



Original article

Syntheses and anti-allergic activity of 2-((bis(trimethylsilyl)methylthio/methylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazoles

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ABSTRACT

A new class of sila-substituted 1,3,4-oxadiazoles was synthesized by a convenient synthetic method. Both silathio/silasulfonyl acetic acids were efficiently condensed with benzohydrazides in the presence of phosphorus oxychloride to give sila-substituted 1,3,4-oxadiazoles in high yields. The compounds displayed variable extent of anti-allergic activity on IgE/Ag-stimulated RBL-2H3 cells at 50 and 100 μM concentrations. Compounds having sulfonyl moiety with bis(trimethylsilyl)-1,3,4-oxadiazoles (**5a–c**), exhibited better anti-allergic activities than those of compounds having sulphur moiety with bis(trimethylsilyl)-1,3,4-oxadiazoles (**4a–c**).

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1. Introduction

Sila-substitution (C/Si exchange) into drug-like scaffolds has attracted considerable attention as a consequence of unique biological activities, chemical reactivities and physicochemical properties of sila-drugs compared with their parent carbon compounds [1–5].

Tacke and Daiss synthesized various sila-substituted drugs such as selective noradrenaline reuptake inhibitors [6,7], muscarinic receptor antagonist [8–10], dopamine receptor antagonist [11–13], retinoid agonist [14,15] etc., which were structurally characterized and biologically evaluated.

In related to sila-substituted drugs, we have interested in sila-substituted five-membered ring heterocycles such as 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles, pyrazoles, isoxazoles, imidazoles as promising candidates for drug discovery. Particularly various substituted 1,3,4-oxadiazoles displayed broad spectra of biological activities [16–20]. 1,3,4-Oxadiazoles derivatives have also been reported as promising anti-allergic agents [21,22].

We previously reported the synthesis of a series of 2-(((trimethylsilyl)methylthio/methylsulfonyl)methyl)-5-aryl-1,3,4-

oxadiazoles (**1a–c**, **11a–c**) and their anti-allergic activities [22]. We observed that **11c** could be a suitable clinical candidate for treatments of atopic disorders (Chart 1). Due to these attractive results, we focused on bis(trimethylsilyl)-1,3,4-oxadiazoles. In this paper we report the syntheses of 2-((bis(trimethylsilyl)methylthio/methylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazoles and their anti-allergic activities.

2. Chemistry

2-(Bis(trimethylsilyl)methylthio)acetic acid (**2**) was synthesized by the reaction of bis(trimethylsilyl)chloromethane (**1**) with mercaptoacetic acid in the presence NaOH/MeOH, and then followed by an oxidation of **2** in the presence of H₂O₂/CH₃COOH to afford compound **3**. The ¹H NMR spectra of both compounds **2** and **3** displayed three singlets at δ 0.08, 1.01, 3.11 and 0.23, 2.21, 4.46 ppm respectively, that are due to methyl, methine and methylene protons. In addition to these chemical shifts **2** and **3** showed broad singlets at δ 10.20 and 9.93 ppm, respectively due to OH, which were disappeared on deuteration process. The desired heterocycles, 2-((bis(trimethylsilyl)methylthio)methyl)-5-aryl-1,3,4-oxadiazoles (**4**) and 2-((bis(trimethylsilyl)-methylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazoles (**5**) were synthesized by the reactions of **2/3** with benzohydrazides in the presence of phosphorus oxychloride [22] (Scheme 1). The ¹H NMR spectra of **4a** and **5a** displayed three

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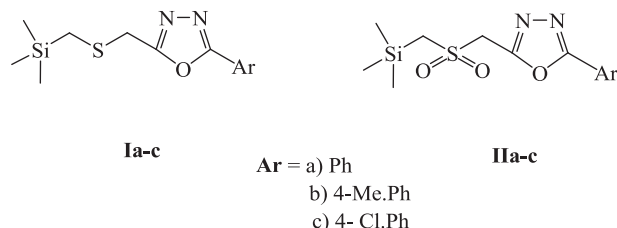


Chart 1. Biologically active sila-substituted 1,3,4-oxadiazoles.

sharp singlets for methyl, methine and methylene protons at δ 0.01, 0.88, 3.80 and at δ 0.26, 2.90, 4.56 ppm, respectively. Apart from these, **4a** and **5a** displayed doublets and multiplet signals at δ 7.48, 8.02, and 7.50–7.62 ppm due to aromatic protons. The ^{29}Si spectra of compounds **4a** and **5a** appeared at δ 3.02, and 2.06 ppm, respectively. The structures of the compounds **4** and **5** were further confirmed by ^{13}C NMR spectra.

3. Anti-allergic activity

Mast cells have a high affinity for immunoglobulin (Ig) E because of having a specific IgE receptor, Fc ϵ R1, on their surface. As the IgE-multivalent allergen complex binds to Fc ϵ R1, the mast cells become activated and release a range of preformed species such as newly synthesized mediators and cytokines that trigger potent allergic reactions [23–27]. We evaluated the release of preformed allergic mediators using β -hexosaminidase as a biomarker of degranulation in the mast cells and then measured cytotoxicity. The release of β -hexosaminidase was first measured following stimulation of the IgE/Ag complex. Compounds **4a**, **4b**, **4c**, **5a**, **5b** and **5c** inhibited IgE/Ag-induced β -hexosaminidase release in concentration-dependent manner (Fig. 1). As shown in Fig. 1, compound 2-((bis(trimethylsilyl)methylsulfonyl)methyl)-5-phenyl-1,3,4-oxadiazole (**5a**) showed much better inhibited IgE/Ag-induced β -hexosaminidase release at 50 μM compared with the other compounds. But in case of 100 μM compounds **5a–c** showed much better inhibition compared with the compounds **4a–c**. From these investigation, compounds having sulfonyl moiety with bis(trimethylsilyl)-1,3,4-oxadiazoles (**5a–c**) unit exhibited good anti-allergic activity compared with the compounds having sulphur moiety with

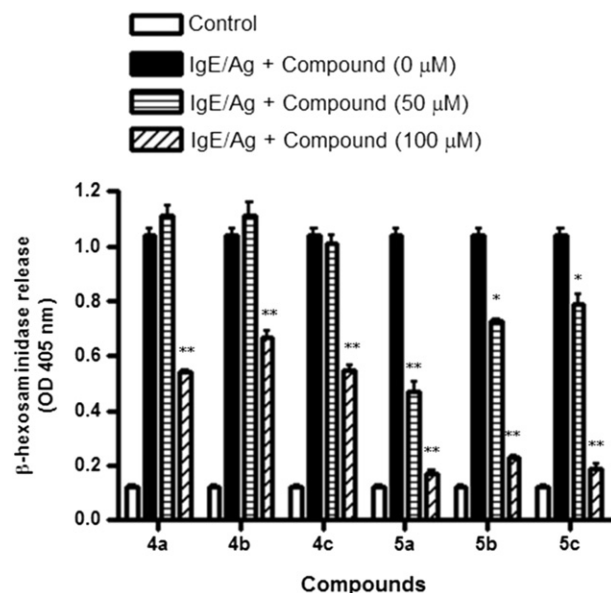
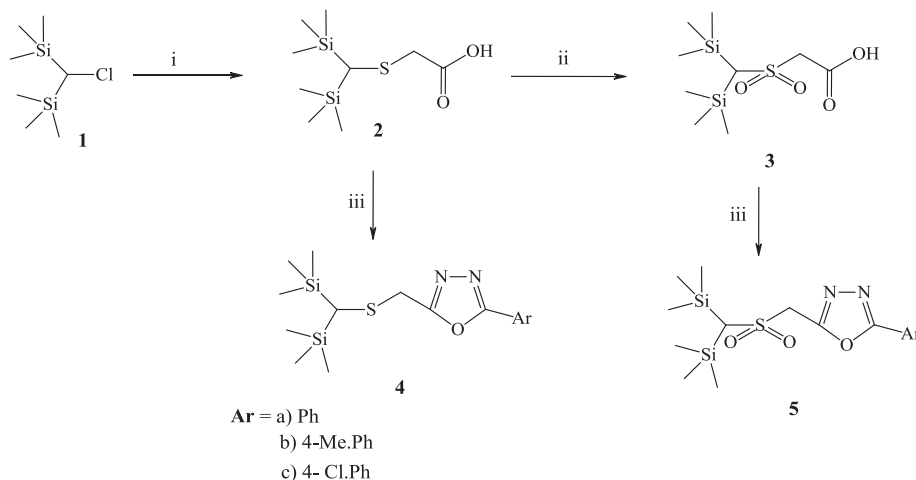


Fig. 1. Effect of **4a**, **4b**, **4c**, **5a**, **5b** and **5c** compounds on β -hexosaminidase release of IgE/Ag-stimulated RBL-2H3 cells. The cells were incubated overnight in 24-well plates with 200 ng/mL of DNP-specific IgE. The medium was replaced with PIPES buffer containing the indicated concentrations of **4a**, **4b**, **4c**, **5a**, **5b** and **5c** compounds, and the cells were then challenged with 25 ng/mL DNP-HSA. After 15 min, β -hexosaminidase release was determined from the absorbance measured at 405 nm. The mean values ($\pm$ S.D.) from three independent experiments are shown, where each was performed in triplicate. * $P < 0.05$, ** $P < 0.01$.

bis(trimethylsilyl)-1,3,4-oxadiazoles (**4a–c**). IC_{50} values of compounds **5a–c** were 37.2 ± 3.3 , 62.3 ± 2.3 , and 75.5 ± 4.1 μM , respectively. In contrast, compound **4a** weakly inhibited IgE/Ag-induced β -hexosaminidase release with an IC_{50} value of 98.8 ± 3.1 μM , whereas **4b–c** failed to inhibit half of IgE/Ag-induced β -hexosaminidase release at a highest concentration of 100 μM .

Additionally, if the compounds have a toxic effect on cells, we must distinguish such an effect from that of β -hexosaminidase release, which also reduces the viability of IgE/Ag-induced cells. Therefore, we compared the cell cytotoxicity in RBL-2H3 cells incubated with **4a**, **4b**, **4c**, **5a**, **5b** and **5c** compounds (0, 50 and 100 μM) for 24 h using the EZ-Cytox kit. As shown in Fig. 2, the RBL-



Reagents and conditions: (i) HSCH_2COOH , MeOH, NaOH, reflux, 4h; (ii) H_2O_2 , AcOH, 24h; (iii) ArCONHNH_2 , POCl_3 , reflux, 6h.

Scheme 1. Synthesis of 2-((bis(trimethylsilyl)methylthio/methylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazoles.

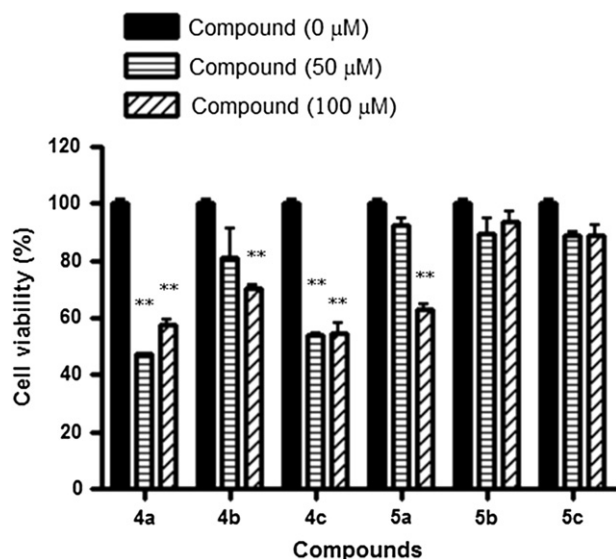


Fig. 2. Cytotoxicity of **4a**, **4b**, **4c**, **5a**, **5b** and **5c** compounds on RBL-2H3 cells. To assess cytotoxicity, RBL-2H3 cells were incubated overnight in medium containing 200 ng/mL of DNP-specific IgE, and then treated with **4a**, **4b**, **4c**, **5a**, **5b** and **5c** compounds at 0, 50 and 100 μ M concentrations for 24 h. Cell viability was determined with the EZ-Cytox kit from the absorbance at 450 nm. The results are expressed as the percentage of surviving cells relative to compound-untreated control cells. The mean values ($\pm$ S.D.) from three independent experiments are shown, where each was performed in triplicate. ** $P < 0.01$.

2H3 cells were weakly affected following treatment with **5a**, **5b** and **5c** at concentrations of 50 and 100 μ M. These results suggest that compound **5a** does not cause cytotoxicity of cells only at 50 μ M, but significantly inhibits β -hexosaminidase release stimulated by IgE/Ag at 50 μ M and 100 μ M. And also compounds **5b** and **5c** do not cause cytotoxicity of cells at 50 μ M and 100 μ M, but significantly inhibit β -hexosaminidase release stimulated by IgE/Ag only at 100 μ M concentration.

4. Conclusion

A novel series of bis(trimethylsilyl)-1,3,4-oxadiazoles was synthesized and investigated their anti-allergic activities in 50 and 100 μ M concentrations. Among them the compounds having sulfonyl moiety, bis(trimethylsilyl)-1,3,4-oxadiazoles (**5a–c**) showed much better inhibited IgE/Ag-induced β -hexosaminidase release at 50 and 100 μ M compared with the compounds having sulphur moiety with bis(trimethylsilyl)-1,3,4-oxadiazoles (**4a–c**). Compound **5a** showed the best anti-allergic activity at 100 μ M concentration among **4a–c** and **5a–c**. However, in cytotoxicity point of view, compound **5a** could be the good candidate for anti-allergy treatment at 50 μ M, and **5b**, **5c** could also be the best candidate for anti-allergy treatment at 100 μ M concentration.

5. Experimental section

5.1. Chemistry

Melting points were determined in open capillaries on a Stuart apparatus and were uncorrected. The purity of the compounds was checked by TLC (silica gel H, BDH, ethyl acetate/hexane, 1:3). The IR spectra were recorded on a Perkin Elmer Spectrum one FT-IR Spectrometer as KBr pellets and the wave numbers were given in cm^{-1} . The ^1H NMR, ^{13}C NMR and ^{29}Si NMR spectra were recorded on a Bruker AMX 400 NMR spectrometer in CDCl_3 . The microanalyses were performed using Perkin Elmer 240C elemental

analyzer. Mass spectra were recorded on a low-resolution (Agilent Technologies GC/MS: 6890N, 5973N mass selective detector) EI mass Spectrometer.

5.1.1. Synthesis of 2-(bis(trimethylsilyl)methylthio)acetic acid (**2**)

A solution of sodium hydroxide (30 mmol) in methanol (30 mL) was taken in a 100 mL two-necked round-bottomed flask, fitted with a reflux condenser, a dropping funnel and equipped over a heating mantle. To this, mercaptoacetic acid (15 mmol) was added slowly through the dropping funnel and followed bis(trimethylsilyl)chloromethane (15 mmol) was added portion-wise. The contents of the flask were refluxed for 3–4 h, cooled and poured into ice-cold water containing concentrated hydrochloric acid. The resultant liquid was extracted with diethyl ether and distilled to afford pure 2-(bis(trimethylsilyl)methylthio)acetic acid (2.15 g, 86%); b.p. 143–145 $^\circ\text{C}$; IR (KBr): 3445 (OH), 1704 ($\text{C}=\text{O}$) cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 0.08 (s, 18H, Si- CH_3), 1.01 (s, 1H, Si-CH), 3.11 (s, 2H, S- CH_2), 10.20 (bs, 1H, OH) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ -1.01 (Si- CH_3), 16.04 (Si-CH), 27.50 (S- CH_2), 174.08 ($\text{C}=\text{O}$); ^{29}Si NMR (CDCl_3 , 79 MHz): δ 2.96.

5.1.2. Synthesis of 2-(bis(trimethylsilyl)methylsulfonyl)acetic acid (**3**)

To an ice-cold solution of 2-(bis(trimethylsilyl)methylthio)acetic acid (**2**) (20 mmol) in 30 mL of glacial acetic acid in a 100 mL round-bottomed flask, 30% hydrogen peroxide (12 mL) was added in portions. The contents were allowed to attain laboratory temperature and kept aside for 36 h. Then, acetic acid was removed under reduced pressure. The residual portion was poured onto crushed ice. The solid separated was filtered, dried and recrystallized from water to get pure 2-(bis(trimethylsilyl)methylsulfonyl)acetic acid (2.28 g, 81%); m.p. 71–73 $^\circ\text{C}$; IR (KBr): 3462 (OH), 1711 ($\text{C}=\text{O}$), 1308, 1133 (SO_2) cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 0.23 (s, 18H, Si- CH_3), 2.21 (s, 1H, Si-CH), 4.46 (s, 2H, SO_2 - CH_2), 9.93 (bs, 1H, OH) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ -0.61 (Si- CH_3), 40.02 (Si-CH), 51.91 (SO_2 - CH_2), 169.38 ($\text{C}=\text{O}$); ^{29}Si NMR (CDCl_3 , 79 MHz): δ 2.04.

5.1.3. General procedure for the synthesis of 2-((bis(trimethylsilyl)methylthio)methyl)-5-aryl-1,3,4-oxadiazole (**4a–c**)

A mixture of 2-(bis(trimethylsilyl)methylthio)acetic acid (**2**) (10 mmol), benzohydrazides (10 mmol) and phosphorus oxychloride (7 mL) was heated under reflux for 5–6 h. The excess POCl_3 was removed under reduced pressure and the residue was poured onto crushed ice. The resulting precipitate was filtered, washed with saturated sodium bicarbonate solution and then with water, dried and recrystallized from ethanol to obtain **4**.

5.1.3.1. 2-((Bis(trimethylsilyl)methylthio)methyl)-5-phenyl-1,3,4-oxadiazole (4a**).** Pale yellow solid (2.90 g, 83%); m.p. 81–83 $^\circ\text{C}$; IR (KBr): 1608 ($\text{C}=\text{N}$) cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 0.01 (s, 18H, Si- CH_3), 0.88 (s, 1H, Si-CH), 3.80 (s, 2H, S- CH_2), 7.48 (d, 3H, Ar-H), 8.02 (d, 2H, Ar-H) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ -0.64 (Si- CH_3), 16.16 (Si-CH), 28.83 (S- CH_2), 163.69 (C-2), 165.48 (C-5), 123.72, 126.92, 129.05, 131.79 (aromatic carbons) ppm; ^{29}Si NMR (CDCl_3 , 79 MHz): δ 3.02; MS m/z (relative intensity) 350 ($\text{M}^+ - 15$, 26.4), 231 (91.9), 163 (33.8), 105 (32.3), 73 (100), 59 (14.7). Anal. Calcd for $\text{C}_{16}\text{H}_{26}\text{N}_2\text{OSSi}_2$: C, 54.81; H, 7.47; N, 7.99; Found: C, 54.74; H, 7.41; N, 8.03.

5.1.3.2. 2-((Bis(trimethylsilyl)methylthio)methyl)-5-p-tolyl-1,3,4-oxadiazole (4b**).** White solid (2.87 g, 79%); m.p. 89–91 $^\circ\text{C}$; IR (KBr): 1616 ($\text{C}=\text{N}$) cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 0.01 (s, 18H, Si- CH_3), 0.87 (s, 1H, Si-CH), 2.40 (s, 3H, Ar- CH_3), 3.79 (s, 2H, S- CH_2), 7.28 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.92 (d, 2H, $J = 8.0$ Hz, Ar-H) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ -0.64 (Si- CH_3), 16.10 (Si-CH), 21.64 (Ar- CH_3), 28.84 (S- CH_2), 163.39 (C-2), 165.64 (C-5),

120.96, 126.88, 129.76, 142.34 (aromatic carbons) ppm; ^{29}Si NMR (CDCl_3 , 79 MHz): δ 3.01; MS m/z (relative intensity) 364 ($\text{M}^+ - 15$, 17.6), 245 (48.5), 174 (17.6), 163 (16.9), 119 (30.1), 91 (26.4), 73 (100). Anal. Calcd for $\text{C}_{17}\text{H}_{28}\text{N}_2\text{OSSi}_2$: C, 55.99; H, 7.74; N, 7.68; Found: C, 55.90; H, 7.81; N, 7.61.

5.1.3.3. 2-((Bis(trimethylsilyl)methylthio)methyl)-5-(4-chlorophenyl)-1,3,4-oxadiazole (4c). White solid (3.22 g, 84%); m.p. 93–95 °C; IR (KBr): 1613 ($\text{C}=\text{N}$) cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 0.01 (s, 18H, Si-CH₃), 0.85 (s, 1H, Si-CH), 3.80 (s, 2H, $\text{SO}_2\text{-CH}_2$), 7.56 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.96 (d, $J = 8.0$ Hz, 2H, Ar-H) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ -0.66 (Si-CH₃), 16.21 (Si-CH), 28.80 (S-CH₂), 163.88 (C-2), 164.69 (C-5), 122.15, 128.19, 129.48, 138.13 (aromatic carbons) ppm; ^{29}Si NMR (CDCl_3 , 79 MHz): δ 3.05. MS m/z (relative intensity) 384 ($\text{M}^+ - 15$, 4.4), 265 (15.4), 163 (11.7), 139 (27.2), 111 (13.2), 73 (100). Anal. Calcd for $\text{C}_{16}\text{H}_{25}\text{ClN}_2\text{OSSi}_2$: C, 49.91; H, 6.54; N, 7.27; Found: C, 49.82; H, 6.45; N, 7.31.

5.1.4. General procedure for the synthesis of 2-((bis(trimethylsilyl)methylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazole (5a–c)

A mixture of aryl acid hydrazide (10 mmol), 2-((bis(trimethylsilyl)methylsulfonyl)acetic acid (**3**) (10 mmol) and phosphorus oxychloride (7 mL) was heated under reflux for 5–6 h. The excess POCl_3 was removed under reduced pressure and the residue was poured onto crushed ice. The resulting precipitate was filtered, washed with saturated sodium bicarbonate solution and then with water, dried and recrystallized from ethanol to obtain **5**.

5.1.4.1. 2-((Bis(trimethylsilyl)methylsulfonyl)methyl)-5-phenyl-1,3,4-oxadiazole (5a). White solid (3.28 g, 86%); m.p. 97–99 °C; IR (KBr): 1611 ($\text{C}=\text{N}$), 1328, 1161 (SO_2) cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 0.26 (s, 18H, Si-CH₃), 2.90 (s, 1H, Si-CH), 4.56 (s, 2H, $\text{SO}_2\text{-CH}_2$) 7.50–7.62 (m, 5H, Ar-H) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ -0.67 (Si-CH₃), 43.36 (Si-CH), 53.44 ($\text{SO}_2\text{-CH}_2$), 157.96 (C-2), 166.45 (C-5), 123.14, 127.20, 129.18, 132.33 (aromatic carbons) ppm; ^{29}Si NMR (CDCl_3 , 79 MHz): δ 2.06; MS m/z (relative intensity) 382 (M^+ , 13.3), 238 (9.6), 159 (62.2), 105 (100), 77 (53.3), 63 (9.6), 51 (9.6). Anal. Calcd for $\text{C}_{16}\text{H}_{26}\text{N}_2\text{O}_3\text{SSi}_2$: C, 50.22; H, 6.85; N, 7.32; Found: C, 50.21; H, 6.91; N, 7.24.

5.1.4.2. 2-((Bis(trimethylsilyl)methylsulfonyl)methyl)-5-p-tolyl-1,3,4-oxadiazole (5b). White solid (3.20 g, 81%); m.p. 103–105 °C; IR (KBr): 1617 ($\text{C}=\text{N}$), 1311, 1158 (SO_2) cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 0.25 (s, 18H, Si-CH₃), 2.41 (s, 3H, Ar-CH₃), 2.90 (s, H, Si-CH), 4.55 (s, 2H, $\text{SO}_2\text{-CH}_2$), 7.30 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.94 (d, $J = 8.0$ Hz, 2H, Ar-H) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ -0.61 (Si-CH₃), 21.31 (Ar-CH₃), 41.83 (Si-CH), 52.03 ($\text{SO}_2\text{-CH}_2$), 157.11 (C-2), 164.21 (C-5), 122.40, 125.01, 128.81, 131.40 (aromatic carbons) ppm; ^{29}Si NMR (CDCl_3 , 79 MHz): δ 2.03; MS m/z (relative intensity) 396 (M^+ , 29.6), 245 (14.0), 173 (50.7), 119 (100), 91 (15.6), 73 (37.5), 59 (14.0). Anal. Calcd for $\text{C}_{17}\text{H}_{28}\text{N}_2\text{O}_3\text{SSi}_2$: C, 51.48; H, 7.12; N, 7.06; Found: C, 51.41; H, 7.21; N, 7.01.

5.1.4.3. 2-((Bis(trimethylsilyl)methylsulfonyl)methyl)-5-(4-chlorophenyl)-1,3,4-oxadiazole (5c). White solid (3.66 g, 88%); m.p. 111–113 °C; IR (KBr): 1619 ($\text{C}=\text{N}$), 1324, 1143 (SO_2) cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ 0.26 (s, 18H, Si-CH₃), 2.86 (s, 1H, Si-CH), 4.58 (s, 2H, $\text{SO}_2\text{-CH}_2$) 7.50 (d, $J = 8.0$ Hz, 2H, Ar-H), 8.01 (d, $J = 8.0$ Hz, 2H, Ar-H) ppm; ^{13}C NMR (CDCl_3 , 100 MHz): δ -0.68 (Si-CH₃), 40.51 (Si-CH), 51.17 ($\text{SO}_2\text{-CH}_2$), 157.32 (C-2), 165.81 (C-5), 121.44, 128.47, 129.66, 138.89 (aromatic carbons) ppm; ^{29}Si NMR (CDCl_3 , 79 MHz): δ 2.09; MS m/z (relative intensity) 416 (M^+ , 25.0), 267 (65.8), 163 (34.1), 105 (32.9), 91 (12.1), 73 (100). Anal. Calcd for $\text{C}_{16}\text{H}_{25}\text{ClN}_2\text{O}_3\text{SSi}_2$: C, 46.08; H, 6.04; N, 6.72; Found: C, 45.98; H, 6.12; N, 6.68.

5.2. Biological assays

5.2.1. Cell culture

Cells from the rat mast cell line RBL-2H3 were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and maintained in Eagle's minimal essential medium (EMEM, Wel-Gene, Inc., Daegu, Korea) supplemented with 10% heat-inactivated foetal bovine serum and antibiotics (100 U/mL of penicillin, 100 μg /mL of streptomycin) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C.

5.3. β -Hexosaminidase release assay

As a marker of degranulation, we measured the release of β -hexosaminidase [22]. Rat basophilic leukaemia (RBL)-2H3 cells were distributed into 24-well plates (2×10^5 cells/well) and were sensitized overnight with anti-dinitrophenylated (DNP)-specific immunoglobulin (Ig) E at 200 ng/mL. The IgE-sensitized cells were washed twice with piperazine- N,N' -bis[2-ethanesulfonic acid] (PIPES) buffer (25 mM PIPES, pH 7.2, 110 mM NaCl, 4 mM KCl, 0.4 mM MgCl_2 , 40 mM HCl, 5.6 mM glucose, a 1 mM CaCl_2 , and 0.1% BSA), then treated for 30 min at 37 °C with **4a**, **4b**, **4c**, **5a**, **5b** and **5c** in PIPES buffer at the indicated concentrations. The cells were then stimulated with dinitrophenylated human serum albumin (DNP-HAS, 25 ng/mL) at 37 °C for 15 min and chilled on ice to stop the stimulation. To measure β -hexosaminidase release, the supernatant from the IgE/Ag-stimulated cells in PIPES buffer and β -hexosaminidase substrate (P-nitrophenyl- N -acetyl- β -D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) were mixed in 96-well plates and incubated at 37 °C for 1 h. This reaction was terminated by adding 0.1 M carbonate buffer (pH 10.5), and the absorbance at 405 nm was measured using a microplate reader.

5.4. Cytotoxicity assay

RBL-2H3 cells were seeded at 2×10^4 cells per mL in 96-well plates and incubated with **4a**, **4b**, **4c**, **5a**, **5b** and **5c** at 50 or 100 μM concentrations for 23 h at 37 °C. After incubation, cells were exposed to 10% of the volume of an EZ-Cytox kit (Daeil Lab Service Co., Ltd., Seoul, Korea) for 1 h at 37 °C. Cytotoxicity was measured using a microplate reader with absorbance at 450 nm. The results are expressed as the percentage of surviving cells relative to compound-untreated control cells.

5.5. Statistical analysis

Experimental results are expressed as means $\pm$ standard deviations. One-way analysis of variance (ANOVA) was followed by Dunnett's test for multiple comparisons. P -values less than 0.05 or less than 0.01 were considered statistically significant, as indicated.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2012.12.035>.

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Electronic Supplementary Information (ESI)

Syntheses and anti-allergic activity of 2-((bis(trimethylsilyl)methylthio/methylsulfonyl)methyl)-5-aryl-1,3,4-oxadiazoles

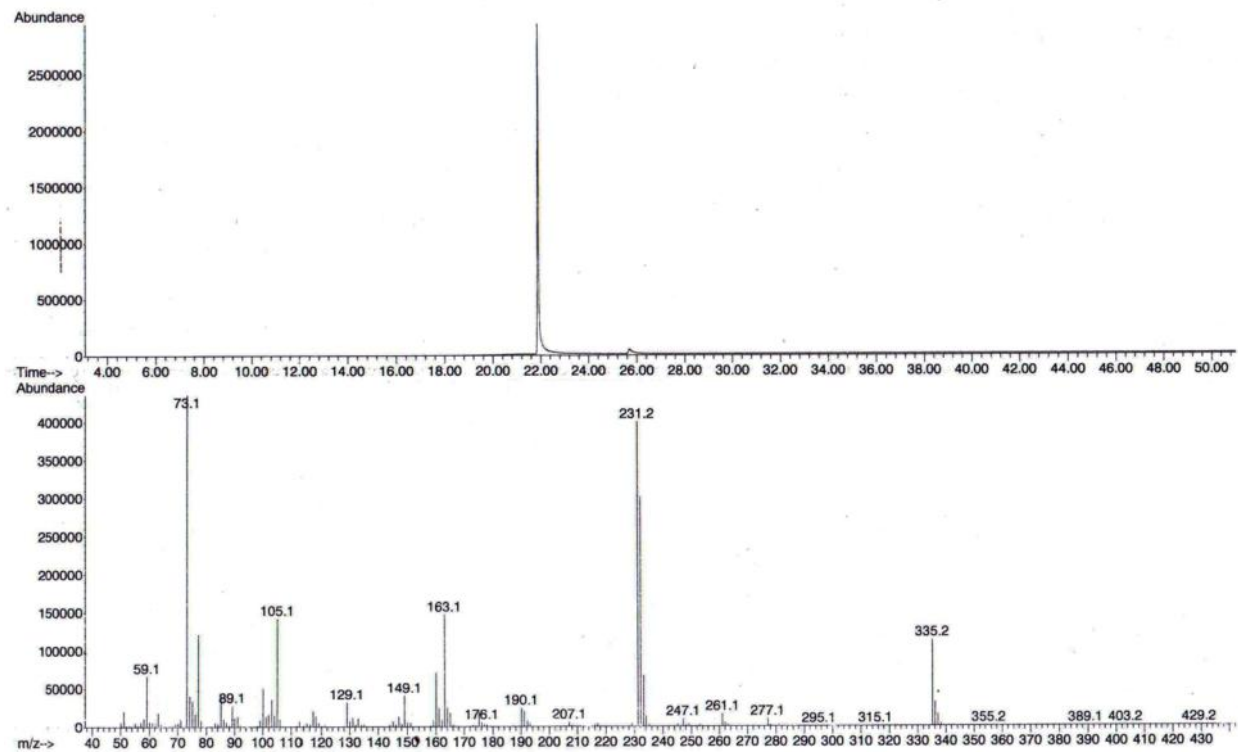
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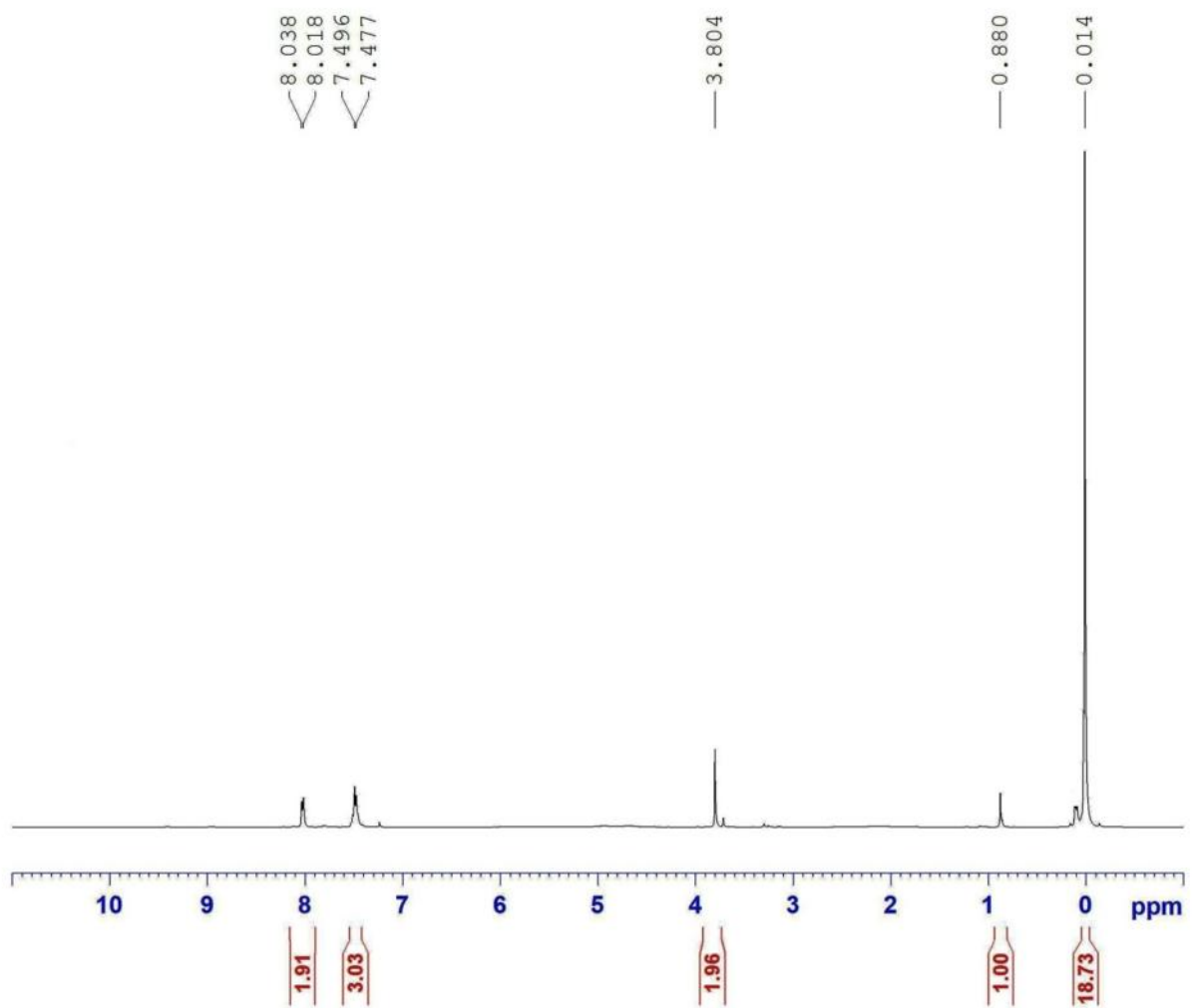
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Yonsei University, Wonju, Gangwon-do 220-710, South Korea

M.E.L: E-mail address: melgg@yonsei.ac.kr

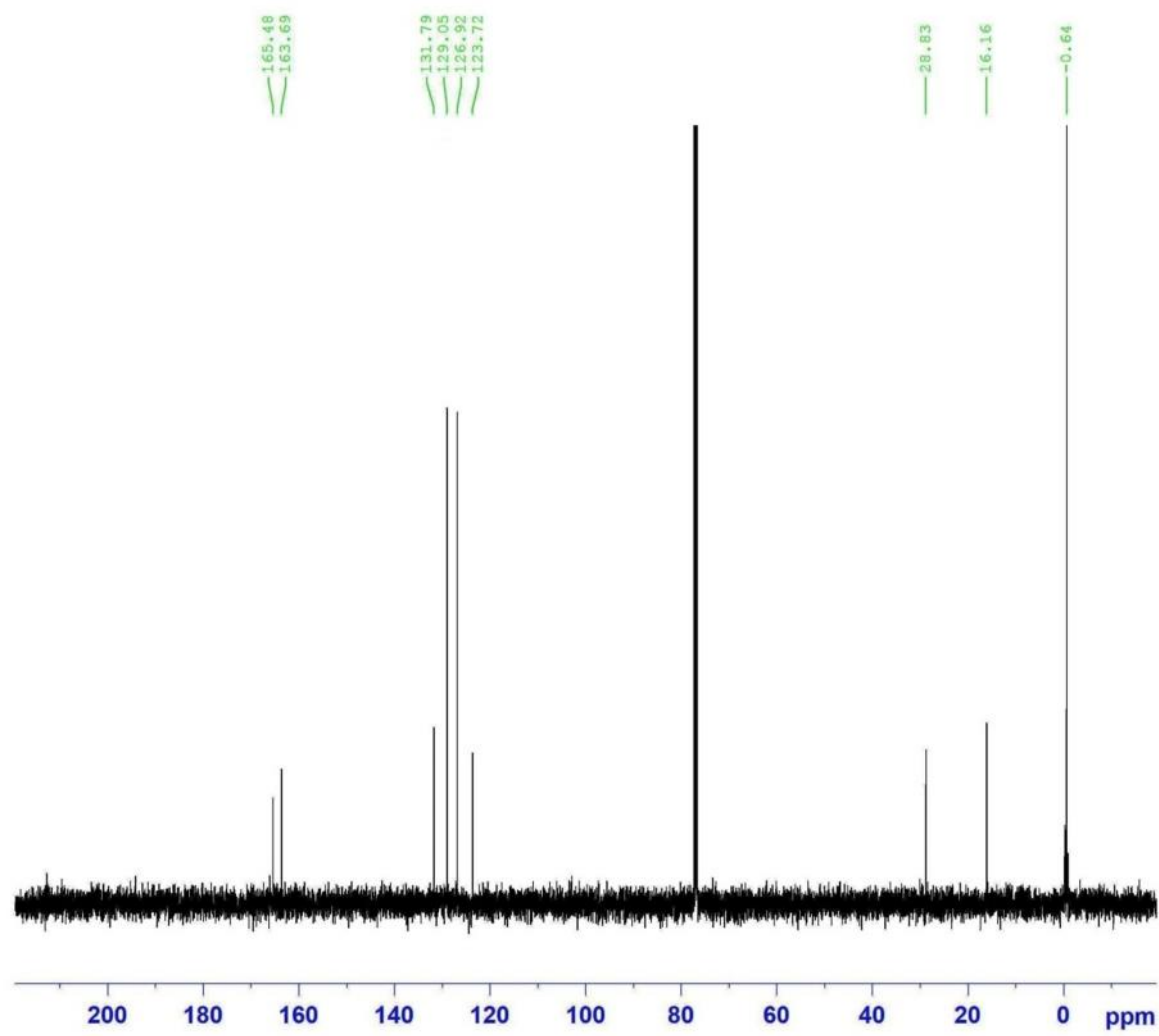
Spectra of Compound 4, 5



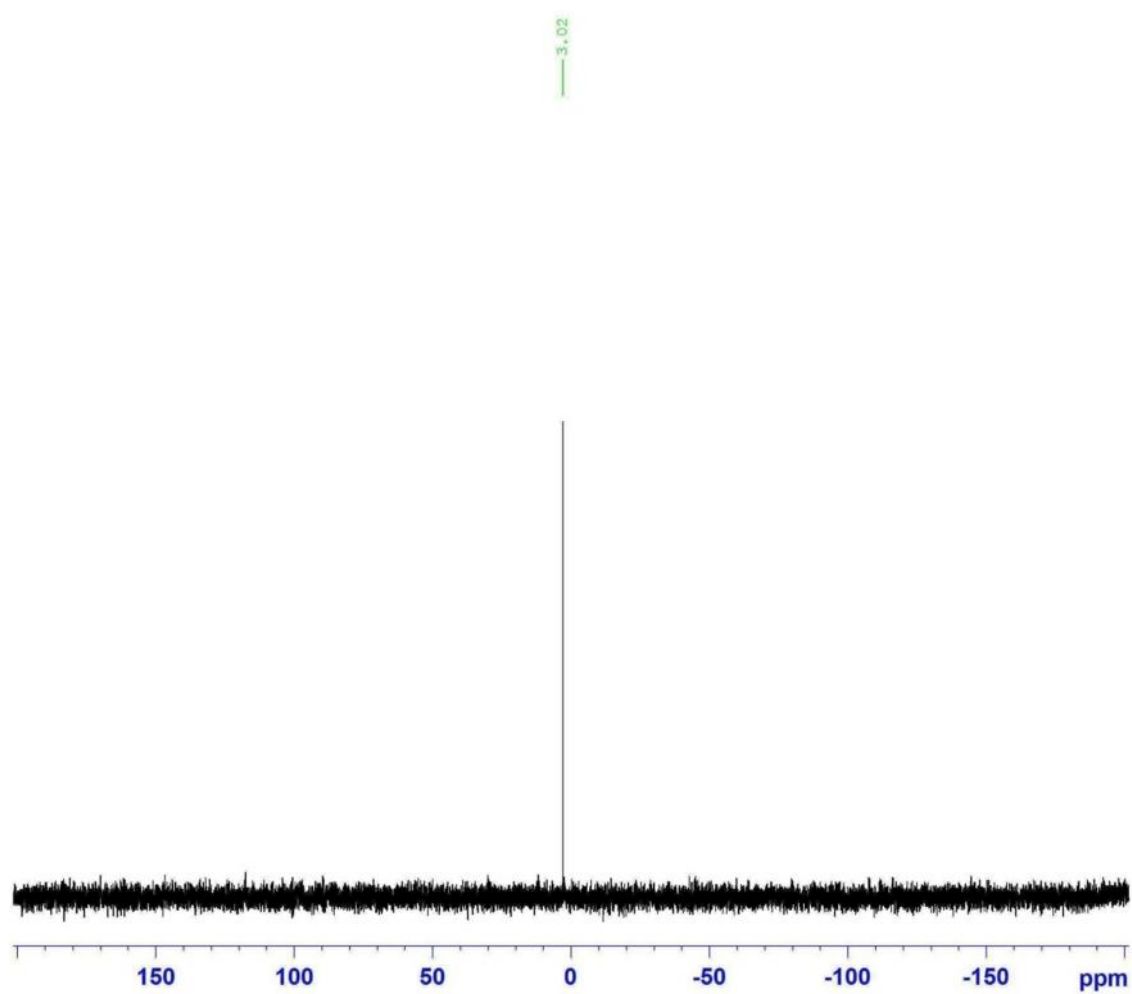
GC- MS of 4a



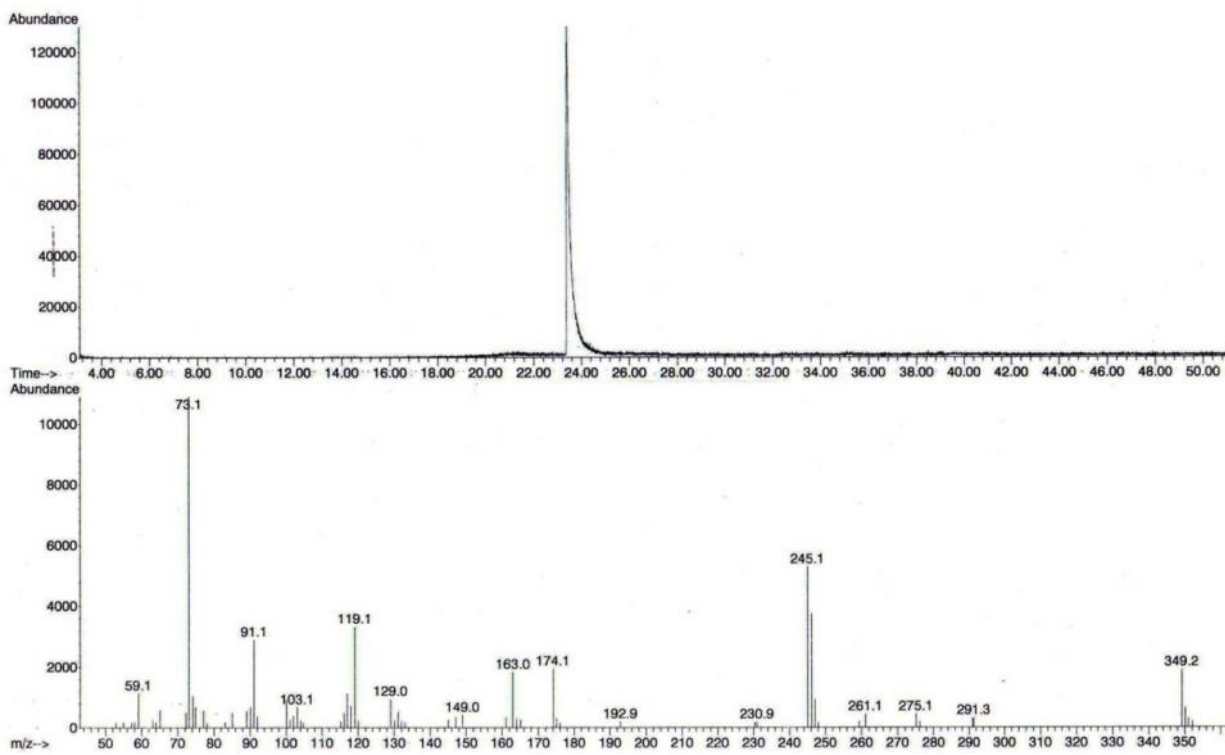
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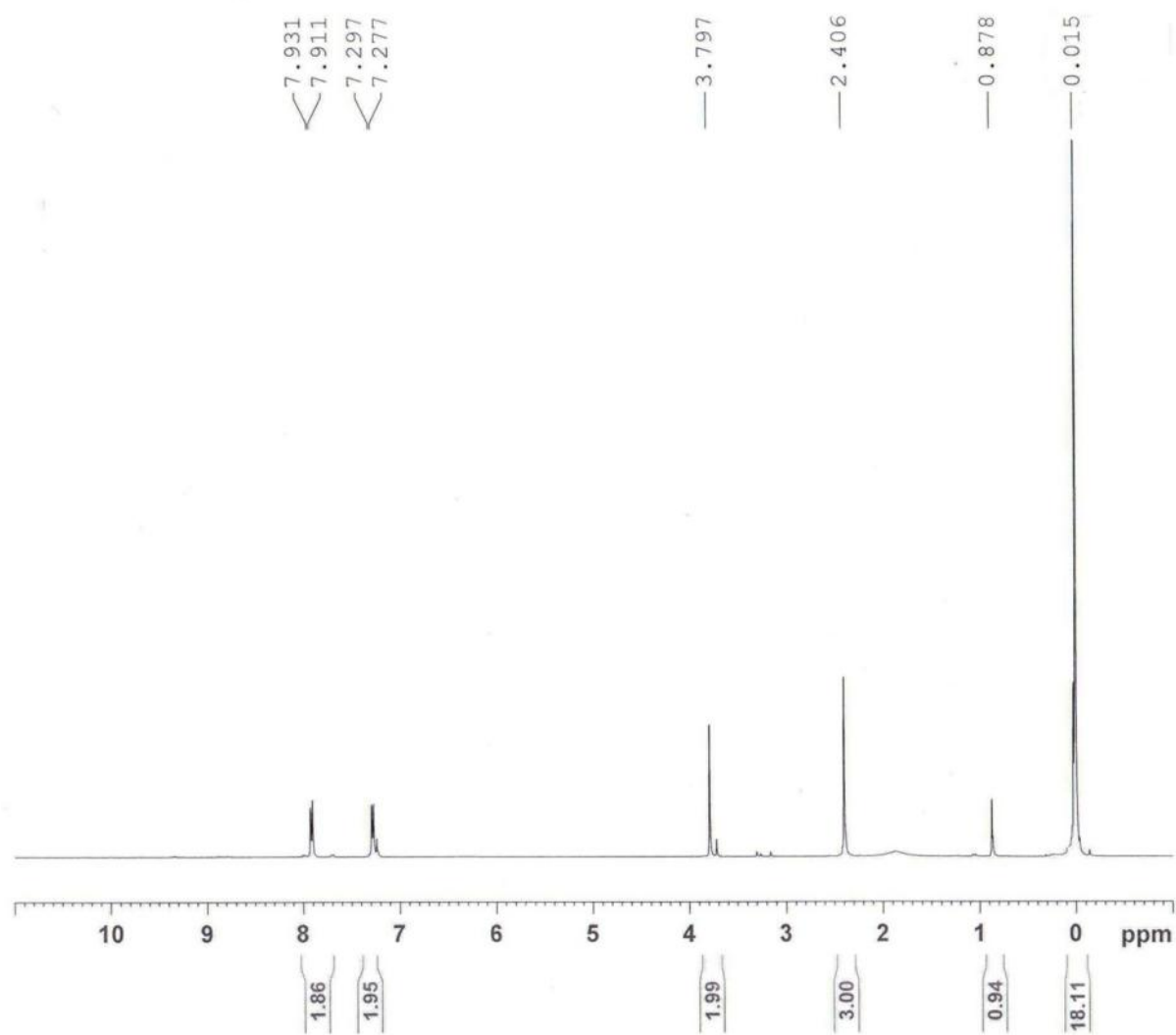
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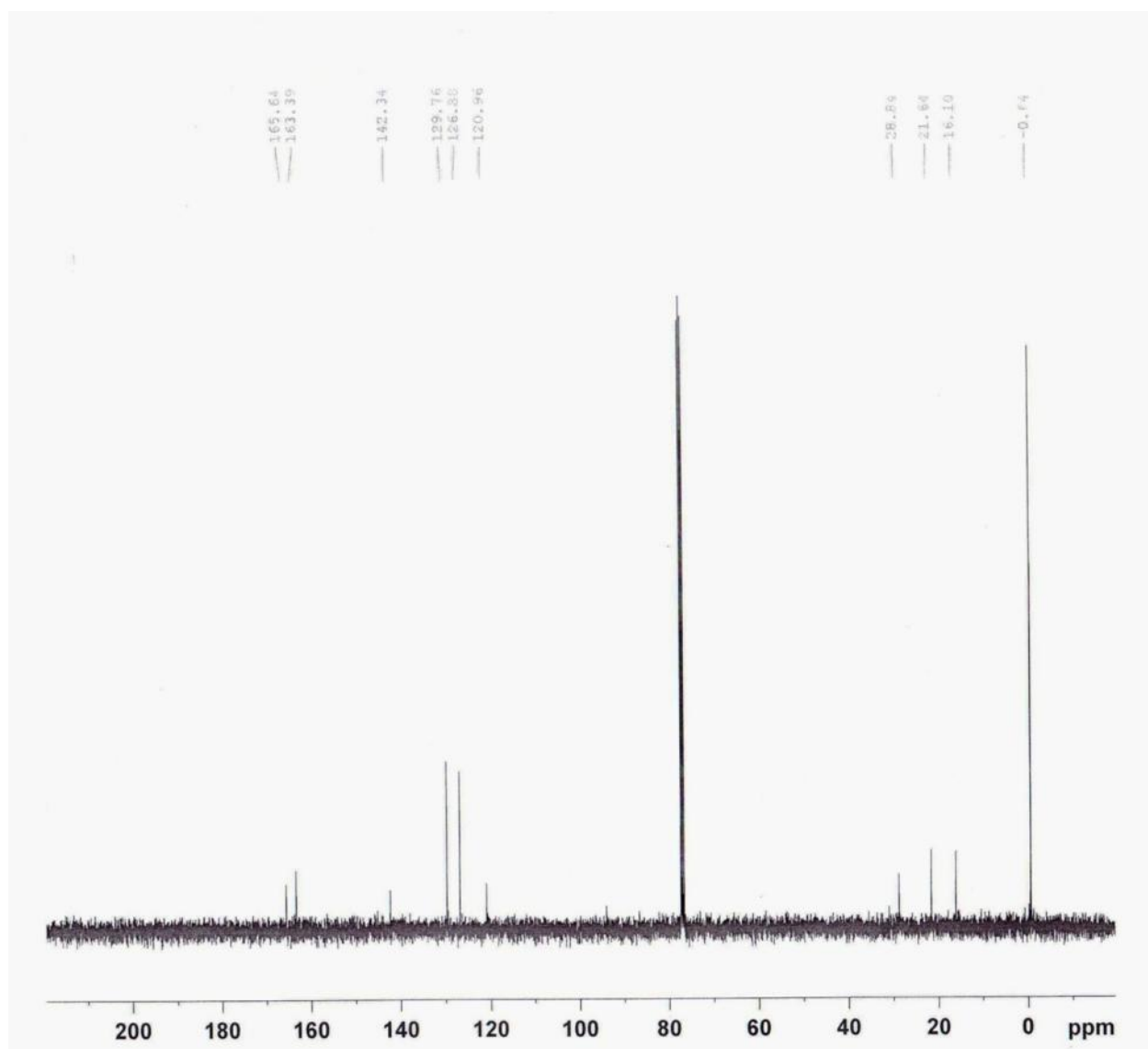
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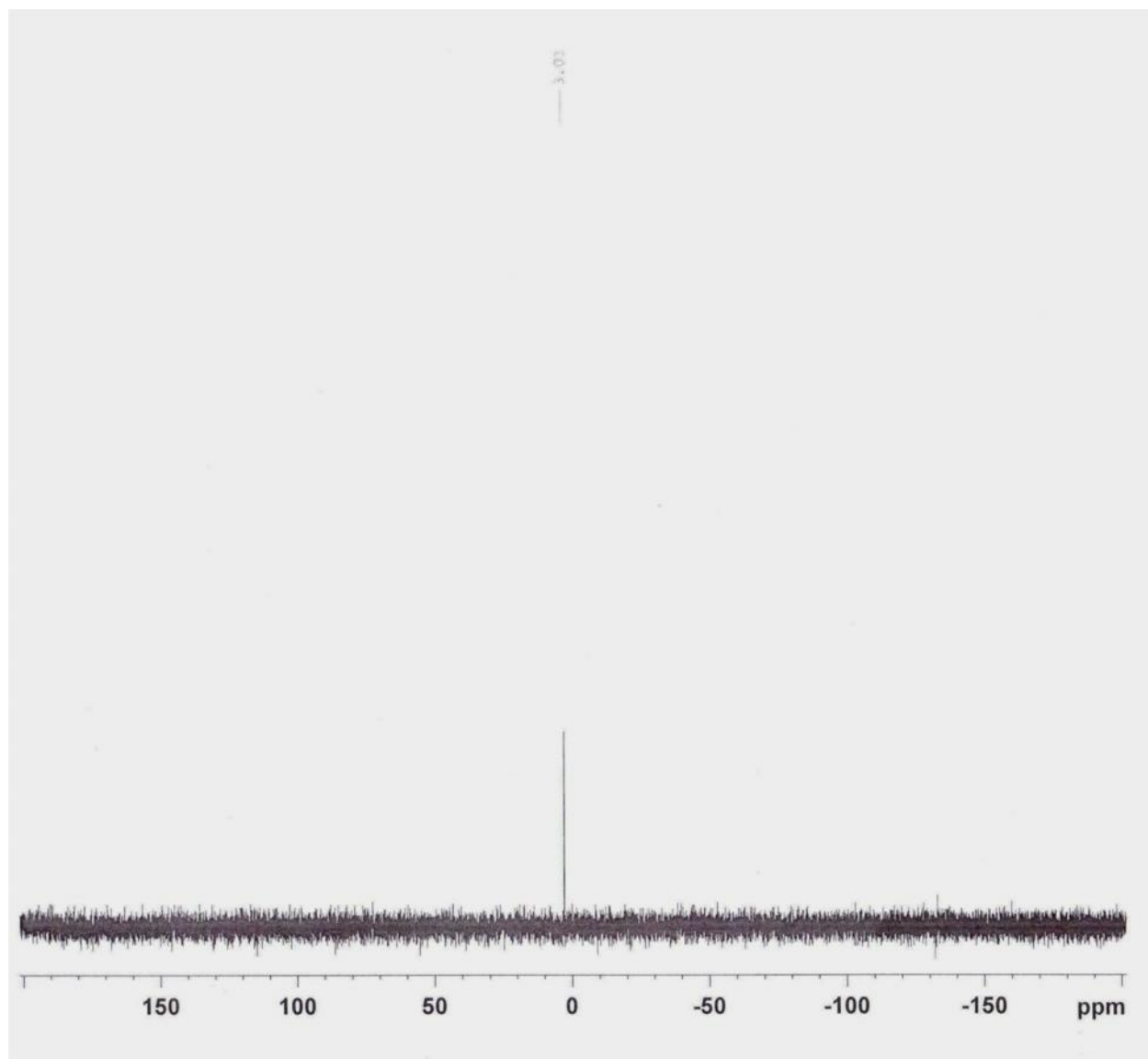
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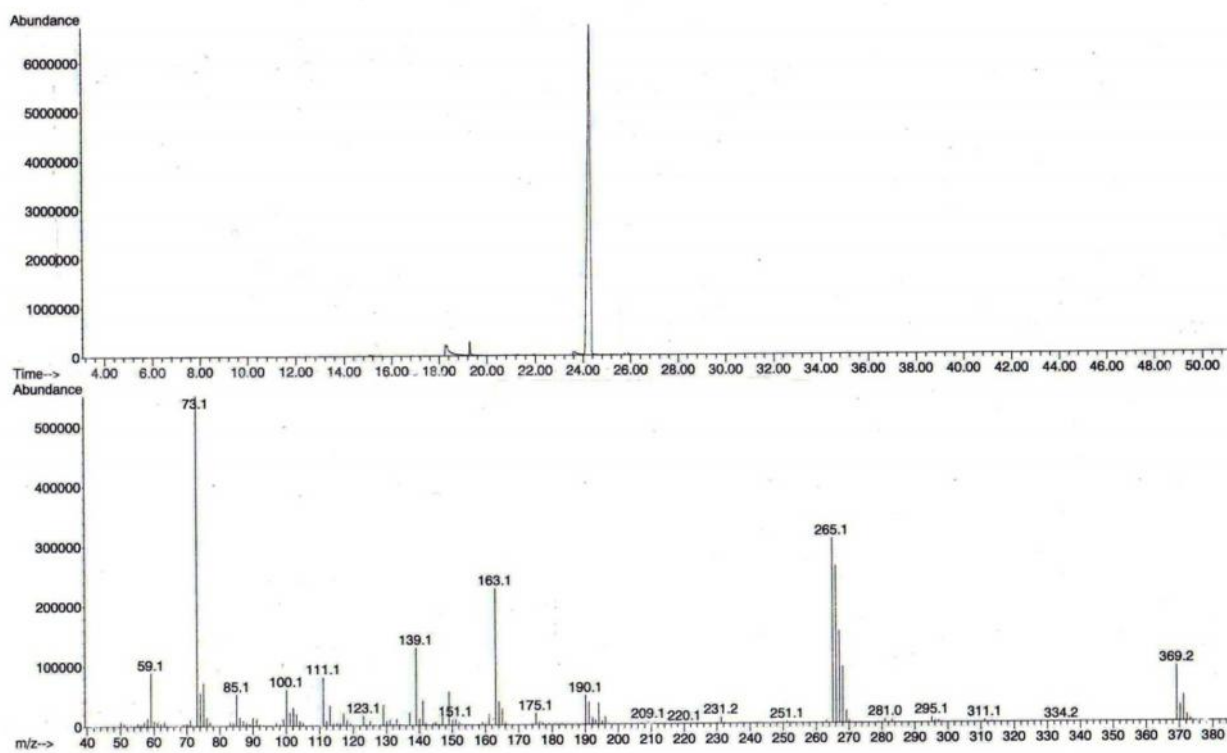
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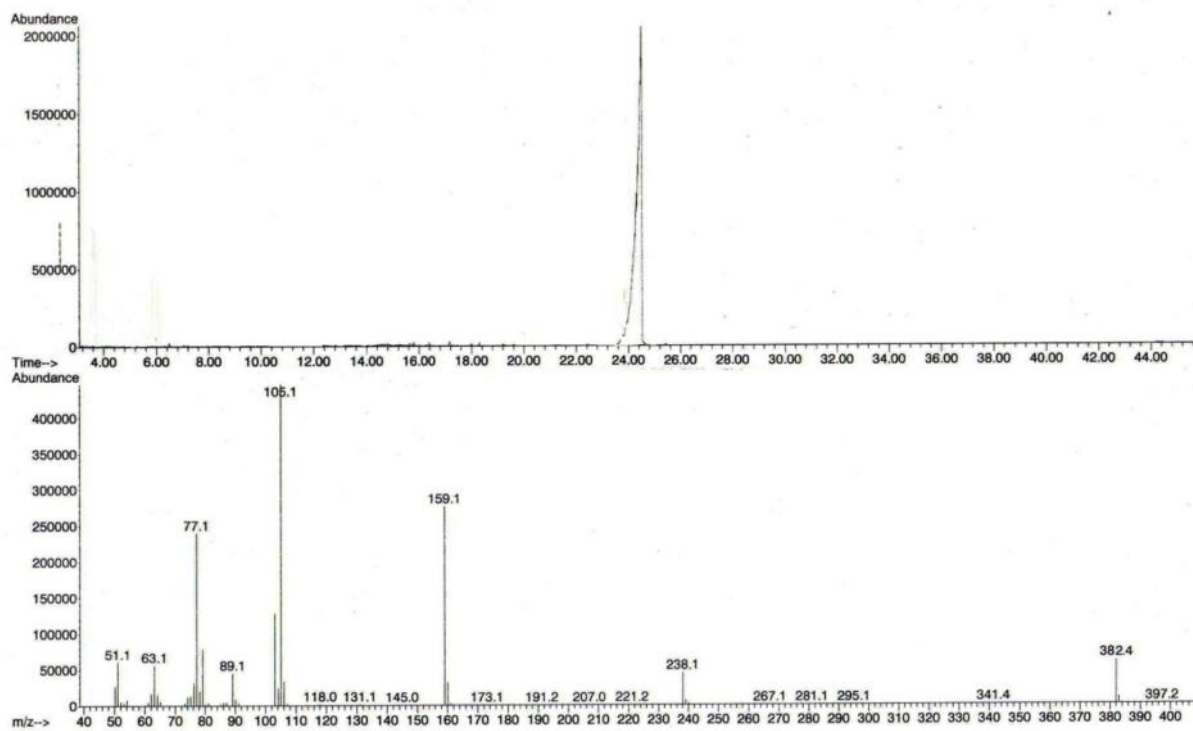
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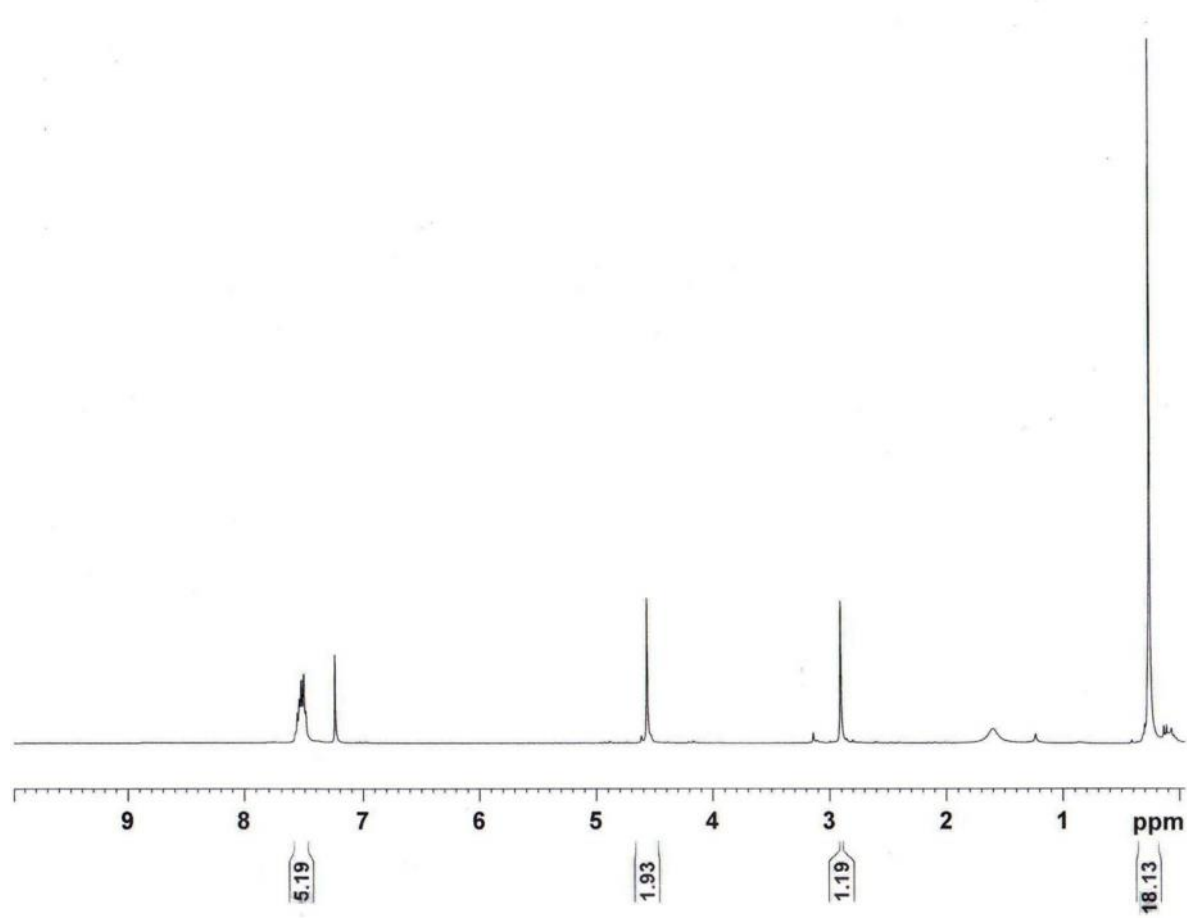
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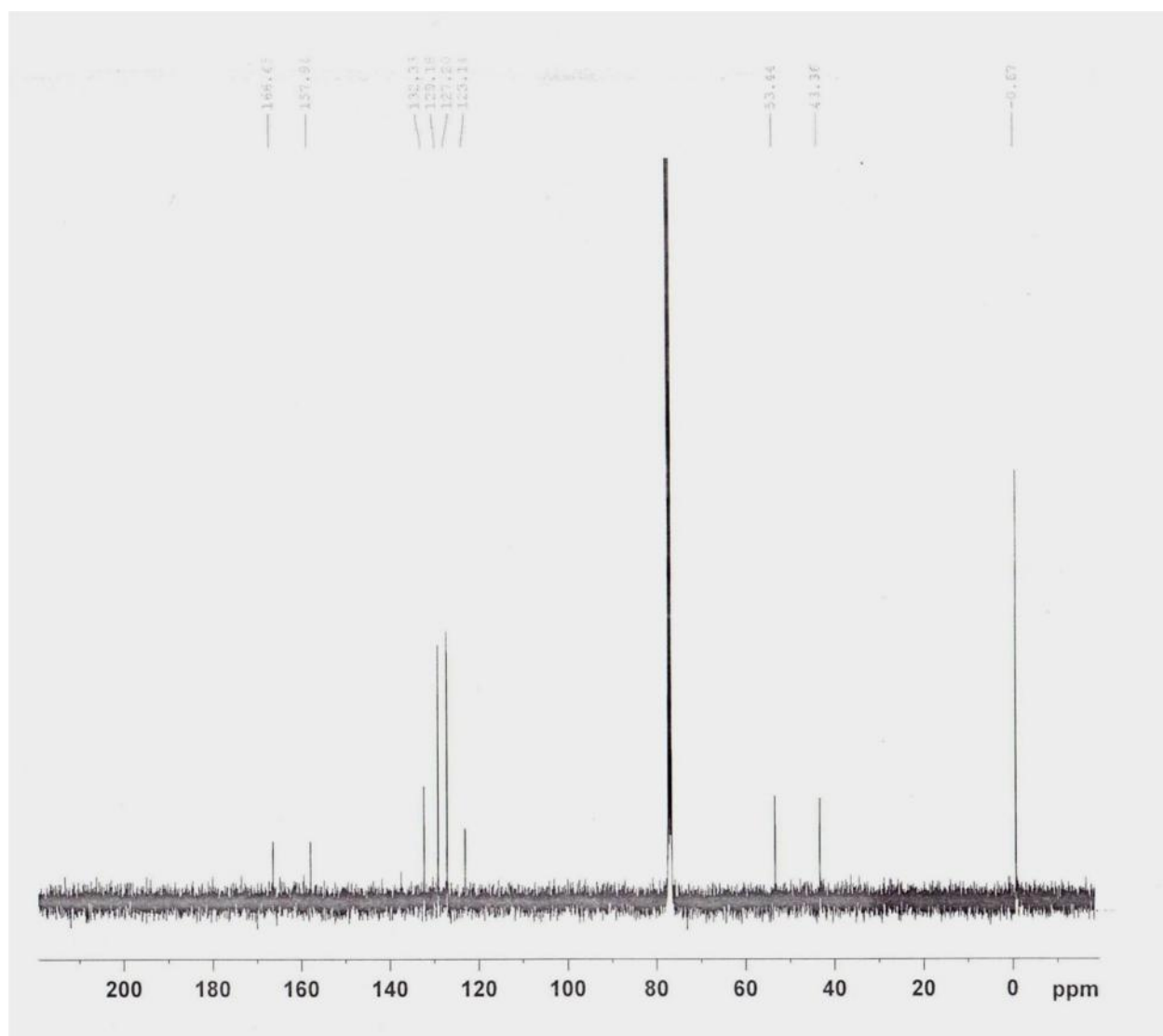
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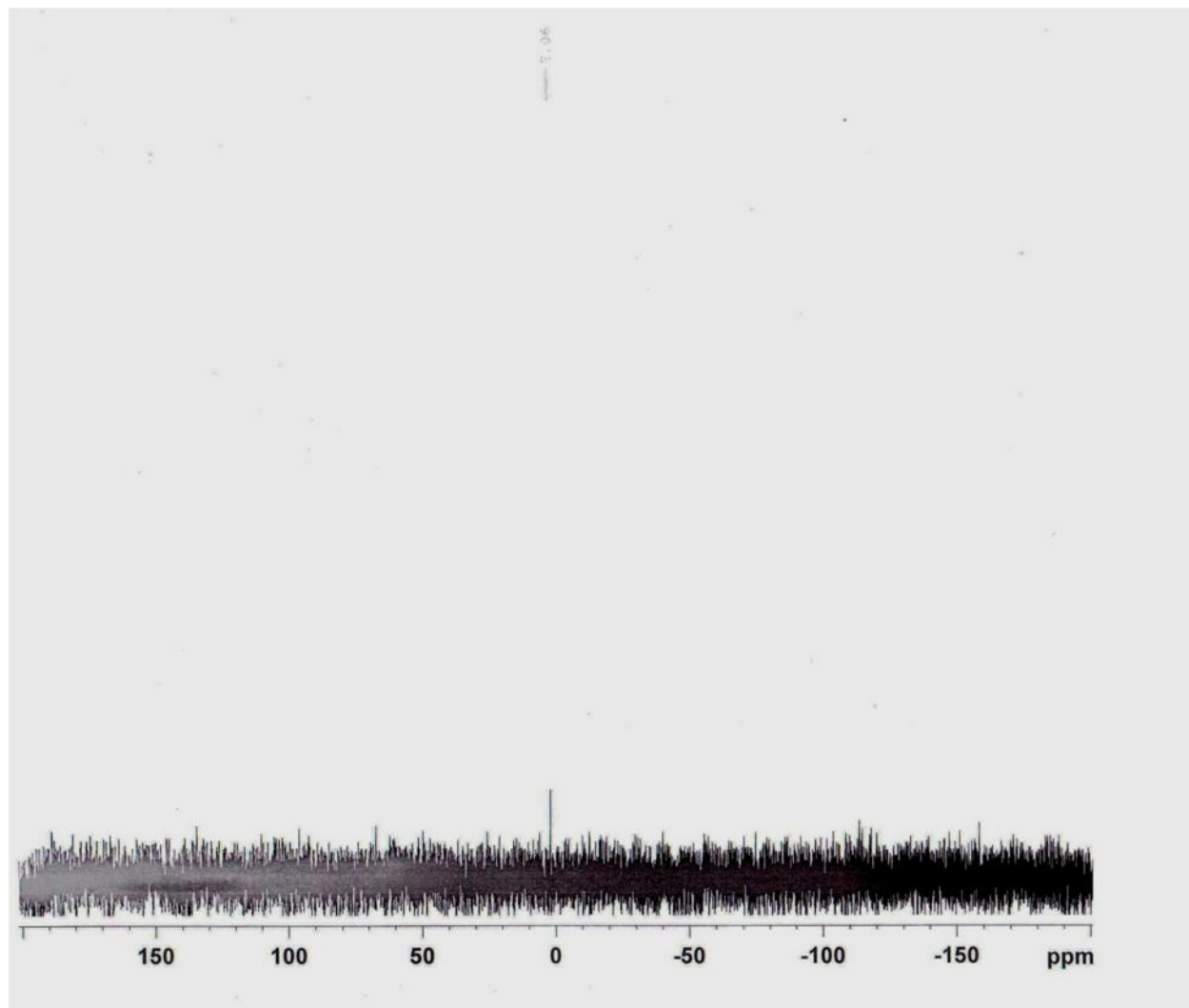
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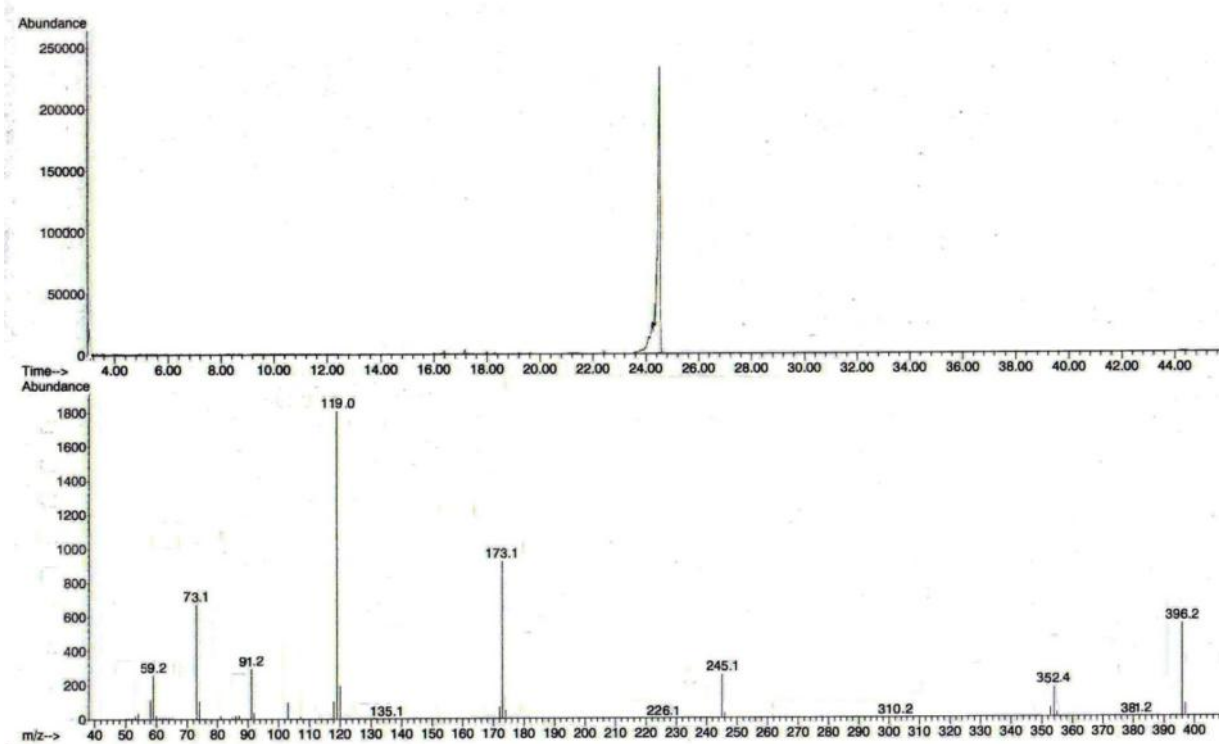
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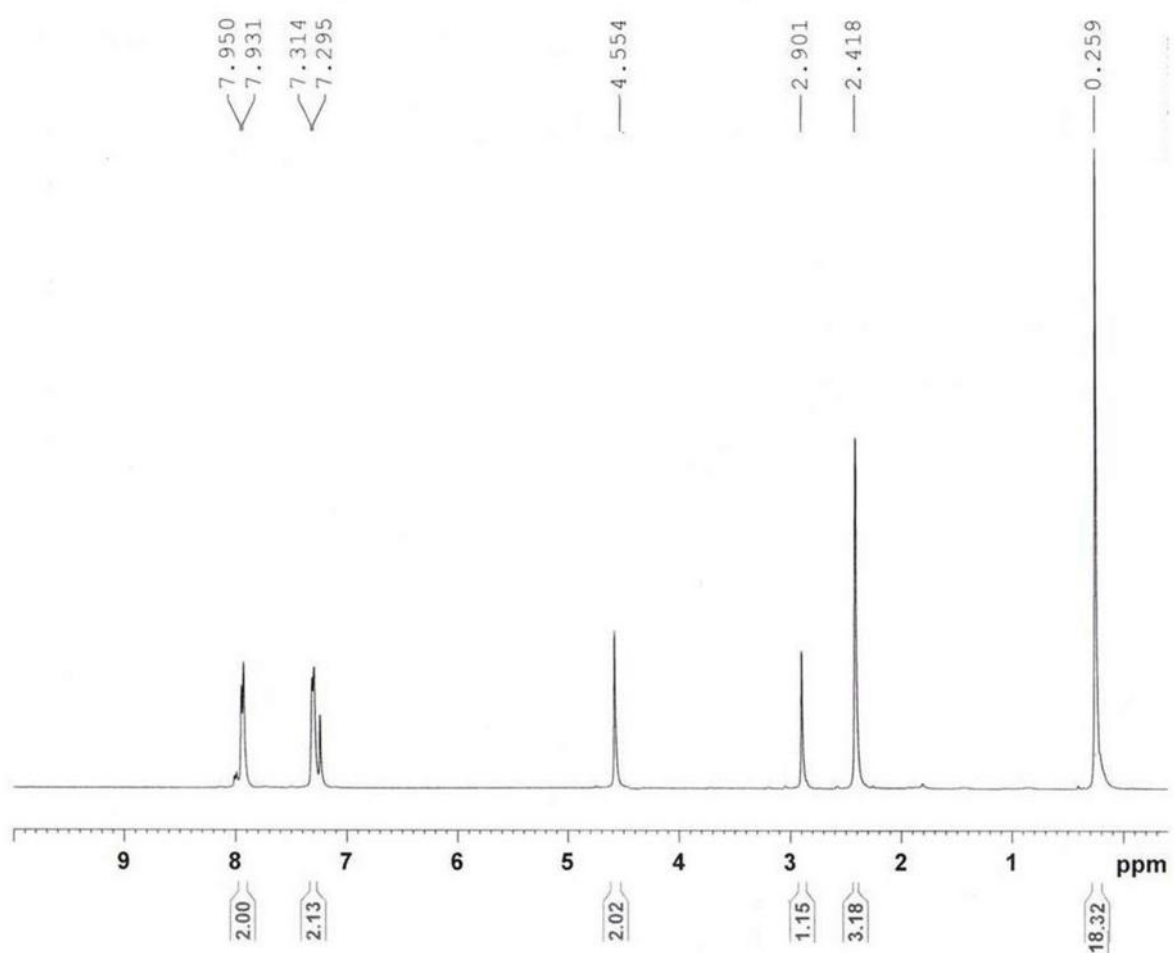
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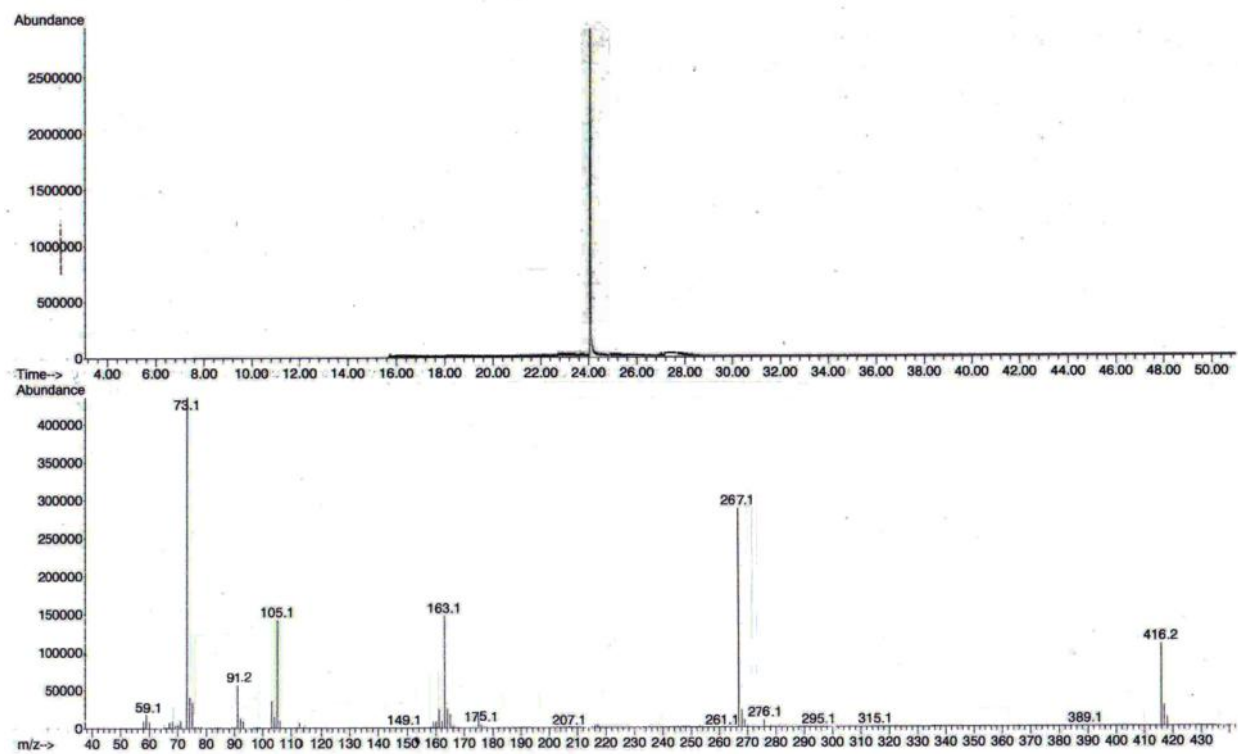
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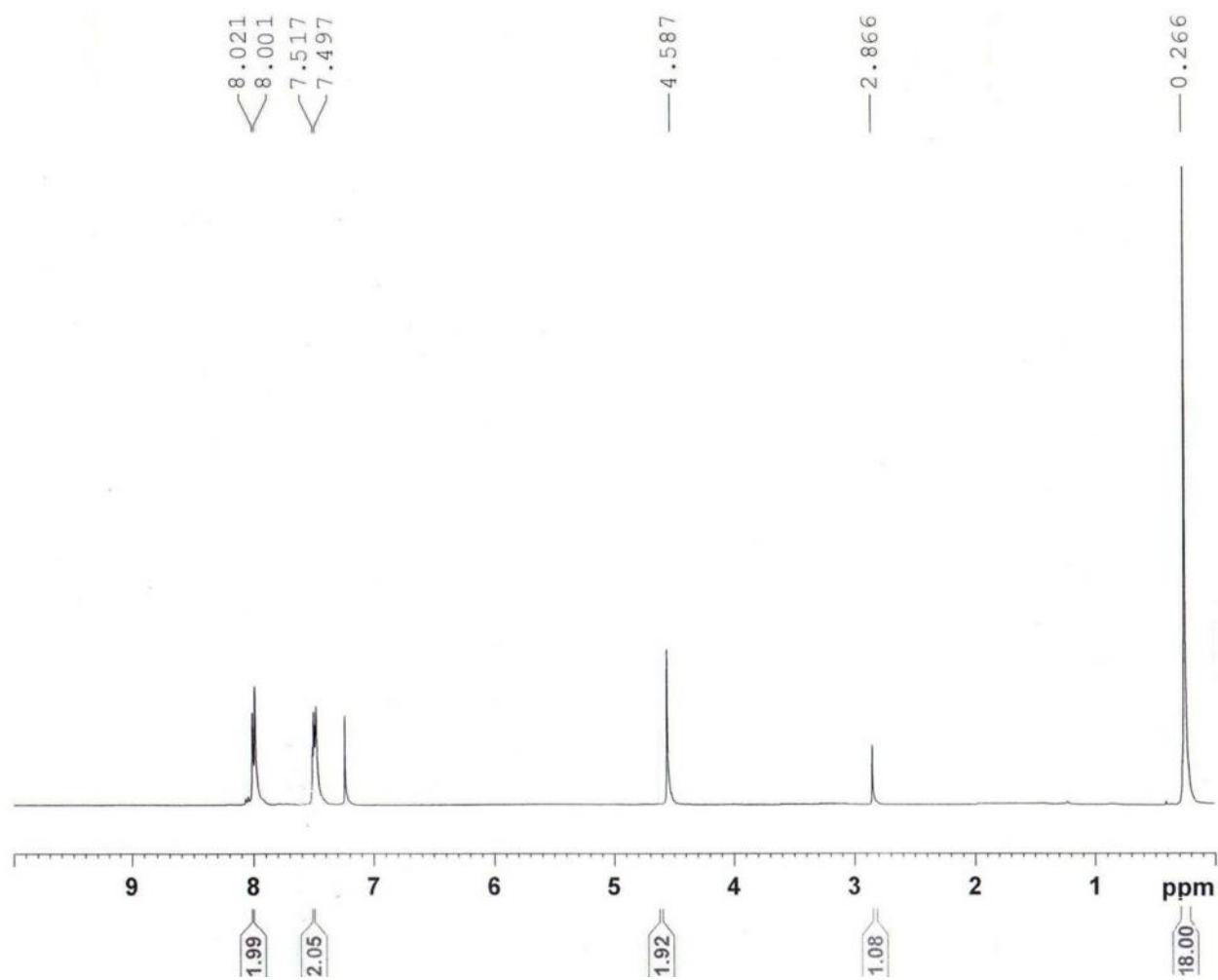
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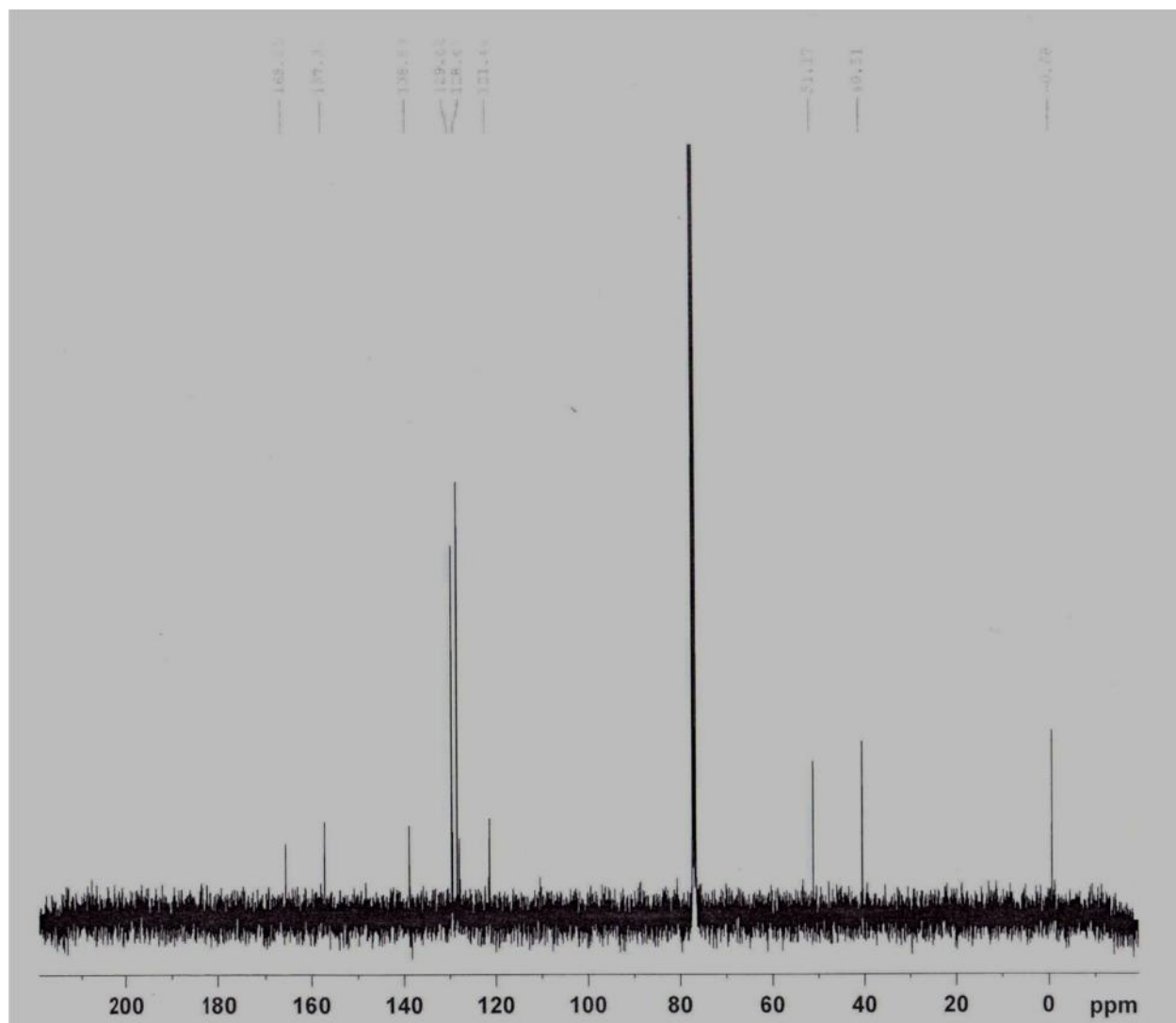
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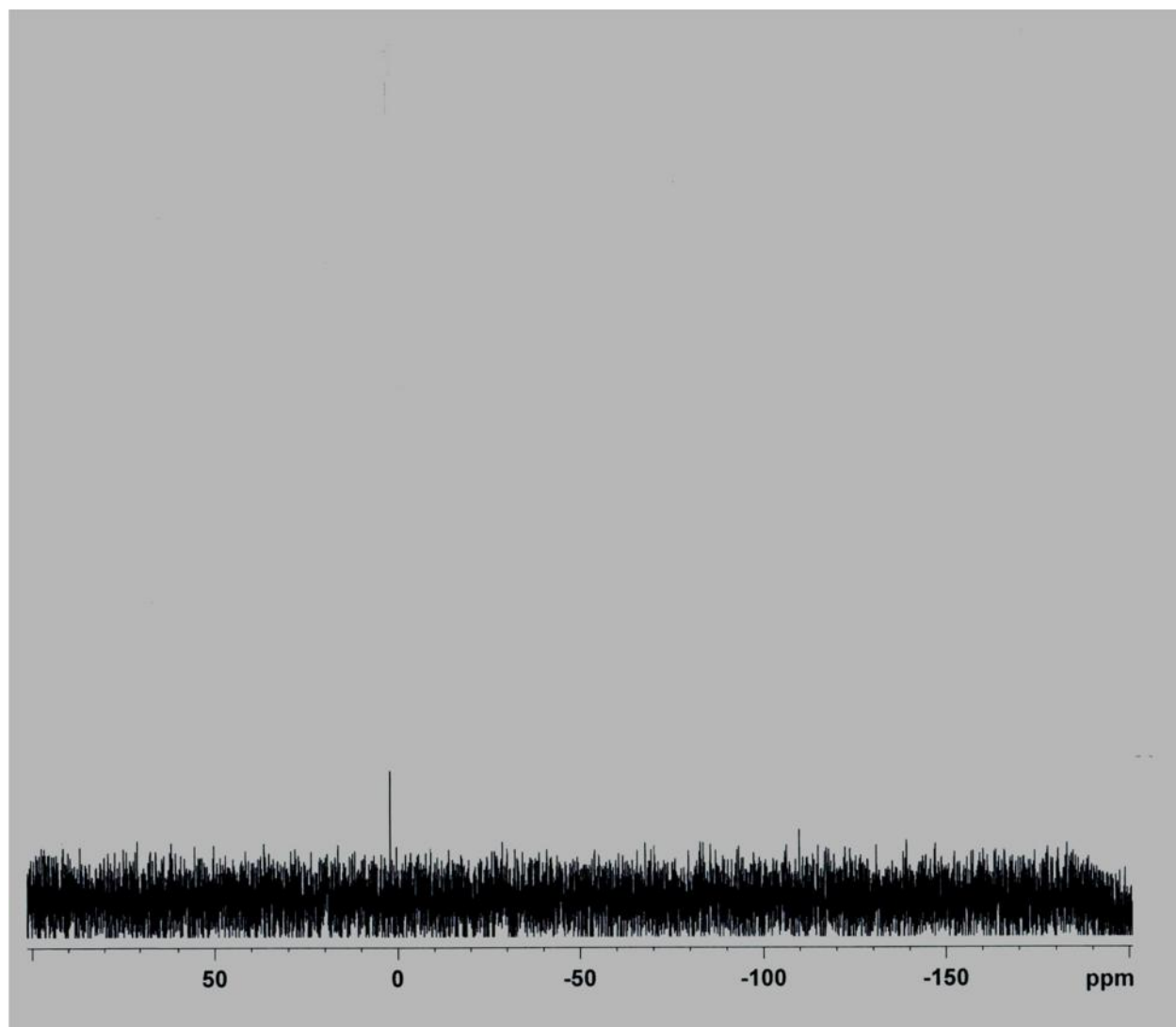
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^1H NMR of 5c



¹³C NMR of 5c



^{29}Si NMR of 5c