

Design, synthesis and biological evaluation of 1,2,3-triazole based 2-aminobenzimidazoles as novel inhibitors of LasR dependent Quorum Sensing in *Pseudomonas aeruginosa*

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Experimental section

1. Materials and methods

All chemical reagents and solvents are purchased from Aldrich, Alfa Aesar, Finar. The solvents and reagents were of LR grade. All the solvents were dried and distilled before use. Thin-layer chromatography (TLC) was carried out on aluminium-supported silica gel plates (Merck 60 F254) with visualization of components by UV light (254 nm). Column chromatography was carried out on silica gel (Merck 100-200 mesh). ¹H NMR and ¹³C NMR spectra were recorded at 400 MHz and 101 MHz respectively using a Bruker AV 400 spectrometer (Bruker CO., Switzerland) in CDCl₃ and DMSO-*d*₆ solution with tetramethylsilane as the internal standard and chemical shift values (δ) were given in ppm. ¹H NMR spectra were recorded in CDCl₃ or DMSO-*d*₆. The following abbreviations are used to designate multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Melting

points were determined on an electro thermal melting point apparatus (Stuart-SMP30) in open capillary tubes and are uncorrected. Elemental analyses were performed by Elementar Analysensysteme GmbH vario MICRO cube CHN Analyzer. Mass spectra (ESI-MS) were recorded on Shimadzu MS/ESI mass spectrometer.

2. General Procedure and Spectral Data

N-(1*H*-benzo[d]imidazol-2-yl)-2-chloroacetamide (**3a**): A stirred solution of 1*H*-benzo[d]imidazol-2-amine (**2a**) (1 g, 7.51 mmol) and pyridine (1.51 mL, 18.76 mmol) in dichloromethane was cooled to 0 °C and chloroacetyl chloride (0.60 mL, 7.51 mmol) was added and stirred for 12h at rt. Once completion of the reaction, as indicated by TLC, the reaction mixture was washed with water and organic layer was dried over sodium sulphate, concentrated under reduced pressure and the crude residue was purified by column chromatography using 60-75% ethyl acetate in hexane as eluents to get compound **3a** (1.3 g, 83%) as White solid. ESI-MS: (*m/z*) calcd for C₉H₈ClN₃O: 209.63, found 210.15 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.08 (s, 2H), 7.09-7.46 (m, 4H), 4.37 (s, 2H) ¹³C NMR (100 MHz, CDCl₃) 167.70, 147.43, 135.76, 121.98, 114.34, 43.92; Anal. calcd for C₁₆H₁₃ClN₂O: (%) C, 51.56; H, 3.85; Cl, 16.91; N, 20.04; O, 7.63 Found: C, 51.61; H, 3.81; Cl, 16.94; N, 20.00; O, 7.59.

2-chloro-*N*-(5,6-dimethyl-1*H*-benzo[d]imidazol-2-yl)acetamide (**3b**): A stirred solution of 5,6-dimethyl-1*H*-benzo[d]imidazol-2-amine (**2b**) (1 g, 6.21 mmol) and pyridine (1.25 mL, 15.52 mmol) in dichloromethane was cooled to 0 °C and chloroacetyl chloride (0.49 mL, 6.21 mmol) was added and stirred for 12 h at rt. Once completion of the reaction, as indicated by TLC, the reaction mixture was washed with water and organic layer was dried over sodium sulphate, concentrated under reduced pressure and the crude residue was purified by column chromatography using 60 % ethyl acetate in hexane as eluents to get compound **3b** (1.1 g, 75%) as White solid. ESI-MS: (*m/z*) calcd for C₁₁H₁₂ClN₃O: 237.69, found 238.20 (M+H)⁺. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.07 (s, 2H), 7.16 (s, 2H), 4.38 (s, 2H), 2.25 (s, 6H) ¹³C NMR (100 MHz, CDCl₃) 167.49, 147.38, 135.37, 121.08, 113.99,

43.85, 20.07; Anal. calcd for C₁₁H₁₂ClN₃O: (%) C, 55.59; H, 5.09; Cl, 14.92; N, 17.68; O, 6.73 Found: C, 55.70; H, 5.01; Cl, 14.87; N, 17.68; O, 6.75.

N-(1*H*-benzo[*d*]imidazol-2-yl)-2-iodoacetamide (**4a**): KI (1.24 g, 7.46 mmol) was added to the stirred solution of compound **3a** (1.3 g, 6.22 mmol) in acetone:water (8:2 ratio, should get clear solution) and heated to 55 °C for 12 h. Once completion of the reaction, as indicated by TLC the reaction mixture, acetone was removed from the reaction mixture and washed with water and organic layer was dried over sodium sulphate, concentrated under reduced pressure and the crude residue was titrated with 3% dichloromethane in hexane to yield compound **4a** (1.5 g, 80%) as off-white solid. The solid compound was directly taken to next step without further purification. ESI-MS: (*m/z*) calcd for C₉H₈IN₃O: 301.08, found 300.15 (M+H). ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.98 (s, 2H), 7.15-7.36 (m, 4H), 4.01 (s, 2H) ¹³C NMR (100 MHz, CDCl₃) 165.13, 147.42, 134.97, 121.87, 114.30, 29.04; Anal. calcd for C₉H₈IN₃O: (%) C, 35.90; H, 2.68; I, 42.15; N, 13.96; O, 5.31 Found: C, 35.97; H, 2.65; I, 42.18; N, 13.99; O, 5.28.

N-(5,6-dimethyl-1*H*-benzo[*d*]imidazol-2-yl)-2-iodoacetamide (**4b**): KI (0.92 g, 5.56 mmol) was added to the stirred solution of compound **3b** (1.1 g, 4.64 mmol) in acetone:water (8:2 ratio, should get clear solution) and heated to 50 °C for 12 h. Once completion of the reaction, as indicated by TLC, acetone was removed from the reaction mixture and washed with water and organic layer was dried over sodium sulphate, concentrated under reduced pressure and the crude residue was titrated with 3% dichloromethane in hexane to yield compound **4b** (1.1 g, 72%) as white solid. The solid compound was directly taken to next step without further purification. ESI-MS: (*m/z*) calcd for C₁₁H₁₂IN₃O: 329.14, found 330.15 (M+H). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.03 (s, 2H), 7.11 (s, 2H), 4.05 (s, 2H), 2.31 (s, 6H) ¹³C NMR (100 MHz, CDCl₃) 165.45, 147.75, 135.12, 121.844, 114.36,

22.28; Anal. calcd for C₁₁H₁₂IN₃O: (%) C, 40.14; H, 3.67; I, 38.56; N, 12.77; O, 4.86 Found: C, 40.21; H, 3.64; I, 38.55; N, 12.73; O, 4.87.

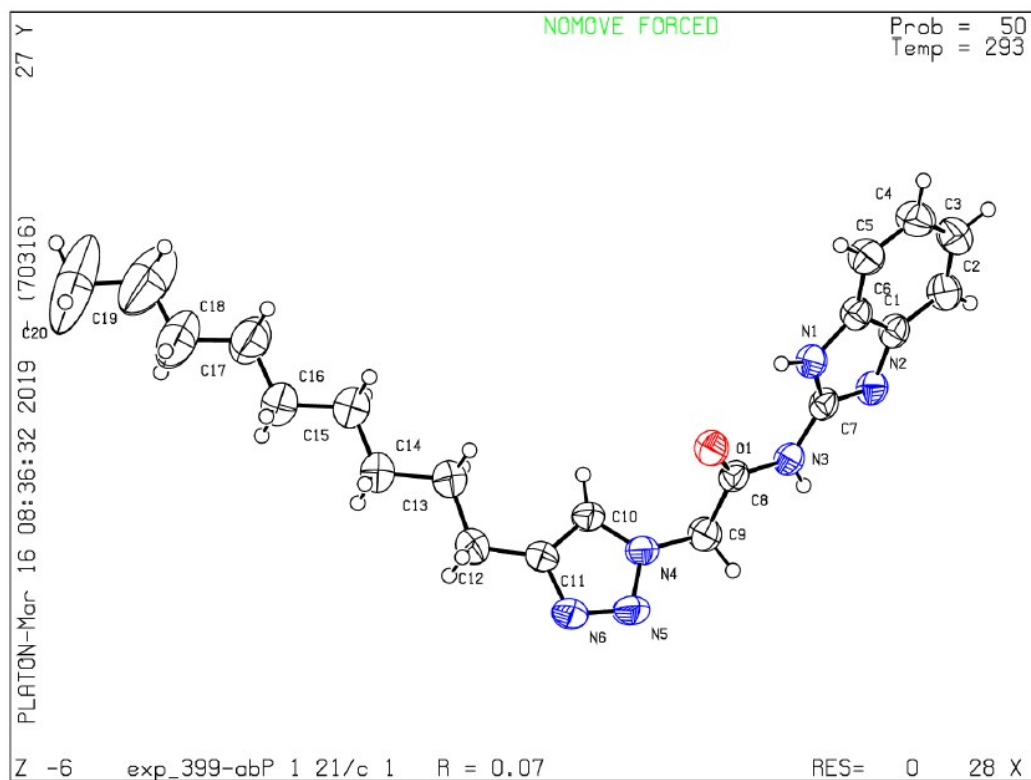
2-azido-N-(1H-benzo[d]imidazol-2-yl)acetamide (5a): NaN₃ (0.49 g, 7.47 mmol) was added to the stirred solution of compound **4a** (1.5g, 4.98 mmol) in DMF:H₂O (8:2) mixture and heated to 110 °C for 20 h. Once completion of the reaction, as indicated by TLC, the reaction mixture was transferred into water. The precipitated product was filtered to yield compound **5a** (0.8 g, 75%) as off-white solid. ESI-MS: (*m/z*) calcd for C₉H₈N₆O: 216.20, found 215.20 (M+H). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.06 (s, 2H), 7.12-7.61 (m, 4H), 3.89 (s, 2H) ¹³C NMR (100 MHz, CDCl₃) 166.74, 147.67, 135.49, 122.53, 114.39, 50.51; Anal. calcd for C₉H₈N₆O: (%) C, 50.00; H, 3.73; N, 38.87; O, 7.40 Found: C, 50.00; H, 3.73; N, 38.87; O, 7.40.

2-azido-N-(5,6-dimethyl-1H-benzo[d]imidazol-2-yl)acetamide (5b): NaN₃ (0.49 g, 7.47 mmol) was added to the stirred solution of compound **4b** (1.1 g, 4.98 mmol) in DMF:H₂O (8:2) mixture and heated to 110 °C for 20 h. Once completion of the reaction, as indicated by TLC, the reaction mixture was transferred into water. The precipitated product was filtered to yield compound **5b** (0.6 g, 74%) as off-white solid. ESI-MS: (*m/z*) calcd for C₁₁H₁₂N₆O: 244.25, found 245.20 (M+H). ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.08 (s, 2H), 7.19 (s, 2H), 4.31 (s, 2H), 2.26 (s, 6H) ¹³C NMR (100 MHz, CDCl₃) 166.44, 147.76, 135.41, 121.25, 113.79, 43.98, 20.14; Anal. calcd for C₁₁H₁₂N₆O: (%) C, 54.09; H, 4.95; N, 34.41; O, 6.55 Found: C, 54.20; H, 4.93; N, 34.42; O, 6.65.

3. Single Crystal data

Single Crystal X-ray Crystallographic Structure of Compound 6d:

Crystallographic data for the compound **6d** have been deposited to the Cambridge Crystallographic Data Center and corresponding deposition number is **CCDC 1904042**.



ORTEP crystal structure diagram of compound **6d**

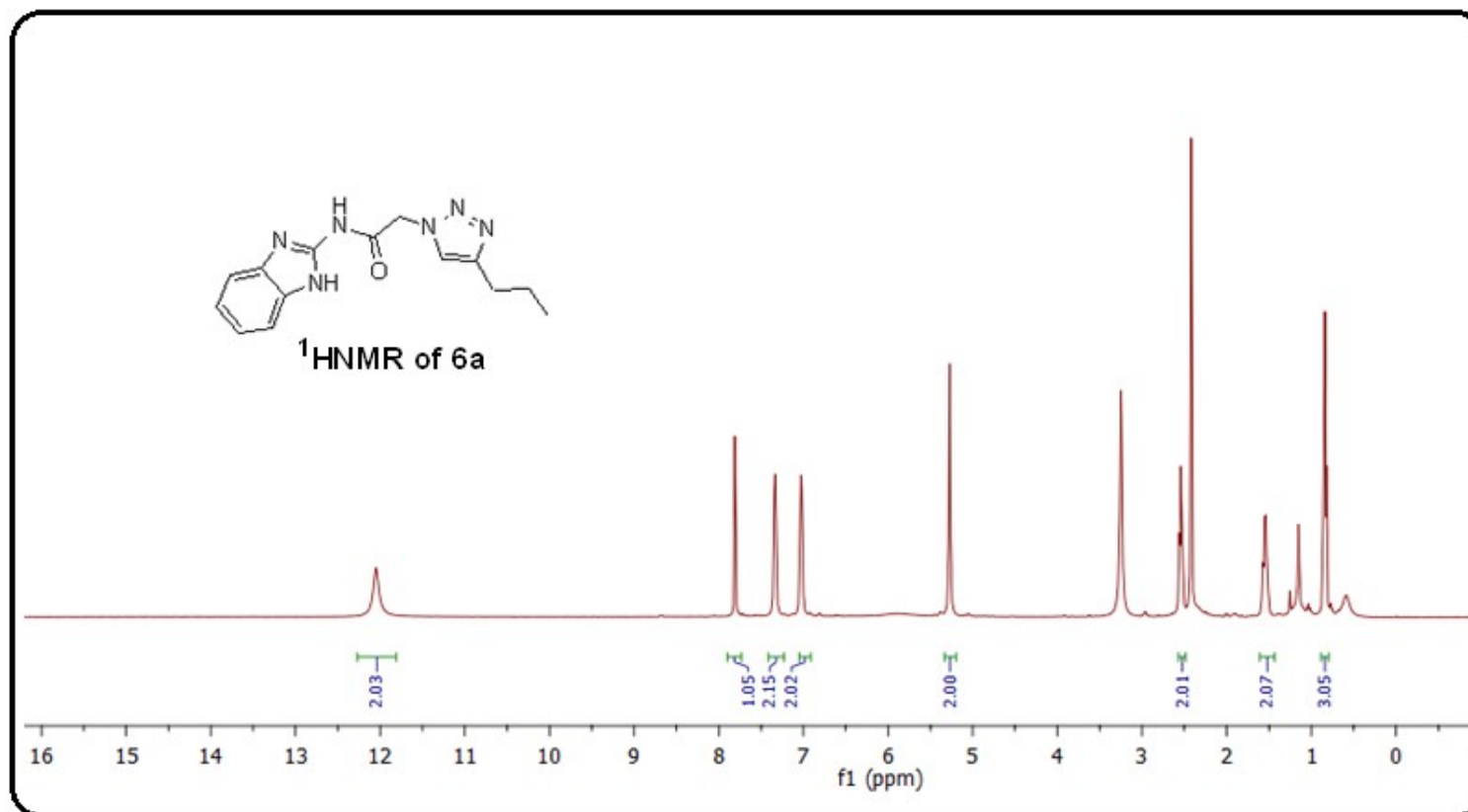
Table of Crystal data and structure refinement for **6d**

Identification code	6d (exp_399-AB-4R)
Empirical formula	C ₂₀ H ₂₈ N ₆ O
Formula weight	368.48
Temperature/K	293(2)
Crystal system	monoclinic
Space group	P2 ₁ /c

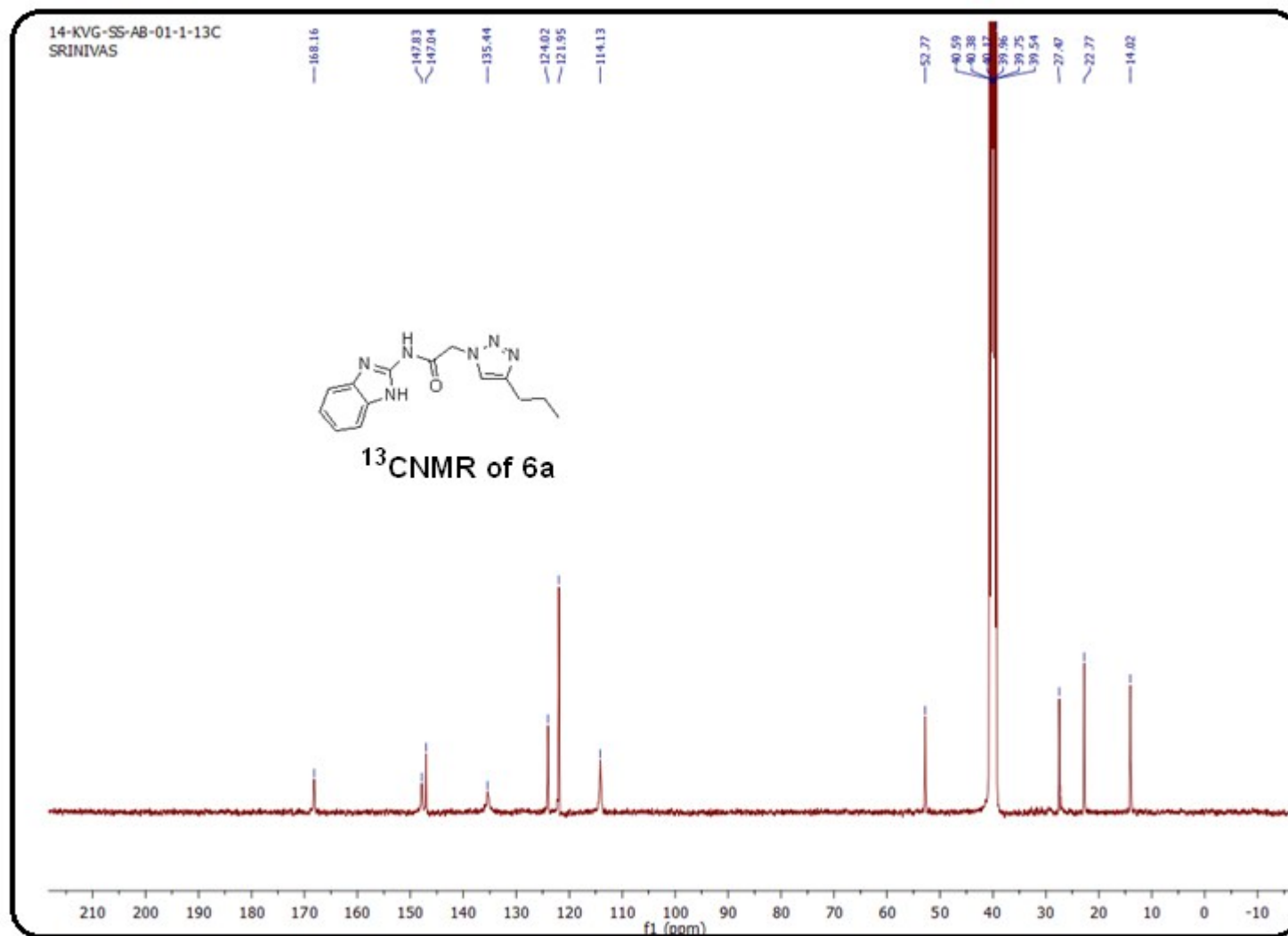
a/Å	18.8646(7)
b/Å	5.4019(2)
c/Å	20.0357(6)
α /°	90
β /°	90.614(3)
γ /°	90
Volume/Å ³	2041.61(12)
Z	4
ρ_{calc} /g/cm ³	1.199
μ /mm ⁻¹	0.619
F(000)	792.0
Crystal size/mm ³	0.5 × 0.02 × 0.01
Radiation	CuK α (λ = 1.54184)
2 Θ range for data collection/°	8.828 to 159.794
Index ranges	-23 ≤ h ≤ 23, -6 ≤ k ≤ 3, -23 ≤ l ≤ 25
Reflections collected	10329
Independent reflections	4308 [R_{int} = 0.0482, R_{sigma} = 0.0635]
Data/restraints/parameters	4308/0/245
Goodness-of-fit on F ²	1.063
Final R indexes [$I \geq 2\sigma(I)$]	R_1 = 0.0671, wR_2 = 0.1867
Final R indexes [all data]	R_1 = 0.0834, wR_2 = 0.2036
Largest diff. peak/hole / e Å ⁻³	0.42/-0.27

4. ^1H and ^{13}C NMR spectra's

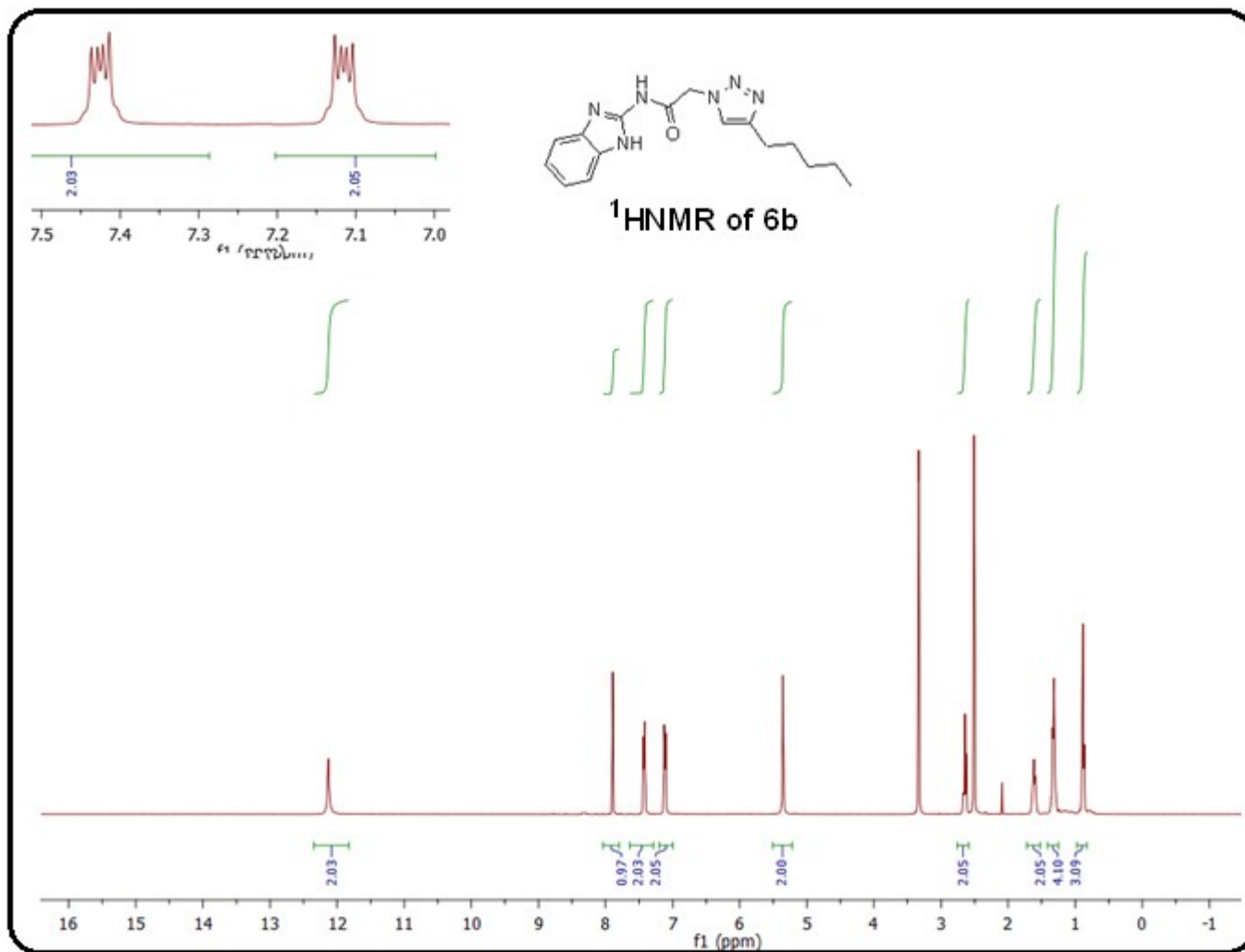
^1H and ^{13}C NMR spectra's of represented final compounds:



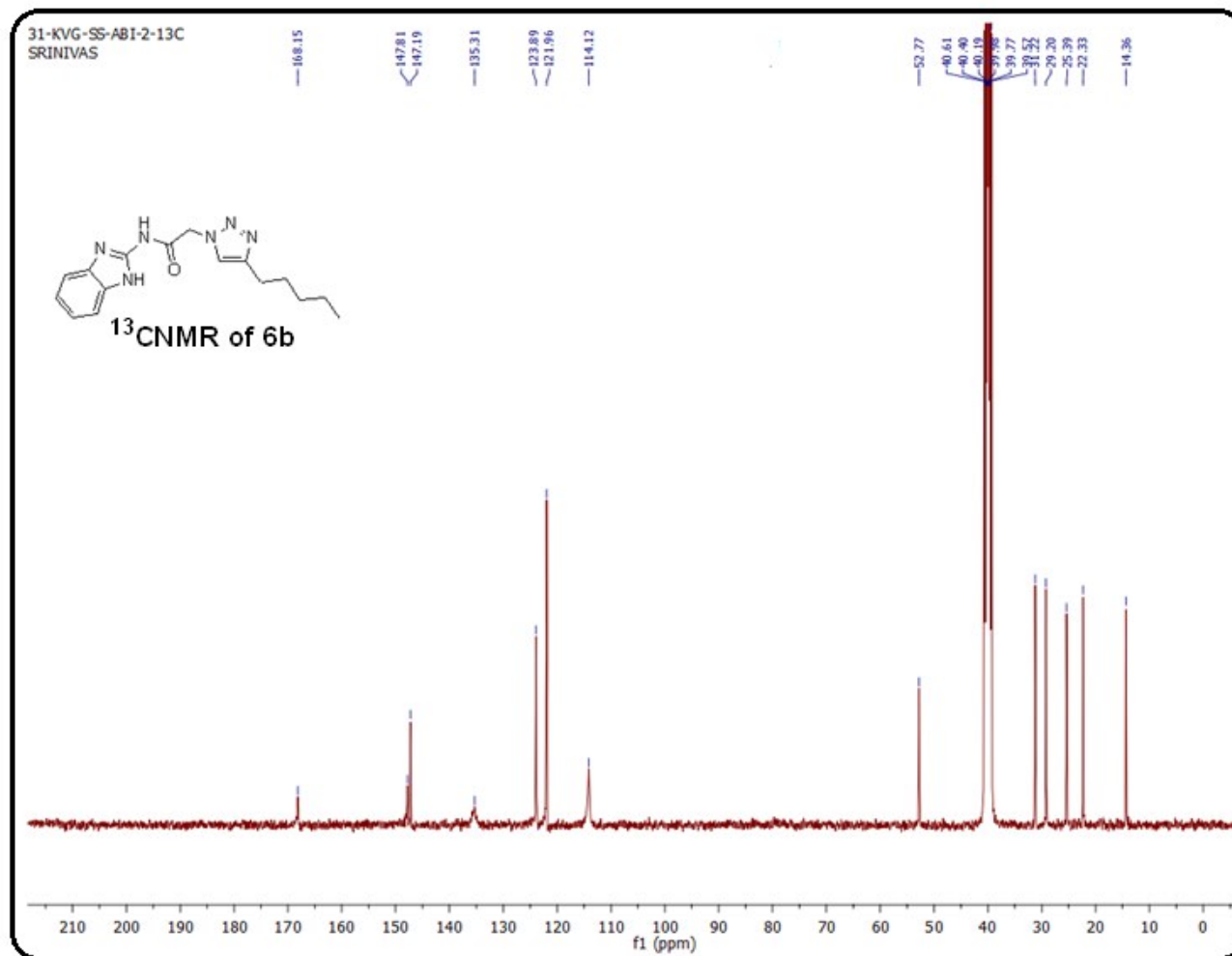
$^1\text{HNMR}$ of 6a



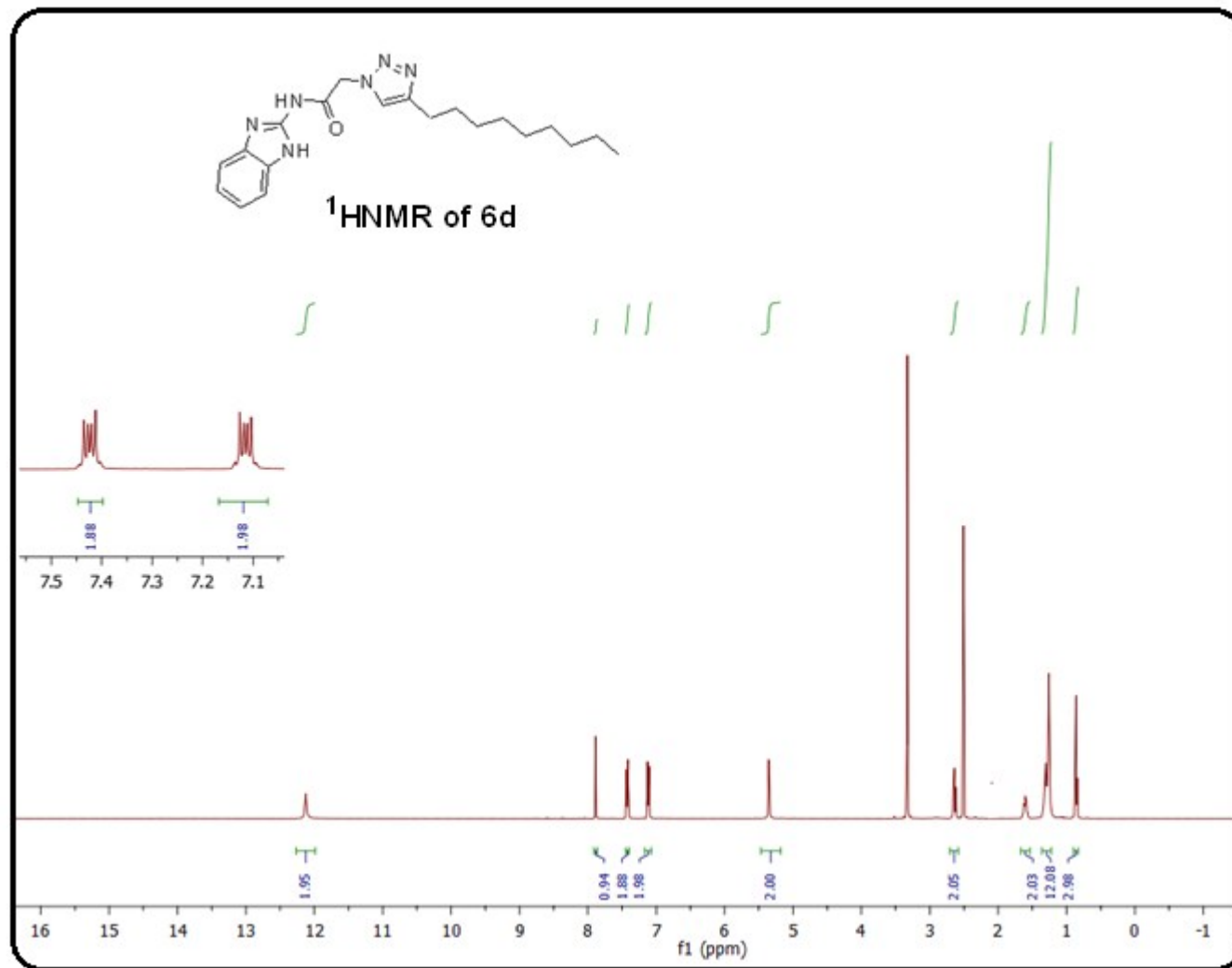
¹³CNMR of 6a



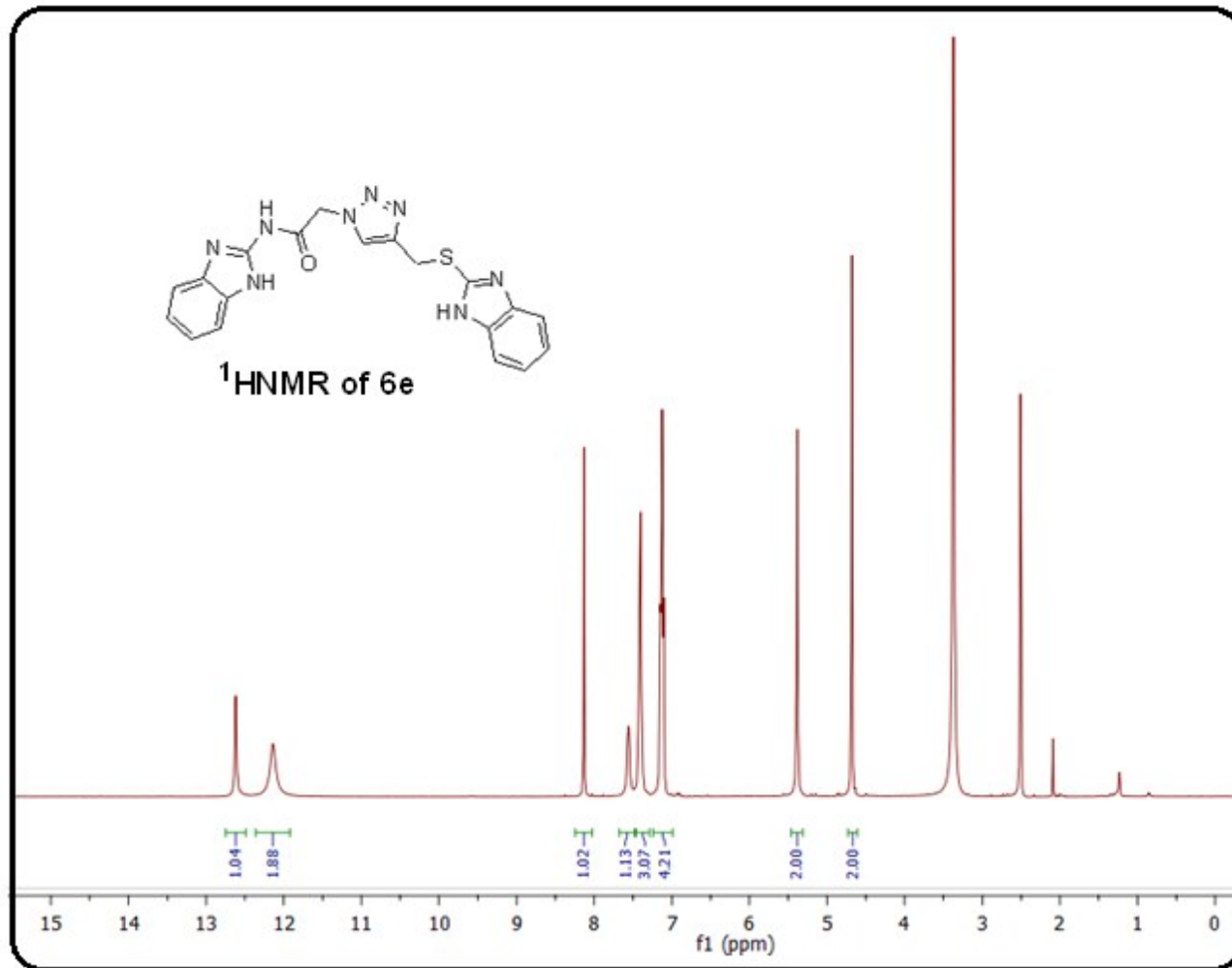
¹H NMR of **6b**



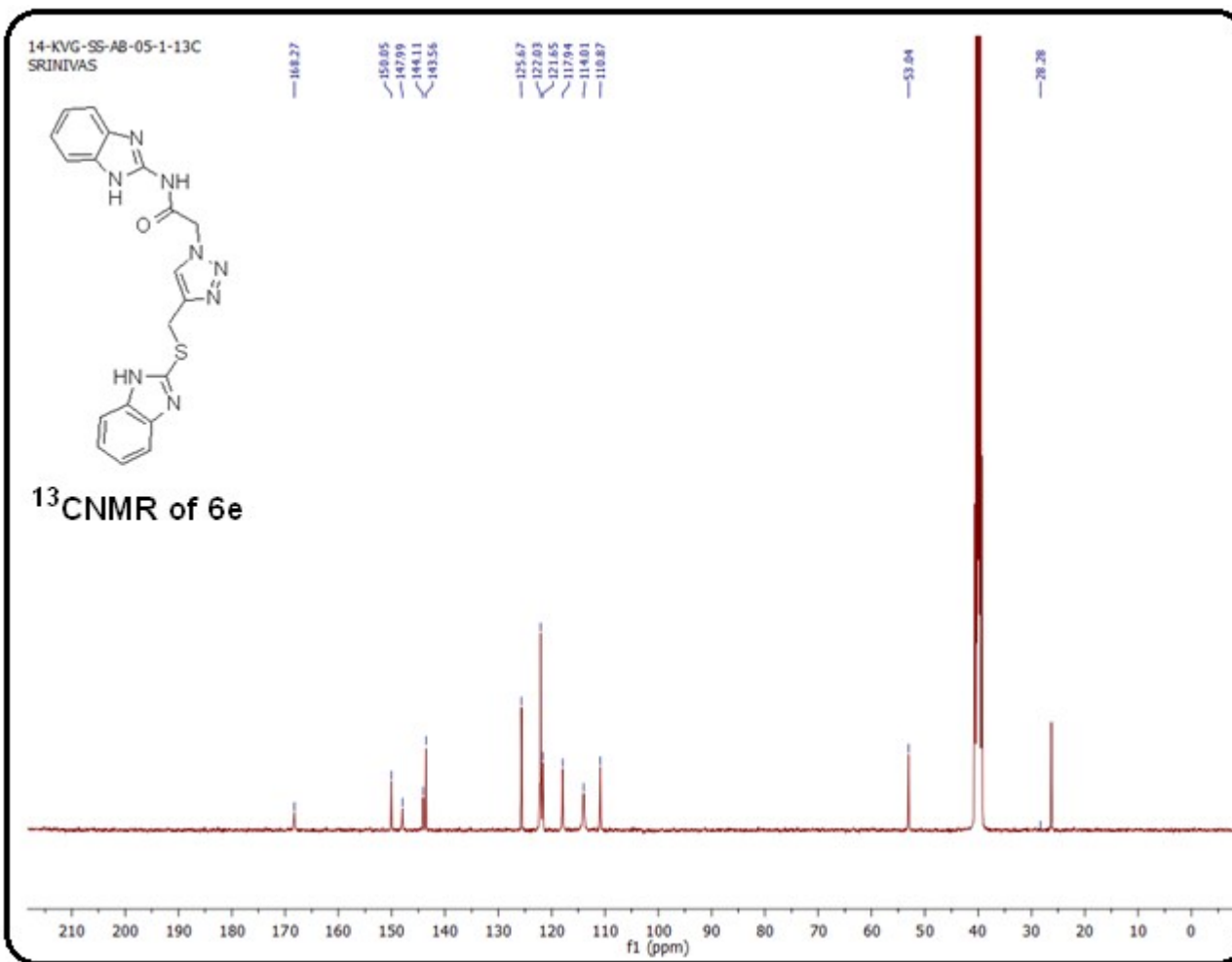
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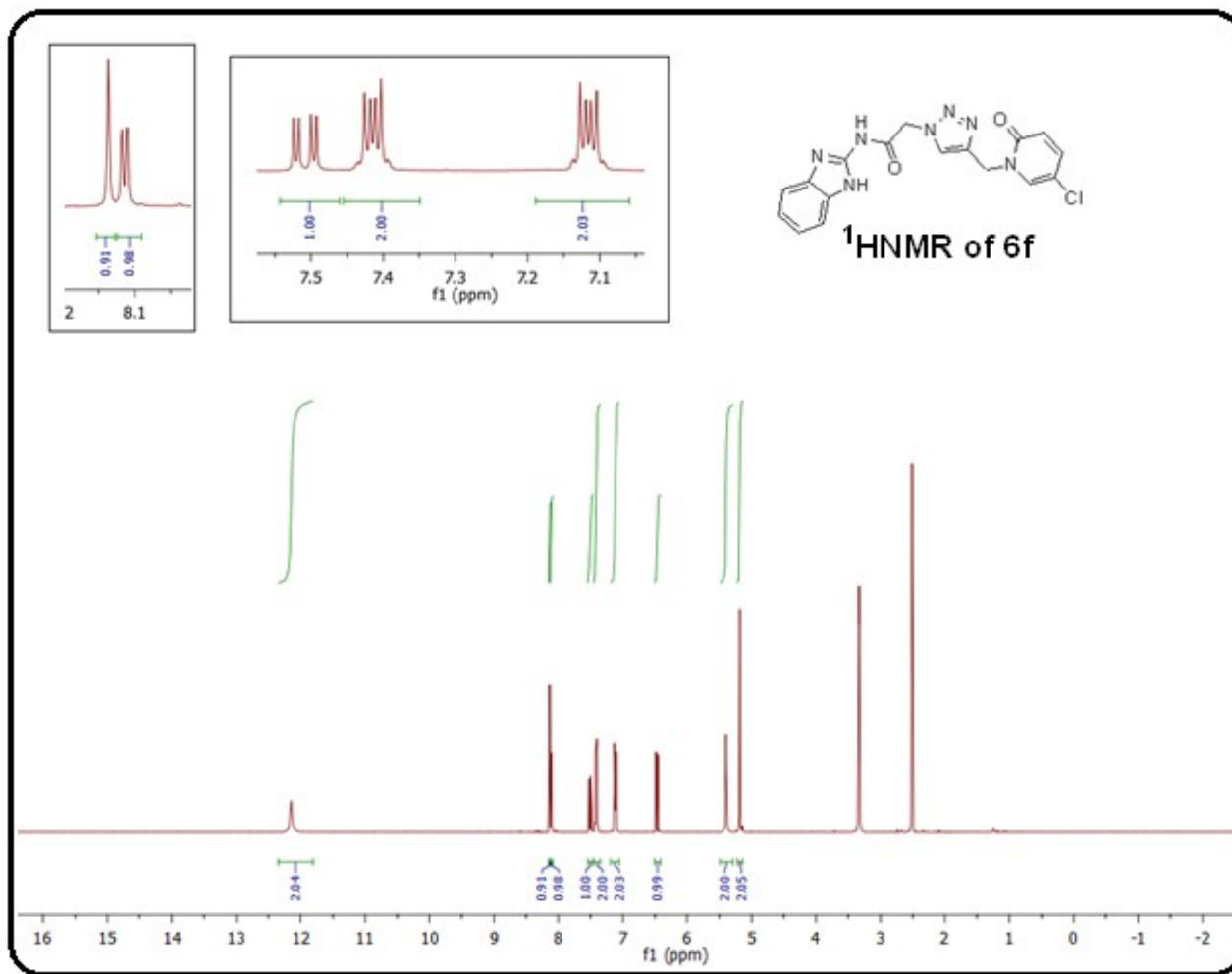
¹H NMR of 6d



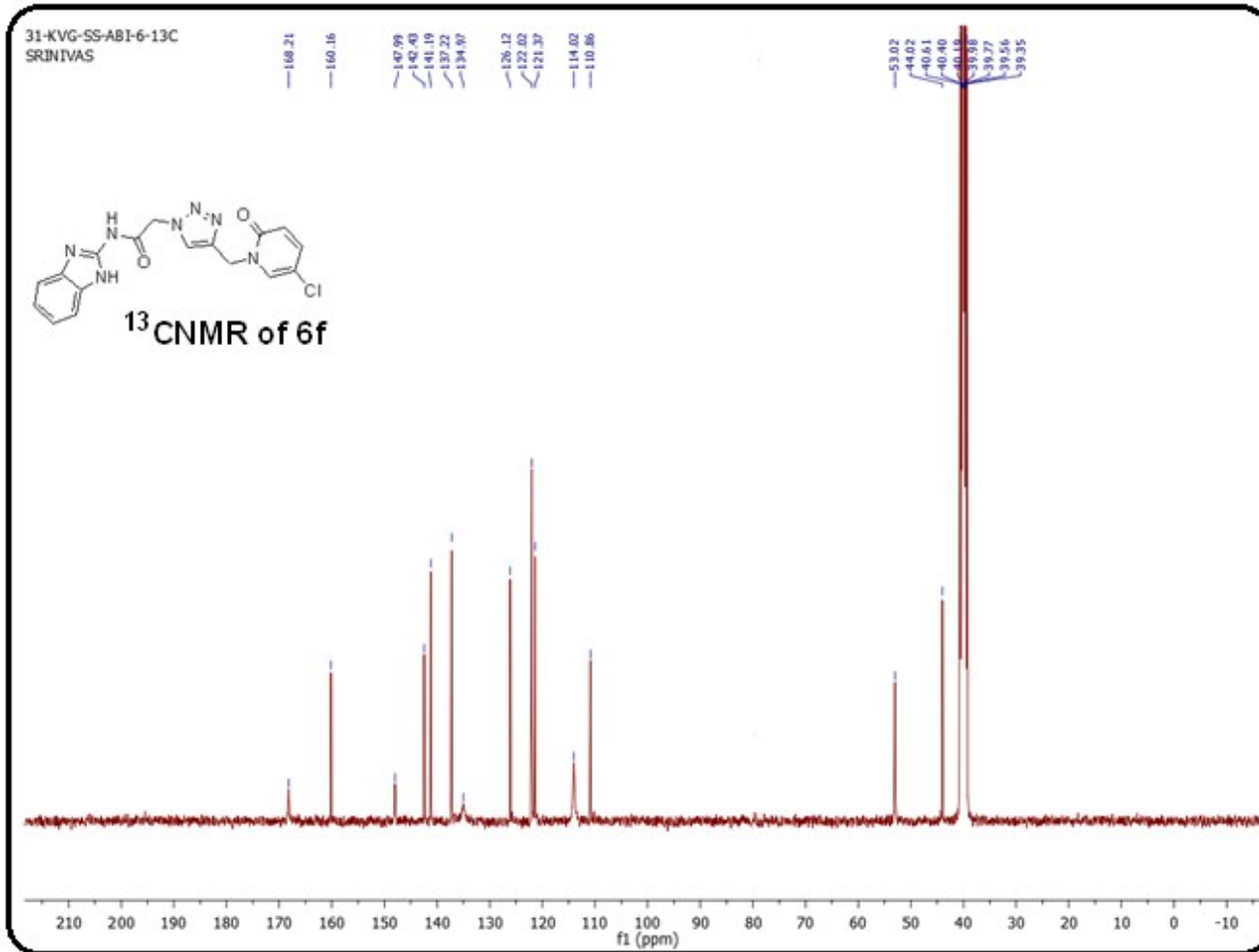
¹HNMR of 6e



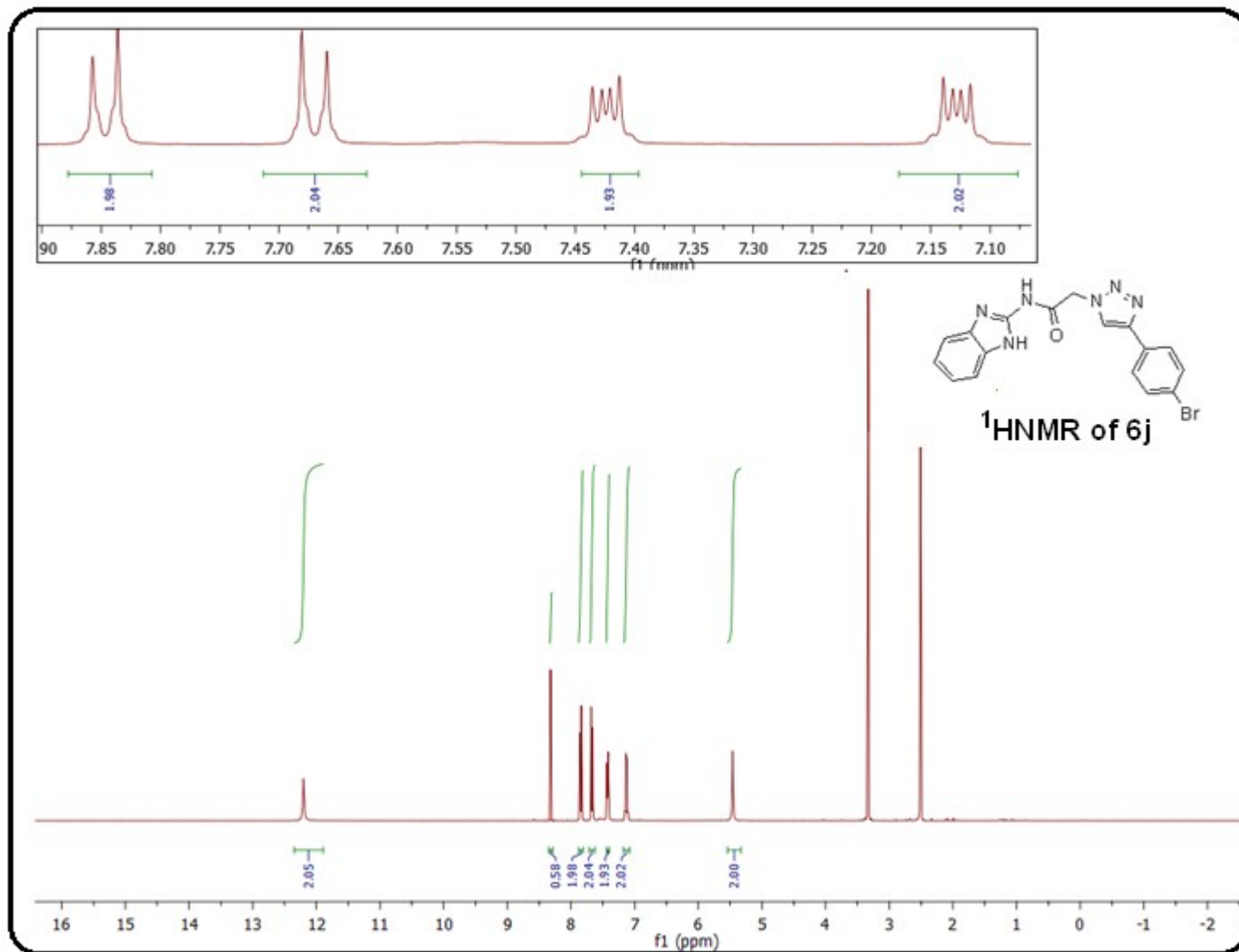
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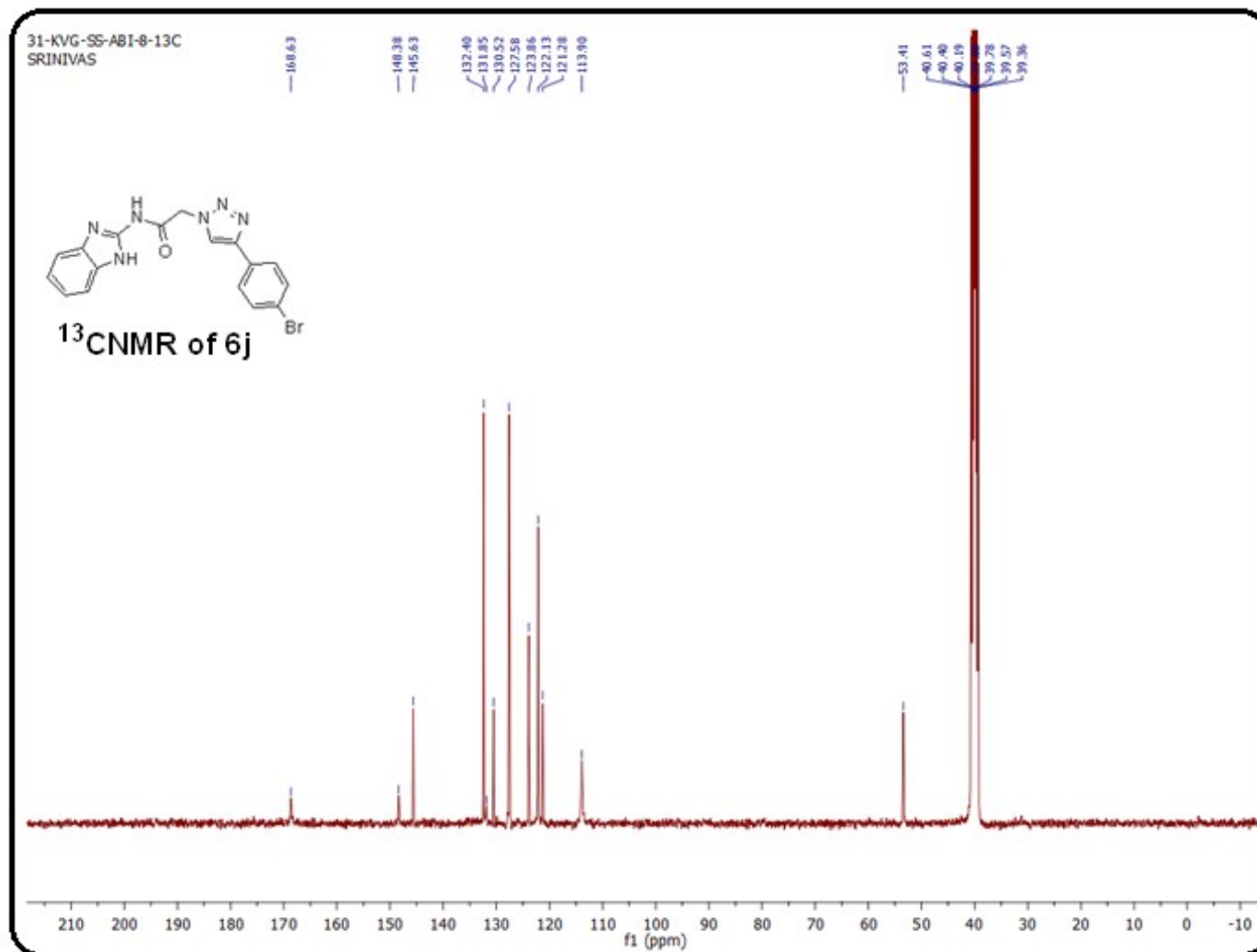
¹³C NMR of 6f



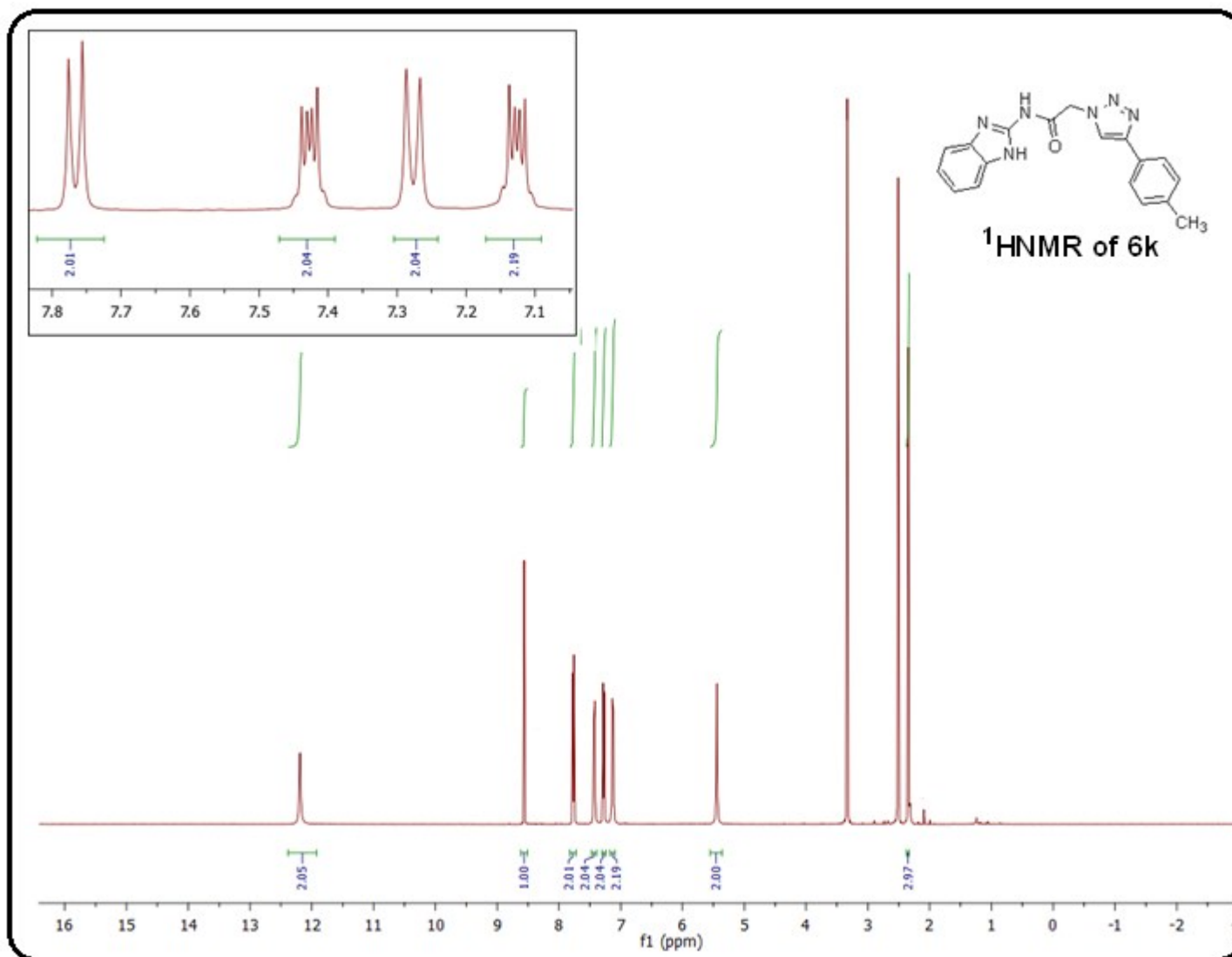
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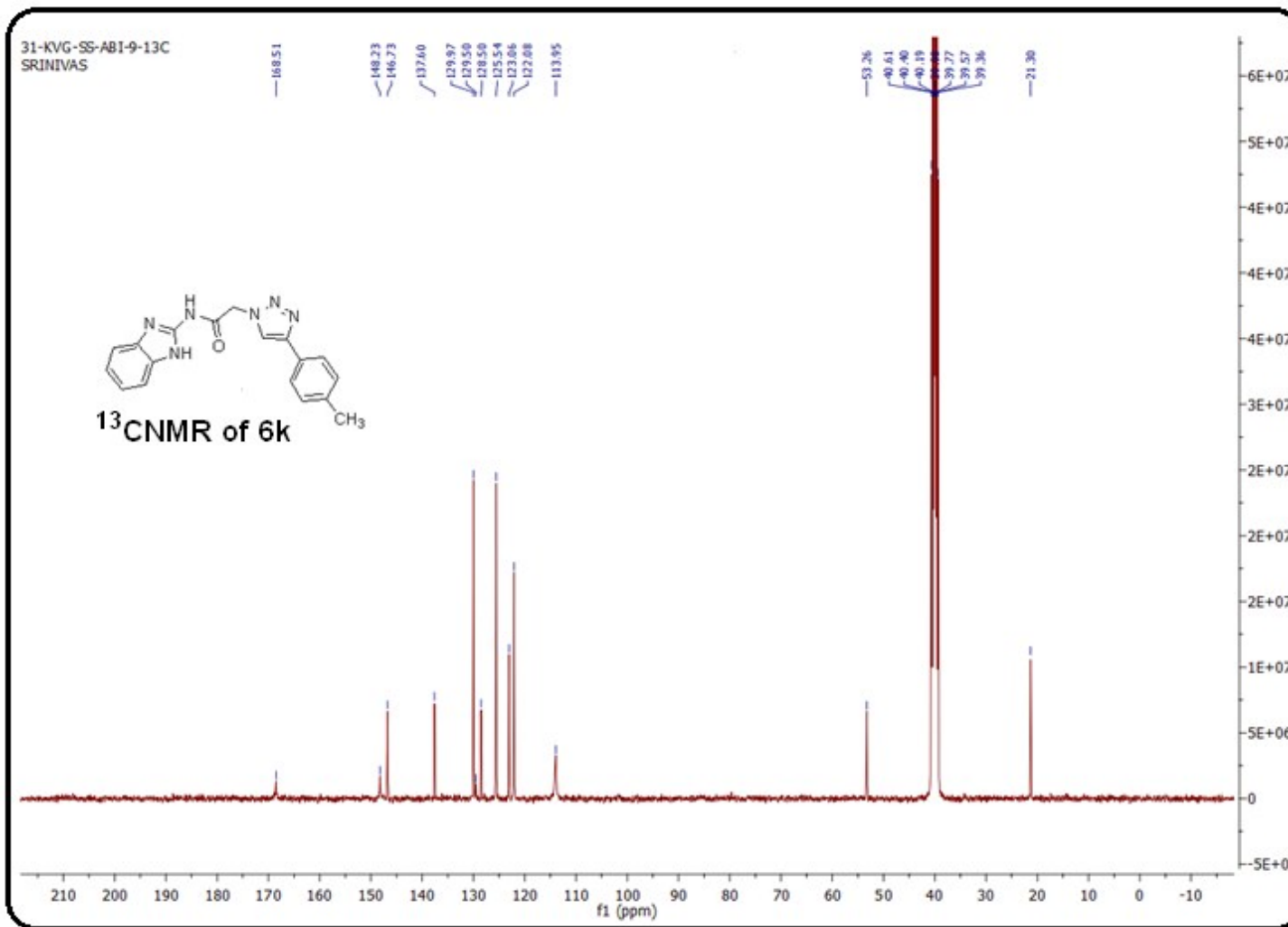
¹H NMR of 6j



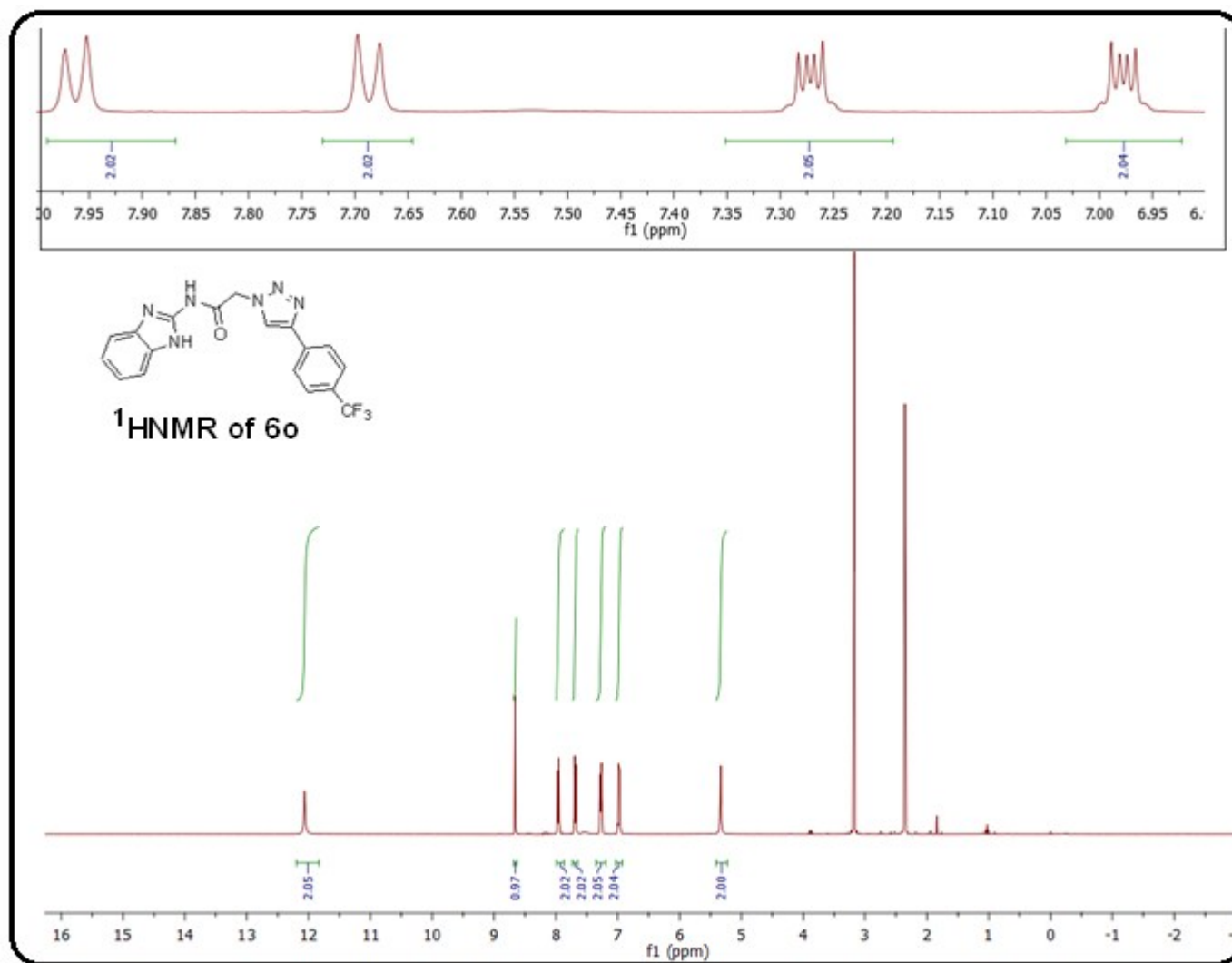
¹³CNMR of 6j



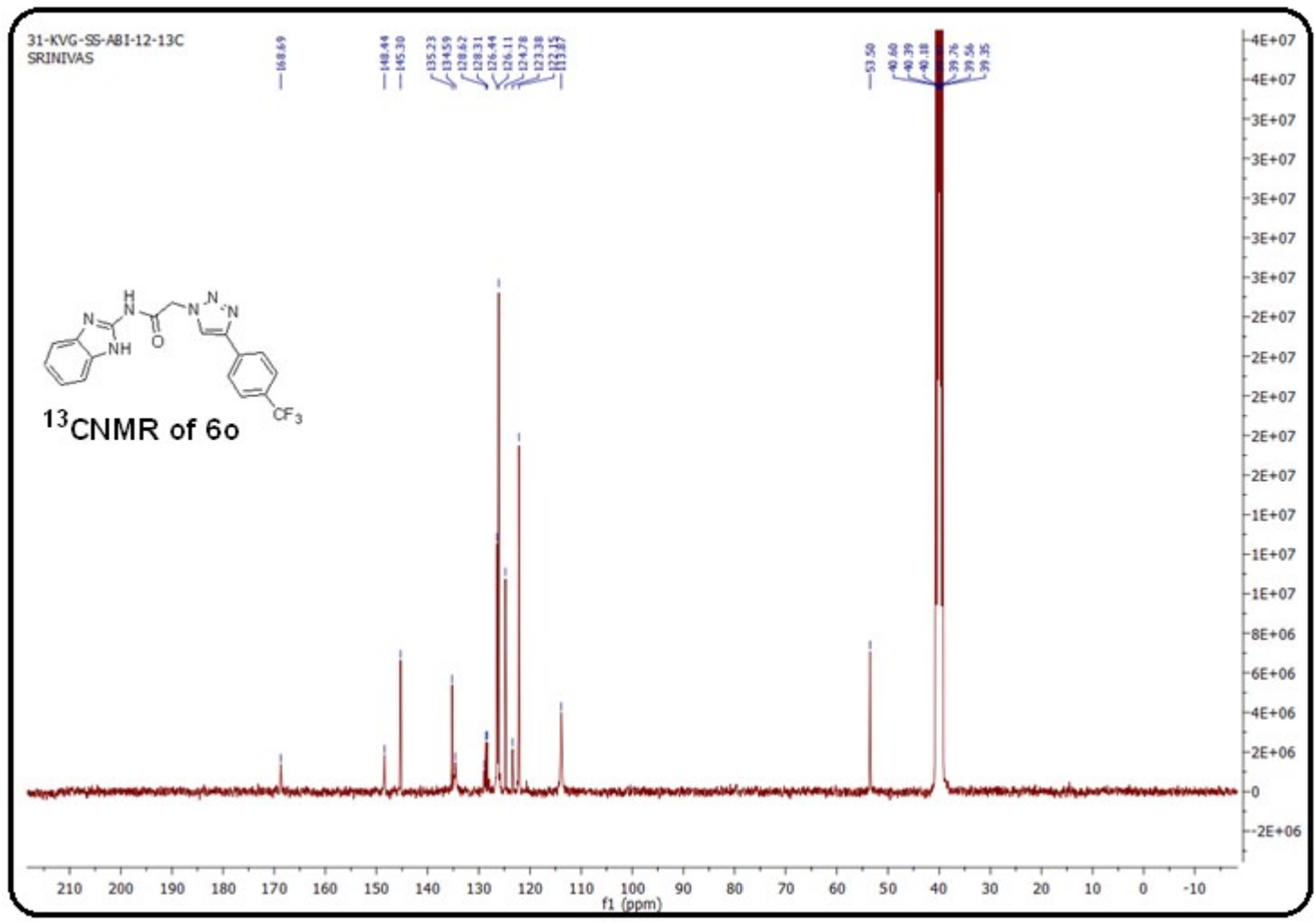
¹H NMR of 6k



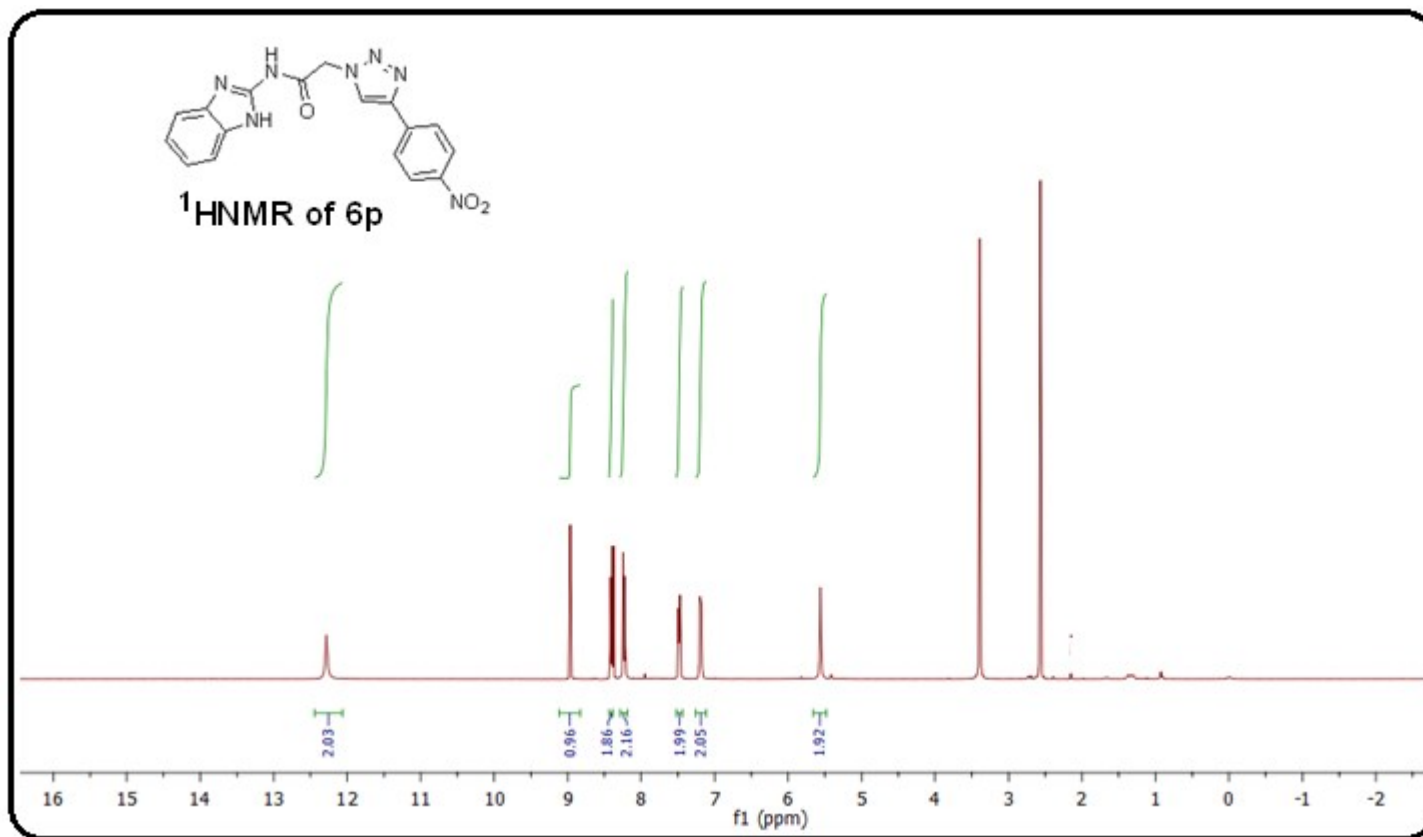
¹³CNMR of 6k



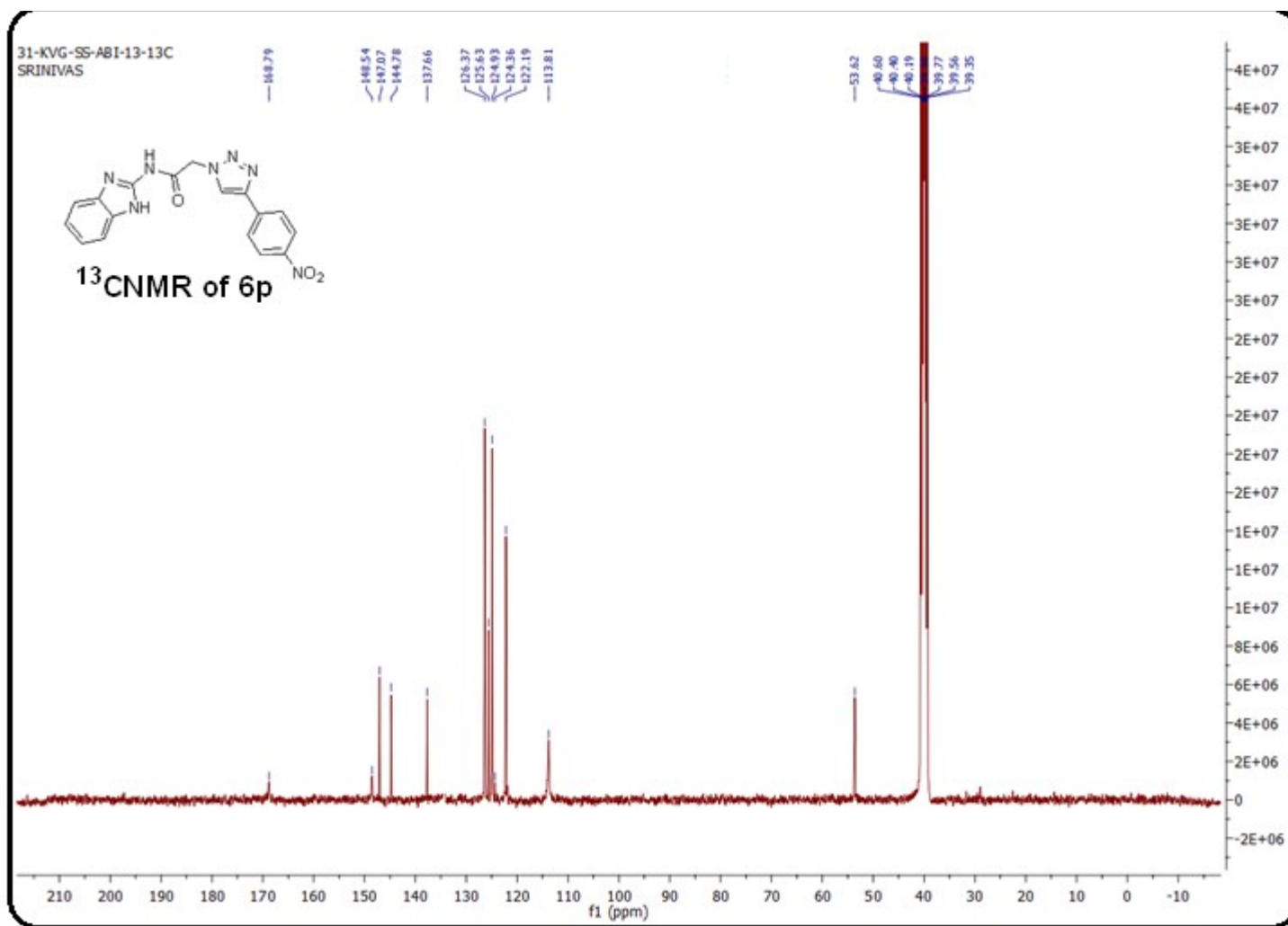
¹H NMR of 60



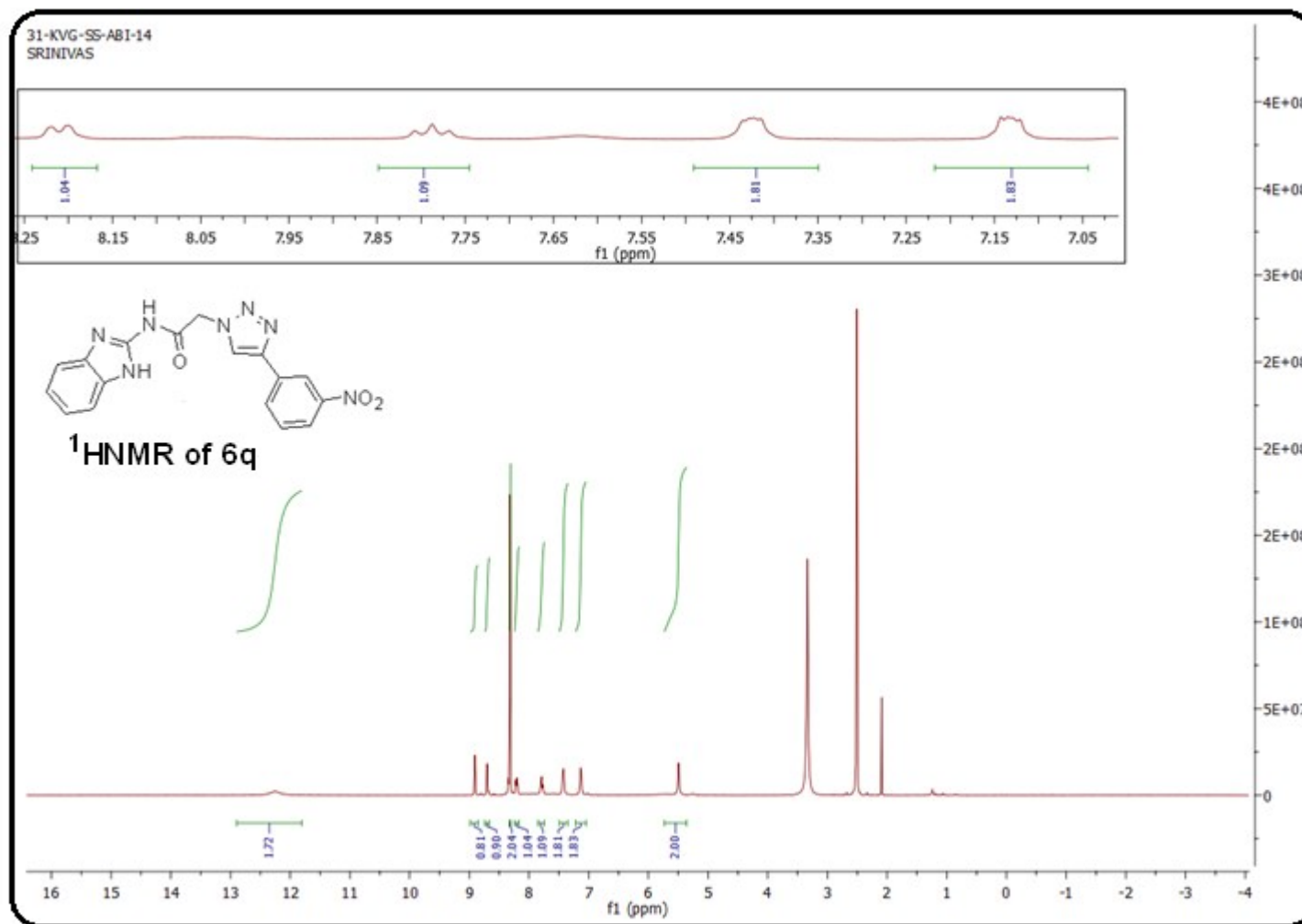
¹³CNMR of 6o



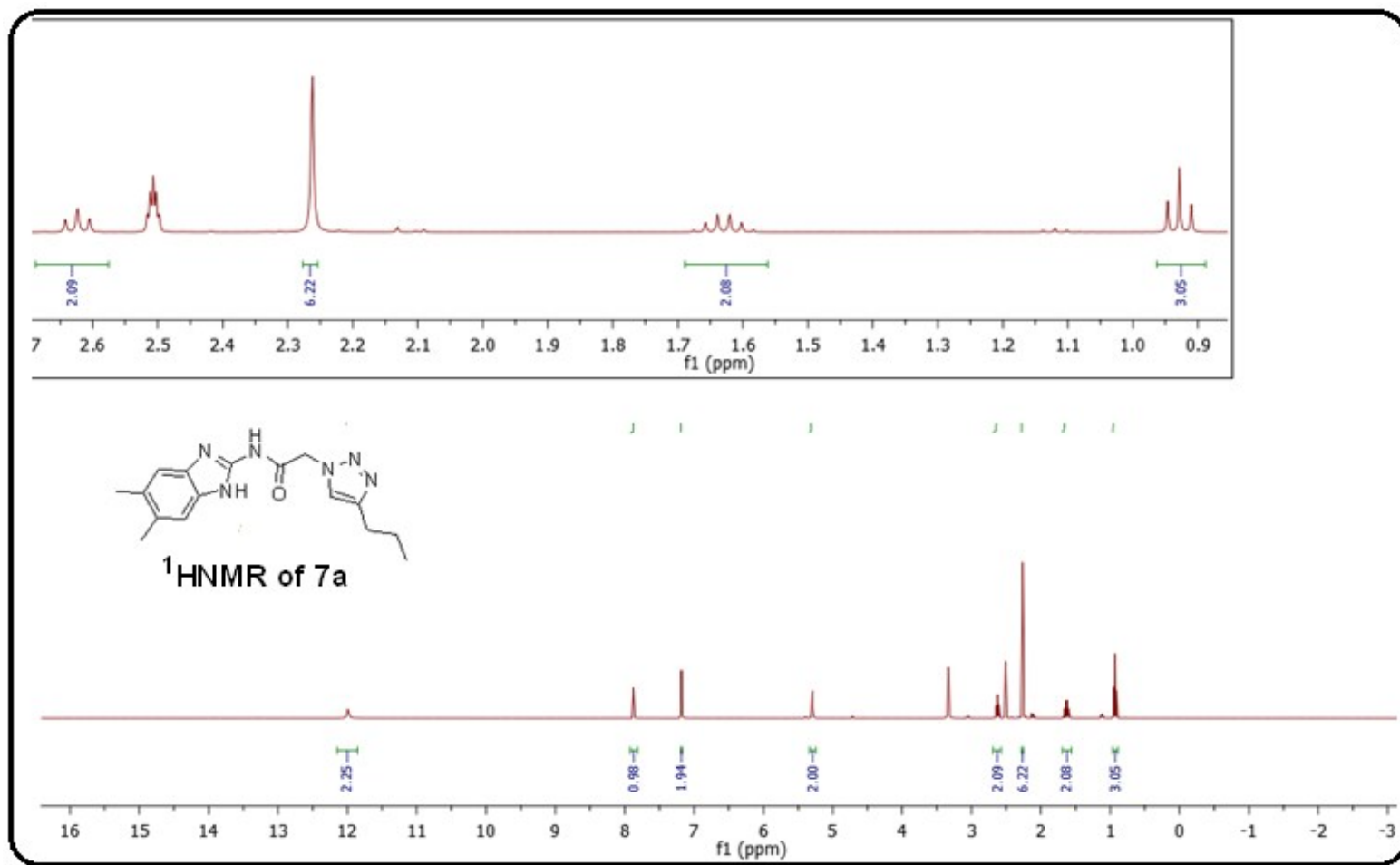
¹HNMR of 6p



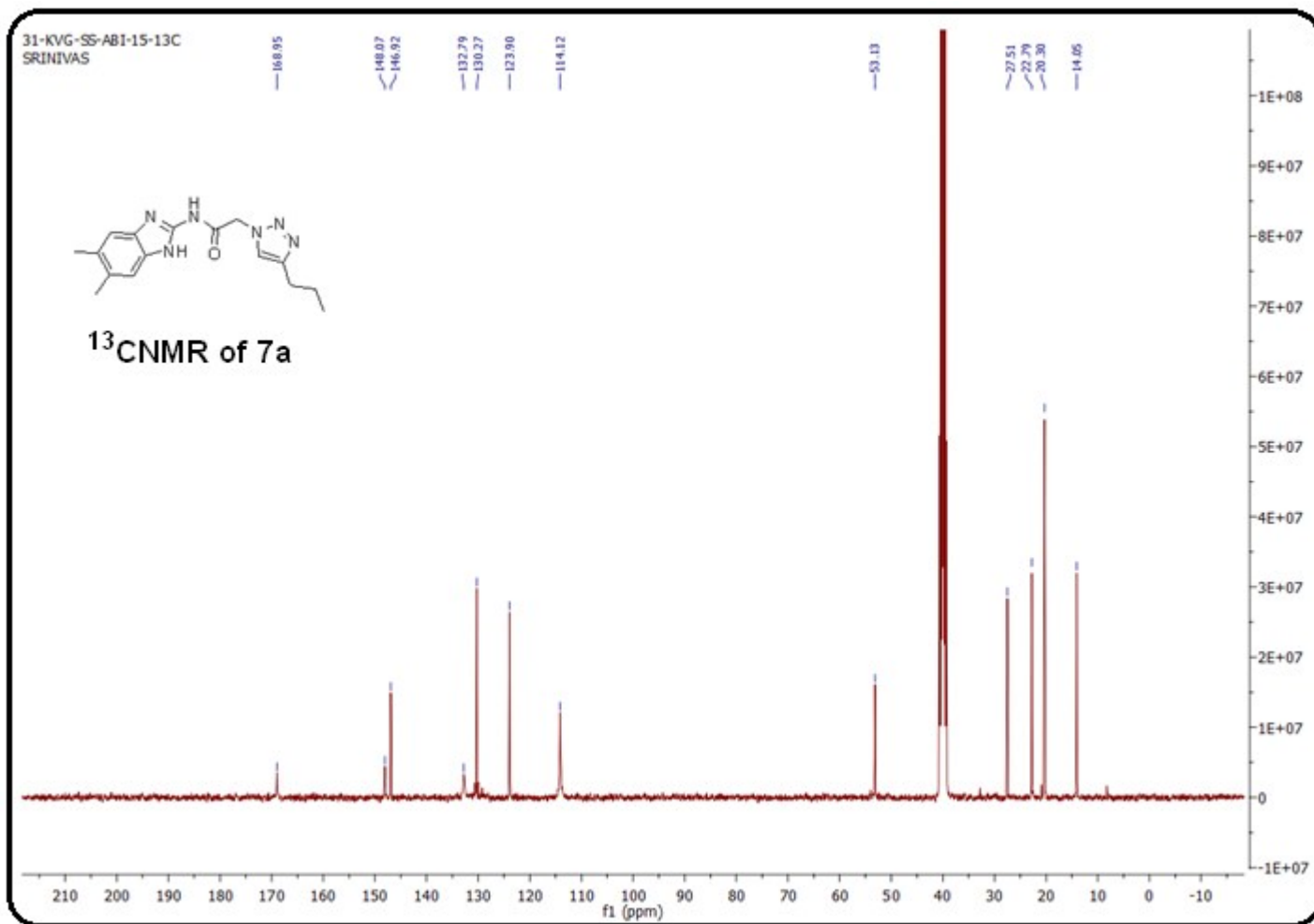
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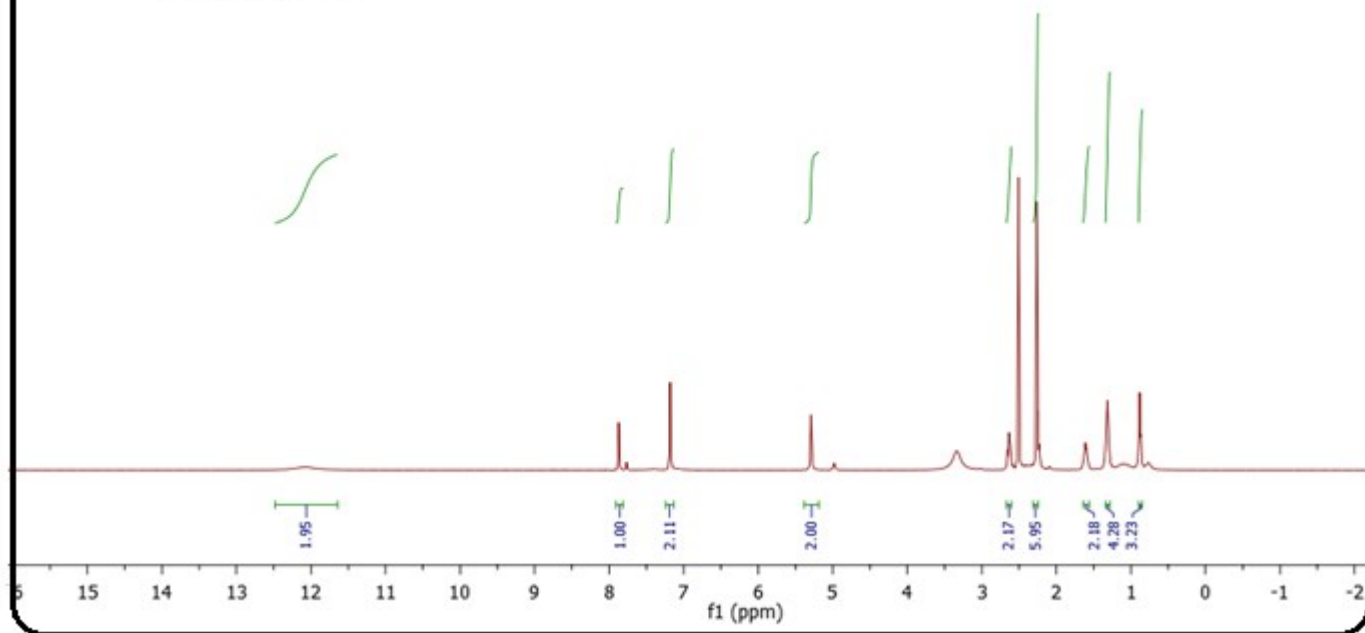
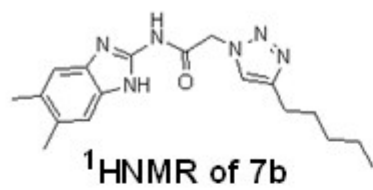
¹H NMR of 6q



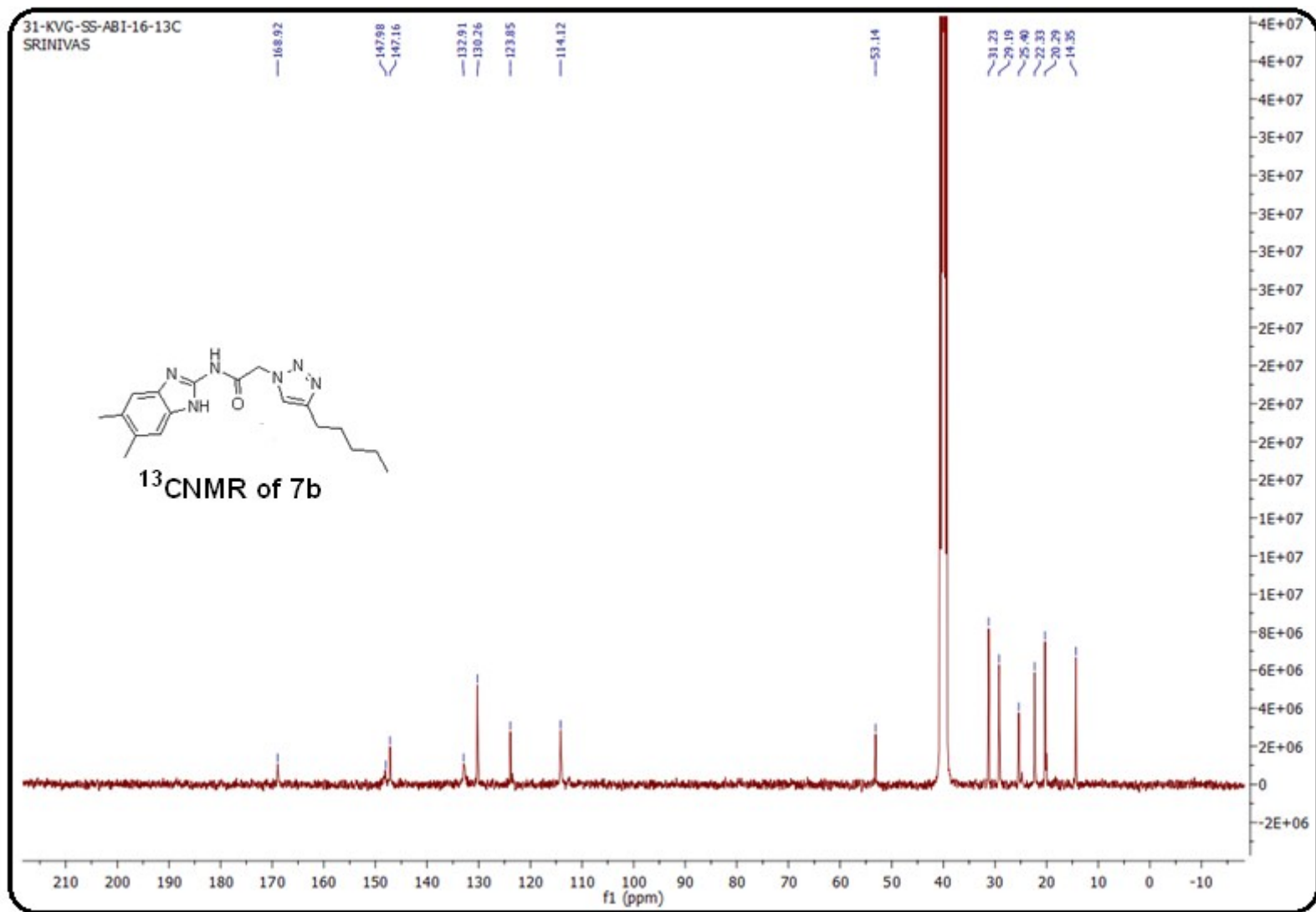
^1H NMR of **7a**



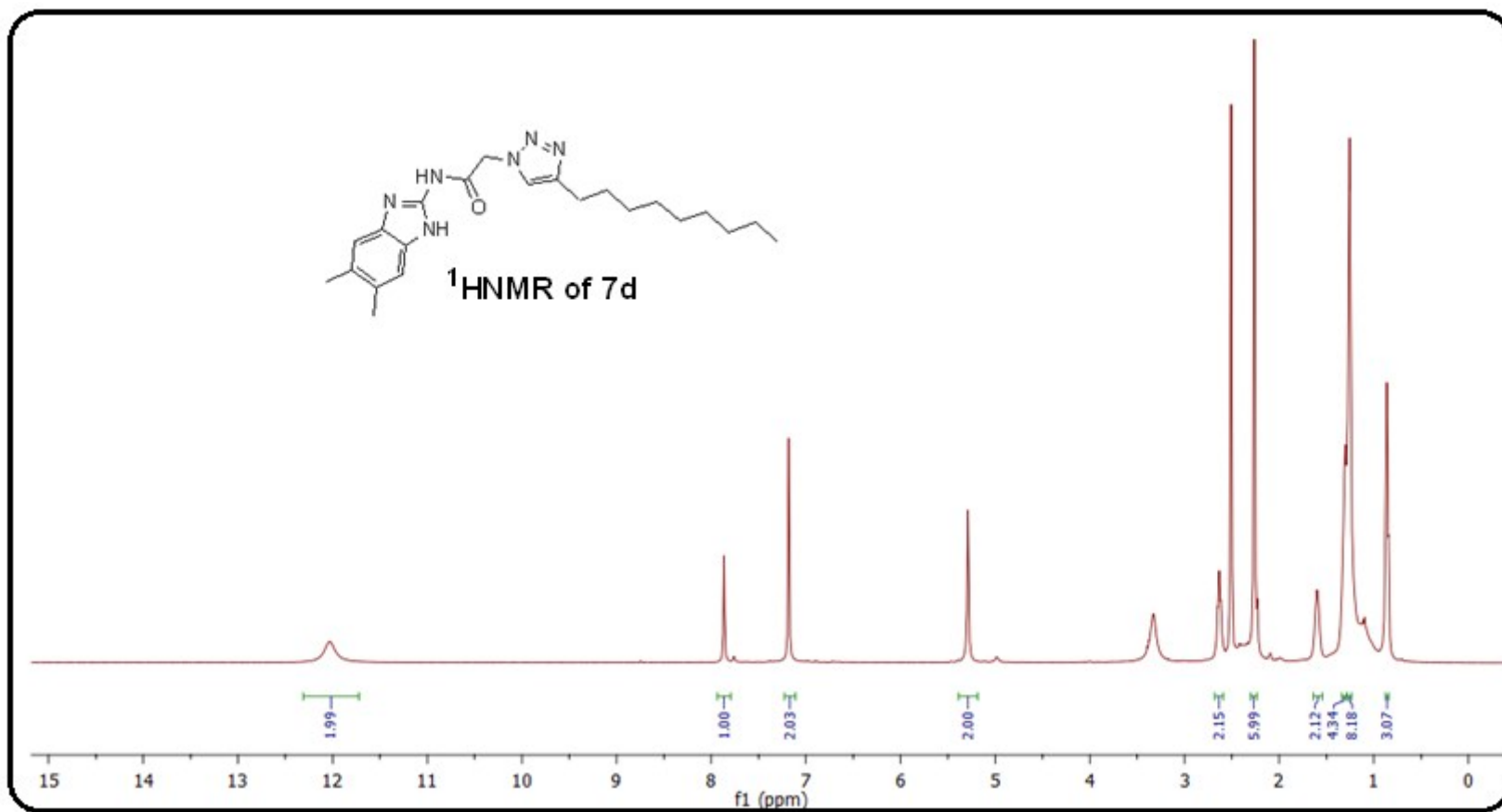
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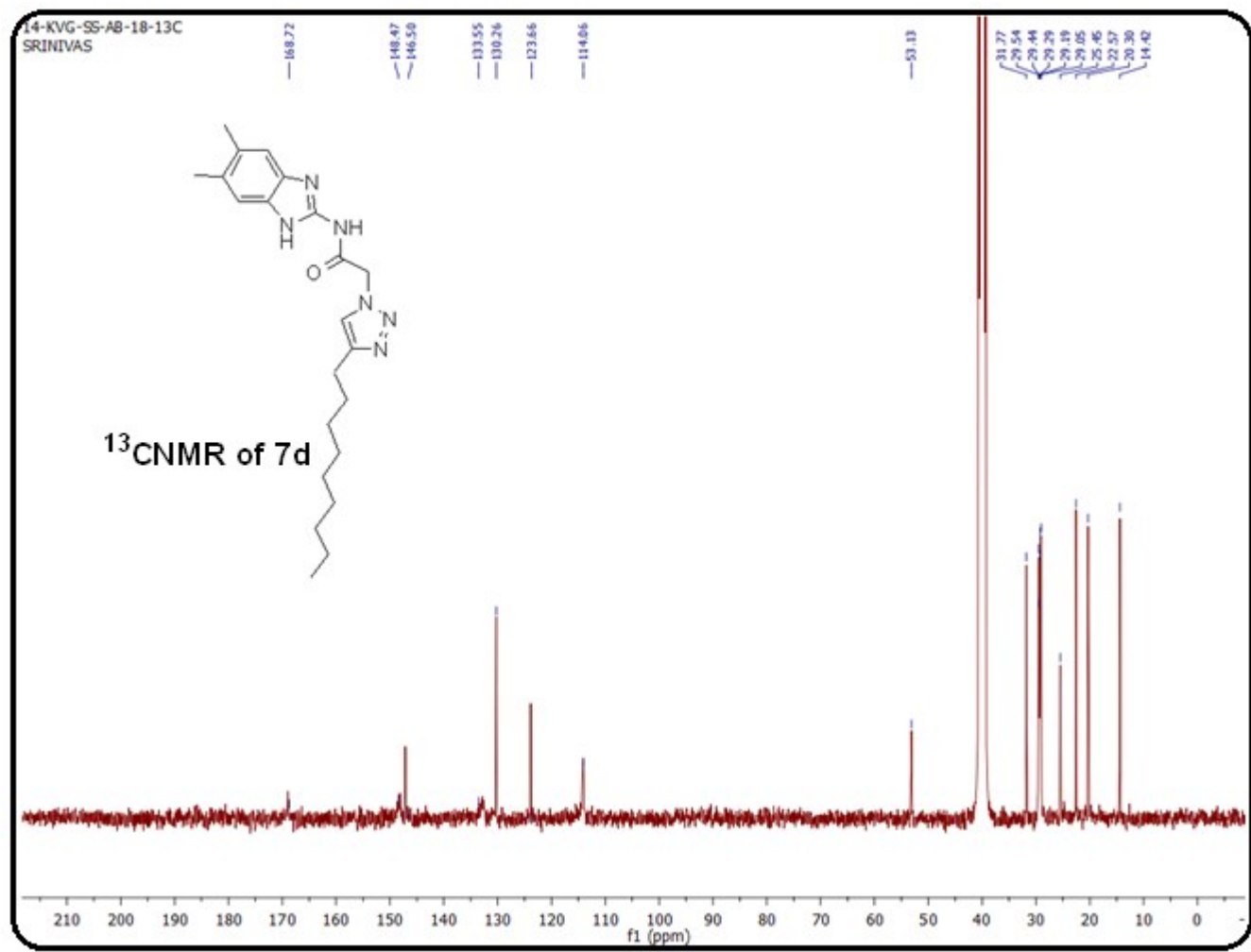
¹HNMR of 7b



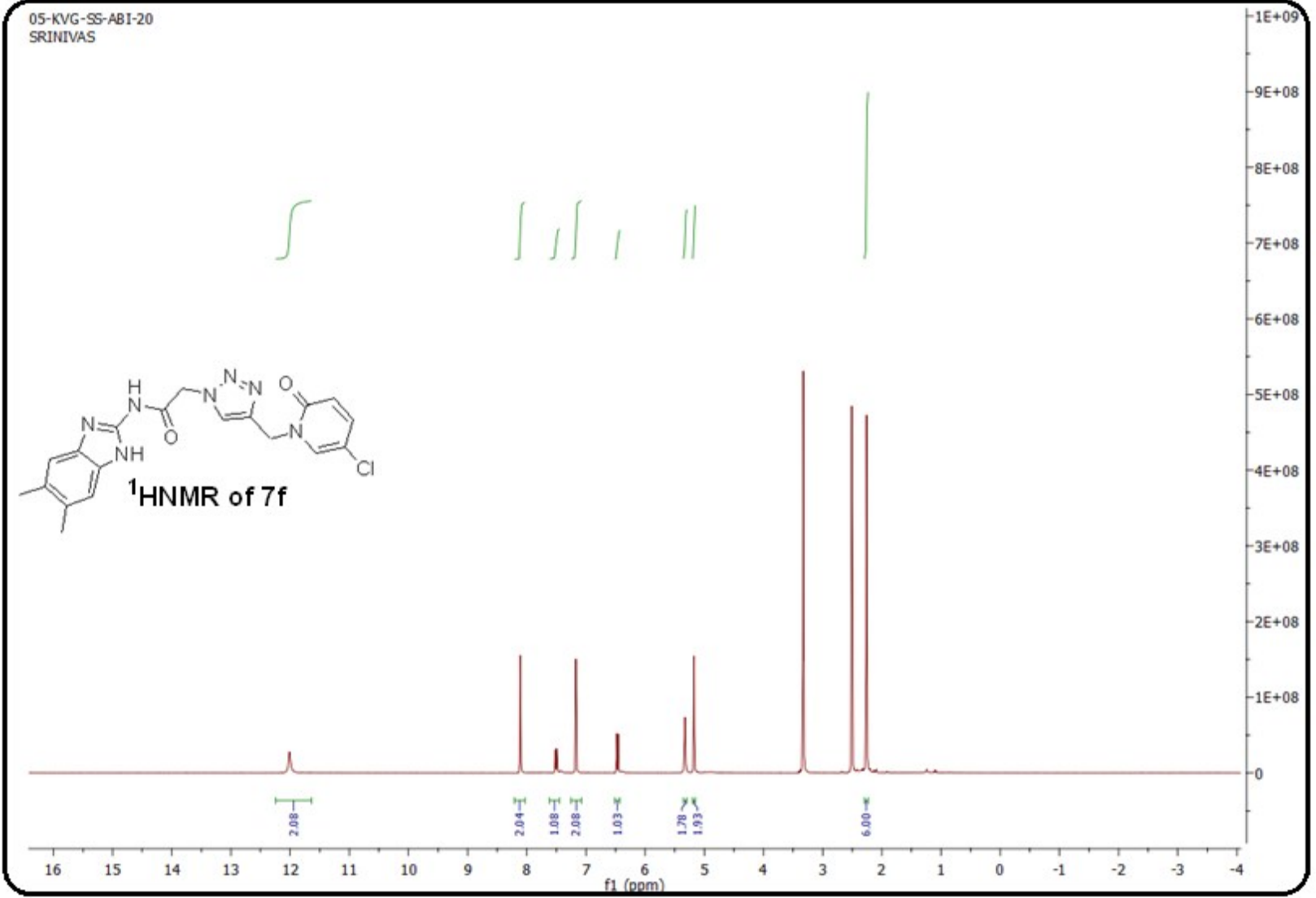
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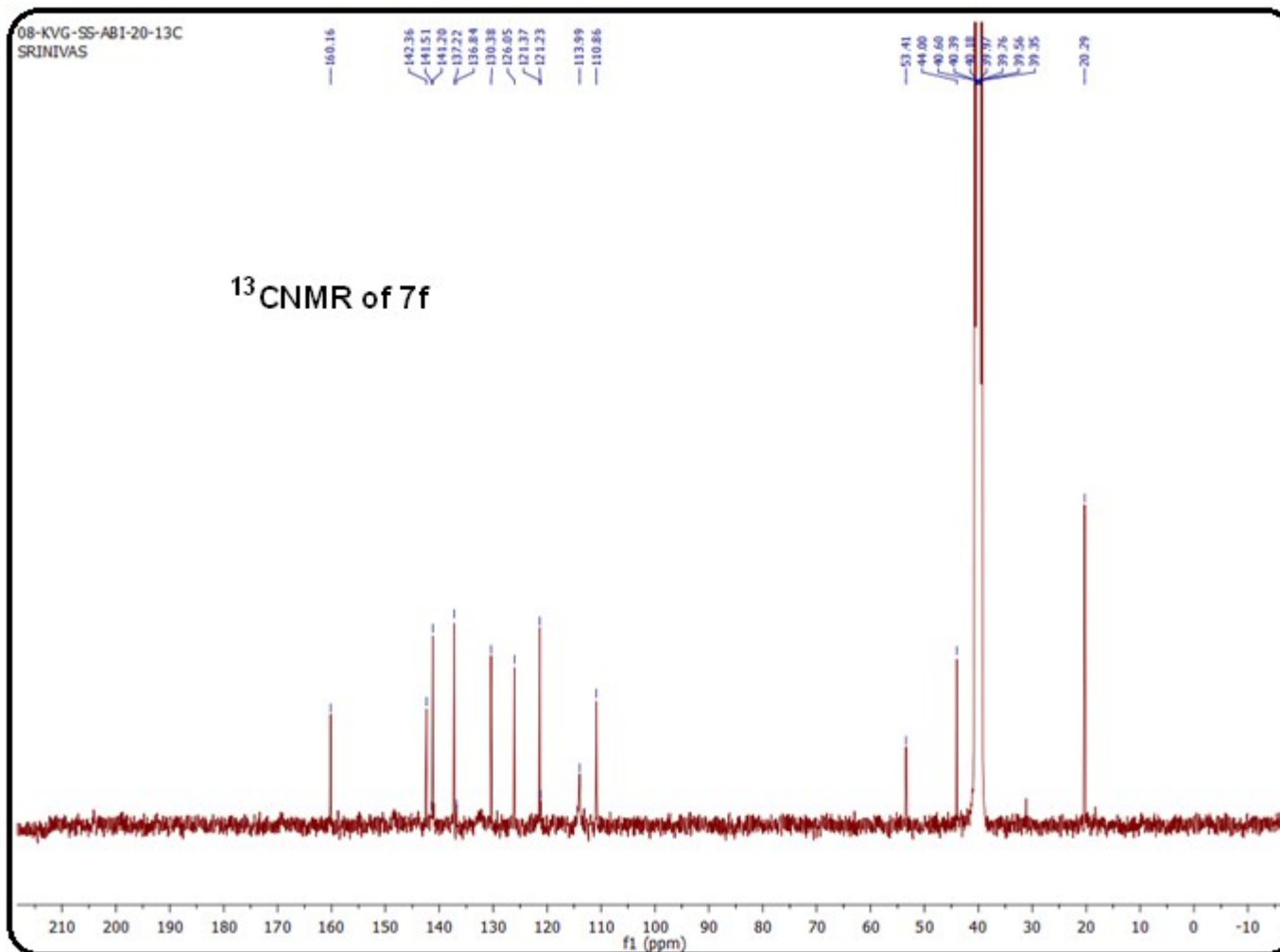
¹HNMR of 7d



¹³CNMR of 7d

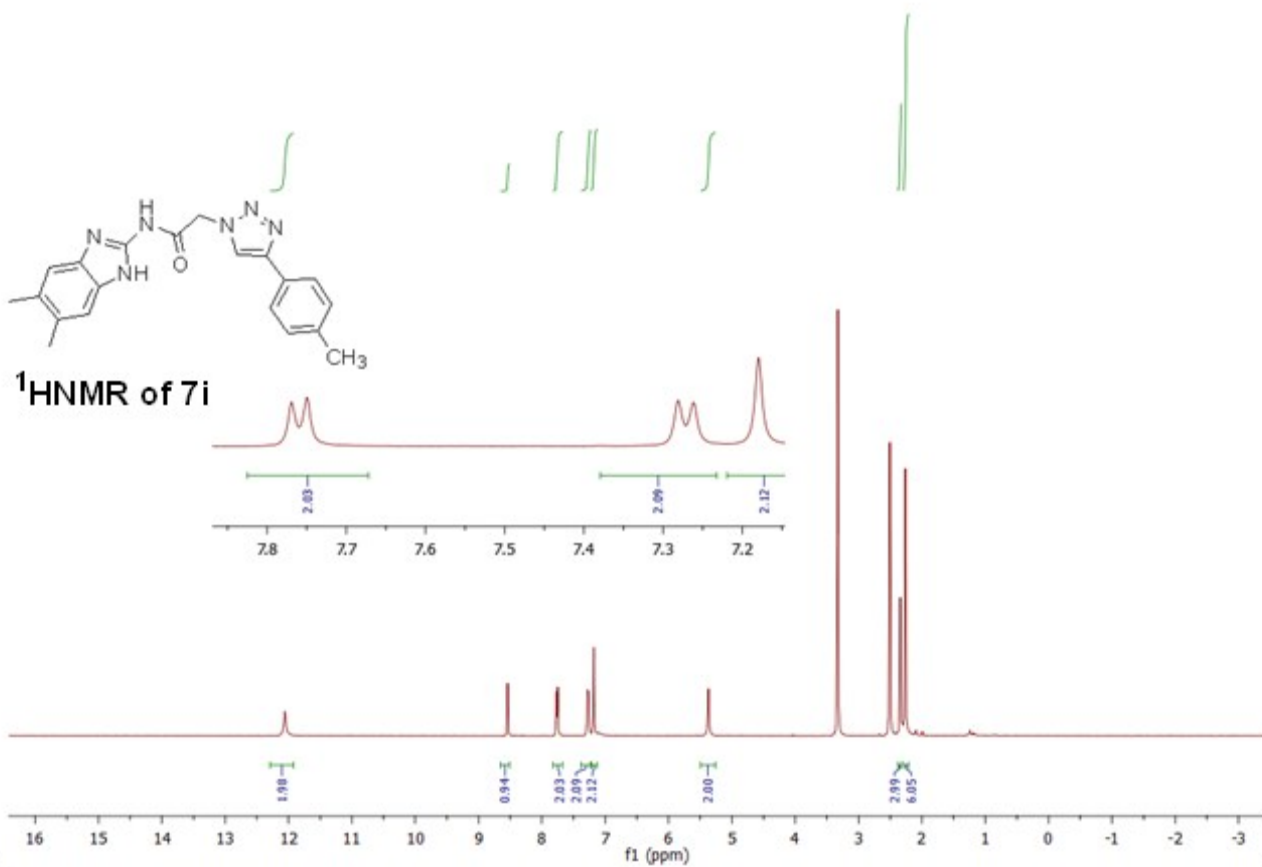


¹HNMR of 7f

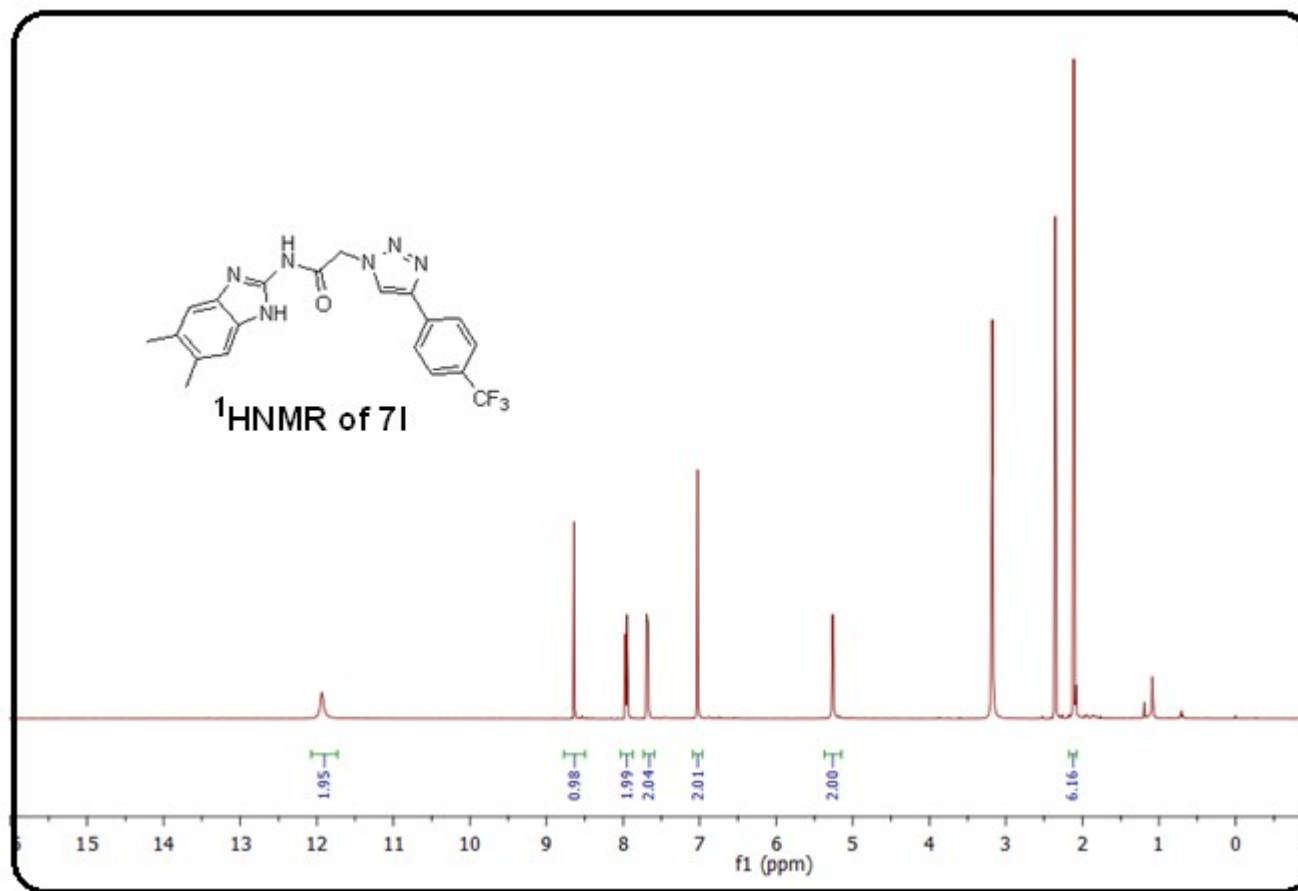


^{13}C NMR of 7f

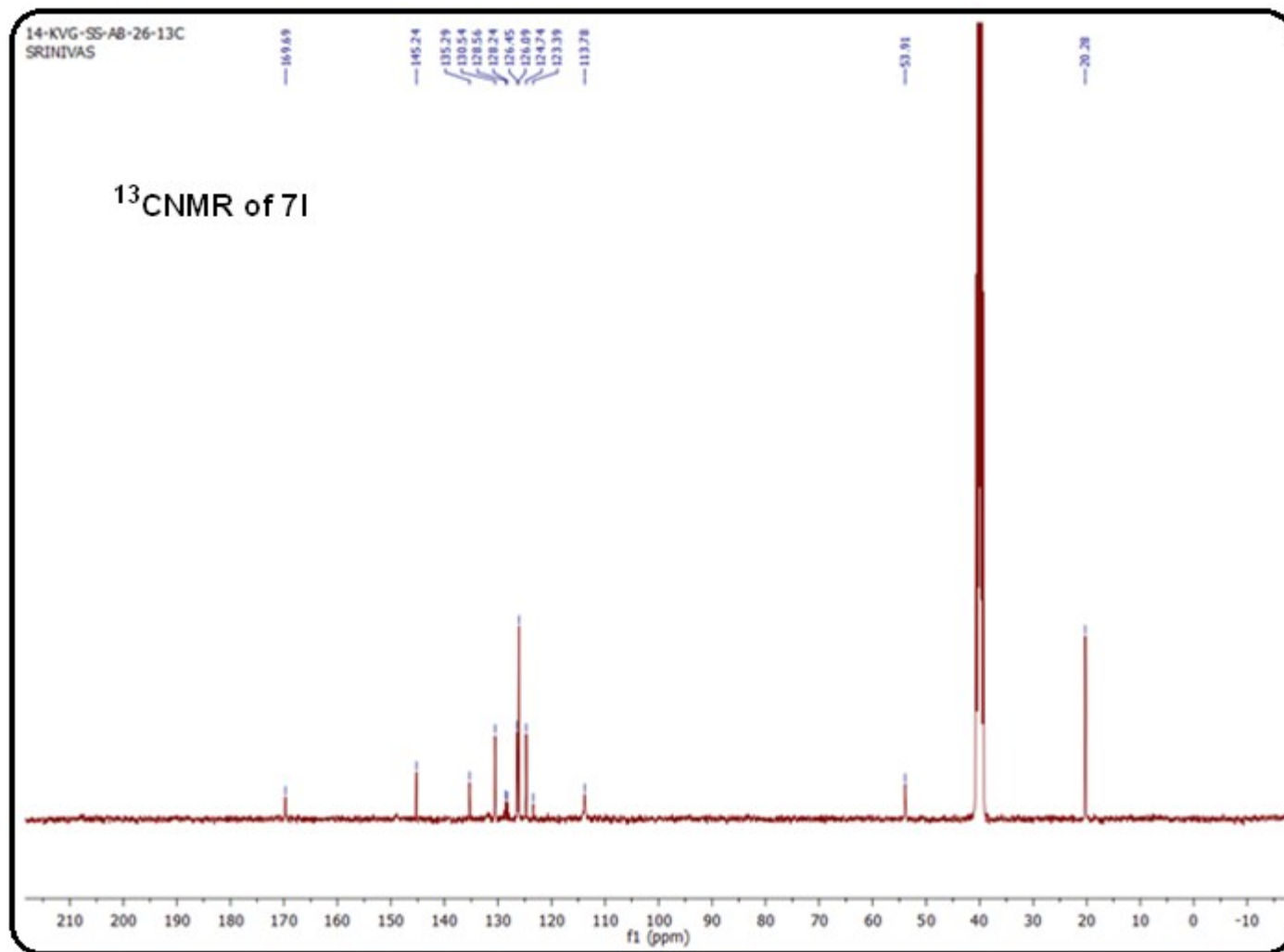
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SRINIVAS



¹H NMR of 7i

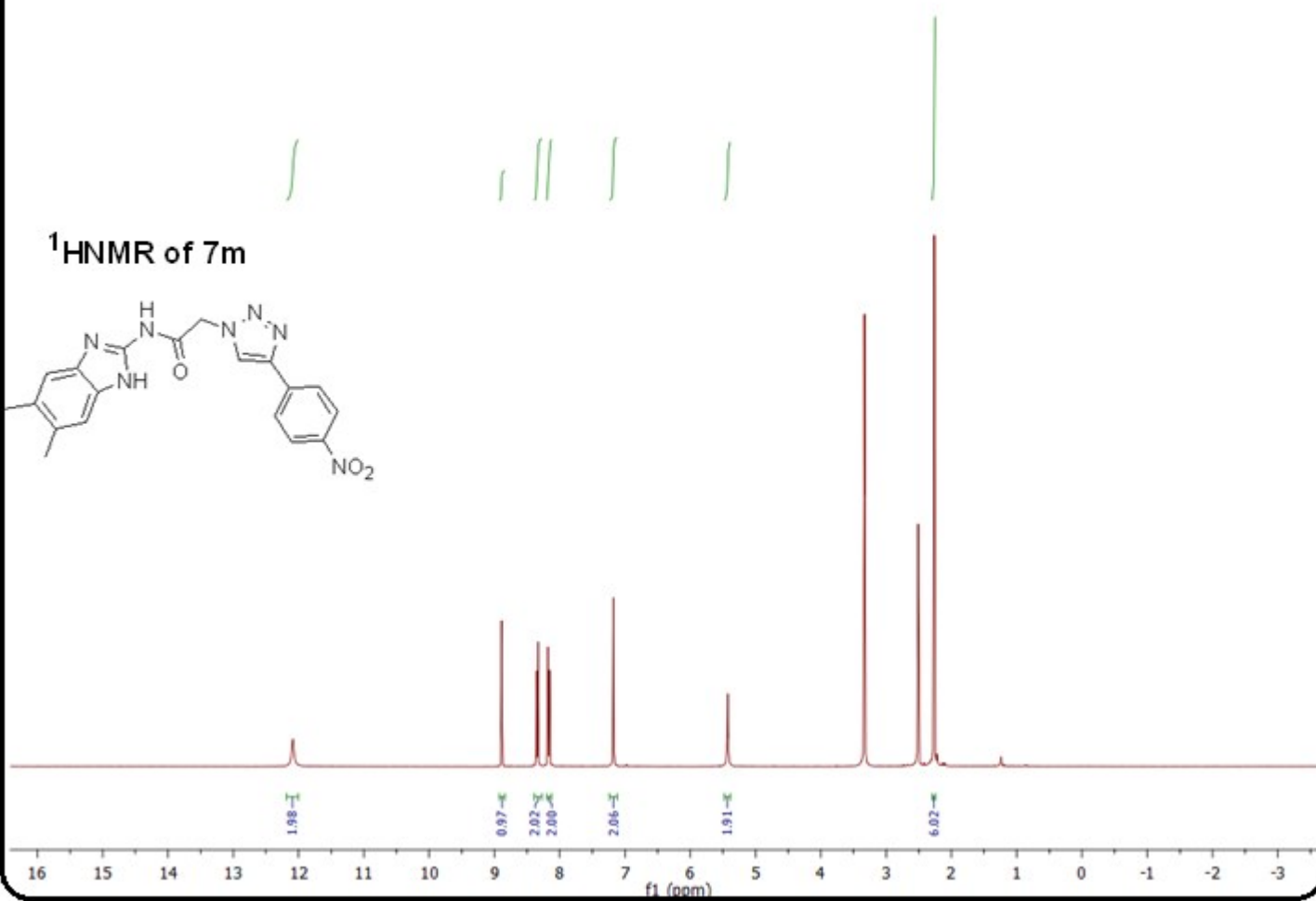


¹H NMR of 71



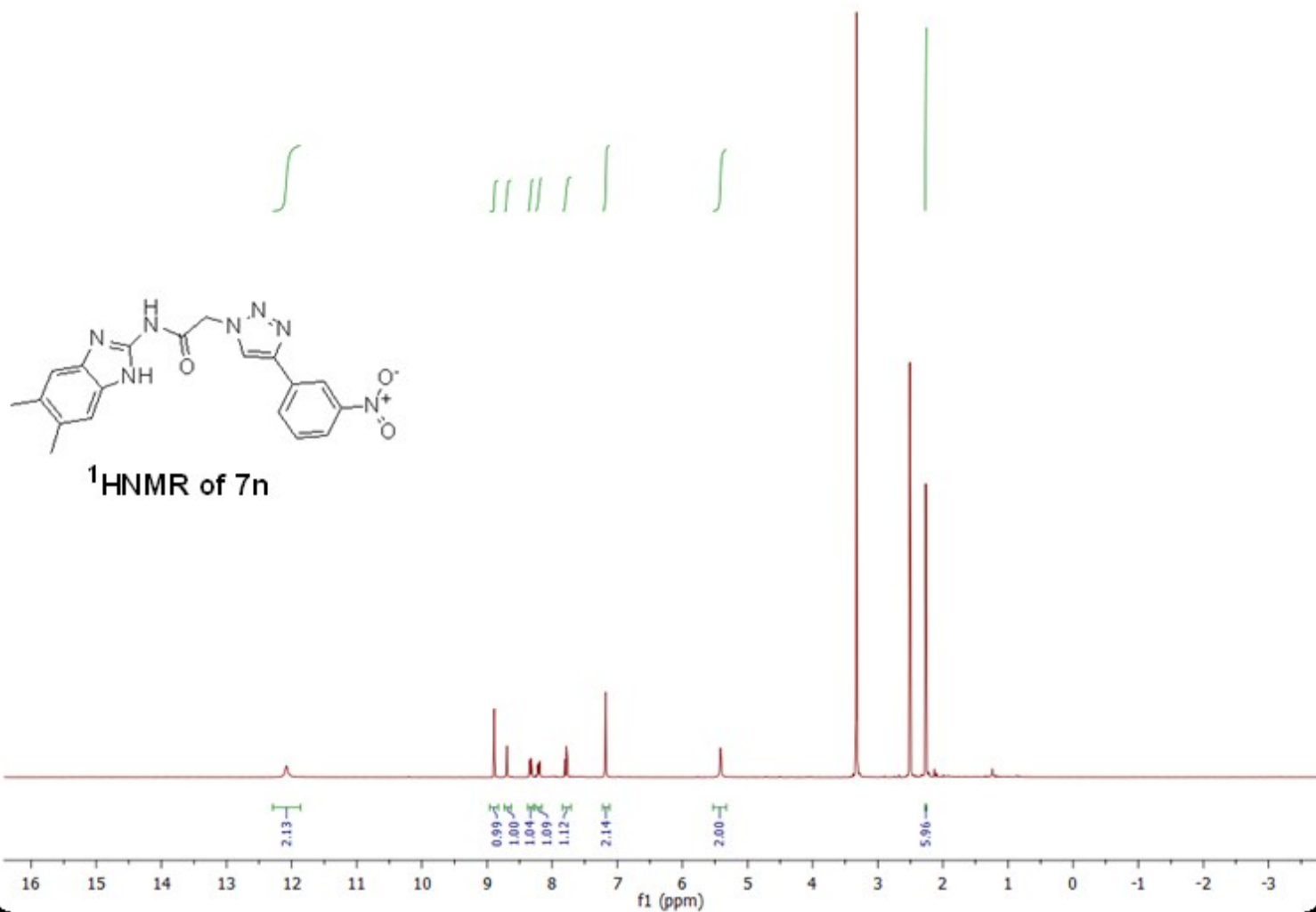
^{13}C NMR of 71

05-KVG-SS-AB1-27
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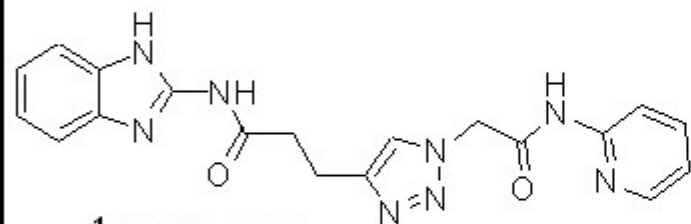
¹HNMR of 7m

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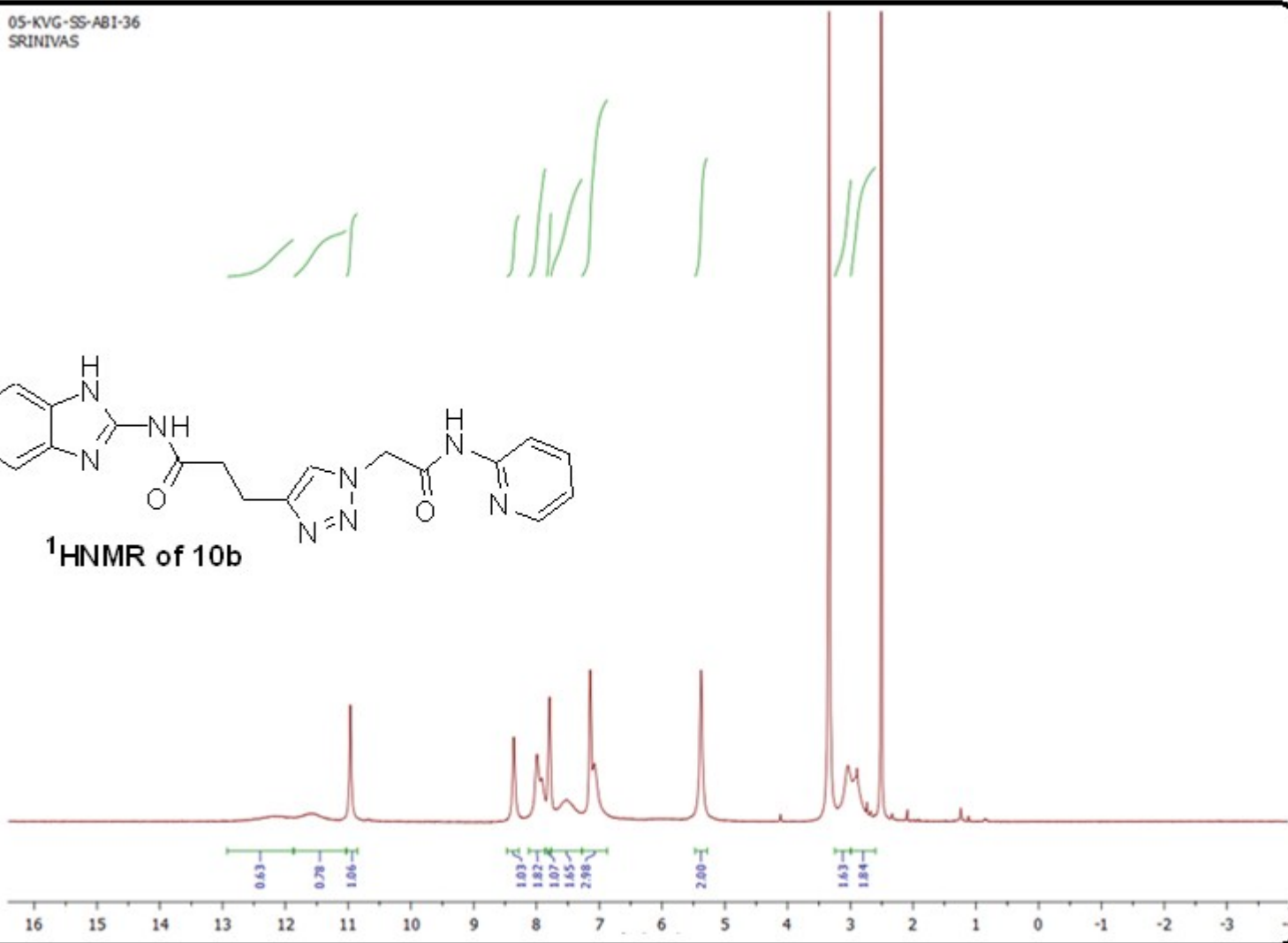


¹H NMR of 7n

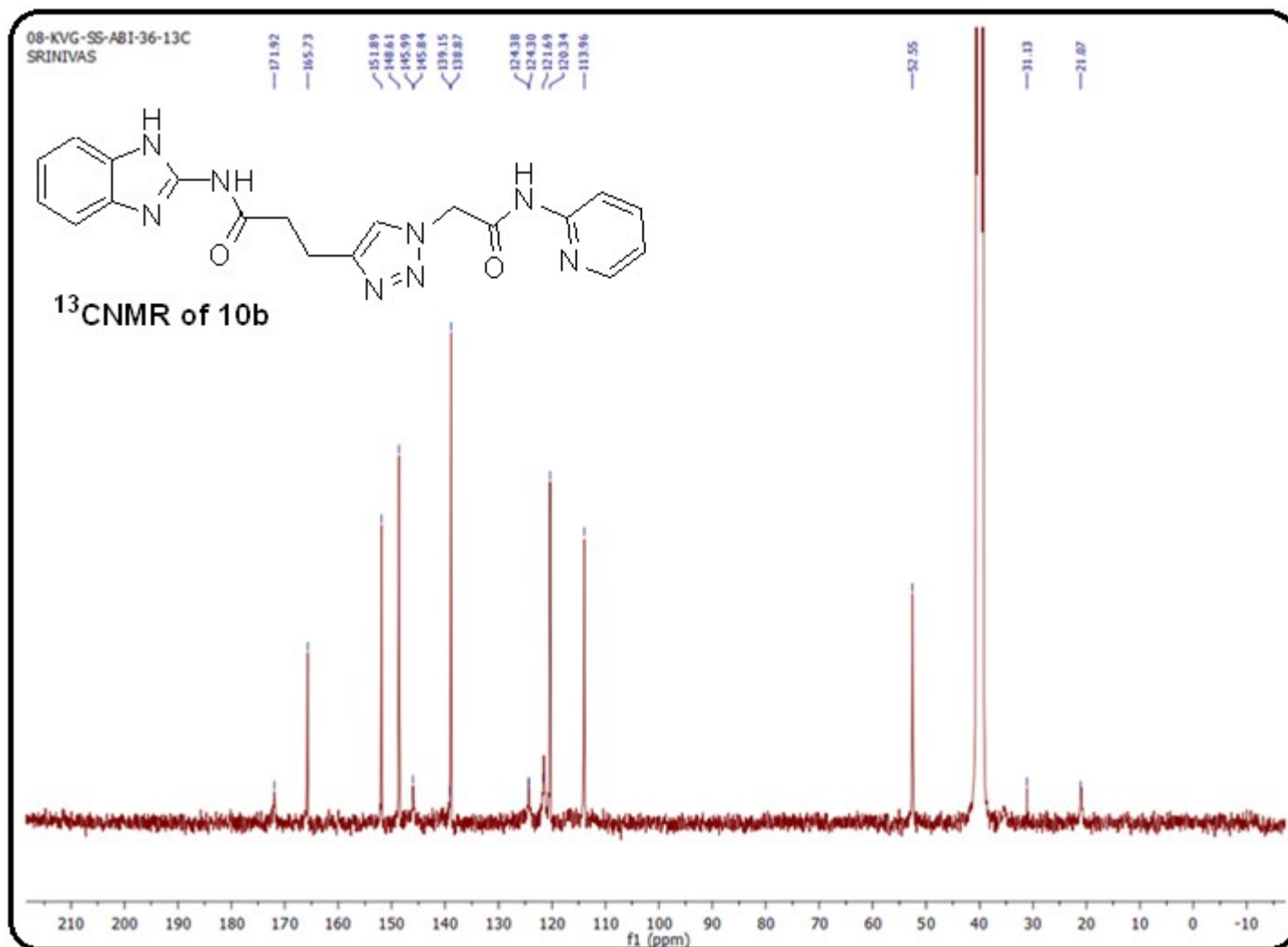
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¹HNMR of 10b

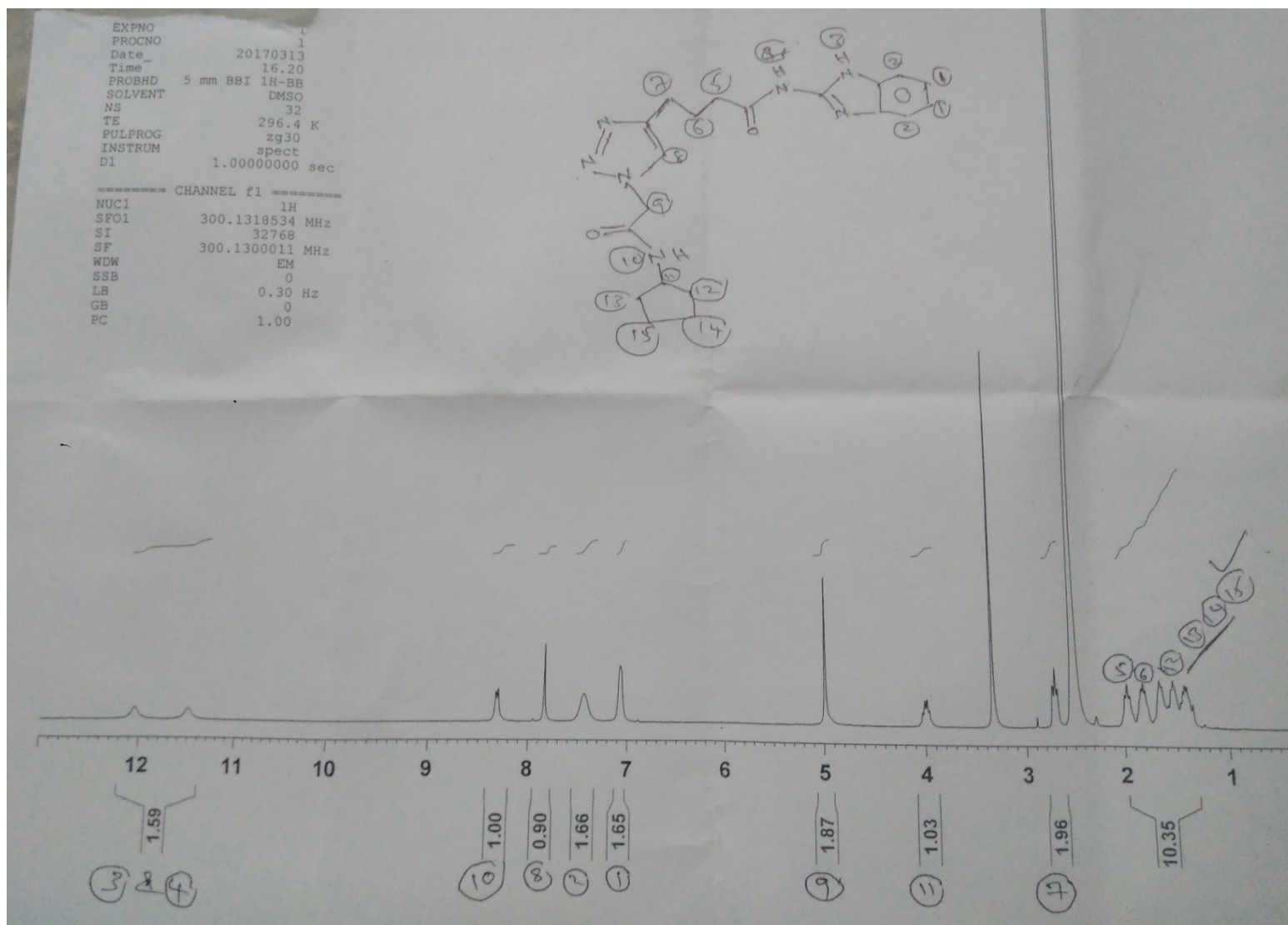


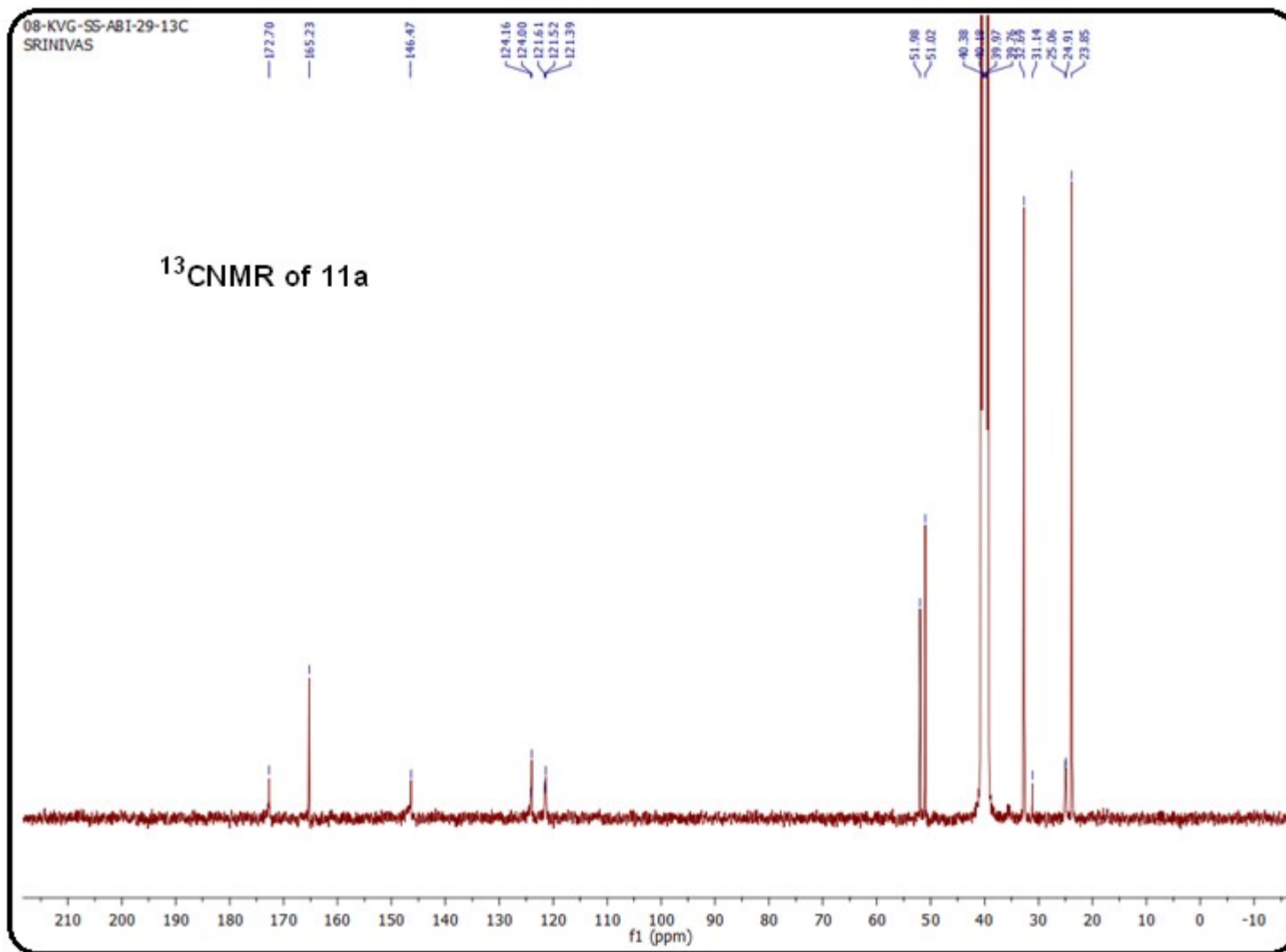
¹HNMR of 10b



¹³CNMR of 10b

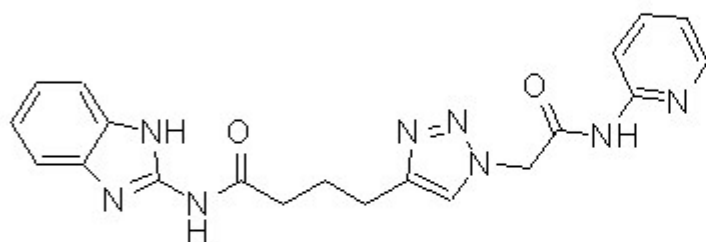
¹H NMR of 11a



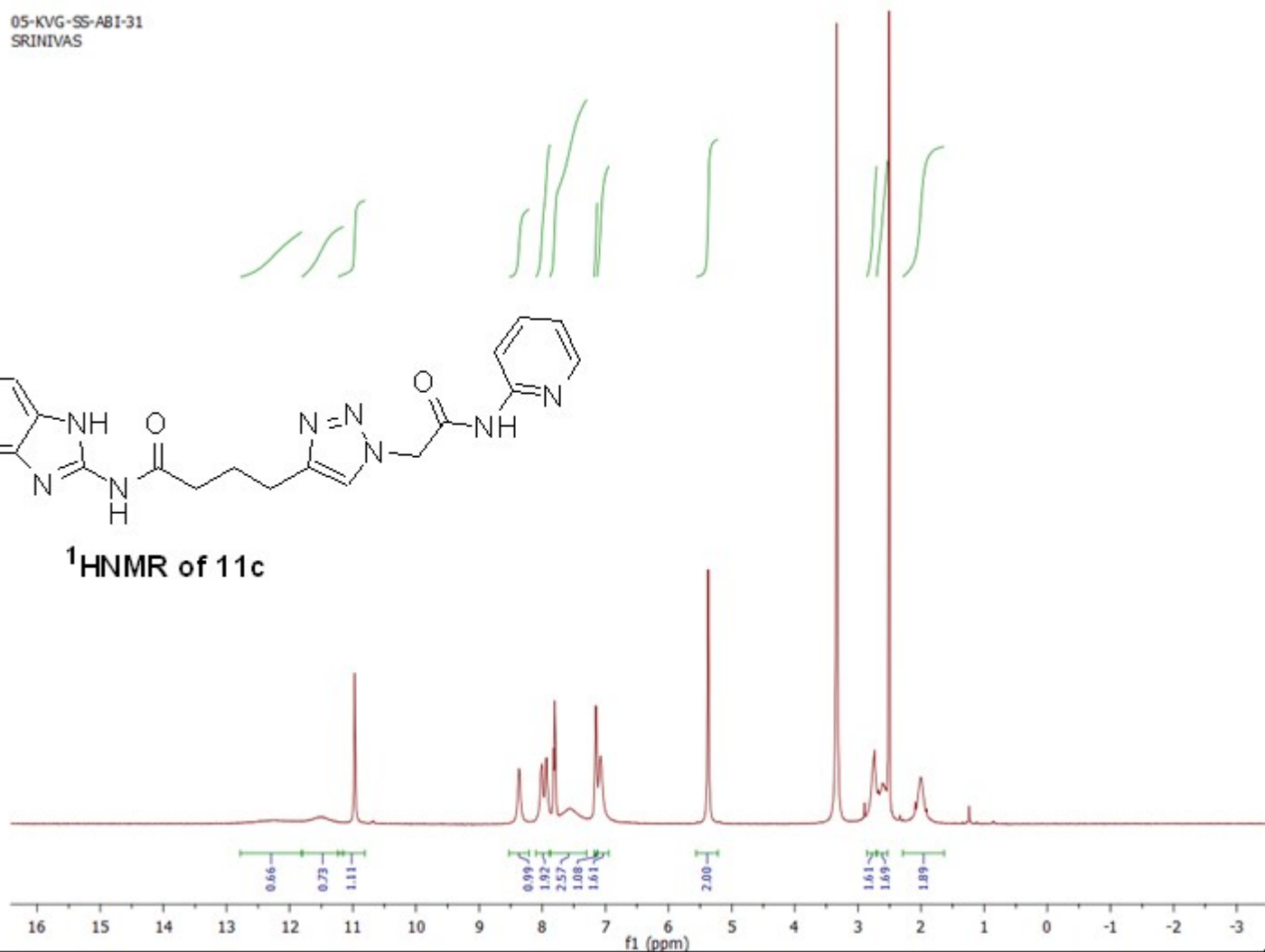


^{13}C NMR of 11a

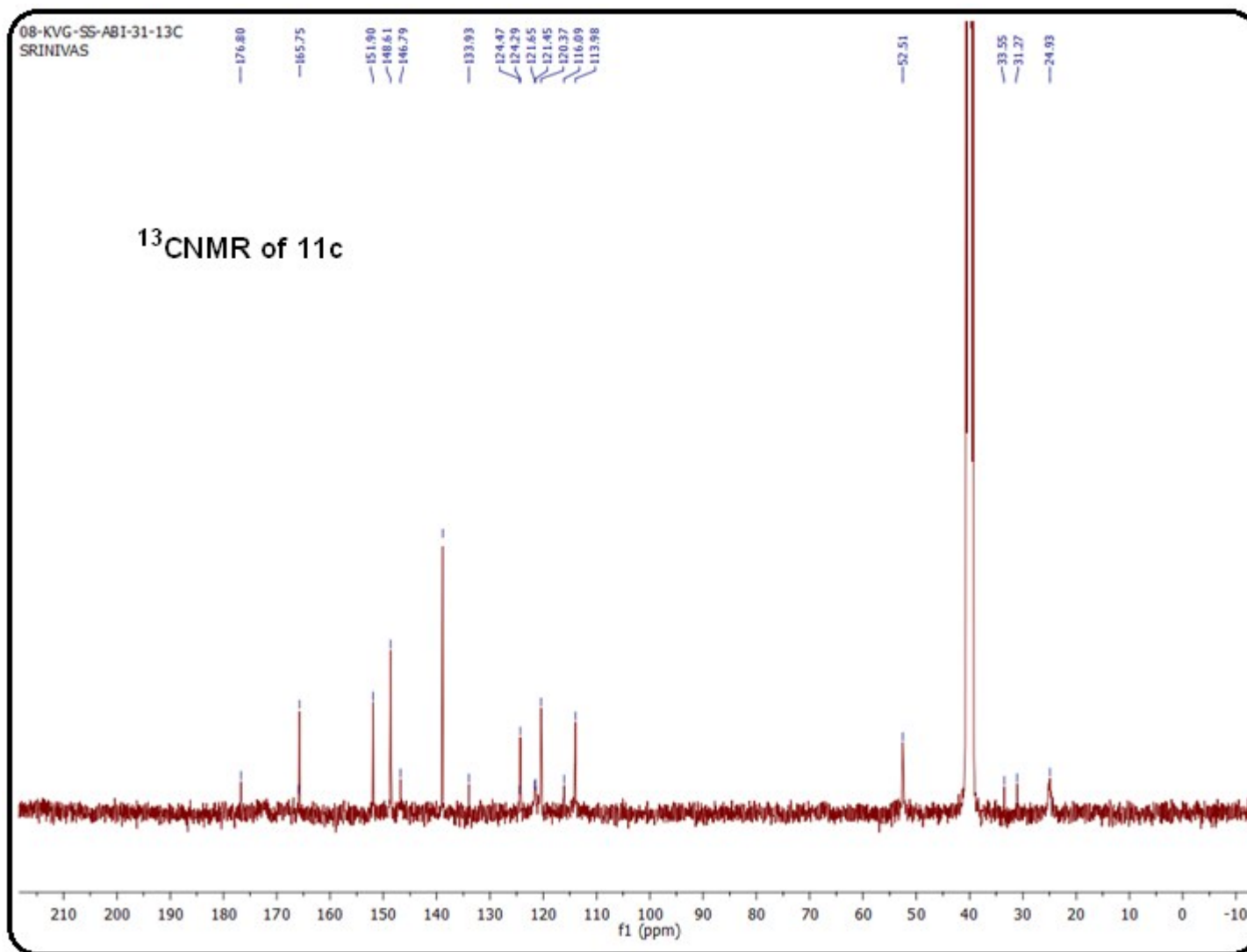
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SRINIVAS



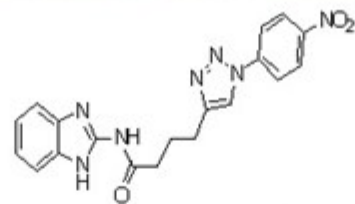
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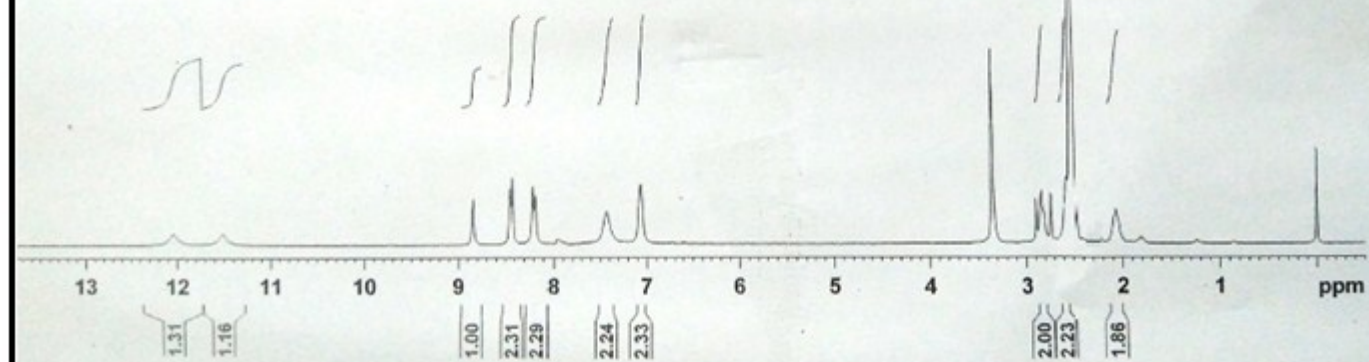
¹HNMR of 11c



^{13}C NMR of 11c

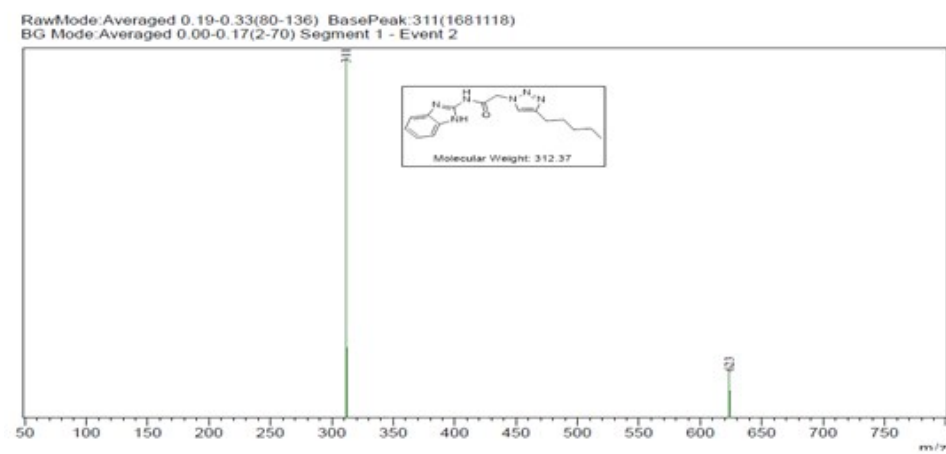
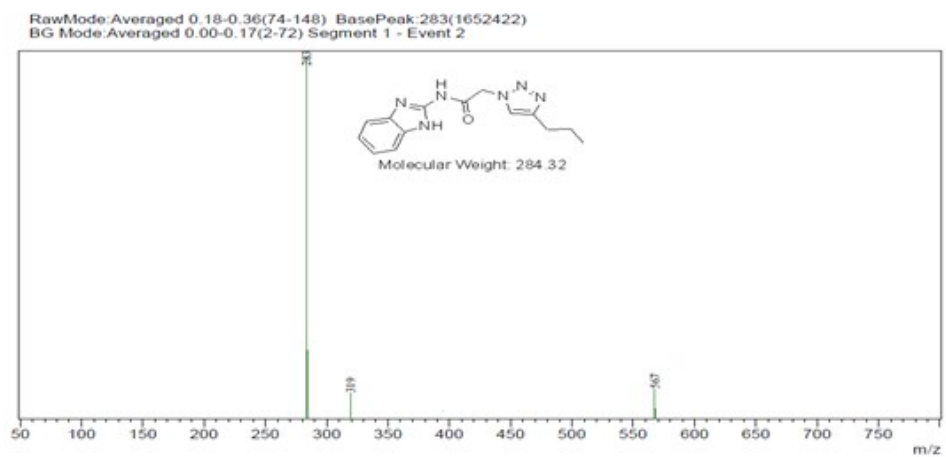


¹HNMR of 11d

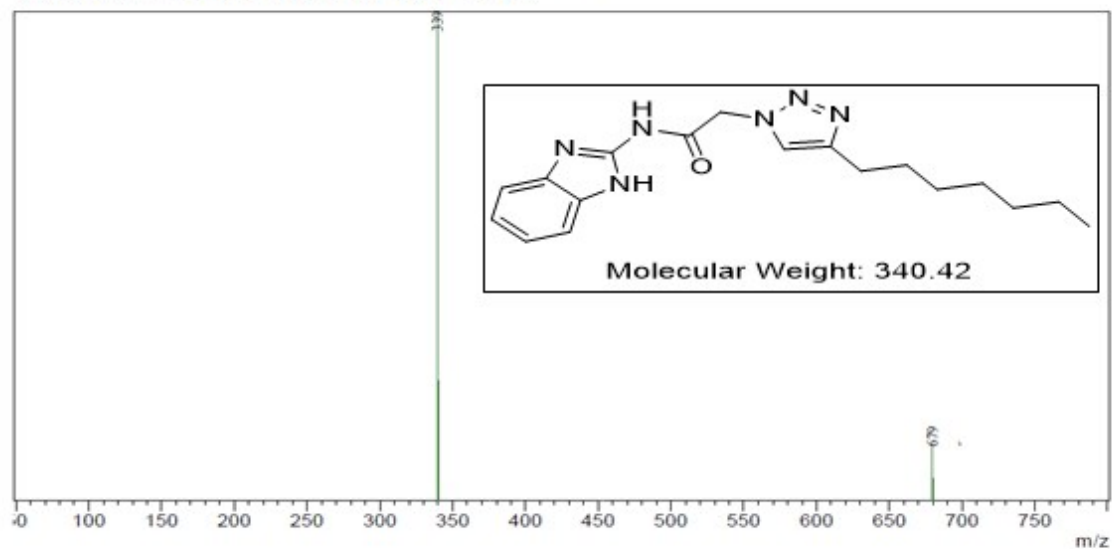


¹HNMR of 11d

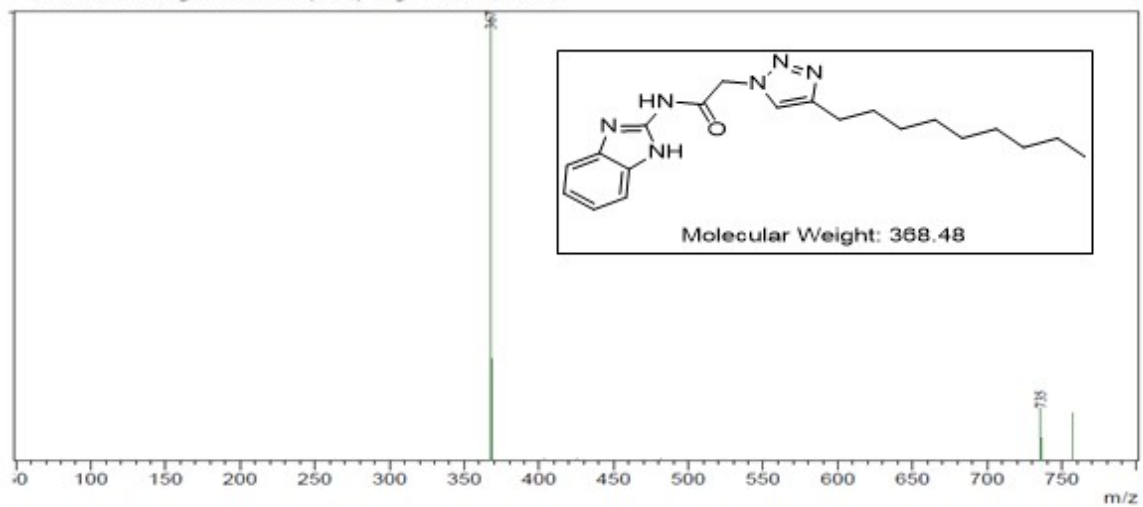
5. Mass spectra's



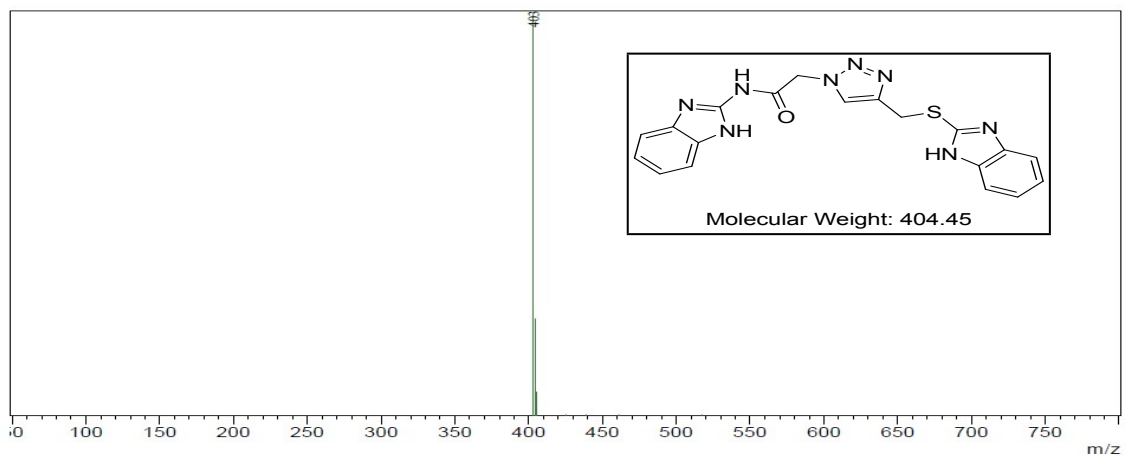
RawMode:Averaged 0.19-0.35(80-144) BasePeak:339(1204665)
BG Mode:Averaged 0.00-0.18(2-74) Segment 1 - Event 2



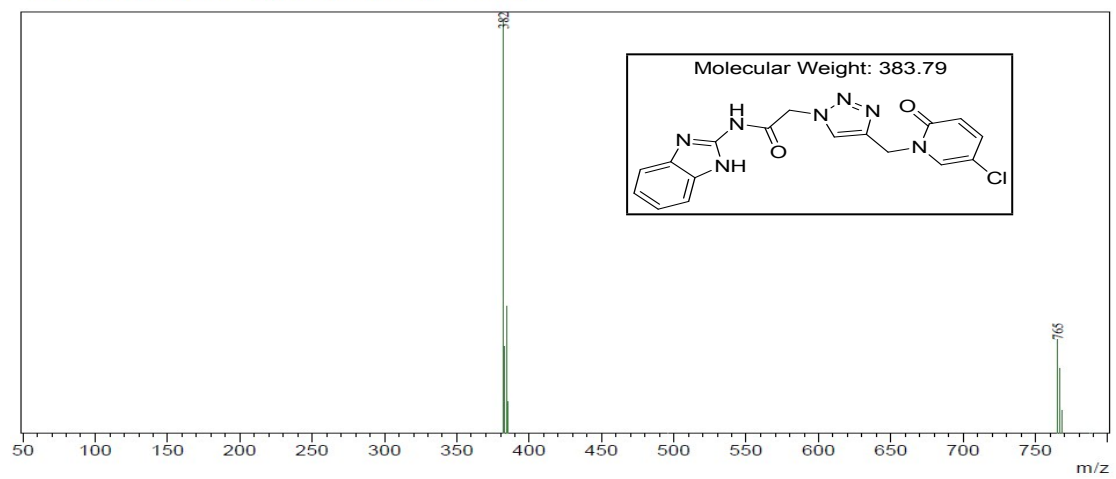
RawMode:Averaged 0.19-0.34(78-142) BasePeak:367(1270533)
BG Mode:Averaged 0.00-0.17(2-72) Segment 1 - Event 2



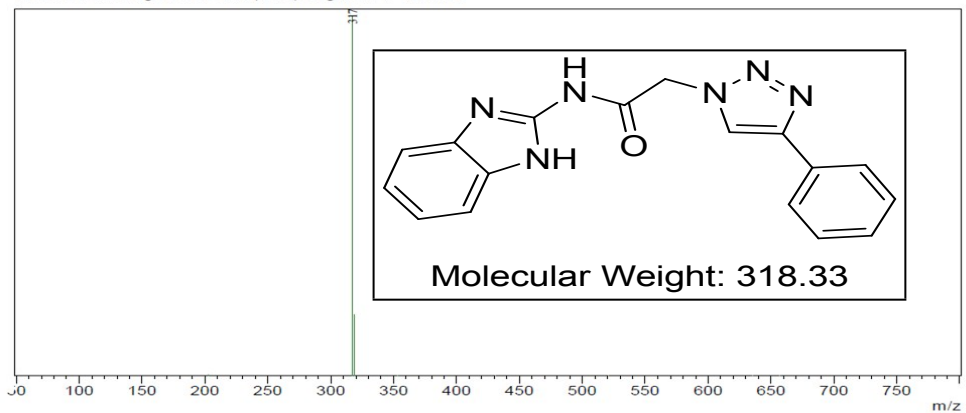
RawMode:Averaged 0.21-0.34(88-140) BasePeak:403(1622703)
BG Mode:Averaged 0.00-0.15(2-62) Segment 1 - Event 2



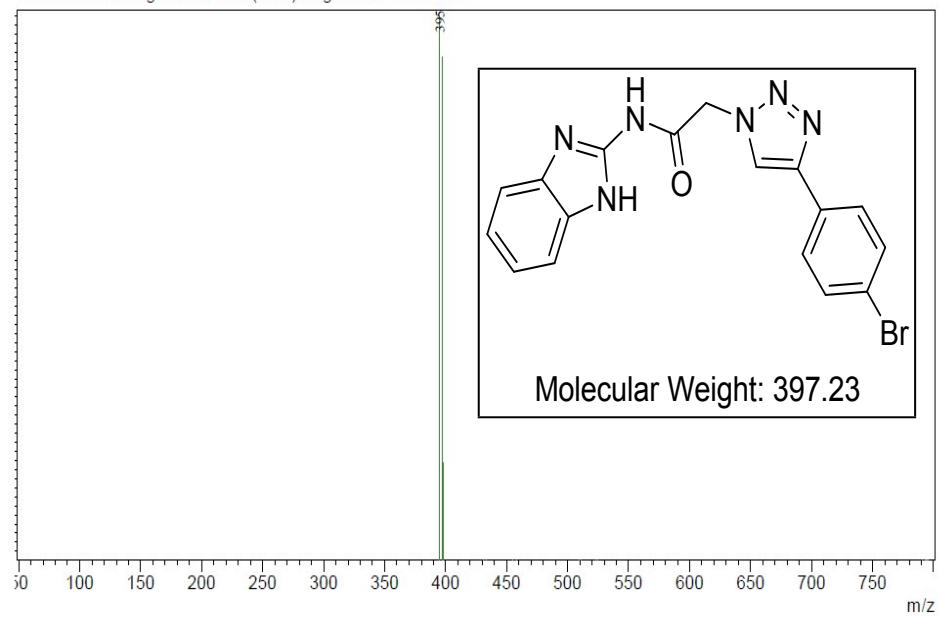
RawMode:Averaged 0.21-0.30(88-124) BasePeak:382(1086296)
BG Mode:Averaged 0.00-0.18(2-74) Segment 1 - Event 2



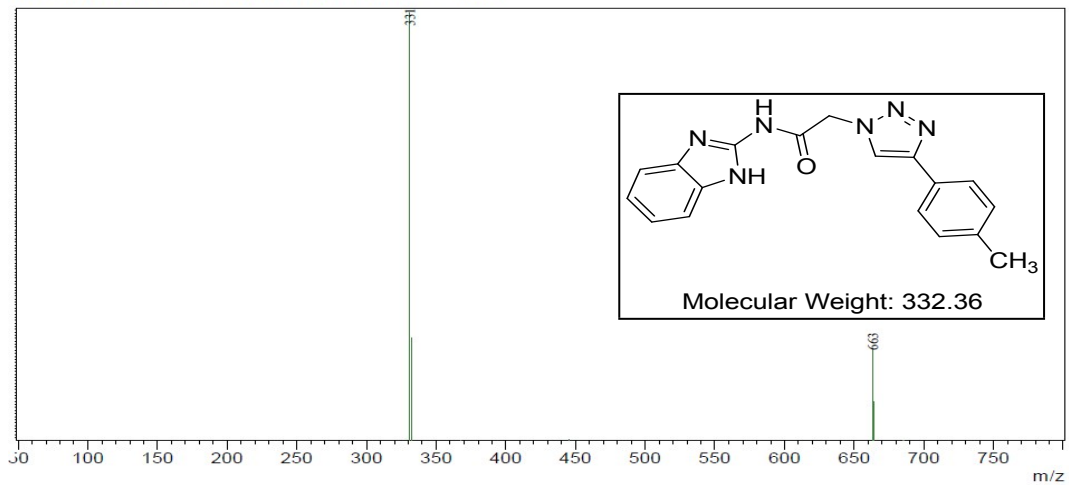
RawMode:Averaged 0.20-0.32(82-132) BasePeak:317(872668)
BG Mode:Averaged 0.00-0.17(2-72) Segment 1 - Event 2



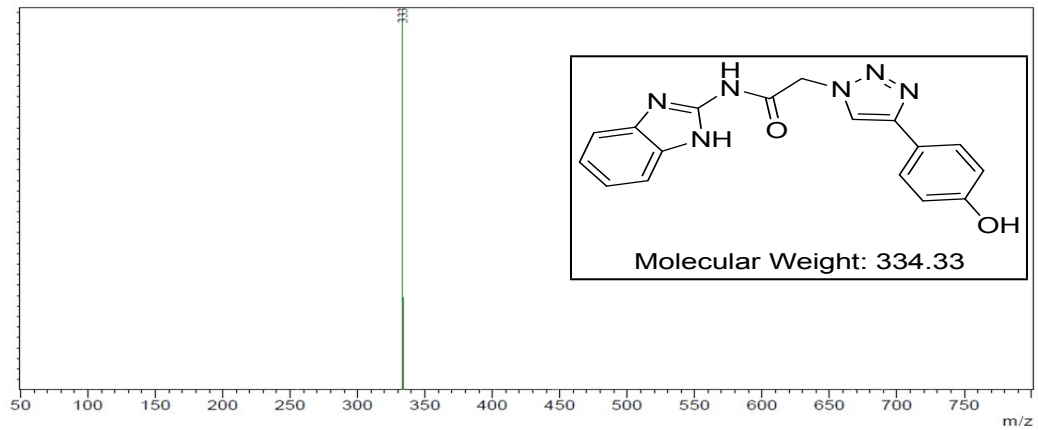
RawMode:Averaged 0.21-0.33(88-136) BasePeak:395(600430)
BG Mode:Averaged 0.00-0.18(2-74) Segment 1 - Event 2



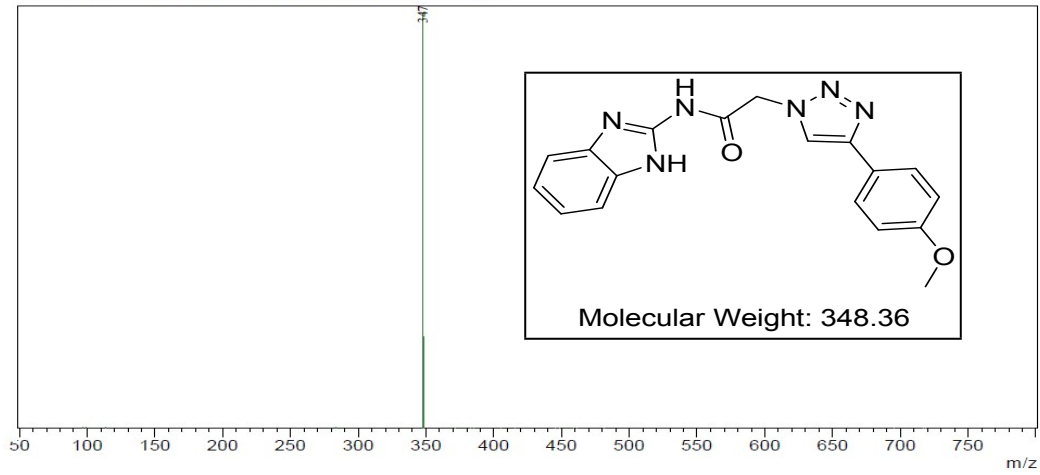
RawMode:Averaged 0.18-0.36(74-148) BasePeak:331(1422643)
BG Mode:Averaged 0.00-0.17(2-72) Segment 1 - Event 2



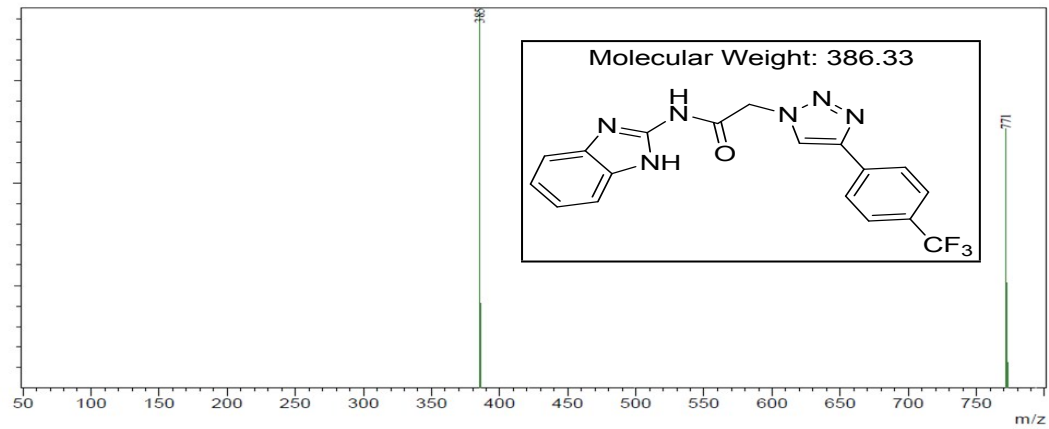
RawMode:Averaged 0.18-0.31(74-128) BasePeak:333(361269)
BG Mode:Averaged 0.00-0.18(2-74) Segment 1 - Event 2



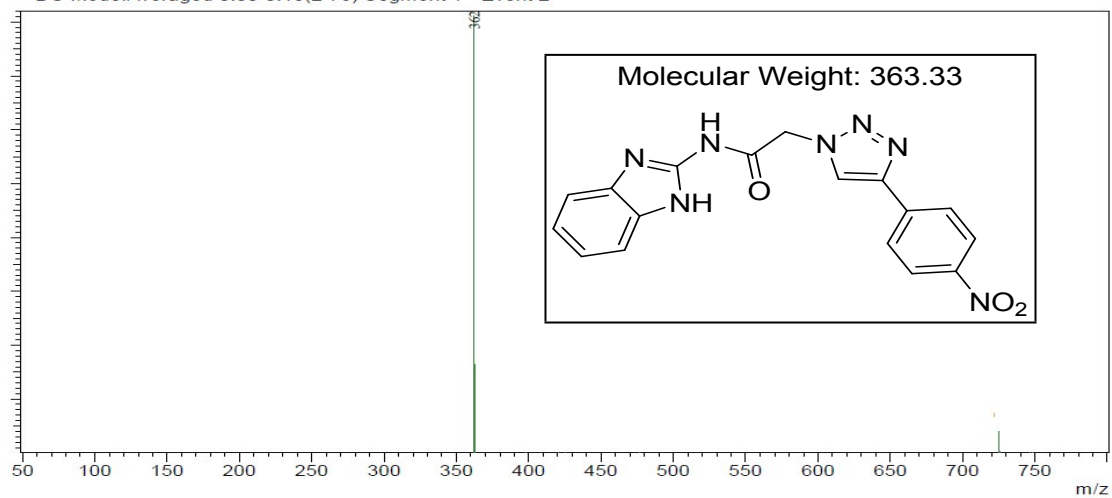
RawMode:Averaged 0.21-0.30(86-126) BasePeak:347(317596)
BG Mode:Averaged 0.00-0.19(2-80) Segment 1 - Event 2



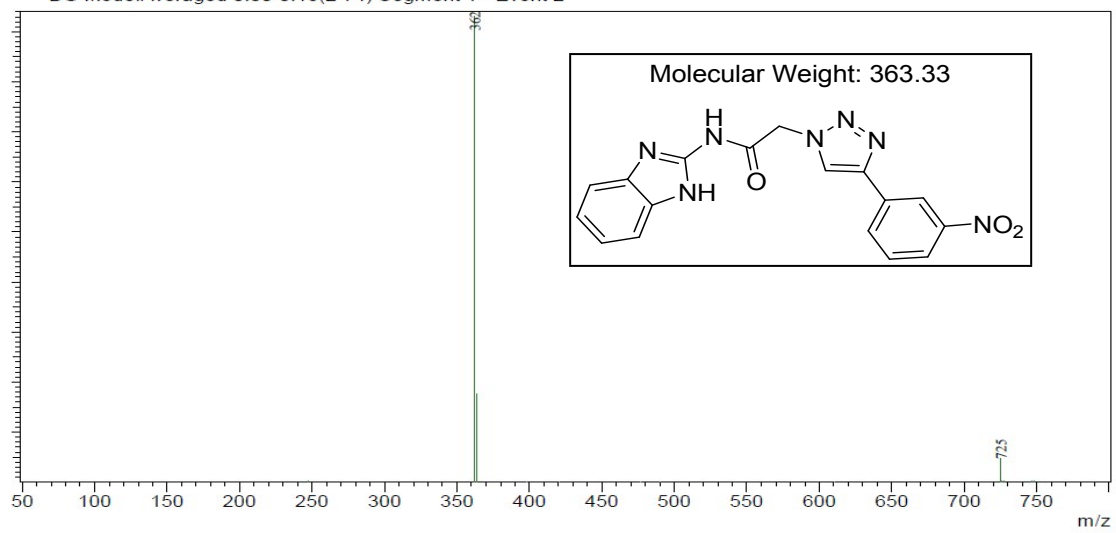
RawMode:Averaged 0.20-0.36(84-148) BasePeak:385(1836587)
BG Mode:Averaged 0.00-0.17(2-70) Segment 1 - Event 2



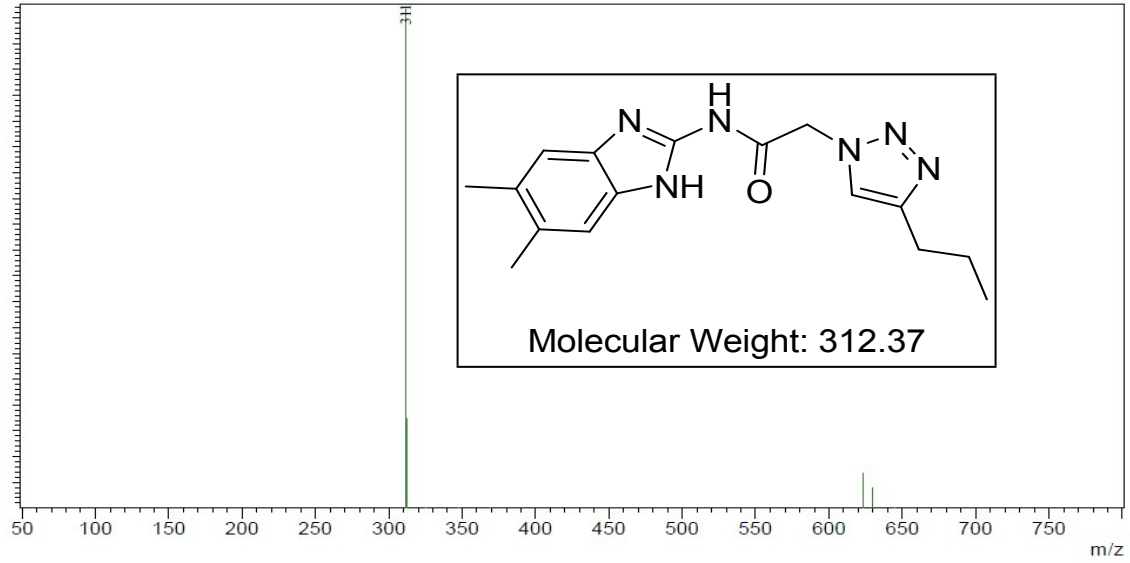
RawMode:Averaged 0.18-0.32(76-132) BasePeak:362(812196)
BG Mode:Averaged 0.00-0.18(2-76) Segment 1 - Event 2



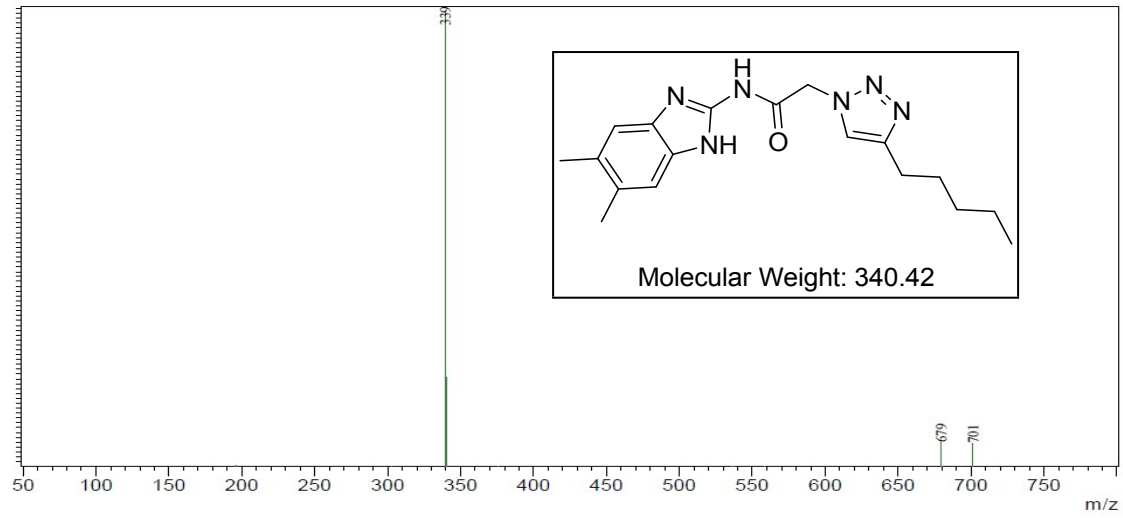
RawMode:Averaged 0.20-0.34(82-142) BasePeak:362(931700)
BG Mode:Averaged 0.00-0.18(2-74) Segment 1 - Event 2



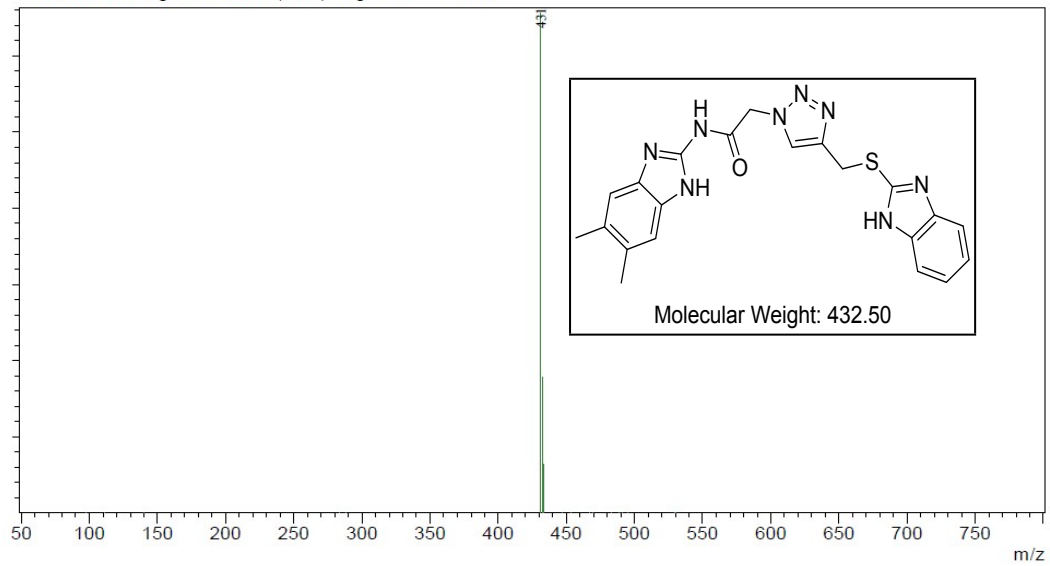
RawMode:Averaged 0.17-0.33(72-136) BasePeak:311(967323)
BG Mode:Averaged 0.00-0.17(2-72) Segment 1 - Event 2



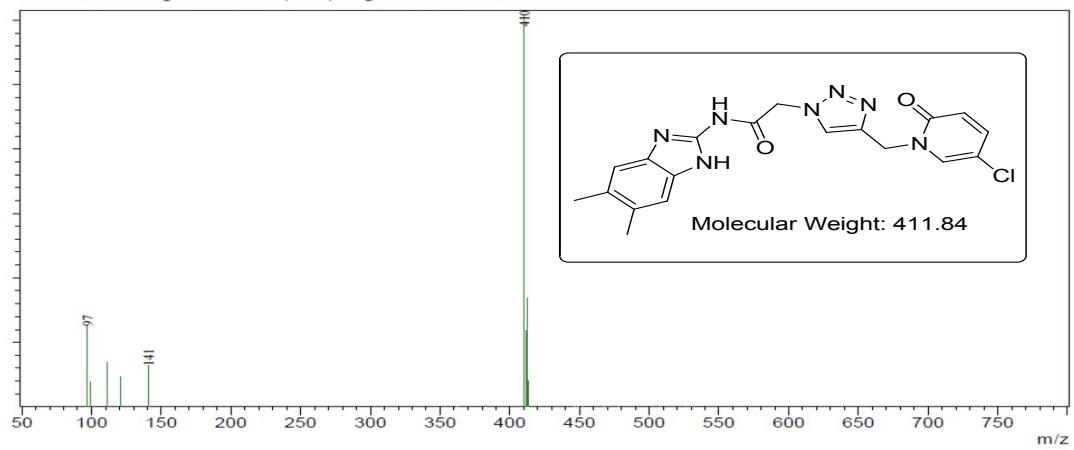
RawMode:Averaged 0.19-0.33(80-138) BasePeak:339(935055)
BG Mode:Averaged 0.00-0.19(2-78) Segment 1 - Event 2



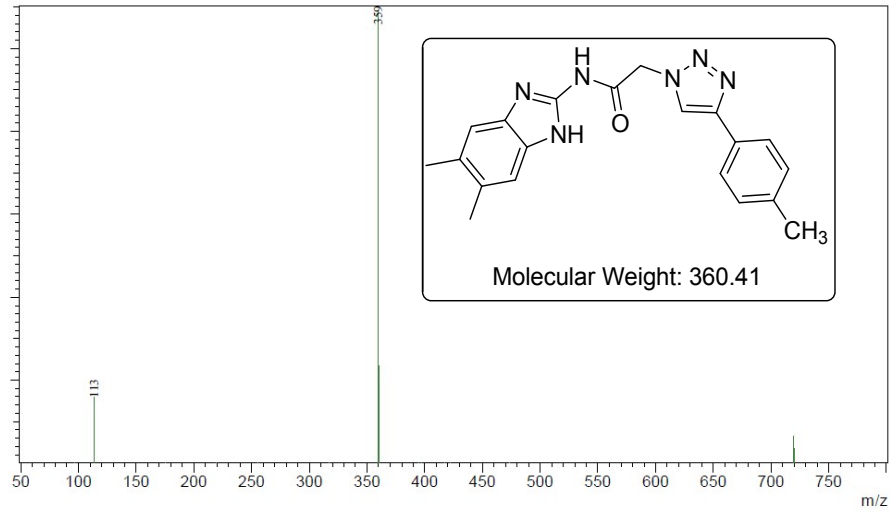
RawMode:Averaged 0.21-0.31(86-130) BasePeak:431(328445)
BG Mode:Averaged 0.00-0.19(2-80) Segment 1 - Event 2



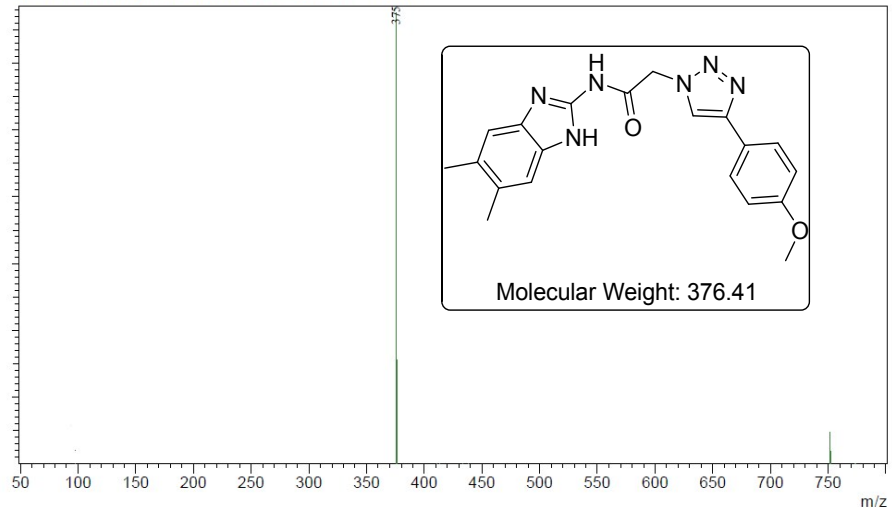
RawMode:Averaged 0.19-0.30(80-126) BasePeak:410(304680)
BG Mode:Averaged 0.00-0.17(2-70) Segment 1 - Event 2



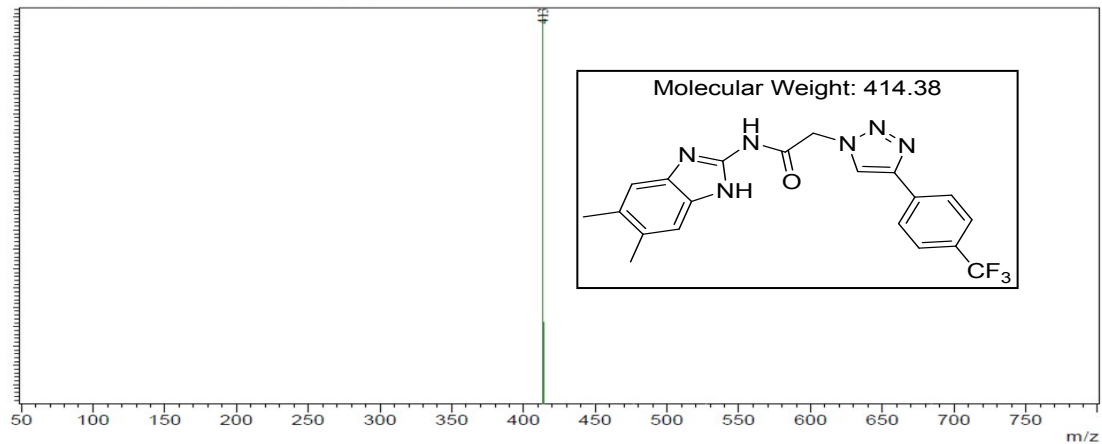
RawMode:Averaged 0.19-0.30(78-126) BasePeak:359(546060)
BG Mode:Averaged 0.00-0.20(2-82) Segment 1 - Event 2



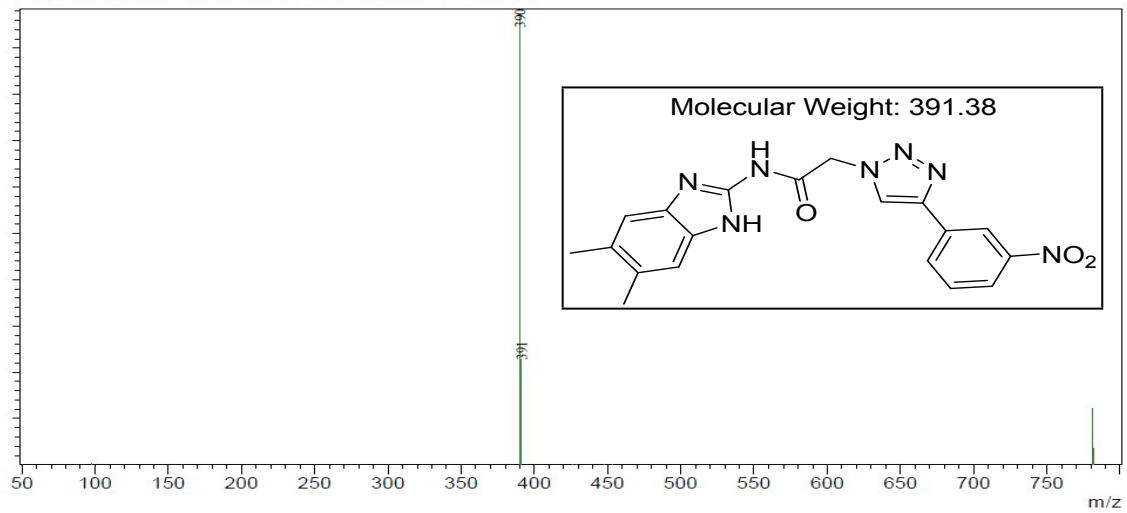
RawMode:Averaged 0.20-0.34(84-140) BasePeak:375(675470)
BG Mode:Averaged 0.00-0.20(2-84) Segment 1 - Event 2

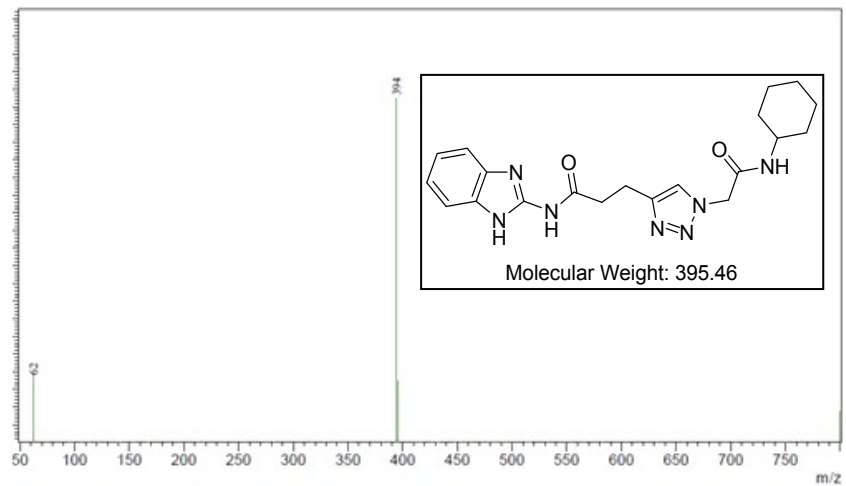


RawMode:Averaged 0.22-0.33(94-138) BasePeak:413(1014285)
BG Mode:Averaged 0.00-0.19(2-80) Segment 1 - Event 2

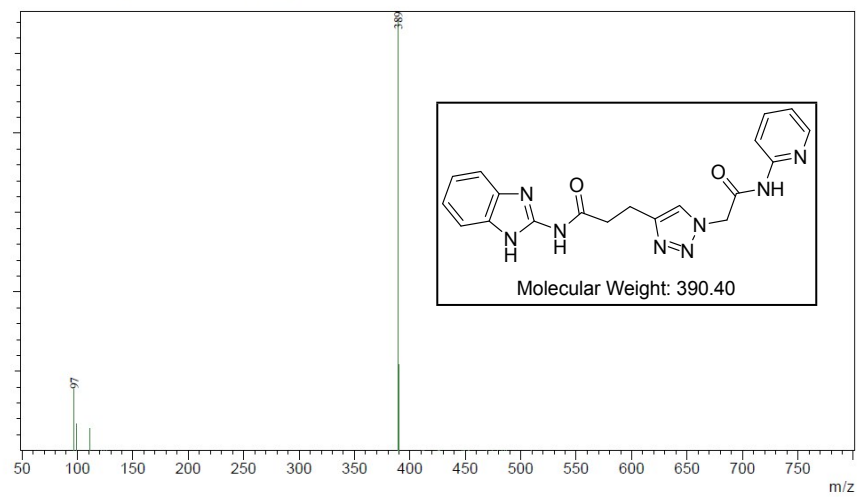


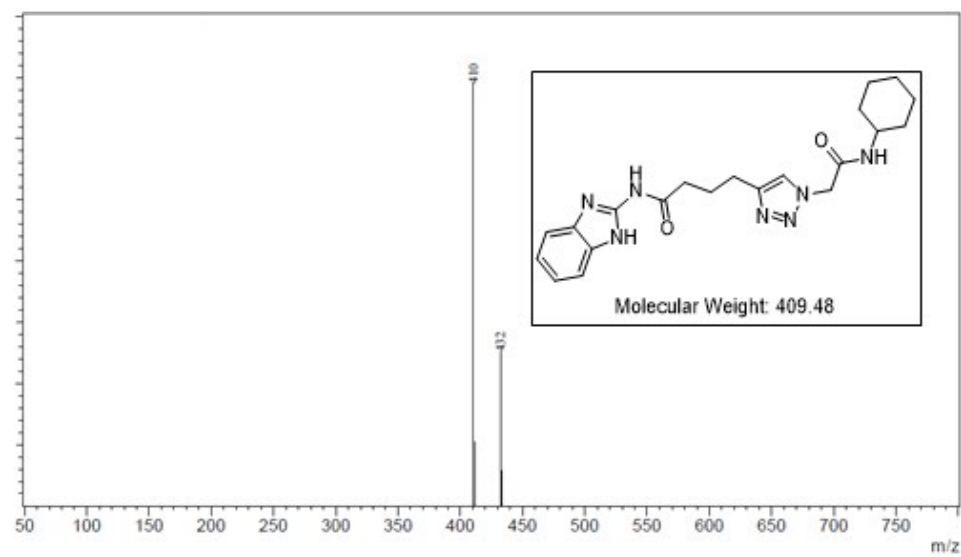
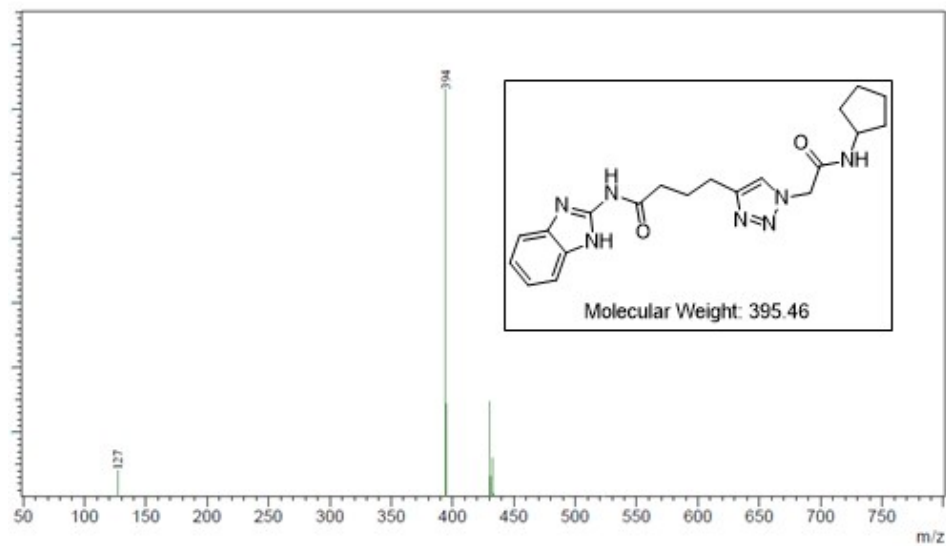
RawMode:Averaged 0.19-0.33(78-136) BasePeak:390(487706)
BG Mode:Averaged 0.00-0.18(2-74) Segment 1 - Event 2



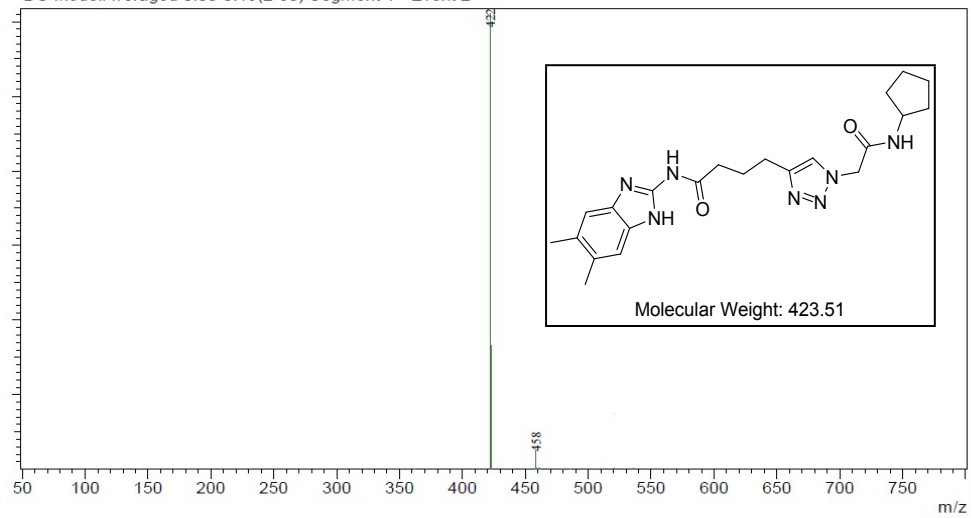


RawMode:Averaged 0.18-0.31(74-128) BasePeak:389(271223)
BG Mode:Averaged 0.00-0.20(2-84) Segment 1 - Event 2





RawMode:Averaged 0.22-0.35(92-146) BasePeak:422(611542)
BG Mode:Averaged 0.00-0.19(2-80) Segment 1 - Event 2



RawMode:Averaged 0.17-0.33(72-138) BasePeak:431(386073)
BG Mode:Averaged 0.00-0.19(2-78) Segment 1 - Event 2

