SUPPORTING INFORMATION

Sulfonamide-Based Inhibitors of Biotin Protein Ligase as New Antibiotic Leads

Kwang Jun Lee,[†#] William Tieu,[†] Beatriz Blanco-Rodriguez,[†] Ashleigh S. Paparella,[‡] Jingxian Yu,[†#] Andrew Hayes,[‡] Jiage Feng,[†] Andrew C. Marshall,[‡] Benjamin Noll,[⁋] Robert Milne,[⁋] Danielle Cini,[§] Matthew C. J. Wilce,[§] Grant W. Booker,[‡] John B. Bruning,[‖] Steven W. Polyak,[‡] and Andrew D. Abell [†#]

[†] Department of Chemistry, School of Physical Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia

[‡] Department of Molecular and Cellular Biology, School of Biological Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia

[⁋] School of Pharmacy & Medical Sciences, University of South Australia, Adelaide, South Australia 5000, Australia

[§] Department of Biochemistry, School of Biomedical Science, Monash University, Clayton, Victoria 3800, Australia

[#] Centre for Nanoscale BioPhotonics (CNBP), University of Adelaide, Adelaide, South Australia 5005, Australia

[‖] Institute of Photonics and Advanced Sensing (IPAS), School of Biological Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia

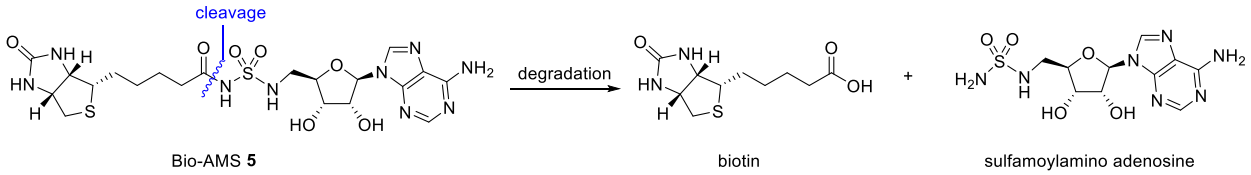


Figure S1. Major metabolites of Bio-AMS **5** in mice.

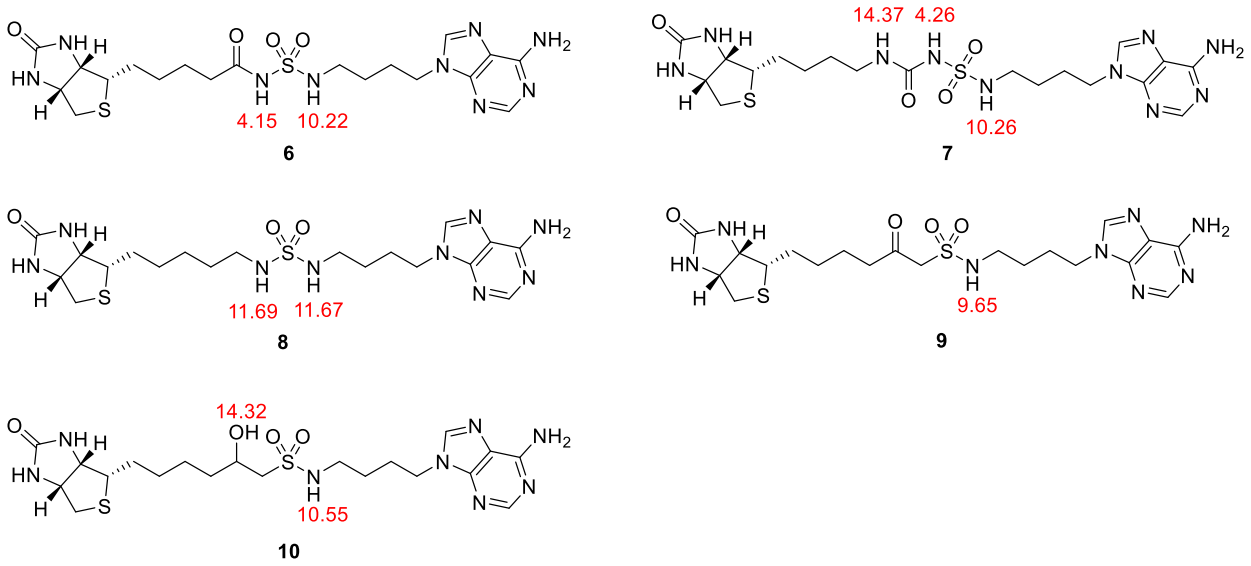
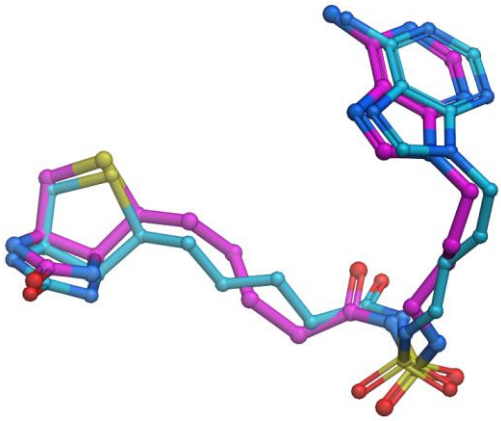


Figure S2. pK_a prediction of sulfonamide linkage of analogues **6** - **10** using Marvin from ChemAxon.[a]

[a] MarvinSketch, version 18.19.0; ChemAxon: Budapest, 2018

A



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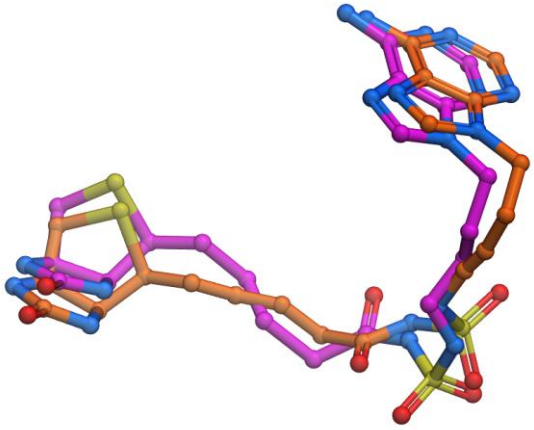


Figure S3. (A) Superposition of the time-averaged conformation of deprotonated form of **6** (cyan) with the cocrystal structure of **6** bound to *Sa*BPL (magenta) - (RMSD - 0.62 Å). (B) Superposition of the time-averaged conformation of protonated form of **6** (orange) with the cocrystal structure of **6** bound to *Sa*BPL magenta) - (RMSD - 1.13 Å).

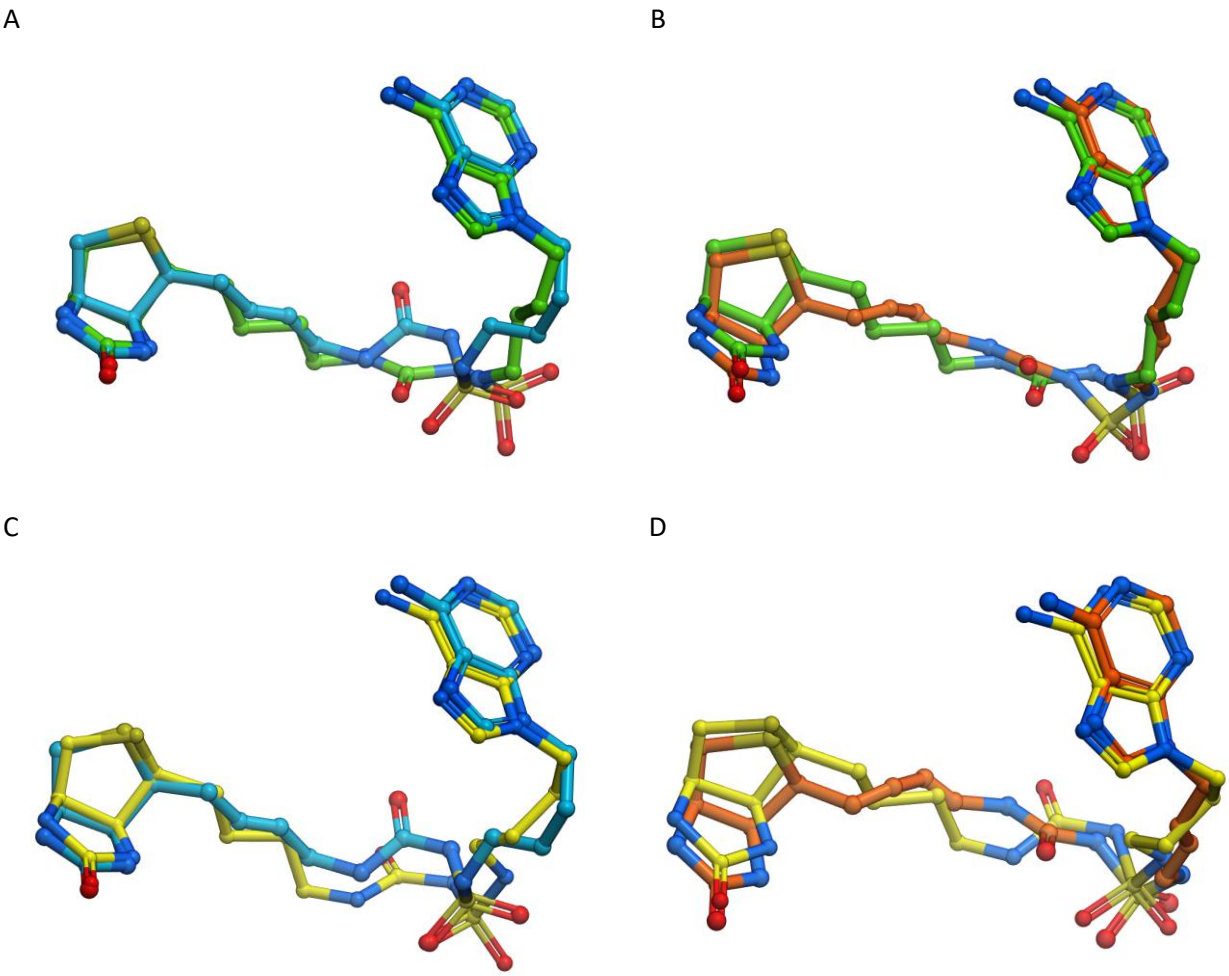


Figure S4. (A) Superposition of the time-averaged conformation of deprotonated form of **7** (cyan) with the major conformer of **7** bound to *Sa*BPL (green) - (RMSD - 1.02 Å). (B) Superposition of the time-averaged conformation of protonated form of **7** (orange) with the major conformer of **7** bound to *Sa*BPL (green) - (RMSD - 0.86 Å). (C) Superposition of the time-averaged conformation of deprotonated form of **7** (cyan) with the minor conformer of **7** bound to *Sa*BPL (yellow) - (RMSD - 0.78 Å). (D) Superposition of the time-averaged conformation of protonated form of **7** (orange) with the minor conformer of **7** bound to *Sa*BPL (yellow) - (RMSD - 0.98 Å).

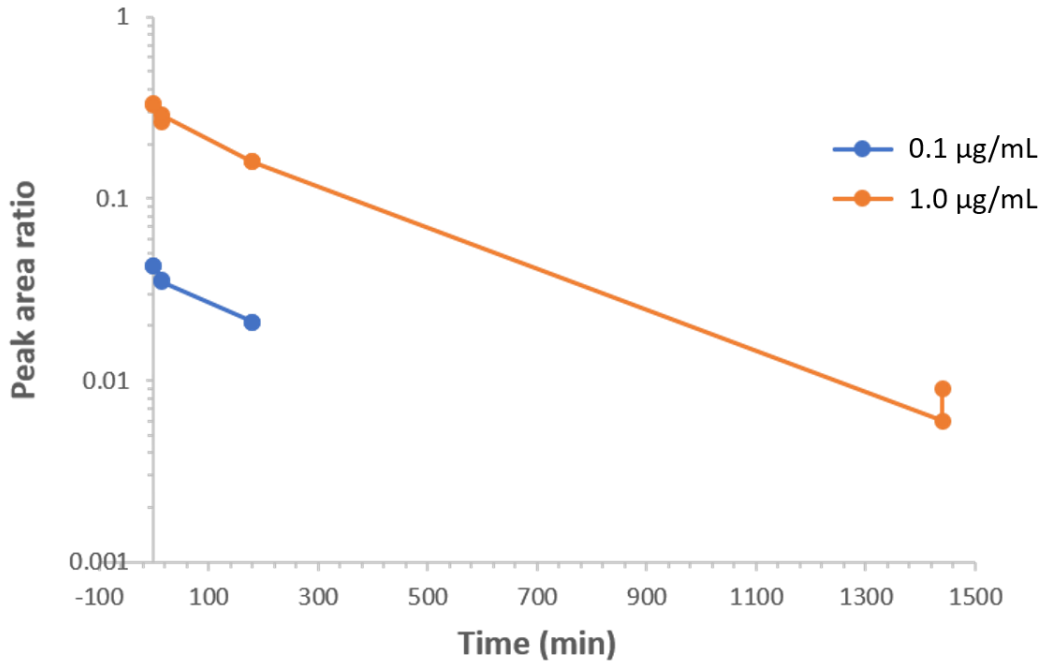
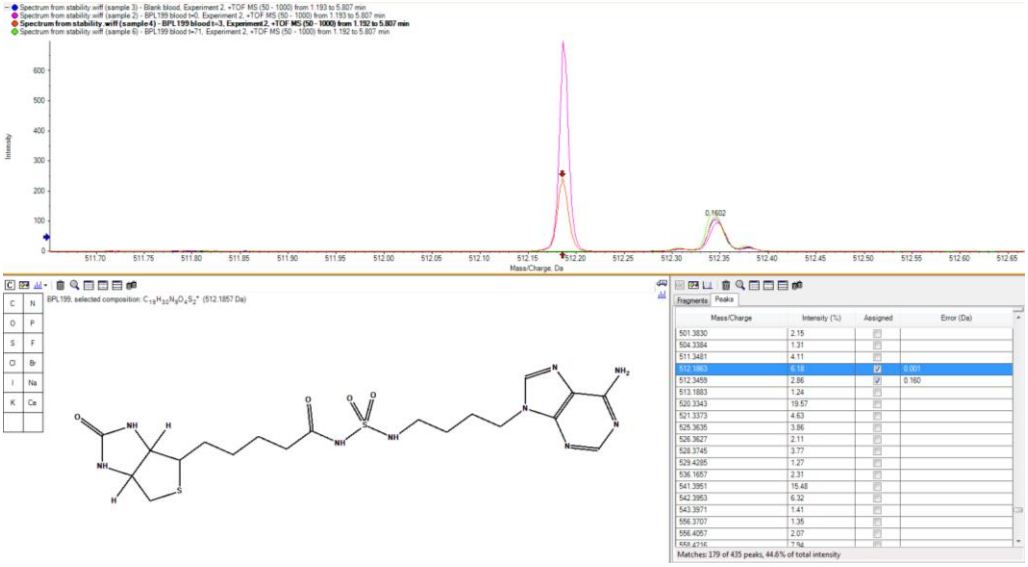


Figure S5. Change in the ratio of the peak responses to acylsulfamide **6** to internal standard against incubation time in blood.

A



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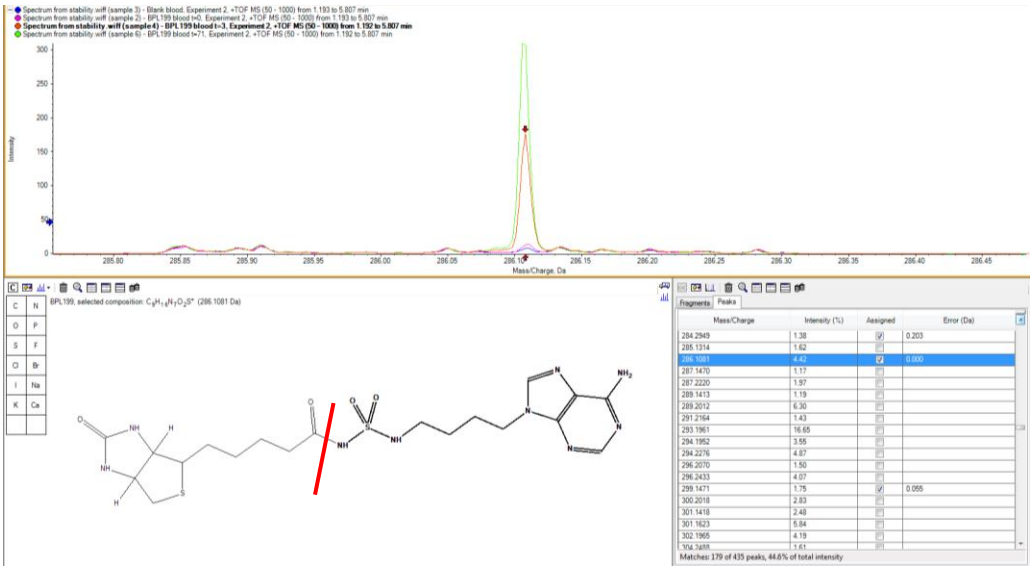


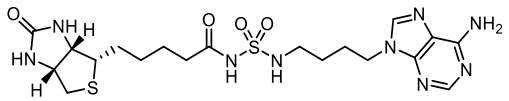
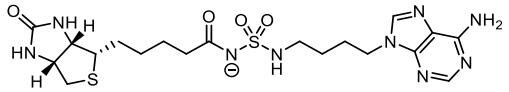
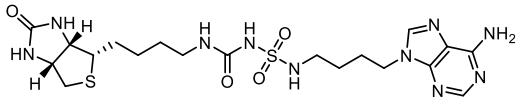
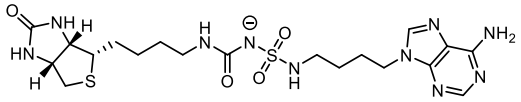
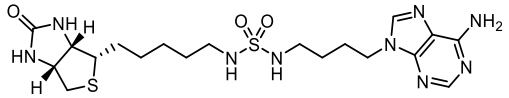
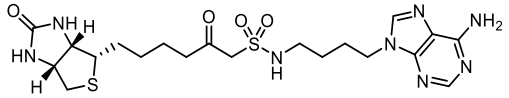
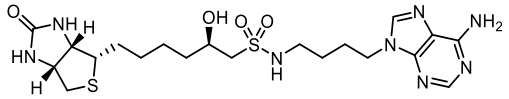
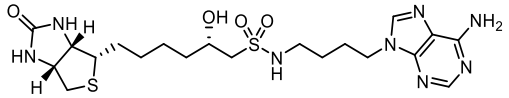
Figure S6. Peak responses for acylsulfamide **6** (A) and its decomposition product (B) at time 0 (purple lines), 3 h (red) and 71 h (green) from the commencement of the incubation of acylsulfamide **6** in rat blood. The solid red line indicates the proposed site for cleavage of acylsulfamide **6**.

Table S1. Data collection and refinement statistics (molecular replacement).

	*Sa*BPL + **6** (PDB: 6ORU)	*Sa*BPL + **7** (PDB: 6NDL)
Data collection[a]	MX1 Beamline Australian Synchrotron	MX1 Beamline Australian Synchrotron
Space group	$P\,4_22_12$	$P4_22_12$
Cell dimensions		
a, *b*, *c* (Å)	92.5, 92.5, 129.0	94.0, 94.0, 131.0
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Wavelength (Å)	0.95	0.95
Resolution (Å)	75.91 - 2.39 (2.478 - 2.392)[b]	46.66 - 2.00 (2.05 - 2.00)[b]
R_{merge}	0.7136 (2.584)	0.129 (6.729)
CC(1/2)	0.881 (0.469)	1.000 (0.204)
$I / \sigma I$	86.72 (3.64)	16.1 (0.7)
Completeness (%)	99.96 (100.0)	100.0 (100.0)
Redundancy	27.0 (25.4)	28.4 (29.5)
Refinement		
Resolution (Å)	75.19 - 2.39	46.66 - 2.00
No. reflections	22801	40340
R_{work} / R_{free}	0.2047 (0.3508) / 0.2582 (0.4341)	0.1693 (0.3650) / 0.2193 (0.3705)
No. atoms		
Protein	2560	2622
Ligand	34	35
Glycerol	-	36
Water	39	347
Average *B*-factors (Å^2)		
Protein	53.20	67.2
Ligand	46.60	51.9
Glycerol	-	101.7
Water	50.60	78.7
RMS deviations		
Bond lengths (Å)	0.019	0.007
Bond angles (°)	1.92	0.870

[a] Diffraction data were collected from one crystal for each structure.
[b] Values in parentheses are for highest-resolution shell.

Table S2. QM/MM-GBSA binding free energy calculations.

Compound	Structure	ΔG_QM/MM-GBSA (kcal/mol)
Protonated **6**		-68.59
Deprotonated **6**		-73.57
Protonated **7**		-62.80
Deprotonated **7**		-81.27
8		-71.28
9		-69.01
R form of **10**		-65.46
S form of **10**		-70.10

HYBRID QM/MM MOLECULAR DYNAMICS SIMULATIONS

Structure preparation. Initial geometry for the complex of *Sa*BPL with **6** was taken from the X-ray crystallographic data revealed in this study. The complexes with other ligands were constructed by modifying the ligand molecule in the *Sa*BPL-**6** crystal structure (PDB code: 6ORU), as these ligands are structurally analogous to **6**. In case of deprotonated **6** and **7**, the involved proton was removed. Charge and spin multiplicity were manually specified for each ligand and their deprotonated state. The LEaP program from Antechamber tools (AmberTools 17) was used to prepare the parameter/topology (.top) and input coordinate (.crd) files. The net charge of each protein-ligand complex was neutralized by adding Na^+ ions at positions of high negative electron potential around the complex. The system was immersed in a truncated octahedral box of pre-equilibrated TIP3P water molecules, ensuring that no atoms in the protein−ligand complex were closer than 12 Å to any of the sides of the water box. The solvent molecules and counter-ions were firstly minimized for 6000 steps (involving 1000 of the steepest decent steps and 5000 of conjugate gradient steps) to remove any bad steric contacts with the complexes, whereby the protein and ligand were position-restrained using a force constant of 100 kcal·mol^{-1}·Å^{-2}. This was followed by additional 6000-step energy minimization without any restriction.

Heating and production phases. MD simulation was carried out for the heating phase, under a constant volume periodic boundary condition with an initial temperature of 0 K, allowing to heat up to 300 K over 40 ps. The final production simulation was carried out under an NVT ensemble condition. The following settings were activated throughout the heating and production phases: The Langevin dynamics was used to control the temperature using a collision frequency of 1.0 ps^{-1} and 0.5 ps coupling time constant. The SHAKE algorithm was used to constrain bonds involving hydrogen, allowing time step of 2 fs, for a total of 2500000 steps (5 ns). The default particle mesh Ewald (PME) method was employed to compute the long range electrostatic interactions using a 1.0 A grid space and a fourth-order spline for interpolation. The non-bonded cutoff value was set to 10 Å in the QM and MM regions. In the production runs, the MD trajectory was written for every 50 steps, resulting in 50000 frames for subsequent time-averaged structures and QM/MM-GBSA analyses.

Time-averaged structures. Time-averaged structures were calculated from 40000 trajectory frames (final 4 ns) of the production phase using the Cptraj program in AmberTools 17, with the water molecules and counter-ions stripped.

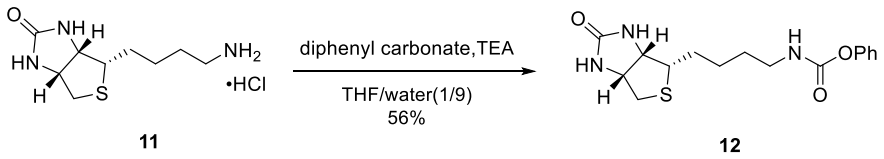
QM/MM-GBSA BINDING FREE ENERGY CALCULATIONS

The binding free energies of ligands to *Sa*BPL were calculated using MMPBSA.py[1] in AmberTools 17. For each complex, we calculated the Δ*G*bind values for the 200 snapshots of the MD trajectory (one snapshot for each 20 ps during the last 4 ns of the stable trajectory) and the final Δ*G*bind value was the average of the calculated Δ*G*bind values for these snapshots. Specifically, the python utility ante-MMPBSA.py was used to prepare the complex, protein and ligand topology files from the topology file of the solvated complex, by setting radii=mbondi2 and stripping water molecules (and counter-ions). The default MM-GBSA parameters α, β, and γ have the values of 1.0, 0.8, and 4.85 respectively. The ligand was treated as the QM region using the semi-empirical PM6-DH+ Hamiltonian theory. As only one MD simulation for each complex was performed, this method is less computationally demanding and leads to an increase of convergence due to cancellation of errors, conformational restraints imposed by the complex geometry, and reduction of noise arising from flexible remote regions relative to the binding site.[2]

CHEMISTRY

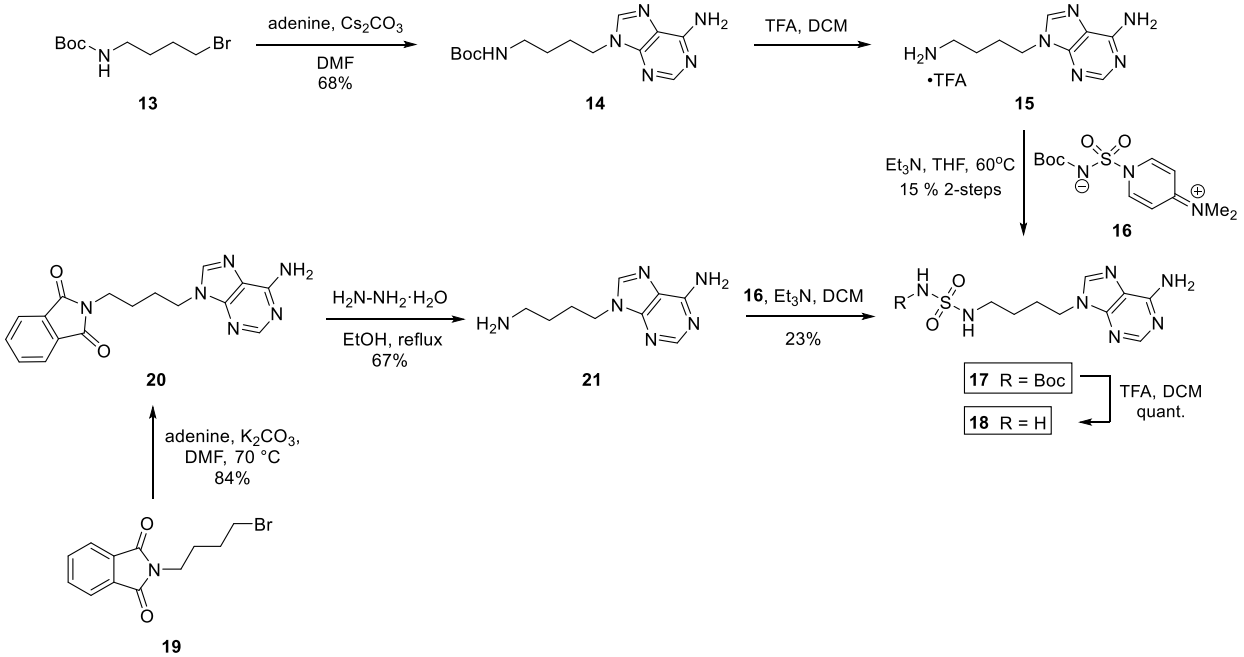
Synthesis

The preparation of **6-8** required preparation of the key intermediates **12**, **17**, **18**, and **25** as outlined in **Schemes S1** – **S3**. Biotin carbamate **12**, required for the preparation of amino sulfonylurea **7**, was prepared as shown in **Scheme S1**. In particular, biotin amine **11**[3] was converted into biotin carbamate **12** on reaction with diphenyl carbonate



Scheme S1. Synthesis of biotin carbamate **12**

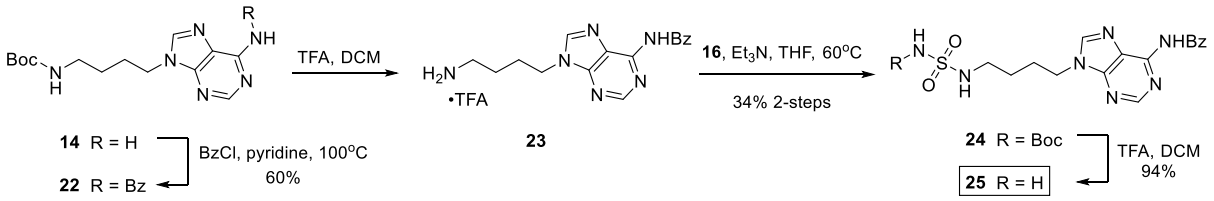
Adenine sulfamides **17** and **18** required for the synthesis of alkylsulfamide **8** and acylsulfamide **6** respectively were prepared as shown in **Scheme S2**. Adenine was alkylated on reaction with caesium carbonate and Boc-protected amino bromide **13**[4] to give Boc-protected adenine amine **14** in 68% yield. Treatment with TFA in DCM gave the amine salt **15**, which was then reacted with **16**[5] in THF to give Boc-protected sulfamide **17**. The key starting material **17** could also be prepared via adenine phthalimide **20** in a superior overall yield of 13% as shown in **Scheme S2**. In particular, Adenine was reacted with N-(4-



Scheme S2. Synthesis of adenine sulfamide building blocks **17** and **18**

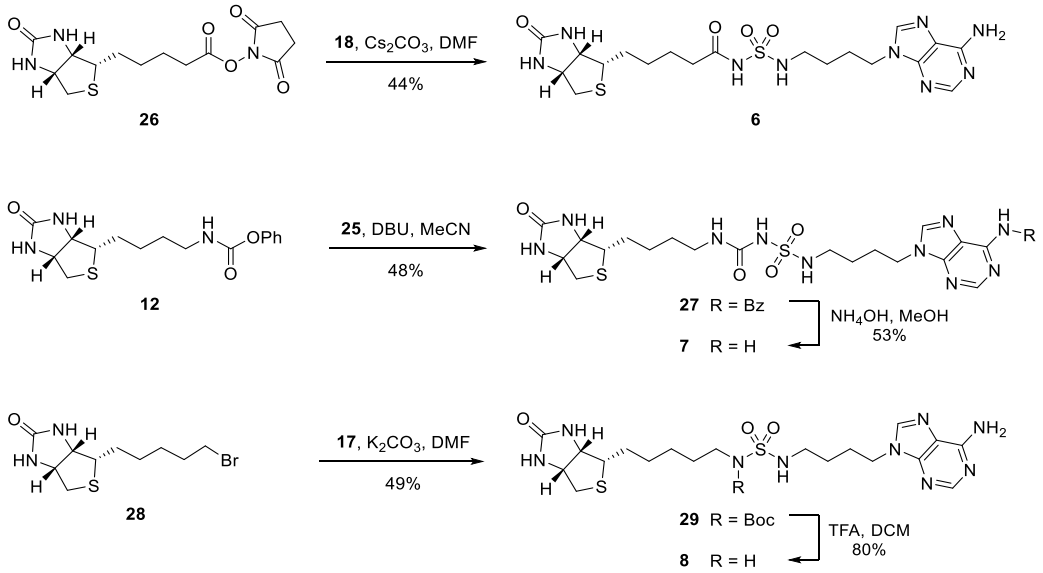
bromobutyl)phthalimide **19** in the presence of K_2CO_3 to give adenine phthalimide **20** in 84% yield. Treatment with hydrazine in ethanol gave adenine amine **21**, which was converted into Boc-protected adenine sulfamide **17** on reaction with **16**[5] in DCM. Removal of the Boc protecting group from **17**, on reaction with 10% TFA in DCM, gave the desired adenine sulfamide **18** as shown.

Adenine sulfamides **25** required for the synthesis of amino sulfonylurea **7** was prepared as shown in **Scheme S3**. Boc-protected adenine amine **14** was reacted with benzoyl chloride, in the presence of pyridine, to give **22.** This was then treated with TFA in DCM to give adenine amine salt **23**, which was reacted with **16**[5] in THF to give sulfamide **24** as per the preparation of **17**, see **Scheme S2.** Removal of the Boc group, on reaction with TFA in DCM, then gave the key benzoyl-protected adenine sulfamide **25**.



Scheme S3. Synthesis of adenine sulfamide building block **25**

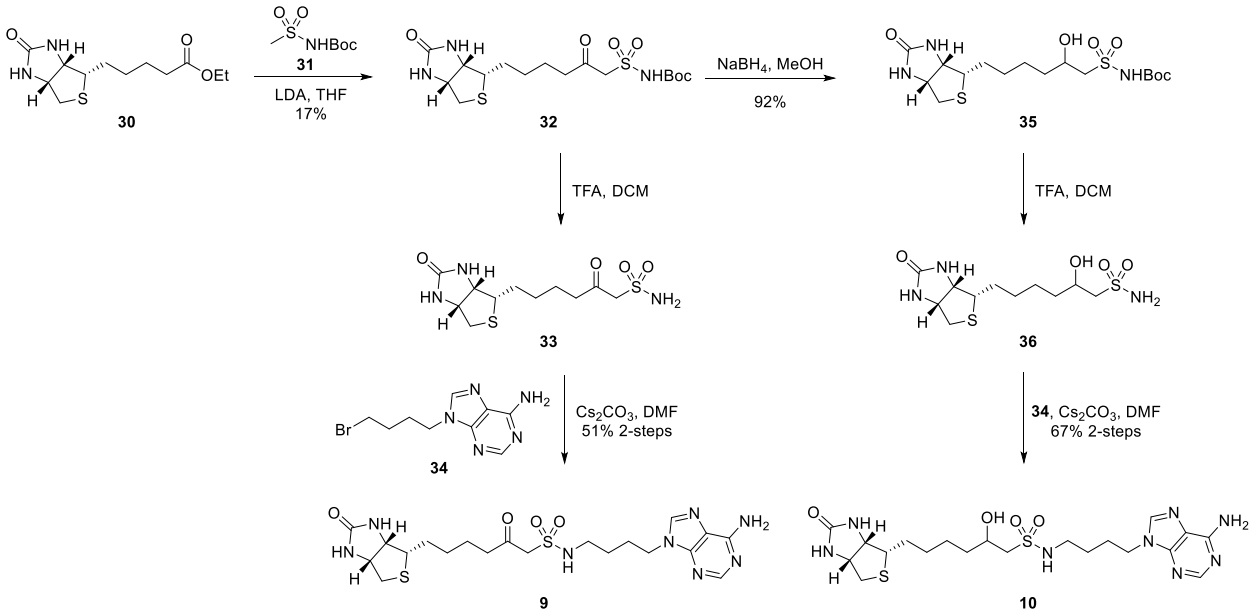
Acylsulfamide **6**, amino sulfonylurea **7**, and alkylsulfamide **8** were next prepared as outlined in **Scheme S4**. In particular, D-(+)-biotin *N*-hydroxysuccinimide ester (Biotin-NHS) **26**[6] was reacted with adenine sulfamide **18**, in the presence of Cs_2CO_3, to give acylsulfamide **6** in 44% yield. Biotin carbamate **12** was coupled to benzoyl-protected adenine sulfamide **25**, in the presence of DBU, to give benzoyl-protected



Scheme S4. Synthesis of acylsulfamide **6**, amino sulfonylurea **7**, and alkylsulfamide **8**

amino sulfonylurea **27**, the benzoyl group of which was removed on treatment with NH_4OH in MeOH to give amino sulfonylurea **7**. Biotin bromide **28**[3] was reacted with boc-protected adenine sulfamide **17** in the presence of K_2CO_3 in DMF to give boc-protected alkylsulfamide **29**, which was then treated with TFA in DCM to give alkylsulfamide **8**.

The final derivatives, β-ketosulfonamide **9** and β-hydroxysulfonamide **10,** were prepared as summarised in **Scheme S5**. Biotin ester **30**[7] was coupled to sulphonamide **31**[8] in the presence of LDA to give *N*-boc-β-ketosulfonamide **32**. Reaction with TFA in DCM then gave the biotin β-ketosulfonamide **33**, which was alkylated with adenine bromide **34**,[9] in the presence of Cs_2CO_3, in DMF to give β-ketosulfonamide **9**. *N*-Boc-β-ketosulfonamide **32** was also reduced with $NaBH_4$ to give **35**, the boc group of which was removed on treatment with TFA in DCM to give the biotin β-hydroxysulfonamide **36**. Treatment with **34** in the presence of Cs_2CO_3 gave β-hydroxysulfonamide **10** as per the synthesis of β-ketosulfonamide **9**.



Scheme S5. Synthesis of β-ketosulfonamide **9** and β-hydroxysulfonamide **10**

Experimental section

Chemistry: general Materials and Methods

All reagents were obtained from commercial sources and are of reagent grade or as specified. Solvents were also obtained from commercial sources, except for anhydrous THF and anhydrous DMF that were dried over solvent purifier (PS-Micro, Innovative Technology, USA). Reactions were monitored by TLC using precoated plates (silica gel 60 F254, 250 µm, Merck, Darmstadt, Germany), spots were visualised under ultraviolet light at 254 nm and with either sulfuric acid-vanillin spray, potassium permanganate dip or Hanessian's stain. Column chromatography was performed with silica gel (40-63 µm 60 Å, Davisil, Grace, Germany). ^{1}H and ^{13}C spectra were recorded on a Varian Inova 500 MHz or a Varian Inova 600 MHz. Chemical shifts are given in ppm (δ) relative to the residue signals, which in the case of DMSO-d_6 were 2.50 ppm for ^{1}H and 39.55 ppm for ^{13}C, $CDCl_3$ were 7.26 ppm for ^{1}H and 77.23 ppm for ^{13}C, and MeOH-d_4 were 3.31 ppm for ^{1}H and 49.00 ppm for ^{13}C. High-resolution mass spectra (HRMS) were recorded on an Agilent 6230 time of flight (TOF) liquid chromatography mass spectra (LC/MS) (Δ < 5 ppm). Infrared mass spectra were recorded on PerkinElmer Spectrum 100 FTIR with a Universal Zinc Selenide crystal ATR attachment.

Synthesis

Phenyl (4-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)butyl)carbamate (**12**)

To a mixture of compound **11**[3] (300 mg, 1.19 mmol) in water/THF (9:1, 3.33 mL) were added diphenyl carbonate (255 mg, 1.19 mmol) and TEA (0.33 mL, 2.38 mmol) and the mixture was stirred for 6 h. The mixture was poured into water and extracted with DCM. The organic layer was washed with cold aqueous 10 % NaOH solution and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography on silica (DCM:MeOH = 10:1) to yield a white solid (223 mg, 56 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.71 (t, *J* = 5.7 Hz, 1H), 7.40 – 7.33 (m, 2H), 7.22 – 7.15 (m, 1H), 7.11 – 7.06 (m, 2H), 6.43 (s, 1H), 6.35 (s, 1H), 4.31 (ddt, *J* = 7.6, 5.0, 1.1 Hz, 1H), 4.14 (ddd, *J* = 7.8, 4.5, 1.9 Hz, 1H), 3.12 (ddd, *J* = 8.2, 6.4, 4.4 Hz, 1H), 3.05 (q, *J* = 6.6 Hz, 2H), 2.83 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.58 (d, *J* = 12.4 Hz, 1H), 1.69 – 1.58 (m, 1H), 1.57 – 1.27 (m, 5H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.68, 154.27, 151.11, 129.18, 124.77, 121.69, 60.93, 59.19, 55.43, 40.22, 39.82, 29.20, 27.98, 25.80.

2-(4-(6-Amino-9*H*-purin-9-yl)butyl)isoindoline-1,3-dione (**20**)

To a suspension of adenine (1.1 g, 8.14 mmol) in DMF (11 mL) was added K_2CO_3 (1.8 g, 13.23 mmol) and *N*-(4-bromobutyl)phthalamide **19** (2.75 g, 9.75 mmol) and the mixture was stirred at 70 °C overnight. The mixture was allowed to cool down to room temperature and partitioned between EtOAc and water. The

aqueous phase was extracted with EtOAc. The combined organics were washed with brine and water, dried over Na_2SO_4, filtrated and concentrated under reduced pressure. The solid obtained was triturated from diethyl ether affording the title compound as a pale yellow solid (2.5 g, 84%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.12 (s, 1H), 8.07 (s, 1H), 7.84 (m, 4H), 7.16 (s, 2H), 4.17 (t, J = 6.7 Hz, 2H), 3.61 (t, J = 6.7 Hz, 2H), 1.83 (dd, J = 14.7, 6.9 Hz, 2H), 1.56 (dt, J = 13.8, 6.9 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.9, 155.9, 152.3, 149.5, 140.8, 134.3, 131.6, 123.0,118.7, 42.4, 36.8, 26.7, 25.1; IR (ATR) υ: 3113 (NH_2), 1698 (C=O), 1600 (N=C and C=C) cm^{-1}.

9-(4-Aminobutyl)-9*H*-purin-6-amine (**21**)

To a suspension of 2-(4-(6-amino-9H-purin-9-yl)butyl)isoindoline-1,3-dione **20** (2.5 g, 7.43 mmol) in ethanol (125 mL) was added hydrazine hydrate (3.5 mL, 114.45 mmol) and the mixture was stirred at reflux overnight. The mixture was allowed to cool down to room temperature and concentrated under reduced pressure. A solid was formed which was filtrated and washed with DCM. Trituration from MeOH afforded the title compound (1.0 g, 67%) as a colourless solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.12 (s, 1H), 8.11 (s, 1H), 7.14 (2, 2H), 4.11 (t, *J* = 7.1 Hz, 2H), 3.08 (br s, 2H), 2.51 (t, *J* = 6.9 Hz, 2H), 1.79 (dd, *J* = 14.9, 7.3 Hz, 2H), 1.28 (dd, *J* = 14.9, 7.3 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.9, 152.3, 149.5, 140.8, 118.7, 42.8, 41.0, 30.0, 26.9; HRMS calcd. for [M + H^+] $C_9H_{15}N_6$: requires 207.1358, found 207.1365; IR (ATR) υ: 3149 (NH_2), 2932 (=C–H), 1677 (N=C and C=C) cm^{-1}.

tert-Butyl (4-(6-amino-9*H*-purin-9-yl)butyl)carbamate (**14**)

To a suspension of adenine (1.10 g, 8.14 mmol) in DMF (12 mL) was added Cs_2CO_3 (3.98 g, 12.2 mmol) and bromide **13**[4] (2.45 g, 9.77 mmol). The mixture was stirred overnight, poured onto water and extracted with EtOAc twice. The combined organic layers were washed with brine and water. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography on silica (DCM:MeOH = 15:1 → 10:1) to yield a white solid (1.70 g, 68 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.12 (s, 1H), 8.11 (s, 1H), 7.16 (s, 2H), 6.80 (t, *J* = 5.8 Hz, 1H), 4.12 (t, *J* = 7.0 Hz, 2H), 2.92 (q, *J* = 6.6 Hz, 2H), 1.76 (m, 2H), 1.38 – 1.28 (m, 11H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 155.93, 155.58, 152.31, 149.52, 140.78, 118.74, 77.38, 42.57, 28.23, 26.83, 26.65.

tert-Butyl (*N*-(4-(6-amino-9*H*-purin-9-yl)butyl)sulfamoyl)carbamate (**17**)

Method 1. To a solution of amine **21** (1.6 g, 7.75 mmol) in DCM (16 ml) was added TEA (2.1 mL, 15.5 mmol), followed by addition of sulfamoylating agent **16**[5] (2.6 g, 7.75 mmol). The reaction mixture was stirred at

room temperature for 12 h. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (5% MeOH in DCM) to give a crystalline white solid (664 mg, 23%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.12 (s, 1H), 8.11 (s, 1H), 7.58 (t, J = 5.8 Hz, 1H), 7.16 (s, 2H), 4.12 (t, J = 7.0 Hz, 2H), 2.90 (q, J = 6.6 Hz, 2H), 1.80 (q, J = 7.4 Hz, 2H), 1.41 (m, 2H), 1.38 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.93, 152.32, 150.58, 149.53, 140.72, 118.71, 81.07, 42.41, 42.18, 27.71, 26.74, 25.82.; IR (ATR) υ: 3455 (NH_2), 3358 (N–H), 2980 (=C–H), 1714 (C=O), 1643 (N=C and C=C), 1329 (SO_2) cm^{-1}.

Method 2. To a mixture of compound **14** (613 mg, 2.00 mmol) in DCM (9 mL) was added TFA (0.83 mL, 10.8 mmol) and the mixture was stirred for 3 hours. TEA (1.5 mL) was added dropwise to the reaction mixture to form a white solid. The solid was collected by filtration and washed with DCM. To a mixture of the collected solid in THF (20mL) were added TEA (0.45 mL, 4.00 mmol) and sulfamoylating agent **16**[5] (602 mg 2.00 mmol) and the mixture was stirred at 60°C overnight. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (DCM:MeOH = 20:1 → 15:1) to yield a white sold (116 mg, 15 %).

N-(4-(6-Amino-9*H*-purin-9-yl)butyl)-sulfamide (**18**)

A solution of compound **17** (664 mg, 1.72 mmol) in DCM (12 mL) cooled down to 0 °C was treated with TFA (1.2 mL) and stirred at room temperature. After 2 h TFA (1.2 mL) was added. After being stirred at room temperature overnight the solvent was removed under reduced pressure. The title compound was obtained as a colourless solid (641 mg) and was used without further purification. HRMS (ESI) calcd. for $C_9H_{15}N_7NaO_2S$ [M + Na^+]: 308.0906, found 308.0883; IR (ATR) υ: 3324 (NH_2), 3212 (N–H), 3011 (=C–H), 1626 (N=C and C=C), 1332 (SO_2) cm^{-1}.

tert-Butyl (4-(6-benzamido-9*H*-purin-9-yl)butyl)carbamate (**22**)

To a mixture of compound **14** (1.03 g, 3.36 mmol) in pyridine (5 mL) was slowly added BzCl (0.39 mL, 3.36 mmol) and the mixture was stirred at 100 °C for 1 h. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (DCM:MeOH = 20:1) to yield a white solid (0.83 g, 60 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.30 (s, 1H), 8.74 (s, 1H), 8.06 – 7.93 (m, 3H), 7.59 – 7.52 (m, 1H), 7.47 (t, *J* = 7.8 Hz, 2H), 4.71 (t, *J* = 6.2 Hz, 1H), 4.28 (t, *J* = 7.3 Hz, 2H), 3.16 (q, *J* = 6.7 Hz, 2H), 2.40 (s, 1H), 1.92 (m, 2H), 1.50 (m, 2H), 1.39 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.93, 156.21, 152.56, 152.23, 149.60, 143.12, 133.74, 132.81, 128.88, 128.02, 123.14, 79.46, 43.69, 39.55, 28.48, 27.36, 27.22.

tert-Butyl (*N*-(4-(6-benzamido-9*H*-purin-9-yl)butyl)sulfamoyl)carbamate (**24**)

To a mixture of compound **22** (820 mg, 2.00 mmol) in DCM (9 mL) was added TFA (0.83 mL, 10.8 mmol) and the mixture was stirred for 3 hours. TEA (1.5 mL) was added dropwise to the reaction mixture to form a white solid. The solid was collected by filtration and washed with DCM. To a mixture of the collected solid in THF (20mL) were added TEA (0.45 mL, 4.00 mmol) and sulfamoylating agent **16**[5] (602 mg 2.00 mmol) and the mixture was stirred at 60°C overnight. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (DCM:acetone:MeOH = 50:25:1) to yield a white sold (320 mg, 34 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.26 (br s, 1H), 8.79 (s, 1H), 8.10 – 7.99 (m, 3H), 7.66 – 7.57 (m, 1H), 7.52 (t, *J* = 7.6 Hz, 2H), 5.81 (br s, 1H), 4.33 (t, *J* = 7.1 Hz, 2H), 3.14 (q, *J* = 5.9 Hz, 2H), 2.03 (m, 2H), 1.69 – 1.55 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.55, 152.43, 150.60, 149.74, 143.23, 133.69, 132.92, 128.92, 128.24, 123.26, 83.93, 68.10, 43.56, 43.14, 28.14, 27.34, 26.15.

N-(9-(4-(Sulfamoylamino)butyl)-9*H*-purin-6-yl)benzamide (**25**)

To a mixture of compound **24** (140 mg, 0.286 mmol) in DCM (5 mL) was added TFA (0.5 mL) and the mixture was stirred for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (DCM:MeOH = 6:1) to yield a white solid (105 mg, 94 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (br s, 1H), 8.73 (s, 1H), 8.50 (s, 1H), 8.09 – 8.00 (m, 2H), 7.69 – 7.60 (m, 1H), 7.55 (t, *J* = 7.7 Hz, 2H), 6.47 (m, 3H), 4.28 (t, *J* = 7.1 Hz, 2H), 2.91 (q, *J* = 6.5 Hz, 2H), 1.91 (m, 2H), 1.52 – 1.40 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 152.42, 151.31, 150.01, 144.66, 133.46, 132.34, 128.42, 128.40, 125.28, 42.96, 41.87, 26.73, 26.11.

N-(*N*-(4-(6-Amino-9*H*-purin-9-yl)butyl)sulfamoyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (**6**)

To a mixture of sulfamide **18** (258 mg, 1.00 mmol) in DMF (3 ml) was added Cs_2CO_3 (232 mg, 1.20 mmol) and biotin-NHS **26**[6] (375 mg, 1.10 mmol). The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (10% MeOH in DCM) to give the title compound as a crystalline solid (178 mg, 35%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.25 (br s, 1H), 8.13 (s, 1H), 8.11 (s, 1H), 7.59 (t, *J* = 5.7 Hz, 1H), 7.20 (s, 2H), 6.50 (s, 1H), 6.38 (s, 1H), 4.30 (dd, *J* = 7.6, 5.2 Hz, 1H), 4.20 – 4.07 (m, 3H), 3.12 – 3.04 (m, 1H), 2.88 (dd, *J* = 12.9, 6.7 Hz, 2H), 2.80 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.57 (d, *J* = 12.4 Hz, 1H), 2.18 (t, *J* = 7.4 Hz, 2H), 1.91 – 1.73 (m, 2H), 1.67 – 1.55 (m, 1H), 1.55 – 1.36 (m, 5H), 1.35 – 1.21 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6): 171.55, 162.79, 155.95, 152.38, 149.55, 140.78, 118.69, 61.08, 59.23,

55.36, 48.61, 42.45, 42.21, 34.96, 28.03, 26.82, 25.78, 24.35; HRMS (ESI) calcd. for $C_{19}H_{30}N_9O_4S_2$ [M + H^+]: 512.1857, found 512.1834; IR (ATR) υ: 3315 (NH_2), 3283 (N–H), 1638 (C=O), 1342 (SO_2) cm^{-1}.

N-(9-(4-((*N*-((4-((3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)butyl)carbamoyl)sulfamoyl)amino)butyl)-9*H*-purin-6-yl)benzamide (**27**)

To a mixture of compound **12** (77 mg, 0.23 mmol) and compound **25** (74 mg, 0.19 mmol) in MeCN (10 mL) was added DBU (43 µL, 0.29 mmol) and the resulting mixture was refluxed for 17 h. The solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc and the organic layer was washed with 0.1N HCl, brine, and water. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography on silica (DCM:MeOH = 9:1) to yield a white solid (58 mg, 48%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (br s, 1H), 9.74 (br s, 1H), 8.72 (s, 1H), 8.47 (s, 1H), 8.09 – 8.00 (m, 2H), 7.70 – 7.60 (m, 1H), 7.55 (t, *J* = 7.7 Hz, 2H), 7.35 (br s, 1H), 6.41 (s, 1H), 6.34 (s, 1H), 6.26 (m, 1H), 4.27 (m, 3H), 4.11 (ddd, *J* = 7.5, 4.5, 1.7 Hz, 1H), 3.07 (ddd, *J* = 8.4, 6.3, 4.4 Hz, 1H), 3.01 (q, *J* = 6.6 Hz, 2H), 2.93 (m, 2H), 2.79 (dd, *J* = 12.5, 5.1 Hz, 1H), 2.56 (d, *J* = 12.5 Hz, 1H), 1.89 (m,2H), 1.62 (m, 1H), 1.54 – 1.24 (m, 7H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.68, 152.43, 152.30, 152.29, 151.33, 150.02, 144.62, 132.33, 128.42, 128.40, 125.30, 60.97, 59.17, 55.43, 42.81, 42.11, 39.81, 29.37, 27.94, 26.59, 25.86, 25.79.

(3a*S*,4*S*,6a*R*)-4-(4-((((4-(6-Amino-9*H*-purin-9-yl)butyl)sulfamoyl)carbamoyl)amino)butyl)tetrahydro-1*H*-thieno[3,4-*d*]imidazol-2(3*H*)-one (**7**)

To a solution of compound **27** (50 mg, 0.079 mmol) in MeOH (1.6 mL) was added 30 % NH_4OH (2.4 mL) and the mixture was stirred overnight. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (DCM:MeOH = 4:1) to yield a white solid (22 mg, 53 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.74 (br s, 1H), 8.13 (s, 1H), 8.11 (s, 1H), 7.33 (br s, 1H), 7.17 (s, 2H), 6.47 (s, 1H), 6.35 (s, 1H), 6.21 (br s, 1H), 4.29 (dd, *J* = 7.7, 5.0 Hz, 1H), 4.17 – 4.09 (m, 3H), 3.08 (ddd, *J* = 8.6, 6.2, 4.4 Hz, 1H), 3.01 (q, *J* = 6.6 Hz, 2H), 2.90 (q, *J* = 6.6 Hz, 2H), 2.80 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.57 (d, *J* = 12.4 Hz, 1H), 1.87 – 1.77 (m, 2H), 1.67 – 1.56 (m, 1H), 1.51 – 1.19 (m, 7H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.72, 155.91, 152.33, 149.51, 140.71, 118.67, 61.01, 59.17, 55.45, 42.42, 42.14, 38.81, 29.38, 27.97, 26.75, 25.82, 25.78; HRMS (ESI) calcd. for $C_{19}H_{30}N_{10}O_4S_2$ [M + H^+]: 527.1966, found 527.1966.

tert-Butyl (*N*-(4-(6-amino-9*H*-purin-9-yl)butyl)sulfamoyl)(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentyl)carbamate (**29**)

To a mixture of sulfamide **17** (50 mg, 0.13 mmol) in DMF (2 mL) was added K_2CO_3 (27 mg, 0.20 mmol) and biotin bromide **28**[3] (42 mg, 0.14 mmol). The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (DCM:MeOH = 15:1) to give a white solid (38 mg, 49%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.12 (s, 1H), 8.11 (s, 1H), 7.61 (br s, 1H), 7.16 (s, 2H), 6.47 (s, 1H), 6.34 (s, 1H), 4.35 – 4.25 (m, 1H), 4.12 (m, 3H), 3.48 (t, J = 7.5 Hz, 2H), 3.14 – 3.04 (m, 1H), 2.88 (m, 2H), 2.81 (dd, J = 12.4, 5.1 Hz, 1H), 2.57 (d, J = 12.4 Hz, 1H), 1.82 (m, 2H), 1.59 (m, 1H), 1.55 – 1.19 (m, 18H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.74, 155.94, 152.34, 151.06, 149.55, 140.71, 118.71, 82.44, 61.01, 59.20, 55.48, 47.39, 42.35, 42.32, 29.12, 28.25, 28.21, 27.60, 26.65, 26.06, 25.68.

N-(4-(6-Amino-9*H*-purin-9-yl)butyl)-*N*-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentyl)sulfuric diamide (**8**)

To a mixture of compound **29** (21 mg, 0.035 mmol) in DCM (0.5 mL) was added TFA (24 µL, 0.32 mmol) and the mixture was stirred overnight. The mixture was quenched with TEA and concentrated under reduced pressure. The residue was purified by flash chromatography on silica (DCM:MeOH = 5:1) to yield a white solid (14 mg, 80 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.12 (d, 2H), 7.18 (s, 2H), 6.75 (t, J = 5.9 Hz, 1H), 6.71 (t, J = 5.9 Hz, 1H), 6.48 (s, 1H), 6.36 (s, 1H), 4.30 (dd, J = 7.7, 5.1 Hz, 1H), 4.13 (m, 3H), 3.08 (ddd, J = 8.5, 6.1, 4.3 Hz, 1H), 2.87 – 2.77 (m, 3H), 2.73 (m, 2H), 2.57 (d, J = 12.4 Hz, 1H), 1.82 (m, 2H), 1.58 (m, 1H), 1.51 – 1.18 (m, 9H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.75, 155.94, 152.34, 149.54, 140.76, 118.70, 61.03, 59.20, 55.47, 42.54, 42.08, 41.61, 39.81, 28.74, 28.25, 28.24, 26.97, 26.33, 26.12; HRMS (ESI) calcd. for $C_{19}H_{31}N_9O_3S_2$ [M + H^+]: 498.2064, found 498.2069.

tert-Butyl ((2-oxo-6-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)hexyl)sulfonyl)carbamate (**32**)

To a mixture of sulfonamide **31**[8] (0.90 g, 4.59 mmol) in anhydrous THF (30 mL) was added dropwise LDA (1.0 M in THF/hexane; 14.22 mL, 14.22 mmol) at 0°C and the mixture was stirred for 1 h at 0°C. Biotin ester **30**[7] (1.38 g, 5.07 mmol) was added and the reaction mixture was stirred for 3 h at 0°C. The mixture was warmed to room temperature and stirred overnight. The reaction mixture was quenched with saturated aq NaCl (15 mL) and 0.5 M aq NaH_2PO_4 (15 mL) and extracted with EtOAc (2 X 15 mL). The aqueous layer was extracted with 10 % MeOH/DCM (6 X 15 mL) and the combined DCM extracts were dried over Na_2SO_4. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (DCM:MeOH = 15:1) to yield a white solid (223 mg, 17 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.45 (br s, 1H), 6.41 (s, 1H), 6.34 (s, 1H), 4.52 (s, 2H), 4.30 (dd, J = 7.7, 5.1 Hz, 1H), 4.12

(ddd, J = 7.5, 4.5, 1.8 Hz, 1H), 3.09 (ddd, J = 8.5, 6.3, 4.4 Hz, 1H), 2.81 (dd, J = 12.4, 5.1 Hz, 1H), 2.64 (td, J = 7.0, 4.1 Hz, 2H), 2.57 (d, J = 12.4 Hz, 1H), 1.60 (m, 1H), 1.54 – 1.21 (m, 14H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 198.92, 162.68, 61.80, 60.97, 59.17, 55.30, 42.87, 31.29, 28.06, 27.82, 27.65, 22.58.

2-Oxo-6-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)hexane-1-sulfonamide (**33**)

To a solution of compound **32** (50 mg, 0.12 mmol) in DCM (1 mL) was added TFA (81 µL) and the mixture was stirred overnight. The mixture was concentrated under reduced pressure and was used without further purification. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.10 (br s, 2H), 4.30 (ddd, *J* = 7.7, 5.1, 1.0 Hz, 1H), 4.17 (s, 2H), 4.12 (dd, *J* = 7.7, 4.4 Hz, 1H), 3.09 (ddd, *J* = 8.5, 6.3, 4.4 Hz, 1H), 2.82 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.72 – 2.59 (m, 2H), 2.57 (d, *J* = 12.4 Hz, 1H), 1.66 – 1.56 (m, 1H), 1.55 – 1.41 (m, 3H), 1.38 – 1.23 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 200.13, 162.69, 65.55, 60.99, 59.18, 55.34, 42.54, 28.11, 27.89, 22.66.

N-(4-(6-Amino-9*H*-purin-9-yl)butyl)-2-oxo-6-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)hexane-1-sulfonamide (**9**)

To a mixture of compound **33** (27 mg, 0.084 mmol) and adenine bromide **34**[9] (25 mg, 0.093 mmol) in DMF (1 mL) was added Cs_2CO_3 (33 mg, 0.10 mmol) and the mixture was stirred overnight. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (DCM:MeOH = 5:1) to yield a white solid (22 mg, 51 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.13 (s, 1H), 8.12 (s, 1H), 7.34 (t, *J* = 5.8 Hz, 1H), 7.18 (s, 2H), 6.44 (s, 1H), 6.36 (s, 1H), 4.32 – 4.27 (m, 1H), 4.21 (s, 2H), 4.17 – 4.10 (m, 3H), 3.08 (ddd, *J* = 8.5, 6.2, 4.4 Hz, 1H), 2.96 (m, 2H), 2.81 (dd, *J* = 12.5, 5.1 Hz, 1H), 2.62 (td, *J* = 7.1, 2.8 Hz, 2H), 2.57 (d, *J* = 12.4 Hz, 1H), 1.87 – 1.77 (m, 2H), 1.59 (m, 1H), 1.52 – 1.20 (m, 7H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 200.07, 162.73, 155.94, 152.36, 149.53, 140.80, 118.71, 62.11, 61.00, 59.19, 55.34, 42.71, 42.41, 41.96, 28.10, 27.88, 26.70, 26.55, 22.65; HRMS (ESI) calcd. for $C_{20}H_{30}N_8O_4S_2$ [M + H^+]: 511.1904, found 511.1910.

tert-Butyl ((2-hydroxy-6-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)hexyl)sulfonyl)carbamate (**35**)

To a mixture of compound **32** (84 mg, 0.20 mmol) in MeOH (2 mL) was added $NaBH_4$ (8 mg, 0.21 mmol) and the mixture was stirred for 4 h. The reaction mixture was quenched with adding HCl to adjust pH of around 5. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (DCM:MeOH = 5:1) to yield a white solid (78 mg, 92 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.05 (br s, 1H), 6.41 (s, 1H), 6.34 (s, 1H), 4.93 (br s, 1H), 4.30 (dd, *J* = 7.7, 5.1 Hz, 1H), 4.16 – 4.10 (m, 1H), 3.87 (br s, 1H), 3.45 – 3.33 (m, 2H), 3.10 (ddd, *J* = 8.5, 6.1, 4.4 Hz, 1H), 2.82 (dd, *J* = 12.4, 5.1

Hz, 1H), 2.57 (d, *J* = 12.4 Hz, 1H), 1.60 (m, 1H), 1.55 – 1.20 (m, 16H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.69, 150.62, 81.88, 65.49, 65.47, 61.03, 61.00, 60.71, 59.19, 58.42, 55.48, 55.44, 39.81, 36.41, 36.38, 29.18, 28.46, 28.41, 28.26, 28.24, 27.69, 24.70, 24.67.

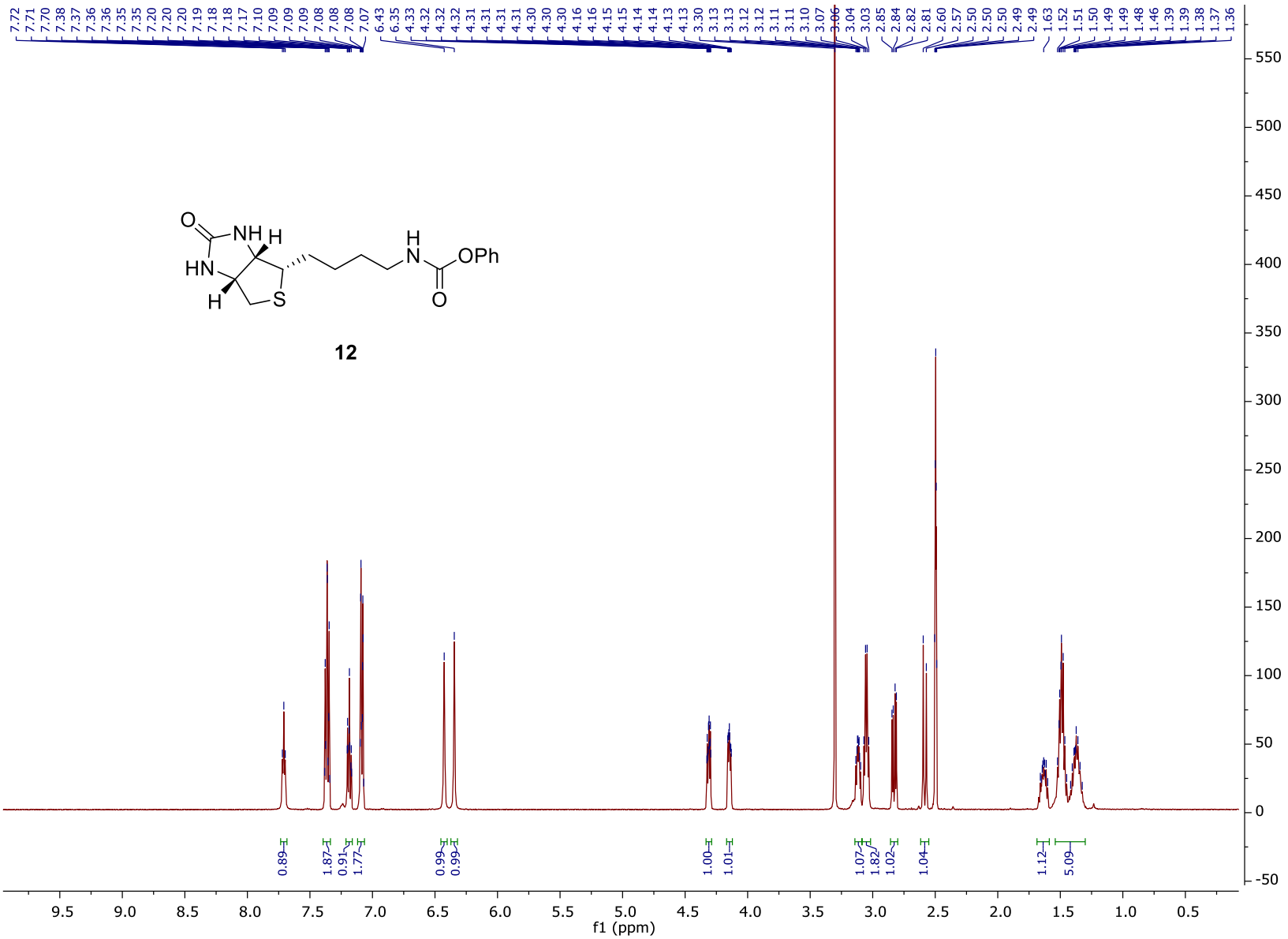
2-Hydroxy-6-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)hexane-1-sulfonamide (**36**)

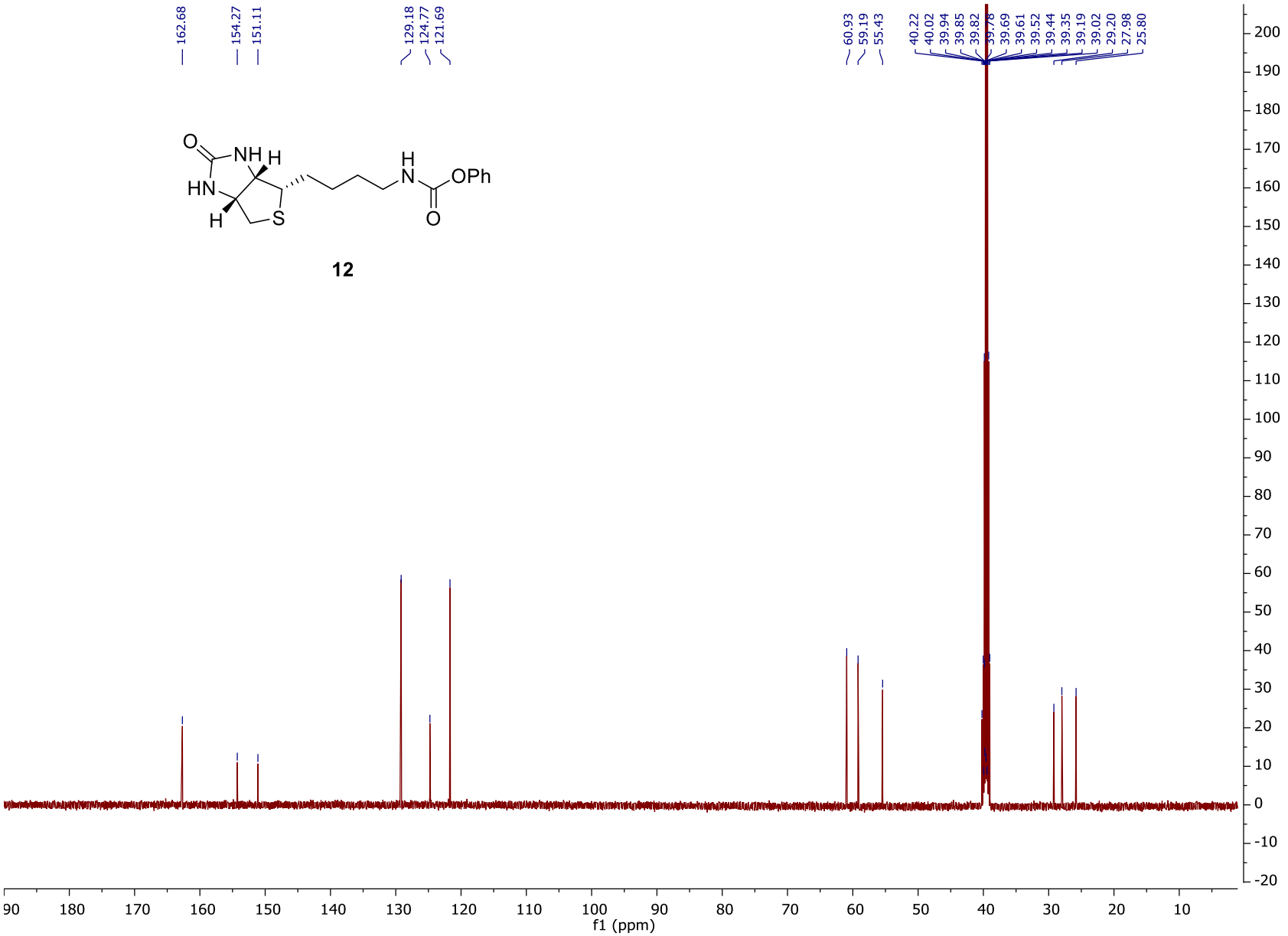
To a solution of compound **37** (56 mg, 0.13 mmol) in DCM (1 mL) was added TFA (81 µL) and the mixture was stirred overnight. The mixture was concentrated under reduced pressure and was used without further purification.

N-(4-(6-Amino-9*H*-purin-9-yl)butyl)-2-hydroxy-6-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)hexane-1-sulfonamide (**10**)

To a mixture of compound **36** (21 mg, 0.064 mmol) and adenine bromide **34**[9] (19 mg, 0.070 mmol) in DMF (1 mL) was added Cs_2CO_3 (25 mg, 0.077 mmol) and the mixture was stirred overnight. The mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica (DCM:MeOH = 10:1 → 5:1) to yield a white solid (22 mg, 67 %). ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.22 (s, 1H), 8.15 (d, J = 2.0 Hz, 1H), 4.55 (br s, 1H), 4.50 (dd, J = 7.9, 5.0 Hz, 1H), 4.35 – 4.25 (m, 2H), 4.06 (m, 1H), 3.22 (dt, J = 9.8, 5.3 Hz, 1H), 3.17 – 3.08 (m, 3H), 2.97 – 2.90 (m, 1H), 2.71 (d, J = 12.7 Hz, 1H), 2.02 – 1.94 (m, 2H), 1.79 – 1.70 (m, 1H), 1.65 – 1.37 (m, 7H); ^{13}C NMR (126 MHz, Methanol-d_4) δ 166.14, 157.33, 153.68, 150.67, 142.77, 120.05, 68.06, 68.01, 67.91, 63.43, 63.41, 63.39, 61.64, 59.57, 59.02, 58.97, 57.10, 57.08, 44.42, 43.27, 41.03, 37.78, 37.72, 37.47, 30.07, 30.01, 30.00, 29.75, 29.70, 29.64, 28.25, 28.21, 26.24.; HRMS (ESI) calcd. for $C_{20}H_{32}N_8O_4S_2$ [M + H^+]: 513.2061, found 513.2066.

^{1}H AND ^{13}C NMR OF COMPOUNDS

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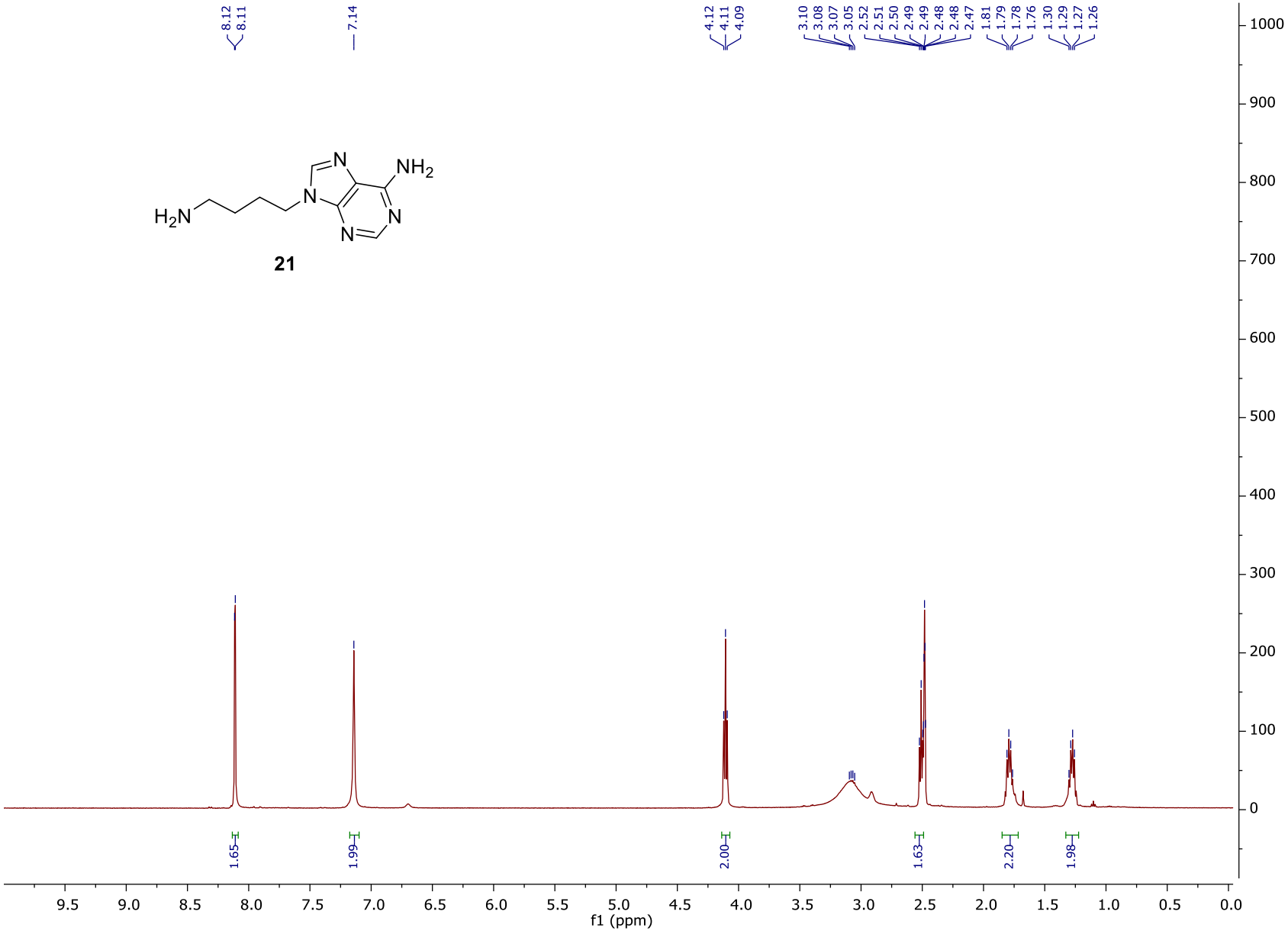
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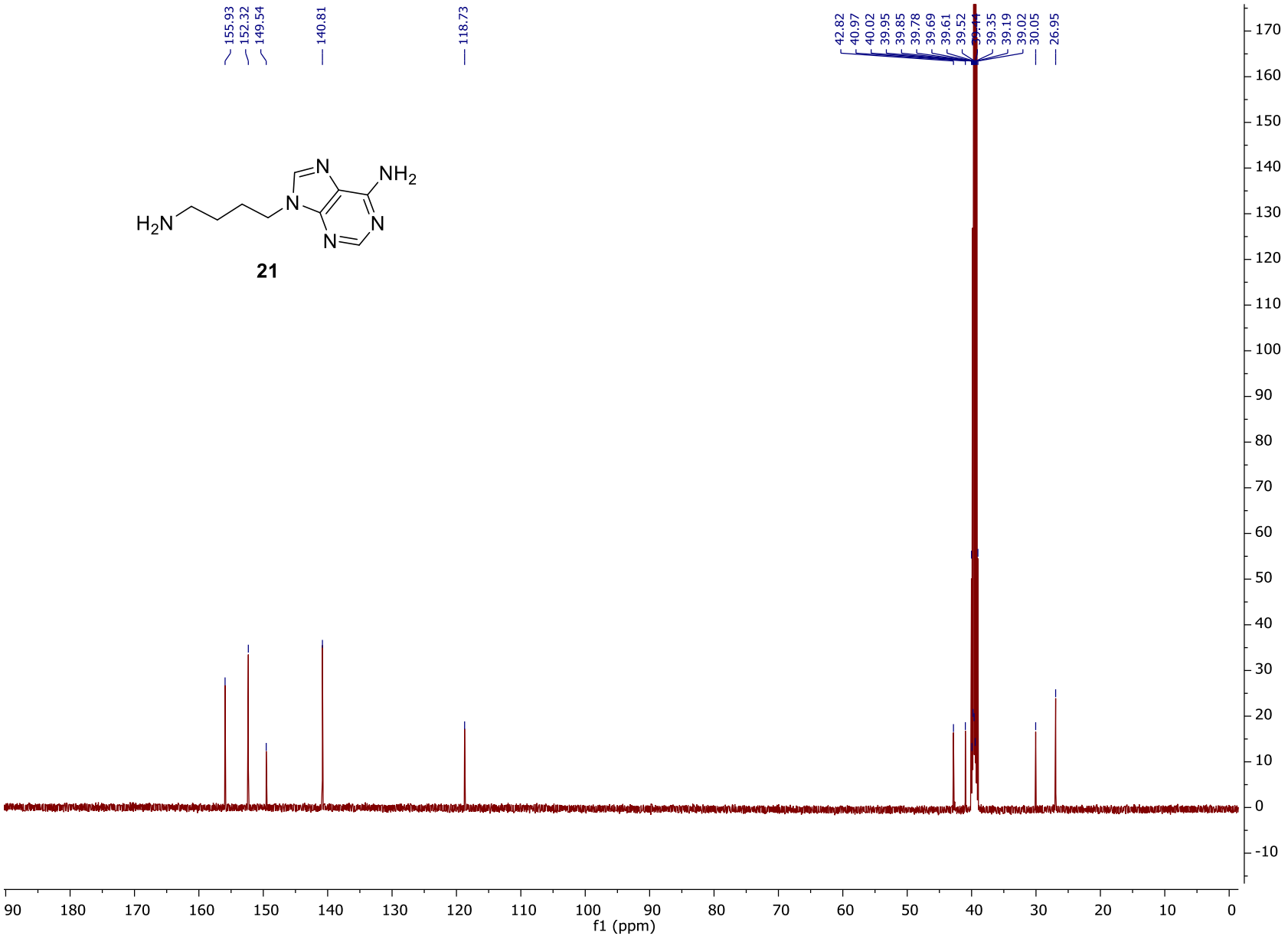
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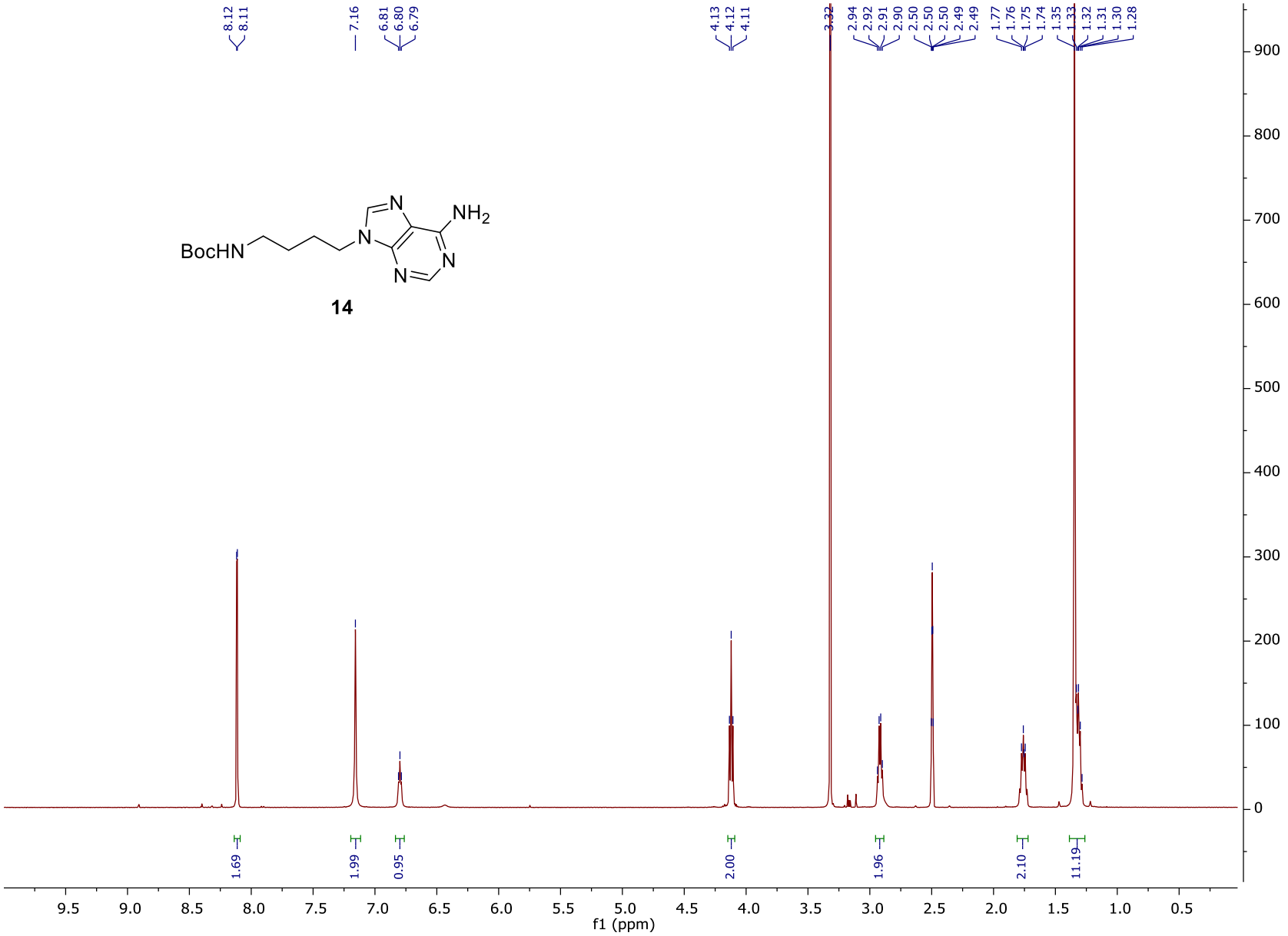
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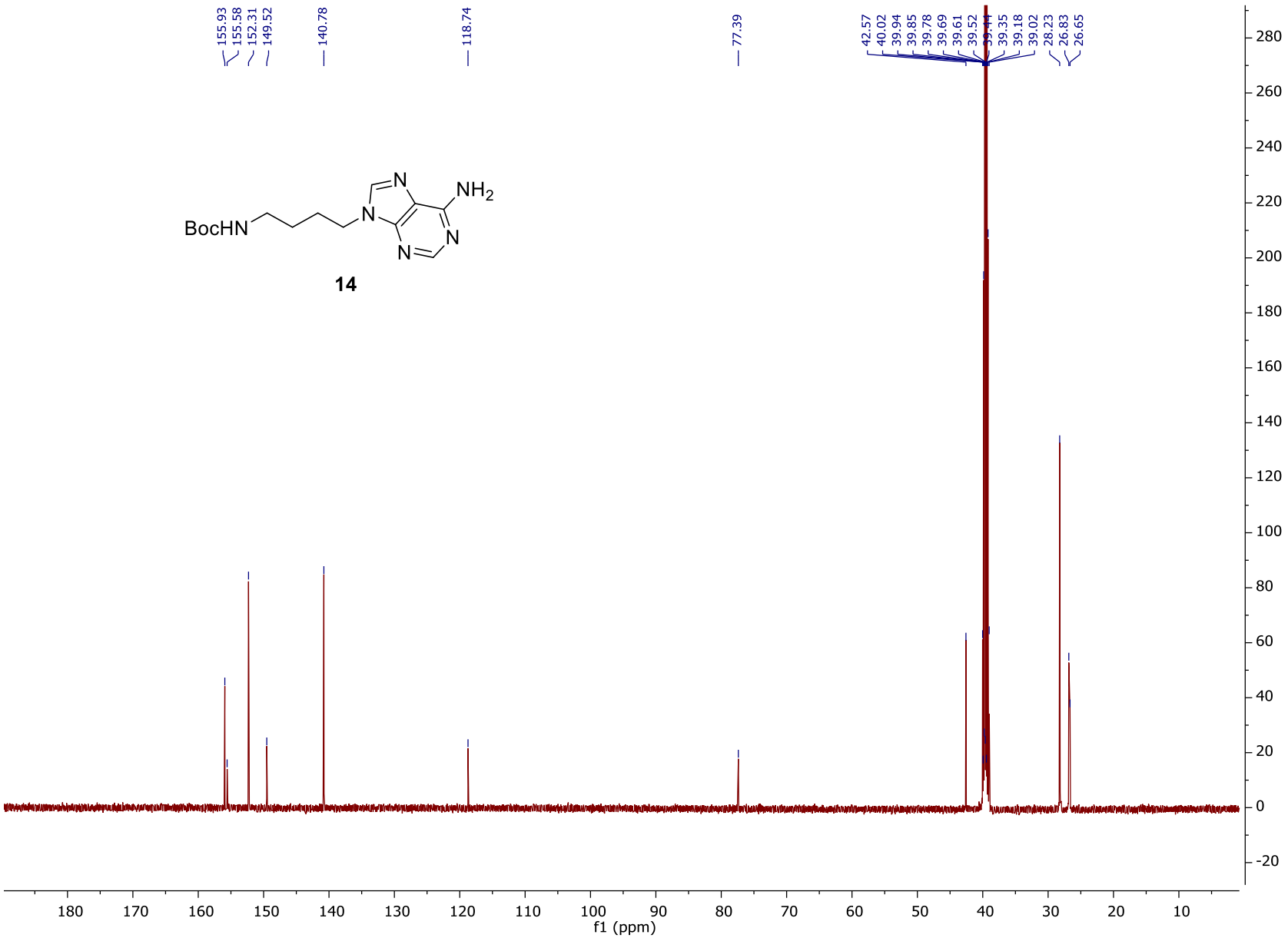
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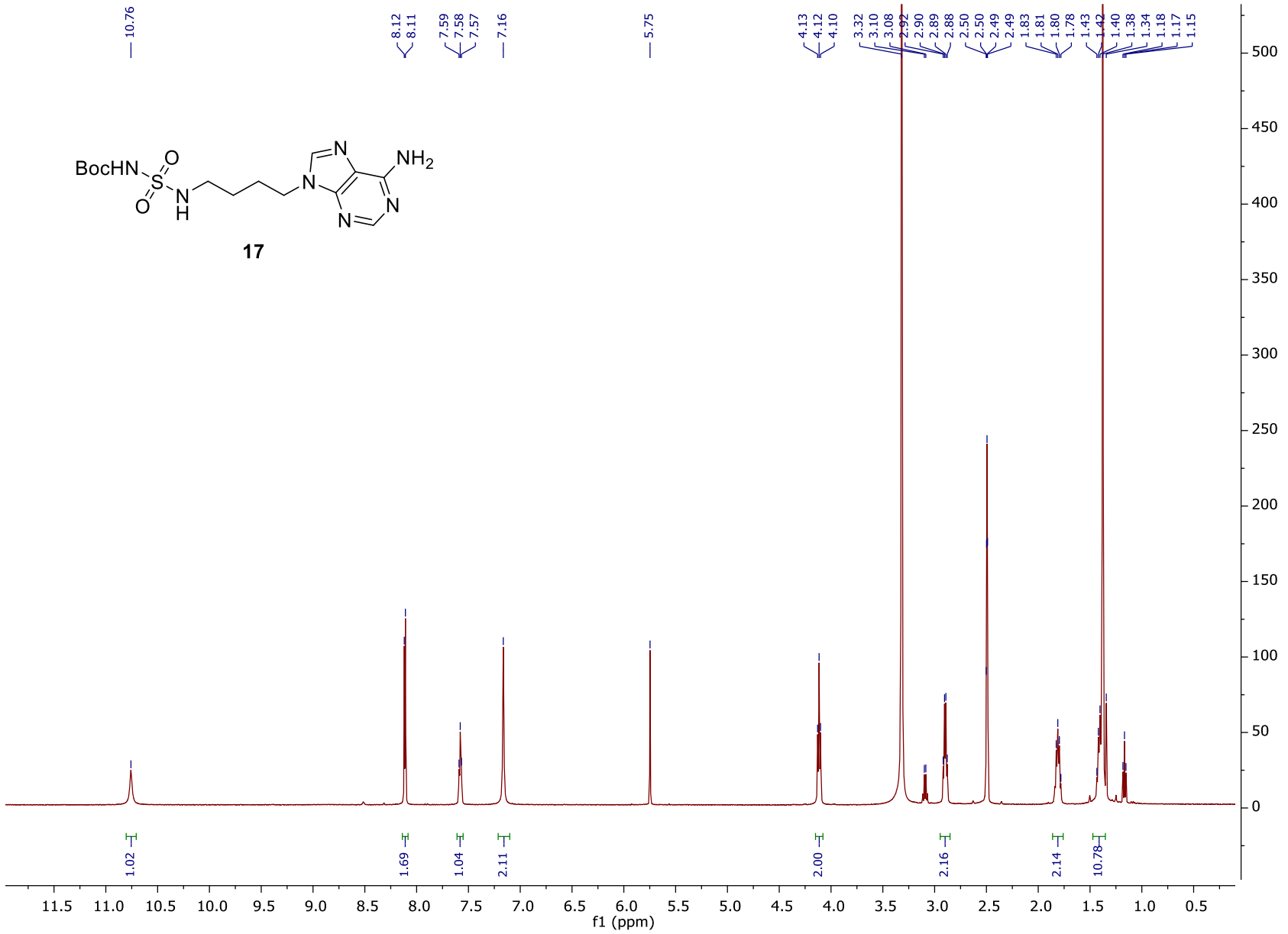
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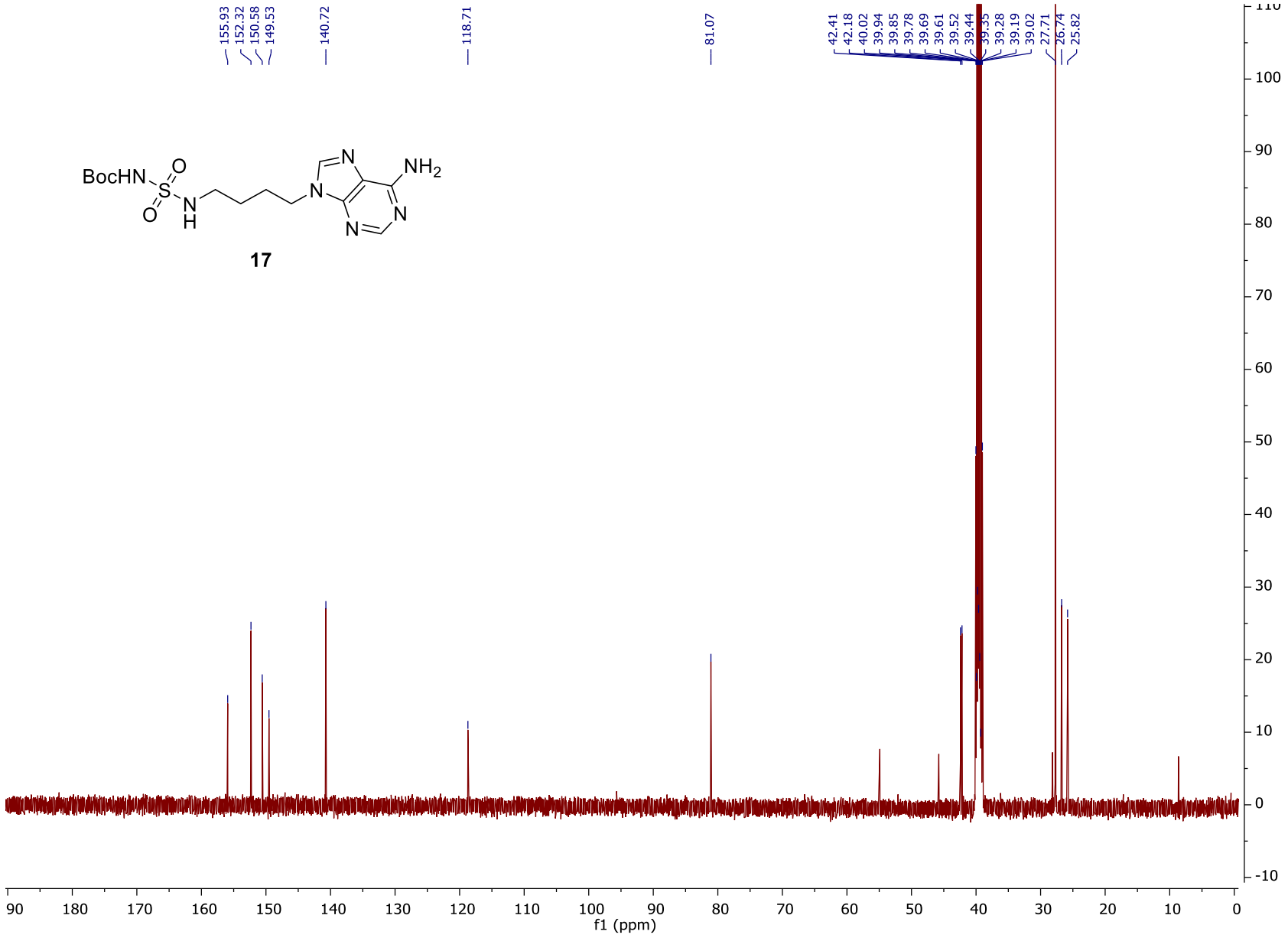
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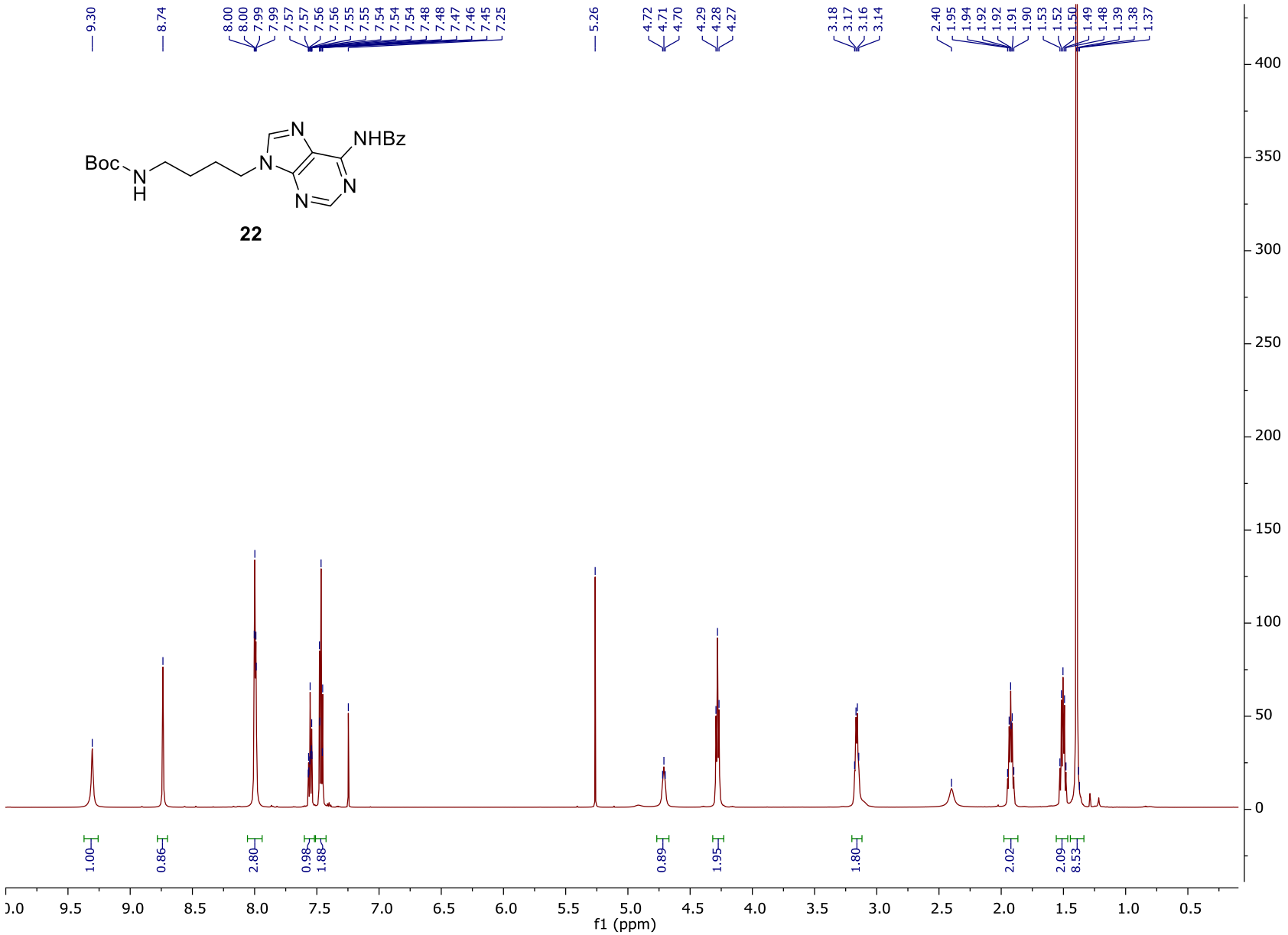
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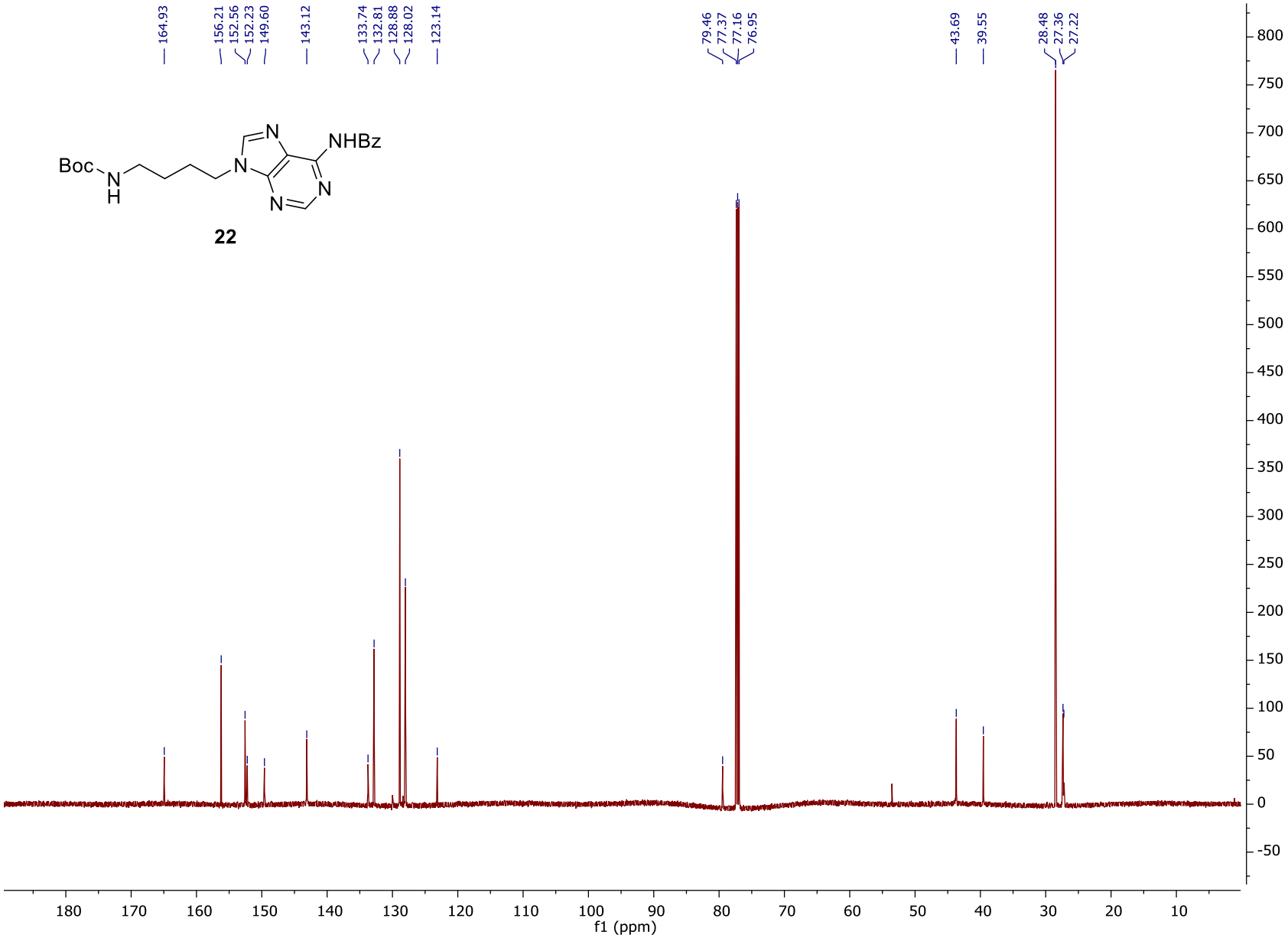
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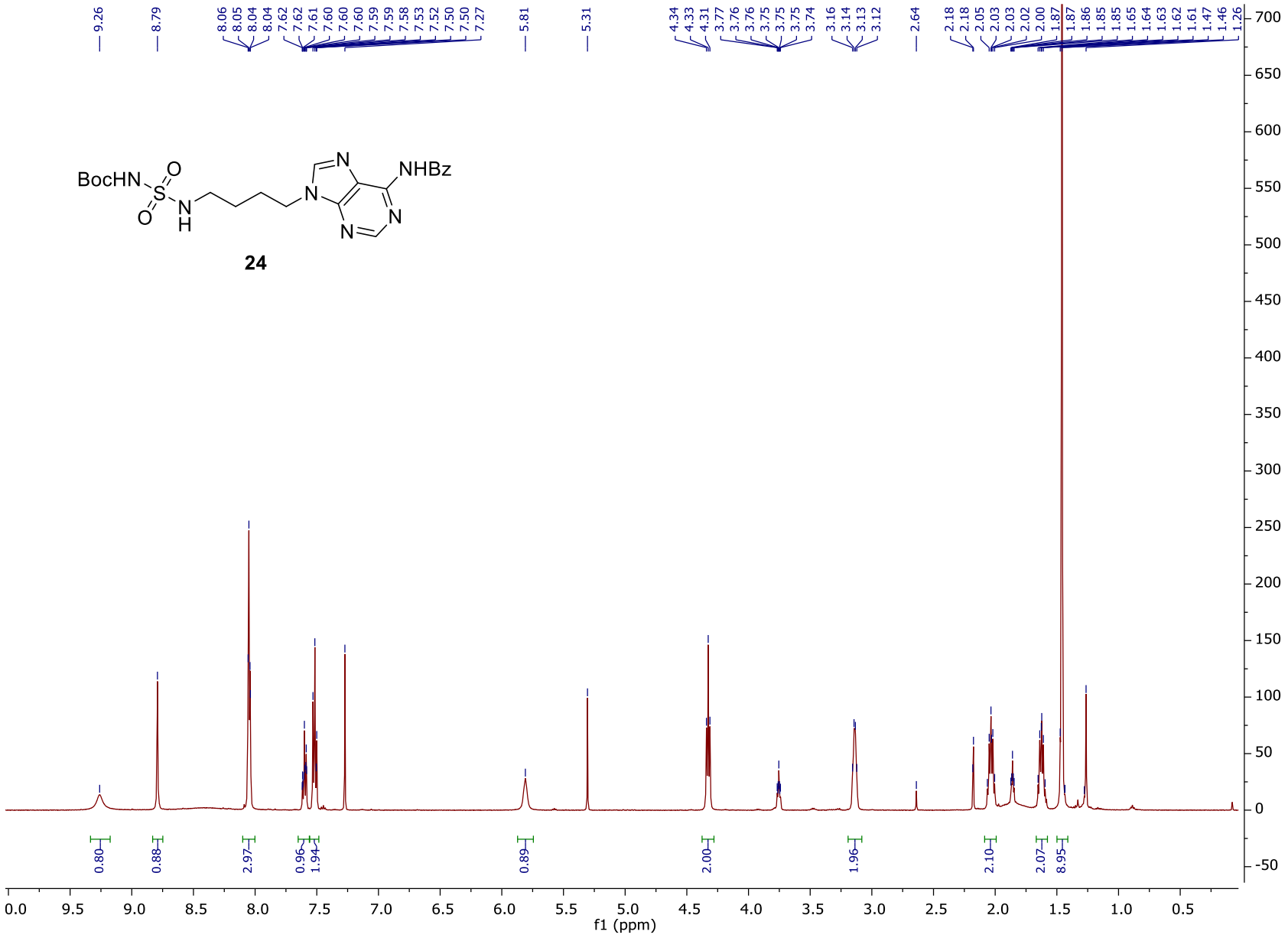
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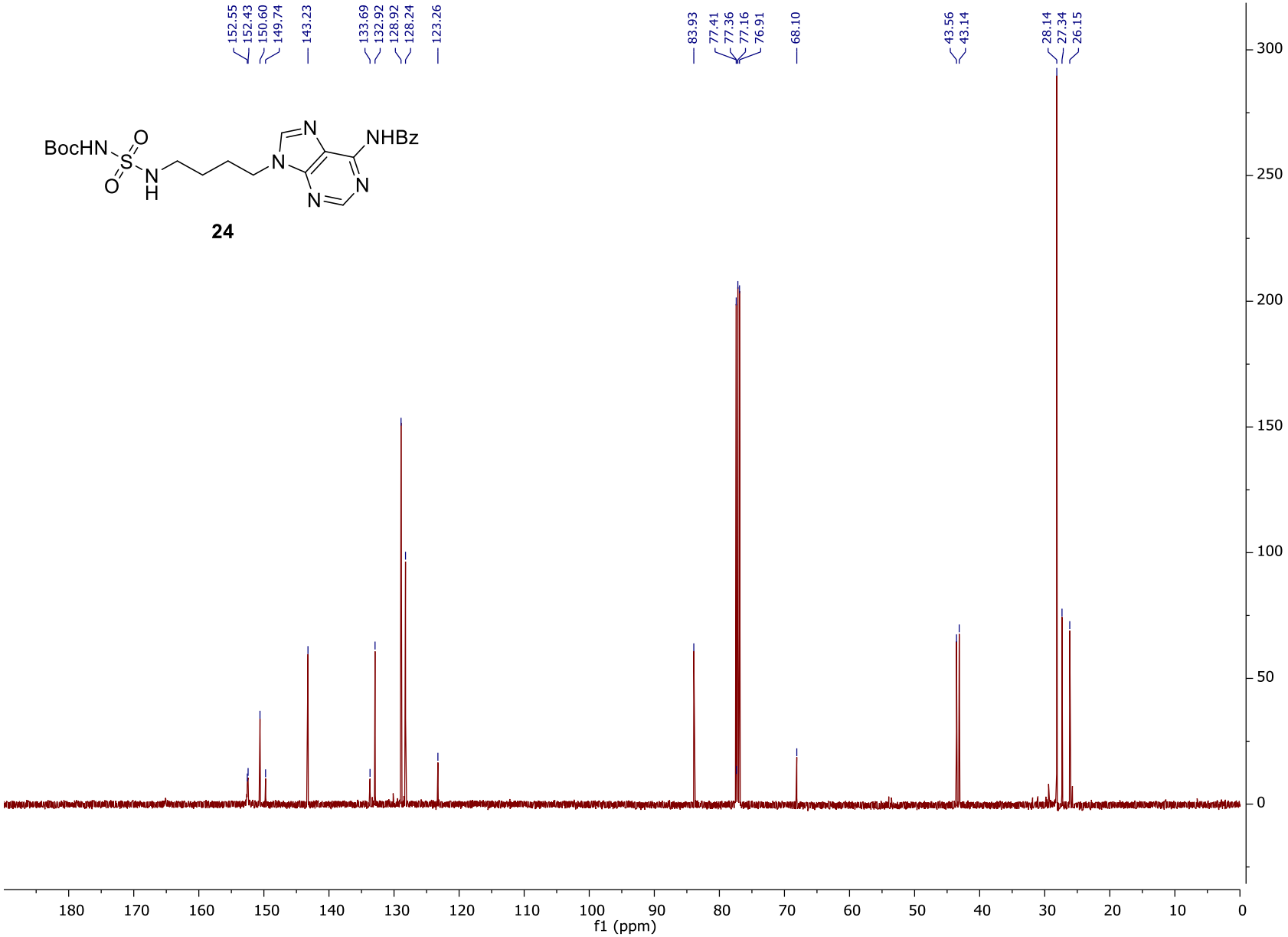
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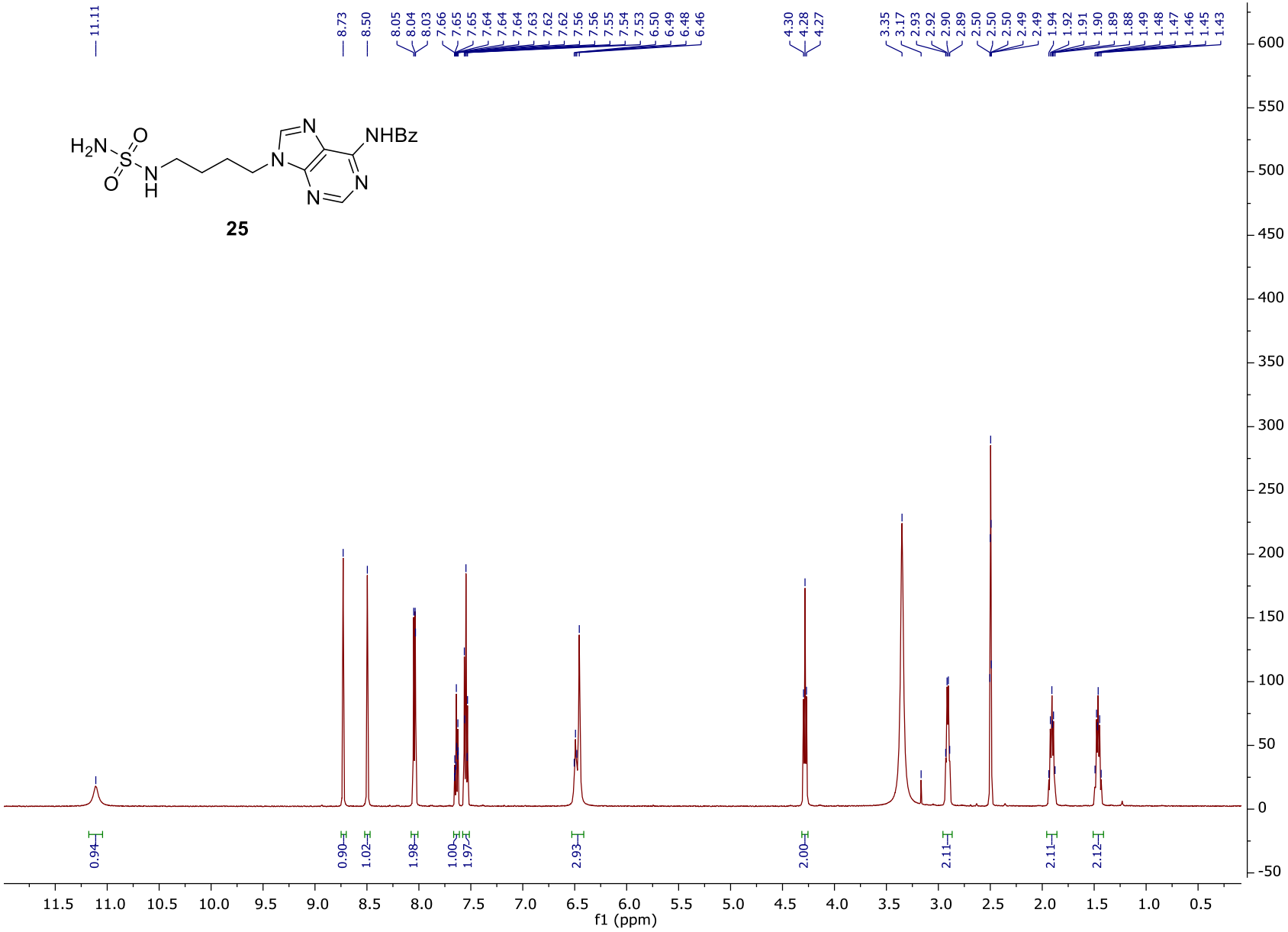
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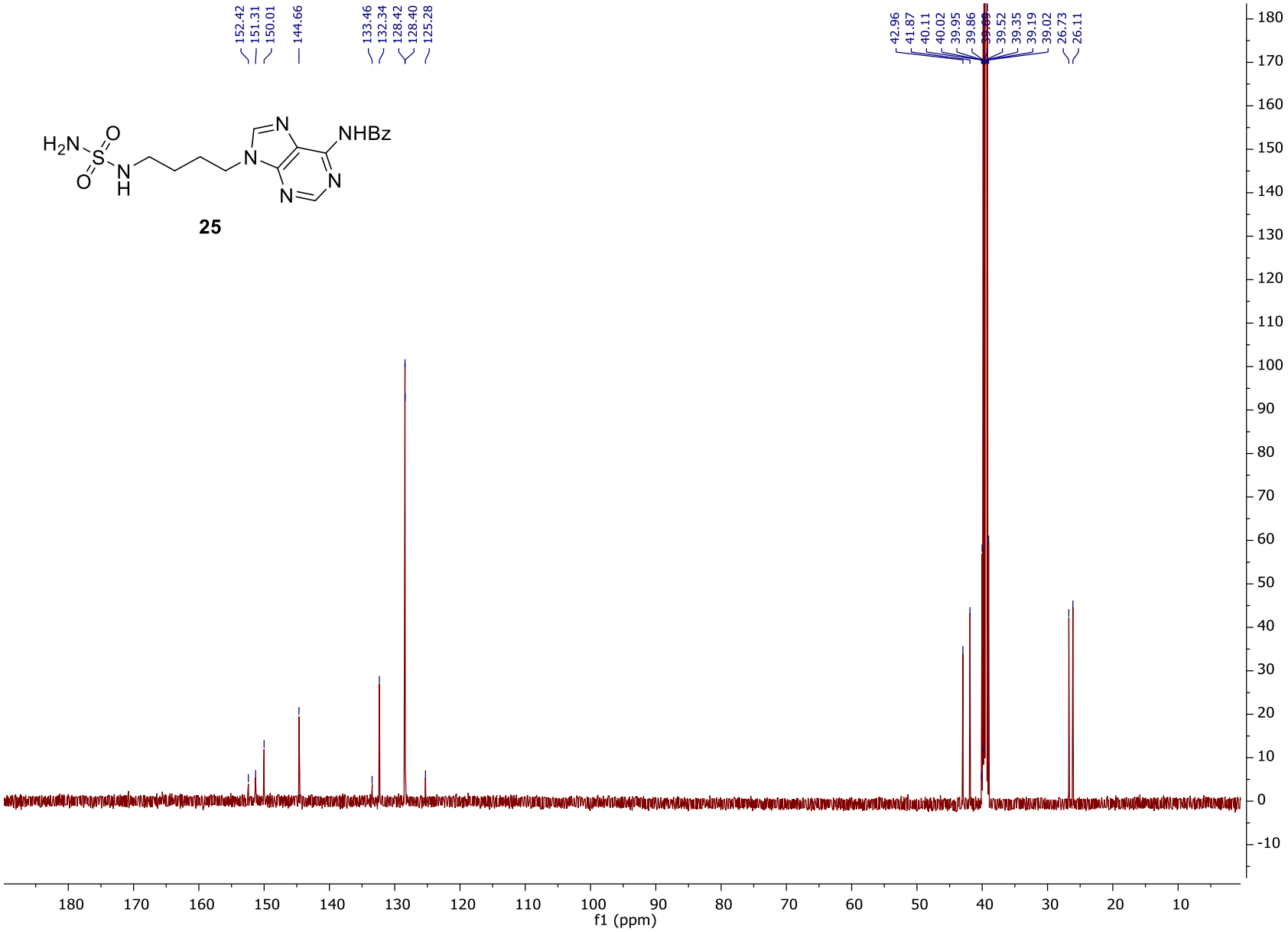
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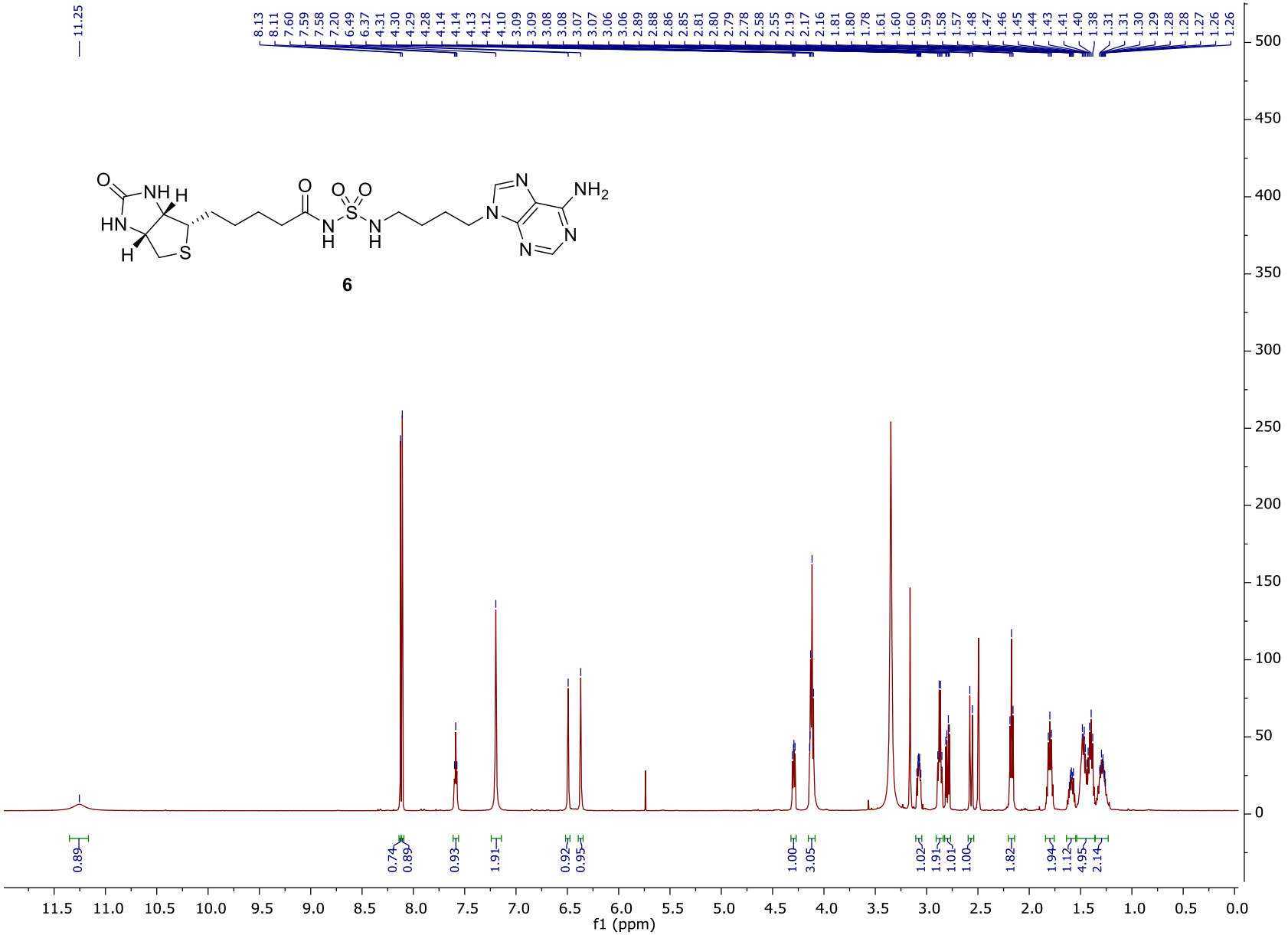
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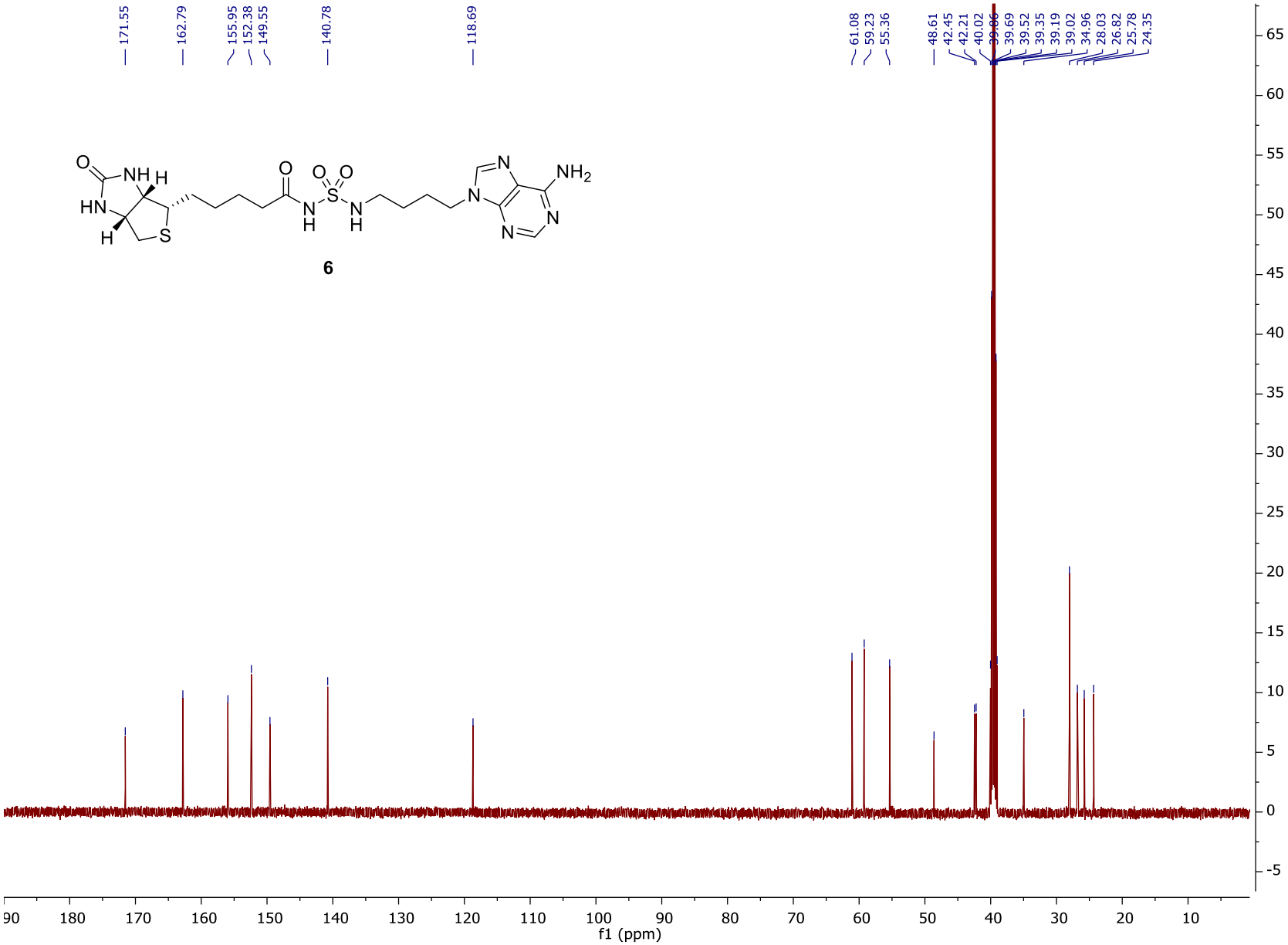
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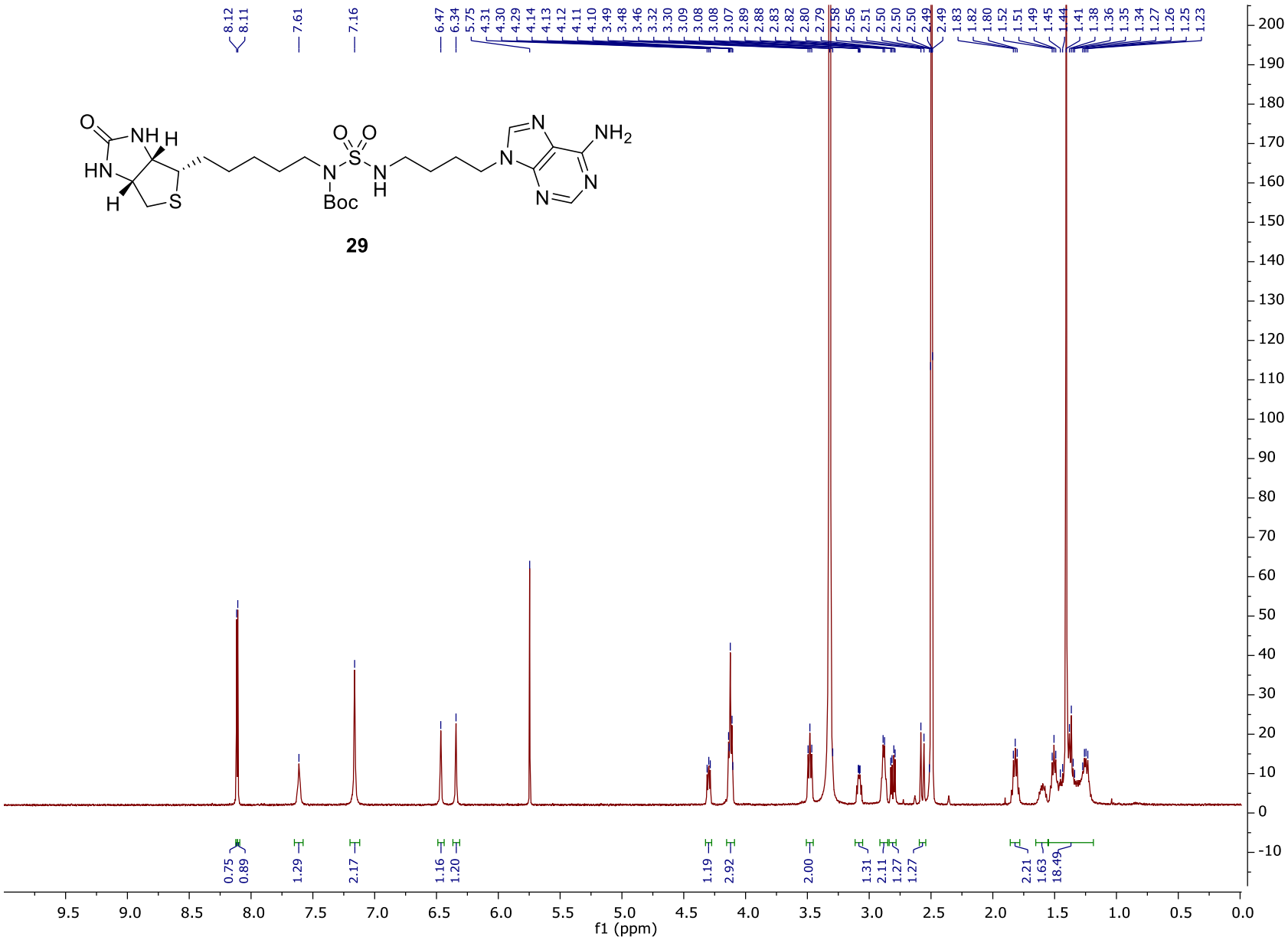
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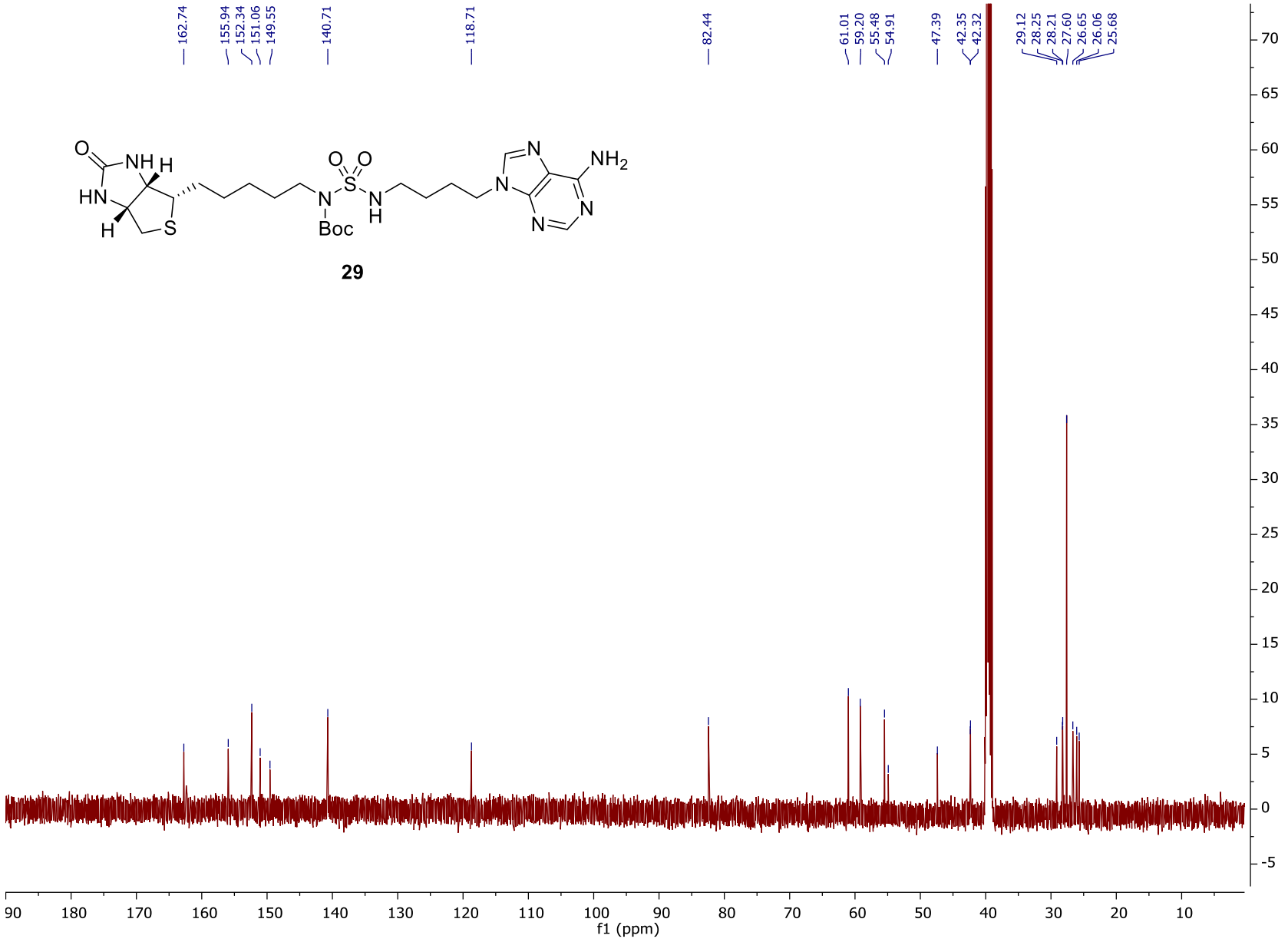
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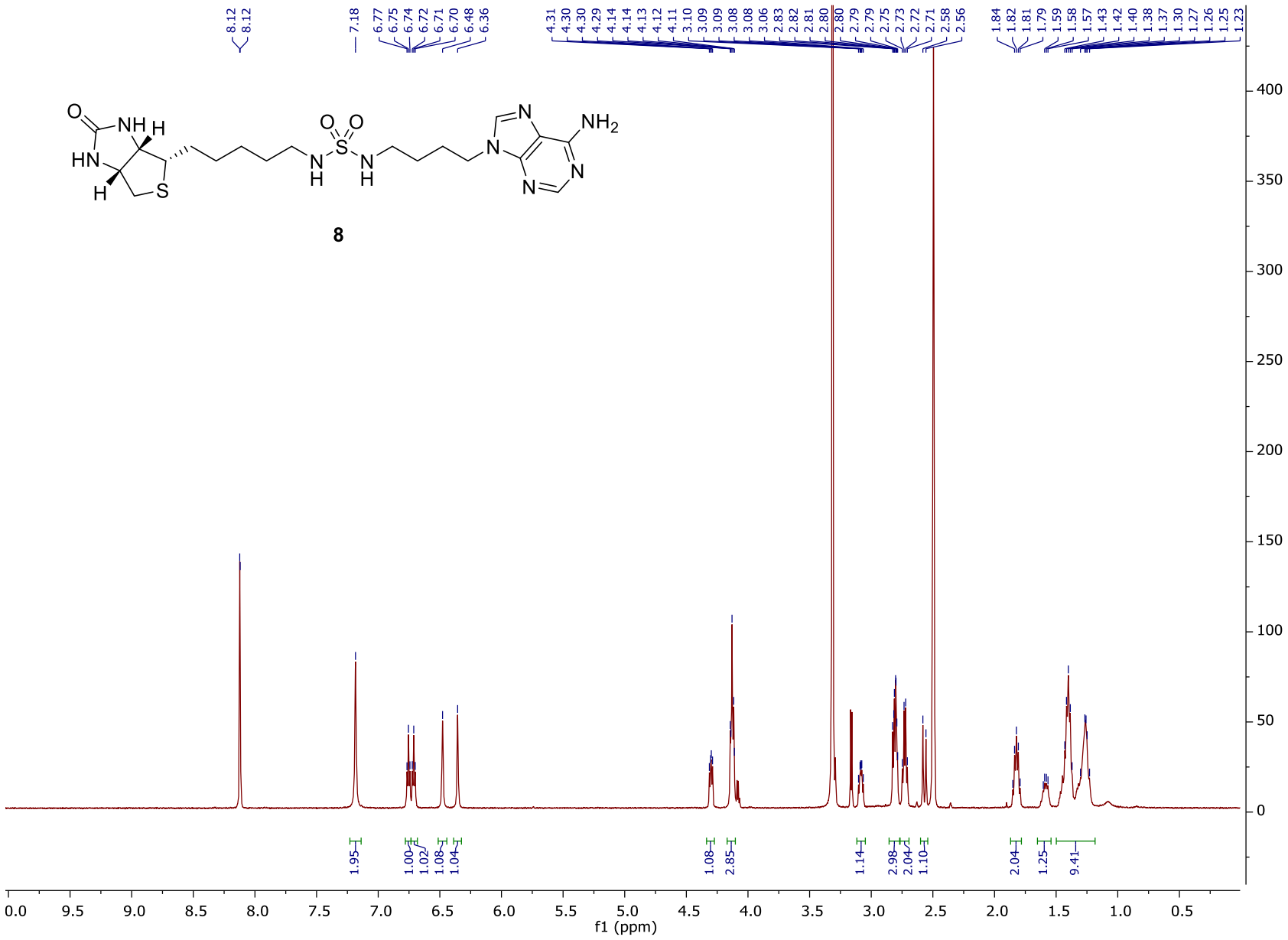
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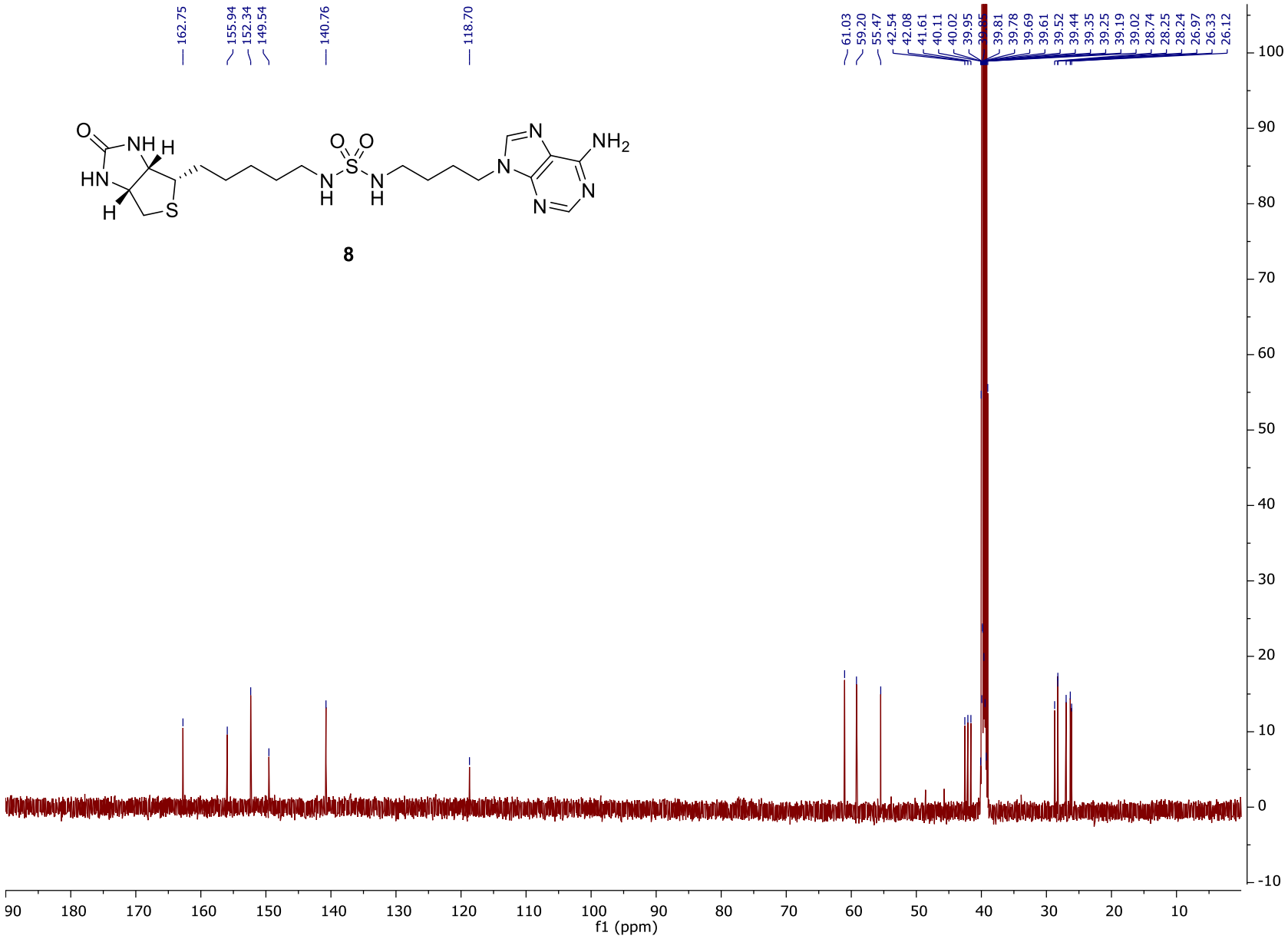
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80
70
60
50
40
30
20
10
0
-10
90
180
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20
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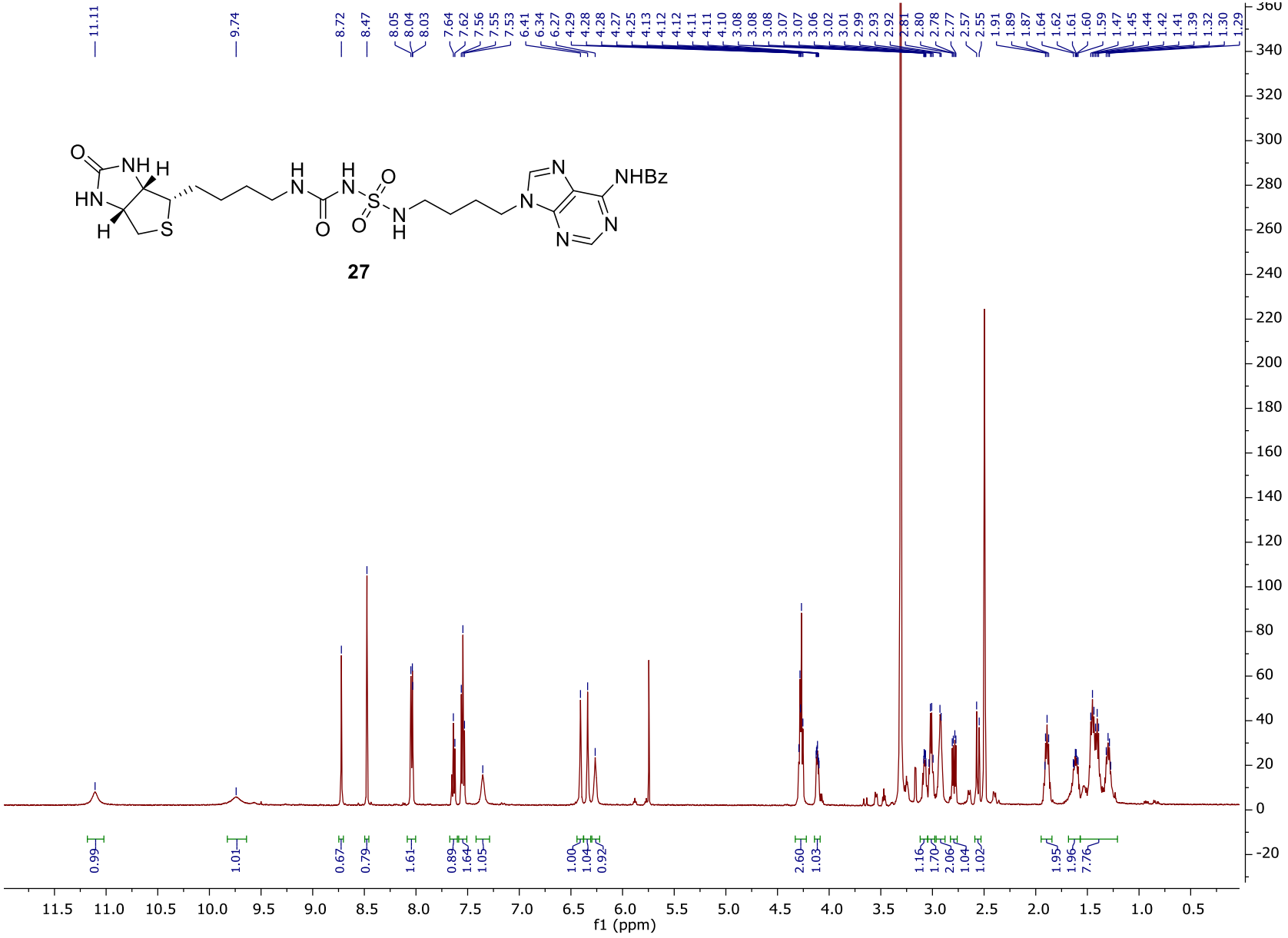
f1 (ppm)

8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6.34
6.27
4.29
4.28
4.28
4.27
4.25
4.13
4.12
4.12
4.11
4.11
4.10
3.08
3.08
3.08
3.07
3.07
3.06
3.02
3.01
2.99
2.93
2.92
2.81
2.80
2.78
2.77
2.57
2.55
1.91
1.89
1.87
1.64
1.62
1.61
1.60
1.59
1.47
1.45
1.44
1.42
1.41
1.39
1.32
1.30
1.29
NHBz
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1.01
0.67
0.79
1.61
0.89
1.64
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1.00
1.04
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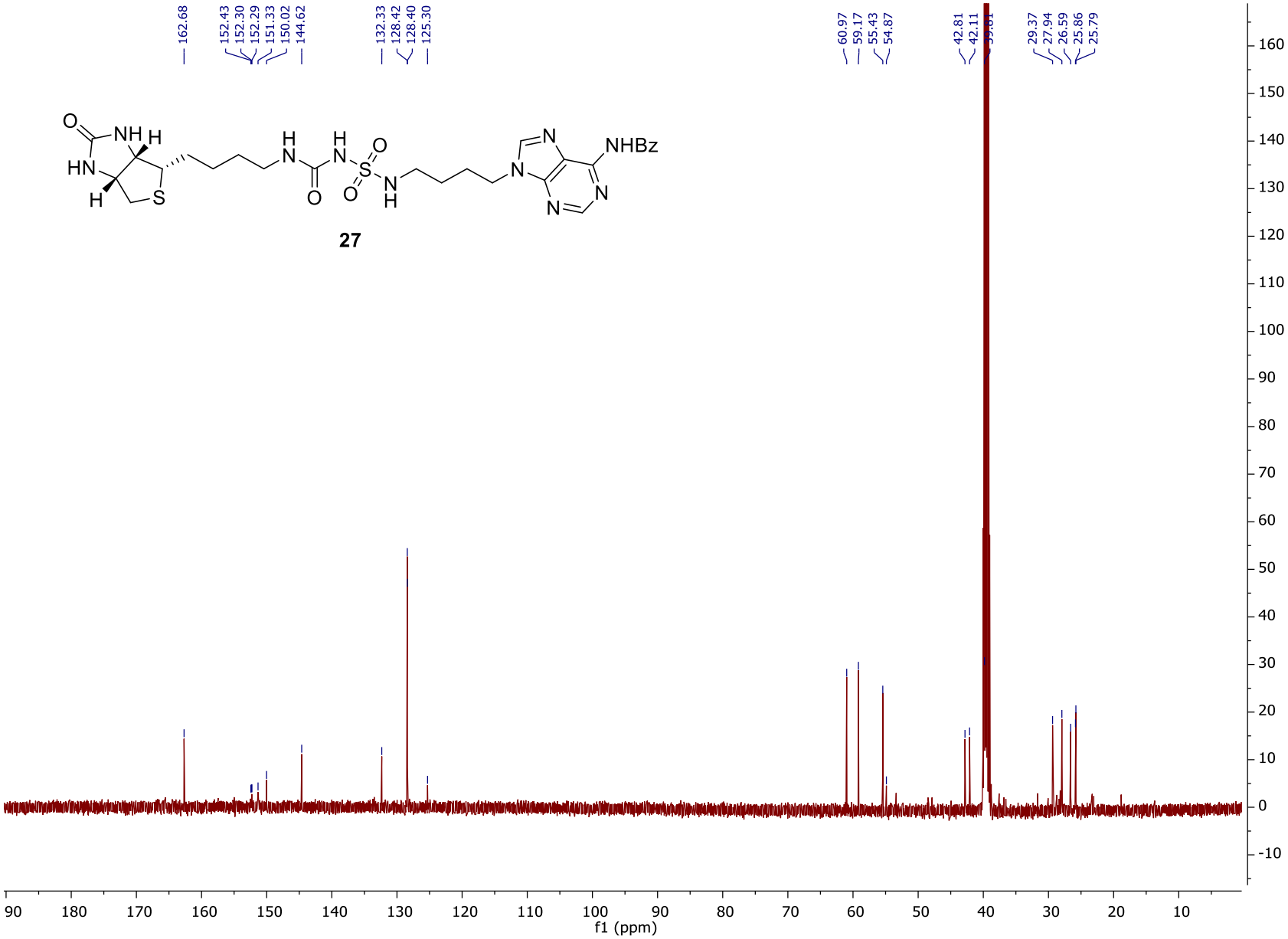
7.76
f1 (ppm)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128.40
125.30
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59.17
55.43
54.87
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39.81
29.37
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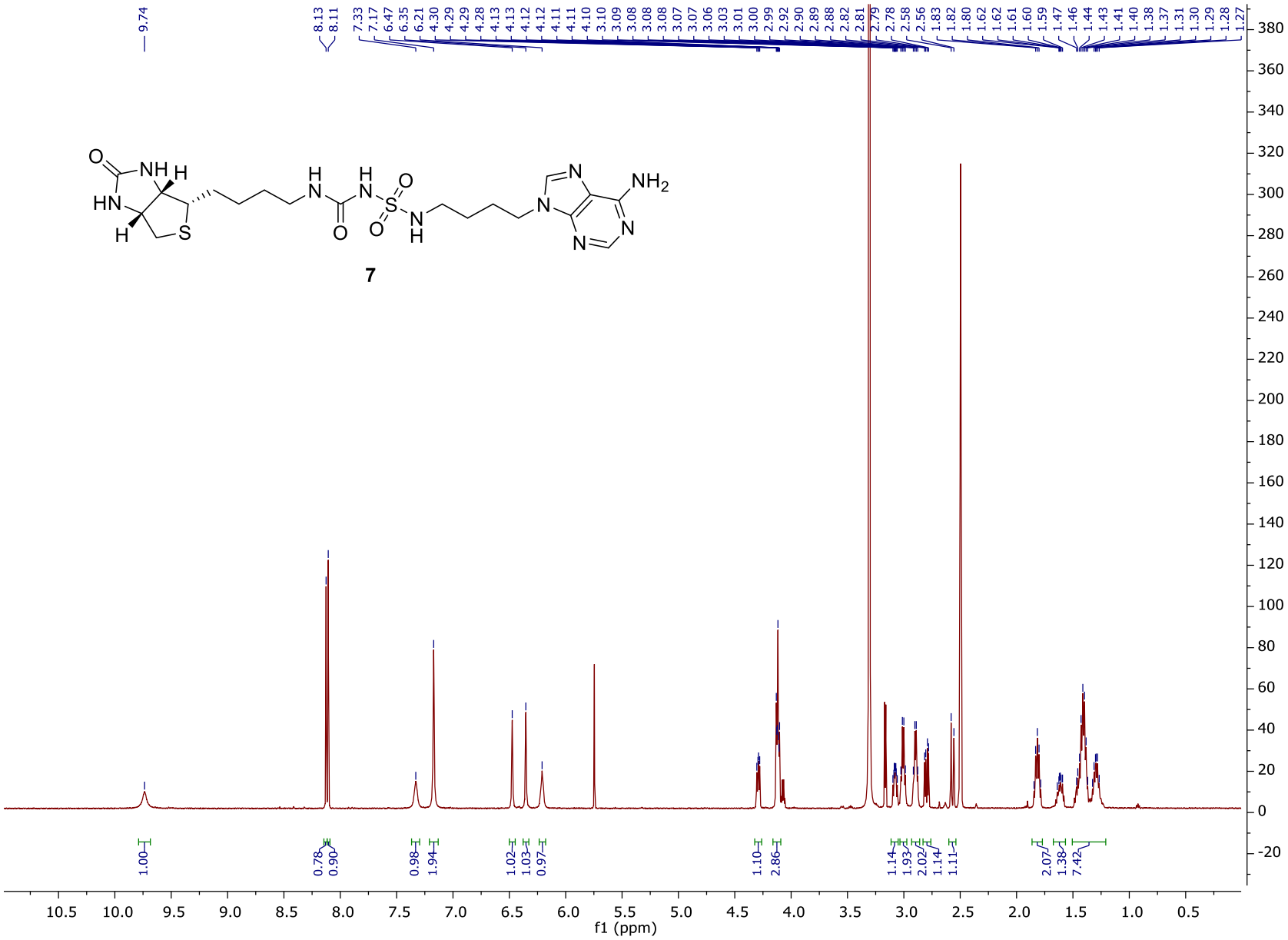
26.59
25.86
25.79
NHBz
27
f1 (ppm)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4.29
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4.12
4.12
4.11
4.11
4.10
3.10
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3.08
3.08
3.08
3.07
3.07
3.06
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3.01
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2.82
2.81
2.79
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2.56
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1.41
1.40
1.38
1.37
1.31
1.30
1.29
1.28
1.27
7
1.00
0.78
0.90
0.98
1.94
1.02
1.03
0.97
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1.14
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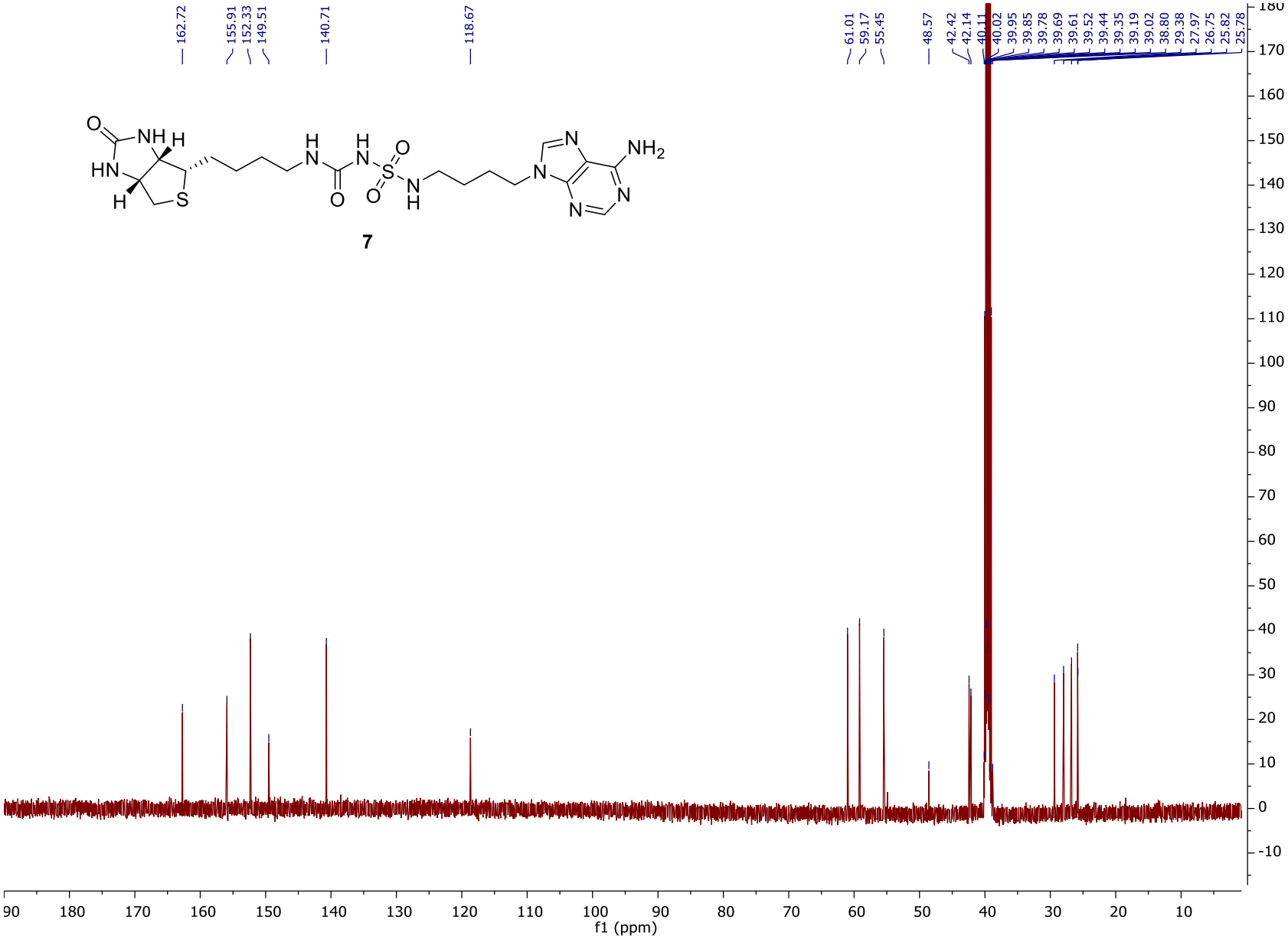
2.07
1.38
7.42
f1 (ppm)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42.42
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40.02
39.95
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39.78
39.69
39.61
39.52
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39.02
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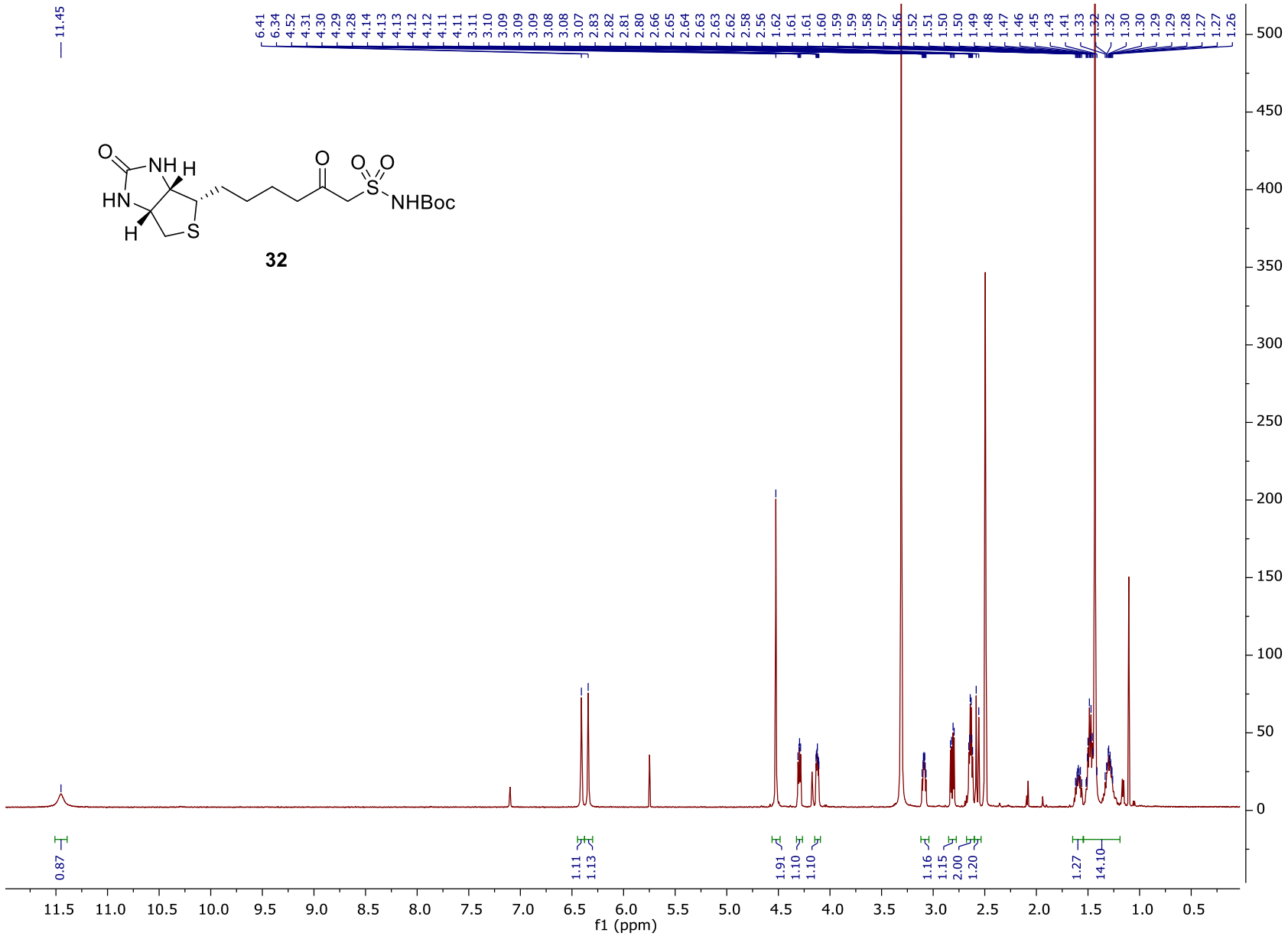
25.78
7
f1 (ppm)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O
NH H
HN
H
S
O
O O
S
NHBoc
32
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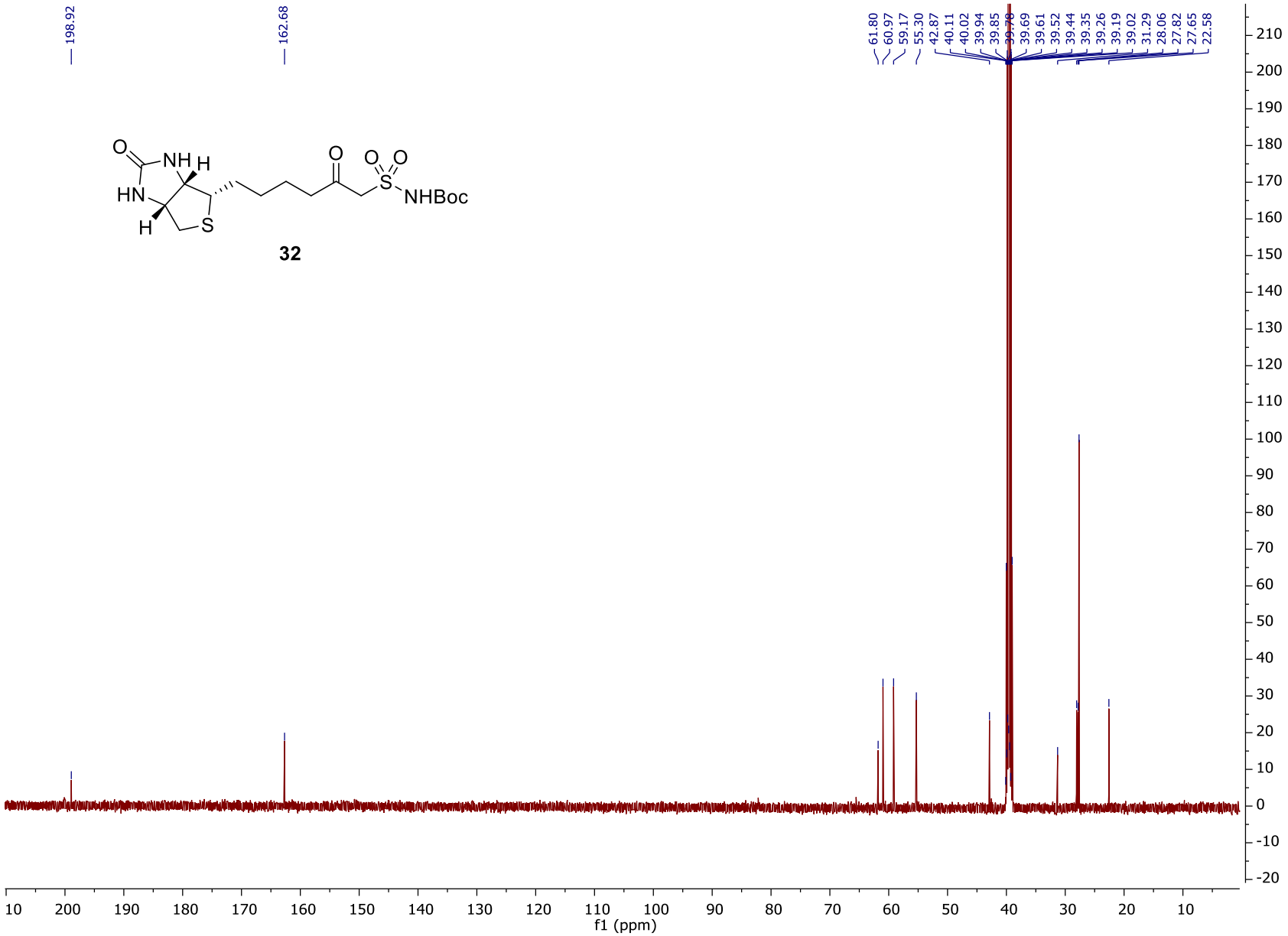
1.11 1.13
1.91 1.10 1.10
1.16 1.15 2.00 1.20
1.27 14.10
f1 (ppm)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162.68
61.80
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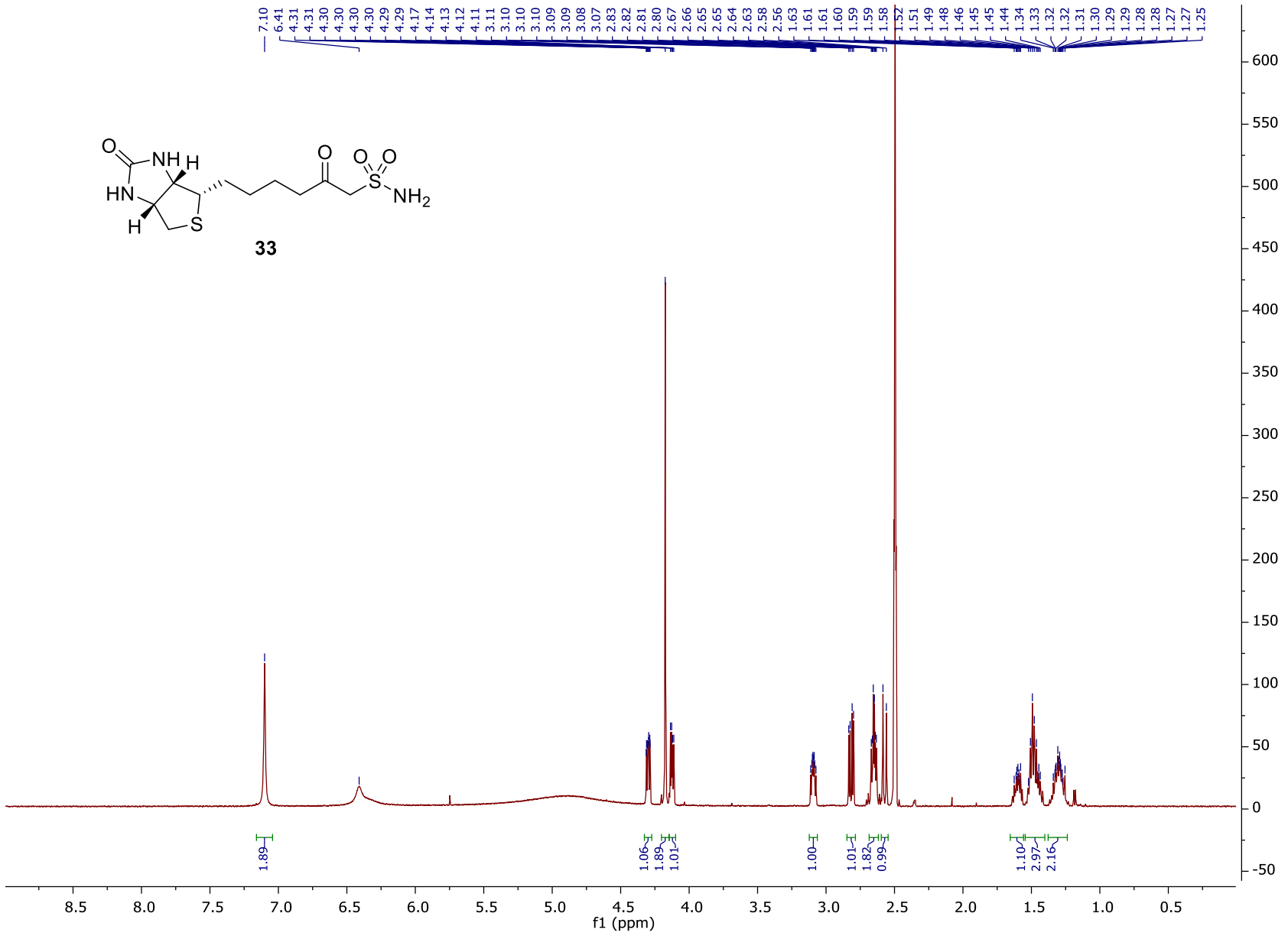
55.30
42.87
40.11
40.02
39.94
39.85
39.78
39.69
39.61
39.52
39.44
39.35
39.26
39.19
39.02
31.29
28.06
27.82
27.65
22.58
O
NH
H
HN
H
S
O
O
O
S
NHBoc
32
f1 (ppm)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4.31
4.31
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4.30
4.30
4.30
4.29
4.29
4.17
4.14
4.13
4.12
4.11
3.11
3.10
3.10
3.10
3.09
3.09
3.08
3.07
2.83
2.82
2.81
2.80
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2.66
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2.65
2.64
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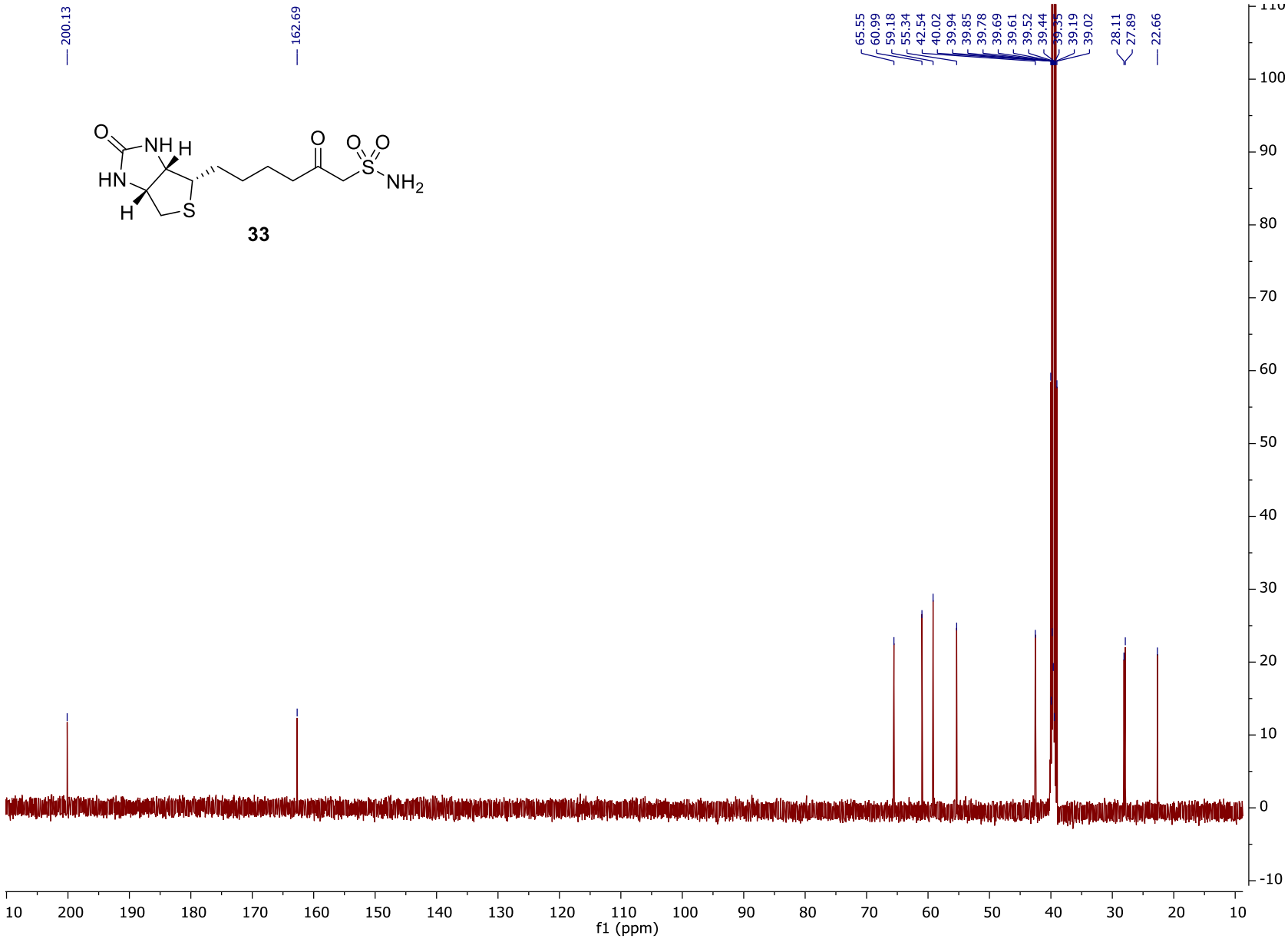
1.59
1.59
1.58
1.52
1.51
1.49
1.48
1.46
1.45
1.45
1.44
1.34
1.33
1.32
1.32
1.31
1.30
1.29
1.29
1.28
1.28
1.27
1.27
1.25
O
NH
H
HN
H
S
O
O
O
S
NH2
33
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
1.89
1.06
1.89
1.01
1.00
1.01
1.82
0.99
1.10
2.97
2.16
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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55.34
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40.02
39.94
39.85
39.78
39.69
39.61
39.52
39.44
39.33
39.19
39.02
28.11
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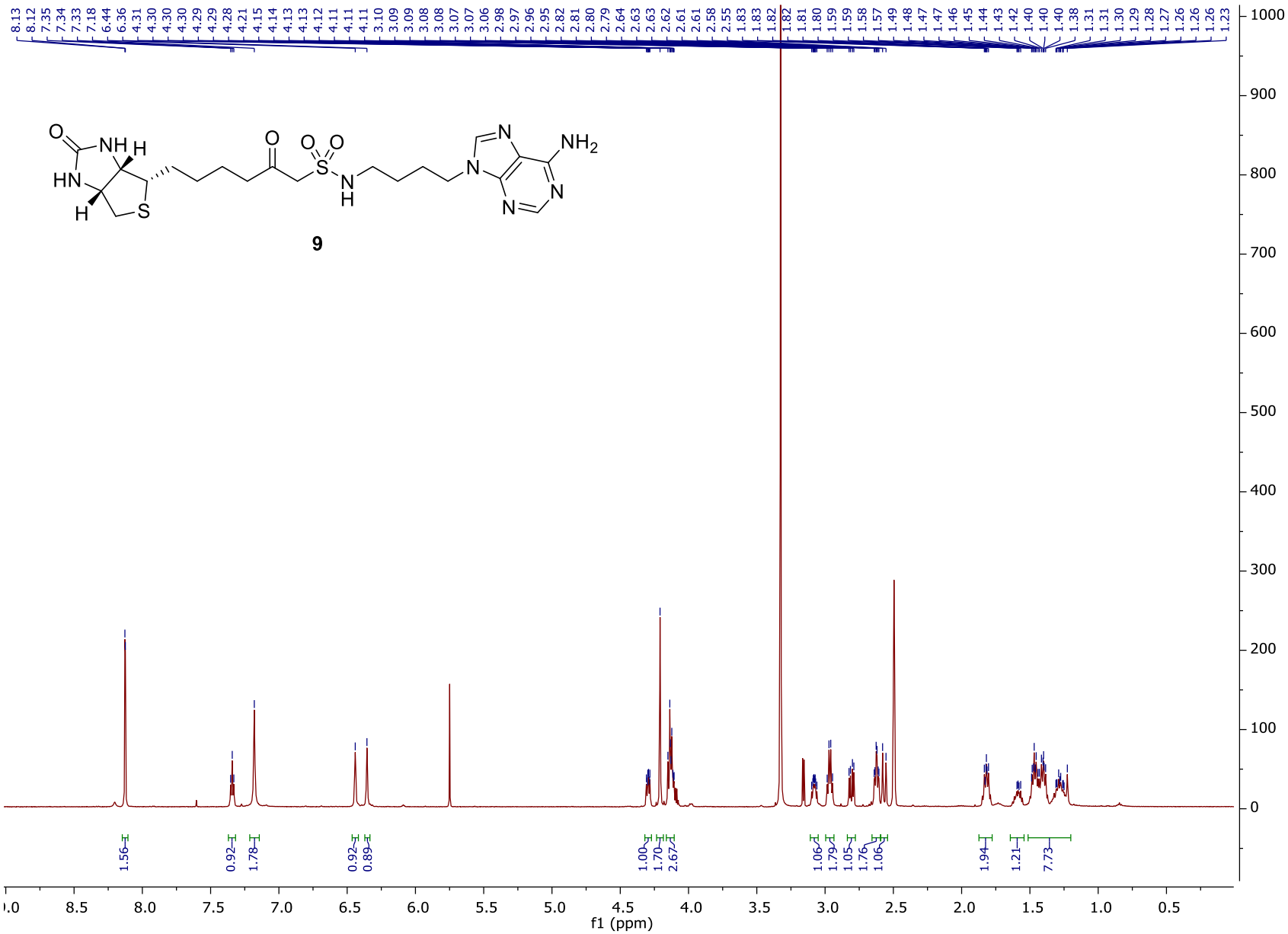
22.66
O
NH
H
HN
H
S
O
O
O
S
NH2
33
f1 (ppm)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O
NH H
HN
H
S
O
O O
S
N
H
N
N
N
NH2
9
1000
900
800
700
600
500
400
300
200
100
0
1.56
0.92
1.78
0.92
0.89
1.00
1.70
2.67
1.06
1.79
1.05
1.76
1.06
1.94
1.21
7.73
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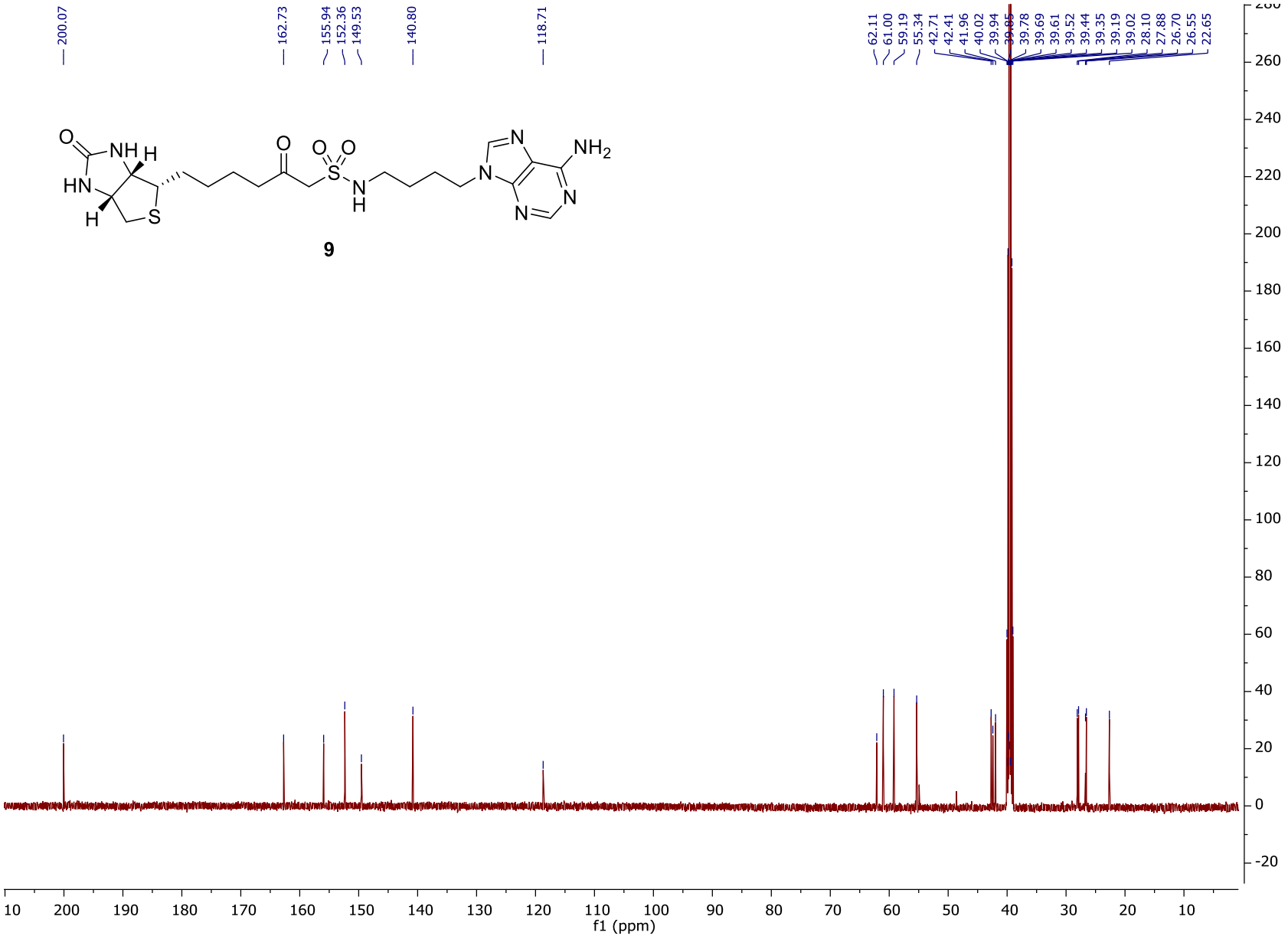
f1 (ppm)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62.11
61.00
59.19
55.34
42.71
42.41
41.96
40.02
39.94
39.88
39.78
39.69
39.61
39.52
39.44
39.35
39.19
39.02
28.10
27.88
26.70
26.55
22.65
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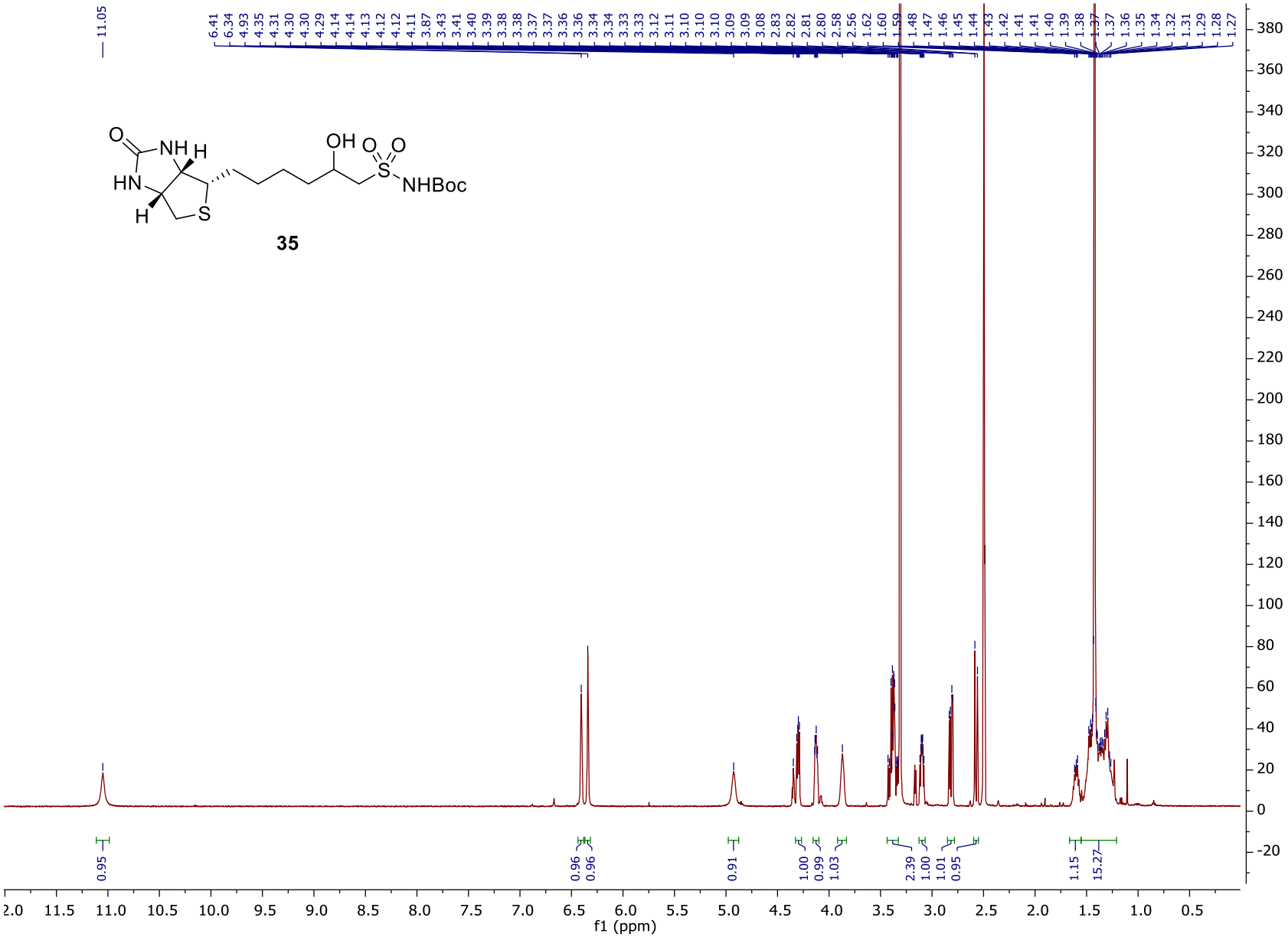
H
HN
H
S
O
O
O
S
N
H
N
N
N
N
NH2
9
f1 (ppm)


11.05
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O
NH H
HN
H
S
OH
O O
S
NHBoc
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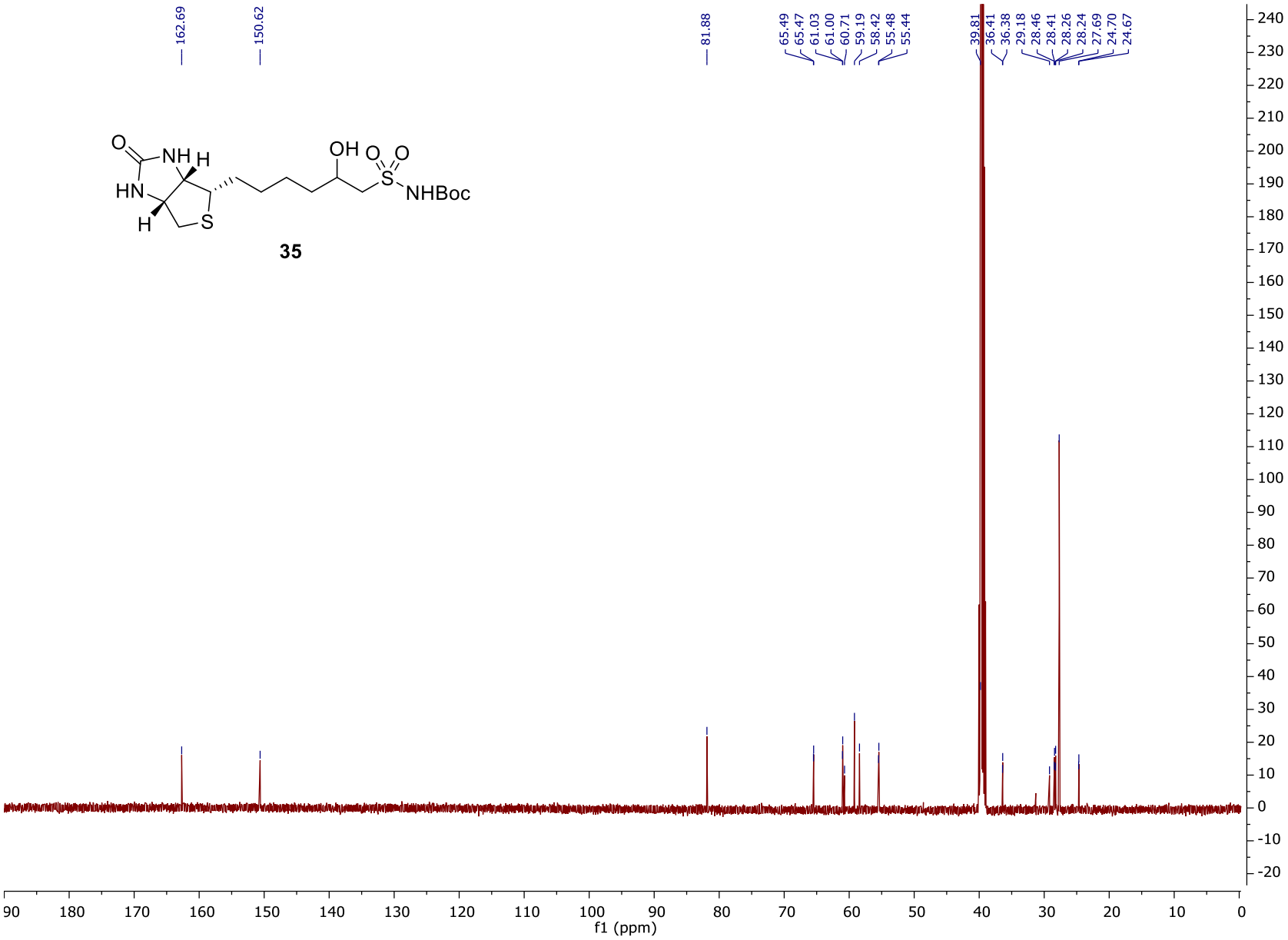
0.95
0.96 0.96
0.91
1.00 0.99 1.03
2.39 1.00 1.01 0.95
1.15 15.27
f1 (ppm)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60.71
59.19
58.42
55.48
55.44
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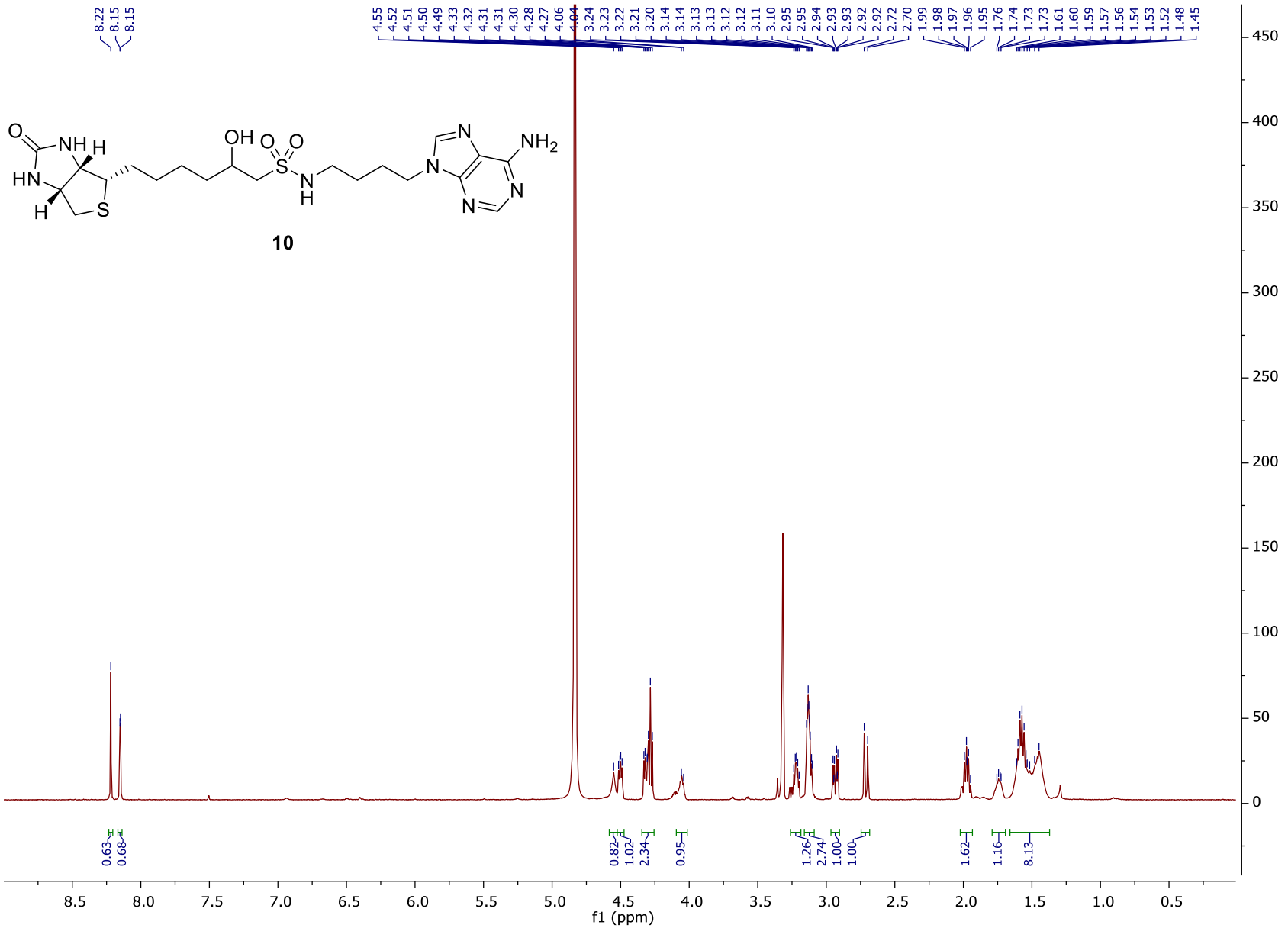
36.41
36.38
29.18
28.46
28.41
28.26
28.24
27.69
24.70
24.67
O
NH H
HN
H
S
OH
O
O
S
NHBoc
35
f1 (ppm)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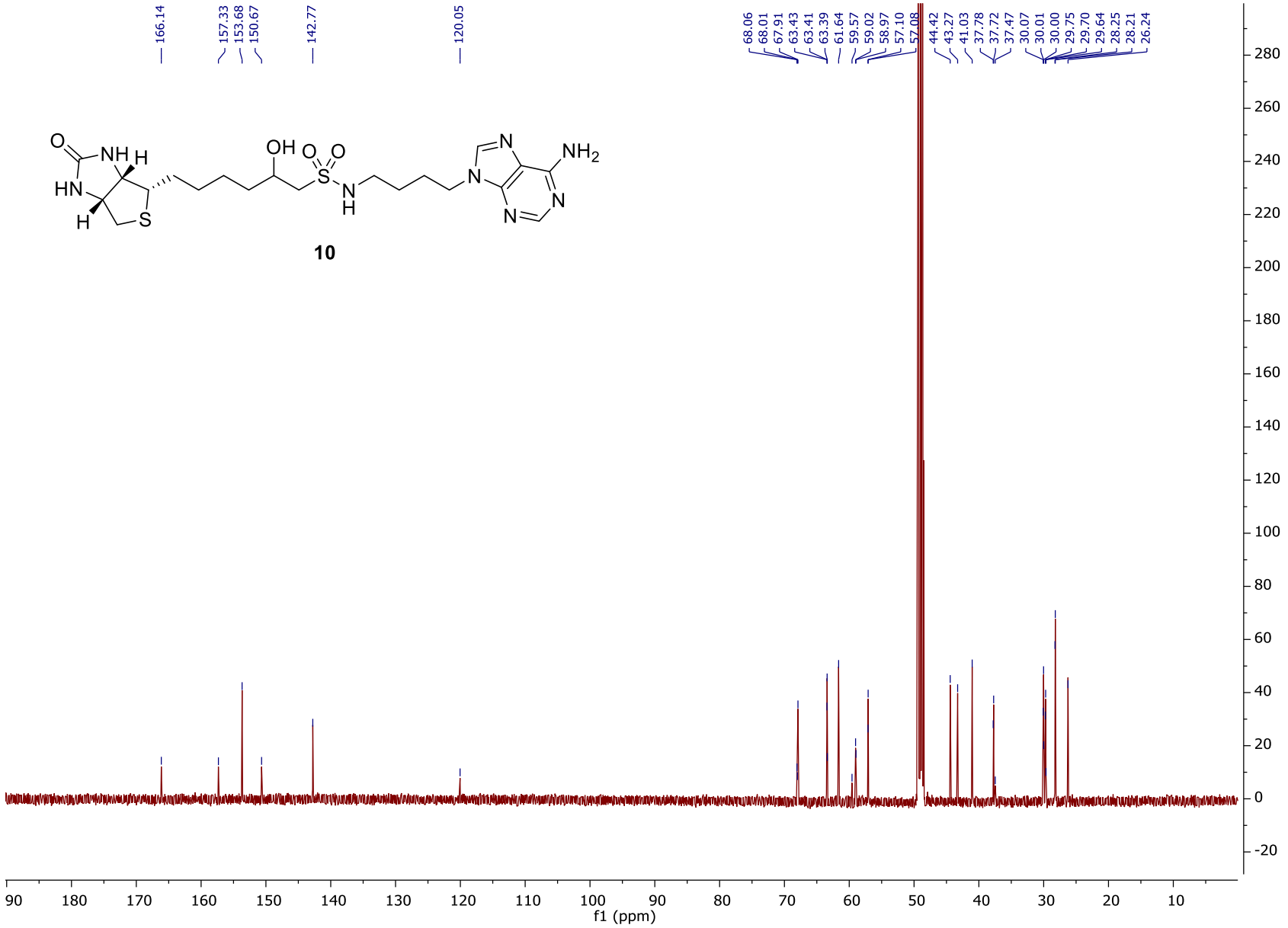
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157.33
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150.67
142.77
120.05
68.06
68.01
67.91
63.43
63.41
63.39
61.64
59.57
59.02
58.97
57.10
57.08
44.42
43.27
41.03
37.78
37.72
37.47
30.07
30.01
30.00
29.75
29.70
29.64
28.25
28.21
26.24
f1 (ppm)

10

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