KJE-3900

Master thesis in organic chemistry

Attempted syntheses of 1,3,4,6–tetrasubstituted-2,5–diketopiperazines using microwave assisted heating

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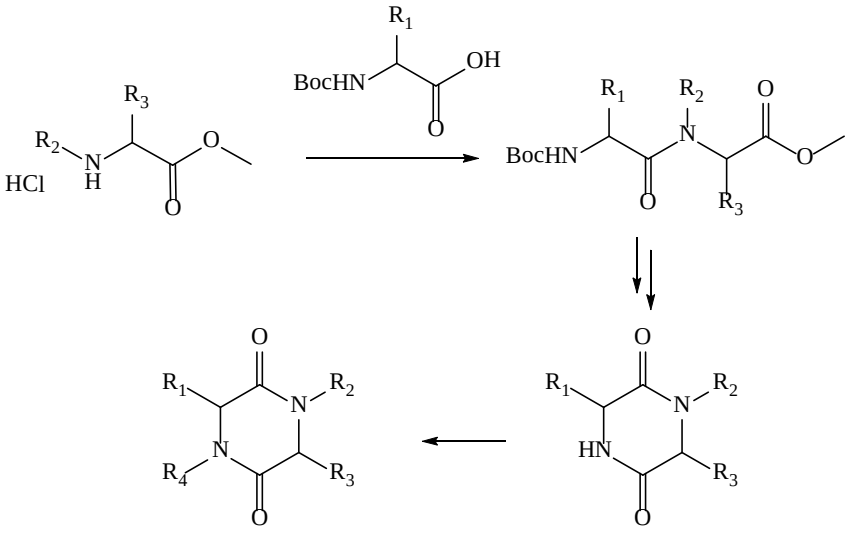
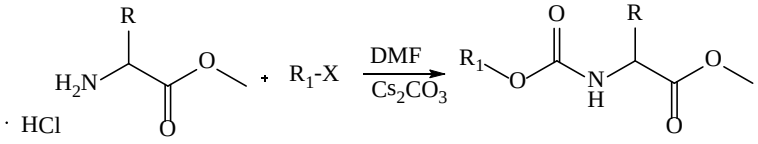
Summary

This project was aimed at synthesis of 1,3,4,6-tetrasubstituted diketopiperazines incorporating both hydrophobic and cathionic hydrophilic groups as a substituents on the diketopiperazine scaffold. In order to check the possibility of a proposed synthesis (Scheme 1), a range of disubtituted diketopiperazines were synthesized by using different amino acids of D and L configurations. In the study it has been investigated the N-alkylation and reductive amination of amino acids, amino acids methyl esters and dipeptides.



Scheme 1. Proposed synthesis pathway for synthesizing 1,3,4,6–tetrasubstituted–2,5-DKPs (R=ethyl, benzyl).

By synthesizing DKPs a new carbamation reaction was discovered (Scheme 2). This way of synthesis of carbamates was explored more extensively during this project.



Scheme 2. Synthesis of carbamates (R_1=ethyl, isopropyl and t–butyl).

Abbrevations

BEMP - 2-tert-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine

Boc – tert - butyloxycarbonyl

DIC - *N*,*N*′-diisopropylcarbodiimide

DCM - dichloromethane

DKP – diketopiperazine

DMF –dimethylformamide

DIPEA – *N*, *N* – diisopropylethylamine

EtOAc – ethyl acetate

EDC – 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

HBTU - O-(Benzotriazol-1-yl)-*N*, *N*, *N*′, *N*′-tetramethyluronium hexafluorophosphate

IR – infrared spectrometry

MBHA - *p*–methylbenzhydrylamine

MCR – multicomponent reaction

NMM - *N*-methylmorpholine

NMR – nuclear magnetic resonance spectroscopy

PEGA – poly[acryloyl-bis(aminopropyl)polyethylene glycol]

PMB - *p* – methoxybenzyl

SDS – solvent drying system

HRMS – high resolution mass spectrometry

UDAC – ugi deprotection+activation/cyclization

THF – tetrahydrofuran

TFA – trifluoracetic acid

1. Background

Science is progressing every day. Together with it, new routes for the synthesis of natural product and potential drug candidates continue to emerge. Today there are a lot of different synthetic routes to diketopiperazines (DKPs), which are the smallest cyclic peptides. These cyclic compounds possess two amide groups with acceptor and donor properties. [1] DKPs are commonly found in a nature (plants, animals or microoganisms) or might be easily synthesized.[2, 3]

The strategies for the synthesis of three isomeric DKP (Figure 1) are different depending on which positions the keto groups are situated, even though they share piperazine core. [2]

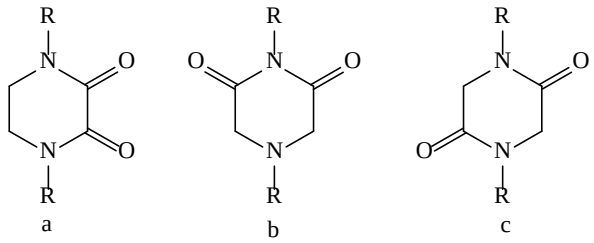


Figure 1. Isomers (a) 2,3-DKP; (b) 2,6-DKP; (c) 2,5-DKP.

Why are DKPs important for science nowadays? First of all, a large variety of DKPs possess an ability to bind to a variety of biologically important receptors with a high affinity. [4] Secondly, they can be synthesized from different kinds of amino acids using simple chemical reactions and the most import reason is that DKPs exhibit numerous medicinally and biologically significant properties like antifungal, [5-7] antibacterial, [8-13] antitumor[14, 15] and antiviral activity. [16] All those features make DKPs an incredibly interesting research object.

1.1. 2,3–DKPs

2,3–DKPs are not investigated that deeply compared to 2,5–DKPs, though there exist some very important compounds belonging to this isomeric group of DKPs. One of them is piperacillin, [17] which is similar to penicillin, but has a wider activity against Gram – negative, Gram – positive in particular many anaerobic species of bacteria.(Figure 2)

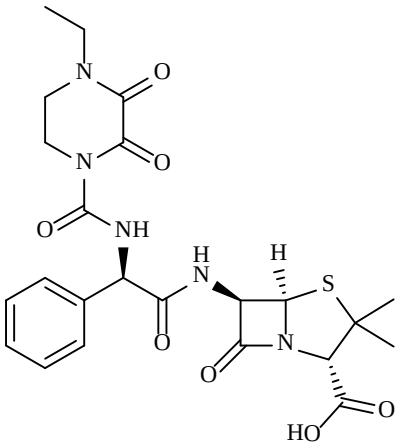
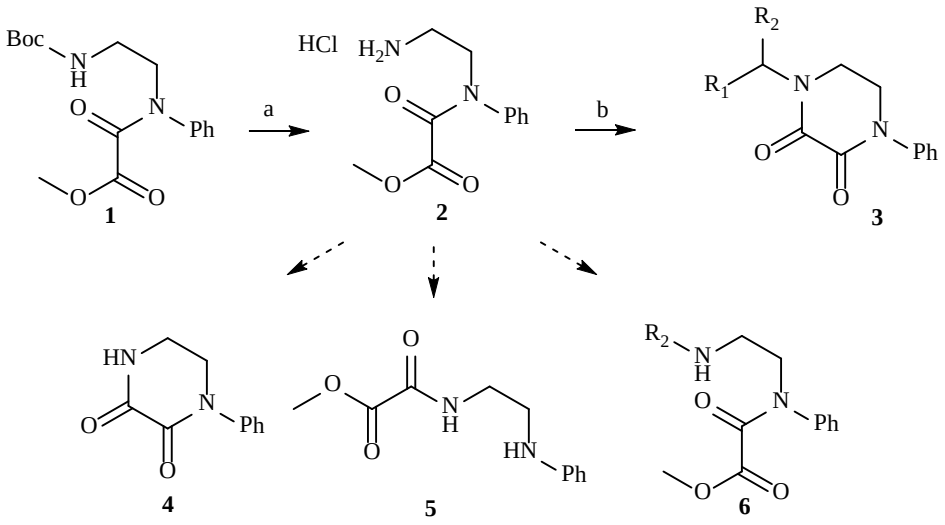


Figure 2. Chemical structure of piperacillin.

There are many ways to synthesize 2,3–diketopiperazines and most important are the ones discussed below.

1.1.1. Tandem reductive alkylation – cyclization reaction

In order to synthesize 2,3–DKPs an intramolecular cyclization reaction was used. This strategy was developed by Dinsmore and Bergman in 1998.[18] As shown in Scheme 3, the mechanism involves the transformation of protected N-(2-aminoethyl) oxamates to stable amine hydrochlorides by HCl in ethylacetate. The next step in this reaction is a reductive amination and cyclization, which results in 1, 4, - disubstituted 2,3–DKPs. For this step sodium triacetoxyborohydride and molecular sieves were used to produce the compound 3.



Scheme 3, Reductive alkylation - cyclization reaction; reagents: (a) HCl, EtOAc, 0°C; (b) R_1COR_2, $Na(OAc)_3BH$, $ClCH_2$-CH_2Cl, 4Å molecular sieves, 0°C to rt, ca. 10 h.

As it is shown in Scheme 3, there are some byproducts in this reaction like 4, 5 and 6, which have arisen through cyclization, acyl transfer or bisalkylation. The yields of the reaction with substituted benzaldehydes were sufficiently high, starting from 61% and higher. The reactions with less hindered aldehydes gave the result of bis - alkylation over reductive amination – cyclization and byproduct 6. The study was continued by using branched aldehydes, which again gave yields over 60%. The results with ketones were unfavorable, the products of cyclization 4 and acyl transfer 5 were formed. Though the cyclic ketones led to high yields.

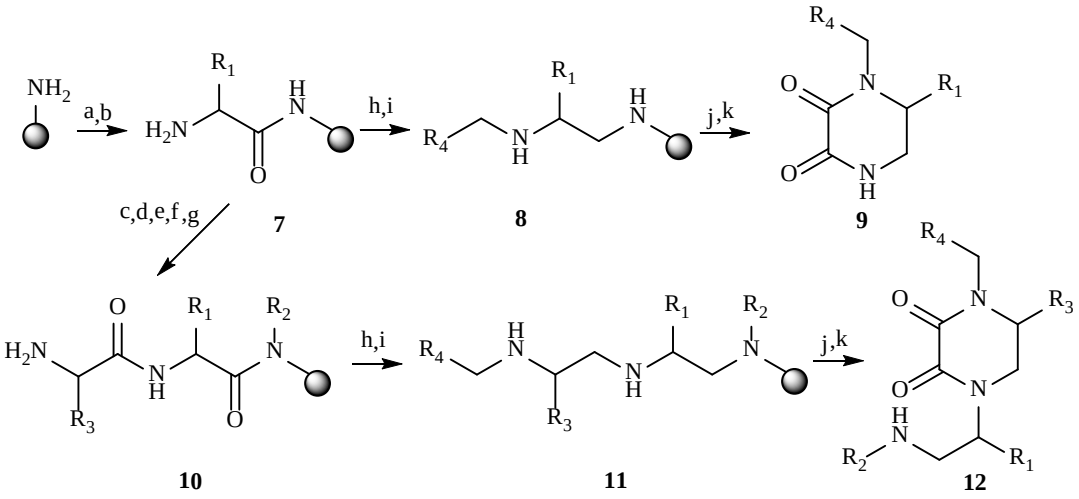
So this suggest that tandem reductive alkylation – cyclization reaction for the preparation of unsymmetrical 1,4,-disubstituted-2,3–DKPs gives very nice outcome by using aromatic aldehydes, branched aliphatic aldehydes and cyclic ketones.

In the literature intramolecular cyclization synthesis reactions for obtaining 2,3–DKPs also can be found. Those methods were reported by Lewis et al.,[19] and Polniaszek and Bell.[20]

1.1.2. Solid phase synthesis from reduced polyamides

This was the first approach to make 2,3–DKPs using solid phase synthesis reported by Houghten et al.[21] Before that the solid phase synthesis was widely used in order to produce 2,5–DKPs. A *p*–methylbenzhydrylamine (MBHA) resin bound acylated

amino acid 7 is introduced as starting material. The amides 7 and 10 were reduced to amines 8 and 11 by borane in THF. It was continued with an impact of the 1, 1 - oxalyldiimidazole to produce a bis–acylated product 1,6–disubstituted–2,3–DKP 9 as well as 1,4,5–trisubstituted–2,3–DKP 12 as final compound after cleavage of the resin by treatment with HF/anisole (Scheme 4).



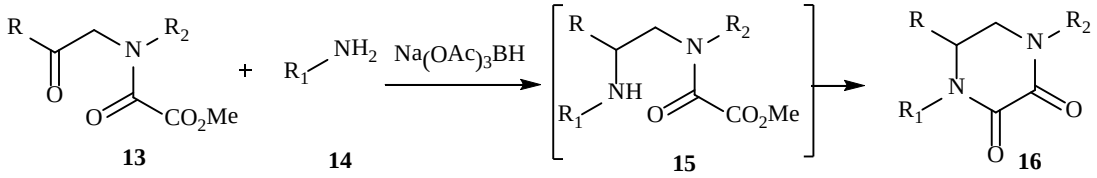
Scheme 4. Solid phase synthesis of 1,6-disubstituted 2,3-DKPs and 1,4,5-trisubstituted 2,3-DKPs from resin-bound polyamines; reagents: (a) Fmoc-Xaa-OH, DIPCDI, HOBt, DMF; (b) 20% piperidine and DMF; (c) Trt-Cl, DIPEA, DCM; (d) R_2–X, BuOLi, DMSO; (e) 2% TFA in DCM, DIPEA/DCM; (f) Fmoc–Xaa–OH, DIPCDI, DMF; (g) 20% piperidine and DMF; (h) R_4–COOH, DIPCDI, DMF; (i) BH_3, THF, 65°C; (j) Oxalyldiimidazole, DMF; (k) HF, anisole.

After purification of the compounds 9 and 12 the yields of this reaction were around 75%. Many different kinds of amino acids were investigated together with alkylating reagents and carboxylic acids that were used for this reaction. Using this method 24 compounds were synthesized in high yields and high purities.

1.1.3. Tandem reductive amination – cyclization

This approach can be compared with the one mentioned in section 2. 1. 1., but instead of reductive alkylation–cyclization, reductive amination-cyclization was used, which was also reported by the Beshore and Dinsmore[22] in 2000. Reductive amination conditions are used in order to couple the starting material 13 with a primary amine and

first give and intermediate 15, which converts to the main product of the reaction; 1,4,5-trisubstituted–2,3–DKP 16 (Scheme 5).



Scheme 5. One pot reductive amination – cyclization reaction.

The reactions with aliphatic amines resulted in moderate to good yields of 2,3–DKP. The rather unhindered amines gave yields of 64 – 75%, meanwhile, when more branched amines gave an efficient result as their yields were 76 – 88%. The reactions with aromatic amines also succeed and gave rather good yields 71 – 80%, while electron withdrawing and sterically hindered anilines did not cyclize without heating.

1.2. 2, 6–DKPs

2,6–DKP is another isomer of DKPs. In contrast to 2,3 and 2,5 DKPs, there was not that much of attention paid to 2,6–DKPs. Though, over the years some useful compounds that belong to the 2,6–DKPs library have been discovered, like further discussed Flutimide,[23] which has similar framework DKP and plays an inhibitor role of influenza virus (Figure 3).

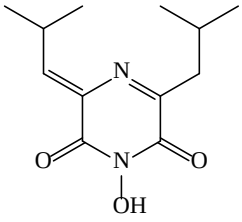
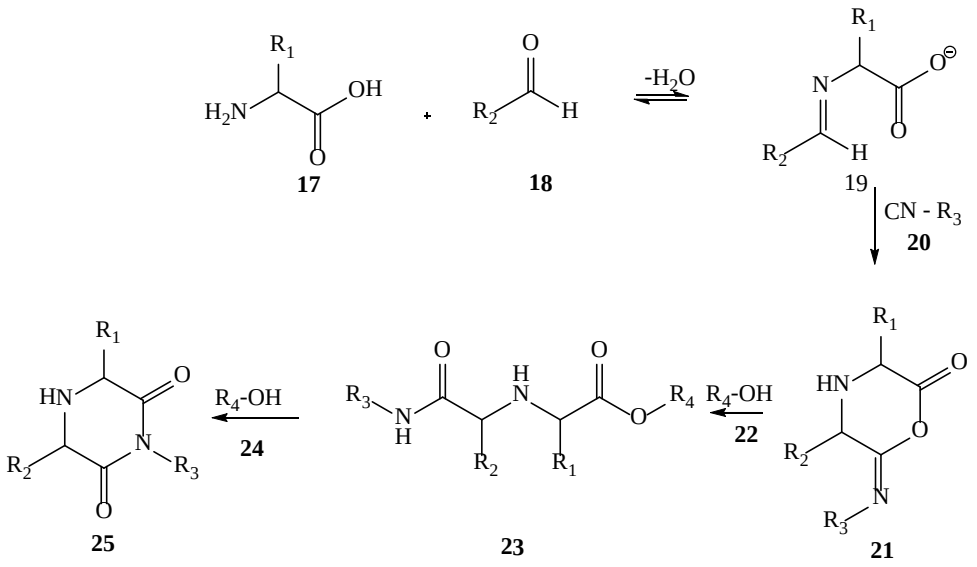


Figure 3. Chemical structure of Flutimide.

1.2.1. Ugi five – center – four – component reaction.

A one–pot multicomponent reaction (MCR) is a very effective way for the preparation of DKPs. For the synthesis of 2,6–DKPs Ugi five–center–four–component reaction[24] was used. It is a simple procedure, which gives high yields in addition to excellent selectivity. As is shown in Scheme 6, first of all, the amino acid 17 reacts with

an aldehyde 18 and after loss of water there is an imine formed, which after treatment with cyanide 20 is converted into an O–acylamide 21. By nucleophilic attack of solvent (alcohol 22) the amino ester 23 is formed. After solvent removal and refluxing in THF in a presence of base, trisubstituted 2,6–DKP was achieved.



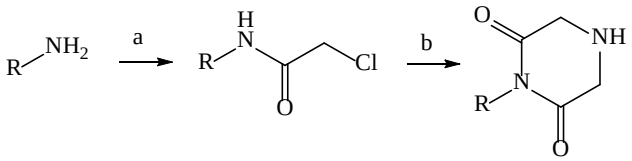
Scheme 6. Ugi five–center–four–component reaction.

This reaction results in very high yields (usually around 95%) with most trifunctional α–amino acids, except lysine, glutamic and aspartic acids, where side chains participated in the MCR reaction or acted as nucleophiles instead of alcohol 22.

It is also possible to synthesize 2,6–DKPs using solid phase synthesis methods as earlier described as one of the synthetic ways for making 2,3–DKPs. This way of synthesis of DKPs from amino acids using solid phase or solution phase was reported by Altamura et al.[25]

1.2.2. Tandem reaction forming N_1-C_2/N_4–C_5 bonds

This is a practical method for preparation of 2,6–DKPs, which involves the formation of N_1 - C_2 and N_4 – C_5 bonds in succession. This synthetic pathway was reported by Abdel – Hamide et al. [26] in 1997. The method was used for synthesis of new antimicrobial agents.

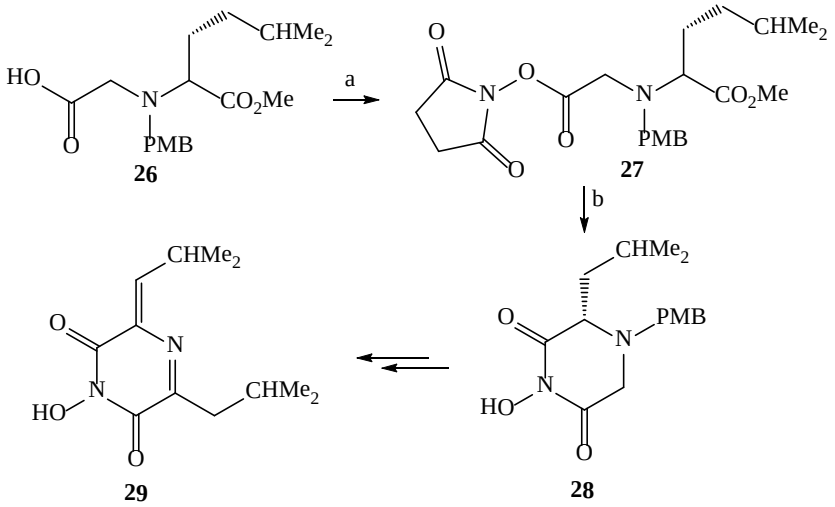


Scheme 7. Tandem reaction forming N_1- C_2/N_4–C_5 bonds via formation of chloraceamide; reagents: (a) $ClCOCH_2Cl$, DMF; (b) $EtO_2CH_2NH_2.HCl$, C_5H_5N, Δ, 8h.

As it can be seen from a Scheme 7, the first step of this reaction is acylation using chloroacetyl chloride, which gives amide from amine and affords good yields of the reaction. The result of reaction with ethylglycinate leads to 2,6–DKP as a product in moderate yields.

1.2.3. Tandem multiple bond formation

One of the most common reactions in synthesizing 2,6–DKPs is via multiple bond formation by forming N_1–C_2 and N_1–C_6 of the imides. This simultaneous method was used in a report by Singh et al. [23] and deals with the synthesis of Flutimide, which in Scheme 8 is the final product. The compound of interest is 2,6–DKP (28 in Scheme 8).



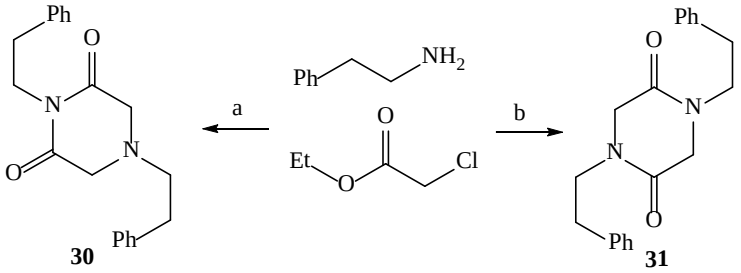
Scheme 8. Tandem N_1–C_2 and N_1–C_6 bond formation; reagents: (a) N–OH–succinimide, DCC, Et_3N, DCM; (b) NH_2OH•HCl, NaOH, H_2O/EtOH; 80 -100°C.

During the first step hydroxamide is activated with N–OH–succinimide and gives an active ester 27. The second step of the reaction gives a 2,6–DKP by treatment with

hydroxylamine and heat. In this case 80% yield was achieved. The reaction was successful and the 2,6–DKP was used as an intermediate product.

1.2. 4. Concurrent 4 C–N bonds formation method for synthesizing the 2,6-DKPs

The more attractive method of all previously mentioned ones would be a concurrent 4 C-N bonds formation in a single reaction. Unfortunately this reaction pattern have not been investigated and developed so far as it could be. In the literature there are only symmetrical 2,6–DKP examples according to this method. In 1968 Basu et al.[27] published a reaction in which phenethylamine and ethyl chloroacetoacetate were heated under solvent free conditions (see Scheme 9).



Scheme 9. 4C-N bonds formation via chloracetamide; reagents: (a) 170 - 175°C, 3h; (b) 195 – 200°C 4h.

The outcome of this reaction depended on a heating. When the sample was heated to 170-175°C for 3 hours 2,6–DKP was obtained as the main product, giving 97% yield. After heating to 195-200°C and prolonging the reaction time to 4 hours 2,5–DKP was produced as a major product (25%) and 2, 6–DKP (4%) as a minor one.

1.3 2,5–DKPs

The most widely investigated and most significant group of the three isomers are 2,5–DKPs. As was mentioned before, 2,5–DKPs exhibit antiviral,[16] antibacterial,[10] antifungal[6] and other properties (Figure 4). Compound on a left was observed to have a use as antiviral agent against vesicular stomatitis virus, coxsackie virus and respiratory

syncytial virus. Compound in the middle of a Figure 4 showed antimicrobial properties against mycobacterium tuberculosis H37Ra and compound on the right proved to exhibit antifungal features.

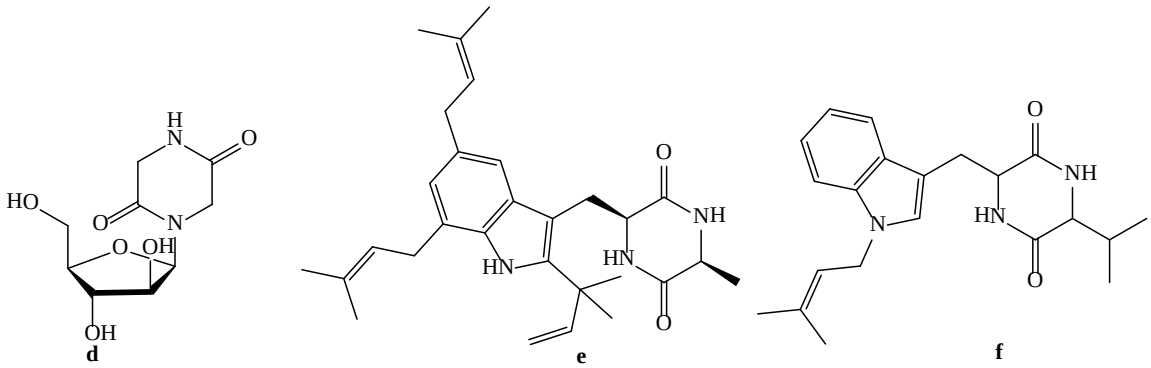
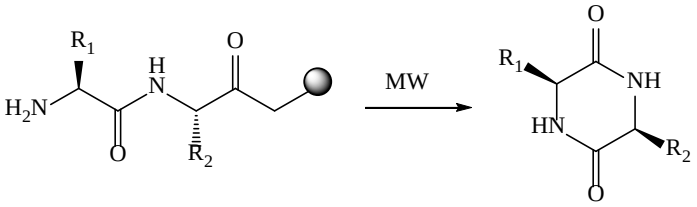


Figure 4. Chemical structure of compounds that exhibit (d), antiviral (e) antibacterial (f) antifungal properties.

1.3.1. Microwave – assisted solid – phase synthesis

Several methods have been reported using solid phase synthesis of 2,5–DKPs. Most of the research was based on specific amino acids, Bianco et al. [28] reported the synthesis of DKP containing hydroxyproline derivatives and Papini et al. [29] about the cyclization of histidine containing peptides on solid phase. Grøtli et al.[30] reported in 2006 a wide investigation about microwave assisted solid phase synthesis of 2,5–DKPs using various combinations of resins and solvents.



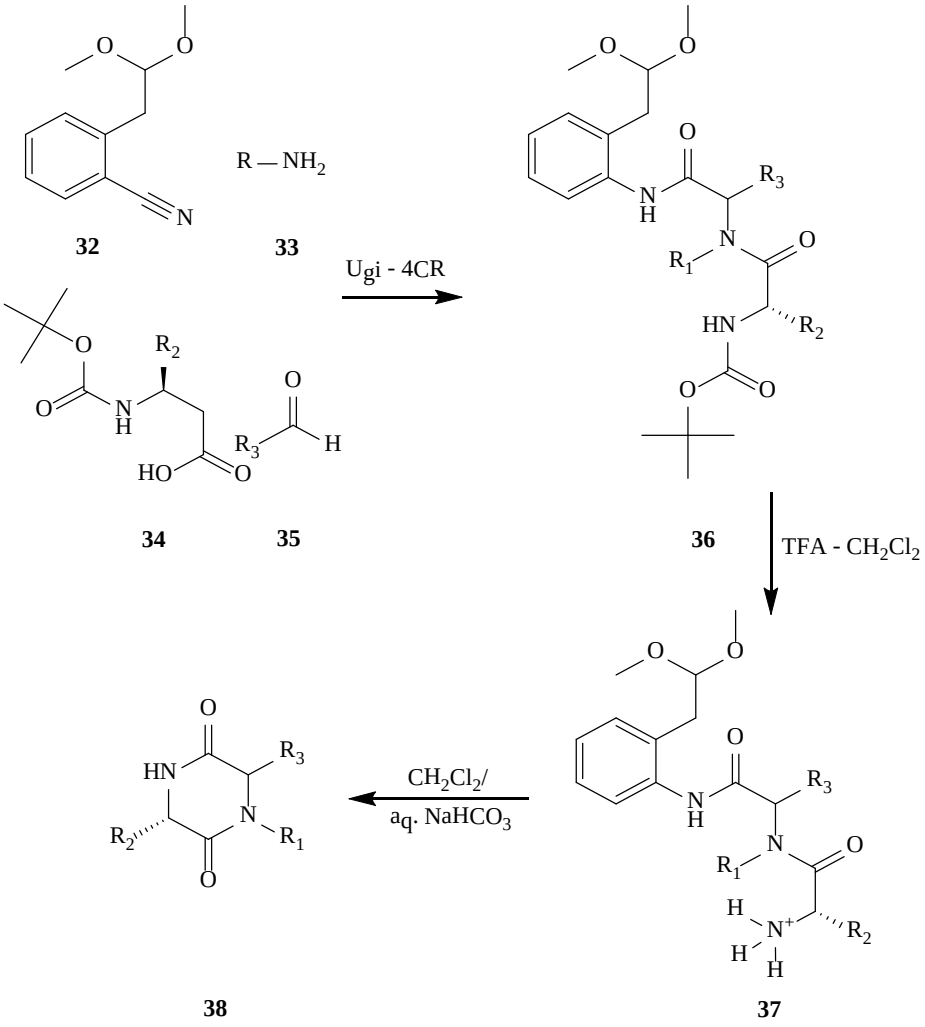
Scheme 10. Microwave assisted solid phase cyclization resulting in 2,5-DKP formation.

By employing the solid phase synthesis method for the preparation of 2,5–DKPs some factors need attention. One of them is the conformations of the amino acids. As can be seen in Scheme 10 the most efficient way is to use a combination of D and L amino acids, because of the minimal steric interference of side chains during the cyclization step. Also, it is very important to choose the right peptide – resin linkage. When

cyclization was done in water, the best choice was PEGA – Ser resin, while using organic solvents the results were similar with ArgoGel – MB OH, PS – Ser and TentaGel S Ac resins. Taking into consideration all the listed factors, there were achieved very high yields for microwave assisted solid phase synthesis of 2,5–DKPs.

1.3.2. Rapid synthesis of N-substituted DKPs by one pot Ugi – 4CR/Deprotection + Activation/Cyclization (UDAC)

One reaction type, that has been mentioned before, is the multicomponent Ugi – 4CR/deprotection + activation/cyclization reaction. This method for synthesis of DKPs was published by Wessjohann et al. [31] in 2009. It describes a simple procedure which leads to surprisingly high yields of the reaction products and short reaction times with no heating demand.



Scheme 11. Synthesis of trisubstituted DKPs via Ugi–4CR/deprotection + activation/cyclization (UDAC) method.

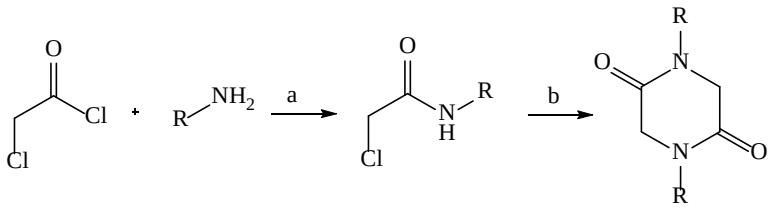
It is a unique method in that it combined two reactions, i.e. Ugi–4CR/ deprotection/cyclization and Ugi–4CR/activaction/cyclization. Here, as can be seen in Scheme 11, the deprotection comes together with the activation of the electrophile.

This one pot reaction was used with different kinds of protected amino acids, primary amines and aldehydes. It is continued by removal of the N-terminal Boc protecting group under acidic conditions using TFA-CH_2Cl_2. After the removal of the protecting group, the final cyclization is reached by adding a base into the solution. For those reactions biphasic mixture containing CH_2Cl_2/aqueous $NaHCO_3$ was used, which was employed in order to keep the product in the organic phase.

In general this method for making DKPs is fast, works under mild conditions and gives moderate to high yields.

1.3.3. One pot synthesis of symmetrical 1, 4- disubstituted piperazine-2, 5-diones

This method was published by Su-Dong Cho et al. [32] in 2003. The first step of this reaction was already mentioned in 1992 by Sandri et al.[33]



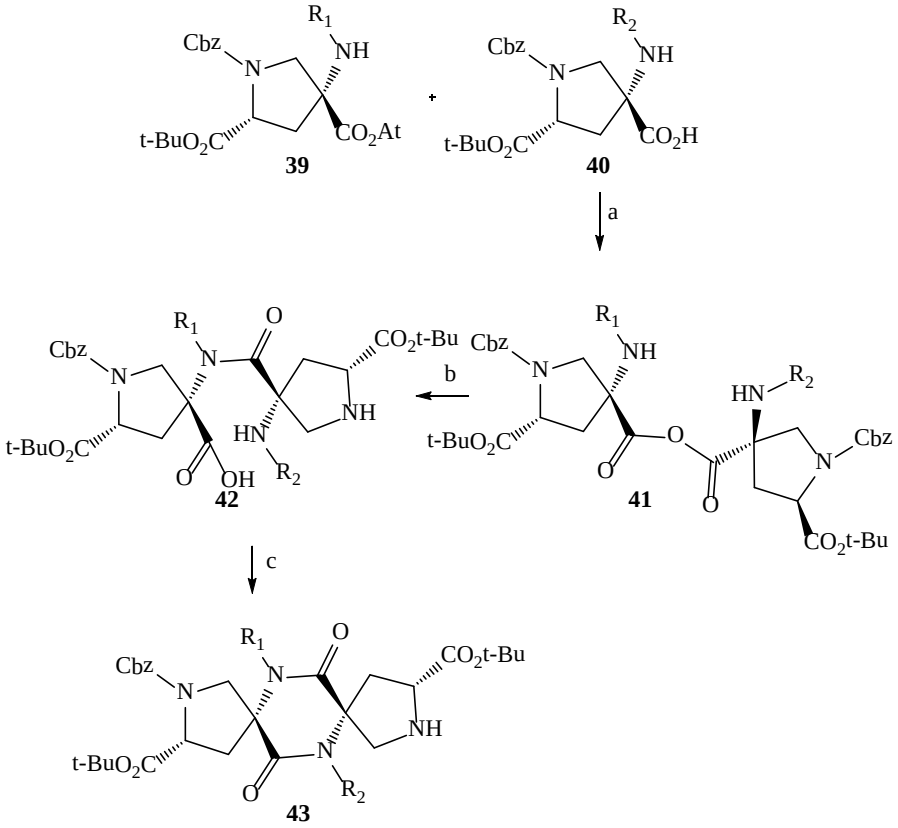
Scheme 12. Cyclization via Chloracetamide; reagents (a) K_2CO_3, CH_2Cl_2; (b) NaOH, CH_3CN, 82°C.

The first step of this reaction is the preparation of chloroacetamide of specific amine. It was done by mixing 2-chloroacetyl chloride with the appropriate amine in CH_2Cl_2 with addition of a base. The second step is the cyclization between two molecules of chloracetoamide initiated by strong base in CH_3CN with heating (see Scheme 12). The yields of the reactions were varying from moderate to good depending on base and substituents on nitrogen. For example, cyclization reaction using alkyl, cycloalkyl, heterocyclic groups together with benzylic groups were giving good yields of the reactions. Though cyclization reactions with sterically hindered aromatic substituents worked very well, electron withdrawing groups on the aromatic ring gave negative results.

Combining the results of the first and second step of this reaction suggests that this reaction is giving very high yields and is extremely fast for synthesizing symmetrical DKPs.

1.3.4. Synthesis of hexa- and pentasubstituted DKPs from sterically hindered amino acids

Synthesis of hexa and pentasubstituted DKPs can sound like a big challenge, but according to Brown and Schafmeister[34] it was possible. In the same year it was also published a solid – phase synthesis method for making hexa substituted DKP by the group of Brown, Alleva and Schafmeister et al.[35] (Scheme 13).



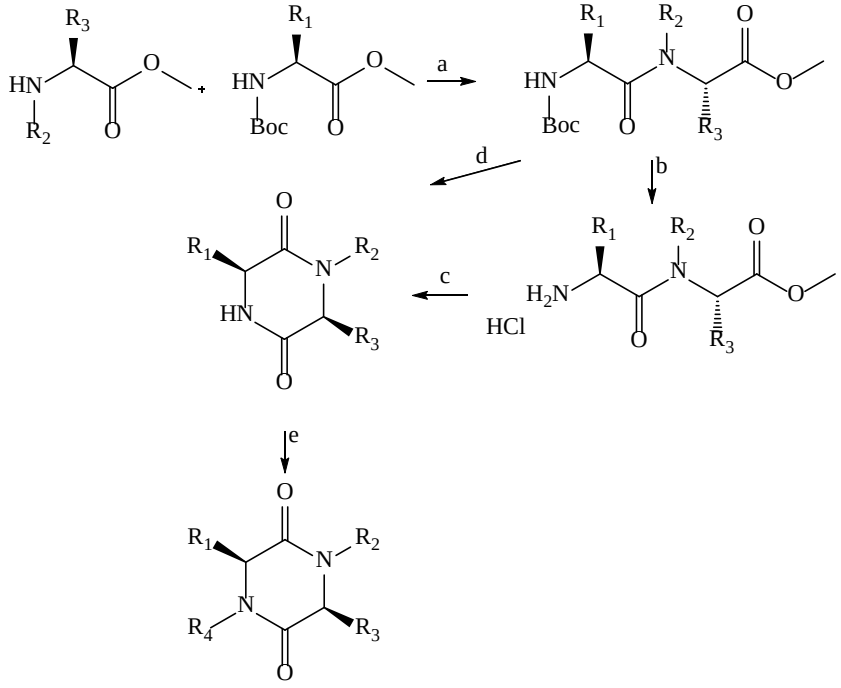
Scheme 13. Synthesis of hexa- and pentasubstituted DKP; reagents: (a) DIPEA, DCM/DMF; (c), DIC/DCM.

This was essentially a very similar reaction, but instead of solution phase solid phase was used. During the first step of the reaction N–alkylamino acid 40 serves as the nucleophile and couples with N–alkyl amino–OAt ester 39 forming an anhydride that spontaneously rearranges through an acyl transfer. 2,5-hexa substituted–DKPs are formed after dehydration assisted by the addition of a dehydrating agent N,N'-

Diisopropylcarbodiimide (DIC). In order for the cyclization to take place, it is very important that amide 42 must be in the cis conformation.

1.3.5. Synthesis of functionalized, unsymmetrical 1,3,4,6 – tetrasubstituted 2,5–DKPs

This synthesis method allows a synthesis of tetrasubstituted DKPs involving the cyclization of N–alkylated dipeptides. It was published by Luthman et al. [36] in 2007.



Scheme 14. Synthesis of functionalized, unsymmetrical 1,3,4,6–tetrasubstituted-2,5–DKPs via base – catalyzed dipeptide ester cyclization. Reagents: (a) EDC/NMM, CH_2Cl_2; (b) HCl (g)/CH_3OH; (c) H_2O, 200°C, 10min. (MW) microwave heating; (d) Et_3N, H_2O, 140°C, 10min., MW heating; (e) BEMP, R_4-Br, CH_2Cl_2 for 24h at rt or DMF for 30min. at 60°C using MW heating.

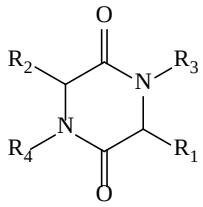
From Scheme 14 the main coupling steps of this reaction can be seen. The first one is the condensation reaction between N–alkylated amino ester and N–protected amino acid, which can be converted to DKP through 2 steps (deprotection and cyclization) or 1 step (deprotection/cyclization) under microwave heating. In order to alkylate the nitrogen and produce the tetrasubstituted DKP, a strong base 2-*tert*-

Butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP) was used.

The yields are varying depending on substituents on the ring DKPs. More bulky or aromatic substituents gave moderate yields, while linear and branched alkyl groups gave rather good results.

2. The aim of the project

The goal of this project was to synthesize 1,3,4,6–tetrasubstituted-2,5-DKPs using different kinds of amino acids with the help of microwave radiation. In a Figure 5 is shown the structure of the 2,5-DKPs. The challenge of this project was the introduction of hydrophilic groups into the DKP scaffold, because of the additional reactive side groups. Though, the introduction of hydrophilic groups is important, because it increases a water solubility and activity of a molecule, which allows us to produce potential therapeutic compounds.



R_1 and R_2 – hydrophobic groups

R_3 and R_4 – hydrophilic groups

Figure 5. General structure of 1,3,4,6–tetrasubstituted-2,5-DKPs.

To be investigated if substituents R_4 and R_3 could be introduced on amino acid methyl ester hydrochlorides, Boc protected amino acids, dipeptides by using alkylation reactions or reductive amination.

3. Results and discussion

3.1. Preparation of 2,5–disubstituted DKPs using a microwave assisted heating

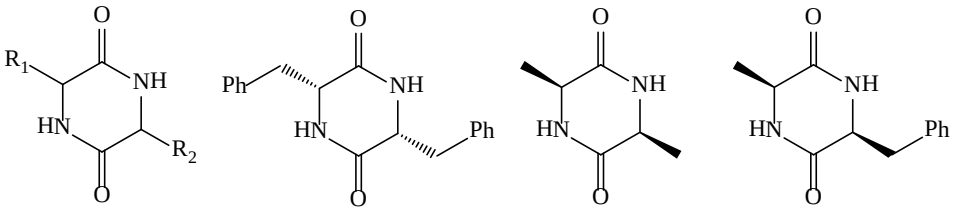


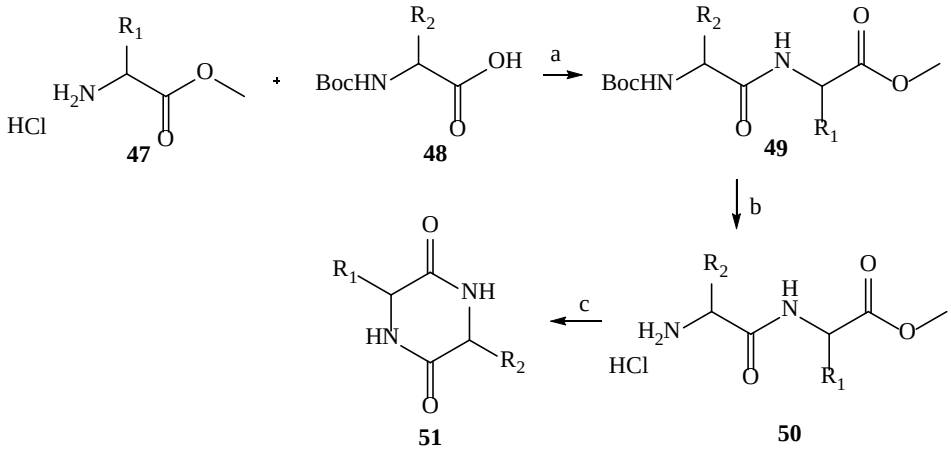
Figure 6. General structure of DKP and examples. (44) (3R,6R)-3,6-dibenzylpiperazine-2,5-dione; (45) (3S,6S)-3,6-dimethylpiperazine-2,5-dione; (46) (3S,6S)-3-benzyl-6-methylpiperazine-2,5-dione.

All of the produced disubstituted DKPs already have been synthesized before. A selection of naturally occurring and synthetic amino acids was used, which included L and D phenylalanine together with L and D alanine in various combinations. R_1 and R_2 substituents thus depended on the amino acid employed. (Table 1)

Table 1. Synthesized disubstituted-2,5-DKPs.

R_1	R_2
D-Phe	D-Phe
L-Phe	L-Phe
D-Phe	L-Phe
L-Ala	L-Ala
L-Ala	L-Phe

In order to synthesize 2,5-disubstituted DKP a known synthetic pathway was chosen. (Scheme 15)



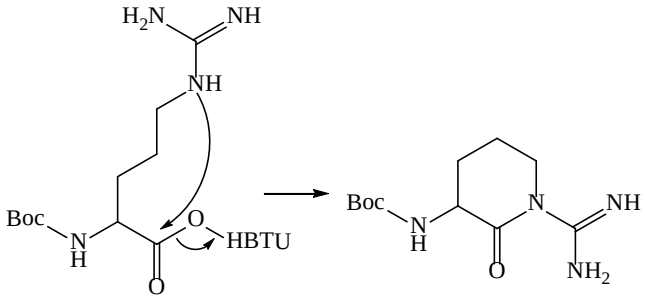
Scheme 15. Synthesis of disubstituted 2,5 DKP via base catalyzed dipeptide ester cyclization; Reagents: (a) HBTU, DIPEA, DMF overnight at rt; (b) HCl, THF 1h; (c) Et_3N, H_2O under microwave heating 140°C, 10 min.

This synthetic procedure consisted of three steps. First, a coupling of an N–Boc–protected acid with amino acid methyl ester hydrochloride to form a dipeptide methyl ester. In this reaction two coupling reagents EDC/NMM and HBTU were tested. The coupling with EDC/NMM as it was described in the literature [37, 38] did not give the planned result, though the results with HBTU were satisfying and gave 85% yields. For purification of compound 3 liquid/liquid extraction with citric acid (10%) and sodium bicarbonate aqueous solution was used. The protecting group removal was carried out smoothly using HCl/THF and gave a dipeptide methyl ester hydrochloride salt, which in the last step of the reaction in the presence of water as solvent and triethylamine as base using microwave heating gave 2,5–DKP as final compound with yields of 25-50%. The yields depended on amino acid configuration and size of substituents. The best result was achieved for compound 45, where for the starting material were used L and L alanine, which does not cause steric hindrance in a molecule. Because of the steric hindrance in the molecule, compound 44 was synthesized in lowest yield. This was the reaction that

needed to be tested before synthesizing 1,3,4,6–tetrasubstituted–2,5–DKP. This synthesis worked well on disubtituted DKPs so it could be used for more substituted ones. The compounds synthesized are not included the experimental part as they were earlier published, but spectral data were in accordance. [39-42]

3.2 Attempts to synthesize arginine containing dipeptides

The previous mentioned conditions for coupling Boc amino acid with amino acid methyl ester hydrochlorides were applied for coupling the arginine. As is known guanidino side chain can function as a nucleophile[43] and as long as there was no additional protection on it the reaction did not lead to a successful result. There were several attempts to produce diarginine dipeptide and phenylalanine and arginine containing dipeptide.



Scheme 16. Possible mechanism for the self-cyclization of N-boc arginine.

It is likely, that the addition of base, in our case it was used DIPEA, led to the self-cyclization[44] (see Scheme 16) of deprotonated amine group on the unprotected side chain on the carbonyl group. It was an undesirable reaction as it prevented arginine to couple to the unprotected N terminus of the amino acid methyl ester hydrochloride. In order to prevent it, the additional protection by a nitro group on the side chain could be used.

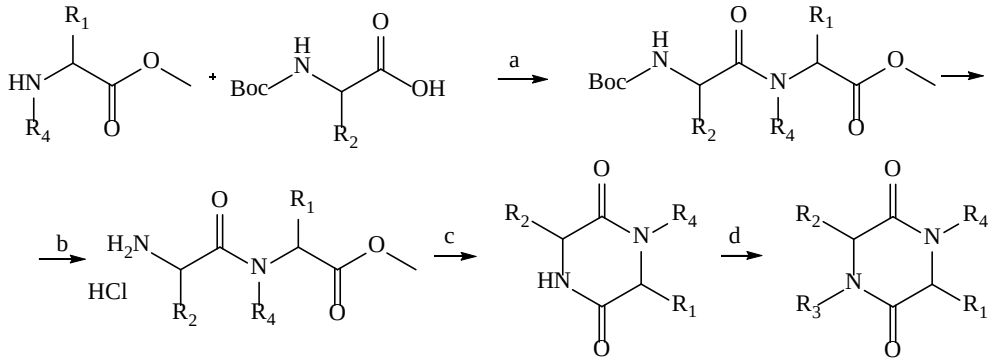
3.3. Attempts towards synthesis of 1,3,4,6–tetrasubstituted-2,5-DKPs

For the synthesis of tetrasubstituted-2,5-DKPs three possible pathways were studied. They are described more widely in a further section. The idea of the reaction was

to cyclize N–alkylated dipeptide and to introduce the last substituent via N–alkylation to get the final compound 1,3,4,6–tetrasubstituted–2,5–DKP using microwave irradiation. In all cases there were encountered some problems in the first steps of the reaction.

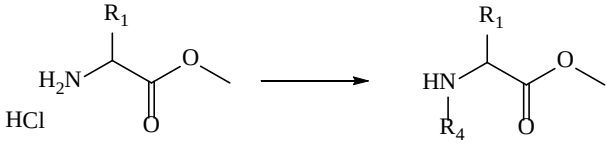
3.3.1. Alkylation of amino acid methyl ester hydrochloride

This was the first and the most common method that was tested in order to produce DKP (see Scheme 17).



Scheme 17. Synthesis of tetrasubstituted-2,5-DKP via base catalyzed dipeptide ester cyclization; Reagents: (a) HBTU, DIPEA, DMF; (b) HCl/THF; (c) Et_3N, H_2O, MW heating 10 min., 140°C; (d) BEMP, R_3-Br, MW heating 30min. at 60°C.

Nonetheless, it seemed to be an easy way to carry out this reaction, but a problem occurred in making the starting compound N–alkylated amino ester.

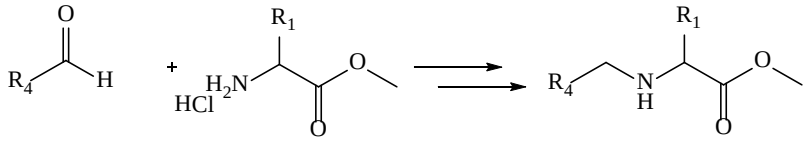


Scheme 18. N-alkylation of amino acid methyl ester hydrochloride.

For that reason, the N-alkylation reaction was tested with NaHMDS[45], Cs_2CO_3[46], K_2CO_3, Et_3N[47], KOH, NaH[48] as bases (Scheme 18). In no case did it lead to the desired result. In most cases the result was dialkylated amino ester, though, using such a strong base as NaH it led, as ^1H NMR showed, to a doubly alkylated amino acid. Though mono alkylation was possible using benzylbromide as an alkylating agent, but this did not give the final product, because the alkylated amino ester hydrochloride was

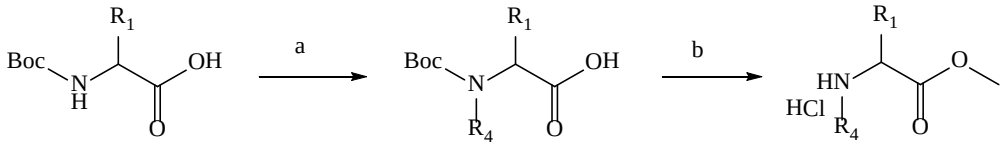
too hindered to couple to Boc protected amino acid. As was mentioned before, for the alkylation reaction Cs_2CO_3 was used, which first seemed to give a mono alkylated amino acid methyl ester. From the ^1H NMR spectra a mono alkylated amino acid methyl ester could be seen, but after the results of MS and ^{13}C NMR spectra it could be seen that the reaction product was a carbamate. This was also confirmed by IR spectra. The reaction was investigated more extensively and is more described in chapter 3.4.

For synthesizing the starting material other methods were tried, like reductive amination (see Scheme 19). It was carried out by using aldehyde and primary amine to produce and imine and was continued with imine reduction by using $NaBH(OAc)_3$[49] or $NaBH_4$[50] as a reducing agent. This did not lead to a mono N - alkylated compound and MS analysis indicated that dialkylation of amino acid methyl ester had occurred. The reason for it is not clear.



Scheme 19. Reductive amination of amino acid methyl ester.

In addition to all attempts to synthesize N–monoalkylated amino ester, one more way to synthesize it was used through Boc–N–alkylated amino acid, which after esterification and deprotection did not give the wanted N–alkylated amino ester, but N–alkylated amino acid (Scheme 20).

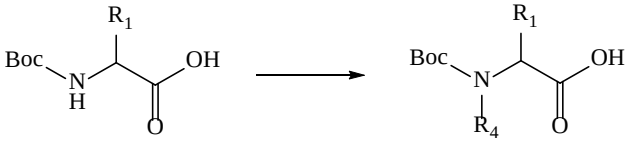


Scheme 20. Synthesis of N-alkylated amino acid methyl ester; reagents (a) Cs_2CO_3, R_4-Br, DMF; (b) Dry MeOH and $SOCl_2$.

This was because of water, that was left after alkylation of Boc amino acid and it was difficult to remove it by drying it with brine, $MgSO_4$, under vacuum or even using azeotropic water removal with benzene as a drying agent. It is likely that ester has formed, but due to the large amount of water it has been hydrolyzed and the acid was the final product.

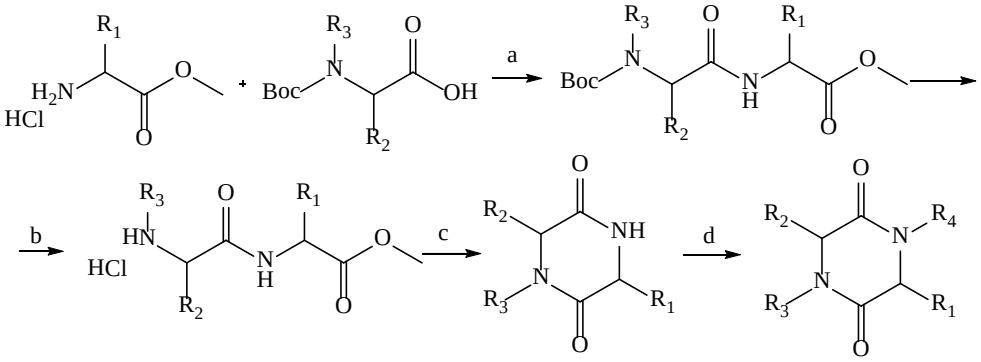
3.3.2. Coupling of N–alkylated Boc–amino acid with amino acid methyl ester hydrochloride

This was one more possible method for synthesis of DKPs. It was easy to alkylate Boc – amino acid using Cs_2CO_3 as base, only monoalkylation was possible because of the protecting group (Scheme 21). Reactions were tested by using different R_4 groups (ethyl, isopropyl, t - butyl) together with different Boc protected amino acids (phenyl alanine, alanine, valine). Reactions were successful and yielded 85-95 %, depending on the size of substituents and steric hindrance of the reaction product.



Scheme 21. Monoalkylation of Boc protected amino acid.

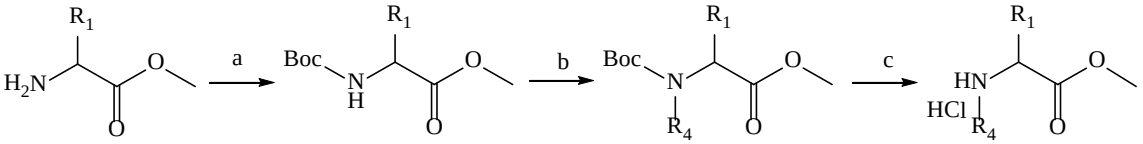
Even though the starting material was synthesized successfully, the coupling reaction of N-alkylated Boc protected amino acid with amino acid methyl ester hydrochloride was not achieved (shown in a Scheme 22). It might be because of the change of a conformation of the Boc–alkylated amino acid or when the alkylation appeared it might be difficult for NH_2 to attack at the right angle because of steric reasons.



Scheme 22. Proposed DKP synthesis via coupling of Boc N-alkylated amino acid with amino acid methyl ester hydrochloride and base catalyzed cyclization; reagents: (a) HBTU, DIPEA, DMF; (b) HCl/THF; (c) Et_3N, H_2O, MW heating 10min., 140°C; (d) BEMP, R_4-Br, MW heating 30 min. at 60°C.

3.3.3. Alkylation of Boc–amino acid methyl ester

The last proposed synthesis was to protect the amino ester hydrochloride using Boc, and then try the N–mono alkylation reaction (see Scheme 23).

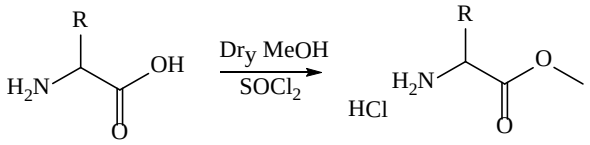


Scheme 23. Reagents (a) $(Boc)_2O$, $NaHCO_3$, Dioxane; (b) Cs_2CO_3, R_4 - Br, DMF; (c) HCl/THF.

After this step it was planned to continue with the deprotection of Boc alkylated amino ester and coupling reaction with a Boc amino acid. Though, the alkylation process of Boc amino ester caused difficulties. The alkylated Boc amino ester was only a minor product of the reaction. The MS analysis of the N alkylated Boc protected amino acid methyl ester showed that there was starting material Boc protected amino acid methyl ester, and N–alkylated Boc protected amino acid methyl ester, just the last one was the minor, and the starting material was the major product of the reaction. This method could be used after optimization of the reaction conditions that would allow getting higher yields of the reaction product. Due to the lack of time, because this was the last attempt to synthesize N–alkylated amino acid ester hydrochlorides, this was not managed to be done.

3.4. Synthesis of the starting material

In order to synthesize methyl esters as a starting material, simple and well known procedures were used (Scheme 24). According to which, dry methanol was cooled in an ice bath and thionyl chloride was introduced slowly, over 5 minute period. Then the amino acid was added to the solution. After dissolving by heating, tert–butyl methyl ether was added in a solution, which thereafter crystalize straight away in most of the cases. In our case it was used for making phenylalanine, valine and alanine methyl esters hydrochlorides. The reaction gave extremely pure compounds in high yield (85-95%), which were the starting materials for the synthesis of DKPs.

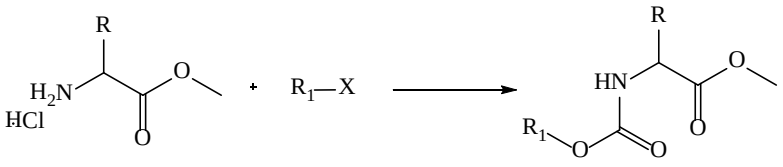


Scheme 24. Synthesis of amino acid methyl ester hydrochloride.

3.5. One–pot synthesis of carbamates

As it was mentioned before in a chapter 3.2.1., a new carbamation reaction during this project was discovered by attempting to alkylate amino acid methyl esters hydrochlorides. It was proved in our study that it is possible to synthesize a carbamate by adding Cs_2CO_3 and alkylating agent into a solution of amino acid methyl ester hydrochloride in DMF (see Scheme 25). This was an unexpected result that was discovered when trying to alkylate the amino acid methyl ester hydrochlorides. Similar procedures were used by Kyung Woon Jung et al.[51] in 2001. But indeed, to produce a carbamate Cs_2CO_3 an alkylating agent in addition to CO_2 and tetrabutylammonium iodide was needed.

In our case was used a very simple procedure, which was giving pure compounds with moderate to high yields of the reaction. The most surprising fact in this reaction is the insertion of CO_2 on the skeleton of amino acid methyl ester hydrochloride without an addition of it. This reaction was therefore investigated. Phenylalanine methyl ester hydrochloride, valine methyl ester hydrochloride and threonine methyl ester hydrochloride together with one aromatic amine–anisidine were chosen as a starting material. Also different alkylating agents like bromoethane, isopropyliodide and t–butylbromide were studied. Using this method, 11 different carbamates were produced. In addition to that, two different bases, Cs_2CO_3 and K_2CO_3 were tested to produce the carbamates.

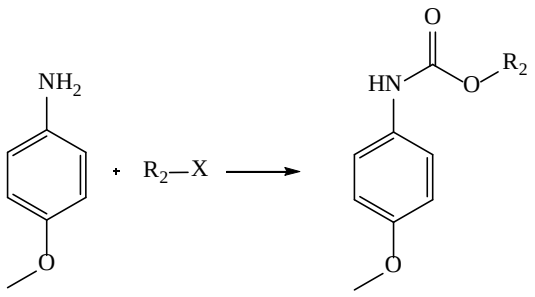


Scheme 25. Synthesis of carbamates via alkylation of amino acid methyl ester hydrochloride; Reagents: Cs_2CO_3 or K_2CO_3 as a base in DMF, overnight stirring at rt.

Some conclusions can be drawn from the results, which are displayed in a Table 2 and Table 3. First of all, in all aspects it was easier to synthesize the ethyl carbamates. It might be because of steric hindrance in a molecule, as changing to isopropyl and t–butyl groups the yields were lower. Also, in most cases Cs_2CO_3 gave better yields in the reactions, though sometimes reactions, in which K_2CO_3 was used as a base, gave products with a better purity. From the data in Table 2 it can be seen that the yields are decreasing with addition of bulkier alkylating agents. For that reason, after purification the water phase was tested and apparently most of the carbamate was staying in it. In order to recover it, the water phase was saturated with sodium chloride and after extraction the yields increased. For example for compound 55a the yield went from 25% to 88%. So it may be that the other compounds yields might be a lot higher by choosing a better extraction procedure. Due to the lack of time this part was left for further investigation.

Table 2. Yields of produced carbamates using Cs_2CO_3 and K_2CO_3 as a base.

Entry	R	R_1	Yield (%) Cs_2CO_3	Yield (%) K_2CO_3
52a	benzyl	ethyl	84	67
52b	benzyl	isopropyl	75	54
52c	benzyl	t–butyl	15	10
53a	isopropyl	ethyl	45	32
53b	isopropyl	isopropyl	34	-
53c	isopropyl	t–butyl	8	-
54a	1-hydroxy ethyl	ethyl	37	32
54b	1-hydroxy ethyl	isopropyl	25	-
55a	methyl indole	ethyl	88	-



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Scheme 26. Synthesis of carbamates via N-alkylation of anisidine; reagents: Cs_2CO_3 or K_2CO_3 as a base in DMF, overnight stired at rt.

Table 3. Yields of produced carbamates using Cs_2CO_3 and K_2CO_3 as a base.

Entry	R_2	Yield (%) Cs_2CO_3	Yield (%) K_2CO_3
56a	ethyl	15	-
56b	isopropyl	-	12

The proof of the reaction product was made according to the ^{13}C NMR, MS, which are displayed in a Figure 7, 8 for a compound 52a. The numbering in the following discussion is used as assigned. The ^{13}C NMR spectrum has one carbonyl peak C7 at 172.6 ppm and the C2 at 157.18 ppm, which proved that the compound consist of 2 carbonyl groups. The aromatic carbons are at 139.9 ppm, 128.80 ppm, 128.03 ppm, 126.41 ppm. The carbons at 60.60 ppm, 55.47 ppm can be assigned to the carbons that are close to the oxygen C9 and C12. The peak at 51.22 ppm belongs to C3, which is close to the nitrogen and the last two peaks at 37.23, 13.46 belong to aliphatic carbons C10 and C6 in that order.

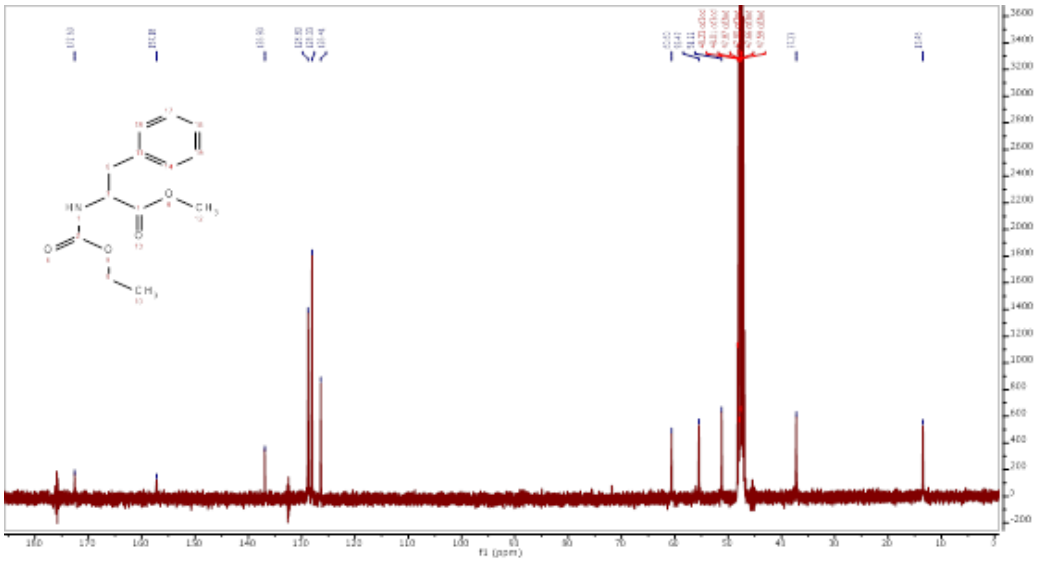


Figure 7. ^{13}C NMR spectra of a compound 52a.

The MS results proved that the product of the reaction is a carbamate.

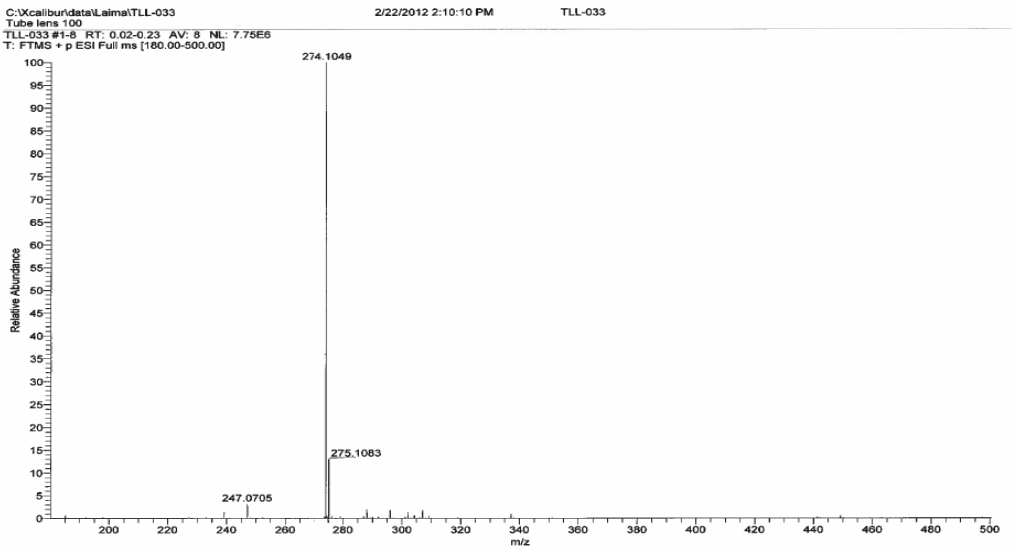
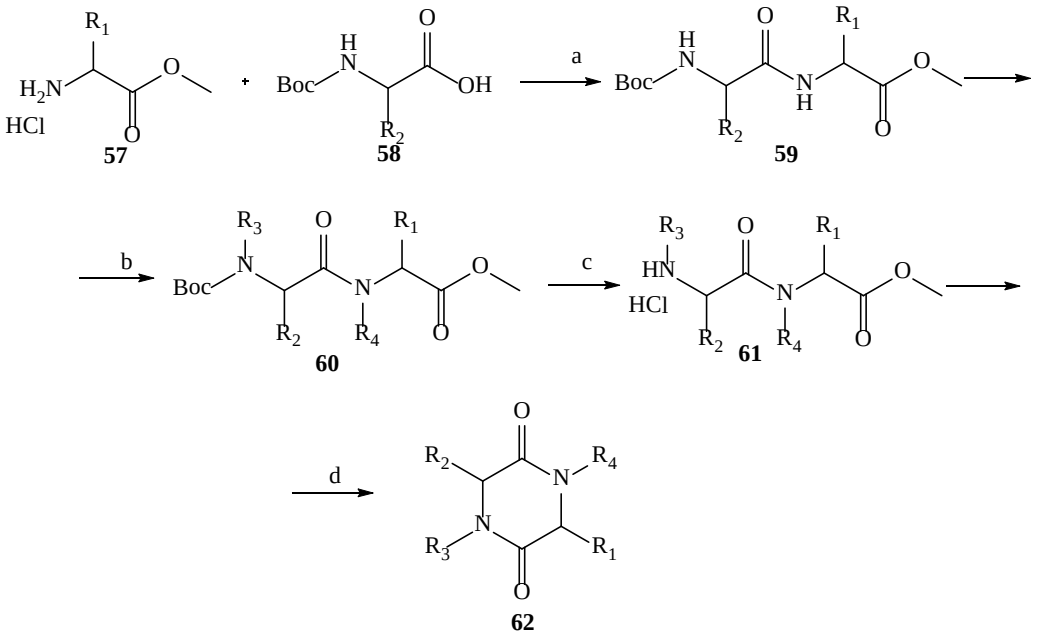


Figure 8. MS spectra of a compound 52a.

4. Future outlook

As a future prospect of this project, I would see the continuation of experiments on attempts to introduce substituents into different peptide precursors, e.g. the alkylation or reductive amination of Boc protected dipeptide.



Scheme 27. Proposed synthesis method for the synthesis of tetrasubstituted-2,5-DKP.

As it was difficult to find a good procedure for introduction of substituents on nitrogen either on amino acids, amino acid methylester hydrochlorides or dipeptides it was not done too much on testing the coupling reagent, only EDC and HBTU were tested. As well the conditions for cyclizing the dipeptides to DKP can also be improved by changing the reaction time, solvents and variation of the substituents. It would be interesting to investigate the possible cyclization of compound 61, which has 4 substituents, two of them are on a nitrogen.

In the chapter 3.2 was discussed the coupling reaction of arginine. My proposed synthesis way for that reaction would be the additional orthogonal protection on the side chain, which would let to produce dipeptide and the use of orthogonal protection would give us an opportunity to control the coupling and cyclization reactions. For example, it can be used N–boc N$^{'}$-nitro arginine. Due to the lack of time this reaction was not tested.

In addition to that, I believe that the yields and purities of carbamation reaction can be improved by applying better solvents for extraction part and it would be a possibility to recover the whole amount of produced carbamate. It was mentioned before that as far as the reaction was used, the yields were decreasing and after the water phase test, the major part of the product was still left in a water phase.

5. Conclusions

For the growing need and use of natural products and new potential drug candidates the new routes of synthesis arise. It will continue to emerge until more economical and versatile synthesis way for making DKPs will be reported.

During this study no new method for DKP synthesis was discovered, but the introduction of substituents into the DKP ring was studied. It was tried to introduce different kinds of R groups on nitrogen by using alkylation and reductive amination methods. The groups were tested to introduce on amino acid methyl ester hydrochlorides, Boc protected amino acids and dipeptides.

By attempting to introduce the substituents using alkylation reaction, a new carbamation reaction was discovered. This was an unexpected result. Thereafter this reaction was investigated more extensively using different amino acid methyl ester hydrochlorides together with different alkylating agents. The use of different bases in the reaction: Cs_2CO_3 and K_2CO_3 was also tested. Results varied from moderate to high yields depending on the substituents, which caused a steric hindrance in the molecule, and the base. The better results were achieved using Cs_2CO_3 though reaction products using K_2CO_3 were showing more pure results.

6. Experimental

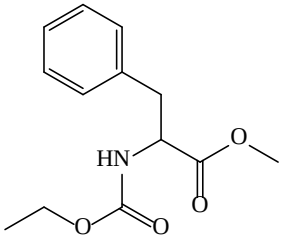
6.1. General

All the solvents and chemicals were purchased from commercial suppliers like Fluka, Aldrich, Merck and used for the reactions without further purification. Dry solvents were taken from dry solvent system (SDS). Reactions were monitored by using TLC 60 F_{254} silica gel plates, visualized by using UV light or iodine vapor. Flash column chromatography was performed on silica gel (35-70), which was supplied by Merck.

The general procedures of the reactions are described further. All spectra are included in the appendixes section, which include spectra of ^{1}H NMR, ^{13}C NMR and MS and IR. All of the samples were run in deuterated methanol. Spectra of ^{1}H NMR and ^{13}C NMR were recorded on a Varian Mercurry 400 Plus (399.65/100.54 Mhz) spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) TMS (δ = 0.00 ppm) used as an internal standard. Coupling constants (J) are measured in Hertz (Hz). Signal multiplicity is assigned as s (singlet), d (doublet), t (triplet), m (multiplet). Infrared spectra were obtained on a Varian 7000e FT – IR spectrometer, with frequencies (ν) reported in centimeter (cm^{-1}). Mass spectra were recorded on a Thermo electron LTQ Orbitrap + Electrospray ion source (Ion - Max).

6.2. General procedure for synthesis of carbamates

The methyl ester of the C terminal amino acid (1 eq.) or an amine (1 eq.) was dissolved in a DMF (4ml per 1mmol) and cesium carbonate (2 eq.) was added into the reaction mixture. After stirring for 5min. an alkylating agent (1.3 eq.) was added dropwise and the reaction left for stirring overnight at room temperature. The reaction mixture was washed by using 4% potassium bisulfate aqueous solution (2x4ml per 1mmol) and the organic layer was washed with brine (3x30ml) and dried over anhydrous $MgSO_4$ and concentrated in vacuo.



Methyl 2-(ethoxycarbonylamino)-3-phenylpropanoate (52a):

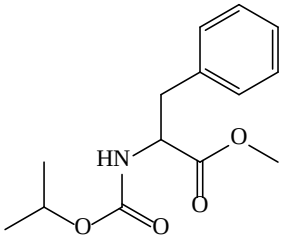
Phenylalanine methyl ester hydrochloride (1eq, 1.10 mmol, 0.20g) and ethyl bromide (1.30 eq, 1.45 mmol, 0.11 ml) gave 52a (0.23g, 0.94 mmol, 84%) as a white solid;

HRMS (ESI): $(M+Na)^+$ calculated for $C_{13}H_{17}NNaO_4$ 274,1055; found 274,1049 (100 %).

^{1}H NMR (400 MHz, CD_3OD)δ 3.12 (dd, *J* = 13.8, 5.6 Hz, 1H), 2.92 (dd, *J* = 13.8, 9.0 Hz, 1H), 1.18 (t, *J* = 7.1 Hz, 3H), 7.34 – 7.09 (m, 5H), 3.68 (s, 3H), 4.41 (dd, *J* = 9.1, 5.6 Hz, 1H), 4.01 (q, *J* = 7.2 Hz, 2H).

^{13}C NMR (400 MHz, CD_3OD) δ 172.60, 157.18, 136.90, 128.80, 128.03, 126.41, 60.60, 55.47, 51.22, 37.23, 13.46.

IR: 675.987, 700.703, 747.042, 777.974, 862.036, 1056.890, 1175.20, 1214.757, 1252.403, 1338.941, 1373.251, 1431.647, 1699.588, 2336.255, 2361.353, 2483.465, 2953.333, 2982.019, 3346.052, 3744.369 cm^{-1}.



Methyl 2-(isopropoxycarbonylamino)-3-phenylpropanoate (52b):

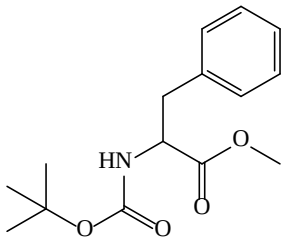
Phenylalanine methyl ester hydrochloride (1 eq, 1.10 mmol, 0.20 g) and isopropyl iodide (1.3 eq, 1.45 mmol, 0.15 ml) gave 52b (0.22 g, 0.83 mmol, 75%) as a yellow oil;

HRMS (ESI): $(M+Na)^+$ calculated for $C_{14}H_{19}NNaO_4$ 288,1212; found 288,1204 (100 %).

^{1}H NMR (400 MHz, CD_3OD) δ 7.35 – 7.12 (m, 5H), 4.74 (h, *J* = 6.0 Hz, 1H), 4.40 (dd, *J* = 9.4, 5.5 Hz, 1H), 3.68 (s, 3H), 3.11 (dd, *J* = 13.7, 5.4 Hz, 1H), 2.91 (dd, *J* = 13.9, 8.8 Hz, 1H), 1.17 (dd, *J* = 21.4, 6.1 Hz, 6H).

^{13}C NMR (400 MHz, CD_3OD) δ 172.64, 136.90, 128.80, 128.01, 126.38, 68.15, 55.41, 51.20, 37.26, 20.88.

IR: 701.017, 747.110, 779.617, 27.714, 915.764, 952.014, 1048.194, 1081.689, 1111.111, 1145.375, 1213.326, 1257.324, 1342.707, 1373.618, 1438.397, 1512.950, 1498.740, 1743.358, 1730.561, 2337.474. 2362.154, 2876.524, 2935.215, 2980.014, 3030.328, 3064.294, 3346.097, 3488.423 cm^{-1}.



Methyl 2-(tert-butoxycarbonylamino)-3-phenylpropanoate (52c):

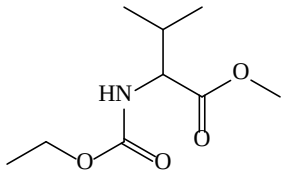
Phenylalanine methyl ester hydrochloride (1eq, 1.10 mmol, 0.20 g) and t – butyl bromide (1.3 eq, 1.45 mmol, 0.16 ml) gave 52c (0.05 g, 0.17 mmol, 15%) as a yellowish oil;

HRMS (ESI): $(M+Na)^+$ calculated for $C_{15}H_{21}NNaO_4$ 302,1368; found 302,1365 (100 %).

^1H NMR (400 MHz, CD_3OD) δ 7.38 – 6.99 (m, 5H), 4.35 (t, *J* = 8.9, 5.7 Hz, 1H), 3.67 (s, 3H), 3.08 (dd, *J* = 13.8, 5.6 Hz, 1H), 2.89 (dd, *J* = 13.8, 8.9 Hz, 1H), 1.37 (s, 9H).

^{13}C NMR(400 MHz, CD_3OD) δ 172.77, 156.34, 136.95, 128.83, 128.01, 126.37, 79.18, 55.13, 51.17, 37.28, 27.24.

IR: 700.328, 745.528, 777.704, 857.845, 914.631, 1051.622, 1117.384, 1248.164, 1279.799, 1365.229, 1390.858, 1441.749, 1498.213, 1603.991, 1712.372, 1744.796, 2285.695, 2386.118, 2857.766, 3011.198, 3209.077, 3454.233, 3695.148 cm^{-1}.



Methyl 2-(ethoxycarbonylamino)-3-methylbutanoate (53a):

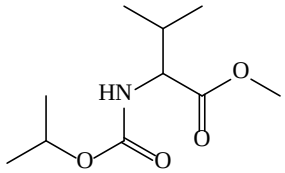
Valine methyl ester hydrochloride (1eq, 1.50 mmol, 0.20 g) and ethyl bromide (1.3 eq, 1.98 mmol, 0.15 ml) gave 53a (0.14 g, 0.65 mmol, 45%) as a yellowish oil;

HRMS (ESI): $(M+Na)^+$ calculated for $C_9H_{17}NNaO_4$ 226,1055; found 226,1045 (100 %).

^1H NMR (400 MHz, CD_3OD)δ 4.13 – 4.01 (m, 3H), 3.71 (s, 3H), 2.11 (td, *J* = 14.6, 13.4, 7.9 Hz, 1H), 1.24 (t, *J* = 7.1 Hz, 3H), 0.93 (dd, *J* = 9.1, 6.8 Hz, 6H).

^{13}C NMR (400 MHz, CD_3OD) δ 172.75, 157.65, 60.63, 59.58, 50.99, 30.39, 18.06, 16.96, 13.50.

IR: 668.458, 778.615, 837.446, 907.212, 1033.689, 1099.029, 1115.827, 1162.271, 1207.511, 1233.724, 1268.875, 1310.787, 1372.443, 1437.011, 1467.358, 1523.205, 1720.152, 2361.116, 2493.898, 2875.081, 2971. 553, 3345.439 cm^{-1}.



Methyl 2-(isopropoxycarbonylamino)-3-methylbutanoate (53b):

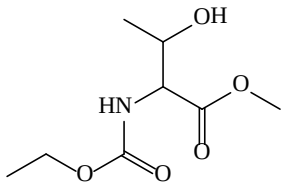
Valine methyl ester hydrochloride (1eq, 1.50 mmol, 0.20 g) and isopropyl iodide (1.3 eq, 1.98 mmol, 0.20 ml) gave 53b (0.11 g, 0.51 mmol, 34%) as a yellow oil;

HRMS (ESI): $(M+H)^+$ calculated for $C_{10}H_{20}NO_4$ 218,1387; found 218,1387 (90 %).

^{1}H NMR (400 MHz, CD_3OD) δ 4.05 (d, *J* = 5.9 Hz, 1H), 3.71 (s, 3H), 3.31 – 3.30 (m, 1H), 2.10 (h, *J* = 6.5 Hz, 1H), 1.23 (d, *J* = 6.2 Hz, 6H), 0.98 – 0.89 (m, 6H).

^{13}C NMR (400 MHz, CD_3OD) δ 172.15, 156.63, 67.49, 58.87, 50.28, 29.72, 20.24, 17.36, 16.29.

IR: 667.843, 779.511, 45.156, 932.524, 1023.913, 1151.321, 1308.888, 1381.670, 1444.253, 1506.074, 1722.551, 2863.790, 2976.458, 3342.413 cm^{-1}.



Methyl 2-(ethoxycarbonylamino)-3-hydroxybutanoate (54a):

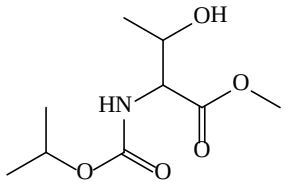
Threonine methyl ester hydrochloride (1eq, 1.50 mmol, 0.20 g) and ethyl bromide (1.3 eq, 1.98 mmol, 0.15 ml) gave 54a (0.12 g, 0.56 mmol, 37%) as a colourles oil;

HRMS (ESI): $(M+Na)^+$ calculated for $C_8H_{15}NNaO_5$ 228,0848; found 228,0839 (100 %).

^{1}H NMR (400 MHz, CD_3OD) δ 4.25 (q, *J* = 6.1, 2.9 Hz, 1H), 4.19 (d, *J* = 2.5 Hz, 1H), 4.11 (q, *J* = 7.3 Hz, 2H), 3.74 (s, 2H), 1.26 (t, *J* = 7.2 Hz, 3H), 1.19 (d, *J* = 6.5 Hz, 3H).

^{13}C NMR (400 MHz, CD_3OD) δ 171.62, 157.73, 67.01, 60.83, 59.61, 51.32, 18.79, 13.48.

IR: 692.647, 844.852, 1021.590, 1071.124, 1159.347, 1207.947, 1272.482, 1381.407, 1443.210, 1513.045, 1728.943, 2863.985, 2976.877, 3441.758.



Methyl 3-hydroxy-2-(isopropoxycarbonylamino)butanoate (54b):

Threonine methyl ester hydrochloride (1eq, 1.50 mmol, 0.20 g) and isopropyl iodide (1.3 eq, 1.98 mmol, 0.2 ml) gave 54b (0.08 g, 0.38 mmol, 25%) as a colourles oil;

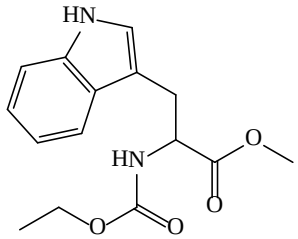
HRMS (ESI): $(M+Na)^+$ calculated for $C_9H_{17}NNaO_5$ 242,1004; found 242,1001 (100 %).

$M+H)^+$ calculated for $C_{19}H_{18}NO_4$ 220,1179; found 220,1181 (75 %).

^{1}H NMR (400 MHz, CD_3OD) δ 4.88 – 4.81 (m, 1H), 4.29 – 4.21 (m, 1H), 4.19 (d, *J* = 3.1 Hz, 1H), 3.74 (s, 3H), 1.25 (d, *J* = 6.9 Hz, 6H), 1.19 (d, *J* = 5.7 Hz, 3H).

^{13}C NMR (400 MHz, CD_3OD) δ 171.67, 157.34, 68.44, 67.06, 59.55, 51.36, 20.97, 20.93, 18.87.

IR: 691.924, 781.517, 844.608, 922.142, 1067.128, 1150.574, 1175.234, 1208.279, 1271.921, 1381.107, 1438.737, 1509.656, 1723.349, 2865.975, 2977.512, 344.475 cm^{-1}.



Methyl 2-(ethoxycarbonylamino)-3-(1H-indol-3-yl)propanoate (55a):

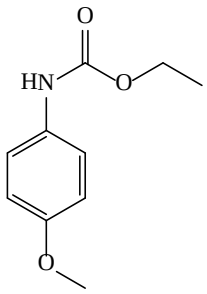
Tryptophan methyl ester hydrochloride (1eq, 0.92 mmol, 0.20 g) and ethyl bromide (1.3 eq, 1.19 mmol, 0.09 ml) gave 55a (0.23 g, 0.81 mmol, 88%) as a colorless oil;

HRMS (ESI): $(M+Na)^+$ calculated for $C_{15}H_{18}N_2NaO_4$ 313,1164; found 313,1159 (100%).

^{1}H NMR (400 MHz, CD_3OD) δ 7.95 (s, 2H), 7.53 (dd, *J* = 8.4, 3.0 Hz, 1H), 7.34 (dd, *J* = 8.2, 2.8 Hz, 1H), 7.08 (d, 2H), 7.02 (d, *J* = 7.2 Hz, 1H), 4.49 (d, *J* = 4.9 Hz, 1H), 4.03 (q, *J* = 6.7, 6.2 Hz, 2H), 3.65 (s, 3H), 3.26 (t, *J* = 5.7 Hz, 1H), 3.14 (d, *J* = 7.3 Hz, 2H), 1.19 (t, *J* = 6.7 Hz, 3H).

^{13}C NMR (400 MHz, CD_3OD) δ 173.05, 163.41, 136.62, 127.28, 123.09, 121.05, 118.44, 117.72, 110.95, 109.38, 60.61, 54.99, 51.25, 35.53, 30.24, 27.32, 13.52.

IR: 744.677, 1065.022, 1096.859, 1214.165, 1340.160, 1374.917, 1521.144, 1616.353 1700.151, 2336.784, 2361.681, 2977.557 cm^{-1}.



Ethyl 4-methoxyphenylcarbamate (56a):

Anisidine (1eq, 1.63 mmol, 0.20 g) and ethyl bromide (1.3 eq, 2.12 mmol, 0.12 ml) gave 56a (0.05 g, 0.25 mmol, 15%) as a dark brown oil;

HRMS (ESI): $(M+Na)^+$ calculated for $C_{10}H_{13}NNaO_3$ 218,0793; found 218,0784 (100%).

^1H NMR (400 MHz, CD_3OD) δ 7.19 (d, 2H), 6.74 (dd, 2H), 4.05 (q, *J* = 7.1 Hz, 2H), 3.65 (s, 3H), 1.19 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (400 MHz, CD_3OD) δ 155.84, 128.34, 120.30, 113.81, 113.58, 60.35, 54.43, 13.53.

IR: 769.188, 933.923, 1036.663, 1068.416, 1077.993, 1382.117, 1443.730, 1513.26, 1601.338, 1731.153, 2864.746, 2976.272, 3309.135 cm^{-1}.

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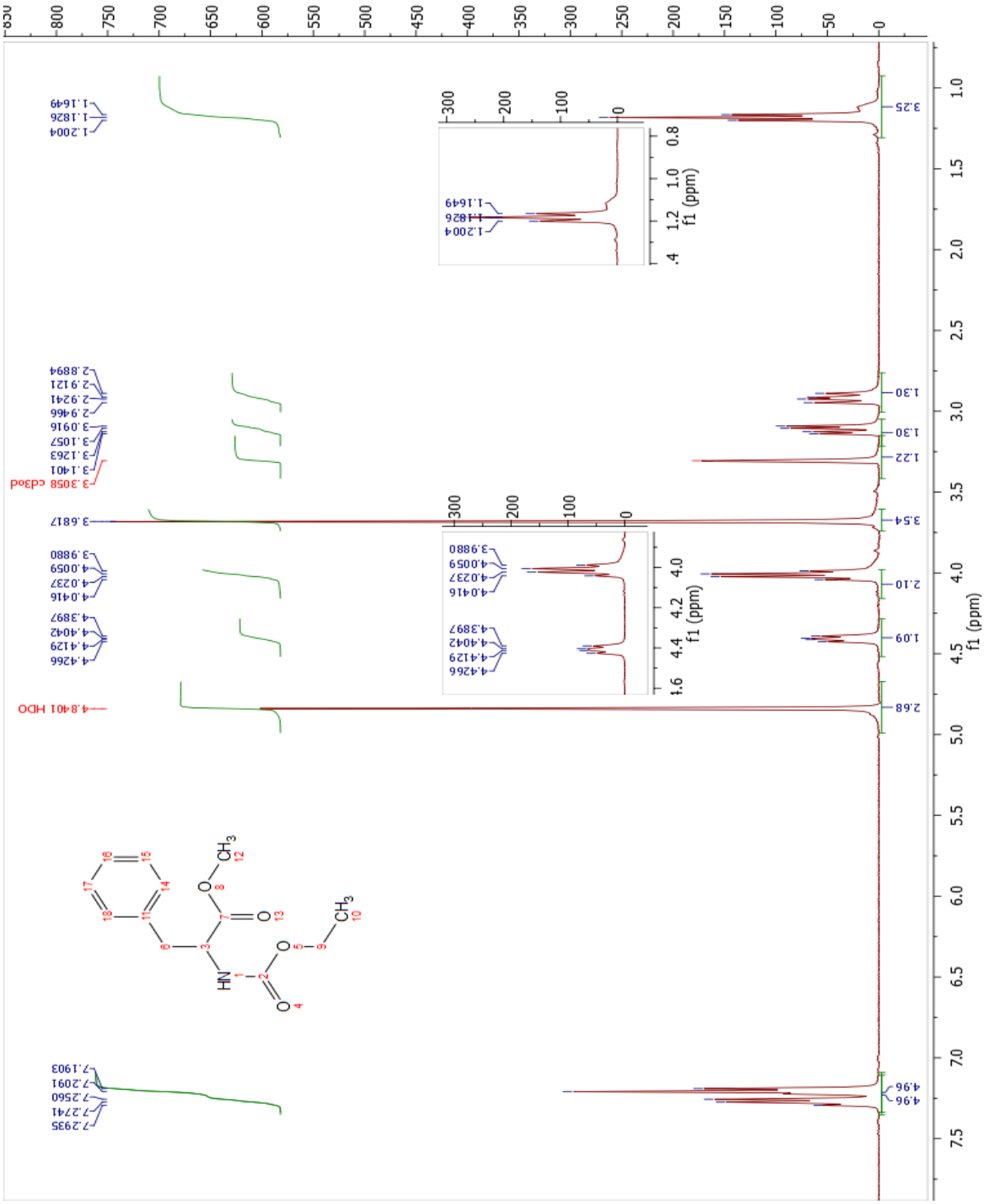
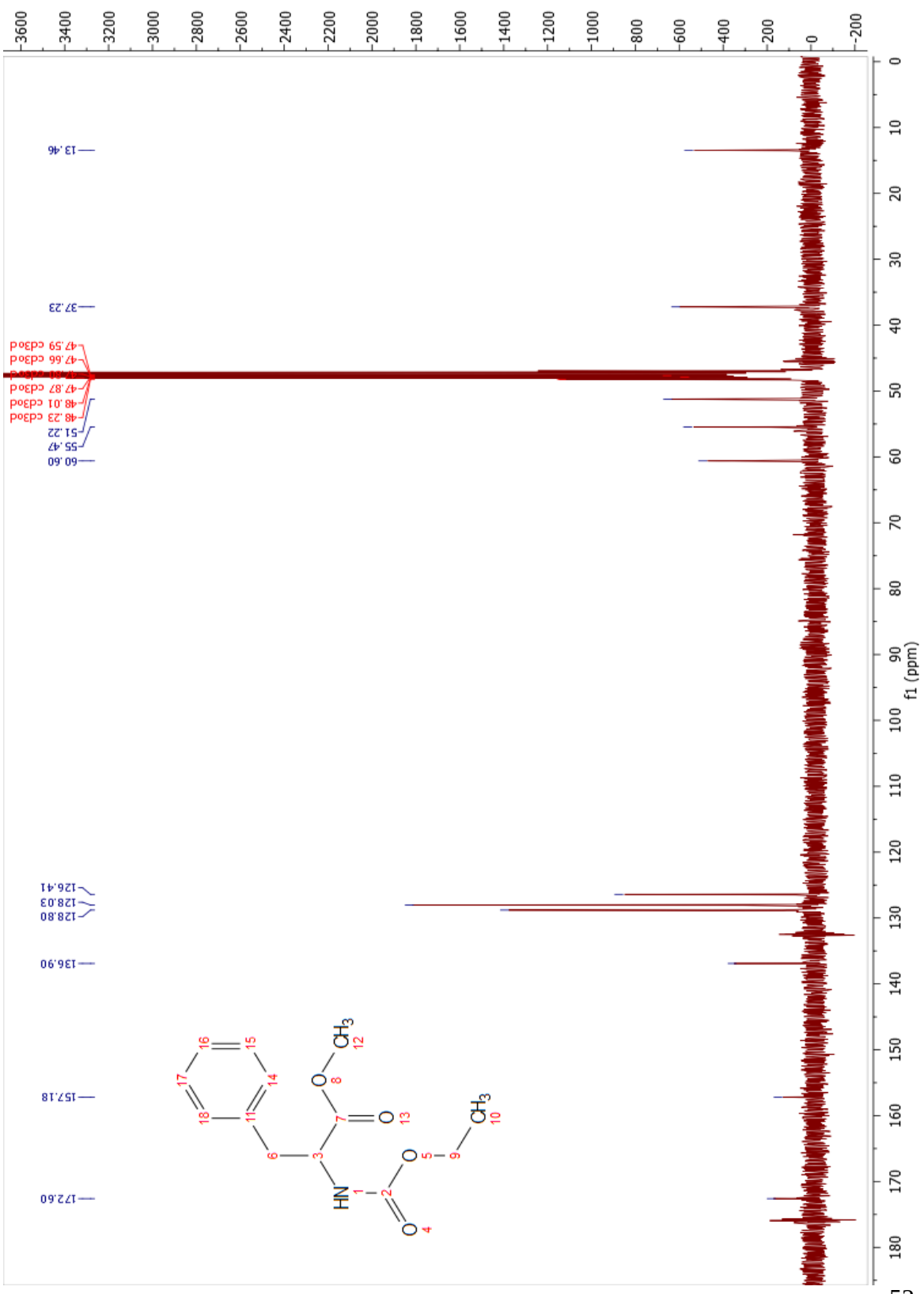
7. Appendix

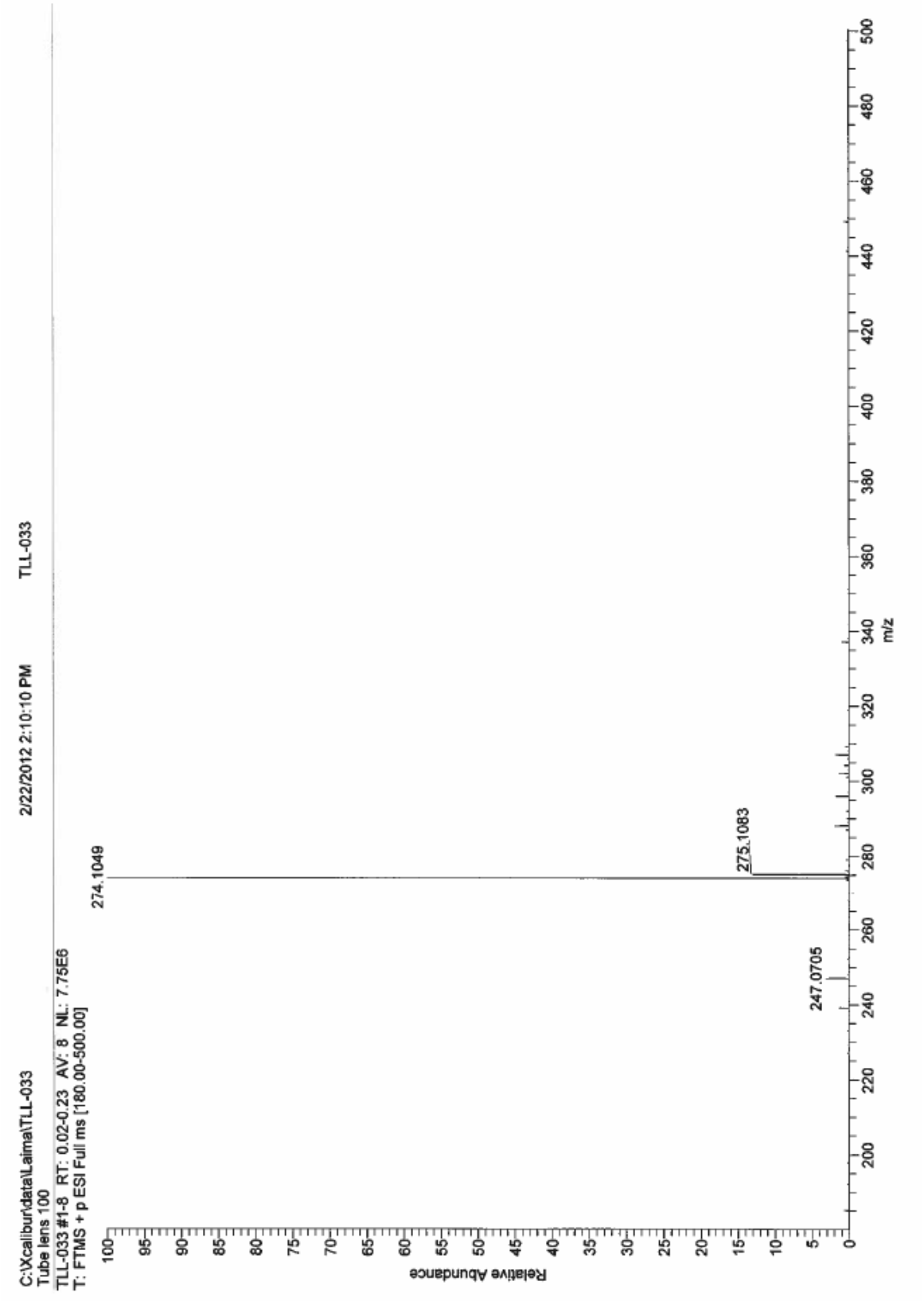
1H NMR of methyl 2-(ethoxycarbonylamino)-3-phenylpropanoate 52a:



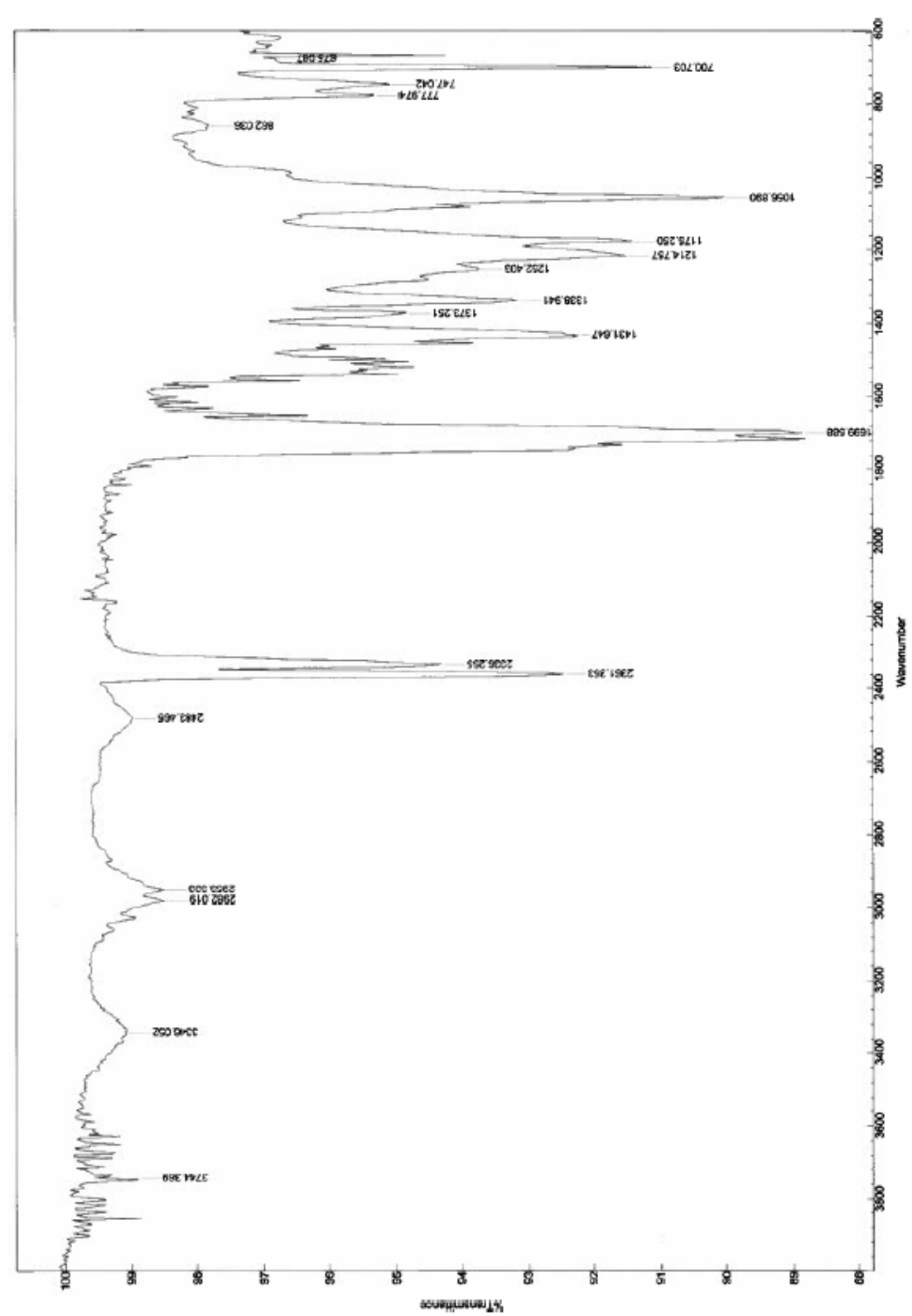
^{13}C NMR of methyl 2-(ethoxycarbonylamino)-3-phenylpropanoate **52a:**



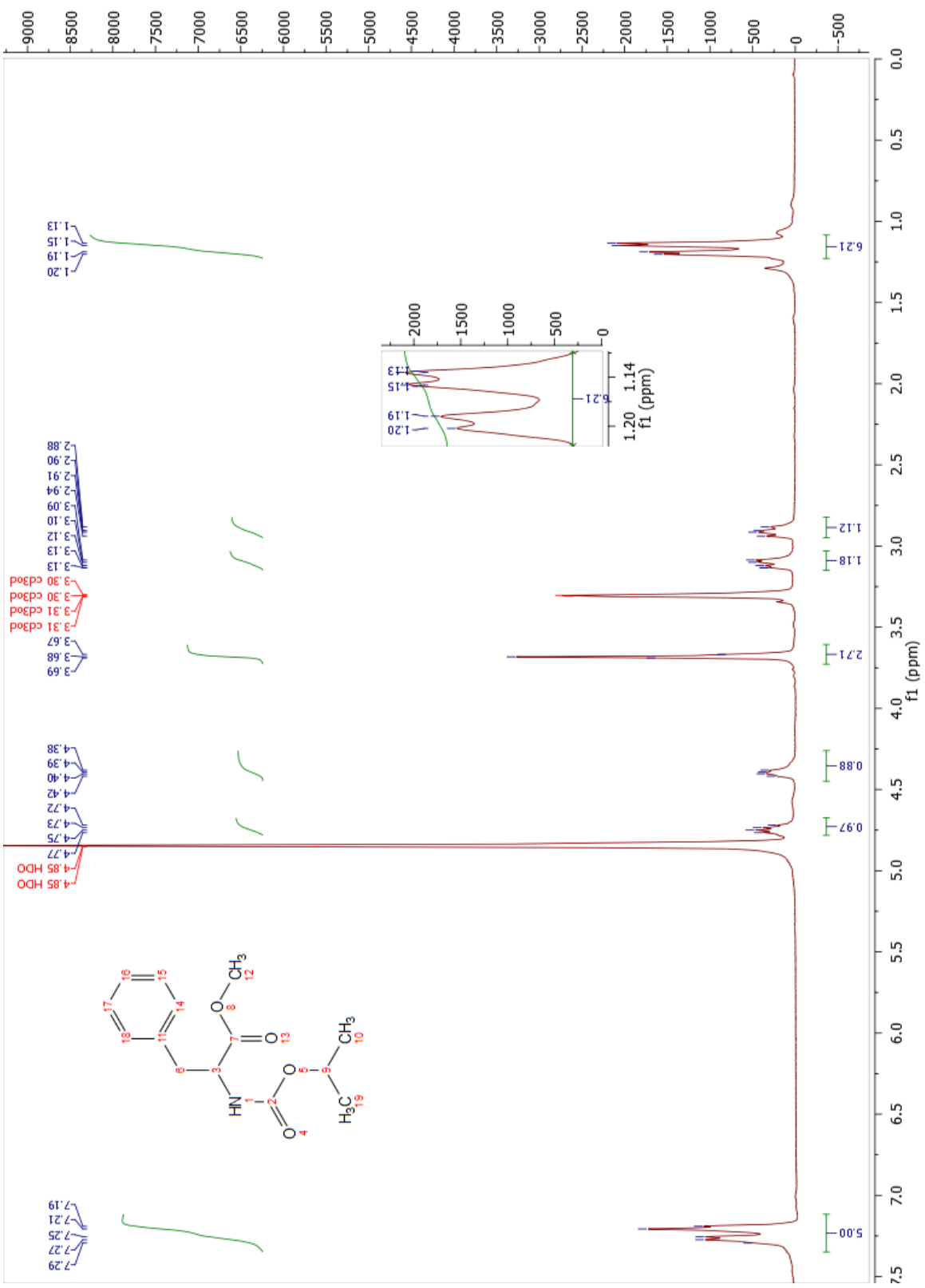
MS of methyl 2-(ethoxycarbonylamino)-3-phenylpropanoate **52a:**



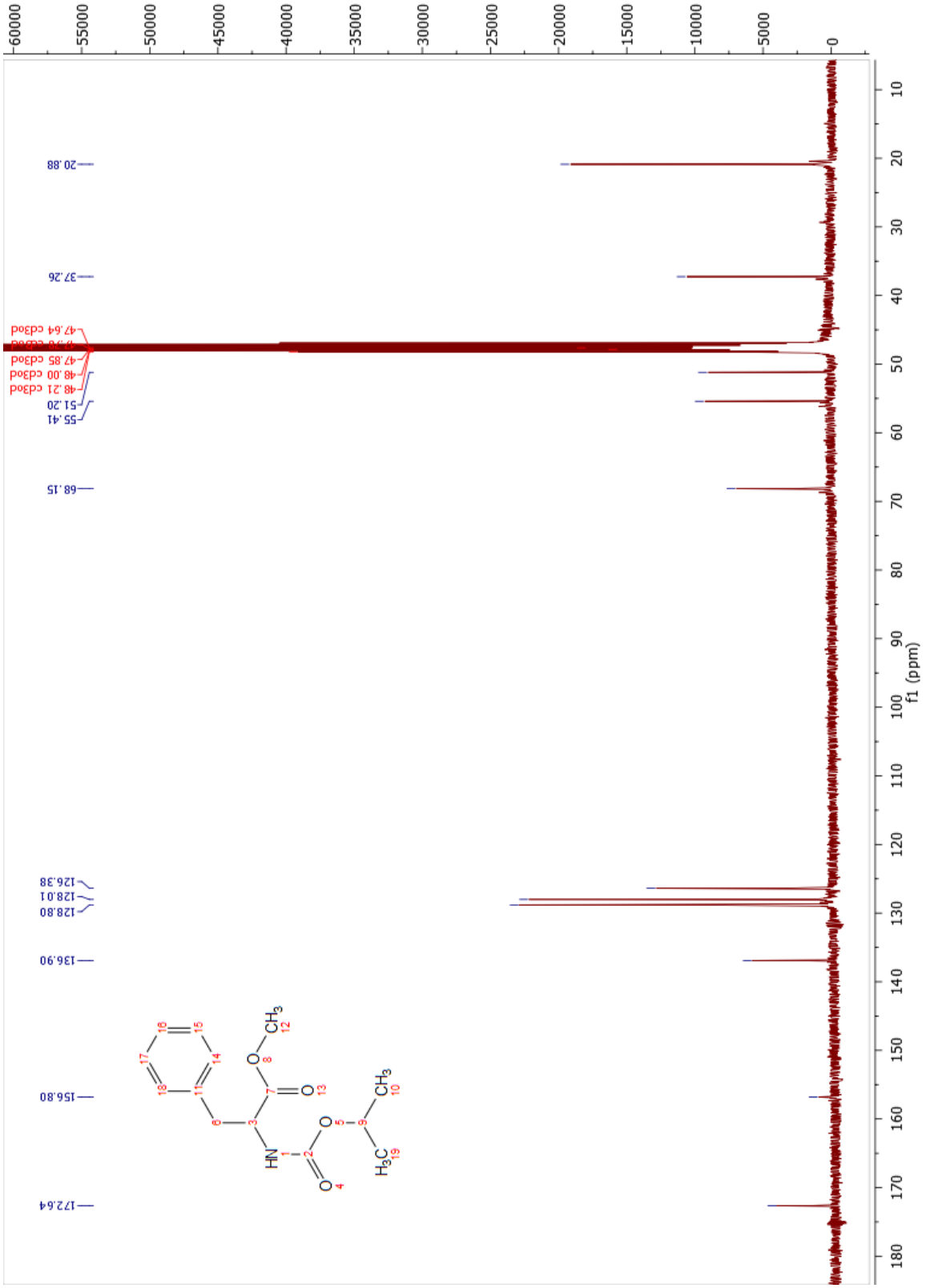
IR of methyl 2-(ethoxycarbonylamino)-3-phenylpropanoate **52a:**



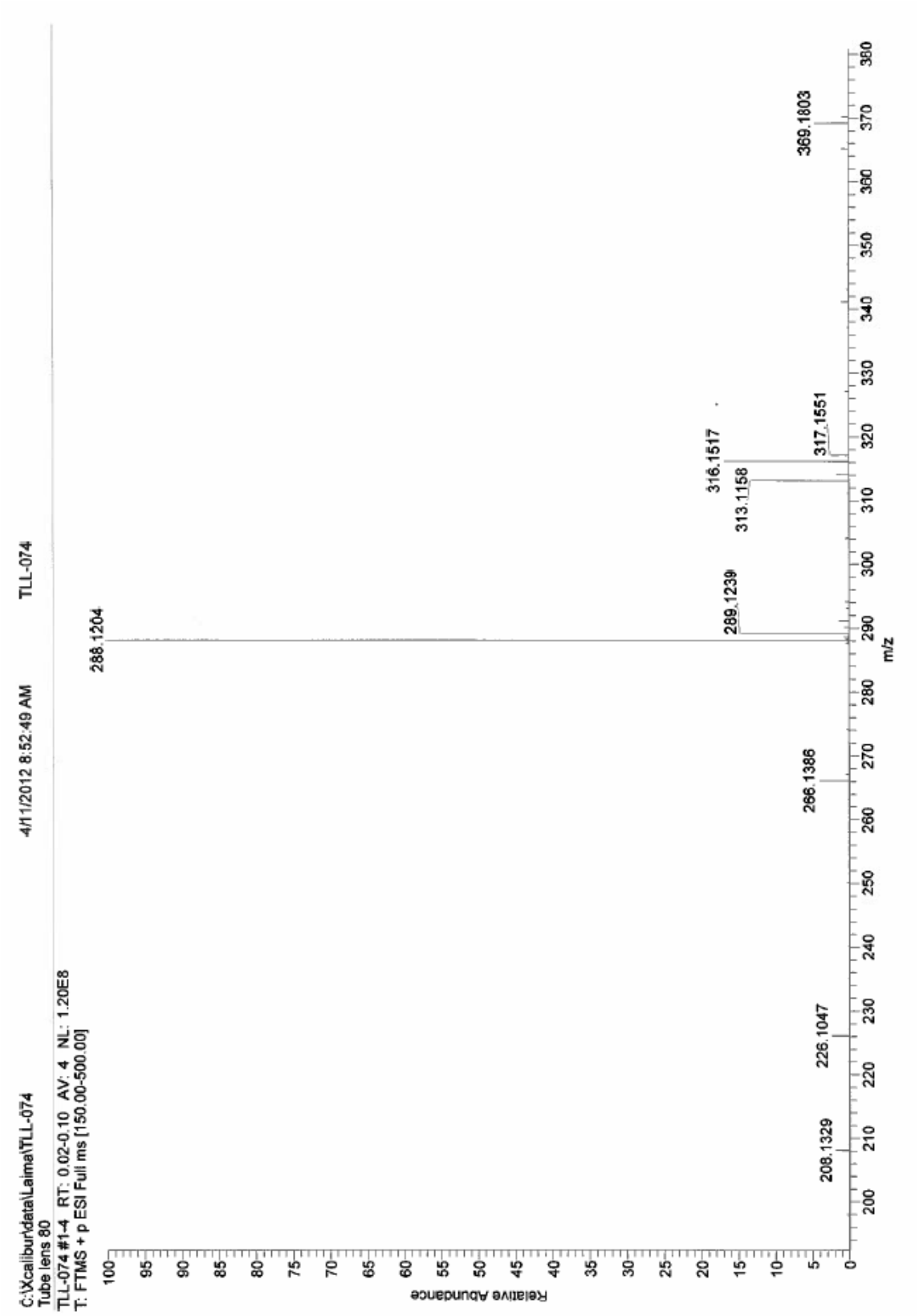
^{1}H NMR of methyl 2-(isopropoxycarbonylamino)-3-phenylpropanoate **52b**:



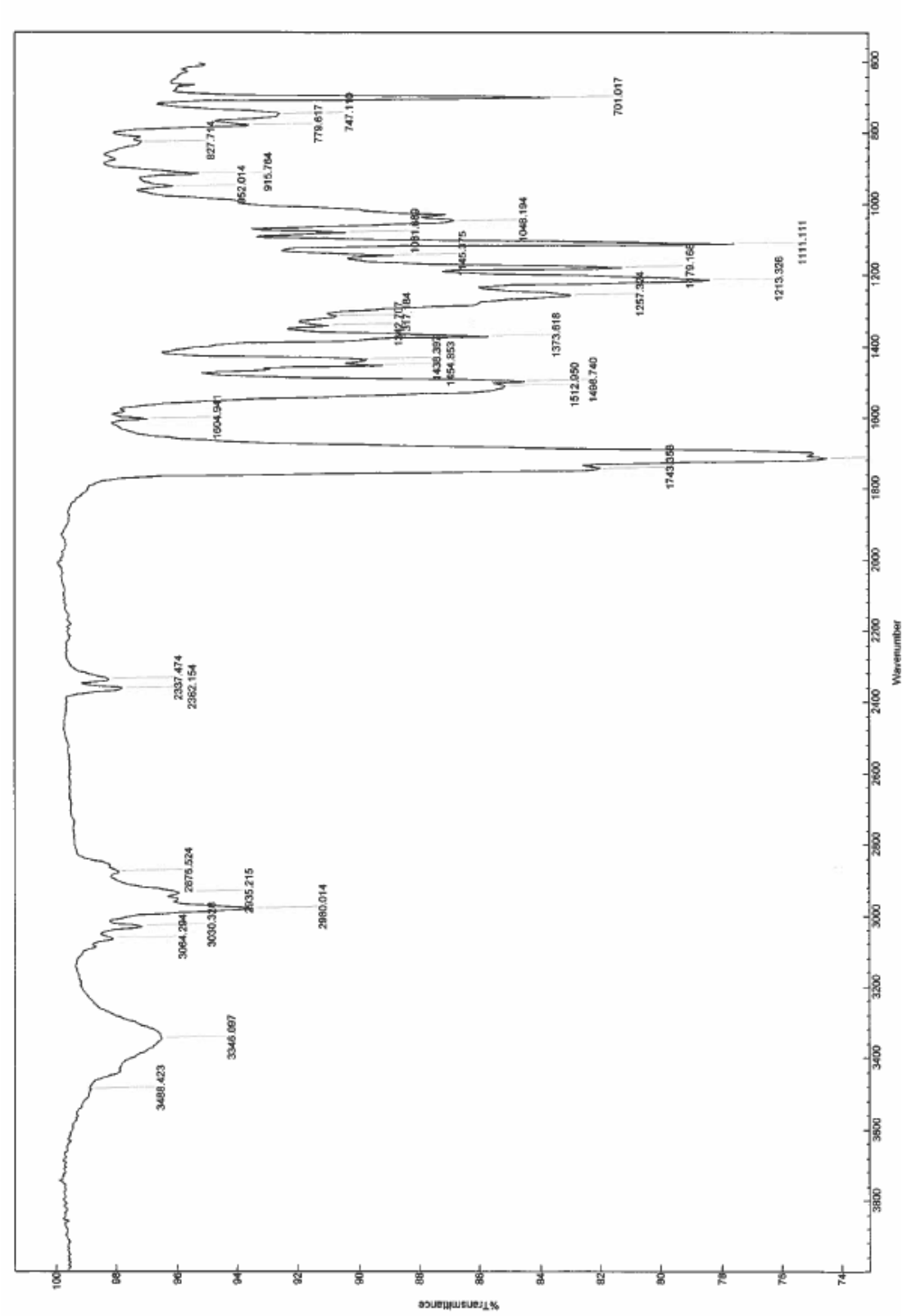
^{13}C of methyl 2-(isopropoxycarbonylamino)-3-phenylpropanoate **52b**:



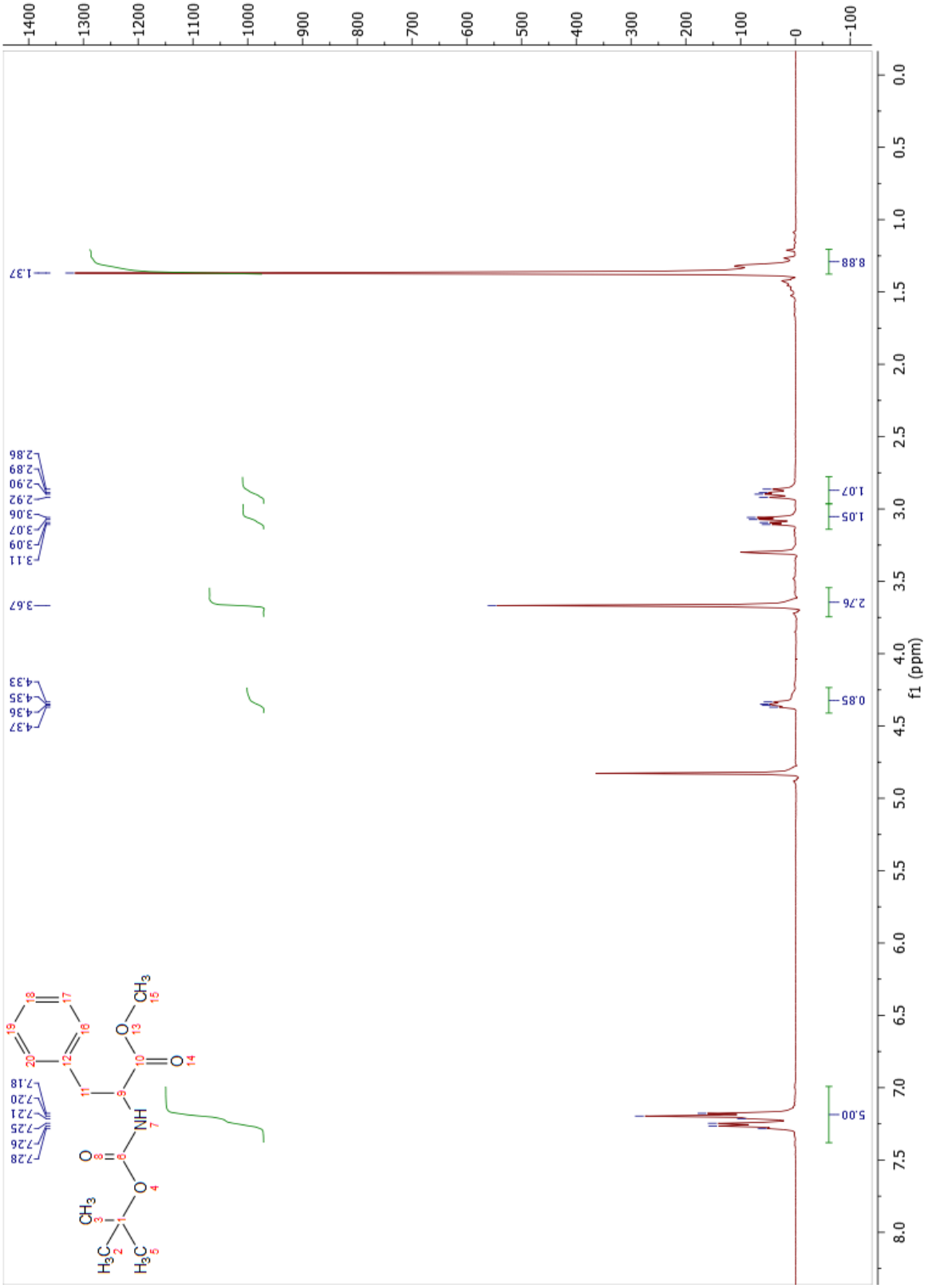
MS of methyl 2-(isopropoxycarbonylamino)-3-phenylpropanoate **52b**:



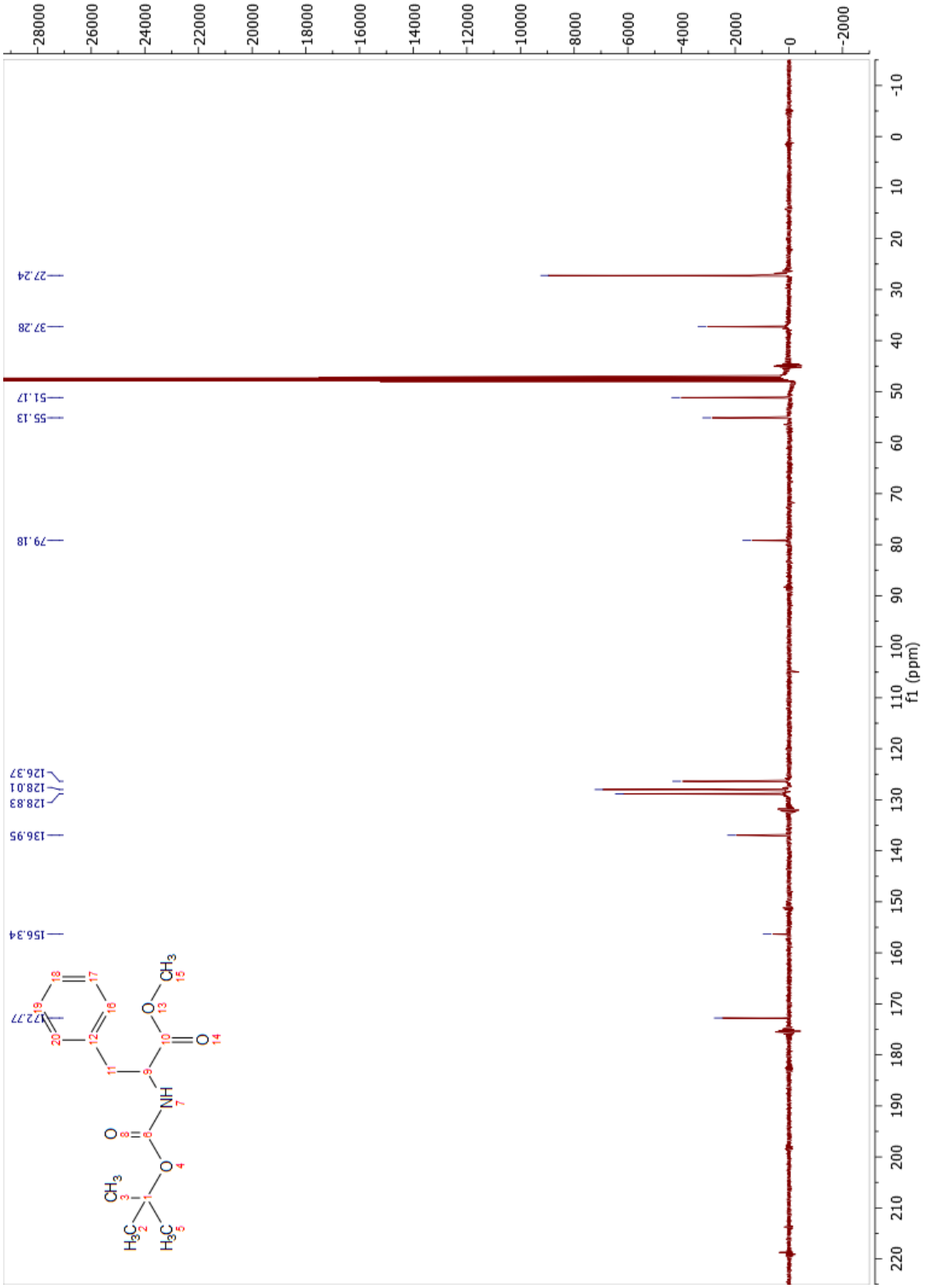
IR of methyl 2-(isopropoxycarbonylamino)-3-phenylpropanoate **52b**:



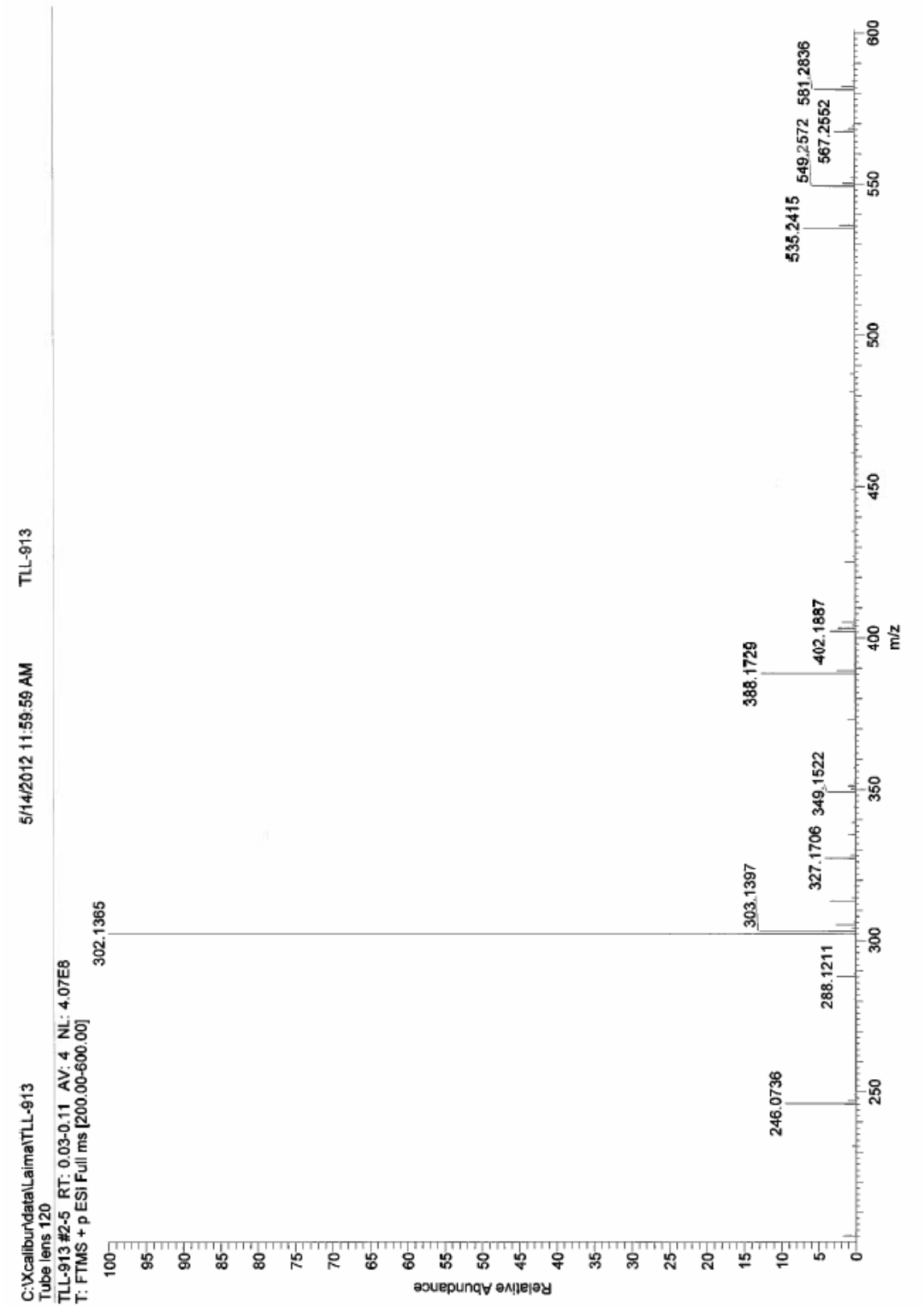
^{1}H NMR of methyl 2-(tert-butoxycarbonylamino)-3-phenylpropanoate **52c**:



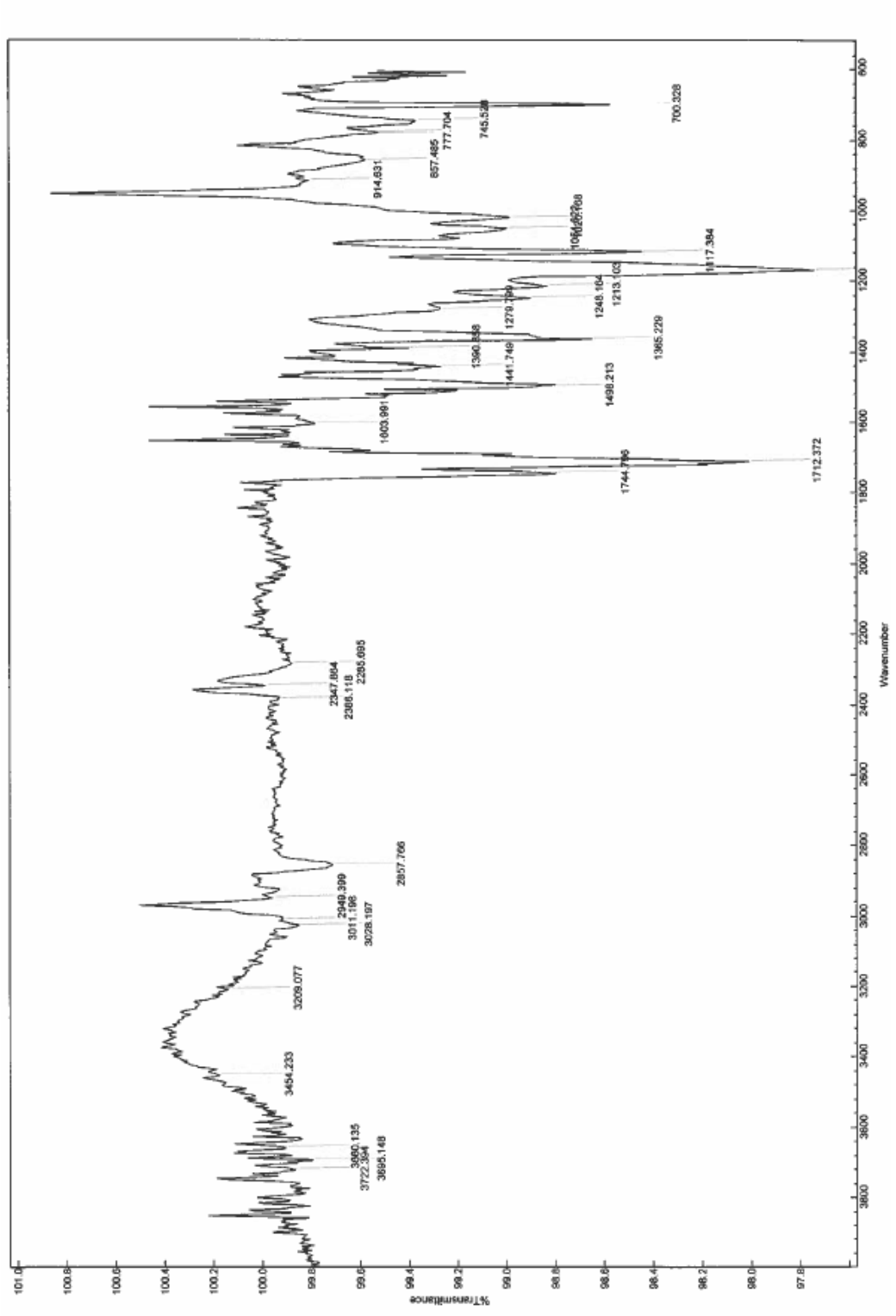
^{13}C NMR of methyl 2-(tert-butoxycarbonylamino)-3-phenylpropanoate **52c**:



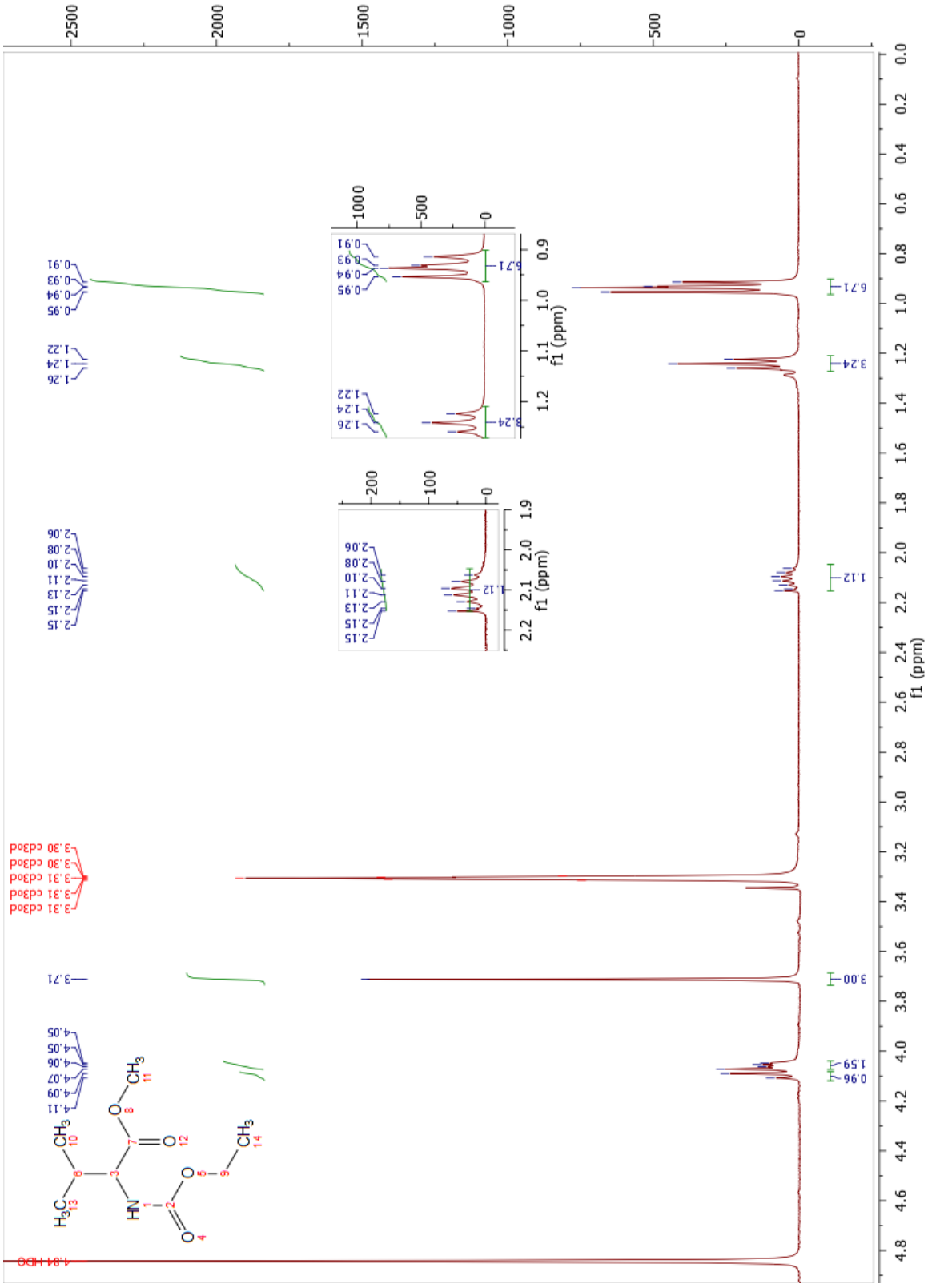
MS of methyl 2-(tert-butoxycarbonylamino)-3-phenylpropanoate **52c**:



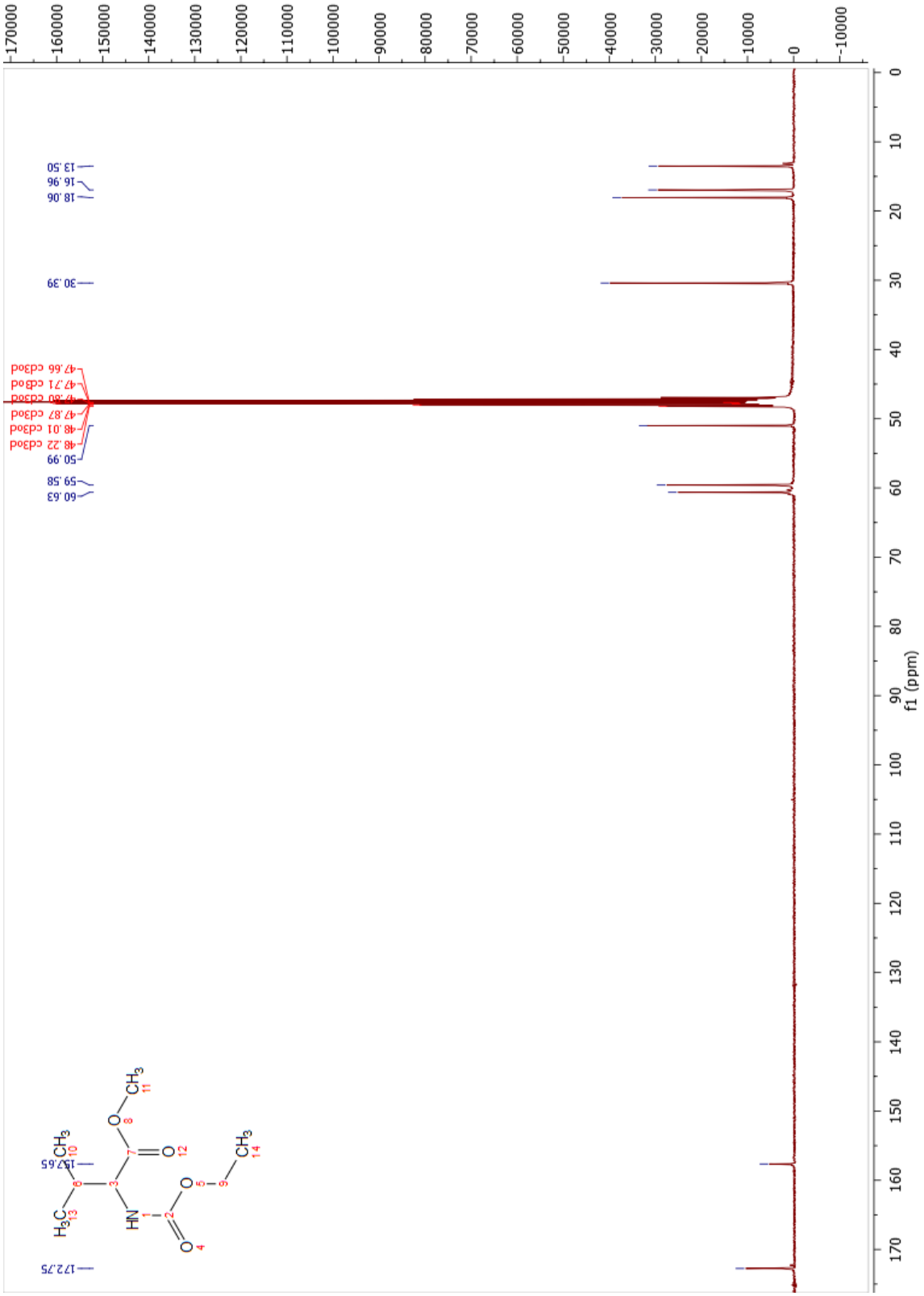
IR of methyl 2-(tert-butoxycarbonylamino)-3-phenylpropanoate **52c**:



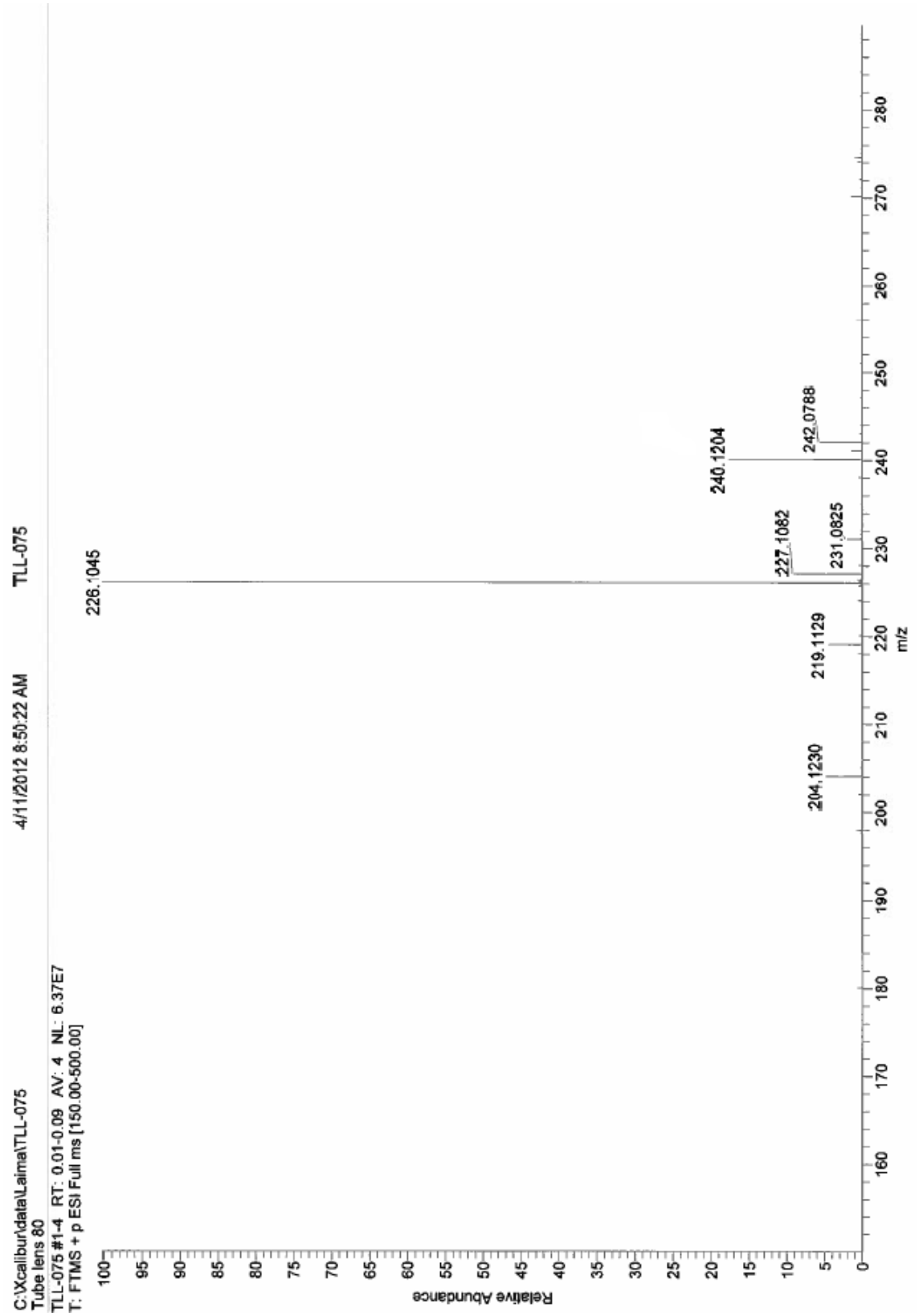
^{1}H NMR of methyl 2-(ethoxycarbonylamino)-3-methylbutanoate **53a**:



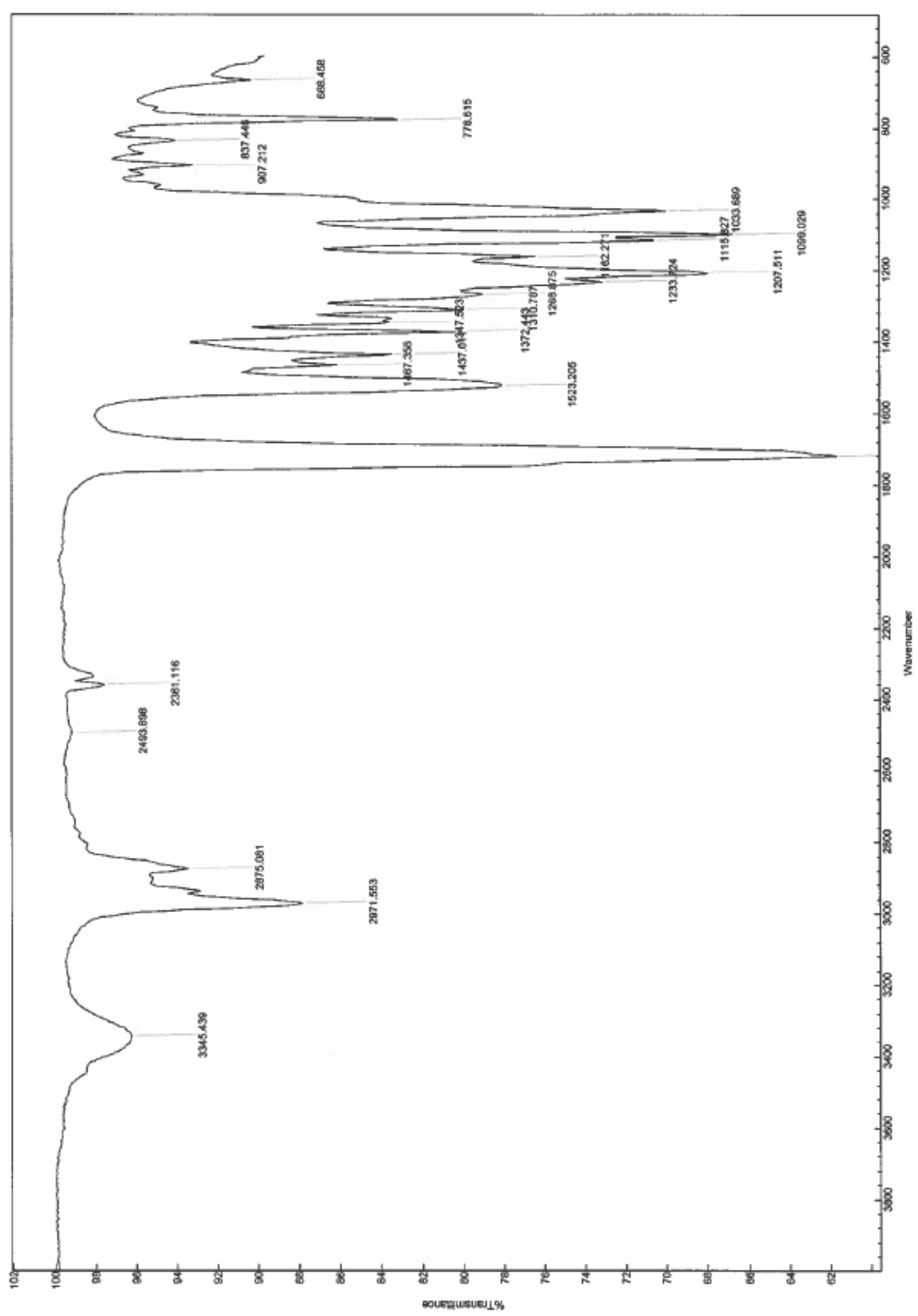
^{13}C NRM of methyl 2-(ethoxycarbonylamino)-3-methylbutanoate **53a**:



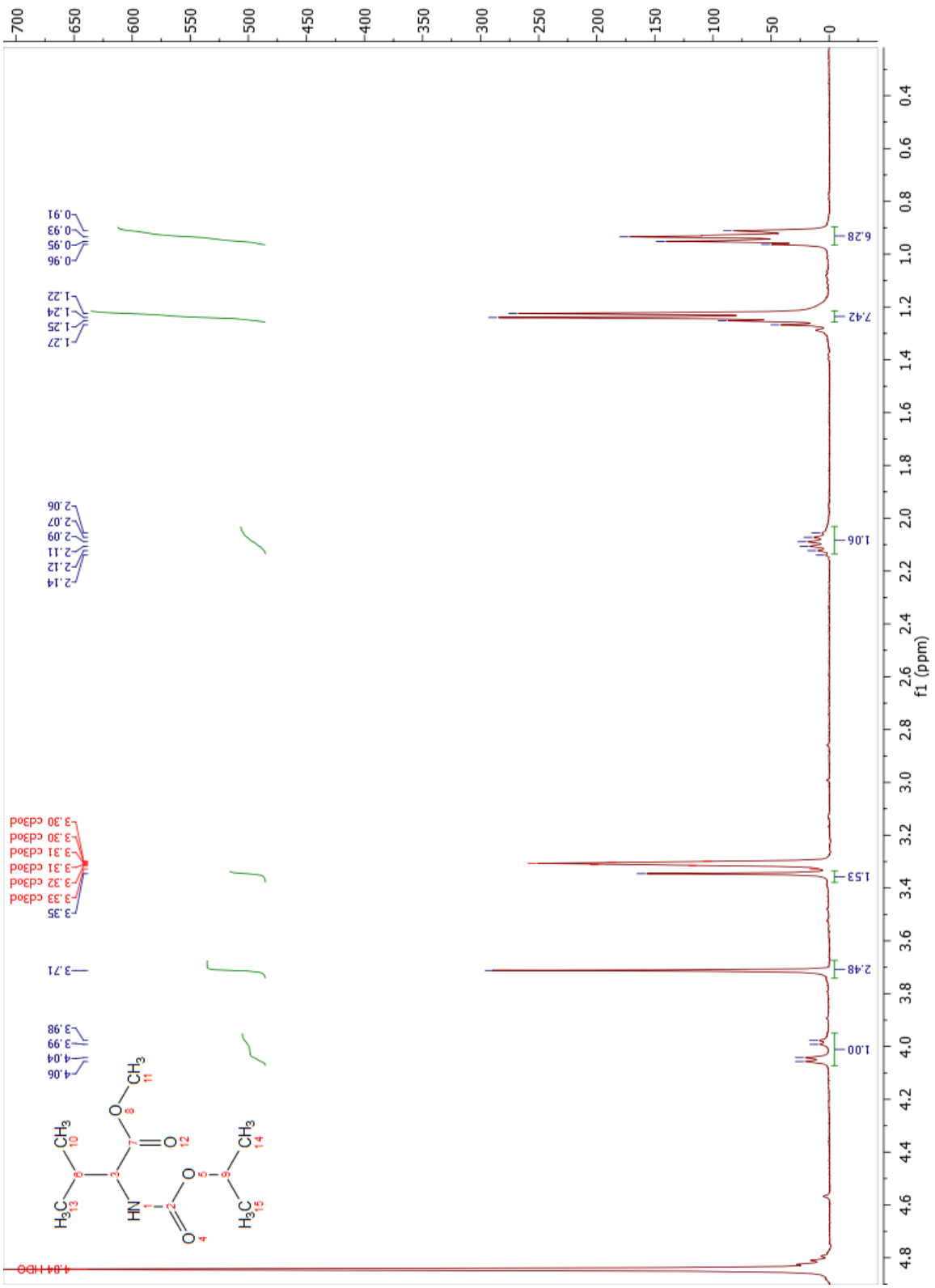
MS of methyl 2-(ethoxycarbonylamino)-3-methylbutanoate **53a**:



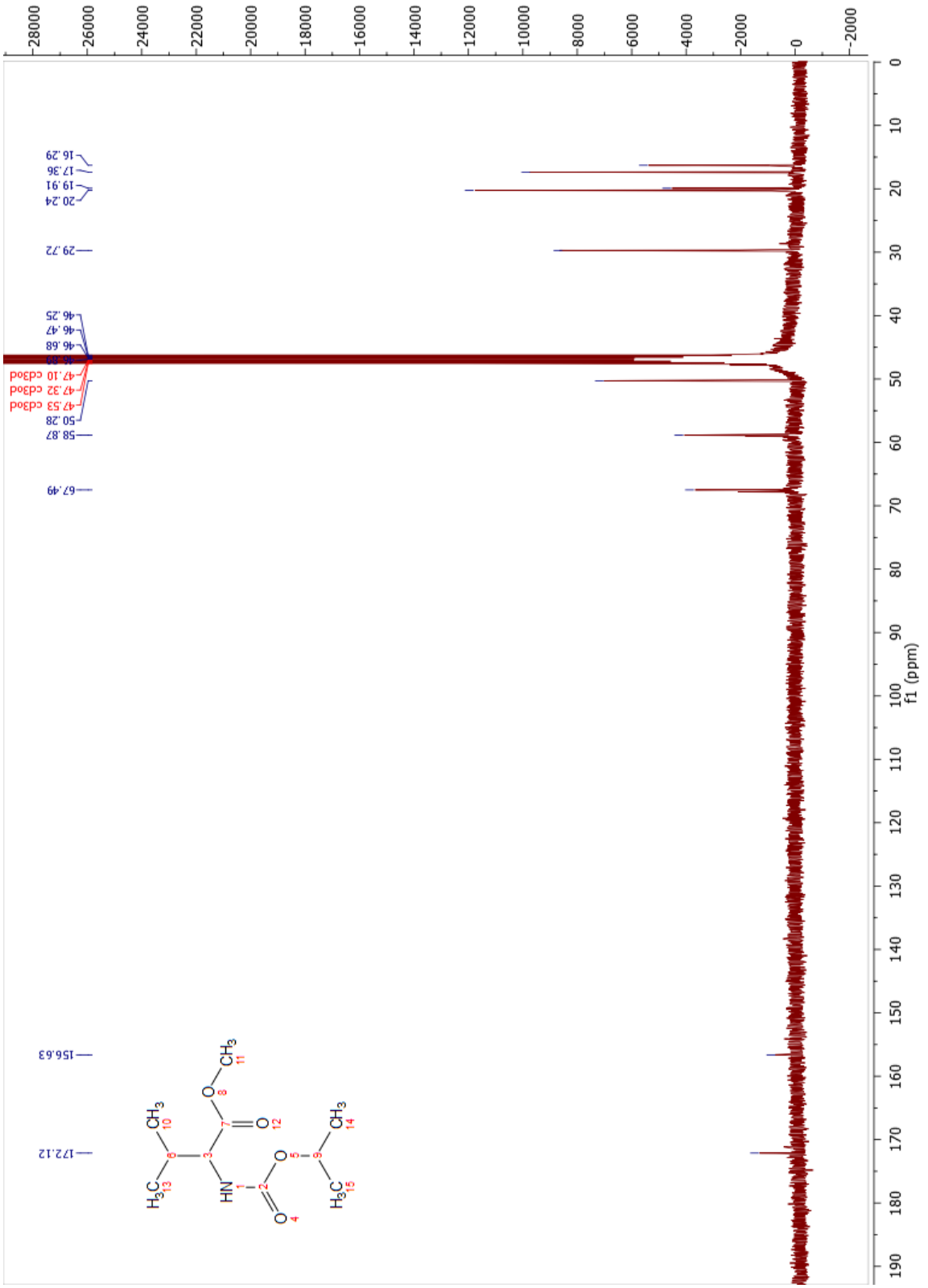
IR of methyl 2-(ethoxycarbonylamino)-3-methylbutanoate **53a**:



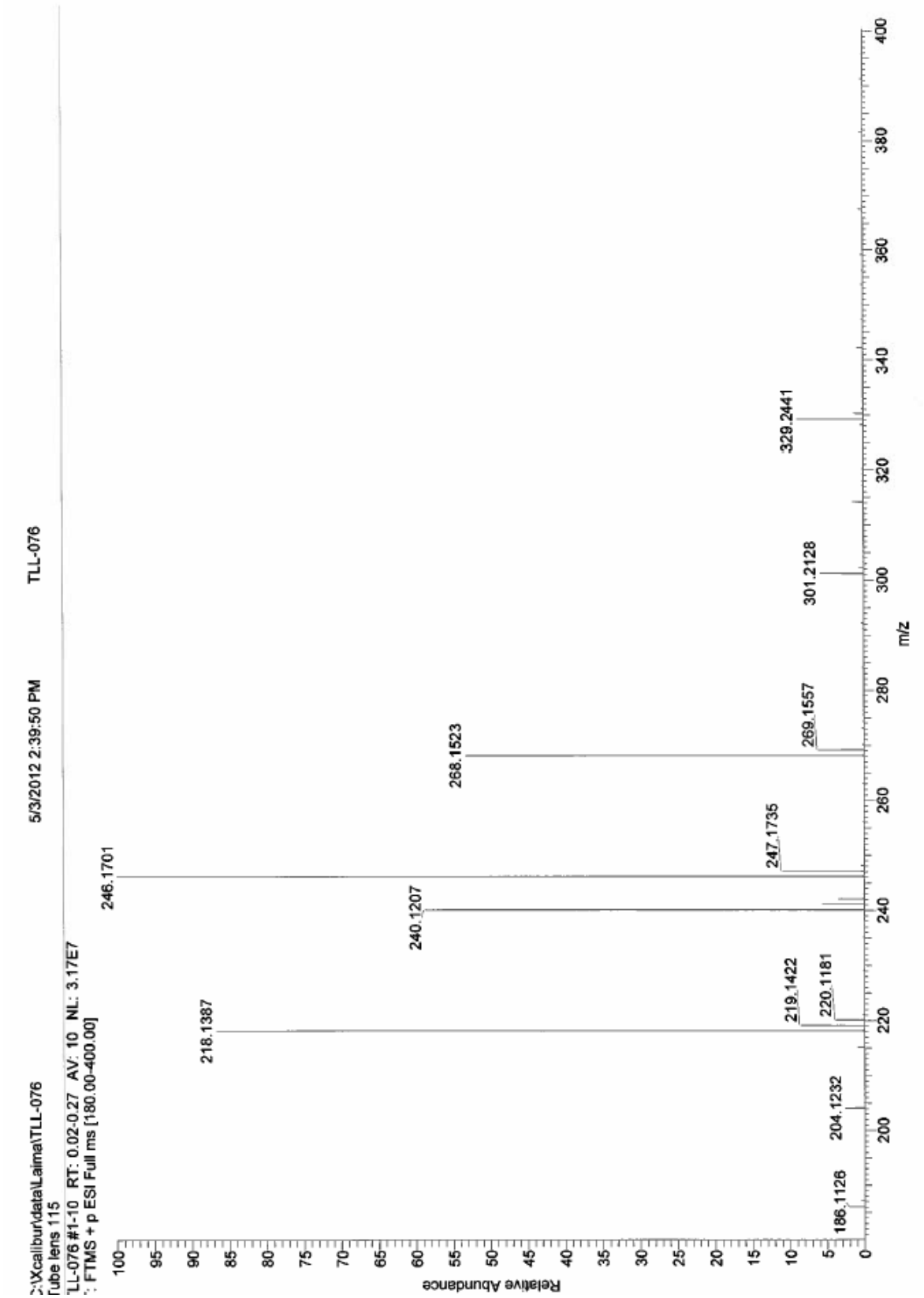
^{1}H NMR of methyl 2-(isopropoxycarbonylamino)-3-methylbutanoate **53b**:



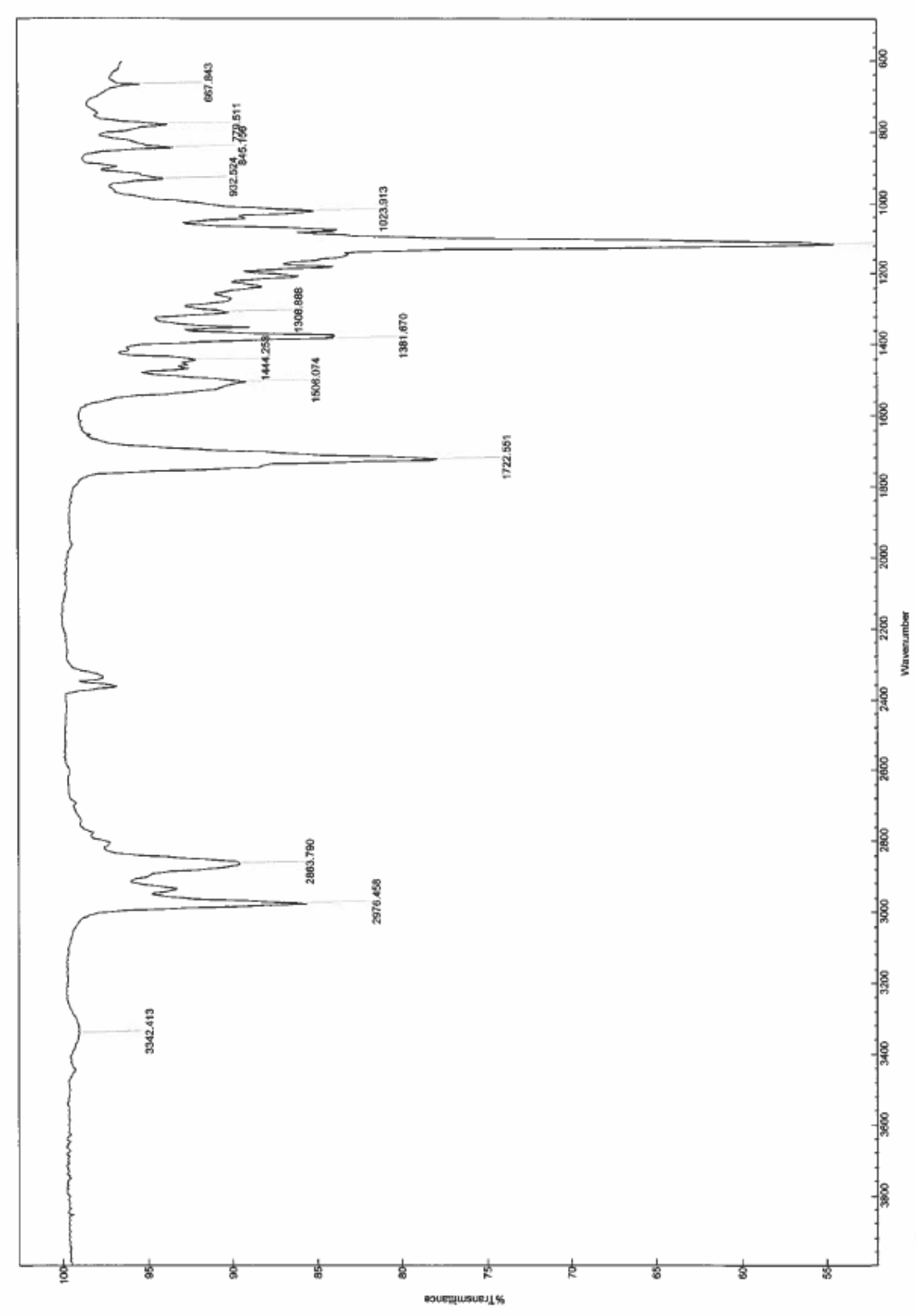
^{13}C NMR Methyl 2-(isopropoxycarbonylamino)-3-methylbutanoate **53b**:



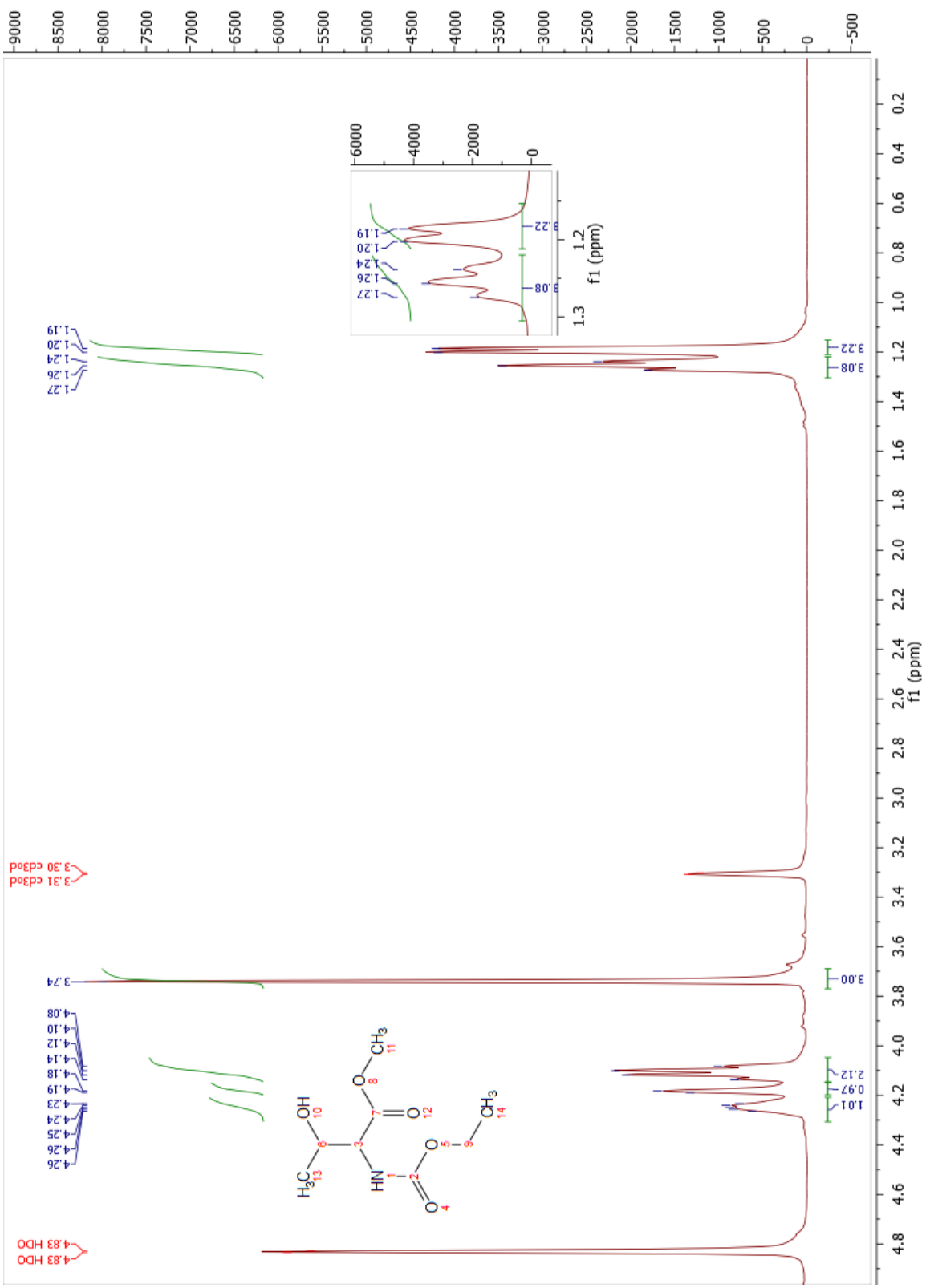
MS of methyl 2-(isopropoxycarbonylamino)-3-methylbutanoate **53b**:



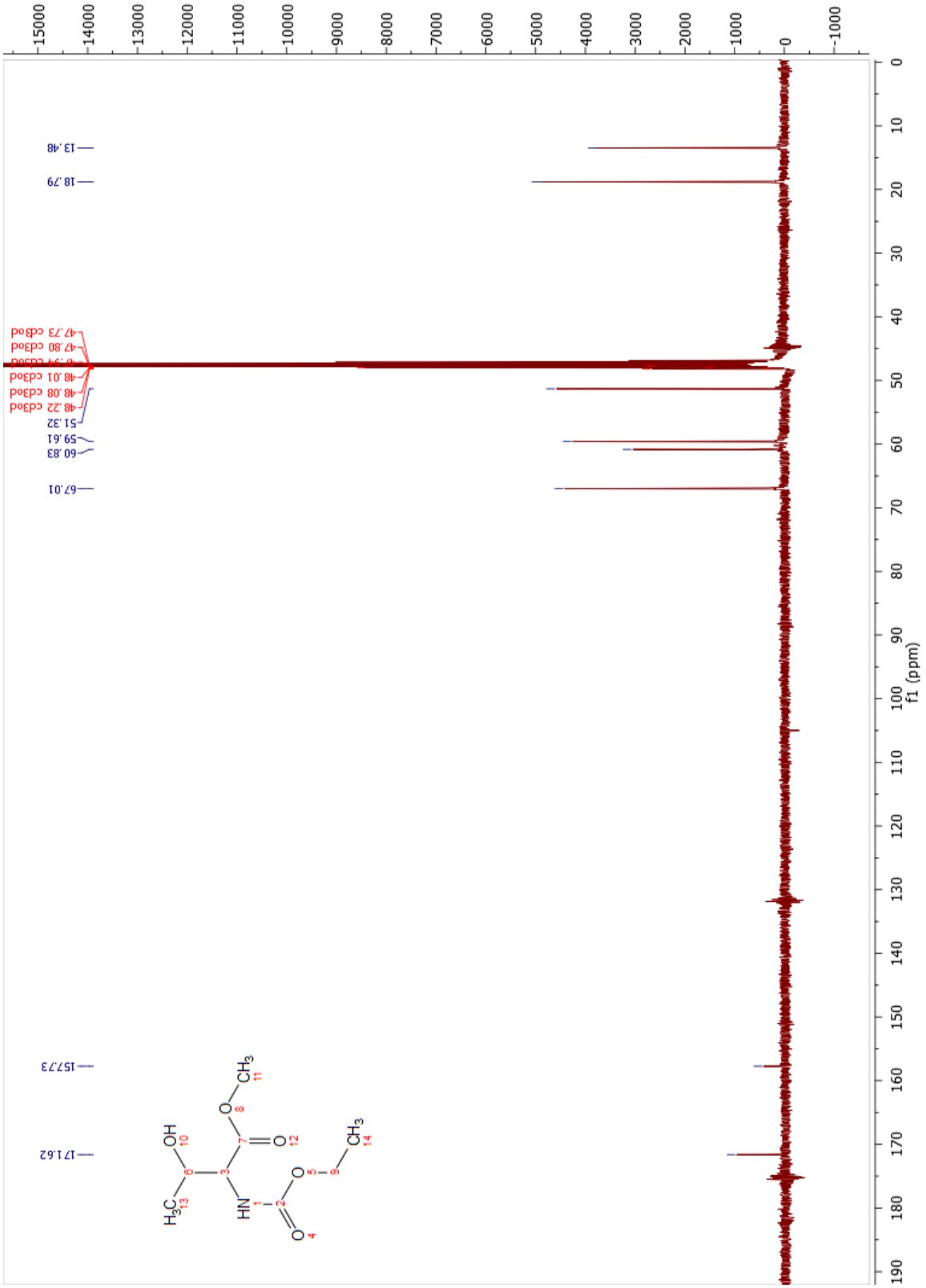
IR of methyl 2-(isopropoxycarbonylamino)-3-methylbutanoate **53b**:



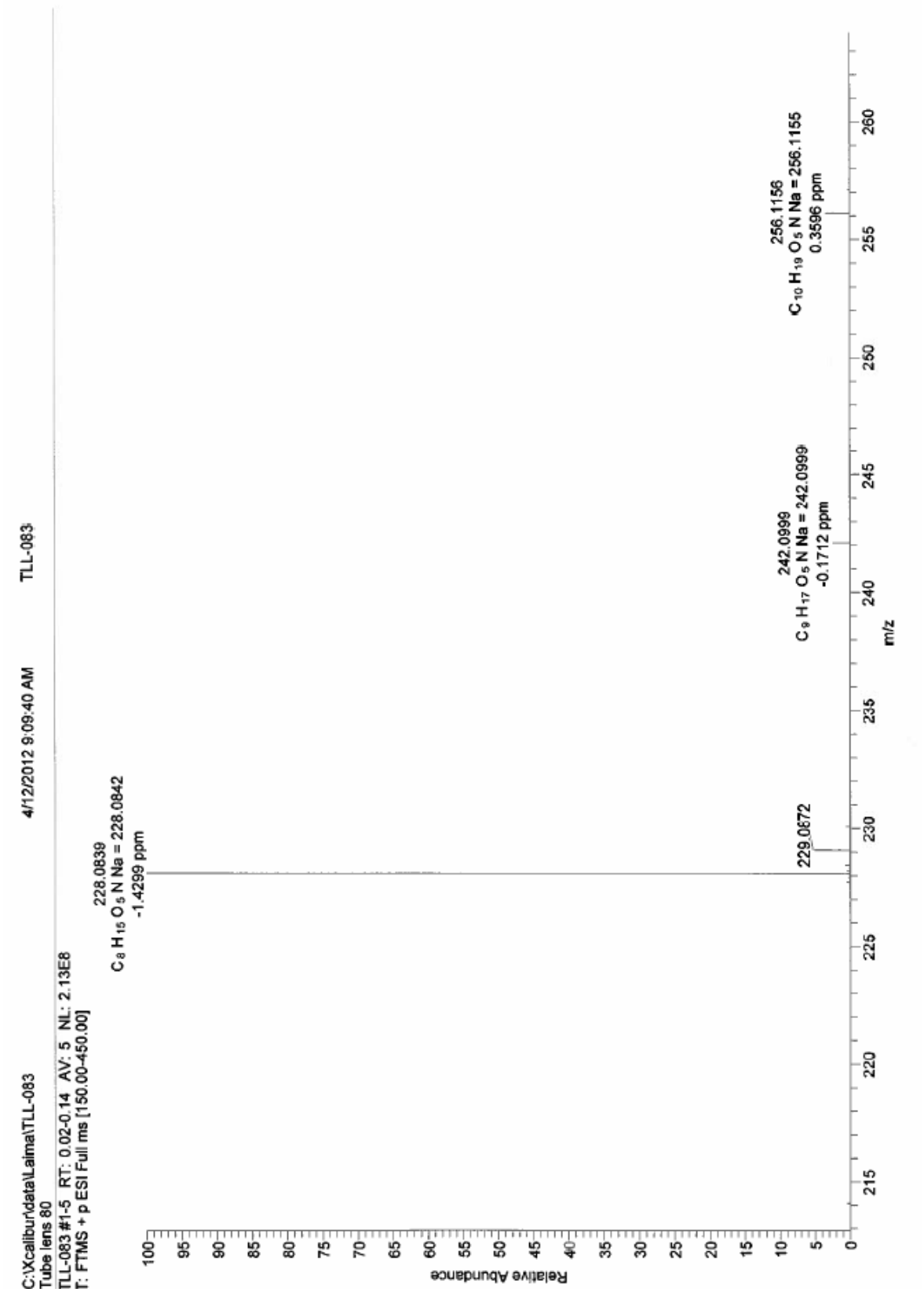
^{1}H NMR of methyl 2-(ethoxycarbonylamino)-3-hydroxybutanoate **54a**:



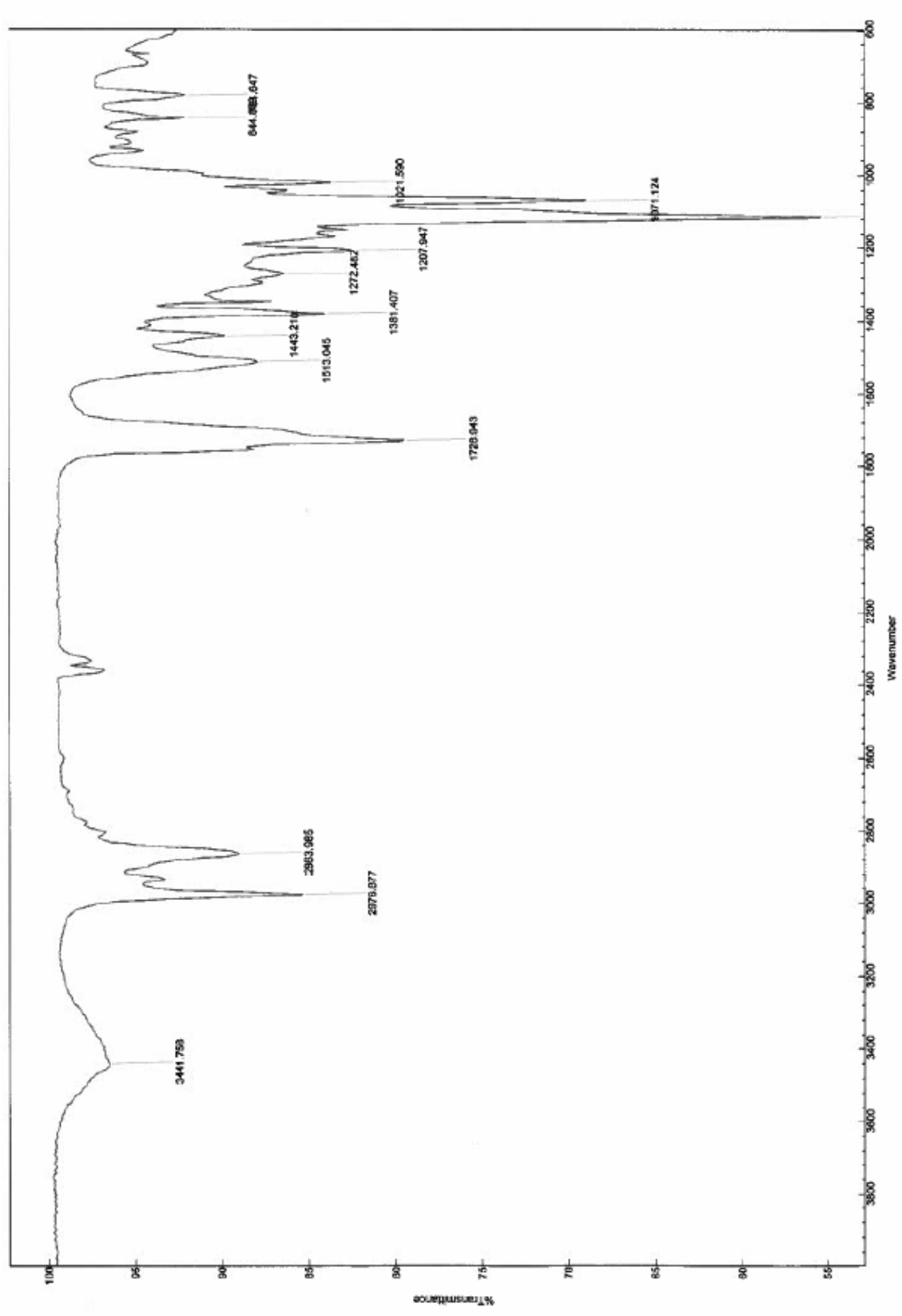
^{13}C NMR of methyl 2-(ethoxycarbonylamino)-3-hydroxybutanoate **54a**:



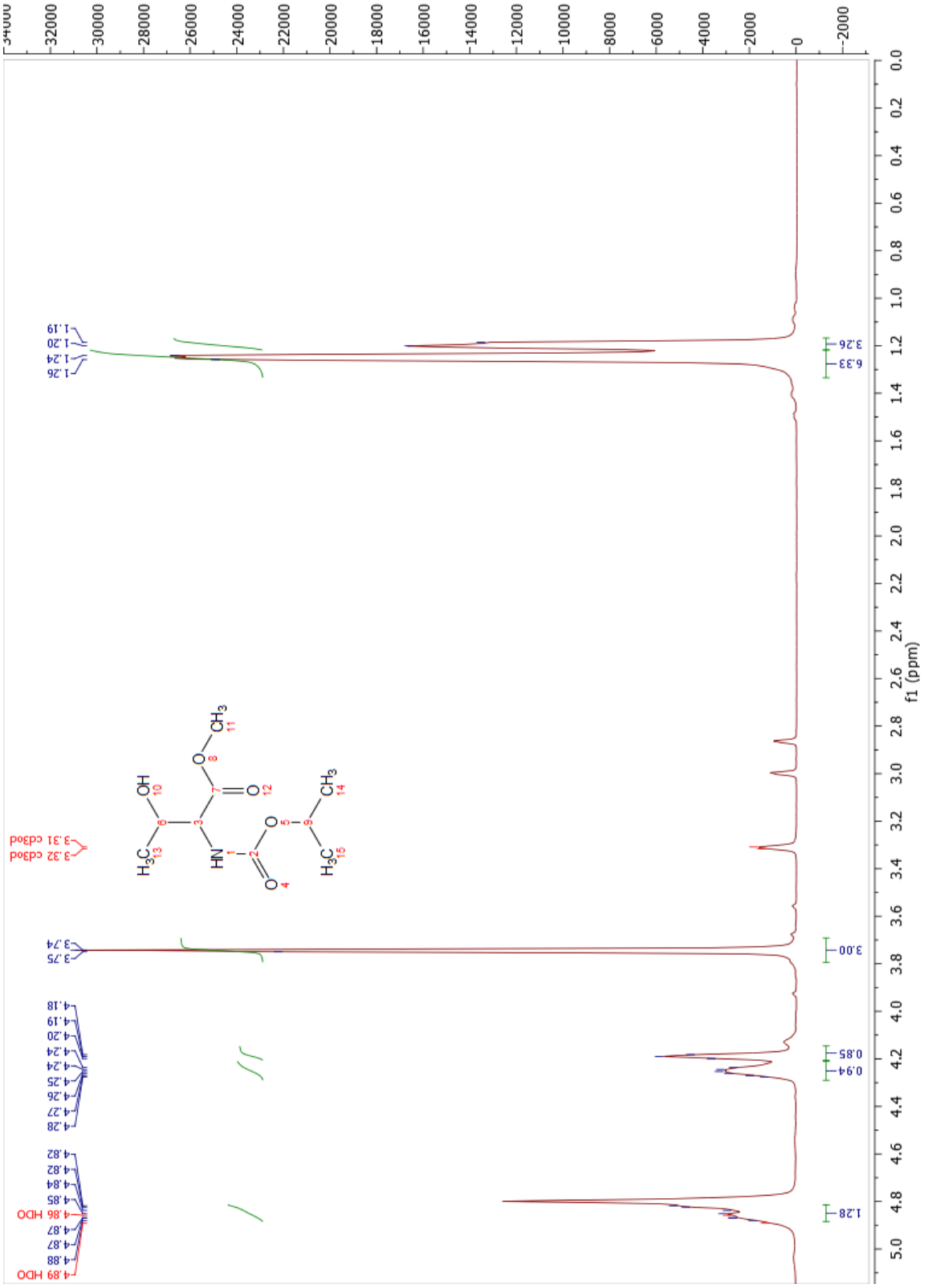
MS of methyl 2-(ethoxycarbonylamino)-3-hydroxybutanoate **54a**:



IR of methyl 2-(ethoxycarbonylamino)-3-hydroxybutanoate **54a**:

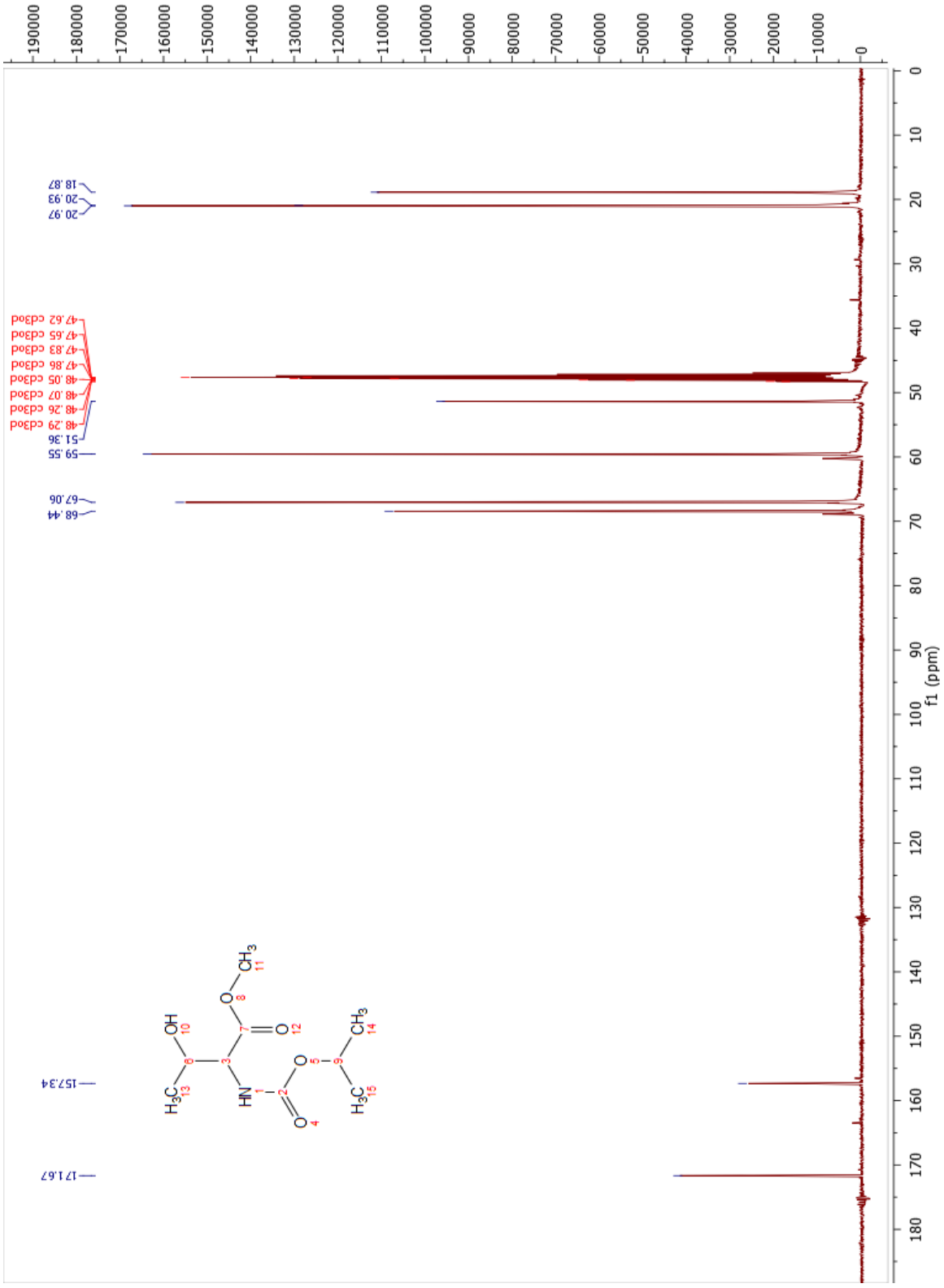


^{1}H NMR of methyl 3-hydroxy-2-(isopropoxycarbonylamino)butanoate **54b**:

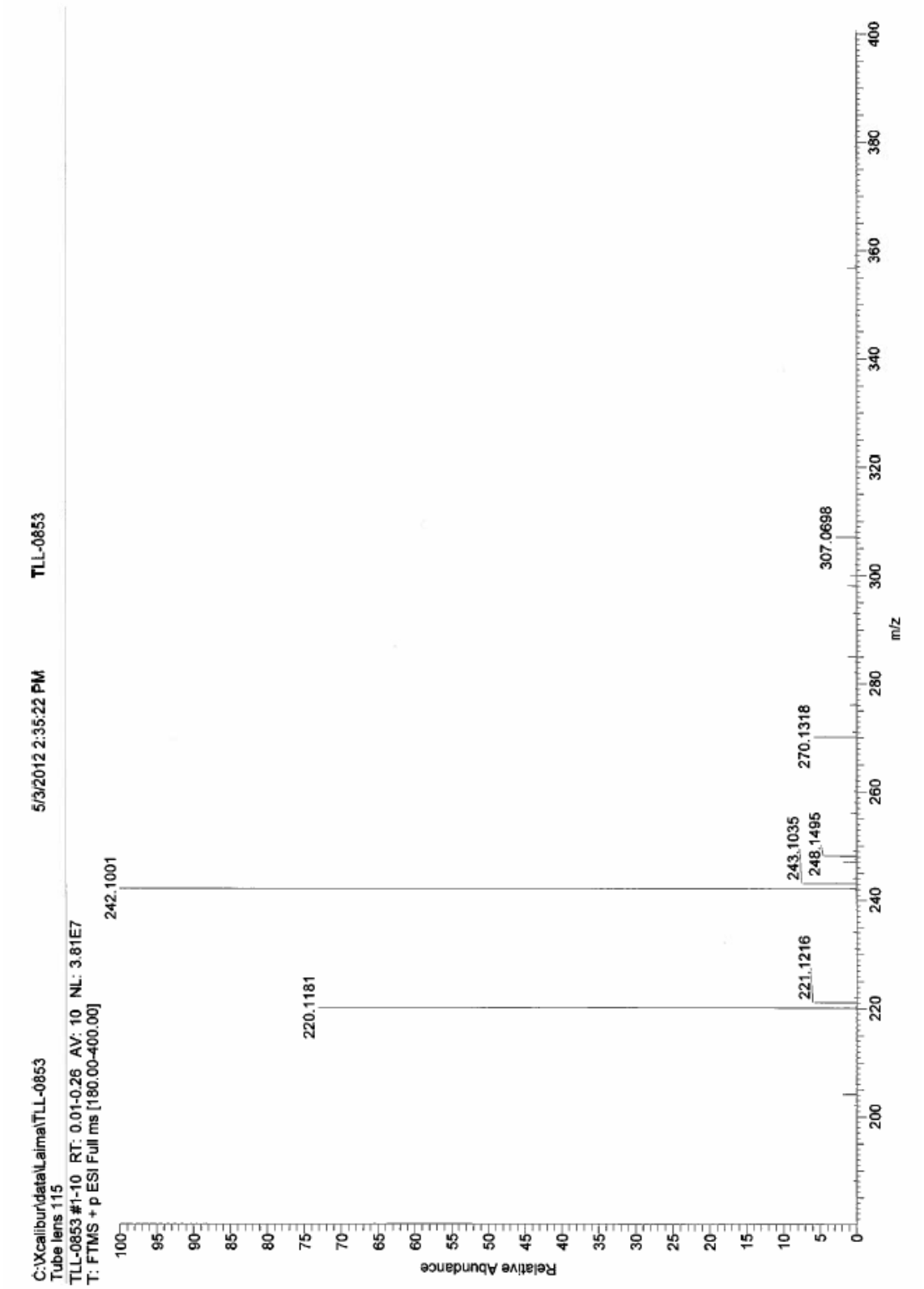


^{13}C NMR of methyl 3-hydroxy-2-(isopropoxycarbonylamino)butanoate

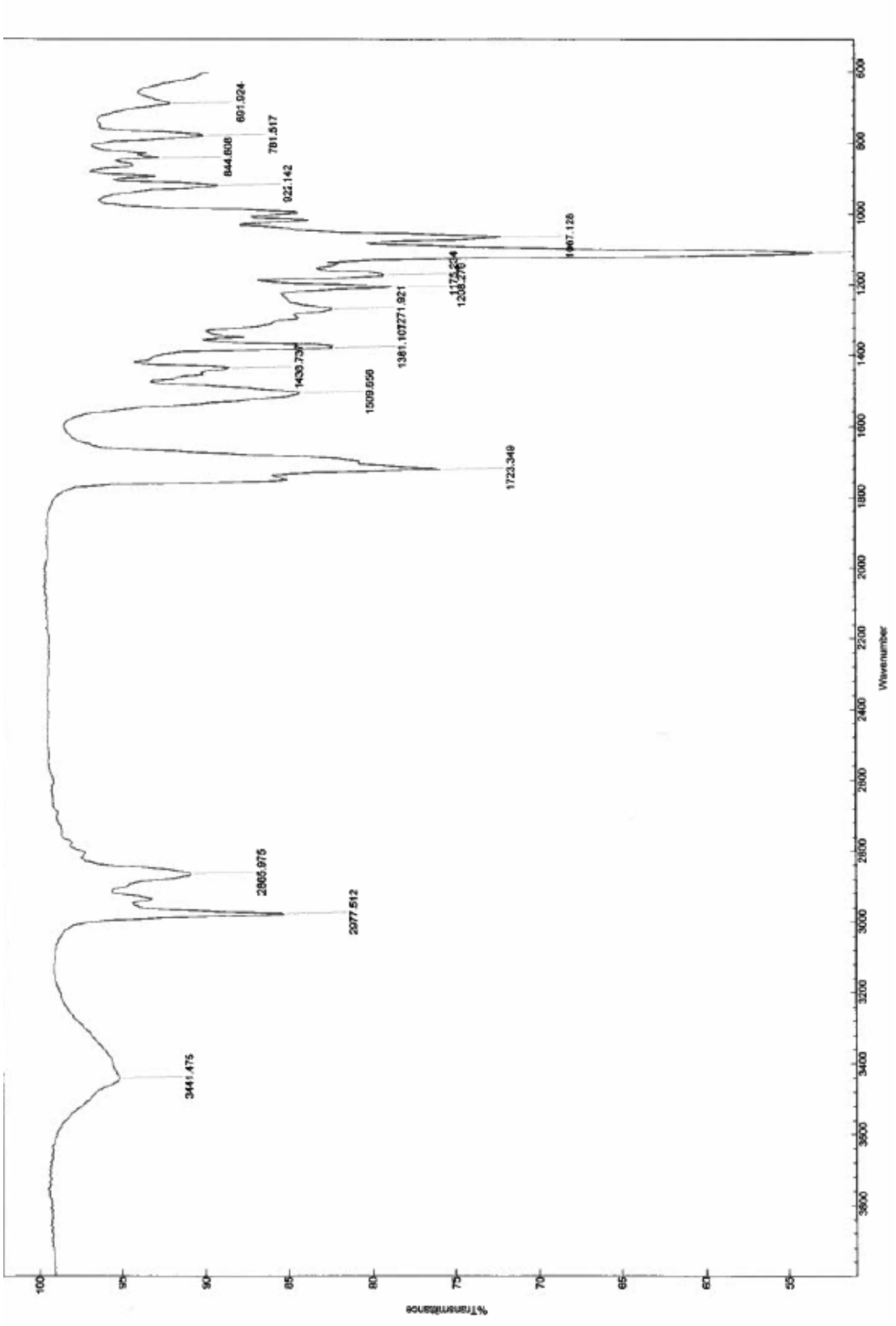
54b:



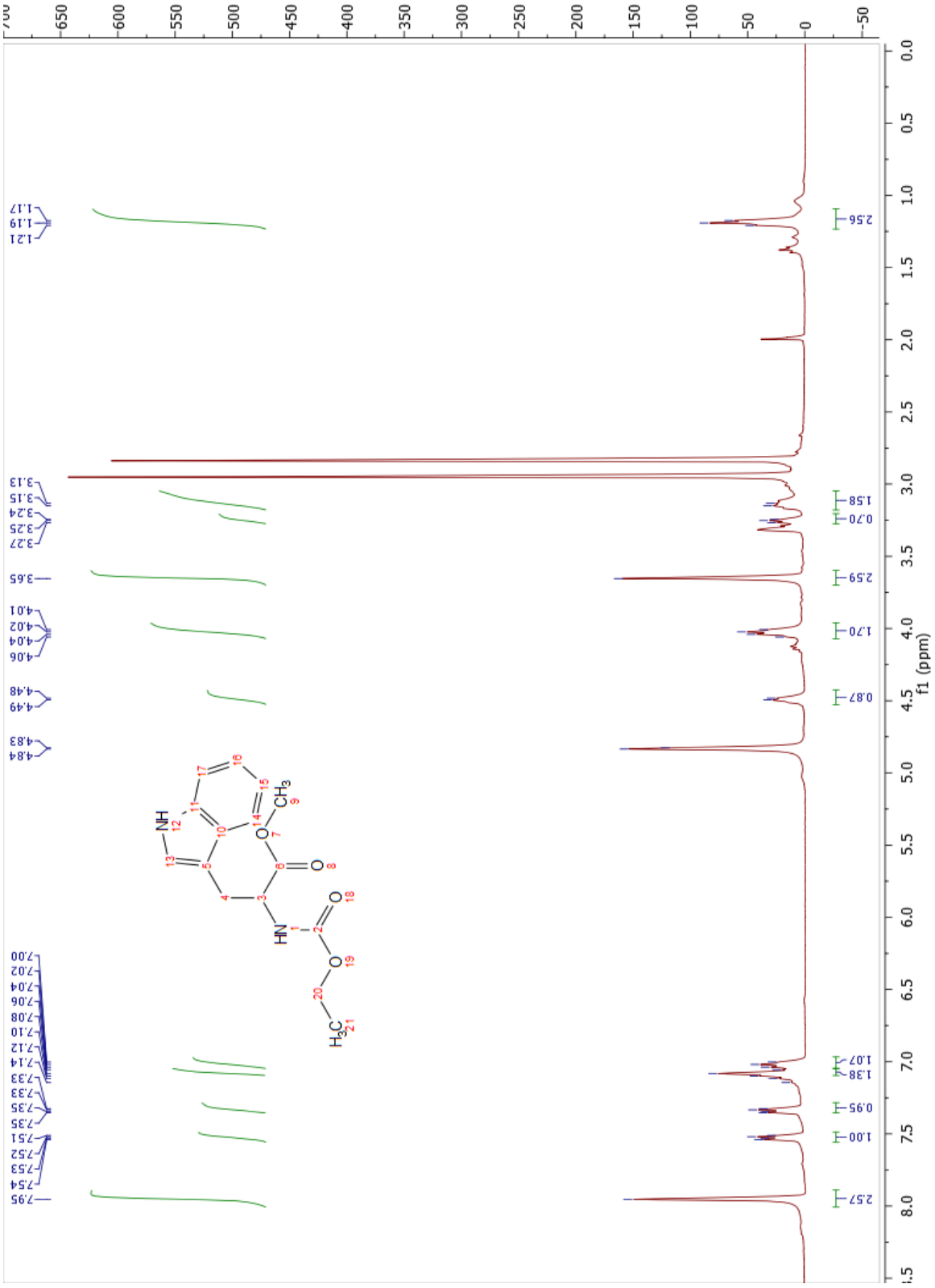
MS of methyl 3-hydroxy-2-(isopropoxycarbonylamino)butanoate **54b**:



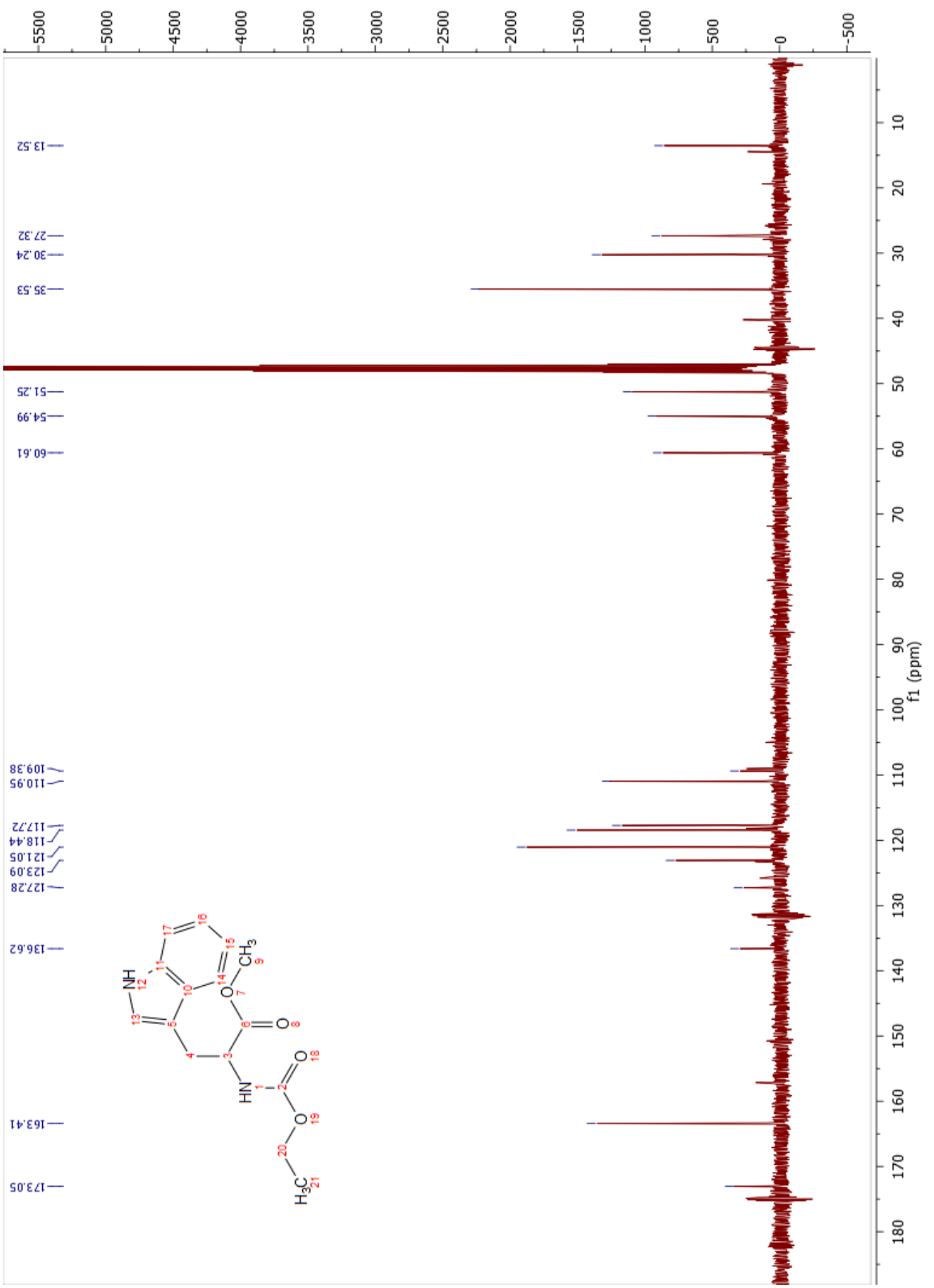
IR of methyl 3-hydroxy-2-(isopropoxycarbonylamino)butanoate **54b**:



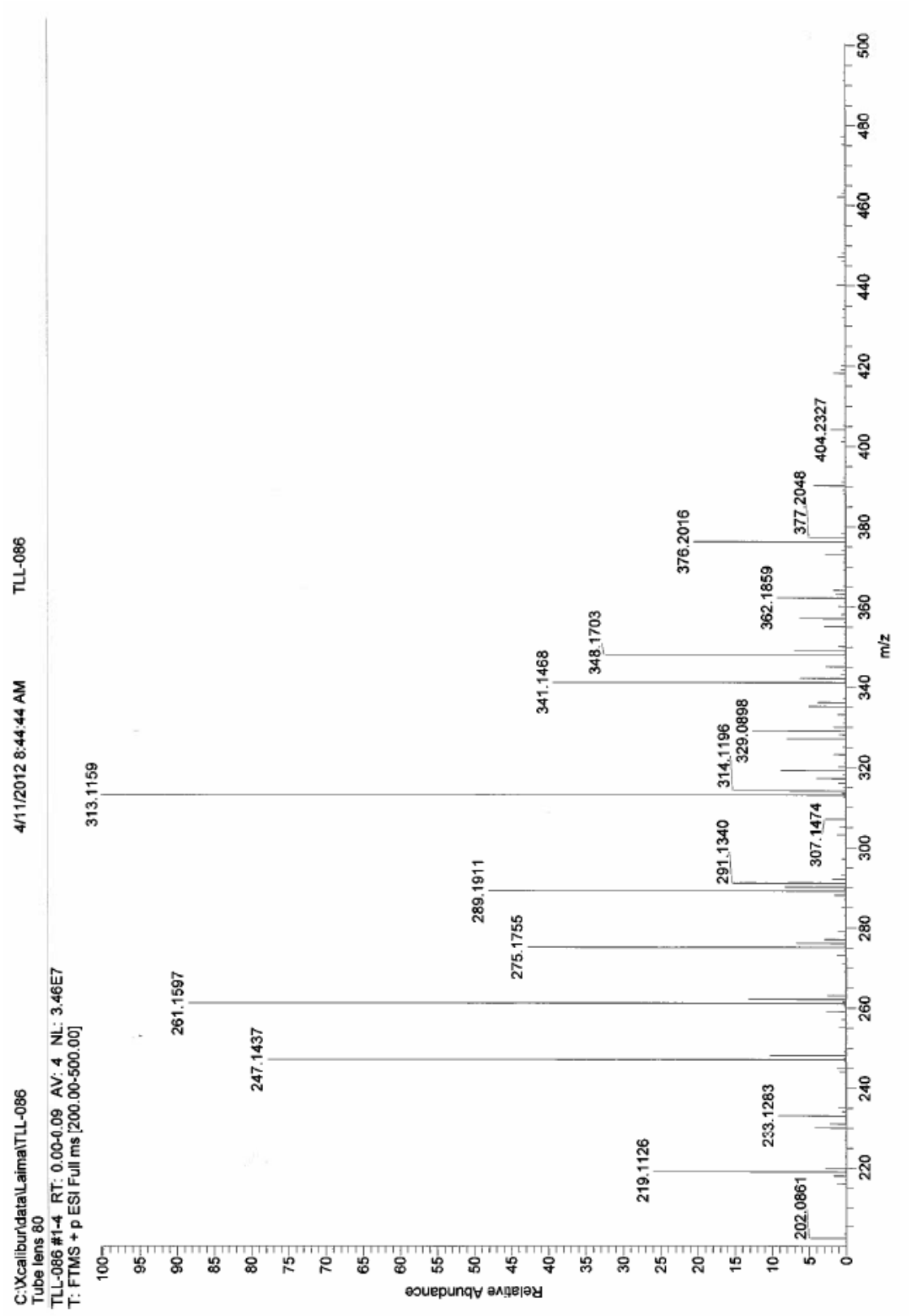
^{1}H NMR of methyl 2-(ethoxycarbonylamino)-3-(1H-indol-3-yl)propanoate **55a:**



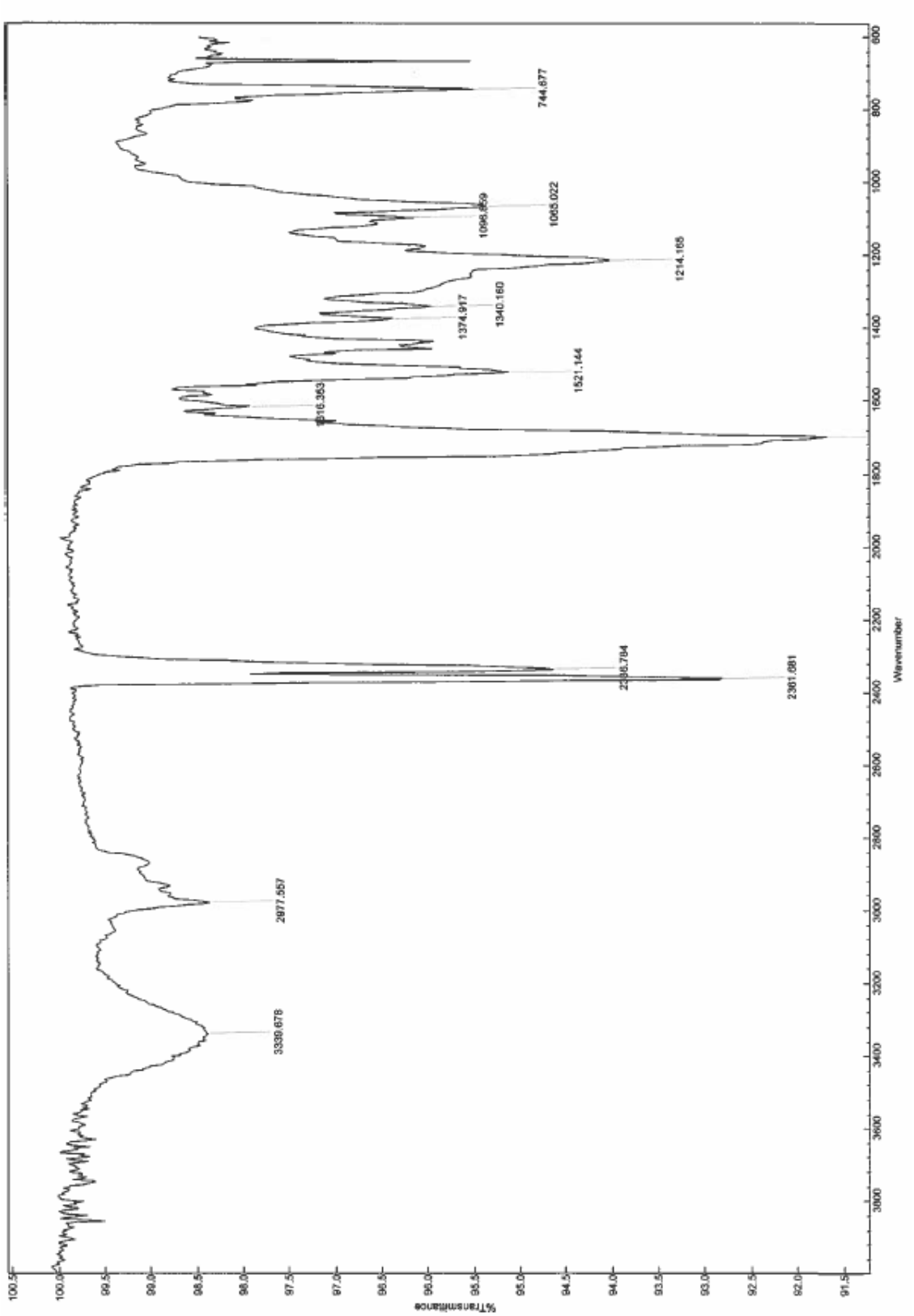
^{13}C NMR of methyl 2-(ethoxycarbonylamino)-3-(1H-indol-3-yl)propanoate **55a:**



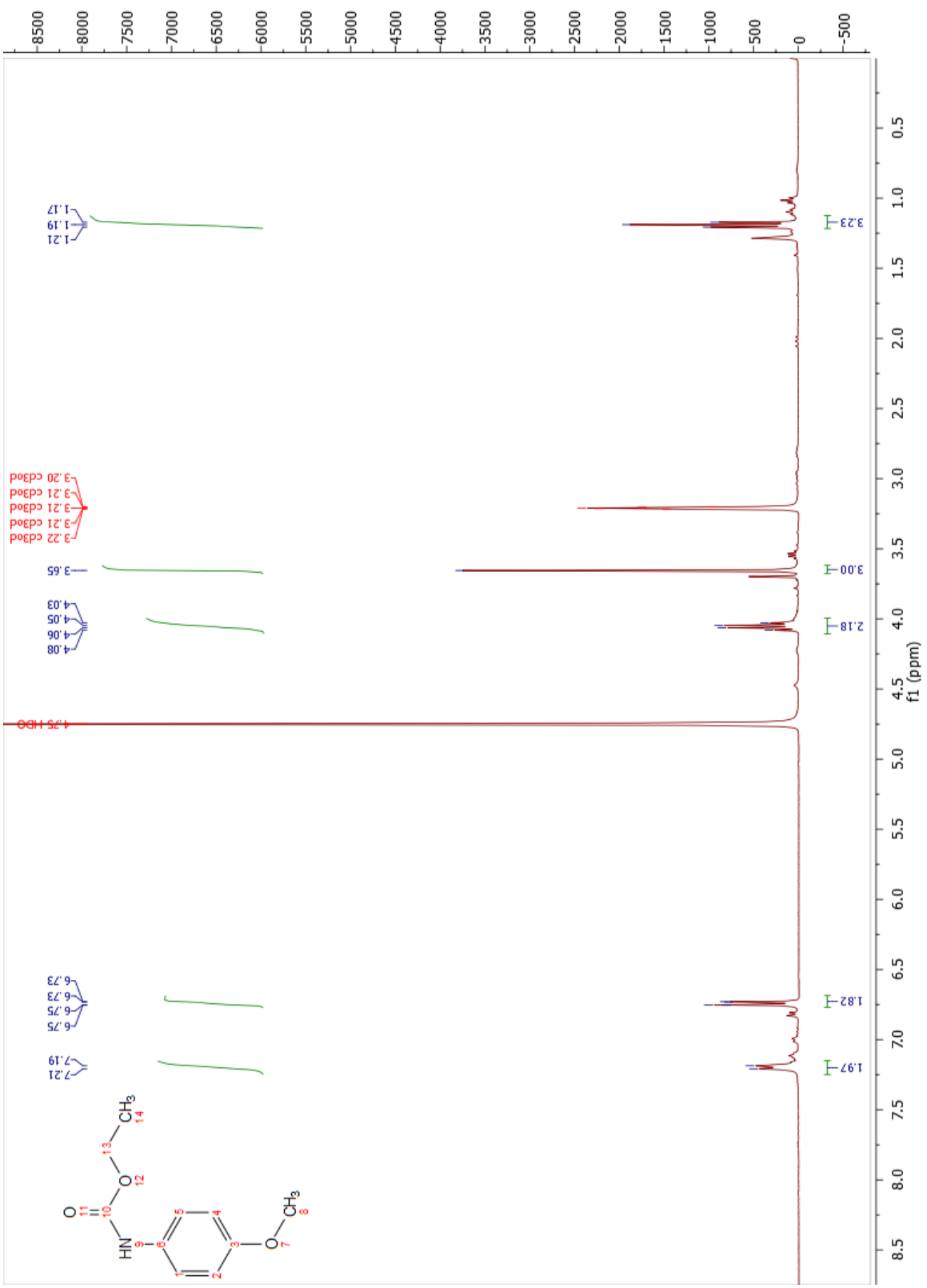
MS of methyl 2-(ethoxycarbonylamino)-3-(1H-indol-3-yl)propanoate **55a:**



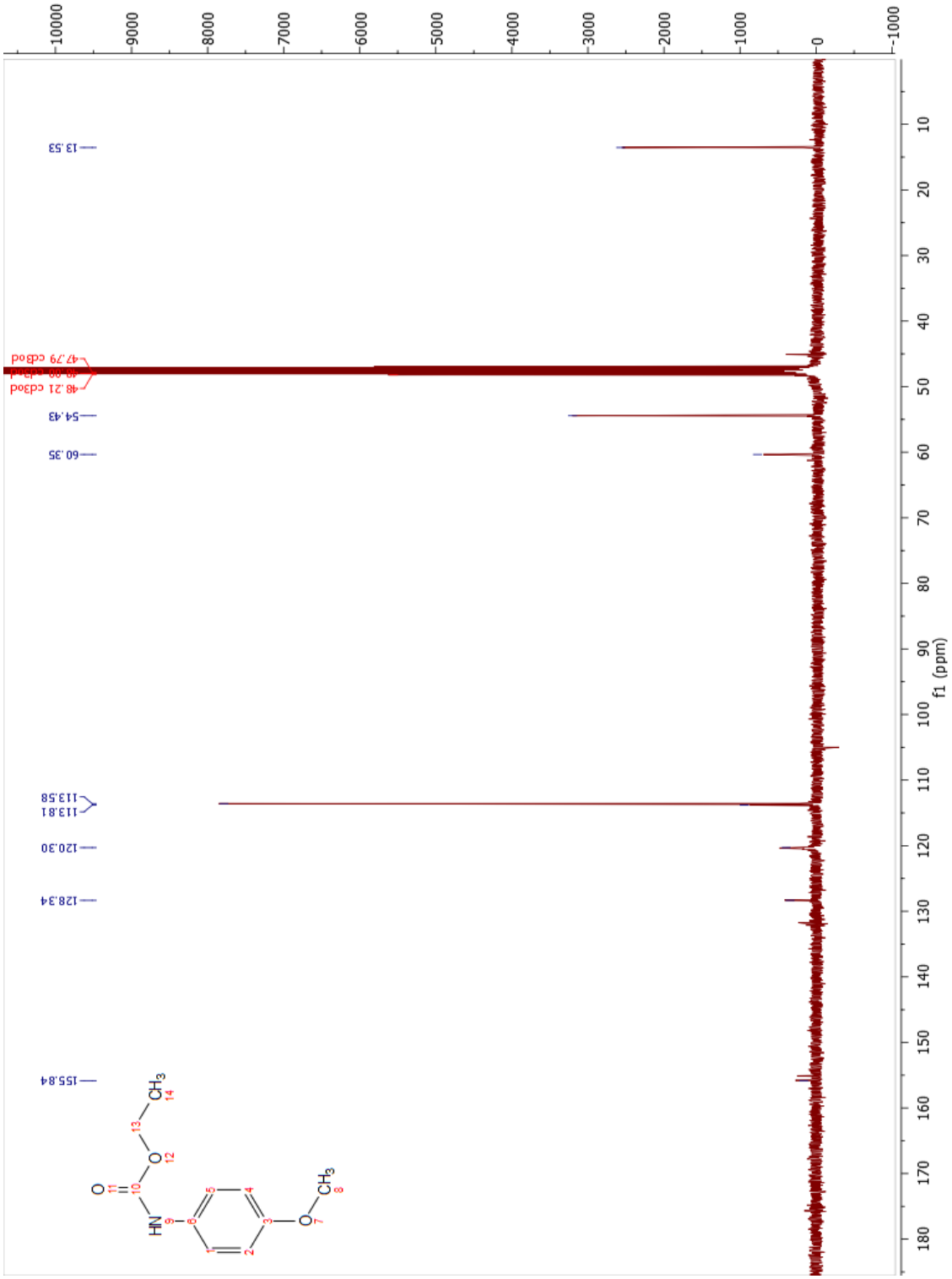
IR of methyl 2-(ethoxycarbonylamino)-3-(1H-indol-3-yl)propanoate **55a:**



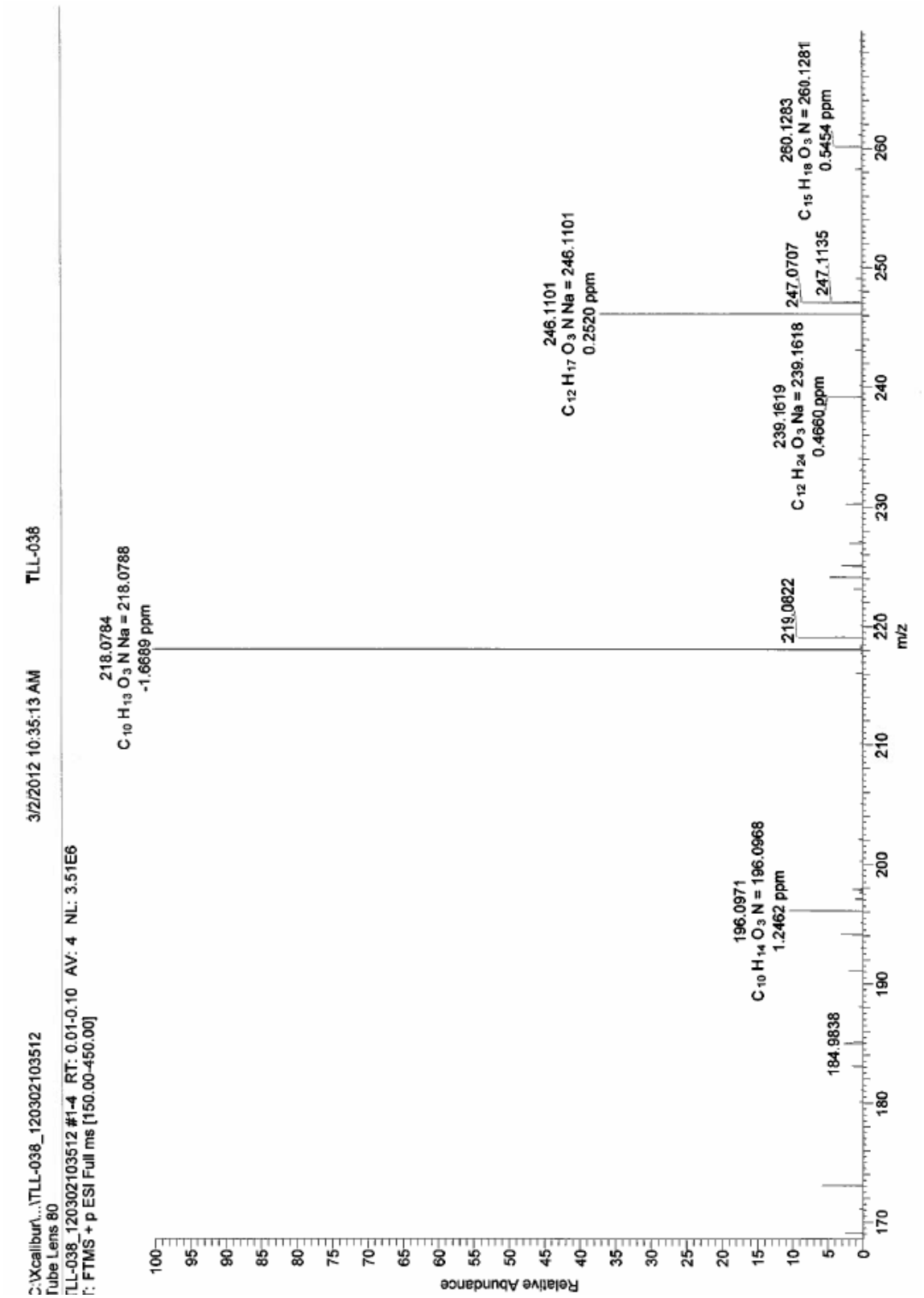
^{1}H NMR of ethyl 4-methoxyphenylcarbamate **56a**:



^{13}C NMR of ethyl 4-methoxyphenylcarbamate **56a**:



MS of ethyl 4-methoxyphenylcarbamate **56a**:



IR of ethyl 4-methoxyphenylcarbamate **56a**: