was stirred for one hour at 0 °C and then 3.4730 g of 1-bromo-3-methylbutane (23 mmol) were added. The reaction was stirred for an additional hour and then quenched with 10 ml of 6M aq. HCl. The resulting solution was extracted with ether (3 x 60 ml). The combined organic extracts were dried and then concentrated under reduced pressure. The residue was purified by chromatography (8% EA/Hex) to yield 0.7466 g (18%) of **1b** as a yellow oil. R_f: 0.31 (10% EA/Hex); ^{1}H NMR (400 MHz): δ 0.896 (d, 6H, J=8.8), 1.18 (q, 2.2H, J=10.8), 1.534-1.64 (overlapping peaks, 3H), 2.53 (t, 1.9H, J= 9.6), 3.46 (s, 1.9H), 3.76 (s, 3H); ^{13}C NMR (100 MHz): δ 21.55 (CH2), 22.65 (2 x CH3), 28.03 (CH), 38.42 (CH2), 43.50 (CH2), 49.25 (CH2), 52.53 (CH3), 167.91 (C), 203.03 (C); IR: 2954, 1746, 1715, 1236, 1152; HRMS calculated for $C_{10}H_{18}O_3$ $(M + Na)^+$: 209.1154; found: 209.1147.

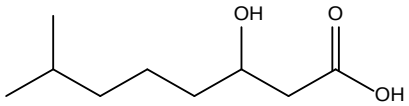
(*R*)-Methyl 3-hydroxy-7-methyloctanoate (**2b**)



To a 0 °C solution of 1b (0.7400 g, 3.98 mmol) in 20 ml of THF was added 0.398 ml of 1M (*R*)-(+)-2-methyl-CBS-oxazaborolidine (0.40 mmol) in toluene, followed by addition

of 2.98 ml of a solution of borane dimethyl sulfide complex (nominally 2M, 5.97 mmol) in THF over 1 hour. The reaction was stirred for 2 h at 0°C and then quenched by slow addition of aq. 6M HCl (5 ml). The resulting solution was extracted with ether (3 x 60 ml) and the combined organic layers were dried and then concentrated under reduced pressure. The residue was purified by chromatography (10% EA/Hex) to yield 0.1942 grams (45%) of *R*-**2b** as a yellow viscous oil. R_f: 0.18 (20% EA/Hex); ^{1}H NMR (300 MHz): δ 0.88 (d, 6H, J=6.6), 1.19-1.55 (overlapping peaks, 7H), 2.53(dd, 1H, J=16.2, 3), 2.42 (dd, 1H, J=16.5, 9), 2.85 (d, OH, J=4.2), 3.73 (s, 3H), 4.02 (m, 1H); ^{13}C NMR (75 MHz): δ 22.78 (2 x CH3), 23.49 (CH2), 28.14 (CH), 36.99(CH2), 39.02 (CH2), 41.33 (CH2), 51.95 (CH3), 68.26 (CH), 173.73 (C); IR: 3461, 2952, 1724, 1166; α_D^{20} -19.1 (5.00, $CHCl_3$); 84% (±3) e.e. based upon chiral GC; HRMS calculated for $C_{10}H_{20}O_3$ (M + Na)$^+$: 211.1310; found: 211.1305.

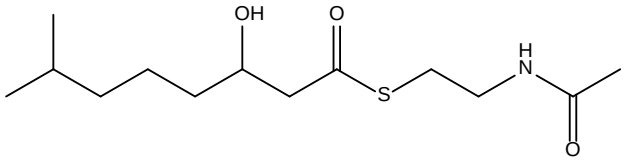
(R)-3-Hydroxy-7-methyloctanoic acid (**3b**)



To a 0 °C solution of 2b (0.1600 g, 0.85 mmol) in 10 ml of MeOH/THF/H_2O (2:2:1) was added LiOH (0.714 g, 17 mmol). The mixture was allowed to warm to rt and stirred for 3 hours. The solution was then quenched with 1M aq. HCl until the solution reached pH 3. The solution was then extracted with EA (3 x 60 ml). The combined organic extracts were dried and then concentrated under reduced pressure to yield 0.1438 grams of **3b** as a white solid that required no further purification (99%). R_f: 0.29 (10% EA/89% Hex/1% acetic acid); mp 37-39 °C; ^{1}H NMR (400 MHz): δ 0.90 (d, 6H, J=5.1), 1.21-1.56 (overlapping peaks, 7H), 2.60 (dd, 1.1H, J=16.8, 3.2), 2.50 (dd, 1.1H, J=16.4, 8.8), 4.061

(m, 1H); ^{13}C NMR (100 MHz): δ 22.54 (2 x CH3), 23.22 (CH2), 27.91 (CH), 36.75(CH2), 38.74 (CH2), 40.97 (CH2), 67.98 (CH), 177.49 (C); IR: 2952, 1707; HRMS calculated for $C_9H_{18}O_3$ $(M + Na)^+$: 197.1154; found: 195.1146.

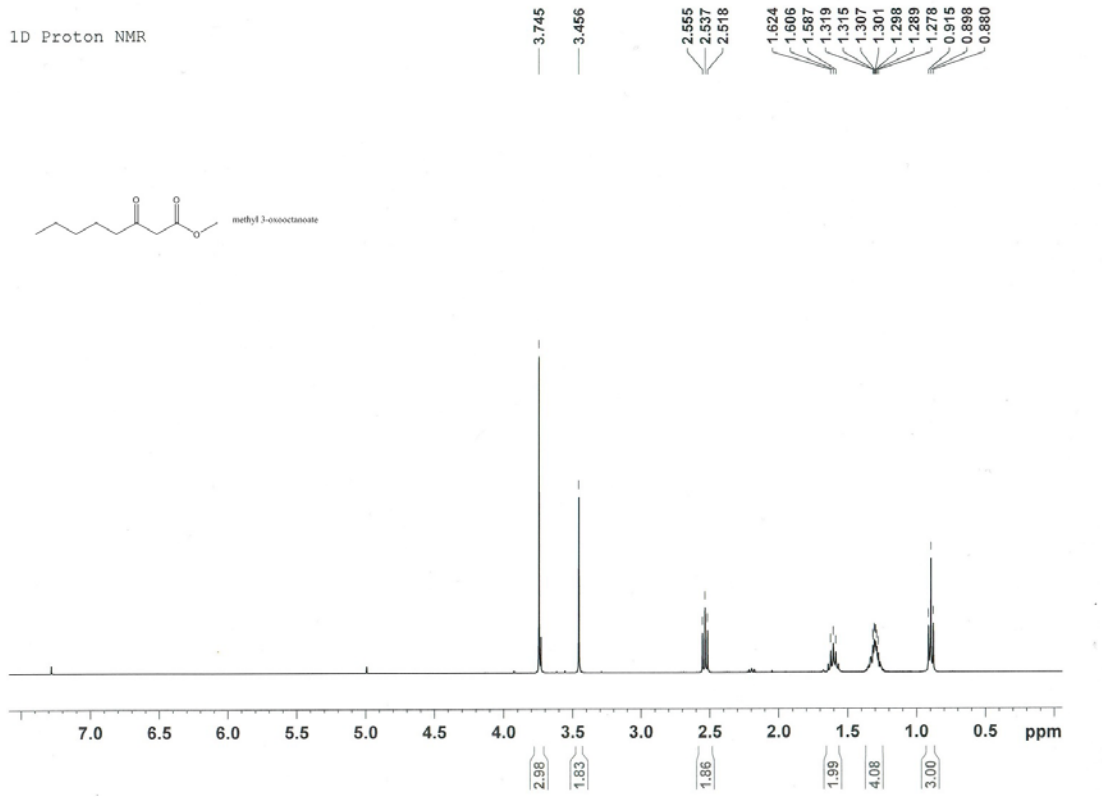
***(R)*-3-Hydroxy-7-methyloctanethioic acid, *S*-[2-(acetylamino)ethyl] ester (4b)**



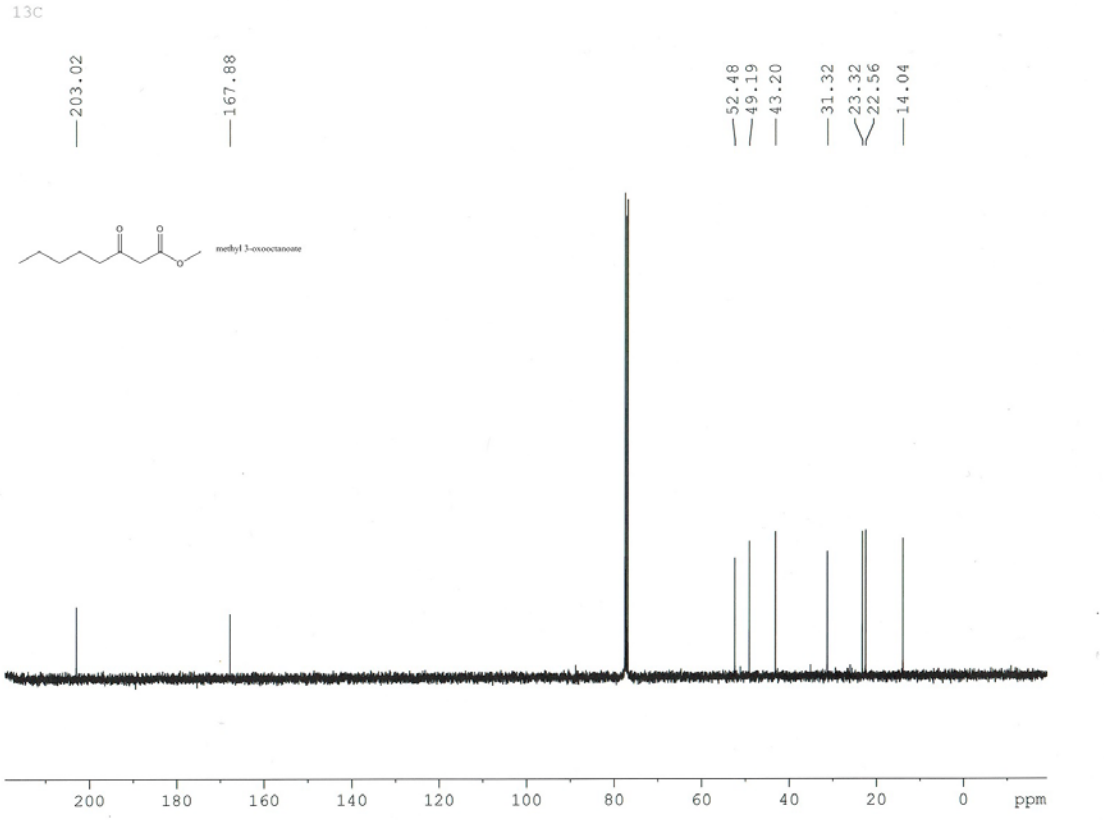
To a 0 °C stirred solution of **3b** (0.1430 g, 8.2 mmol) in DCM (10 ml) was added *N*-acetylcysteamine (0.1190 g, 1.29 mmol) and 4-dimethylaminopyridine (0.0210 g, 0.1724 mmol), followed by *N*,*N*'-dicyclohexylcarbodiimide (0.2311g 1.12 mmol). The reaction was allowed to warm to rt and stirred for 4 hours. The reaction was quenched with 10 ml of 1M aq. HCl and then filtered. The combined DCM extracts (3 x 30ml) were washed with aq. 0.5 M HCl (10ml), washed with sat. aq. $NaHCO_3$ (10ml) and finally washed with 20ml sat. aq. NaCl. The combined organic extracts were dried and then concentrated under reduced pressure. The residue is then purified by chromatography (3% MeOH/DCM) to yield 0.1846 grams (79%) of *R*-**4b** as a white/yellow solid. R_f: 0.13 (2% methanol/DCM); mp 43-45 °C, ^{1}H NMR (300 MHz): δ 0.89 (d, 6H, J=6.6), 1.20-1.63 (overlapping peaks, 7H), 1.99 (s, 3H), 2.78 (dd, 1H, J=15.6, 3.9), 2.71 (dd, 1H, J=15.3, 6.9), 3.07 (t, 2H, J= 6.3), 3.47 (q, 2H, J= 4.5), 4.08 (m, 1H), 5.820 (s, 0.9H); ^{13}C NMR (75 MHz): δ 22.56 (2 x CH3), 23.21 (CH3), 27.91 (CH2), 28.86 (CH), 36.98 (CH2), 38.76 (CH2), 39.30 (CH2), 51.04 (CH2), 68.84 (CH), 170.38 (C), 199.60 (C); IR: 3288, 2930, 1652, 1038; α_D^{20} -7.0 (6.00, $CHCl_3$), HRMS calculated for $C_{13}H_{25}NO_3S$ $(M + Na)^+$: 298.1453; found: 298.1453.

Supplementary figures

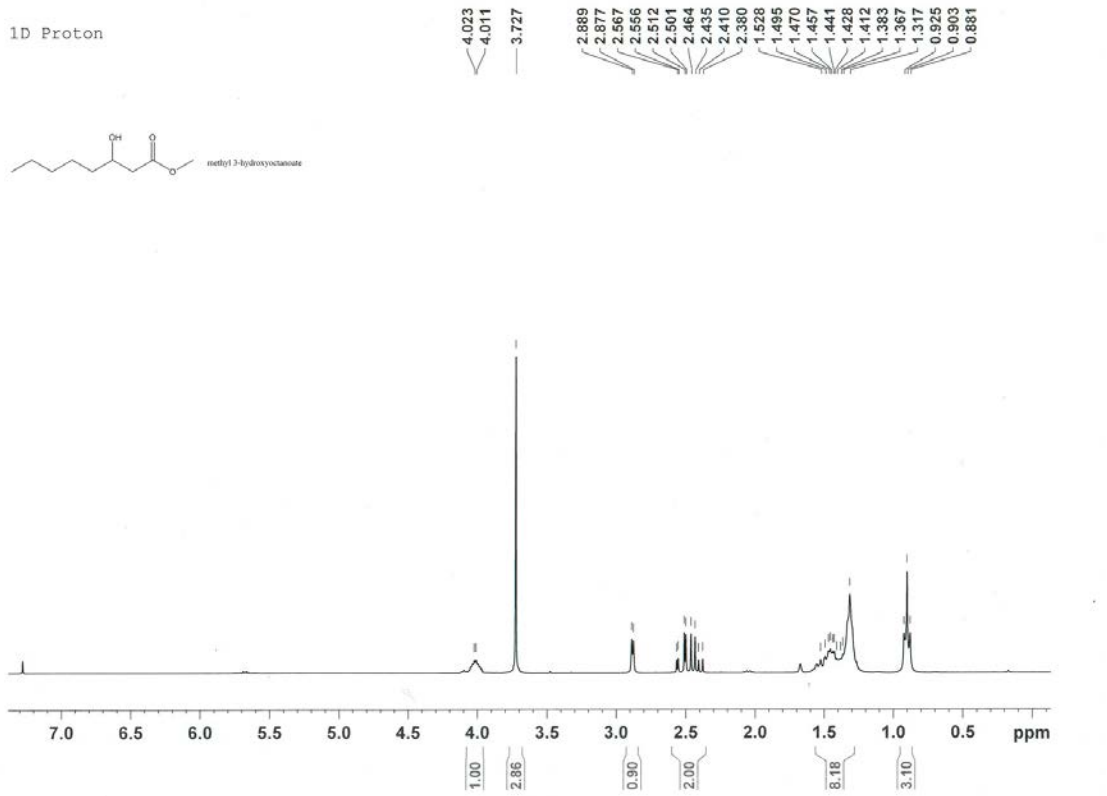
^{1}H NMR spectrum of 1a



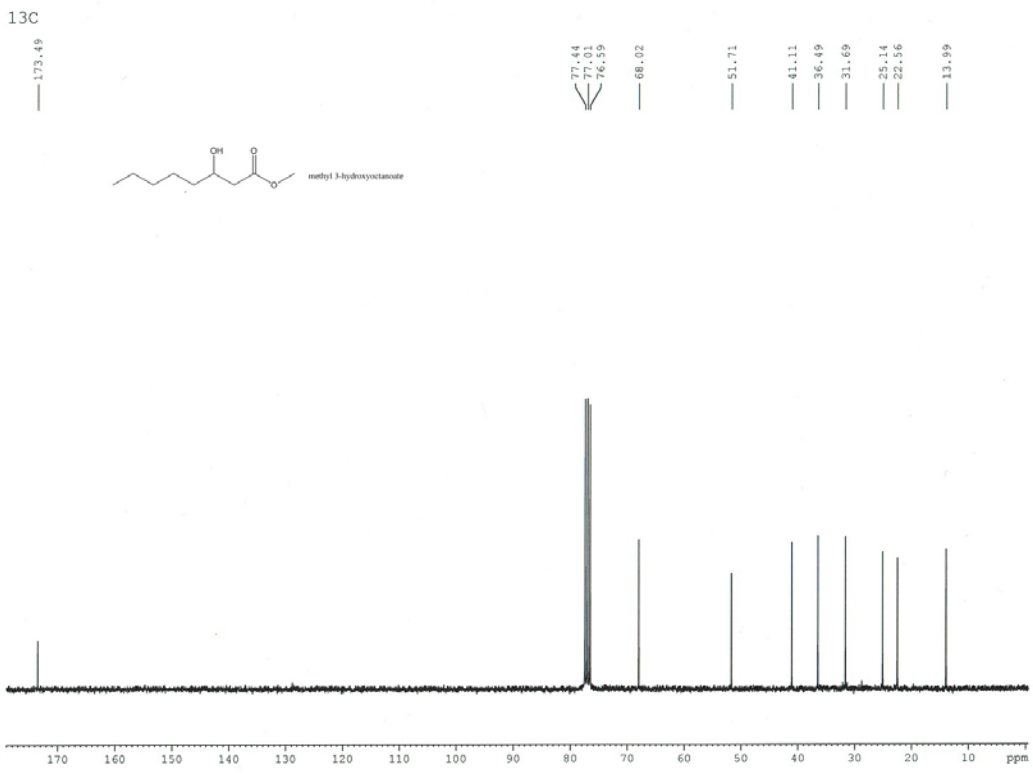
^{13}C NMR Spectrum of 1a



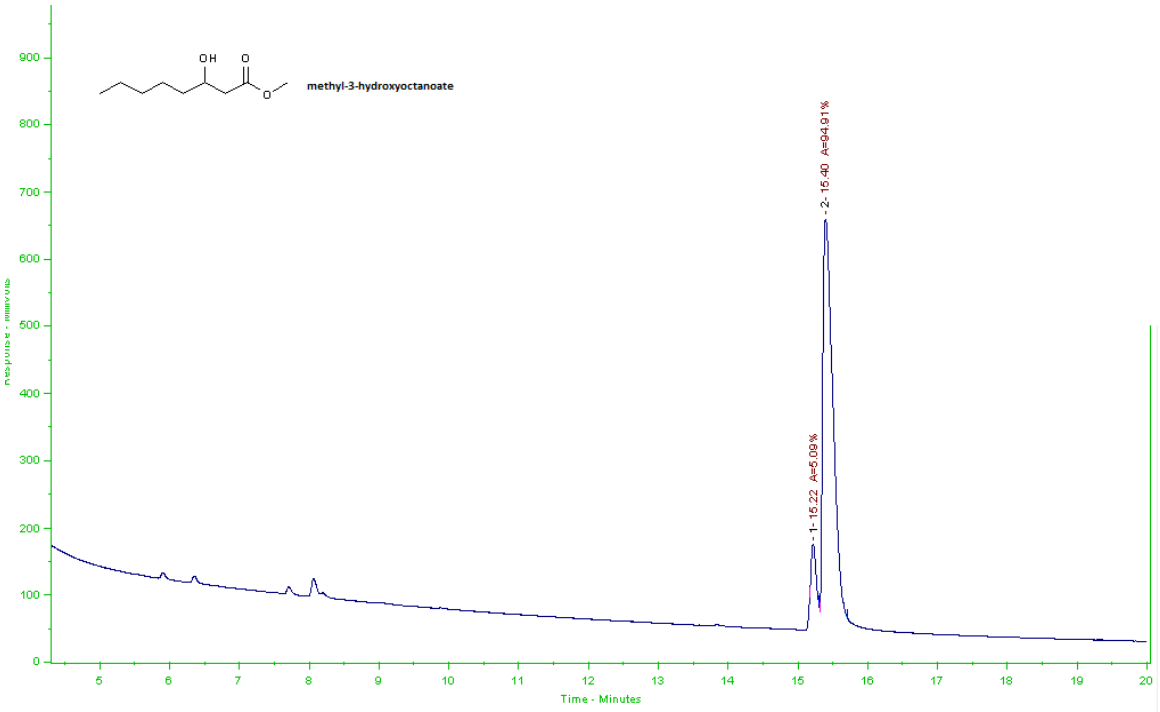
^{1}H NMR spectrum of 2a



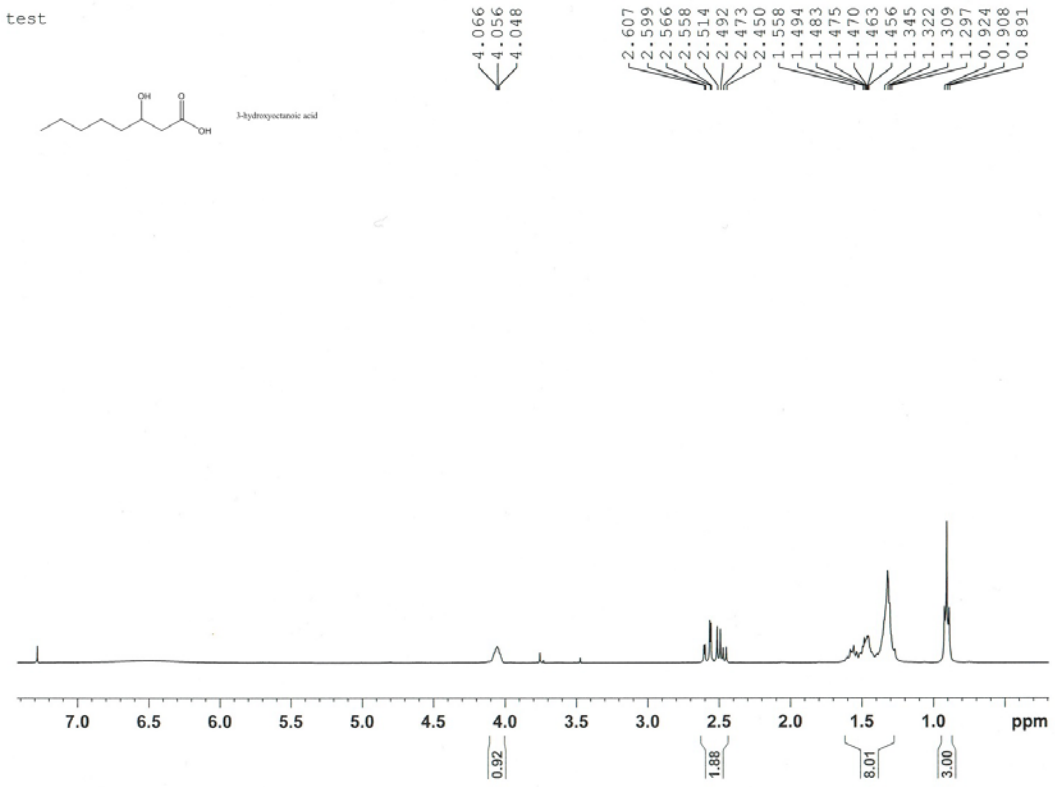
^{13}C NMR Spectrum of 2a



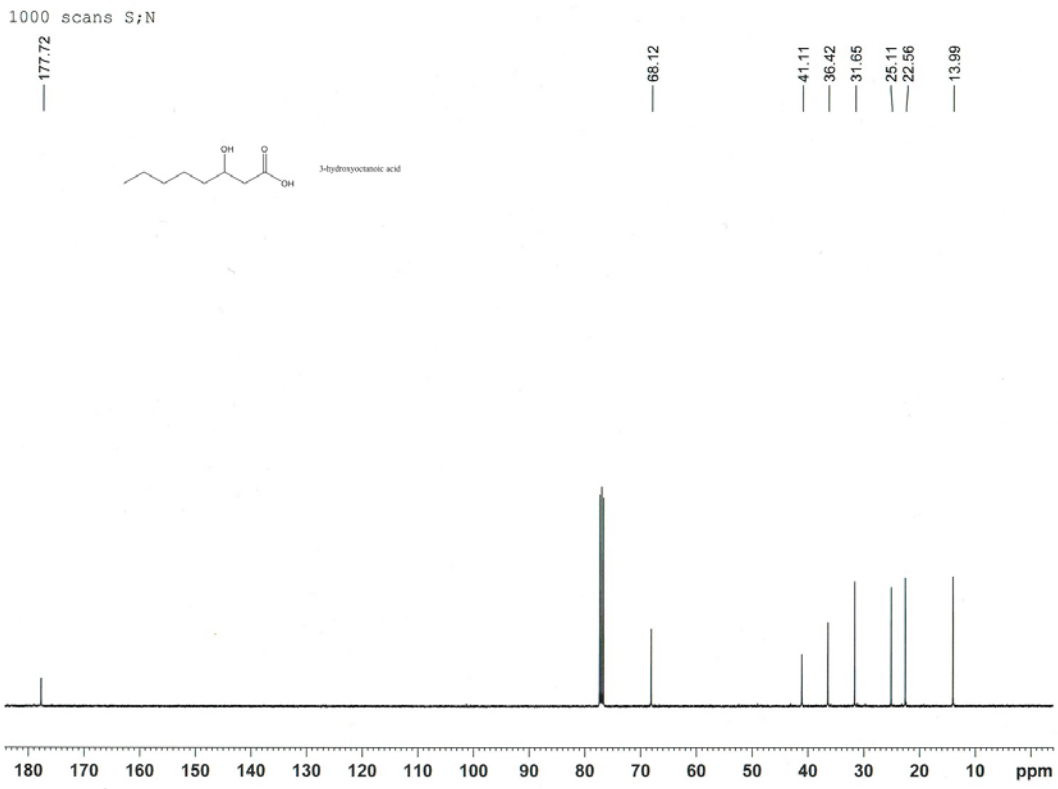
Chiral GC trace of 2a



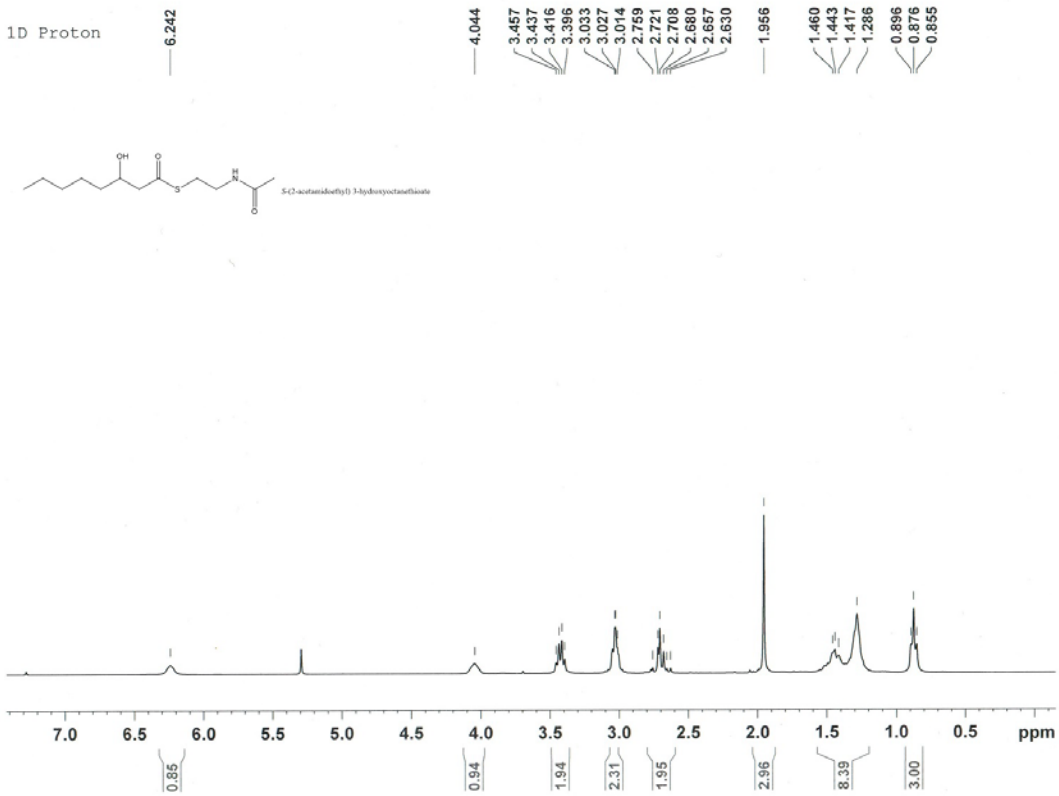
^{1}H NMR spectrum of 3a



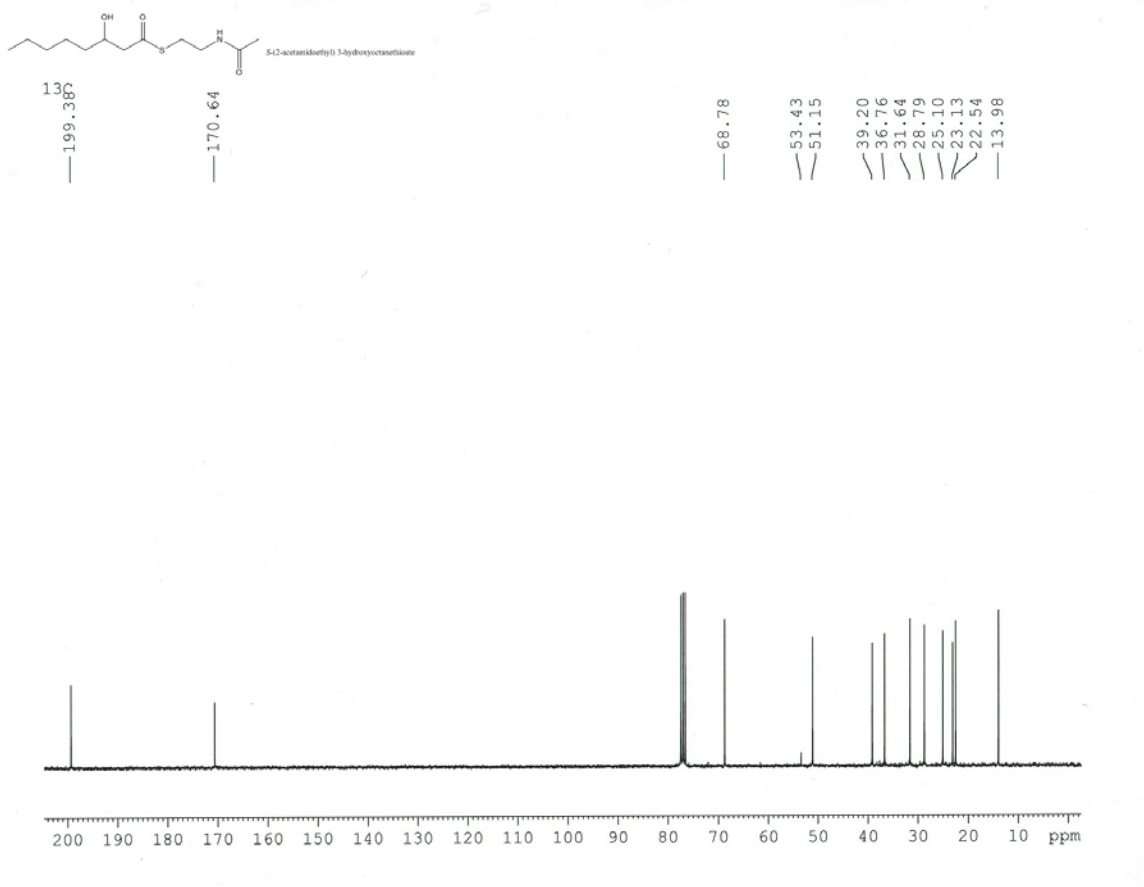
^{13}C NMR Spectrum of 3a



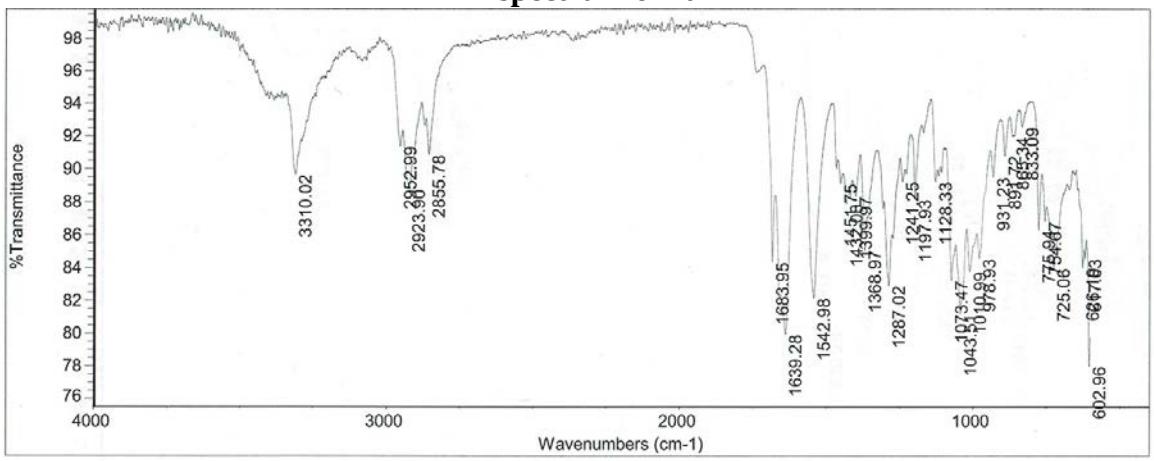
^{1}H NMR spectrum of 4a



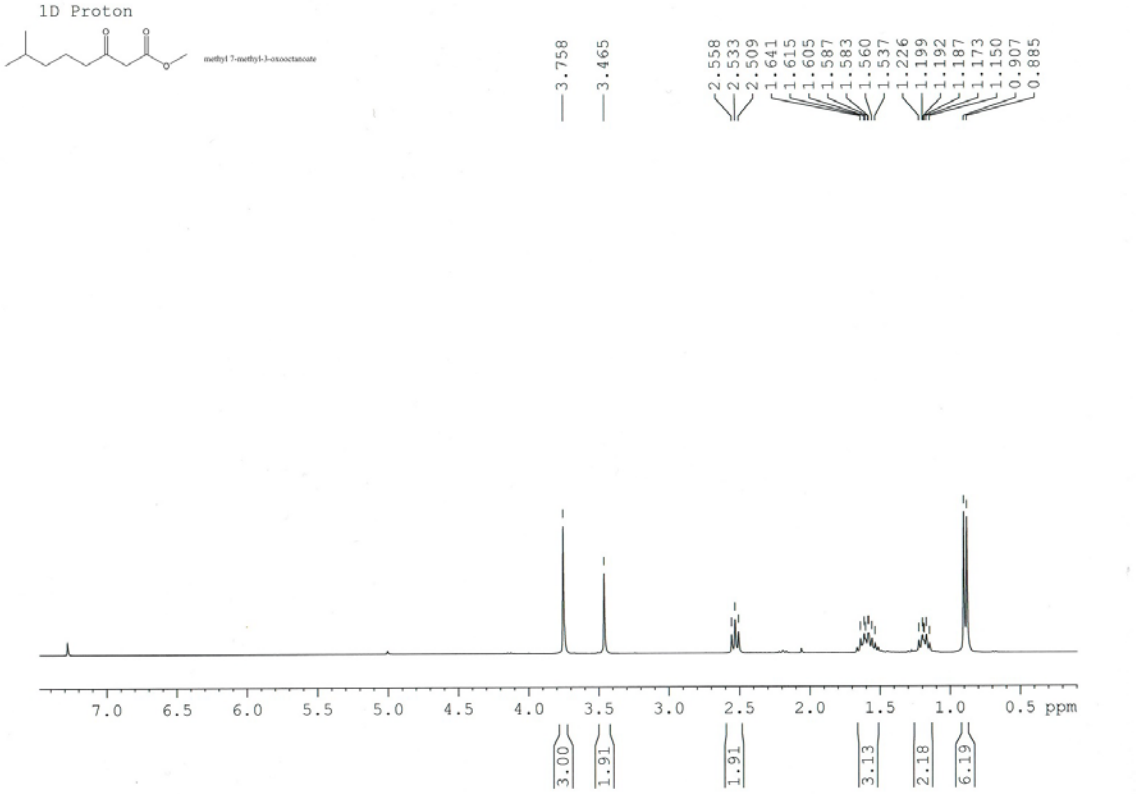
^{13}C NMR Spectrum of 4a



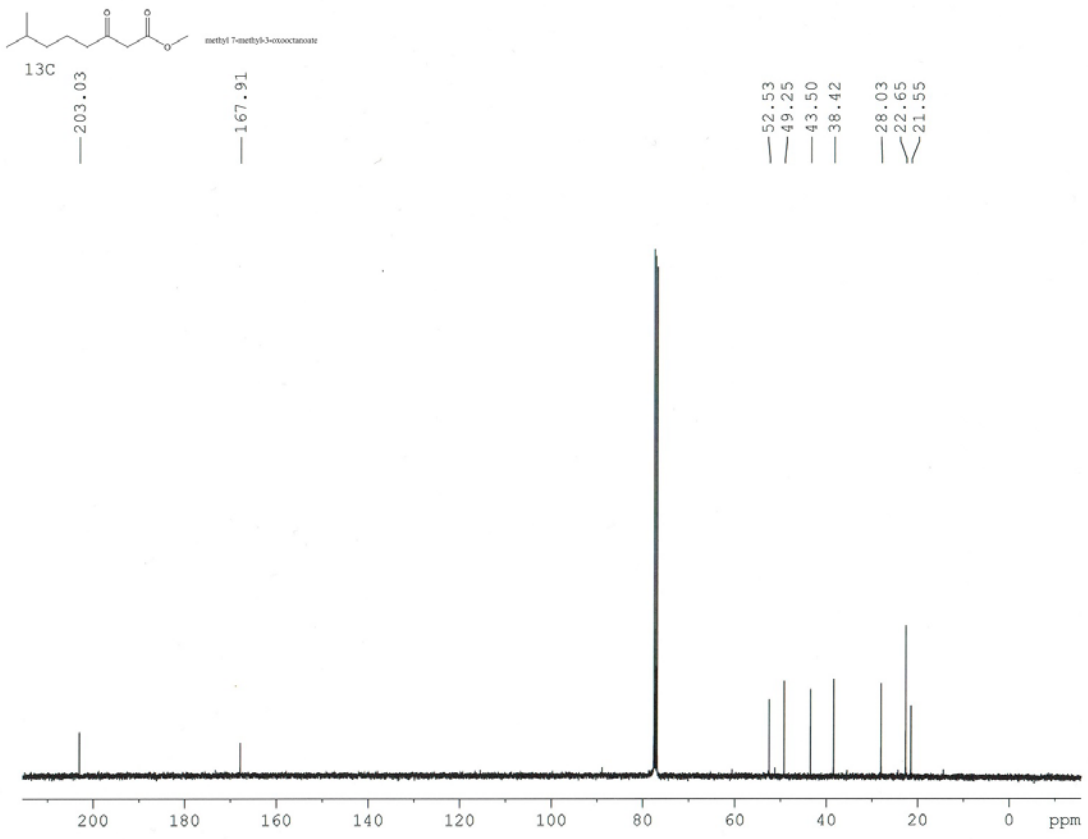
IR spectrum of 4a



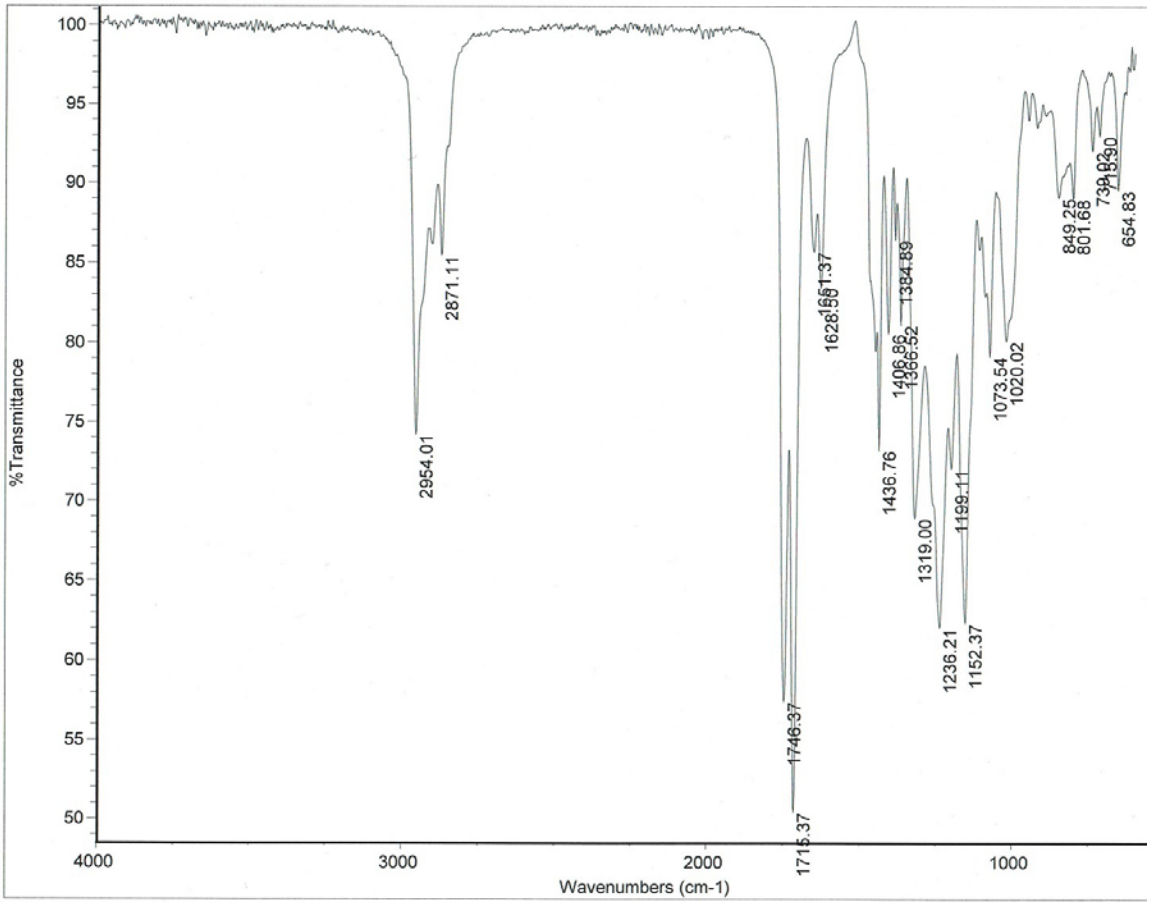
^{1}H NMR spectrum of 1b



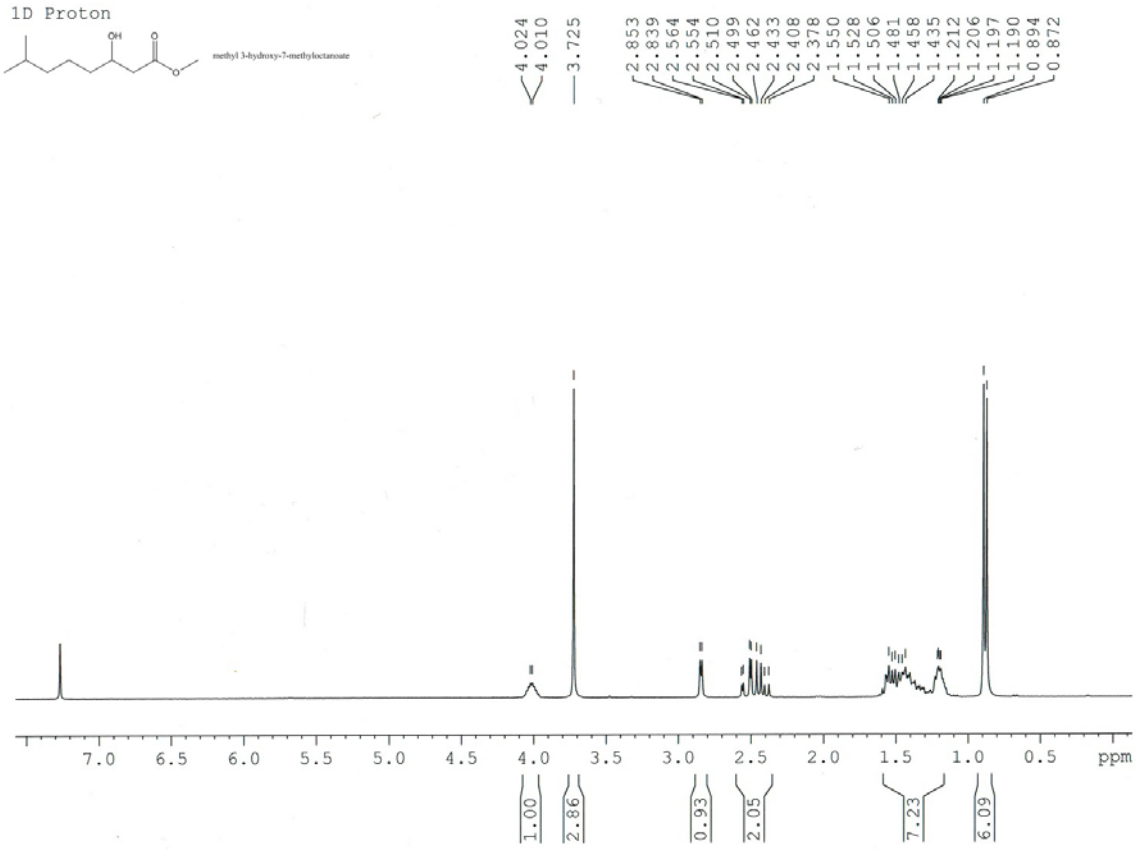
^{13}C NMR Spectrum of 1b



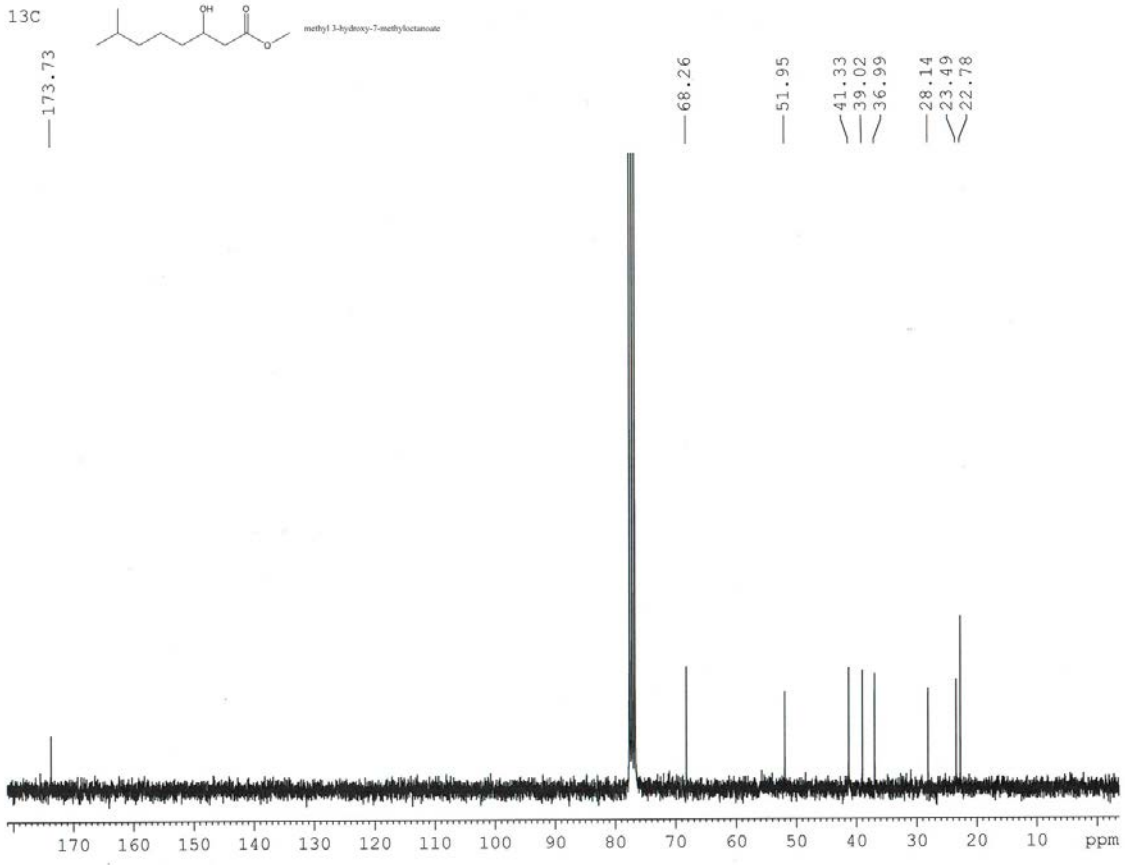
IR spectrum of 1b



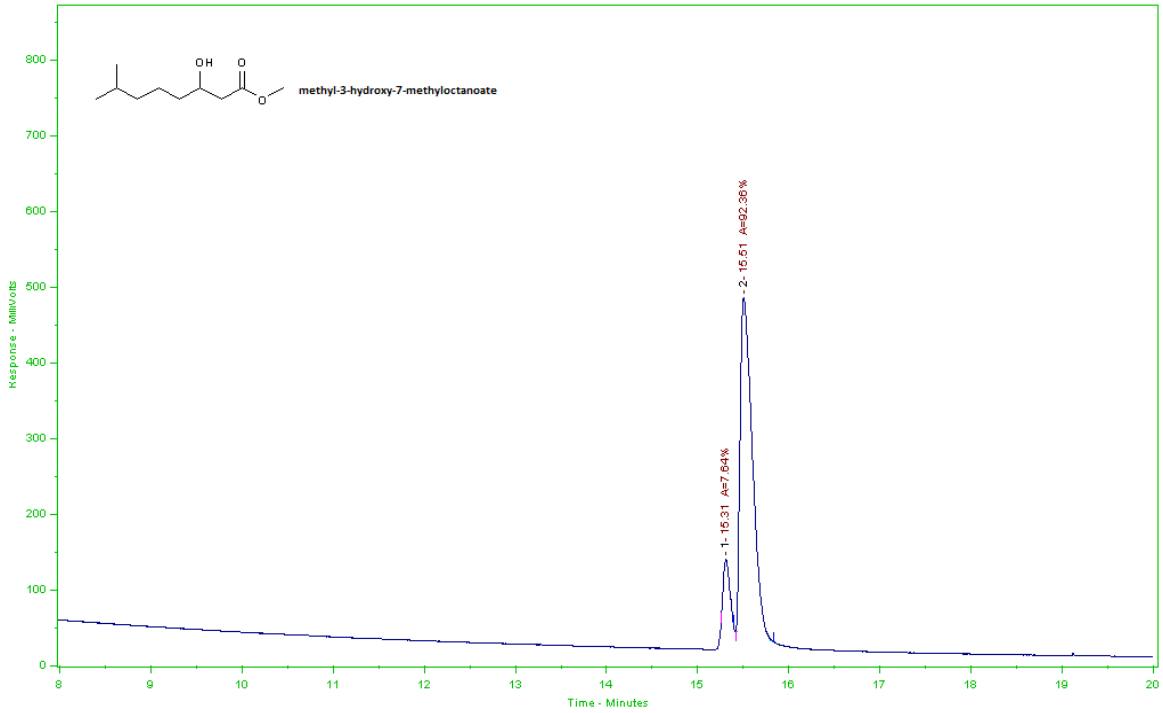
^{1}H NMR spectrum of 2b



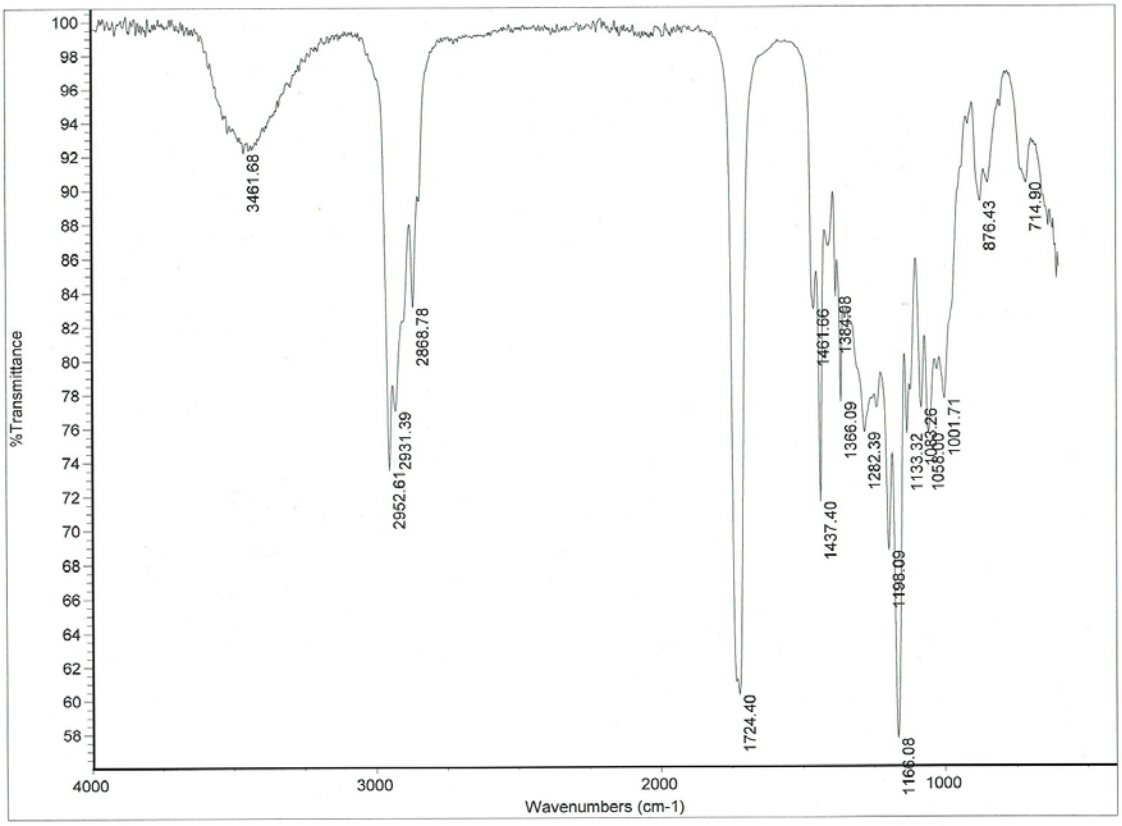
^{13}C NMR Spectrum of 2b



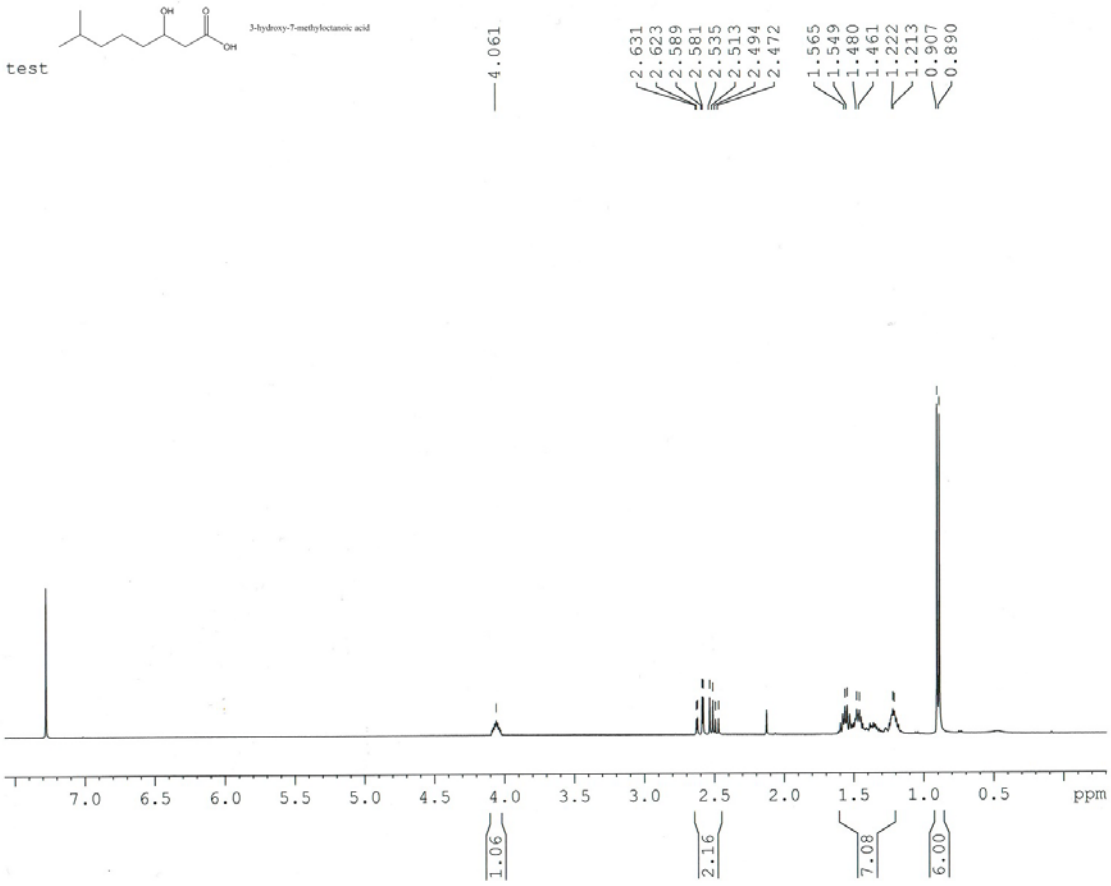
Chiral GC Trace of 2b



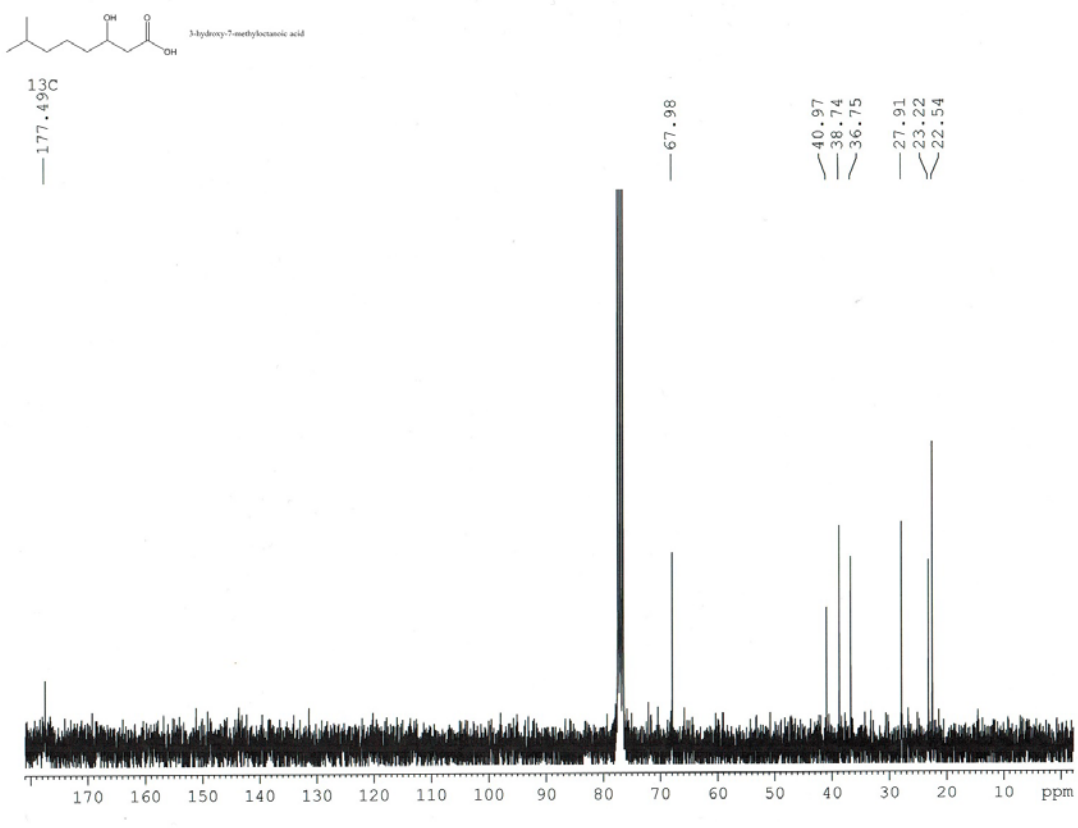
IR spectrum of 2b



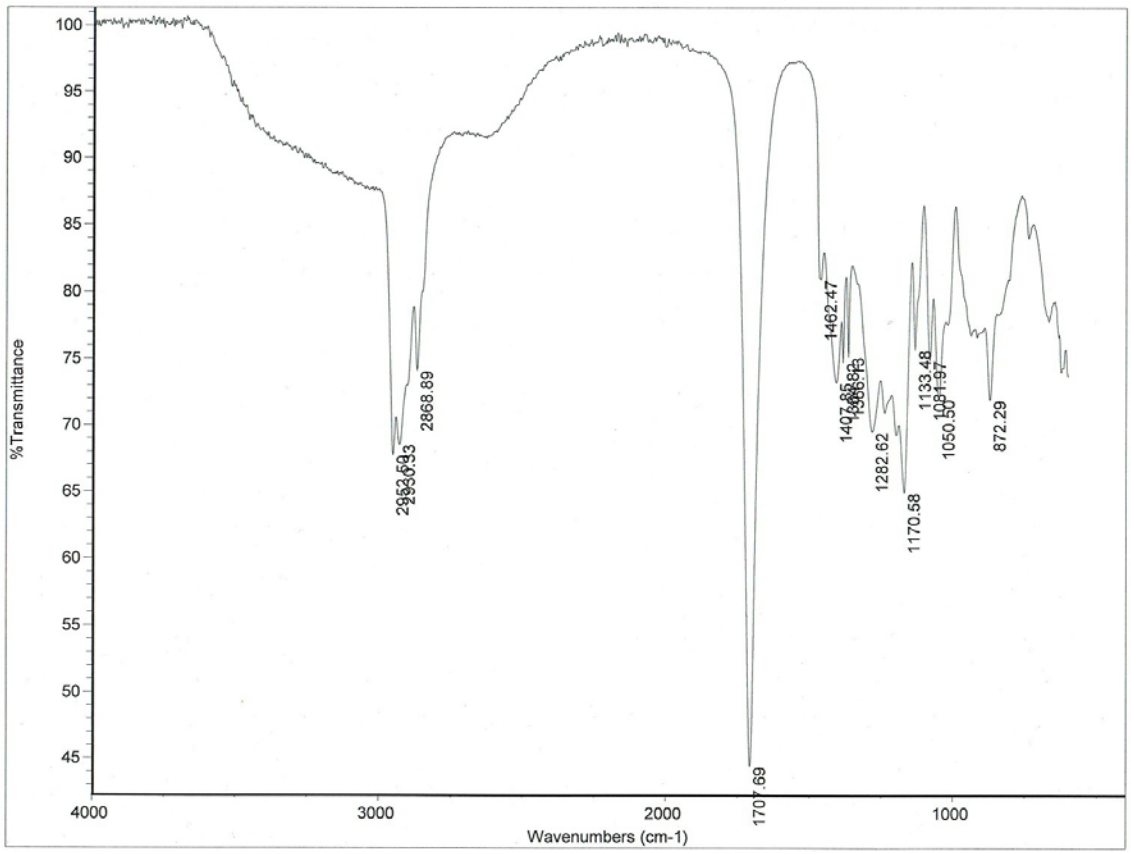
^{1}H NMR spectrum of 3b



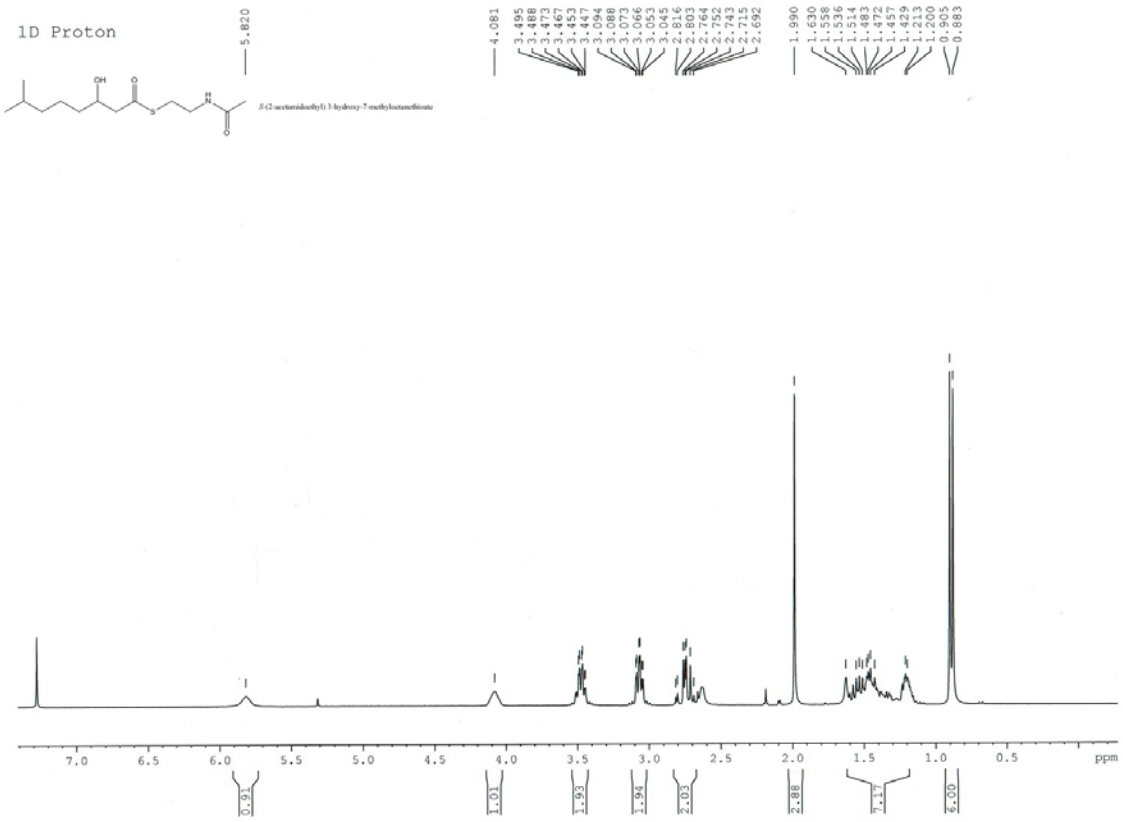
^{13}C NMR Spectrum of 3b



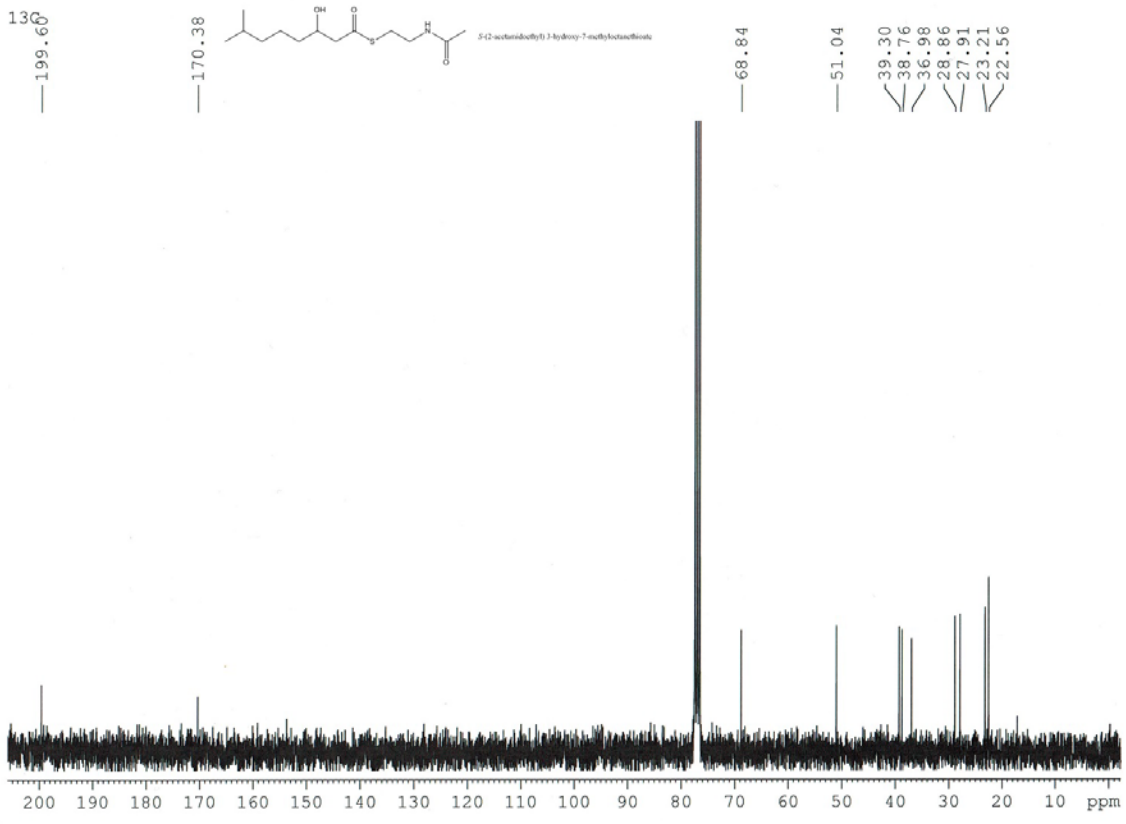
IR spectrum of 3b



^{1}H NMR spectrum of 4b



^{13}C NMR Spectrum of 4b



IR spectrum of 4b