# Novel forms of a potent Cannabinoid receptor modulator

## 1 Introduction

Obesity is widely recognized as the largest and fastest growing public health problem in the developed and developing world [1]. Since the last decade, obesity has received major attention globally. It is no more considered as a cosmetic concern, rather a serious global epidemic, because of its wide impact on public health. The International Obesity Task Force estimates that more than 300 million individuals worldwide are obese and an additional 800 million are over-weight [2]. According to an estimate by WHO, at least 2.8 million people die each year as a result of being overweight or obese, and an estimated 35.8 million (2.3%) of Disability Adjusted Life Years are lost by overweight or obesity globally. The worldwide prevalence of obesity has nearly doubled between 1980 and 2008. In 2008, 10% of men and 14% of women in the world were obese (BMI ≥30 $kg/m^2$), compared with 5% for men and 8% for women in 1980 [3].

Sedentary life style and uncontrolled dietary habits is a common tendency in most of the developed and many developing societies which contribute to the obesity epidemic. The problem is compounded with the advent of junk foods, ready to eat and processed food; modern lifestyles with their various stresses as well as increasing disposable income also play a part in this problem. Although food is necessary for survival, increased exposure to processed food is overwhelming people [4], and effective strategies to reduce body-mass index (BMI) in populations are scarce. Obesity is associated with substantial increases in morbidity, premature mortality, impaired quality of life and large healthcare costs [5]. The problem with obesity are compounded due to the major comorbidities associated with it that include type 2 diabetes, metabolic syndrome, hypertension, dyslipidemia, myocardial infarction, stroke, certain cancers, sleep apnea and osteoarthritis [6]. Surprisingly, however, treatment options remain quite limited. Lifestyle changes in the form of dieting and/or exercise per se do not generally

produce marked or sustainable weight loss [7], whereas psychological therapies are difficult to deliver on a mass scale [8] and long-term results are disappointing. Bariatric surgery though more effective are limited by possible complications in surgery and requirement of reoperation. Hence, prevalence of obesity pharmacotherapy has become a popular choice, especially among the younger generations [9].

Till date, obesity pharmacotherapy, though pursued for over half a century has yielded very little success. Earlier attempts to treat obesity using various agents such as centrally acting sympathomimetic desoxyephedrine, phentermine, or, combination of phentermine and fenfluramine, or the dual monoamine (noradrenaline and serotonin)-reuptake inhibitor sibutramine etc. have all been plagued with serious adverse effects, most important being cardiovascular risks. Such adverse effects have led to the withdrawal of most of them from the market.

Cannabinoids are present in Indian hemp *Cannabis sativa* and have been well known for their medicinal properties for ages. Use of Cannabinoids as a therapeutic agent is however a recent phenomenon [10]. Over the last decade, research in this area has provided very important information on the cannabinoid receptors and their agonists and antagonists. Development of various central cannabinoid (CB) receptor ligands [11] has been attempted. Further cloning and isolation of two different subtypes of cannabinoid receptors - $CB_1$ (central subtype) and $CB_2$ (peripheral subtype) and the identification of the first endogenous ligand, N-arachidonyl ethanolamine amide (AEA) (anadamide) [12, 13] have stimulated research in this field. $CB_1$ is a member of the G-protein coupled receptor (GPCR) family. The $CB_2$ receptor has 44% amino acid identity with $CB_1$, and a distinct yet similar binding profile, and thus represents a receptor subtype [14].

### 1.1 Mechanisms of Cannabinoid Receptor Regulation

Despite the growing list of diseases that show cannabinoid receptor expression changes, relatively little is known about the mechanisms underlying these changes. In primary hepatocytes, RAR-γ (Retinoic acid

receptor γ] binds the -500 to +50 region of the $CB_1R$ promoter and increases its expression which results in Retinoic acid being released from hepatic stellate cells induces $CB_1R$ expression in hepatocytes, which in turn induces lipogenesis. Further, CNS-specific knockout of SF-1 (steroidogenic factor-1) leads to loss of $CB_1R$ expression in the ventromedial hypothalamus (VMH) and CNS-specific SF-1 knockouts therefore do not show the appetite-stimulating effects of $CB_1R$ agonists. SF-1 regulation of $CB_1R$ expression in the VMH is thus required for cannabinoid effects on food intake [15].

### 1.2 $CB_1$ and $CB_2$-Selective Cannabinoid Receptor Agonists

Tetrahydrocannabinol (THC) found in cannabis is an agonist for both $CB_1$ and $CB_2$. Compounds that are significantly more potent at activating $CB_1$ than $CB_2$ receptors include three synthetic analogs of anandamide (Fig. 1): R-(+)-methanandamide, arachidonyl-2'-chloroethylamide (ACEA), and arachidonyl cyclopropylamide [16]. Each of these compounds possesses significant potency and relative intrinsic activity as a $CB_1$ receptor agonist. Noladin ether (2-arachidonyl glyceryl ether) [17] is also a $CB_1$-selective agonist. It has been reported to possess $CB_1$ receptor relative intrinsic activity but has been found to be less potent as a $CB_1$ receptor agonist [18]. As far as $CB_2$-selective agonists are concerned, the most frequently used pharmacological tools are (6a*R*,10a*R*)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran (JWH-133; a classical cannabinoid), {4-[4-(1,1-dimethylheptyl)-2,6-dimethoxy-phenyl]-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl}-methanol (HU-308; a non-classical cannabinoid), and (2-methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenylmethanone (JWH-015) and *R*-3-(2-iodo-5-nitrobenzoyl)-1-methyl-2-piperidinylmethyl)-1*H*-indole (aminoalkylindoles)(AM1241) [19].

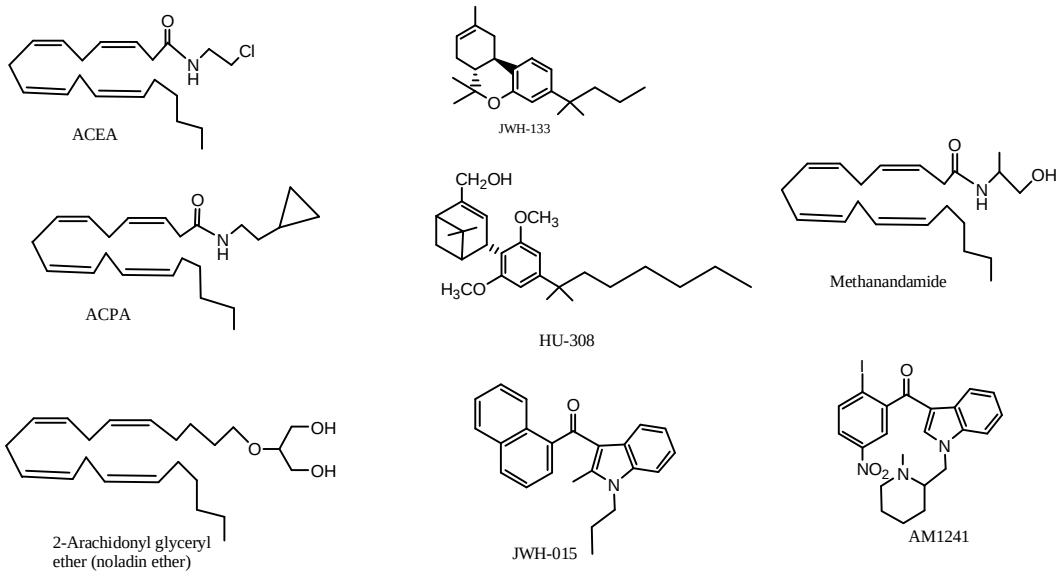



**Figure 1:** The structures of ACEA, arachidonyl cyclopropylamide (ACPA), methanandamide, and noladin ether JWH-133, HU-308, JWH-015, and AM1241 [19].

### 1.2.1 $CB_1$-Selective Competitive Antagonists:

Rimonabant [5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide, SR141716A], *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251), 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide (AM281), 4-[[6-methoxy-2-(4-methoxyphenyl)-3-benzofuranyl]carbonyl]benzonitrile (LY320135) and Taranabant [N-[(2S,3S)-4-(4-chlorophenyl)-3-(3-cyanophenyl)-2-butanyl]-2-methyl-2-{[5-(trifluoromethyl)-2-pyridinyl]oxy}propanamide](Fig. 2) can all block agonist-induced activation of cannabinoid $CB_1$ receptors in a competitive manner and bind with significantly greater affinity to $CB_1$ than $CB_2$ receptors.

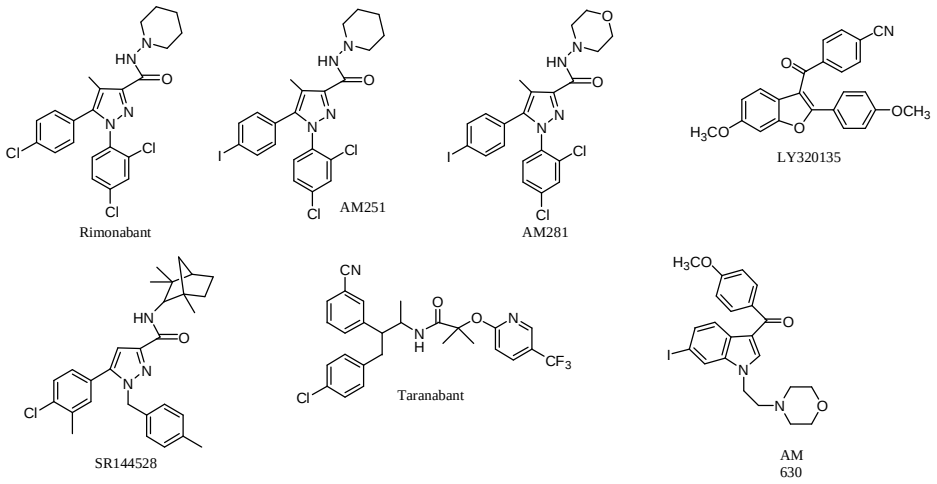



**Figure 2**: The structures of the CB1-selective antagonists/inverse agonists, rimonabant, AM251, AM281, LY320135, and taranabant and of the $CB_2$-selective antagonists/inverse agonists SR144528 and AM630 [19].

Some $CB_1$ receptor competitive antagonists have also been developed that lack any detectable ability to induce signs of inverse agonism at the $CB_1$ receptor when administered alone. One example of such a "neutral" antagonist is *N*-piperidinyl-[8-chloro-1-(2,4-dichlorophenyl)-1,4,5, 6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide] (NESS O327) (Fig. 3), which is a structural analog of Rimonabant and displays markedly higher affinity for $CB_1$ than for $CB_2$ receptors. This compound behaves as $CB_1$ receptor antagonist both *in vitro* and *in vivo* and yet, by itself, does not affect [$^{35}$S]GTPγS binding to rat cerebellar membranes [20]. Several other compounds have been reported to behave as neutral cannabinoid $CB_1$ receptor antagonists. These include (6a*R*,10a*R*)-3-(1-methanesulfonylamino-4-hexyn-6-yl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran (O-2050) (Fig. 3)

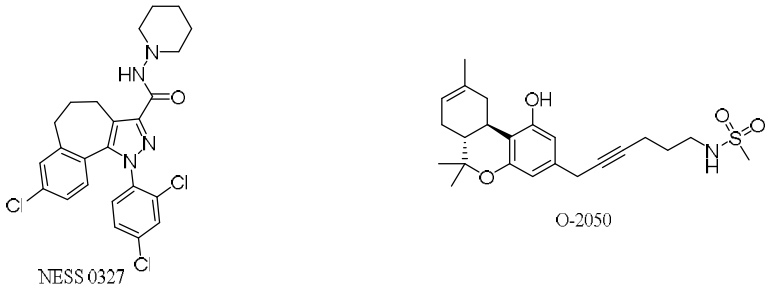



**Figure 3**: The structures of NESS 0327 and O-2050.

Rimonabant hydrochloride (Figure 4 below) is the first therapeutically relevant, potent and selective $CB_1$ receptor inverse agonist, recently approved in Europe as anti-obesity drug, which belongs to diaryl pyrazole family [21]. It was marketed as Acomplia™ by Sanofi-Aventis.

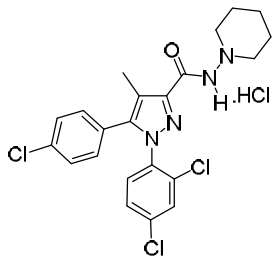



**Figure 4**: Rimonabant hydrochloride

Rimonabant resulted in a significantly greater benefit than placebo for all primary weight loss outcomes. At 1 year, Rimonabant showed a statistically significant beneficial effect on systolic blood pressure, high-density lipoprotein cholesterol, triglycerides and fasting plasma glucose in diabetics and non-diabetics, and glycosylated hemoglobin in diabetics. Improvements were maintained over 2 years with Rimonabant; withdrawal of Rimonabant treatment at 1 year resulted in a reduction in weight loss until there was no difference from placebo at 2 years. However, psychiatric adverse events were experienced by 26% and 14% of Rimonabant and placebo patients respectively [22].

On 23 October 2008, the European Medicines Agency (EMEA) issued a press release stating its Committee for Medical Products for Human Use (CHMP) had concluded the benefits of Rimonabant no longer outweighed its risks, and subsequently recommended the product be suspended from the market. Sanofi-Aventis later released a press statement stating the drug had been suspended. Approval of the drug was officially withdrawn by the EMEA on 16 January 2009.

This decision in turn rapidly led to the termination of several $CB_1$-receptor-antagonist-based anti-obesity drug development programmes [including those for Taranabant, Otenabant, Surinabant and Ibipinabant] [23].

Despite the withdrawal of Rimonabant and the demise of several $CB_1$ receptor antagonist development programmes, many researchers believe

that one has not yet reached the end of the line for anti-obesity treatments targeting the $CB_1$ receptor [24, 25]. It is now believed that neutral antagonists might retain the weight loss advantages of, but will be devoid of reported adverse effects for e.g. of Rimonabant [26]. Another promising approach is to develop agents which do not cross the blood-brain barrier [27].

Srivastava et. al. [28] discloses certain novel compounds of the class tricyclic pyrazole derivatives useful as modulators of cannabinoid receptors having the following general formula (A) (Figure 5):

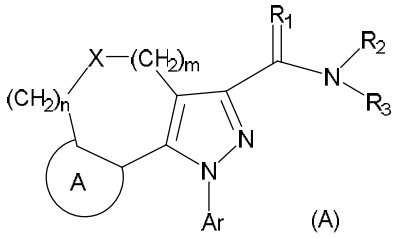



**Figure 5**: General structure of $CB_1$ antagonist disclosed in WO 2006/025069

wherein 'Ar' represents single or fused groups selected from aryl, aralkyl, heterocyclyl, heteroaryl, heterocyclyl($C_1$-$C_{12}$)alkyl & heteroaryl($C_1$-$C_{12}$)alkyl group, each of them independently may optionally be substituted; 'A' represents optionally substituted heteroaromatic or aromatic groups; "X' is selected from $-CH_2-$, O, S, SO, $SO_2$, or NR'; where R' represents H, optionally substituted groups selected from linear or branched alkyl and cycloalkyl groups and, m & n represents integers such that $1 \leq m+n \leq 3$; $R_1$ represents O, S; $R_2$ is either H or ($C_1$-$C_6$) alkyl; $R_3$ is $\overset{\oplus}{\text{—}}NRaRbRc$ or $-NR_bR_c$ where $R_a$ is ($C_1$-$C_6$)alkyl or $R_a$ forms a bridge containing 1-2 atoms, with one of the atoms of the heterocyclic radical formed by $-NR_bRc$; $R_b$ & $R_c$ represents optionally substituted groups selected from alkyl, aralkyl or alkenyl or $R_b$ & $R_c$ together with the nitrogen atom to which they are bonded, form a saturated or unsaturated heterocyclic or heteroaromatic radical which may be optionally substituted and may be fused.

One of the compound disclosed therein is 1H-[1] benzoxepino[5,4-c]pyrazole-3-carboxamide, 8-chloro-1-(2,4-dichlorophenyl)-4,5-dihydro-N-1-piperidinyl, Compound (1) (Figure 6).

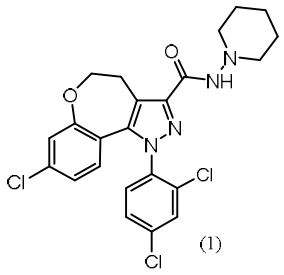



**Figure 6**: Compound of formula (1)

## 2 Studies on 1H-[1]Benzoxepino[5,4-c]pyrazole-3-carboxamide, 8-chloro-1-(2,4-dichlorophenyl)-4,5-dihydro-N-1-piperidinyl, Compound (1)

### 2.1 Rationale & Development strategy

The compound (**1**) exhibited an $EC_{50}$ 14.5 µM in human $CB_1$ receptor forskolin-induced cAMP assay, compared to an $EC_{50}$ of 17.3 nM for Rimonabant. *In-vivo* the compound reversed $CB_1$ agonist-induced hypothermia in Swiss albino mice, indicating the efficacy of the compound. Interestingly, this compound did not change the forskolin-stimulated cAMP accumulation in $CB_1$-transfected HEK cells up to 10 µM concentration in absence of an agonist indicating that this compound may be a neutral antagonist. Therefore, the compound (**1**) though a weak agonist of $CB_1$, its neutral antagonism made it a potential candidate for further development. This compound was always obtained in crystalline form. The crystalline form suffered from poor oral bioavailability and pharmacokinetic profile, like many other promising molecules in the $CB_1$ class. Further, no commercially viable process for the preparation of this compound was developed earlier. Therefore, the compound, though showing promise as a possible neutral agonist was not selected for further development.

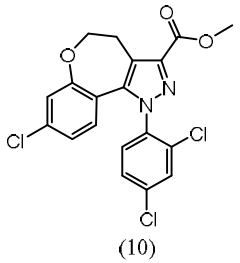
This compound (**1**) has therefore been selected for:

a) Developing a new scalable process for preparation of the compound;
b) Improve the oral bioavailability of the compound through preparation of salts and alternate polymorphic forms.

## 2.2 Chemistry

A scalable process for preparation of the compound (**1**) was developed. The process is described in Scheme 1 below. In order to improve the pharmaceutical properties of the compound, initial attempts were directed at changing the polymorphic form of the compound (**1**) in order to alter the bioavailability. These attempts were limited by the insolubility of the compound in most organic solvents. The compound was found to be soluble upon heating in DMF, DMSO, dichloromethane and acetone. When these solutions were cooled and kept for 48 hours, crystals was obtained from DMF only, while a pasty material was obtained from the DMSO solution. No crystalline material were obtained when acetone or dichloromethane were used as solvents. Upon characterization, the crystal was found to be solvate of DMF. Since, acute exposure to dimethyl formamide has been observed to damage the liver in animals and in humans [29], the crystalline DMF solvate was not tested any further.

Subsequently, attempts were made to prepare various inorganic and organic salts of compound (**1**). The hydrochloride, bisulfate, oxalate and methyl iodide salts were prepared. Interestingly, the hydrochloride salt upon storage is hydrolyed wherein the piperidine ring gets cleaved to form the corresponding methyl ester (**10**) (Figure 7). The structure was confirmed through single crystal analysis.



(10)

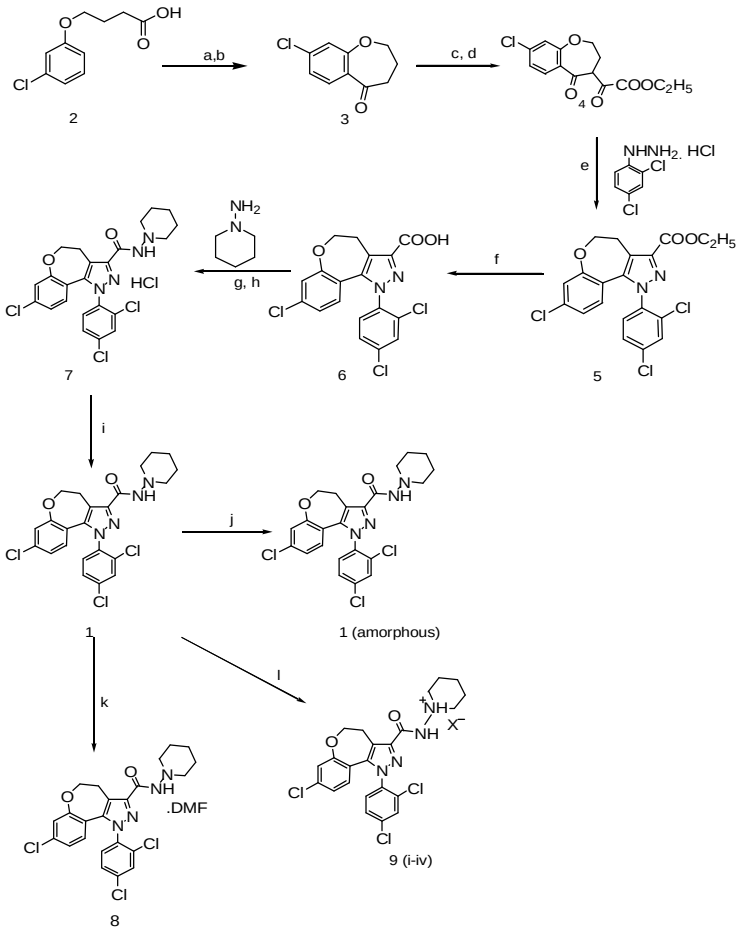
**Figure 7**: Compound of formula (10)

The other salts remained stable. Salt formation did not improve the bioavailability of compound (**1**) and therefore was not developed further.

In a third strategy, the amorphous form was prepared and its efficacy and bioavailability was compared with the crystalline form. *In vitro* $CB_1$ antagonism was measured using a binding assay in CHO cells expressing $hCB_1$ receptors. The $CB_1$ antagonism was also confirmed by reversal of $CB_1$ agonist-induced hypothermia in Swiss albino mice. Anti-obesity effects of crystalline and amorphous forms of compound (**1**) were evaluated using sucrose (5% w/v) consumption models in Zucker fatty rats.

The compound (**1**) was synthesized as described in Scheme 1.

Synthetic scheme for preparing compound 1




**Scheme 1**: Reagents and conditions: (a) $(COCl)_2$, dry DCM, stirred at 0 °C for 30 min and 26-28 °C for 1.5 h; (b) anhydrous $AlCl_3$, DCM, stirred at0-5 °C for 20-25 min. and at 26-27 °C for 30 min; (c) diethyl oxalate, Na metal, ethanol; (d) 2N HCl, $CHCl_3$;(e) ethanol, IPA/HCl, refluxed at 75-77 °C for 2 h; (f) KOH/MeOH; (g) $SOCl_2$, toluene, refluxed at 107-108 °C for 30 min; (h) dry methanol, ethereal HCl, 0-5 °C; (i) NaOH; (j) DCM, reflux under reduced pressure at 50 °C; (k) DMF, heat at 40-50 °C for 2 hours, cooled and kept for 48 hours; (l) acetone, heat at 40 °C till clear solution, added acids corresponding to X= Cl-, $HSO_4$-, $(COO)_2^{-2}$ & $-CH_3I$ in acetone, and the mixture was cooled when necessary and filtered.

Initially the cyclization of 4-(3-chlorophenyl)-butanoic acid **2** was attempted by using polyphosphoric acid. However, the yields were not satisfactory (~25-30%). Subsequently, the process was changed wherein the compound **2** was reacted with oxalyl chloride in dichloromethane at room temperature and subsequently with anhydrous $AlCl_3$ in DCM, and the mixture was

stirred at 0-5 °C for 20-25 minutes and then at 26-27 °C for 30 minutes to provide 8-Chloro-3,4-dihydro-2H-benzo[b]oxepin-5-one **3**. The compound **3** was reacted with sodium metal in ethanol to generate a carbanion and then reacted with diethyl oxalate in ethanol to yield 8-Chloro-5-oxo-2,3,4,5-tetrahydro-benzo[b]oxepin-4-yl)-oxo-acetic acid ethyl ester **4**. Use of other polar solvents such as t-butanol, DMF did not yield good results. Reaction of **4** with 2,4-dichlorophenyl hydrazine hydrochloride in ethanol at 5-7 °C provided 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid ethyl ester **5**. Hydrolysis of **5** using KOH in methanol, yielded 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid **6**. Reduction of **6** with $SOCl_2$/toluene and subsequent reaction with 1-amino piperidine in dichloromethane yielded 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide as an oil which was treated with ethereal HCl when the HCl salt was obtained as a brown solid **7**. Reduction of **6** using $PCl_5$ did not yield any compound. Titration with ethyl acetate and subsequent drying yielded the pure compound **7** as a white solid. Basification of **7** using NaOH provided the compound **1** in crystalline form. Crystalline compound **1** was dissolved in DMF by heating at 40 °C and the solution was cooled and kept for 48 hours when needle shaped crystals of the DMF solvate **8** were formed. The salts were prepared by dissolving **1** in acetone by heating at 40 -50 °C and adding the corresponding acid in acetone and separating the salt formed. The following salts were attempted (Table 2):

Table 1: Salts of compound 1:

| Sl. No. | Salt prepared | Melting point °C (by DSC) | Nature |
|---|---|---|---|
| 1. | Hydrochloride | Onset = 187.4; Peak = 189.0 | Crystalline |
| 2. | Hydrobromide | No salt formation | |

| | | | |
|---|---|---|---|
| 3. | Bisulfate | Onset = 232.7; Peak = 244.4 | Crystalline |
| 4. | Phosphate | A gummy mass was obtained | |
| 5. | Oxalate | Onset = 219.3, Peak = 220.6 (first peak);<br>Onset = 239.7; Peak = 242.1 (second peak) | Crystalline |
| 6. | Citrate | A hazy solution was formed which could not be separated | |
| 7. | Acetate | No solid isolated | |
| 8. | Mesylate | No solid isolated | |
| 9. | Gentisate | No solid isolated | |
| 10. | Benzenesulfonate | Onset = 149.6; Peak = 169.2 | Crystalline |
| 11. | p-toluene sulfonate | A sticky mass was obtained | |
| 12. | Methyl iodide | Onset = 232.7; Peak = 234.5 | Crystalline |

The amorphous form of **1** was prepared by dissolving **1** in dichloromethane at room temperature, stirring and removing the solvent under reduced pressure.

## 3 Results and discussions:

The process of Scheme 1 was found to be scalable and gave the compound (**1**) in high yield (76%). The binding affinity of compound (**1**) was tested in an *in vitro* cAMP assay. The compound (**1**) had an $EC_{50}$ 14.5 µM in human $CB_1$ receptor forskolin-induced cAMP assay. The $CB_1$ antagonism of compound (**1**) was also confirmed by reversal of $CB_1$ agonist-induced hypothermia in Swiss albino mice. Further, as discussed earlier, this compound appeared to

be a neutral antagonist. The anti-obesity effects as well as mean pharmacokinetic effect of both the crystalline and amorphous forms of compound (**1**) were evaluated using sucrose (5% w/v) consumption models in Zucker fa/fa rats. The results are provided in Tables 2 & 3:

**Table 2 :** Mean pharmacokinetic parameters of the crystalline and amorphous form of compound (**1**) in fasted female Zucker fa/fa rats p.o. at 10 mg/kg.*

| Compound | Route | Dose (mg/kg) | $T_{max}$ (h) | $C_{max}$ (ng/mL) | $T_{1/2}$ (h) | AUC (0--∞) (h.ng/mL) |
|---|---|---|---|---|---|---|
| Crystalline | Oral | 10 | 4.5±0.7 | 223±13 | 23.9±6.8 | 3241±125 |
| Amorphous | Oral | 10 | 4.2±1.2 | 575±32 | 22.3±2.9 | 6338±234 |

* Values indicate mean ± SD for n = 6

**Table 3 :** *In vivo* efficacy of the crystalline and amorphous form of the compound (**1**) in 5 % sucrose solution intake model in female Zucker fa/fa rats at single oral dose of 10 mg/kg.*

| ***In-vivo* (in 5% sucrose solution intake)** | | | |
|---|---|---|---|
| **Compound** | **Total consumption in 4hrs** | **% Change vs Control (Sucrose intake)** | **5% Change vs Control (per 100 g body wt.)** |
| Control | 48.00 ± 2.20 | | |
| Rimonabant | 18.4 ± 3.3 | -66.4 ±11.0 | -62.0 ± 15.3 |
| Crystalline Form of 1 | 32.2 ± 6.4 | -38.7 ± 12.2 | -30.2 ± 16.4 |
| Amorphous Form of 1 | 31.9 ± 5.2 | -34.7 ± 11.5 | -34.2 ± 15.3 |

* Values indicate Mean ± SEM for n=6 in 4 h

The crystalline form of the compound (**1**) showed significant appetite suppression in rodent model (Table 3), however its pharmacokinetic profile was poor (Table 2).

The salts of the drug did not alter the pharmacokinetics significantly and therefore, were not studied in details.

Owing to the possibility of amorphous form of the compounds to improve pharmacokinetic and pharmacodynamics properties, the amorphous form of the compound (**1**) was synthesized.

The amorphous form exhibited improved pharmacokinetic and pharmacodynamic parameters (Table 2 & Table 3).

## 4 Conclusion

Amorphous form of compound (**1**) is a $CB_1$ antagonist that shows anti-obesity and anti-dyslipidemic effects in rodent models. The compound (**1**) exhibited acceptable safety profile upto a dose 30 times of the efficacy dose. No changes in bodyweight and weights of the organs (kidney, heart and liver) were observed upto 300 mg/kg dose in a 14 day repeat dose study. The compound also did not bind to hERG upto 3 micromolar concentrations. Also, initial data indicates that the compound is a neutral antagonist and it may be possible that the feeding suppression induced by compound (**1**) will not be accompanied by behavioral signs of nausea. Based on the improved pharmacokinetic and pharmacodynamics profile, the amorphous form of compound (**1**) can be taken up for further pre-clinical and clinical studies.

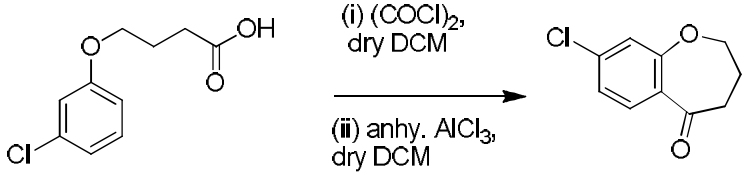
## 5 Experimental Section:

### 5.1 Materials and Methods:

Reagents and solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed using commercial silica gel (230-400 mesh). Melting points were determined on a capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FT IR 8300 spectrophotometer (Vmax in $cm^{-1}$, using KBr pellets or Nujol). The $^1H$ NMR spectra were recorded on a Brucker Avance-300 & 400 spectrometer (300 & 400 MHz). The chemical shifts ($\delta$) are reported in parts per million (ppm) relative to TMS, either in $CDCl_3$ or DMSO-$d_6$ solution. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). $^{13}$CNMR spectra were recorded on Brucker Avance-400 at 100 MHz either in $CDCl_3$ or DMSO-$d_6$ solution. Mass spectra (ESI-MS) were obtained on Shimadzu LC-MS 2010-A spectrometer. HPLC analysis were

carried out at $\lambda_{max}$ 220 nm using column ODS C-18, 150nm * 4.6 nm * 4 $\mu$ on AGILENT 1100 series.

### 5.1.1 8-Chloro-3,4-dihydro-2H-benzo[b]oxepin-5-one (3)




| Reagents | $C_{10}H_{11}ClO_3$ | $C_2O_2Cl_2$ | $AlCl_3$ | $CH_2Cl_2$ | Yield (%) |
|---|---|---|---|---|---|
| Amount | 128.0 g | 74.06 mL | 95.6 g | 2200 mL | 118.0 g (100%) |

4-(3-chlorophenyl)-butanoic acid (128.0 g, 596.74 mmol) was taken in a round bottom flask and dry DCM (1lit) was added to it. The solution was stirred and cooled to -20 °C. To this solution oxalyl chloride (74.06 mL, 835.43 mmol, 1.4 eq.) was added drop wise at –20 °C over a period of 15-20 min. The resulting solution was stirred at 0 °C for 30 min and 26-28 °C for 1.5 h. The progress of the reaction was checked using TLC until all starting material was consumed. The reaction was quenched with ethanol using mobile phase 5 % methanol in chloroform. The solvents were evaporated on a rotatory evaporator under reduced pressure to afford brown oil. Separately, in a 4-necked flask, anhydrous $AlCl_3$ (95.6 g, 716.08 mmol, 1.2 eq) was taken and to it was added dry DCM (1 lit). The suspension was stirred and cooled to 0-5 °C. To this cooled suspension, solution of acid chloride obtained above in dry DCM (200 mL) was added dropwise at 0-5 °C over a period of 20-25 min. The resulting solution was stirred at 26-27 °C for 30 min. TLC was until all starting material was consumed, the mixture was diluted with DM water, extracted with DCM, DCM layer was separated, dried over anhydrous $Na_2SO_4$ using mobile phase 5 % methanol in chloroform. The reaction mixture was poured into mixture of DM water and crushed ice (3 lit) in 5 lit round bottom flask followed by DCM (1 lit). The mixture was

stirred at 26-27 °C for 16 h. The organic layer was separated, washed with DM water, dried over anhydrous $Na_2SO_4$. The organic layer was treated with activated charcoal (3-tea spoon) at 38-39 °C for 15-20 min and filtered hot through hyflow. The organic layer was concentrated on a rotatory evaporator under reduced pressure to yield a brown oil (118 g, 100 %).

IR (KBr $cm^{-1}$): 2970, 2887, 1685, 1595, 1087, 821, 767;
$^1H$ NMR (400 MHz, $CDCl_3$) δ (ppm), 2.22 (m, 2H),2.88 (t, *J* = 6.97 Hz, 2H), 4.25 (t, *J* = 6.60 Hz, 2H),7.06 (m, 2H),7.70 (dd, *J* = 8.72 and 0.77Hz, 1H).
ESI-MS m/z (Relative intensities) (+ve mode) 256.8 $(M+K)^+$

**5.1.2 (8-Chloro-5-oxo-2,3,4,5-tetrahydro-benzo[b]oxepin-4-yl)-oxo-acetic acid ethyl ester (4)**

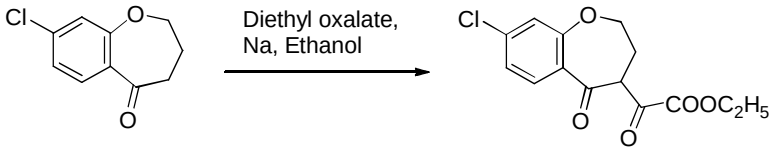



Table 4: Preparation of compound 4

| Reagents | $C_{10}H_9ClO_2$ | $C_6O_{10}O_4$ | Na | $C_2H_6O$ | Yield (%) |
|---|---|---|---|---|---|
| Amount | 150.0 g | 103.67 mL | 35.10 g | 3500 mL | 110.0 g (48.60 %) |

Dry ethanol (1500 mL) was taken in a round bottom flask and to it small pieces of Na metal (31.10 g, 1526.71 mmol, and 2.0 eq) was added with stirring. The solution was stirred till all Na metal was dissolved. The solution was cooled to 25-27 °C. To this diethyl oxalate (103.67 mL, 763.335 mmol, 1.0 eq) was added drop wise at 25-27 °C over a period of 15-20 min and stirred for 25-30 min. at the same temperature. To this solution was added 8-Chloro-3,4-dihydro-2H-benzo[b]oxepin-5-one (150.0 g, 763.335 mmol) in ethanol (2000 mL) drop wise at 25-27 °C over a period of 15-20 min. The resulting yellow solution was stirred at 25-26 °C for 3-4 h. TLC was checked for complete consumption of the starting material, the reaction mixture was diluted with DM water and acidified with dil. HCl, extracted with $CHCl_3$ and using mobile phase 10 % ethyl acetate in petroleum ether. The reaction

mixture was diluted with DM water (2500 mL), acidified with 2 N HCl to pH 4 was and extracted with $CHCl_3$. The chloroform layer was separated, washed with DM water, dried over anhydrous $Na_2SO_4$ and evaporated on a rotatory evaporator under reduced pressure to get yellow oil, which solidifies upon standing. The solid was slurried in diethyl ether (1 L) and filtered to get yellow solid (110 g, 48.6 %).

IR (KBr $cm^{-1}$) 3417, 3109, 1830, 1714, 1614, 1595, 1544, 1207, 1089, 661, 617, 565, 447;

$^1H$ NMR (400 MHz, $CDCl_3$) δ (ppm) 1.44 (t, *J* = 7.11 Hz, 3H), 4.50-4.31 (q, *J* = 8.22 Hz, 2H), 2.88 (m, 2H), 4.37 (m, 2H), 7.26 (m, 2H), 7.93 (d, *J* = 9.3 Hz, 1H).

### 5.1.3 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid ethyl ester (5)

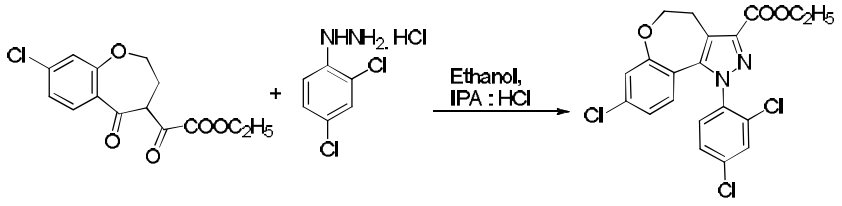



Table 5: Preparation of compound 5

| Reagents | $C_{14}H_{13}ClO_5$ | $C_6H_7Cl_3N_2$ | IPA : HCl | $C_2H_6O$ | Yield (%) |
|---|---|---|---|---|---|
| Amount | 22.0 g | 17.90 g | 2.2 mL | 225 mL | 38.10 g (100 %) |

(8-Chloro-5-oxo-2,3,4,5-tetrahydro-benzo[b]oxepin-4-yl)-oxo-acetic acid ethyl ester (22.0 g, 74.199 mmol) **4** was taken in a round bottom flask and to it was added dry ethanol (225 mL). The suspension was stirred and cooled to 5- 7 °C. To this suspension 2,4-Dichlorophenyl hydrazine hydrochloride (17.90 g, 83.845 mmol, 1.13 eq) was added portion wise at 5-7 °C. The resulting suspension was stirred at 5-7 °C for 15-20 min and brought to 25-26 °C. IPA: HCl (2.2 mL) was added to this mixture and reaction mixture was refluxed at 75-77 °C for 2.0 h. Upon completion of the reaction

(monitored by TLC) the mixture was diluted with DCM using mobile phase 20 % ethyl acetate in petroleum ether. The reaction mixture was cooled to 25-27 $^{o}$C and the solid separated out was filtered on a buchner funnel under suction and dried to afford orange solid (38.0 g, 100 %).

IR (KBr $cm^{-1}$) 3425, 1724, 1679, 1564, 1535, 1454, 1083, 906, 813;

$^{1}$H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.44 (t, *J* = 7.11 Hz, 3H), 3.46 (m, 2H), 4.50-4.31 (m, 4H), 6.66 (d, *J* = 8.58 Hz, 1H), 6.83 (dd, *J* = 8.58 Hz, 2H), 7.34 (d, *J* = 2.25 Hz, 2H), 7.40 (t, 1H).

ESI-MS m/z (Relative intensities) (+ve mode) 458.5 $(M+H)^{+}$

### 5.1.4 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid (6)

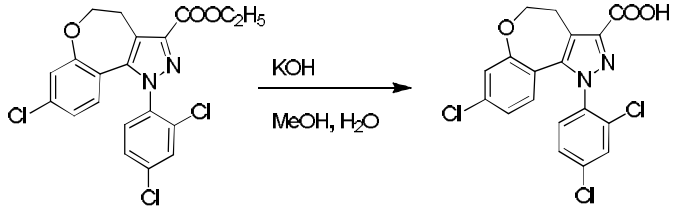



Table 6: Preparation of compound 6

| Reagents | $C_{20}H_{15}Cl_3N_2O_3$ | KOH | $CH_3OH$ | $H_2O$ | Yield (%) |
|---|---|---|---|---|---|
| Amount | 38.0 g | 9.73 g | 400 mL | 100 mL | 20.2 g (56.80 %) |

8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid ethyl ester (38.0 g, 86.857 mmol) **(5)** was taken in a round bottom flask and to it was added methanol (300 mL). To this a solution of KOH (9.73 g, 173.174mmol, 2.0 eq) in methanol: water mixture (1:1) (200 mL) was added and reaction mixture was refluxed at 65-68 $^{o}$C for 2.0 h. TLC was checked for completion, and then the mixture was diluted with DM water, acidified to pH 4 using 10 % HCl solution, extracted with ethyl acetate and using mobile phase 10 % methanol in chloroform. The reaction mixture was cooled to 25-26 $^{o}$C, poured into ice cold water and acidified to pH 4 using 10 % HCl solution. The solid separated out was

filtered on a buchner funnel under suction. The off white solid was washed with water, dried under suction. The solid was further taken in IPA, stirred for 10-15 min and filtered to afford an off white solid (20.2 g, 56.8 %)

IR (KBr $cm^{-1}$) 3450, 3082, 1687, 1596, 1568, 1431, 1386, 1035, 985, 567, 549, 455;

$^1H$ NMR (400 MHz, DMSO-$d_6$) δ (ppm): 4.04 (m, 4H),6.71 (d, *J* = 8.58 Hz, 1H), 7.01 (dd, J = 2.19 Hz, *J* = 8.58 Hz, 1H),7.23 (d, *J* = 2.13 Hz, 1H), 7.72-7.64 (m, 2H), 7.88 (d, *J* = 2.04 Hz, 1H), 13.08 (bs, 1H).

ESI-MS m/z (Relative intensities) (+ve mode) 410.5 $(M+H)^+$

### 5.1.5 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide (1), crystalline

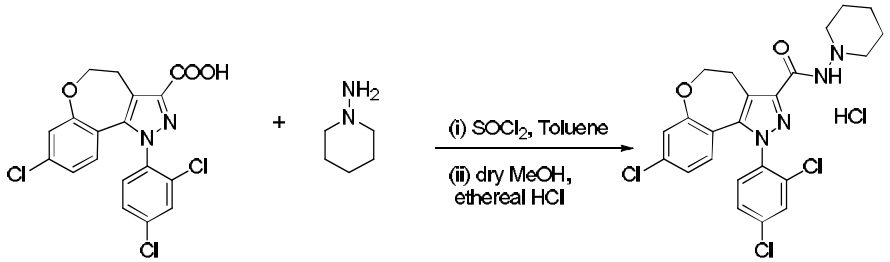



Table 7: Preparation of compound 1

| Reagents | $C_{18}H_{11}Cl_3N_2O_3$ | $C_5H_{12}N_2$ | $SOCl_2$ | $C_7H_8$ | Yield (%) |
|---|---|---|---|---|---|
| Amount | 5.0 g | 1.83 g | 2.91 g | 30 mL | 5.0 g (HCl salt)<br>(99. 5 % by HPLC) |

8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid (5.0 g, 12.25 mmol) was taken in a round bottom flask and toluene (30 mL) was added to it. To this mixture thionyl chloride (2.91 g, 24.5 mmol) was added and the mixture was refluxed at 107-108 °C for 30 min. The reaction was quenched with amino piperidine using mobile phase 50 % EtOAc in hexane. Subsequently, the reaction mixture was cooled to 30-35 °C and transferred into single neck round

bottom flask. The solvents were evaporated on a rotatory evaporator under reduced pressure to afford oil. The oil was further diluted in dry DCM (20 mL), cooled to 0-5 $^{o}$C in an ice bath and treated with 1-Amino piperidine (1.83 g, 18.38 mmol). The resulting mixture was stirred at 25- 26 $^{o}$C for 15-20 min. The mixture was diluted with DM water and extracted with ethyl acetate using mobile phase 50 % EtOAc in hexane. The reaction mixture was again diluted with DM water (200 mL) and extracted with toluene. The organic layer was separated, washed with DM water (100 mL), brine solution, dried over anhydrous $Na_2SO_4$ and solvents were evaporated on a rotatory evaporator under reduced pressure to afford oil. The oil was diluted with dry methanol (25 mL), cooled to 0-5 $^{o}$C in an ice bath and treated with ethereal HCl (5-6 mL). The solvents were evaporated on a rotatory evaporator under reduced pressure to afford brown solid. The crude solid was triturated in ethyl acetate, filtered on a Buchner funnel under suction and dried to afford an off white solid (5.0 g, 77.6 %)

$^{1}$HNMR: (DMSO-$D_6$, 300 MHz): δ1.31(m, 2H), 1.53 (m, 4H), 2.7 (m, 4H), 3.23 (bs, 2H), 4.25 (bs, 1H), 4.45 (bs, 1H), 6.72 (d, *J*= 8.61 Hz, 1H), 7.03 (dd, *J*= 10.56, 1.95 Hz, 1H), 7.26 (d, *J*= 1.95 Hz, 1H), 7.68 (dd, *J*= 10.47, 1.95 Hz, 1H), 7.78 (d, *J*= 8.49 Hz, 1H), 7.89 (d, *J*= 2.01 Hz, 1H), 9.1(s, 1H).
Basification with NaOH gave the compound of formula 4 in crystalline form

$^{1}$HNMR: (DMSO-$D_6$, 300 MHz): δ 1.31(m, 2H), 1.53 (m, 4H), 2.7 (m, 4H), 3.23 (bs, 2H), 4.25 (bs, 1H), 4.45 (bs, 1H), 6.72 (d, *J*= 8.61 Hz, 1H), 7.03 (dd, *J*= 10.56, 1.95 Hz, 1H), 7.26 (d, *J*= 1.95 Hz, 1H), 7.68 (dd, *J*= 10.47, 1.95 Hz, 1H), 7.78 (d, *J*= 8.49 Hz, 1H), 7.89 (d, *J*= 2.01 Hz, 1H), 9.1(s, 1H)
Melting point (by DSC): Onset = 195.3 °C, peak = 196.6 °C.

XPRD: 6.97, 16.42, 17.53, 18.19, 18.35, 19.8, 20.93, 21.4, 23.69, 27.62$^{o}$ ± 0.2 degrees 2Θ.

### 5.1.6 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide (1), dimethyl formamide solvate (8)

IR (KBr $cm^{-1}$) 3412, 2941, 1678, 1595, 1564, 1492, 1290, 1265, 1209, 1153, 1026, 983, 956, 869, 817, 810;

$^1H$ NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45-1.43 (m, 2H),1.78-1.72 (m, 4H), 2.85(s, 3H), 2.95-2.85 (m, 4H), 2.95 (s, DMF), 3.49 (bs, 2H), 4.38 (bs, 2H), 6.60 (d, *J* = 8.4 Hz, 1H), 6.79 (dd, *J* = 8.8 Hz, *J* = 2.0 Hz, 1H), 7.14 (d, *J* = 2.0 Hz, 1H),7.33 (dd, *J* = 9.2 Hz, *J* = 4.4 Hz, 1H), 7.39 (dd, *J* = 8.6 Hz, *J* = 2.0 Hz, 1H), 7.53 (d, *J* = 2.4 Hz, 1H),7.63 (s, 1H), 8.01 (s, DMF).

ESI-MS m/z (Relative intensities) (+ve mode) 493.0 $(M+H)^+$ (100%)

### 5.1.7 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide (1), dimethyl sulfoxide solvate (8a)

IR (KBr $cm^{-1}$) 3412, 2941, 1678, 1595, 1564, 1492, 1290, 1265, 1209, 1153, 1026, 983, 956, 869, 817, 810;

$^1H$ NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45-1.43 (m, 2H),1.78-1.72 (m, 4H), 2.85(s, 3H), 2.95-2.85 (m, 4H), 2.61 (s, DMSO), 3.49 (bs, 2H), 4.38 (bs, 2H), 6.60 (d, *J* = 8.4 Hz, 1H), 6.79 (dd, *J* = 8.8 Hz, *J* = 2.0 Hz, 1H), 7.14 (d, *J* = 2.0 Hz, 1H),7.33 (dd, *J* = 9.2 Hz, *J* = 4.4 Hz, 1H), 7.39 (dd, *J* = 8.6 Hz, *J* = 2.0 Hz, 1H), 7.53 (d, *J* = 2.4 Hz, 1H),7.63 (s, 1H).

ESI-MS m/z (Relative intensities) (+ve mode) 493.0 $(M+H)^+$ (100%)

### 5.1.8 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide oxalate {9 (i)}

Placed 0.50 g (1.017 mmol) of compound **1** into round bottom flask, followed by 10 ml acetone. The suspension was warmed upto 45-50°C using hot water to get clear solution. To this solution of 0.075 g (1.017 mmol) oxalic acid into 3 ml acetone was added slowly. The resulting clear solution was cooled to 0-5°C and stirred for 35-40 mins. The solid started separating out from the reaction mixture. The solid was filtered, washed with acetone and dried under vacuum.

Weight of white solid substance = 0.267 g

% yield = 45.9%

IR (KBr $cm^{-1}$) 3412, 2951, 1899, 1691, 1597, 1564, 1492, 1236, 817, 704;

$^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) 1.4-1.3 (m, 2H),1.64-1.58 (m, 4H), 2.82-2.80 (m, 4H), 3.28 (bs, 2H), 3.40 (bs, 2H), 4.26 (bs, 1H), 4.49 (bs, 1H), 6.74 (d, *J* = 8.4 Hz, 1H), 7.04 (dd, *J* = 8.6 Hz, *J* = 2.4 Hz, 1H), 7.27 (d, *J* = 2.4 Hz, 1H), 7.71 (dd, *J* = 8.4 Hz, *J* = 2.4 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 2.4 Hz, 1H), 9.19 (s, 1H).

ESI-MS m/z (Relative intensities) (+ve mode) 493.05 $(M+H)^+$ (80%)

### 5.1.9 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylicacid piperidin-1-ylamide benzene sulfonate salt {9 (ii)}

Placed 0.50 g (1.017 mmol) of **1** into a round bottom flask and to it was added 10 ml acetone. The suspension was warmed upto 45-50 °C using hot water to get clear solution. To this solution of 0.160 g (1.017 mmol) benzene sulfonic acid in 3 ml acetone was added slowly. The resulting clear solution was cooled to 0-5 °C and stirred for 35-40 mins. The solid started separating out from the reaction mixture. The solid was filtered, washed with acetone and dried under vacuum.

Weight of White solid substance = 0.350 g

% yield = 53.0%

IR (KBr $cm^{-1}$) 3383, 3153, 2945, 1896, 1701, 1697, 1606, 1552, 1219, 1151, 981, 956, 896, 731;

$^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm) 1.5-1.4 (m, 2H),1.83-1.80 (m, 4H), 3.33-3.32 (m, 5H), 3.46-3.43 (m, 1H), 4.30-4.29 (m, 1H), 4.55-4.52 (m, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 7.07 (dd, *J* = 8.6 Hz, *J* = 2.4 Hz, 1H), 7.37-7.30 (m, 4H), 7.65-7.61 (m, 2H),7.73 (dd, *J* = 8.6 Hz, *J* = 2.4 Hz, 1H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.94 (d, *J* = 2.4 Hz, 1H), 11.05 (bs, 1H).

ESI-MS m/z (Relative intensities) (+ve mode) 493.0 $(M+H)^+$ (100%)

### 5.1.10 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylicacid piperidin-1-ylamide bisulfate {9 (iii)}

Placed 1.0 g (2.034 mmol) of **1** into round bottom flask and to it was added 15 ml acetone. The suspension was warmed upto 45-50 °C using hot water to get clear solution. To this solution of 0.108 ml (2.034 mmol) conc. $H_2SO_4$

was added dropwise. The resulting clear solution was cooled to 0-5 °C and stirred for 15-20 mins. The solid started separating out from the reaction mixture. The solid was filtered, washed with acetone and dried under vacuum.

Weight of White solid substance = 1.03 g
% yield = 85.87%
IR (KBr $cm^{-1}$) 3404, 3153, 2951, 1708, 1599, 1550, 1369, 1215, 1049, 987, 958, 868, 812;
$^1$HNMR: (DMSO-$D_6$, 300 MHz): δ 1.31(m, 2H), 1.53 (m, 4H), 2.7 (m, 4H), 3.23 (bs, 2H), 4.25 (bs, 1H), 4.45 (bs, 1H), 6.72 (d, *J*= 8.61 Hz, 1H), 7.03 (dd, *J*= 10.56, 1.95 Hz, 1H), 7.26 (d, *J*= 1.95 Hz, 1H), 7.68 (dd, *J*= 10.47, 1.95 Hz, 1H), 7.78 (d, *J*= 8.49 Hz, 1H), 7.89 (d, *J*= 2.01 Hz, 1H), 9.1(s, 1H).
ESI-MS m/z (Relative intensities) (+ve mode) 493.0 $(M+H)^+$ (99%)

#### 5.1.11 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylicacid piperidin-1-ylamide methyl iodide {9 (iv)}

Placed 5.0 g (10.17 mmol) of **1** in a round bottom flask and to it was added 15 ml acetone. The suspension was warmed to 45-50 °C using hot water to get clear solution. To this solution 14.435 (6.322 mmol) methyl iodide was added. The resulting clear solution was refluxed overnight at 42 °C. The solid was dissolved in distilled acetone and resulting solution was concentrated under reduced pressure at 45 °C to get yellowish solid. The solid was filtered, washed with acetone and dried under vacuum.

Weight of solid substance = 5.3 g
% yield = 82.81%
IR (KBr $cm^{-1}$) 3419, 2958, 1593, 1564, 1496, 1309, 1294, 1209, 1030, 954, 920, 869, 812;
$^1$HNMR: (DMSO-$D_6$, 300 MHz): δ 1.31(m, 2H), 1.53 (m, 4H), 2.2 (s. 3H, methyl iodide), 2.7 (m, 4H), 3.23 (bs, 2H), 4.25 (bs, 1H), 4.45 (bs, 1H), 6.72 (d, *J*= 8.61 Hz, 1H), 7.03 (dd, *J*= 10.56, 1.95 Hz, 1H), 7.26 (d, *J*= 1.95 Hz, 1H), 7.68 (dd, *J*= 10.47, 1.95 Hz, 1H), 7.78 (d, *J*= 8.49 Hz, 1H), 7.89 (d, *J*= 2.01 Hz, 1H), 11.2 (s, 1H)

ESI-MS m/z (Relative intensities) (+ve mode) 507.0 $(M+H)^+$ (100%)

### 5.1.12 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylicacid piperidin-1-ylamide (1), amorphous

The crystalline 8-Chloro-1-(2,4-dichlorophenyl)-4,5-dihydro-1H-6-oxa-diaza-benzo[c]azulene-3-carboxylic acid-piperidin-1-ylamide (5.0 g, 10.17 mmol) was placed in a round bottom flask and added 100 mL of dichloromethane. The resulting solution was stirred at 27-29 °C for 10 min. The solvents were removed under reduced pressure at 50 °C to afford white solid whose XRD pattern established it to be in amorphous form.

The DSC thermogram showed two peaks one at 182.9 °C and the other at 196.3 °C.

## 5.2 Biological studies:

### 5.2.1 *In vitro* cAMP assay:

Fatty acid-free BSA, IBMX (isobutyl methyl xanthine), RO20-1724 {4-[(3-butoxy-4-methoxyphenyl) methyl]-2-imidazololidinone}, forskolin and DMSO (hybrimax) were purchased from Sigma Chemical Co. cAMP detection ELISA kit was from Assay Designs, USA. Tissue culture reagents were purchased from Sigma and Hi-media. Other reagents used were all of analytical grade. The cAMP assay was carried out in Chinese Hamster Ovarian (CHO) cells (CHOK1) stably expressing human $CB_1$ receptor following the method of Rinaldi-Carmona et. al [30]. Cells grown to 80% confluence were maintained in HAM'S F12 medium containing 10% heat inactivated dialyzed fetal bovine serum and 0.8 mg/mL G-418. Cells were seeded at a density of 50,000 cells/well in 24-well plate, grown for 16–18 h, washed once with PBS and incubated for 30 min at 37 °C in plain HAM'SF12 containing 0.25% free fatty acid BSA, IBMX (0.1 mM) and RO20-1724 (0.1 mM). IBMX, the pan phosphodiesterase inhibitor and RO20-1724, the specific phosphodiesterase-4 inhibitor were added to restore cAMP up to the detection limit. After 5 min incubation with the drugs, forskolin was added at a final concentration of 10 mM and incubation was carried out for

another 20 min at 37 °C. The reaction was terminated by washing once with PBS and adding 200 Llysis buffer comprising 0.1 N HCl and 0.1% Trition X-100. The lysates were centrifuged and aliquotes from supernatants were used for detection of cAMP by ELISA as per the manufacturer's protocol.

### 5.2.2 5% Sucrose Solution Intake in Zuckerfa/fa rats:

All the animals used in the study were procured from the Animal Breeding Facility of Zydus Research Center. Institutional Animal Ethical Committee approved all the study protocols. Female Zucker fa/fa rats (age of 10–12 weeks and 300–350 g of weight) were used for *in vivo* experiments. Compounds were suspended with 0.5% carboxymethyl cellulose sodium salt in distilled water. The test compounds were administered at the dose of 10 mg/kg and by oral route in a volume of 2 mL/kg body weight. The obese Zucker fa/fa rats were housed individually and subjected to training for consuming 5% sucrose solution over a period of 4 h, by allowing access to the 5% sucrose solution in the bottles. Food and water were withdrawn during this time. This training was given for six consecutive days, at the same time of the day. On seventh day, the animals were randomized into groups of six animals each and treated with the test compounds. After one hour of treatment, the animals were exposed to the 5% sucrose solution for 4 h as that of the training schedule. The amount of sucrose solution consumed by each animal was calculated. Difference between the control and treatment groups were analyzed by performing one way ANOVA followed by Dunnett's test on sucrose solution consumption using Graphpad Prism software.

### 5.2.3 Pharmacokinetics experiment:

Pharmacokinetics of the test compound was studied via per-oral route of administration in wistar rats of 8 to 10 weeks of age. Animals were fasted for 18 hours and food was supplied after 4 hours of administration of the test compound. There was free access to water throughout the study. A homogenous suspension of the test substance was prepared in 0.5 % w/v CMC in normal saline and a per-oral dose of 30 mg/kg was administered.

After the administration of the test compounds, blood samples were withdrawn at various time intervals through retro-orbital plexus and collected into heparinized micro centrifuge tubes. Plasma was separated by centrifugation at 4000 rpm for 5 min at ambient temperature and analyzed immediately. Remaining samples were stored at -20 °C until analyzed.

Analysis was carried out by taking an aliquots of 180 µL plasma and 20 µL of internal standard (Atorvastatin) and was extracted with 2.5 mL of extracting solvent (ethyl acetate: acetonitrile 80:20, v/v) in glass test-tube by vortexing with spinix vortex mixture for a minute. This was then centrifuged at 2000 rpm for 2.0 min. The supernatant was transferred to another glass test-tube and the solvent was evaporated under nitrogen using Zymark evaporator at 40 °C. Finally, the tubes were reconstituted with 0.1 mL diluent (acetonitrile: methanol: water 40: 40: 20, v/v/v). The reconstituted samples were analyzed on Agilent 1100 Series HPLC system with a mobile phase of 0.05 % v/v trifluoroacetic acid in water: acetonitrile (32:68, v/v); flowing at a flow rate of 1.0 mL/min through a Kromasil 250 mm x 4.6 mm x 5 µ column maintained at 30 °C. Chromatographic separation was achieved within 15 min. Agilent software version Chemstation Rev.A.09.01. (1206) was used to acquire and process all chromatographic data. Quantification was based on a series of calibrators ranging from 0.031 to 32 µg/mL, prepared by adding test compound to drug free rat plasma. Quality control samples were analyzed in parallel to verify that the system performs in control. Pharmacokinetic parameters namely - maximum plasma concentration ($C_{max}$), time point of maximum plasma concentration ($t_{max}$), area under the plasma concentration - time curve from 0 hour to infinity ($AUC_{0-\infty}$) and half-life of drug elimination during the terminal phase ($t_{1/2}$) were calculated from plasma concentration versus time data, by standard non-compartmental methods, using the WinNonLin software version 4.0.1 procured from Pharsight Corporation, USA.

## 6 References

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# Characterization Data

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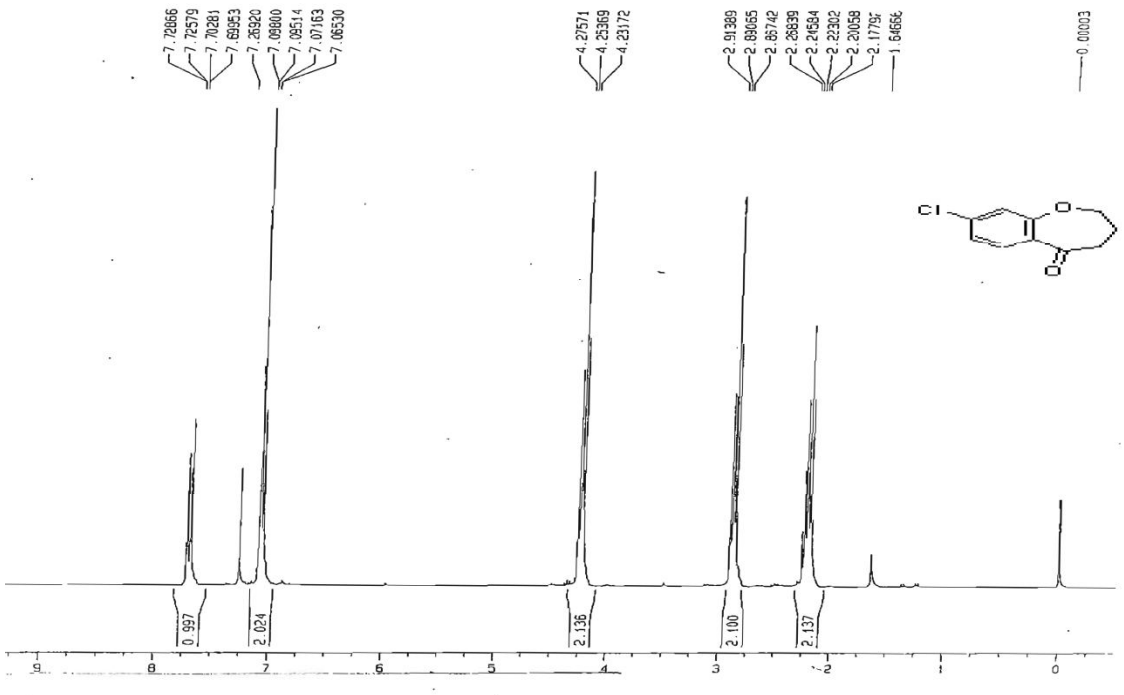
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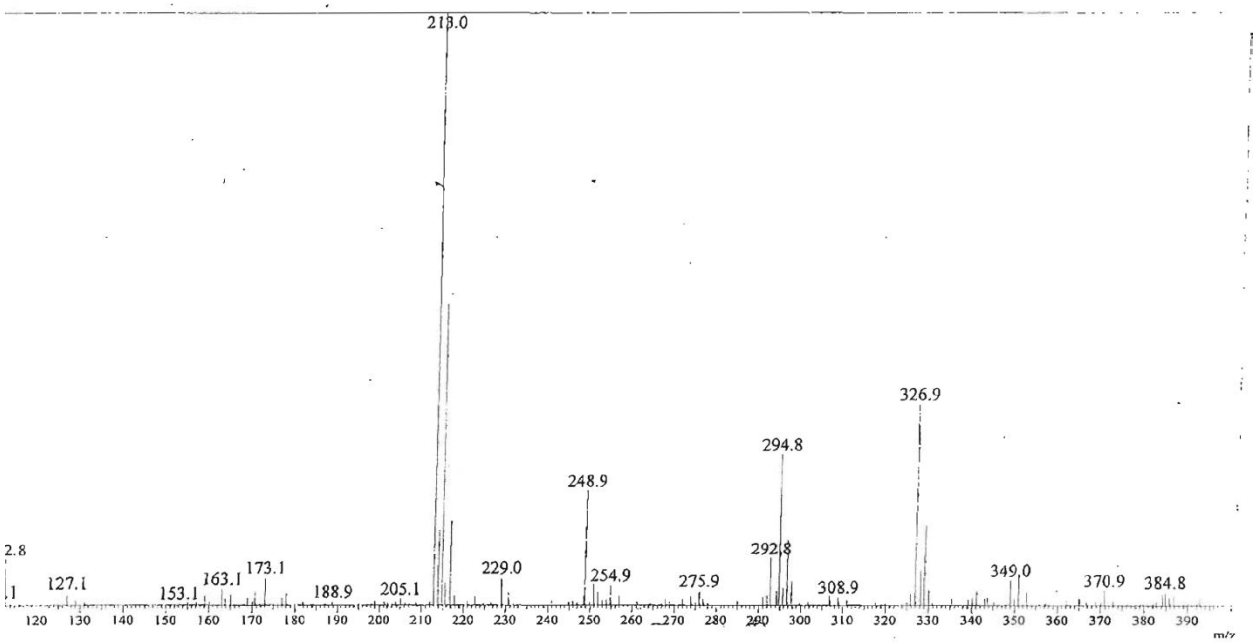
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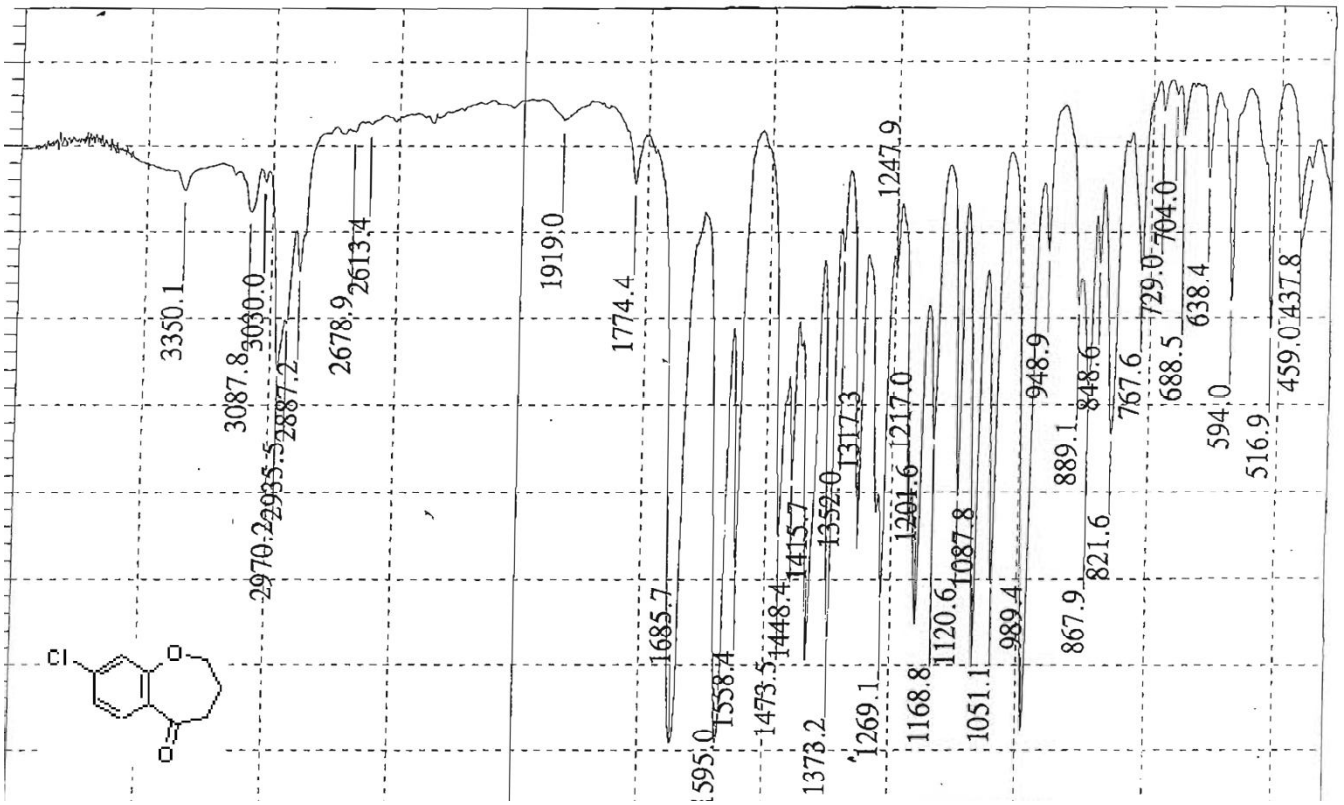
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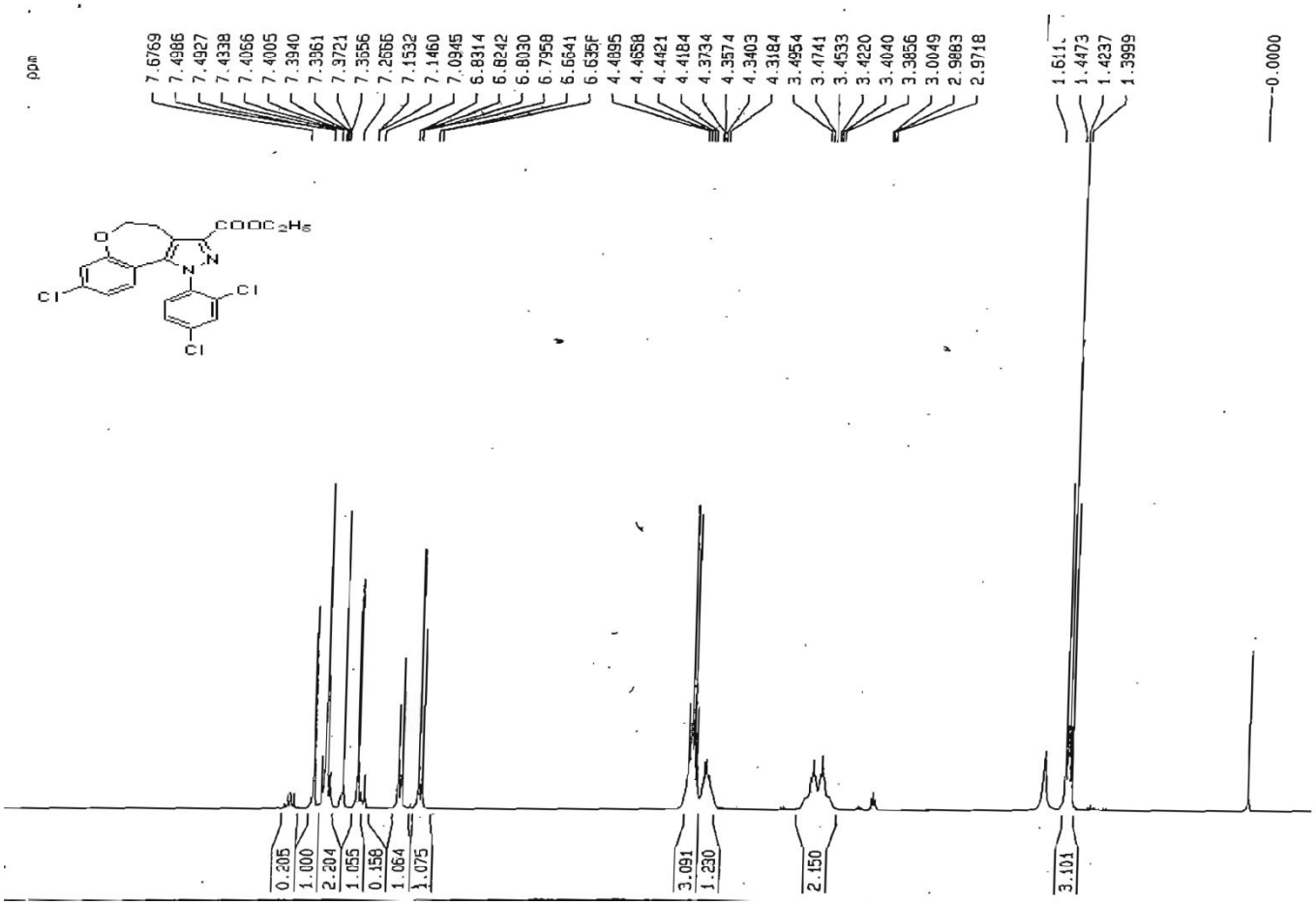
## 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid ethyl ester (5)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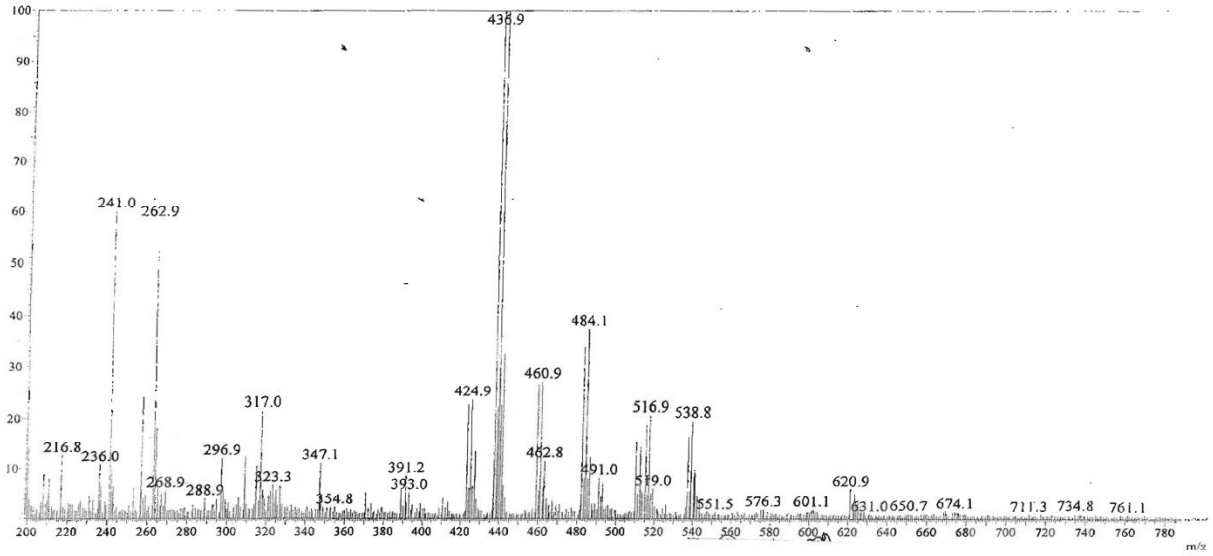
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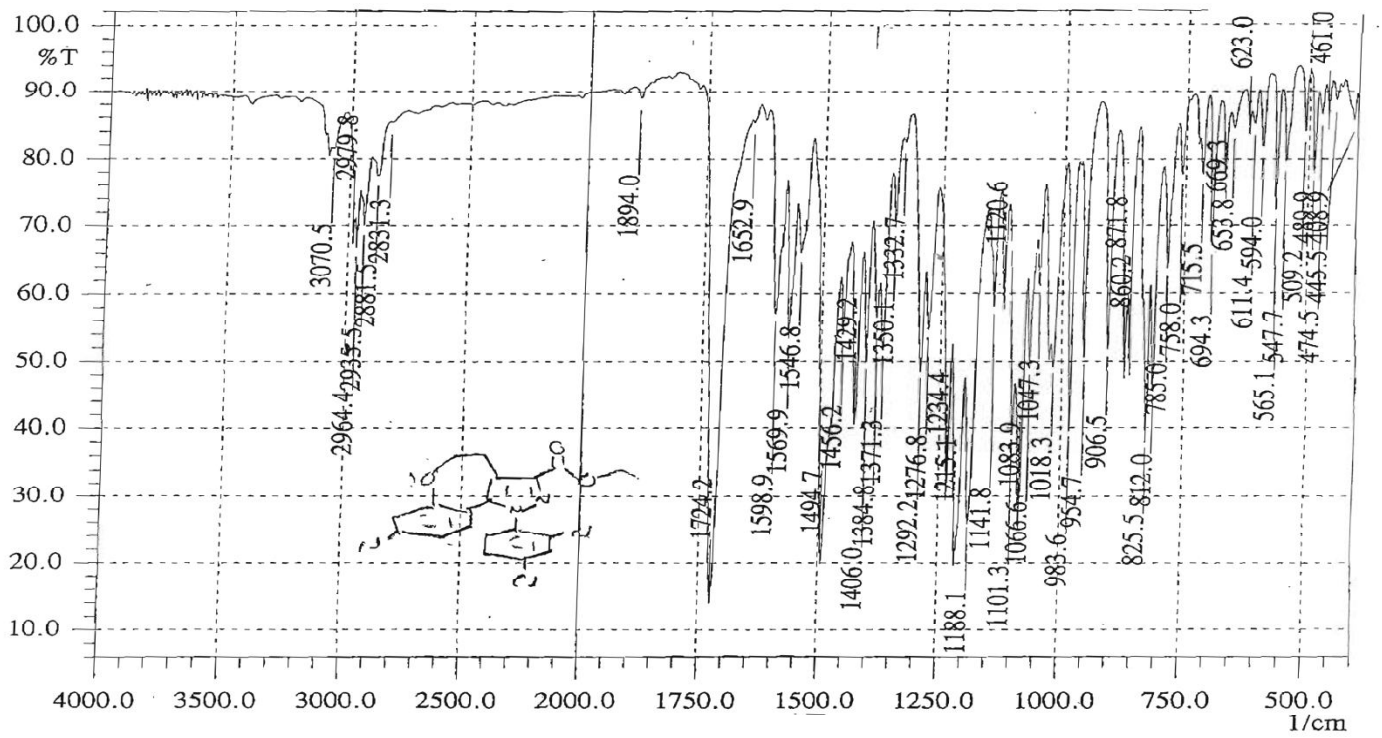
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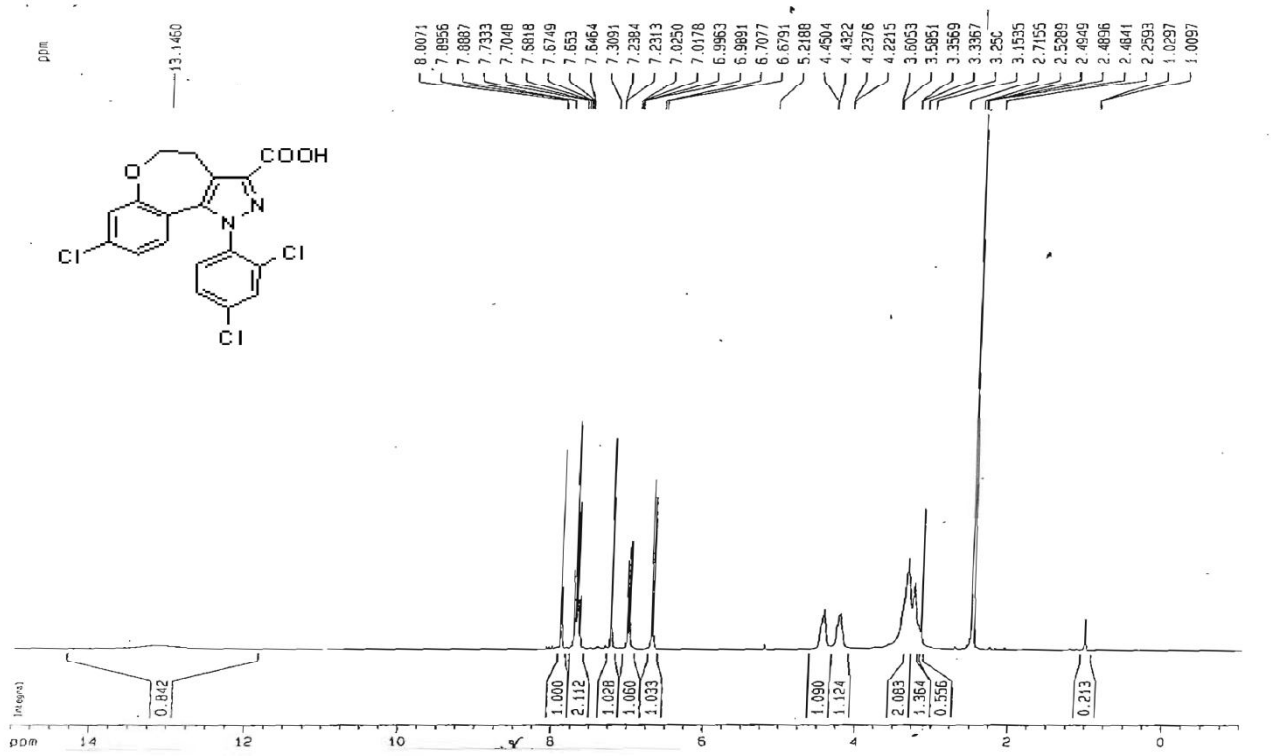
## 8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid (6)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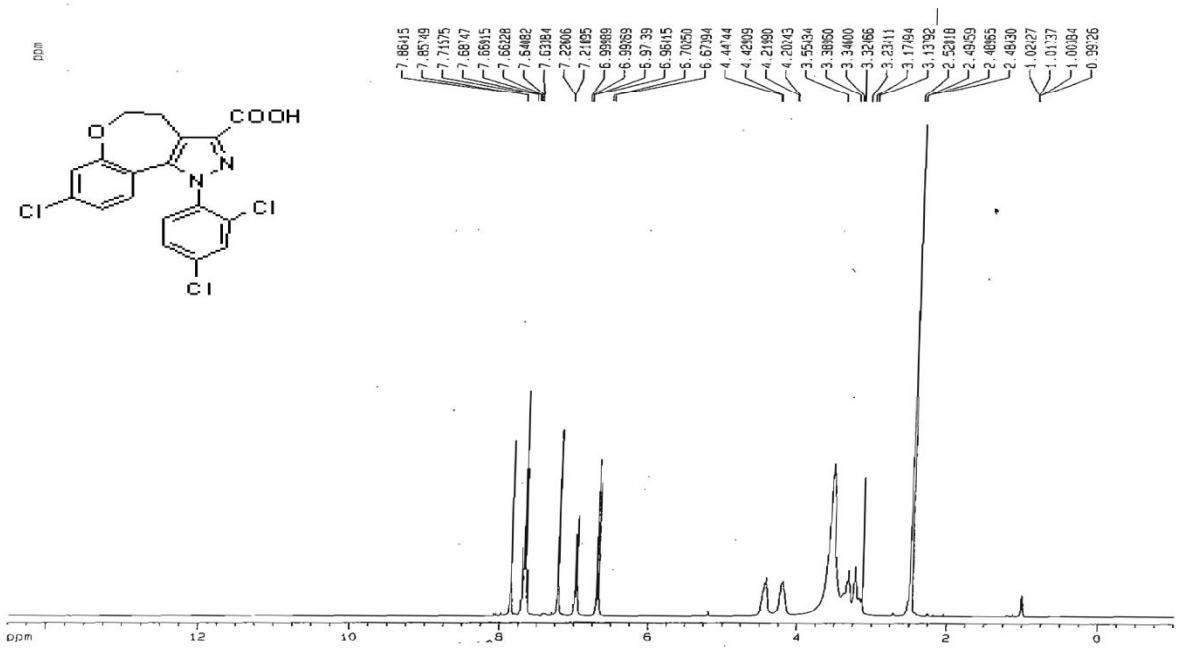
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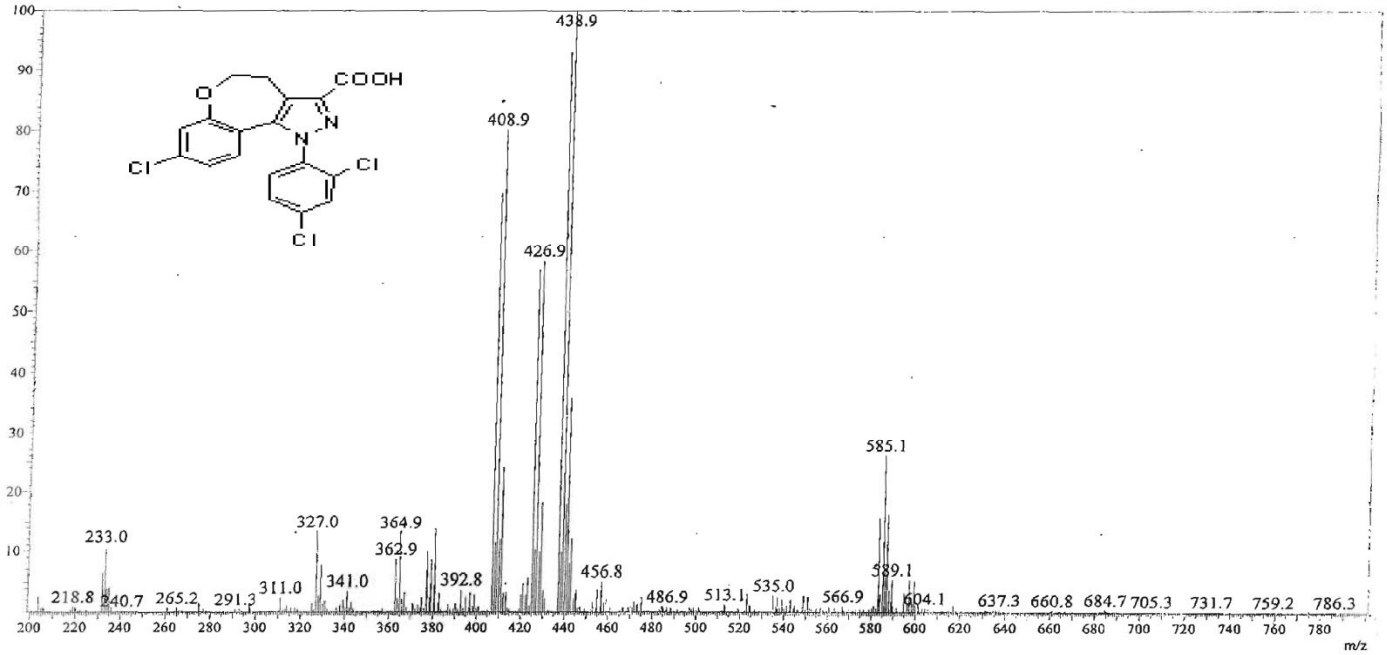
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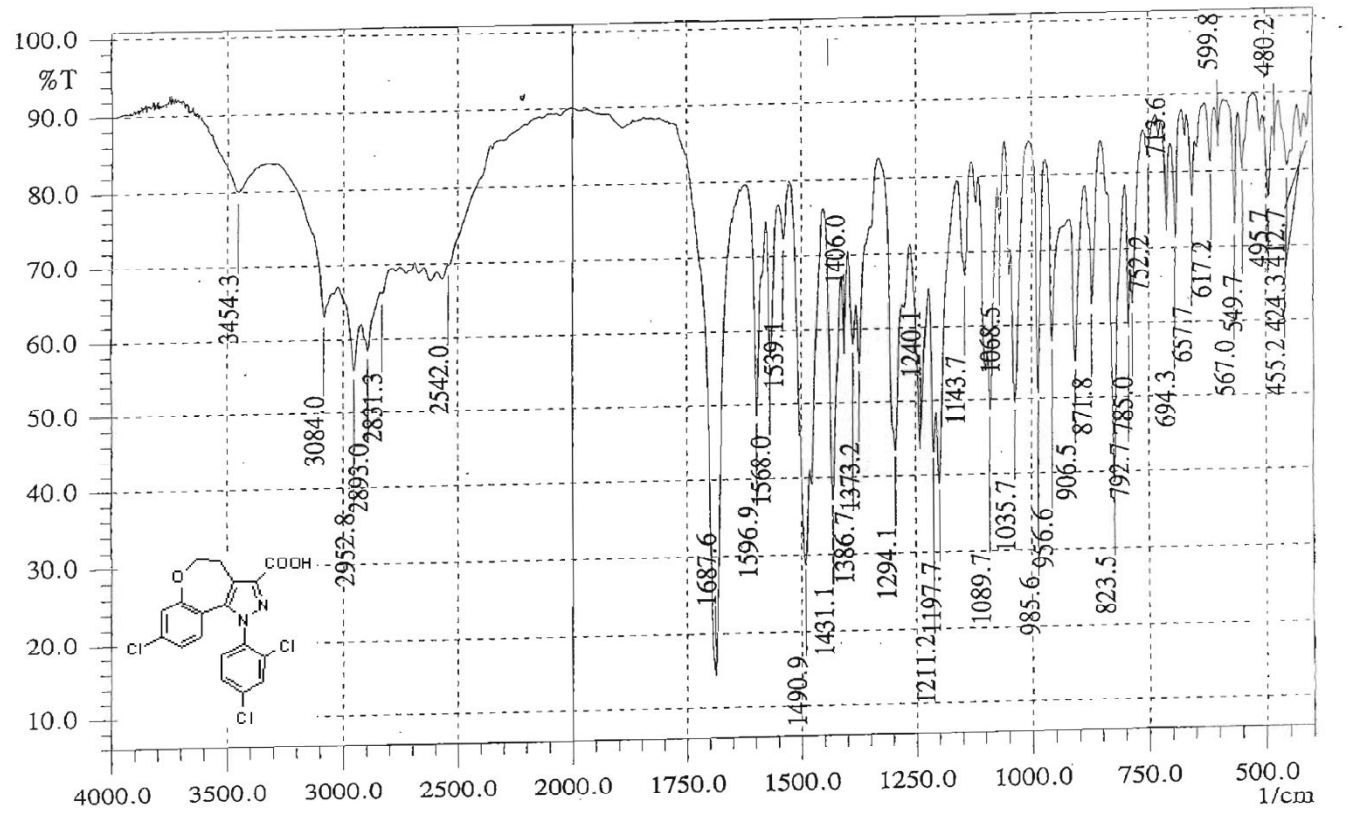
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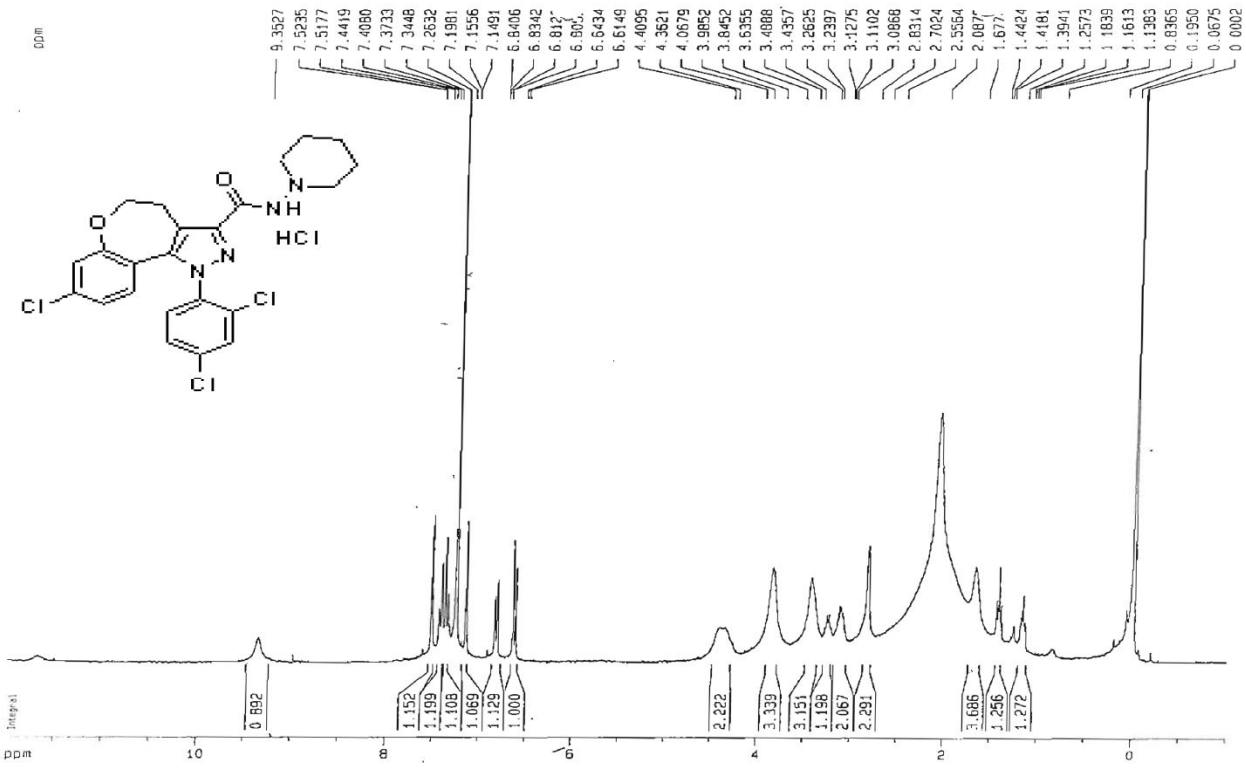
**8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide.HCl**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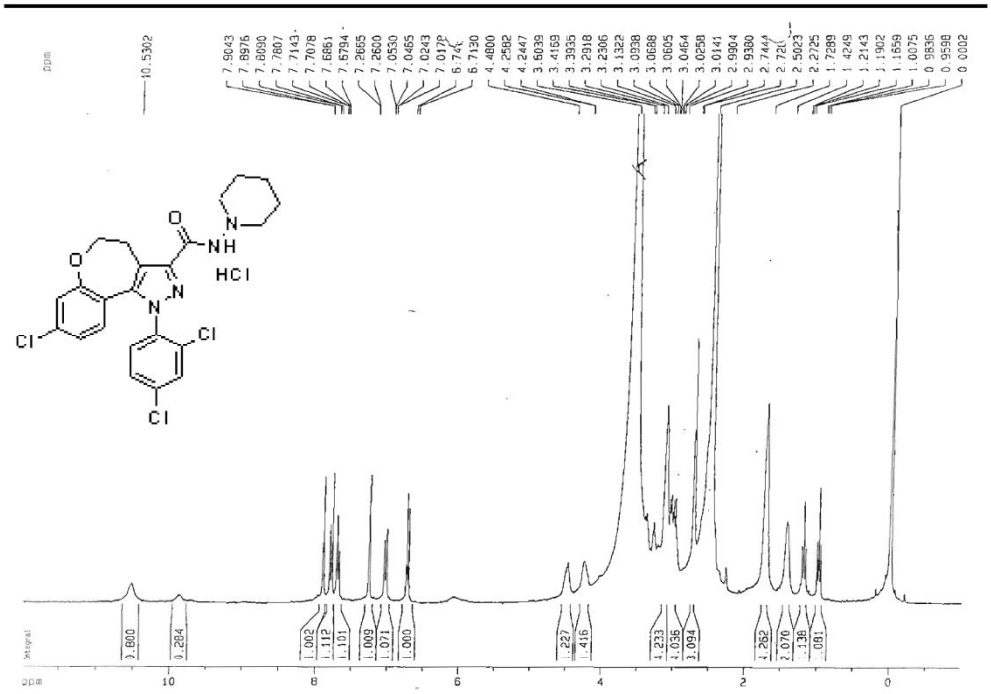
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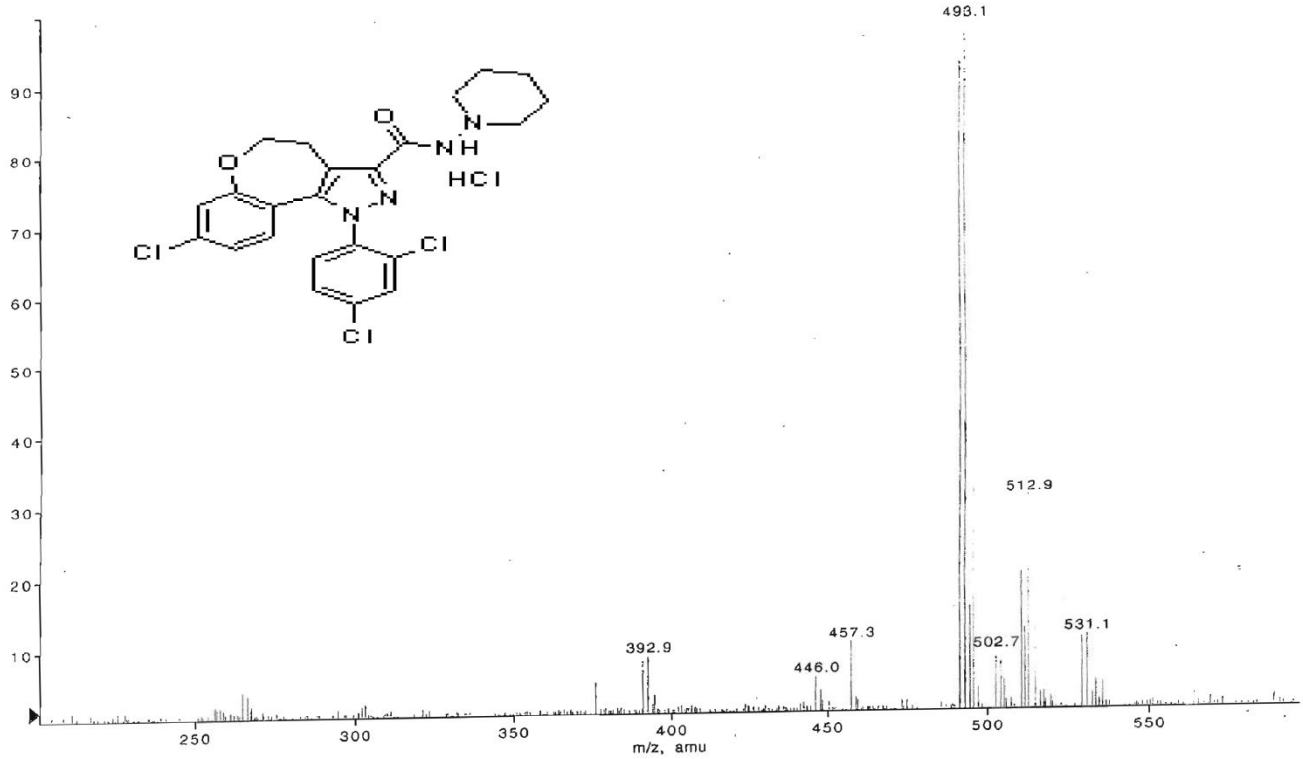
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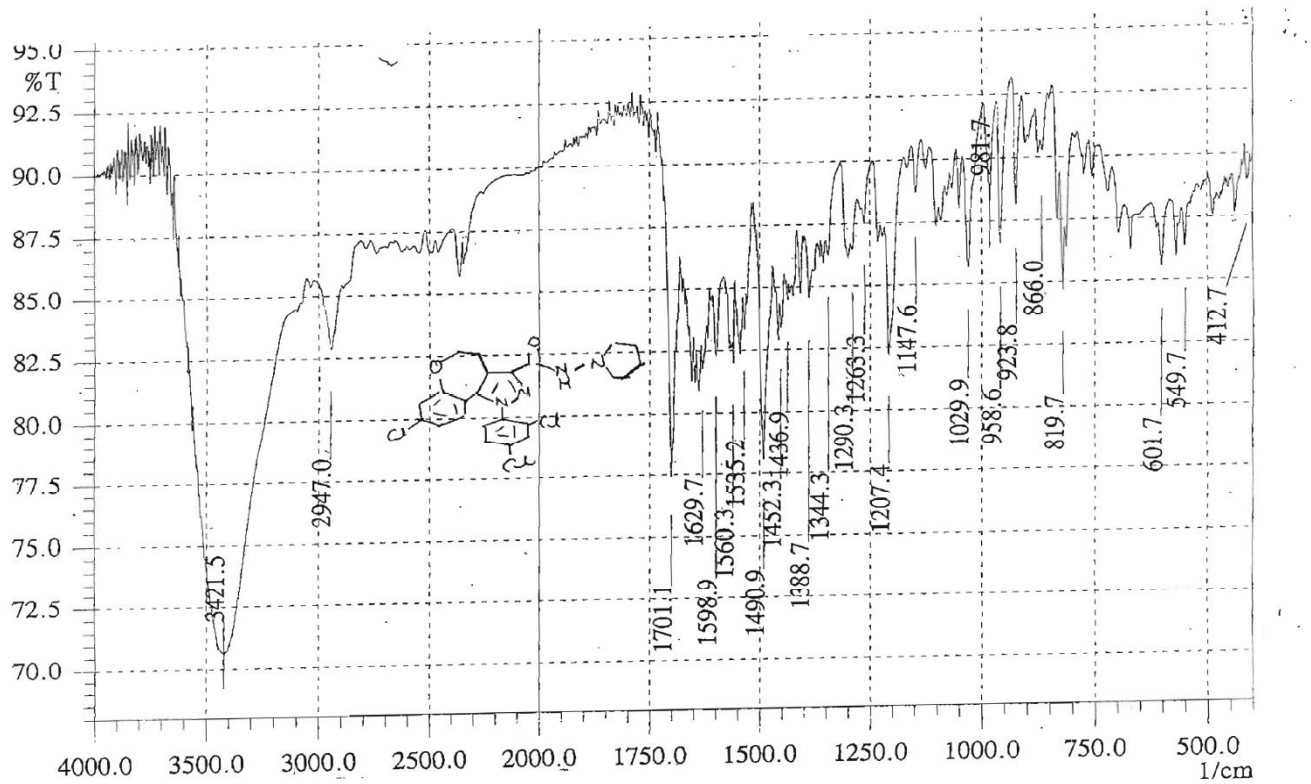
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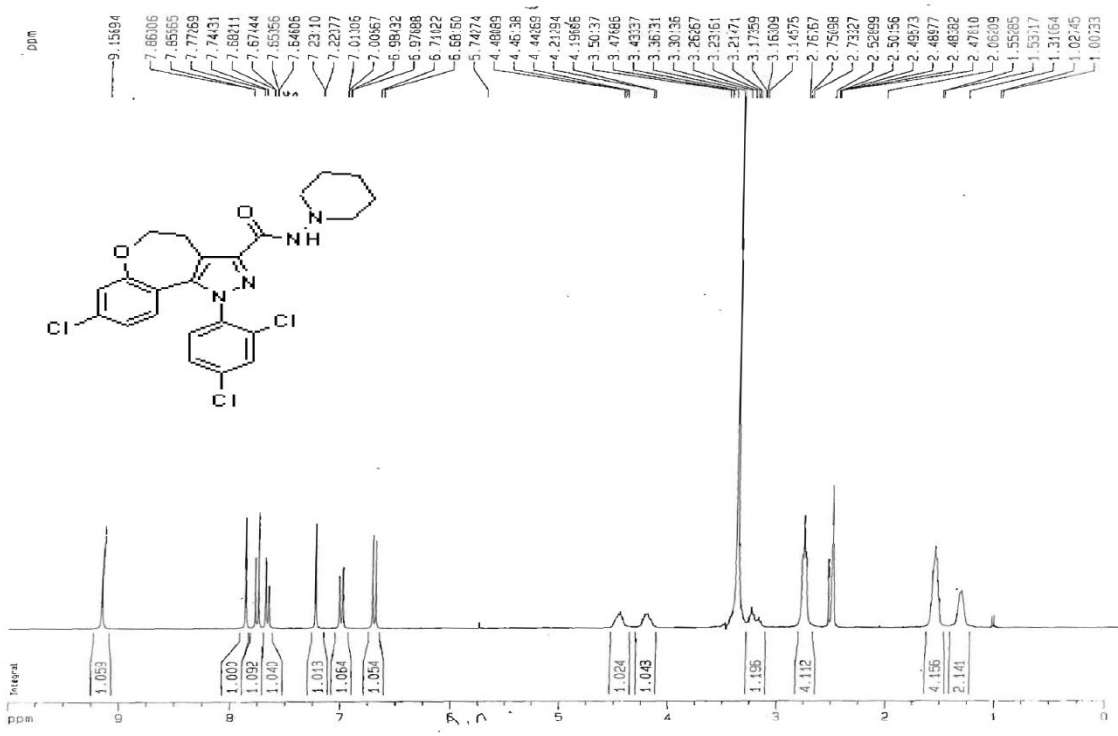
**8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide (1), crystalline**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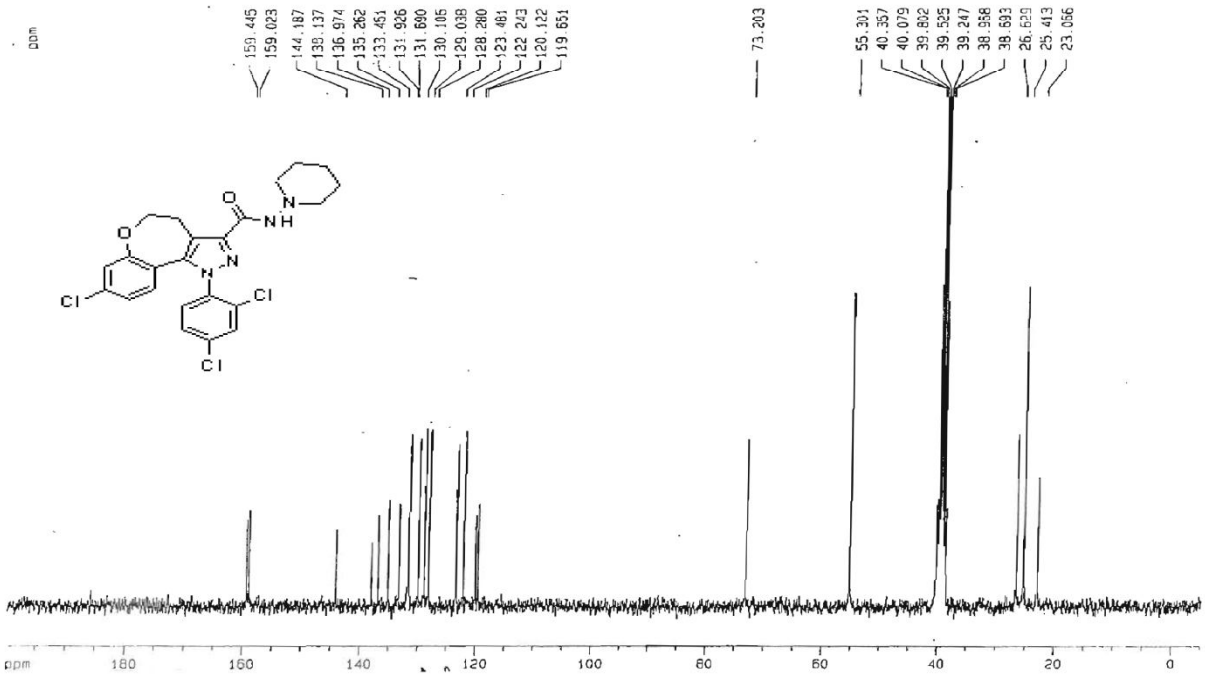
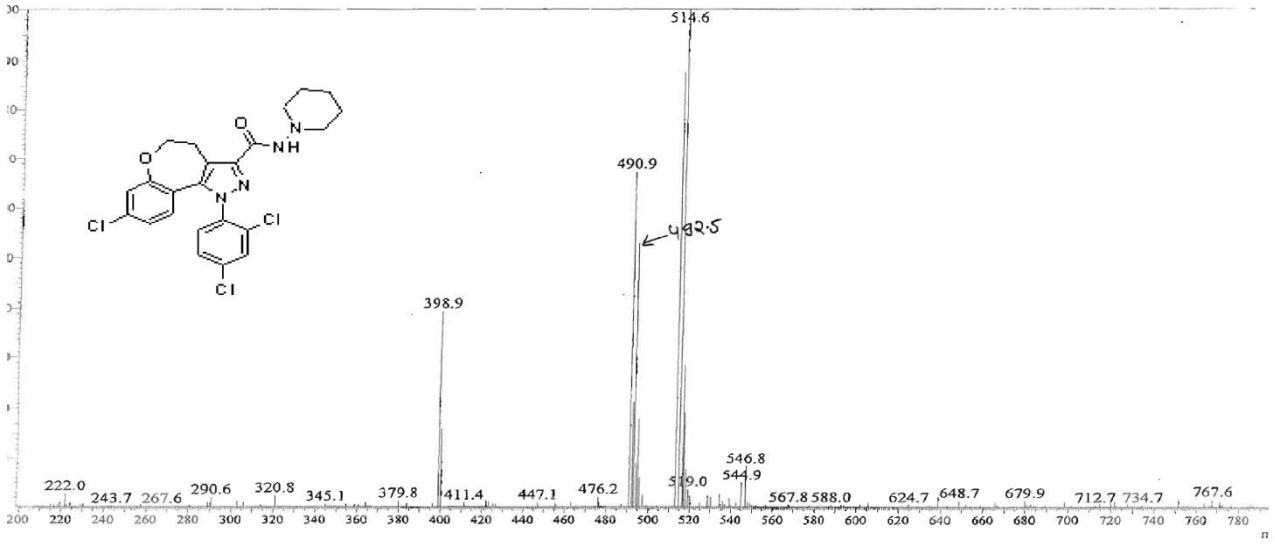
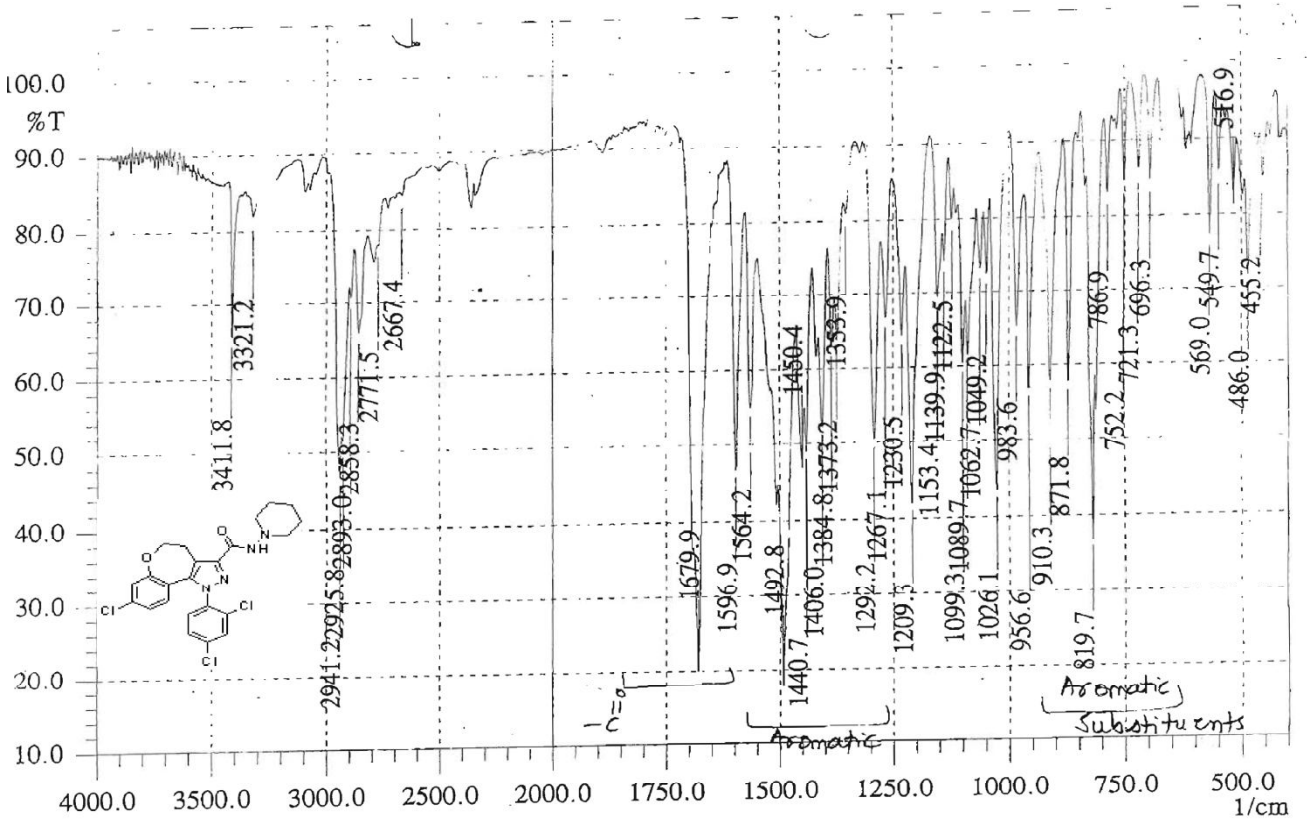
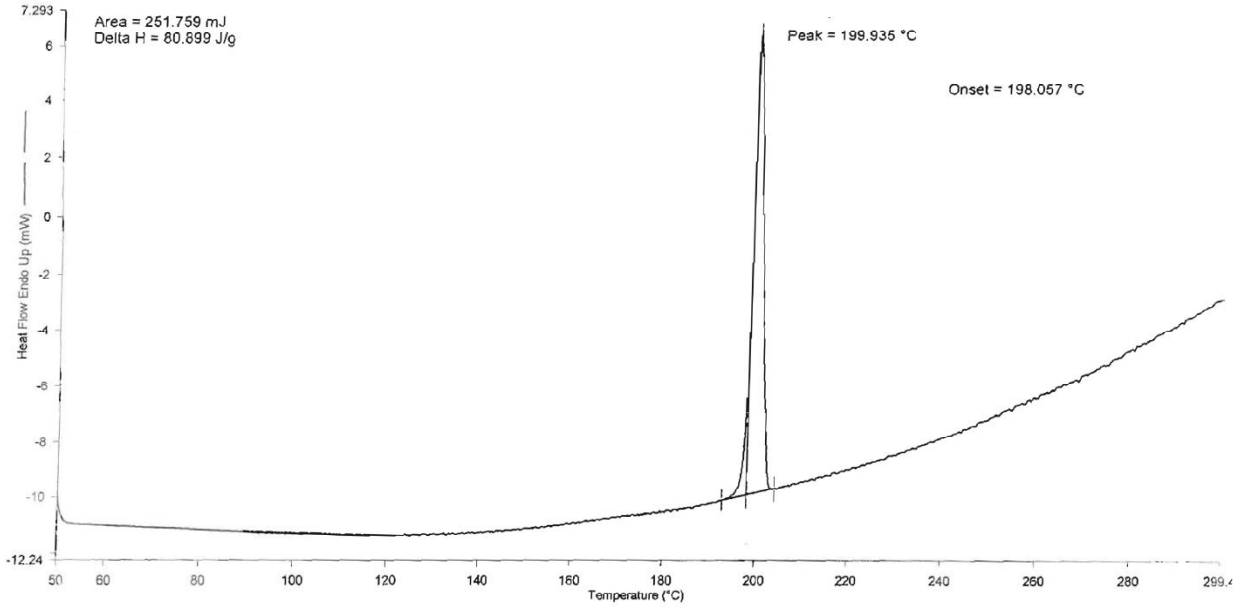
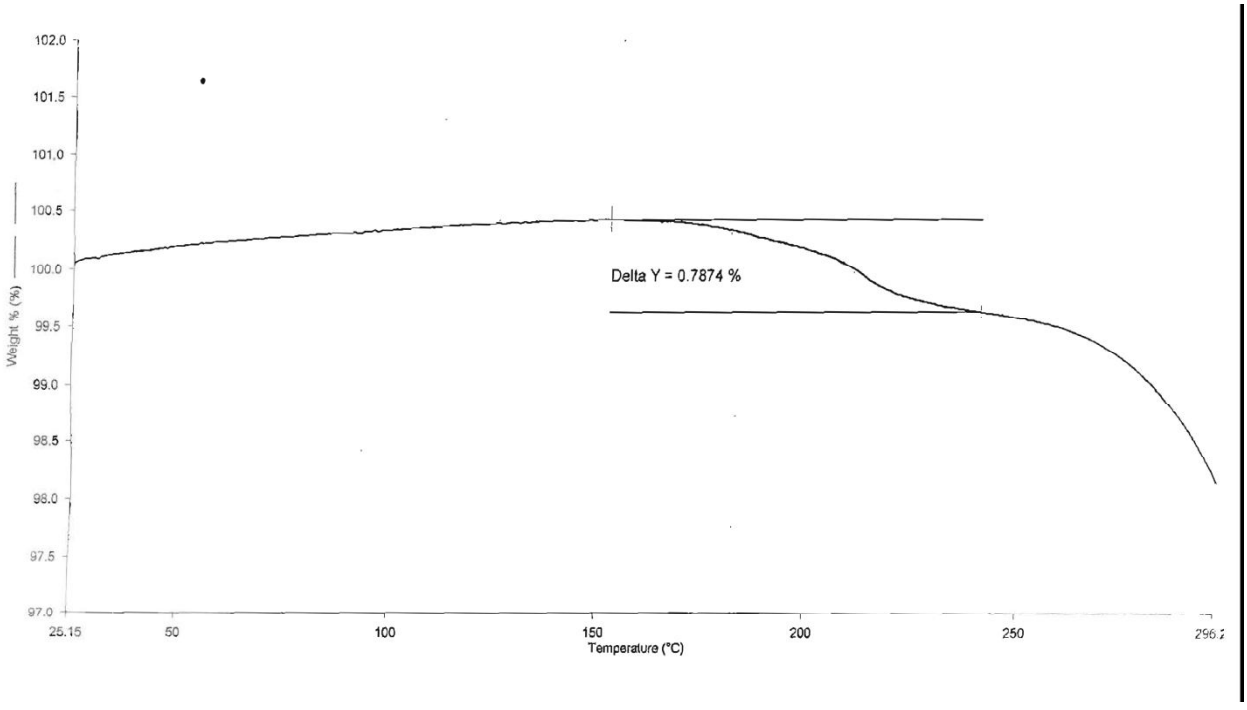
2.50156
2.49573
2.48677
2.48382
2.47410
2.06009
1.55285
1.53317
1.31164
1.02145
1.00233
Integral
1.059
1.000
1.092
1.040
1.013
1.064
1.054
1.024
1.043
1.196
4.112
4.156
2.141
Cl
Cl
Cl
O
N
NH
O
N
N

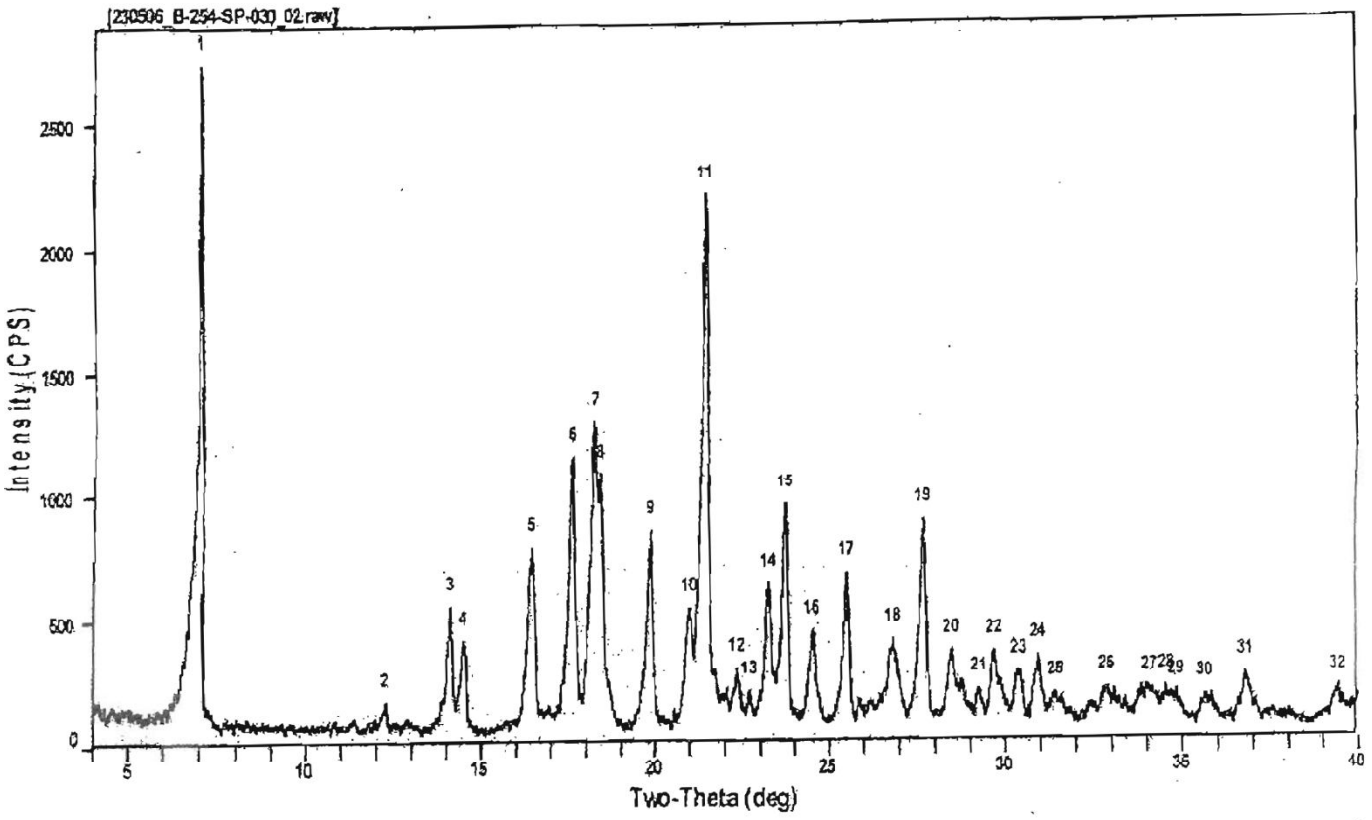

ppm
159.445
159.023
144.187
138.137
136.974
135.262
133.451
131.926
131.690
130.105
129.038
128.280
123.461
122.243
120.122
119.651
73.203
55.301
40.357
40.079
39.802
39.525
39.247
38.958
38.693
26.629
25.413
23.056
Cl
Cl
Cl
O
N
NH
O
N
N


514.6
490.9
492.5
398.9
546.8
544.9
519.0
222.0
243.7
267.6
290.6
320.8
345.1
379.8
411.4
447.1
476.2
567.8
588.0
624.7
648.7
679.9
712.7
734.7
767.6

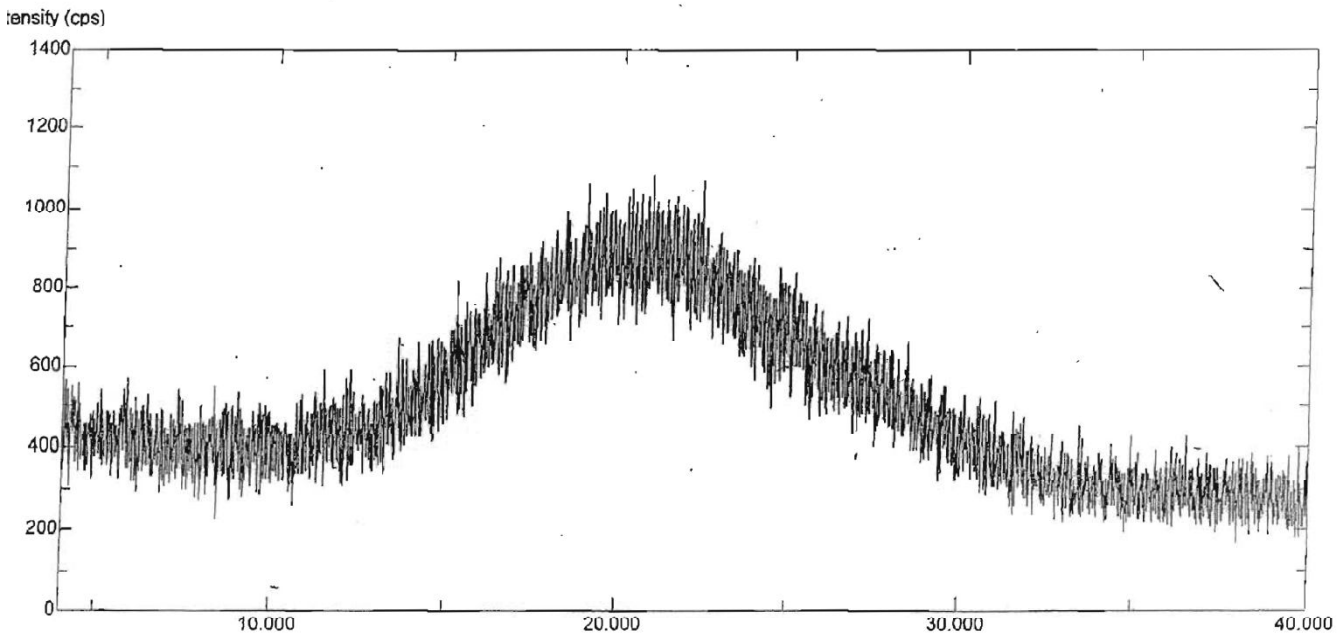

100.0
%T
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
4000.0
3500.0
3000.0
2500.0
2000.0
1750.0
1500.0
1250.0
1000.0
750.0
500.0
1/cm
3411.8
3321.2
2941.2
2925.8
2893.0
2858.3
2771.5
2667.4
1679.9
1596.9
1564.2
1492.8
1450.4
1440.7
1406.0
1384.8
1373.2
1353.9
1295.2
1267.1
1230.5
1209.3
1153.4
1139.9
1122.5
1099.3
1089.7
1062.7
1049.2
1026.1
983.6
956.6
910.3
871.8
819.7
786.9
752.2
721.3
696.3
569.0
549.7
516.9
486.0
455.2
-C=O
Aromatic
Aromatic
Substituents


Area = 251.759 mJ
Delta H = 80.899 J/g
Peak = 199.935 °C
Onset = 198.057 °C
Heat Flow Endo Up (mW)
Temperature (°C)

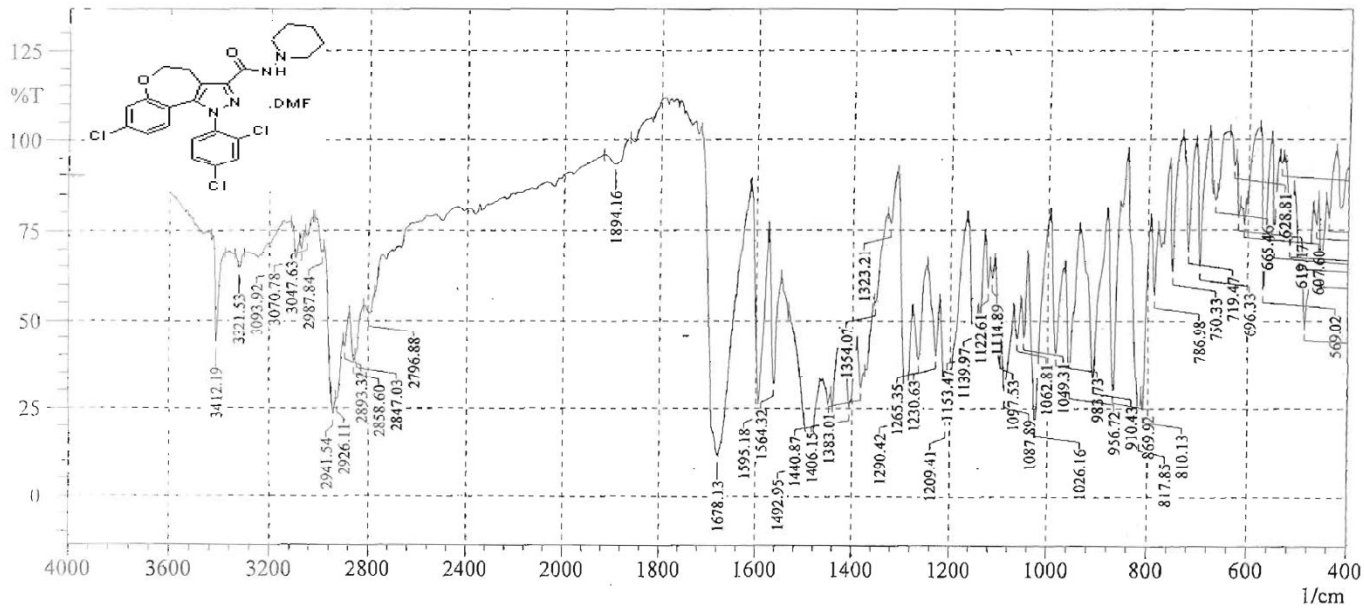

Delta Y = 0.7874 %
Weight % (%)
Temperature (°C)

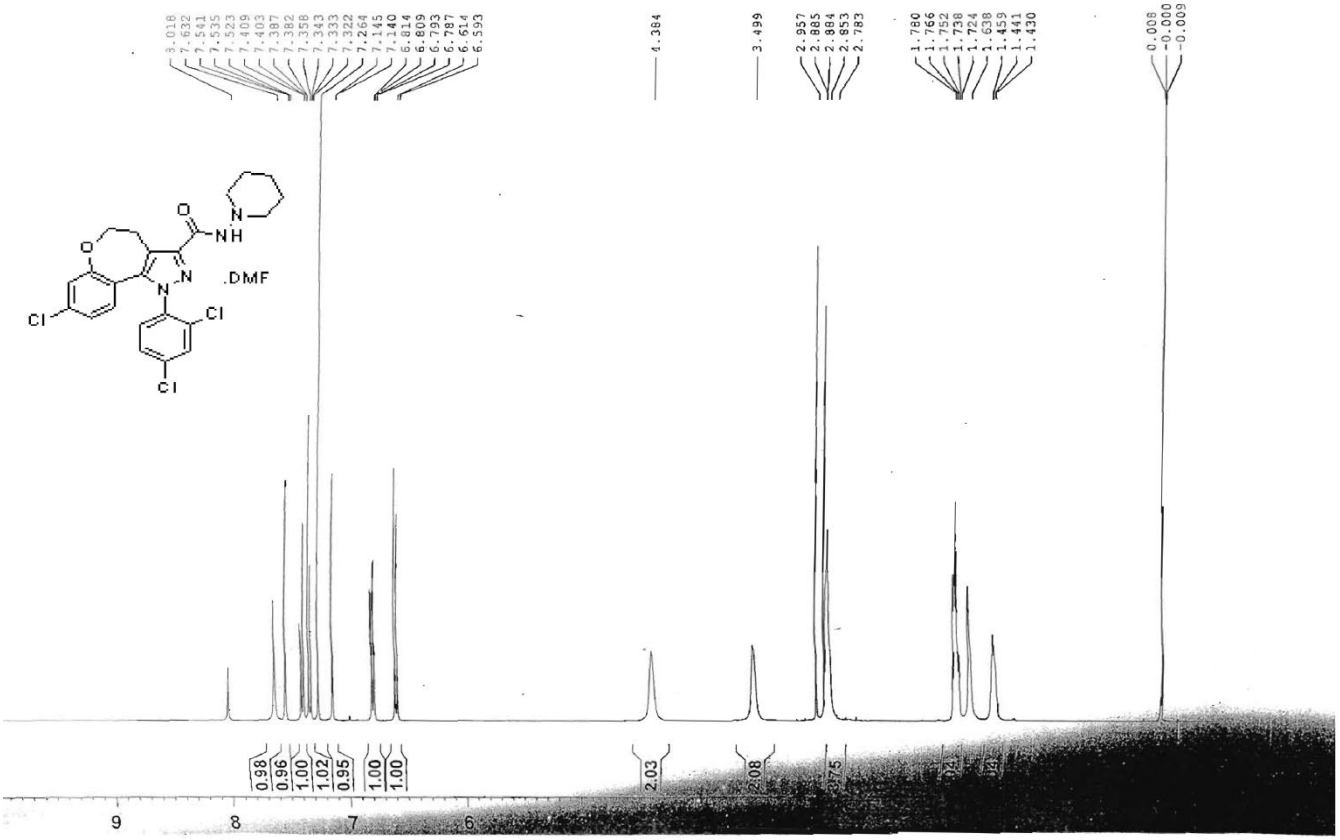

[230506_B-254-SP-030_02.raw]
Intensity (CPS)
Two-Theta (deg)

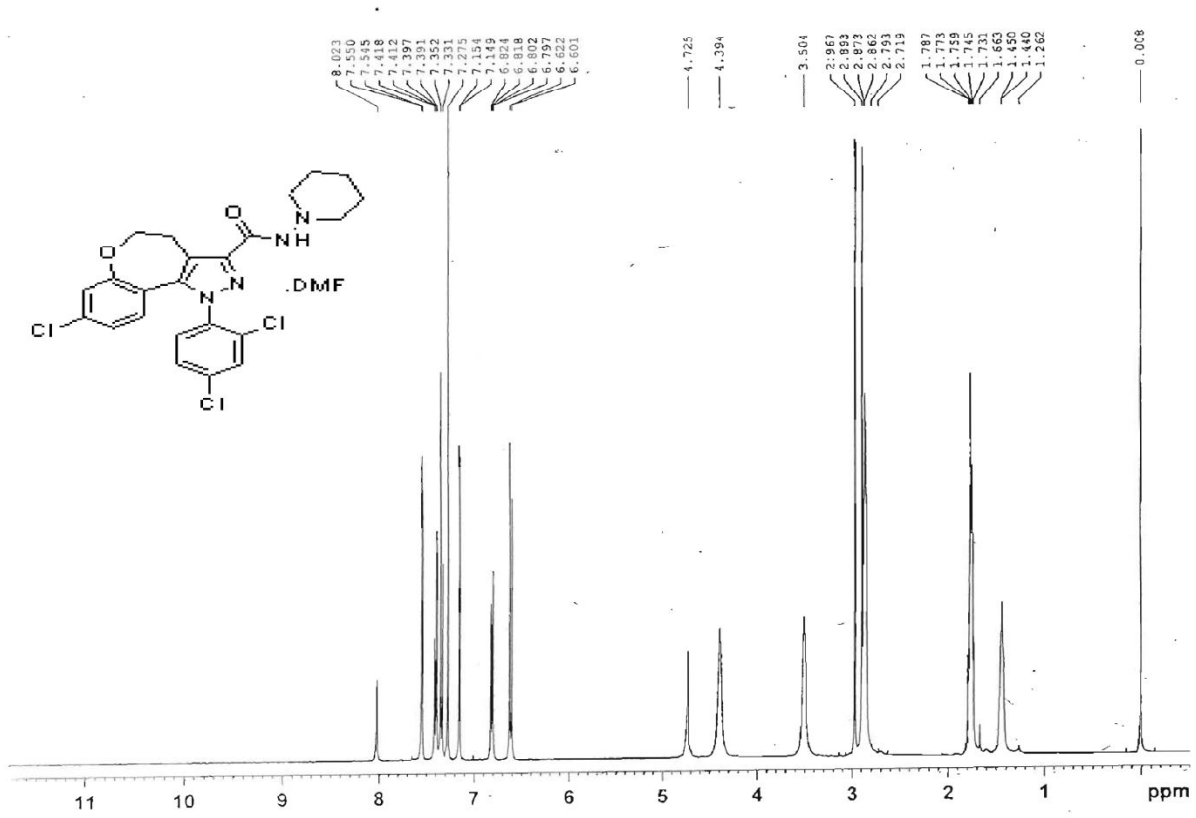
**8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide (1), amorphous**

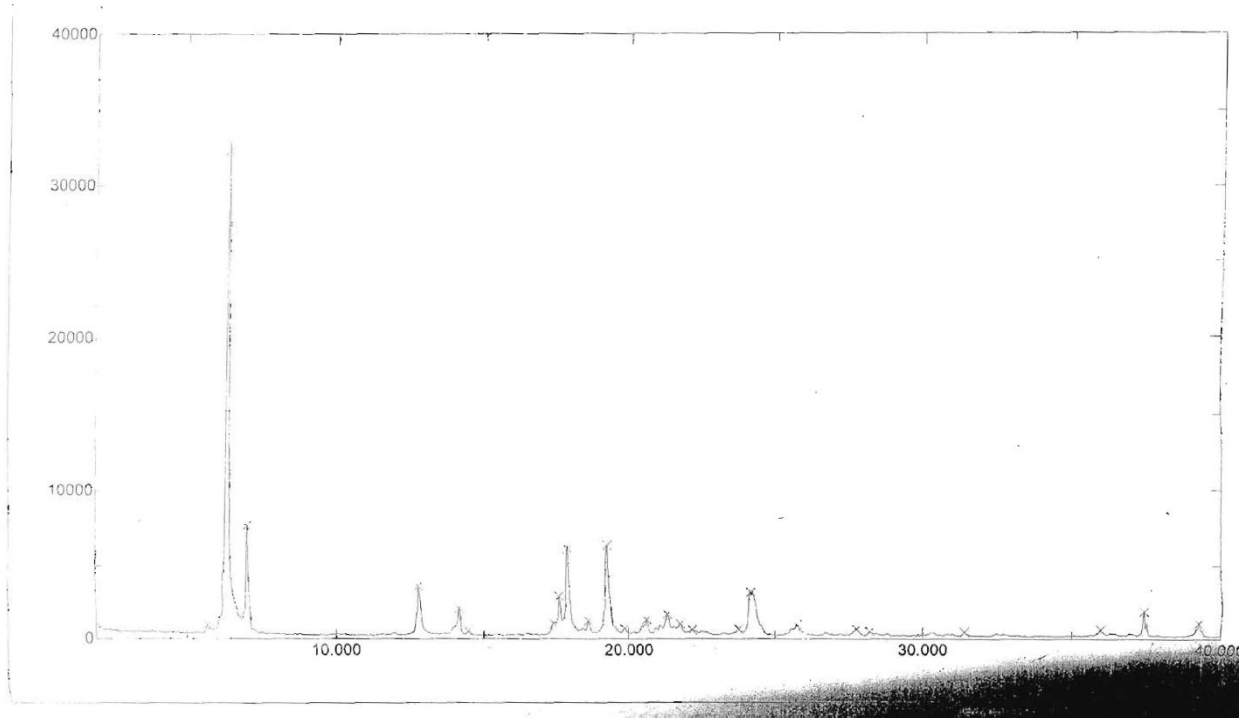

tensity (cps)
1400
1200
1000
800
600
400
200
0
10.000
20.000
30.000
40.000

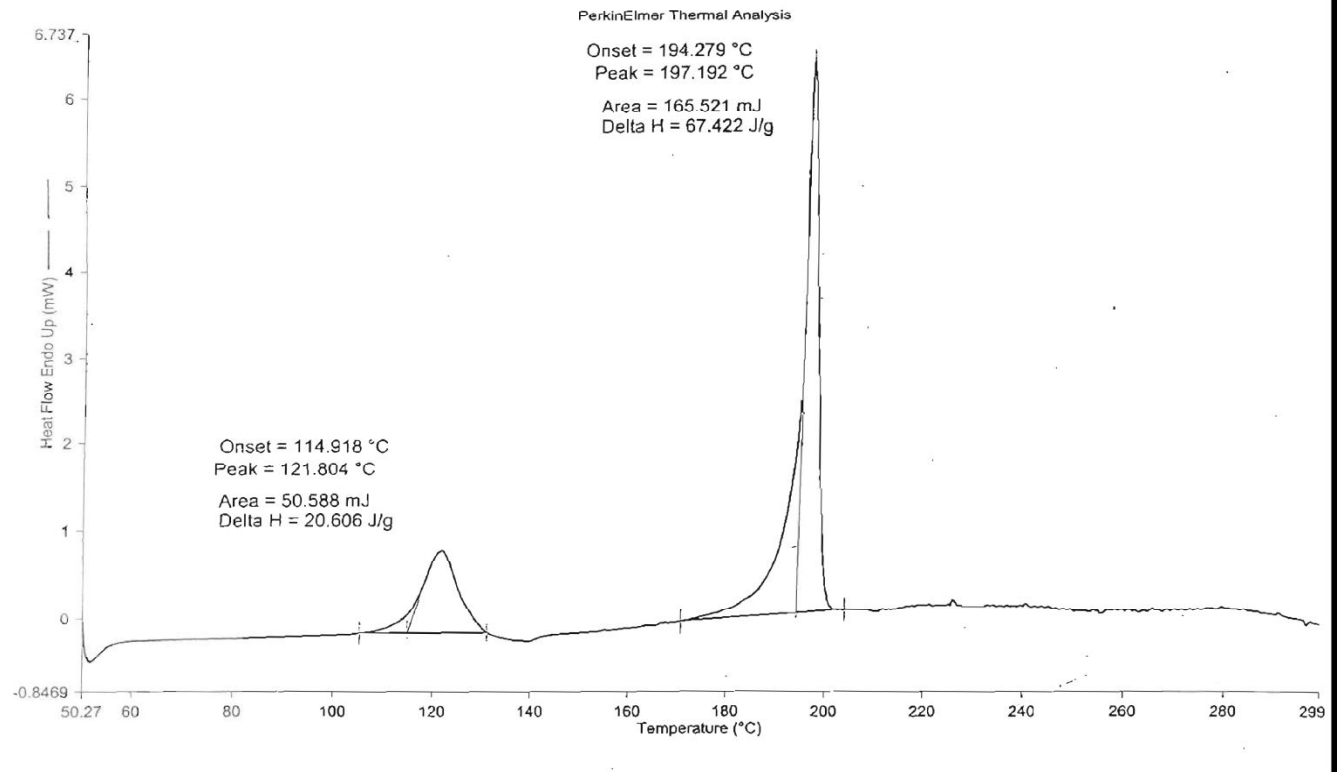
**8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide. (DMF solvate)**

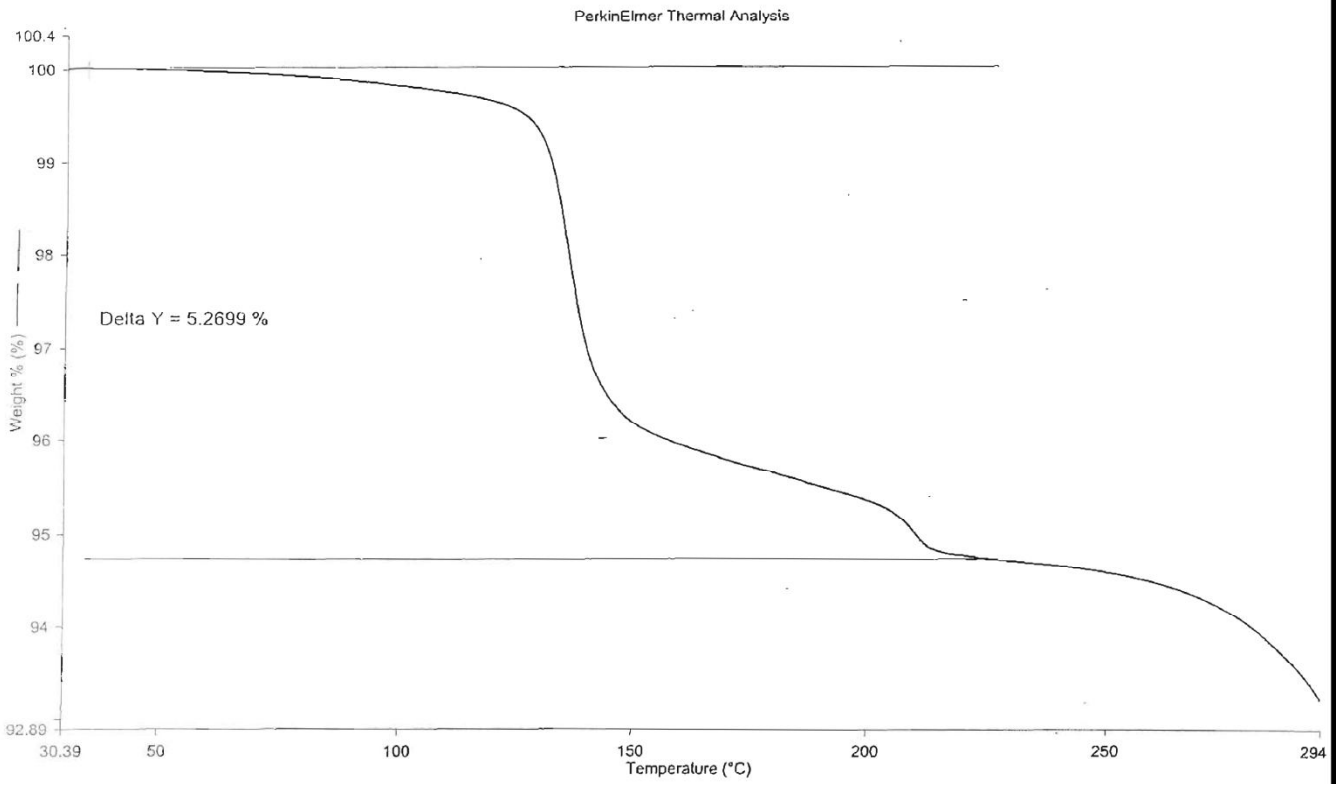

%T
1/cm
.DMF
3412.19
3221.53
3093.92
3070.78
3047.63
2987.84
2941.54
2926.11
2893.32
2858.60
2847.03
2796.88
1894.16
1678.13
1595.18
1564.32
1492.95
1440.87
1406.15
1383.01
1354.07
1323.21
1290.42
1265.35
1230.63
1209.41
1153.47
1139.97
1122.61
1114.89
1097.53
1087.89
1062.81
1049.31
1026.16
983.73
956.72
910.43
869.92
817.85
810.13
786.98
750.33
719.47
696.33
665.46
628.81
619.17
607.60
569.02


.DMF
7.632
7.541
7.535
7.523
7.409
7.403
7.397
7.382
7.356
7.343
7.333
7.322
7.264
7.145
7.140
6.814
6.809
6.793
6.787
6.614
6.593
4.384
3.499
2.957
2.885
2.884
2.853
2.783
1.780
1.766
1.752
1.738
1.724
1.638
1.459
1.441
1.430
0.008
-0.000
-0.009
0.98
0.96
1.00
1.02
0.95
1.00
1.00
2.03
2.08
2.75

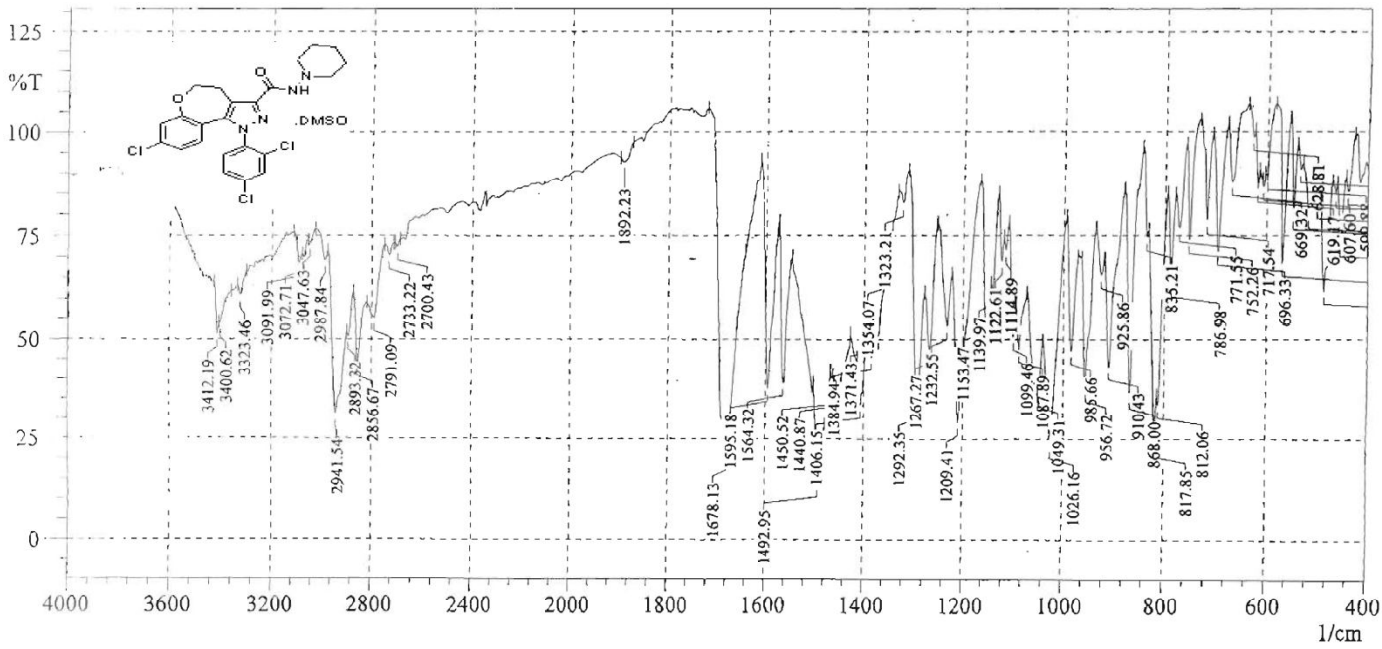

8.023
7.550
7.545
7.418
7.412
7.397
7.391
7.352
7.331
7.275
7.154
7.149
6.824
6.818
6.802
6.797
6.622
6.601
4.725
4.394
3.504
2.967
2.893
2.873
2.862
2.793
2.719
1.787
1.773
1.759
1.745
1.731
1.663
1.450
1.440
1.262
0.008
O
N
NH
O
N
N
.DMF
Cl
Cl
Cl
11
10
9
8
7
6
5
4
3
2
1
ppm

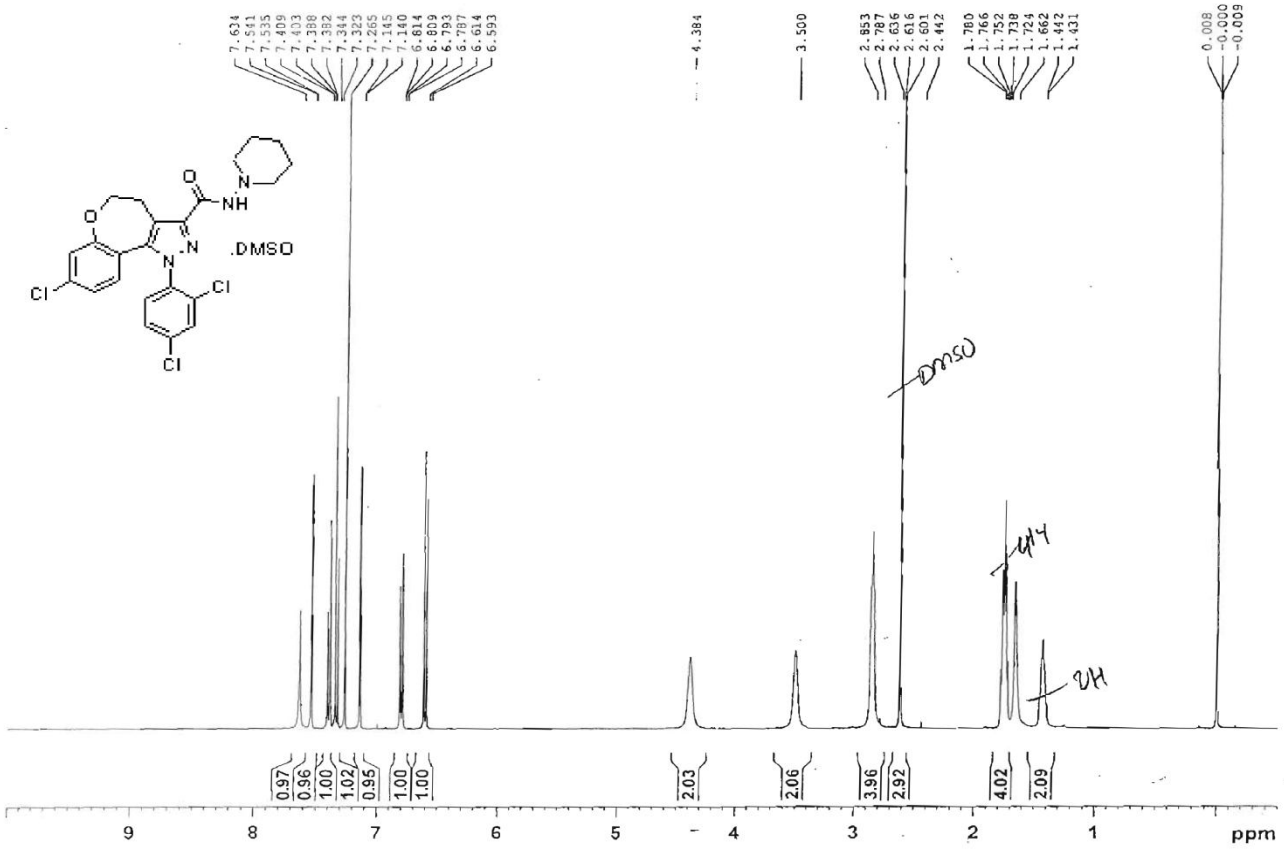

40000
30000
20000
10000
0
10.000
20.000
30.000

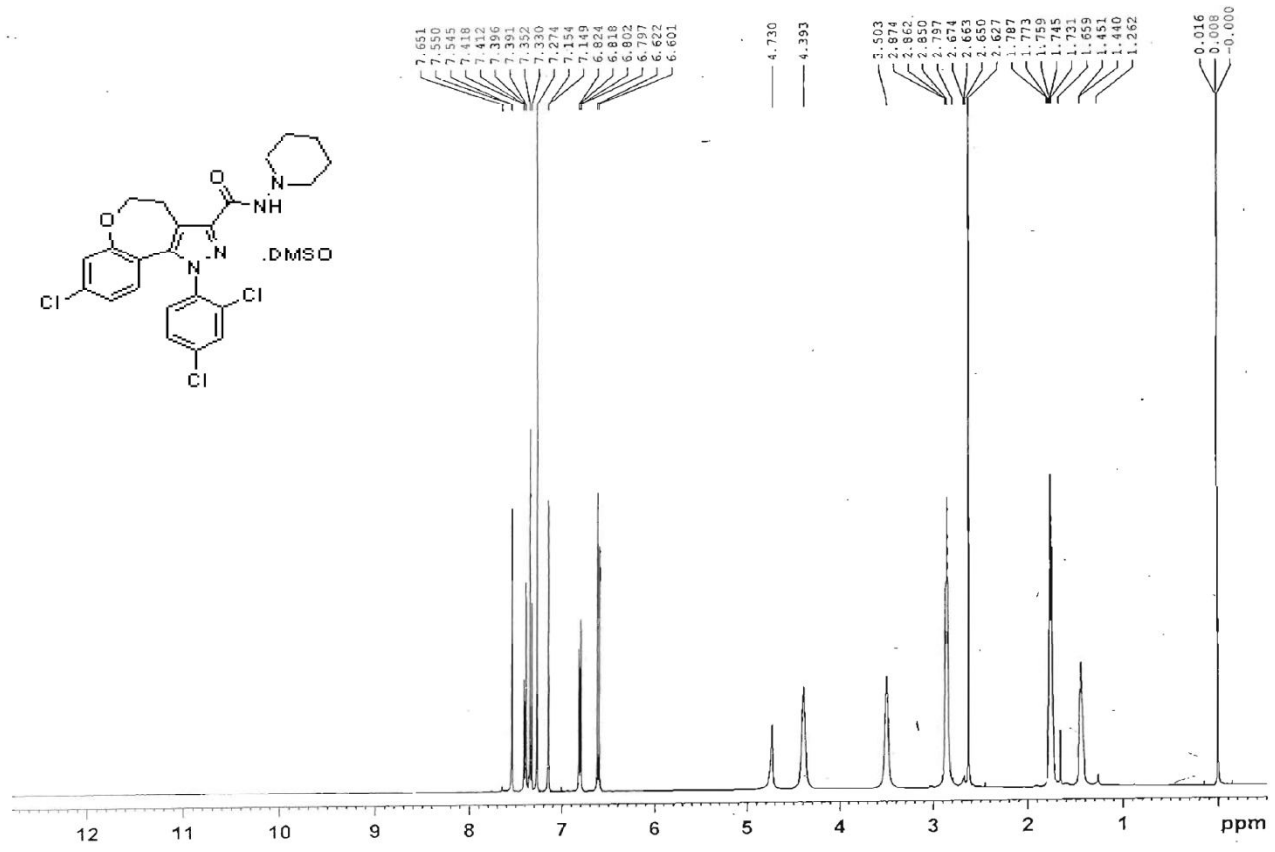

PerkinElmer Thermal Analysis
Onset = 194.279 °C
Peak = 197.192 °C
Area = 165.521 mJ
Delta H = 67.422 J/g
Onset = 114.918 °C
Peak = 121.804 °C
Area = 50.588 mJ
Delta H = 20.606 J/g
Heat Flow Endo Up (mW)
6.737
6
5
4
3
2
1
0
-0.8469
50.27
60
80
100
120
140
160
180
200
220
240
260
280
299
Temperature (°C)

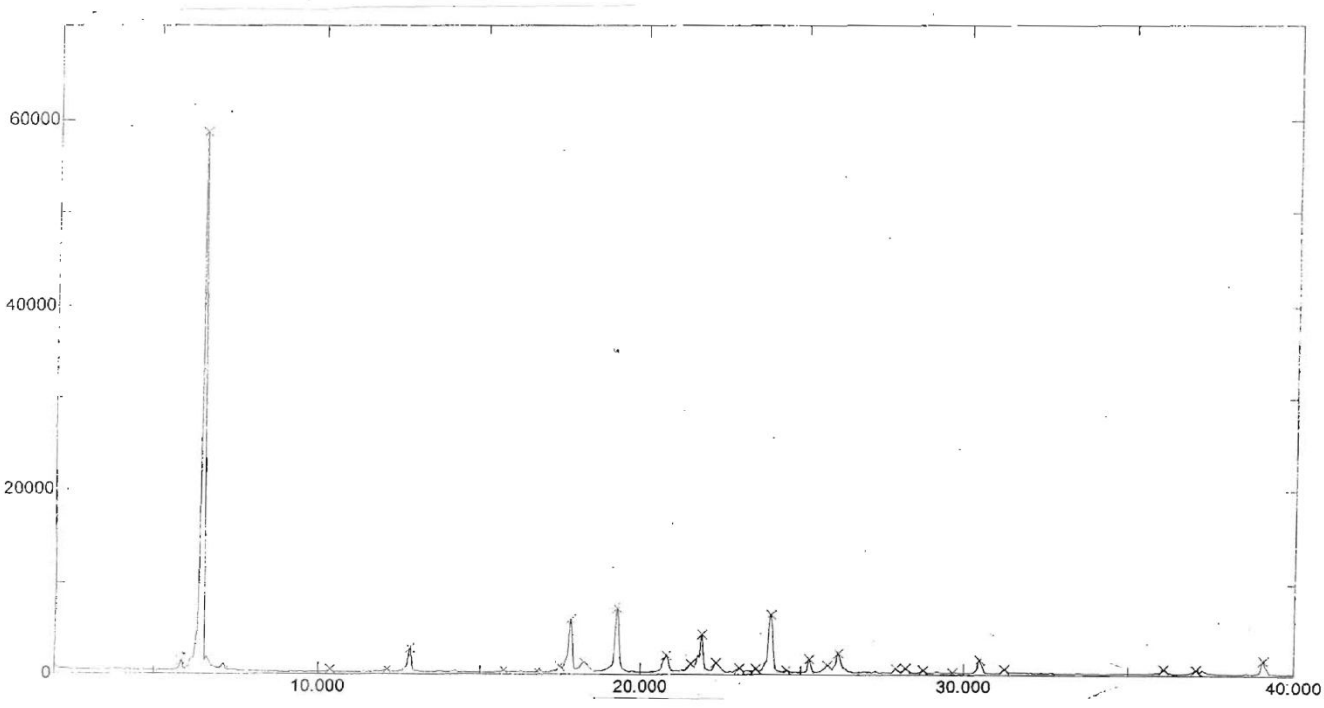

PerkinElmer Thermal Analysis
Delta Y = 5.2699 %
Weight % (%)
100.4
100
99
98
97
96
95
94
92.89
30.39
50
100
150
200
250
294
Temperature (°C)

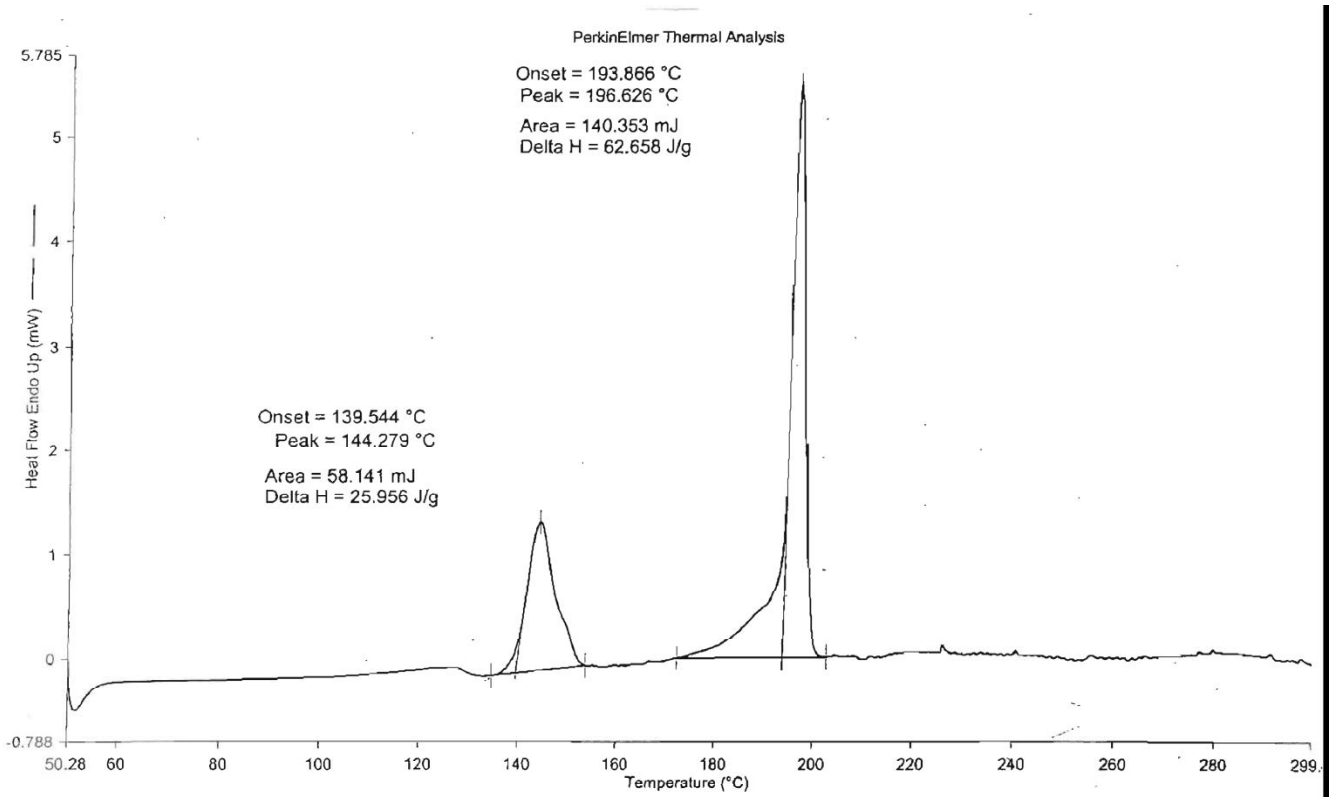
**8-Chloro-1-(2,4-dichloro-phenyl)-4,5-dihydro-1H-6-oxa-1,2-diaza-benzo[e]azulene-3-carboxylic acid piperidin-1-ylamide. (DMSO solvate)**

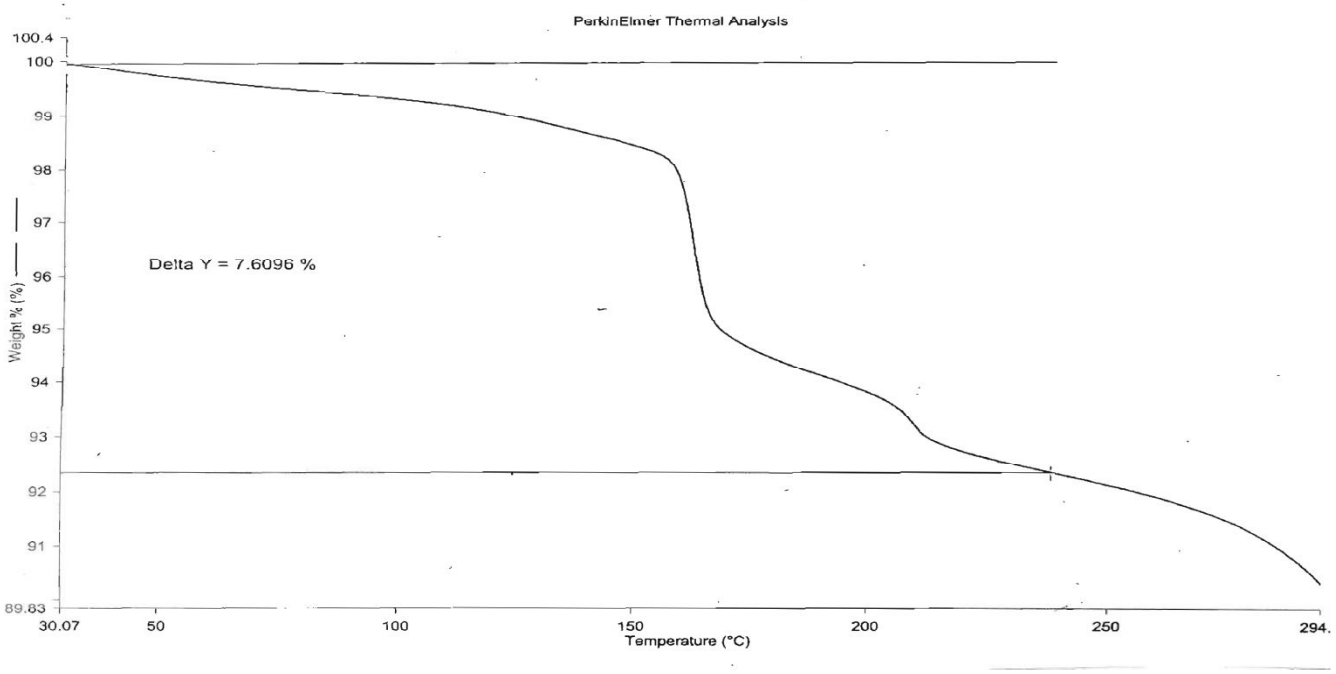

%T
1/cm
.DMSO
3412.19
3400.62
3323.46
3091.99
3072.71
3047.63
2987.84
2941.54
2893.32
2856.67
2791.09
2733.22
2700.43
1892.23
1678.13
1595.18
1564.32
1492.95
1450.52
1440.87
1406.15
1384.94
1371.43
1354.07
1323.21
1292.35
1267.27
1232.55
1209.41
1153.47
1139.97
1122.61
1114.89
1099.46
1087.89
1049.31
1026.16
985.66
956.72
925.86
910.43
868.00
835.21
817.85
812.06
786.98
771.53
752.28
717.54
696.33


.DMSO
DMSO
ppm

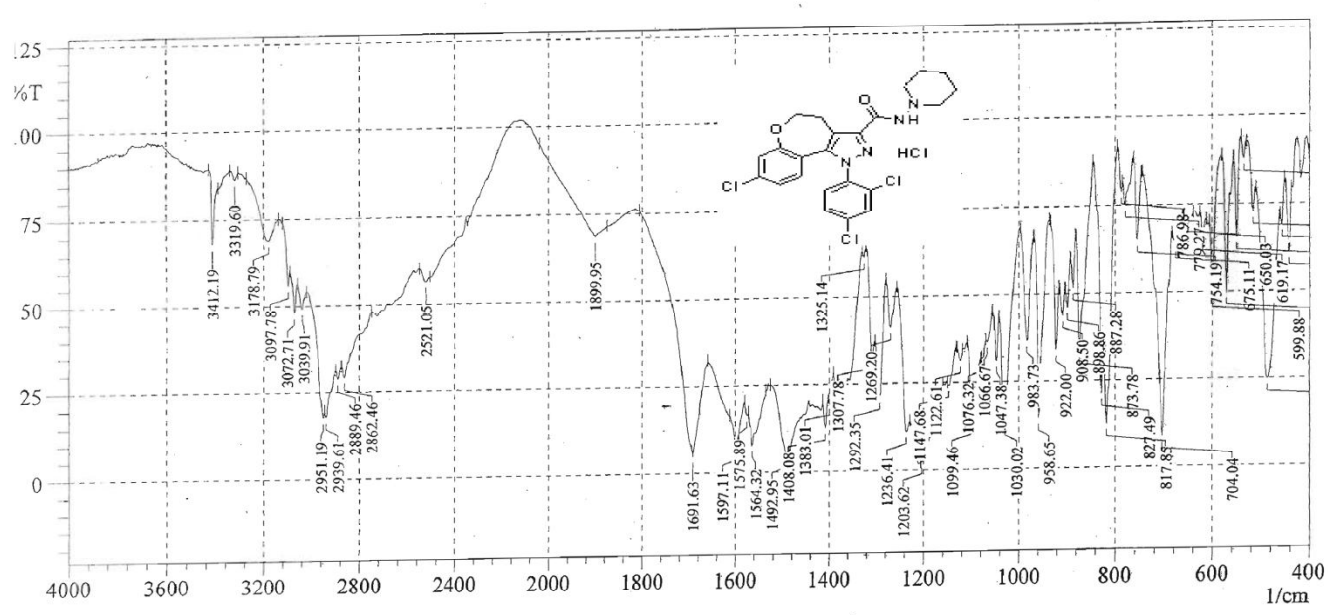

7.651
7.550
7.545
7.418
7.412
7.396
7.391
7.352
7.330
7.274
7.154
7.149
6.824
6.818
6.802
6.797
6.622
6.601
4.730
4.393
3.503
2.874
2.862
2.850
2.797
2.674
2.663
2.650
2.627
1.787
1.773
1.759
1.745
1.731
1.659
1.451
1.440
1.262
0.016
0.008
-0.000
O
N
NH
O
N
N
.DMSO
Cl
Cl
Cl
12
11
10
9
8
7
6
5
4
3
2
1
ppm

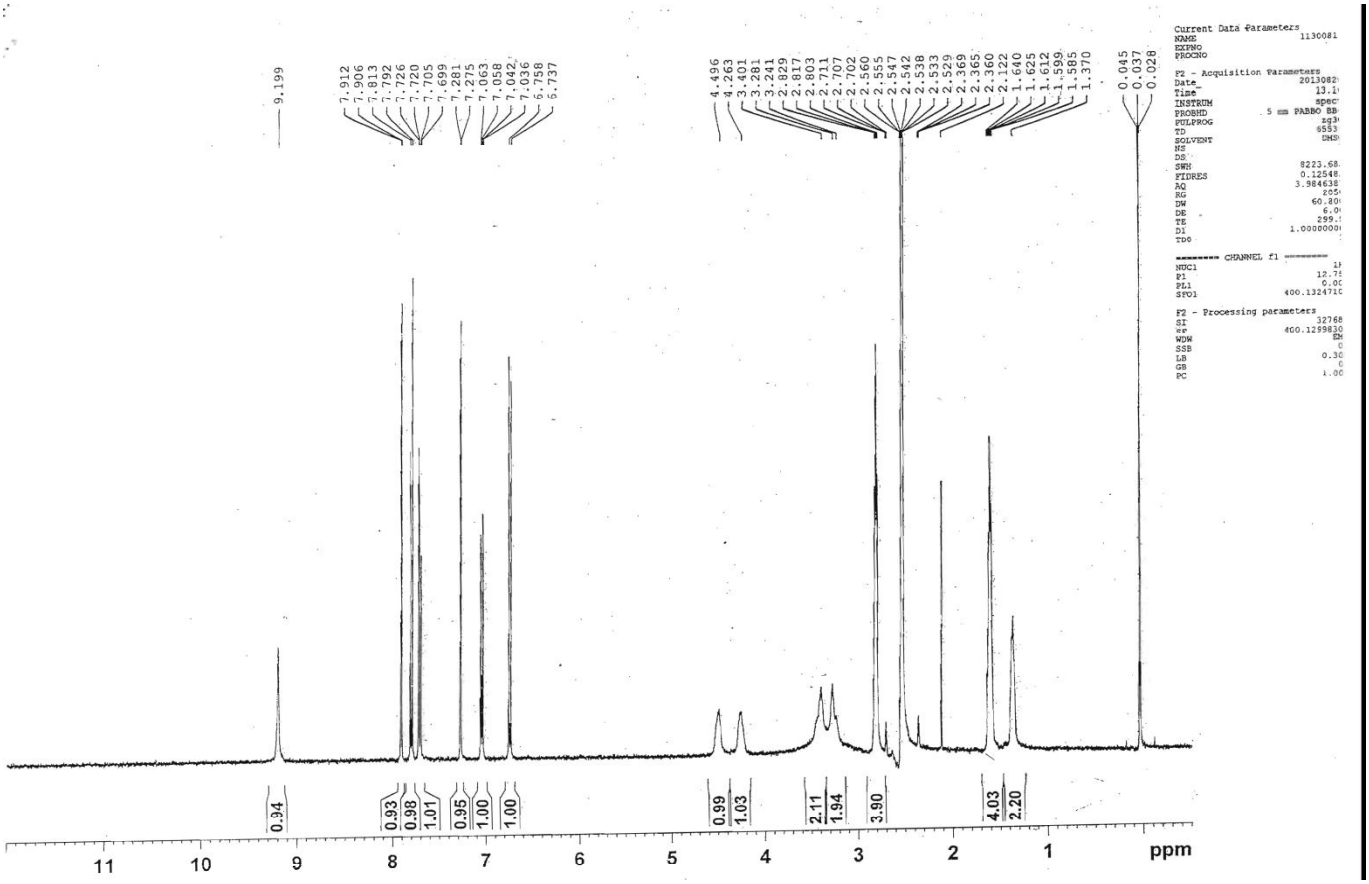

60000
40000
20000
0
10.000
20.000
30.000
40.000

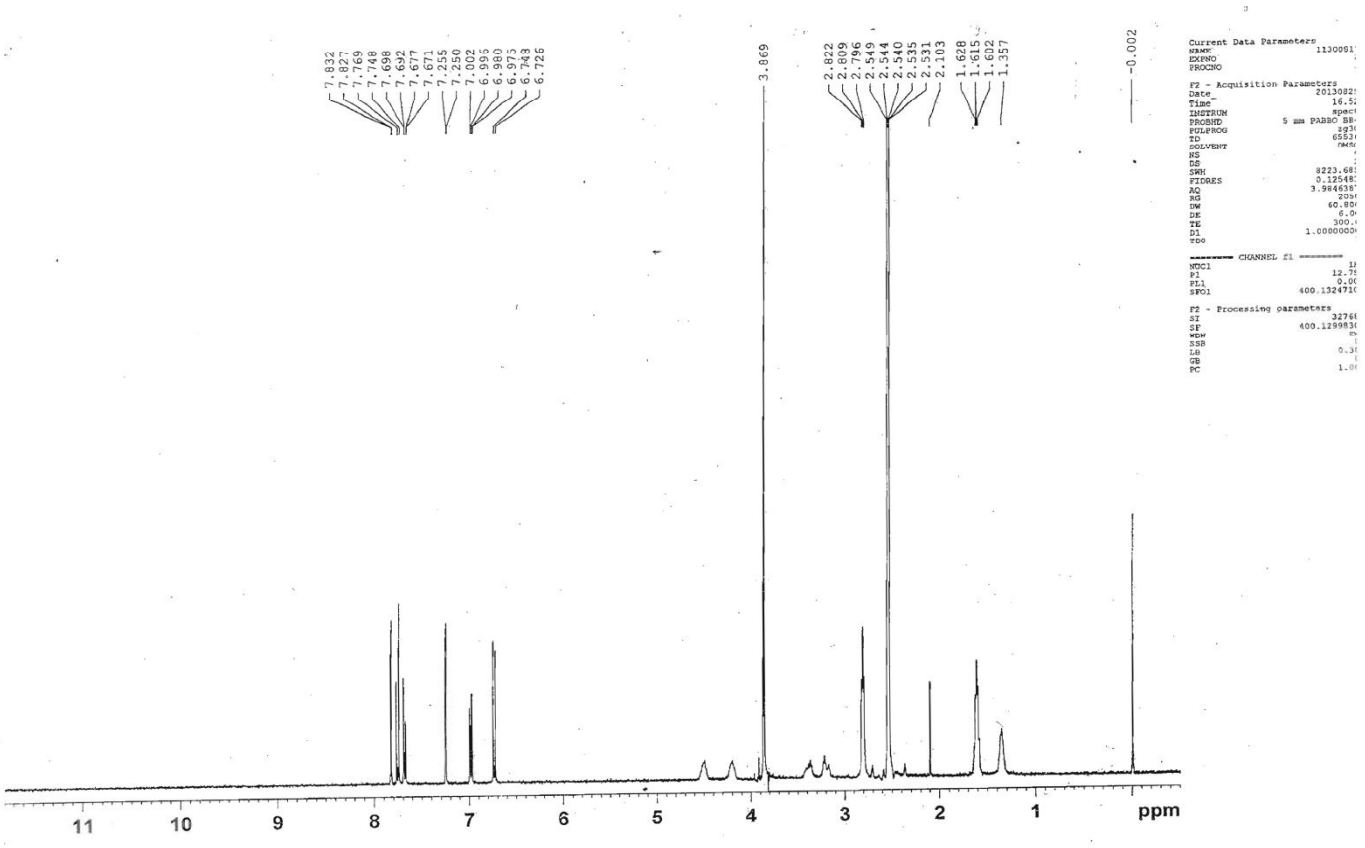

PerkinElmer Thermal Analysis
Onset = 193.866 °C
Peak = 196.626 °C
Area = 140.353 mJ
Delta H = 62.658 J/g
Onset = 139.544 °C
Peak = 144.279 °C
Area = 58.141 mJ
Delta H = 25.956 J/g
Heat Flow Endo Up (mW)
5.785
5
4
3
2
1
0
-0.788
50.28
60
80
100
120
140
160
180
200
220
240
260
280
299.
Temperature (°C)


PerkinElmer Thermal Analysis
Delta Y = 7.6096 %
Weight % (%)
100.4
100
99
98
97
96
95
94
93
92
91
89.83
30.07
50
100
150
200
250
294.
Temperature (°C)

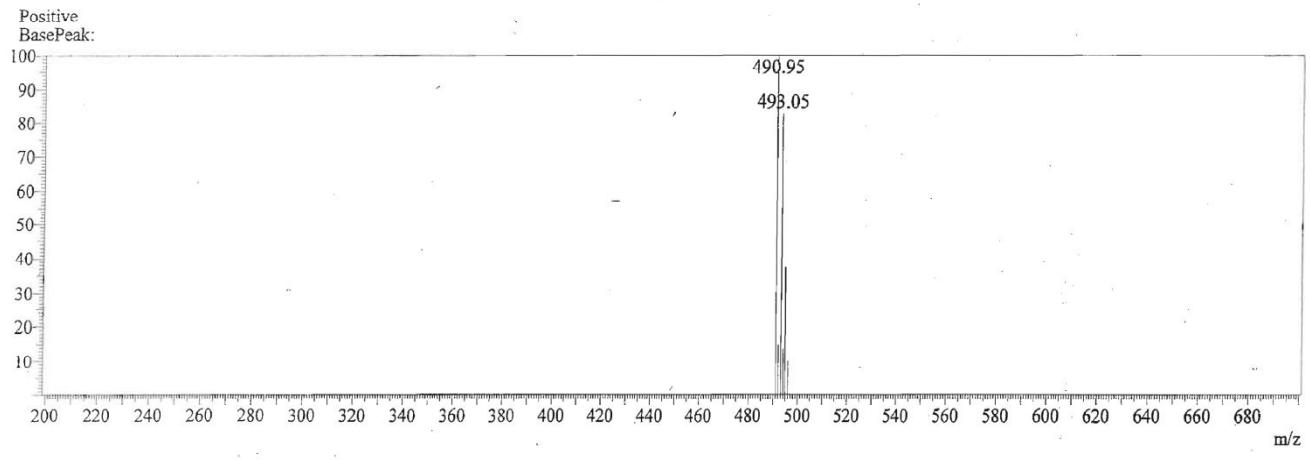
**HCl salt of Compound 1**

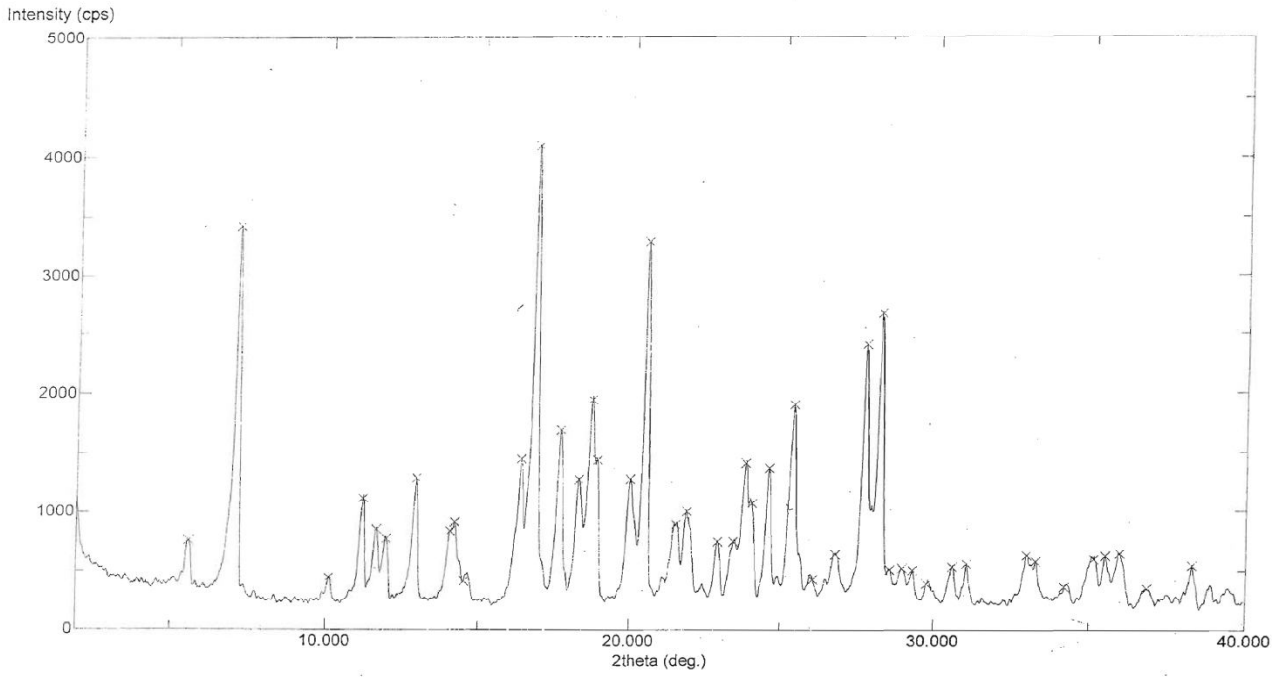

%T
125
100
75
50
25
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm
3412.19
3319.60
3178.79
3097.78
3072.71
3039.91
2951.19
2939.61
2889.46
2862.46
2521.05
1899.95
1691.63
1597.11
1575.89
1564.32
1492.95
1408.08
1383.01
1325.14
1307.78
1292.35
1269.20
1236.41
1203.62
1147.68
1122.61
1099.46
1076.32
1066.67
1047.38
1030.02
983.73
958.65
922.00
908.50
898.86
887.28
873.78
827.49
817.85
786.98
754.19
704.04
675.11
650.03
619.17
599.88
HCl
Cl
Cl
Cl
O
N
NH
N

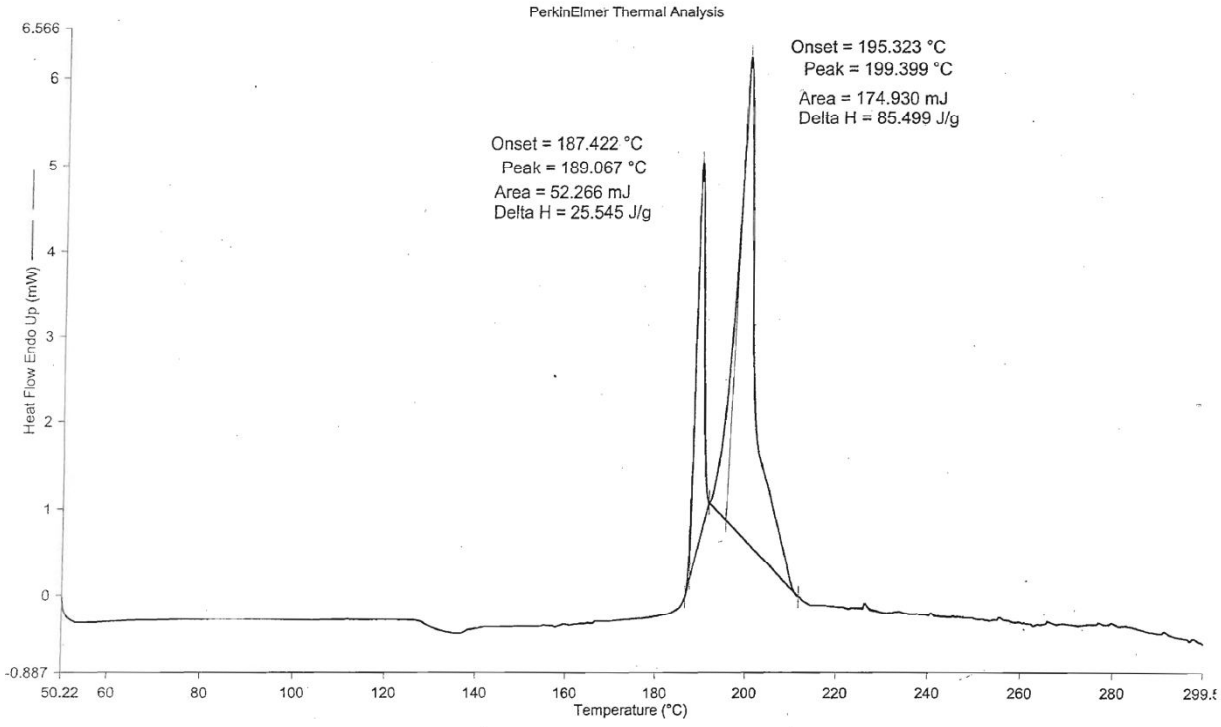

9.199
7.912
7.906
7.813
7.792
7.726
7.720
7.705
7.699
7.291
7.275
7.063
7.058
7.042
7.036
6.758
6.737
4.496
4.263
3.401
3.281
3.241
2.829
2.817
2.803
2.711
2.707
2.702
2.560
2.555
2.547
2.542
2.538
2.533
2.529
2.369
2.365
2.360
2.122
1.640
1.625
1.612
1.599
1.585
1.370
0.045
0.037
0.028
0.34
0.93
0.98
1.01
0.95
1.00
1.00
0.99
1.03
2.11
1.94
3.90
4.03
2.20
11
10
9
8
7
6
5
4
3
2
1
ppm
Current Data Parameters
NAME
1130081
EXPNO
PROCNO
F2 - Acquisition Parameters
Date_
Time
INSTRUM
PROBHD
PULPROG
TD
SOLVENT
NS
DS
SWH
FIDRES
AQ
RG
DW
DE
TE
D1
TD0
CHANNEL f1
NUC1
P1
PL1
SFO1
F2 - Processing parameters
SI
SF
WDW
SSB
LB
GB
PC

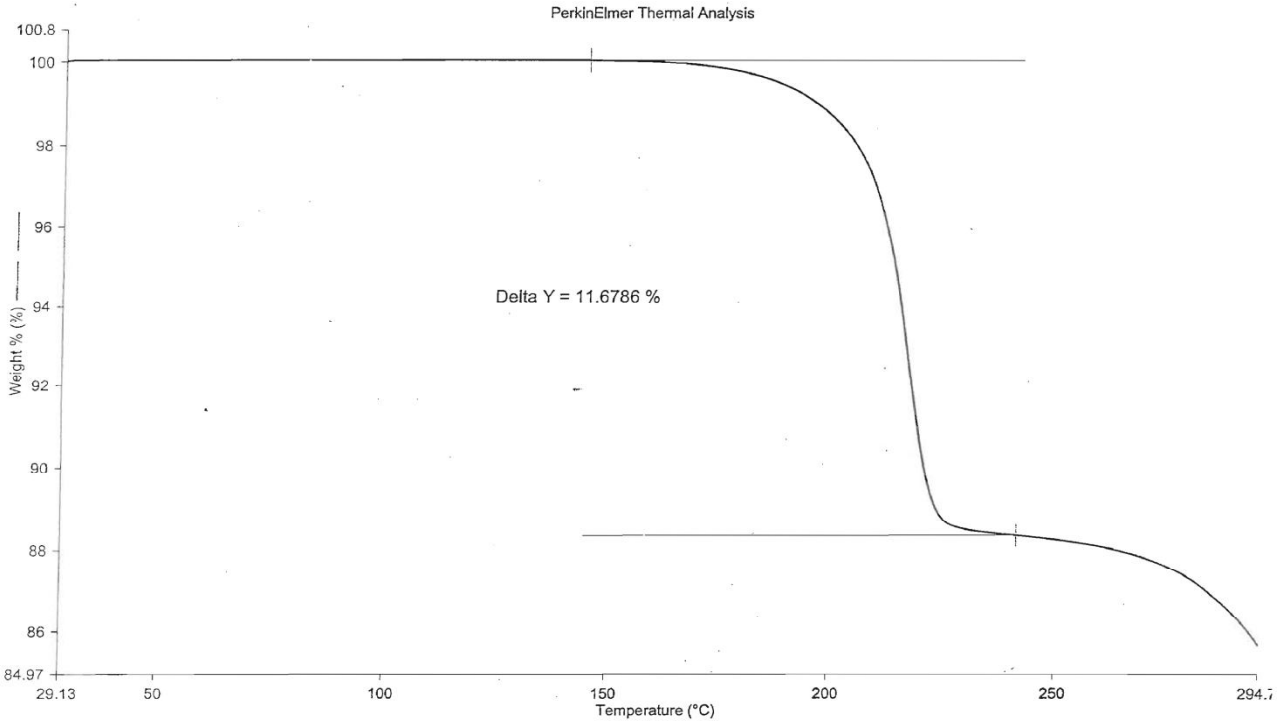



Mass Spectrum

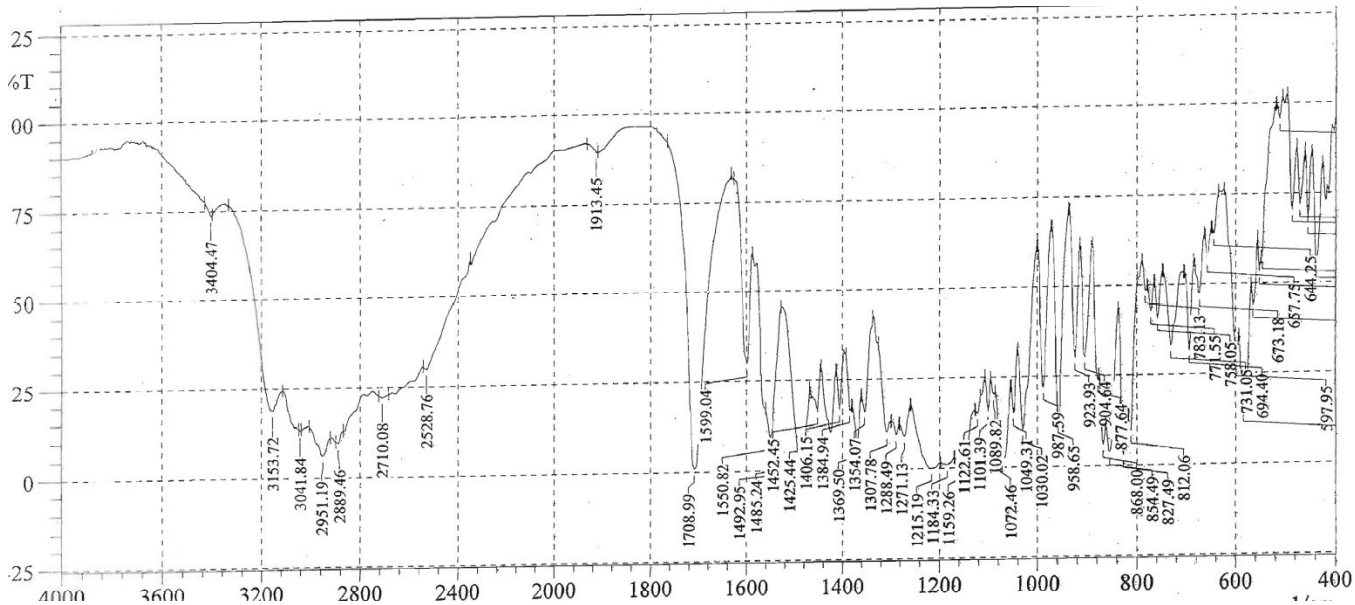


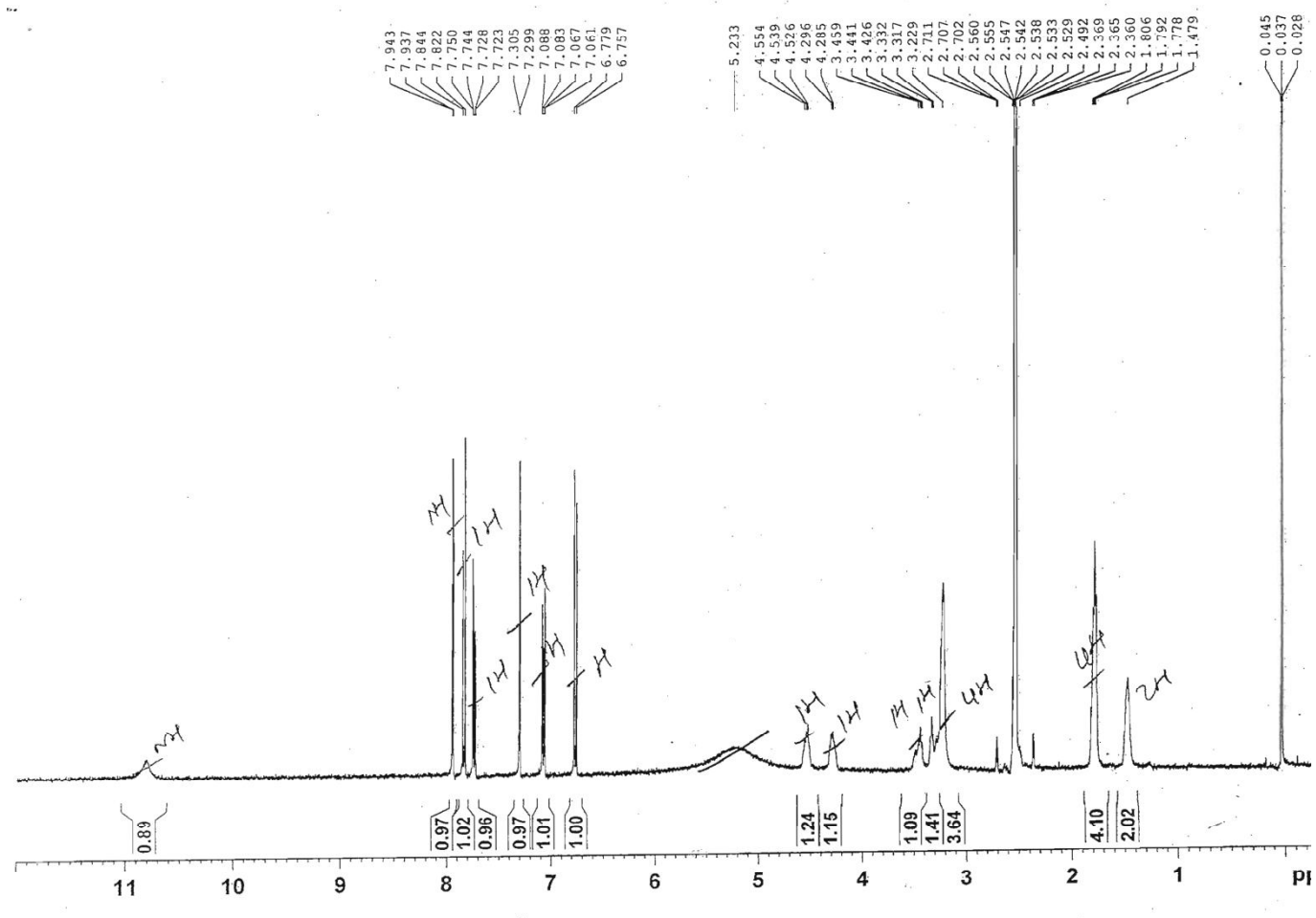

Intensity (cps)
5000
4000
3000
2000
1000
0
10.000
20.000
30.000
40.000
2theta (deg.)

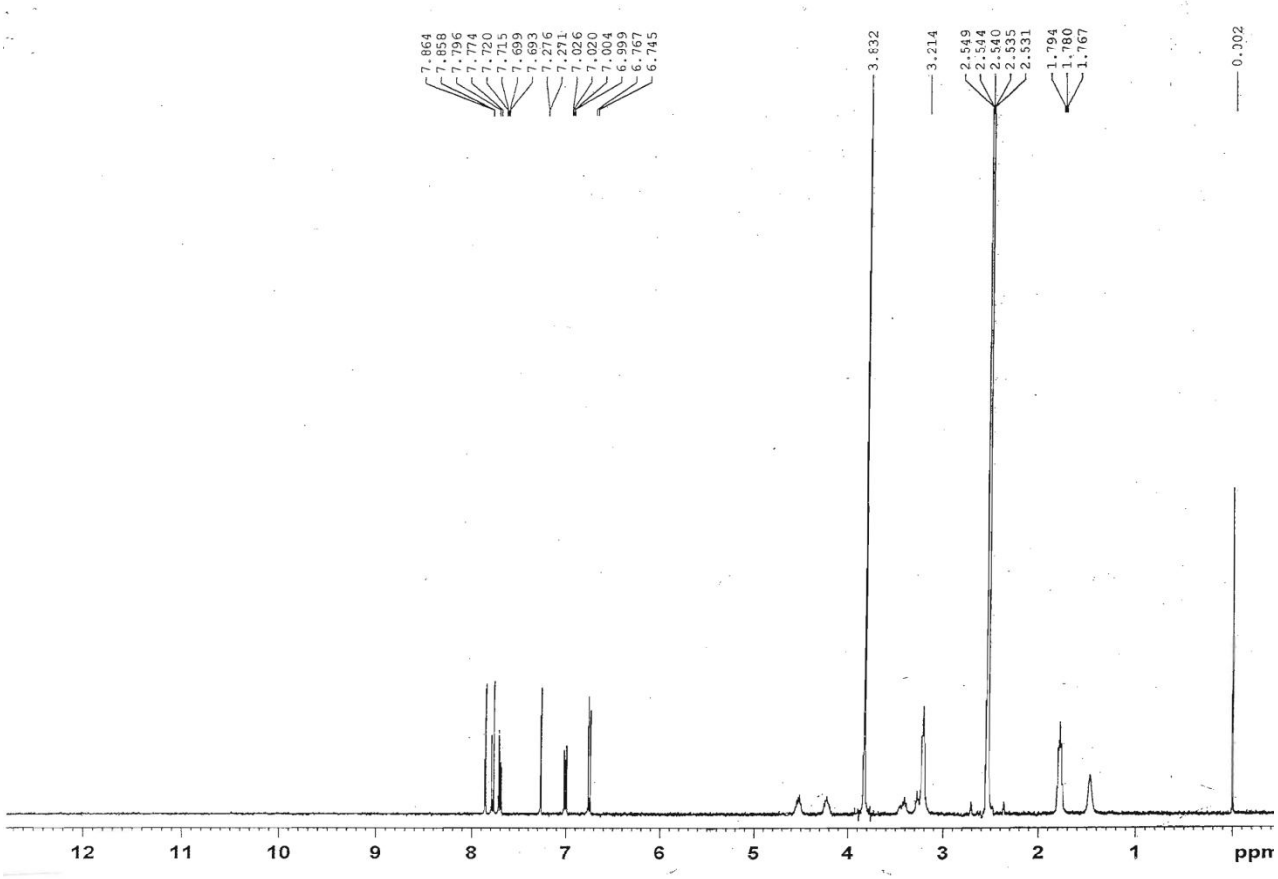

PerkinElmer Thermal Analysis
Onset = 195.323 °C
Peak = 199.399 °C
Area = 174.930 mJ
Delta H = 85.499 J/g
Onset = 187.422 °C
Peak = 189.067 °C
Area = 52.266 mJ
Delta H = 25.545 J/g
Heat Flow Endo Up (mW)
6.566
6
5
4
3
2
1
0
-0.887
50.22
60
80
100
120
140
160
180
200
220
240
260
280
299.5
Temperature (°C)

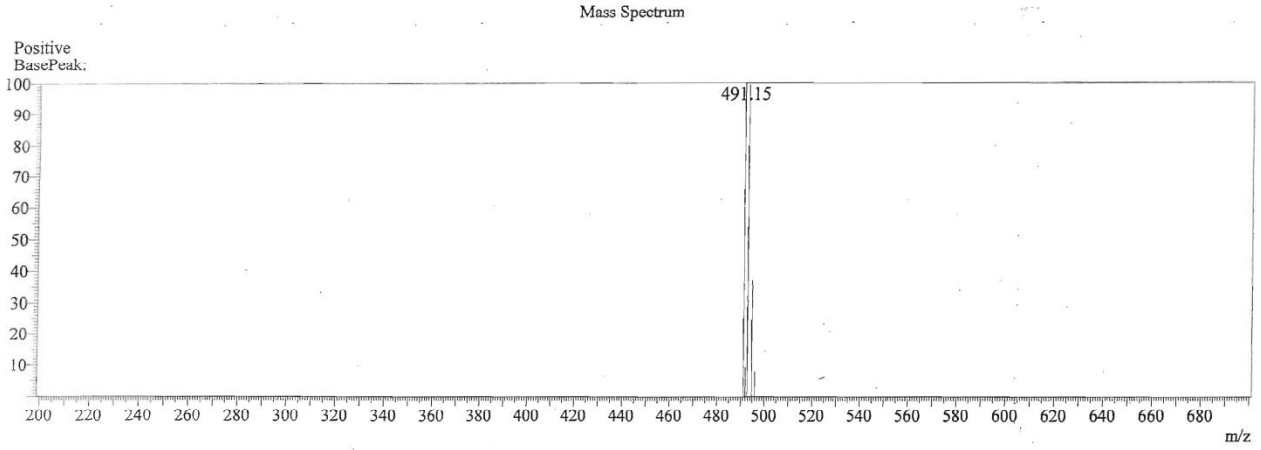

PerkinElmer Thermal Analysis
100.8
100
98
96
94
92
90
88
86
84.97
Weight % (%)
Delta Y = 11.6786 %
29.13
50
100
150
200
250
294.7
Temperature (°C)

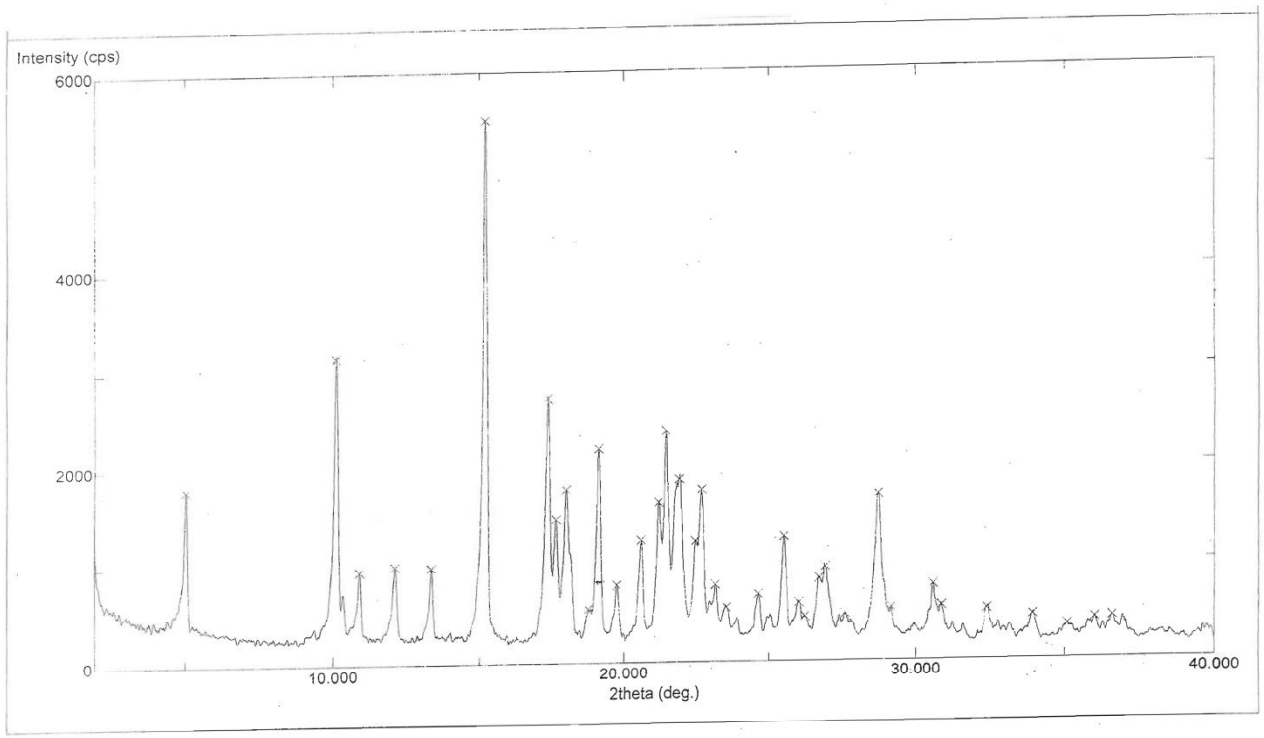
## Bisulfate salt of Compound 1

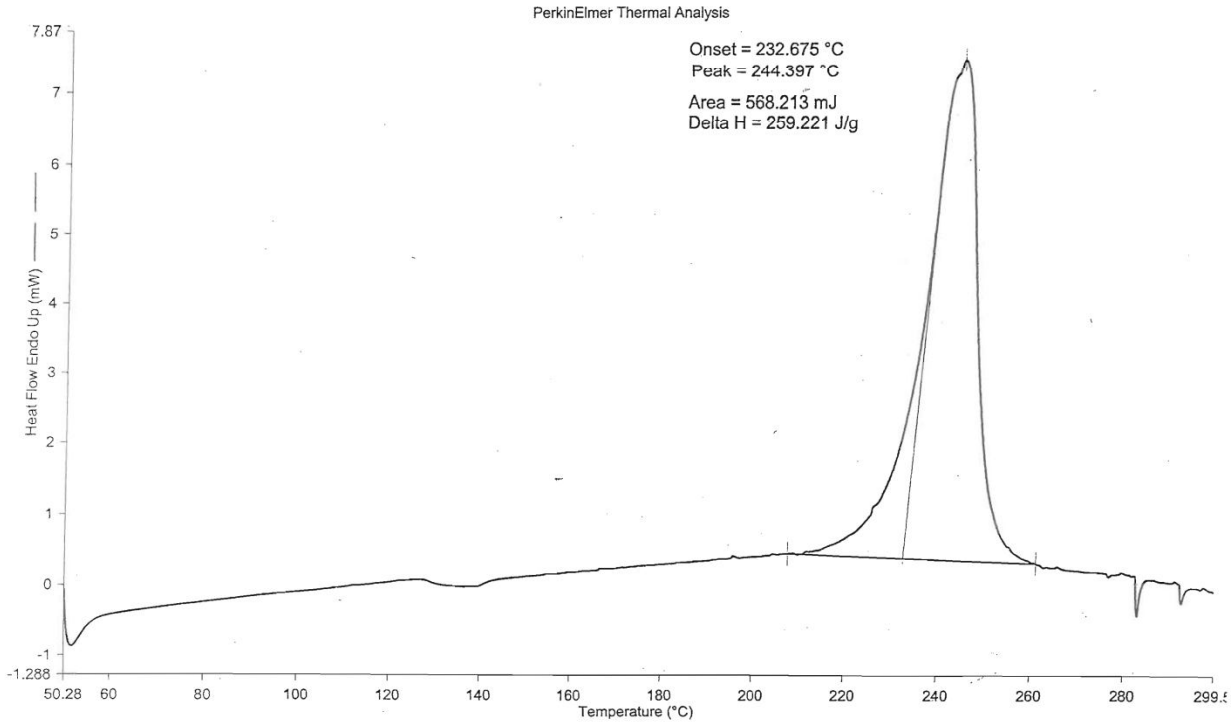

%T
3404.47
3153.72
3041.84
2951.19
2889.46
2710.08
2528.76
1913.45
1708.99
1599.04
1550.82
1492.95
1485.24
1452.45
1425.44
1406.15
1384.94
1369.50
1354.07
1307.78
1288.49
1271.13
1215.19
1184.33
1159.26
1122.61
1101.39
1089.82
1072.46
1049.31
1030.02
987.59
958.65
923.93
904.64
877.64
868.00
854.49
827.49
812.06
783.13
771.55
758.05
731.05
694.40
673.18
657.73
644.25
597.95

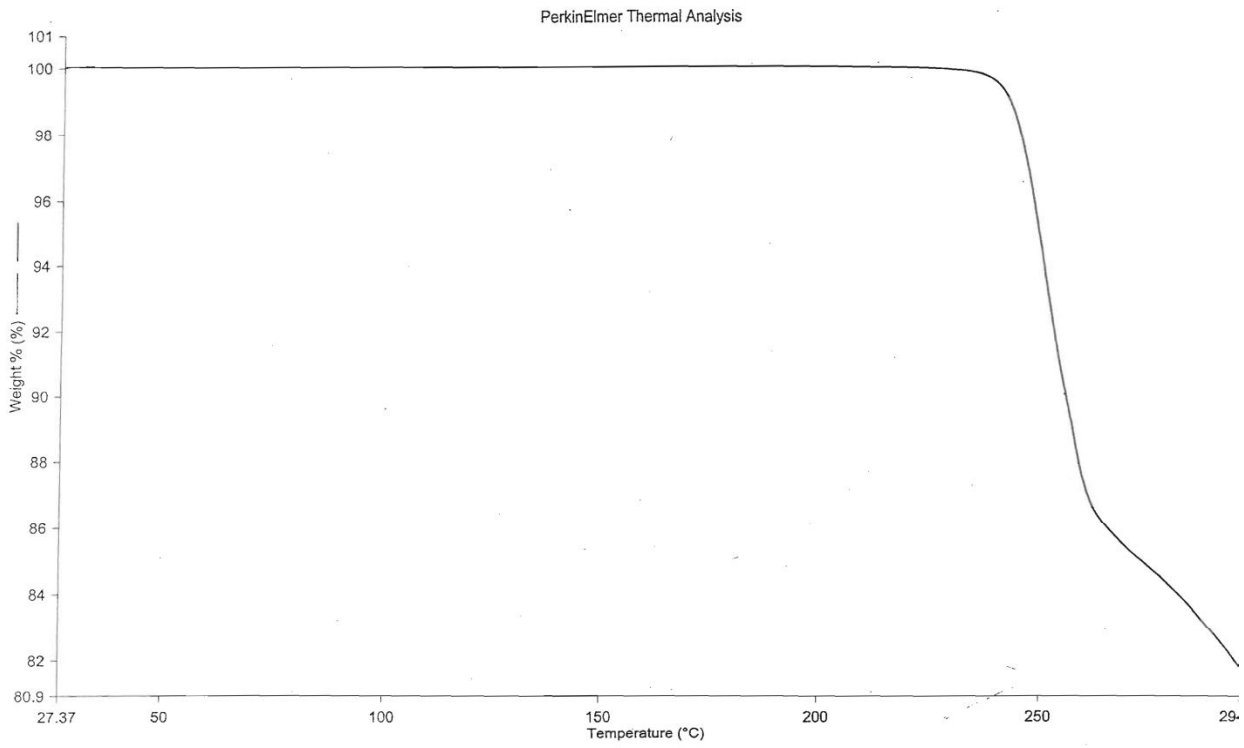

7.943
7.937
7.844
7.822
7.750
7.744
7.728
7.723
7.305
7.299
7.088
7.083
7.067
7.061
6.779
6.757
5.233
4.554
4.539
4.526
4.296
4.285
3.459
3.441
3.426
3.332
3.317
3.229
2.711
2.707
2.702
2.560
2.555
2.547
2.542
2.538
2.533
2.529
2.492
2.369
2.365
2.360
1.806
1.792
1.778
1.479
0.045
0.037
0.028
0.89
0.97
1.02
0.96
0.97
1.01
1.00
1.24
1.15
1.09
1.41
3.64
4.10
2.02


7.864
7.858
7.796
7.774
7.720
7.715
7.699
7.693
7.276
7.271
7.026
7.020
7.004
6.999
6.767
6.745
3.632
3.214
2.549
2.544
2.540
2.535
2.531
1.794
1.780
1.767
0.302
12
11
10
9
8
7
6
5
4
3
2
1
ppm

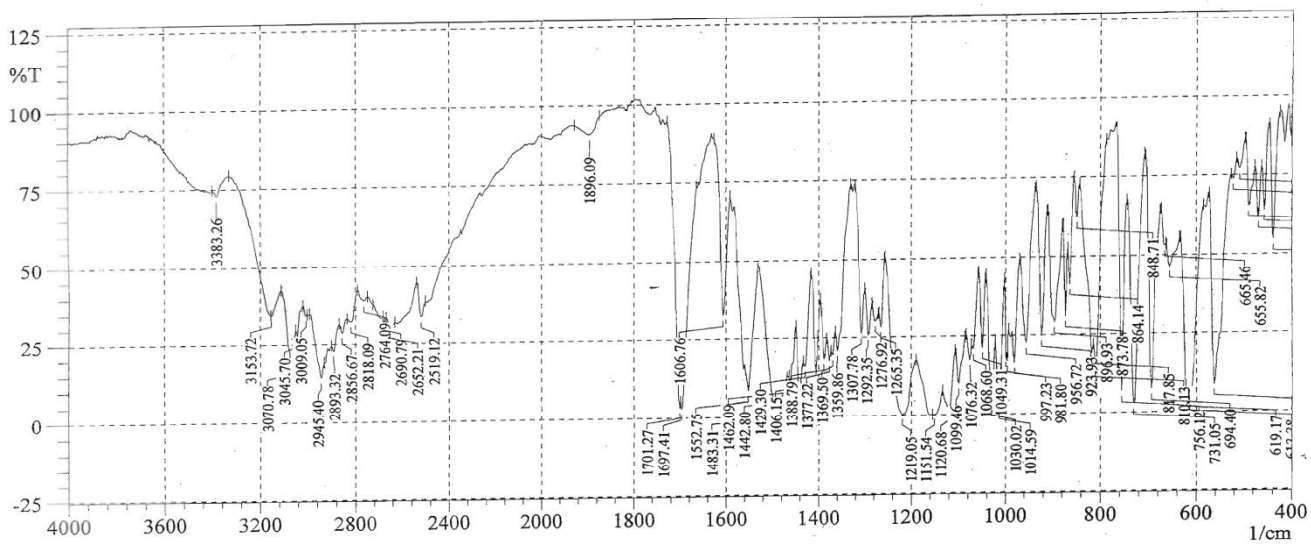

Mass Spectrum
Positive
BasePeak:
491.15
100
90
80
70
60
50
40
30
20
10
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
560
580
600
620
640
660
680
m/z

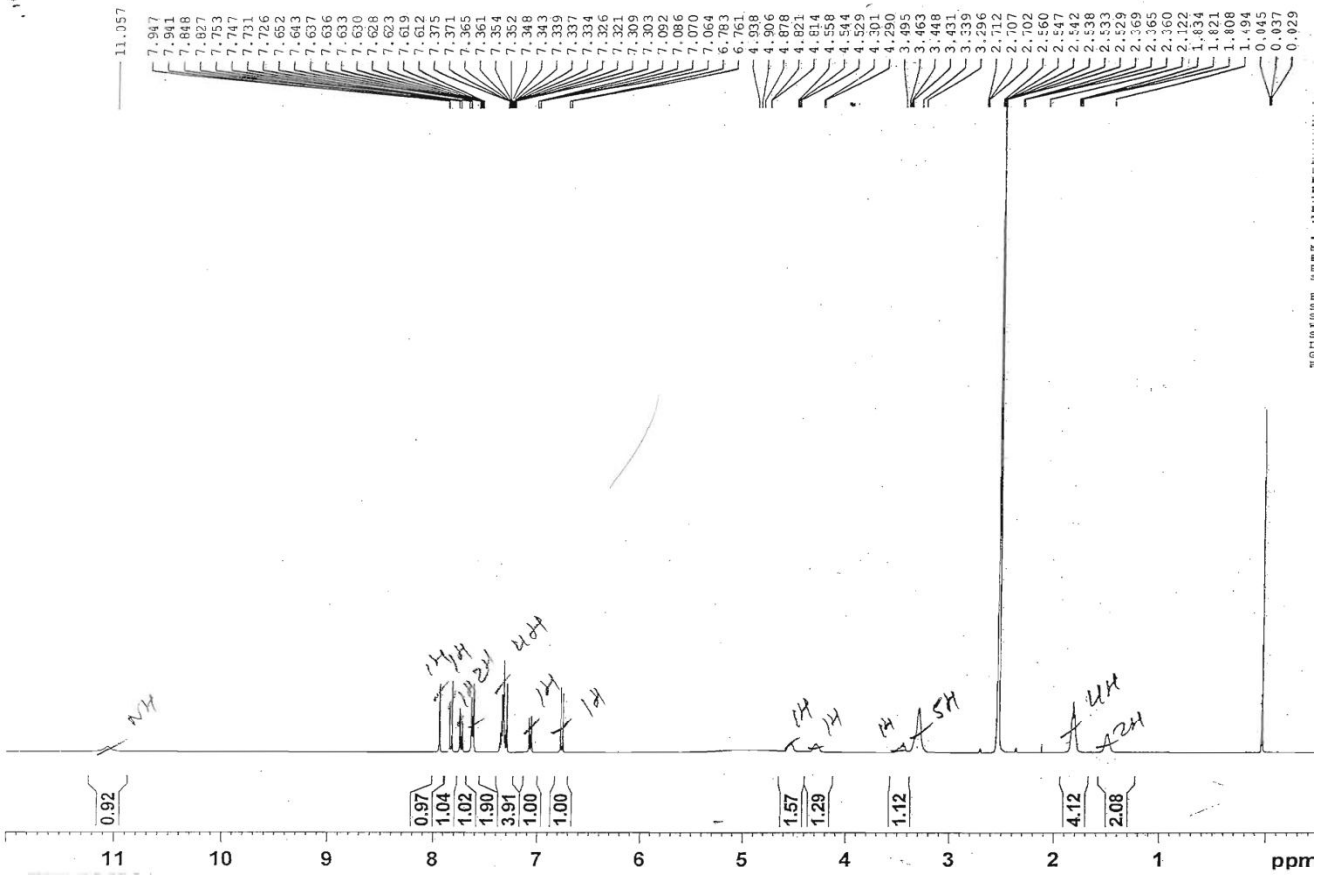

Intensity (cps)
6000
4000
2000
0
10.000
20.000
30.000
40.000
2theta (deg.)

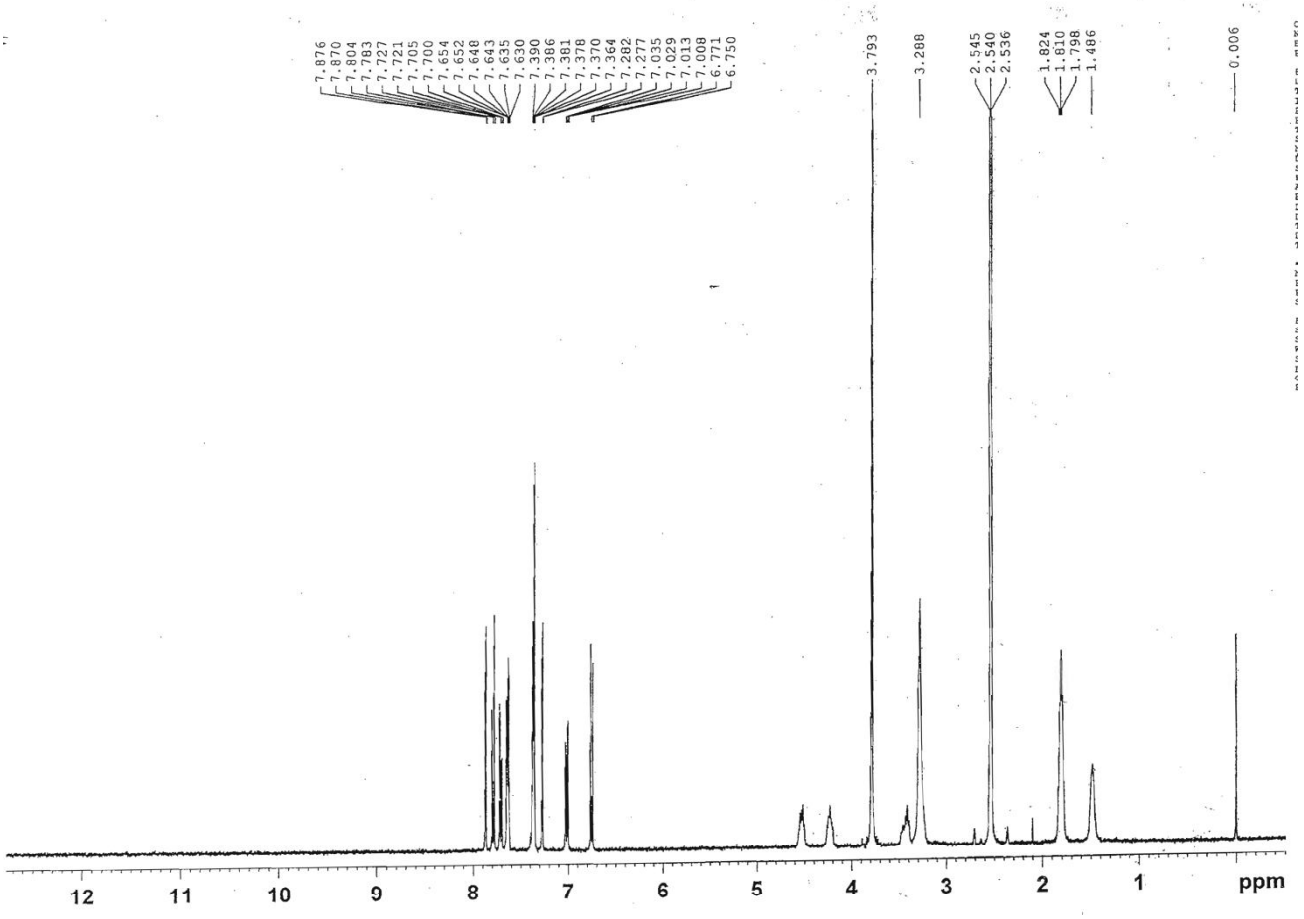

PerkinElmer Thermal Analysis
Onset = 232.675 °C
Peak = 244.397 °C
Area = 568.213 mJ
Delta H = 259.221 J/g
Heat Flow Endo Up (mW)
7.87
7
6
5
4
3
2
1
0
-1
-1.288
50.28
60
80
100
120
140
160
180
200
220
240
260
280
Temperature (°C)


PerkinElmer Thermal Analysis
101
100
98
96
94
92
90
88
86
84
82
80.9
Weight % (%)
27.37
50
100
150
200
250
Temperature (°C)

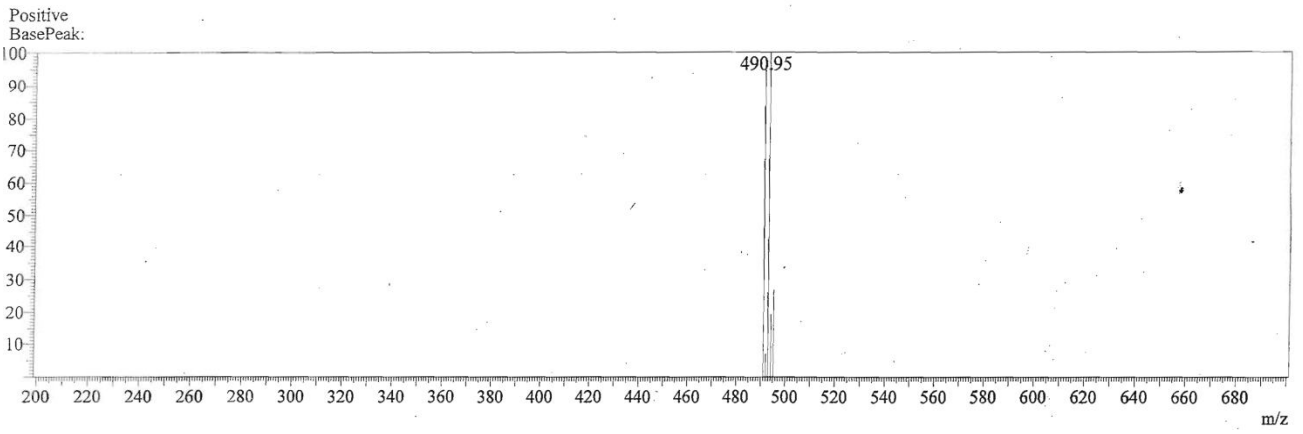
## Oxalate salt of Compound 1

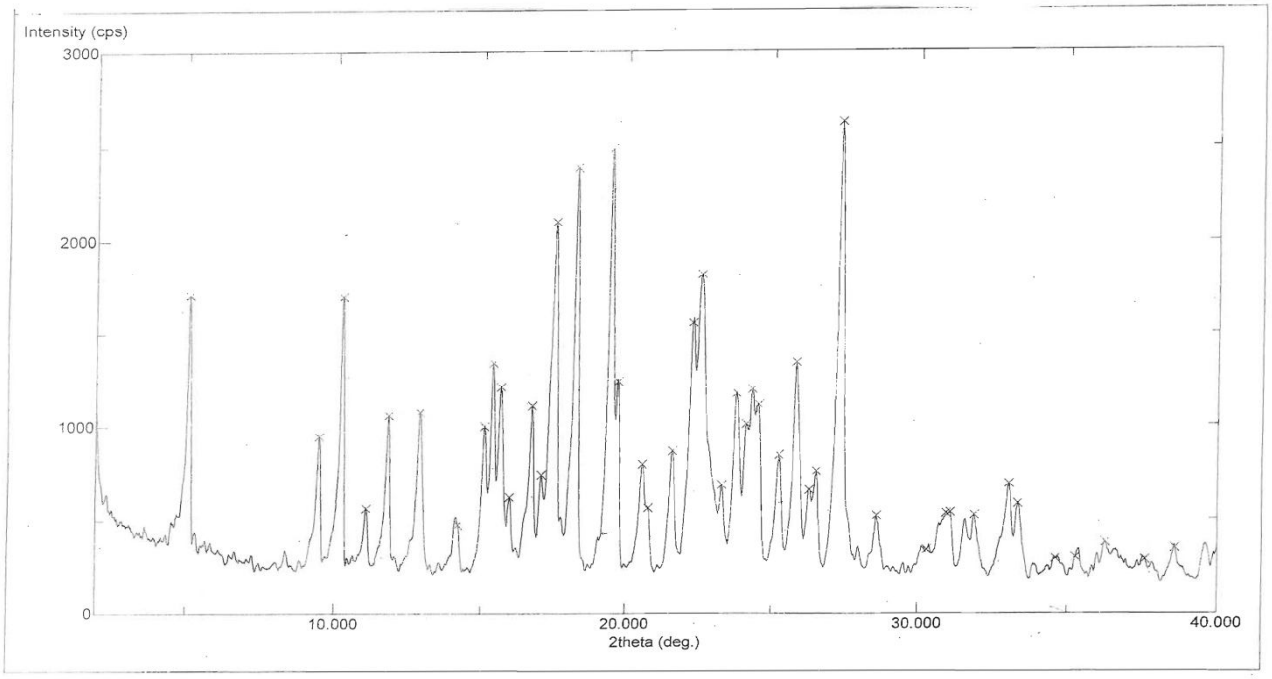

%T
1/cm
3383.26
3153.72
3070.78
3045.70
3009.05
2945.40
2893.32
2856.67
2818.09
2764.09
2690.79
2652.21
2519.12
1896.09
1606.76
1701.27
1697.41
1552.75
1483.31
1462.09
1442.80
1429.30
1406.15
1388.79
1377.22
1369.50
1359.86
1307.78
1292.35
1276.92
1265.35
1219.05
1151.54
1120.68
1099.46
1076.32
1068.60
1049.31
1030.02
1014.59
997.23
981.80
956.72
923.93
896.93
873.78
864.14
848.71
847.85
810.13
756.19
731.05
694.40
665.46
655.82
619.17

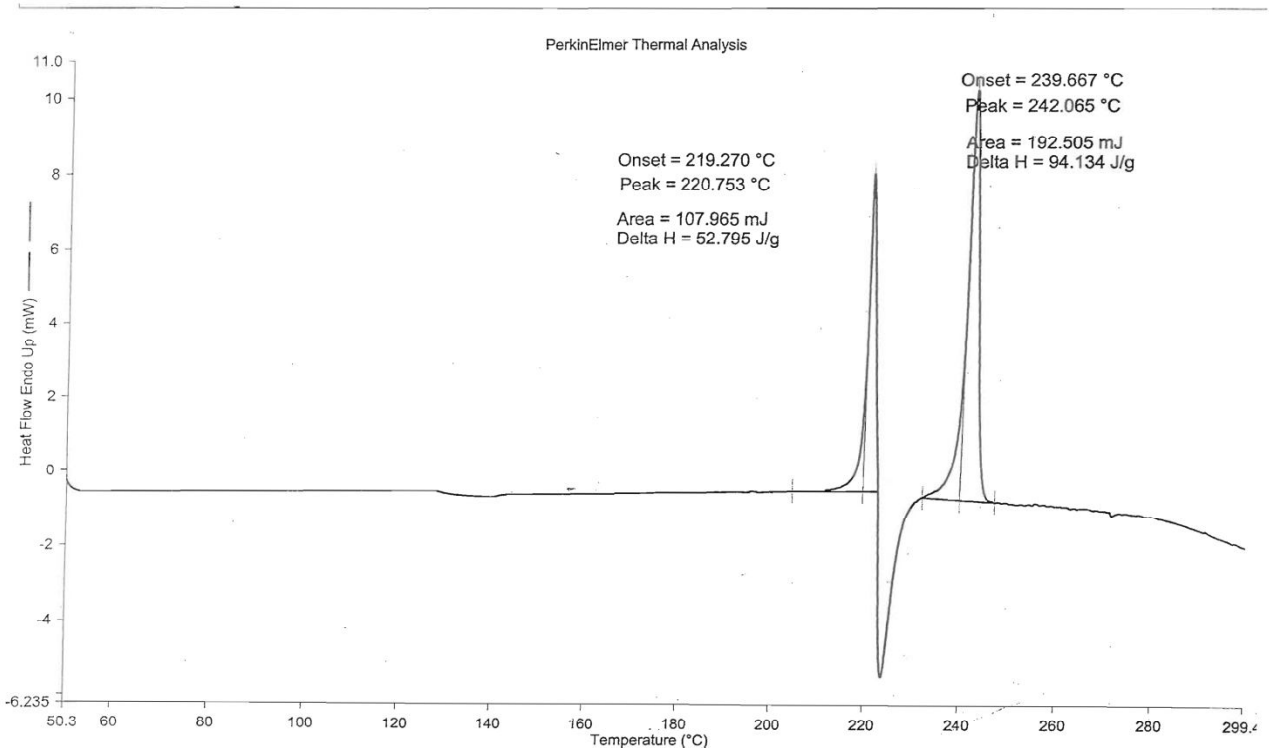

11.057
7.947
7.941
7.848
7.827
7.753
7.747
7.731
7.726
7.652
7.643
7.637
7.636
7.633
7.630
7.628
7.623
7.619
7.612
7.375
7.371
7.365
7.361
7.354
7.352
7.348
7.343
7.339
7.337
7.334
7.326
7.321
7.309
7.303
7.092
7.086
7.070
7.064
6.783
6.761
4.938
4.906
4.878
4.821
4.814
4.558
4.544
4.529
4.301
4.290
3.495
3.463
3.448
3.431
3.339
3.296
2.712
2.707
2.702
2.560
2.547
2.542
2.538
2.533
2.529
2.369
2.365
2.360
2.122
1.834
1.821
1.808
1.494
0.045
0.037
0.029
0.92
0.97
1.04
1.02
1.90
3.91
1.00
1.00
1.57
1.29
1.12
4.12
2.08
ppm

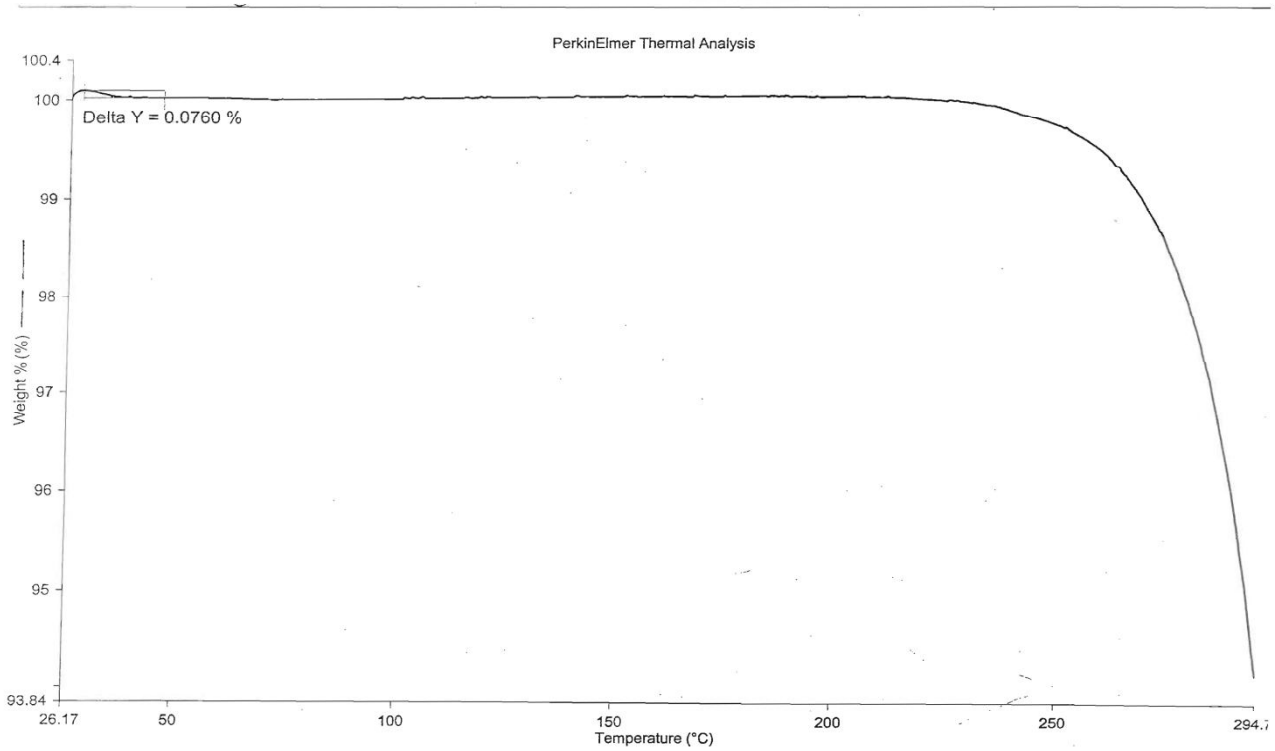



Mass Spectrum

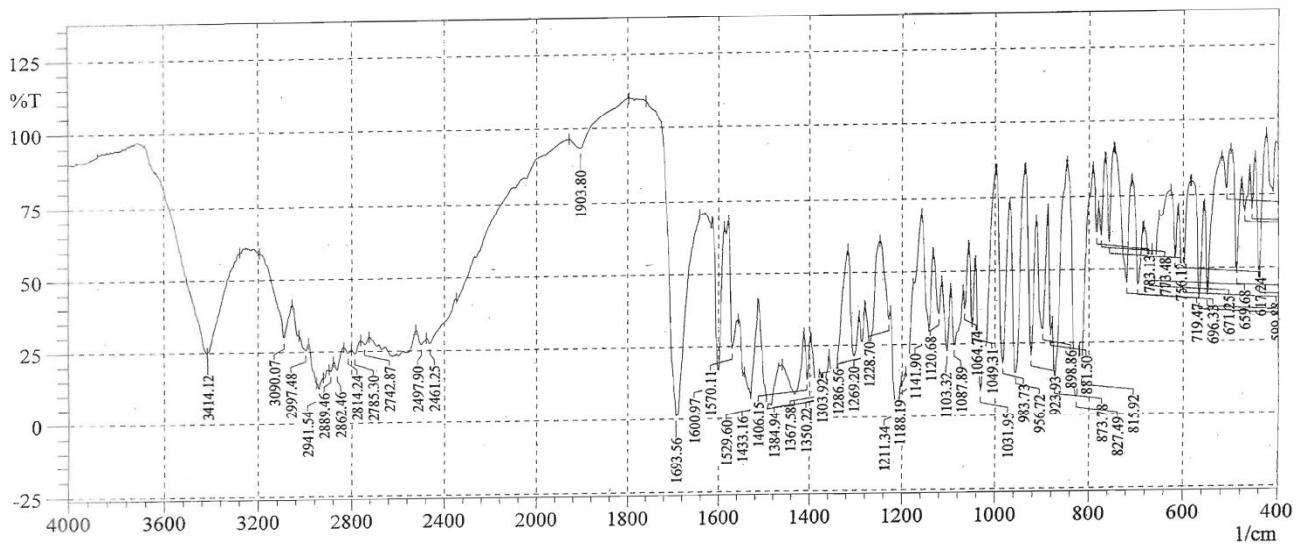


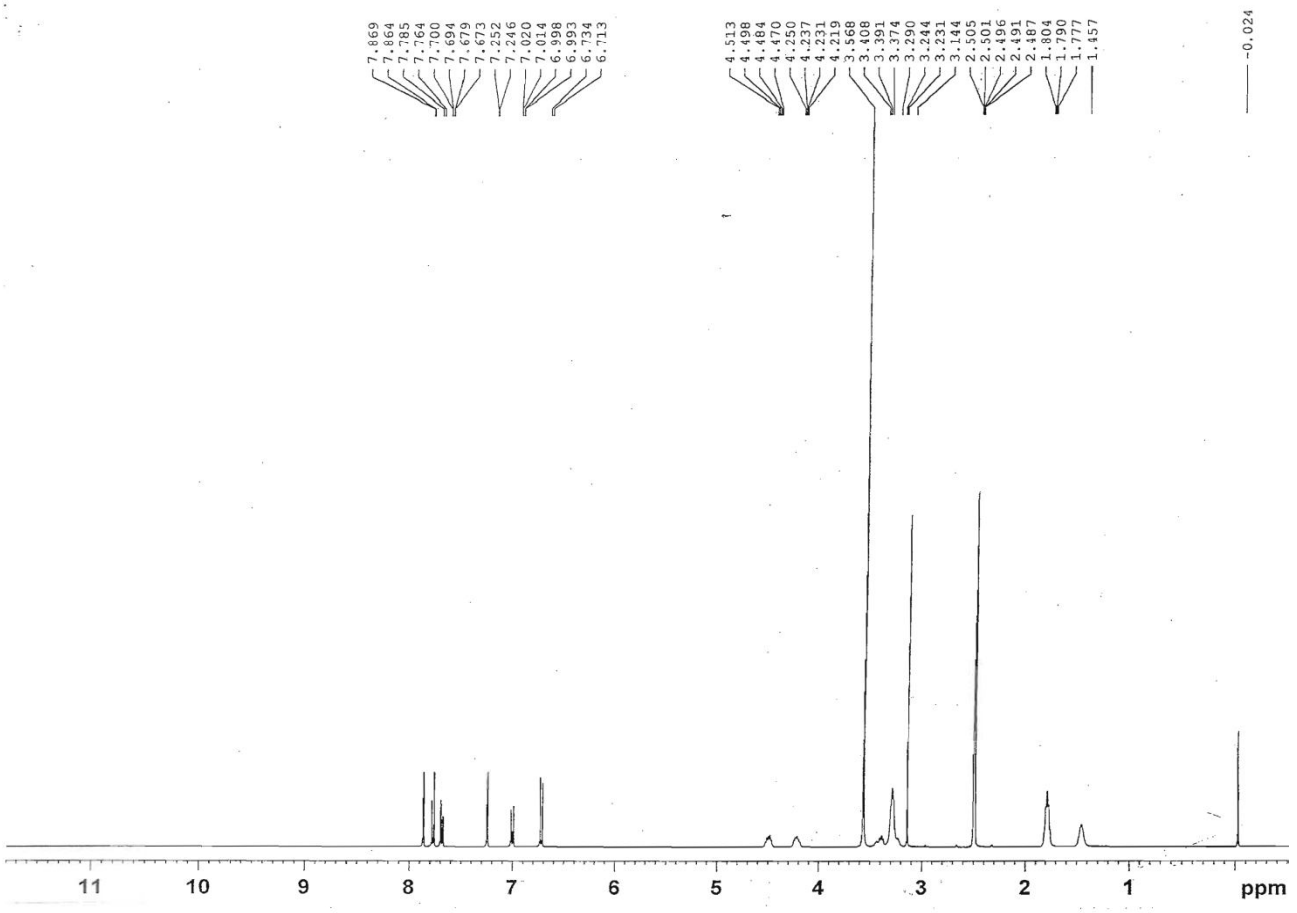

Intensity (cps)
3000
2000
1000
0
10.000
20.000
30.000
40.000
2theta (deg.)

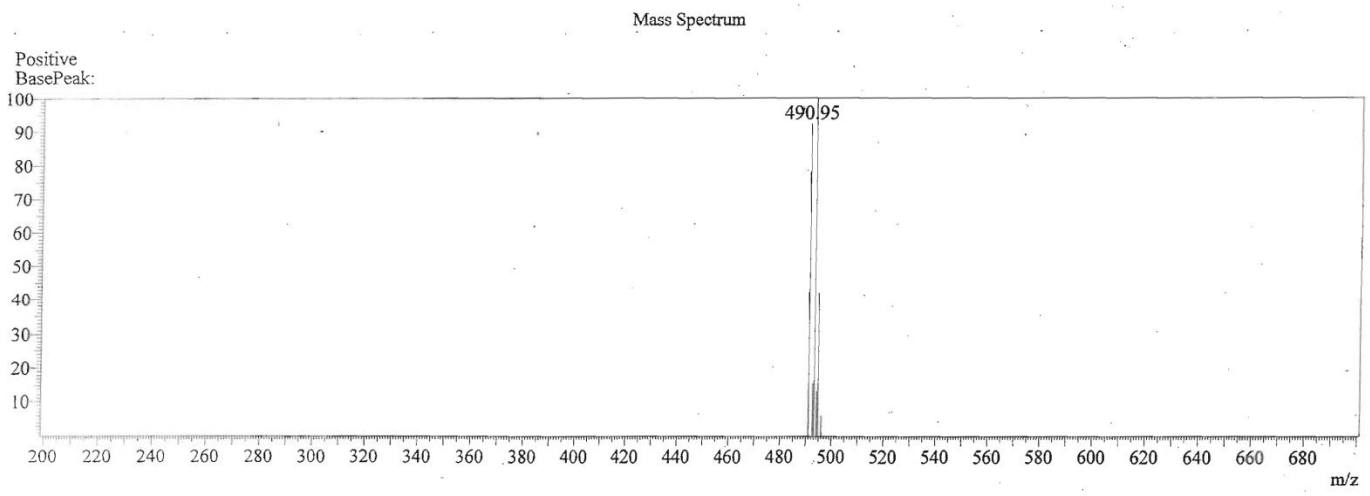

PerkinElmer Thermal Analysis
Onset = 239.667 °C
Peak = 242.065 °C
Area = 192.505 mJ
Delta H = 94.134 J/g
Onset = 219.270 °C
Peak = 220.753 °C
Area = 107.965 mJ
Delta H = 52.795 J/g
Heat Flow Endo Up (mW)
11.0
10
8
6
4
2
0
-2
-4
-6.235
50.3
60
80
100
120
140
160
180
200
220
240
260
280
Temperature (°C)

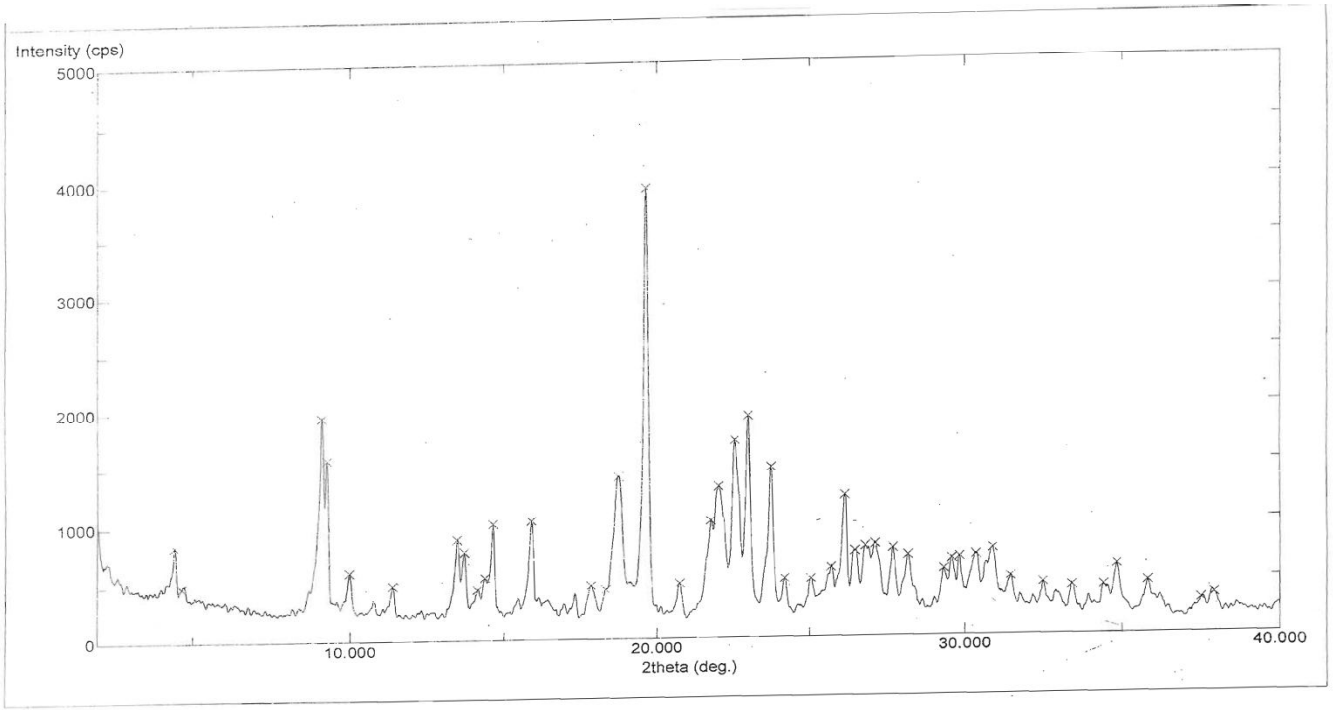

PerkinElmer Thermal Analysis
100.4
100
99
98
97
96
95
93.84
Delta Y = 0.0760 %
Weight % (%)
26.17
50
100
150
200
250
Temperature (°C)

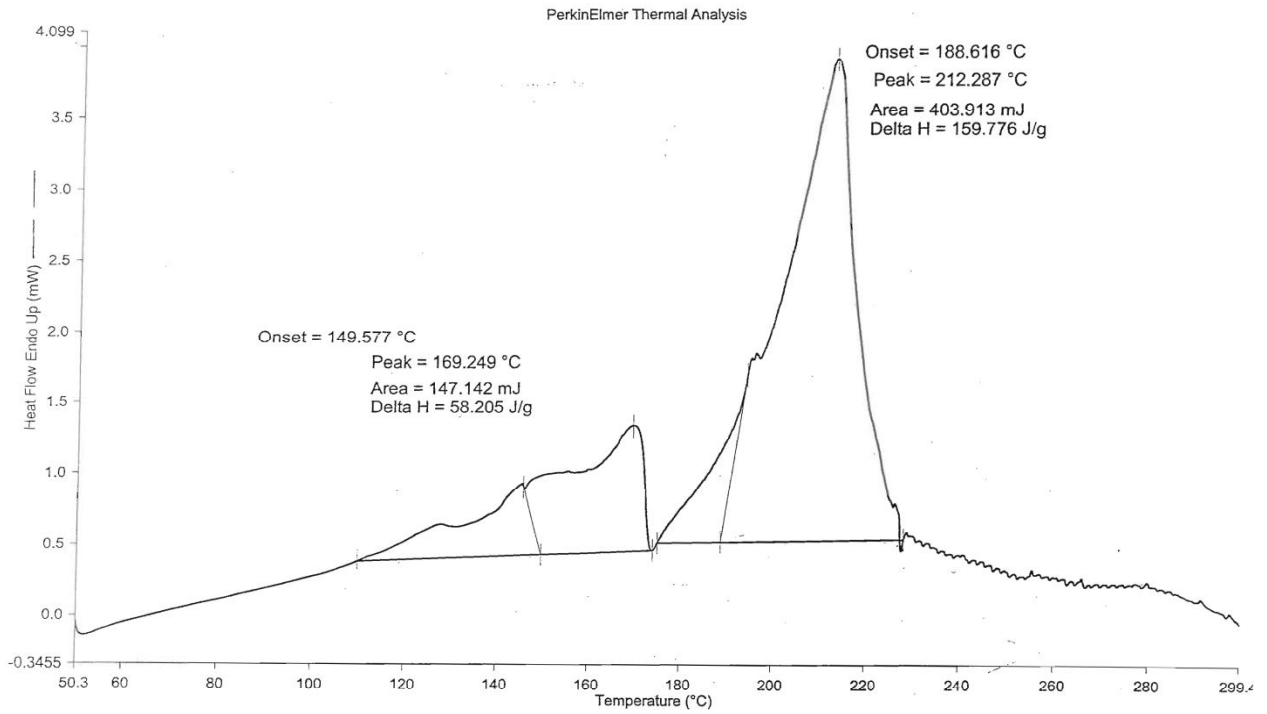
# Besylate salt of Compound 1

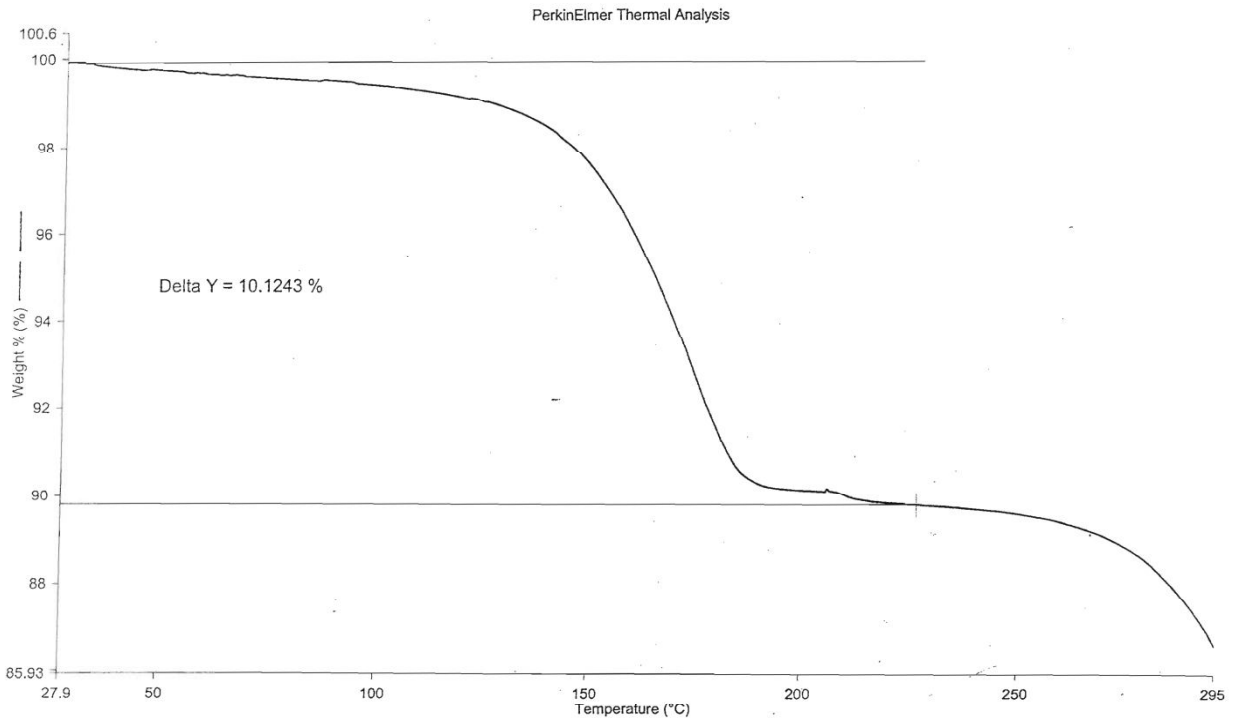

125
%T
100
75
50
25
0
-25
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm
3414.12
3090.07
2997.48
2941.54
2889.46
2862.46
2814.24
2785.30
2742.87
2497.90
2461.25
1903.80
1693.56
1600.97
1570.11
1529.60
1433.16
1406.15
1384.94
1367.58
1350.22
1303.92
1286.56
1269.20
1228.70
1211.34
1188.19
1141.90
1120.68
1103.32
1087.89
1064.74
1049.31
1031.95
983.73
956.72
923.93
898.86
881.50
873.78
827.49
815.92
783.13
719.47
696.38
671.25
659.68


7.869
7.864
7.785
7.764
7.700
7.694
7.679
7.673
7.252
7.246
7.020
7.014
6.998
6.993
6.734
6.713
4.513
4.498
4.484
4.470
4.250
4.237
4.231
4.219
3.568
3.408
3.391
3.374
3.290
3.244
3.231
3.144
2.505
2.501
2.496
2.491
2.487
1.804
1.790
1.777
1.457
-0.024
11
10
9
8
7
6
5
4
3
2
1
ppm

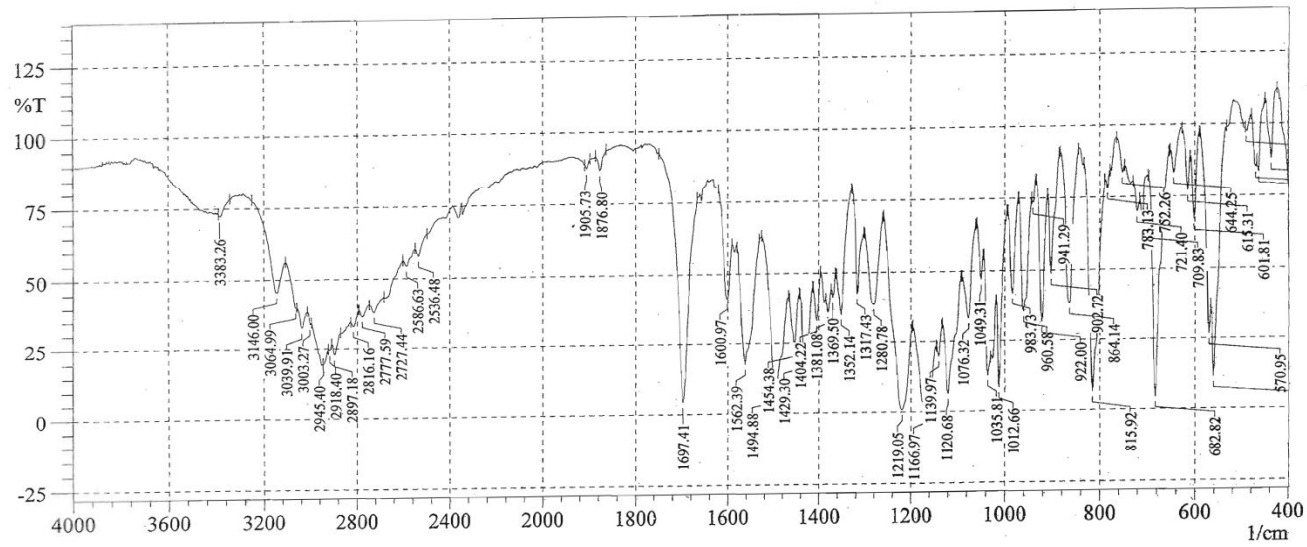

Mass Spectrum
Positive
BasePeak:
490.95
100
90
80
70
60
50
40
30
20
10
200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600 620 640 660 680
m/z

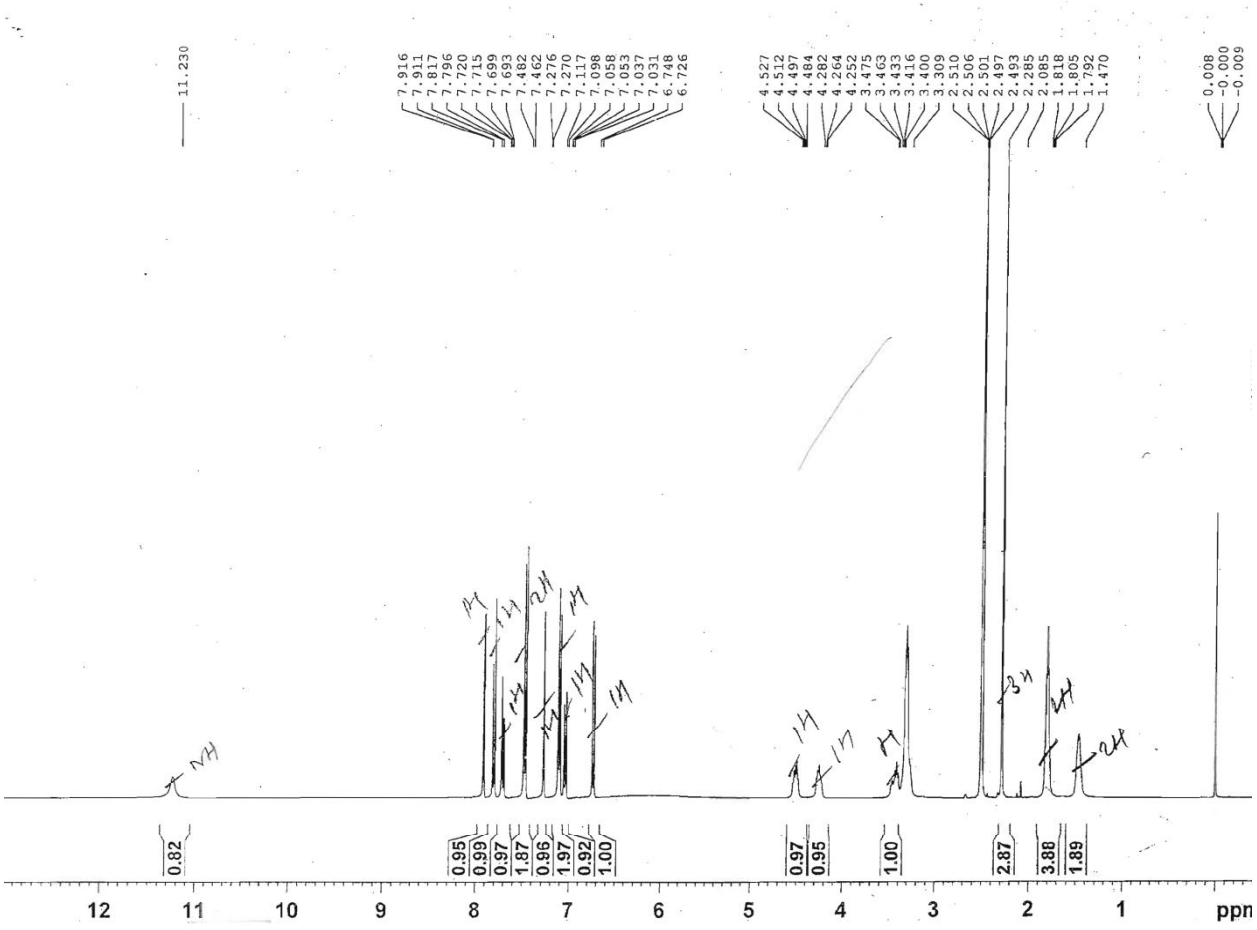

Intensity (cps)
5000
4000
3000
2000
1000
0
10.000
20.000
30.000
40.000
2theta (deg.)

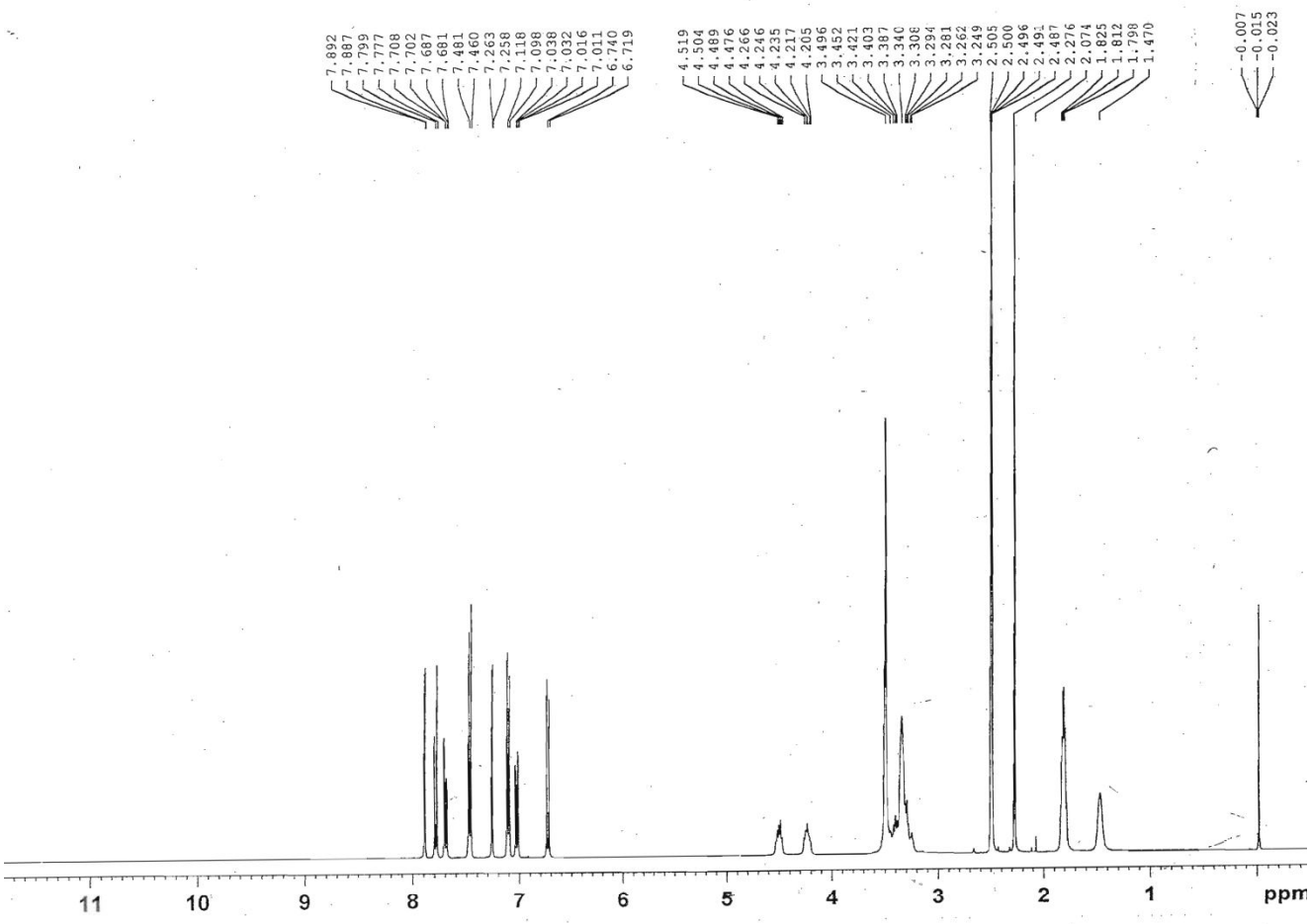

PerkinElmer Thermal Analysis
Onset = 188.616 °C
Peak = 212.287 °C
Area = 403.913 mJ
Delta H = 159.776 J/g
Onset = 149.577 °C
Peak = 169.249 °C
Area = 147.142 mJ
Delta H = 58.205 J/g
Heat Flow Endo Up (mW)
Temperature (°C)

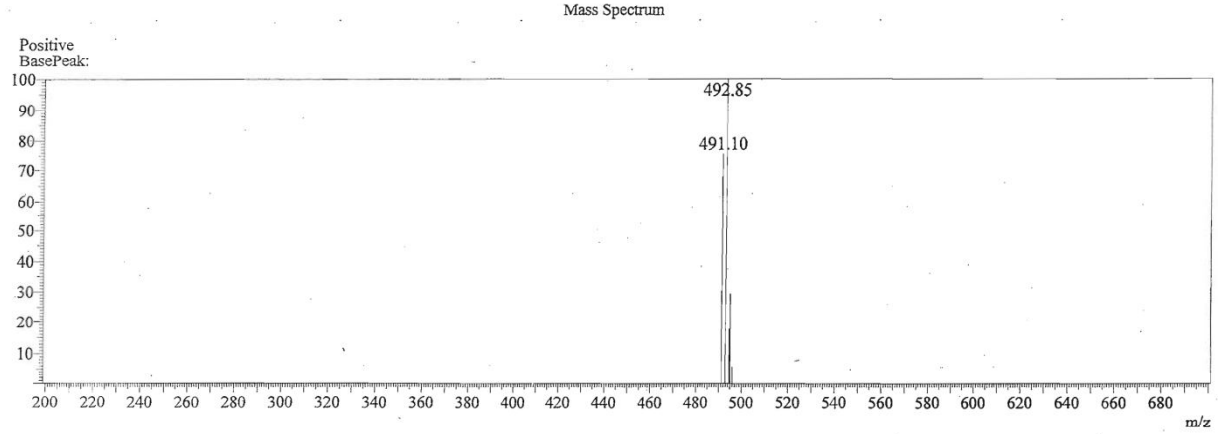

PerkinElmer Thermal Analysis
Delta Y = 10.1243 %
Weight % (%)
Temperature (°C)

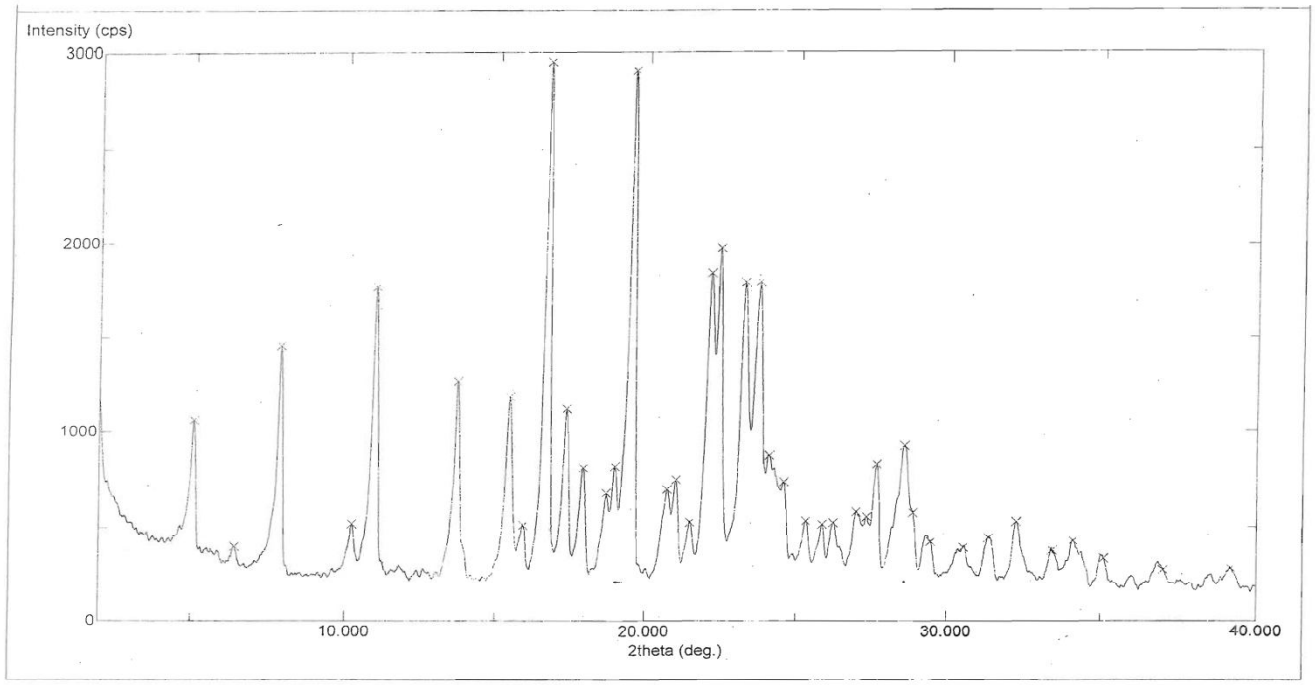
# Methyl iodide salt of Compound 1

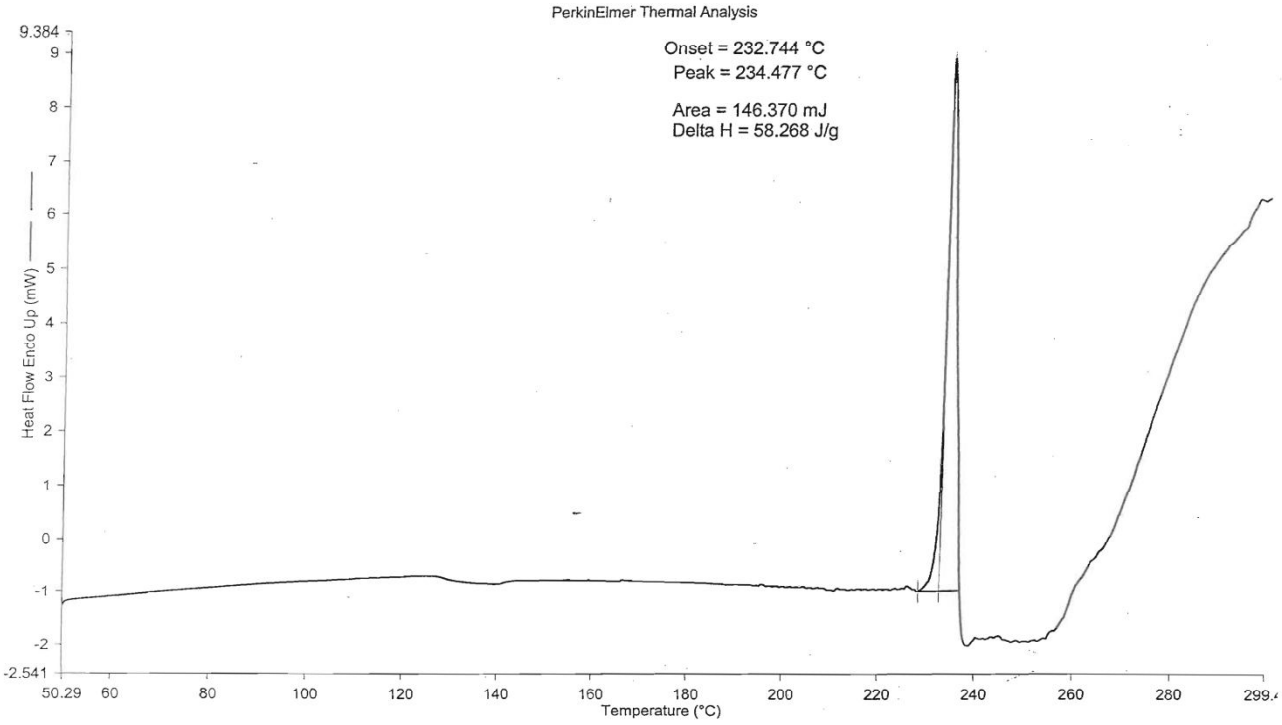

%T
1/cm
3383.26
3146.00
3064.99
3039.91
3003.27
2945.40
2918.40
2897.18
2816.16
2777.59
2727.44
2586.63
2536.48
1905.73
1876.80
1697.41
1600.97
1562.39
1494.88
1454.38
1429.30
1404.22
1381.08
1369.50
1352.14
1317.43
1280.78
1219.05
1166.97
1139.97
1120.68
1076.32
1049.31
1035.81
1012.66
983.73
960.58
941.29
922.00
902.72
864.14
815.92
783.13
762.26
721.40
709.83
682.82
644.25
615.31
601.81
570.95

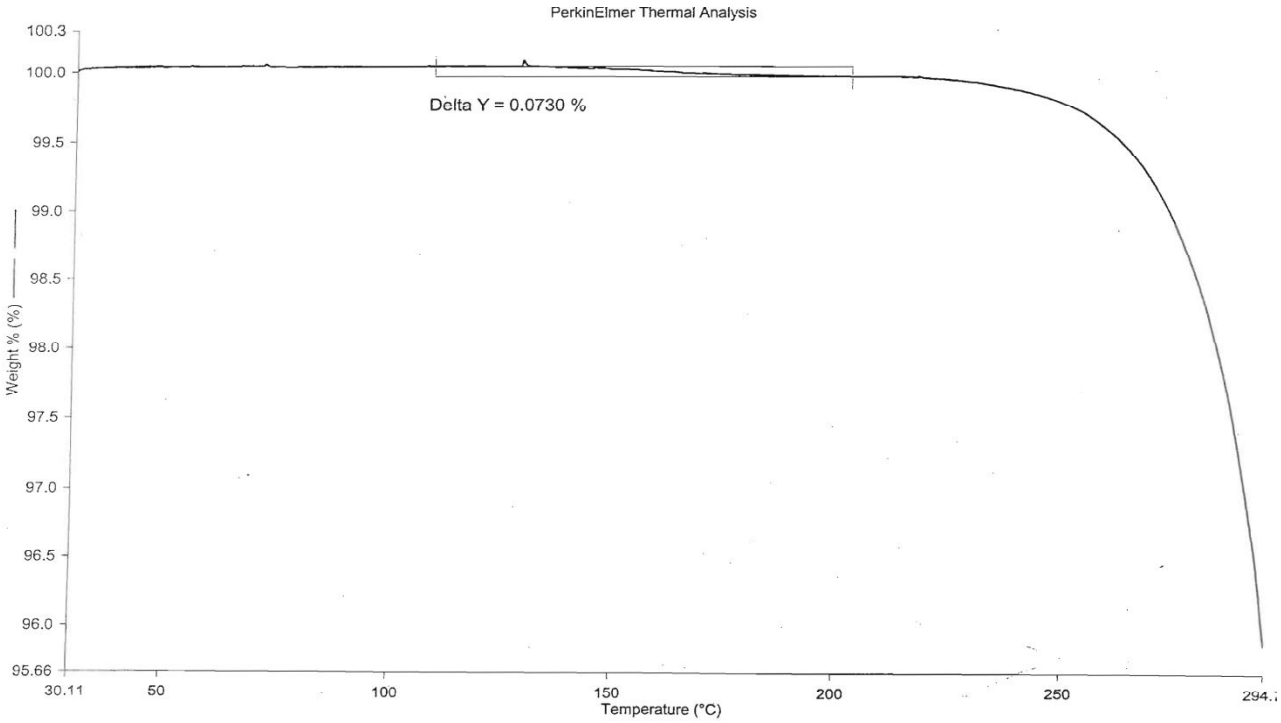

11.230
7.916
7.911
7.817
7.796
7.720
7.715
7.699
7.693
7.482
7.462
7.276
7.270
7.117
7.098
7.058
7.053
7.037
7.031
6.748
6.726
4.527
4.512
4.497
4.484
4.282
4.264
4.252
3.475
3.463
3.433
3.416
3.400
3.309
2.510
2.506
2.501
2.497
2.493
2.285
2.085
1.818
1.805
1.792
1.470
0.008
-0.000
-0.009
NH
0.82
0.95
0.99
0.97
1.87
0.96
1.97
0.92
1.00
0.97
0.95
1.00
2.87
3.88
1.89
ppm


7.892
7.887
7.799
7.777
7.708
7.702
7.687
7.681
7.481
7.460
7.263
7.258
7.118
7.098
7.038
7.032
7.016
7.011
6.740
6.719
4.519
4.504
4.489
4.476
4.266
4.246
4.235
4.217
4.205
3.496
3.452
3.421
3.403
3.387
3.340
3.249
2.505
2.500
2.496
2.487
2.276
2.074
1.825
1.812
1.798
1.470
-0.007
-0.015
-0.023
11
10
9
8
7
6
5
4
3
2
1
ppm


Mass Spectrum
Positive
BasePeak:
100
90
80
70
60
50
40
30
20
10
492.85
491.10
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
520
540
560
580
600
620
640
660
680
m/z


Intensity (cps)
3000
2000
1000
0
10.000
20.000
30.000
40.000
2theta (deg.)


PerkinElmer Thermal Analysis
Onset = 232.744 °C
Peak = 234.477 °C
Area = 146.370 mJ
Delta H = 58.268 J/g
Heat Flow Endo Up (mW)
9.384
-2.541
50.29
60
80
100
120
140
160
180
200
220
240
260
280
Temperature (°C)


PerkinElmer Thermal Analysis
Delta Y = 0.0730 %
Weight % (%)
100.3
100.0
99.5
99.0
98.5
98.0
97.5
97.0
96.5
96.0
95.66
30.11
50
100
150
200
250
Temperature (°C)