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Organoselenocyanates and Symmetrical Diselenides Redox Modulators: Design, Synthesis and Biological Evaluation

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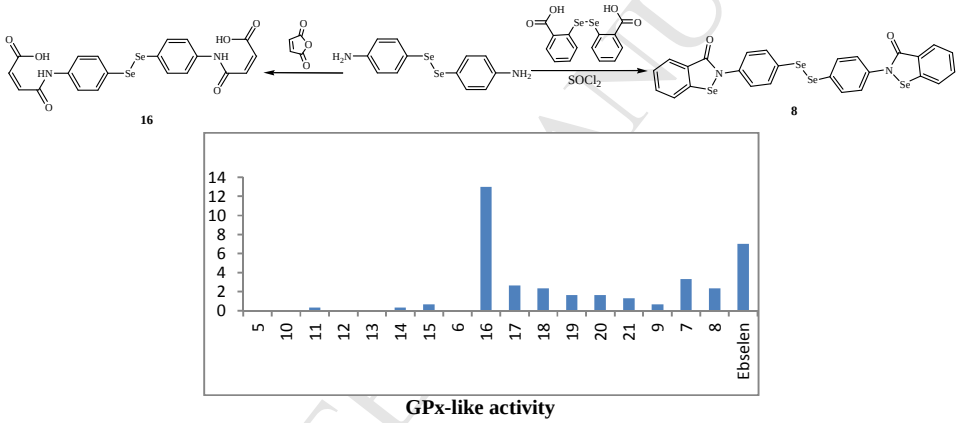
Graphical abstract



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ABSTRACT

Oxidative stress (OS) and disturbed intracellular redox balance have been predominantly observed in different types of cancer, including hepatocellular carcinoma (HCC). Agents which can stop OS multi-stressor events and modulate the intracellular redox state are becoming a major focus in HCC prevention. Among them, compounds with glutathione peroxidase (GPx)-like activity are of particularly concern. We herein report the synthesis of novel series of organoselenocyanates and symmetrical diselenide antioxidants, inspired by the natural redox enzyme, GPx and the synthetic organoselenium ebselen antioxidants. Their cytotoxic activity was evaluated against Hep G2 cells and their antimicrobial activities were evaluated against *Candida albicans* (*C. albicans*) fungus as well as against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), gram-negative and gram-positive bacteria, respectively. These compounds were also tested for their antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH), GPx-like activity and bleomycin dependent DNA damage assays and a basic structure-activity relationship was subsequently established. The physicochemical parameters and drug-likeness were computed employing the Molinspiration online property calculation toolkit and MolSoft software. Interestingly, some compounds proved to be more cytotoxic than ebselen and the known anticancer drug 5-Fu and in the same time they showed

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similar, sometime even more, antifungal activity than the reference antifungal drugs. Among these compounds, compound **16** was considered to be the most interesting with free radical-scavenging activity comparable to ascorbic acid and a GPx-like activity similar to ebselen. As most of these compounds comply with Lipinski's Rule of Five, they promise good bioavailability, which needs to be studied as part of future investigations.

Keywords: ebselen; organoselenocyanates; diselenides; GPx mimics; multicomponent reactions.

Abbreviations: Oxidative stress (OS); hepatocellular carcinoma (HCC); glutathione peroxidase (GPx); reactive oxygen species (ROS); reactive nitrogen species (RNS); isocyanide based multicomponent reactions (IMCRs).

1. Introduction

Overproduction of reactive oxygen (ROS) and nitrogen species (RNS) and diminished antioxidant capacity have been observed in wide range of diseases ranging from neurodegenerative diseases and inflammation to cancer. The role of antioxidant therapeutics is to scavenge free radicals, recover the cellular "redox homeostasis" and therefore they are widely believed to reduce the risk of such diseases. This is where organoselenium compounds come into play. These compounds attract the continuous interest of chemists and medicinal biologists due to their largely untapped pharmacological applications. Selenium compounds are being used as antioxidants, neuroprotectives, nootropics, anxiolytics, antinociceptives and antidepressants.[1,2] It is noteworthy that not only the amount but also the nature of the selenium containing compounds is essential for its pharmacological potency, or toxicity. In this context, organoselenium compounds have been found to be less toxic than some inorganic selenium compounds. Therefore, there has been a growing interest in the synthesis of organoselenium compounds.

Symmetrical diorganyl diselenides have become a hot spot in selenium chemistry since they are stable, easy to prepare and depending on the substituents can have reduced toxicity.[3] Some compounds play a leading role in reducing the risk of cancer incidence and mortality in many tumors.[4,5] In this context, various diselenides have shown promising hepatoprotective properties as they were able to reduce hepatotoxicity induced by heavy metals.[4,6] Considering

the fact that the difference between prevention and treatment are strictly dose dependent, diselenides in this context can be model for cancer therapy and particularly for HCC.[7]

Recently, we have reported the synthesis of selenium-containing peptoids and peptide-like compounds employing isocyanide based multicomponent reactions (IMCRs).[8-10] Some of these compounds were able to induce cell cycle delay, reduction of glutathione levels and apoptosis *via* dose-dependent activation of caspase 3 and 7 in melanoma (A-431) and breast cancer (MCF-7) cells.[7,10-15] Based on these result and in continuation of our previous work on organoselenium compounds and HCC, we would like to develop further sophisticated diorganyl diselenides and evaluate their antitumor activity on HCC cells.

Twenty years ago, it was found that ebselen behaved against OS in a similar manner as the common enzyme GPx (Figure 1).[16-18][19,20] More recently, it was reported that diphenyl diselenide was able to increase both the cellular GPx expression and showed activity that was two times higher than that of ebselen.[21] Another important factor is the relatively low toxicity of diphenyl diselenide which is attributed to its *in vivo* stability. Recent evidence suggested that diphenyl diselenide is a good substrate of hepatic and cerebral thioredoxin reductase (TrxR) proposing another rational for its potent antioxidant action (Figure 1).[22-24] These studies stimulated the quest for new organic diselenides that would be better soluble, more efficient and less toxic.

<Figure 1 >

The objective of this study was to synthesize novel series of organoselenocyanates and symmetrical diselenides with the aim to find out more efficient GPx mimics with a potent anti-HCC activity.

2. Results and Discussions

2.1. Chemistry

Diverse trials were made to enhance ebselen catalytic activity.[25,26] These were only focused to modulate the substituent effects which in turn have been marred with limited success. Keeping this in mind and taking in considerations that diphenyl diselenide potentially is more efficient mimetic of native GPx than ebselen and can be re-reduced by thioredoxin reductases, our primary objective was to devise a rapid synthesis of modified diphenyl diselenides, ideally

combining beneficial features found in diselenides, ebselen and yet unexplored of selenocyanates.

The key intermediate 2-(chloroseleno)benzoyl chloride **(2**), obtained from the reaction of 2,2`-diselanediyldibenzoic acid (**1**) with thionyl chloride, possesses two electrophilic centers suitable for *N*-nucleophilic substitution.[27] The primary aromatic amines 4-aminophenylselenocyanate (**3**) and 4-(2-(4-aminophenyl)diselanyl)benzenamine (**4**) were selected as selenium-based key synthons that enable access to symmetrical diselenides. In this context, the selenenylationacylation of **3** and **4** with **2** afforded the corresponding ebselen selenocyanate **5** and ebselen diselenide **6** in good yields (71% and 68%, Scheme 1).[9,28,29]

Interestingly, 2,2`-diselanediyldibenzoic acid (**1**) has not been involved in any isocyanide based multicomponent reaction (IMCR) before. This promoted us to investigate it in Ugi four component reaction (U4CR) which in turn would give access to highly functionalized diaryldiseleno-peptidomimetic scaffolds (Scheme 1), different in stability and redox properties than selenocystein-based diselenide.

The U4CR comprises the one pot synthesis of α-aminoacyl amides from an aldehyde, amine, acid and isonitrile.[30] The amine components employed in this exploratory investigation were tryptamine and benzylamine, whereas in this initial study, *tert*-butylisocyanide, was chosen because it is highly reactive and commercially available. Isobutyraldehyde and 4-methylbenzaldehyde were used as examples of aliphatic and aromatic aldehyde components (Scheme 1).

<Scheme 1 >

Further efforts were directed into the preparation of organoselenium cyclic imides which recently received much attention in drug discovery and development.[31] Organoselenium cyclic imides have been used for the synthesis of different prodrugs and drug delivery systems, to estimate the characteristics of dual-acting drugs and to evaluate the receptor-binding with respect to their relative spatial arrangements.[32][31,33-35]

Cyclic imides form an integral part of different naturally occurring compounds (*e.g.*, alkaloids like rebeccamycin) and therapeutically or otherwise biologically interesting compounds (*e.g*., thalidomide, chlorophthalim, isogranulatimide) (Figure 1).[36] Among these are herbicidal, antimicrobial, anti-inflammatory, anticancer and antidepressant compounds.[37] The observed biological properties usually refer to their ability to inhibit protoporphyrinogen IX oxidase which

in turn is essential for chlorophyll and heme biosynthesis.[38,39] Furthermore, these compounds are easily absorbed by cells (*in vivo*) due to their hydrophobic and neutral nature. Moreover, the electrophilicity and size of the imide ring substituent(s) are pivotal in determining the bioactivity *via* modulation of their steric properties.[40,41]

Regardless of the vast application of *N*-substituted cyclic imides, only few synthetic procedures are available to date. Condensation of an amine with anhydrides usually is the typical method of choice. Herein we report the synthesis of selenium containing cyclic imides by dehydrative condensation of an anhydride (maleic, succinic or glutaric anhydride) and selenium-containing amines namely: 4-aminophenylselenocyanate **3** and 4-(2-(4-aminophenyl)diselanyl)benzenamine **4** (Schemes 2 & 3). The later amines **3** and **4** were selected as selenium-based key synthons that enable access to selenocyanate- and symmetrical diselenide-containing cyclic imides.[11]

<Scheme 2 >

The synthesis of selenium-containing maleimides, succinimides and glutarimides was carried out in two steps. Reactions of maleic, succinic and glutaric anhydrides with **3** and **4** in toluene and at room temperature afforded the corresponding *N*-substituted maleanilic (**10** and **16**), succinanilic (**12** and **18**) and glutaranilic acids (**14** and **20**) in approximately quantitative yields. Cyclic imides (**11**, **13**, **15**, **17**, **19** and **21**) were obtained from their corresponding monoamidic acids (**10**, **12**, **14**, **16**, **18** and **20**) *via* dehydration and subsequent ring-closure up on gentle heating with acetic anhydride and sodium acetate (Schemes 2 & 3). The reaction was accomplished in 30 minutes and the product was easily isolated by ice-water precipitation. Purification was achieved by simple washing with water. If necessary, further purification can be accomplished by column chromatography.

<Scheme 3 >

2.2. Pharmacology, toxicity and antioxidant profiles

2.2.1. Cytotoxic activity of redox active compounds in Hep G2 cancer cells

The cytotoxic activity of the synthesized compounds was assayed using Hep G2 cells in order to check if any of them would be useful for in-depth biochemical investigations. It's worth noting that Hep G2 cells are a suitable model for the studying of intracellular trafficking, hepatocarcinogenesis and drug targeting in vitro.[42] Recently, these cells have been employed

extensively as they exhibit large part of cellular functions similar to those of normal hepatocytes, such as expression of hepatocyte-specific cell surface receptors and synthesis and secretion of plasma proteins.[43,44] The cytotoxicity of the compounds was determined using an MTT assay using 5-fluorouracil (5-Fu) and ebselen as positive control. The IC_{50} values were estimated from the respective dose response curves and are summarized in Table 1.

<Table 1 >

Interestingly, the substitution pattern plays a distinct role in the activity of the diselenides, showing that it is not general selenium cytotoxicity. The compounds under investigation could be divided into two classes: 1) cytotoxic compounds, able to reduce the viability of Hep G2 tumor cells (with higher IC_{50} than ebselen) and 2) non-cytotoxic compounds.[*] Class 1 compounds were further divided into two subclasses based on their extent of cytotoxicity *i.e.* compounds with good cytotoxicity (IC_{50} < 8 µM)[†] (**10**, **11**, **16** and **14**) and compounds with modest to very low cytotoxicity (**17**, **19** and **21**).

In culture, cells became rounded, cell layer partially condensed forming cell-free areas, and cells were detached from the culture plate. On the other hand, compounds falling under the second class i.e. non-cytotoxic ones (**5**, **6**, **7**, **8**, **9**, **12**, **13**, **15**, **18** and **20**) were unable to reduce the survival of Hep G2 cells at all.

A basic SAR shows a correlation between chemical structures and the cytotoxic activities. Maleimides (**11** and **17**) and their corresponding *N*-substituted maleanilic acids (**10** and **16**) were among the most potent cytotoxic compounds compared to their succinimides (**13** and **19**), glutarimides (**15** and **21**), *N*-substituted succinanilic (**12** and **18**) and glutaranilic acids (**14** and **20**) analogues.

Contrary to our expectations, ebselen like compounds **5** and **6** showed moderate to low cytotoxicity. This may be attributed to their poor solubility and lipophilicity. The same holds true in case of peptidomimetic compounds **7**, **8** and **9**. The degree of bulkiness and low solubility together with their high molecular weight and large molecular volumes may affect their action on various levels, cell penetration and localization or at the receptors/enzymes binding sites.

2.2.2. Assessment of antioxidant activity

[*] The cytotoxicity threshold is at IC_{50} < 14 µM.
[†] Having lower IC_{50} than 5-Fu *i.e.* IC_{50} < 8 µM.

The last decade has witnessed a growing interest in the development of selenium and selenium- based quinones redox modulators because of their role in the etiology of tumorigenesis *via* modulation of oxidative stress (OS). Interestingly, the mechanism of action of organoselenium compounds depends on their environment, as they have the possibility to function as antioxidant catalysts in normal cells and pro-oxidants in cells exposed to oxidative stress. These agents do not themselves change the redox balance, but their activities are depending upon the cellular redox state in which they are placed.

Mount evidence suggested that regular uptake of antioxidants is required to scavenge ROS and RNS to reduce the risk of cancer, diabetes as well as aging and neurodegeneration related diseases. This ultimately accentuates the role of antioxidants in disease prevention.[45,46] The same holds true for electrophiles (diselenides and selenocyanates are electrophiles, the latter not unlike isothiocyanates responsible for the broccoli anticancer effect).

As part of our research, we herein aim to evaluate the potency of the newly synthesized organoselenium compounds as reliable antioxidants. DPPH, GPx-like activity and bleomycin dependent DNA damage assays were used for the assessment of the compounds antioxidant activities.

2.2.2.1. DPPH free radical scavenging assay

The DPPH method is a standard to estimate the (chemical) antioxidant property of nutritional products and chemicals like novel organoselenium compounds. It is one of the most efficient methods for evaluating the radical-scavenging action by a chain-breaking mechanism.[47,48] This assay is based on the ability of $DPPH^{\cdot}$ to be decolorized in the presence of antioxidants. The corresponding DPPH radical-scavenging activity was determined by the decrease in absorbance at 517 nm due to reduction by the antioxidant (AH) or reaction with a radical species:$DPPH^{\cdot} + R^{\cdot} \rightarrow$ DPPH-R.

The method is fast, efficient and as being standard, comparative data are abundant. However, one must be aware that chemical antioxidant activity does not necessarily translate into *in vivo* action, and that ADME factors are not covered either. The results of the DPPH free radical scavenging activity compiled in Figure 2.

Compound **16** was the most active compound in this assay with free radical scavenging activity higher than ebselen and similar activity to ascorbic acid. Furthermore, compounds **10**, **18**, **20**, **5**, **6**, **12** and **17** showed a moderate radical scavenging activity. The rest of the compounds showed low radical scavenger activity *i.e*. **8**, **7**, **9**, **11**, **13**, **14**, **15**, **19** and **21**.

<Figure 2>

In general, it was found that the amide acids (**10, 12, 14**, **16**, **18** and **20**) show considerable better free radical-scavenging activity more than their corresponding cyclic imides (**11**, **13**, **15**, **17**, **19** and **21**). Surprisingly, the corresponding ebselen selenocyanate **5** and ebselen diselenide **6** show only moderate antioxidant activity in this assay.

2.2.2.2. Glutathione peroxidase-like activity assay

GPx probably is the best studied selenoenzyme. In conjunction with its thiol cofactor glutathione (GSH), it catalyzes the reduction of peroxides and protects cellular components and lipid membranes against OS. The enzyme catalytic site includes selenium which undergoes a redox cycle affording the selenol (RSeH) active form that is able to reduce peroxides (hydrogen peroxides and organic peroxides). It's also worth mentioning that all the GPx family [classical cytosolic GPx (cGPx), phospholipid hydroperoxide GPx (PHGPx), plasma GPx (pGPx) and gastrointestinal GPx (giGPx)] all require selenium at their active sites to exercise their catalytic activity.[49]

Ever since the initial observations by Wendel *et al.*[50,51] and Sies *et al*. that ebselen mimics the activity of GPx and many organoselenium compounds have been devised as synthetic GPx mimics.[52][16,53][54,55] This includes benzoselenazolinones, selenenamides, selenosubtilisin and diaryl diselenides. The latter have recently attracted considerable attention as it behaved as good mimics of GPx in biological systems *in vitro*.[56-60][61,62] Therefore we examined novel symmetrical diselenides and their selenocyanate analogues for their GPx mimicking activity.

Different methods are reported in the literature to estimate catalytic activities of organoselenium compounds as the GPx mimics, these include high-performance liquid chromatography (HPLC) and NMR based dithiol-disulfide conversion assays.[63,64]

In this study, we have evaluated the GPx activity by the NADPH-reductase coupled assay.[65] The principle of this assay relies on the transformation of the oxidized glutathione

(GSSG), which is produced upon reduction of peroxides by GPx, to its reduced state by glutathione reductase. This results in the oxidation of NADPH to $NADP^+$ which is accompanied by decrease in the cofactors absorbance at 340 nm providing a spectrophotometric means for monitoring GPx like activity of the investigated compounds (Figure 3).[65] The values represented in figure 3 are expressed after background correction for the reaction with H_2O_2 and GSH.

<Figure 3>

An inspection of the data indicates that compound **16** exhibited the most potent GPx-like activity, compared to ebselen, followed by **17**, **7**, **8**, **18**, **19**, **20** and **21**. On the other hand, compounds **9**, **11**, **14** and **15** showed moderate GPx activity.

In contrast to expectation, ebselen selenocyanate **5** and ebselen diselenide **6** were not active in this assay. This unexpected behavior may be due to the electronic nature of the substituents at the phenyl groups in the *para* position that, unlikely as it seems, diminish the GPx-like activity.

2.2.2.3. Bleomycin DNA damage Assay

The pro-oxidant activities of the aforementioned compounds were assessed *via* bleomycin induced DNA damage assay. This assay has been endorsed as a specific, yet sensitive, method to estimate the pro-oxidant activity of drugs and food antioxidants.[66,67] The bleomycins are family of antibiotics that exhibit antitumor activity by Fe^{2+} assisted cleavage of DNA. Briefly, If the compounds are able to reduce the bleomycin- Fe^{+3} to bleomycin-Fe^{+2}, DNA degradation in this system would be stimulated, resulting in a positive test for pro-oxidant activity.

Among the tested compounds (Table 2), compounds **10**, **12** and **17** manifest the lowest DNA degradation potency induced by bleomycin-Fe complex. Furthermore, compounds **6, 13** and **14** show activity similar to ascorbic acid. This ultimately reveals that these manifested poor prooxidant activity.

<Table 2>

2.2.3. Antimicrobial evaluation

To study the cytotoxic activity beyond a human cell line we also studied the effect on lower organisms i.e. fungi and bacteria. Thus the antimicrobial activity of the compounds was evaluated against gram-negative *Escherichia coli* (*E. coli*) and gram-positive *Staphylococcus aureus* (*S. aureus*) as well as against the pathogenic fungus *Candida albicans* (*C. albicans*). A standard agar diffusion assay was used and the diameters [mm] of inhibition zones are summarized in Table 3.

<Table 3>

In general, most compounds exhibited toxicity against gram-positive (*S. aureus*) bacteria, more than the gram-negative (*E. coli*) bacteria. In this context, compounds **13** and **16** were the most active ones against *S. aureus* with up to 95 % relative activity (compared to the known drug, ampicillin). Compounds **14**, **18**, **20** and **21** showed good-moderate activity (90 % activity index). Against gram-negative *E. coli* only compound **17** showed a moderate toxicity.

In the case of *C. albicans*, compounds **10** and **13** exhibited activity similar to the antifungal drug colitrimazole with activity indices of 96 and 100%, respectively.

2.2.4. Physicochemical parameters and drug-likeness assessment

Whilst the spectrum of activities associated with some of the compounds against Hep G2 cell, bacteria but especially against *C. albicans* are promising, it should be further checked whether these compounds might have the required bioavailability. One such test is compliance with the traditional Lipinski Rule of Five which indicates if a chemical substance can be orally active in humans by estimating molecular properties which are important for drug's pharmacokinetics.[68] The overall physicochemical properties of the test compounds and the drug-likeness model score (collective property of physicochemical properties, pharmacokinetics and pharmacodynamics of a compound is represented by a numerical value) were computed employing the Molinspiration online property calculation toolkit and MolSoft software (MolSoft, 2007).[69,70]

Lipinski's Rule states that most molecules with good membrane permeability should have partition **c**oefficient values (logP) of the compounds in the octanol-water system ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10 and number of hydrogen bond donors ≤ 5.[71]

In a physicochemical perspective (Table 4), all of the studied compounds have hydrogen bond acceptors (HBA) far fewer than 8 and hydrogen bond donors (HBD) far fewer than 4. This in turn fits with the Lipinski's rule of five indicating that these compounds may have good absorption or permeability properties through the biological membranes. Furthermore, most of the compounds' lipophilicity, expressed as logP, was found to be below 5 confirming their drug-relevant properties.

<Table 4>

Only compounds **6**, **7**, **8** and **9** violate the rule as they were found to be too lipophilic (logP >5) and their molecular masses were beyond the limit i.e. > 700 Daltons.

3. Conclusion

The facile synthesis of novel organoselenocyanates and symmetrical diselenides was described. Most compounds were readily synthesized in two steps in good to moderate yields and were evaluated for their *in vitro* cytotoxic activity against Hep G2 cell line, gram-negative, gram-positive bacteria and a pathogenic yeast. In general, compound **16** was considered to be the most interesting with a free radical-scavenging activity comparable to ascorbic acid and a GPx-like activity similar to ebselen. On the other hand, the simple amides and cyclic imides proved to be better toxins than the ebselen and Ugi-derivatives. Compounds **10**, **11** and **14** were able to reduce the viability of Hep G2 tumor cells already after 48 h incubation at concentrations below 10 µM, especially the activity of some cyclic imide selenocyanates (**10**, **14**) against *Candida* fungus is of relevance, as the activity values are similar to those of reference antimycotics. These initial results suggest further investigations using a wider arsenal of cancer and primary cells and against different organisms, especially humanopathogenic fungi.

Also the free radical scavenging ability, as measured by DPPH assay, was more enhanced in the simple amides and imides (schemes 2 & 3). Compounds **10**, **18** and **20** were the strongest radical scavengers among the tested compounds, followed by compounds **5**, **6**, **12** and **17**. Furthermore, the pro-oxidant activity was estimated using bleomycin dependent DNA damage. Compared to the standard antioxidant ascorbic acid, compounds **10**, **12** and **17** showed the highest protection. Compounds **13**, **14** and **6** showed activity similar to ascorbic acid. The GPx-like activity was evaluated in a NADPH-reductase coupled assay. Compound **16** was the most

potent one, followed by **7**, **8**, **17**, **18**, **19**, **20** and **21**. The larger and more lipophilic molecules synthesized initially (Scheme 1) are mostly outside the realm guarded by Lipinski's Rule of Five, whereas the simpler amides and imides (schemes 2 & 3) comply with Lipinski's Rule of Five. However, this cannot explain the equally lower activity in the non cell-based assays.

While it is premature to estimate why the diseleno-*N*-substituted maleanilic acid **16** was particularly the most active organoselenium compound in the most of performed assays, one may speculate that this compound may hit specific cellular target(s) and cause a widespread modification of proteins and enzymes for the benefit of activation. Furthermore, it's likely that that such compound might also be taken up by cells and modified *in vivo* into active metabolic intermediates. As this is an immensely unknown class of compounds and hence it is too early to speculate over details on their exact metabolism, pharmacokinetics in animals and enrichment of such compounds in specific tissues or degradation, although these issues are clearly important and will form part of future studies. Ultimately, as the structure of this compound provides considerable scope for modifications, and the synthesis of derivatives is now straightforward, this will become a promising starting point to future modulations of its activity through the design, synthesis and evaluation of structural variants of compound **16.** It will also be necessary to produce a wider range of such compounds, including some tellurium-containing analogues, and to screen for further activities and selectivity, also in order to derive reliable structure–activity relationships.

Our studies clearly demonstrate differential behavior of diselenides, dependent on the substitution pattern. We see evidence that such compounds can not only play a role in anticancer redox processes but also may have even more value in antifungal, but clearly at this point the compound base is still too meager to jump to conclusions. Structurally it may be wise to not stick any longer to the old gold standard of ebselen, which had the advantage of a first player. A clear QSAR will require a larger and diverse set of compounds to obtain a better understanding of the mode(s) of action. Eventually, this justifies the realization of more in-depth studies and additional experiments to investigate the exact mode(s) of action responsible for the pronounced biological activity apparently exhibited by this compound and to identify possible intracellular targets (such as specific organelles, membranes or proteins). Additionally, we are fully aware that these findings raise wealth of more questions. For example, what are the possible

applications and the corresponding pharmacological and pharmacokinetic properties of such compound?

We see plenty of room for further, multi-disciplinary studies involving synthetic, bioorganic and medicinal chemistry, cell biology, and pharmacology in order to develop a strategy to treat cancer or yeast infections by applying organoselenium compounds.

4. Experimental protocols

4.1. Material and methods

All chemical reagents for the synthesis of compounds were purchased from Sigma-Aldrich-Fluka or Merck (AMD) and used without further purification unless stated otherwise. Reactions in inert atmosphere were carried out under argon (4.6) using standard Schlenck techniques. Silica gel 60 (Macherey-Nagel, 50-200 μm) was used for column chromatography. Unless noted otherwise, the dimensions of columns used were 2.5 cm (diameter) and 25-30 cm (height of silica gel). TLC plates (silica gel 60 F_{254}, 0.20 mm) were purchased from Merck. NMR spectroscopy*:* ^{1}H NMR spectra were recorded at 400 MHz, ^{13}C NMR spectra at 100 MHz on a Bruker DRX 500 or Avance 500 spectrometer. Chemical shifts are reported in δ (ppm), expressed relative to the solvent signal at 7.26 ppm ($CDCl_3$, ^{1}H NMR) and at 77.16 ppm ($CDCl_3$, ^{13}C NMR), as well as 3.31 ppm (^{1}H NMR, CD_3OD) and 49.00 ppm (^{13}C NMR, CD_3OD). Coupling constants (*J*) are given in Hz. MS analysis: analyses were performed using a TSQ quantum mass spectrometer equipped with an ESI source and a triple quadrupole mass detector (Thermo Finnigan). HRMS: high-resolution mass spectrometry was performed on an Accela UPLC-system (Thermo-Fisher) coupled to a linear trap-FT-Orbitrap combination (LTQ-Orbitrap), operating in positive ionization mode. These spectra indicated ≥ 99% MS-purity of the prepared compounds.

DNA (Calf Thymus type1), bleomycin sulfate, thiobarbituric acid (TBA), 1,1-diphenyl-1-picrylhydrazyl (DPPH), ethylenediaminetetraacetic acid (EDTA), and ascorbic acid were obtained from Sigma. All other chemicals were of analytical grade. Compound 1, 3 and 4 were synthesized according to the literature reported method.[72-74]

4.2.Synthesis and characterization

Preparation of sodium diselenide

A mixture of sodium hydroxide (4.4 g, 110 mmol), selenium powder (8.8 g, 111.40 mmol) and rongalite (sodium formaldehyde sulfoxylate) (8.8 g, 74.50 mmol) in 60 cm^3 of water was stirred under nitrogen for 30 min. After the selenium had completely dissolved a brownish red solution was generated.

Synthesis of 2,2'-diselenobis[benzoic acid] (**1**)

A stirred solution containing *o*-aminobenzoic acid (14.0 g, 34.91 mmol), 20 cm^3 of conc. hydrochloric acid and 20 cm^3 of water in an ice bath (under 5 ^oC), was added dropwise to sodium nitrite (9.0 g, 134.31 mmol) in 20 cm^3 of water. A solution of 2- carboxybenzodiazonium chloride resulted; this was added dropwise to a stirred solution containing sodium diselenide which was prepared in advance by the reaction of selenium powder (8.8 g, 111.40 mmol), rongalite (8.8 g, 74.50 mmol) and sodium hydroxide (4.4 g, 110 mmol) in 60 cm^3 of water. The mixture was stirred for an additional 2 h after an initial evolution of nitrogen gas stopped. It was confirmed that the solution was basic using litmus paper. The reaction mixture was acidified with hydrochloric acid and the precipitated solid was collected by filtration, washed with water, and dried in a desiccators to give 2,2'-diselenobis[benzoic acid]. Yield: 20.0 g, 90%. M.p. 293 ^oC.

General procedure for the preparation of ebselen derivatives **5** and **6**

Thionyl chloride (2.5 mL, 34.0 mmol) was added to diselenide **1** (1.0 g, 2.5 mmol) in a three necked round bottomed flask. The reaction mixture was heated to reflux for 6 h. Excess thionyl chloride was removed under vacuum. The obtained residue was dissolved in dichloromethane (10 mL). A solution of amine (5.0 mmol) and triethylamine (2.1 mL, 15 mmol) in dichloromethane (15 mL) was added to the reaction mixture at 0 ^oC. The ice bath was removed and the reaction mixture was stirred for additional 3 h. The reaction mixture was poured into water and extracted with dichloromethane (2x20 mL). The organic layer was washed with HCl (1 N), an aqueous solution of $NaHCO_3$ (5 %w/v), and water. The organic layer was dried over sodium sulfate and evaporated to give a dark brown solid, which was purified by column chromatography on silica gel.

General procedure for the preparation of peptide derivatives **7**, **8** and **9**

As a general procedure, a mixture of **1** (1 mmol), aldehyde (2 mmol), amine (2 mmol) and isonitrile (2.2 mmol) in 2 mL methanol was stirred at room temperature overnight. Upon completion (monitored by TLC), 10 mL water were added to dissolve the sticky product. The aqueous layer was extracted three times with CH_2Cl_2, the organic layers were combined, dried over Na_2SO_4 and concentrated to yield a sticky product which was purified by chromatography on silica gel.

General procedure for the preparation of amide-acids **10**, **12**, **14**, **16**, **18** and **20**

To a stirring solution of anhydride (1 mmol) in dry toluene (5 mL) was added dropwise a solution of amine (1 mmol) at room temperature. The mixture was vigorously stirred for 3 h, and the formed precipitate was separated by s filtration. The cake was washed with toluene and dried under reduced pressure.

General procedure for the preparation of cyclic imides **11**, **13**, **15**, **16**, **19** and **21**.

A reaction mixture containing the appropriate acid (cf. schemes 2, 3) in 5 mL of acetic anhydride and 100 mg of sodium acetate was heated for 2 h under reflux. The reaction was cooled and quenched with water. The aqueous solution was extracted with CH_2Cl_2, dried with Na_2SO_4, filtered, and the solvent was evaporated.

4.2.1. 2-(4-selenocyanatophenyl)benzo[d][1,2]selenazol-3(2H)-one (5)

Yield: 0.266 g (71%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 7.46-7.53 (m, 1H, phenyl SeCN), 7.68-7.73 (d, 1H, J= 8.06, PhSeCN), 7.73-7.82 (d, 4H, J= 7.6), 7.89- 7.94 (m, 1H, PhSeCN), 8.06-8.12 (m, 1H, phenyl SeCN); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 165.23, 140.97, 138.70, 134.58, 132.56, 128.37, 128.04, 126.39, 125.87, 125.57, 119.93, 105.31; MS (ESI): *m/z* = found 381.2 [M^++1], 403 [M^++Na]; calcd. 379.9; HRMS calcd. for $C_{14}H_8N_2OSe_2$ [M^++Na]: 402.8898, found 402.88586 [M^++Na].

4.2.2. 2, 2`(4-(diseleno)phenyl)benzo[d][1,2]selenazol-3(2H)-one (6)

Yield: 0.481 g (68%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 7.44-7.50 (m, 2H, PhSe), 7.53-7.64 (m, 2H, PhSe), 7.66-7.75 (m, 8H), 7.88-7.93 (d, 2H, J= 7.80), 8.08-8.14 (d, 2H, J= 8.00); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 165.12, 139.88, 138.80, 133.60, 132.35, 132.32, 128.51, 127.95, 126.58, 126.28, 125.93, 125.40, 125.18; MS (ESI): *m/z* = found 709.0 [M^++1], 731.8 [M^++Na]; calcd. 707.79; HRMS calcd. for $C_{26}H_{16}N_2O_2Se_4$ [M^++Na]: 730.7798, found 730.77648 [M^++Na].

4.2.3. N,N`-(1-(tert-butylcarbamoyl)-2-methylpropyl)-N-(2-(1H-indol-3-yl)ethyl)-2-(diseleno)benzamide (7)

Yield: 0.697 g (70%). ^{1}HMNR (400 MHz, $CDCl_3$) δ ppm: 0.89-0.95 (d, 6H, J= 5.04, *iso*-propyl), 1.03- 1.07 (d, 6H, J= 6.05, *iso*-propyl), 1.28-1.31 (s, 9H, *t*-butyl), 1.32-1.34 (s, 9H, *t*-butyl), 2.49-2.78 (m, 6H, CH_2), 2.98-3.19- (m, 4H, CH_2), 3.39-3.47- (t, 2H, J= 10.70), 3.57-3.65- (t, 2H, J= 11.60, CH), 4.27-4.40 (s, 2H), 6.50-6.62 (s, 1H), 6.79-6.86 (m, 3H), 7.02-7.11 (m, 5H), 7.13-7.22 (m, 3H), 7.48-7.55 (s, 1H), 7.84-7.92 (t, 2H, J= 7.96), 8.11-8.19 (d, 2H, 5.49), 8.23-8.32 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 172.22, 169.85, 136.04, 131.71, 131.65, 130.68, 130.23, 127.00, 126.83, 126.74, 122.12, 121.87, 119.32, 118.36, 118.31, 111.01, 60.37, 51.20, 28.61, 28.56, 28.52, 26.16, 25.55, 21.03, 20.07, 18.89; MS (ESI): *m/z* = found 997.5 [M^++1], 1035.4 [M^++K]; calcd. 996.33; HRMS calcd. for $C_{52}H_{64}N_6O_4Se_2$ [M^++Na]: 1019.3198, found 1019.3112 [M^++Na].

4.2.4. N,N-((tert-butylcarbamoyl)(p-tolyl)methyl)-N-(2-(1H-indol-3-yl)ethyl)-2-(diseleno)benzamide (8)

Yield: 0.753 g (69%). ^{1}HMNR (400 MHz, $CDCl_3$) δ ppm: 1.27-1.35 (s, 18H, *t*-butyl), 2.29-2.36 (s, 6H, *P*-CH_3), 4.40 -4.71 (m, 4H, CH_2), 5.24-5.49 (m, 4H, CH_2), 5.71-5.91 (s, 2H, CH), 6.94-7.18 (m, 22H, Ph), 7.35-7.41 (s, 2H), 7.62-7.77 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 171.61, 171.56, 168.36, 138.41, 138.38, 136.52, 136.46, 132.20, 132.16, 132.10, 132.07, 130.47, 129.65, 129.61, 129.40, 128.24, 128.21, 127.42, 126.72, 64.82, 64.77, 60.36, 52.84, 52.78, 51.59, 28.55, 28.34, 21.11, 21.02, 14.17; MS (ESI): *m/z* = found 1114.7 [M^++Na]; calcd. 1092.33; HRMS calcd. for $C_{60}H_{64}N_6O_4Se_2$ [M^++Na]: 1115.3198, found 1013.7488.

4.2.5. N,N-(1-(tert-butylcarbamoyl)-2-methylpropyl)-N-benzyl-2-(diseleno)benzamide (9)

Yield: 0.685 g (77%). ^{1}HMNR (400 MHz, $CDCl_3$) δ ppm: 0.96-1.04 (d, 12H, J= 6.3, *iso*-propyl), 1.31- 1.38 (s, 18H, *t*-butyl), 2.69-2.77 (s, 2H), 3.87-4.02 (m, 2H), 4.44-4.65 (m, 4H, phenyl), 6.95- 7.24 (m, 16H), 7.59-7.72 (s, 2H, phenyl); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 172.32, 169.22, 136.23, 136.17, 135.83, 131.81, 130.94, 130.67, 130.02, 128.35, 128.29, 128.18, 128.14, 127.51, 126.71, 126.67, 126.47, 51.08, 28.71, 28.63, 27.14, 19.91, 19.63; MS (ESI): *m/z* = found 891.4 [M^++1], 913.5 [M^++Na]; calcd. 890.28; HRMS calcd. for $C_{46}H_{58}N_4O_4Se_2$ [M^++Na]: 913.2698, found 913.2681 [M^++Na].

4.2.6. (Z)-3-(4-selenocyanatophenylcarbamoyl)acrylic acid (10)

Yield: 0.269 g (91%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 6.30-6.35 (dd, 1H, J= 11.95, CH=), 6.46-6.50 (dd, 1H, J= 12.01, =CH), 7.68-7.70 (s, 4H, Ph), 10.32-10.72 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 166.81, 163.41, 139.82, 134.78, 131.53, 130.18, 120.67,

120.58, 117.11, 114.99, 105.34; MS (ESI): *m/z* = found 297.1 [M^++1]; calcd. 295.97; HRMS calcd. for $C_{11}H_8N_2O_3Se$ [M^++1]: 296.97794, found 296.97709 [M^++1].

4.2.7. 1-(4-selenocyanatophenyl)-1H-pyrrole-2,5-dione (11)

Yield: 0.144 g (52%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 7.23-7.31- (s, 2H, CH=CH), 7.43-7.55 (d, 2H, J=8.43, Ph), 7.84-7.96 (d, 2H, J= 8.35, Ph); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 169.59, 143.05, 134.81, 134.22, 134.01, 132.52, 129.88, 127.97, 124.76, 123.10, 105.28; MS (ESI): *m/z* = found 279.2 [M^++1]; calcd. 277.96; HRMS calcd. for $C_{11}H_6N_2O_2Se$ [M^++NH_4]: 295.99894, found 296.96283 [M^++1+ NH_4].

4.2.8. 3-(4-selenocyanatophenylcarbamoyl)propanoic acid (12)

Yield: 0.265 g (89%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 2.51-2.65 (m, 4H, CH_2), 7.53-7.76 (s, 4H, Ph), 9.94-10.42 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 173.66,170.54, 170.45, 140.45, 134.85, 120.15, 120.06, 115.99, 105.35, 31.00, 28.58; MS (ESI): *m/z* = found 299.3 [M^++1]; calcd. 297.99; HRMS calcd. for $C_{11}H_{10}N_2O_3Se$ [M^++1]: 298.99794, [M^++Na]: 320.9798, found 298.99273 [M^++1], 320.97473 [M^++Na].

4.2.9. 1-(4-selenocyanatophenyl)pyrrolidine-2,5-dione (13)

Yield: 0.134 g (48%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 2.77-2.80 (s, 4H, CH_2), 7.25-7.42 (d, 2H, J= 8.49, Ph), 7.74-7.88 (d, 2H, J= 8.56, Ph); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 176.69, 133.89, 133.57, 128.52, 123.75, 105.28, 28.52; MS (ESI): *m/z* = found 280.30 [M^++1]; calcd. 279.98; HRMS calcd. for $C_{11}H_8N_2O_2Se$ [M^+]: 279.98, found 278.96779 [M^+].

4.2.10. 4-(4-selenocyanatophenylcarbamoyl)butanoic acid (14)

Yield: 0.290 g (93%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 1.75-1.86 (p, 2H, J_1=7.38, J_2= 14.70, CH_2), 2.20-2.32 (t, 2H, J= 7.28, CH_2), 2.33-2.42 (t, 2H, J=7.41, CH_2), 7.51-7.79 (m, 4H, Ph), 9.66-10.49 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 174.05, 171.08, 140.44, 136.41, 134.83, 120.31, 120.22, 116.05, 105.34, 35.36, 32.87, 20.25; MS (ESI): *m/z* = found 313.00 [M^++1], 335.2 [M^++Na]; calcd. 312.00; HRMS calcd. for $C_{12}H_{12}N_2O_3Se$ [M^++Na]: 334.9898, found 334.99078 [M^++Na].

4.2.11. 1-(4-selenocyanatophenyl)piperidine-2,6-dione (15)

Yield: 0.126 g (43%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 2.08-2.28 (m, 6H, CH_2), 7.72-7.91 (d, 2H, J=8.42, CH_2), 7.24-7.46 (d, 2H, J=8.45, Ph); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 172.26, 172.14, 140.48, 134.07, 131.45, 130.86, 130.76, 130.11, 124.44, 105.21, 26.73, 21.00; MS (ESI): *m/z* = found 311.1 [M^++NH_4], 318.1 [M^++Na]; calcd. 293.99; HRMS calcd. for $C_{12}H_{10}N_2O_2Se$ [M^++1]: 294.99794, [M^++K]: 334.09624, found 294.00063 [M^++1], 334.99084 [M^++K].

4.2.12. (Z, Z)-3, 3`-(4-(diseleno)phenylcarbamoyl)acrylic acid (16)

Yield: 0.489 g (91 %). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 3.32-3.43 (s, 2H, NH), 6.43-6.53 (d, 2H, J=11.95, CH=), 6.19-6.37 (d, 2H, J=12.01, =CH), 7.08-7.32 (m, 2H, Ph), 7.52-7.62 (m, 6H, Ph), 10.02-10.88 (s, 2H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 166.79, 163.23, 136.01, 133.04, 131.56, 130.23, 124.41, 120.14; MS (ESI): *m/z* = found 540.5 [M^++1]; calcd. 539.93; HRMS calcd. for $C_{20}H_{16}N_2O_6Se_2$ [M^++1]: 540.93794, [M^++Na]: 562.9198, found 540.94191 [M^++1], 562.92402 [M^++Na].

4.2.13. 1, 1`-(4-(diseleno)phenyl)-1H-pyrrole-2,5-dione (17)

Yield: 0.227 g (45%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 7.22-7.26 (s, 4H, CH=CH), 7.36-7.41 (d, 4H, J=8.42, Ph), 7.59-7.67 (m, 2H, Ph), 7.79-7.84 (d, 2H, J= 8.40, Ph); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 169.69, 134.74, 131.37, 130.10, 127.53, 119.69; MS (ESI): *m/z* = found 521.2 [M^++NH_4]; calcd. 521.2 [M^++NH_4]; HRMS calcd. for $C_{20}H_{12}N_2O_4Se_2$ [M^++1+NH_4]: 522.95688, found 522.94966 [M^++1+NH_4].

4.2.14. 3, 3`-(4-(diseleno)phenylcarbamoyl)propanoic acid (18)

Yield: 0.511 g (94%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 2.51-2.65 (m, 8H, CH_2), 7.40-7.65 (m, 8H, Ph), 10.03-10.19 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 173.70, 170.31, 170.22, 139.59, 139.48, 133.29, 123.38, 119.65, 119.56, 31.05, 31.00, 28.67; MS (ESI): *m/z* = found 543.2 [M^+]; calcd. 543.97; HRMS calcd. for $C_{20}H_{20}N_2O_6Se_2$ [M^++1]: 544.97794, [M^++Na]: 566.9598, found 544.97367 [M^++1], 566.95533 [M^++Na].

4.2.15. 1, 1`-(4-(diseleno)phenyl)pyrrolidine-2,5-dione (19)

Yield: 0.208 g (41%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 2.70-2.80 (s, 8H, CH_2), 7.20-7.29 (d, 4H, J= 8.48, Ph), 7.69-7.82 (d, 4H, J= 8.46, Ph); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 176.76, 132.31, 131.14, 129.68, 127.98, 28.46; MS (ESI): *m/z* = found 531.1 [M^++Na]; calcd. 507.94; HRMS calcd. for $C_{20}H_{16}N_2O_4Se_2$ [M^++Na]: 530.9298, found 530.93427 [M^++Na].

4.2.16. 4, 4`-(4-(methylselanyl)phenylcarbamoyl)butanoic acid (20)

Yield: 0.526 g (92%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 1.76-1.84 (m, 4H, CH_2), 2.25-2.29 (m, 4H, CH_2), 2.33-2.39 (m, 4H, CH_2), 6.42-6.59 (s, 1H), 7.16-7.29 (s, 1H), 7.32-7.41 (s, 1H), 7.45- 7.64 (m, 8H, Ph), 9.99-10.10 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 174.07, 174.03, 170.95, 170.86, 139.56, 139.45, 136.06, 134.22, 134.06, 133.32, 133.19, 123.45, 119.81, 119.72, 119.63, 114.39, 35.41, 35.36, 32.91, 32.72, 20.33, 19.94; MS (ESI): *m/z* = found 573.4 [M^++1], 594.8 [M^++Na]; calcd. 572.00; HRMS calcd. for $C_{22}H_{24}N_2O_2Se$ [M^++1]: 573.00794, [M^++Na]: 594.9898, found 573.00407 [M^++1], 594.98679 [M^++Na].

4.2.17. 1, 1`-(4-(methylselanyl)phenyl)piperidine-2,6-dione (21)

Yield: 0.493 g (92%). ^{1}HMNR (400 MHz, DMSO-d_6) δ ppm: 2.01-2.25 (m, 12H, CH_2), 7.14-7.33- (d, 4H, J= 8.35, Ph), 7.68-7.86- (d, 4H, J= 8.39, Ph); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 172.24, 139.21, 131.44, 130.10, 119.77, 119.68, 38.86, 26.69; MS (ESI): *m/z* = found 537.8 [M^++1], 558.7 [M^++Na]; calcd. 535.98; HRMS calcd. for $C_{22}H_{20}N_2O_4Se_2$ [M^++K]: 575.0783, found 574.9896 [M^++K].

4.3. Biological assays

4.3.1. Cytotoxicity assay

The Hep G2 human liver carcinoma cell line was purchased from American Type Culture Collection (HTB‑37; Rockville, MD, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) calf serum (Hyclone Laboratories, Ogden, UT), 60mg/mL penicillin G and 100mg/mL streptomycin sulfate maintained at 37 ^{0}C in a humidified atmosphere containing about 15% (v/v) CO_2 in air.

MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide] (Sigma) was used to measure the metabolic activity of cells which are capable of reducing it by dehydrogenases to a violet colored formazan product. Briefly, 120 µL aliquots of a cell suspension (50,000 cells mL^{-1}) in 96-well microplates were incubated at 37 °C and 10% CO_2 and allowed to grow for two days. Then 60 µL of serial dilutions of the test compounds were added. After 48h of incubation at 37 ^{o}C and 10% CO_2, 75 µL MTT in phosphate buffered saline (PBS) were added to a final concentration of 0.5 mg mL^{-1}. After 2 h the precipitate of formazan crystals was centrifuged and the supernatant discarded. The precipitate was washed with 100 µL PBS and dissolved in 100 µL DMSO. The resulting color was measured at 590 nm using an ELISA plate reader. All investigations were carried out in two parallel experiments. The IC_{50} values were determined as the concentrations of tested materials, which showed 50% of the absorbance of untreated control cells as estimated from the dose-response curves. 5-Fluorouracil (5-Fu) and ebselen were used as a positive control.

4.3.2. *In vitro* studies

4.3.2.1. DPPH free radical scavenging activity

The hydrogen atom or electron donation ability of the corresponding compounds was measured by the bleaching of the purple color of a methanolic solution of diphenylpicrylhydrazyl (DPPH). This spectrophotometric assay uses stable radical diphenylpicrylhydrazyl (DPPH) as a reagent.

The test compounds were dissolved in methanol to obtain a final concentration of 1 mg/ml. 200 µL of each sample were added to 0.4 ml of 0.1 mM DPPH in methanol. After 30 min of incubation in the dark, the absorbance was read against a blank at 517 nm. Ascorbic acid (vitamin C) and ebselen were used as standard antioxidant (positive control). Blank sample was run without DPPH and using methanol instead of sample. Negative control sample was run with methanol instead of tested compound. The radical scavenging activity was calculated from the following equation:

$$I\% = (A_{blank} - A_{sample})/(A_{blank}) \times 100$$

4.3.2.2. Glutathione peroxidase like activity

GPx kit (Biodiagnostic, Egypt) was used for the determination of GPx according to Paglia *et al.*[71] The reaction mixture contained 1 ml assay buffer (50 mM phosphate buffer containing 0.1 % triton X-100) and 0.1 ml NADPH reagent (24 µmol Glutathione, 12 unit Glutathione reductase and 4.8 µmol NADPH) and 0.01 ml (41 µM) tested compounds and the reaction was started by the addition of H_2O_2 (0.8 mM). The contents were mixed well and the absorbances were recorded at 340 nm over a period of 3 min against deionized water. The change of absorbance per minute (A_{340nm} / min) was estimated using ebselen (41 µM) as the positive control. The values represented in figure 3 are expressed after background correction for the reaction with H_2O_2 and GSH. In case of colored compounds, their activities were estimated after subtracting their own absorbances at the used wave length.

4.3.2.3. Bleomycin-dependent DNA damage

The reaction mixture contained calf thymus DNA (0.5 mg/ml), bleomycin sulfate (0.05 mg/ml), $MgCl_2$ (5 mM), $FeCl_3$ (50 mM), samples to be tested (2 mM) and L-ascorbic acid was used as positive control. The mixture was incubated at 37 ^oC for 1 hour. The activity of test compounds was evaluated as malondialdehyde (MDA) equivalents. Thiobarbituric reactive substances which arose from deoxyribose degradation of DNA were assessed. The reaction was terminated by addition of 0.05 ml EDTA (0.1 M). The color was developed by adding 0.5 ml thiobarbituric acid (1% w/v) and 0.5 ml HCl (25% v/v). The tube was capped with a screw cap and heated at 80 ^0C for 30 min. After cooling in ice water, the mixture was then shaken and centrifuged and the extent of DNA damage was measured by increase in absorbance at 532 nm.

4.3.2.4. Antimicrobial activity

Chemical compounds were individually tested against gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacterial pathogens as well as *Candida albicans* fungus (yeast) strain. Antimicrobial tests were carried out by the agar well diffusion method using 100 µL of suspension containing $1x10^8$ CFU/mL of pathological tested bacteria and $1x10^4$ spores/mL of fungi spread on nutrient agar (NA), and potato dextrose agar (PDA) medium respectively. After the media had cooled and solidified, paper discs of 6 mm diameter soaked with 20 µl of the test compounds (1mg/ml) were added to the agar plates and incubated at 30°C. After incubation time, antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The antibacterial activity of a common standard antibiotic ampicillin and the antifungal coltrimazole were chosen as positive control using the same procedure as above at the same concentration. The relative (%) activity index was calculated as shown below:

% activity index= (inhibition zone of the test compounds/ inhibition zone of the standard drug) ×100.

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Figures captions

Figure 1. Chemical structures of most important organoselenium and cyclic imides.

Figurer 2. In vitro DPPH free radical scavenging assay.

Figure 3. Glutathione peroxidase-like activity assay.

Tables

Table 1 Influence of the compounds **5** - **21** on the viability of Hep G2 cells.

Compd. No.	IC_{50} (µM)
5-Fu	8
Ebselen	25
5	64
6	40
7	45
8	a
9	a
10	5
11	0.6
12	a
13	a
14	7
15	47
16	3
17	13
18	a
19	14
20	67
21	35

The metabolic activity of the cells was measured after 48h of incubation with different concentrations of the investigated compounds by means of an MTT assay. The IC_{50} was determined from the dose-response curves as the mean of two parallel experiments; 5-fluorouracil (5-Fu) was used as a positive control; [a] no growth inhibition was recorded.

Table 2. Bleomycin-dependent DNA damage assay

Compd. No.	Absorbance of samples[b]
vitamin C[a]	0.062
5	0.090
6	0.060
7	0.070
8	0.073
9	0.073
10	0.051
11	0.082
12	0.053
13	0.065
14	0.068
15	0.078
16	0.089
17	0.055
18	0.088
19	0.074
20	0.083
21	0.090

[a] ascorbic acid is used as standard for antioxidant activity

[b] values are means of 3 replicates.

Table 3. Diameters (in mm) of inhibition zones of agar diffusion assays against a variety of fungi and bacteria (growth was quantified after 1 and 2 days).[a] Please note that compounds not diffusing well in agar may be underestimated in their activity.

Compd. No.	Diameter inhibition zone in mm (% activity index)		
	E. coli	*S. aureus*	*C. albicans*
5	-	-	-
7	-	-	-
8	-	12 (55)	-
9	-	-	-
10	-	16 (73)	27 (96)
11	-	-	-
12	-	15 (68)	-
13	-	21 (95)	28 (100)
14	-	20 (91)	-
15	-	-	-
16	-	21 (95)	-
17	7 (29)	-	-
18	-	20 (91)	19 (68)
19	-	14 (64)	-
20	-	20 (91)	16 (57)
21	-	21 (90)	-
Ampicillin	24 (100)	22 (100)	-
Colitrimazole	-	-	28 (100)

[a] Diameters (mm) of zones of inhibition (agar diffusion assay) are provided. In each case, 6 mm disks with 20 µg of the test compounds were incubated. Ampicillin and colitrimazole were used as the positive control. Values below 6 mm (25 %) are of limited value as they refer either to inactive or non-diffusing compounds.

Table 4. Calculated pharmacokinetic parameters.

Compd. No.	MW	LogP	HBA	HBD	Drug-likeness score	Lipinski violations
5	378.15	3.4	3	0	- 0.52	0
6	704.26	6.9	4	0	- 0.22	2
7	995.04	8.9	10	4	- 0.28	2
8	1091.1	9.4	10	4	0.31	2
9	888.91	8.3	8	2	0.07	2
10	295.15	1.3	5	2	- 0.23	0
11	277.14	1.5	4	0	- 1.72	0
12	297.17	1.3	5	2	- 0.16	0
13	279.15	0.6	4	0	- 1.67	0
14	311.19	1.8	5	2	- 0.24	0
15	293.18	0.7	4	0	- 1.53	0
16	542.30	2.8	8	4	0.08	1
17	502.24	3.2	6	0	- 1.54	1
18	542.30	2.8	8	4	0.08	1
19	506.27	1.4	6	0	- 1.50	1
20	570.36	3.9	8	4	0.00	1
21	534.33	1.5	6	0	- 1.36	1

MW, molecular weight; logP, logarithm of compound partition coefficient between *n*-octanol and water; HBA, number of hydrogen bond donors; HBD, number of hydrogen bond acceptors.

Figures

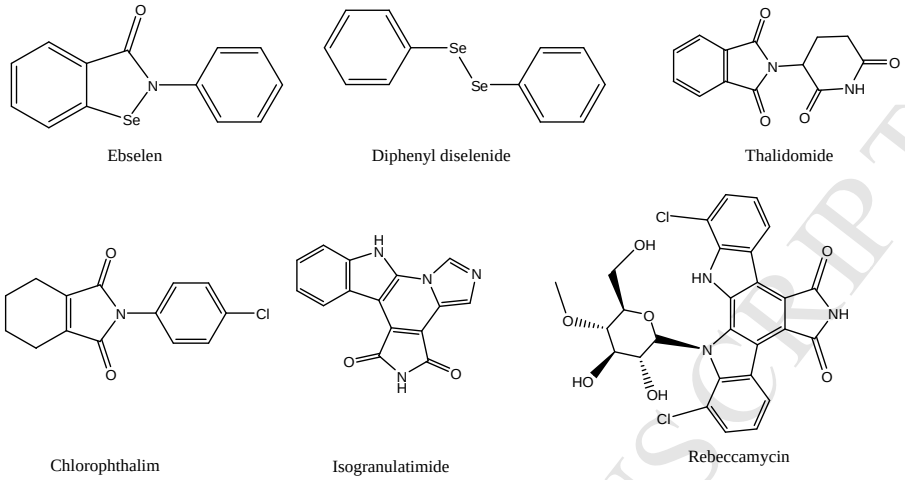
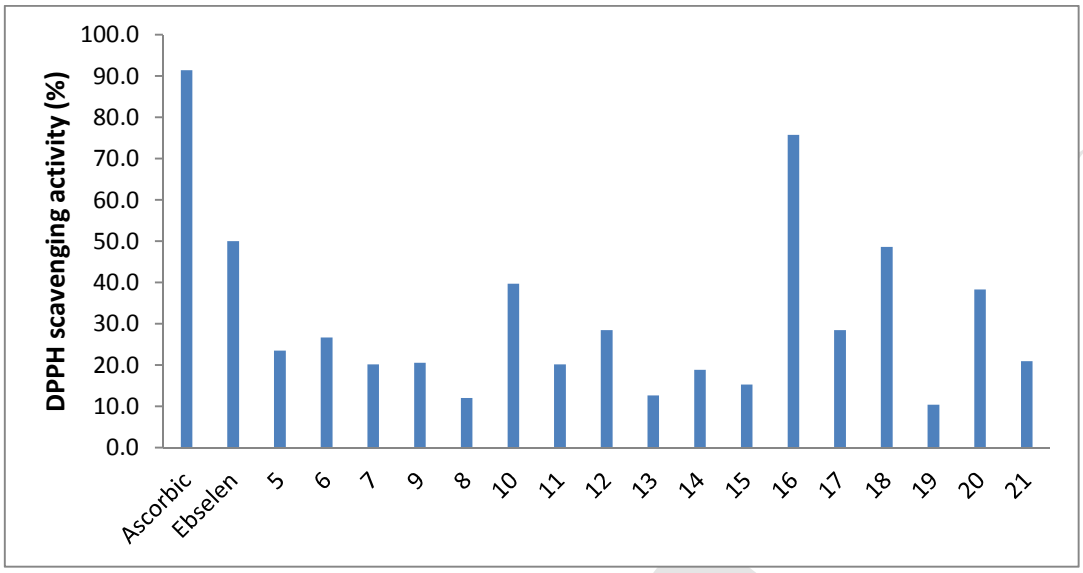


Figure 1. Chemical structures of selected organoselenides and cyclic imides of biological or pharmaceutical relevance



Figurer 2. In vitro DPPH free radical scavenging assay.

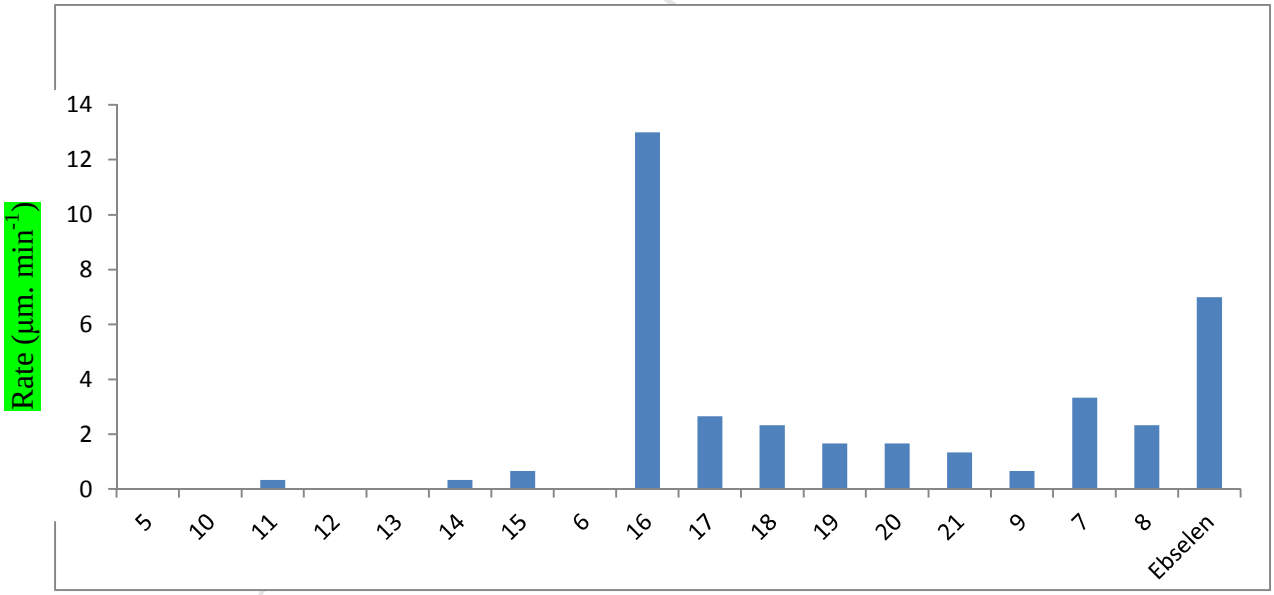
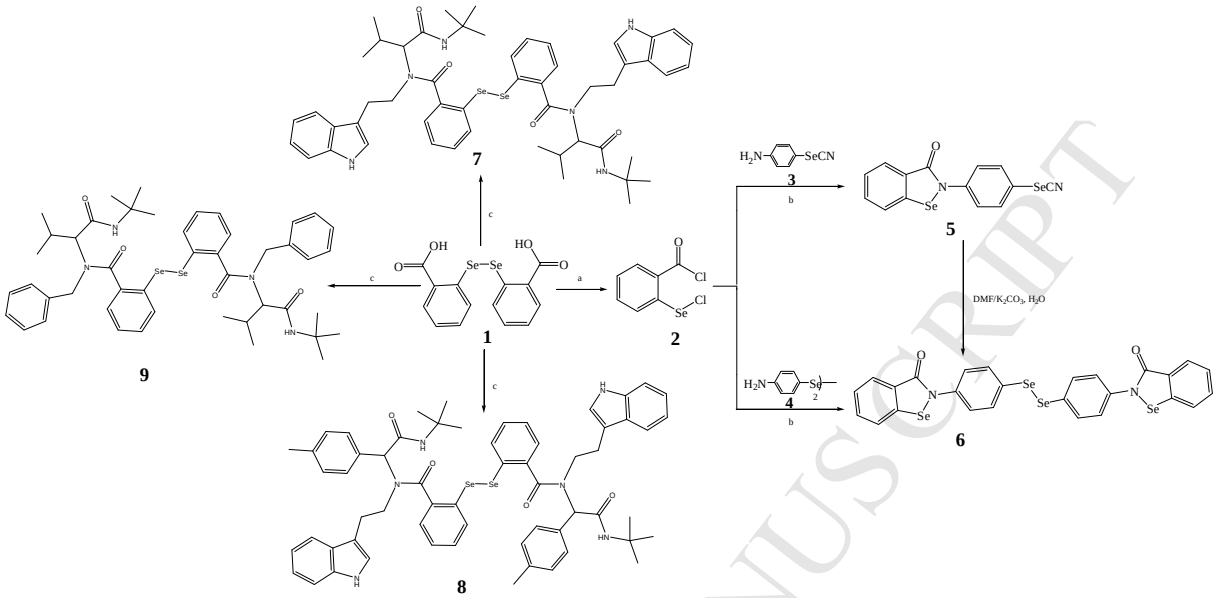
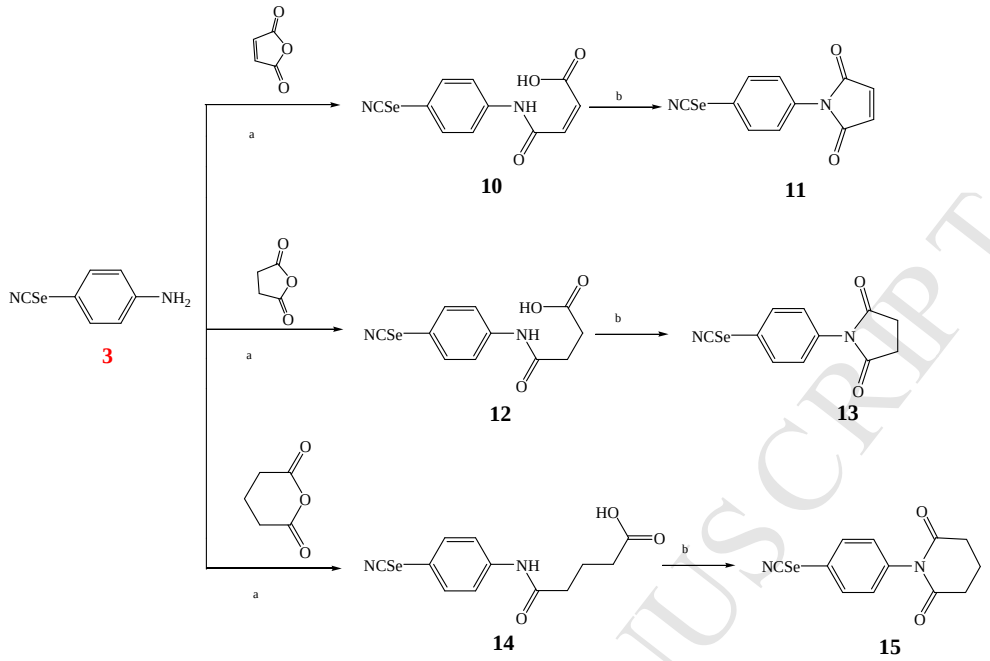


Figure 3. Glutathione peroxidase-like activity assay. The reaction was monitored to completion and the reaction rate was linear throughout the entire time course.

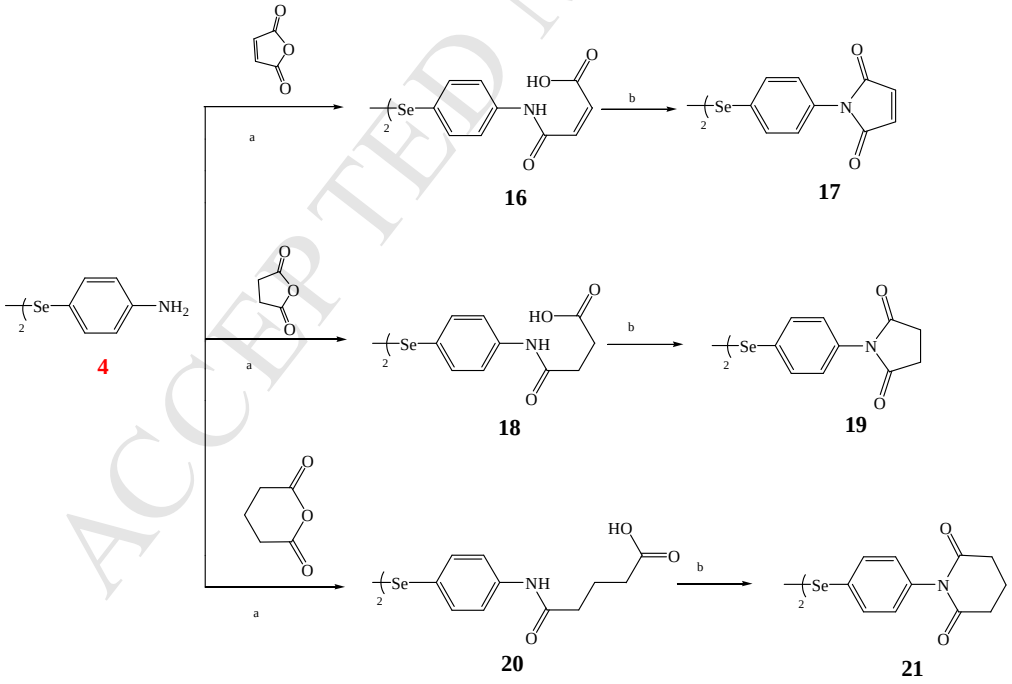
Schemes



Scheme 1. Synthesis of *o*-selenobenzoic acid derivatives (peptoid and ebselen type). Reagent and reaction conditions: (a) $SOCl_2$; (b) dichloromethane, triethylamine, rt, Yields 68-71%; (c) aldehyde (2 mmol), amine (2 mmol), isonitrile (2.2 mmol), methanol, rt, Yields 69-77%.



Scheme 2. Synthesis of *N*-(4-selenoisocyanato-phenyl)-substituted amido-acids and cyclic imides. Reagent and reaction conditions: (a) toluene, rt, Yields 89-93%; (b) acetic anhydride, sodium acetate, reflux, Yields 43-52%.



Scheme 3. Synthesis of *N*-(4-amino-phenyl)-substituted amido-acids and cyclic imides. (a) toluene, rt, Yields 91-94%; (b) acetic anhydride, sodium acetate, reflux, Yields 41-92%.

Organoselenocyanates and Symmetrical Diselenides Redox Modulators: Design, Synthesis and Biological Evaluation

Saad Shaaban,[a,d*] Amr Negm,[b] Mohamed A. Sobh[c] and Ludger A. Wessjohann[d**]

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Highlights

- Novel series of organoselenocyanates and symmetrical diselenides were synthesized.
- Some compounds proved to be more cytotoxic than 5-Fu.
- Their antifungal activity was similar to reference antifungal drugs.
- Some compounds manifested a good free radical-scavenging activity comparable to ascorbic acid.
- They have also shown GPx-like activity similar to ebselen.

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E-mail address: wessjohann@ipb-halle.de

SUPPLEMENTARY MATERIALS

Organoselenocyanates and Symmetrical Diselenides Redox Modulators: Design, Synthesis and Biological Evaluation

Saad Shaaban,[a,d*] Amr Negm,[b] Mohamed A. Sobh[c] and Ludger A. Wessjohann[d**]

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[b]*Biochemistry Division, Department of Chemistry, Faculty of Science, Mansoura University, El-Gomhorya Street, 35516 Mansoura, Egypt.*

[c] *Experimental Biology, Urology & Nephrology Center, Faculty of Medicine, Mansoura University, El-Gomhorya Street, 35516 Mansoura, Egypt.*

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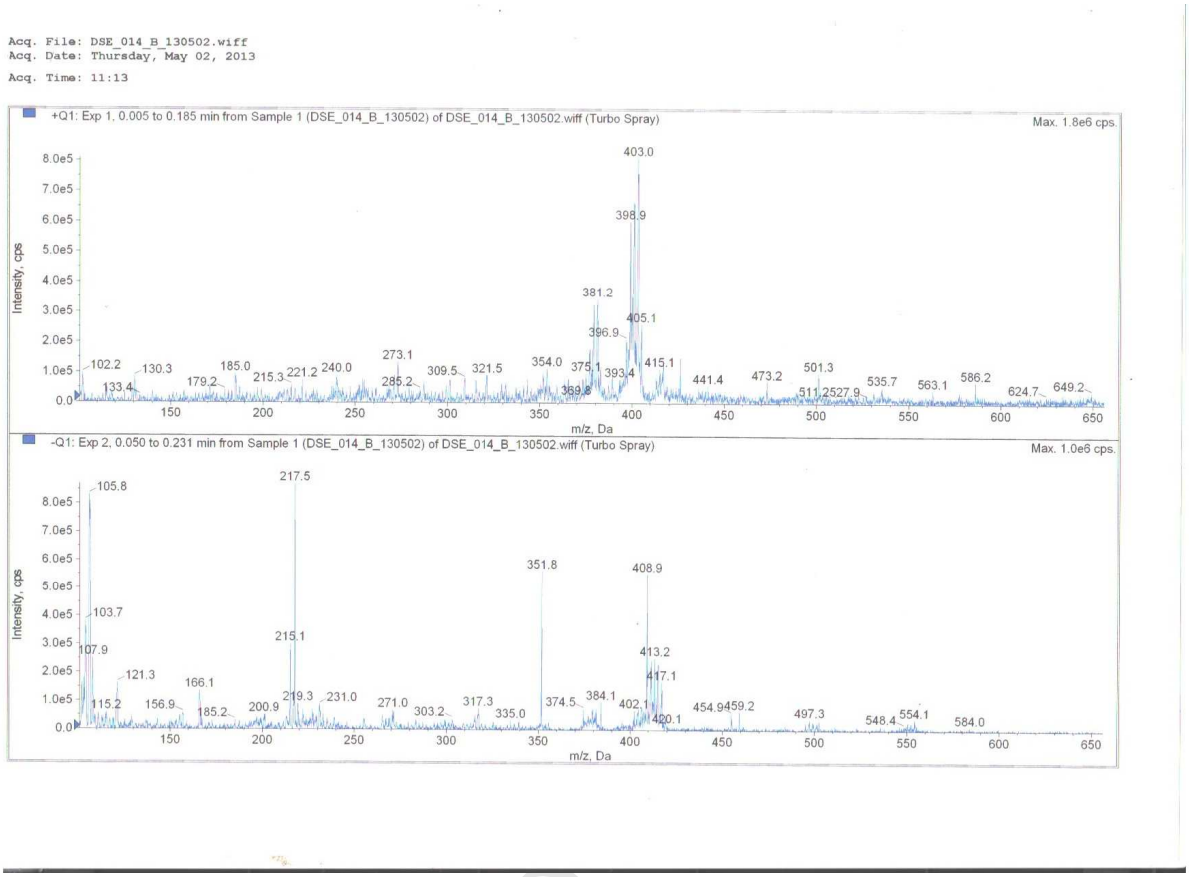
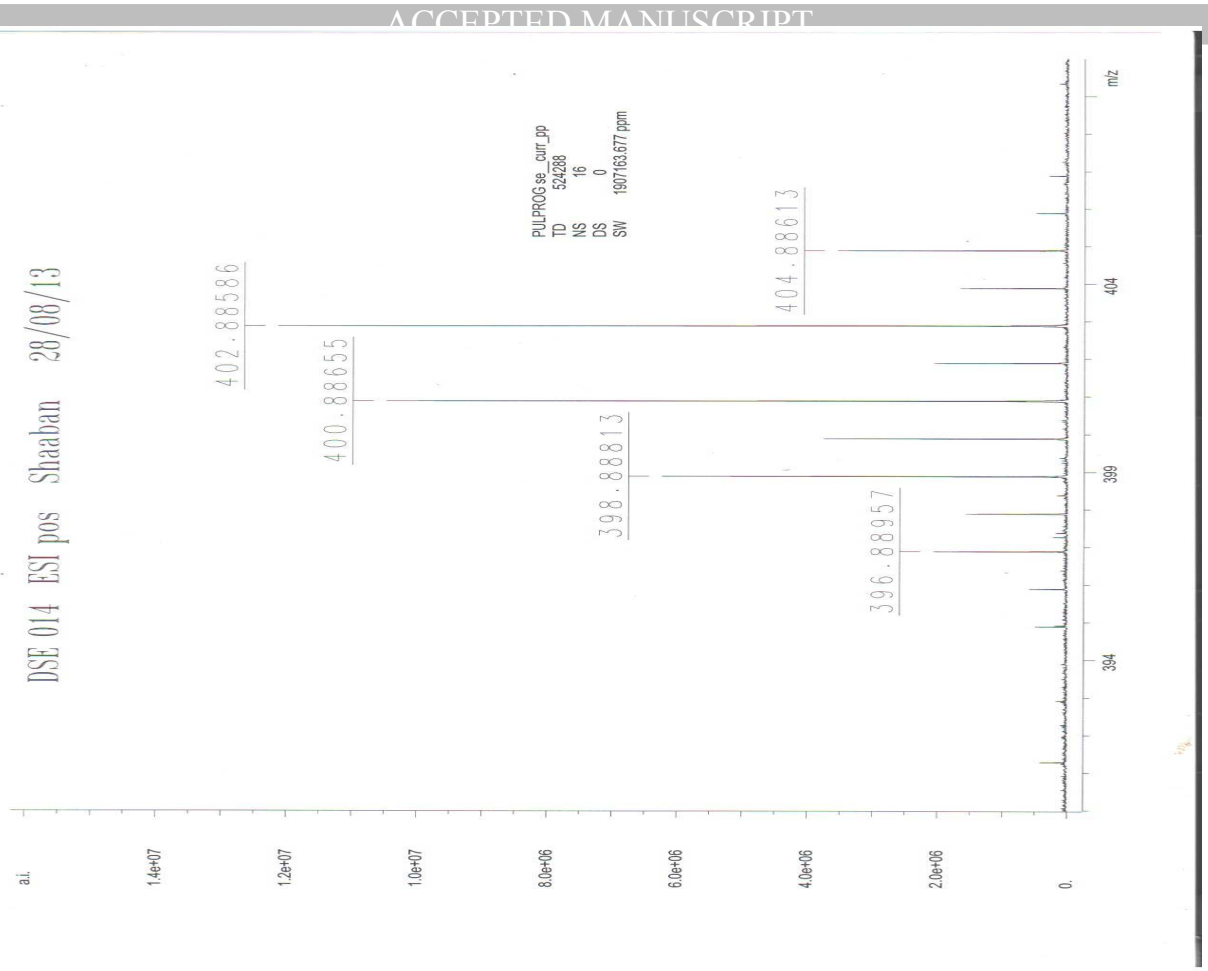
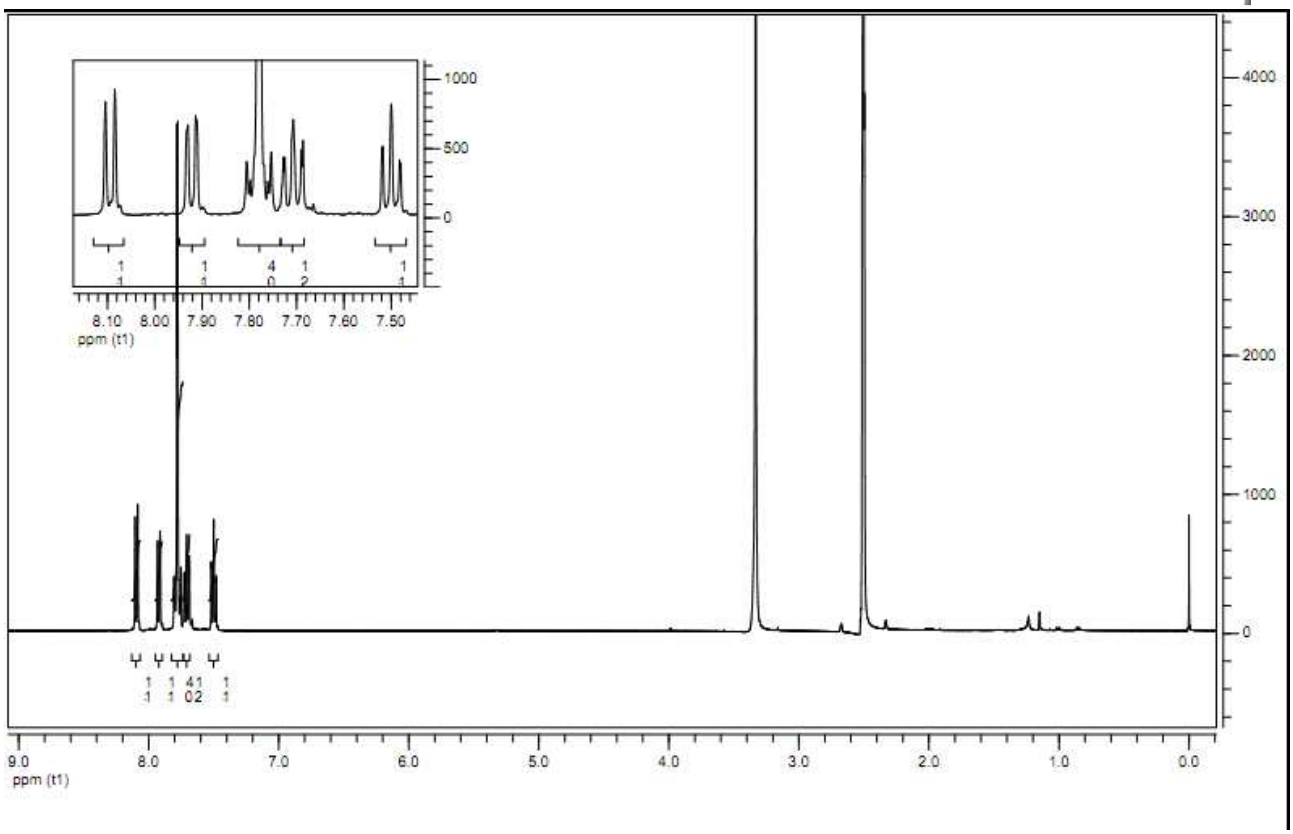
E-mail address: wessjohann@ipb-halle.de

S1. ^{1}H NMR, ^{13}C NMR, ^{13}C DEPT-135 and high resolution mass spectra of novel compounds.

Compound 5




DSE 014 ESI pos Shaaban 28/08/13
402.88586
400.88655
398.88813
396.88957
404.88613
PULPROG se_curr_pp
TD 524288
NS 16
DS 0
SW 1907163.677 ppm
m/z
a.i.
1.4e+07
1.2e+07
1.0e+07
8.0e+06
6.0e+06
4.0e+06
2.0e+06
0.
394
399
404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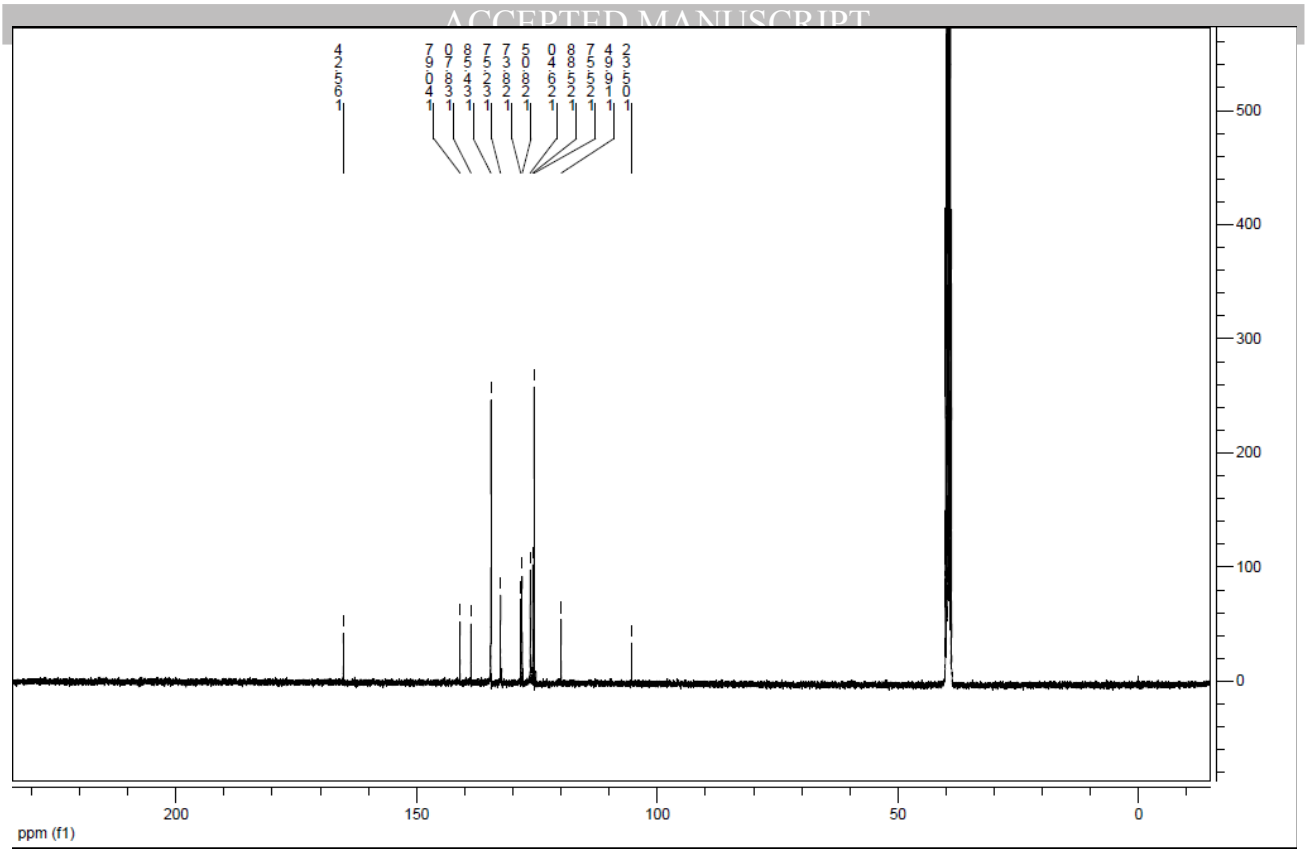
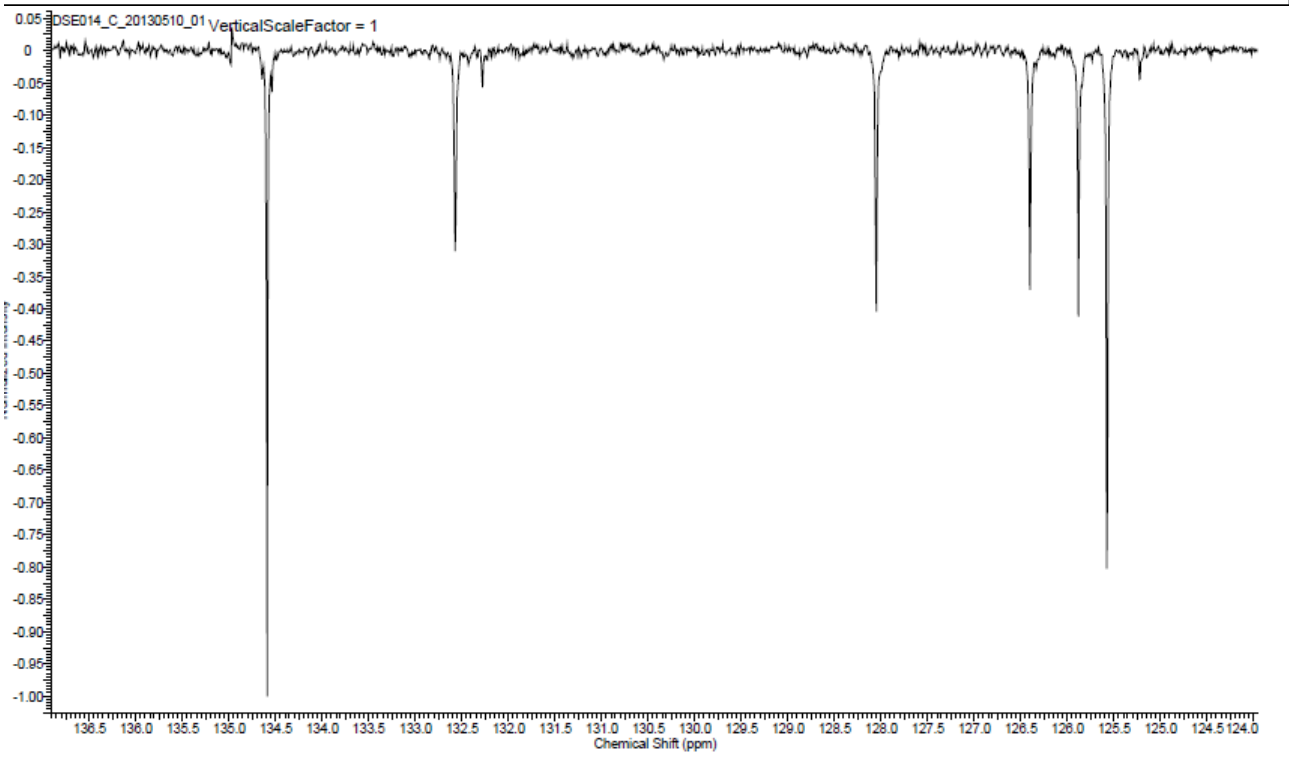
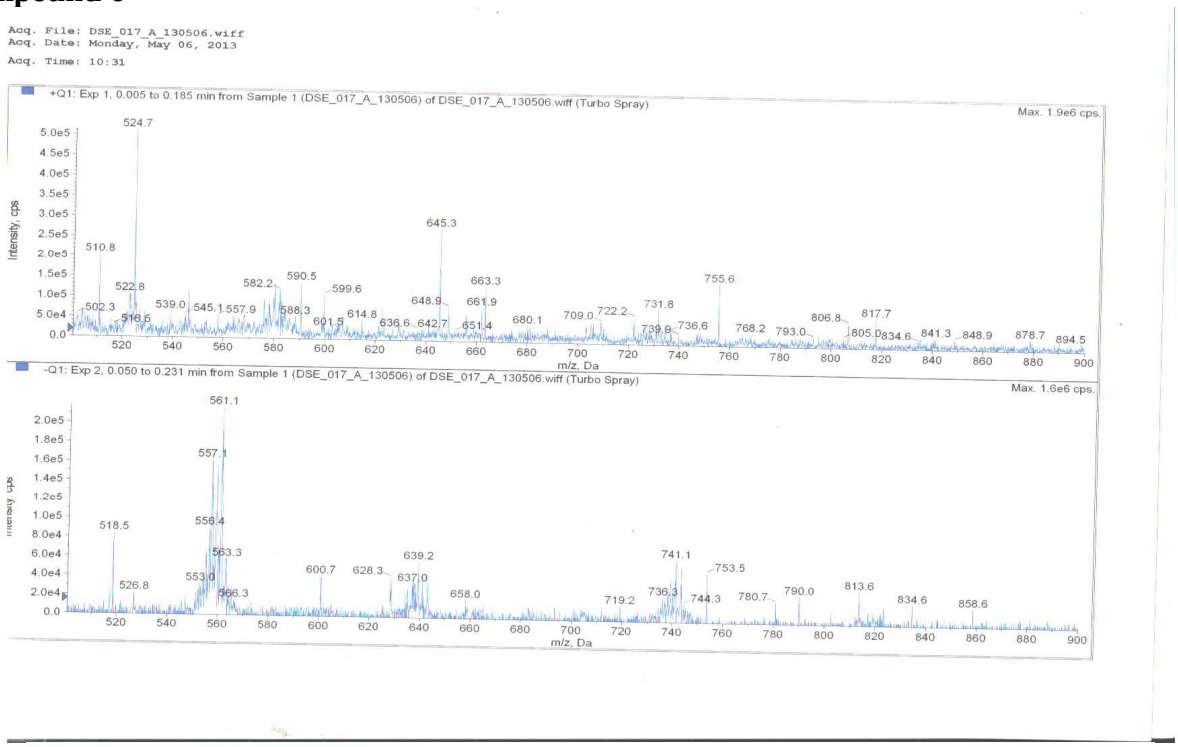
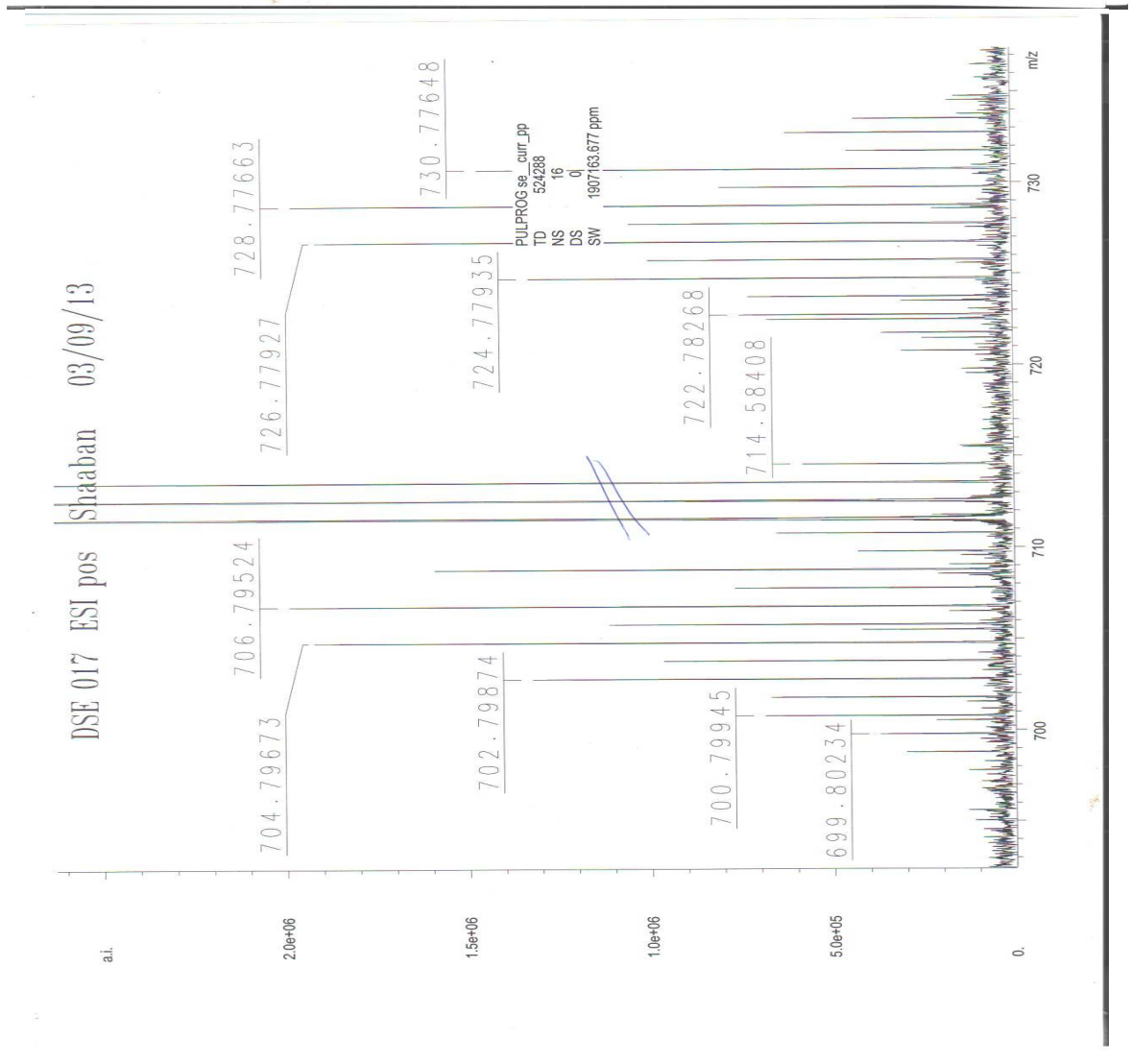
500
0
8.10 8.00 7.90 7.80 7.70 7.60 7.50
ppm (t1)
1 1 4 1
1 2
1
1
4000
3000
2000
1000
0
1 1 4 1 1
1 1 0 2 1
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
ppm (t1)

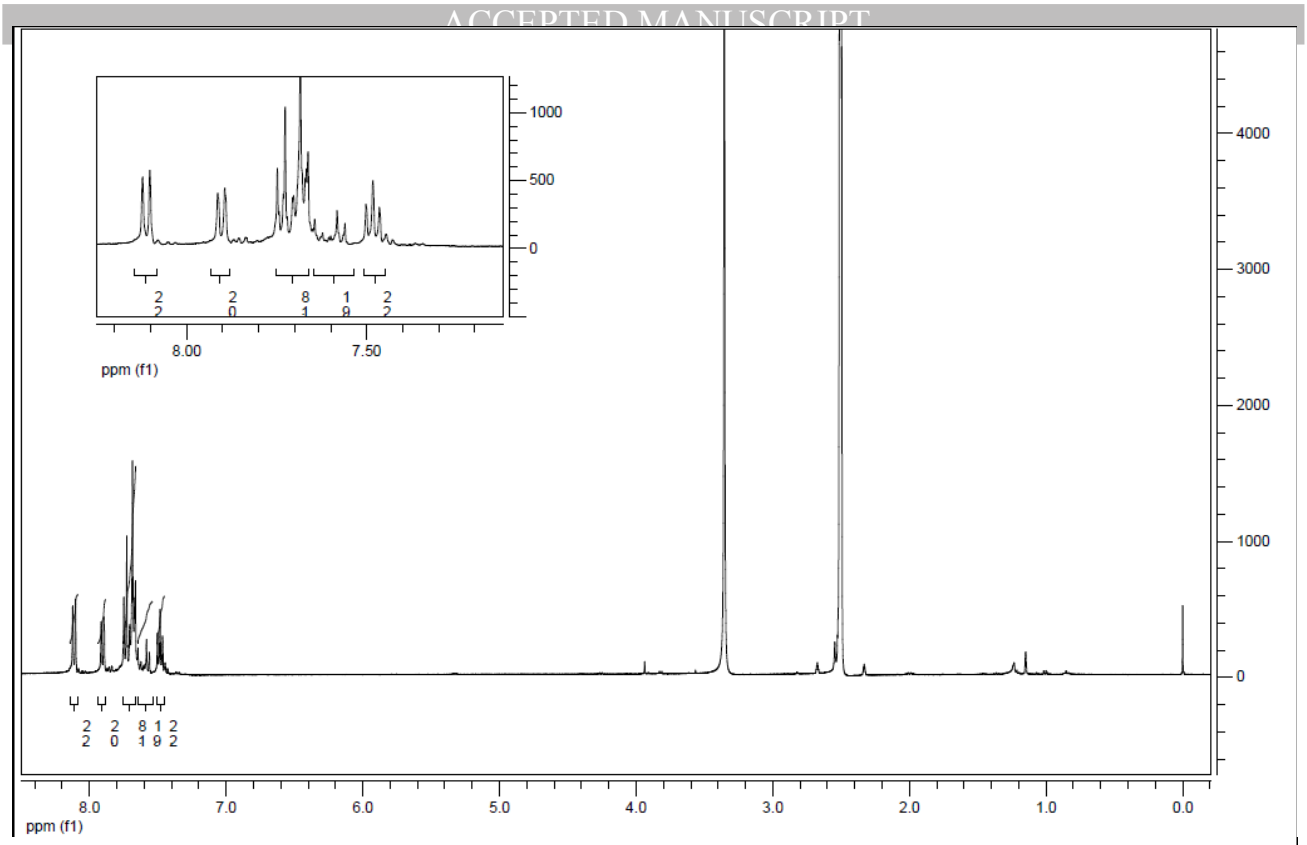


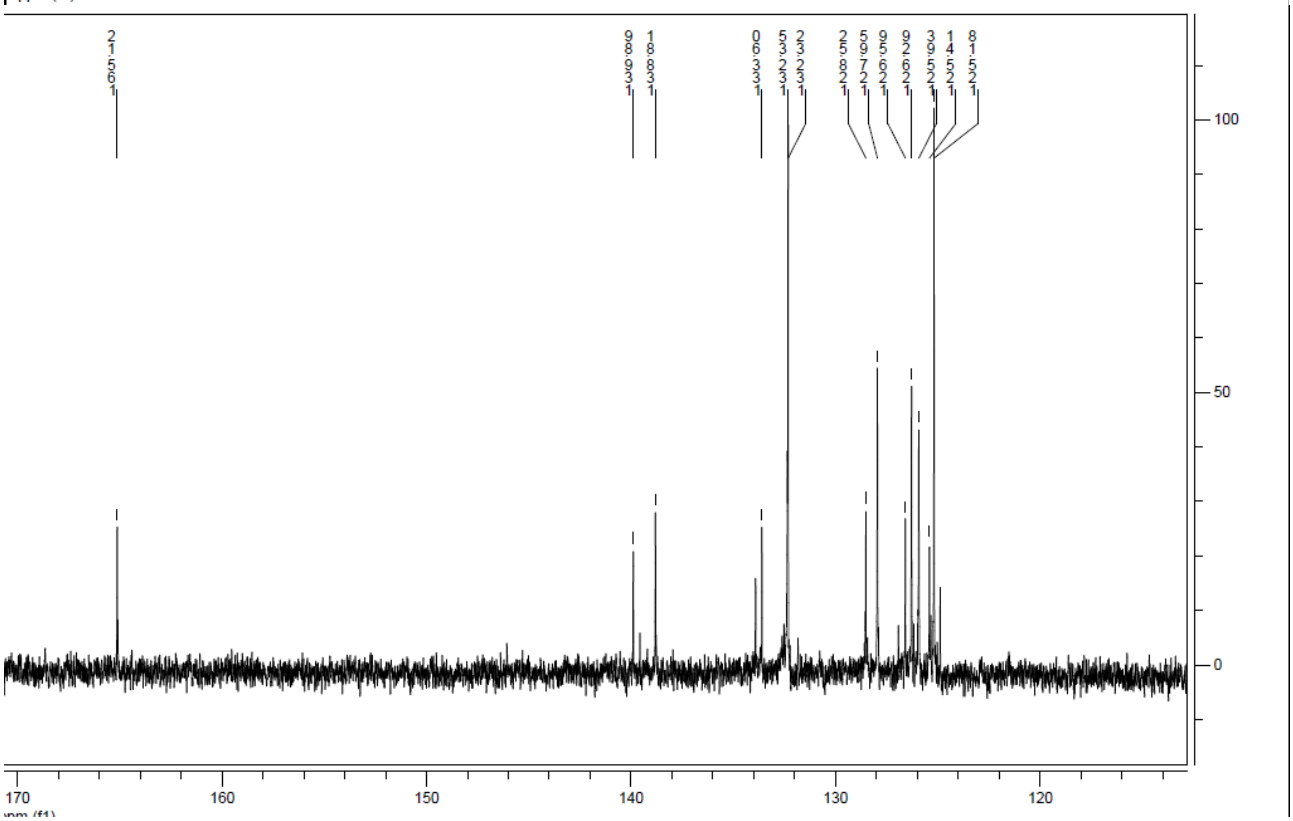


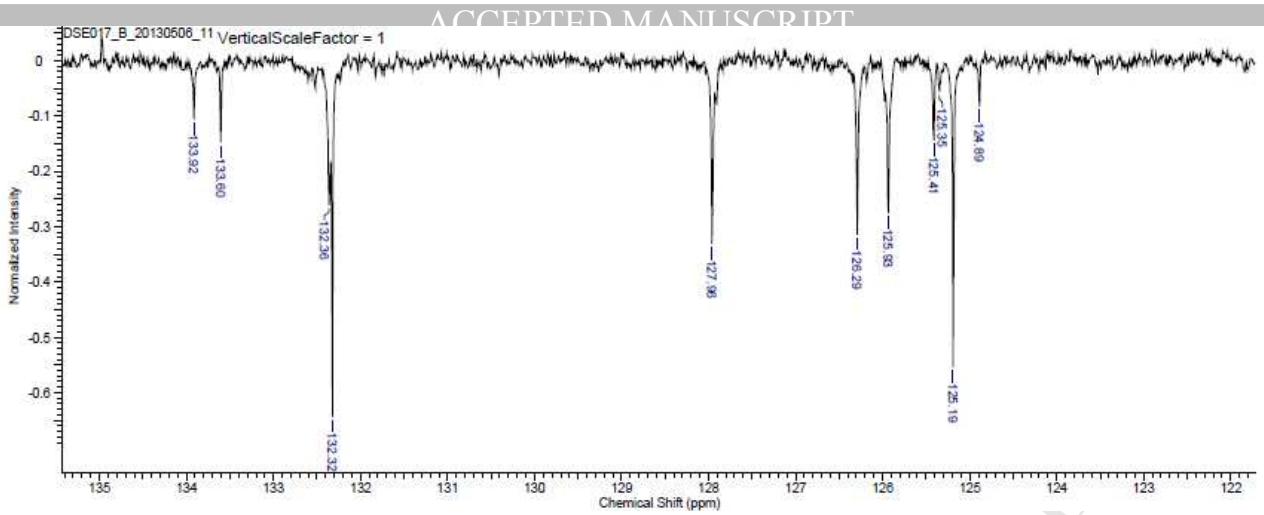
Compound 6


Acq. File: DSE_017_A_130506.wiff
Acq. Date: Monday, May 06, 2013
Acq. Time: 10:31
+Q1: Exp 1, 0.005 to 0.185 min from Sample 1 (DSE_017_A_130506) of DSE_017_A_130506.wiff (Turbo Spray)
Max. 1.9e6 cps.
Intensity, cps
m/z, Da
-Q1: Exp 2, 0.050 to 0.231 min from Sample 1 (DSE_017_A_130506) of DSE_017_A_130506.wiff (Turbo Spray)
Max. 1.6e6 cps.

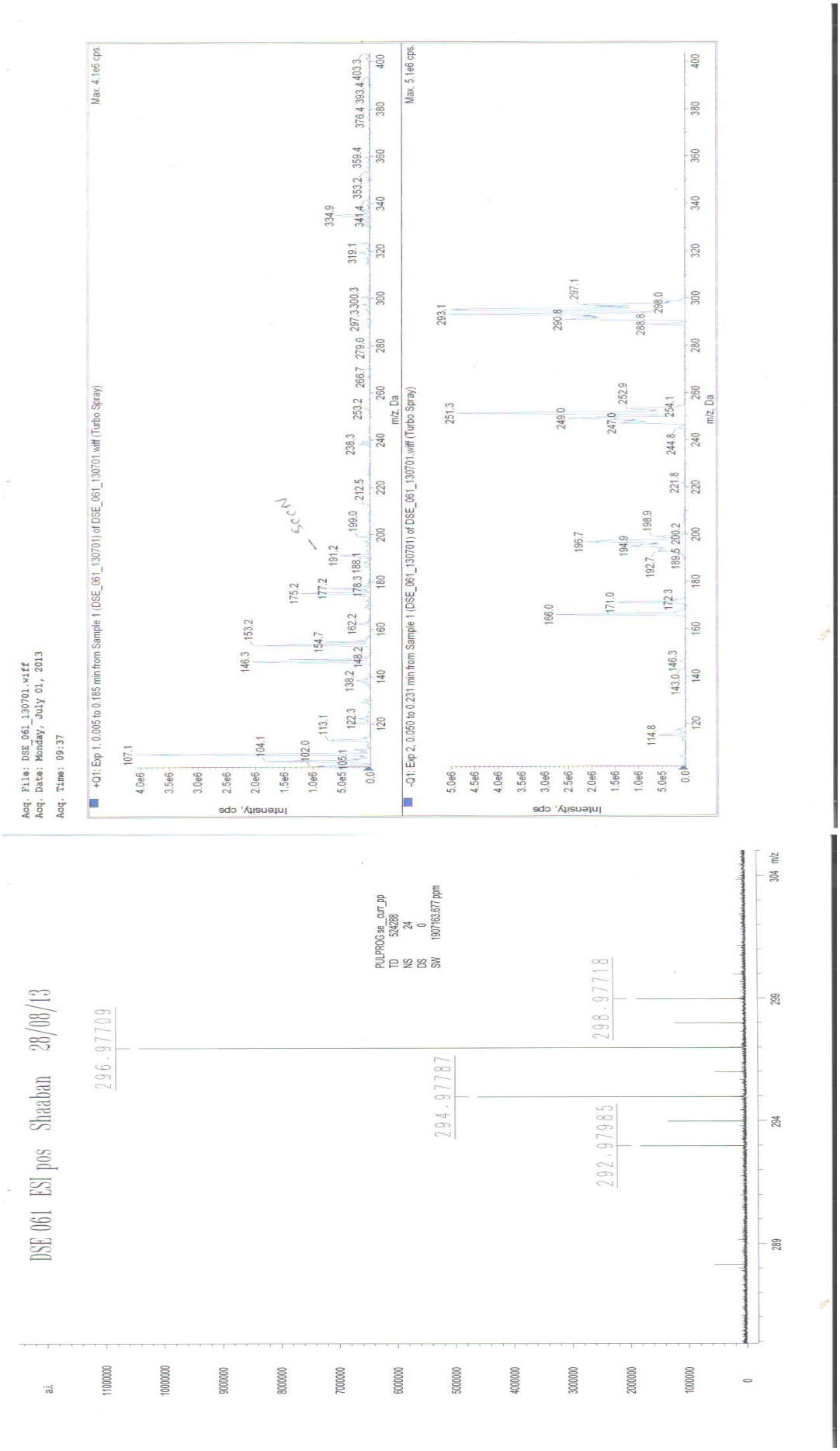

DSE 017 ESI pos
Shaaban 03/09/13
PULPROG se__curr_pp
TD 524288
NS 16
DS 0
SW 1907.63.677 ppm
730.77648
728.77663
726.77927
724.77935
722.78268
714.58408
706.79524
704.79673
702.79874
700.79945
699.80234
m/z
a.i.

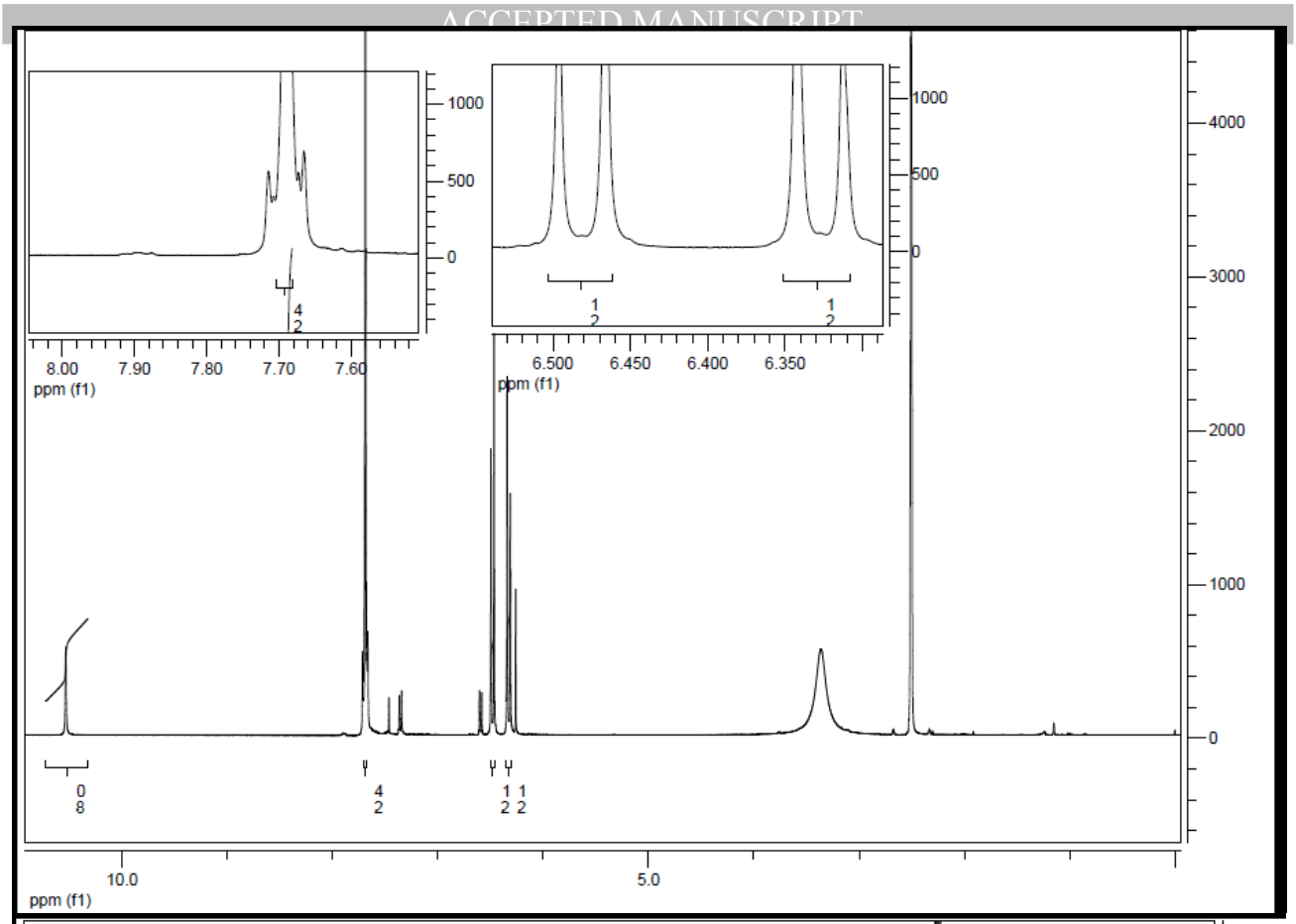


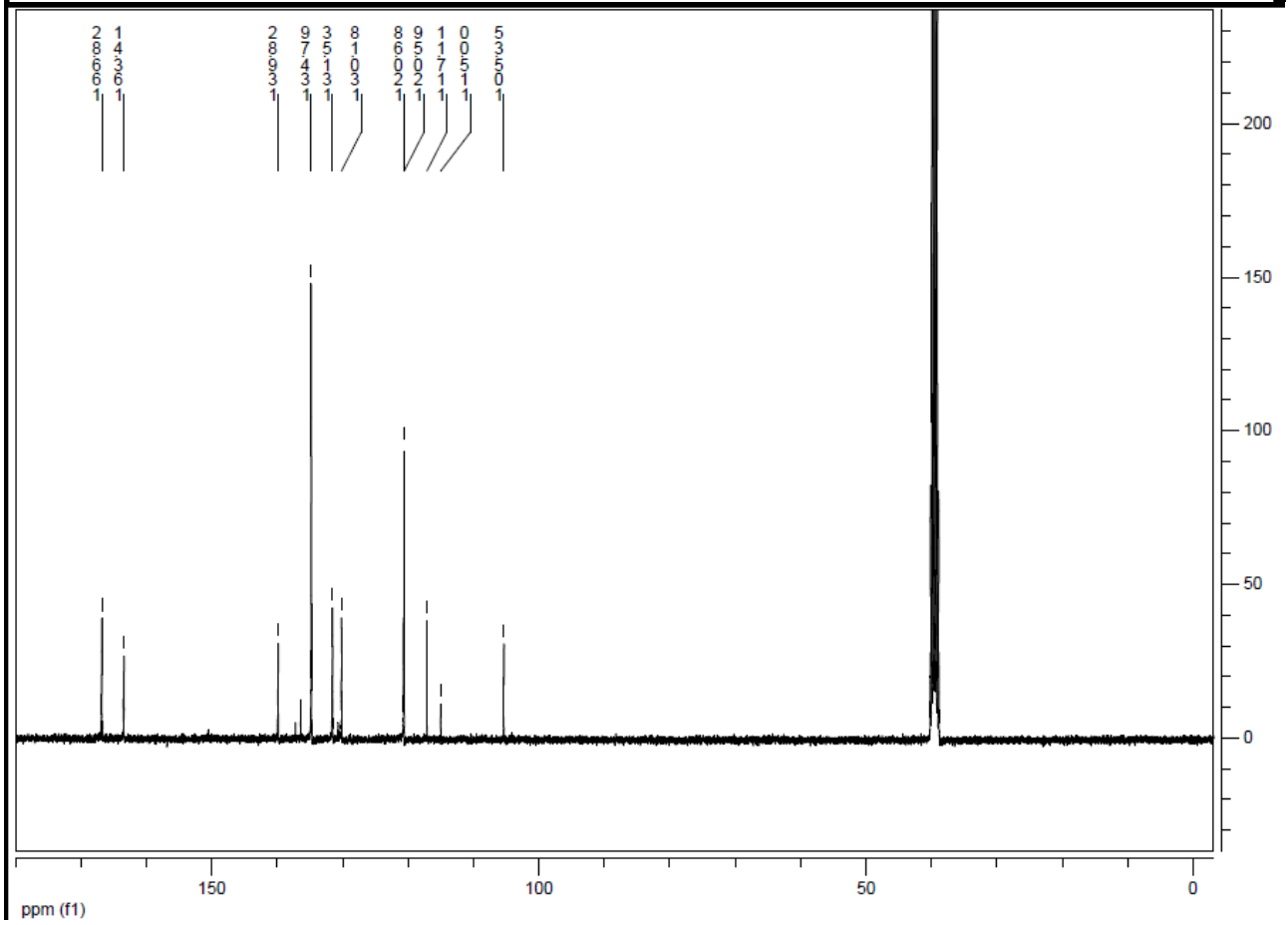


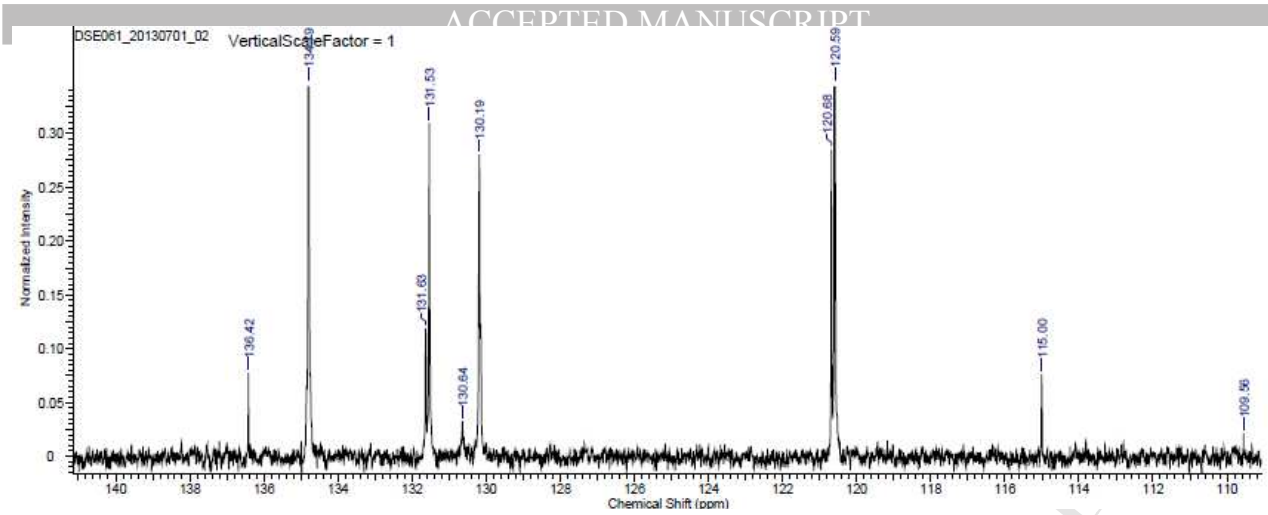


Compound 10

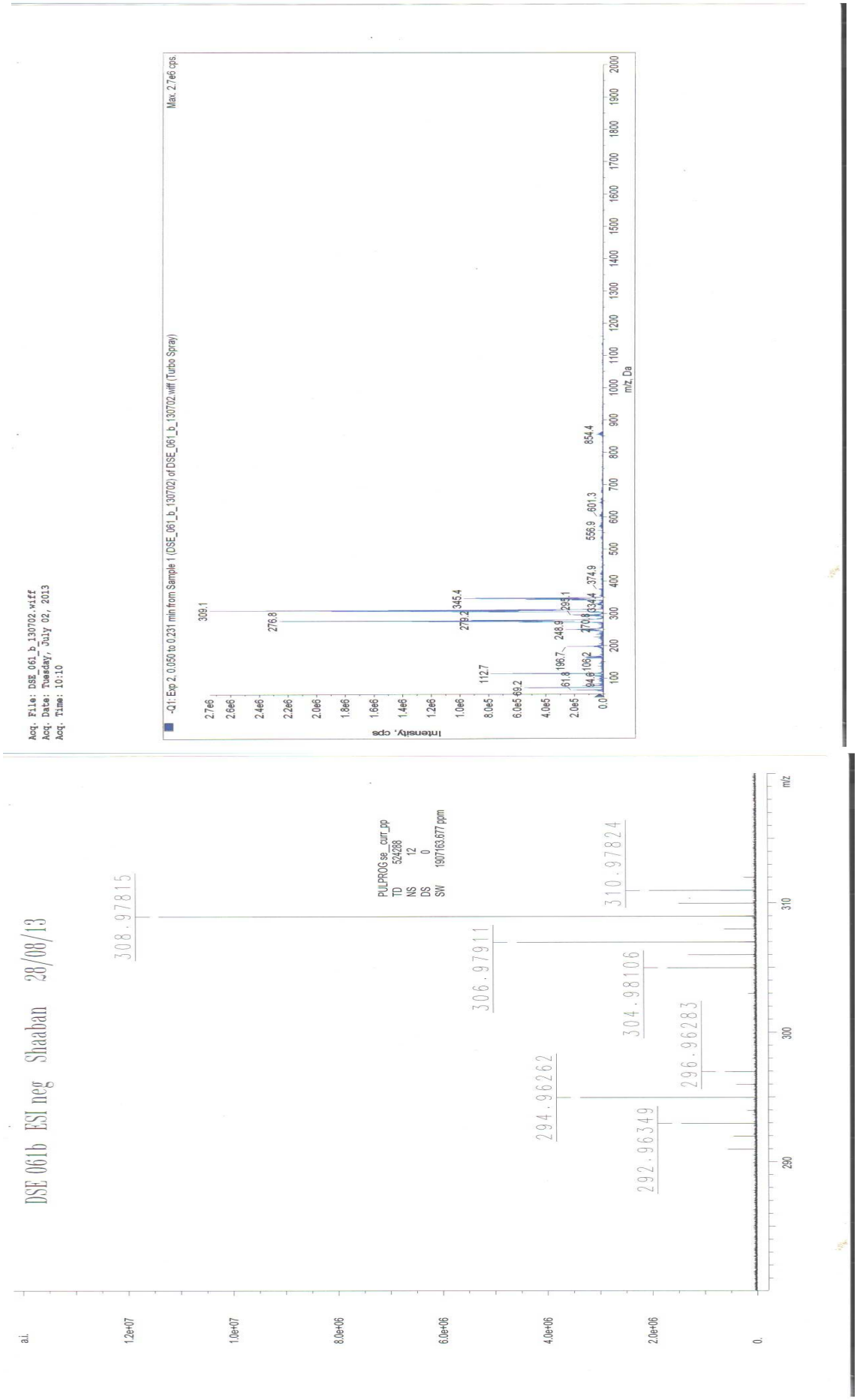

Acq. File: DSE_061_130701.wiff
Acq. Date: Monday, July 01, 2013
Acq. Time: 09:37
+Q1: Exp 1, 0.005 to 0.185 min from Sample 1 (DSE_061_130701) of DSE_061_130701.wiff (Turbo Spray)
Max. 4.1e6 cps.
-Q1: Exp 2, 0.050 to 0.231 min from Sample 1 (DSE_061_130701) of DSE_061_130701.wiff (Turbo Spray)
Max. 5.1e6 cps.
Intensity, cps
m/z, Da
DSE 061 ESI pos Shaaban 28/08/13
296.97709
294.97787
298.97718
292.97985
PULPROG se_curr_pp
TD 524288
NS 24
DS 0
SW 19071163.877 ppm
a.i.
m/z

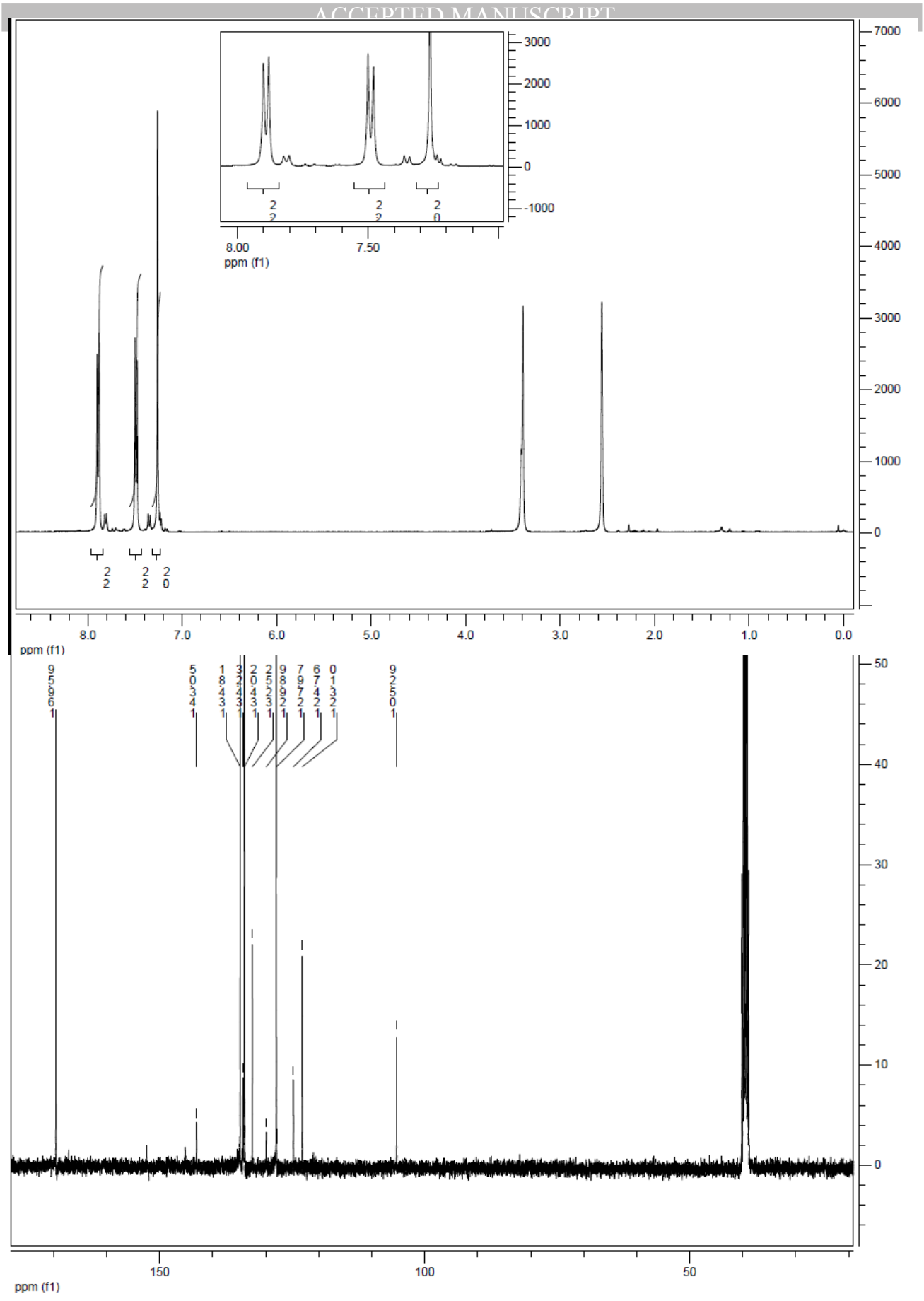


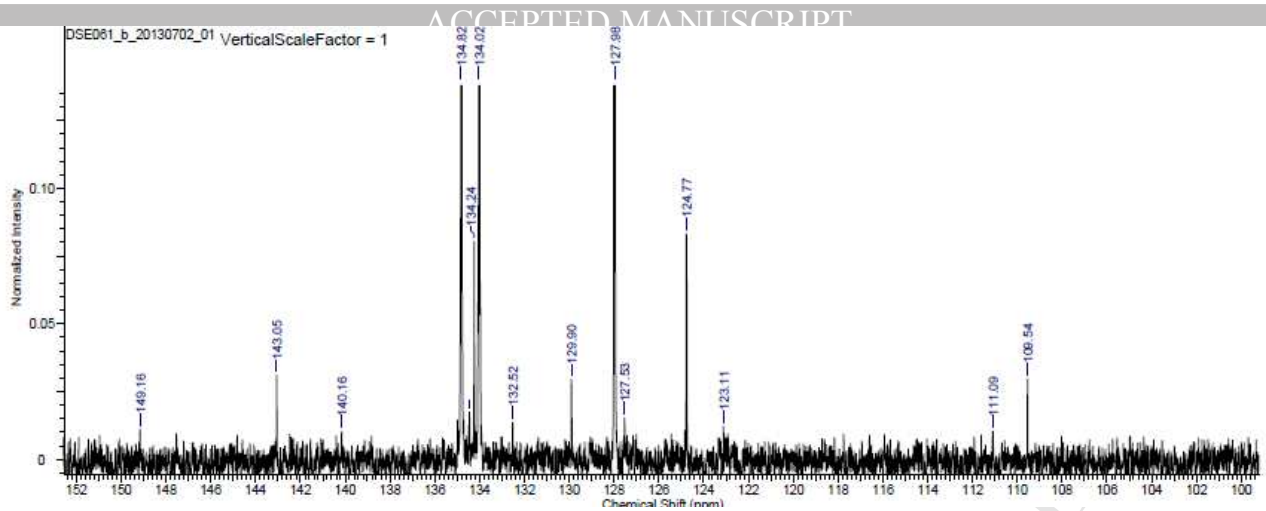




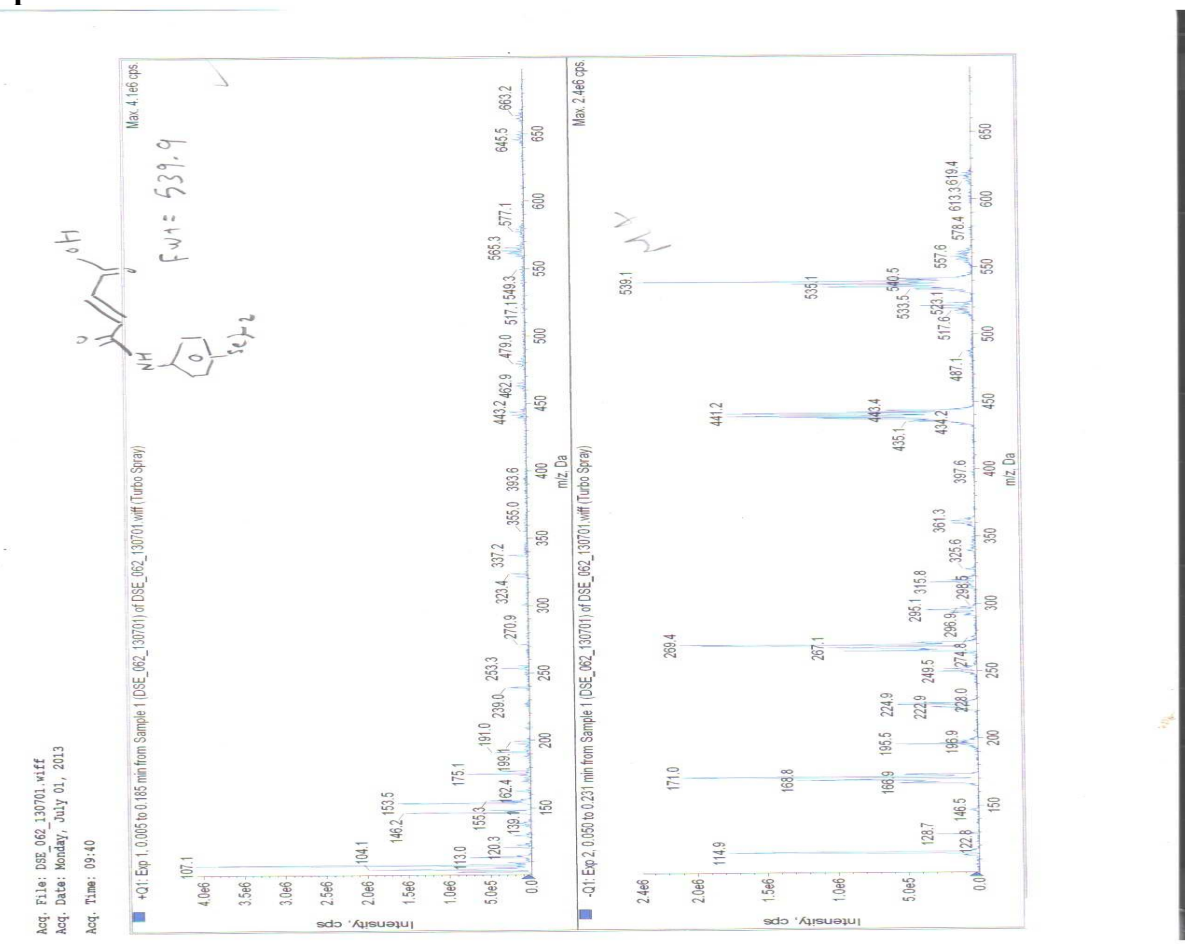
Compound 11

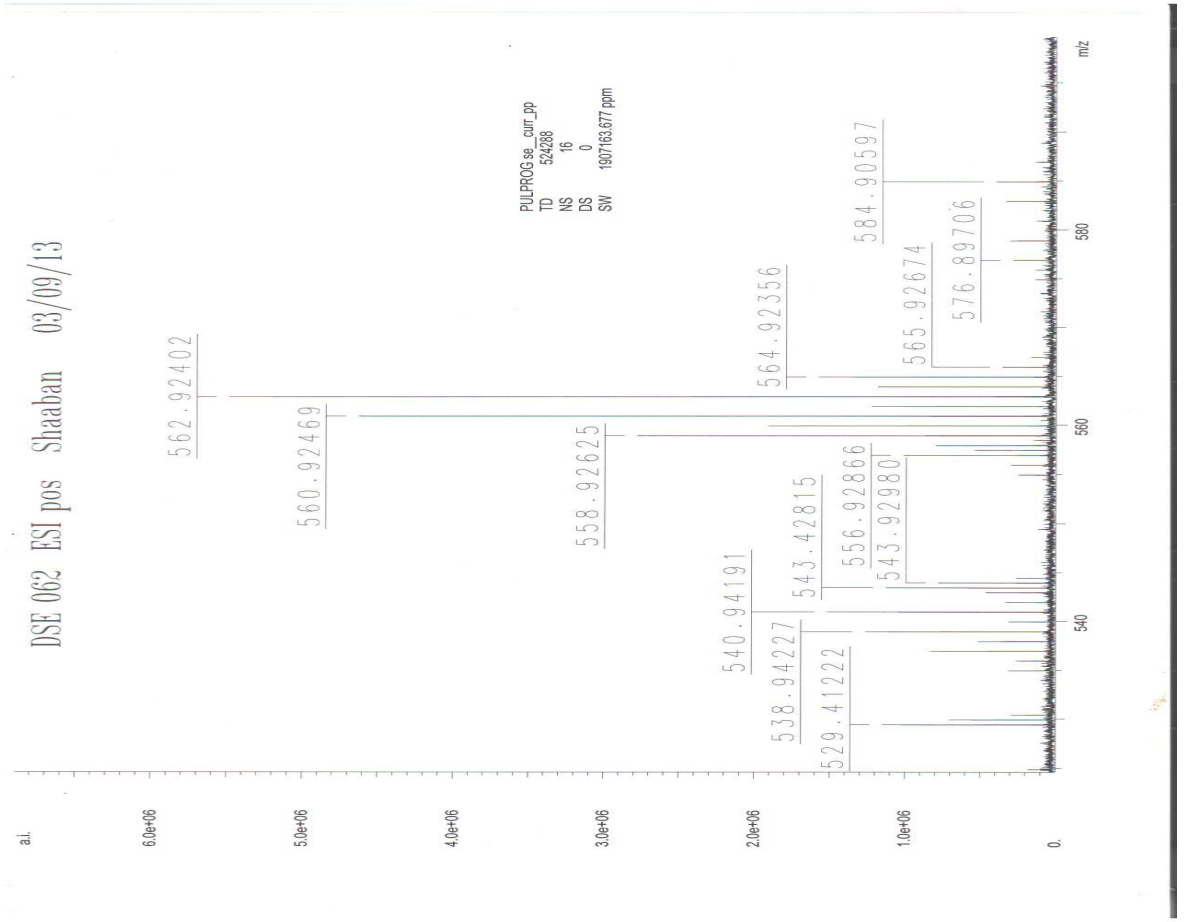

Acq. File: DSE_061_b_130702.wiff
Acq. Date: Tuesday, July 02, 2013
Acq. Time: 10:10
-Q1: Exp 2, 0.050 to 0.231 min from Sample 1 (DSE_061_b_130702) of DSE_061_b_130702.wiff (Turbo Spray)
Max. 2.7e6 cps.
Intensity, cps
m/z, Da
DSE 061b ESI neg Shaaban 28/08/13
PULPROG se_curr_pp
TD 524288
NS 12
DS 0
SW 19071163.677 ppm
308.97815
306.97911
310.97824
294.96262
304.98106
292.96349
296.96283
m/z





Compound 16


Acq. File: DSE_062_130701.wiff
Acq. Date: Monday, July 01, 2013
Acq. Time: 09:40
+Q1: Exp 1, 0.005 to 0.185 min from Sample 1 (DSE_062_130701) of DSE_062_130701.wiff (Turbo Spray)
Max. 4.1e6 cps.
Intensity, cps
m/z, Da
-Q1: Exp 2, 0.050 to 0.231 min from Sample 1 (DSE_062_130701) of DSE_062_130701.wiff (Turbo Spray)
Max. 2.4e6 cps.
Intensity, cps
m/z, Da


DSE 062 ESI pos Shaaban 03/09/13
PULPROG se_curr_pp
TD 524288
NS 16
DS 0
SW 19071163.677 ppm
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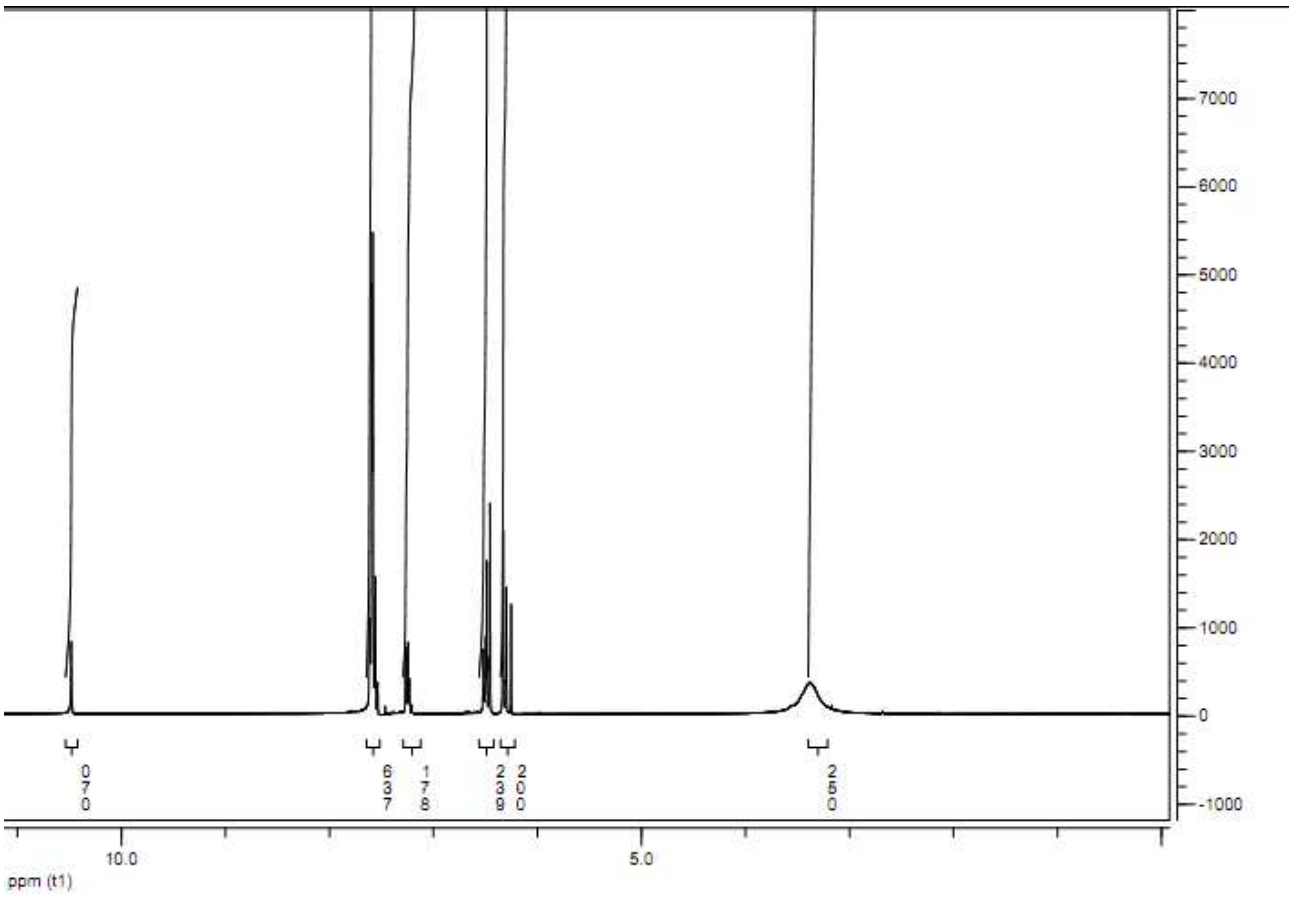
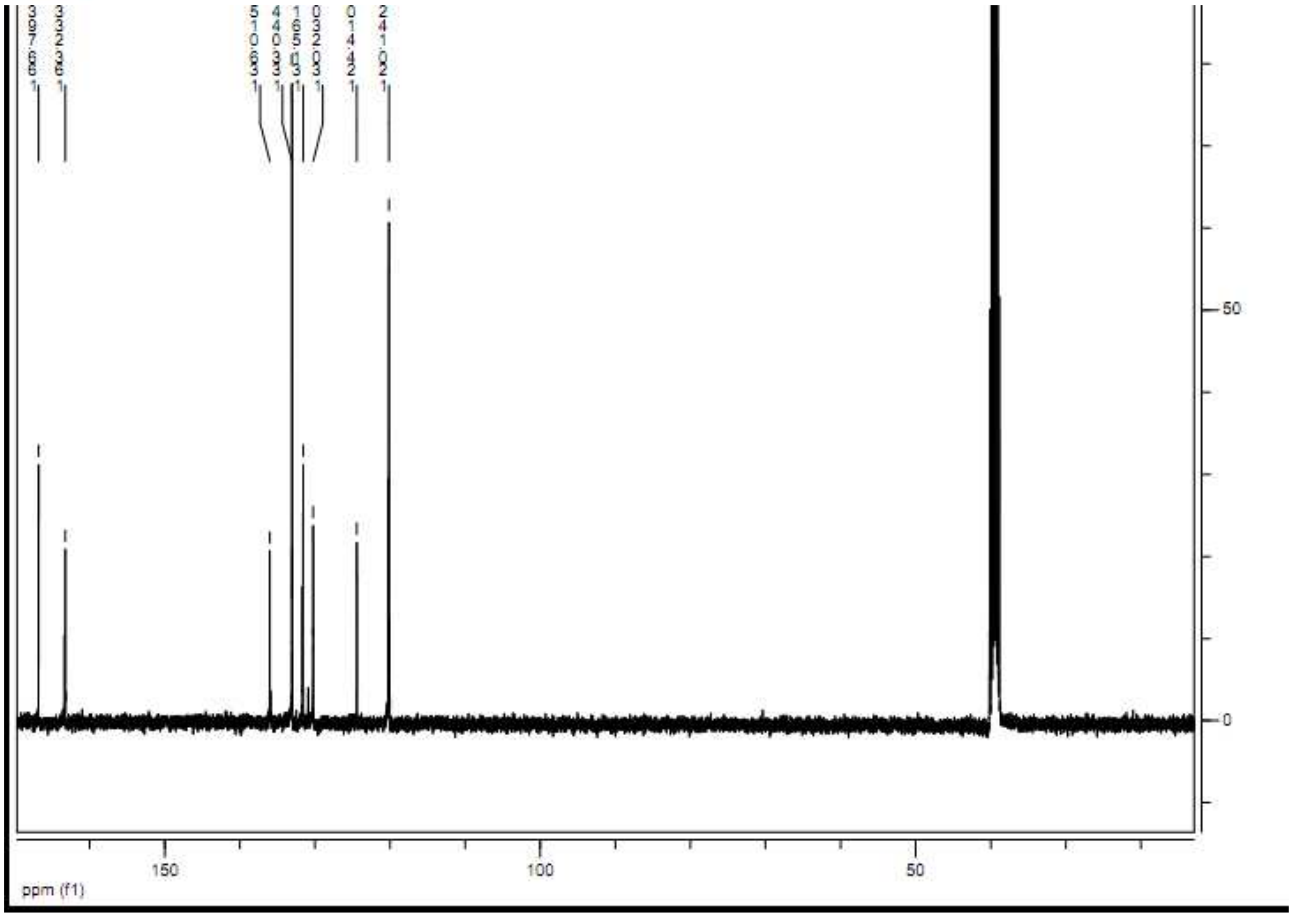
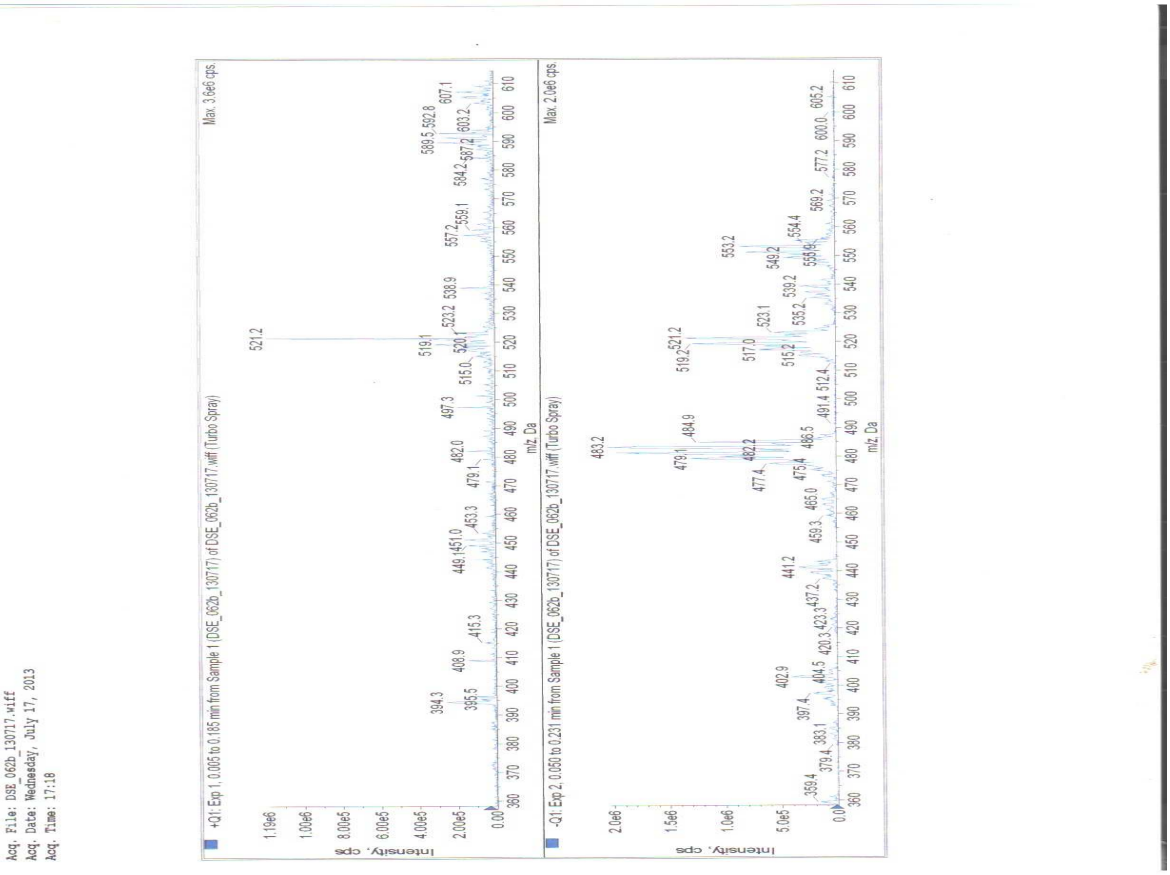
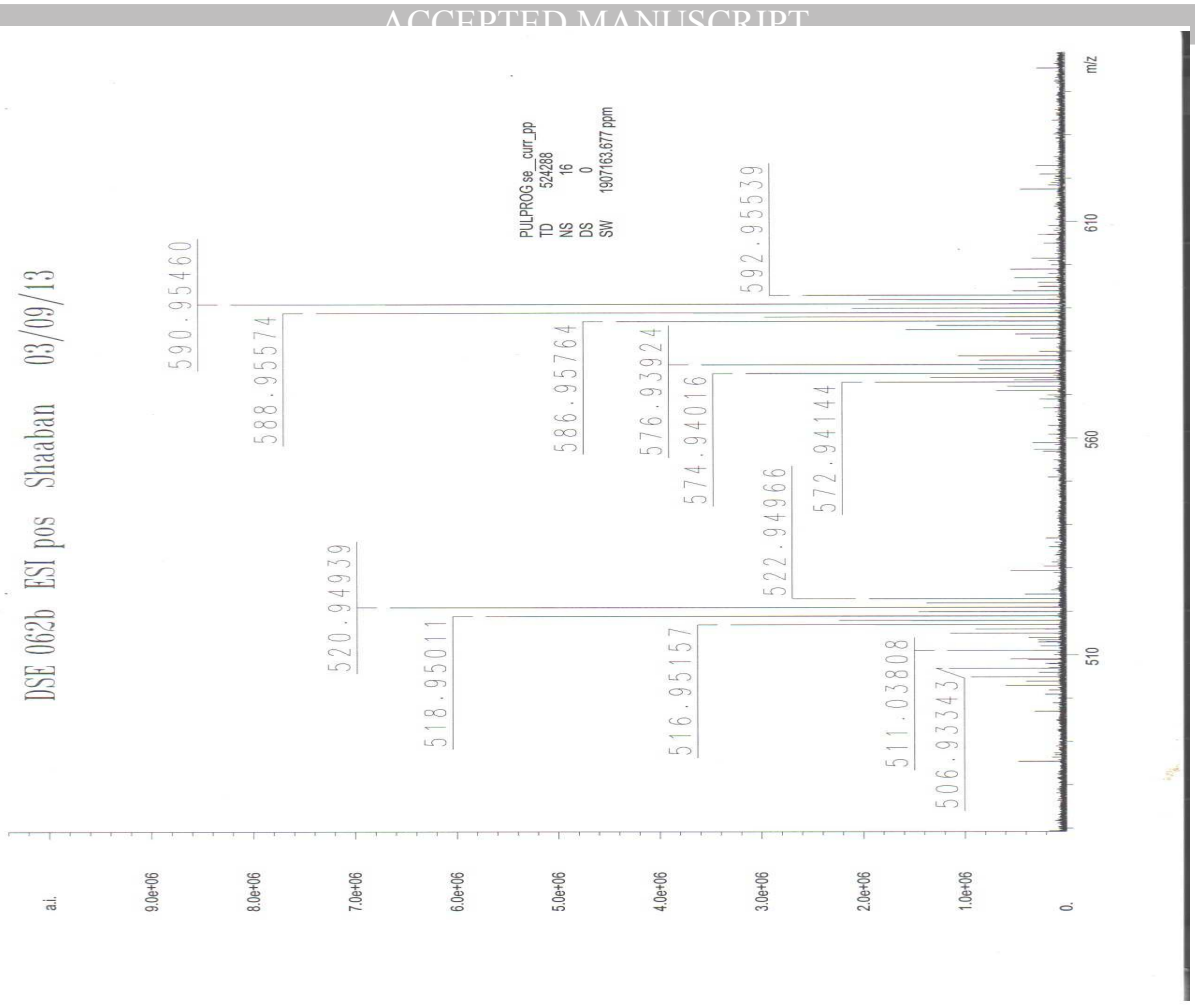
560.92469
558.92625
564.92356
584.90597
565.92674
576.89706
540.94191
543.42815
556.92866
543.92980
538.94227
529.41222
a.i.
m/z

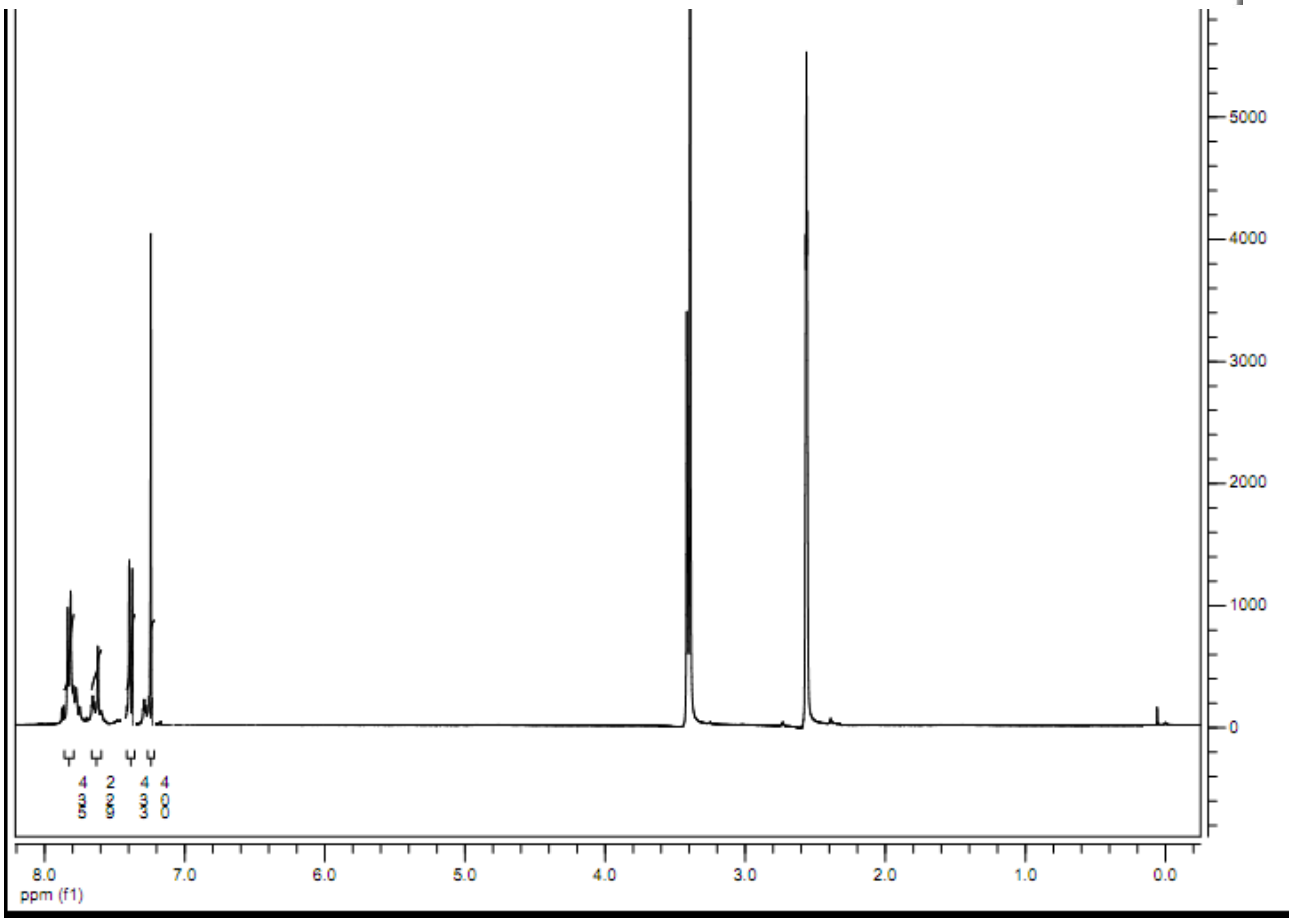

7000
6000
5000
4000
3000
2000
1000
0
-1000
0 7 0
6 3 7
1 7 8
2 3 9
2 0 0
2 5 0
10.0
5.0
ppm (t1)

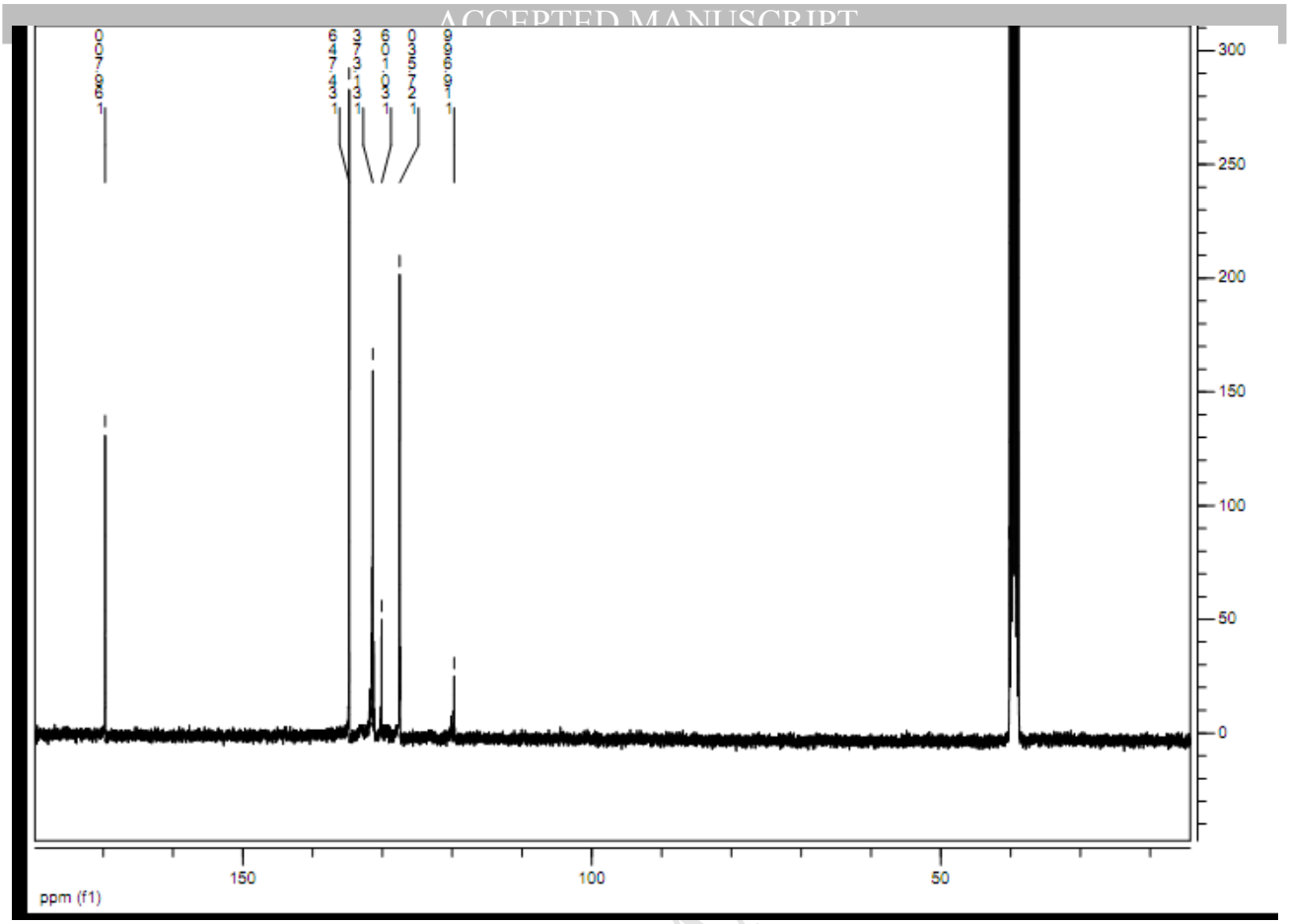

50
0
150
100
50
ppm (f1)

Compound 17

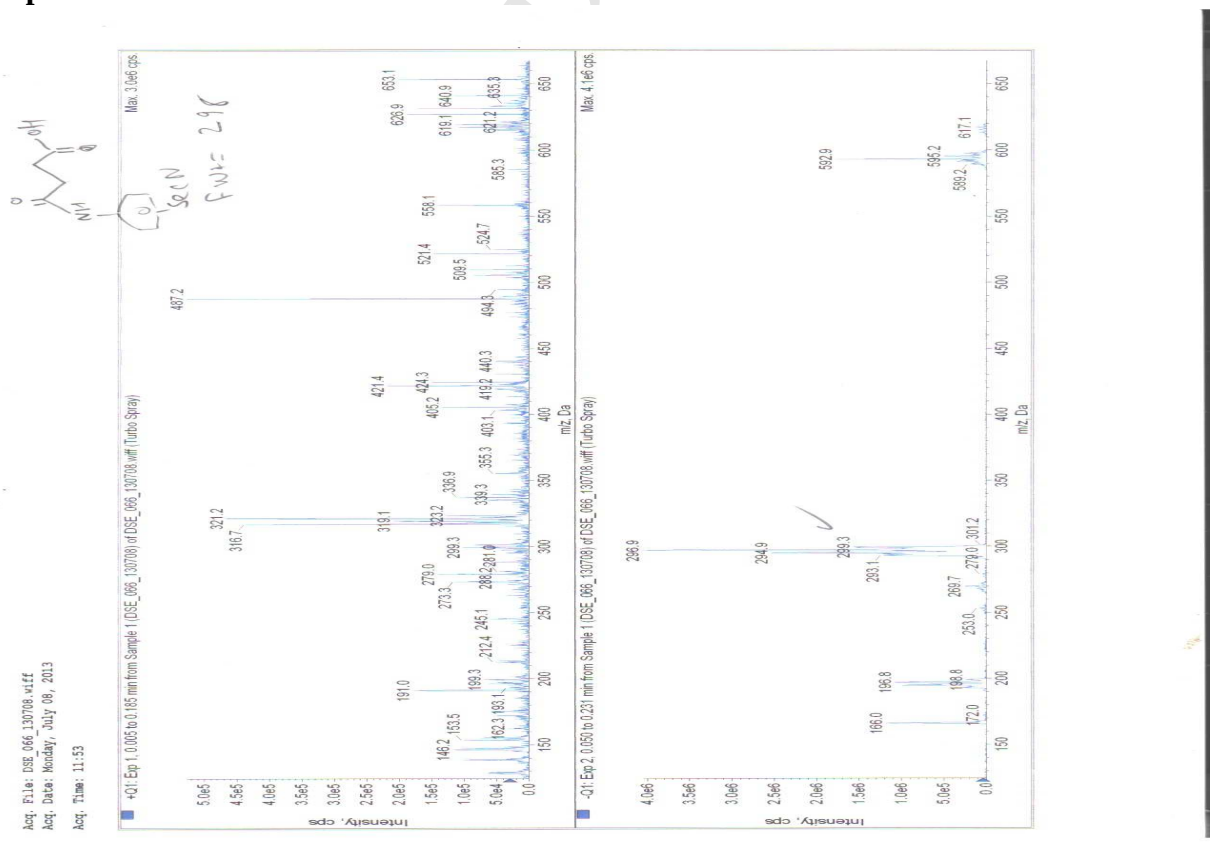


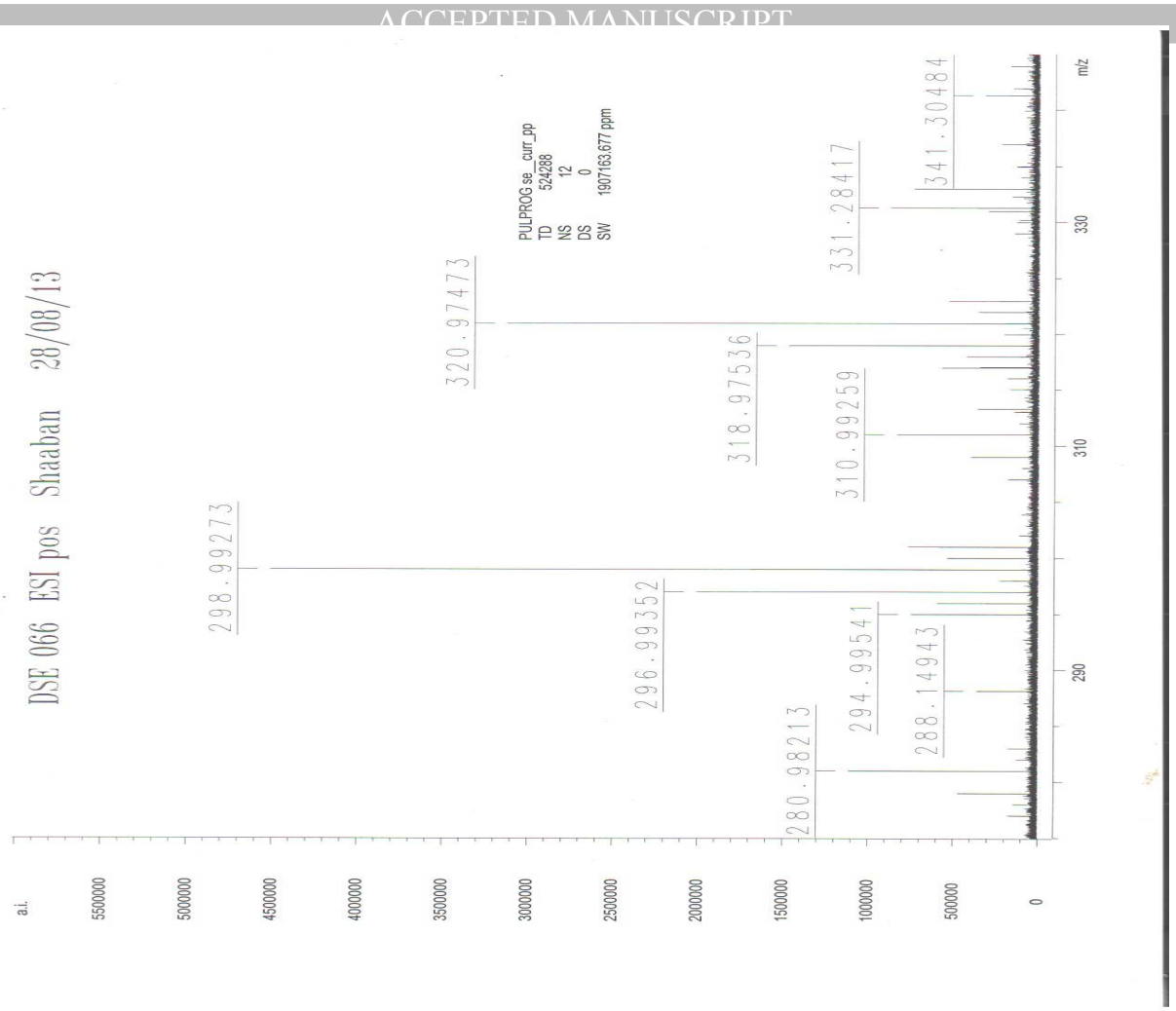

DSE 062b ESI pos Shaaban 03/09/13
PULPROG se_curr_pp
TD 524288
NS 16
DS 0
SW 1907163.677 ppm
590.95460
588.95574
586.95764
592.95539
576.93924
574.94016
572.94144
520.94939
522.94966
518.95011
516.95157
511.03808
506.93343
m/z
a.i.


ppm (f1)



Compound 12




DSF 066 ESI pos Shaaban 28/08/13
PULPROG se_curr_pp
TD 524288
NS 12
DS 0
SW 1907163.677 ppm
298.99273
320.97473
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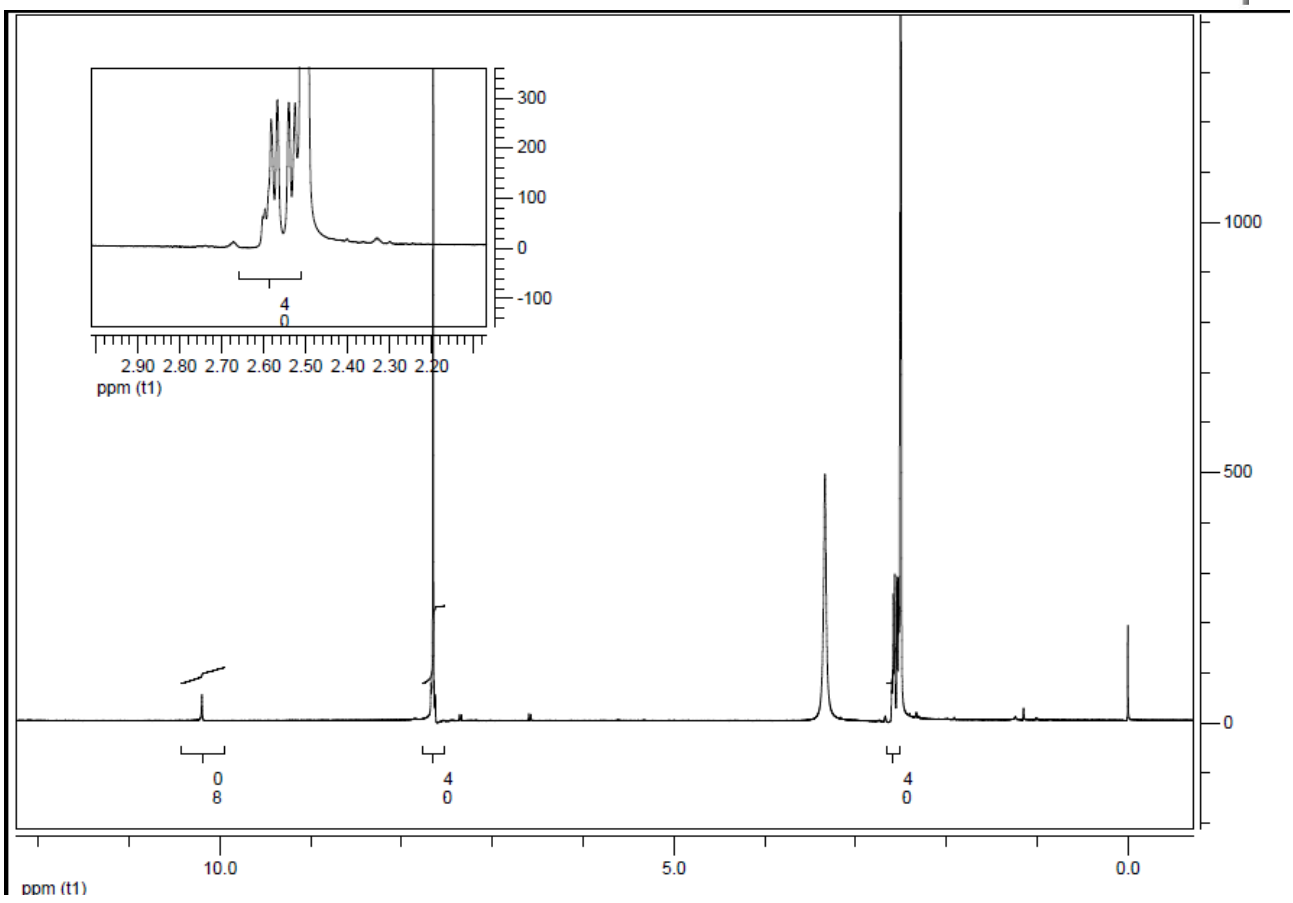
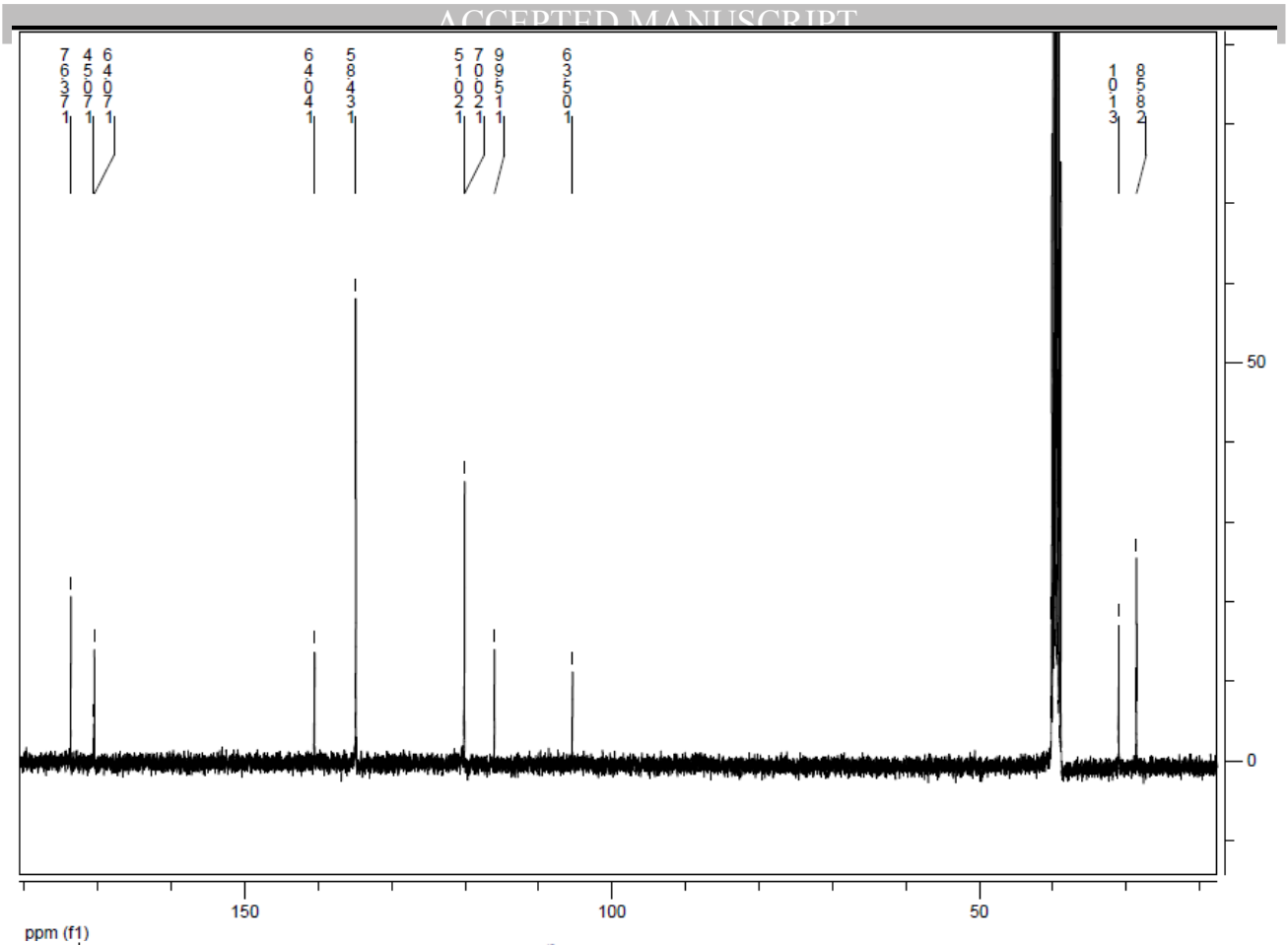
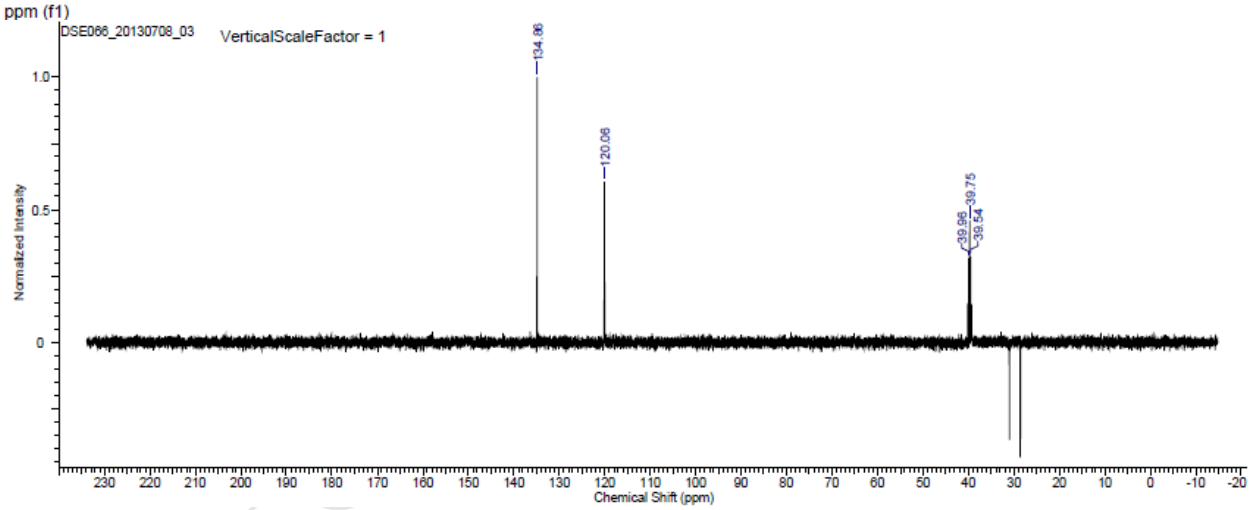
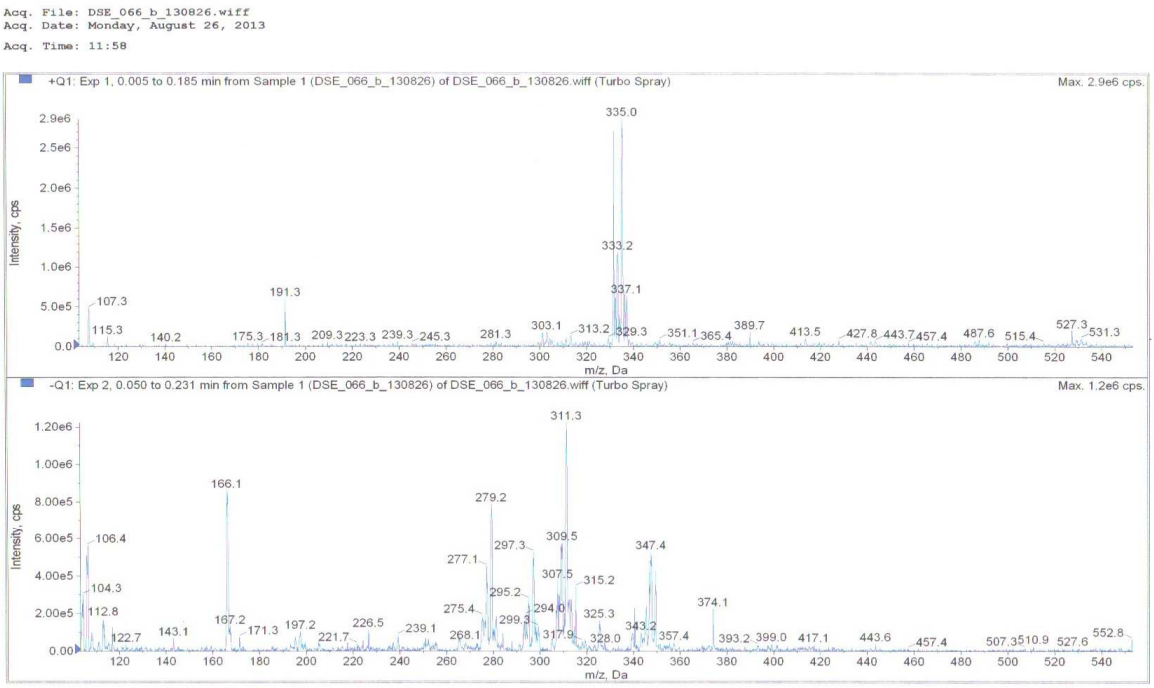
310.99259
294.99541
280.98213
288.14943
331.28417
341.30484
m/z
a.i.

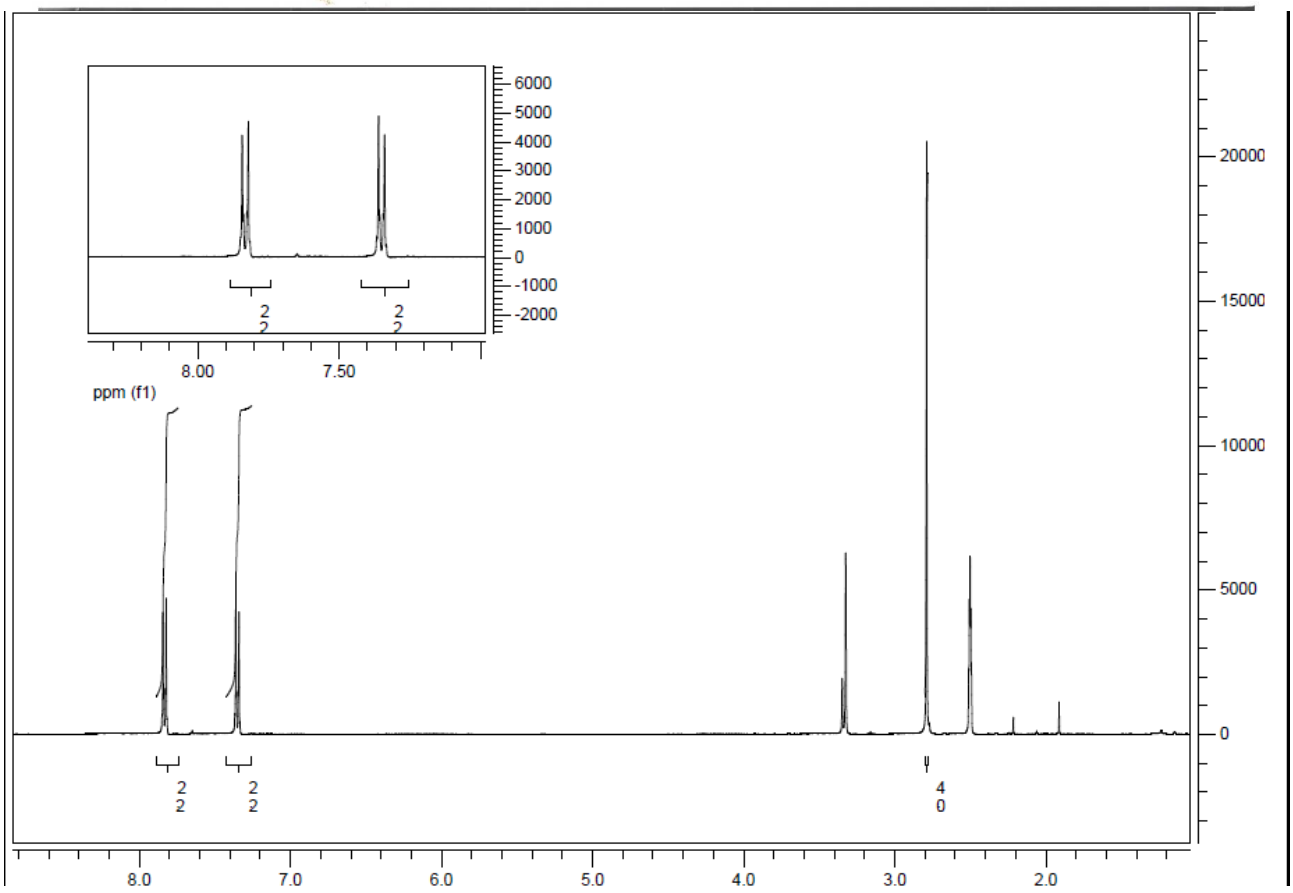

ppm (t1)
10.0
5.0
0.0
2.90 2.80 2.70 2.60 2.50 2.40 2.30 2.20

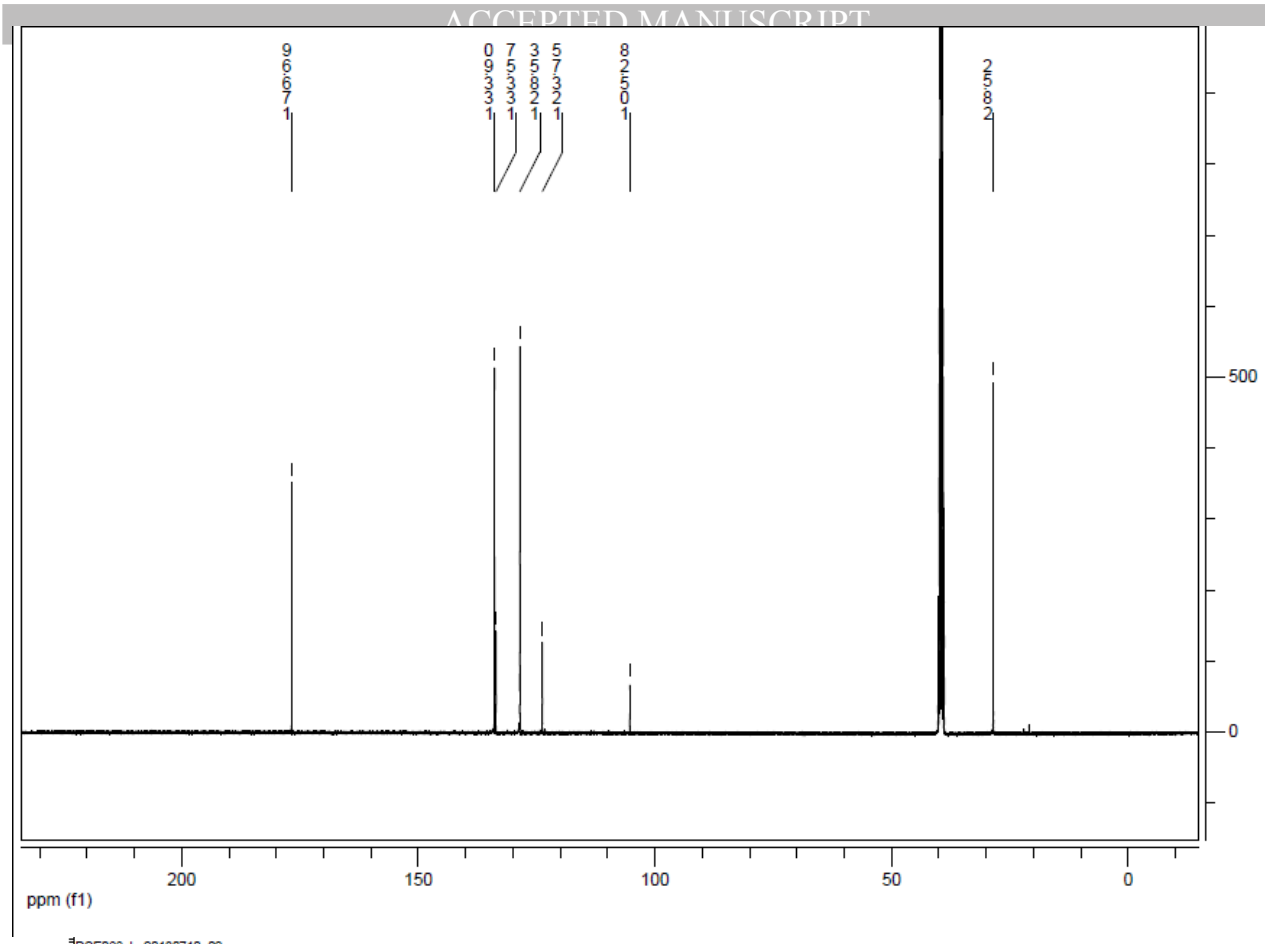


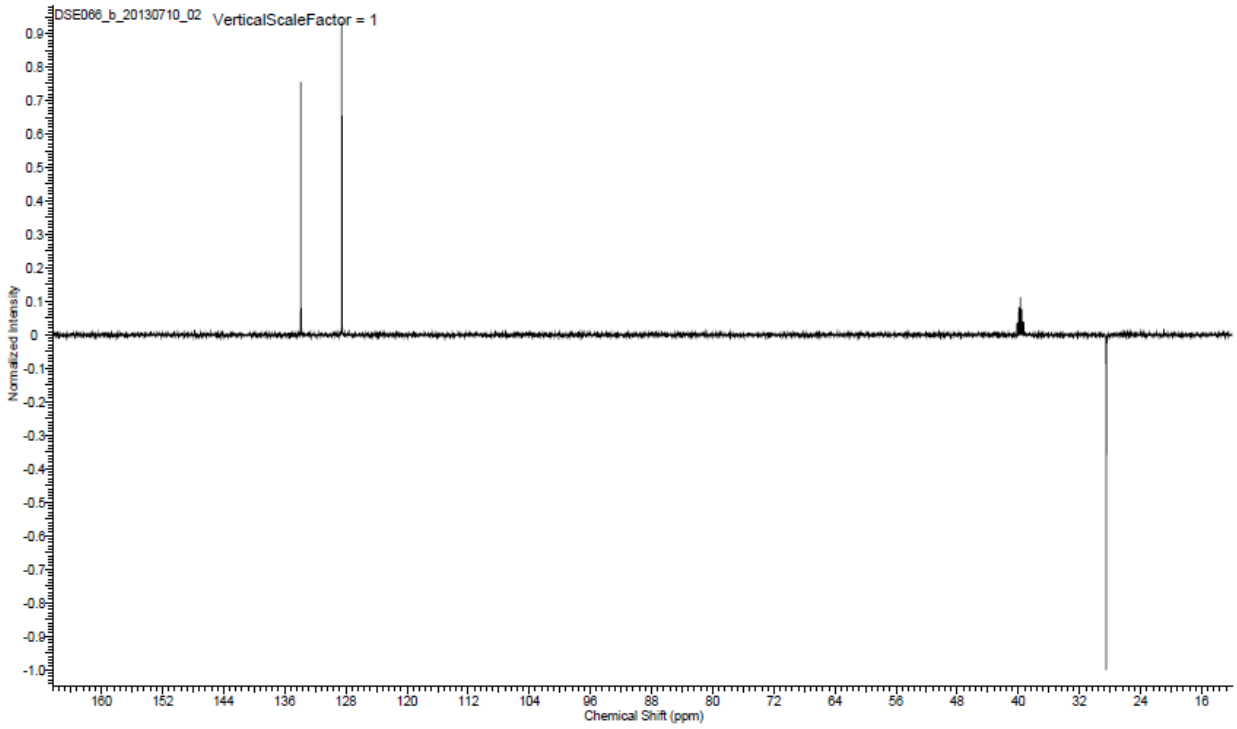


Compound 13

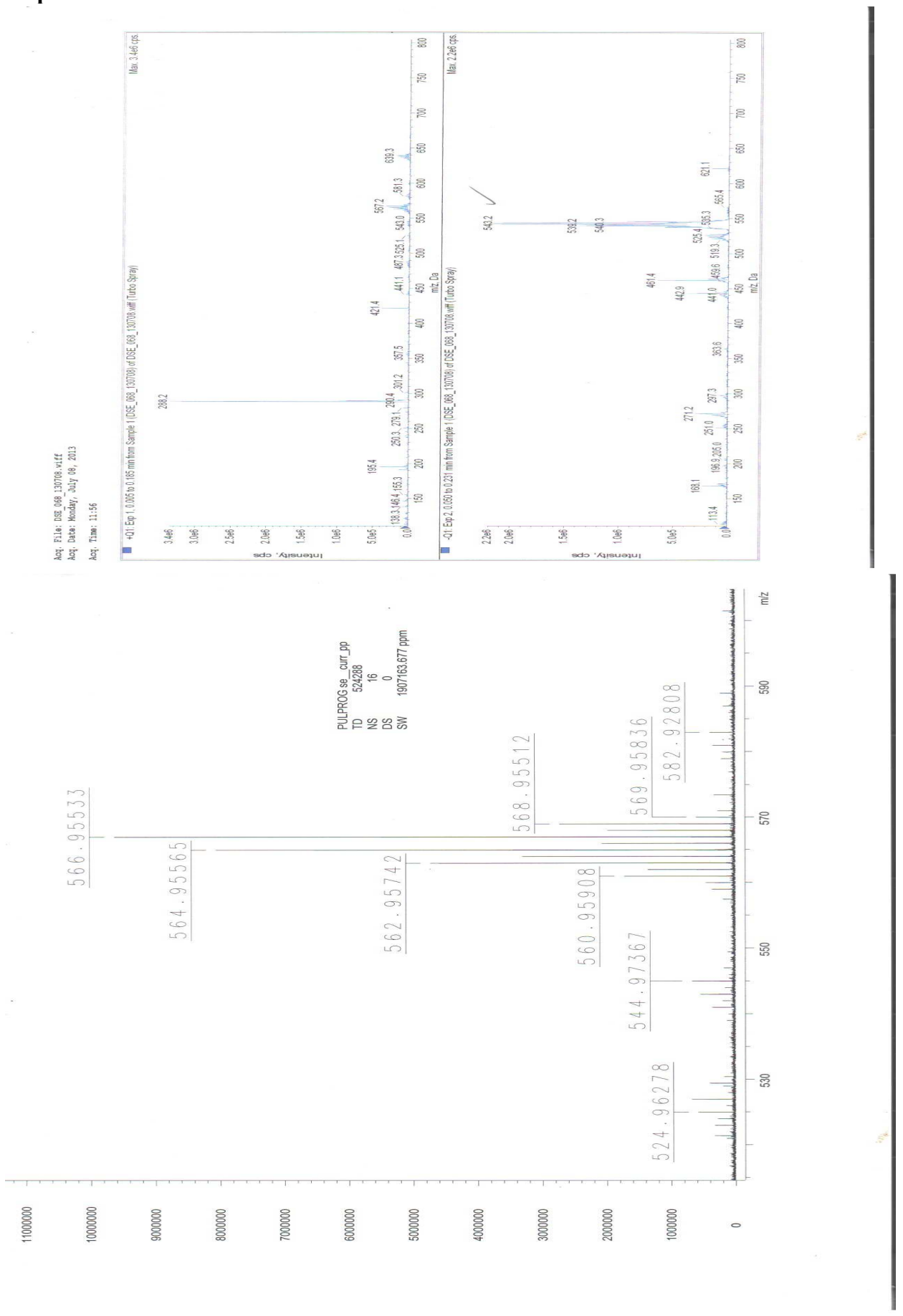

Acq. File: DSE_066_b_130826.wiff
Acq. Date: Monday, August 26, 2013
Acq. Time: 11:58
+Q1: Exp 1, 0.005 to 0.185 min from Sample 1 (DSE_066_b_130826) of DSE_066_b_130826.wiff (Turbo Spray)
Max. 2.9e6 cps.
Intensity, cps
m/z, Da
-Q1: Exp 2, 0.050 to 0.231 min from Sample 1 (DSE_066_b_130826) of DSE_066_b_130826.wiff (Turbo Spray)
Max. 1.2e6 cps.
Intensity, cps
m/z, Da

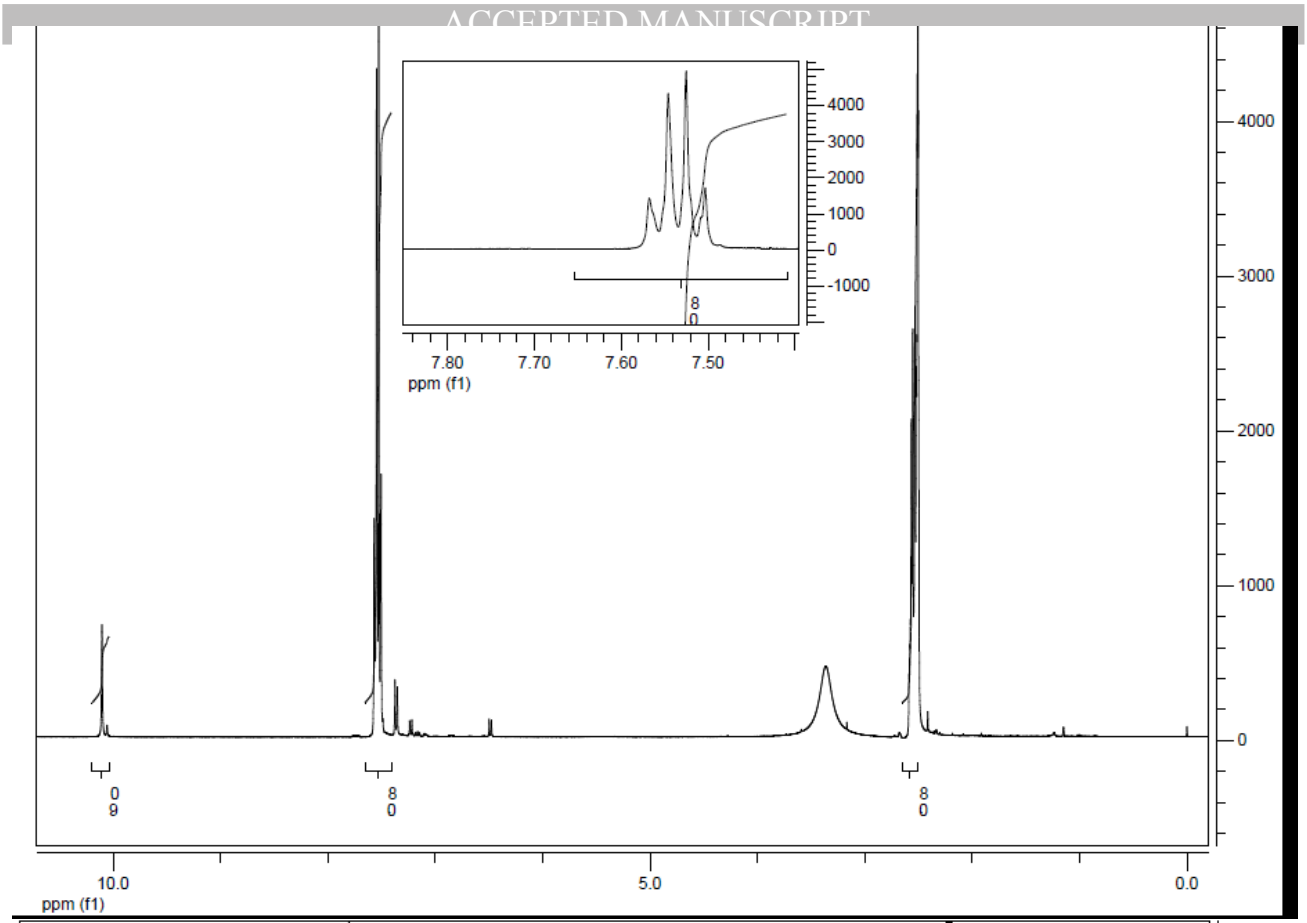

ppm (f1)
8.00
7.50
8.0
7.0
6.0
5.0
4.0
3.0
2.0

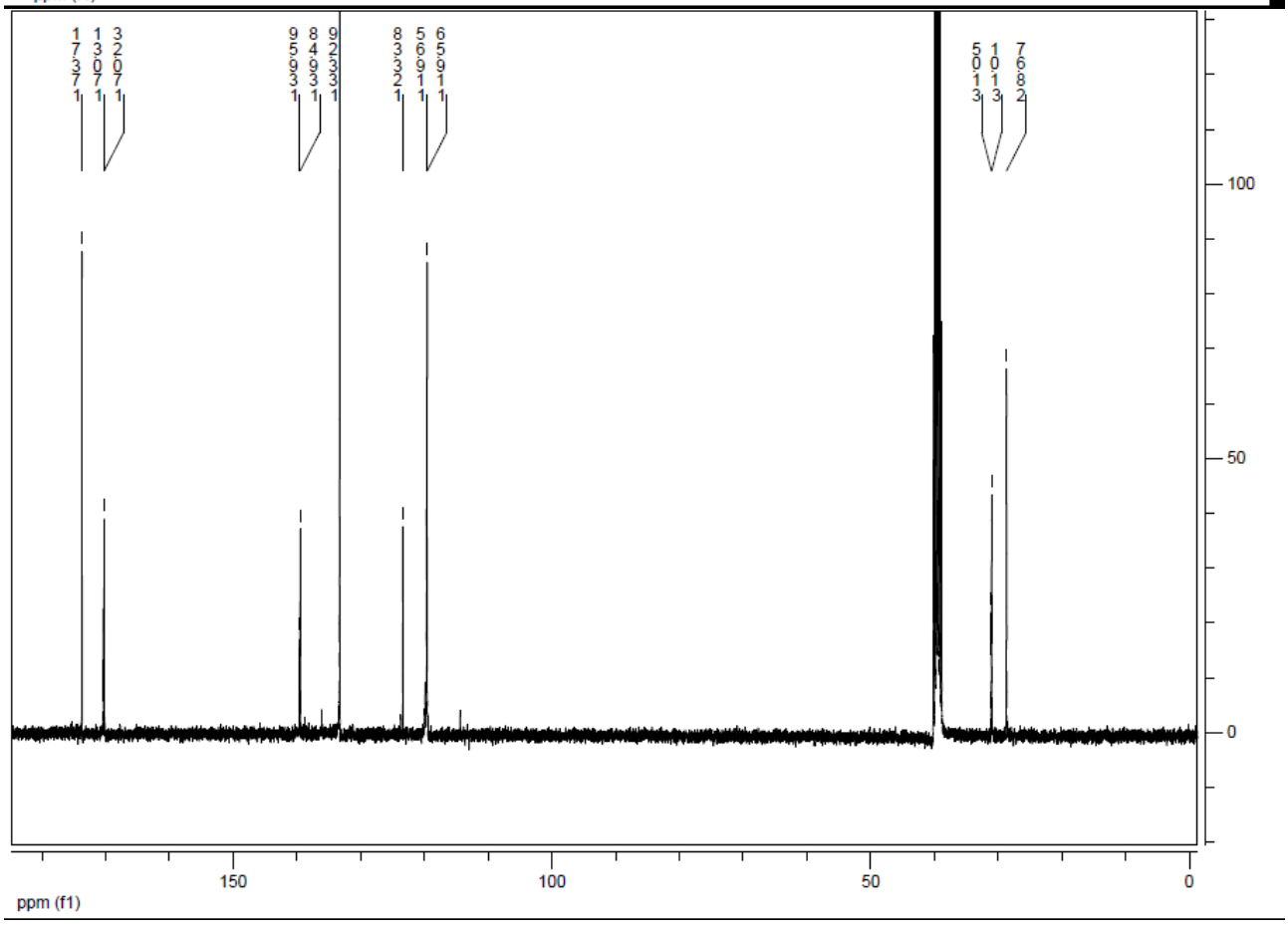


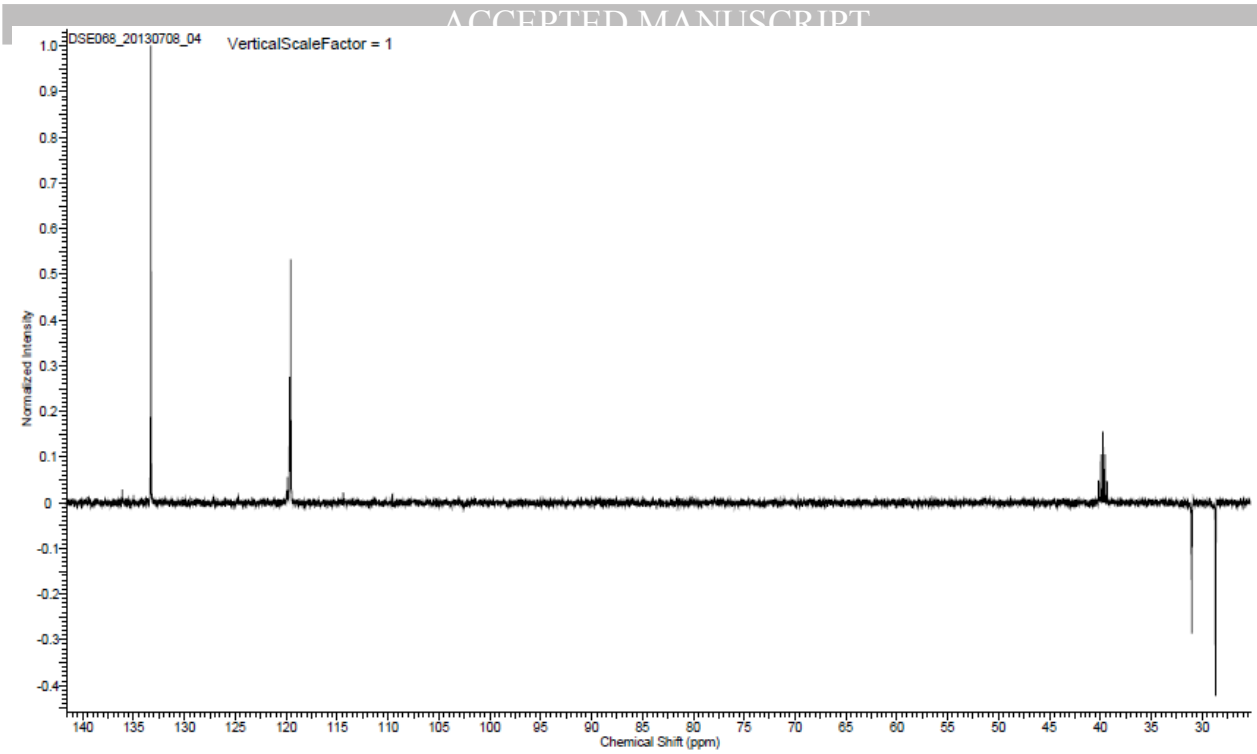


Compound 18

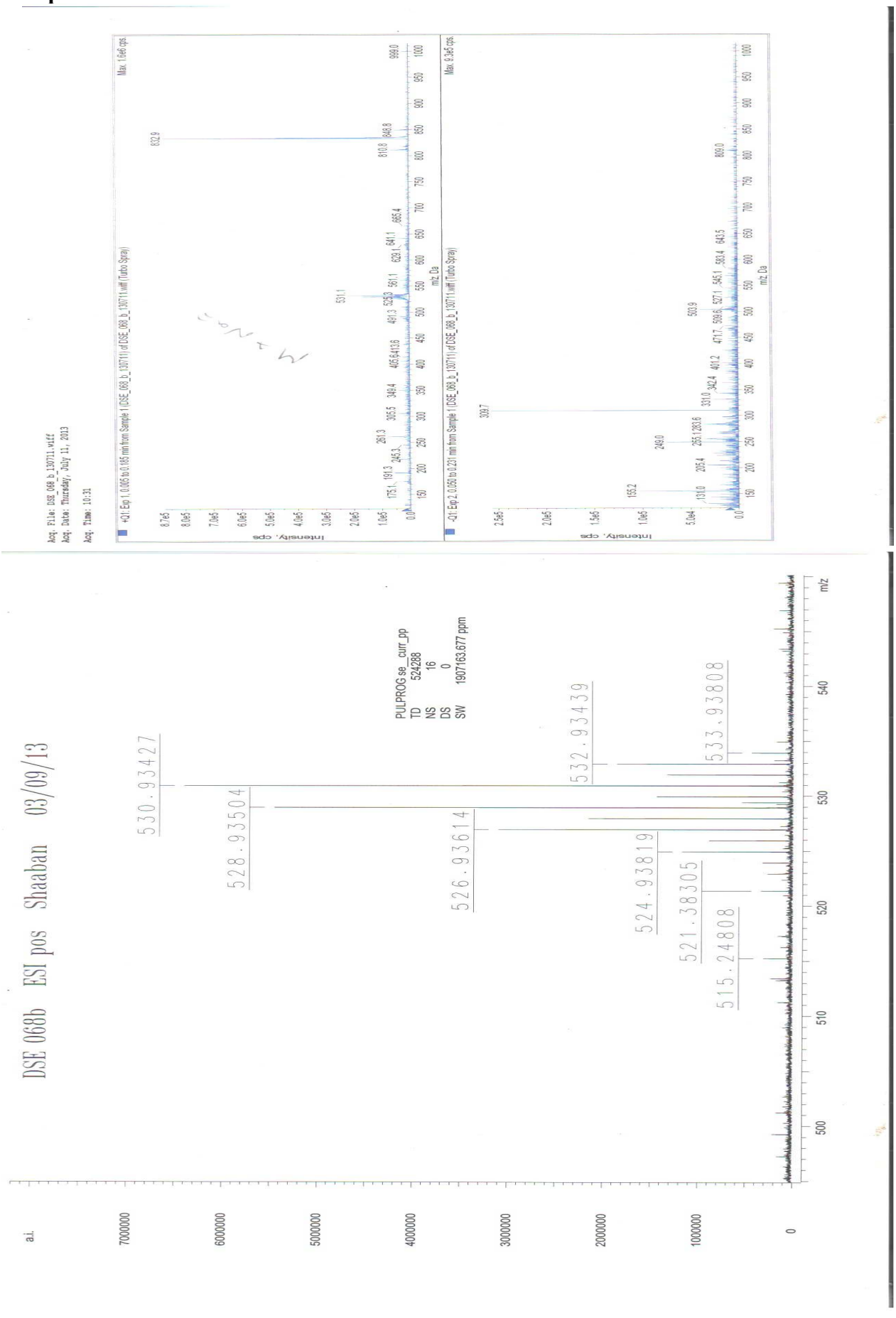

Acq. File: DSE_068_130708.wiff
Acq. Date: Monday, July 08, 2013
Acq. Time: 11:56
+Q1 Exp 1, 0.005 to 0.165 min from Sample 1 (DSE_068_130708) of DSE_068_130708.wiff (Turbo Spray)
Max. 3.4e6 cps.
Intensity, cps
m/z, Da
-Q1 Exp 2, 0.050 to 0.231 min from Sample 1 (DSE_068_130708) of DSE_068_130708.wiff (Turbo Spray)
Max. 2.2e6 cps.
PULPROG se_curr_pp
TD 524288
NS 16
DS 0
SW 19071163.677 ppm
566.95533
564.95565
562.95742
568.95512
569.95836
582.92808
560.95908
544.97367
524.96278
m/z

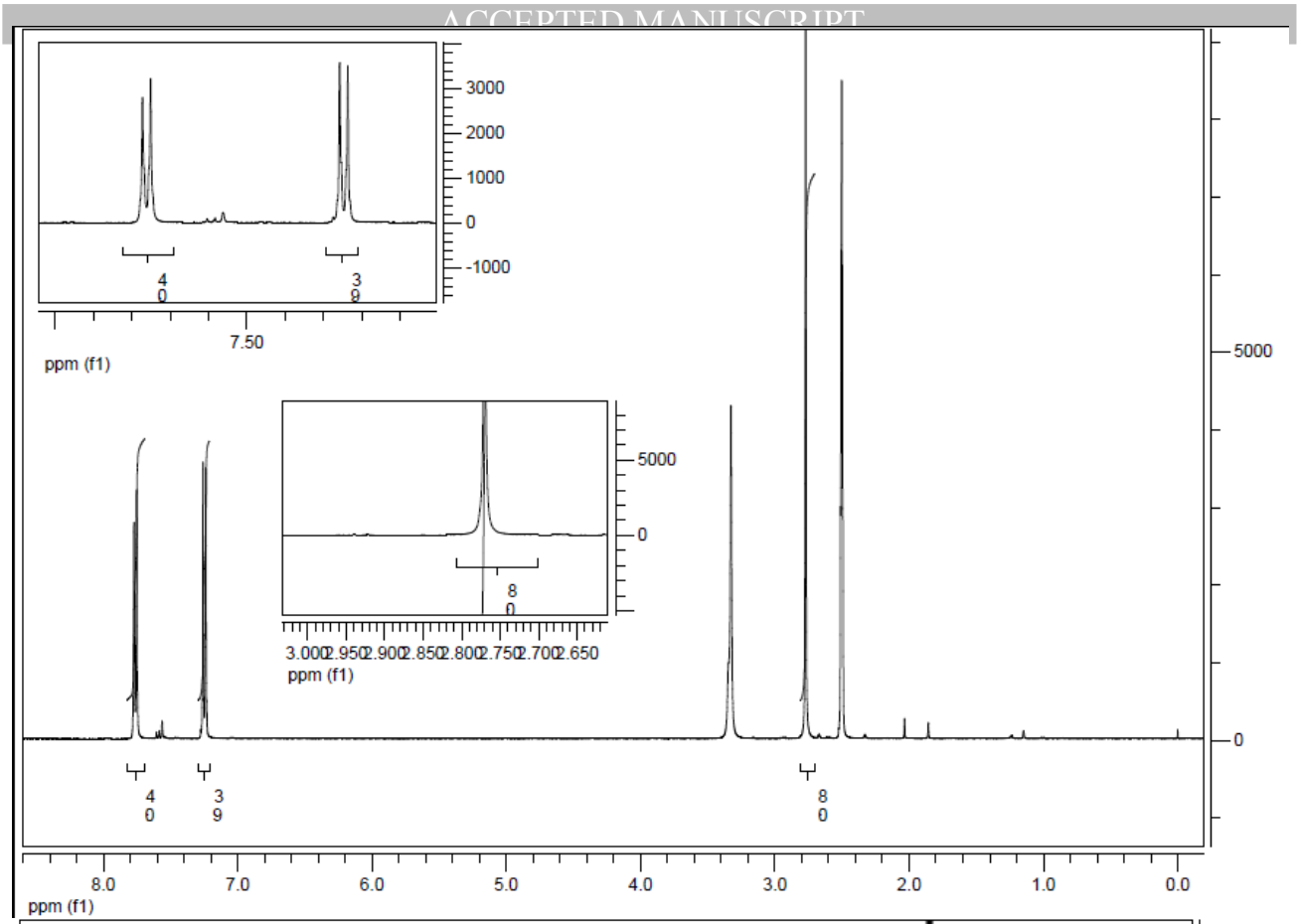


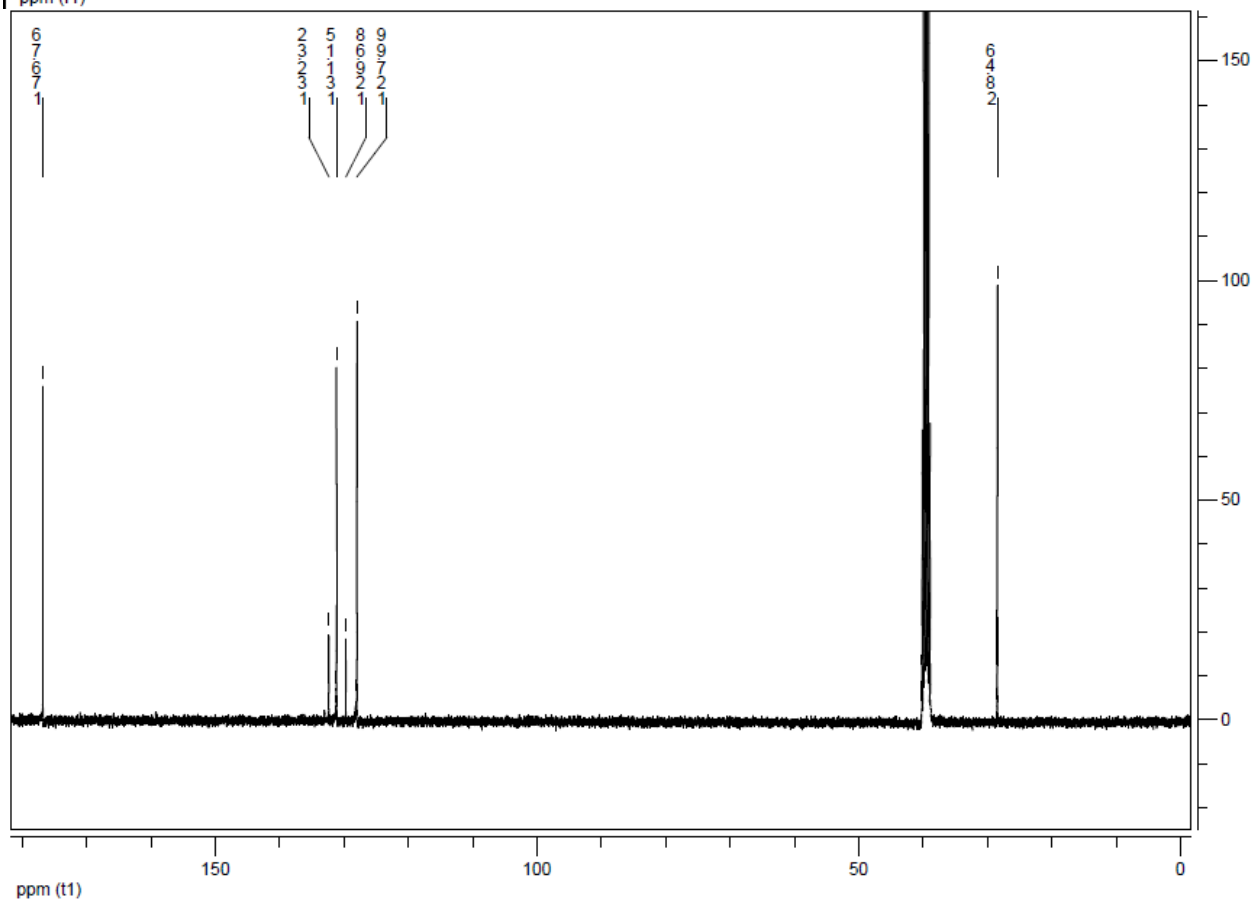


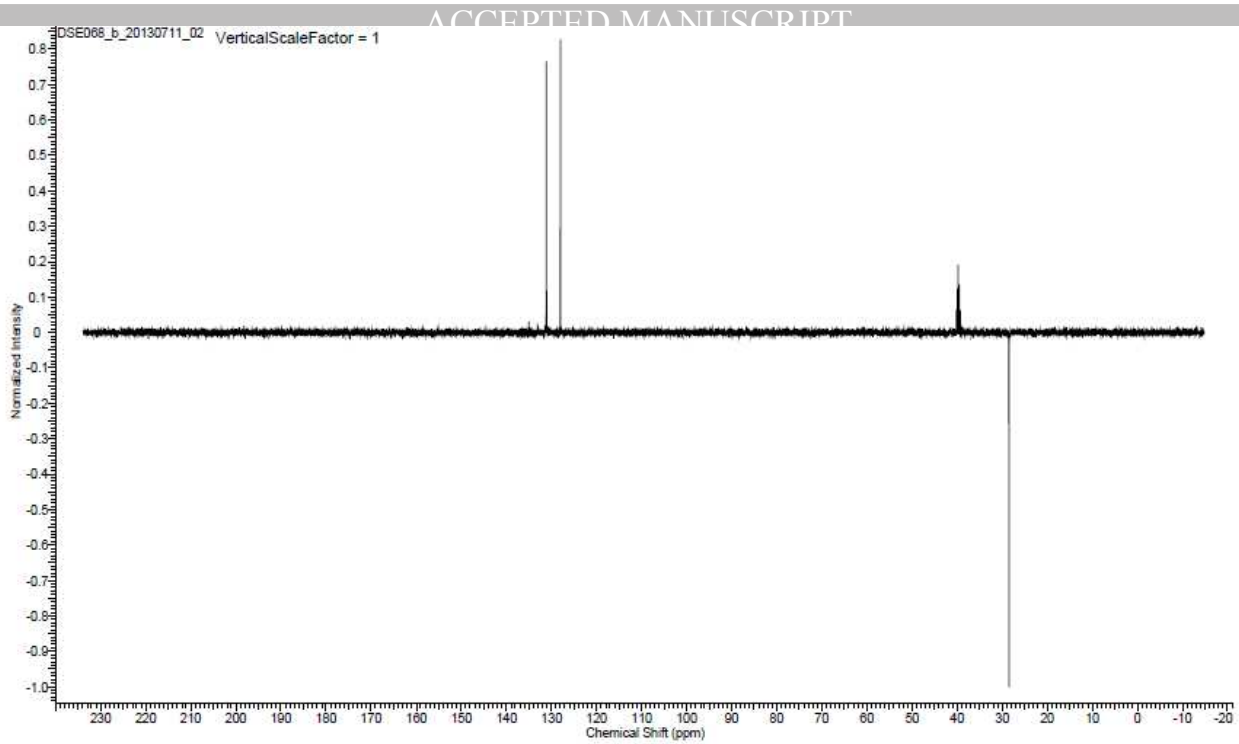


Compound 19

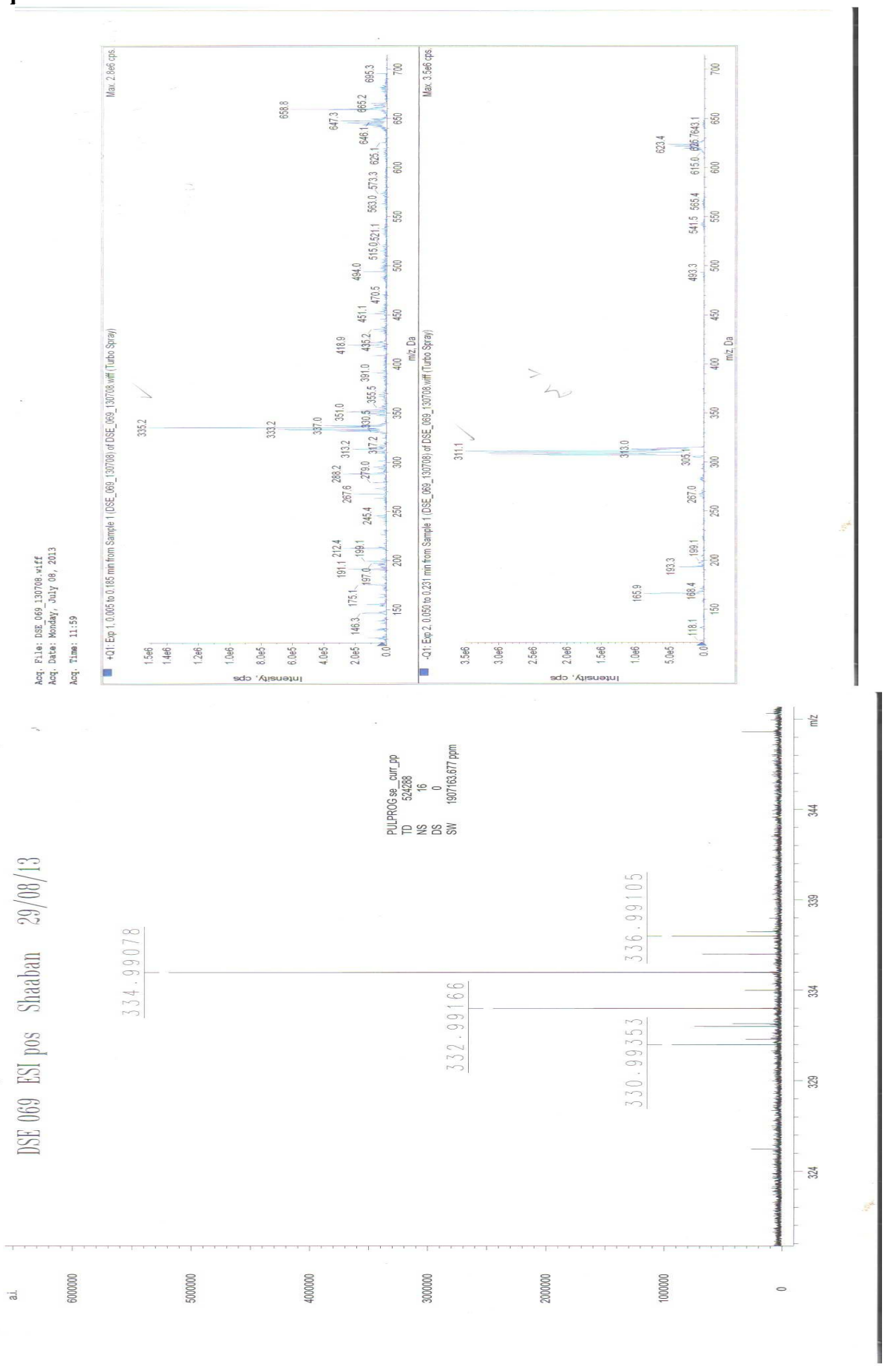

DSE 068b ESI pos Shaaban 03/09/13
530.93427
528.93504
526.93614
532.93439
533.93808
524.93819
521.38305
515.24808
PULPROG se_curr_pp
TD 524288
NS 16
DS 0
SW 1907163.677 ppm
a.i.
m/z
Acq. File: DSE_068_b_130711.wiff
Acq. Date: Thursday, July 11, 2013
Acq. Time: 10:31
Intensity, cps
mz, Da

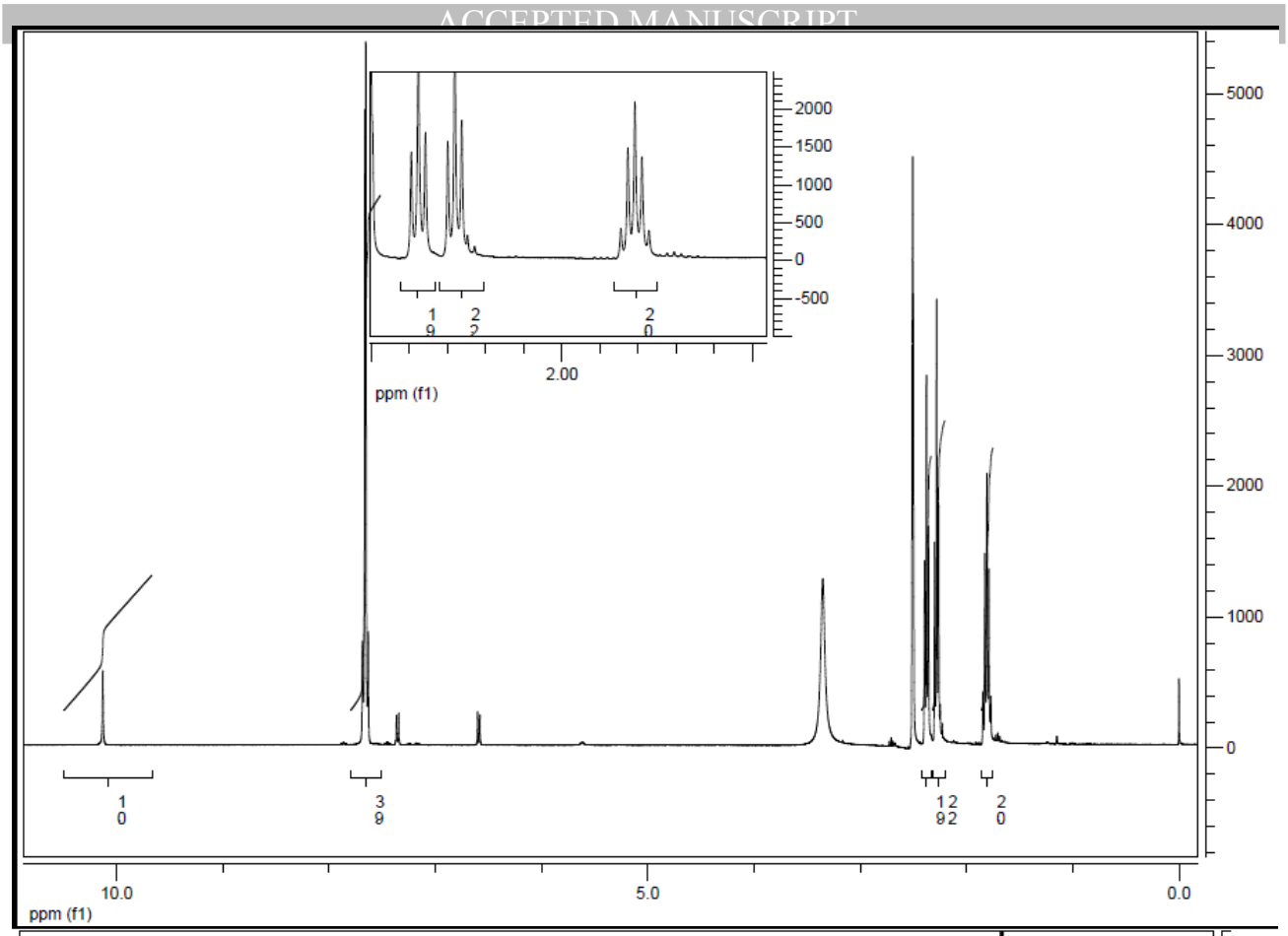


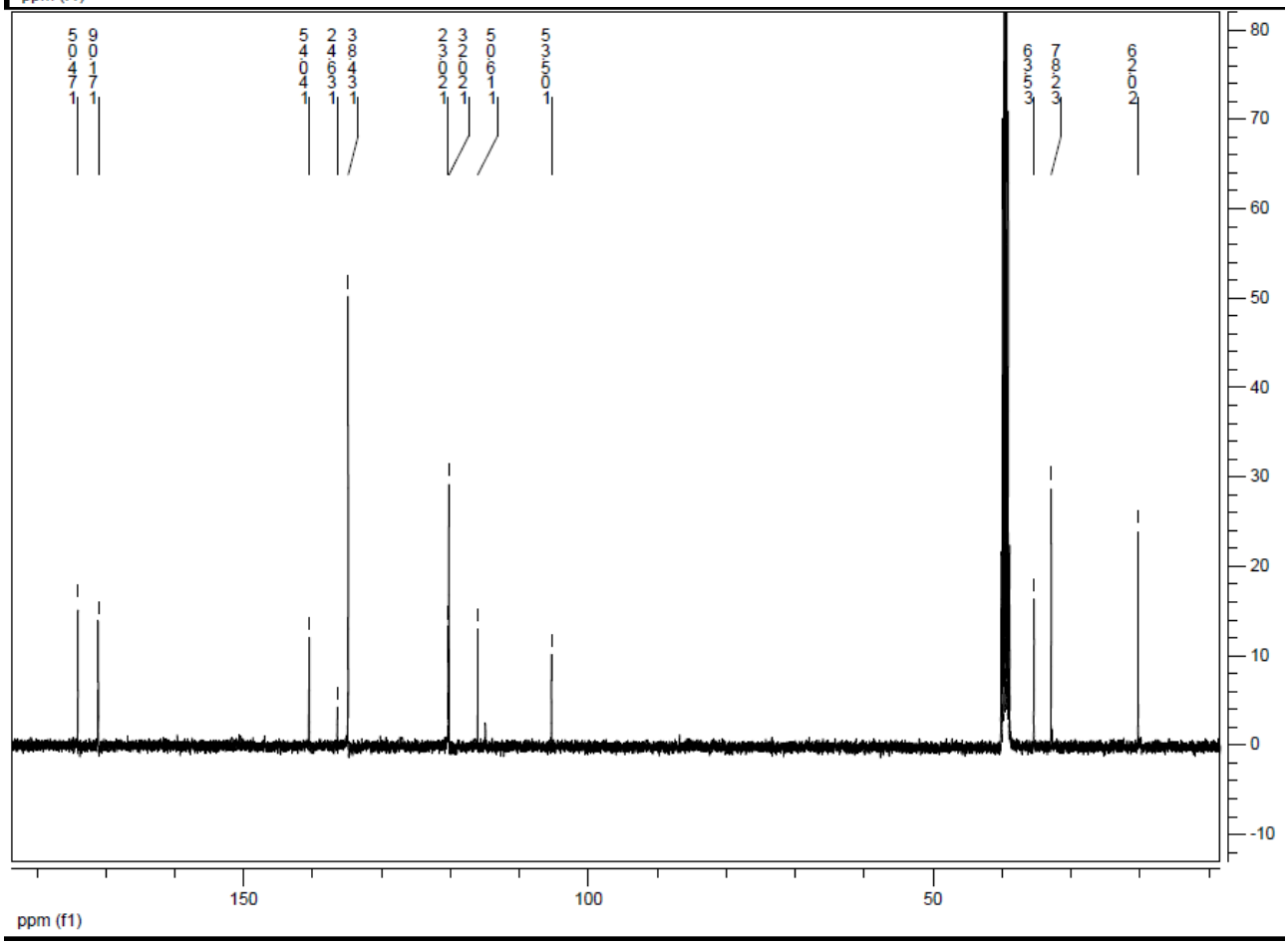


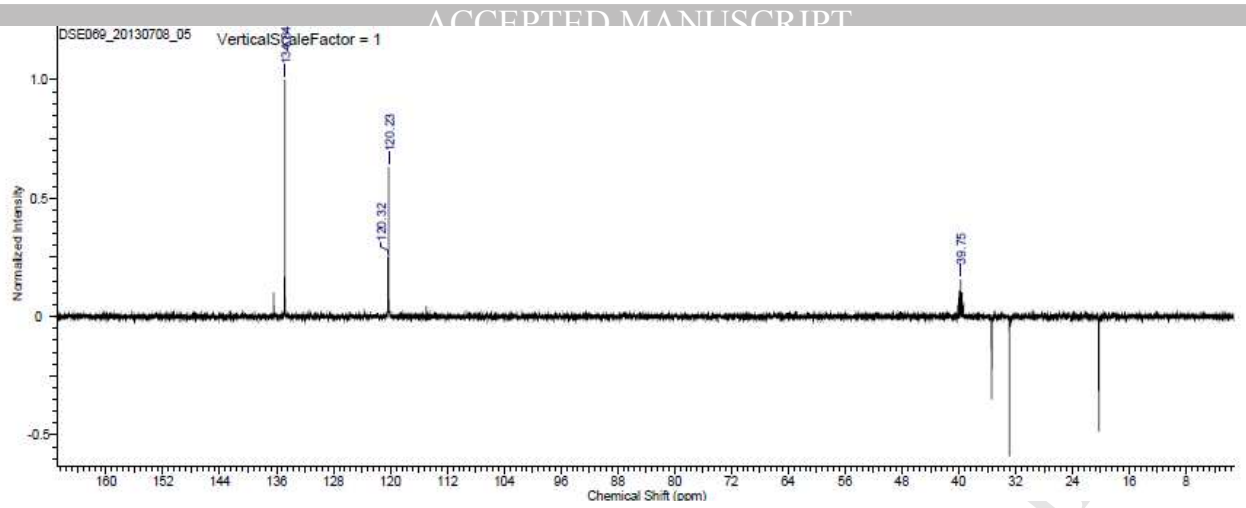


Compound 14

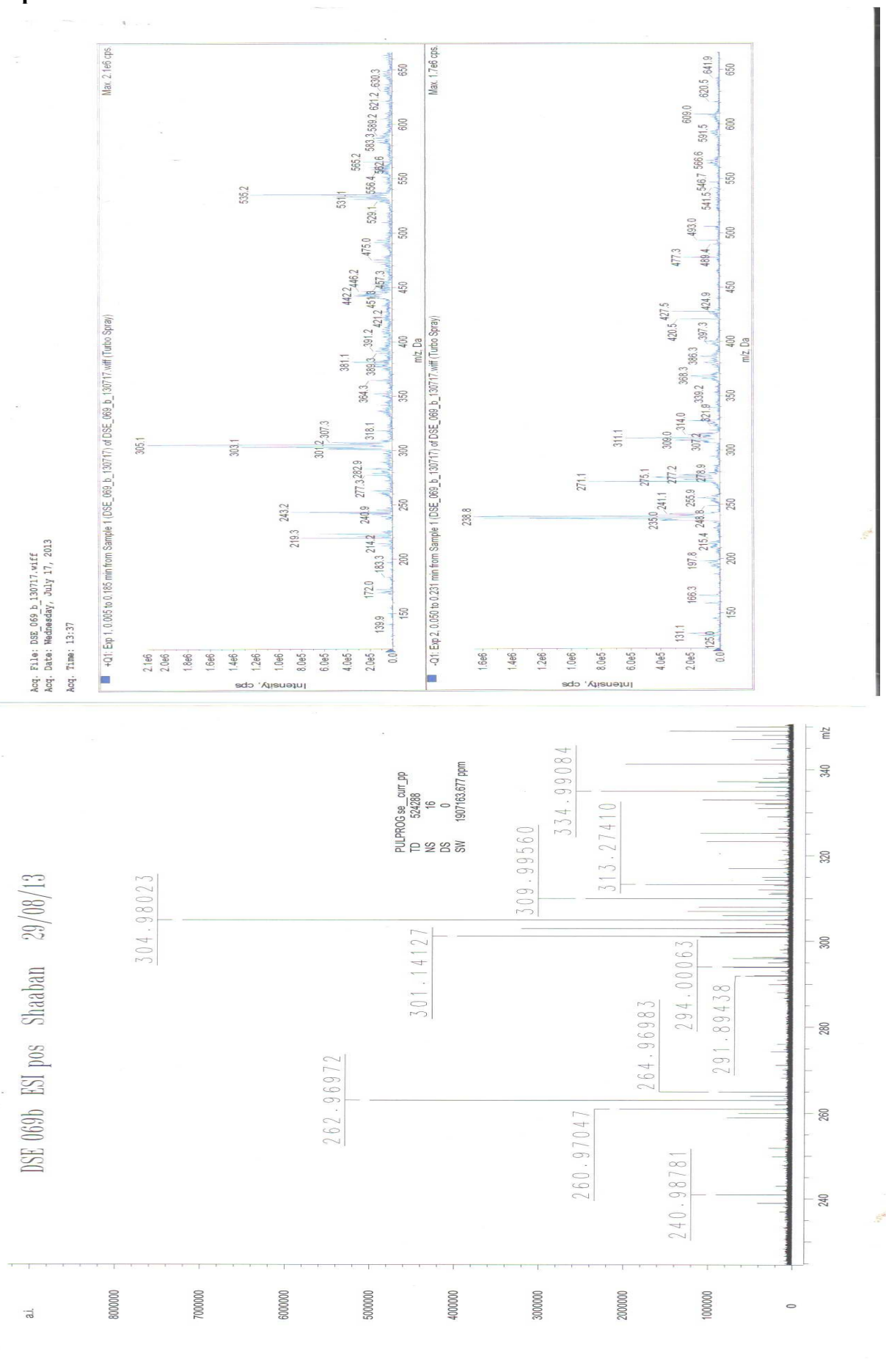

Acq. File: DSE_069_130708.wiff
Acq. Date: Monday, July 08, 2013
Acq. Time: 11:59
+Q1: Exp 1, 0.005 to 0.185 min from Sample 1 (DSE_069_130708) of DSE_069_130708.wiff (Turbo Spray)
Max. 2.6e6 cps.
Intensity, cps
m/z, Da
-Q1: Exp 2, 0.050 to 0.231 min from Sample 1 (DSE_069_130708) of DSE_069_130708.wiff (Turbo Spray)
Max. 3.5e6 cps.
DSE 069 ESI pos Shaaban 29/08/13
334.99078
332.99166
330.99353
336.99105
PULPROG se_curr_pp
TD 524288
NS 16
DS 0
SW 1907163.677 ppm
a.i.
m/z

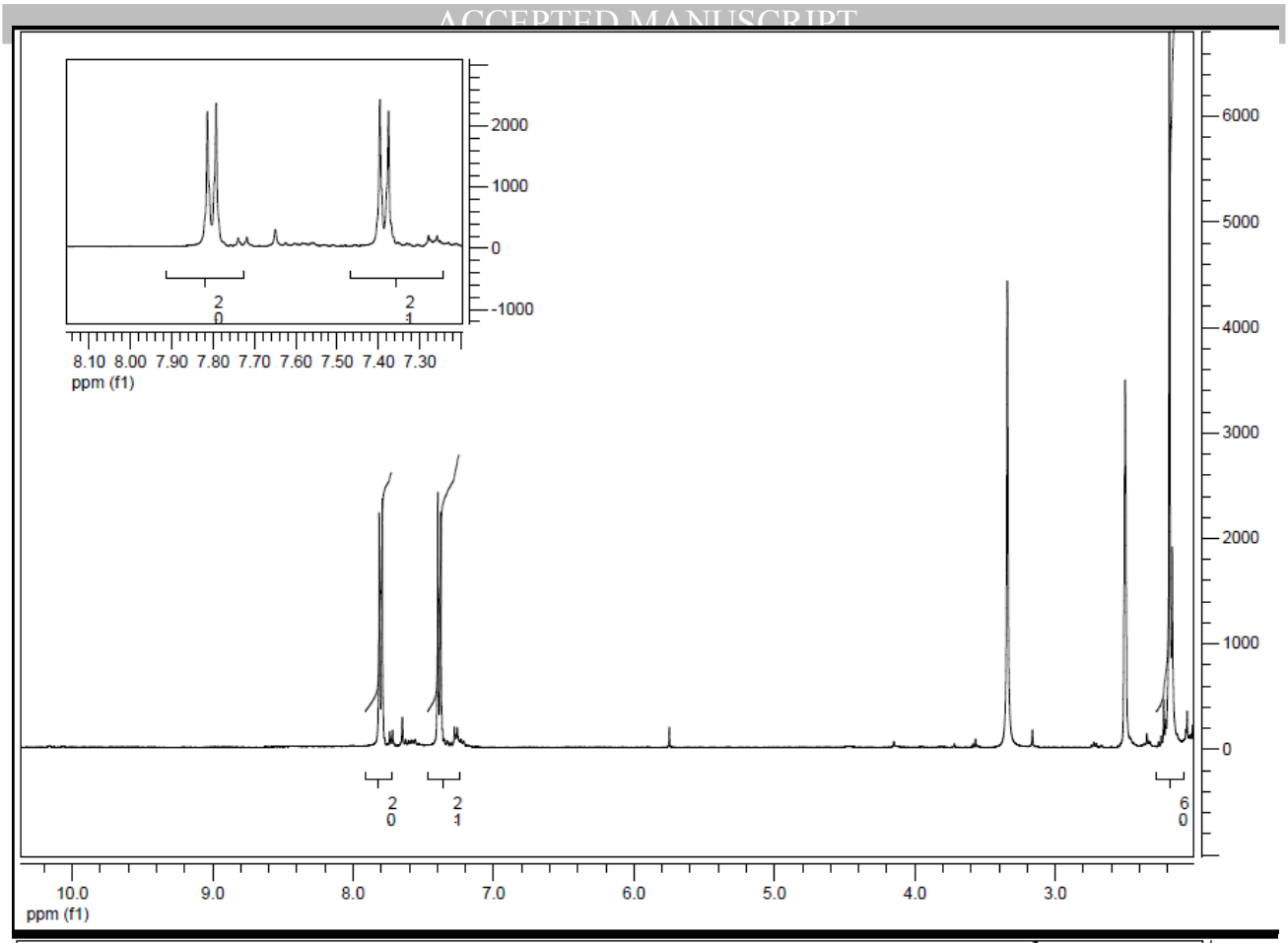


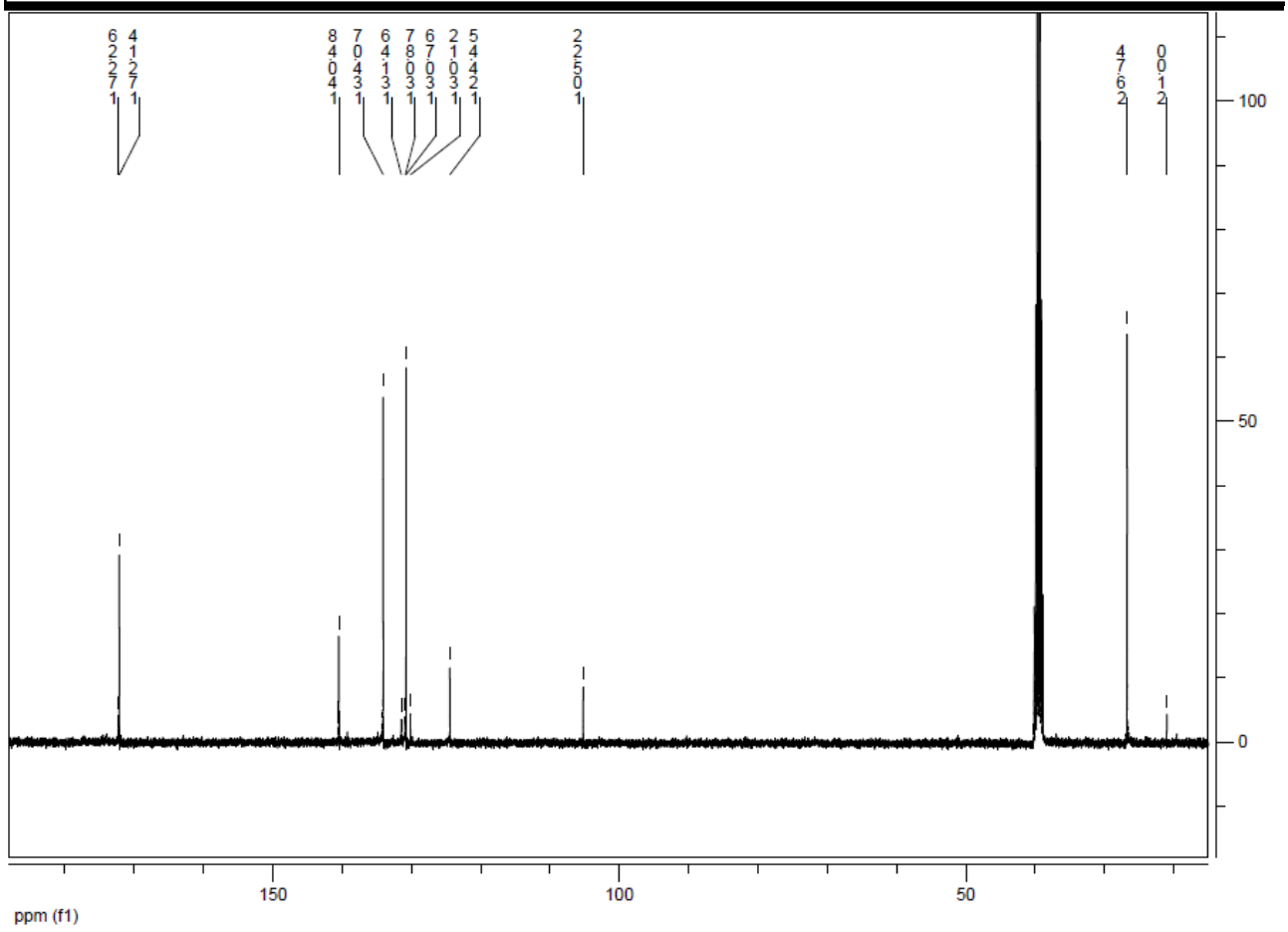


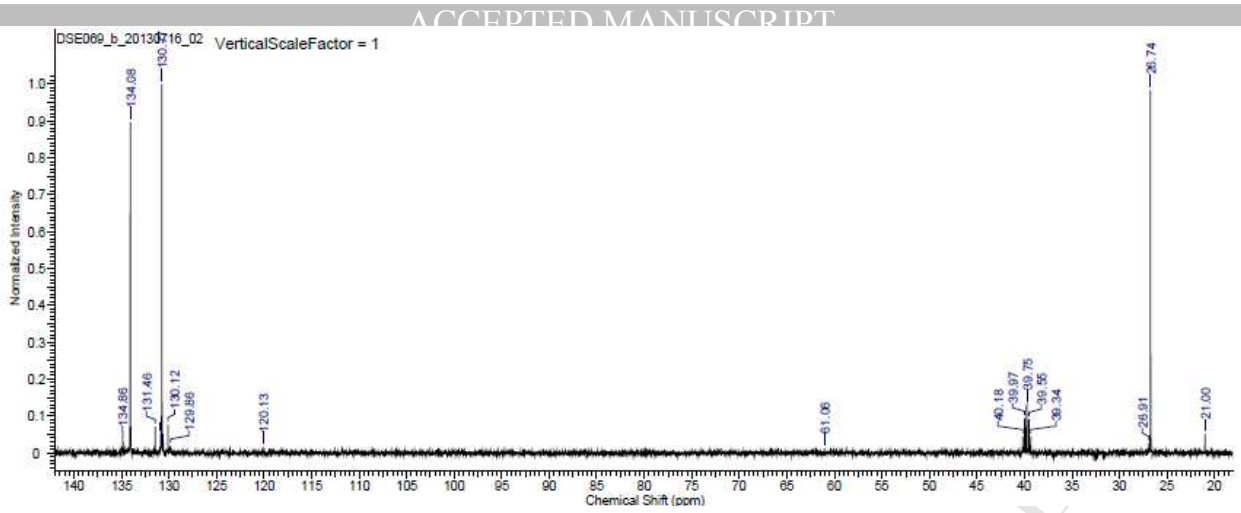


Compound 15

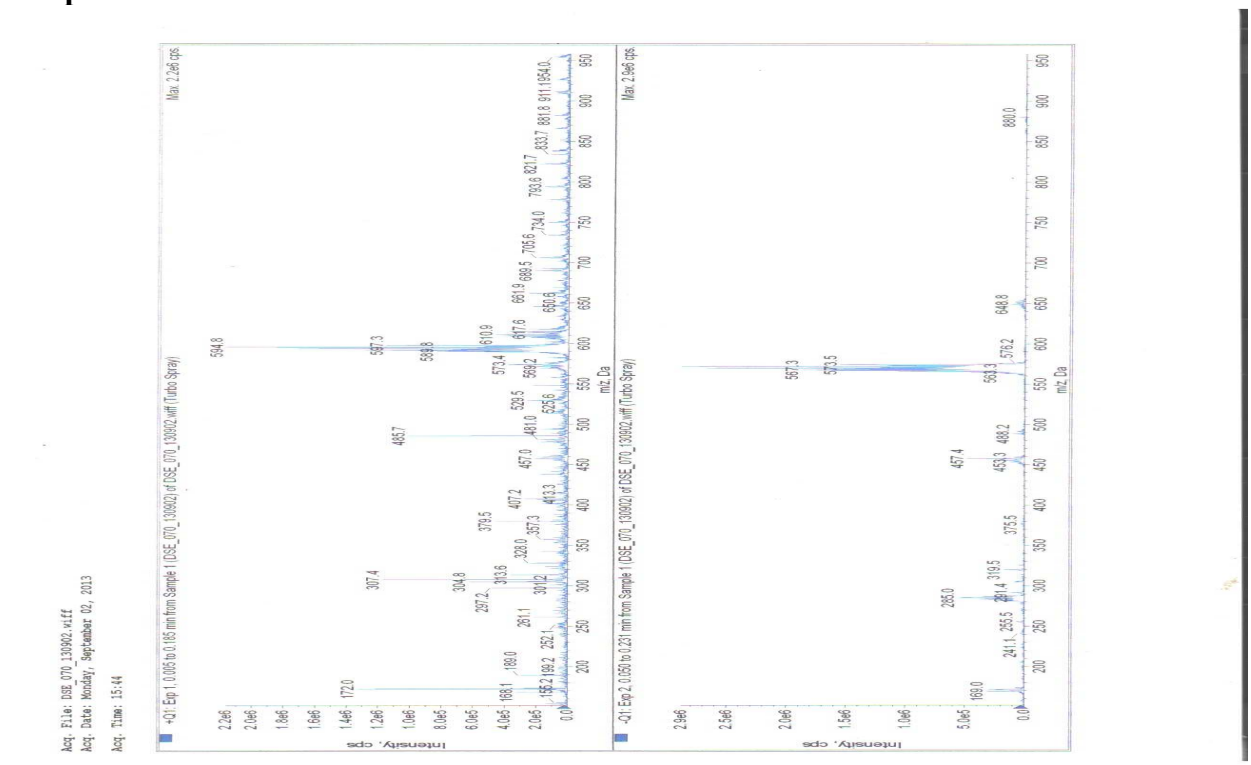

Acq. File: DSE_069_b_130717.wiff
Acq. Date: Wednesday, July 17, 2013
Acq. Time: 13:37
+Q1, Exp 1, 0.005 to 0.185 min from Sample 1 (DSE_069_b_130717) of DSE_069_b_130717.wiff (Turbo Spray)
Max. 2.1e6 cps.
Intensity, cps
m/z, Da
-Q1, Exp 2, 0.050 to 0.231 min from Sample 1 (DSE_069_b_130717) of DSE_069_b_130717.wiff (Turbo Spray)
Max. 1.7e6 cps.
Intensity, cps
m/z, Da
DSE 069b ESI pos Shaaban 29/08/13
PULPROG se_curr_pp
TD 524288
NS 16
DS 0
SW 1907163.677 ppm
304.98023
301.14127
262.96972
309.99560
334.99084
313.27410
260.97047
264.96983
294.00063
291.89438
240.98781
a.i.
m/z

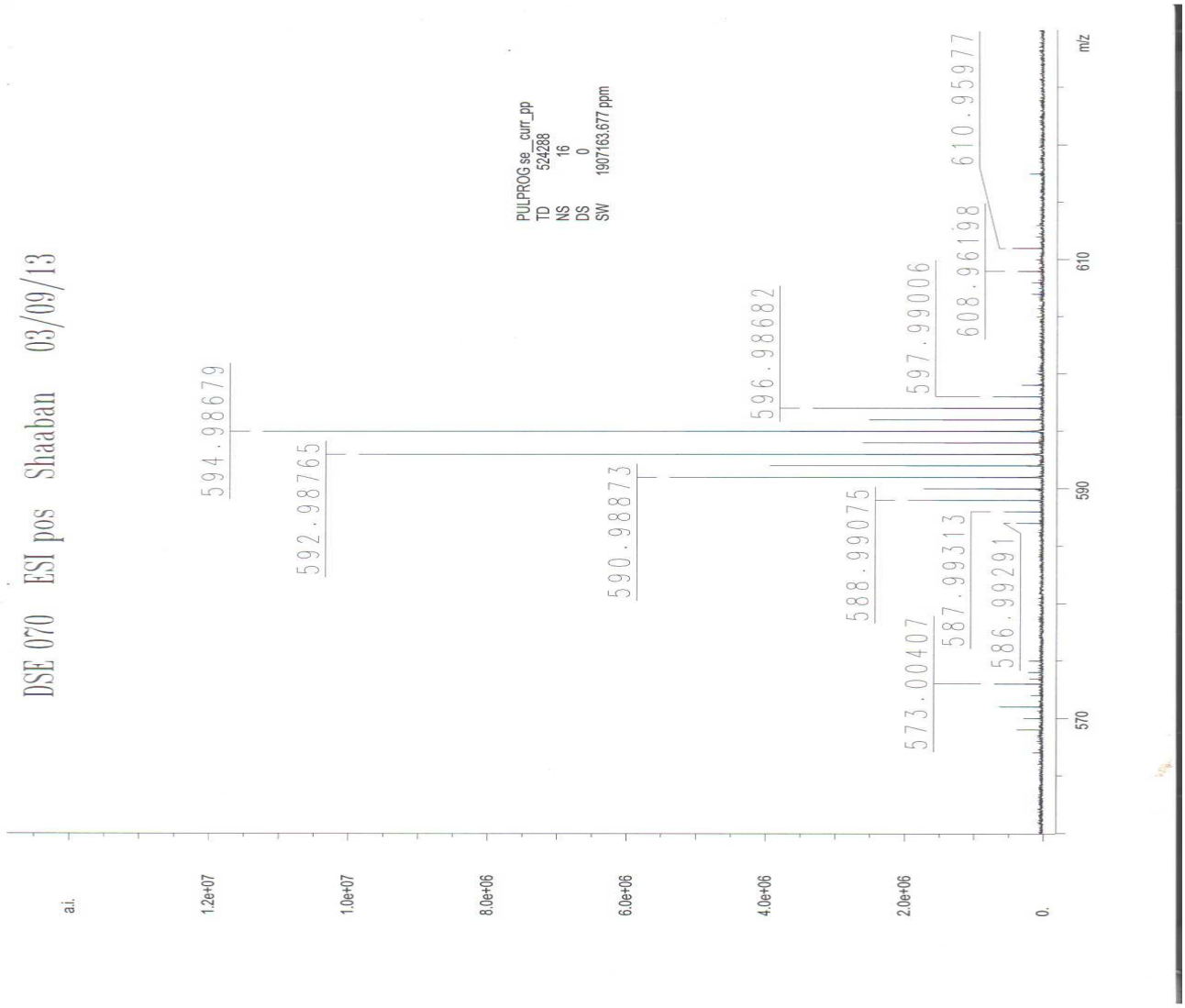


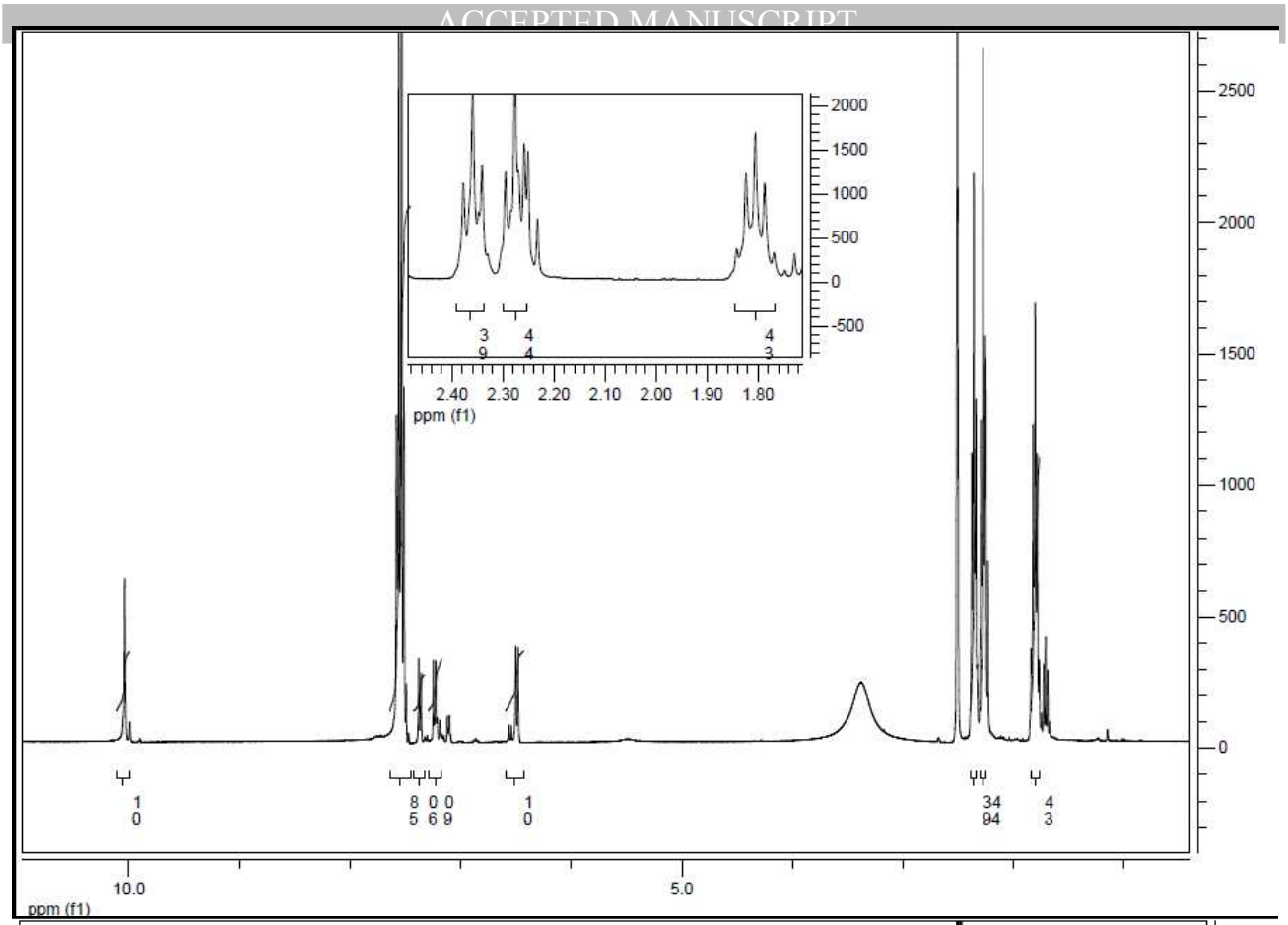


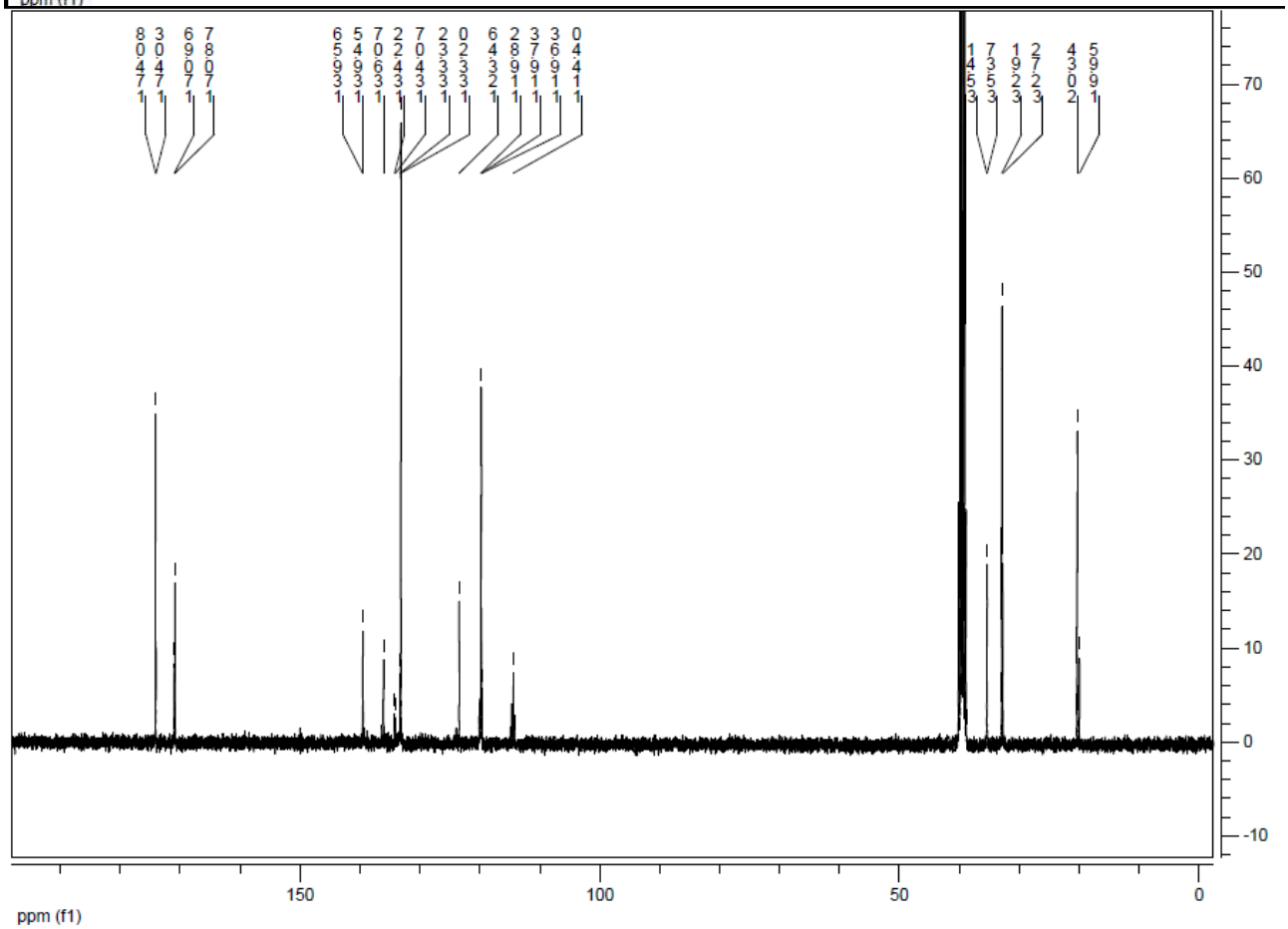


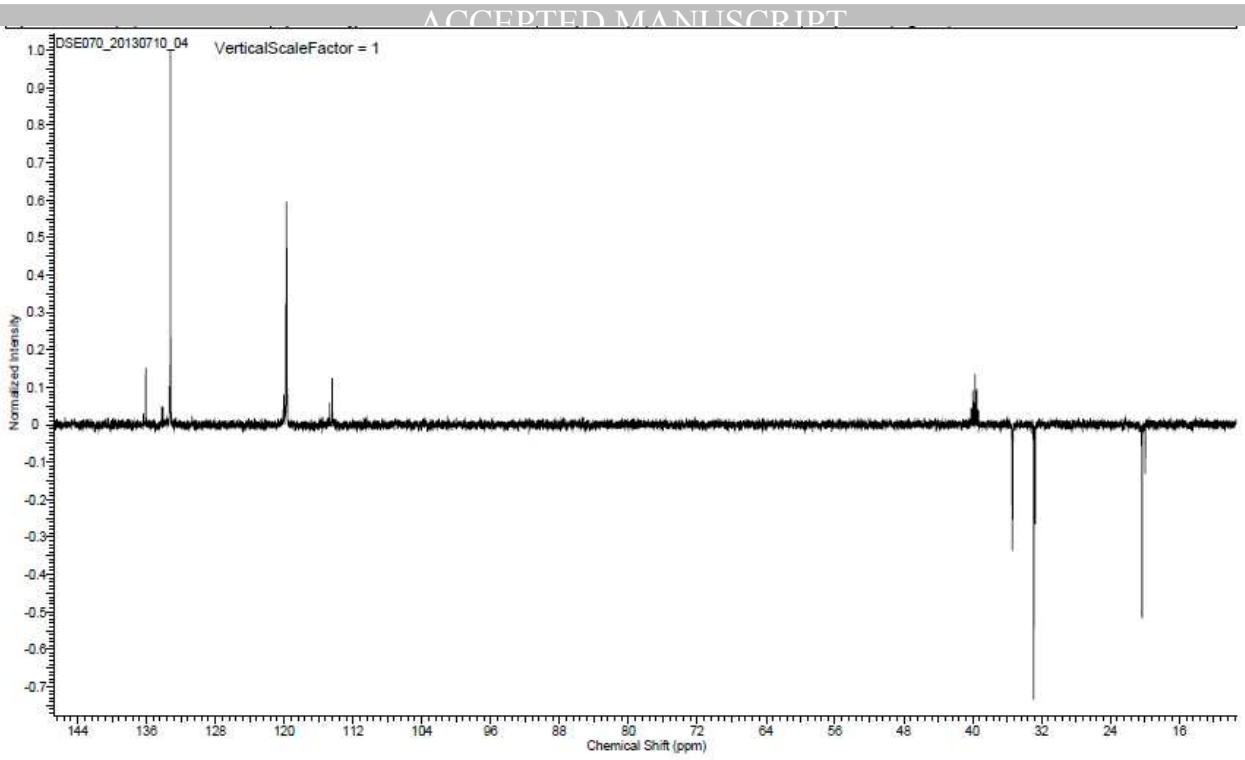
Compound 20


Acq. File: DSE_070_130902.wiff
Acq. Date: Monday, September 02, 2013
Acq. Time: 15:44
Intensity, cps
m/z, Da

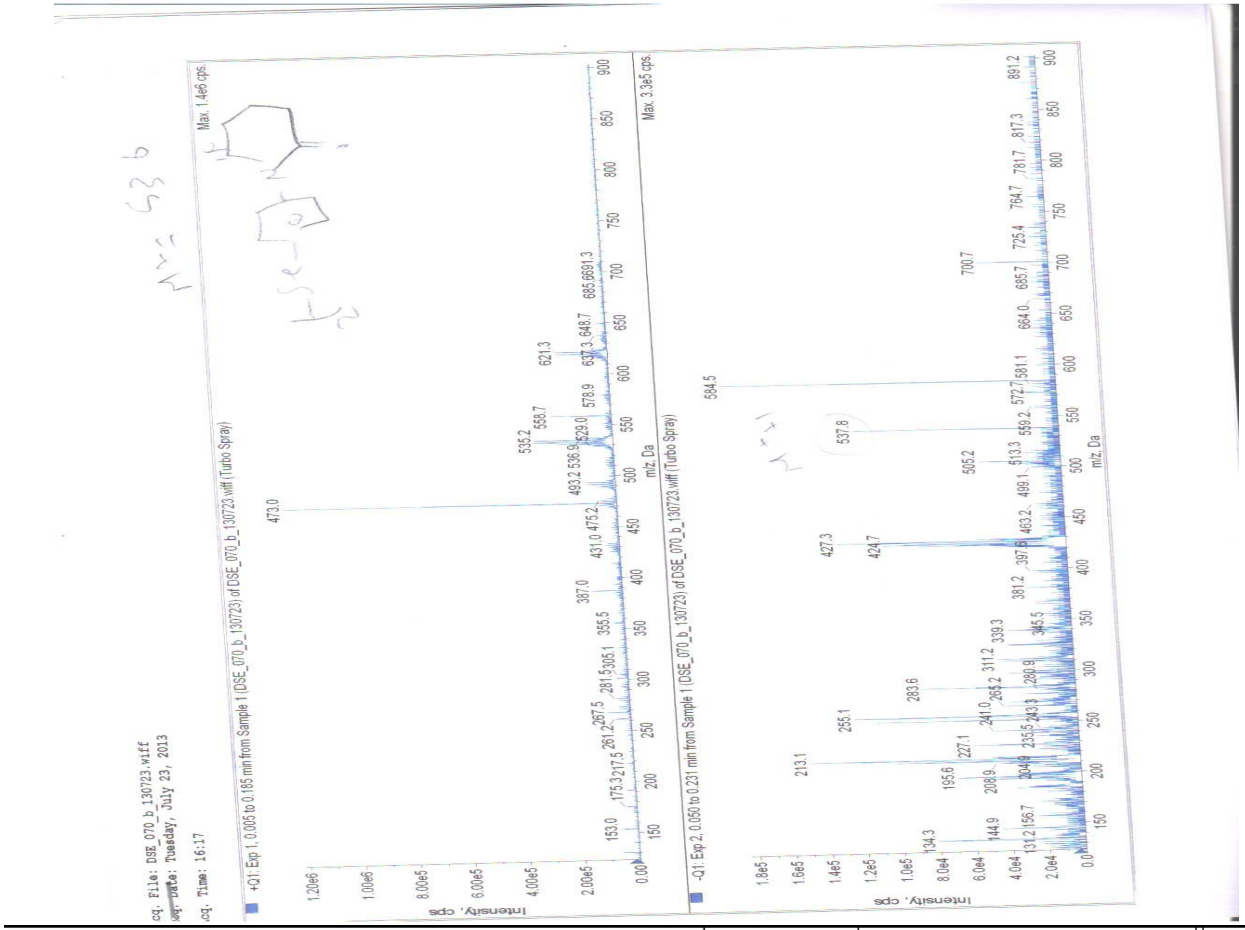

DSE 070 ESI pos Shaaban 03/09/13
PULPROG se_curr_pp
TD 524288
NS 16
DS 0
SW 1907163.677 ppm
a.i.
m/z
594.98679
592.98765
590.98873
596.98682
597.99006
608.96198
610.95977
573.00407
588.99075
587.99313
586.99291

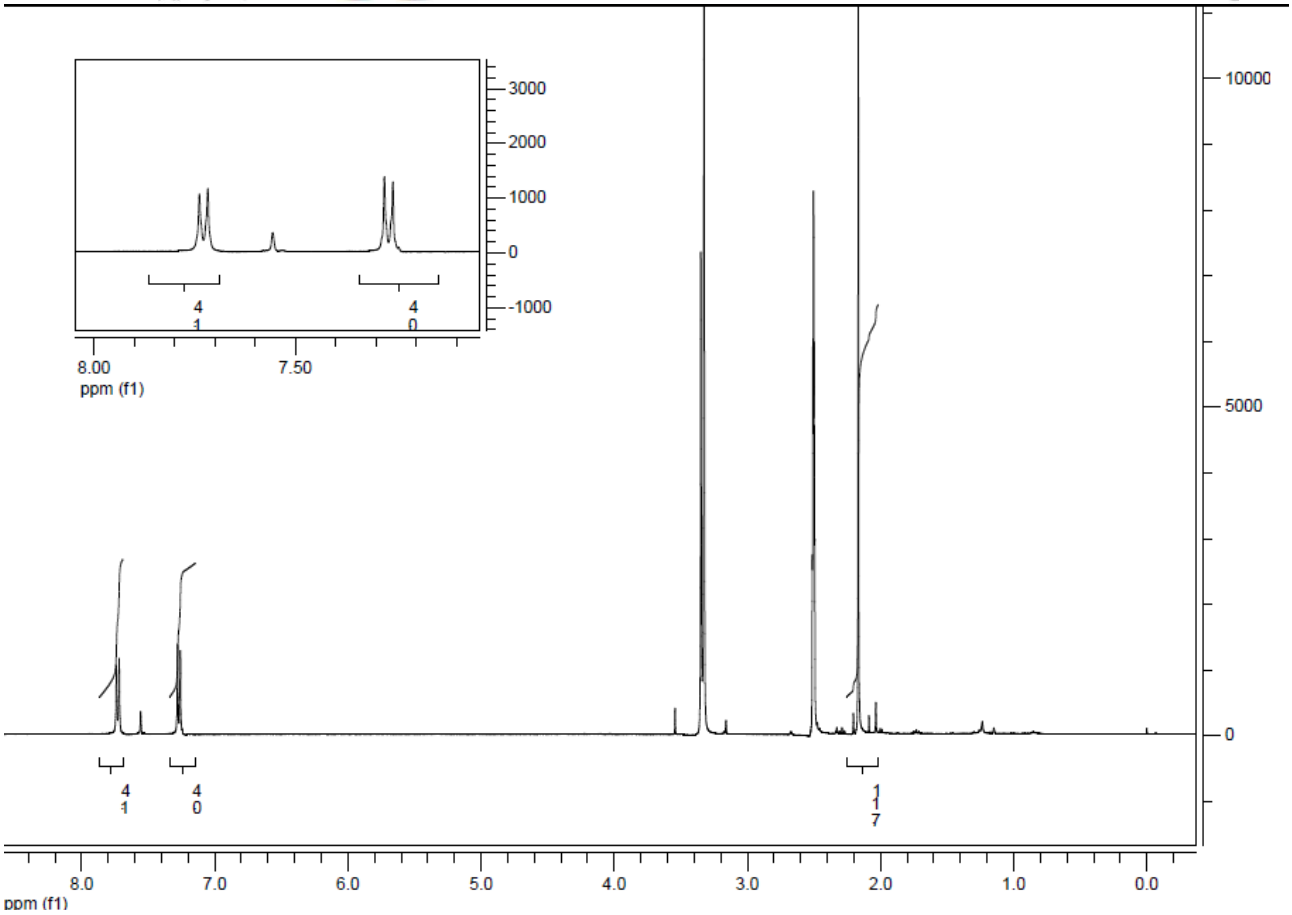


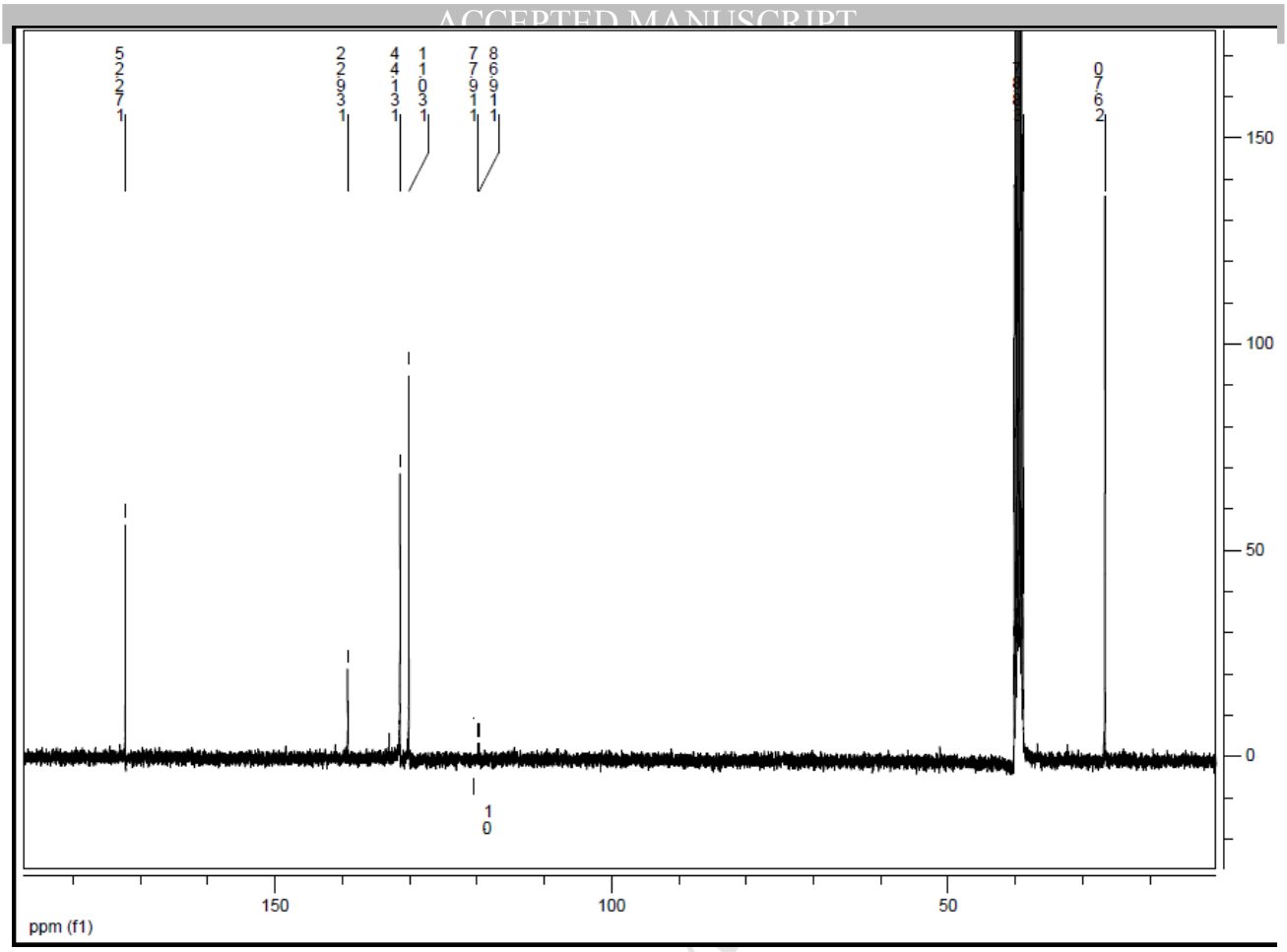




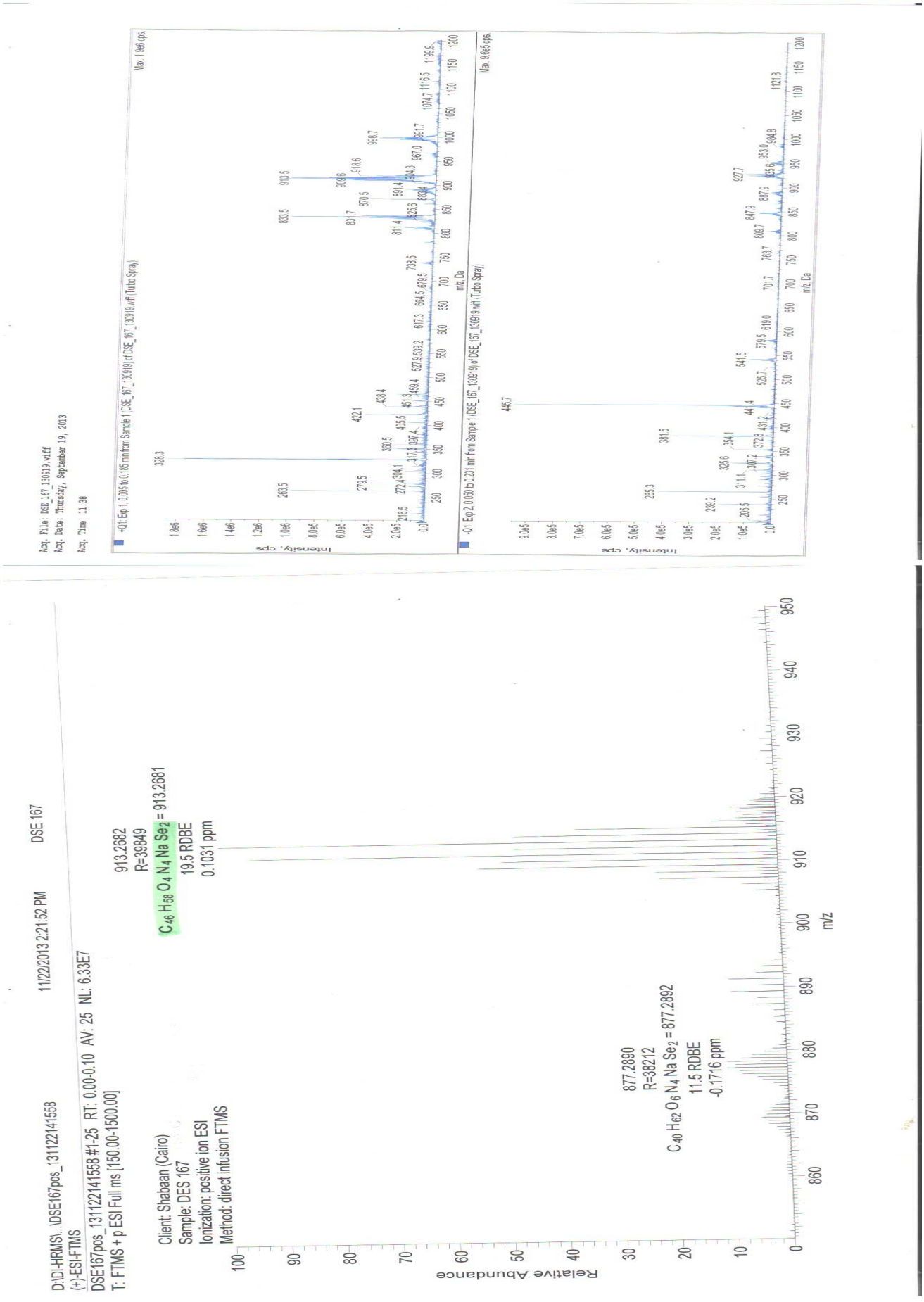
Compound 21

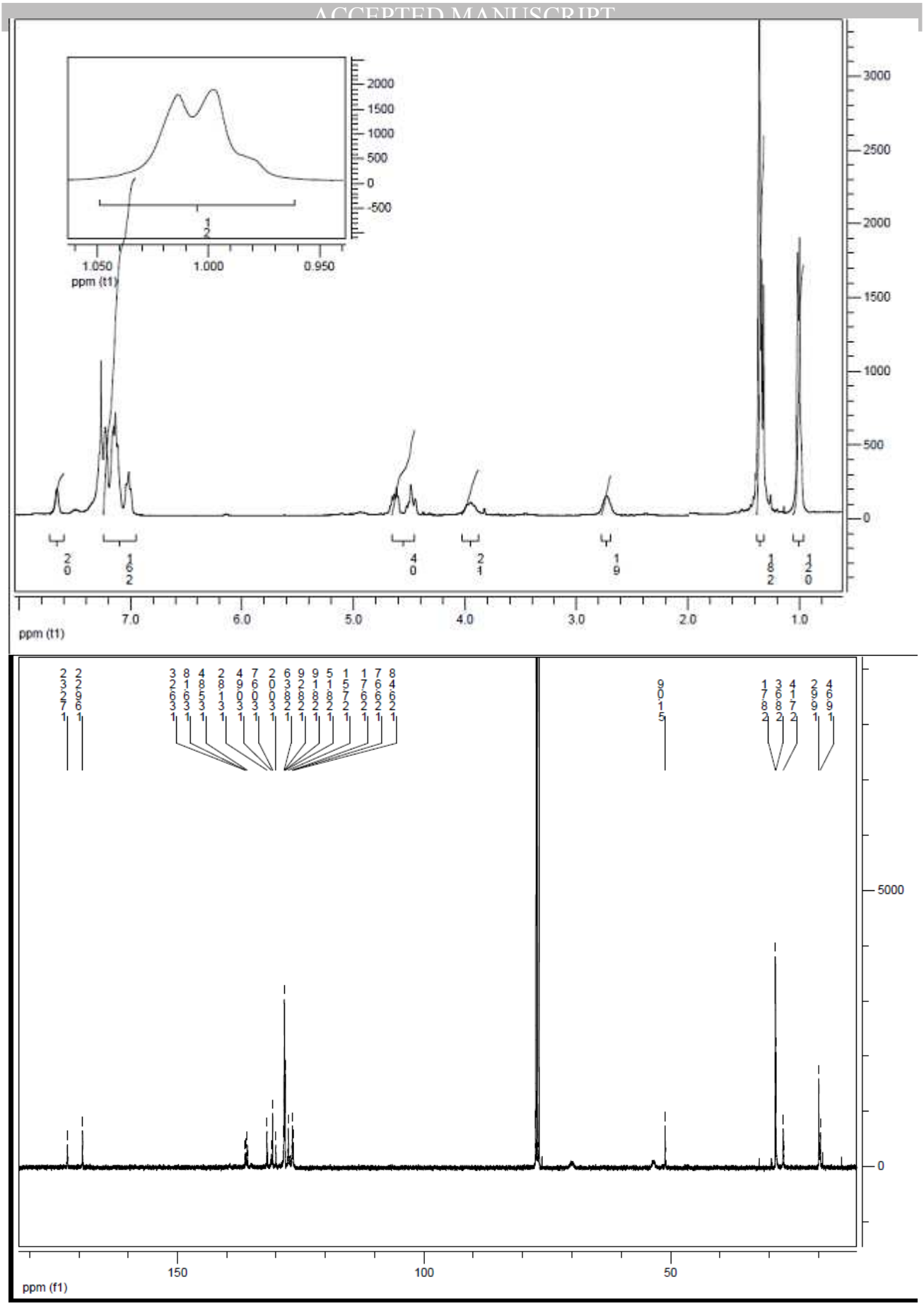

Max. 1.4e6 cps.
Max. 3.3e5 cps.
+Q1: Exp 1, 0.005 to 0.165 min from Sample 1 (DSE_070_b_130723) of DSE_070_b_130723.wiff (Turbo Spray)
-Q1: Exp 2, 0.050 to 0.231 min from Sample 1 (DSE_070_b_130723) of DSE_070_b_130723.wiff (Turbo Spray)
Intensity, cps
m/z, Da
cq: File: DSE_070_b_130723.wiff
cq: Date: Tuesday, July 23, 2013
cq: Time: 16:17


4
1
4
0
1
1
7
8.00
7.50
ppm (f1)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
10000
5000
0
3000
2000
1000
-1000

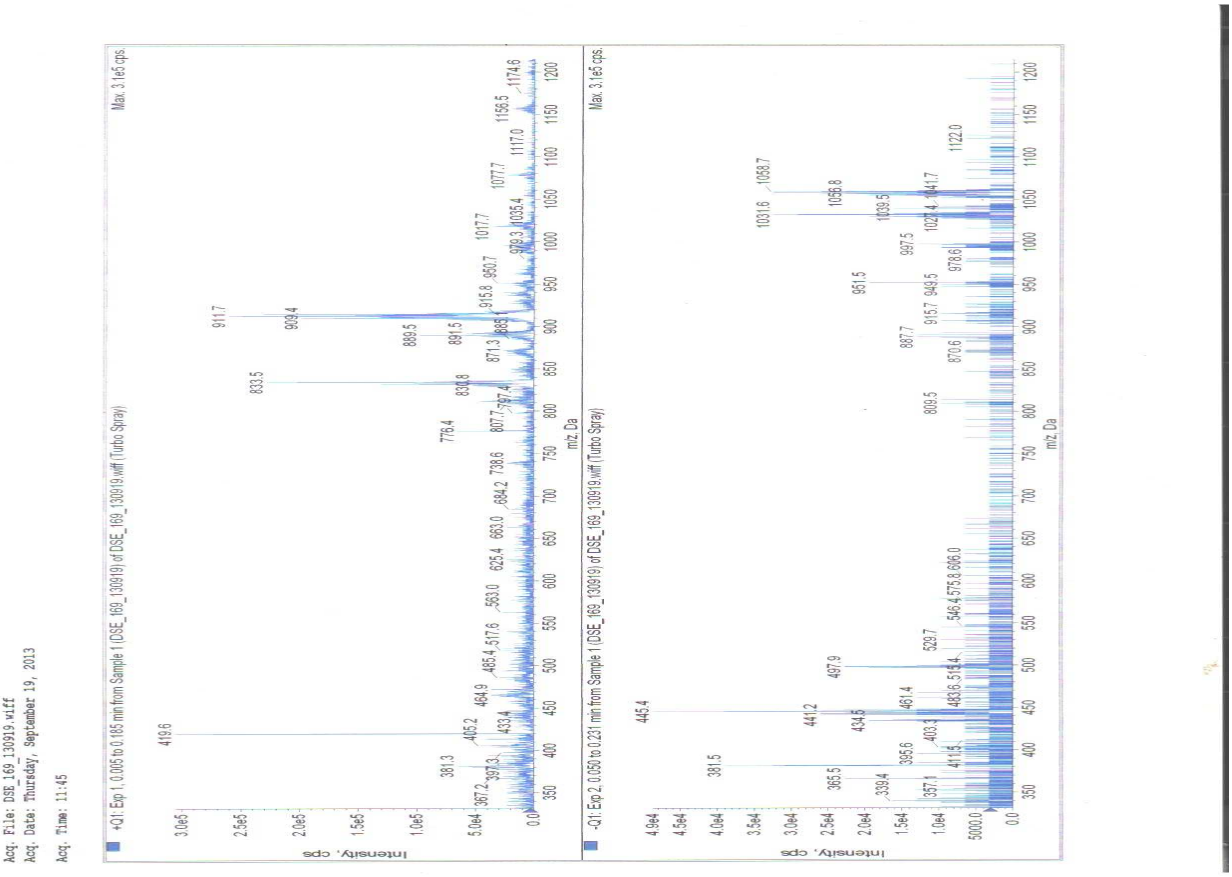


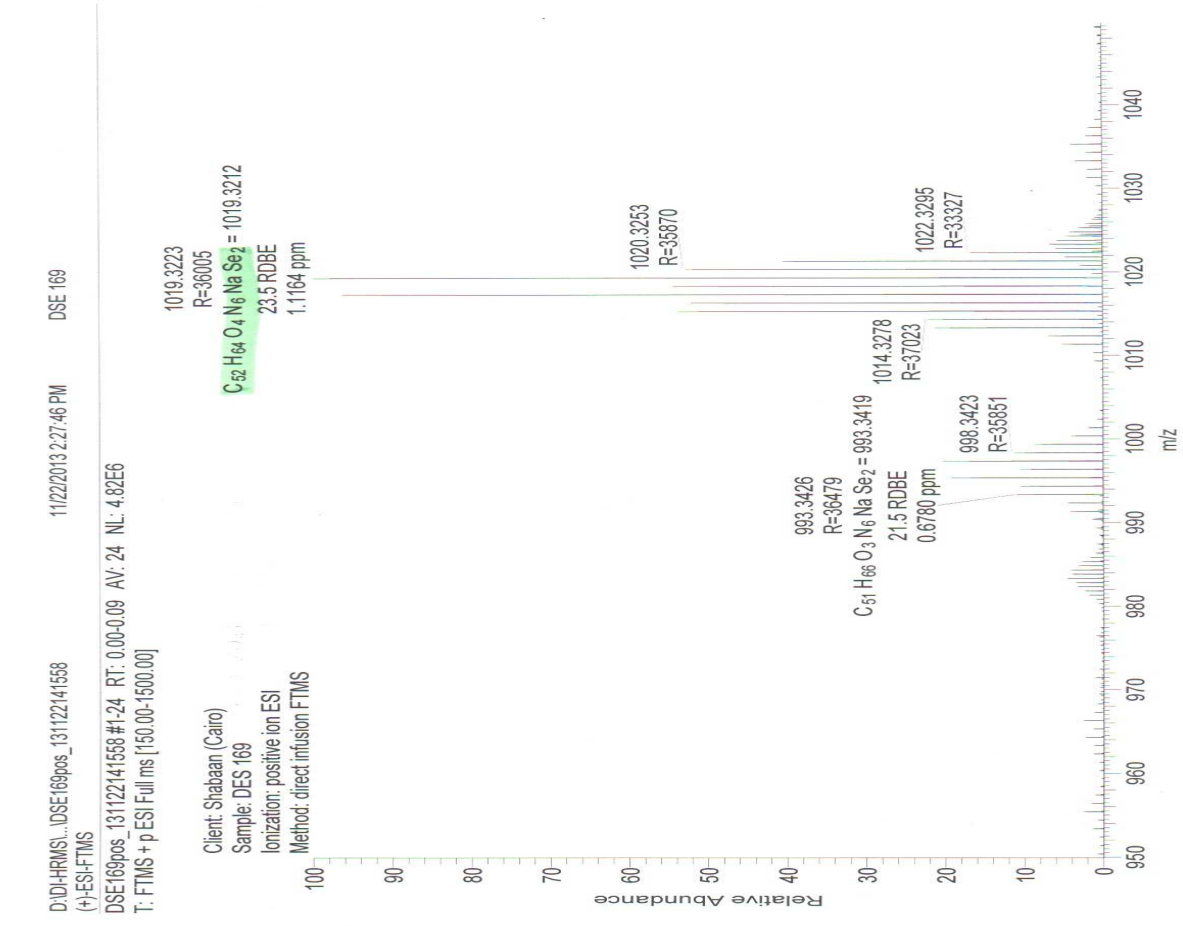
Compound 9

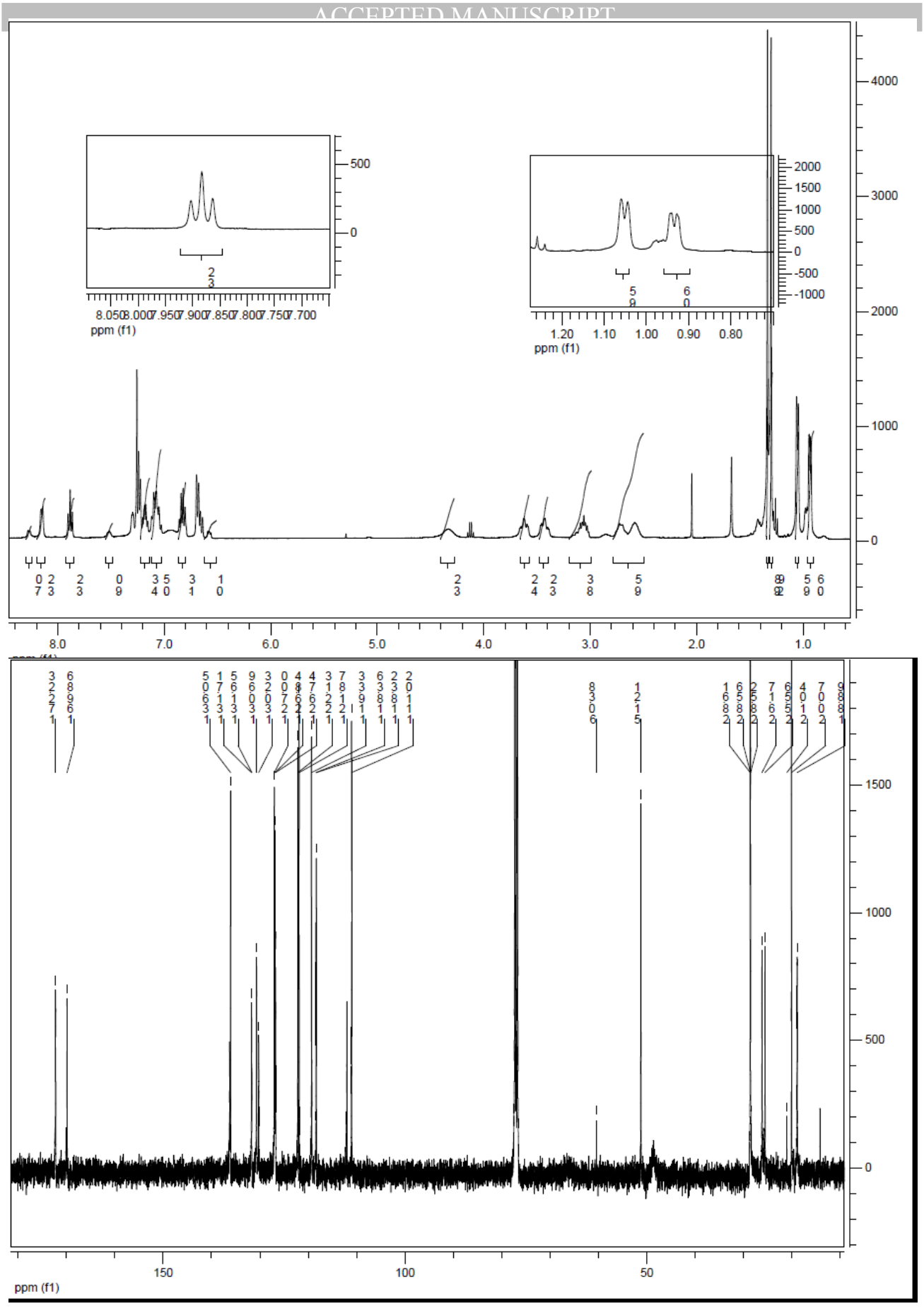

Acq. File: DSE_167_130919.wiff
Acq. Date: Thursday, September 19, 2013
Acq. Time: 11:30
+Q1: Exp 1, 0.005 to 0.165 min from Sample 1 (DSE_167_130919) of DSE_167_130919.wiff (Turbo Spray)
-Q1: Exp 2, 0.050 to 0.231 min from Sample 1 (DSE_167_130919) of DSE_167_130919.wiff (Turbo Spray)
Intensity, cps
m/z, Da
D:\DI-HRMS\...\DSE167pos_131122141558
(+)-ESI-FTMS
DSE167pos_131122141558 #1-25 RT: 0.00-0.10 AV: 25 NL: 6.33E7
T: FTMS + p ESI Full ms [150.00-1500.00]
11/22/2013 2:21:52 PM
DSE 167
Client: Shabaan (Cairo)
Sample: DES 167
Ionization: positive ion ESI
Method: direct infusion FTMS
913.2682
R=39849
C46 H58 O4 N4 Na Se2 = 913.2681
19.5 RDBE
0.1031 ppm
877.2890
R=38212
C40 H62 O6 N4 Na Se2 = 877.2892
11.5 RDBE
-0.1716 ppm
Relative Abundance
m/z

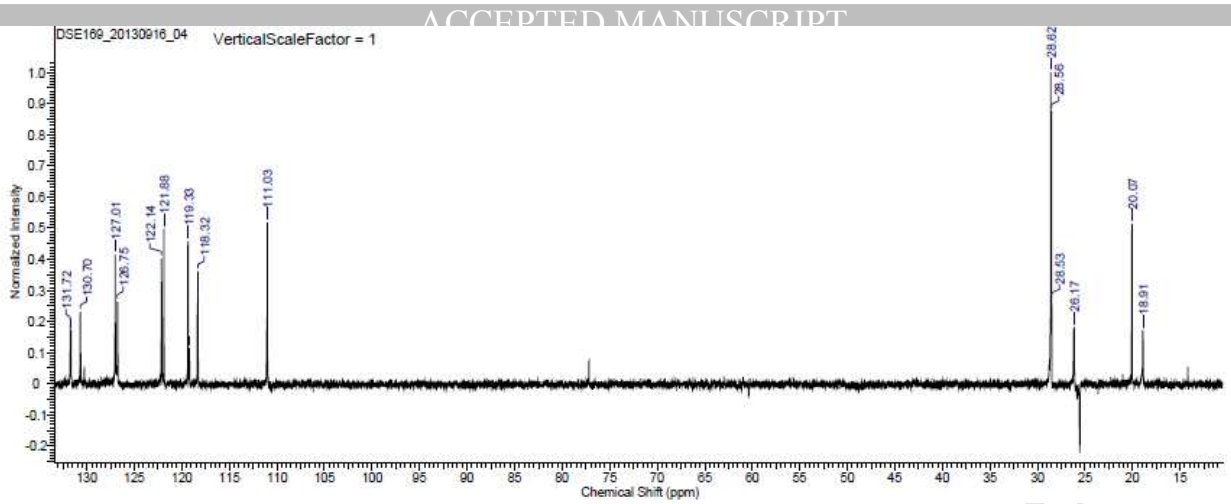

ppm (t1)
ppm (f1)

Compound 7

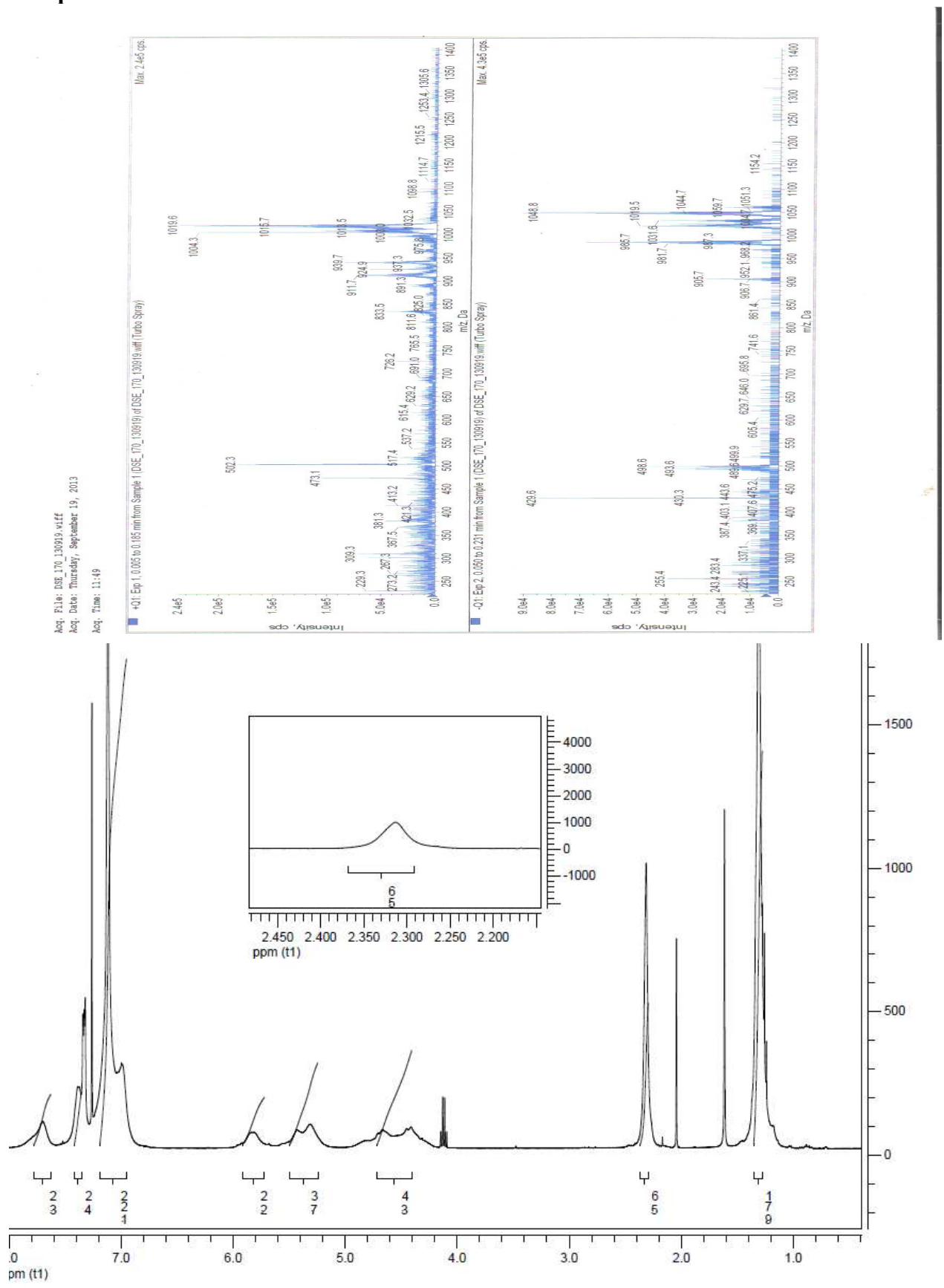

Acq: File: DSE_169_130919.wiff
Acq: Date: Thursday, September 19, 2013
Acq: Time: 11:45
+Q1: Exp 1, 0.005 to 0.185 min from Sample 1 (DSE_169_130919) of DSE_169_130919.wiff (Turbo Spray)
Max. 3.1e5 cps.
Intensity, cps
m/z, Da
-Q1: Exp 2, 0.056 to 0.231 min from Sample 1 (DSE_169_130919) of DSE_169_130919.wiff (Turbo Spray)
Max. 3.1e5 cps.
Intensity, cps
m/z, Da


D:\DI-HRMS\...\DSE169pos_131122141558
(+)-ESI-FTMS
11/22/2013 2:27:46 PM
DSE 169
DSE169pos_131122141558 #1-24 RT: 0.00-0.09 AV: 24 NL: 4.82E6
T: FTMS + p ESI Full ms [150.00-1500.00]
Client: Shabaan (Cairo)
Sample: DES 169
Ionization: positive ion ESI
Method: direct infusion FTMS
1019.3223
R=36005
C52 H64 O4 N6 Na Se2 = 1019.3212
23.5 RDBE
1.1164 ppm
1020.3253
R=35870
993.3426
R=36479
C51 H66 O3 N6 Na Se2 = 993.3419
21.5 RDBE
0.6780 ppm
1014.3278
R=37023
1022.3295
R=33327
998.3423
R=35851
Relative Abundance
m/z





Compound 8


Acq. File: DSE_170_130919.wiff
Acq. Date: Thursday, September 19, 2013
Acq. Time: 11:49
+Q1: Exp 1, 0.005 to 0.185 min from Sample 1 (DSE_170_130919) of DSE_170_130919.wiff (Turbo Spray)
Max. 2.4e5 cps.
-Q1: Exp 2, 0.050 to 0.231 min from Sample 1 (DSE_170_130919) of DSE_170_130919.wiff (Turbo Spray)
Max. 4.3e5 cps.
Intensity, cps
m/z, Da
4000
3000
2000
1000
0
-1000
2.450 2.400 2.350 2.300 2.250 2.200
ppm (t1)
1500
1000
500
0
7.0
6.0
5.0
4.0
3.0
2.0
1.0