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Downregulation of Gene Expression in Hypoxic Cancer Cells by an Activatable G-Quadruplex Stabiliser

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Experimental Procedures

General Methods

All reagents and solvents were purchased from commercial sources and used without further purification. Column chromatography was carried out using silica stationary phase (230–400 mesh, SiliCycle Inc., Canada) and compounds were visualized under UV light Analytical thin layer chromatography was performed on 0.25 mm thick precoated silica gel plates (60F254, Merck, Germany). Nitroreductase enzyme was purchased from Sigma-Aldrich (E. Coli, N9284). All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova instrument (400 MHz) at Selcuk University and Ataturk University respectively. Chemical shifts (δ) are reported in parts per million (ppm) and referenced to the residual solvent peak. Coupling constants (J) are reported in hertz (Hz). Standard abbreviations indicating multiplicities are given: b = broad, d = doublet, m = multiplet, s = singlet, t = triplet. High-resolution mass spectrometry was carried out using Agilent 6530 Accurate-Mass Q-TOF LC/MS of the Eastern Anatolia Advanced Technology Research and Application Centre (DAYTAM, Erzurum, Turkey). Mass spectrometry data were recorded using Schimadzu LCMS-2020 Single Quadrupole Liquid Chromatography Mass Spectrometer at KIT-ARGEM Konya Food and Agriculture University. For cell culture experiments MCF7 human breast adenocarcinoma cell line was used. Cells were visualized with Zeiss Inverted Microscopy. UV-Vis Absorbance spectra are recorded using Agilent Cary 60 UV-Vis Spectrophotometer. FRET-melting experiments and gene expression analysis was performed using Bio-rad CFX96 Real Time System qPCR instrument. Primers of PCR and G-quadruplex forming oligonucleotides labelled with FAM at 5' region and TAMRA dyes at 3’ region as donor and acceptor probes respectively, were purchased from Sentebiolab, Ankara, Turkey. HPLC retention times were obtained by Shimadzu Prominence-i, LC-2030C Plus.

Synthesis

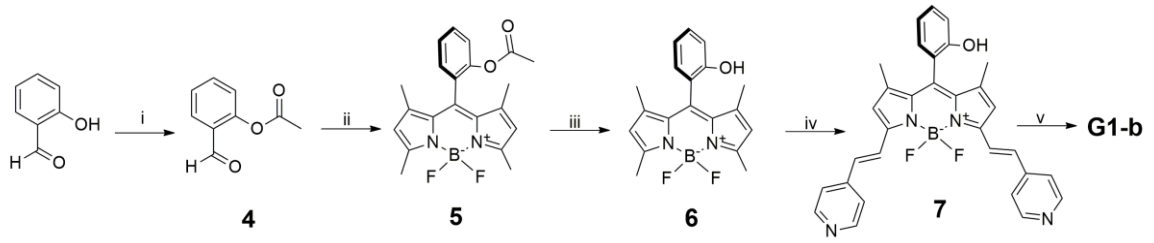
Compound **G1-a** was synthesized using the reaction steps shown in Scheme 1.

Compound 1. 2-hydroxy benzaldehyde (1.5 g, 12.3 mmol) and 1.1 equivalent of 4-nitrobenzyl bromide (2.92 g, 13.5 mmol) were dissolved in 15 ml dimethylformamide (DMF). 2 equivalent of potassium carbonate (24.6 mmol; 3,39 g) was added. Reaction mixture was stirred 16 h at room temperature. DMF was evaporated and the reaction mixture was extracted with dichloromethane (DCM) and water. Organic layer was collected and dried over sodium sulfate. Sample was purified by silica column chromatography using DCM as mobile phase. Product was obtained as white solid with the yield of 87%.
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 10.58 (s, 1H), 8.29 (d, J = 8.69 Hz, 2H), 7.90 (d, J = 7.69 Hz, 1H), 7.66 (d, J = 8.74 Hz, 2H), 7.57 (t, J = 7.85 Hz,1H), 7.12 (t, J = 7.46 Hz, 1H), 7.02 (d, J = 8.33 Hz, 1H), 5.33 (s, 2H).
^{13}C NMR ($CDCl_3$, 101 MHz, δ ppm) 69.1, 112.7, 121.6, 124.0, 125.2, 127.5, 129.2, 136.0, 143.4, 147.7, 160.1, 189.2.
High resolution ESI-MS values are 258.0766 for theoretical m/z of $(M+H)^+$ and 258.0764 for experimental (Δ: 0.77 ppm).

Compound 2. DCM (150 ml) was purged with N_2 for 20 minutes and then compound **1** (1.2 g, 4.7 mmol) was added. 2.2 equivalent of 2,4-dimethyl pyrrole (1.05 ml, 10.3 mmol) was added to the reaction mixture. A few drops of trifluoroacetic acid (TFA) were added. The reaction mixture was stirred for 16 h under inert atmosphere at room temperature. 1.1 equivalent of p-chloranil (1.27 g, 5.17 mmol) was added and the reaction mixture was stirred for 3 hours at room temperature. Then, triethylamine (Et_3N, 6 ml) and boron trifluoride diethyl etherate ($BF_3.OEt_2$, 6 ml) were slowly added, reaction was stirred further for 3 hours at room temperature. Mixture was extracted with DCM/H_2O and purified by two separate silica column chromatography using with petroleum ether: ethyl acetate (3/1; v/v) first and then hexane: DCM (3/1; v/v) mobile phases. Orange solid product was obtained with the yield of 22%.
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): δ 8.10 (d, J = 8.7 Hz, 1H), 7.42 (ddd, J = 8.3, 7.4, 1.8 Hz, 1H), 7.33 (d, J = 9.0 Hz, 2H), 7.26 (s, 1H), 7.21 (dd, J = 7.5, 1.8 Hz, 1H), 7.13 (td, J = 7.5, 1.0 Hz, 1H), 7.01 (dd, J = 8.4, 1.0 Hz, 1H), 5.96 (s, 2H), 5.17 (s, 2H), 2.58 (s, 6H), 1.40 (s, 6H).
^{13}C NMR ($CDCl_3$, 101 MHz, δ ppm) 14.2, 14.8, 69.5, 113.6, 121.2, 122.8, 123.9, 124.9, 127.4, 130.1, 130.9, 131.6, 138.5, 142.6, 144.4, 147.7, 155.2, 155.5.
High resolution ESI-MS values are 476.1952 for theoretical m/z of $(M+H)^+$ and 476.1937 for experimental (Δ: 5.3 ppm).

Compound 3. Compound **2** (200 mg, 0.42 mmol) was dissolved in benzene (5 ml). 3 equivalent of 4-pyridine carboxaldehyde (119 μl, 1.26 mmol) was added and then 0.3 ml piperidine and 0.3 ml acetic acid were added to the reaction mixture which was stirred and refluxed by using Dean-Stark apparatus at 90 °C. By using thin layer chromatography (TLC), formation of green product was followed. The reaction was cooled to room temperature, the mixture was extracted with DCM/H2O and purified with silica column chromatography using 7% MeOH in DCM as mobile phase and the collected fraction is further purified with silica column chromatography using 50% acetone in DCM as mobile phases. The yield of green compound **3** was 16%.
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 8.65 (d, J = 6.0 Hz, 4H), 8.10 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 16.3 Hz, 2H), 7.49 (d, J = 6.1 Hz, 4H), 7.35 (d, J = 8.6 Hz, 2H), 7.30 – 7.26 (m, 1H), 7.17 (d, J = 16.3 Hz, 2H), 7.08 (d, J = 8.3 Hz, 1H), 6.68 (s, 2H), 5.21 (s, 2H), 1.50 (s, 6H) ppm.
^{13}C NMR (CDCl3, 101 MHz, δ ppm) 155.2, 151.9, 150.3, 144.0, 143.9, 142.7, 133.5, 131.4, 130.2, 127.5, 124.4, 124.0, 123.5, 122.9, 121.6, 118.6, 113.6, 69.6, 29.9, 14.5.
High resolution ESI-MS values are 654.2488 for theoretical m/z of $(M+H)^+$ and 654.24950 for experimental (Δ: 1.07 ppm).

Compound G1-a. Compound **3** (21 mg, 32 μmol) was dissolved in DMF (1 ml) and then excess amount of 0.1 ml iodomethane was added. The reaction mixture was stirred for 3 days at room temperature. Formation of the product was followed with thin layer chromatography. DMF was evaporated under vacuum. Solid product was washed with ethyl acetate. Dark green compound was obtained with quantitative yield.
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (d, J = 6.4 Hz, 4H), 8.17 (d, J = 6.4 Hz, 4H), 8.05 – 7.98 (m, 4H), 7.91 (s, 1H), 7.48 (d, J = 15.4 Hz, 4H), 7.27 (d, J = 9.0 Hz, 3H), 7.19 – 7.13 (m, 3H), 7.07 (d, J = 8.4 Hz, 1H), 6.91 (s, 2H), 5.15 (s, 2H), 4.41 (s, 6H), 1.47 (s, 6H).
High resolution ESI-MS values are 341.64395 for theoretical m/z of $(M)^{2+}$ and 341.6428 for experimental (Δ: 2.84 ppm).



Scheme S1. Synthesis of compound **G1-b**. Reaction conditions: i) Acetyl chloride, Et_3N, DCM; ii) a. N_2, 2,4-dimethyl pyrrole, TFA, CH_2Cl_2, R.T. b. p-chloranil, c. Et_3N, $BF_3.OEt_2$; iii) KOH, THF, MeOH; iv) 4-pyridine carboxaldehyde, piperidine, acetic acid, benzene 90°C; v) methyl iodide, DMF, R.T.

Compound 4. 2-hydroxybenzaldehyde (2.44 g, 20 mmol) was dissolved in 25 ml of DCM. 4.2 ml triethylamine was added. And then 2.14 ml acetylchloride was added slowly. The reaction mixture was stirred 16 h at room temperature. Extraction was done with DCM and saturated sodium bicarbonate solution. Compound was purified with silica column chromatography using DCM as mobile phase to obtain colourless oil product. The yield of compound 4 was 78%.
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.09 (s, 1H), 7.87 (dt, J = 7.7, 1.3 Hz, 1H), 7.62 (tdd, J = 7.4, 1.8, 0.9 Hz, 1H), 7.42-7.37 (m, 1H), 7.17 (d, J = 8.2 Hz, 1H), 2.38 (s, 3H) ppm.
^{13}C NMR (101 MHz, $CDCl_3$) δ 188.9, 169.4, 151.7, 135.5, 131.5, 128.2, 126.6, 123.72, 21.0 ppm.
High resolution ESI-MS values are 165.0552 for theoretical m/z of $(M+H)^+$ and 165.05459 for experimental (Δ: 3.7 ppm).

Compound 5. DCM (150 ml) was purged with N_2 for 15 min. Compound **4** (1 g, 6 mmol) was added. 2,4-dimethyl pyrrole (2.4 ml, 22.5 mmol) was added. Catalytic amount of TFA (a few drops) was added. The reaction mixture was stirred 16 h at RT under inert atmosphere. p-Chloranil (2.2 g, 9 mmol) was added to the reaction mixture and stirred for 3.5 hours, followed by the addition of triethylamine (8 ml) and boron trifluoride diethyl etherate complex (8 ml). The reaction mixture was stirred for additional 3.5 hours at RT. Extraction was done with DCM/H_2O. Silica column chromatography with hexane: acetone (2/1; v/v) was done to have partial purification. Then a new column chromatography was performed with DCM mobile phase to purify the compound further. The yield of orange solid compound **5** was 20%.
^{1}H NMR (400 MHz, Chloroform-d) δ 7.51 (td, J = 7.8, 1.8 Hz, 1H), 7.36 (td, J = 7.5, 1.2 Hz, 1H), 7.33 – 7.21 (m, 2H), 5.97 (s, 6H), 2.09 (s, 6H), 1.47 (s, 6H) ppm.
^{13}C NMR (101 MHz, $CDCl_3$) δ 169.0, 155.7, 148.0, 143.1, 136.3, 131.1, 130.3, 129.9, 127.7, 126.5, 123.8, 121.3, 20.8, 14.6, 13.9 ppm.
High resolution ESI-MS values are 383.1742 for theoretical m/z of $(M+H)^+$ and 383.17283 for experimental (Δ: 3.58 ppm).

Compound 6. Compound **5** (76 mg, 0.2 mmol) was dissolved in 8 ml tetrahydrofuran (THF) and 2 ml methanol. And then, 2 equivalent of potassium hydroxide (23 mg, 0.4 mmol) was added. The reaction was followed by TLC. After 2 h, total consumption of reactant was observed. Extraction was done with saturated sodium bicarbonate solution and DCM. Organic layer was dried with sodium sulfate. Compound was purified by silica column chromatography using DCM:Hexane (2/1; v/v) solvent mixture as mobile phase. Orange solid product was obtained quantitatively.
^{1}H NMR (400 MHz, Chloroform-d) δ 7.36 (ddd, J = 8.2, 7.2, 1.8 Hz, 1H), 7.10 (dd, J = 7.5, 1.7 Hz, 1H), 7.09 – 6.94 (m, 2H), 5.99 (s, 2H), 5.42 (b, 1H), 2.53 (s, 6H), 1.50 (s, 6H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 156.4, 152.3, 143.3, 135.3, 131.3, 131.1, 129.1, 121.6, 121.5, 120.9, 116.5, 14.6, 13.7.

High resolution ESI-MS values are 341.1637 for theoretical m/z of $(M+H)^+$ and 341.16168 for experimental (Δ: 5.92 ppm).

Compound 7. Compound **6** (200 mg, 0.39 mmol) was dissolved in 5 ml benzene. 4 equivalent 4-pyridine carboxaldehyde (200 µl, 1.6 mmol) was added. And then, 0.4 ml piperidine and 0.4 ml acetic acid were added. The reaction mixture was refluxed at 90°C by using Dean-Stark apparatus. As the green product was formed, the reaction was cooled to room temperature. Extraction was performed with DCM and water. The organic phase was dried over sodium sulfate. Silica column chromatography was performed by using Acetone: Hexane (2/1; v/v) solvent mixture as mobile phase. Due to stability concerns, compound **7** was used immediately after synthesis in further step without further purification and full characterization.
High resolution ESI-MS values are 519.20890 for theoretical m/z of $(M+H)^+$ and 519.21533 for experimental (Δ: 12.38 ppm).

Compound G1-b. 25 mg of compound **7** (25 mg, 48 µmol) was dissolved in 2 ml DMF and 0.5 ml methyl iodide. The reaction was mixed for 2 days at room temperature. The solid product was washed with ethyl acetate to yield dark green G1-b with quantitative yield.
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (bs, 1H, OH), 8.92 (bs, 4H), 8.22 (bs, 4H), 7.94 (d, J = 14.0 Hz, 2H), 7.85 (d, J = 16.4 Hz, 2H), 7.44 – 7.40 (m, 1H), 7.19 (s, 3H), 7.07 (d, J = 8.5 Hz, 2H), 4.33 (s, 6H), 1.61 (s, 6H).
High resolution ESI-MS values are 274.1279 for theoretical m/z of $(M)^{2+}$ and 274.1218 for experimental (Δ: 22.25 ppm).

FRET-Melting Analysis

FRET Melting analysis was performed with HPLC-purified 5' FAM and 3' TAMRA-labelled 0.2 µM oligonucleotides (c-*myc*, c-*kit*, h-*tert*, *bcl-2* and h-*telo*) in a buffer solution (10 mM sodium cacodylate, 0.1 M LiCl, pH 7.2) prepared with MiliQ purified water as in the literature.[1] Sequences of the oligomers are given In Table S1. The experiment was conducted with 1 µM or 10 µM ligand concentrations. The experiment was performed in 96-well plate between the temperatures 25-95 °C by using qPCR (Bio-rad CFX96 Real Time System). After an initial incubation at 25°C for 5 minutes and the temperature was raised by 1 °C for every minute until 95 °C. Labelled oligonucleotides was excited at 492 nm and the donor emission was followed at 516 nm as described in literature.[1]

Table S1. Sequences of oligonucleotides used in FRET-melting assay [1] bcl2 sequence is mapped to -58 to -19 bp upstream of promoter of this gene.[2] c-myc sequence is mapped to -142 to -115 base pairs upstream of the P1 promoter of this gene.[3] hTERT is sequence is located at core promoter region.[4] c-kit sequence used to analyse DNA oligomer stability is mapped to - 87 bp upstream of transcription start site of this gene.[5] h-telo is human telomeric DNA.

Oligomer	Sequence (5' to 3')
h-*telo*	A GGG TTA GGG TTA GGG TTA GGG
c-*myc*	TGG GGA GGG TGG GGA GGG TGG GGA AGG
c-*kit1*	AGG GAG GGC GCT GGG AGG AGG G
h-*tert*	GGG GGC TGG GCC GGG GAC CCG GGA GGG GTC GGG ACG GGG CGG GG
bcl-2	AGG GGC GGG CGC GGG AGG AAG GGG GCG GGA GCG GGG CTG
ds-DNA	CAAAAATTTTTGCAAAAATTTTTG

UV-Vis Spectrophotometric Titration Analysis

To determine selective binding to G-quadruplex bearing nucleic acid fragments 2 µM active ligand (**G1-b**) was dissolved in a buffer solution (10 mM sodium cacodylate, 0.1 M LiCl, pH 7.2) prepared with MiliQ purified water and titrated with either bcl-2 oligonucleotide or ds-DNA. Oligonucleotides have the sequence given in Table S1 however they lack modifications at 3' or 5' ends. UV-Vis Absorbance spectrum of the solution was recorded and change in the absorbance of **G1-b** at 692 nm was plotted. For Bovine Serum Albumin (BSA) titration, protein is added in small aliquots to a 10 µM solution of the stabilizer. Binding constants are calculated from Benesi-Hildebrand plot using inverse slope of the linear equation given in Equation 1

$$A_o/\Delta A = 1/(K_a \cdot \Delta A_0)\,[DNA] + 1 \qquad \text{Equation 1}$$

where ΔA is the change in Absorbance at 692 nm, A_o is the initial absorbance value at the same wavelength.[6]

Cell Viability Assay

MCF-7 cells ($10x10^3$ cells/well) were seeded in 96-well plate using RPMI 1640 media supplemented with 10% FBS and 1% gentamicin at 37 °C in 5% CO_2 in the incubator (Thermo Scientific, Steri-cycle i160 CO_2 Incubator). Cells were treated with different concentrations (0-40 µM) of **G1-a** and incubated for 24 h. Each experiment was repeated four times. After 24 h incubation with the reagent, 10 µl MTT solution (5 mg/ml) was added to each well and mixed gently. After 4 hours, the medium was removed and 100 µl DMSO was added to each well to solubilize the precipitate. The cells viability was estimated by measuring absorbance at 570 nm using a Quant ELISA plate reader (Bio-tek Instruments, USA). Cell viability under low oxygen level is determined using a hypoxia chamber (Modular Incubator Chamber, MIC-101, Billups-Rothenberg Inc.). Cells are fluxed with 0.5% O_2, 5% CO_2, and 94.5% N_2 gas mixture for 6 minutes with 17 L/min flux.[7] Then the chamber was sealed and incubated at 37 °C for 24 h. **G1-a** was introduced at

different concentrations (0-40 μM). Following 1h hypoxic gas flux as described above, chamber was sealed, and cells are incubated further at 37 °C. MTT solution was added, and viability analysis was performed using the same procedure.

Gene Expression Analysis

MCF-7 cells ($5x10^6$) were incubated under normoxic or hypoxic conditions as described above prior to experiment. Cells were treated with (10 μM and 20 μM) or without **G1-a** and then incubated under normoxic or hypoxic atmosphere for additional 24 h. RNAs were isolated from the cells by using GeneJET RNA purification kit (Thermo Scientific, GeneJet, K0731) following the recommended protocol. The amount and purity of isolated RNAs was calculated by using NanoDrop (Epoch 2 Microplate Reader). cDNAs were synthesized prior to RT-qPCR analysis by using PrimeScript 1st strand cDNA synthesis kit (Takara: 6110A-50) following the protocol provided by the kit. RT-qPCR was performed with synthesized cDNA samples using primers for *hif-1α*, c-*myc*, *bcl-2* and GAPDH listed in Table S2 and SYBR Green Super mix (Bio-Rad).[4] RT-qPCR protocol was performed as initial denaturation at 95°C for 30 seconds, denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. Annealing and extension steps were performed for 39 cycles. RT-qPCR analysis was evaluated using Bio-Rad CFX ConnectTM Real-Time System. All experimental data were repeated three times. The RT-qPCR analysis was performed using GAPDH under normoxia as internal control by using the $2^{(-\Delta\Delta Ct)}$ method. The comparisons between groups have been assessed in RT2ProfilesTM PCR Array Data Analysis, which is statistically evaluated using 'Student t-test'.

Table S2. Sequences of primers used in PCR [8,9]

Gene	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
c-*myc*	CCT GGT GCT CCA TGA GGA GAC	CAG ACT CTG ACC TTT TGC CAG G
bcl-2	ATC GCC CTG TGG ATG ACT GAG T	GCC AGG AGA AAT CAA ACA GAG GC
hif-1α	TAT GAG CCA GAA GAA CTT TTA GGC	CAC CTC TTT TGG CAA GCA TCC TG
TBP	TGCCTCCAGAATATGCCTCT	CAATGGTTTTCAAGCTTTCCA
GAPDH	GAA GGT GAA GGT CGG AGT C	GAA GAT GGT GAT GGG ATT TC

Results and Discussion

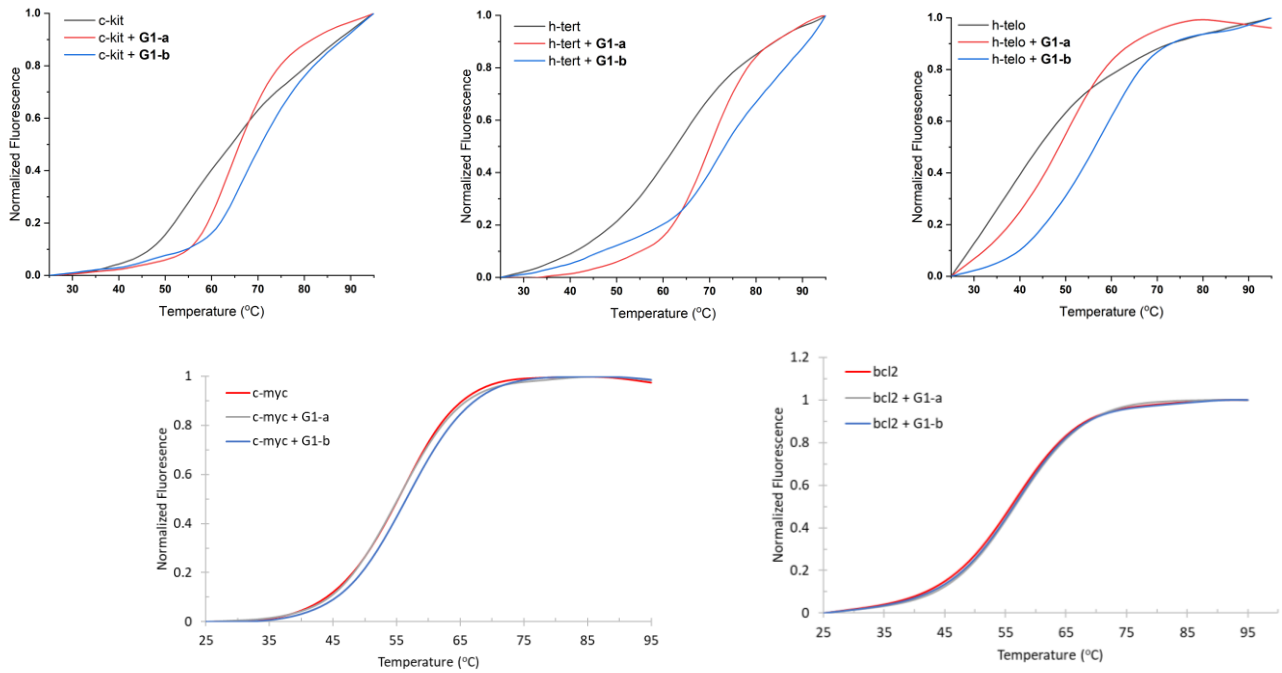


Figure S1. FRET-melting curves of 0.2 μM DNA oligomer c-kit, h-tert or h-*telo* alone (black) or in the presence of 10 μM inactive (G1-a) or active forms (G1-b) of G-quadruplex stabilizers (red and blue respectively). 1 μM of active and inactive forms are used to obtain the curves of 0.2 μM DNA oligomer. T_m of the c-myc increases by 1°C whereas no change in bcl2 T_m was detected at this lower concentration. Results suggest that to have significant binding higher concentrations of the stabilizers are necessary. Phenyl moiety present at the *meso* position of BODIPY may still interfere with G-quadruplex binding even the bulky substituent is removed.

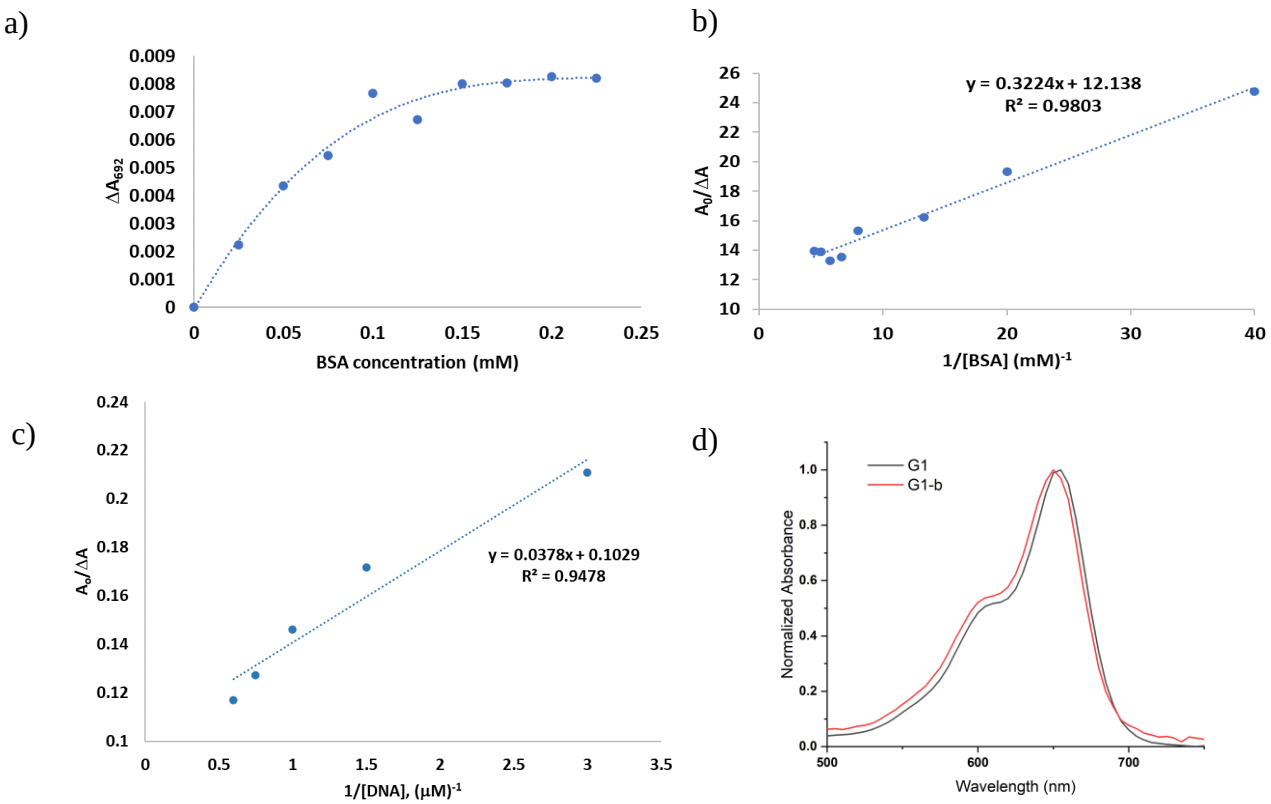


Figure S2. Change in the absorption of active ligand **G1-b** (10 μM) upon titration with BSA (a) and Benesi-Hildebrand plot of the same titration (b). Benesi-Hildebrand plot of **G1-b** (2 μM) titrated with bcl-2 oligomer (c). All experiments are done in in cacodylate buffer at pH 7.2. UV-Vis absorbance spctra of **G1-a** (black) and **G1-b** (red) in water (d).

When change in T_m values shown in Table 1 are considered, c-myc is the most stabilized oligomer and c-kit is the least stabilized one. This difference cannot be attributed to topologies of G-quadruplexes because both of them are reported to have parallel type topologies.[10] So, change in stabilization can be a result of exact G-quadruplex sequence, sequences at the loop regions instead of topology.

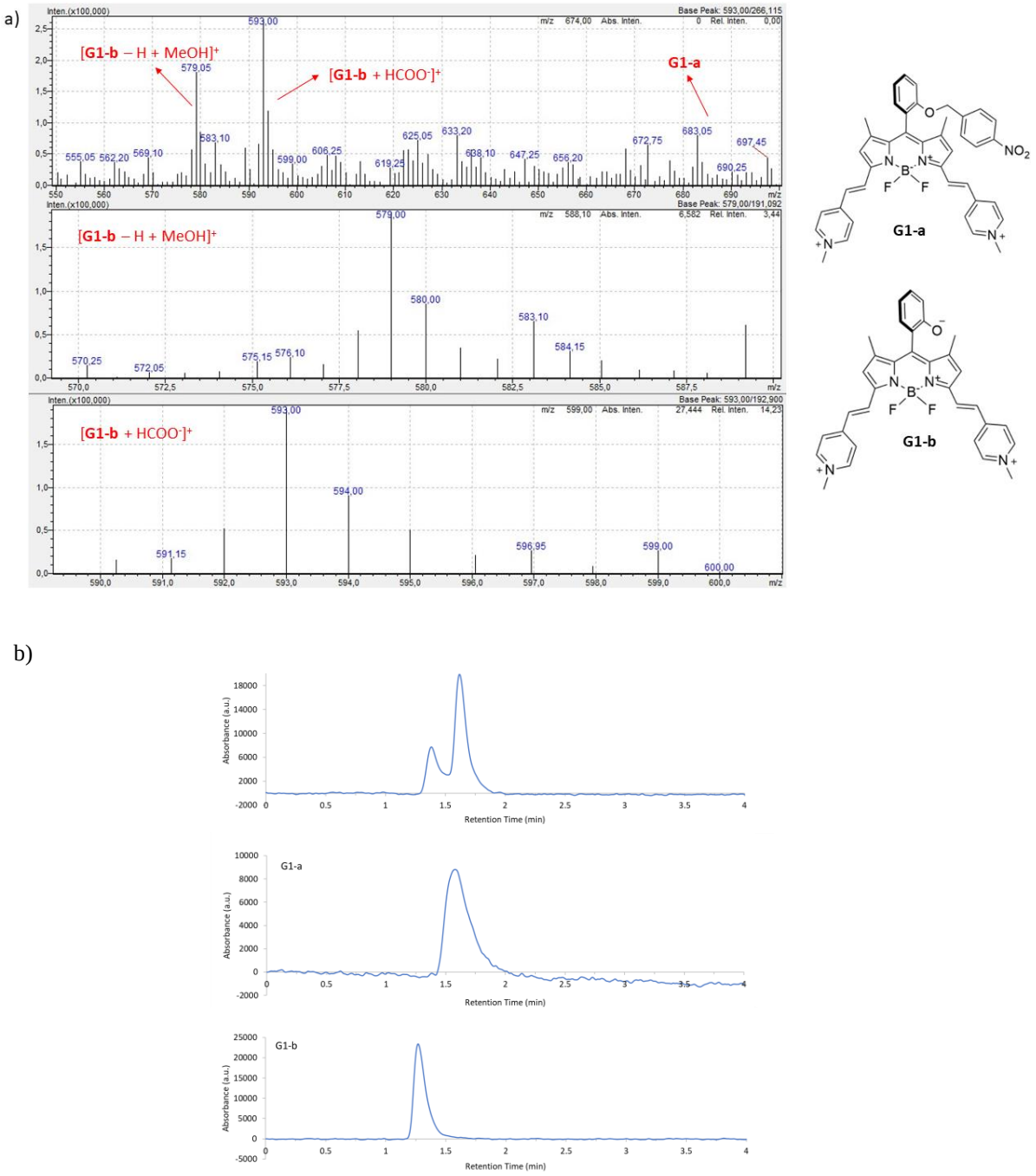


Figure S3. a) MS spectra (left) of **G1-a** (0.5 mM) incubated with nitroreductase enzyme (3 U/ml) and NADH (3 mM) at 37°C in PBS buffer (pH 7.4) for 2h. Sample was diluted with methanol and filtered using 0.45 μm PTFE syringe filter. Peaks corresponding to parent compound (**G1-a**) and reduced-eliminated product (**G1-b)** are labelled. b) HPLC (Shimadzu Prominence-i, LC-2030C Plus) retention times of **G1-a**, **G1-b** and **G1-a** (100 μM) incubated with nitroreductase enzyme (40 U/ml) in the presence of 100 μM NADH for 2 h at 37°C (right). After incubation, sample was diluted to 10 μM in acetonitrile and filtered before analysis. 60% acetonitrile, 39.8% H_2O and 0.2% acetic acid was used as mobile phase on a C18 column (GL Sciences AQ-C18). Sample was run using 1 ml/min flow rate and detected by following absorption at 650 nm.

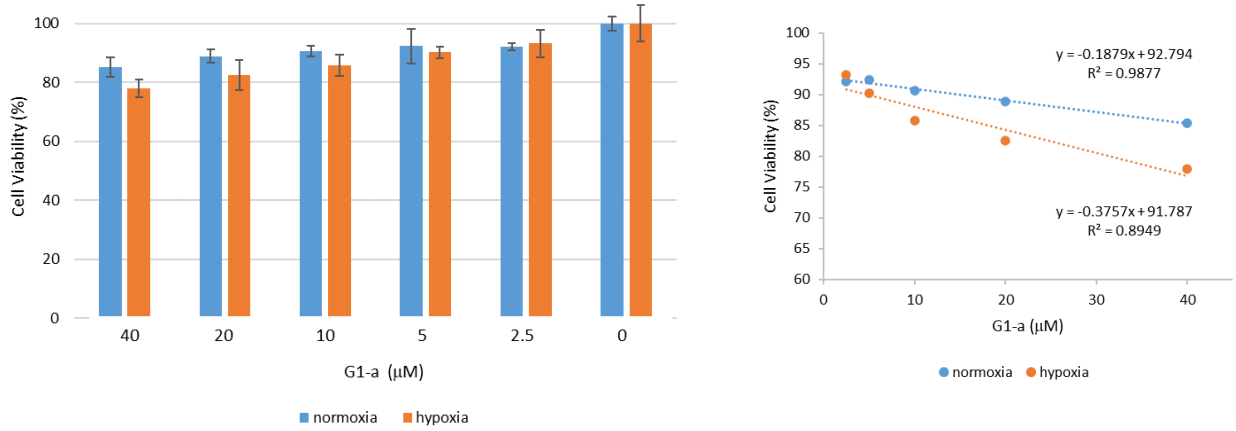


Figure S4. Viability of MCF7 cells incubated under normoxic (blue) and hypoxic (orange) conditions in the presence of various concentrations of **G1-a** (a) and linear regression of the same result (n=4).

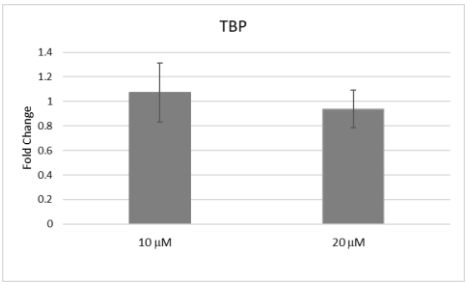
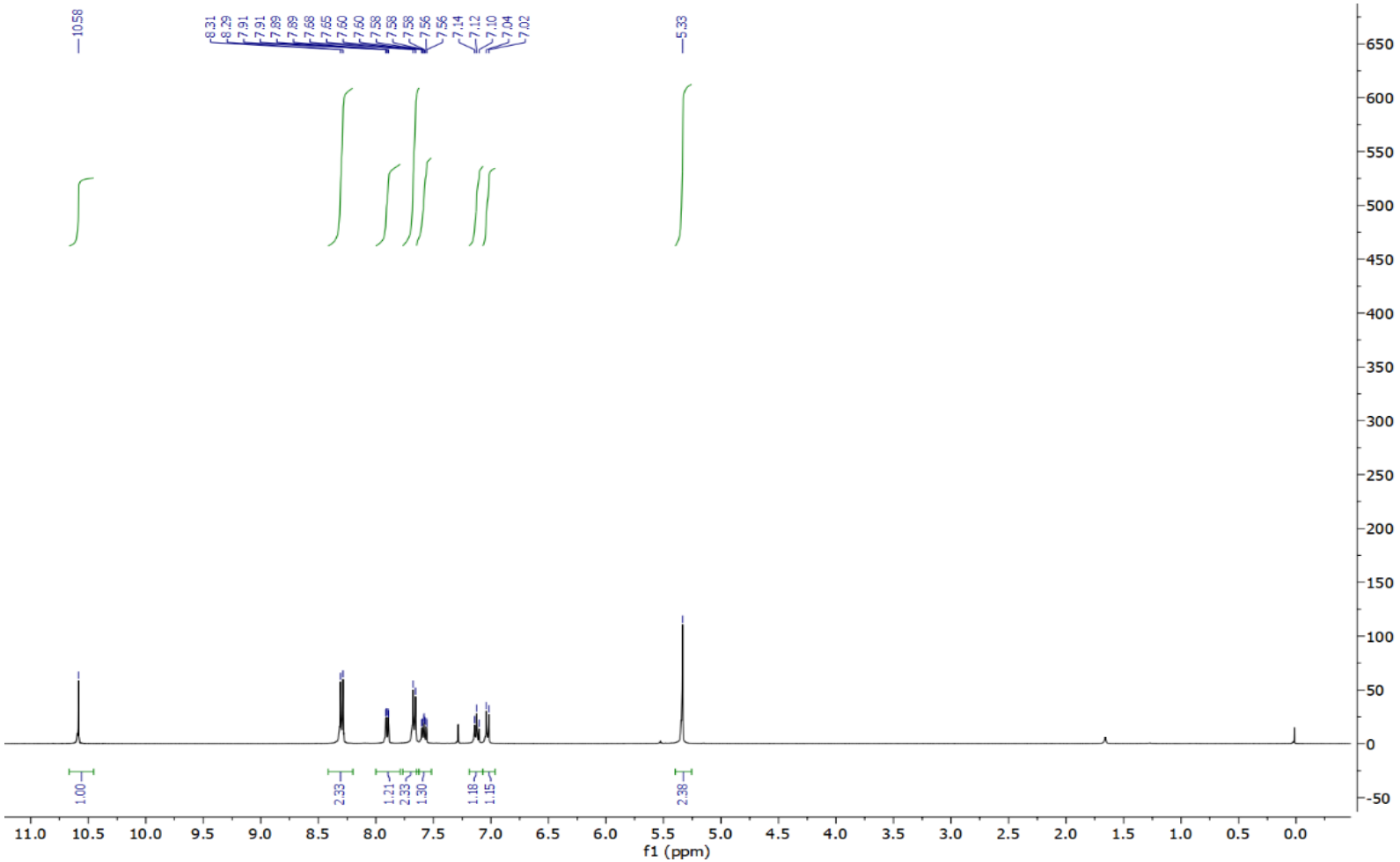
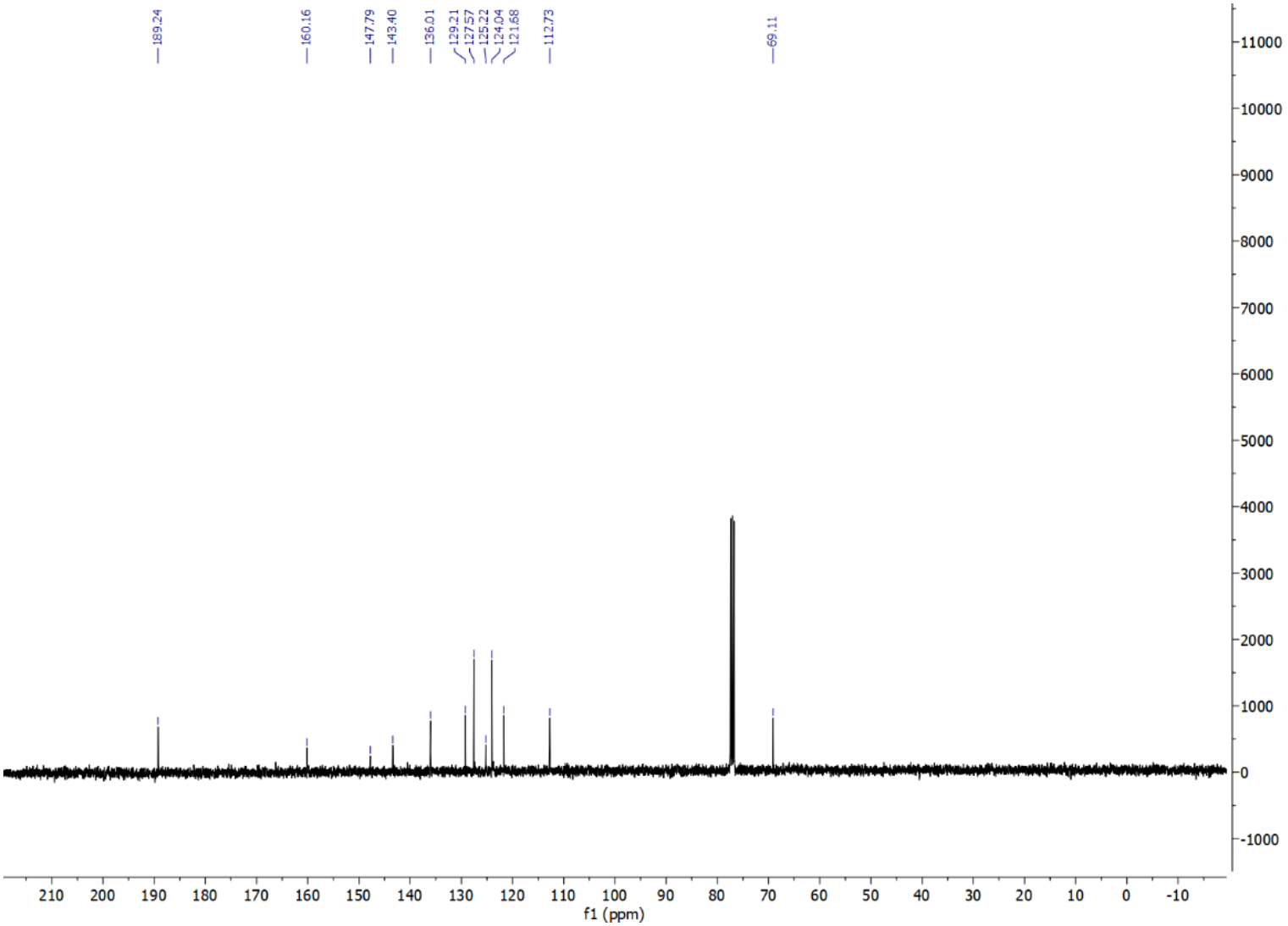


Figure S5. Fold increase in the expression TBP in hypoxic MCF7 cells in the presence of **G1-a** (10 μM or 20 μM). To investigate the effect of **G1-a** on the genes lacking G-quadruplex structure TBP expression level was analysed. Expression of the gene is also reported to unaffected under hypoxia.[9] GAPDH is used as internal control. All values are normalized to untreated hypoxic sample results. There is not a significant change in the expression of the gene in the presence of the compound.

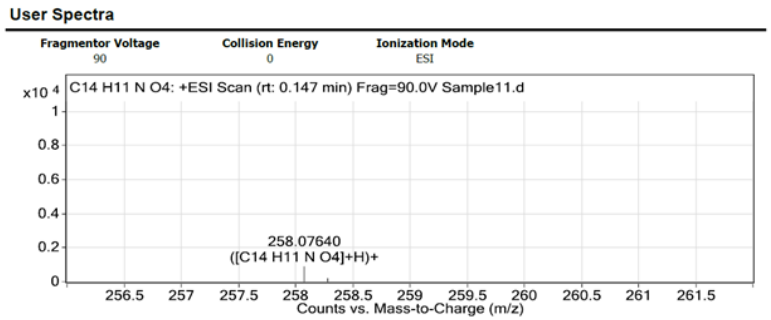
NMR and High-Resolution Mass Characterization of Compounds



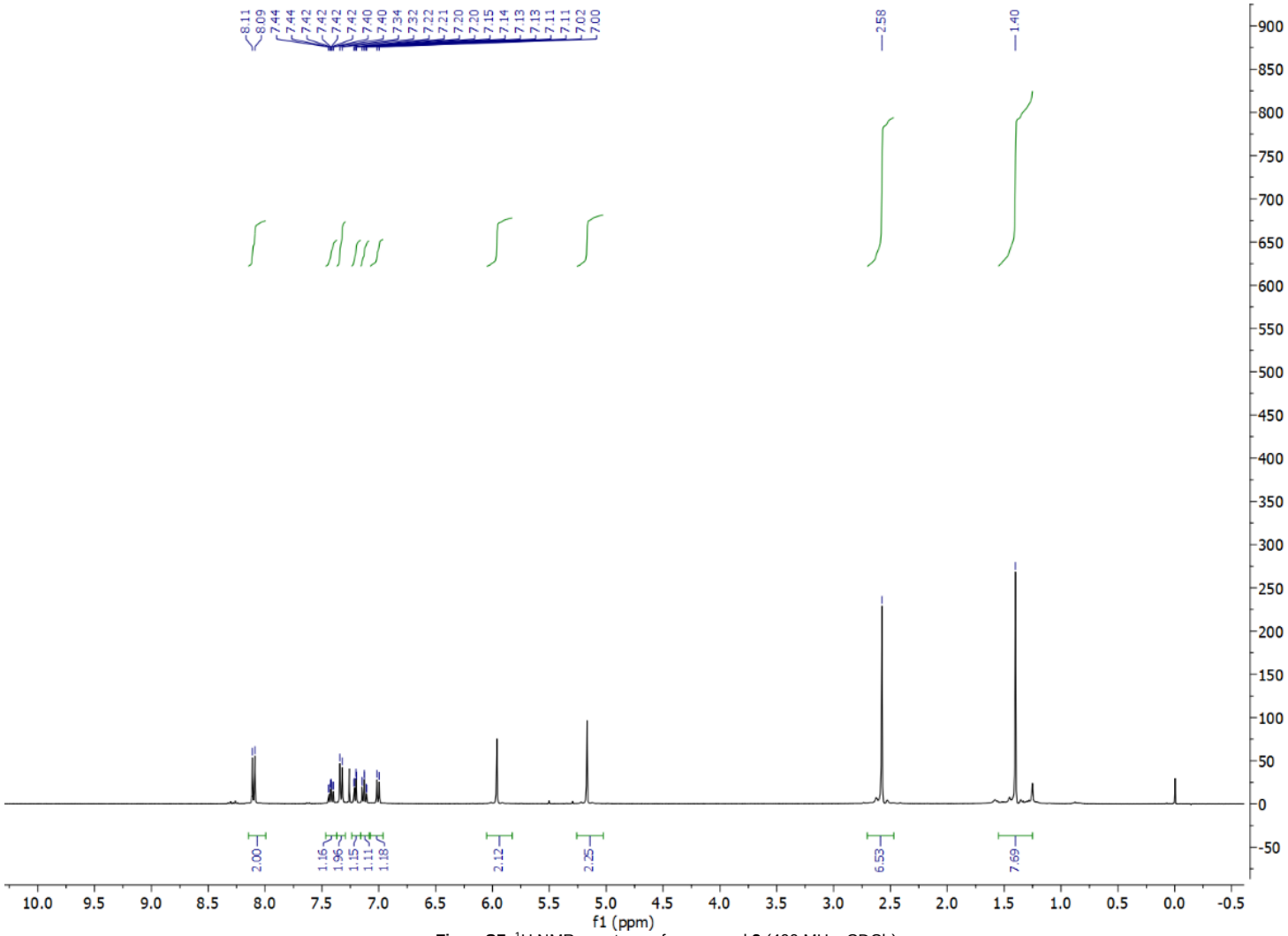
FigureS4. ^{1}H NMR spectrum of compound **1** (400 MHz, $CDCl_3$).



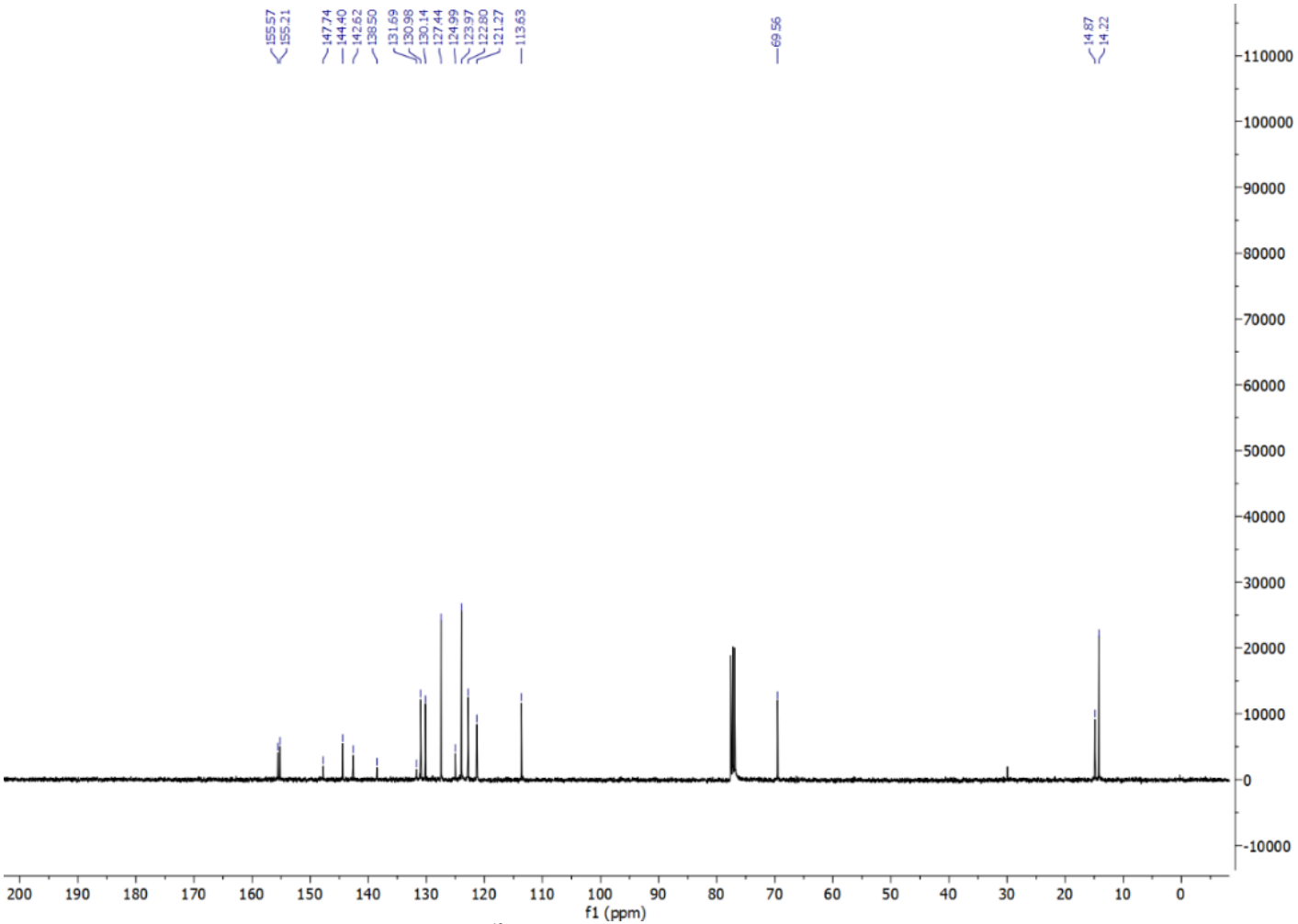
FigureS5. ^{13}C NMR spectrum of compound **1** (101 MHz, $CDCl_3$).



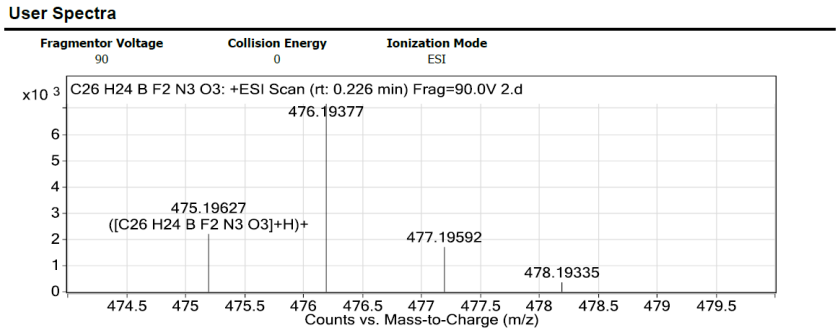
FigureS6. High-Resolution ESI-MS spectrum of compound **1**.



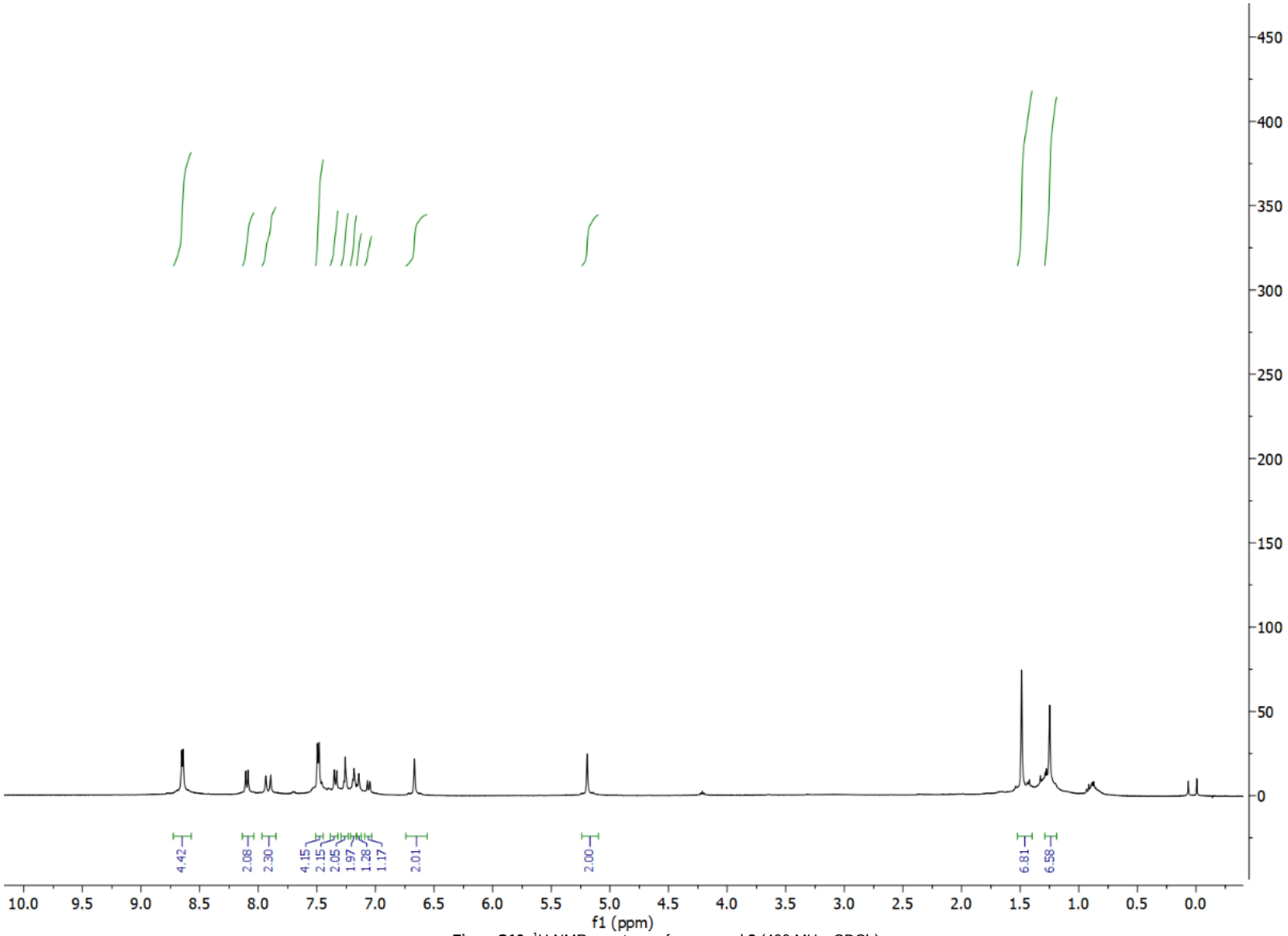
FigureS7. ^{1}H NMR spectrum of compound **2** (400 MHz, $CDCl_3$).



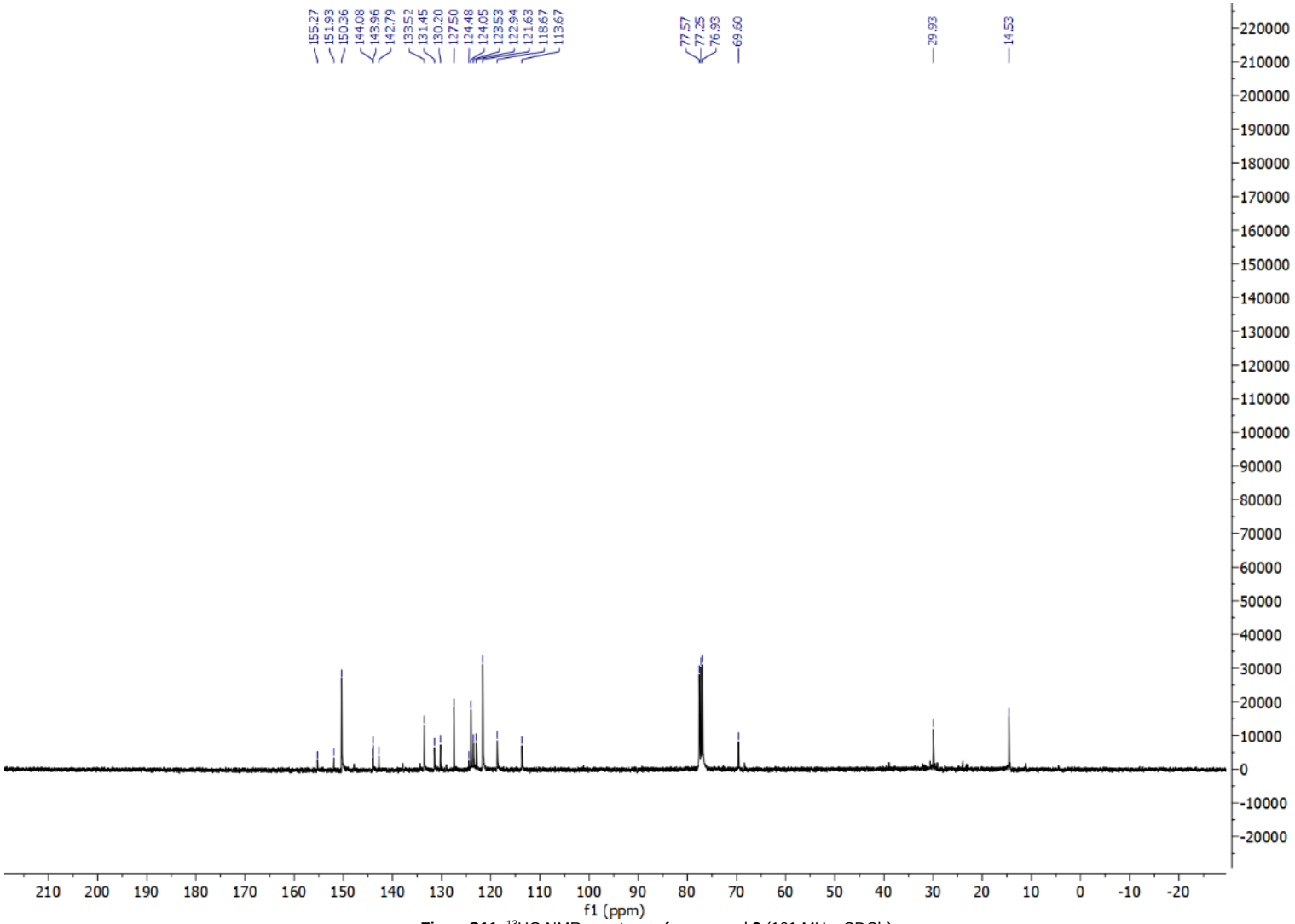
FigureS8. ^{13}HC NMR spectrum of compound **2** (101 MHz, $CDCl_3$).



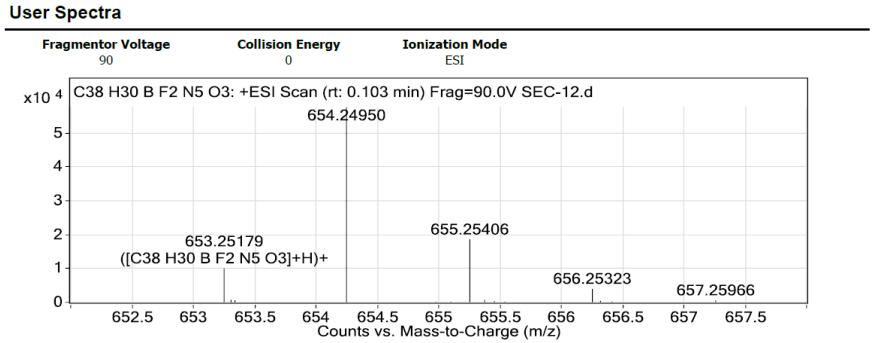
FigureS9. High-Resolution ESI-MS spectrum of compound **2**.



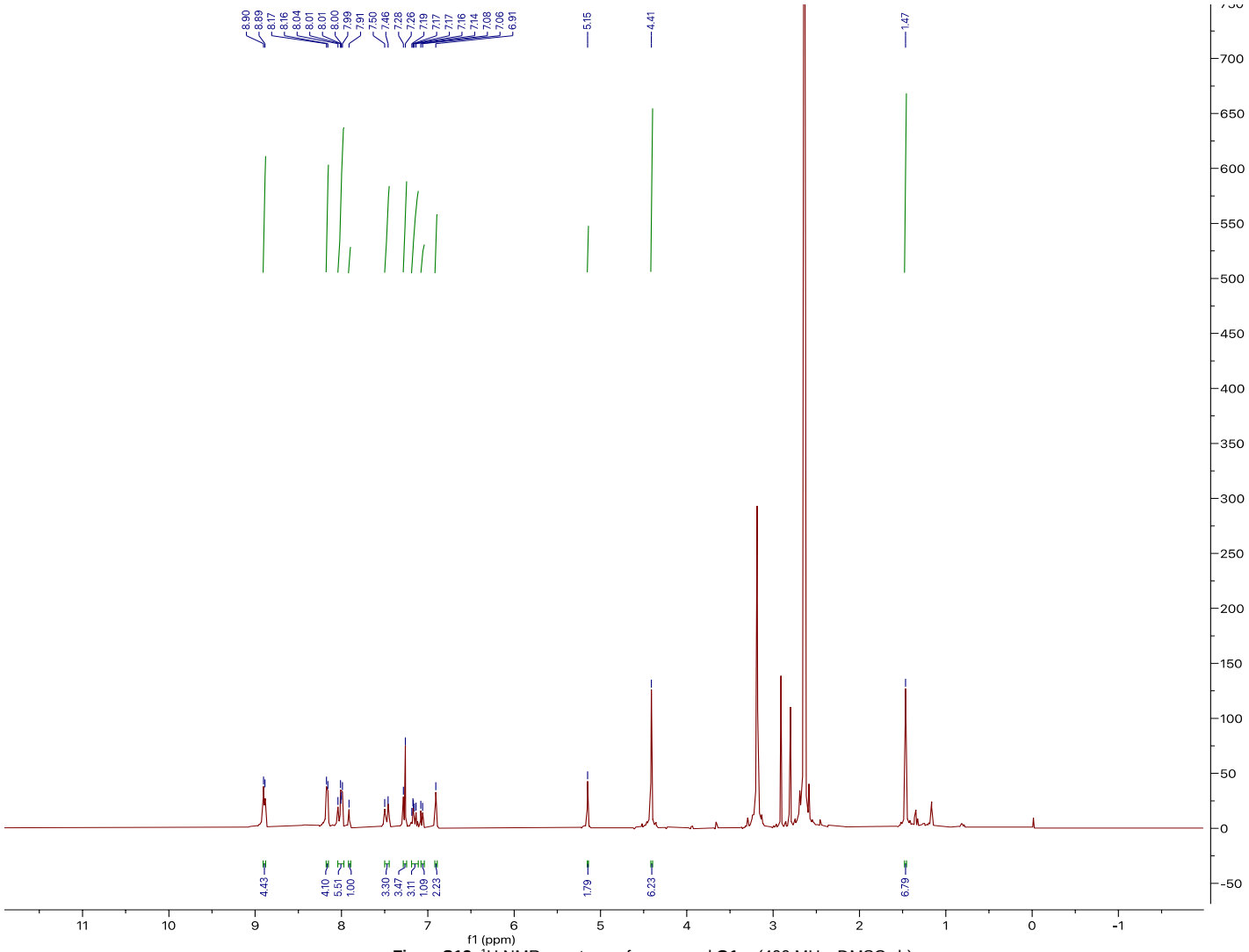
FigureS10. ^{1}H NMR spectrum of compound **3** (400 MHz, $CDCl_3$).



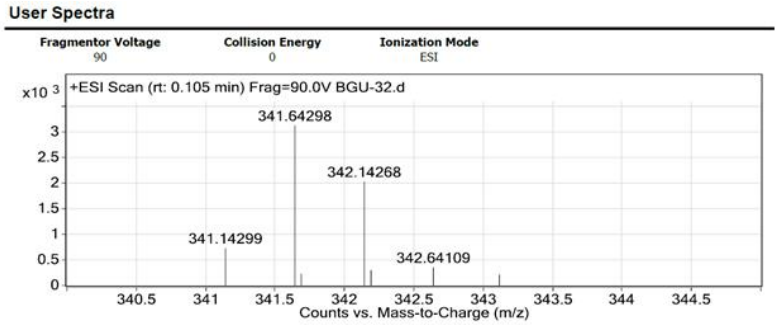
FigureS11. ^{13}HC NMR spectrum of compound **3** (101 MHz, $CDCl_3$).



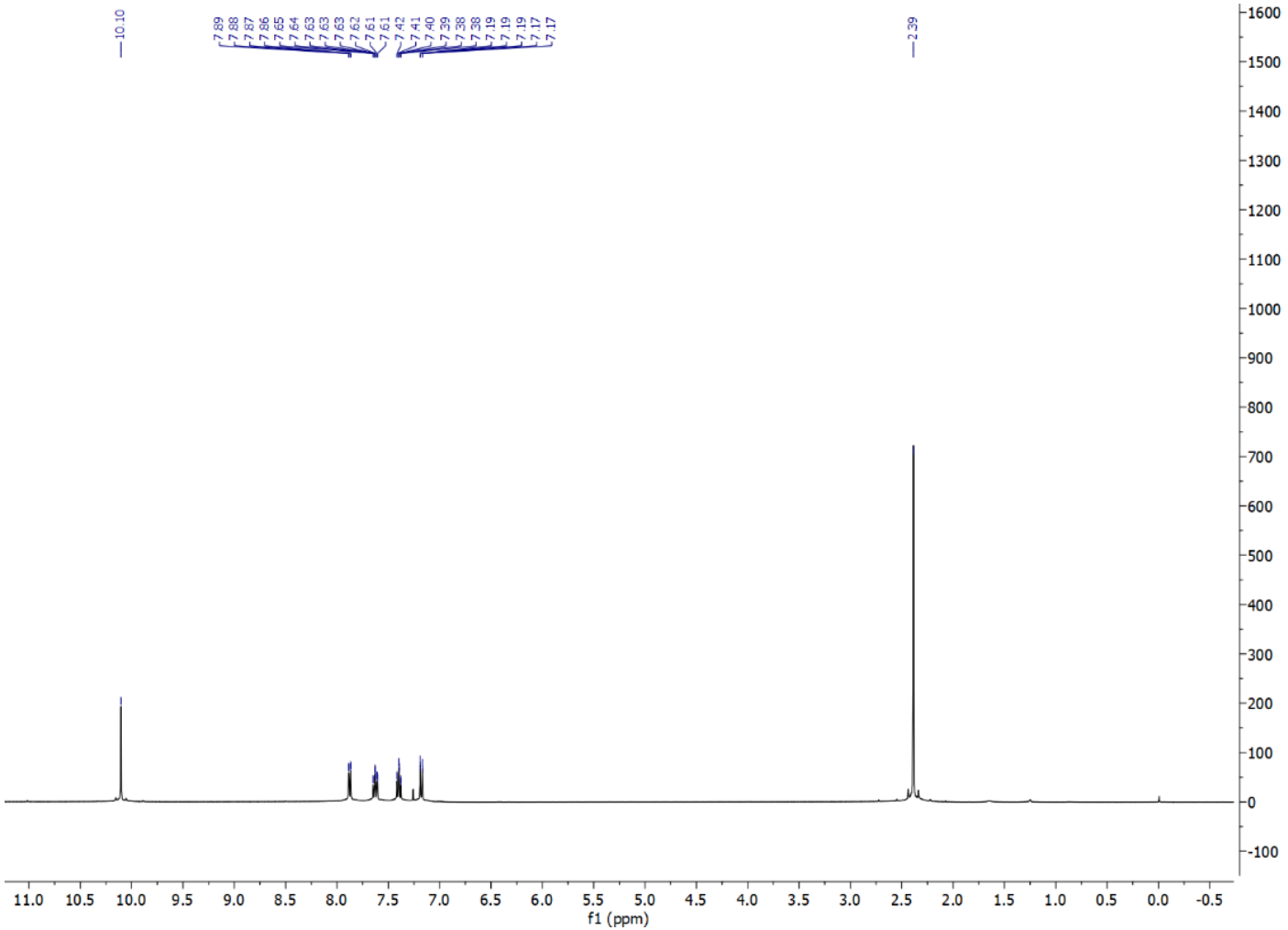
FigureS12. High-Resolution ESI-MS spectrum of compound **3**.



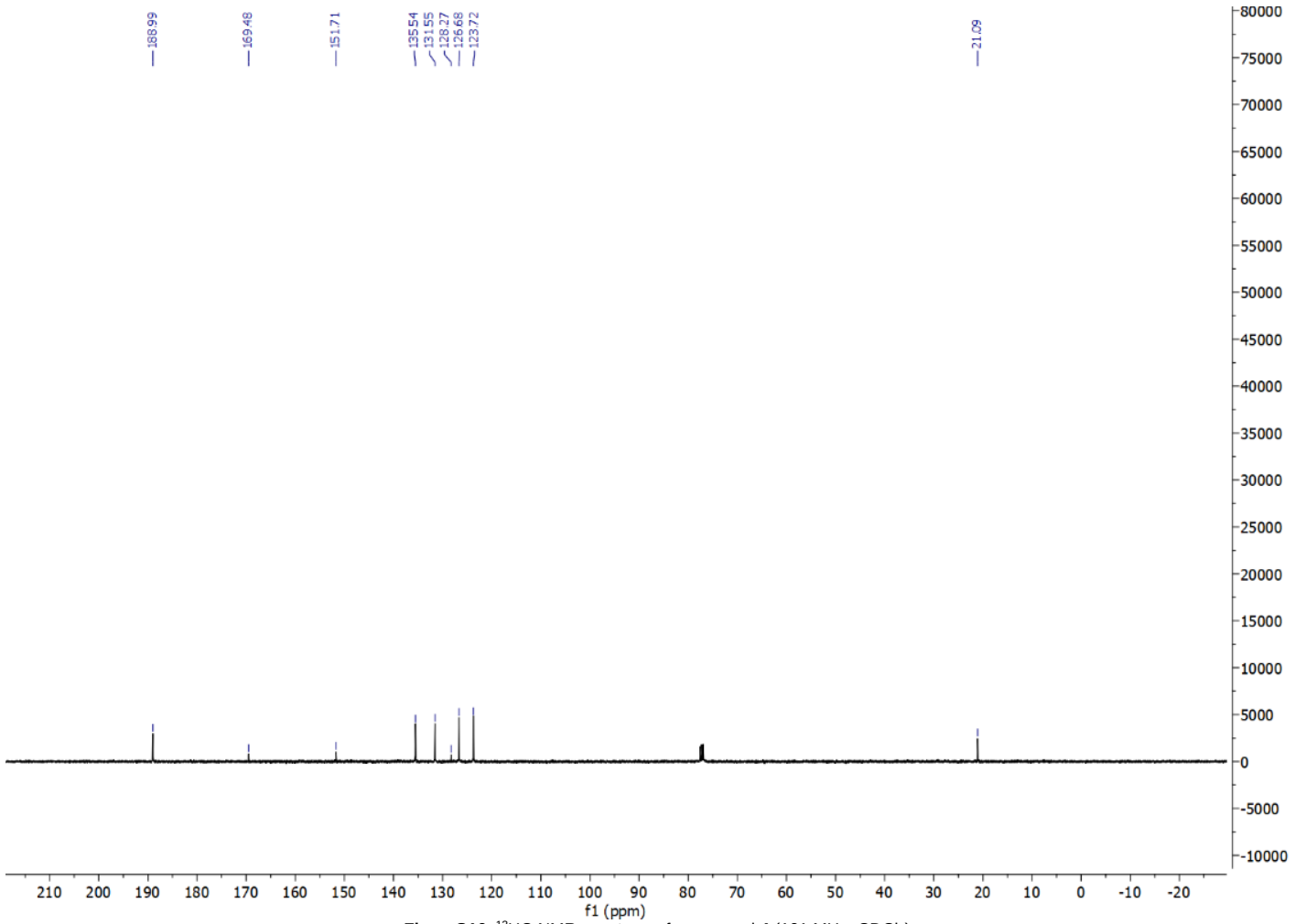
FigureS13. ^{1}H NMR spectrum of compound **G1-a** (400 MHz, DMSO-d_6).



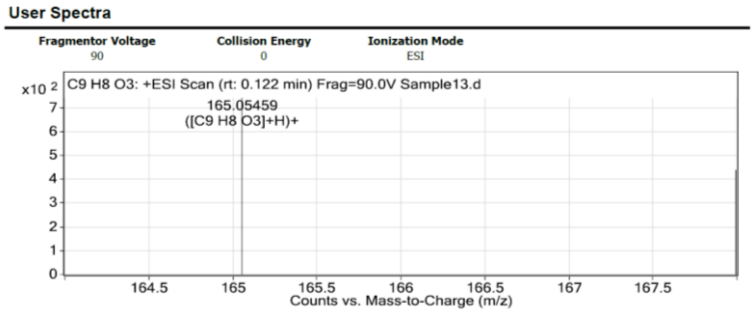
FigureS14. High-Resolution ESI-MS spectrum of compound **G1-a**.



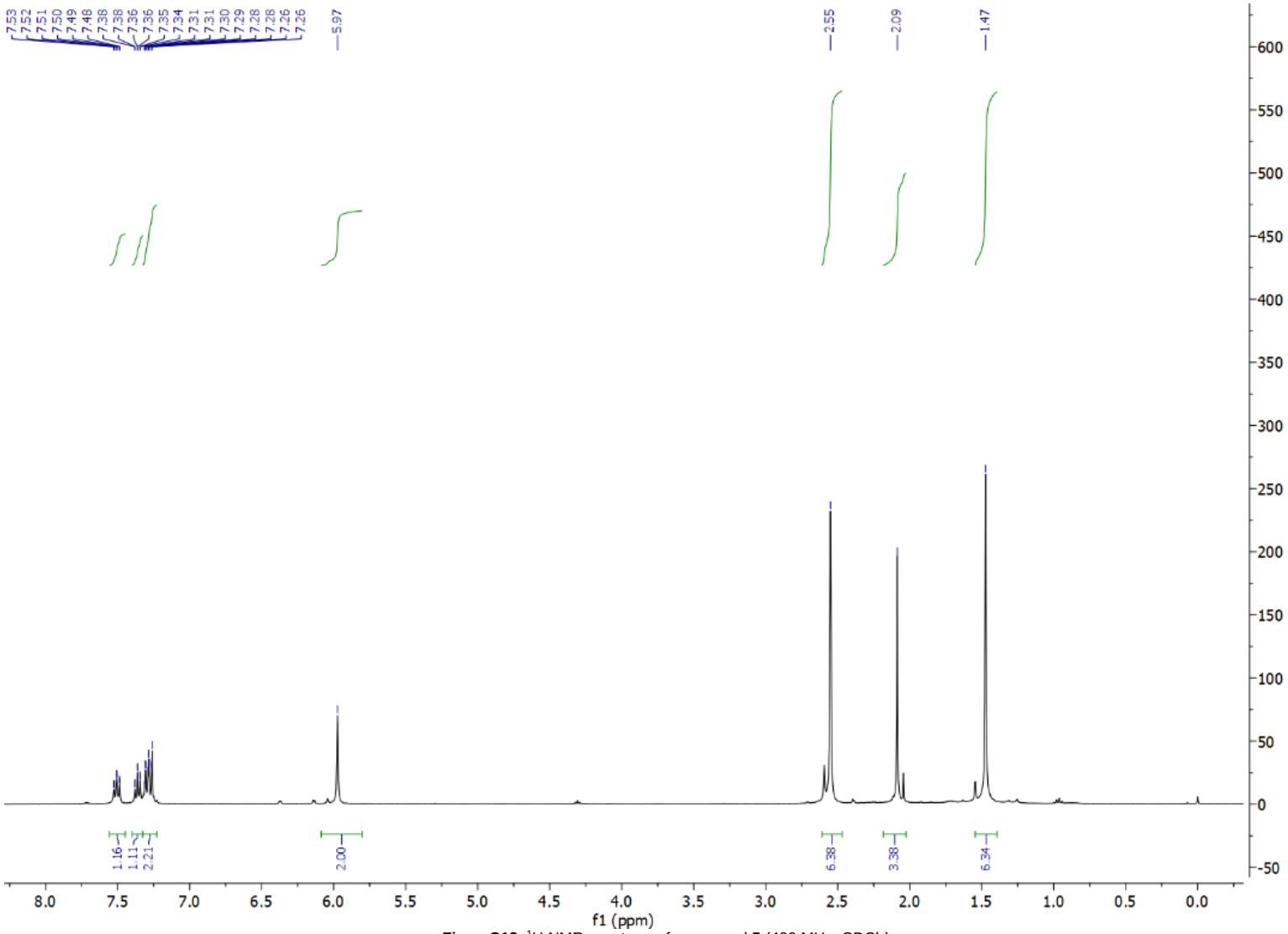
FigureS15. ^{1}H NMR spectrum of compound **4** (400 MHz, $CDCl_3$).



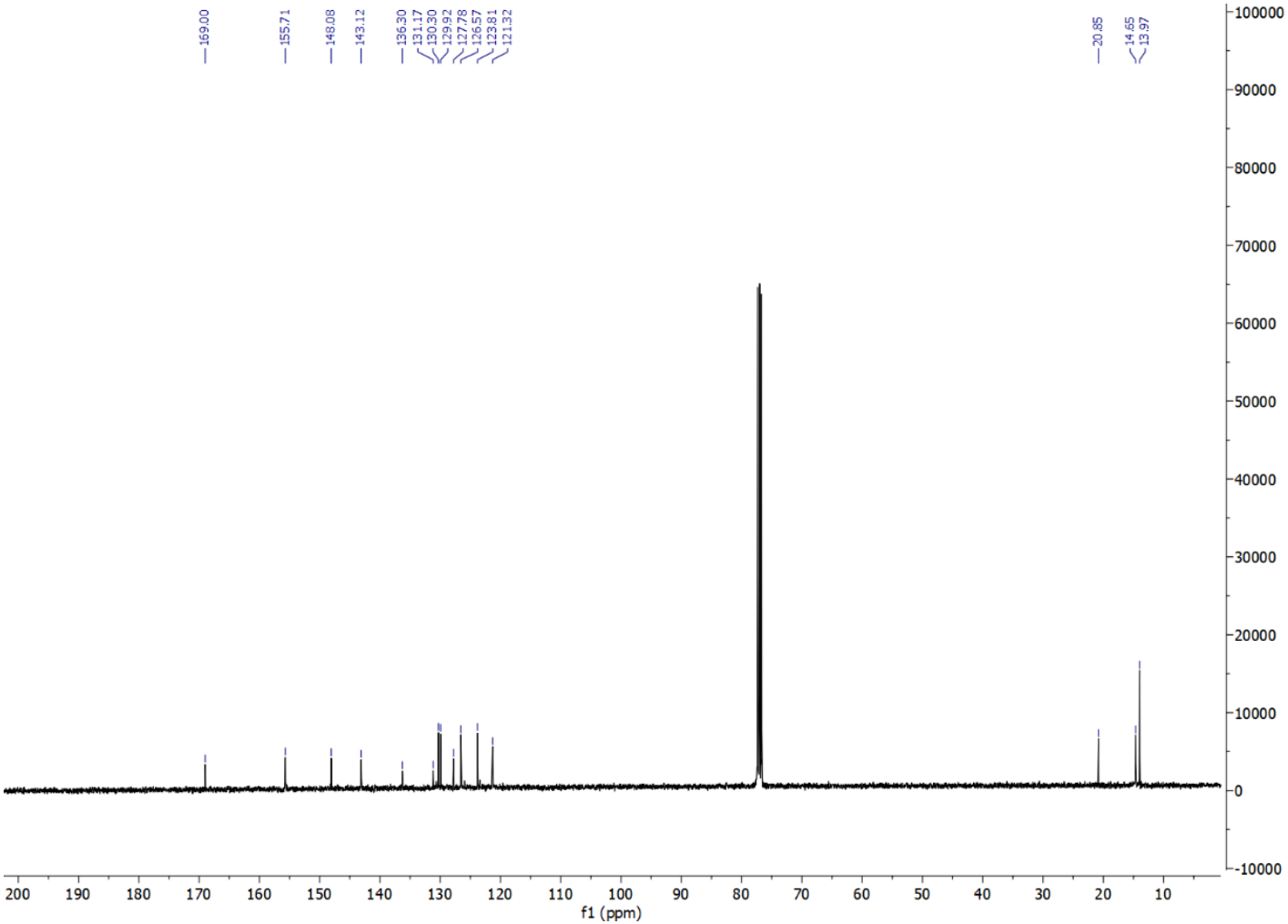
FigureS16. ^{13}HC NMR spectrum of compound **4** (101 MHz, $CDCl_3$).



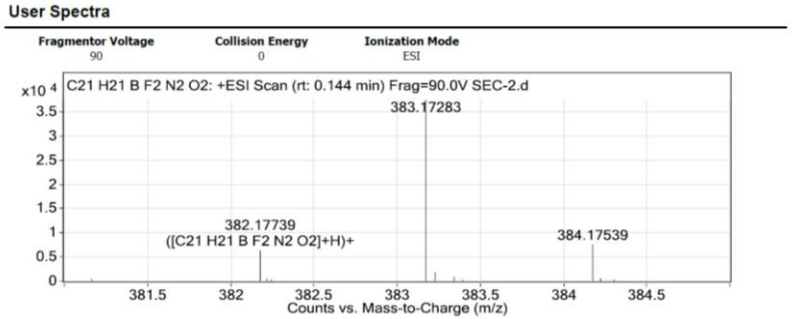
FigureS17. High-Resolution ESI-MS spectrum of compound **4**.



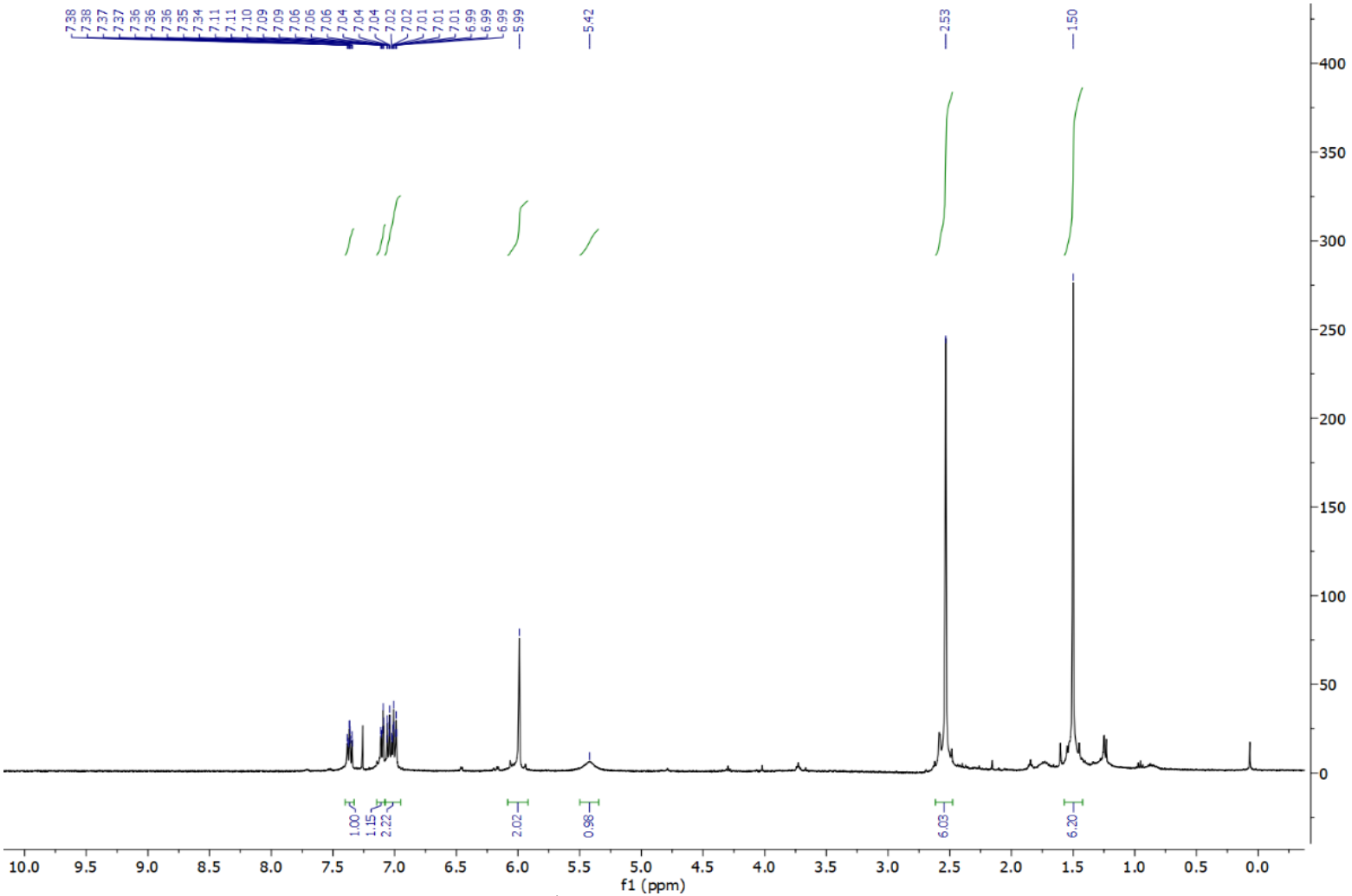
FigureS18. ^{1}H NMR spectrum of compound **5** (400 MHz, $CDCl_3$).



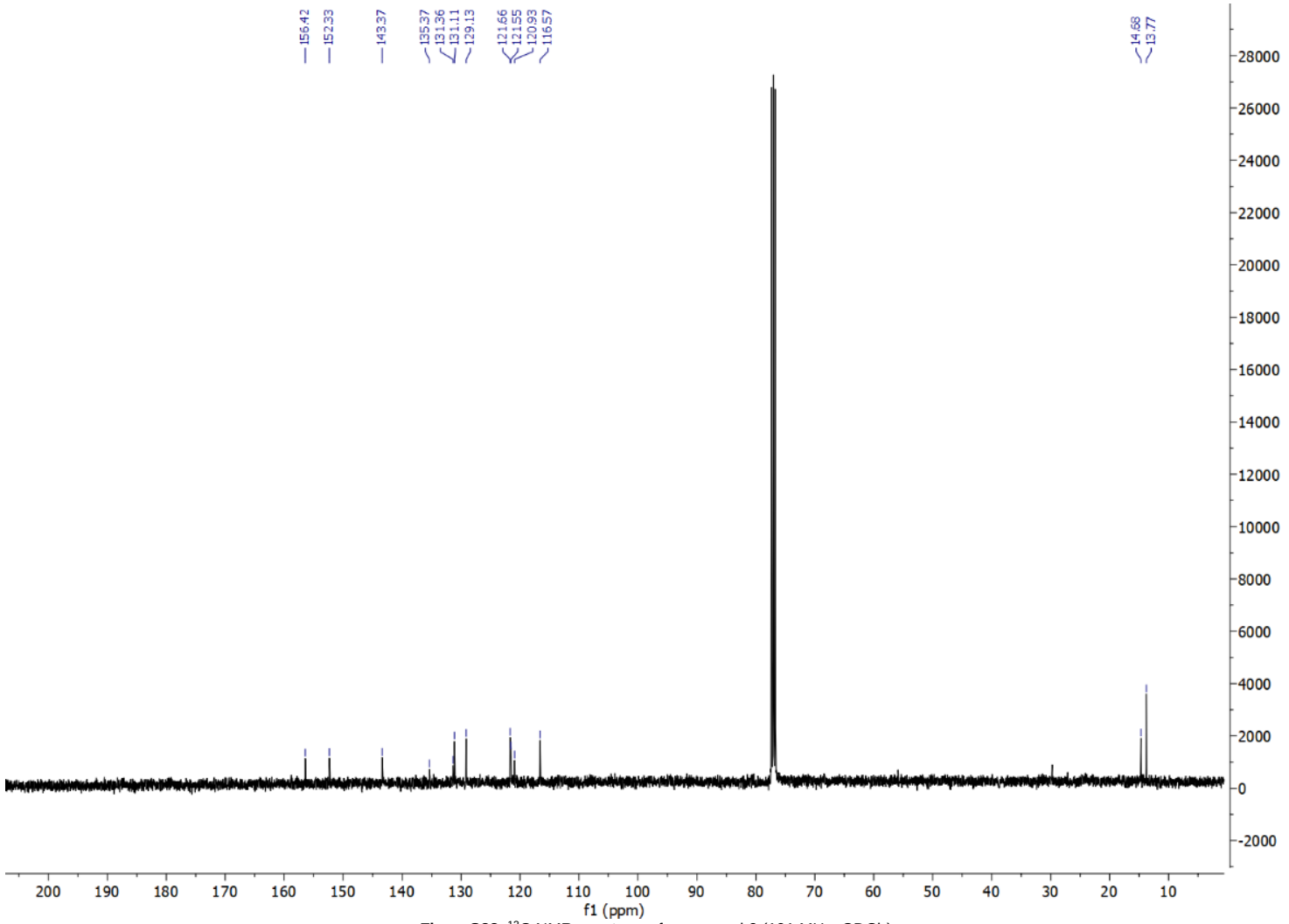
FigureS19. ^{13}C NMR spectrum of compound **5** (101 MHz, $CDCl_3$).



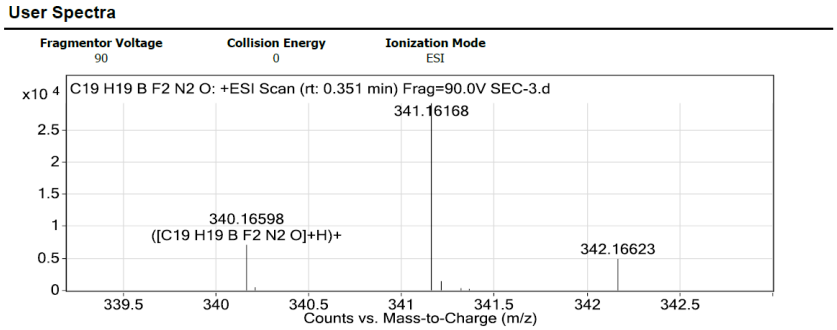
FigureS20. High-Resolution ESI-MS spectrum of compound **5**.



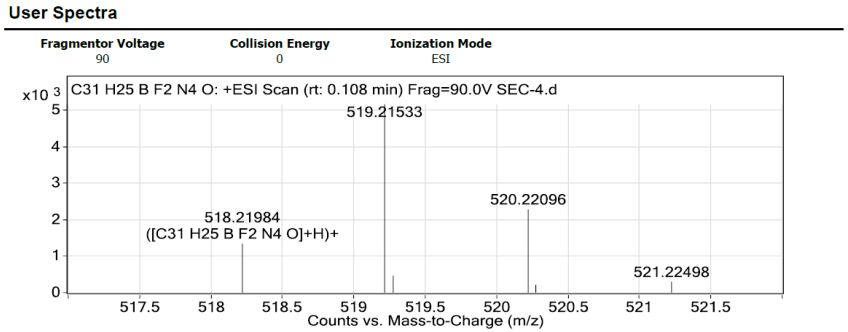
FigureS21. ^{1}H NMR spectrum of compound **6** (400 MHz, $CDCl_3$).



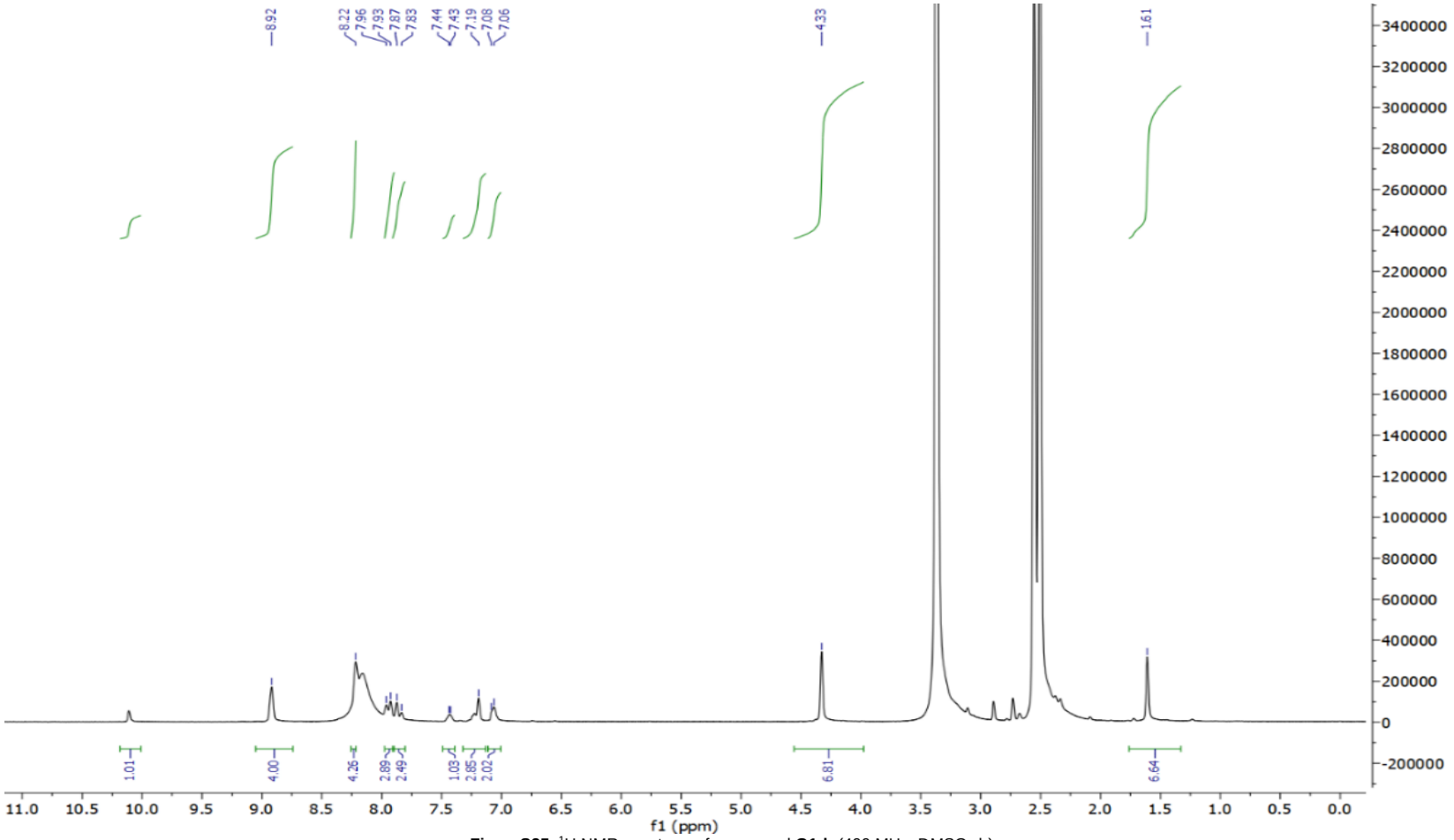
FigureS22. ^{13}C NMR spectrum of compound **6** (101 MHz, $CDCl_3$).



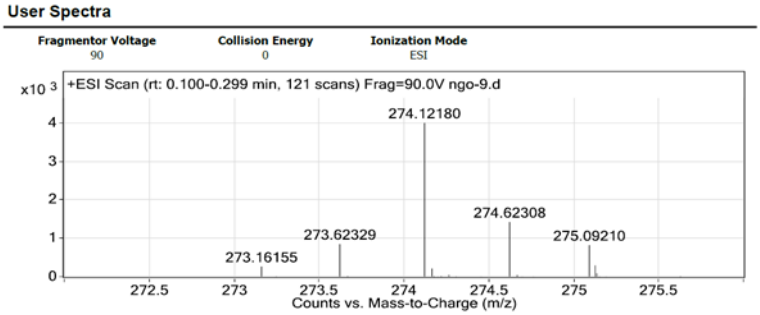
FigureS23. High-Resolution ESI-MS spectrum of compound **6**.



FigureS24. High-Resolution ESI-MS spectrum of compound **7**.



FigureS25. ^{1}H NMR spectrum of compound **G1-b** (400 MHz, DMSO-d_6).



FigureS26. High-Resolution ESI-MS spectrum of compound **G1-b**.

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Author Contributions

B.U., N.G.O. synthesised the compounds and performed FRET-melting analysis. N.G.O, F.S.C., I.O. performed cell culture studies. S.E.C. coordinated the research and prepared the manuscript. All authors discussed the results. B.U., N.G.O. and F.S.C. contributed equally. Authors acknowledge G. Kayadibi Koygun for the support in preliminary experiments.