SUPPORTING INFORMATION

Synthesis, Antioxidant and Antitumoral Activities of 5-ArylChalcogeno-AminoThymidine (ACAT) derivatives

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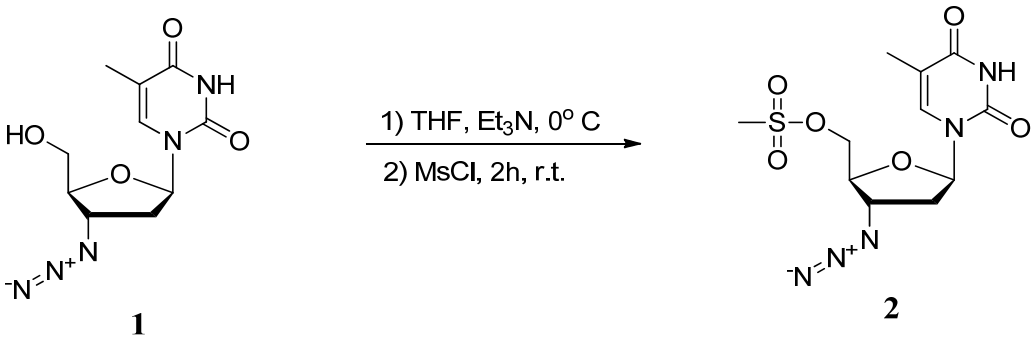
1. GENERAL METHODS

Chemistry: All reactions were run under an atmosphere of dry nitrogen or argon unless otherwise noted. DMSO, Thiobarbituric Acid (TBA), Malondialdehyde was obtained from Sigma (St. Louis, MO). All other chemicals were of analytical grade and obtained from standard commercial suppliers. The NMR spectra were recorded with a Bruker DPX-400 spectrometer with chemical shifts expressed in parts per million (in $CDCl_3$ and Me4Si as internal standard). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, brs = broad singlet, m = multiplet), coupling constants, and number of protons. High-resolution mass spectra (ESI) and High-resolution mass spectra (APPI) were obtained on a XEVO G2 Q-TOF spectrometer.

2. SYNTHETIC PROCEDURES

5'-*O*-(mesyl)zidovudine (2)[1]



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In the two-necked round-bottom flask under an argon atmosphere, 1 mmol zidovudine (1) was added in 7 mL THF. After the dissolution of zidovudine, the system was cooled to 0° C and triethylamine (1.5 mmol) added. After 10 min, while still at 0° C, dropwise mesyl chloride (1.1 mmol) diluted in 3 ml of THF was added. Then, the ice bath was removed and the system allowed to react for 2 h at room temperature. After this period, the reaction was extracted with a saturated solution of NH_4Cl (~ 20 mL) and the organic phase extracted with dichloromethane (3x 20 mL). Organic phases were combined and dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the product crystallized in ethyl acetate and dried in high vacuum pump. Mesylate (2) yield obtained was of 92%.

RMN ^{1}H (DMSO-d_6, 400 MHz), δ (ppm):11.28 (s, 1H), 7.47 (d, J = 1.1 Hz, 1H), 6.15 (t, J = 6.7 Hz, 1H), 4.47 – 4.41 (m, 2H), 4.10 – 4.04 (m, 1H), 3.23 (s, 3H), 2.53 – 2.43 (m, 3H), 1.80 (d, J = 0.8 Hz, 3H).

RMN ^{13}C (DMSO-d_6, 100 MHz), δ (ppm): 164.42, 151.18, 136.72, 110.81, 84.87, 81.34, 69.72, 60.86, 37.75, 36.37, 12.73. Physical state: White solid. Yield: 92%

[1] D. Souza, D. O. C. Mariano, F. Nedel, E. Schultze, V. F. Campos, F. Seixas, R. S Silva, T. S. Munchen, V. Ilha, L. Dornelles, A. L.Braga, J. B. T. Rocha, T.Collares, O. E. D. Rodrigues *J. Med. Chem*. 2015, **58**, 3329.

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Representative Procedure for the Preparation of chalcogeno-aminothymidines 3a-m.

In a two-necked round-bottom flask under argon atmosphere, the following items were added: diaryl dichalcogenide (0.5 mmol), THF (4 mL) and ethanol (3 mL). Afterwards, $NaBH_4$ (5,0 eq., 5mmol, 0,185g,) was added and the reaction was stirred until the disappearance of the color. Subsequently, the mesylate 2 (1 mmol) dissolved in THF (3 mL) was added dropwise to the reaction flask. The system was heated at reflux for 24 h. After completion of the reaction, the mixture was quenched with a saturated solution of NH_4Cl and extracted with ethyl acetate. The crude product was purified by chromatographic column employing a gradient of a mixture of dichloromethane and ethanol (90:5, 90:10 and 70:30) as solvent. Compounds **3a-3m** were obtained with yields ranging from 20 to 82%.

5'-Se-(phenyl)-3-(amino)-thymidine (3a)

RMN ^1H ($CDCl_3$, 400 MHz), δ (ppm): 7.57 – 7.46 (m, 2H), 7.34 (d, J = 1.2 Hz, 1H), 7.29 – 7.18 (m, 3H), 6.16 (dd, J^1 = 7.2, J^2 = 5.6 Hz, 1H), 3.93 – 3.84 (m, 1H), 3.55 – 3.48 (m, 1H), 3.29 – 3.24 (m, 2H), 2.31 – 2.13 (m, 2H), 1.85 (d, J = 1.2 Hz, 3H).

RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 163.7, 150.3, 135.5, 132.3, 129.9, 129.2, 127.2, 110.9, 85.7, 84.4, 54.9, 41.4, 30.2, 12.3.

HRMS calcd for $C_{16}H_{19}N_3O_3Se$ $[M+H]^+$: 382.0670. Found: $[M+H]^+$: 382.0688. Melting Point: 132-134°C. Physical state: light yellow solid. Yield: 78%

5'-Se-(2-methyl- phenyl) -3-(amino)-thymidine (3b)

RMN ^1H ($CDCl_3$, 400 MHz), δ (ppm): 7,35 (d, J = 1.2 Hz, 1H), 7.21 – 7.15 (m, 2H), 7.14 – 7.08 (m, 2H), 6.18 (dd, J^1 = 6.4, J^2 = 5.6 Hz, 1H), 3.95 – 3.90 (m, 1H), 3.58 – 3.51 (m, 1H), 3.27 – 3.23 (m, 2H), 2.43 (s, 3H), 2.33 – 2.17 (m, 2H), 1.84 (d, J = 1.2 Hz, 3H). RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 163.9, 150.4, 135.7, 135.5, 135.1, 129.1, 129.0, 126.4, 115.0, 110.8, 84.9, 84.4, 53.9, 41.0, 36.3, 31.3, 12.3.

HRMS calcd for $C_{17}H_{21}N3O_3Se$ $[M+H]^+$: 396.0826. Found: $[M+H]^+$: 396.0826. Melting Point: 82-85°C. Physical state: dark orange solid. Yield: 70%

5'-Se-(4-methoxy-phenyl) -3-(amino)-thymidine (3c)

RMN ^1H ($CDCl_3$, 400 MHz), δ (ppm): 7.52 – 7.44 (m, 2H), 7.37 (d, J = 1.2 Hz, 1H), 6.85 – 6.77 (m, 2H), 6.17 (dd, J^1 = 6.8, J^2 = 5.6 Hz, 1H), 3.90 – 3.82 (m, 1H), 3.78 (s, 3H), 3.55 – 3.44 (m, 1H), 3.17 (d, J = 5.6 Hz, 2H), 2.31 – 2.14 (m, 2H), 1.87 (d, J = 1.2 Hz, 3H). RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 164.1, 159.3, 150.4, 135.5, 135.0, 119.4, 114.9, 110.7, 85.6, 84.1, 55.1, 54.6, 41.1. 31.2, 12.4.

HRMS calcd for $C_{17}H_{21}N_3O_4Se$ $[M+H]^+$: 412.0775. Found: $[M+H]^+$: 412.0765. Melting Point: 124-127°C. Physical state: beige solid. Yield: 72%

5'-Se-(4-chloro- phenyl) -3-(amino)-thymidine (3d)

RMN ^1H ($CDCl_3$, 400 MHz), δ (ppm): 7.51 – 7.43 (m, 2H), 7.30 (d, J = 1.2 Hz, 1H), 7.26 – 7.20 (m, 2H), 6.17 (dd, J^1= 6.8, J^2 = 5.2 Hz, 1H), 3.90 – 3.79 (m, 1H), 3.56 – 3.42 (m, 1H), 3.33 – 3.11 (m, 2H), 2.33 – 2.12 (m, 2H), 1.87 (d, J = 1.2 Hz, 3H). RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 163.5, 150.3, 135.5, 133.7, 133.6, 129.4, 128.0, 110.9, 85.5, 84.3, 54.9, 41.5, 30.5, 12.5.

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HRMS calcd for $C_{16}H_{18}ClN_3O_3Se$ $[M+H]^+$: 416.0280. Found: $[M+H]^+$: 416.0273. Melting Point: 144-146°C. Physical state: beige solid. Yield: 68%.

5'-Se-(4-methyl- phenyl) -3-(amino)-thymidine (3e)

RMN ^1H ($CDCl_3$, 400 MHz), δ (ppm): 7.46 – 7.41 (m, 2H), 7.37 (d, J = 1.2 Hz, 1H), 7.12 – 7.02 (m, 2H), 6.17 (dd, J^1 = 6.8, J^2 = 5.2 Hz, 1H), 3.90 – 3.79 (m, 1H), 3.56 – 3.45 (m, 1H), 3.28 – 3.11 (m, 2H), 2.31 (s, 3H), 2.28 – 2.15 (m, 2H), 1.87 (d, J = 0.8 Hz, 3H). RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 163.8, 150.3, 137.4, 135.6, 132.81, 130.1, 125.9, 110.9, 85.7, 84.3, 54.8, 41.4, 30.5, 21.0, 12.4.

HRMS calcd for $C_{17}H_{21}N_3O_3Se$ $[M+H]^+$: 396.0826. Found: $[M+H]^+$: 396.0826. Melting Point: 146-149°C. Physical state: light yellow solid. Yield: 40%

5'-Se-(2-chloro- phenyl) -3-(amino)-thymidine (3f)

RMN ^1H ($CDCl_3$, 400 MHz), δ (ppm): 7.51 – 7.48 (m, 1H), 7.37 – 7.33 (m, 1H), 7.31 (d, J = 1.2 Hz, 1H), 7.19 – 7.13 (m, 2H), 6.20 (dd, J^1 = 7.2, J^2 = 5.6 Hz, 1H), 3.98 – 3.92 (m, 1H), 3.61 – 3.52 (m, 1H), 3.39 – 3.28 (m, 2H), 2.34 – 2.18 (m, 2H), 1.82 (d, J = 1.2 Hz, 3H). RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 163.9, 150.5, 135.6, 134.91, 131.2, 130.5, 129.5, 127.7, 127.3, 110.9, 85.0, 84.1, 54.7, 41.13, 28.7, 12.3.

HRMS calcd for $C_{16}H_{18}ClN_3O_3Se$ $[M+H]^+$: 416,0280. Found: $[M+H]^+$: 416,0273. Melting Point: 92-95°C. Physical state: orange dark solid. Yield: 62%

(5l) 5'-Se-(4-fluor- phenyl) -3-(amino)-thymidine (3g)

RMN ^1H ($CDCl_3$, 400 MHz), δ (ppm): 7.56 – 7.50 (m, 2H), 7.32 (d, J = 1.2 Hz, 1H), 7.00 – 6.94 (m, 2H), 6.18 (dd, J^1 = 6.8, J^2 = 5.2 Hz, 1H), 3.92 – 3.83 (m, 1H), 3.57 – 3.46 (m, 1H), 3.29 – 3.17 (m, 2H), 2.31 – 2.20 (m, 2H), 1.87 (d, J^1 = 1.2 Hz, 3H). RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 163.8, 150.4, 135.5, 135.0, 134.9, 116.6, 116.4, 111.0, 85.4, 84.4, 54.9, 41.3, 31.1, 12.5.

HRMS calcd for $C_{16}H_{19}FN_3O_3Se$ $[M+H]^+$: 400,0576. Found: $[M+H]^+$: 400,0573. Melting Point: 94-97°C. Physical state: dark orange solid. Yield: 62%

5'-Se-(naphtyl) -3-(amino)-thymidine (3h)

RMN ^1H ($CDCl_3$, 400 MHz), δ (ppm): 8.39 – 8.32 (m, 1H), 7.89 – 7.75 (m, 3H), 7.61 – 7.44 (m, 2H), 7.40 – 7.32 (m, 1H), 7.30 (d, J = 1.2 Hz, 1H), 6.15 (dd, J^1 = 6.4, J^2 = 5.6 Hz, 1H), 3.95 – 3.82 (m, 1H), 3.57 – 3.46 (m, 1H), 3.34 – 3.17 (m, 2H), 2.28 – 2.14 (m, 2H), 1.80 (d, J = 1.2 Hz, 2H). RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 164.0, 150.4, 135.4, 133.8, 133.7, 131.8, 128.8, 128.5, 128.3, 126.88, 126.6, 126.1, 125.6, 110.7, 85.4, 84.1, 54.7, 40.7, 30.2, 12.2.

HRMS calcd for $C_{20}H_{21}N_3O_3Se$ $[M+H]^+$: 432,0826. Found: $[M+H]^+$: 432,0836. Melting Point: 27-30°C. Physical state: beige solid. Yield: 42%

5'-S-(phenyl) -3-(amino)-thymidine (3i)

RMN ^1H ($CDCl_3$, 400 MHz), δ (ppm): 7.42 – 7.38 (m, 2H), 7.34 (d, J = 0.8 Hz, 1H), 7.32 – 7.28 (m, 2H), 7.23 – 7.17 (m, 1H), 6.20 (dd, J^1 = 6.8, J^2 = 5.2 Hz, 1H), 3.96 – 3.80 (m, 1H), 3.65 – 3.50 (m, 1H), 3.33 (d, J = 5.0 Hz, 2H), 2.33 – 2.16 (m, 2H), 1.82 (d, J = 0.8 Hz, 3H). RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 163.9, 150.4, 135.6, 135.5, 129.1, 128.9, 126.4, 110.9, 84.9, 84.2, 53.9, 41.2, 36.1, 12.5.

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HRMS calcd for $C_{16}H_{19}N_3O_3S$ [M+H] +: 334.1225. Found: [M + H] +: 334.1240. Melting Point: 140-143°C. Physical state: light orange solid. Yield: 82%

5'-Te-(phenyl) -3-(amino)-thymidine (3j)

RMN ^{1}H ($CDCl_3$, 400 MHz), δ (ppm): 7.78 – 7.73 (m, 2H), 7.45 (s, 1H), 7.31 – 7.16 (m, 3H), 6.20 – 6.14 (m, 1H), 3.93 – 3.85 (m, 1H), 3.45 – 3.22 (m, 3H), 2.38 – 2.18 (m, 2H), 1.87 (d, *J* = 2.1 Hz, 3H). RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 164.3, 150.3, 137.4, 135.9, 128.8, 127.3, 111.2, 110.3, 85.5, 83.5, 55.4, 40.0, 11.0, 10.9.

Melting Point: 199°C. HRMS calcd for $C_{16}H_{19}N_3O_3Te$ $[M+H]^+$: 432.0567. Found: $[M+H]^+$: 432.0572. Physical state: beige solid. Yield: 48%

5'-S-(4-chloro-phenyl) -3-(amino)-thymidine (3k)

RMN ^{1}H ($CDCl_3$, 400 MHz), δ (ppm): 7.35 – 7.30 (m, 2H), 7.27 (m, 1H), 7.26 – 7.22 (m, 2H), 6.17 (dd, J^1 = 6.8, J^2 = 5.2 Hz, 1H), 4.28 (sl, 2H), 3.95 – 3.79 (m, 1H), 3.63 – 3.44 (m, 1H), 3.39 – 3.17 (m, 2H), 2.37 – 2.06 (m, 2H), 1.83 (d, *J* = 1.2 Hz, 3H). RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 163.9, 150.4, 135.5, 134.3, 132.4, 130.4, 129.1, 110.9, 84.8, 84.4, 54.0, 41.0, 36.5, 12.3.

HRMS calcd for $C_{16}H_{18}ClN_3O_3S$ $[M+H]^+$: 368.0835. Found: $[M+H]^+$: 368.0828. Melting Point: 133-136°C. Physical state: light yellow solid. Yield: 20%

5'-S-(4-methoxi-phenyl) -3-(amino)-thymidine (3l)

RMN ^{1}H ($CDCl_3$, 400 MHz), δ (ppm): 7.42 – 7.38 (m, 2H), 7.37 (d, *J* = 0.8 Hz, 1H), 6.88 – 6.82 (m, 2H), 6.16 (dd, *J1*= 6.4, *J2* = 5.2 Hz, 1H), 3.84 – 3.77 (m, 4H), 3.59 – 3.49 (m, 1H), 3.23 – 3.15 (m, 2H), 2.30 – 2.12 (m, 2H), 1.88 (d, *J* = 1.2 Hz, 3H). RMN ^{13}C ($CDCl_3$, 100 MHz), δ (ppm): 164.1, 158.8, 150.4, 135.4, 132.5, 125.7, 114.6, 110.5, 84.2, 55.1, 53.9, 40.7, 38.2, 12.2.

Melting Point: 133-136°C. HRMS calcd for $C_{17}H_{21}N_3O_4S$ $[M+H]^+$: 364.1331. Found: $[M+H]^+$: 364.1349. Physical state: light rose solid. Yield: 60%

5'-S-(4-methyl-phenyl) -3-(amino)-thymidine (3m)

RMN ^{1}H ($CDCl_3$, 400 MHz), δ (ppm): 7.36 (s, 1H), 7.33 – 7.28 (m, 2H), 7.13 – 7.07 (m, 2H), 6.17 (t, *J* = 6.0 Hz, 1H), 3.91 – 3.82 (m, 1H), 3.63 – 3.50 (m, 1H), 3.33 – 3.21 (m, 2H), 2.31 (s, 3H), 2.29 – 2.19 (m, 2H), 1.85 (s, 3H). RMN ^{13}C (DMSO-d_6, 100 MHz), δ (ppm): 163.5, 150.2, 135.9, 135.2, 132.4, 129.5, 128.66, 109.4, 84.7, 83.3, 54.2, 35.8, 20.3, 11.9.

HRMS calcd for $C_{17}H_{21}N_3O_3S$ $[M+H]^+$: 348.1382. Found: [M + H] $^+$: 348.1376. Melting Point: 150-152°C. Physical state: Dark orange solid. Yield: 47%

3 ANTITUMORAL STUDIES

3.1 Preparation of use solutions.

Compounds were previously diluted in DMSO and added in solutions with medium supplemented with 10% fetal bovine serum (FBS) (DMSO concentration in solutions was up to 0.5%). Un-treated cells were used as negative controls and DMSO 0.5% was used as vehicle control.

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3.2 Cell culture and proliferation assay.

Human Bladder Carcinoma (5637 cell line) was purchased from ATCC, maintained in DMEM, supplemented with 10% FBS and 1× antibiotic–antimycotic (Gentamicin sulfate 50 mg/L; Amphotericin B 2 mg/L), and incubated at 37 °C with 5% CO2. Cell viability assay was performed by seeding 2 x 104 cells per well in a volume of 100 µL in 96-well plates. Cells were treated with the relevant drug for 24 and 48 h, then incubated with 20 µL/well (5 mg/mL) of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide) for 3 h at 37°C. Differences in total cellular metabolism were detected at a wavelength of 492 nm using a microplater reader. Inhibition (%) of cell proliferation was determined as follows: inhibitory growth = (1- Abs492treated cells/Abs492controlcells) x 100. Cells in logarithmic growth phase were used in all experiments.

3.3 Data analysis.

Data analysis and graphics were performed in Graphpad Prism 6.0 software. Data sets from MTT were analyzed using two-way ANOVA followed by Tukey's test for multiple comparisons. Different concentrations and times used during treatments were the factors considered. Significance of $p < 0.05$ was considered in all analyzes. Data are presented as mean ± SEM.

3.4 Cell cycle assay

The evaluation of DNA content to cell cycle analysis of cell strain 5637 was based on the use of fluorescent DNA intercalator, propidium iodide (PI), enters the cell after permeabilization. The detection was performed in Guava® Flow Cytometry easyCyte™ System plus flow cytometer. Briefly, $1x10^5$ (100 µL) cells were seeded in 12-well plates after 24 h serum-free medium was added to cells were synchronized for later addition of the treatments. The administered treatment was chosen based on the MTT assay. Cell cycle evaluation according to the manufacturer's protocol was performed by propidium iodide staining using Guava® Cell Cycle (Merck KGaA) reagent kit, after 24 h treatment with the compound **3j** in concentration 12,5 µM.

4. ANTIOXIDANT STUDIES

4.1 Thiobarbituric acid reactive substances (TBARS)

For this analysis, the method used was proposed by Ohkawa, Ohishi and Yagi (1979). Phosphatidylcholine (0.4 mg) was incubated for 30 min at 37 °C with iron sulfate (55 µM), Tris-HCl buffer pH 7.4 (1.85 mM) to induce lipid peroxidation. Compounds derived from azidothymidine were tested with the aim of inhibiting lipid peroxidation at final concentrations of 0-200 µM. The vehicle was DMSO. Incubation with phosphatidylcholine, buffer and distilled water was used as a blank. Subsequently, acetic acid buffer pH 3.4 and thiobarbituric acid (0.22%) were added. Samples were then incubated for one hour at 100 °C. Finally, samples were cooled and 400 µL of N-butanol was added. Then, the tubes were stirred for 30 s and centrifuged for 10 min at 6000 rpm. The supernatant was read at 532 nm. MDA was used as standard. Diphenyl diselenide (0-400 µM) and α-tocopherol (0-200 µM) were used as positive control.

4.2 DPPH radical scavenging assay

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The method proposed by Pereira et al (2014) was used. One milimolar of azidothymidine derivative compounds was mixed with 0.3 mM DPPH in ethanol. For the concentration curve, compound **3j** was used at the final concentrations 0 – 1 mM. Absorbance was read at 518 nm every 30 min for 180 min, and BHT was used as positive control.

4.3 Thiol peroxidase-like activity

This method determines whether the derivatives of azidothymidine derivative compounds can mimic enzyme glutathione peroxidase activity (GPx). For this analysis, the method proposed by Iwaoka and Tomoda (1994) with few modifications was used. Benzenethiol was used as an alternative to glutathione. The incubation system consisted of ethanol, benzenethiol (2.5 mM), DMSO (blank) or compounds (final concentrations of 0 - 450 μM) and hydrogen peroxide (2.3 mM). The reaction was monitored at 305 nm for 20 min.

4.4 Data analysis

Experiments were repeated three times. Data were analyzed using one or two-way ANOVA, followed by Newman-Keuls test or by Student's t Test when appropriate. Differences were considered significant when $p < 0.05$. Data analysis was performed with the software GraphPad v. 6 for Windows.

5 CELL TOXICITY

5.1 Cell viability

Sample preparation and cell viability were carried out as previously described in the literature (Bueno et al., 2013; Waczuk et al., 2015). Heparinized venous blood was obtained from healthy donors (ages 25–52) from The University Hospital of Santa Maria (HUSM), Santa Maria, RS, Brazil. The protocol used in this study was approved by the Ethical Committee of UFSM (n. 089.0.243.000-07). Isolated leukocytes (2 x 106 /mL-1) were then incubated for 3 h with DMSO (0.5%), 1 mM t-butyl hidroperoxide (positive control) or indicated compounds in a Hank's buffer solution containing 10% human plasma.

5.2 Data analysis.

Experiments were repeated three times. Data were analyzed using one-way ANOVA, followed by Newman-Keuls test. The difference was considered to be significant when $p < 0.0001$. Data analysis was performed with the software GraphPad v. 6 for Windows.

6 TOXICITY *IN VIVO*

All experiments are in accordance with Law 11.794, of October 8 2008, Decree 6899, of July 15, 2009, with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was approved by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 03/31/2016 (Protocol number ***CEUA 4622031115).***

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6.1 Treatments

The toxicity studies were conducted using adults Swiss male mice. Animals were randomized in two groups (n = 4 per group) and injected subcutaneously (s.c.) The control group received DMSO (1 mL/kg of body weight) and the second group received 100 μmol/kg of **3j** s.c..

6.2 Evaluation of toxic effects:

During 168 hours mice were monitored by a closed circuit tv. The consumption of food, water and the body weigh were record daily. After this period mice were euthanized by decapitation and liver, brain, spleen and kidneys and blood were collected.

6.3 Evaluation of kidney and liver function

The urea, creatinine, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were performed using commercial kits (Labtest Diagnostica SA, Lagoa Santa, Minas Gerais, Brazil).

6.4 Data analysis.

Experiments were conducted with four mice per group. Data were analyzed using Log-rank (Mantel-Cox) test for analysis of survival, unpaired t test for biochemistry analysis. The difference was considered to be significant when $p < 0.05$. Data analysis was performed with the software GraphPad v. 6 for Windows.

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7. NMR and HRMS Charts

Figure 1. ^{1}H and ^{13}C NMR of the compound **3a** in $CDCl_3$ at 400 MHz and 100 MHz respectively

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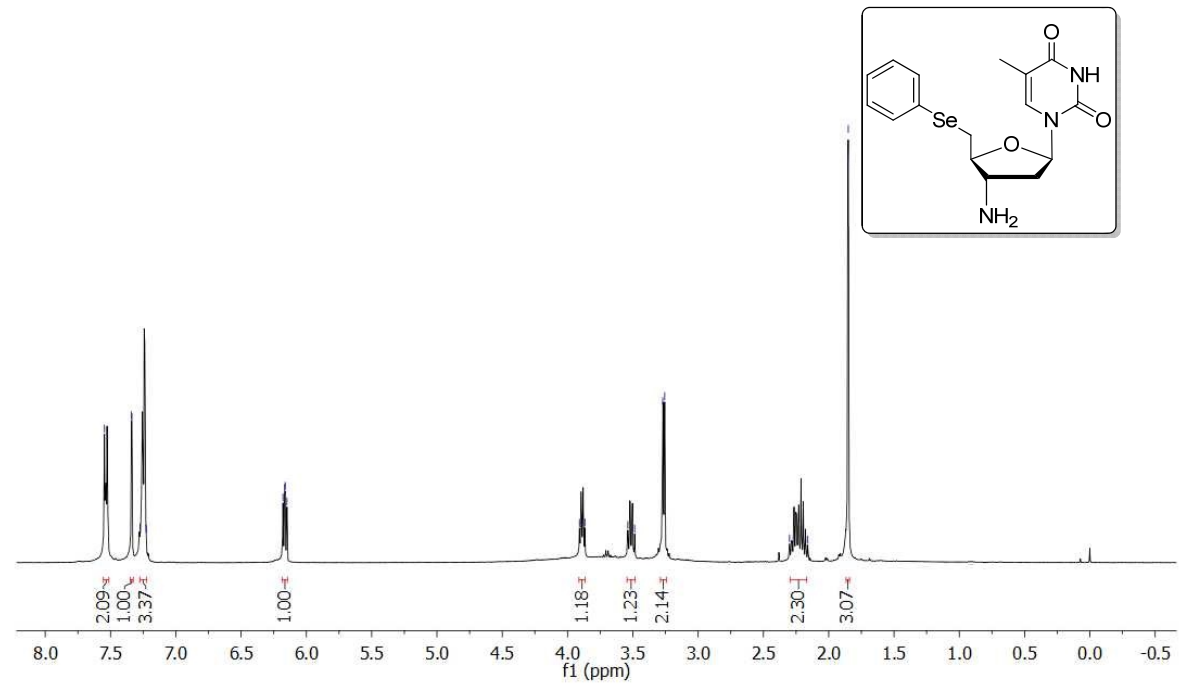

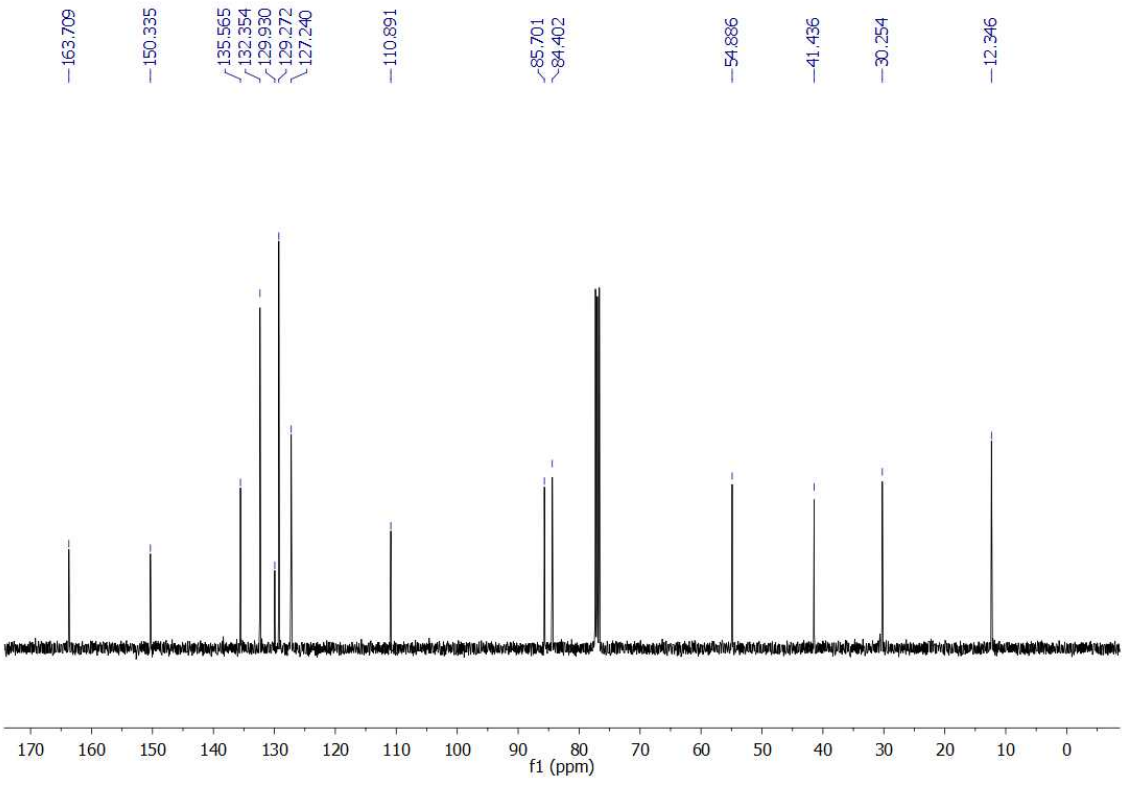

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Figure 2. ^{1}H and ^{13}C NMR of the compound **3b** in $CDCl_3$ at 400 MHz and 100 MHz respectively

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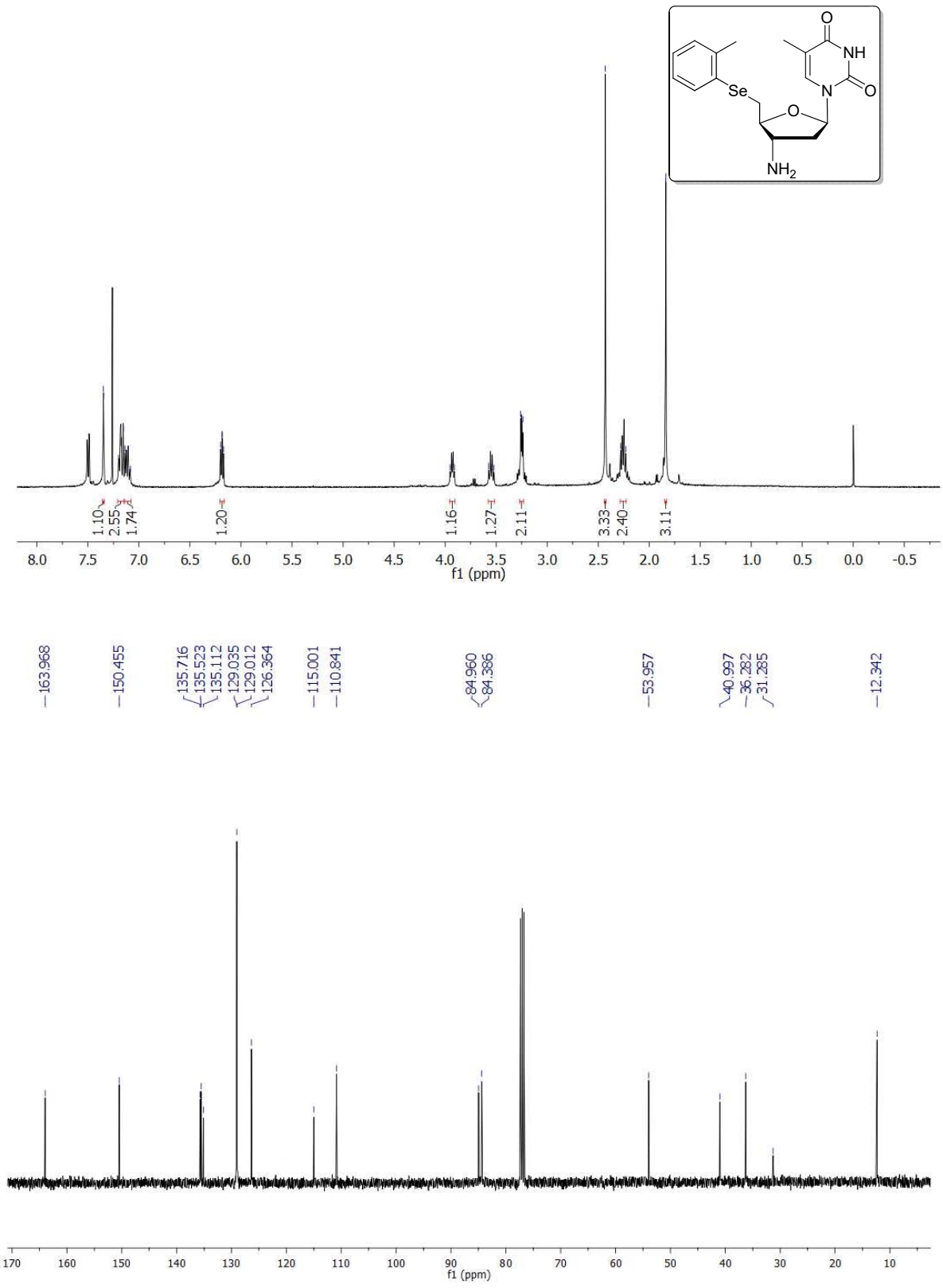

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Figure 3. ^{1}H and ^{13}C NMR of the compound **3c** in $CDCl_3$ at 400 MHz and 100 MHz respectively

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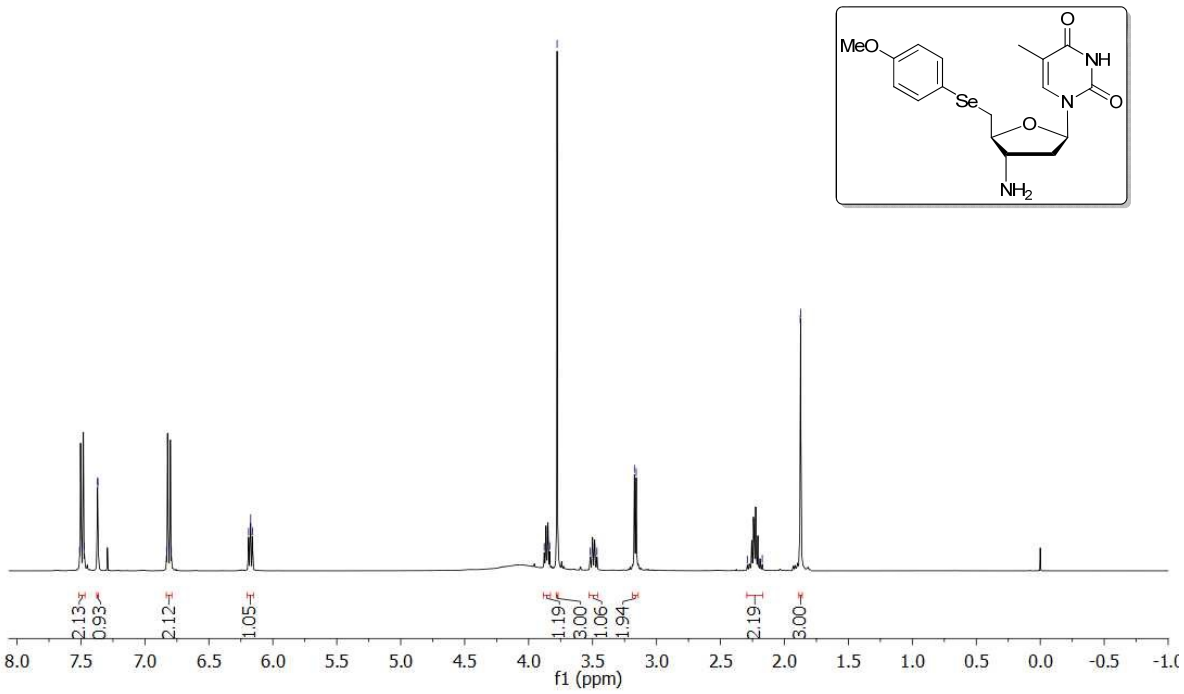

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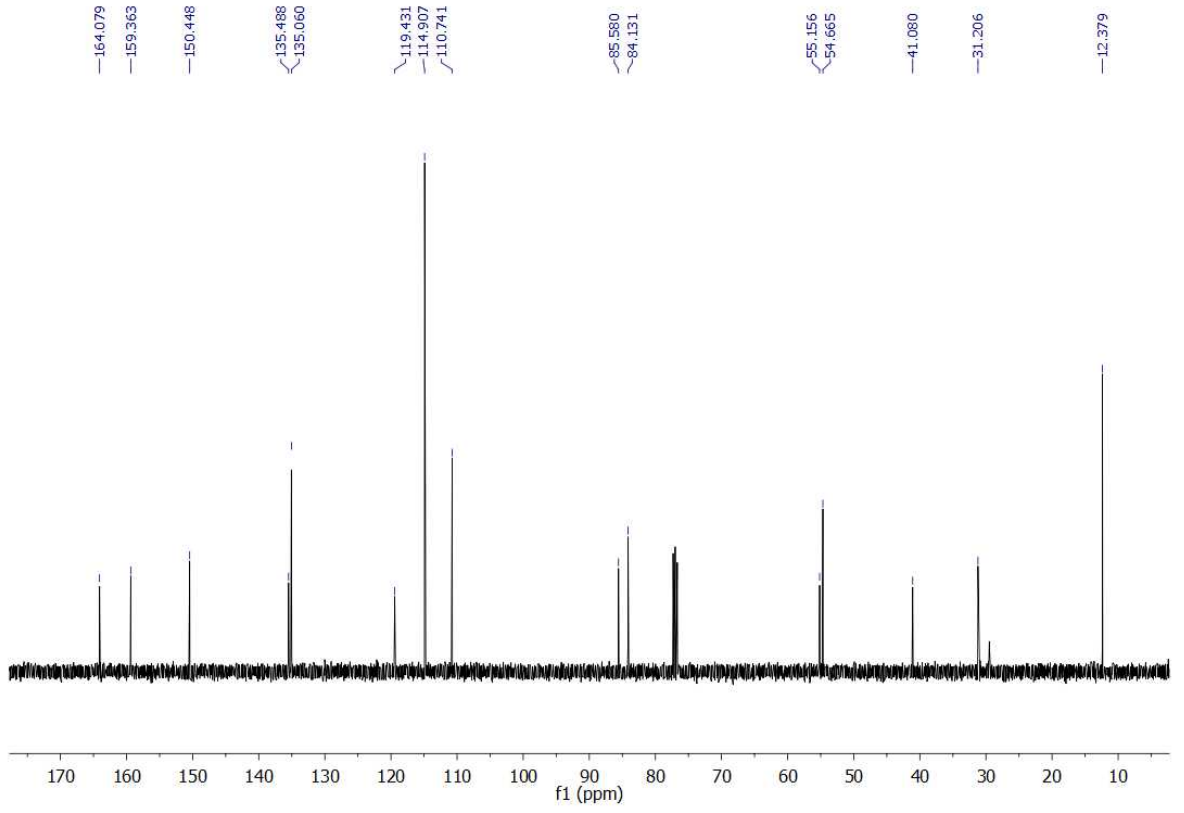

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Figure 4. ^{1}H and ^{13}C NMR of the compound **3d** in $CDCl_3$ at 400 MHz and 100 MHz respectively

#

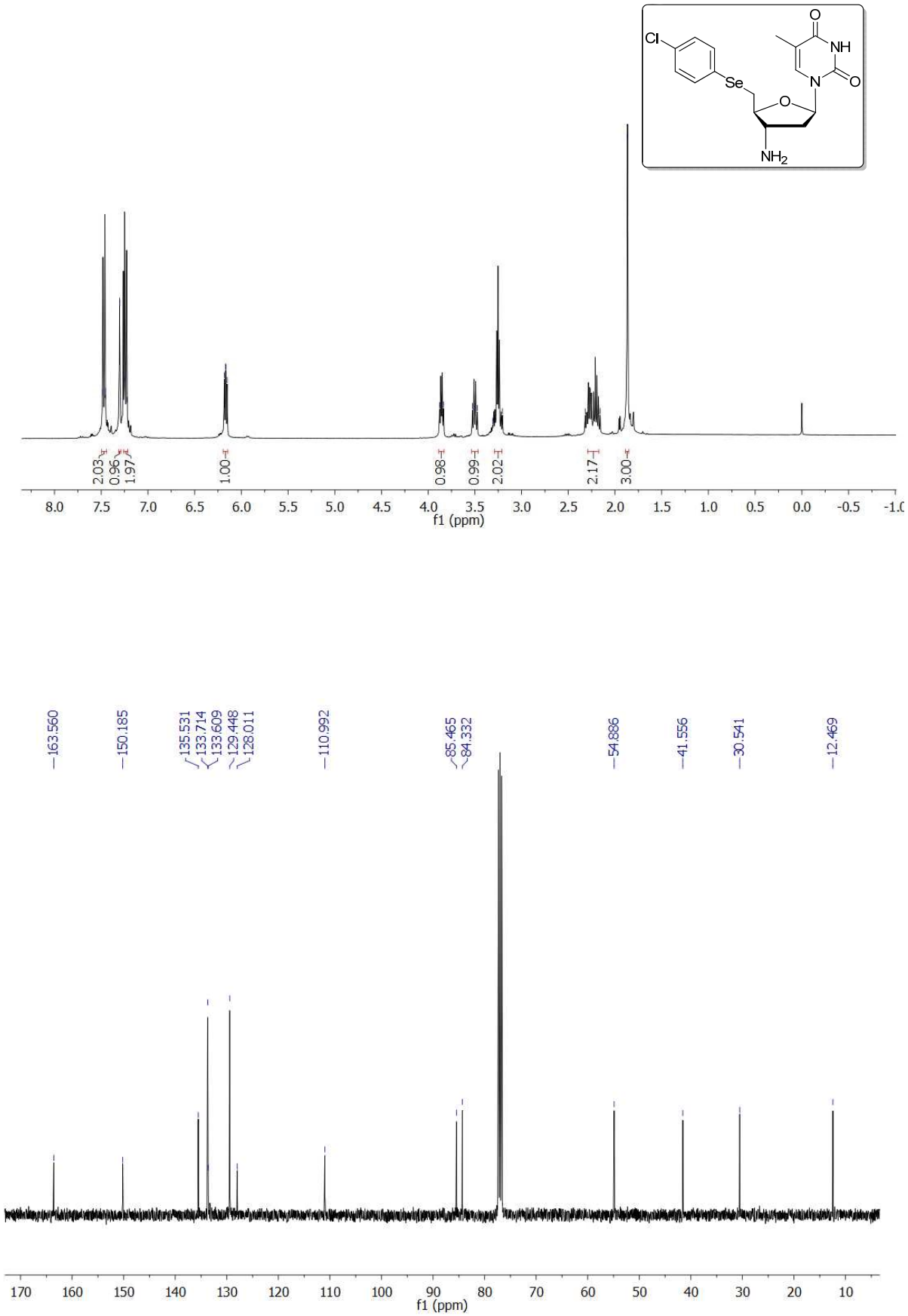

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Figure 5. ^{1}H and ^{13}C NMR of the compound **3e** in $CDCl_3$ at 400 MHz and 100 MHz respectively

#

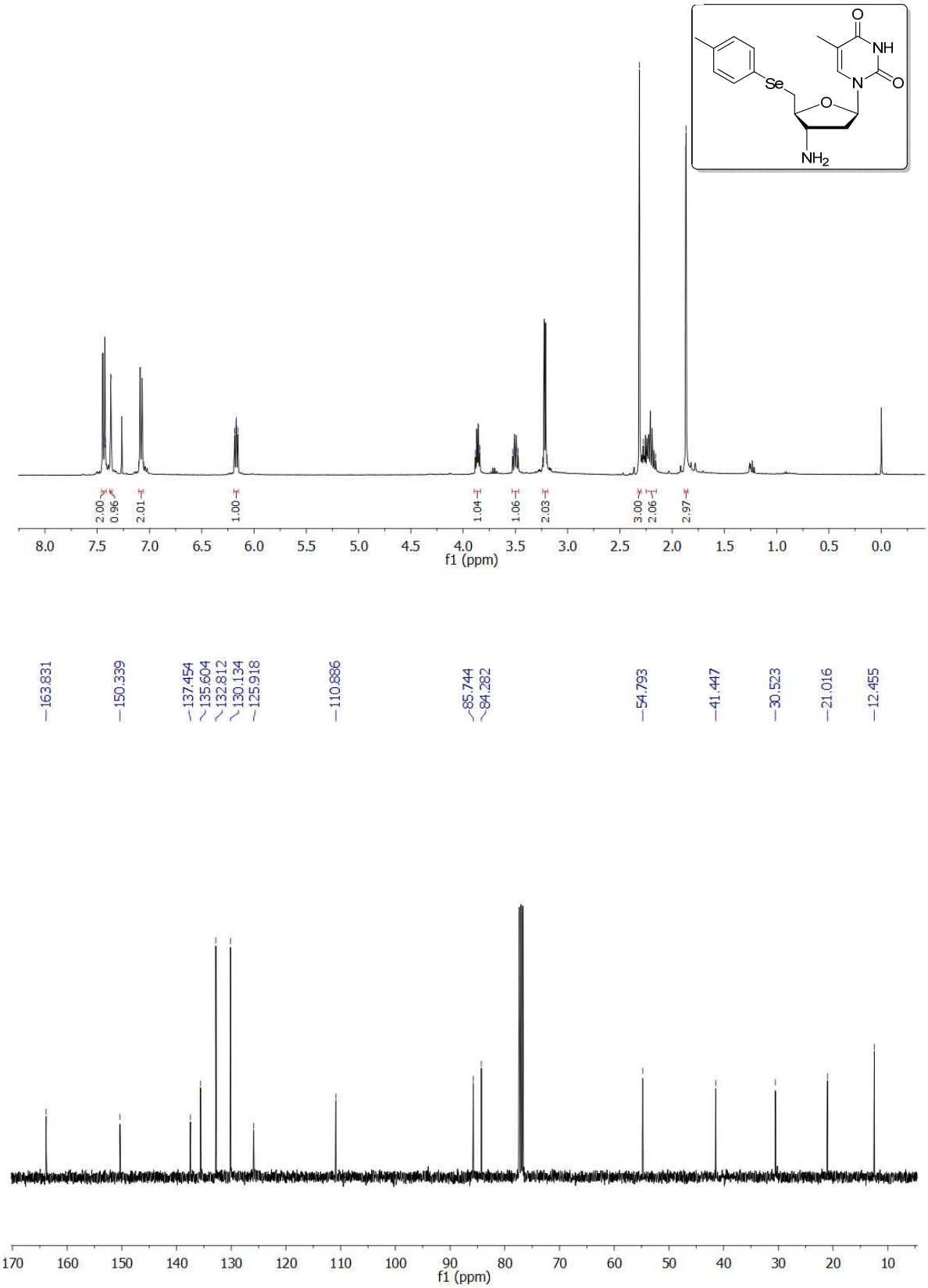

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Figure 6. ^{1}H and ^{13}C NMR of the compound **3f** in $CDCl_3$ at 400 MHz and 100 MHz respectively

#

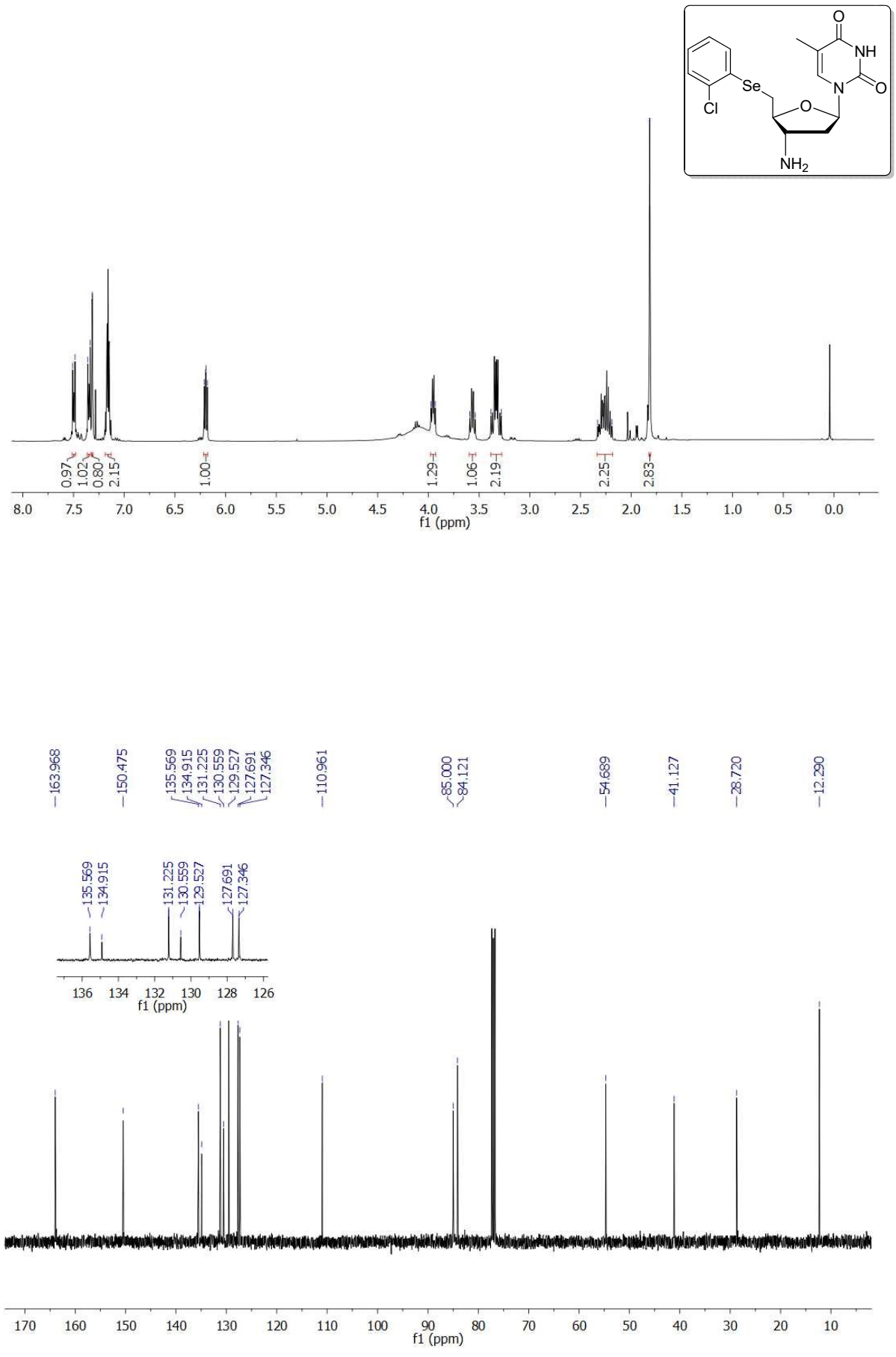

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Figure 7. ^{1}H and ^{13}C NMR of the compound **3g** in $CDCl_3$ at 400 MHz and 100 MHz respectively

#

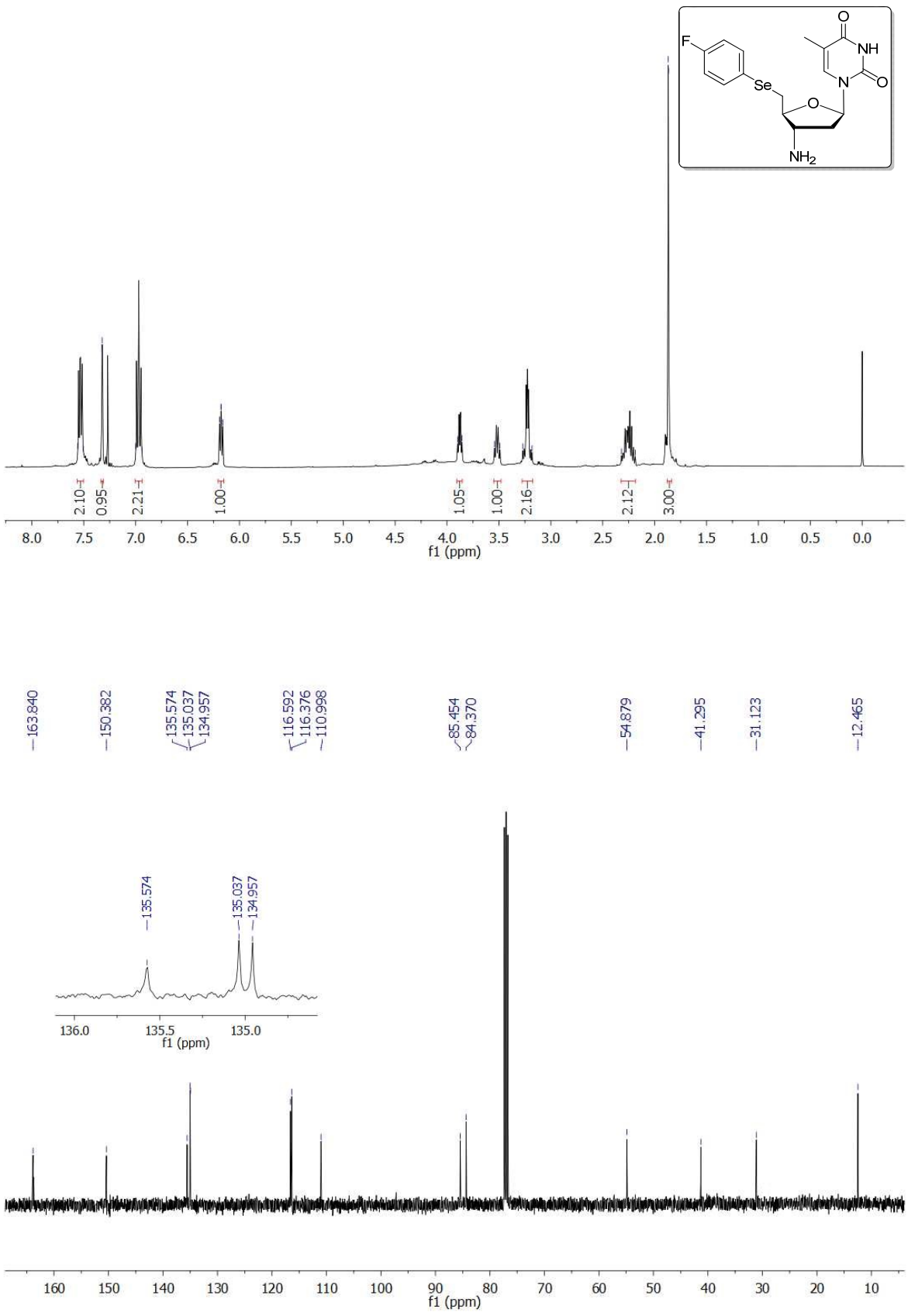

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Figure 8. ^{1}H and ^{13}C NMR of the compound **3h** in $CDCl_3$ at 400 MHz and 100 MHz respectively

#

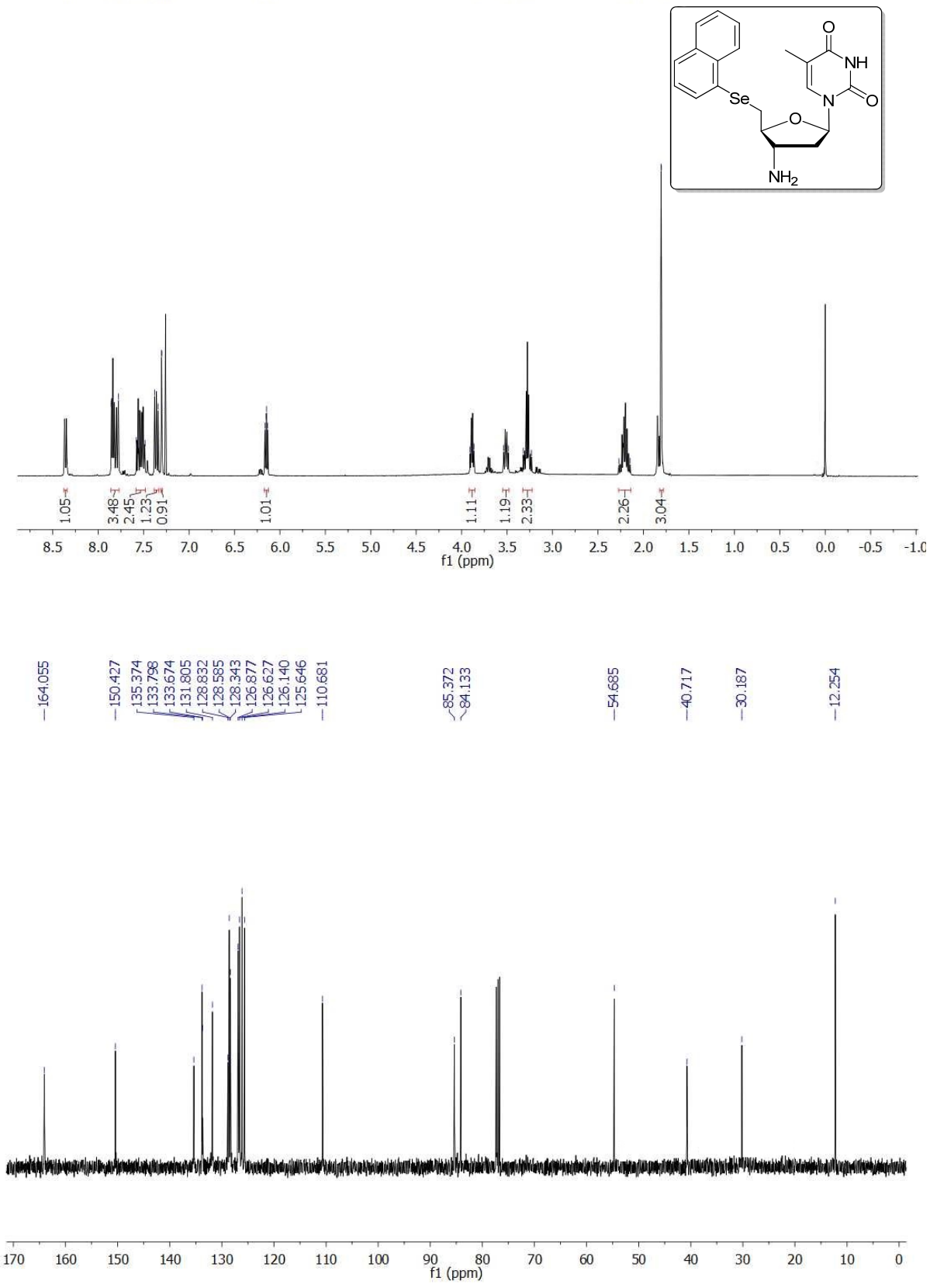

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Figure 9. ^{1}H and ^{13}C NMR of the compound **3i** in $CDCl_3$ at 400 MHz and 100 MHz respectively

#

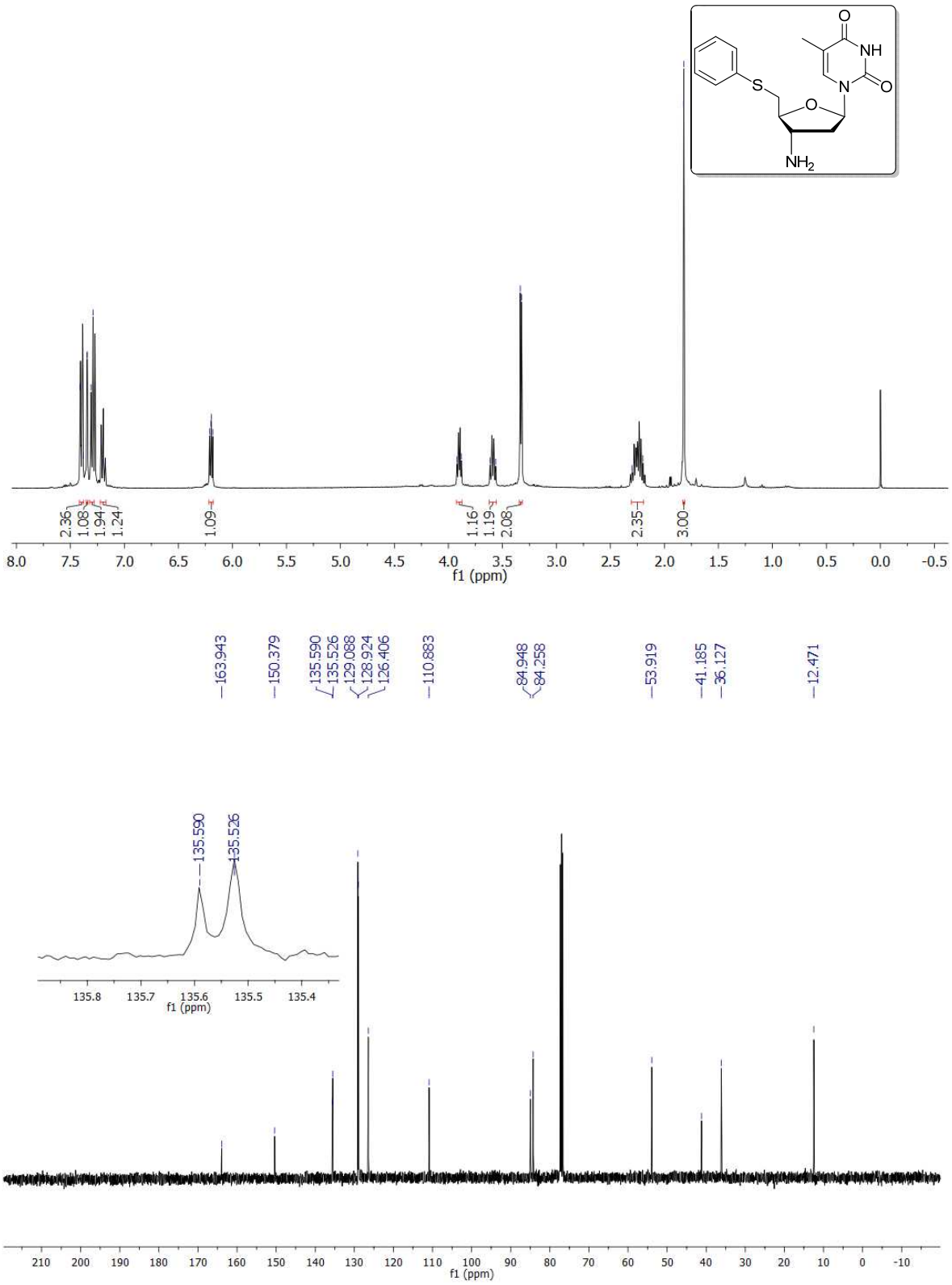

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Figure 10. [1]H and [13] C NMR NMR of the compound **3j** in $CDCl_3/CD_3OD$ at 400 MHz and 100 MHz respectively

#

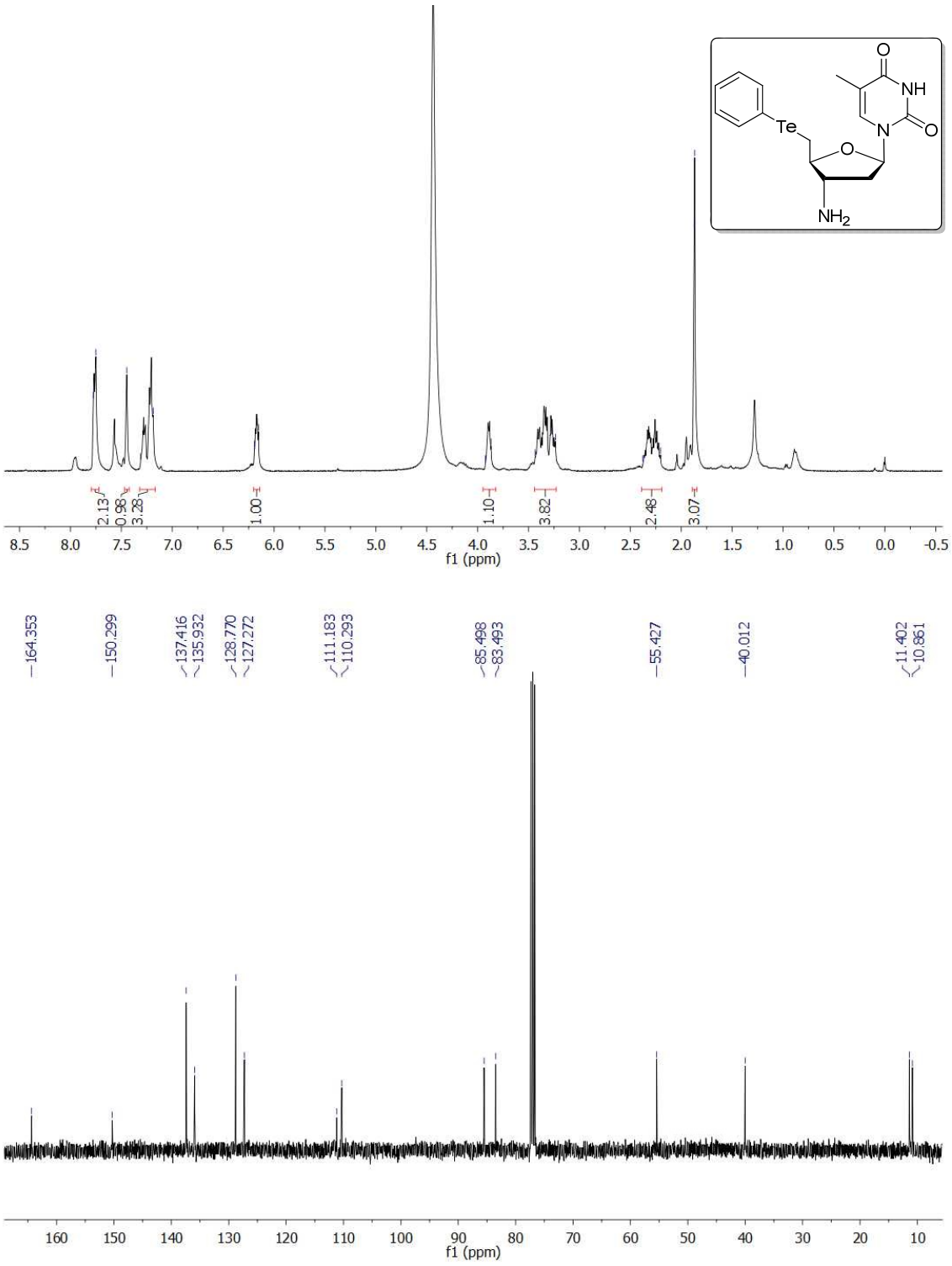

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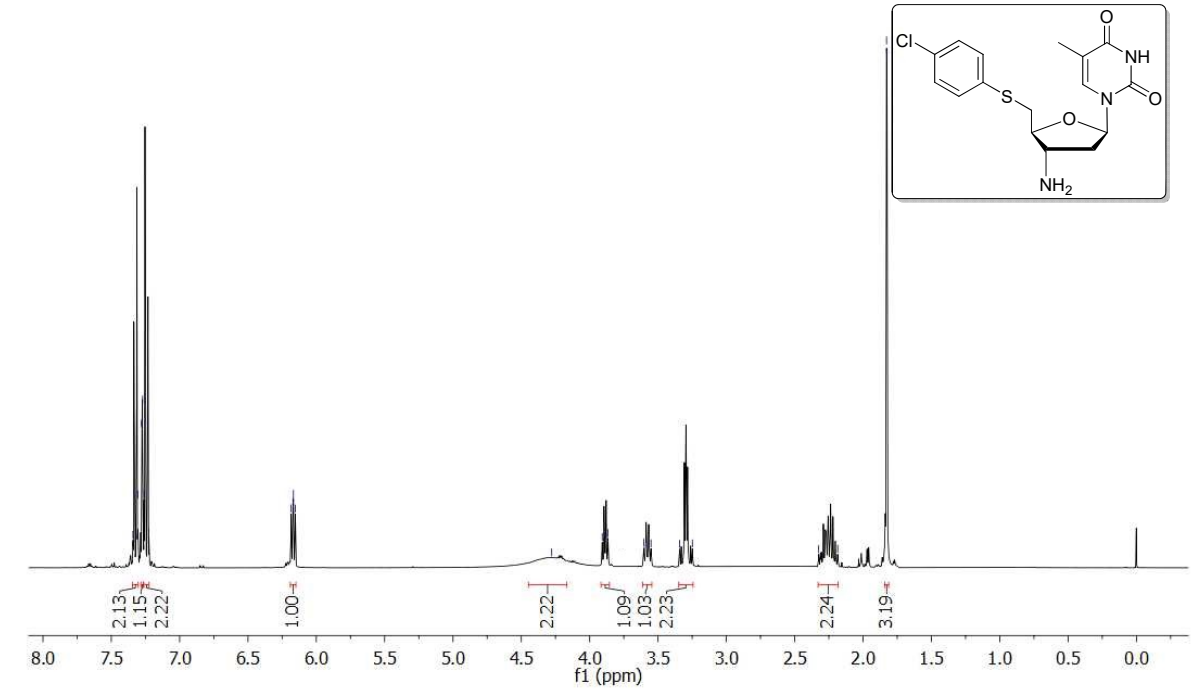
Figure 11. ^{1}H and ^{13}C NMR of the compound **3k** in $CDCl_3$ at 400 MHz and 100 MHz respectively

#



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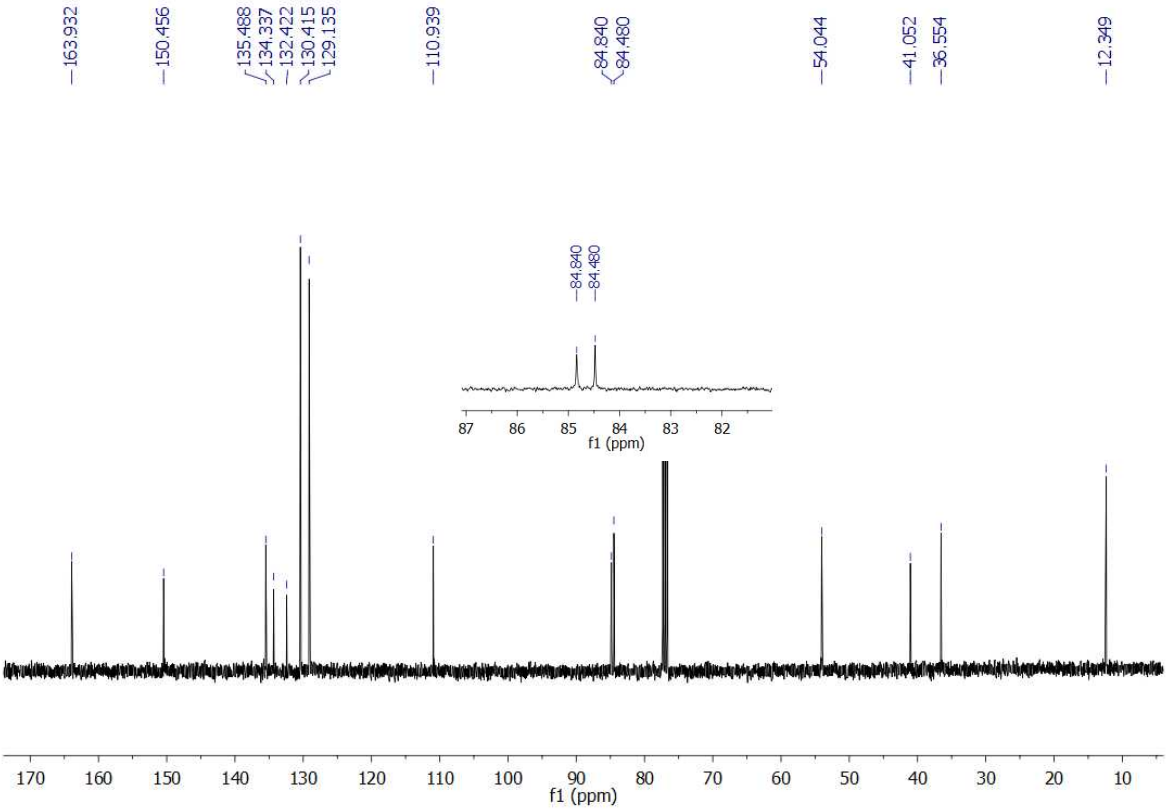

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Figure 12. [1]H and [13] C NMR of the compound **3l** in $CDCl_3$ at 400 MHz and 100 MHz respectively

#

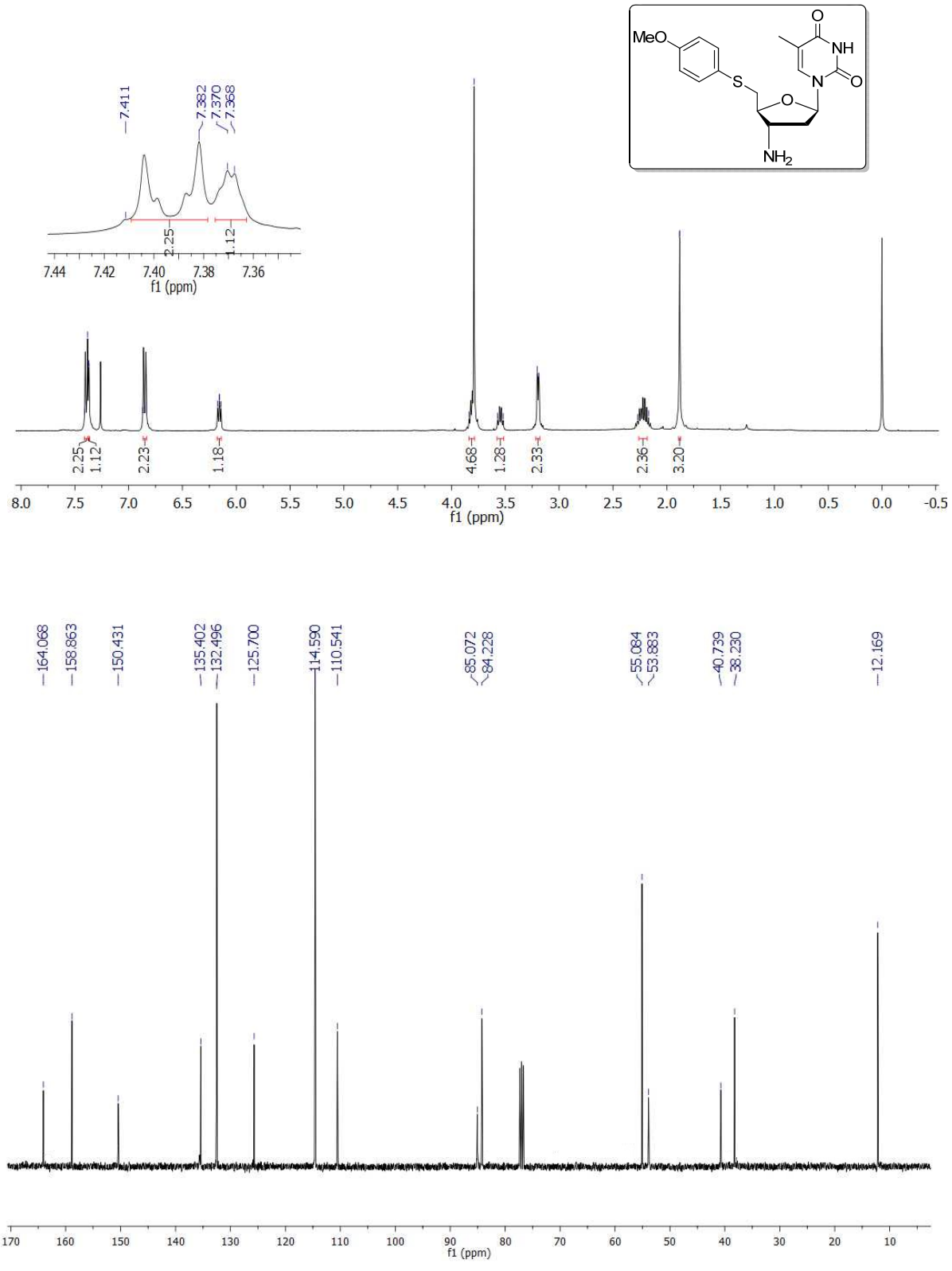

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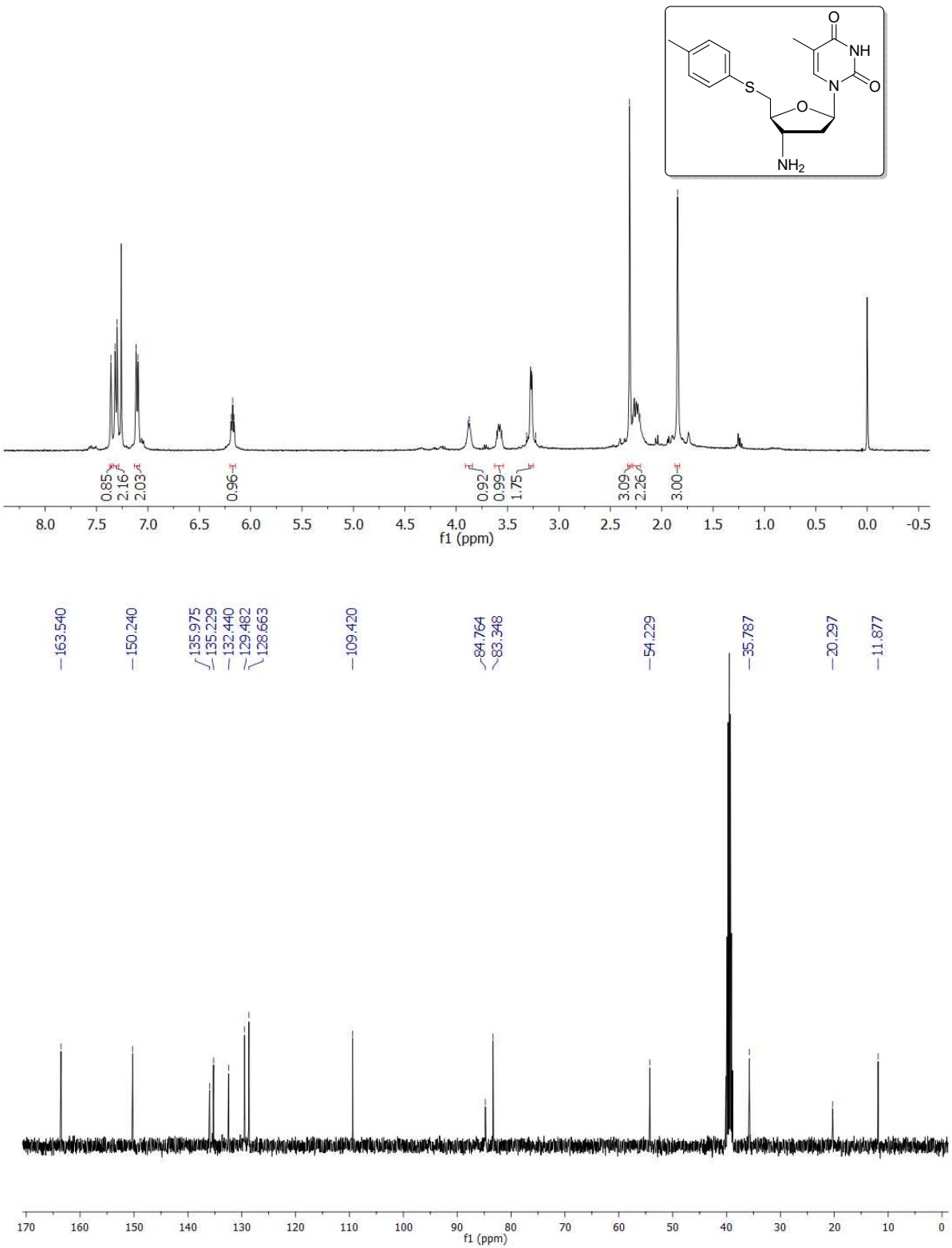
Figure 13. [1]H and [13] C NMR of the compound **3m** in DMSO at 400 MHz and 100 MHz respectively

#



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Selected HRMS

Figure 14. HRMS of **3a** (calculated for $M^{+}H$: 382,0670).

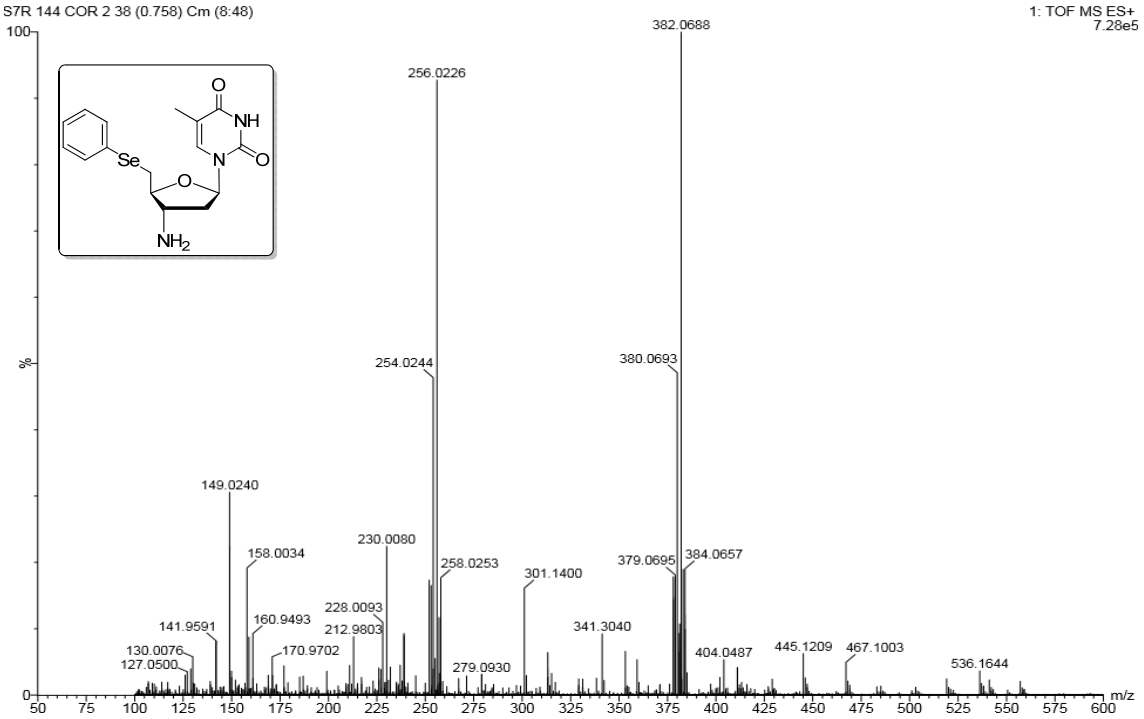

Figure 15. HRMS of **3b** (calculated for $M^{+}H$: 396,0826).

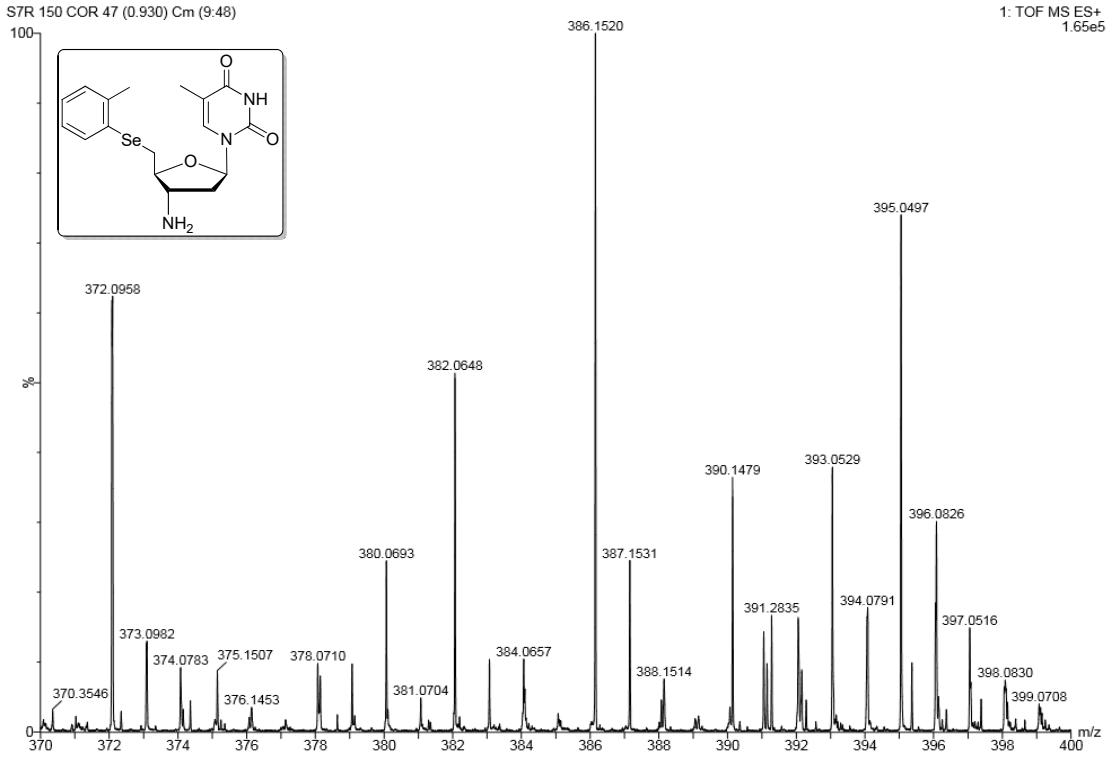

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Figure 16. HRMS of **3c** (calculated for $M^{+}H$: 412,0775).

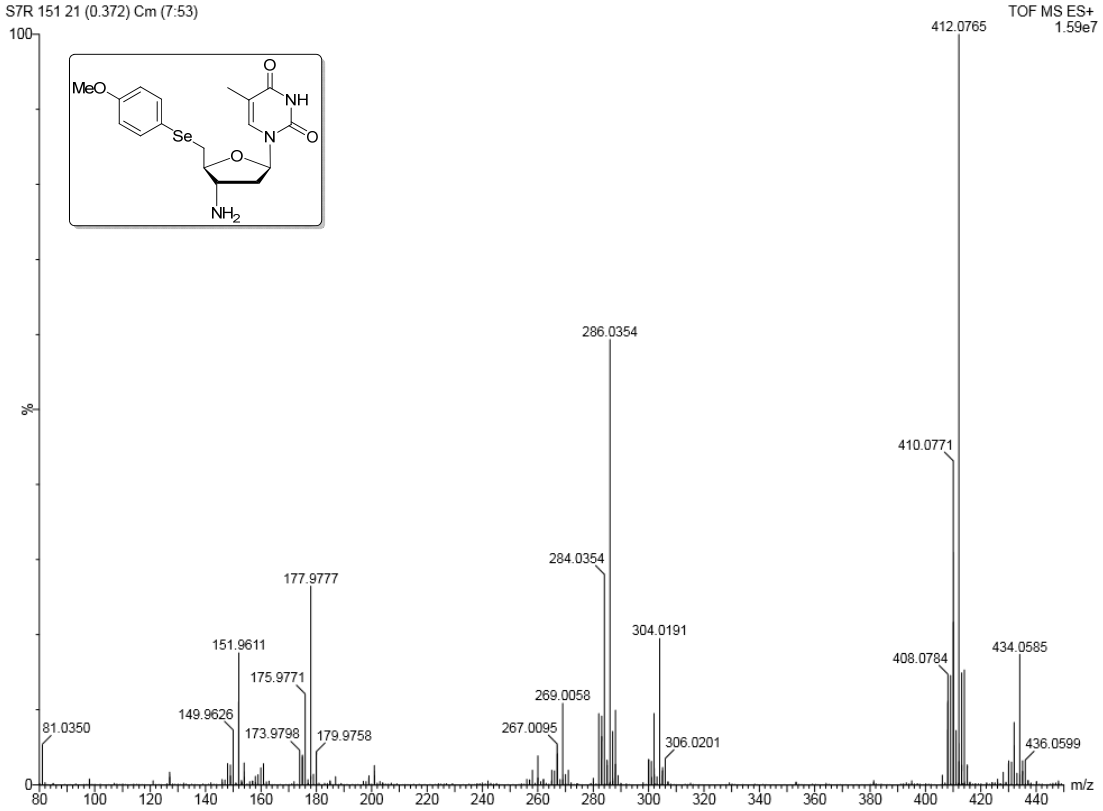

Figure 17. HRMS of **3d** (calculated for $M^{+}H$: 416,0280).

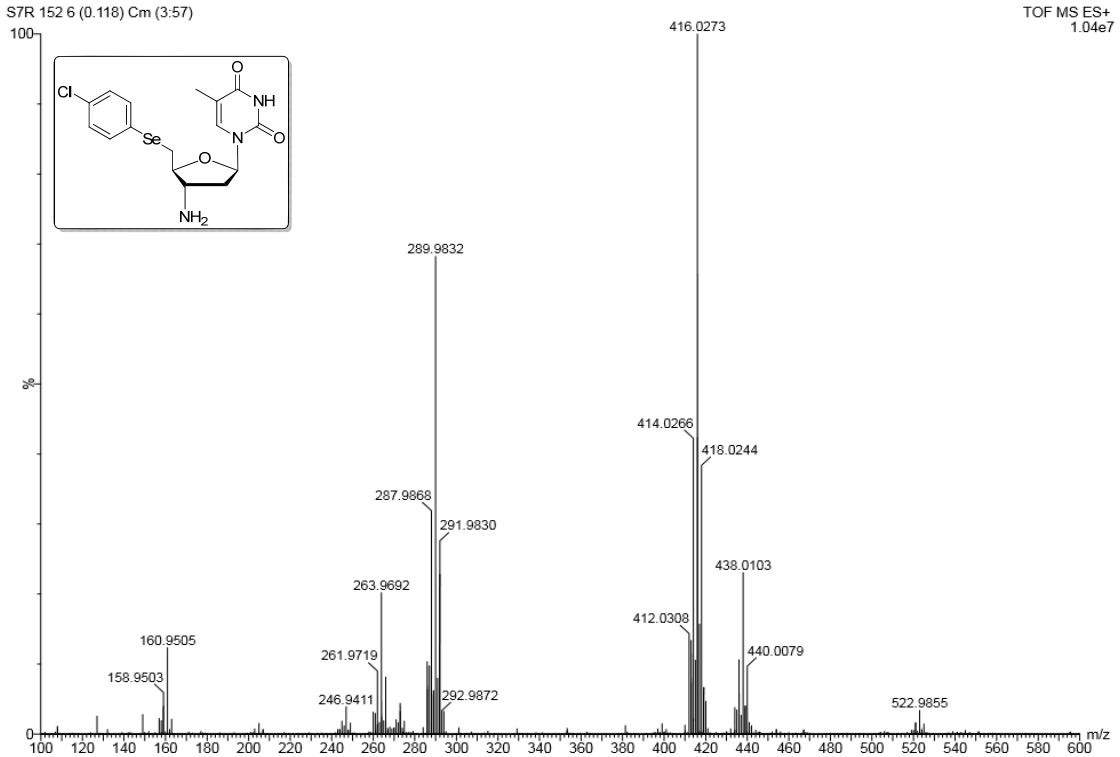

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Figure 18. HRMS of **3e** (calculated for $M^{+}H$: 396,0826).

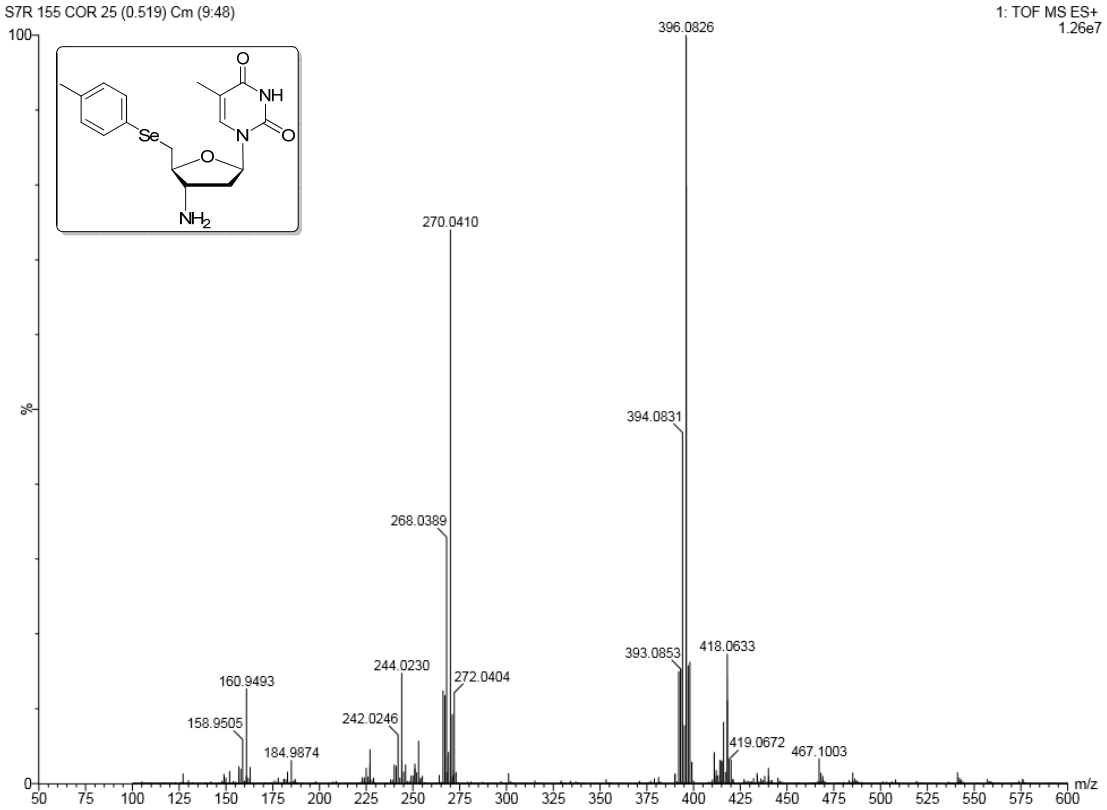

Figure 19. HRMS of **3f** (calculated for $M^{+}H$: 416,0280).

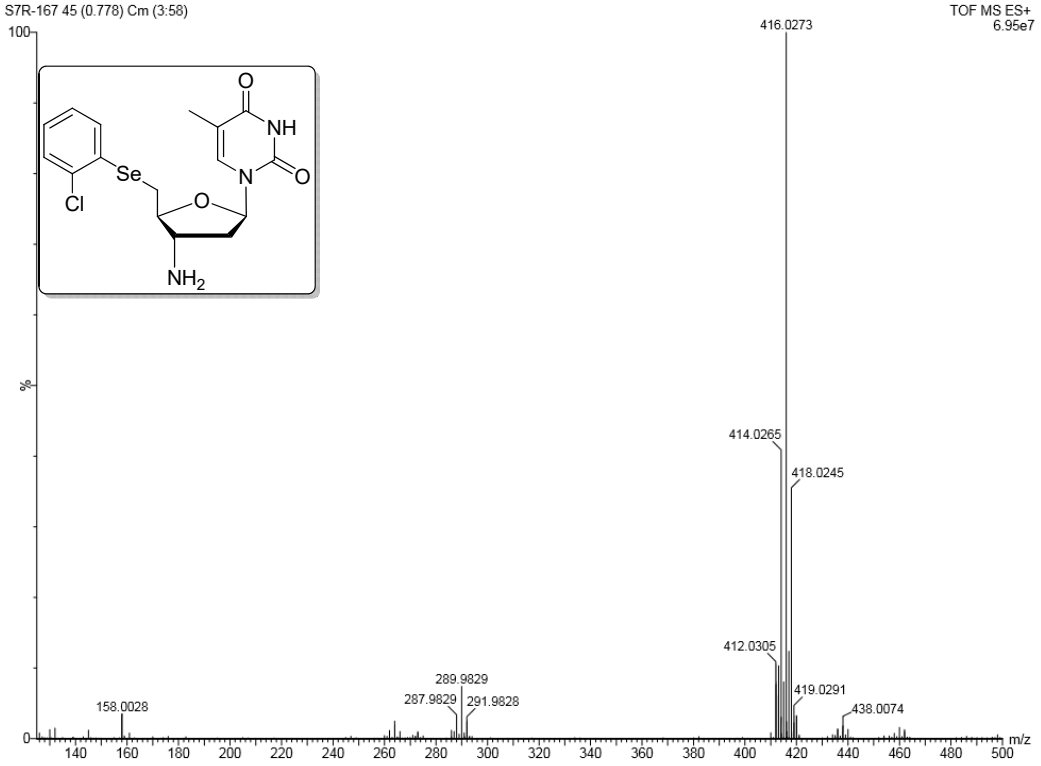

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Figure 20. HRMS of **3g** (calculated for M^+H: 400,0575).

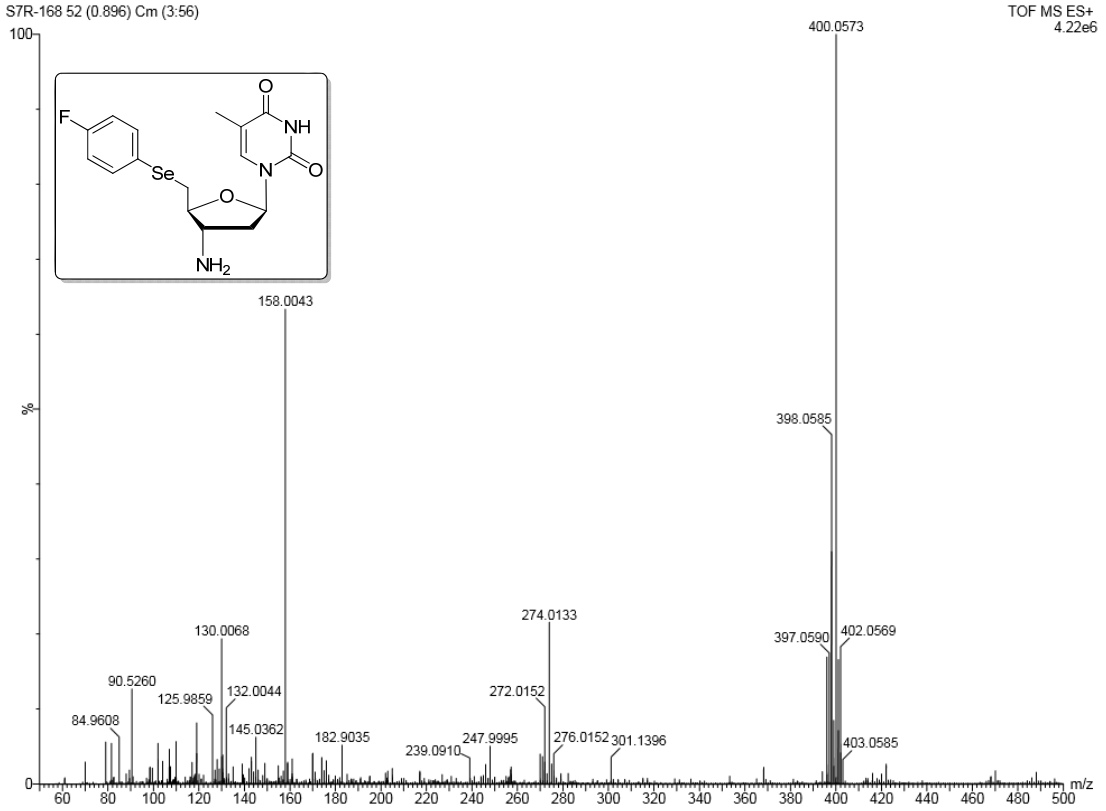

Figure 21. HRMS of **3h** (calculated for M^+H: 432,0826).

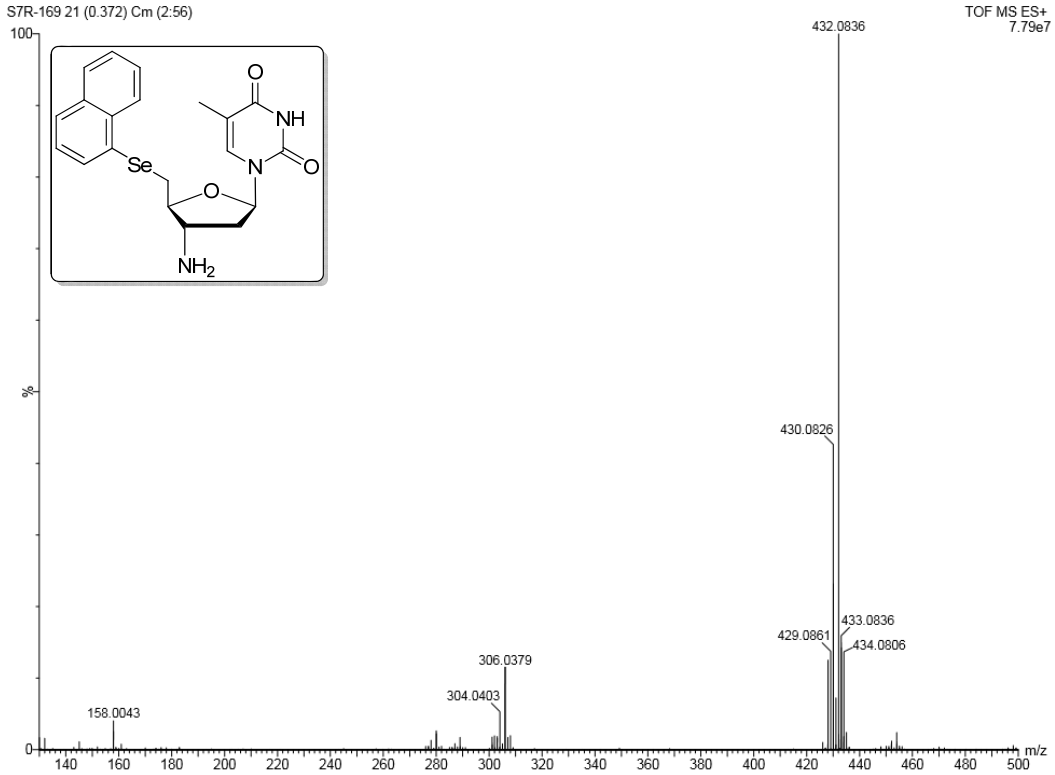

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Figure 22. HRMS of **3i** (calculated for $M^{+}H$: 334,1225).

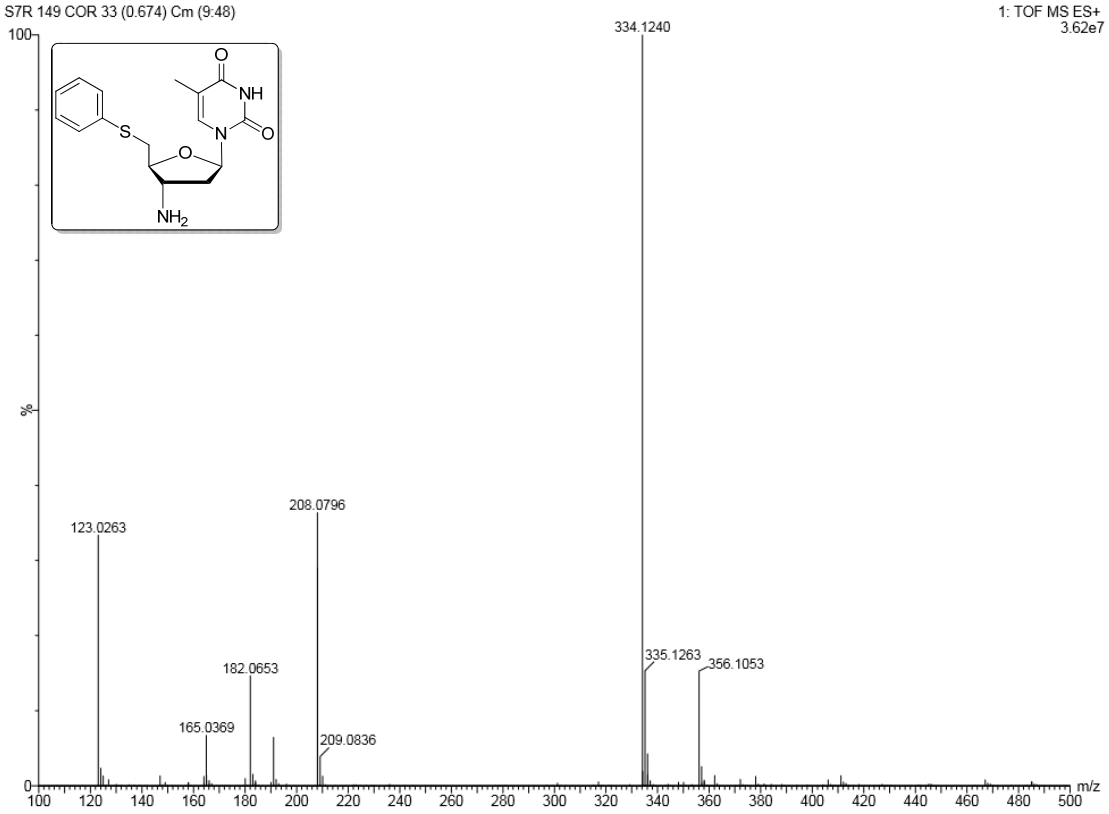

Figure 23. HRMS of **3j** (calculated for $M^{+}H$: 432,0567).

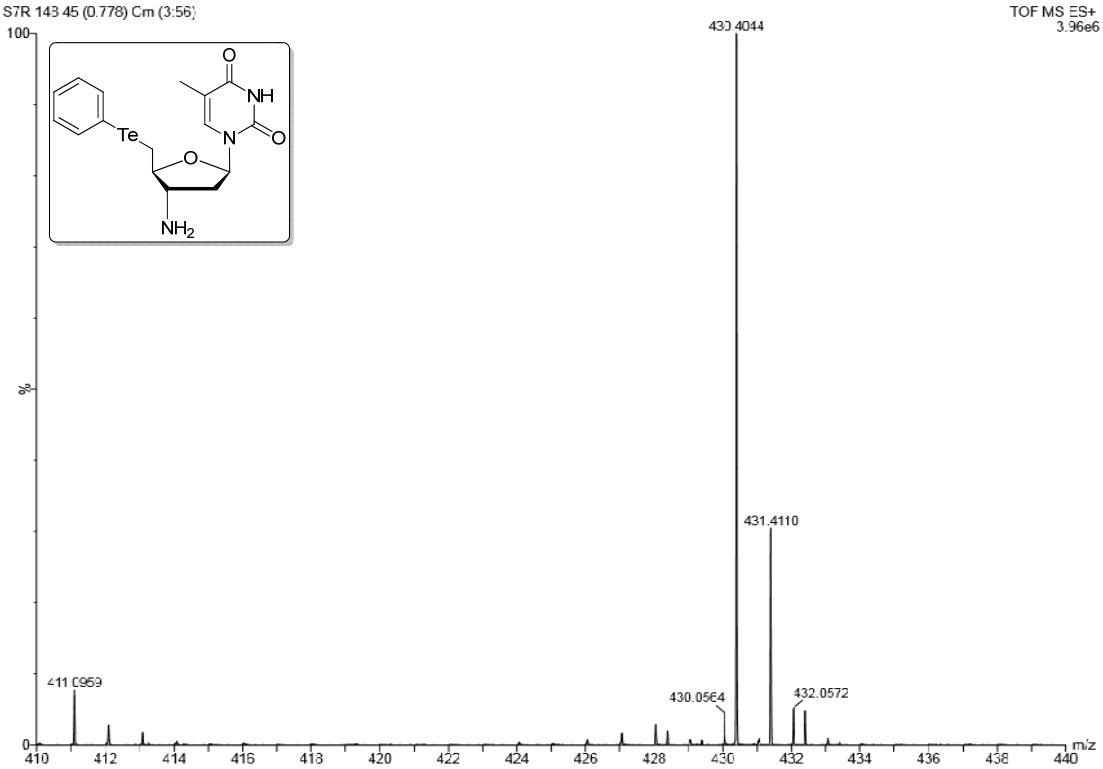

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Figure 24. HRMS of **3k** (calculated for $M^{+}H$: 368,0835).

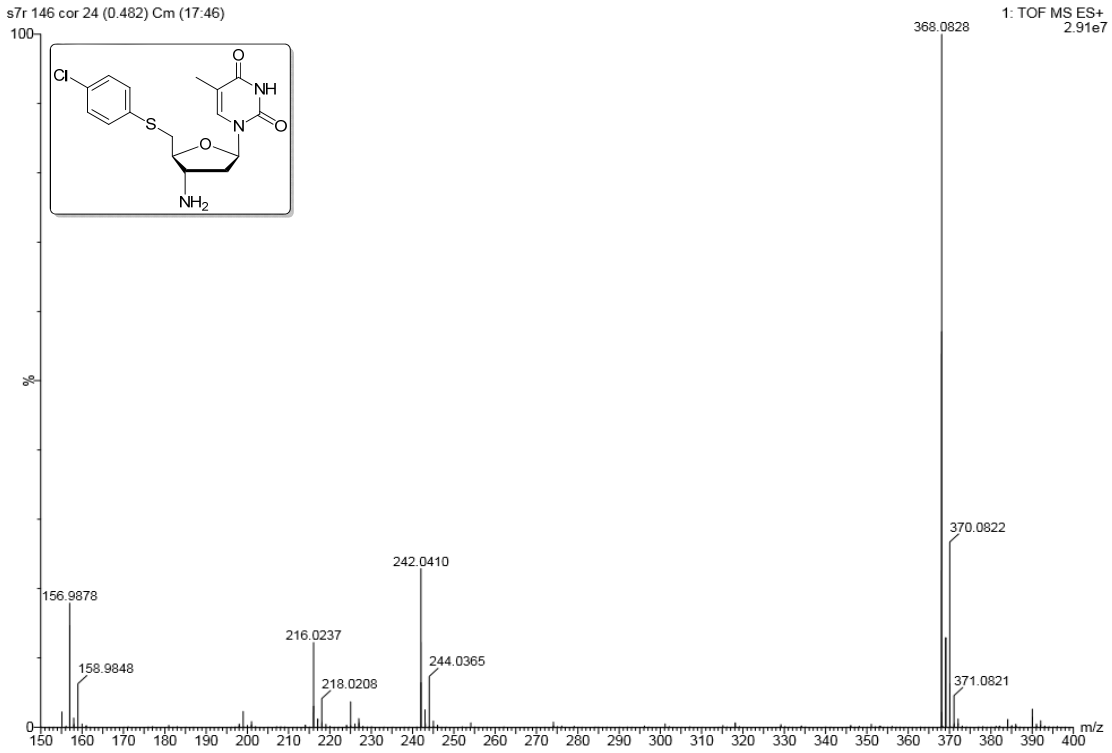

Figure 25. HRMS of **3l** (calculated for $M^{+}H$: 364,1331).

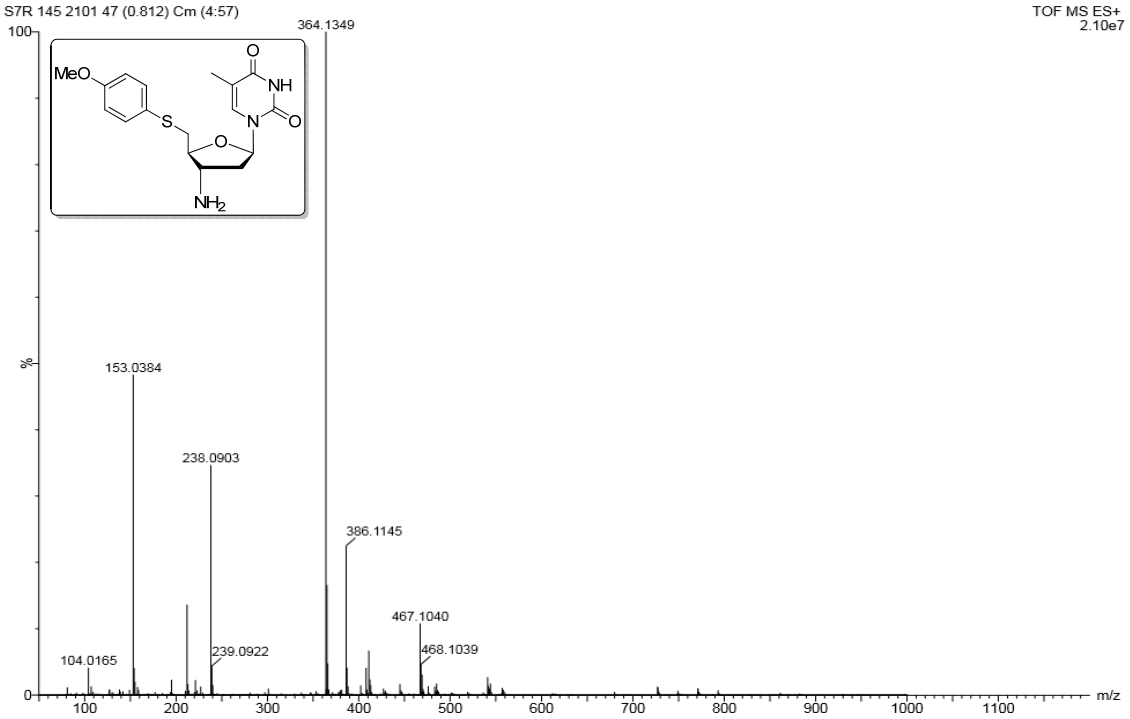

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Figure 26. HRMS of **3m** (calculated for $M^{+}H$: 348,1382).