Supplementary Data

Syntheses of fluorescent imidazoquinoline conjugates as probes of Toll-like receptor 7

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Chemistry. All of the solvents and reagents used were obtained commercially and used as such unless noted otherwise. Moisture- or air-sensitive reactions were conducted under nitrogen atmosphere in oven-dried (120 ^{o}C) glass apparatus. The solvents were removed under reduced pressure using standard rotary evaporators. Flash column chromatography was carried out using RediSep Rf 'Gold' high performance silica columns on CombiFlash Rf instruments unless otherwise mentioned, while thin-layer chromatography was carried out on silica gel CCM pre-coated aluminum sheets. Purity for all final compounds was confirmed to be greater than 97% by LC-MS using a Zorbax Eclipse Plus 4.6 mm x 150 mm, 5 μm analytical reverse phase C_{18} column with H_2O-isopropanol or H_2O-CH_3CN gradients and an Agilent ESI-TOF mass spectrometer (mass accuracy of 3 ppm) operating in the positive ion acquisition mode.

Synthesis of Compound 4a: 2-Butyl-1-(naphthalen-1-ylmethyl)-1*H*-imidazo[4,5-c]quinolin-4-amine. To a solution of **1** (100 mg, 0.41 mmol) in 5 mL of anhydrous dichloromethane, were added triethylamine (54 mg, 0.53 mmol) and naphthalen-1-ylmethanamine (71 mg, 0.45 mmol). The reaction mixture was refluxed at 45 ^{0}C for 30 minutes. The solvent was then evaporated under vacuum and product was isolated using column chromatography to obtain the intermediate compound **2a**. To a solution of **2a** in 10 mL of EtOAc, were added a catalytic amount of Pt/C and Na_2SO_4. The reaction mixture was subjected to hydrogenation at 55 psi hydrogen pressure for 4 hours. The reaction mixture was then filtered through celite and the filtrate was evaporated under vacuum to obtain the compound **3a** (90 mg). To a solution of **3a** (90 mg, 0.27 mmol) in anhydrous THF, were added triethylamine (41 mg, 0.41 mmol) and valeryl chloride (39 mg, 0.32 mmol). The reaction mixture was stirred at room temperature for 6 hours. The solvent was then removed under vacuum, and the residue was dissolved in ethyl acetate and washed with water. The ethyl acetate fraction was then dried using Na_2SO_4 and evaporated under vacuum to obtain the intermediate amide compound, which was then dissolved in 2 ml of 2M solution of ammonia in MeOH. The sealed reaction vessel was heated 150 ^{0}C for 24 hours. The solvent was then removed under vacuum and the residue was purified using column chromatography (7% MeOH/dichloromethane) to obtain the compound **4a** (62 mg; 40%). ^{1}H NMR (500 MHz, DMSO) δ 8.40 (d, *J* = 8.3 Hz, 1H), 8.05 (d, *J* = 8.1 Hz, 1H), 7.86 (d, *J* = 8.2 Hz, 1H), 7.77 (t, *J* = 7.2 Hz, 1H), 7.69 (t, *J* = 7.5 Hz, 1H), 7.62 (d, *J* = 8.2 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 1H), 7.33 (t, *J* = 7.5 Hz, 1H), 7.27 (t, *J* = 7.7 Hz, 1H), 7.08 (s, 2H), 6.90 (t, *J* = 7.5 Hz, 1H), 6.39 (d, *J* = 7.1 Hz, 1H), 6.35 (s, 2H), 2.92 (t, *J* = 7.6 Hz, 2H), 1.69 (dt, *J* = 15.2, 7.6 Hz, 2H), 1.32 (dt, *J* = 14.6, 7.4 Hz, 2H), 0.80 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 154.39, 151.16, 133.62, 133.25, 131.77, 129.64, 128.74, 127.88, 126.91, 126.80, 126.48, 126.15, 125.58, 123.07, 121.57, 121.42, 119.87, 114.11, 46.60, 29.45, 26.08, 21.70, 13.61. MS (ESI) calculated for $C_{25}H_{24}N_4$, m/z 380.20, found 381.21 $(M + H)^+$.

Compound 4b was synthesized similarly as described for compound 4a.

4b: 1-(Biphenyl-4-ylmethyl)-2-butyl-1*H*-imidazo[4,5-c]quinolin-4-amine. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (d, *J* = 7.9 Hz, 1H), 7.78 – 7.73 (m, 1H), 7.60 – 7.56 (m, 2H), 7.55 – 7.52 (m, 2H), 7.51 – 7.47 (m, 1H), 7.45 – 7.40 (m, 2H), 7.35 (ddt, *J* = 8.5, 6.5, 1.4 Hz, 1H), 7.30 – 7.25 (m, 1H), 7.10 (d, *J* = 8.3 Hz, 2H), 5.81 (s, 2H), 2.94 – 2.91 (m, 2H),

1.84 (dt, *J* = 15.4, 7.6 Hz, 2H), 1.51 – 1.41 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.27, 149.52, 141.25, 139.59, 135.17, 132.65, 129.02, 128.64, 127.93, 127.52, 126.72, 125.52, 124.77, 124.58, 120.31, 120.23, 112.54, 48.72, 29.17, 26.80, 22.15, 13.47. MS (ESI) calculated for $C_{27}H_{26}N_4$, m/z 406.22, found 407.22 $(M + H)^+$.

Synthesis of Compound 5c: 1-(3-(Aminomethyl)benzyl)-2-butyl-1*H*-imidazo[4,5-c]quinolin-4-amine. To a solution of **1** (200 mg, 0.83 mmol) in 5 mL of anhydrous dichloromethane, were added triethylamine (92 mg, 0.91 mmol) and *tert*-butyl 3-(aminomethyl)benzylcarbamate (215 mg, 1.06 mmol) dissolved in 2 ml of anhydrous MeOH. The reaction mixture was refluxed at 45 ^{0}C for 30 minutes. The solvent was then evaporated under vacuum and product was isolated using column chromatography to obtain the intermediate compound **2c**. To a solution of **2c** in 10 mL of EtOAc, were added a catalytic amount of Pt/C and Na_2SO_4. The reaction mixture was subjected to hydrogenation at 55 psi hydrogen pressure for 4 hours. The reaction mixture was then filtered through celite and the filtrate was evaporated under vacuum to obtain the compound **3c** (202 mg). To a solution of **3c** (202 mg, 0.49 mmol) in anhydrous THF, were added triethylamine (64 mg, 0.64 mmol) and valeryl chloride (73 mg, 0.54 mmol). The reaction mixture was stirred at room temperature for 6 hours. The solvent was then removed under vacuum, and the residue was dissolved in ethyl acetate and washed with water. The ethyl acetate fraction was then dried using Na_2SO_4 and evaporated under vacuum to obtain the intermediate amide compound, which was then dissolved in 2 ml of 2M solution of ammonia in MeOH. The sealed reaction vessel was heated 150 ^{0}C for 24 hours. The solvent was then removed under vacuum and the residue was purified using column chromatography (9% MeOH/dichloromethane) to obtain the compound **4c** (44 mg; 12%). This was then dissolved in 10 ml of HCl/dioxane solution and stirred for 12 hours. The solvent was then removed to obtain the compound **5c** (52 mg, 15%). ^{1}H NMR (500 MHz, MeOD) δ 7.85 (s, 1H), 7.67 (d, *J* = 7.0 Hz, 1H), 7.52 (s, 1H), 7.39 – 7.18 (m, 4H), 7.02 (s, 1H), 5.92 (s, 2H), 4.01 (s, 2H), 2.94 (s, 2H), 1.80 (s, 2H), 1.41 (d, *J* = 4.4 Hz, 2H), 0.88 (t, *J* = 6.1 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 159.02, 150.28, 137.51, 135.84, 135.26, 131.34, 131.06, 129.95, 127.66, 127.46, 126.67, 125.73, 123.01, 119.66, 114.08, 50.24, 44.18, 30.32, 27.98, 23.45, 14.25. MS (ESI) calculated for $C_{22}H_{25}N_5$, m/z 359.21, found 360.22 $(M + H)^+$.

Compound 5d was synthesized similarly as described for compound 5c.

5d: 1-(4-(Aminomethyl)benzyl)-2-butyl-1*H*-imidazo[4,5-c]quinolin-4-amine. ^{1}H NMR (500 MHz, MeOD) δ 7.85 (d, *J* = 8.2 Hz, 1H), 7.69 (d, *J* = 8.3 Hz, 1H), 7.54 (t, *J* = 7.7 Hz, 1H), 7.40 (d, *J* = 7.7 Hz, 2H), 7.26 (t, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 5.93 (s, 2H), 4.01 (s, 2H), 2.94 (t, *J* = 7.6 Hz, 2H), 1.83 – 1.71 (m, 2H), 1.43 – 1.32 (m, 2H), 0.86 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 159.02, 150.27, 137.51, 137.47, 135.33, 134.59, 131.17, 131.11, 127.54, 126.51, 125.53, 122.95, 119.66, 114.03, 49.93, 43.81, 30.31, 27.78, 23.35, 14.12. MS (ESI) calculated for $C_{22}H_{25}N_5$, m/z 359.21, found 360.22 $(M + H)^+$.

Synthesis of Compound 6: 2-Butyl-1-(4-(isothiocyanatomethyl)benzyl)-1*H*-imidazo[4,5-c]quinolin-4-amine. To a solution of **5d** (150 mg, 0.35 mmol) in anhydrous dichloromethane, were added carbon disulfide (266 mg, 3.5 mmol) and triethylamine (106 mg, 1.05 mmol). The reaction mixture was stirred for an hour and then was cooled to 0^{o} C. Di-*tert*-butyl dicarbonate (76 mg, 0.35 mmol) and a catalytic amount of DMAP were added to the reaction mixture. The reaction mixture was stirred for 18 hours and then the solvent was removed under vacuum. The residue was purified using column chromatography (7% MeOH/ dichloromethane) to obtain the compound **6** (105 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.83 (m, 1H), 7.68 (dd, *J* = 8.3, 0.8 Hz, 1H), 7.51 – 7.45 (m, 1H), 7.32 (d, *J* = 8.2 Hz, 2H), 7.23 – 7.17 (m, 1H), 7.10 (d, *J* = 8.2 Hz, 2H), 6.52 (s, 2H), 5.78 (s, 2H), 4.71 (s, 2H), 2.94 – 2.86 (m, 2H), 1.82 (dt, *J* = 15.5, 7.6 Hz, 2H), 1.52 – 1.41 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.19, 150.27, 135.10, 134.65, 134.57, 128.20, 127.95, 126.10, 125.92, 124.08, 123.59, 119.94, 113.99, 48.74, 48.21, 29.71, 27.12, 22.47, 13.76. MS (ESI) calculated for $C_{23}H_{23}N_5S$, m/z 401.17, found 402.18 $(M + H)^+$.

Synthesis of Compound 7: 2-(3-(4-((4-Amino-2-butyl-1*H*-imidazo[4,5-c]quinolin-1-yl)methyl)benzyl)thioureido)-6-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoic acid. To a solution of fluorescein isothiocyanate (17 mg, 0.043 mmol) in anhydrous MeOH, were added triethylamine (13 mg, 0.13 mmol) and **5d** (20mg, 0.043 mmol). The reaction mixture was then heated at 45 ^{o}C for 18 hours and then the solvent was removed under vacuum. The residue was then purified using column chromatography (22% MeOH/dichloromethane) to obtain the compound **7** (3 mg, 10%). ^{1}H NMR (500 MHz, DMSO) δ 10.13 (s, 3H), 8.44 (s, 1H), 8.21 (s, 1H), 7.88 – 7.67 (m, 3H), 7.62 – 7.53 (m, 1H), 7.33 (t, *J* = 8.1 Hz, 2H), 7.26 – 7.13 (m, 2H), 7.07 – 6.94 (m, 3H), 6.67 (d, *J* = 2.1 Hz, 2H), 6.57 (tt, *J* = 5.4, 4.0 Hz, 5H), 5.87 (s, 2H), 4.74 (s, 2H), 2.97 – 2.84 (m, 2H), 1.77 – 1.67 (m, 2H), 1.45 – 1.33 (m, 2H), 0.91 – 0.85 (m, 3H). MS (ESI) calculated for $C_{43}H_{36}N_6O_5S$, m/z 748.25, found 749.26 $(M + H)^+$.

Synthesis of Compound 8: *N*-(9-(4-(3-(4-((4-amino-2-butyl-1*H*-imidazo[4,5-c]quinolin-1-yl)methyl)benzyl)thioureido)-2-carboxyphenyl)-6-(diethylamino)-3*H*-xanthen-3-ylidene)-*N*-ethylethanaminium. To a solution of rhodamine B isothiocynate (50 mg, 0.12 mmol) in anhydrous dichloromethane, were added triethylamine (47 mg, 0.47 mmol) and **5d** (64 mg, 0.12 mmol). The reaction mixture was then stirred for 14 hours and then the solvent was removed under vacuum. The residue was then purified using column chromatography (50% MeOH/dichloromethane) to obtain the compound **8** (16 mg, 16%). ^{1}H NMR (500 MHz, DMSO) δ 10.06 (s, 1H), 8.49 (s, 2H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.81 – 7.73 (m, 2H), 7.56 (dd, *J* = 8.4, 1.0 Hz, 2H), 7.33 – 7.29 (m, 1H), 7.24 (d, *J* = 8.1 Hz, 2H), 7.03 – 6.95 (m, 3H), 6.51 (dd, *J* = 12.7, 8.4 Hz, 4H), 6.46 – 6.41 (m, 4H), 5.82 (s, 2H), 4.63 (s, 2H), 3.36 (dd, *J* = 11.9, 4.8 Hz, 8H), 2.92 – 2.83 (m, 2H), 1.70 (dt, *J* = 15.3, 7.6 Hz, 2H), 1.36 (dq, *J* = 14.7, 7.4 Hz, 2H), 1.10 (t, *J* = 7.0 Hz, 12H), 0.85 (t, *J* = 7.4 Hz, 3H). MS (ESI) calculated for $C_{51}H_{55}N_8O_3S^+$, m/z 859.41, found 859.41 $(M)^+$.

Synthesis of Compound 9: BODIPY®-TR cadaverine conjugated to Compound 6. To a solution of BODIPY® TR cadaverine [5-(((4-(4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-

diaza-s-indacene-3-yl)phenoxy)acetyl)amino)pentylamine] hydrochloride (Invitrogen, Inc., 10 mg, 0.02 mmol) in anhydrous pyridine, was added **6** (11 mg, 0.03 mmol). The reaction mixture was then heated at 45 ^{o}C for 18 hours and the solvent was then removed under vacuum. The residue was purified using column chromatography (8% MeOH/dichloromethane) to obtain the compound **9** (2.34 mg, 15%). ^{1}H NMR (400 MHz, MeOD) δ 7.99 – 7.93 (m, 3H), 7.82 (d, *J* = 8.3 Hz, 1H), 7.66 (d, *J* = 8.4 Hz, 1H), 7.56 (t, *J* = 6.7 Hz, 2H), 7.42 (s, 1H), 7.26 (t, *J* = 7.3 Hz, 3H), 7.17 (dd, *J* = 8.5, 4.3 Hz, 2H), 7.06 (dd, *J* = 8.9, 2.6 Hz, 3H), 6.95 (d, *J* = 8.0 Hz, 2H), 6.83 (d, *J* = 4.3 Hz, 1H), 6.74 (d, *J* = 4.1 Hz, 1H), 5.73 (s, 2H), 4.57 (s, 2H), 3.22 (ddd, *J* = 25.7, 16.1, 9.0 Hz, 4H), 2.88 – 2.83 (m, 2H), 1.76 (dt, *J* = 15.3, 7.6 Hz, 3H), 1.51 (dt, *J* = 18.8, 9.6 Hz, 4H), 1.39 (dd, *J* = 15.1, 7.5 Hz, 2H), 1.36 – 1.24 (m, 3H), 0.90 (t, *J* = 7.4 Hz, 3H). MS (ESI) calculated for $C_{49}H_{50}BF_2N_9O_2S_2$, m/z 909.36, found 910.37 $(M + H)^+$.

NF-κB induction in human TLR7-expressing reporter gene assays: The induction of NF-κB was quantified using HEK-Blue-7 cells as previously described by us.[1;2] HEK293 cells were stably transfected with human TLR7, MD2, and secreted alkaline phosphatase (sAP), and were maintained in HEK-Blue™ Selection medium containing zeocin and normocin. Stable expression of secreted alkaline phosphatase (sAP) under control of NF-κB/AP-1 promoters is inducible by the TLR7 agonists, and extracellular sAP in the supernatant is proportional to NF-κB induction. HEK-Blue cells were incubated at a density of ~10^5 cells/ml in a volume of 80 µl/well, in 384-well, flat-bottomed, cell culture-treated microtiter plates until confluency was achieved, and subsequently graded concentrations of stimuli. sAP was assayed spectrophotometrically using an alkaline phosphatase-specific chromogen (present in HEK-detection medium as supplied by the vendor) at 620 nm.

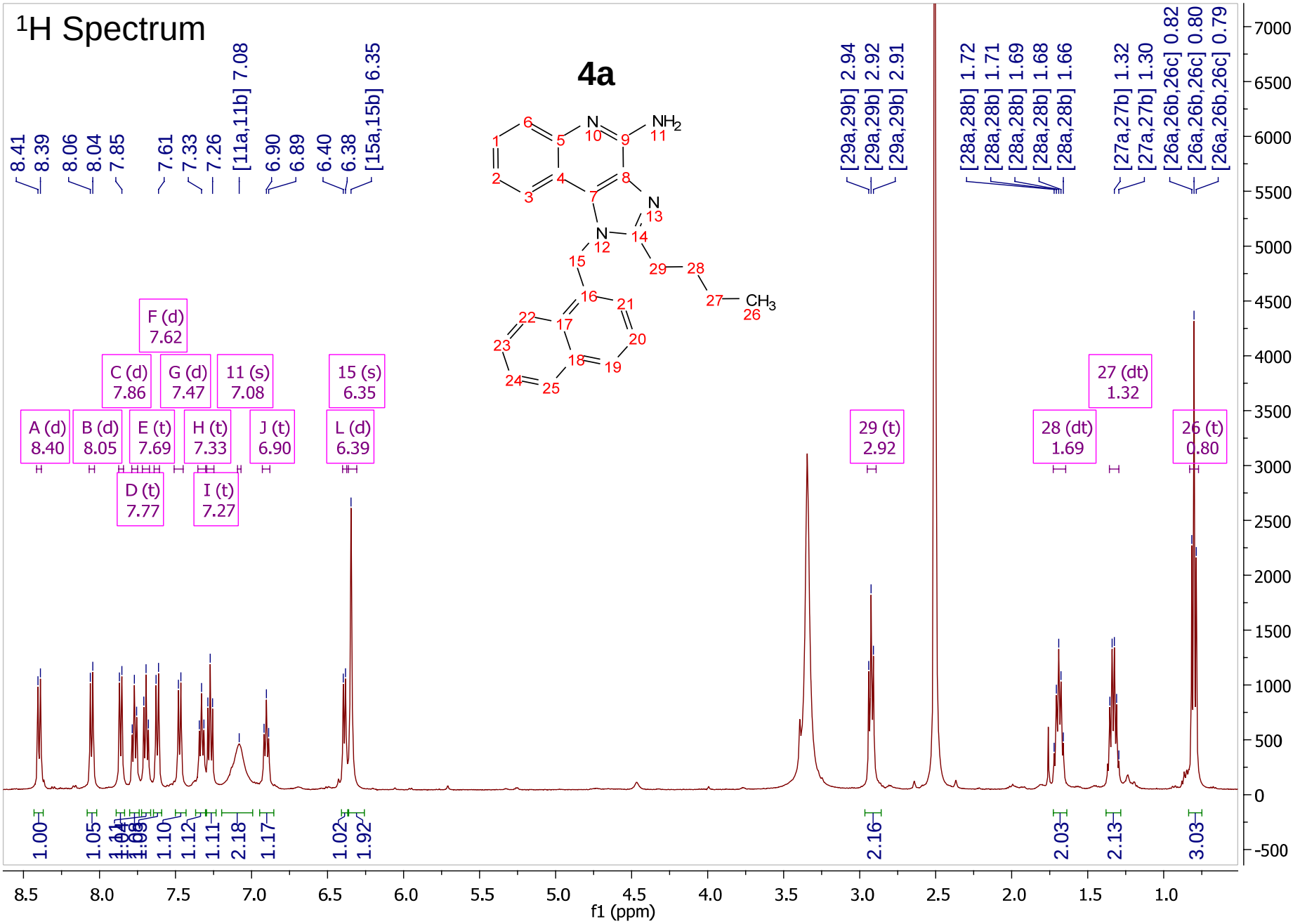
Fluorescence microscopy: Murine macrophage J774.A1 cells were grown to confluency in optical-grade flat-bottomed 96 well plates as described earlier.[3;4] The cells were then exposed to graded concentrations of the fluorescently labeled compounds for 4h at 37^{o}C. Intravital epifluorescence and phase contrast images were obtained directly from the plated cells using an inverted Olympus IX-71 microscope equipped with long working-distance air objectives and temperature-controlled stage, using appropriate filter sets for the various fluorescent analogues. Images were processed on Image-J software.

Flow-cytometric immunostimulation experiments: Detailed methods have been published by us.[1] Heparin-anticoagulated whole blood samples were obtained by venipuncture from healthy human volunteers with informed consent and as per guidelines approved by the University of Kansas Human Subjects Experimentation Committee. Two mL aliquots of whole human blood samples were stimulated with graded concentrations of **7** 1 in a 6-well polystyrene plate and incubated at 37^{o}C in a rotary (100 rpm) incubator for 30 min. Negative (endotoxin free water) controls were included in each experiment. Following incubation, 200 µL aliquots of anticoagulated whole blood were stained with 20 µL of fluorochrome-conjugated antibodies (anti-CD3-PE, and anti-CD56-APC) at 37^{o}C in the dark for 30 min. Following staining, erythrocytes were lysed and leukocytes fixed in one step by mixing 200 µL of the samples in 4 mL pre-warmed Whole Blood Lyse/Fix Buffer (Becton-Dickinson Biosciences, San Jose, CA). After washing the cells

twice at 200 g for 8 minutes in saline, the cells were transferred to a 96-well plate. Flow cytometry was performed using a Becton-Dickinson LSR II instrument in the tri-color mode. The primary gate for the lymphocytic population was obtained on FSC and SSC channels (100,000 gated events). Secondary gating included natural killer lymphocytes (NK cells: $CD3^{-}CD56^{+}$), nominal B lymphocytes ($CD3^{-}CD56^{-}$), and nominal T lymphocytes ($CD3^{+}CD56^{-}$). Post-acquisition analyses were performed using FlowJo v 7.0 software (Treestar, Ashland, OR). Compensation for spillover was computed for each experiment on singly-stained samples.

References

1. Hood, J. D.; Warshakoon, H. J.; Kimbrell, M. R.; Shukla, N. M.; Malladi, S.; Wang, X.; David, S. A. *Hum.Vaccin.* **2010**, *6*, 1-14.
2. Shukla, N. M.; Kimbrell, M. R.; Malladi, S. S.; David, S. A. *Bioorg.Med.Chem.Lett.* **4-15-2009**, *19*, 2211-2214.
3. David, S. A.; Silverstein, R.; Amura, C. R.; Kielian, T.; Morrison, D. C. *Antimicrob.Agents Chemother.* **1999**, *43*, 912-919.
4. Miller, K. A.; Suresh Kumar, E. V. K.; Wood, S. J.; Cromer, J. R.; Datta, A.; David, S. A. *J.Med.Chem.* **2005**, *48*, 2589-2599.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6.89
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B (d) 8.05
C (d) 7.86
D (t) 7.77
E (t) 7.69
F (d) 7.62
G (d) 7.47
H (t) 7.33
I (t) 7.27
J (t) 6.90
11 (s) 7.08
15 (s) 6.35
L (d) 6.39
29 (t) 2.92
28 (dt) 1.69
27 (dt) 1.32
26 (t) 0.80
1.00
1.05
1.10
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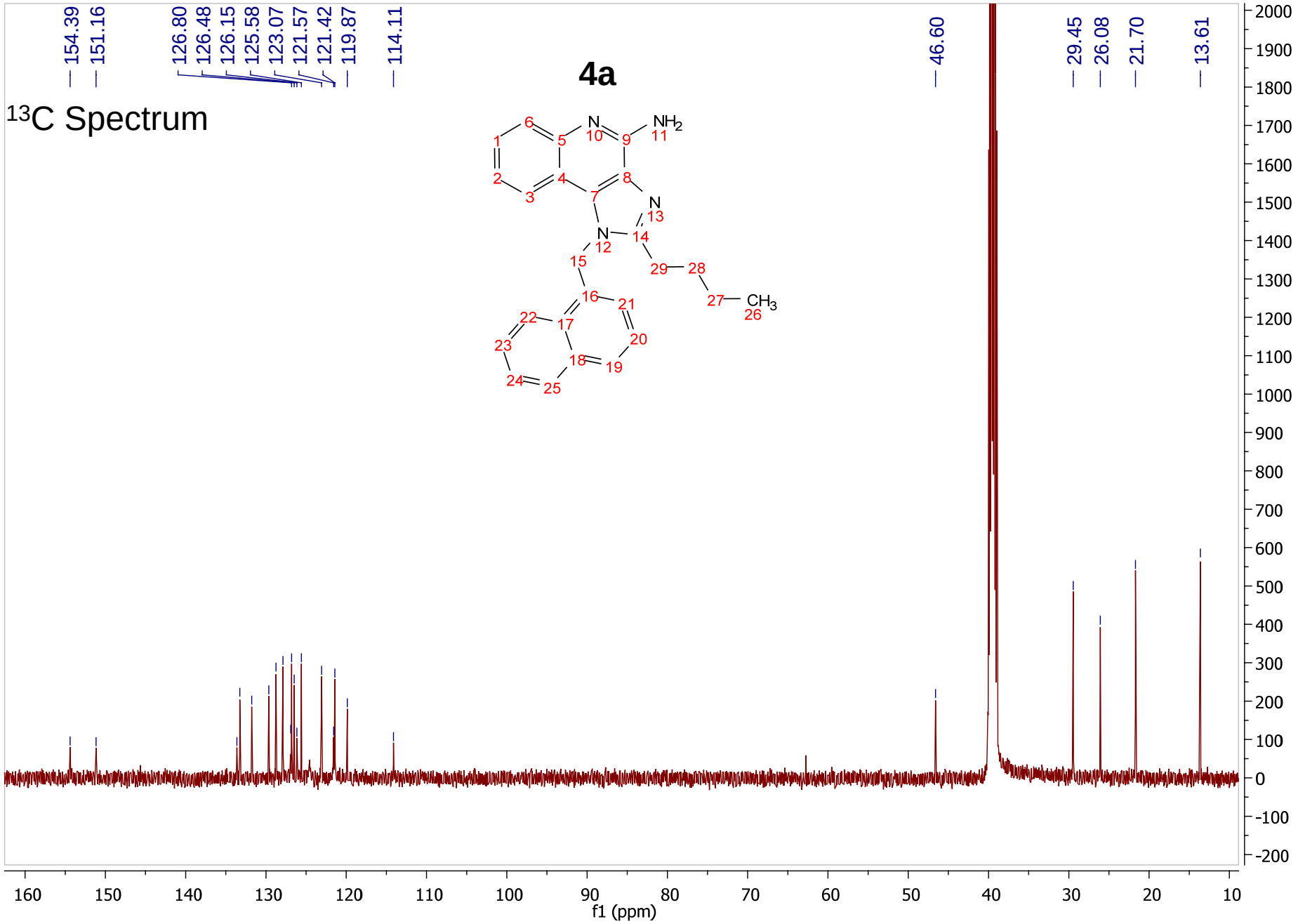
1.17
1.02
1.92
2.16
2.03
2.13
3.03
f1 (ppm)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29.45
26.08
21.70
13.61
4a
13C Spectrum
NH2
CH3
f1 (ppm)

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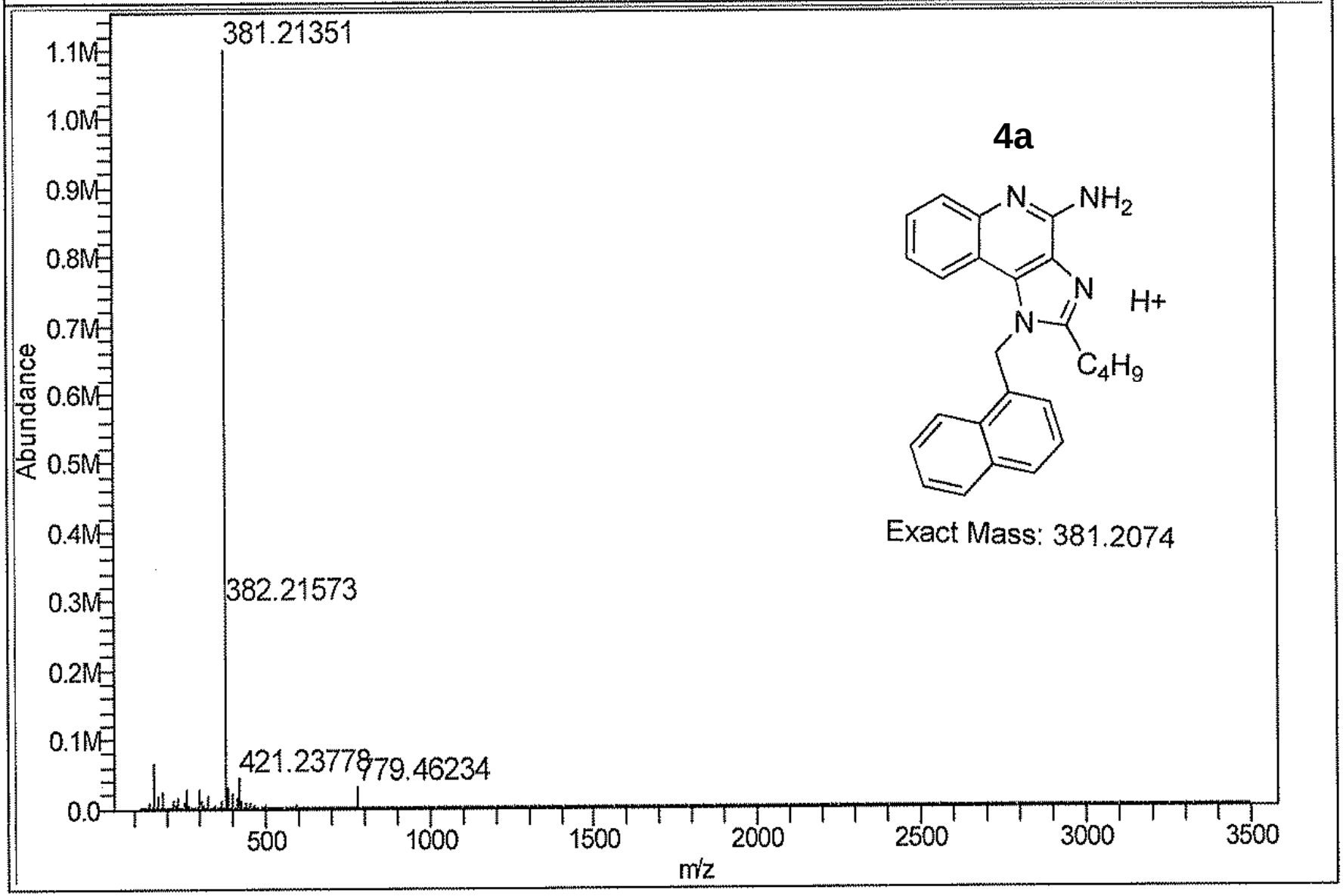

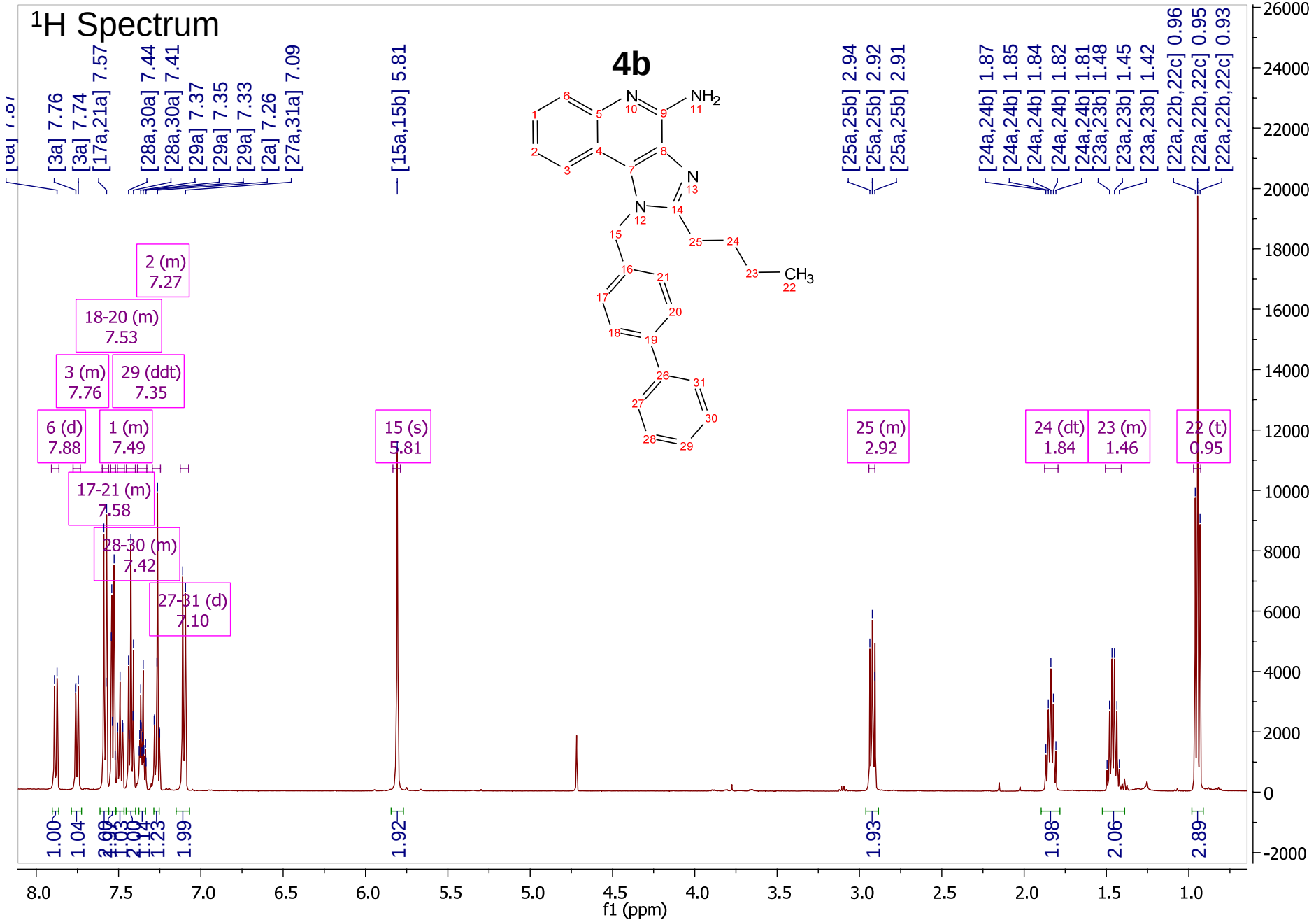
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[29a] 7.37
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[29a] 7.33
[2a] 7.26
[27a,31a] 7.09
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18-20 (m) 7.53
3 (m) 7.76
29 (ddt) 7.35
6 (d) 7.88
1 (m) 7.49
15 (s) 5.81
25 (m) 2.92
24 (dt) 1.84
23 (m) 1.46
22 (t) 0.95
17-21 (m) 7.58
28-30 (m) 7.42
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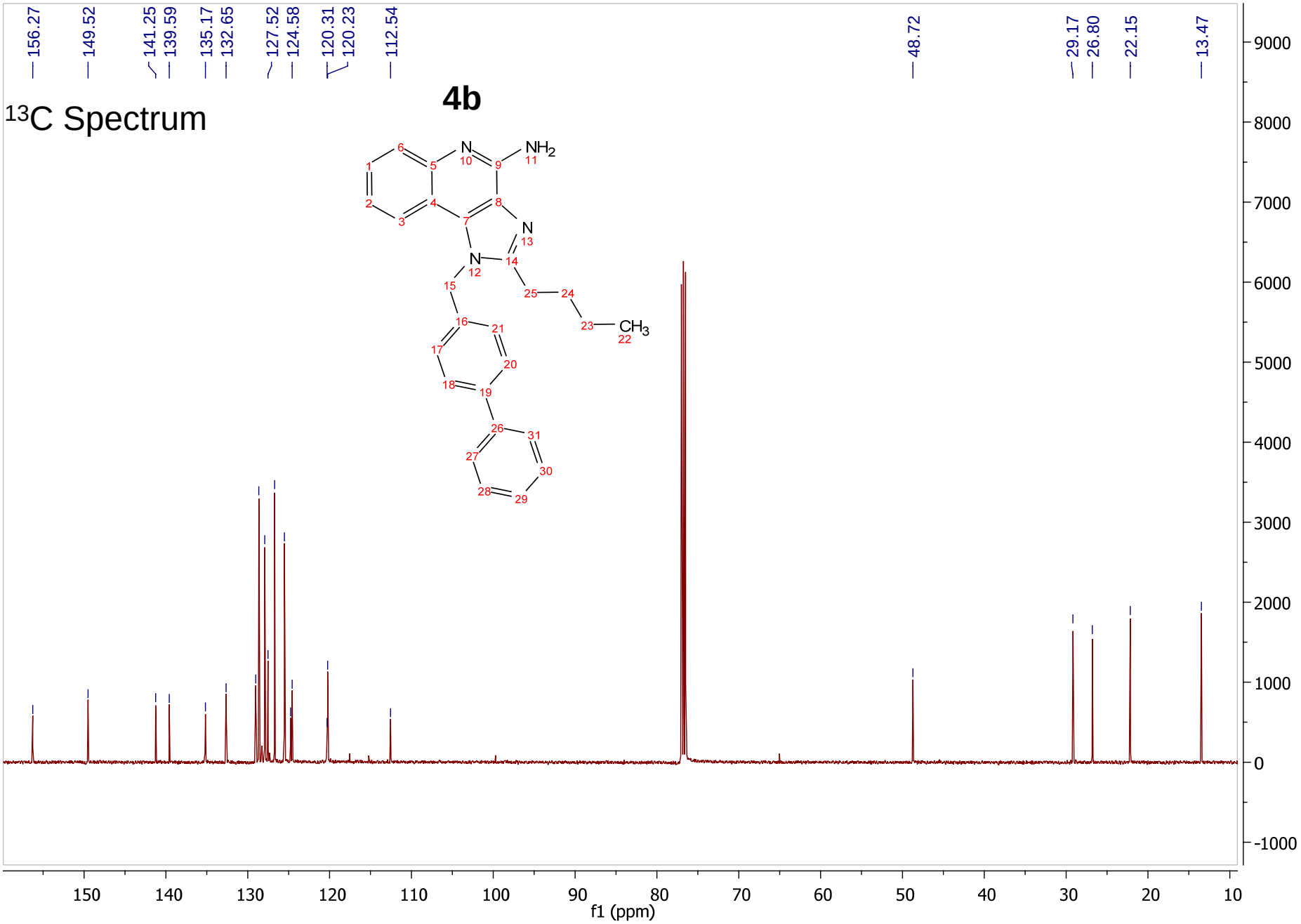
2.89
f1 (ppm)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149.52
141.25
139.59
135.17
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124.58
120.31
120.23
112.54
48.72
29.17
26.80
22.15
13.47
4b
13C Spectrum
NH2
CH3
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

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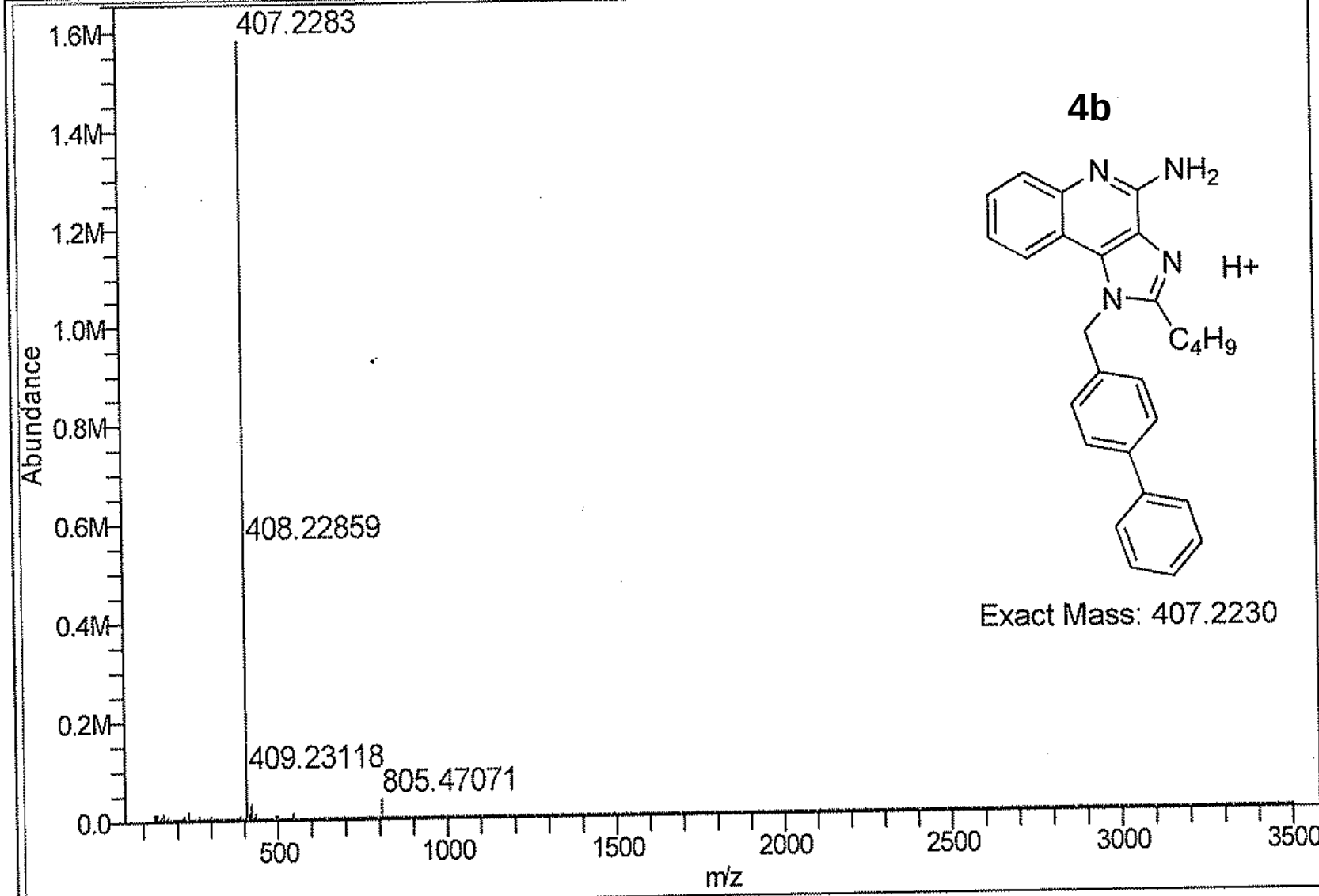
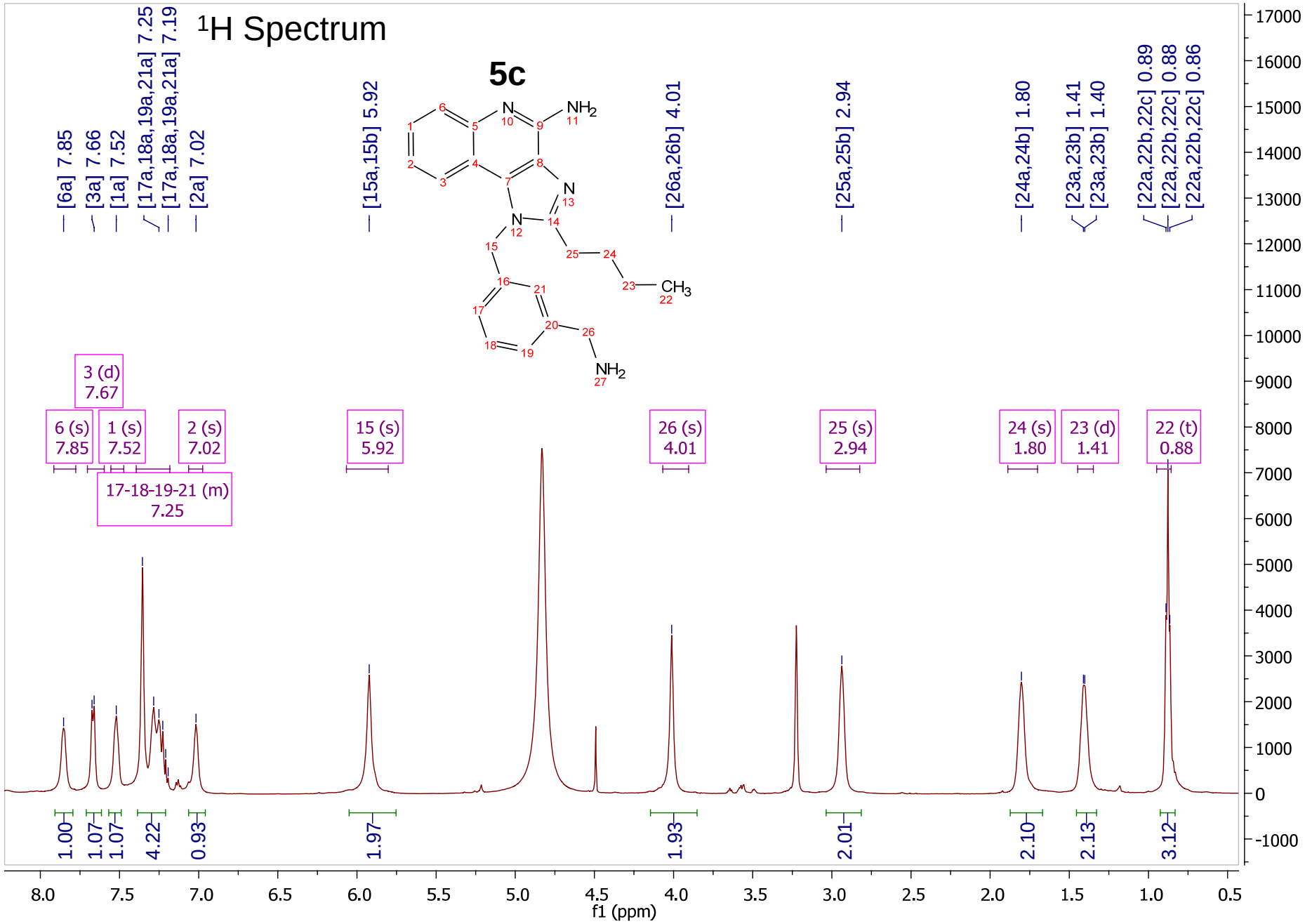


4b

Exact Mass: 407.2230


1H Spectrum
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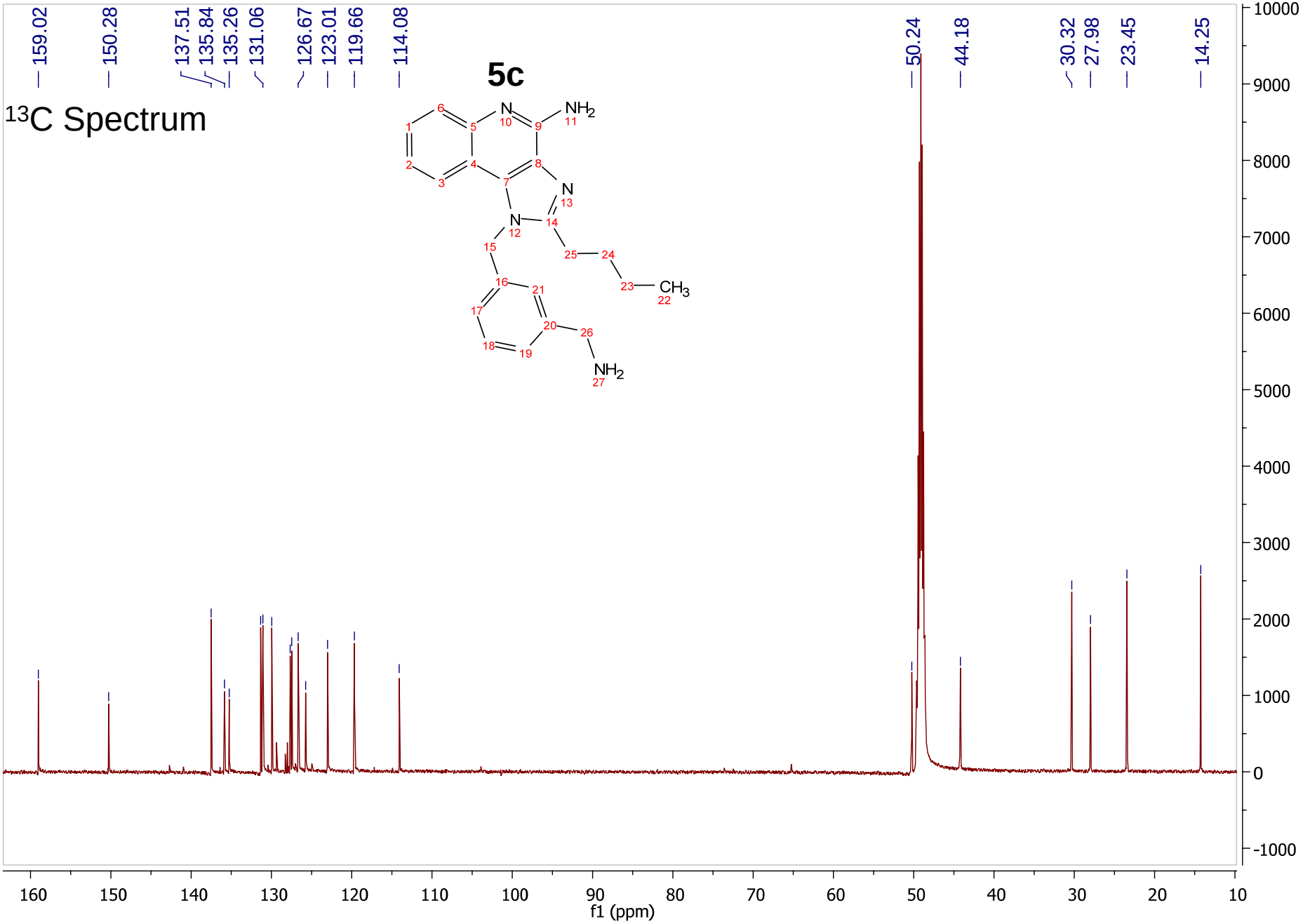
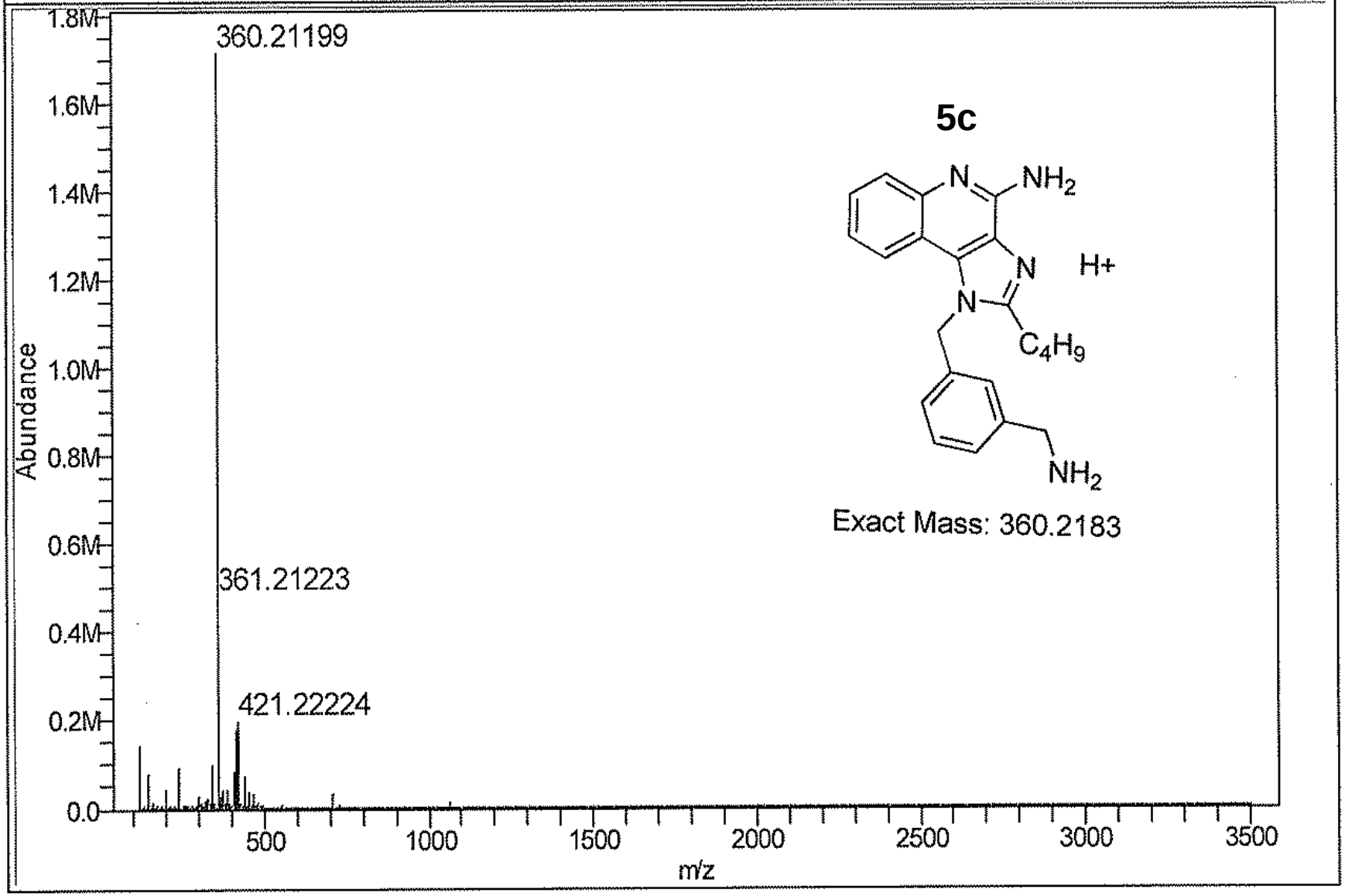
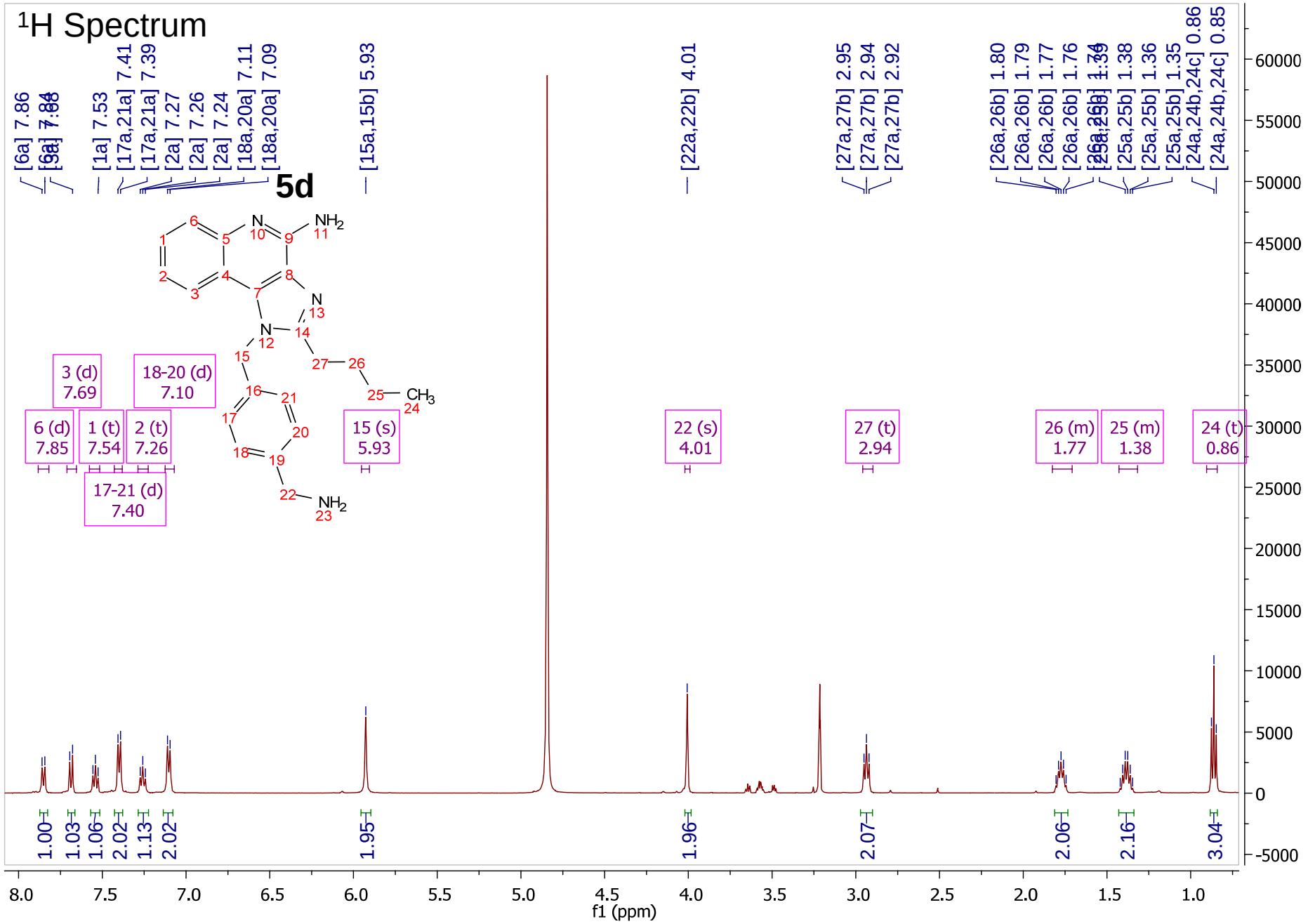
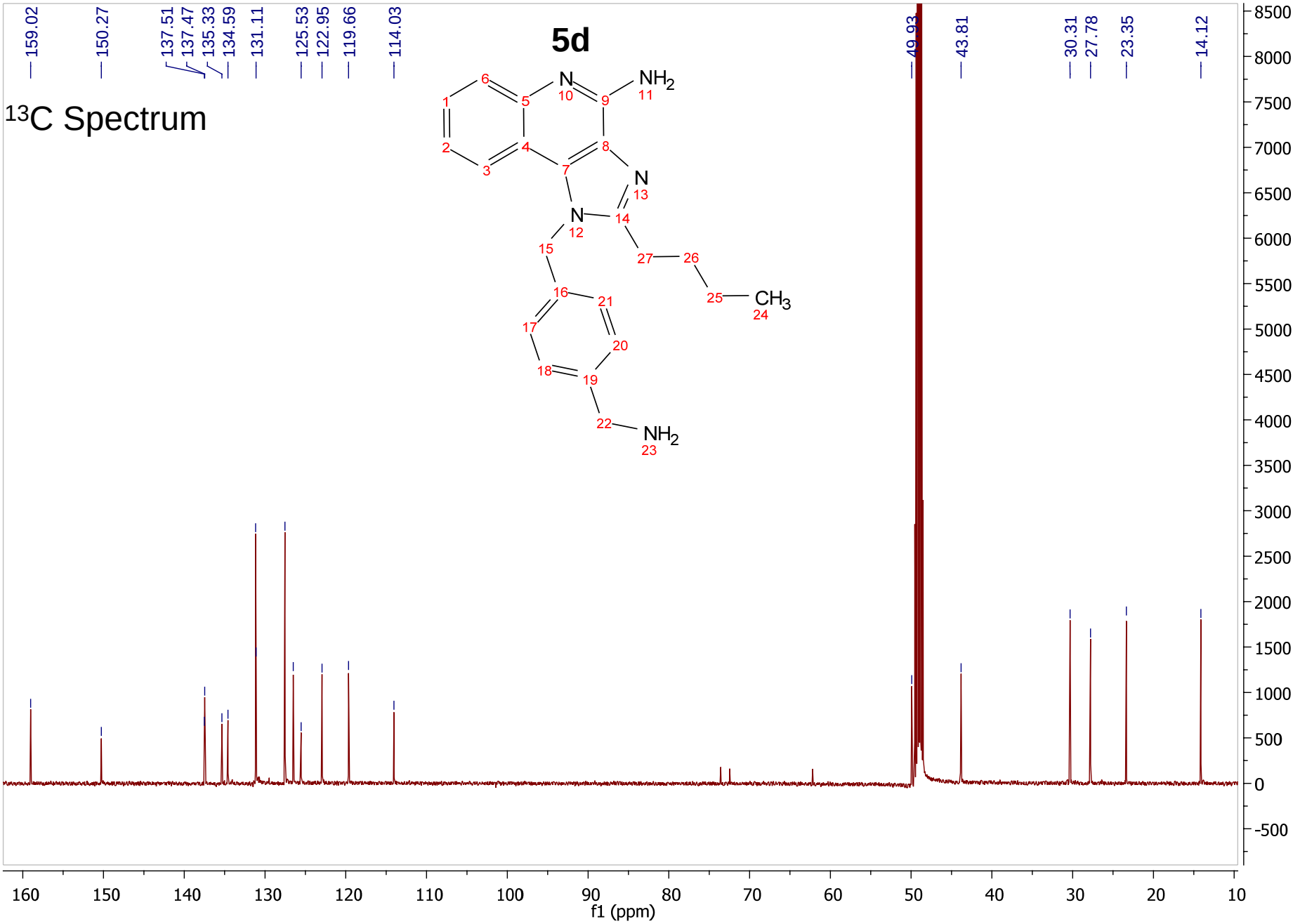
[1a] 7.52
[17a,18a,19a,21a] 7.25
[17a,18a,19a,21a] 7.19
[2a] 7.02
[15a,15b] 5.92
[26a,26b] 4.01
[25a,25b] 2.94
[24a,24b] 1.80
[23a,23b] 1.41
[23a,23b] 1.40
[22a,22b,22c] 0.89
[22a,22b,22c] 0.88
[22a,22b,22c] 0.86
3 (d) 7.67
6 (s) 7.85
1 (s) 7.52
2 (s) 7.02
17-18-19-21 (m) 7.25
15 (s) 5.92
26 (s) 4.01
25 (s) 2.94
24 (s) 1.80
23 (d) 1.41
22 (t) 0.88
1.00
1.07
1.07
4.22
0.93
1.97
1.93
2.01
2.10
2.13
3.12
f1 (ppm)


159.02
150.28
137.51
135.84
135.26
131.06
126.67
123.01
119.66
114.08
50.24
44.18
30.32
27.98
23.45
14.25
13C Spectrum
5c
NH2
CH3
NH2
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

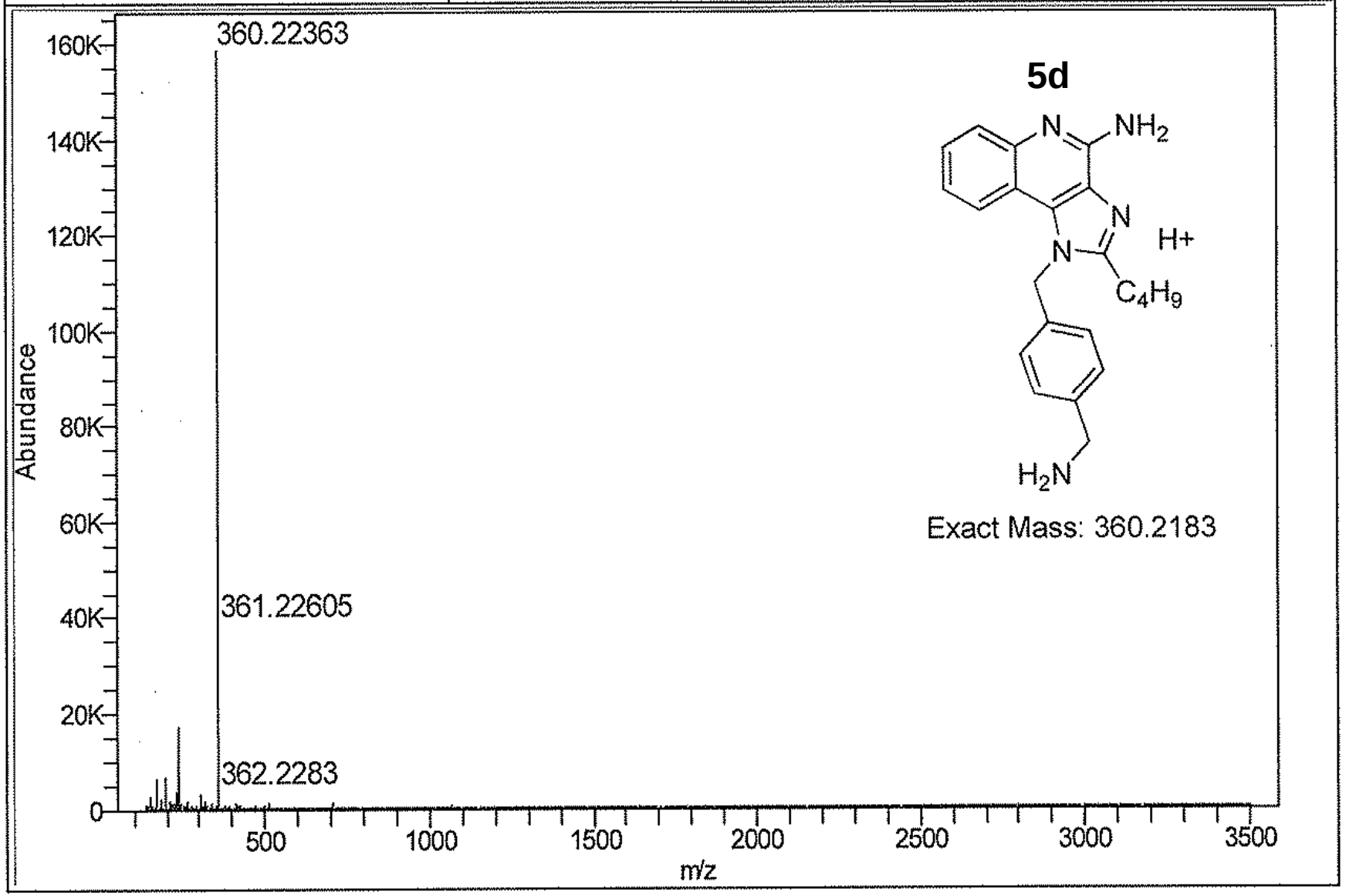
TOF Spectrum	TOF	Profile Spectrum at 1243.58 min (scan 1)
Baseline = 8.33491799381982	Abundance Min = 0	Abundance Max = 1716003

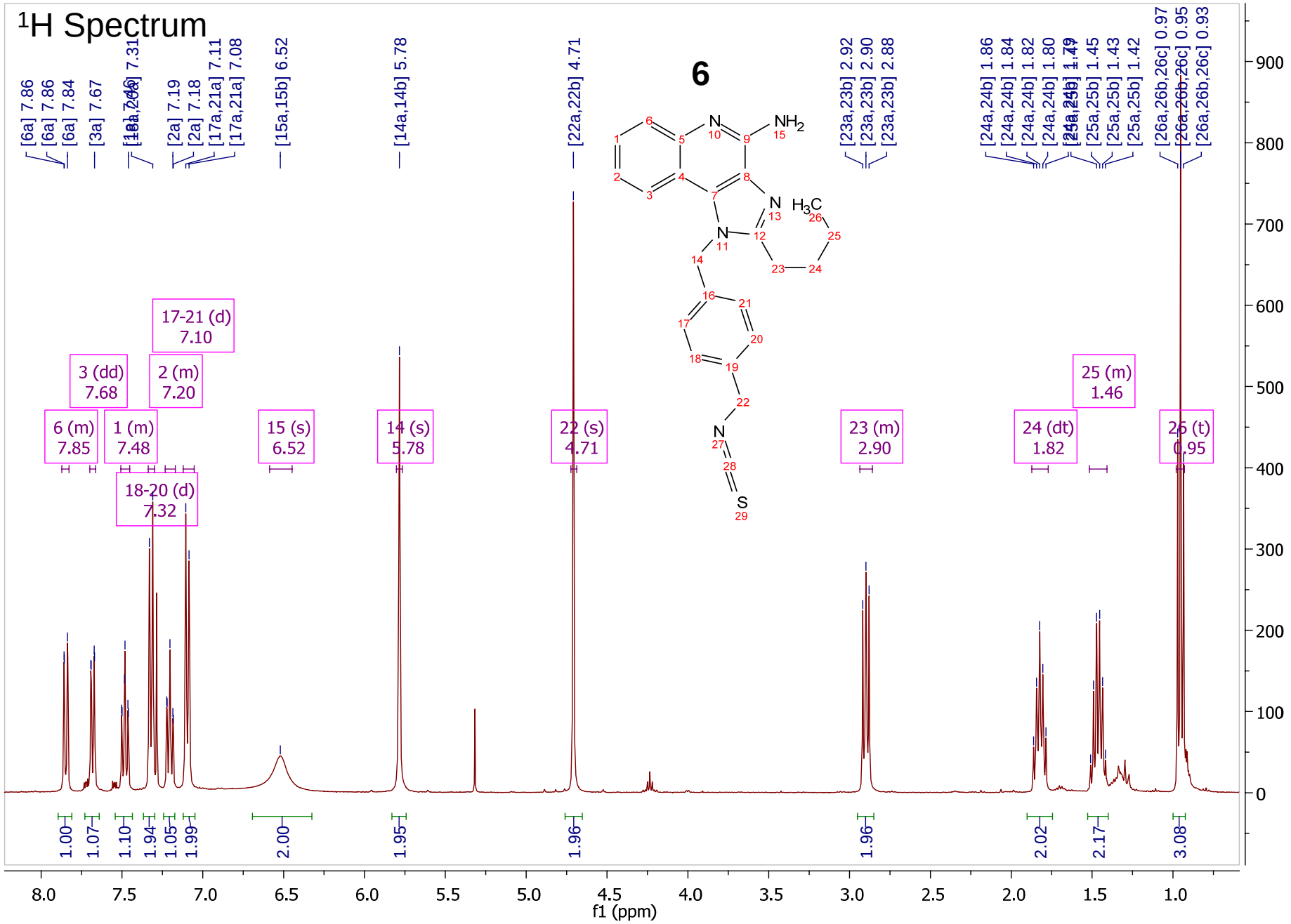


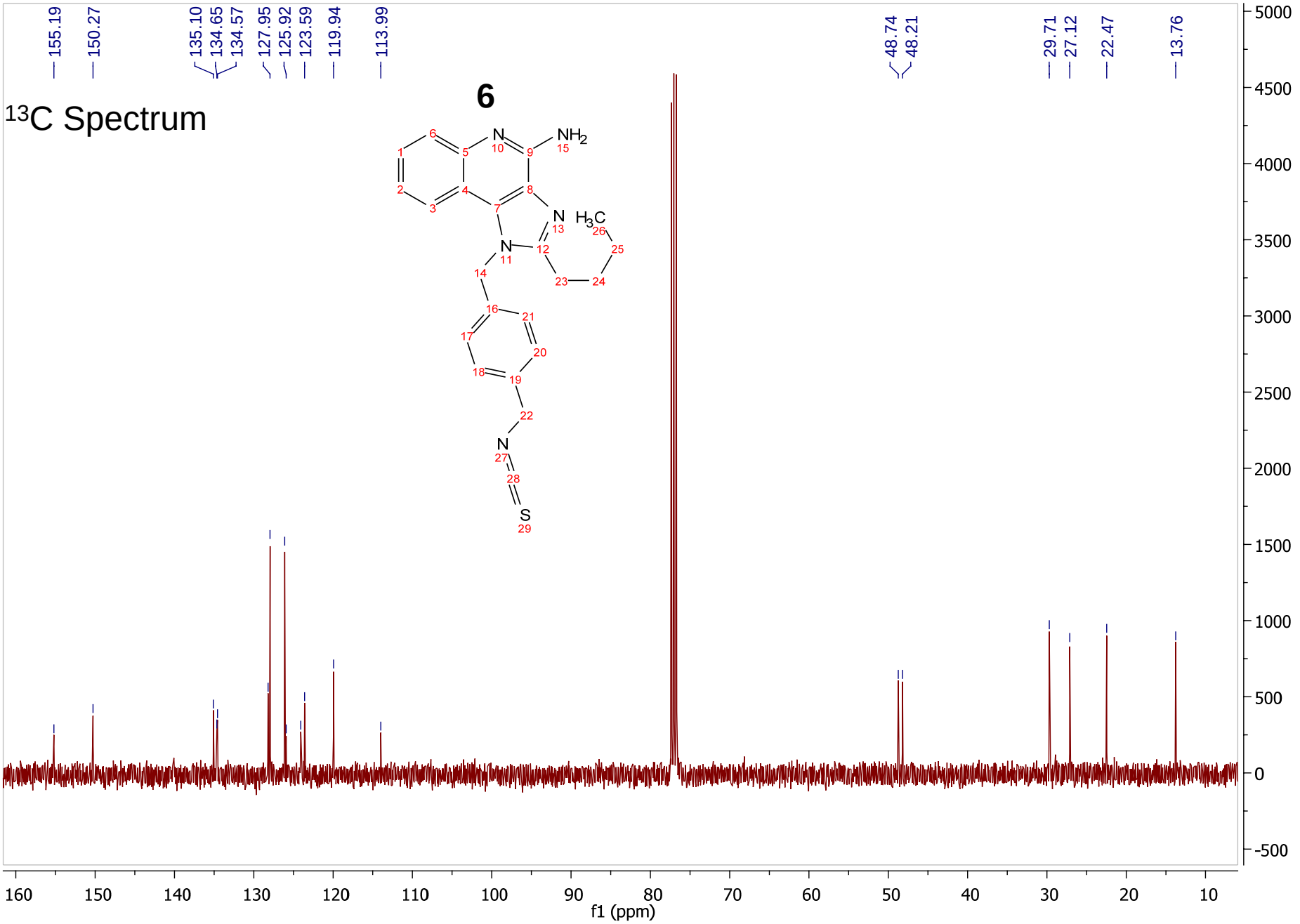

1H Spectrum
5d
[6a] 7.86
[1a] 7.53
[17a,21a] 7.41
[17a,21a] 7.39
[2a] 7.27
[2a] 7.26
[2a] 7.24
[18a,20a] 7.11
[18a,20a] 7.09
[15a,15b] 5.93
[22a,22b] 4.01
[27a,27b] 2.95
[27a,27b] 2.94
[27a,27b] 2.92
[26a,26b] 1.80
[26a,26b] 1.79
[26a,26b] 1.77
[26a,26b] 1.76
[25a,25b] 1.38
[25a,25b] 1.36
[25a,25b] 1.35
[24a,24b,24c] 0.86
[24a,24b,24c] 0.85
3 (d) 7.69
18-20 (d) 7.10
6 (d) 7.85
1 (t) 7.54
2 (t) 7.26
17-21 (d) 7.40
15 (s) 5.93
22 (s) 4.01
27 (t) 2.94
26 (m) 1.77
25 (m) 1.38
24 (t) 0.86
1.00
1.03
1.06
2.02
1.13
2.02
1.95
1.96
2.07
2.06
2.16
3.04
f1 (ppm)


159.02
150.27
137.51
137.47
135.33
134.59
131.11
125.53
122.95
119.66
114.03
49.93
43.81
30.31
27.78
23.35
14.12
13C Spectrum
5d
NH2
CH3
f1 (ppm)

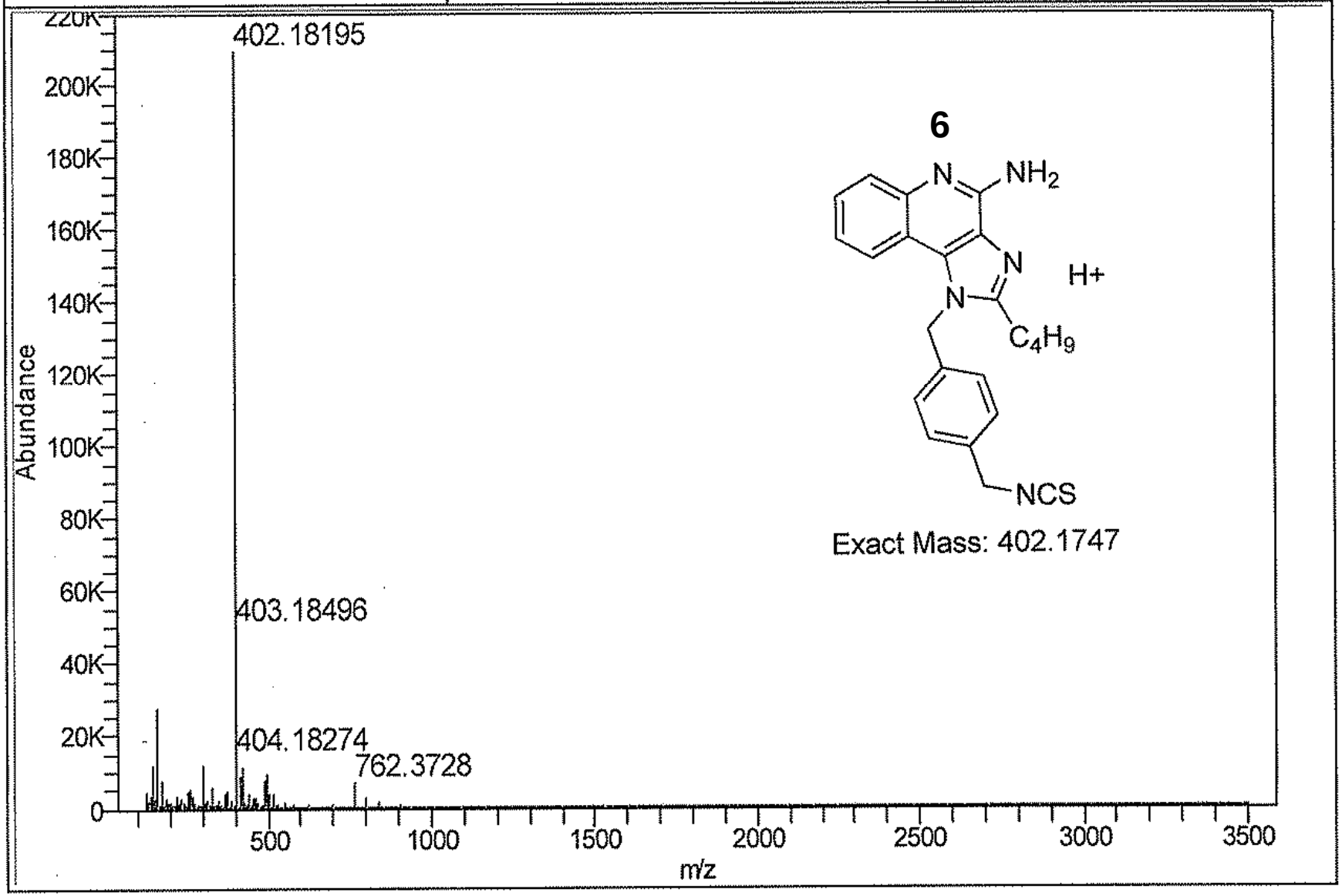
TOF Spectrum	TOF	Profile Spectrum at 5989.16 min (scan 1)
Baseline = 0	Abundance Min = 0	Abundance Max = 158961

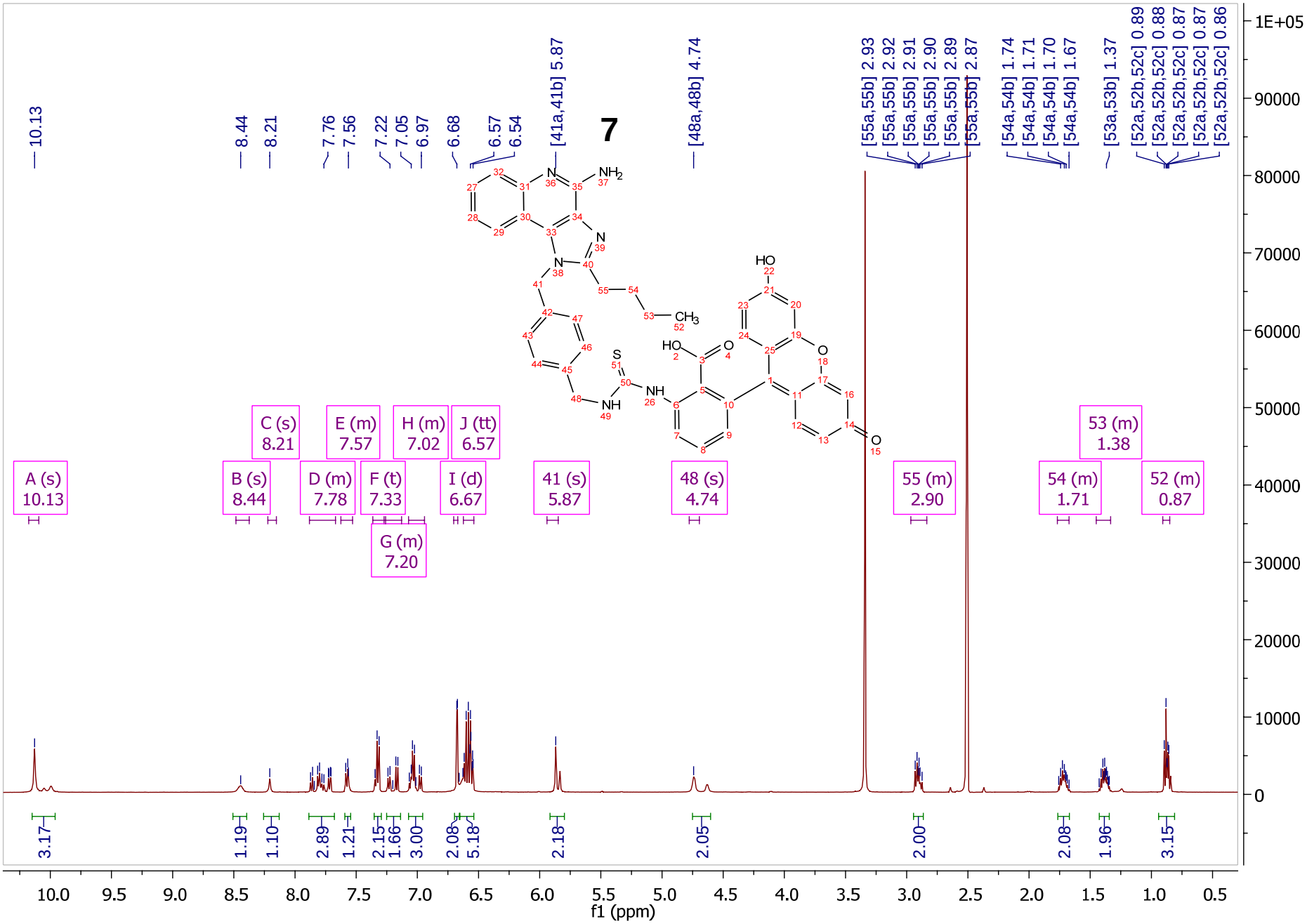



1H Spectrum
6
[6a] 7.86
[6a] 7.86
[6a] 7.84
[3a] 7.67
[1a] 7.46
[1a] 7.31
[2a] 7.19
[2a] 7.18
[17a,21a] 7.11
[17a,21a] 7.08
[15a,15b] 6.52
[14a,14b] 5.78
[22a,22b] 4.71
[23a,23b] 2.92
[23a,23b] 2.90
[23a,23b] 2.88
[24a,24b] 1.86
[24a,24b] 1.84
[24a,24b] 1.82
[24a,24b] 1.80
[25a,25b] 1.45
[25a,25b] 1.43
[25a,25b] 1.42
[26a,26b,26c] 0.97
[26a,26b,26c] 0.95
[26a,26b,26c] 0.93
17-21 (d) 7.10
3 (dd) 7.68
2 (m) 7.20
6 (m) 7.85
1 (m) 7.48
18-20 (d) 7.32
15 (s) 6.52
14 (s) 5.78
22 (s) 4.71
23 (m) 2.90
24 (dt) 1.82
25 (m) 1.46
26 (t) 0.95
1.00
1.07
1.10
1.94
1.05
1.99
2.00
1.95
1.96
1.96
2.02
2.17
3.08
f1 (ppm)

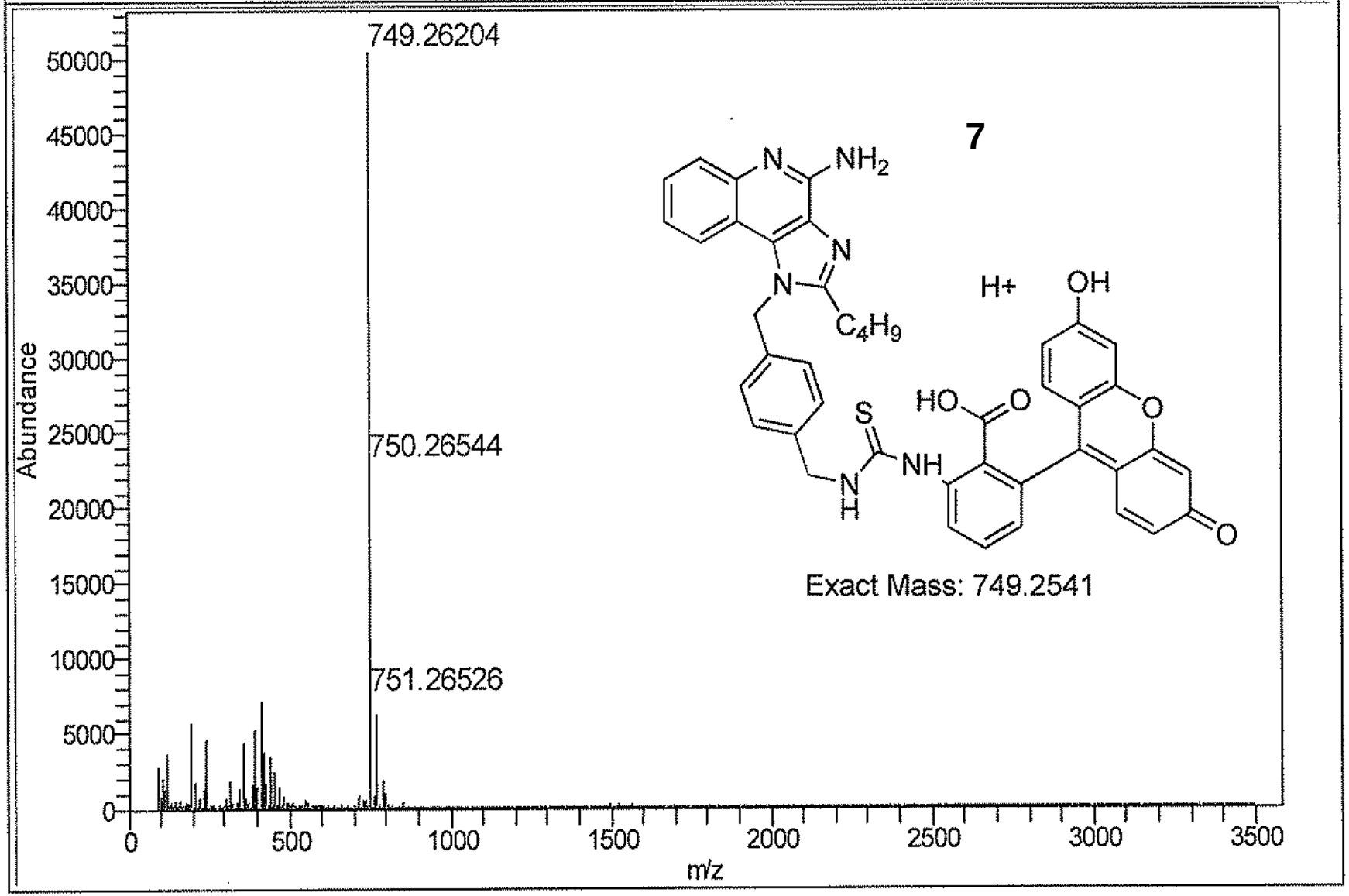

155.19
150.27
135.10
134.65
134.57
127.95
125.92
123.59
119.94
113.99
48.74
48.21
29.71
27.12
22.47
13.76
13C Spectrum
6
NH2
H3C
S
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

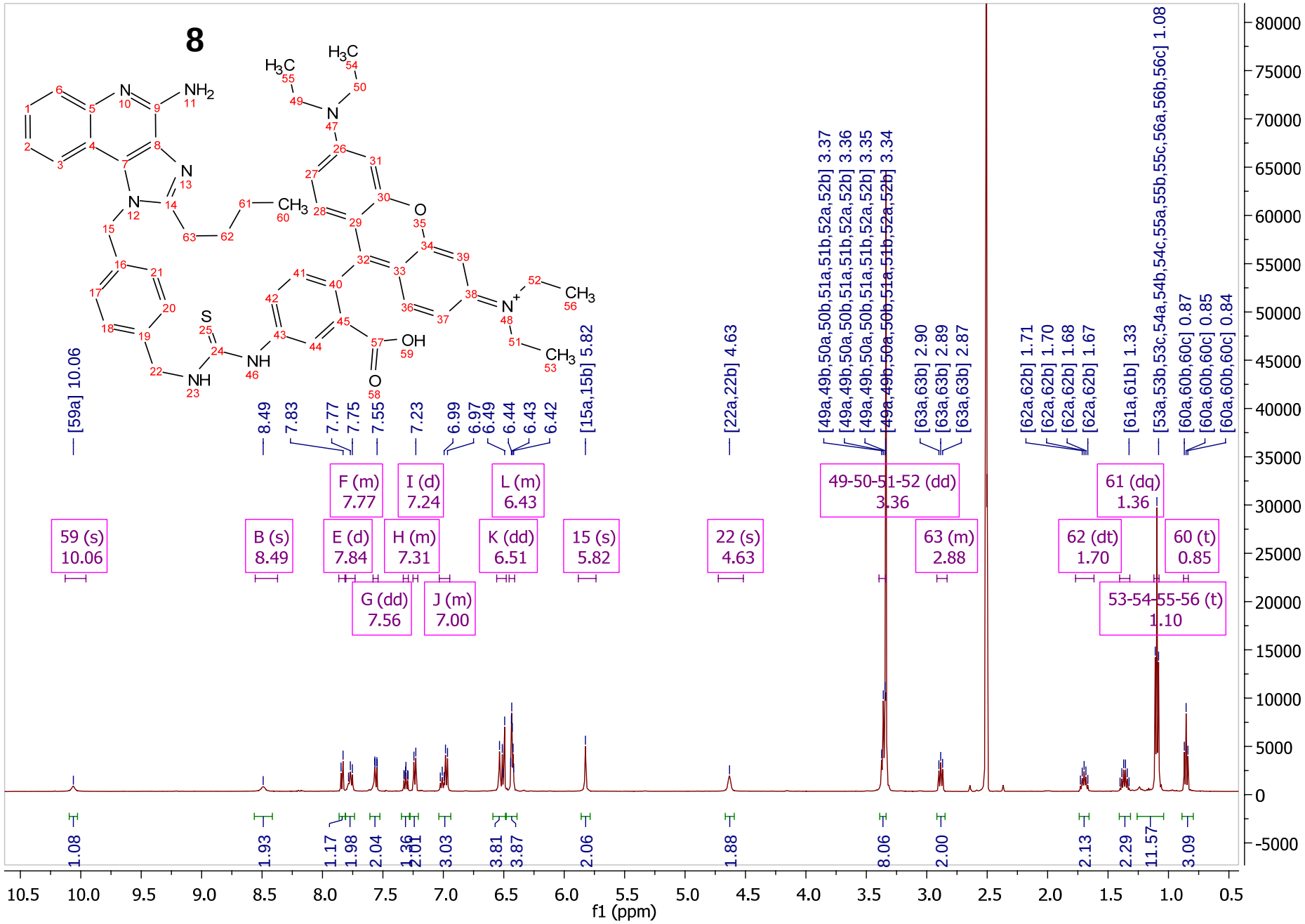
TOF Spectrum	TOF	Profile Spectrum at 15920.91 min (scan 1)
Baseline = 0	Abundance Min = 0	Abundance Max = 209622



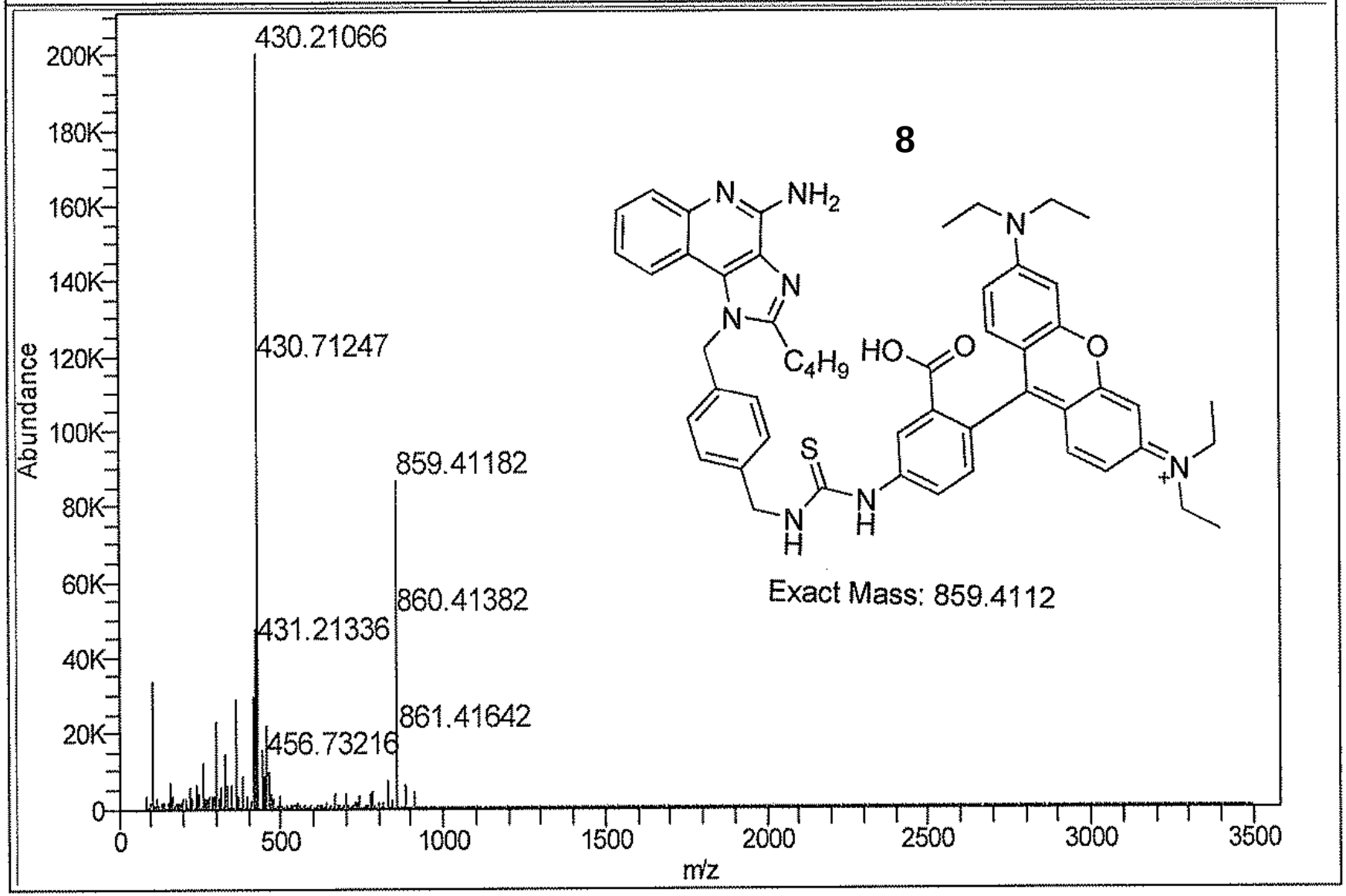

7
10.13
8.44
8.21
7.76
7.56
7.22
7.05
6.97
6.68
6.57
6.54
[41a,41b] 5.87
[48a,48b] 4.74
[55a,55b] 2.93
[55a,55b] 2.92
[55a,55b] 2.91
[55a,55b] 2.90
[55a,55b] 2.89
[55a,55b] 2.87
[54a,54b] 1.74
[54a,54b] 1.71
[54a,54b] 1.70
[54a,54b] 1.67
[53a,53b] 1.37
[52a,52b,52c] 0.89
[52a,52b,52c] 0.88
[52a,52b,52c] 0.87
[52a,52b,52c] 0.87
[52a,52b,52c] 0.86
A (s) 10.13
B (s) 8.44
C (s) 8.21
D (m) 7.78
E (m) 7.57
F (t) 7.33
G (m) 7.20
H (m) 7.02
I (d) 6.67
J (tt) 6.57
41 (s) 5.87
48 (s) 4.74
55 (m) 2.90
54 (m) 1.71
53 (m) 1.38
52 (m) 0.87
3.17
1.19
1.10
2.89
1.21
2.15
1.66
3.00
2.08
5.18
2.18
2.05
2.00
2.08
1.96
3.15
1E+05
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

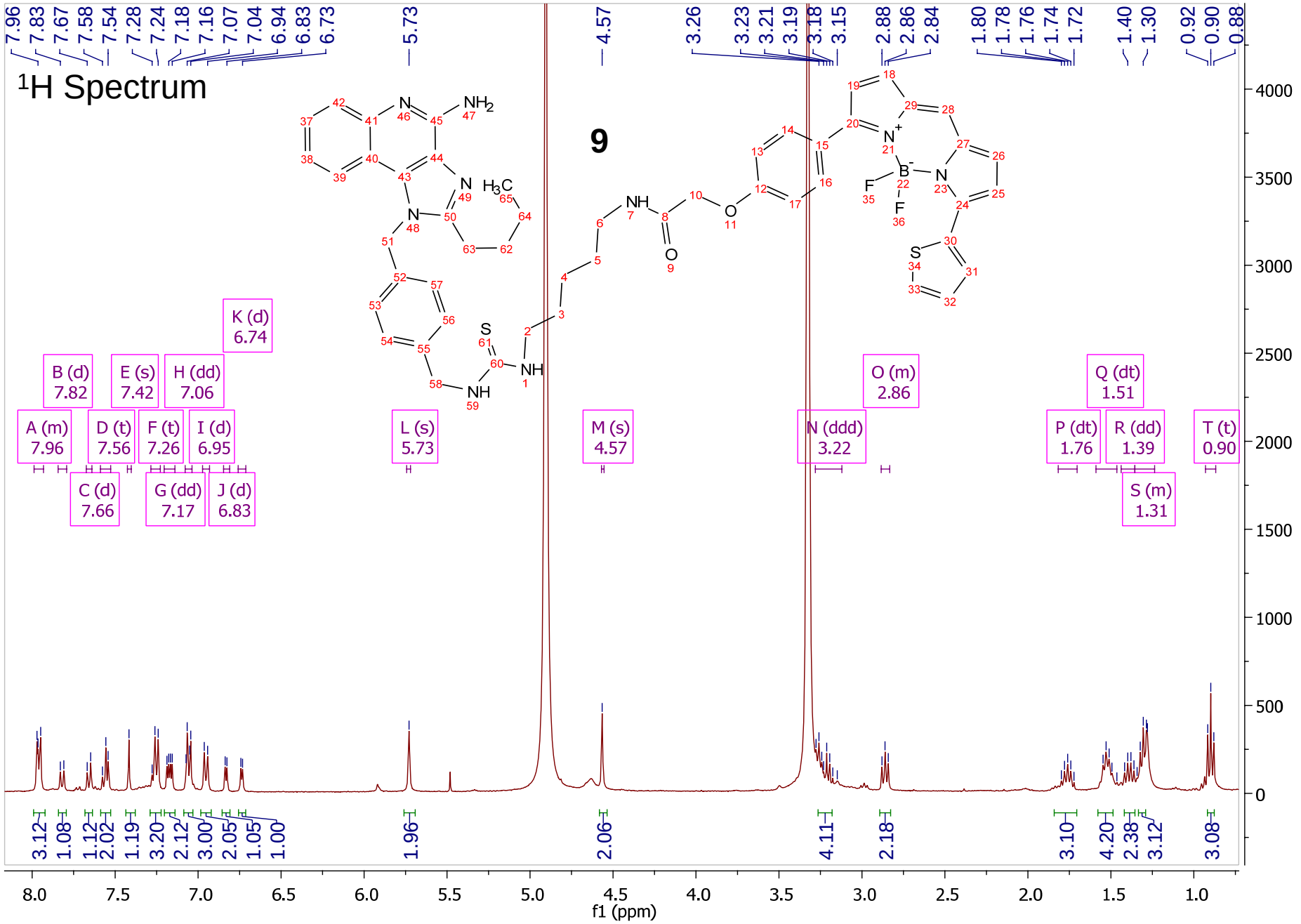
TOF Spectrum	TOF	Profile Spectrum at 1430.34 min (scan 1)
Baseline = 0	Abundance Min = 0	Abundance Max = 50506




8
59 (s) 10.06
B (s) 8.49
E (d) 7.84
F (m) 7.77
G (dd) 7.56
H (m) 7.31
I (d) 7.24
J (m) 7.00
K (dd) 6.51
L (m) 6.43
15 (s) 5.82
22 (s) 4.63
49-50-51-52 (dd) 3.36
63 (m) 2.88
62 (dt) 1.70
61 (dq) 1.36
60 (t) 0.85
53-54-55-56 (t) 1.10
f1 (ppm)

TOF Spectrum	TOF	Profile Spectrum at 1380.19 min (scan 1)
Baseline = 1.03464892152363	Abundance Min = 0	Abundance Max = 200380




^{1}H Spectrum
9
7.96
7.83
7.67
7.58
7.54
7.28
7.24
7.18
7.16
7.07
7.04
6.94
6.83
6.73
5.73
4.57
3.26
3.23
3.21
3.19
3.18
3.15
2.88
2.86
2.84
1.80
1.78
1.76
1.74
1.72
1.40
1.30
0.92
0.90
0.88
A (m) 7.96
B (d) 7.82
C (d) 7.66
D (t) 7.56
E (s) 7.42
F (t) 7.26
G (dd) 7.17
H (dd) 7.06
I (d) 6.95
J (d) 6.83
K (d) 6.74
L (s) 5.73
M (s) 4.57
N (ddd) 3.22
O (m) 2.86
P (dt) 1.76
Q (dt) 1.51
R (dd) 1.39
S (m) 1.31
T (t) 0.90
3.12
1.08
1.12
2.02
1.19
3.20
2.12
3.00
2.05
1.05
1.00
1.96
2.06
4.11
2.18
3.10
4.20
2.38
3.12
3.08
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)
4000
3500
3000
2500
2000
1500
1000
500
0

TOF Spectrum	TOF	Profile Spectrum at 999.91 min (scan 1)
Baseline = 0.007097232079489	Abundance Min = 0	Abundance Max = 117354