
Supplementary information

A strategic Ser/Cys exchange in the catalytic triad unlocks an acyltransferase-mediated synthesis of thioesters and tertiary amides

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A strategic Ser/Cys exchange in the catalytic triad unlocks an acyltransferase-mediated synthesis of thioesters and tertiary amides

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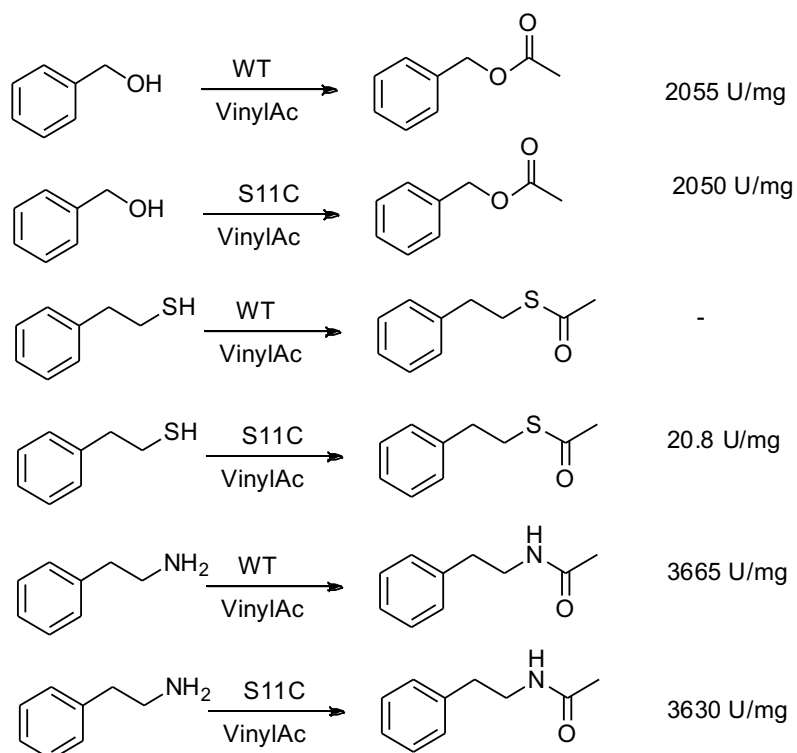
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Supplementary methods

S11C MsAcT activity assay

Activity measurements were performed as reported in Contente *et al.*¹ Specific activity was 1.8 U/mg. A comparison between the mutated enzyme and the wild-type was carried out. Activity measurements through three different condensation reactions were performed.



Supplementary Table: 1 Reactions were performed at 250 mM using 0.1 mg/mL of enzyme. Samples at 5, 10, 15 min were analysed by GC or HPLC following the procedure reported in Chiarelli *et al.*² and Contente *et al.*¹ The specific activity was calculated for each compound.

WT MsAcT batch reactions

Batch reactions testing the catalytic performance of WT MsAcT were carried out in 10 mL screw cap tubes; 1 mL reaction mixture in 100 mM phosphate buffer pH 8.0, containing 250 mM substrate (thiols or secondary amines), 1 or 5 mg/mL enzyme, and 10% v/v vinyl acetate were left under magnetic stirring at 25 °C. 50 µL aliquots were collected at different reaction times. For thiol biotransformations, samples were extracted with 100 µL of EtOAc. In the case of amine substrates before the extraction, samples were quenched with NaOH 1 M. The reactions were monitored by TLC (synthesis of thioesters: *n*-Hexane/EtOAc 8:2; synthesis of amides: CH₂Cl₂/MeOH 9:1 + 0.1% TEA). Samples, after anhydrication were prepared for GC analysis or after evaporation, resuspended in the mobile phase for HPLC analysis. No transformation was observed in any case.

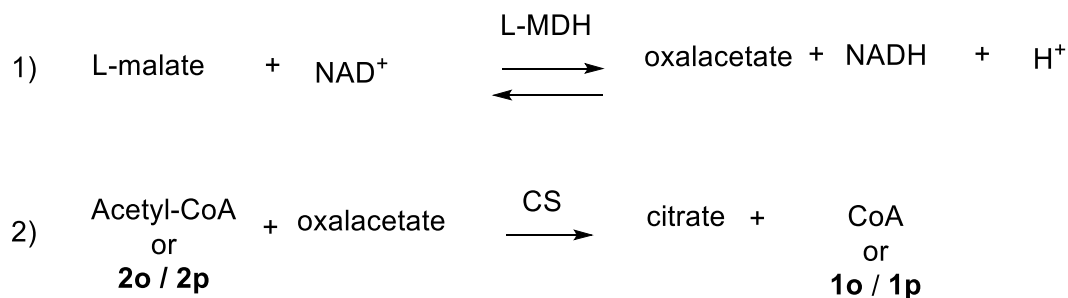
Thiol inhibition effect

To verify any inhibition effect due to the presence of thiol substrates, the WT MsAcT was incubated with increasing concentrations of **1a** (10 mM, 50 mM, 100 mM, 250 mM). Samples were analysed at different

times (2, 4, 6, 24 h) and the activity assay performed as reported in Contente *et al.*¹ No effect on the enzyme activity was observed.

Assay of acetyl-CoA and its mimetics.

The formation and employment of acetyl-CoA and analogues as starting material for more complex reactions was demonstrated through the “Determination of acetic acid Kit” (r-Biopharm). Samples were prepared and assayed following the kit instructions. Measurements were performed spectrophotometrically at 340 nm observing the formation of NADH at 25 °C in a half-microcuvette at determined times. The determination is based on the formation of NADH employing the following reactions:



Supplementary Figure 1. *In-vitro* assay of acetyl-CoA and surrogates.

Reactions with two enzymes of the Krebs cycle were carried out to test the new formed acetyl-CoA and analogues. L-MDH: L-Malate Dehydrogenase, CS: Citrate Synthase.

In the assay, acetyl-CoA or the analogues react with oxalacetate to form citrate and CoA in the presence of citrate synthase (CS). The oxalacetate required for reaction 2 is prepared starting from L-malate and NAD⁺ through L-malate dehydrogenase (L-MDH). In this reaction NAD⁺ is reduced to NADH. When reaction 1 is at equilibrium, all the reagents of reaction 2 are added. CS, in the presence of acetyl-CoA will start depleting the oxalacetate, prompting L-MDH to re-equilibrate reaction 1 producing more oxalacetate and NADH.

GC and HPLC analysis

To determine the conversion of enzymatic reactions GC or HPLC analysis were carried out.

Carlo Erba Fractovap GC equipped with a fused-silica capillary column MEGA-DEX DMP-Beta (dimethyl pentyl-β-cyclodextrin; 25 m x 0.25 mm i.d.), with the injector temperature at 200 °C was employed. Temperature gradient: from 80 °C to 180 °C with 5 °C/min.

The retention times were:

2-Phenylethanethiol (**1a**) 6.00 min, S-Phenethyl thioacetate (**2a**) 11.6 min; Thiophenol (**1b**) 2.7 min, S-Phenyl thioacetate (**2b**) 6.9 min; Benzyl mercaptan (**1c**) 4.2 min, S-Benzyl thioacetate (**2c**) 9.4 min; Cyclohexanethiol (**1d**) 2.7 min, S-Cyclohexyl thioacetate (**2d**) 6.7 min; 2-Furfurylthiol (**1e**) 2.4 min, S-Furfuryl thioacetate (**2e**) 6.1 min; Cyclopentathiol (**1f**) 2.6 min, S-Cyclopentyl thioacetate (**2f**) 6.5 min; Butanthiol (**1g**) 5.2 min, S-Butyl thioacetate (**2g**) 7.3 min; Hexanthiol (**1h**) 6.3 min, S-Hexyl thioacetate (**2h**) 8.4 min.

S-Phenethyl thiopropionate (**2i**) 14.1 min, S-Phenethyl thiomethacrylate (**2j**) 20.6 min; S-Phenethyl thiochloroacetate (**2k**) 17.0 min; S-Phenyl thiopropionate (**2l**) 11.7 min; S-Phenyl thiomethacrylate (**2m**) 18.1 min; S-Phenyl thiochloroacetate (**2n**) 14.9 min.

N-Acetylcysteamine (**1o**) 6.7 min, *S*-(2-Acetamidoethyl) ethanethioate (**2o**) 8.9 min; 2-(BOC-amino)ethanthiol (**1p**) 7.8 min, *S*-(2-((Tert-butoxycarbonyl)amino)ethyl) ethanethioate (**2p**) 10.0 min.

N-Methyl-2-phenylethanamine (**3a**) 7.2 min, *N*-Methyl-*N*-phenethylacetamide (**4a**) 9.6 min; *N*-Methylaniline (**3c**) 4.8 min, *N*-Methyl-*N*-phenylacetamide (**4c**) 6.7 min; *N*-Methylcyclohexanamine (**3d**) 4.4 min, *N*-Cyclohexyl-*N*-methylacetamide (**4d**) 6.9 min; Piperidine (**3e**) 4.2 min, 1-(Piperidin-1-yl)ethanone (**4e**) 6.2 min; Pyrrolidine (**3f**) 3.5 min, 1-(Pyrrolidin-1-yl)ethanone (**4f**) 5.4 min.

CoA, acetyl-CoA and ADP reactions were analysed through HPLC (LiChroCART 250-4.6 Purospher STAR RR-18e (5 µm) HPLC cartridge) using (100 mM NaHPO₄ + 75 mM C₂H₃NaO₂ pH 4.6)/ACN 88:12, λ = 259 nm, flow rate: 0.5 mL/min and (100 mM NaHPO₄ + 75 mM C₂H₃NaO₂ pH 4.6)/MeOH 90:10, λ = 259 nm, flow rate: 0.8 mL/min, respectively.

The retention times were:

CoA: 7.4 min, acetyl-CoA: 9.6 min, ADP: 17.8 min.

HPLC analysis of biotransformation of *N*-Methylbenzylamine (**3b**) and 3-Methoxy-*N*-Methylbenzylamine (**3g**), was performed using a Chiralpack IC column, *n*-Hexane/EtOH 70:30 + 0.1% TEA, λ = 254 nm, flow rate: 1 mL/min.

The retention times were:

N-Methylbenzylamine (**3b**): 4.7 min, *N*-Methyl-*N*-benzylacetamide (mixture of rotamers) (**4b**): rotamer₁ 5.9 min, rotamer₂ 8.0 min; 3-Methoxy-*N*-methylbenzylamine (**3g**): 5.2 min, 3-Methoxy-*N*-methyl-*N*-benzylacetamide (mixture or rotamers) (**4g**): rotamer₁ 6.5 min, rotamer₂ 9.0 min.

Molar conversion of the biotransformations with 1-Phenylethylthiol (**5a**), 1-Phenylethylamine (**5b**), (*R*)-1-Phenylethanol (**5f**) and 2-Phenylpyrrolidine (**5h**) were analysed through HPLC (XBridge C18 150-4.6 (5 µm) using a DAD detector at 280, 265, 250 or 210 nm after a 10 min gradient run (A: 0.1% TFA in water, B: Acetonitrile. Gradient: 0 min 95% A 5% B; 1-5 min 5% A 95% B; 5-7 min 0% A 100% B; 7-10 min 95% A 5% B. Injection volume 2 µL) at 40 °C with a flow rate of 0.8 mL/min.

The retention times were:

1-Phenylethylthiol (**5a**): 5.9 min, *S*-(1-Phenylethyl) thioacetate(**6b**): 6.9 min; 1-Phenylethylamine (**5b**): 4.6 min, *N*-(1-Phenylethyl)acetamide (**6b**): 5.4 min; (*R*)-1-Phenylethanol (**5f**): 5.6 min, (*R*)-1-phenylethyl acetate (**6f**): 6.6 min; 2-Phenylpyrrolidine (**5h**): 4.9 min, *S*-1-(2-Phenyl-1-pyrrolidinyl)ethanone (**6h**): 5.4 min.

The enantiomeric excess was evaluated through Agilent GC equipped with a CP-Chirasil-Dex CB column (25 m, 0.25 mm i.d.), with the injector temperature at 250 °C. Temperature gradient: from 80 °C to 180 °C with 5 °C/min.

The retention times were:

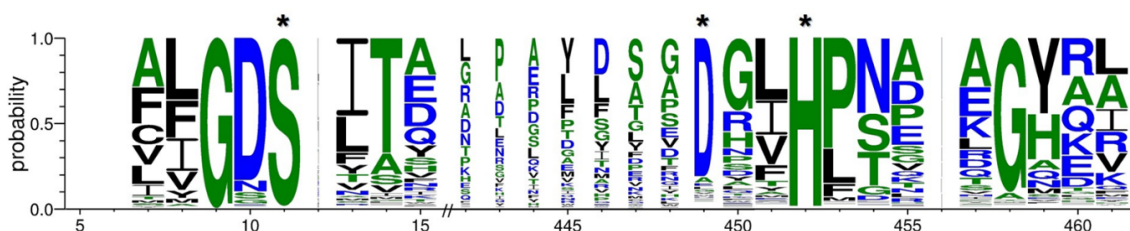
(*S*)-*S*-(1-Phenylethyl) thioacetate: 10.0 min, (*R*)-*S*-(1-Phenylethyl) thioacetate: 10.5 min; (*S*)-*N*-(1-Phenylethyl)acetamide: 8.2 min, (*R*)-*N*-(1-Phenylethyl)acetamide: 8.7 min; (*S*)-1-Phenylethyl acetate: 5.2 min, (*R*)-1-Phenylethyl acetate: 6.5 min; (*S*)-*S*-1-(2-Phenyl-1-pyrrolidinyl)ethanone: 11.2 min (*R*)-*S*-1-(2-Phenyl-1-pyrrolidinyl)ethanone: 11.9 min.

Retention times were compared with commercially available compounds or chemically synthesised as previously reported by *Contente et al.*¹

Supplementary Figures

Representation of residue conservation in GDSL-like family

Sequence alignment for the protein family PF13472 (Lipase_GDSL_2) was retrieved from <https://pfam.xfam.org>. The alignment was analysed using Clustal X to calculate residue conservation.



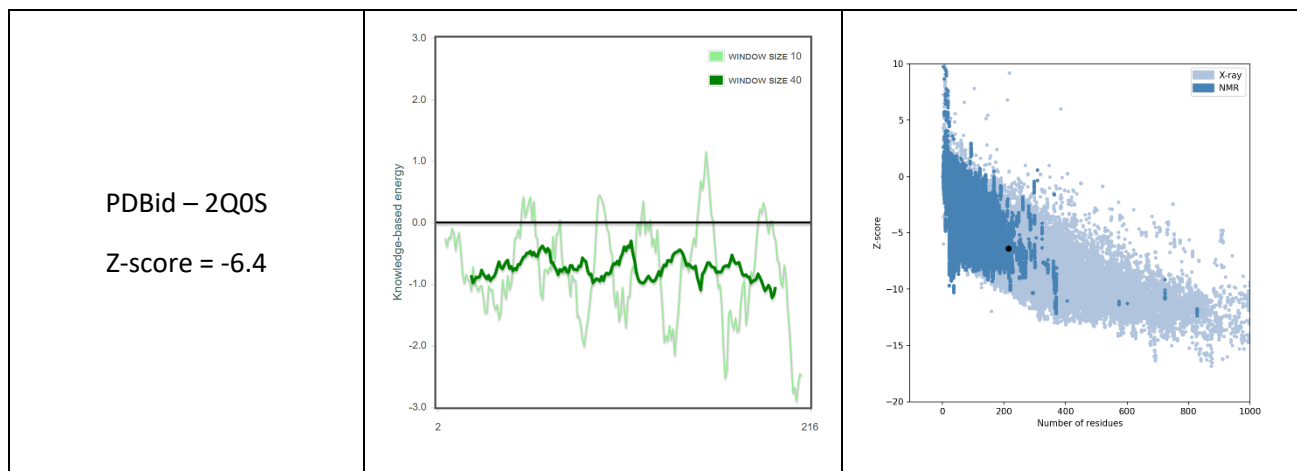
Supplementary Figure 2: Graphical representation of the residue conservation in GDSL-family.

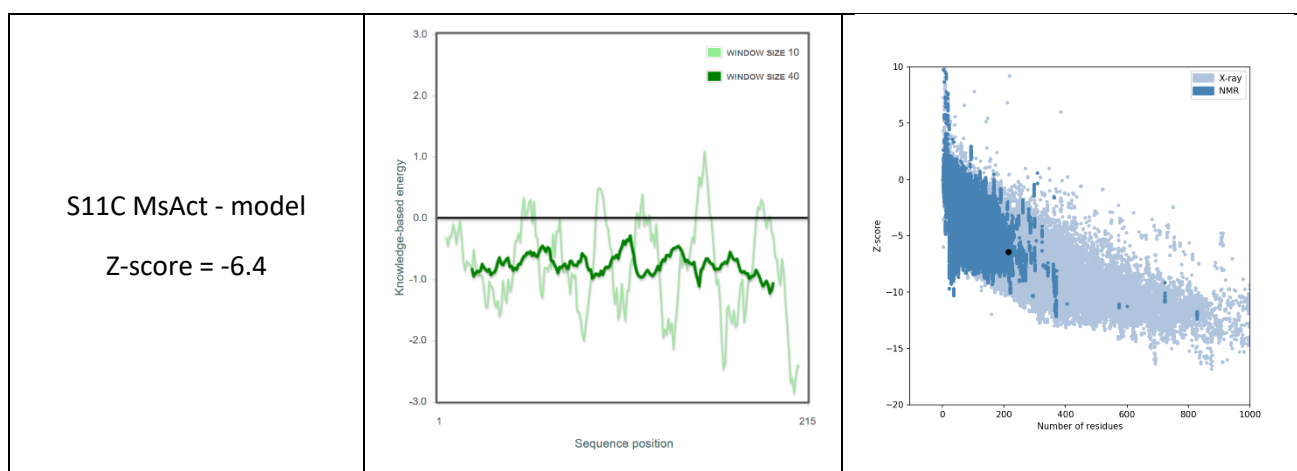
Seqlogo representation of the seed alignment from GDSL-family from pfam (<https://pfam.xfam.org>) was created using Weblogo (<https://weblogo.berkeley.edu/>). The catalytic residues are indicated with an asterisk on top.

Docking of acetyl-CoA mimetics

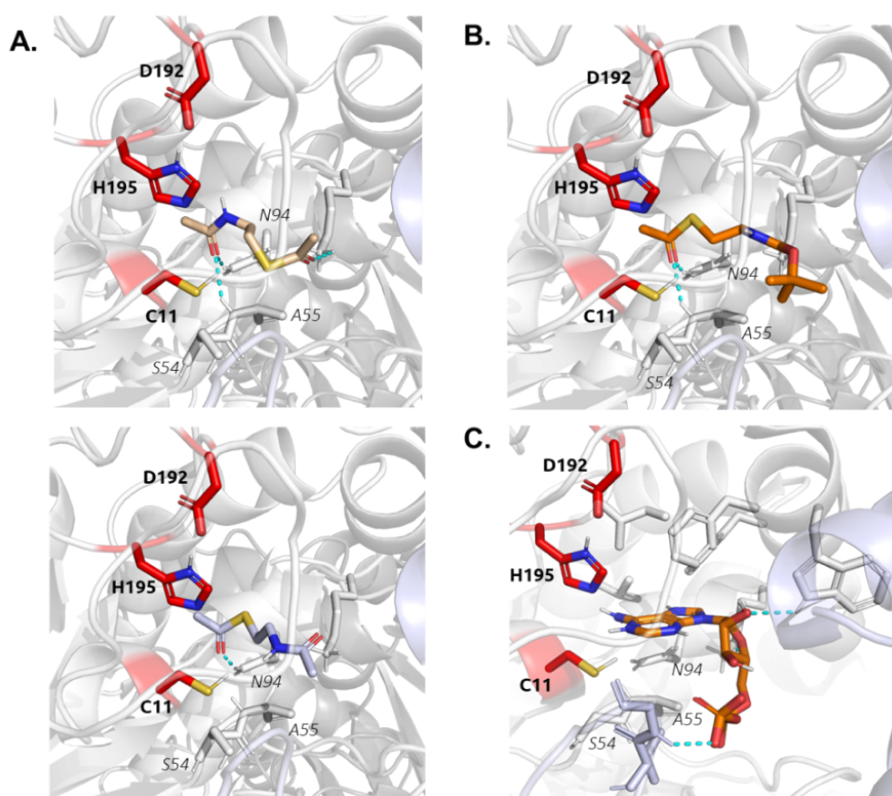
A crystal structure for MsAct with an inhibitor on the active site, mimicking a substrate bound conformation³ was obtained from the Protein Data Bank (<https://www.rcsb.org/>). Starting from this structure, the catalytic serine was mutated to a cysteine computationally using USCF Chimera.⁴ After *in-silico* mutation of S11 to cysteine, hydrogens were added, using antechamber Gasteiger charges calculated in the AMBER ff14SB force field and minimisation performed via PARMCHK for 150 steepest descent minimisation steps and 10 conjugate gradient minimisation steps. The overall quality of the resulting structure was evaluated using the ProSA server. No difference was observed in the quality as compared to the crystal structure.

Docking studies were performed using AutoDock Vina Software.⁵ For ADP and HS-NAC the box was redimensioned to 15 Å centered in the catalytic cysteine. The conformations with lower binding energy and in a potential catalytically active position were selected.



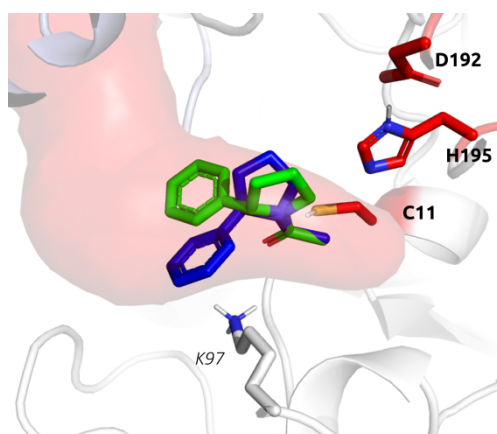


Supplementary Figure 3. Quality assessment of the initial crystal structure and the S11C model created. The energy of each position is shown in green. The black dot indicates the overall quality of the structure, either the crystal structure or the model, with the Z-score.



Supplementary Figure 4: **A.** Docking of **2o** with a score of -4.9 kcal/mol (up) and -4.4 kcal/mol (down). Both the poses confirm the product in a catalytically active position, with no major difference in binding energy. **B.** Docking of **2p**, with a score of -5.3 kcal/mol. The product is in a catalytically active conformation when the thioester group faces the oxyanion hole. **C.** Representation of the best pose for the adenine dinucleotide phosphate (ADP) with a score of -1.6 kcal/mol, which is not in catalytically active conformation. In all the images, grey cartoon indicates the different chains of MsAct, its catalytic triad is shown in red and bold letters while the oxyanion hole residues indicated in italics. Hydrogen bonds are depicted in light blue.

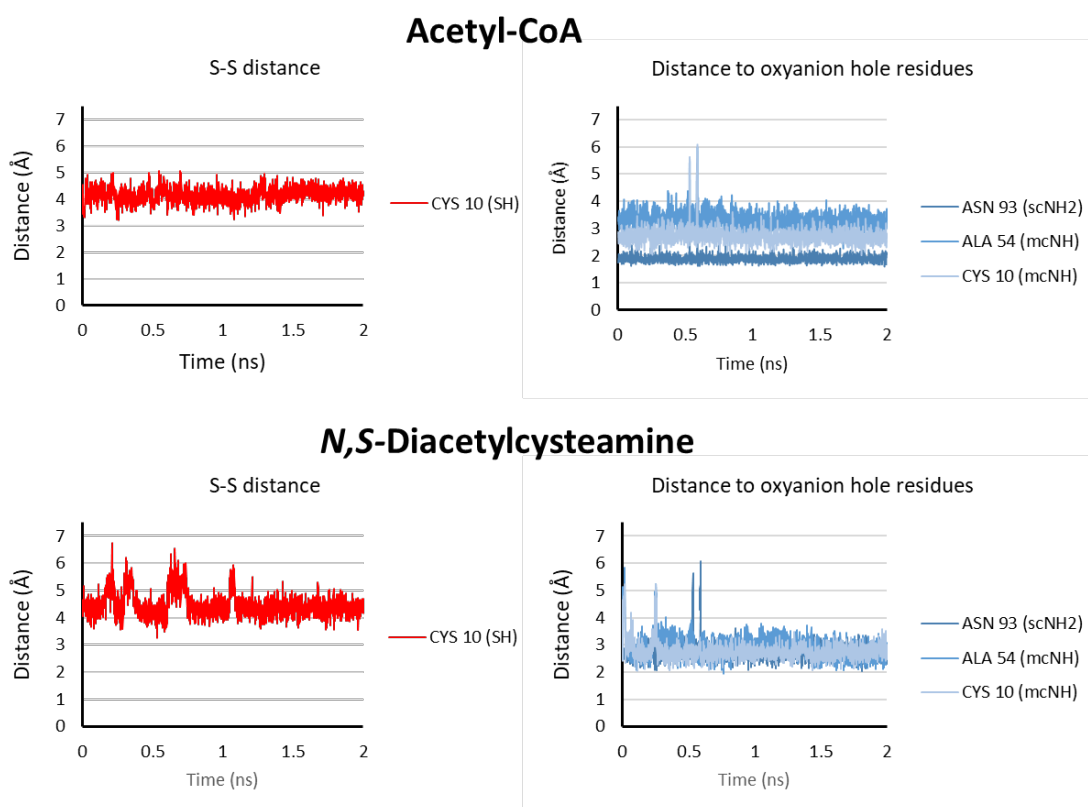
Docking of (*S*)- 2-Phenylpyrrolidine



Supplementary Figure 5. Docking of (*S*)-2-Phenylpyrrolidine (green) in its catalytic conformation. In blue, the (*R*)-enantiomer is forced in the catalytic conformation, showing evident clashes in the active site pocket.

Molecular dynamics of MsAcT and acetyl-CoA

The structure with docked acetyl-CoA or *N,S*-Diacetyl cysteamine with the lowest energy from the docking was used as the starting point. Molecular dynamics (MD) simulations were carried out with Enlighten2 plugin for PyMOL.⁶ In short, Enlighten2 uses the ff14SB⁷ force field and TIP3P water model with AmberTools20.⁸ A spherical boundary of 20 Å centered in the ligand was used and the substrate parameterisation was performed with GAFF. For the system, Enlighten2 protocols were used for the hydrogen and solvent addition (PREP protocol) as well as the minimisation and temperature equilibration. After that, 2 ns simulation were performed using a 20 Å solvent shell surrounding the substrate. MM/GBSA analysis were implemented using the MMPBSA.py⁹ script from AmberTools20 package (See Supplementary Data).



Supplementary Figure 6. Distance of the thiol group of C11 to the sulphur of the thioester in both tested substrates along the 2 ns simulation (left) and evolution of the distance of the carbonyl oxygen of the thioester to the different side chains (sc) and main chains (mc) atoms known forming the oxyanion hole.

The contribution of the different forces governing the binding is shown in Table S2

	Van der Waals contribution	Electrostatic energy	Electrostatic contribution	Non-Polar contribution	Binding Energy
1o	-20.82 ± 2.24	-26.87 ± 2.24	28.18 ± 2.24	-3.38 ± 0.09	-22.89 ± 2.65
acetyl-CoA	-35.86 ± 3.42	-56.58 ± 9.96	78.51 ± 9.60	-5.97 ± 0.29	-19.91 ± 4.37

Supplementary Table 2. Binding Free Energy components in kcal/mol and the standard deviation calculated with MMPBSA.py using AmberTools20 with 2 ns MD simulation in water.

Representation of S11C MsAcT tunnels

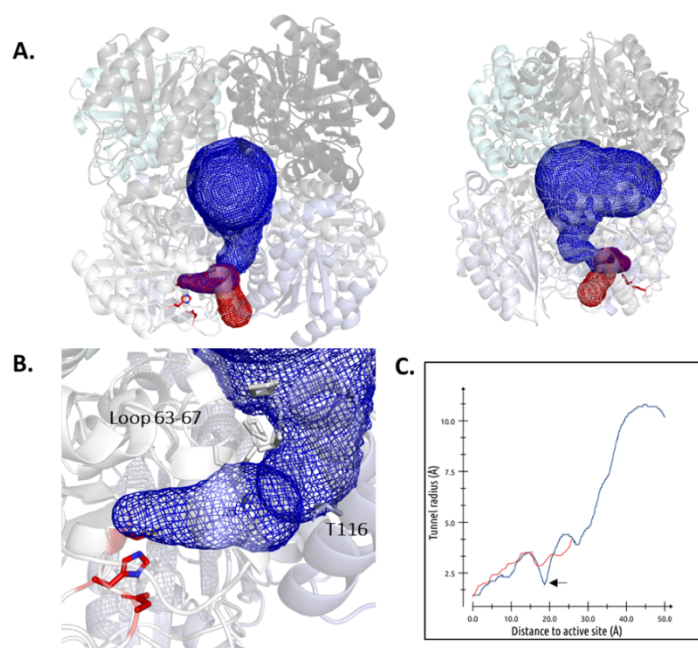
Computational of the tunnels:

The tunnels were identified using CAVER plugin¹⁰ in Pymol molecular visualization software.¹¹ The default values were kept for all variables with exception for the shell radius set to 10 Å to allow for relevant tunnel identification. The starting point was set to the residue α -carbon of the catalytic cysteine.

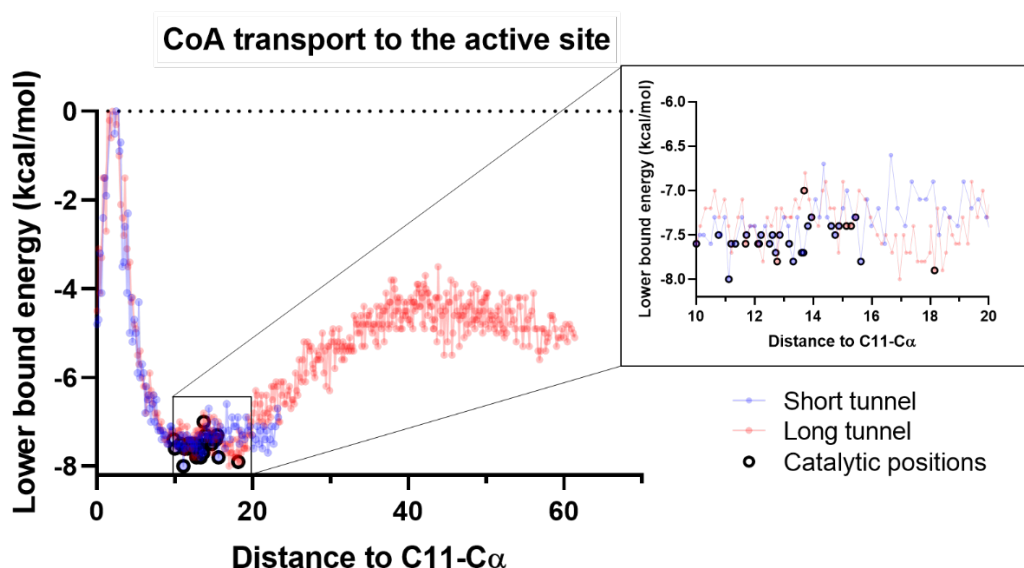
Ligand transport analysis:

To identify the effect of the tunnel (nature and length) to CoA-acetylation, its movement through the tunnel was studied by CAVERDOCK.^{12,13} Default values were used for its docking and the results were analysed using Pymol. The catalytic pocket was defined as a region of 10 Å surrounding the catalytic cysteine and part of the tunnel. Due to CoA and Acetyl-CoA size, attempts to dock the substrate in more

constricted box space and closer to the catalytic cysteine, resulted in non-catalytic or highly improbable binding modes (binding energies > 0).



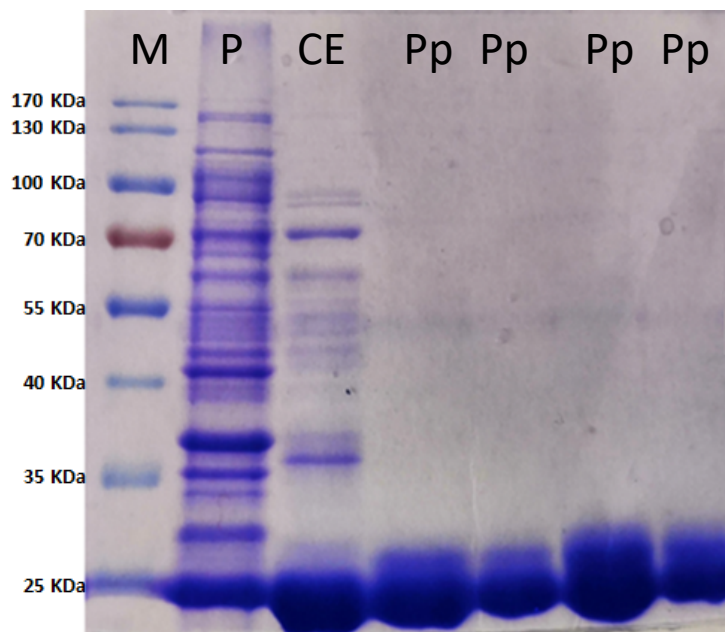
Supplementary Figure 7: **A.** Depiction of the tunnels as a red and blue mesh calculated using Caver. The residues composing the active site are coloured in red. **B.** Detail of the main bottleneck from the two analysed tunnels. **C.** Evolution of the tunnel radius as the distance from the active site increases. The main bottleneck, only present in the blue tunnel, is indicated by an arrow and a detailed view of the residues forming.



Supplementary Figure 8: Docking scores for CoA travelling through the tunnels into the active site of S11C MsAcT. The common part for both tunnels is highlighted inside the square and the CoA potential catalytically active conformations indicated with black circles. All data was obtained using CaverDock.

1. SDS-PAGE S11C MsAcT

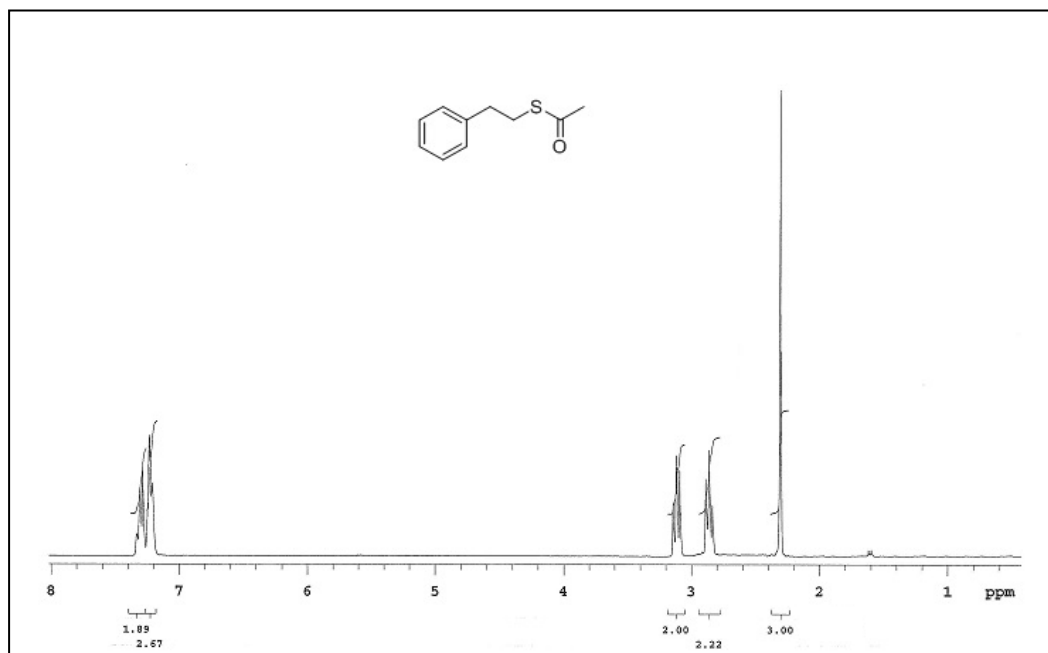
Figure S5 shows pellet crude extract, and pure protein analysed by SDS-PAGE. The monomer of S11C MsAcT is 25.6 kDa. Typically, starting from 5 g of wet cell paste, it was possible to obtain 100 mg of pure protein (5 mg/mL).



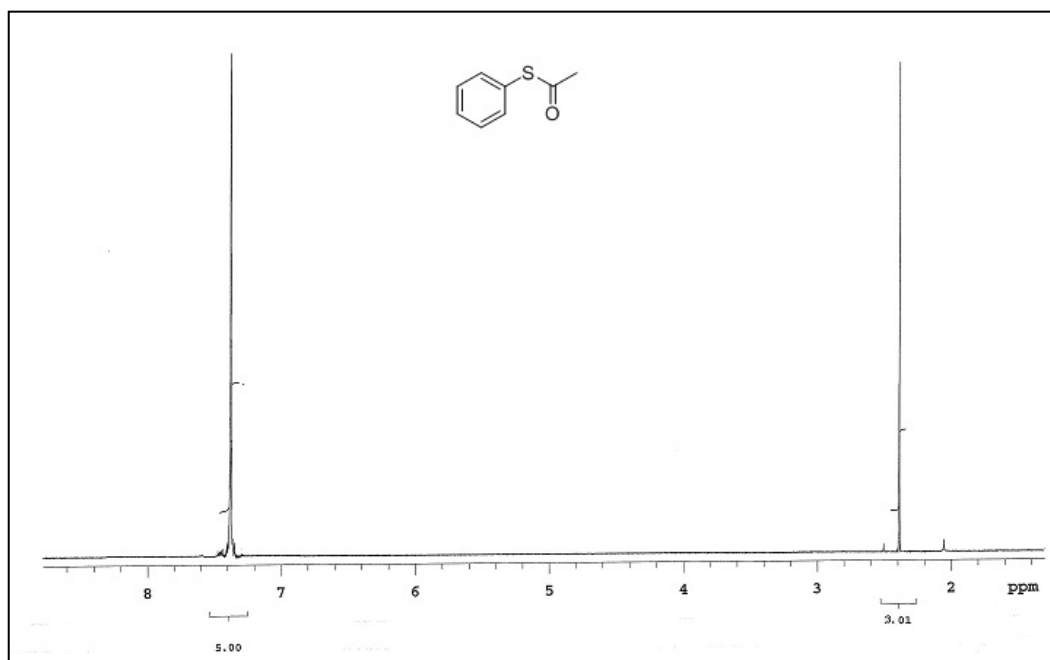
Supplementary Figure 9. Lane 1: Marker (M), Lane 2: Pellet (P), Lane 3: Crude Extract (CE), Lane 4-7: Pure Protein (pP).

¹H-NMR spectra of the small scale reactions

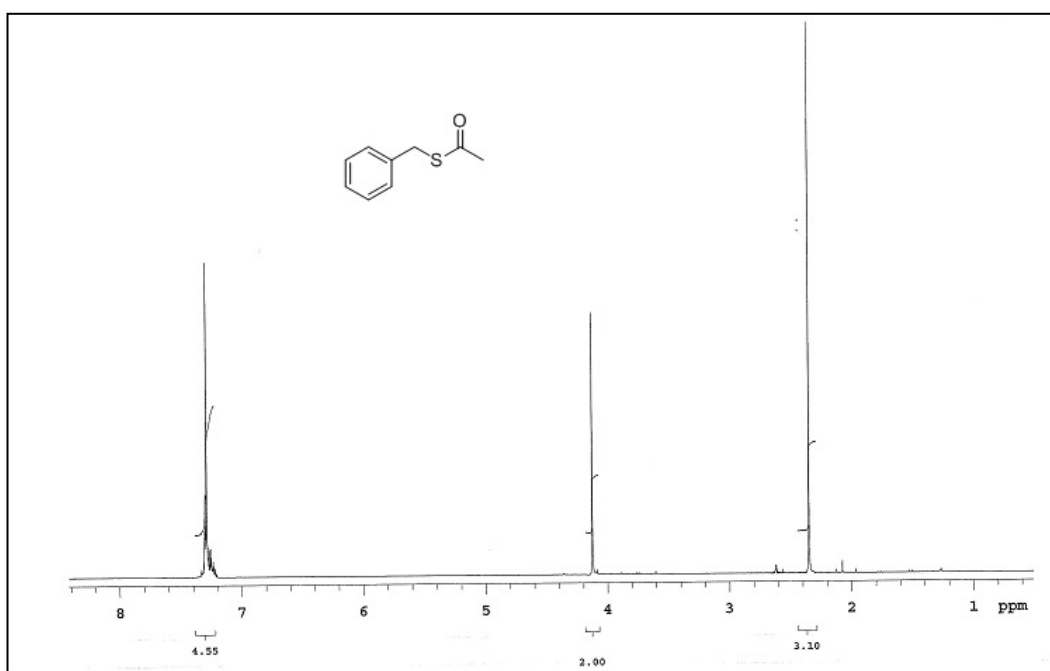
S-Phenethyl thioacetate (**2a**): colourless oil; ¹H-NMR (300 MHz, CDCl₃) δ = 7.30- 7.38 (m, 2H), 7.19-7.29 (m, 3H), 3.08-3.17 (m, 2H), 2.82-2.91 (m, 2H), 2.32 (s, 3H) ppm.¹⁴



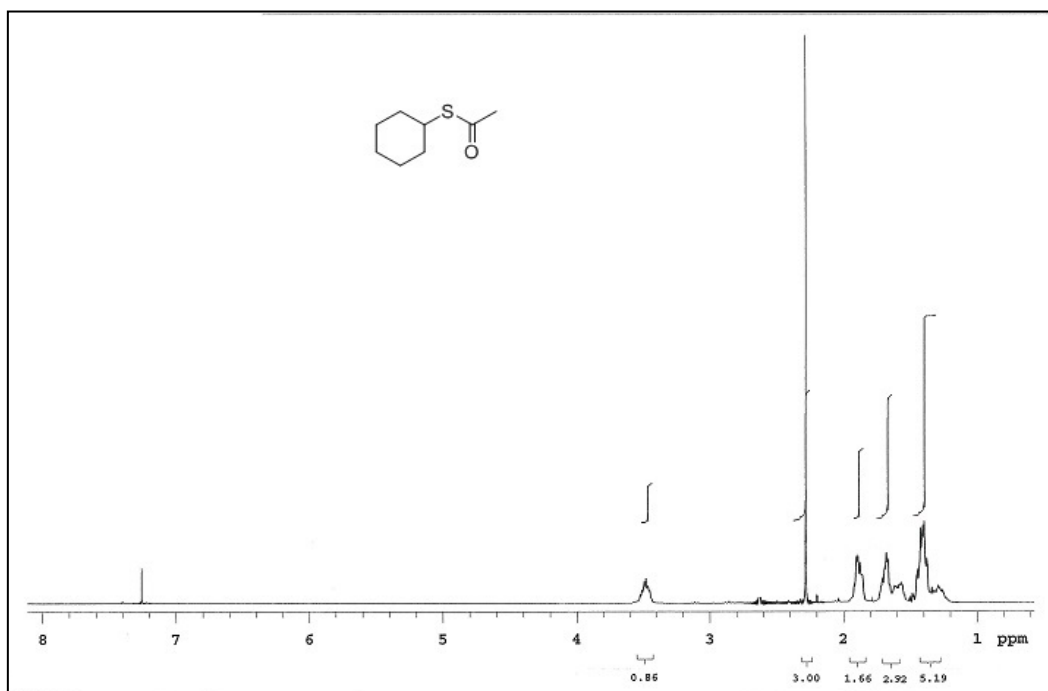
S-Phenyl thioacetate (**2b**): pale yellow oil; ¹H-NMR (300 MHz, CDCl₃) δ = 7.37–7.43 (m, 5H), 2.39 (s, 3H) ppm.¹⁵



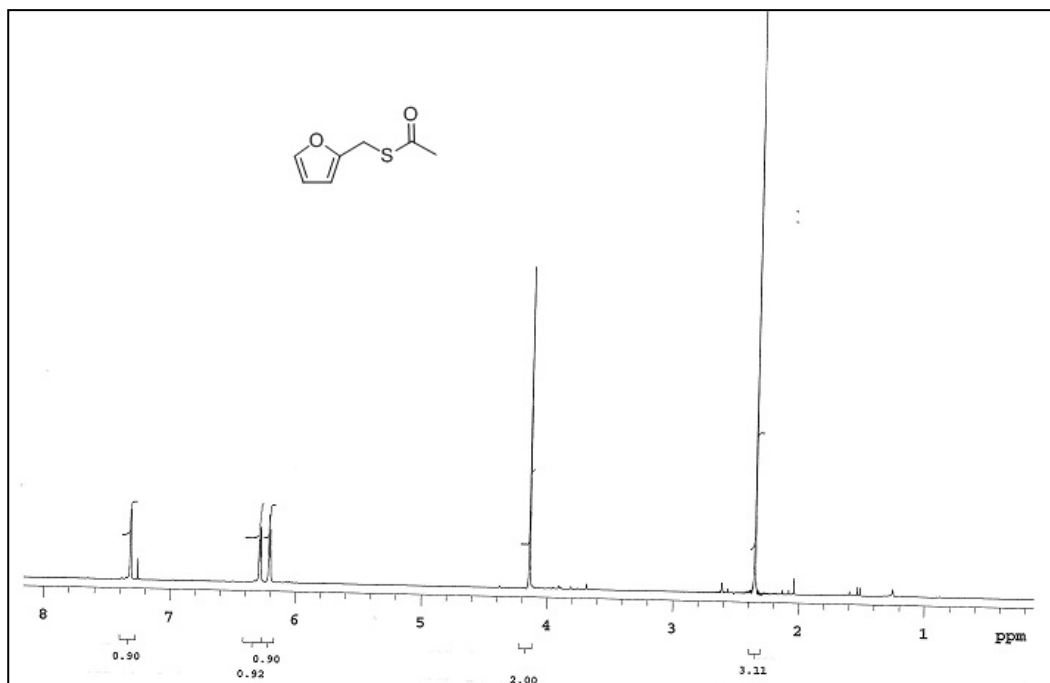
S-Benzyl thioacetate (**2c**): pale yellow oil; ¹H-NMR (300 MHz, CDCl₃) δ = 7.19–7.38 (m, 5H), 4.15 (s, 2H), 2.37 (s, 3H) ppm.¹⁶



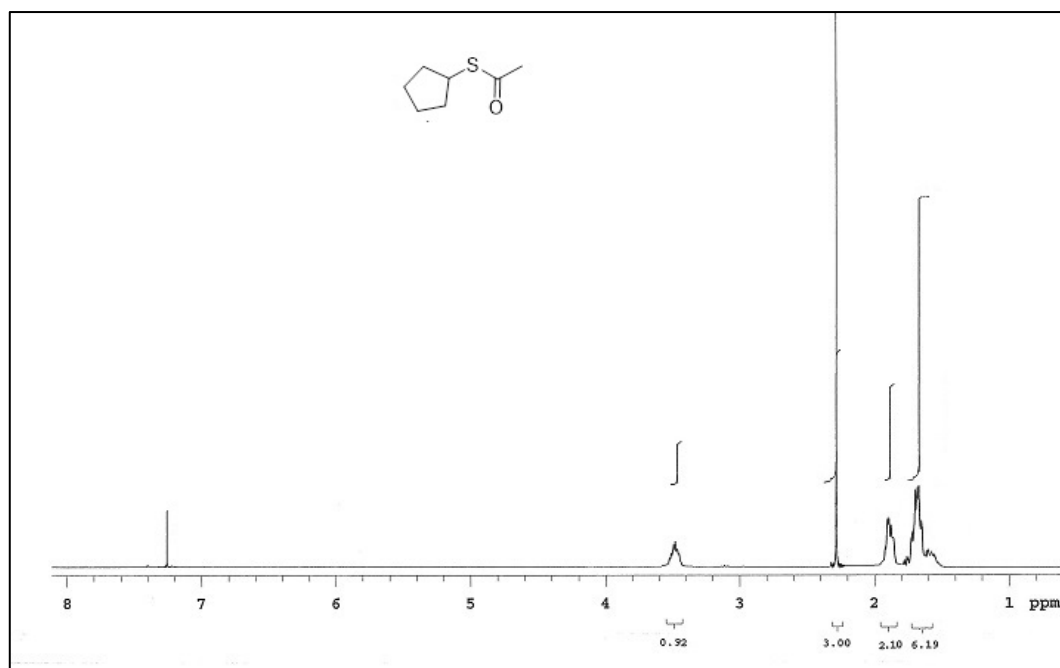
S-Cyclohexyl thioacetate (**2d**): pale yellow oil; ¹H-NMR (300 MHz, CDCl₃) δ = 3.43–3.58 (m, 1H), 2.28 (s, 1H), 1.86–1.95 (m, 2H), 1.65–1.78 (m, 3H), 1.36–1.44 (m, 5H) ppm.¹⁵



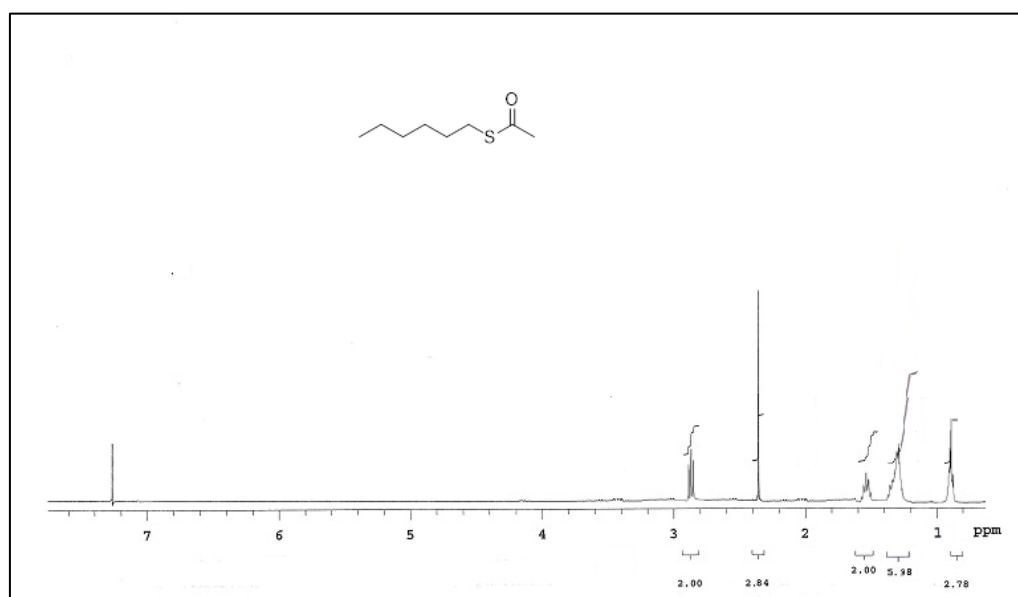
S-Furfuryl thioacetate (**2e**): pale yellow oil; ¹H-NMR (300 MHz, CDCl₃) δ = 7.33 (s, 1H), 6.30 (s, 1H), 6.21 (s, 1H), 4.16 (s, 2H), 2.36 (s, 3H) ppm.¹⁷



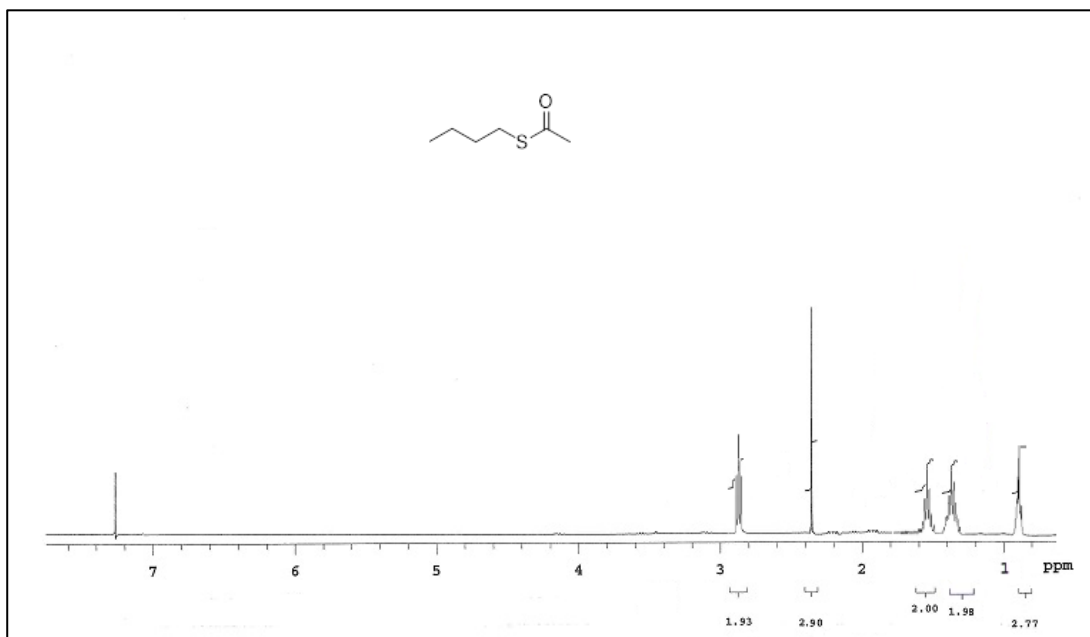
S-Cyclopentyl thioacetate (**2f**): pale yellow oil; ¹H-NMR (300 MHz, CDCl₃) δ = 3.42-3.57 (m, 1H), 2.28 (s, 1H), 1.82-1.94 (m, 2H), 1.60-1.78 (m, 6H), ppm.¹⁵



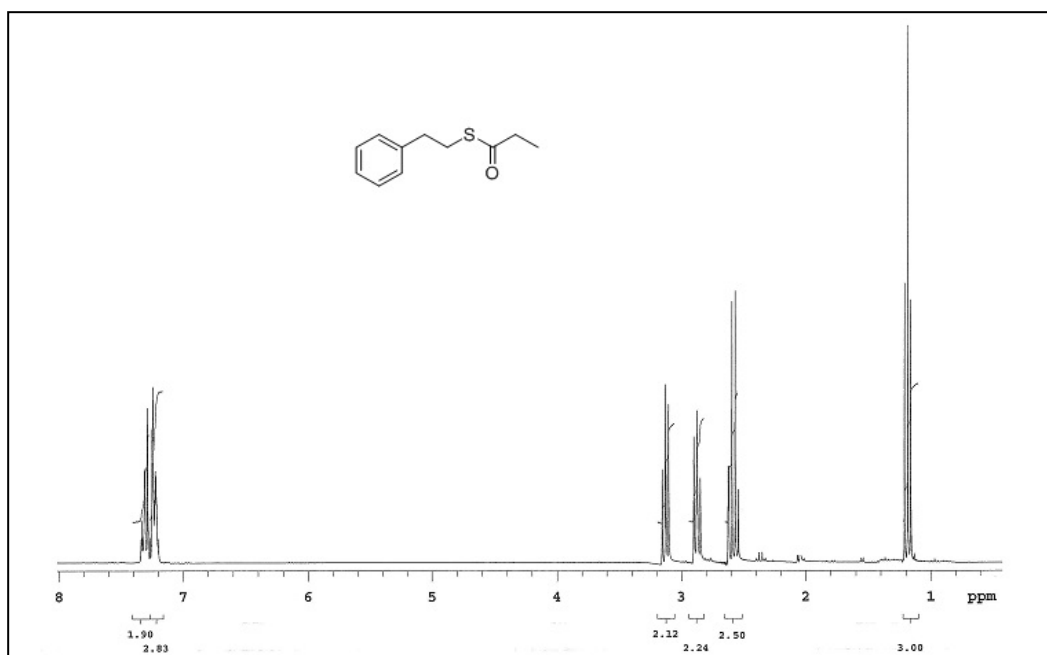
S-Hexylthioacetate (**2g**): yellow oil; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ = 2.83 (t, J = 7.4 Hz, 2H), 2.36 (s, 3H), 1.48-1.58 (m, 2H), 1.22-1.38 (m, 6H) 0.82-0.94 (m, 3H) ppm.¹⁸



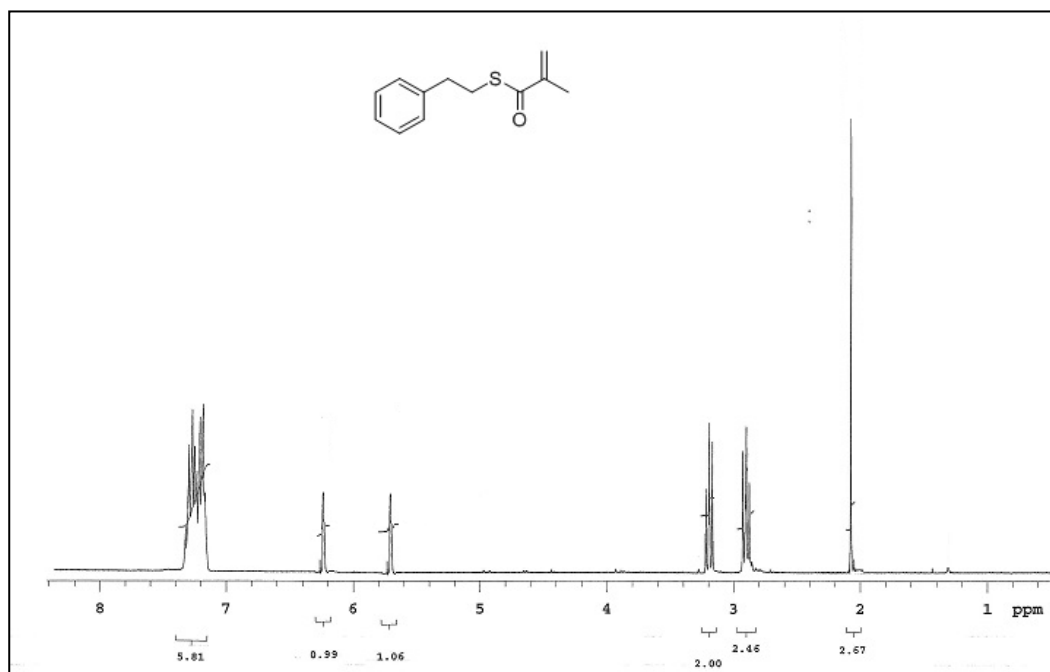
S-Butylthioacetate (**2h**): yellow oil; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ = 2.83 (t, J = 7.4 Hz, 2H), 2.36 (s, 3H), 1.50 (quintet, J = 7.5 Hz, 2H), 1.38 (sex, J = 7.4 Hz, 2H), 0.90 (t, J = 7.5 Hz, 3H) ppm.¹⁸



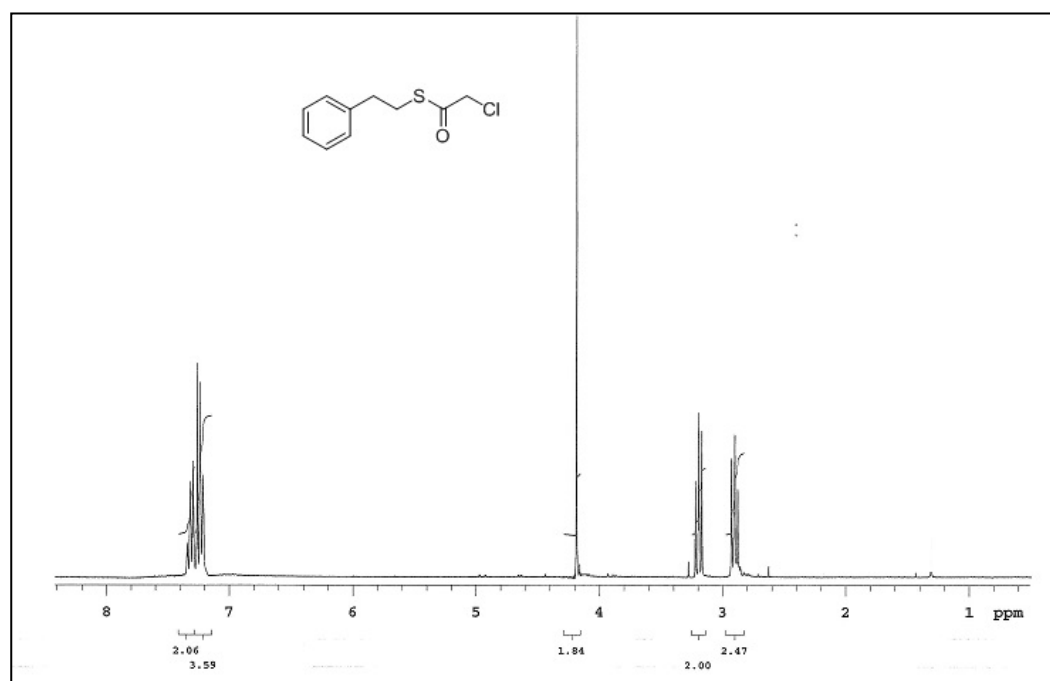
S-Phenethyl thiopropionate (**2i**): pale yellow oil; ¹H-NMR (300 MHz, CDCl₃) δ = 7.28-7.36 (m, 2H), 7.18-7.28 (m, 3H), 3.15 (t, J = 7.5 Hz, 2H), 2.88 (t, J = 7.5 Hz, 2H), 2.58 (q, J = 7.8 Hz, 2H), 1.15 (t, J = 7.8 Hz, 3H) ppm.¹⁴



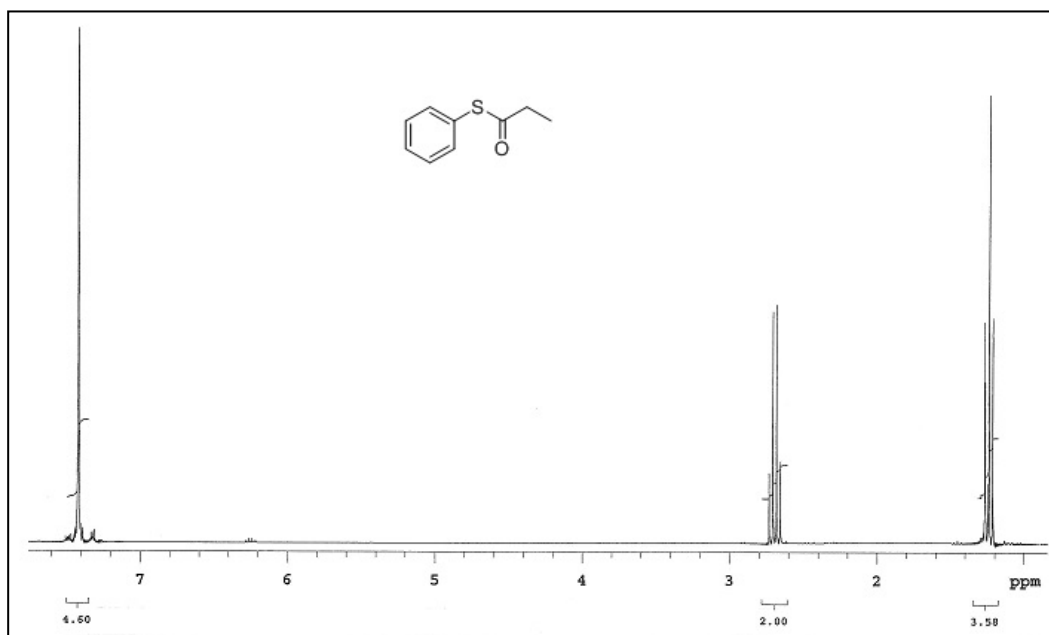
S-Phenethyl thiomethacrylate (**2j**): colourless oil; ¹H-NMR (300 MHz, CDCl₃) δ = 7.14–7.41 (m, 5H), 6.22 (s, 1H), 5.68 (s, 1H), 3.18 (t, J = 7.5 Hz, 2H), 2.88 (t, J = 7.5 Hz, 2H), 2.08 (s, 3H) ppm.¹⁹



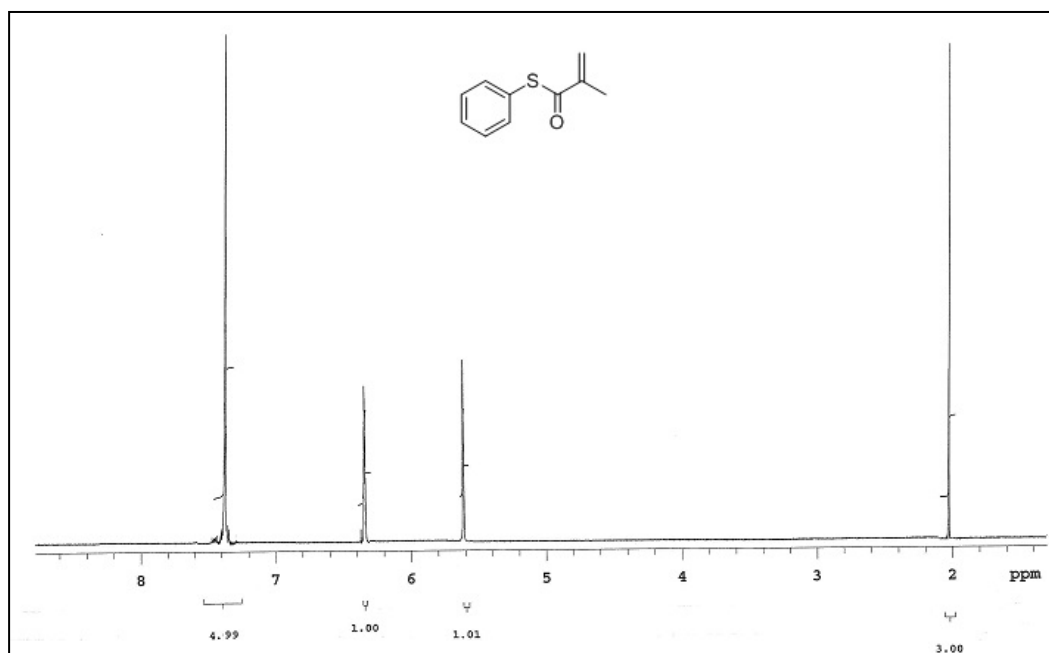
S-Phenethyl thiochloroacetate (2k): colourless oil; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ = 7.14–7.41 (m, 5H), 6.22 (s, 1H), 4.18 (s, 1H), 3.18 (t, J = 7.5 Hz, 2H), 2.88 (t, J = 7.5 Hz, 2H) ppm.²⁰



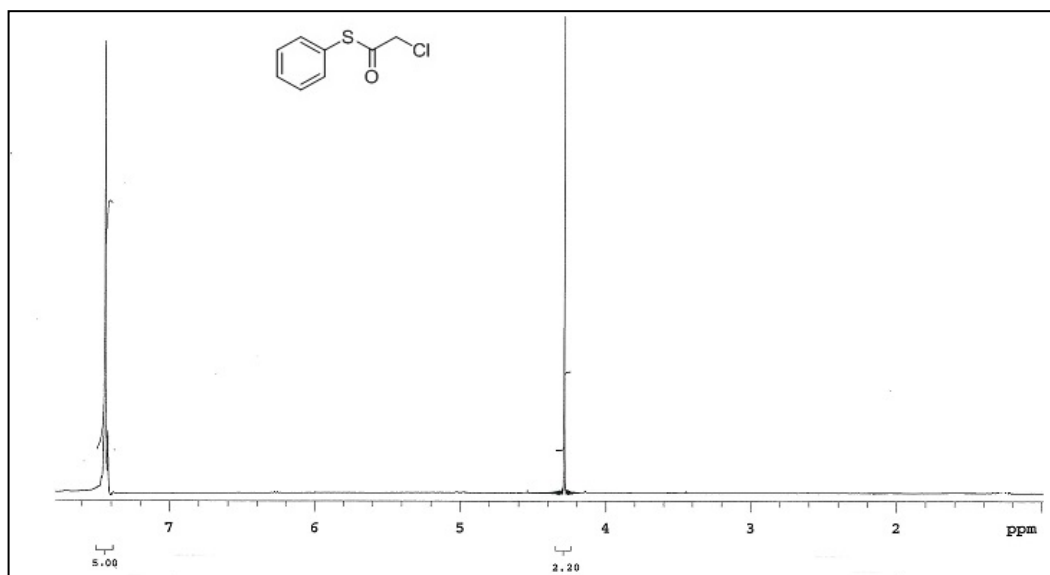
S-Phenyl thiopropionate (2l): pale yellow oil; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ = 7.37–7.43 (m, 5H), 2.68 (q, 2H, J = 7.80 Hz), 1.23 (t, J = 7.8 Hz, 3H) ppm.²¹



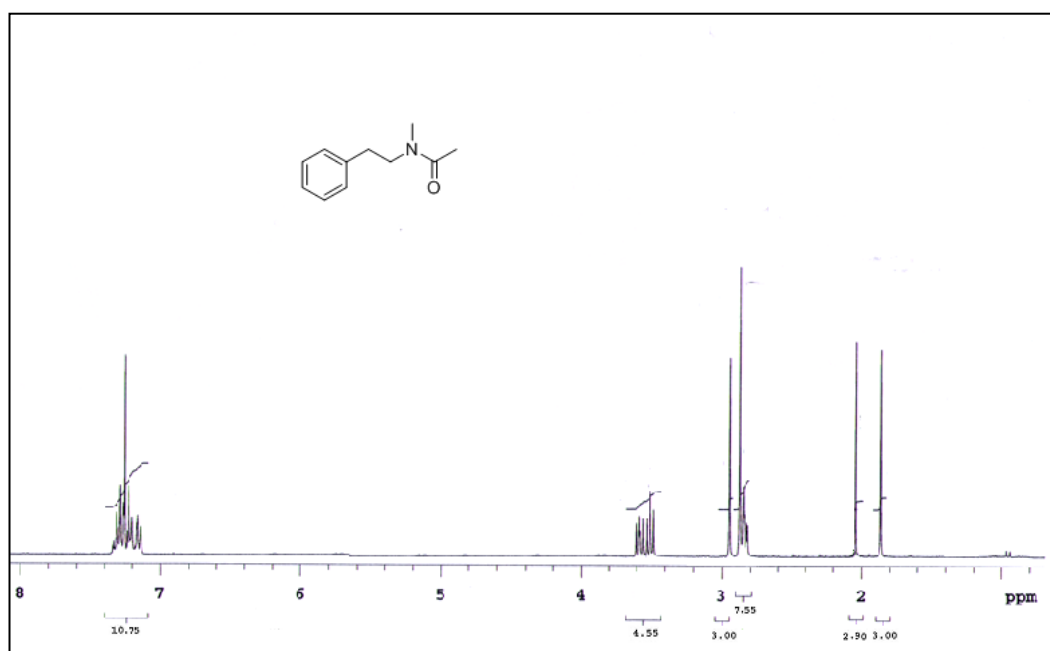
S-Phenyl thiomethacrylate (**2m**): pale yellow oil; ^1H -NMR (300 MHz, CDCl_3) δ = 7.37–7.43 (m, 5H), 6.36 (s, 1H), 5.74 (s, 1H), 2.05 (m, 3H) ppm.²²



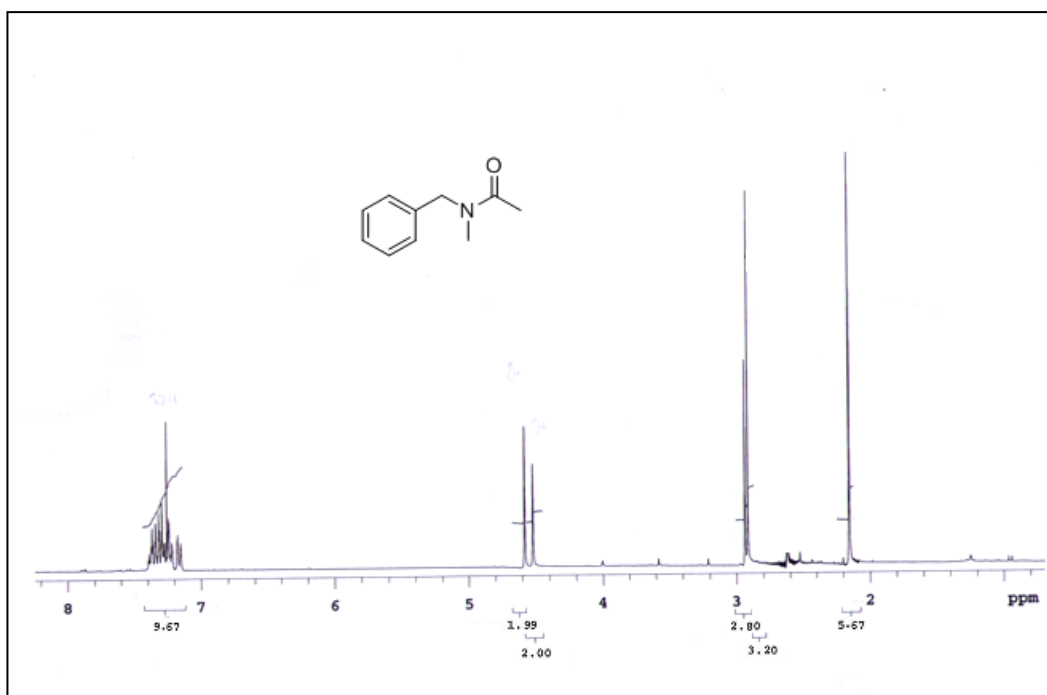
S-Phenyl thiochloroacetate (**2n**): pale yellow oil; ^1H -NMR (300 MHz, CDCl_3) δ = 7.37–7.43 (m, 5H), 4.25 (s, 2H) ppm.²⁰



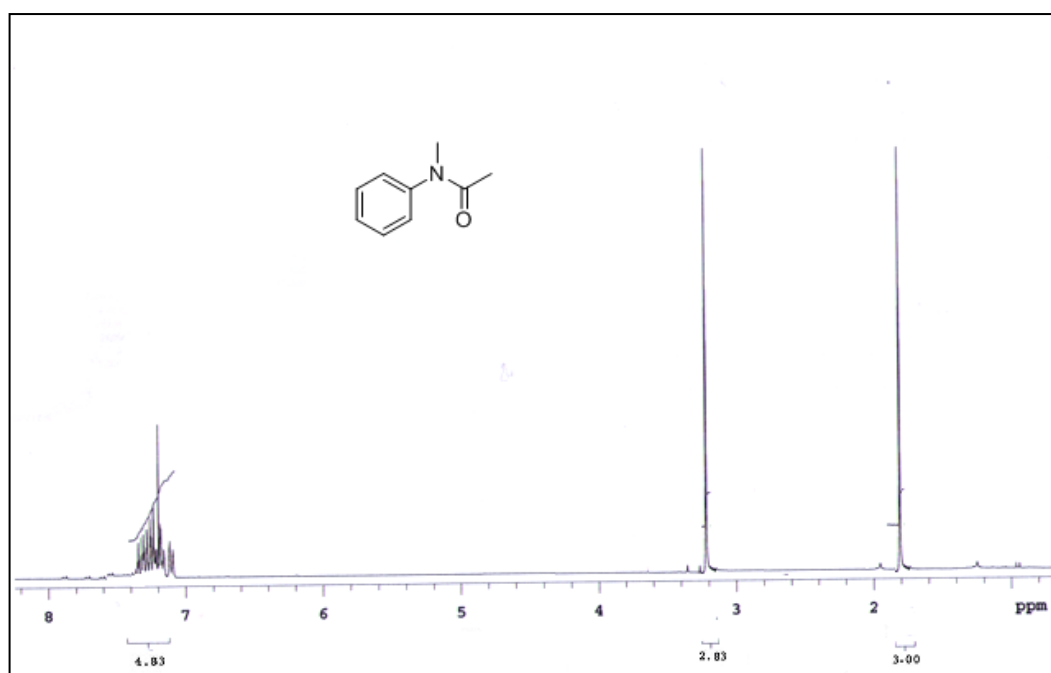
N-Methyl-*N*-phenethylacetamide (**4a**): pale yellow oil; the product was obtained as a mixture of two rotamers. ^1H -NMR (300 MHz, CDCl_3) δ = 7.13–7.36 (m, 10H), 3.46–3.64 (m, 4H), 2.95 (s, 3H), 2.86 (s, 3H), 2.78–2.90 (m, 4H), 2.05 (s, 3H), 1.84 (s, 3H) ppm.²³



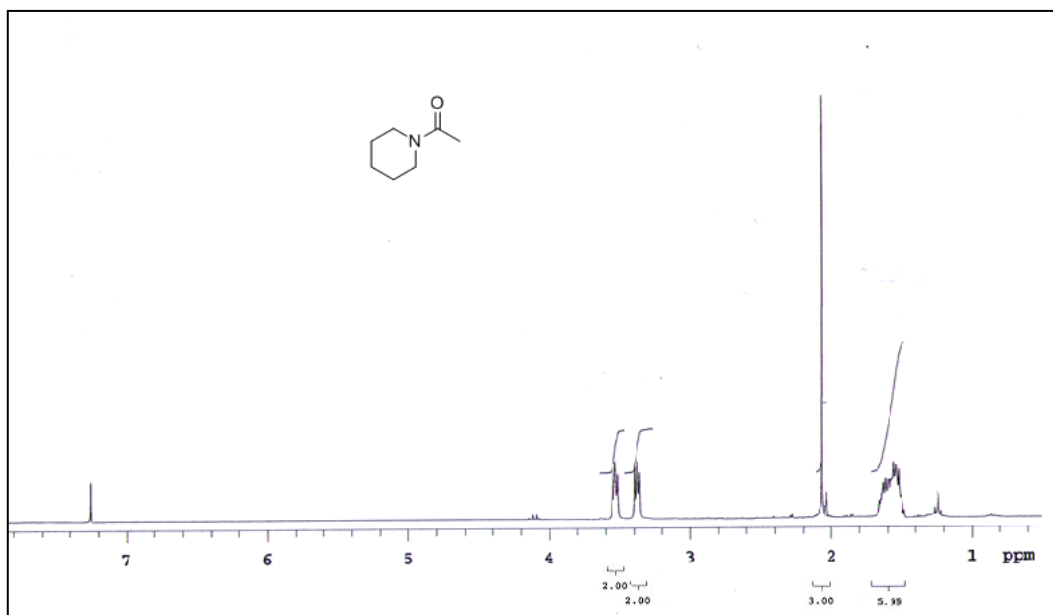
N-Methyl-*N*-benzylacetamide (**4b**): brown oil; the product was obtained as a mixture of two rotamers. ^1H -NMR (300 MHz, CDCl_3) δ = 7.13–7.39 (m, 10H), 4.58 (s, 2H), 4.52 (s, 2H), 2.94 (s, 3H), 2.92 (s, 3H), 2.90–2.79 (m, 4H), 2.16 (s, 6H) ppm.²³



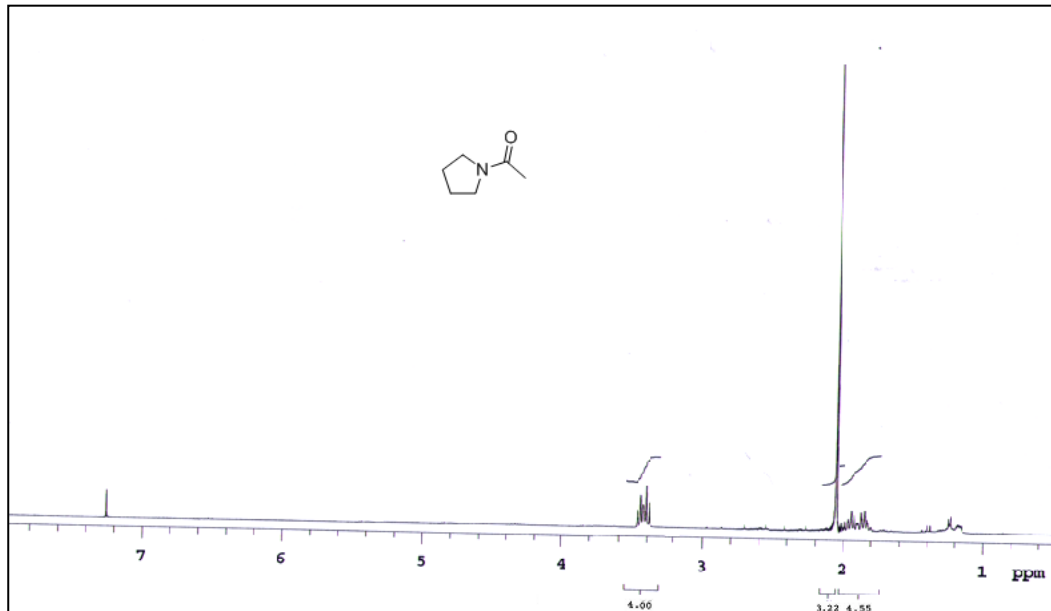
N-methyl-*N*-phenylacetamide (**4c**) White solid (mp 98-100 °C); $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ = δ 7.10–7.38 (m, 5H), 3.20 (s, 3H), 1.80 (s, 3H) ppm.²³



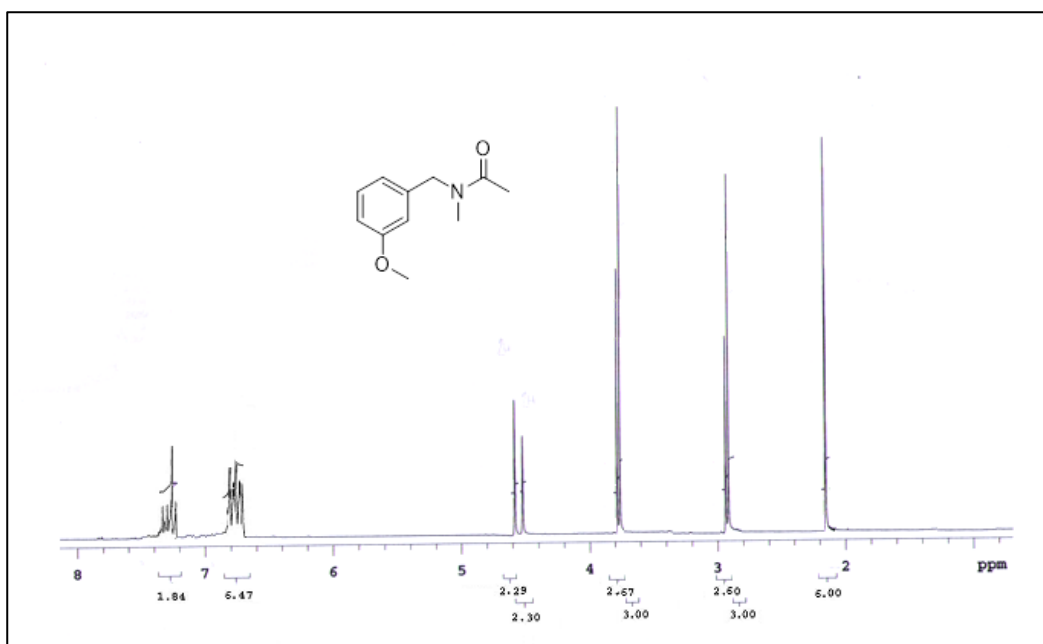
1-(Piperidin-1-yl)ethanone (**4e**): Brown oil; $^1\text{H-NMR}$ (300 MHz, CDCl_3) δ = 3.52-3.58 (m, 2H), 3.33-3.42 (m, 2H), 2.05 (s, 3H), 1.48-1.75 (m, 6H) ppm.²⁴



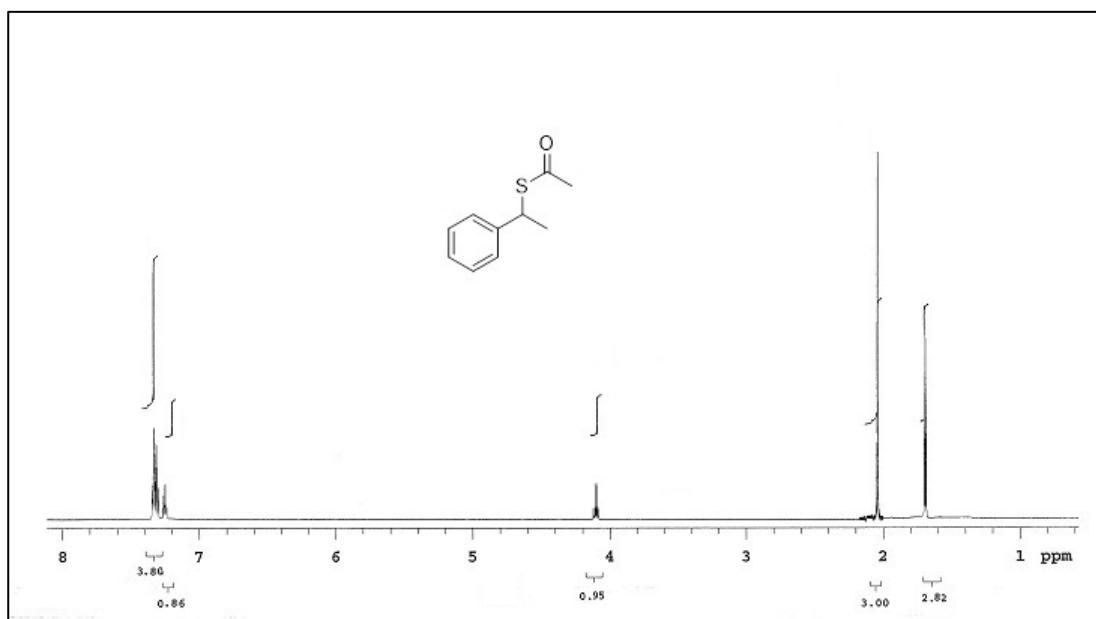
1-(Pyrrolidin-1-yl)ethanone (**4f**): Brown oil; ¹H-NMR (300 MHz, CDCl₃) δ = 3.38-3.48 (m, 4H), 2.05 (s, 3H), 1.80-2.02 (m, 4H) ppm.²⁴



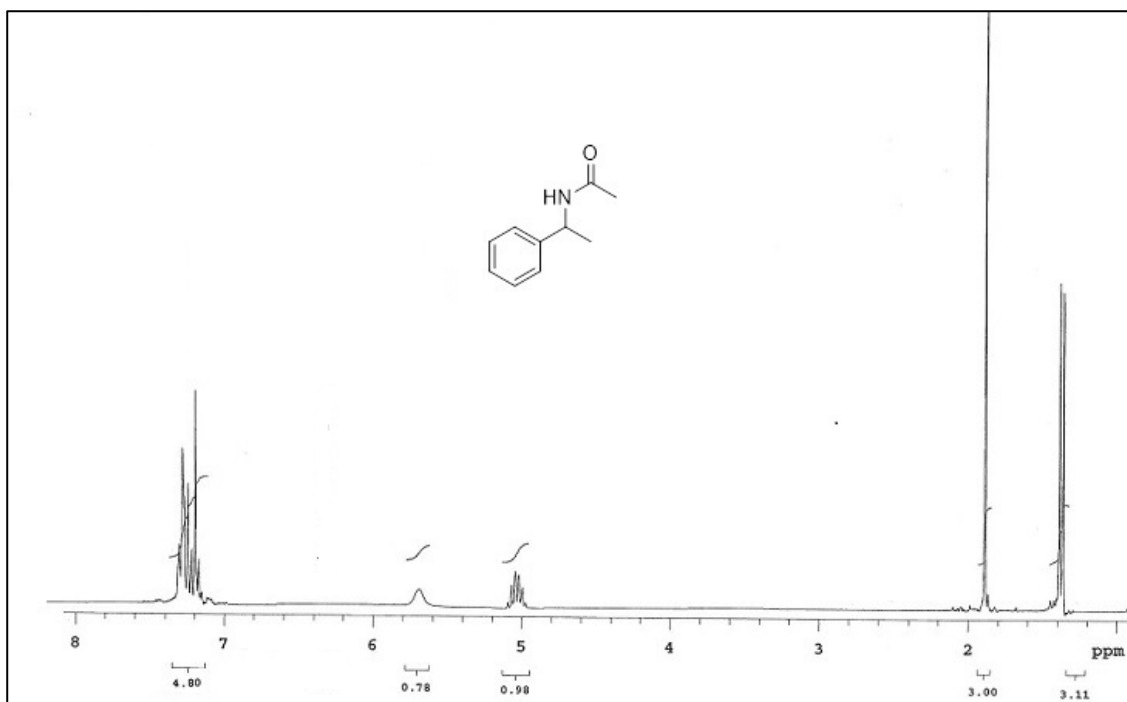
3-Methoxy-N-Methyl-N-benzylacetamide (**4g**): colourless oil; the product was obtained as a mixture of two rotamers. ¹H-NMR (300 MHz, CDCl₃) δ = 7.21-7.28 (m, 2H), 6.68-6.82 (m, 6H), 4.57 (s, 2H), 4.50 (s, 2H), 3.78 (s, 3H), 3.76 (s, 3H), 2.93 (s, 3H), 2.91 (s, 3H), 2.16 (s, 6H) ppm.²⁵



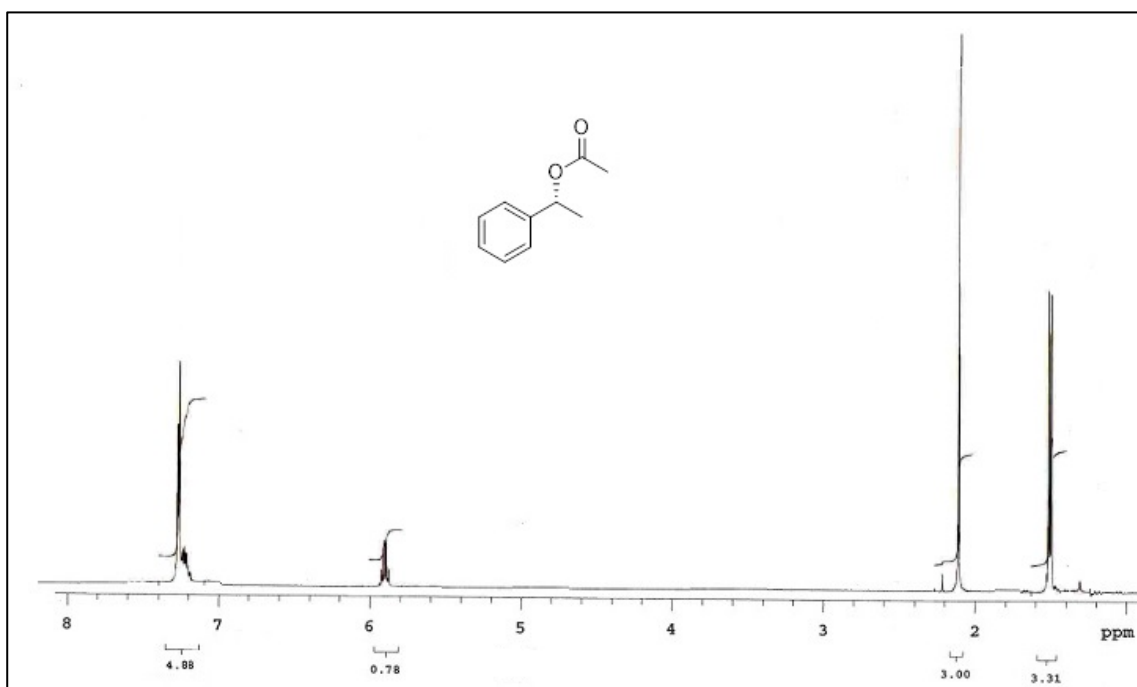
S-(1-Phenylethyl) thioacetate (**6a**): colourless oil; ¹H-NMR (300 MHz, CDCl₃) δ = 7.31-7.37 (m 4H), 7.25-7.29 (m, 1H), 4.05-4.14 (m, 1H), 2.05 (s, 3H), 1.68 (d, *J* = 7.1, 3H) ppm.²⁶



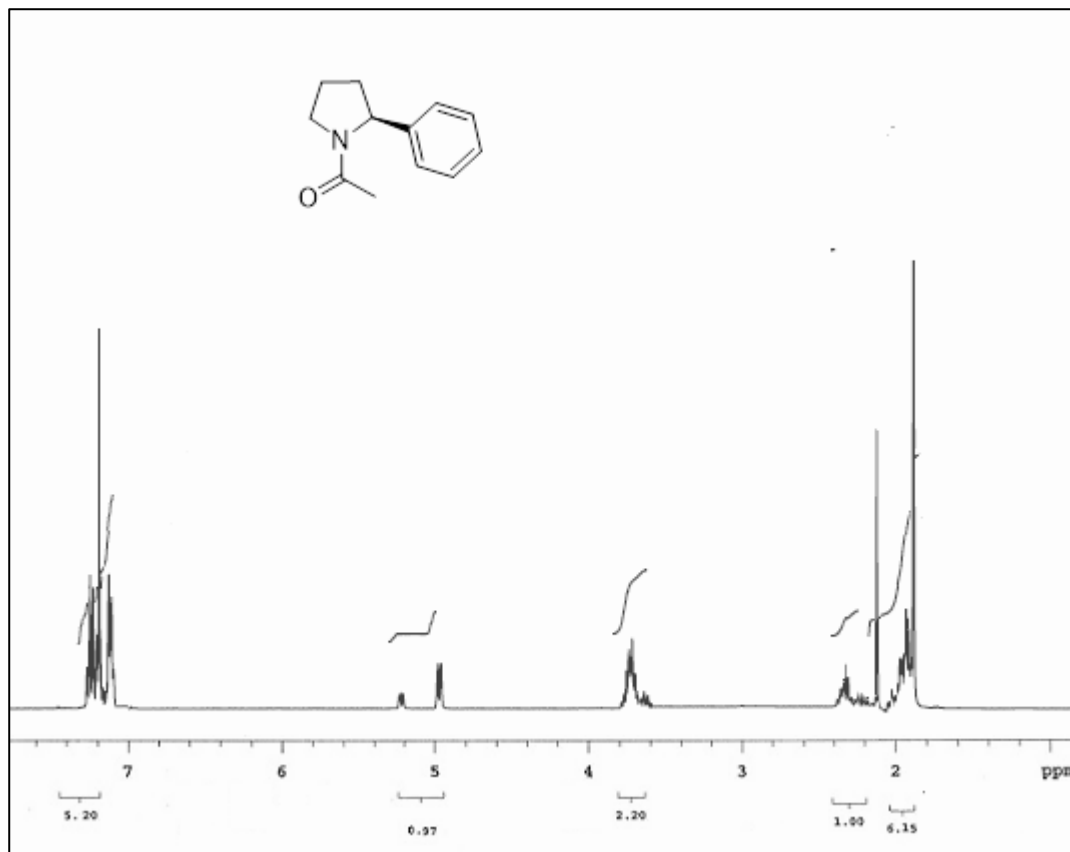
N-(1-Phenylethyl)acetamide (**6b**): colourless oil; ¹H-NMR (300 MHz, CDCl₃) δ = 7.18-7.32 (m, 5H), 5.70 (br, s, 1H), 5.05 (q, *J* = 6.0 Hz, 1H), 1.90 (s, 3H), 1.42 (d, *J* = 6.0 Hz, 3H) ppm.²⁷



(*R*)-1-Phenylethyl acetate (**6f**): colourless oil; ¹H-NMR (300 MHz, CDCl₃) δ = 7.29-7.38 (m, 5H), 5.92 (q *J* = 6.6 Hz, 1H), 2.15 (s, 3H), 1.55 (d, *J* = 6.6 Hz, 3H) ppm. ²⁸

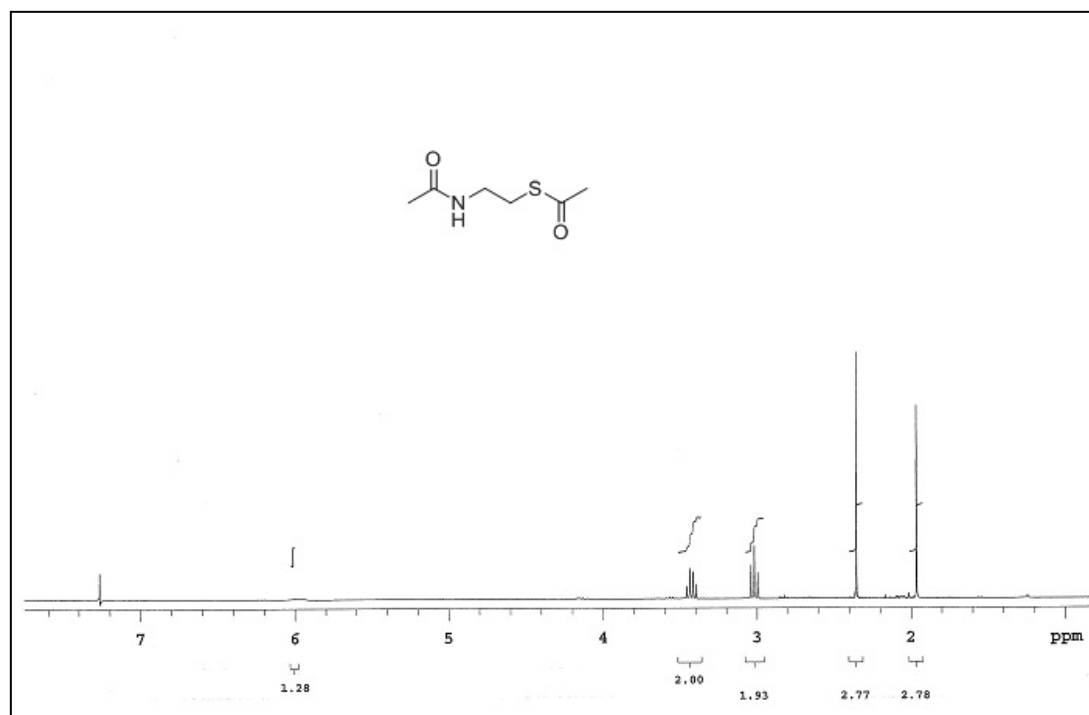


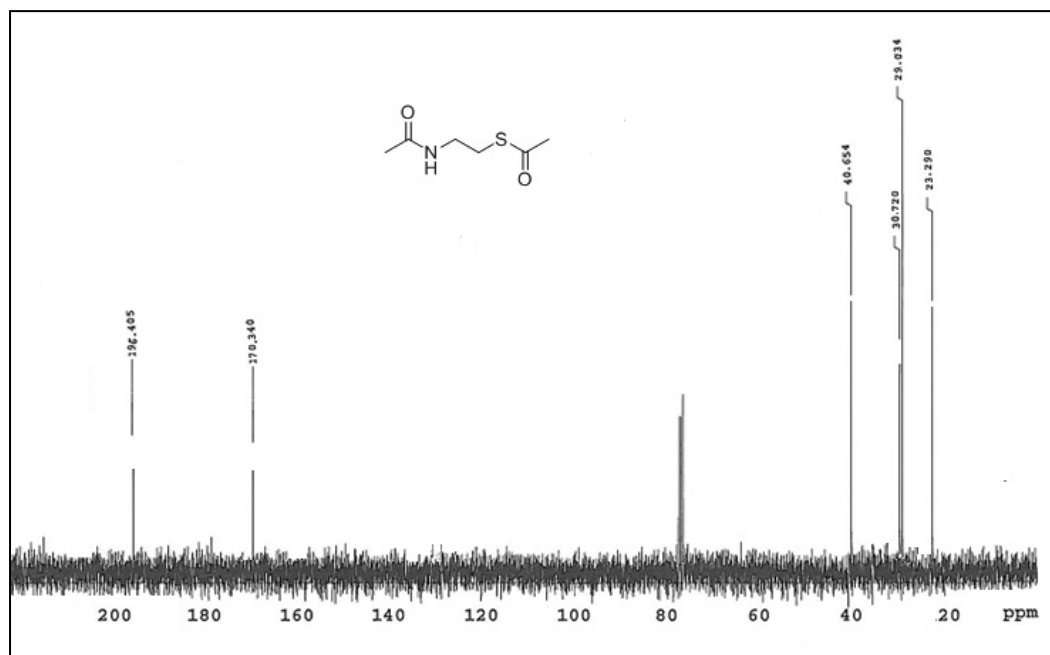
(*S*)-1-(2-Phenyl-1-pyrrolidinyl)ethanone (**6h**): colourless oil; the product was obtained as a mixture of two rotamers. ¹H-NMR (300 MHz, CDCl₃) δ = 7.10-7.38 (m, 5H), 4.98-5.22 (m, 1H), 3.58-3.81 (m, 2H), 2.20-2.41 (m, 1H), 1.83-2.10 (m, 6H) ppm. ²⁹



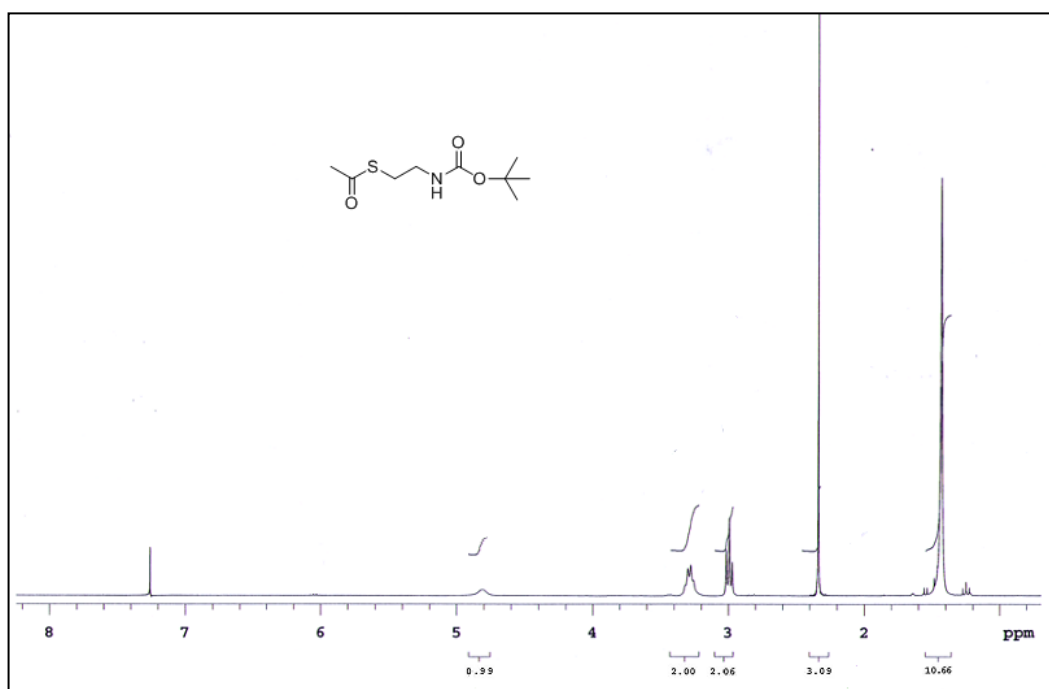
¹H- and ¹³C-NMR Spectra of the 10-mL scale reactions

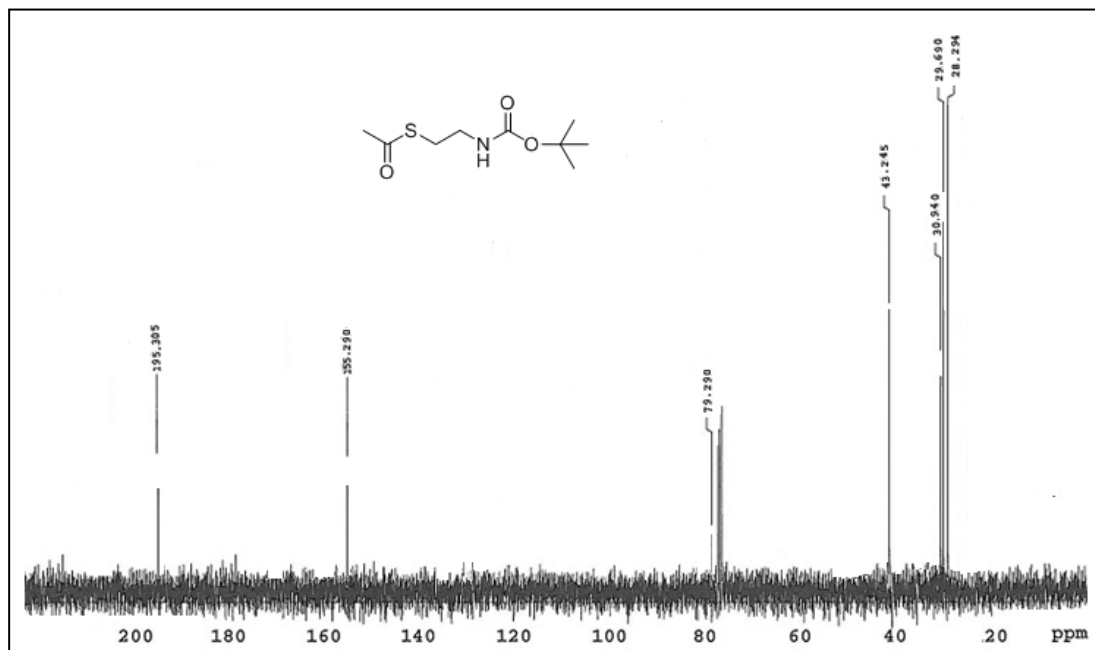
S-(2-acetamidoethyl) ethanethioate (**2o**): colourless oil; ¹H-NMR (300 MHz, CDCl₃) δ = 6.02 (br, s, 1H), 3.43 (q, J = 6.5 Hz, 2H), 3.03 (t, J = 6.5 Hz, 2H), 2.37 (s, 3H), 1.97 (s, 3H) ppm. ¹³C-NMR (75 MHz, CDCl₃) δ 196.4, 170.3, 40.6, 30.7, 29.0, 23.3 ppm.³⁰





S-(2-((tert-butoxycarbonyl)amino)ethyl) ethanethioate (**2p**): colourless oil; ¹H-NMR (300 MHz, CDCl₃) δ = 4.85 (s, br, 1H), 3.22-3.37 (m, 2H), 2.97 (t, *J* = 6.5 Hz, 2H), 2.28 (s, 3H), 1.43 (s, 9H).
¹³C-NMR (75 MHz, CDCl₃) δ 195.3, 155.3, 79.3, 40.2, 30.9, 29.7, 28.3 ppm. ³¹





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