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Nonaqueous platination for synthesis of platinated 7 alpha-substituted estradiol

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Nonaqueous Platination for Synthesis of Platinated 7α-Substituted Estradiol

(TITLE)

BY

Sarah A. Mazzella

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I would also like to thank the agencies which made this research possible with generous external funding. The estrogen synthesis was funded by grants from NIH and Research Corporation. A grant from The Penny Severns Breast and Cervical Cancer Research Fund supported the nonaqueous platination method development; this grant was my primary financial support while completing the masters' program. I was also the fortunate recipient of the Sidney R. Steele Summer Stipend Award from the EIU Chemistry Department.

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List of Abbreviations

DACH	(R,R)-*trans*-1,2-Diaminocyclohexane
DMF	*N,N*-Dimethylformamide
ER	Estrogen Receptor
EN	Ethylenediamine
IR	Infrared Spectroscopy
MEM	Methoxyethoxymethyl
MS (HRESI)	Mass Spectrometry High Resolution Electrospray Ionization
MS (LRESI)	Mass Spectrometry Low Resolution Electrospray Ionization
MS (LRFAB)	Mass Spectrometry Low Resolution Fast Atom Bombardment
NMR	Nuclear Magnetic Resonance
RBA	Relative Binding Affinity
TBDMS	*tert*-Butyldimethylsilyl
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TLC	Thin Layer Chromatography

Abstract

Cisplatin, carboplatin, and oxaliplatin are used clinically for treating some cancers, but are they are not the preferred treatment for breast cancer. The usefulness of these platinum-containing drugs is limited by poor concentration of the active platinum moiety within the tumor before dose-limiting side effects are reached. One way to selectively concentrate a platinum antitumor compound in breast cancer cells is to conjugate the platinum moiety via a malonate linkage to an estrogen which selectively binds to the estrogen receptor (ER) protein. The major obstacle to linking a lipophilic estrogen to a diamino-platinum compound is the lack of solubility of estrogens in water, the typical solvent for malonate platination reactions.

The development of a nonaqueous platination method was explored with two simple malonic acid derivatives that served as models of an estrogen. The model compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, mass spectrometry, and elemental analysis. In DMF solution, the models were successfully coordinated to either a diaminoplatinum(II) and *trans*-1,2-diaminocyclohexaneplatinum(II) species. Each model complex was characterized by at least three of the following techniques: ^{1}H NMR, ^{13}C NMR, ^{195}Pt NMR, mass spectrometry, and elemental analysis.

The developed nonaqueous platination method was applied to synthesis of a platinated estrogen. The DACH platinated estrogen was characterized by ^{1}H NMR, ^{195}Pt NMR, mass spectrometry, and elemental analysis. The *cis*-dimainoplatinated estrogen was characterized by mass spectrometry.

Chapter I
Introduction

1.1 Overview

According to the American Cancer Society, an estimated 211,300 new cases of breast cancer were expected in the United States in 2004.[1] The majority of these cases are estrogen receptor positive (ER+), meaning that the estrogen receptor protein is overexpressed in the tumor cells. In such cases, ER offers a target for selective delivery of antitumor drugs.[2] Synthesis of two such antitumor compounds (**1**, **2**) with high affinity for ER was the goal of this project. The target compounds are novel in that each is a platinum complex coordinated through a malonate linkage to the 7α position of estradiol. The structures of the compounds are shown in Figure 1.1. The rationale for the design of these compounds is the subject of this chapter.

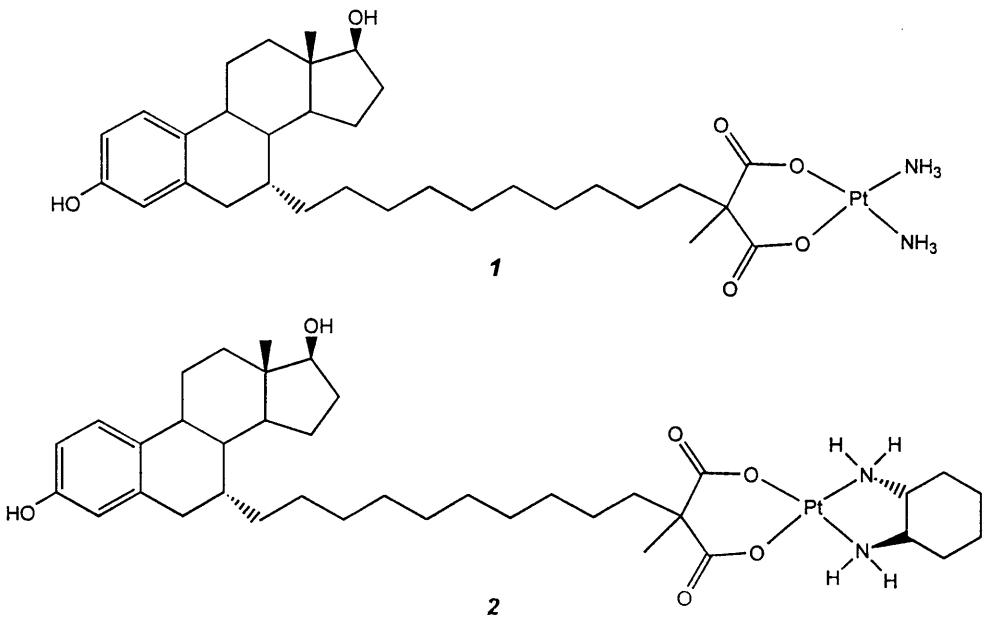


Figure 1.1. Target Molecules

1.2 Some Currently Approved Platinum Drugs

Platinum compounds in current clinical use as antitumor compounds share two common structural features, as emphasized in Figure 1.2. (1) The metal is coordinated to a bidentate amine ligand or two monodentate amine ligands which remain attached when the platinum coordinates to DNA, its ultimate physiological target. The structure of the amine is known to affect the antitumor activity of the compound. (2) The platinum is also coordinated to a leaving group or to two leaving groups. Schwartz et al. found that the more labile the leaving group, the more toxic the platinum compound. For example, *cis*-diamminodinitroplatinum(II) caused death within hours of administration to mice in a dose equivalent to a normal therapeutic dose of cisplatin. The toxicity of the compound was reduced, and the antitumor activity was increased by exchanging the amines with diaminocyclohexane (DACH).[3]

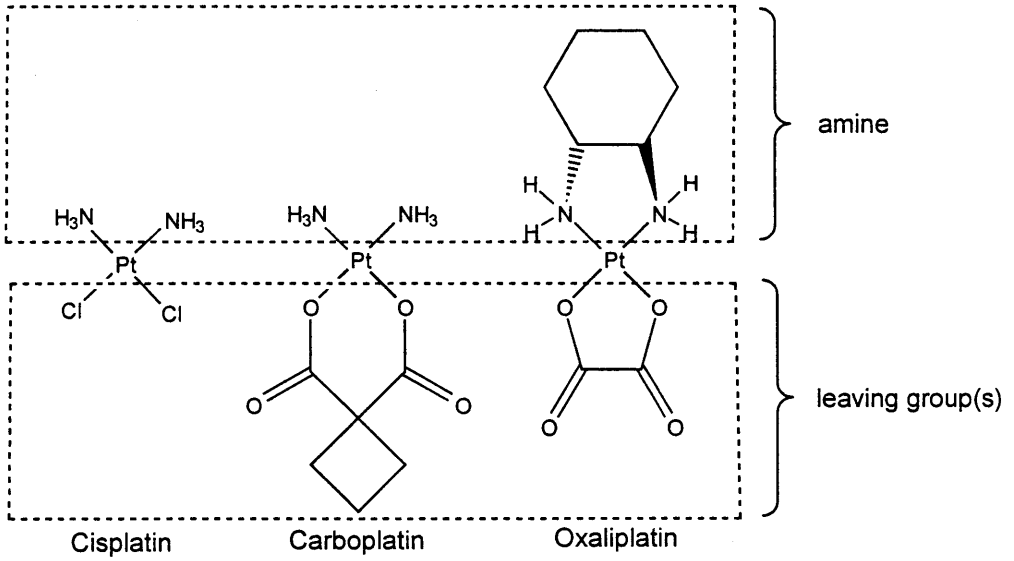


Figure 1.2. Currently Approved Platinum Drugs

Cisplatin [*cis*-diamminedichloroplatinum(II)] is currently one of the most important compounds in the treatment of a variety of cancers such as head, neck, testicular and ovarian cancers. Like all platinum compounds, cisplatin exerts its toxicity by crosslinking of DNA, thereby inhibiting DNA replication. Cisplatin produces the diamminoplatinum moiety that binds preferentially to the N(7) of guanine, forming an inter- or intrastrand cross link. The intrastrand crosslink is believed to be the critical lesion. Figure 1.3 shows a diagram of interstrand cross-linking (l) and intrastrand cross-linking (r) of cisplatin.

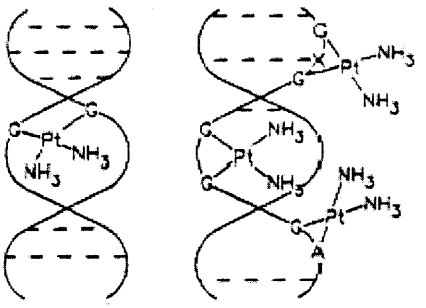


Figure 1.3. Platinum-DNA adduct.

(l)interstrand cross link, (r)intrastrand cross link[4]

A platinum-bridged crosslink alone is insufficient to guarantee inhibition of DNA replication, since DNA repair enzymes are capable of excising the cross-linked nucleotides and replacing them with normal nucleotides. This repair process is effectively blocked by a protein containing a high mobility group (HMG) that binds preferentially to DNA at the crosslink, perhaps as a result of recognizing the change in conformation of the DNA at the site where platinum

binds. The resulting protein-DNA complex is very stable. The HMG protein thus masks the damage from DNA repair processes.[5-10] In general, malignant cells that express the highest levels of HMG proteins are the most susceptible to inhibition by clinically-used platinum drugs.[11,12]

In producing the diamminoplatinum moiety that ultimately binds to DNA, cisplatin is believed to pass through an aquated intermediate, shown in Figure 1.4. This aquated intermediate can give rise to μOH oligomers which are highly toxic.[4]

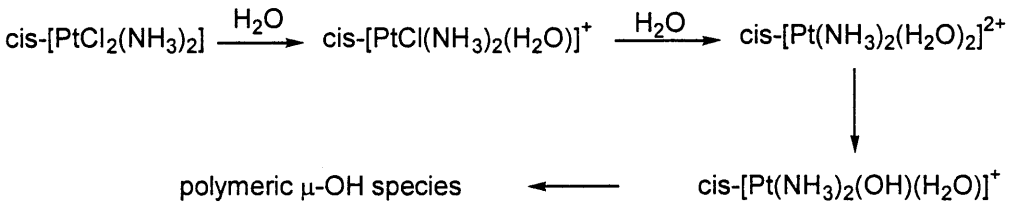


Figure 1.4. Aquation of Cisplatin

Recent studies have shown that doses of cisplatin below that needed to retard DNA synthesis still inhibit cell proliferation. Cisplatin may exert some action without binding to DNA—perhaps by binding to proteins which trigger cell death by apoptotic pathways.[13,14]

Cisplatin's major drawback is its dose-limiting side effects, which include neurotoxicity and nephrotoxicity. Another drawback to cisplatin is that some malignant cells are resistant or become resistant after a single treatment with the drug.[13] This resistance can result from several mechanisms: (1) Conjugation to glutathione preventing the platinum from coordinating to the DNA. (2) An

increase in concentration of DNA repair enzymes that repair the damaged DNA. (3) An increased tolerance to damaged DNA by the cell.[13]

Carboplatin [*cis*-diamminocyclobutanedicarboxylatoplatinum(II)] is considered to be a second-generation platinum drug for cancer treatment. The main advantage of carboplatin over cisplatin is that it causes fewer side effects. Carboplatin doses can be greater than those of cisplatin doses before dose limiting side effects are reached.[15] The leaving group for carboplatin is cyclobutanedicarboxylate. Since the aquation of this leaving group is slower than the aquation of the chlorides in cisplatin, carboplatin has the lower toxicity. Once aquated, carboplatin binds to DNA in the same manner as cisplatin. Carboplatin is given in doses of two orders of magnitude greater than that of cisplatin to achieve the same level of DNA platination.[13] Another advantage to carboplatin is the reduced conjugation to glutathione.[14]

Oxaliplatin [1,2-diaminocyclohexane(oxalato)platinum(II)] is considered to be a third-generation platinum drug. This compound has shown effectiveness in treating some cisplatin-resistant tumors.[16] There are some advantages to using oxaliplatin. (1) The DACH group increases the molecule's lipophilicity for better cellular uptake. (2) It is more effective than cisplatin at inhibiting DNA chain elongation, a phase of replication.[14] The size of the DACH group allows it to fit into the major grove of the DNA strand; consequently, some DNA repair enzymes are unable to detect the platinum lesion.[13,17] The therapeutic advantages of oxaliplatin include low nephrotoxicity, as well as reduction of other

side effects caused by cisplatin.[18] The dose limiting side effect for oxaliplatin is neurotoxicity, as well.[14]

Currently approved platinum drugs such as cisplatin and carboplatin are not very effective at reducing the reoccurrence rates in breast cancer patients especially if they have been previously exposed to other chemotherapies.[18] The current platinum treatments are not successful due to low concentrations of the drugs reaching the tumors. One method that could increase the platinum concentration in tumor cells is the use of a carrier molecule that can improve the specificity of drug delivery to the tumor. The result would enhance the usefulness of platinum compounds in treating breast cancer. ER offers a means of selectively concentrating antitumor compounds in ER+ breast tumors. Carrier molecules that have some estrogenic properties have been linked to platinum compounds.[19-27] Some of these compounds have been investigated for their antitumor activity and binding affinity for the estrogen receptor with little success.

1.3 The estrogen receptor protein

The estrogen receptor protein is member of the steroid receptor superfamily. These proteins are located within the cell's nucleus and regulate gene expression. Some other members of this family include progesterone receptor, glucocorticoid receptor, mineralocorticoid receptor and androgen receptor. There are three domains found in these proteins. (1) One domain is a conserved DNA binding domain of 66-68 residues. (2) The second is the hormone binding domain, which is not conserved in length or amino acid

sequence. (3) The third domain is the transcriptional activation domain that is also variable in length and in sequence.

In its natural role, ER is a transcription factor of genes that regulate development, differentiation, and metabolism in female reproductive tissues. The estrogen receptor is activated by binding of its natural ligand, estradiol. The binding of estradiol results in a change of protein conformation so that the protein can then interact with other transcription factors and with DNA. If no ligand is present this conformation change does not occur.

Any estrogen's ability to bind to ER is measured by its relative binding affinity (RBA). The value of RBA is a ratio of dissociation constants, as shown in Figure 1.5. The RBA value for estradiol is therefore defined as 100. A synthetic estrogen with an RBA value of 20 is considered a good estrogen, whereas an RBA of 5 or less indicates relatively poor estrogenic character.

$$\text{RBA} = \frac{k_D \text{ of estrogen of interest}}{k_D \text{ of estradiol}} \times 100$$

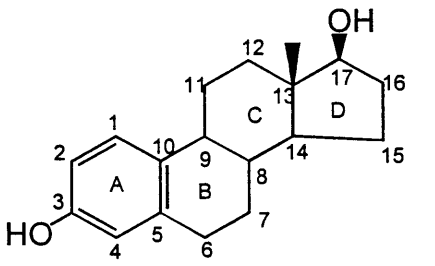
where k_D is the equlibrium dissociation constant of the reaction:

$$\text{ER} \bullet \text{estrogen} \rightleftharpoons \text{ER} + \text{estrogen}$$

Figure 1.5. Definition of RBA

There are two isoforms of ER, ER(α) and ER(β). A ligand that acts as an antagonist to the ER(α) can act as an agonist to the ER(β). The isoform found in ER+ breast cancer cells is predominantly ER(α).[28]

ER tolerates substituents at several locations on estradiol, whose structure and standard steroid numbering are shown in Figure 1.6. The protein also has high affinity for a number of completely synthetic estrogens. Thus ER has motivated the synthesis of "cytotoxic estrogens" intended for use as therapeutic agents. Appropriate substituents can be added to estradiol at the 17α and 7α positions without destroying the binding affinities of ER for the ligand.[29] This fact is due to pockets in the ER binding site at these positions when estradiol is bound to the protein. The 17α position requires substitution via an ethynyl linkage, since a linear geometry at the 17α carbon is required to maintain high affinity.[29] The pocket at the 17α position is smaller in size than the pocket at the 7α position, and both pockets are hydrophobic in nature.[29]



◄ = above the plane of the ring = β

IIIIII = below the plane of the ring = α

Figure 1.6 Structure of Estradiol and Steroid Carbon Numbering

Estradiol derivatives with substituents in the 17α position act as ER agonists[30], while substituting at the 7α position produces ER antagonists.[29] Affinity for ER is known to vary with the nature of the group at the end of a

hydrocarbon chain at the 7α position.[31] The more polar the group at the end of the hydrocarbon chain, the greater the binding affinity. The long chain may allow the polar substituent at the end of the chain to remain outside the receptor-hormone complex.

1.4 Current Platinated Estrogens

The use of an estrogen as the carrier molecule for platinum could provide several benefits. The first would be concentration of the platinum compound in malignant cells that over-express the estrogen receptor. Other benefits could result from hormonal actions of the estrogen. If the estrogen (either platinated or unplatinated), were an ER agonist, the result might be an increase in cellular concentrations of the HMG1 protein. Cotreatment of MCF-7 cells (a common ER+ cell culture line of breast tumor cells) with estradiol and either cisplatin or carboplatin increases the number of platinum-DNA adducts by twofold.[16] Sensitization of the cells to cisplatin is most marked when the timing of treatments allows estradiol to reach the cell first and upregulate HMG1 production before the platinum drugs are aquated to their active form.

Nonsteroidal Platinated Estrogens

One of the first published platinated estrogens was nonsteroidal, as shown in Figure 1.7A. The compound displayed relatively low binding affinity for the estrogen receptor. However, the compound caused less damage to kidneys in mice than did cisplatin.[20] Later work placed chlorines meta to the phenol, and the resulting platinum complex showed some estrogenic and cytotoxic properties. The compound remained a poor ligand for ER.[21]

A second type of platinated estrogen consists of a 2-phenylindole derivative connected by a linking group to an amine that coordinates to platinum.[22] Various linkers were evaluated. The compounds with the highest binding affinity are shown in Figure 1.7B, C. Compound 1.7B had an RBA of 6.5 and compound 1.7C an RBA of 4.4. These are low binding affinities but extend the concept of linking an estrogenic moiety to a platinum compound.

A similar platinated 2-phenylindole derivative with estrogenic properties was found to have some effectiveness against ER+ breast cancer cells *in vitro*. This derivative is shown in Figure 1.7D. The binding affinity for this complex was low at 5.2. The lack of effectiveness against tumor cells was attributed to lack of platinum release from the carrier molecule to cross link with the DNA.[23] Later work by demonstrated this that this compound was not concentrated in ER-rich tissues but was primarily deposited in the liver of rats during *in vivo* studies.[19]

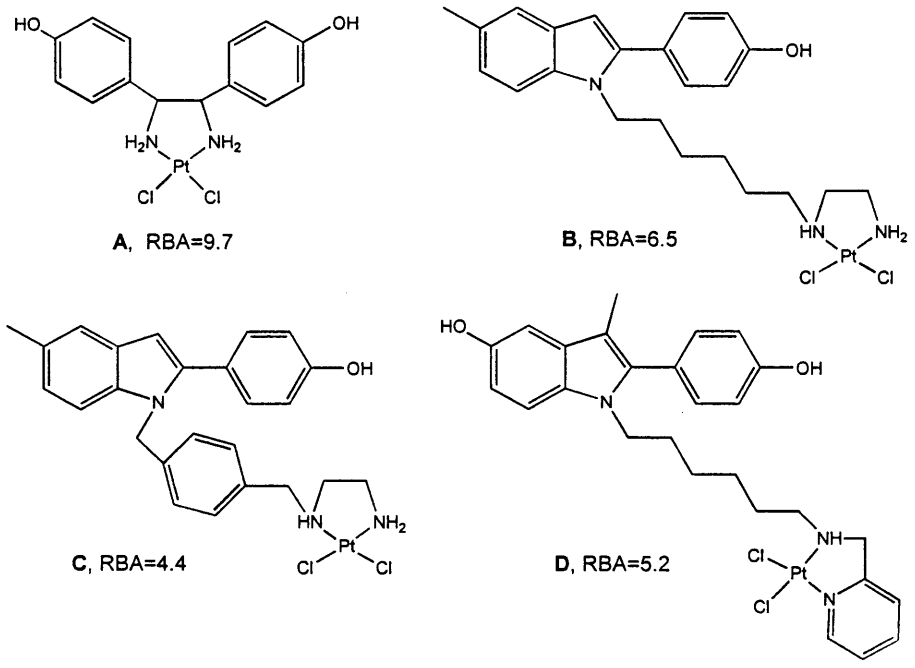


Figure 1.7. Nonsteroidal Platinated Estrogens

Steroidal

One of the first reported steroidal platinated estrogens is shown in Figure 1.8A. Although the compound's RBA was not reported, a low value could reasonably be anticipated, since the O3, which is required for high affinity for ER, is functionalized. At 5 μM, the antitumor activity of 1.8A was measured at 55.05±0.40% inhibition of MCF-7 cells, an ER+ line of breast cancer cells. Cisplatin, at the same concentration, displayed 68.46±0.50% inhibition of the MCF-7 cells. This value makes the compound approximately 80% as effective as cisplatin.[24]

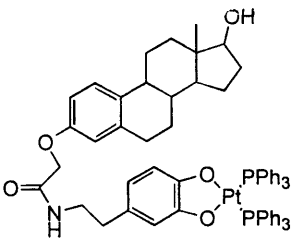
Like 1.8A, the steroidal platinated estrogen shown as Figure 1.8B employs an amine linkage from the estrogen to platinum. Compound 1.8B had a very low binding affinity for ER. This fact is not surprising, since neither OH group of estradiol has been preserved in the estrogen-platinum conjugate.[25]

The platinated steroidal estrogen shown in Figure 1.8C is the first example of a malonate linkage from the estrogen to platinum; this design thus places the estrogen in the leaving group.[26] No biological data has been published on this complex since it was first described in 1989.

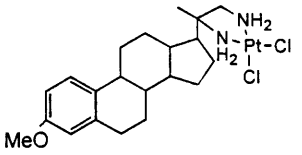
The pair of platinated steroidal estrogens shown as 1.8D and 1.8E are similar to 8C in that all three compounds link platinum to estradiol's 17α position. Both 8D and 8E displayed low affinity for ER; perhaps the polarity of the substituent is higher than optimal. However, both compounds were readily taken up into whole cells.

Compound 1.8F is actually a series of complexes that differ in lengths of two linkers. The design is unusual in the sense that conjugation is from the 16α position of estradiol, rather than the more typical 17α substitution site. No RBA data are reported for these compounds. However, IC_{50} values were measured in both ER+ and ER- cell lines. Some complexes were essentially inactive, and some displayed IC_{50} values in the low micromolar range. The complex in which m = 2 and n = 8 is the most significant, since its IC_{50} values are 3 – 4 fold lower than that of cisplatin.[32]

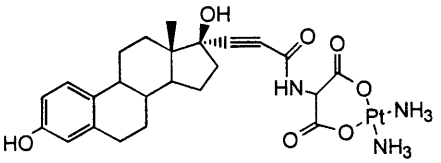
The platinated estrogen shown as series 1.8G is unique in several respects, the most obvious of which is use of platinum (IV). After uptake into the cell, compounds in the 1.8G series are designed to yield cisplatin upon exposure to the intracellular reducing environment. Hydrolysis of the remaining estradiol ester should release two equivalents of estradiol, which is known to upregulate production of HMGB1, a protein that prevents excision of platinum-crosslinked nucleotides from DNA. Thus 1.8F represents a significant departure from typical strategy, since the estrogen moiety is designed to increase sensitivity to cisplatin rather than to effect concentration of the entire complex in a malignant cell by virtue of binding to ER. Hence, no RBA was reported for 1.8F. The compounds proved comparably toxic to ER+ and ER- cell lines, and IC_{50} values were in the low micromolar range, i.e. 2 – 4 fold lower than the value of cisplatin.[33]



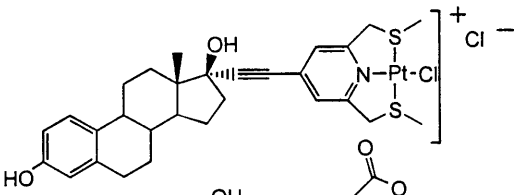
A, RBA=NA



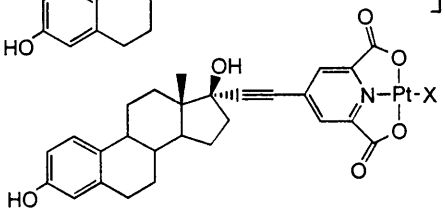
B, RBA=0.12



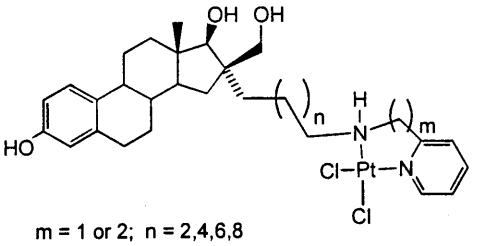
C, RBA=NA



D, RBA=3.3

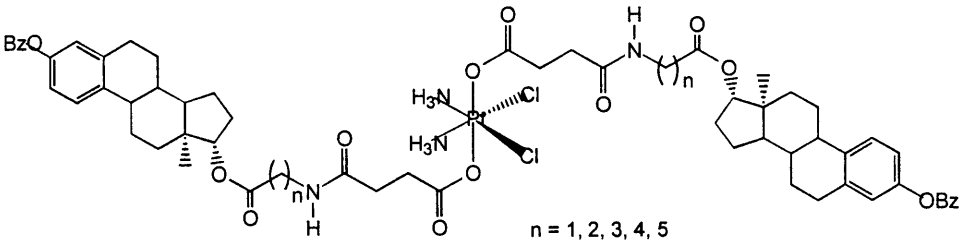


E, RBA=0.027



m = 1 or 2; n = 2,4,6,8

F, RBA = NA



n = 1, 2, 3, 4, 5

G, RBA = NA

Figure 1.8. Steroidal Platinated Estrogens

Previously published platinated estrogens all share the common feature of poor binding to the estrogen receptor. A general explanation for this fact may be that excessive bulk in the amine ligand is known to lower antitumor activity.[34] In most published cases, the estrogen moiety adds significantly to the bulk of the amine ligand.

1.5 Target Compounds

A prominent feature of compounds **1** and **2** is the use of conjugation to the 7α position of estradiol. Although this position is the most tolerant of bulky, polar substituents, there is no precedent for using it as the point of conjugation to platinum. This design is deemed the single most important factor favoring high affinity of the platinum complex for ER.

Although delivery of estrogen and platinum together can sensitize a cell to platinum, the target compounds are unlikely to act via this mechanism. Because the estrogen is substituted at the 7α position, it is most likely to function as an ER antagonist. Consequently, the compound's action is not expected to include upregulation of genes that encode HMG-containing proteins that cause sensitization to platinum. However, by the mere property of ER-antagonism, the target compounds should inhibit cell proliferation in addition to any action by platinum.

The design of **1** and **2** allows complete freedom for use of amines that are known to allow optimal antitumor activity of platinum. Building the estrogen into the leaving group should leave the amine completely unencumbered. Furthermore, joining the estrogen to a malonate unit in the metal's coordination

sphere should yield a molecule sufficiently stable to hydrolysis to suppress the nonspecific binding to serum proteins that has plagued some previous platinated estrogens.

References

1. http://www.cancer.org/downloads/STT/CAFF2003BrFPWSecured.pdf (Accessed 01/17/05).
2. von Angerer, Erwin *The Estrogen Receptor as a Target for Rational Drug Design*, R.G. Landes Company, 1995.
3. Schwartz, P.; Meischen, S.J.; Gale, G.R.; Atkins, L.M.; Smith, A.B.; Walker Jr, E.M. *Cancer Treatment Reports* **1977**, *61*, 1519.
4. Pascini, A.; Zunino, F. *Angew. Chem. Int. ed. Engl.* **1987**, *26*, 615.
5. Zamble, D.B.; Mu, D.; Reardon, J.T.; Sancar, A.; Lippard, S.J. *Biochemistry*, **1996**, *35*, 10004.
6. Brown, S.J.; Kellet, P.J.; Lippard, S.J. *Science*, **1993**, *261*, 603.
7. McA'Nulty, M.M.; Whitehead, J.P.; Lippard, S.J. *Biochemistry*, **1996**, *35*, 6089.
8. Wei, M.; Cohen, S.M.; Silverman, A.P.; Lippard, S.J. *J. Biol. Chem.*, **2001**, *276*, 38774.
9. Jung, Y.; Lippard, S.J. *Biochemistry*, **2003**, *42*, 2664.
10. Wei, M.; Burkenkova, O.; Lippard, S.J. *J. Biol. Chem.*, **2003**, *278*, 1796.
11. Ohndorf, U.M.; Whitehead, J.P.; Raju, N.L.; Lippard, S.J. *Biochemistry*, **1997**, *36*, 14807.

12. Zamble, D.B.; Mikata, Y.; Eng, C.H.; Sandman, K.E.; Lippard, S.J. *J. INorg. Biochem.* **2002**, *91*, 451.

13. Brabec, V. *Progress in Nucleic Acid Research And Molecular Biology*, **2002**, *71*, 1.

14. Fuertes, M.A.; Castilla, J.; Alonso, C.; Perez, J.M. *Curr. Med. Chem. Anti-Can Agents* **2002**, *2*, 539

15. Knox, R.J.; Friedlos F.; Lydall, D.A.; Roberts, J.J. *Cancer Research* **1986**, *46*, 1972.

16. Ho, Y.; Au-Yeung, S.C.F.; To, K.K.W. *Medicinal Research Reviews* **2003**, *23*, 633.

17. Spingler, B.; Whittington, D.A.; Lippard, S.J. *J. Inorg. Chem.* **2001**, *40*, 5596.

18. Martin, M. *Clinical Breast Cancer* **2001**, *2*, 190.

19. DiZio, J.P.; Carlson, K.E.; Bannochie, C.J.; Welch, M.J.; von Angerer, E.; Katzenellenbogen, J.A. *J. Steroid Biochem. Molec. Biol.* **1992**, 363.

20. Wappes, B.; Jennerwein, M.; von Angerer, E.; Schonenberger, H.; Engel, J.; Berger, B.; Worbel, K. *J. Med. Chem.* **1984**, *27*, 1280.

21. Karl, J.; Gust, R.; Spruss, T.; Scheider, M.R.; Schonenberger, H.; Engel, J.; Wrobel, K.; Lux, F.; Haeberlin, S.T. *J. Med. Chem.* **1988**, *31*, 72.

22. Knebel, N.; von Angerer, E. *J. Med. Chem.* **1988**, *31*, 1675.

23. Knebel, N.; von Angerer, E. *J. Med. Chem.* **1991**, *34*, 2145.

24. Gandolfi, O.; Blum, J. *Inorg. Chem. Acta,* **1984**, *91*, 257.

25. Georgiadis, M.P.; Haroutounian, S.A. *Inorg. Chem. Acta.* **1987**, *138*, 249.

26. Gandolfi, O.;Apfelbaum, H.C.; Mogron, Y.; Blum, J. *Inorg. Chem. Acta.* **1989**, *161*, 113.

27. Jackson, A.; Davis, J.; Pither, R.J.; Rodger, A.; Hannon, M.J. *Inorg. Chem.* **2001**, *40*, 3964.

28. Paech, K.; Webb, P.; Kuiper, G.G.J.M.; Nilsson, S.; Gustafsson, J.; Kushner, P.J.; Scanlan, T.S. *Science*, **1997**, *277*, 1508.

29. Anstead, G.M.; Carlson, K.E.; Katzenellenbogen, J.A. *Steroids* **1997**, *62*, 268.

30. Pomper, M.G.; VanBrocklin, H.; Thieme, A.M.; Thomas, R.D.; Kieseweyer, D.O.; Carlson, K.E.; Mathias, C.J.; Welch, M.J.; Katzenellenbogen, J.A. *J. Med. Chem.* **1990**, *33*, 3143.

31. DaSilva, J.N.; van Lier, J.E. *J.Med. Chem.* **1990**, *33*, 430.

32. Descoteaux, C.; Provencher-Mandeville, J.; Mathieu, I.; Perron, V.; Mandal, S.K.; Asselin, E.; Berube, G. *Bioorganic and Medicinal Chemistry Letters* **2003**, *13*, 3927.

33. Barnes, K.R.; Kutikov, A.; Lippard, S.J. *Chemistry and Biology* **2004**, *11*, 557.

34. Gibson, D.; Gean, K.; Ben-Shoshan, R.; Ramu, A.;, Ringel, I.; Katzhendler, J. *J. Med. Chem.* **1991**, *34*, 414

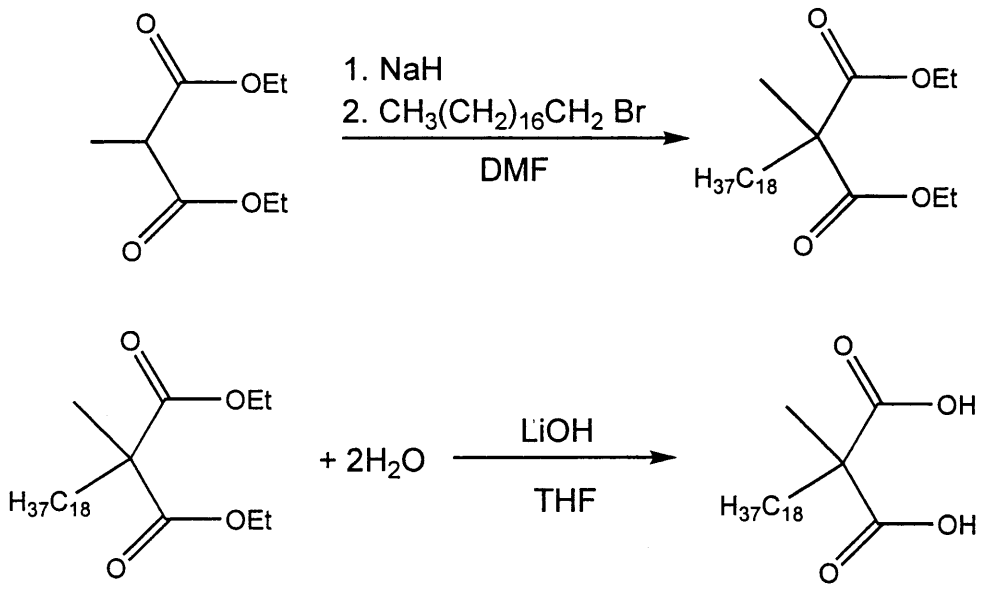
Chapter II: Synthesis of Substituted Malonic Acids as Models for Platination Reactions

Section I: Introduction

Development of a method of linking platinum to an estrogen via a malonate group was undertaken with simple derivatives of malonic acid for two reasons: (1) The 7-α substituted estradiol compound is made by a multistep synthesis and is in short supply. (2) The lipophilic character of the synthetic estrogen made it a poor prospect for convenient platination by traditional aqueous methods, such as displacement of barium from a barium salt.[1] The model lipophilic malonic acids were chosen due to their ease of synthesis, purification and their resemblance to the synthetic estrogen.

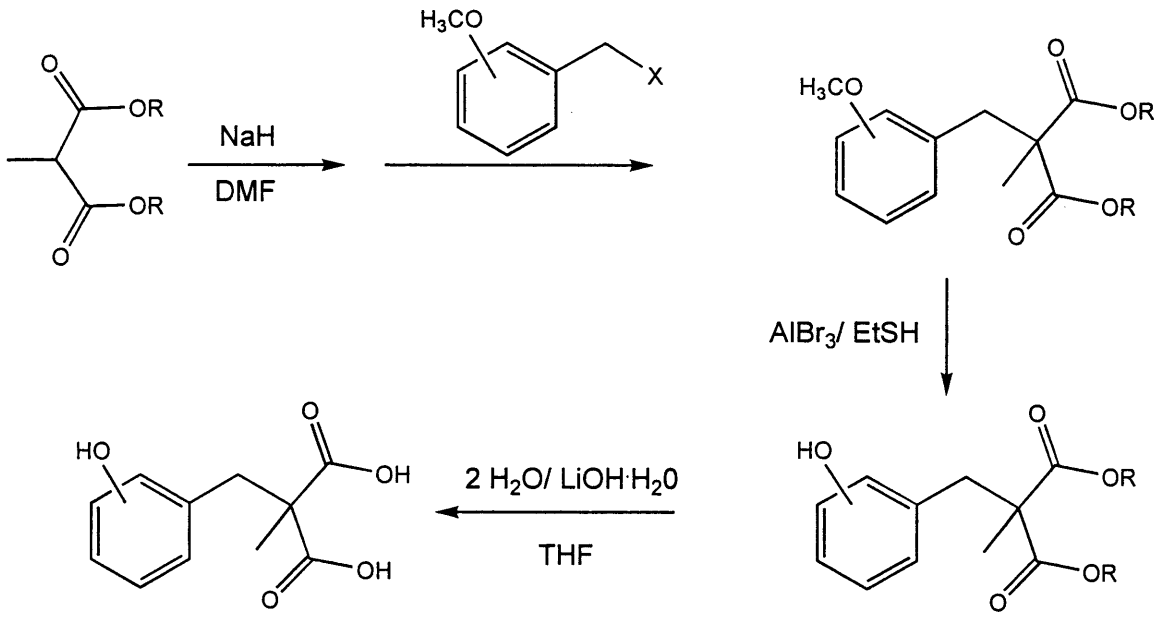
The first model malonic acid was designed based on lipophilicity and ease of synthesis. The proposed synthesis for this model is shown in Scheme 2.1. The synthesis of the first model is accomplished in two steps. The first step is the alkylation of diethyl (methyl) malonate with 1-bromooctadecane. The second step is the saponification of the esters with aqueous lithium hydroxide. The main disadvantage of this model is that it does not share any functional groups with the synthetic estrogen. The only shared characteristic of this model with the synthetic estrogen is lipophilicity. The main advantage of this model is its ease of synthesis and purification. The product of the second step could be isolated as the lithium salt, which only required acidification to obtain the final pure product.

Scheme 2.1 Proposed Synthesis of 2-Methyl-2-octadecylmalonic Acid



The second model malonic acid was developed to introduce a shared functional group with the synthetic estrogen. The functional group is a phenol. The synthesis for the second model is shown in Scheme 2.2. The second model is synthesized in three steps. The first step is the alkylation of the malonate ester with 3-methoxybenzyl bromide or 4-methoxybenzyl chloride. The second is the removal of the methyl ether with aluminum bromide and ethane thiol. The third step is saponification of the esters with aqueous lithium hydroxide to afford the product. The main disadvantages of this model are the more complex synthesis and difficulty in isolation of the final pure product. The main advantage of this model is its shared functional group with the synthetic estrogen.

Scheme 2.2 Proposed Synthesis of 2-(3-Hydroxybenzyl)-2-methylmalonic Acid

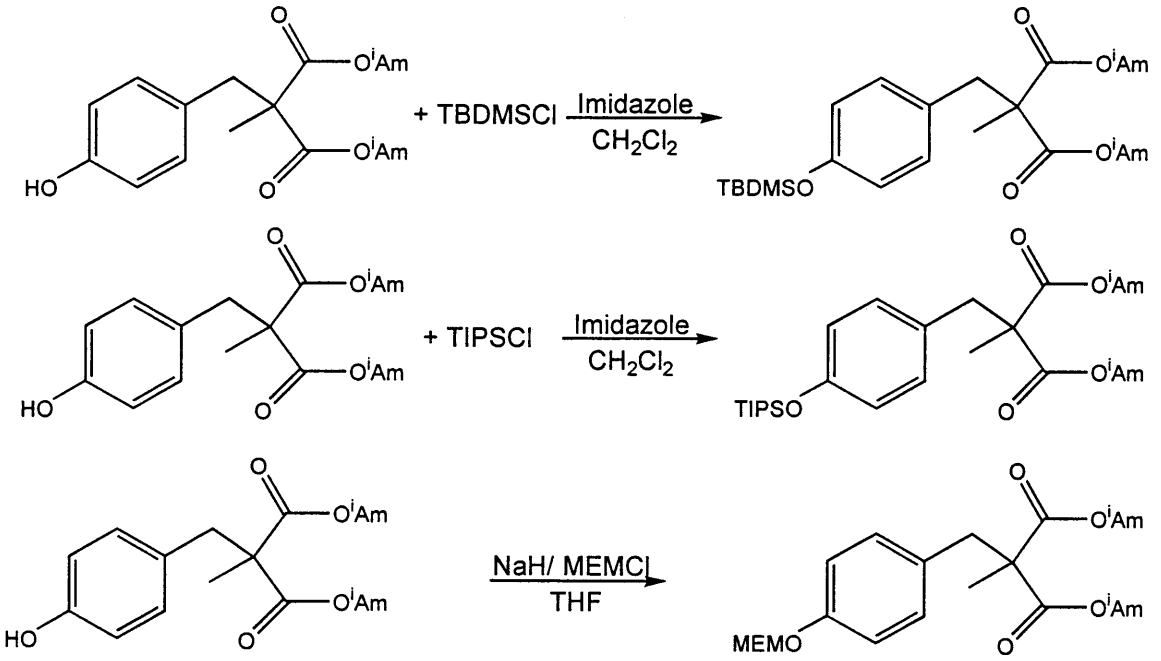


R = ethyl, benzyl, isoamyl
X = Br, Cl

The variety in the esters used was due to a probable need of a protecting group for the phenol during the final platination procedure. Although the initial ester used was the diethyl ester, isoamyl groups were emphasized since they are used in the synthetic route to the 7α-substituted estrogen. Several different protecting groups for the phenolic oxygen were investigated. The protecting groups investigated were *tert*-butyl-dimethyl silyl (TBDMS), triisopropyl silyl (TIPS), and methoxyethoxymethyl (MEM). The protecting group was added before saponification of the isoamyl esters. The proposed syntheses of these protected models are shown in Scheme 2.3. The silyl ethers are formed using imidazole as the catalyst, and the MEM ether is formed using sodium hydride to

deprotonate the phenol. The protecting groups were chosen due to their ease of removal. The silyl ethers can be removed by exposure to a fluoride source and the MEM ether can be removed by exposure to triflouroacetic acid.[2] These deprotecting agents are not expected to react with the desired platinum functionality in the target product, the platinated estrogen.

Scheme 2.3 Proposed Syntheses of Protected Phenol Compounds



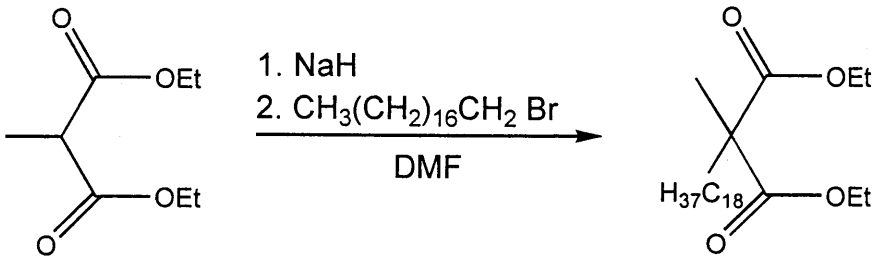
Chapter II

Section II: Results and Discussion

A. Nonfunctionalized Lipophilic Model

A.1 Synthesis of diethyl-2-methyl-2-octadecyl malonate

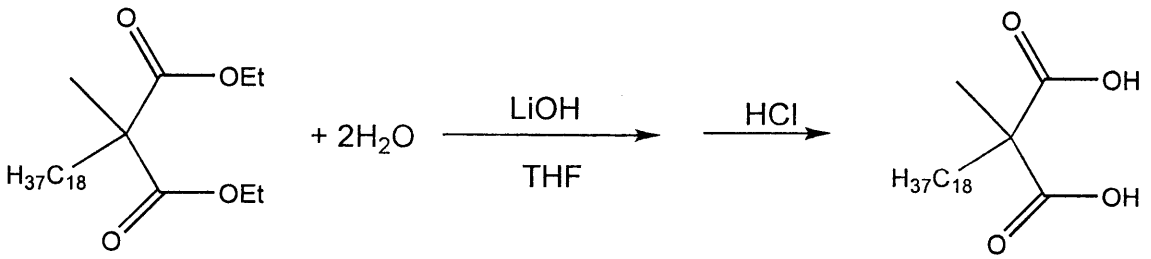
Scheme 2.4 Synthesis of Diethyl-2-methyl-2-octadecyl Malonate



Scheme 2.4 shows the synthesis of diethyl-2-methyl-2-octadecyl malonate. The enolate was formed by exposing diethyl(methyl) malonate to sodium hydride and was alkylated with 1-bromooctodecane. The pure product was a pale yellow oil that was characterized with TLC, ^{1}H NMR (Figure 2.1), ^{13}C NMR (Figure 2.2), MS (LRFAB) (Figure 2.3), IR (Figure 2.4) and elemental analysis. Each spectrum supports the formation of the desired product.

A.2 Synthesis of 2-methyl, 2-octadecylmalonic acid

Scheme 2.5 Synthesis of 2-Methyl-2-octadecylmalonic Acid

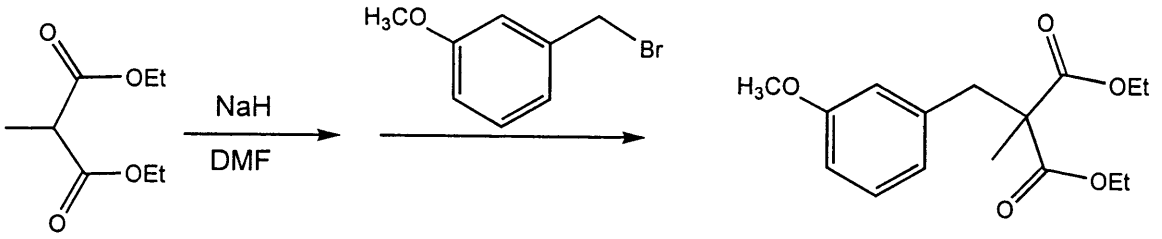


Scheme 2.5 shows the synthesis of 2-methyl-2-octadecylmalonic acid. The ester groups were converted to carboxylic acids via saponification with lithium hydroxide as the catalyst. The pure product was a while solid that was characterized with TLC, ^{1}H NMR (Figure 2.5), ^{13}C NMR (Figure 2.6), MS (LRFAB) (Figure 2.7), IR (Figure 2.8) and elemental analysis. Each spectrum supports the formation of the desired product.

B. Functionalized 3' Substituted Model

B.1 Synthesis of diethyl-2-(3-methoxybenzyl)-2-methyl malonate

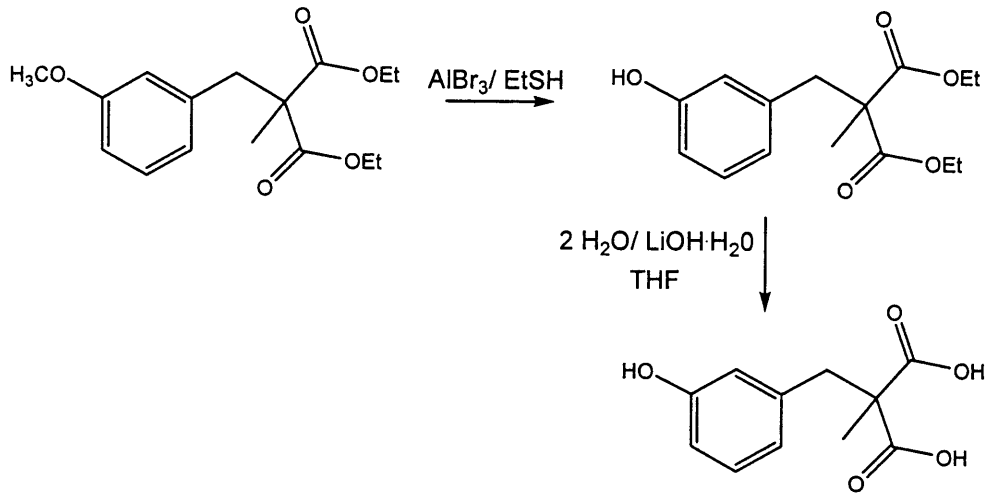
Scheme 2.6 Synthesis of Diethyl- 2-(3-methoxybenzyl)-2-methyl Malonate



Scheme 2.6 depicts the synthesis diethyl-2-(3-methoxybenzyl)-2-methyl malonate. The enolate formed by exposing diethyl (methyl) malonate to sodium hydride was alkylated with 3-methoxybenzyl bromide. The product was a colorless liquid that was characterized by ^{1}H NMR (Figure 2.9), ^{13}C NMR (Figure 2.10), MS (LRESI) (Figure 2.11), IR (Figure 2.12) and elemental analysis. The spectra support the formation of the desired product.

B.2 Synthesis of 2-(3-hydroxybenzyl)-2-methylmalonic acid

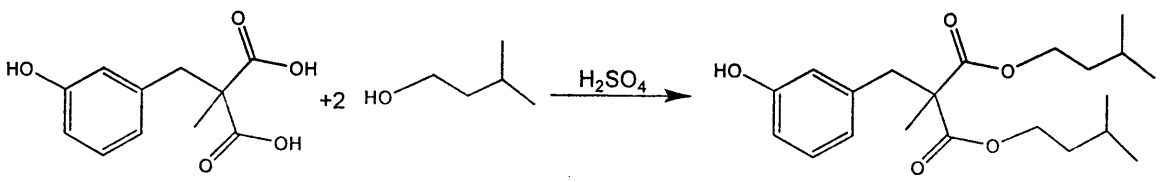
Scheme 2.7 Synthesis of 2-(3-Hydroxybenzyl)-2-methylmalonic Acid



Scheme 2.8 depicts the synthesis of 2-(3-hydroxybenzyl)-2-methylmalonic acid. The phenol was isolated by acidification followed by extraction with ethyl acetate. The crude product was converted from the diester to the dicarboxcylic acid via saponification. The final product was purified by flash chromatography. The final product, a white solid, was characterized by TLC analysis, ^{1}H NMR (Figure 2.13), ^{13}C NMR (Figure 2.14), MS (LRESI) (Figure 2.15), and IR (Figure 2.16). The spectra support the formation of the desired product.

B.3 Synthesis of diisoamyl-2-(3-hydroxybenzyl)-2-methyl malonate

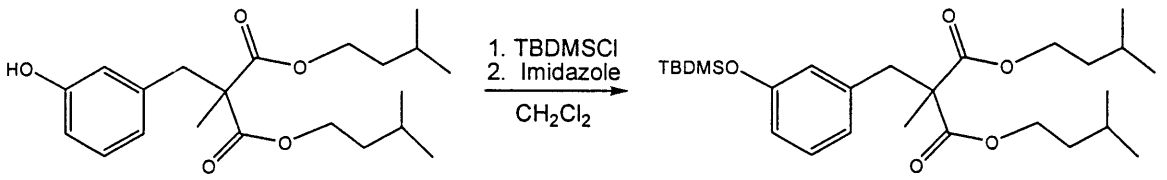
Scheme 2.8 Synthesis of Diisoamyl-2-(3-hydroxybenzyl)-2-methyl Malonate



This new malonate ester was the first of two designed to include a free phenol that could be protected with various groups. The reaction is a simple Fisher esterification. The final product was purified by flash chromatography. The final product, a viscous, yellow oil was characterized by TLC and ^{1}H NMR (Figure 2.17). The results are consistent with the desired product.

B.4 Synthesis of diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-methyl malonate

Scheme 2.9 Synthesis of Diisoamyl-2-(3-*tert*-butyl-dimethylsilyloxy benzyl)-2-methylMalonate



Scheme 2.9 depicts the synthesis for the addition of the *tert*-butyl-dimethylsilyl (TBDMS) protecting group to the phenol. This protecting group was chosen due to its stability to base-catalyzed hydrolysis.[2] The final product was isolated by flash chromatography as a clear liquid analyzed by TLC, elemental analysis, ^{1}H NMR (Figure 2.18), ^{13}C NMR (Figure 2.19), and MS (LRESI) (Figure 2.20). The results are consistent with the desired product.

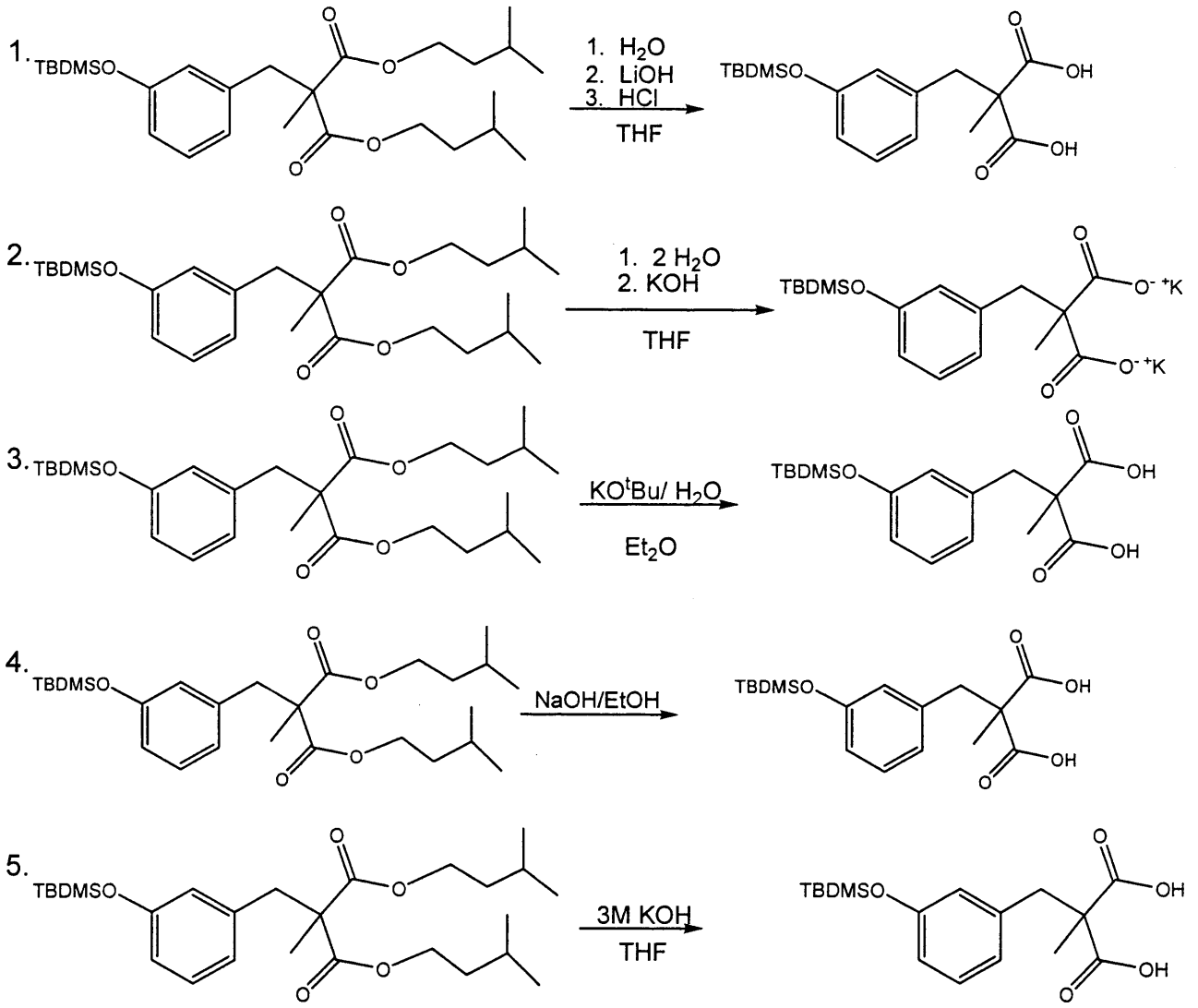
Attempts at selective ester hydrolysis

The general purpose of this set of experiments was to hydrolyze the diisoamyl ester to the free carboxylic acid and leave the *tert*-butyl dimethylsilyl

protecting group in place. Three methods were evaluated: (1) Several reaction sequences were simple base-catalyzed hydrolysis. (2) A second attempted method to remove the esters was reduction followed by re-oxidation. (3) A third approach involved converting the ester to an anhydride, since anhydrides react directly with some platinum compounds to yield platinum carboxylates.[7]

B.5 Attempted base-catalyzed hydrolysis syntheses of 2-(3-*tert*-butyldimethylsilyloxybenzyl)-2-methylmalonic acid

Scheme 2.10 Attempted Basic Hydrolysis Syntheses of 2-(3-*tert*-Butyl-dimethylsilyloxy benzyl)-2-methylmalonic Acid



The first attempted synthesis is shown as reaction 1 in Scheme 2.10. This attempt is the common saponification route for converting an ester to a carboxylic acid using an inorganic base and water under reflux conditions. The ^{1}H NMR spectrum (Figure 2.21) showed that the protecting group was lost. It is unknown

if the group was removed during the saponification or during the work up when the reaction mixture was acidified to a pH of 1. The second attempt, reaction 2 in Scheme 2.10 was designed to rule out the acidification during the work up as the cause for the loss of the TBDMS protecting group. The pH was decreased to a pH of 5.5 since that is the pH required for eventual ligand exchange with platinum. A ^{1}H NMR spectrum (Figure 2.22), showed the loss of the protecting group after workup at this pH as well.

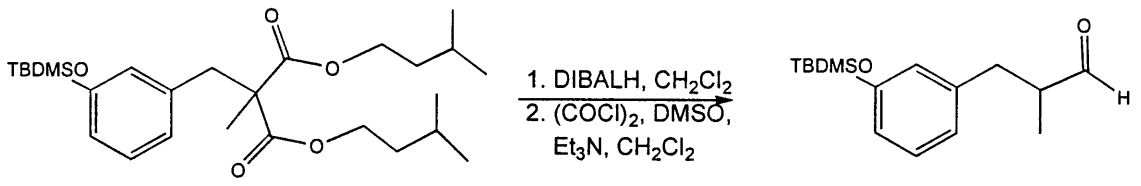
The third attempt, reaction 3 in Scheme 2.10, involved the generation of "nonaqueous hydroxide" reported by Gassman and Schenk.[3] The hydroxide is generated by the hydrolysis of potassium *tert*-butoxide to form *tert*-butanol and hydroxide. This method was employed in order to avoid the excess water that might have been responsible for the removal of the *tert*-butyl dimethyl silyl protecting group. The Gassman and Schenk work involved successful conversions of benzoate esters to their respective acids at room temperature. Some of these esters contained acid sensitive components as well that remained intact. The results of ^{1}H NMR spectrum (Figure 2.23) were inconclusive but the MS (LRESI) (Figure 2.24) showed the desired product. This reaction was discarded due to extremely low yields.

The fourth attempt, reaction 4 in Scheme 2.10, involved use of a solution of ethanolic sodium hydroxide at reflux for one hour.[4] The reaction product was acidified to a pH of 3 and the organic extract was analyzed by ^{1}H NMR. The spectrum (Figure 2.25) showed the loss of the TBDMS protecting group.

The final attempt at base-catalyzed hydrolysis, reaction 5 in Scheme 2.10, was based on the precedent of Skaddan et al.[5] This reaction occurred at room temperature and the work up of the reaction was divided into two methods. Half of the reaction product was acidified to a pH of three and the second half was not acidified. The ^{1}H NMR spectrum (Figure 2.26) of the organic extraction from the acidified portion showed loss of the protecting group. The ^{1}H NMR spectrum (Figure 2.27) of the organic extract from the non-acidified portion showed no reaction. The aqueous extracts, acidified and nonacidified, were also analyzed by ^{1}H NMR (Figures 2.28 and 2.29, respectively). Neither spectrum shows the desired product.

B.6 Attempted deprotection by reduction and re-oxidation of diisoamyl-(3-*tert*-butyl-dimethylsilyloxy benzyl)-2-methyl malonate to 2-(3-*tert*-butyl-dimethylsilyloxy benzyl)-2-methylmalonaldehyde

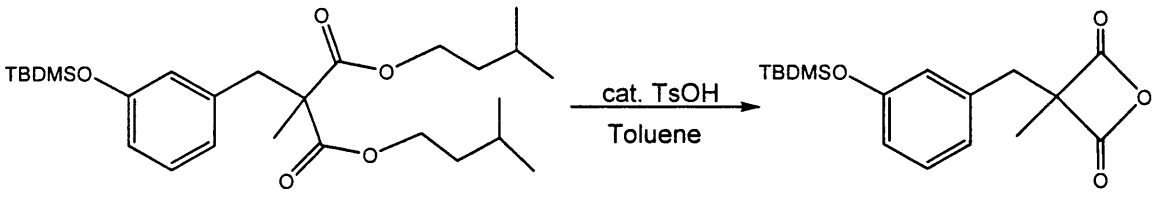
Scheme 2.11 Attempted Conversion of Diisoamyl-2-(3-*tert*-butyl-dimethylsilyloxy benzyl)-2-methyl Malonate to 2-(3-*tert*-Butyl-dimethylsilyloxy benzyl)-2-methylmalonaldehyde



This synthesis was attempted due to the success of converting a benzylic ethyl ester to the parent carboxylic acid as reported by Heckrodt and Mulzer.[6] The diester was to be converted to the diol by the reducing agent, diisobutyl aluminum hydride. The alcohol would then be oxidized to the aldehyde via a Swern oxidation. This synthesis was unsuccessful. The ^{1}H NMR (Figure 2.30) and MS (LRESI) (Figure 2.31) analyses did not show the desired product.

B.7 Attempted conversion of diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl-2-methyl malonate to 2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl malonic anhydride

Scheme 2.12 Attempted Conversion of Diisoamyl-2-(3-*tert*-butyl-dimethylsilyloxy benzyl)-2-methyl Malonate to 2-(3-*tert*-Butyldimethylsilyloxy benzyl)-2-methyl Malonic Anhydride



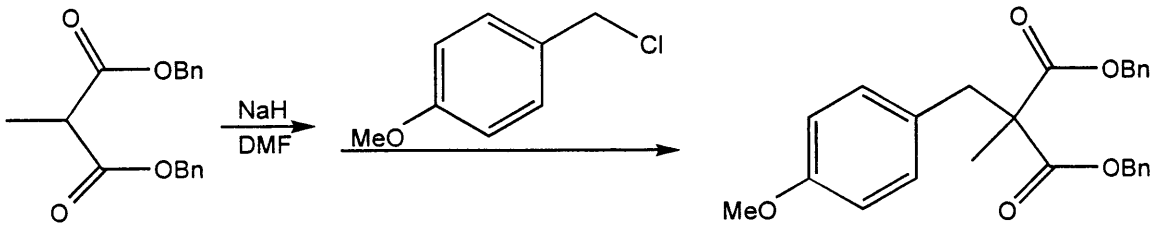
This synthesis was attempted due to a paper by Ho et al.[7] which detailed the reaction of an anhydride with a diammino, nitro platinum complex to form a carboplatin derivative. The attempt to convert a diester to an anhydride was based on the precedent by Graham and Paquette.[8] This attempt resulted in no reaction, as indicated by ^{1}H NMR spectrum (Figure 2.32). This could be due to

the less crowded structure the isoamyl ester compared to the *tert*-butyl ester used by Graham and Paquette.[8]

C Functionalized 4' Substituted Model

C.1 Synthesis of dibenzyl-2-(4-methoxybenzyl)-2-methyl malonate

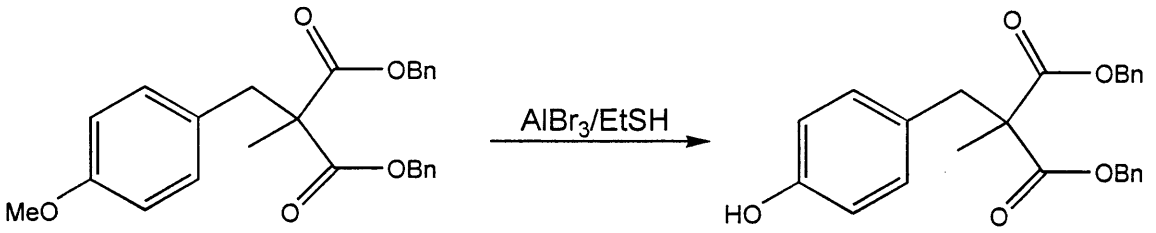
Scheme 2.13 Synthesis of Dibenzyl-2-(4-methoxybenzyl)-2-methyl Malonate



Scheme 2.13 shows the synthesis of dibenzyl-2-(4-methoxybenzyl)-2-methyl malonate. The enolate was formed by exposing dibenzyl(methyl) malonate to sodium hydride and was alkylated with 4-methoxybenzyl chloride. The product was a white solid that was characterized with TLC and ^{1}H NMR (Figure 2.33). The spectrum was consistent with the desired product and with a published spectrum of this compound. The aim of this compound was to form a model with esters whose alkyl groups could easily be removed by hydrogenation.

C.2 Attempted Synthesis of dibenzyl-2-(4-hydroxybenzyl)-2-methyl malonate

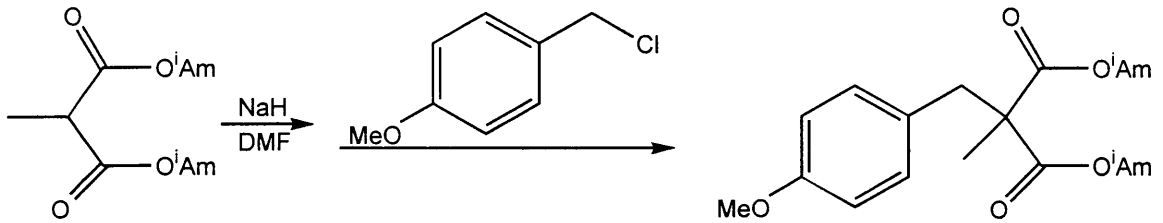
Scheme 2.14 Attempted Synthesis of Dibenzyl-2-(4-hydroxybenzyl)-2-methyl Malonate



Scheme 2.14 depicts the attempted synthesis of dibenzyl-2-(4-hydroxybenzyl)-2-methyl malonate. This route is commonly used to convert aromatic methyl ethers to phenols. The ^{1}H NMR spectrum (Figure 2.34) showed the benzyl groups were also lost. The benzyl esters were easily removed but unfortunately at the wrong stage of the synthetic sequence. The IR spectrum (Figure 2.35) and MS (LREI) (Figure 2.36) are also consistent with the loss of the benzyl groups.

C.3 Synthesis of diisoamyl-2-(4-methoxybenzyl)-2-methyl malonate

Scheme 2.15 Synthesis of Diisoamyl-2-(4-methoxybenzyl)-2-methyl Malonate

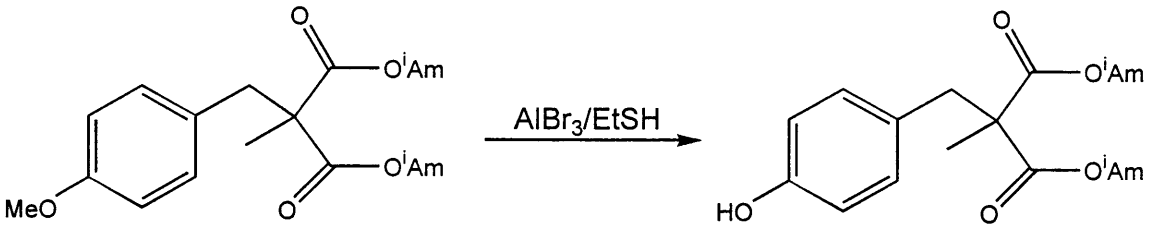


Scheme 2.15 shows the synthesis of diisoamyl-2-(4-methoxybenzyl)-2-methyl malonate. The enolate was formed by exposing diisoamyl(methyl)

malonate to sodium hydride and was alkylated with 4-methoxybenzyl chloride. The product was a pale yellow liquid that was characterized with TLC and ^{1}H NMR (Figure 2.38), ^{13}C NMR (Figure 2.39), MS (LRESI) (Figure 2.40), IR (Figure 2.37) and elemental analysis. The results are consistent with the desired product. This compound is very similar to a previous model, with the difference being the location of the methoxy group. The previous methoxy group was meta and this compound's methoxy group is para to the malonate linkage. The advantage of this compound is a simpler aromatic region in ^{1}H NMR analysis and possibly greater ease of purification by recrystallization due to the symmetry of the molecule.

C.4 Synthesis of diisoamyl-2-(4-hydroxybenzyl)-2-methyl malonate

Scheme 2.16 Synthesis of Diisoamyl-2-(4-hydroxybenzyl)-2-methyl Malonate

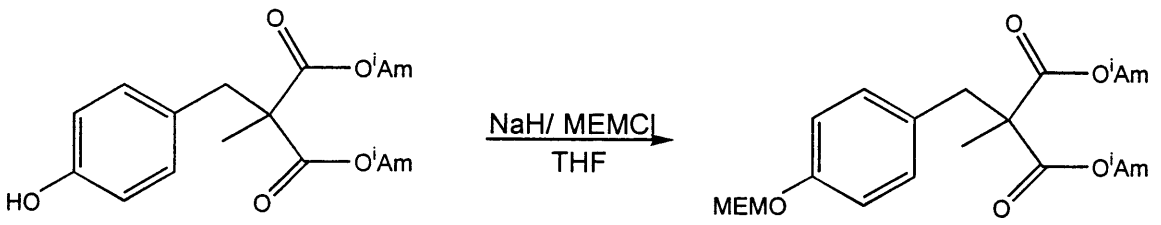


Scheme 2.16 depicts the synthesis of diisoamyl-2-(4-hydroxybenzyl), 2-methyl malonate. The phenol was isolated by acidification followed by extraction with ethyl acetate. The pure product was a pale yellow oil that was characterized with TLC and ^{1}H NMR (Figure 2.42), ^{13}C NMR (Figure 2.43), MS (LRESI) (Figure

2.44), IR (Figure 2.41) and elemental analysis. The results are consistent with the desired product.

C.5 Synthesis of diisoamyl-2-(4-methoxyethoxymethylbenzyl)-2-methyl malonate

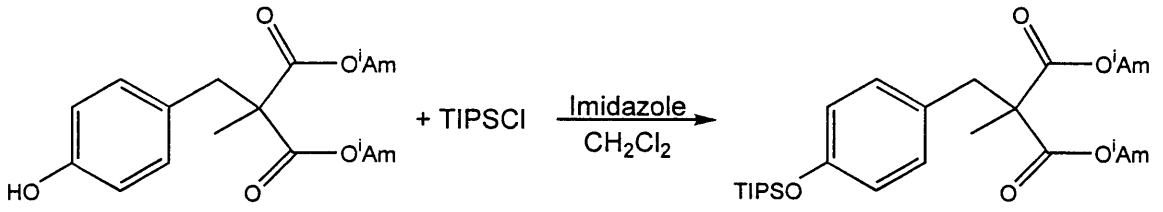
Scheme 2.17 Synthesis of Diisoamyl-2-(4-methoxyethoxymethylbenzyl)-2-methyl Malonate



Scheme 2.17 shows the synthesis of diisoamyl-2-(4-methoxyethoxymethylbenzyl)-2-methyl malonate. The phenolic anion was formed by exposing diisoamyl-2-(4-hydroxybenzyl)-2-methyl malonate to sodium hydride and alkylated with methoxyethoxymethyl chloride. The MEM protecting group was chosen due to its stability to basic conditions and ease of removal with triflouroacetic acid.[2] The methoxyethoxymethyl (MEM) protecting group has been used successfully as a protecting group on the phenolic oxygen of a synthetic estrogen as performed by Skadden et al.[5] The pure product was a colorless liquid that was characterized with TLC and ^{1}H NMR (Figure 2.46), ^{13}C NMR (Figure 2.47), MS (LRESI) (Figure 2.48) and IR (Figure 2.45).

C.6 Synthesis of diisoamyl-2-(4-triisopropylsilyloxybenzyl)-2-methyl malonate

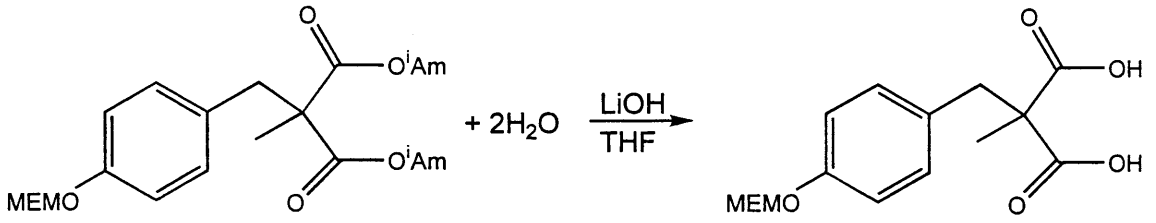
Scheme 2.18 Synthesis of Diisoamyl-2-(4-triisopropylsilyloxybenzyl)-2-methyl Malonate



Scheme 2.18 depicts the synthesis of diisoamyl-2-(4-triisopropylsilyloxybenzyl)-2-methyl malonate. The triisopropylsilyl (TIPS) protecting was evaluated because it is even more stable to basic and acidic conditions than is TBDMS.[4] The final product, a pale yellow liquid, was characterized by TLC and ^{1}H NMR (Figure 2.50), ^{13}C NMR (Figure 2.51), MS (LRESI) (Figure 2.52), IR (Figure 2.49) and elemental analysis. The results are consistent with the desired product.

C.7 Attempted synthesis of 2-(4-methoxyethoxymethylbenzyl)-2-methylmalonic acid

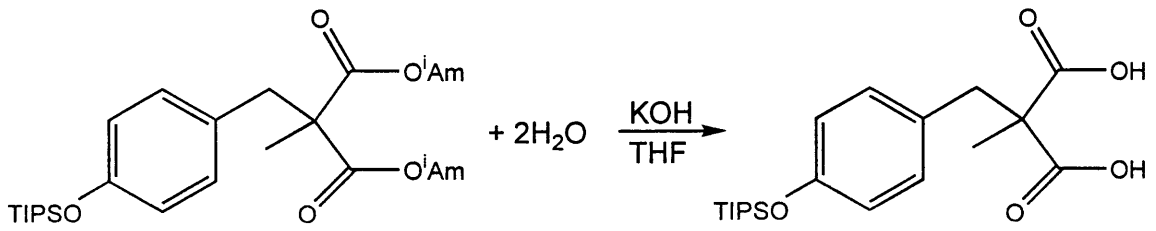
Scheme 2.19 Attempted Synthesis of 2-(4-Methoxyethoxymethylbenzyl)-2-methylmalonic Acid



Scheme 2.19 shows the attempted synthesis of 2-(4-methoxyethoxymethylbenzyl)-2-methylmalonic acid. The saponification of diisoamyl-2-(4-methoxyethoxymethylbenzyl)-2-methyl malonate was unsuccessful according to the ^{1}H NMR spectrum (Figure 2.53), which shows none of the desired product. In the work up of the reaction, the pH was lowered to 5, since this pH is required for deprotonation of the product prior to coordination to platinum.

C.8 Attempted synthesis of 2-(4-triisopropylsilyloxybenzyl)-2-methylmalonic acid

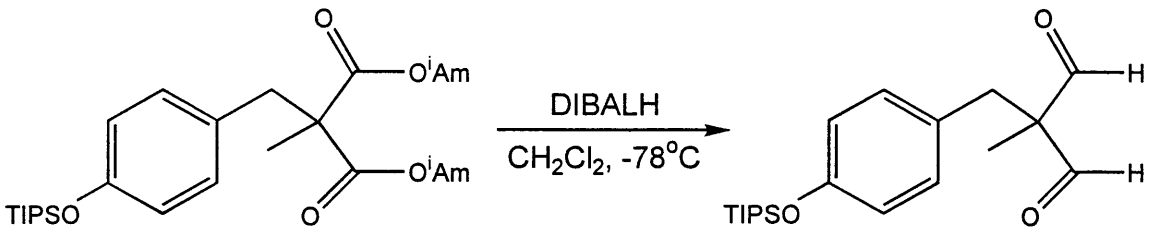
Scheme 2.20 Attempted Synthesis of 2-(4-Triisopropylsilylbenzyl)-2-methylmalonic Acid



Scheme 2.20 shows the attempted synthesis of 2-(4-triisopropylsilyloxybenzyl)-2-methylmalonic acid. The saponification of diisoamyl-2-(4-triisopropylsilyloxybenzyl)-2-methyl malonate was unsuccessful. The ^{1}H NMR spectrum (Figure 2.54) shows the loss of the TIPS protecting group.

C.9 Attempted synthesis of 2-(4-triisopropylsilyloxybenzyl)-2-methylmalonaldehyde

Scheme 2.21 Attempted Synthesis of 2-(4-Triisopropylsilyloxybenzyl)-2-methylmalonaldehyde



Scheme 2.21 depicts the attempted synthesis of 2-(4-triisopropylsilyloxybenzyl)-2-methylmalonaldehyde. The difference of these reactions from those in section B.6 is the constant reaction temperature of -78°C. The goal was to arrest the reduction at the aldehyde instead of complete reduction to the alcohol. If the reduction were stopped at the aldehyde, then the aldehyde could be oxidized to the carboxylic acid in a single step. The ^{1}H NMR spectrum (Figure 2.55) was consistent with a reduction of only one of the esters to the aldehyde. The ^{1}H NMR (Figure 2.55) and IR spectra (Figure 2.56) do show an aldehyde signal.

Chapter II:

Section III: Conclusions

The synthesis of 2-methyl-2-octadecylmalonic acid was successful. The compound was characterized by ^{1}H NMR, ^{13}C NMR, MS (LRFAB), IR and elemental analysis. The synthesis of 2-(3-hydroxybenzyl)-2-methylmalonic acid was also successful and, the product was characterized by TLC, ^{1}H NMR, ^{13}C NMR, MS (LRESI), and IR analysis. The synthesis of 2-(4-hydroxybenzyl)-2-methylmalonic acid was also successful and, the product was characterized by TLC, ^{1}H NMR, ^{13}C NMR, MS (LRESI), and IR analysis.

Several other compounds were also successfully synthesized: diisoamyl-2-(3-*tert*-butyldimethylsilyloxybenzyl)-2-methyl malonate, diisoamyl-2-(4-methoxyethoxymethylbenzyl)-2-methyl malonate, and diisoamyl-2-(triisopropylsilyloxybenzyl)-2-methyl malonate. These compounds were synthesized due to a perceived need for a protected phenol prior to coordination to platinum. Many different attempts were made to selectively convert the esters into carboxylic acids, and maintain the protected phenol. None of these attempts were successful.

Chapter II

Section IV: Experimental

The 1-bromooctadecane, diethyl (methyl) malonate, 3-methoxybenzyl chloride, isoamyl alcohol, aluminum bromide, ethanethiol, *tert*-butyl-dimethylsilyl chloride, imidazole, 85% potassium hydroxide, 95% potassium tert-butoxide, diisobutyl aluminum hydride, oxalyl chloride, dimethyl sulfoxide, triethylamine, methoxyethoxymethyl chloride, and triisopropylsilyl chloride were used as received from Aldrich. The sodium hydride, 60% in mineral oil and 4-methoxybenzyl chloride were used as received from Acros. The lithium hydroxide, concentrated hydrochloric acid, concentrated sulfuric acid, and glacial acetic acid were used as supplied from the Eastern Illinois University chemistry stock room. The tetrahydrofuran was purchased from Fisher Scientific and distilled from sodium benzophenone. The dimethylformamide was purchased from Aldrich and distilled over calcium hydride. Hexane, dichloromethane, and ethyl acetate were used as supplied from Fisher Scientific. Silica GF from Analtech was used as the solid for all TLC; staining was done with iodine visualization. The dibenzyl-2-methyl malonate and diisoamyl-2-methyl malonate were prepared according to Bendler.[9] Flash chromatography was conducted with silica gel, Merck grade 9385, 200–400 mesh, 40Å. NMR spectra were collected on a QE 300 instrument. Mass spectra were obtained with either Fast Atom Bombardment (FAB) or Electrospray Ionization (ESI) on a Quattro instrument at the School of Chemical Sciences, University of Illinois at Urbana-Champaign.

Elemental analyses were conducted by the Microanalytical Laboratory at the School of Chemical Sciences, University of Illinois at Urbana-Champaign.

Synthesis of diethyl-2-methyl-2-octadecyl malonate A 250 mL, three neck flask with a stir bar was evacuated and charged with argon. Sodium hydride, 60% in mineral oil (1.999 g, 49.98 mmol) was added to the reaction flask in an N_2 filled glove bag. The reaction flask was placed in an ice bath and DMF (30 mL) was added. Diethyl(methyl)malonate (7.8 mL, 46 mmol) was added drop wise resulting in a large amount of bubbles (H_2), forming an enolate solution. 1-Bromooctadecane (16.70 g, 50.11 mmol) was placed in a 300 mL three neck flask, which was evacuated and charged with argon, and DMF (110 mL). Gentle heating was required for the solution to become homogeneous. This solution was transferred to an addition funnel and added drop wise to the enolate solution. Initially the solution had a milky appearance, then after approximately three hours the solution became a mostly clear brown homogeneous solution. The solution was stirred for two days.

The reaction was quenched with the addition of deionized water (50 mL). The mixture was transferred to a separatory funnel with CH_2Cl_2 (50 mL). The lower brown organic layer was removed. The aqueous layer was extracted with CH_2Cl_2 (75 mL, three times). The combined organic layers were washed with deionized water (100 mL, three times) and brine (100 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was diluted in methanol to precipitate unreacted 1-bromooctodecane.

This precipitate was removed by vacuum filtration and the methanol was removed *in vacuo*.

The final product, a pale yellow liquid, was purified by flash chromatography in 9:1 hexane/ ethyl acetate. Yield = 13.5568 g (69.4%). R_f: 0.65 in 9:1 hexanes/ethyl acetate. IR (neat): 1734 cm^{-1} (s, C=O), Figure 2.4. ^1H NMR ($CDCl_3$) δ: 4.147 (q, J = 7.12 Hz, 4H), 1.838-1.784 (m, 2H), 1.364 (s, 3H), 1.239-1.278 (m, 38H), 2.77 (t, J = 6.53, 1H), Figure 2.1. ^{13}C NMR ($CDCl_3$) δ: 172.632, 60.964, 53.642, 35.210, 31.914, 29.847, 29.676, 29.649, 29.618, 29.538, 29.360, 24.193, 22.678, 19.788, 14.105, 14.028, Figure 2.2. MS (LRFAB) (m/z) (rel. intensity): 427.4 (M+1, 100%), Figure 2.3. Anal. Calcd for $C_{26}H_{50}O_4$: C, 73.11; H, 11.81. Found C, 73.26; H, 11.94.

Synthesis of 2-methyl-2-octadecylmalonic acid A stir bar, deionized water (51 mL), THF (50 mL) and $LiOH•H_2O$ (11.9 g, 284 mmol) were combined in a 500 mL round bottom flask open to the atmosphere. This mixture was stirred as diethyl 2-methyl-2-octadecyl malonate (4.00 g, 9.40 mmol) was added. This solution was stirred at reflux overnight resulting in a large amount of white precipitate.

The precipitate was removed from the reaction mixture by vacuum filtration. The solid was transferred to a new filter paper and rinsed with ether under vacuum filtration. These crystals are believed to be the lithium salt of the desired compound. The crystals from the filter paper were dissolved in 8 M HCl (20 mL) and ethyl acetate (150 mL). This mixture was washed with deionized

water (100 mL, two times) and brine (100 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo*. Yield of fine white powder: 2.56 g (74.4%). IR (KBr pellet): 3200-2800 cm^{-1} (s, O-H), 1693 cm^{-1} (s, C=O), Figure 2.8. ^1H NMR (d-6 acetone) δ: 1.382, (s, 3H), 1.280 (s, 33H), 0.871 (t, J = 6.28, 3H), Figure 2.5. ^{13}C NMR (d-6 acetone) δ: 172.84 (2C), 35.65 (1C), 31.83 (1C), 30.03 (1C), 29.61 (1C), 29.41 (3C), 29.23(1C), 24.81 (1C), 24.55 (2C), 24.27 (3C), 24.12 (2C), 23.73 (1C), 22.53 (1C), 19.42 (1C), 13.41 (1C), Figure 2.6 MS (LRFAB) (m/z) (rel. intensity): 371.4 (M+1, 10.72%), 119.0 (McLafferty, 100%), Figure 2.7. Anal. Calcd. For $C_{22}H_{42}O_4$: C, 71.11; H, 11.66. Found C, 71.30; H, 11.47. Melting point: 101.8-102.8 °C.

Synthesis of diethyl-2-(3-methoxybenzyl)-2-methyl malonate A 50 mL, 3-neck flask was charged with argon. Sodium hydride, 60% in mineral oil (1.352 g, 33.80 mmol) was placed in the flask in an N_2 filled glove bag. The flask was placed under argon and in an ice bath where DMF (10 mL) was added. Diethyl(methyl) malonate (5.90 mL, 34.2 mmol) was added to the reaction flask, resulting in the evolution of gas. 3-Methoxybenzyl bromide (4.3 mL, 31 mmol) was added to the solution, resulting in the formation of a white precipitate. The mixture was stirred at room temperature for 1.5 hours.

The reaction was quenched with deionized water (10 mL) that dissolved the precipitate. The mixture was transferred to a separatory funnel with ether (30 mL). The lower aqueous layer was removed and extracted with ether (30 mL, two

times). The organic extracts were washed with brine (60 mL) and dried over $MgSO_4$. Solvent was removed *in vacuo*.

The final product, a colorless liquid, was purified by flash chromatography in 9:1 hexanes/ ethyl acetate. Yield = 5.9578 g (65.93%). TLC: R_f=0.35 in 9:1 hexanes/ ethyl acetate. IR (neat): 1732 cm^{-1} (C=O), 1602 cm^{-1} (C=C) 1240 cm^{-1} (C-O), Figure 2.12. ^1H NMR ($CDCl_3$) δ:7.14 (t, J=7.94 Hz, 1H), 6.76-6.64 (m, 3H), 4.17 (q, J=6.88 Hz, 4H), 3.74 (s, 3H), 3.18 (s, 2H), 1.31 (s, 3H), 1.23 (t, J=7.09 Hz, 6H), Figure 2.9. ^{13}C NMR ($CDCl_3$) δ: 171.81 (2C), 159.25 (1C), 137.64 (1C), 129.98 (1C), 122.49 (1C), 115.95 (1C), 111.98 (1C), 61.19 (2C), 54.95 (1C), 54.61 (1C), 40.97 (1C), 19.61 (1C), 13.92 (2C), Figure 2.10. MS (LRESI) (m/z) (rel. intensity): 294.3 (M+, 70.67%), Figure 2.11. Analysis Calculated for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53. Found C, 65.39; H, 7.49.

Synthesis of 2-(3-hydroxybenzyl)-2-methylmalonic acid A three neck, 500 mL with a stir bar was evacuated and charged with argon. In an N_2 filled glove bag $AlBr_3$ (36.0 g, 135 mmol) was added to the flask. The flask was placed in an ice bath for the addition of ethanethiol (100 mL, 1.35 mol) forming a vibrant yellow/orange solution. Diethyl-2-(3-methoxybenzyl)-2-methyl malonate (3.46 g, 11.8 mmol) was added with stirring to the reaction flask. A large amount of precipitate formed and the ice bath was removed. The reaction was stirred for one hour. The reaction flask was placed in an ice bath for the slow addition of 20 mL of deionized water to quench. A large amount of gas and solid formed. The ice bath was removed and the ethanethiol was removed by air purge over a 24-

hour period. The reaction mixture was diluted in ethyl acetate (80 mL) and acidified to pH of 1 with 2 M HCl (100 mL). The reaction flask contents were transferred to a separatory funnel. The aqueous layer was extracted with ethyl acetate (80 mL, three times). The combined organic layers were dried over magnesium sulfate. The magnesium sulfate was removed by gravity filtration and the ethyl acetate was removed *in vacuo*. The crude product, diethyl-2-(3-hydroxybenzyl)-2-methyl malonate was a dark brown oil, 2.5407 g.

A three neck, 250 mL round bottom flask with a stir bar was fitted with a condenser and charged with argon. Crude diethyl-2-(3-hydroxybenzyl)-2-methyl malonate (2.54 g, 9.06 mmol) dissolved in 8 mL of tetrahydrofuran was transferred to the reaction flask followed by deionized water (50 mL, 2.8 mol) and lithium hydroxide (13.5 g, 322 mmol). The resulting mixture was a heterogeneous pale yellow. The mixture was heated at reflux for five hours.

The reaction flask was then cooled in an ice bath and concentrated hydrochloric acid (25 mL) was added to the reaction flask to bring the pH of the reaction mixture to 2. The reaction flask contents were transferred to a separatory funnel. The aqueous layer was extracted with ethyl acetate (40 mL, two times). Thin layer chromatography was performed on silica in 10:30:1 hexane/ ethyl acetate/ glacial acidic acid with iodine visualization. The results showed some of the desired product was present in the aqueous layer. The aqueous layer was reextracted with ethyl acetate (80 mL, six times). The combined organic extractions were dried over magnesium sulfate, which was removed by gravity filtration. The ethyl acetate was removed *in vacuo*.

The product, a white solid, was purified by flash chromatography on an SiO_2 column with 10:30:1 hexane/ ethyl acetate/ glacial acidic acid as the mobile phase. Attempts were made to purify by recrystallization but were unsuccessful. Yield = 1.2278 g (51.7%). TLC: R_f=0.35 in 10:30:1 hexane/ ethyl acetate/ glacial acidic acid. IR (nujol): 3349 cm^{-1} (O-H), 1704 cm^{-1} (C=O), 1614 cm^{-1} (C=C) Figure 2.16. ^1H NMR (d-6 acetone, 23 °C): δ 8.188 (s, 1H), 7.073 (t, J=7.8 Hz, 1H), 6.739-6.677 (m, 3H), 3.151 (s, 2), 1.314 (s, 3H), Figure 2.13. ^{13}C NMR (d-6 acetone) δ: 207.02, 173.52, 157.95, 139.05, 129.87, 122.37, 118.05, 114.57, 54.86, 41.63, 20.09, Figure 2.14. MS (LRESI) (m/z) (rel. intensity): 224.2 (M+, 34%), 448.4 (2M+, 93%), Figure 2.15. Anal. Calcd for $C_{11}H_{12}O_5$: C, 58.93; H, 5.39. Found: C, 58.15; H, 5.40.

Synthesis of diisoamyl-2-(3-hydroxybenzyl)-2-methyl) malonate 2-(3-Hydroxybenzyl)-2-methylmalonic acid (0.47g, 1.79 mmol), and isoamyl alcohol (4.0 mL, 36.7 mmol) were combined in a 50 mL flask and stirred until a homogeneous pale yellow solution was formed. A catalytic amount of 18 M sulfuric acid was added to the reaction flask. The solution was heated at reflux for 2.5 hours.

The reaction solution was transferred to a separatory funnel with deionized water (15 mL) and ethyl acetate (15 mL). The lower aqueous layer was extracted with ethyl acetate (20 mL, two times). The organic extract was washed with sodium bicarbonate (20 mL), water (20 mL), and brine (20 mL). The organic extract was dried over $MgSO_4$ and the solvent was removed *in vacuo.*

The final product, a viscous, yellow oil, was isolated by flash chromatography in 8:2 hexanes/ethyl acetate. Yield = 0.5067 g (71.6%). TLC: R_f=0.54 in 8:2 hexanes/ethyl acetate. ^{1}H NMR ($CDCl_3$, 23 °C): δ 7.050 (t, J=8 Hz,1H), 6.672-6.583 (m, 3H), 4.187-4.045 (m, 4H), 3.137(s, 2H), 1.678(sep, J=6.7 Hz, 2H), 1.500 (q, 6.9 Hz, 4H), 1.306(s, 3H), 0.867(dd, J_3=6.6 Hz, J_4=2.2 Hz, 12H), Figure 2.17.

Synthesis of diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl malonate A 100 mL, three neck flask with a stir bar was evacuated and charged with argon. *Tert*-butyl-dimethylsilyl chloride (2.78 g, 18.4 mmol) was added to the reaction flask in an N_2 filled glove bag. The reaction flask was placed under argon and a solution of diisoamyl-2-(3-hydroxybenzyl)-2-methyl malonate (2.43 g, 6.16 mmol) and dichloromethane (12 mL) was added to the reaction flask. The reaction solution was a clear, yellow/orange color. Imidazole (2.52 g, 37.0 mmol) was added. The reaction mixture was heterogeneous and pale yellow. Dichloromethane (5 mL) was added to maintain a stirred solution. The reaction mixture was stirred overnight under static argon. The reaction mixture maintained the white precipitate and pale yellow supernatant.

The reaction mixture was quenched with deionized water (20 mL), and most of the precipitate dissolved. The organic supernatant became a beige color. The mixture was transferred to a separatory funnel with ethyl acetate (40 mL). An emulsion formed which was dispersed with the addition of brine (10 mL). The lower aqueous layer was removed and extracted with ethyl acetate (50

mL, two times). The organic extracts were washed with brine (100 mL) and dried over magnesium sulfate. Solvent was removed *in vacuo*.

The final product, a colorless liquid, was purified by flash chromatography in 19:1 hexanes/ ethyl acetate. Yield = 2.3182 g (81%). TLC: R_f=0.54 in 19:1 hexanes/ ethyl acetate. ^{1}H NMR ($CDCl_3$) δ: 7.07 (t, J= 7.9 Hz, 1H), 6.68-6.59 (m, 3H), 4.16-4.08 (m, 4H), 3.15 (s, 2H), 1.70-1.57 (m, 2H), 1.49 (q, J= 6.8 Hz, 4H), 1.30 (s, 3H), 0.95 (s, 9H), 0.89 (dd, J_3= 6.5Hz, J_4= 2.1Hz, 12H), 0.15 (s, 6H) Figure 2.18. ^{13}C NMR ($CDCl_3$) δ: 171.85 (2C), 155.30 (1C), 137.61 (1C), 128.93 (1C), 123.15 (1C), 121.92 (1C), 118.50 (1C), 63.87 (2C), 54.76 (1C), 40.91 (1C), 37.04 (2C), 25.59 (4C), 24.88 (3C), 22.34 (2C), 19.63 (1C), -4.52 (2C), Figure 2.19. MS (LRESI) (m/z) (rel. intensity): 479.5 (M+, 100%), Figure 2.20. Anal. Calcd. for $C_{27}H_{46}O_5Si$: C, 67.74; H, 9.68. Found C, 67.33; H, 9.61.

Attempted base-catalyzed hydrolysis synthesis of 2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methylmalonic acid using lithium hydroxide

A 50 mL, three neck flask with a condenser and stir bar was evacuated and charged with argon. A solution of diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl malonate (0.282 g, 0.589mmol) and THF (2.6 mL) was added to the reaction flask. Lithium hydroxide monohydrate, (1.17 g, 39.6 mmol) and deionized water (10.5 mL, 580 mmol) were added to the reaction flask. The mixture was heated at reflux, under argon for five hours. The reaction mixture was cooled to room temperature and placed in an ice bath. The mixture was acidified to a pH of 1 with concentrated hydrochloric acid.

The reaction mixture was transferred to a separatory funnel with ethyl acetate (40 mL). The lower aqueous layer was extracted with ethyl acetate (50 mL, three times). The organic extracts were washed with brine (80 mL) and dried over magnesium sulfate. Solvent was removed *in vacuo*. ^{1}H NMR, Figure 2.21, was obtained on the crude product and demonstrated the loss of the silyl proton signals.

Attempted base-catalyzed hydrolysis synthesis of 2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methylmalonic acid using potassium hydroxide A 50 mL, three neck flask with a condenser and stir bar was evacuated and charged with argon. A solution of diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl malonate (0.424 g, 0.884mmol) and THF (6 mL) was added to the reaction flask. Potassium hydroxide, 85%, (1.7 g, 27 mmol) and deionized water (15 mL, 880 mmol) were added to the reaction flask. The mixture was heated at reflux, under argon for five hours. The reaction mixture was cooled to room temperature and placed in an ice bath. The mixture was acidified to a pH of 5.5 with dilute nitric acid.

Solvent was removed *in vacuo*. Methanol (45 mL) was added to the reaction flask and stirred for one hour. Any undissolved solid was removed by gravity filtration. The solvent was removed *in vacuo* from the filtrate. ^{1}H NMR of the residue (Figure 2.22) was obtained on the crude product and demonstrated the loss of the silyl proton signals.

Attempted base-catalyzed hydrolysis synthesis of 2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methylmalonic acid using potassium *t*-butoxide A 100 mL, three neck flask with a stir bar was evacuated and charged with argon. Potassium *t*-butoxide, 95% (2.16 g, 18.3 mmol) was added to the reaction flask in an N_2 filled glove bag. The flask was placed under argon and in an ice bath. Diethyl ether (50 mL) was added to the reaction flask and most of the potassium *t*-butoxide dissolved. Deionized water (0.10 mL, 5.6 mmol) was added drop wise to the reaction flask. Diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl malonate (0.499 g, 1.07 mmol) was added dropwise to the reaction mixture. The reaction mixture was stirred at room temperature for 90 minutes. The reaction flask was placed in an ice bath for the quench with the addition of deionized water (40 mL). The precipitate dissolved, and the mixture separated into two phases.

The mixture was transferred to a separatory funnel, and the lower aqueous layer was removed. The aqueous layer was extracted with diethyl ether (40 mL, three times). The combined organic extracts were washed with brine (50 mL) and dried over magnesium sulfate. ^{1}H NMR spectrum (Figure 2.23) was not consistent with the desired product. MS (LRESI) spectrum (m/z) (rel. intensity): 339.2 (M+, 55%), 225.0 (M-TBDMS, 100%), Figure 2.24.

Attempted base-catalyzed hydrolysis synthesis of 2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methylmalonic acid using ethanolic sodium hydroxide A solution of ethanolic sodium hydroxide was prepared from sodium

hydroxide (1.05 g, 26.3 mmol) and 95% ethanol (23.5 mL, 396 mmol). Diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl malonate (0.46 g, 0.99 mmol) and the sodium hydroxide/ ethanol solution (10 mL) were placed in a 25 mL flask. The reaction mixture was heated at reflux for one hour. A white precipitate formed after 30 minutes of being heated at reflux. The reaction mixture was cooled to room temperature and acidified to a pH of 3 with 1 M HCl (aq.).

The reaction flask contents were transferred to a separating funnel. The lower aqueous layer was removed and extracted with ethyl acetate. The organic extracts were dried over magnesium sulfate. The solvent was removed *in vacuo* from the organic and aqueous extracts. A ^{1}H NMR spectrum (Figure 2.25) was obtained for the organic extract. The spectrum did not show the desired product.

Attempted base-catalyzed hydrolysis synthesis of 2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methylmalonic acid using 3 M potassium hydroxide Diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl malonate (0.460 g, 0.989 mmol), THF (20 mL) and 3 M KOH (aq.) (4.2 mL) were combined in a 100 mL flask. The reaction mixture was stirred overnight. The reaction mixture remained as two phases. The phases became miscible upon the addition of 95% ethanol.

The reaction flask contents were transferred to a separatory funnel, where the total volume was divided into two parts. The first part was acidified to a pH of 3 with 1 M HCl (aq.). The acidified half was extracted with ethyl acetate. The

organic extracts were dried over magnesium sulfate, and the solvent was removed *in vacuo*. A ^{1}H NMR spectrum (Figure 2.26) was obtained on this extract, and it did not reveal the desired product. A ^{1}H NMR spectrum (Figure 2.28) was obtained on the residue of the acidified aqueous residue, and the spectrum did not show the desired product.

The second half of the original reaction solution was not acidified. Ethyl acetate and deionized water were added to the non-acidified product. The lower aqueous layer was removed and extracted with ethyl acetate. The organic extracts were dried over magnesium sulfate. The solvents were removed *in vacuo* from the organic and aqueous extracts. A ^{1}H NMR spectrum (Figure 2.27) was obtained on the non-acidified organic extract, and the spectrum was not consistent with the desired product. A ^{1}H NMR spectrum (Figure 2.29) was obtained on the non-acidified aqueous extract, and the spectrum was also inconsistent with the desired product.

Attempted deprotection by reduction and re-oxidation diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl malonate to 2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methylmalonaldeyde Diisoamyl-2-(3-*tert*-butyldimethylsilyl ether benzyl)-2-methyl malonate (0.51 g, 1.1 mmol) was placed in a three neck, 50 mL flask. The flask was evacuated, charged with argon and cooled to –78 °C. Diisobutyl aluminum hydride, 1.0 M in dichloromethane (6.5 mL, 6.5 mmol) was added drop wise to the reaction flask. The reaction solution was clear and colorless and stirred at –78 °C for one hour. The reaction solution

was stirred at 0 °C for an additional six hours. A solution of 1:1 tetrahydrofuran/ deionized water (5 mL) was added drop wise to quench the reaction, and the solution became gelatinous. Ethyl acetate (25 mL) was added to the reaction flask, and a white precipitate formed which was removed by vacuum filtration through celite. The celite was rinsed with ethyl acetate (100 mL). The filtrate was transferred to a separatory funnel with deionized water (80 mL). The lower aqueous layer was removed and extracted with ethyl acetate (50 mL, two times). The organic extracts were combined and washed with brine (100 mL) and dried over magnesium sulfate. The solvent was removed *in vacuo*, affording a pale yellow oil.

A 100 mL, three neck flask was evacuated, charged with argon and cooled to –78 °C. Oxalyl chloride, 2.0 M in dichloromethane (0.81 mL, 1.6 mmol) was added to the reaction flask along with dichloromethane (1.0 mL). Dimethyl sulfoxide (0.23 mL, 3.2 mmol) was added to the reaction flask. The clear, colorless solution was stirred for two minutes before the addition of the oil from the previous reduction as a solution in dichloromethane (1 mL). A white precipitate formed after five minutes of stirring. The mixture was stirred for 15 minutes when triethylamine (1.1 mL, 7.9 mmol) was added. The mixture became a pale yellow/ orange and was stirred for five minutes. The mixture was allowed to warm to room temperature, and deionized water (10 mL) was added resulting in two orange layers.

The mixture was transferred to a separatory funnel and the lower organic layer was removed. The aqueous layer was extracted with dichloromethane (20

mL, three times). The combined organic extracts were washed with brine (50 mL) and dried over magnesium sulfate. Solvent was removed *in vacuo*, affording an orange oil. The oil was diluted in dichloromethane (20 mL) and filtered through a one inch plug of silica that was rinsed with 4:6 hexanes/ ethyl acetate (100 mL). Solvent was removed *in vacuo*, affording a vibrant yellow oil. The oil was analyzed by ^{1}H NMR (Figure 2.30) and MS (LRESI) (Figure 2.31). The spectra were inconsistent with the desired product.

Attempted conversion of diisoamyl-2-(3-*tert*-butyldimethylsilyloxybenzyl)-2-methyl malonate to 2-(3-*tert*-butyldimethylsilyloxybenzyl)-2-methylmalonic anhydride A 25 mL, three neck flask with a condenser was evacuated and charged with argon. Diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl malonate (0.46 g, 1.0 mmol), toluene (15 mL) and a catalytic amount of p-toluene sulfonic acid monohydrate were added to the reaction flask. The reaction was heated at reflux for four hours. The flask was cooled to room temperature, and the solvent was removed *in vacuo*. A ^{1}H NMR spectrum (Figure 2.32) was obtained on the residue. The spectrum was consistent with diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl malonate

Synthesis of dibenzyl-2-(4-methoxybenzyl)-2-methyl malonate A 100 mL, three neck flask with a stir bar was evacuated and charged with argon. Sodium hydride, 60% in mineral oil (0.44 g, 11 mmol) was added to the reaction flask in an N_2 filled glove bag. The reaction flask was placed in an ice, and DMF (14 mL)

was added. Dibenzyl (methyl) malonate (3.0 g, 10 mmol) was added dropwise, resulting in a large amount of bubbles (H_2), forming the enolate solution. 4-Methoxybenzyl chloride (1.6 mL, 11 mmol) was added to the reaction flask drop wise. A precipitate formed during the addition. The reaction mixture was stirred over night at room temperature. A large amount of white precipitate had formed.

The reaction was quenched with the addition of deionized water (50 mL). The amount of white precipitate increased. The solid was removed by gravity filtration. The solid was not soluble in ethyl acetate or deionized water. The mixture was transferred to a separatory funnel with ethyl acetate (50 mL). The lower aqueous layer was extracted with ethyl acetate (50 mL, three times). The combined organic layers were washed with deionized water (50 mL) and brine (75 mL). The organic layer was dried over magnesium sulfate, and the solvent was removed *in vacuo*.

The final product, the white solid that had precipitated out, was not purified. Yield = 2.7898 g (66%). TLC: R_f = 0.45 in benzene. ^{1}H NMR, ($CDCl_3$) δ: 7.34-7.27 (m, 10H), 6.93 (d, J=8.7 Hz, 2H), 6.71 (d, J=6.7 Hz, 2H), 5.15 (s, 4H), 3.75 (s, 3H), 1.30 (s, 3H), Figure 2.33.

Attempted synthesis of dibenzyl-2-(4-hydroxybenzyl)-2-methyl malonate A 250 mL, three neck flask was evacuated and charged with argon. Aluminum bromide (18 g, 69 mmol) was added to the reaction flask in an N_2 filled glove bag. The reaction flask was placed in an ice bath and ethanethiol (20 mL, 27 mmol) was added. Dibenzyl-2-(4-methoxybenzyl)-2-methyl malonate (2.62 g,

6.26 mmol) was suspended in ethanethiol (20 mL, 267 mmol). This mixture was added to the reaction flask via pipette. Additional ethanethiol (10 mL, 130 mmol) ensured complete transfer of the dibenzyl-2-(4-methoxybenzyl)-2-methyl malonate. The solution became a homogeneous vibrant yellow. The reaction was stirred at room temperature for one hour.

The reaction flask was placed in an ice bath, and the reaction was quenched with deionized water (50 mL). A white solid and clear supernatant formed after a robust evolution of gas. The ethanethiol was removed by air purge. Ethyl acetate (50 mL) and 2 M hydrochloroic acid (50 mL) were added to the reaction flask. The solution had a pH of 1, and most of the solid dissolved. The reaction flask contents were transferred to a separatory funnel. The lower aqueous layer was extracted with ethyl acetate (80 mL, three times). The combined organic layers were dried over magnesium sulfate, and the solvent was removed *in vacuo*. ^{1}H NMR spectrum (Figure 2.34) was not consistent with the desired product, since it indicates removal of both benzyl groups. The final product, a white solid, was purified by flash chromatography in 10:30:1 hexane/ ethyl acetate/ glacial acetic acid. TLC: R_f = 0.45 in 10:30:1 hexane/ ethyl acetate/ glacial acetic acid. IR (nujol): 3293 cm^{-1} (O-H), 1713 cm^{-1} (C=O), 1612 (C=C), Figure 2.35. ^{1}H NMR (CD_3OD) δ: 6.99 (d, J=8.5 Hz, 2H), 6.65 (d, J=8.5 Hz, 2H), 3.08 (s, 2H), 1.28 (s, 3H), Figure 2.34. MS (LREI) (m/z) (rel. intensity): 224.1 (M+, 3%), Figure 2.36. MS (HREI) calc for $C_{11}H_{12}O_5$ 224.0685, found 224.0687.

Synthesis of diisoamyl-2-(4-methoxybenzyl)-2-methyl malonate A 100 mL, three neck flask with a stir bar was evacuated and charged with argon. Sodium hydride, 60% in mineral oil (1.14 g, 28.5 mmol) was added to the reaction flask in an N_2 filled glove bag. The reaction flask was placed in an ice bath, and DMF (20 mL) was added. Diisoamyl (methyl) malonate (5.3 g, 20 mmol) was added dropwise, resulting in a large amount of bubbles (H_2), forming the enolate solution. 4-Methoxybenzyl chloride (3.9 mL, 29 mmol) was added to the reaction flask dropwise, and a precipitate formed during the addition. The reaction mixture was stirred for three and a half hours at room temperature.

The reaction was quenched with the addition of deionized water (35 mL). The mixture was transferred to a separatory funnel with diethyl ether (80 mL). The lower aqueous layer was extracted with ethyl acetate (70 mL, two times). The combined organic layers were washed with brine (40 mL, two times). The organic layer was dried over magnesium sulfate, and the solvent was removed *in vacuo*.

The final product, a pale yellow liquid, was purified by flash chromatography in 8:2 hexanes/ ethyl acetate. Yield = 6.3146 g (82%). TLC: R_f = 0.71 in 8:2 hexanes/ ethyl acetate. IR(Neat): 1732 cm^{-1} (s, C=O), 1621 cm^{-1} (C=C), Figure 2.37. ^{1}H NMR, ($CDCl_3$) δ: 7.00 (d, J=8.6 Hz, 2H), 6.76 (d, J=8.6 Hz, 2H), 4.26 (m, 4H), 3.74 (s, 3H), 3.14 (s, 2H), 1.70-1.57 (m, 2H), 1.48 (q, J=6.8 Hz, 4H), 1.30 (s, 3H), 0.88 (d, J=6.6 Hz, 12H) Figure 2.38. ^{13}C NMR δ: 171.89 (2C), 131.00 (2C), 128.02 (1C), 113.42 (2C), 69.71 (1C) 63.76 (1C), 54.95 (1C), 40.22 (1C), 37.01 (1C), 33.94 (1C), 24.80 (1C), 22.27 (4C), 19.59

(1C), 16.20 (1C), 11.05 (1C), Figure 2.39. MS(LRESI) (m/z) (rel. intensity): 379.6 (M+1, 100%), Figure 2.40. Anal Calc for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found C, 69.57; H, 8.95.

Synthesis of diisoamyl-2-(4-hydroxybenzyl)-2-methyl malonate A 250 mL, three neck flask was evacuated and charged with argon. Aluminum bromide (32.1 g, 120 mmol) was added to the reaction flask in an N_2 filled glove bag. The reaction flask was placed in an ice bath, and ethanethiol (90 mL, 1.2 mol) was added. Diisoamyl-2-(4methoxybenzyl)-2-methyl malonate (4.5 g, 12 mmol) was added to the reaction flask dropwise. The solution became a homogeneous vibrant yellow. The reaction was stirred at room temperature for one hour.

The reaction flask was placed in an ice bath and the reaction was quenched with deionized water (35 mL). The ethanethiol was removed by air purge. Ethyl acetate (40 mL) and 2 M hydrochloroic acid (25 mL) were added to the reaction flask. The solution had a pH of 1. The reaction flask contents were transferred to a separatory funnel. The lower aqueous layer was extracted with ethyl acetate (50 mL, three times). The combined organic layers were washed with brine (100 mL) and dried over magnesium sulfate, and the solvent was removed *in vacuo*.

The final product, a pale yellow oil, was purified by flash chromatography in 9:1 benzene/ ethyl acetate. Yield = 2.7766 g (63.7%). TLC: R_f = 0.40 in 9:1 benzene/ ethyl acetate. IR (neat): 3439 cm^{-1} (O-H), 1731 cm^{-1} (s, C=O), 1615 cm^{-1} (C=C), Figure 2.41. ^1H NMR, ($CDCl_3$) δ: 6.92 (d, J=8.6 Hz, 2H), 6.66 (d,

J=8.6 Hz, 2H), 4.19-4.06 (m, 4H), 3.12 (s, 2H), 1.70-1.57 (m, 2H), 1.49 (q, J=6.4 Hz, 4H), 1.31 (s, 3H), 0.88 (d, J=6.7 Hz, 12H), Figure 2.42. ^{13}C NMR ($CDCl_3$) δ: 172.33, 154.81, 131.20, 127.77, 115.07, 64.04, 55.06, 40.33, 37.04, 24.90, 22.35, 19.68, Figure 2.43. MS (LRESI) (m/z) (rel. intensity): 365.2 (M+1, 100%), Figure 2.44. Anal Cald for $C_{21}H_{32}O_5$: C, 69.20; H, 8.65. Found C, 68.51; H, 8.78.

Synthesis of diisoamyl-2-(4-methoxyethoxymethylbenzyl)-2-methyl malonate In 100 mL, three neck flask with a stir bar was evacuated and charged with argon. Sodium hydride, 60% in mineral oil (0.71 g, 18 mmol) was added to the reaction flask in an N_2 filled glove bag. The reaction flask was placed in an ice bath, and THF (10 mL) was added. A solution of diisoamyl-2-(4-hydroxybenzyl)-2-methyl malonate (2 g, 6 mmol) and THF (4 mL) was added to the reaction flask drop wise. A large amount of bubbles formed, and the mixture became a vibrant yellow. Additional THF (5 mL) was used to ensure complete transfer. Methoxethoxymethyl chloride (2.9 mL, 24 mmol) was added drop wise to the reaction flask. The yellow color faded, and the precipitate became finer in nature. The reaction mixture was stirred overnight at room temperature.

The reaction flask was placed in an ice bath, and the reaction mixture was quenched with the addition of deionized water (10 mL), which resulted in formation of bubbles. The precipitate dissolved. The mixture was transferred to a separatory funnel with ethyl acetate (10 mL). The lower aqueous layer was extracted with ethyl acetate (15 mL, three times). The combined organic layers

were washed with brine (50 mL) and dried over magnesium sulfate. The solvent was removed *in vacuo*.

The final product, a colorless liquid, was purified by flash chromatography in 8:2 hexanes/ ethyl acetate. Yield = 2.1337 g, (80.5%). TLC: R_f = 0.54 in 8:2 hexanes/ ethyl acetate. IR (neat): 1731 cm^{-1} (s, C=O), 1611 cm^{-1} (C=C), Figure 2.45. ^1H NMR, ($CDCl_3$) δ: 6.99 (d, J=8.8 Hz, 2H), 6.54 (d, J=8.8 Hz, 2H), 5.20, (s, 2H), 4.17-4.04 (m, 4H), 3.79-3.76 (m, 2H), 3.53-3.50 (m, 2H), 3.33 (s, 3H), 3.13 (s, 2H), 1.69-1.56 (m, 2H), 1.48 (q, J=6.9 Hz, 4H), 1.29 (s, 3H), 0.88 (d, J=6.7 Hz, 12H), Figure 2.46. ^{13}C NMR ($CDCl_3$) δ: 171.94, 156.16, 131.09, 115.81, 93.36, 71.46, 67.49, 63.87, 58.91, 54.87, 40.27, 37.04, 24.86, 22.32, 19.61, Figure 2.47. MS (LRFAB) (m/z) (rel. intensity): 453.3 (M+1, 100%), Figure 2.48.

Synthesis of diisoamyl-2-(4-triisopropylsilyloxybenzyl)-2-methyl malonate

In a 50 mL, three neck flask with a stir bar was evacuated and charged with argon. A solution of diisoamyl-2-(4-hydroxybenzyl)-2-methyl malonate (2.3 g, 6.3 mmol) and CH_2Cl_2 (6 mL) was added to the reaction flask. Triisopropylsilyl chloride (4.2 mL, 19 mmol) was added to the reaction flask. The solution was a homogeneous pale yellow. Imidazole (2.6 g, 38 mmol) was added to the reaction flask and a large amount of precipitate formed. The reaction mixture was stirred over night.

The reaction was quenched with the addition of deionized water (20 mL), and the precipitate dissolved. The mixture was transferred to a separatory funnel

with ethyl acetate (20 mL). The lower aqueous layer was extracted with ethyl acetate (25 mL, two times). The combined organic layers were washed with brine (50 mL) and dried over magnesium sulfate. The solvent was removed *in vacuo*.

The final product, a pale yellow liquid, was purified by flash chromatography in benzene. Yield = 2.1220 g (65%). TLC: R_f = 0.80 in benzene. IR (neat): 1735.41 cm^{-1} (C=O), 1609 cm^{-1} (C=C), Figure 2.49. ^1H NMR, ($CDCl_3$) δ: 6.93 (d, J=8.4 Hz, 2H), 6.74 (d, J=8.4 Hz, 2H), 4.17-4.04 (m, 4H), 3.12, (s, 2H), 1.70-1.57 (m, 2H), 1.48 (q, J=6.7 Hz, 4H), 1.28 (s, 3H), 1.26-1.13 (m, 3H), 1.06 (d, J=7.0 Hz, 18H), 0.81 (d, J=6.4 Hz, 12H), Figure 2.50. ^{13}C NMR ($CDCl_3$) δ: 172.11, 154.95, 131.06, 128.53, 119.55, 63.92, 55.00, 40.41, 37.09, 24.94, 22.39, 19.68, 17.88, 12.60, Figure 2.51. MS (LRFAB) (m/z) (rel. intensity): 521.4 (M+1, 22%), Figure 2.52. Anal. Calcd for $C_{30}H_{52}O_5Si$: C, 69.18; H, 10.06. Found C, 68.66; H, 9.97.

Attempted synthesis of 2-(4-methoxyethoxymethylbenzyl)-2-methylmalonic acid Diisoamyl-2-(4methoxyethoxymethylbenzyl)-2-methyl malonate (0.38 g, 0.84 mmol), THF (4 mL), lithium hydroxide monohydrate (1.1 g, 25 mmol) and deionized water (4.8 mL, 270 mmol) were combined in a 25 mL flask. The reaction mixture was heated at reflux for 13 hours. Nitric acid (3 M) was added to the mixture until the solution pH was 5. The solvent was removed *in vacuo* affording a white solid. The solid was stirred with ethyl acetate (50 mL) for 30 minutes. The mixture was filtered and the solvent was removed *in vacuo* from

the filtrate affording a white solid. The ^{1}H NMR spectrum (Figure 2.53) of the solid was inconsistent with the desired product.

Attempted synthesis of 2-(4-triisopropylsilyloxybenzyl)-2-methylmalonic acid Diisoamyl-2-(triisopropylsilyloxybenzyl)-2-methyl malonate (0.51 g, 0.97 mmol), THF (10 mL), potassium hydroxide, 85% (1.9 g, 35 mmol) and deionized water (5.4 mL, 300 mmol) were combined in a 50 mL flask. The reaction mixture was heated at reflux for 5 hours. Nitric acid (3 M) was added to the mixture until the solution pH was 5.5. The solvent was removed *in vacuo,* affording an orange solid. The solid was washed with absolute ethanol (200 mL, three times). The ethanol was removed *in vacuo*, affording an orange solid. The ^{1}H NMR spectrum (Figure 2.54) of the solid was inconsistent with the desired product.

Attempted synthesis of 2-(4-triisopropylsilyloxybenzyl)-2-methyl malonaldehyde Diisoamyl-2-(triisopropylsilylbenzyl)-2-methyl malonate (0.41 g, 0.78 mmol) and a stir bar were placed in a 50 mL, three neck flask. The flask was evacuated and charged with argon. Dichloromethane (1 mL) was added to the reaction flask, which was cooled to -78 °C. Diisobutyl aluminum hydride, 1.0 M in CH_2Cl_2 (4.7 mL, 4.7 mmol) was added to the reaction flask. The solution was clear and colorless. The solution was stirred at –78 °C for five and a half hours.

The reaction was quenched with the addition of 1:1 THF/deionized water (5 mL), and a large amount of white precipitate formed. The solid was

suspended in ethyl acetate (20 mL) and vacuum filtered through celite. The solid was rinsed with ethyl acetate (50 mL). The filtrate was transferred to a separatory funnel. The lower aqueous layer was removed and extracted with ethyl acetate (30 mL, two times). The combined organic layers were washed with brine (40 mL) and dried over magnesium sulfate. The solvent was removed *in vacuo*. The ^{1}H NMR spectrum (Figure 2.55) of the solid was inconsistent with the desired product but does show an aldehyde proton at 9.72 ppm. The IR spectrum (Figure 2.56) also shows an aldehyde, C-H, stretch at 2752 cm^{-1}.

References

1. Rochon, F.R.; Gruia, L.M. *Inorg Chim. Acta*. **2000**, *306*, 193.
2. Greene, T.W. *Protective Group in Organic Synthesis*, John Wiley & Sons, 1981.
3. Gassman, P.G.; Schenk, W.N., *J. Org. Chem.* **1977**, *42*, 918.
4. Cunico, R.F.; Bedell, L., *J. Org. Chem.* **1980**, *45*, 4797.
5. Skaddan, M.B.; Wust, F.R.; Katzenellenbogen, J.A. *J. Org. Chem.* **1999**, *64*, 8108.
6. Heckrodt, T.J.; Mulzer, J. *J. Am. Chem. Soc.* **2003**, 4680.
7. Ho, Y.P.; To, K.K.W.; Au-Yeung, S.C.F.; Wang, X.; Lin, G.; Han, X. *J. Med. Chem.* **2001**, 2065.
8. Graham, R.J., Paquette, L.A. *J. Org. Chem.* **1995**, 5770
9. Bendler, S.E.; Bundy, J.S.; Clark, S.A.; Davis, D.N.; Orwar, N.A.; Chesnut, R.W. *Synth. Comm.* **2003**, *33*, 3365.

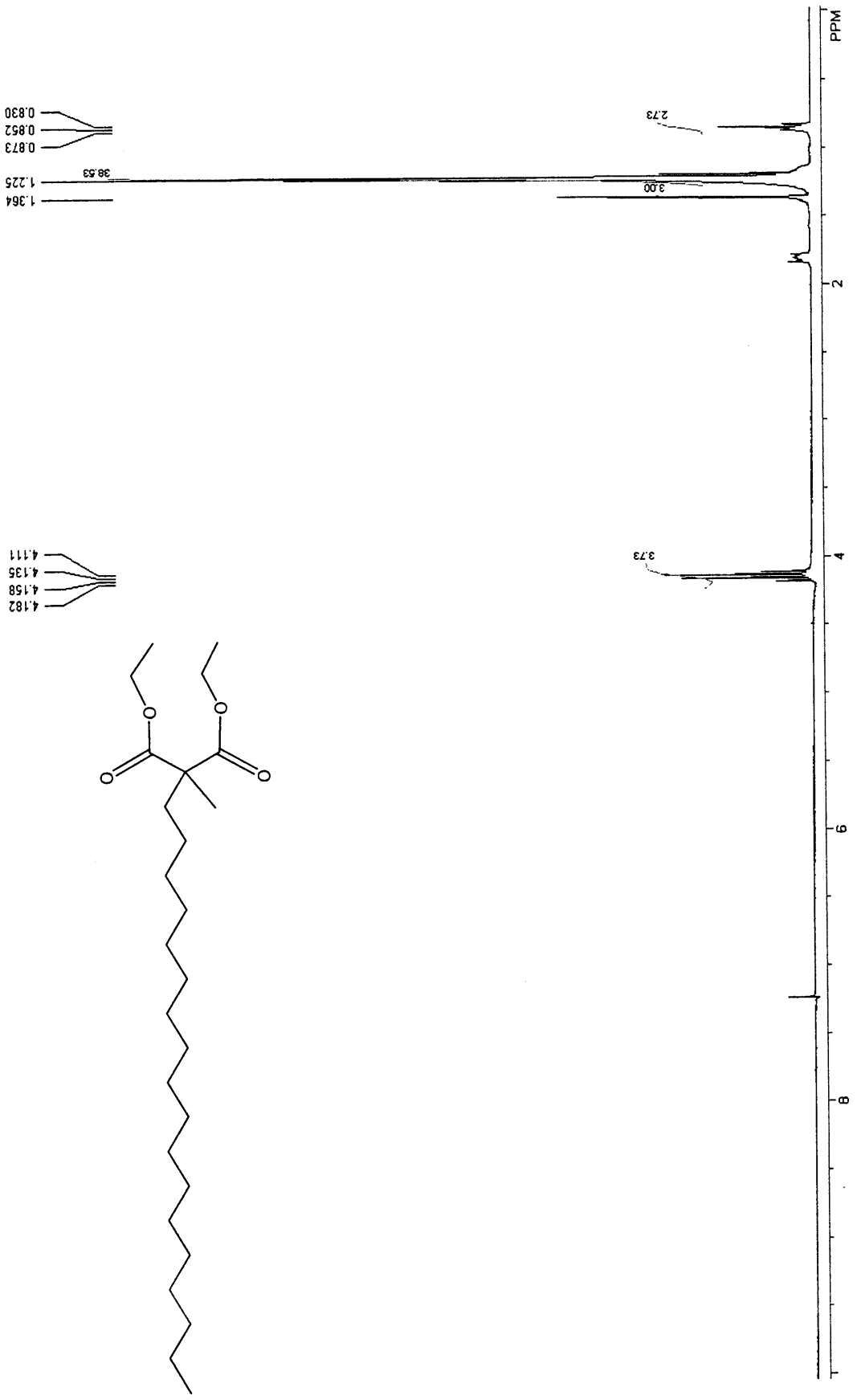


Figure 2.1 ^{1}H NMR Spectrum of Diethyl-2-methyl-2-octadecyl Malonate

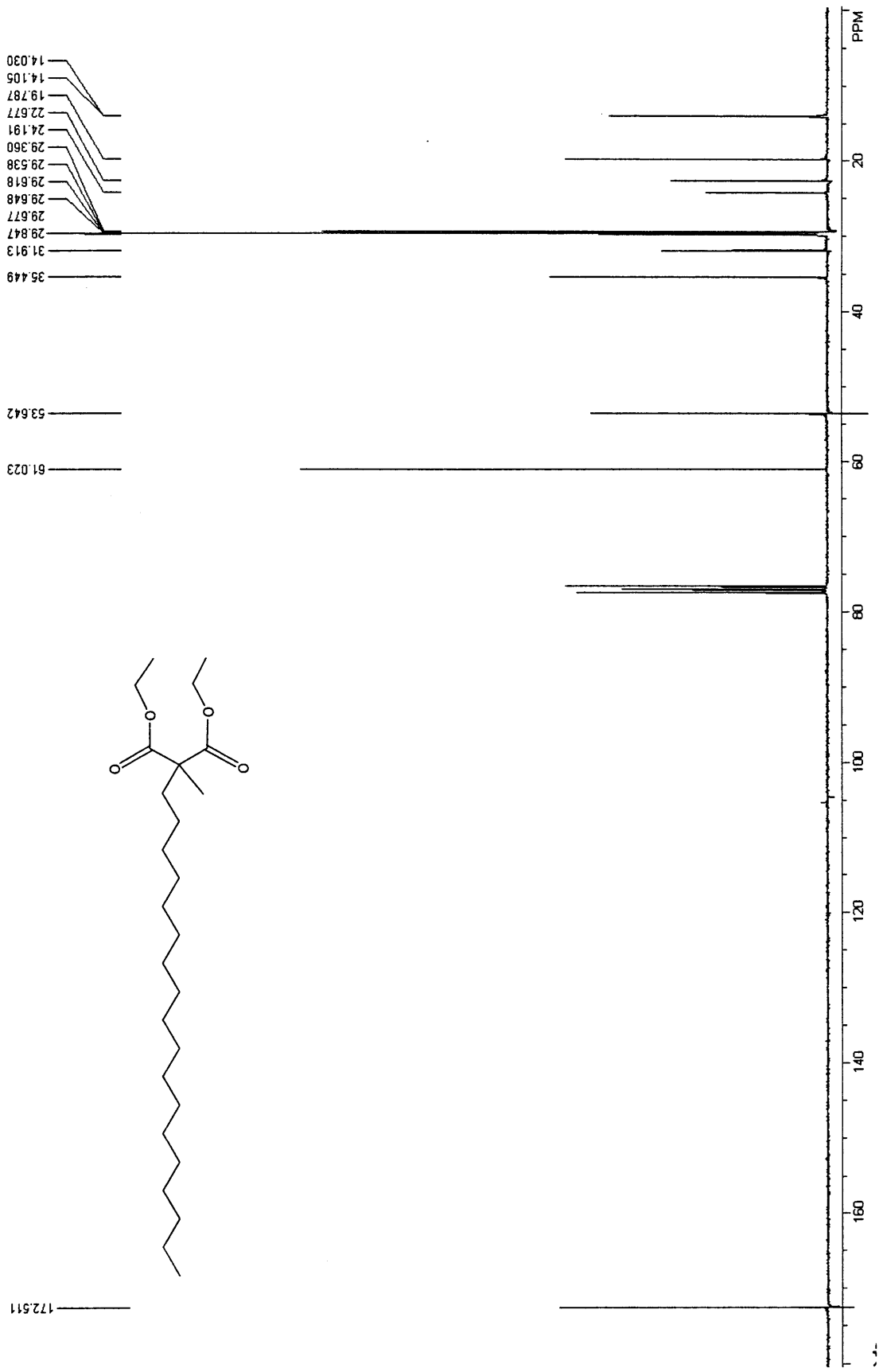


Figure 2.2 ^{13}C NMR Spectrum of Diethyl-2-methyl-2-octadecyl Malonate

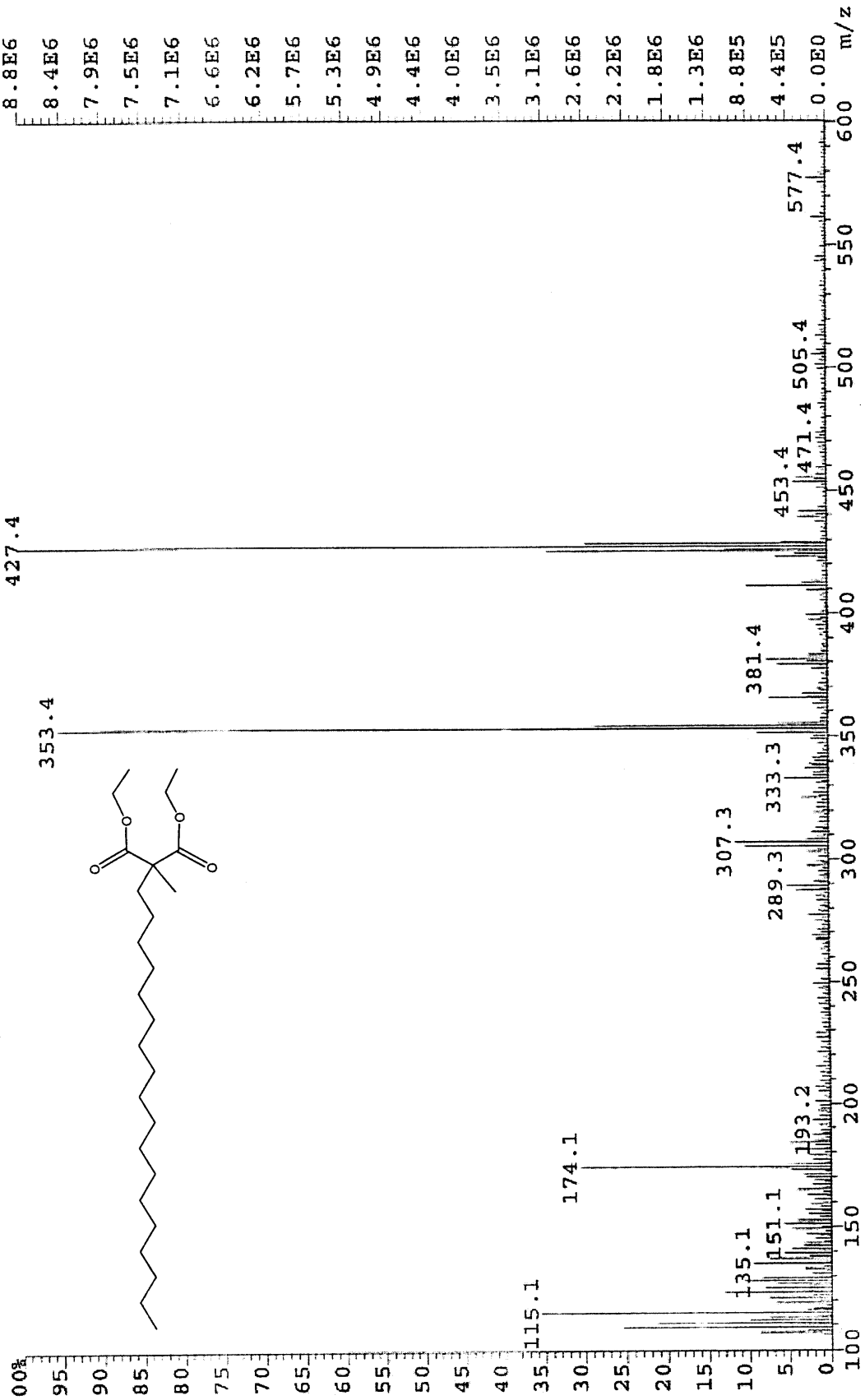


Figure 2.3 MS (LRFAB) of Diethyl-2-methyl-2-octadecyl Malonate

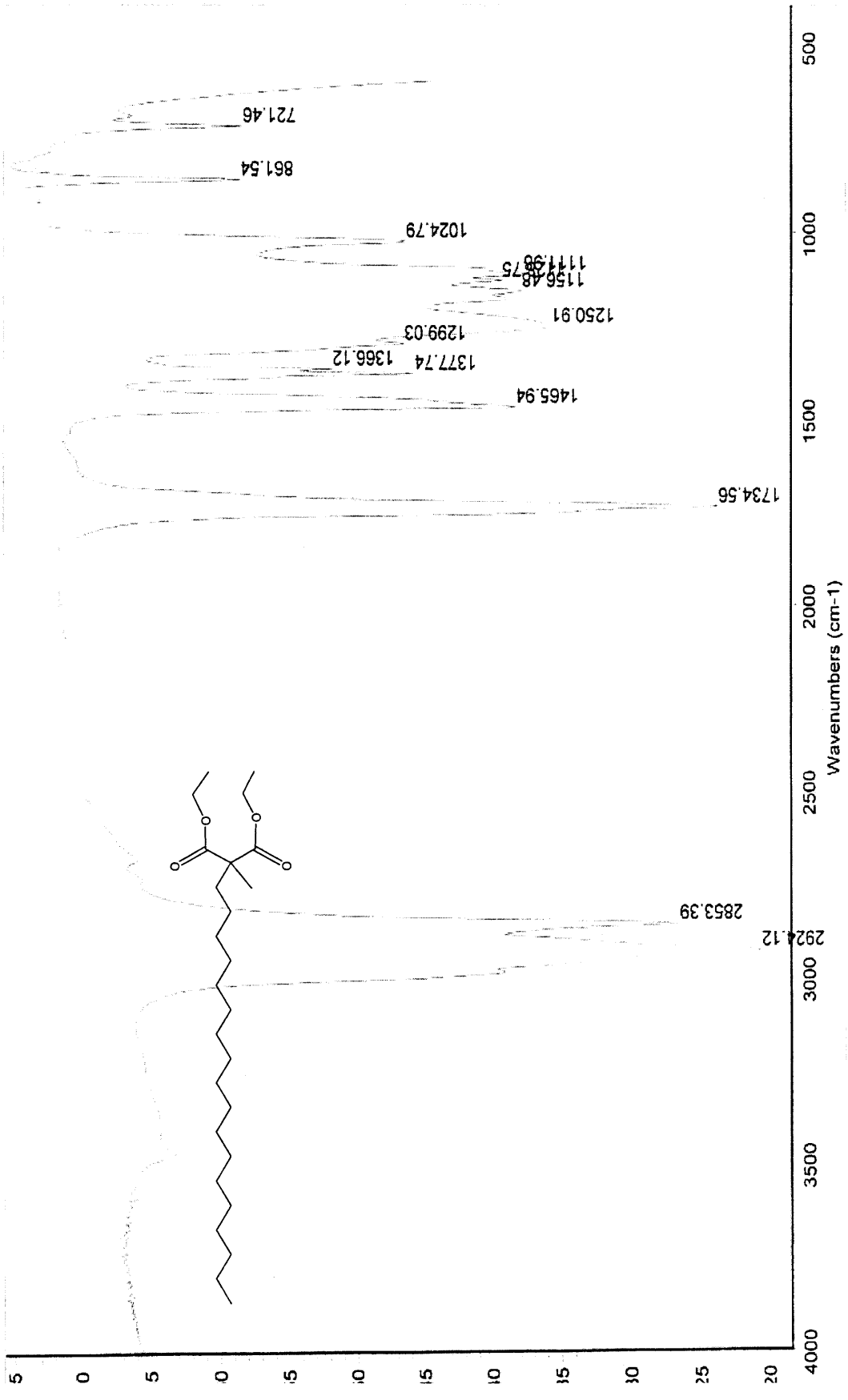


Figure 2.4 IR Spectrum of Diethyl-2-methyl-2-octadecyl Malonate

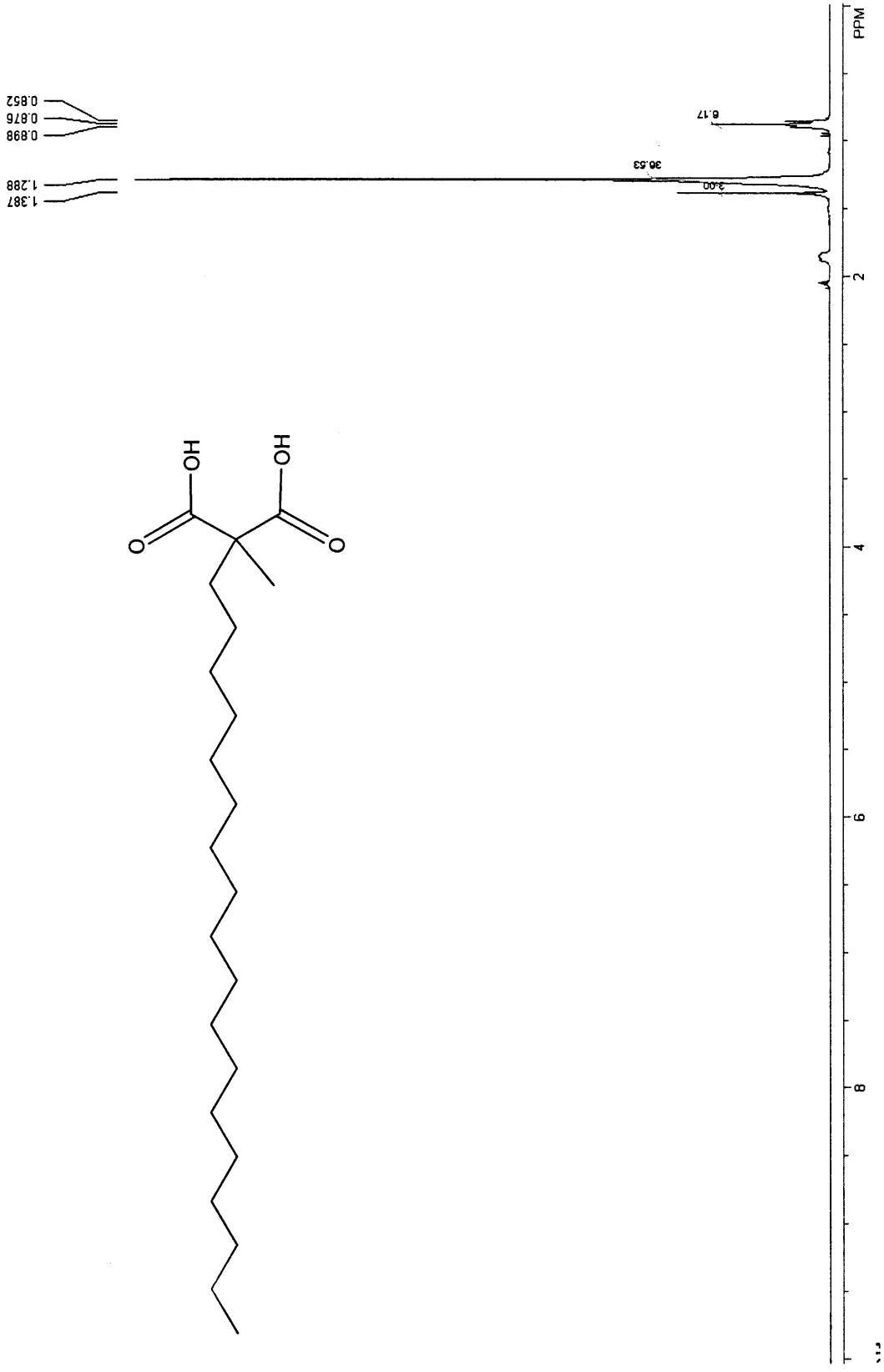


Figure 2.5 ^{1}H NMR Spectrum of 2-Methyl-2-octadecylmalonic Acid

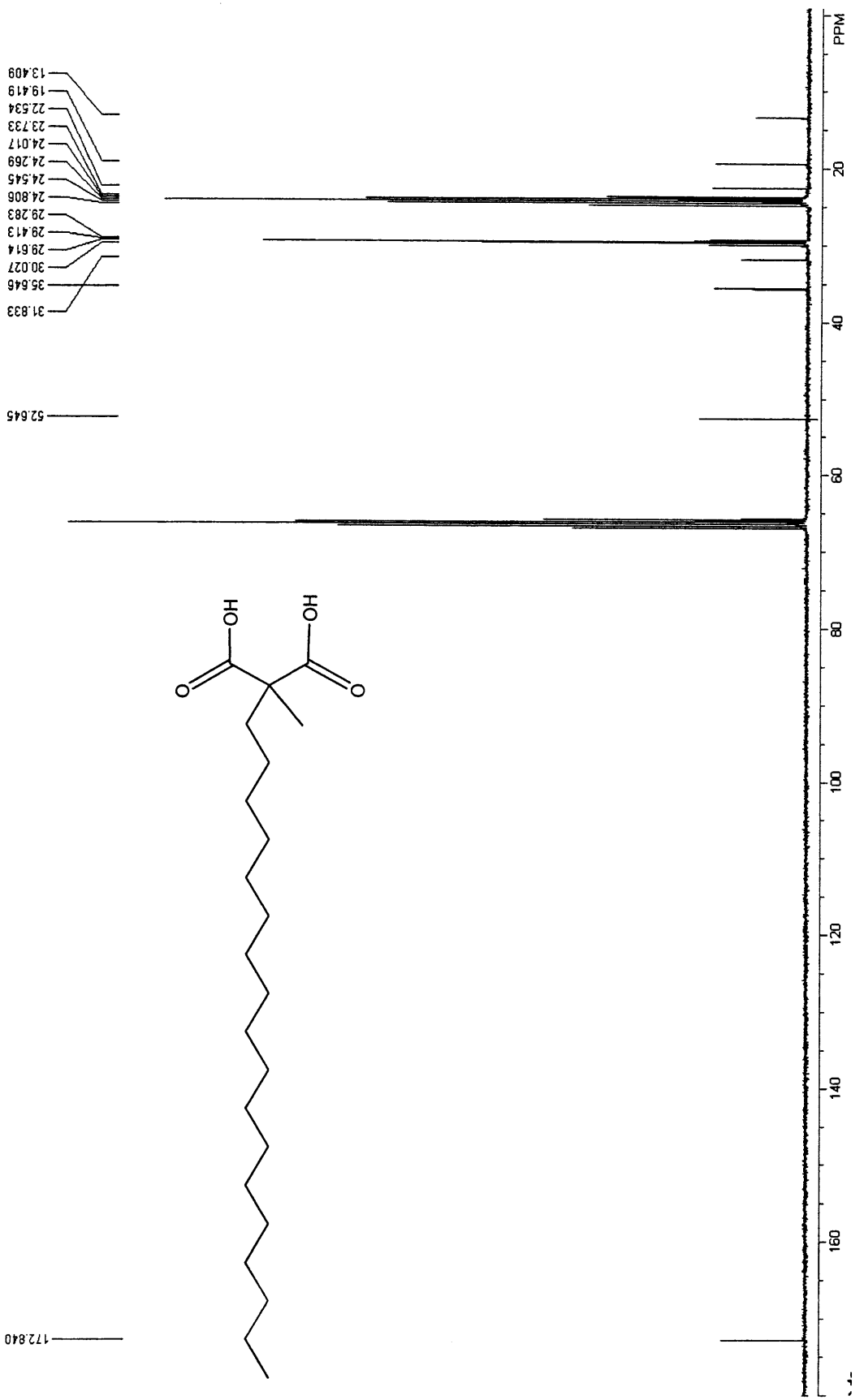


Figure 2.6 ^{13}C Spectrum of 2-Methyl-2-octadecylmalonic Acid

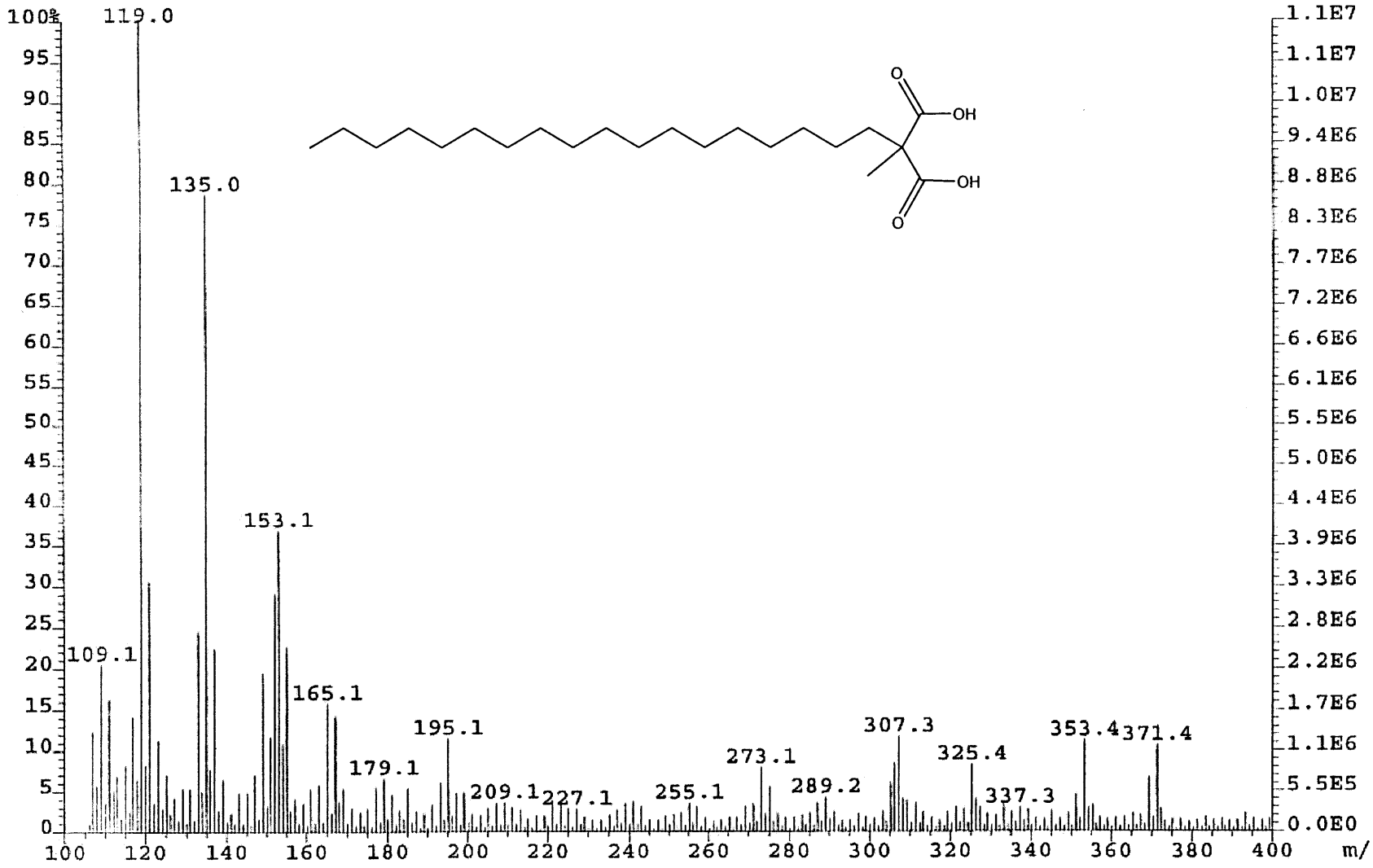


Figure 2.7 MS (LRFAB) of 2-Methyl-2-octadecylmalonic Acid

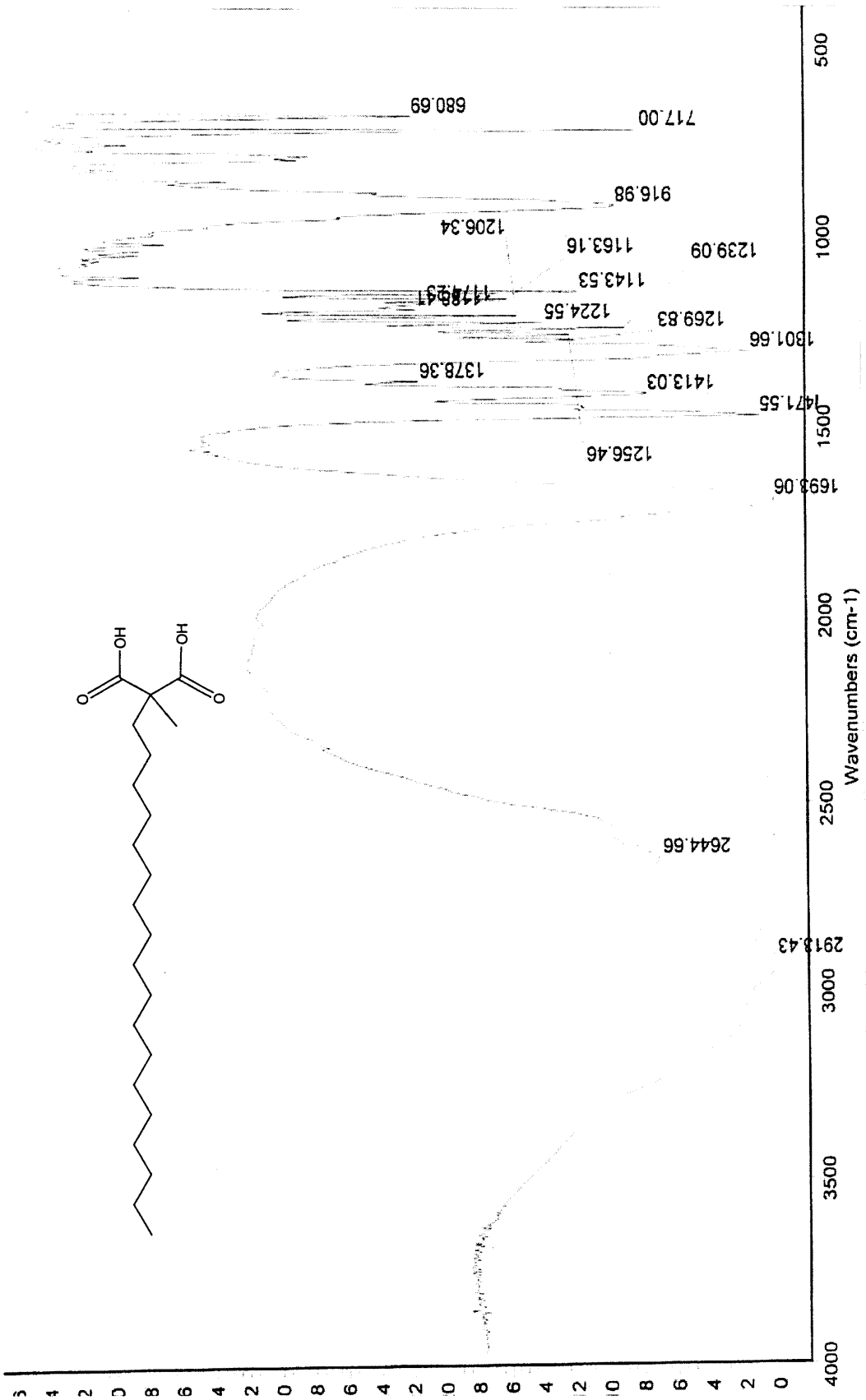


Figure 2.8 IR Spectrum of 2-Methyl-2-octadecylmalonic Acid

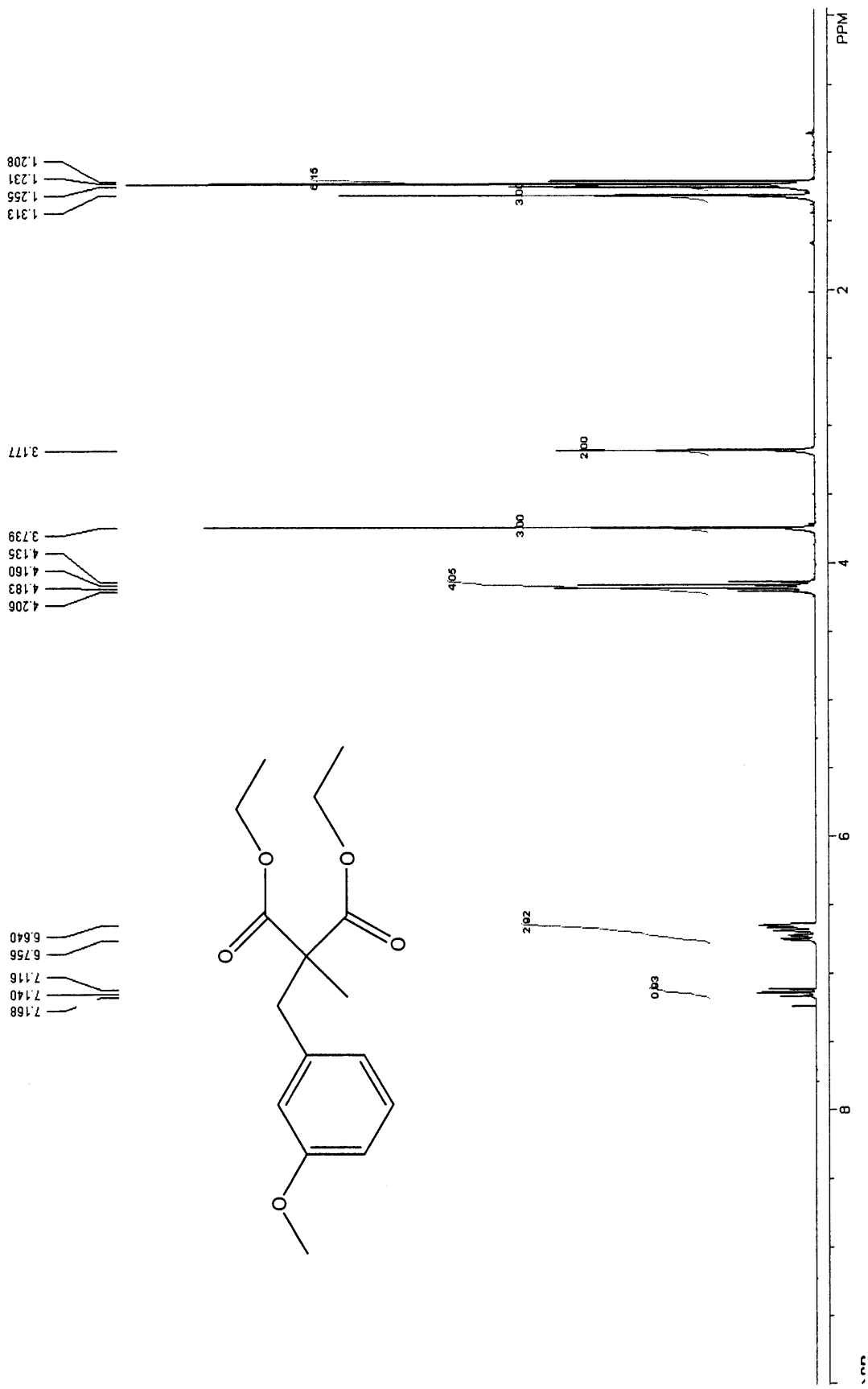


Figure 2.9 ^{1}H NMR Spectrum of Diethyl-2-(3-methoxybenzyl)-2-methyl Malonate

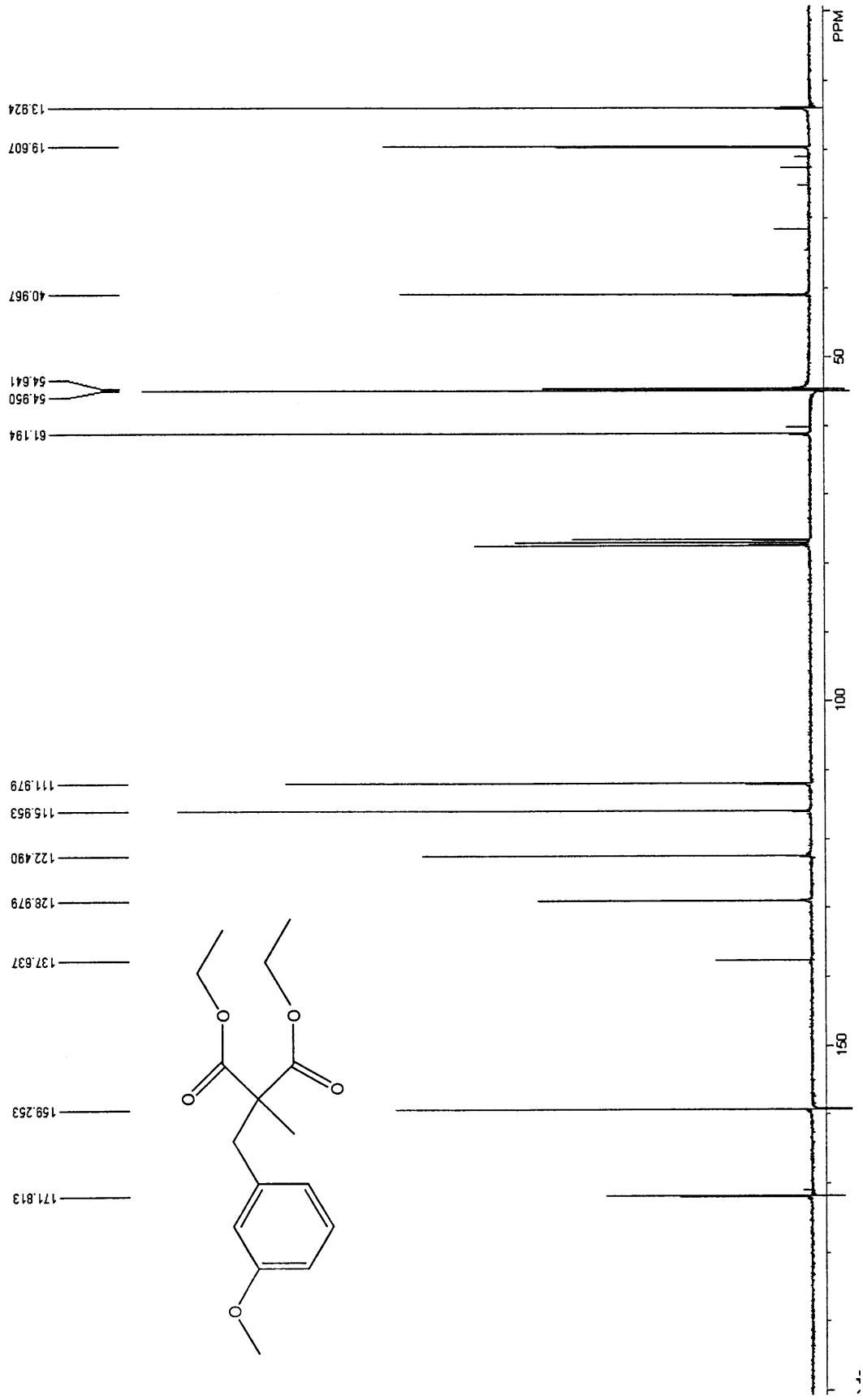


Figure 2.10 ^{13}C NMR Spectrum of Diethyl-2-(3-methoxybenzyl)-2-methyl Malonate

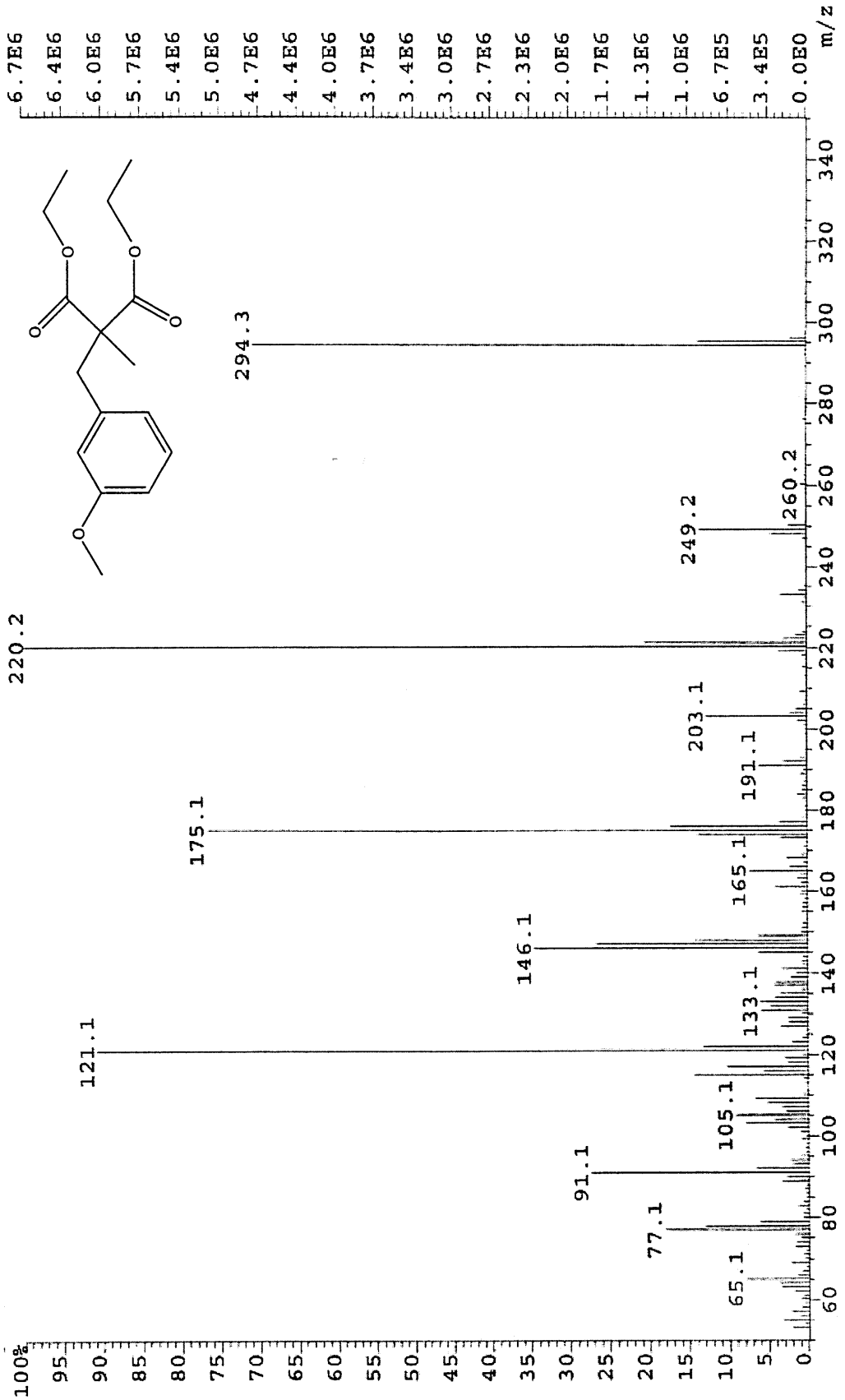


Figure 2.11 MS (LREI) of Diethyl-2-(3-methoxybenzyl)-2-methyl Malonate

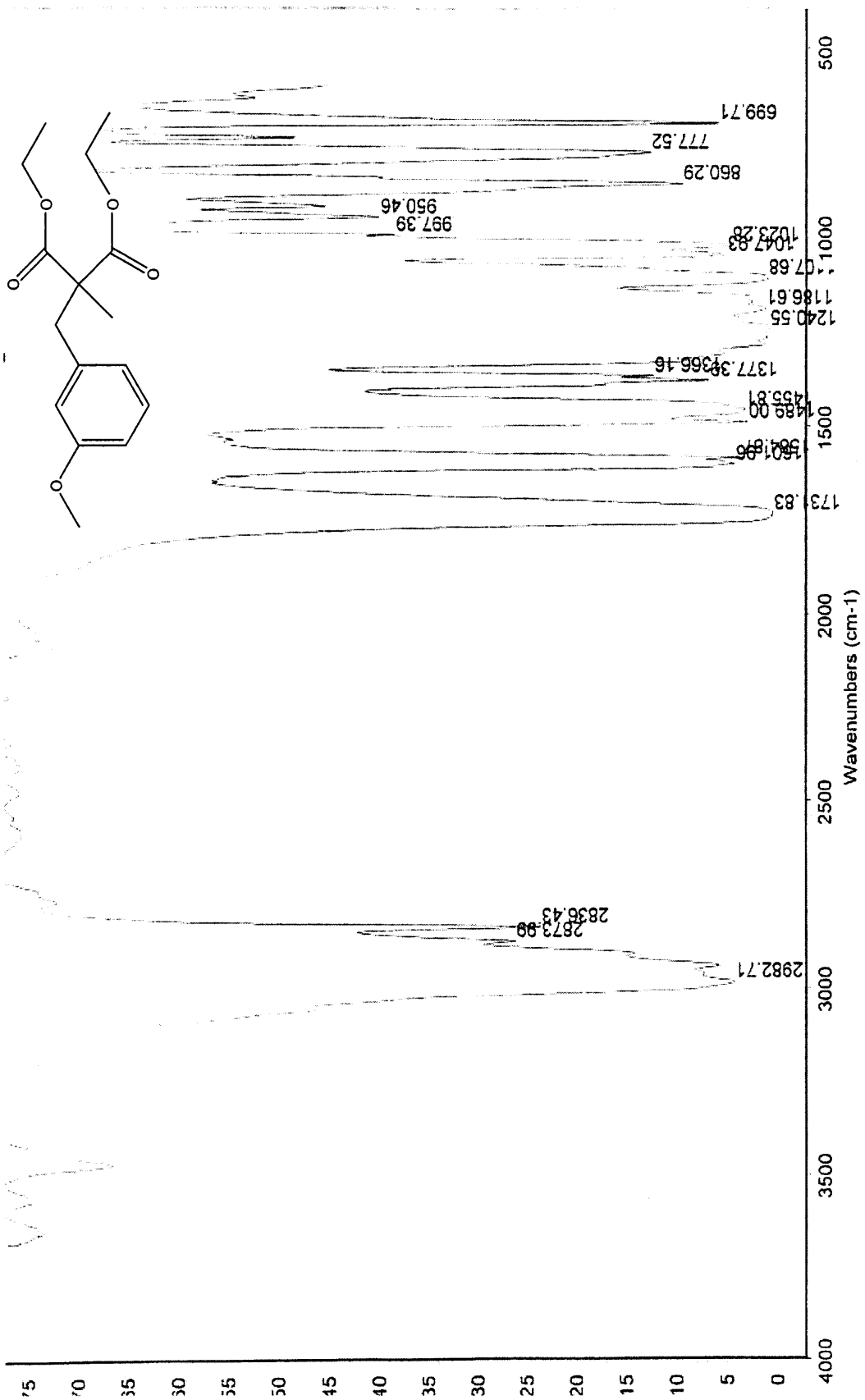


Figure 2.12 IR Spectrum of Diethyl-2-(3-methoxybenzyl)-2-methyl Malonate

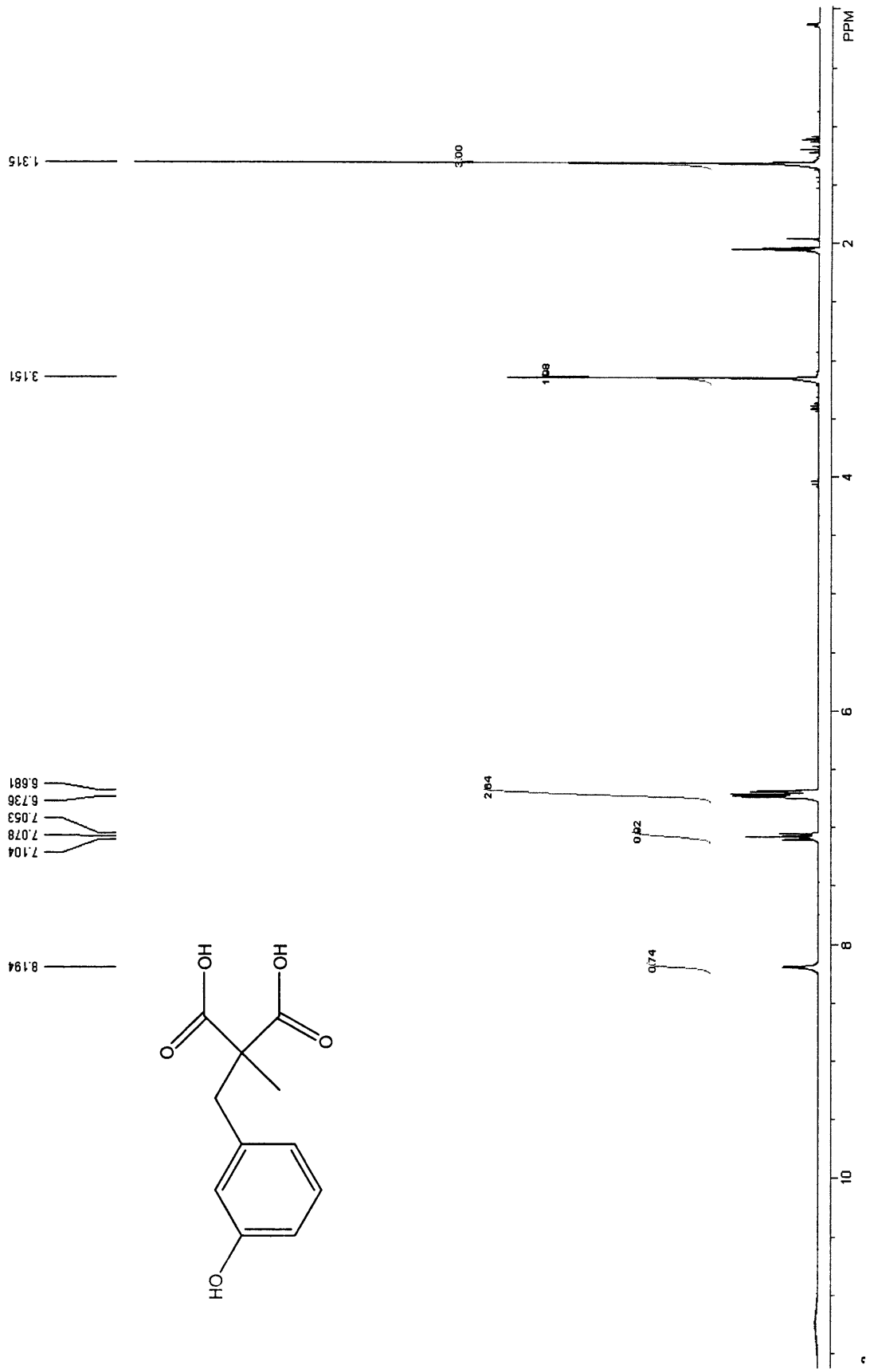


Figure 2.13 ^{1}H NMR Spectrum of 2-(3-Hydroxybenzyl)-2-methylmalonic Acid

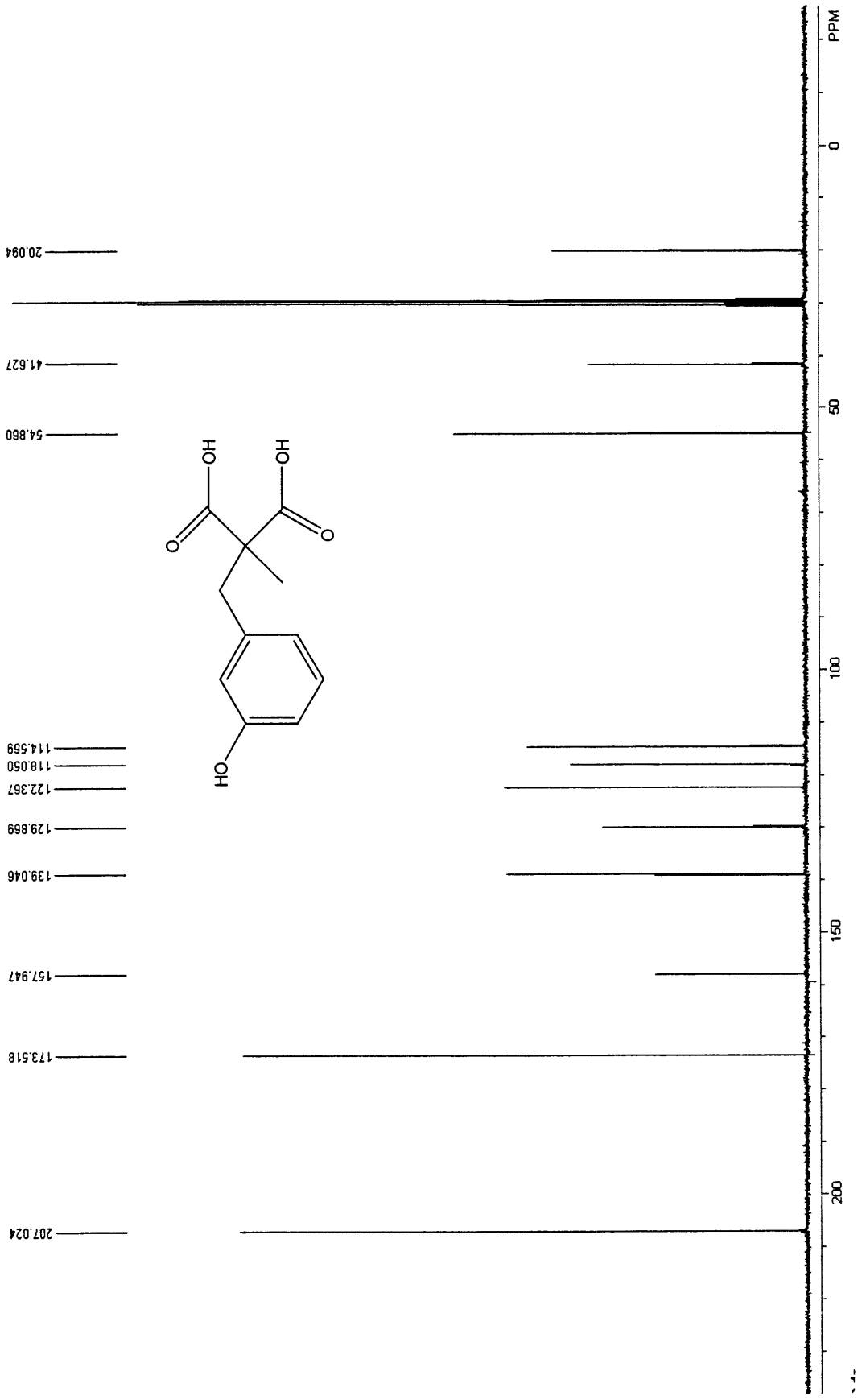


Figure 2.14 ^{13}C Spectrum of 2-(3-Hydroxybenzyl)-2-methylmalonic Acid

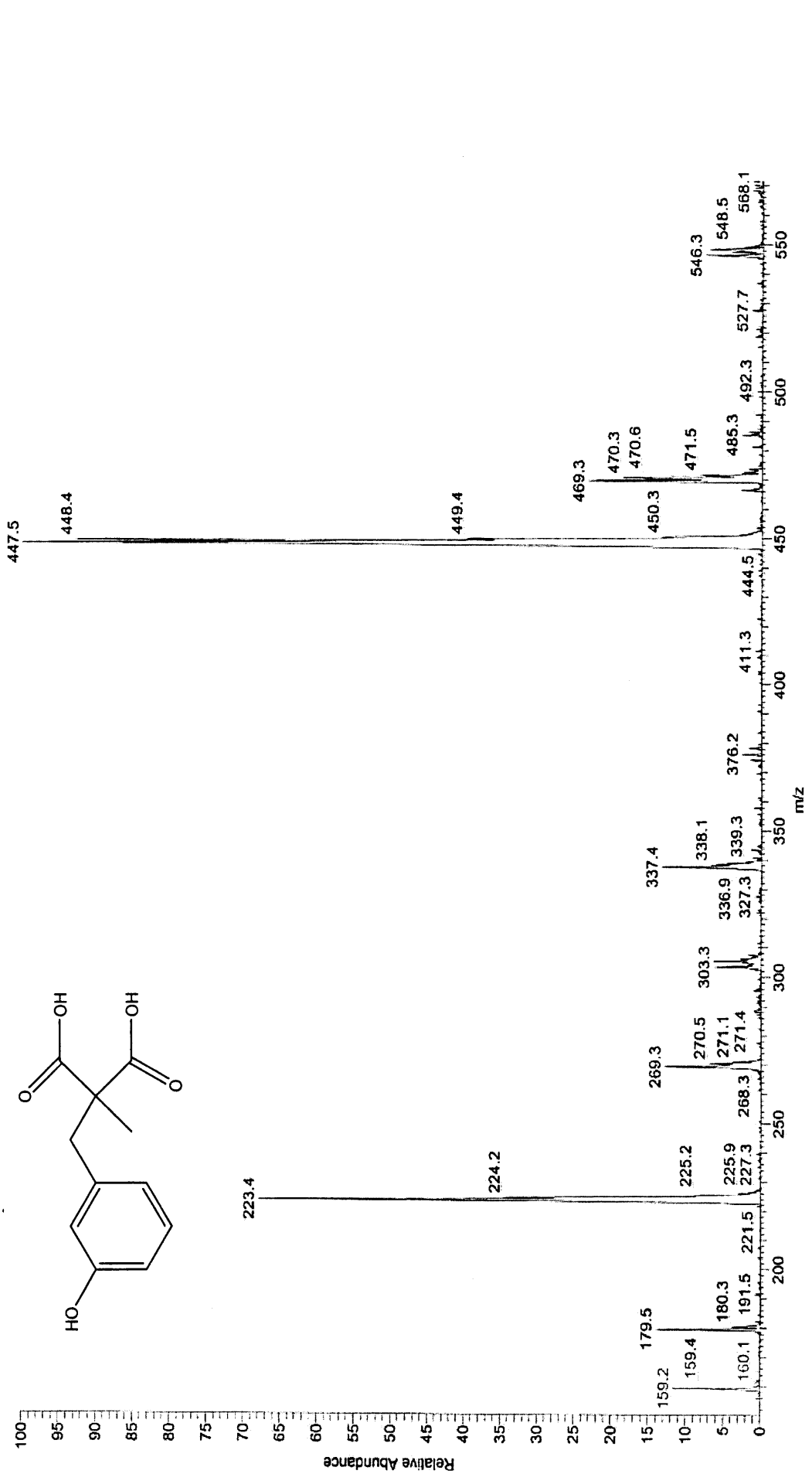


Figure 2.15 MS (LRESI) of 2-(3-Hydroxybenzyl)-2-methylmalonic Acid

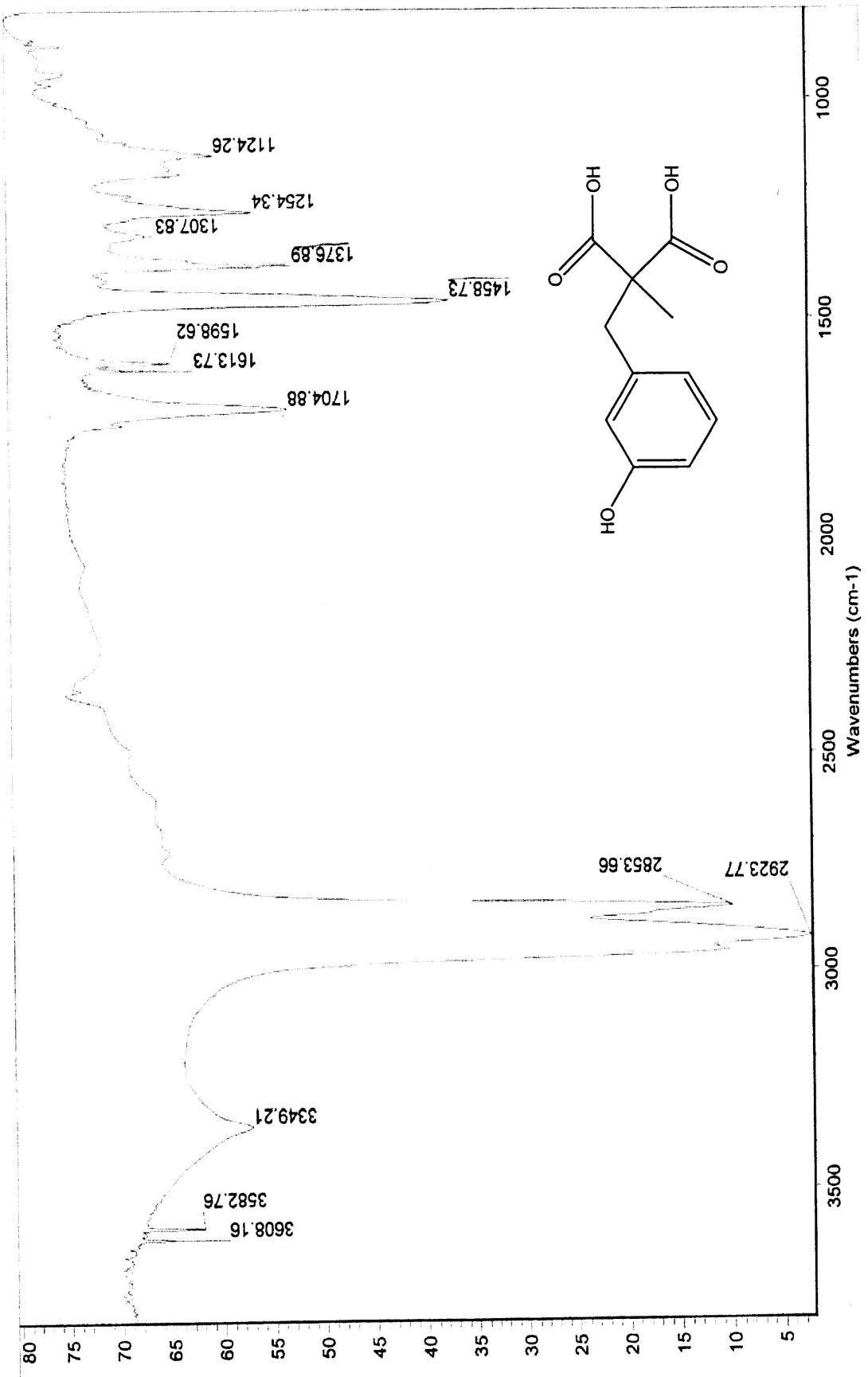


Figure 2.16 IR Spectrum of 2-(3-Hydroxybenzyl)-2-methylmalonic Acid

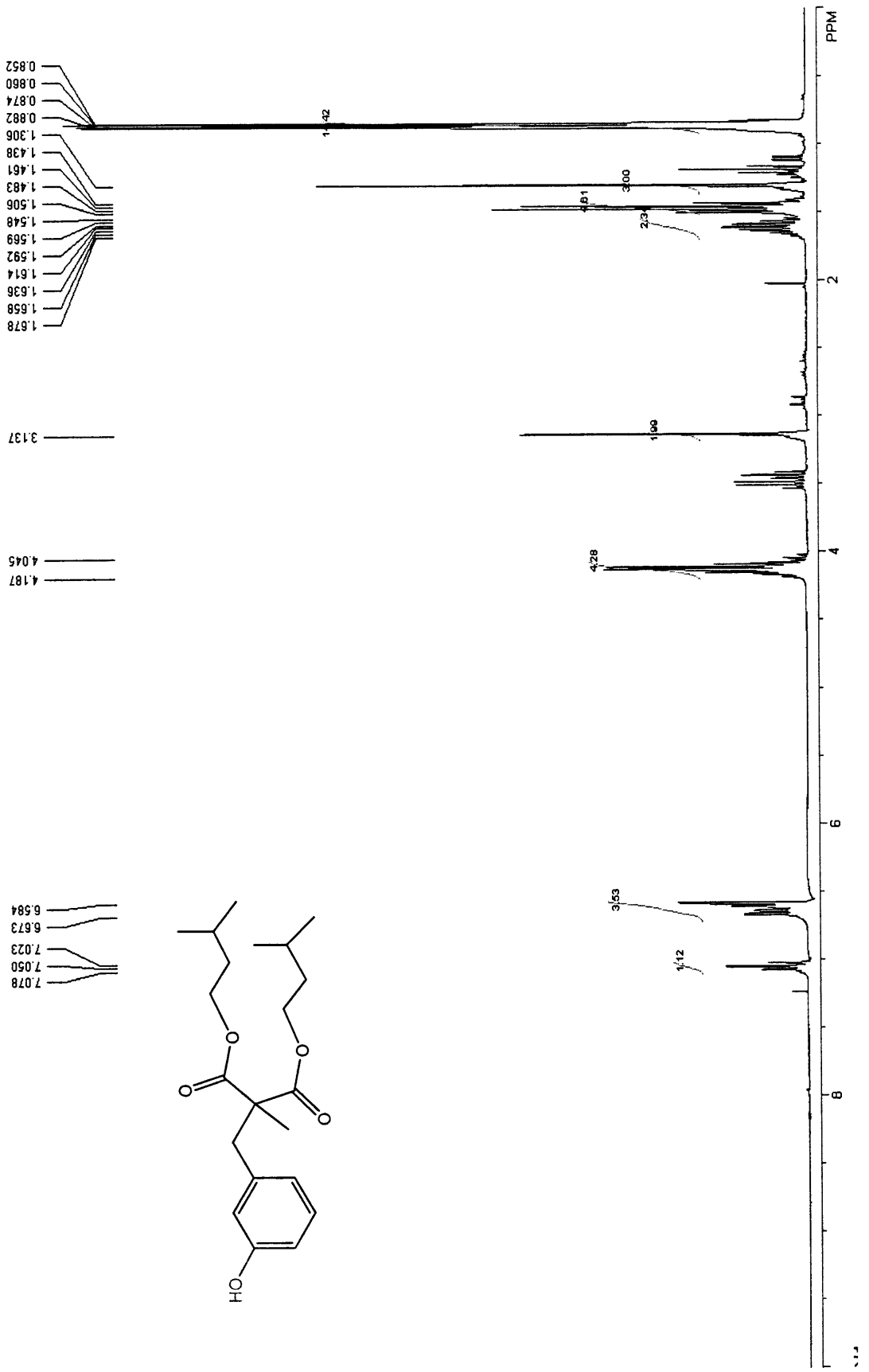


Figure 2.17 ^{1}H NMR Spectrum of Diisoamyl-2-(3-hydroxybenzyl)-2-methyl Malonate

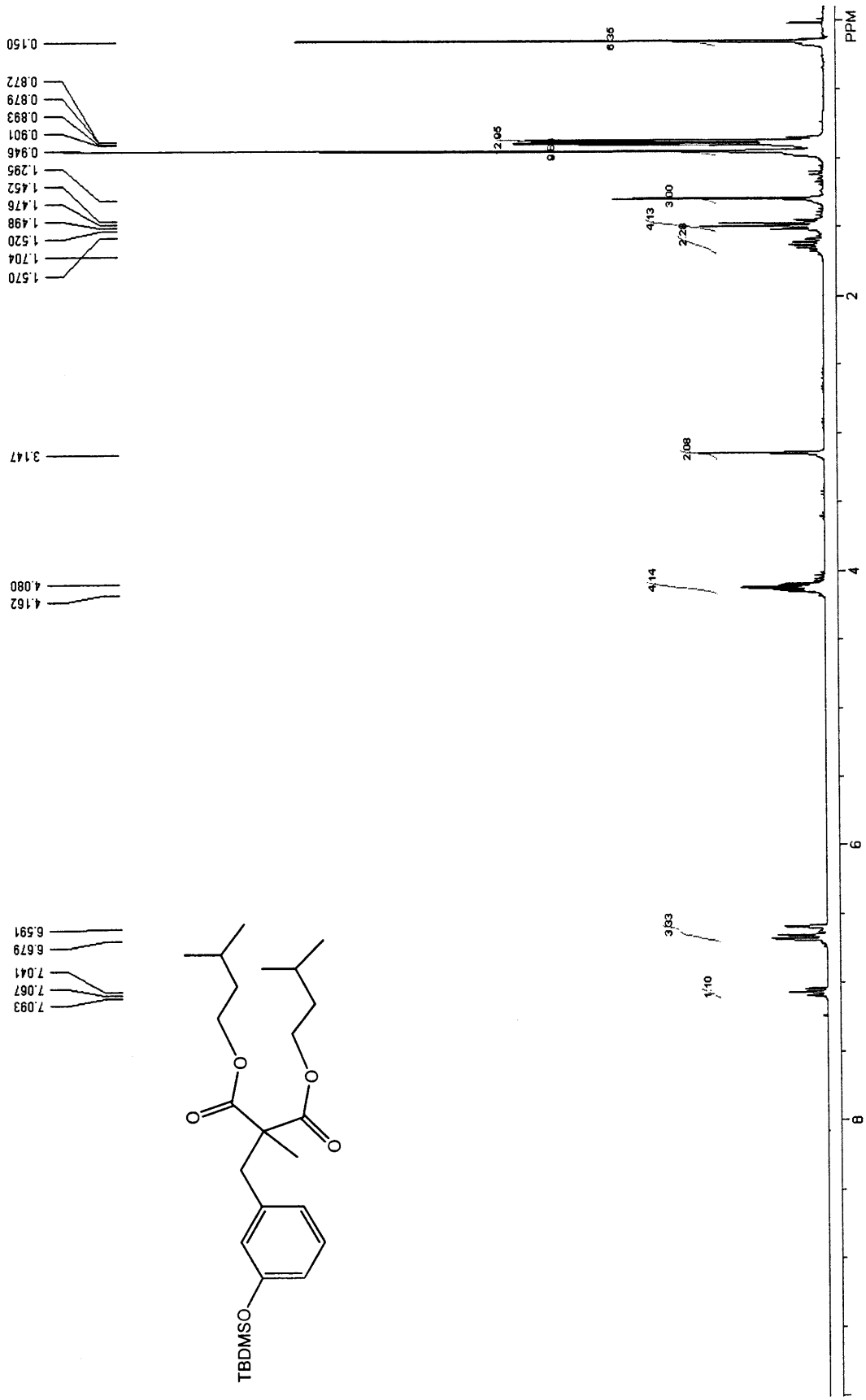


Figure 2.18 ^{1}H NMR Spectrum of Diisoamyl-2-(3-*tert*-butyldimethyloxybenzyl)-2-methyl Malonate

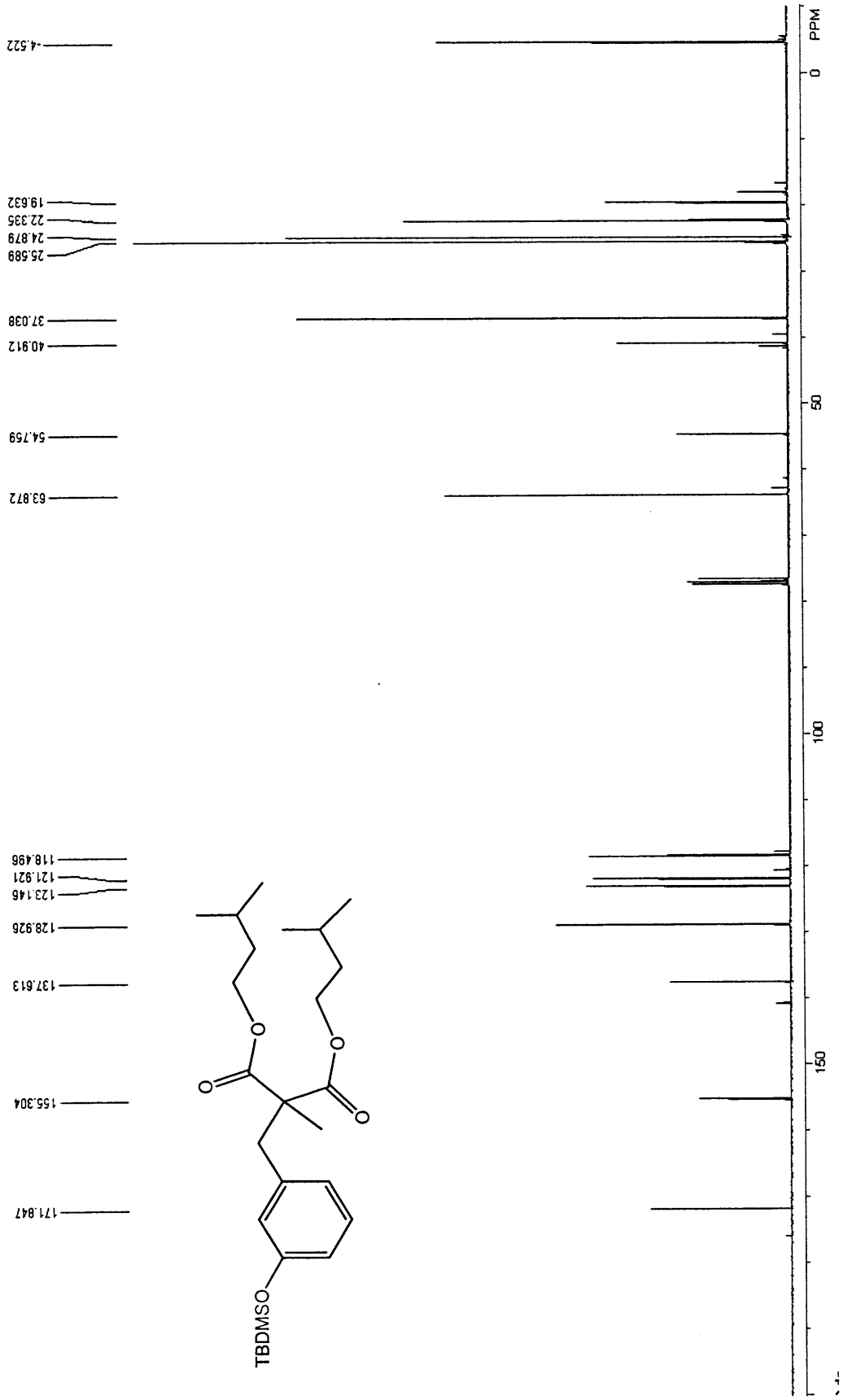


Figure 2.19 ^{13}C NMR Spectrum of Diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl Malonate

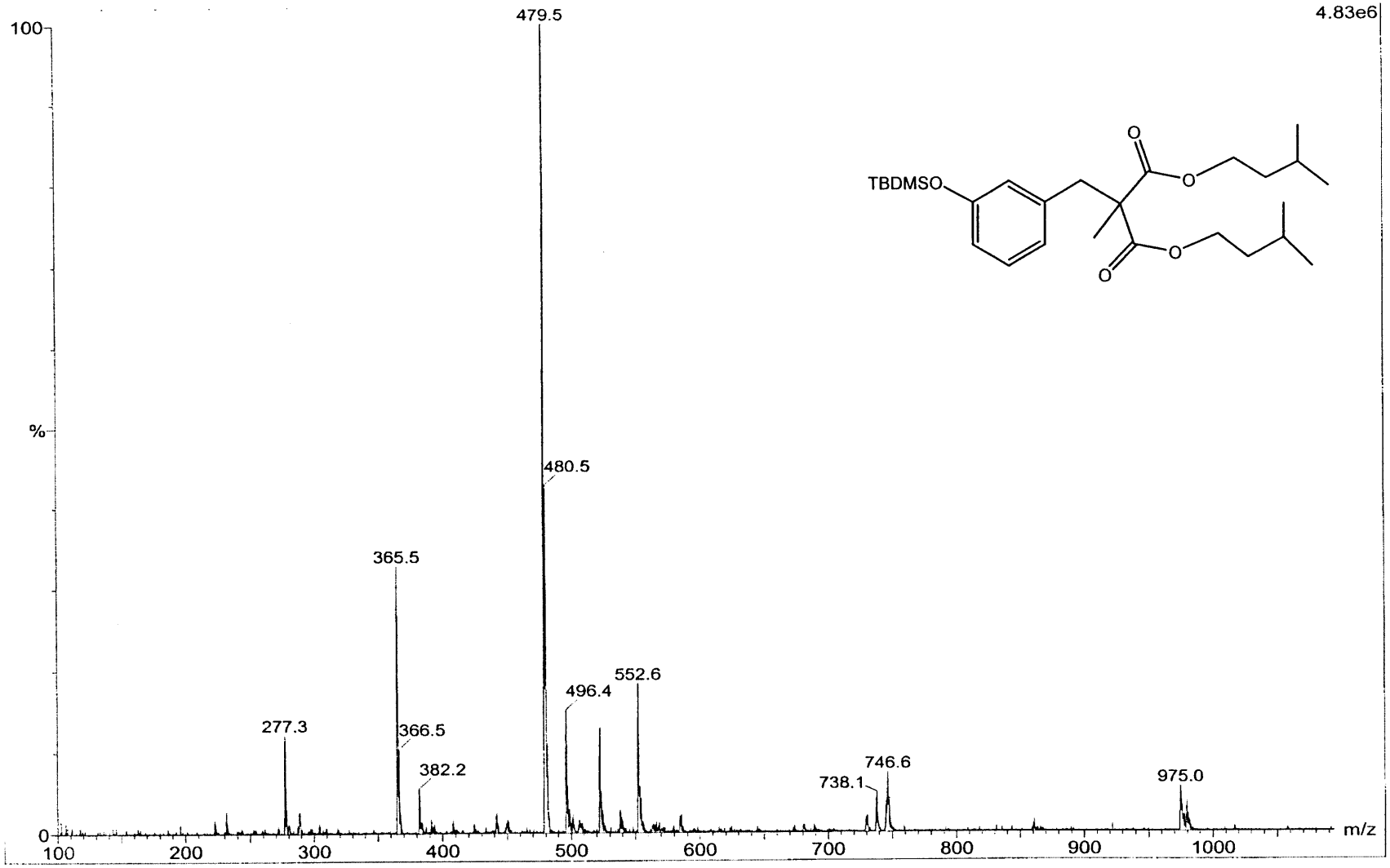


Figure 2.20 MS (LRESI) Spectrum of Diisoamyl-2-(3-*tert*-butyldimethylsilyloxy benzyl)-2-methyl Malonate

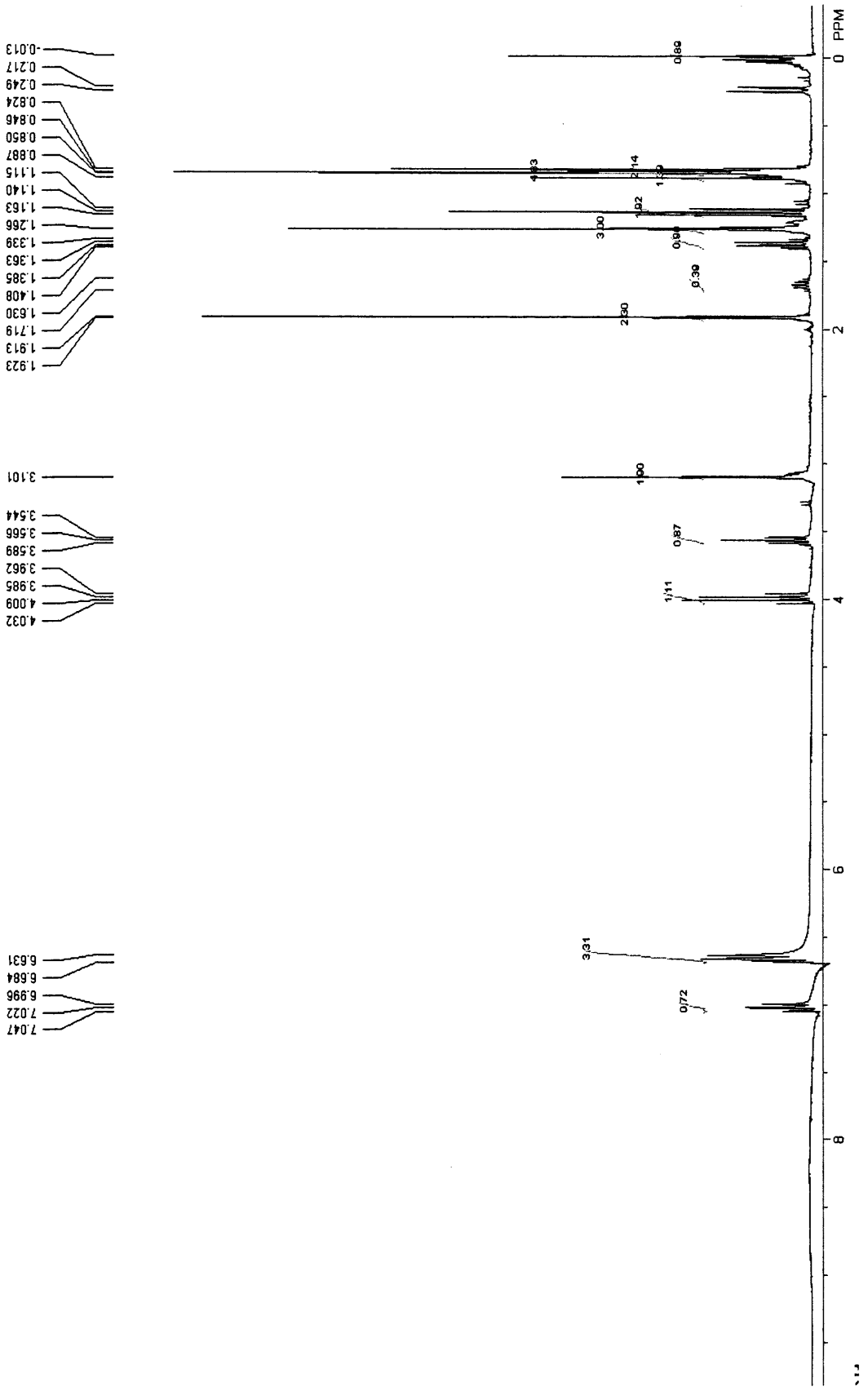


Figure 2.21 ^{1}H NMR Spectrum of Saponification Product of Reaction 1 Product

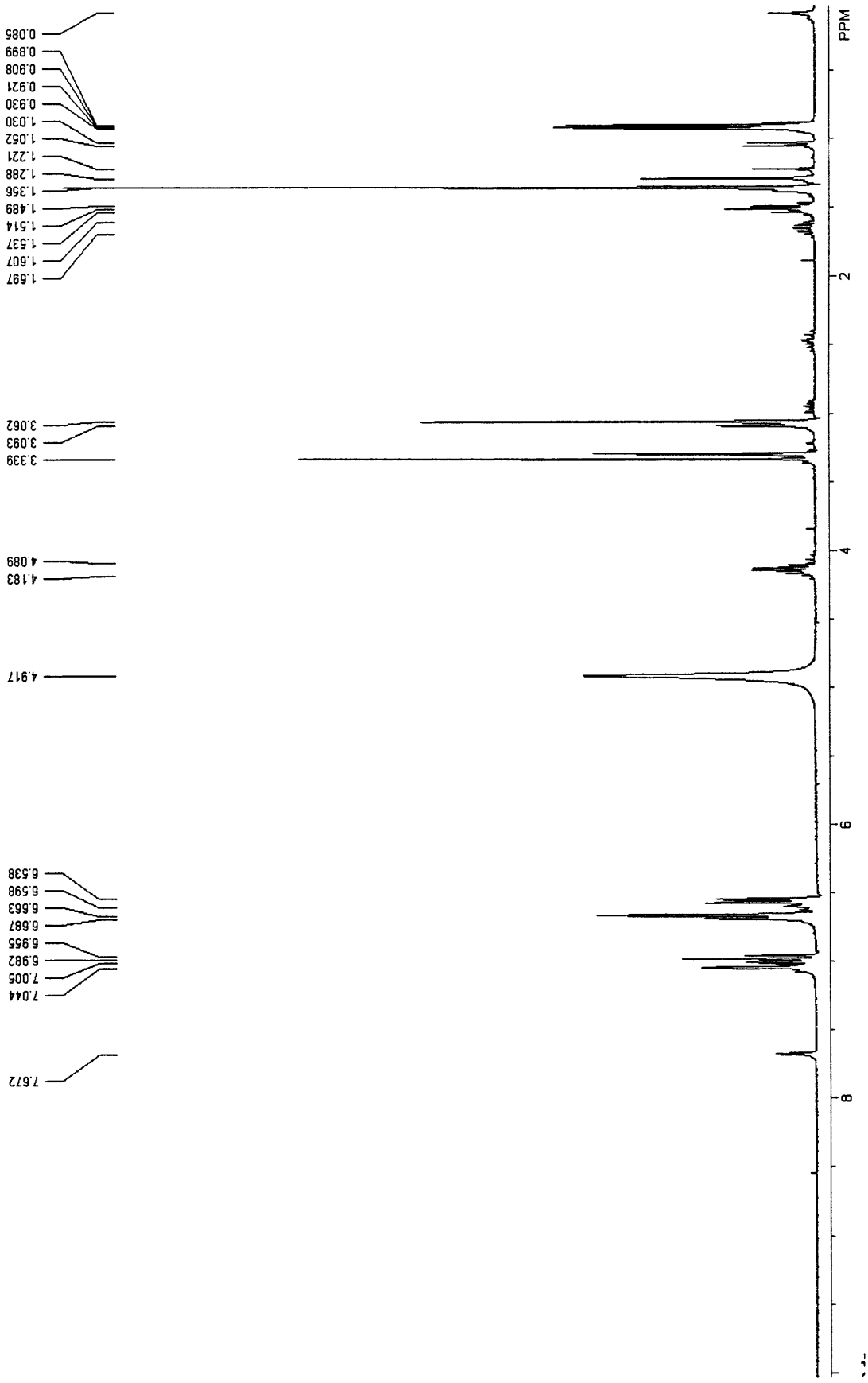


Figure 2.22 ^{1}H NMR Spectrum of Saponification Product from Reaction 2 Product

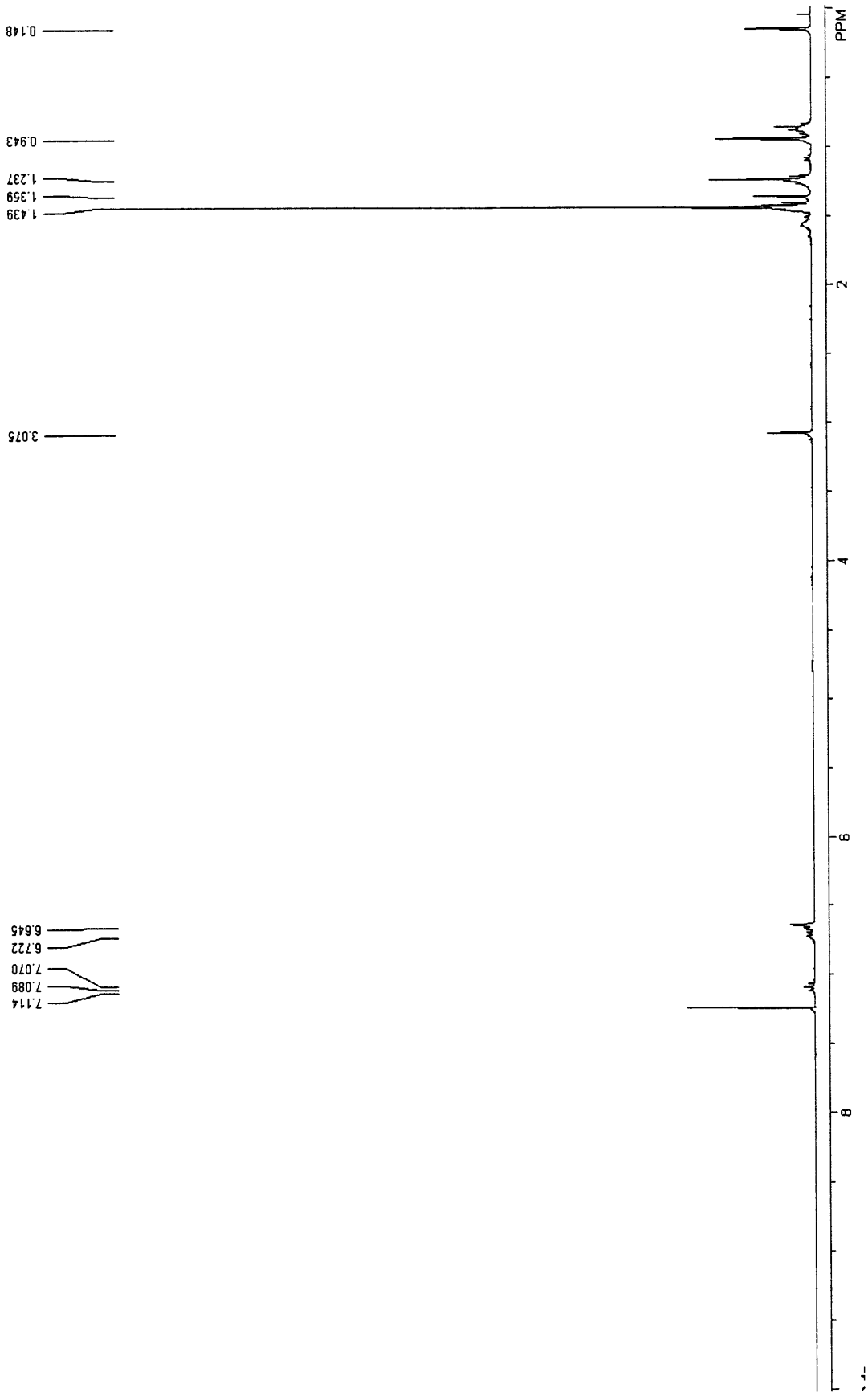


Figure 2.23 ^{1}H NMR Spectrum of Reaction 3 Product

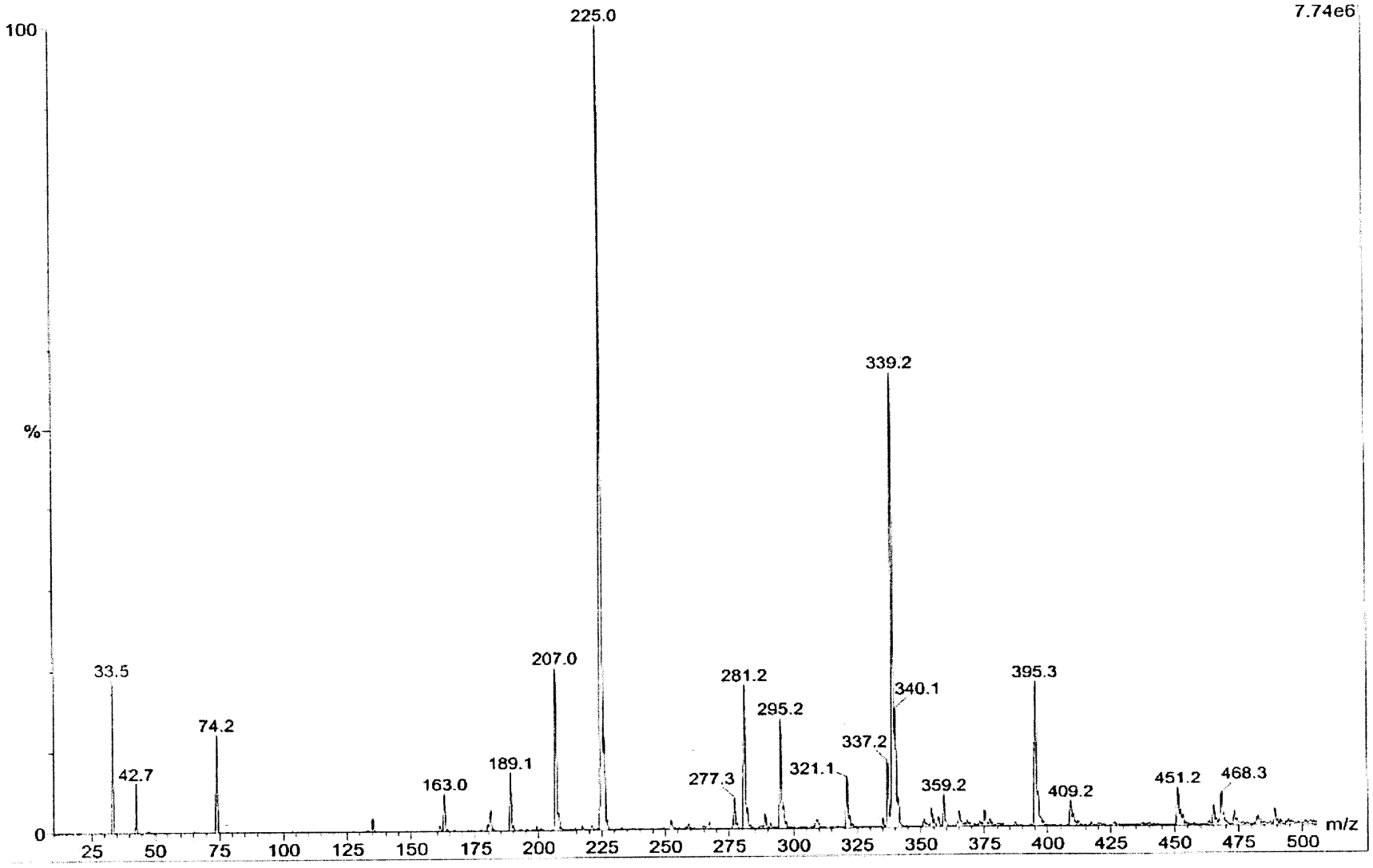


Figure 2.24 MS (LRESI) Spectrum of Reaction 3 Product

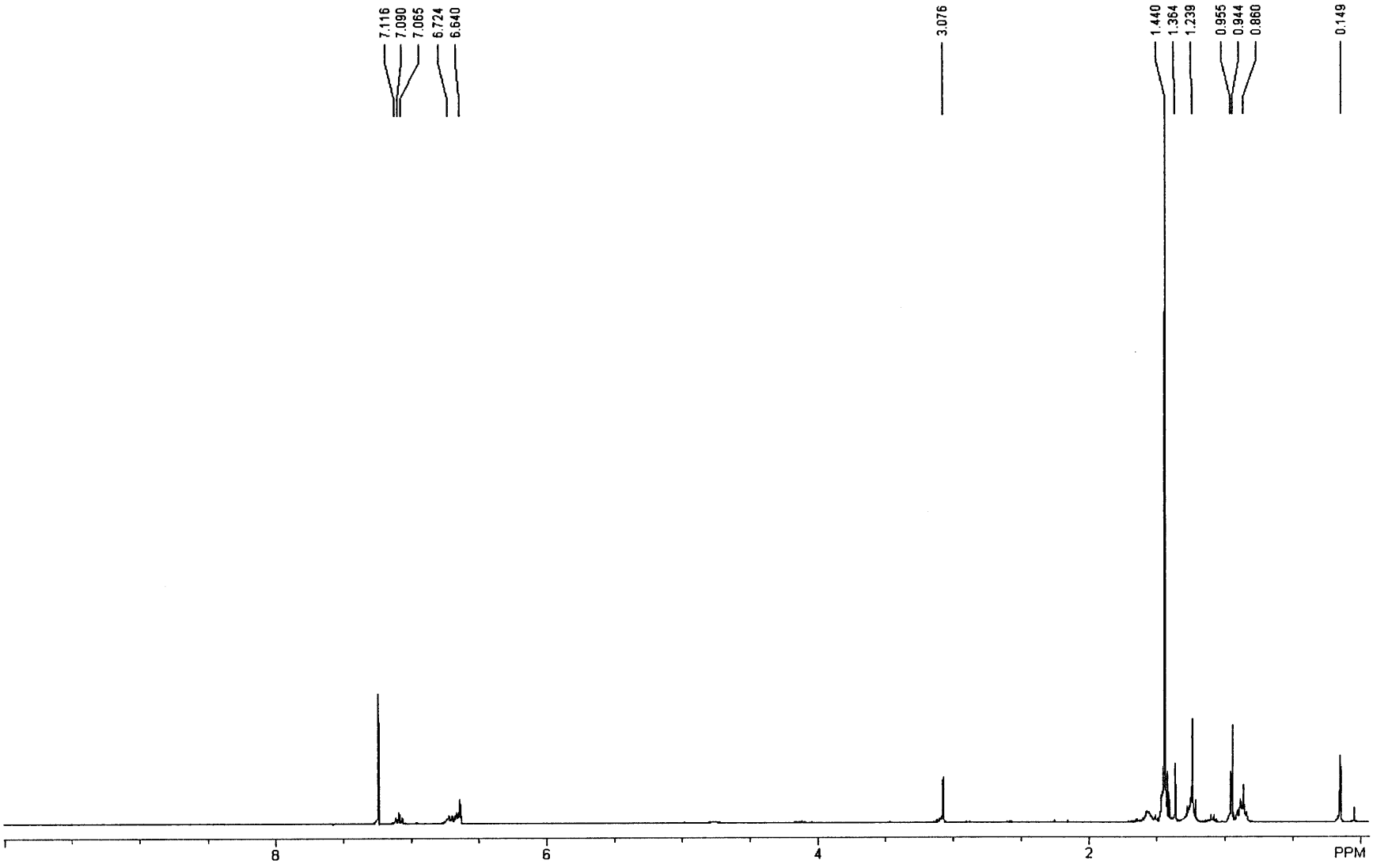


Figure 2.25 ^{1}H NMR Spectrum of Reaction 4 Product

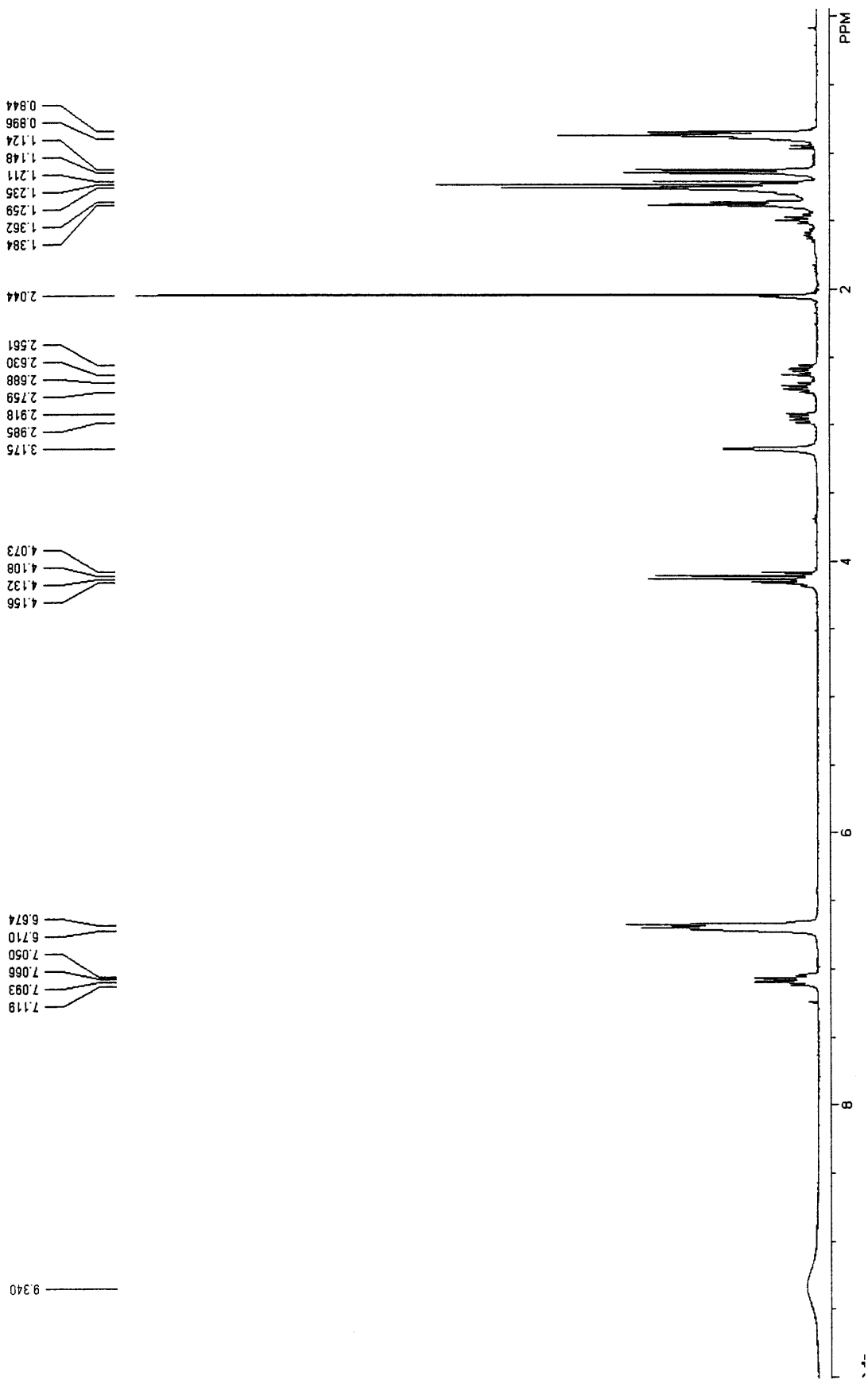


Figure 2.26 ^{1}H NMR Spectrum of Acidified, Organic Extract of Reaction 5 Product

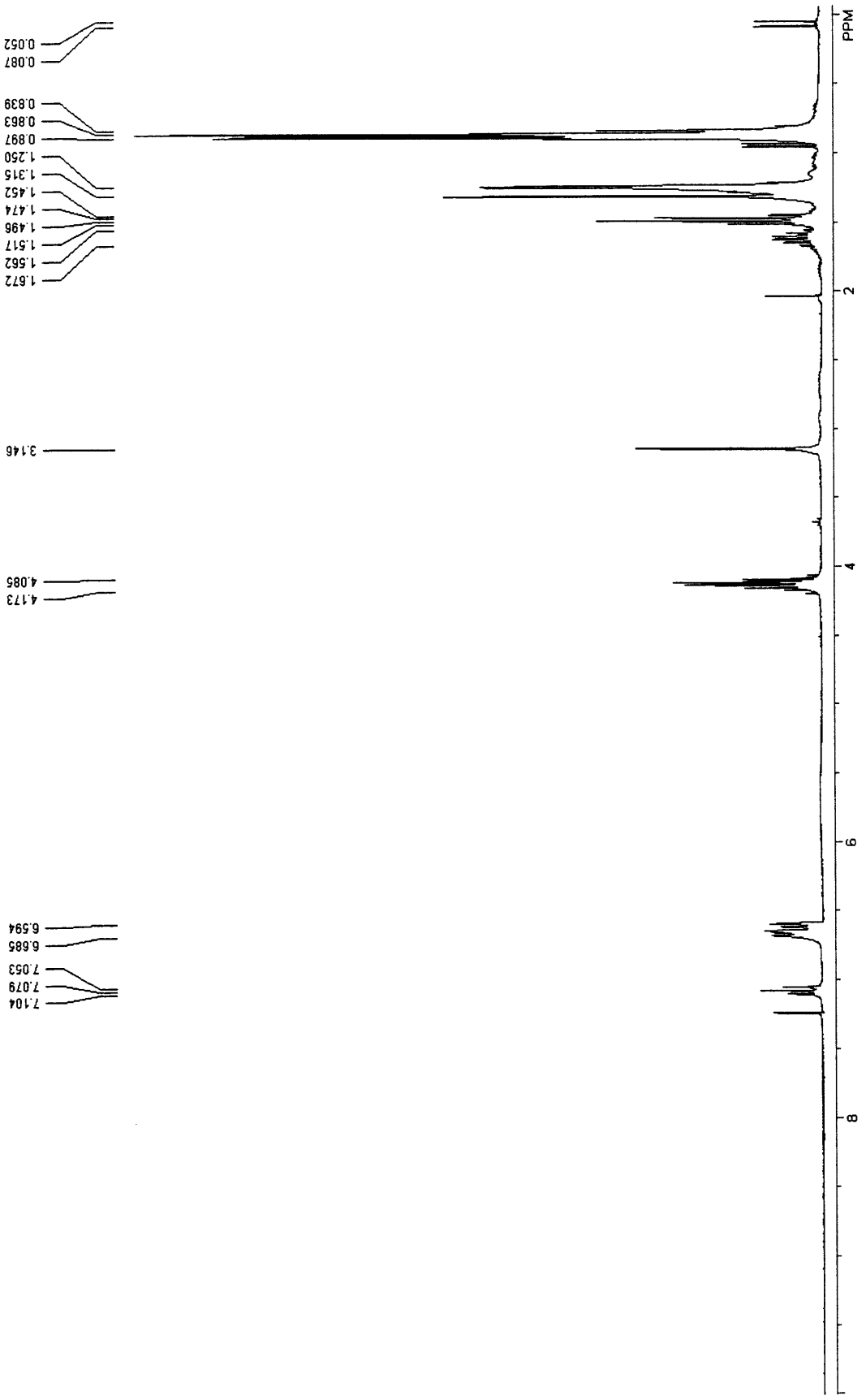


Figure 2.27 ^{1}H NMR Spectrum of Nonacidified, Organic Extract of Reaction 5 Product

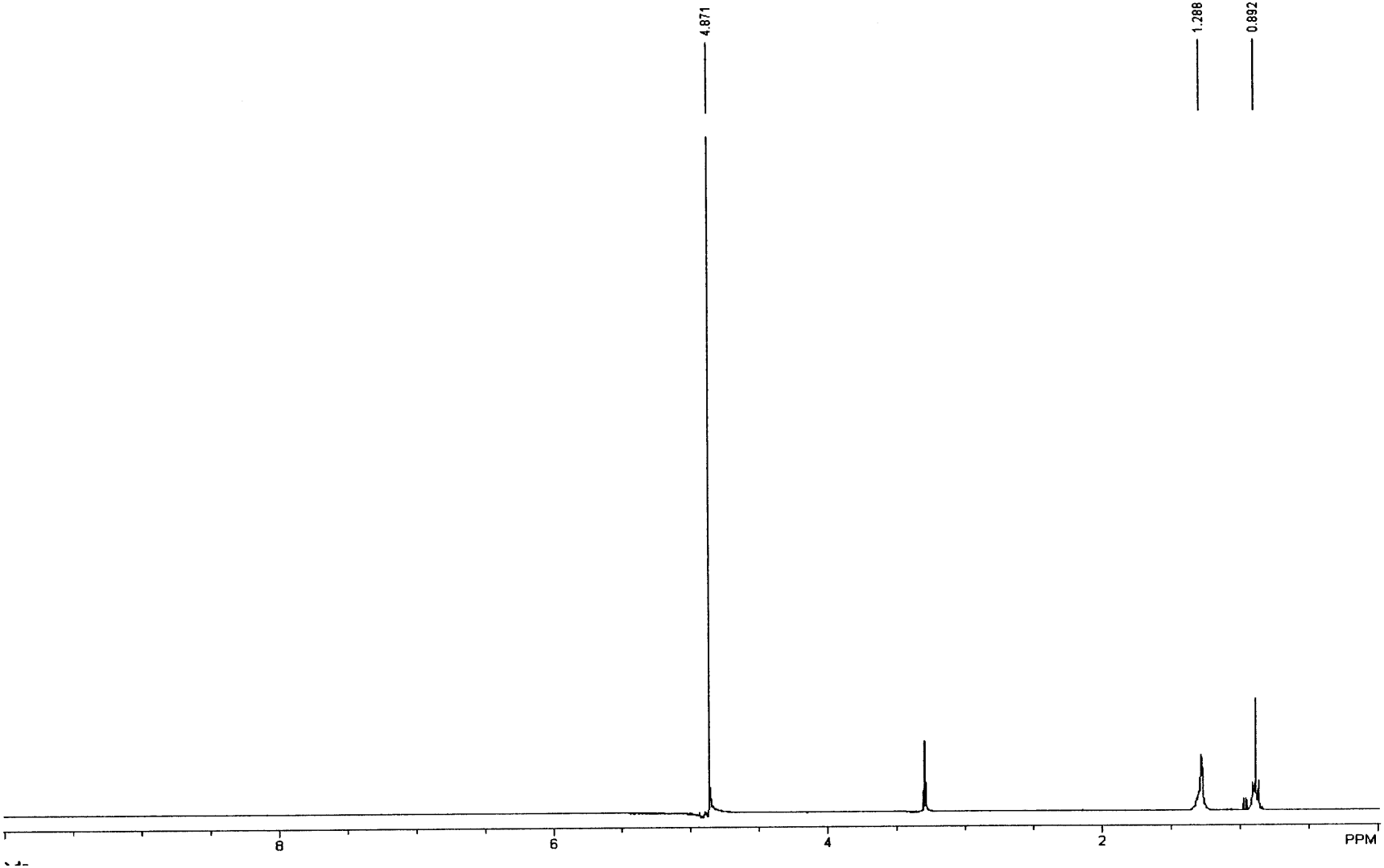


Figure 2.28 ^{1}H NMR Spectrum of Acidified, Aqueous Extract of Reaction 5 Product

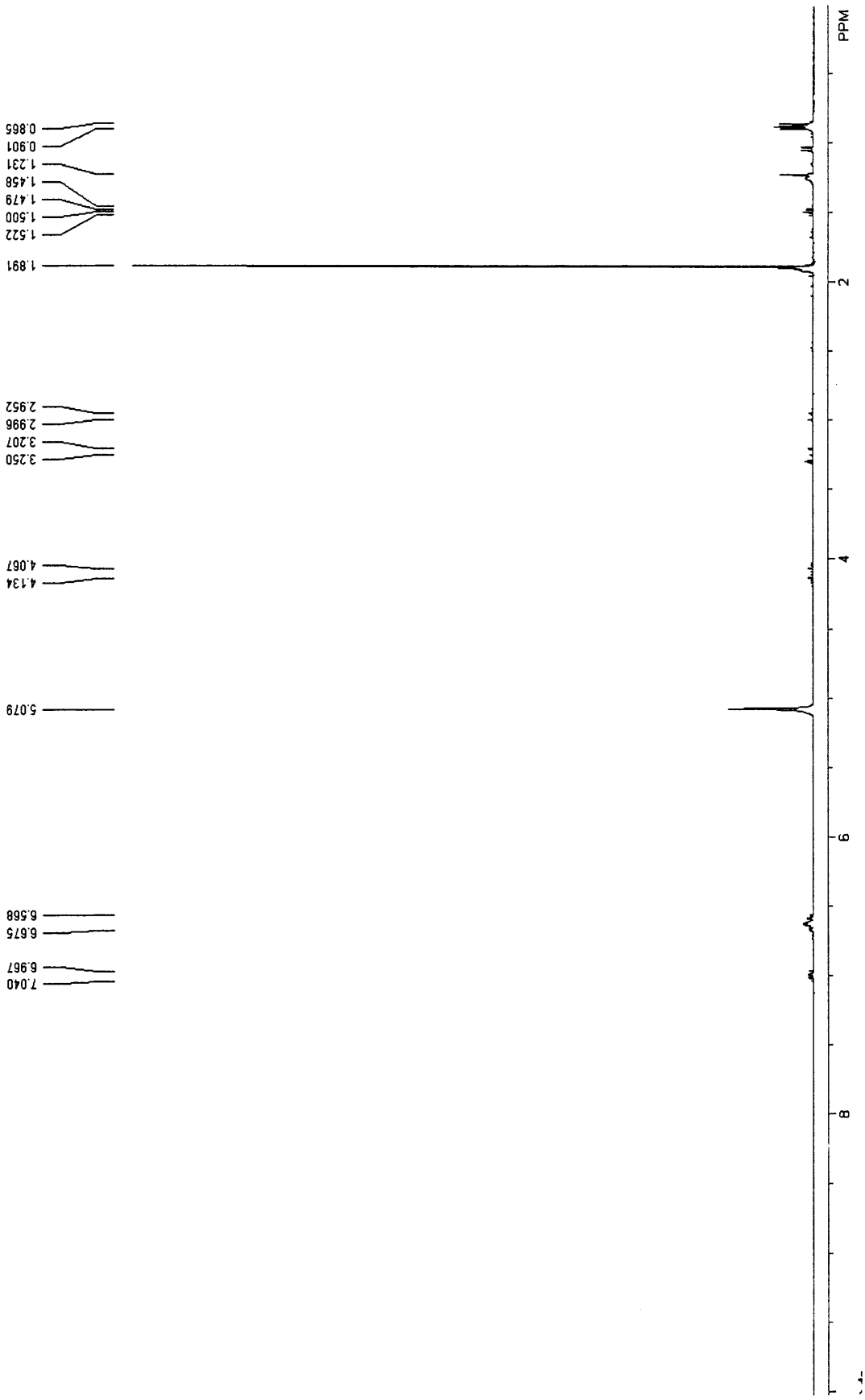


Figure 2.29 1H NMR Spectrum of Nonacidified, Aqueous Extract of Reaction 5 Product

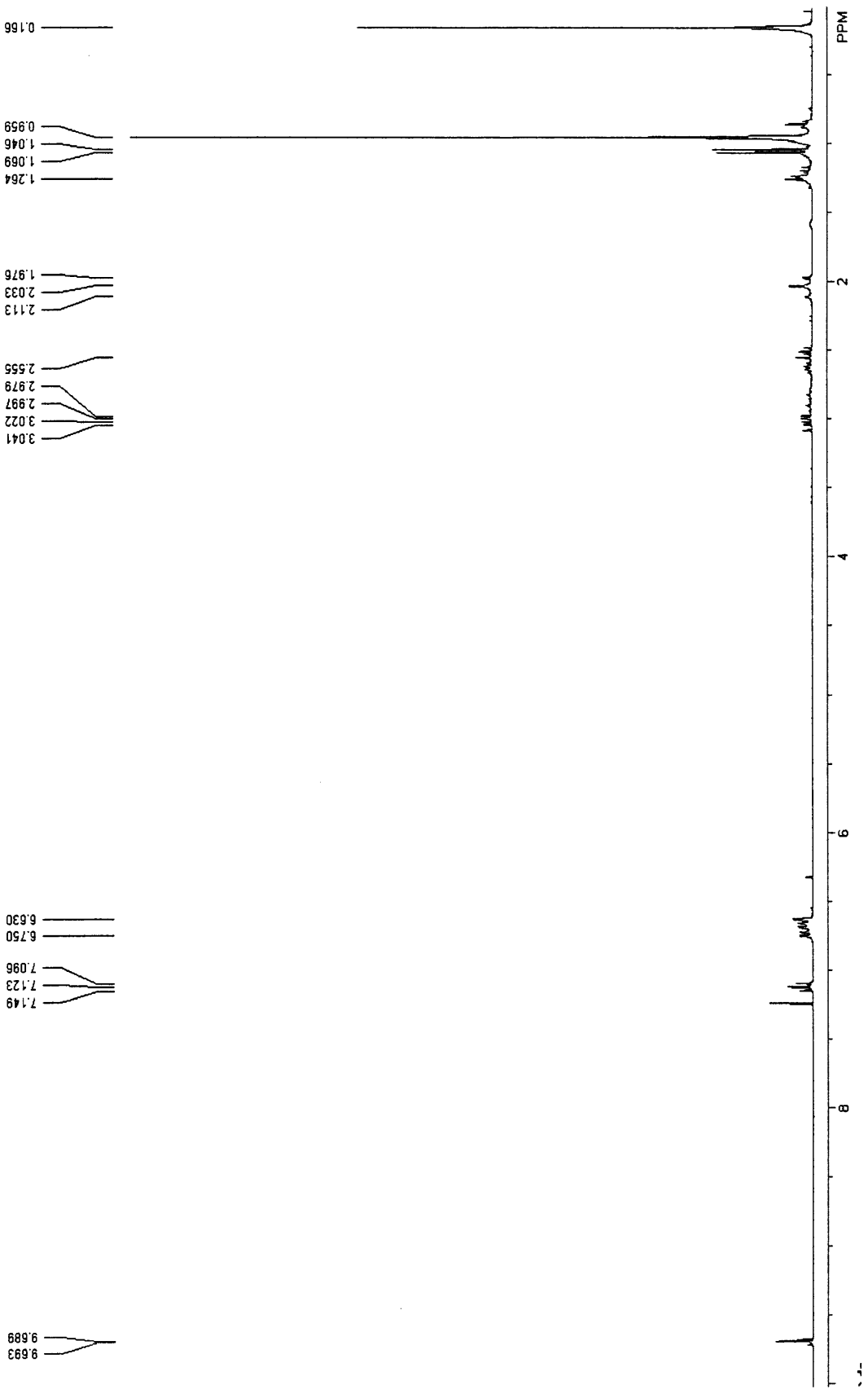


Figure 2.30 ^{1}H NMR Spectrum of Reduction/ Swern Product

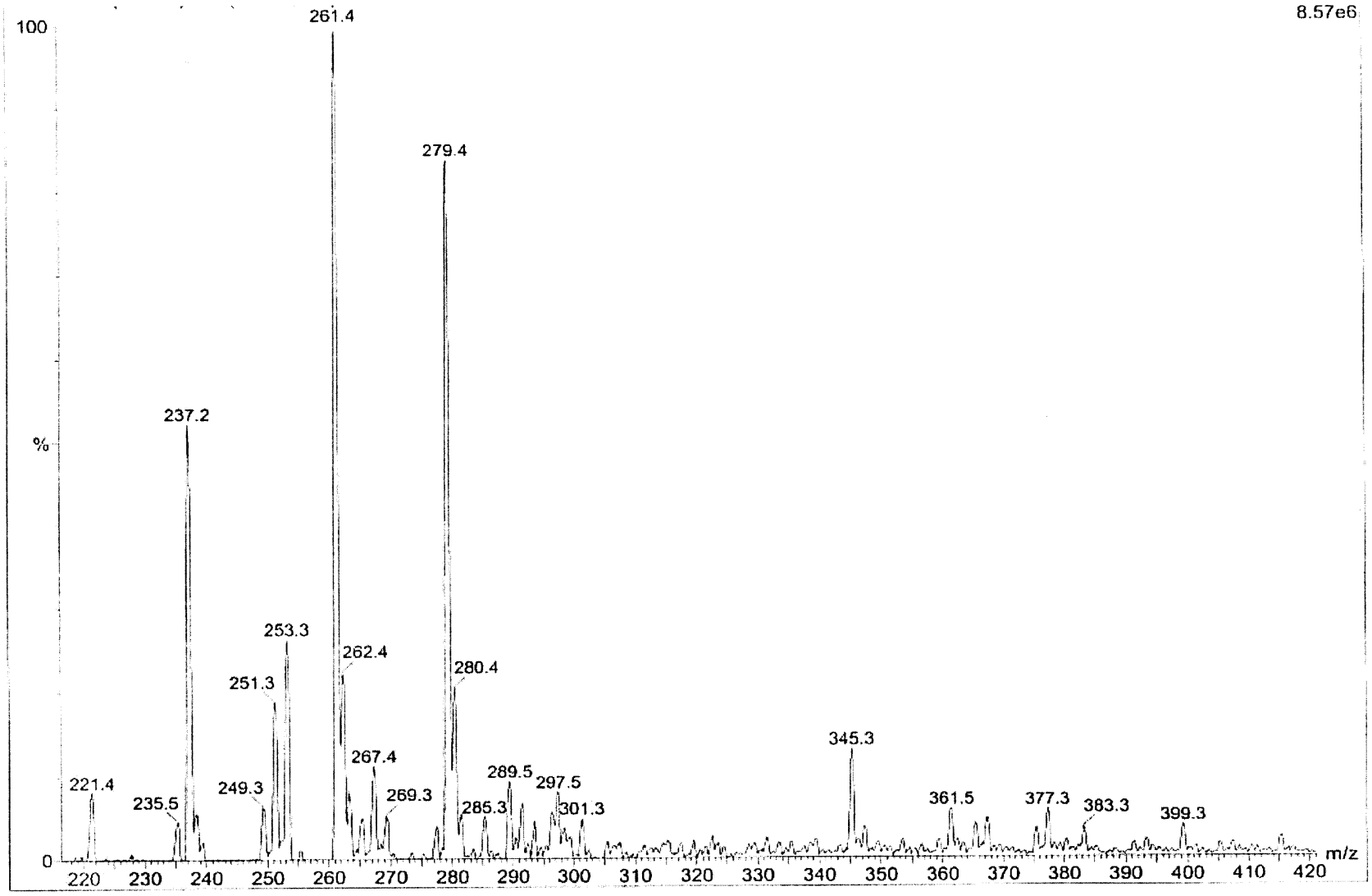


Figure 2.31 MS (LRESI) Spectrum of Reduction/Swern Product

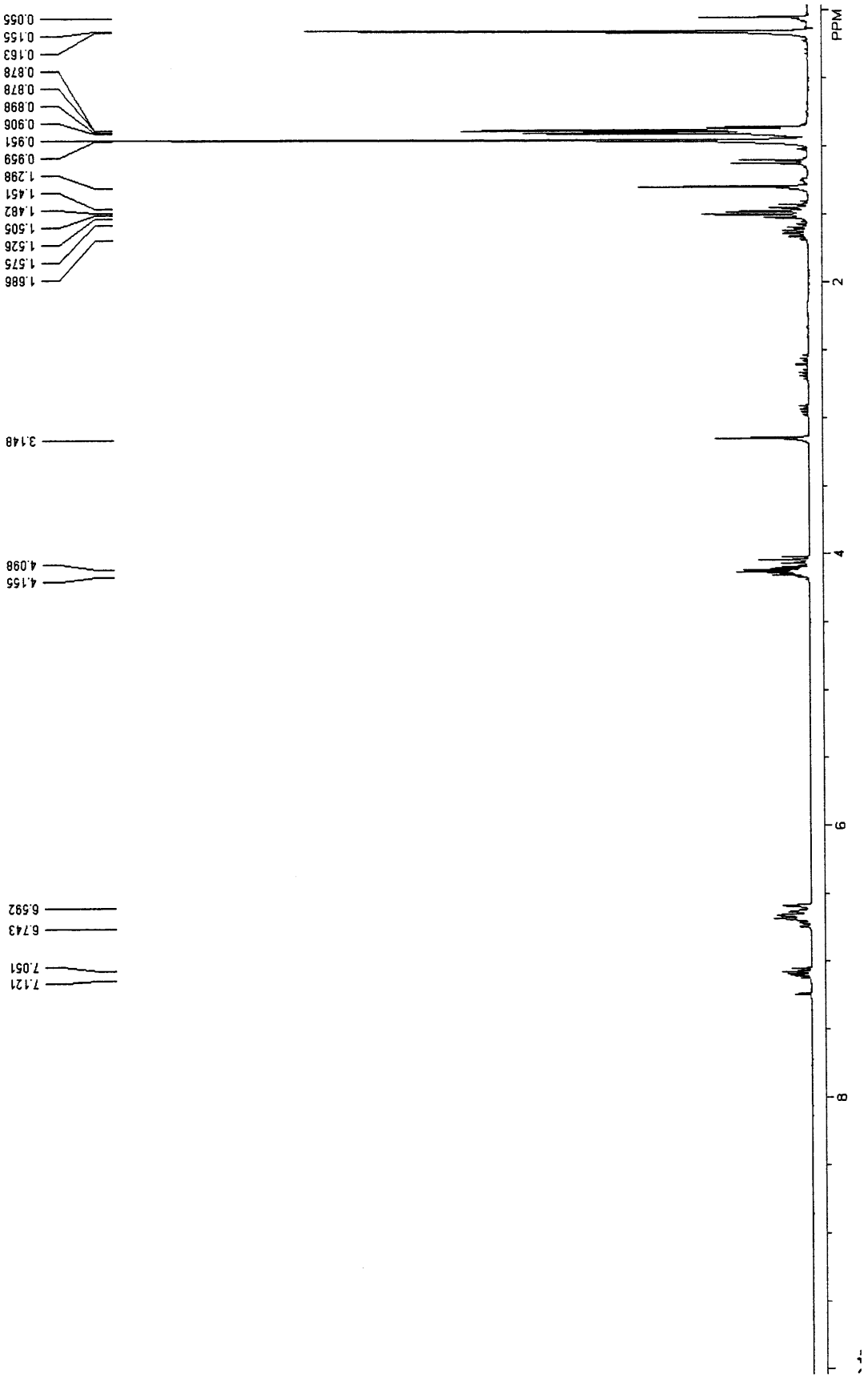


Figure 2.32 ^{1}H NMR Spectrum of Failed Anhydride Reaction

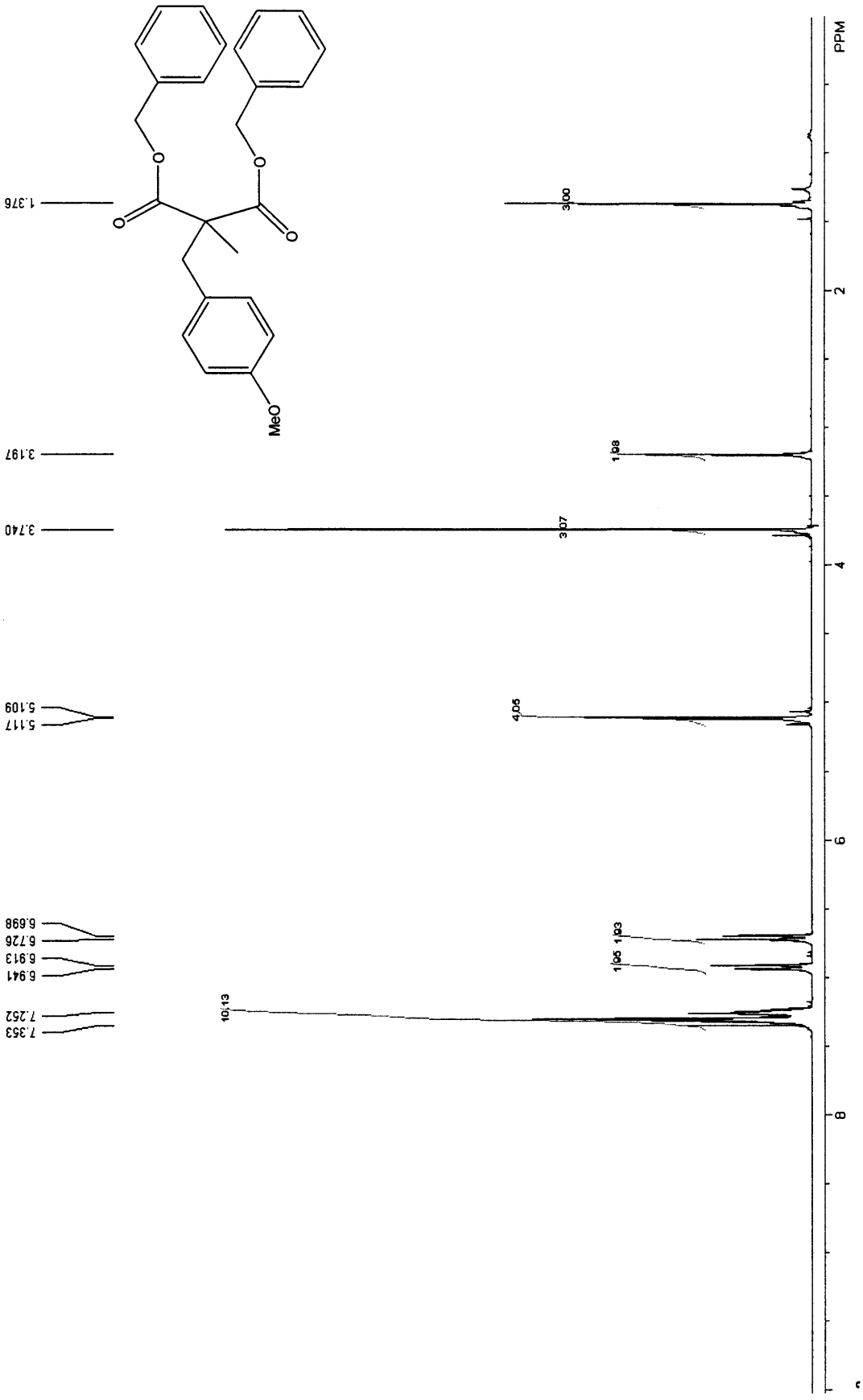


Figure 2.33 ^{1}H NMR Spectrum of Dibenzyl-2-(4-methoxybenzyl)-2-methyl Malonate

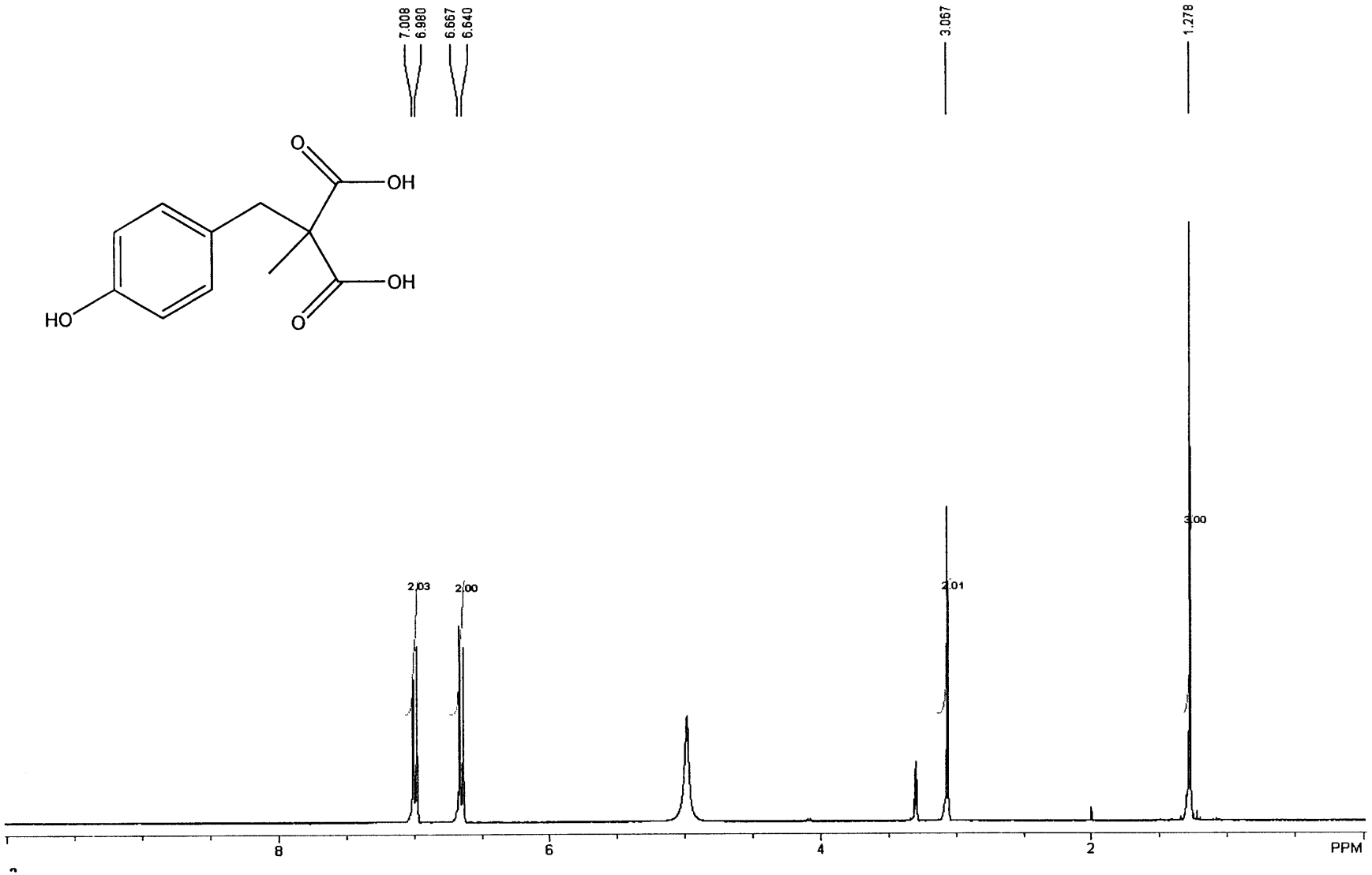


Figure 2.34 ^{1}H NMR Spectrum of Failed Demethylation Reaction

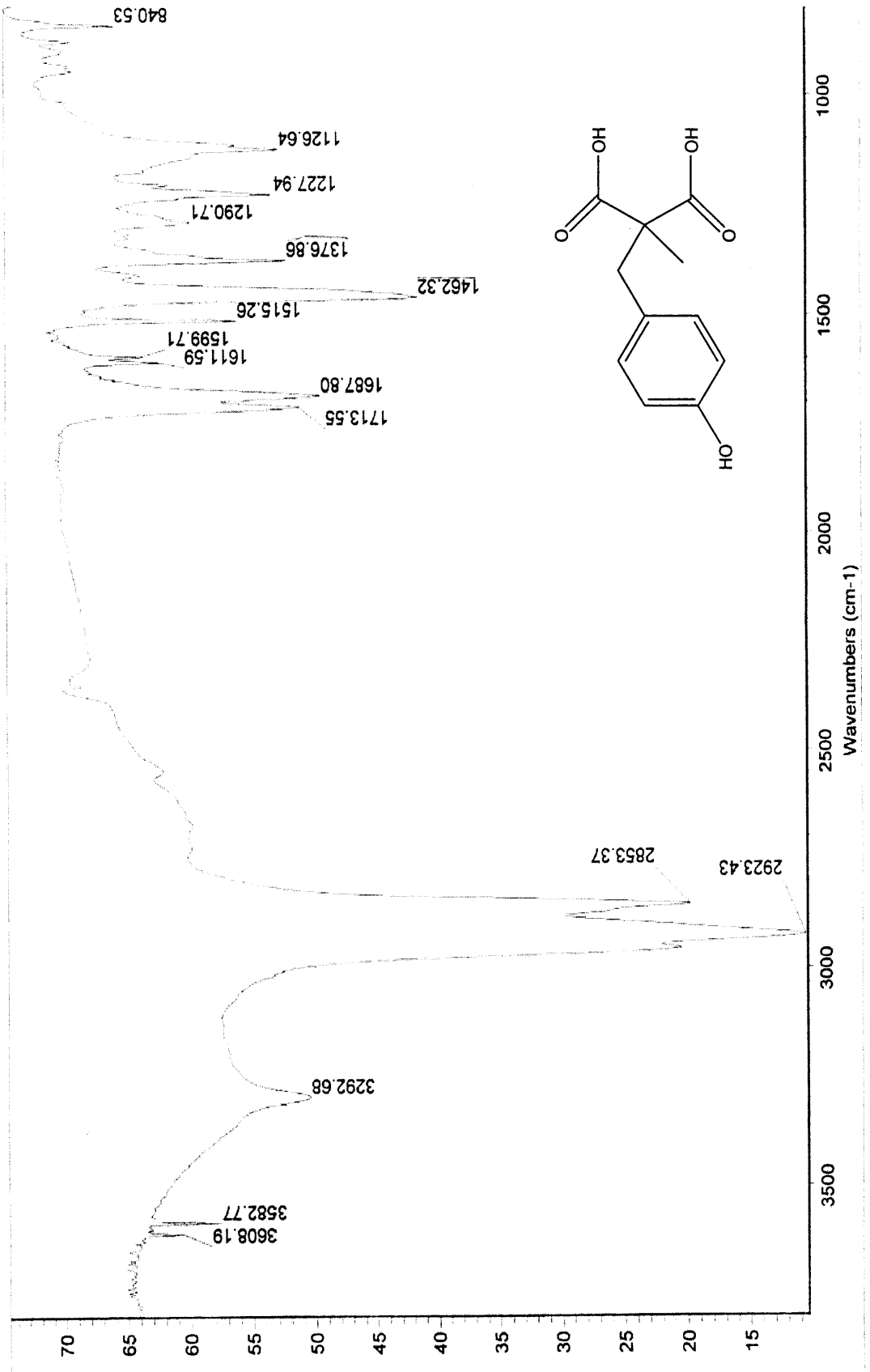


Figure 2.35 IR Spectrum of Failed Demethylation Reaction

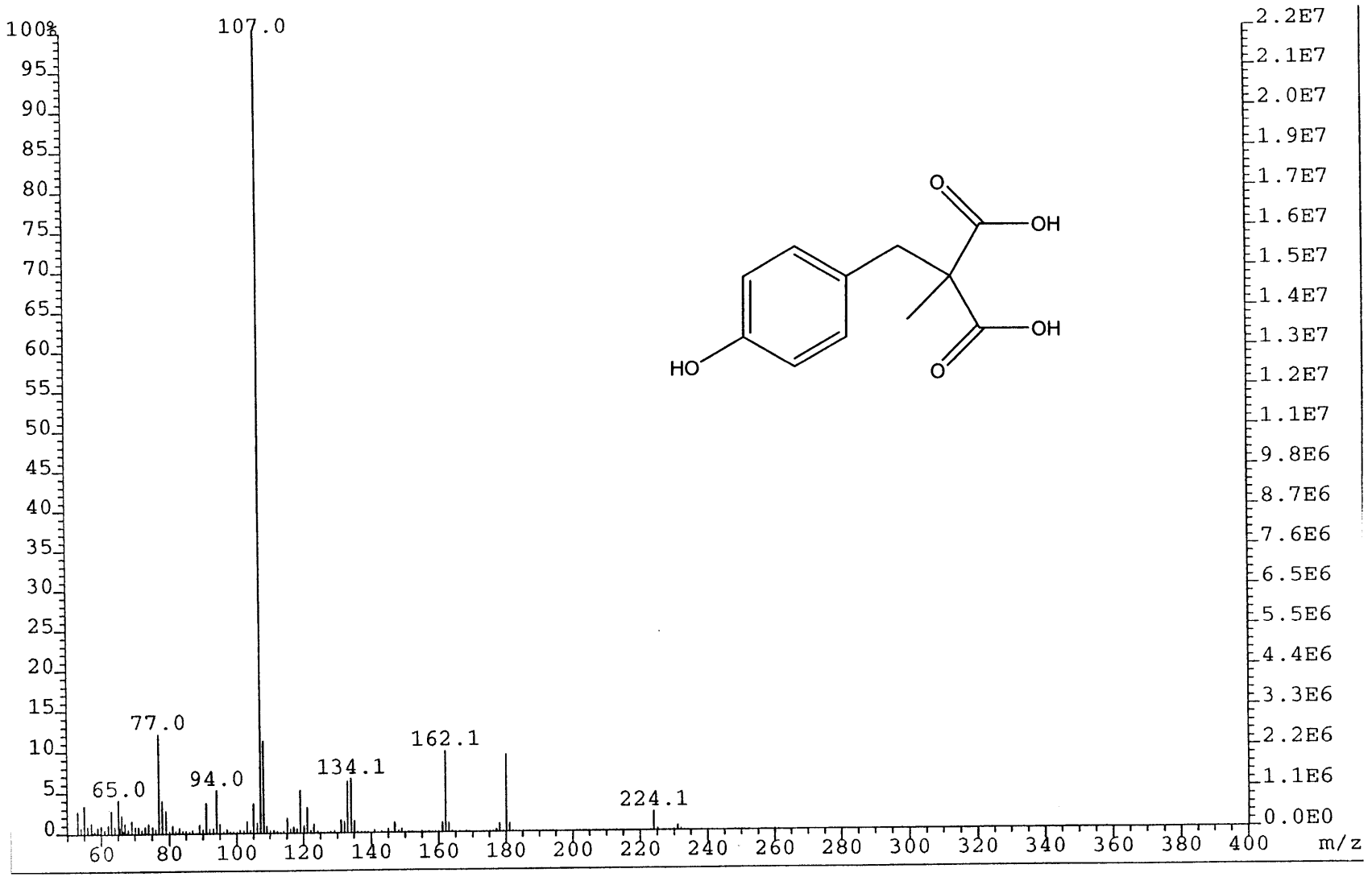


Figure 2.36 MS (LREI) Spectrum of Failed Demethylation Reaction

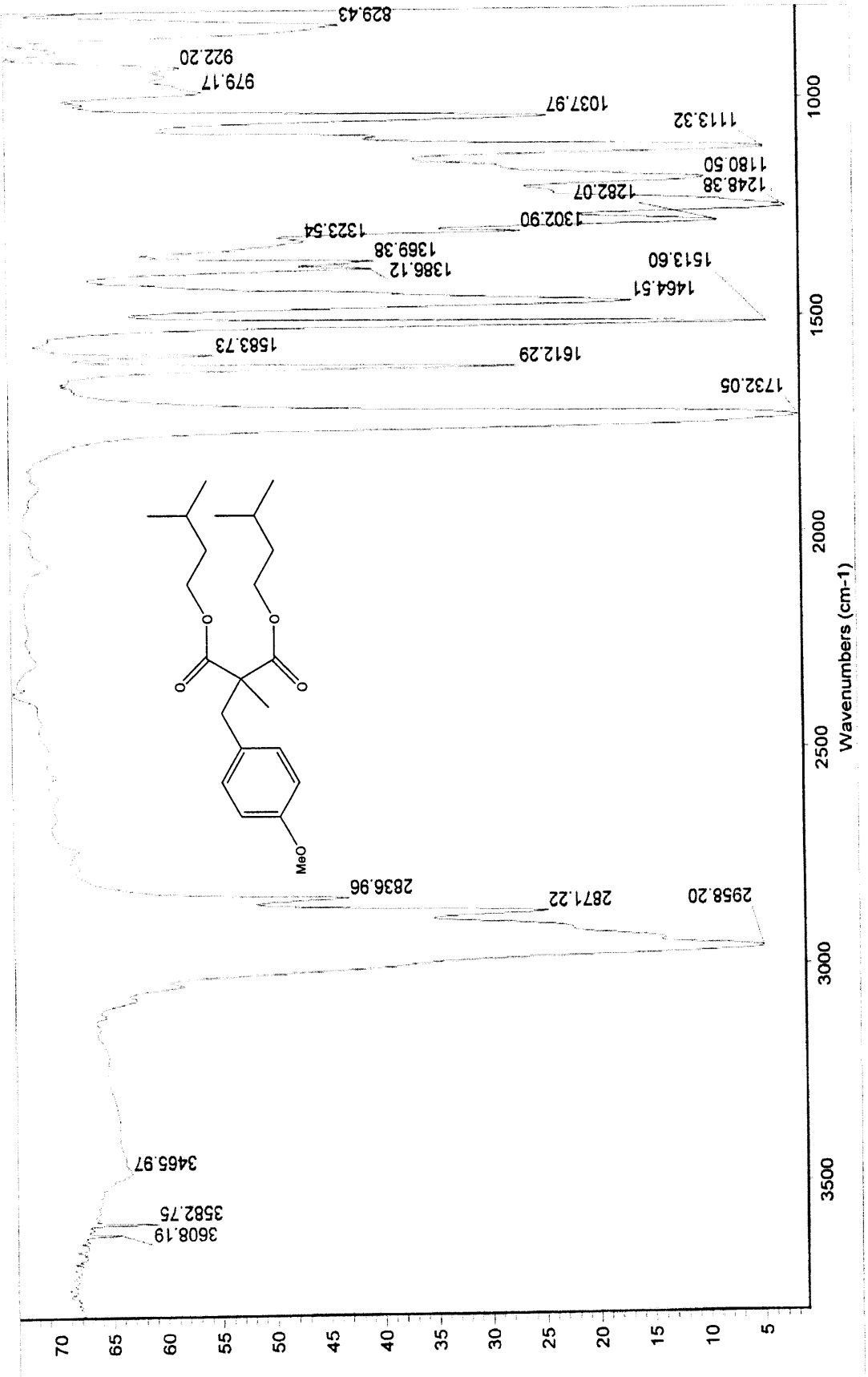


Figure 2.37 IR Spectrum of Diisoamyl-2-(4-methoxybenzyl)-2-methyl Malonate

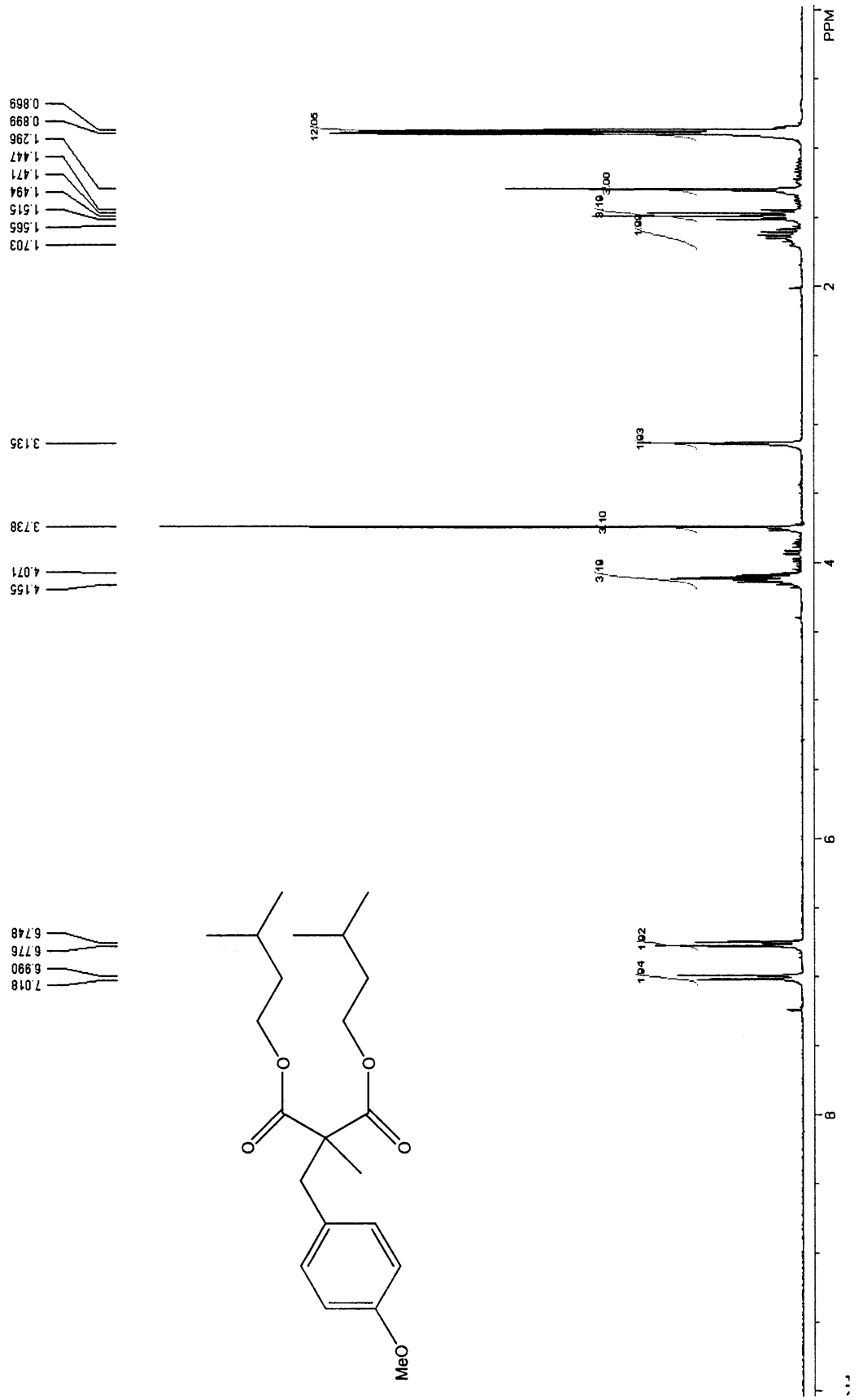


Figure 2.38 ^{1}H NMR Spectrum of Diisoamyl-2-(4-methoxybenzyl)-2-methyl Malonate

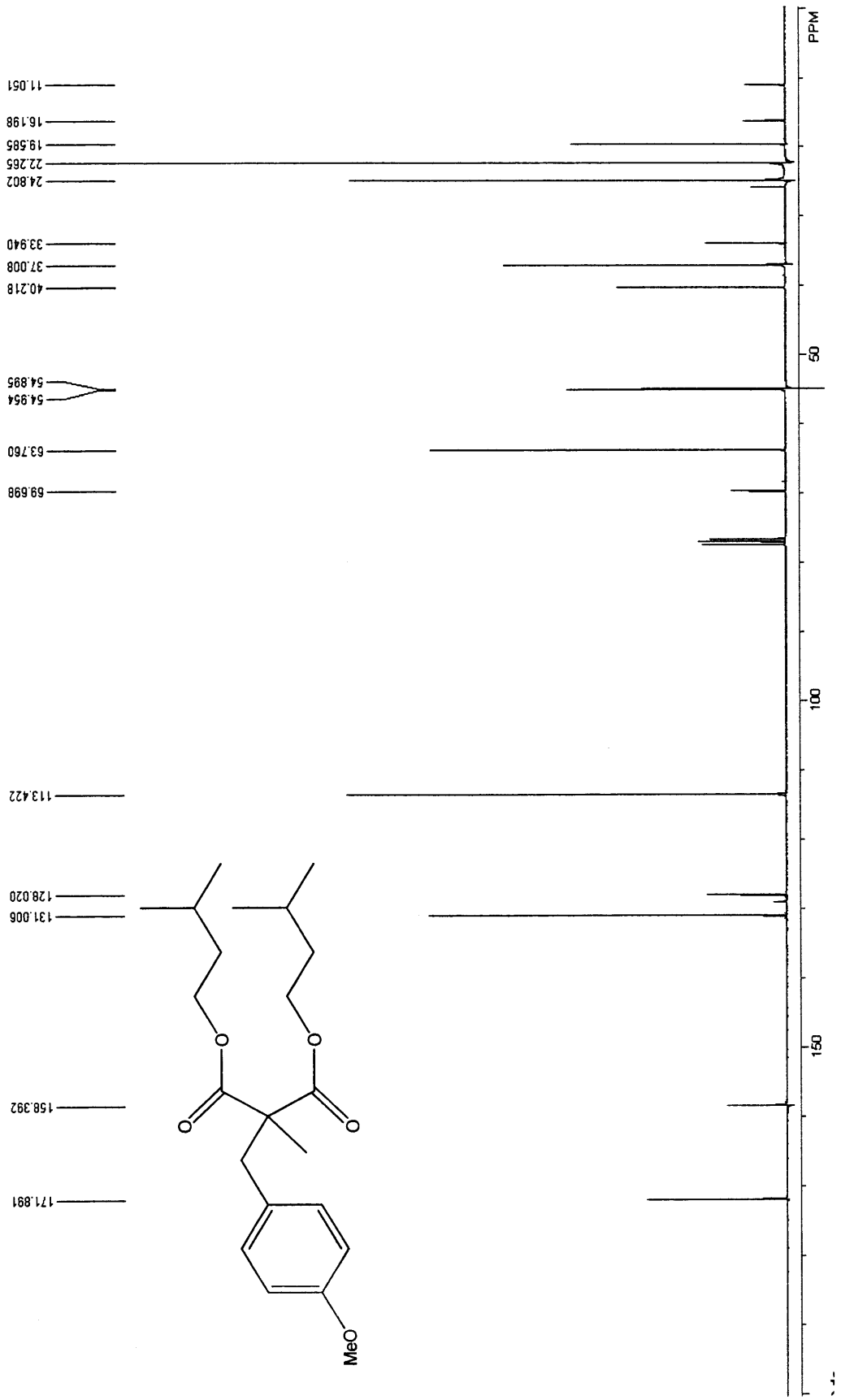


Figure 2.39 ^{13}C NMR spectrum of Diisoamyl-2-(4-methoxybenzyl)-2-methyl Malonate

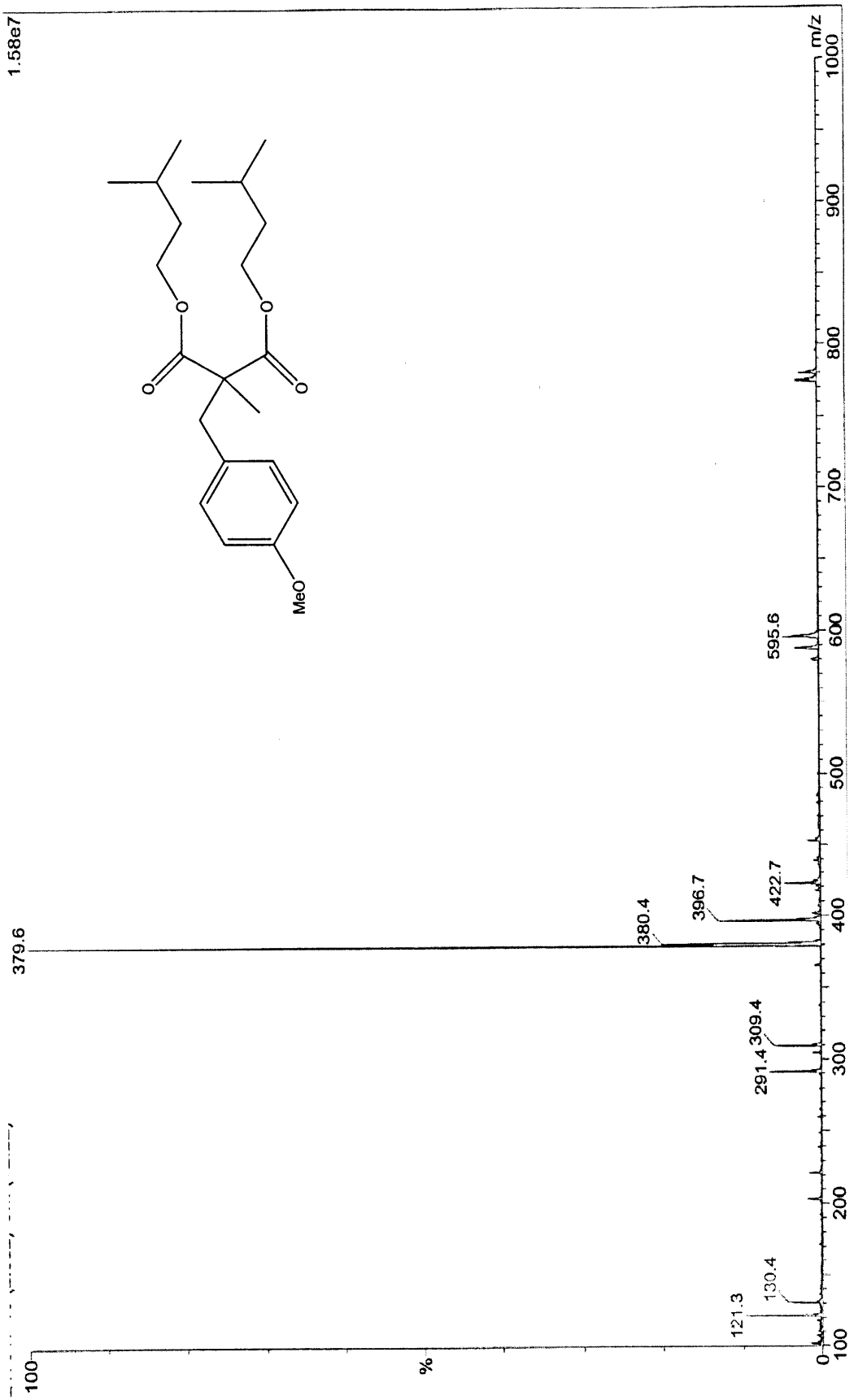


Figure 2.40 MS (LRESI) of Diisoamyl-2-(4-methoxybenzyl)-2-methyl Malonate

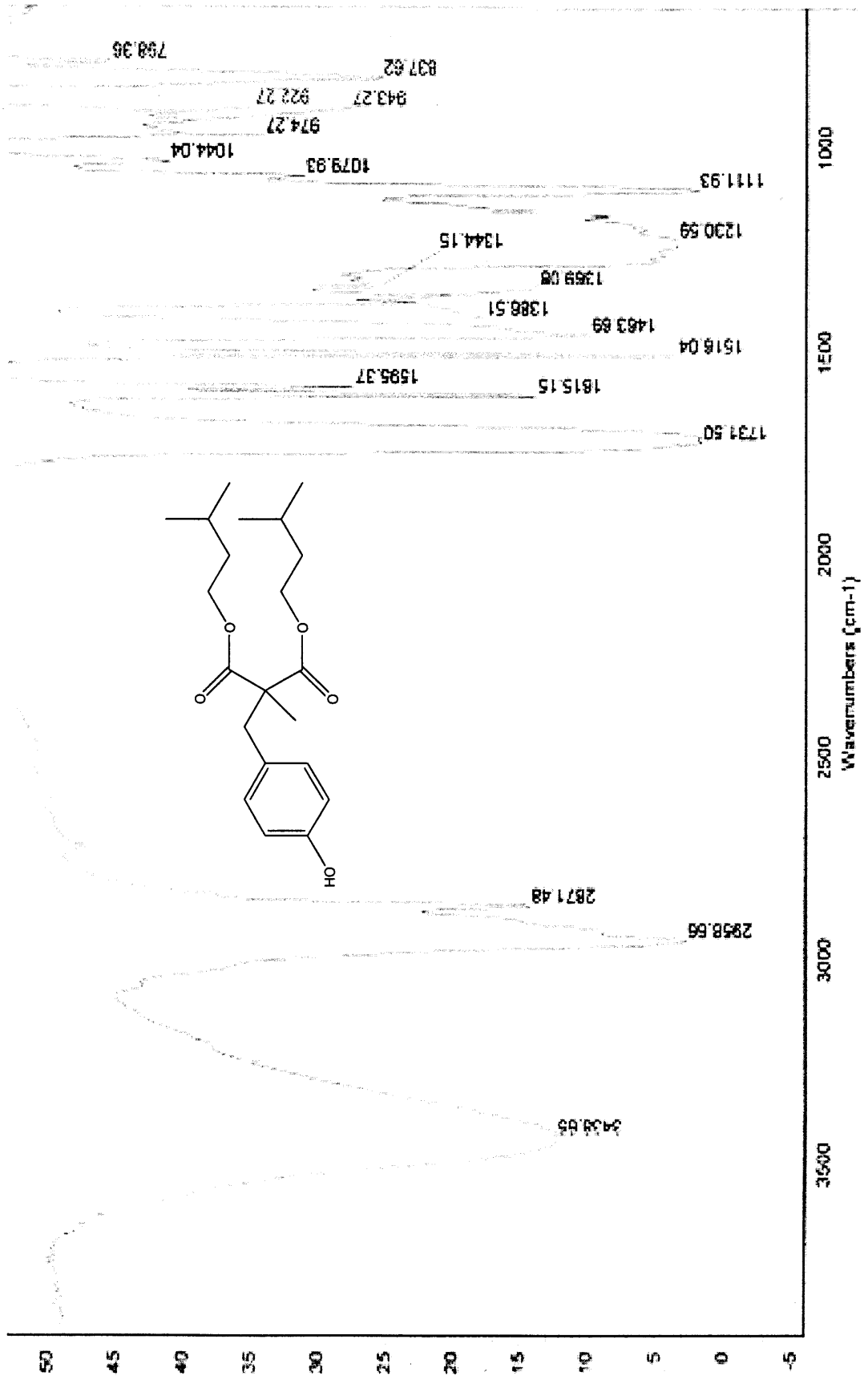


Figure 2.41 IR Spectrum of Diisoamyl-2-(4-hydroxybenzyl)-2-methyl Malonate

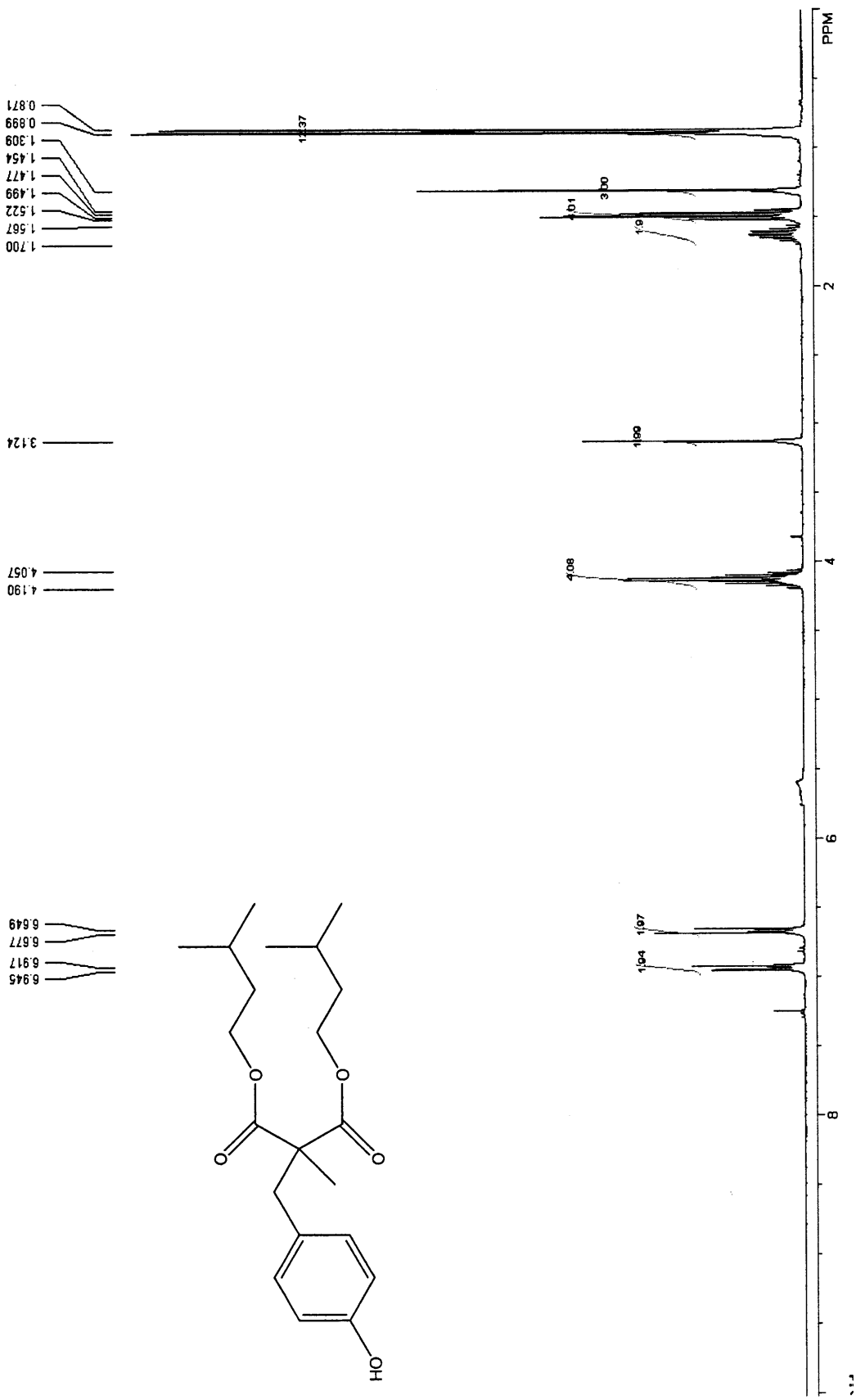


Figure 2.42 ^{1}H NMR Spectrum of Diisoamyl-2-(4-hydroxybenzyl)-2-methyl Malonate

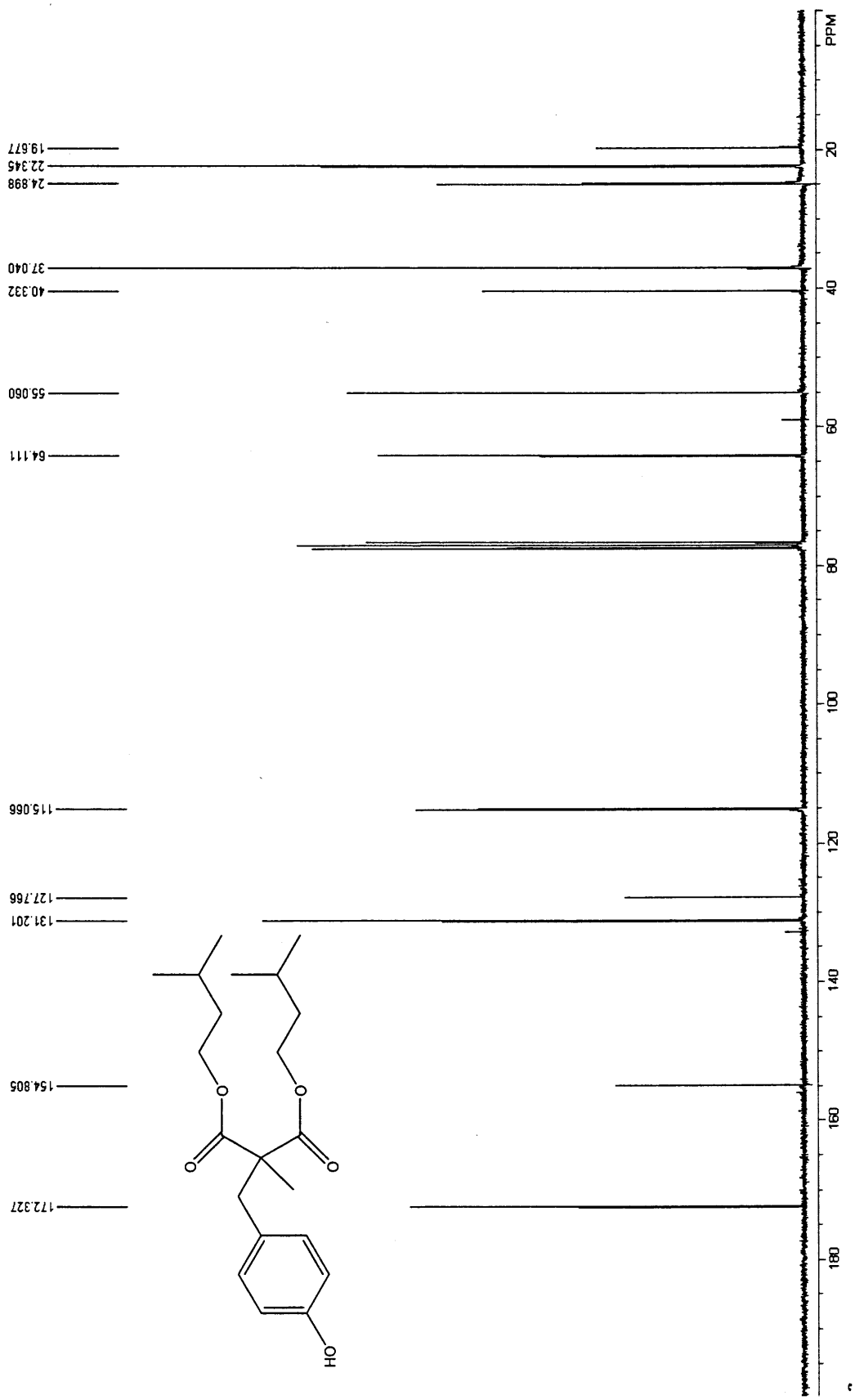


Figure 2.43 ^{13}C NMR Spectrum of Diisoamyl-2-(4-hydroxybenzyl)-2-methyl Malonate

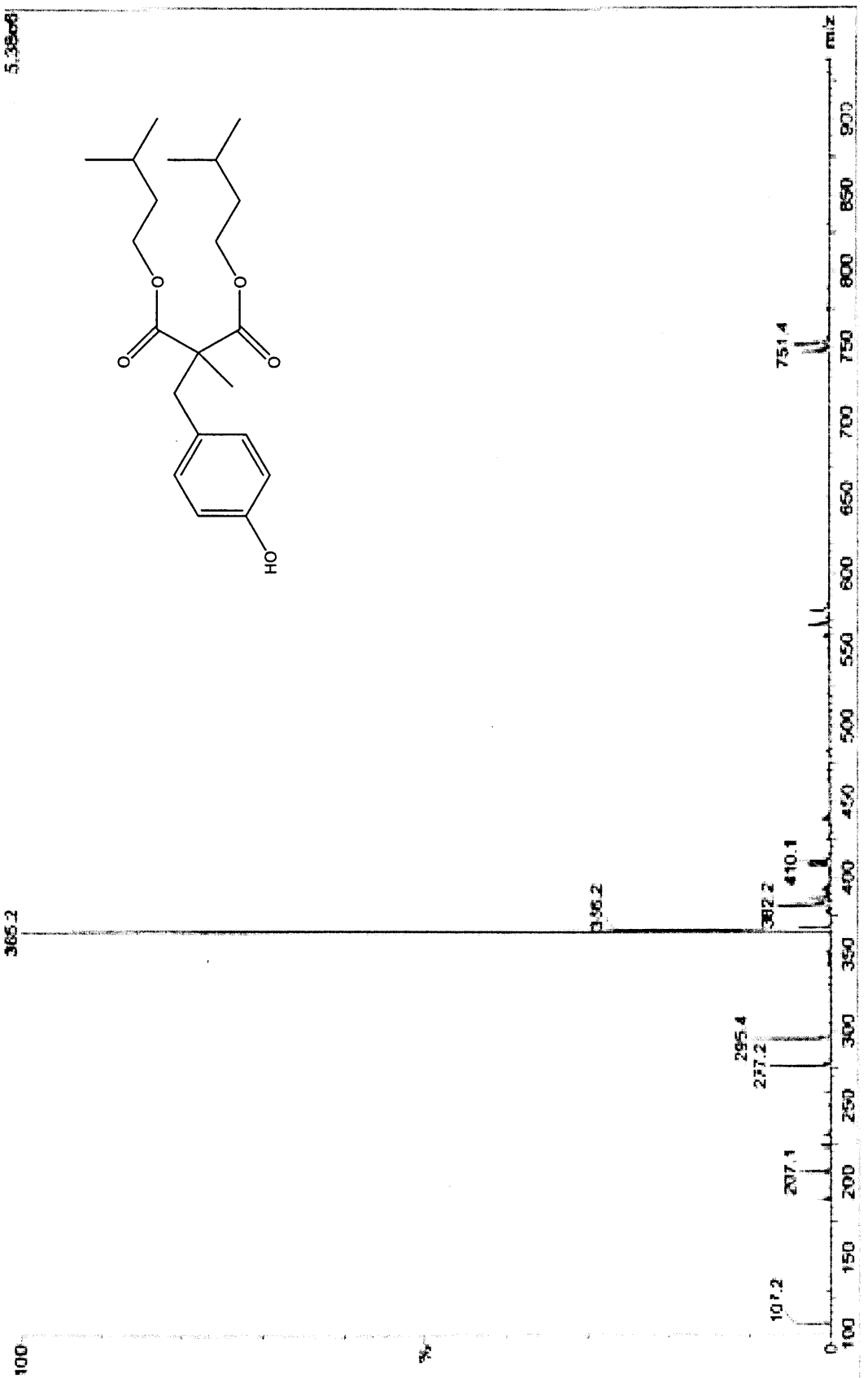


Figure 2.44 MS (LRESI) of Diisoamyl-2-(4-hydroxybenzyl)-2-methyl Malonate

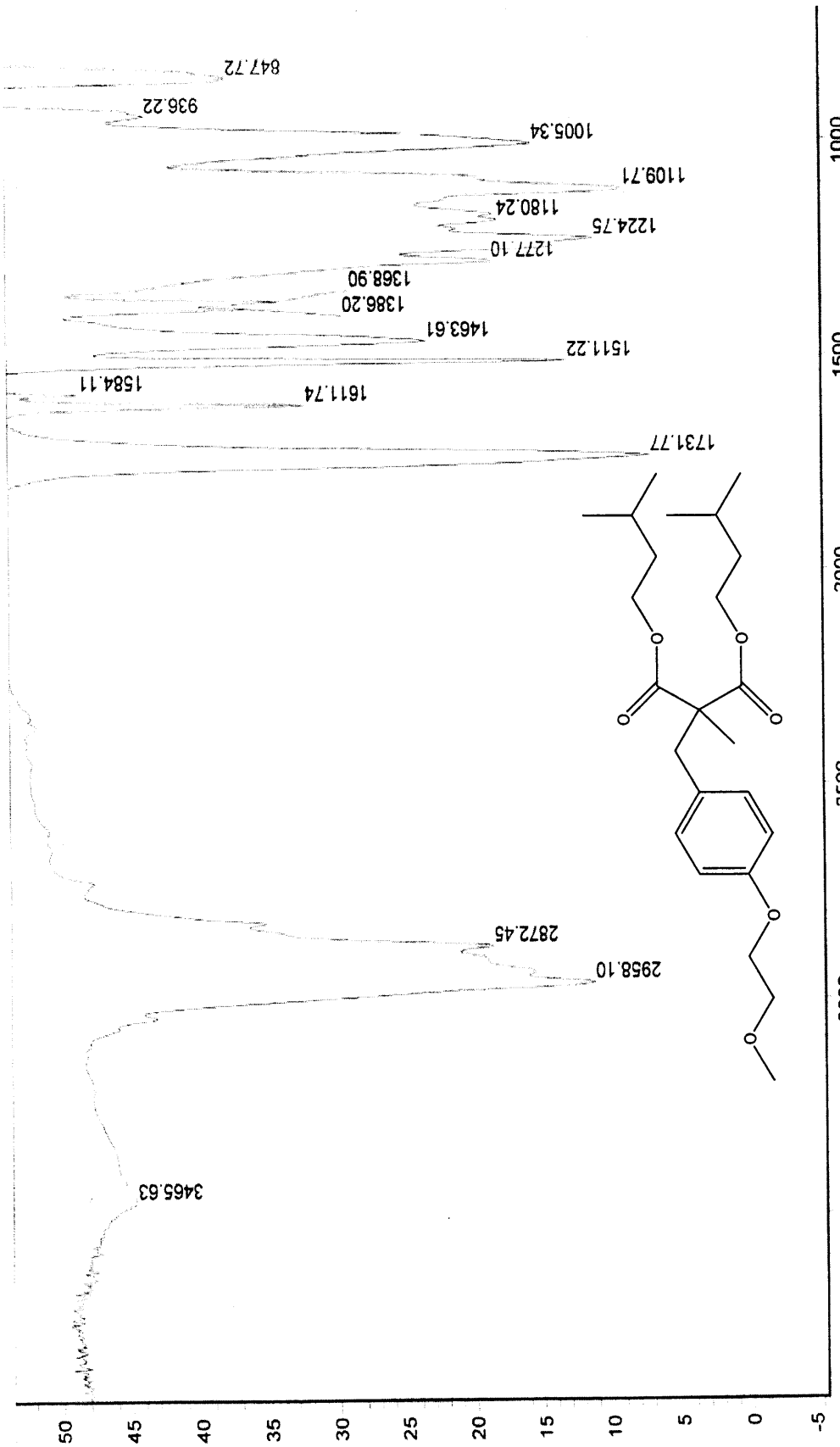


Figure 2.45 IR Spectrum of Diisoamyl-2-(4-methoxyethoxymethylbenzyl)-2-methyl Malonate

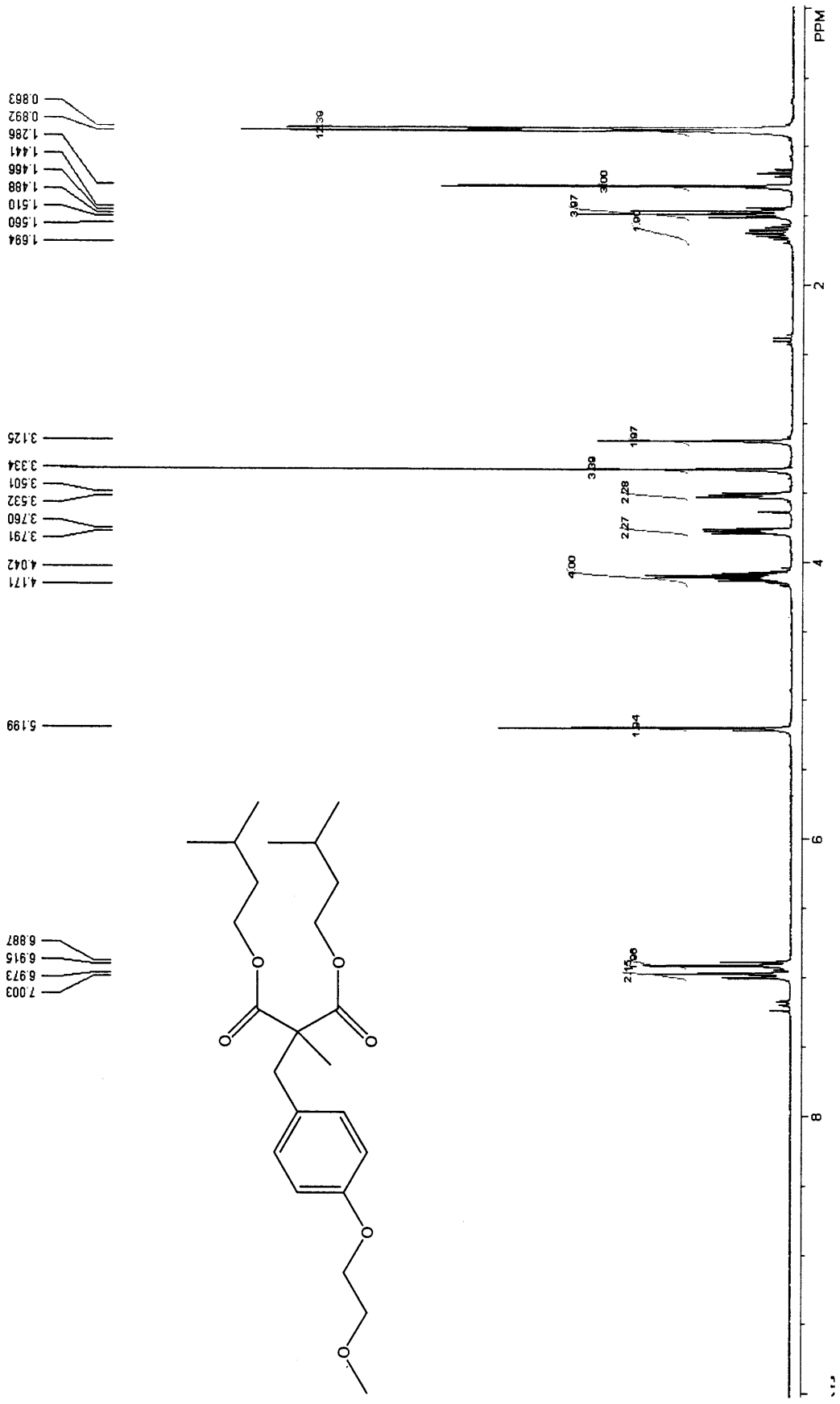


Figure 2.46 ^{1}H NMR Spectrum of Diisoamyl-2-(4-methoxyethoxymethylbenzyl)-2-methyl Malonate

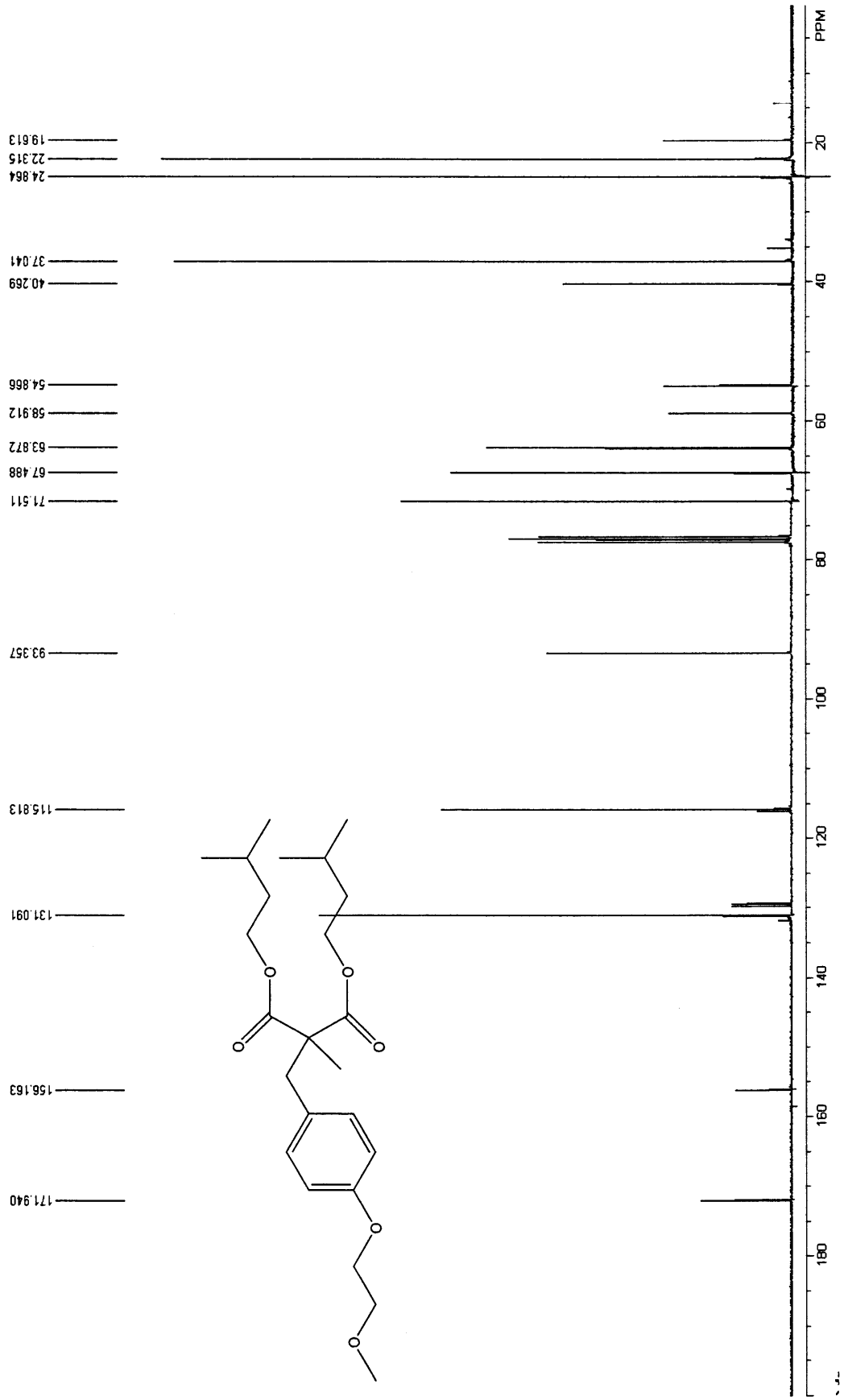


Figure 2.47 ^{13}C NMR Spectrum of Diisoamyl-2-(4-methoxyethoxymethylbenzyl)-2-methyl Malonate

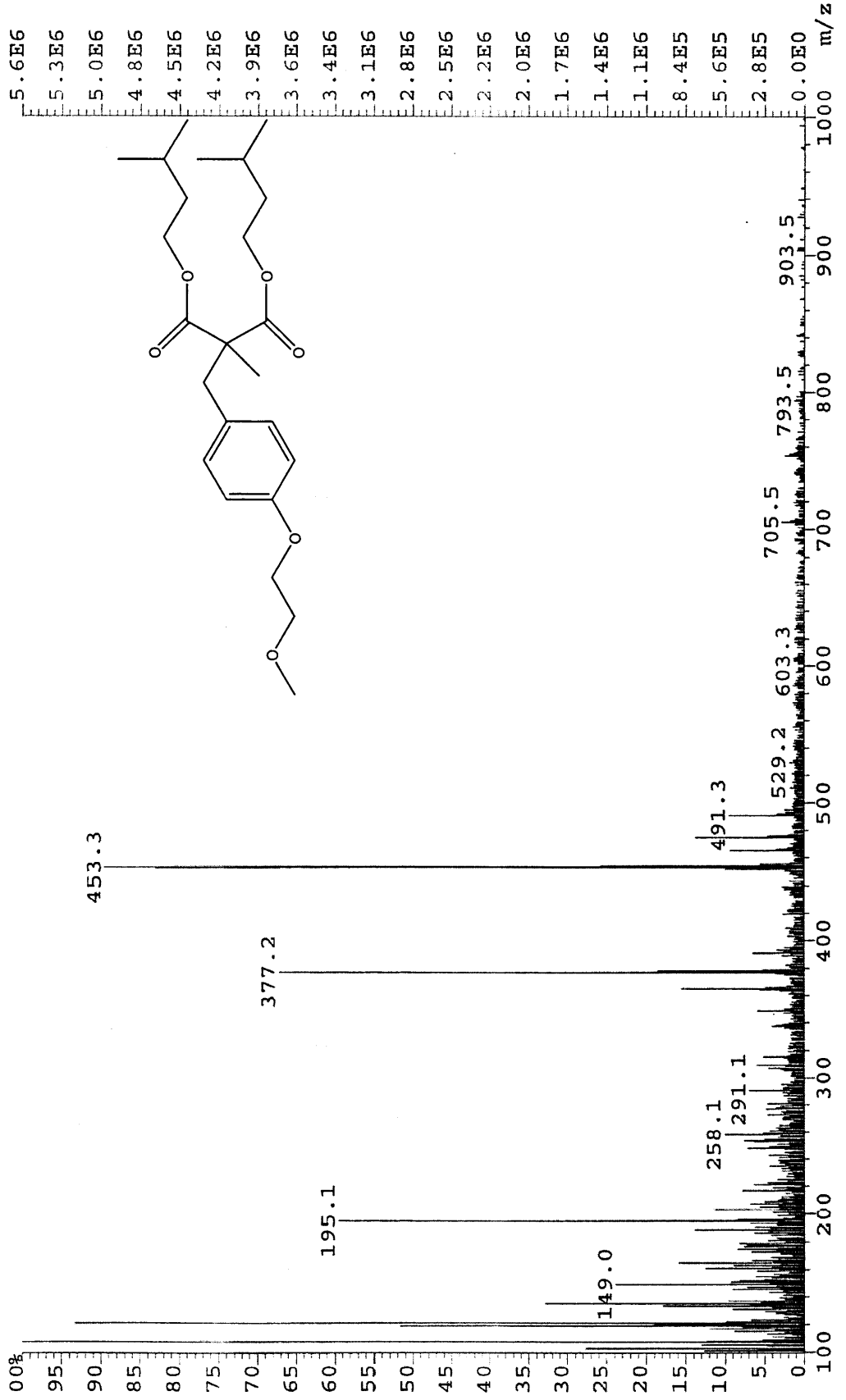


Figure 2.48 MS (LRFAB) of Diisoamyl-2-(4-methoxyethoxymethylbenzyl)-2-methyl Malonate

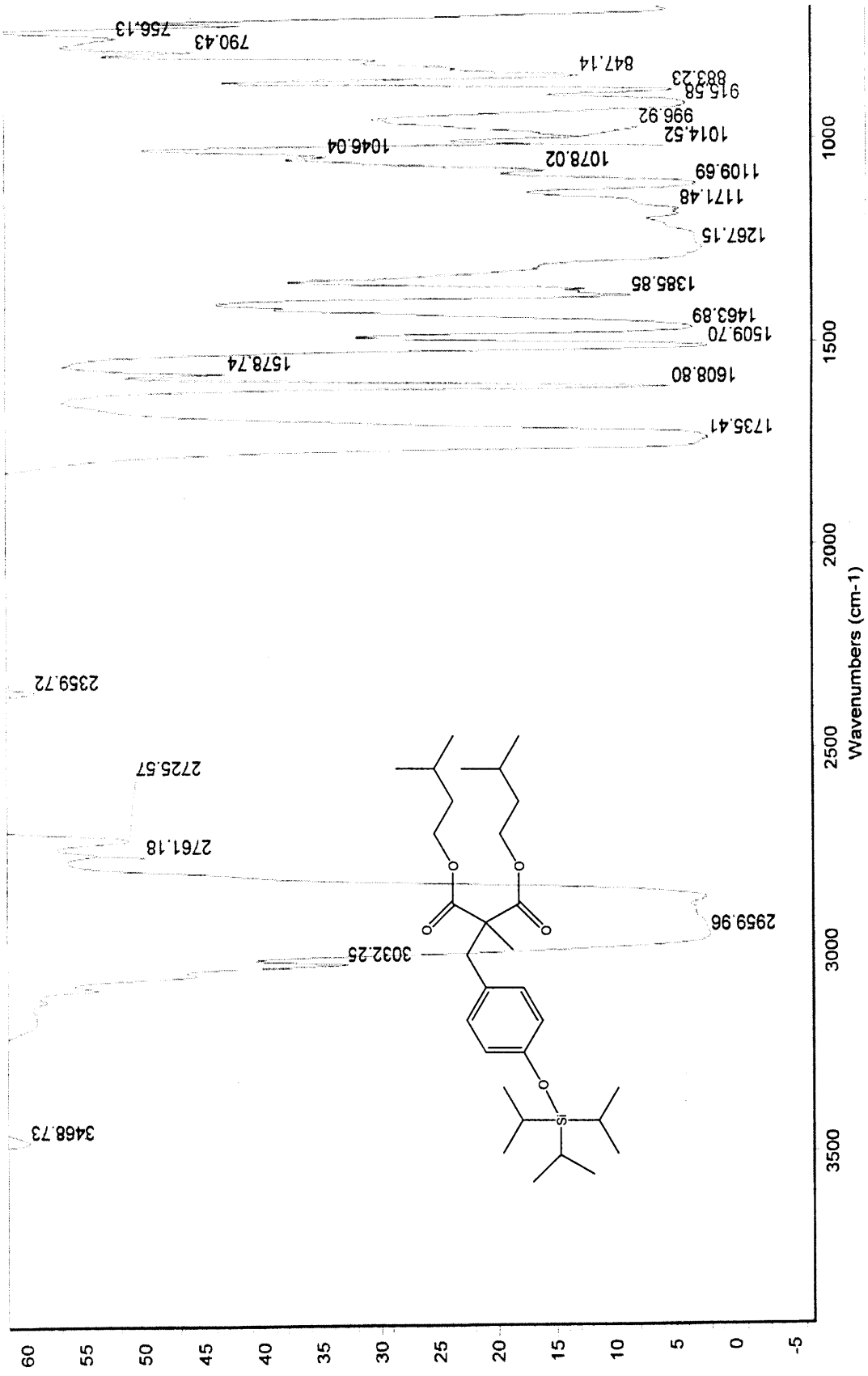


Figure 2.49 IR Spectrum of Diisoamyl-2-(4-triisopropylsilylbenzyl)-2-methyl Malonate

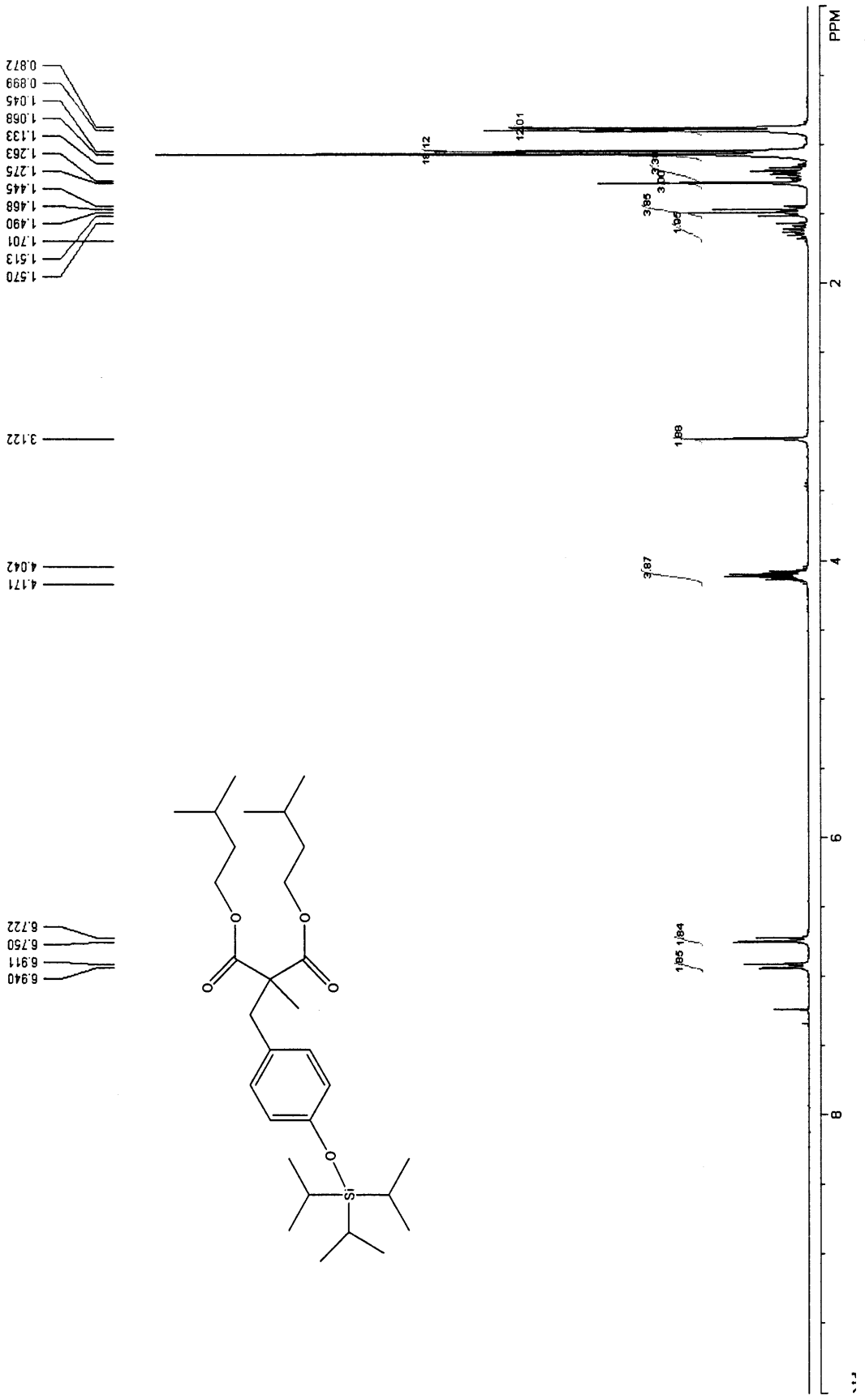


Figure 2.50 ^{1}H NMR Spectrum of Diisoamyl-2-(4triisopropylsilylbenzyl)-2-methyl Malonate

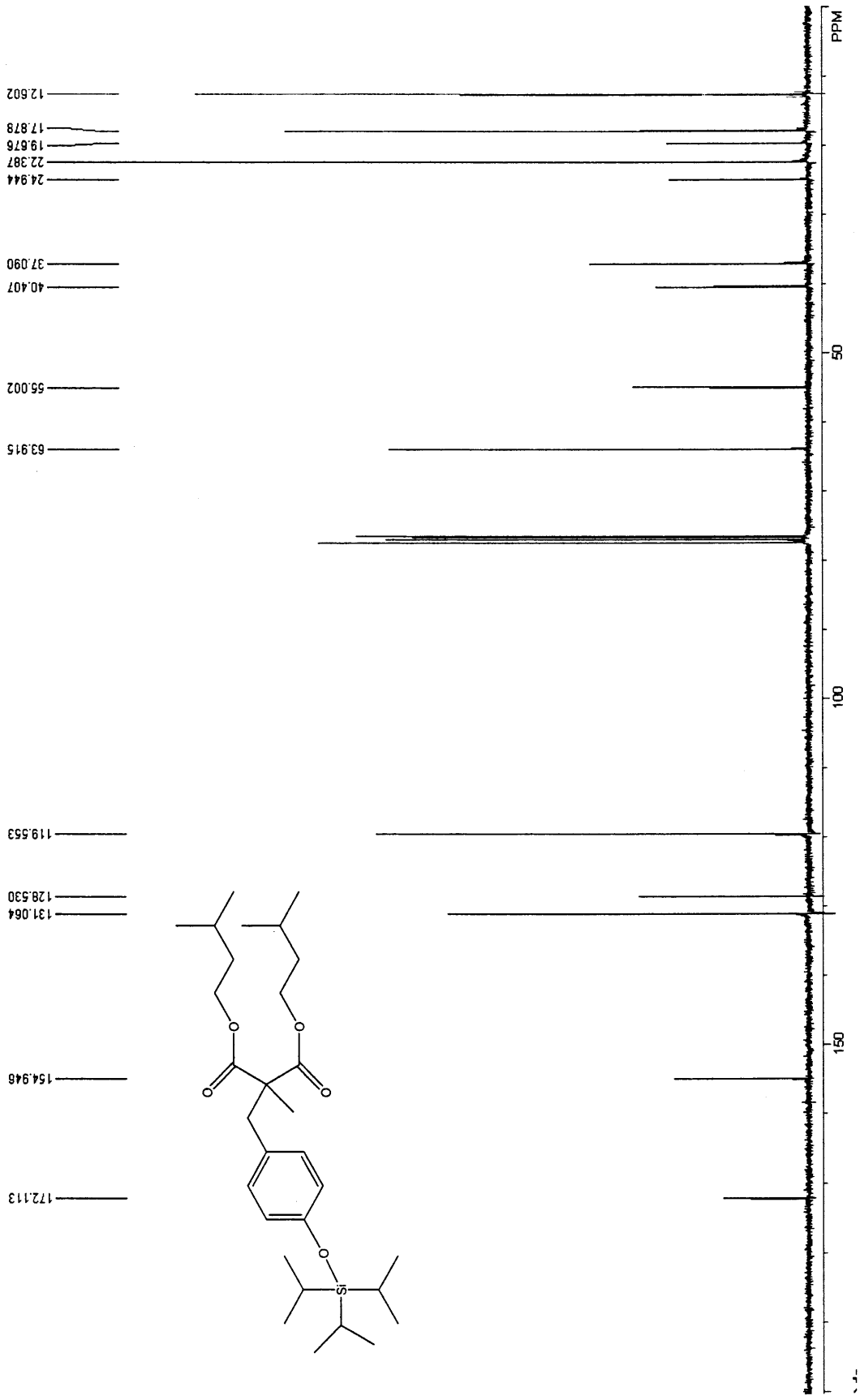


Figure 2.51 ^{13}C NMR Spectrum of Diisoamyl-2-(4-triisopropylsilylbenzyl)-2-methyl Malonate

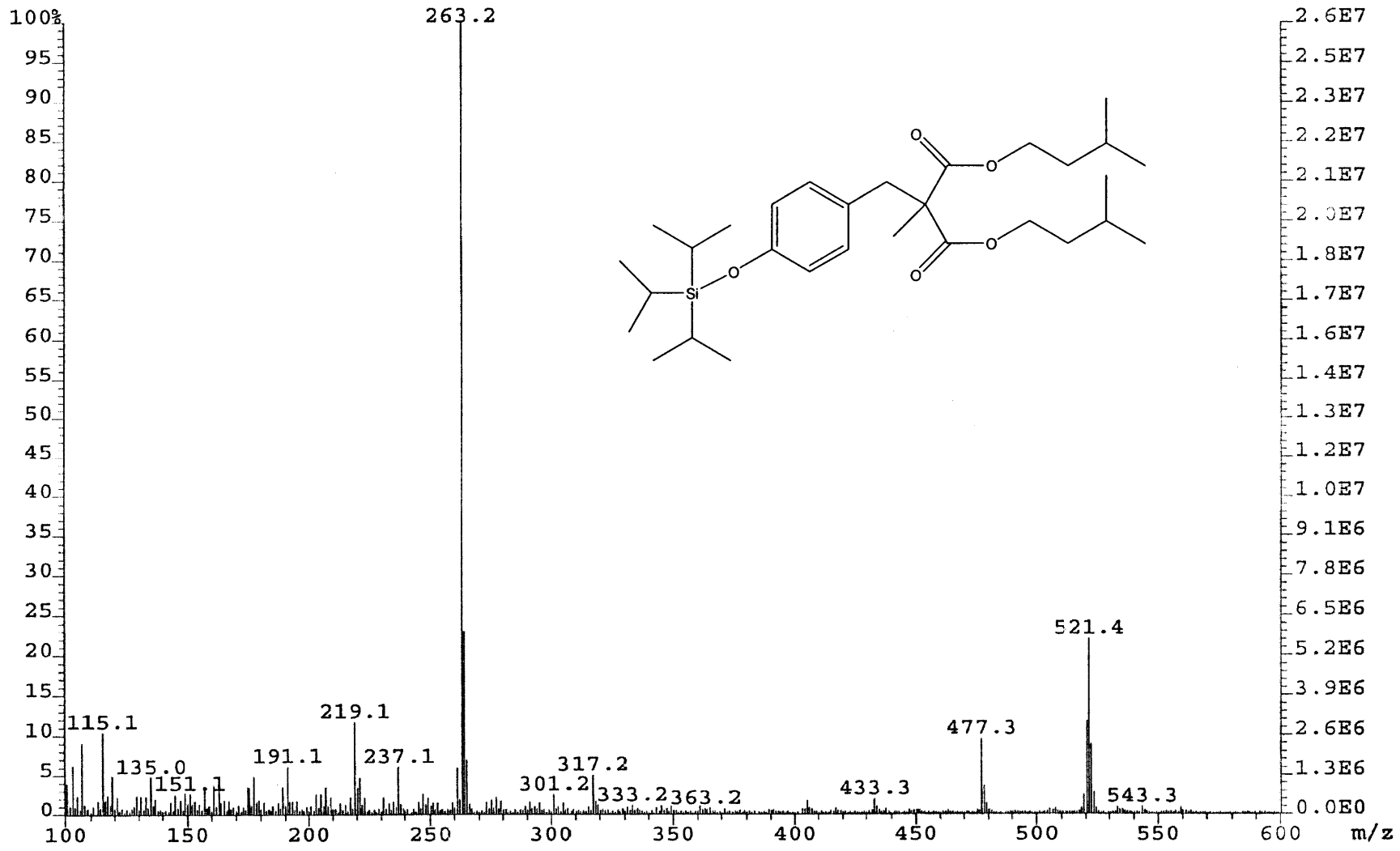


Figure 2.52 MS (LRFAB) of Diisoamyl-2-(4-triisopropylsilylbenzyl)-2-methyl Malonate

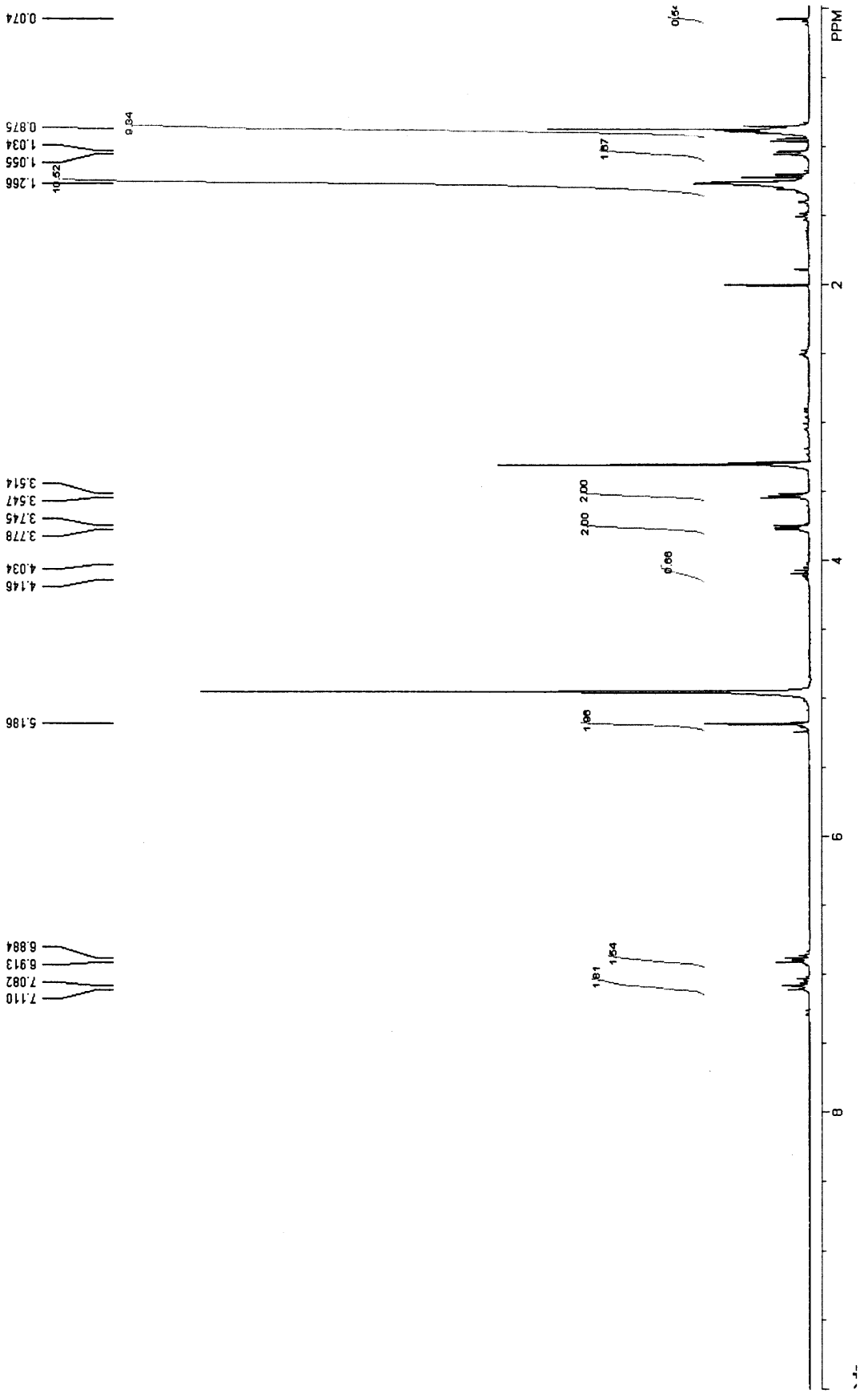


Figure 2.53 ^{1}H NMR Spectrum of Failed Saponification Product with MEM

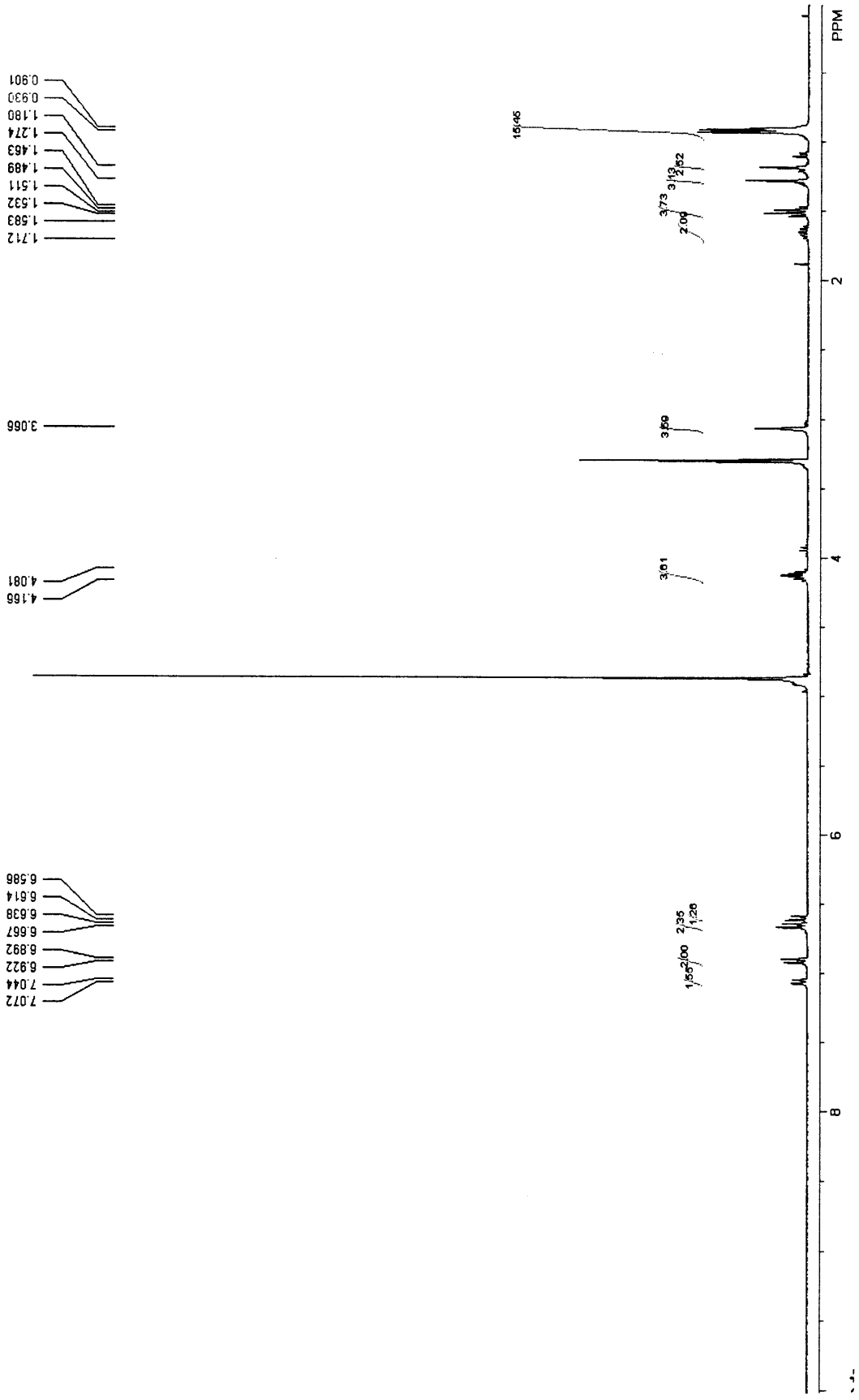


Figure 2.54 ^{1}H NMR Spectrum of Failed Saponification Product with TIPS

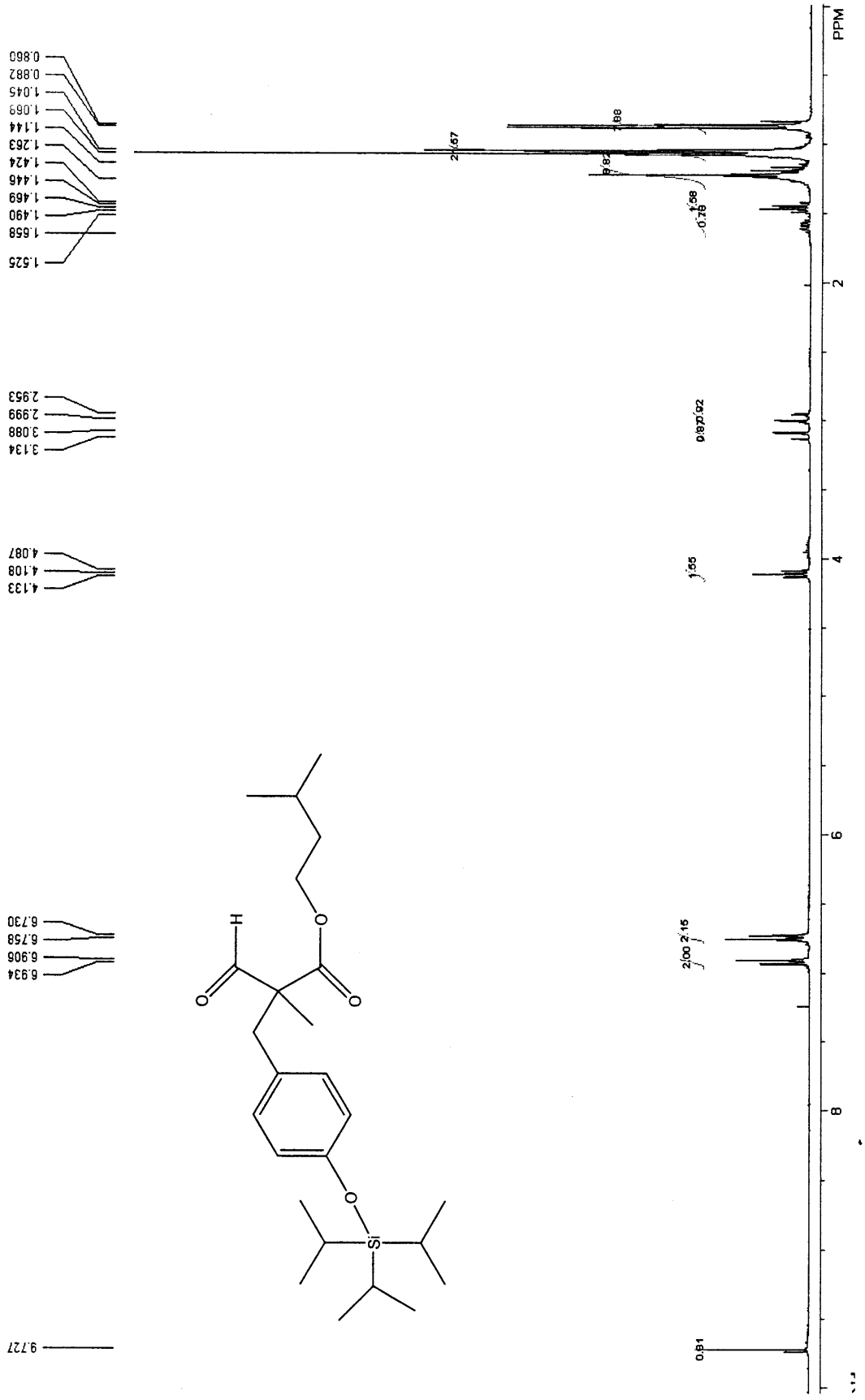


Figure 2.55 ^{1}H NMR Spectrum of Failed Reduction Product with TIPS

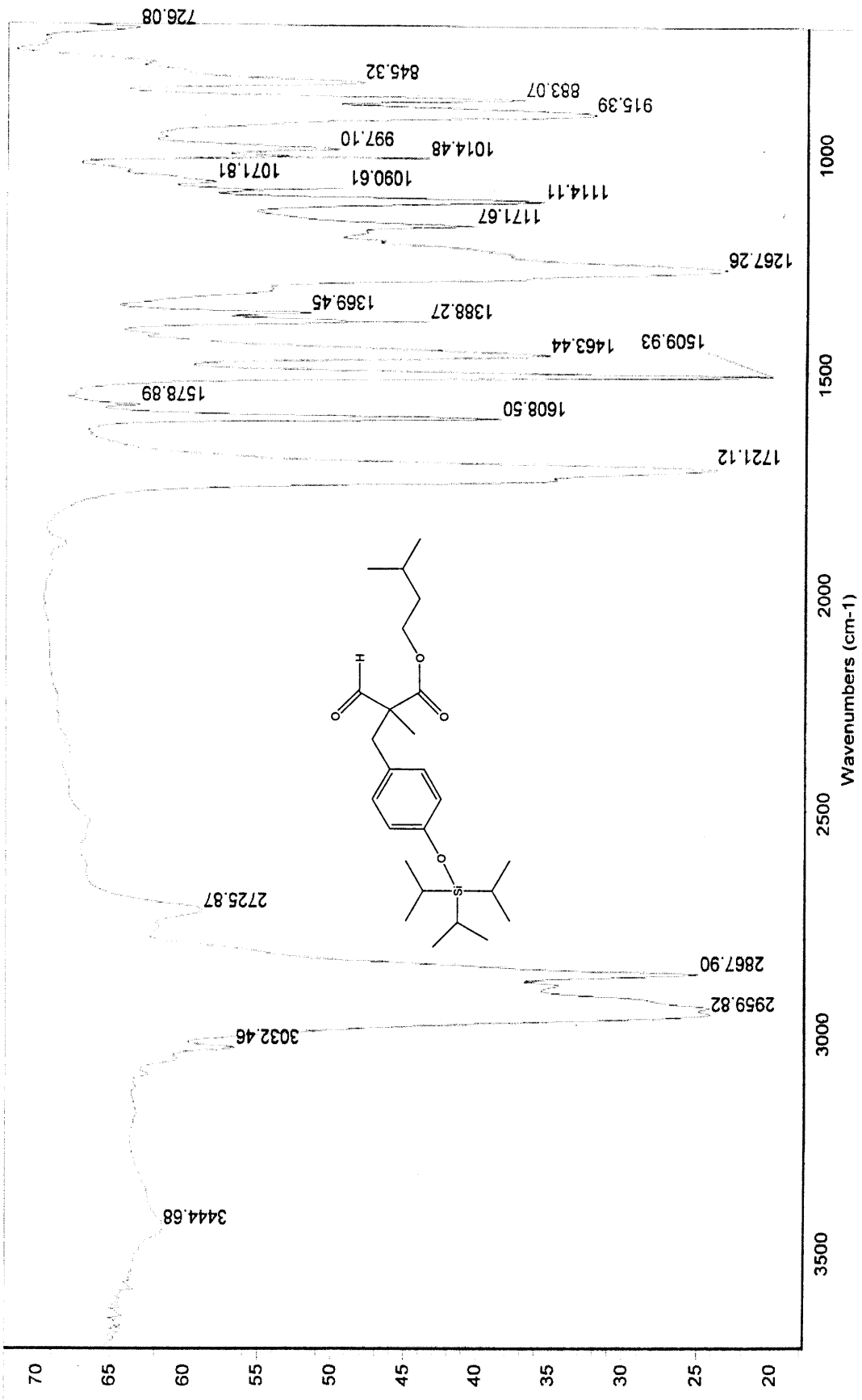


Figure 2.56 IR Spectrum of Failed Reduction Product with TIPS

Chapter III: Platination of Model Substitued Malonic Acids

Section I: Introduction

Traditional Platination Methods

The platinum compounds commonly used to treat cancer, cisplatin and carboplatin, are commonly synthesized in water. The standard synthetic route to cisplatin with high yields and purity was published by Dhara.[1] This method avoids the formation of the Magnus salt by reacting $K_2[PtCl_4]$ with excess KI to form the salt $K_2[PtI_4]$. This salt is converted by ammonium hydroxide to *cis*-$[Pt(NH_3)_2I_2]$. This compound is isolated and converted to *cis*-$[Pt(NH_3)_2(NO_3)_2]$ by treatment with $AgNO_3$. The nitrate complex is then treated with solid KCl to form the desired cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$. This series of reactions was performed using water as the reaction solvent. The reaction sequence is shown in Scheme 3.1.

Scheme 3.1 Synthesis of Cisplatin

$$K_2PtCl_4 + \text{excess KI} \xrightarrow[H_2O]{\text{heat}} K_2PtI_4(aq) + 4\,KCl(aq)$$

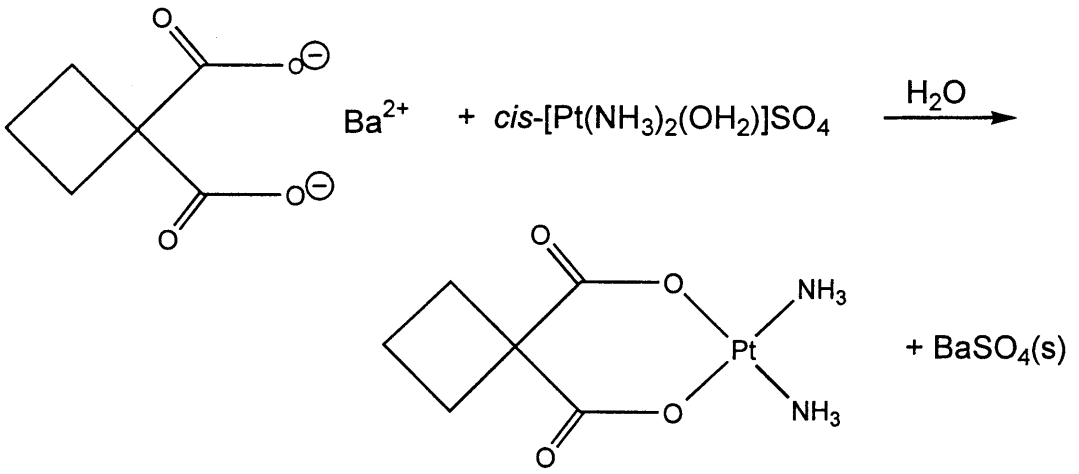
$$K_2PtI_4 + 2\,NH_3\,(aq) \longrightarrow \textit{cis}\text{-}[Pt(NH_3)_2I_2](s) + 2\,KI\,(aq)$$

$$\textit{cis}\text{-}[Pt(NH_3)_2I_2] + 2\,AgNO_3(aq) \longrightarrow \textit{cis}\text{-}[Pt(NH_3)_2(NO_3)_2](aq) + 2\,AgI(s)$$

$$\textit{cis}\text{-}[Pt(NH_3)_2(NO_3)_2](aq) + KCl\,(s) \xrightarrow{\text{heat}} \textit{cis}\text{-}[Pt(NH_3)_2Cl_2](s) + KNO_3(aq)$$

The common synthetic route for the formation of carboplatin was published by Harrison and McAuliffe.[2] The synthesis was performed by adding *cis*-$[Pt(NH_3)_2I_2]$ to a silver sulfate solution to form a platinum sulfate intermediate. A solution of the barium salt of 1,1-cyclobutanedicarboxylic acid was added to the platinum sulfate solution to form the desired product with 88% yield. The reaction series is shown in Scheme 3.2

Scheme 3.2 Synthesis of Carboplatin



The development of malonate-platinum complexes has been investigated by many different groups. Harrison and McAuliffe formed the malonate-platinum complexes by performing the coordination in water with the sodium salt of the malonate.[2] Rochon and Gruia as well as Young et al. used a similar tactic to form malonate-platinum complexes.[3,4] They were able to coordinate small organic molecules to platinum by using the malonate barium salts. Both methods were tried with synthetic estrogens developed in the Chesnut lab with no success. A new platination method was required to coordinate the aqueous insoluble estrogen with platinum.

Use of Platinum Intermediates

A significant review article by Pascini et al. summarizes methods for coordinating malonates to platinum.[5] The malonates that the Pascini group investigated were very water soluble, but other reaction solvents such as acetone and DMF were investigated. Some of their work involved the formation of platinum intermediates shown in the following reaction sequences. (A = amine; X = Cl, I.)

(1) $cis\text{-}[PtA_2X_2] + 2\ AgNO_3 \rightarrow cis\text{-}[PtA_2(NO_3)_2] + 2\ AgX$

(2) $cis\text{-}[PtA_2X_2] + Ag_2SO_4 \rightarrow cis\text{-}[PtA_2SO_4] + 2\ AgX$

(3) $cis\text{-}[PtA_2X_2] + {}_2\ AgNO_3 \xrightarrow{H_2O} cis\text{-}[PtA_2(NO_3)_2] + 2AgX$

$$cis\text{-}[PtA_2(NO_3)_2] \xrightarrow{NaOH\ (aq)} \xrightarrow[(OH\ form)]{ion\ exchange\ resin} cis\text{-}[PtA_2(OH)_2]$$

Each of these platinum intermediates was formed to yield a better leaving group from the platinum for the next reaction of coordinating the malonate to the metal. The benefit of the first two reactions is that the silver halide product is a solid and can easily be removed by filtration. The amount of silver halide formed can also be used as an indicator to the success of the dehalogenation of platinum. A variety of reaction conditions and times have been reported for the nitrate intermediate as formed in Reaction (1).[5] The reaction times and temperatures have varied from 20 minutes at 50 °C to three days at 5 °C. The stoichiometry of halogenated platinum amine to silver nitrate is 1:1.95. The slight

excess in platinum limits silver impurities in the final product. The reaction solvents included water, acetone and DMF.

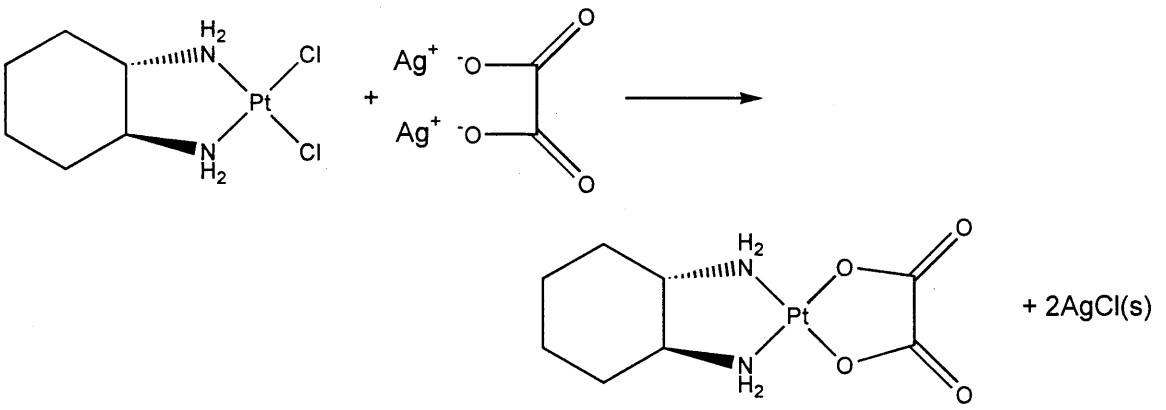
Formation of the sulfate intermediate by Reaction (2) has also been investigated under a variety of reaction conditions and times similar to the platinum nitrate conditions. The isolation of pure platinum-sulfate intermediate can be difficult to achieve due to silver impurities. The purification can be difficult since "analytically pure" samples darken with aging.[5] This intermediate has also only been formed using water as the reaction solvent. The platinum-sulfate intermediate contains a water molecule as well. The sulfate is coordinated through one oxygen and the water fills the other vacant coordination site.

The advantage of the hydroxy-platinum intermediate formed in Reaction (3) is that upon coordination to malonate, the only byproduct is water. The drawback to using this platinum intermediate is the extra time required with its synthesis. The platinum amine nitrate complex is first formed and isolated. The reaction solvent, water, is removed *in vacuo*. The second step requires dissolving the platinum amine nitrate in an aqueous sodium hydroxide solution followed by elution through an anion exchange column. This intermediate is synthesized with water as the reaction solvent, which needs to be removed *in vacuo*. Unlike the platinum nitrate and platinum sulfate intermediate, the hydroxy-platinum intermediate has only been formed with the DACH ligand as the coordinated amine.

Ligand Exchange Reactions

The coordination of the malonates to platinum can occur with the use of the previously described platinum intermediates or with direct exchange as shown in Scheme 3.3

Scheme 3.3 Synthesis of Oxaliplatin by Displacement of Chloride



This direct exchange simplifies the coordination of dicarboxylic acids to platinum in one reaction step. This reaction was performed by Swartz et al. as a means of evaluating new platinum compounds for antitumor activity.[6] A variety of (di)carboxylic acids were evaluated using water as the reaction solvent. Each of their organic ligands were water soluble when the acids were deprotonated and their respective salts were coordinated to platinum DACH compounds.

Use of Nonaqueous Solvents

The vast majority of the published platinum coordination chemistry uses water as the reaction solvent. An exception is a procedure by Pascini and Cardirola for the synthesis of carboplatin reported using *N,N*-dimethylformamide

as an alternate reaction solvent.[7] The procedure included the use of various aqueous base solutions of LiOH, KOH, and NaOH. The bases were used to ensure deprotonation of the carboxylic acids. The reaction for this method is shown in Scheme 3.4. The exchange was direct replacement of halogens by a malonate. The yield for this reaction method was reported as ~40% and varied with the type of amine and malonate used.

Scheme 3.4 Formation of $[PtA_2Mal]$

$$cis\text{-}[PtA_2X_2] + H_2Mal \xrightarrow[DMF]{Base(aq)} cis\text{-}[PtA_2Mal]$$

A= amine
Mal= malonic acid

Platinum Coordination and pH

Coordinating malonic acids to platinum in aqueous solutions is highly dependent on the pH of the reaction. The platinum intermediate, *cis*-$[Pt(NH_3)_2(NO_3)_2]$, in aqueous solutions is converted to *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ before coordination to a new ligand.[1,2,5,8,9] The pH of the reaction solutions can lead to deprotonation of the coordinated water molecule(s). The hydroxide ligand can then become a bridging ligand. The bridged platinum compounds are less reactive and can result in lower yields.[8] Table 3.1 lists the pK_a values of the coordinated water protons with various platinum amine complexes.

Table 3.1. Acid dissociation constants of the complexes *cis*-$[PtA_2(H_2O)_2]^{2+}$ [8]

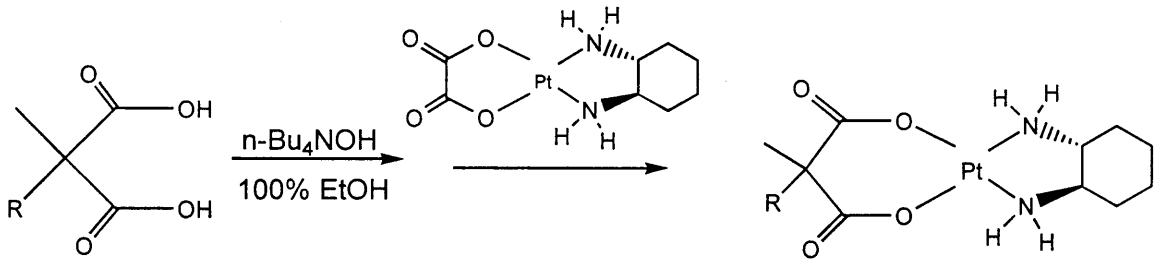
Amine	pK_1	pK_2
NH_3	5.6	7.3
EN	5.8	7.6
DACH	6.1	7.6

The pH of the reaction solutions of malonates and platinum nitrate intermediates must be carefully balanced. The pH needs to be high enough for complete deprotonation of the carboxylic acids but low enough to prevent oligimerzation of the platinum intermediates.

Proposed Platination Methods

Several possible methods appear to be adaptable to coordination of a lipophilic malonate ligand to a platinum amine complex. The first method is the direct reaction of a lipophilic malonate with oxaliplatin. The proposed reaction is shown in Scheme 3.5. This is potentially the simplest and most convenient method since oxaliplatin can be purchased. Precedent for this reaction has been reported by Schwartz, who displaced oxalate from oxaliplatin with a calcium malonate.[6]

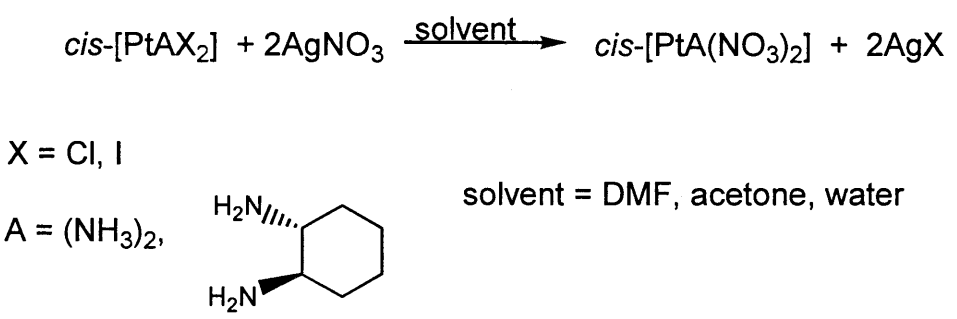
Scheme 3.5 Direct Exchange Method



The second possible method is the use of a platinum nitrate intermediate. The synthesis of *cis*-$[Pt(NH_3)_2(NO_3)_2]$ from *cis*-$[Pt(NH_3)_2I_2]$ and $AgNO_3$ with DMF as the reaction solvent has been reported by Pascini.[5] The reaction scheme for forming the platinum-nitrate intermediate is shown in Scheme 3.6. The *cis*-

$[Pt(NH_3)_2I_2]$ complex is known to be soluble in DMF, and AgI will precipitate readily.[10] The AgI can be easily removed by filtration and the desired product, *cis*-$[Pt(NH_3)_2(NO_3)_2]$, will be present in the filtrate. Acetone also had potential as a solvent, since some platinum amine complexes are known to be soluble in acetone.[11] As a control experiment, water was also investigated as a solvent in an effort to replicate results published by Maeda et al.[11]; this work coordinates a lipophilic molecule, lauric acid, with platinum amine complexes.

Scheme 3.6 Proposed Synthesis of *cis*-$[Pt(NH_3)_2(NO_3)_2]$



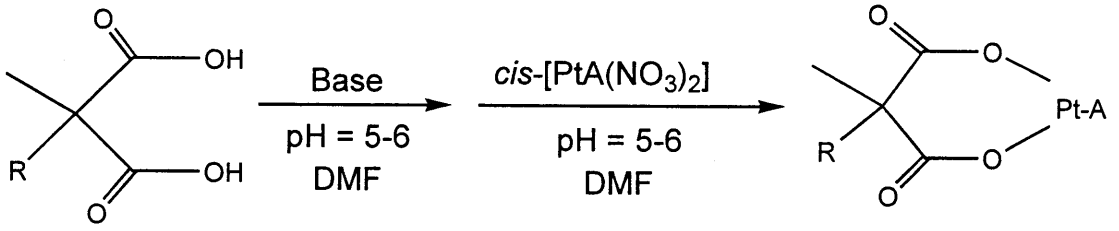
In addition to nitrate, sulfate was evaluated as a leaving group from platinum in DMF. Sulfate was chosen because it has been successfully used in the formation of platinum intermediates in aqueous solutions.[5] These intermediates are known to react with malonate salts to produce amino platinum malonate complexes.

Other platinum intermediates considered worthy of investigation were the *cis*-$[PtA_2(B(C_6F_5)_4)_2]$ and *cis*-$[PtA_2(OSO_2CF_3)_2]$. The potential advantages of using BARF or triflate as a leaving group is that they are lipophilic and coordinate to metals weakly. These weakly coordinated ligands could be easily displaced by malonate, just as triflate has proven to be a good leaving group in the

synthesis of bridged osmium compounds.[13] The intermediate *cis*-$[PtA_2(B(C_6F_5)_4)_2]$ is formed from *cis*-$[PtA_2X_2]$ and $AgB(C_6F_5)_4$ (silver BARF). The intermediate *cis*-$[PtA_2(OSO_2CF_3)_2]$ is formed from *cis*-$[PtA_2X_2]$ and $AgOSO_2CF_3$ (silver triflate). The silver BARF ligand can easily be prepared from LiBARF and silver nitrate in ether.[14]

The coordination of the malonate ligand to the platinum intermediate requires deprotonation of the conjugate acid. The acid was dissolved in a minimal amount of DMF and the pH of the solution was adjusted to 5-6 to ensure complete deprotonation. The base was used as a methanolic solution. The bases evaluated include tetrabutylammonium hydroxide, sodium hydroxide and potassium hydroxide. The resulting salt solution was added to a solution of *cis*-$[Pt(NH_3)_2(NO_3)_2]$ in DMF. This proposed reaction is shown in Scheme 3.7.

Scheme 3.7 Proposed Coordination of Malonic Salt to *cis*-$[Pt(NH_3)_2(NO_3)_2]$

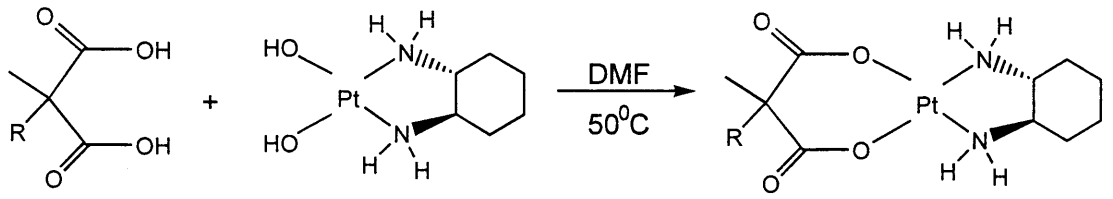


In previously published aqueous reactions, water molecules coordinate to the platinum and the nitrates are spectator anions. The proposed reaction in Scheme 3.7 will not have water available for this coordination with DMF as the solvent. Raudashl and Lippert isolated and obtained crystal structures of DMF solvated *cis*-$[Pt(NH_3)_2Cl_2]$ and *cis*-$[Pt(NH_3)_2I_2]$ compounds.[10] Their work suggests

that DMF weakly coordinates to the platinum-nitrate intermediate and assumes the water molecule's role with the traditional platination.

The final proposed method for coordinating a lipophilic malonate to a platinum amine complex is shown in Scheme 3.8. This method requires a more lengthy preparation of the platinum compound. The main benefit is the products are the desired compound and water. The water can easily be removed by evaporation.

Scheme 3.8 Proposed Coordination of Malonate to *cis*-[$(HO)_2$PtDACH]

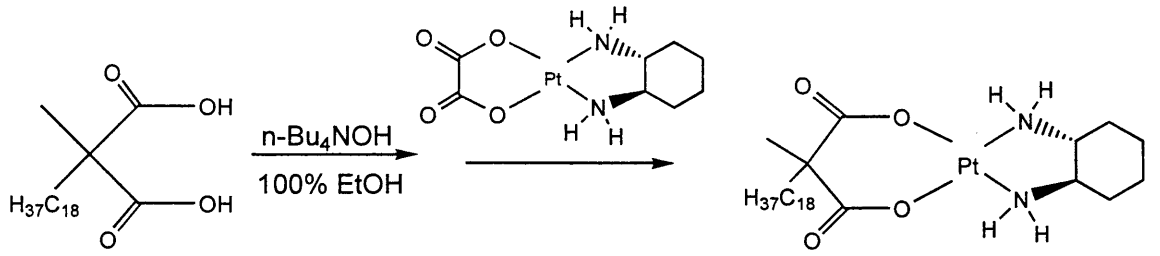


Chapter III

Section II: Results and Discussion

A. Attempted substitution of oxalate

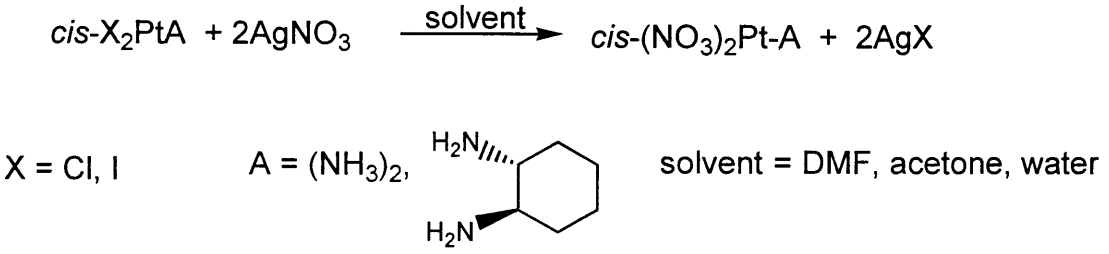
Scheme 3.9 Attempted Substitution of Oxalate



The attempt to substitute oxalate with 2-methyl-2-octadecylmalonic acid is shown in Scheme 3.9. This substitution reaction was attempted because of published precedent.[6] The tetrabutyl ammonium hydroxide was used to deprotonate the acid and establish a pH of 5. Reaction progress was monitored by reverse phase thin layer chromatography. The reaction was stirred at reflux for a total of eight hours and then at room temperature overnight. Only the starting materials were observed by TLC, and the experiment was terminated. Apparently formation of the tetrabutyl ammonium oxalate was not a sufficiently strong driving force for the reaction. There was also a lack in solubility of oxaliplatin in ethanol. The work performed by Swartz had the driving force of forming a precipitate.[6]

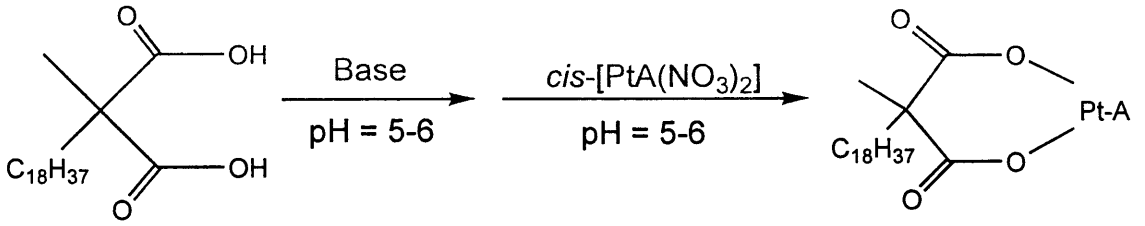
B. Formation of amino platinum nitrate complexes followed by dicarboxylate ligand exchange

Scheme 3.10 Nitration of Platinum Amine Complexes



Scheme 3.10 shows the formation of a nitro platinum complex. This step is required due to nitrate's behaving as a better leaving group than the halides in the next step of the reaction sequence, ligand exchange. The halides, chloride and iodide were both investigated as the leaving groups for nitration of the platinum with DMF as the reaction solvent. The iodide was determined to be the better leaving group, since yields of silver iodide were greater than yields of silver chloride. As a reaction solvent, acetone did not allow formation of *cis*-$[Pt(NH_3)_2(NO_3)_2]$, because *cis*-$[Pt(NH_3)_2I_2]$ was insoluble in acetone.[11] (Water was used successfully in the nitration step but was unsuccessful in the subsequent ligand exchange with the dicarboxylate ligand.) Dimethyl formamide was the most used solvent for method development, since it was successful for both reaction steps. Table 3.4 summarizes the results of the nitration experiments.

Scheme 3.11 Exchange of Nitrate and 2-Methyl-2-octadecyl Malonate Ligands



Base: 1.0M n-Bu_4NOH in MeOH, 1.0M KOH in MeOH, 0.172M NaOH in MeOH
A = $(NH_3)_2$ or DACH

Scheme 3.11 shows the ligand exchange reaction of nitrate with 2-methyl-2-octadecylmalonic acid. The solution pH was adjusted to 5-6 in order to assure deprotonation of the ligand. Successful ligand exchange was demonstrated with each base. The largest amount of crude product was formed when the methanol was removed from the bases (n-Bu_4NOH, KOH and NaOH) immediately after formation of the salt complex. The malonic salts were dissolved in DMF before addition to the amino platinum nitrate solutions. The reaction was stirred at room temperature over night and protected from light. Table 3.2 summarizes the series of reactions performed.

Table 3.2 Summary of platination reaction conditions with 2-methyl-2-octadecylmalonic acid

Reaction Number	*cis*-Pt Source	% Yield AgX	Solvents	Base	Stoich. Equiv. of Malonate
1	$Cl_2Pt(NH_3)_2$	80%	DMF, MeOH	1.0 M n-BuNOH in MeOH	1
2	$Cl_2Pt(NH_3)_2$	88%	DMF, MeOH	1.0 M n-BuNOH in MeOH	1
3	$I_2Pt(NH_3)_2$	93%	DMF, MeOH	1.0 M n-BuNOH in MeOH	1
4	$I_2Pt(NH_3)_2$	99%	DMF, MeOH	1.0 M n-BuNOH in MeOH	2
5	$I_2Pt(NH_3)_2$	90%	DMF	1.0 M n-BuNOH in MeOH	1
6	$I_2Pt(NH_3)_2$	93%	DMF	1.0 M n-BuNOH in MeOH	1
7	$I_2Pt(NH_3)_2$	96%	DMF, MeOH	1.0 M KOH in MeOH	1
8	$I_2Pt(NH_3)_2$	88%	DMF, MeOH	0.172 M NaOH in MeOH	1
9	$I_2Pt(NH_3)_2$	150%	Acetone	1.0 M n-BuNOH in MeOH	1
10	I_2Pt-DACH	67%	DMF, MeOH	1.0 M n-BuNOH in MeOH	1
11	I_2Pt-DACH	95%	DMF, MeOH	1.0 M n-BuNOH in MeOH	2
12	I_2Pt-DACH	a	DMF, MeOH	1.0 M n-BuNOH in MeOH	1
13	I_2Pt-DACH	90%	DMF	1.0 M n-BuNOH in MeOH	1
14	$I_2Pt(NH_3)_2$	91%	Water	1.0 M n-BuNOH in MeOH	1

[a] AgI precipitate was too fine to remove by filtration with filter paper. Celite was required, so yield was undeterminable

The work up of the reactions typically involved removing the reaction solvent *in vacuo* followed by a series of extractions of the crude solid. First, the reaction residue was suspended in deionized water (10 mL) and filtered. Then the solid was extracted with diethyl ether and then with absolute ethanol. In some instances a solid remained after the extractions. The solvent was removed from each extract *in vacuo*. In two instances the crude solid was analyzed

directly, since it proved entirely soluble in at least one solvent. No further attempts were made to purify products after recovery of solid from an extraction procedure. Purification was not pursued with platinum complexes of this malonate model since it lacks functional groups such as a phenol and secondary alcohol that are present in an estrogen.

Analysis of the products was conducted in order to determine whether the expected products formed. These products are shown as compounds **3** and **4** in Figure 3.1. The only difference between these two compounds is the type of amine coordinated to the platinum.

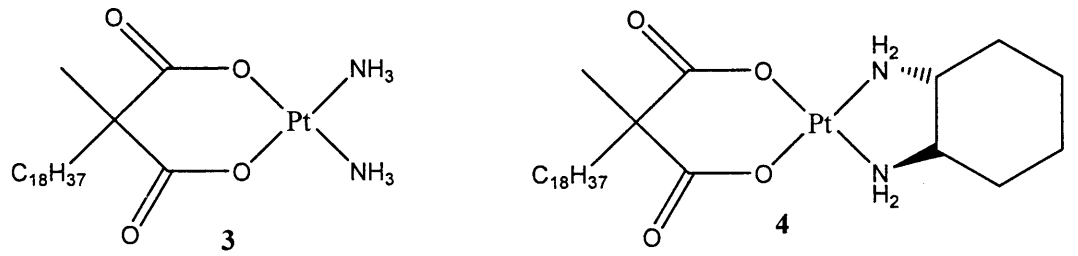


Figure 3.1 Platinated Alkyl Malonates

The residue of the ether and ethanol extracts as well as the completely insoluble solid were analyzed by MS (LRFAB or LRESI). The spectra for the reactions (Figures 3.2-3.17) show the characteristic isotope pattern of a platinum-containing species. Table 3.3 summarizes mass spectrometry data of various reaction products.

Table 3.3 Mass spectrometry analysis of reaction products:

Reaction Number	Amine	Figure	Resolution and Ionization method	M^+ or M+1 observed	Other Pt species
2, ether extract	$(NH_3)_2$	3.2	Low-Res FAB	No	No
2, ethanol extract	$(NH_3)_2$	3.3	Low-Res FAB	No	No
2, insoluble solid	$(NH_3)_2$	3.4	Low-Res FAB	Yes	Yes
3, ethanol extract	$(NH_3)_2$	3.5	Low-Res ESI	Yes	Yes
3, insoluble solid	$(NH_3)_2$	3.6	Low-Res ESI	Yes	Yes
4, ethanol extract	$(NH_3)_2$	3.7	Low-Res ESI	Yes	Yes
5, ethanol extract	$(NH_3)_2$	3.8	Low-Res ESI	Yes	Yes
5, insoluble solid	$(NH_3)_2$	3.9	Low-Res ESI	Yes	Yes
6, insoluble solid	$(NH_3)_2$	3.10	Low-Res ESI	Yes	Yes
7, insoluble solid	$(NH_3)_2$	3.11	Low-Res ESI	No	Yes
8, insoluble solid	$(NH_3)_2$	3.12	Low-Res ESI	Yes	Yes
14, insoluble solid	$(NH_3)_2$	3.13	Low-Res ESI	No	No
10, ether extract	$(NH_3)_2$	3.14	Low-Res ESI	No	No
10, ethanol extract	$(NH_3)_2$	3.15	Low-Res ESI	No	No
12, crude product	DACH	3.16	Low-Res ESI	Yes	Yes
13, crude product	DACH	3.17	Low-Res ESI	Yes	Yes

The desired product was consistently found in the ethanol extract and the insoluble solid, implying that the desired product has some solubility in ethanol but not in ether or water. The majority of the mass was also found in the ethanol extract and insoluble solid. Percent yields (based on the assumption that the products were pure) were approximately 40% for the combined masses of the ethanol extract and insoluble solid.

Elemental analysis was also performed on many of the reaction extracts. A summary of the elemental analysis results is listed in Table 3.4. The desired product appears to be present in the ethanol extract and the insoluble solid but not in the ether extract. This finding is consistent with the MS analysis.

Table 3.4 Elemental analysis of reaction products:

Reaction Number	% C	% H	% N
Theoretical, A=$(NH_3)_2$	**44.21**	**7.76**	**4.69**
2, ether extract	73.79	11.39	0.76
2, insoluble solid	42.63	7.85	5.48
3, ethanol extract	43.90	7.85	6.55
3, insoluble solid	43.86	7.84	5.14
4, ethanol extract	44.39	8.13	5.54
5, ethanol extract	43.15	7.83	6.99
5, insoluble solid	41.85	7.76	6.07
14, insoluble solid	50.58	8.55	3.29
Theoretical, A=DACH	**49.62**	**8.03**	**4.13**
10, ether extract	52.06	9.14	5.91
10, ethanol extract	44.87	7.81	6.53
12, crude solid	46.15	8.21	7.51

High resolution mass spectrometry (HRESI) was used to confirm the identity of three example products. The insoluble solid isolated from reaction 2 and DACH a product from reaction 12 and reaction 13 were chosen as representatives. Table 3.5 summarizes the results. There is good agreement between each calculated and measured value of molecular weight.

Table 3.5 Summary of MS (HRESI) results

Reaction Number	Formula	Calculated MW (g/mol)	Measured MW (g/mol)
2, insoluble solid	$C_{22}H_{46}N_2O_4Pt$	598.3183	598.3182
12	$C_{28}H_{54}N_2O_4Pt$	677.3789	677.3779
13	$C_{28}H_{54}N_2O_4Pt$	677.3789	677.3768

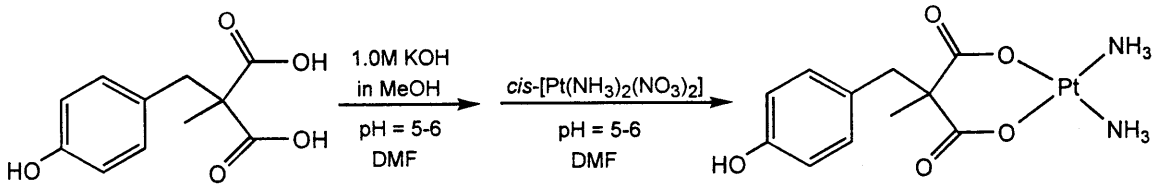
The product from reaction 12 was also analyzed by ^{195}Pt NMR (Figure 3.18). A single signal was observed at δ= -2,363 ppm. This chemical shift is in reference to the Na_2PtCl_6 signal. The observed chemical shift is consistent with published ^{195}Pt NMR spectra of DACH-platinum(II) malonates, which display chemical shifts in the -1840 to -1860 ppm range.[4] The published malonate

complexes were less lipophilic than compounds **3** and **4**.. Another contributing factor to the different observed chemical shift is the solvent. Published ^{195}Pt NMR spectra were obtained from deuterated DMSO solutions, whereas compounds **3** and **4** were analyzed as deuterated methanol solutions.

Taken together the results of analysis by mass spectrometry, microanalysis, and ^{195}Pt NMR are very strong evidence that the desired products, **3** and **4**, in fact formed. Exchange of nitrate for malonate was successful.

Laurate and 2-cyclobutanyl malonate were investigated as coordinating ligands. Both of these attempts were used as controls for method development. The laurate attempts were repetitions of a published method, in which nitrate was displaced from *cis*-$[Pt(NH_3)_2(NO_3)_2]$ and *cis*-$[PtDACH(NO_3)_2]$ by laurate in water as the reaction solvent.[12] The reported reaction conditions were stirring for two weeks in the dark at room temperature. No characterization data was reported. This method was not found to be repeatable with lauric acid or with 2-methyl, 2-octadecyl malonic acid. By contrast, laurate was successfully coordinated to the platinum diamine moiety in DMF as the reaction solvent. Similarly, the 2-cyclobutanyl malonic acid was used to determine whether the nonaqueous procedure formed the known compound carboplatin. According to TLC evidence this method successfully formed carboplatin.

Scheme 3.12 Exchange of Nitrate and 2-(4-Hydroxybenzyl)-2-methylmalonic Acid



The versatility of nitrate exchange was probed with a second malonate ligand with functional groups that are common in estrogens. Scheme 3.12 shows the exchange of the nitrates of *cis*-$[Pt(NH_3)_2(NO_3)_2]$ with 2-(4-hydroxybenzyl)-2-methylmalonic acid. This coordination is similar to the coordination of *cis*-$[Pt(NH_3)_2(NO_3)_2]$ with 2-methyl-2-octadecylmalonic acid. The solution of the 2-(4-hydroxybenzyl)-2-methylmalonic acid and DMF was adjusted to a pH of 5-6 with 1.0 M KOH in methanol. This base was the only one used in an attempt to simplify the purification, since KNO_3 is not soluble in alcohol. The reaction solution was protected from light and stirred overnight at room temperature.

The solvent was removed *in vacuo* affording the crude product, a tan oil with some fine dark solid (probably a silver impurity). The oil was diluted in a minimum amount of methanol and filtered through celite to remove the dark solid. Diethyl ether was added to the filtrate and a light colored precipitate formed. The precipitate was removed by vacuum filtration. During purification the precipitate was dissolved in methanol. This solution was not stable and always formed a blue/green color, suggesting oxidation or oligomerization of the crude product.

The light colored precipitate was analyzed by MS (LRESI) (Figure 3.19). The mass spectrum showed an M+46 signal at 100% intensity with an isotopic

pattern of a platinum species. The M+46 species is interpreted as a noncovalent complex between the desired product and ethanol, which is the solvent used for dissolving samples for analysis by mass spectrometry. The M+ signal was not observed.

Three significant conclusions come from the platination of the second malonate. (1) The phenol did not interfere with the coordination of the malonate to the platinum. (2) The previously determined reaction conditions were successful. Apparently the solvent, DMF, and leaving group, nitrate, are generally useful even with functionalized malonates. (3) More work will be required before applying this technique to the synthetic estrogen, due to the apparent instability of the final product.

C. Formation of other ammino platinum intermediates

Scheme 3.13 Formation of other Ammino Platinum Intermediates

(1) *cis*-$I_2Pt(NH_3)_2$ + Ag_2SO_4 $\xrightarrow{DMF}$ *cis*-$O_3SOPt(NH_3)_2$ + 2AgI

(2) *cis*-$I_2Pt(NH_3)_2$ + $2AgB(C_6F_5)_4$ $\xrightarrow{DMF}$ *cis*-$(B(C_6F_5)_4)_2Pt(NH_3)_2$ + 2AgI

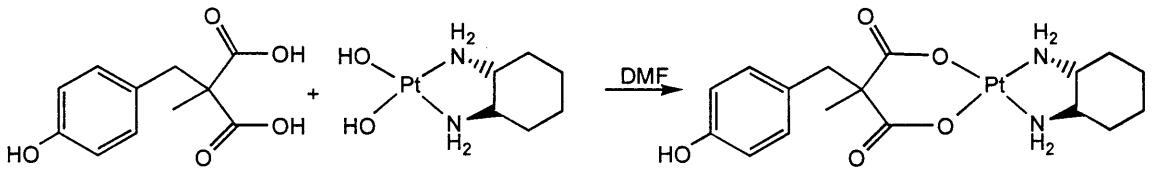
(3) *cis*-$I_2Pt(NH_3)_2$ + $2AgOSO_2CF_3$ $\xrightarrow{DMF}$ *cis*-$(F_3CO_2SO)_2Pt(NH_3)_2$ + 2AgI

Scheme 3.13 shows the formation of other amino platinum intermediates that were investigated. The sulfato platinum complex was investigated with no success in forming the complex with DMF as the reaction solvent. There was also no success in forming a BARF-platinum amine complex. The formation of cis-$[Pt(NH_3)_2(OTf)_2]$ was successful, and triflate was successfully displaced in the

coordination of the functionalized model to the diammino platinum. The final complex was unstable, but the triflate is not the likely cause of the instability.

D. Coordination of *cis*-[PtDACH] to 2-(4-hydroxybenzyl)-2-methylmalonic acid

Scheme 3.14 Coordination of *cis*-[PtDACH] to 2-(4-hydroxybenzyl)-2-methylmalonic acid.



Scheme 3.14 shows the reaction of *cis*-[$Pt(OH)_2$DACH] with 2-(4-hydroxybenzyl)-2-methyl malonate. The complex is formed by combining the starting materials in DMF and heating at 50^{0}C for five hours. The gentle heating results in a homogeneous pale yellow reaction solution. Dissolving in methanol and precipitation with diethyl ether isolated the final product. The final product was a tan solid.

The product was analyzed by ^{1}H NMR analysis (Figure 3.19) and MS (LRESI) (Figure 3.21). The ^{1}H NMR spectrum does not show a pure product. The ^{1}H NMR spectrum shows signals consistent with the desired product with the exception of the integration of the benzylic hydrogens. The DACH ^{1}H signals are present and consistent with published platinated DACH compounds.[15] The mass spectrum shows the M+1 signal and the M+46 signal with the platinum species isotopic pattern.

Chapter III

Section III: Conclusion

A successful nonaqueous procedure has been developed for coordinating a lipophilic compound, 2-methyl-2-octadecyl malonate to platinum. The method used includes (1) DMF as the solvent and (2) amino platinum nitrate as the active intermediate. The final crude product was a yellow/ brown solid that is slightly soluble in ethanol. Results of elemental analysis, mass spectrometry and ^{195}Pt NMR are consistent with the desired product. The product is produced in yields of approximately 40%, though this result does not represent completely purified products.

A second nonaqueous platination method was also successful for the coordination of a functionalized malonate to a platinum DACH moiety. This method used DMF as the solvent, but used a *cis*-dihydroxo(*trans*-1,2-diaminocyclohexane)platinum (II) intermediate. The ^{1}H NMR spectrum and mass spectrum are both consistent with the desired product. The nonaqueous platination method was also successful for the coordination of a functionalized malonic acid to a platinated diamine complex.

Chapter III

Section IV: Experimental

The oxaliplatin was used as supplied from Strem. The 1.0 M tetrabutyl ammonium hydroxide in methanol, cisplatin, carboplatin, lauric acid, potassium tetrachloroplatinate, 5.07 M (aq) ammonium hydroxide diluted to 2.54 M, acetone, 85% potassium hydroxide, (1R,2R)-(-)-1,2 diaminocyclohexane, deuterated methanol, silver sulfate and silver triflate were used as supplied by Aldrich. The absolute ethanol was used as supplied by AAPER. The silver nitrate was used as supplied from Acros. The dimethyl formamide was distilled from calcium hydride from various sources. Diethyl ether, potassium iodide and methanol were used as supplied by Fisher. The lithium BARF was used as supplied by Boulder Scientific. The *cis*-dihydroxo(*trans*-1,2-diaminocyclohexane) platinum(II) was prepared by the published procedure.[16] Mass spectra were obtained with either Fast Atom Bombardment (FAB) or Electrospray Ionization (ESI) on a Quattro instrument at the School of Chemical Sciences, University of Illinois at Urbana-Champaign. The ^{195}Pt NMR spectrum was obtained on a Varian Unity Inova 600 MHz at the School of Chemical Sciences, University of Illinois at Urbana-Champaign. Elemental analyses were conducted by the Microanalytical Laboratory at the School of Chemical Sciences, University of Illinois at Urbana-Champaign.

Attempted substitution oxalate within oxaliplatin A stock solution of 2-methyl-2-octadecylmalonic acid (0.165g, 0.222 mmol), absolute ethanol (1 mL), and 1.0 M nBu_4NOH (0.2 mL, 0.1 mmol) was combined in a 5 mL round bottom flask. The pH of this solution was 5, and half of this solution was used for the reaction. Oxaliplatin (0.0888 g, 0.224 mmol) was added to the reaction flask. Additional absolute ethanol (2 mL) was used in an attempt to dissolve the oxaliplatin. The oxaliplatin did not dissolve. The reaction mixture was stirred at room temperature for one hour. Reaction progress was monitored with reverse phase TLC in methanol with I_2 visualization. The R_f's were 0.83 (oxaliplatin), and 0.63 (2-methyl- 2-octadecylmalonic acid and tetrabutyl ammonium salt), indicating only the presence of starting materials. The reaction was then heated at reflux for three hours. The TLC was repeated with the same conditions and showed no change. The reaction sat overnight and the solid settled to the bottom. According to TLC evidence the solid was oxaliplatin, and the supernatant was a solution of 2-methyl-2-octadecylmalonic acid/ tetrabutyl ammonium salt. This mixture was transferred to a 25 mL round bottom flask. Since no reaction had occurred, the remaining stock solution of 2-methyl-2-octadecylmalonic acid/ tetrabutyl ammonium salt was added to the reaction flask. The reaction was heated at reflux for an additional 5 hours. TLC analysis continued to show no reaction progress.

Substitution of nitrate in platinum amine complexes. The nitration of the platinum compound was carried out under dim light. The platinum starting

material with the general formula of *cis*-[PtA_2X_2] (0.23 mmol) was dissolved in the solvent (0.5 mL). Silver nitrate (1.95-1.98 equiv), a stir bar and solvent (0.5 mL) were combined in a 5 mL round bottom flask. The platinum solution was added drop wise and the solution became yellow. The reaction flask was wrapped in aluminum foil and heated at 50 °C for 50 minutes. A precipitate (presumably AgX) formed and was removed by vacuum filtration. The filtrate was transferred to a 10 mL round bottom flask. This solution was assumed to contain the nitrated platinum ammine, *cis*-$(NO_3)_2Pt(NH_3)_2$ or *cis*-$(NO_3)_2Pt$(DACH).

Ligand exchange of nitrated platinum compounds with 2-methyl-2-octadecylmalonic acid A malonate salt solution was made by dissolving 1-2 equivalents of the 2-methyl-2-octadecylmalonic acid in 1.0 mL of solvent and adjusting the pH to 5-6 with base. The malonate salt solution was added drop wise to the solution of *cis*-$(NO_3)_2Pt(NH_3)_2$ or *cis*-$(NO_3)_2Pt$(DACH). The initial reaction solution was homogeneous. The reaction flask was wrapped in foil and stirred overnight. Reverse phase TLC was then performed with methanol as the solvent with iodine stain. The only new spot that had formed was at R_f of 0.0. Some reactions had spots that correlated with the malonate salt.

The reaction solvent was removed *in vacuo*. A brown oil or solid remained as the crude product. Deionized water (10 mL) was added to the reaction flask. Any remaining oil solidified and was isolated by vacuum filtration. The solid was extracted with diethyl ether (10 mL), and the mixture was vacuum filtered. Any solid that remained was then extracted with absolute ethanol, and the mixture

was vacuum filtered. Any solid that remained after the extractions was dried *in vacuo*.

Ligand exchange of nitrated platinum diamine compounds with 2-(4-hydroxbenzyl)-2-methylmalonic acid A malonate salt solution was formed by dissolving one equivalent of 2-(4-hydroxybenzyl)-2-methylmalonic acid in DMF and adjusting the pH to 5-6 with 1 M KOH in methanol. This solution was added to *cis*-$(NO_3)_2Pt(NH_3)_2$ dissolved in DMF. The initial reaction solution was homogeneous. The reaction flask was wrapped in foil and stirred overnight. The reaction solvent was removed *in vacuo*. A thick, brown oil and sometimes a fine gray solid remained as the crude product. The oil was diluted with a minimum amount of methanol, and the dark solid was removed by vacuum filtration through celite. Diethyl ether was added to the filtrate, and a light colored precipitate formed. The precipitate was removed by vacuum filtration. The final product was analyzed by MS (LRESI) (m/z) (rel. intensity): 497.0 (M+46, 100%), Figure 3.19. The precipitate was dissolved in methanol to allow TLC analysis required for identifying a chromatography solvent. The color of the solution changed from a yellow to a blue/ green in a short amount of time under dim light conditions.

Coordination of *cis*-[PtDACH] to 2-(4-hydroxybenzyl)-2-methylmalonic acid *Cis*-dihydroxo(*trans*-1,2-diaminocyclohexane) platinum(II) (0.2 mmol) was suspended in DMF (1mL) in a 25 mL flask. A solution of 2-(4-hydroxybenzyl)-2-

methylmalonic acid (0.2 mmol) and DMF (12 mL) was added to the reaction flask. The flask was wrapped in aluminum foil. The reaction mixture was heated at 50 °C for five hours. After heating the reaction solution was a homogeneous pale yellow. The solvent was removed *in vacuo*, affording a tan solid. The solid was dissolved in methanol, and diethyl ether was added. A light tan precipitate formed and was removed by vacuum filtration. The crude product yield was approximately 40%.

^{1}H NMR, (CD_3OD) δ: 7.031 (m, 2 H), 6.619 (m, 2H), 3.047 (s, 2H), 2.474 (m, 2H), 2.019 (m, 2H), 1.589 (m, 2H), 1.282 (s, 2H), 1.205 (m, 4H) Figure 3.20. MS (LRESI) (m/z) (rel. intensity): 532.1 (M+1, 36%), 577.1 (M+46, 100%), Figure 3.21.

Reference:

1. Dhara, S.C. *Indian J. Chem.* **1970**, *8*, 193.
2. Harrison, R.C.; McAuliffe, C.A.; Zaki, A.M. *Inorg. Chim. Acta*, **1980**, *46*, L15.
3. Rochon, F.R.; Gruia, L.M. *Inorg. Chim. Acta*, **2000**, *306*, 193.
4. Young, A.L.; Chung, Y.K.; Sohn, Y.S. *J. Inorg. Biochem.*, **1997**, *68*, 289.
5. Pascini, A.; Caldirola, C.; Spinelli, S.; Valsecchi, M. *Synth. React. Inorg. Met.-Org. Chem.* **1993**, *6*, 1021.
6. Shwartz, P.; Meischen, S.J.; Gale, G.R.; Atkins, L.M.; Smith, A.B.; Walker Jr, E.M. *Cancer Treatment*, **1977**, *61*, 1519.
7. Pascini, A.; Caldirola, C. *Inorg. Chim. Acta* **1988**, *151*, 19.

8. Wimmer, S.; Castan, P.; Wimmer, F.L.; Johnson, N.P. *J. Chem. Soc. Dalton Trans.* **1989**, 403.

9. Appleton, T.G.; Berry, R.D.; Davis, C.A.; Hall, J.R.; Kimlin, H.A. *Inorg. Chem.*, **1984**, *23*, 3514.

10. Raudaschl, G.; Lippert, B.; Hoeschele, J.D.; Howard-Lock, H.E.; Lock, C.J.; Pilon, P. *Inorg. Chim. Acta*, **1985**, *106*, 141.

11. Souchard, J.P.; Wimmer, F.L.; Ha, T.T.B.; Johnson, N.P. *J. Chem. Soc. Dalton Trans.* **1990**, 307.

12. Maeda, M.; Uchida, N.A.; Sasaki, T. *Jpn. J. Cancer Res.*, **1986**, 532.

13. Anson, C.E.; Sheppard, N.; Powell, D.B.; Norton, J.R.; Fischer, W.; Keiter, R.L.; Johnson, B.F.G.; Lewis, J.; Bhattacharrya, A.K.; Knox, S.A.R.; Turner, M.L. *J. Am. Chem. Soc.* **1994**, *116*, 3058.

14. Mukaiyama, T.; Maeshima, H.; Jona, H. *Chem. Soc. Jpn.*, **2001**, 388.

15. Hoeschele, J.D.; Farrell, N.; Turner, W.R.; Rithner, C.D. *Inorg. Chem.* **1988**, *27*, 4106.

16. Gill, D.S.; Rosenberg, B. *J. Am. Chem. Soc.* **1982**, *104*, 4598.

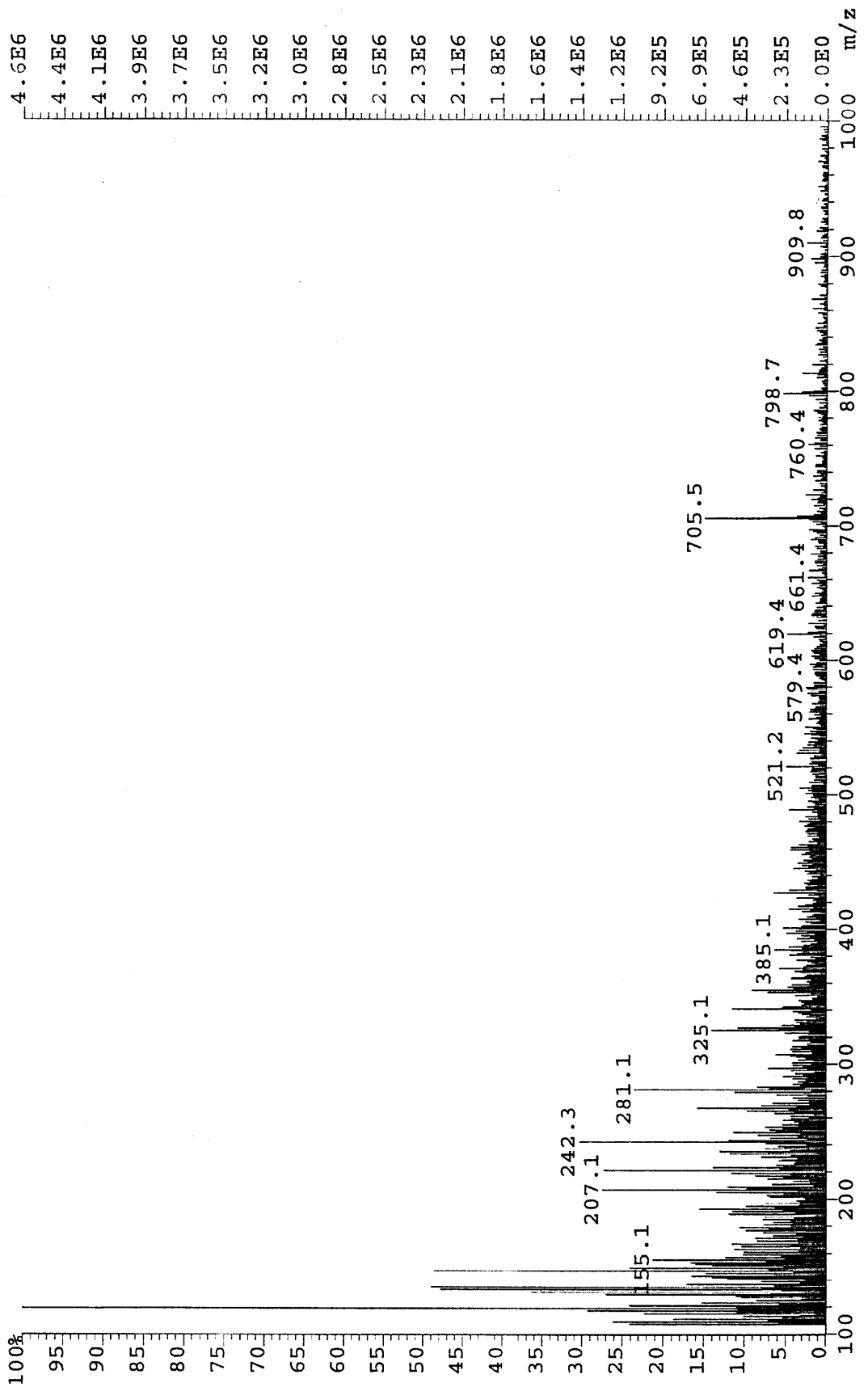


Figure 3.2 MS (LRFAB) of Reaction 2, Ether Extract

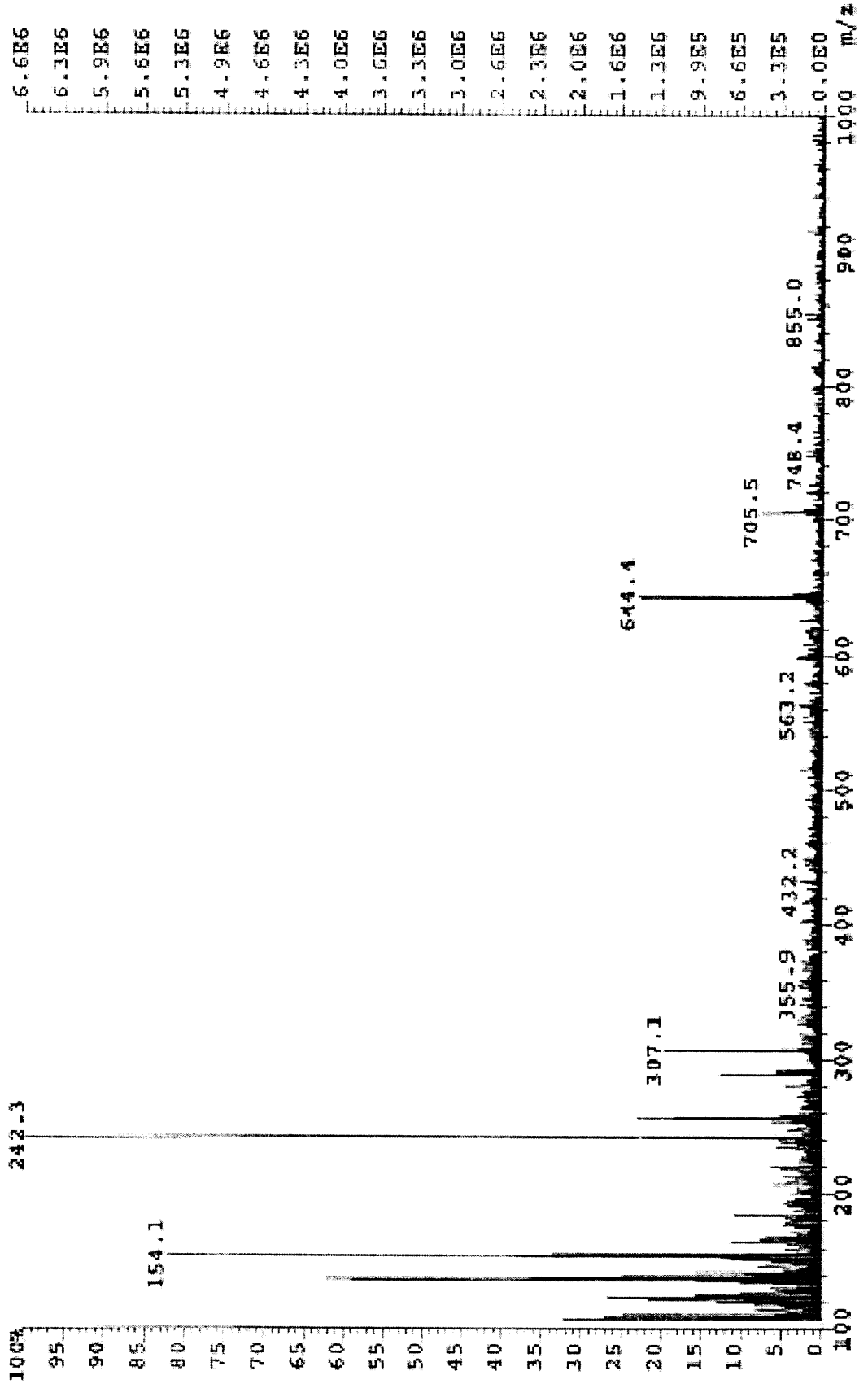


Figure 3.3 MS (LRFAB) of Reaction 2, Ethanol Extract

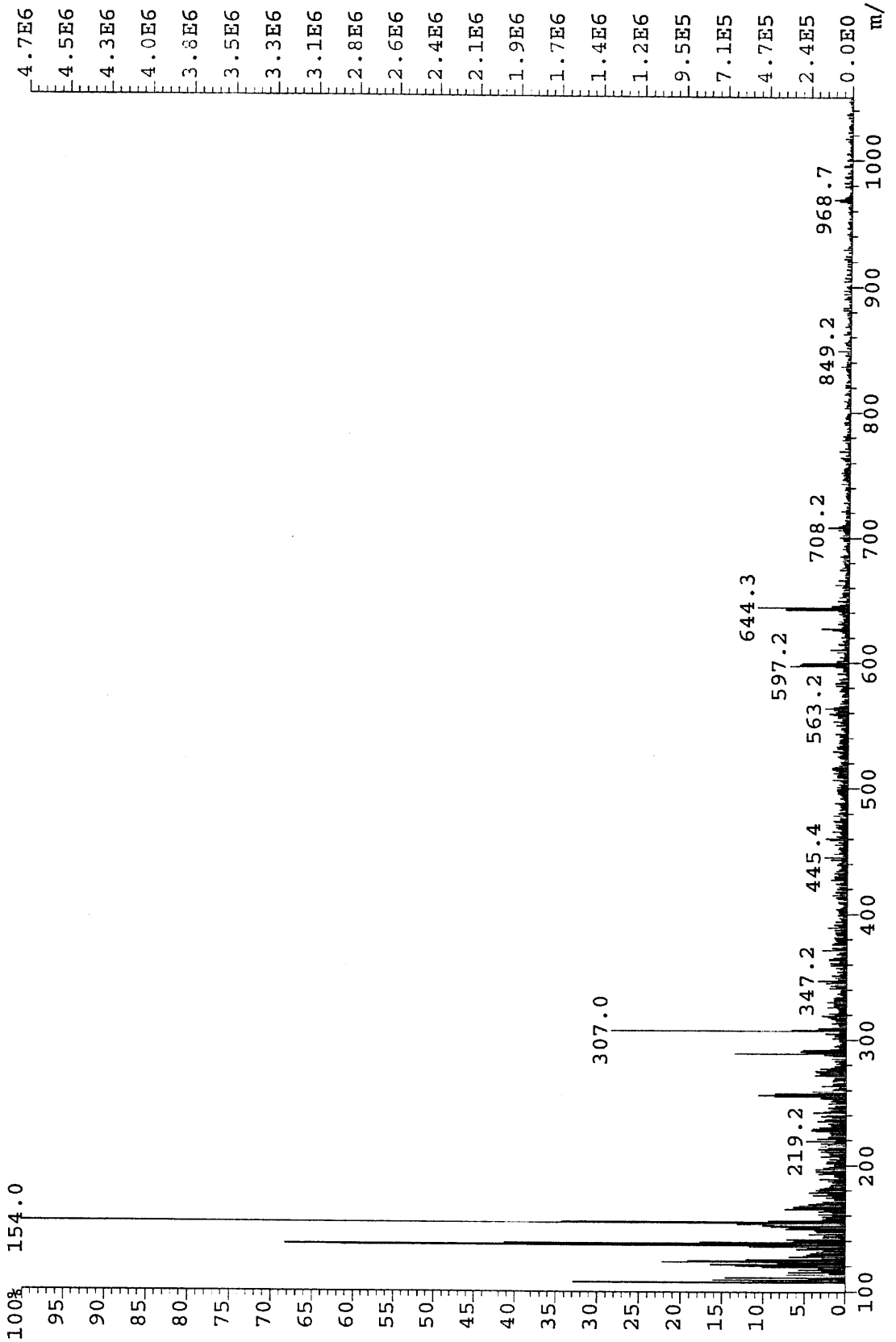


Figure 3.4 MS (LRFAB) of Reaction 2, Insoluble Solid

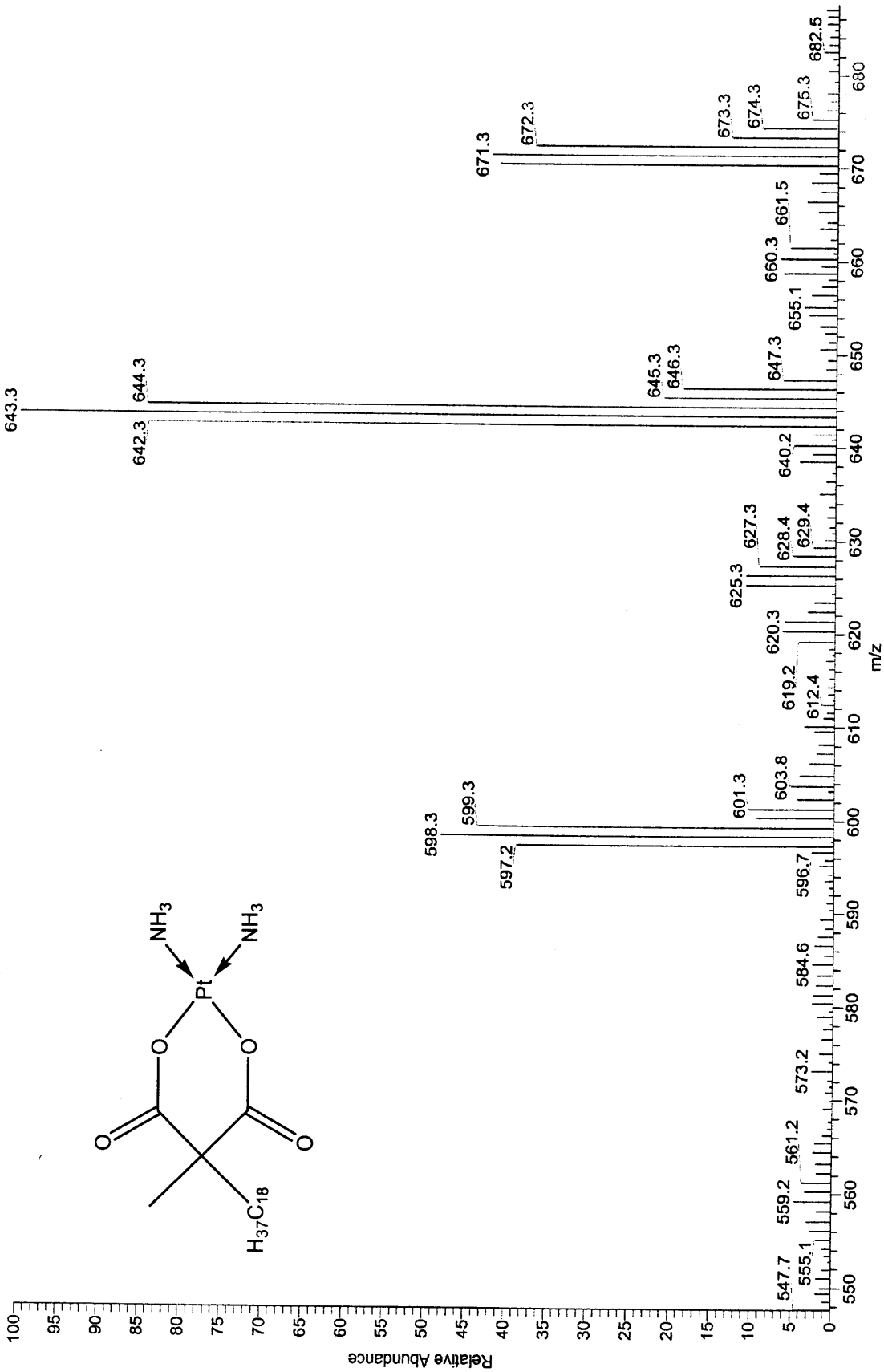


Figure 3.5 MS (LRESI) of Reaction 3, Ethanol Extract

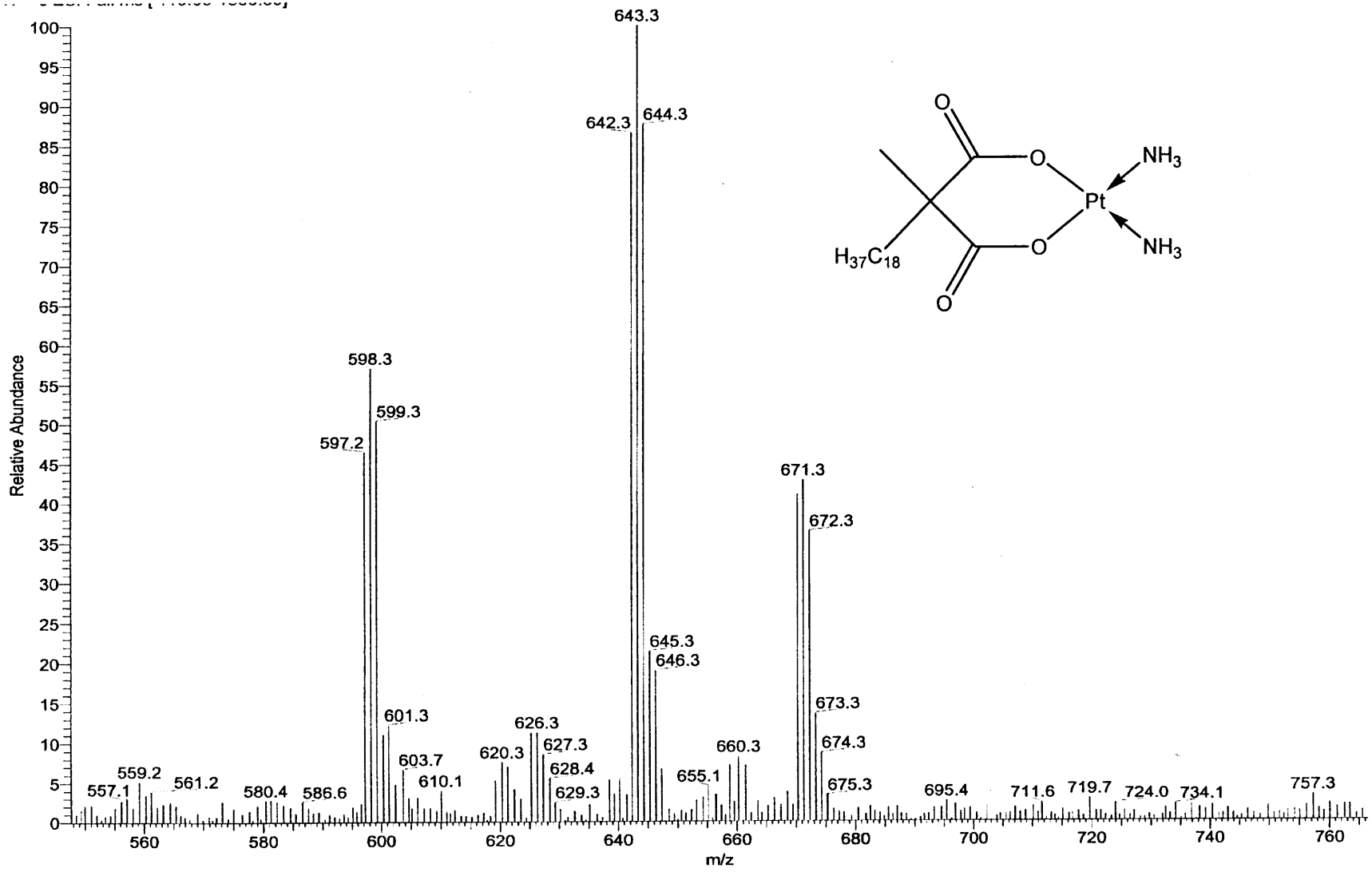


Figure 3.6 MS (LRESI) of Reaction 3, Insoluble Solid

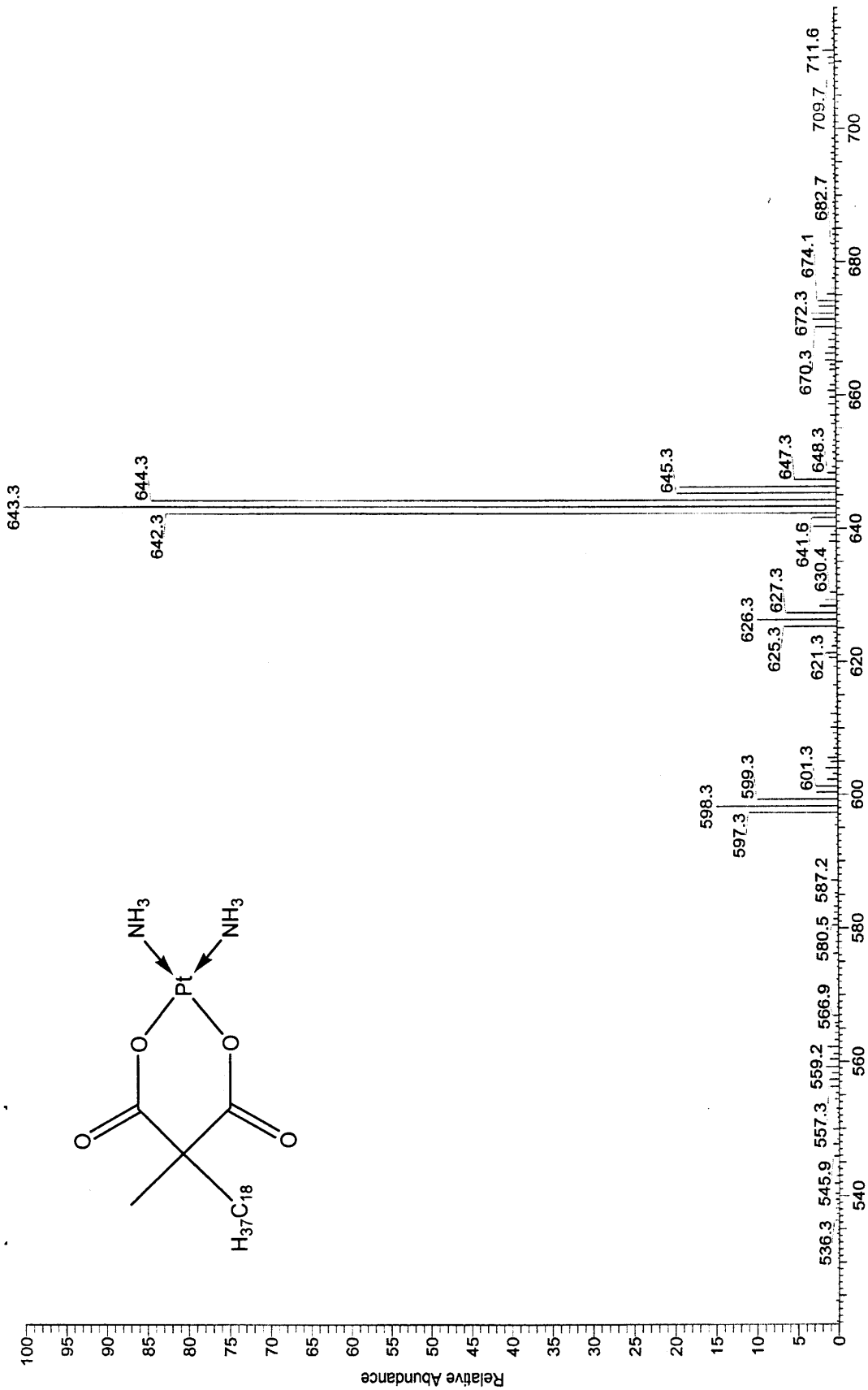


Figure 3.7 MS (LRESI) of Reaction 4, Ethanol Extract

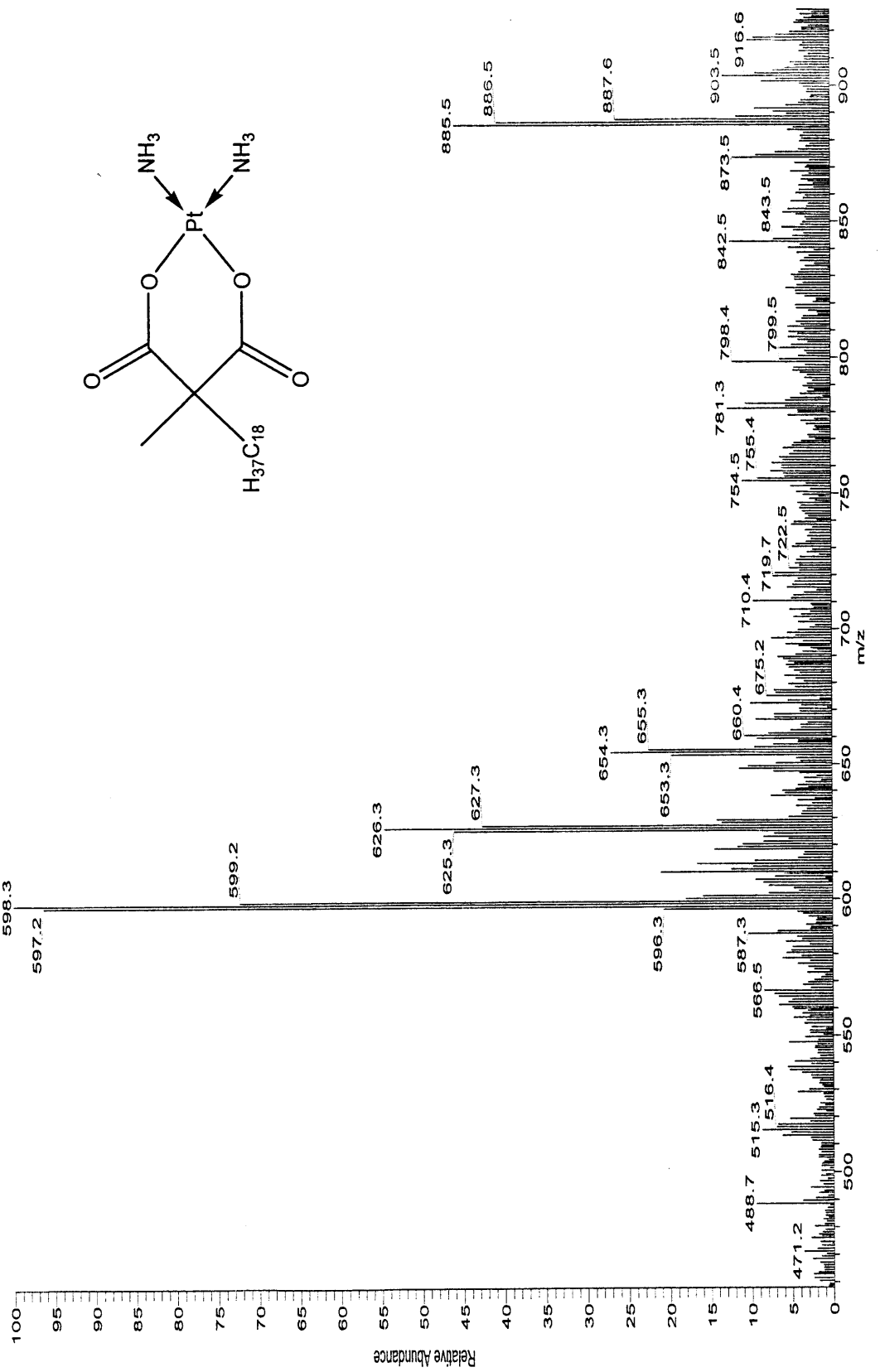


Figure 3.8 MS (LRESI) of Reaction 5, Ethanol Extract

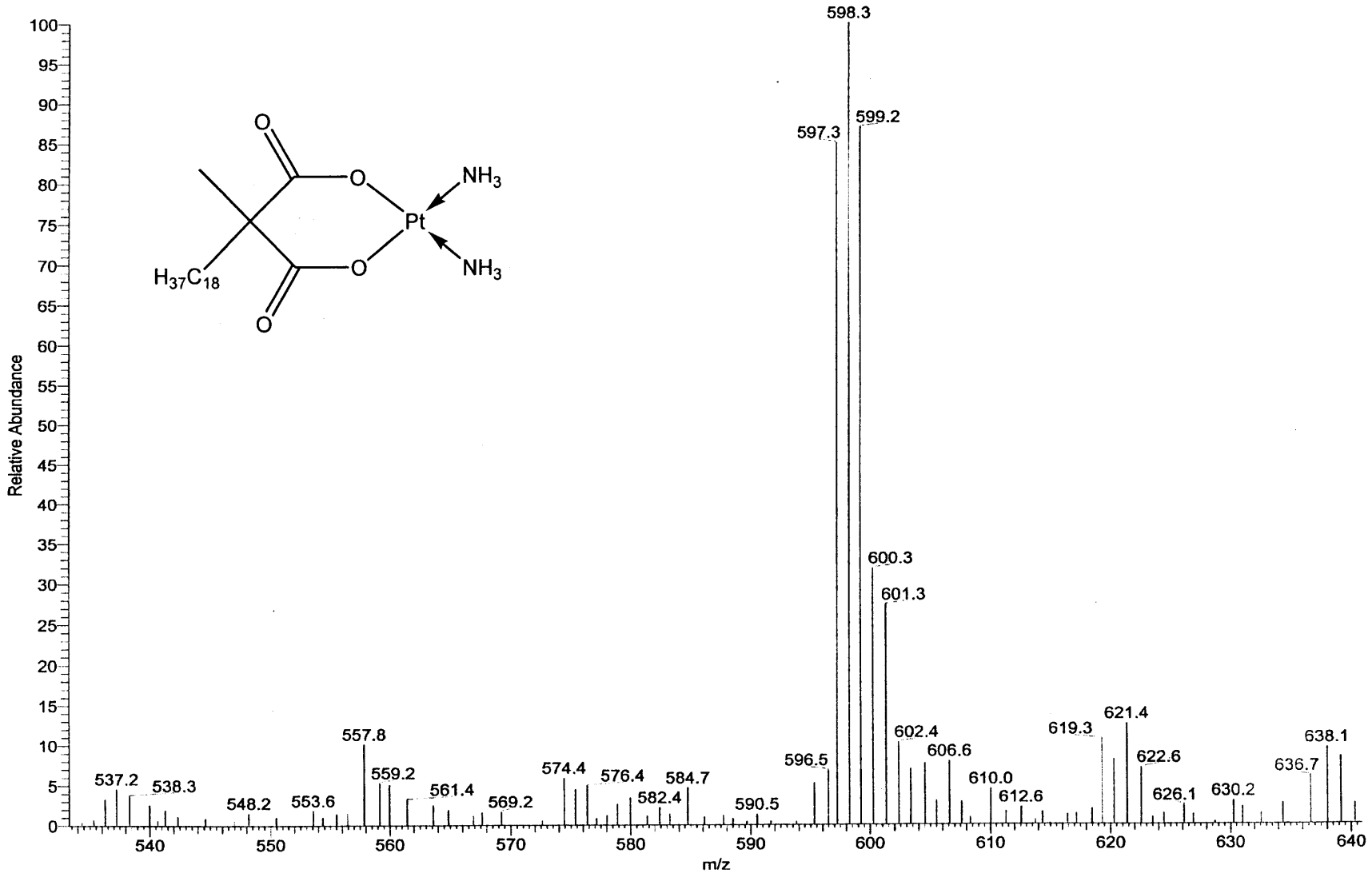


Figure 3.9 MS (LRESI) of Reaction 5, Insoluble Solid

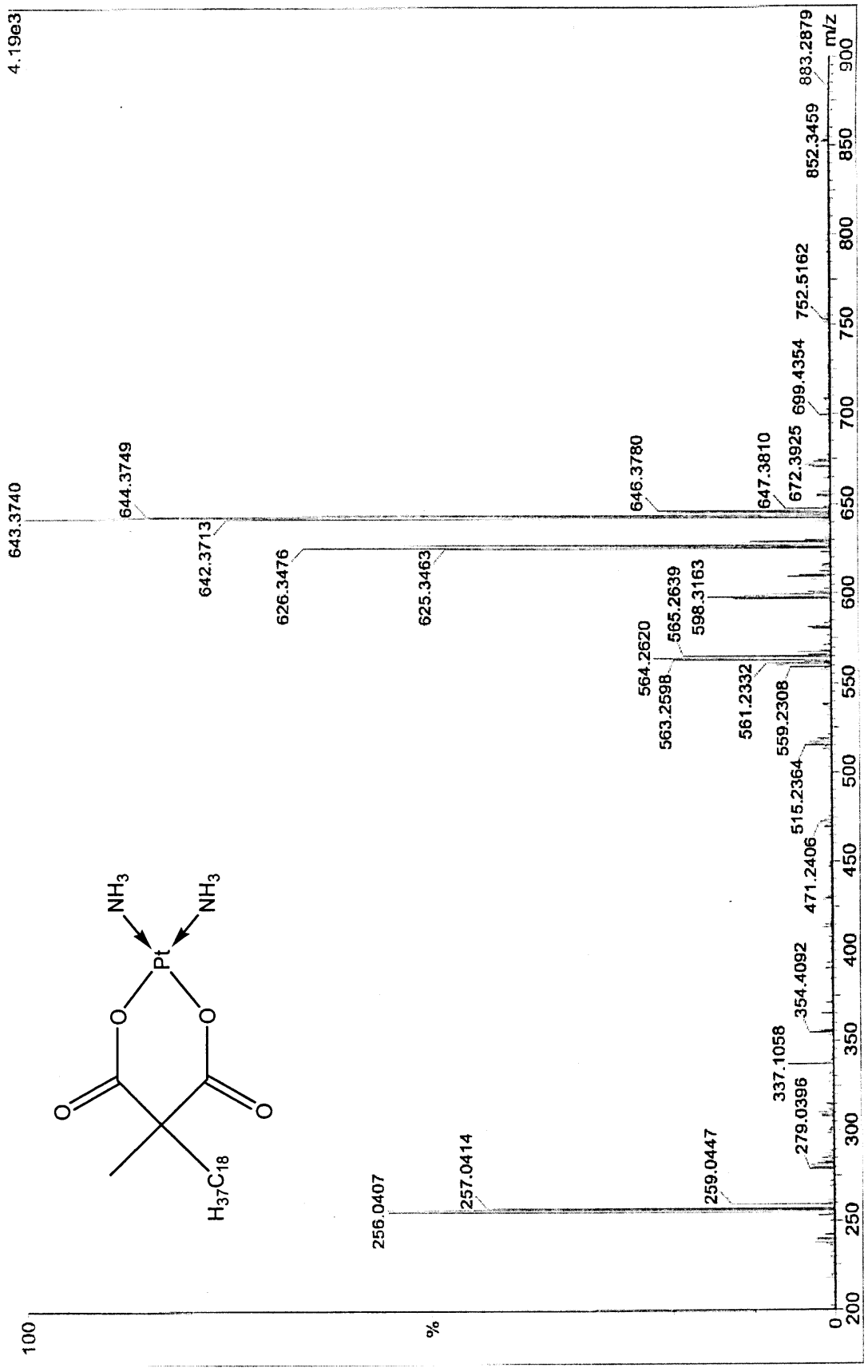


Figure 3.10 MS (LRESI) of Reaction 6, Insoluble Solid

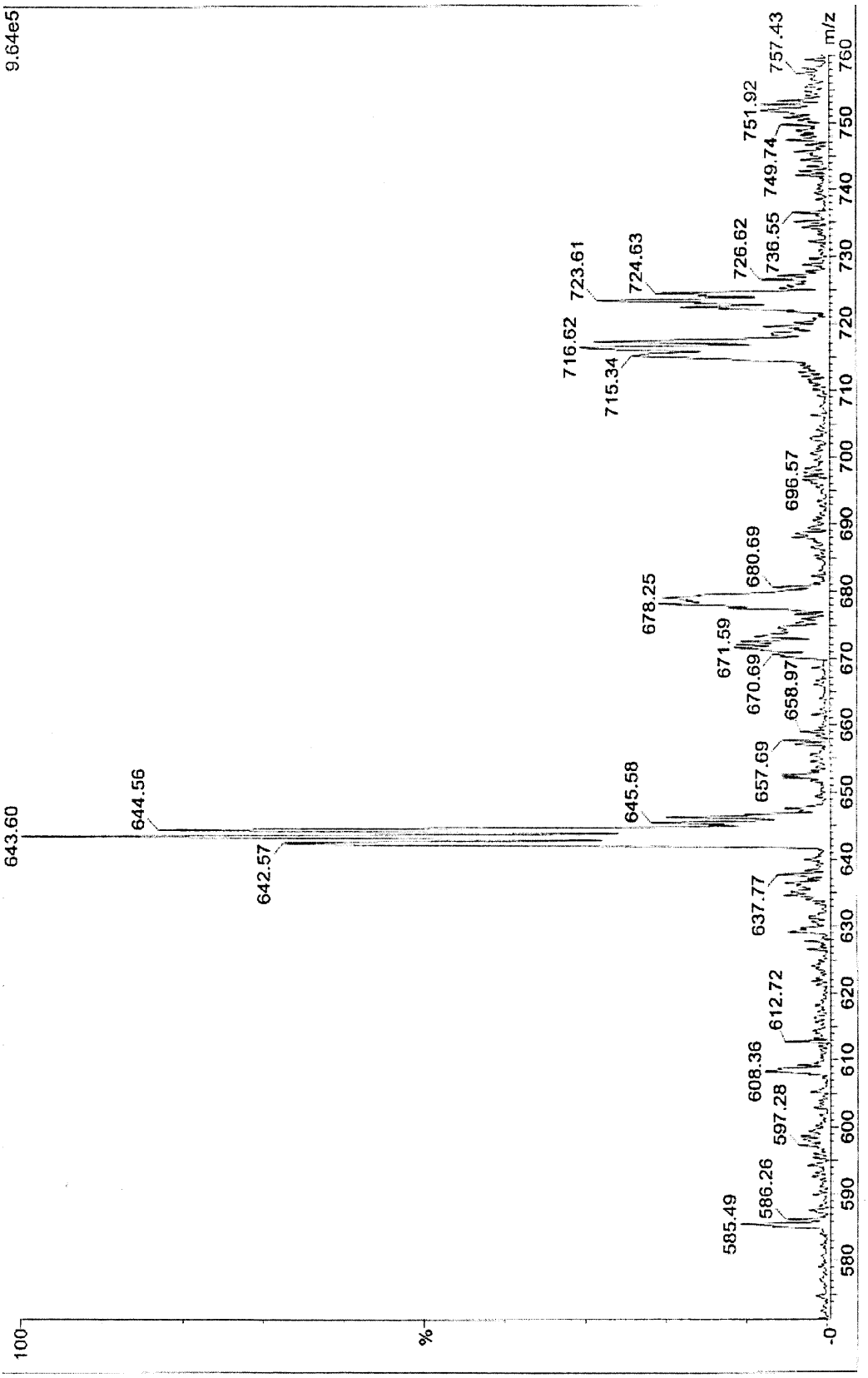


Figure 3.11 MS (LRESI) of Reaction 7, Insoluble Solid

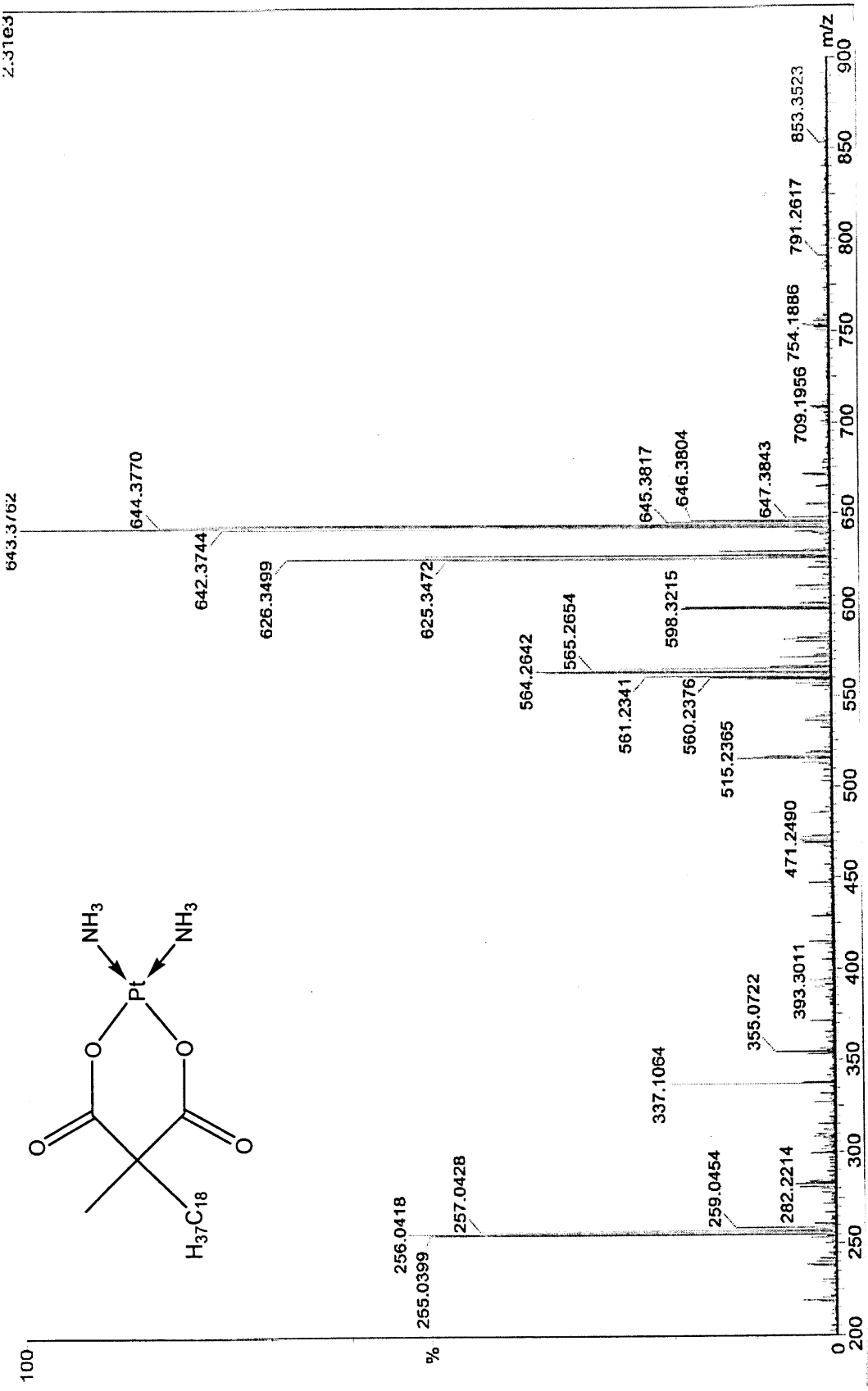


Figure 3.12 MS (LRESI) of Reaction 8, Insoluble Solid

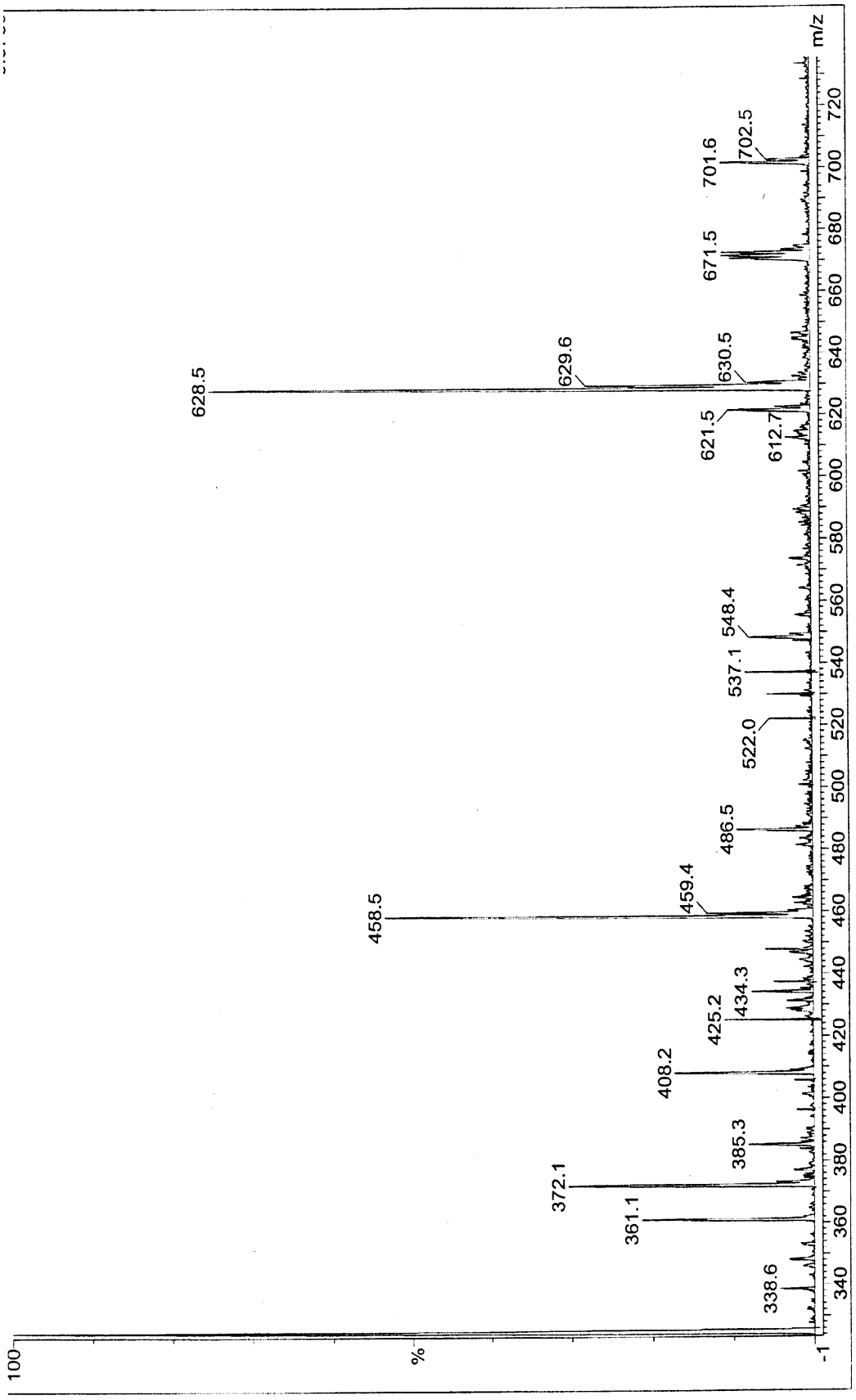


Figure 3.13 MS (LRESI) of Reaction 14, Insoluble Solid

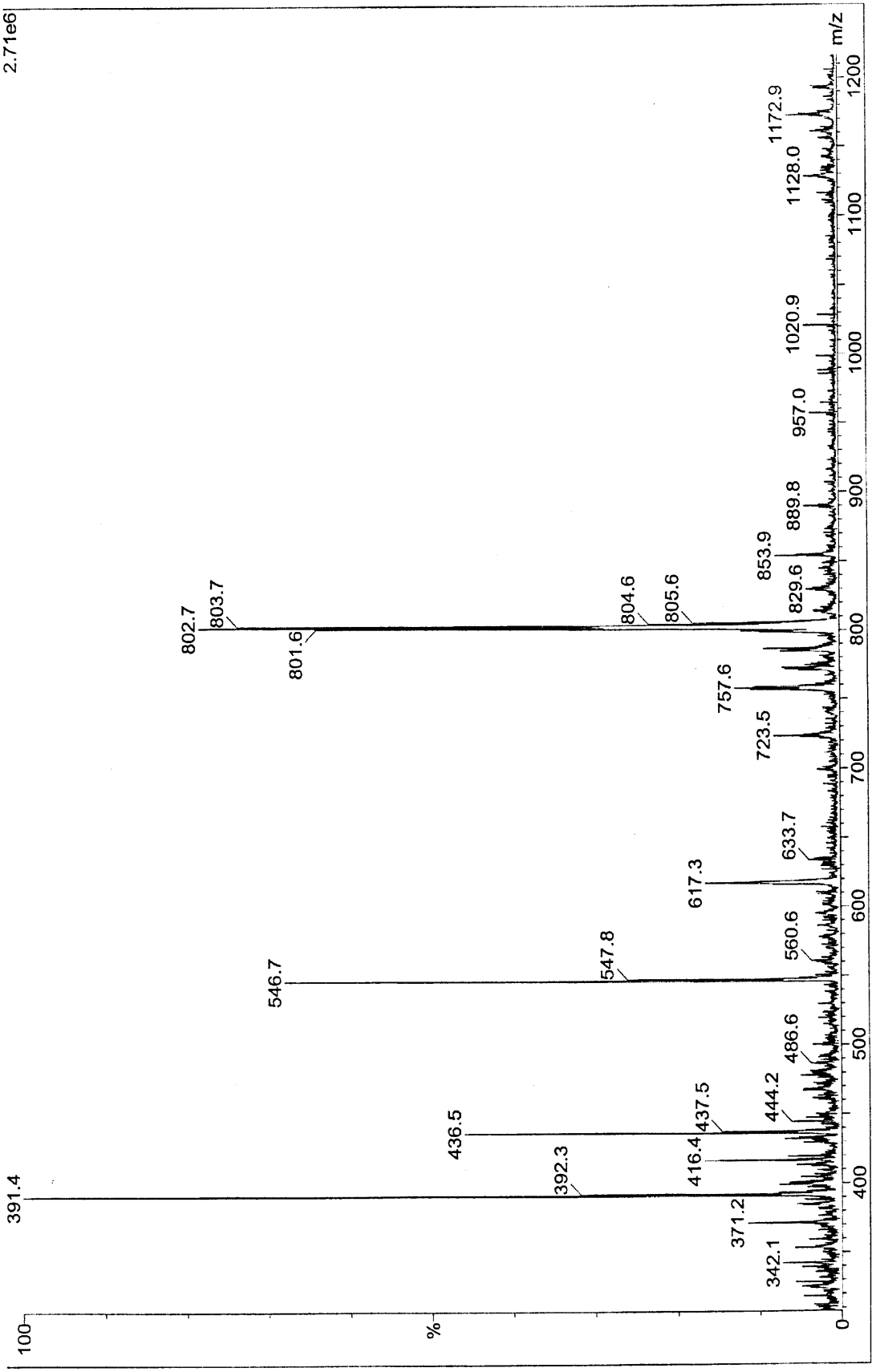


Figure 3.14 MS (LRESI) of Reaction 10, Ether Extract

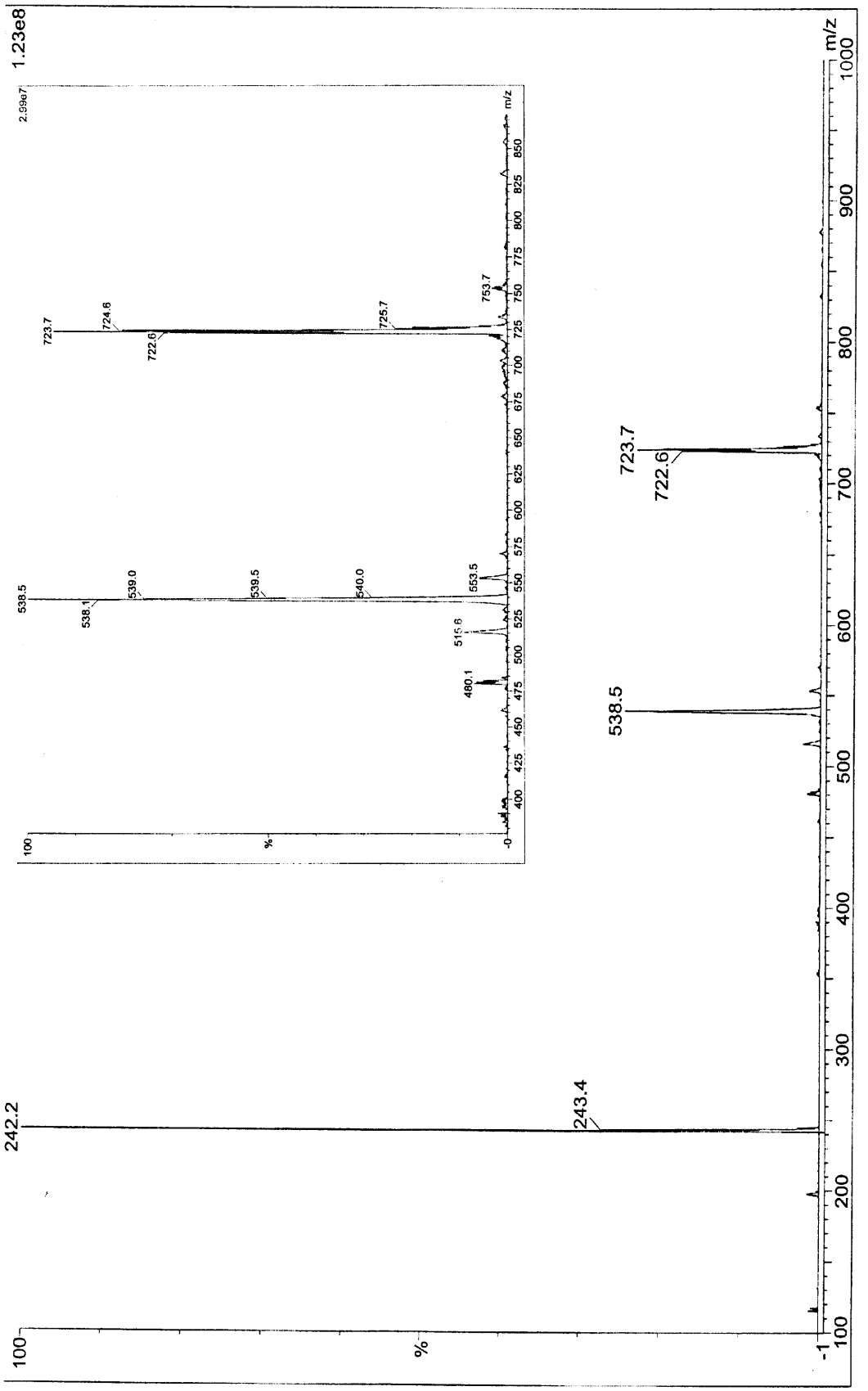


Figure 3.15 MS (LRESI) of Reaction 10, Ethanol Extract

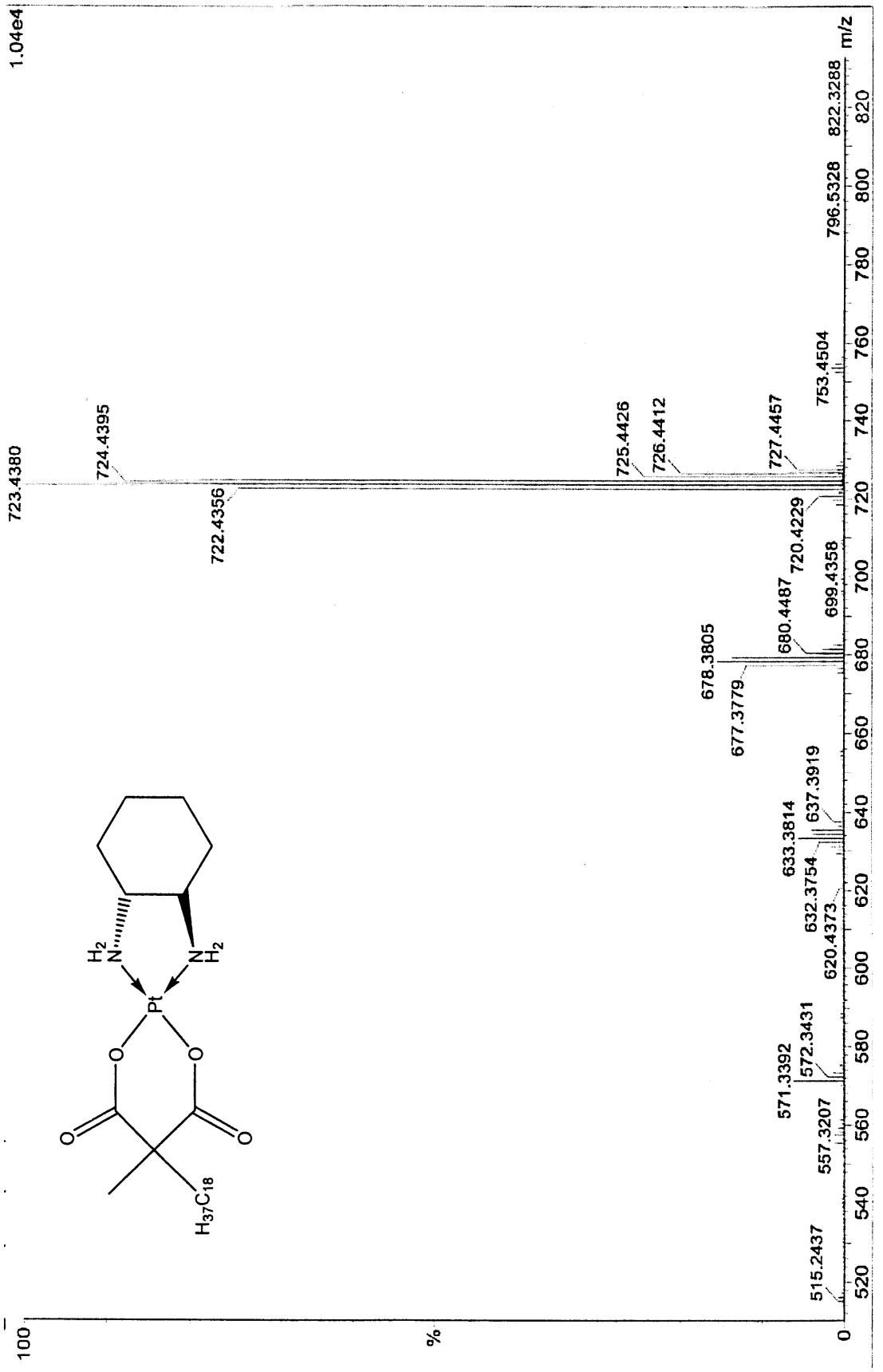


Figure 3.16 MS (LRESI) of Reaction 12, Crude Product

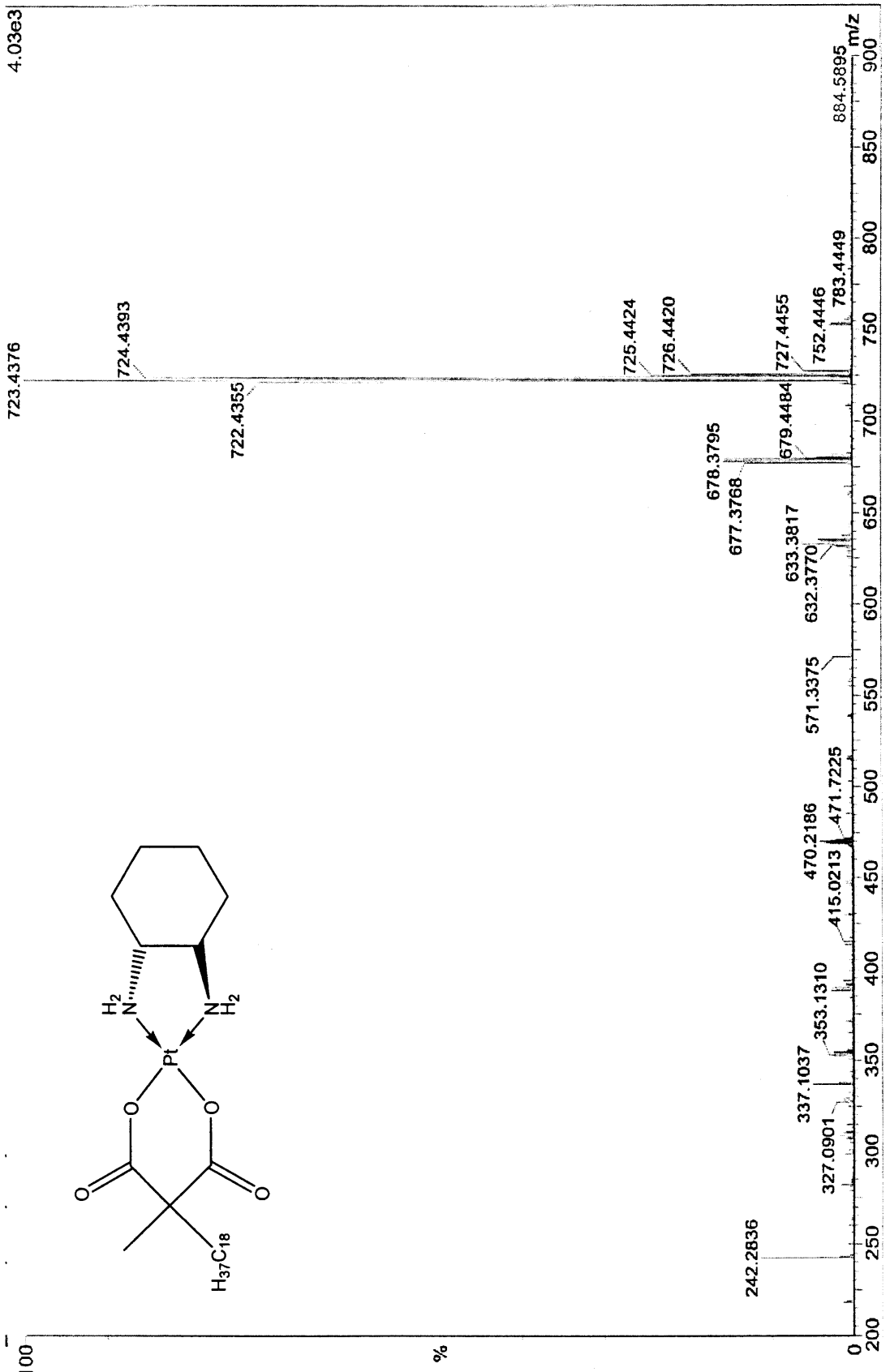


Figure 3.17 MS (LRESI) of Reaction 13, Crude Product

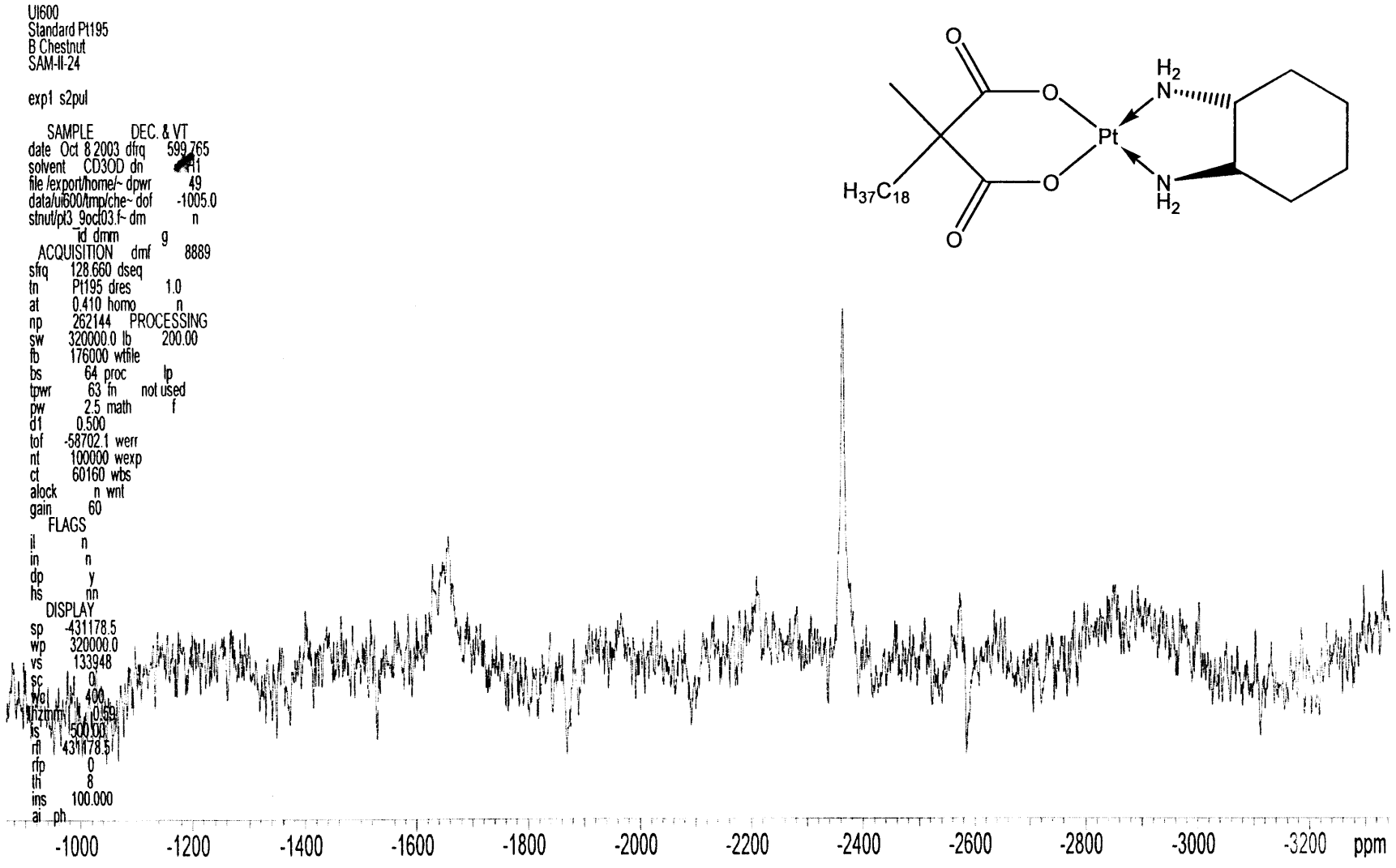


Figure 3.18 ^{195}Pt NMR Spectrum of Reaction 12, Crude Product

Figure 3.19 MS (LRESI) Spectrum of 2-(4-Hydroxybenzyl)-2-methyl Malonate Coordinated to Platinum Diamine

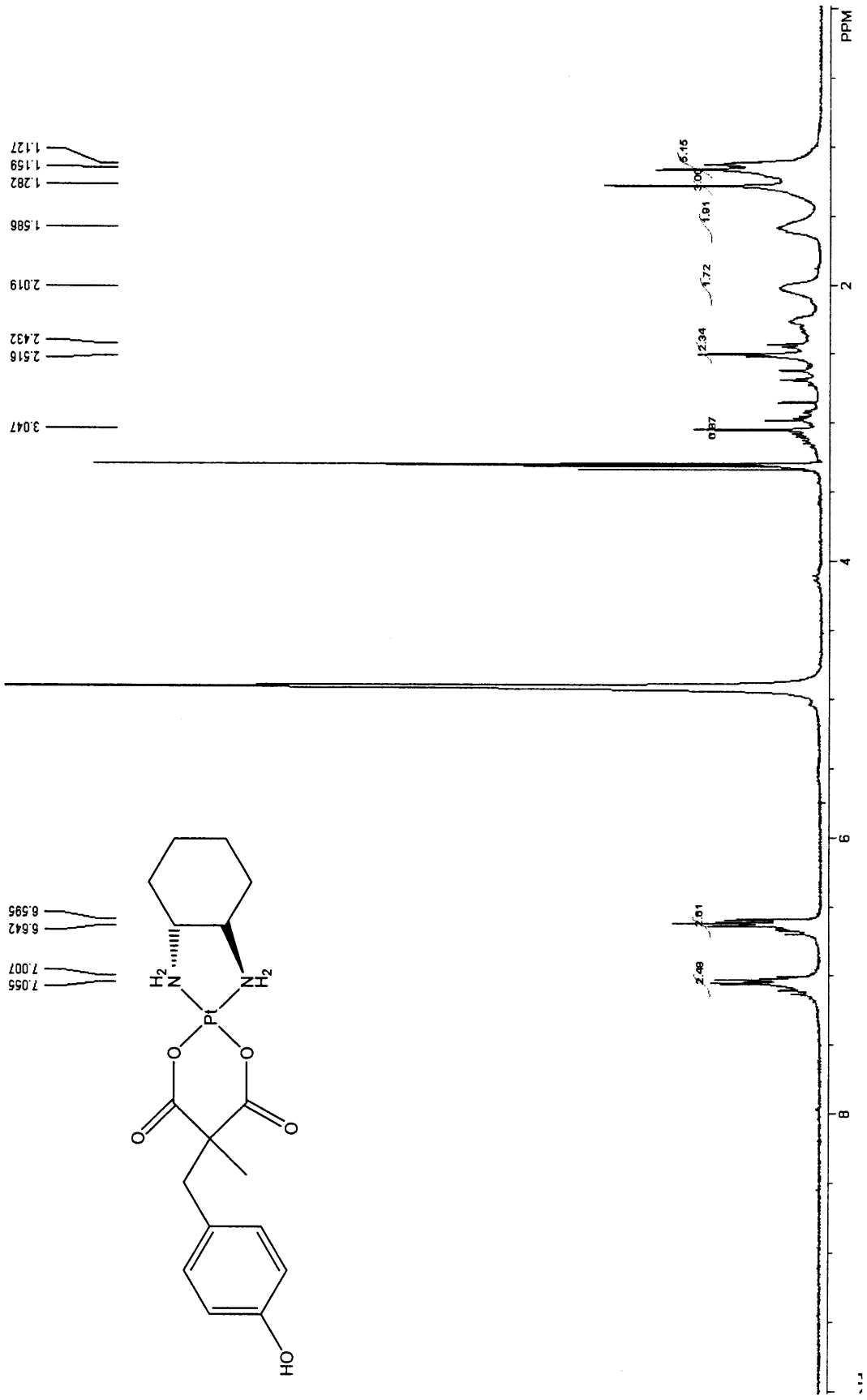


Figure 3.20 ^{1}H NMR Spectrum of 2-(4-Hydroxybenzyl)-2-methyl Malonate Coordinated to Platinum DACH

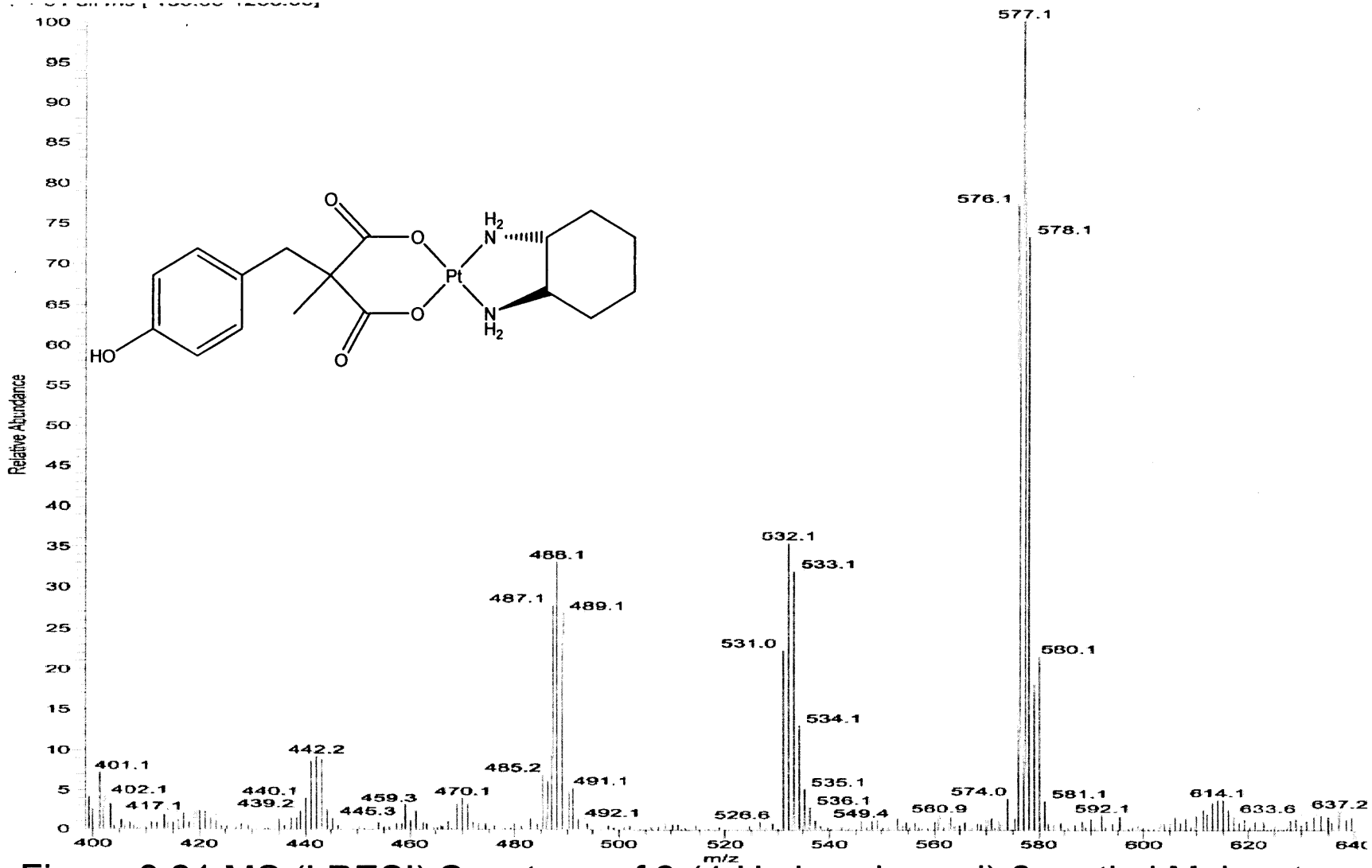


Figure 3.21 MS (LRESI) Spectrum of 2-(4-Hydroxybenzyl)-2-methyl Malonate Coordinated to Platinum DACH

Chapter IV

7α-Estradiol-Malonate Conjugates: Synthesis and Affinity for the Estrogen Receptor

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Abstract

We have prepared two Estradiol derivatives that are conjugated through the 7α position to malonate substituents. One compound, **4a**, contains a dimethyl(decyl)(methyl)malonate substituent, while the second compound, **5**, contains a (decyl)(methyl)malonic acid substituent. Both compounds have significant affinity for purified α and β isoforms of the estrogen receptor (ER). The receptor binding affinity (RBA) values of **4a** are 89.1(α) and 70.9(β). The RBA values of **5** are 20.7(α) and 46.3(β). The lower affinity of **5** can be explained by its free carboxylic acid groups, which allow nonspecific interactions with basic residues in ER. In whole cytosol preparations, the RBA of **4a** is 86.2, whereas

the RBA of **5** is only 2.1 This large difference is also attributable to nonselective interactions between cytosol proteins and the free carboxylic acid groups of **5**. Compound **5** may be a viable platform for delivering transition metals of diagnostic or therapeutic value to ER+ breast tumor cells.

1. Introduction

The estrogen receptor (ER) is a natural target for selective delivery of diagnostic or therapeutic compounds to ER+ breast tumor cells. One such category of compounds exploits the fact that ER retains high affinity for estradiol derivatives with large substituents at the 7α position. Substituents that have been attached to this position via hydrocarbon linkage include a wide variety of functional groups, some of which contain transition metals. [2-10]

We have recently begun exploration of 7α-substituted estradiol compounds in which the substituent includes malonate functionality—both as the methyl ester and the free acid. Ultimately, the new (deprotonated) malonate conjugate is designed to coordinate to metals that may be of diagnostic or therapeutic significance in breast cancer. We report here the synthesis of these compounds and their affinity for ER.

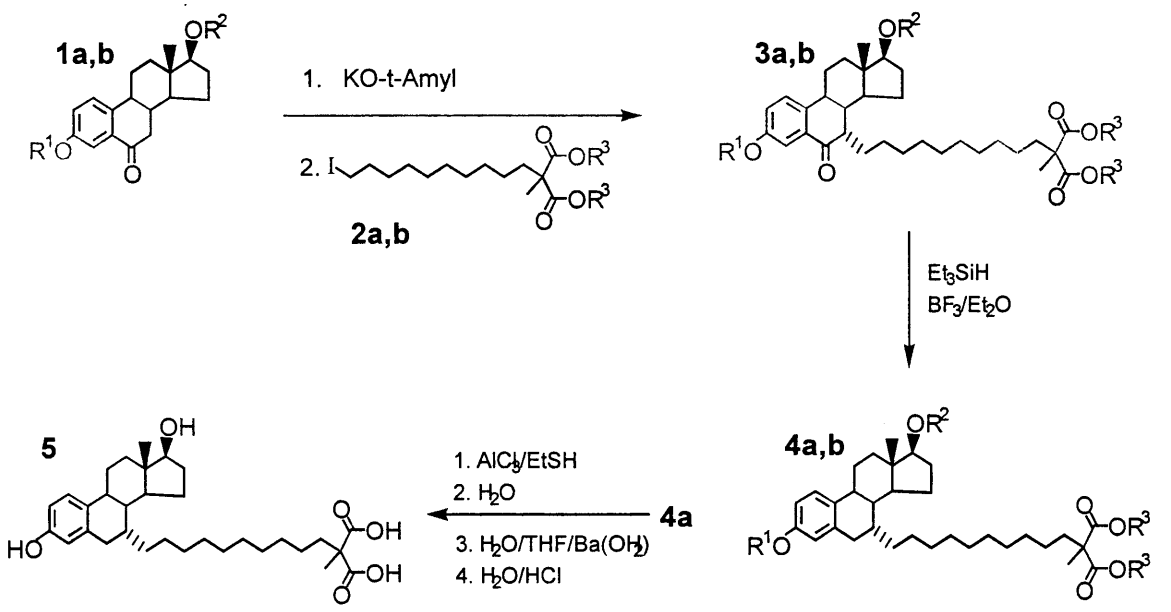
2. Experimental

All synthetic operations were carried out under Argon. Published procedures were used to prepare **1a**, **1b**,[11] **2a**, **2b**,[12] and KO-t-amyl.[11] Dimethoxyethane (DME) was purchased from Aldrich or Fisher Scientific and distilled from sodium benzophenone. Dichloromethane was purchased from Fisher Scientific and distilled from CaH_2. Triethylsilane, boron trifluoride diethyl

etherate, aluminum tribromide, and ethanethiol were purchased from Aldrich and used as received. Silica GF from Analtech was used as the solid for all TLC; staining was done with phosphomolybdic acid. Flash chromatography was conducted with silica gel, Merck grade 9385, 230 – 400 mesh, 40Å. NMR spectra were collected on a QE 300 instrument. Mass spectra were obtained with Fast Atom Bombardment (FAB) ionization on a VSE-70 instrument, or with Electrospray Ionization (ESI) on a Quattro instrument at the School of Chemical Sciences, University of Illinois at Urbana-Champaign. Elemental analyses were conducted by the Microanalytical Laboratory at the School of Chemical Sciences, University of Illinois at Urbana-Champaign. RBA values were measured by Cathryn Carlson at the University Illinois at Urbana-Champaign.

2.1 Synthesis

Figure 4.1 shows the scheme used in synthesis of the new Estradiol-malonate conjugates.



Compound	R^1	R^2	R^3
1a	CH_3	THP	
1b	THP	THP	
2a			$CH_2CH_2CH(CH_3)_2$
2b			CH_3
3a	CH_3	THP	$CH_2CH_2CH(CH_3)_2$
3b	THP	THP	CH_3
4a	CH_3	H	$CH_2CH_2CH(CH_3)_2$
4b	H	H	CH_3

Figure 4.1 Synthetic Route to 7α-Estradiol-Malonate Conjugates

2.1.1 **17α-(Bis(3-methylbutyl)(methyl)(1-decyl)malonate)-3-methoxy-17b-(2-tetrahydropyranyloxy)estra-1,3,5-trien-6-one (3)a**

A 50 mL 3-neck flask was charged with 2 mL of DME and 473.1 mg (1.233 mmol) of **1a**. The solution was chilled to -78 °C, and 1.50 mL of 2.60 M KO-t-amyl (3.84 mmol) in cyclohexane was added gradually. The solution was stirred at 0 °C for 1 hr, then 1.9642 g (3.746 mmol) of **2a** was added. A precipitate formed almost immediately, and the mixture was stirred at room temperature for 20 min. Addition of 20 mL of water and 10 mL of Et_2O produced two layers. The organic

layer was washed with 10 mL of brine and dried over $MgSO_4$. Following removal of solvent *in vacuo*, the crude product was purified by flash chromatography. The product with R_f = 0.62 (8:2 hexane/EtOAc) was isolated. Yield of colorless oil: 258.7 mg (33.0% corrected). IR (Neat): 1,732 cm^{-1} (s, ester C=O), 1,684 cm^{-1} (s, ketone C=O). ^1H NMR ($CDCl_3$): δ 7.48 (d, J = 2.84 Hz, 1 H), 7.26 (dd, J_1 = 7.82 Hz, J_2 = 3.58 Hz,1H), 7.02 (dd, J_1 = 8.44 Hz, J_2 = 2.93 Hz, 1H), 4.61 (d, J = 11.73 Hz, 1H), 4.08 (td, J_1 = 6.81 Hz, J_2 = 1.32 Hz, 3H), 3.79 (s, 3H), 3.71 (q, J = 8.20 Hz, 1H), 3.39 – 3.50 (m, 1H), 2.59 – 2.71 (m, 1H), 2.37 – 2.45 (m, 1H), 2.26 – 2.36 (m, 1H), 1.28 – 2.12 (m, 28 H), 1.12 – 1.28 (m, 18H), 0.85 (d, J = 6.56 Hz, 12H), 0.82 (d, J = 3.34 Hz, 1H), 0.76 (d, J = 4.80 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 200.94 (ketone C=O), 172.36 (ester C=O). LRMS (FAB) *m/z* (rel. intensity): 781.7 (M, 16), 697.6 (M - THP, 100). Anal. Calcd for $C_{48}H_{77}O_8$: C, 73.77; H, 9.93. Found: C, 73.81; H, 10.17.

2.1.2 **4a**

A 50 mL, 3-necked flask was charged with 3 mL of CH_2Cl_2 and 412.1 mg (0.5273 mmol) of **3a**. This solution was cooled to 0 °C, and 3.0 mL (19 mmol) of $HSiEt_3$ and 6.0 mL (47 mmol) of BF_3/Et_2O were added slowly. The 0 °C bath was removed, and the solution stirred at room temperature for 4.5 hrs. Addition of 15 mL of 2.5 M Na_2CO_3 (aq) caused formation of a white precipitate. Addition of 25 mL of H_2O and 50 mL of EtOAc produced two layers. The organic layer was washed with 50 mL of H_2O and 50 mL of brine and dried over $MgSO_4$. Removal of solvent *in vacuo* afforded a crude product that was dissolved in 8:2 hexane/EtOAc; the resulting solution was filtered through a 2.5 cm plug of silica.

The silica was washed with an additional 1 L of 8:2 hexane/EtOAc. Removal of solvent *in vacuo* afforded a colorless oil with a single TLC spot (R_f = 0.37 in 8:2 Hex/EtOAc). Yield: 275.4 mg (76.4%). IR (Neat): 1,732 cm^{-1} (s, ester C=O). ^1H NMR ($CDCl_3$): δ 7.17 (d, J = 8.69 Hz, 1 H), 6.68 (dd, J_1 = 8.49 Hz, J_2 = 2.58 Hz, 1H), 6.59 (d, J = 2.49 Hz, 1H), 4.11 (t, J = 5.61 Hz, 2H), 3.74 (s, 4H), 3.71 (q, J = 9.11 Hz, 1H), 2.70 – 2.91 (m, 1H), 2.27 – 2.33 (m, 1H), 1.58 – 2.14 (m, 13 H), 1.48 (q, J = 10.1 Hz, 6H), 1.37 (m, 4H), 1.37 (s, 3H), 1.12 – 1.30 (m, 18H), 0.89 (d, J = 6.6 Hz, 12H), 0.76 (s, 3H). ^{13}C NMR: δ 172.39 (ester C=O). LRMS (FAB) *m/z* (rel. intensity): 682.5 (M, 39), 171.1 (100). HRMS (FAB) Calc. for $C_{43}H_{70}O_6$: 682.5172. Found: 682.5173. Anal. Calcd for $C_{43}H_{70}O_6$: C, 75.62; H, 10.33. Found: C, 75.91; H, 10.75.

2.1.3 **5**

A 50 mL, 3-neck flask was charged with 2.9818 g (11.18 mmol) of $AlBr_3$ and 15 mL of EtSH. This solution was stirred at 0 °C as 486.0 mg of **4a** (0.7105 mmol) in 10 mL of CH_2Cl_2 was added slowly. The solution was stirred for 15 minutes at 0 °C and for 45 minutes at room temperature. The solution was re-cooled to 0 °C, and 15 mL of H_2O was added slowly. A white precipitate formed immediately. The mixture was then purged with air at room temperature for 12 hours. To the residue, 15 mL H_2O and 15 mL EtOAc were added. The aqueous layer was extracted with two additional 15 mL portions of EtOAc. The pooled EtOAc layers were dried over $MgSO_4$. Removal of solvent *in vacuo* afforded an oil with mass 273.2 mg. ^1H-NMR showed that the O3 methyl group had been completely removed, but the isoamyl protecting groups were still present.

The oil described above was dissolved in 5 mL of THF in a 50 mL 3-necked flask. To this stirred solution, 1.2876 g of $Ba(OH)_2 \cdot 8H_2O$ and 5 mL of H_2O were added. The mixture was stirred and refluxed for 3.5 hours. The mixture was cooled to room temperature, and the pH was adjusted to 1 by addition of dilute HCl (aq). Addition of 15 mL of EtOAc and 15 mL of H_2O produced two layers. The aqueous layer was further extracted with two 15 mL portions of EtOAc. Removal of solvent *in vacuo* afforded a solid that was purified by flash chromatography (R_f = 0.46 in 20:20:1 THF/hexane/HOAc). Yield of colorless solid: 94.9 mg (25.3%). ^{1}H NMR (acetone-d_6): δ 7.99 (s, 1H), 7.08 (d, J = 8.52 Hz, 1H), 6.58 (dd, J_1 = 8.44 Hz, J_2 = 2.57 Hz, 1H), 6.52 (d, J = 2.63 Hz, 1H), 3.66 (t, J = 8.77 Hz, 2H), 2.70 – 2.80 (m, 2H), 2.24 – 2.31 (m, 2H), 1.48 – 2.08 (m, 10H) 1.12 – 1.44 (m, 20H) 1.36 (s, 3H), 0.77 (s, 3H). ^{13}C NMR (acetone-d_6): δ 172.01 (acid C=O). Anal. Calcd for $C_{32}H_{48}O_6$: C, 72.69; H, 9.15. Found: C, 70.30; H, 8.89. LRMS (FAB) *m/z* (rel. intensity): 528.2 (M, 90), 371.0 (100). HRMS (FAB) Calc. for $C_{32}H_{48}O_6$: 528.3451. Found: 528.3451.

2.1.4 **7α-((Dimethyl)(methyl)(1-decyl)malonate)-3-methoxy-17β-(2-tetrahydropyranyloxy)estra-1,3,5-trien-6-one (3b)**

A 50 mL 3-neck flask was charged with 1.99 g (4.37 mmol) of **1b** and 4.5 mL of DME. The solution was cooled to –78 °C, and 7.4mL (17.46mmole) of 2.36 M KO-t-amyl (in cyclohexane) was added slowly. The solution was allowed to warm to 0 °C and then stirred for 1 hour. Then 5.37 g (13.0 mmol) of **2b** was added slowly. A white precipitate resulted. The mixture was stirred at room temperature for 1 hour, then addition of 200 mL of H_2O and 100 mL of diethyl

ether produced two layers. The organic layer was washed with 100 mL of brine and dried over $MgSO_4$. Removal of solvent *in vacuo* afforded an oil that was purified by flash chromatography. A mixture of diastereomers with R_f = 0.33 and 0.29 (20:1 benzene/EtOAc) were isolated. Yield of colorless oil: 997.6 mg (31.3%). ^{1}H NMR ($CDCl_3$): δ 7.59 (d, J = 2.51 Hz, 1 H), 7.21 (dd, J_1 = 8.61 Hz, J_2 = 3.15 Hz,1H), 7.09 (dd, J_1 = 8.73 Hz, J_2 = 2.31 Hz, 1H), 5.36 (d, J = 2.95 Hz, 1H), 4.56 (m, 1H), 3.80 (m, 2H), 3.64 (m, 1H), 3.60 (s, 6H), 3.50 (m, 1H), 3.38 (m, 1H), 2.60 (m, 2H), 2.24 – 2.36 (m, 2H), 1.95 – 1.98 (m, 3H), 1.84 – 1.87 (m, 2H), 1.73 – 1.76 (m, 5H), 1.41 – 1.58 (m, 13H), 1.30 (s, 3H), 1.12 – 1.20 (m, 16H), 0.70 (d, J = 4.64 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 200.64 (ketone C=O), 172.73 (ester C=O). LRMS (FAB) *m/z* (rel. intensity): 571.3 (M- 2 THP, 40), 118.9 (100). HRMS (FAB) Calc. for $C_{44}H_{65}O_9$: 737.4629; Found: 737.4626.

2.1.5 **4b**

A 100mL, 3-neck flask was charged with 997.6 mg (1.367 mmole) of **3b** and 7 mL of CH_2Cl_2. The solution was cooled to 0 °C, and 8.5 mL (53 mmole) of $HSiEt_3$ and 17.3 mL (137 mmole) of BF_3/Et_2O were slowly added. The 0 °C bath was removed and the reaction stirred for 4.5 hours at room temperature. Addition of 50 mL of 3.8 M Na_2CO_3 (aq) caused formation of a white precipitate. Addition of 25 mL of H_2O and 50 mL of EtOAc produced two layers. The organic layer was washed with 50 mL of H_2O and 50 mL of brine and dried over $MgSO_4$. Removal of solvent *in vacuo* afforded a colorless solid that was triturated with 8:2 hexane/EtOAc and then re-dried *in vacuo*. Yield: 518.4 mg (68.1%). ^{1}H NMR ($CDCl_3$): δ 7.13 (d, J = 8.47 Hz, 1H), 6.60 (dd, J_1 = 8.65 Hz, J_2 = 2.81 Hz, 1H),

6.54 (d, J = 3.06 Hz, 1H), 3.69 (s, 6H), 2.76 – 2.84 (m, 1H), 2.24 – 2.34 (m, 2H), 2.02 – 2.20 (m, 2H), 1.78 – 1.96 (m, 4H), 1.80 – 1.96 (m, 3H), 1.52 – 1.76 (m, 6H), 1.42 – 1.50 (m, 3H), 1.38 (s, 3H), 1.28 – 1.36 (m, 3H), 1.12 – 1.28 (m, 12H), 0.85 – 1.01 (m, 1H), 0.76 (s, 3H). ^{13}C NMR (acetone-d_6): 172.83 (ester C=O). LRMS (FAB) *m/z* (rel. intensity): 556.3 (M, 30), 119.0 (100). HRMS (FAB) Calcd for $C_{34}H_{52}O_6$: 556.3764. Found: 556.3764. Anal. Calcd for $C_{34}H_{52}O_6$: C, 73.34; H, 9.41. Found: C, 73.70; H, 9.29.

3. Results and Discussion

The synthesis of **3a** and **3b** is a straightforward adaptation of published methods that have been used to introduce substituents into the 7α position of estradiol.[13] Chromatographic separation of **3b** from the ketone starting material, **1b**, was feasible but difficult due to the fact that **3b** and **1b** have nearly identical R_f values on silica with a variety of solvents. Purification was simplified by a choice of protecting groups that widened the difference in polarity between ketone and alkylated ketone. Replacing a THP with a methyl group increased the polarity of the (unalkylated) ketone, **1a**, and replacing methyl with isoamyl protecting groups decreased the polarity of the alkylated ketone, **3a**. The yields for the alkylation reaction are similar to reported yields. In addition to the method shown in Figure 1, we were able to effect alkylation by using potassium t-butoxide as the base and triethylborane to suppress O-alkylation, as has been reported in published accounts.[8]

Reduction of the ketones, **3a** and **3b**, to prepare compounds **4a** and **4b**, followed a literature method; yields were comparable to reported yields.[13]

Removal of all THP groups during this procedure occurred as expected. Deprotection of **4a** to afford **5** was accomplished in two stages. First, the methyl group was removed by treatment with $AlBr_3$/EtSH, a reagent that has proven generally useful in demethylation of steroid methyl ethers. Then the esters were hydrolyzed under basic conditions.

Receptor binding affinities are reported in Table 1. The values demonstrate that both **4a** and **5** have moderate to high affinity for purified ER. Compound **5** has lower affinity for ER than does **4a**, presumably because **5** is less lipophilic and contains acidic hydrogens that allow for more nonspecific interactions with ER. Although each compound exhibits a greater affinity for one isoform of the receptor than for the other, neither difference in affinity is remarkable. In whole cytosol the difference between **4a** and **5** is further accentuated by the very small RBA of **5**; this difference can be explained by the large number of nonspecific interactions possible between **5** and basic residues on the surfaces of cytosol proteins.

Table 4.1 Receptor Binding Affinities of 7α-Estradiol Conjugates

Compound	ER (α)	ER(β)	Whole Cytosol
4a	89.1	70.9	86.2
5	20.7	46.3	2.1

Taken together, the receptor binding affinities of compounds **4a** and **5** suggest a significant basis for new transition metal complexes with high affinity for ER. The metal could be coordinated to a malonate group which is itself conjugated to Estradiol at the 7α position. The entire ligand set of the metal must

be chosen so as to minimize nonspecific interactions with ER or any other protein.

References

*to whom correspondence should be addressed

1. A preliminary report of part of this work was presented at a national meeting of the American Chemical Society in March, 2003: Division of Medicinal Chemistry Paper 91 : (Poster) *Novel 7-α-substituted estradiol derivatives.* R. W. Chesnut , L. J. Bailey , J. S. Bundy , S. E. Bendler , S. A. Clark , D. N. Davis , N. A. Orwar , J. A. Katzenellenbogen , K. E. Carlson

2. Bowler, J.L., T.J.; Pittman, J.D.; Wakeling, A.E., *Novel Steroidal Pure Antiestrogens.* Steroids, 1989. **54**: p. 71 - 99.

3. Bucort, R.V., M.; Torelli, V.; Richard-Foy, H.; Geynet, C.; Secco-Millet, C.; Redeuilh, G.; Baulieu, E.E., *New Biospecific Adsorbents for the Purification of Estradiol Receptor.* J. Biol. Chem., 1978. **253**: p. 8221 - 8226.

4. DaSilva, J.N.v.L., Johan E., *Synthesis and Structure-Affinity of a Series of 7α-Undecylestradiol Derivatives: A Potential Vector for Therapy and Imaging of Estrogen-Receptor-Positive Cancers.* J. Med. Chem., 1990. **33**: p. 430 - 434.

5. Wakeling, A.E., Dukes, M., Bowler, J., *A Potent Specific Pure Antiestrogen with Clinical Potential.* Cancer. Res., 1991. **51**: p. 3867 - 3873.

6. French, A.N.W., Scott R.; Welch, Michael J.; Katzenellenbogen, John A., *A Synthesis of 7α-Substituted Estradiols: Synthesis and Bio.* Steroids, 1993. **58**: p. 157 - 169.

7. Muhlenbruch, B.K., F.; Roth, H.J., *Syntheses and Properties of Fluorescent Estradiol Derivatives.* Arch Pharm (Weinheim), 1986. **319**: p. 430 - 434.

8. Anstead, G.M.C., Carlson, Cathryn E.; Katzenellenbogen, John A., *The Estradiol Pharmacophore: Ligand Structure-Estrogen Receptor Binding Affinity Relationships and a Model for the Receptor Binding Site.* Steroids, 1997. **62**: p. 268- 303.

9. Skaddan, M.B., Wust, F. R., Katzenellenbogen, J. A.;. *Novel Rhenium-Containing Estrogen Mimics as Potential Imaging Agents for Breast Cancer.* in *American Chemical Society National Meeting.* 1998. Boston: American Chemical Society.

10. Mitra, K.M., John C.; Hillier, Shawn M.; Rye, Peter T.; Zayas, Beatriz; Lee, Annie S.; Essigman, John M.; Croy, Robert G., *A Rationally Designed Genotoxin that Selectively Destroys Estrogen-Positive Breast Cancer Cells.* J. Am. Chem. Soc., 2002. **124**: p. 1862 - 1863.

11. Tedesco, R., Fiaschi, R., Napolitano, E., *6-Oxoestradiols from Estradiols: Exploiting Site-Selective Metalation of Aralkyl Systems with Superbases.* SYNTHESIS, 1995: p. 1493 - 1495.

12. Bendler, Sara E.; Bundy, Jason S.; Clark, Sarah A.; Davis, Diana N.; Orwar, Nicole A.; Chesnut, Robert W., *ω-Iodoalkyl(methyl)malonate Esters.* Synth. Commun., 2003. **33**(19): p. 3365 - 3371.

13. Tedesco, R.K., John A.; Napolitano, Elio, *An Expeditious Route to 7α-Substituted Estradiol Derivatives.* Tetrahedron Lett., 1997. **38**(46): p. 7997 - 8000.

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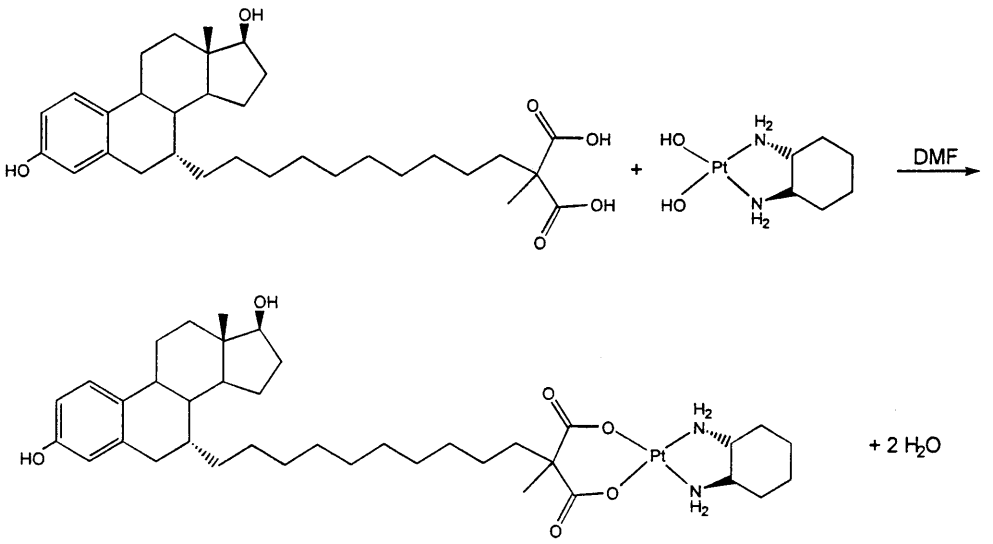
Chapter V: Synthesis of Platinated 7α-Substituted Estrogen

Section I: Introduction

The nonaqueous method of platinating model malonic acids was applied to the platination of the 7α-substituted estradiol compound whose synthesis was described in Chapter 4.

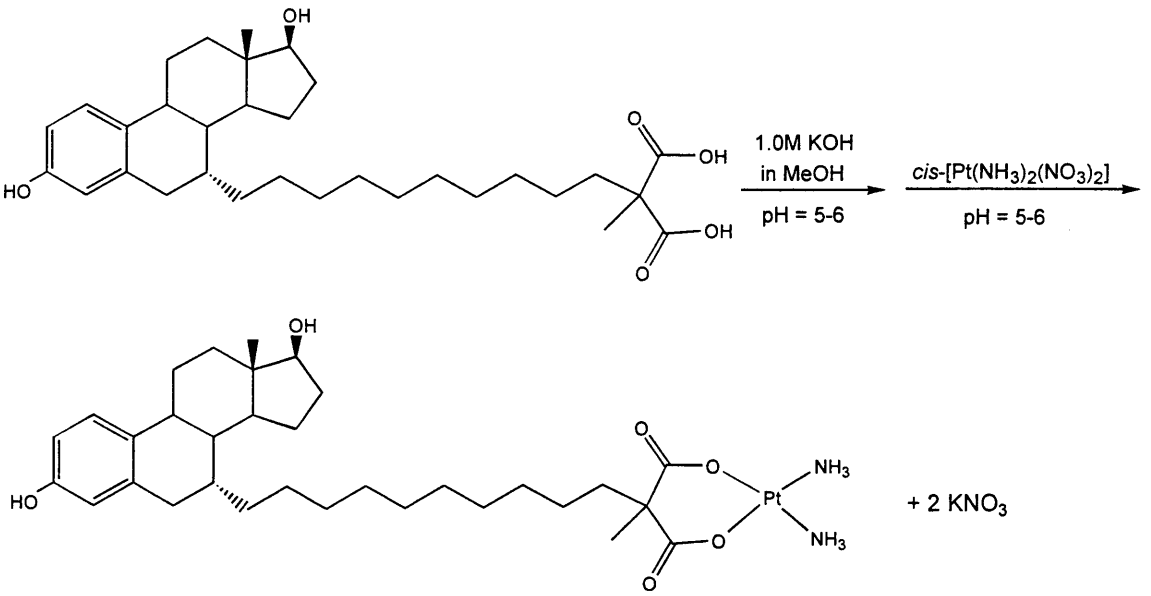
In model studies described in Chapter 3, the *cis*-PtDACH moiety was introduced by condensation of *cis*-[$Pt(OH)_2$DACH] with 2-(4-hydroxybenzyl)-2-methylmalonic acid. This method was considered directly applicable to the platination of 7α-substituted estradiol. The proposed synthesis, as shown in Scheme 5.1, depends on DMF as the solvent. Compared to platination of model compounds, this reaction had the potential advantage of yielding a water-insoluble product that might be purified by extraction or recrystallization.

Scheme 5.1. Proposed Coordination of 7α-Substituted Estradiol to *cis*-[PtDACH]



Coordination of the 7α-substituted estradiol to the platinum moiety, *cis*-$Pt(NH_3)_2$, was also considered feasible on the basis of platination of model malonic acid compounds, as described in Chapter 3. This method offered potential ease of purification, since potassium nitrate, the by-product of the platination reaction, should be convenient to remove by extraction with water. The proposed synthesis in DMF solution is shown in Scheme 5.2.

Scheme 5.2 Proposed Coordination of 7α-Substituted Estradiol to *cis*-$[Pt(NH_3)_2]$

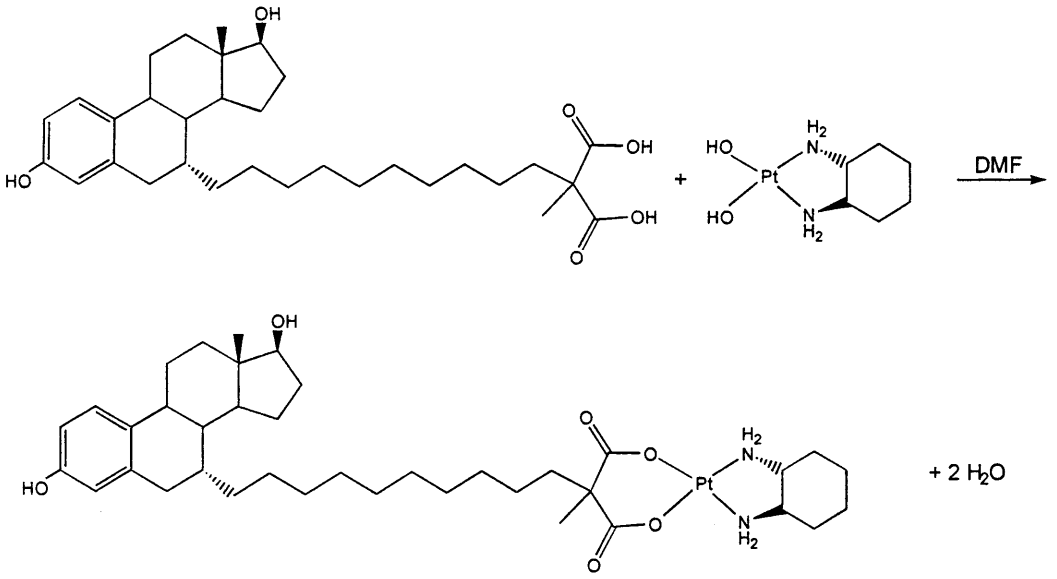


Chapter V

Section II: Results and Discussion

A. Coordination of 7α-Substituted Estradiol to *cis*-PtDACH

Scheme 5.3. Coordination of 7α-Substituted Estradiol to *cis*-PtDACH



The reaction of 7α-Substituted Estradiol with *cis*-Pt(OH)$_2$DACH is shown in Scheme 5.3. The complex was formed by combining the starting materials in DMF and heating at 50 °C for five hours. The gentle heating resulted in a homogeneous pale yellow solution. Removal of solvent afforded a crude product that was triturated with water in order to remove any unreacted *cis*-Pt(OH)$_2$DACH. The residue was then extracted with absolute ethanol in order to remove the target product from any residual silver-containing compounds. The solvent was concentrated *in vacuo* and the final product was precipitated out with acetone. The final product was an ecru solid.

When the isolated product was completely dried, its solubility became very low. If trace amounts of DMF or ethanol were retained, then the compound was quite soluble in polar organic solvents such as methanol. The completely dried product retains only slight solubility for absolute ethanol. This complication might be avoided by purification via HPLC before complete drying.

The final product was characterized by ^{1}H NMR spectroscopy (Figure 5.1), MS (LRESI) (Figure 5.2), MS (HRESI) and elemental analysis. The ^{1}H NMR spectrum was not conclusive on the formation of the desired product. This was due to several reasons: (1) the overlap of the DACH proton signals and the steroid ring proton signals, (2) poor resolution, and (3) poor solubility of the isolated product. The integration suggests additional protons are present from the DACH ring. The integration differences between the free ligand and the isolated product are summarized in Table 5.1. There are slight differences in chemical shifts between the free ligand and the isolated product due to different solvents. Two of the five protons gained in the 2.70-2.80 ppm region can be attributed to DACH. The four protons gained in the 1.48-2.08 ppm can also be assigned to DACH. The large increase in the 1.12-1.44 ppm range is due to overlap with impurities of deuterated ethanol as well as four protons from the DACH ring.

Table 5.1 Integration differences between free ligand and isolated product.

δ, ppm	Free Ligand, H	Isolated Product, H	Difference
2.70-2.80	2	7	+5
2.24-2.31	2	4	+2
1.48-2.08	10	14	+4
1.12-1.44	23	43	+20

The ^{195}Pt NMR (Figure 5.2) spectrum shows two signals. The most intense signal is at -2800 ppm. This signal is probably the desired product due to its intensity. The less intense signal is at -2360 ppm and could be a platinum impurity. An additional ^{195}Pt NMR spectrum would need to be obtained after purification for conclusive assignment.

The mass spectrum (Figure 5.3) is consistent with the desired product. As in the mass spectra of model compounds of platinum reported in Chapter 3, both the M+1 and M+ 46 peaks are clearly present. Each of these signals is part of a pattern of three peaks whose intensities closely approximate the ratios of the natural abundance of platinum isotopes. The M+46 peak is assigned to a noncovalent complex between the desired product and ethanol, which is the solvent used for dissolving the samples for analysis by mass spectrometry. High-resolution mass spectrometry of the M+1 peak measured a mass of 836.4177 g/mol for this ion. The calculated mass of $C_{38}H_{61}O_6N_2Pt$ as 836.4177 g/mol. This agreement is strong evidence that the correct product was made.

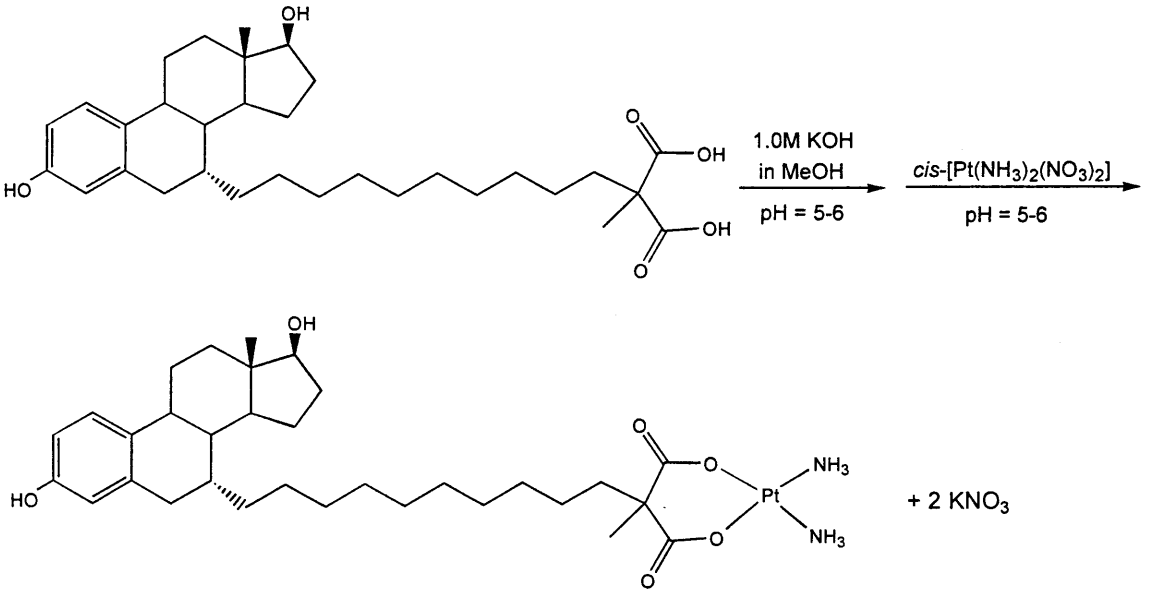
According to elemental analysis, the isolated product was not pure. The results are summarized in Table 5.2. The product of reaction 37B was analyzed by ^{1}H NMR and found to contain ethanol; this fact could explain the high percentages of carbon and hydrogen with low nitrogen. In reaction numbers 58E and 66D, the low values for carbon and high values for nitrogen are similar to those measured for the products of model reactions reported in Chapter 3.

Table 5.2 Elemental analysis results of isolated product

Reaction Number	%C	%H	%N
Theoretical	**54.60**	**7.23**	**3.35**
37B	62.07	8.35	2.81
58E	47.00	7.02	4.65
66D	46.91	6.94	4.53

B. Coordination of 7α-Substituted Estradiol to *cis*-[$Pt(NH_3)_2$]

Scheme 5.4 Coordination of 7α-Substituted Estradiol to *cis*-[$Pt(NH_3)_2$]



The reaction of 7α-substituted estradiol with *cis*-[$Pt(NO_3)_2(NH_3)_2$] is shown in Scheme 5.4. A solution of the free ligand in DMF was adjusted to a pH of 6 with 1.0 M KOH in methanol. This solution was added to the platinum intermediate solution and stirred overnight protected from light. The solvent was removed *in vacuo* affording a tan solid. The crude solid was triturated with water. The remaining solid was dissolved in absolute ethanol and vacuum filtered to remove any silver impurities. The solvent was removed *in vacuo* affording a pale orange solid. The mass spectrum (Figure 5.4) is consistent with the desired product with the M+46 peak observed. The M+46 peak is assigned to a

noncovalent complex between the desired product and ethanol, which is the solvent used for dissolving the samples for analysis by mass spectrometry.

C. Purification of platinated estrogens

The purification of these compounds has been a challenge. When the isolated products were completely dried, their solubility became very low. If trace amounts of DMF or ethanol were retained, then the compounds were quite soluble in polar organic solvents such as methanol. The completely dried product only retains slight solubility for absolute ethanol. This complication may be avoided by purification before complete drying. Purification by HPLC will be attempted.

Chapter V

Section III: Conclusion

The nonaqueous platination of the 7α-substituted estradiol with the DACH amines was successfully used for five reactions. The use of the *cis*-dihydroxo(*trans*-1,2-diaminocyclohexane)platinum (II) intermediate and DMF as a solvent were as successful with the steroid as with the model malonic acids. The desired product was formed according to ^{1}H NMR and mass spectrometry. The elemental analysis results also suggest the desired product was made, but not purified. The crude product yields were approximately 25% for the DACH compound.

The nonaqueous platination of the 7α-substituted estradiol with the amines as $(NH_3)_2$ was also successful with three reactions. The platinum intermediate was a DMF solution of *cis*-$[Pt(NO_3)_2(NH_3)_2]$. The base used to insure deprotonation was a methanolic potassium hydroxide solution. The crude product yields were 60% for the diamine compound.

Chapter V

Section IV: Experimental

The absolute ethanol was used as supplied from AAPER. The dimethyl formamide was distilled from calcium hydride from various sources. Acetone, 85% potassium hydroxide, and deuterated ethanol were used as supplied from Aldrich. The methanol was used as supplied from Fisher. ^{1}H NMR spectra were collected on a QE 300 instrument. Mass spectra were obtained with Electrospray Ionization (ESI) on a Quattro instrument at the School of Chemical Sciences, University of Illinois at Urbana-Champaign. Elemental analyses were conducted by the Microanalytical Laboratory at the School of Chemical Sciences, University of Illinois at Urbana-Champaign. ^{195}Pt NMR spectra were collected on a 500 MHz Varian instrument at the School of Chemical Sciences, University of Illinois at Urbana-Champaign.

Coordination of 7α-Substituted Estradiol to *cis*-PtDACH *Cis*-dihydroxo(*trans*-1,2-diaminocyclohexane)platinum (II) (0.2 mmol) was suspended in DMF (5 mL) in a 50 mL flask. A homogeneous pale yellow solution of the 7α-substituted estradiol (0.2 mmol) and DMF (10 mL) was added to the reaction flask. The flask was wrapped in aluminum foil. The reaction mixture was heated at 50 °C for five hours. After heating, the reaction solution was a homogeneous pale yellow. The solvent was removed *in vacuo*, affording a brown solid. The

solid was triturated with water. The remaining solid was extracted with absolute ethanol. There was often a small amount of an insoluble gray solid (a silver impurity) that was removed by vacuum filtration. The ethanol was concentrated *in vacuo* to a volume of approximately 5 mL. Acetone (25 mL) was added, and a light colored precipitate formed. This precipitate was removed by vacuum filtration. The isolated product was an ecru solid. The exact yields are summarized by product number in Table 5.3.

Table 5.3 Isolated product yields 7α-Substituted Estradiol to *cis*-PtDACH

Product Number	Yield
58E	32%
66D	18%
70B	25%

^{1}H NMR, (CD_3CD_2OD) δ: 7.03 (d, J=8.3 Hz, 1H), 6.59 (d, J=7.0 Hz, 1H), 6.50 (s, 1H), 3.67, (t, J=8 Hz, 2H), 2.84-2.63 (m, 7H), 2.35-2.19 (m, 3H), 2.09-1.49 (m, 14H), 1.48-0.98 (m, 43H), 0.77 (s, 3H), Figure 5.1. ^{195}Pt NMR, (CD_3CH_2OD) δ: -2800, Figure 5.2. MS (LRESI) (m/z, rel. intensity): 836.4 (M+1, 18%), 881.5 (M+46, 100%), Figure 5.3.

Coordination of 7α-Substituted Estradiol to *cis*-[$Pt(NH_3)_2$] A salt solution was formed by dissolving the 7α-substituted estradiol (0.2 mmol) in DMF (6 mL) and adjusting the pH to 6 with 1 M KOH in methanol. This solution was added to *cis*-$(NO_3)_2Pt(NH_3)_2$ (0.2 mmol) in DMF (3 mL) in a 25 mL flask. The reaction solution was homogeneous and stirred overnight. The reaction flask was wrapped in foil. The solvent was removed *in vacuo*, affording an orange/tan solid. The solid was triturated with water. The remaining solid was dissolved in

absolute ethanol and vacuum filtered to remove any silver impurities. The solvent was removed *in vacuo*, affording a pale orange solid. The isolated product yields are summarized in Table 5.4.

Table 5.4 Isolated product yields

Product Number	Yield
82A	58%
85B	63%

MS (LRESI) (m/z, rel. intensity): 801.4 (M+46, 100%), Figure 5.4.

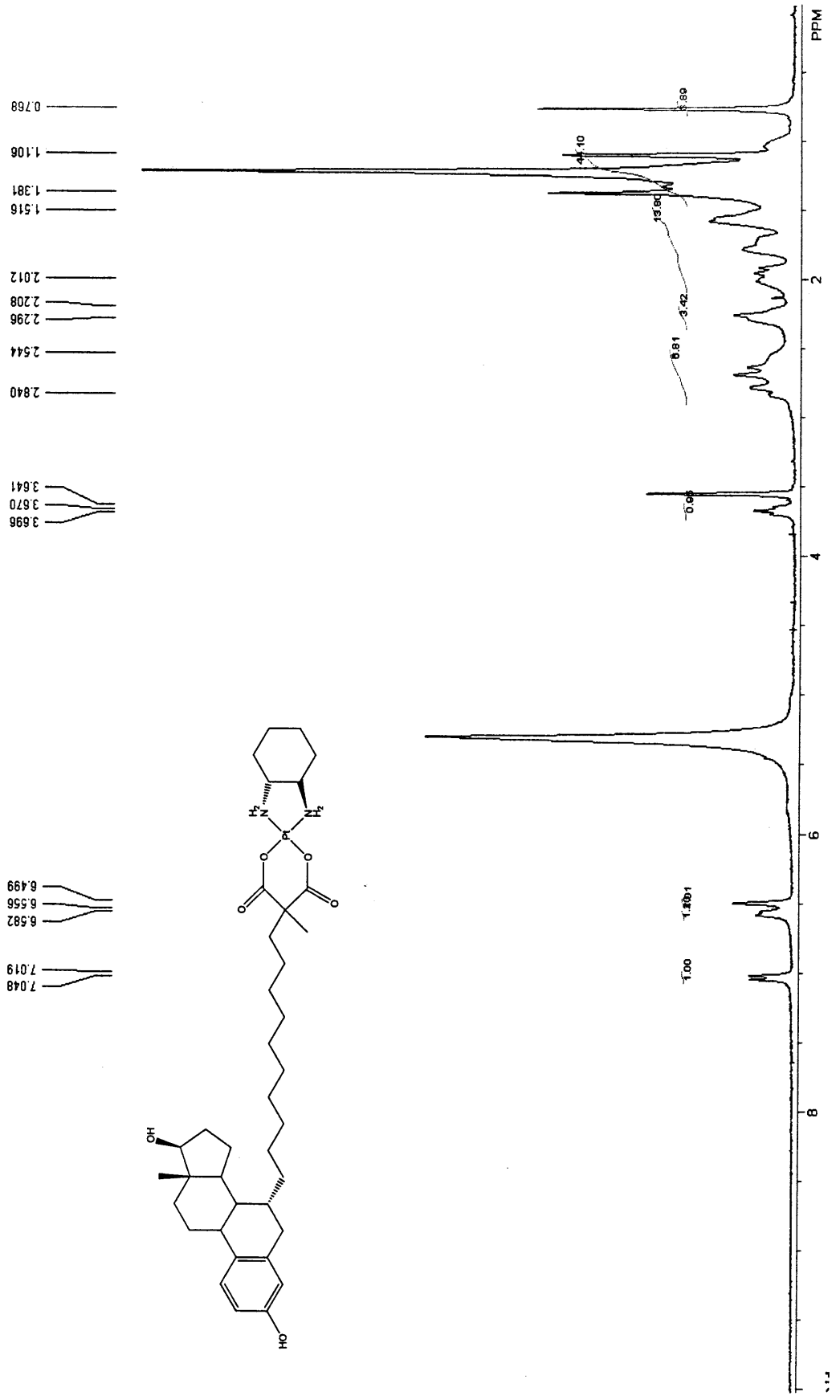


Figure 5.1 ^{1}H NMR Spectrum of 7α-Substituted Estradiol-Platinum DACH

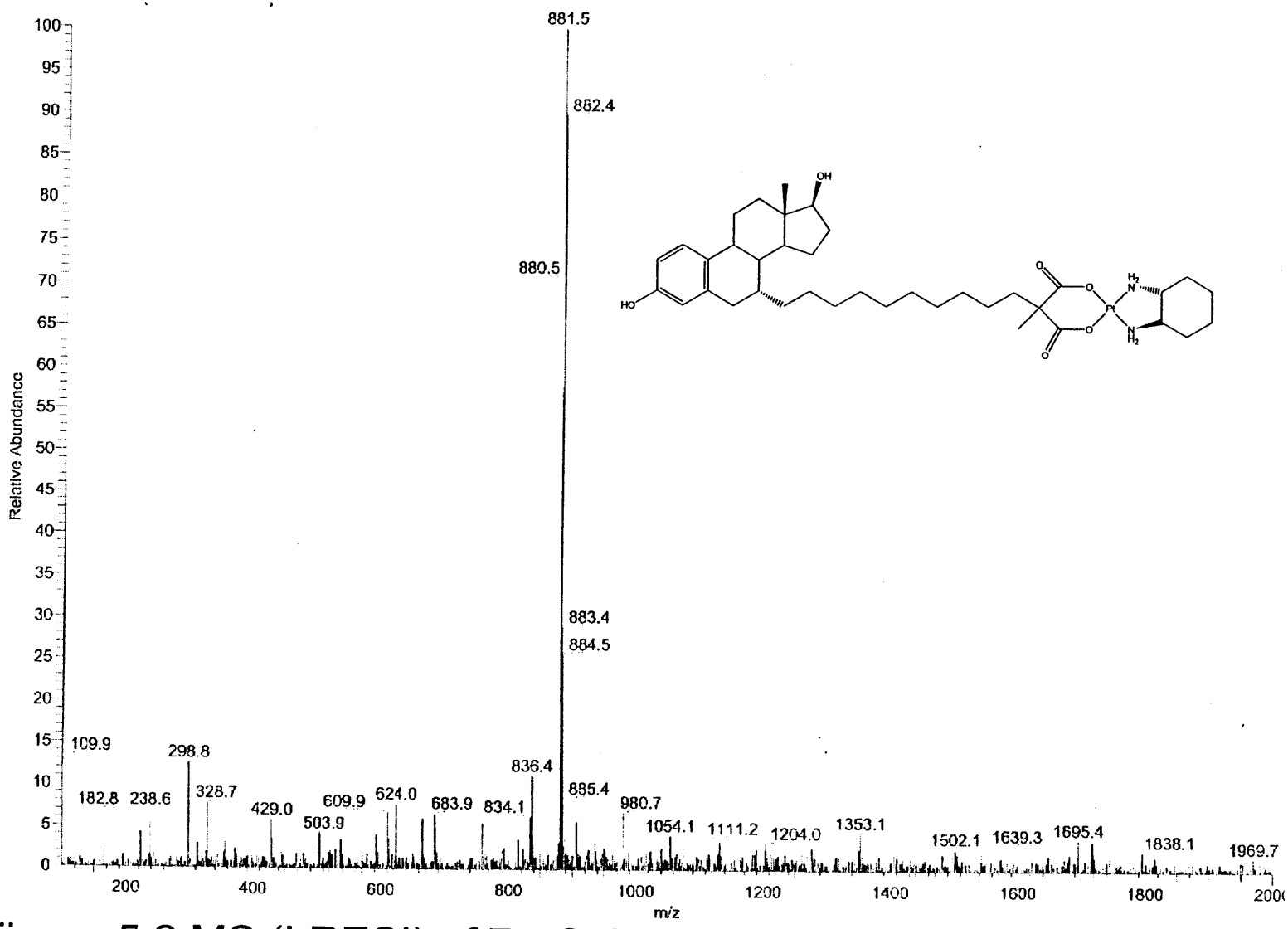
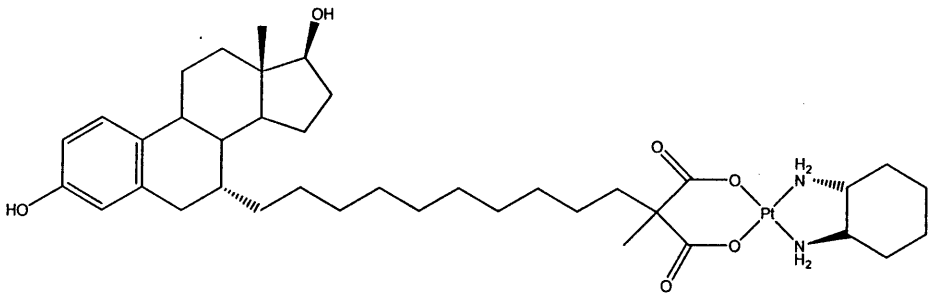


Figure 5.2 MS (LRESI) of 7α-Substituted Estradiol-Platinum DACH



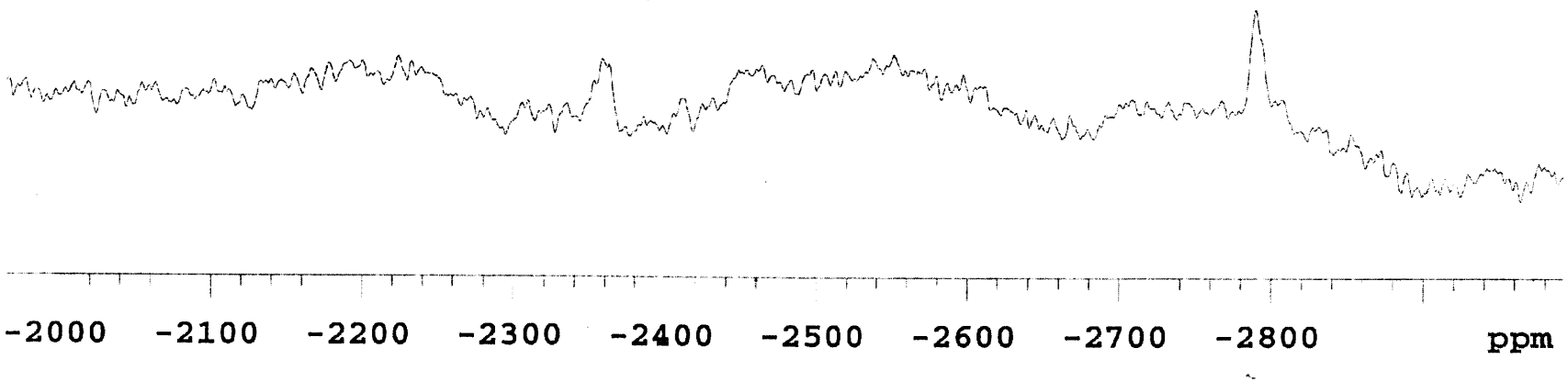


Figure 5.3 ^{195}Pt NMR of 7α-Substituted Estradiol-Platinum DACH

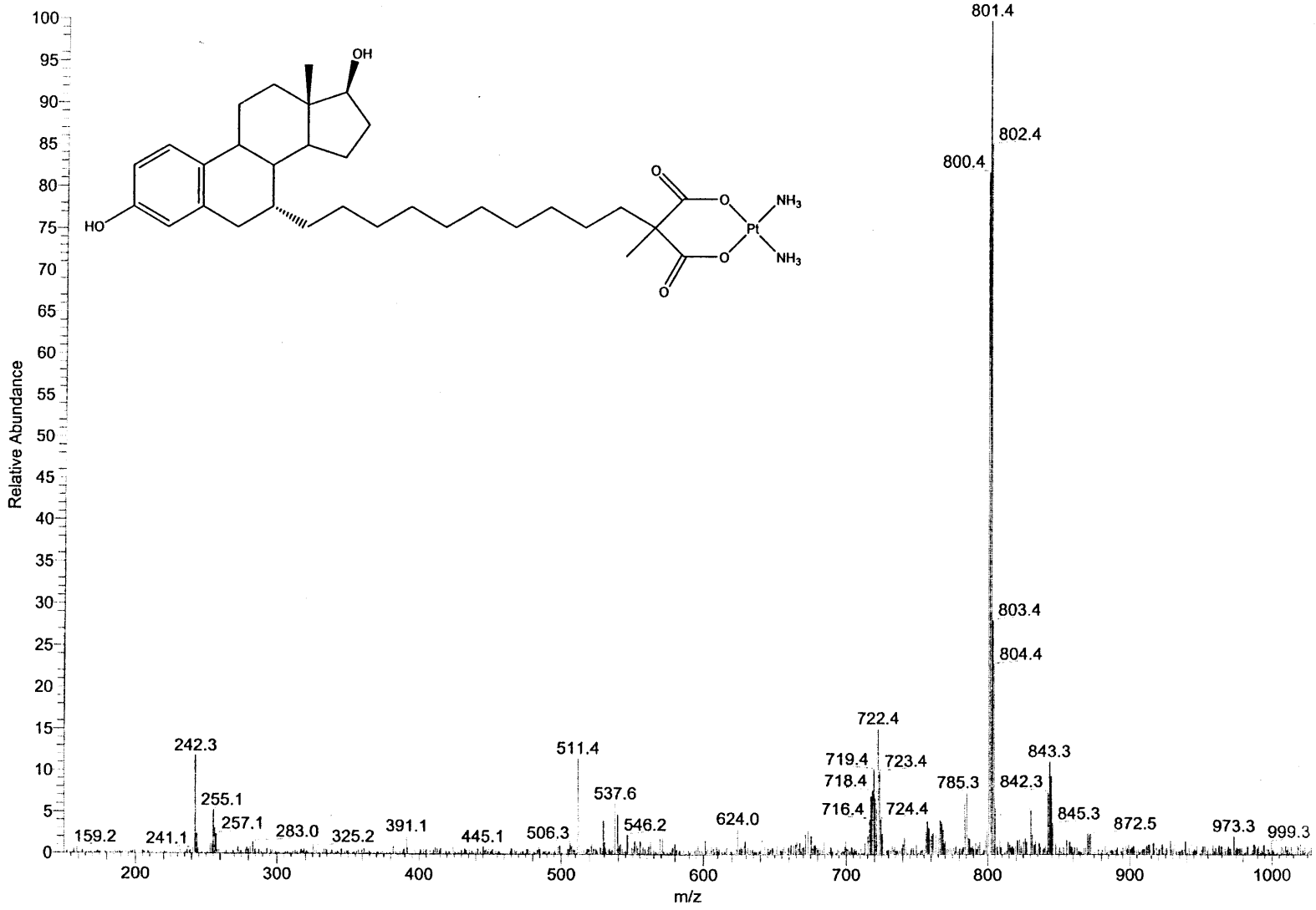


Figure 5.4 MS (LRESI) of 7α-Substituted Estradiol-Platinum Diamine