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Synthesis of Potential Metallo-β-Lactamase Inhibitors

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1 Introduction

Bacterial resistance against antibiotics has in the last decades become an increasing problem worldwide. One cause of antibiotic resistance is the bacterial production of enzymes that hydrolyze the β-lactam ring found in many antibiotics. These enzymes, called β-lactamases, are classified into four main groups, one of them being metallo-β-lactamases (MBL). The MBLs subgroup currently has no clinically developed inhibitors, and due to the more recent development of resistance against antibiotics due to MBLs the search for inhibitors is a highly important strategy. The structural complexity and heterogeneity of MBLs have posed challenges to find a common inhibitor to treat infections caused by MBL-producing bacteria.

The purpose of this project is to create different kinds of MBL inhibitors, and test for their activities on two specifically selected Verona integron-borne metallo-β-lactamase (VIM) enzymes, VIM-2 and VIM-7. VIM-2 is one of the most clinically relevant MBLs. One of the characteristics of the VIM enzymes, is the presence of two Zn(II) ions in the catalytic site, that are important for both enzymatic and inhibitory activity.

Only a few MBL inhibitors have been reported so far, and even fewer have been described for the VIM class. No VIM-7 inhibitors have been found. For VIM-2, some inhibitors have been described in literature, and among them, 2-substituted-3-mercaptopropanoic acids (Figure 1) have inhibitory activity for VIM-2. To date, this is the only inhibitor described as co-crystallized with the VIM-2 enzyme. This forms the basis for the current project.

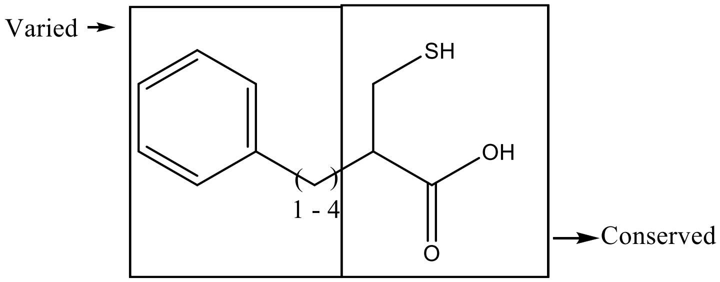
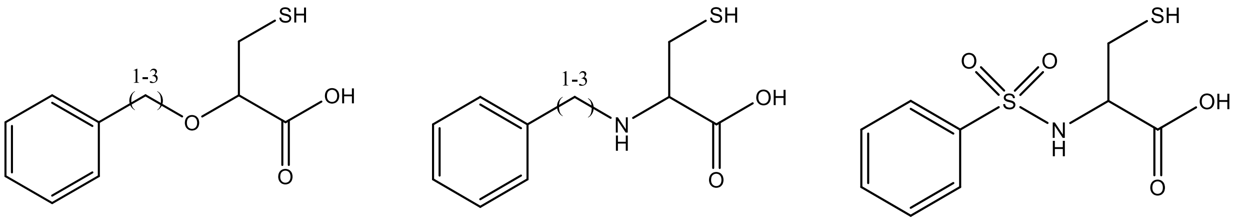


Figure 1 The 2-substituted-3-mercaptopropanoic acid inhibitor. The sidechain in the brackets on the left is of variable lengths, whereas the functional groups on the right is fixed.

The synthesis plan is to keep the 3-mercaptopropanoic acid moiety unchanged as it possess a thiol-group (Figure 1) described to be an important group for binding to the Zn(II) ions in the catalytic site, and because the carboxylic acid may confer electrostatic interactions with the enzyme. The side chain in position two will be envisioned with ethers, amines and sulfonamides. The carbon chain connecting the ethers, amines or sulfonamides will subsequently vary in length from one to three carbons. Special considerations to the hydrophobic pocket in the active site of the enzyme will be taken, varying the length of the side-chain. The additional side-chain extensions include addition of extra functional groups. This will be done to explore the possibilities of higher avidity due to polar interactions between the inhibitor and the active site domain.

To synthesize the compounds (see Figure 2) different strategies can be used. Ethers (Figure 2, left) may be prepared by ring opening of ethyl oxirane-2-carboxylate (ethyl glycidate) with a sulfur nucleophile, followed by a Mitsunobu reaction on the alcohol group by different alcohols to obtain the desired ether functionality. This reaction is shown in Scheme 1.

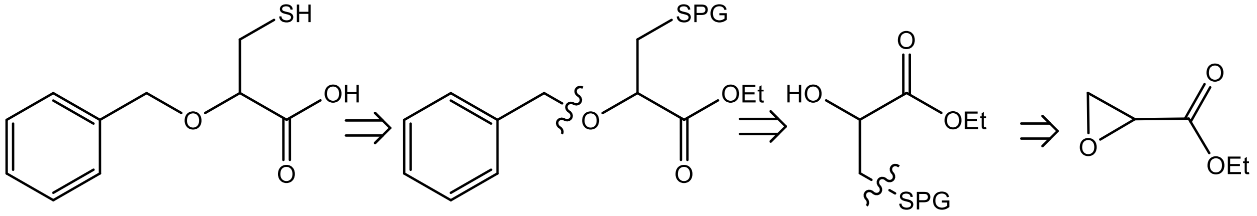


2-substituted-3-mercaptopropanoic acids ethers

2-substituted-3-mercaptopropanoic acids amines

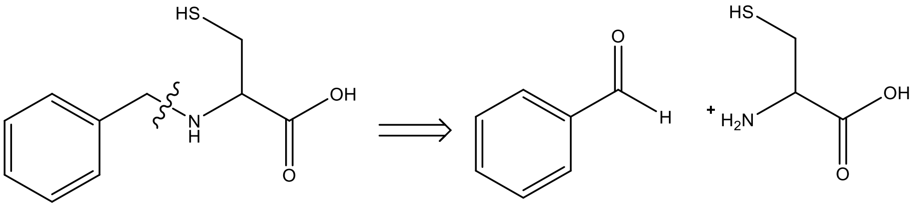
2-substituted-3-mercaptopropanoic acids sulfonamides

Figure 2 Overview over the 2-substituted-3-mercaptopropanoic acids that are the focus of this project.



Scheme 1 Retro synthesis of 2-substituted-3-mercaptopropanoic acids ethers.

The amines (see Figure 2, center) are planned to be made by reductive amination of the appropriate aldehydes with cysteine (Scheme 2).


HS
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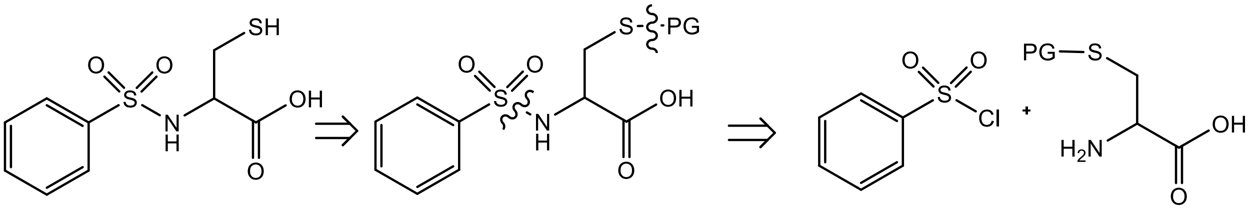
H
OH
O
O
H
HS
+
H_2N
OH
O

Scheme 2 Retro synthesis of 2-substituted-3-mercaptopropanoic acid amines.

The sulfonamide variants (see Figure 2, at the right) will be synthesized by reacting a protected cysteine with different sulfonyl chlorides. This reaction is shown in Scheme 3.


SH
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Cl
+
PG
S
H_2N
OH
O

Scheme 3 Retro synthesis showing sulfonylation of a protected amino acid.

2 Background information

2.1 *β –Lactamases and bacterial resistance*

The bacterial resistance against β-lactam antibiotics poses a continuous challenge, thus it is highly relevant and important to develop general and more specific inhibitors conferring to the antibiotic resistance. The β-lactam antibiotics hold a beta-lactam ring as its key feature (see Figure 3). The β-lactam antibiotics works by inhibition of bacterial peptidyltransferase by forming a stable acyl-enzyme intermediate after a nucleophilic attack governed by a serine residue in the active site of peptidyltransferase, where the attack results in a cleavage of the β-lactam ring[1]. Peptidyltransferase is critical for the peptidoglycan biosynthesis of bacterial cell wall. Similar to peptidyltransferase, most beta-lactamases (not MBLs) contain a serine residue within its active site, which performs a nucleophilic attack on the amide bond and cleaves the β-lactam ring. These types of enzymes are called serine β-lactamases (SBLs), and on the basis of sequence and structural homology, they have been suggested to be grouped into classes A, C and D by Ambler[5]. β-lactamases that employ one or two active-site Zn(II) ions to catalyze the cleavage of β-lactams are called metallo-β-lactamases and belong to class B[2]. Based on amino acid sequence identity and structural features, MBLs can be further classified into three subclasses: B1, B2 and B3[3]. Similar for them all is that they have an αβ/βα-fold[4], where the two β-sheets are in between the two α-helices. This is called the metallo-β-lactamase fold[5]. The active site with one or two Zn(II) ions is located at the edge of the two β-sheets, and the positions of the Zn(II) ions are essential for substrate binding and hydrolysis[6].

All three subclasses of MBLs have a binuclear active site, which requires one or two zinc ions for full activity. For some binuclear B1 and B3 MBLs, two Zn(II) ions are not necessary for activity. The second Zn(II) ion in the Zn2 binding site serves to stabilize the built-up negative charge on the anionic N atom during hydrolysis of the peptide bond, thereby increasing the activation free energy for nucleophilic attack. As a result, the binuclear enzyme has an overall better catalytic efficiency than the mononuclear variant[2]. Subclass B2 enzymes are catalytically active with one Zn(II) ion binding to the Zn2 binding site. MBLs hydrolyze all β-lactam antibiotics, except for the monobactam aztreonam[2].

The VIM enzymes are categorized into subclass B1[3]. As shown by Oelschlaeger et al[2] (2010) VIM enzymes bind two Zn(II) ions in a binuclear center formed by the conserved amino acids His116, His118, and His196; the Zn2 binding site consists of Asp120, Cys221, and His263. The two Zn(II) ions activate a water/hydroxide ion nucleophile by coordination. Mutations at amino acid position 120 have shown that the Zn2 binding site and the Zn1-Zn2 distance are flexible[7]. Wang et al[8] (1999) proposed a mechanism for nitrocefin hydrolysis of CcrA, and it is reasonable to assume that the reaction mechanism is closely related to that of VIM. According to this mechanism, the Zn(II) ions are coordinated by a bridging water/hydroxide molecule that performs a nucleophilic attack on the carbonyl carbon in the β-lactam ring of the substrate, resulting in cleavage of the amide bond and deactivation of the antibiotic activity.

So far, these types of MBLs have been identified[9]: IMP, VIM, GIM, SPM, SIM, KHM, AIM, DIM, TMB, and NDM. The genes encoding these enzymes are associated with mobile genetic elements, and are often carried together with other important antibiotic resistance genes as gene cassettes in integrons, resulting in multidrug resistance that further limits treatment options[10]. These integrons are frequently isolated from patients suffering from antibiotic resistant infections. To this date, twenty-seven VIM variants have been reported[9]. The VIM enzymes are the globally most widespread MBLs, and the VIM-2 was first isolated from *Pseudomonas aeruginosa*[11]. VIM-7 is the most divergent VIM type in terms of amino acid sequence, sharing only 74 % identity with VIM-2[9]. It was first isolated in the United States in 2001, and its gene was found on an integron in a *Pseudomonas aeruginosa* isolate. Due to the low sequence identity with VIM-2, it has lead to the belief that VIM-7 has evolved independently in the US where it first was isolated, rather than having been transferred from Europe or Asia[2].

The two VIM enzymes that are used for testing in the current project possess a number of different features. VIM-7 is more positively charged than VIM-2, carrying an overall charge of -9, compared to -14 for VIM-2[9]. Some of this negative charge in VIM-2 is confined to the active site suggesting a possible influence in interactions with substrates. Because of this charge difference, VIM-7 has a lower affinity and a reduced catalytic efficiency against cephalosporins relative to VIM-2, particularly those with a bulky positively charged C3 substituent (see Figure 3). On the other hand, penicillinase and carbapenemase activities are comparable with that of VIM-2. The different substituents on the cephalosporins, penicillins and carbapenemases result in differences in affinity and activity. This can be explained by the differences in the active site of the enzymes; In VIM-7, loop 1 is positioned further away from the active site than in VIM-2, which makes the active site more open[9]. Moreover, VIM-7 has many residue substitutions in loop 1 and in the active site, which may influence specificity and activity.

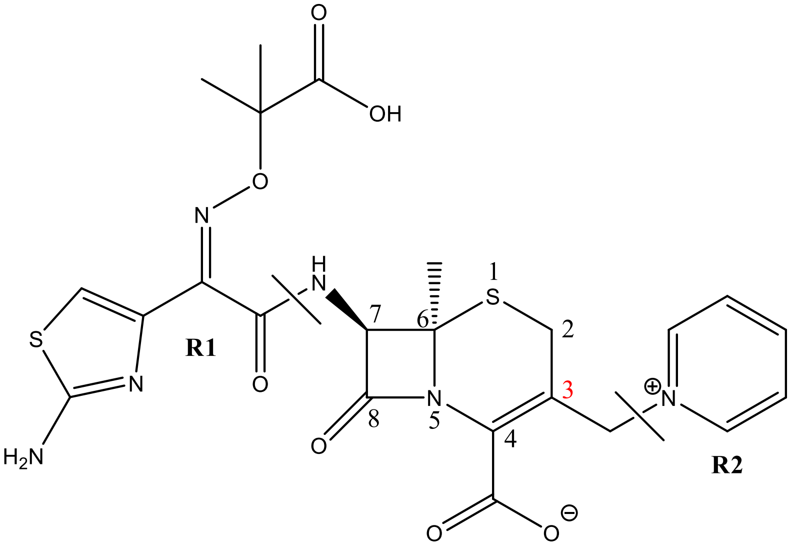


Figure 3 The chemical structure of ceftazidime: A typical example on an antibiotic with a bulky positively charged C3 substituent that lowers the affinity to VIM-7 but not to VIM-2.

To develop potent and common inhibitors, it is necessary to focus on several aspects simultaneously. The most important aspects are the structure and dynamics of both Zn(II) ions binding sites, loops 1 and 2 in each MBL, and its complex with a lead compound. Efforts to develop effective inhibitors have been hampered by the lack of structural information on how these enzymes recognize and modify the beta-lactam substrates. Relevant for inhibitor design, the VIM turn-over mechanism differs from the one catalyzed of serine β-lactamases in that it lacks a covalent enzyme-substrate intermediate. In general, inhibitors for MBLs feature two functional groups: hydrophobic moieties that bind in a predominantly hydrophobic pocket around loop 1, and a metal-ligating group that interacts with the Zn(II) ions.

Jin et al[12] have found a series of inhibitors for VIM-2; 2-substituted-3-mercaptopropanoic acid (Figure 4, left) and N-substituted 2-mercaptoacetamide derivatives (Figure 4, right). For both of these, the inhibitory effect was greatest for $n = 4$.

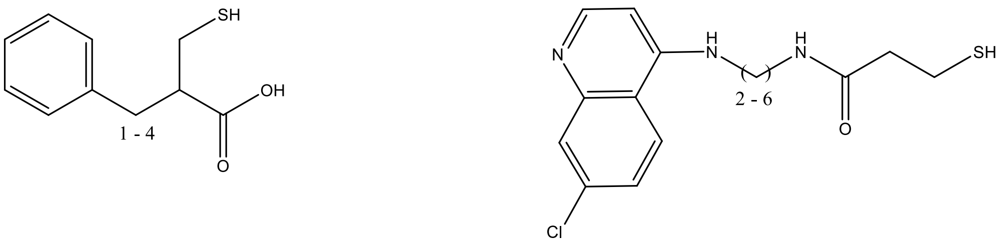


Figure 4 Chemical structure of potent inhibitors for VIM-2.

Jin et al[6] recognized, by a structure analysis of the co-crystallized enzyme-inhibitor complex, that the thiol group of 2-(mercaptomethyl)-5-phenylpentanoic acid replaced the water molecule between the two Zn(II) ions in the active site due to the high affinity sulfur holds for Zn(II). Furthermore, the phenyl group interacted with Tyr67 (47) on loop 1 close to the active site by π-π-stacking interactions. The methylene group interacted with Phe61 (42) located at the bottom of loop 1 through CH-π interactions. The carboxyl group was involved in hydrogen bonding and electrostatic interactions with preserved residues, such as Lys. Arg228 (185) and Asn233 (190) on loop 2 were moved compared with the native structure. These results suggested that the above-mentioned four residues play important roles in the binding and recognition of inhibitors or substrates, and in stabilizing a loop in the VIM-2 enzyme.

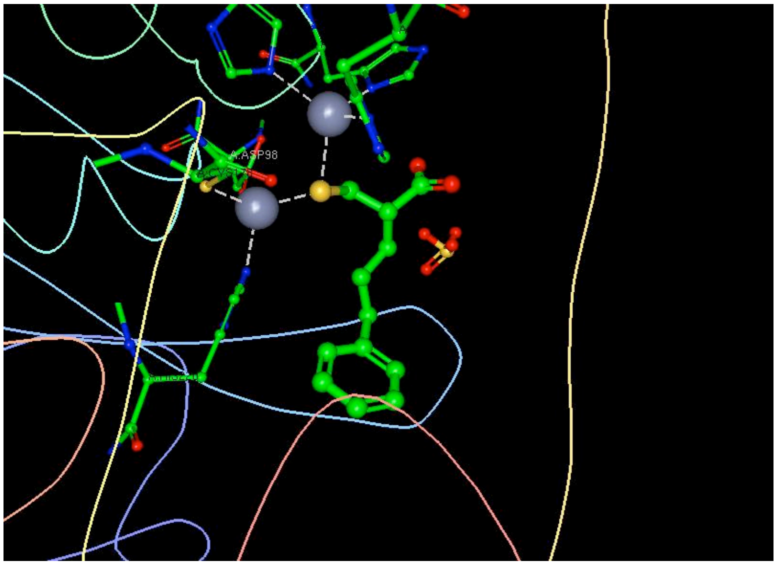


Figure 5 The figure shows how the sulfur atom may coordinate with the sink ions.

D. Minod et al[13] (2009) has described two potent first generation sulfonyltriazole inhibitors for the enzyme, see Figure 6**.**

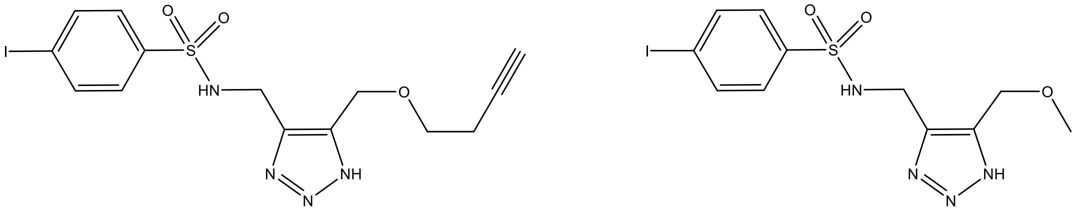


Figure 6 Chemical structures of the first-generation sulfonyltriazole inhibitors.

The predicted mode of binding (by docking) for these molecules (Figure 6) orients the molecule such that the arylsulfonamide is exposed to the solvent while the alkoxyl group is buried into a hydrophobic sub-pocket in the active site. Out of these two, the one with the largest alkoxyl-group appears to exploit the hydrophobic cavity best. Both arylsulfonamides and triazoles may make strong interactions with aromatic groups through π-stacking as well as other hydrophobic types of binding.

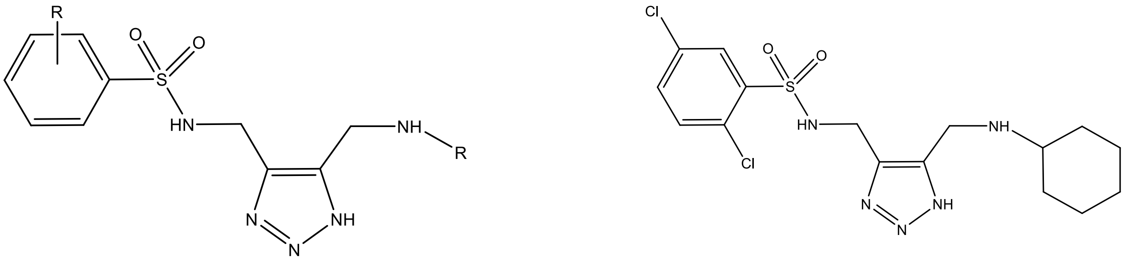
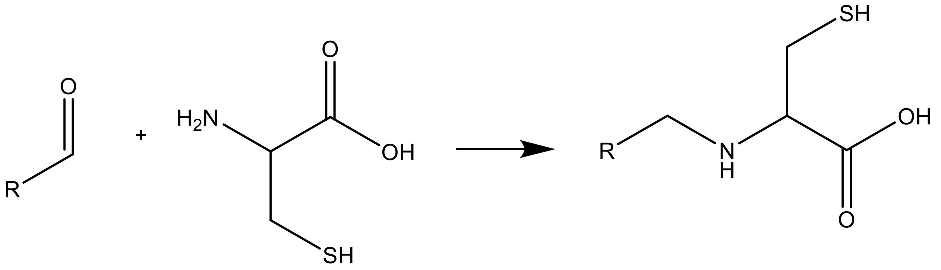


Figure 7 The chemical structures of the second-generation sulfonyltriazole inhibitors.

Second-generation sulfonyltriazoles[14] (see Figure 7) have been developed by Weide et al[14] (2010) with a starting point being the most potent inhibitor from the study by Minod et al (see Figure 6, left substance). With the new inhibitors the modifications were done with respect to the aromatic substituents, and on the C4 methyl of the triazole. They discovered that the most potent inhibitors contained a dichlorobenzene group, and a hydrogen acceptor of the C4 methyl of the triazole was important for inhibitor-protein interactions, with nitrogen being superior to oxygen.

2.2 Reductive amination


SH
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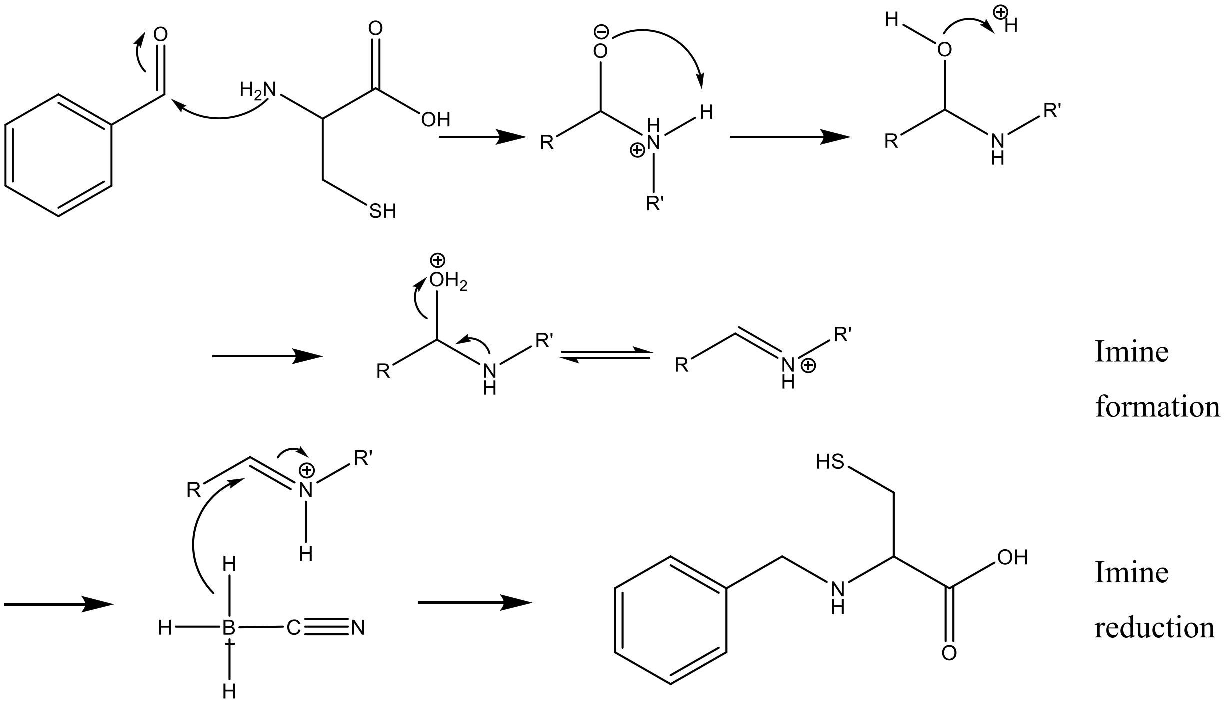
H2N
OH
OH
R
R
N
H
O
SH

Scheme 4 Scheme showing reductive amination of cysteine.

Reductive amination[15] of aldehydes/ketones is a useful reaction in organic chemistry and is an important tool in the synthesis of amines. The aldehyde/ketone condensates with an amine and generates an imine followed by reduction to furnish the desired amines. The reaction mechanism for reductive amination is shown in Scheme 5.


O
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H2N
OH
SH
O
R
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N
H
R'
H
O
H
R
R'
N
H
OH2
R
R'
N
H
R
R'
N
H
Imine
formation
R
R'
N
H
H
H
B
C
N
H
HS
OH
N
H
O
Imine
reduction

Scheme 5 Reaction mechanism of reductive amination with $NaBH_3CN$ as the reducing agent.

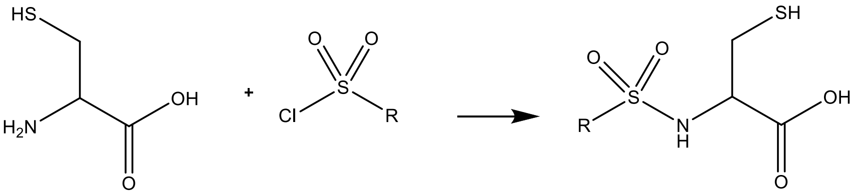
The reaction mechanism for reductive amination involves an initial formation of an intermediate hydroxylamine followed by elimination of water which furnishes the imine. The reaction is reversible and the imine formation is the slow step. Subsequent reduction of the imine with a suitable reducing agent produces the amine.

Among the various reduction methods at hand are catalytic hydrogenation, reduction with amine borane complexes[16] or by using metallo-hydride reducing agents such as sodium cyanoborohydride[17], sodium triacetoxyborohydride[18], sodium[19]- or zinc borohydride[20]. Among the latter, sodium cyanoborhydride and sodium triacetoxyborhydride are the most common because of their versatility and will be described later. The success of the reaction depends on the choice of the reducing agent, as it might have to reduce imines selectively over ketones/aldehydes under the same reaction conditions. The rate of the reduction of iminium ions is in addition, much faster than that for aldehydes or ketones, but requires catalytic amount of acid. As a result, reductive amination can be carried out as a one-pot procedure by introducing the reducing agent into a mixture of the amine and carbonyl compound at the start of the reaction.

$NaBH_3CN$[17] is a mild reducing agent that reduces a variety of organic functional groups[21] with good selectivity and will readily reduce imines to amines. The successful use of $NaBH_3CN$ is due to its pH depending selectivity[22]. At pH 3-4 $NaBH_3CN$ reduces aldehydes and ketones efficiently, and at pH 6-8, imines are preferentially protonated and reduced faster than aldehydes and ketones. $NaBH_3CN$ is stabile in relatively strong acids (pH 3) and show good solubility in hydroxylic solvents such as methanol. Limitations of this method are that the reaction may require up to a fivefold excess of the amine in relation to the carbonyl compound[22]. For aromatic ketones and with weakly basic amines, conversion can be slow. A drawback for the use of $NaBH_3CN$ in reductive amination is that the highly toxic gas HCN may be liberated under strong acidic conditions. Furthermore the products may be contaminated by HCN or a cyanide salt that might follow the product through work up. Such contaminants have reportedly[18] been hard to remove.

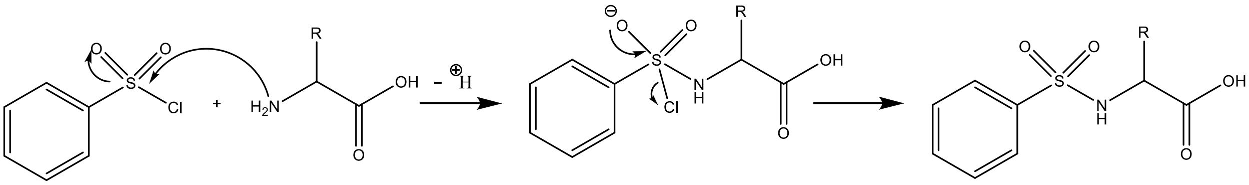
An other mild reducing agent, is $Na(OAc)_3BH$[18]. The boron-hydride bond is stabilized by the steric and electron-withdrawing influence of the acetoxy groups. $Na(OAc)_3BH$ works well in reduction of aliphatic acyclic and cyclic ketones, aliphatic and aromatic aldehydes, and primary and secondary amines including a variety of weakly basic and nonbasic amines. Some challenging substrates for the reductive amination with $Na(OAc)_3BH$ include aromatic and unsaturated ketones and some sterically hindered ketones and amines.

2.3 Sulfonation of amino acids



Scheme 6 Preparation of a sulfonamide with Cysteine using sulfonyl chloride

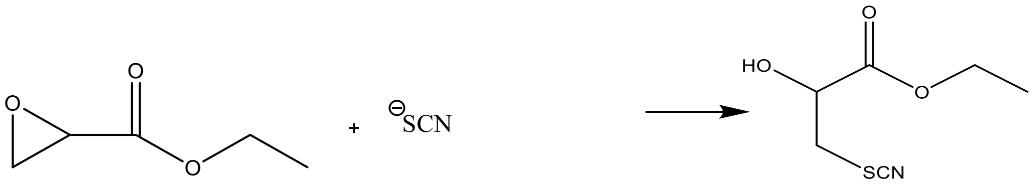
The most common way of preparing sulfonamide derivatives of unprotected amino acids is by nucleophile acyl substitution at suitable sulfonyl chloride in aqueous solution in the presence of a base (Scheme 6). Schröder et al[25] have proposed a general procedure for preparing N-sulfonylated amino acids involves addition of the sulfonyl chloride in a solution of the amino acid in aqueous base before heating the mixture with vigorous stirring. This procedure works for most of the natural amino acids but they tend to favor different bases for optimal yields. NaOH[23], DIEA[24], K_2CO_3[25], and Na_2CO_3[26] are the most frequently used bases. The sulfonation requires a reaction time between 10 min up to 2 hours. For some amino acids, heating the reaction mixture up to 70° C is necessary for achieving an acceptable conversion. The differences in reaction conditions required might be explained by solubility issues or steric hindrance among the amino acids with charge carrying or bulkier side chains. There are no reported methods for sulfonation of a cysteine unless its thiol group functionality is protected. For the protected cysteine[27] the sulfonyl chloride is dissolved in 1,4-dioxane prior to its addition to a solution of protected cysteine in aqueous sodium hydroxide and the reaction is stirred at room temperature for 16 hours. The reaction mechanism for sulfonation of amino acids is shown in Scheme 7.



Scheme 7 Reaction mechanism of sulfonation of amino acids.

The amine will react as a nucleophile and attack the electron poor sulfur and displace the leaving group, in this case a chloride ion. The resulting amine cation will be deprotonated by the base present in the system yielding the sulfonamide.

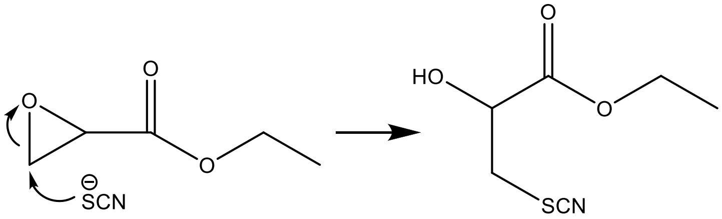
2.4 Ring opening of epoxides with sulfur nucleophiles



Scheme 8 Reaction scheme of epoxide ring opening with thiocyanate.

Oxirane ring-opening with various nucleophiles is well recognized as a useful starting point for the synthesis of multi functionalized organic compounds. Among such reactions, the one with sulfur nucleophiles plays an important part in classical organosulfur chemistry.

The reaction mechanism for epoxide ring opening with thiocyanate is shown in Scheme 9.



Scheme 9 Reaction mechanism for epoxide ring opening with thiocyanate.

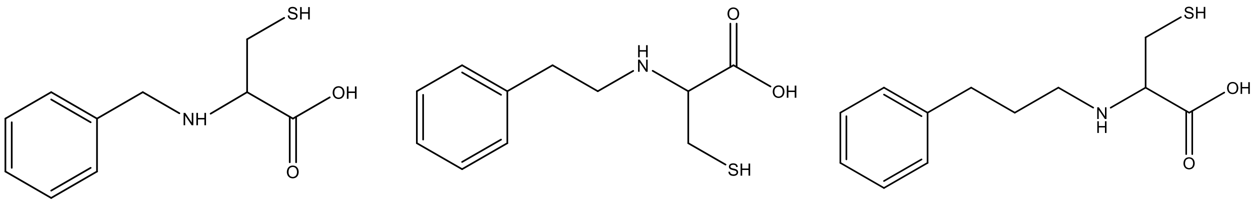
As shown in Scheme 9, the thiocyanate anion attacks the carbon on the epoxide and opens the ring, creating an alkoxide anion. Thiocyanate anion may attack both carbons, generating two isomers, but the predominantly attack is on the least substituted carbon. To promote the reaction a variety of catalysts can be used. For example, a Lewis acid can be used to increase the reactivity of the epoxide, coordinating to the oxygen, making the carbons attached even more electron deficient.

In the literature many different ring openings of compounds similar to ethyl oxirane-2-carboxylate (ethyl glycidate; see the starting material in Scheme 8) are reported. In many of these ring openings aromatic and aliphatic thiolates are employed as sulfur nucleophiles. These sulfur nucleophiles were not relevant for the purpose of the current project, since they are difficult to deprotect and the free thiol would not be available. Other nucleophiles at interest described are KSAc[28], NH_4SCN/KSCN/HSCN[29], and TMSNCS[30].

One of the most described protection groups for sulfur is the cyano group. There are three methods reported in literature for the synthesis of beta-hydroxythiocyanates, and the most frequently used method is the epoxide ring opening with a thiocyanate anion. The thiocyanate salts were used in both presence and absence[31] of catalyst. Catalysts that has been used are; crown ethers[42], tetrabutylammonium fluoride (TBAF)[32], $Ti(OPr^{i})_4$[33], various Lewis acids like titanium-, zinc,- indium,- and gallium chlorides[34], 2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ)[35], triphenylphosphine-thiocyanogen (TPPT)[36], triphenylphosphinepalladium (article not found), polyacryloamides[37], selectfluor[38], 2-phenyl-2-(pyridyl)imidazolidine (PPI)[39], 2,6-bis[2-(*o*-aminophenoxy)-methyl]-4-bromo-1-methoxybenzene (BABMB)[40], Schiff-base metal(II) complexes (article not available), metalloporphyrins[41], phenol-containing macrocyclic diamides[42] and tetraarylporphyrins[43]. It has been reported that the presence of hydroquinone[29b] is required to stabilize the beta-hydroxy thiocyanate to slow down the rapid conversion into thiiranes. This is only valid if the beta-hydroxythiocyanate is contaminated in the reaction mixture. If it is in its pure form, the addition of hydroxyquinone will catalyze the thiirane formation. Alternative methods for making beta-hydroxythiocyanates include opening of a cyclic sulfate with NH_4SCN followed by hydrolysis of the corresponding beta-sulfate to form the thiocyanohydrins[44], and nucleophilic substitution of a bromine atom in bromohydrin by a thiocyanate anion[45].

3 Results and Discussion

3.1 Preparation of N-Alkyl substituted cysteine derivatives by reductive amination



1a **1b** **1c**

Figure 8 Chemical structure of the produced amines.

The synthesis of compounds **1a-c** has been described in literature. The described procedure by Park et al[17d] (2002) reported the reductive amination of cysteine with $NaBH_3CN$. The procedure had relatively low reported yield, about 30 %. According to the procedure, cysteine and $NaBH_3CN$ were mixed in methanol, before adding the aldehyde. In our hands, this gave only 5 % yield for 2-(benzylamino)-3-mercaptopropanoic acid (**1a**), and even less for 3-mercapto-2-(phenethylamino)propanoic acid (**1b**) and 3-mercapto-2-(3-phenylpropylamino)propanoic acid (**1c**). When the procedure was scaled up 4 times for **1b** the yield was raised to 11 %. **1a** and **1b** gave relatively pure products, but **1c** contained approximately 80 % unreacted cysteine. This indicated a need for longer reaction time than the two other compounds, **1a** and **1c**. The yield did not however improve when the reaction time was increased from 26 hours to 50 hours.

The overall poor yield can be explained by attempting to condensate the aldehyde with cysteine in its zwitter ionic form. Due to the protonation of cysteines' amine functionality in this state, its nucleophilicity will be non existing unless a base can abstract the proton and leave the free amine to react with the aldehyde. If there is equilibrium for the zwitter ion form, it has to be strongly shifted to the charged form where its reactivity is lowest, which would be in line with the isolated yields. To avoid this protonation problem, two alternatives are possible; a methyl ester of cysteine could be utilized, or running the reaction in the presence of a base to deprotonate the charged amine.

These compounds could not be purified by silica chromatography because of the low solubility in any tested solvents, eg H_2O, MeOH, $CHCl_3$, dioxane, MeOH/H_2O, THF, acetonitrile, acetonitril+TFA, DMSO, DMF, Et_2O, pyridine, and benzene. Several means of reducing reaction time and increasing yield are reported in literature, such as the use of molecular sieves, azeotropic removal of water and addition of acid[22]. We investigated the use of a dehydrating agent Na_2SO_4, and catalytic addition of acid, HCl, in the synthesis of **1c**. Due to the low solubility of **1c**, the dehydrating agent, Na_2SO_4, could not be separated from target compound.

The procedure reported in literature[49] for compound **1a-1c** has reported that the ^1H NMR and ^{13}C NMR analysis were run in D_2O. It was impossible to solve the target compounds **1a-1c** in this solvent. Therefore the use of NaOH was utilized, and the ^1H NMR and ^{13}C NMR analysis were run with NaOH in D_2O. The spectra from literature and the ones obtained can therefore not be compared. ^1H NMR and ^{13}C NMR reference spectra with cysteine in NaOH/D_2O were prepared for comparison for the contamination in the spectra for **1b** and **1c**.

The ^1H NMR spectrum of target compound **1a** is shown in Figure 9. The numbering in the following discussion is used as assigned. The aromatic protons (position 1-4 and 6) are in the expected region at 7.19-7.26 ppm, and integrated to five. The two doublets at 3.47 ppm and 3.62 ppm, integrated to one each, are the two protons in the benzylic position, 7a and 7b. The appearing double doublet at 2.91 ppm belong to the α-proton (position 9). The two double doublets at 2.64 ppm and 2.44 ppm, are the two diastereotopic protons, 11a and 11b. The ^{13}C NMR spectrum validates the ^1H NMR results. The peak at 181.66 ppm, correspond to the carbonyl carbon (C10), number ten. There are four signals, 158.55 ppm, 128.59 ppm, 127.12 ppm and 125.79 ppm, in the aromatic region as expected due to symmetry. The signal at 51.24 ppm arises from the carbon in the benzylic position (C7), while the two other peaks at 67.78 ppm and 28.37 ppm arises from C9 and C11, respectively.

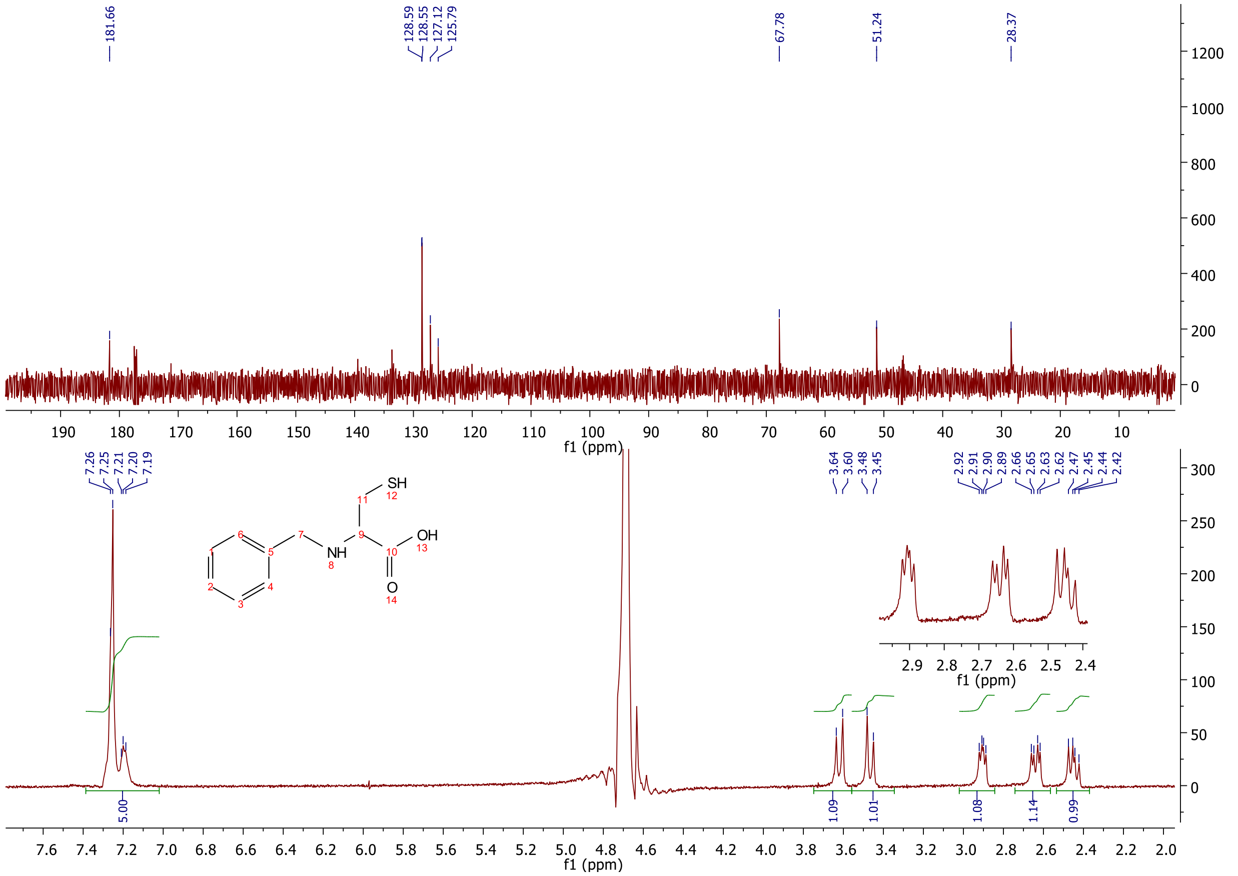


Figure 9 ^{1}H NMR and ^{13}C NMR spectra of compound 1a.

The ^{1}H NMR spectrum (Figure 10) for target compound **1b** shows a small amount of unreacted starting material and the target compound. The aromatic protons resonate at 7.12-7.26 ppm, and are integrated to five. The appearing double doublet at 2.86 ppm belongs to the α-proton at position five, and is integrated to one. The mutiplet at 2.64-2.71 ppm is integrated to three, belonging to three of four protons in the aliphatic sidechain (position 1 and 3). The multiplet in the range from 2.55-2.60 ppm is the overlapping signals from one of the protons from the aliphatic side chain and one of the diasterotopic protons in position 7. The last signal is a double doublet integrated to one, and is the second proton at position 7. The ^{13}C NMR spectrum has one signal at 181.84 ppm which belongs to the carbonyl carbon (C6). The aromatic protons are resonates at 140.31 ppm, 128.81 ppm, 128.63 ppm and 126.24 ppm. The peaks at 68.60 ppm and 28.19 belongs to carbon 5 and 7, respectively. The last two peaks at 48.87 ppm and 35.11 ppm, belong to the two aliphatic carbons C3 and C1, in that order.

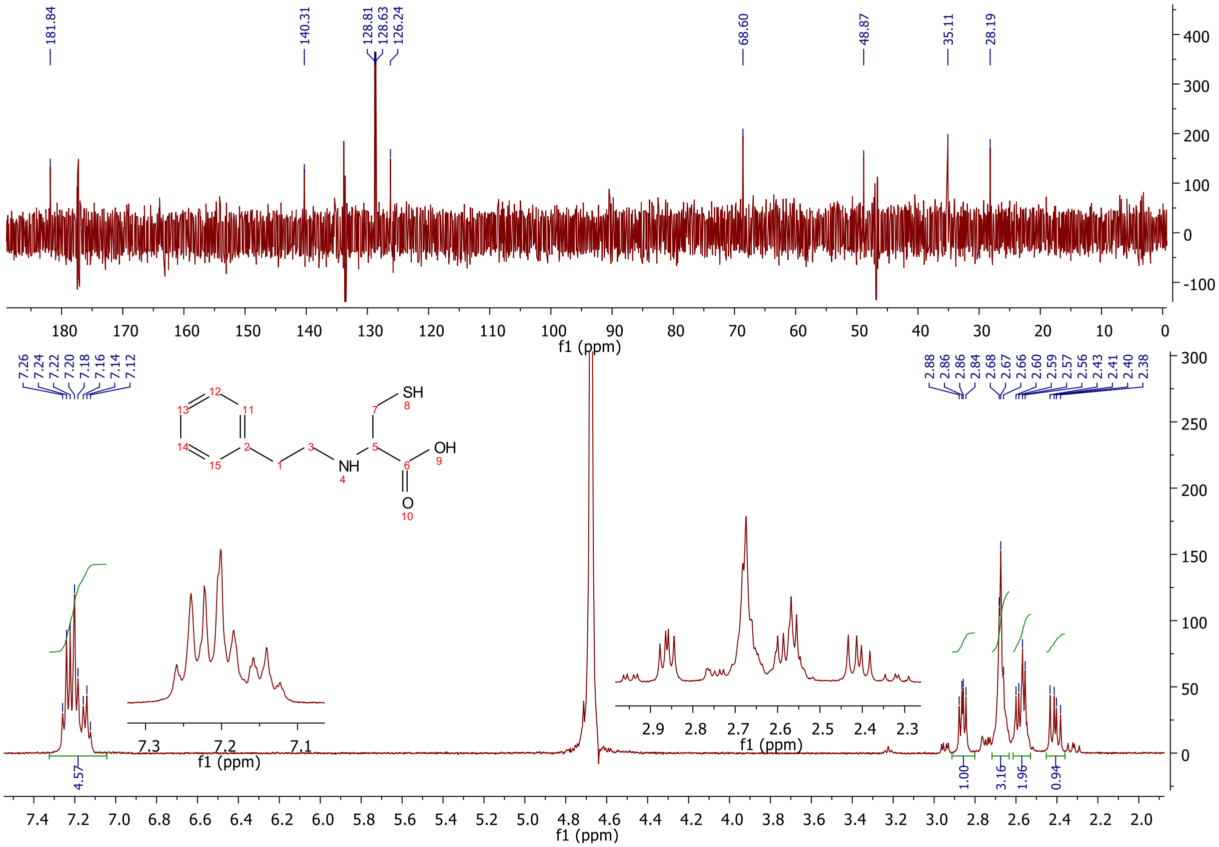


Figure 10 ^{1}H NMR and ^{13}C NMR spectra of compound 1b.

The ^{1}H NMR spectra (Figure 11) after reductive amination of 3-phenylpropionaldehyde shows two compounds, unreacted starting material and the target compound **1c**. The aromatic protons are as expected at 7.12-7.26 ppm as a multiplet integrated to five. The appearing double doublet at 2.97 ppm and the two double doublets at 2.76 ppm and 2.34 ppm are cysteine (see reference spectra of cysteine in the appendix). These three peaks are in a 1:1:1 relationship. The appearing double doublet at 2.85 ppm has arisen from the α-proton (position 11). The double doublet at 2.62 ppm belongs to one of the diastereotopic protons (position 13a). The other diastereotopic proton in position 13b, is in overlap with the two protons in position 9, in a multiplet from 2.44-2.48 ppm. The triplet integrated to two at 2.55 pmm is the benzylic protons at position 7, and the multiplet at 1.64-1.72 ppm is the two protons in position 8. The ^{13}C NMR spectrum underlines the fact that it is two different compounds. The cysteine peaks are found at 182.28 ppm, 60.29 ppm and 31.92 ppm. Due to **1c** poor solubility even in basic water, the sample was too weak for a clear view of the aromatic protons. Therefore, these are not all accounted for in a precise manner, but there are two clear signals at 128.56 ppm and 128.51 ppm. The peak at 177.15 ppm is from the carbonyl carbon (C12). The peak at 68.53 ppm arises from C11 and the peak at 46.90 from C9. The three left at 32.87 ppm, 30.71 ppm and 28.27 ppm arises from C7, C8 and C13, but can not be correctly assigned without further analysis.

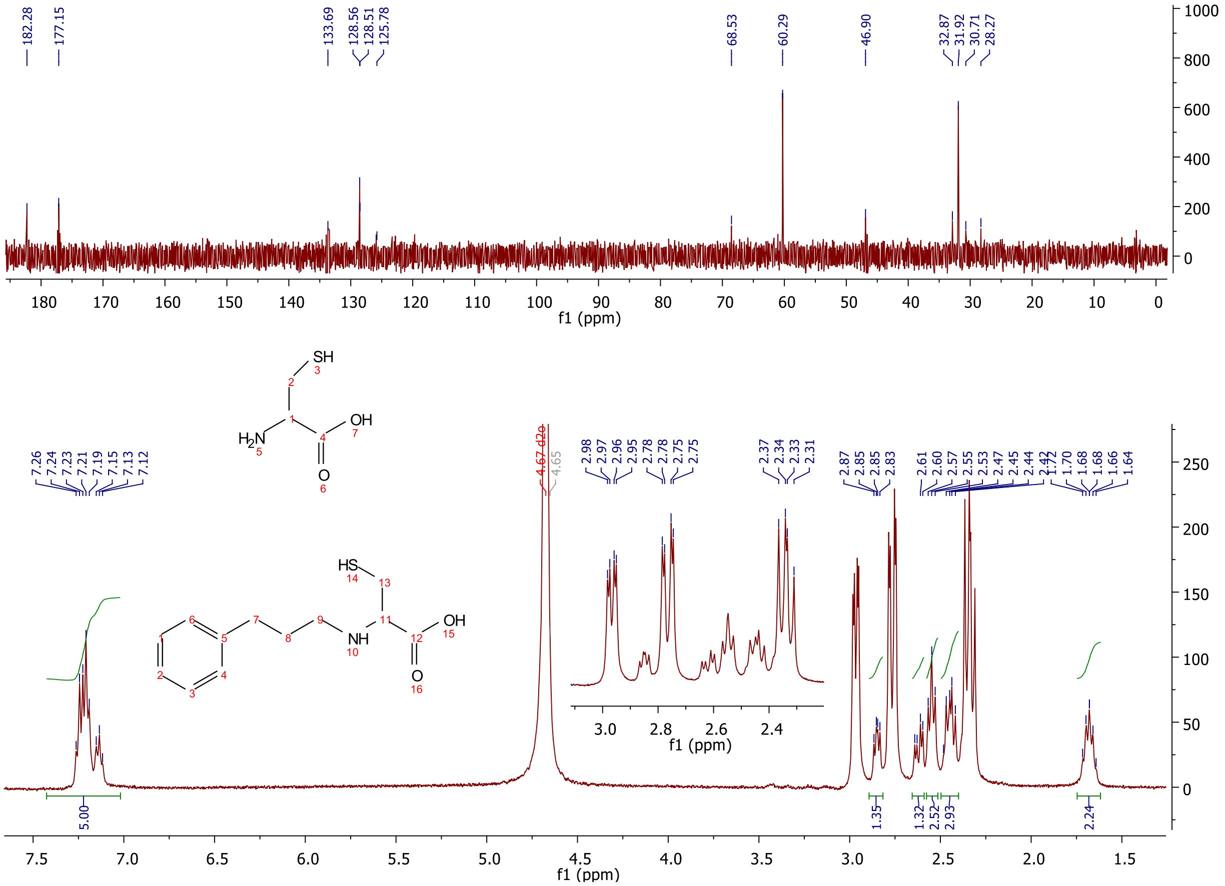


Figure 11 ^{1}**H NMR and** ^{13}**C NMR spectra of compound 1c.**

Since attempts to improve the yield using $NABH_3CN$ failed, the use of $Na(OAc)_3BH$ was explored. This reducing agent did not provide the desired product. MS analysis indicated dialkylation of the amine had occurred. In literature, the occurrence of dialkylation products is reported, especially with aldehydes and primary amines[18]. In comparison to the primary amine, the secondary amine holds a higher degree of reactivity, which can result in overalkylation. This challenge can be suppressed by the addition of up to 5 % molar excess of the primary amine, or by a stepwise reductive amination with preformed imines. Normally the formation of overalkylated products can be monitored by TLC, but since the compounds **1a**-**1c** had poor solubility TLC analysis could not be used.

3.2 Preparation of N-sulfonyl substituted cysteine derivatives

The synthesis of sulfonation of unprotected amino acids has been reported in literature[23]. However, for unprotected cysteine only sulfonylation with dansyl chloride has been described.[46] The dansylation of cysteine differed from the common procedure for sulfonation of unprotected amino acids in the use of a carbonate buffer instead of base.

The use of the dansylation procedure with sulfonyl chloride and unprotected cysteine gave an unsolvable product. Due to the low solubility, it was not possible to make any analysis, and it is therefore not known what the product was. Using the general procedure for sulfonation of amino acids for the sulfonation of cysteine did not generate the target compound **2a**. The ^{1}H NMR spectrum (Figure 12) was somewhat similar to cysteine, but the ^{13}C NMR spectrum was different; the peak at 43.37 ppm had shifted from 31.85 ppm (cysteine reference spectrum), which indicates that the dimer of cysteine had formed[47]. MS analysis was not suited for this compound. Further tests are necessary before it is verified that this is a dimerization-reaction of cysteine. To the best of our knowledge, a new way of synthesizing cystine with sulfonyl chloride in water has been discovered.

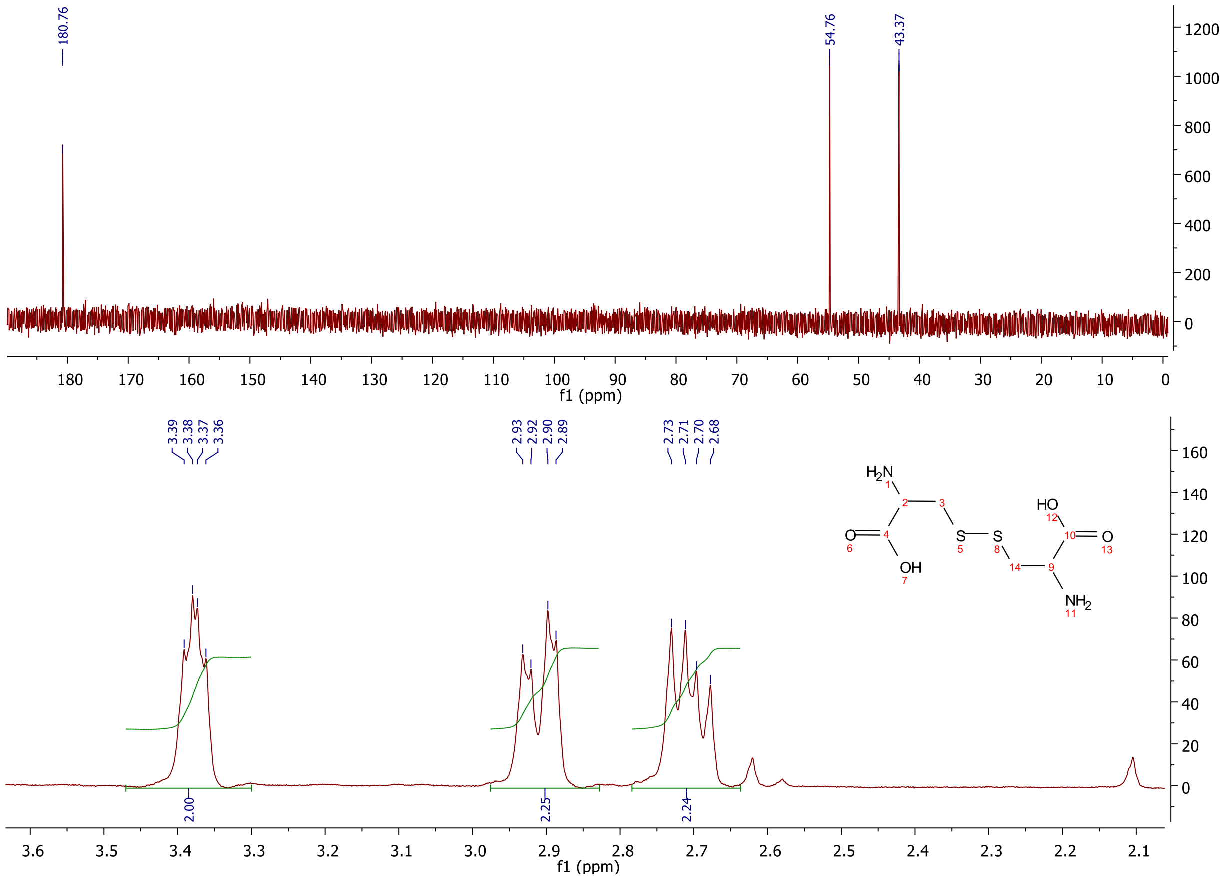


Figure 12 ^{1}H NMR and ^{13}C NMR spectra of cystine.

The general sulfonation procedure was applied to serine in order to test our hypothesis that the free thiol was not compatible with the reaction conditions. This reaction produced the expected sulfonamide, **2b**. The ^{1}H NMR and ^{13}C NMR spectra demonstrate that the target compound **2b** was achieved. The ^{1}H NMR spectrum shows a doublet at 7.90 ppm arising from the sulfonamide proton (position nine). The aromatic protons appeared as two doublets at 7.65 ppm and 7.35 ppm. The multiplet at 3.42-3.50 ppm could be the α-proton and the two diastereotopic protons (position 10 and 12). The singlet integrated to three at 2.34 ppm is the methyl-group at the benzene ring (position 7). The ^{13}C NMR spectra of **2b** show a peak at 171.76 ppm, belonging to the carbonyl carbon (C11). The aromatic carbons are located at 140.00 ppm, 138.66 ppm, 129.83 ppm, and 126.98 ppm. The two peaks at 62.48 ppm and 58.41 ppm belong to C10 and C12, respectively. The last peak at 21.41 ppm has arisen from C7. MS analysis verified that compound **2b** was prepared (see appendix).

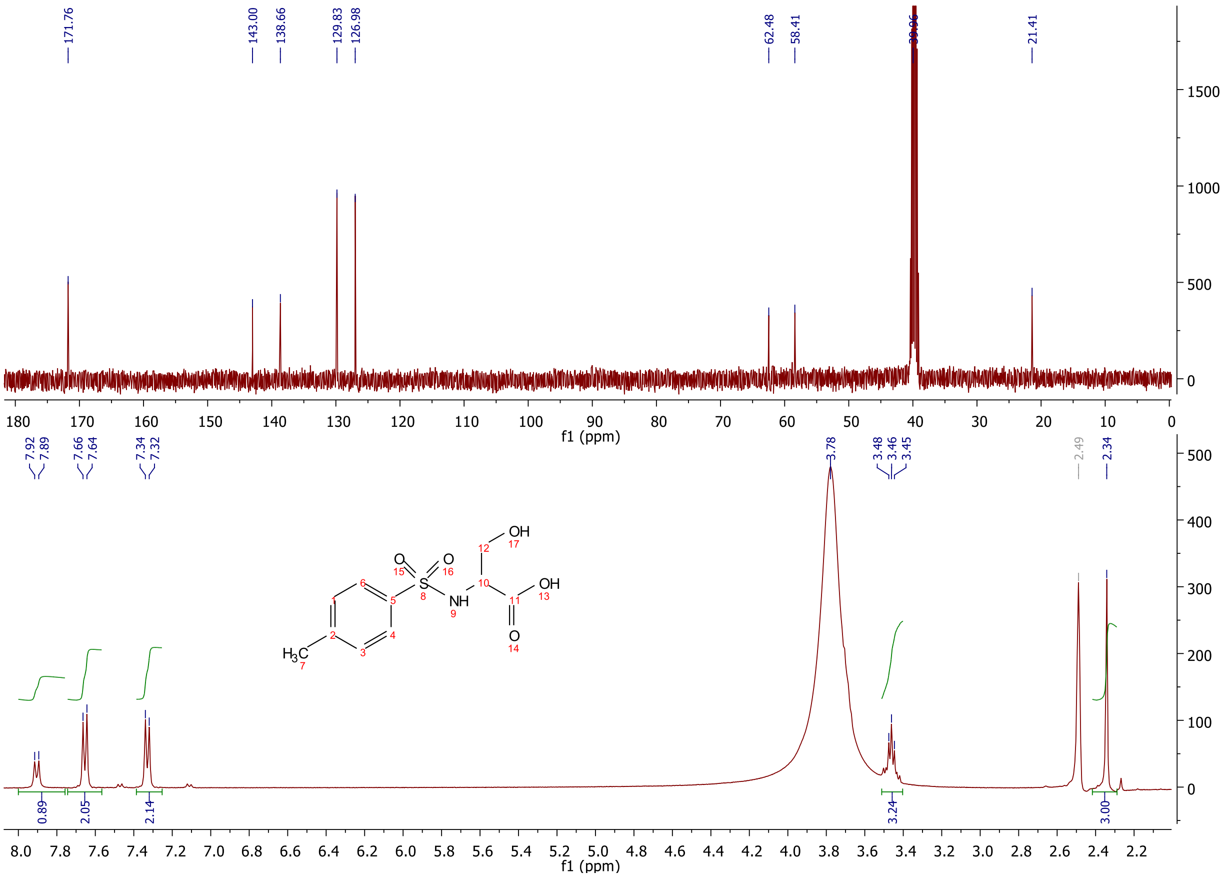


Figure 13 The ^{1}H NMR and ^{13}C NMR spectra of 2b.

The successful sulfonation of serine indicated the need for protection of the thiol group. An acetamidomethyl-protected cysteine was reacted under the same conditions as serine to give the *S*-protected derivative of **2a**. The reaction conditions were the same as in the synthesis of **2b,** but afforded a lower yield of 30 %. The ^{1}H NMR and ^{13}C NMR spectra (Figure 14) prove that the target compound was achieved. The ^{1}H NMR spectrum has two appearing doublets at 7.75 ppm and 7.34 ppm, which belong to the protons on the aromatic ring. The singlet at 4.25 ppm, belong to the two protons at position 15. The α-proton at position 9 appeared as a triplet. The two diastereotopic protons at position 13 appear as two double doublets at 2.96 ppm and 2.85 ppm. The two singlets at 2.41 ppm and 1.95 ppm has arisen from the two methyl-groups in position 22 and 18. The ^{13}C NMR spectra of protected **2a** show two peaks at 171.63 ppm and 171.62 ppm, which belong to the two carbonyl carbons C12 and C17. Further, the aromatic carbons are located at 143.30 ppm, 137.74 ppm, 129.13 ppm, and 126.85 ppm. The three peaks at 55.98 ppm, 40.74 ppm, and 33.53 ppm can not be as easily assigned without further analysis, but they belong to C9, C13 and C15. The two peaks at 21.20 ppm and 20.05 ppm belong to C18 and C22, but can not be differentiated only by using this spectrum.

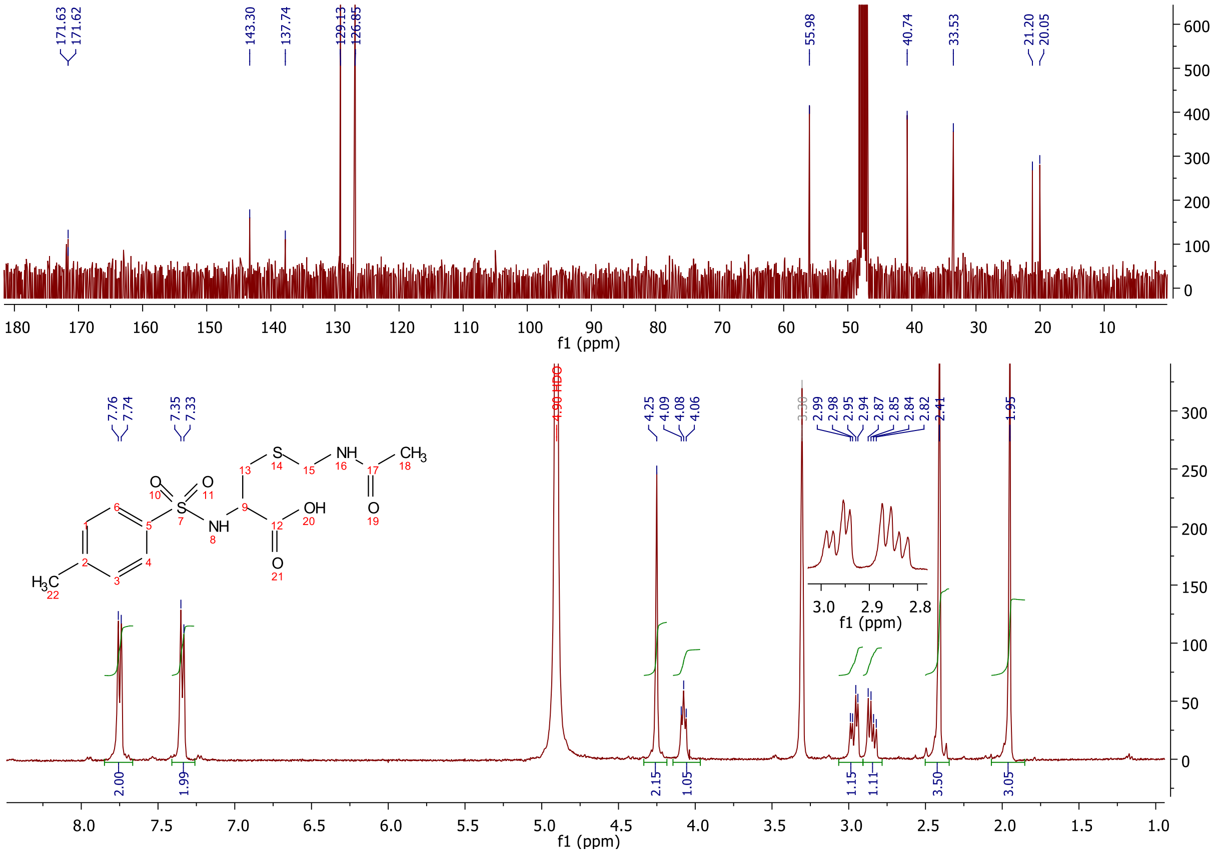


Figure 14 ^{1}H NMR and ^{13}C NMR spectra of 2a.

3.3 Attempts towards 2-alkoxy derivatives of cysteine by ring opening of epoxide with sulfur nucleophiles

3.3.1 Ring opening with thioacetate nucleophiles

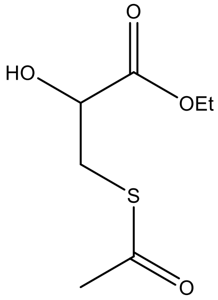


Figure 15 Chemical structure of ethyl 3-(acetylthio)-2-hydroxypropanoate, 3a.

Using KSAc as a nuclophile for ring opening of an epoxide has not been reported for structures similar to ethyl glycidate in literature. This particular protection group for the thiol is of special interest because of its ability to be hydrolyzed under the same conditions as the ester. Many different approaches were tried. The first reaction was conducted by mixing KSAc in DMF before adding the solution slowly to a mixture of ethyl glycidate in DMF. The reaction mixture was left in room temperature with stirring for two hours. This was not successful, and variations in the reaction conditions with factors such as temperature, amount of Lewis acid, addition of crown ether, and the addition speed of KSAc (in DMF) to the reaction mixture, was applied. BF_3*Et_2O and 18-crown-6 was used to catalyze the reaction, and DMF was used as solvent. This lead to a successful reaction with achievement of the target compound **3a**. In this reaction the ethyl glycidate, BF_3*Et_2O and 18-crown-6 was solved in DMF before the KSAc in DMF was added slowly and left at room temperature with stirring for two hours. A fractional factorial design was employed to get a feeling for the reaction. It underlined the successful reaction with indications it was important to carry out the reaction at 0° C, with stociometric amount BF_3*Et_2O, and slow addition of the KSAc solution. During work-up, the product, **3a**, decomposed rapidly and was impossible to isolate and store, even at low temperature, as a pure compound. Tanabe et al[32] have reported that acetylthiohydrin is unstable due to the weakness of the acetyl-sulfur bond adjacent to the hydroxy group.

An attempt to trap the β-hydroxythioacetate with an acid chloride/ benzyl bromide before work-up was unsuccessful. MS and NMR analysis did not indicate that the target compound had been formed. In literature, trapping of an acetylthiohydrin has not been reported, only trapping of a β-hydroxythiocyanate has been reported by Łukowska-Chojnacka et al[48] (2011).

HSAc was also tested as nucleophile. The ^1H NMR and ^{13}C NMR spectra showed only unreacted start material, which was not surprising considering its poor nucleophilic abilities.

3.3.2 Ring opening with thiocyanate nucleophiles

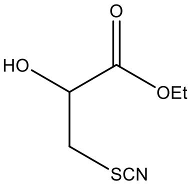


Figure 16 Chemical structure of ethyl 2-hydroxy-3-thiocyanatopropanoate, 3b.

Two thiocyanate nucleophiles were used; KSCN and TMSNCS. It was executed by dissolving KSCN in DMF before adding in to a solution of ethyl glycidate and 18-crown-6 in DMF. The ^{1}H NMR and ^{13}C NMR and MS analysis showed no conversion of the epoxide to the ethyl 2-hydroxy-3-thiocyanatopropanoate.

Tanabe et al[32] had reported a mild, effective and regioselective ring opening of oxiranes using TBAF as catalyst. The article gave a general procedure for ring opening suggesting that the TBAF and TMSNCS should be added successively to a stirred solution of the epoxide in DMF at room temperature, before heating the reaction mixture to 50°C for three hours. Additionally, for methyl 3-methyloxirane-2-carboxylate as substrate more specific reaction conditions were reported asking for benzene as solvent and increased reaction time (24 hours). Since ethyl glycidate is very similar to methyl 3-methyloxirane-2-carboxylate both approaches were tested. The application of TMSNCS was successful. Both reactions gave a mixture of two isomers (see Figure 17), but the general procedure gave the best result with respect to the purity and isomer ratio. The ethyl 2-hydroxy-3-thiocyanatopropanoate was highly unstable, and due to these stability difficulties the yield was not obtainable. The ethyl 2-hydroxy-3-thiocyanatopropanoate could not be stored in room temperature or over a period of time because of its degradation to its corresponding thiirane (see Figure 18).

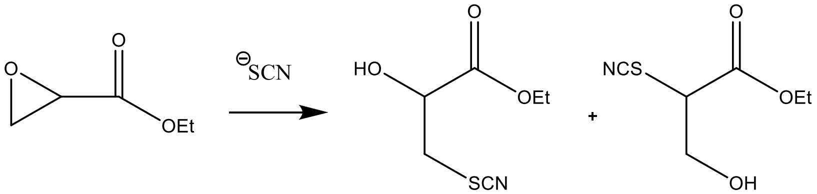


Figure 17 Chemical structure of the two isomers formed during ring opening of epoxide with TMSNCS.

After a silica gel flash chromatography had been employed the desired region-isomer was less contaminated with the unwanted region-isomer, but it was impossible to fully separate the isomers.

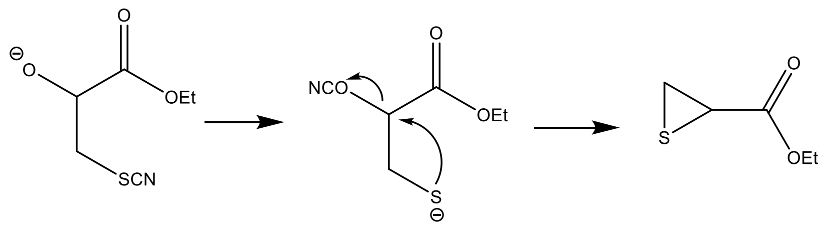


Figure 18 Reaction mechanism for the formation of thiirane.

^{1}H NMR and ^{13}C NMR analysis indicates the presence of two isomers. The following interpretation of the ^{1}H NMR spectrum will be given for the compound **3b**, and not the isomer ethyl 3-hydroxy-2-thiocyanatopropanoate. The appearing triplet at 4.24 belongs to the proton in position 1. The multiplet at 3.97-4.06 ppm is integrated to two, and belongs to the protons at position 7. The two double doublets at 3.13 ppm and 3.00 ppm belong to the two protons in position 4. The triplet at 1.03 ppm arises from the three protons in position 9. The ^{13}C NMR spectrum can not as easily be interpreted. Using a spectrum with a larger isomer ratio, the peaks most that most likely belong to **3b** are the following: 171.40 ppm (C4), 111.83 ppm (C10), 69.00 ppm (C1), 62.04 ppm (C7), 37.92 ppm (C2), 14.04 ppm (C8).

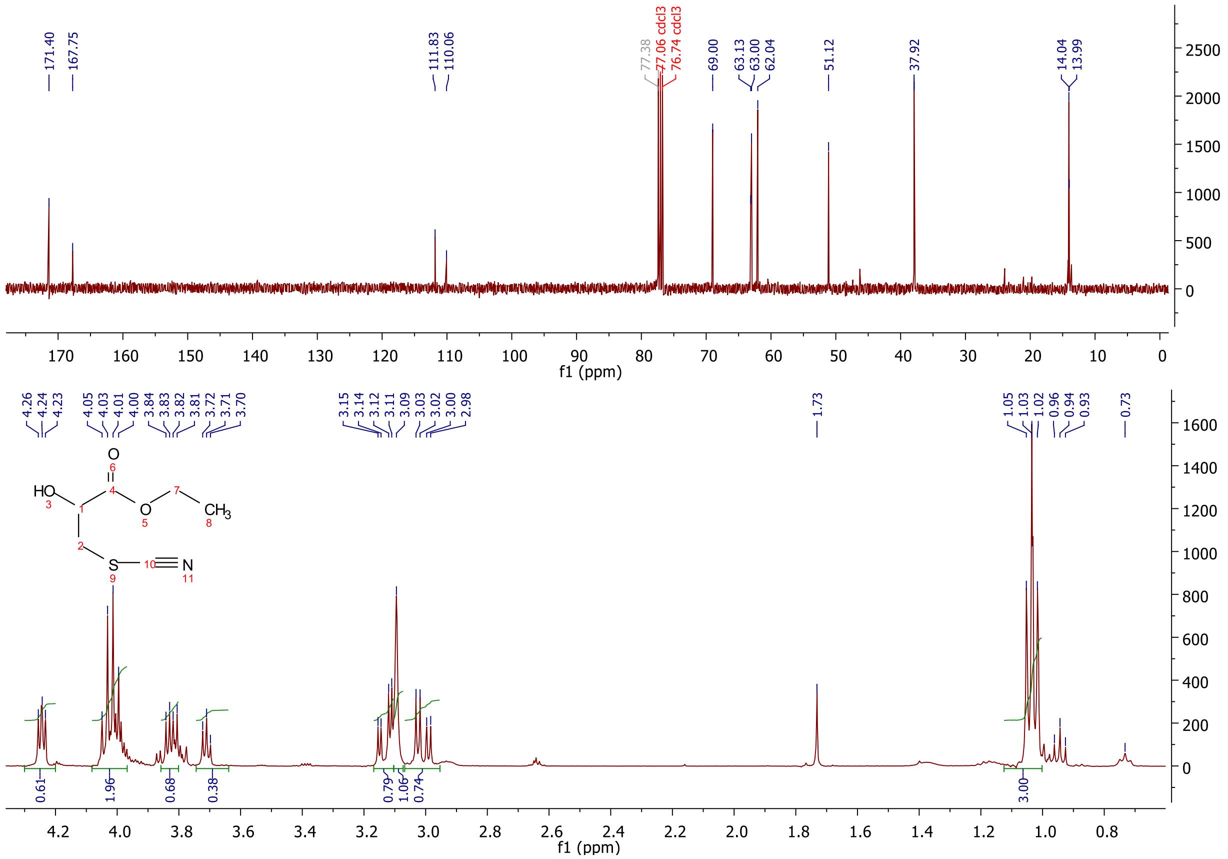


Figure 19 ^{1}H NMR and ^{13}C NMR spectra for the ring opening of ethyl glycidate with TMSNCS.

Two different approaches of the nuclophile were possible: $^{-}$NCS or $^{-}$SCN. To determine which of these two nucleophiles had reacted, the ^{13}C NMR spectrum was crucial. S-CN gives a signal at approximately 112 ppm, while N=C=S gives signal a broad signal at 130 ppm[49]. The signals from the ^{13}C NMR spectrum for the ring opening of ethyl glycidate with TMSNCS are at 111.83 ppm, 110.06 ppm. This indicates clearly that the wanted S-CN product was obtained.

3.3.3 Different strategy

A different strategy that was utilized was to make the ethyl ester of glycolic acid and then make the ether with benzyl bromide. However, the Williamson ether synthesis of the glycolic ester and benzyl bromide yielded benzyl ethyl ether, and the strategy was therefore not pursued further.

3.3.4 Starting material; ethyl glycidate

The starting material ethyl glycidate was made by reacting serine with KBr/HBr and $NaNO_2$ using water as solvent. The crude oil from the first step was reacted with base in absolute ethanol giving the epoxide salt. The epoxide salt was reacted with EtBr in DCM to give the ethyl glycidate. First time the reaction was run the results were according to literature. Second time around however, the end result was contaminated with a byproduct, in a 2:1 relationship, and suspected formation of polymers since the yield was very low. The distillation gave no more than approx. 4 grams, and it should have yielded 18 grams. The reason for the differences in yield and purity has no obvious explanation.

4 Future Outlook

4.1 Reductive amination

In an attempt to improve yields in this reaction, the use of not only catalytic amount of HCl but stociometric amount of glacial acetic acid could be interesting approach as it has been described.[18] Molecular sieves (3 Å) [22] or azeotropic removal of water should also be considered as a mean to drive the equilibrium in the imine formation in the desired direction.

To utilize the methyl ester of cysteine in the reductive amination with $NaBH_3CN$ would be an interesting approach to investigate the suspicion of the formation of the zwitter ion causing the low yields in this reaction.

Lowering of the pH with HCl to the isoelectric point upon the completion of the reduction has been reported to cause the product to precipitate in methanol and should be considered as a way to isolate the product[18b].

4.2 Sulfonylation of amino acids

The next step in the sulfonation of amino acids would be to remove the acetamidomethyl-protection group on cysteine in product **2a** with $MeSiCl_3$, Ph_2SO, TFA at 4°C for 30 minutes. Other protection groups such as S-CN or S-Ac would be interesting to test, given that they would be easier to detach. Another interesting suggestion is by reacting the appropriate sulfonyl chloride with serine (as done) and then perform a Mitsunobu on the alcohol with a sulfur nucleophile with an appropriate protection and then cleave the protection group to generate the target compound.

4.3 Ring opening of epoxides with thio nucleophiles

The last step in the synthesis of the target ethers is performing a Mitsunobu with an alcohol, such as 3-phenyl propan-1-ol, 2-phenylethanol and phenylmethanol, on the betahydroxythiocyanate alcohol after the ring opening with a thio nuclophile. This must be preformed right after the ring opening due to the short lifetime of the beta-hydroxythiocyanate. After the Mitsunobu reaction, cleavage of the S-CN protection group is desirable.

5 Conclusion

In the reductive amination with $NaBH_3CN$ three target amines (**1a-1c)** was obtained. The yields were very low; less than 5 % except for one reaction with 11 %. The solubility was poor which can pose a challenge in the testing for inhibitor activity. **1a** and **1b** can be tested for inhibitory activity without further purification; **1c** needs to be purified before testing can be conducted.

Two sulfonamide compounds, protected **2a** and **2b,** were prepared. Sulfonation of serine with *para*-toluene sulfonyl chloride gave **2b** in good yields (85%). Acetamidomethyl-protected cysteine gave the protected target compound **2a** in acceptable yield (30%). Even though it has not been deprotected, it is interesting to test the compound for inhibitor activity. **2b** will also be tested for inhibitor activity.

The ring opening of ethyl glycidate with KSAc and TMSNCS was successful, and gave the two compounds 3a and 3b. Isolation of the products was possible, but due to their instability it was not possible to obtain yields or store 3a and 3b over time. These two compounds were difficult to work with, and in order to reach the ultimate goal of synthesizing 2-substituted-3-mercaptopropanoic acids ethers there are one step left. The Mitsunobu reaction should be performed in order to obtain the target ethers.

Three amines and two sulfonamides were prepared for VIM-2 and VIM-7 inhibitor testing.

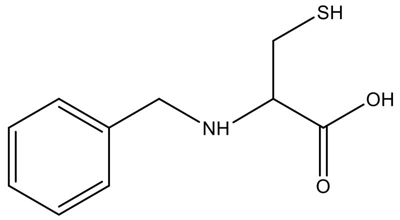
6 Experimental section

The solvents and reagents were purchased from commercial suppliers and used without further purification (except from *para*-toluene sulfonyl chloride). The reactions monitored by TLC was on run on 60 F_{254} silica gel plates and visualized with UV light and staines. Column chromatography was performed on silica gel (35-70 mesh) purchased from Merck. The IR spectra were recorded on a Varian 7000e FT-IR spectrometer. Mass spectra were recorded on a LTQ Orbitrap XL in a positive and negative Electron Spray Ionization mode. The samples were run in methanol with some basic water in it. The ^{1}H NMR and ^{13}C NMR were recorded on a Varian Mercury 400 MHz at room temperature. Significant ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, dublet; dd, double dublet; t, triplet; m, multiplet) and number of protons.

6.1 Reductive amination

6.1.1 General procedure:

The aldehyde (1.5 eq) was added to a suspension of cysteine (1.0 eq) and $NaBH_3CN$ (1.0 eq) in methanol (5 ml per 2 mmol) at room temperature and stirred for 22-26 hours. A white precipitate formed and was filtered of and washed with methanol. During the reaction, the evolving gas was bubbled through a mixture of 6 ml household chlorine bleach (a solution of approximately 3-6 % sodium hypochlorite, NaClO) and 5 ml 10 % NaOH.



2-(benzylamino)-3-mercaptopropionic acid **(1a):**

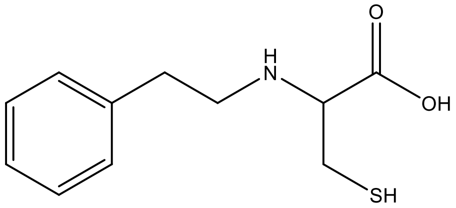
Benzaldehyde (1.5 eq, 3.1 mmol, 0.33 g) and cysteine (1.0 eq, 2.1 mmol, 0.25 g) gave **1a** (0.084 mmol, 0.02 g, 4.0 %) as a white solid.

High-resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{10}H_{13}NO_2S$, 212.0740; found 212.0736 (100 %). $(M+Na)^+$: calculated for $C_{10}H_{13}NO_2SNa$, 234.0559; found 234.0554 (40 %).

^{1}H NMR (D_2O with NaOH, 400 MHz): δ 7.46 – 7.01 (m, 5H), 3.62 (d, *J* = 12.7 Hz, 1H), 3.47 (d, *J* = 12.7 Hz, 1H), 2.90 (dd, *J* = 8.3, 5.0 Hz, 1H), 2.64 (dd, *J* = 12.6, 5.0 Hz, 1H), 2.45 (dd, *J* = 12.7, 8.4 Hz, 1H).

^{13}C NMR (D_2O with NaOH, 400 MHz): δ 181.7, 128.6, 128.5, 127.1, 125.8, 67.8, 51.2, 28.4.

IR: 3027 cm^{-1}, 1606 cm^{-1}



3-mercapto-2-(phenethylamino)propionic acid (**1b**):

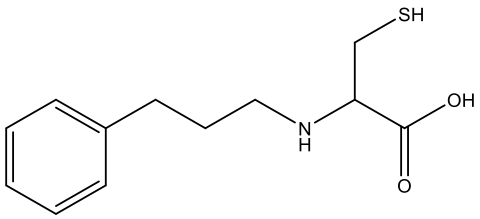
Phenylacetaldehyde (1.5 eq, 3.1 mmol, 0.37 g) and cysteine (1.0 eq, 2.1 mmol, 0.25 g) gave **1b** (0.098 mmol, 0.02 g, 4.7 % (11.4 %)) as a white solid.

High-resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{11}H_{15}NO_2S$, 226.0896; found 226.0893 (100 %). $(M+Na)^+$: calculated for $C_{11}H_{15}NO_2S$ Na, 248.0716; found 248.0714 (20 %).

^{1}H NMR (D_2O with NaOH, 400 MHz): δ 7.19 (m, 5H), 2.86 (dd, *J* = 8.0, 5.4 Hz, 1H), 2.67 (t, *J* = 4.3 Hz, 4H), 2.58 (dd, *J* = 12.5, 5.3 Hz, 2H), 2.41 (dd, *J* = 12.5, 8.0 Hz, 1H).

^{13}C NMR (D_2O with NaOH, 400 MHz): δ 181.84, 140.31, 128.81, 128.63, 126.24, 68.60, 48.87, 35.11, 28.19.

IR: 2803 cm^{-1}, 1581 cm^{-1}



3-Mercapto-2-(3-phenylpropylamino)propionic acid (**1c**):

3-Phenyl-propionaldehyde (1.5 eq, 3.1 mmol, 0.42 g) and cysteine (1.0 eq, 2.1 mmol, 0.25 g) gave **1c** (0.095 mmol, 0.02 g, 4.5 %) as a white solid.

High-resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{12}H_{18}NO_2S$, 240.1052; found 240.1053 (100 %). $(M+Na)^+$: calculated for $C_{12}H_{18}NO_2SNa$, 262.0872; found 262.0871 (3%).

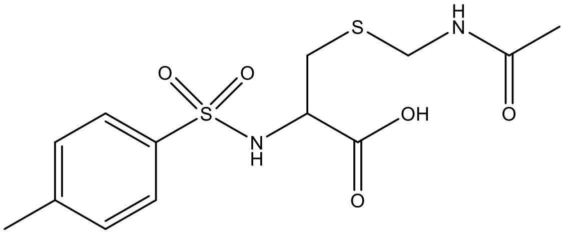
^{1}H NMR (D_2O with NaOH, 400 MHz): δ 7.43 – 7.02 (m, 5H), 2.85 (dd, *J* = 8.0, 5.1 Hz, 1H), 2.62 (dd, *J* = 12.6, 5.1 Hz, 1H), 2.55 (t, *J* = 7.7 Hz, 2H), 2.45 (m, 3H), 1.69 (m, 2H).

^{13}C NMR (D_2O with NaOH, 400 MHz): δ 182.28, 177.15, 133.69, 128.56, 128.51, 125.78, 68.53, 60.29, 46.89, 46.87, 46.78, 32.87, 31.92, 28.27.
IR: 2973 cm^{-1}, 1587 cm^{-1}

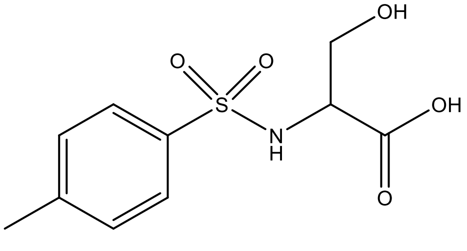
6.2 Sulfonation of amino acids

6.2.1 General procedure:

The sulfonyl chloride (1 eq) was added to a solution of the amino acid (1.2 eq) in aqueous K_2CO_3 (2-3 eq). Under vigorous stirring, the mixture was heated to 70° C for 30 min, cooled in an icebath, and acidified (HCl) to pH 2.5 by dropwise addition of concentrated HCl under stirring. The precipitate was collected by filtration and washed with a minimum of cold water.



3-(acetamidomethylthio)-2-(4-methylphenylsulfonamido)propanoic acid (**2a**):
Para-toluene sulfonyl chloride (1.0 eq, 0.84 mmol, 0.15 g) and S-acetamidomethyl-L-cysteine*HCl (1.2 eq, 1.1 mmol, 0.25 g) gave **2a** (0.25 g, 0.72 mmol, 86 %)
High-resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{13}H_{19}N_2O_5S_2$, 347.0730; found 347.0732 (37%). $(M+Na)^+$: calculated for $C_{13}H_{19}N_2O_5S_2Na$, 369.0549; found 369.0549 (100%). $(M+K)^+$: calculated for $C_{13}H_{19}N_2O_5S_2K$, 385.0289; found 385.0286 (75%).
^{1}H NMR (CD_3OD, 400 MHz): δ 7.75 (d, J = 7.6 Hz, 2H), 7.34 (d, J = 7.8 Hz, 2H), 4.25 (s, 2H), 4.08 (t, J = 6.4 Hz, 1H), 2.96 (dd, J = 14.0, 5.5 Hz, 1H), 2.85 (dd, J = 13.9, 7.3 Hz, 1H), 2.41 (s, 3H), 1.95 (s, 3H).
^{13}C NMR ($CDCl_3$, 400 MHz): δ 171.63, 171.62, 143.30, 137.74, 129.13, 126.85, 55.98, 40.74, 33.53, 21.20, 20.05.
IR: 3402 cm^{-1}, 3280 cm^{-1}, 2959 cm^{-1}, 1732 cm^{-1}



3-hydroxy-2-(4-methylphenylsulfonamido)propanoic acid (**2b**):

Para-toluene sulfonyl chloride (1.0 eq, 2.5 mmol, 0.48 g) and serine (1.2 eq, 3.0 mmol, 0.32 g) gave **2b** (1.08 g, 4.4 mmol, 85 %).

High-resolution mass spectroscopy (ESI): $(M+K)^+$: calculated for $C_{10}H_{13}NO_5SK$, 298.0146, found 298.0149 (100%).$(M-H)^-$: calculated for $C_{10}H_{12}NO_5S$, 258.0.442; found 258.2 (100 %).

^{1}H NMR (DMSO, 400 MHz): δ 7.90 (d, J = 8.5 Hz, 1H), 7.65 (d, J = 8 Hz 2H), 7.33 (d, J = 8.0 Hz, 2H), 3.46 (m, 3H), 2.34 (s, 3H).

^{13}C NMR (DMSO, 400 MHz): δ 171.76, 143.00, 138.66, 129.83, 126.98, 62.48, 58.41, 39.96, 21.41, 3.78.

IR: 2916 cm^{-1}, 1579 cm^{-1}

6.2.2 Dimerization reaction

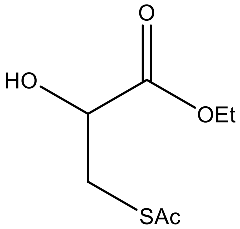
Methanesulfonyl chloride (1.0 eq, 5.0 mmol, 0.57 g) and cysteine (1.2 eq, 6.0 mmol, 0.73 g) gave cystine.

^{1}H NMR (D_2O, 400 MHz): δ 3.45 – 3.26 (m, 2H), 2.91 (dd, J = 13.6, 4.4 Hz, 2H), 2.70 (dd, J = 13.6, 7.6 Hz, 2H).

^{13}C NMR (D_2O, 400 MHz): δ 180.76, 54.76, 43.37.

6.3 Ring opening of epoxide with sulfur nucleophiles

6.3.1 Representative procedure for ring opening of epoxides with KSAc



Ethyl 3-(acetylthio)-2-hydroxypropanoate (**3a**):

The ethyl glycidate (1.0 eq, 4.3 mmol, 0.5 g) was diluted in DMF (9 ml) and the 18-crown-6 ether (0.1 eq, 0.43 mmol, 0.11 g) and BF_3*Et_2O (1.05 eq, 4.5 mmol, 0.64 g) was added. KSAc (1.25 eq, 5.4 mmol, 0.61 g) in DMF (11 ml) was slowly added and left with stirring for 2 hours. The solution was hydrolyzed with ice-water, and extracted with EtOAc. The combined EtOAc extracts were shaken with brine, dried ($NaSO_4$) and concentrated.

High-resolution mass spectroscopy (ESI): $(M+Na)^+$: calculated for $C_7H_{12}O_4SNa$, 215.0349: found 215.0346.

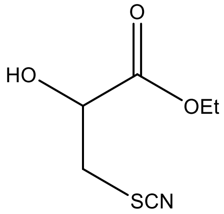
^1H NMR ($CDCl_3$, 400 MHz): δ 4.34 (dd, *J* = 6.3, 4.9 Hz, 1H), 4.17-4.27 (m, 4H), 3.97 – 3.81 (m, 1H), 3.37 (dd, *J* = 13.7, 4.7 Hz, 1H), 3.24 (dd, *J* = 13.8, 6.5 Hz, 1H), 2.37 (d, *J* = 4.2 Hz, 2H), 2.35 (s, 3H), 2.13 (s, 1H), 1.39 – 1.16 (m, 14H).

^{13}C NMR ($CDCl_3$, 400 MHz): δ 194.19, 172.04, 170.38, 162.17, 70.46, 69.24, 62.05, 60.98, 59.79, 47.83, 46.80, 45.69, 35.95, 32.77, 30.81, 29.91, 29.29, 20.43, 19.92, 13.74.

Table 1 overview of the ring opening reactions with KSAc as nucleophile

	catalytic BF_3*Et_2O	stociometric BF_3*Et_2O	time	RT	0° C	18-crown-6	fast addition of KSAc/DMF	slow addition of KSAc/DMF
1			2 h	x				x
2			4 h	x				x
3			20 h	x		x		x
4		x	2 h	x		x		x
5	X		1 h		x	x	x	
6		x	1 h		x	x		x
7		x	1 h	x		x	x	
8		x	4 h		x	x		x

6.3.2 Ring opening of epoxides with thiocyanate



Ethyl 2-hydroxy-3-thiocyanatopropanoate (**3b**):

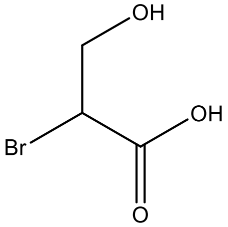
TBAF (0.02 eq, 0.08 mmol, 0.021 g) and TMSNCS (1.0 eq, 0.43 mmol, 0.53 g) were added to a stirred solution of ethyl glycidate (1.0 eq, 0.43 mmol, 0.5 g) in DMF (6+3 ml) at RT. The mixture was stirred at 50° C for 3 hours, and then diluted with water and extracted with EtOAc. The extract was washed with water and brine, dried (Na_2SO_4) and concentrated. The resulting crude oil was purifid by silica gel column chromatography (heptane. EtOAc 4:1).

High-resolution mass spectroscopy (ESI): $(M+Na)^+$: calculated for $C_6H_9NO_3SNa$, 198.0195: found 198.0194.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.54 (t, J = 4.2 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.43 (dd, J = 13.7, 4.0 Hz, 1H), 3.30 (dd, J = 13.7, 5.3 Hz, 1H), 1.33 (t, J = 7.2 Hz, 4H), 1.25 (s, 1H), 0.86 (t, J = 6.9 Hz, 1H).

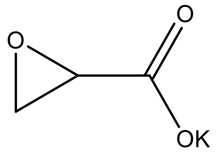
^{13}C NMR (DMSO, 400 MHz): δ 171.53, 168.33, 113.46, 111.65, 69.32, 62.45, 61.32, 61.25, 51.71, 37.88, 31.71, 28.82, 22.56, 14.42.

6.3.3 Synthesis of epoxide starting material: ethyl (RS)-2,3-epoxypropanoate[50]



(SR)-2-Bromo-3-hydroxypropanoic acid: DL-serine (1.0 eq, 0.5 mol, 52.5 g,) and KBr 3.4 eq, 1.7 mol, 200 g) was dissolved in water and HBr 48 % (2.2 eq, 1.09 mol, 123 ml,) was added at room temperature. The mixture was cooled to -13°C with stirring. Nitrogen was bubbled through the solution and sodium nitrite (1.2 eq, 0.62 mol, 42.8 g) was added in small portions (5 g every 15 minutes, total time 2.5 hours). The solution was allowed to warm to 0°C, the N_2 purge stopped,

and left with stirring for 6 hours. Excess nitrogen oxides were removed by bubbling N_2 through the solution for 1 hour. The pale green solution was extracted with 6*300 ml ether, dried over $MgSO_4$ and concentrated by rotary evaporation to give pale green oil (75.0 g, 89 %).

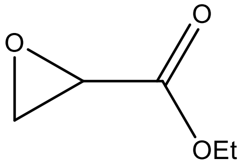


Potassium (RS)-2,3-epoxypropanoate: The crude acid (1.0 eq, 0.44 mol, 75.0 g) from the previous step was dissolved in absolute ethanol (300 ml) and cooled to -20°C. Under N_2, a solution of potassium hydroxide (1.9 eq, 0.85 mol, 55.5 g) in absolute ethanol (300 ml) was slowly added. After 1.5 hours, the mixture was allowed to warm to 0°C, and stirred for this temperature for 12 hours. The white, thick solution was filtered and concentrated, and filtered again to isolate an additional crop of salt. The combined salts were dried under vacuum to give 105 g 1:1 mixture of KBr and potassium glycidate. NMR data were in accordance with already published data.

^1H NMR (D_2O, 400 MHz): δ 3.25 (dd, $J = 4.8, 2.9$ Hz, 1H), 2.86 – 2.80 (m, 2H), 2.67 (dd, $J = 5.8, 2.8$ Hz, 2H).

^{13}C NMR (D_2O, 400 MHz): δ 176.69, 49.40, 45.96.

Crystallization: one third of the 1:1 mixture of KBr and potassium glycidate was extracted by refluxing in a mixture of ethanol (585 ml) and water (15 ml) with good stirring for 45 minutes. The hot suspension was filtered, and the glycidate crystallized. This was done with the entire batch of KBr-glycidate mixture, all in the same ethanol mother liquid. The solids collected from the three hot filtrations were combined and extracted a fourth time with the same solution, and crystallized to give additional product.



Ethyl (RS)-2,3-epoxypropanoate: A suspension of dry potassium glycidate (1.0 eq , 0.157 mol, 19.5 g of an 90:10 potassium glycidate:KBr mixture), benzyltriethylammonium chloride (1.0 eq, 0.157 mol, 38.8 g) and ethyl bromide (3.7 eq, 0.58 mol, 56.5 g) in methylene chloride (250 ml) was heated to 50°C with reflux for 16 hours with good stirring. It was left to cool, and extracted with 3*100 ml ether, filtrated of the solids and dried with $MgSO_4$. The solution was concentrated and distilled at $2.6*10^{-1}$ mbar at 32°C with cold water circulating through the condenser. The yield was 48 %, 53 % and 16 %.

^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.34 – 4.14 (m, 2H), 3.42 (dd, X of ABX, 1 H, J_{AX} = 4.1 Hz, J_{BX} = 2.5 Hz), 2.94 (B of ABX, 1 H, J_{AB} = 6.5 Hz, J_{BX} = 3.3 Hz), 2.89 (A of ABX, 1 H, J_{AB} = 6.8, J_{AX} = 4.4)1.30 (t, J = 7.2 Hz, 3H).

^{13}C NMR ($CDCl_3$, 400 MHz): δ 169.18, 61.62, 47.34, 46.23, 14.08.

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8 Appendices

8.1 Abbreviations

DCM - dichloromethane

ddd - double double dublet

DMF - dimethylformamide

Ethyl glycidate - ethyl oxirane-2-carboxylate

IMP, GIM, SPM, SIM, KHM, AIM, DIM, TMB, NDM - different types of MBLs

IR - infrared spectroscopy

MBL - metallo-β-lactamase

$MgSO_4$ - magnesium sulfate

MS - mass spectra

Na_2SO_4 - sodium sulfate

Nitrocefin (- 6R,7R)-3-[(2,4-dinitro)styryl]-8-oxo-7-[(2-thienylacetyl) amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

NMR - nuclear magnetic resonance

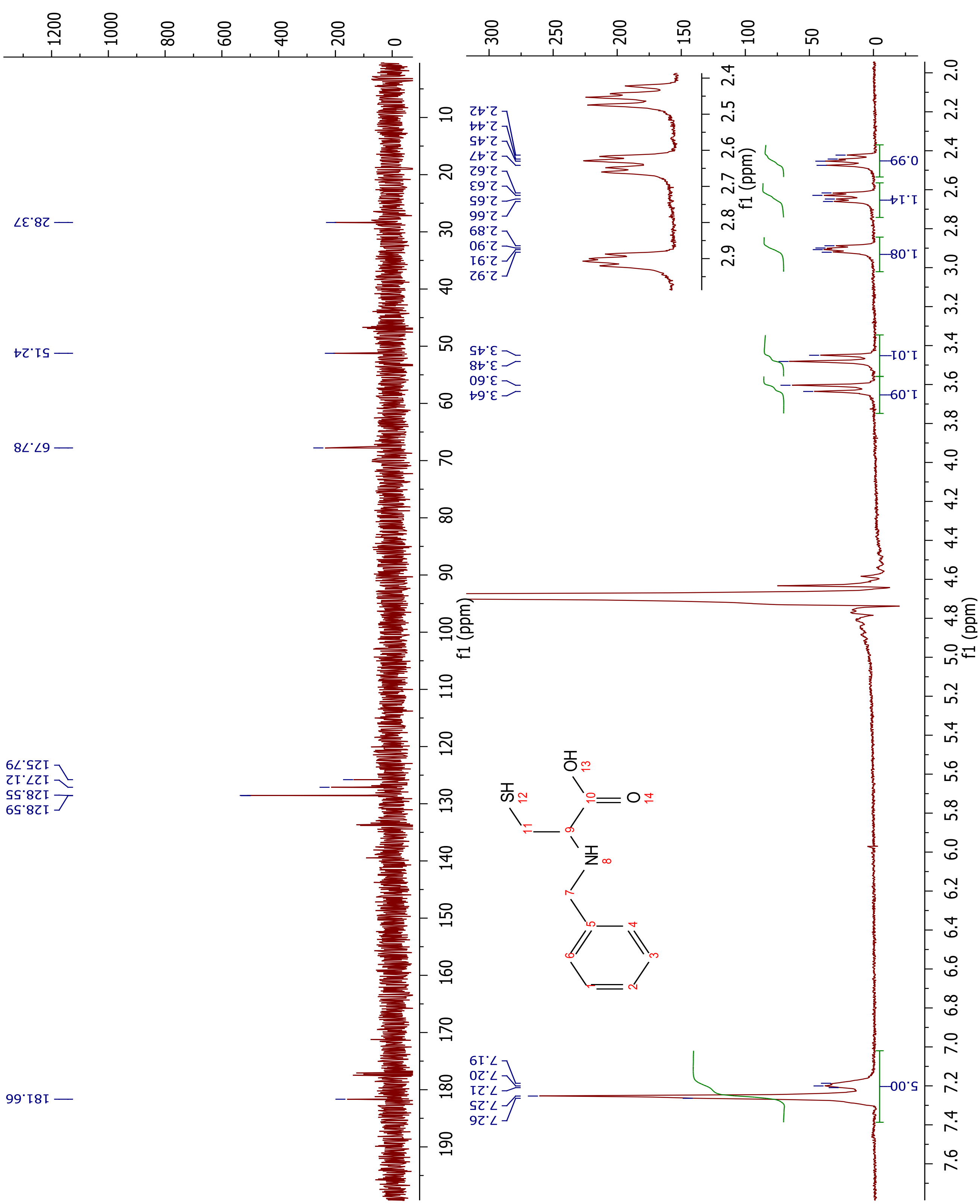
Quinoline - N-((7-chloroquinolin-4-ylamino)alkyl)-3-mercaptopropanamide

TLC - thin layer chromatography

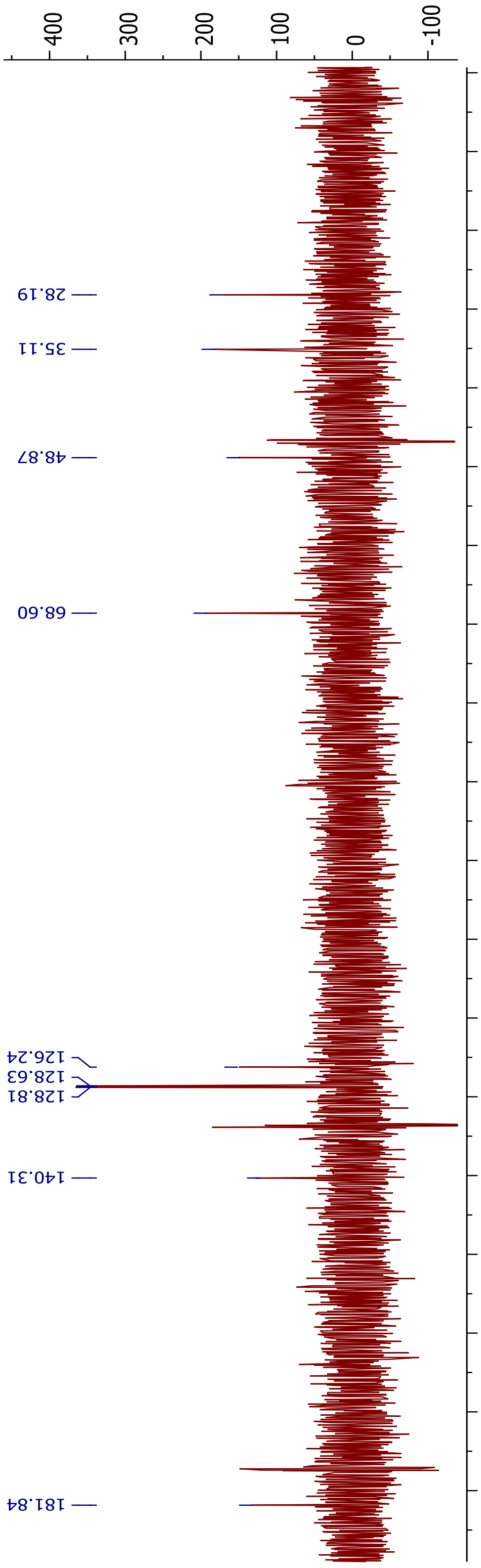
VIM - verona integron-borne metallo-β-lactamase

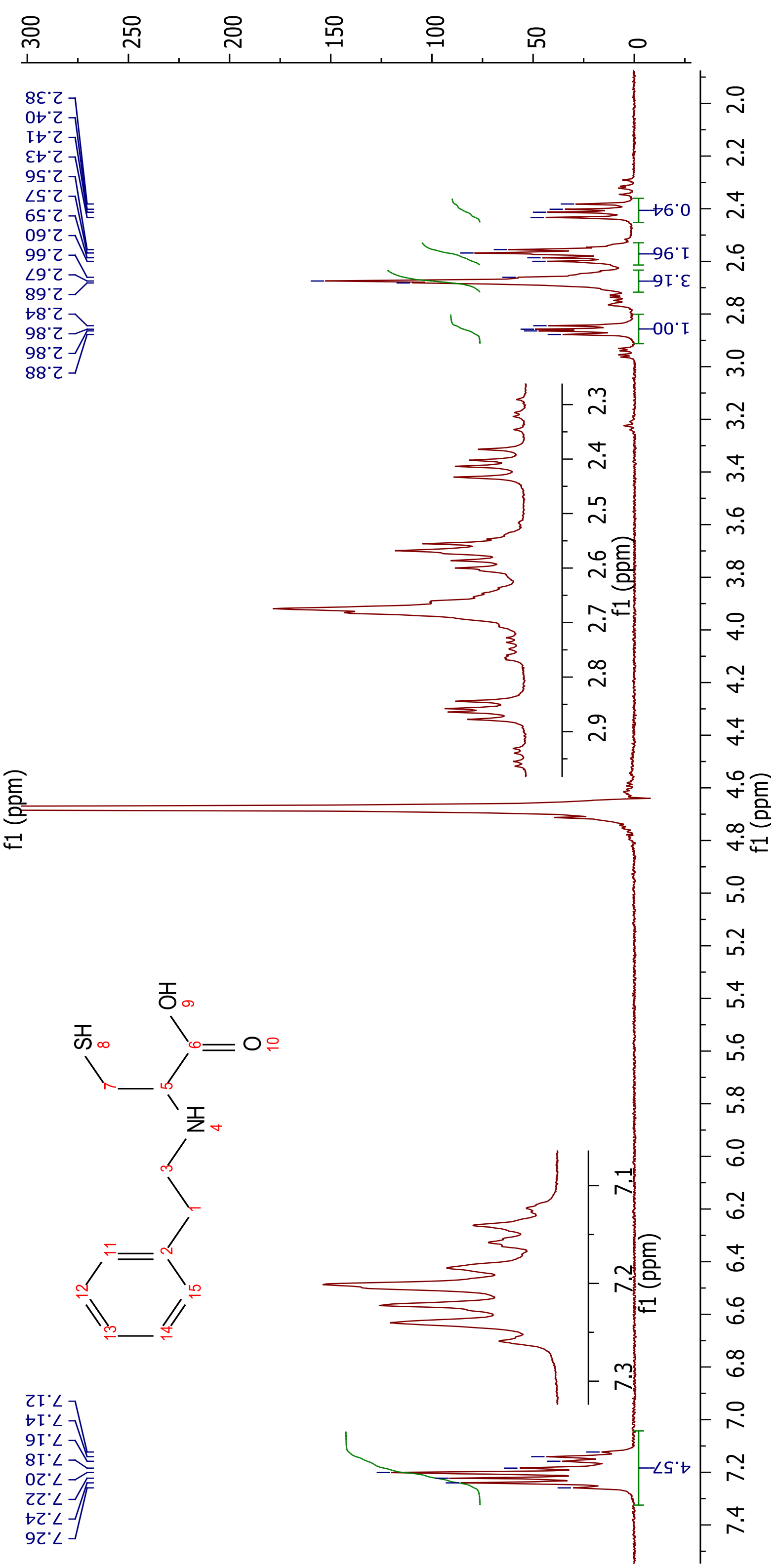
8.2 NMR spectra of 1a-1c

1a, 2-(benzylamino)-3-mercaptopropanoic acid

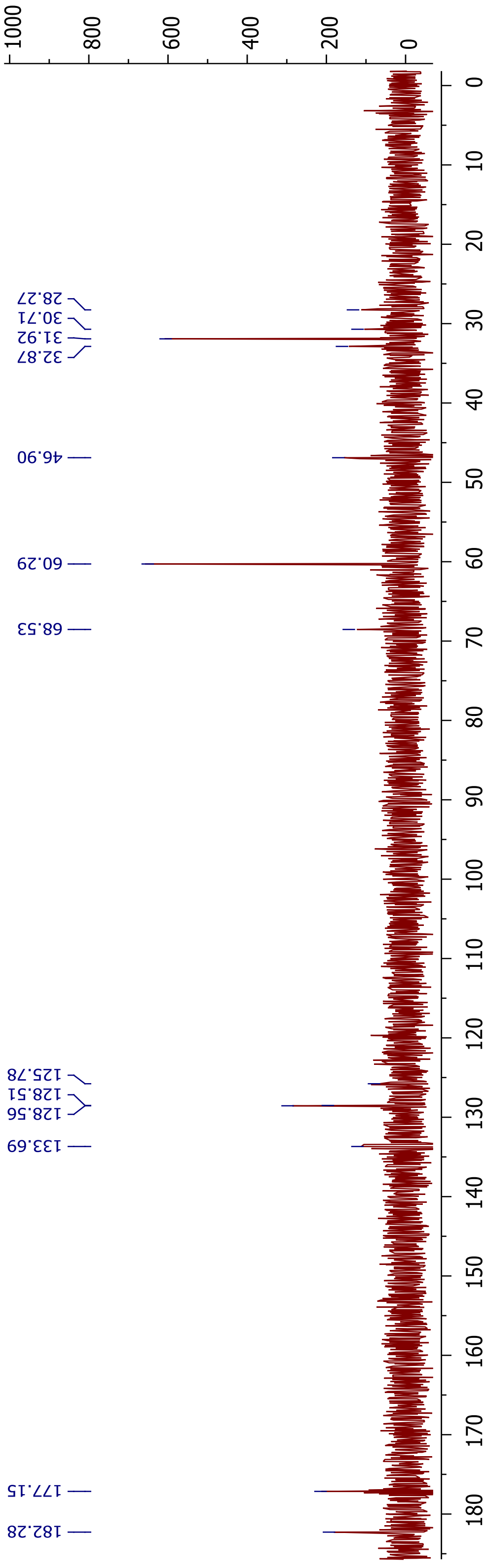


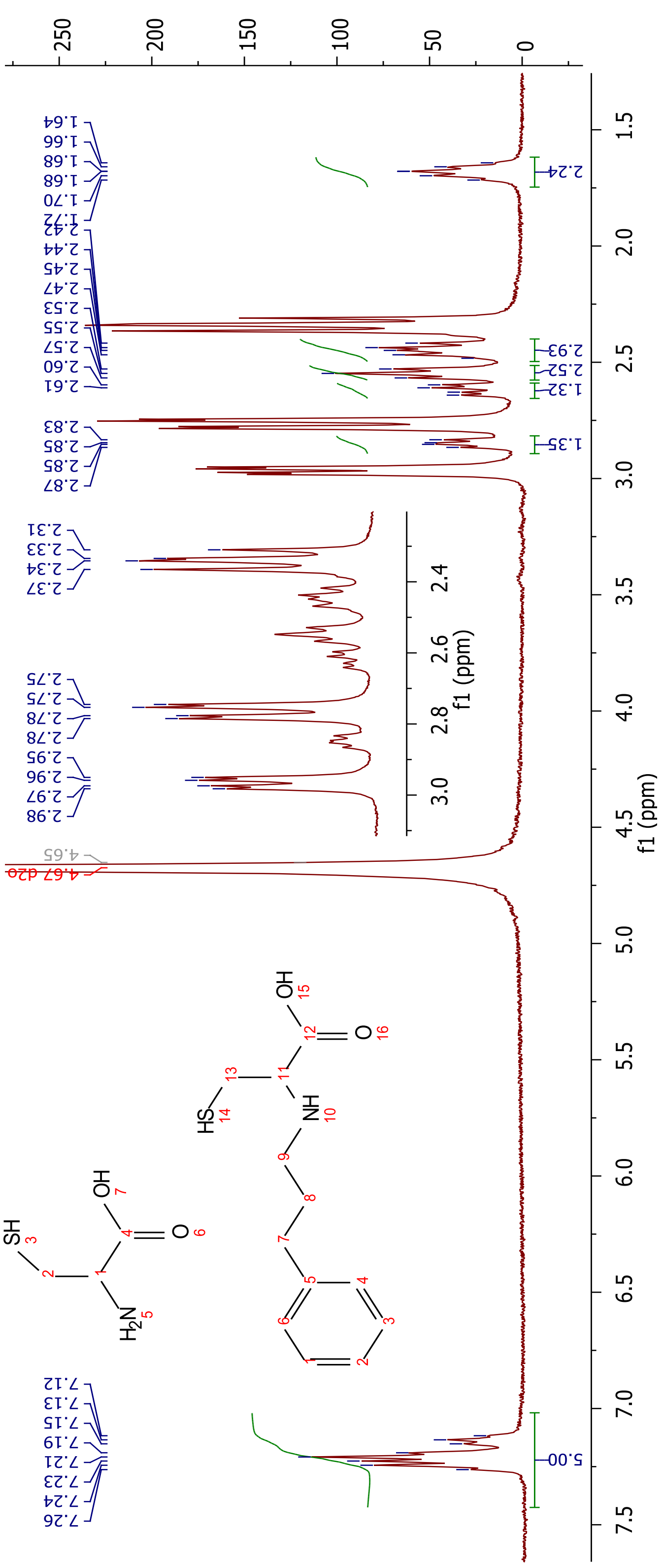
1b, 3-mercapto-2-(phenethylamino)propanoic acid





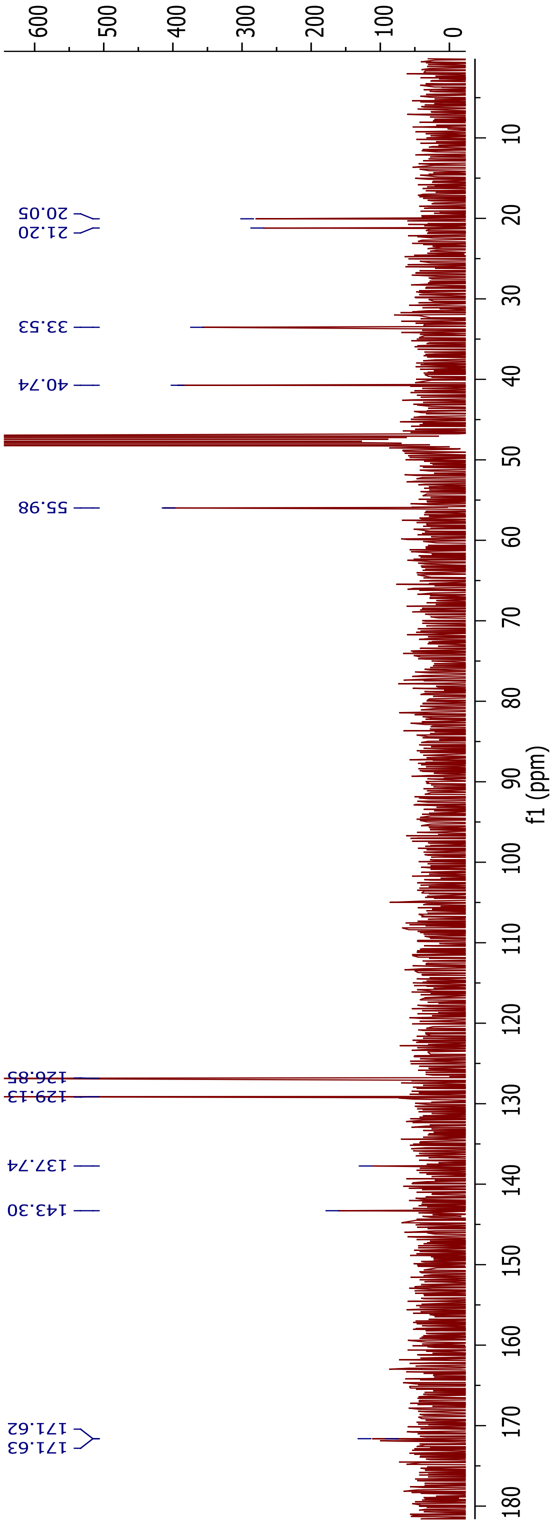
1c, 3-mercapto-2-(3-phenylpropylamino)propanoic acid

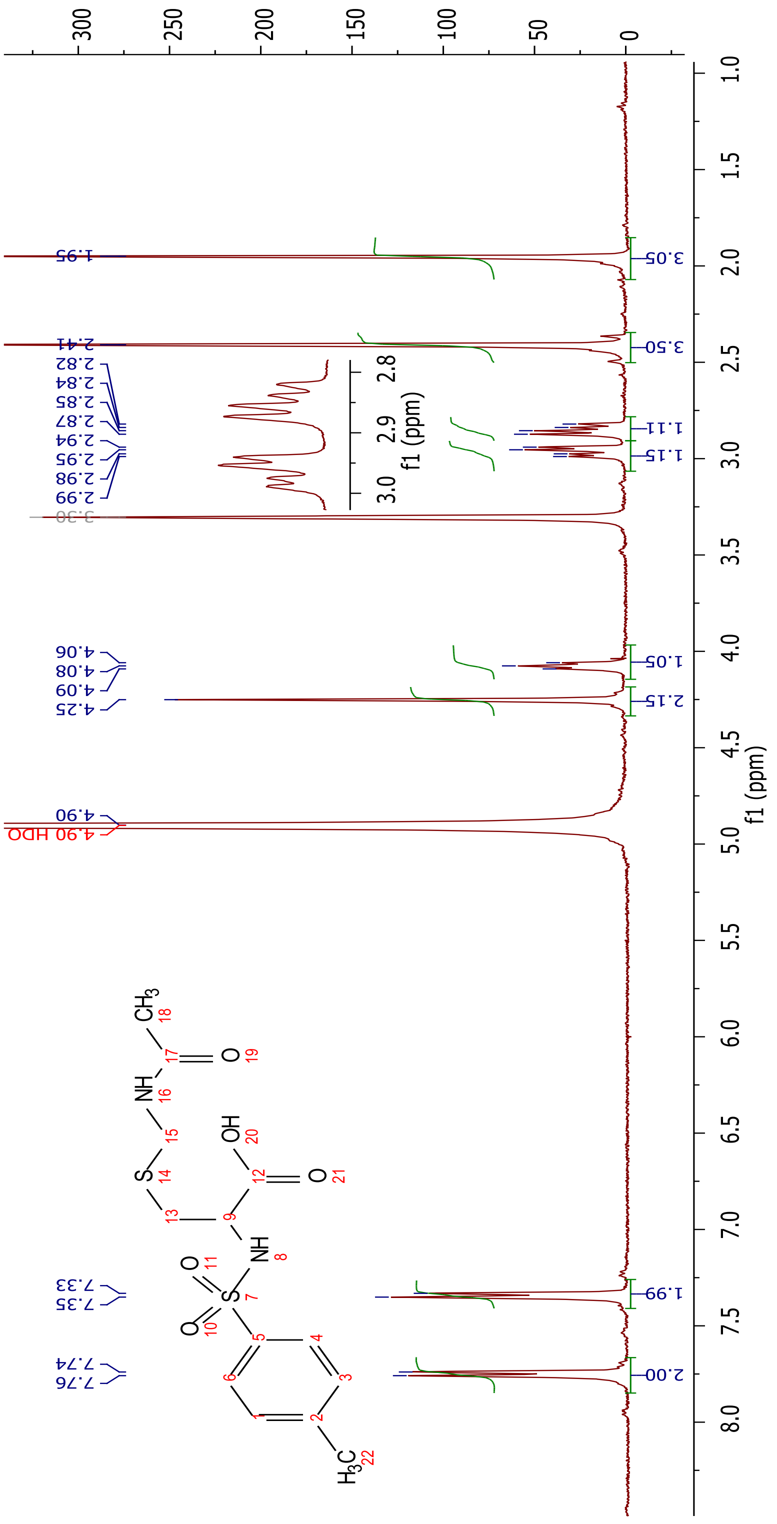




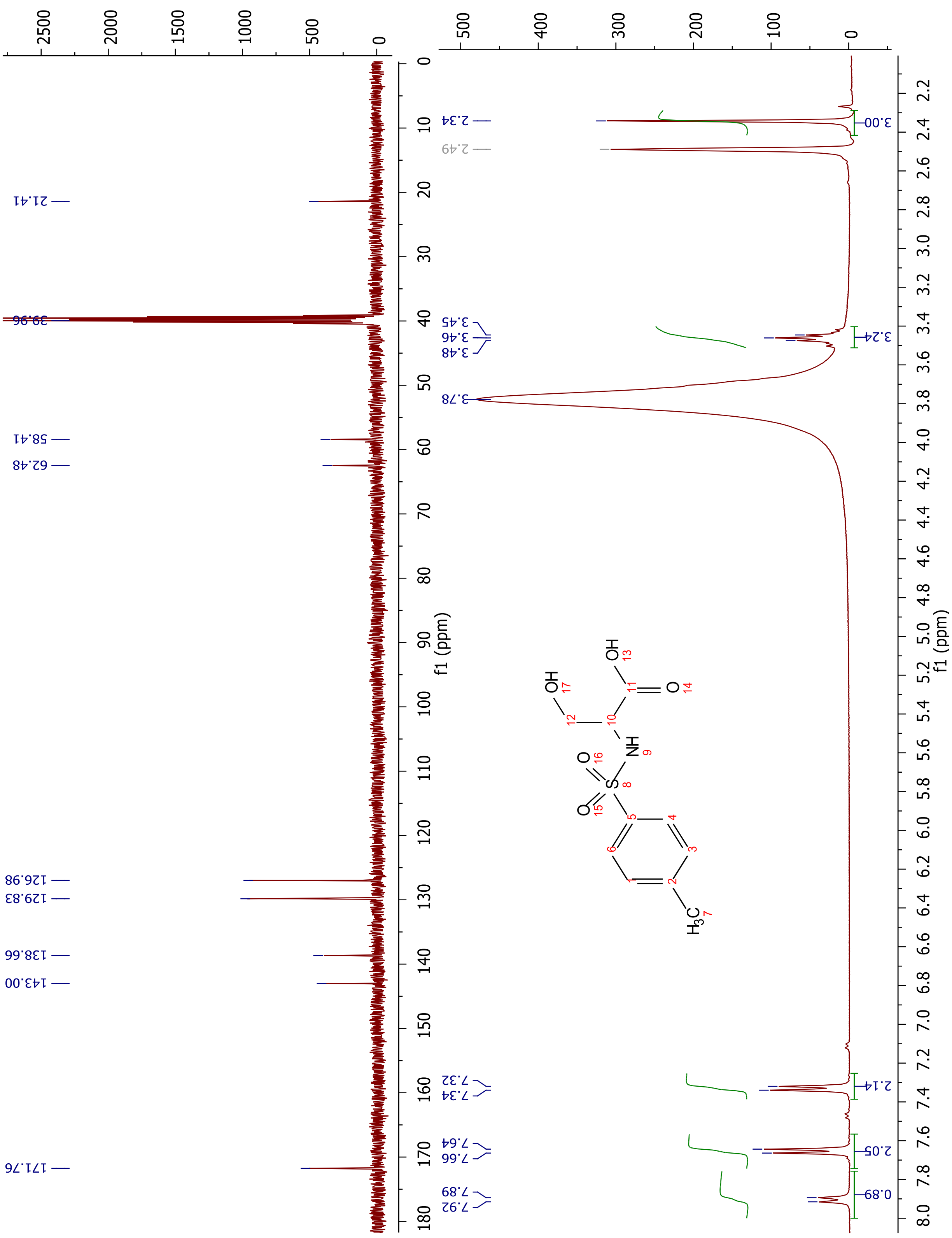
8.3 NMR spectra of protected 2a, 2b and cysteine

Protected 2a, 3-(acetamidomethylthio)-2-(phenylsulfonamido)propanoic acid

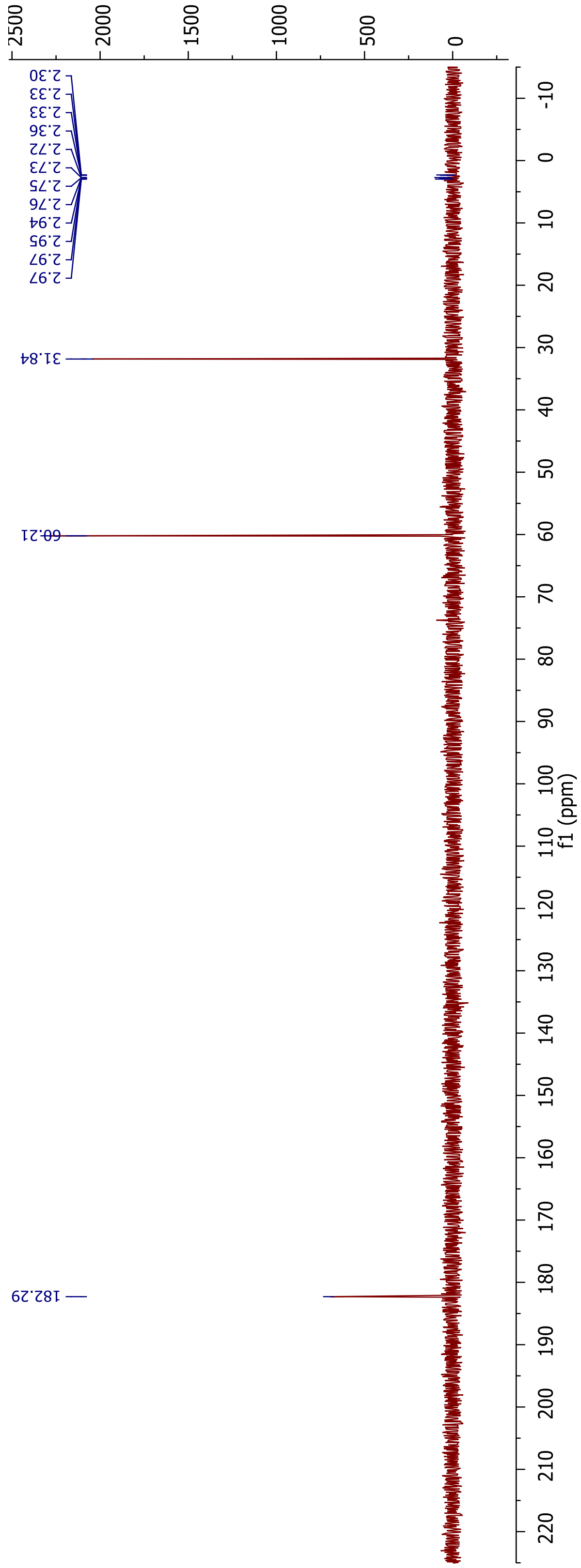




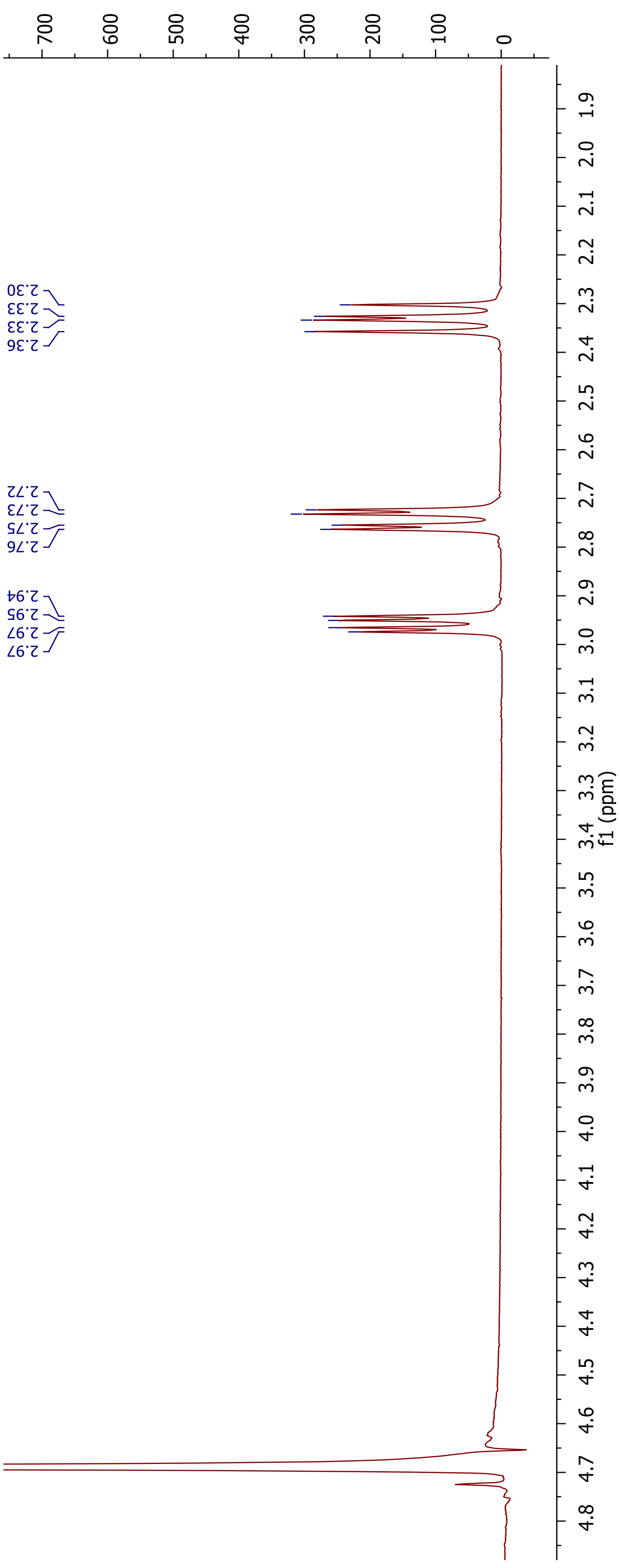
2b, 3-hydroxy-2-(phenylsulfonamido)propanoic acid



Cysteine



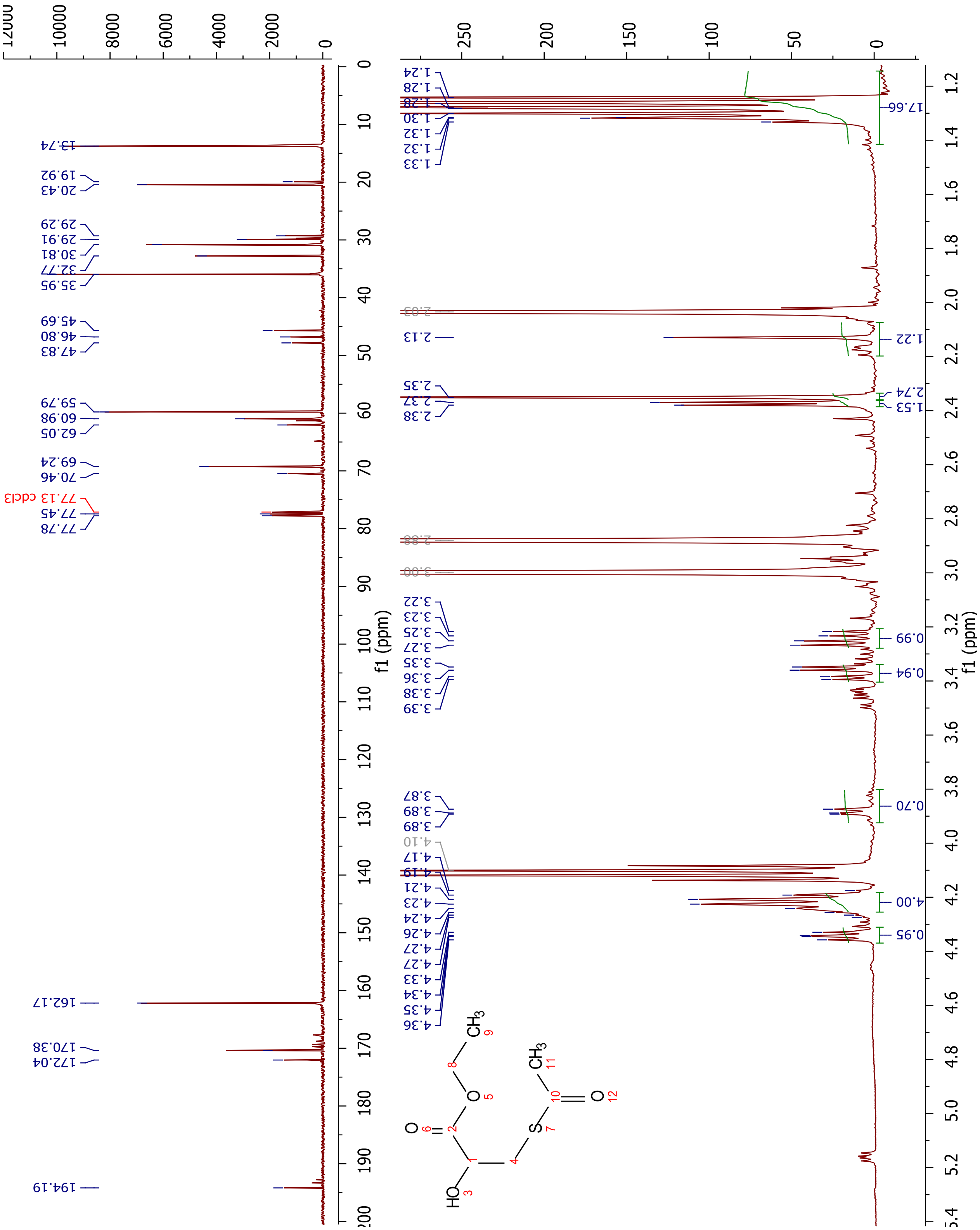
2500
2000
1500
1000
500
0
2.30
2.33
2.33
2.36
2.72
2.73
2.75
2.76
2.94
2.95
2.97
2.97
31.84
60.21
182.29
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
f1 (ppm)



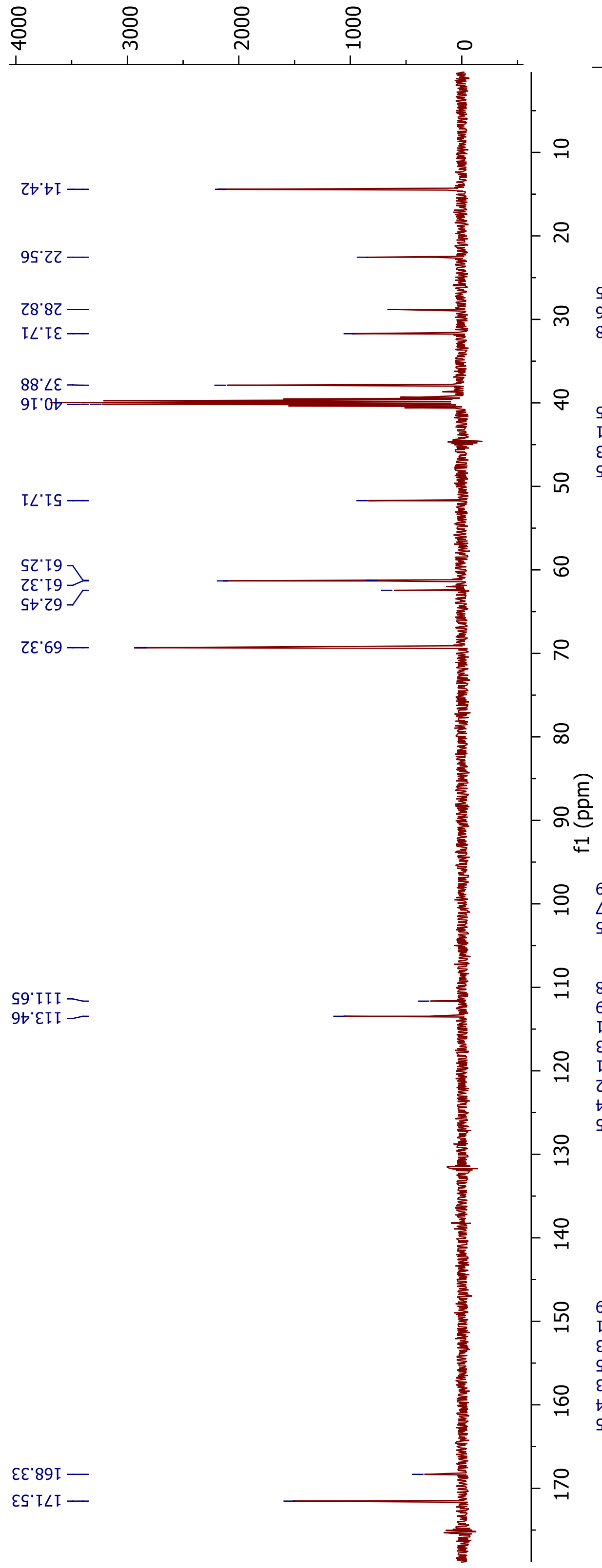
700
600
500
400
300
200
100
0
2.30
2.33
2.33
2.36
2.72
2.73
2.75
2.76
2.94
2.95
2.97
2.97
1.9
2.0
2.1
2.2
2.3
2.4
2.5
2.6
2.7
2.8
2.9
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
f1 (ppm)

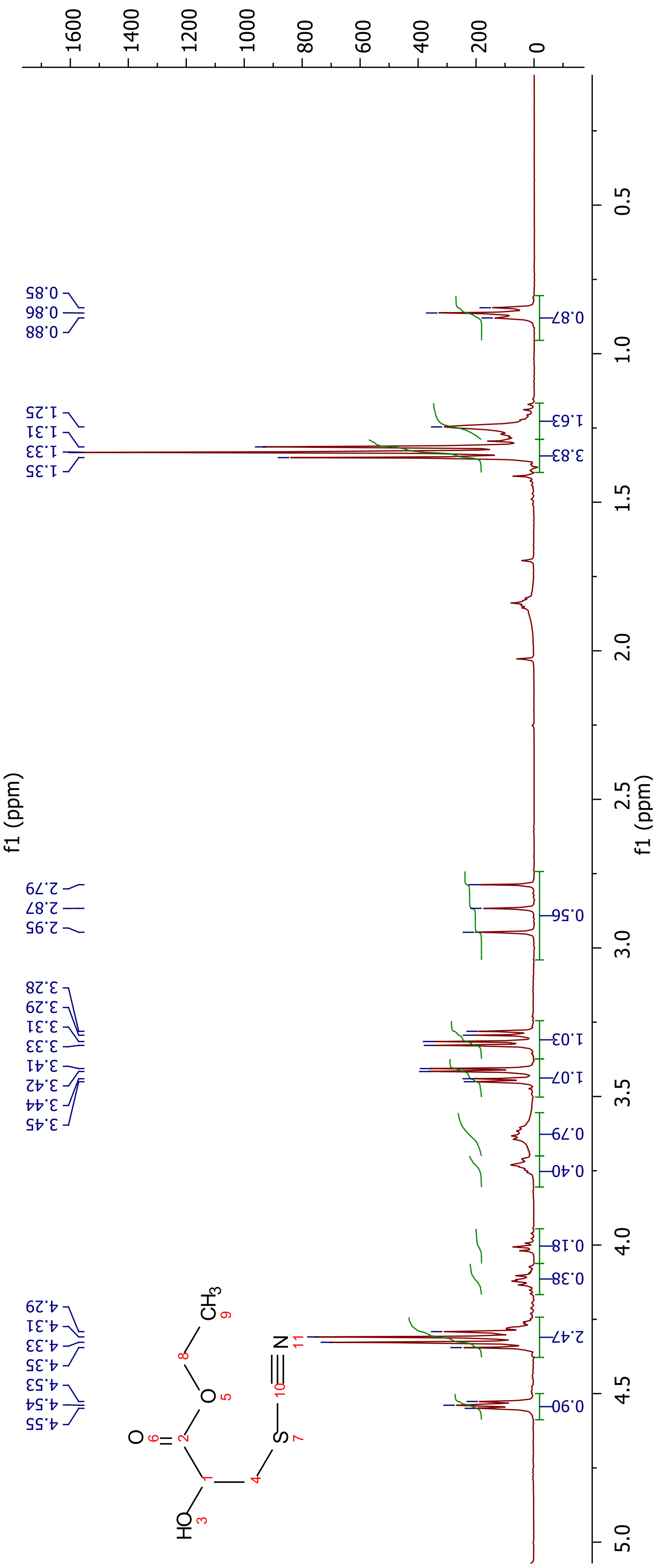
8.4 NMR spectra for 3a, 3b and ethyl glycidate

3a, ethyl 3-(acetylthio)-2-hydroxypropanoate

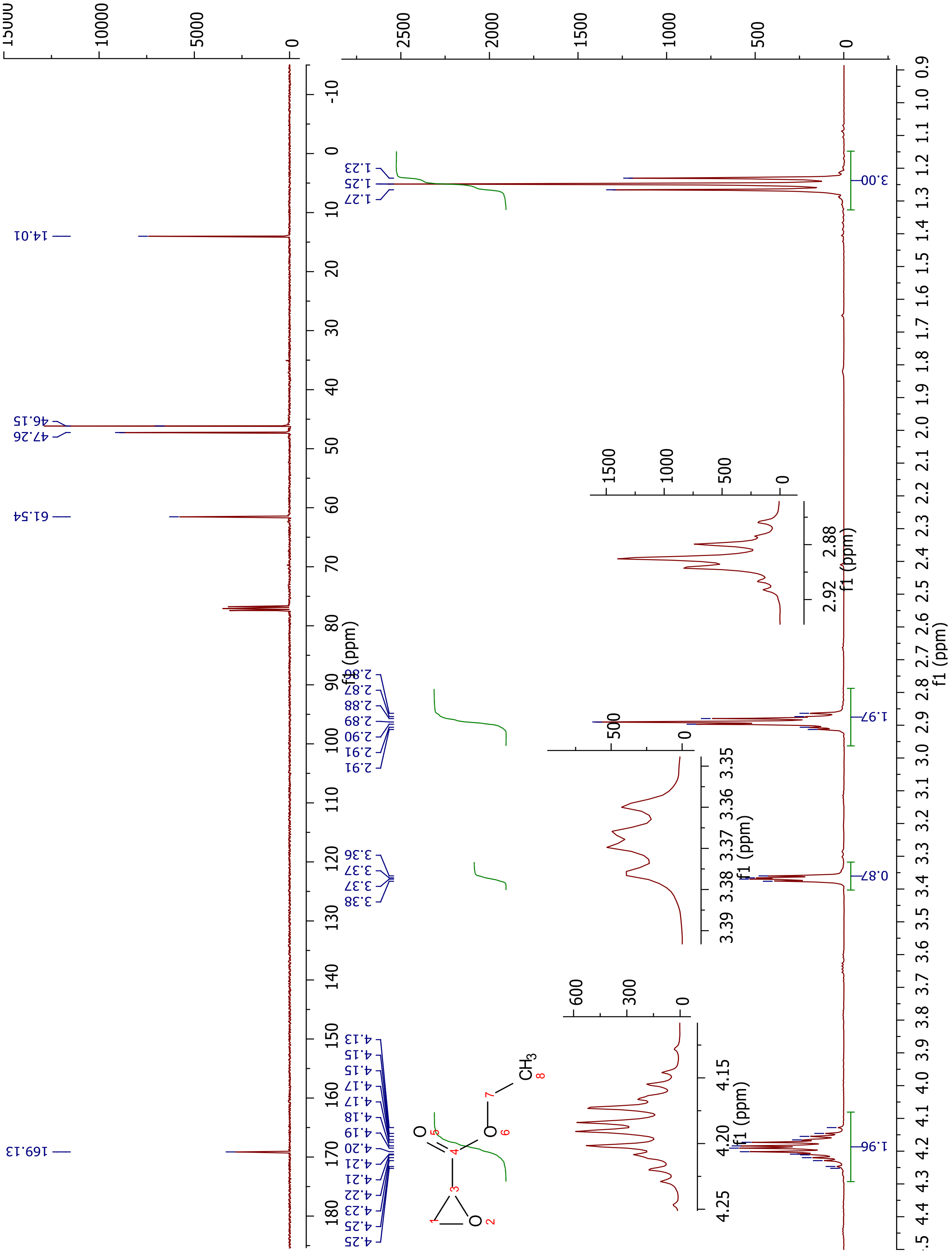


3b, ethyl 2-hydroxy-3-thiocyanatopropanoate





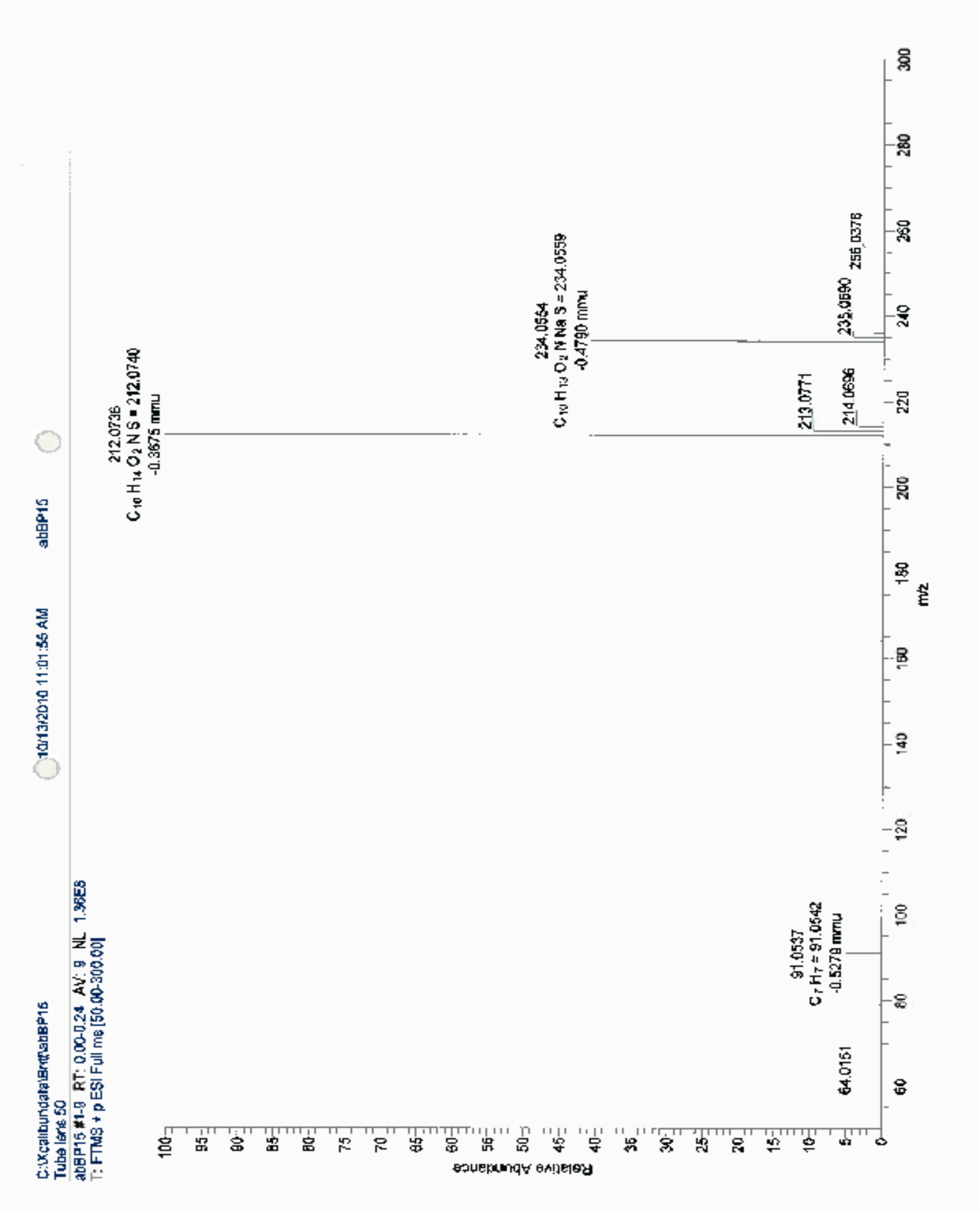
Ethyl glycidate



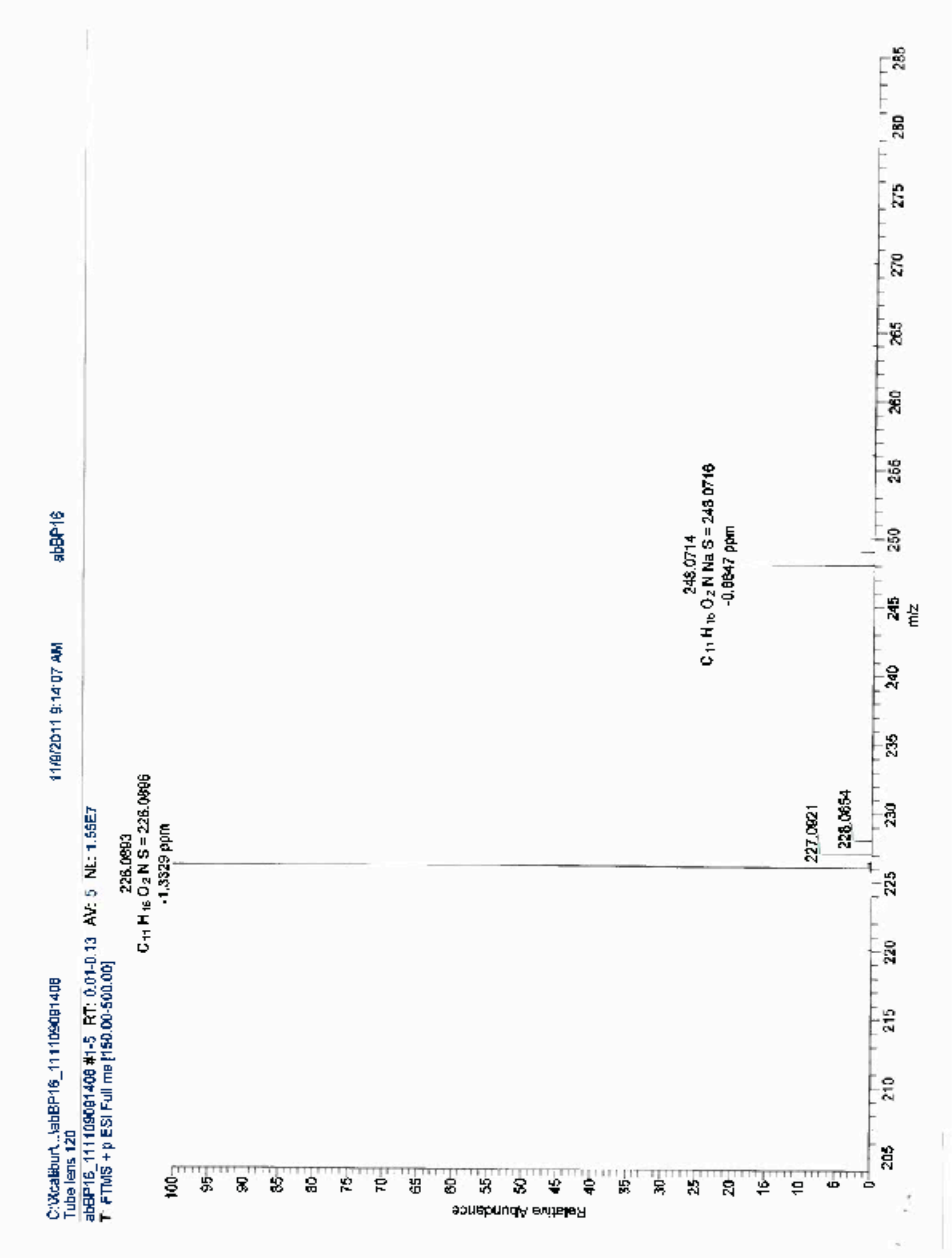
9 Mass spectra

9.1 Mass spectra

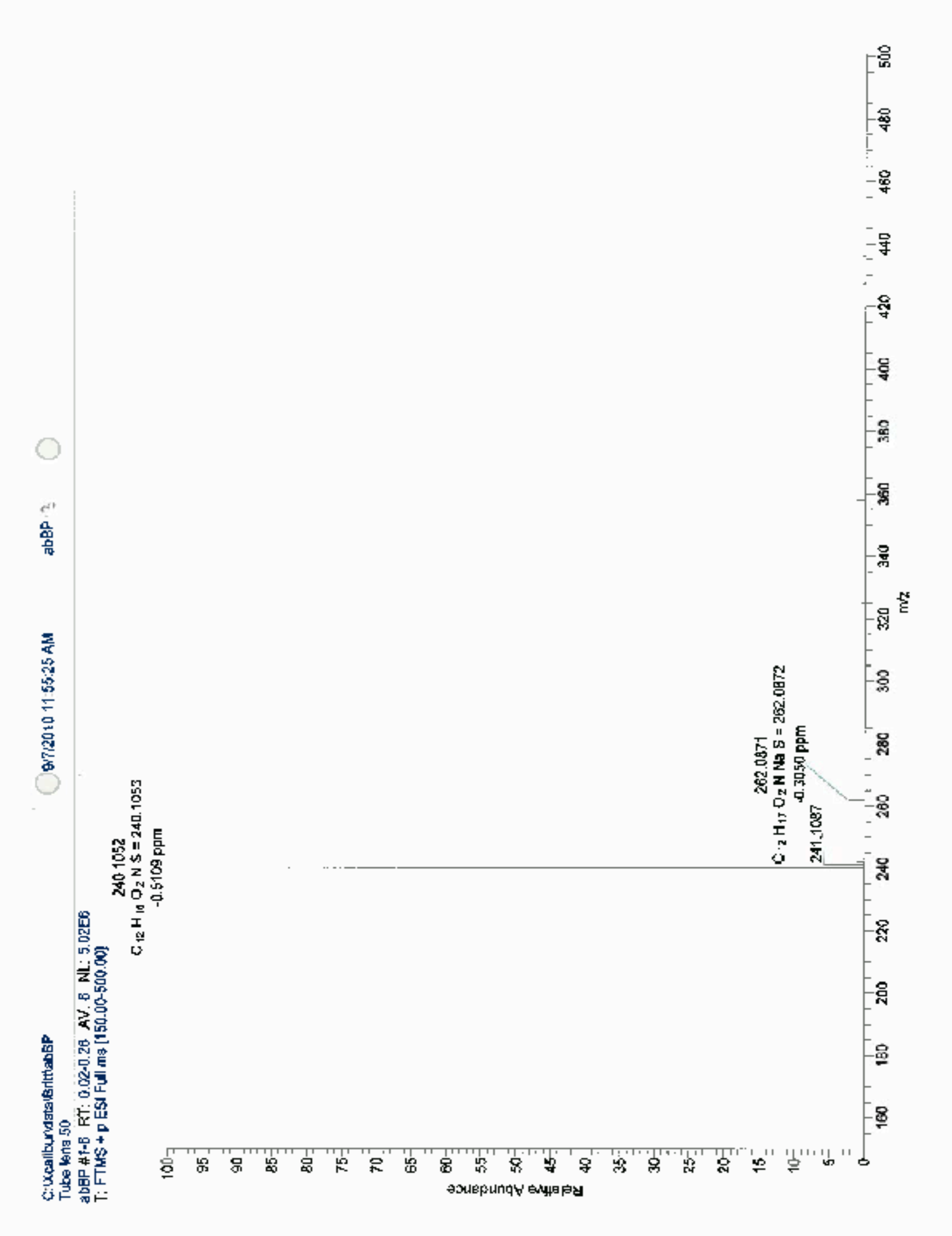
1a, 2-(benzylamino)-3-mercaptopropanoic acid



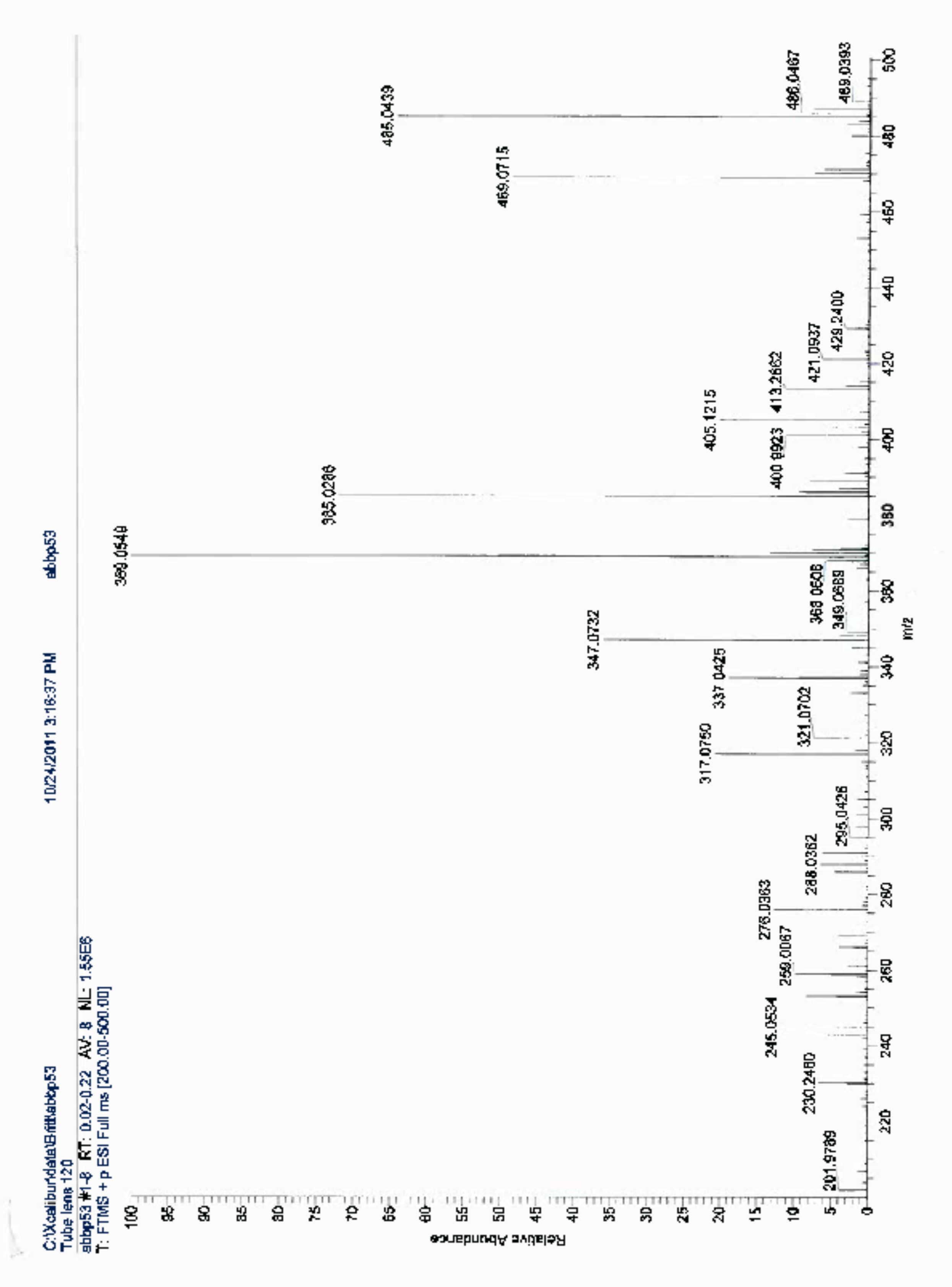
1b, 3-mercapto-2-(phenethylamino)propanoic acid



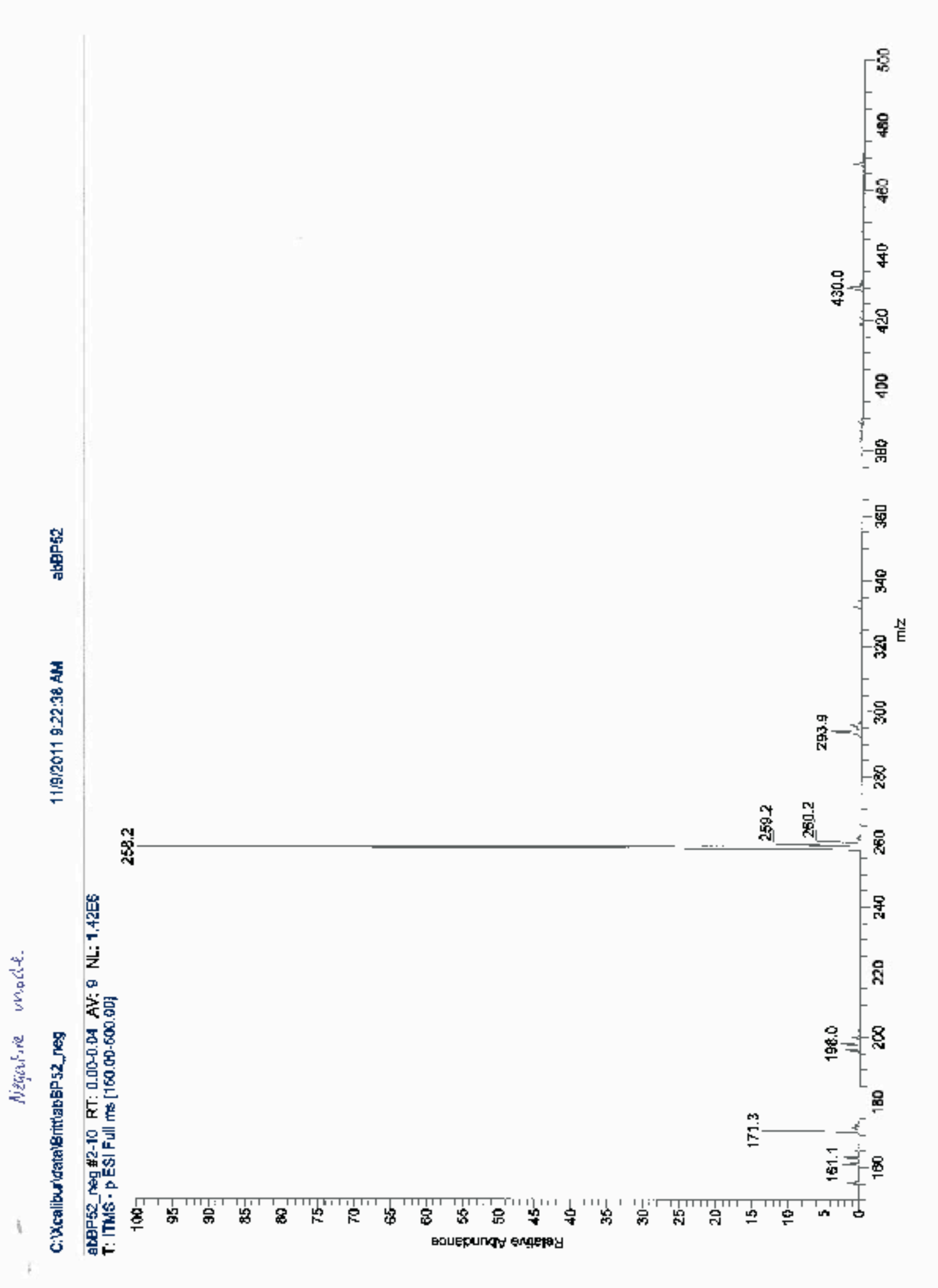
1c, 3-mercapto-2-(3-phenylpropylamino)propanoic acid



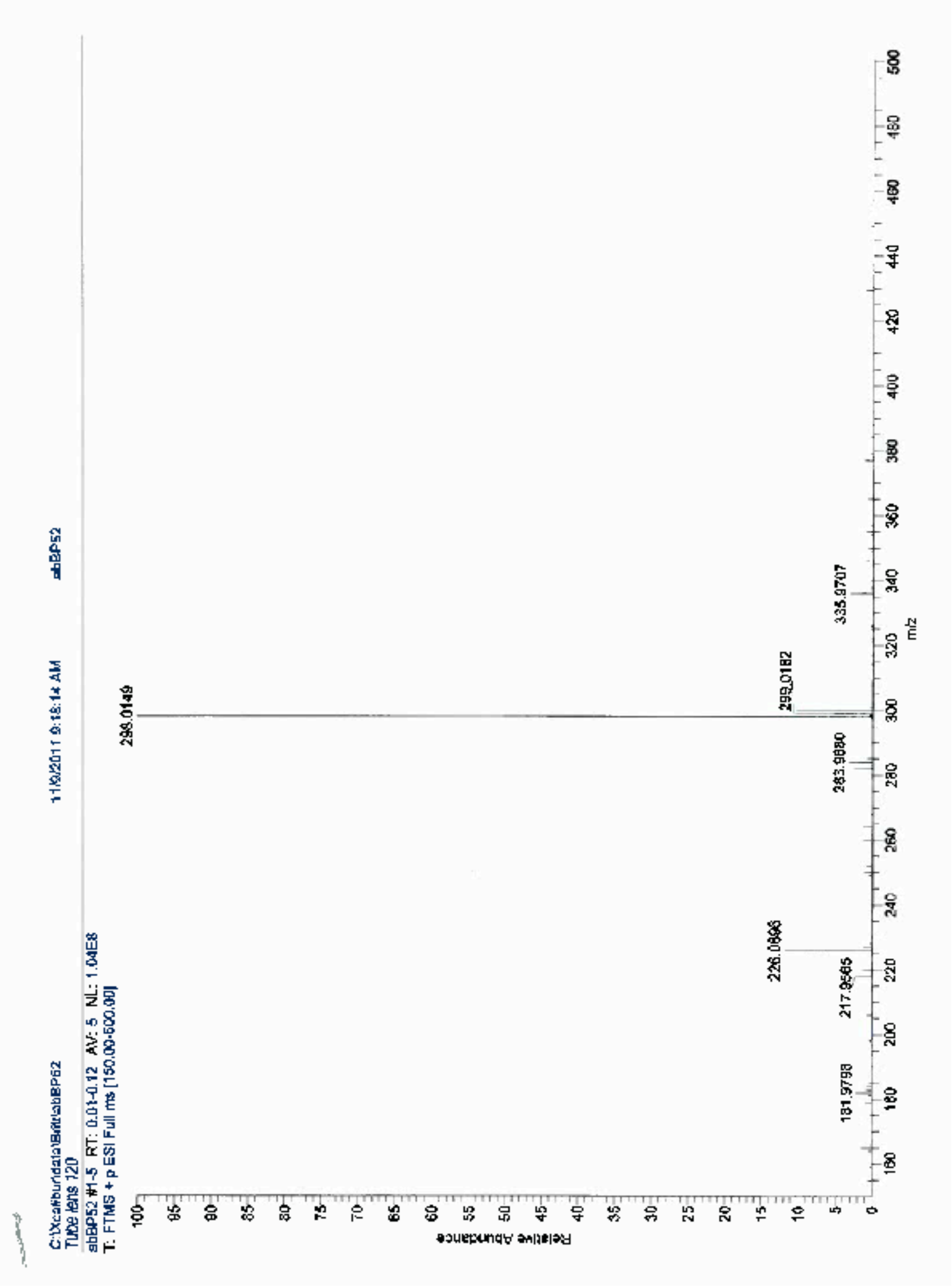
Protected 2a, 3-(acetamidomethylthio)-2-(phenylsulfonamido)propanoic acid



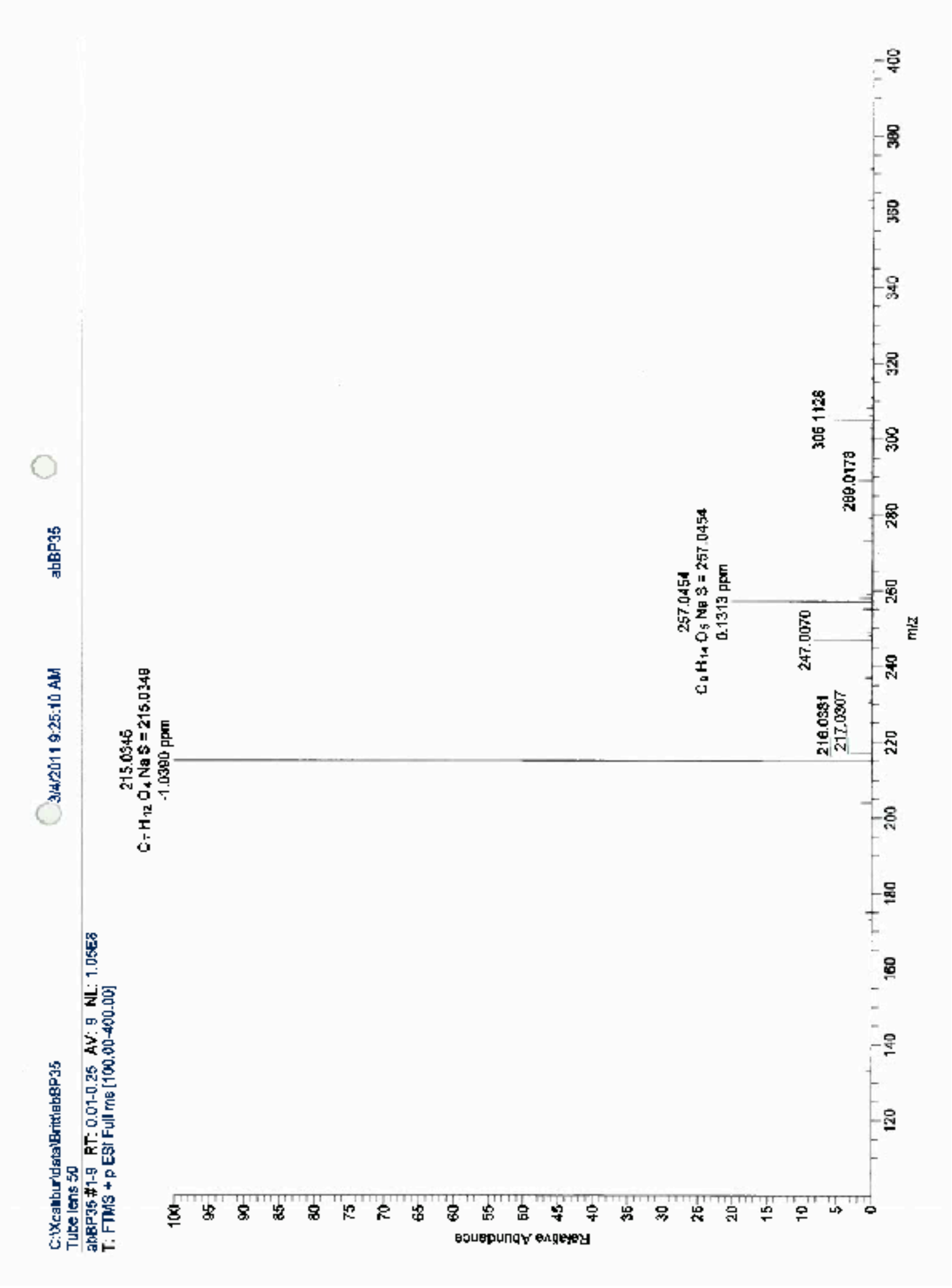
2b, 3-hydroxy-2-(phenylsulfonamido)propanoic acid



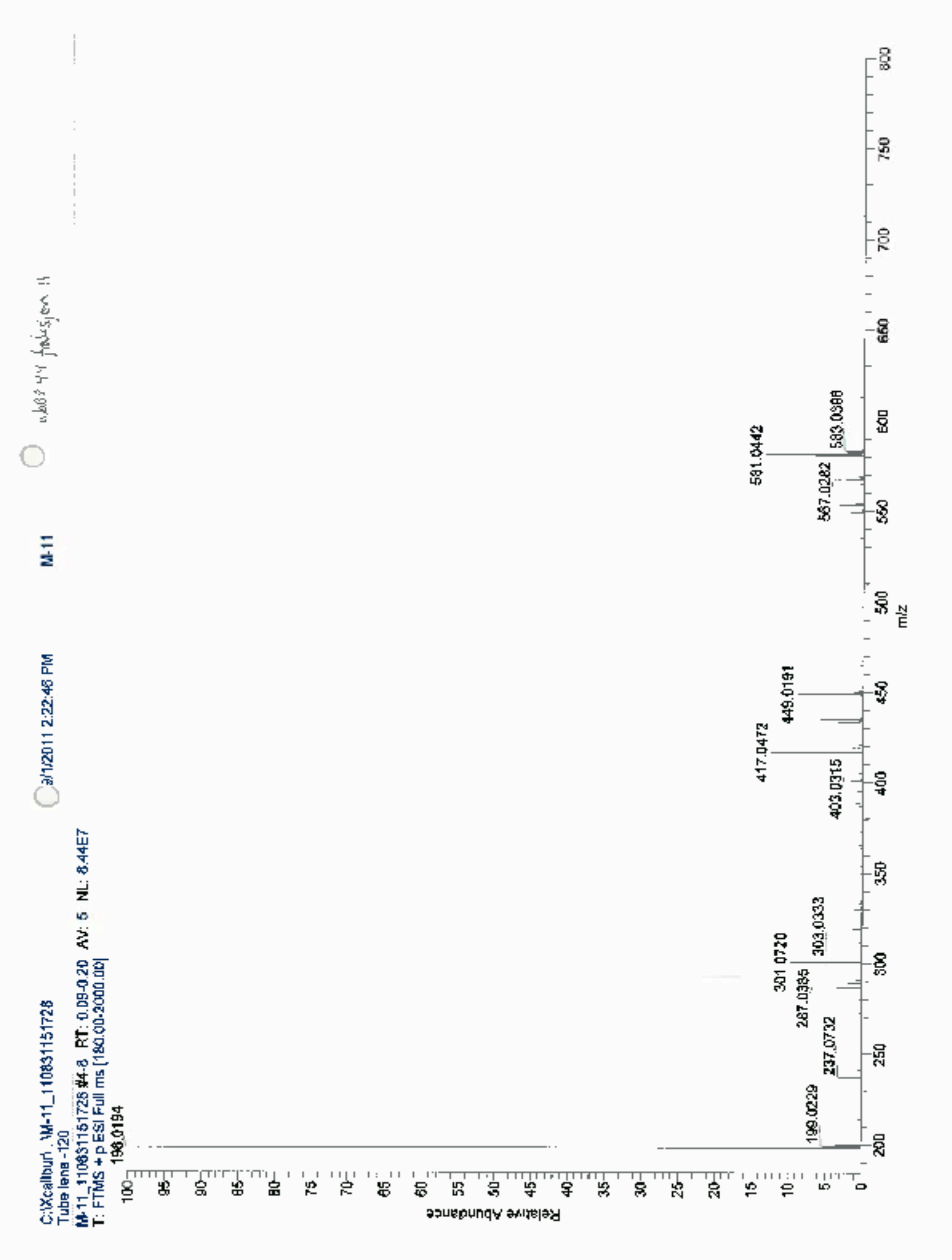
2b, 3-hydroxy-2-(phenylsulfonamido)propanoic acid



3a, ethyl 3-(acetylthio)-2-hydroxypropanoate

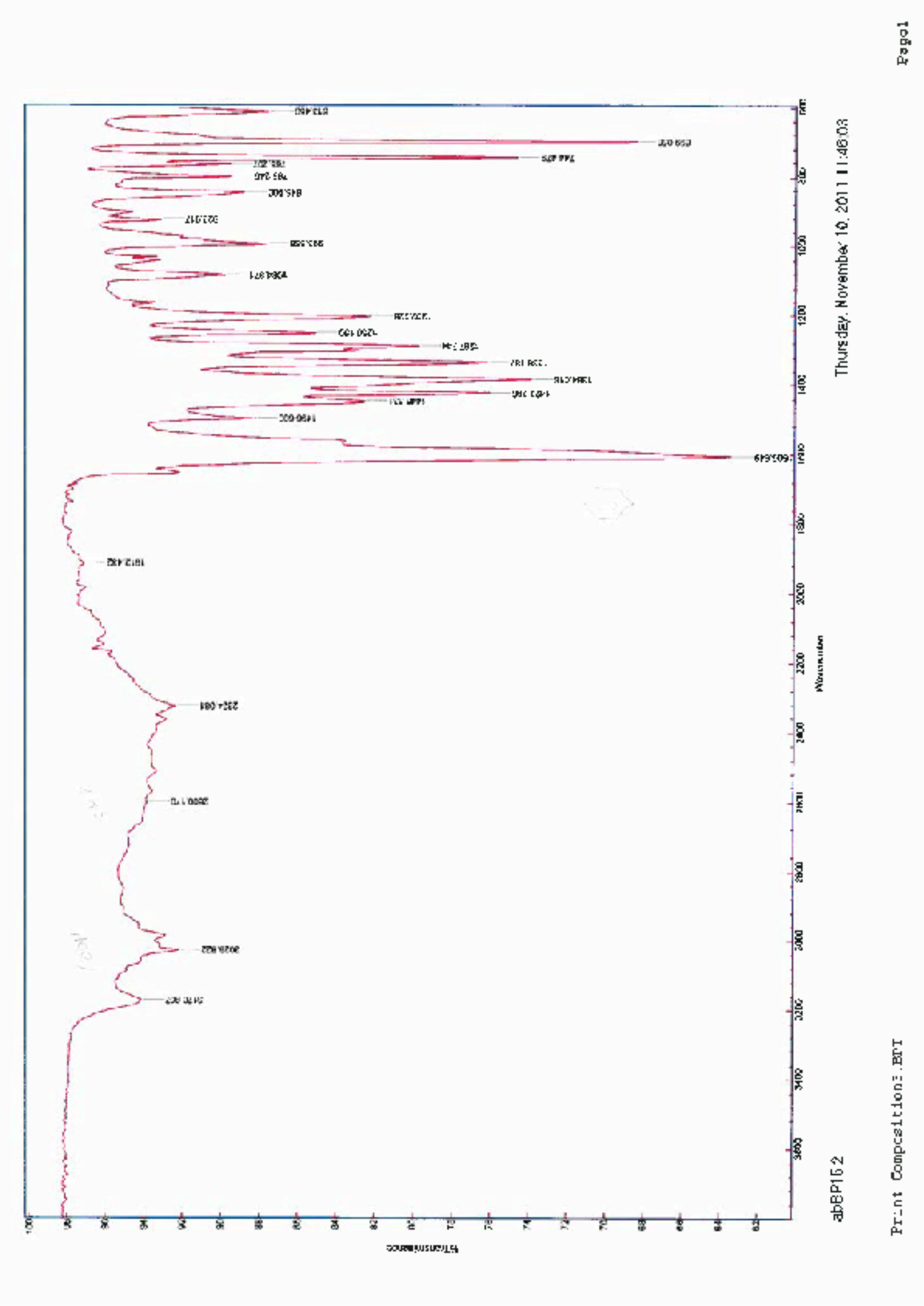


3b, ethyl 2-hydroxy-3-thiocyanatopropanoate

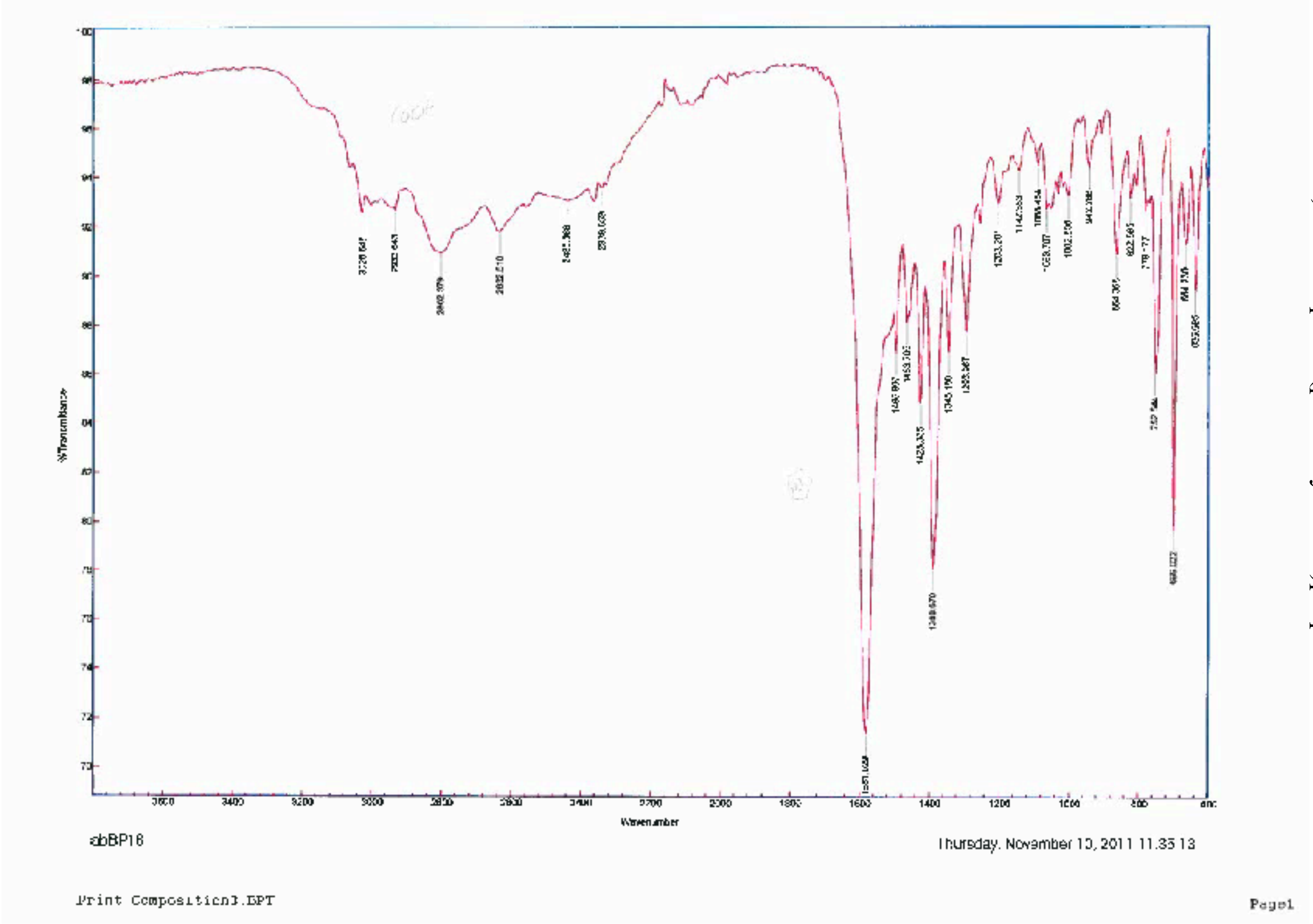


9.2 IR spectra

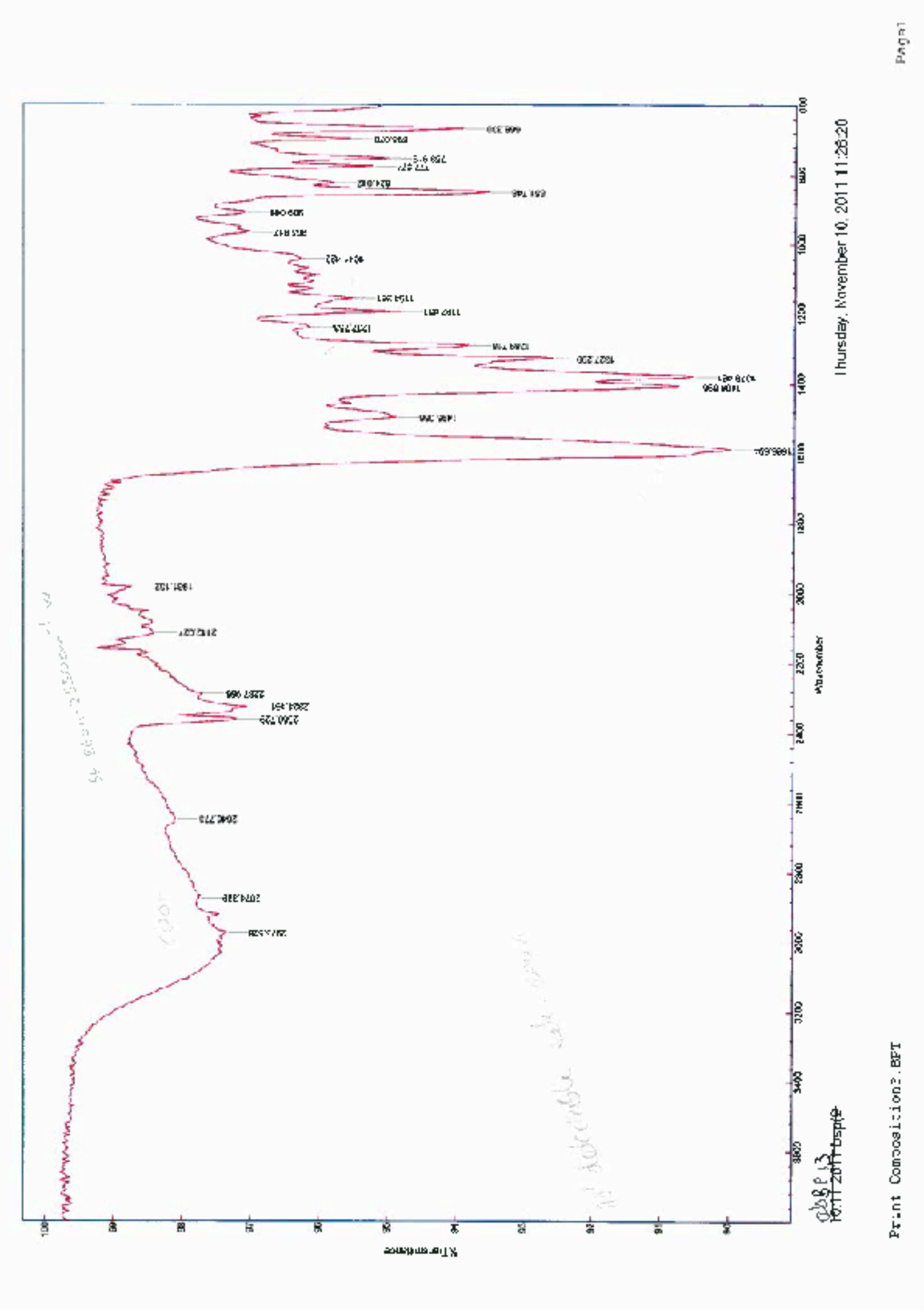
1a, 2-(benzylamino)-3-mercaptopropanoic acid



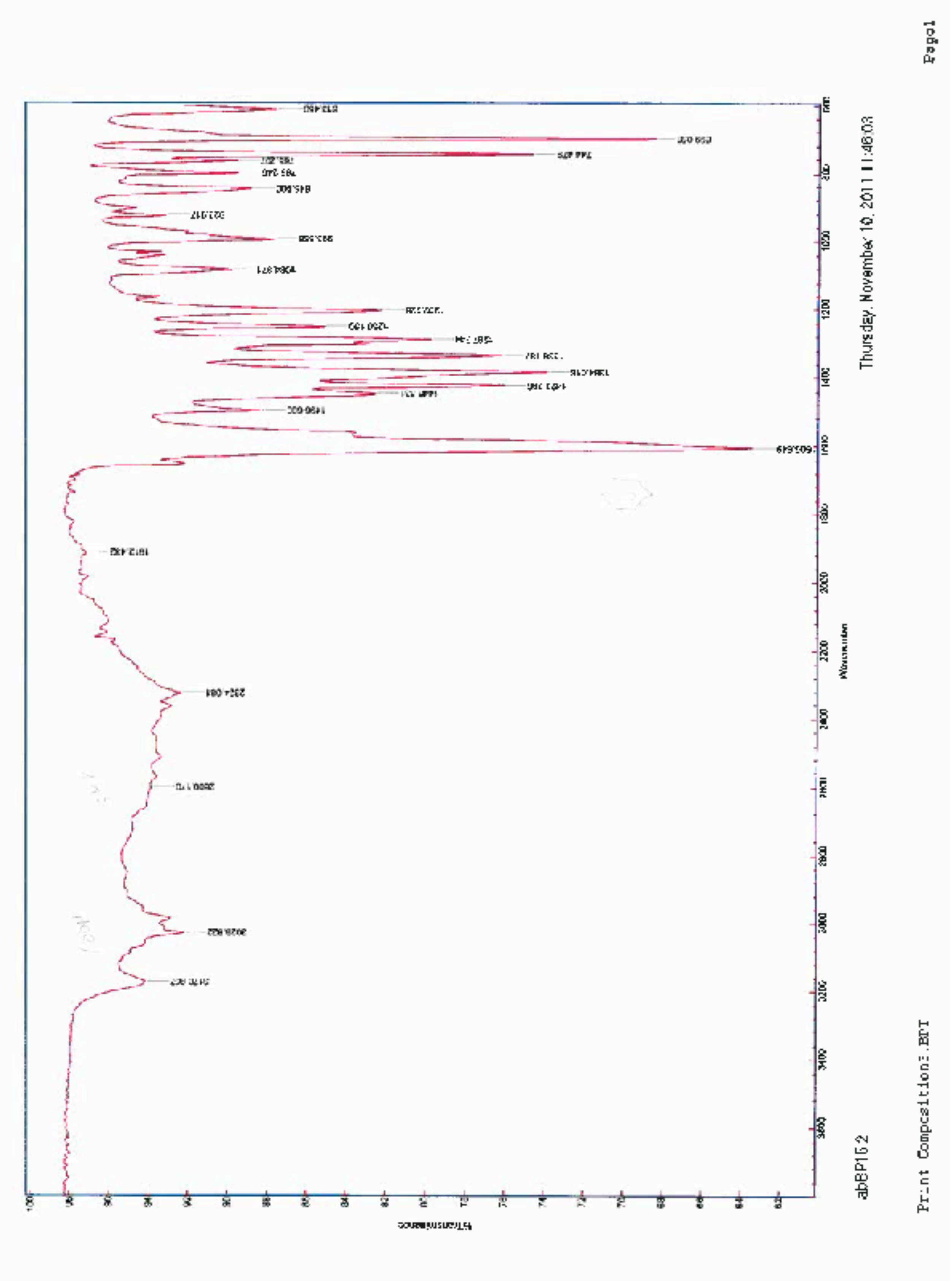
1b, 3-mercapto-2-(phenethylamino)propanoic acid

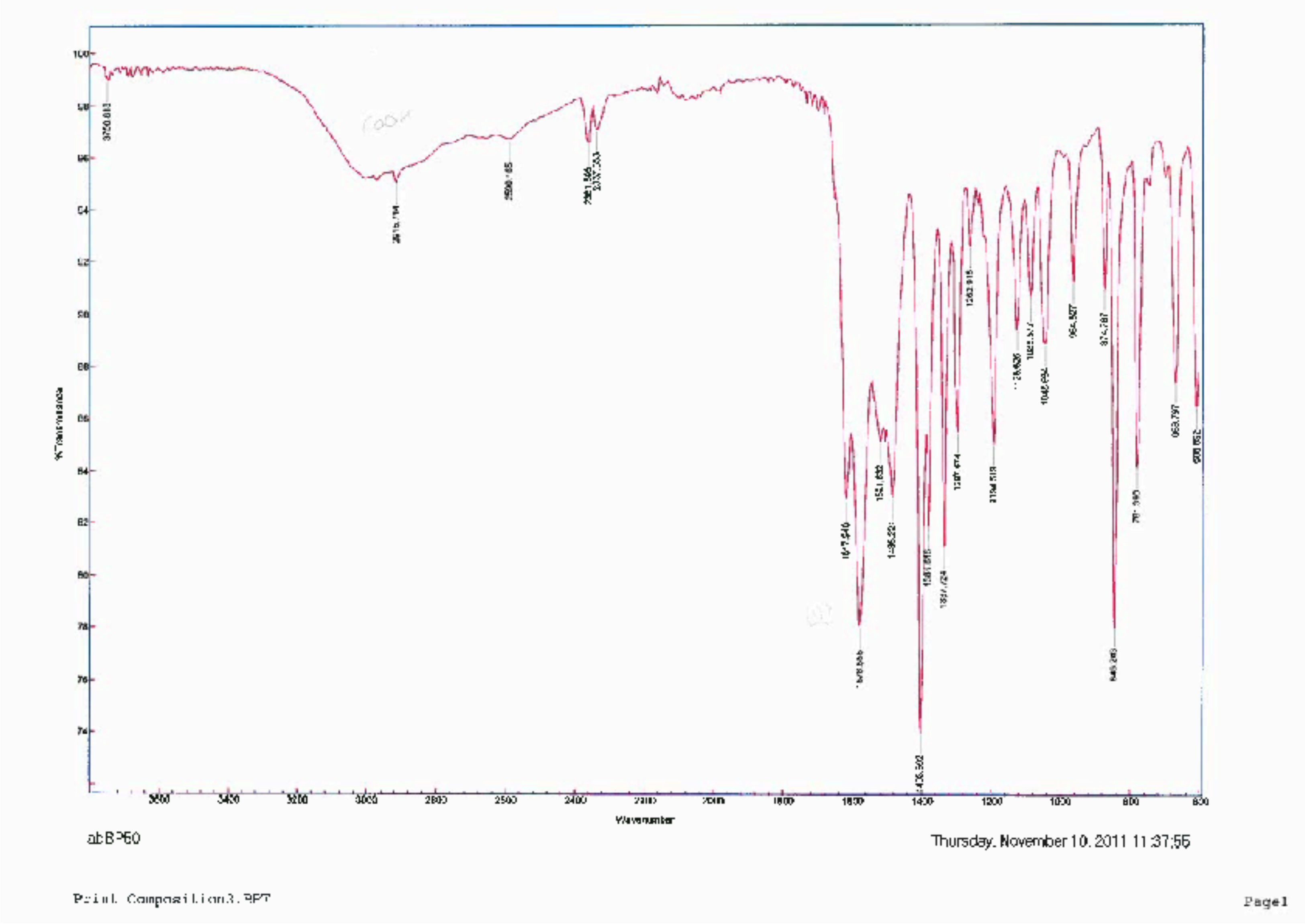


1c, 3-mercapto-2-(3-phenylpropylamino)propanoic acid



Protected 2a, 3-(acetamidomethylthio)-2-(phenylsulfonamido)propanoic acid



2b, 3-hydroxy-2-(phenylsulfonamido)propanoic acid