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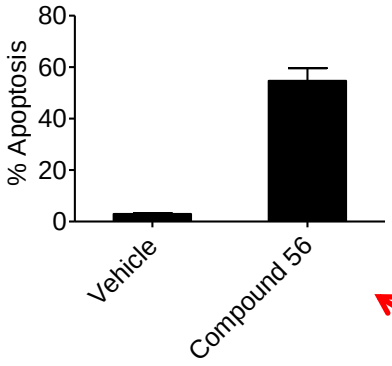
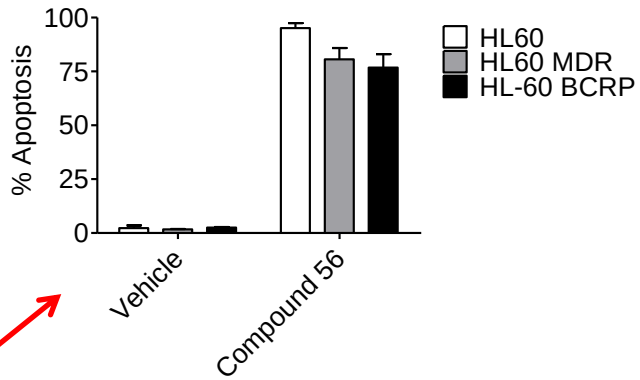
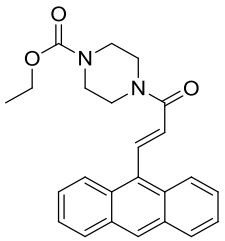
Apoptosis-inducing,
Anti-cancer agent



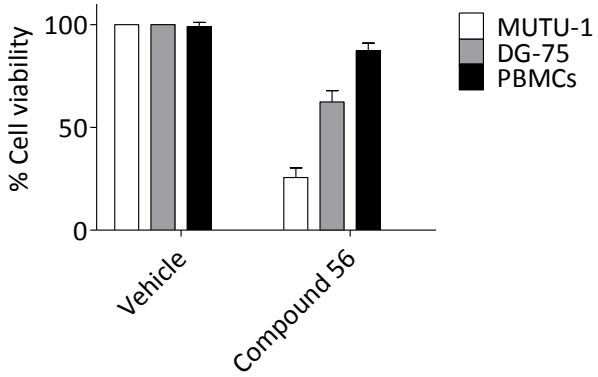
Overcomes multi-drug
resistance



Maprotiline analogue =



A novel lead compound



Low toxicity against
normal cells

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Synthesis and antiproliferative action of a novel series of maprotiline analogues.

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Abstract

The synthesis of a diverse library of compounds structurally related to maprotiline, a norepinephrine reuptake transporter (NET) selective antidepressant which has recently been identified as a novel *in vitro* antiproliferative agent against Burkitt's lymphoma (BL) cell lines is reported. A series of 9,10-dihydro-9,10-ethanoanthracenes was synthesised with modifications to the bridge of the dihydroethanoanthracene structure and with alterations to the basic side chain. A number of compounds were found to reduce cell viability to a greater extent than maprotiline in BL cell lines. In addition a related series of novel 9-substituted anthracene compounds was investigated as intermediates in the synthesis of 9,10-dihydro-9,10-ethanoanthracenes. These compounds proved the most active from the screen and were found to exert a potent caspase-dependant apoptotic effect in the BL cell lines, while having minimal effect on the viability of peripheral blood mononuclear Cells (PBMCs). Compounds also displayed activity in multi-drug resistant (MDR) cells.

1. Introduction

Burkitt's lymphoma (BL) is a rare but aggressive B-cell malignancy that was first documented in 1958 by Dennis Burkitt[1]. There are three main forms of BL, the sporadic form found in developed countries, the more common endemic form found in the malarial belt of equatorial Africa and a HIV-associated form[2, 3]. BL is the most frequent childhood cancer in equatorial Africa while in developed countries, the sporadic form accounts for 1-2% of adult lymphomas. The disease can manifest as tumours in the jaw and facial bones, kidneys, ovaries and abdomen. Endemic BL is usually associated with the oncogenic Epstein Barr virus (EBV)[2, 3]. EBV acts to interrupt cellular pathways that regulate cell proliferation and prevent apoptosis of the cell. In this way, EBV maintains proliferation of the tumour cells[4, 5].

BL malignancies proliferate rapidly and as such require intensive combination chemotherapy treatments including a combination of cyclophosphamide, doxorubicin, vincristine (oncovin), and prednisone (CHOP) and more recently rituximab, a monoclonal

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antibody which targets the CD20 antigen on the surface of malignant and normal B-lymphocytes. Rituximab, in conjunction with chemotherapeutic drugs such as vincristine, doxorubicin, methotrexate and cyclophosphamide can allow up to 60 % survival rates in children[6], [7]. However, due to a growing incidence of HIV-associated BL and increased resistance to treatments there is a vital need to develop more potent, selective and economical treatments for this disease.

Antidepressants are a class of compounds used to treat the symptoms of depression[8, 9] which target the monoamine transporters: NET, serotonin reuptake transporter (SERT) and dopamine reuptake transporter (DAT) by mimicking the effects of naturally occurring neurotransmitters. Different types of antidepressants vary in their affinities for different transporters. Recent discoveries of the presence of these transporters in some malignancies[10] originally led to the study of monoamine transporter ligands as pro-apoptotic agents including citalopram, fluoxetine, tricyclic antidepressants (TCA) imipramine and clomipramine[11-15] and amphetamine related compounds such as MDMA (ecstasy) and fenfluramine. BL cells have also been shown to overexpress the monoamine transporters SERT and NET to various degrees. However their involvement in the antiproliferative effect of monoamine transporter ligands has been disputed and is unlikely to play an important role[16].

Maprotiline is an atypical antidepressant compound, characterised by its tetracyclic structure and secondary amine side chain. Maprotiline was first patented in 1969 by Wilhelm and Schmidt and a subsequent publication reported its synthesis[17]. Maprotiline selectively targets NET over SERT and DAT transporters[18] (norepinephrine selective reuptake inhibitor, NSRI) but is also known to have moderate effects on β-noradrenergic receptors, α-adrenergic and muscarinic receptors and histaminic receptors[19, 20]. Side effects from the use of maprotiline include seizures, drowsiness, sweating, headache, arrhythmia and memory impairment[19]. Although maprotiline is not used clinically as an antidepressant due to the emergence of more efficient drugs such as serotonin reuptake inhibitors (SSRI), other effects of maprotiline have recently been discovered.

Previous research from our groups identified the antidepressant maprotiline (Fig. 1) as a potential antiproliferative agent against BL cell lines, in particular, the resistant lymphoma cell line DG-75 had an EC_{50} value in the low micromolar range following a treatment time of 72 hours with maprotiline[16, 21]. It was found that maprotiline (and the SSRI fluoxetine) induce Type II autophagic cell death in the resistant DG-75 cell line[21]. This antiproliferative effect was not observed for other NSRIs nisoxetine and reboxetine and is thought to occur independently of the NET transporter. Also, when the normal activity of the transporters was blocked with the NSRI nisoxetine, the autophagic death induced by maprotiline was not prevented [16].

Maprotiline-induced anti-multi drug resistance (MDR) effects in both cancer cell lines and the malarial strain *Plasmodium falciparum* have recently been reported[22] [23]. MDR is a major problem in drug treatment of all cancers. Proteins such as P-glycoprotein (P-gp) and breast cancer resistant protein (BCRP) are drug efflux pumps commonly overexpressed in many cancers and are responsible for eliminating therapeutic drugs from a target cancer cell. Maprotiline has previously been shown to sensitise resistant malarial strains and resistant cancer cell lines overexpressing P-gp toward anti-malarial and chemotherapeutic

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drugs[24, 25]. A study on strains of *P. falciparum* known to be resistant and sensitive to the anti-malarial drug chloroquine found several functional moieties of 9,10-dihydro-9,10-ethanoanthracene compounds including aromatic groups, the nature of a basic side chain and a cationic charge were important for an anti-MDR effect. It was found that most of the successful compounds contained amine substituted ethanoanthracene structures compared to compounds which contained amide side chains which were not as potent[25, 26]. The anti-MDR effect is thought to have an inhibitory effect on the P-gp mediated efflux pump but the exact mechanism of activity is unknown. Further studies demonstrated the ability of these compounds to inhibit anti-MDR activity in a leukaemic MDR cell line[24].

Based on this evidence, it was decided to design a library of analogues related in structure to maprotiline **1** (Fig. 1), with modifications to both the bridgehead of the 9,10-dihydro-9,10-ethanoanthracene structure and to the C-9 substituent. These compounds were then evaluated in a series of malignant cell lines including BL cell lines, MUTU-I and DG-75 cell lines, and MDR cells overexpressing P-gp and BCRP proteins, in order to investigate the structure-activity relationships of these maprotiline analogues and to attempt to improve potency.

2. Chemistry

In the present work, modifications of the dihydroethanoanthracene bridgehead structure and also to the C-9 basic substituent of the maprotiline were investigated to produce a varied library of compounds for evaluation. Synthesis of novel maprotiline analogues was achieved as illustrated in Schemes 1-4. The initial approach required formation of the dihydroethanoanthracene and dihydroethenoanthracene structures from an anthracene precursor, by way of a Diels-Alder reaction, to give products with varied functional groups on the bridgehead (Series 1 and 2). An alternative route involved building the basic side chain from anthraldehyde, followed by a Diels-Alder reaction to form the bridged dihydroethanoanthracene structure, (Series 4). A further series of related anthracene compounds was also prepared to allow the effects of the presence or absence of the ethylene bridge on the biochemical activity of the products to be assessed, (Series 3).

Series 1: The 9,10-dihydro-9,10-ethanoanthracenes **2** and **3** were obtained *via* a Diels-Alder reaction of the diene anthracene with diethyl fumarate and ethyl acrylate[27]. Esters **2** and **3** were then hydrolysed to give the corresponding carboxylic acids **4** and **5** which were then coupled to a series amines (EDCI/HOBt) to provide the 11-substituted- and 11,12-disubstituted-dihydroethanoanthracene amides (**6-20**), (Scheme 1). In the ^{1}H NMR spectrum of **7**, the alkyl protons H11 and H12 appear as a singlet at δ 4.30 ppm, integrating for two protons. Interestingly, the alkyl protons H9 and H11 do not show coupling to each other to give the expected doublet, perhaps a result of a small dihedral angle calculated as 65.9 ˚ [28]. Instead, both signals appear as singlets; however, the H-H COSY spectrum indicates the presence of coupling interaction between the two protons. This small angle would predict a small coupling constant of approximately <1.0 Hz, which was not observed in the ^{1}H NMR spectrum. The appearance of protons H9 and H11 as singlets in the ^{1}H NMR spectrum is in agreement with literature reports of related 9,10-dihydroethanoanthracene compounds[29, 30].

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The ^{1}H NMR spectrum for the novel amide **9** shows the diastereotopic methylene protons $H12_a$ and $H12_b$ resonating as two signals at δ 1.95 ppm and δ 2.15 ppm. The signal at δ 2.15 ppm appears as a multiplet , while the signal at δ 1.95 ppm ($H12_a$) resonates with coupling to the diastereotopic $H12_b$ (J = 11.9 Hz). *Cis* coupling with H11 (J = 17.8 Hz) and coupling to H10 (J = 2.5 Hz) is also clearly seen in the H-H COSY spectrum. A multiplet at δ 2.96 ppm represents the alkyl proton H11 due to the interaction with both diastereotopic H12 protons and H9. H10 is found as a singlet at δ 4.89 ppm. Interestingly, even though coupling was observed for $H12_a$ with H10 (J = 2.5 Hz), this is not obvious from its singlet signal. Similarly, H9, which is further downfield than H10 due to its relative proximity in space to the amide group, does not show any coupling to H11, as it resonates as a singlet at δ 4.51 ppm. However, the H-H COSY clearly demonstrates the interaction of both H10 and H9 with and H12 and H11 protons respectively.

The X-Ray crystal structure of the novel compound **9** was obtained (Fig. 2B) and confirmed the *trans* configuration of the compound. The three six membered rings adopt a boat conformation, as previously observed for related 9,10-dihydro-9,10-ethano- and etheno-anthracenes[31]. The out-of-plane angle between the two aromatic rings for compound **9** was determined to be 55.20 ^{o}, compared with the dihedral angle of 54.91 ^{o} observed for the 11, 12-bis-disubstituted **6** determined in the present work (Fig. 2A), and the value of 63.11 ^{o} previously reported for the diester **2**[31].

The amines **21-31** were obtained by reduction of the corresponding amides with $LiAlH_4$ in moderate yields. The amino acid ester coupled compounds **19** and **20** were subsequently converted to their corresponding amino acids **32** and **33** by base hydrolysis. Deprotection of **16** with TFA afforded the piperazine **34**, which was reduced to afford the amine **35** (Scheme 1). The acids **4** and **5** were also reduced to the alcohols **36** and **37** respectively. Compound **40** was obtained by reaction of anthracene with acrylonitrile under microwave conditions.

Series 2: The 9,10-ethenoanthracene compounds **38** and **39** were obtained as previously reported[32]′ [33] by reaction of anthracene with the dienophiles dimethyl acetylene dicarboxylate and ethyl propiolate respectively using both conventional sealed tube and/or microwave methods[34, 35]. Compound **39** was hydrolysed to the acid **41**, which was then coupled with the amines piperidine and N-methyl-N-cyclohexylamine to afford the novel amides **42** and **43** respectively. Subsequent reduction with $LiAlH_4$ afforded the corresponding amines **44** and **45** (Scheme 2). These compounds were prepared to investigate the effect of the alkene structure on the bridgehead of the dihydroethenoanthracene structure. The specific amines used, *N*-methyl-*N*-cyclohexylamine and piperidine, were chosen due to the positive result obtained from their corresponding dihydroethanoanthracene analogues when initially evaluated in BL cell lines, (see biochemical evaluation).

Series 3: The preparation of a related series of substituted anthracenes was next investigated, (Scheme 3). Following successful modification of the anthraldehyde **46**, a Diels-Alder reaction allowed construction of the bridged dihydroethanoanthracene structures, (Scheme 4). Anthraldehyde **46** was reacted with carbethoxymethylene

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triphenylphosphorane to afford **47** in high yield as the *E*-isomer (Scheme 3), identified due to the characteristic coupling constant (J = 16.0 Hz),[36]. The alkenes **48** and **49** were similarly obtained on reaction of anthraldehyde with the appropriate ylides. The ester **47** was then hydrolysed in basic conditions to produce the corresponding acid **50**[36]**.** A series of novel amides (**51-57**) were obtained from the acid **50** in a coupling reaction with a variety of amines, while the N-methylamide **58** was obtained by heating **47** with methylamine (2M in THF) in a sealed tube at 110˚C for 24 hours, (Scheme 3).

The generation of a ketone functional group in compound **48** allowed progression to compound **59** *via* a reductive amination reaction with methylamine hydrochloride and $NaCNBH_3$. A Knoevenagel reaction was next used to obtain the nitrile **60** by treatment of anthraldehyde with cyanoacetic acid while the alternative product **61** was isolated on reaction at 90 ^{o}C for 1 h.[37] The nitrostyrene **62** was obtained from 9-anthraldehyde and nitromethane in a Henry- Knoevenagel reaction in 66% yield[38] (Scheme 3).

Series 4: These dihydroethanoanthracene compounds contain substitutents at both the bridgehead C-9 and the bridge C-11 positions, (scheme 4). Reaction of anthranaldehyde with the dienophile acrylonitrile under microwave conditions at 160 ^{o}C afforded the adduct **63** in 70% yield, (compared with 48% yield after 24 h at 130 ^{o}C under conventional conditions). ^{1}H NMR spectroscopy for compound **63** indicated the exclusive formation of the *ortho* adduct, attributed to the stabilising overlap of molecular orbitals from carbonyl and nitrile groups[39]. Surprisingly, the X-Ray crystal structure obtained by XRD was not as expected for **63** as initially indicated by NMR, IR and mass spectrometry. Instead of an aldehyde group at the C-9 position of the dihydroethanoanthracene structure, a methyl hemiacetal structure **63'** is present as shown in Fig. 2C. The three six membered rings are seen to adopt a boat conformation[31]. The out-of-plane dihedral angle between the two aromatic rings for compound **63'** was determined to be 56.60 ^{o}. It was demonstrated that this novel acetal structure (**63'**) was formed during the slow crystallisation of **63** from methanol and is reversible. The structure of compound **63'** was confirmed by high resolution mass spectrometry of the crystal which detected a molecular ion of 292.1329, [M^{+} + H]; (molecular formula $C_{19}H_{18}NO_2$ requires 292.1338). An IR spectrum also identified absorption bands at 2238 cm^{-1} and 3448 cm^{-1}, corresponding to a nitrile and a hydroxyl group respectively. A ^{1}H NMR spectrum of **63'** could not be obtained as a shift in equilibrium towards formation of the aldehyde **63** was observed. The dimethylacetal of **63** had been previously reported[40]. The adducts **64** [41] and **65** [39, 42] were obtained on reaction of anthranaldehyde with dimethylacetylene dicarboxylate and acrylic acid respectively. Compound **65** was coupled to *N*-methyl-*N*-cyclohexylamine using HOBt and ECDI, to afford the amine **66,** Scheme 4.

The *ortho* adducts **67**, **68** and **69** were isolated in high yields, by reaction of the anthracenes **47**, **58** and **60** respectively with acrylonitrile**,** at high pressure in a sealed tube at 130 ^{o}C, using catalytic amounts of hydroquinone as an inhibitor. Reduction (H_2/Pd) of the alkenes **67**, **68** and **69** afforded products **70**, **71** and **72** respectively. The alcohol **73** was obtained from **63** *via* $NaBH_4$ reduction while reductive amination of **63** with $NaCNBH_3$ and methylamine led to the formation of **74** in high yield (87 %). Esterification of the alcohol **73**

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afforded the esters **76** and **77**, while the carbamate products **78**, **79** and **80** were similarly obtained from the oxime **75** in high yields, (Scheme 4).

3. Results and Discussion

3.1 Biochemical Evaluation

The library of synthesised 9,10-dihydroanthracene and 9-anthracenyl compounds was evaluated for antiproliferative activity on the BL cell lines MUTU-I (chemosensitive cell line) and DG-75 (chemoresistant cell line). Antiproliferative activity was measured with an Alamar Blue dye which is used to determine the percentage of cell viability when treated with a test sample. The BL cell lines were chosen for evaluation as previous results from our group have shown maprotiline to reduce viability in such cell lines. Cells were treated over a range of concentrations for each compound for 24 (MUTU-I) and 72 (DG-75) hours (Table 1).

The results for selected dihydroethanoanthracene ester, carboxylic acid, carboxyamide and nitrile compounds in Series 1, Scheme 1 (**2-20**, **32-33, 40**), showed that compounds **2, 3, 19** displayed anti-proliferative effects in MUTU-I cells following 24 h treatment with EC_{50} values of 89.4 µM to 55.4 µM. All other dihydroethanoanthracene carboxyamide compounds were found to have no effect on the MUTU-I cell line (**4, 5, 6, 7, 11, 20, 32, 33**: EC_{50} > 100µM). Despite their activity in MUTU-I cells, compound **19** was the only compound in this series that exhibited a toxic effect on the drug resistant DG-75 cell line with an EC_{50} value of 54.6 µM.

Results for selected 9,10-dihydro-9,10-ethanoanthracene methanamine compounds (**22**-**31**), Series 1, Scheme 1, demonstrated compound **29** to be the most potent compound of this series with EC_{50} values of 23.5 µM in MUTU-I cells and 8.8 µM in DG-75 cells; other active compounds from this series included **22, 24** and **27** (EC_{50} values of 65.0, 65.6, 23.0 µM, and 51.8, 69.9, 35.5 µM in MUTU-I and DG-75 cells respectively). Other compounds were not active (**23, 28, 30, 31:** EC_{50} > 100 µM). Importantly, these results show that compounds with an amide group (**4**-**6**, **11**, **20**, **32**, **33**) or nitrile (**40**) at C-11, do not possess any antiproliferative effect. This is consistent with a previous reports that a series of 9,10-dihydro-9,10-ethanoanthracenes with amine groups were much more active than their amide analogues at reducing efflux of rhodamine through the P-gp efflux pump, in an MDR leukaemia cell line (EC_{50} range of 0.25 µM - 970 µM)[24].

9,10-Dihydro-9,10-ethenoanthracene compounds (**38, 39, 44, 45**), Series 2, Scheme 2 were similarly evaluated for their antiproliferative effects using an Alamar Blue dye. **45** was found to exhibit a potent antiproliferative effect on the DG-75 cell line with an EC_{50} value of 10.2 µM while other similar compounds (**38**, **39**, **44**) had no effect. This antiproliferative effect was found to be much more potent than the effect of **24** (which is the saturated analogue of **45**), (EC_{50}: 70 µM). Compound **45** also displayed the strongest antiproliferative effect in the MUTU-I cell line with an EC_{50} value of 31.5 µM. In contrast, the unsaturated dihydroethenoanthracene compound **44** displayed a weaker antiproliferative effect than the corresponding saturated compound **27** (EC_{50}: 73.3 µM versus 23 µM) on the MUTU-I cell

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line. **38** and **39** (EC_{50} > 100 μM) were found to have no effect on either of the BL cell lines, while their saturated analogues **2** and **3** displayed moderate to weak antiproliferative effects on the MUTU-I cell line (EC_{50} values of 89 μM and 55 μM respectively), suggesting that the double bond accounts for the lack of activity of **38** and **39**.

For the 9,11-disubstituted-9,10-dihydro-9,10-ethanoanthracene compounds evaluated (**63, 66-80**), Series 4, Scheme 4, the diethylcarbamate compound **79** was found to be the most effective compound for inducing an antiproliferative effect in the resistant DG-75 cell line with an EC_{50} value of 3.1 μM. This value is more potent than the EC_{50} value of maprotiline determined in the present study (37.5 μM). The benzoyl oxime derivative **80** was also found to weakly inhibit proliferation of the DG-75 cell line with an EC_{50} value of 95.3 μM after treatment for 72 hours.

The acetate ester **76** was found to have the most potent effect on the MUTU-I cell line with an EC_{50} value of 12.8 μM. The oxime **75** was also found to display a slightly less potent antiproliferative effect, with an EC_{50} value of 20.4 μM. Interestingly, the effect of the acetoxyimino compound **78** on the viability of MUTU-I cells is much less potent than either **75** or **76** (EC_{50}: 34.6 μM). Compound **79**, contains an ethylamide group and was also found to have a moderate effect on the MUTU-I cell line with an EC_{50} value of 28.4. All other 11-cyano-dihydroethanoanthracene compounds evaluated were found to have only weak antiproliferative effects on the MUTU-I cell line (EC_{50} values > 80 μM). Interestingly the saturated ester compound **70** has a more effective antiproliferative activity than its unsaturated analogue **67** (EC_{50} value of 29.3 μM versus 45.6 μM respectively), possibly due to the restricted conformation of the unsaturated ester **67**. (Compounds **68, 69, 71, 73, 74** had no effect on either the DG-75 or MUTU-I cell line (EC_{50} > 100 μM).

However, by far the most potent group of compounds synthesised were the 9-substituted anthracenyl compounds **47-49**, **51-60** and **62** (Series 3, Scheme 3) with antiproliferative activities in the low micromolar range the BL cell lines. Compound **53** was found to exert the most potent antiproliferative effect on the MUTU-I cell line, with an EC_{50} value of 1.9 μM. The structurally related **55** and **56** also displayed potent activities with EC_{50} values of 7.6 μM and 5.4 μM, respectively. The nitrostyrene compounds **49** and **62** also exhibited potent antiproliferative effects with approximate EC_{50} values of 8.8 μM and 3.0 μM respectively. Most of the remaining anthracene related compounds were found to have antiproliferative activities in the low micromolar range, with EC_{50} values in the range of 18.7-38.5 μM. **58** and **59** displayed only moderate effects on the MUTU-I cell line (EC_{50}: 62.5 and 62.9 μM). Interestingly, both **58** and **59** have secondary *N*-methylamine groups compared to the other, more potent compounds, which for the most part have tertiary amine groups, which may be influencing their relative potencies on the MUTU-I cell line.

The lead compound maprotiline **1** also contains a secondary amine and was found to be less potent than a number of the 9-anthracenyl compounds (**49**, **53**, **55**, **56**, **62**) in this cell line. Several of the 9-substituted anthracene compounds displayed potent antiproliferative activity on the resistant DG-75 cell line. The nitrostyrene **62** compound, displayed the most potent activity for the DG-75 cell line with an EC_{50} value of 1.5 μM. The potent antiproliferative effect of compound **62** on BL cell lines further suggests nitrostyrene based compounds as potential lead compounds in the development of new anticancer agents[43]. The styrene **49** was found to have no effect on the viability of DG-75 cells (>50 % cell viability at 100 μM), compared to the nitrostyrene **62**, which displayed the most potent

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effect against the DG-75 cell line. Structurally related piperazines **55** and **56** were also found to induce a significant antiproliferative effect, with EC_{50} values of 62.1 μM and 11.6 μM. It is noteworthy that both of these potent compounds (**56**, **55**) contain *N*-substituted piperazine groups, and while they exhibit a potent toxic effect the BL cell lines, the structurally similar piperazine compound, **57**, was found to have no antiproliferative effect on the DG-75 cell line and only a moderate effect on the MUTU-I cell line (38.5 μM). The amides **51** and **53** were inactive in DG-75 cells. However, the N,N-diethylamide **52** displayed a significant effect after 72 hours (EC_{50}: 9.3 μM), while the pyrrolidine **54** induced an EC_{50} value of 32.5 μM.

3.2 Investigations into the effects of 9,10-dihydro-9,10-ethanoanthracenes and 9-anthracenyl compounds on peripheral blood mononuclear cells and apoptosis

Representative 9,10-dihydro-9,10-ethanoanthracenes and 9-anthracenyl compounds were chosen for further biochemical investigation based on their antiproliferative effects and analysis of their drug-like (Lipinski) properties[44] from a Tier-1 profiling together with predictions of permeability, metabolic stability, blood-brain barrier partition, plasma protein binding and human intestinal absorption properties (see supplementary information). The most active 9,10-dihydro-9,10-ethanoanthracene, **79**, and 9-anthracenyl compounds (**53**, **55**, **56, 62**) were evaluated at 10 μM over a 24 hour treatment time in MUTU-I, DG-75 and PBMC cells (Fig. 3A). Only compound **62** significantly reduced the viability of PBMCs. Compared to PBMCs, compounds **55** and **79** had a more potent effect on the malignant cell lines, while compounds **56** and the positive control vinblastine significantly reduced the cell viability of BL cell lines whilst but did not significantly reduce the cell viability of PBMCs. This suggests that the 9-substituted anthracene compounds exert a selectively toxic effect on BL cell lines.

Designing drugs that can induce programmed cell death (PCD), namely apoptosis, of a cancer cell, whilst ignoring the 'normal' cells of the body is imperative to the future development of safe effective anticancer agents. In order to investigate the possible apoptotic effect of this subset of potent compounds, PI FACS analysis was carried out at 10 μM in the MUTU-I cell line. The MUTU-I cell line was chosen for this study because these cells tend to die by classical apoptosis. This is in contrast to the chemoresistant DG-75 cell line which is more likely to die by the autophagic route of PCD[21]. Vinblastine (10 μM) was used as a positive control with 52.89 ± 5.1% of cells in the pre-G_1 phase of the cell cycle, after 24 hours. FACS analysis determined that compounds **56** and **62** had 54.70 ± 4.9% and 31.43 ± 4.1% of cells in the pre-G_1 phase of the cell cycle respectively (Fig. 3B), indicating that they are inducing significant apoptosis in the MUTU-I cell line. Compound **53** was found to have an average of 15.44 ± 5.8% of cells in the pre-G_1 phase, again indicating the formation of apoptotic cells. Compounds **55** and **79** did not induce apoptosis in the MUTU-I cell line at 10 μM.

Apoptosis is an energy-dependant process which involves activation of specific caspases and a complex cascade of biochemical signalling events to allow the cell to die. The apoptotic cell exhibits several structural and biochemical modifications including protein cleavage, DNA fragmentation, membrane disruption and caspase activation[45]. As activation of caspases is one of the hallmarks for apoptosis, it was decided to carry out

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caspase activation experiments in order to confirm the results from the FACS analysis. Caspases 3 and 7 are two of ten major caspases identified and are categorised as effectors or executioner caspases[45]. Caspase 3/7 activity was assessed using the Apotox-Glo Triplex Assay, results showed that compounds **53, 56** and **62** all significantly activate caspases 3 and 7, compared to the untreated control, consistent with the results of the FACS analysis, suggesting that these compounds do in fact induce apoptosis in the MUTU-I cell line (Fig. 3C). In contrast **55** and **79** do not seem to induce caspase activation in this cell line, again, consistant with results from the FACS analysis suggesting that these compounds do not have an apoptotic effect.

3.3 Multi-drug resistance activity of maprotiline analogues

9,10-Dihydro-9,10-ethanoanthracenes have been previously evaluated by Alibert *et al.* and were found to decrease the multi-drug resistance of a leukaemia cell line *via* possible inhibition of the P-gp efflux pump, allowing increased cellular accumulation of rhodamine[24]. As a number of the novel dihydroethanoanthracenes evaluated in the present study were found to have antiproliferative effects at low μM concentrations, it was of interest to this study to determine if these compounds (**27**, **66**), and the cohort of compounds tested above, could demonstrate an ability to overcome MDR. For this, normal (parental) HL-60 cells and HL-60 cells overexpressing the drug efflux pumps P-gp and BCRP were acquired[46]. Parental HL-60 cells were sensitive to both paclitaxel (taxol, a microtubule stabiliser) and SN-38 (a topoisomerase I inhibitor), common chemotherapeutics (Fig. 4A). HL-60 P-gp cells are resistant to taxol (but not SN-38) and this resistance can be overcome by administration of the P-gp inhibitor verapamil (Fig. 4B). Likewise, HL-60 BCRP are resistant to SN-38 (but not taxol), this resistance can be reversed by administration of the BCRP inhibitor KO143 (Fig. 4C). All three cell lines were treated with the selected compounds and evaluated for apoptotic effects. Results showed that compounds **56, 62** and **66** induced apoptosis in all 3 HL-60 cell lines. These results suggest that these particular maprotiline analogues are not substrates for drug efflux pumps. Compound **53**, which induced the lowest level of apoptosis in the MUTU-I cell line, was not active in the HL-60 cells, whilst compounds **27**, **55** and **79** displayed no activity (Fig. 4D).

4. Conclusion

Previous research identified the NSRI maprotiline as a pro-autophagic antiproliferative agent in BL cell lines[16, 21]. Based on this evidence, a diverse library of 9,10-dihydro-9,10-ethanoanthracenes, 9,10-dihydro-9,10-ethenoanthracenes and related anthracenes the were synthesised that were related in structure to maprotiline. Biochemical evaluation of these analogues revealed a number of compounds that displayed potent antiproliferative effects and induced apoptosis and caspase activation in BL cell lines. Many of these compounds were more potent than maprotiline. A representative group of compounds were evaluated in PBMCs and it was revealed that many of the compounds had no effect on their viability, implying that these maprotiline analogues are selectively toxic to BL cell lines. Three of these compounds **56**, **62** and **66** also displayed the ability to overcome

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chemotherapeutic resistance due to drug efflux pumps. However, the significant antiproliferative and apoptotic effects of compound **56** on BL cell lines, together with a lack of toxicity against normal PBMCs suggests this nitrostyrene based compound as a potential lead compound in the development of new anticancer agents and warrants further investigation[43]. These results also demonstrate the importance of defining structure-activity relationships for novel compounds.

5. Acknowledgements

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6. Figure legends

Figure 1. Maprotiline **1.**

Figure 2. Ortep representation of the X-ray crystal structure of (A) **6**, (B) **9** and (C) **63'** (acetal) with the thermal ellipsoids set at 30% probability.

Scheme 1: Reagents and conditions: (a) EtO_2C-CH=CH-CO_2Et, or CH_2=CH-CO_2Et, $AlCl_3$, rt, 18-24 h (b) KOH, EtOH, reflux, 3 h; (c) EDCI, HOBt, Et_3N, NHR_1R_2, 0 °C, 18-24 h; (d) $LiAlH_4$, THF, rt, 18-24 h; (e) $LiAlH_4$, THF, reflux, 3 h; (f)TFA, CH_2Cl_2, 0°-23 °C, 6 h; (g) CH_2=CHCN, hydroquinone, microwave irradiation, 200 °C, 10 min.

Scheme 2: Reagents and conditions: (a) Ethyl propiolate, hydroquinone, microwave irradiation, 160 °C, 45 min (b) 5M KOH, EtOH, reflux, 3 h; (c) EDCI, HOBt, NEt_3, NHR_2, CH_2Cl_2, 18-24 h; (d) $LiAlH_4$, THF, 18-24 h; (e) Dimethyl acetylenedicarboxylate, sealed tube, 120 °C, 24 h, (f) Dimethyl acetylenedicarboxylate, microwave irradiation, 160 °C, 45 min.

Scheme 3: Reagents and conditions: ; (a) CH_3NO_2, CH_3CO_2H, cyclohexylamine, 6 h, reflux; (b) $NO_2C_6H_4CH_2PPH_3Br$, NaH, THF, 12 h, reflux; (c) $CH_3CH_2OCOCHPPh_3$, CH_2Cl_2, reflux 6-7 h; (d) $NaCNBH_3$, CH_3OH, CH_3NH_2HCl, pH5-6, 72 h, rt; (e) $CH_3CH_2OCOCHPPh_3$, CH_2Cl_2, reflux 6-7 h; (f) 5M KOH, EtOH, 3h, reflux; (g) EDCI, HOBt, NEt_3, NHR_1R_2, CH_2Cl_2, 18 h, rt.; (h) CH_3NH_2, 110 °C, 24h, sealed tube; (i) $CNCH_2CO_2H$, morpholine, DMF, reflux, 7 h; (j) $CNCH_2CO_2H$, morpholine, DMF, 90 °C, 1 h.

Scheme 4: Reagents and conditions: (a) Dimethyl acetylenedicarboxylate, microwave irradiation, 160 °C, 45 min; (b) CH_2=$CHCO_2H$, xylene, CH_3CO_2H, microwave irradiation, 250 °C, 10 min; (c) $NH(CH_3)(C_6H_{11})$, EDCI, HOBt, CH_2Cl_2, 0 °C - rt, 24 h; (d) CH_2=CHCN, hydroquinone, sealed tube, 130 °C, 24 h; (e) $NH_2OH.HCl$, pyridine, EtOH, reflux, 3 h; (f) RCOCl, Et_3N, CH_2Cl_2, reflux, 3 h; (g) $NaCNBH_3$, $CH_3NH_2.HCl$, CH_3OH, rt, 72 h; (h) $HCl/CHCl_3$, 10 min, rt; (i) CH_3OH, 4 weeks; (j) $NaBH_4$, MeOH, rt, 2h; (k) H_2/Pd, ethyl acetate, 48 h, rt.

Figure 3. Investigations into the effects of analogue compounds on PBMCs, apoptosis and caspase activation. A) The effect of each compound at 10 μM on the viability of the MUTU-I, DG-75 and PBMC cells was analysed. Compounds were evaluated using the Alamar Blue

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cell viability assay in three independent experiments. Compounds were screened for the induction of B) programmed cell death or apoptosis by FACS analysis and C) caspase activation. D) Compounds **53, 55, 62, 66** and **79**. Statistical analysis was performed using a Student's t-test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 4. Selective compounds induce apoptosis in multidrug resistant (MDR) cells. (A) Parental HL-60 cells are sensitive to both taxol and SN-38 while drug-resistant cells are resistant to either **(B)** taxol (p-glycoprotein-expressing MDR cells) or **(C)** SN-38 (BCRP-expressing cells). **(D)** Maprotiline and analogues **56, 62** and **66** show activity in both taxol and SN-38-resistant HL-60 cells. Statistical analysis was performed using a Student's t-test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

7. Legends for tables

Table 1. The antiproliferative effects of maprotiline analogues on BL cell lines

EC_{50} values were estimated from log-concentration sigmoidal dose response curves where the cytotoxic potency of each compound was evaluated with an alamar blue assay. Experiments were performed in triplicate on three independent days using four test compound concentrations. Data was subjected to non-linear regression analysis using a sigmoidal dose response (Hill slope=1) using GRAPHPAD Prism4 software (Graphpad software Inc., San Diego, CA). Miconazole (10 µM) was used as a positive control and resulted in >90 % cytotoxicity to all cell lines. Of the compounds selected for biological screening, data is only shown for those compounds which exhibited activity (EC_{50} value < 100) in at least 1 of the BL cell lines.

8. Experimental section

8.1 Chemistry: experimental methods

All reagents were commercially available and were used without further purification unless otherwise indicated. Tetrahydrofuran (THF) was distilled immediately prior to use from Na/Benzophenone under a slight positive pressure of nitrogen, toluene was dried by distillation from sodium and stored on activated molecular sieves (4Å) and dichloromethane was dried by distillation from calcium hydride prior to use. Uncorrected melting points were measured on a Gallenkamp apparatus. IR spectra were recorded as thin films on NaCl plates or as KBr discs on a Perkin-Elmer Paragon 100 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance DPX 400 instrument at 20^{o}C, 400.13MHz for ^{1}H spectra, 100.61MHz for ^{13}C spectra, in either $CDCl_3$, CD_3COCD_3 or CD_3OD (internal standard tetramethylsilane). Low resolution mass spectra were run on a Hewlett-Packard 5973 MSD GC–MS system in an electron impact mode, while high resolution accurate mass determinations for all final target compounds were obtained on a Micromass Time of Flight mass spectrometer (TOF) equipped with electrospray ionization (ES) interface operated in the positive ion mode at the High Resolution Mass Spectrometry Laboratory by Dr. Martin Feeney in the School of Chemistry, Trinity College Dublin. Thin layer chromatography was performed using Merck Silica gel 60 TLC aluminium sheets with fluorescent indicator visualizing with UV light at 254nm. Flash chromatography was carried out using standard

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silica gel 60 (230-400 mesh) obtained from Merck. All products isolated were homogenous on TLC. The purity of the tested compounds was determined by high-performance liquid chromatography (HPLC) or combustion analysis and unless otherwise stated, the purity level was ≥95%. Elemental analyses were performed on an Exetor Analytical CE4400 CHN analyser in the microanalysis laboratory, Department of Chemistry, University College Dublin. Analytical HPLC was performed using a Waters 2487 Dual Wavelength Absorbance detector, a Waters 1525 binary HPLC pump and a Waters 717plus Autosampler. The column used was a Varian Pursuit XRs C18 reverse phase 150 x 4.6mm chromatography column. Samples were detected using a wavelength of 254 nm. All samples were analyzed using acetonitrile (70%): water (30%) over 10 min and a flow rate of 1 mL/min.

The following compounds were prepared as previously reported: *Trans*-11,12-Diethoxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**2**)[47], 11-Ethoxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**3**)[48], *trans*-11,12-Dihydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**4**)[31], 11-Hydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**5**)[49], *trans*-11,12-(*N,N,N',N'*-tetramethyl)-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxyamide (**6**)[31], 9,10-dihydro-*N*-methylpiperazinyl-9,10-ethanoanthracene-11-carboxyamide (**11**)[50], 9,10-Dihydro-11-*N*-ethylamino-9,10-ethanoanthracene-11-carboxyamide (**14**)[51], 9,10-dihydromorpholinyl-9,10-ethanoanthracene-11-carboxyamide (**15**)[52], *trans*-9,10-dihydro-*N, N, N', N'*-tetramethyl-9,10-ethanoanthracene-11,12-dimethan amine (**21**)[31], 9,10-dihydro-*N*-piperidinyl-9,10-ethanoanthracene-11-methanamine (**24**)[53], 9,10-dihydro-*N*-methylpiperazinyl-9,10-ethanoanthracene-11-methanamine (**25**)[53], 9,10-Dihydromorpholinyl-9,10-ethanoanthracene-11-methanamine (**29**)[52], 9,10-Dimethyl-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**38**)[32], 9,10-dihydro-9,10-ethenoanthracene-11-ethylcarboxylate (**39**)[33], 9,10-Dihydro-9,10-ethanoanthracene-11-carbonitrile (**40**)[54], 9,10-Dihydro-9,10-ethenoanthracene-11-carboxylic acid (**41**)[31], 3-(9-Anthracenyl)acrylic acid ethyl ester (**47**)[36], (*E*)-4-(9-anthracenyl)but-3-en-2-one (**48**)[55], 3-(9-Anthracenyl)acrylic acid (**50**)[36], 3-(9-Anthracenyl)acrylonitrile (**60**)[38], (*E*)-9-(2-Nitrovinyl)anthracene (**62**)[38], 9-Formyl-9,10-dihydro-11-cyano-9,10-ethanoanthracene (**63**)[56], Dimethyl-9-formyl-9,10-dihydro-9,10-ethenoanthracene-11,12-dicarboxylate (**64**)[41], 9-Formyl-9,10-dihydro-9,10-ethanoanthracene-11-carboxylic acid (**65**)[39, 42] 9-((*E*)-(Hydroxyimino)methyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (**75**)[34].

8.1.1 General procedure 1: preparation of amides

To a solution of the appropriate acid (10 mmol) in dry DCM at 0 ˚C, were added HOBt (36 mmol), EDCI (36 mmol) and triethylamine (4.4 mmol). The reaction mixture was stirred for 10 min before adding the appropriate amine (36 mmol). This solution was stirred overnight at room temperature. The solvent was then evaporated and water (50 mL) was added and the product extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine (3 x 50 mL) and dried over anhydrous Mg_2SO_4. The solvent was evaporated *in vacuo* to afford the amide

8.1.1.1 *trans*-11,12-(*N,N,N',N'*-Tetraethyl)-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxyamide (7)

trans-11,12-Dihydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**4**) (1.70 mmol, 0.50 g) was treated with dimethylamine hydrochloride (6.12 mmol, 0.25 g) according to general procedure 1 above. The product was then purified using flash column chromatography over silica gel (5 % MeOH/DCM) to afford the product as colourless crystals (75 %), M.p**.** 120-123 ˚C. IRν_{max} (KBr) 1636 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.08 (6H, t, J = 7.0 Hz, CH_3), 1.23 (6H, t, J = 7.0 Hz, CH_3), 3.08 (2H, m, CH_2), 3.41 (8H, m, CH_2, H9/H10), 4.30 (2H, s, H11/H12), 7.12-7.19 (8H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 12.76, 14.52 (CH_3), 39.93, 41.63 (CH_2), 44.65 (C11/C12), 47.95 (C9/C10), 122.05, 124.61, 125.65, 125.80 (ArCH), 139.49, 142.27 (C_4), 170.83 (C=O). HRMS (ESI) calculated for $C_{26}H_{33}N_2O_2$: $[M^+ + H]$ 405.2549: found 405.2542.

8.1.1.2 *trans*-11,12-*N*-Piperidinyl-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxyamide (8)

trans-11,12-Dihydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**4**) (1.7 mmol, 0.50 g) was treated with piperidine (6.12 mmol, 0.52 g) according to general procedure 1 above. The product was then purified using flash column chromatography over silica gel (5 % MeOH/DCM) to afford the product as colourless crystals (89 %), M.p. 144-145 ˚C. IRν_{max} (KBr**)** 1623 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.53 (12H, m, CH_2), 3.42 (2H, H9), 3.57 (4H, m, CH_2), 3.76 (4H, m, CH_2), 4.32 (2H, s, H11), 7.12 (4H, m, ArH), 7.21 (2H, d, J = 8.0 Hz, ArH), 7.32 (2H, d, J = 8.0 Hz, ArH). ^{13}C NMR ppm ($CDCl_3$) 24.27, 25.45, 26.57, 42.93, 44.53 (CH_2), 46.35 (C9), 47.45 (C11), 122.32, 124.41, 125.81, 139.59, 142.01 (ArCH), 169.83 (C=O). HRMS (ESI) calculated for $C_{28}H_{32}N_2O_2Na$: $[M^+ + Na]$ 451.2361: found 451.2357.

8.1.1.3 9,10-Dihydro-*N,N*-dimethylamino-9,10-ethanoanthracene-11-carboxyamide (9)

11-Hydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**5**) (1.8 mmol, 0.50 g) was reacted with dimethylamine hydrochloride (3.24 mmol, 0.26 g) according to general procedure 1 above. The product was then purified by flash column chromatography over silica gel (eluent: 85:15, hexane/ethyl acetate) to afford the product as a colourless semi-solid (78 %). IR ν_{max} (film) 1655 (C=O) cm^{-1} ^{1}H NMR δ ($CDCl_3$) 1.95 (1H, ddd, J = 17.8 Hz, J = 11.9 Hz, J = 2.5 Hz, $H12_a$), 2.15 (1H, m, $H12_b$), 2.86 (3H, s, CH_3), 2.96 (1H, m, H11), 3.02 (3H, s, CH_3), 4.89 (1H, s, H10), 4.51 (1H, s, H9), 7.13 (4H, m, ArH), 7.25 (3H, m, ArH), 7.39 (1H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 31.79 (CH_2), 35.34 (CH_3), 36.48 (CH_3), 41.40 (C10), 43.61 (C9), 46.31 (C10), 122.17, 122.68, 123.27, 125.25, 125.30, 125.66 (ArCH), 140.14, 143.30 (C_4), 173.00 (C=O). HRMS (ESI) calculated for $C_{19}H_{20}NO$: $[M^+ + H]$ 278.1545: found 278.1539.

8.1.1.4 9,10-Dihydro-11-piperidinyl-9,10-ethanoanthracene-11-carboxyamide (10)

11-Hydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**5**) (1.8 mmol, 0.50 g) was reacted with piperidine (3.24 mmol, 0.28 g) according to general procedure 1 above. The product was then purified by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate) to afford a colourless powder (70 %), M.p. 138-140 ˚C. IR ν_{max} (KBr) 1672 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.58 (6H, br m, CH_2), 2.00 (1H, ddd, J = 18.0 Hz, J = 11.0 Hz, J = 2.4 Hz, $H12_a$), 2.11 (1H, m, $H12_b$), 2.95 (1H, ddd, J = 16.0 Hz, J = 10.0 Hz, J = 2.4 Hz, H11), 3.46 (4H, br s, CH_2), 4.37 (1H, s, H10), 4.48 (1H, s, H9), 7.11 (4H, m, ArH), 7.31 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 24.51 (C12), 41.63 (C10), 42.00 (CH_2), 43.98 (C9), 44.40 (CH_2), 46.39 (C11), 47.10 (CH_2), 47.25 (CH_2) 122.43, 122.94, 123.48, 125.53, 125.57, 125.78, 125.89 (ArCH), 133.37, 134.59, 140.45, 143.64 (C_4), 171.58. HRMS (ESI) calculated for $C_{21}H_{23}NONa$: $[M^+ + Na]$ 340.1677: found 340.1673.

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8.1.1.5 9,10-Dihydro-11-pyrrolidinyl-9,10-ethanoanthracene-11-carboxyamide (12)

11-Hydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**5**) (1.8 mmol, 0.50 g) was reacted with pyrrolidine (3.24 mmol, 0.23 g) according to general procedure 1 above. The product was then purified by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate) to afford a colourless powder (58 %), M.p. 74-75 ˚C. IR ν_{max} (KBr) 1643 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.67 (2H, br s, CH_2), 1.98 (3H, br m, CH_2, $H12_a$), 2.09 (1H, m, $H12_b$), 2.87 (1H, ddd, J = 16.0 Hz, J = 10.0 Hz, J = 2.4 Hz, H11), 3.41 (4H, br s, CH_2), 4.38 (1H, s, H9), 4.52 (1H, s, H10), 7.12 (4H, m, ArH), 7.27 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 23.76 (CH_2), 25.87 (CH_2), 31.30 (C12), 43.05 (C9), 43.59 (C10), 45.50 (CH_2), 46.10 (C11), 122.13, 122.62, 123.24, 125.23, 125.29, 125.46, 125.53 (ArCH), 143.25, 143.40 (C_4), 171.68 (C=O). HRMS (ESI) calculated for $C_{21}H_{21}NO$: [M^+ + Na] 326.1521: found 326.1512.

8.1.1.6 9,10-Dihydro-11-*N*-methyl-*N*-cyclohexanyl-9,10-ethanoanthracene-11-carboxyamide (13)

11-Hydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**5**) (1.8 mmol, 0.50 g) was reacted with *N*-methyl-*N*-cyclohexylamine (3.24 mmol, 0.36 g) according to general procedure 1 above. The product was then purified by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate) to afford a colourless powder (94 %), M.p. 70-74 ˚C. IR ν_{max} (KBr) 1640 (C=O), 2927 (C-H) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.28-1.92 (10H, m, $5CH_2$), 1.98 (1H, m, $H12_a$), 2.15 (1H, m, $H12_b$) 2.72 (1H, br s, NCH), 2.88 (1H, br s, NCH), 2.97 (1H, m, H11), 4.37 (1H, s, H10), 4.85 (1H, s, H9), 7.11 (4H, m, ArH), 7.29 (2H, m, ArH), 7.35 (2H, dd, J = 3.5 Hz, J = 8.0 Hz, ArH). ^{13}C NMR ppm ($CDCl_3$) 25.32, 25.63, 25.82, 25.97 (CH_2), 27.70 (CH), 29.20 (CH_3), 29.86 (C12), 31.04, 31.26, 32.32, 32.66 (CH_2), 42.29 (C11), 44.15 (C10), 46.98 (C9), 143.83, 140.72 (C_4), 173.00 (C=O). HRMS (ESI) calculated for $C_{24}H_{27}NONa$: 368.1990 [M^+ + Na]: found 368.1974.

8.1.1.7 *tert*-Butyl-4-(9,10-dihydro-9,10-ethanoanthracene-11-carbonyl)piperazine-1-carboxylamide (16)

11-Hydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**5**) (0.3 mmol, 0.08 g) was reacted with 1-^{t}Boc-piperazine (0.5 mmol, 0.11 g) according to general procedure 1 above. The product was then purified by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate) to afford a colourless powder (85 %), M.p. 192-195 ˚C. IR ν_{max} (KBr) 1630 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.48 (9H, s, $3xCH_3$), 1.96 (1H, dd, J = 5.0 Hz, J = 10.7 Hz, $H12_a$), 2.13 (1H, m, $H12_b$), 2.94 (1H, m, H11), 3.39 (8H, br m, CH_2 piperizine), 4.38 (1H, s, H10), 4.47 (1H, s, H9), 7.12 (4H, m, ArH), 7.14 (3H, m, ArH), 7.40 (1H, d, J = 3.34 Hz, ArH). ^{13}C NMR ppm ($CDCl_3$) 27.92 (CH_3), 32.02 (C12), 41.32 (C10), 43.51 (C11), 44.73 (CH_2 piperazine), 46.35 (C9), 79.90 (C_4 O^{t}Bu), 122.26, 122.71, 123.27, 125.34, 125.37, 125.43, 125.59, 125.66 (ArCH), 139.91, 142.91, 143.10, 143.14 (C_4), 154.11 (C=O ^{t}Bu), 171.83 (C=O amide). HRMS (ESI) calculated for $C_{26}H_30N_2O_3Na$: [M^+ + Na] 441.2154: found 441.2148.

8.1.1.8 9,10-Dihydro-11-*N*-anilinyl-9,10-ethanoanthracene-11-carboxyamide (17)

11-Hydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**5**) (1.8 mmol, 0.50 g) was reacted with aniline (3.24 mmol, 0.31 g) according to general procedure 1 above. The product was then purified by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate) to afford a colourless powder (60 %), M.p. 230-232 ˚C. IR ν_{max} (KBr)

1660 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.80 (1H, ddd, J = 14.5, J = 12.4, J = 2.9 Hz, $H12_a$), 2.07 (1H, m, $H12_b$), 2.87 (1H, ddd, J = 14.9 Hz, J = 9.9 Hz, J = 2.5 Hz H11), 4.43 (1H, s, H10), 4.69 (1H, s, H9), 6.99-7.16 (4H, m, ArH), 7.24-7.41 (4H, m, ArH), 7.49 (2H, d, J = 7.5 Hz, ArH). ^{13}C NMR ppm ($CDCl_3$) 29.69 (CH_2), 42.89 (C11), 44.85 (C10), 47.45 (C9), 119.22 (C15), 122.95, 123.43, 124.69, 125.28, 125.58 (ArCH), 128.62 (C15), 139.45, 140.03, 143.32 (C_4), 143.97 (NH), 170.89 (C=O). HRMS (ESI) calculated for $C_{23}H_{19}NONa$: [M^+ + Na] 348.1364: found 348.1362.

8.1.1.9 9,10-Dihydro-11-4-(4-chlorophenyl)piperazinyl-9,10-ethanoanthracene-11-carboxyamide (18)

11-Hydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**5**) (1.8 mmol, 0.50 g) was reacted with 4-(4-chlorophenyl)-piperazine (3.24 mmol, 0.64 g) according to general procedure 1 above. The product was then purified by flash column chromatography over silica gel (eluent: 95 %, DCM/MeOH) to afford a colourless powder (87 %), M.p. 111-113 ˚C. IR ν_{max} (KBr) 1648 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.98 (1H, ddd, J = 17.7 Hz, J = 11.9 Hz, J = 2.5 Hz, $H12_a$), 2.01 (1H, m, $H12_b$), 2.99 (1H, ddd, J = 16.3 Hz, J = 10.2 Hz, J = 2.1, H11), 3.00, 3.69 (8H, 2br m, CH_2), 4.39 (1H, s, H10), 4.50 (1H, s, H9), 6.86 (2H, d, J = 1.9 Hz, H15), 7.14-7.38 (10H, 2m, ArH, H16). ^{13}C NMR ppm ($CDCl_3$) 32.03 (CH_2 C12), 41.26 (C9), 43.56 (C10), 45.00 (CH_2), 46.40 (C11), 49.05, 49.50, 53.06 (CH_2), 117.43, 122.30, 122.74, 122.74, 123.31, 125.37, 125.39, 125.47, 125.62, 125.69, 128.69 (ArCH), 139.95 (C_4 C-N), 142.9, 143.14, 143.23 149.05 (C-Cl), 171.65 (C=O). HRMS (ESI) calculated for $C_{27}H_{25}N_2OClNa$: [M^+ + Na] 451.1553: found 451.1541.

8.1.1.10 Methyl 2-(9,10-dihydro-9,10-ethanoanthracene-11-carboxamido)-3-(4-hydroxyphenyl) propanoate (19)

11-Hydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**5**) (1.12 mmol, 0.31 g) and DL-tyrosine methyl ester (2.2 mmol, 0.51 g) were reacted together according to general procedure 1 above. No further purification was required. The product was obtained as pale crystals (73 %), M.p. 102-104 ˚C. IRν_{max} (KBr) 1745 (CH_3OC=O), 1614 (NHC=O), 3320 (OH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.71 (1H, m, $H12_a$), 1.86* (1H, m, $H12_a$), 2.08 (1H, m, 12_b), 2.20* (1H, m, $H12_b$), 2.73 (2H, m, CH_2), 2.78 (1H, d, J = 5.6 Hz, H11), 3.03* (2H, m, CH_2), 3.83 (3H, s, CH_3), 4.33 (1H, s, H10), 4.37* (1H, s, H10), 4.47 (1, s, H9), 4.58* (1H, s, H9), 4.67 1H, dd J = 16.0 Hz, J = 7.0 Hz, H13), 4.76* (1H, dd, J = 14.2 Hz, J = 5.4 Hz, H13), 5.72 (1H, d, J = 7.6 Hz, NH), 5.77* (1H, dd, J = 7.6 Hz, NH), 6.69 (2H, m, ArH phenol), 6.77* (2H, d, J = 8.0 Hz, ArH phenol), 7.17 (4H, m, ArH), 7.31 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 30.54 (CH_3), 31.30, 31.68* (C12), 36.37, 36.88* (CH_2), 43.69, 43.75* (C10), 45.39, 45.46* (C9), 46.88, 46.90* (C11), 53.15, 53.35* (C13), 115.45, 115.47* (Ar<u>CH</u>COH), 122.70, 122.88, 123.01, 123.18, 123.27, 125.00, 125.41, 125.57, 125.68, 125.86, 126.06, 126.13 (ArH), 130.11, 130.22* (Ar<u>CH</u>CCH_2), 143.70, 143.49, 142.70, 142.53, 142.38, 139.16, 138.91 (C_4), 154.80, 154.85 (C_4) 171.78 (CH_3C=O), 172.80 (NHC=O). HRMS (ESI**)** calculated for $C_{27}H_{26}NO_4$: [M^+ + H] 428.1863: found 428.1848

8.1.1.11 Methyl-2-(9,10-dihydro-9,10-ethanoanthracene-11-carboxyamido)-4-methylpentoate (20)

11-Hydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**5**) (1.8 mmol, 0.50 g) and L-leucine methyl ester (3.24 mmol, 0.49 g) were reacted together according to general procedure 1 above. The product was then purified by flash column chromatography over

silica gel (2:1, hexane/ethyl acetate) to afford a colourless powder (55 %), M.p. 143-144 ˚C. IRν_{max} (KBr) 1648 (C=O), 1748 (C=O), 3288 (NH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.90 (6H, d, J = 8.0 Hz), 1.33 (1H, m, CH), 1.50 (2H, m, CH_2), 1.98 (1H, m, H11), 2.14 (1H, m, H11), 2.82 (1H, ddd, J = 2.0 Hz, J = 5.0 Hz, J = 16.0 Hz, H12), 3.73 (3H, s, OCH_3), 4.41 (1H, s, H10), 4.52 (1H, m, NHCH), 4.61 (1H, s, H9), 5.44 (1H, d, J = 8.0 Hz, NH), 7.13 (4H, m, ArH), 7.33 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 21.42, 22.32 (CH_3), 24.24 (C16), 41.04 (C14), 43.35 (C11), 45.21 (C9), 46.98 (C10), 50.10 (C13), 51.79 (OCH_3), 122.88, 123.01, 123.16, 124.93, 124.97, 125.43, 125.69, 125.97 (ArCH), 139.28, 142.35, 142.82, 143.32 (C_4), 172.88, 173.18 (C=O). HRMS (ESI) calculated for $C_{24}H_{27}NO_3$: [M^+ + H] 378.2070: found 378.2053.

8.1.1.12 9,10-Dihydro-11-piperazinyl-9,10-ethanoanthracene-11-carboxyamide (34)

To a solution of *tert*-butyl-4-(9,10-dihydro-9,10-ethanoanthracene-11-carbonyl)piperazine-1-carboxylate (**16**) (0.095 mmol, 0.04 g) in DCM (3 mL) was added trifluoroacetic acid (0.956 µmol, 0.11 mg) at 0 ˚C. The solution was stirred at 23 ˚C for 6 h. After this time the solution was diluted with DCM (10 mL) and basified by the slow addition of aq. satd. $NaHCO_3$. The aqueous layer was extracted with ethyl acetate, dried over anhydrous Na_2SO_4 and the solvent removed *in vacuo*. The resulting residue required no further purification and the product was isolated as a pale solid (80 %), M.p. 130-133 ˚C. IR ν_{max} (KBr) 1668 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.26 (1H, m, H11), 1.97 (1H, m, $H12_a$), 2.13 (1H, m, $H12_b$), 2.91-3.60 (8H, 3 x br s, CH_2 piperizine), 4.38 (1H, s, H10), 4.46 (1H, s, H9), 7.16 (4H, m, ArH), 7.26 (3H, m, ArH), 7.40 (1H, d, J = 7.6 Hz, ArH). ^{13}C NMR ppm ($CDCl_3$) 31.95 (C12), 41.15 (C10), 43.49 (C11), 46.30 (C9), 122.27, 122.72, 123.29, 125.34, 125.38, 125.45, 125.28, 125.67 (ArCH), 139.88142.89, 143.11, 143.15 (C_4), 171.68 (C=O). HRMS (ESI) calculated for $C_{21}H_{23}N_2O$: [M^+ + H] 319.1810: found 319.1800.

8.1.2 General procedure 2: preparation of amines

The appropriate amide (1 mmol) was added slowly to a slurry of $LiAlH_4$ (8 mmol) in dry THF. The solution was stirred overnight. H_2O (2 mL) was added slowly to quench the reaction. The suspension was filtered over celite. Diethyl ether (50 mL) was added to the solution and extracted with 2N HCl (3 x 20 mL). The aqueous phase was basified and extracted with EtOAc (3 x 20 mL). The solution was dried over anhydrous Mg_2SO_4. The solvent was evaporated *in vacuo* to give the product.

8.1.2.1 *trans*-9,10-Dihydro-*N,N,N',N'*-tetraethyl-9,10-ethanoanthracene-11,12-dimethanamine (22)

trans-11,12-*N,N,N',N'*-Tetraethyl-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxyamide (**7**) (1 mmol, 0.4 g) was added to $LiAlH_4$ according to general procedure 2 above. No further purification was required. The resulting residue required no further purification and the product was isolated as a brown solid (35 %), M.p. 60-64 ˚C. IR ν_{max} (KBr) 1466 (Ar C=C) 2973 (ArCH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.98 (12H, t, J = 7.0 Hz, $4CH_3$), 1.44 (2H, br m, C11/C12), 1.94 (2H, dd, J = 9.5 Hz, J = 12.5 Hz, C13), 2.05 (2H, dd, J = 4.0 Hz, J = 12.5 Hz, C14), 2.41 (4H, 2t, J = 7.0 Hz, $2CH_2$), 2.54 (4H, 2t, J = 7.0 Hz, $2CH_2$), 7.10 (4H, m, ArH), 7.28 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 11.37 (CH_3), 42.84 (C11), 46.19 (C9), 47.03 (CH_2 C13/C14), 57.00 (CH_2), 122.55, 124.69, 125.13, 125.37 (ArCH), 141.21, 144.33 (C_4). HRMS (ESI) calculated for $C_{26}H_{37}N_2$: [M^+ + H] 377.2961: found 377.2961.

8.1.2.2 9,10-Dihydro-*N,N*-dimethyl-9,10-ethanoanthracene-11-methanamine (23)

9,10-Dihydro-*N,N*-dimethylamino-9,10-ethanoanthracene-11-carboxyamide (**9**) (1 mmol, 0.27 g) was added to $LiAlH_4$ according to general procedure 2 above and purified by flash column chromatography over silica gel (eluent: 95 % DCM/MeOH) to afford the product as colourless crystals (86 %), M.p. 83-84 ˚C[52]. IR ν_{max} (KBr) 1456 (ArC=C) 2940 (ArH) cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.21 (1H, ddd, J = 16.8 Hz, , J = 12.3 Hz, J = 2.5 Hz, H11), 2.01, 2.19 (2H, 2m, 2H12), 2.37 (6H, s, $2CH_3$), 4.29 (1H, s, H9), 4.51 (1H, s, H10), 7.26 (4H, m, ArH), 7.32 (2H, m, ArH), 7.43 (2H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 32.84 (CH_2, C12), 35.52 (C11), 43.90 (C9), 44.90 (CH_3), 46.38 (C10), 63.75 (CH_2, C13), 123.18, 123.54, 123.66, 125.73, 125.77, 125.79, 125.93, 126.17 (ArCH), 139.48, 142.84, 143.13, 143.30 (C_4). HRMS (ESI) calculated for $C_{19}H_{22}N$: $[M^+ + H]$ 264.1752: found 264.1749.

8.1.2.3 9,10-Dihydro-*N*-pyrrolidinyl-9,10-ethanoanthracene-11-methanamine (26)

9,10-Dihydro-11-*N*-pyrrolidinyl-9,10-ethanoanthracene-11-carboxyamide (**12**) (1 mmol, 0.29 g) was reacted with $LiAlH_4$ according to general procedure 2 above. The crude product was then purified by flash column chromatography over silica gel (eluent: 95 % DCM: MeOH) to afford a pale brown solid (50 %), M.p. 92-94 ˚C. IR ν_{max} (KBr) 1457 (Ar C=C), 2941(Ar C-H) cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.23 (1H, m, H11), 1.80 (4H, br s, H15), 1.96 (2H, m, H12), 2.15 (2H, m, H13), 2.53 (4H, br m, H14), 4.27 (1H, s, H9), 4.39 (1H, s, H10), 7.10 (4H, m, ArCH), 7.26 (4H, m, ArCH). ^{13}C NMR ppm ($CDCl_3$) 23.01 (CH_2), 33.04 (C12), 36.79 (C9), 43.67 (C10), 46.59 (C11), 54.04 (CH_2), 64.47 (C13) 122.55, 122.97, 125.04, 125.18 (ArH), 140.05, 143.44 (C_4). HRMS (ESI) calculated for $C_{21}H_{24}N$: $[M^+ + H]$ 290.1909: found 290.1898.

8.1.2.4 9,10-Dihydro-*N*-methyl-*N*-cyclohexanyl-9,10-ethanoanthracene-11-methanamine (27)

9,10-Dihydro-11-*N*-methyl-*N*-cyclohexanyl-9,10-ethanoanthracene-11-carboxyamide (**13**) (1 mmol, 0.33 g) was reacted with $LiAlH_4$ according to general procedure 2 above. The crude product was then purified by flash column chromatography over silica gel (eluent: 95 % DCM/MeOH) to afford a pale brown solid (50%), M.p. 80-82 ˚C. IR ν_{max} (KBr) 1456 (ArC=C), 2927 (ArCH) cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.05-1.23 (6H, m, H16, H17), 1.62 (1H, d, J = 12.5 Hz, H11), 1.79 (4H, br s, H15), 2.00 (3H, m, CH_3), 2.15-2.40 (5H, 2m, H12, H13, H14), 4.30 (1H, s, H10), 4.46 (1H, s, H9), 7.13-7.17 (4H, m, ArH), 7.25-7.40 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 25.62, 25.81, 27.41, 28.19 (CH_2), 32.80 (CH_2 C12), 37.81 (C11), 43.79 (C10), 46.09 (C9), 57.98 (CH_2 C13), 63.34 (CH_3), 122.41, 122.93, 122.99, 124.88, 125.04 (ArCH), 125.08, 125.24, 125.38 143.71 (C_4). HRMS (ESI) calculated for $C_{24}H_{30}N$: $[M^+ + H]$ 332.2378: found 332.2373

8.1.2.5 9,10-Dihydro-*N*-ethyl-9,10-ethanoanthracene-11-methanamine (28)

9,10-Dihydro-11-*N*-ethylamino-9,10-ethanoanthracene-11-carboxyamide (**14**) (1 mmol, 0.26 g) was reacted with $LiAlH_4$ according to general procedure 2 above and the product was purified by flash column chromatography over silica gel (eluent: 95 % DCM/MeOH) to afford a brown oil (33 %). IR ν_{max} (film) 1466 (ArC=C), 2943 (ArCH) cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.12 (3H, t, J = 7.0 Hz, CH_3), 1.19 (1H, m H11), 2.01 (H, m, H12), 2.18 (1H, m, H12), 2.25 (2H, d, J = 7.0 Hz, H13), 2.60 (2H, m, CH_2), 4.30 (1H, s, H10), 4.34 (1H, s, H9), 7.12 (4H, m, ArH), 7.30 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 14.59 (CH_3), 32.73 (C12), 38.07 (C11), 43.64 (CH_2), 43.69 (C10), 46.42 (C9), 54.14 (C13), 122.60, 122.92, 123.00, 124.74, 125.03, 125.14, 125.39,

125.42 (ArCH), 143.22, 143.38, 143.58 (C_4). HRMS (ESI) calculated for $C_{19}H_{22}N$: [M^+ + H] 264.1752: found 264.1758

8.1.2.6 9,10-Dihydro-*N*-anilinyl-9,10-ethanoanthracene-11-methanamine (30)

9,10-Dihydro-11-*N*-anilinyl-9,10-ethanoanthracene-11-carboxyamide (**17**) (1 mmol, 0.33 g) was reacted with $LiAlH_4$ according to general procedure 2 above. The crude product was then purified by flash column chromatography over silica gel (eluent: 95 % DCM: MeOH) to afford a brown resin (57 %). IR ν_{max} (KBr) 2853 (ArCH), 3410 (NH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.05 (2H, m, H13), 2.25 (1H, m, H11), 2.70 (1H, m, $H12_a$), 2.79 (m, $H12_b$), 3.70 (1H, NH), 4.33 (1H, t, J = 3.0 Hz, H10), 4.38 (1H, d, J = 2.0 Hz, H9), 6.58 (2H, d, J = 7.5 Hz, H17), 6.72 (2H, t, J = 7.5 Hz, H15), 7.13-7.22 (6H, m, ArH, H16), 7.30 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 32.28 (CH_2 C12), 37.90 (C11), 43.68 (C10), 46.31 (C9), 48.43 (CH_2 C13), 112.36 (C17), 122.76 (C15), 128.81 (C16), 123.04, 124.93, 125.25, 125.30, 125.64 (ArCH), 139.99, 143.39, 143.21 (C_4), 147.79 (C_4 NH). HRMS (ESI) calculated for $C_{23}H_{22}N$: [M^+ + H] 312.1752: found 312.1748.

8.1.2.7 9,10-Dihydro-11-4-(4-chlorophenyl)piperazinyl-9,10-ethanoanthracene-11-methanamine (31)

9,10-Dihydro-11-(4-{4-chlorophenyl}-piperazinyl)-9,10-ethanoanthracene-11-carboxyamide (**18**) (0.49 mmol, 0.20 g) was reacted with $LiAlH_4$ according to general procedure 2 above. The crude product was then purified by flash column chromatography over silica gel (eluent: 95 % DCM/MeOH) to afford a pale brown oil (66 %). IR ν_{max} (film) 751 (C-Cl), 1598 (ArC=C), 2941 (ArCH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.25 (1H, br m, H11), 2.01 (3H, br s, CH_2, $H12_a$), 2.25 (1H, br s, $H12_b$), 2.51 (2H, br s, CH_2), 2.62 (2H, br s, CH_2), 3.23 (4H, br s, CH_2), 4.34 (1H, br s, H10), 4.45 (1H, br s, H9), 6.91 (2H, br m, ArH), 7.10 (4H, m, ArH), 7.28 (6H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 32.69 (C12), 35.25 (C11), 43.79 (C10), 46.74 (C10), 48.78 (CH_2), 52.81, 52.98 (NCH_2), 63.07, 63.15 (NCH_2), 115.61, 116.75, 119.20, 122.63, 123.01, 125.03, 125.07, 125.16, 125.21, 125.35, 128.50, 128.68 (ArCH), 140.46, 143.43, 143.51, 143.84 (C4), 149.61 (C_4N), 150.99 (C_4Cl). HRMS (ESI) calculated for $C_{27}H_{27}NCl$: [M^+ + H] 415.1941: found 415.1939.

8.1.2.8 9,10-Dihydropiperazinyl-9,10-ethanoanthracene-11-methanamine (35)

Preparation from 9,10-dihydro-11-piperizinyl-9,10-ethanoanthracene-11-carboxyamide **(34)** (0.047 mmol, 13 mg) was reacted with $LiAlH_4$ following the general procedure 2 above. The crude product was then purified by flash column chromatography over silica gel (eluent: 95 % DCM/MeOH) to afford the product as a pale resin (48 %). IR ν_{max} (film) 3418.38(NH), 2926.50(CH),1651.38,1457.45 cm^{-1}. ^{1}H NMR δ ($CDCl_3$): 0.91(1H, m, $H12_a$), 1.15(1H, m, $H12_b$),1.93(1H, m, H11), 2.10(2H, m, CH_2), 2.48(4H, br s, CH_2), 2.72(4H, br s, CH_2), 3.14(1H, s br, NH), 4.27(1H, s br, H-9), 4.32(1H, s br, H-10), 7.12(4H, m, ArH), 7.29(4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 29.41(CH_2), 32.38(CH_2), 35.01(C12), 35.16(C11), 43.63(C9), 46.42(C10), 52.69(CH_2), 62.77(CH_2), 122.65, 122.97, 124.89, 124.96, 125.05, 125.17, 125.25, 125.31(ArCH), 140.50, 144.00(C_4). HRMS (ESI) calculated for $C_{21}H_{25}N_2$: [M^+ + H] 305.2018: found 305.2018.

8.1.3 General Procedure 3: hydrolysis of esters

The appropriate ester (10 mmol) was dissolved in EtOH (100 mL) and an aqueous solution of KOH (5M, 150 mL) and heated at reflux for 3 hours. After this time, the solution was diluted with water (50 mL) and washed with diethyl ether. The aqueous layer was acidified with HCl

(2M) and the product was extracted using ethyl acetate (3 x 50 mL). The organic phase wash washed with brine (3 x 25 mL), dried over anhydrous Mg_2SO_4 and the solvent was evaporated *in vacuo*. The product required no further purification.

8.1.3.1 2-(9,10-Dihydro-9,10-ethanoanthracene-11-carboxamido)-3-(4-hydroxyphenyl) propanoic acid (32)

Methyl 2-(9,10-dihydro-9,10-ethanoanthracene-11-carboxamido)-3-(4-hydroxyphenyl) propanoate (**19**) (1.45 mmol, 0.62 g) was reacted with KOH (5M, 15 mL) according to general procedure 3 above. The product was obtained as pale brown crystals and no further purification was required, (98 %), M.p. 161-163 ˚C. IRν_{max} (KBr) 1723 (OHC=O), 1650 (NHC=O), 3022, 3308 (OH) cm^{-1}. ^{1}H NMR δ (CD_6SO) 1.70 (1H, m, $H12_a$), 1.72* (1H, m, $H12_a$), 1.85* (1H, m, $H12_b$), 1.99 (1H, m, $H12_b$) 2.64 (1H, m, H11), 2.66* (1H, m, H11), 2.73 (1H, m, H14), 2.77* (1H, m, H14), 2.90 (1H, m, H14), 2.98* (1H, m, H14), 4.18 (1H, m, H13), 4.20* (1H, m, H13), 4.32 (1H, m, H10), 4.34 (1H, m, H10), 4.36* (1H, m, H9), 4.95* (1H, m, H9), 6.61* (2H, d, J = 8.0 Hz, Ar<u>CH</u>COH), 6.80 (2H, d, J = 8.0 Hz, Ar<u>CH</u>COH), 6.96* (2H, d, J = 8.0 Hz, Ar<u>CH</u>CCH_2), 7.14 (2H, d, J = 8.0 Hz, Ar<u>CH</u>CCH_2), 7.10-7.35 (16H, m, ArH). ^{13}C NMR ppm (CD_6SO) 26.68, 30.12* (C12), 36.63, 36.44* (CH14), 44.00, 44.20* (C11), 54.54 (C13), 43.30, 43.40* (C10), 47.70, 47.90* (C9), 130.40*, 130.60 (Ar<u>CH</u>CCH_2), 115.30*, 115.50 (Ar<u>CH</u>COH), 128.20, 127.80* ($\underline{C_4}CH_2$), 120-140 (ArCH), 155.20, 156.0 ($\underline{C_4}$OH). HRMS (ESI) calculated for $C_{26}H_{23}NO_4Na$: [M^+ + Na] 436.1525: found 436.1523.

8.1.3.2 9,10-Dihydro-9,10-ethanoanthracene-11-carboxamido-4-methylpentanoic acid (33)

Methyl-2-[9,10-dihydro-9,10-ethanoantracene-11-carboxyamido]-4-methyl-pentoate (**20**) (0.52 mmol, 0.2 g) was treated with KOH (5M, 5 mL) according to the general procedure 3 above. The product was obtained as colourless crystals and no further purification was required, (89 %), M.p. 169-172 ˚C. IRν_{max} (KBr) 3105 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.86 (6H, s, CH_3), 1.49 (1H, br s, $CH_3\underline{CH}CH_3$), 1.96 (1H, m, $H12_a$), 2.12-2.16 (3H, br m, CH_2, $H12_b$), 2.85 (1H, m, H11), 5.56, 5.75 (1H, 2 s, NH), 7.14 (4H, m, ArH), 7.28 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 20.43, 21.23*, 21.29, 22.37* (CH_3), 24.23, 24.33* ($CH_3\underline{CH}CH_3$), 31.60, 31.89* (C12), 40.53, 40.48* (CH_2), 43.29, 43.35* (C10), 44.97, 45.19* (C9), 46.73*, 46.95 (C11), 50.34, 50.42* (CH), 122.95, 123.00*, 123.08, 123.20*, 123.27, 125.00*, 125.54, 125.76* (ArCH), 126.04, 126.16*, 138.96, 139.16*, 142.22, 142.23*, 142.66, 142.75* (C_4), 173.93 (COOH), 175.00 (NHC=O). HRMS (ESI) calculated for $C_{23}H_{25}NO_3Na$: [M^+ + Na] 386.1732: found 386.1721.

8.1.3.3 *trans*-9-10-Dihydroxymethyl-9,10-ethanoanthracene (36)

A solution of compound *trans*-11,12-dihydroxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**4**) (5.7 mmol, 2.00 g) in dry THF (20 mL) was added dropwise to a solution of $LiAlH_4$ (18.3 mmol, 0.695 g) in dry THF (20 mL). The mixture was refluxed for 3 hours and then quenched with the careful addition of water (25 mL) and then HCl (1M, 25 mL). The aqueous phase was extracted with diethyl ether. The organic phase was then washed with water, dried over anhydrous Mg_2SO_4 and solvent evaporated *in vacuo* to give a colourless powder. The product was then purified using flash column chromatography over silica gel (eluent: DCM) and washed with methanol to elute the product, colourless needles (40 %), M.p. 194-198 °C [57]. IRν_{max} (KBr) 3280 (OH), 1076 (C-O) cm^{-1}. ^{1}H NMR δ (DMSO-d) 1.27 (2H, m, H11), 2.75 (2H, m, H12), 3.10 (2H, m, H12), 4.34 (2H, s, H9), 4.68 (2H, t, J = 5.0 Hz, OH), 7.08-7.27 (8H, m, ArH). ^{13}C NMR ppm (DMSO-d**)** 45.28 (C11), 45.96 (C9/C10), 64.42

(C12), 123.45, 125.61, 125.84, 126.07 (C1-C8), 141.68, 144.44 (C_4). HRMS (ESI) calculated for $C_{18}H_{18}O_2Na$: [M^+ + Na] 289.1204: found 289.1218.

8.1.3.4 11-Hydroxymethyl-9,10-ethanoanthracene (37)

A solution of compound 11-ethoxycarbonyl-9,10-dihydro-9,10-ethanoanthracene (**5**) (1.7 mmol, 0.50 g) in dry THF (20 mL) was added dropwise to a solution of $LIAlH_4$ (8.16 mmol, 0.31 g) in dry THF (20 mL). The mixture was refluxed for 3 hours and then quenched with the careful addition of water (25 mL) and then HCl (1M, 25 mL). The aqueous phase was extracted with diethyl ether. The organic phase was then washed with water, dried over anhydrous Mg_2SO_4 and solvent was evaporated *in vacuo*. The product was purified by flash column chromatography (eluent: 1:1 hexane/ethyl acetate) and recrystallised from methanol as a colourless powder (38 %), M.p. 96-98 °C[50]. IR ν_{max} (KBr) 3290 (OH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.08 (1H, ddd, J = 17.0 Hz, J = 12.1 Hz, J = 2.5 Hz, H11) 1.93 (1H, m, $H12_a$), 2.15 (1H, m, $H12_b$), 2.98 (1H, t, J = 10.0 Hz, H13), 3.35 (1H, q, J = 5.0 Hz, H13), 4.29 (1H, t, J = 2.5 Hz, H10), 4.46 (1H, d, J = 2.0 Hz, H9), 7.15 (4H, m, ArH), 7.28 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 30.55 (C12), 40.48 (C10), 43.56 (C11), 45.05 (C9), 65.63 (CH_2), 122.68, 122.99, 123.11, 124.86, 125.18, 125.23, 125.25, 125.53 (ArCH), 140.02, 143.35, 143.40 (C_4). HRMS (ESI) calculated for: $C_{17}H_{17}O$ [M^+ + H] 237.3163: found 237.3182

8.1.3.5 (*E*)-3-(9-Anthracenyl)-*N,N*-dimethylacrylamide (51)

3-(9-Anthracenyl)acrylic acid (**50**) (2.01 mmol, 0.50 g) and dimethylamine hydrochloride (3.60 mmol, 1.12 g) were reacted according to the general procedure 1 above. The residue was purified by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate) to afford the product as a dark brown solid (87 %), M.p**.** 77-80 ˚C. IRν_{max} (KBr) 1645 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 3.17 (6H, s, CH_3), 6.86 (1H, d, J = 15.5 Hz, H12), 7.51 (4H, m, ArH), 8.29 (2H, d, J = 9.5 Hz, H4/H5), 8.29 (2H, d, J = 9.5 Hz, H1/H8), 8.60 (1H, s, H10), 8.59 (1H, d, J = 15.5 Hz, H11). ^{13}C NMR ppm ($CDCl_3$) 34.43 (C9), 36.00 (CH_3), 37.47 (CH_3), 125.26, 125.33, 125.59, 126.00, 126.70, 127.24, 127.51, 128.47, 128.76, 132.84 (ArCH, C12), 139.68 (C11), 166.16 (C=O). HRMS (ESI) calculated for $C_{19}H_{18}NO$: [M^+ + H] 276.1388: found 276.1380.

8.1.3.6 (*E*)-3-(9-Anthracenyl)-*N,N*-diethylacrylamide (52)

3-(9-Anthracenyl)acrylic acid (**51**) (2.01 mmol, 0.50 g) and *N,N*,-diethylamine hydrochloride (3.60 mmol, 1.22 g) were reacted according to the general procedure 1 above. The residue was purified by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate) to afford an orange solid (78 %), M.p**.**78-81 ˚C. IRν_{max} (KBr) 1649 (C=O), 2974 (Ar C=C) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.29 (6H, s, CH_3), 3.46-3.63 (4H, br d, J = 6.0 Hz, CH_2), 6.80 (1H, d, J = 15.5 Hz, H12), 7.50 (4H, m, ArH), 8.03 (2H, m, H4/H5), 8.30 (2H, m, H1/H8), 8.49 (1H, s, H10), 8.65 (1H, d, J = 15.5 Hz, H11). ^{13}C NMR ppm ($CDCl_3$) 12.88, 14.80 (CH_2), 40.78, 41.88 (CH_3), 124.87, 125.17, 125.49, 125.64, 128.29 (ArCH), 126.98 (C12), 128.95, 130.65, 130.83 (C_4), 139.15 (C11), 164.68 (C=O). HRMS (ESI) calculated for $C_{21}H_{22}NO$: [M^+ + H] 304.1714: found 304.1701.

8.1.3.7 (*E*)-3-(9-Anthracenyl)-1-(piperidinyl)prop-2-en-1-one (53)

3-(9-Anthracenyl)acrylic acid (**50**) (2.01 mmol, 0.50 g) and piperidine (3.60 mmol, 1.13 g) were reacted according to the general procedure 1 above. The residue was purified by flash

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column chromatography over silica gel (eluent: 85.15, hexane/ethyl acetate) to afford the product as a yellow solid (54 %), M.p. 92-98 ˚C. IRν_{max} (KBr) 1655 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.68 (6H, br s, CH_2), 3.56 (4H, br s, CH_2), 6.90 (1H, d, J = 15.0 Hz, H12), 7.54 (4H, m, ArH), 8.04 (2H, d, J = 8.0 Hz, ArH), 8.31 (2H, d, J = 8.0 Hz, ArH), 8.48 (1H, s, H10), 8.59 (1H, d, J = 15.0 Hz, H11). ^{13}C NMR ppm ($CDCl_3$) 24.19 (CH_2), 25.89 (CH_2), 124.85, 125.16, 125.50, 128.14, 128.29 (ArCH), 127.33 (C12), 128.93, 130.83 (C_4), 139.07 (C11), 164.32 (C=O). HRMS (ESI) calculated for $C_{22}H_{22}NO$: [M^+ + H] 316.1701: found 316.1692.

8.1.3.8 (*E*)-3-(9-Anthracenyl)-1-(pyrrolidinyl)prop-2-en-1-one (54)

3-(9-Anthracenyl)acrylic acid (**50**) (2.01 mmol, 0.50 g) and pyrrolidine (3.60 mmol, 1.08 g) were reacted according to the general procedure 1 above. The residue was purified by flash column chromatography over silica gel (eluent: 85:15, hexane/ethyl acetate) to afford the product as an orange powder (63 %), M.p. 94-95 ˚C. IRν_{max} (KBr) 1656 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.00(4H, br s, CH_2), 3.66 (4H, 2s, CH_2), 6.73 (1H, d, J = 16.0 Hz, H12), 7.50 (4H, m, ArH), 8.02 (2H, m, H4/H5), 8.29 (2H, m, H1/H8), 8.45 (1H, s, H10), 8.63 (1H, d, J = 16.0 Hz, H11). ^{13}C NMR ppm ($CDCl_3$) 46.49 (CH_2), 53.01 (CH_2), 125.25, 125.34, 125.61, 125.80, 126.02, 127.60, 127.85, 128.76 (ArCH) 127.02 (C12), 129.43, 130.84, 131.28 (C_4), 139.36 (C11), 164.28 (C=O). HRMS (ESI) calculated for $C_{21}H_{20}NO$: [M^+ + H] 302.1545: found 302.1555.

8.1.3.9 (*E*)-3-(9-anthracenyl)-1-(*N*-methylpiperazinyl)prop-2-en-1-one (55)

3-(9-Anthracenyl)acrylic acid (**50**) (2.01 mmol, 0.50 g) and *N*-methylpiperazine (3.60 mmol, 1.18 g) were reacted according to the general procedure 1 above. The residue was purified by flash column chromatography over silica gel (eluent: 85:15, hexane/ethyl acetate) to afford the product as a brown solid (67 %), M.p. 120-125 ˚C. IRν_{max} (KBr) 1644 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.48 (3H, s, CH_3), 2.66 (4H, 2 x br s, CH_2), 3.79 (2H, br s, CH_2), 4.01 (2H, br s, CH_2), 6.84 (1H, d, J = 15.5 Hz, H12), 7.52 (4H, m, ArH), 8.03 (2H, m, H5/H4), 8.25 (2H, m, H1/H8), 8.48 (1H, s, H10), 8.73 (1H, d, J = 15.5 Hz, H11). ^{13}C NMR ppm ($CDCl_3$) 30.52 (CH_3), 45.24 (CH_2), 49.50 (CH_2), 124.89, 124.98, 125.55, 125.65, 127.24 (C12), 130.82, 128.91, 128.35 (C_4), 140.08 (C11), 164.38 (C=O).. HRMS (ESI) calculated for $C_{22}H_{23}N_2O$: [M^+ + H] 331.1810: found 331.1810.

8.1.3.10 (*E*)-Ethyl-4-(3-(9-anthracenyl)acryloyl)piperazine-1-carboxylate (56)

3-(9-Anthracenyl)acrylic acid (**50**) (2.01 mmol, 0.50 g) and ethyl piperazine-1-carboxylate (3.6 mmol, 0.56 g) were reacted according to the general procedure 1 above. The residue was purified by flash column chromatography over silica gel (eluent: 85:15, hexane/ethyl acetate) to afford the product as orange crystals (65 %), M.p. 80-85 ˚C. IRν_{max} (KBr) 1648 (C=O), 1693 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.30 (3H, m, CH_3), 3.60 (6H, 2br s CH_2), 4.82 (2H, br s, CH_2), 4.17 (4H, m, CH_2), 6.85 (1H, d, J = 15.5 Hz, H11), 7.55 (4H, m, ArH), 8.04 (2H, d, J = 7.0 Hz, ArH), 8.25 (2H, d, J = 7.0 Hz, ArH), 8.49 (1H, s, H10), 8.68 (1H, d, J = 15.5 Hz, H12). ^{13}C NMR ppm ($CDCl_3$) 40.55 (CH_2), 20.62 (CH_3), 59.96, 61.01 (CH_2), 124.73, 128.48, 129.07, 130.63 (C_4), 124.90, 125.00, 125.72, 127.28, 127.57, 127.81, 128.33 (ArCH), 131.87 (C11). HRMS (ESI) calculated for $C_{24}H_{24}N_2O_3Na$: [M^+ + Na] 411.4487: found 411.1691.

8.1.3.11 (*E*)-3-(9-Anthracenyl)-1-(4-(*p*-tolyl)piperazinyl)prop-2-en-1-one (57)

3-(9-Anthracenyl)acrylic acid (**50**) (2.01 mmol, 0.50 g) and *p*-toluylpiperazine (3.60 mmol, 0.63 g) were reacted according to general procedure above. The residue was purified by

flash column chromatography over silica gel (eluent: 85:15, hexane/ethyl acetate) to afford the product as orange crystals (67 %), M.p**.** 76-78 ˚C. IRv_{max} (KBr) 1643 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.61 (3H, s, CH_3), 3.24 (4H, br d, J = 30.0 Hz, CH_2), 3.86 (2H, br s, CH_2), 4.04 (2H, br s, CH_2), 6.90 (1H, d, J = 15.5 Hz, H11), 7.13 (2H, d, J = 8.0 Hz, ArH), 7.51 (4H, m, ArH), 8.04 (2H, dd, J = 5.0 Hz, J = 8.0 Hz, ArH), 8.28 (2H, dd, J = 5.0 Hz, J = 8.0 Hz, ArH), 8.47(1H, s, H10), 8.69 (1H, d, J = 15.5 Hz, H12). ^{13}C NMR ppm ($CDCl_3$) 20.62 (CH_3), 59.96, 61.01 (CH_2), 124.73, 124.90, 125.00, 125.72, 127.01, 127.28, 128.48 (ArCH), 127.57 (C12), 131.84 (C11), 129.07, 130.63 (C_4), 164.98 (C=O). HRMS (ESI**)** calculated for $C_{28}H_{27}N_2O$: [M^+ + H] 407.2123: found 407.2174.

8.1.3.12 3-(9-Anthracenyl)-*N*-methylacrylamide (58)

Methylamine (10 mL of 2M solution in THF) was added to 3-(9-anthryl)-acrylic acid ethyl ester (**47**) (5.71 mmol, 2 g) and stirred at 110 °C in a sealed tube for 24 hours. Yellow needles precipitated during reaction were filtered off and washed with ethyl acetate (10 mL). The filtrate was evaporated to remove the excess methylamine. Water (25 mL) was added to the residue and the product was extracted using ethyl acetate (3 x 25 mL). The organic phase was washed with brine (3 x 25 mL), dried over anhydrous Mg_2SO_4 and the solvent evaporated *in vacuo*. The product was then purified flash column chromatography over silica gel (eluent: 85:15, hexane/ethyl acetate) followed by recrystallisation from dichloromethane as yellow needles (95 %), M.p. 240 ˚C. IRv_{max} (KBr) 3296 (N-H, s), 1563 (N-H, b), 1360 (C-N), 1651 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 3.05 (3H, s, CH_3), 5.75 (1H, br s, NH), 6.34 (1H, d, J = 14.5 Hz, H12), 7.49 (4H, m, H2/H7, H3/H6), 8.02 (2H, m, H4/H5), 8.23 (2H, m, H1/H8), 8.45 (1H, s, H10), 8.55 (1H, d, J = 14.5 Hz, H11). ^{13}C NMR ppm ($CDCl_3$) 26.16 (CH_3), 125.30 (C3/C6), 125.5 (C1/C8), 126.00 (C2/C7), 128.8 (C4/C5), 129.20 (C_4), 129.50 (C12), 130.00 (C9), 131.30 (C_4), 137.85 (C11), 165.50 (C=O). HRMS (ESI) calculated for $C_{18}H_{15}NONa$: [M^+ + Na] 284.1051: found 284.1052.

8.1.3.13 (*E*)-9-(4-Nitrostyryl)anthracene (49)

(4-Nitrophenyl)triphenlphosphonium bromide (2.18 mmol, 1.04 g) and 60 % NaH in oil (2.9 mmol, 0.12 g) were stirred for 30 min in anhydrous THF (20 mL), in an inert atmosphere, at 0 ˚C. 9-Anthraldehyde **46** (1.45 mmol, 0.3 g) in dry THF (10 mL) was added dropwise to the solution and it was heated at reflux for 12 h. After this time the reaction was quenched with water (1-5 mL). The product was diluted with water (20 mL) and the product extracted with dichloromethane (3 x 20 mL). The solvent was removed *in vacuo* and the residue was purified by flash column chromatography over silica gel (eluent: 85:15 hexane/ethyl acetate) to afford the product as orange crystals (35 %), M.p. 89-91 ˚C. IRv_{max} (KBr) 1339, 1512 (NO_2) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 7.05 (1H, d, J = 16.5 Hz, H12), 7.34 (2H, d, J = 8.8 Hz, H13), 7.53 (4H, m, ArH), 7.83 (2H, d, J = 8.8 Hz, H14), 8.15 (1H, d, J = 17.0 Hz, H11), 8.32 (4H, m, ArH), 8.49 (1H, s, H10). ^{13}C NMR ppm ($CDCl_3$) 124.28 (C15), 125.30, 125.46, 126.03, 127.40 (ArCH) 127.02 (C14), 128.92 (ArCH), 129.87 (C11), 129.64, 131.27, 131.46 (C_4), 135.15 (C12), 143.63 (C_4), 148.04 (C_4 NO_2). HRMS (ESI) calculated for $C_{22}H_{15}NO_2Na$: [M^+ + Na] 348.1000: found 348.1007.

8.1.3.14 (E)-4-(9-Anthracenyl)-*N*-methylbut-3-en-2-amine (59)

To a solution of (*E*)-4-(9-anthracenyl)but-3-en-2-one (**48**) (1.12 mmol, 0.27 g) in dry MeOH (20 mL) was added $NaCNBH_3$ (1.57 mmol, 0.10 g) and methylamine hydrochloride (8.96

mmol, 0.60 g). This solution was stirred under an atmosphere of N_2 for 72 hours as indicated by TLC for completion of the reaction. The pH was adjusted to 5-6 with 4M methanolic HCl. When the reaction was complete, excess hydride was quenched using 10 % aq. HCl (25 mL). The aqueous solution was washed with dichloromethane (3 x 25 mL). The aqueous phase was basified with 2M NaOH and extracted with dichloromethane (3 x 25 mL). The organic phases were combined and dried with anhydrous Mg_2SO_4 and the solvent was evaporated *in vacuo*. The crude product was purified by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate) to afford a pale oil (89 %). IRv_{max} (film) 3109 (NH) cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.55 (3H, d J = 6.5 Hz, CH_3), 3.59 (3H, s, $\underline{CH_3}NH$), 4.24 (1H, q, J = 6.5 Hz, H13), 6.00 (1H, dd, J = 16.0 Hz, J = 7.0 Hz, H12), 7.35 (1H, d, J = 16.5 Hz, H11), 7.50 (4H, m, ArH), 8.03 (2H, m, ArH), 8.30 (2H, m, ArH), 8.45 (1H, s, H10). ^{13}C NMR ppm ($CDCl_3$) 13.69 (CH_3), 56.08 ($\underline{CH_3}NH$), 78.06 (C13), 124.67, 125.01, 125.23, 125.33, 125.92, 128.23 (ArCH), 126.67 (C11), 128.98, 130.94, 131.76 (C_4, C9), 139.74 (C12). HRMS (ESI) calculated for $C_{19}H_{20}N$: [M^+ + H] 262.1596: found 262.1601.

8.1.3.15 3-(9-Anthracenyl)propanenitrile (60)

Morpholine (0.70 mL) was added to a solution of anthraldehyde (5 mmol, 1 g) and cyanoacetic acid (5.8 mmol, 0.49 g) in DMF (10 mL). The mixture was refluxed for 7 h and then left at -20 °C overnight to allow precipitation of the product. The filtrate was diluted with water (15 mL) to allow further precipitation of the product. The product was combined, filtered and recrystallised from toluene to afford yellow crystals (30 %), M.p. 150-155 °C[38] IRv_{max} (KBr) 2217 (CN) cm^{-1}. ^1H NMR δ ($CDCl_3$) 5.65 (1H, d, J = 17.0 Hz, H12), 7.56 (4H, 2t, J = 6.5 Hz, ArH), 8.06 (2H, d, J = 8.5 Hz, H4/H5), 8.17 (2H, d, J = 8.5 Hz, H1/H8), 8.35 (1H, d, J = 17.0 Hz, H11), 8.53 (1H, s, H10). ^{13}C NMR ppm ($CDCl_3$) 105.14 (C12), 117.18 (CN), 123.96, 125.12, 126.58, 128.89 (ArCH), 124.28, 126.27 (C_4), 127.62 (C9), 130.66 (C10) 148.09 (C11). HRMS (ESI) calculated for $C_{17}H_{11}NNa$: [M^+ + Na] 252.0789: found 252.0791.

8.1.3.16 3-(9-Anthracenyl)-2-cyanoacrylic acid (61)

Morpholine (0.70 mL) was added to a solution of anthraldehyde **46** (5.00 mmol, 1.00 g) and cyanoacetic acid (5.80 mmol, 0.49 g) in DMF (10 mL). The mixture was heated at 90 °C for 1 hour. A solution of KOH (1 g) in water: methanol (1:2) (1.5 mL) was added, followed by diethyl ether (5 mL) which caused a yellow precipitate to form. The mixture was filtered and washed with ether and recrystallised from methanol. The crystals were dissolved in water and then acidified using 10 % aq. HCl. This caused a bright orange solid to precipitate. The orange crystals were recrystallised from dichloromethane, (76 %), M.p**.** 70 °C. IRv_{max} (KBr) 2224 (CN), 3351 (OH) cm^{-1}. ^1H NMR δ ($CDCl_3$) 7.56-7.65 (4H, dt, J = 7.0 Hz, H2/H7, H3/H6), 8.00 (2H, d, J = 8.5 Hz, H5/H4), 8.14 (2H, d, J = 8.5 Hz, H5/H4), 8.68 (1H, s, H10), 9.31 (1H, s, H11). ^{13}C NMR ppm ($CDCl_3$) 114.65, 114.8 (CN, C12), 124.87, 125.90, 127.32, 128.16 (ArCH), 128.99, 129.78 (ArC_4), 130.56 (C10), 154.79 (C11), 162.20 (C=O). HRMS (ESI) calculated for: (M^+ - H) 272.0717: found 272.0732.

8.1.4 General procedure 4 - Preparation of dihydroethanoanthracenes

The appropriate anthracenyl compound (10 mmol) and acrylonitrile (14 mmol), with hydroquinone (0.2 mmol) were heated together in a sealed tube, at 130 ˚C for 24 h (method A) or heated by microwave irradiation at 160 ˚C for 45 min (method B). The reaction mixture was then decanted into a large beaker with ethyl acetate (10-20 mL). This was allowed to

evaporate using air and N_2. This process was repeated to allow the excess acrylonitrile to evaporate. The solid that remained was filtered and washed with hexane. The crude product was then purified flash column chromatography over silica gel and recrystallised from methanol.

8.1.4.1 9-(2-Cyanovinyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (69) was obtained from 3-(9-anthracenyl)acrylonitrile (**60**) (2.18 mmol, 0.5 g) according to general procedure 4 above (method A). The product was purified by flash column chromatography over silica gel (eluent: 2:1 hexane/ethyl acetate) to afford a colourless powder (67 %), M.p. 222-226 °C. IRν_{max} (KBr) 2228 (CN), 3055 (C=C) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.93 (1H, ddd, J = 3.4 Hz, J = 14.8 Hz, H12$_a$), 2.30 (1H, m, H12$_b$), 3.57 (1H, dd, J = 3.9 Hz, J = 10.3 Hz, H11), 4.60 (1H, s, H10), 6.46 (1H, d, J = 17.1 Hz, H14), 7.25 (5H, m, ArH), 7.49 (3H, m, ArH), 8.08 (1H, d, J = 16.6 Hz, H13). ^{13}C NMR ppm ($CDCl_3$) 30.51 (C10), 39.75 (C9), 41.81 (C11), 105.23 (CN vinyl), 115.62 (CN alkyl), 122.99, 123.30, 123.79, 124.16, 126.05, 126.14, 127.17, 127.24 (ArCH, C14), 138.99, 140.20, 142.26, 142.64 (C_4), 151.32 (C13). HRMS (ESI) calculated for $C_{20}H_{14}N_2Na$: [M^+ + Na] 305.1055: found 305.1047.

8.1.4.2 9-Formyl-9,10-dihydro-11-cyano-9,10-ethanoanthracene (63)

Anthraldehyde (91.39 mmol, 4 g), acrylonitrile (164.82 mmol, 8.75 g) were reacted together according to general procedure 4 above, (method A or method B). The product was then purified by flash column chromatography over silica gel (eluent: 2:1 hexane/ethyl acetate), followed by recrystallisation from methanol to afford pale yellow crystals (48 %)^A (70 %)^B, M.p. 160 °C (lit[56] M.p. 172-176 ˚C). IRν_{max} (KBr) 2239 (CN), 1727 (C=O), 2831, 2728 (C-H aldehyde) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.10 (1H, m, H12$_a$), 2.35 (1H, m, H12$_b$), 3.21 (1H, dd, J = 4.7 Hz, J = 10.5 Hz, H10), 4.47 (1H, s, H11), 7.19-7.5 (8H, ArH), 10.93 (1H, s, H13). ^{13}C NMR ppm ($CDCl_3$) 28.61 (C12), 33.53 (C10), 42.87 (C11), 58.09 (C9), 119.84 (CN), 121.58, 122.58, 123.86, 124.03, 126.00, 126.34, 127.38, 127.42, 136.13, 137.54, 141.61, 142.02 (ArCH, C_4), 199.80 (C13). HRMS (ESI) calculated for $C_{18}H_{13}NONa$: [M^+ + Na] 282.0895: found 282.0921.

8.1.4.3 9-(1'-Hydroxy-1'-methoxymethyl)-9,10-dihydro-11-cyano-9,10-ethanoanthracene (63'), crystals obtained by slow crystallisation of **63** from methanol over a period of 4-8 weeks. IRν_{max} (KBr) 3448 (OH), 2238 (CN), 1727 (C=O) cm^{-1}**.** HRMS (ESI) calculated for $C_{19}H_{18}NO_2$: [M^+ + H] 292.1338: found 292.1329.

8.1.4.4 9,10-Dihydro-11-cyano-9,10-ethanoanthracene-(9-acrylic acid ethyl ester) (67)

3-(9-Anthracenyl)acrylic acid ethyl ester (**47**) (10 mmol, 2.76 g) was reacted according to general procedure 4 above (method A). The product was then purified by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate), followed by recrystallisation from methanol, colourless crystals (90 %)**,** M.p. 70 °C. IRν_{max} (KBr) 1190 (C-O), 1722 (C=O), 2237 (CN) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.43 (3H, t, J = 7.0 Hz, CH_3), 2.15 (1H, m, H12$_a$), 2.35 (1H, m, H12$_b$), 3.10 (1H, dd, J = 4.0 Hz, J = 10.5 Hz, H10), 4.38 (2H, q, J = 7.5 Hz, CH_2), 4.46 (1H, s, H11), 6.50 (1H, d, J = 16.5 Hz, H13), 7.15-7.28 (8H, m, ArH), 8.01 (1H, d, J = 16.5 Hz, H14). ^{13}C NMR ppm ($CDCl_3$) 13.87 (CH_3), 31.04 (C11), 34.49 (C10), 42.58 (CH_2, C12), 50.23 (C9), 60.63 (CH_2), 120.07 (CN), 122.70-127.02 (C14, ArCH), 138.72, 140.15, 141.38, 142.12 (C_4), 143.17 (C13), 165.28 (C=O)**.** HRMS (ESI) calculated for $C_{22}H_{19}NO_2Na$: [M^+ + Na] 352.1313: found 352.1325.

8.1.4.5 9,10-dihydro-9,10-ethanoanthracen-11-cyano-*N*-methylacrylamide (68)

3-(9-Anthracenyl)-*N*-methyl-acrylamide (**58**) (0.77 mmol, 0.2 g) was reacted according to general procedure 4 above (method A). The product was purified by flash column chromatography over silica gel (eluent: 2:1 hexane/ethyl acetate). Colourless powder (88 %), M.p. 180-182 °C. IRν_{max} (KBr) 1675 (C=O), 2240 (CN) 3289 (NH), cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.20 (1H, m, $H12_a$), 2.38 (1H, m, $H12_b$), 3.06 (4H, t, J = 8.0 Hz, CH_3, H11), 4.45 (1H, s, H10), 5.85 (1H, br s, NH), 6.42 (1H, d, J = 16.0 Hz, H14), 7.17 (2H, m, ArH), 7.25 (3H, m, ArH), 7.33 (1H, d, J = 7.0 Hz, ArH), 7.39 (2H, d, J = 7.0 Hz, ArH), 7.95 (1H, d, J = 16.0 Hz, H13). ^{13}C NMR ppm ($CDCl_3$) 26.16 (CH_3), 30.99 (C11), 34.59 (CH_2), 42.54 (C10), 49.95 (C9), 120.42 (CN), 122.85, 123.04, 123.29, 123.43, 125.80, 126.10, 127.16 (ArCH), 126.83 (C14), 138.97 (C13), 139.45, 140.42, 141.35, 142.09 (C_4), 164.67 (C=O). HRMS (ESI) calculated for $C_{21}H_{18}N_2ONa$: [M^+ + Na] 337.1317: found 337.1329.

8.1.5 General procedure 5 - Hydrogenation of alkenes

The appropriate unsaturated compound was dissolved in ethyl acetate (10 mL) and added to 10% palladium on charcoal (1 g). The flask was filled with H_2 and stirred for 48 h, while being monitored by TLC. After this time, the solution was filtered through celite and solvent was evaporated *in vacuo*. No further purification was necessary.

8.1.5.1 Ethyl 3-(11-cyano-9,10-dihydro-9,10-ethanoanthracenyl)-9-propanoate (70)

9,10-Dihydro-9,10-ethanoanthracene-11-cyano-*N*-methylacrylamide (**68**) (1.52 mmol, 0.5 g) was reacted according to general procedure 5 above. The product was isolated as colourless crystals (95 %) and no further purification was required. M.p. 132-138 °C. IRν_{max} (KBr) 2233 (CN) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.38 (3H, t, J = 7.0 Hz, CH_3), 2.10 (1H, m, $H12_a$), 2.40 (1H, m, $H12_b$), 2.83 (2H, m, CH_2), 2.89 (1H, m, H11), 3.13 (1H, m, CH_2), 4.30 (2H, q, J = 7.0 Hz, OCH_2), 4.38 (1H, s, C10), 7.18-7.30 (4H, m, ArH), 7.35 (3H, m, ArH), 7.49 (1H, d, J = 8.0 Hz, ArH). ^{13}C NMR ppm ($CDCl_3$) 13.86 (CH_3), 23.78 (C12), 29.45 (CH_2), 33.79 (CH_2), 38.50 (C11), 42.82 (C10), 46.41 (C9), 60.59 (OCH_2), 119.97 (CN), 121.94, 122.54, 123.10, 123.70, 125.73, 126.06, 126.32, 126.45 (ArCH), 139.99, 142.79, 143.43 (C_4), 172.54 (C=O). HRMS (ESI) calculated for $C_{22}H_{21}NO_2Na$: [M^+ + Na] 354.1470: found 354.1463.

8.1.5.2 9,10-Dihydro-9,10-ethanoanthracen-11-cyano-*N*-methylpropanamide (71)

9,10-Dihydro-9,10-ethanoanthracen-12-cyano-*N*-methylacrylamide (**68**) (0.95 mmol, 0.3 g) was reacted according to general procedure 5 above. The product was isolated as a grey solid (98 %) and no further purification was required. IRν_{max} (KBr) 2244 (CN) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.05 (1H, m, $H12_a$), 2.10 (1H, m, 12_b), 2.60 (1H, m, CH_2), 2.98 (3H, s, CH_3), 3.01 (1H, m, H11), 4.37 (1H, s, H10), 5.85 (1H, br s, NH), 7.20 (4H, m, ArH), 7.25 (2H, m, ArH), 7.42 (1H, d, J = 8.0 Hz, ArH), 7.50 (1H, d, J = 8.0 Hz, ArH). ^{13}C NMR ppm ($CDCl_3$) 24.60, (CH_2), 26.18 (CH_3), 31.35 (CH_2), 31.44, 33.64 (C12), 42.79 (C10), 46.50 (C9), 120.22 (CN), 122.31, 122.52, 123.04, 123.62, 125.83, 126.03, 126.26, 126.39 (ArCH), 140.14, 142.92, 143.28 (C_4), 172.16 (C=O). HRMS (ESI) calculated for $C_{21}H_{21}N_2O$: [M^+ + H] 339.1471: found 339.1462.

8.1.5.3 9-(2-Cyanoethyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (72)

9-(2-Cyanovinyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (**69**) (0.7 mmol, 0.2 g) was reacted according to general procedure 5 above. The residue was purified by flash

column chromatography over silica gel (eluent: 2:1 hexane/ethyl acetate) to afford the product as a pale solid (75 %)**,** M.p. 149-151 °C**.** IRv_{max} (KBr) 2237 (CN) cm^{-1}**.** ^{1}H NMR δ ($CDCl_3$) 2.07 (1H, ddd, J = 17.0 Hz, J = 10.2 Hz, J = 3.0 Hz, $H12_a$), 2.34 (1H, m, $H12_b$), 2.74 (1H, dd, J = 10.0 Hz, J = 4.0 Hz, H11), 2.83 (1H, dd, J = 17.0 Hz, J = 9.0 Hz, $H13_a$), 2.96 (1H, dd, J = 16.8 Hz, J = 8.0 Hz, $H13_b$), 3.17 (2H, t, J = 7.5 Hz, H14), 4.42 (1H, J = 2.7 Hz, H10), 7.25 (4H, m, ArH), 7.40 (4H, m, ArH)**.** ^{13}C NMR ppm ($CDCl_3$) 12.97 (C12), 25.39 (C13), 31.62 (C11), 33.68 (C14), 42.71 (C10), 46.52 (C9), 118.71, 119.37 (CN), 121.58, 122.55, 123.84, 126.39, 124.54, 126.39, 126.68, 127.27, 127.31 (ArCH), 138.79, 142.49, 143.21 (C_4)**.** HRMS (ESI) calculated for $C_{20}H_{16}N_2Na$: [M^+ + Na] 307.1211: found 307.1195.

8.1.5.4 9-(Hydroxymethyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (73)

To a solution of 9-formyl-9,10-dihydro-12-cyano-9,10-ethanoanthracene (**63**) (1.93 mmol, 0.5 g) in MeOH (30 mL) and DCM (10 mL), was added $NaBH_4$ (2.32 mmol, 0.88 g) in portions. The mixture was stirred at room temperature and monitored by TLC. After 2 hours, the solvent was evaporated *in vcauo*. Chloroform (50 mL) was added to the residue and the solution washed with water (3 x 25 mL). The organic phase was dried over anhydrous Mg_2SO_4, and the solvent was evaporated *in vacuo* to afford the desired product which required no further purification, colourless solid (89 %), M.p. 192-195 °C. IRv_{max} (KBr) 2238 (CN), 3485 (OH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.09 (1H, m, $H12_a$), 2.37 (1H, m, $H12_b$), 3.20 (1H, dd, J = 10.5 Hz, J = 4.5 Hz, H11), 4.39 (1H, s, H10), 4.94 (1H, d, J = 11.0 Hz, $H13_a$), 5.05 (1H, d, J = 11.0 Hz, $H13_b$), 7.22 (8H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 29.70 (C11), 33.41 (C12), 42.96 (C10), 48.38 (C9), 60.65 (CH_2), 120.49 (CN), 121.96, 122.67, 123.26, 123.34, 125.76, 125.96, 126.28, 126.52 (ArCH), 138.26, 139.69, 142.87, 143.16 (C_4). HRMS (ESI) calculated for $C_{18}H_{15}NONa$: [M^+ + Na] 284.1051: found 284.1041.

8.1.6 General procedure 6: preparation of amines

To a solution of 9-formyl-9,10-dihydro-12-cyano-9,10-ethanoanthracene (**63**) (0.77 mmol, 0.2 g) in dry methanol (50 mL) was added the appropriate amine (6.16 mmol, 0.42 g) and $NaCNBH_3$ (1.09 mmol, 0.07 g). The mixture was stirred at room temperature for 72 h and monitored by TLC. The pH was adjusted occasionally to pH 5-6 using 4M methanolic HCl. When the reaction was complete, excess hydride was quenched using 10 % aq. HCl (50 mL). The aqueous solution was washed with dichloromethane (3 x 25 mL). The aqueous phase was basified with 2M NaOH and extracted with dichloromethane (3 x 25 mL). The organic phases were combined and dried with anhydrous Mg_2SO_4 and the solvent was evaporated *in vacuo* to afford the desired product

8.1.6.1 9-((Methylamino)methyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (74)

9**-**Formyl-9,10-dihydro-12-cyano-9,10-ethanoanthracene (**63**) (0.77 mmol, 0.2 g) in dry methanol (50 mL) was treated with methylamine HCl (6.16 mmol, 0.42 g) and $NaCNBH_3$ (1.09 mmol, 0.07 g) according to the general procedure 6 above to afford the desired product as a brown oil (87 %) which required no further purification. IRv_{max} (KBr**)** 2333 (CN) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.72 (1H, ddd, J = 18.0 Hz, J = 12.4 Hz, J = 2.0 Hz, $H12_a$), 2.16 (1H, m, $H12_b$), 2.71 (1H, dd, J = 12.0 Hz, J = 8.0 Hz, H11), 3.08 (3H, s, CH_3), 4.09 (1H, br s, NH), 4.30 (2H, s, CH_2), 4.42 (1H, d, J = 1.5 Hz, H10), 7.12 (5H, m, ArH), 7.20 (1H, m, ArH), 7.29 (1H, m, ArH), 7.38 (1H, ArH). ^{13}C NMR ppm ($CDCl_3$) 27.95 (C12), 31.36 (CH_3), 44.25 (C10), 49.76 (C9),

50.12 (CH_2), 50.24 (C11), 118.99 (CN), 121.20, 122.27, 124.31, 125.43, 125.57, 125.90 (ArCH), 137.56, 141.87, 143.18, 145.67 (C_4). HRMS (ESI) calculated for $C_{19}H_{19}N_2$: [M^+ + H] 275.1548: found 275.1537.

8.1.6.2 11-*N*-Cyclohexyl-*N*-methyl-9-formyl-9,10-dihydro-9,10-ethanoanthracene-11-carboxyamide (66)

9-Formyl-9,10-dihydro-9,10-ethanoanthracene-11-carboxylic acid (**65**) (0.1 mmol, 0.03 g) was reacted with *N*-methyl-*N*-cyclohexylamine (180.0 µmol, 0.02 g) according to general procedure 6 above. The desired product was obtained as a pale brown resin (56 %). IRv_{max} (KBr) 1660 (C=O), 1708 (C=O) cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.13-3.16 (15H, m, CH_2, CH_3, $H12_a$, $H12_b$), 3.49 (1H, m, H11), 4.52 (1H, m, H10), 7.18 (8H, m, ArH), 10.91 (1H, s, CHO). ^{13}C NMR ppm ($CDCl_3$) 24.78, 25.10, 25.31, 29.02, 29.29 (CH_2), 27.33 (CH_3), 42.07 (C11), 46.99 (C10), 52.46 (NCH), 119.81, 121.39, 123.35, 125.31, 125.60, 126.07, 126.14, 126.56 (ArCH), 140.20, 140.44, 140.51, 143.22 (C_4), 171.91 (C=O), 202.90 (CHO). HRMS (ESI) calculated for $C_{25}H_{27}NO_2Na$: [M^+ + Na] 396.1939: found 396.1926.

8.1.6.3 9,10-Dihydro-11-*N*-methyl-*N*-cyclohexanyl-9,10-ethenoanthracene-11-carboxyamide (42)

9,10-Dihydro-9,10-ethenoanthracene-11-carboxylic acid (**41**) (0.8 mmol, 0.2 g) was reacted with *N*-methyl-*N*-cyclohexylamine (1.41 mmol, 0.16 g) according to general procedure 6 above. Purification by flash column chromatography over silica gel (eluent: 2:1 hexane/ethyl acetate) afforded the desired product as colourless crystals (34 %), M.p. 118-119 ˚C. IRv_{max} (KBr) 1635 (C=O), 3399 (ArCH) cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.20-2.00 (6H, m, CH_2), 2.01 (3H, s, NCH_3), 2.70 (5H, br s, NCH, CH_2), 5.22 (2H, s, H9, H10), 6.98 (5H, m, ArH, H12), 7.46 (2H, m, ArH), 7.45 (2H, m, ArH).^{13}C NMR ppm ($CDCl_3$) 14.24, 21.09, 25.36, 30.56 (CH_2), 50.93 (C10), 53.36 (C9), 60.43 (CH_3), 123.22, 123.48, 124.81, 124.85 (ArCH), 145.13 (C12), 147.91 (C_4), 165.43 (C=O). HRMS (ESI) calculated for $C_{24}H_{25}NONa$: [M^+ + Na] 366.1834: found 366.1822.

8.1.6.4 9,10-Dihydro-11-*N*-piperidinyl-9,10-etheneoanthracnene-11-carboxyamide (43)

9,10-Dihydro-9,10-ethenoanthracene-11-carboxylic acid (**41**) (0.8 mmol, 0.2 g) was reacted with piperidine (1.41 mmol, 0.12 g) according to general procedure 6 above. The product was purified by flash column chromatography over silica gel (eluent: 2:1 hexane/ethyl acetate) to afford a brown solid (66 %), M.p**.** 123-124 ˚C. IRv_{max} (KBr) 1626 (C=O), 2937 (ArCH) cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.64 (6H, br s, CH_2), 3.40 (4H, br s, CH_2), 5.22 (1H, d, J = 6.0 Hz, H10), 5.35 (1H, s, H9), 6.99 (4H, m, ArH), 7.07 (1H, d, J = 6.0 Hz, H12), 7.32 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 24.18 (CH_2), 50.53 (C10), 52.85 (C9), 122.78, 122.96, 124.34, 124.41 (ArCH), 138.93 (C12), 144.65, 144.92, 146.72 (C_4), 167.44 (C=O). HRMS (ESI) calculated for $C_{22}H_{21}NONa$: [M^+ + Na] 338.1521: found 338.1512.

8.1.6.5 9,10-Dihydro-*N*-methyl-*N*-cyclohexanyl-9,10-ethenoanthracene-11-methanamine (44)

9,10-Dihydro-11-*N*-methyl-*N*-cyclohexane-9,10-etheneoanthracnene-11-carboxyamide (**42**) (0.29 mmol, 0.11 g) was added to $LiAlH_4$ according to general procedure 2 above. The crude product was then purified by flash column chromatography over silica gel (eluent: 95 % DCM: MeOH) to afford a colourless solid (87 %). M.p**.** 160-163 ˚C. IRv_{max} (KBr) 3034 (ArCH) cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.27-1.69 (6H, m, CH_2), 1.80 (3H, s, NCH_3), 2.60 (5H, br s, NCH, CH_2),

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3.19 (2H, s, CH_2), 5.21 (1H, d, J = 6.0 Hz, H10), 5.28 (1H, s, H9), 7.00 (5H, m, ArH, H12), 7.49 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 14.26, 20.99, 25.60, 29.63 (CH_2), 50.24 (C10), 54.90 (C9), 61.65 (CH_2), 60.57 (CH_3), 123.48, 123.98, 124.22, 124.99 (ArCH), 145.01, 145.23, 146.11 (C_4), 149.98 (C12). HRMS (ESI) calculated for $C_{24}H_{28}N$: [M^+ + H] 330.2222: found 330.2229.

8.1.6.6 9,10-Dihydro-*N*-piperidinyl-9,10-ethenoanthracene-11-methanamine (45)

9,10-Dihydro-11-*N*-piperidinyl-9,10-etheneoanthracnene-11-carboxyamide (**43**) (0.8 mmol, 0.2 g) was added to $LiAlH_4$ according to general procedure 2 above. The crude product was then purified by flash column chromatography over silica gel (eluent: 95 % DCM: MeOH) to afford the desired product as a brown resin (85 %). IRν_{max} (film) 2937 (ArCH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.53 (6H, br m, CH_2), 1.97 (4H, m, CH_2), 3.11 (2H, s, CH_2), 5.05, (1H, d, J = 6.0 Hz, H10), 5.26 (1H, s, H9), 6.66 (1H, d, J = 6.0 Hz, H12), 6.95 (2H, m, ArH), 6.96 (2H, m, ArH), 7.10 (4H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 24.18 (CH_2), 25.42 (CH_2) 50.53 (C10), 52.85 (C9), 60.88 (CH_2), 64.12 (CH_2), 122.73, 122.96, 124.34, 124.40 (ArCH), 138.93 (C12), 144.65, 144.91, 146.72 (C_4),145. HRMS (ESI) calculated for $C_{22}H_{24}N$: [M^+ + H] 302.1909: found 302.1911

8.1.7 General procedure 7: preparation of esters and carbamates

The appropriate acid chloride (1.4 mmol) was dissolved in anhydrous DCM (10 mL) and added dropwise to a stirring solution of 9-((*E*)-(hydroxyimino)methyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (**75**) or 11-hydroxymethyl-9,10-ethanoanthracene (**73**) (1 mmol) and triethylamine (3 mmol) in anhydrous DCM (10 mL). The solution was heated at reflux for 3 h. After this time, the solution was cooled, diluted with DCM (50 mL) and washed with water (3 x 25 mL) and brine (3 x 25 mL). The organic phase was then dried over anhydrous Na_2SO_4 and the solvent evaporated *in vacuo*. The product was then purified by flash column chromatography over silica gel to afford the pure product.

8.1.7.1 9-((*E*)-(Acetoxyimino)methyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (78)

9-((*E*)-(Hydroxyimino)methyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (**75**) (1 mmol, 0.26 g) and acetyl chloride (1.2 mmol, 0.09 g) were reacted according to general procedure 7 above. Purification by flash column chromatography (eluent: 2:1, hexane/ethyl acetate) afforded the product as colourless crystals (74 %), M.p**.** 64-68 ˚C. IRν_{max} (KBr) 2251 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.13 (1H, m, $H12_a$), 2.36 (1H, m, $H12_b$), 2.46 (3H, s, CH_3), 3.41 (1H, dd, J =10.5 Hz, J = 4.0 Hz, H11), 4.49 (1H, s, H10), 7.19-7.30 (5H, m, ArH), 7.37 (2H, d, J = 7.0 Hz, ArH), 7.43 (1H, m, ArH), 8.85 (1H, s, HCN). ^{13}C NMR ppm ($CDCl_3$) 13.76 (CH_3), 30.53 (C11), 33.70 (C12), 42.63 (C10), 59.97 (C9), 120.10 (CN), 122.31, 122.52, 123.43, 123.91, 125.99, 126.32, 127.32, 127.30 (ArCH), 137.18, 138.84, 141.35, 141.91 (C_4), 155.07 (C=N), 168.73 (C=O). HRMS (ESI) calculated for $C_{20}H_{16}N_2O_2Na$: [M^+ + Na] 339.1109: found 339.1109.

8.1.7.2 9-((*E*)-(((1-(Diethylamino)vinyl)oxy)imino)methyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (79)

9-((*E*)-(Hydroxyimino)methyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (**75**) (0.73 mmol, 0.2 g) and diethylcarbamoyl chloride (0.87 mmol, 0.12g) were reacted according to general procedure 7 above. Purification by flash column chromatography (eluent: 2:1, hexane/ethyl acetate) afforded the product as a colourless solid (70 %), M.p. 189-193 ˚C. IRν_{max} (film) 1625 (C=O), 2250 (CN), 3364 (C=N) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.23,

1.26 (6H, 2x t, J = 6.0 Hz, CH_3), 2.14 (1H, m, $H12_a$), 2.32 (1H, m, $H12_b$), 3.26 (1H, dd, J = 10.5 Hz, J = 4.0 Hz, H11), 3.43 (2H, q, J = 7.0 Hz, CH_2), 3.50 (2H, q, J = 7.0 Hz, CH_2), 4.49 (1H, s, H10), 7.25 (6H, m, ArH), 7.35 (1H, d, J = 7.5 Hz, ArH), 7.41 (1H, s, ArH), 8.55 (1H, s, HC=N). ^{13}C NMR ppm ($CDCl_3$) 12.41, 13.29 (CH_3), 30.42 (C11), 33.90 (C12), 42.65 (C10), 44.00, 45.28 (CH_2), 49.61 (C9), 120.76 (CN), 122.41, 122.80, 123.20, 123.66, 125.82, 126.20, 126.98, 127.07 (ArCH), 138.08, 139.89, 141.39, 142.14 (C_4), 149.01 (C=O). HRMS (ESI) calculated for $C_{23}H_{23}N_3O_2Na$: [M^+ + Na] 396.1688: found 396.1751.

8.1.7.3 ((*E*)-((Benzoyloxy)imino)methyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (80)

9-((*E*)-(Hydroxyimino)methyl)-9,10-dihydro-9,10-ethanoanthracene-11-carbonitrile (**75**) (0.36 mmol, 0.1 g) and benzoyl chloride (0.5 mmol, 0.07 g) were reacted according to general procedure 7 above. Purification by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate) affordrd the product as a colourless solid (74 %), M.p. 180-183 ˚C. IRν_{max} (KBr) 1741 (C=O) 2242 (CN) 3065 (C=N) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.16 (1H, m, $H12_a$), 2.34 (1H, m, $H12_b$), 3.55 (1H, dd, J = 10.5 Hz, J = 4.0 Hz, H11), 4.51 (1H, s, H10), 7.21-7.33 (6H, m, ArH), 7.39 (1H, d, J = 7.0 Hz, ArH), 7.49 (2H, m, ArH), 7.57 (2H, t, J = 7.0 Hz, ArH), 7.69 (1H, t, J = 7.0 Hz), 8.24 (2H, d, J = 8.0 Hz, ArH), 9.10 (1H, s, HCN). ^{13}C NMR ppm ($CDCl_3$) 30.87 (C11), 33.75 (C12), 42.72 (C10), 50.41 (C9), 120.14 (CN), 122.58, 123.38, 123.94, 126.01, 126.30, 127.29, 127.38, 128.22, 129.48, 133.25 (ArCH), 137.26, 138.90, 141.42, 141.94 (C_4), 156.58 (HCN), 162.97 (C=O). HRMS (ESI) calculated for $C_{25}H_{18}N_2O_2Na$: [M^+ + H] 401.1266: found 401.1309.

8.1.7.4 11-Cyano-9,10-dihydro-9,10-ethanoanthracenyl-9-methyl acetate (76)

11-Hydroxymethyl-9,10-ethanoanthracene (**73**) (0.24 mmol, 0.06 g) and acetyl chloride (0.29 mmol, 0.02 g) were reacted according to the general procedure 7 above. Purification by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate) afforded the product as colourless crystals (67 %), M.p. 66-69 ˚C. IRν_{max} (KBr) 1640 (C=O), 2234 (CN) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.08 (1H, m, $H12_a$), 2.24 (3H, s, CH_3), 2.39 (1H, m, $H12_b$), 3.15 (1H, dd, J = 10.5 Hz, J = 4.0 Hz, H11), 4.42 (1H, s, H10), 5.23 (1H, d, J = 12.0 Hz, CH_2), 5.48 (1H, d, J = 12.0 Hz, CH_2), 7.19-7.35 (8H, m, ArH). ^{13}C NMR ppm ($CDCl_3$) 29.27 (CH_3), 30.39 (C11), 33.55 (C12), 42.85 (C10), 46.55 (C9), 62.34 (OCH_2), 119.84 (CN), 121.89, 122.04, 123.42, 123.44, 125.81, 126.16, 126.55, 126.80 (ArCH), 137.49, 139.13, 142.42, 142.95 (C_4), 170.36 (C=O). HRMS (ESI) calculated for $C_{20}H_{17}NO_2Na$: [M^+ + Na] 326.1157: found 326.1149.

8.1.7.5 9-(11-Cyano-9,10-dihydro-9,10-ethanoanthracenyl)methyl benzoate (77)

11-Hydroxymethyl-9,10-ethanoanthracene (**73**) (0.38 mmol, 0.1 g) and benzoyl chloride (0.53 mmol, 0.08 g) were reacted according to the general procedure 7 above. Purification by flash column chromatography over silica gel (eluent: 2:1, hexane/ethyl acetate) afforded the product as a colourless solid (74 %), M.p. 169-172 ˚C. IRν_{max} (KBr) 2238 (CN), 1540 (C=O), 3454 (ArCH) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.08 (1H, m, $H12_a$), 2.39 (1H, m, $H12_b$), 3.21 (1H, dd, J = 11.0 Hz, J = 4.0 Hz, H11), 4.39 (1H, s, H10), 4.95 (1H, d, J = 11.0 Hz, CH_2), 5.06 (1H, d, J = 11.0 Hz, CH_2), 7.20 (12H, m, ArH), 7.60 (1H, d, J = 7.0 Hz, H12). ^{13}C NMR ppm ($CDCl_3$) 30.71 (C11), 33.61 (C12), 42.27 (C10), 48.35 (C9), 59.24 ($O\underline{CH_2}$), 122.63 (CN), 123.37, 123.67, 123.80, 125.50, 125.78, 126.09, 126.33, 127.80, 127.80, 129.20, 129.36, 130.38, 130.81 (ArCH),

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135.18, 139.87, 144.89, 145.01 (C_4). HRMS (ESI) calculated for $C_{25}H_{19}NO_2Na$: [M^+ + Na] 388.1313: found 388.1321.

8.2 X-Ray Crystallography

Crystals of compounds **6, 9** and **63'** were obtained by slow crystallisation from a dilute solution of methanol over a period of 4-8 weeks. The data for the crystal structures 74, 78, 81 and 127' were collected on a Rigaku Saturn 724 CCD Diffractometer. A suitable crystal from each crystal compound was selected and mounted using inert oil on a 0.3mm diameter glass fiber tip or loop and placed on the goniometer head in a 150K N2 gas stream. Each data set was collected using Crystalclear-SM 1.4.0 software. Data integration, reduction and correction for absorption and polarization effects were all performed using Crystalclear-SM 1.4.0 software. Space group determination, structure solution and refinement were obtained using Bruker Shelxtl Ver. 6.14 software. [58] Each structure was solved with Direct Methods using the SHELXTL program and refined against IF2I with the program XL from SHELX-97 using all data. Non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were placed into geometrically calculated positions and refined using a riding model.

8.2.1 Crystal Data for compound 6:

Cambridge Database Deposition number: CCDC 920112. $C_{72}H_{64}N_8O_8$, M = 1104.8, Triclinic, a = 9.4200(19), b = 12.123(2), c = 14.275(3)Å, α = 99.13(3)°, β = 10151(3)°, γ = 110.87(3)°, U = 1444.9(5)Å3, T = 150 K, space group P-1, Z = 1, μ (Mo Kα) = 0.086 mm−1, ρ = 1.270g/cm3, 22667 reflections collected, 5061 unique, (Rint = 0.0609), aR1 = 0.0808, wR2 [I > 2σ(I)] = 0.2413, Gof = 1.198, aR1 = ||Fo| − |Fc||/ |Fo|, wR2 = [w(Fo2 − Fc2)2/ w(Fo2)2]1/2.

8.2.2 Crystal Data for compound 9

Cambridge Database Deposition number: CCDC 920111. $C_{76}H_{76}N_4O_4$, M = 277.35, Monoclinic, a = 10.622(2), b = 9.7423(19), c = 14.343(3)Å, β = 97.02(3)°, U = 1473.10(5)Å3, T = 150 K, space group P2(1)/c, Z = 1, μ (Mo Kα) = 0.077 mm−1, ρ = 1.251g/cm3, 12367 reflections collected, 2588 unique, (Rint = 0.061), aR1 = 0.0673, wR2 [I > 2σ(I)] = 0.1487, Gof = 1.263, aR1 = ||Fo| − |Fc||/ |Fo|, wR2 = [w(Fo2 − Fc2)2/ w(Fo2)2]1/2.

8.2.3 Crystal Data for compound 63'

Cambridge Database Deposition number: CCDC 920114. $C_{76}H_{68}N_4O_8$, M = 1096.8, Monoclinic, a = 9.920(2), b = 17.200(3), c = 8.3340(17)Å, β = 94.39(3)°, U = 1417.8(5)Å3, T = 150 K, space group P2(1)/c, Z = 1, μ (Mo Kα) = 0.086mm−1, ρ = 1.285g/cm3, 11149 reflections collected, 2456 unique, (Rint = 0.0355), aR1 = 0.0530, wR2 [I > 2σ(I)] = 0.1839, Gof = 1.197, aR1 = ||Fo| − |Fc||/ |Fo|, wR2 = [w(Fo2 − Fc2)2/ w(Fo2)2]1/2.

8.3 Biochemistry: Experimental methods

8.3.1 Materials

DG-75 and MUTU-I (c179) BL cell lines were gifts from Dr. Dermot Walls (School of Biotechnology, Dublin City University, Ireland) and Professor Martin Rowe (Division of Cancer Studies, The University of Birmingham, UK) respectively. HL-60 cells were originally obtained from Prof. Balazs Sarkadi's research group (National Medical Center, Hungary).

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RPMI-1640, IMDM, FBS, HEPES, sodium pyruvate and glutamine were from Gibco (Invitrogen). Alamar Blue was obtained from BioSource, Belgium, LymphoPrep from Biosciences Ltd, Ireland. The Apotox-Glo Triplex assay was provided by Promega, U.K. All other chemicals were purchased through Sigma–Aldrich Inc., Ireland.

8.3.2 Cell lines

The DG-75 cell line is a B-lymphocyte, Burkitt's lymphoma line derived from a metastatic pleural effusion (lung) of a sporadic case of Burkitt's lymphoma[59]. The MUTU-I (c179) cell line is an isogenic stable group I BL cell line derived from a BL biopsy[60]. The parental HL-60 cell line is an acute promyelocytic leukemia line derived from peripheral blood leukocytes obtained by leukopheresis from a 36 year old Caucasian female with acute promyelocytic leukaemia[61]. The HL60-P-gp cells had been drug-selected by chronic exposure to adriamycin, while the HL60-BCRP cells had been retrovirally transduced then further selected by exposure to mitoxantrone[46]. Cell lines were cultured in RPMI-1640 medium containing phenol red and supplemented with 10% (v/v) foetal bovine serum (FBS), L-glutamine (2 mM), penicillin and streptomycin (100 µg/ml). The MUTU-I cell line also required the additional supplements of alpha-thioglycerol (5mM in phosphate buffered saline (PBS) with 20 uM bathocuprione disulfonic acid), sodium pyruvate (100 mM) and HEPES (1 mM). Cells were maintained at 37°C in a humidified atmosphere of 95% oxygen, and 5% carbon dioxide.

8.3.3 Generation of human peripheral blood mononuclear cells (PBMCs)

Blood was obtained from a healthy donor, transferred into a 50 mL falcon tube and diluted 1:2 with PBS. LymphoPrep was used to separate the blood into red blood cells, white blood cell ring and serum. The blood was slowly added to 20 mL of ficoll pague plus. The tubes were centrifuged at 1700g for 30 min. The white blood cell ring was transferred into a new 50 mL tube. The volume was adjusted to 50 mL and the samples were centrifuged again at 1700g for 10 min. The supernatant was removed. This step was repeated again, the pellet was then resuspended in 10 mL of complete IMDM media (10% FBS, 0.1% Ciprofloxacin (10 mg/mL)).

8.3.4 Alamar Blue viability assay

1–5 x 10^4 cells/well were seeded in a 96-well plate and treated with the respective drug for the desired length of time. Each well was then treated with 20 µl of Alamar Blue and left to incubate at 37 °C in the dark for 4–6 h. Fluorescence was read using at 590 nm (excitation 544 nm). The background fluorescence of the media without cells + Alamar Blue was taken away from each group, and the control untreated cells represented 100% cell viability. The antifungal agent miconazole (10 µM) was used as a positive control for cell death in each of the cell lines, resulting in 90% cytotoxicity.

8.3.5 Statistical analysis

Non-linear regression analysis. Each compound was screened over a 1 µM–1 mM concentration range in triplicate on two independent days with activity expressed as percentage cell viability compared to vehicle treated controls. All data points (mean ± SEM) were analysed using GRAPHPAD Prism (San Diego, CA).

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8.3.6 Quantification of apoptosis

Propidium iodide FACS analysis. 7.5 x 10^5 cells/5mL were treated with the appropriate amount of compound and incubated for a specified time. Cells were harvested by centrifugation at 300g for 5 min and washed with 5 mL of ice-cold PBS. The pellet was resuspended in 200 µl PBS and 2 mL of ice-cold 70% ethanol and cells were fixed overnight at 4 °C. After fixation, the cells were pelleted by centrifugation at 300g for 5 min and the ethanol was carefully removed. The pellet was resuspended in 400 µl of PBS with 25 µl of RNAse A (10 mg/mL stock) and 75 µl of propidium iodide (1 mg/mL). The tubes were incubated in the dark at 37 °C for 30 min. Cell cycle analysis was performed using appropriate gates counting 10,000 cells and analysed using CELLQUEST software package. Untreated cells had <5% cells in the pre-G1 phase of the cell cycle and 10 µM Taxol was used as a positive control for cell death.

8.3.7 Caspase activation assay

Activation of caspase 3 and 7 was assessed using an Apotox-Glo Triplex assay (Promega). Cells were seeded at a density of 10,000 cells/well in opague 96-well plates and treated with the appropriate compound (10 µM) for 8 h. After this time, Caspase-Glo reagent (100 µL) was added to each well and the mixture was mixed by orbital shaking (500 rpm for 30 s). This was incubated at room temperature for 30 mins and then the luminescence was read on a luminescence plate reader. Vinblastine (100 nM) was used as a positive control.

8.3.8 Statistical analysis

Statistical analysis was performed using the Student's t-test comparing vehicle versus treated samples. For illustrative purposes p values are presented as * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$.

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		MUTU-I (24hrs)	DG-75 (72hrs)			MUTU-I (24hrs)	DG-75 (72hrs)
Compound		**EC_{50}**	**EC_{50}**	**Compound**		**EC_{50}**	**EC_{50}**
Maprotiline	**1**	15.8	37.5	9-Substituted anthracenes (Series 3)	**55**	7.6	62.1
9,10-Dihydro-9,10-ethanoanthracenes (Series 1)	**2**	89.4	>100		**56**	5.4	11.6
	3	55.4	>100		**57**	38.5	>100
	19	63.0	54.6		**58**	62.5	>100
	22	65.0	51.8		**59**	62.9	>100
	24	65.6	69.9		**60**	21.2	>100
	27	23.0	35.5		**62**	3.0	1.5
	29	23.5	8.8	9,11-Disubstituted-9,10-dihydro-9,10-ethanoanthracene (Series 4)	**63**	31.7	>100
9,10-Dihydro-9,10-etheneoanthracenes (Series 2)	**44**	73.3	>100		**66**	40.6	>100
	45	31.5	10.2		**67**	45.6	>100
9-Substituted anthracenes (Series 3)	**47**	27.1	>100		**70**	29.3	>100
	48	21.8	7.6		**75**	20.4	>100
	49	8.8	>100		**76**	12.8	>100
	51	24.9	>100		**77**	24.5	>100
	52	21.5	9.3		**78**	34.6	>100

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	53	1.9	>100		**79**	28.4	3.1
	54	18.7	32.5		**80**	80.5	95.3

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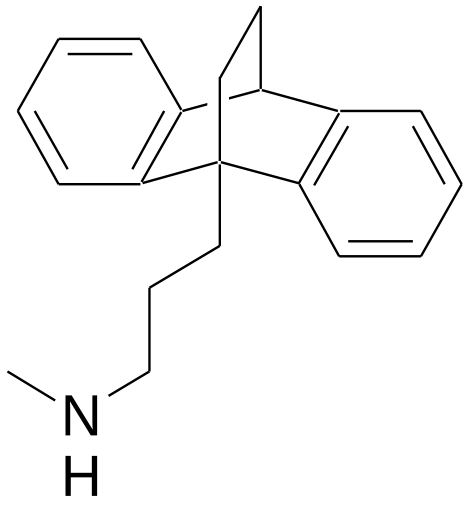
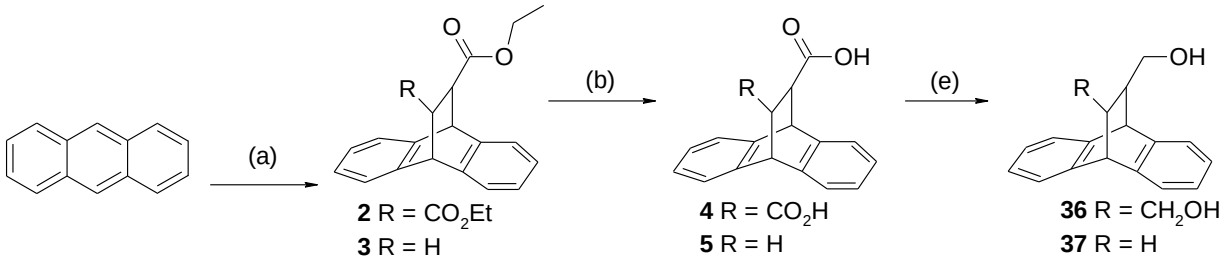
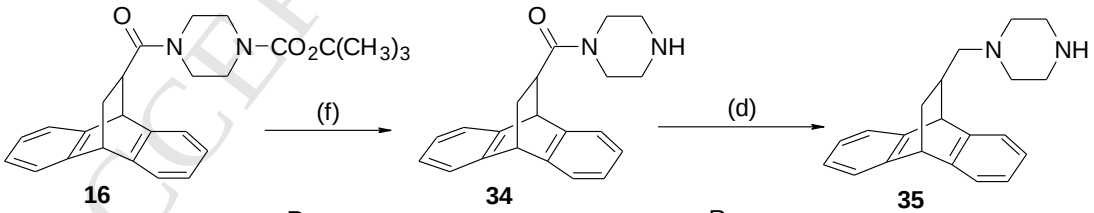

N
H



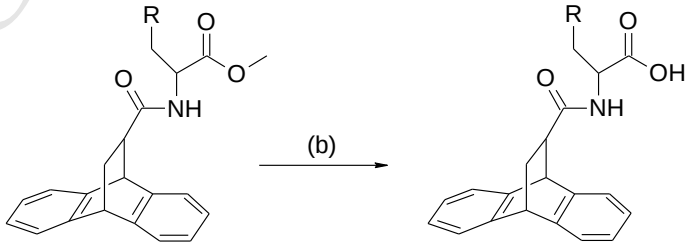
2 R = CO_2Et
3 R = H
4 R = CO_2H
5 R = H
36 R = CH_2OH
37 R = H
40

21 $R_1 = CH_2NR_2R_3$, $R_2 = R_3 = CH_3$
22 $R_1 = CH_2NR_2R_3$, $R_2 = R_3 = CH_2CH_3$
23 R_1 = H, $R_2 = R_3 = CH_3$
24 R_1 = H, $R_2 = R_3 = (C_5H_{10})$-
25 R_1 = H, $R_2 = R_3$= -$(C_4H_8)NCH_3$
26 R_1 = H, $R_2 = R_3$ = -(C_4H_8)-
27 R_1 = H, $R_2 = CH_3$, $R_3 = C_6H_{11}$
28 $R_1 = R_2$ = H, $R_3 = CH_2CH_3$
29 R_1 = H, $R_2 = R_3$ = -$(C_2H_4OC_2H_4)$-
30 $R_1 = R_2$ = H, $R_3 = C_6H_5$
31 R_1 = H, $R_2 = R_3$= $(C_2H_4)N(C_6H_4)Cl$

6 $R_1 = CONR_2R_3$, $R_2 = R_3 = CH_3$
7 $R_1 = CONR_2R_3$, $R_2 = R_3 = CH_2CH_3$
8 $R_1 = CONR_2R_3$, $R_2 = R_3$ = -(C_5H_{10})-
9 R_1 = H, $R_2 = R_3 = CH_3$
10 R_1 = H, $R_2 = R_3$ = -(C_5H_{10})-
11 R_1 = H, $R_2 = R_3$= -$(C_4H_8)NCH_3$
12 R_1 = H, $R_2 = R_3 = (C_4H_8)$-
13 R_1 = H, $R_2 = CH_3$, $R_3 = C_6H_{11}$
14 $R_1 = R_2$ = H, $R_3 = CH_2CH_3$
15 R_1 = H, $R_2 = R_3$ = -$(C_2H_4OC_2H_4)$-
16 R_1 = H, $R_2 = R_3 = (C_4H_8)NCO_2C(CH_3)_3$
17 $R_1 = R_2$ = H, $R_3 = C_6H_5$
18 R_1 = H, $R_2 = R_3$= $(C_2H_4)N(C_6H_4)Cl$
19 $R_1 = R_2$ = H, $R_3 = CH(COOCH_3)(CH_2C_6H_4OH)$
20 $R_1 = R_2$ = H, $R_3 = HC(CO_2CH_3)CH_2CH(CH_3)_2$



16
34
35



19 R = 4-C_6H_4OH
20 R = $CH(CH_3)_2$
32 R = 4-C_6H_4OH
33 R = $CH(CH_3)_2$

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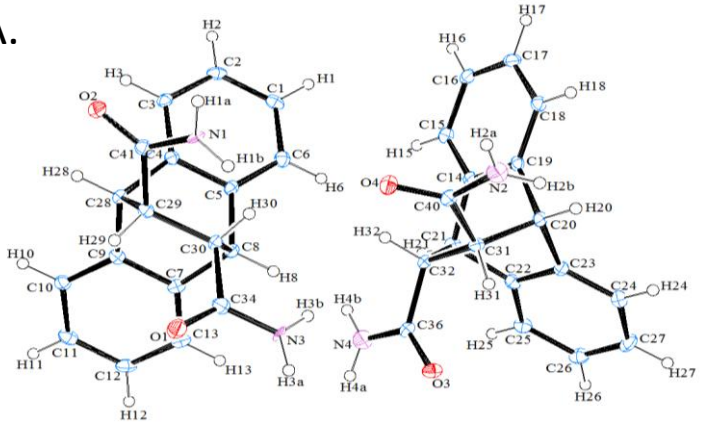
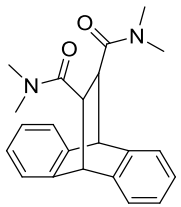
A.





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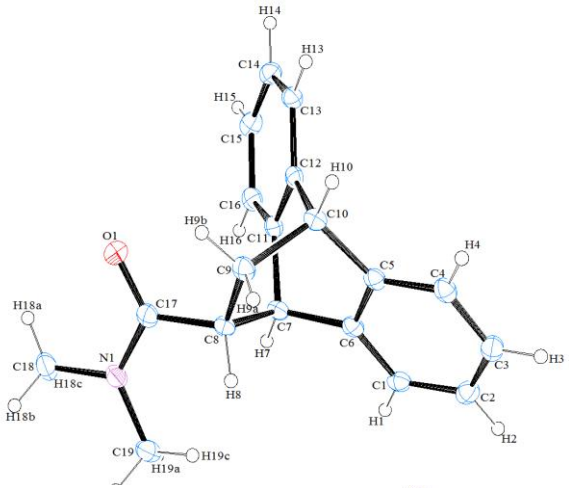
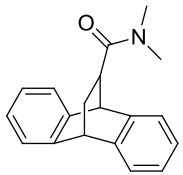
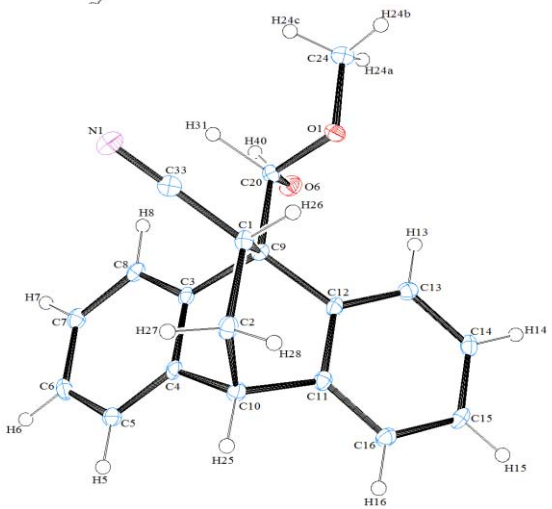
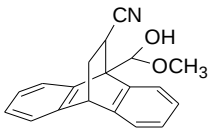
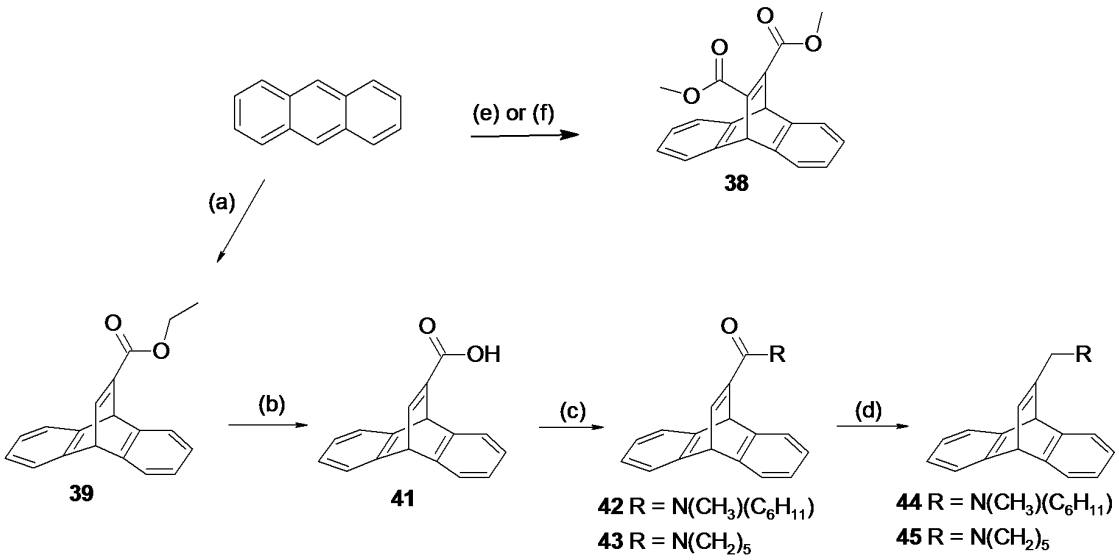




C.







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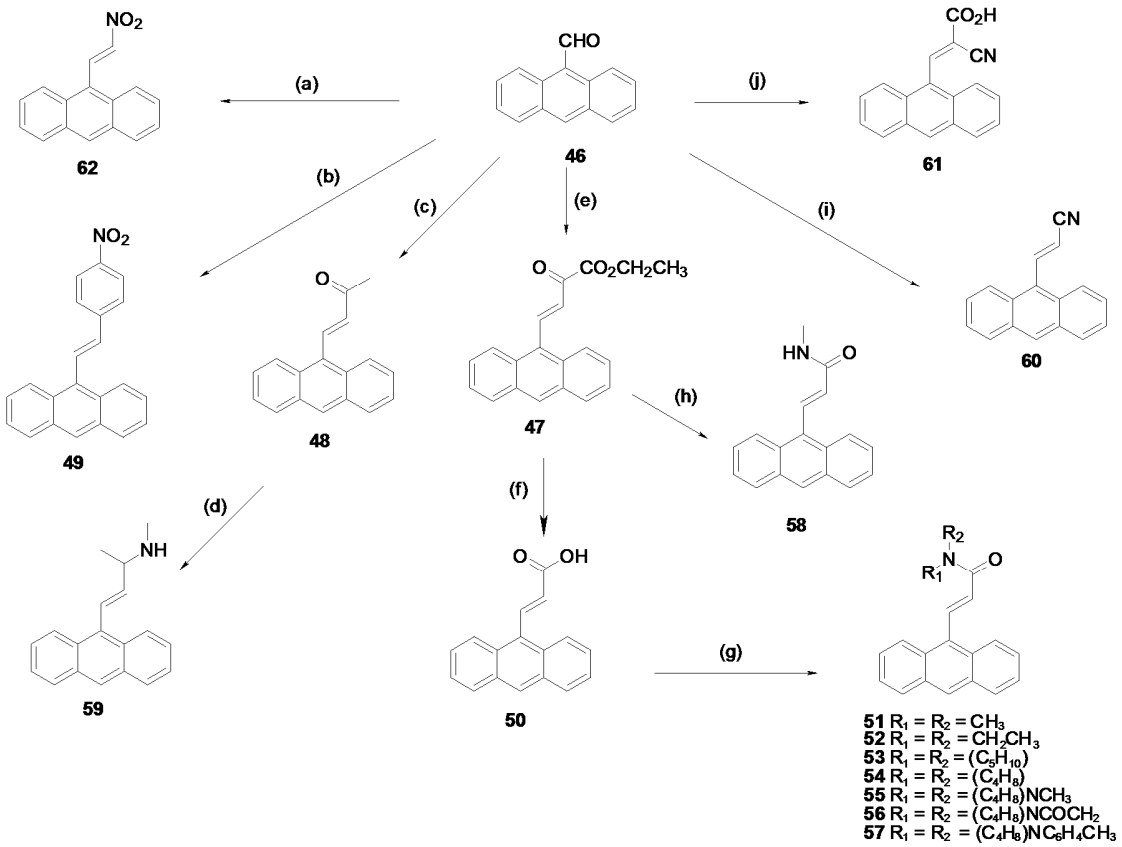

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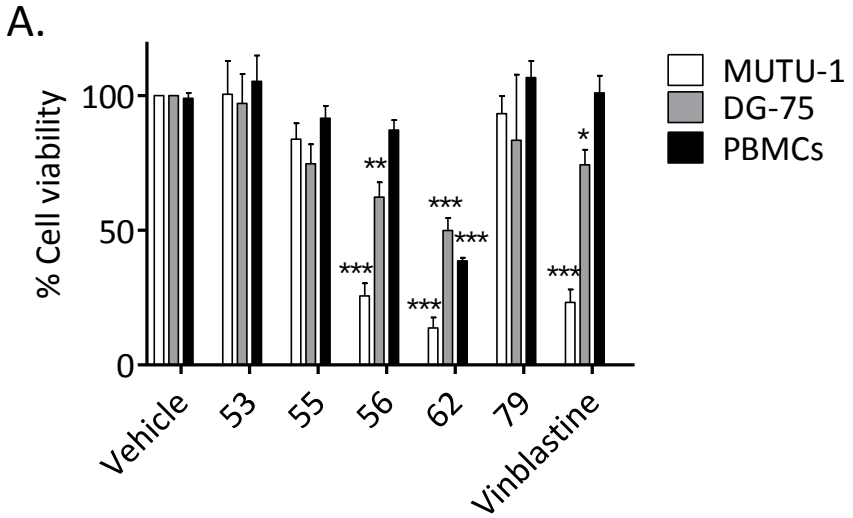
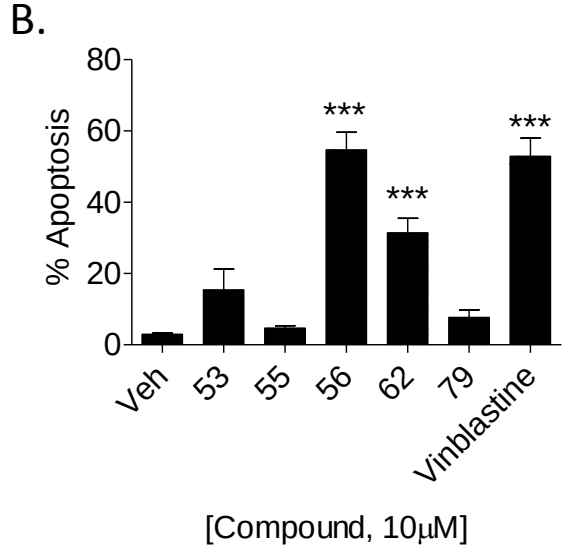
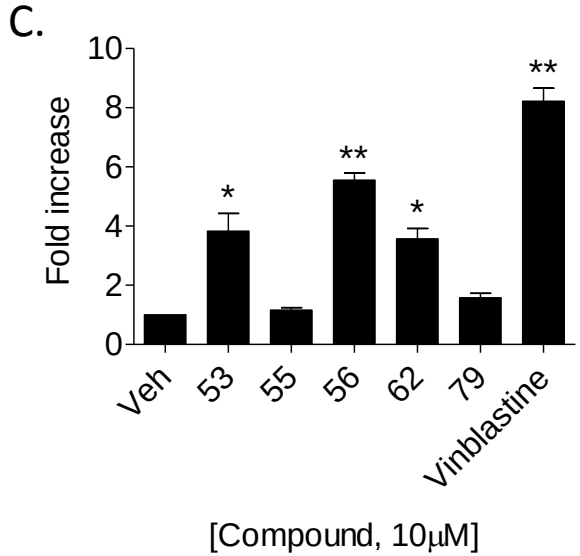
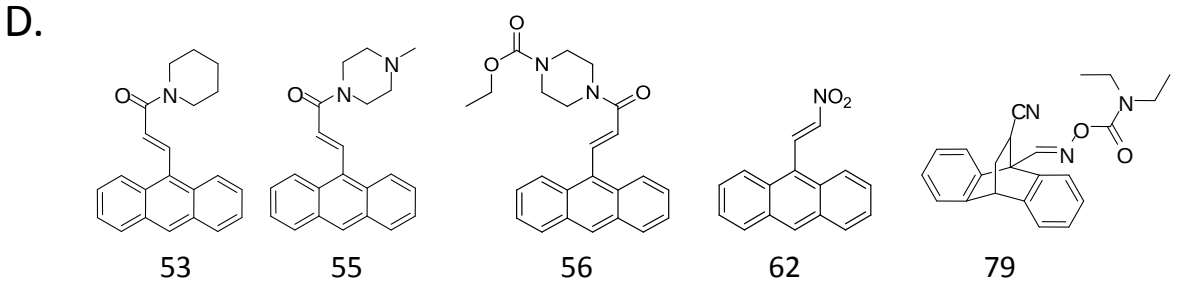
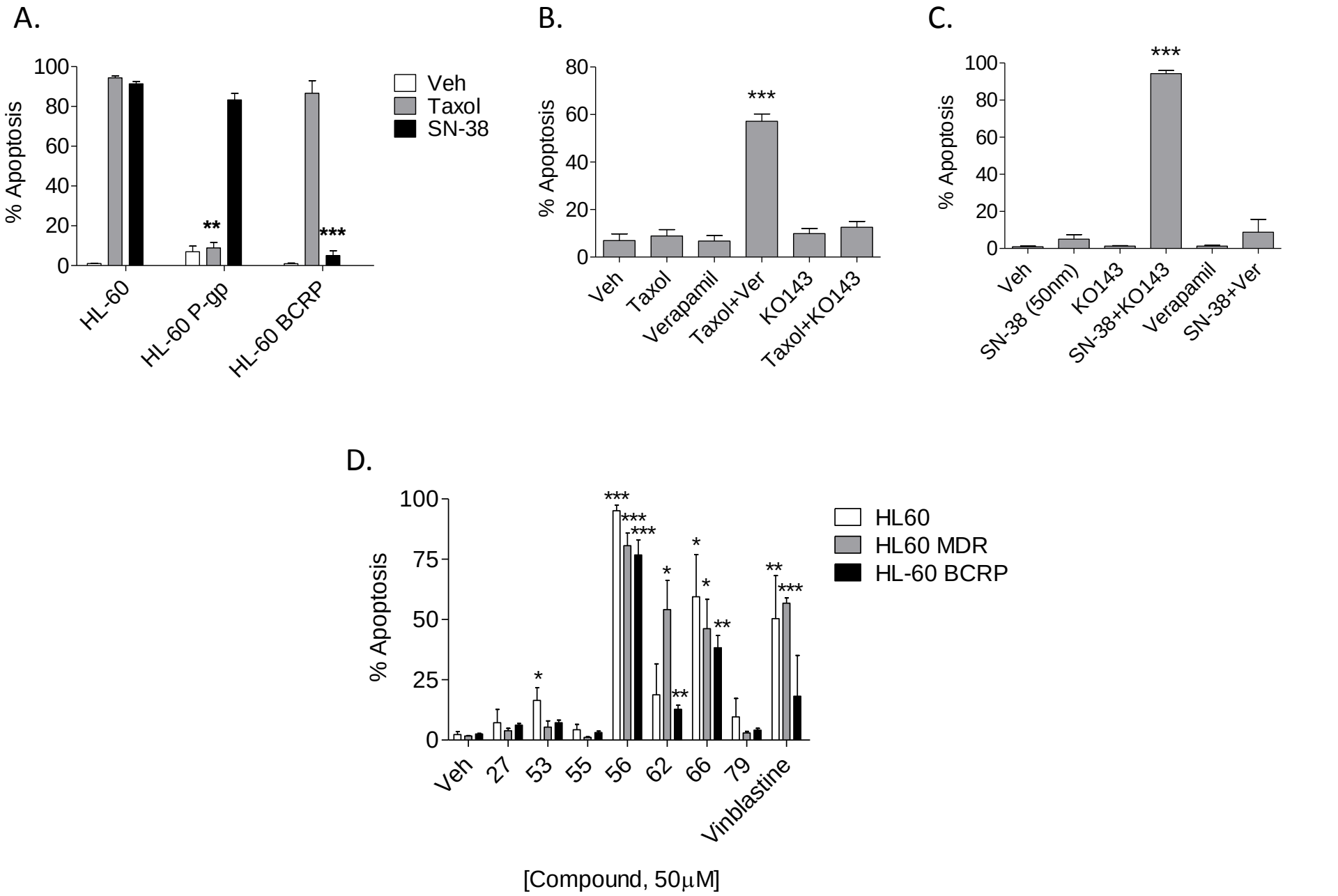
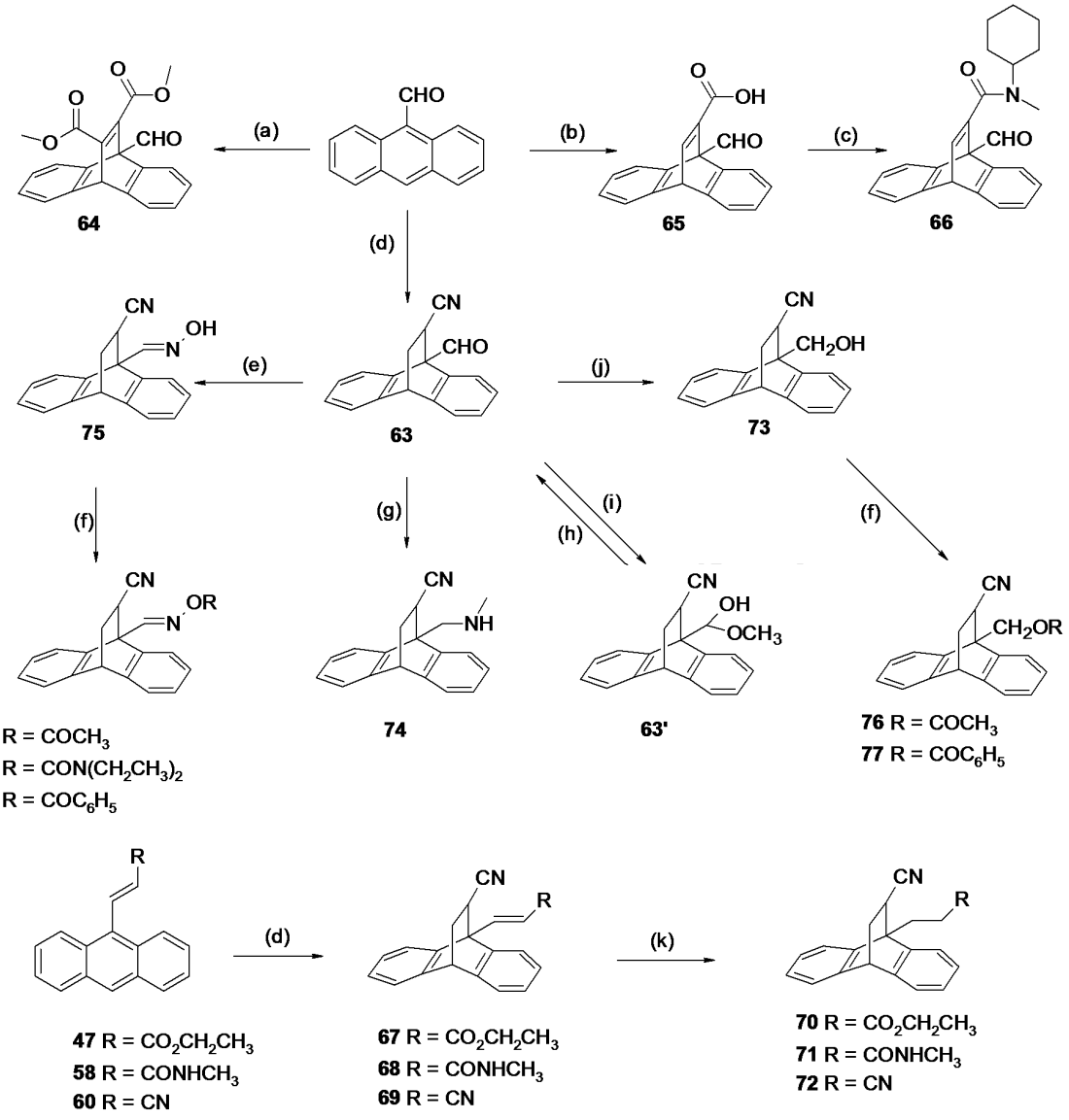

A.
% Cell viability
100
50
0
MUTU-1
DG-75
PBMCs
Vehicle
53
55
56
62
79
Vinblastine


B.
% Apoptosis
80
60
40
20
0
Veh
53
55
56
62
79
Vinblastine
[Compound, 10μM]


C.
Fold increase
10
8
6
4
2
0
Veh
53
55
56
62
79
Vinblastine
[Compound, 10μM]


D.
53
55
56
62
79


A.
% Apoptosis
100
80
60
40
20
0
**
***
HL-60
HL-60 P-gp
HL-60 BCRP
Veh
Taxol
SN-38
B.
% Apoptosis
80
60
40
20
0
***
Veh
Taxol
Verapamil
Taxol+Ver
KO143
Taxol+KO143
C.
% Apoptosis
100
80
60
40
20
0
***
Veh
SN-38 (50nm)
KO143
SN-38+KO143
Verapamil
SN-38+Ver
D.
% Apoptosis
100
75
50
25
0
HL60
HL60 MDR
HL-60 BCRP
Veh
27
53
55
56
62
66
79
Vinblastine
[Compound, 50μM]


CHO
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
64
65
66
63
75
73
74
63'
78 R = COCH3
79 R = CON(CH2CH3)2
80 R = COC6H5
76 R = COCH3
77 R = COC6H5
47 R = CO2CH2CH3
58 R = CONHCH3
60 R = CN
67 R = CO2CH2CH3
68 R = CONHCH3
69 R = CN
70 R = CO2CH2CH3
71 R = CONHCH3
72 R = CN

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Highlights

- Synthesis of a diverse library of novel anti-depressant analogues
- Antiproliferative effects of maprotiline analogues in Burkitt's lymphoma cell lines
- Apoptotic cell death caspase-dependant
- Antiproliferative effects in multi-drug resistant cells

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Supplementary Information:

Synthesis and antiproliferative action of a novel series of maprotiline analogues.

Y. M. McNamara[1], S. A. Bright[2], A. J. Byrne[1], Suzanne M. Cloonan[2], T. McCabe[3], D. C. Williams[2], M. J. Meegan[1].

[1]School of Pharmacy & Pharmaceutical Sciences, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland

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[3]School of Chemistry, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland

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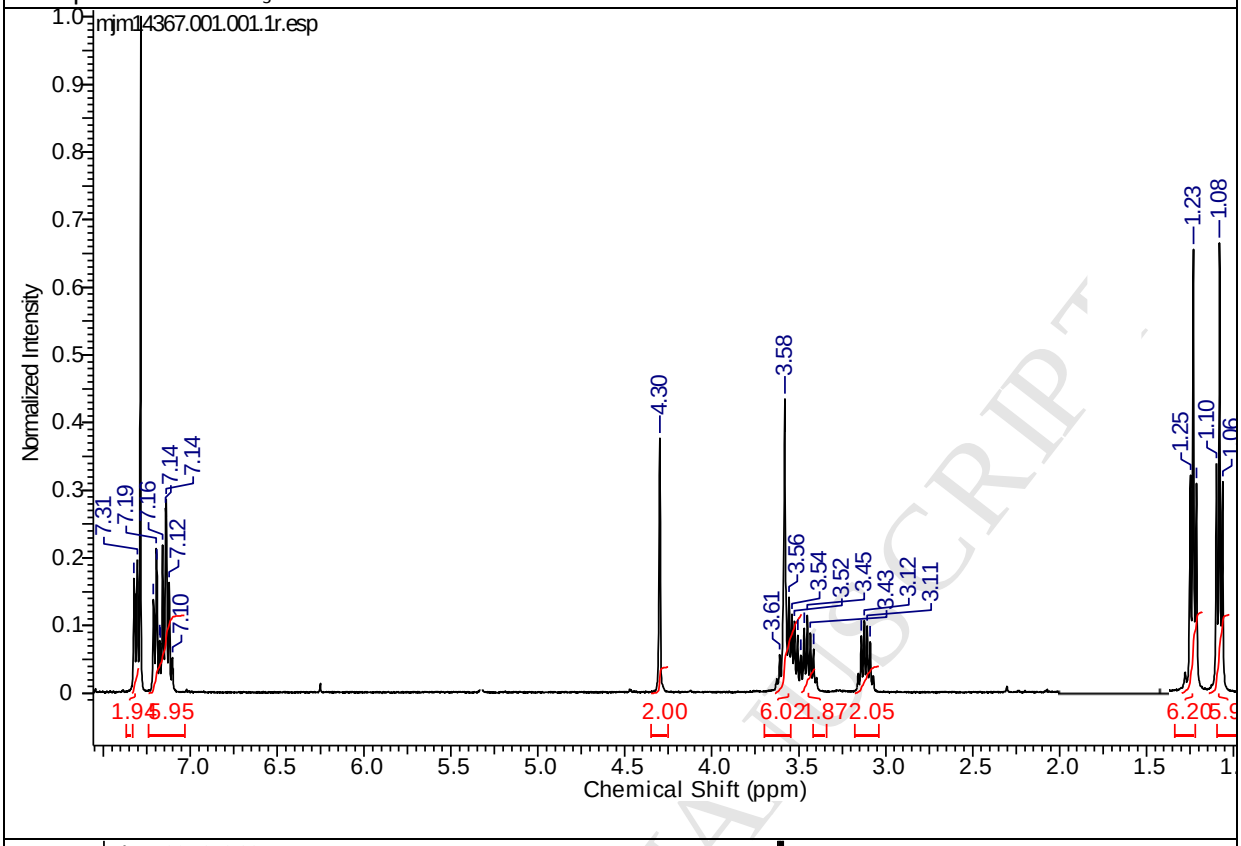
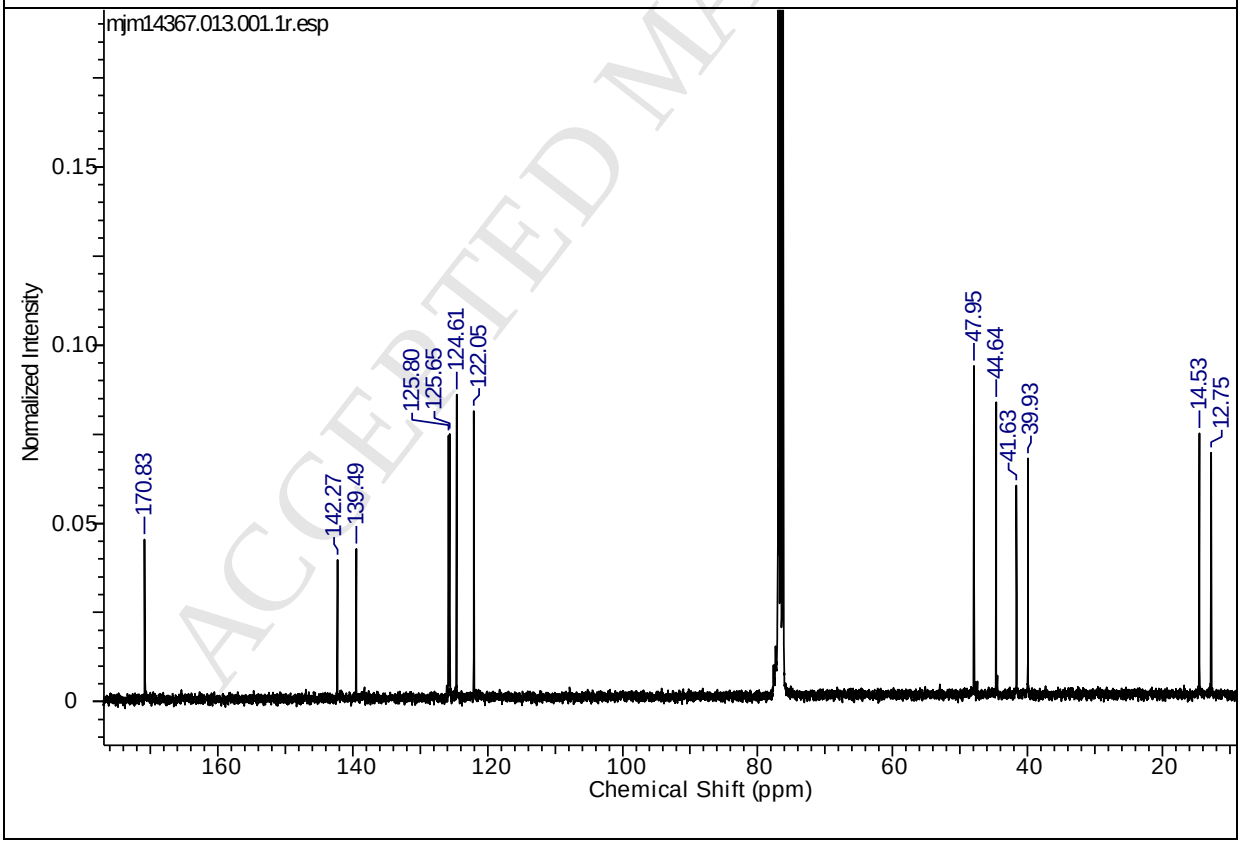
Compound 7 – $CDCl_3$





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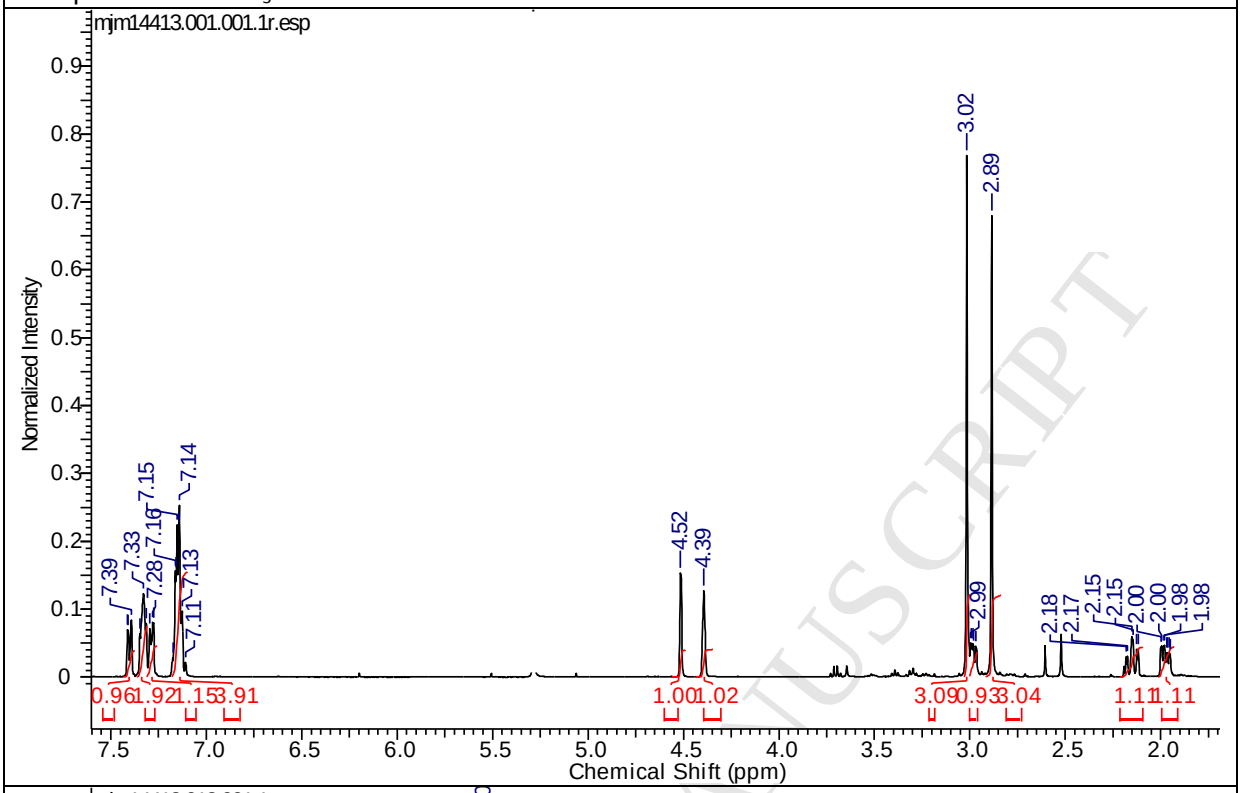
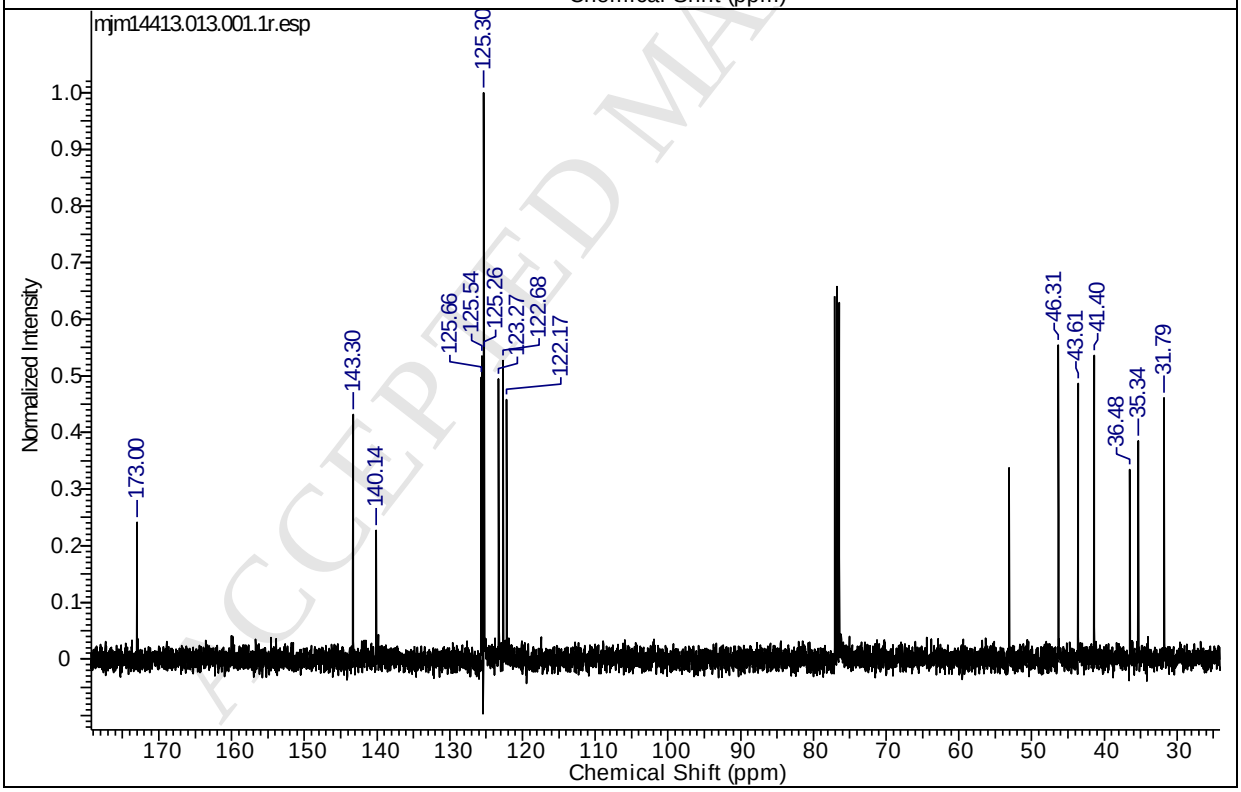
Compound 9 – $CDCl_3$





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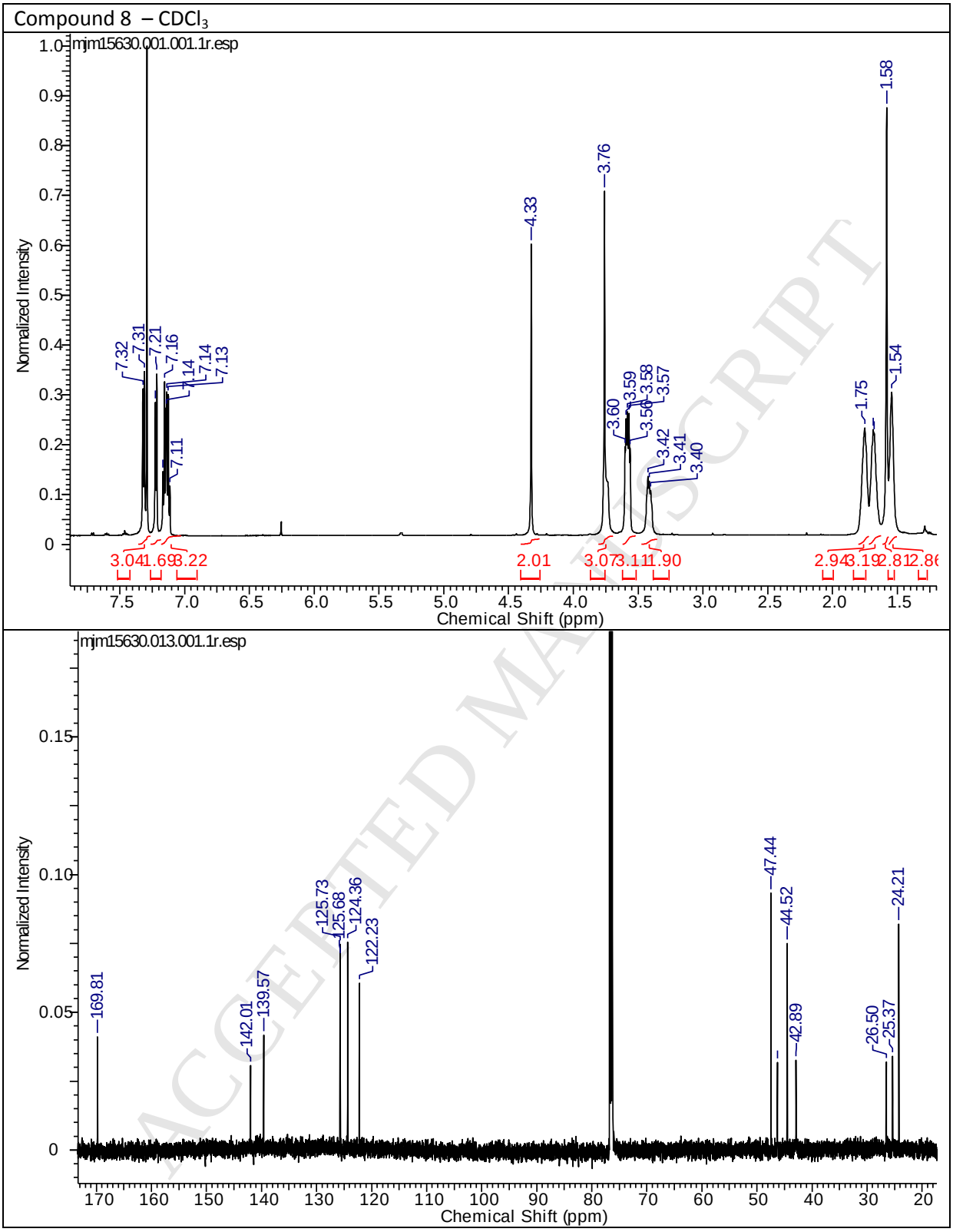

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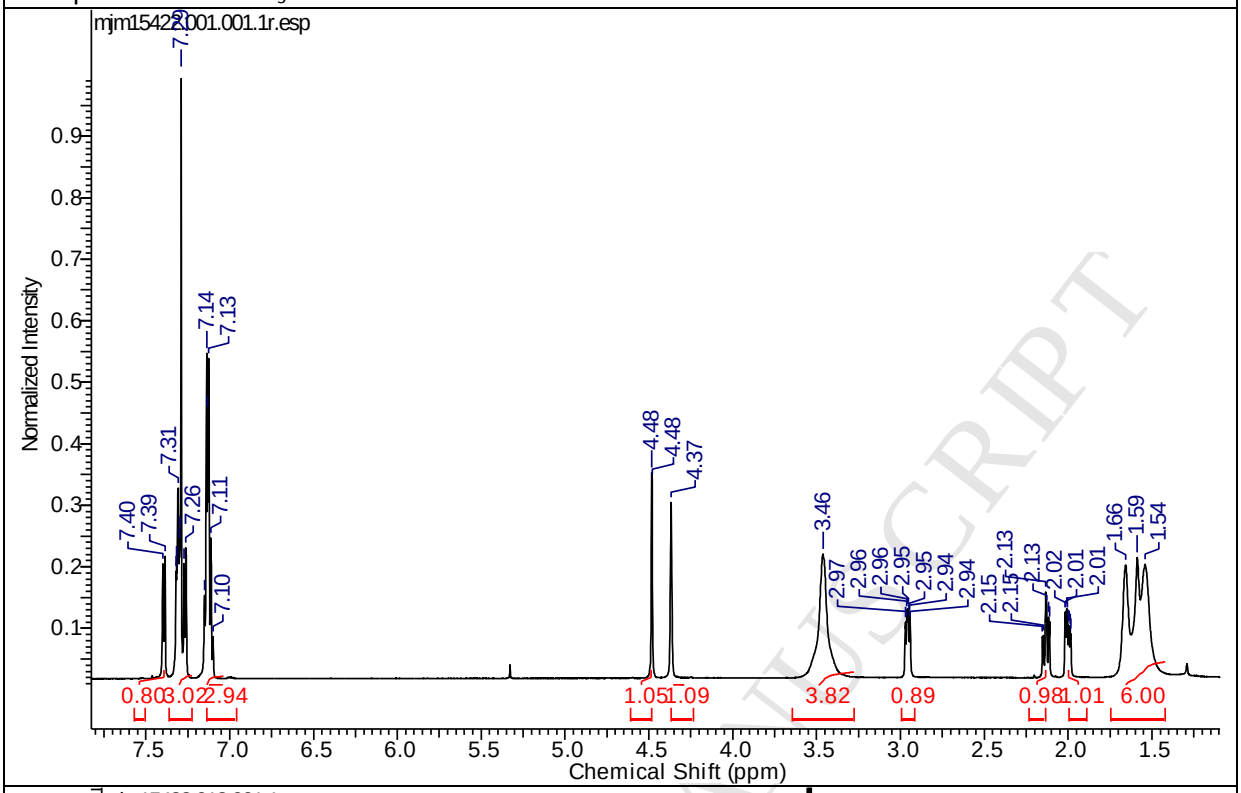
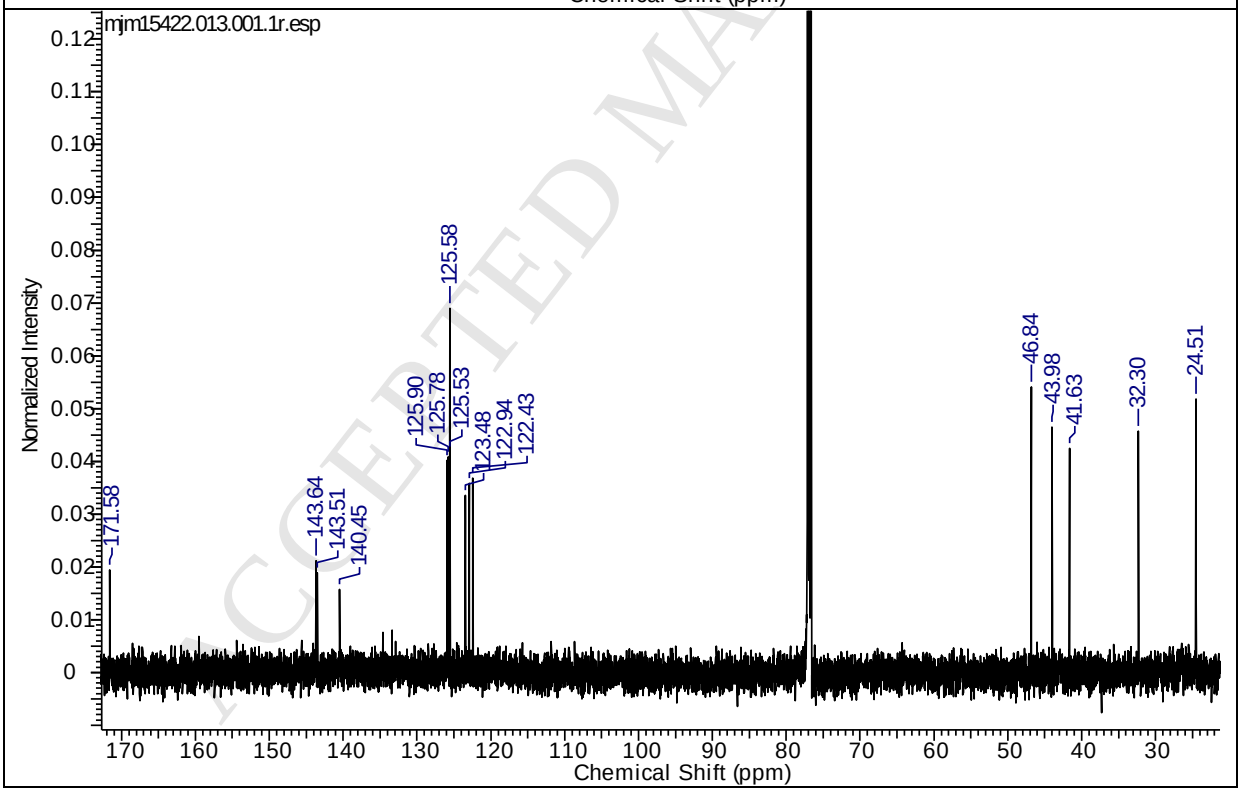
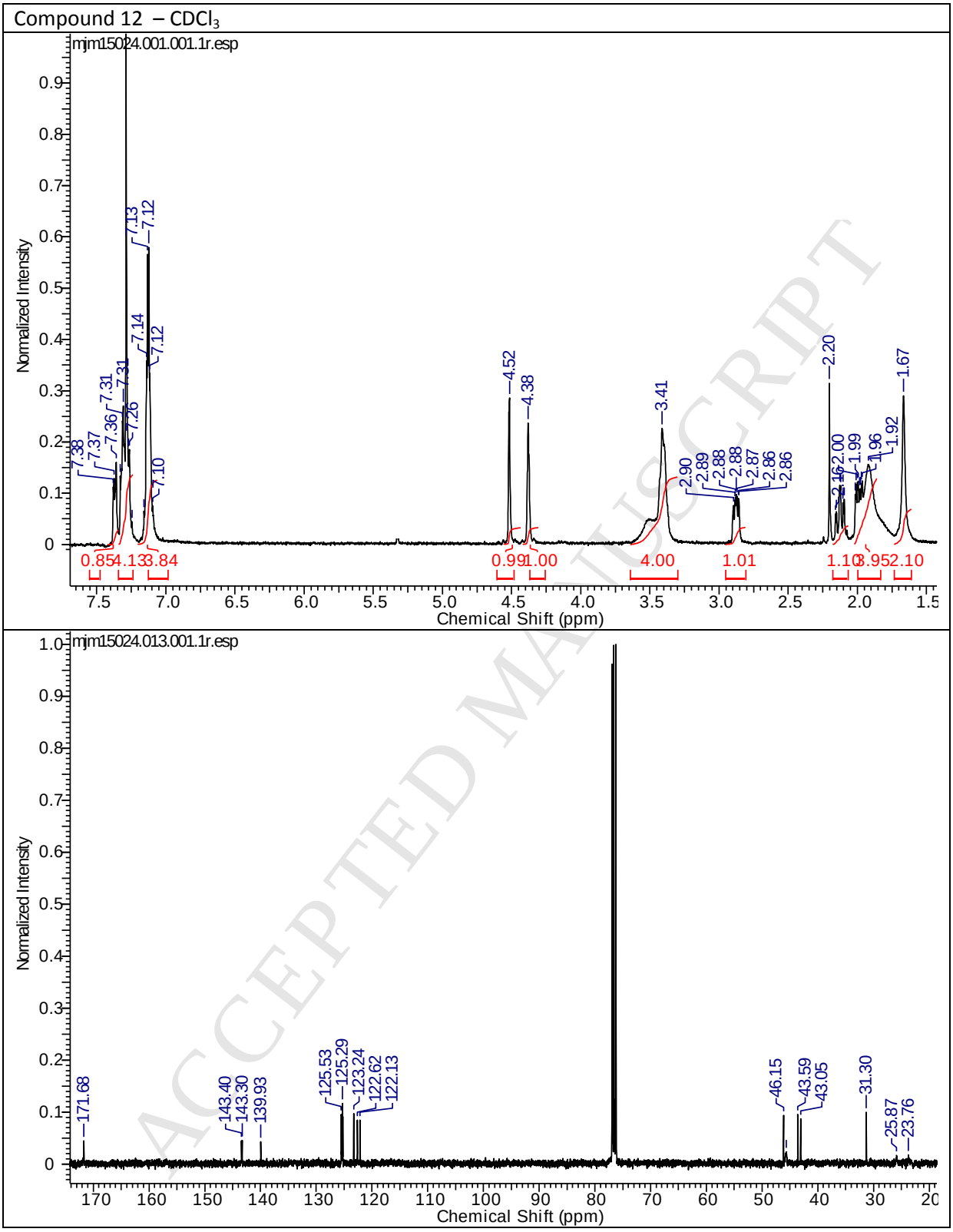
Compound 10 – $CDCl_3$







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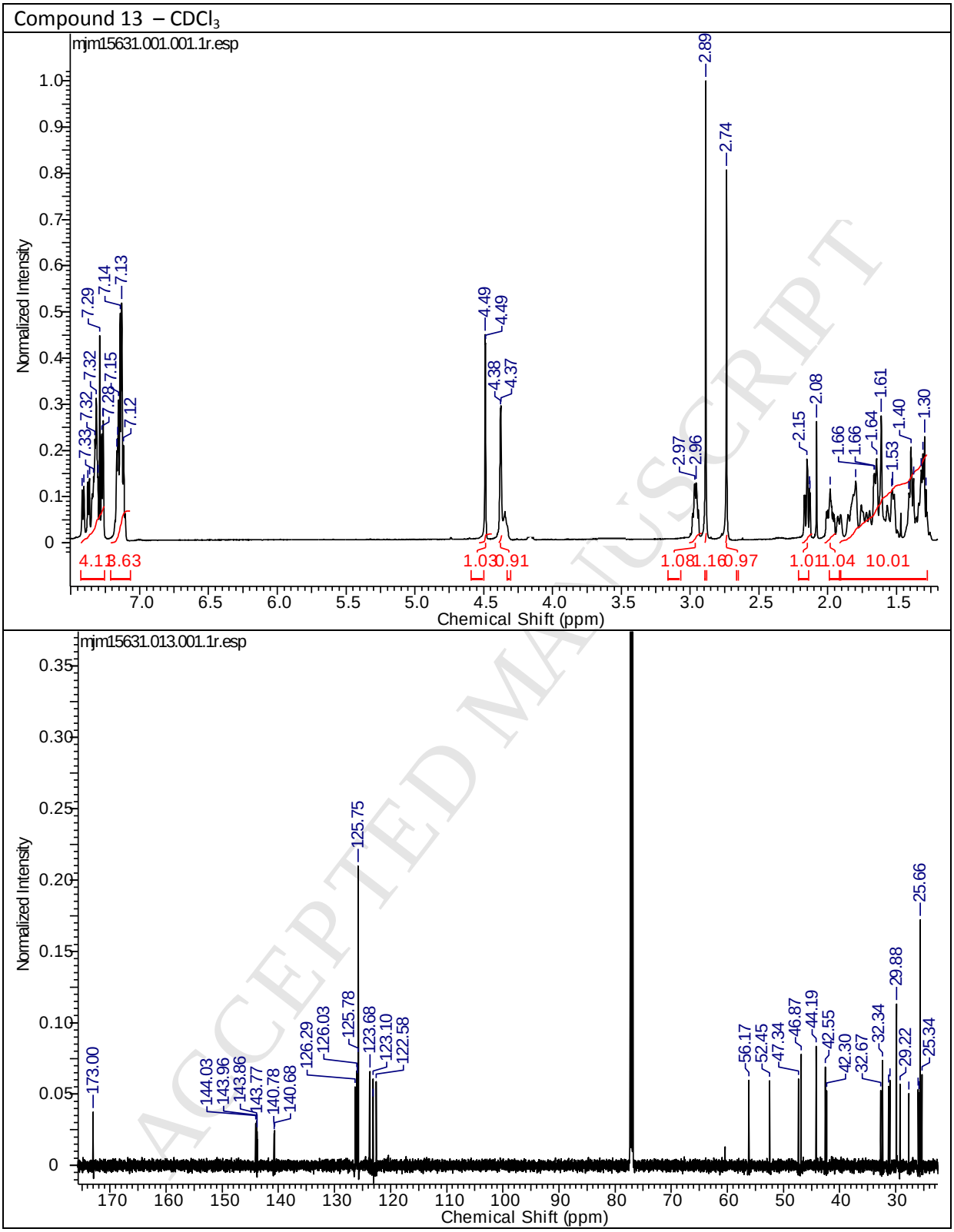

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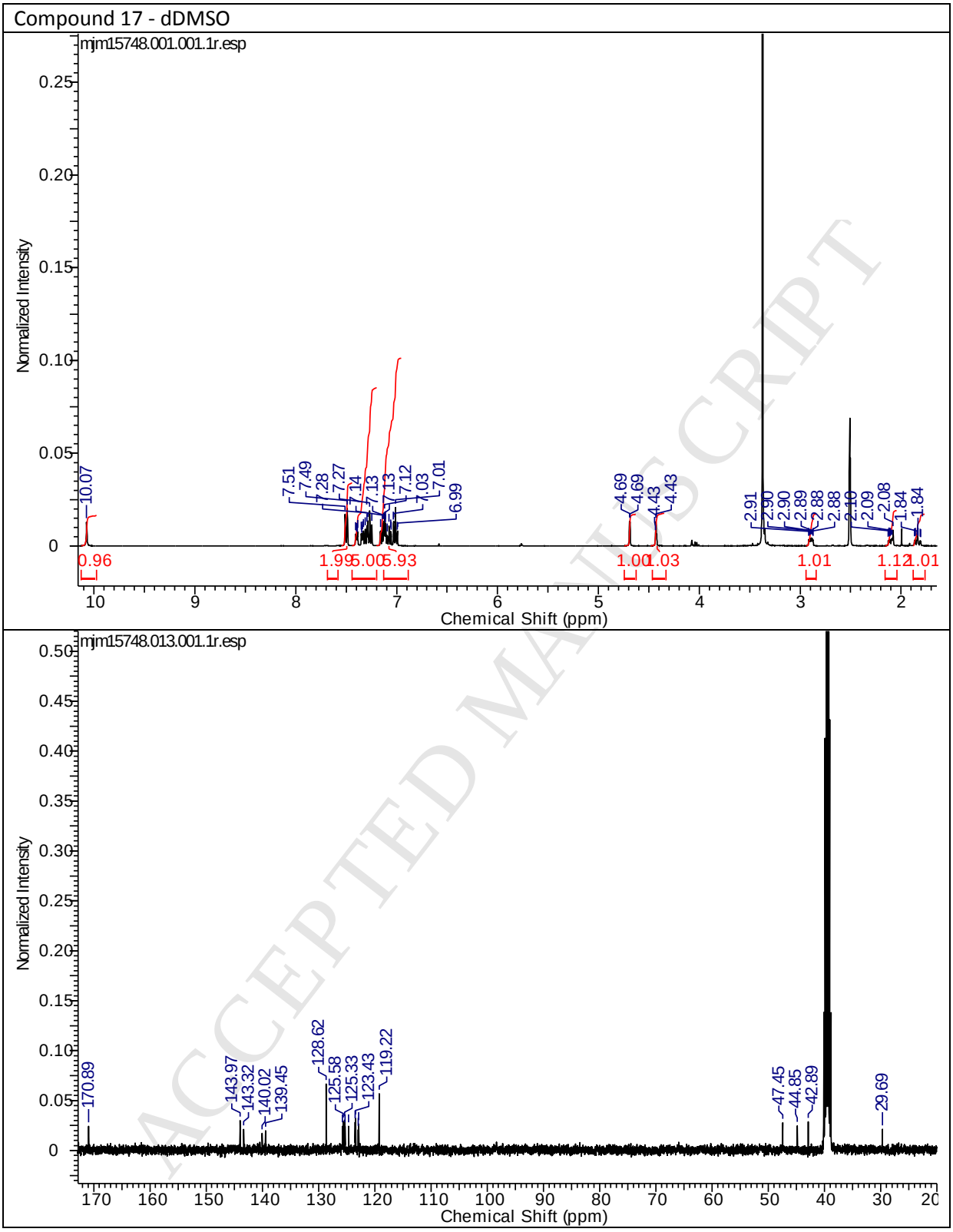

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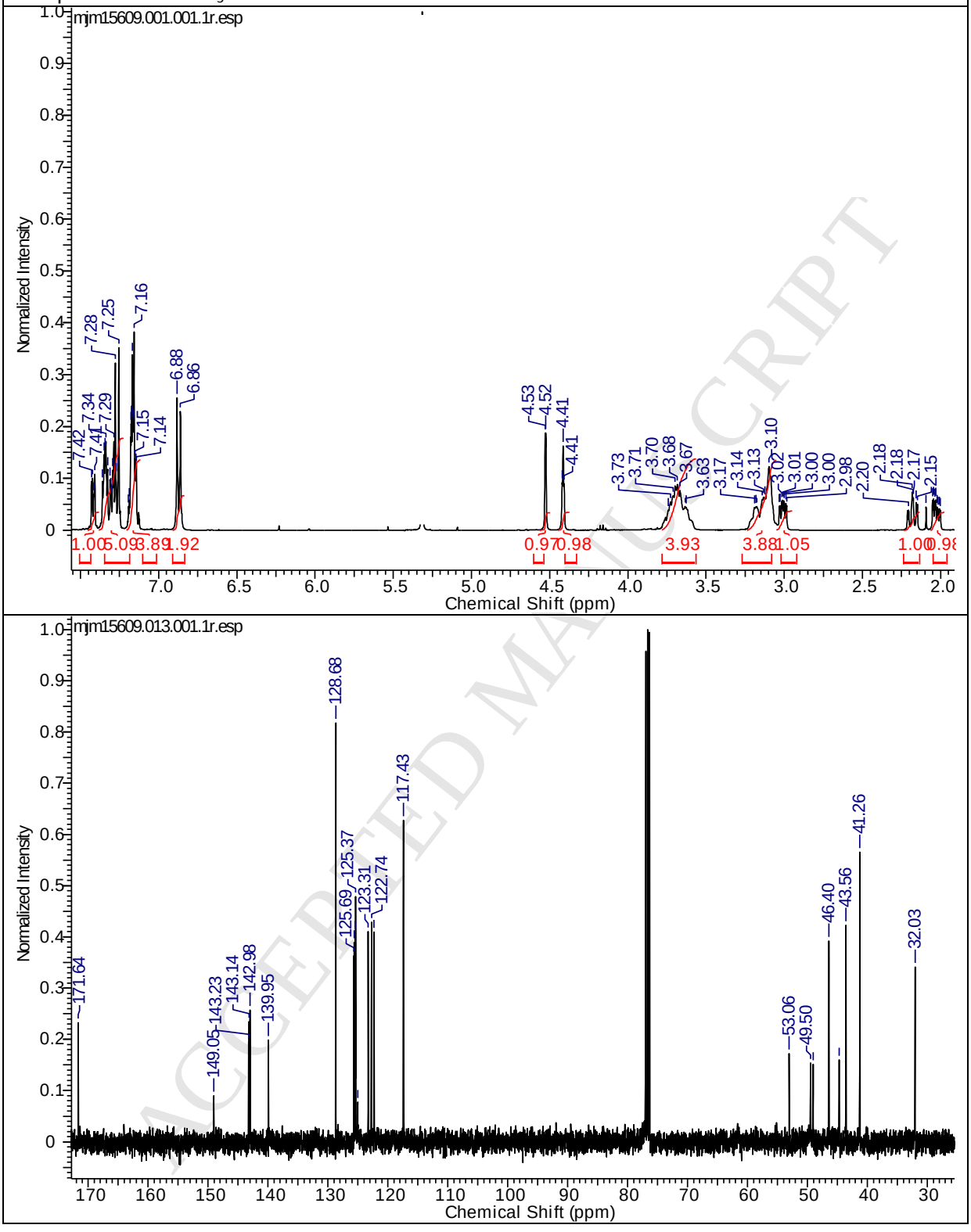
Compound 18 – $CDCl_3$



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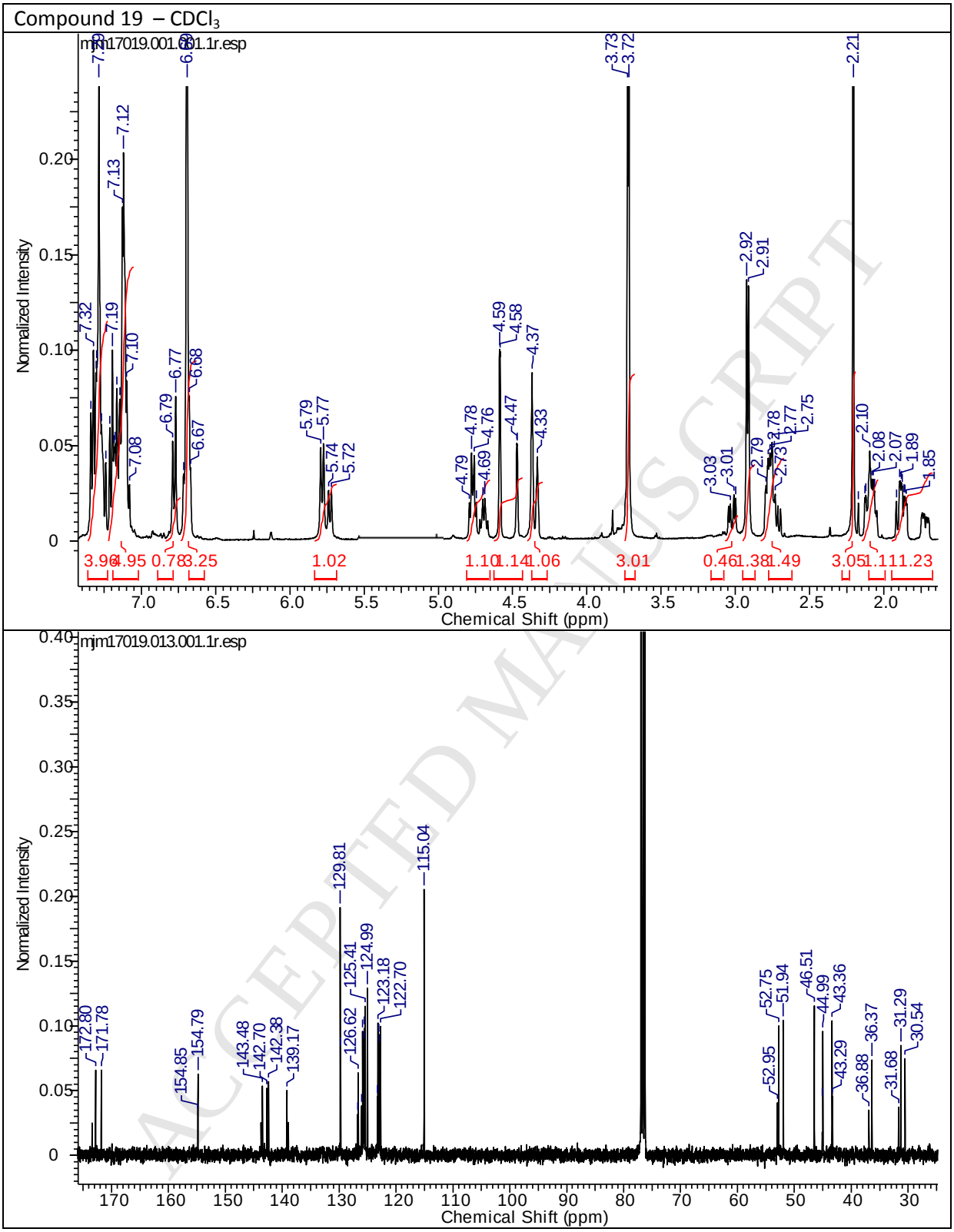

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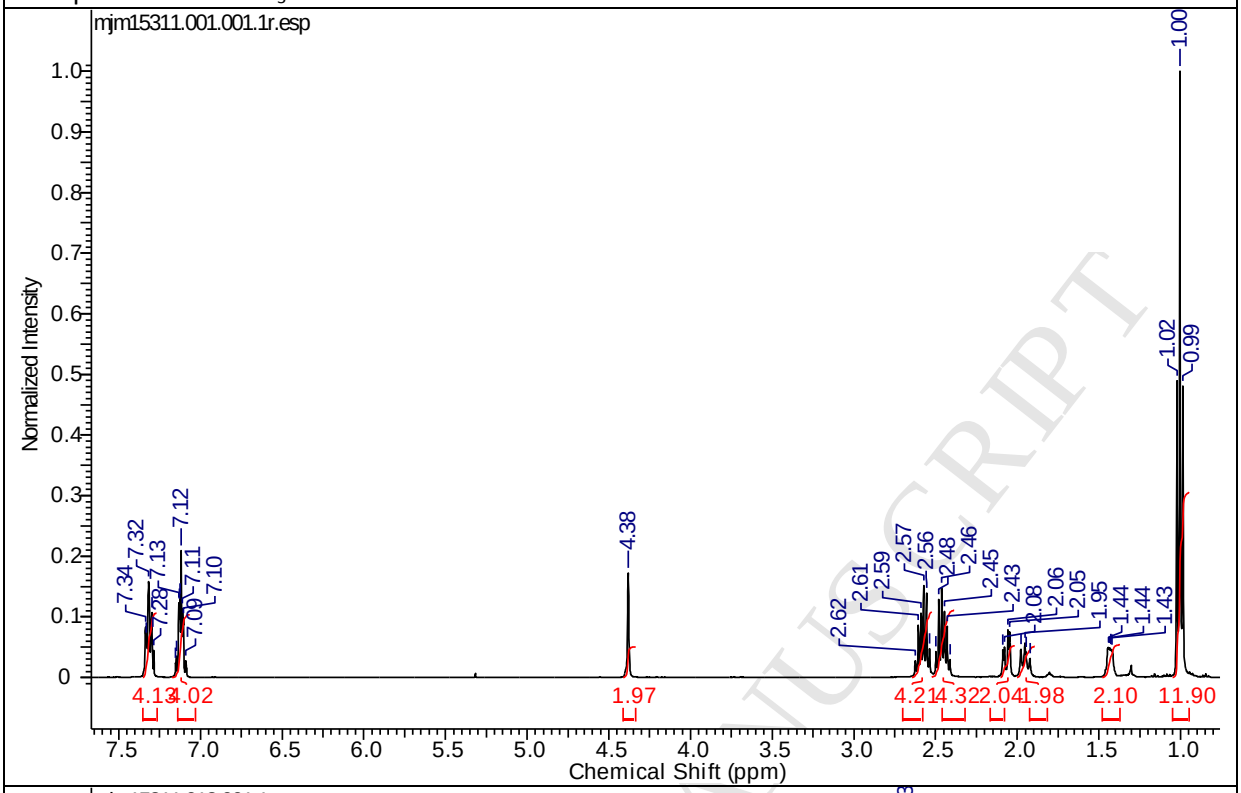
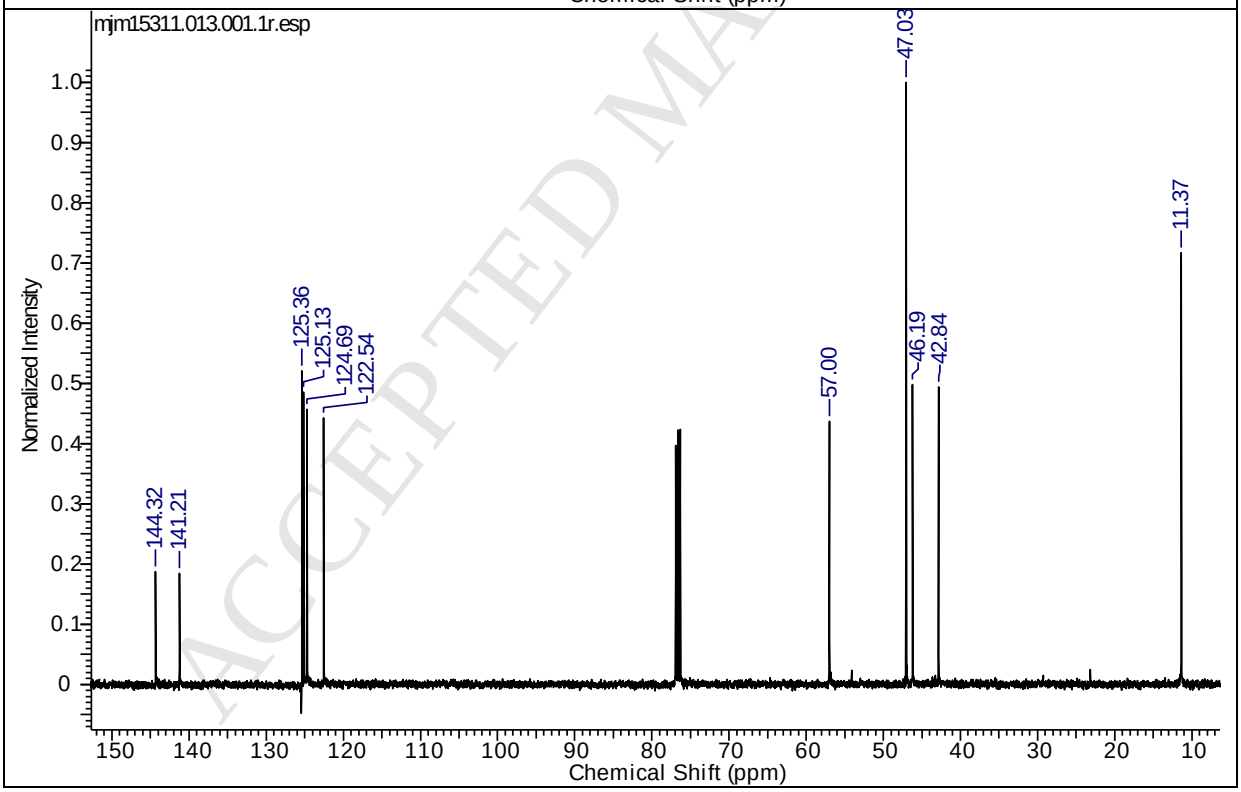
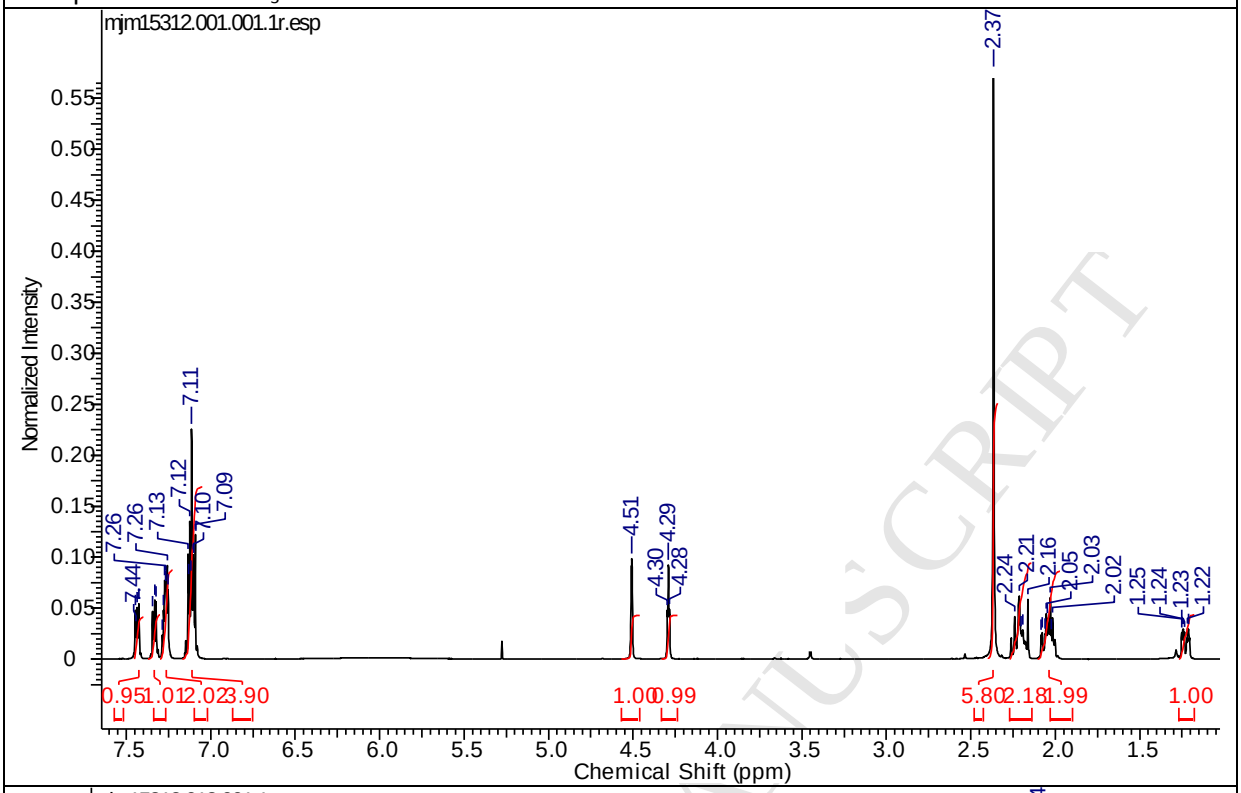
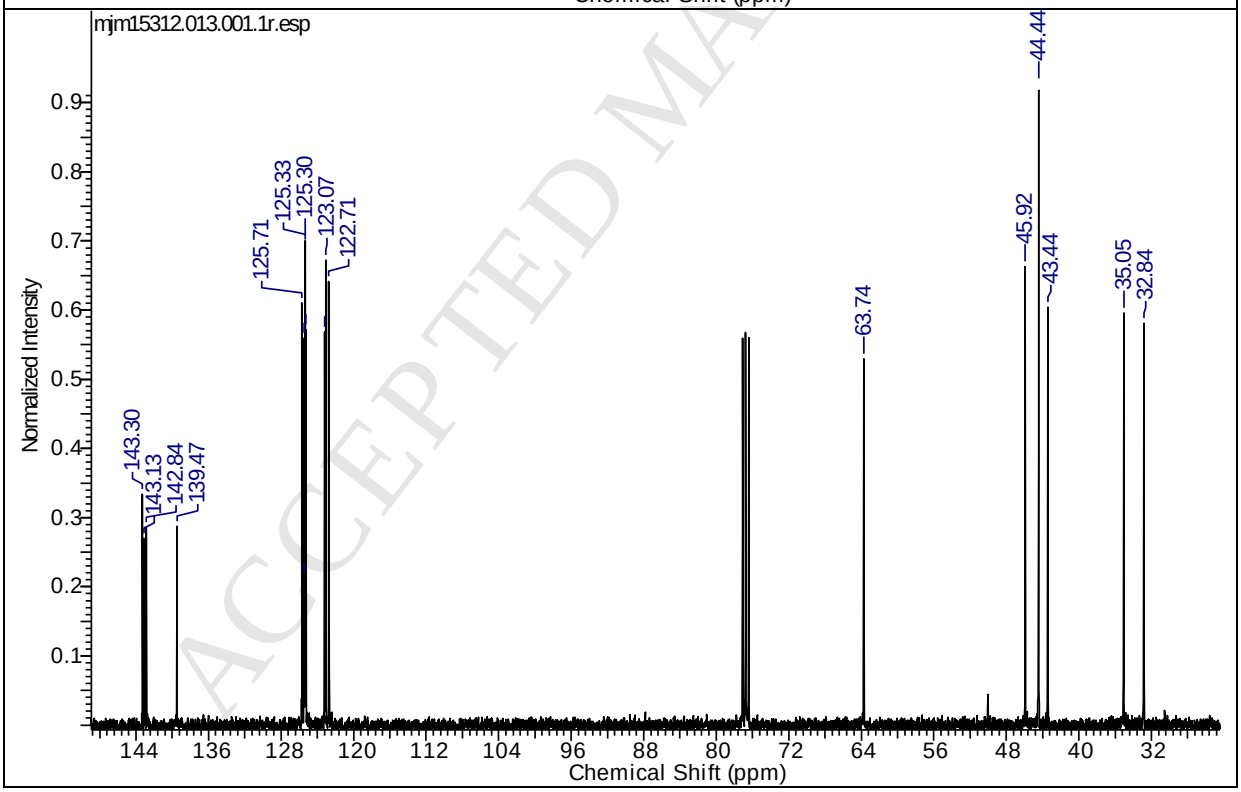
Compound 22 – $CDCl_3$





Compound 23 – $CDCl_3$

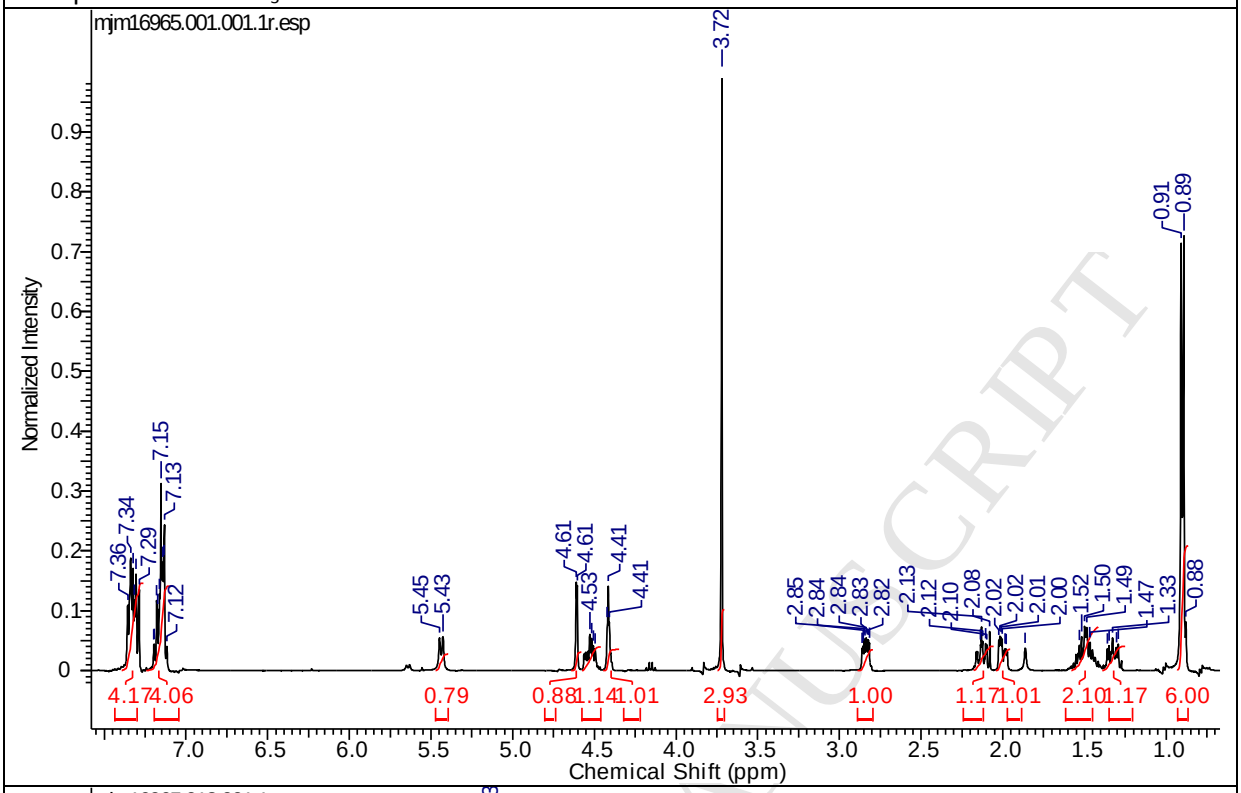


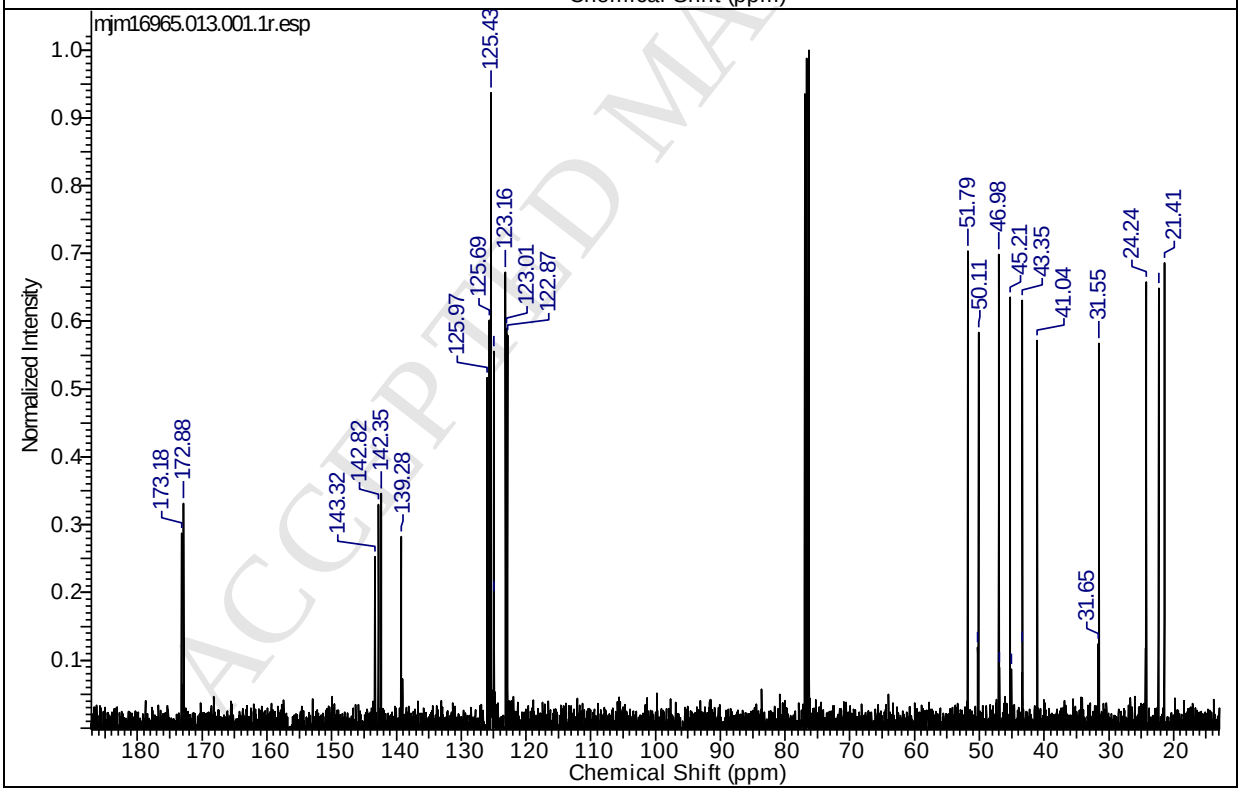


ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

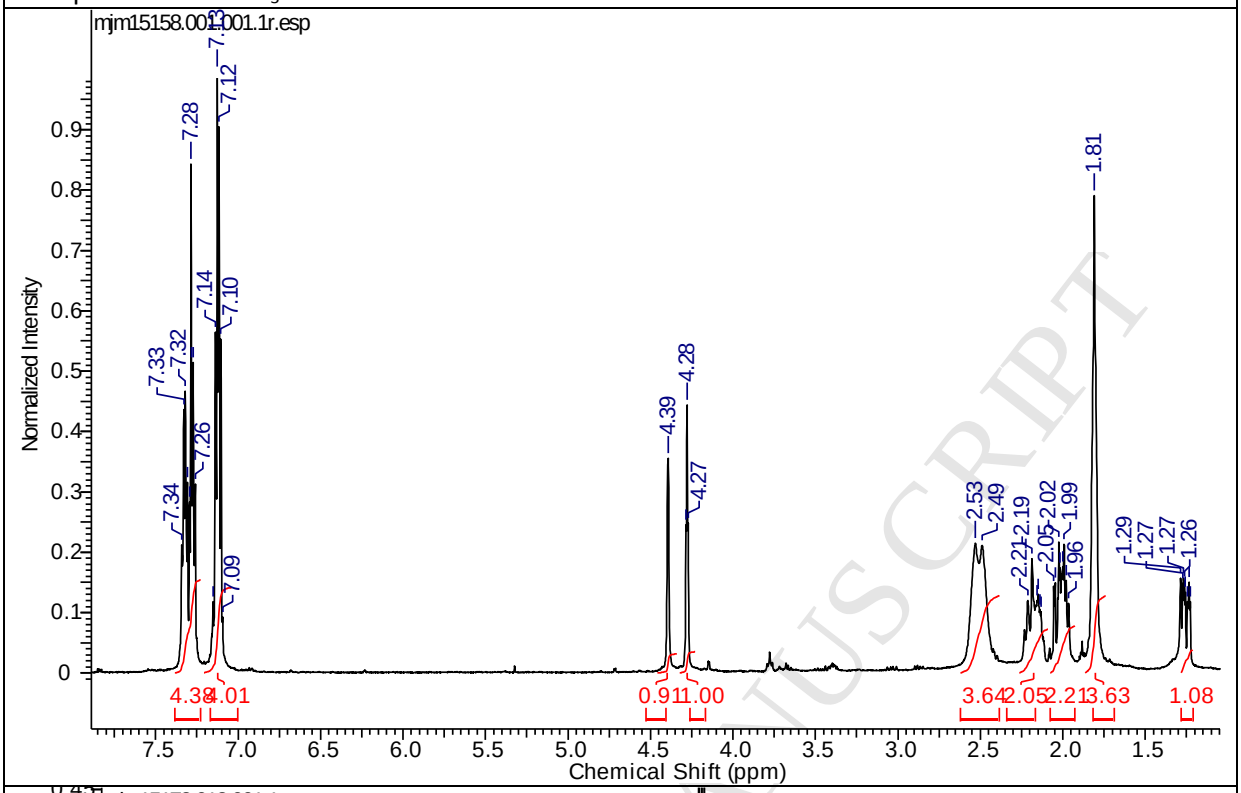
Compound 20 – $CDCl_3$

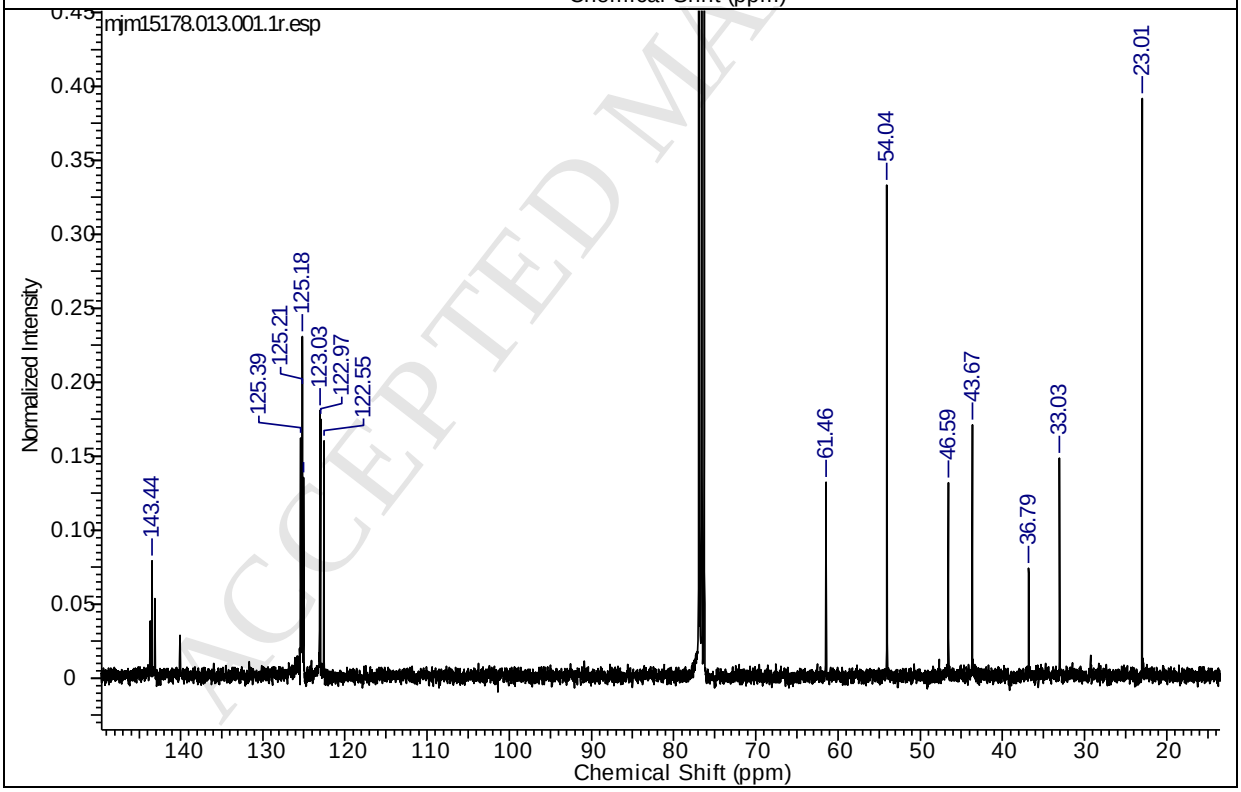




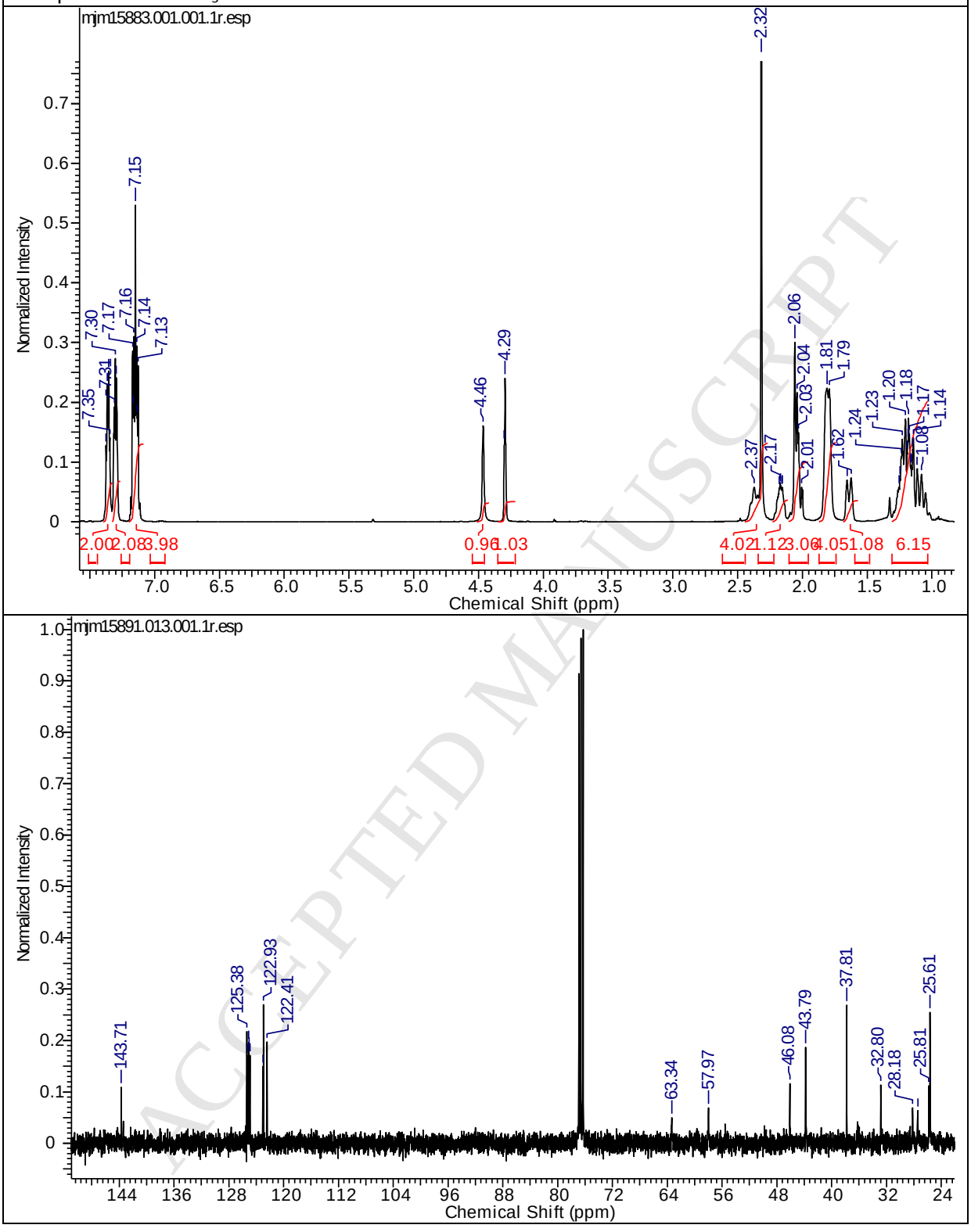
ACCEPTED MANUSCRIPT

Compound 26 – $CDCl_3$





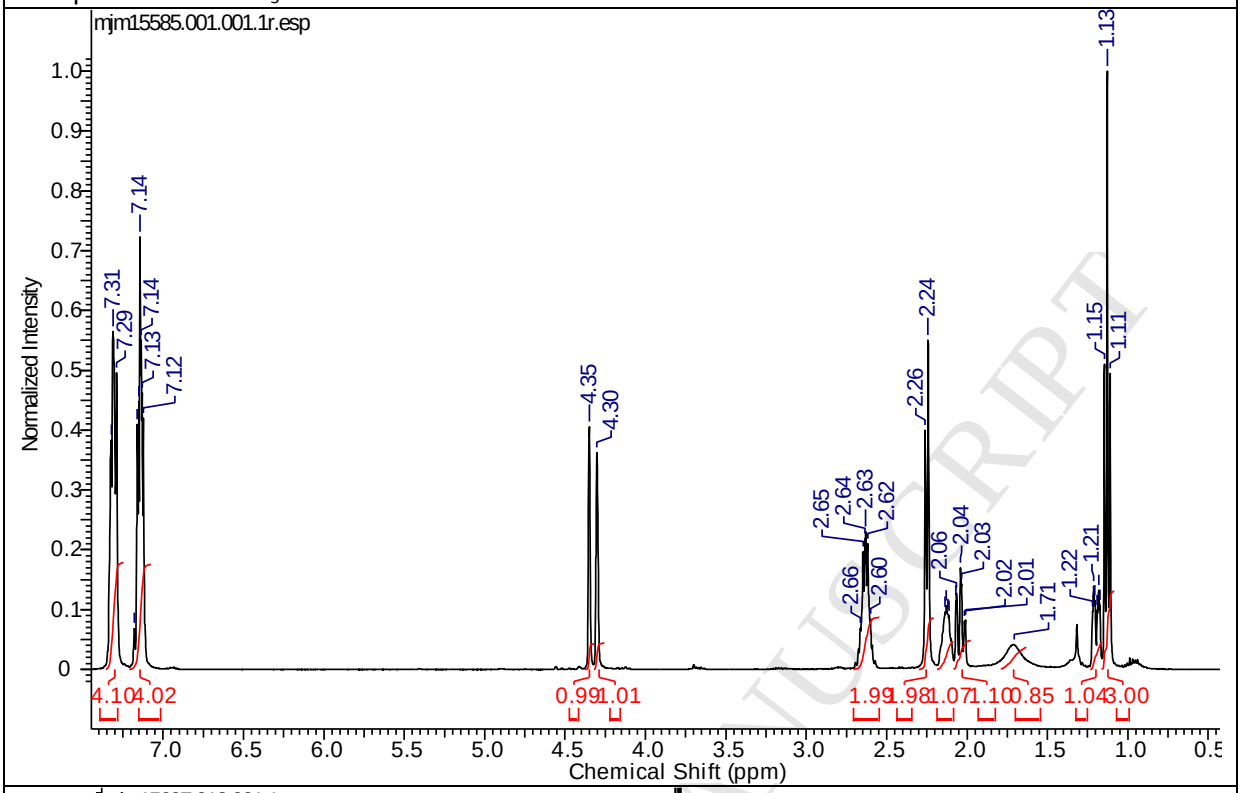
Compound 27 – $CDCl_3$

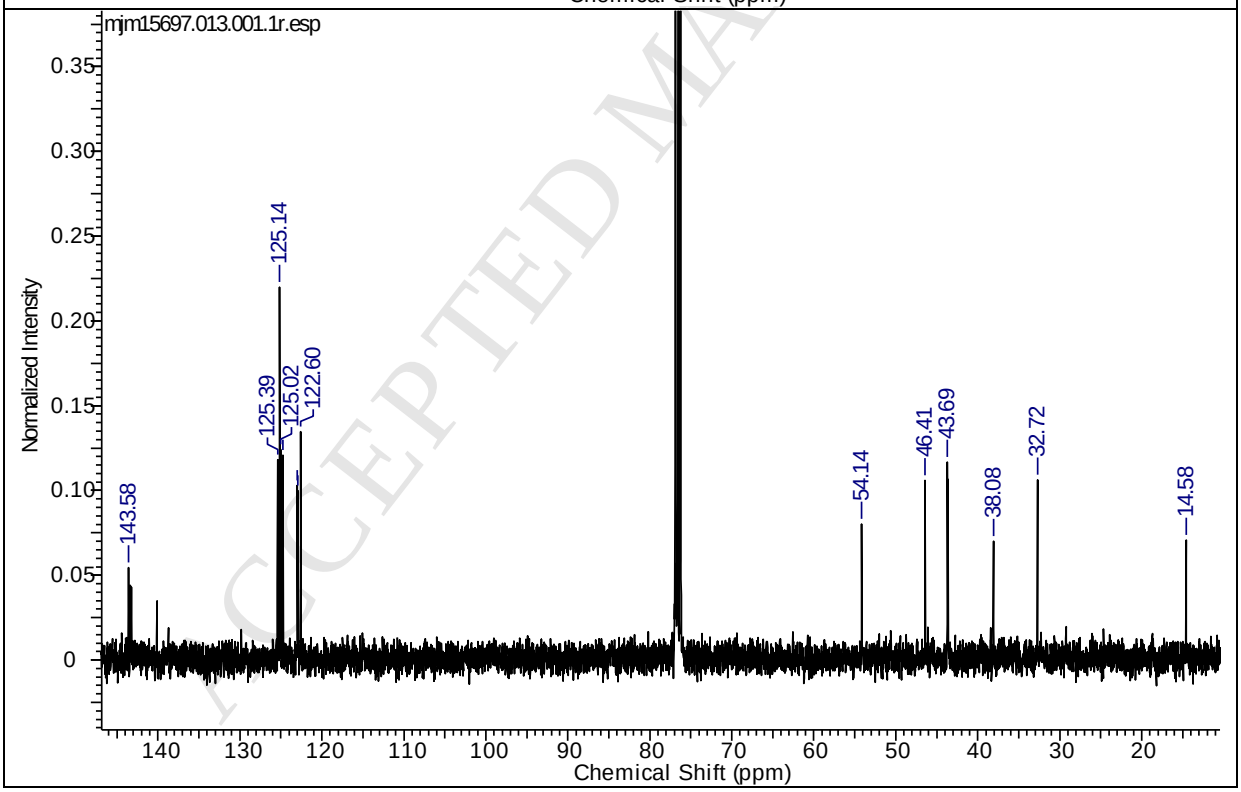


ACCEPTED MANUSCRIPT

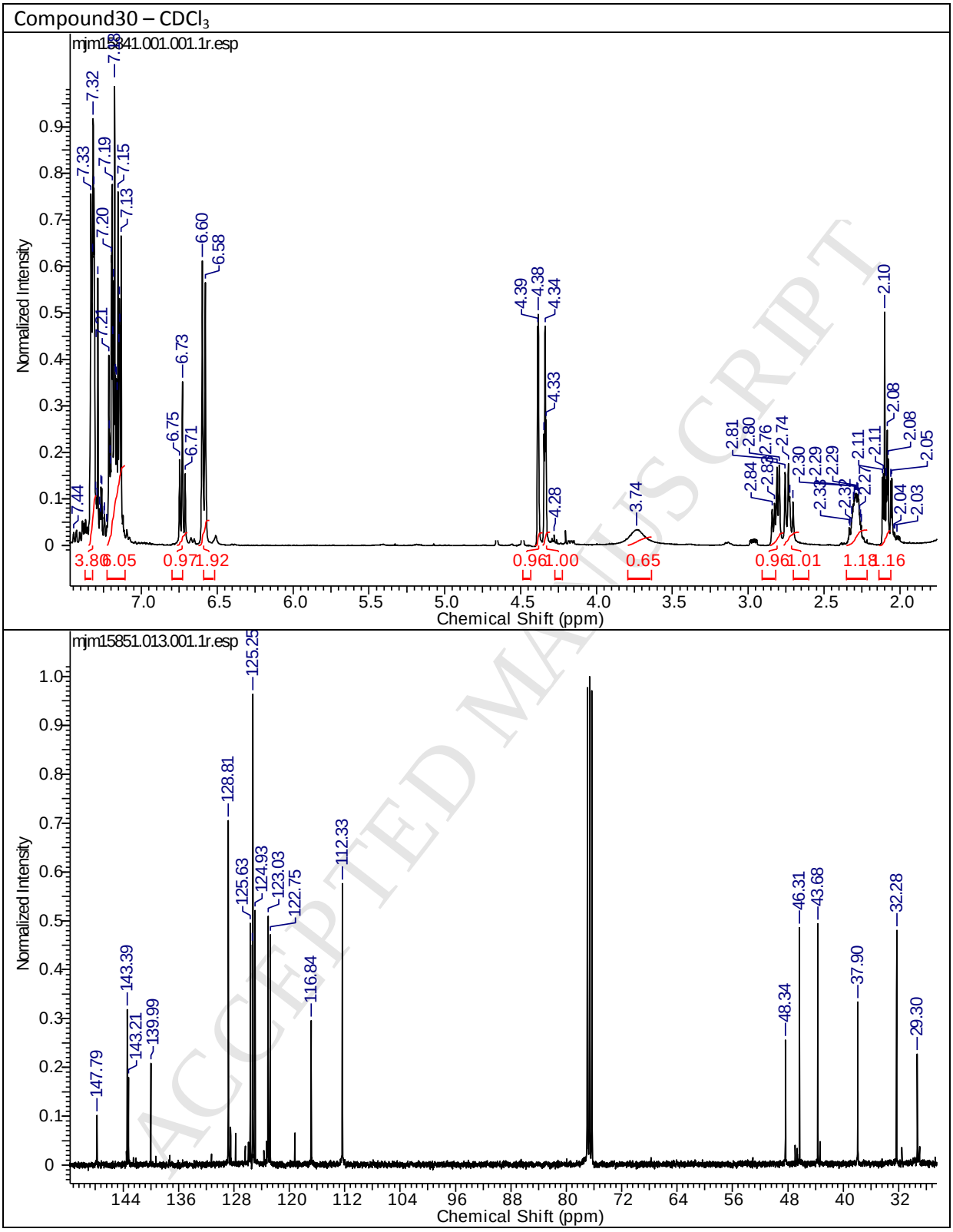
ACCEPTED MANUSCRIPT

Compound 28 – $CDCl_3$



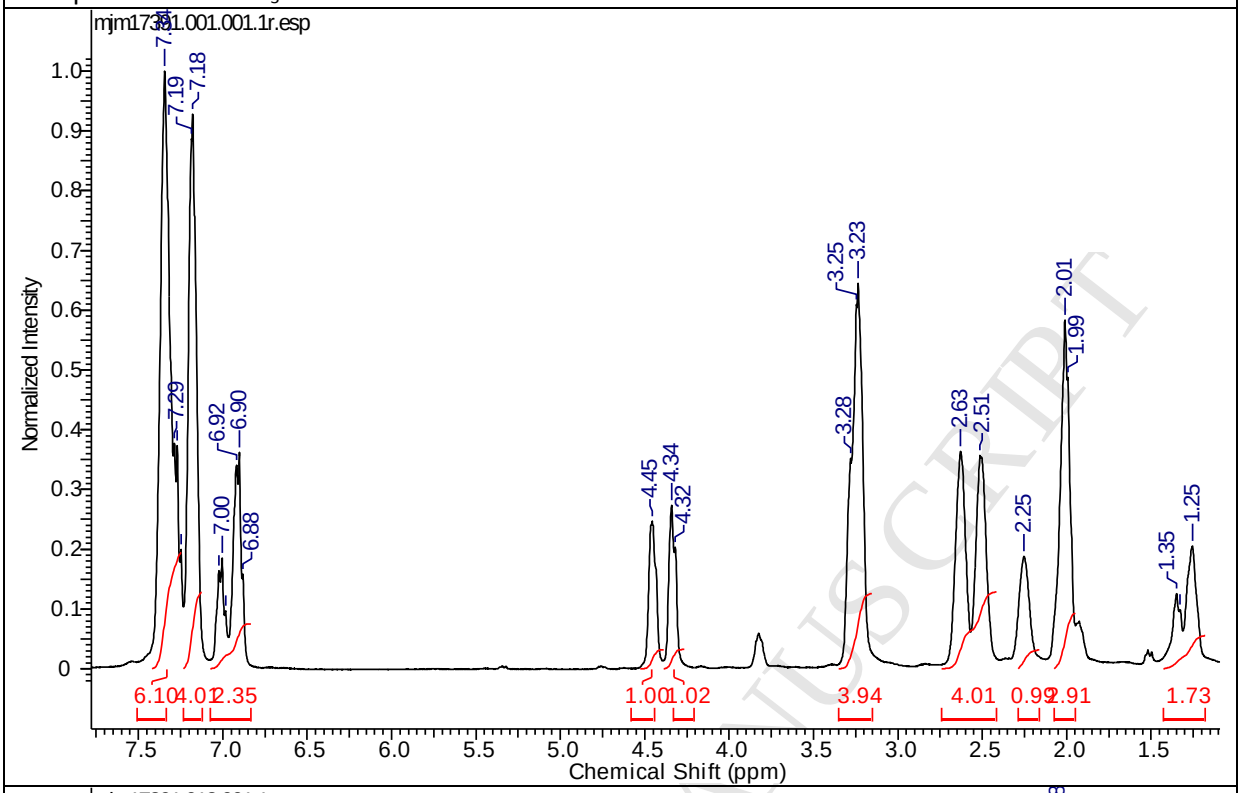


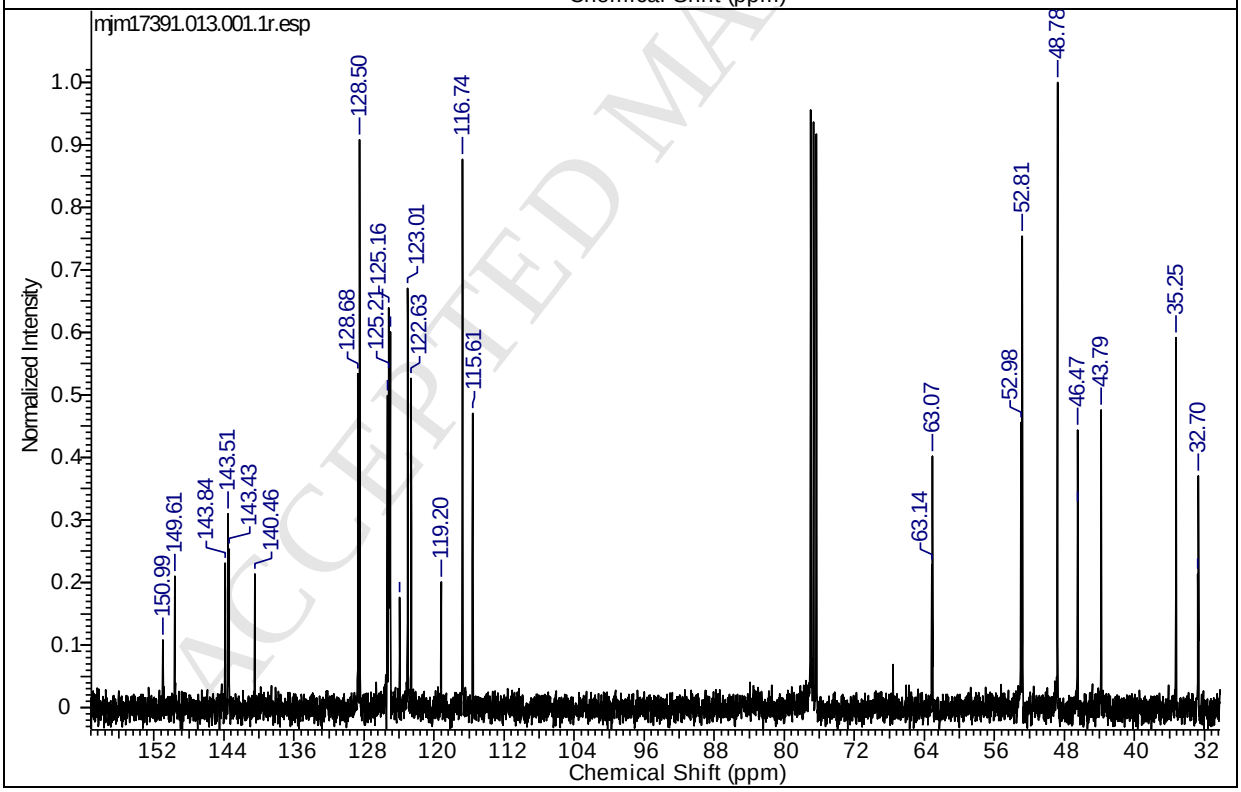
ACCEPTED MANUSCRIPT

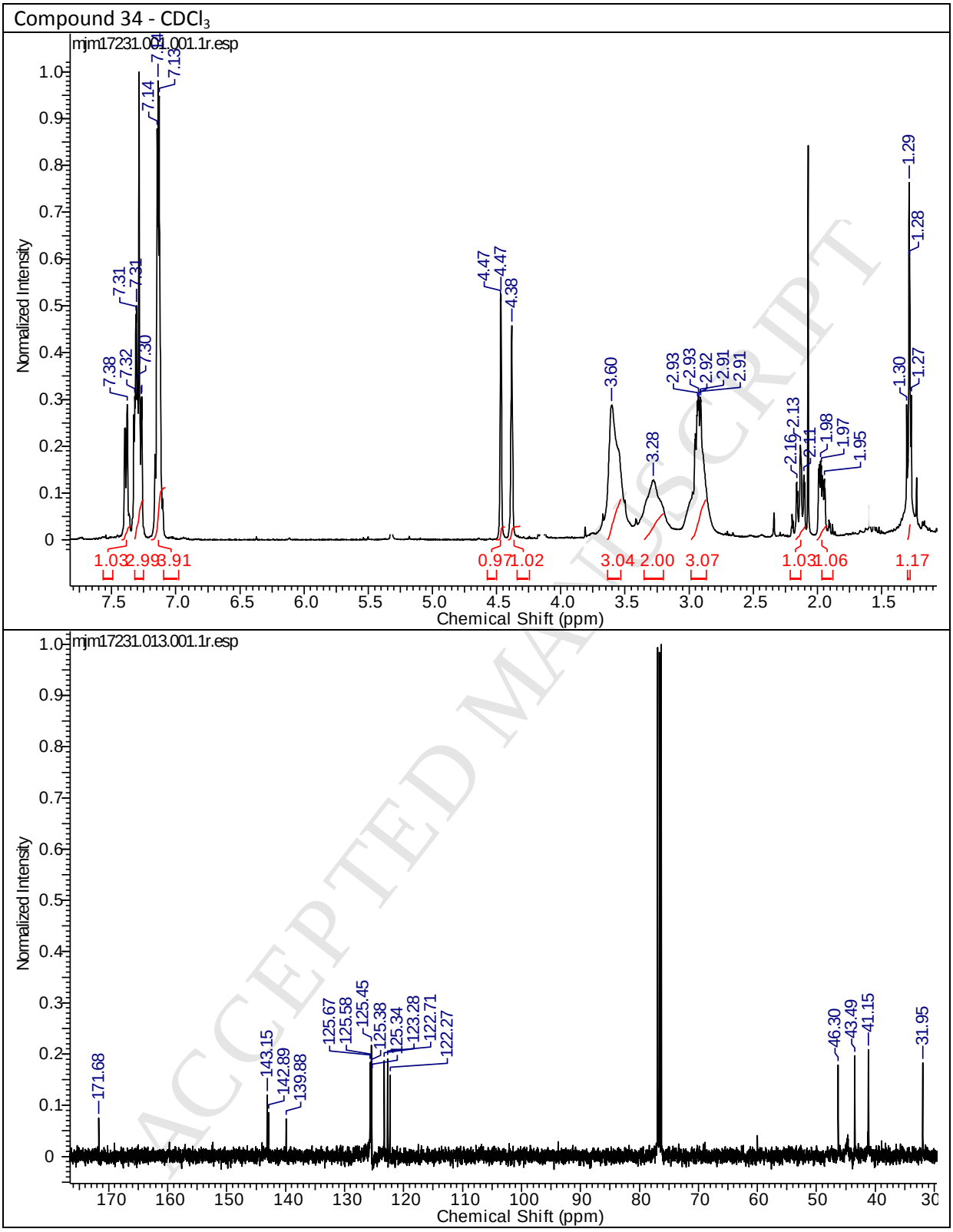


ACCEPTED MANUSCRIPT

Compound 31 – $CDCl_3$

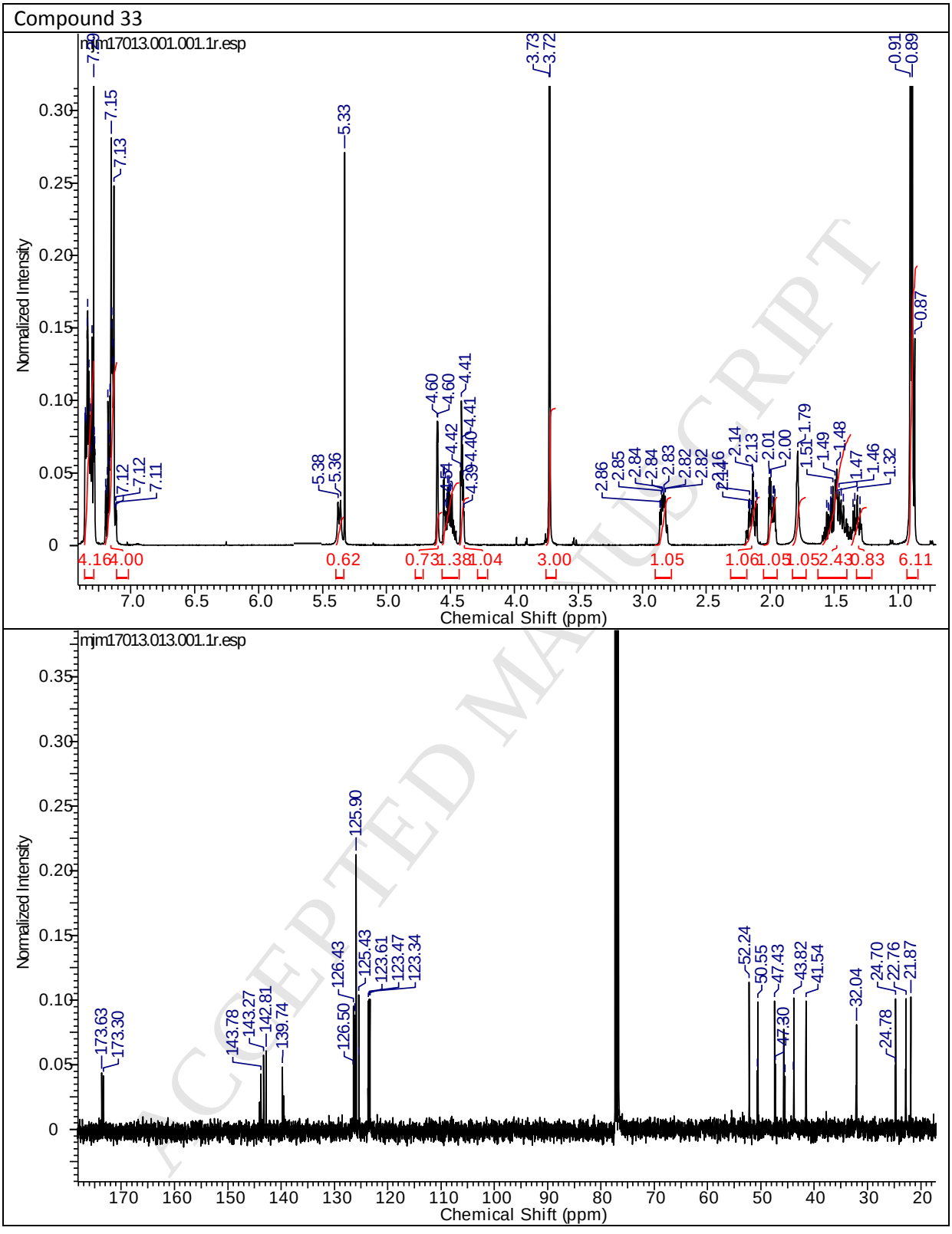






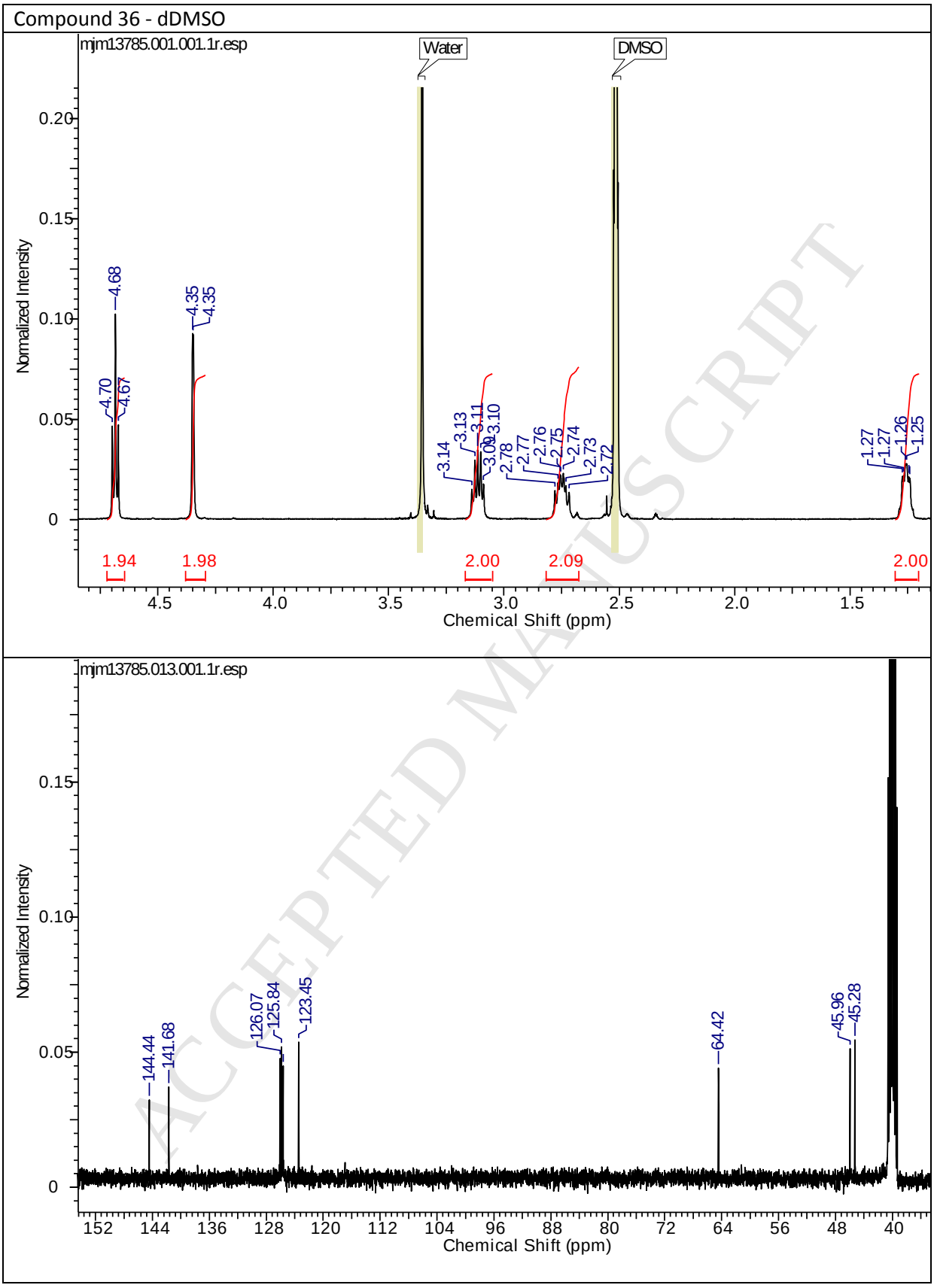
ACCEPTED MANUSCRIPT

Compound 33

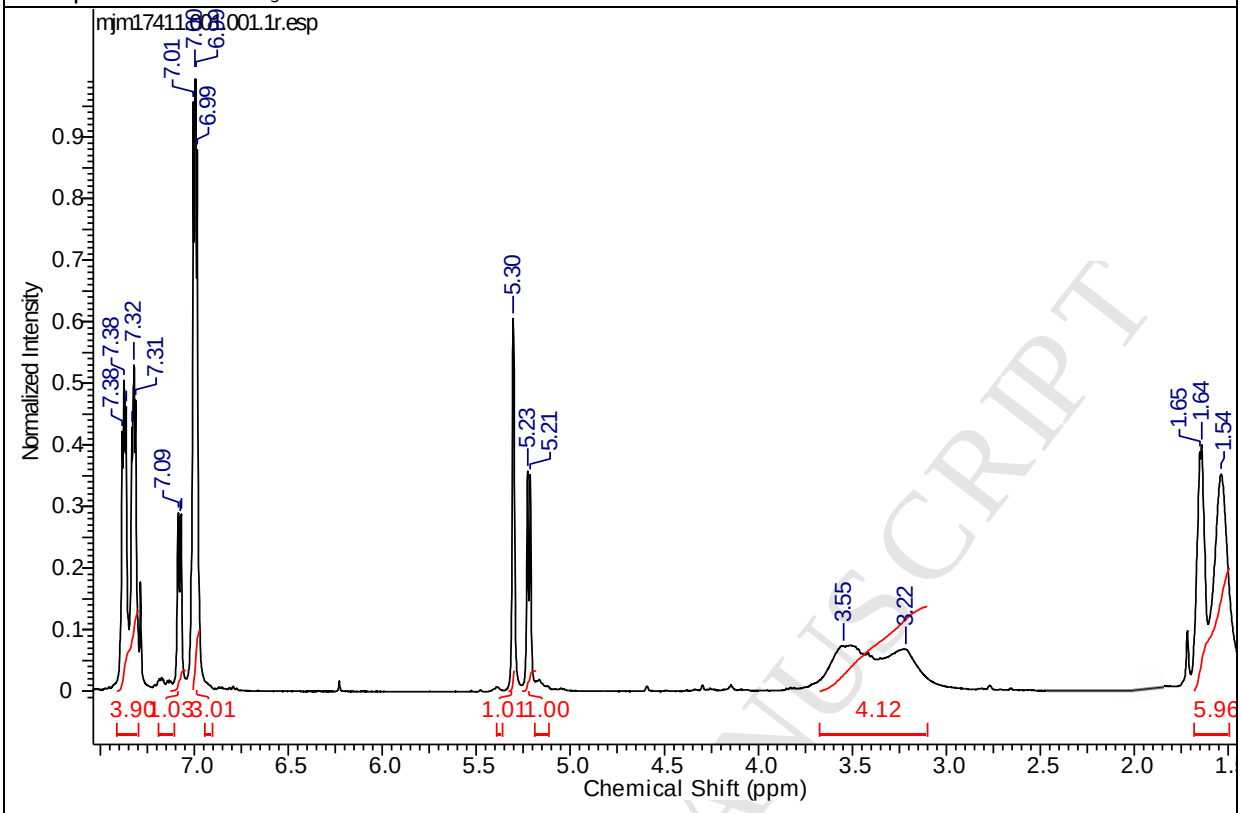


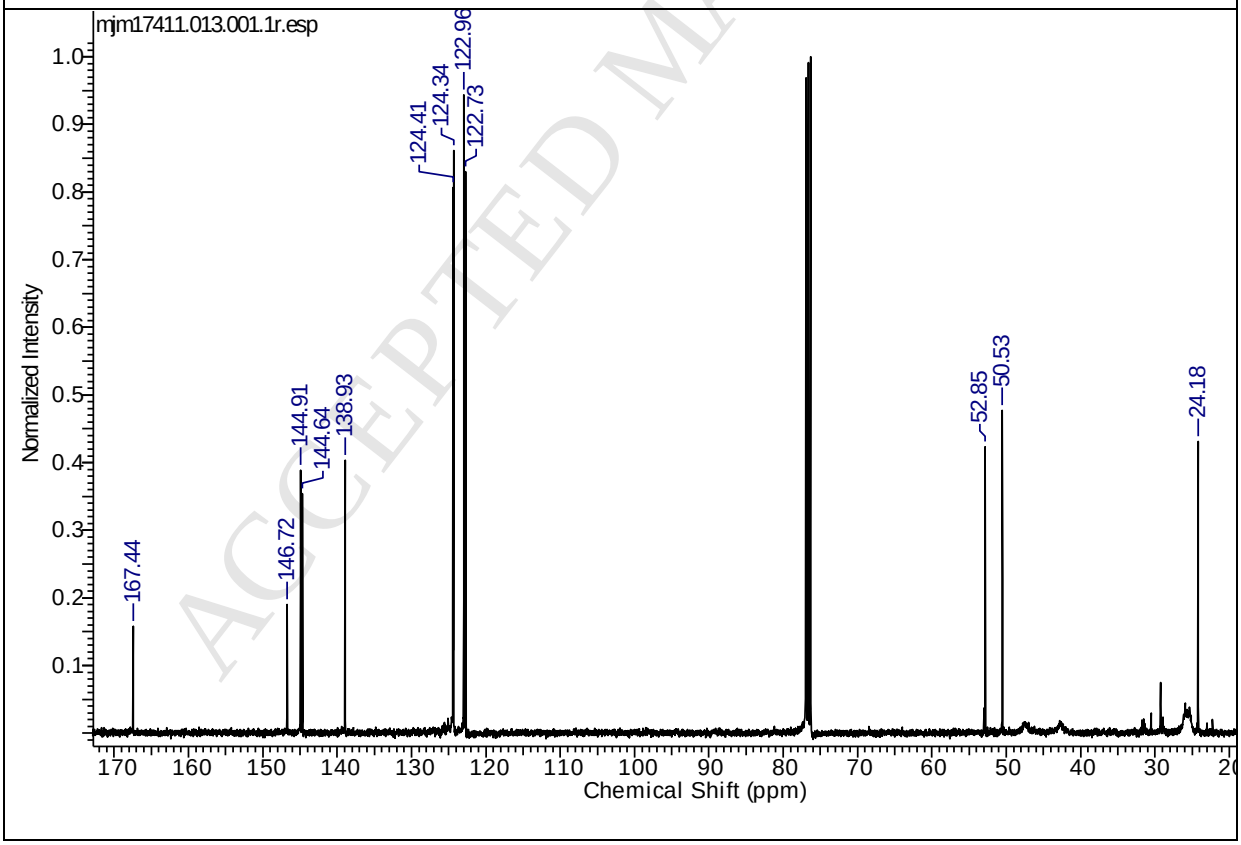
ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT



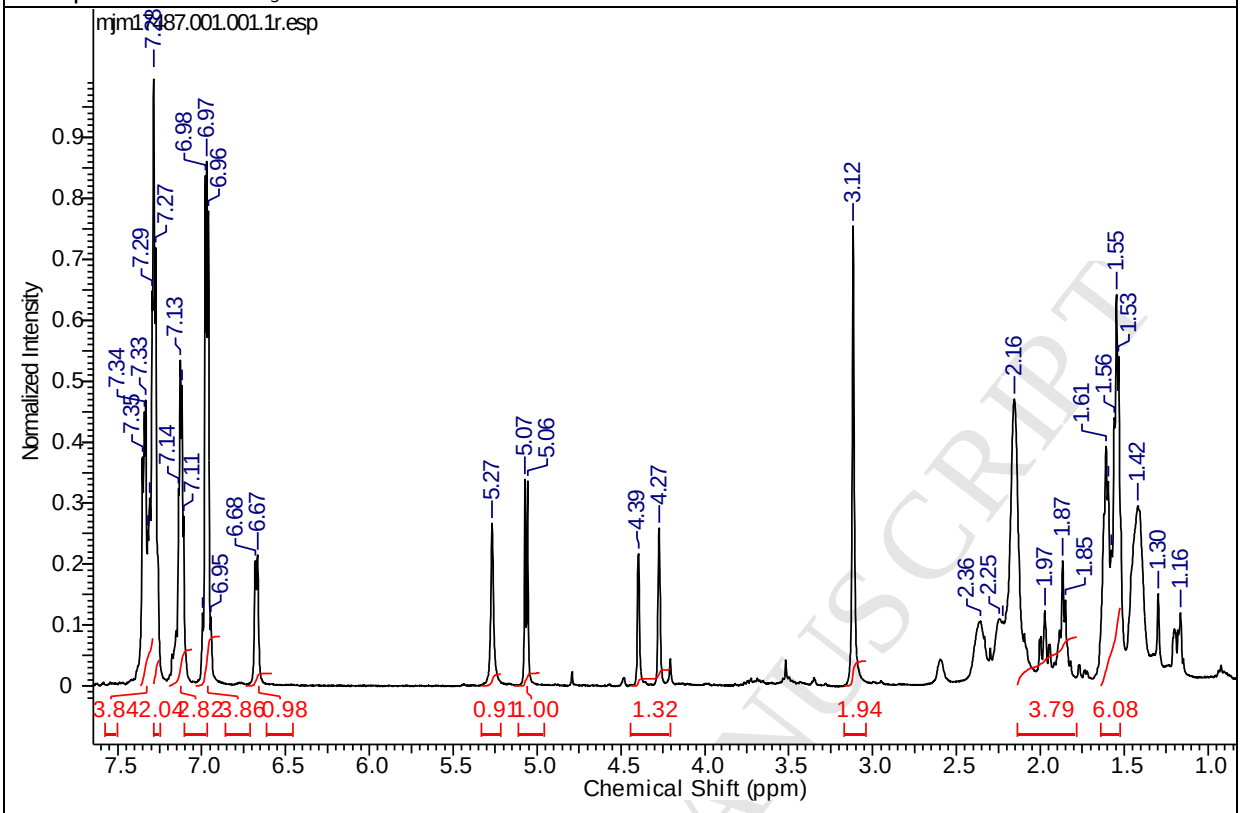
Compound 43 – $CDCl_3$

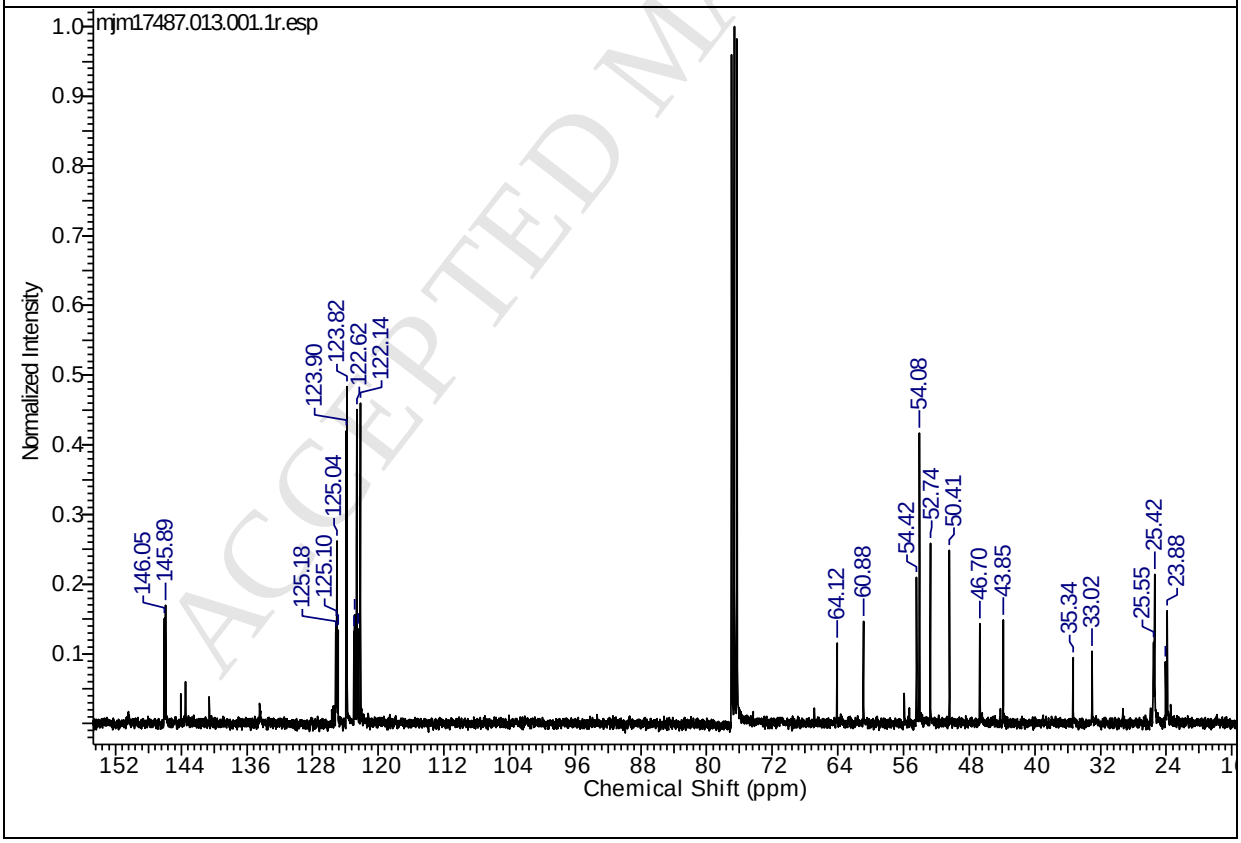




ACCEPTED MANUSCRIPT

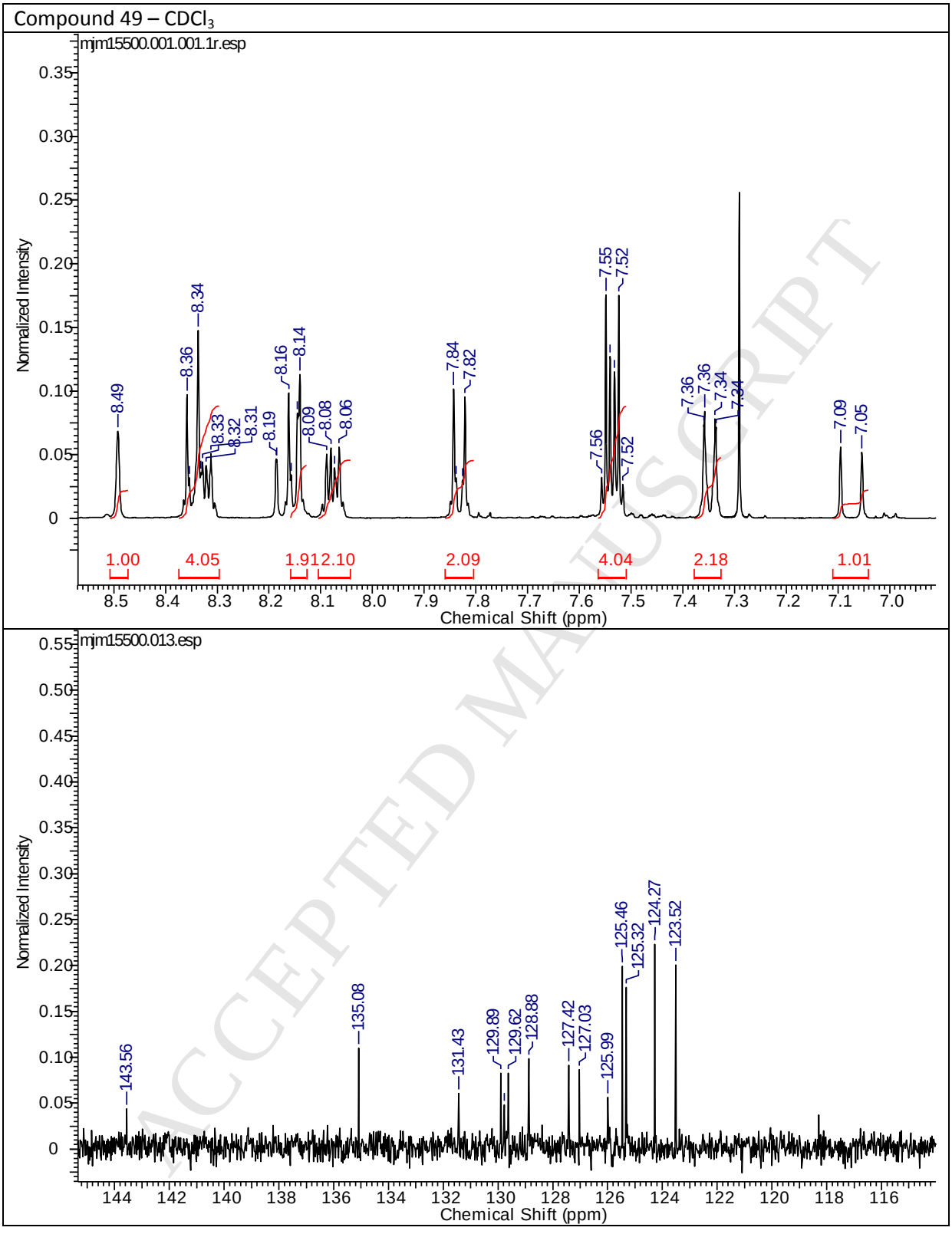
Compound 45 – $CDCl_3$





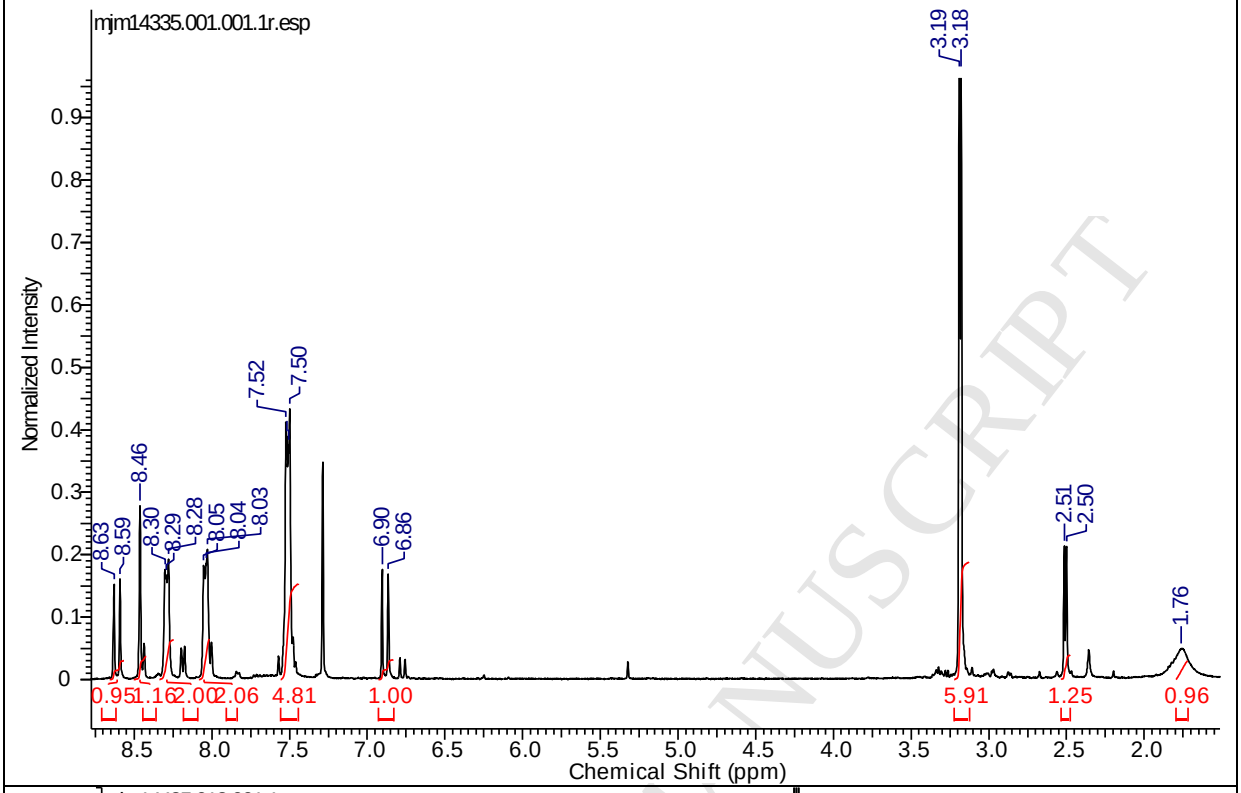
ACCEPTED MANUSCRIPT

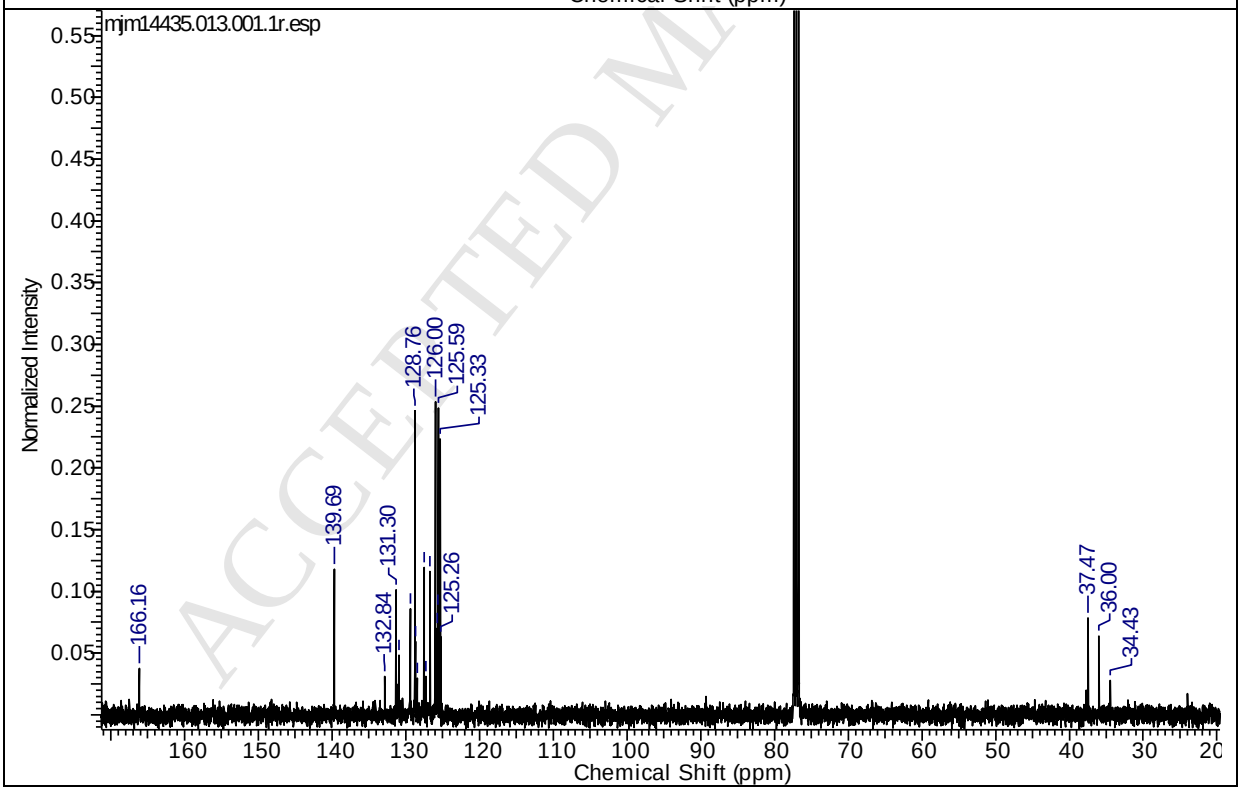
Compound 49 – $CDCl_3$



ACCEPTED MANUSCRIPT

Compound 51 – $CDCl_3$

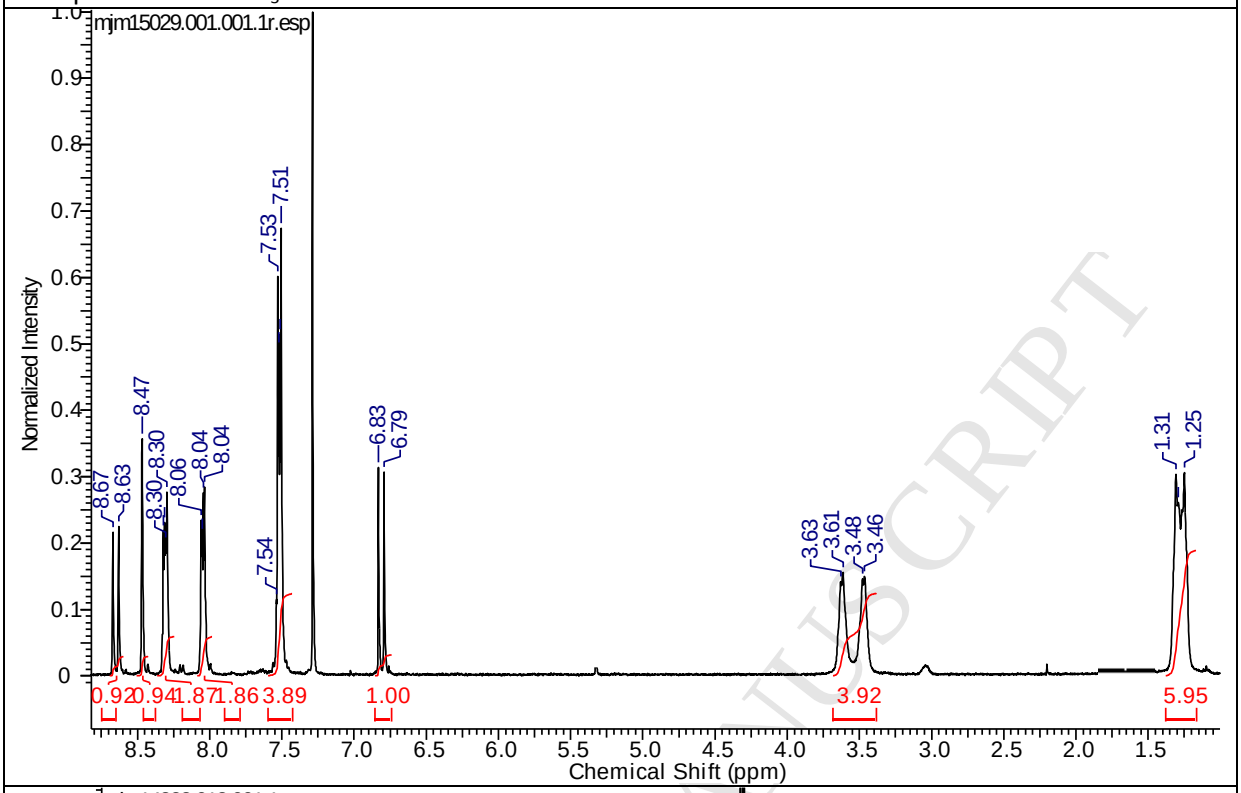


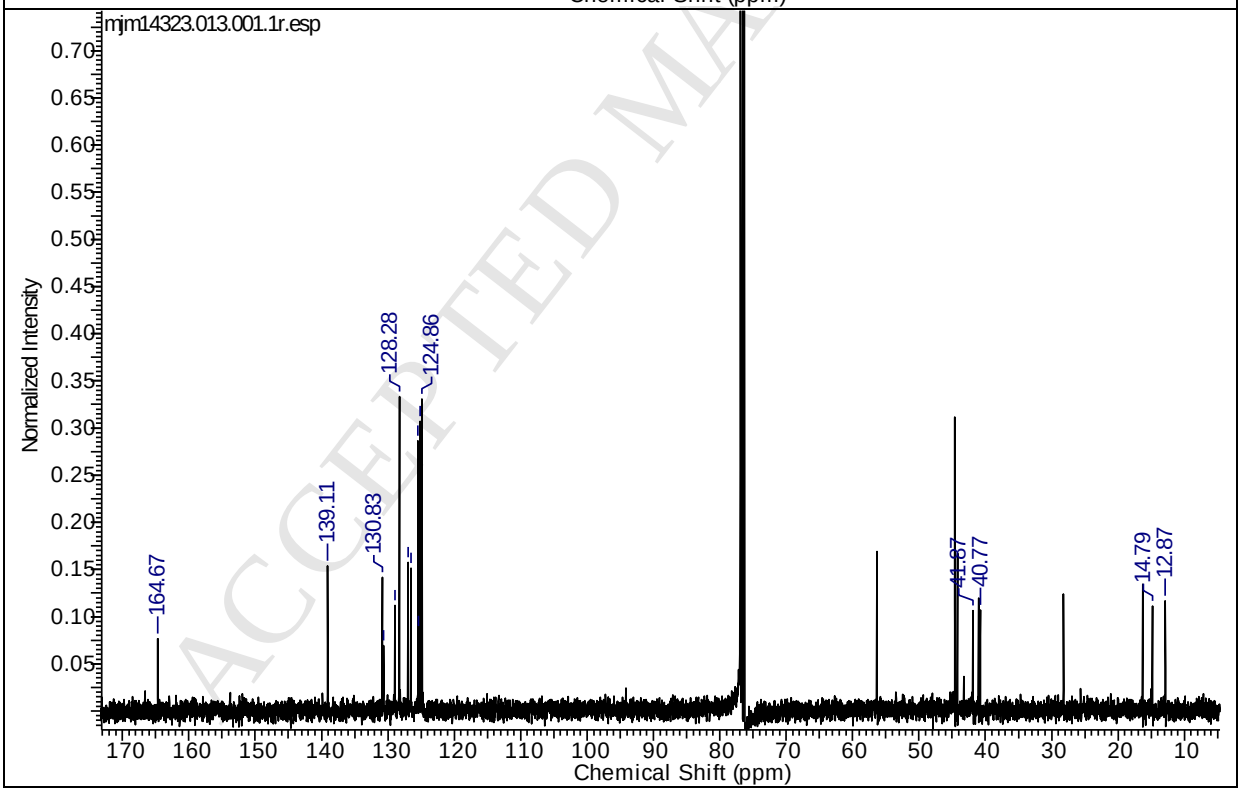


ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

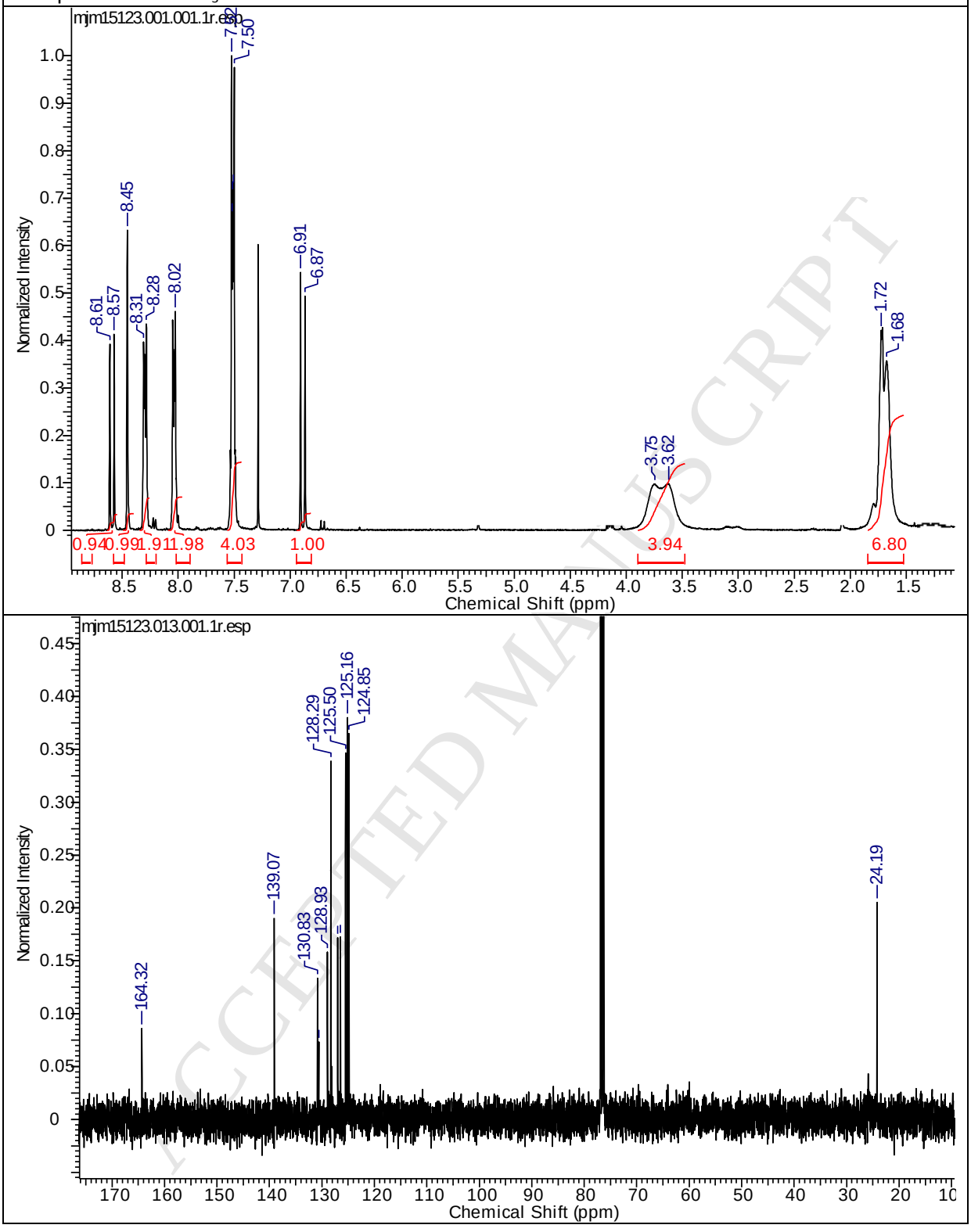
Compound 52 – $CDCl_3$





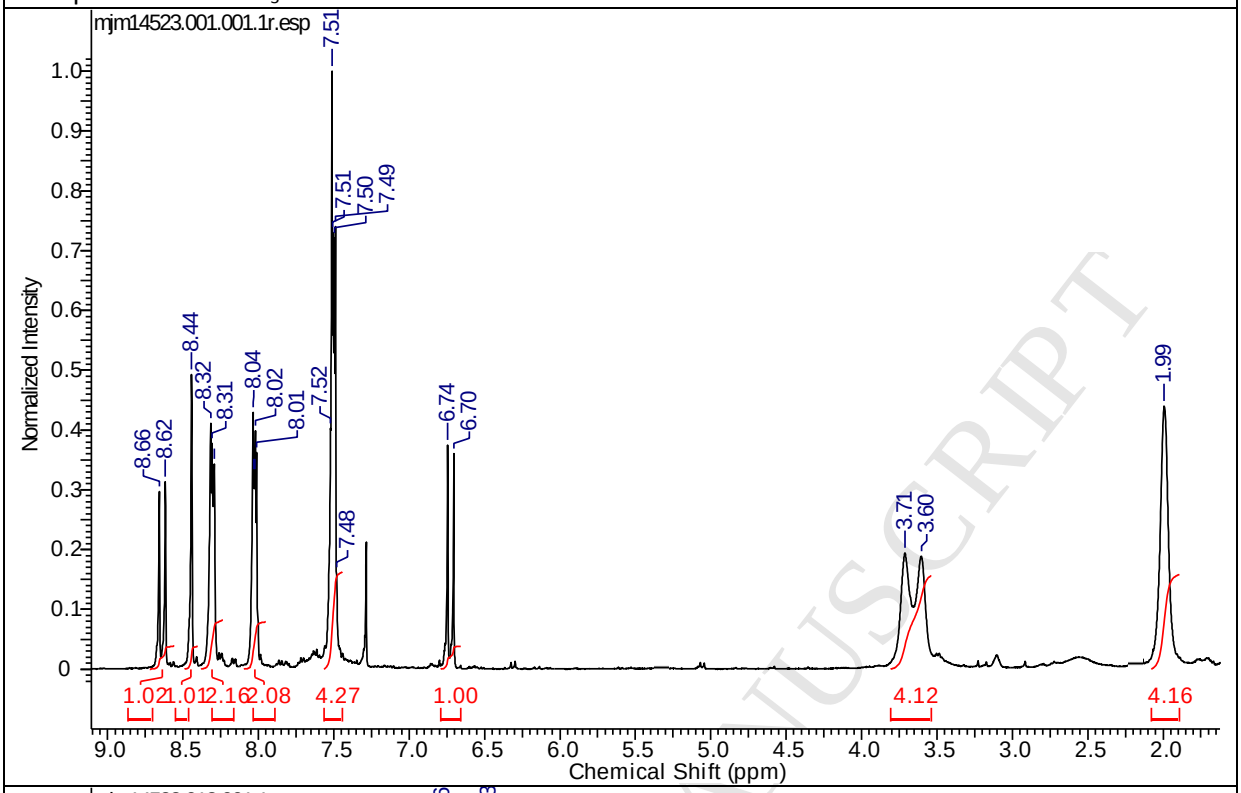
ACCEPTED MANUSCRIPT

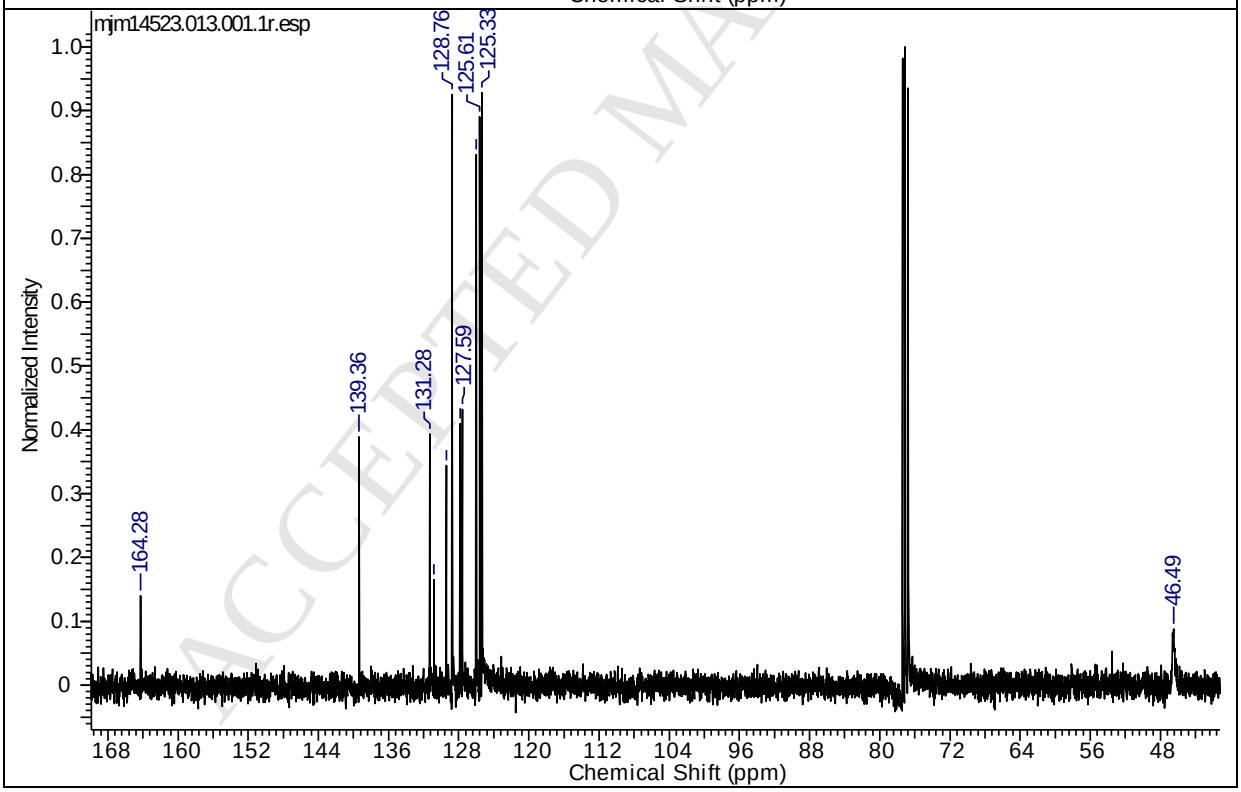
Compound 53 – $CDCl_3$



ACCEPTED MANUSCRIPT

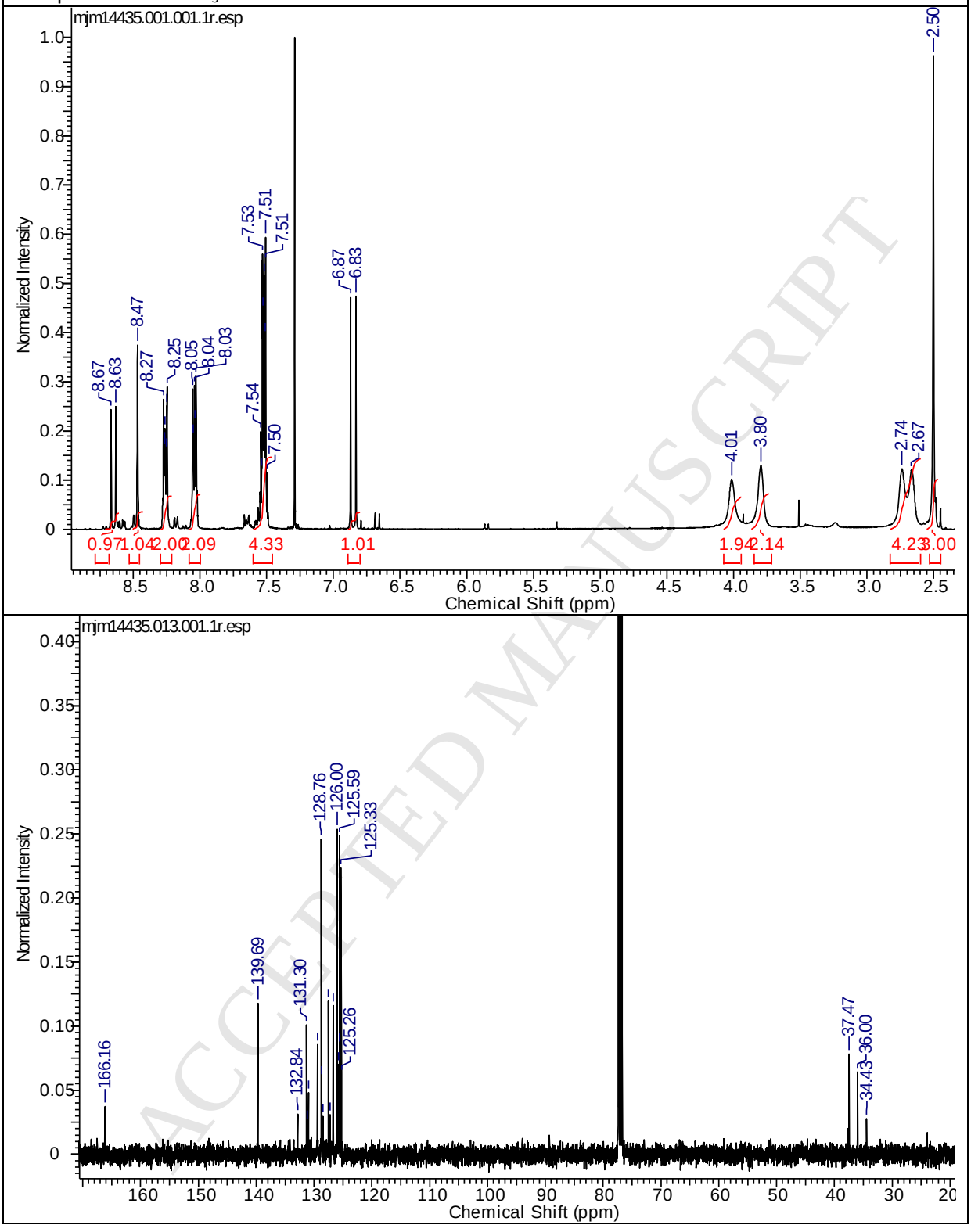
Compound 54 – $CDCl_3$





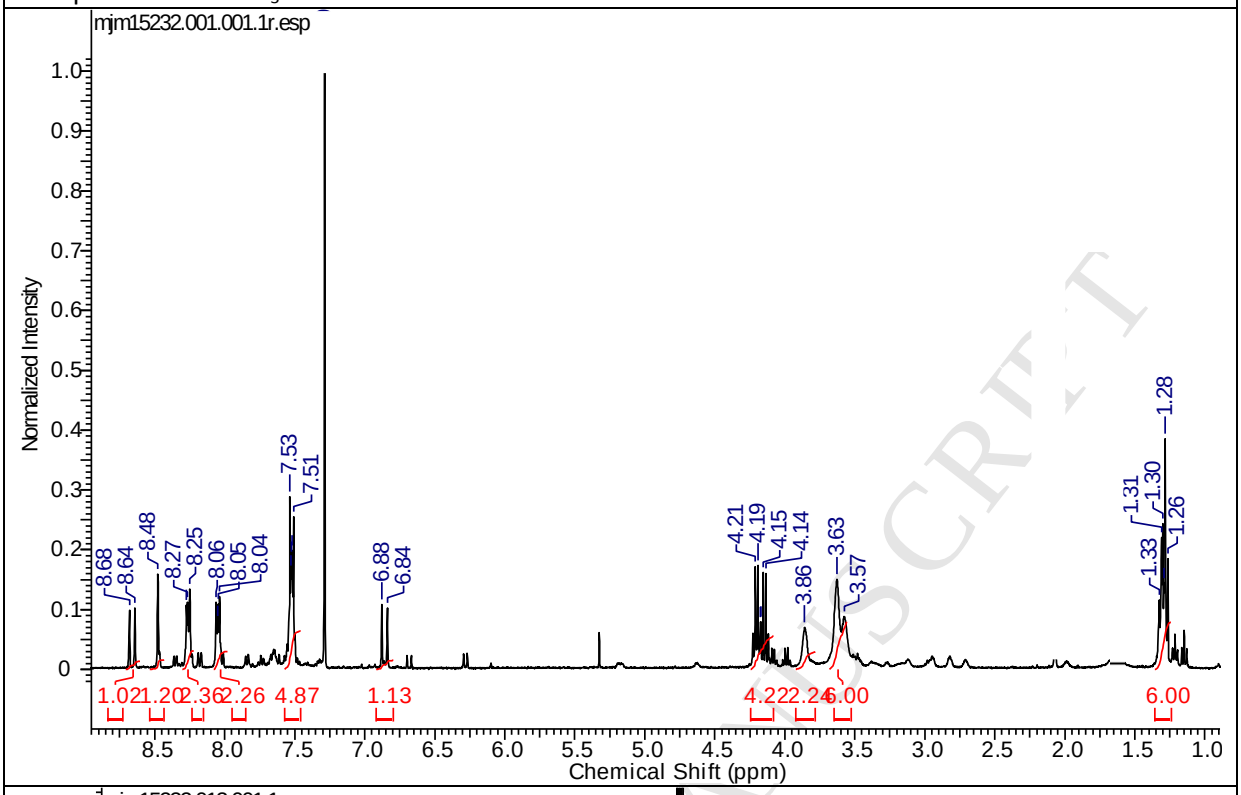
ACCEPTED MANUSCRIPT

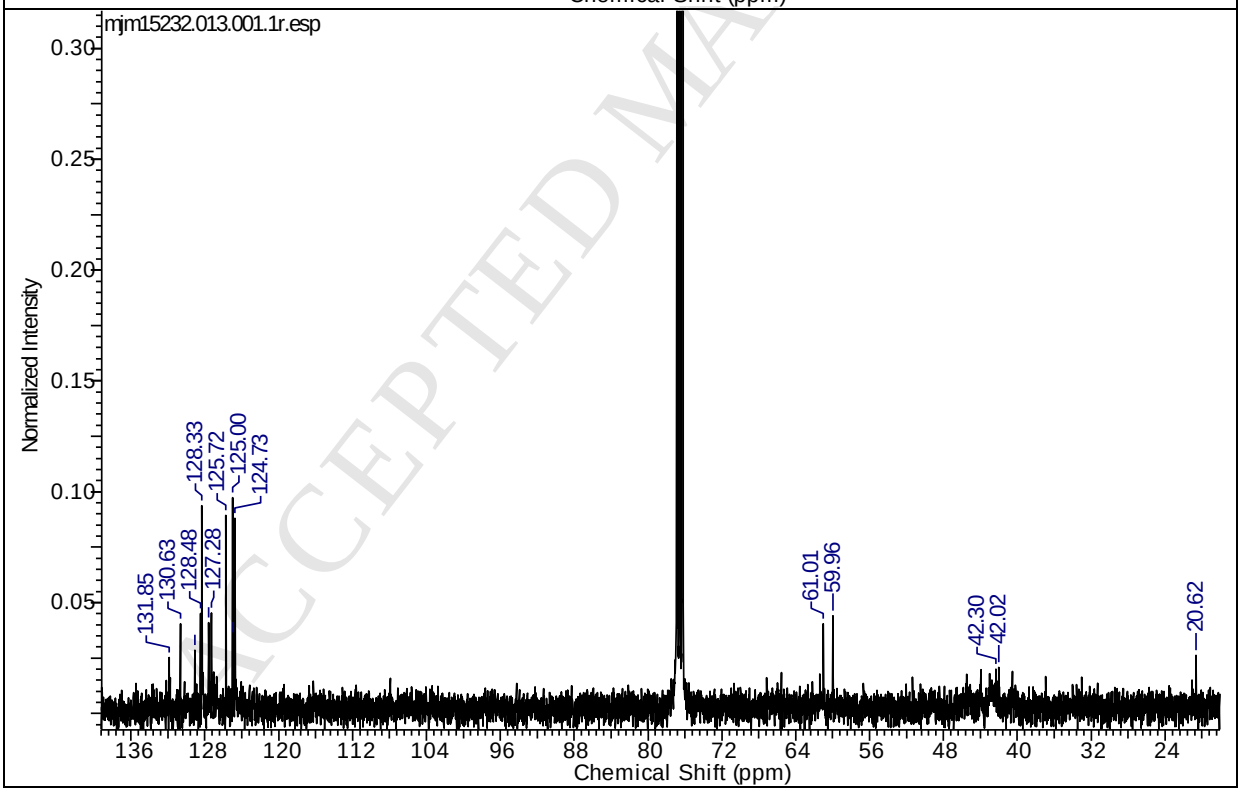
Compound 55 – $CDCl_3$



ACCEPTED MANUSCRIPT

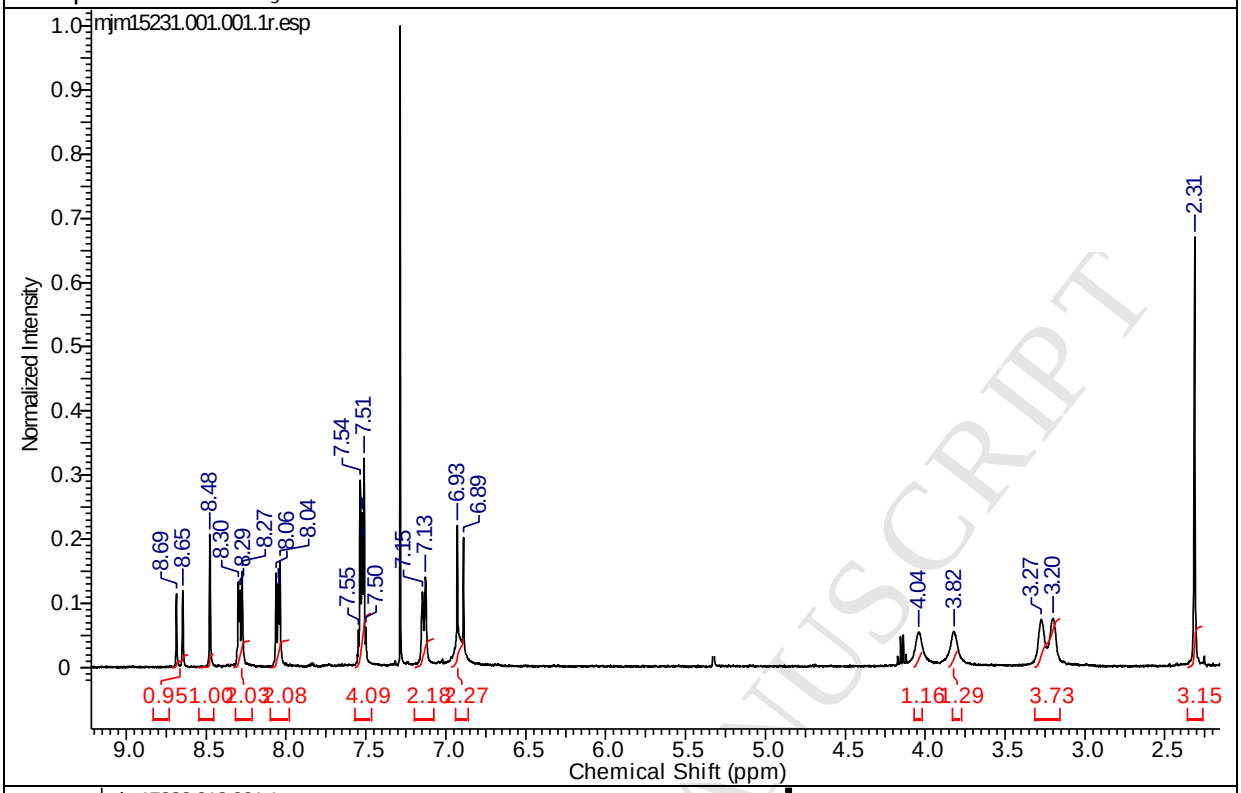
Compound 56 – $CDCl_3$

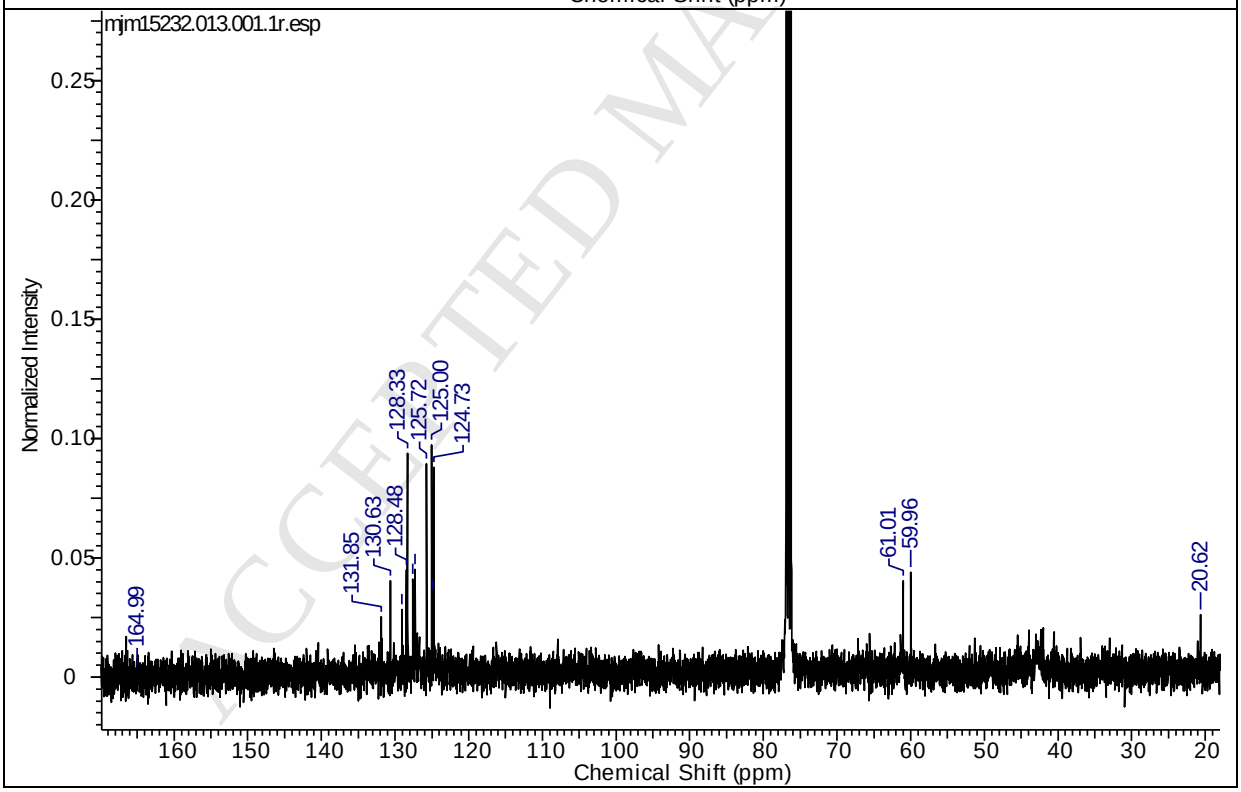




ACCEPTED MANUSCRIPT

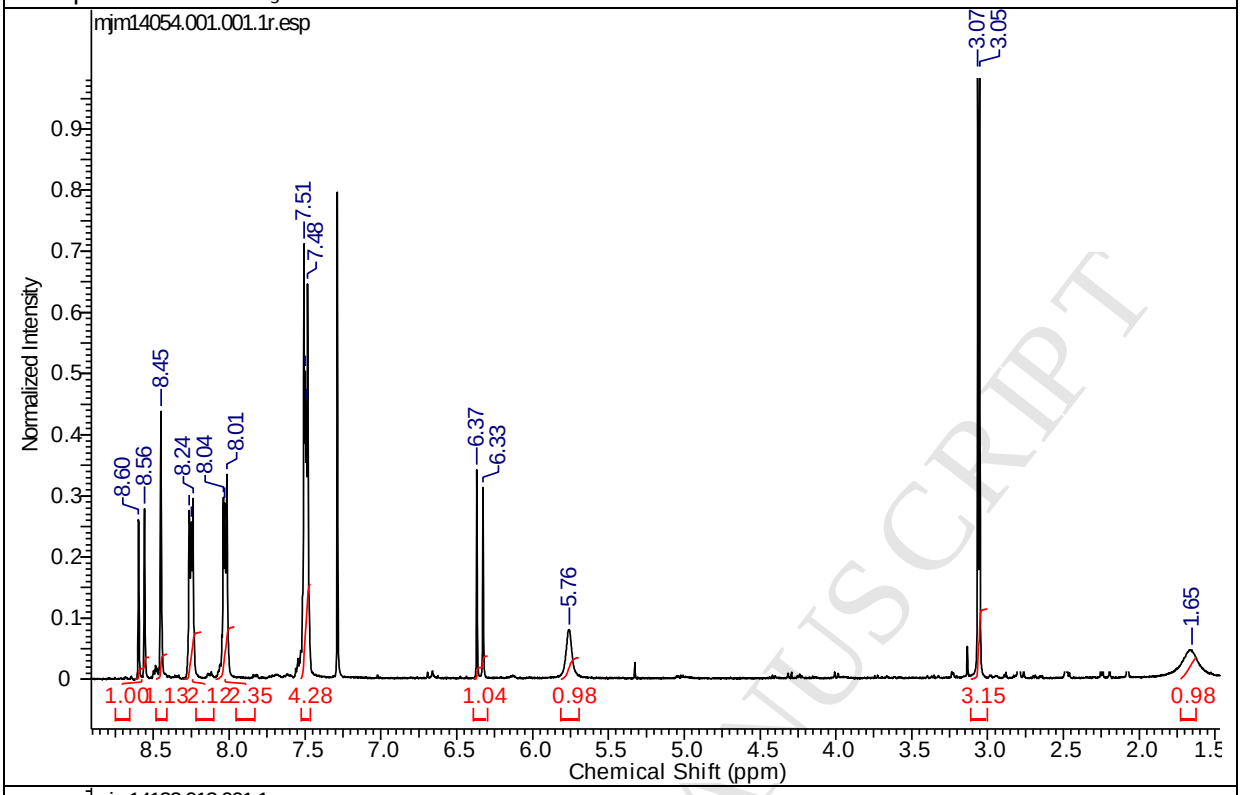
Compound 57 – $CDCl_3$

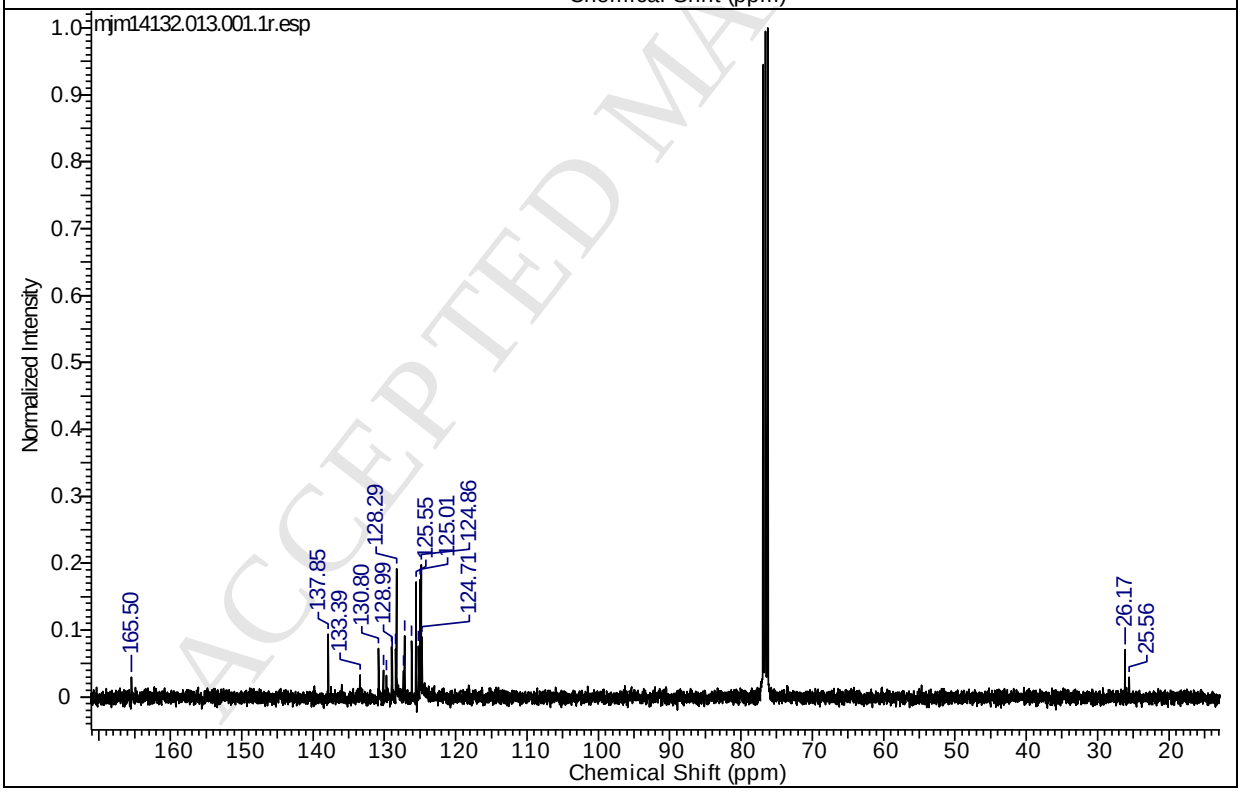




ACCEPTED MANUSCRIPT

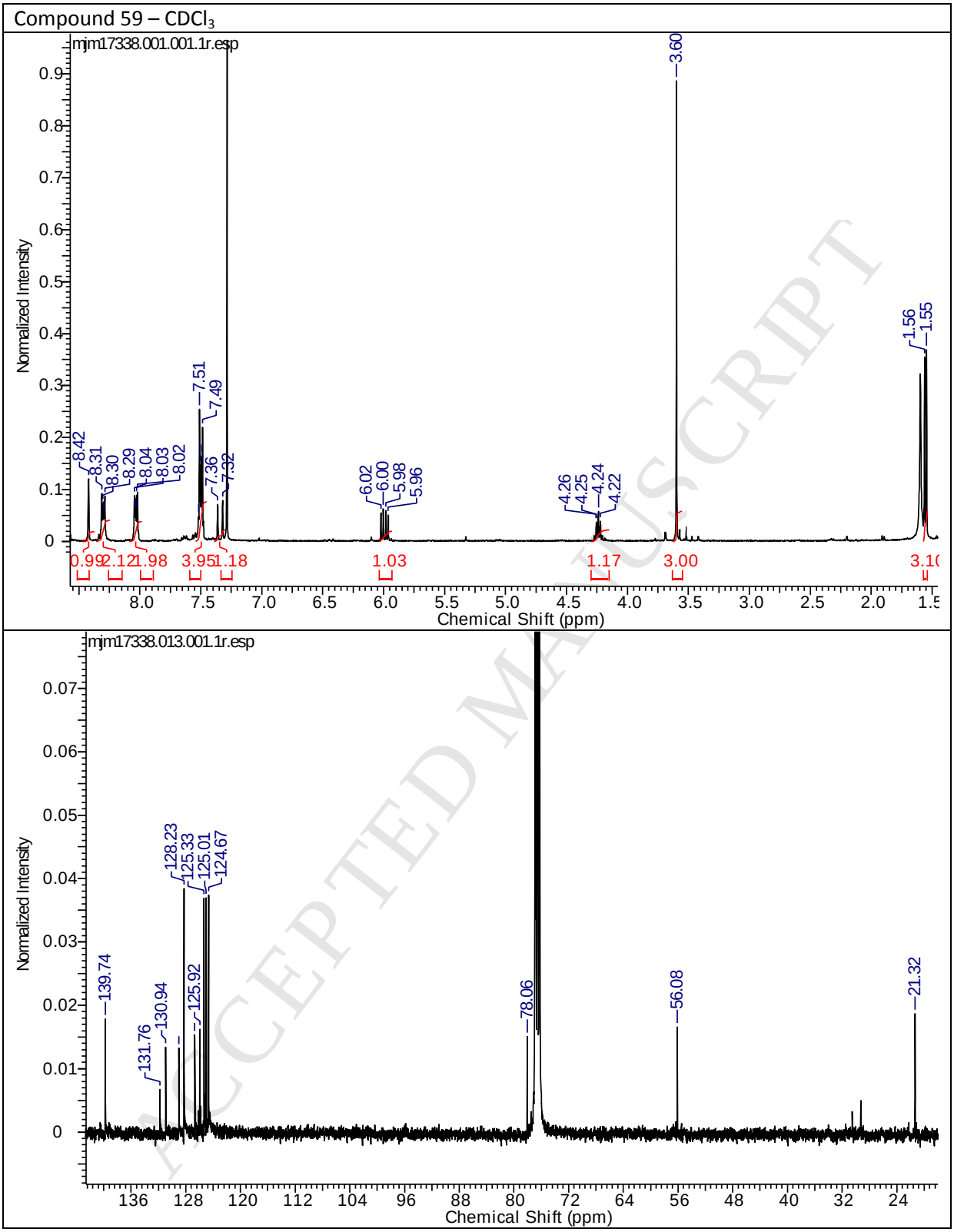
Compound 58 – $CDCl_3$



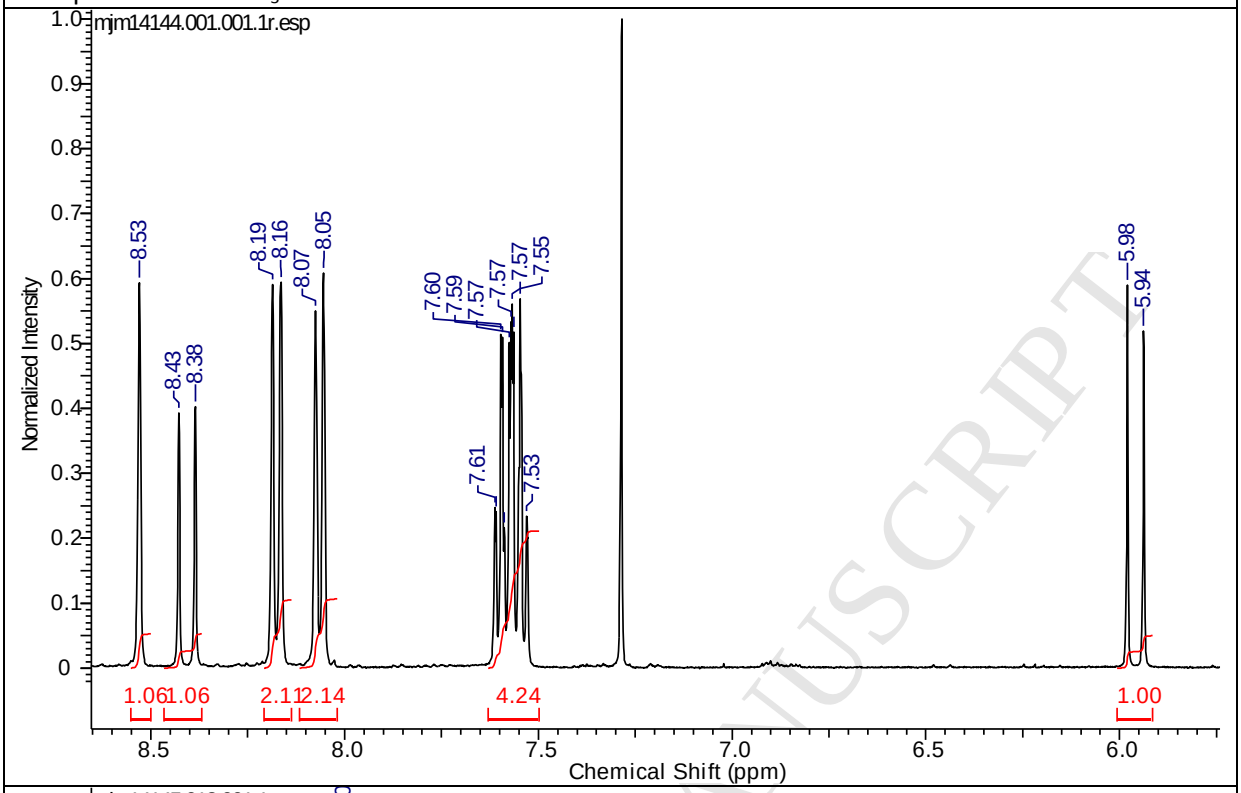


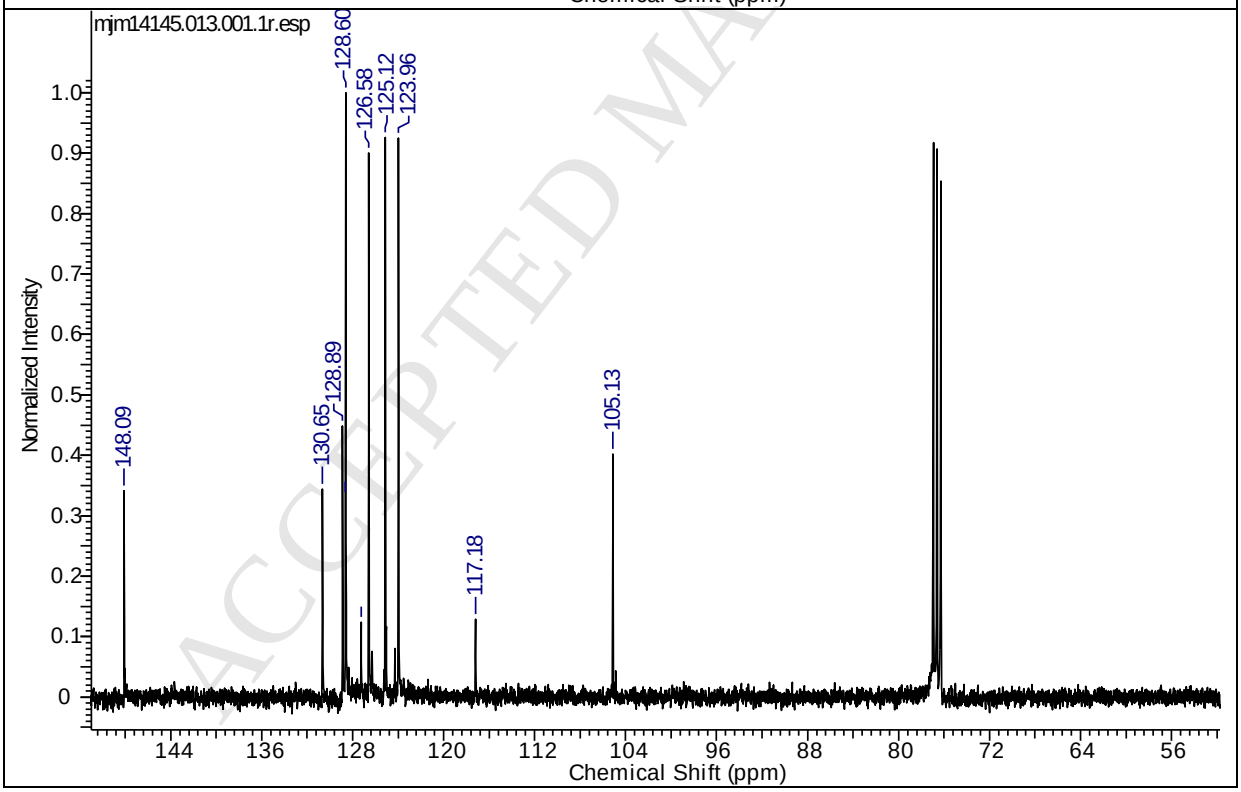
ACCEPTED MANUSCRIPT

Compound 59 – $CDCl_3$



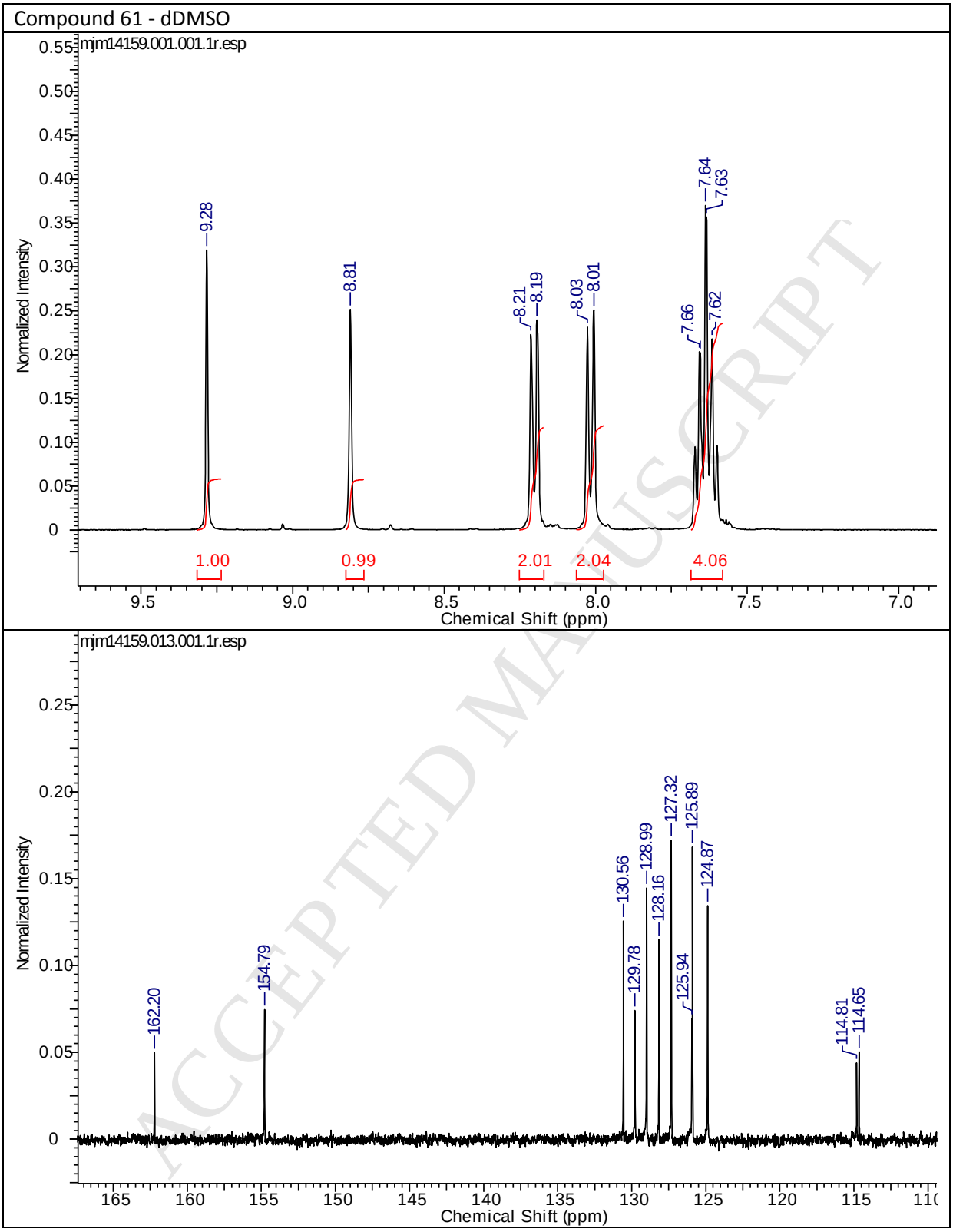
Compound 60 – $CDCl_3$





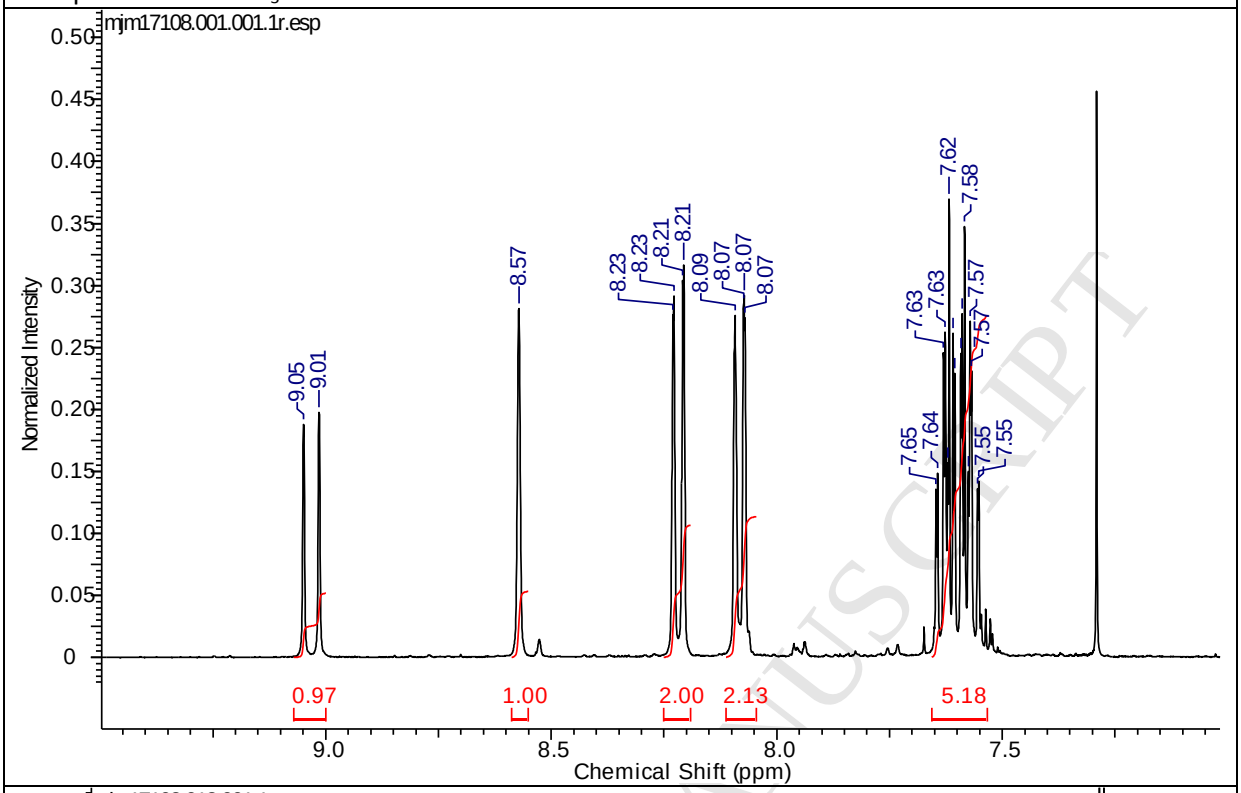
ACCEPTED MANUSCRIPT

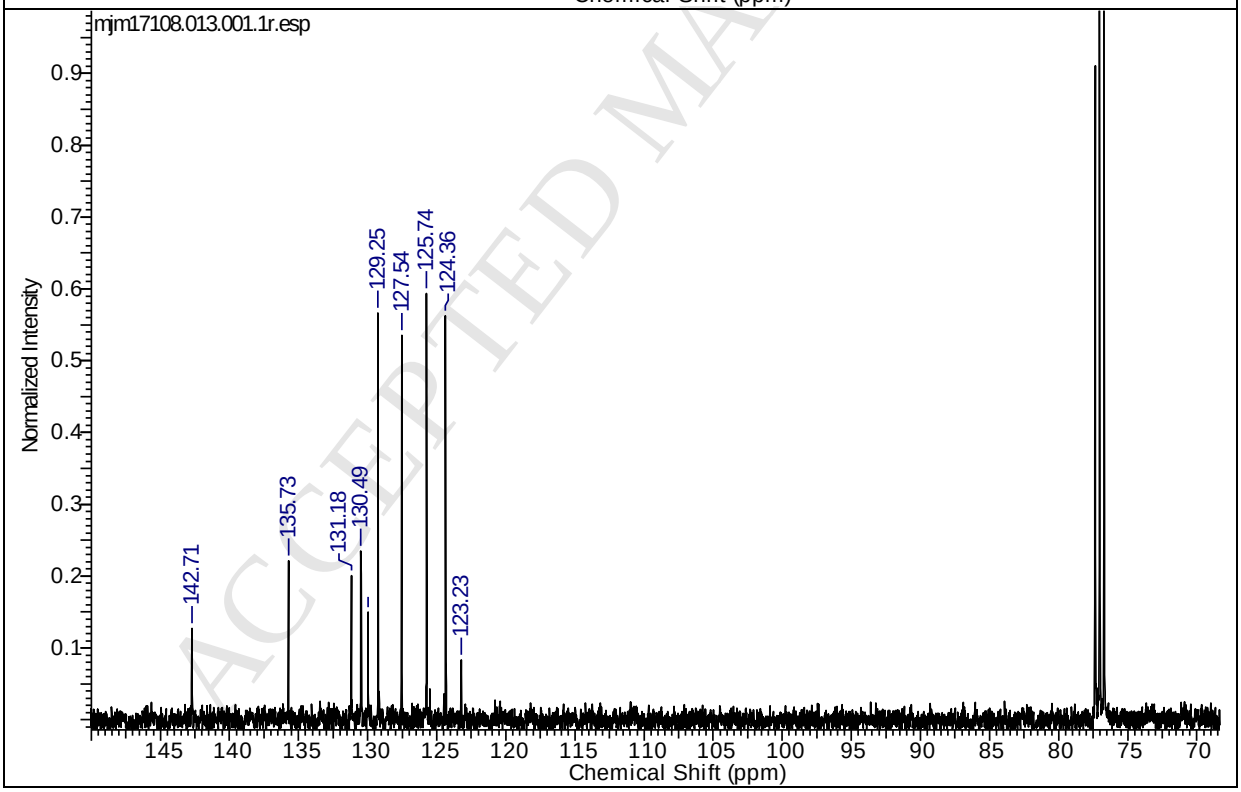
ACCEPTED MANUSCRIPT



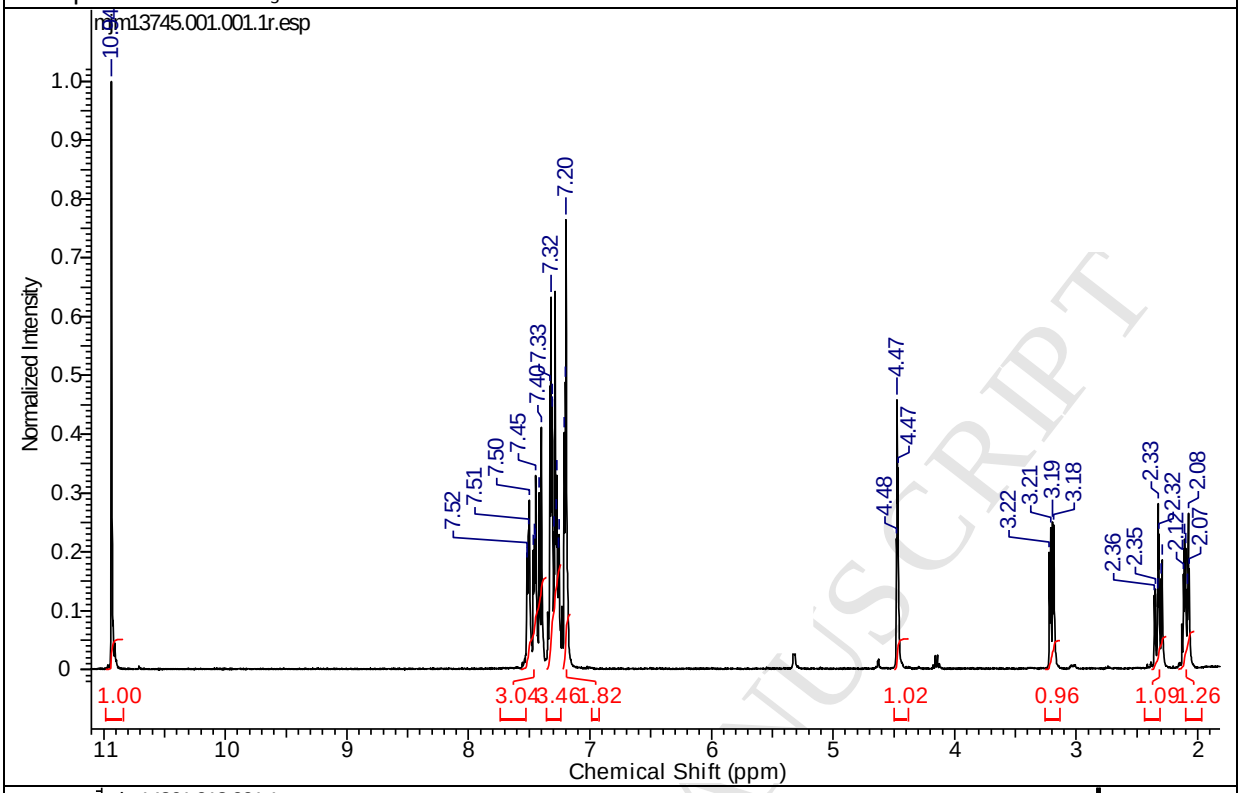
ACCEPTED MANUSCRIPT

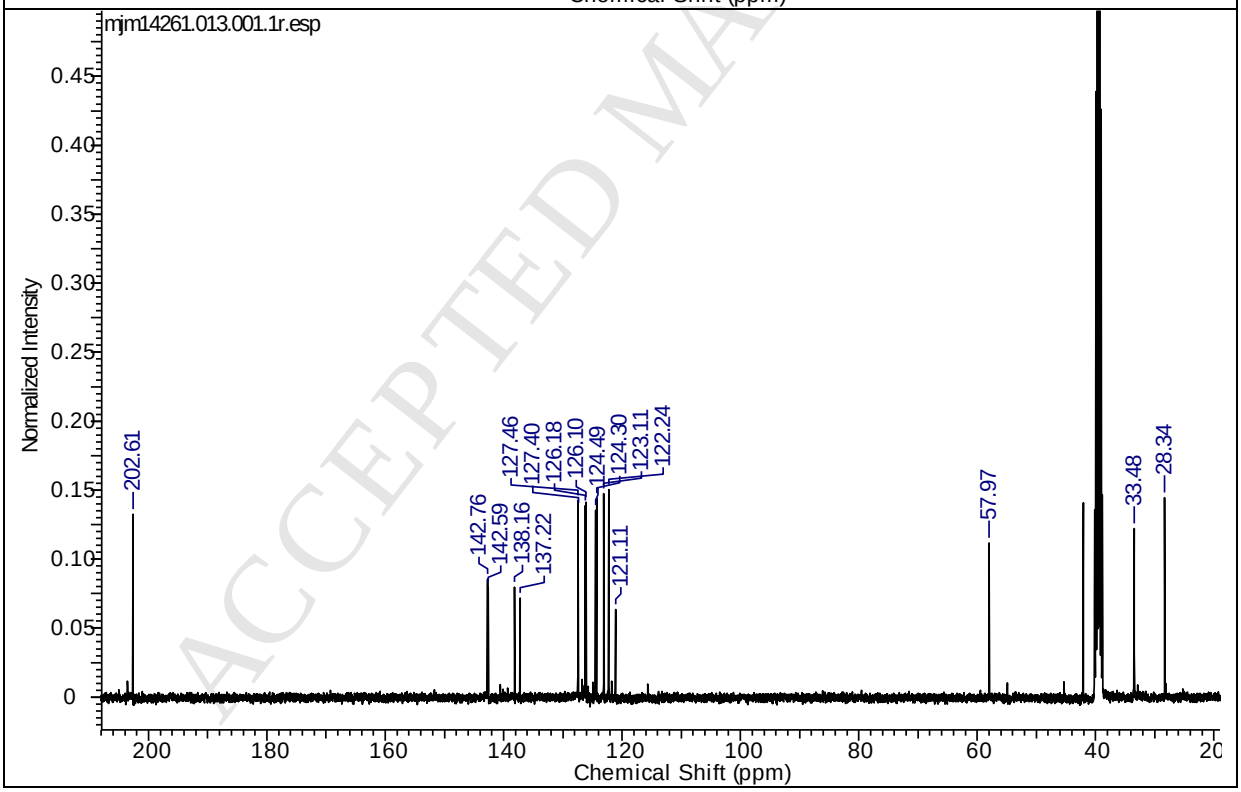
Compound 62 – $CDCl_3$





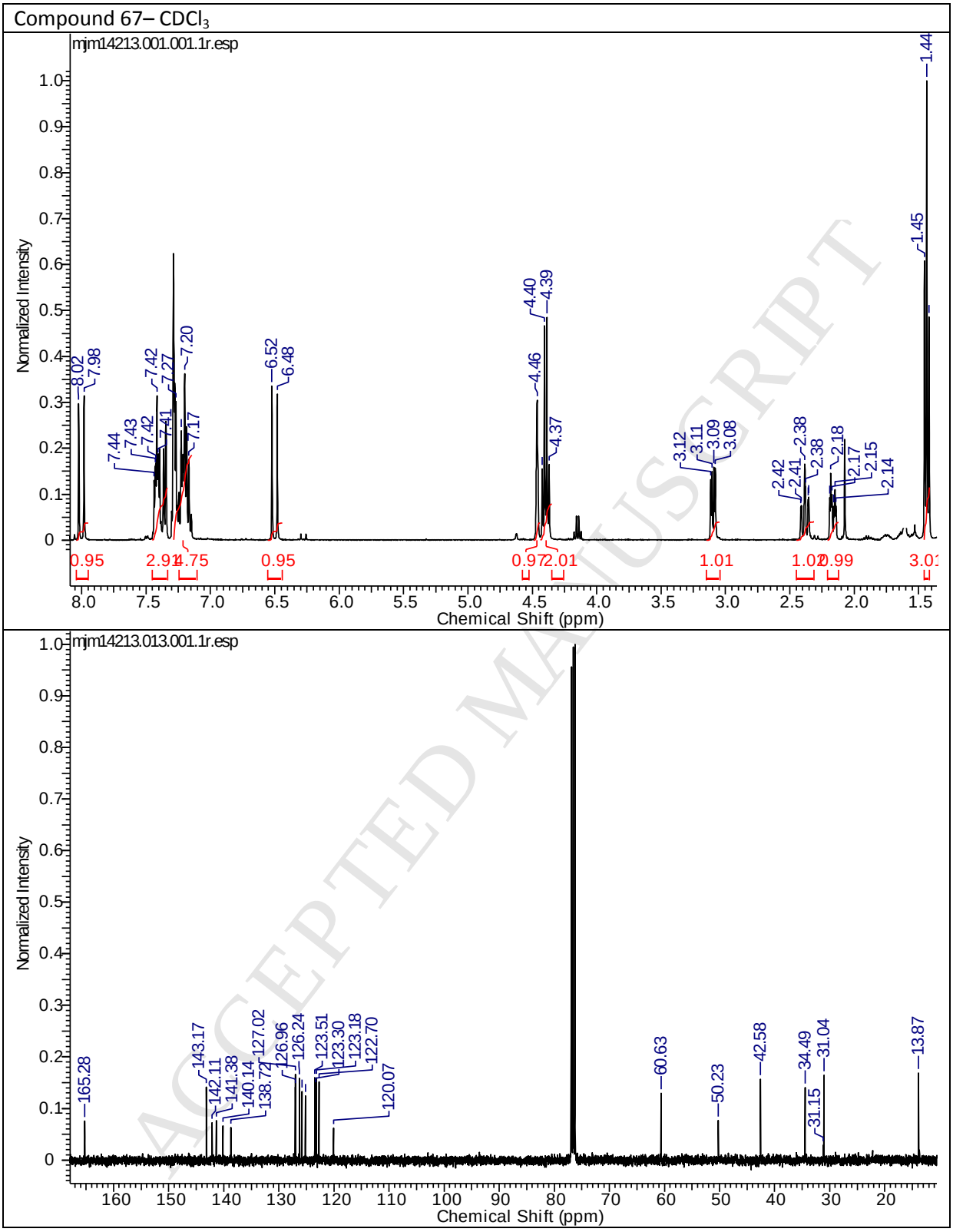
Compound 63 – $CDCl_3$





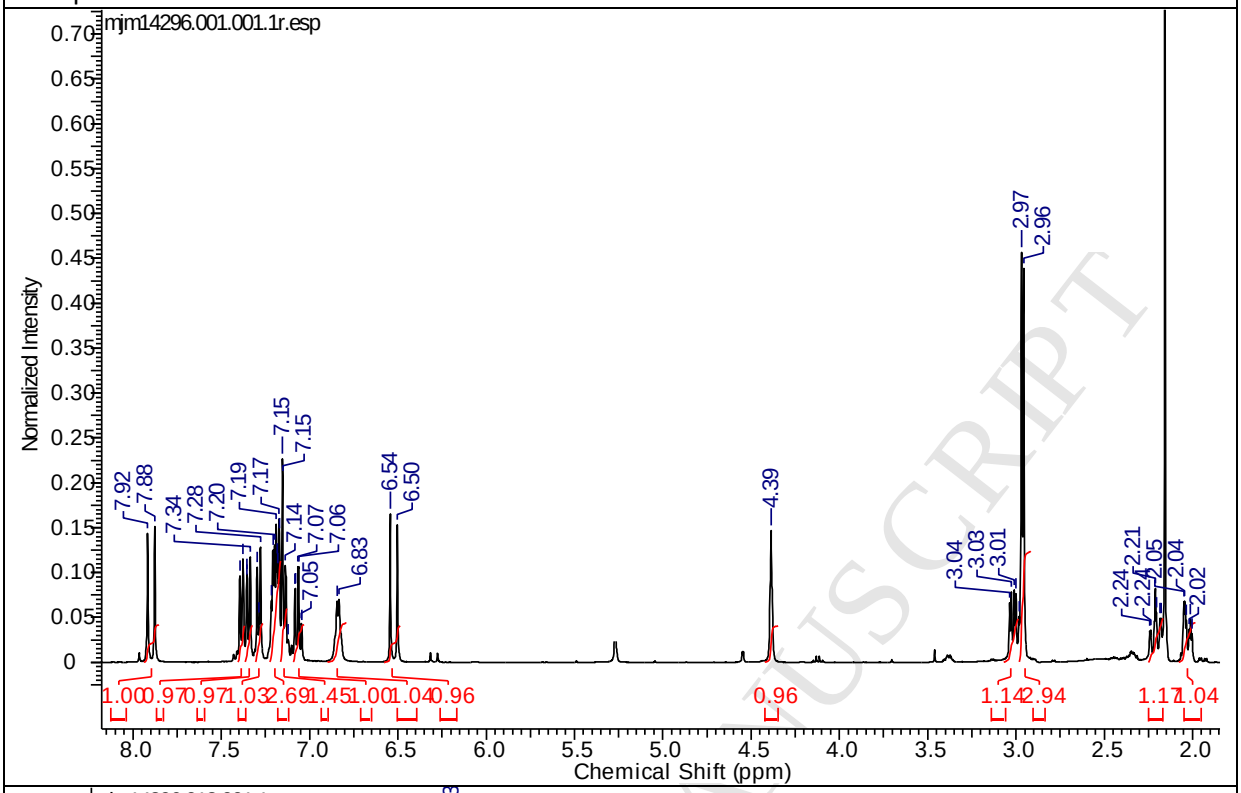
ACCEPTED MANUSCRIPT

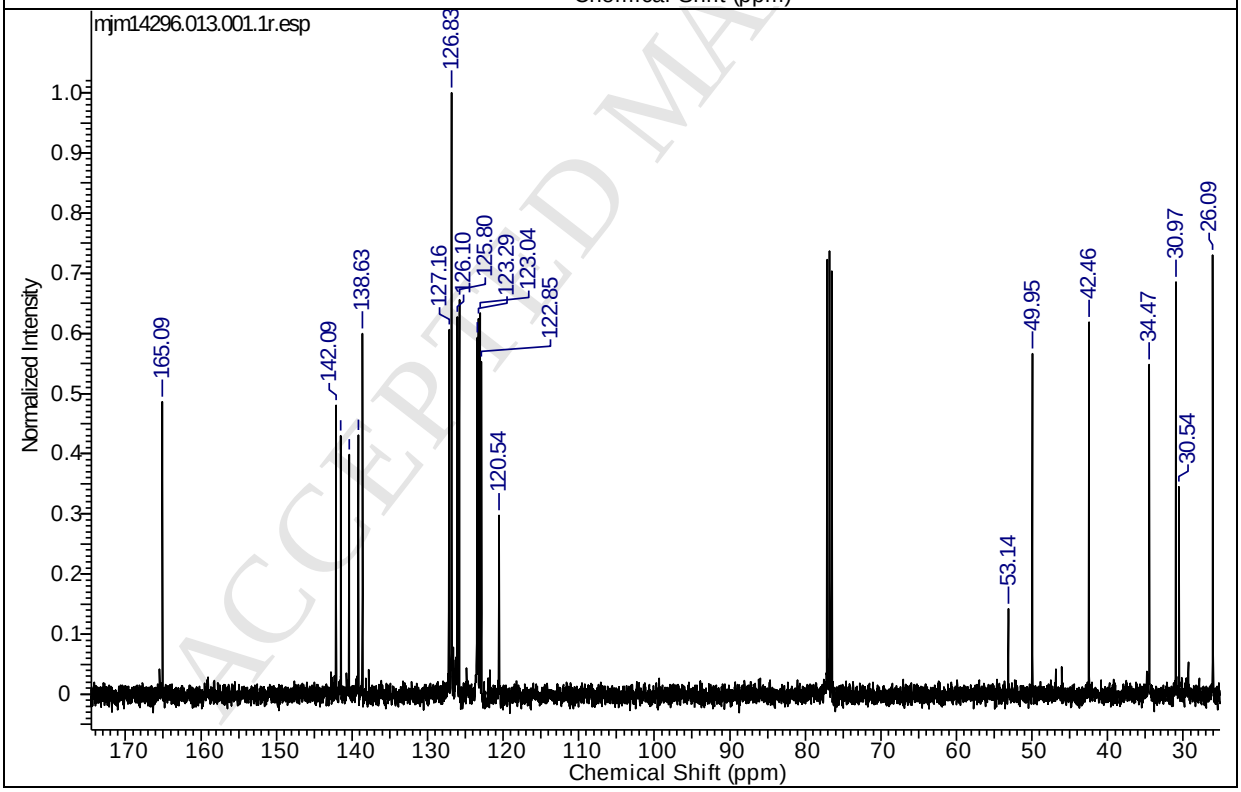
ACCEPTED MANUSCRIPT



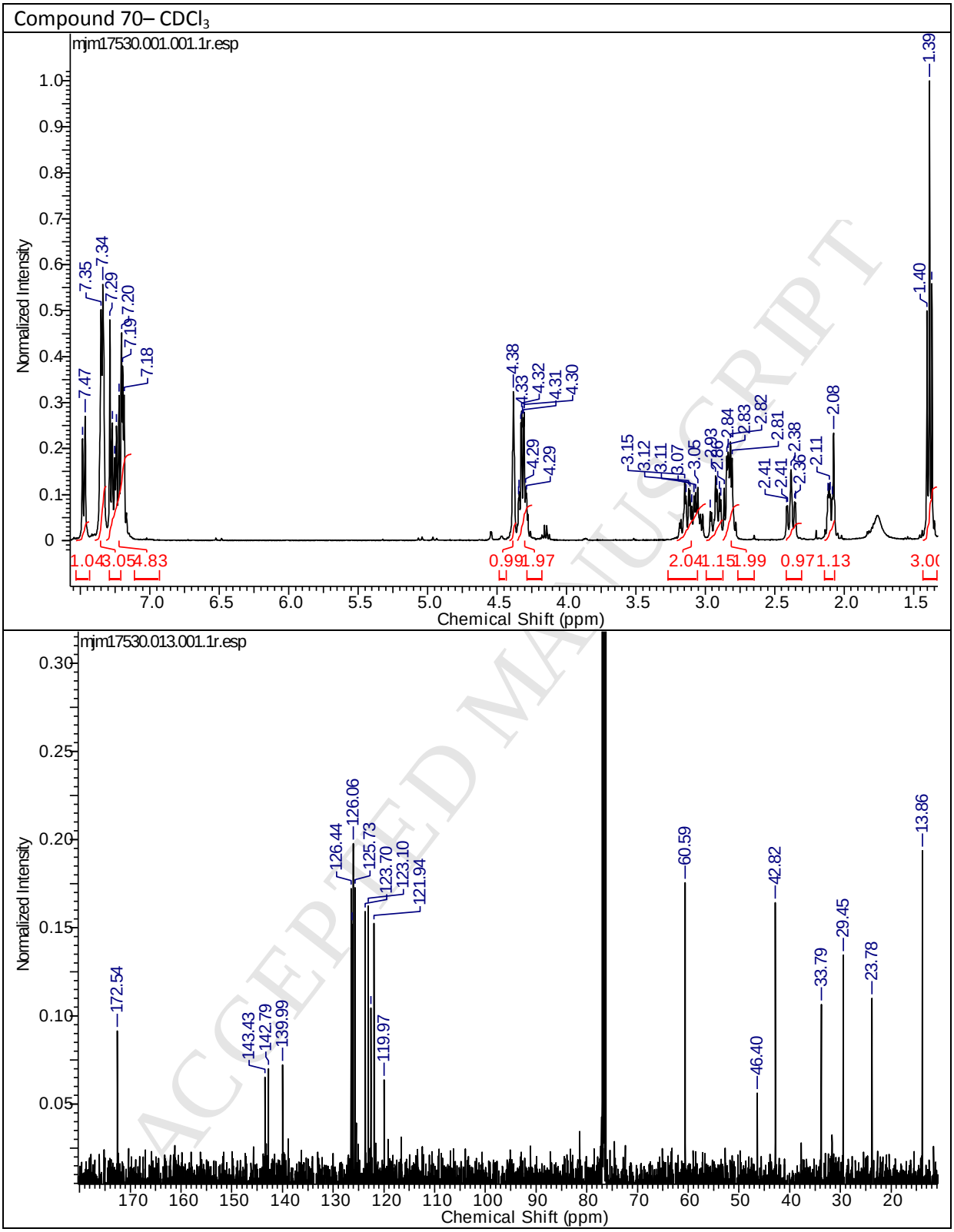
ACCEPTED MANUSCRIPT

Compound 68 – CDCl3



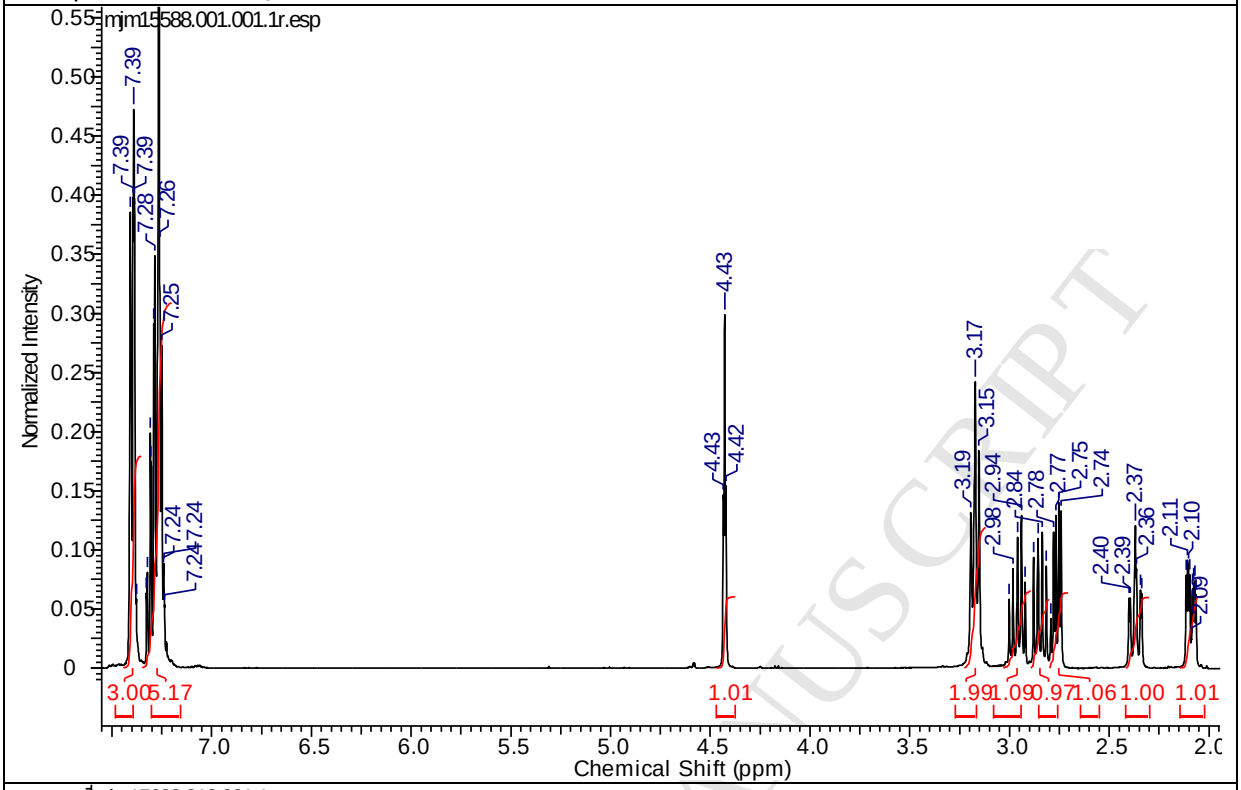


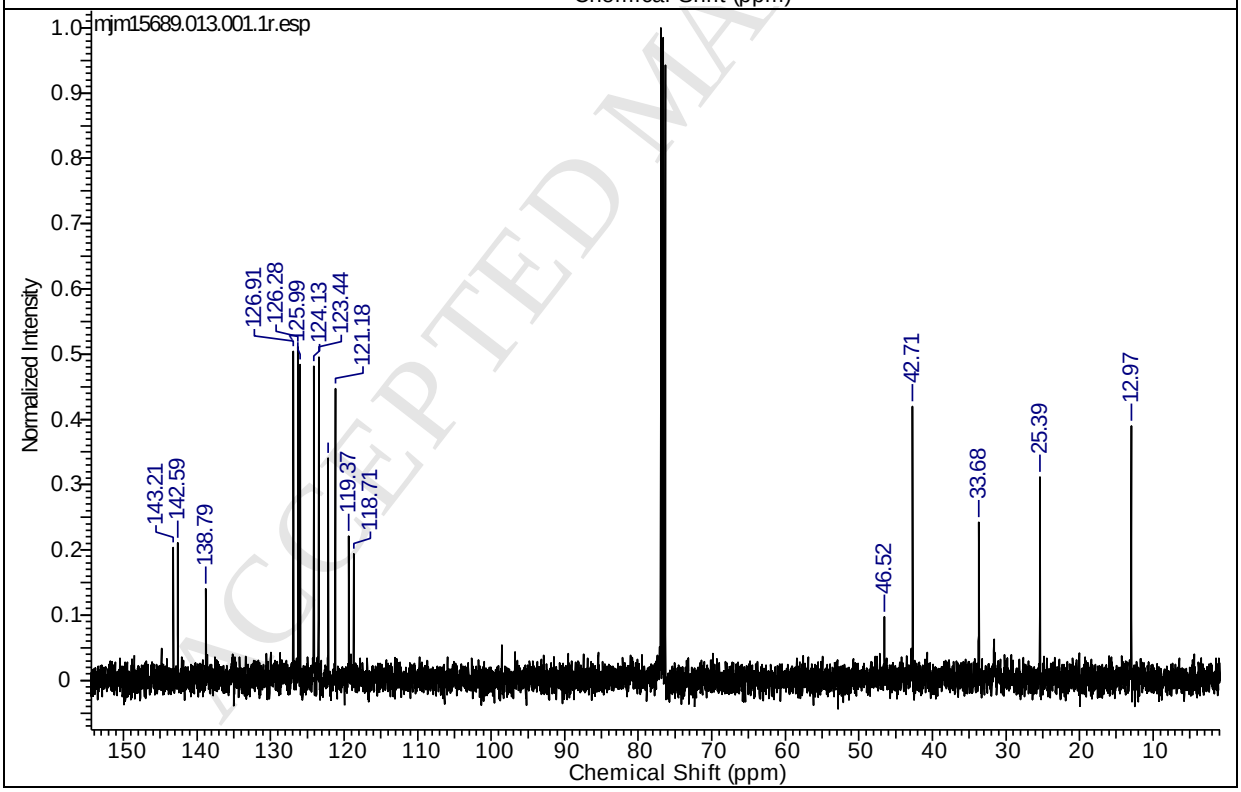
ACCEPTED MANUSCRIPT



ACCEPTED MANUSCRIPT

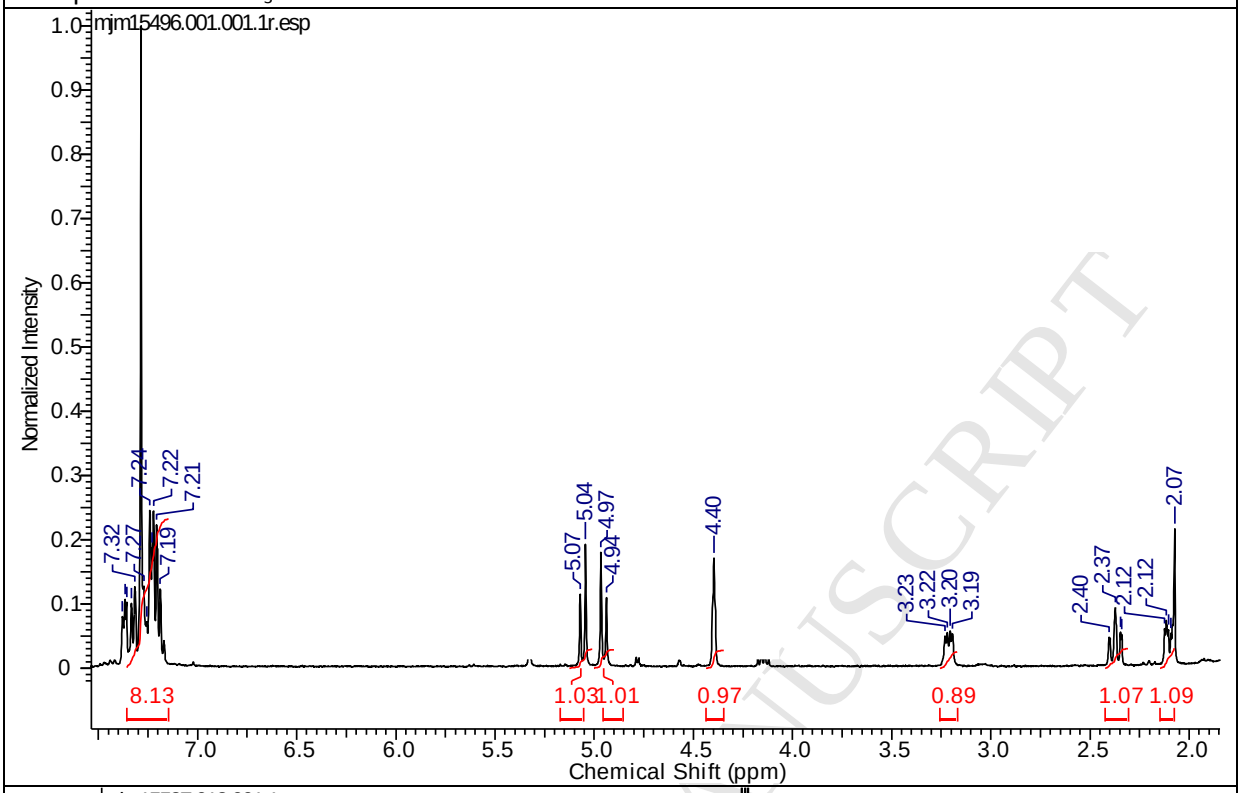
Compound 72– $CDCl_3$

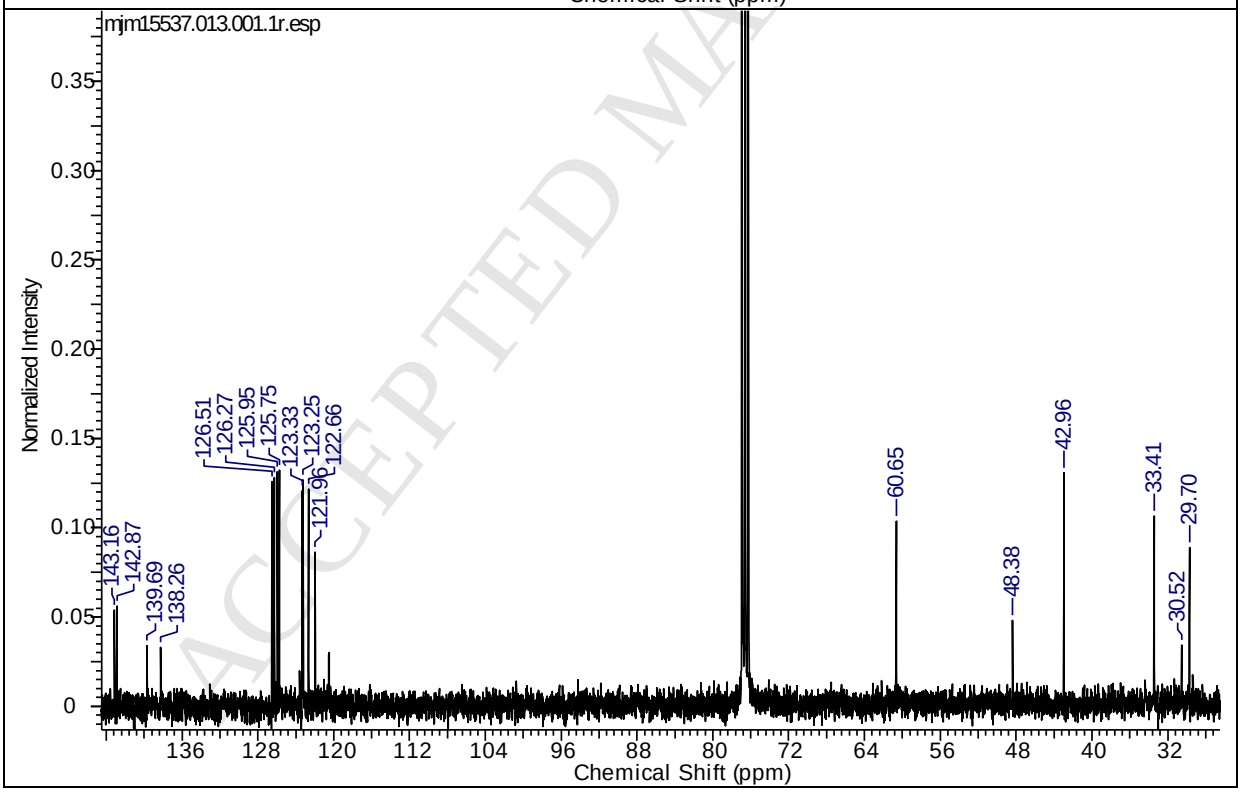


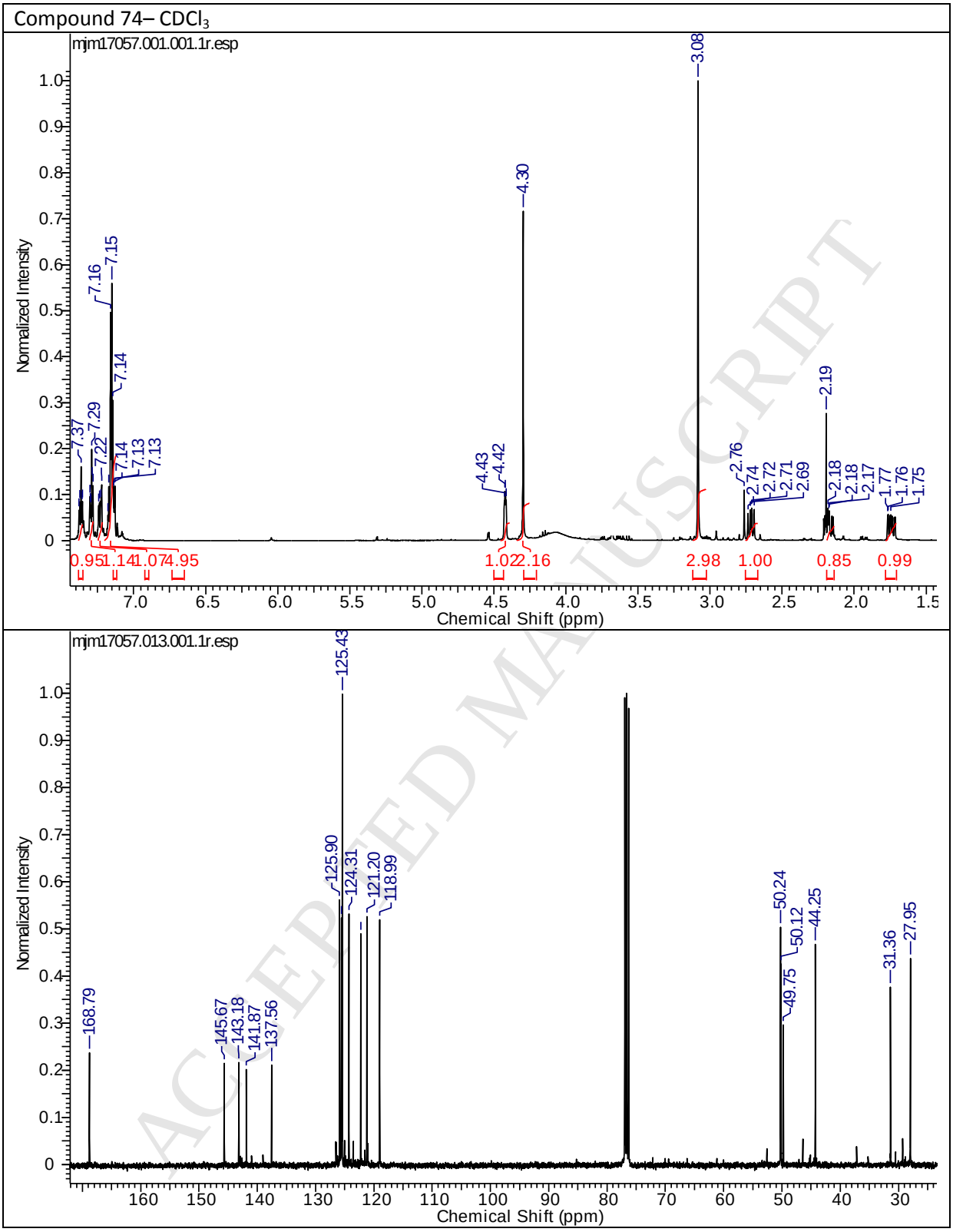


ACCEPTED MANUSCRIPT

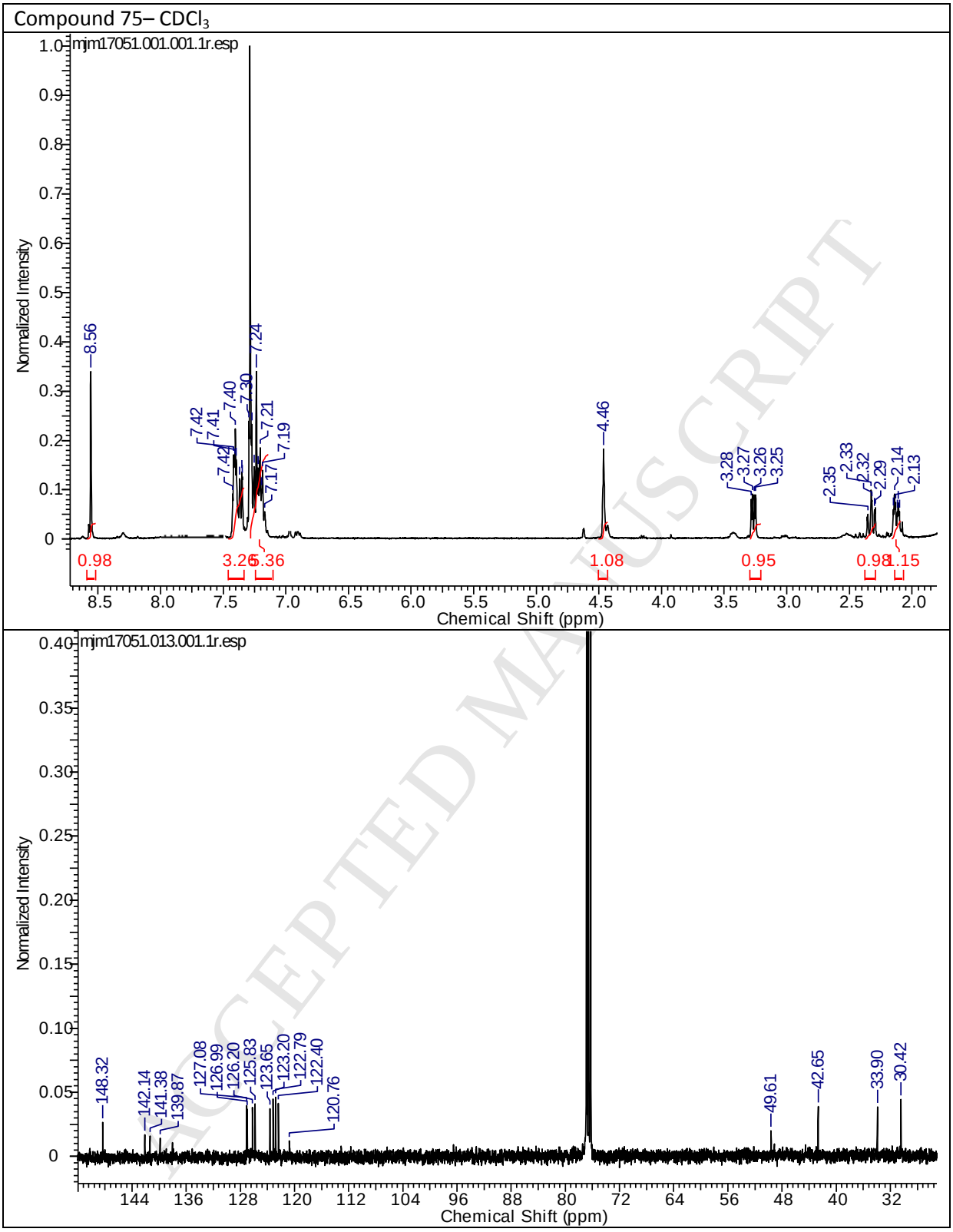
Compound 73– $CDCl_3$



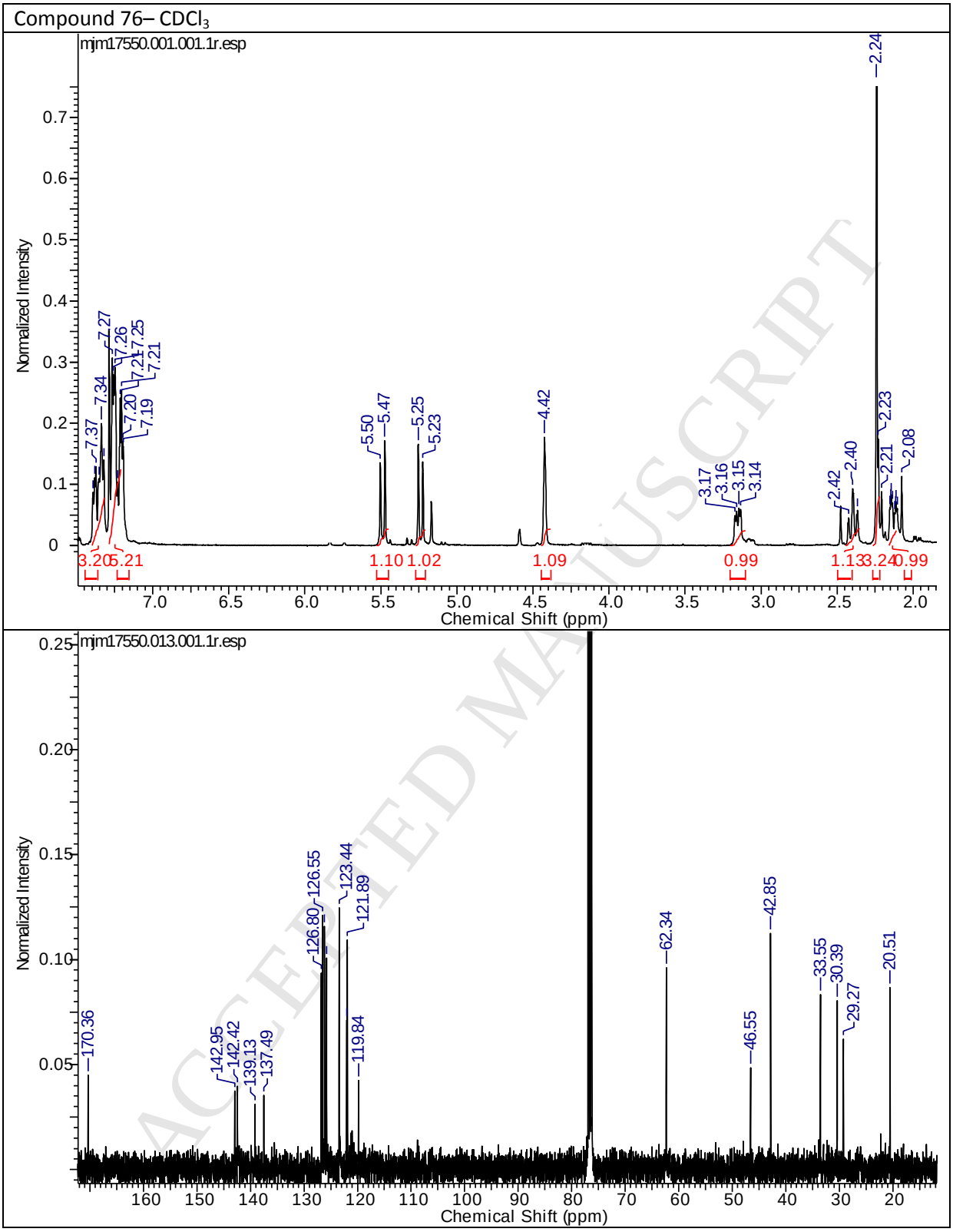




ACCEPTED MANUSCRIPT



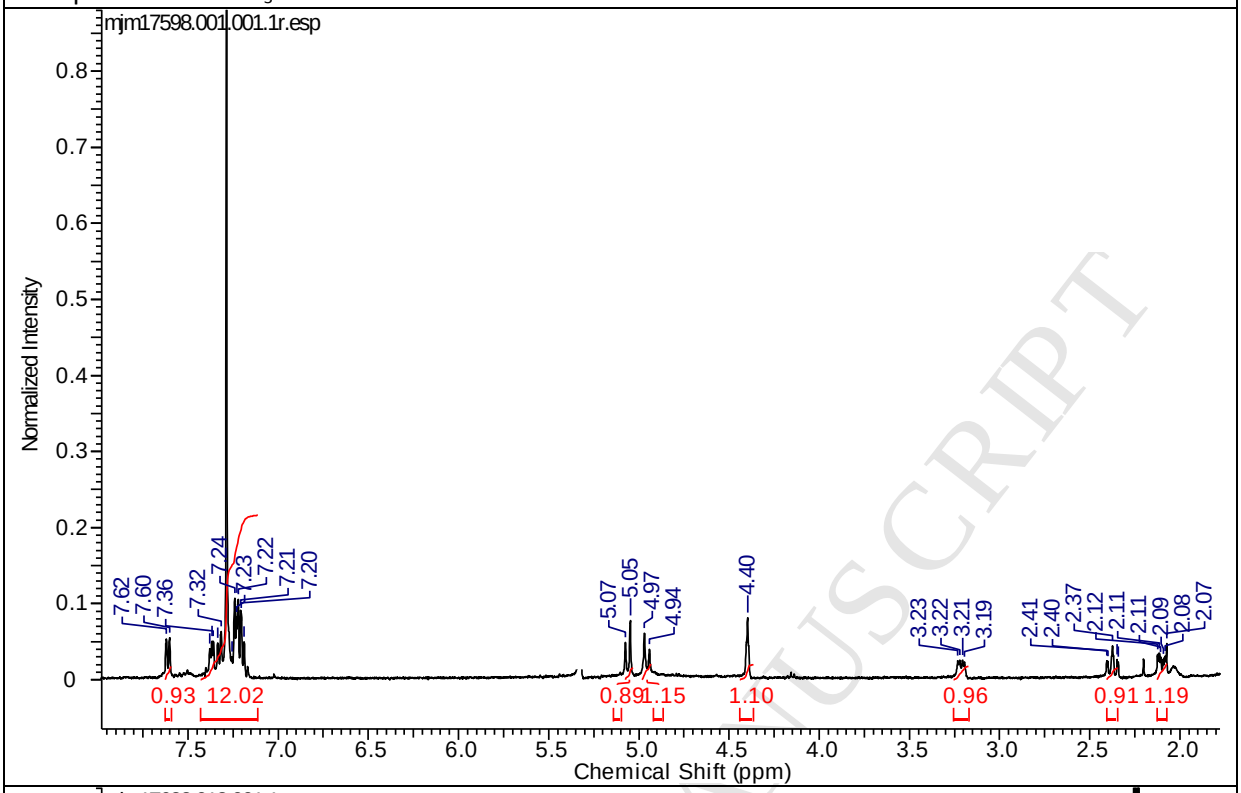
ACCEPTED MANUSCRIPT

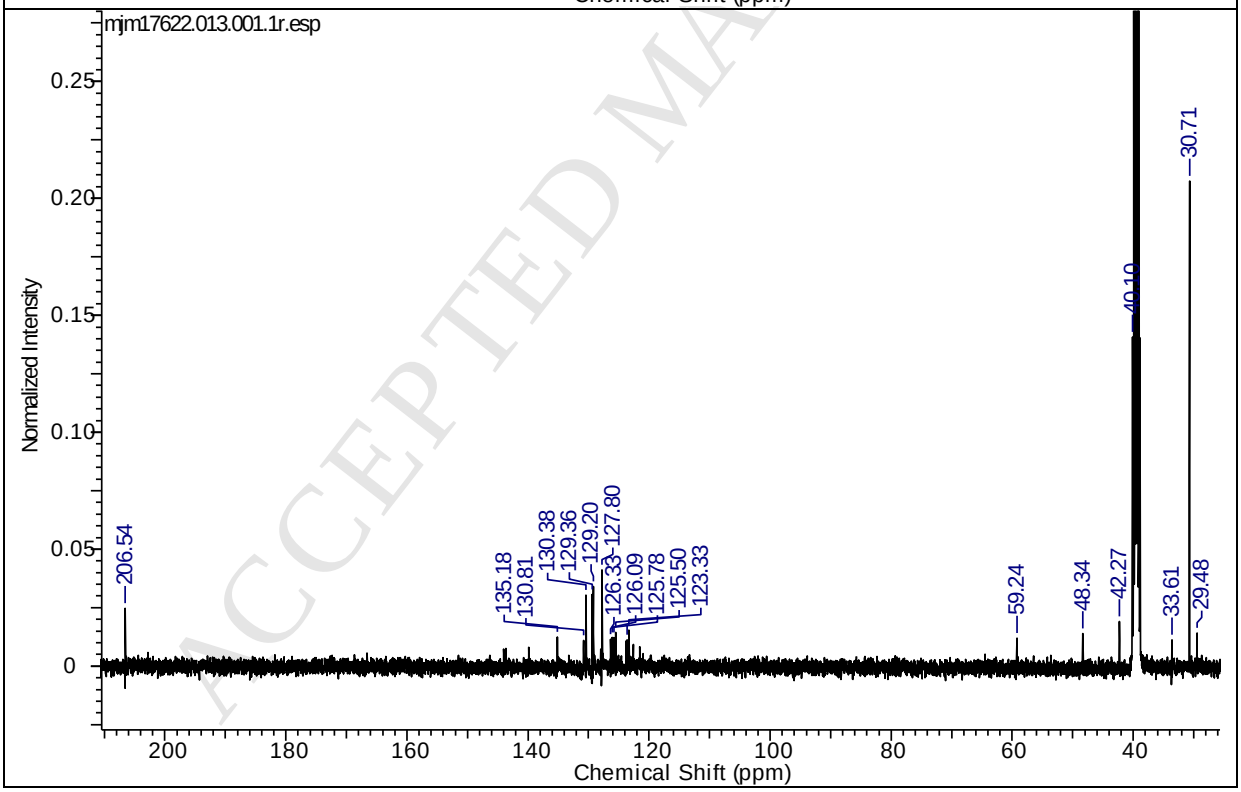


ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

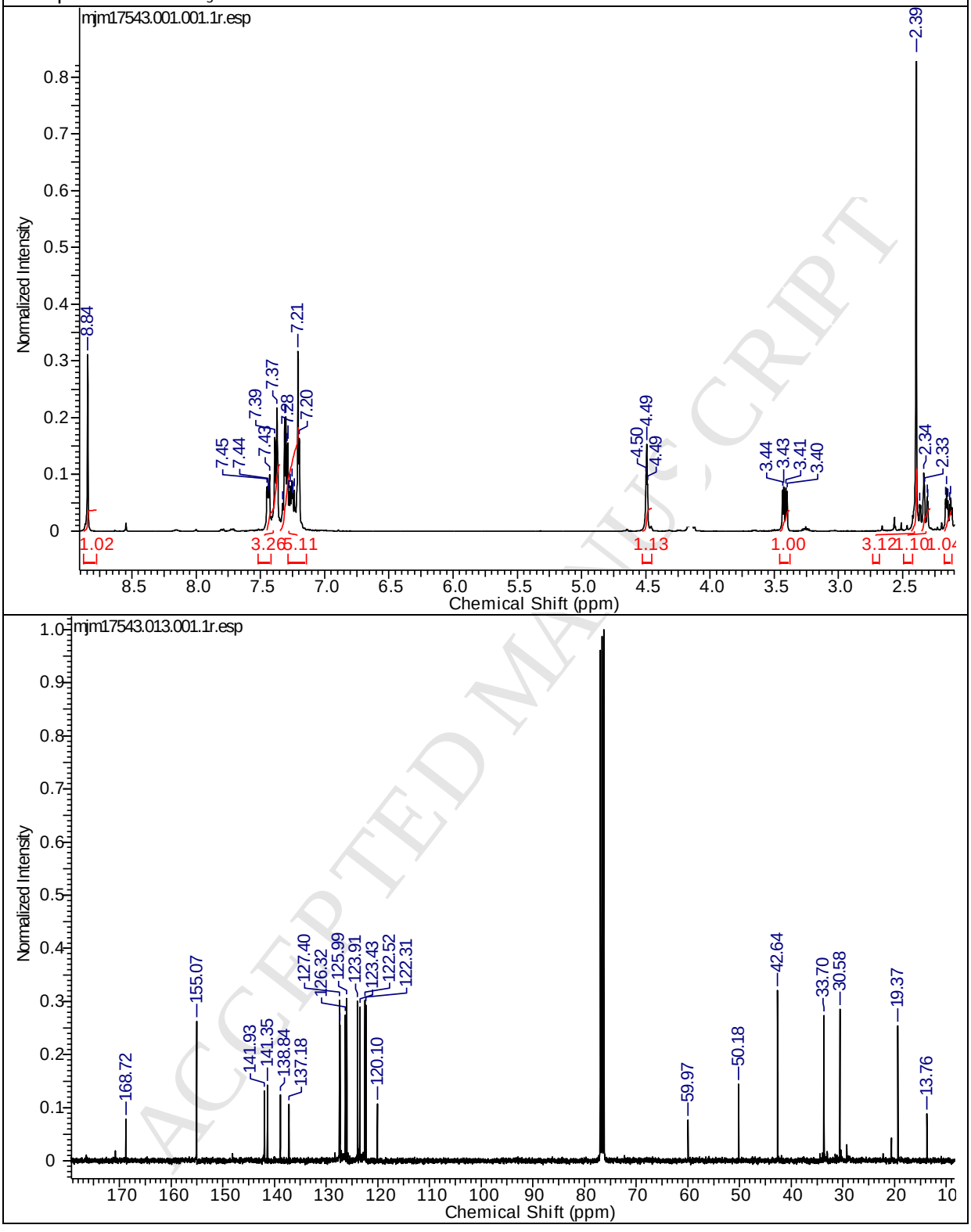
Compound 77– $CDCl_3$



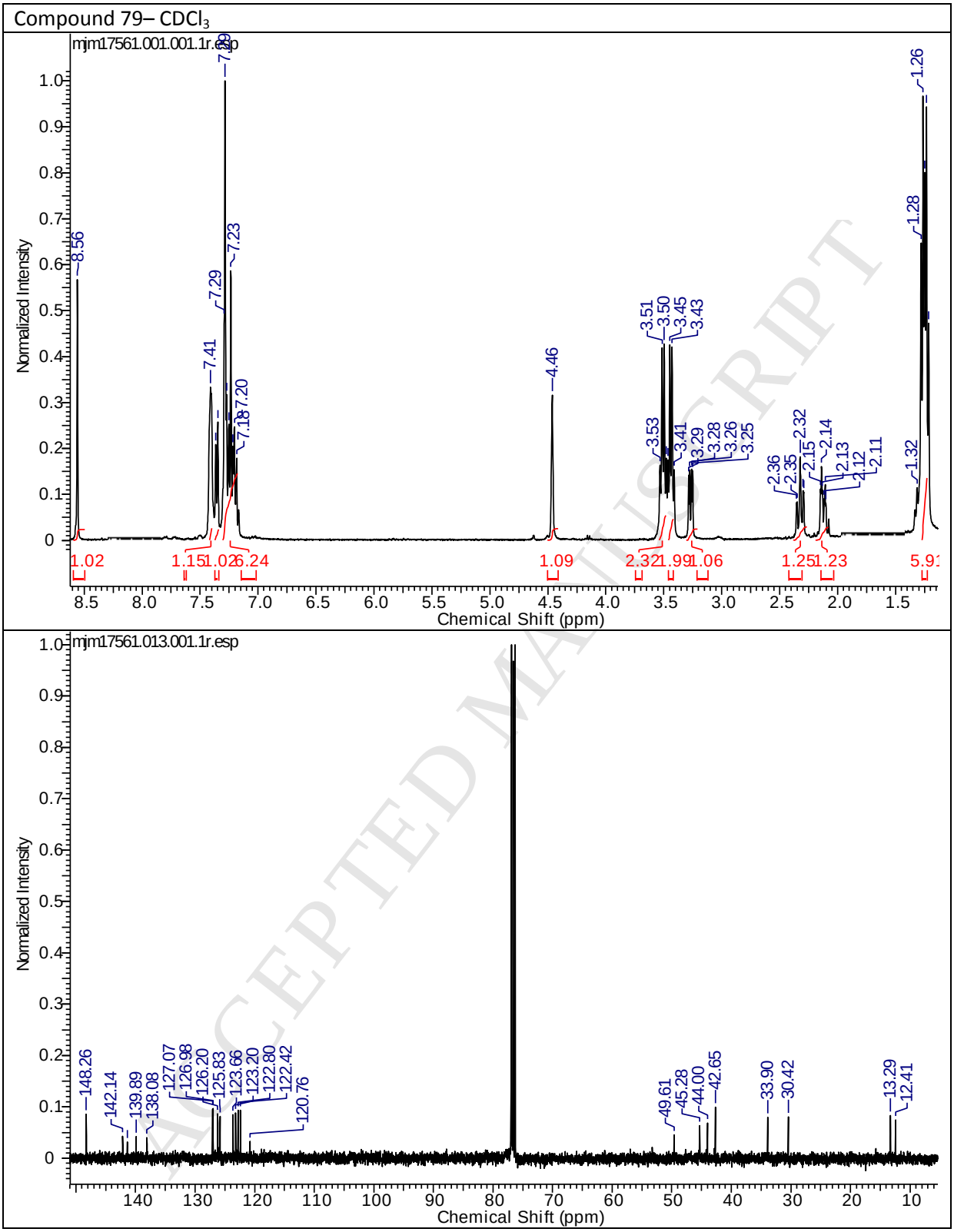


ACCEPTED MANUSCRIPT

Compound 78– $CDCl_3$



ACCEPTED MANUSCRIPT



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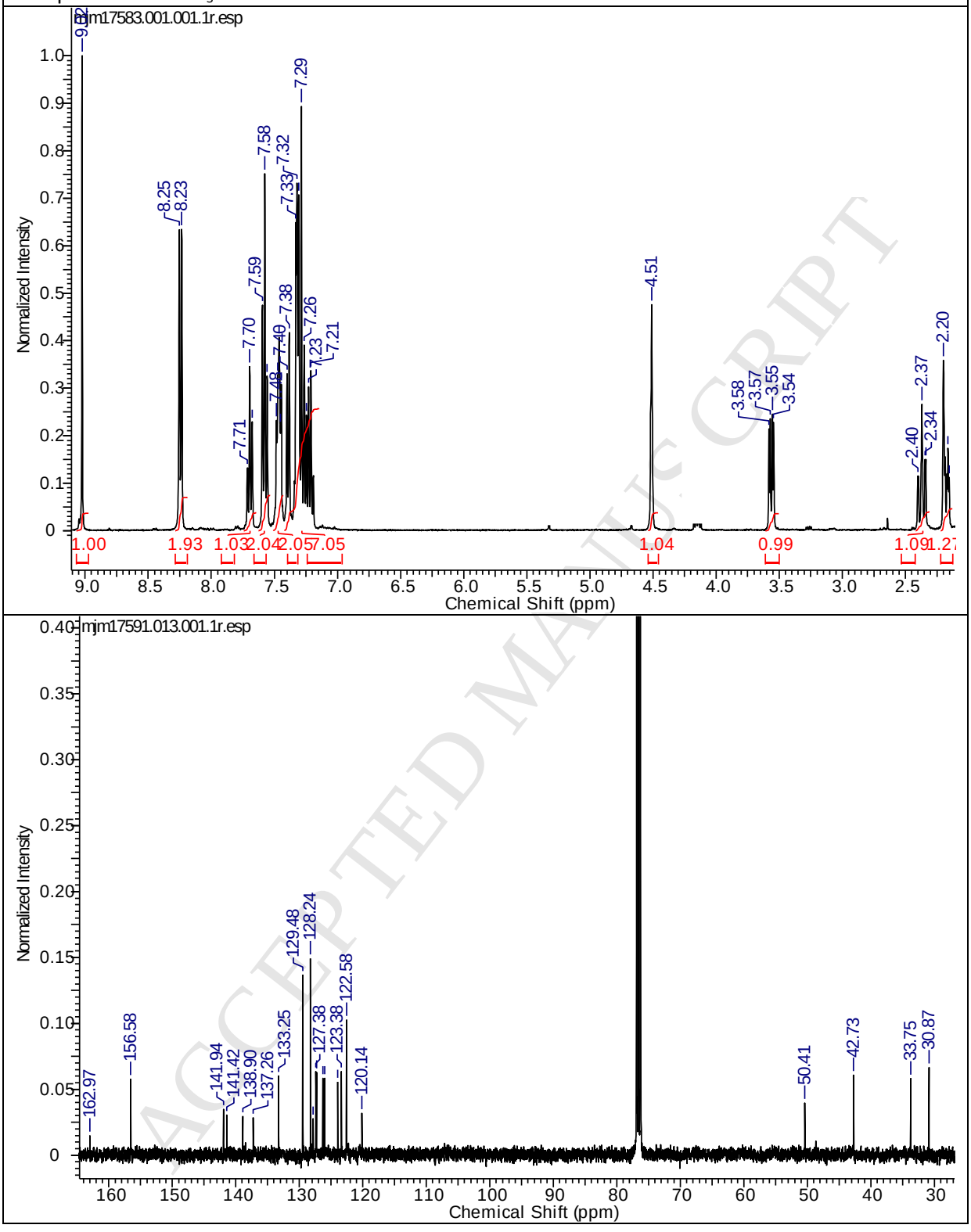
Compound 80– $CDCl_3$



Drug-like properties of 9,10-dihydro-9,10-ethanoanthracenes and 9-anthracenyl compounds

Representative 9,10-dihydro-9,10-ethanoanthracenes and 9-anthracenyl compounds were chosen for analysis of their drug-like properties from a Tier-1 profiling screen using Molinspiration Chemoinformatics (v2010.01) (www.molinspiration.com). These compounds satisfy Lipinski's 'rule of five' for drug-like properties, for example molecular weights are less than 500, the number of oxygen/nitrogen atoms is less than 10, the number of hydrogen bond donors is less than 5. All compounds have less than 7 rotatable bonds[1] and the cLogP values are less than 5 (except for compound **27** cLogP 5.711), implying that they are moderate lipophilic–hydrophobic drugs and are suitable candidates for further investigation. Only compound **79** is slightly above the 60 Å limit for BBB permeability. The Pipeline Pilot Professional (v8.0.1.100) screen includes predictions of permeability, metabolic stability, hepatotoxicity, blood-brain barrier (BBB) partition[2, 3], plasma protein binding (PPB) and human intestinal absorption properties which indicated the suitability of these compounds for further development[2, 3]. All of the compounds examined are predicted to bind to plasma proteins and have poor intestinal absorption. Compounds **27** and **66**, active in MDR cells, are not predicted to cause hepatoxicity, while potent anti-proliferative 9-anthracenyl compounds (**53**, **55**, **56** and **62**) and compound **79** were not predicted to inhibit CYP2D6.

Predicted parameters evaluated using Pipeline Pilot Professional (v8.0.1.100) and Molinspiration Chemoinformatics (v2010.01). Aqueous solubility: at 25 ˚C, scale of 0-5 where 0 represents low solubility and 5 represents high solubility. Human intestinal absorption: Scale of 0-3, where 0 is poor absorption. Blood-brain barrier partition: Scale of 0-5, where 0 represents a high probability of BBB permeability and 5 represents a low probability).

Table 2. Predicted molecular parameters for drug-likeness of a representative group of 9,10-dihydro-9,10-ethanoanthracene and 9-anthracenyl compounds.

Compound	**27**	**53**	**55**	**56**	**62**	**66**	**79**
Total polar surface area Å^2	3.24	20.31	23.55	49.85	45.82	37.38	65.69
No. of rotational bonds	3	2	2	4	2	3	5
No. of H bond donors	0	0	0	0	0	0	0
No. of H bonds acceptors	1	1	2	3	2	2	4
Molecular weight, Mr	331.49	315.41	330.42	388.46	249.26	371.47	373.45
LogP	5.711	4.445	3.485	3.737	3.626	4.24	3.676
Molecular volume (Å^3)	245.58	218.14	228.09	261.02	151.60	253.13	251.76
Aqueous Solubility	1	1	2	2	2	2	2
ADMET/Intestinal	1	0	0	0	0	0	0

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Absorption							
Plasma protein binding	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Blood-brain barrier permeability	1.558	0.893	0.543	0.206	0.289	0.556	0.03
CYP2D6 inhibition probability	Yes	No	No	No	No	Yes	No
Hepatotoxicity probability	No	Yes	Yes	Yes	Yes	No	Yes

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