SYNTHESIS OF VINYLGLYCINE AND HALOENOL LACTONES

by

SRAVAN KUMAR PATEL

(Under the Direction of Timothy E. Long)

ABSTRACT

Vinylglycine (VG) is a naturally occurring, non-proteinogenic amino acid and exists in both L- and D- isomeric forms. The D-VG was first extracted in 1974 from carpophores of a fungus, *Rhodophyllus nidrosorus* and the L-antipode was observed as an intermediate in a variety of biosynthetic mechanisms. Owing to its intriguing biological properties and use as a chiral synthetic molecule, VG has attracted many chemists to asymmetrically synthesize and utilize it in building pharmaceutically active compounds. The traditional problems of isomerization and racemization during synthesis as reported in the preceding research, described in chapter **I**, made us undertake a study and report a high yielding method to enantiomerically pure VG by a mild toluene reflux of a 2-nitrophenyl homocysteine analog (illustrated in chapter **II**).

The second project deals with the synthesis of bromoenol lactone (BEL) analogs or haloenol lactones (HELs) as candidate anticancer and antibacterial agents. BEL was shown to inhibit multiple types of tumor cells through $iPLA_2$ cascade mechanism, PAP-1 inhibition and mitochondria mediated apoptosis. Therefore three different analogs of BEL were synthesized and sent to Dr. Cummings' laboratory to test their anticancer activity. Also these compounds were

assumed, in part, to exhibit their activity against bacterial cell growth through the above mentioned mechanisms (as similar pathways exist in prokaryotes). Also, a β-lactam antibiotic inactivating enzyme, serine β-lactamase was thought to be a major target for these molecules. Growth inhibition study of *E. coli* and *S. aureus* was conducted at three different concentrations of BEL analogs as described in chapter **III** and a ring activated amine containing compound showed partial inhibition of *S. aureus*. In the near future, experiments to test the β-lactamase activity through synergistic studies with BEL analogs in combination with β-lactam antibiotics shall be conducted.

INDEX WORDS: Vinylglycine, Olefin synthesis, Syn elimination, Homocysteine, Peptidomimetics, Haloenol lactones, $iPLA_2$, Bromoenol lactones, Clavulanic acid, β-lactamase

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The University of Georgia
August 2009

DEDICATION

To my parents

Mr. Veeresham Patel and Mrs. Sree Devi Patel

for their love, support, guidance and belief in me.

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CHAPTER 1

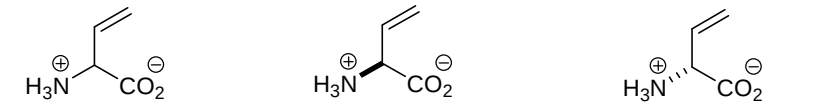
VINYLGLYCINE

INTRODUCTION AND LITERATURE REVIEW

Vinylglycine (VG) **1** is a naturally occurring, non-proteinogenic amino acid and exists in both L- and D- isomeric forms (Fig. 1.1). The D-VG **3** was first extracted in 1974 from carpophores of a fungus, *Rhodophyllus nidrosorus*[1] and the L-antipode was observed as an intermediate in a variety of biosynthetic mechanisms. For instance, L-VG **2** was observed as a mechanistic intermediate in the conversion of homoserine to threonine by threonine synthetase[2] and also in the biosynthesis of cystathionine catalyzed by cystathionine γ-synthase.[3] The L- and D-antipodes have proven to act as inhibitors and substrates towards a plethora of enzymes that are involved in biosynthetic pathways. Vinylglycine irreversibly inhibits several pyridoxal phosphate-dependent enzymes such as aspartate aminotransferase, serine glyoxylate aminotransferase, alanine racemase, heart α-ketoglutarate dehydrogenase, cystathionine γ-synthase, etc. L-VG **2** acts as a suicide substrate for *E. coli* tryptophan synthetase, sheep liver threonine deaminase, rat liver cystathionine γ-lyase and *Salmonella typhimurium* cystathionine γ-synthetase.[4]

Antibacterial activity against *Bacillus subtilis, E. coli, S. flexneri and C. freundii* has been displayed by vinylglycine.[5] The antibacterial effect observed was mostly bacteriostatic, since no morphological damage was detected for the different species of bacteria investigated. The growth inhibitory action of vinylglycine was attributed to the variations in physiological

metabolism. No further studies were carried out along these lines due to the high concentration of VG (10 μg/ml) required to produce significant antibacterial activity. A methoxy derivative of VG was shown to possess antimicrobial activity against *E. coli* and *Bacillus* species.[6-7] VG was also implicated in the inhibition of the first enzyme of sphingolipid pathway, bacterial and microsomal 3-ketodihydrosphingosine synthetases (3KDS), which ultimately showed the effect on the sphingolipid biosynthesis in the bacterial membranes thereby rendering VG antimicrobial activity against *Bacteroides levii.*[8]



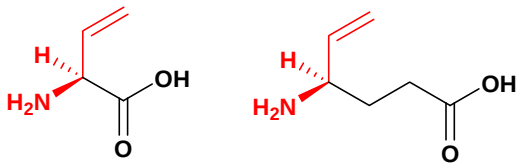
rac-Vinylglycine (VG) **1** L-Vinylglycine (L-VG) **2** D-Vinylglycine (D-VG) **3**

Fig. 1.1: Racemic and optical isomers of vinylglycine

Owing to its biological activity and use as a chiral synthon, vinylglycine **1** has been a favorite compound for many synthetic chemists. It has been implicated both as an intermediate and starting material in the synthesis of compounds like alkaloids, azasugars, novel amino acids, antibiotics, polymers and many pharmaceutically active compounds. For instance, L-VG was used as an intermediate in the synthesis of acivin, an antitumor agent[9] and as a starting material in the synthesis of the antitumor antibiotic (+)-FR900482.[10] Shaw et al. proposed the synthesis of the antitumor antibiotic mitosen, which also possesses antibacterial activity, utilizing protected vinylglycine.[11] *N*-Cbz protected vinylglycine benzyl ester was used as a synthon by Townsend to construct β-hydroxy ornithine which was proposed to be an intermediate in the biosynthesis of β-lactamase inhibitor clavulanic acid.[12] More recently, the Gert-Jan Boons group utilized protected vinylglycines to synthesize protected diaminopimelic acid (DAP), a component of peptidoglycan

of Gram-negative bacteria.[13] The chirality of vinylglycine was effectively utilized by Bartley and Coward to construct phosphinic acid phosphapeptide based compounds that act as folylpoly-ç-glutamate synthetase (FPGS) inhibitors.[14] FPGS, an attractive target for anticancer drugs, is essential for cell viability and hence its inhibition leads to cell death.

The structurally comparable antiepileptic drug Vigabatrin **4** was considered to be designed based on the anti-GABA aminotransferase activity of vinylglycine (Fig. 1.2).[4, 15-17] L-VG inactivates GABA aminotransferase, a PLP dependent enzyme responsible for catabolism of GABA (γ-Gamma Amino Butyric Acid). GABA is a major inhibitory neurotransmitter in the brain[18] and hence the inhibition of GABA aminotransferase results in increased GABA levels in the neurons thereby acting against the abnormal firing of neurons (epilepsy). This mechanism is utilized in designing a structural analog of vinylglycine, Vigabatrin, which also acts by mechanistically inhibiting GABA aminotransferase.



L-VG (2) **(*S*)-Vigabatrin (4)**

Fig. 1.2: Vinylglycine and Vigabatrin (structural analogs)

VG belongs to a subclass of amino acid group called α-vinylic amino acids (Fig 1.3). α-Vinylic amino acids are β,γ-unsaturated compounds with C-C double bond directly attached to the α-carbon along the side chain. The members of this class are obtained from natural sources like fungi, bacteria, plants and all of them possess enzyme inhibitory and substrate activity towards different enzymes. VG, with simple structural features, is treated as the parent member of the α-vinylic amino acid class. Besides being useful as chiral auxiliaries and cheap synthetic

starting materials to construct biologically and pharmaceutically significant compounds, they also serve as conformationally restricted analogs of naturally occurring amino acids, which, when incorporated into peptides, can provide insight into the biologically active conformation of such molecules.[19]

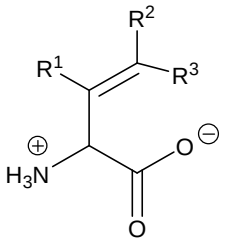


Fig. 1.3: α-Vinylic amino acid general structure

As discussed previously, vinylglycine (an α-amino acid) acts as an inhibitor of several PLP-dependent enzymes. Majority of the PLP-dependent enzymes initiate their chemistry on the substrate amino acid by extracting the α-proton. In addition to this, racemases and β- and γ-eliminases act by subsequent β-deprotonation whereas the β- and γ-replacement enzymes exhibit their action by expelling the leaving group attached to the β- and γ-carbon respectively (Fig. 1.4).

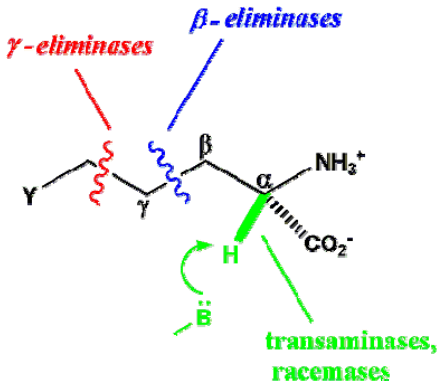


Fig. 1.4: Sites on vinylglycine acted upon by different enzymes

MECHANISM OF VINYLGLYCINE

A) Inhibitory mechanism

Vinylglycine's enzyme inhibitory and substrate mechanism is similar towards all the PLP-dependent enzymes. For instance, vinylglycine mechanistically inhibits pyridoxal phosphate cofactor dependent fruit enzyme 1-aminocyclopropane 1-carboxylate (ACC) synthase.[20] ACC synthase converts *(S,S)*-S-adenosyl-L-methionine (SAM) to ACC utilizing the cofactor pyridoxal 5'-phosphate, which is subsequently oxidized to ethylene in the presence of ACC oxidase (Fig. 1.5). Ethylene is a plant hormone that is involved in fruit ripening, senescence, wound healing and other developmental processes.[21]

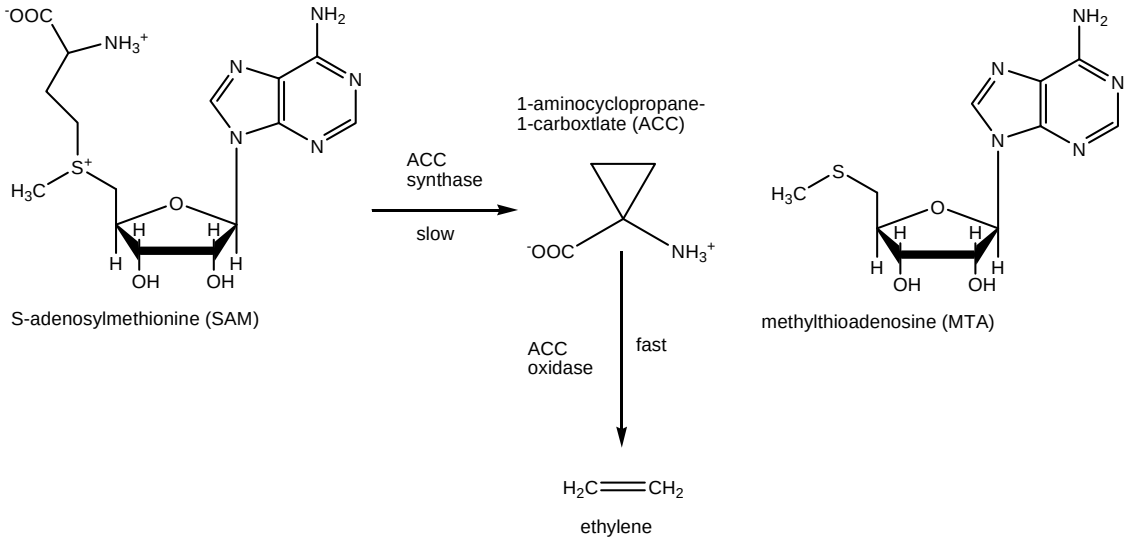


Fig. 1.5: Ethylene biosynthesis

The mechanism-based inhibition of ACC synthase is initiated with L-VG binding to the cofactor PLP of the enzyme followed by the irreversible nucleophilic attack of the base, ε-amine moiety of Lys-273 expressed at the active site, at either the β- or γ- carbons of the cofactor bound L-VG to yield B or A, respectively (Fig. 1.6). From the crystal structure, the γ-carbon of

L-VG (A) was identified as a major site of the nucleophilic attack; however nucleophilic attack at β-carbon to yield species B was also proposed. The nucleophilic addition was proposed to occur by Michael addition of ε-amine moiety of Lys-273 to a vinylglycine ketimine intermediate that was formed at an earlier stage by C_α-proton abstraction by the same ε-amine moiety of Lys-273. The Michael adduct is the inactivated form of ACC synthase.[22]

B) Substrate Mechanism

L-VG is not only an inhibitor but also acts as an alternate substrate for many PLP-dependent enzymes. The mechanism-based inhibitory activity of L-VG on ACC synthase was discussed earlier but ACC synthase was also proven to catalyze the inactivation of L-VG (Fig.1.6). Several enzymes like threonine deaminase, tryptophan synthase of the β-family, and methionine γ-lyase and cystathionine γ-synthase of the γ-family have demonstrated enzymatic deamination of L-VG to α-ketobutyrate and ammonia. The ability of L-VG to act as a substrate is 500 fold more than its inhibitory activity against ACC synthase.

The ε-amine group of active site Lys-273 abstracts the C_α-proton from external aldimine (VG-PLP), Abs_{max} at 430 nm, and generates the quinonoid species at 530 nm (Fig. 1.6). This intermediate may form 2-aminocrotonate (~480 nm) directly by γ-carbon protonation or through the intermediate species ketimine (330 nm). Subsequent hydrolysis of the 2-aminocrotonate releases α-ketobutyrate, ammonia and free PLP-bound enzyme (E-PLP).[23]

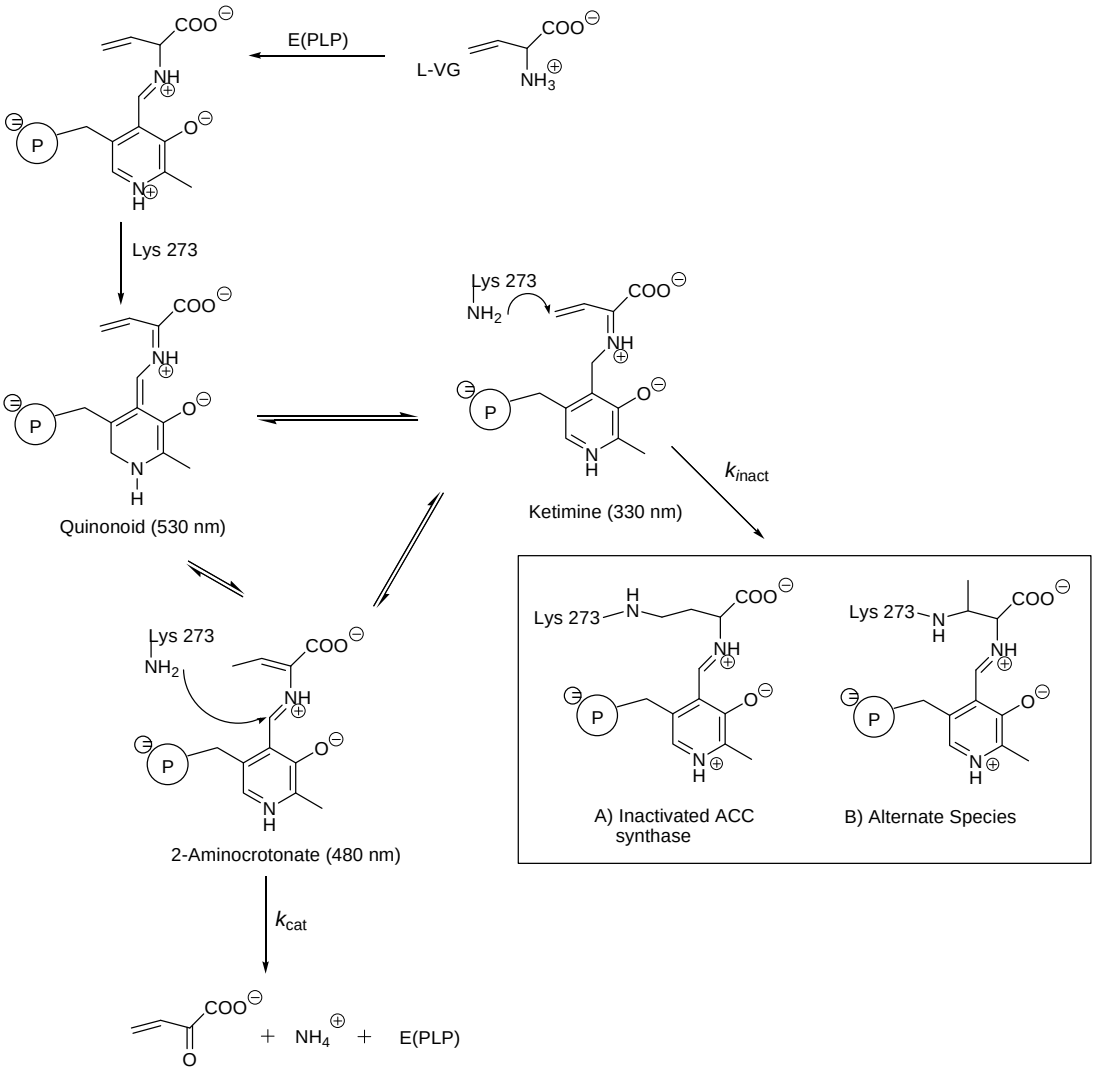
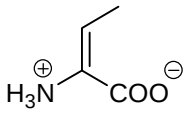


Fig. 1.6: Inhibitory and substrate mechanisms of vinylglycine

EXISTING METHODS TO SYNTHESIZE VG

Synthesis of optically pure vinylglycine poised many challenges to synthetic chemists for decades. Instability towards heat, acidic and basic conditions, which are typically employed to obtain alkenes, resulted in decreased yields by forming α,β-unsaturated isomer **5** (Fig. 1.7). Racemization under these conditions was also a major challenge faced in the synthesis of this

simple α-amino acid. This section provides a summary of the methodologies reported to synthesize vinylglycine starting from the year it was discovered.

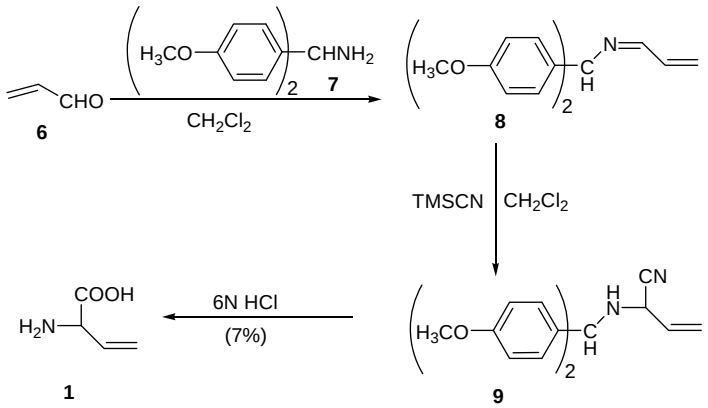


5

Figure 1.7: α,β-Unsaturated isomer

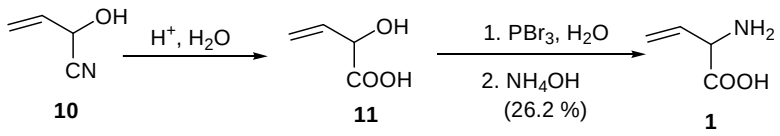
The first synthesis was reported in the same year, 1974, it was extracted from the carpophores of a mushroom. DL-Vinylglycine was synthesized by a Strecker synthesis from acrolein.[24] Optically pure D-VG was resolved by treating the racemic mixture with L-amino oxidase (baker's yeast). The yield was only 1.1% after extraction using ion exchange chromatography. In 1984, W. J. Greenlee also used Strecker reaction to synthesize VG from acrolein with very low yield of 7% (Scheme 1.1).[25] Treatment of acrolein **6** with 4,4'-dimethoxybenzhydrylamine **7** afforded imine **8**, which upon treating with trimethylsilylcyanide (TMSCN) produced β,γ-unsaturated aminonitriles **9**. Refluxing **9** in 6N HCl afforded racemic vinylglycine. Though the VG yield was low, the overall yield for this protocol was quite good.

Scheme 1.1: Synthesis of *rac*-VG by Strecker reaction[25]



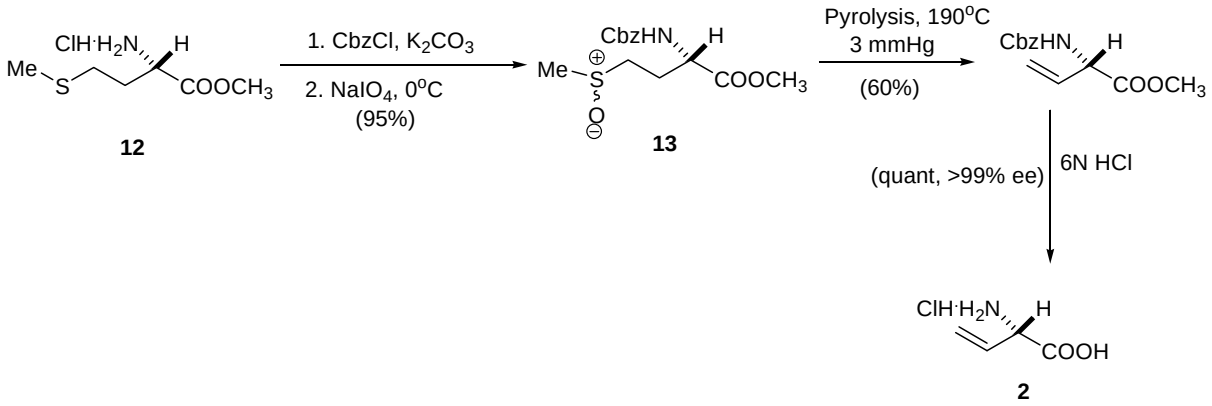
In 1977, Baldwin group developed a method to *rac*-VG **1** from 2-hydroxy-3-butenoic acid **11** (obtained from acrolein cyanohydrin **10**) in 29% overall yield (Scheme 1.2).[26]

Scheme 1.2: Baldwin's synthesis of *rac*-VG from acrolein cyanohydrin[26]



In 1980, Rapoport and Afzali-Ardakani reported for the first time a practical way to synthesize vinylglycine with an acceptable overall yield of 54% (Scheme 1.3).[27] *N*-Cbz protected methionine methyl ester **12** was subjected to controlled oxidation (sulfoxide **13**), followed by pyrolysis under Kugelrohr conditions (148°C, 3 mmHg) to distill off the protected L-VG **2** as formed.

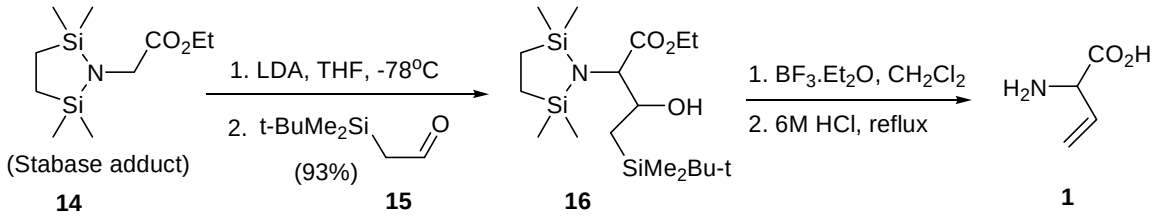
Scheme 1.3: Rapoport's asymmetrical synthesis of L-VG from (*S*)-Met-OMe•HCl [27]



Racemic vinylglycine was also synthesized by P. F. Hudrlik and coworkers in 1981 as part of their effort to introduce vinyl group α to the carbonyl moiety to produce biologically active and synthetically useful intermediates.[28] The STABASE adduct of ethyl glycinate **14** was converted to β-hydroxysilane compound **16** when treated with α-silylated acetaldehyde **15**

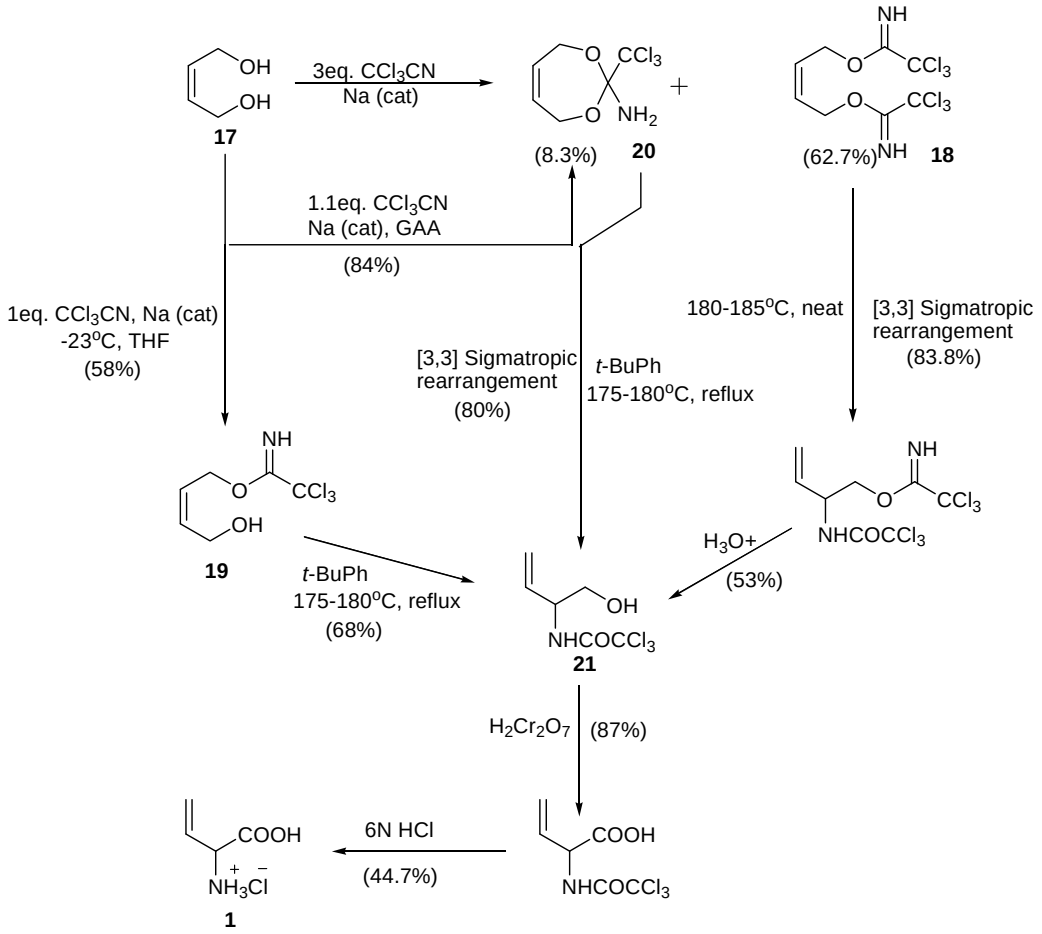
(Scheme 1.4). Elimination and deprotection of amino group was achieved by treating **16** with $BF_3{\cdot}Et_2O$. Hydrolysis using 6M HCl produced racemic vinylglycine **1** in 48% overall yield.

Scheme 1.4: Hudrlik's synthesis of *rac*-VG from ethyl glycinate **14**[28]



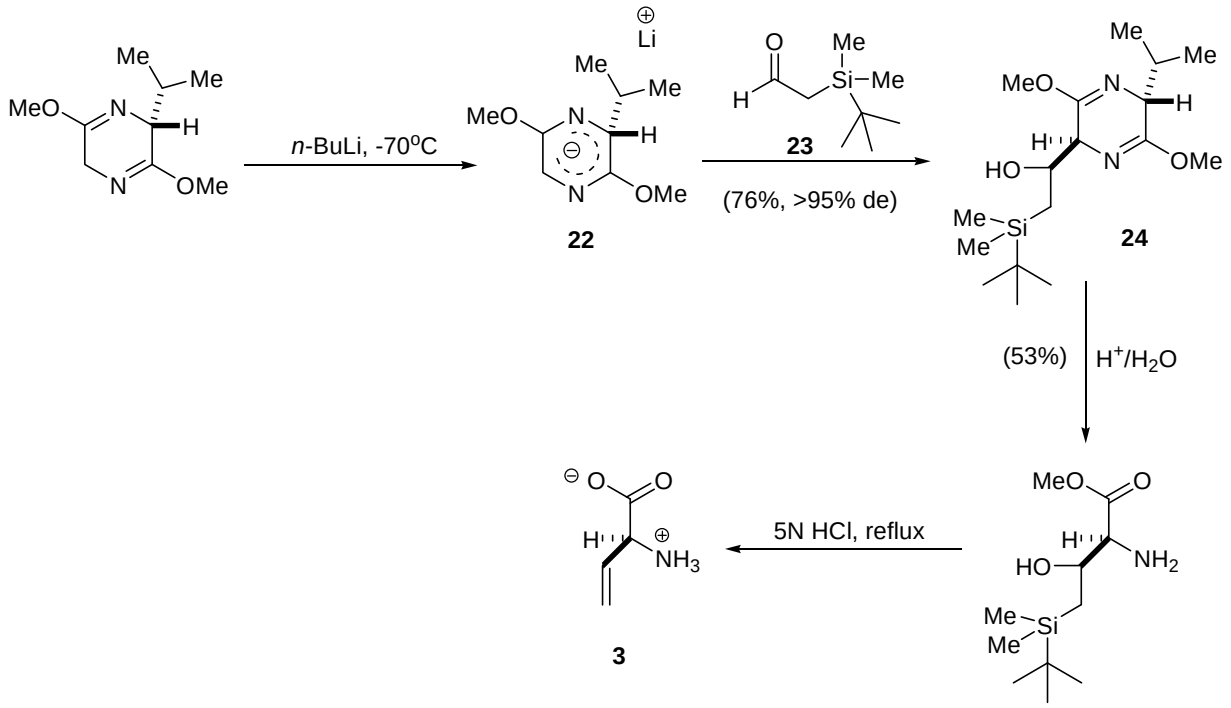
In 1984, extensive research was carried out in different laboratories and several methods to synthesize vinylglycine were published. Bristol-Myers workers have developed a method that utilizes [3,3] sigmatropic rearrangements to afford the target through different routes (Scheme 1.5).[29] (*Z*)-2-butene-1,4-diol **17** was used as a starting material and imidation reaction was performed using trichloroacetronitrile (CCl_3CN). Three different products, *bis*-imidate **18**, mono-imidate **19** and dioxepine **20**, were observed under mildly varied reaction conditions. This was followed by the formation of alcohol **21** from all three intermediates when heated and subjected to different conditions. Alcohol **21** was latter oxidized and acid hydrolyzed to give racemic vinylglycine in 26% overall yield.

Scheme 1.5: Bristol-Myers' synthesis of *rac*-VG by [3,3] sigmatropic rearrangements[29]



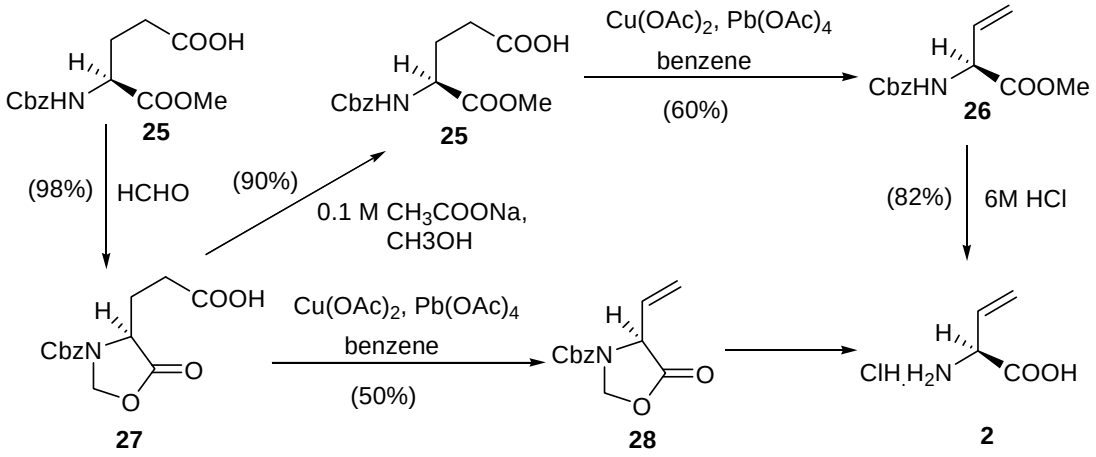
SchÖllkopf et al.[30] have utilized chiral auxiliary based approach to demonstrate a high yielding method (Scheme 1.6) by α-vinylation using a vinyl cation equivalent **23** developed by Hudrlik.[28] Condensation of lithiated *bis*-lactim ether **22**, a chiral auxiliary, with silylated aldehyde **23** afforded **24** in 76% yield with >95% diastereomeric excess (de). The rigidity of heterocyclic intermediate **24** was utilized to induce a high degree of asymmetry. Subsequent treatment of **24** with HCl rendered β-eliminated (silanol elimination) product D-VG **3** in 62 % yield with high enantiomeric purity.

Scheme 1.6: SchÖllkopf's enantioselective synthesis of D-VG[30]



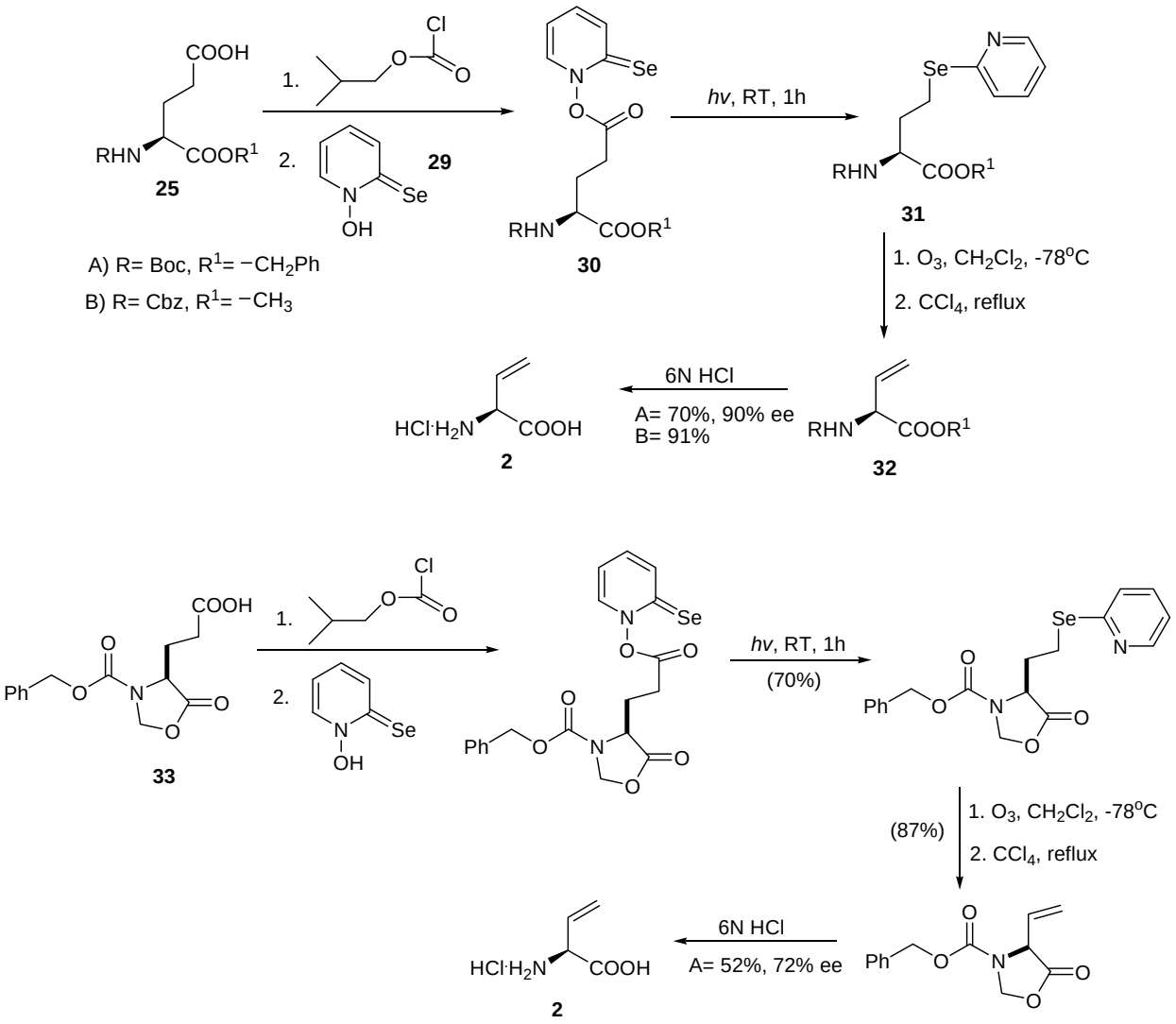
In the same year, 1984, the Hanessian group published a practical method to obtain optically pure protected L-VG **26** in 60 % yield from readily available L-glutamic acid (Scheme 1.7).[31] Several chemists used this inexpensive starting material and demonstrated different methods to vinylglycine. The method involved oxidative decarboxylation of *N*-Cbz protected L-glutamic acid monomethyl ester **25** with lead tetra-acetate and catalyzed by cupric acetate to introduce vinyl group. 6N HCl reflux of ester **26** gave pure L-VG in 82 % yield. Hanessian was successful in tackling the racemization and isomerization problems, which are efficiently answered with milder conditions.

Scheme 1.7: Hanessian's asymmetrical synthesis of L-VG from L-Glu[31]



D. H. R. Barton, 1985, reported two routes to obtain optically pure L-VG in 45% yield using seleno chemistry from L-glutamic acid (Scheme 1.8).[32] *O*-ester of selenohydroxamic acid **29** (from 2-bromopyridine-*N*-oxide and sodium hydrogen selenide) with *N*-Cbz protected glutamic acid monomethyl ester **25B** gave alkyl-selenides **31** upon exposure to heat or light. The decarboxylative rearrangement to **31** was expected to occur through free radical mechanism. The alkyl-selenide obtained was subjected to ozonolysis to yield protected vinylglycine **32** through oxidative elimination of selenopyridine compound. Two other protected glutamic acid compounds (**25A, 33**) were also used and subjected to similar conditions to obtain optically pure L-VG.

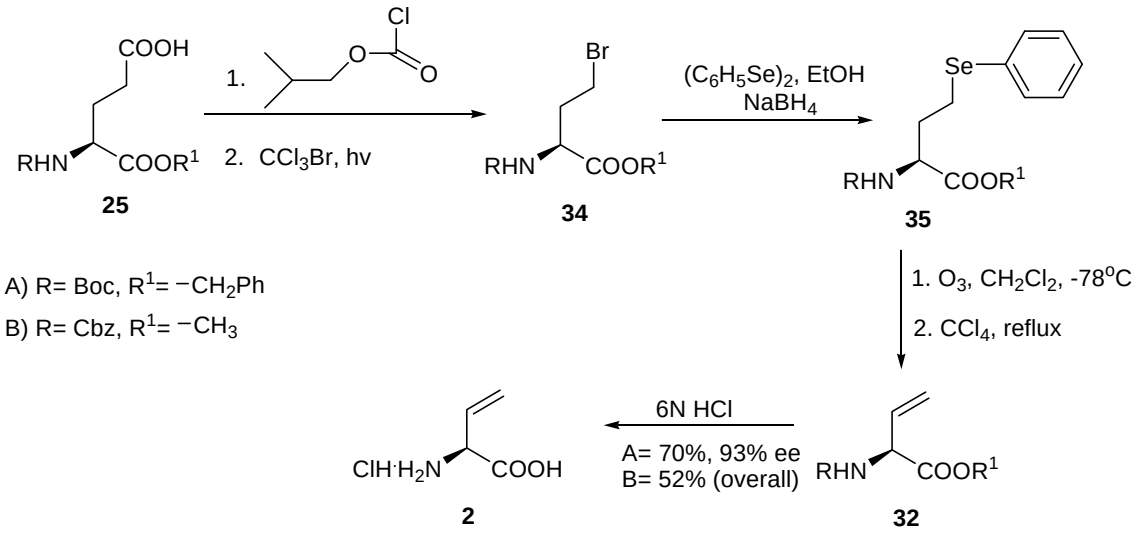
Scheme 1.8: Barton's asymmetrical synthesis of L-VG from glutamic acid ester **25** and **33**[32]



The instability of selenohydroxamic acid **29** slightly limited the use of these approaches. Therefore, an alternate route for a comparative study was tried by Barton and coworkers. Slight modification of the Hunsdiecker reaction on the terminal carboxyl group of *N*-Cbz protected glutamic acid monomethyl ester **25A** gave the bromide **34** which readily afforded the phenyl-selenide compound **35** on treatment with phenylselenide anion (Scheme 1.9).[32] Subsequent oxidative elimination reaction with ozone yielded protected vinylglycine **32** in high optical

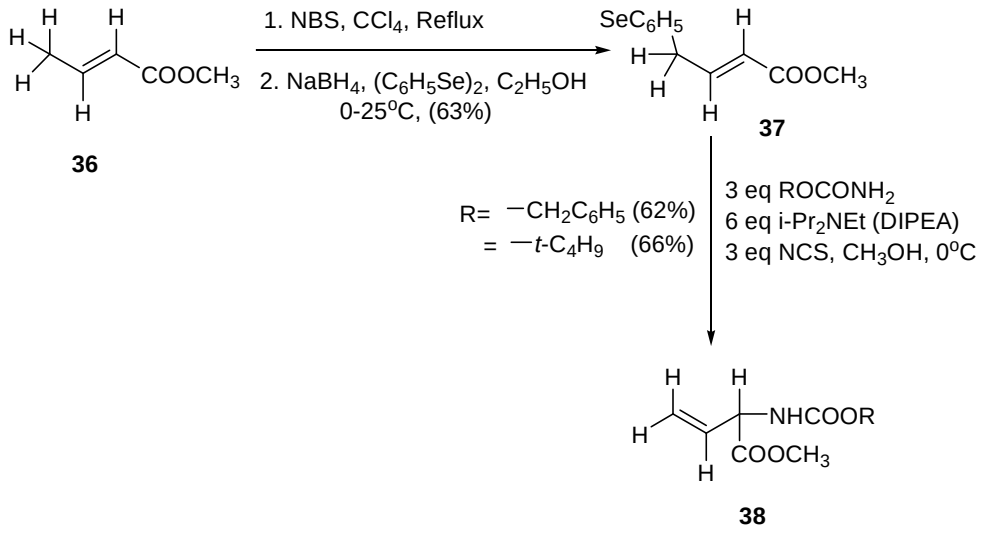
purity. *N*-Boc protected benzyl ester compound **25B** was also used following the same protocol to produce protected L-VG in overall yield of 78% making it a practically efficient procedure.

Scheme 1.9: Barton's modified asymmetrical synthesis of L-VG[32]



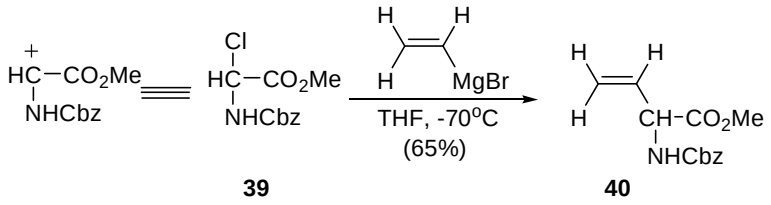
In the same year, Fitzner was successful in synthesizing protected DL-VG through a mild oxidative rearrangement of γ-phenylseleno-α,β-unsaturated ester **37** in ~60% yields (Scheme 1.10).[33] A two step method to synthesize **37** was employed involving allylic bromination of **36** with *N*-bromosuccinimide (NBS), followed by introduction of phenyl selenide moiety through displacement of bromide with phenyl selenide anion. Rearrangement of allylic selenide **37** to protected β,γ-unsaturated compound **38** was achieved with *N*-chlorosuccinimide (NCS), *N,N*-diisopropylethylamine (DIPEA) and a carbamate reagent. Use of hindered base DIPEA ceased the base catalyzed rearrangement of **38** to α,β-unsaturated isomer thereby limiting the isomerization problem.

Scheme 1.10: Fitzner's synthesis of *rac*-VG from phenylseleno-ester **37**[33]



Utilizing the classical Grignard reaction, A.L. Castelhano in 1986 developed a new approach from protected glycine cation equivalent **39**.[34] A vinylic Grignard was condensed with **39** at -70°C in THF to give protected VG **40** in racemic form with an isolated yield of 65%.

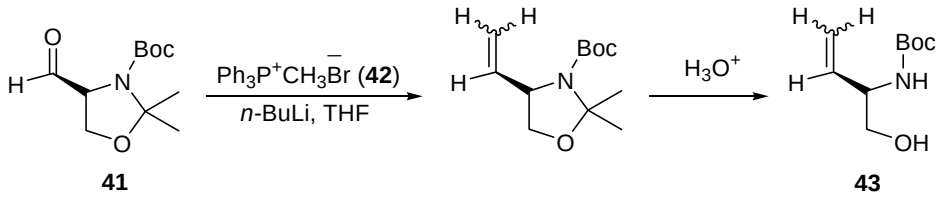
Scheme 1.11: Castelhano's synthesis of *rac*-VG ester **40** *via* Grignard reaction[34]



Many methods were demonstrated in the literature to synthesize racemic vinylglycine but synthetic routes to make enantiomerically pure VG in defined doubled bond geometry (*E/Z*) are less common. In 1991, Beaulieu and coworkers were successful in providing a synthetic route towards enantiomerically pure vinylglycine from chiral starting material serine.[19] L-Vinylglycine derivatives were obtained from D-serine and similarly D-vinylglycine derivatives from L-serine. Beaulieu did not succeed in disclosing a high yielding method to synthesize vinylglycine but was

successful in making other vinylglycine derivatives in high yields and optical purity. The method utilizes Wittig condensation of methyltriphenylphosphorane **42** with protected form of aldehyde **41** obtained from serine to afford protected olefinic product **43** in 27% yield upon acid hydrolysis (Scheme 1.12). Boc-deprotection and oxidation would yield VG. Oxidation was never attempted on **43** and we presume that a strong oxidizing agent would isomerize in to an α,β-unsaturated amino acid. The stability of Wittig ylide would effect the *E/Z* configuration thereby giving a chance to control the double bond stereoisomerism.

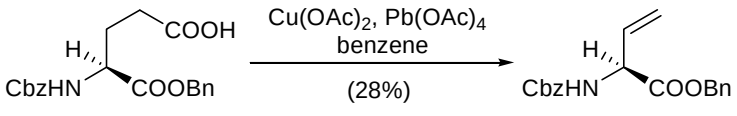
Scheme 1.12: Beaulieu's synthesis of vinyl glycinol **43** *via* Wittig reaction[34]



41 **43**

Townsend et al.[12] reported a modification of Hanessian method[32] to obtain an alternately protected VG in optically pure, crystalline form (Scheme 1.13). This method was developed while working to synthesize L-β-hydroxy ornithine, which was thought to be implicated in the biosynthesis of β-lactamase inhibitor clavulanic acid. Switching the methyl ester with benzyl ester **44** helped to obtain optically pure protected L-VG **45** as fine white needles.

Scheme 1.13: Townsend's synthesis of L-VG ester **45** by Pb-mediated decarboxylation.[12]

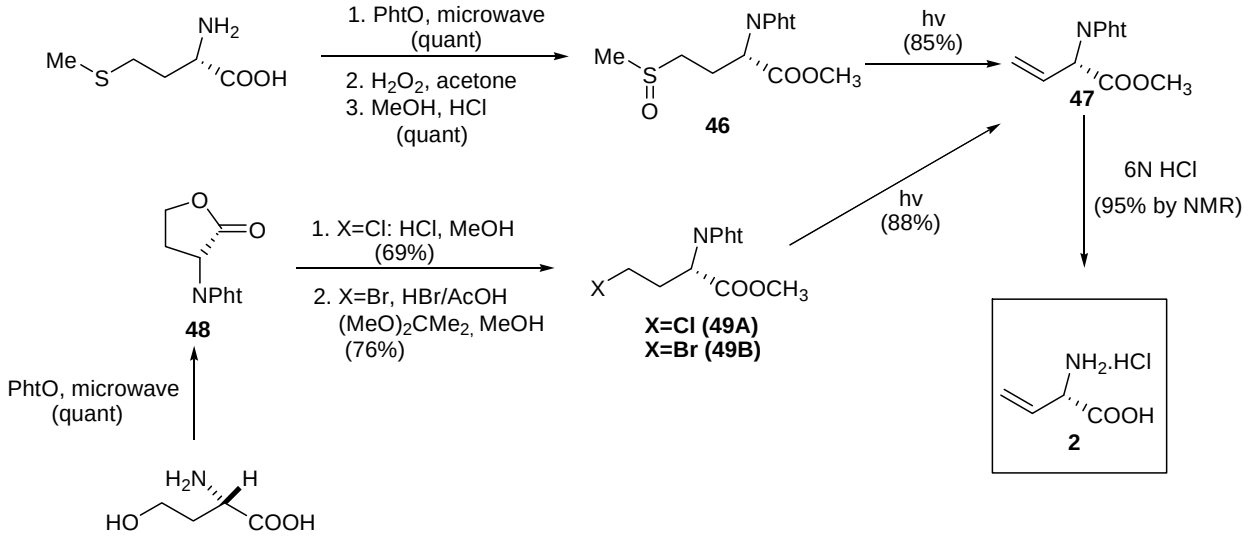


44 **45**

Thus far, several routes involving thermal processes on different amino acids (methionine, glutamic acid, serine and homoserine) leading to enantiomerically pure and racemic vinylglycine were discussed. In 1995, Griesbeck experimented on photochemistry and proposed two routes to synthesize optically pure VG in acceptable yields from L-methionine.[35] However, the first route produced poor yields when more concentrated substrate solutions (>10 mM) were used because of the secondary photochemical reactions, which made this work less attractive. Nevertheless, the second route was proposed to be used for scale-up processes but has not been attempted to date.

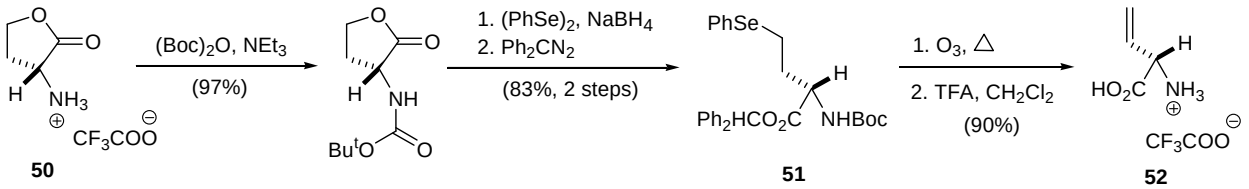
The first photochemical approach used *N*-phthaloyl protected methionine sulfoxide **46** which upon irradiation at 300 nm using photoreactor (800 W) in non-sensitizing solvent acetonitrile produced protected VG **47** in 85% yield (Scheme 1.14).[35] Deprotection of unpurified **47** using 6N HCl yielded vinylglycine **2** in 95% by NMR. The second approach is more straightforward using the chloro and bromo derivatives (**49A, 49B**) of photo stable *N*-phthaloyl homoserine lactone **48**, available from methionine or homoserine. Irradiation of these derivatives gave quantitative conversion to protected vinylglycine **47**. Deprotection using 6N HCl effectively produced vinylglycine with an overall yield of 50-70%. Griesbeck applied this photochemical route to a high molecular weight dipeptide and produced significant conversion to the elimination product (alkene) which was supposed to be less accessible through thermal processes. Inability to reproduce this protocol for scale-up synthesis has made the work less promising.

Scheme 1.14: Griesbeck's synthesis of L-VG by photolysis of esters **46** and **49**[35]



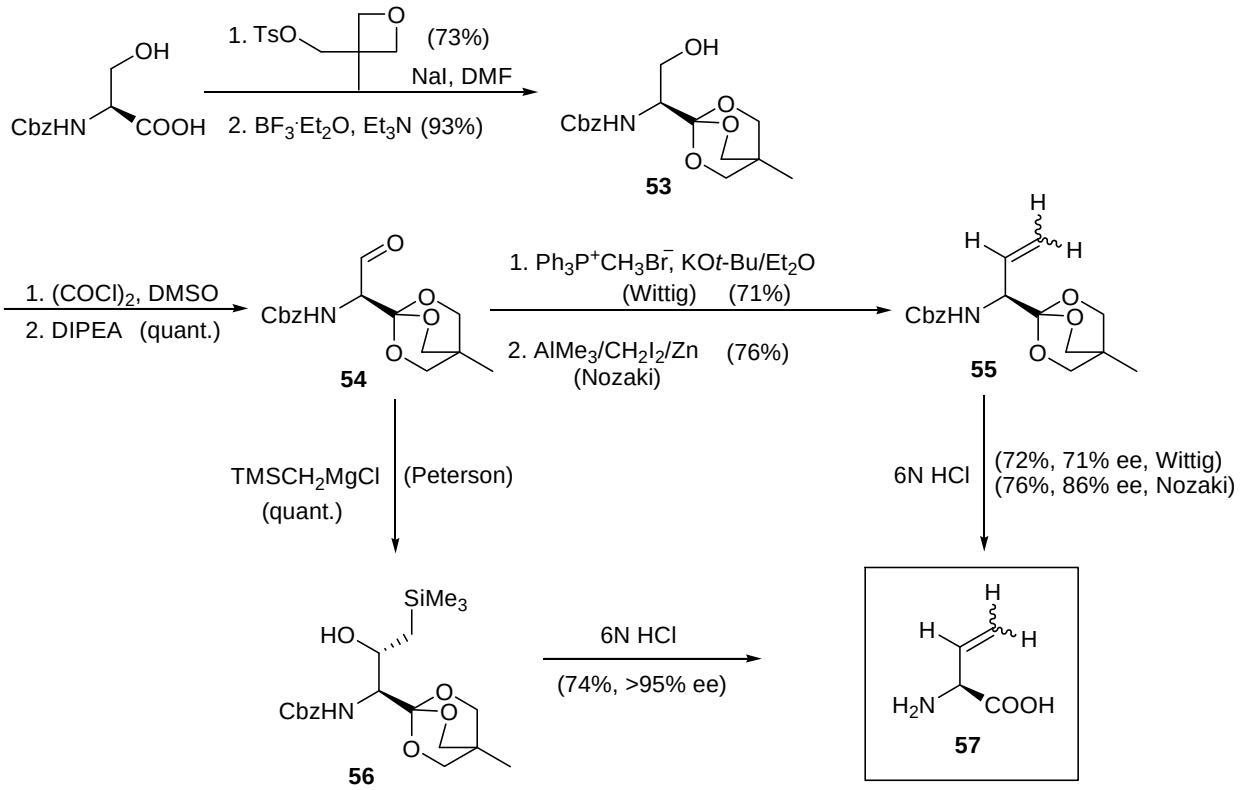
Pelliciari et al. have proposed a route employing L-homoserine as the chiron and aryl selenoxide elimination chemistry to obtain L-vinylglycine in good yields.[36] Using a similar protocol, Berkowitz et al. (1996) developed a high yielding practical route based on selenoxide elimination chemistry starting from L-homoserine lactone (HSL) **50.**[37] Boc protection of amine **50** followed by ring opening with the introduction of phenyl selenium group to form protected selenium compound **51** in good yield. Syn elimination preceded by selenium oxidation and subsequent deprotection afforded L-vinylglycine TFA salt **52** in high yields (Scheme 1.15).

Scheme 1.15: Berkowitz's asymmetrical synthesis of L-VG from L-Hsl (**50**)[37]



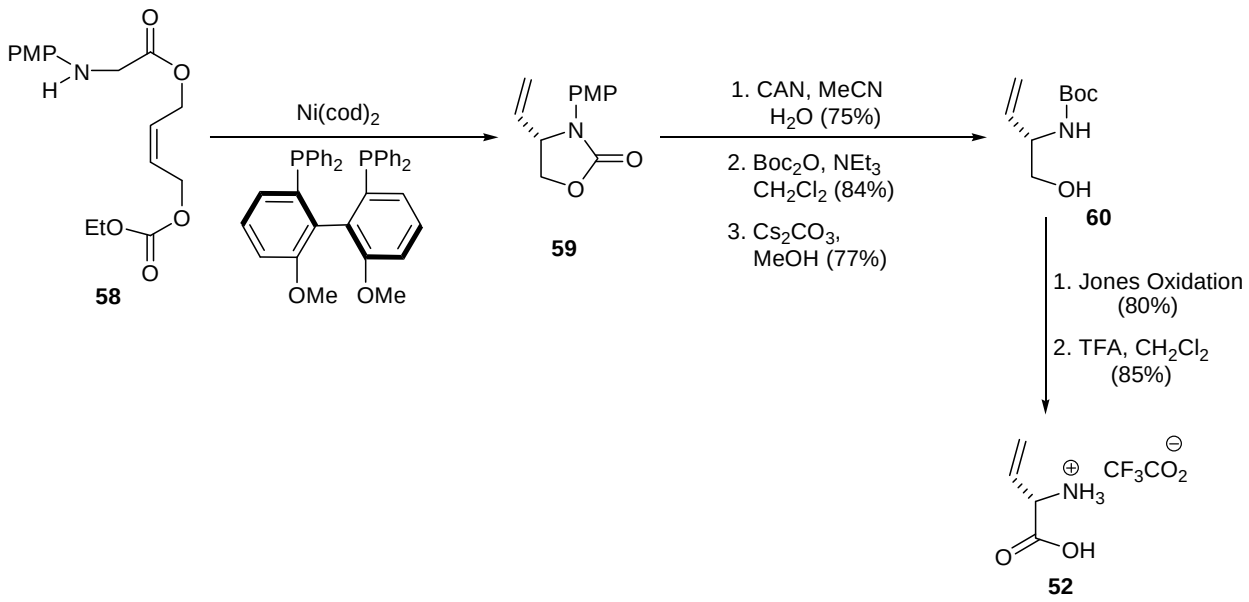
Following the paths of Beaulieu[19], N. G. W. Rose (2001) reported the application of Peterson, Wittig and Nozaki conditions to a protected serinal aldehyde **54**, similar to Beaulieu's protected serine derivative **41**, to yield vinylglycine (Scheme 1.16).[38] *N*-Cbz protected 4-methyl-2,6,7-trioxabicyclo[2.2.2] ortho (OBO) ester **53** was oxidized to aldehyde **54**. Wittig reaction to methylenate the aldehyde was carried out using triphenylmethylphosphorane to yield protected VG **55** in 71 % yield from **53**. ^{13}C-labeled methylenetriphenylphosphorane was used for isotopic induction which produced **55** in 71% yield with 72% ee. Configuration of double bond protons depends on the type of ylide used (stable or unstable). Nozaki conditions (Zn, $AlMe_3$, CH_2I_2) were investigated on the serine aldehyde **54** to yield protected VG **55** in 76% yield. Application of Peterson olefination to the protected serine aldehyde yielded vinylglycine **57** (74% yield, >95% ee). Grignard conditions were used to generate β-hydroxyalkylsilane **56** from the aldehyde **54** using trimethylsilylmethylene magnesium chloride. With the failure of base catalyzed hydrolysis, acidic conditions were screened, which provided elimination and concomitant deprotection to yield **57**. This method also provided a route to isotopically labeled vinylglycine by labeling the carbon in trimethylsilylmethylene magnesium chloride. Deprotection of Cbz-vinylglycine-OBO ester **55** using 6N HCl provided VG **57** in 72% yield with 71% ee *via* Wittig olefination and 76% yield with 86% ee *via* Nozaki olefination. The final olefinic product was obtained in ~40% yield employing all these methods.

Scheme 1.16: Rose's asymmetrical synthesis of L-VG *via* Wittig reaction[38]



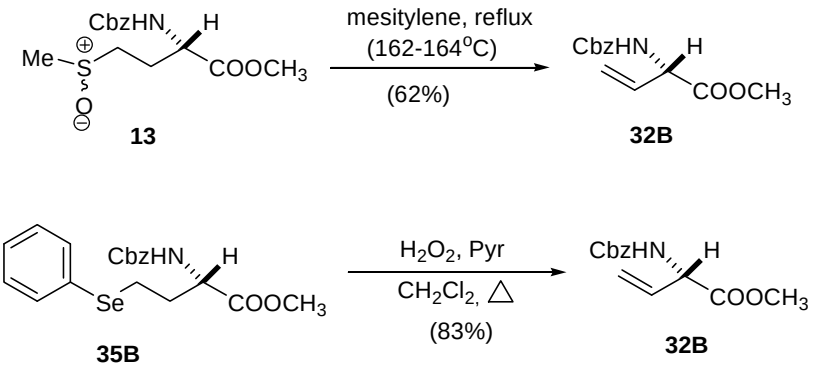
In 2004, Berkowitz et al.[39, 4] reported asymmetric Ni(0)-mediated allylic amination chemistry, which then allowed for a practical entry in to L-vinylglycine (Scheme 1.17). An *N*-protected vinyloxazolin-2-one intermediate **59** generated from **58** *via* an enantioselective, intramolecular allylic amination with a Ni(0)-BIPHEP catalyst system was transformed in to **60**. Jones oxidation and deprotection using trifluoroacetic acid in CH_2Cl_2 yielded the L-vinylglycine TFA salt **52**.

Scheme 1.17: Berkowtiz's synthesis of L-VG via the Ni(0)-BIPHEP chiral catalyst complex[39]



D.M. Bartley and J.K. Coward,[14] in 2005, have proposed slight modifications to the established methods of Rapoport (Scheme 1.3)[27] and Barton (Scheme 1.15).[32] The modifications were made and optimized to utilize VG as a starting material in the synthesis of phosphonic acid containing pseudopeptide to study as inhibitors of FPGS (folylpoly-γ-glutamate synthetase) in tumor control. Rapoport method was modified by using mesitylene (bp 162-164^{o}C) as a solvent to foster the elimination of the protected methionine sulfoxide **13** affording protected VG **32B** in 62 % yield (Scheme 1.18). Use of lower temperature (162-164^{o}C), in contrast to 190^{o}C used by Rapoport's method, helped in reducing the α,β-dehydro isomer **5**. Barton's ozonolysis of selenium compound **35B** and subsequent elimination was modified to a single pot oxidation/elimination reaction using H_2O_2. After screening several reaction conditions and oxidants, oxidation of **35B** with H_2O_2 and pyridine in refluxing dichloromethane was confirmed as the most optimum condition to yield protected VG **32B** in 83% yield.

Scheme 1.18: Coward's modified synthesis of L-VG from sulfoxide **13** and selenide **35B**[14]



The methods presented so far are only a few among the myriad of protocols developed since the discovery of VG. Other methods were also used to yield optically pure, racemic or isotope labeled VG such as Crout's[40] enzymatic kinetic resolution of racemic vinylglycine *via* papain-mediated esterification in biphasic system to yield both antipodes, Sawada's[41] deuterium labeled VG for stereochemical study of different enzymes inhibited by vinylglycine, etc. A number of methods employing chiral compounds were reported that includes Diaz-de-Villegas's[42] stereo communication protocol to induce chirality at an adjacent center utilizing D-glyceraldehyde as the starting material, Meffre's[43] thermolysis of L-methionine sulfoxide in *o*-dichlorobenzene, Mulzer's[44] protocol to obtain D-VG from D-mannitol and Duhamel's[45] induction of asymmetry from external chiral acid source.

Vinylglycine's significance as a chemically and biologically useful compound has prompted many syntheses of **1** both as a racemate and as a pure enantiomer. The disadvantages associated with the methods reported such as poor yields, use of toxic reagents, highly uneconomical schemes in terms of number of steps, purification and inability to simulate the protocol for scale up processes, and use of cost ineffective enzymes, catalysts or starting materials to make optically pure VG has continuously prompted chemists to come up with new

protocols for commercial synthesis. Rapoport's L-methionine sulfoxide method and Berkowitz's seleno chemistry serves as the best protocols to date for practical purposes. Rapoport's method faces the disadvantage of low yield, isomerization and racemization at high temperatures which in turn makes the separation process tedious. Berkowitz's seleno chemistry employs costly and toxic reagents which limits its use for large scale synthesis. The seleninic acid byproduct produced in the final elimination step is highly toxic and requires an extra effort to dispose without harming the environment.

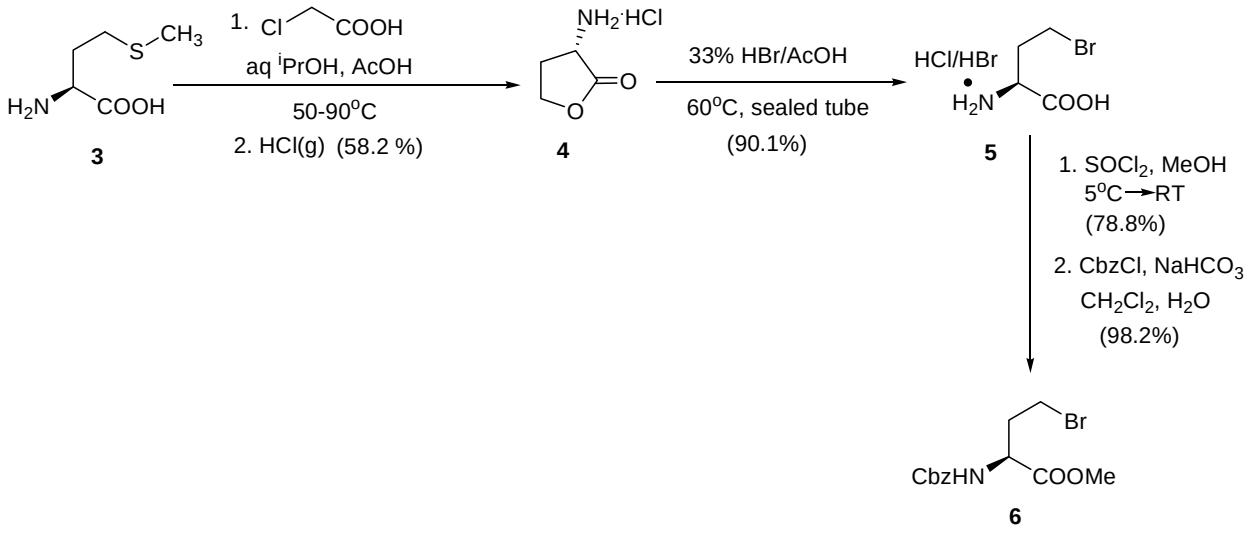
To efficiently answer these limitations, our lab has undertaken a study to provide a cost-effective, yet high yielding protocol that employs non-toxic reagents and avoids problems of side products formation, namely α,β-unsaturated isomer **5**. The following chapter describes the study on the thermolysis of alkyl and aryl substituted homocysteine analogs employed in synthesizing optically pure vinylglycine.

CHAPTER II

RESULTS AND DISCUSSION

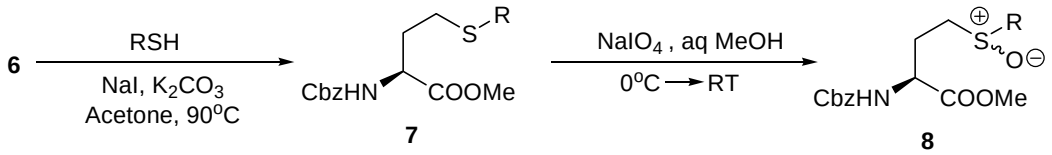
As discussed in chapter I, Rapoport's methionine method is the best practical protocol to date to synthesize optically pure vinylglycine with relatively cheap starting material in acceptable yields. The harsh conditions used (190^{o}C, 3 mmHg) were thought to be a reason for a considerable decrease in the yields by forming an α,β-isomer **2** and other degradation products. The novel route involving the change of substituent (-CH_3) on methionine's sulfur atom with longer alkyl chains was never attempted before and we thought that this route would solve the limitations of Rapoport's method by giving more resistant analogs **(8a-g)** to withstand the harsh conditions. Retrosynthetic analysis led to a route starting with a salt of bromide **5**. As reported previously,[46] **5** was obtained from homoserine lactone **4** by hydrobromination in 90.1% yield (Scheme 2.1). The reaction was performed in a sealed tube to avoid loss of HBr gas. Lactone, on the other hand, was synthesized from L-Met **3** when reacted with chloroacetic acid and HCl (gas) in 58.2% yield. The literature procedure[47] utilized bromoacetic acid and bromine being a better leaving group rendered good results in terms of yield. Though the yield was compromised, the inexpensive chloroacetic acid made our method economically advantageous. The carboxylic acid group of **5** was then protected with methyl ester in 78.8% yield and amine protection with Cbz proceeded with 98.2% yield. The amine of bromide was also protected using Boc and phthalamido groups, but the non-chromophoric nature of the former (for monitoring the reaction using TLC and chromatographic separations) and low yield in the latter case precluded their use.

Scheme 2.1: Synthesis of *N,O* protected bromide **6**



Our attempt to make the unreported homocysteine analogs, sulfides (Hcy) and sulfoxides (HcyO), was successful with good yields. Nucleophilic thiolation of bromide **6** under Finkelstein's conditions[48] using catalytic amount of sodium iodide afforded Hcy(alkyl) esters **(7a-h)**. The mechanism proceeds with *in situ* replacement of bromine with iodine and the latter being a better leaving group aids in the acceleration of the reaction. The reactions were performed in a sealed tube to prevent thiol and solvent (acetone) evaporation when subjected to high temperature (90°C) to yield alkyl sulfides **(7a-h)** in 52-88% yields (Scheme 2.2, table 2.1). Ester **7e** was failed to form by this method as steric hindrance from the *t*-butyl group was thought to be a plausible reason. This reason also provided an explanation for low yield (52.3%) when *i*-propyl thiol **7c** was used. Oxidation of the alkyl sulfides by aqueous sodium periodate (mild) provided alkyl sulfoxides **(8a-g)** as a mixture of diastereomers in good yields (Scheme 2.2, Table 2.1). The low yield in case of protected HcyO(*i*-propyl) **8c** was not surprising because of the possible steric hindrance.

Scheme 2.2: Synthesis of alkyl homcysteine sulfides and sulfoxides



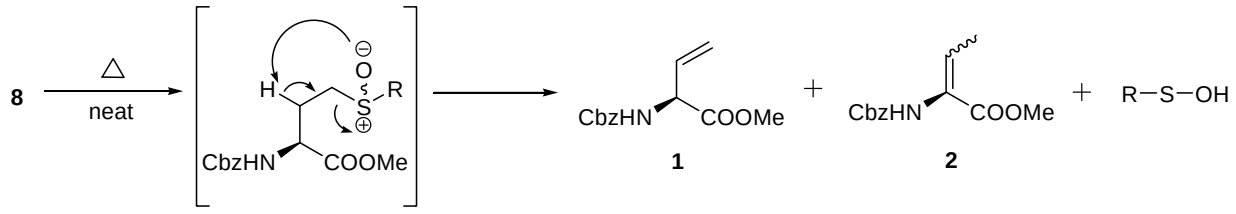
R= Et,*n*-Pr, *i*-Pr, *n*-Bu, *t*-Bu, *n*-Hex, *n*-Oct, *n*-Dec

Table 2.1: Yields of protected Hcy(alkyl) **(7a-h)** and HcyO(alkyl) **(8a-g)**

R=	Yield, % (**7**)	Yield, % (**8**)
ethyl	76.3 (**7a**)	92.6 (**8a**)
n -propyl	81.8 (**7b**)	74.2 (**8b**)
i-propyl	52.3 (**7c**)	11.4 (**8c**)
n -butyl	66.7 (**7d**)	87.4 (**8d**)
t-butyl	0 (**7e**)	-
n -hexyl	88.1 (**7f**)	99.6 (**8e**)
n-octyl	81.9 (**7g**)	68.1 (**8f**)
n-decyl	70.2 (**7h**)	83.6 (**8g**)

Alkyl analogs, subjected to different temperatures and reaction times (Scheme 2.3, Table 2.2), were studied for the best R group in terms of yield, purity and optimum reaction conditions. The reactions were conducted for each sulfoxide on a 0.1 mmol scale at 145°C, neat with agitation over 3 days. ^{1}H NMRs were taken every day to monitor the progress of the reaction and to provide ratio estimates of (*S*)-Cbz-VG-OMe **1**, α,β-unsaturated isomer **2**, and starting materials **(8a-g)** (Table 2.2). Not surprisingly, the syn elimination rates were very slow for all the analogs. A mechanism of syn elimination is illustrated in Scheme 2.3.

Scheme 2.3: Syn elimination of alkyl sulfoxides **(8a-g)** under thermolytic and pyrolytic conditions



Met- and Et- analogs showed considerable amounts of isomer formation after 3 day agitation with very less desired product and a significant amount of starting material. The best results were obtained for Bu- group **8d** (49.8% yield), but slow elimination rates and substantial amounts of isomer formation when exposed to prolonged heat were the common notion among all the sulfoxides. In an attempt to decrease isomer formation, the duration was decreased by exposure to high temperature 190^{o}C and low pressure (3 mmHg). Though the ^{1}H NMR ratio showed a low amount of the isomer and complete depletion of starting material, the isolated yield was low in case of HcyO(*n*-Bu) **8d**, when exposed to 190^{o}C under vacuum (3mm Hg), presumably due to the decomposition on silica. Overall, the alkyl analogs were not effective in preventing the traditional problems associated with VG synthesis (i.e. isomerization and low yields). Results for alkyl substituents are tabulated in Table 2.2. Our attention was next turned to the search for better leaving groups to enhance elimination under thermolytic conditions.

Table 2.2: Syn elimination results of HcyO(alkyl) esters **(8a-g)**

R=	°C	time (h)	mmHg	**1:2:8**[a]	yield, %[b]
Me	145	72	760	0.3:0:1	12.3[c]
Me	190	2	760	1:0.7:0.7	1.2[c]
Et (**8a**)	145	72	760	0.8:0.3:1	-
n-Pro (**8b**)	145	42	760	0.6:0.1:1	-
n-Pro (**8b**)	145	72	760	0.5:0.4:1	47.1
i-Pro (**8c**)	145	72	760	0.2:0:1	-
n-Bu (**8d**)	145	42	760	0.8:0.1:1	-
n-Bu (**8d**)	145	72	760	0.7:0.3:1	49.8
n-Bu (**8d**)	190	5	3	1:0:0.2	30.3
n-Hex (**8e**)	145	72	760	1:0:0.8	35.1
n-Oct (**8f**)	145	72	760	1:0.1:0.7	18.2
n-Dec (**8g**)	145	72	760	1:0.1:1.4	41.3

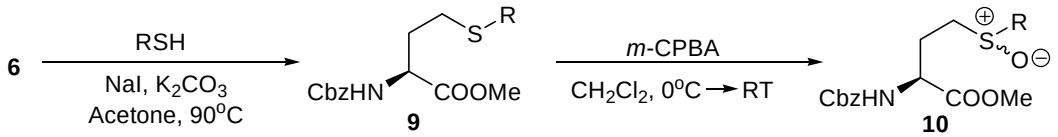
[a] Est. based on integrations in crude ^{1}H NMR. [b] Isolated yield.

[c] Contained α,β-unsaturated isomer **2**

As noted from the results in Table 2.2, the alkyl sulfoxides were not effective, as presumed, to yield enantiomerically pure vinylglycine by syn elimination. Significant amount of isomer formation and the presence of starting material after 3 days of reaction time were few of the limitations of alkyl analogs. As a continuum to this study, aromatic substituents were examined hoping to yield better results. Alkyl and aryl substituents share a different electronic arrangement, chemical reactivities and leaving group capabilities, which we thought would help in enhancing the syn elimination.

The *(S)*-Hcy(aryl) analogs (**9a-f**) were prepared in the same manner as described for alkyl analogs. The benzyl analog **9a** was obtained as a mixture of closely spaced spots from TLC which was difficult to isolate, therefore it was carried to the next reaction without purification. Oxidation was carried out using a stronger oxidizing agent *m*-CPBA, since sodium periodate gave low yields (Scheme 2.4, Table 2.3). Benzoic acid is the byproduct in *m*-CPBA assisted oxidation reactions. Almost all the Hcy(aryl) analogs were oxidized within one hour. Careful monitoring of the reaction using TLC was required as longer reaction times gave a double oxidized sulfur compound.

Scheme 2.4: Synthesis of aryl Homocysteine sulfides and sulfoxides



R= Bn, Ph, *p*-MeOPh, *p*-ClPh, *p*-NO_2Ph, *o*-NO_2Ph

Table 2.3: Yields of protected Hcy(aryl) **(9a-f)** and HcyO(aryl) **(10a-f)**

R=	Yield, % (**9**)	Yield, % (**10**)
Benzyl	Not isolated	54.1 (**10a**, after 2 steps)
Phenyl	89.0 (**9b**)	89.1 (**10b**)
p-MeOPh	45.6 (**9c**)	89.6 (**10c**)
p -ClPh	77.8 (**9d**)	95.8 (**10d**)
p-NO_2Ph	62.2 (**9e**)	62.7 (**10e**)
o-NO_2Ph	94.9 (**9f**)	95.3 (**10f**)

Phenyl sulfoxide compound **10b** was the first analog used to evaluate elimination conditions (Scheme 2.5, Table 2.4). Sulfoxide **10b** was subjected to thermolysis at 145^{o}C under neat conditions, which after 18 h converted to olefin **1**, trace amounts of deoxygenated Hcy(Ph) **9b** and α,β-isomer **2** quantified from relative peak areas in crude ^{1}H NMR. However, chromatographic purification on silica yielded only 54.1% of pure *(S)*-Cbz-VG-OMe, in part, due to the decomposition on the column and increased degradation products (undetected on TLC or NMR) when exposed to high temperatures. Therefore additional conditions were screened including reduced pressure (3 mmHg) and higher temperature (190^{o}C/0.25 h). ^{1}H NMR after 10 h of pyrolysis (3 mmHg) on **10b** at 145^{o}C showed the formation of side product **9b** while some amount of the starting material still remained unreacted conveying the fact that pyrolysis leads to more deoxygenated product though the exposure time is relatively less (10 h) compared to thermolysis (18 h). Chromatographic separation at this stage yielded 57.9% of pure protected VG **1**. In the latter case (190^{o}C, 0.25 h), isolated yield was less and contaminated with isomer. At temperatures less than 140^{o}C, the elimination rate decreased substantially and the reduced side product increased in proportion to longer durations of heat exposure.

Scheme 2.5: Syn elimination of aryl sulfoxides **(10a-f)** under thermolytic and pyrolytic conditions

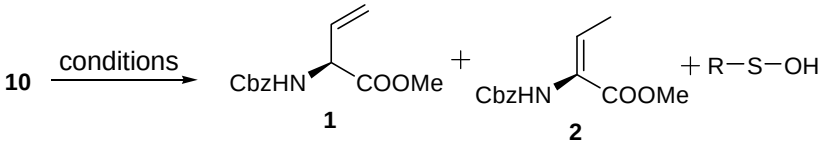


Table 2.4: Syn elimination results of HcyO(aryl) esters **(10a-f)**

R=	^{o}C	time (h)	mmHg	**1:9:10**[a]	yield, %[b]
Bn (**10a**)	145	72	760	0.6:0:1	29.9
Bn (**10a**)	145	10	3	0.1:0:1	-
Ph (**10b**)	145	18	760	1:0.1:0	54.1
Ph (**10b**)	145	10	3	1:0.3:0.3	57.9
Ph (**10b**)	190	0.25	760	1:0:0.3[c]	25.0
p-MeOPh (**10c**)	145	18	760	1:0.4:0.2	35.5
p-MeOPh (**10c**)	190	0.25	760	1:0.4:0.2[c]	9.2
p-ClPh (**10d**)	145	18	760	1:0:0[c]	41.0
p-ClPh (**10d**)	190	0.25	760	1:0.1:0[c]	67.0
p-NO_2Ph (**10e**)	145	19	760	1:0:0[c]	53.9
p-NO_2Ph (**10e**)	190	0.25	760	1:0:0	62.9
o-NO_2Ph (**10f**)	145	0.25	760	1:0:1.3	-
o-NO_2Ph (**10f**)	145	1	760	1:0:0	35.5
o-NO_2Ph (**10f**)	100	18	760	1:0:2.8	-

[a] Est. based on integrations in crude 1H NMR. [b] Isolated yield.

[c] Contained *α,β*-unsaturated isomer **2**

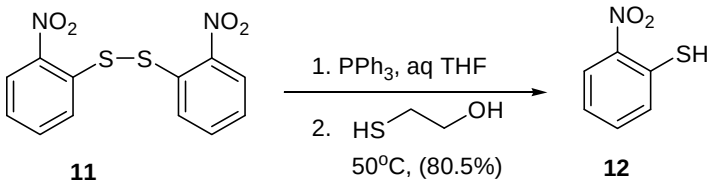
HcyO(Bn) **10a** was the next analog evaluated against thermolysis at 145^{o}C for 72 h. The reaction was monitored every day using TLC and it did not confirm the presence of the olefinic product **1** until 72 h. Crude ^{1}H NMR showed the presence of product and appreciable amounts of starting material. Chromatographic separation at this stage yielded only 29.9% of pure Cbz-VG-OMe **1**. Even the pyrolysis conditions (145^{o}C, 10 h) were not effective towards syn elimination of **10a** and therefore further studies were not continued on this compound.

Attention was next turned to substituted phenyl analogs **(10c-10f)** with electron withdrawing and electron donating groups. Except HcyO(*p*-MeOPh) **10c**, all the analogs revealed comparable results. Sulfoxide **10c** appeared to have greater susceptibility to form deoxygenated product **9c** at both lower (145^{o}C, 18 h) and higher temperatures (190^{o}C, 15 min) with isolated yields of 35.5% and 9.2% respectively. The low yield in case of the latter was due to a combination of factors like the presence of α,β-isomer, starting material, deoxygenated product and decomposition on silica. This is the only compound among the substituted analogs with sulfoxide remaining unconverted after 18 h. This result was not unexpected as the research from Emerson[49] established that *para*-substituted electron donating groups slowed the pyrolysis of aryl *n*-propyl sulfoxides while electron withdrawing groups responded in an opposite way.

Thermolysis on HcyO(ClPh) **10d** and HcyO(*p*-NO_2Ph) **10e** revealed that the reactions were complete within 18-19 h at 145^{o}C in acceptable yields and trace amounts of isomer. Also we determined that the time of thermolysis could be reduced to 15 min by increasing the temperature to 190^{o}C by compromising a trace amount of deoxygenated product formation. Yields in the latter case were good with the chloro substituted analog affording 67% of pure VG **1**.

Sharpless and Young[50] proposed that the electron-withdrawing nitro group located *ortho* would accelerate the syn elimination of aryl selenoxides to yield *o*-nitrophenyl selenenic acid and 1-dodecene at 25^{o}C. This study prompted us to screen the conditions for syn elimination of HcyO(*o*-NO_2Ph) **10f** which required the synthesis of 2-nitrothiophenol **12** from the reduction of disulfide **11** (Scheme 2.6).

Scheme 2.6: Synthesis of 2-nitrothiophenol



When **10f** was subjected to heat (145°C, neat), decomposition was observed within minutes (monitored based on the color change from colorless to dark brown) and after 1 h, ^{1}H NMR analysis confirmed the complete conversion to VG **1** with no isomerized and deoxygenated products. The crude material was chromatographed on silica to provide pure Cbz-VG-OMe in 35.5% yield. Temperatures as low as 100°C were also found to catalyze the syn elimination for this particular analog. One limitation of the study was that all the analogs (alkyl and aryl) decomposed on silica during purification, which became a major determinant in decreasing the yields.

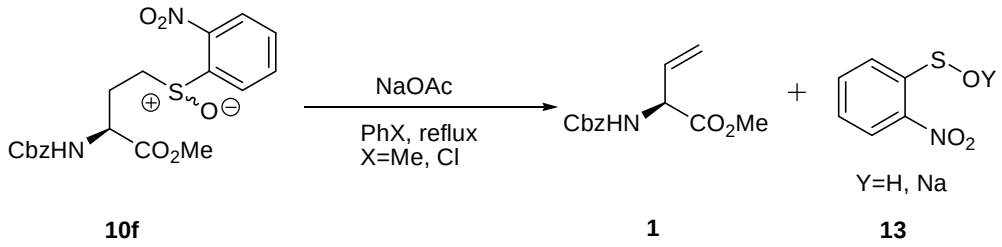
Therefore, in an effort to tackle this drawback, a novel idea to reflux HcyO(*o*-NO_2Ph) **10f**, which gave good results among aryl sulfoxides, in organic solvents to precipitate the water soluble sulfenic acid **13** and removal by filtration was explored. Different solvents were screened including chloroform, benzene, *n*-butanol, ethylbutyrate and xylenes however; higher boiling non polar, aprotic solvent such as toluene was required to complete the transformation (Table 2.5). Unfortunately, a small portion of the sulfenic acid decomposed into several toluene-soluble impurities and we began to explore the conditions to limit this outcome.

Table 2.5: Effect of different solvents on syn elimination of **10f**

Solvent	Result
Chloroform	No conversion
Benzene	Florescent impurities
n-butanol	Transesterification
Ethylbutyrate	Transesterification
Xylenes	Fluorescent impurity
Toluene	Total conversion, sulfenic acid impurity

Reactions supplemented with different bases were screened to neutralize the sulfenic acid product **13** and prevent its decomposition. Pyridine, $NaHCO_3$, and K_2CO_3 were effective but each catalyzed partial isomerization. Sodium acetate (NaOAc), on the other hand, prevented impurity formation by precipitating the sulfenic acid/salt **13** which was removable by filtration (Fig. 2.1, Scheme 2.7). ^{1}H NMR analysis of the toluene filtrate revealed >99% conversion to VG **1** and the absence of side products. Substitution with chlorobenzene (higher boiling point) gave an added advantage of reduced reaction time to under 6h. However, prolonged reflux (>10 h) with chlorobenzene resulted in trace amounts of isomer **2**.

Scheme 2.7: Thermolysis of sulfoxide **10f** under mild toluene reflux



10f **1** **13**

Before reflux

NaOAc suspended in a PhMe solution of the 2-nitrosulfoxide. Yellow coloration is due to the nitrophenyl group.

After reflux

Acid **13** absorbs onto the NaOAc turning it brown. The solution containing the olefin is colorless due to precipitation of the nitrophenyl sulfenic acid byproduct.

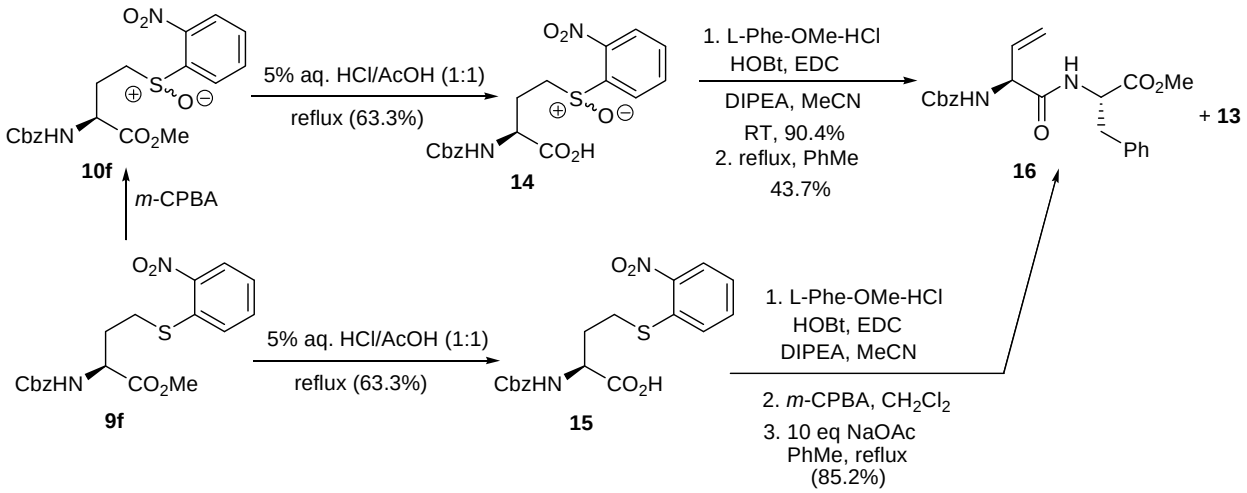
Fig. 2.1: Precipitation of sulfenic acid byproduct

A utility of this protocol was next applied towards the synthesis of the dipeptide **16** (Scheme 2.8). Two routes were evaluated to optimize and infer the differences in the yields with the presence and absence of sodium acetate. First method started with *S*-oxidation of sulfide **9f** followed by saponification yielding the acid product **14** in 63.3% yield. Dipeptide formation was achieved by EDC-mediated coupling of the acid **14** with L-Phe-OMe. Thermolysis of the so formed dipeptide by toluene reflux in the absence of the base NaOAc provided vinyl dipeptide **16** in 43.7% yield after filtration of sulfenic acid byproduct **13**. Some amount of dissolved sulfenic acid byproducts was observed considerably decreasing the yield.

To increase the yield, an alternate method was evaluated whose preparation began with saponification of sulfide **9f**, followed by an EDC-mediated coupling of the resulting acid with L-Phe-OMe (Scheme 2.8). Coupling reaction *via* the *S*-oxidized saponified product gave low yield, therefore oxidation after peptide bond formation was preferred. Subsequent *S*-oxidation with *m*-CPBA and thermolysis of the sulfoxide formed under toluene reflux with 10 eq of NaOAc

resulted in the precipitation of the acid **13** while yielding 85.2% of pure vinyl dipeptide **16**. The latter method clearly states the significance of using NaOAc to precipitate even the toluene soluble byproducts and produce pure VG derivative **16** in good yield.

Scheme 2.8: Synthesis of vinyl dipeptide **16**



CONCLUSIONS

These studies on homocysteine alkyl and aryl derivatives led to a novel route using inexpensive starting materials to incur enantiomerically pure VG in appreciable yields. The utility of the *o*-nitrophenyl sulfoxide derivative **10f** was a vertical advancement which can serve as an alternative to toxic and expensive aryl selenoxides. Isomerization, racemization and use of toxic and expensive chemicals were some of the limitations surrounding the large scale synthesis of optically pure VG, which are efficiently answered with our method of mild toluene reflux. Additionally, the simple filtration method to remove the sulfenic acid byproduct thereby avoiding the tedious chromatographic separations that are expensive, time consuming and yield decreasing, adds to the efficacy of this method. The protocol is of high synthetic utility to obtain compounds with heat- or oxidant-sensitive olefin moieties. This method serves as an efficient

replacement to selenoxide chemistry and Rapoport's pyrolysis which served as practical entries to large scale synthesis of VG.

FUTURE DIRECTIONS

The ultimate goal of this project would be to synthesize novel cephalosporins utilizing the protocol developed to synthesize olefinic compounds by mild toluene reflux (Fig. 2.2). The idea is to start from a hydroxylamine protected *o*-nitrophenyl sulfoxide derivative and convert it to an olefinic compound by toluene reflux. This olefin can be subjected to cross metathesis using Grubbs catalyst or Heck reaction to afford a diene compound. Hetero-Diels-Alder chemistry on this compound leads to a cyclized product which upon deprotection and conjugation with other compounds to form peptide bonds would yield novel cephalosporin derivatives. These derivatives will be screened against different strains of bacteria to test their antibacterial potency.

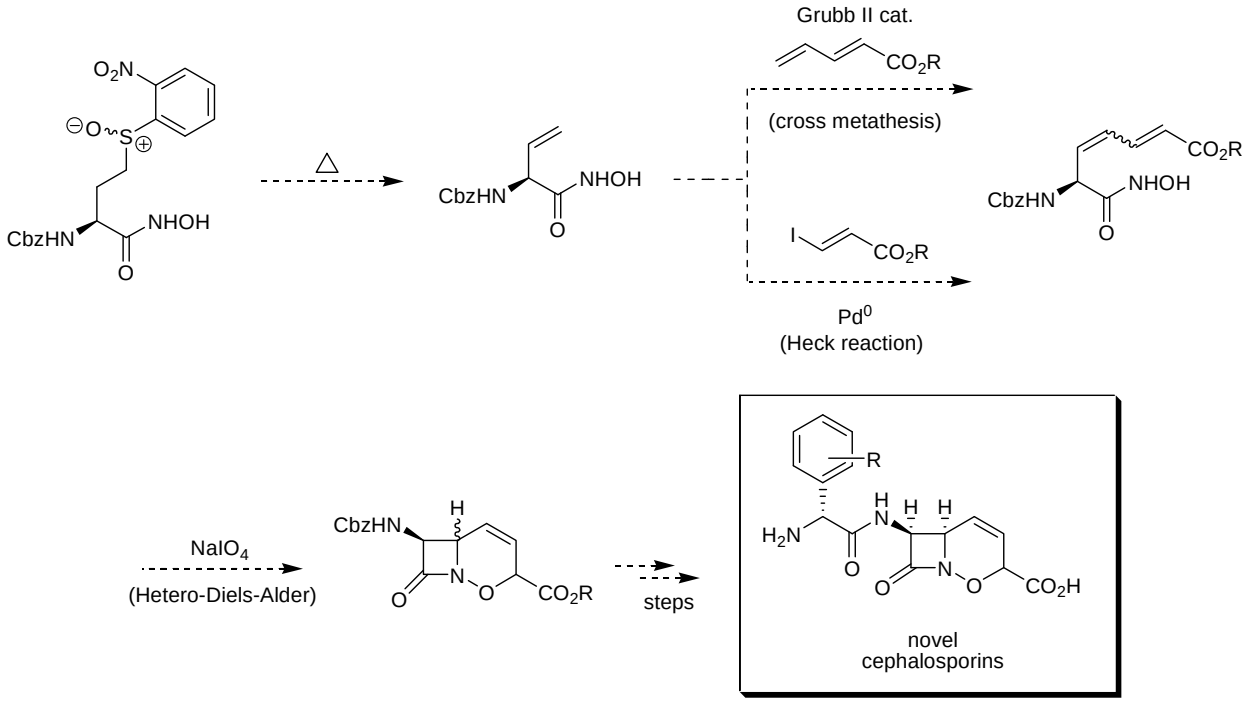


Fig. 2.2: Proposed synthesis of cephalosporins

CHAPTER III

HALOENOL LACTONES

Haloenol lactones (HEL) are family of compounds with 5- or 6-membered lactone ring structure. They have been shown to be suicide inhibitors of serine proteases like α-chymotrypsin, trypsin and human neutrophil elastase (HNE).[51] The former two proteolytic enzymes were shown to be oncogenic in different cancers, whereas, over expression and genetic mutation of the latter may lead to emphysema and congenital neutropenia respectively.

The target protease enzyme acts by revealing the latent reactivity of these molecules through the exposure of reactive electrophilic species for alkylation of the enzyme.[52] The high degree of target specificity of these molecules lies in the potential to generate reactive electrophilic moiety exclusive to the active site. A haloenol lactone acts by hydrolysis of the lactone by the enzyme and subsequent acyl transfer to the active site hydroxyl group (serine's hydroxyl group) releasing an α-haloketone electrophilic moiety that would alkylate the accessible nucleophilic residues at the active site (Fig. 3.1).

HEL also acts as a site-directed inactivator of glutathione S-transferase π isozyme (GST-π).[53] GST, a detoxifying phase-II metabolizing enzyme, which has been proposed to be one of the biochemical mechanisms for acquired drug resistance, a serious impediment towards the use of cancer chemotherapy.

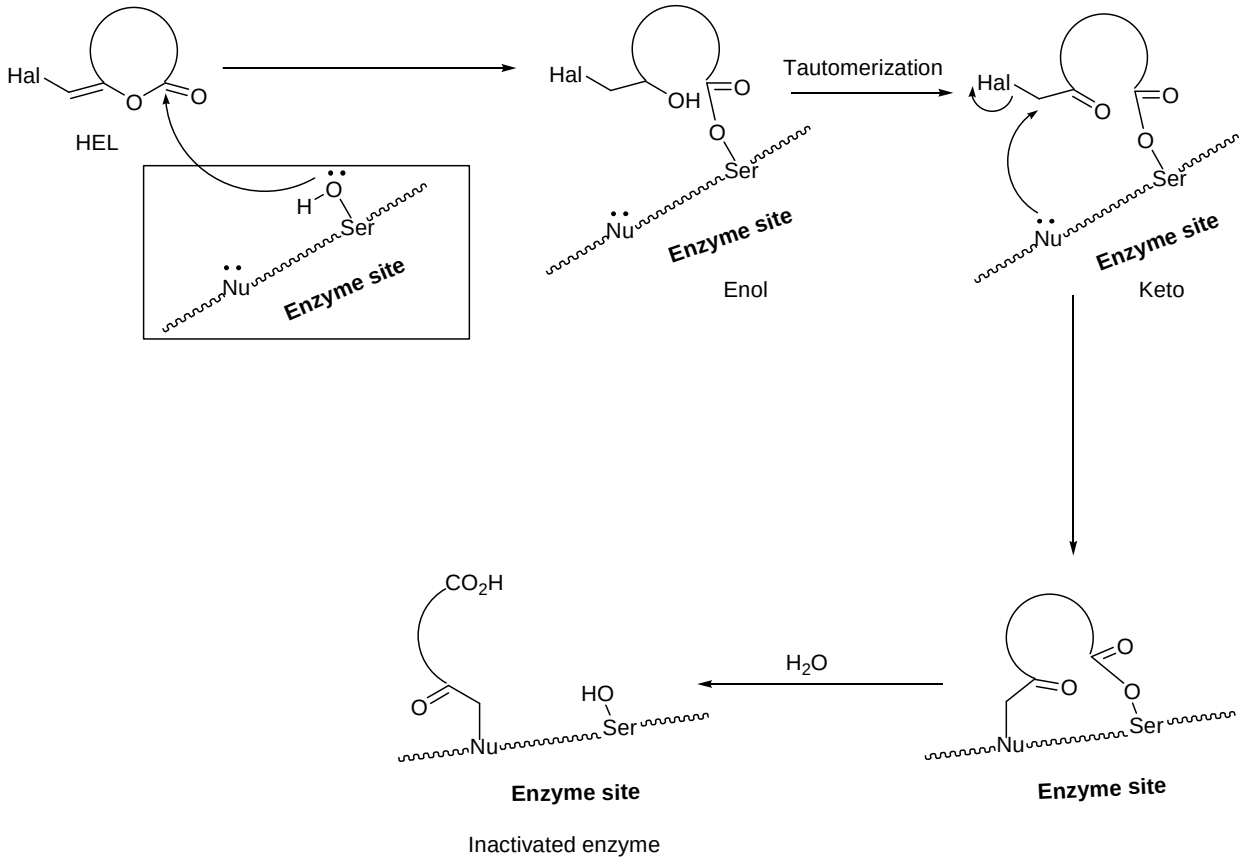


Fig. 3.1: Mechanism-based inhibition of protease enzyme by HEL

Bromoenol lactone (BEL), a member of the haloenol lactone family, is a suicide substrate of different enzymes namely serine proteases like chymotrypsin, trypsin, HLE and also an important enzyme involved in cancer cell growth, group VI phospholipase ($iPLA_2$). $iPLA_2$ acts on membrane phospholipids to release arachidonic acid and lysophosphatidic acid (LPA) which are involved in cell proliferation and angiogenesis through the EGFR and GPCR pathways. Metabolites of arachidonic acid (AA), called eicosanoids, were described as mitogens in one or more cell types particularly of tumor origin.[54] Herbert hypothesized that $iPLA_2$ plays a central role in the regulation of endothelial cell proliferation and angiogenesis.[55]

PAP-1, a key enzyme in cellular phospholipid metabolism that helps maintain phosphatidyl choline (PC) homeostasis[56] was also studied as a target for anticancer agent, BEL. At high concentrations of 10-25 μM, BEL was proved to inhibit PAP-1 leading to cell death.[56] Another mechanism by which BEL was proposed to inhibit cell growth is through its effect on mitochondrial membrane leading to cell death. In mitochondria-dependent apoptosis, mitochondrial membrane permeabilization resulting in the release of proteins. Interference of the inner mitochondrial membrane subsequently altering the mitochondrial membrane resulting in irreversible DNA degradation also occur leading to cell death. $iPLA_2$ inhibition leads to apoptosis through a cascade of biochemical reactions and this aspect of BEL's activity interested us to research further and apply the findings to design novel BEL analogs as candidate anticancer and antibacterial agents.

From the recent findings, it came to our attention that BEL's use is implicated in the control of prostate cancer. Sun et al.[57] confirms the role of BEL in inhibiting $iPLA_2$ and thereby controlling the prostate tumor growth through different cell signaling pathways. The study proves that racemic BEL acts as a selective suicide inhibitor of Ca^{2+} independent $iPLA_2$ thereby impeding the cell growth in both LNcap (androgen sensitive, p53-dependent) and PC-3 (androgen insensitive, p53-independent) cell lines. G1/G0 arrest (p53-dependent) in the cell cycle was proposed to be the mechanism of cell death in the former, while the latter is effected by G2/M (p53 independent) arrest. Similar to other HELs, bromoenol lactone compounds are acted upon by $iPLA_2\beta$ to expose the bromo methyl ketone moiety, which acts as an electrophilic center to alkylate the thiol group of the cysteine residue at the enzyme site causing irreversible inhibition of the enzyme (Fig. 3.2).[58] The BEL analogs synthesized were sent to Dr. Cummings' lab for further study on prostate cancer cell lines.

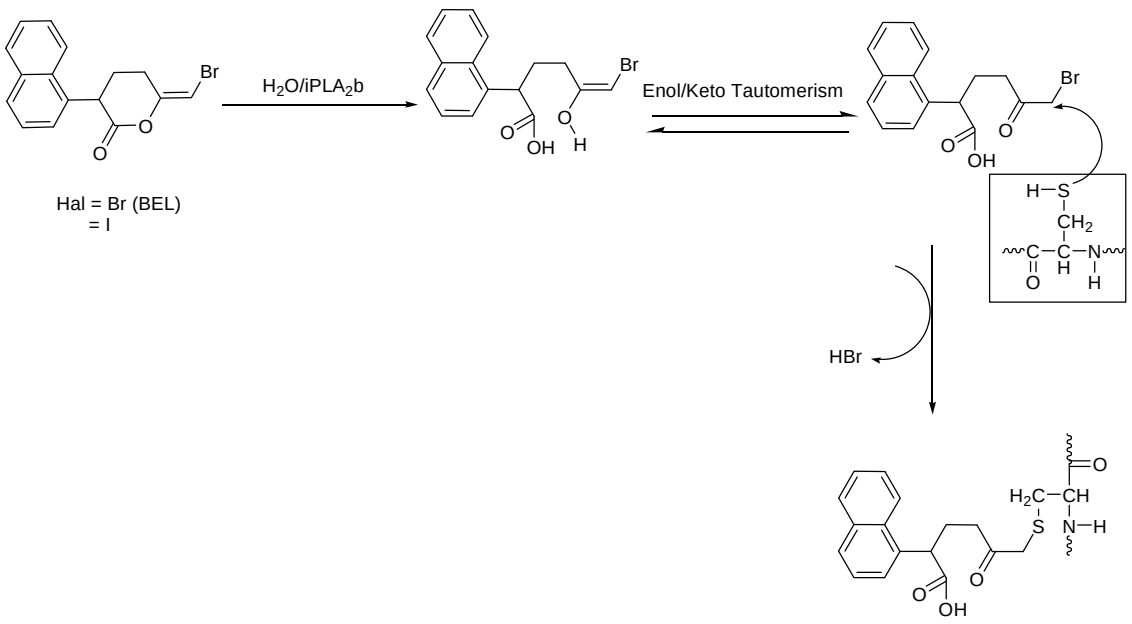


Fig. 3.2: Mechanism of $iPLA_2\beta$ inhibition by BEL[58]

The aforementioned biological activity of BEL triggered a thought that its analogs might act as candidate antibacterial agents. BEL's activity against different tumor cells through $iPLA_2$ mechanism might possibly be simulated in bacterial cells as similar biochemical pathways exist in prokaryotic organisms. Membrane bound and secretory phospholipases in different strains of bacteria like *E. coli, M. tuberculosis, H. pylori*, etc. has been proven to be involved in pathogenesis.[59-61] The exact mechanism of bacterial phospholipase action *in vivo* has not been determined. The toxicity was related to the cytolytic activity by membrane-destabilized products or by the destruction of membrane phospholipids. They may additionally trigger the release of eicasanoids, lysophospholipids and other phospholipid metabolism products which activate many destructive processes in the host tissue. Bacterial PLA_2 was also shown to be involved in bacterial activation and survival in the host tissue. Outer membrane bound PLA (OMPLA) in *E. coli* and other strains of Gram-negative bacteria was shown as a virulence factor and pathogenic

in many diseases like peptic ulcer, lung diseases, etc. Crystallographic studies confirmed that OMPLA is a dimeric protein with serine-144 as the active site residue.

We hypothesize that BEL analogs can act as OMPLA and other bacterial phospholipase inhibitors. Similar to eukaryotic $iPLA_2$, bacterial PLA's active site residue, Ser-144, can hydrolyze the lactone ring of BEL analogs and the other proximal serine moiety (Ser-152) can be involved in nucleophilic attack at the halo methyl group and results in irreversible inhibition.

Apart from this, our major hypothesis for BEL analogs to be a part of antibacterial effect is through the inhibition of β-lactamases, specifically serine β-lactamase, a major family of resistant factors against β-lactam antibiotics. Serine β-lactamase uses an active site serine to catalyze the hydrolysis of the β-lactam bond and make the β-lactam antibiotics inactive.[62] Several β-lactamase inhibitors were developed and given in combination with β-lactam antibiotics to increase the efficacy of the latter. Clavulanic acid, a mechanism based inhibitor, is the first compound used clinically to combat several types of β-lactamases. [63] Though it contains a β-lactam ring similar to β-lactam antibiotics, the intrinsic antimicrobial activity of this compound is negligible due to the absence of side chain peptide bond, which is present in β-lactam antibiotics. The absence of the exocyclic peptide results in poor binding to PBPs (Penicillin binding proteins), targets for penicillin and other β-lactam antibiotics, and hence negligible inhibition.

Clavulanic acid acts as a suicide inhibitor of the enzyme as shown in Fig. 3.3.[63-65] The hydroxyl group of Ser-70 residue hydrolyzes the β-lactam ring and forms an acyl bond. Oxazolidone ring opening assisted by a proton transfer from an adjacent water molecule exposes an electrophilic imine group. The hydroxyl group of Ser-130, lying in close proximity, attacks

the imine group and forms an enol ether functionalized enzyme adduct, which is the irreversibly inhibited form of the enzyme.

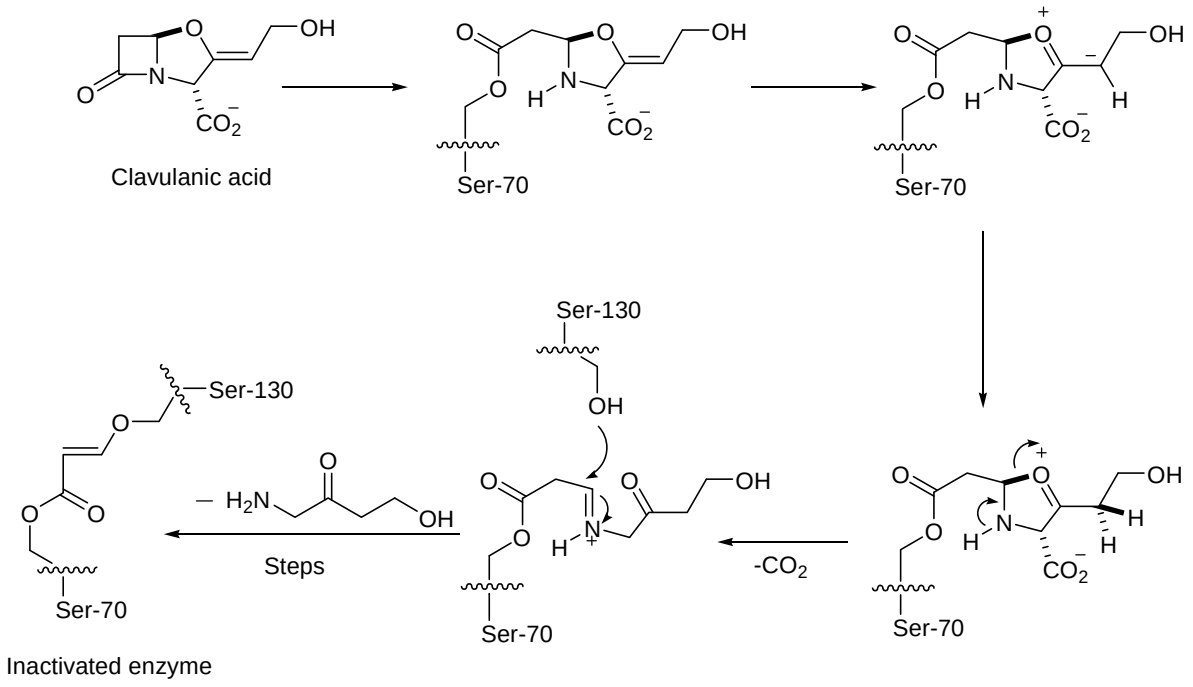


Fig. 3.3: Clavulanic acid inhibition of β-lactamase[63-65]

A similar mechanism of inactivation may be realized in the case of BEL analogs when encountered with β-lactamases. Fig. 3.4 illustrates a proposed mechanism of serine β-lactamase inhibition through acyl transfer to Ser-70 and nucleophilic attack at the α-carbon of halo methyl ketone moiety by Ser-130 to form an inactivated enzyme adduct.

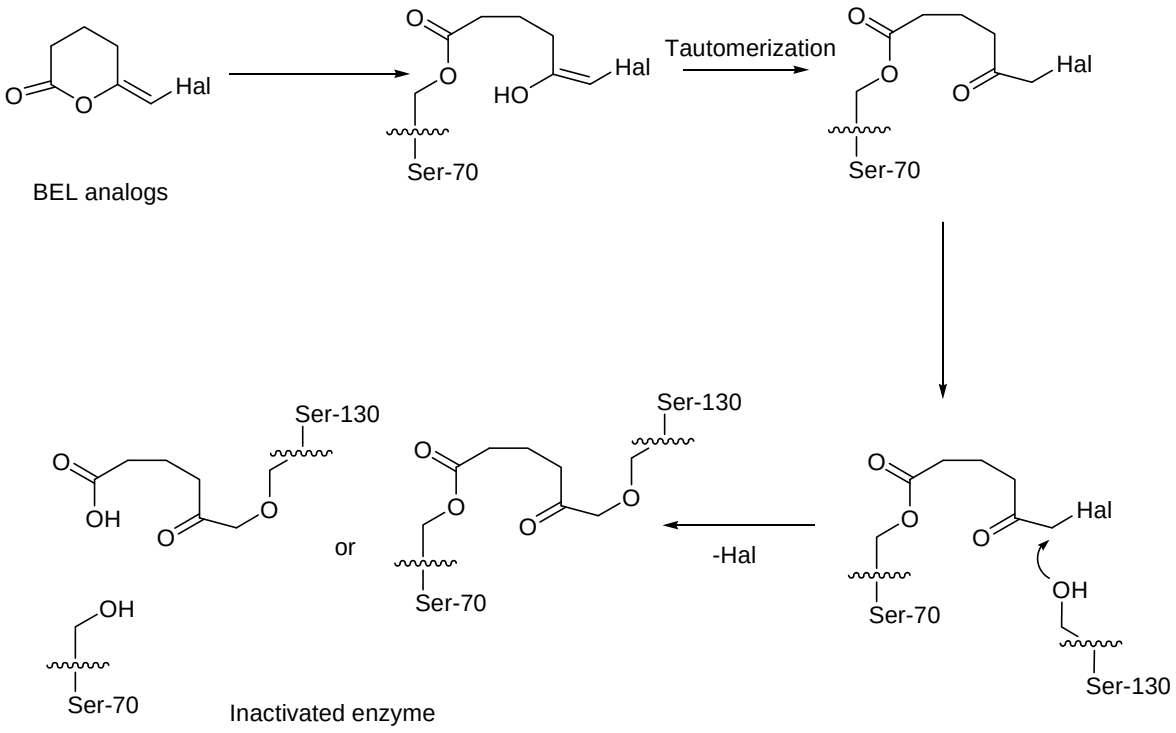


Fig. 3.4: Proposed inhibition of β-lactamase by BEL analogs

To test these hypotheses, three different analogs of BEL with structural variations inside and outside the ring were synthesized and tested against *E. coli* and *S. aureus* for preliminary data on growth inhibition. Later, experiments on β-lactamase inhibition through synergistic studies using penicillin G were also conducted. The following sections elaborate the synthesis, antibacterial screening and future directions of this research.

SYNTHESIS

Synthesis of BEL analogs was carried out starting from readily available amino acids. Amino acids gives us a chance to make multiple analogs of BEL with variations at X, R, R^1 and R^2 positions and control the stereochemistry of the molecules (Fig. 3.5). One analog starting from L-phenylalanine and two from 5-hexynoic acid were synthesized, but the yields for these

reactions were low. Optimization of the reaction conditions for better yields was given less priority as preliminary data against *E. coli* and *S. aureus* was needed to continue further study.

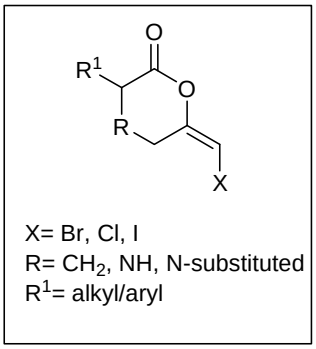
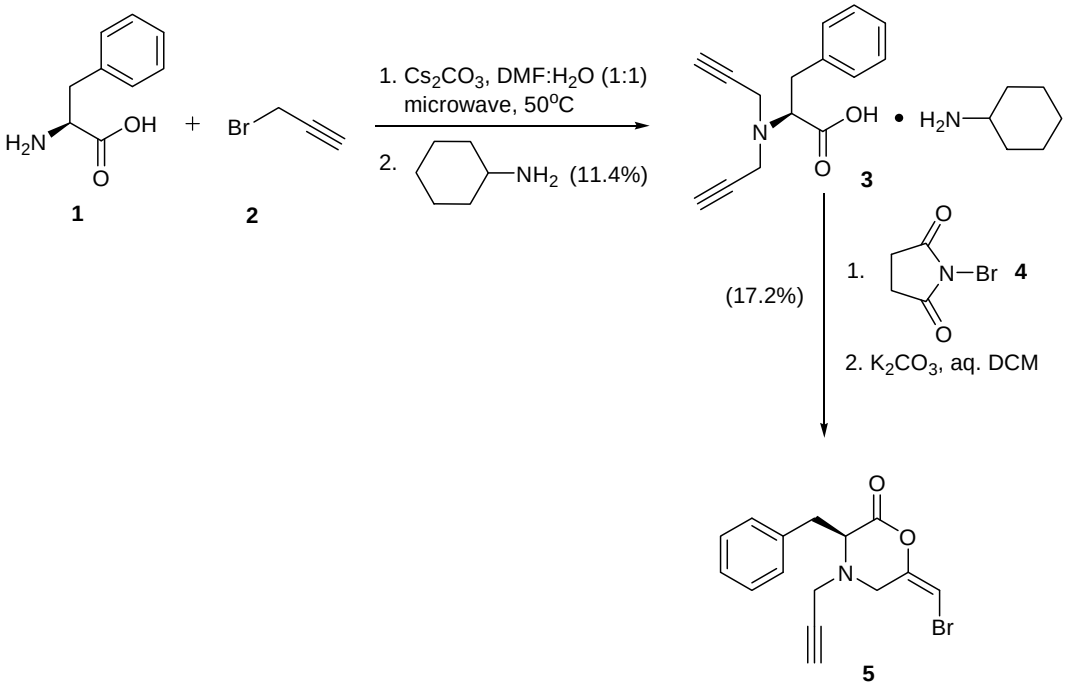


Fig. 3.5: General structure of BEL analogs

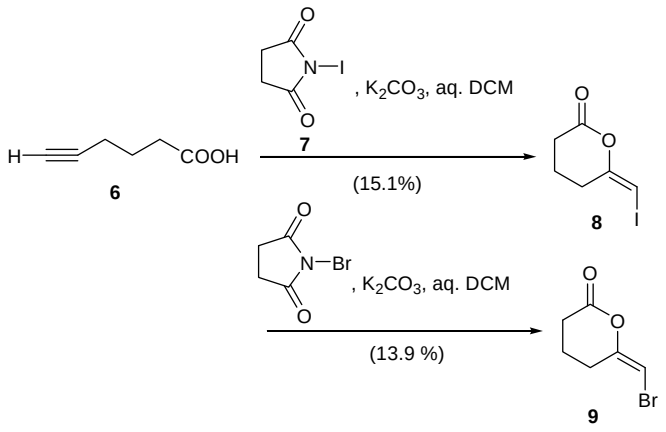
Phenylalanine **1** was reacted with propargyl bromide **2** and cesium carbonate was chosen as the base for selective *N*-mono-alkylation through cesium effect (Scheme 3.1).[66] Aprotic polar solvent like DMF was used to aid in the coordination of cesium with amine by decreasing the cesium ion's affinity towards carbonate. The mono-alkylated amine is more nucleophilic and can subsequently involve in further alkylation. But the coordination of cesium results in steric hindrance at the available proton and hence di-alkylation is not favored. In our case, use of water resulted in di-alkylated product which was extracted as cyclohexylamine salt **3**. Acid **3** was reacted with *N*-bromosuccinimide (NBS) **4** as electrophilic bromine source, potassium carbonate as the base in DCM and water. As water helps to increase the solubilities of K_2CO_3 and NBS,[67] it will accelerate the deprotonation of the acid and protonation of NBS to release Br^+. The mechanism of lactone formation involves initial nucleophilic attack by carboxylate anion at the alkyne and subsequent bromination at terminal unsaturated carbon. The lactone **5** was obtained in 17.5% yield after purification.

Scheme 3.1: Synthesis of BEL analog **5** from phenylalanine



The final two analogs, lactones **8** and **9,** were prepared from 5-hexynoic acid **6** in accordance with the literature procedure (Scheme 3.2).[67] *N*-iodosuccinimide (NIS) **7** and *N*-bromosuccinimide **4** used as halogen donors resulted in lactones **8** and **9** in 15.1 % and 13.9% yields, respectively.

Scheme 3.2: Synthesis of BEL analogs **8** and **9** from 5-hexynoic acid[67]



ANTIBACTERIAL SCREENING

All four analogs were tested against *E. coli* and *Staphylococcus aureus* using Kirby-Bauer diffusion experiment. Compounds, in concentrations of 20, 40, and 60 μM (in DMSO), were incubated in bacteria streaked agar plate for one day and inhibition was based on zone of inhibition. Lactones **8** and **9** had no effect at any given concentration on bacterial growth after 24 hours. Bromoenol **5** showed partial inhibition of bacterial cell growth after 24 hours, with the highest concentration having the greatest amount of inhibition against Gram-positive *S. aureus*.

Synergistic studies were also conducted to examine if the lactones could target a serinase common to many penicillin insensitive bacteria, β-lactamases. All the compounds were incubated in combination with a known β-lactam antibiotic, penicillin G, against methicillin resistant *S. aureus* (MRSA). Equal amounts of penicillin G (either 1 μg or 5 μg) dissolved in dH_2O were filled in five wells drilled in the agar plates. Different amounts of the test compounds 0, 20, 40, 60 and 80 μg dissolved in DMSO were filled in the wells containing penicillin G. The plates were incubated overnight and examined for the zone of inhibition. Out of all the compounds, only the iodoenol **8** demonstrated synergism with penicillin G which was evident by the increase in the diameter of the zone of inhibition as the amount of lactone increased. As shown in the Fig. 3.6, lactone **8** was successful in increasing the activity of penicillin G (1 μg and 5 μg) against MRSA and the exact mechanism involved in the synergistic effect observed needs to be studied further.

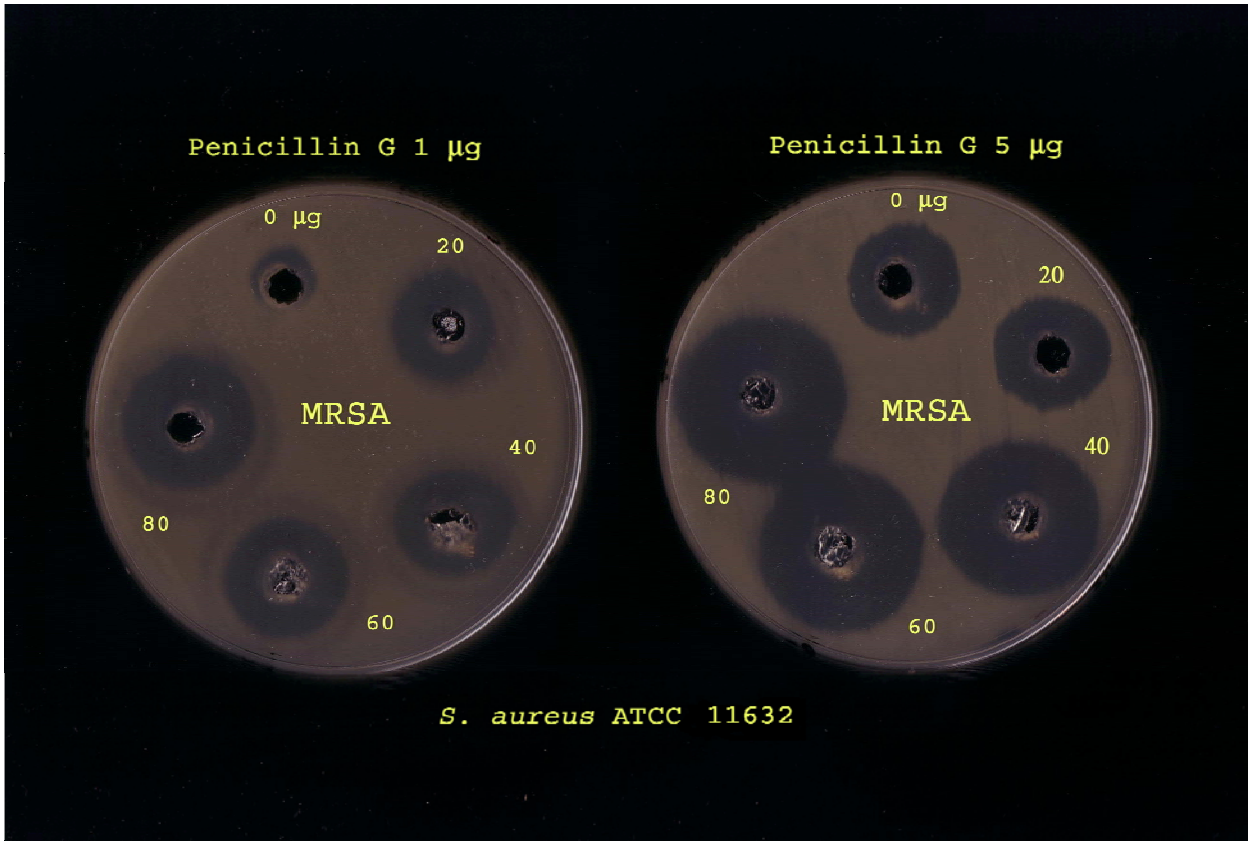


Fig 3.6: Synergistic study of iodoenol lactone **8** in combination with penicillin G (1 and 5 µg) against methicillin resistant *S. aureus* (MRSA)

CONCLUSIONS AND FUTURE DIRECTIONS

Growth inhibition studies with the three compounds **5, 8,** and **9** conducted against Gram-negative *E. coli* and Gram-positive *S. aureus* revealed interesting results. None of the compounds displayed activity against *E. coli*. Lactone **5** with the ring amine showed partial inhibition of *S. aureus* significantly at 60 µM concentration. A faint zone of inhibition with some bacteria still alive inside the zone was observed. The partial inhibition observed could be, in part, because of the inability of lactone **5** to diffuse through hydrophilic agar medium. This reason also gives an explanation for complete inactivity of analogs **8** and **9**. The decreased lipophilicity of the lactone **5**, containing a ring amine, would also account for the weak penetration of the compound

through the hydrophobic layers of the bacterial cell causing decreased activity. Thus, higher concentrations of these compounds need to be tested to confirm the bioactivity. In order to overcome the effect of hydrophilic agar media on the activity, broth studies should be conducted. An important conclusion from the screening would be the possibility of an alternate mechanism by which these compounds might act (i.e. β-lactamase inhibition).

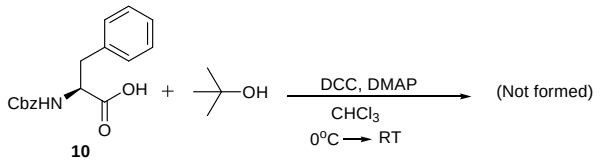
The results from synergistic studies explain the importance of these compounds in possible β-lactamase inhibition. Possibly, the lactone **8** was successful in protecting penicillin G from β-lactamase inactivation and helped increase the activity. Another mechanism that might be attributed to the effect observed is the entry of compound **8** in to the cell through the weakened cell wall of MRSA by the action of penicillin G. After entering the cell, the inhibition of essential biochemical processes such as DNA and protein synthesis by the lactone may result in the observed antibacterial effect. Inhibition of phospholipases cannot be confirmed from these studies as their role in bacterial cell growth is not yet proven. To gain insight in to the biochemical mechanisms involved in the growth inhibition, further studies need to be conducted. The future goals of the project involves synthesizing new BEL analogs to test against different strains of bacteria and conduct bioactivity studies in cancer cell lines. Structure activity relationship (SAR) studies and the determination of minimum inhibitory concentration (MIC) also constitute our goals in the near future.

FAILED REACTIONS

A plethora of reactions were performed to optimize the reaction conditions and synthesize multiple analogs of BEL. The following schemes shows the synthetic strategies implemented that resulted in very low yields or no progress at all. The carboxyl group of Cbz-

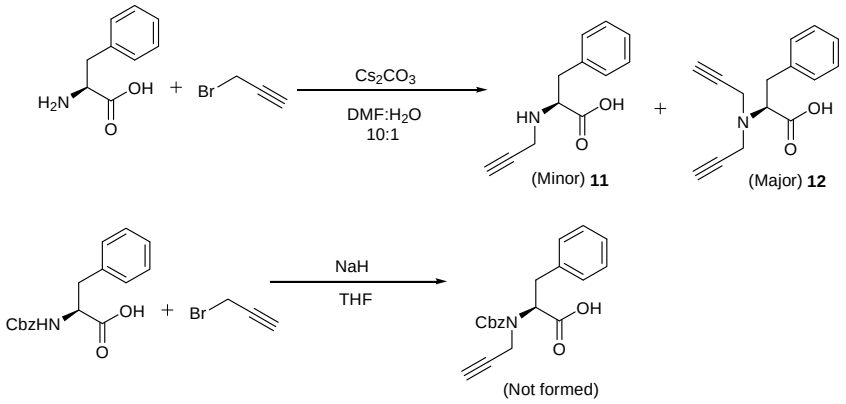
protected phenylalanine **10** was tried protecting using *t*-butyl group, as the deprotection of the same in the later stages to yield acid for cyclization would be an easy reaction to carry out in comparison to methyl or benzyl ester deprotection (Scheme 3.3).

Scheme 3.3: *t*-Butyl esterification of Cbz-Phe-OH



In our attempts to make *N*-mono-alkylated products, multiple approaches were explored but none yielded the desired product. The first reaction (Scheme 3.4) using cesium carbonate as a base resulted in majority of di-alkylated compound **12** and a trace amount of mono-alkylated analog **11**. The second reaction with sodium hydride as a base did not yield any product.

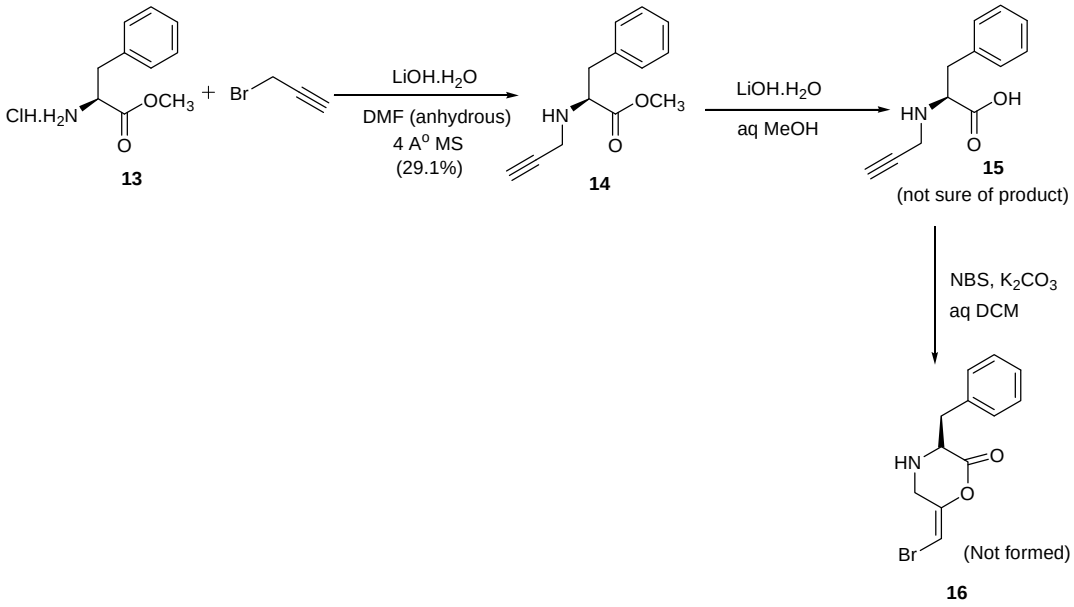
Scheme 3.4: Mono-alkylation of phenylalanine using different bases



After experimenting with different bases, Jung Hyun Cho's procedure to selectively mono-alkylate using lithium hydroxide monohydrate ($LiOH \cdot H_2O$) as the base was evaluated but the yields were not promising and small amounts of the dialkylated product was also observed.[68] Lithium, being smaller than other metals, possesses strong covalent character to bind the

nitrogen and oxygen atoms of α-amino esters facilitating alkylation and exhibits steric interference for dialkylation. A salt of Phe-OMe **13** was treated with propargyl bromide in dry DMF to yield **14**. Acid hydrolysis of the methyl ester did not yield the product, therefore base hydrolysis with $LiOH{\bullet}H_2O$ in aqueous methanol was attempted. We observed that the product remained in the water layer during extraction and failure to extract the product out of the water layer with pH adjustments resulted in trace amounts of compound which showed a streaked UV active spot on TLC corresponding to the acid group. NMR analysis could not confirm the product was obtained. However, the crude acid **15** was carried on to the next stage i.e. lactonization, which resulted in the failure of the formation of **16** (Scheme 3.5).

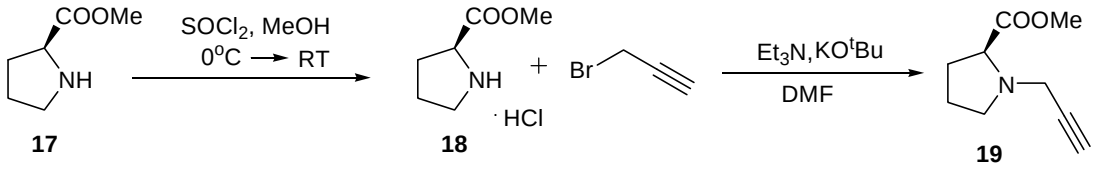
Scheme 3.5: Mono-alkylation of Phe-OMe **13** and methyl ester hydrolysis



We have also succeeded in alkylating proline methyl ester salt **18** using a strong base KOtBu (Scheme 3.6). Triethylamine (1.1 eq) was added to neutralize the acid (HCl) and the alkylation proceeded in dry DMF to yield alkylated product **19** (R_f 0.59, 2:1 hexanes: EtOAc) and free base of **18** (R_f 0.43). Acid and base catalysed the methyl ester hydrolysis was not

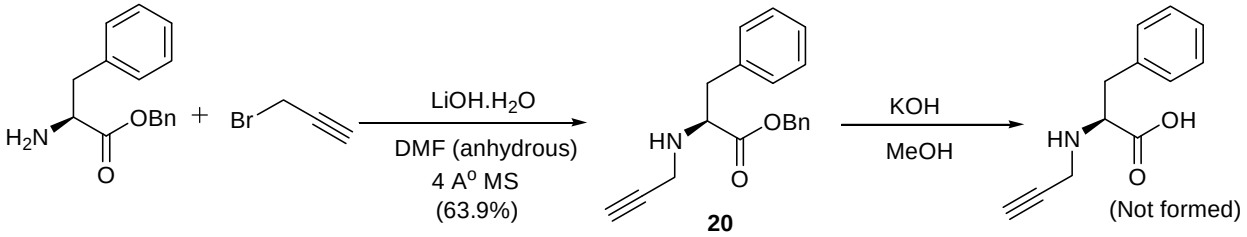
successful in the above protocol (Scheme 3.5), therefore it was not attempted on the proline analog.

Scheme 3.6: Alkylation of proline methyl ester salt **18**



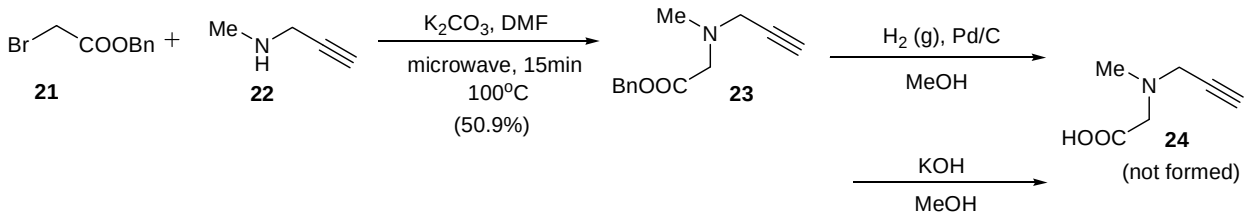
Since methyl ester hydrolysis was not dealt successfully, benzyl esters were tried to actuate the process of hydrolysis. Phe-OBn **20** was synthesized in high yield and attempted to mono-alkylate using $LiOH{\cdot}H_2O$ as the base. The reaction proceeded with 63.9% yield, but the benzyl ester hydrolysis in MeOH and KOH afforded no acid product (Scheme 3.7).

Scheme 3.7: Mono-alkylation of Phe-OBn and benzyl ester hydrolysis



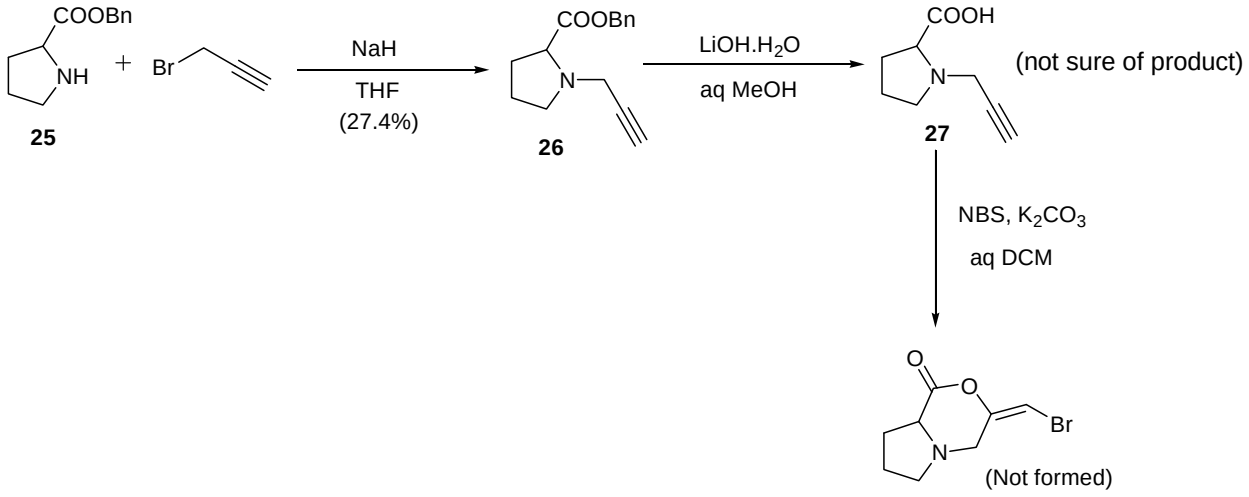
Palladium catalyzed hydrogenation serves as an alternate route to cleave the benzyl ester (Scheme 3.8). Therefore, benzyl ester of bromoacetic acid **21** was reacted with **22** under microwave at 100°C to obtain ester **23**. Hydrogenation in MeOH with Pd/C as a catalyst to hydrolyze the benzyl ester however; did not provide proceed to the desired product **24**. Hydrogenation resulted in methyl elimination and saturation of alkyne. Also, base (KOH) hydrolysis in MeOH was attempted with no success.

Scheme 3.8: Palladium catalyzed hydrogenation and base hydrolysis of **23**



Simultaneously, proline benzyl ester **25** was alkylated with propargyl group in THF using NaH as the base to afford **26** in 27.4% yield. An alternate method with LiOH•H_2O in aqueous methanol was attempted to saponify the benzyl ester. Despite the fact that TLC showed a streaked spot corresponding to the acid group, the NMR spectrum did not clearly confirm the presence of the product **27**. Nonetheless, the crude material was used for lactonization with NBS but yielded no product (Scheme 3.9).

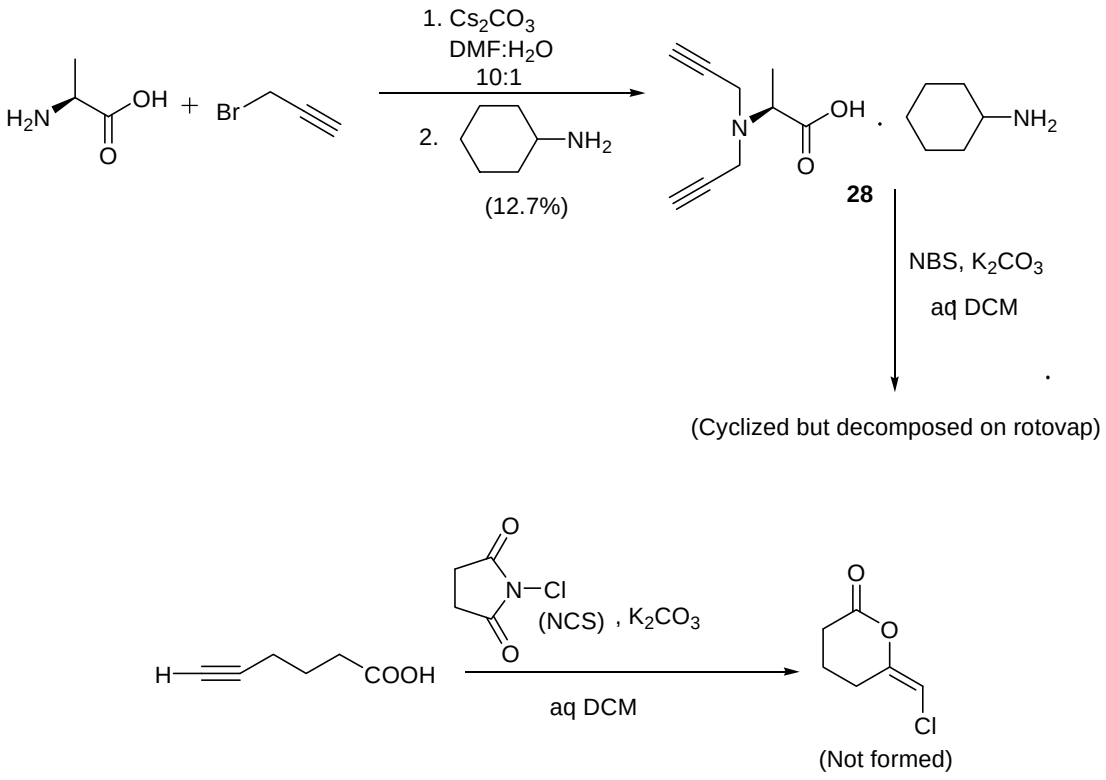
Scheme 3.9: Lactonization of proline analog **27**



The following two schemes show our failed attempts to make other BEL analogs. Di-alkylated alanine **28** failed to undergo the enol lactone formation and 5-hexynoic acid's reaction

with *N*-chlorosuccinimide (NCS) did not turn positive indicating the difficulties associated with the synthesis of these simple molecules (Scheme 3.10).

Scheme 3.10: Synthesis of two BEL analogs from alanine and 5-hexynoic acid



Though we were able to mono-alkylate with careful selection of bases, hydrolysis of the methyl or benzyl ester was never successful. A common theme among the acid and base hydrolysis is the inability to extract the acid products from the water layer. Extractions with organic solvents by adjusting the water layer to acidic, basic and neutral pH were performed with no success.

CHAPTER IV

MATERIALS AND METHODS

Section-I

This section illustrates the methods and materials used in the schemes described in chapter-II.

Chemicals were purchased from commercial sources and used as received. Products were purified by flash chromatography on 60-100 mesh silica and visualized by UV on TLC plates (silica gel 60 F_{254}). Melting points were determined on a melting point apparatus and are uncorrected. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. High resolution mass spectrometry (HRMS) was performed by electrospray ionization (ESI). ^1H and ^{13}C NMRs were recorded on a 500 MHz NMR and referenced to residual $CDCl_3$ or CD_3OD.

(*S*)-3-Amino-dihydrofuran-2(3H)-one • HCl (4): L-Methionine (30 g, 0.201 mol) was suspended in ^{i}PrOH (100 mL), water (100 mL), and AcOH (40 ml). Chloroacetic acid (18.9 g, 0.201 mol) was added and the solution was heated to an internal temperature of 50^{o}C with stirring for 2 h. The fully dissolved mixture was then heated for 5 h to an internal temperature of 90^{o}C. The liquid was then evaporated and the oil concentrate was heated at 90^{o}C for 2 h under reduced pressure with flask rotation on a rotary evaporator. The orange, semi-solid slurry was next suspended in anhydrous dioxane (30 mL), chilled 2 h in an ice bath, and HCl was bubbled into the solution for 2 min. The flask was capped, removed from the ice bath, and stirred at RT for 3h. The fine, white precipitate was then filtered, washed with EtOAc, and dried under

vacuum. Crude lactone **4** (16.1 g, 0.117 mol) was obtained in 58.2% yield and used without further purification.

(*S*)-2-Amino-4-bromobutanoic acid • HCl/Br (5): In a 150 mL sealed tube was suspended lactone **2** (14.7 g, mmol) in 40 mL of 30% HBr/AcOH. The container was sealed tightly and the solution stirred in a heated oil bath at 60^{o}C (CAUTION: high pressure buildup) for 15 h. The tube was removed from the oil bath and allowed to stand at RT for 2 h. After careful removal of the lid (CAUTION: HBr(g) is released), the salt was filtered, washed with EtOAc, and dried under vacuum. The off-white product with a m.p. of 179-182^{o}C was obtained in 90.1% yield. ^{1}H NMR (500 MHz, CD_3OD) δ 3.99 (t, 1H, J = 6.5 Hz), 3.51-3.46 (m 2H), 2.36 (m, 1H), 2.20 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 171.1, 52.6, 34.9, 28.5.

Benzyl (*S*)-1-(methoxycarbonyl)-3-bromopropyl-carbamate (6): Bromide **5** (24 g, 0.091 mol) was dissolved in anhydrous MeOH (150 mL) and the solution was chilled in an ice bath. Thionyl chloride (9.94 mL, 0.137 mol) was added dropwise with stirring over 0.5 h and the resulting mixture was allowed to warm the RT. After 18 h, the solvent was evaporated under reduced pressured. EtOAc was then added to the orange oil concentrate to precipitate the methyl ester product. The fine white solid was collected by filtration and the liquor was subsequently evaporated giving again a orange viscous oil. EtOAc was added to precipitate additional product and this process was repeated 6 times to provide 19.2 g (78.8%) of intermediate. The esterified salt (19.0 g, 0.069 mol) was then added to an ice-chilled 2:1 mixture of dH_2O:DCM (90 mL) containing $NaHCO_3$ (13.8 g, 0.165 mol) and CBzCl (13.82 g, 0.081 mol) was introduced dropwise to the vigorously stirred biphasic solution. After 15h, the DCM was evaporated and the pH of the aqueous layer was adjusted to 2.0 with 0.5 M HCl. The mixture was extracted 3 times with EtOAc, dried over $MgSO_4$, filtered, and concd to highly viscous oil. Carbamate **6** was

purified by silica gel chromatography using first 9:1 hexanes:EtOAc to remove excess CBzCl and BnOH decomposed byproduct followed by 2:1 hexanes:EtOAc that produced the product (R_f 0.40) as a viscous oil (22.6 g, 0.068 mol, 98.2%) that slowly solidified to a waxy solid with a m.p. of 57-60°C and a $[\alpha]^{26}D$ = -40.0 (*c* 1, DMF) [lit[10c]: m.p.= 61°C; $[\alpha]^{25}D$ -40.3° (*c* 1, DMF)]. ^1H NMR (500 MHz, $CDCl_3$) δ 7.36-7.29 (m, 5H), 5.43 (d, 1H, *J* = 7.5 Hz), 5.10 (s, 2H), 4.52-4.48 (m, 1H), 3.74 (s, 3H), 3.40 (t, 2H, *J* = 7 Hz), 2.44-2.40 (m, 1H), 2.24-2.20 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 156.0, 136.1, 128.5, 128.2, 128.1, 67.1, 52.8, 52.6, 35.3, 28.4; ESI-HRMS calcd for $C_{13}H_{16}BrNO_4$ $[M+Na]^+$ 352.0160, found 352.0164.

***S*-Alkyl Homocysteine Sulfide Analogs 7; General Procedure:** To a sealed tube containing bromide **6** (0.50 g, 1.50 mmol), NaI (66 mg, 0.44 mmol), and K_2CO_3 (0.24 g, 1.75 mmol) was added 3 mol equiv of thiol (4.5 mmol) in 10 mL of dry acetone. The containers were then tightly capped and stirred in a sand bath heated to 90-95°C for 16-20 h. After cooling to RT, the solutions were filtered, evaporated, redissolved in DCM, washed with brine, dried over Na_2SO_4, and concd. The sulfides were purified by flash chromatography in accordance to product R_f values.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(ethylthio)propyl-carbamate (7a): colorless oil; TLC (SiO_2) R_f 0.41 (3:1 hexanes:EtOAc); $[\alpha]^{26}_D$ +18.5° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.33-7.27 (m, 5H), 5.51 (d, 1H, *J* = 7.5 Hz), 5.08 (ABq, 2H, J_{AB} = 12.5 Hz), 4.49-4.45 (m, 1H), 3.71 (s, 3H), 2.53-2.46 (m, 4H), 2.13-2.07 (m, 1H), 1.96-1.88 (m, 1H), 1.20 (t, 3H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 156.0, 136.3, 128.6, 128.3, 128.2, 67.2, 53.3, 52.6, 32.5, 27.3, 25.9, 14.7; ESI-HRMS calcd for $C_{15}H_{21}NO_4S$ $[M+H]^+$ 312.1269, found 312.1266.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(propylthio)-propylcarbamate (7b): colorless oil; (SiO_2) R_f 0.48 (3:1 hexanes:EtOAc); $[\alpha]^{26}_D$ +18.4° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.27 (m, 5H), 5.51 (d, 1H, *J* = 8 Hz), 5.08 (ABq, 2H, J_{AB} = 12.5 Hz), 4.49-4.44 (m, 1H), 3.71 (s, 3H), 2.50 (t, 2H, *J* = 7.5 Hz), 2.44 (t, 2H, *J* = 7.5 Hz), 2.12-2.09 (m, 1H), 1.94-1.90 (m, 1H), 1.55 (sxt, 2H, *J* = 7.5 Hz), 0.94 (t, 3H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 156.0, 136.3, 128.6, 128.3, 128.2, 67.1, 53.3, 52.6, 34.2, 32.6, 27.7, 22.9, 13.6; ESI-HRMS calcd for $C_{16}H_{23}NO_4S$ $[M+H]^+$ 326.1426, found 326.1426.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(isopropylthio)-propylcarbamate (7c): colorless oil; TLC (SiO_2) R_f 0.43 (3:1 hexanes:EtOAc); $[\alpha]^{26}_D$ +18.1° (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.28 (m, 5H), 5.51 (d, 1H, *J* = 8 Hz), 5.08 (ABq, 2H, J_{AB} = 12.5 Hz), 4.49-4.45 (m, 1H), 3.72 (s, 3H), 2.89-2.85 (m, 1H), 2.53 (t, 2H), 2.14-2.07 (m, 1H), 1.96-1.89 (m, 1H), 1.22 (s, 3H), 1.21 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 156.0, 136.3, 128.7, 128.3, 128.2, 67.2, 53.4, 52.6, 34.9, 32.8, 26.3, 23.4; ESI-HRMS calcd for $C_{16}H_{23}NO_4S$ $[M+H]^+$ 326.1426, found 326.1429.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(butylthio)propyl-carbamate (7d): colorless oil; TLC (SiO_2) R_f 0.48 (3:1 hexanes:EtOAc); $[\alpha]^{26}_D$ +19.5° (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34-7.27 (m, 5H), 5.51 (bs, 1H), 5.08 (app s, 2H), 4.49-4.45 (m, 1H), 3.71 (s, 3H), 2.50 (t, 2H, *J* = 7.5 Hz), 2.46 (t, 2H, *J* = 7.5 Hz), 2.14-2.07 (m, 1H), 1.95-1.88 (m, 1H), 1.51 (qnt, 2H, *J* = 7.5 Hz), 1.36 (sxt, 2H, *J* = 7.5 Hz), 0.88 (t, 3H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 156.0, 136.3, 128.6, 128.3, 128.2, 67.2, 53.4, 52.6, 32.6, 31.9, 31.7, 27.8, 22.1, 13.8; ESI-HRMS calcd for $C_{17}H_{25}NO_4S$ $[M+H]^+$ 340.1582, found 340.1593.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(hexylthio)-propylcarbamate (7f): colorless oil; TLC (SiO_2) R_f = 0.52 (3:1 hexanes:EtOAc); $[\alpha]^{26}_D$ +18.3° (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34-7.27 (m, 5H), 5.53 (d, 1H, *J* = 8 Hz), 5.03 (app s, 2H), 4.44-4.39 (m, 1H), 3.71 (s, 3H), 2.45 (t, 2H, *J* = 7.5 Hz), 2.40 (t, 2H, *J* = 7.5 Hz), 2.09-2.02 (m, 1H), 1.90-1.82 (m, 1H), 1.47 (qnt, 2H, *J* = 7.5 Hz), 1.31-1.16 (m, 6H), 0.86 (t, 3H, *J* = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 156.0, 136.3, 128.6, 128.3, 128.2, 67.1, 53.3, 52.6, 32.5, 32.2, 31.5, 29.5, 28.6, 27.8, 22.6, 14.1; ESI-HRMS calcd for $C_{19}H_{29}NO_4S$ $[M+H]^+$ 368.1896, found 368.1902.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(octylthio)propyl-carbamate (7g): colorless oil; (SiO_2) R_f 0.44 (3:1 hexanes:EtOAc); $[\alpha]^{24}_D$ +17.0° (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.27 (m, 5H), 5.53 (d, 1H, *J* = 8.5 Hz), 5.07 (app s, 2H), 4.48-4.44 (m, 1H), 3.71 (s, 3H), 2.50 (t, 2H, *J* = 7.5 Hz), 2.45 (t, 2H, *J* = 7.5 Hz), 2.14-2.07 (m, 1H), 1.95-1.89 (m, 1H), 1.55-1.49 (m, 2H), 1.33-1.24 (m, 10H), 0.85 (t, 3H, *J* = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 156.0, 136.3, 128.6, 128.3, 128.2, 67.1, 53.3, 52.6, 32.5, 32.2, 31.9, 29.6, 29.3, 29.0, 27.8, 22.7, 14.2; ESI-HRMS calcd for $C_{21}H_{33}NO_4S$ $[M+H]^+$ 396.2209, found 396.2219.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(decylthio)propyl-carbamate (7h): colorless waxy solid; TLC (SiO_2) R_f 0.57 (3:1 hexanes:EtOAc); $[\alpha]^{23}_D$ = +15.7° (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.27 (m, 5H), 5.48 (bs, 1H), 5.08 (s, 2H), 4.49-4.45 (m, 1H), 2.50 (t, 2H, *J* = 7.5 Hz), 2.46 (t, 2H, *J* = 7.5 Hz), 2.14-2.08 (m, 1H), 1.96-1.89 (m, 1H), 1.52 (qnt, 2H, *J* = 7.5 Hz), 1.33-1.24 (m, 14H), 0.86 (t, 3H, *J* = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 156.0, 136.3, 128.7, 128.3, 128.2, 67.2, 53.4, 52.6, 32.6, 32.2, 32.0, 29.7, 29.4, 29.0, 27.9, 22.8, 14.2; ESI-HRMS calcd for $C_{23}H_{37}NO_4S$ $[M+H]^+$ 424.2522, found 424.2532.

***S*-Alkyl Homocysteine Sulfoxide Analogs 8; General Procedure:** To a stirring ice-chilled MeOH (10 mL) solution of sulfides **7** (0.9-1.5 mmol) was added $NaIO_4$ (1.02 mol equiv) in 3 mL of water. After 10 h, the solutions were filtered and the MeOH was evaporated under reduced pressure. Brine was then added and the products were extracted twice with DCM. The combined organic phases were next dried over Na_2SO_4, filtered, and concd. The crude sulfoxides were purified by flash chromatography in accordance to product R_f values.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(ethylsulfinyl)-propylcarbamate (8a): colorless oil (mixture of diastereomers); TLC (SiO_2) R_f 0.57 (9:1 DCM:MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.28 (m, 10H), 5.79 (d, 1H, *J* = 7 Hz), 5.69 (d, 1H, *J* = 7.5 Hz), 5.08 (s, 4H), 4.48-4.45 (m, 2H), 3.74 (s, 6H), 2.75-2.60 (m, 8H) 2.37-2.33 (m, 2H), 2.17-2.14 (m, 12H), 1.29-1.26 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 156.2, 136.2, 128.8, 128.7, 128.5, 128.4, 128.3, 67.4, 53.3, 53.0, 47.5, 46.0, 26.5, 26.2, 7.0; ESI-HRMS calcd for $C_{15}H_{21}NO_5S$ $[M+H]^+$ 328.1219, found 328.1227.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(propylsulfinyl)-propylcarbamate (8b): pale yellow oil (mixture of diastereomers); TLC (SiO_2) R_f 0.28 (100% EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28-7.23 (m, 10H), 6.41 (d, 1H, *J* = 8 Hz), 6.38 (d, 1H, *J* = 8 Hz), 5.04 (app s, 4H), 4.39-4.36 (m, 2H), 3.66 (s, 6H), 2.68-2.59 (m, 6H), 2.5-2.45 (m, 2H), 2.29-2.25 (m, 2H), 2.11-2.05 (m, 2H), 1.7-1.66 (m, 4H), 0.97 (t, 6H, *J* = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 156.2, 136.1, 128.3, 128.0, 127.9, 66.8, 54.0, 53.9, 53.1, 52.8, 52.4, 47.9, 47.8, 25.5, 25.3, 16.1, 13.2; ESI-HRMS calcd for $C_{16}H_{23}NO_5S$ $[M+H]^+$ 342.1375, found 342.1377.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(isopropyl-sulfinyl)propylcarbamate (8c): colorless oil (mixture of diastereomers); TLC (SiO_2) R_f 0.21 (1:1 hexanes:EtOAc); ^{1}H NMR

(500 MHz, $CDCl_3$) δ 7.32-7.27 (m, 10H), 5.91 (d, 1H, *J* = 8 Hz), 5.80 (d, 1H, *J* = 8 Hz), 5.07 (app s, 4H), 4.46-4.43 (m, 2H), 3.72 (s, 6H), 2.74-2.64 (m, 4H), 2.60-2.54 (m, 2H), 2.34 (m, 2H), 2.14-2.08 (m, 2H), 1.24 (d, 6H, *J* = 7 Hz), 1.18 (d, 6H, *J* = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 170.8, 156.3, 136.3, 128.6, 128.7, 128.3, 128.2, 67.2, 64.1, 53.4, 53.1, 52.9, 52.4, 50.7, 44.7, 26.6, 26.3, 15.1, 15.0; ESI-HRMS calcd for $C_{16}H_{23}NO_5S$ $[M+H]^+$ 342.1375, found 342.1372.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(butylsulfinyl)-propylcarbamate (8d): colorless oil (mixture of diastereomers); TLC (SiO_2) R_f 0.20 (1:1 hexanes:EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 7.31-7.26 (m, 10H), 6.28 (d, 1H, *J* = 8 Hz), 6.23 (d, 1H, *J* = 8 Hz), 5.07 (s, 4H), 4.45-4.38 (m, 2H), 3.69 (s, 6H), 2.73-2.54 (m, 8H), 2.33-2.30 (m, 2H), 2.16-2.07 (m, 2H), 1.68-1.64 (m, 4H), 1.47-1.34 (m, 4H), 0.91 (t, 6H, *J* = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 156.2, 136.2, 128.4, 128.1, 128.0, 66.9, 53.2, 52.9, 52.6, 52.0, 48.0, 47.9, 25.8, 25.6, 24.5, 21.9, 13.6; ESI-HRMS calcd for $C_{17}H_{25}NO_5S$ $[M+H]^+$ 356.1532, found 356.1544.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(hexylsulfinyl)-propylcarbamate (8e): pale yellow oil; (mixture of diastereomers); TLC (SiO_2) R_f 0.21 (1:1 hexanes:EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 7.34-7.27 (m, 10H), 5.87 (d, 1H, *J* = 8 Hz), 5.77 (d, 1H, *J* = 8 Hz), 5.07 (s, 4 H), 4.48-4.41 (m, 2H), 3.72 (s, 6H), 2.74-2.53 (m, 8H), 2.38-2.31 (m, 2H), 2.17-2.07 (m, 2H), 1.71-1.66 (m, 4H), 1.45-1.23 (m, 12H), 0.86 (t, 6H, *J* = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 156.2, 136.3, 136.2, 128.7, 128.4, 128.3, 67.3, 53.3, 53.0, 52.9, 52.7, 48.2, 48.1, 31.5, 28.6, 26.4, 26.1, 22.7, 22.5, 14.1; ESI-HRMS calcd for $C_{19}H_{29}NO_5S$ $[M+H]^+$ 384.1845, found 384.1846.

Benzyl (*S*)-1-(methoxycarbonyl)-(octylsulfinyl)-propylcarbamate (8f): white waxy solid (mixture of diastereomers); m.p. 50-52°C; TLC (SiO_2) R_f 0.30 (1:1 hexanes:EtOAc); ^1H

NMR (500 MHz, $CDCl_3$) δ 7.32-7.26 (m, 10H), 5.91 (d, 1H, *J* = 8 Hz), 5.82 (d, 1H, *J* = 8 Hz), 5.07 (s, 4H), 4.46-4.42 (m, 2H), 3.71 (s, 6H), 2.71-2.52 (m, 8H), 2.34-2.30 (m, 2H), 2.15-2.09 (m, 2H), 1.68-1.66 (m, 4H), 1.42-1.32 (m, 4H), 1.26-1.23 (m, 16H), 0.84 (t, 6H, *J* = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 156.2, 136.2, 128.7, 128.4, 128.3, 128.2, 67.2, 53.3, 53.0, 52.9, 52.7, 48.2, 48.1, 31.9, 29.3, 29.1, 28.9, 26.3, 26.0, 22.7, 14.2; ESI-HRMS calcd for $C_{21}H_{33}NO_5S$ $[M+H]^+$ 412.2158, found 412.2174.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(decylsulfinyl)-propylcarbamate (8g): soft white solid (mixture of diastereomers); m.p. 66-70°C; TLC (SiO_2) R_f 0.30 (1:1 hexanes:EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 7.29-7.22 (m, 10H), 6.45 (d, 1H, *J* = 8 Hz), 6.41 (d, 1H, *J* = 8 Hz), 5.03 (s, 4H), 4.37-4.34 (m, 2H), 3.64 (s, 6H), 2.68-2.60 (m, 6H), 2.52-2.47 (m, 2H), 2.28-2.25 (m, 2H), 2.10-2.04 (m, 2H), 1.63-1.60 (m, 4H), 1.23-1.20 (m, 28H), 0.82 (t, 6H, *J* = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 156.2, 156.1, 136.1, 128.3, 127.9, 66.7, 53.0, 52.8, 52.3, 52.1, 47.9, 47.8, 31.7, 29.3, 29.2, 29.1, 29.0, 28.6, 25.5, 25.3, 22.5, 22.4, 14.0; ESI-HRMS calcd for $C_{23}H_{37}NO_5S$ $[M+H]^+$ 440.2471, found 440.2482

***S*-Aryl Homocysteine Sulfide Analogs 9; General Procedure:** Aryl sulfides **9** were prepared from bromide **3** in a manner similar to the procedure detailed for sulfides **7** with the exception that 1-1.2 mol equiv of thiophenols were used.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(phenylthio)-propylcarbamate (9b): white waxy solid; m.p. 47-48°C; TLC (SiO_2) R_f 0.37 (3:1 hexanes:EtOAc); $[\alpha]^{22}_D$ +19.2° (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.40-7.30 (m, 8H), 7.23 (tt, 2H, *J* = 7, 1.5 Hz), 5.66 (d, 1H, *J* = 8 Hz), 5.16 (ABq, 2H, J_{AB} = 12.3 Hz), 4.59-4.55 (m, 1H), 3.75 (s, 3H), 2.98 (t, 2H, *J* = 7.5 Hz), 2.25-2.18 (m, 1H), 2.05-1.98 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 156.0, 136.2, 135.6,

129.6, 129.1, 128.6, 128.3, 128.2, 126.4, 67.1, 53.2, 52.6, 32.2, 29.7; ESI-HRMS calcd for $C_{19}H_{21}NO_4S$ $[M+H]^+$ 360.1270, found 360.1281.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-methoxy-phenylthio)propylcarbamate (9c): waxy white solid; m.p. 36-37°C; TLC (SiO_2) R_f 0.37 (3:1 hexanes:EtOAc); $[\alpha]^{26}_D$ +11.3° (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.28 (m, 5H), 6.83-6.80 (m, 2H), 5.41 (d, 1H, *J* = 8.5 Hz), 5.08 (ABq, 2H, J_{AB} = 12.3 Hz), 4.51-4.47 (m, 1H), 3.77 (s, 3H), 3.70 (s, 3H), 2.81 (t, 2H, *J* = 7.5 Hz), 2.12-2.05 (m, 1H), 1.91-1.84 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6, 159.3, 156.0, 136.3, 133.9, 128.7, 128.4, 128.3, 125.5, 114.8, 67.2, 55.5, 53.2, 52.7, 32.5, 32.0; ESI-HRMS calcd for $C_{20}H_{23}NO_5S$ $[M+H]^+$ 390.1375, found 390.1390.

Benzyl(*S*)-1-(methoxycarbonyl)-3-(4-chlorophenylthio)-propylcarbamate (9d): white waxy solid; TLC (SiO_2) R_f 0.42 (3:1 hexanes:EtOAc); m.p. 48-49°C; $[\alpha]^{26}_D$ +15.2° (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41-7.35 (m, 5H), 7.28 (m, 4H), 5.51 (d, 1H, *J* = 8 Hz), 5.15 (ABq, 2H, J_{AB} = 12.5 Hz), 4.58-5.43 (m, 1H), 3.76 (s, 3H), 2.95 (app t, 2H, *J* = 7 Hz), 2.21-2.16 (m, 1H), 2.02-1.96 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4, 156.1, 136.2, 134.2, 132.6, 131.2, 129.3, 128.8, 128.3, 67.4, 53.2, 52.8, 32.4, 30.2; ESI-HRMS calcd for $C_{19}H_{20}ClNO_4S$ $[M+H]^+$ 394.0879, found 394.0874.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-nitropheny-lthio)propylcarbamate (9e): orange semi-solid; TLC (SiO_2) R_f 0.32 (3:1 hexanes:EtOAc); $[\alpha]^{26}_D$ +11.6° (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (d, 2H, *J* = 10.5 Hz), 7.28-7.18 (m, 7H), 5.51 (d, 1H, *J* = 7.5 Hz), 5.07 (ABq, 2H, J_{AB} = 12.3 Hz), 4.49-4.45 (m, 1H), 3.67 (s, 3H), 3.00-2.93 (m, 2H), 2.20-2.16 (m, 1H), 2.01-1.93 (m 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 156.1, 146.7, 145.3, 136.1, 128.7,

128.5, 124.2, 67.4, 53.2, 52.9, 31.9, 28.0; ESI-HRMS calcd for $C_{19}H_{20}N_2O_6S$ $[M+H]^+$ 405.1120, found 405.1126.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(2-nitrophenyl-thiol)propylcarbamate (9f): yellow solid; m.p. 78-80°C; TLC (SiO_2) R_f 0.32 (3:1 hexanes:EtOAc); $[\alpha]^{27}_D$ +37.7° (*c* 0.8, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (d, 1H, *J* = 8.5 Hz), 7.55 (t, 1H, *J* = 7.5 Hz), 7.38-7.36 (m, 6H), 7.28 (t, 1H, *J* = 7.5 Hz), 5.63 (d, 1H, *J* = 7 Hz), 5.17-5.12 (m, 2H), 4.57-4.56 (m, 1H), 3.78 (s, 3H), 3.06-2.96 (m, 2H), 2.34-2.33 (m, 1H) 2.11-2.07 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 156.1, 146.3, 137.0, 136.2, 133.8, 128.7, 128.4, 128.3, 126.6, 126.4, 124.9, 67.4, 53.5, 52.9, 31.2, 28.2; ESI-HRMS calcd for $C_{19}H_{20}N_2O_6S$ $[M+H]^+$ 405.1120, found 405.1122.

***S*-Aryl Homocysteine Sulfoxide Analogs 10; General Procedure:** To a stirring ice-chilled DCM (10 mL) solution of sulfides **9** (0.7-2.1 mmol) was added *m*-CPBA (1.25 mol equiv) in 5 mL of DCM. After 1 h, the reactions were quenched with 5% $NaHCO_3$ (20 mL) and extracted twice with DCM. The combined organic extracts were then dried over $NaSO_4$, filtered, and concd. The crude sulfoxides were then purified by flash chromatography in accordance to product R_f values.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(benzylsulfinyl)-propylcarbamate (10a): thick beige oil (mixture of diastereomers); TLC (SiO_2) R_f 0.48 (100% EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.36 (m, 16H), 7.30-7.28 (m, 4H), 5.72 (d, 1H, *J* = 7.5 Hz), 5.63 (d, 1H, *J* = 7.5 Hz), 5.13 (app s, 6H), 4.52-4.46 (m, 2H), 4.07-4.02 (m, 2H), 3.97-3.95 (m, 2H), 3.77 (s, 6H), 2.77-2.67 (m, 3H), 2.61-2.55 (m, 1H), 2.40-2.37 (m, 2H), 2.22-2.12 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 156.2, 136.2, 130.1, 129.6, 129.0, 128.6, 128.5, 128.3, 128.1, 67.1, 58.0,

53.1, 52.9, 52.7, 46.5, 25.7, 25.4; ESI-HRMS calcd for $C_{20}H_{23}NO_5S$ $[M+H]^+$ 390.1375, found 390.1376.

Benzyl (*S*)-1-(methoxycarbonyl)-(phenylsulfinyl)-propylcarbamate (10b): thick beige oil/solid (mixture of diastereomers); TLC (SiO_2) R_f 0.25 (1:1 hexanes:EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 7.53-7.45 (m, 10H), 7.32-7.26 (m, 10H), 5.89 (d, 1H, J = 8 Hz), 5.82 (d, 1H, J = 8 Hz), 5.05 (ABq, 4 H, J_{AB} = 13 Hz), 4.45-4.41 (m, 1H), 4.37-4.33 (m, 1H), 3.68 (s, 3H), 3.66 (s, 3H), 2.96-2.86 (m, 2H), 2.82-2.68 (m, 2H), 2.34-2.29 (m, 1H), 2.13-2.07 (m, 2H), 1.91-1.87 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 171.9, 156.2, 143.0, 136.2, 131.3, 131.2, 129.4, 128.6, 128.3, 128.2, 124.1, 67.2, 53.1, 52.8, 52.7, 52.6, 25.5, 25.0; ESI-HRMS calcd for $C_{19}H_{21}NO_5S$ $[M+H]^+$ 376.1218, found 376.1217.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-methoxy-phenylsulfinyl)propylcarbamate (10c): pale yellow oil (mixture of diastereomers); TLC (SiO_2) R_f 0.21 (1:1 hexanes:EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 7.48 (d, 4H, J = 8 Hz), 7.32-7.29 (m, 10 H), 6.98 (m, 4H), 5.67 (d, 1H, J = 8 Hz), 5.61 (d, 1H, J = 8 Hz), 5.07 (ABq, 4 H, J_{AB} = 13 Hz), 4.45-4.44 (m, 1H), 4.39-4.38 (m, 1H), 3.83 (s, 6H), 3.72 (s, 3H), 3.70 (s, 3H) 2.9-2.68 (m, 4H), 2.29 (m, 1H), 2.15-2.02 (m, 2H), 1.94-1.87 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 171.9, 162.1, 162.0, 156.2, 136.2, 133.7, 128.6, 128.2, 128.1, 126.0, 115.0, 67.1, 55.6, 53.1, 52.8, 25.4, 25.0; ESI-HRMS calcd for $C_{20}H_{23}NO_6S$ $[M+H]^+$ 406.1324, found 406.1340.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-chlorophenyl-sulfinyl)propylcarbamate (10d): pale yellow oil (mixture of diastereomers); TLC (SiO_2) R_f 0.39 (1:1 hexanes:EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 7.51-7.44 (m, 8H), 7.33 (m, 10H), 6.06 (d, 1H, J = 8 Hz), 6.01 (d, 1H, J = 8.5 Hz), 5.12-5.05 (m, 4H), 4.50-4.46 (m, 1H), 4.42-4.38 (m, 1H), 3.72 (s, 3H), 3.70 (s, 3H),

2.99-2.90 (m, 2H) 2.83-2.71 (m, 2H), 2.38-2.31 (m, 1H), 2.15-2.09 (m, 2H), 1.92-1.88 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 156.1, 141.4, 141.3, 137.1, 136.1, 129.5, 129.4, 128.4, 128.1, 128.0, 125.4, 66.9, 52.9, 52.7, 52.6, 52.5, 25.0, 24.5; ESI-HRMS calcd for $C_{19}H_{20}ClNO_5S$ $[M+H]^+$ 410.0829, found 410.0847.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-nitrophenyl-sulfinyl)propylcarbamate (10e): yellow oil (mixture of diastereomers); TLC (SiO_2) R_f 0.16 (1:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34-8.30 (m, 4H), 7.75-7.71 (m, 4H), 7.34-7.32 (m, 10H), 5.64 (d, 1H, J = 8 Hz), 5.58 (d, 1H, J = 8 Hz), 5.07 (ABq, 4 H, J_{AB} = 11.5 Hz), 4.52 (m, 1H), 4.40 (m, 1H), 3.74 (s, 3H), 3.72 (s, 3H), 3.03-2.98 (m, 2H), 2.83-2.82 (m, 1H) 2.76-2.75 (m, 1H), 2.47-2.45 (m, 1H), 2.18-2.11 (m, 2H), 1.88-1.84 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7, 171.6, 165.8, 156.3, 150.8, 149.7, 136.1, 128.8, 128.6, 128.3, 125.3, 124.6, 67.5, 53.4, 53.1, 52.9, 52.6, 26.3; ESI-HRMS calcd for $C_{19}H_{20}N_2O_7S$ $[M+H]^+$ 421.1070, found 421.1079.

Benzyl (*S*)-1-(methoxycarbonyl)-3-(2-nitrophenyl-sulfinyl)propylcarbamate (10f): yellow solid (mixture of diastereomers); m.p. 103-109°C; TLC (SiO_2) R_f 0.22 (1:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (d, 2H, J = 8 Hz), 8.26 (d, 2H, J = 8 Hz), 7.93 (td, 2H, J = 7.5, 3.5 Hz), 7.72 (t, 2H, J = 7.5 Hz), 7.36-7.30 (m, 10H), 5.86 (d, 1H, J = 8.5 Hz), 5.81 (d, 1H, J = 8.5 Hz), 5.81 (os, 4H), 4.89-4.45 (m, 1H), 4.43-4.38 (m, 1H), 3.74 (s, 3H), 3.38-3.26 (m, 2H), 2.95-2.82 (m, 2H), 2.59-2.54 (m, 1H), 2.34-2.25 (m, 2H), 2.07-2.05 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 156.1, 144.2, 142.5, 136.2, 135.6, 131.8, 128.6, 128.2, 127.0, 125.5, 67.2, 53.0, 52.8, 52.1, 51.6, 26.1, 25.8; ESI-HRMS calcd for $C_{19}H_{20}N_2O_7S$ $[M+H]^+$ 421.1070, found 421.1079.

Preparation of 2-Nitrothiophenol (12): To a suspension of 2-nitrophenyl disulfide **11** (0.805 g, 2.61 mmol) in 20 mL of degassed THF was added PPh_3 (1.03 g, 3.92 mmol), 2-mercaptoethanol (184 μL, 2.61 mmol), and 470 μL of dH_2O (26.1 mmol). The solution was stirred at 50°C for 6 h then cooled to RT, concd, redissolved in DCM, washed with brine, dried over Na_2SO_4, and evaporated. The resulting thick pungent orange oil was subject to flash chromatography (9:1 hexanes:EtOAc) and pure 2-nitrothiophenol (653 mg, 4.21 mmol) was isolated in 80.5% yield from the bright yellow band fractions. m.p. 40-42°C.

Synthesis of L-Cbz-VG-Phe-OMe (16): Two methods were used to synthesize **16**.

Conversion of 10f to Cbz-VG-OMe (1); General Procedure: Sulfoxide **10f** (1 mol equiv) and NaOAc (10 mol equiv) were refluxed with stirring in PhMe for 18 h. The solution was then cooled to RT and the precipitate removed by vacuum filtration through celite. The flask was rinsed with toluene, filtered, and concd to provide VG **1**. Decolorization of the product can be achieved by vacuum filtration of the oil through a plug of silica with 3:1 hexanes:EtOAc or for instances when starting material is still present, the mixture can be refluxed in toluene with fresh 10 equiv NaOAc until the reaction is complete. Spectroscopic data of Cbz-VG-OMe prepared by this method was equivalent to those reported in previous pubications.[27, 31, 36-37, 69]

First method: Sulfoxide **10f** was stirred in a 1:1 solution of 5% aqueous HCl and AcOH for 0.5 h at 100°C. The AcOH was then evaporated and the aqueous portion was extracted with EtOAc. After drying over $MgSO_4$, the product was concd under vacuum and 44.5 mg (0.11 mmol) of the crude acid **14** was transferred to a flask containing HOBt (14.8 mg, 0.11 mmol) and L-Phe-OMe-HCl suspended in acetonitrile. EDC•HCl (22 mg, 0.12 mmol) was then added followed by DIPEA (21 μL, 0.12 mmol) after 10 min. After overnight stirring, the reaction was

quenched with 5% citric acid and the mixture was extracted with EtOAc. The combined EtOAc parts were then washed with 5% $NaHCO_3$, brine, dried over $MgSO_4$, filtered, and concentrated to provided Cbz-HcyO(2-NO_2Ph)-Phe-OMe. Some amount of this sulfoxide (24 mg, 0.04 mmol) was used without purification for conversion to the titled product **16** by refluxing in toluene. The resulting pale white solid was subject to flash chromatography (2:1 hexanes:EtOAc) and pure L-Cbz-VG-Phe-OMe **16** (7.3 mg, 0.02 mmol) was isolated in 43.7% m.p. 89-90°C.

Second method: Sulfide **9f** was stirred in a 1:1 solution of 5% aqueous HCl and AcOH for 0.5h at 100°C. The AcOH was then evaporated and the aqueous portion was extracted with EtOAc. After drying over $MgSO_4$, the product was concd under vacuum and 74 mg (0.19 mmol) of the crude acid was transferred to a flask containing HOBt (28 mg, 0.21 mmol) and L-Phe-OMe-HCl suspended in acetonitrile. EDC•HCl (51 mg, 0.29 mmol) was then added followed by Et_3N (53 μL, 0.38 mmol) after 10 mins. After overnight stirring, the reaction was quenched with 5% citric acid and the mixture was extracted with EtOAc. The combined EtOAc parts were then washed with 5% $NaHCO_3$, brine, dried over $MgSO_4$, filtered, and concentrated to provided Cbz-Hcy(2-NO_2Ph)-Phe-OMe. This sulfide (105 mg, 0.19 mmol) was next oxidized to the sulfoxide with *m*-CPBA (43 mg, 0.25 mmol) in DCM at RT and after workup, was used without purification for conversion to the titled product **16** (60.1 mg, 0.15 mmol) by the general procedure described above. Pale white solid; m.p. 89-90°C; TLC (SiO_2) R_f 0.56 (1:1 hexanes:EtOAc); $[\alpha]^{24}_D$ +66.3° (*c* 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.34 (m, 5H). 7.33-7.36 (m, 3H), 7.11 (d, 2H *J* = 7 Hz), 6.50 (d, 1H, *J* = 5.5 Hz), 5.87 (ddd, 1H, *J* = 17, 10.5, 6.5 Hz), 5.41 (d, 1H, *J* = 17 Hz), 5.33 (d, 1H, *J* = 10 Hz), 5.15 (s, 2H), 4.91-4.87 (m, 1H), 4.76 (m, 1H), 3.76 (s, 3H), 3.19 (dd, 1H, *J* = 14, 6 Hz), 3.13 (dd, 1H, *J* = 14, 6 Hz); ^{13}C NMR

(125 MHz, $CDCl_3$) δ 171.3, 169.2, 155.9, 136.4, 135.7, 133.7, 129.4, 128.8, 128.7, 128.4, 128.3, 127.4, 119.1, 67.3, 57.6, 53.6, 52.6, 37.9.

Section-II

This section illustrates the methods and materials used in the schemes described in chapter III.

Synthesis of di-alkylated cyclohexamine salt of phenylalanine (3): In a 5ml microwave tube, phenylalanine **1** (1 g, 6.05 mmol) was added to a 3 mL 10:1 solution of DMF. To this solution, cesium carbonate (1.9 g, 6.05 mmol) and propargyl bromide (539 µL, 6.05 mmol) were added, sealed the tube and prestirred for 30 sec in Biotage initiator (microwave). The reaction was carried out with stirring for 1 h at 50^{o}C. After 1 h, the tube was cooled, removed from microwave, acidified with 1N HCl and extracted with EtOAc. EtOAc layer was washed 4x with water and the organic layer dried over $MgSO_4$, filtered and evaporated under reduced pressure to a white solid. To this cyclohexamine and DCM were added, heated to form a salt, filtered and collected the white solid of hexamine salt **3**.

Synthesis of haloenol lactones (BEL analogs) 5, 8, 9; General procedure: To K_2CO_3 (1 mmol) was added the acid (1 mmol) and DCM (10 mL) with stirring. After 10 min, *N*-bromosuccinimide or *N*-iodosuccinimide (1 mmol) was added, followed by the addition of dH_2O (4 mmol). The reactions were monitored by TLC (5:1 hexanes:EtOAc) and also observed a brown spot when heated corresponding to the product. After the total disappearance of starting material, dilution with additional DCM, drying over $MgSO_4$, and evaporation under reduced pressure afforded crude lactones. The crude products were subjected to flash chromatography (5:1 hexanes:EtOAc) to obtain pure haloenol lactones **5, 8,** and **9**.

(*S,E*)-3-benzyl-6-(bromomethylene)-4-(prop-2-ynyl)morpholin-2-one (5): brown solid; TLC (SiO_2) R_f 0.48 (5:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35-7.32 (m, 2H), 7.30-7.27 (m, 4H), 6.04 (s, 1H), 3.92 (t, 1H, *J* = 5.75), 3.84 (dd, 1H, *J* = 12.5, 0.5 Hz), 3.66 (app dd, 1H, *J* = 17, ~0.5 Hz), 3.19 (dd, 1H, *J* = 17.5, 2.5 Hz), 3.48 (dd, 1H, *J* = 17.5, 2.5 Hz), 3.28 (dd, 1H, *J* = 15, 7 Hz), 3.19 (dd, 1H, *J* = 14.5, 5 Hz), 2.36 (t, 1H, *J* = 2.5 Hz).

(*E*)-tetrahydro-6-(iodomethylene)pyran-2-one (8): yellow oil; TLC (SiO_2) R_f 0.41 (5:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.89 (s, 1H), 2.66 (t, 2H, *J* = Hz), 2.57 (t, 2H, *J* = 8.8 Hz), 1.93-1.90 (qnt, 2H, *J* = 8.3 Hz).

(*E*)-6-(bromomethylene)-tetrahydropyran-2-one (9): off white solid; TLC (SiO_2) R_f 0.4 (5:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.0 (s, 1H), 2.65 (t, 2H, *J* = 6.3 Hz), 2.60 (t, 2H, *J* = 6.5 Hz), 1.91 (qnt, 2H, *J* = 6.75 Hz).

Synthesis of *N*-monoalkylated Phe-OMe 14: To activated 4 A^o molecular sieve powder (2.0g) in dry DMF (15 mL) was added $LiOH{\cdot}H_2O$ (0.58 g, 13.89 mmol) and then the suspension was stirred for 30 min. L-Phe-OMe.HCl **13** (1 g, 4.63 mmol) was added to the above suspension and stirred for additional 45 min. To this white suspension, propargyl bromide (0.49 mL, 5.55 mmol) was added and allowed to stir for 24 h at room temperature. After filtration through a thick bed of Celite, EtOAc was added and the combined filtrate washed four times with water, dried the organic layer over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The residue was subject to flash chromatography (2:1 hexanes:EtOAc) to yield pure **14** (0.29 g, 1.34 mmol, 29.1% yield) as a pale yellow oil. TLC (SiO_2) R_f 0.65 (1:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29-7.26 (m, 2H), 7.23-7.18 (m, 3H), 3.74 (t, 1H, *J* = 7 Hz), 3.64 (s, 3H), 3.38 (ABdq, 2H, J_{AB} = 16.8, 2.5 Hz), 2.98 (dq, 2H, *J* = 14, 6 Hz), 2.21 (t, 1H, *J* = 2.5

Hz), 1.89 (bs, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.1, 136.8, 129.0, 128.3, 126.7, 81.1, 61.0, 51.6, 39.2, 36.6.

Synthesis of proline methyl ester salt 18: To an ice cold stirred solution of L-proline **17** (2 g, 17.3 mmol) was added thionyl chloride (3.3 mL, 45.1 mmol) in a dropwise manner. The reaction was warmed to room temperature and allowed to stir for 24 h and concentrated to dryness under reduced pressure. The resultant oil was dissolved in toluene and concd. to dryness to remove residual thionyl chloride and methanol to yield **18** as a pale yellow oil. The oil when kept in freezer for one day afforded a hygroscopic off white solid.

Synthesis of *N*-alkylated proline methyl ester 19: Proline salt **18** (0.48 g, 2.92 mol) was suspended in 10 mL DMF and slowly added triethylamine (0.28 mL, 3.22 mmol) while stirring to form a white precipitate. Potassium *tert*-butoxide (0.33 g, 2.92 mmol) was added to the suspension and allowed to stir for 30 min. To this suspension, propargyl bromide (0.4 mL, 2.92 mmol) was added and stirred for 24 h. Afterwards, the suspension was filtered, EtOAc was added, combined filtrate was acidified with 5% citric acid and washed with water for 5 times. Extraction with ethyl acetate, drying over anhydrous magnesium sulfate and concentration under reduced pressure yielded a pale yellow oil. The oil was subjected to flash chromatography (2:1 hexanes:EtOAc) to yield a pale yellow oil as desired product **19**.

Synthesis of *N*-monoalkylated Phe-OBn 20: Using the above mentioned procedure for alkylation of proline methyl ester **19**, Phe-OBn was alkylated to yield **20** in 63.9% yield as a pale yellow oil.

Synthesis of *N*-alkylated methyl propargyl amine 23: Benzyl ester of bromoacetic acid (0.32 g, 1.52 mmol) was added to 5 mL microwave tube containing anhydrous DMF (3 mL) and

potassium carbonate (0.21 g, 1.52 mmol). To this suspension *N*-methyl propargylamine (0.14 mL, 1.68 mmol) was added and prestirred for 30 sec in Biotage Initiator. The reaction was carried out at 100C for 30 min, diluted with EtOAc and washed with water for three times. Organic layer was dried over anhydrous magnesium sulfate, filtered and concd. under reduced pressure to yield pale yellow oil. This was chromatographed on silica (2:1 hexanes:EtOAc) to yield pure **23** (0.168 g, 0.77 mmol, 50.9% yield). TLC (SiO_2) R_f 0.48 (2:1 hexanes:EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.30 (m, 5H), 5.17 (s, 2H), 3.54-3.49 (m, 2H), 3.42 (dd, 1H, J = 17.5, 2.5 Hz), 2.40 (s, 1H), 2.25 (t, 1H, J = 2.5 Hz), 1.35 (d, 1H, J = 7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 135.8, 128.6, 128.5, 128.3, 128.3, 79.1, 73.0, 66.2, 60.5, 43.3, 38.4, 15.8.

^{1}H NMR of benzyl ester of bromoacetic acid, precursor of 23: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 5H), 5.20 (s, 2H), 3.87 (s, 2H).

Synthesis of *N*-alkylated proline benyl ester 26: An HCl salt of proline benzyl ester was converted to a free base using triethylamine. The free base proline benzyl ester **25** (0.27 g, 1.33 mmol) was added to a flask contaning 10 mL anhydrous THF and while stirring sodium hydride (0.032 g, 1.33 mmol) was added slowly. To this suspension propargyl bromide (0.12 mL, 1.33 mmol) was added and stirred overnight, acidified with 5% citric acid and extracted using EtOAc. The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure affording a pale yellow oil. This was subjected to flash chromatography (2:1 hexanes: EtOAc) to yield pure alkylated proline benzyl ester **26** (89 mg, 0.37 mmol, 27.4% yield) as a colorless oil. TLC (SiO_2) R_f 0.5 (2:1 hexanes:EtOAc).

Synthesis of *N*-dialkylated alanine hexylamine salt 28: To a stirring solution of 13 mL DMF:water (10:1) was added cesium carbonate (1.825 g, 5.11 mmol) which partially dissolved.

To this L-alanine was added and stirred. Propargyl bromide (0.99 mL, 11.22 mmol) was then added to the suspension while stirring and allowed to react for 24 h. EtOAc was added and washed with water for 5 times. The organic layer was concd. under reduced pressure to yield a colorless oil (0.27g). To this cyclohexamine (0.93 mL, 8.18 mmol) and DCM were added, heated to form a salt, refrigerated overnight, filtered and collected the white crystalline solid of hexylamine salt **28** (0.37 g, 1.42 mmol, 12.7% overall yield).

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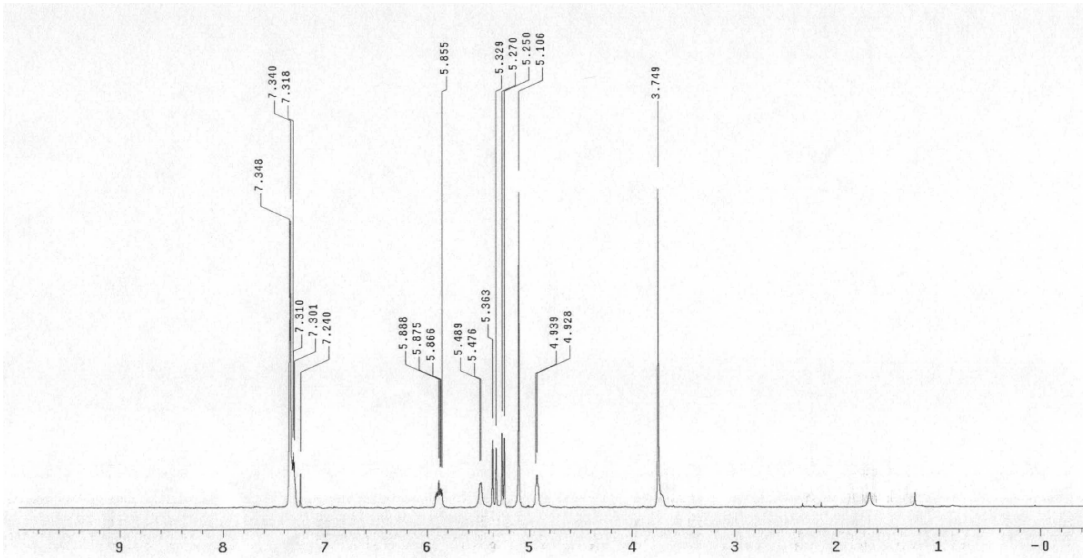
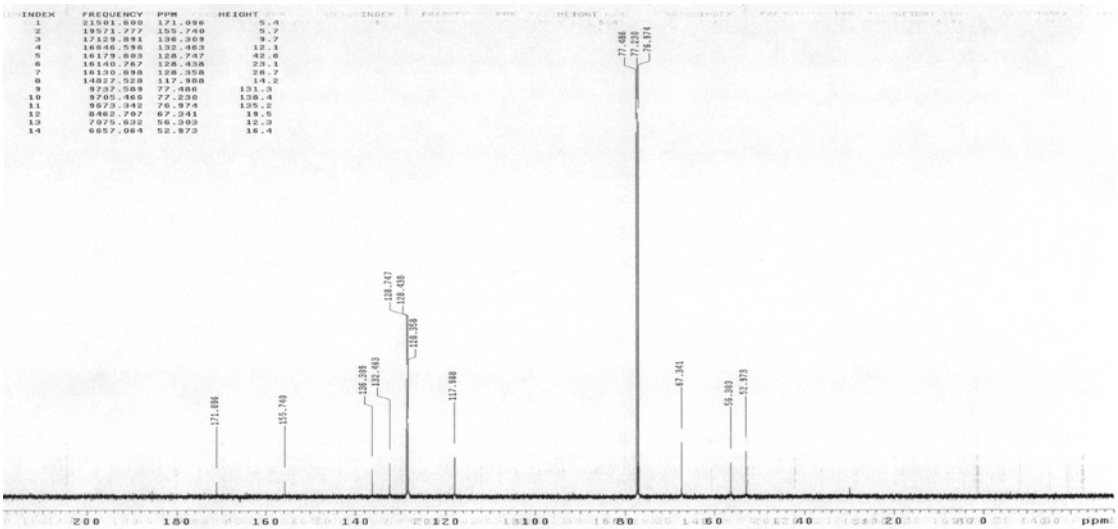
APPENDICES

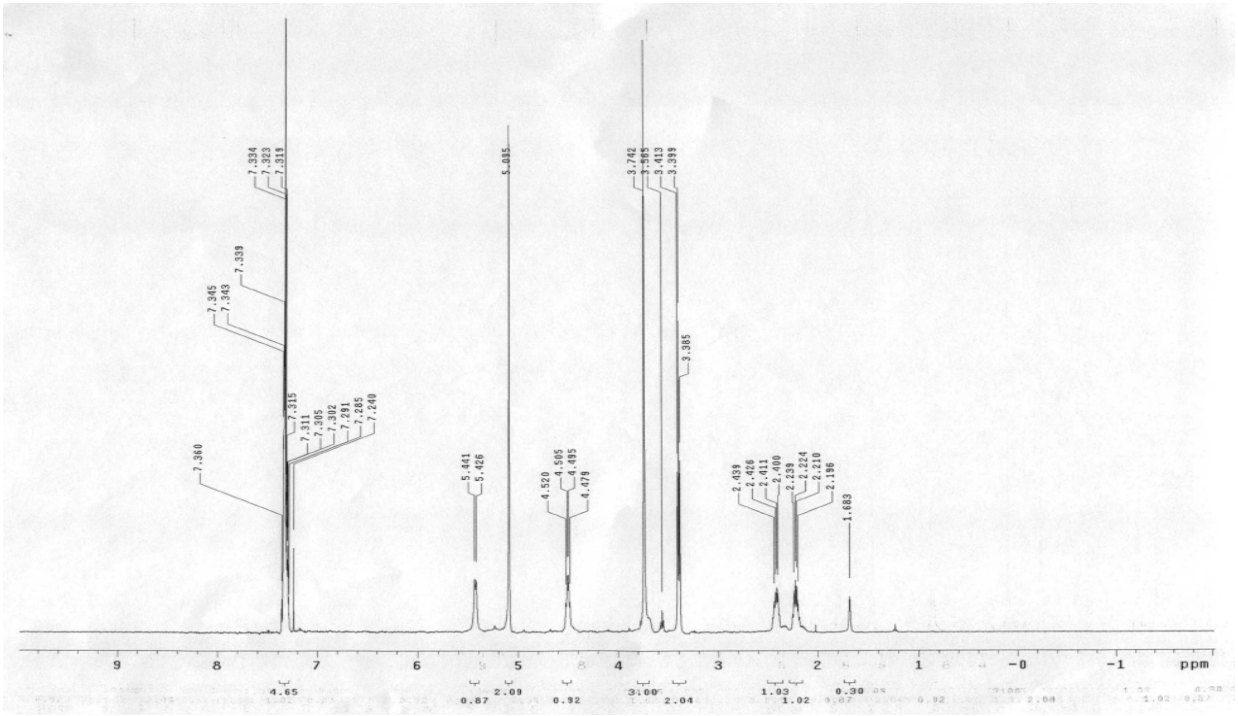
Appendix-I

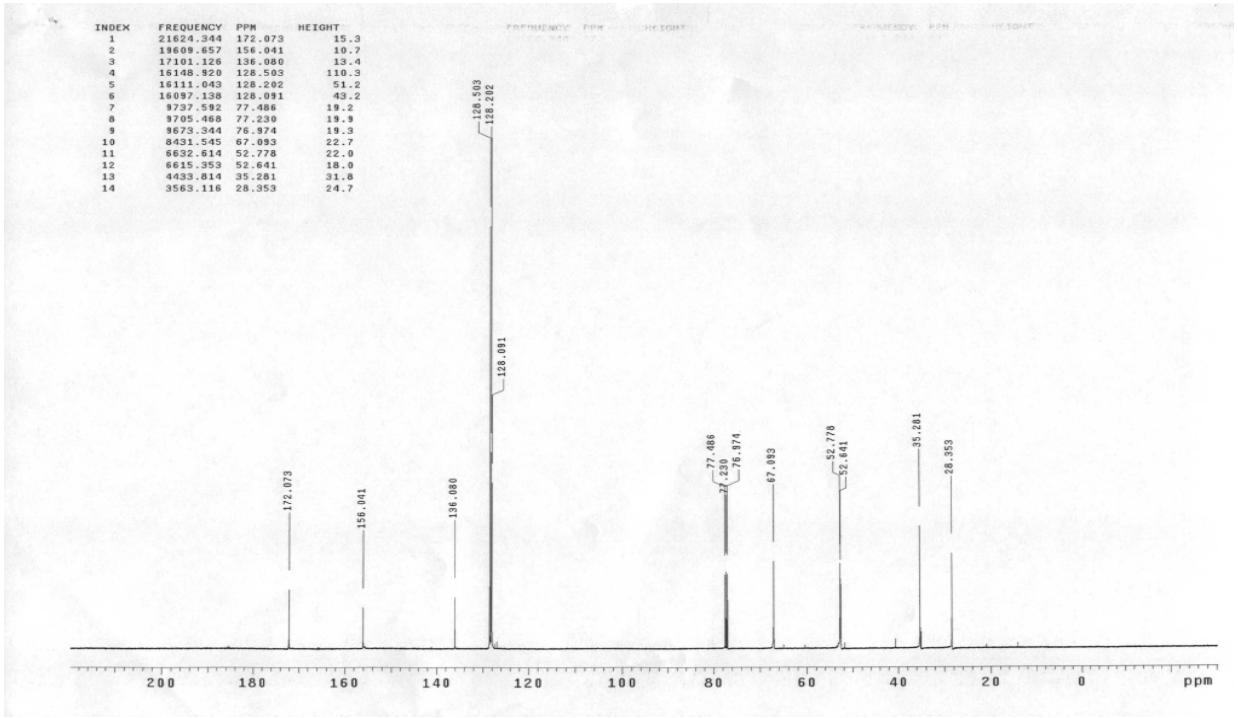
Benzyl (*S*)-1-(methoxycarbonyl)allylcarbamate (1)

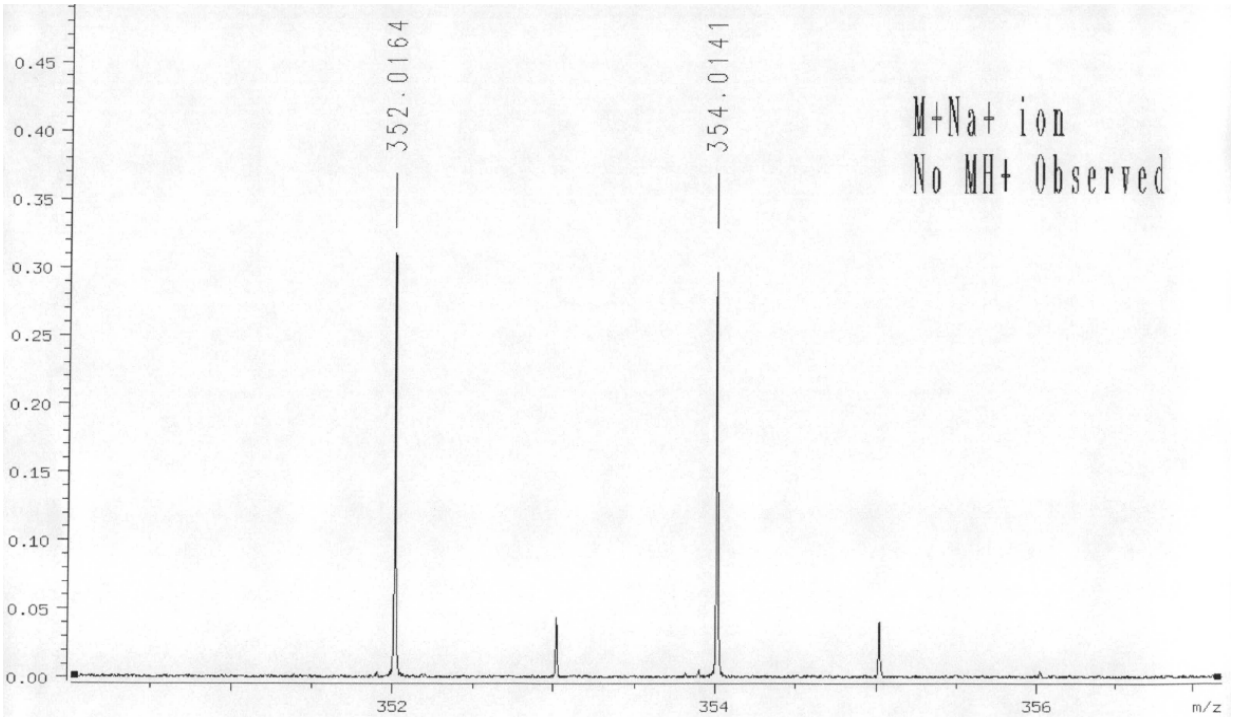




Benzyl (*S*)-1-(methoxycarbonyl)-3-bromopropylcarbamate (6)

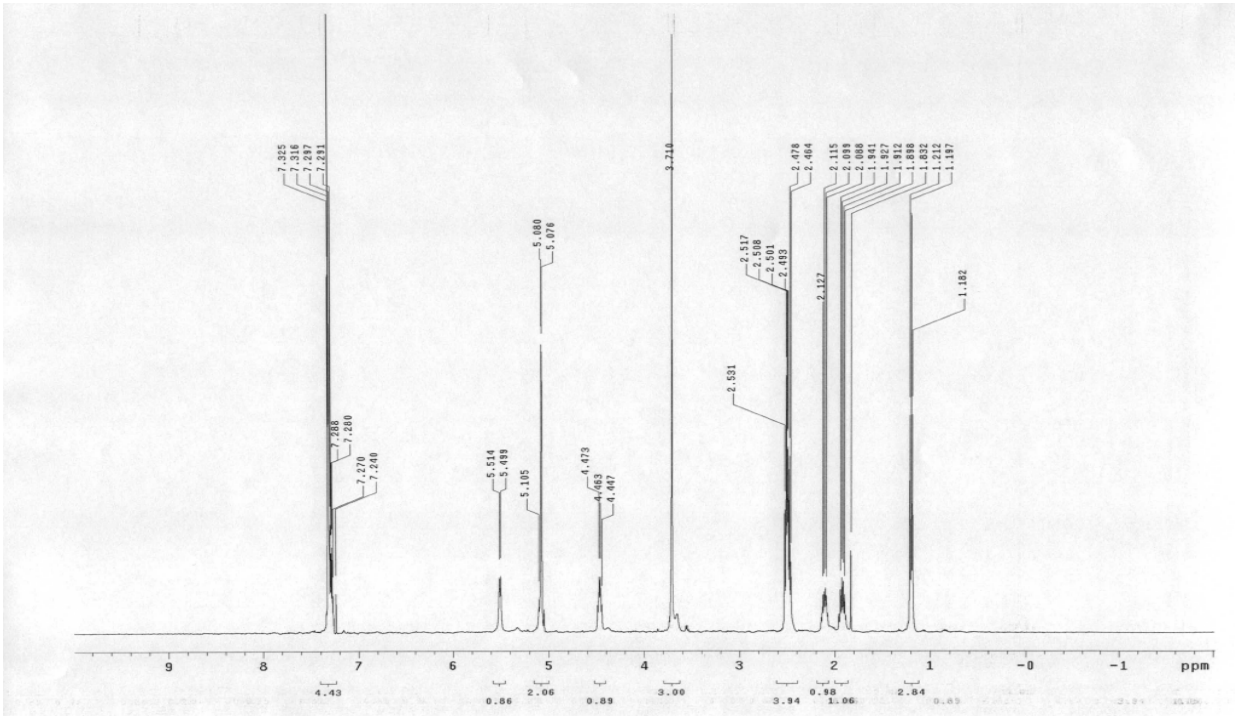


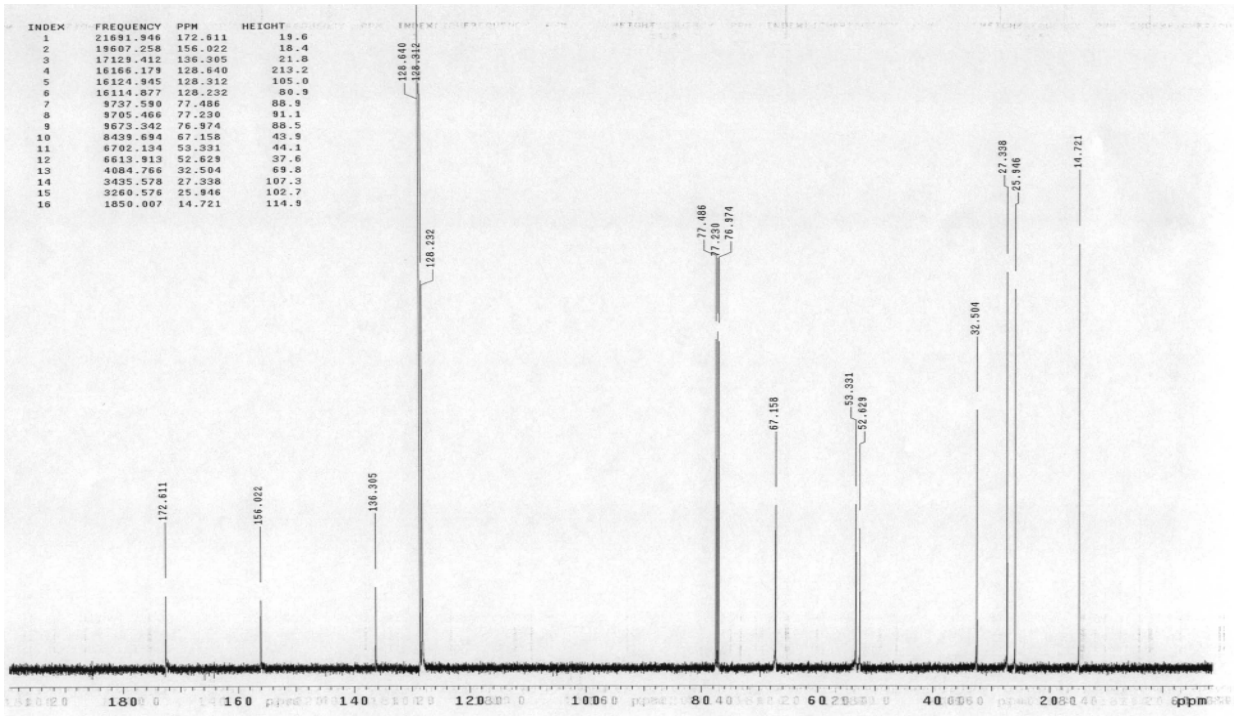


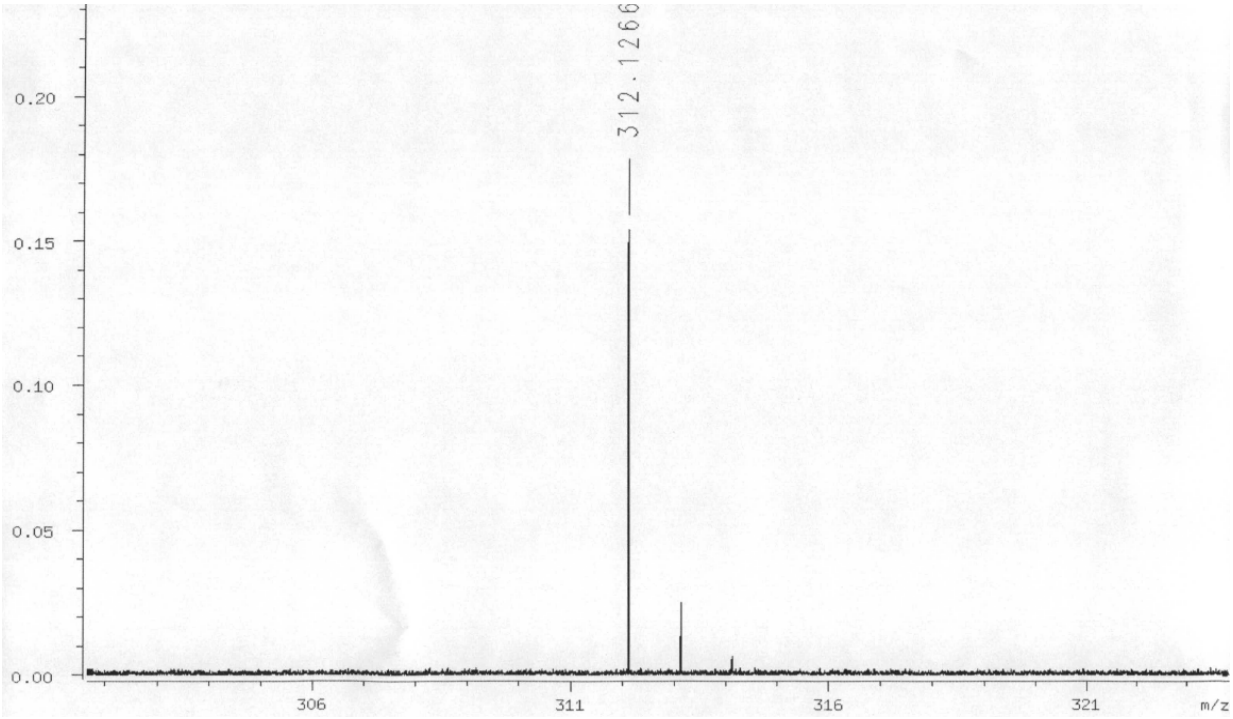


ESI-HRMS calcd for $C_{13}H_{16}BrNO_4$ $[M+Na]^+$ 352.0160, found 352.0164

Benzyl (*S*)-1-(methoxycarbonyl)-3-(ethylthio)propylcarbamate (7a)

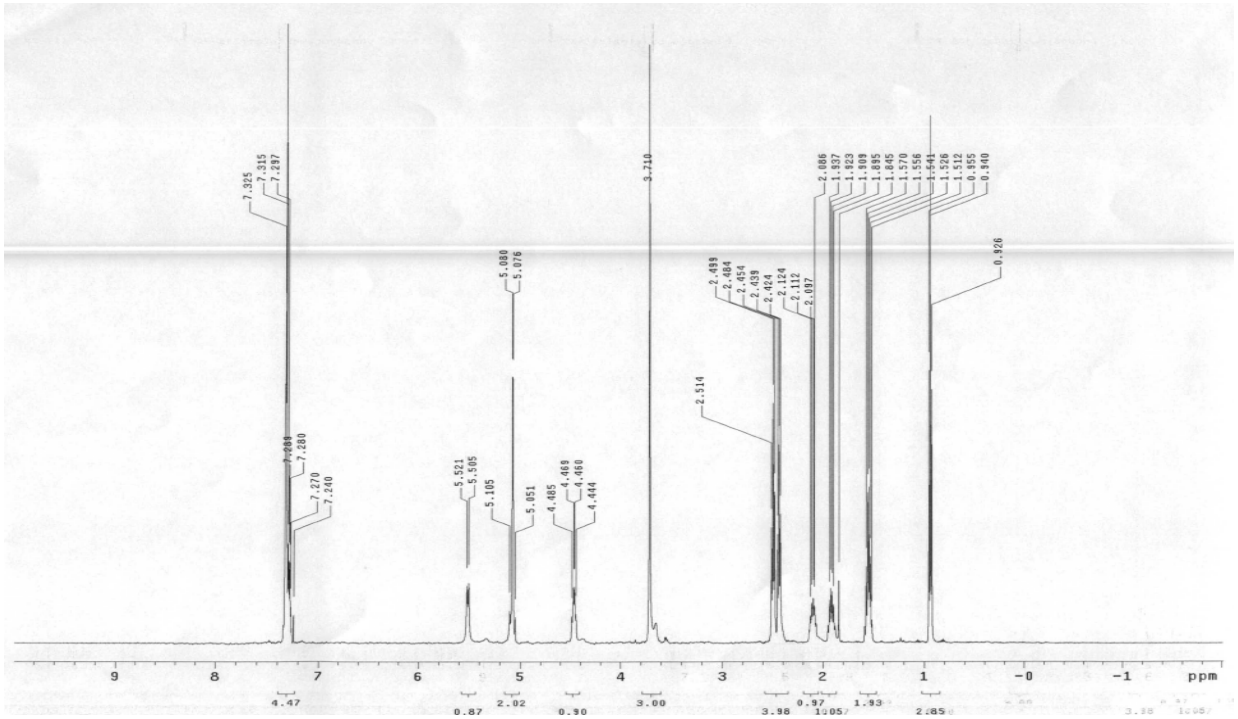


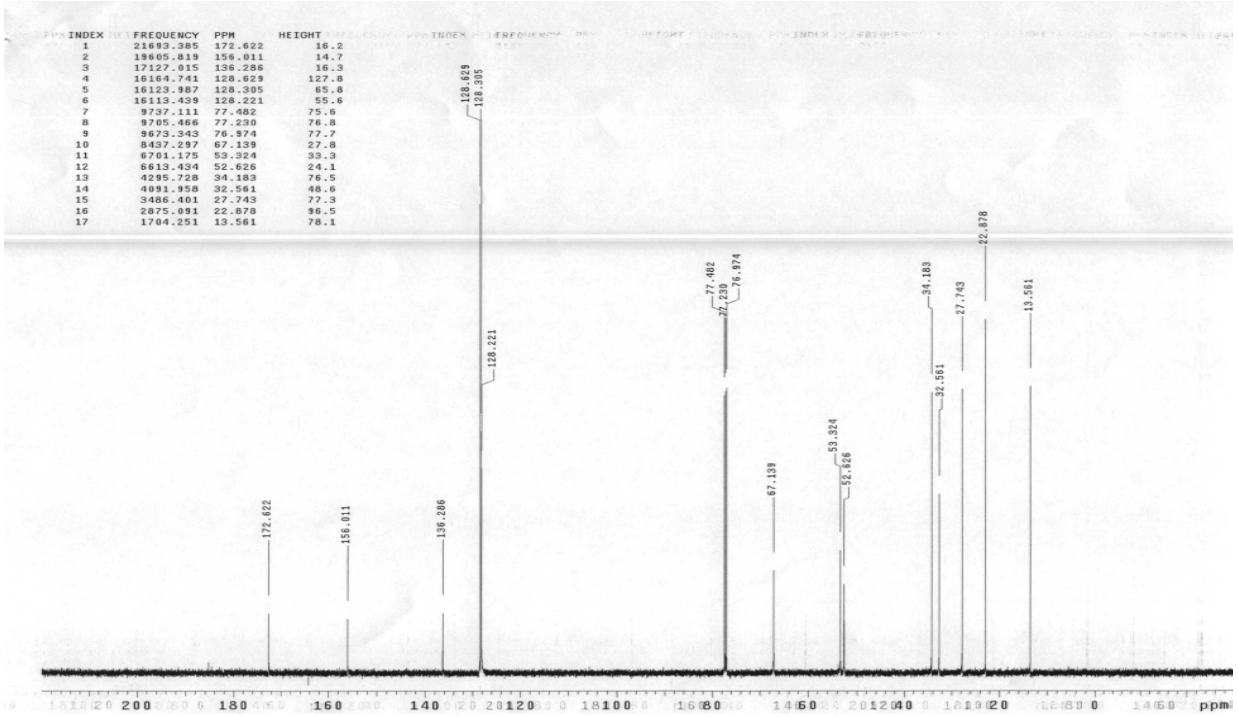


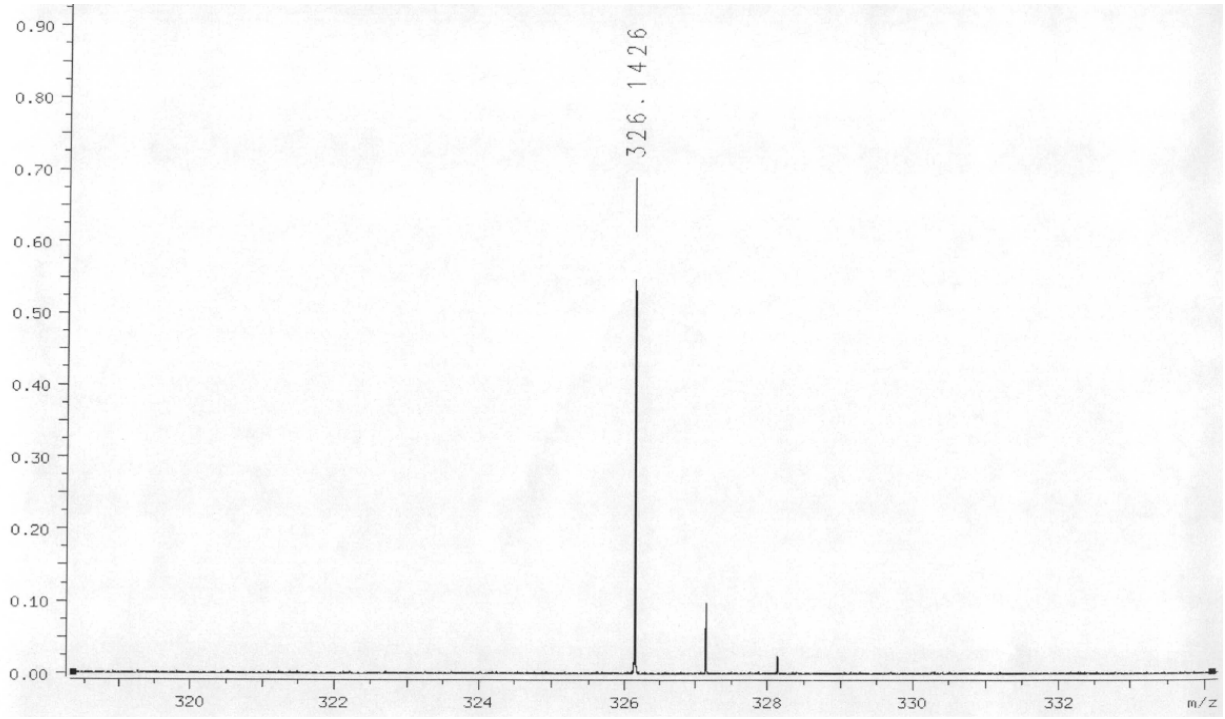


ESI-HRMS calcd for $C_{15}H_{21}NO_4S$ $[M+H]^+$ 312.1269, found 312.1266

Benzyl (*S*)-1-(methoxycarbonyl)-3-(propylthio)propylcarbamate (7b)

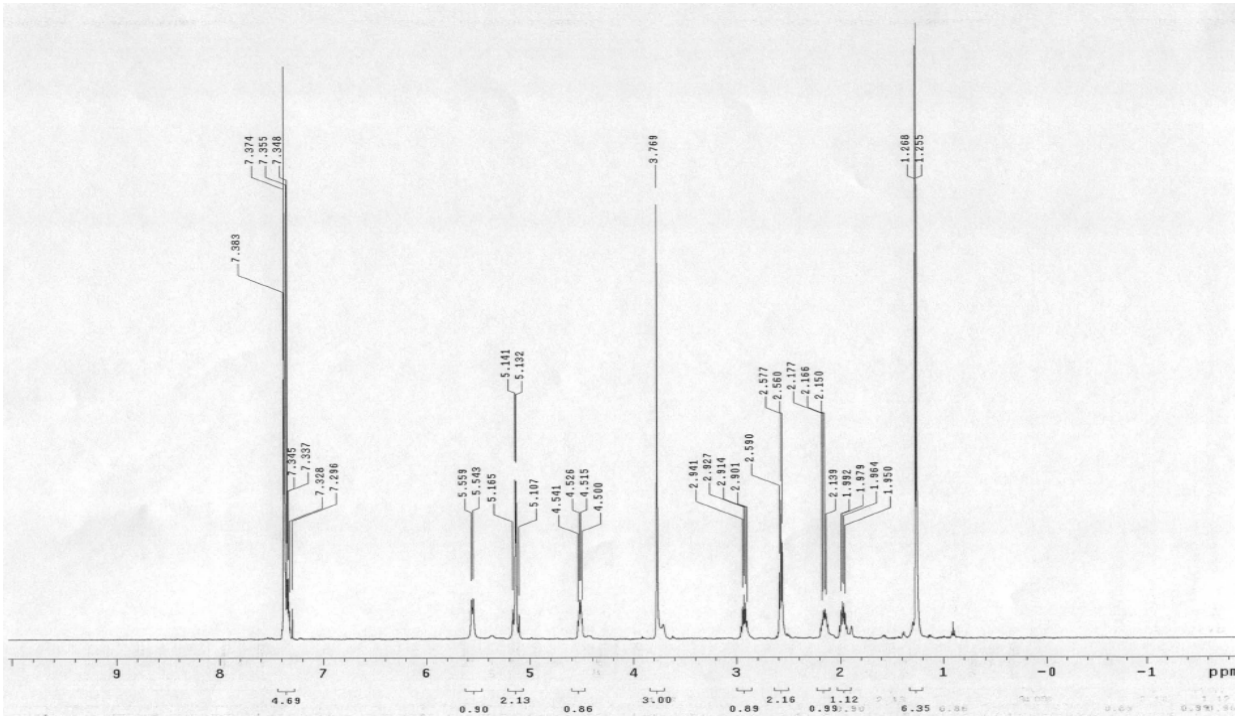


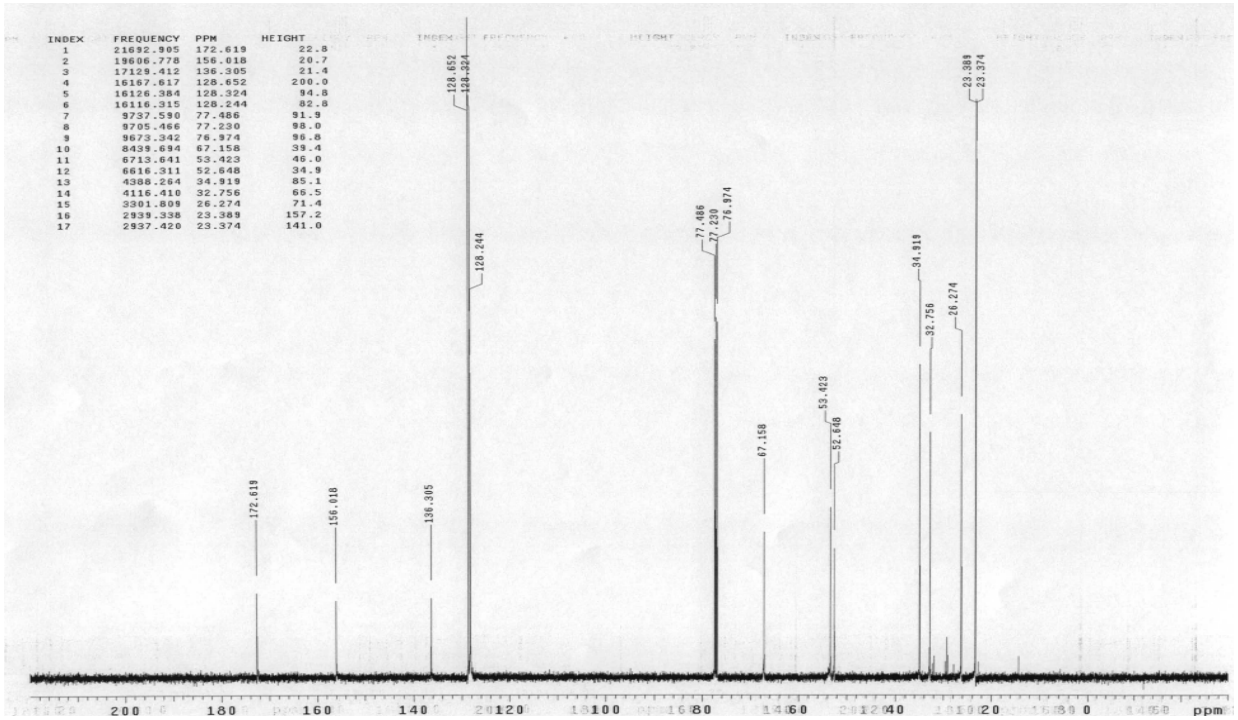


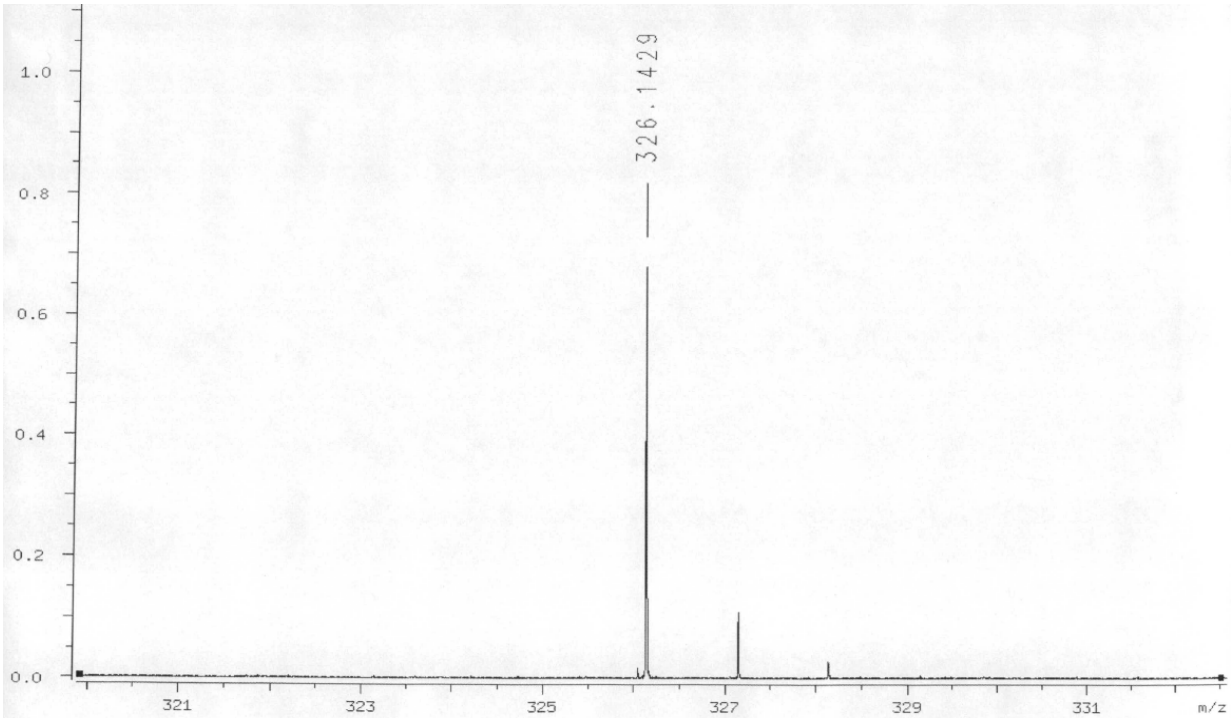


ESI-HRMS calcd for $C_{16}H_{23}NO_4S$ $[M+H]^+$ 326.1426, found 326.1426

Benzyl (*S*)-1-(methoxycarbonyl)-3-(isopropylthio)propylcarbamate (7c)

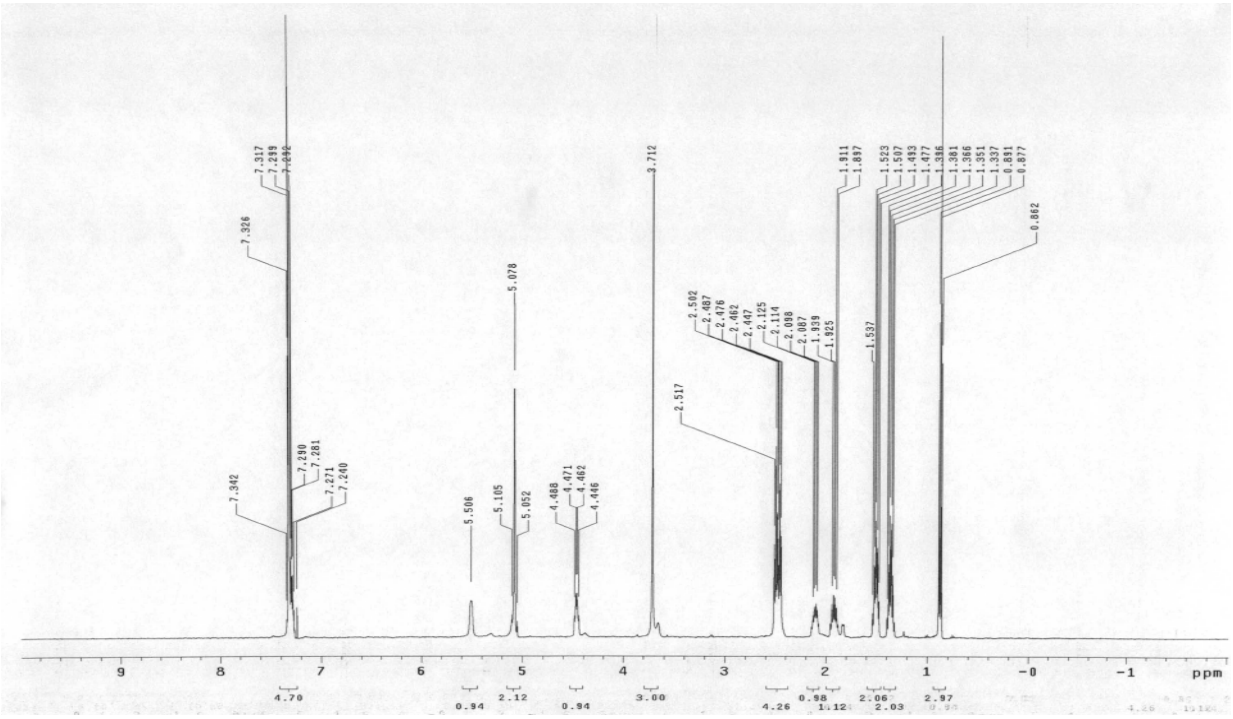


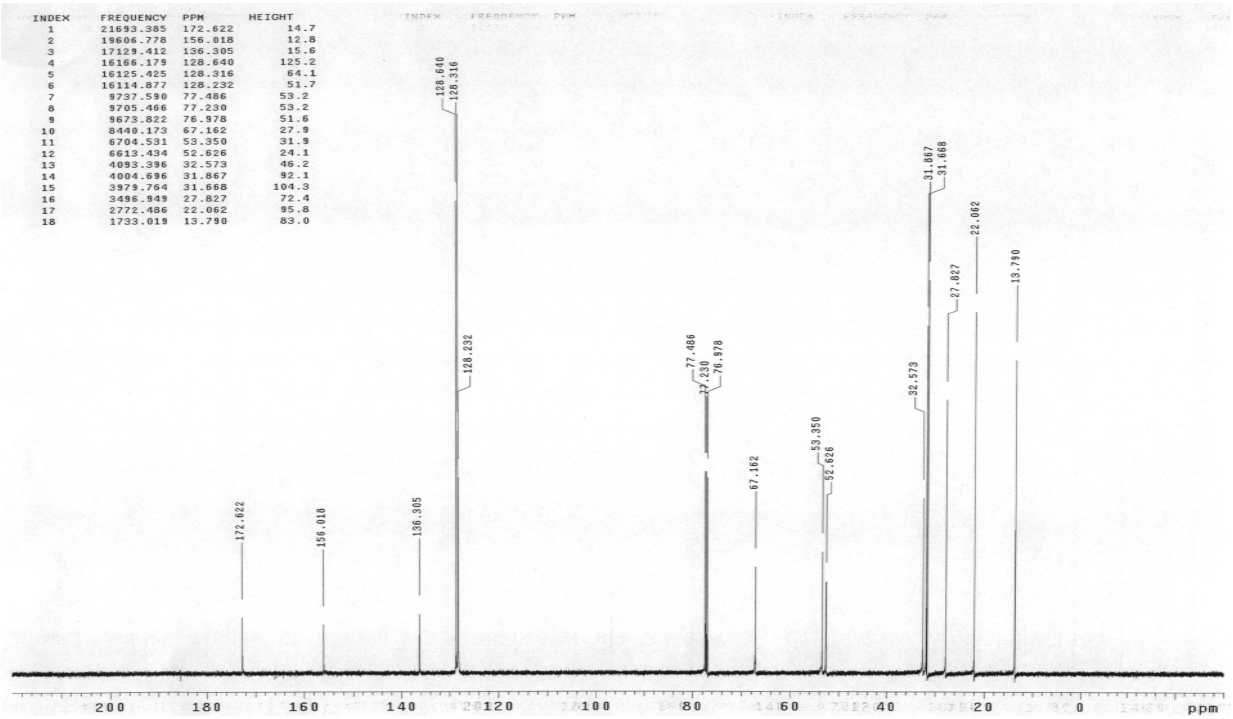


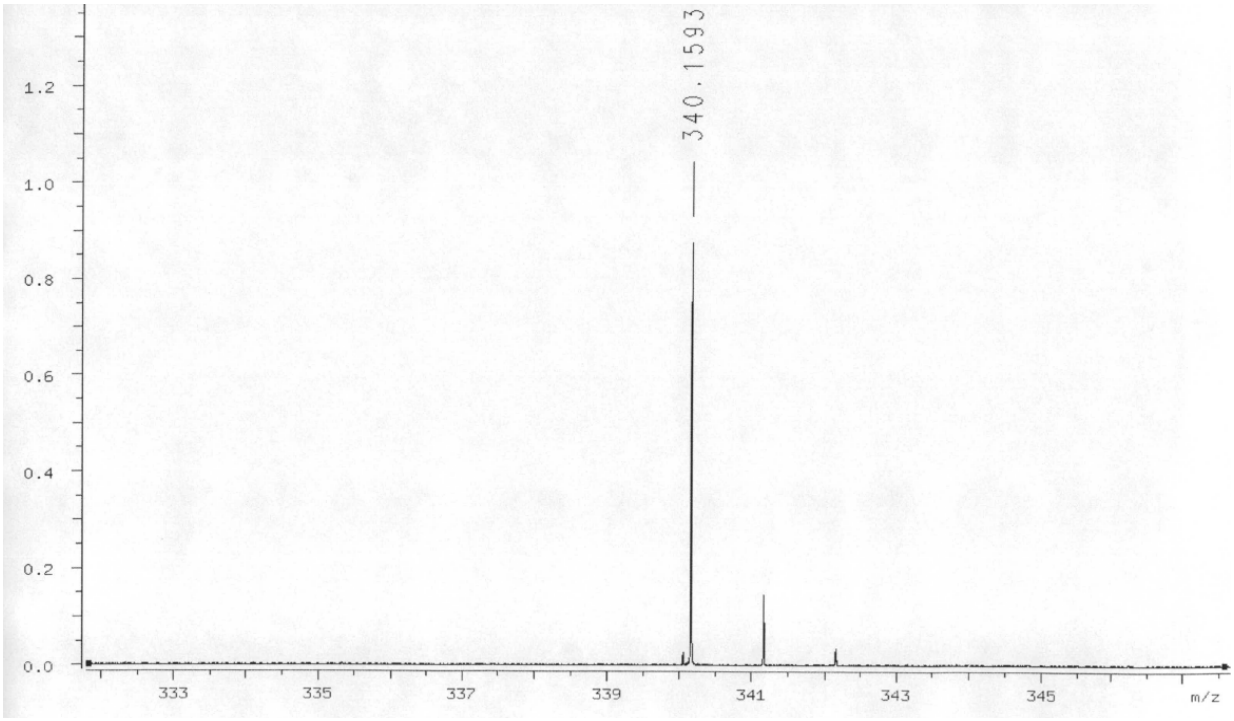


ESI-HRMS calcd for $C_{16}H_{23}NO_4S$ $[M+H]^+$ 326.1426, found 326.1429

Benzyl (*S*)-1-(methoxycarbonyl)-3-(butylthio)propylcarbamate (7d)

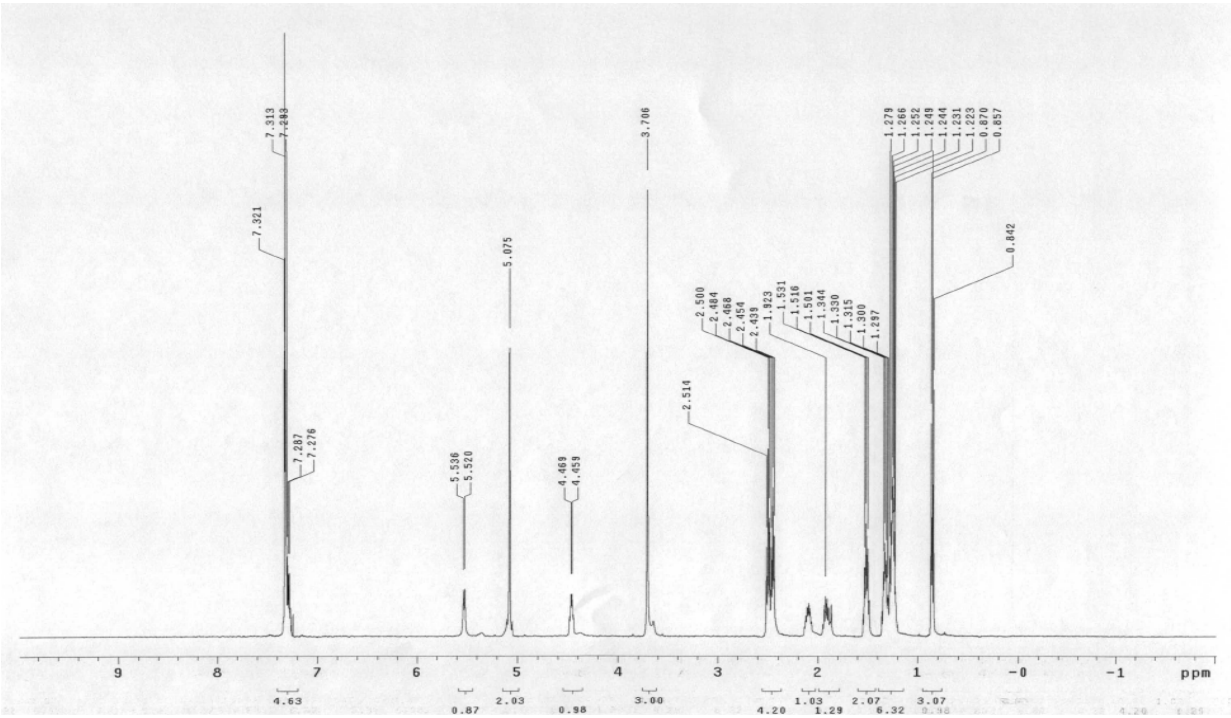


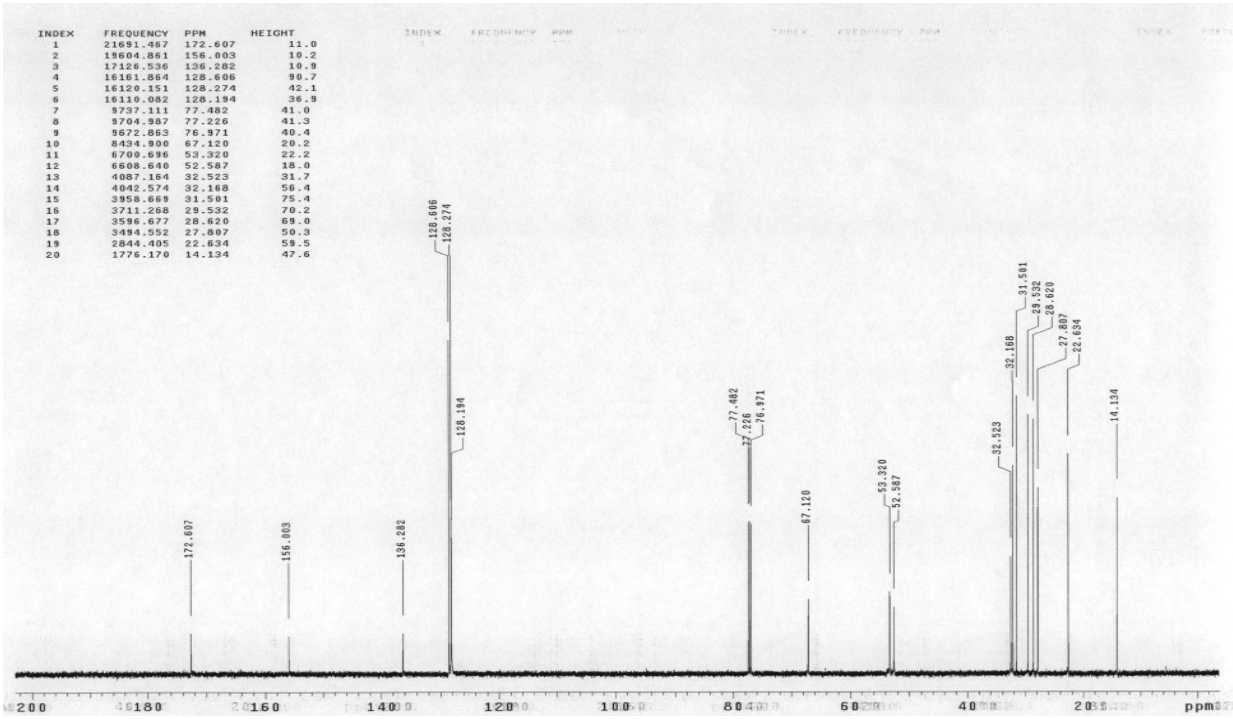


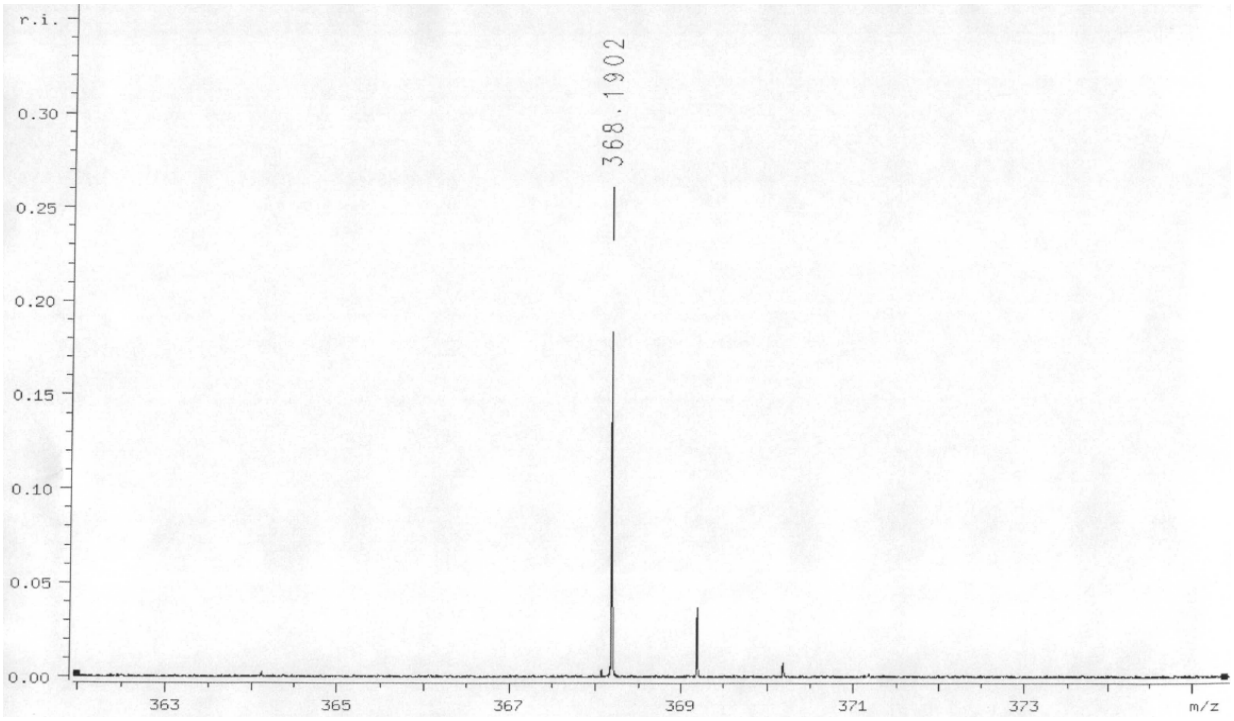


ESI-HRMS calcd for $C_{17}H_{25}NO_4S$ $[M+H]^+$ 340.1582, found 340.1593

Benzyl (*S*)-1-(methoxycarbonyl)-3-(hexylthio)propylcarbamate (7f)

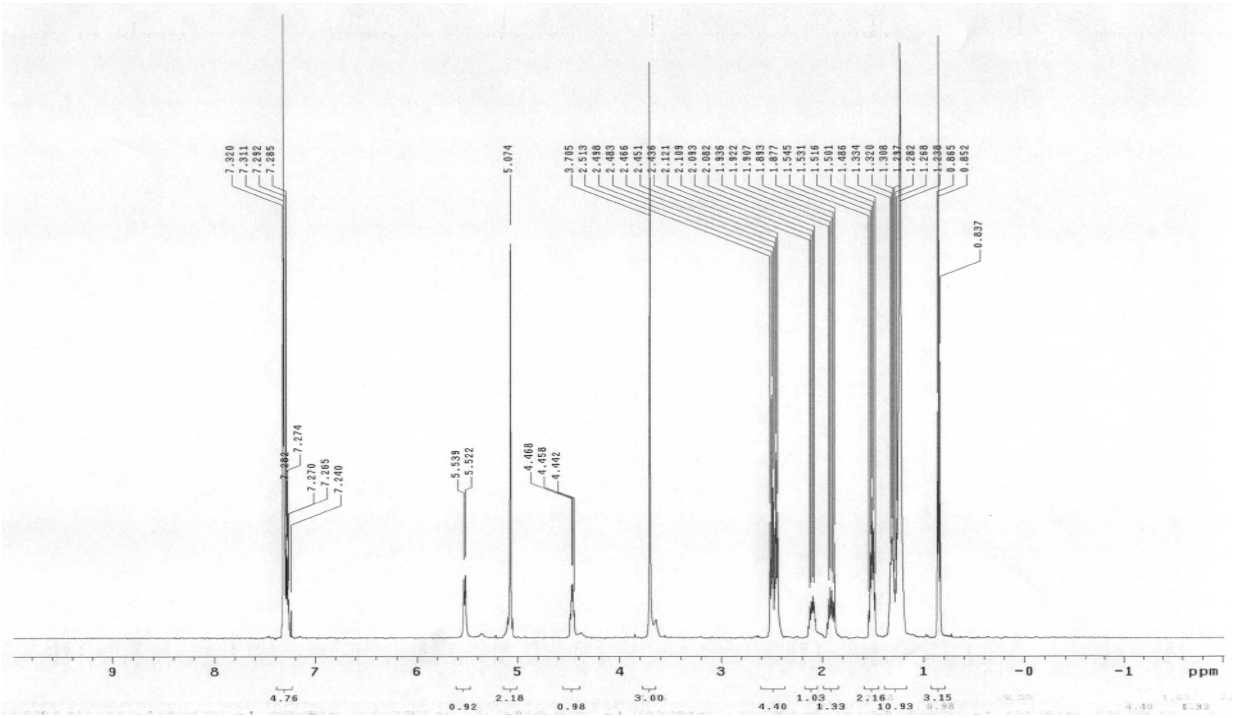


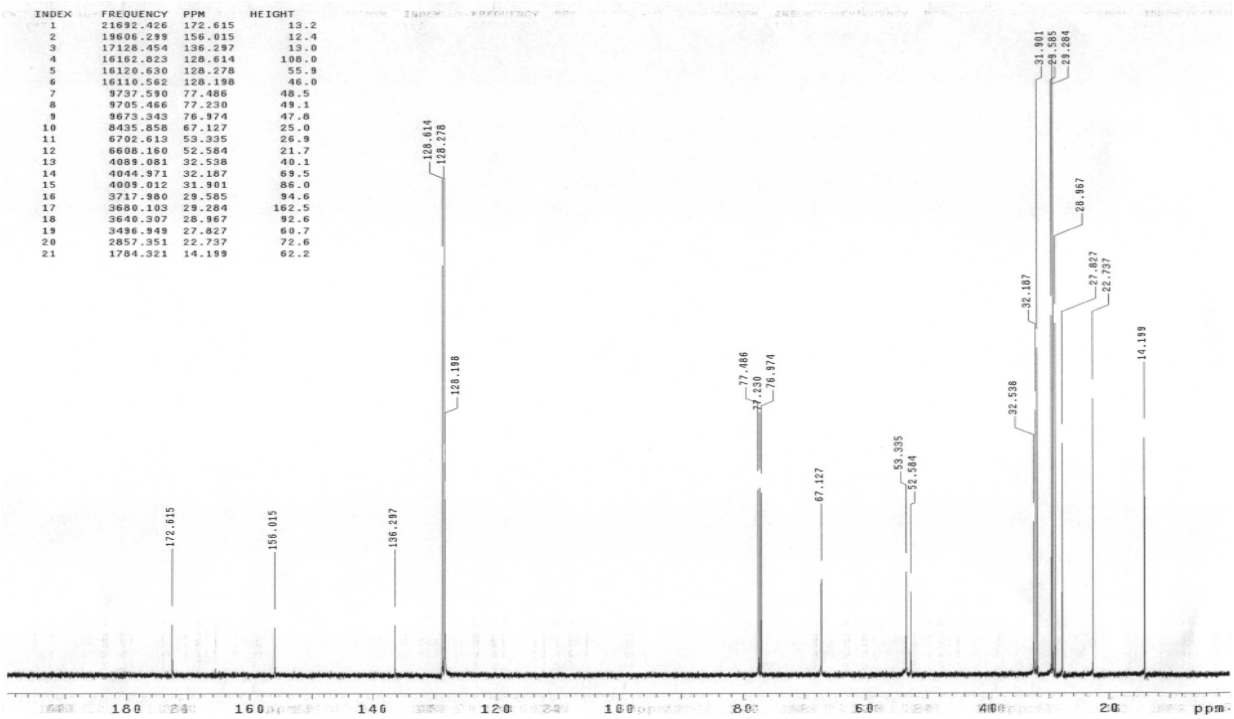


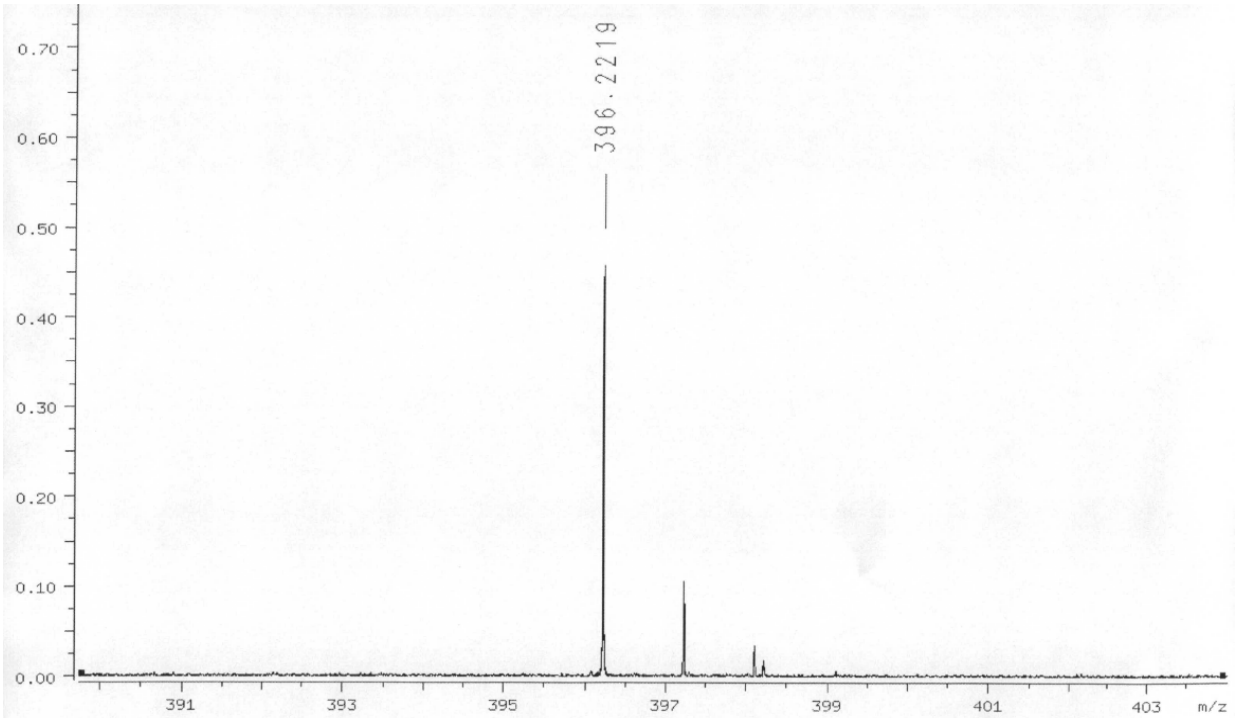


ESI-HRMS calcd for $C_{19}H_{29}NO_4S$ $[M+H]^+$ 368.1896, found 368.1902

Benzyl (*S*)-1-(methoxycarbonyl)-3-(octylthio)propylcarbamate (7g)

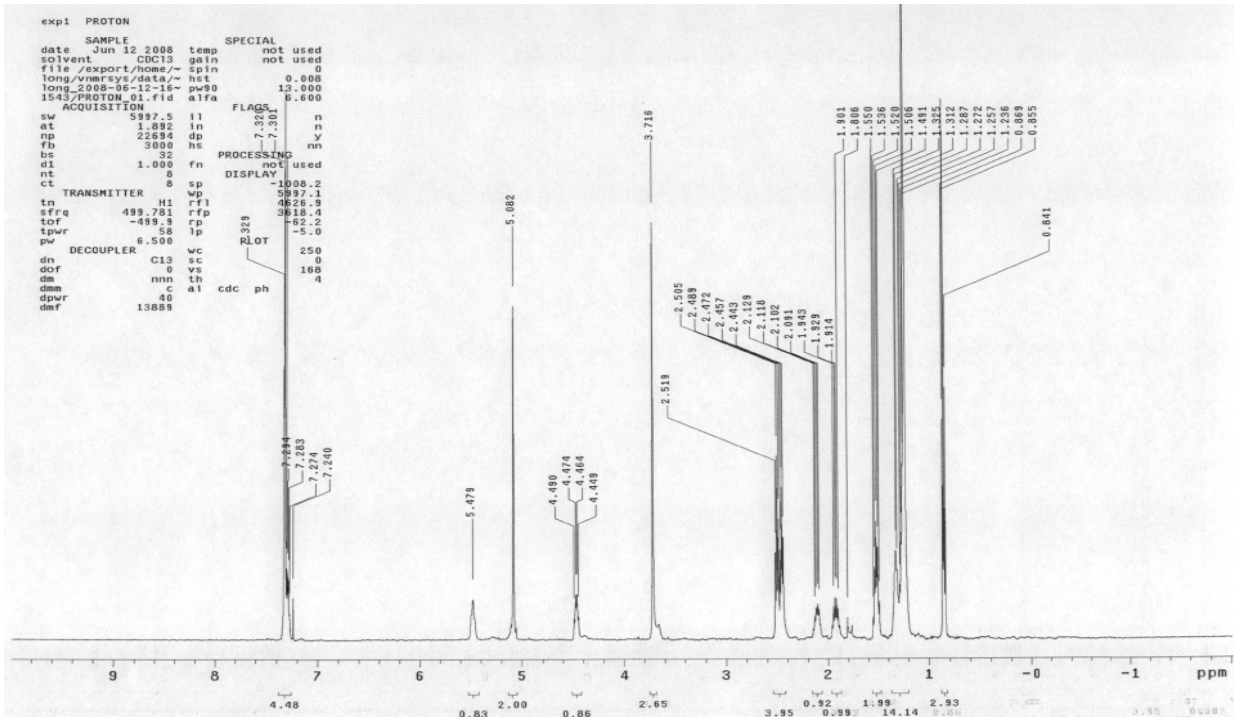


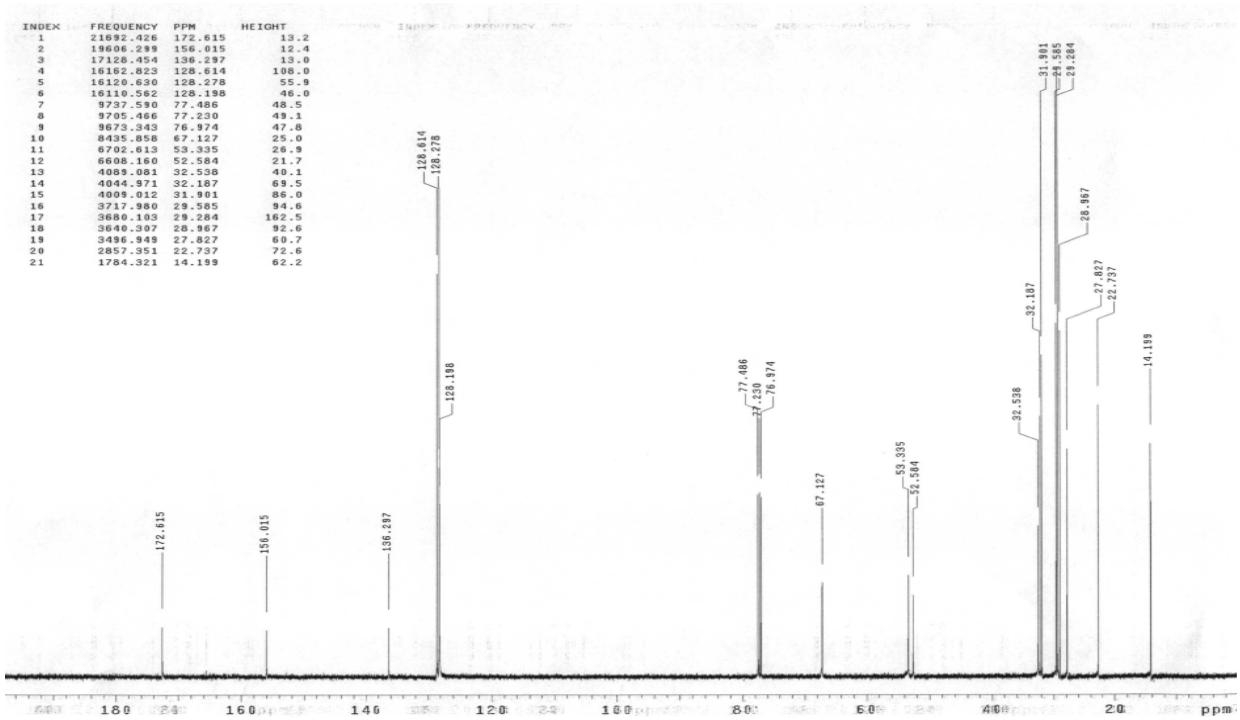


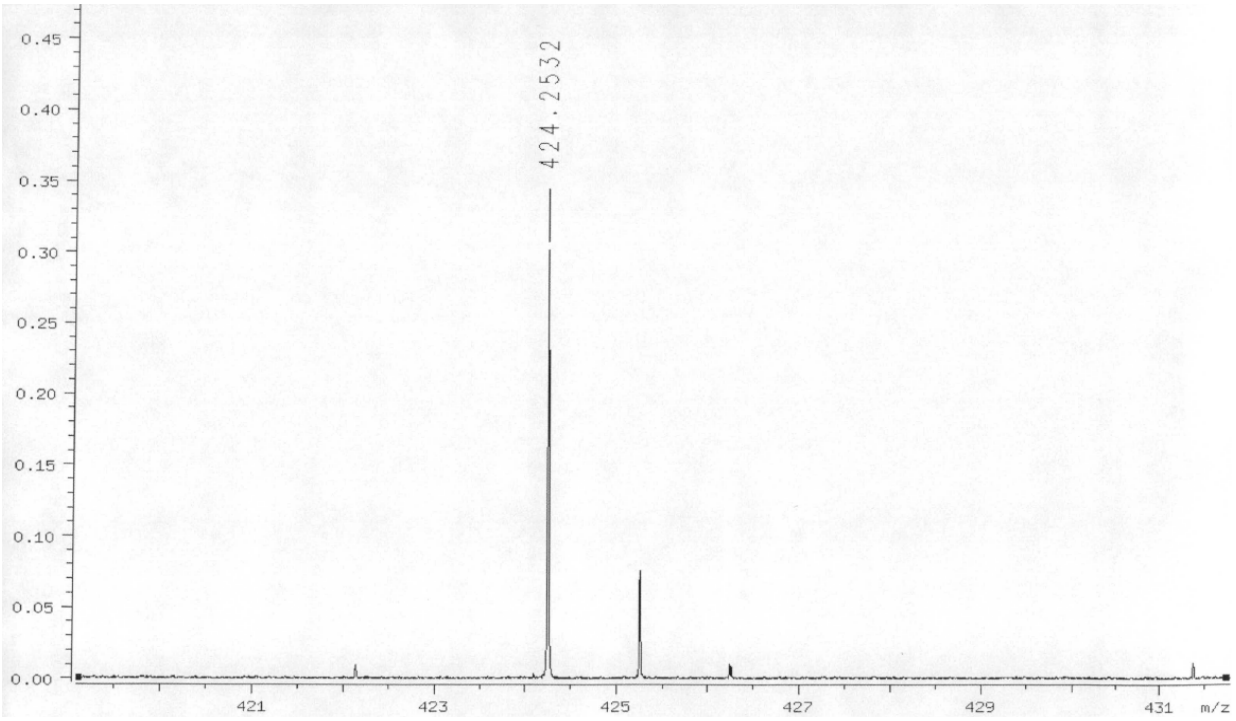


ESI-HRMS calcd for $C_{21}H_{33}NO_4S$ $[M+H]^+$ 396.2209, found 396.2219

Benzyl (*S*)-1-(methoxycarbonyl)-3-(decylthio)propylcarbamate (7h)

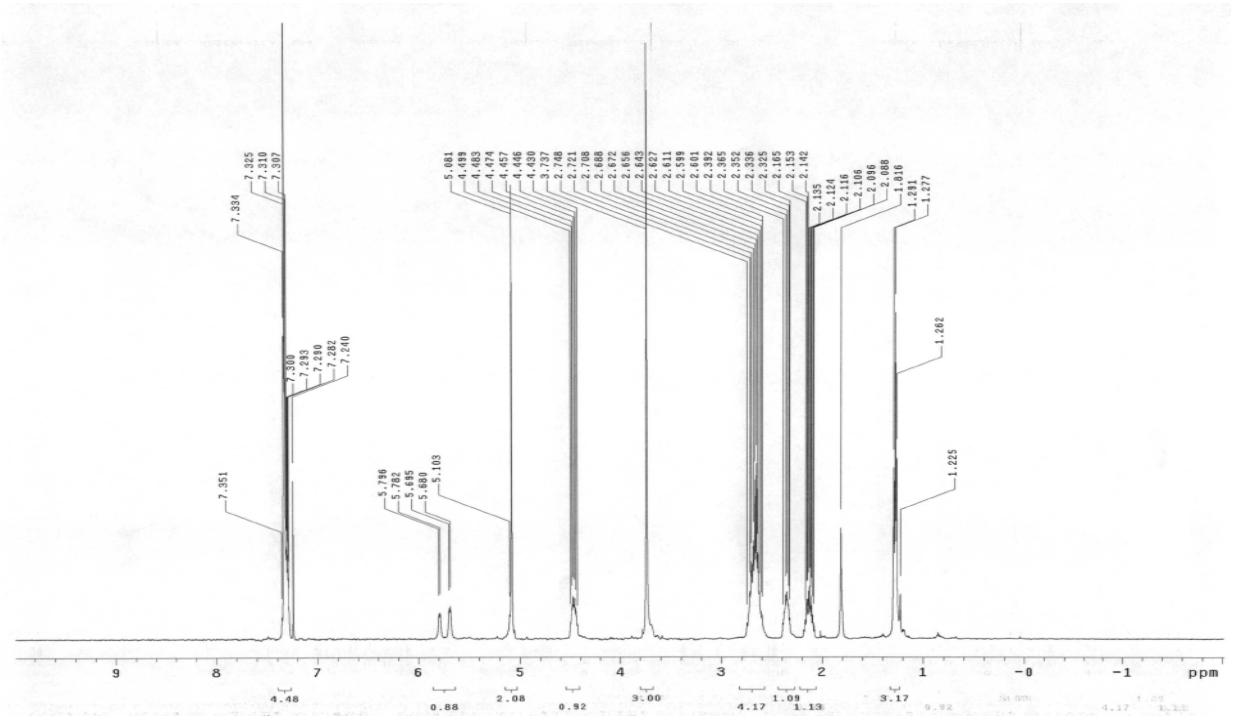


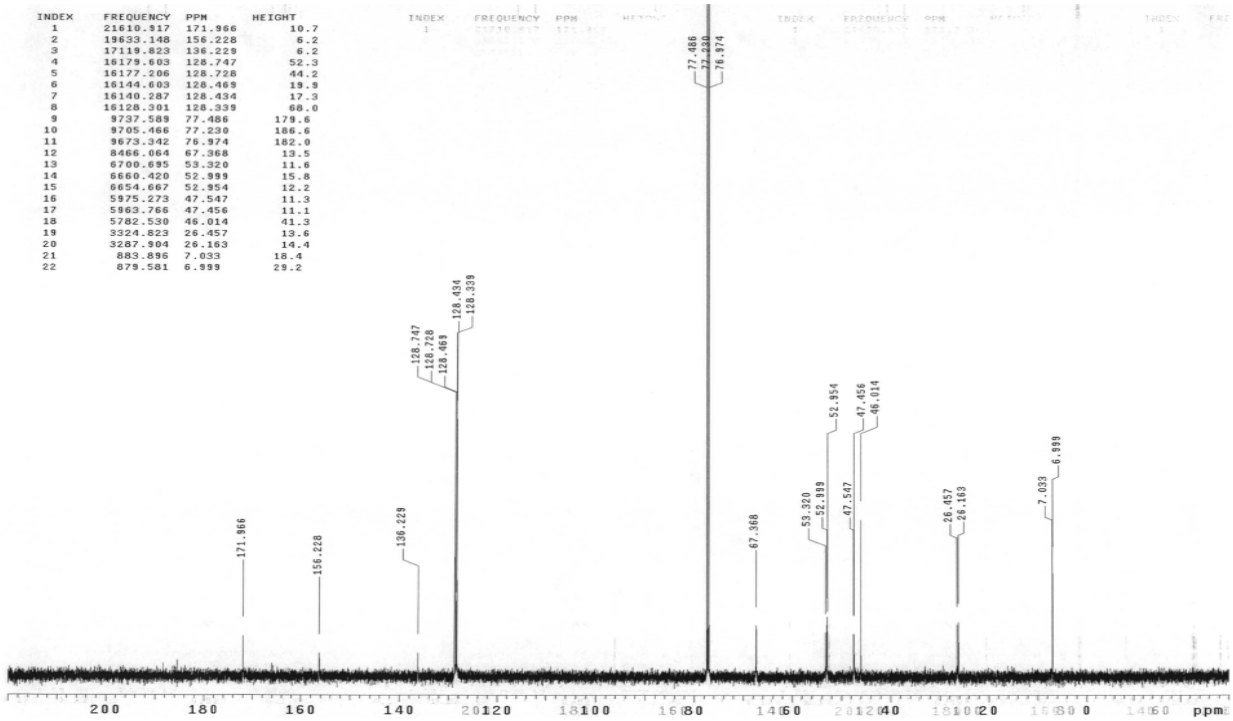


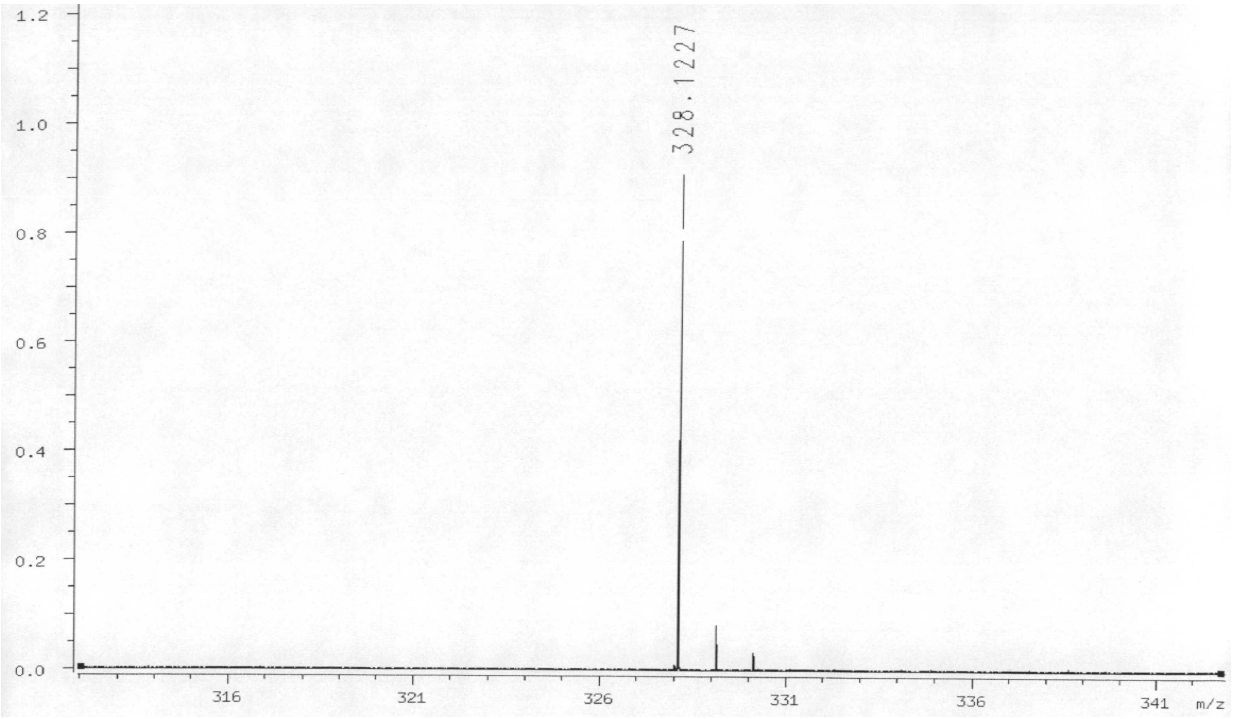


ESI-HRMS calcd for $C_{23}H_{37}NO_4S$ $[M+H]^+$ 424.2522, found 424.2532

Benzyl (*S*)-1-(methoxycarbonyl)-3-(ethylsulfinyl)propylcarbamate (8a)

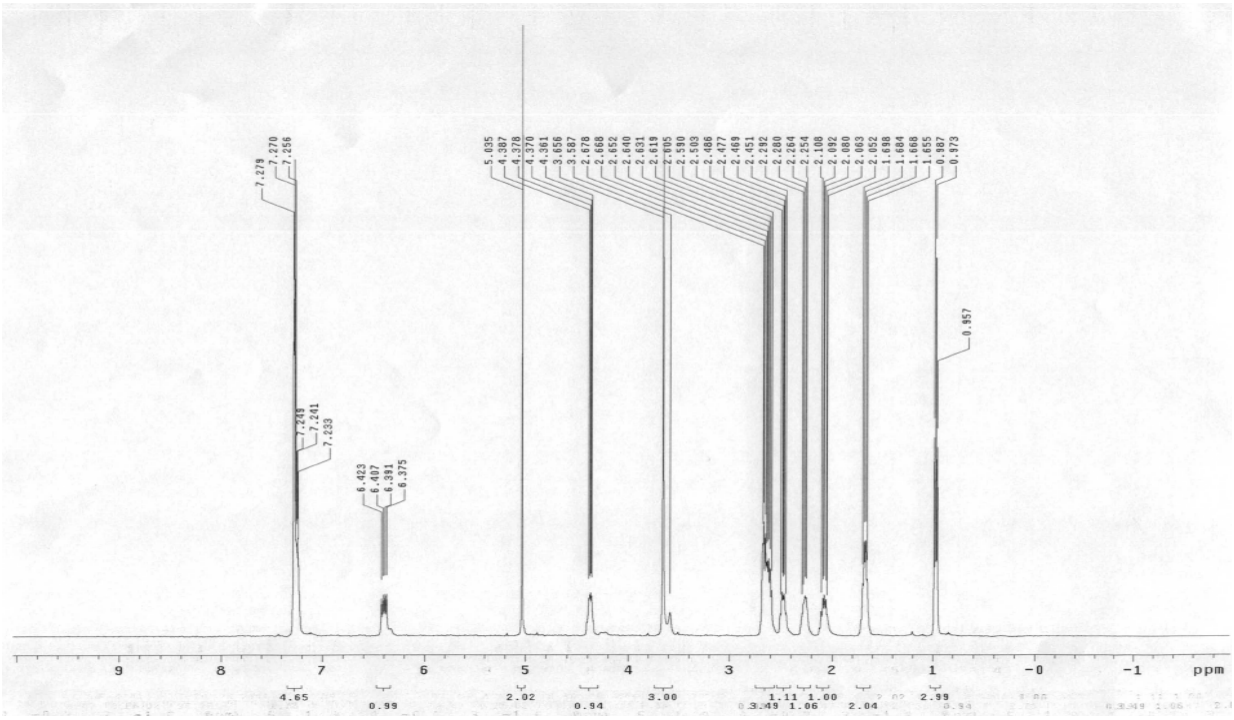






ESI-HRMS calcd for $C_{15}H_{21}NO_5S$ $[M+H]^+$ 328.1219, found 328.1227

Benzyl (*S*)-1-(methoxycarbonyl)-3-(propylsulfinyl)propylcarbamate (8b)



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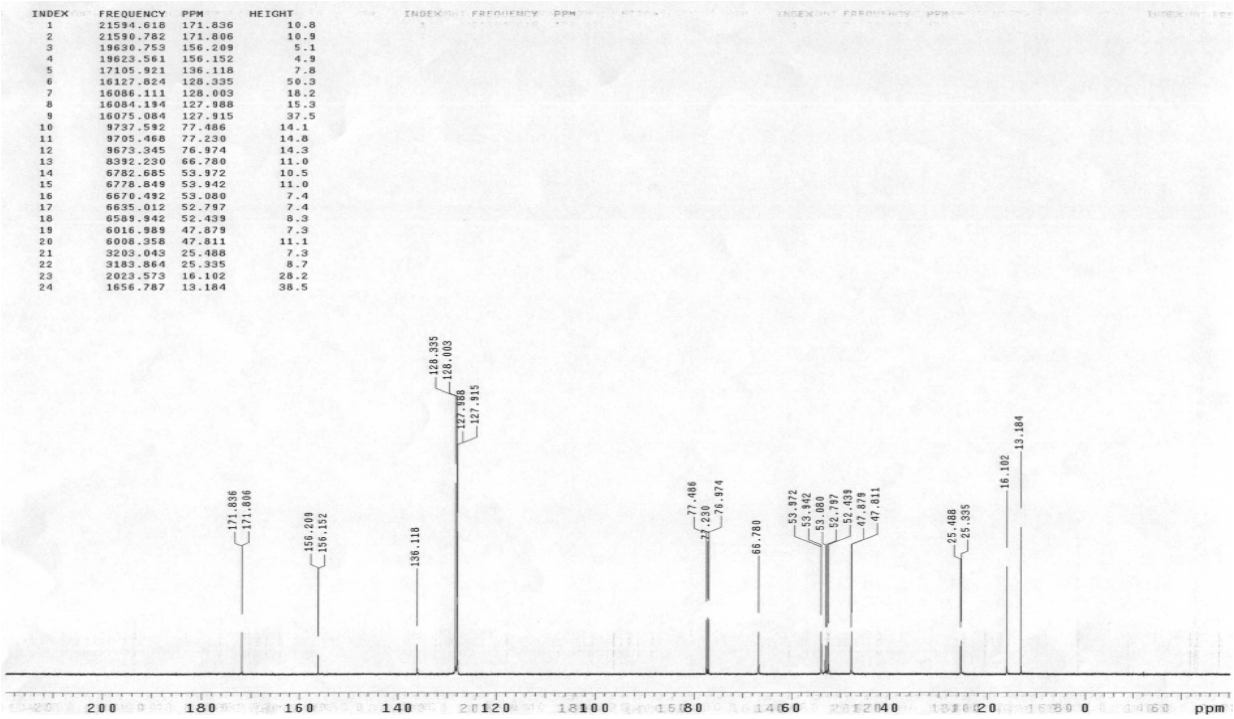
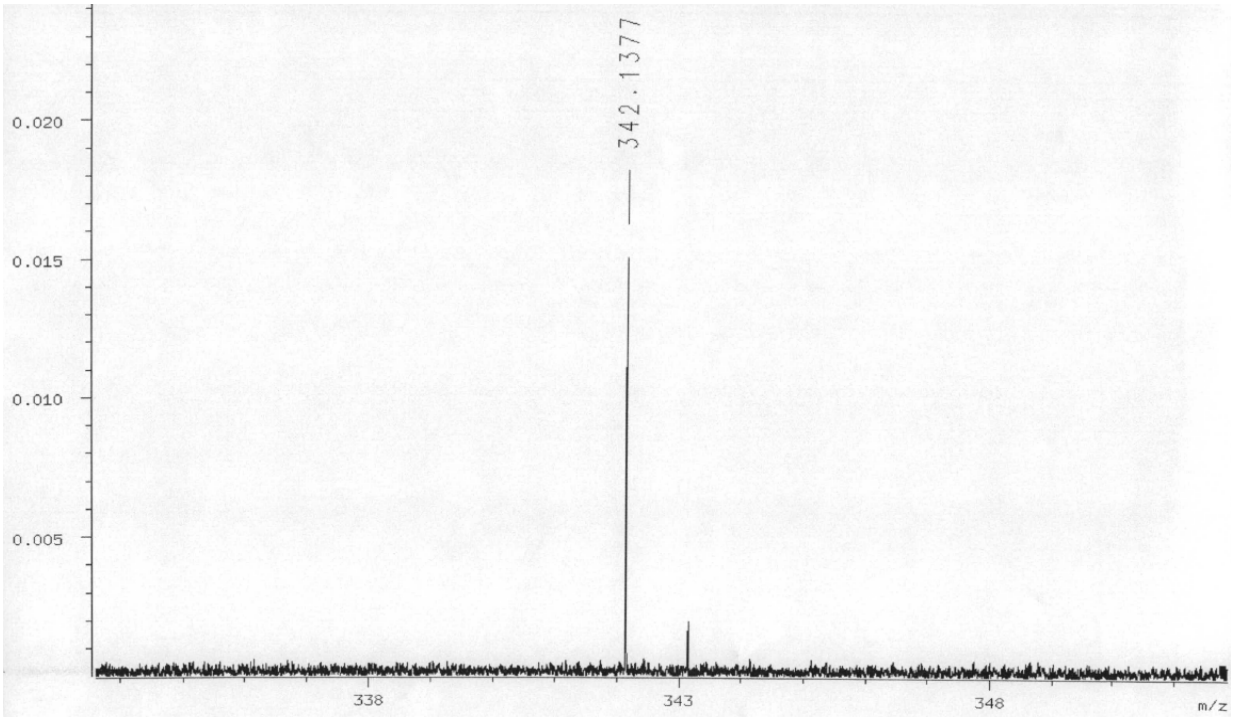
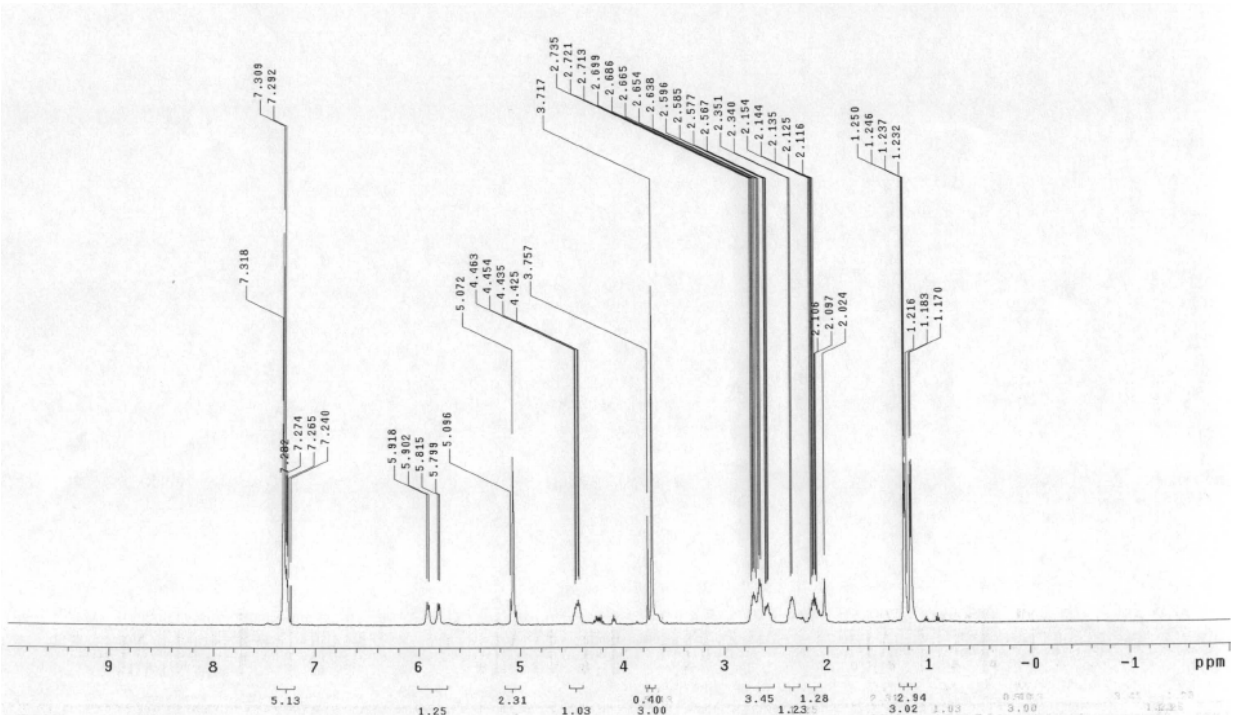
2	21590.782	171.806	10.9
3	19630.753	156.209	5.1
4	19623.561	156.152	4.9
5	17105.921	136.118	7.8
6	16127.824	128.335	50.3
7	16086.111	128.003	18.2
8	16084.194	127.988	15.3
9	16075.084	127.915	37.5
10	9737.592	77.486	14.1
11	9705.468	77.230	14.8
12	9673.345	76.974	14.3
13	8392.230	66.780	11.0
14	6782.685	53.972	10.5
15	6778.849	53.942	11.0
16	6670.492	53.080	7.4
17	6635.012	52.797	7.4
18	6589.942	52.439	8.3
19	6016.989	47.879	7.3
20	6008.358	47.811	11.1
21	3203.043	25.488	7.3
22	3183.864	25.335	8.7
23	2023.573	16.102	28.2
24	1656.787	13.184	38.5

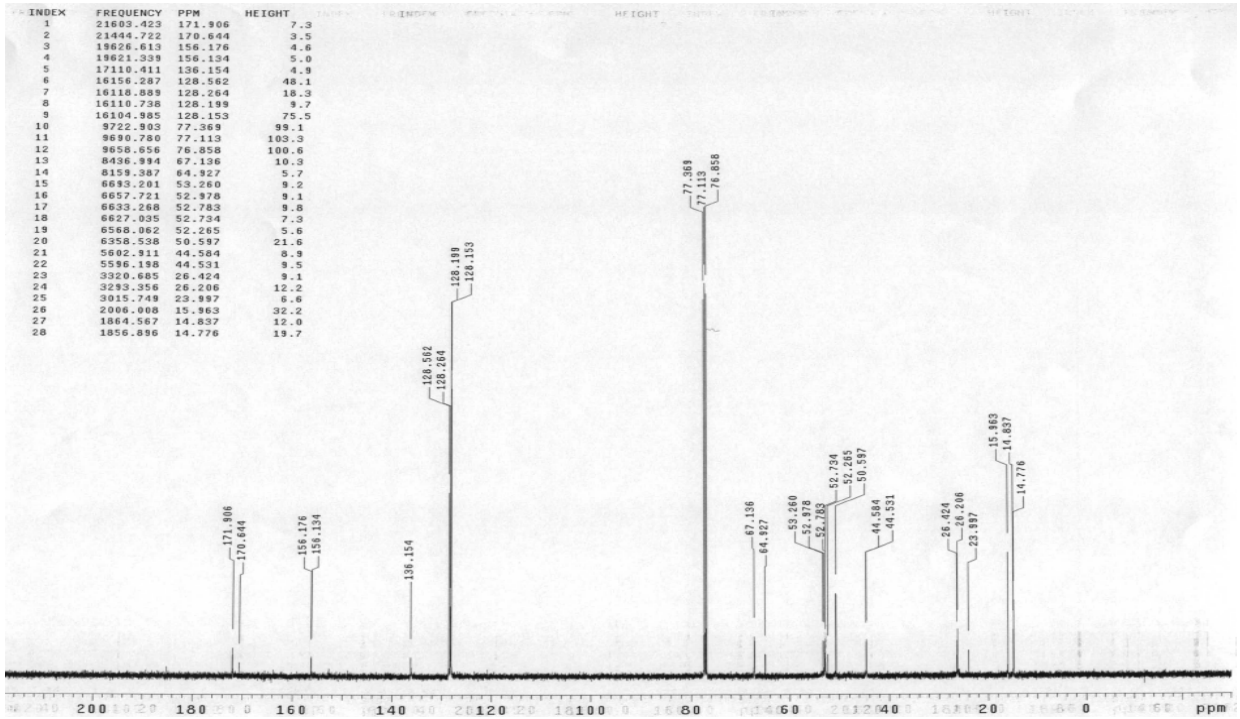




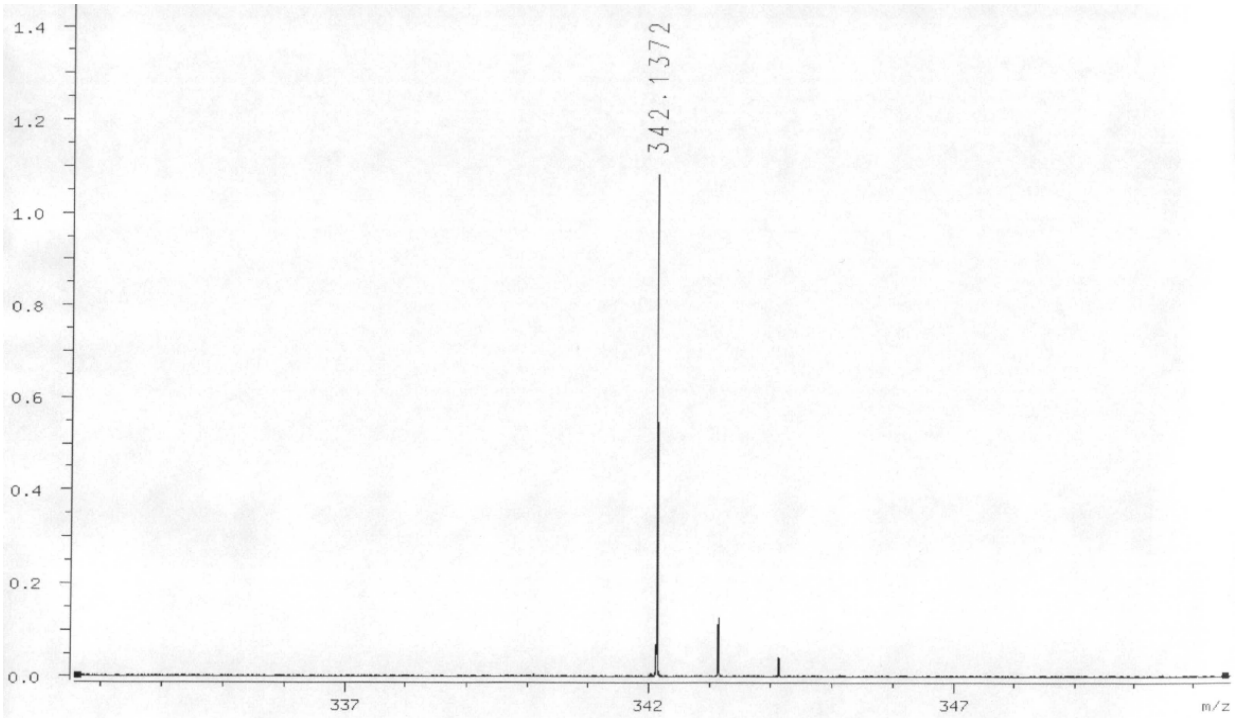
ESI-HRMS calcd for $C_{16}H_{23}NO_5S$ $[M+H]^+$ 342.1375, found 342.1377

Benzyl (*S*)-1-(methoxycarbonyl)-3-(isopropylsulfinyl)propylcarbamate (8c)



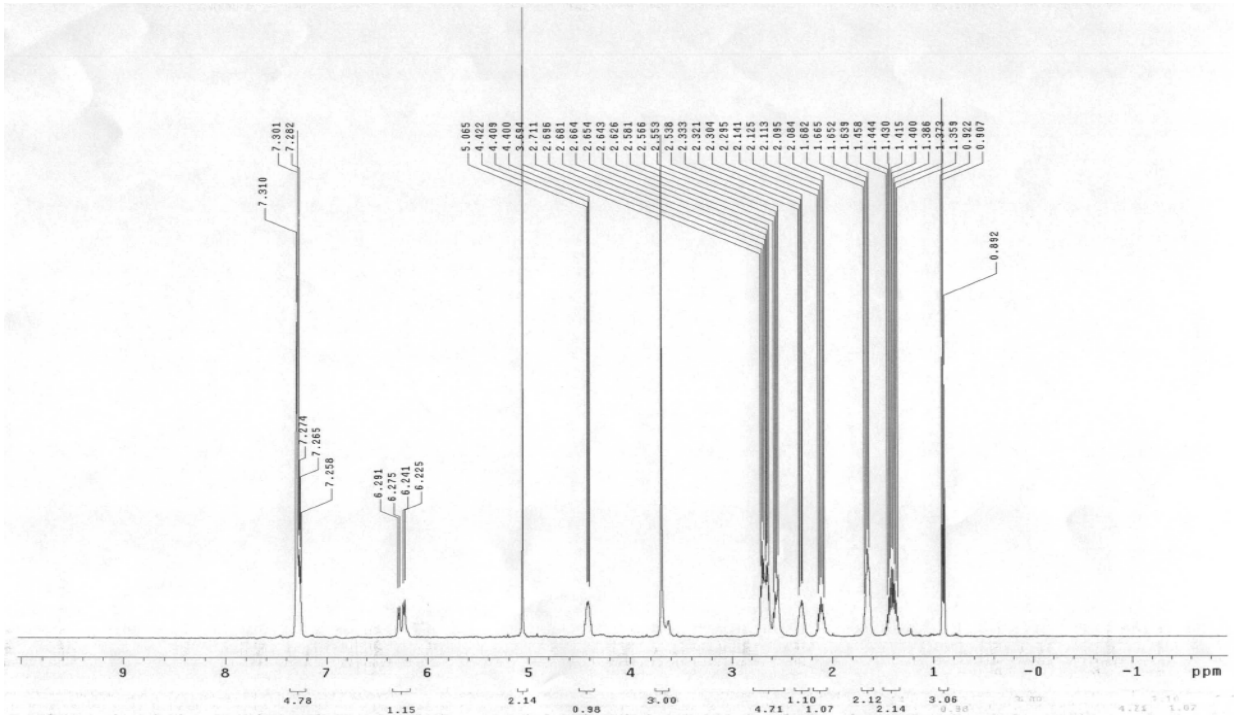


INDEX	FREQUENCY	PPM	HEIGHT
1	21603.423	171.906	7.3
2	21444.722	170.644	3.5
3	19626.613	156.176	4.6
4	19621.339	156.134	5.0
5	17110.411	136.154	4.9
6	16156.287	128.562	48.1
7	16118.889	128.264	18.3
8	16110.738	128.199	9.7
9	16104.985	128.153	75.5
10	9722.903	77.369	99.1
11	9690.780	77.113	103.3
12	9658.656	76.858	100.6
13	8436.994	67.136	10.3
14	8159.387	64.927	5.7
15	6693.201	53.260	9.2
16	6657.721	52.978	9.1
17	6633.268	52.783	9.8
18	6627.035	52.734	7.3
19	6568.062	52.265	5.6
20	6358.538	50.597	21.6
21	5602.911	44.584	8.9
22	5596.198	44.531	9.5
23	3320.685	26.424	9.1
24	3293.356	26.206	12.2
25	3015.749	23.997	6.6
26	2006.908	15.963	32.2
27	1864.567	14.837	12.0
28	1856.898	14.776	19.7

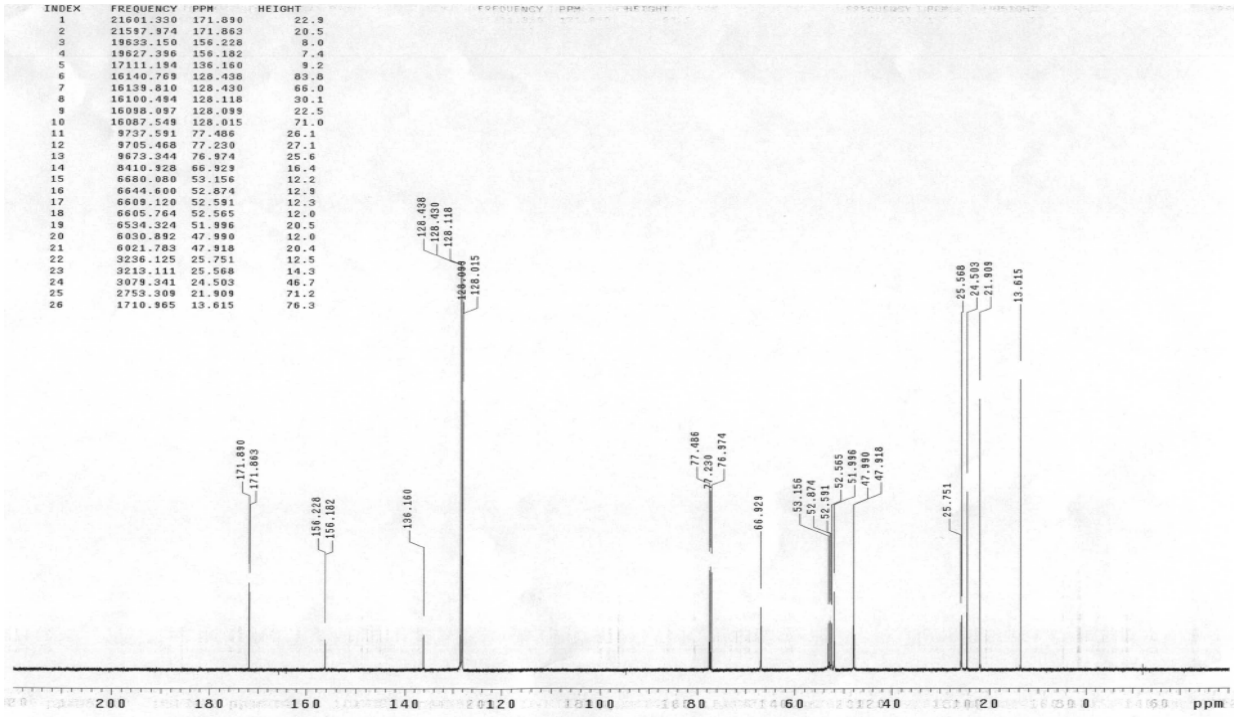


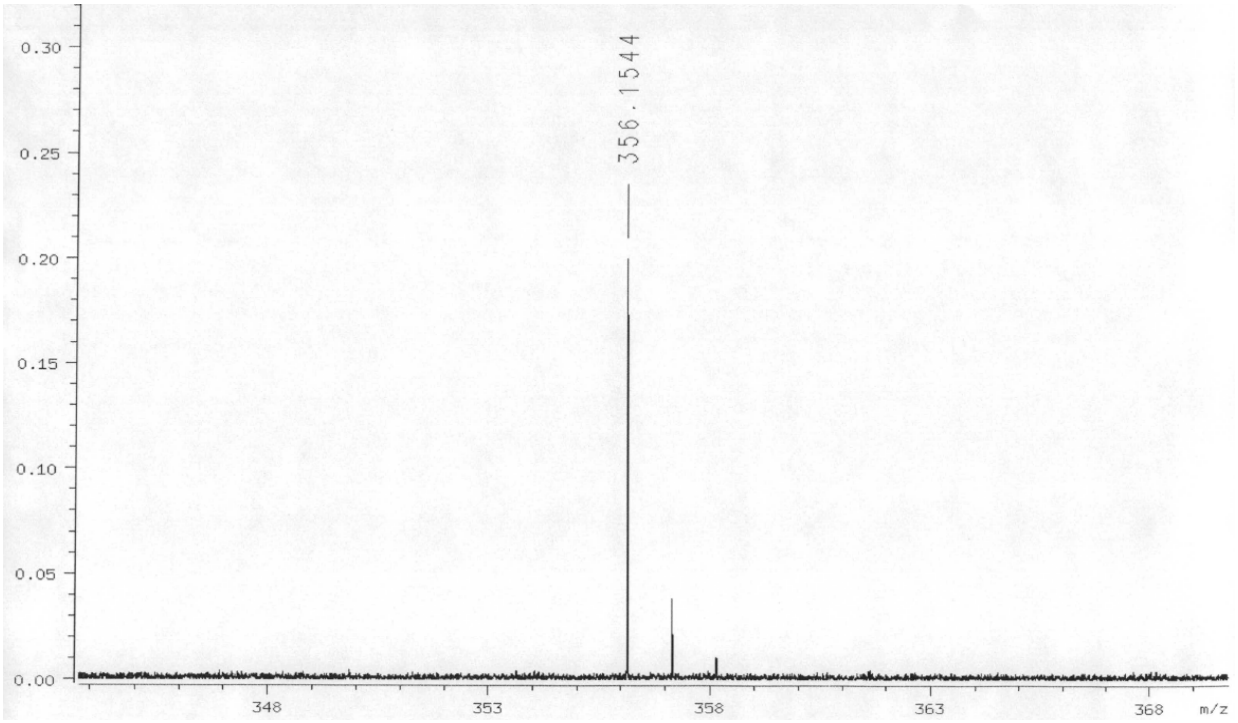
ESI-HRMS calcd for $C_{16}H_{23}NO_5S$ $[M+H]^+$ 342.1375, found 342.1372

Benzyl (*S*)-1-(methoxycarbonyl)-3-(butylsulfinyl)propylcarbamate (8d)



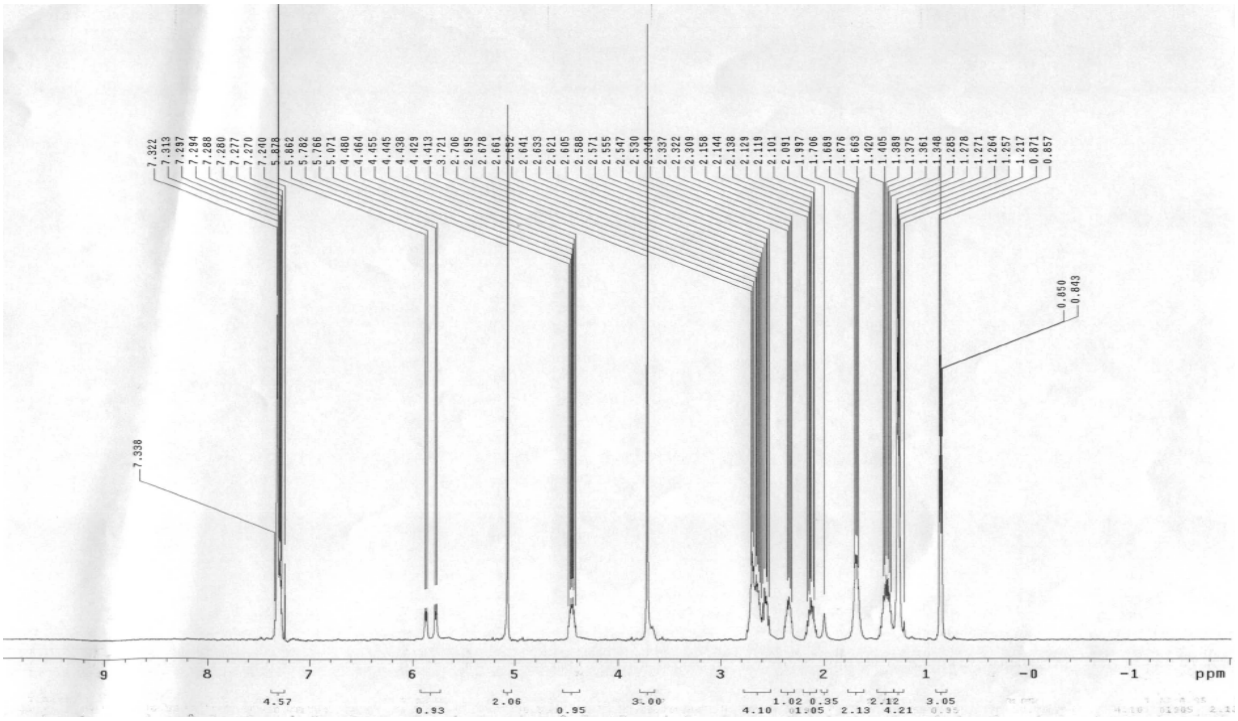
INDEX	FREQUENCY	PPM	HEIGHT
1	21601.330	171.890	22.9
2	21597.974	171.863	20.5
3	19633.150	156.228	8.0
4	19627.396	156.182	7.4
5	17111.194	136.160	9.2
6	16140.769	128.438	83.8
7	16139.810	128.430	66.0
8	16100.494	128.118	30.1
9	16098.097	128.099	22.5
10	16087.549	128.015	71.0
11	9737.591	77.486	26.1
12	9705.468	77.230	27.1
13	9673.344	76.974	25.6
14	8410.928	66.929	16.4
15	6680.080	53.156	12.2
16	6644.600	52.874	12.9
17	6609.120	52.591	12.3
18	6605.764	52.565	12.0
19	6534.324	51.996	20.5
20	6030.892	47.990	12.0
21	6021.783	47.918	20.4
22	3236.125	25.751	12.5
23	3213.111	25.568	14.3
24	3079.341	24.503	46.7
25	2753.309	21.909	71.2
26	1710.965	13.615	76.3



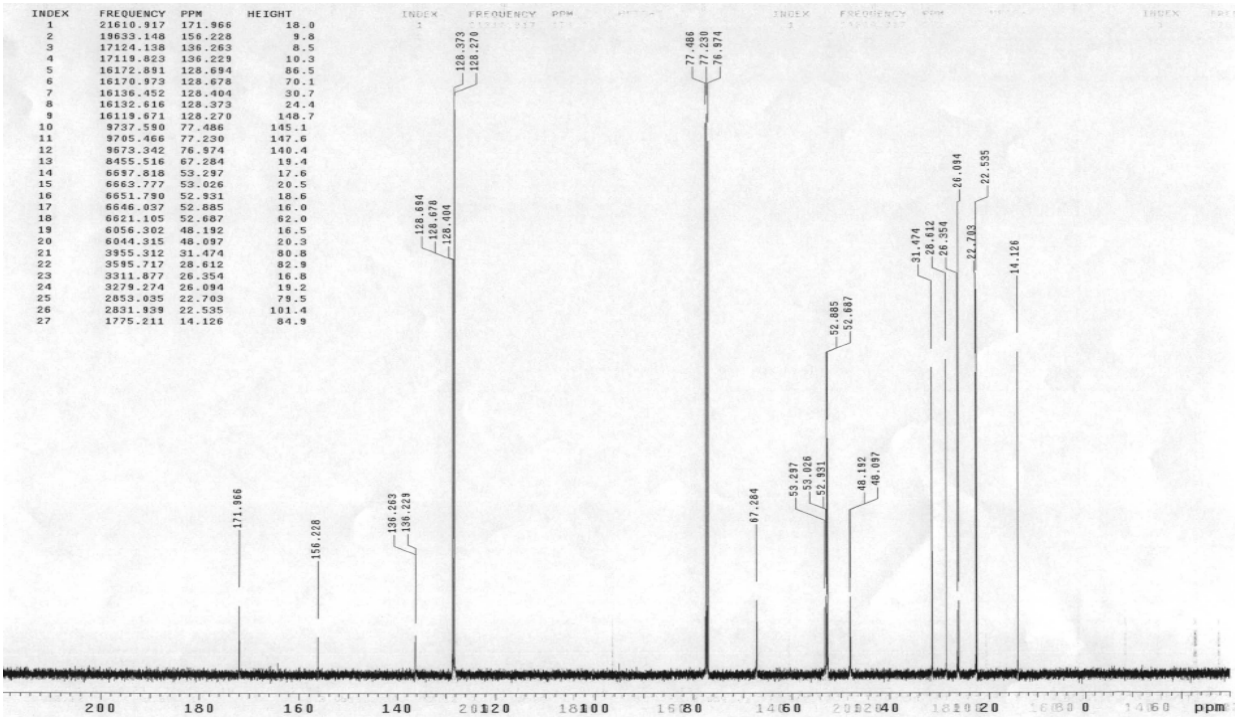


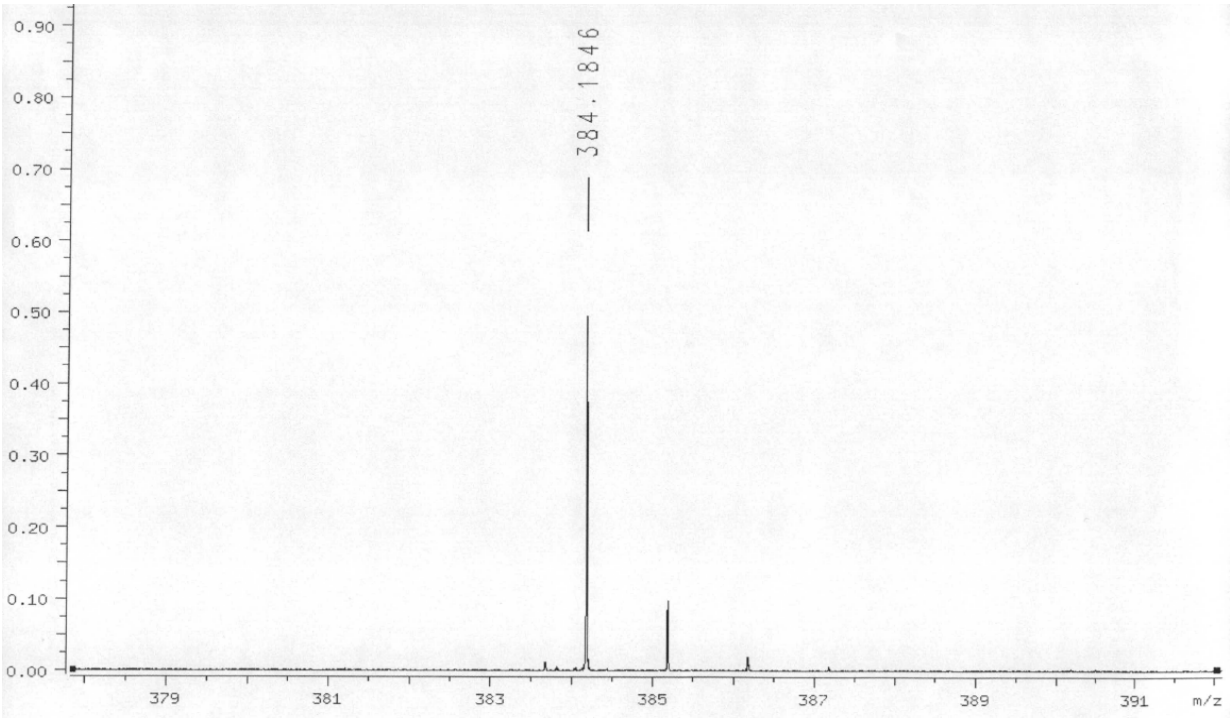
ESI-HRMS calcd for $C_{17}H_{25}NO_5S$ $[M+H]^+$ 356.1532, found 356.1544

Benzyl (*S*)-1-(methoxycarbonyl)-3-(hexylsulfinyl)propylcarbamate (8e)



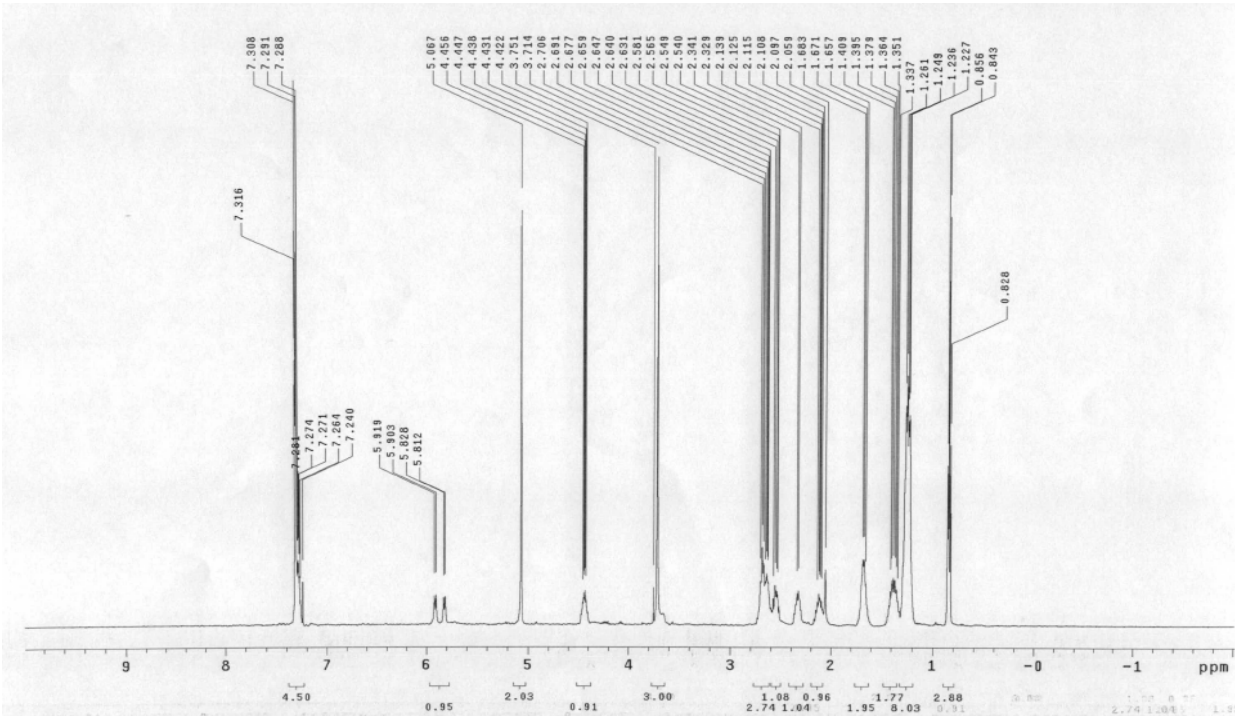
INDEX	FREQUENCY	PPM	HEIGHT
1	21610.917	171.966	18.0
2	19633.148	156.228	9.8
3	17124.138	136.263	8.5
4	17119.823	136.229	10.3
5	16172.891	128.694	86.5
6	16170.973	128.678	70.1
7	16136.452	128.404	30.7
8	16132.616	128.373	24.4
9	16119.671	128.270	148.7
10	9737.590	77.486	145.1
11	9705.466	77.230	147.6
12	9673.342	76.974	140.4
13	8455.516	67.284	19.4
14	6597.818	53.297	17.6
15	6563.777	53.026	20.5
16	6651.790	52.931	18.6
17	6646.037	52.885	16.0
18	6621.105	52.687	62.0
19	6056.302	48.192	16.5
20	6044.315	48.097	20.3
21	3955.312	31.474	80.8
22	3595.717	28.612	82.9
23	3311.877	26.354	16.8
24	3279.274	26.094	19.2
25	2853.035	22.703	79.5
26	2831.939	22.535	101.4
27	1775.211	14.126	84.9



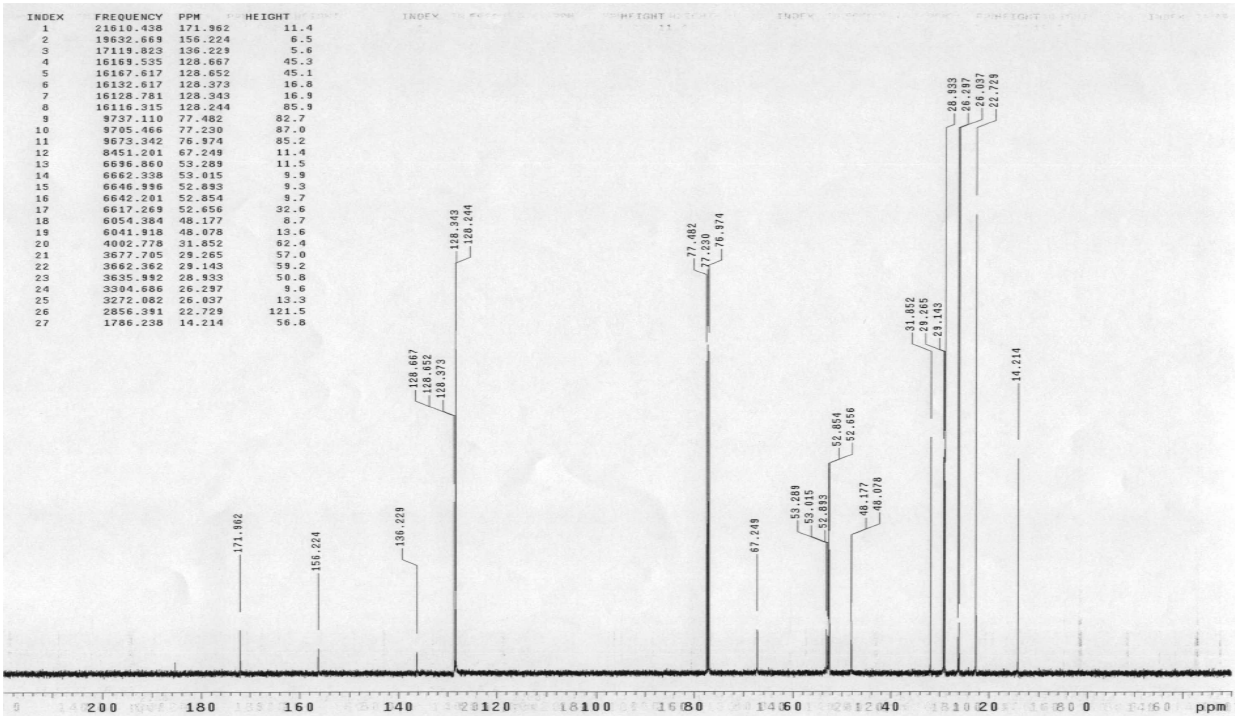


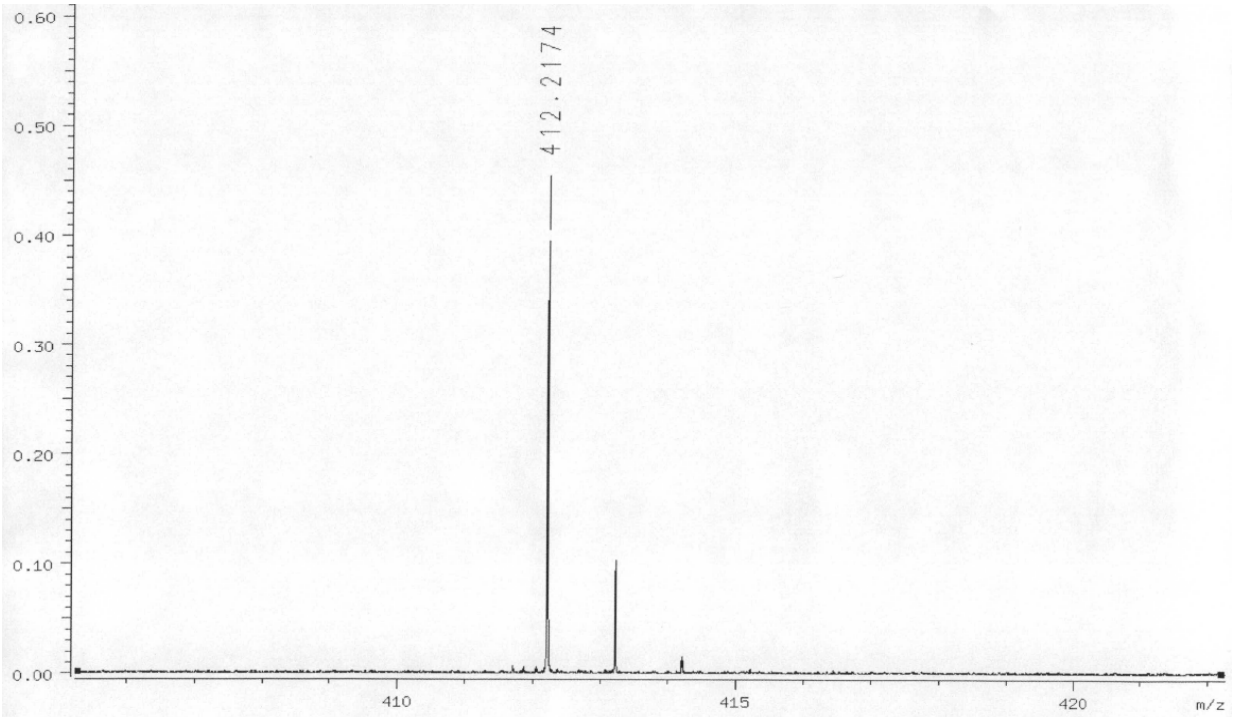
ESI-HRMS calcd for $C_{19}H_{29}NO_5S$ $[M+H]^+$ 384.1845, found 384.1846

Benzyl (*S*)-1-(methoxycarbonyl)-3-(octylsulfinyl)propylcarbamate (8f)



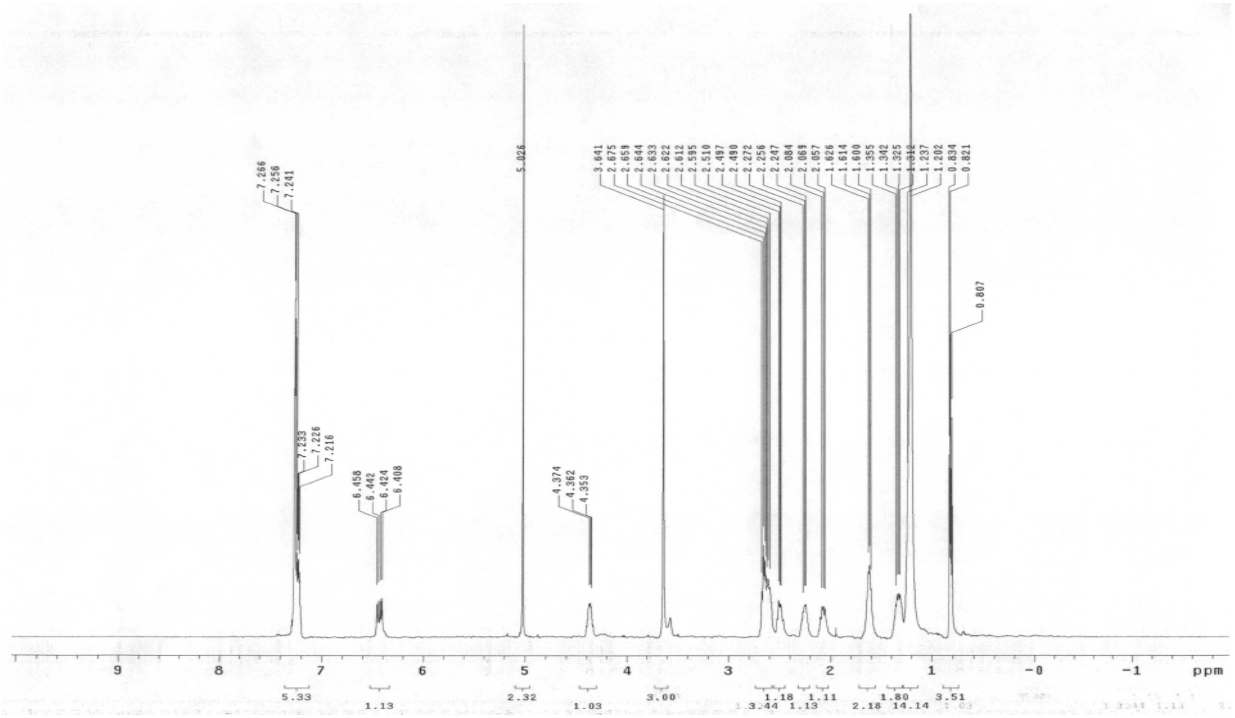
INDEX	FREQUENCY	PPM	HEIGHT
1	21610.438	171.962	11.4
2	19632.669	156.224	6.5
3	17119.823	136.229	5.6
4	16169.535	128.667	45.3
5	16167.617	128.652	45.1
6	16132.617	128.373	16.8
7	16128.781	128.343	16.9
8	16116.315	128.244	85.9
9	9737.110	77.482	82.7
10	9705.466	77.230	87.0
11	9673.342	76.974	85.2
12	8451.201	67.249	11.4
13	6636.860	53.289	11.5
14	6662.338	53.015	9.9
15	6646.936	52.893	9.3
16	6642.201	52.854	9.7
17	6617.269	52.656	32.6
18	6054.384	48.177	8.7
19	6041.918	48.078	13.6
20	4002.778	31.852	62.4
21	3677.705	29.265	57.0
22	3662.362	29.143	59.2
23	3635.982	28.933	50.8
24	3304.686	26.297	9.6
25	3272.082	26.037	13.3
26	2856.391	22.729	121.5
27	1786.238	14.214	56.8

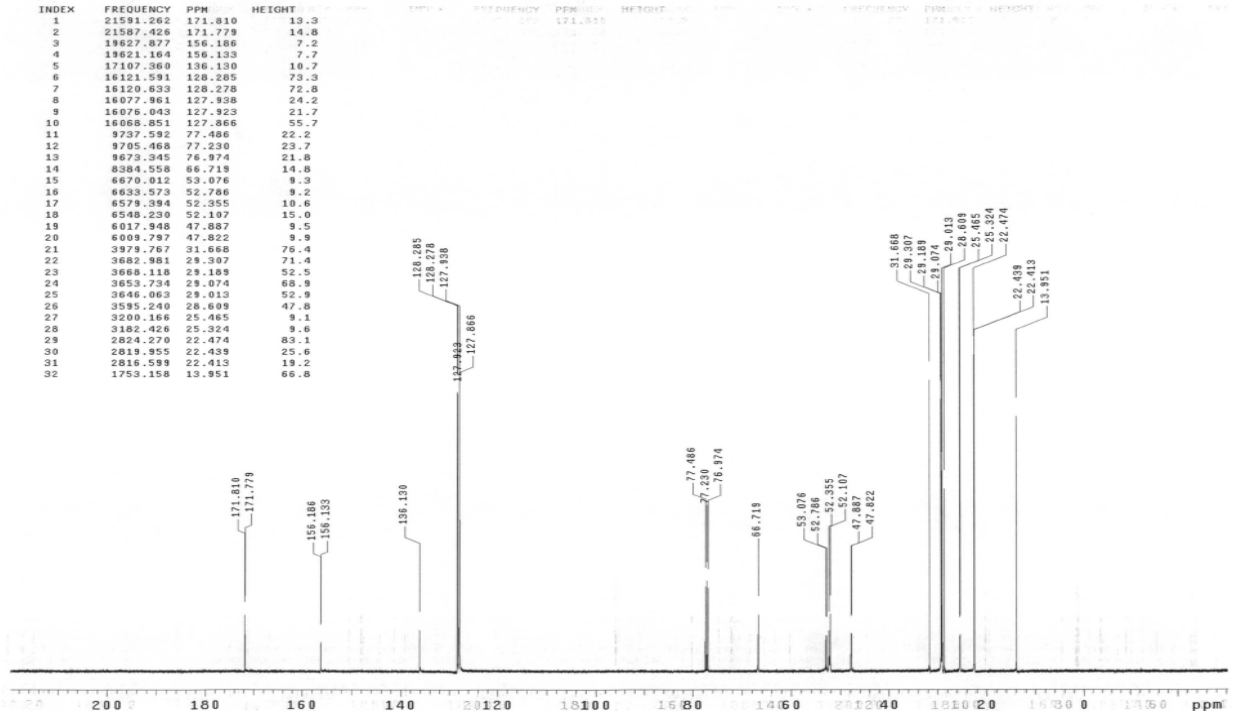


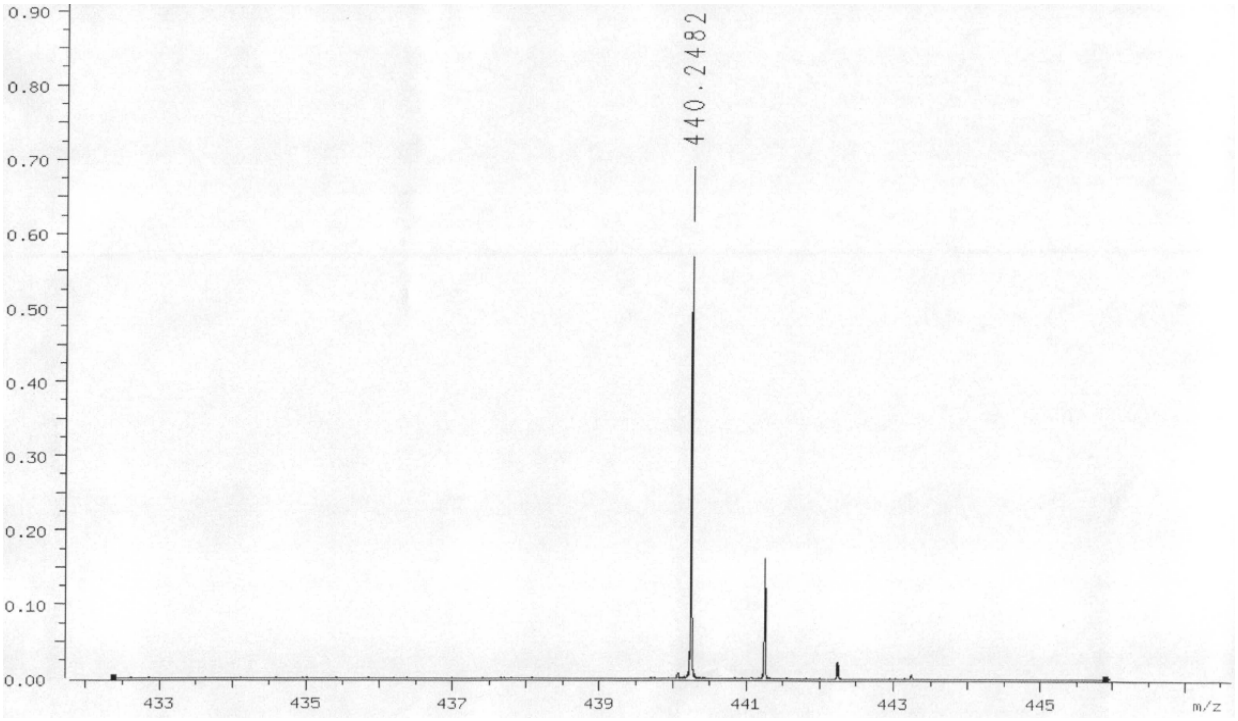


ESI-HRMS calcd for $C_{21}H_{33}NO_5S$ $[M+H]^+$ 412.2158, found 412.2174

Benzyl (*S*)-1-(methoxycarbonyl)-3-(decylsulfinyl)propylcarbamate (8g)

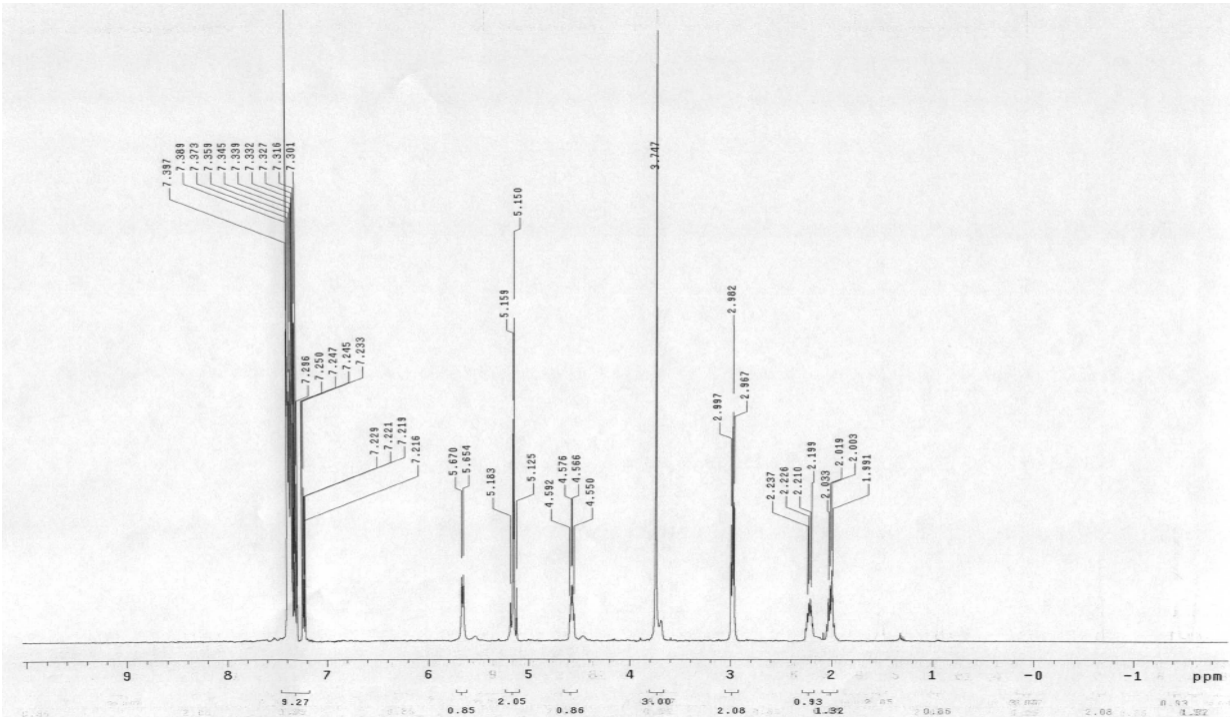


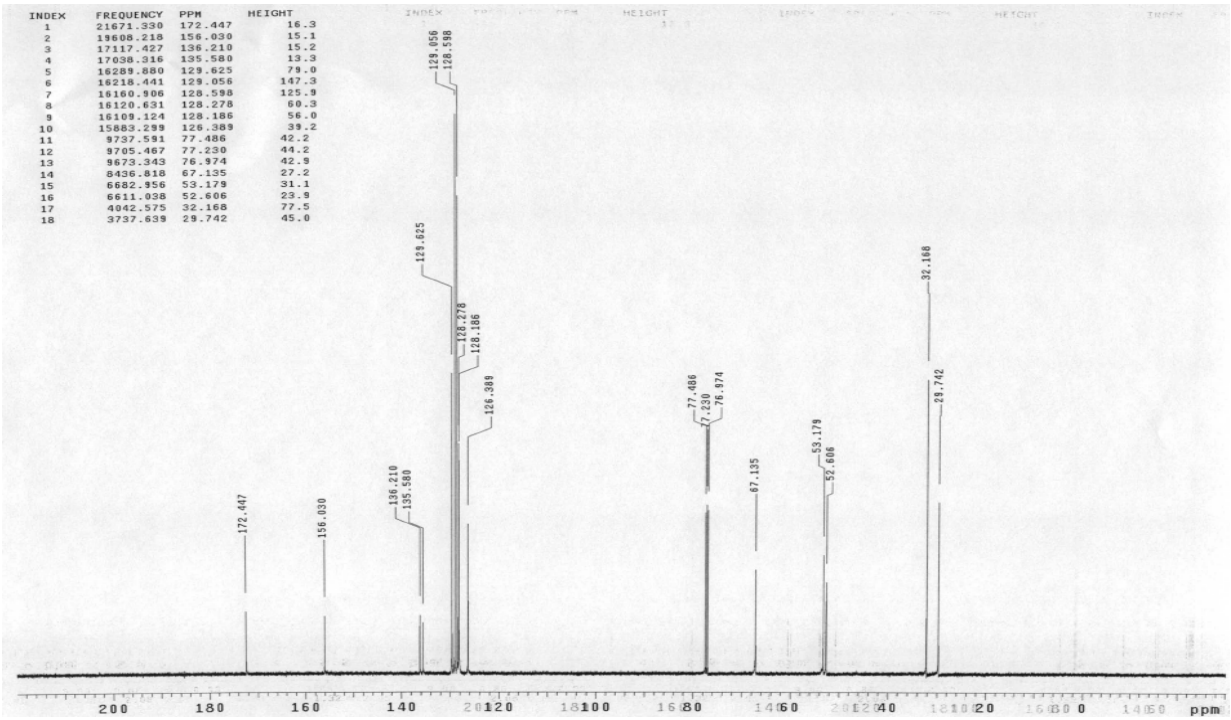




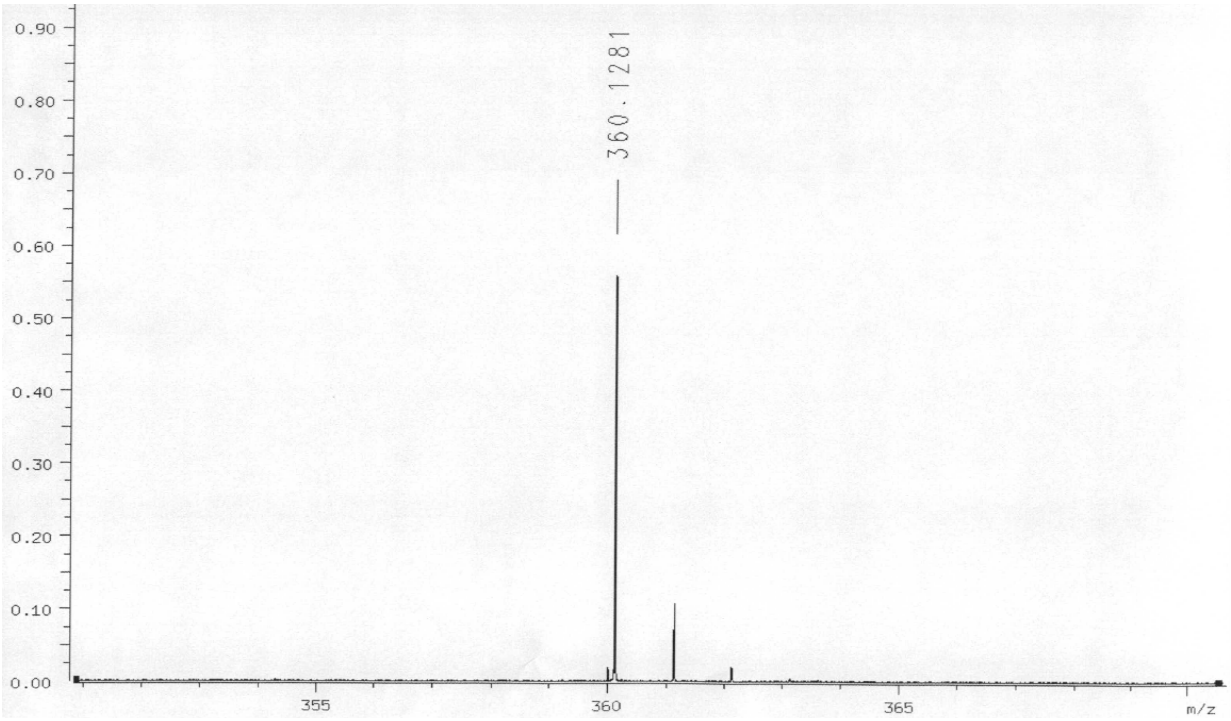
ESI-HRMS calcd for $C_{23}H_{37}NO_5S$ $[M+H]^+$ 440.2471, found 440.2482

Benzyl (*S*)-1-(methoxycarbonyl)-3-(phenylthio)propylcarbamate (9b)



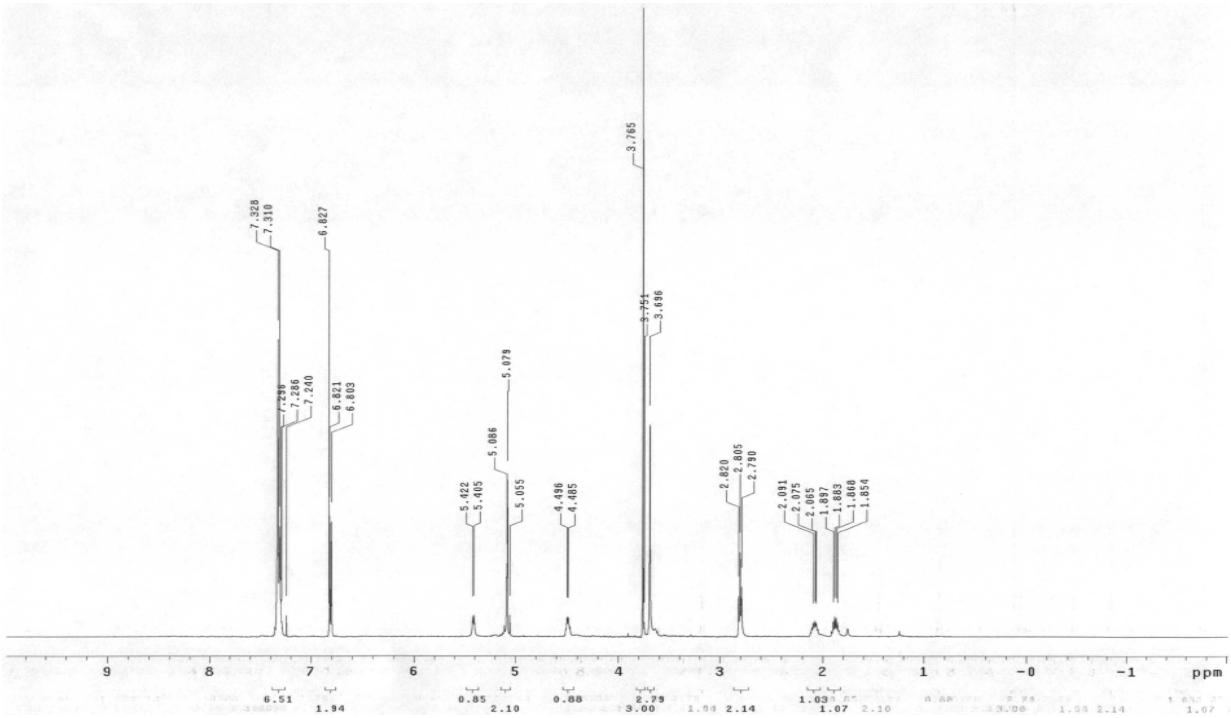


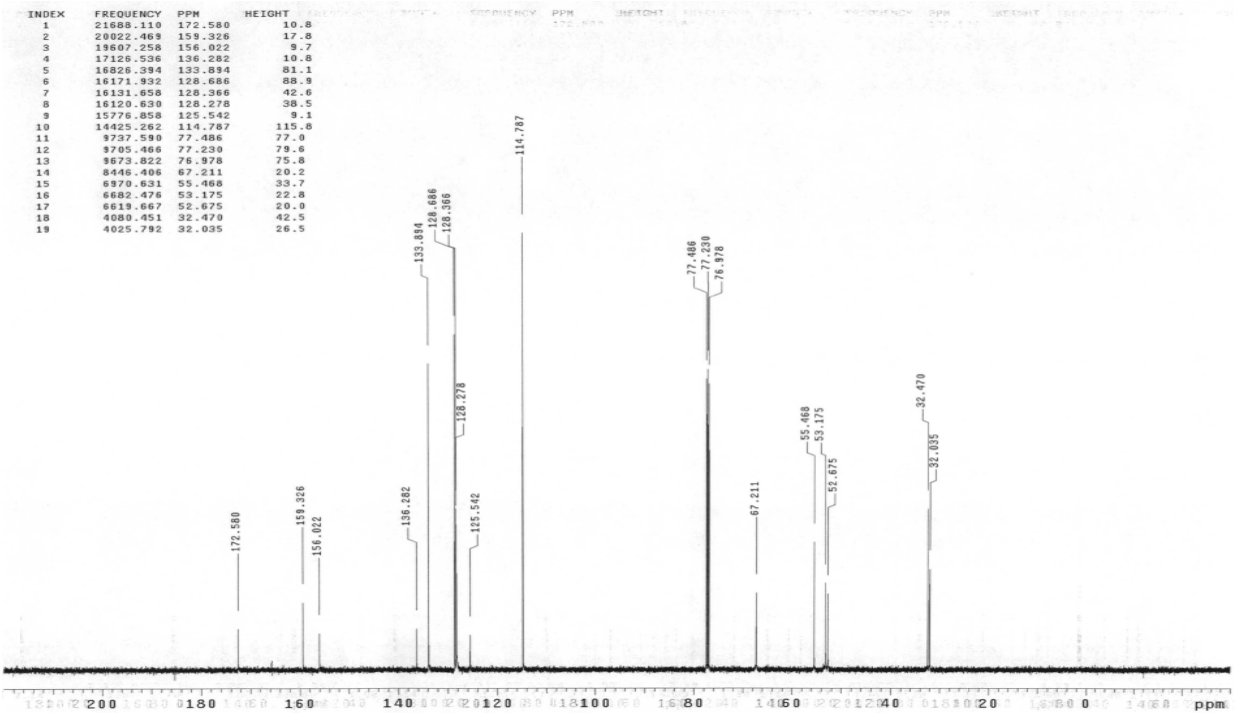
INDEX	FREQUENCY	PPM	HEIGHT
1	21671.330	172.447	16.3
2	19608.218	156.030	15.1
3	17117.427	136.210	15.2
4	17038.316	135.580	13.3
5	16289.880	129.625	79.0
6	16218.441	129.056	147.3
7	16160.906	128.598	125.9
8	16120.631	128.278	60.3
9	16109.124	128.186	56.0
10	15883.299	126.389	39.2
11	9737.591	77.486	42.2
12	9705.467	77.230	44.2
13	9673.343	76.974	42.9
14	8436.818	67.135	27.2
15	6682.956	53.179	31.1
16	6611.038	52.606	23.9
17	4042.575	32.168	77.5
18	3737.639	29.742	45.0

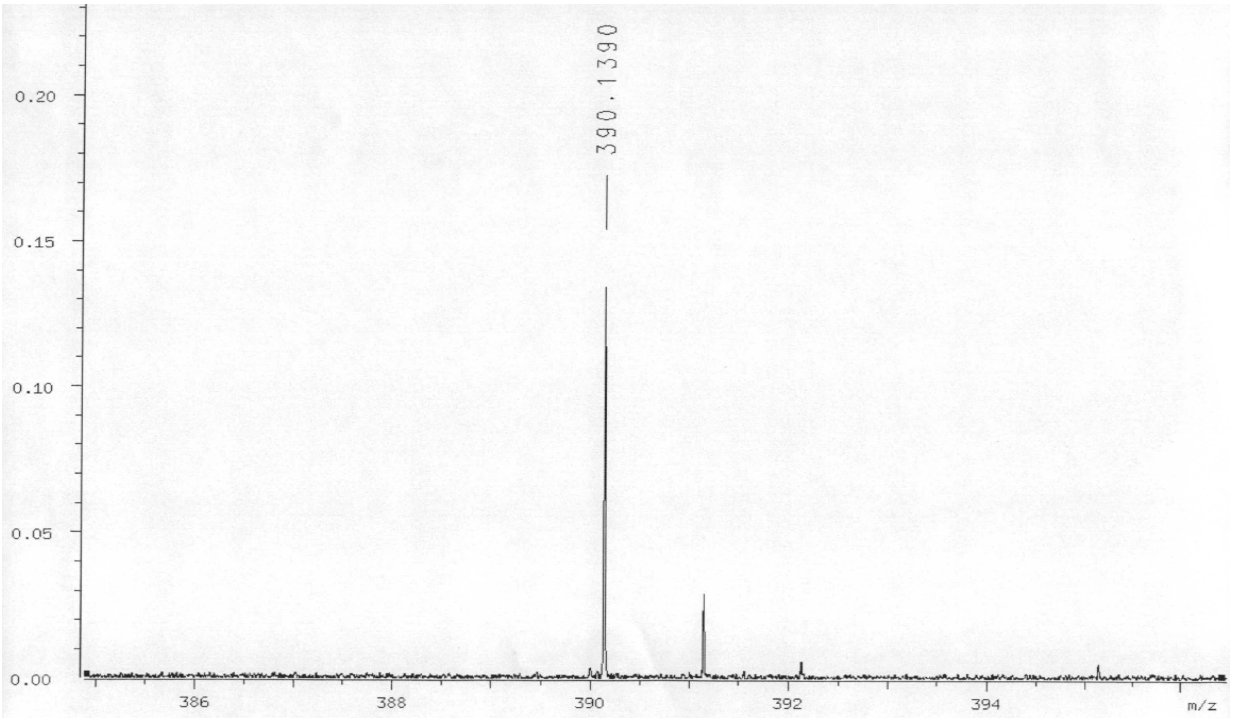


ESI-HRMS calcd for $C_{19}H_{21}NO_4S$ $[M+H]^+$ 360.1270, found 360.1281

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-methoxyphenylthio)propylcarbamate (9c)

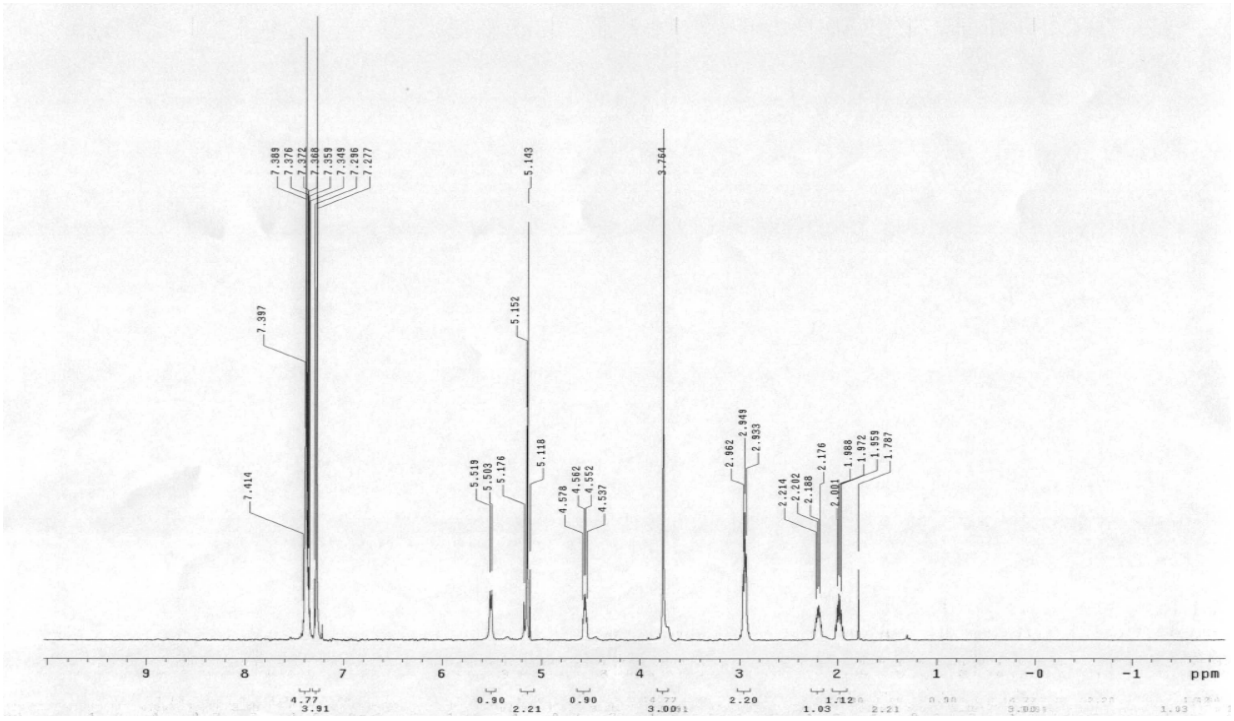


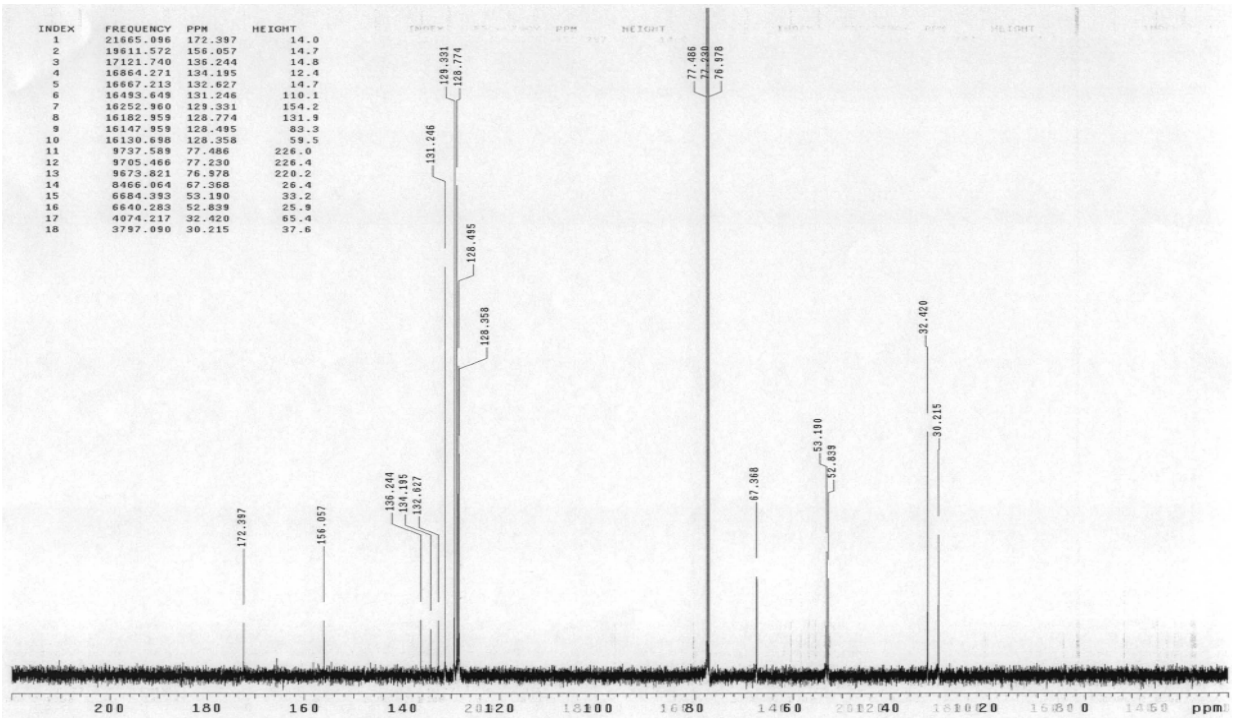




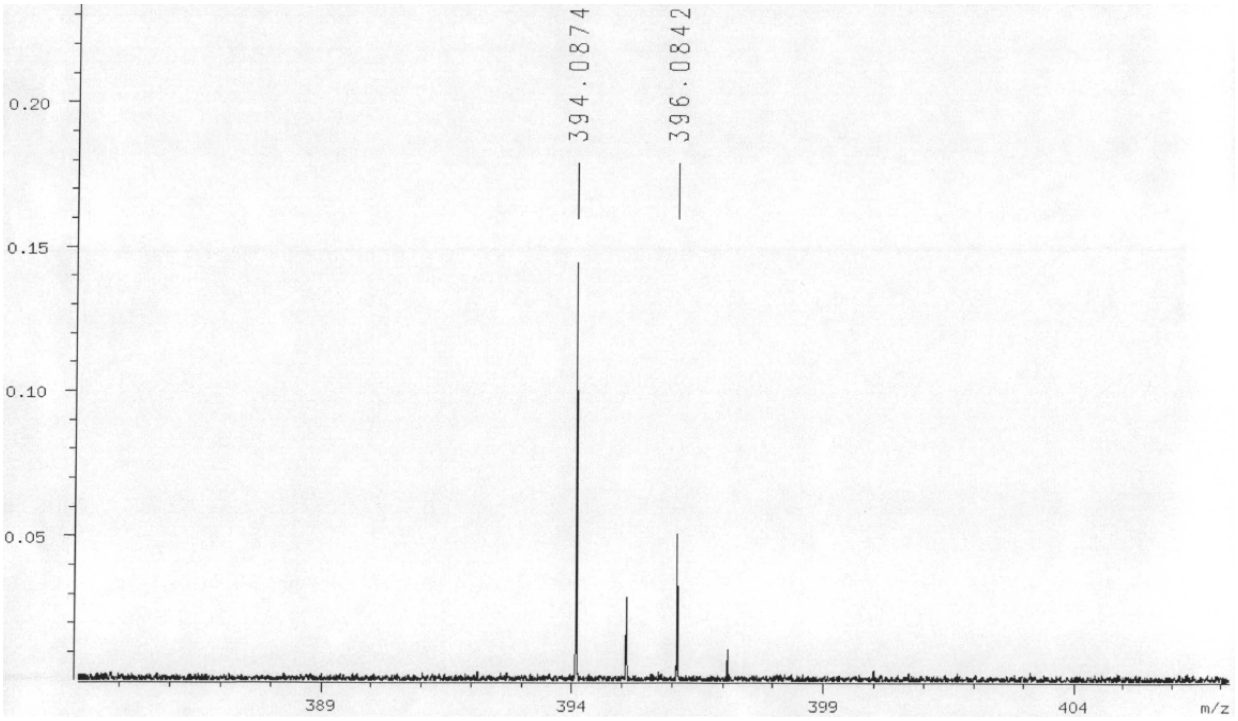
ESI-HRMS calcd for $C_{20}H_{23}NO_5S$ $[M+H]^+$ 390.1375, found 390.1390

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-chlorophenylthio)propylcarbamate (9d)



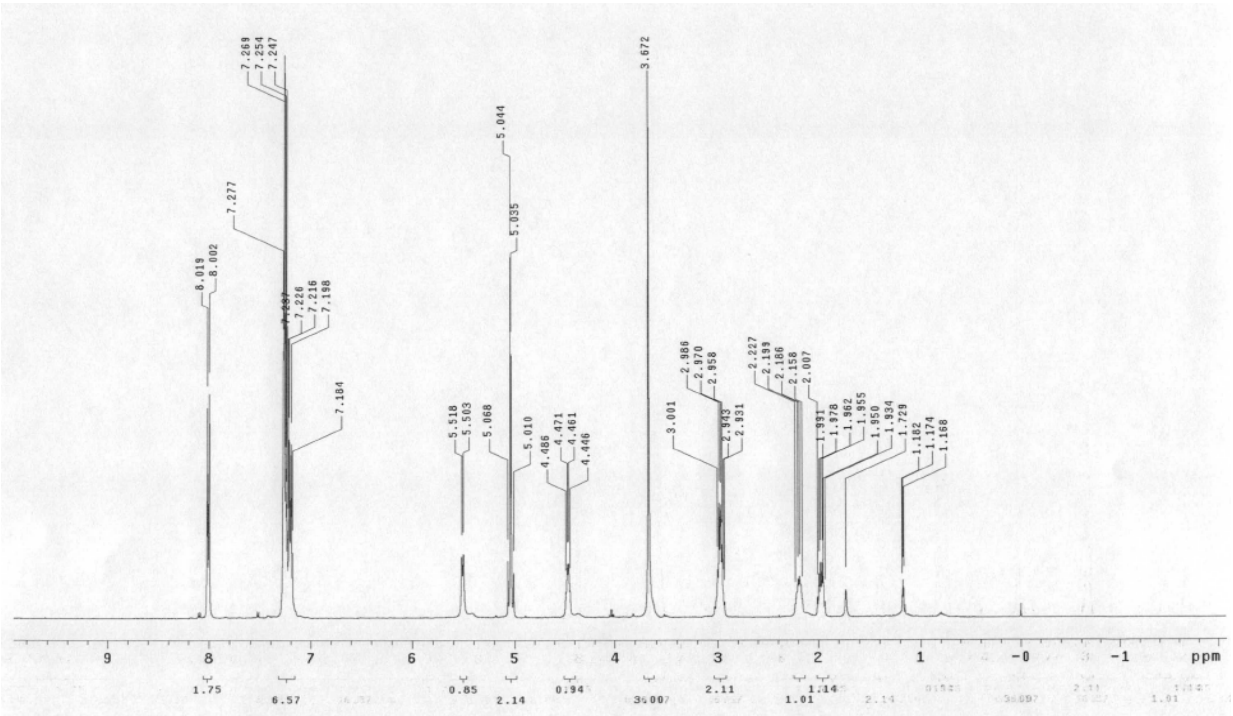


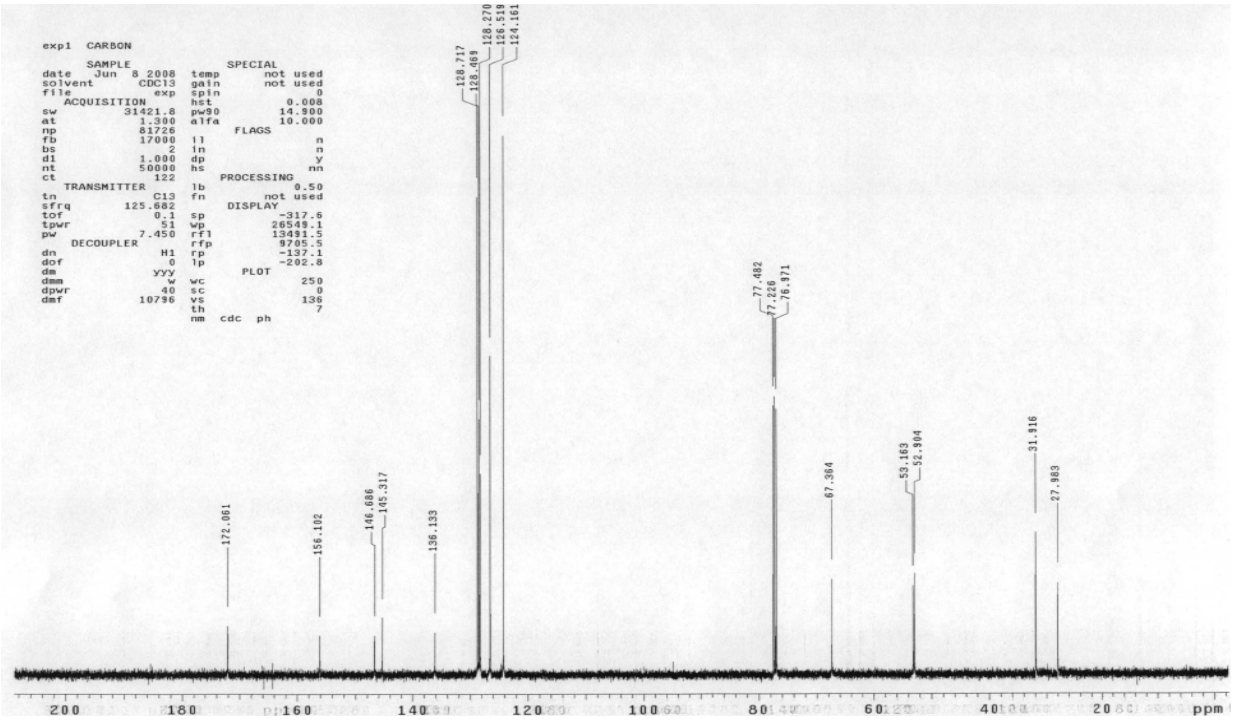
INDEX	FREQUENCY	PPM	HEIGHT
1	21665.096	172.397	14.0
2	19611.572	156.057	14.7
3	17121.740	136.244	14.8
4	16864.271	134.195	12.4
5	16667.213	132.627	14.7
6	16493.649	131.246	110.1
7	16252.960	129.331	154.2
8	16182.959	128.774	131.9
9	16147.959	128.495	83.3
10	16130.698	128.358	59.5
11	9737.589	77.486	226.0
12	9705.466	77.230	226.4
13	9673.821	76.978	220.2
14	8466.064	67.368	26.4
15	6684.393	53.190	33.2
16	6640.283	52.839	25.9
17	4074.217	32.420	65.4
18	3797.090	30.215	37.6

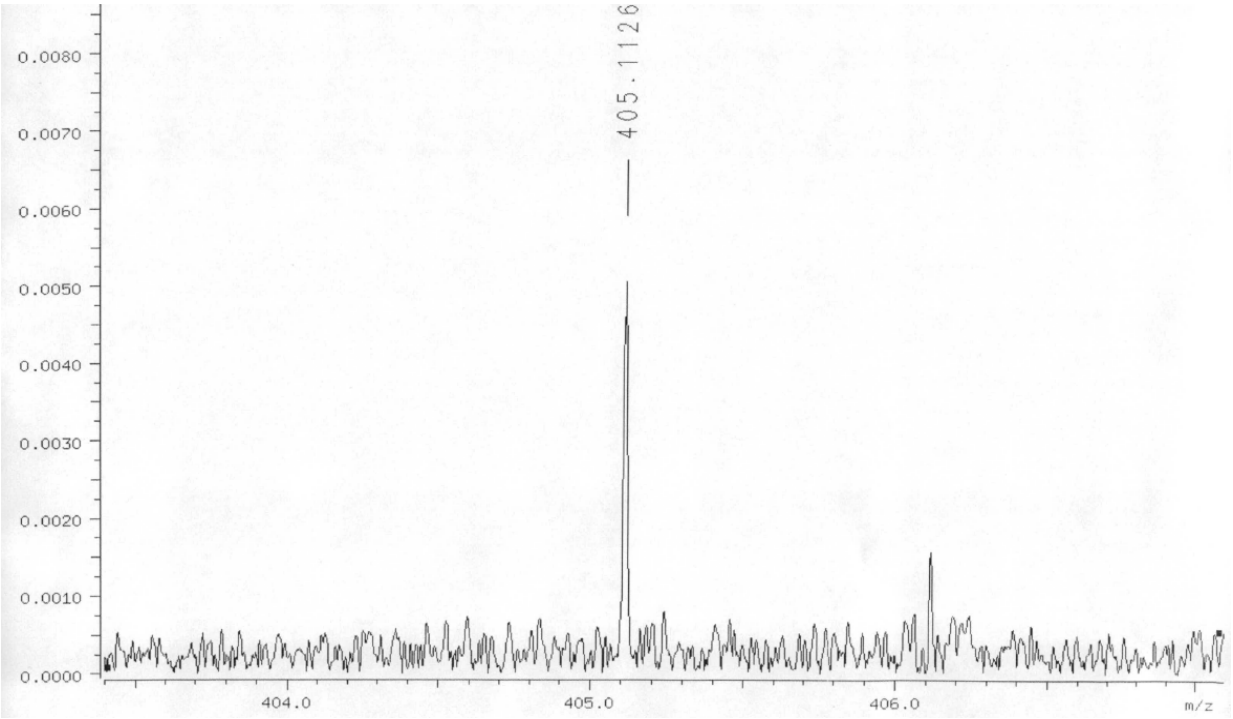


ESI-HRMS calcd for $C_{19}H_{20}ClNO_4S$ $[M+H]^+$ 394.0879, found 394.0874

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-nitrophenylthio)propylcarbamate (9e)

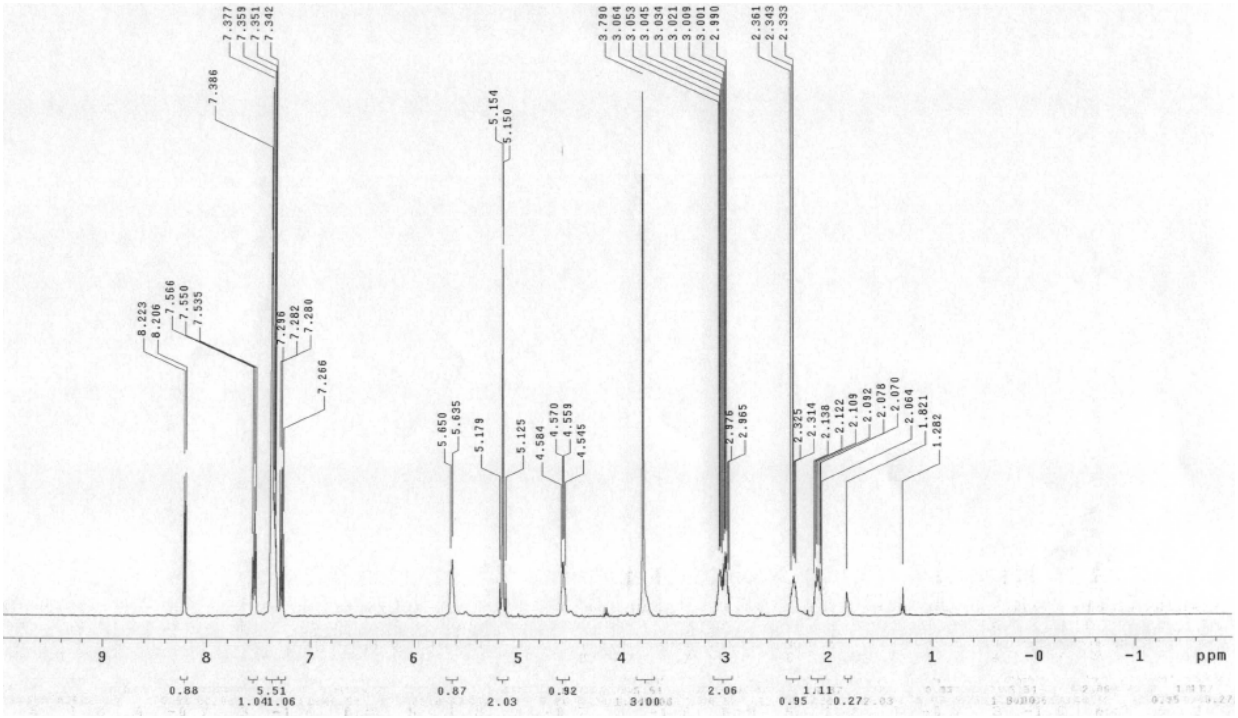


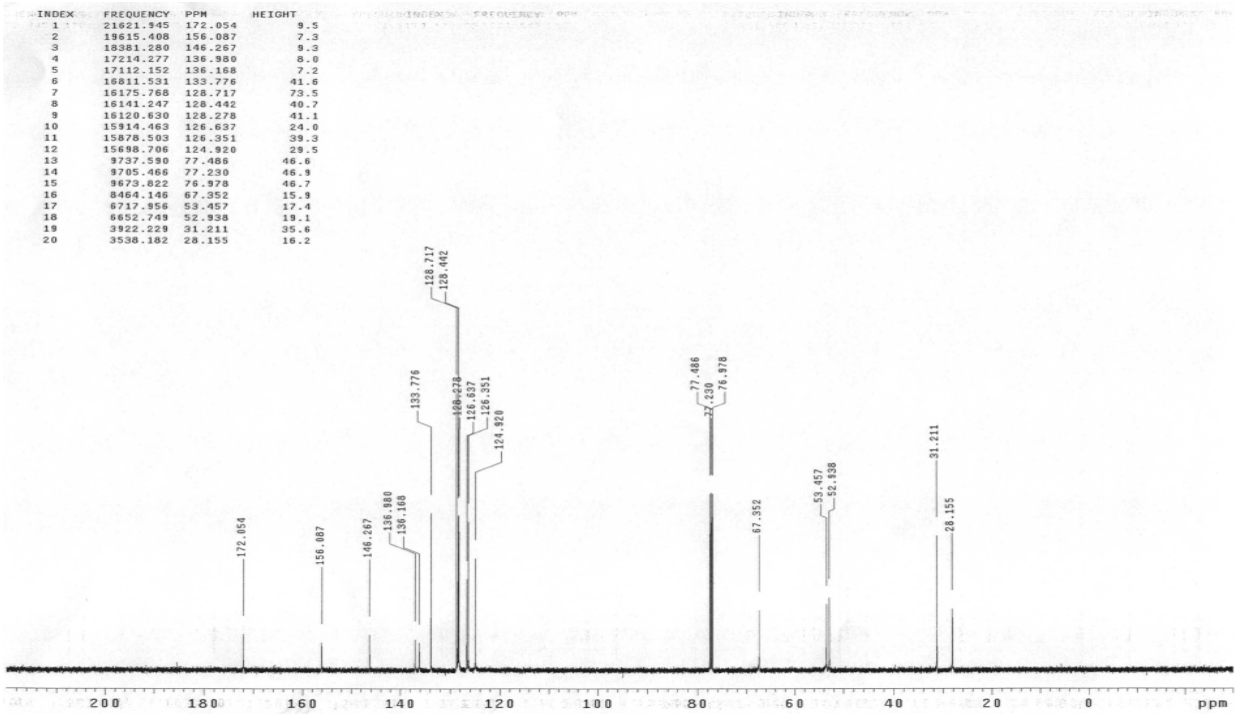


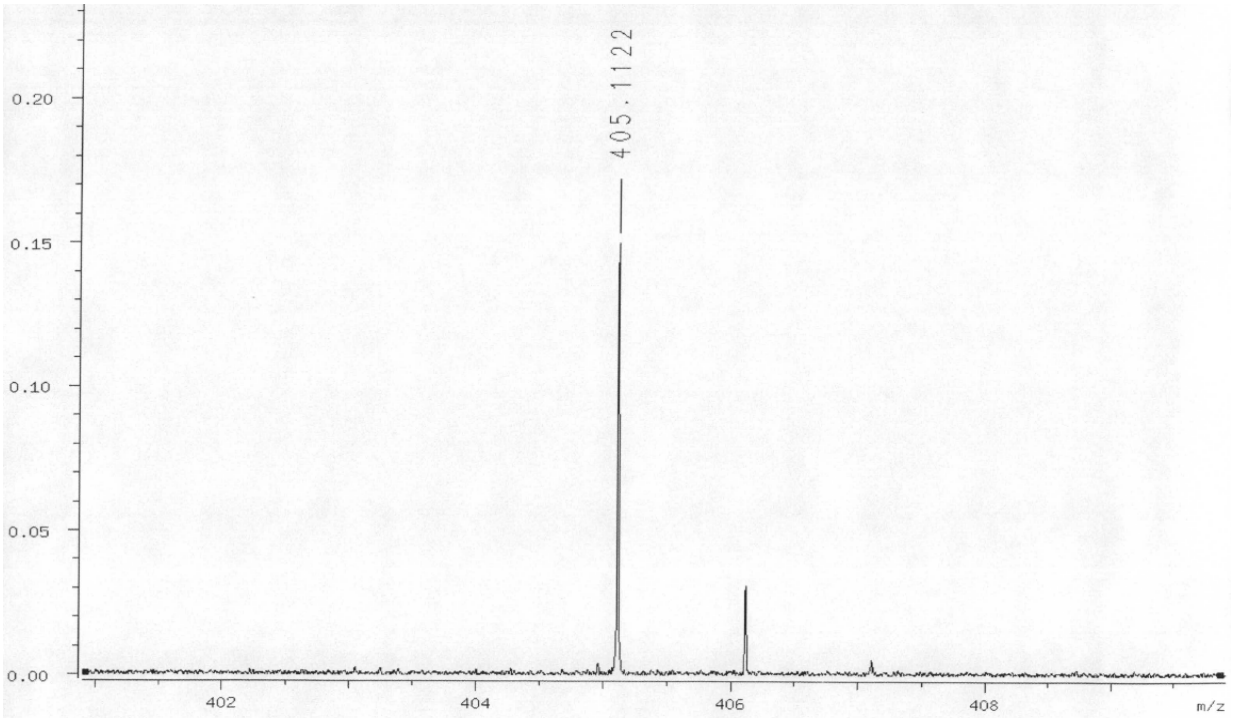


ESI-HRMS calcd for $C_{19}H_{20}N_2O_6S$ $[M+H]^+$ 405.1120, found 405.1126

Benzyl (*S*)-1-(methoxycarbonyl)-3-(2-nitrophenylthio)propylcarbamate (9f)

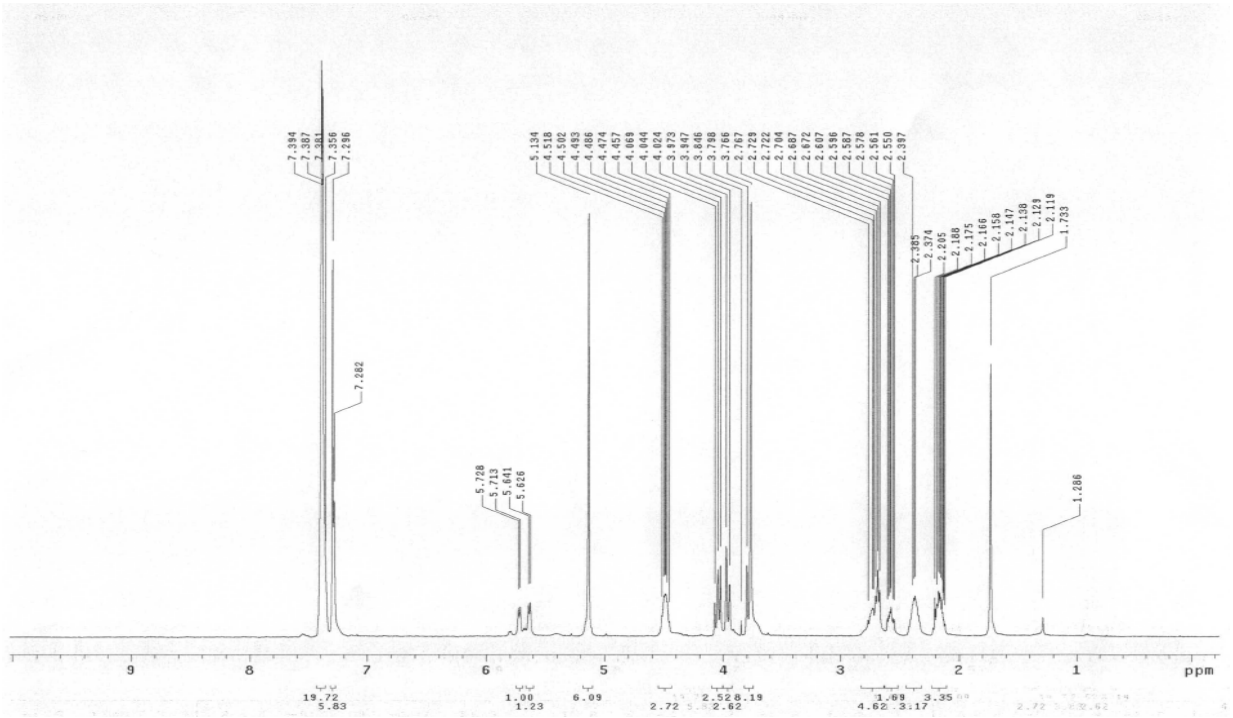


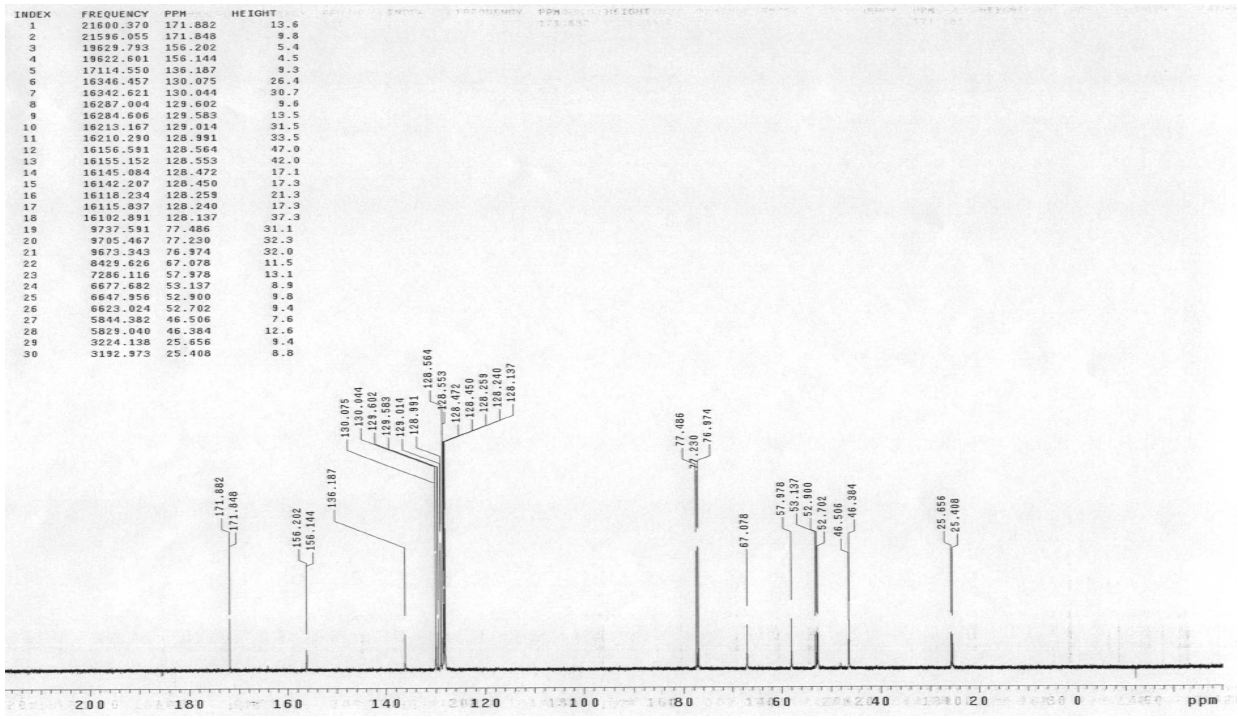




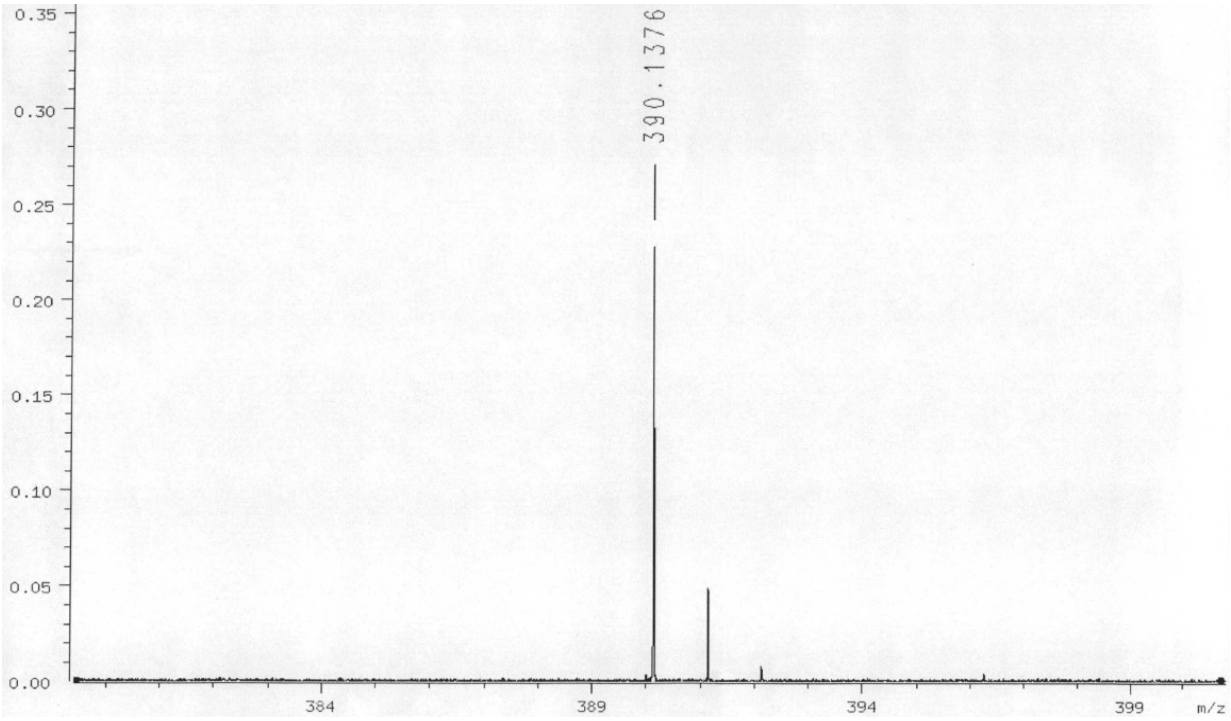
ESI-HRMS calcd for $C_{19}H_{20}N_2O_6S$ $[M+H]^+$ 405.1120, found 405.1122

Benzyl (*S*)-1-(methoxycarbonyl)-3-(benzylsulfinyl)propylcarbamate (10a)



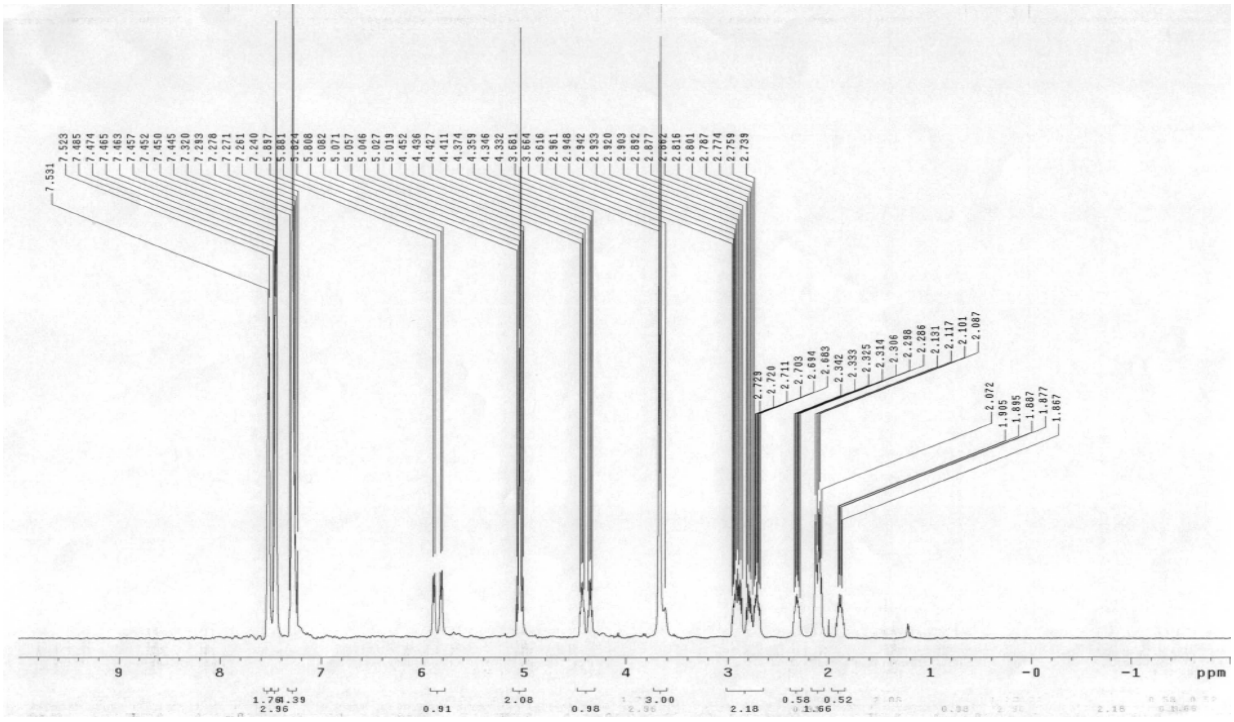


INDEX	FREQUENCY	PPM	HEIGHT
1	21600.370	171.882	13.6
2	21596.055	171.848	9.8
3	19629.793	156.202	5.4
4	19622.601	156.144	4.5
5	17114.550	136.187	9.3
6	16346.457	130.075	26.4
7	16342.621	130.044	30.7
8	16287.004	129.602	9.6
9	16284.606	129.583	13.5
10	16213.167	129.014	31.5
11	16210.290	128.991	33.5
12	16156.591	128.564	47.0
13	16155.152	128.553	42.0
14	16145.084	128.472	17.1
15	16142.207	128.450	17.3
16	16118.234	128.259	21.3
17	16115.837	128.240	17.3
18	16102.891	128.137	37.3
19	9737.591	77.486	31.1
20	9705.467	77.230	32.3
21	9673.343	76.974	32.0
22	8429.626	67.078	11.5
23	7286.116	57.978	13.1
24	6677.682	53.137	8.9
25	6647.956	52.900	9.8
26	6623.024	52.702	9.4
27	5844.382	46.506	7.6
28	5829.040	46.384	12.6
29	3224.138	25.656	9.4
30	3192.973	25.408	8.8

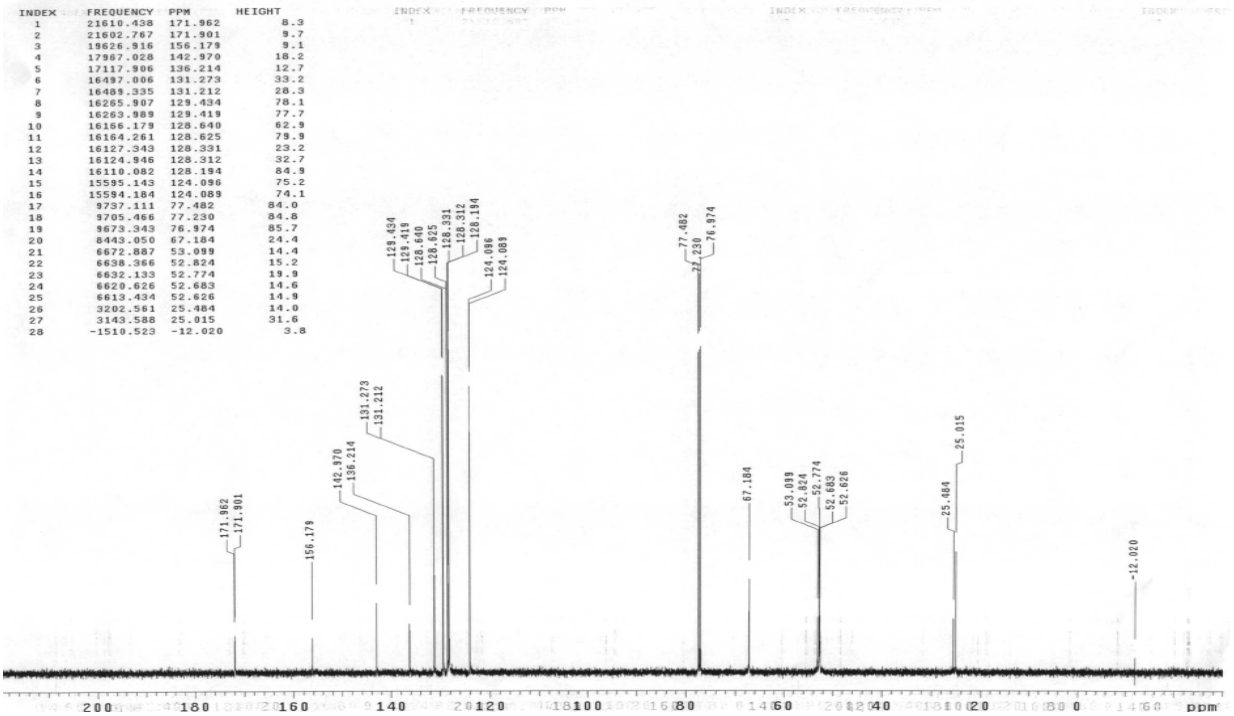


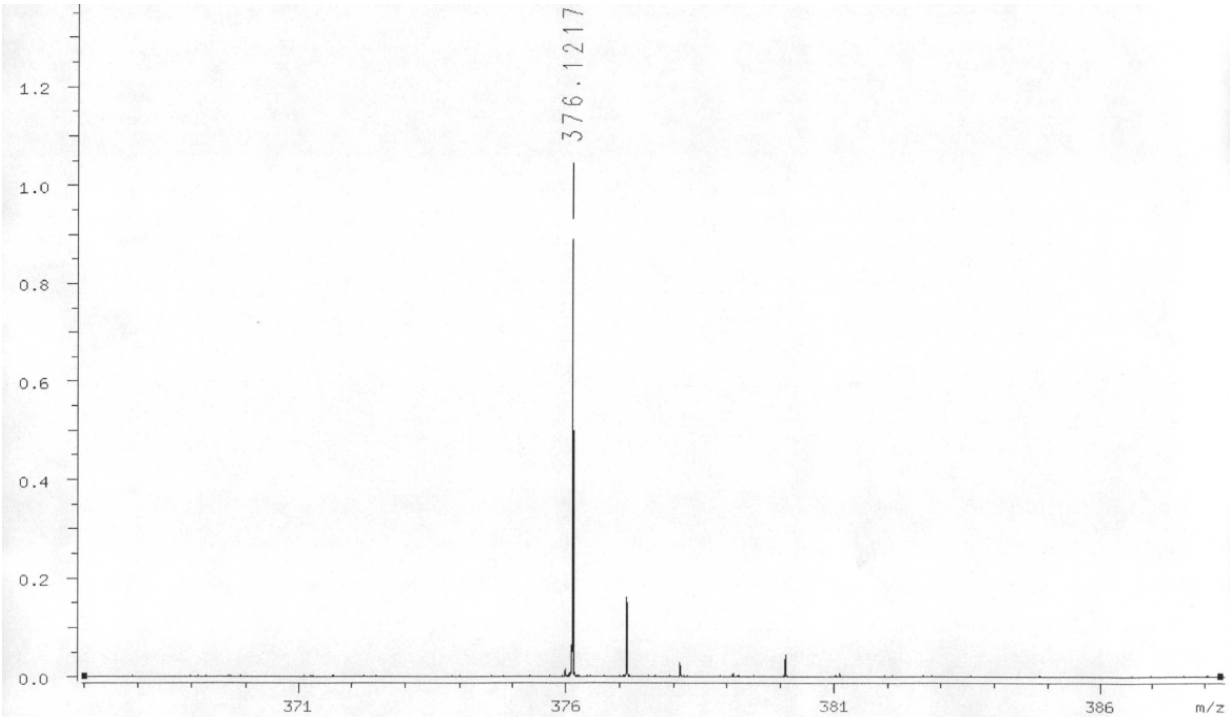
ESI-HRMS calcd for $C_{20}H_{23}NO_5S$ $[M+H]^+$ 390.1375, found 390.1376

Benzyl (*S*)-1-(methoxycarbonyl)-3-(phenylsulfinyl)propylcarbamate (10b)



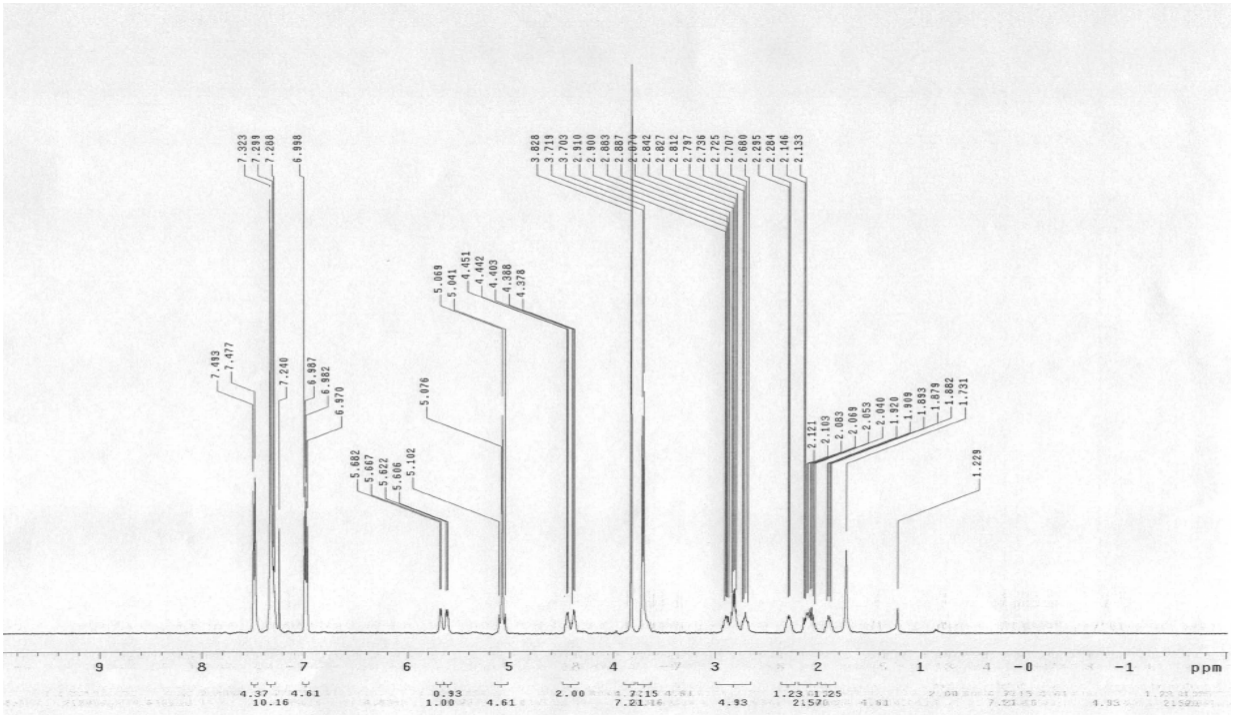
INDEX	FREQUENCY	PPM	HEIGHT
1	21610.438	171.962	8.3
2	21602.767	171.901	9.7
3	19626.916	156.179	9.1
4	17967.028	142.970	18.2
5	17117.906	136.214	12.7
6	16497.006	131.273	33.2
7	16489.335	131.212	28.3
8	16265.907	129.434	78.1
9	16263.989	129.419	77.7
10	16166.179	128.640	62.9
11	16164.261	128.625	79.9
12	16127.343	128.331	23.2
13	16124.946	128.312	32.7
14	16110.082	128.194	84.9
15	15595.143	124.096	75.2
16	15594.184	124.089	74.1
17	9737.111	77.482	84.0
18	9705.466	77.230	84.8
19	9673.343	76.974	85.7
20	8443.050	67.184	24.4
21	6672.887	53.099	14.4
22	6638.366	52.824	15.2
23	6632.133	52.774	19.9
24	6620.626	52.683	14.6
25	6613.434	52.626	14.9
26	3202.561	25.484	14.0
27	3143.588	25.015	31.6
28	-1510.523	-12.020	3.8

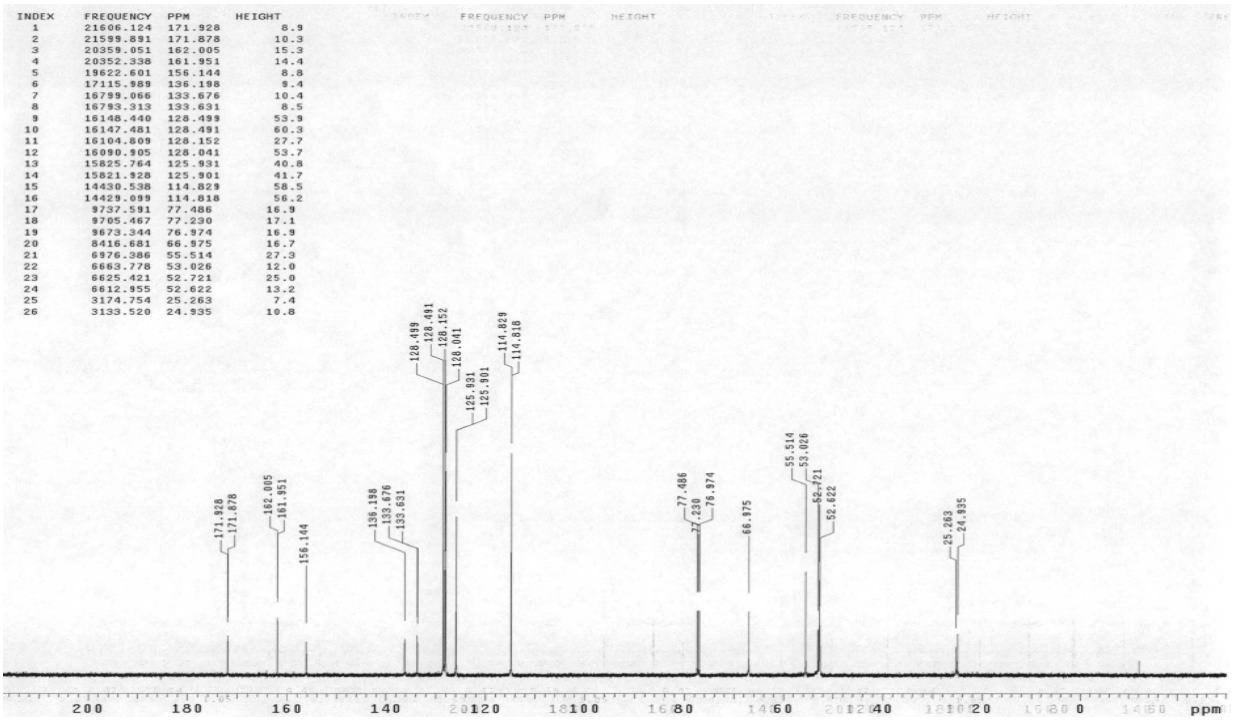


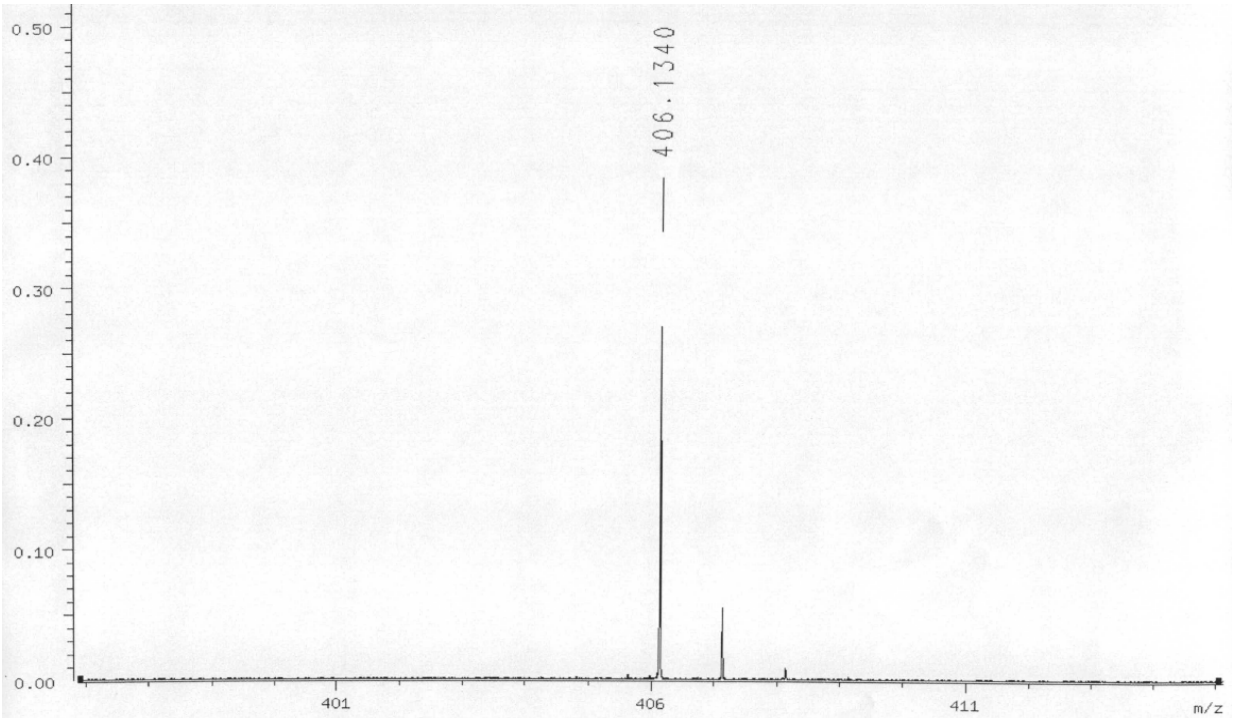


ESI-HRMS calcd for $C_{19}H_{21}NO_5S$ $[M+H]^+$ 376.1218, found 375.1217

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-methoxyphenylsulfinyl)propylcarbamate (10c)

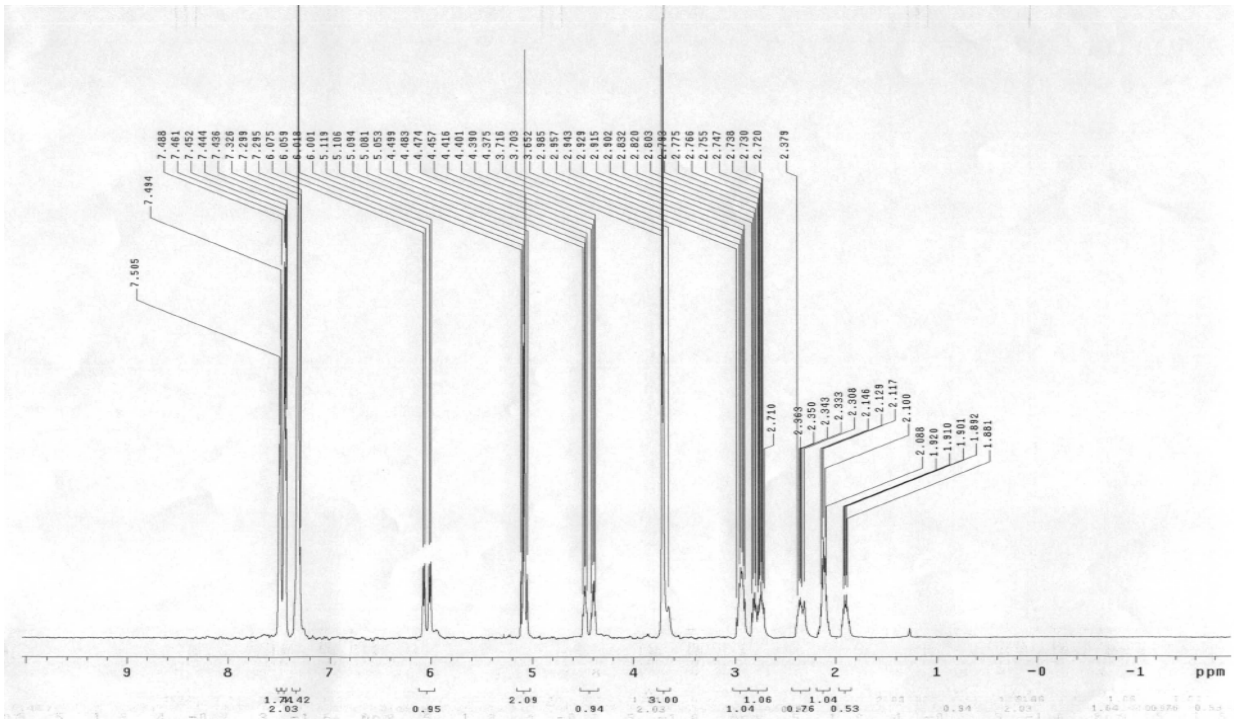




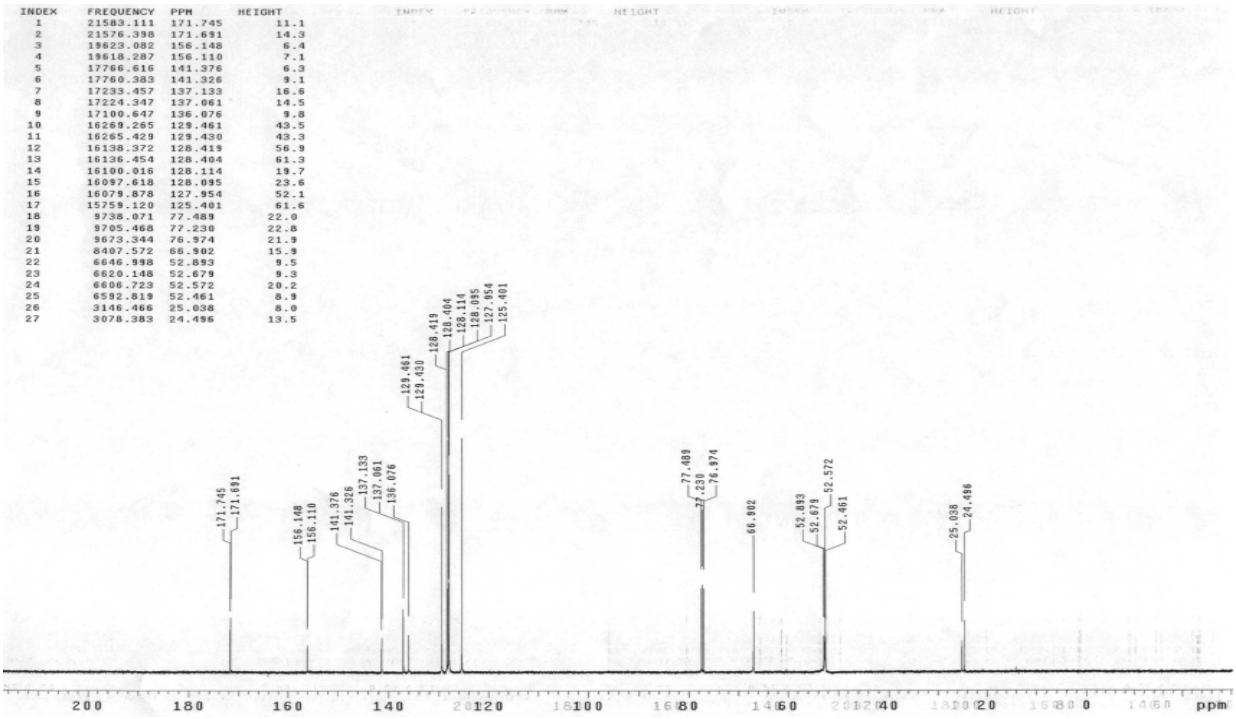


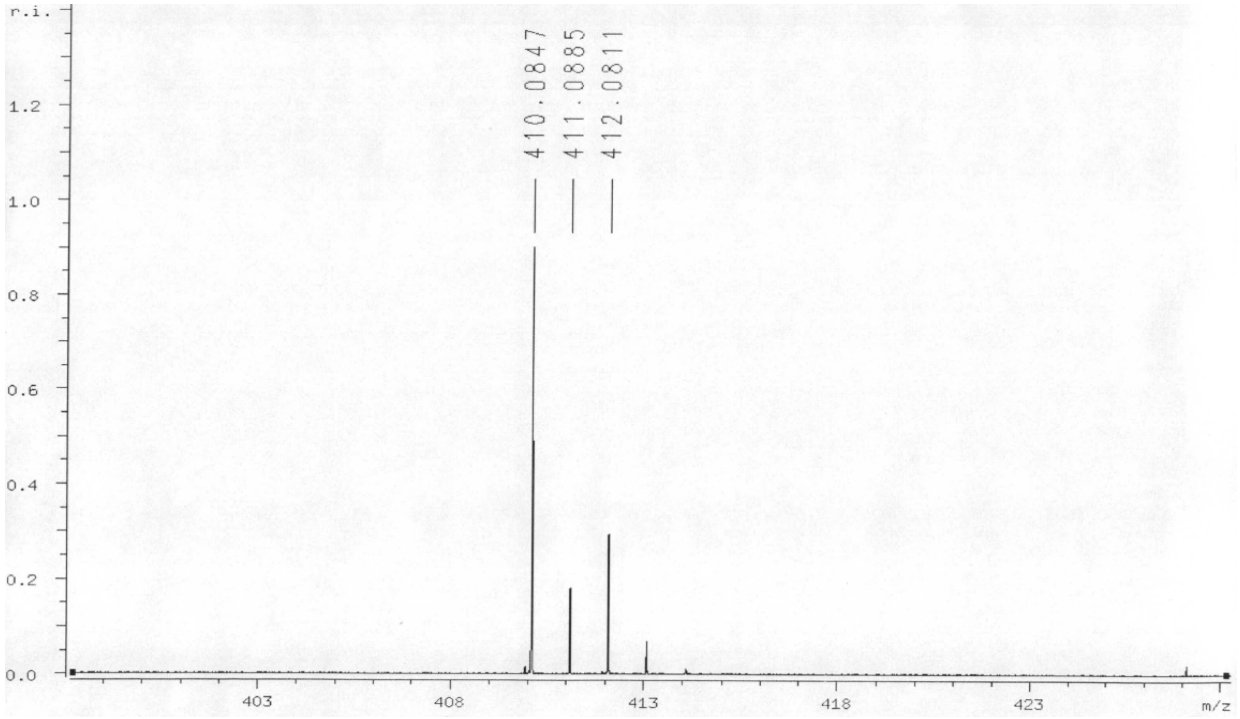
ESI-HRMS calcd for $C_{20}H_{23}NO_6S$ $[M+H]^+$ 406.1324, found 406.1340

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-chlorophenylsulfinyl)propylcarbamate (10d)



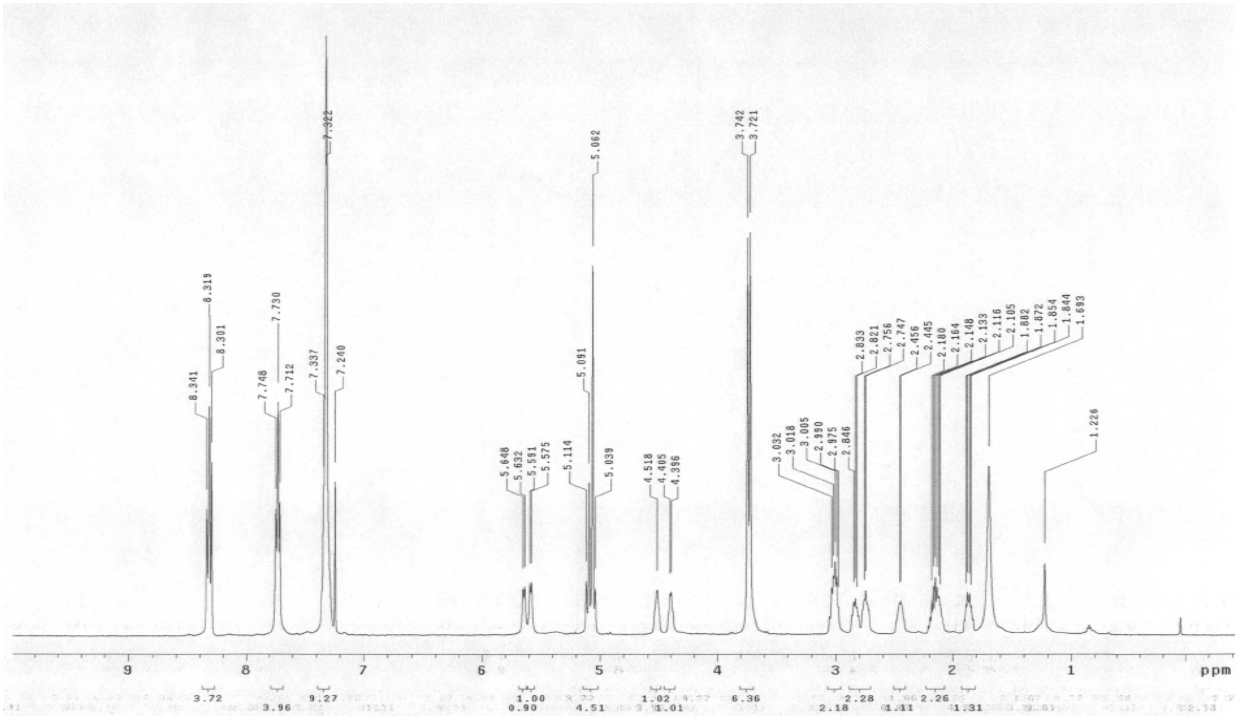
INDEX	FREQUENCY	PPM	HEIGHT
1	21583.111	171.745	11.1
2	21576.398	171.691	14.3
3	19623.082	156.148	6.4
4	19618.287	156.110	7.1
5	17766.616	141.376	6.3
6	17760.383	141.326	9.1
7	17233.457	137.133	16.6
8	17224.347	137.061	14.5
9	17100.647	136.076	9.8
10	16269.265	129.461	43.5
11	16265.429	129.430	43.3
12	16138.372	128.419	56.9
13	16136.454	128.404	61.3
14	16100.016	128.114	19.7
15	16097.618	128.095	23.6
16	16079.878	127.954	52.1
17	15759.120	125.401	61.6
18	9738.071	77.489	22.0
19	9705.468	77.230	22.8
20	9673.344	76.974	21.9
21	6407.572	66.902	15.9
22	6646.998	52.893	9.5
23	6620.148	52.679	9.3
24	6606.723	52.572	20.2
25	6592.819	52.461	8.9
26	3146.466	25.038	8.0
27	3078.383	24.496	13.5

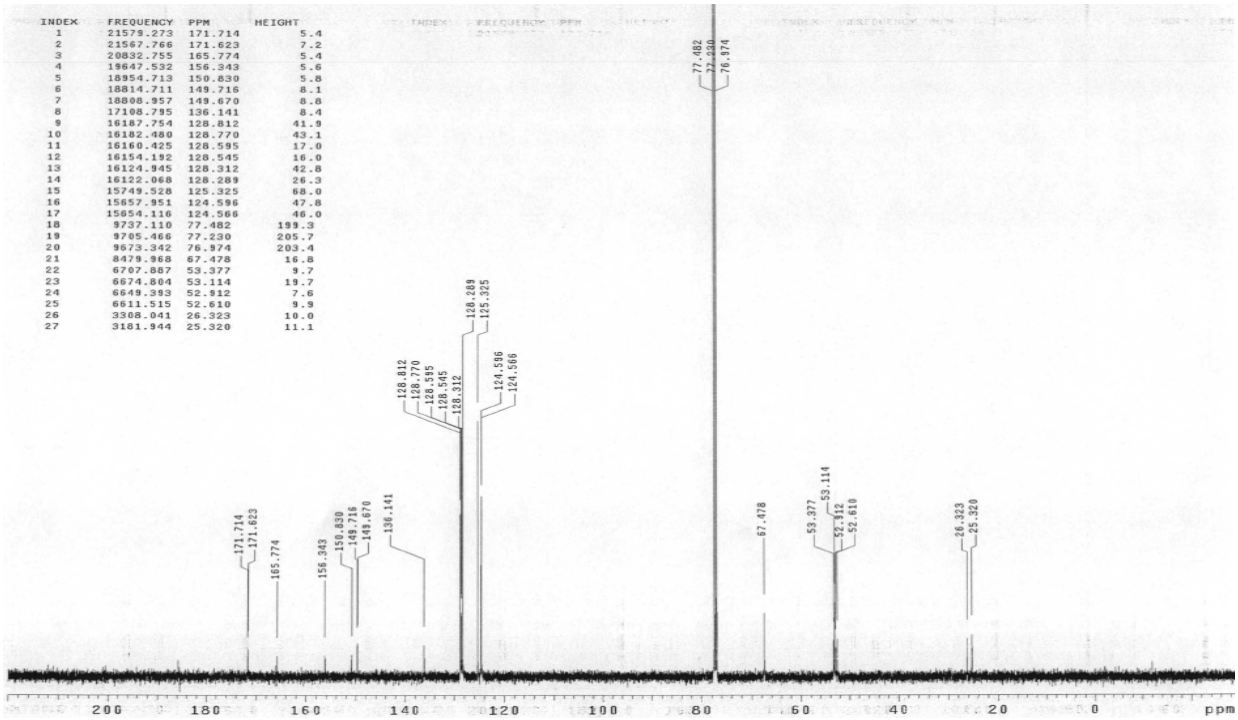


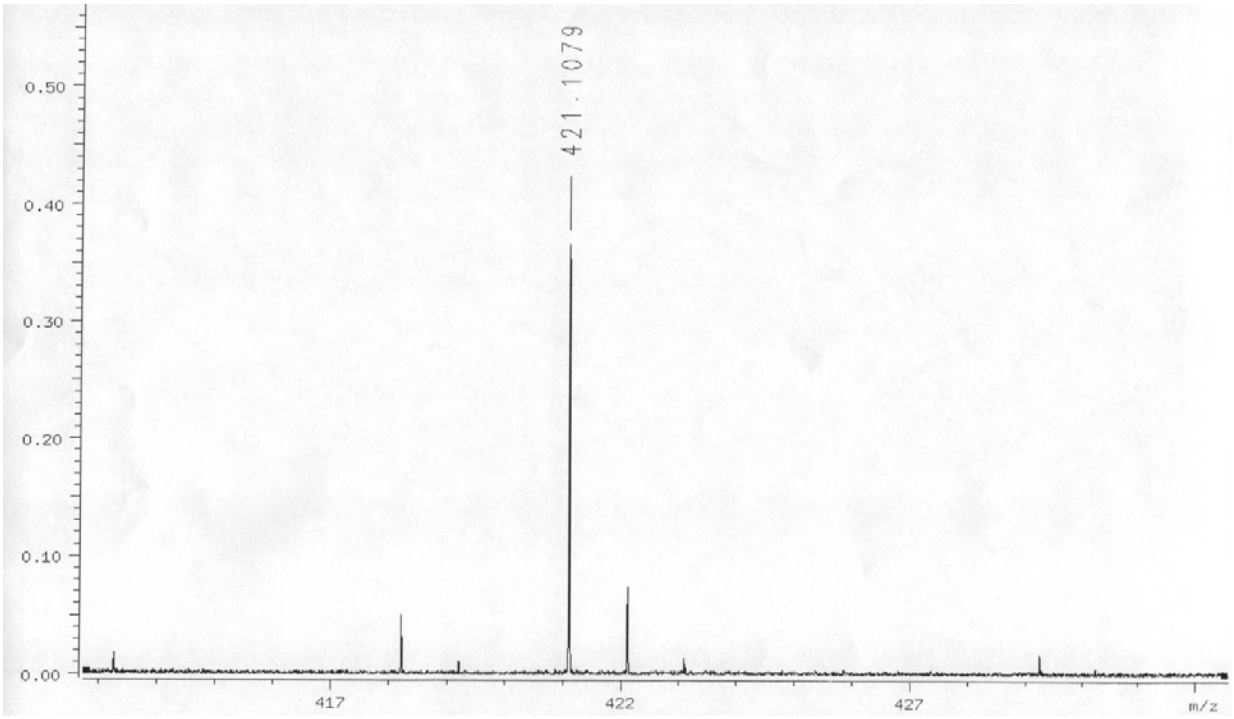


ESI-HRMS calcd for $C_{19}H_{20}ClNO_5S$ $[M+H]^+$ 410.0829, found 410.0847

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-nitrophenylsulfinyl)propylcarbamate (10e)

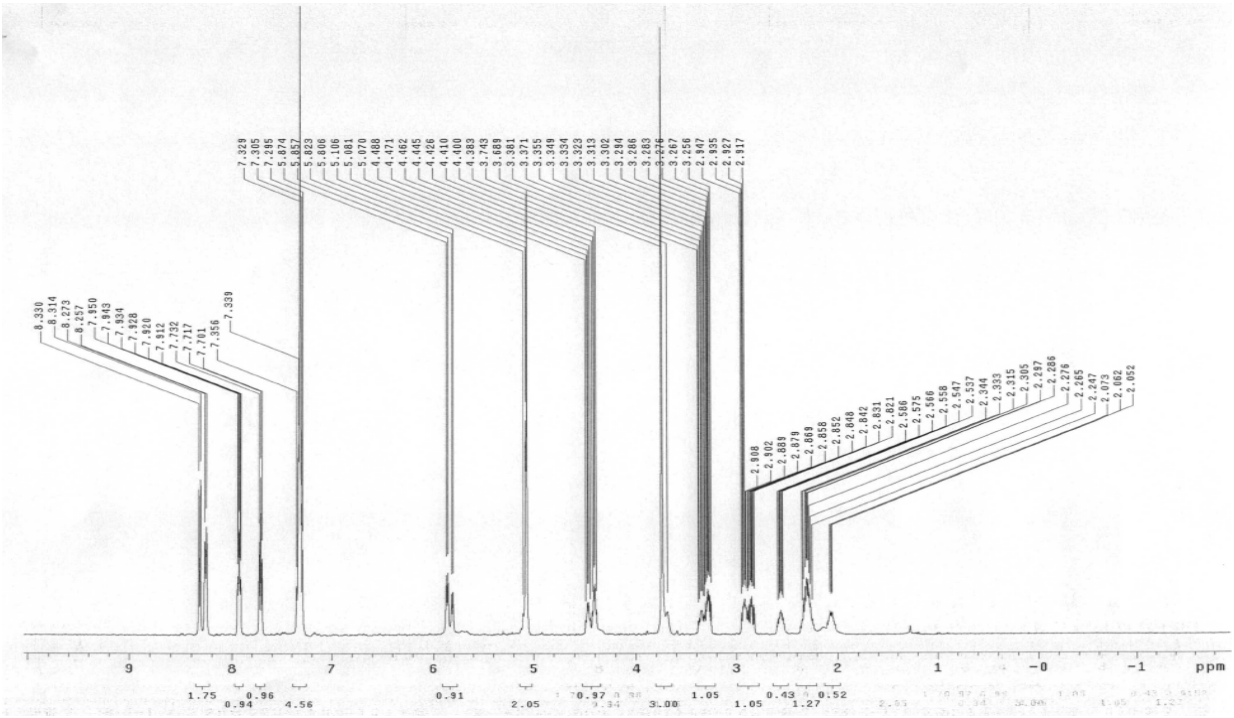




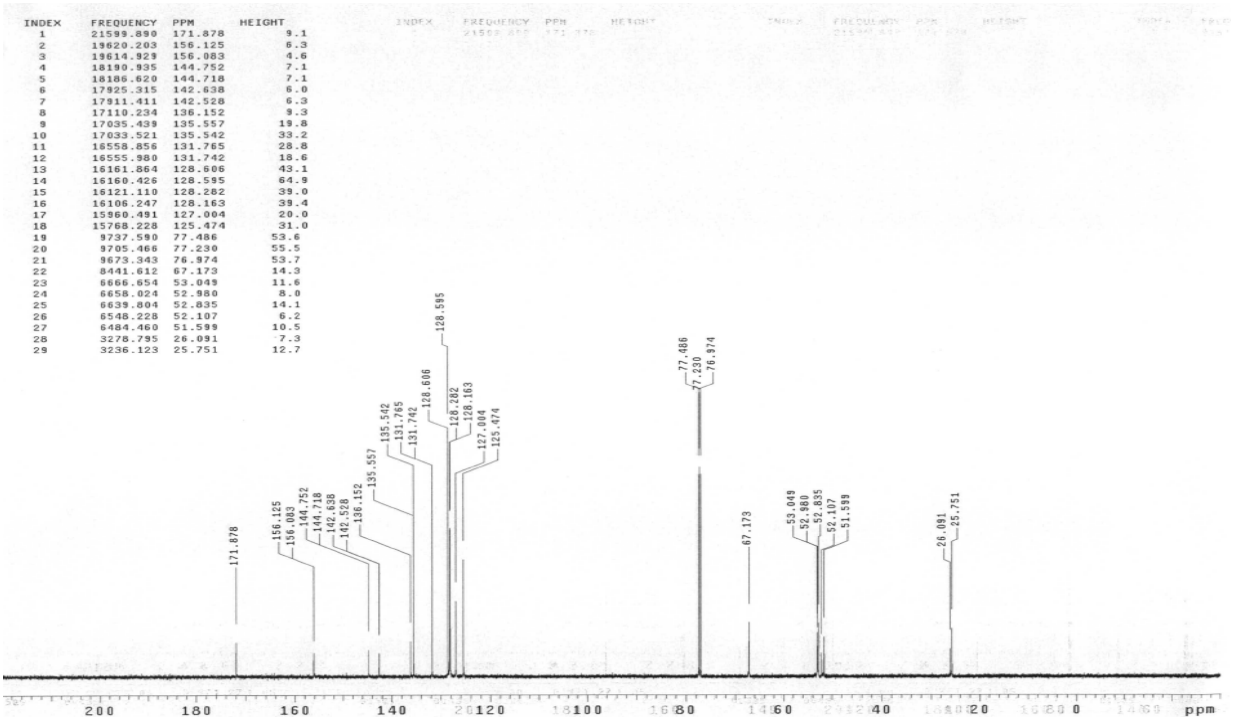


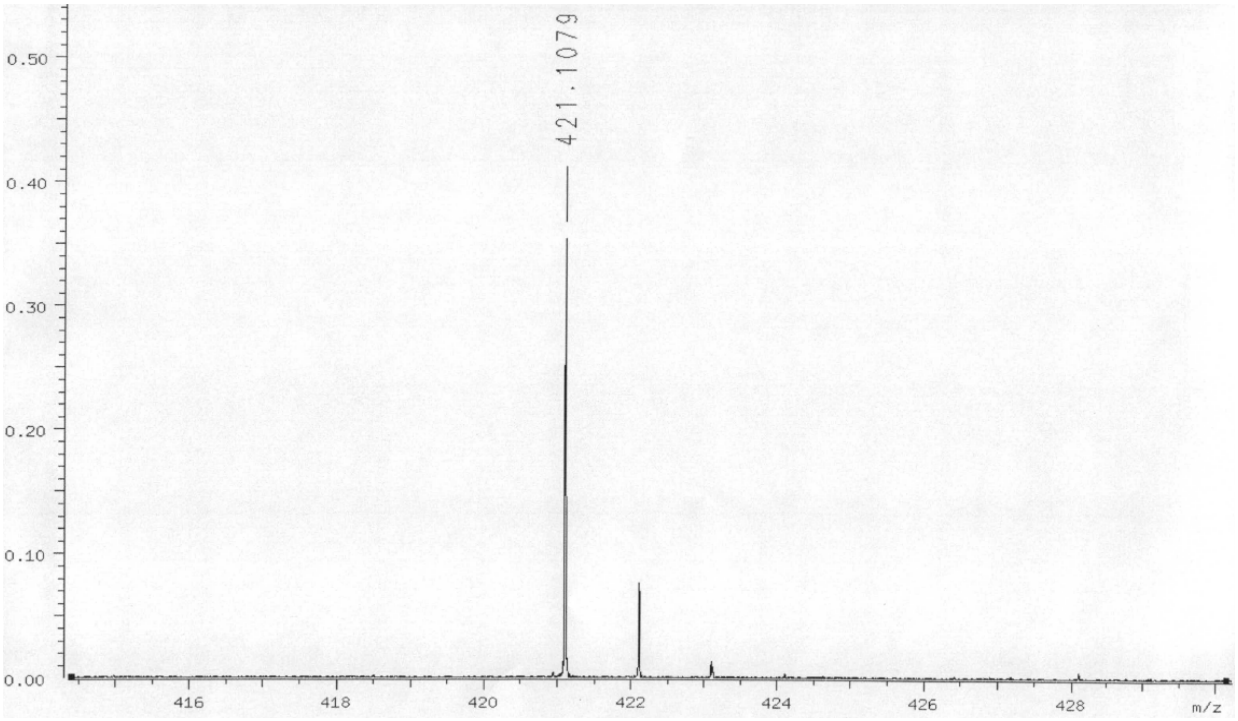
ESI-HRMS calcd for $C_{19}H_{20}N_2O_7S$ $[M+H]^+$ 421.1070, found 421.1079

Benzyl (*S*)-1-(methoxycarbonyl)-3-(4-nitrophenylsulfinyl)propylcarbamate (10f)



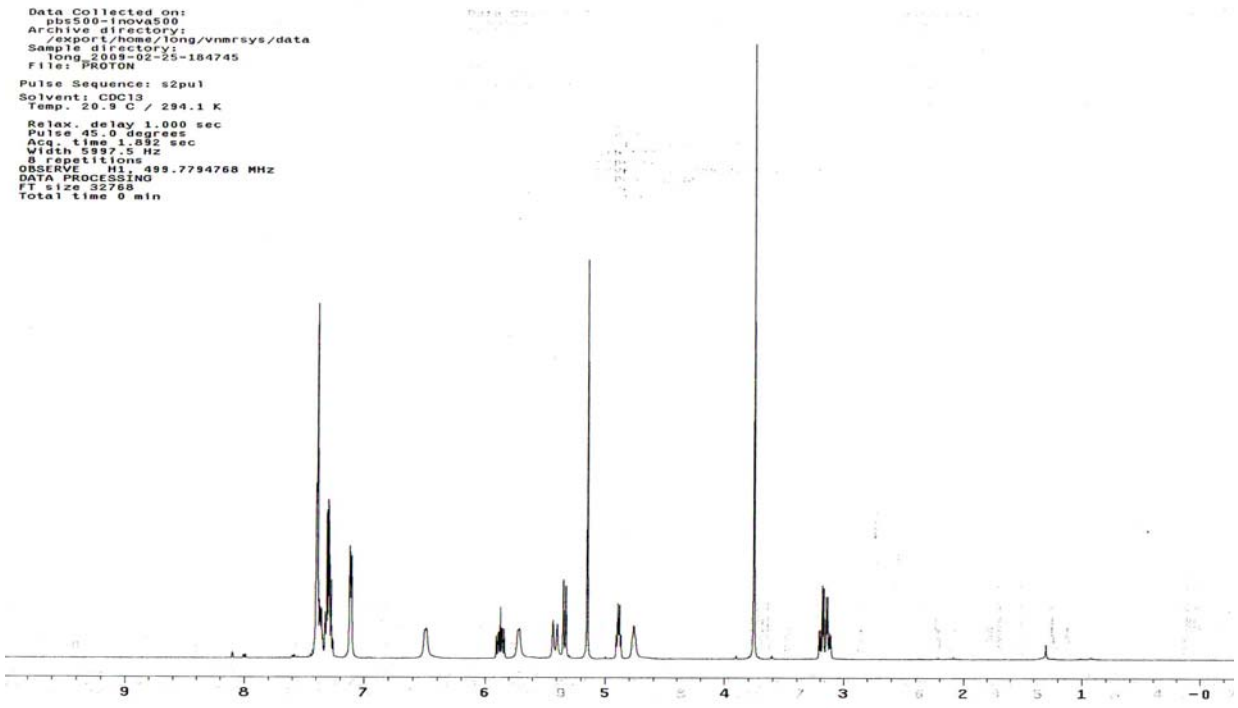
INDEX	FREQUENCY	PPM	HEIGHT
1	21599.890	171.878	9.1
2	19620.203	156.125	8.3
3	19614.929	156.083	4.6
4	18190.935	144.752	7.1
5	18186.620	144.718	7.1
6	17925.315	142.638	6.0
7	17911.411	142.528	6.3
8	17110.234	136.152	9.3
9	17035.439	135.557	19.8
10	17033.521	135.542	33.2
11	16558.856	131.765	28.8
12	16555.980	131.742	18.6
13	16161.864	128.606	43.1
14	16160.426	128.595	64.9
15	16121.110	128.282	39.0
16	16106.247	128.163	39.4
17	15960.491	127.004	20.0
18	15768.228	125.474	31.0
19	9737.590	77.486	53.6
20	9705.466	77.230	55.5
21	9673.343	76.974	53.7
22	8441.612	67.173	14.3
23	6666.654	53.049	11.6
24	6658.024	52.980	8.0
25	6639.804	52.835	14.1
26	6548.228	52.107	6.2
27	6484.460	51.599	10.5
28	3278.795	26.091	7.3
29	3236.123	25.751	12.7

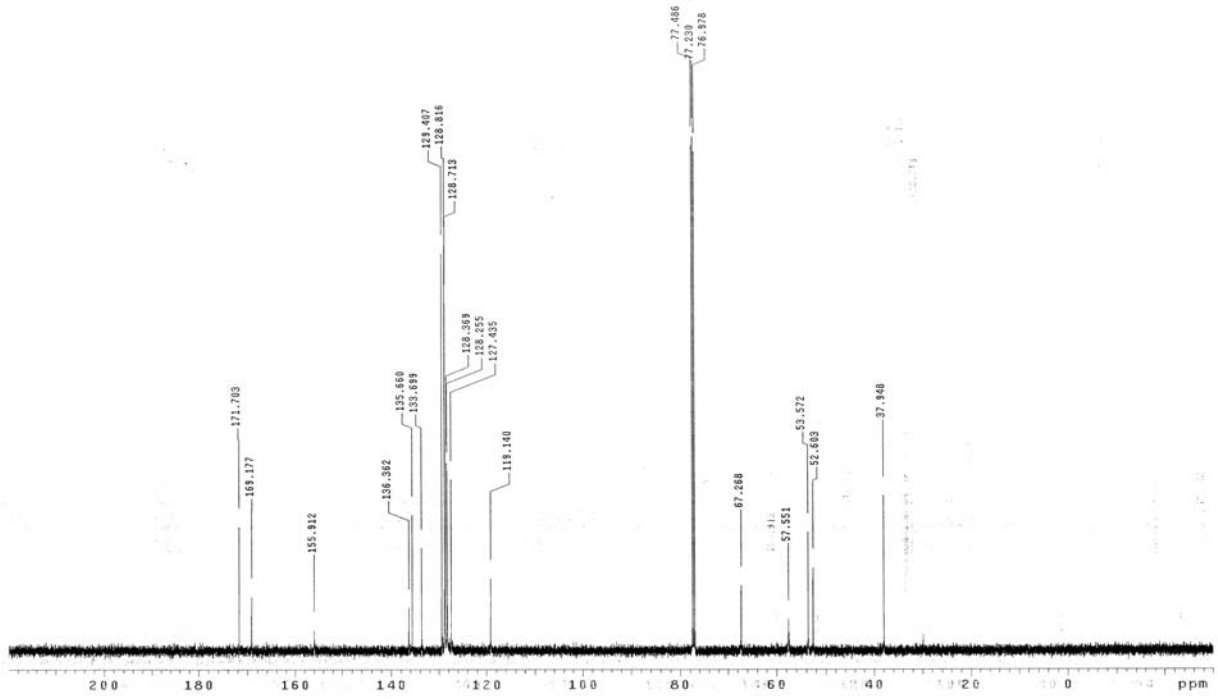




ESI-HRMS calcd for $C_{19}H_{20}N_2O_7S$ $[M+H]^+$ 421.1070, found 421.1079

L-Cbz-VG-Phe-OMe (16)

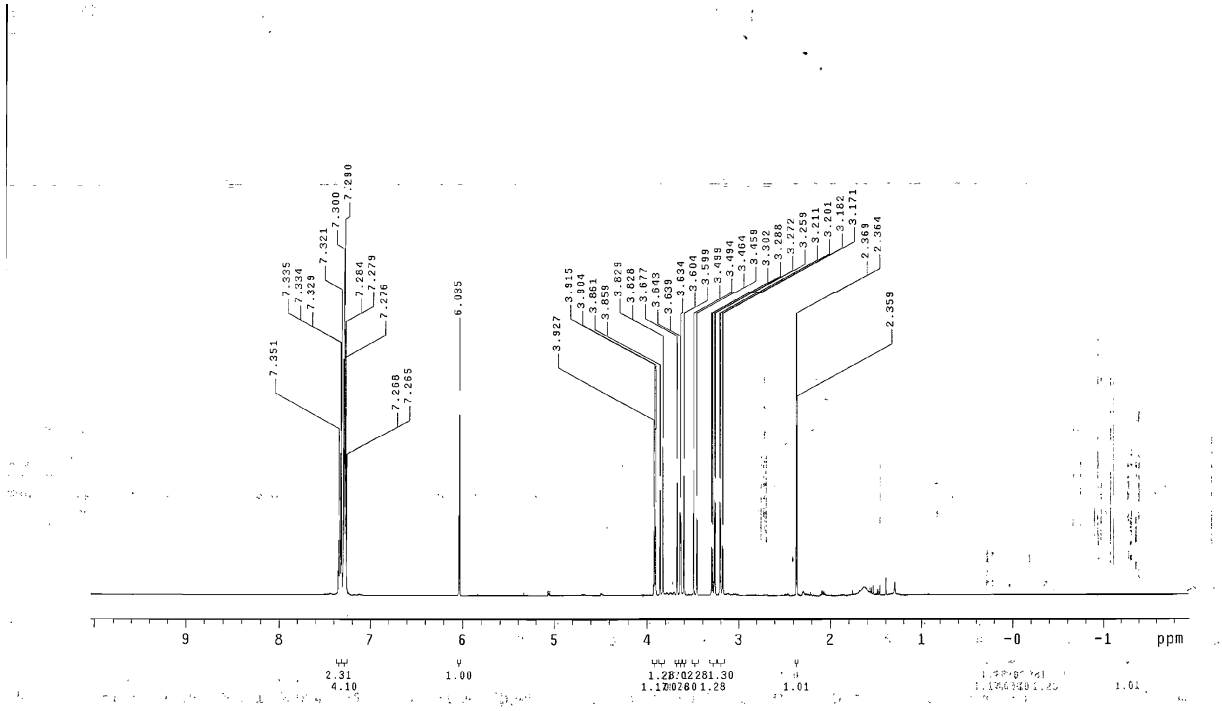


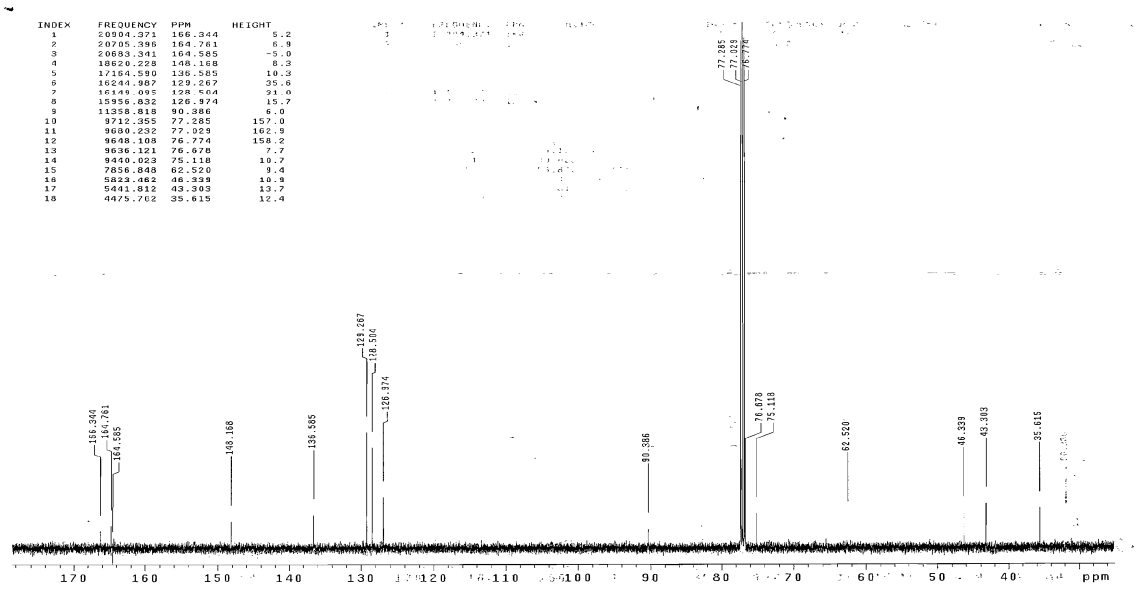


Appendix-II

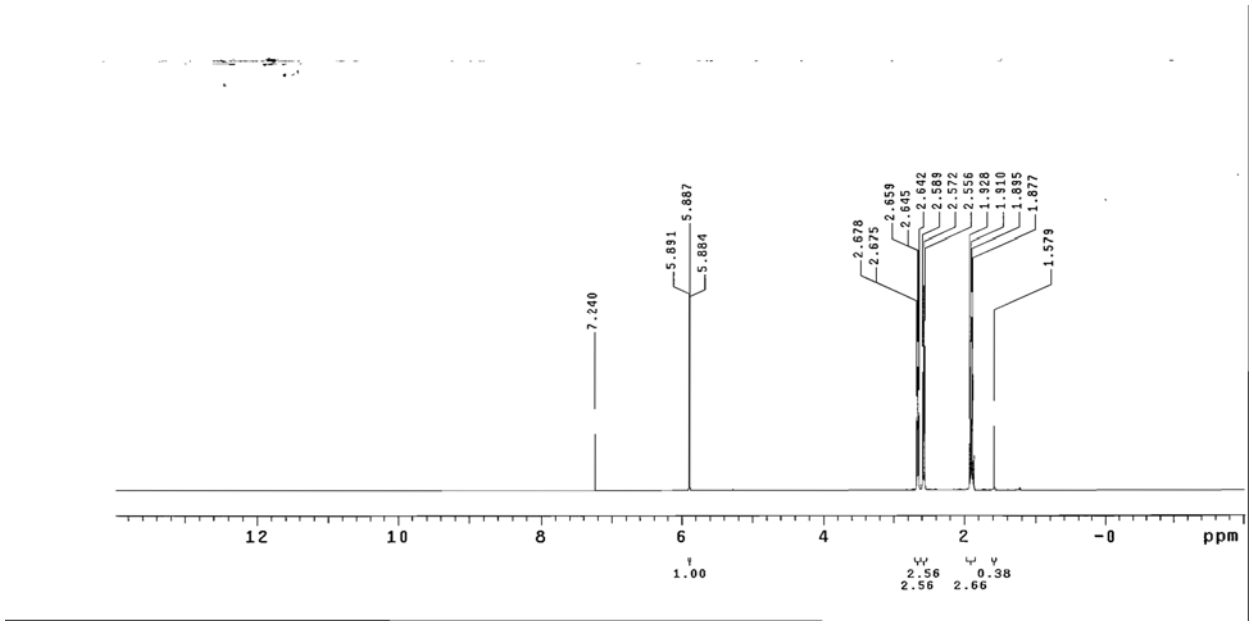
^{1}H and ^{13}C NMRs of compounds synthesized in chapter-III

^{1}H and ^{13}C NMR of (*S*,*E*)-3-benzyl-6-(bromomethylene)-4-(prop-2-ynyl)morpholin-2-one (5)

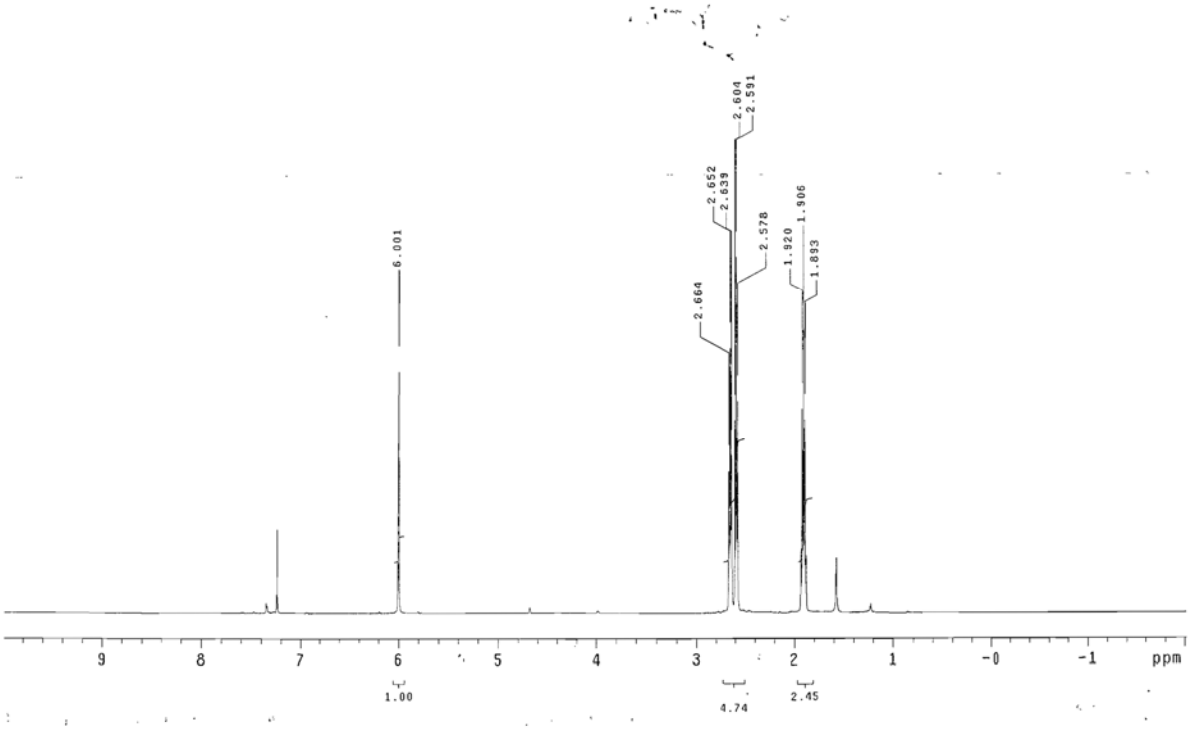




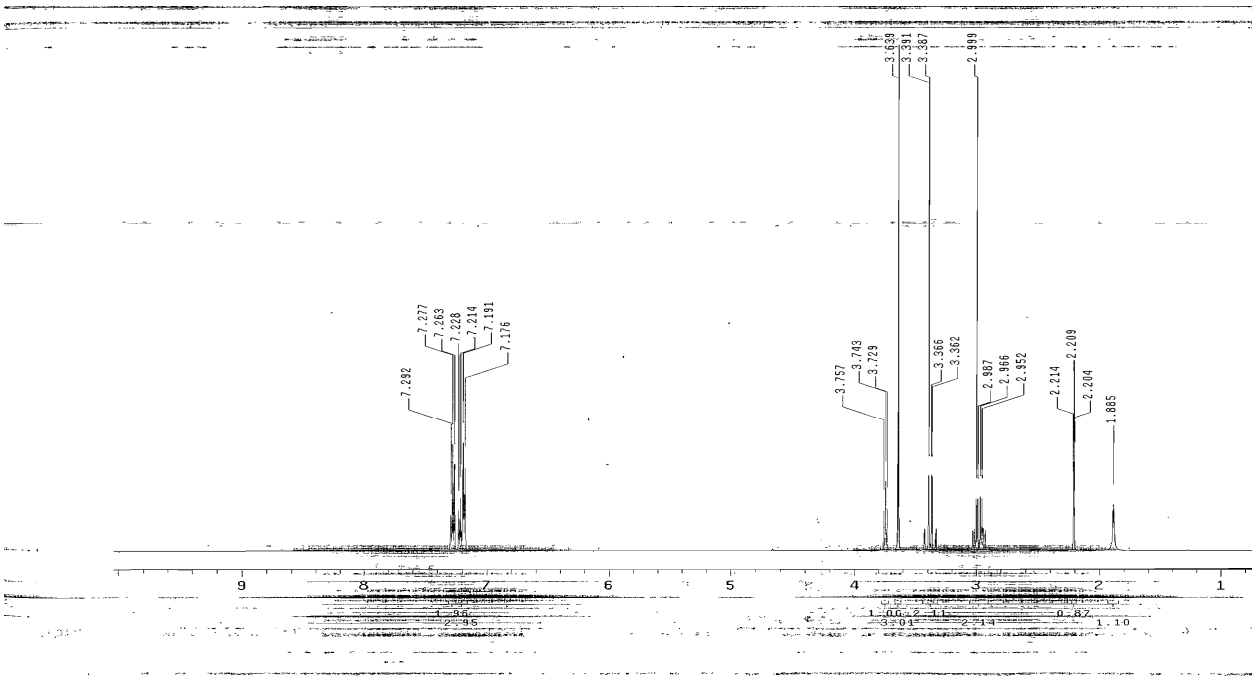
^{1}H NMR of (*E*)-tetrahydro-6-(iodomethylene)pyran-2-one (8)

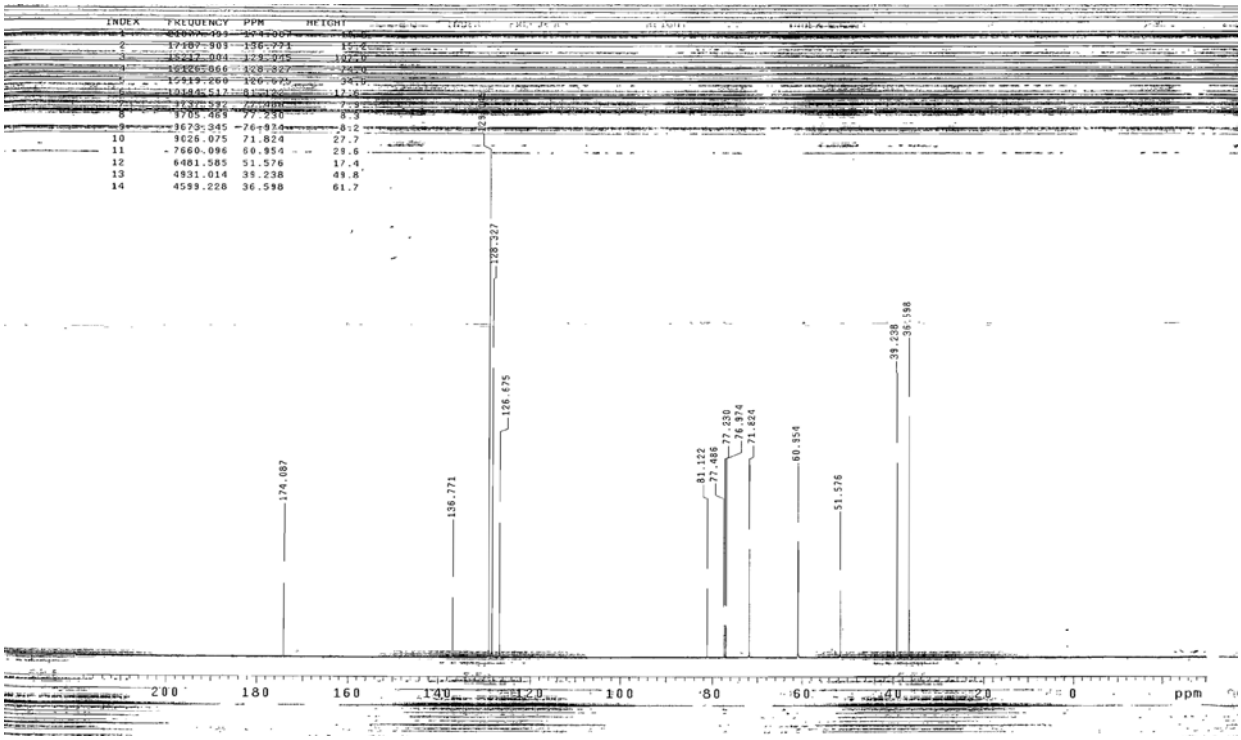


^{1}H of (*E*)-6-(bromomethylene)-tetrahydropyran-2-one (9)

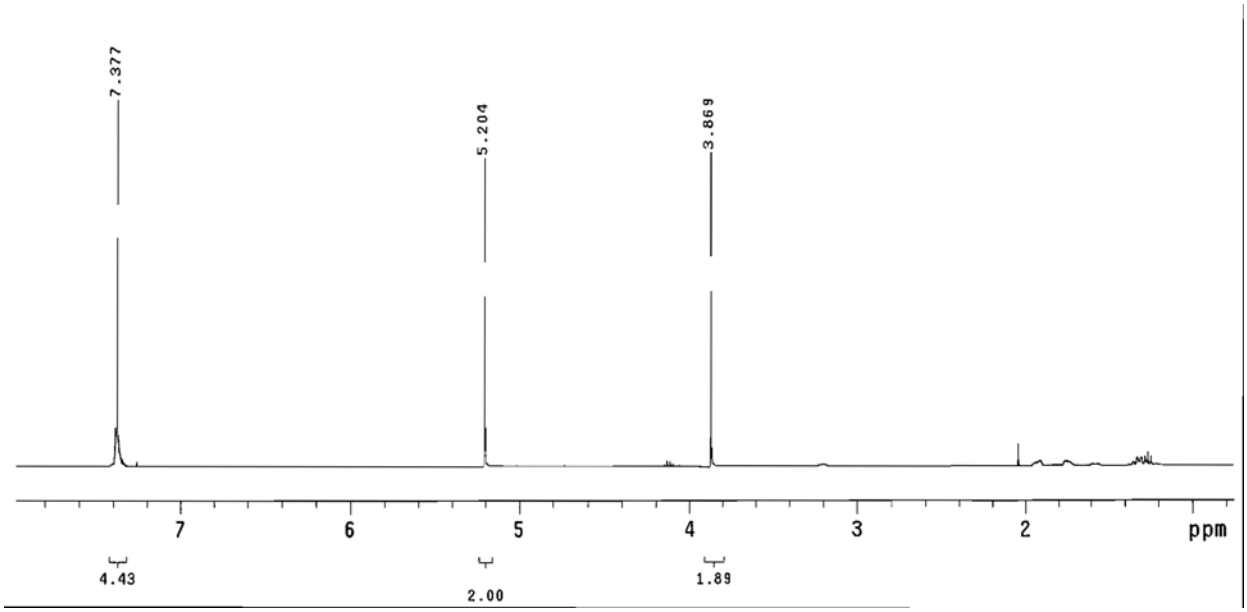


^{1}H and ^{13}C NMR of *N*-monoalkylated Phe-OMe (14)

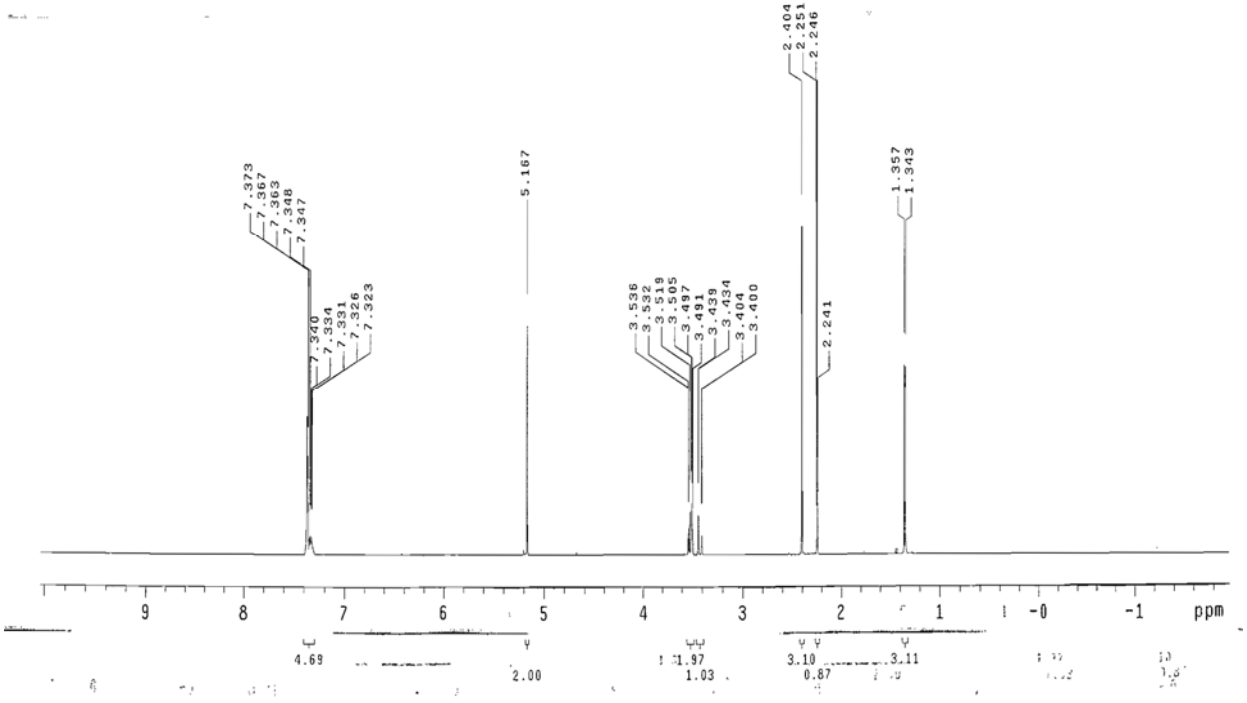




^{1}H NMR of benzyl ester of bromoacetic acid, precursor of 23



^{1}H and ^{13}C NMR of *N*-alkylated methyl propargylamine (23)



INDEX	FREQUENCY	PPM	HEIGHT
1	21754.758	173.111	11.8
2	17071.880	135.847	4.4
3	17067.565	135.813	5.4
4	16157.552	128.572	22.8
5	16154.196	128.545	32.8
6	16121.113	128.282	37.5
7	16117.757	128.255	55.0
8	9938.486	79.084	4.5
9	9933.212	79.042	4.5
10	9738.073	77.489	10.9
11	9705.469	77.230	8.7
12	9673.346	76.974	7.7
13	9182.859	73.071	36.2
14	8325.106	66.246	21.6
15	7604.959	60.515	12.4
16	7601.123	60.485	15.6
17	5446.913	43.343	13.0
18	5443.077	43.313	15.9
19	4830.808	38.441	33.9
20	1988.573	15.824	18.8
21	1986.176	15.805	30.6