

Supporting Information

S-adenosylhomocysteine analogs selectively suppress pan-coronavirus replication by inhibition of nsp14 methyltransferase

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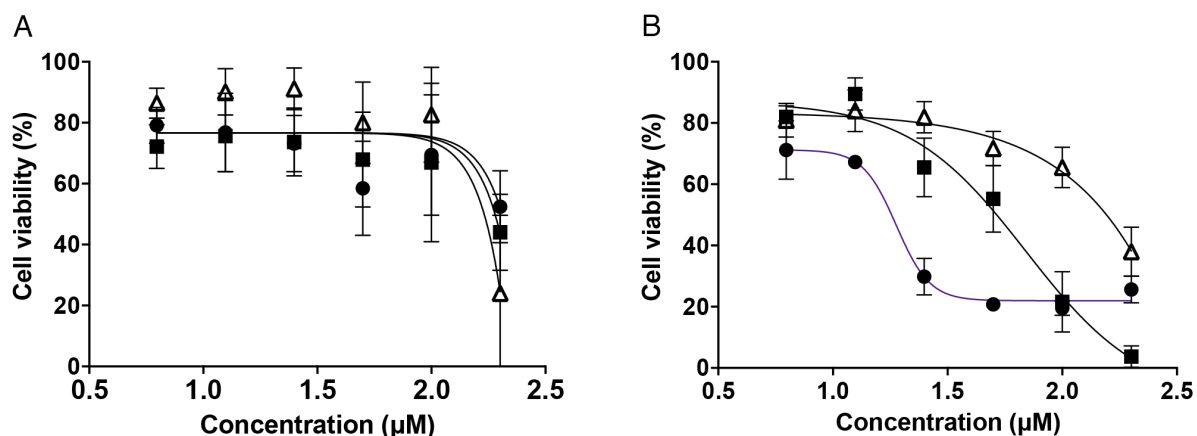
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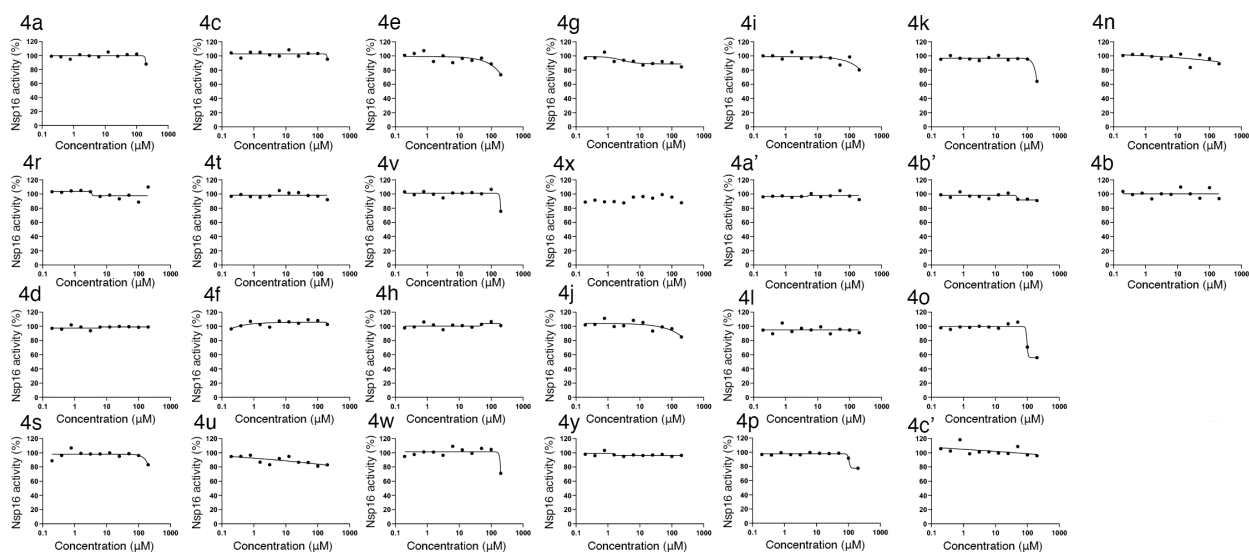
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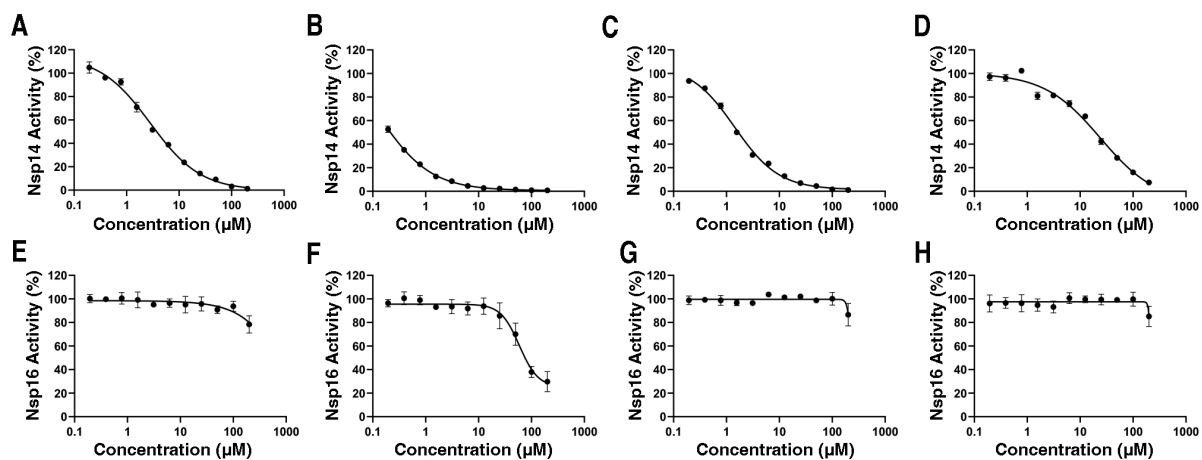
1. Supporting Figures 1-8



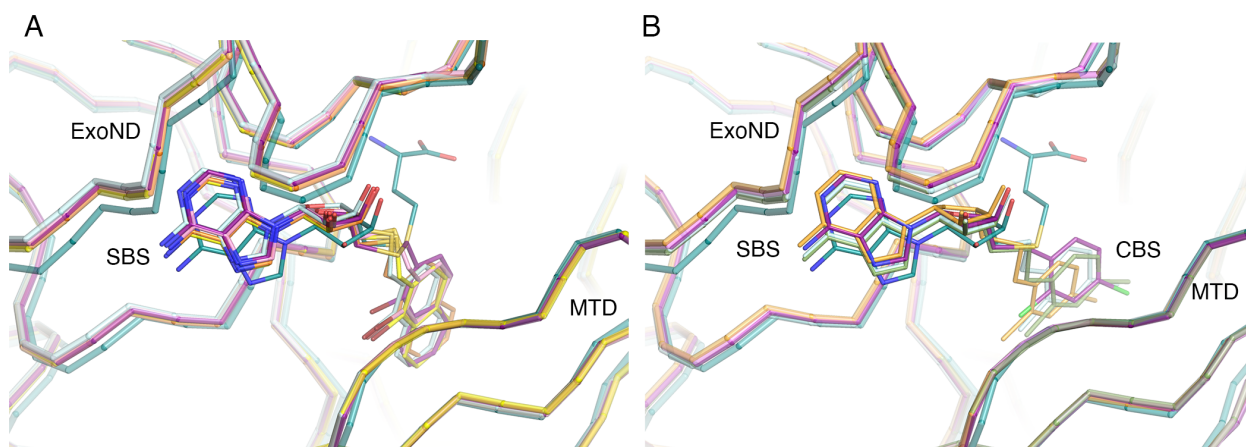
Supporting Figure 1. Compound 4h is more cytotoxic than 4p for Calu-3 cells. The plots show the mean and standard deviation of the dose-response curve to calculate the CC_{50} for (A) L2 and (B) Calu-3 cells. The cells were treated with the compounds **4h** (circles) or **4p** (squares) at the indicated concentrations. Remdesivir was used as a positive (white triangles), and DMSO as a negative control to normalize to 100% infection. The plots show the mean and standard deviation of the dose-response curve to calculate the CC_{50} , $n=4$.



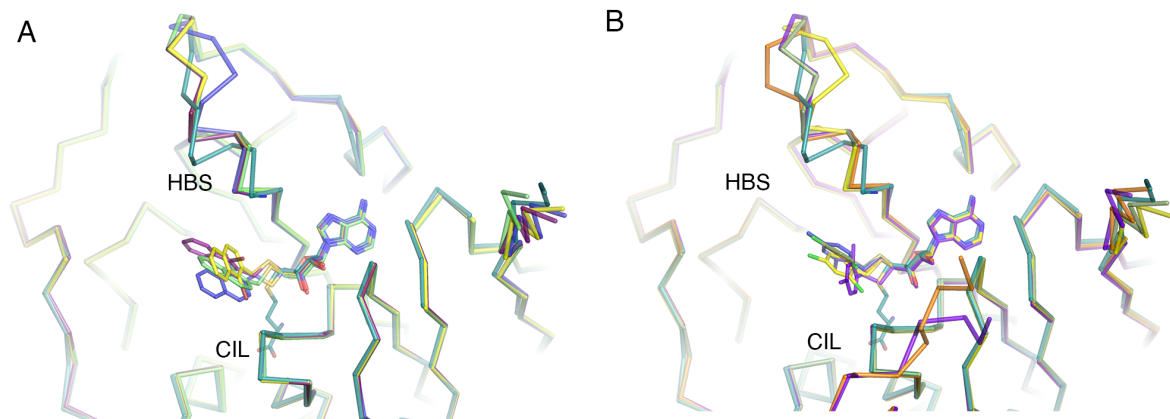
Supporting Figure 2. Compound 4p inhibits nsp16. The plots show a dose-response curve to determine the IC_{50} of using the compounds as indicated.



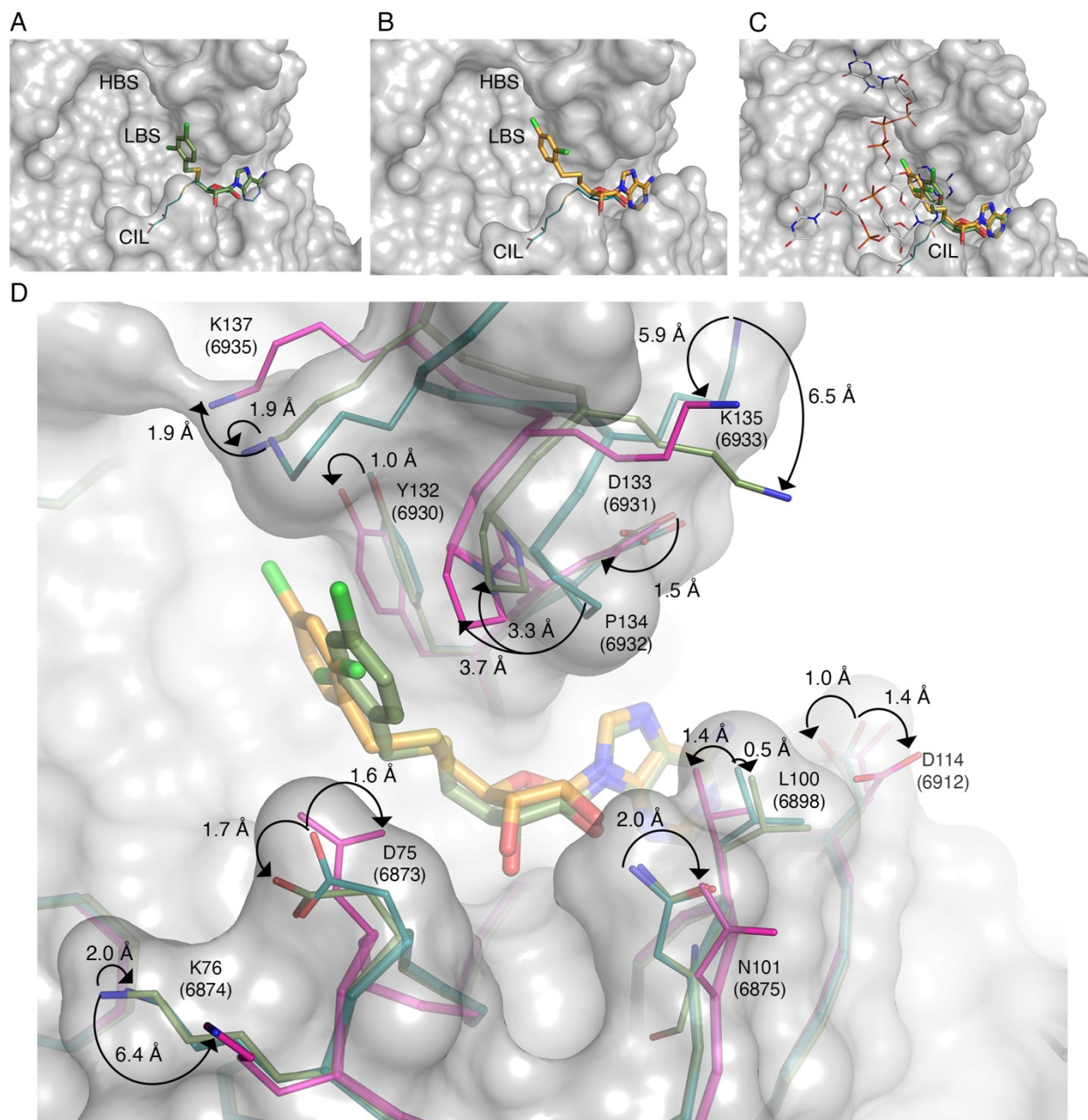
Supporting Figure 3. Compounds 4h and 4p inhibit nsp14 activity preferentially. The plots show a dose-response curve to determine the IC_{50} of nsp14 and nsp16 using the compounds **4h** (A and E), **4p** (B, F), **4v** (C and G) and **4x** (D and H).



Supporting Figure 4. AF3 predictions for nsp14 and compounds 4p and 4h. The superposition of the nsp14/SAH crystal structure (7r2v) with AF3 models for compound **4p** (A) and **4h** (B). Protein chains are represented as ribbons and compounds as sticks with carbons and protein chains colored in deep teal for 7r2v, yellow for the best model, pink for the model_1, cyan for the model_2, orange for the model_3, and purple for the model_4. Atoms are colored: oxygen (red), sulfur (yellow), nitrogen (blue), bromine (deep red), and chlorine (bright green). Structurally important parts of nsp14 are labeled as ExoND for Exonuclease domain, SBS for SAM binding site, and MTD for Methyltransferase domain.



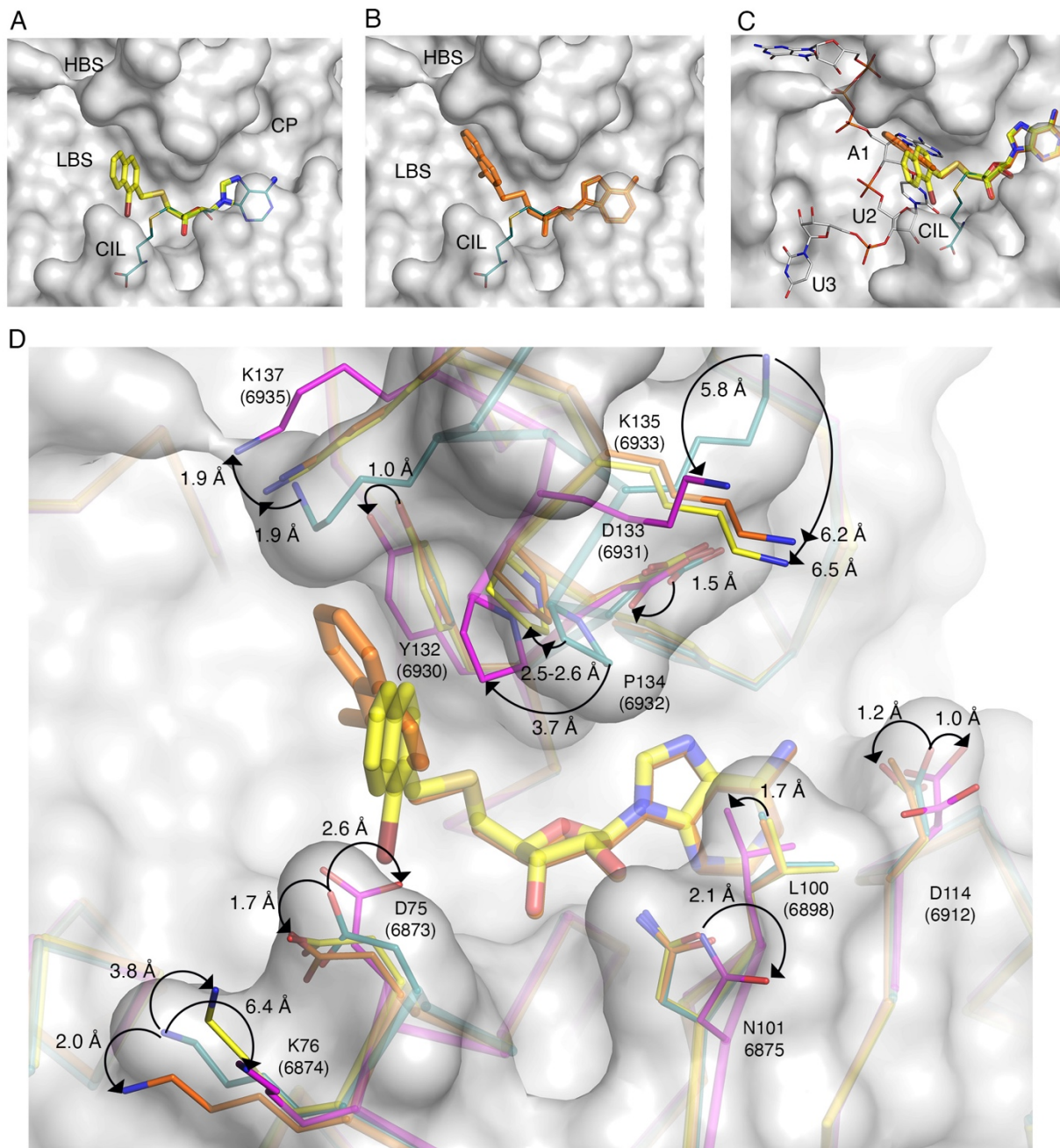
Supporting Figure 5. AF3 predictions for nsp16-nsp10 and compounds 4p and 4h. The superposition of the nsp16-nsp10/SAH crystal structure (7jz0) with AF3 models for compound **4p** (A) and **4h** (B). Protein chains are represented as ribbons and compounds as sticks with carbons and protein chains colored in dark blue-green for 7jz0, yellow for the best model, blue for the model_1, light green for the model_2, and purple for the model_3. Atoms are colored: oxygen (red), sulfur (yellow), nitrogen (blue), bromine (deep red), and chlorine (bright green). Structurally important parts of nsp16 are labeled as HBS for High-affinity binding site and CIL for Coronavirus Insertion loop.



Supporting Figure 6. The AF3 prediction and docking results for compound 4h and SARS-CoV-2 nsp16-nsp10. (A-B) Crystal structure of nsp16-nsp10 (PDB ID 7jz0) protein represented as a grey surface and bound SAH as sticks (carbons in dark green) with modeled position of **4h** overlaid as follows: **(A)** the AF3 best predicted model of bound compound **4h** (carbons in green) and **(B)** the top-ranked QVina2 docking pose (carbons in orange). **(C)** Overlay of the top

ranked AF3 best predicted model (green as in panel A) and top ranked QVina2 pose (orange as in panel B) with bound SAH (dark green) and the Cap-1-RNA.

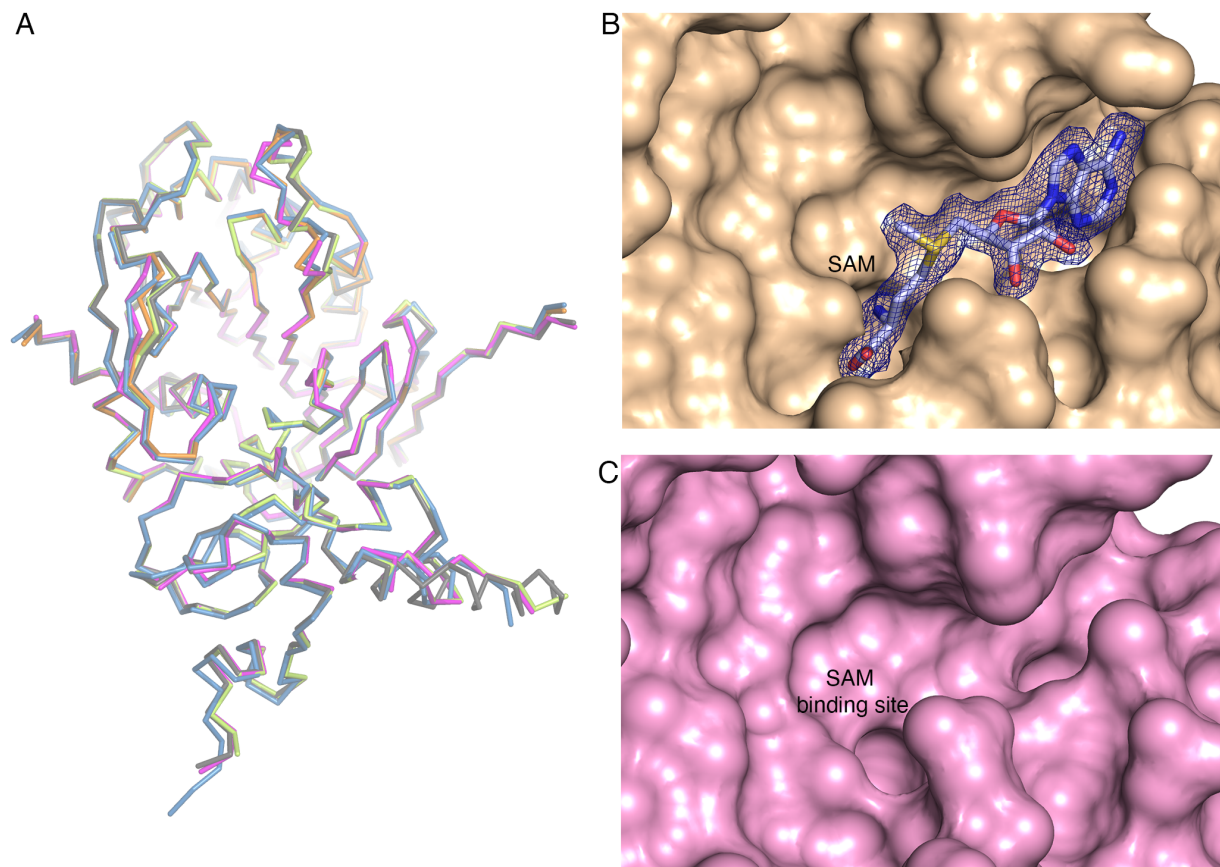
(D) Changes in the interactions and sidechain positions in the SAM/SAH and Cap-RNA binding sites. Superposition of the AF3 best solution, the top ranked docking pose and apo- nsp16-nsp10 crystal structure (7ult). Protein backbones are presented as ribbons in dark green (AF3), orange (QVina-W) and magenta (7ult). Selected residues are shown as sticks and labeled. Movements of sidechains are shown as black arrows. Atoms are colored: oxygen in red, nitrogen in blue, sulfur in yellow; chlorine in bright green. Amino acids are numbered from the nsp5 cleavage site at Orflab residue 6799 as amino acid 1. Amino acids in parentheses are according to the Orflab polypeptide sequence and match residues in PDB deposits 7jz0 and 7ult used for modeling.



Supporting Figure 7. The AF3 prediction and docking results for compound **4p and SARS-CoV-2 nsp16-nsp10. (A-B)** Crystal structure of nsp16-nsp10 (PDB ID 7jz0) protein represented as a grey surface with modeled position of **4p** overlaid as follows: **(A)** the AF3 best predicted model of bound compound **4p** (carbons in yellow) and **(B)** the top-ranked QVina-2 docking pose (carbons in orange). **(C)** Overlay of the top ranked AF3 best predicted model (yellow as in panel

A) and top-ranked QVina2 pose (orange as in panel B) with bound SAH (dark green) and the Cap-1-RNA.

(D) Changes in the interactions and sidechain positions in the SAM/SAH and Cap-RNA binding sites. Superposition of the AF3 best solution, the top ranked docking pose and apo-nsp16-nsp10 crystal structure (7ult). Protein backbones are presented as ribbons in teal SAH-bound (7jz0), yellow (AF3), and magenta (7ult). Selected residues are shown as sticks and labeled. Movements of sidechains are shown as black arrows. Atoms are colored: oxygen in red, nitrogen in blue, sulfur in yellow), and bromine in deep red. Amino acids are numbered from the nsp5 cleavage site at Orflab residue 6799 as amino acid 1. Amino acids in parentheses are according to the Orflab polypeptide sequence and match residues in PDB deposits 7jz0 and 7ult used for modeling.



Supporting Figure 8. Crystal structure of the apo-nsp16-nsp10 complex. **A)** The apo structure of the nsp16-nsp10 complex (7ult) is shown as pink ribbon and structurally aligned with crystal structures 6w4h (orange), 6wvn (gray), 7jyy (green) and 7jz0 (blue). **B)** The SAM binding site of nsp16 (6w4h) is shown with bound SAM represented in sticks (carbons lavender, oxygens red, nitrogens blue, and sulfur yellow) and surrounded by the composite omit electron density map shown as a black mesh. **C)** The SAM binding site of the apo-structure is shown as solvent exposed surface (pink) with no electron density for SAM.

2. Supporting Tables 1-9

Supporting Table 1. Data quality and refinement statistics for SARS-CoV-2 nsp16-nsp10 apo- structure.

PDB Accession Code	7ult
Data Collection	
Space group	<i>P3₂21</i>
Unit cell parameters (Å; °)	<i>a</i> = <i>b</i> = 166.68, <i>c</i> = 98.55; <i>a</i> = <i>b</i> = 90.00, <i>g</i> = 120.00
Wavelength (Å)	1.12723
Resolution range (Å)	30.00 - 1.90 (1.93 - 1.90) ^a
No. of unique reflections	123,235 (5,985)
<i>R</i> _{pim} (%)	4.1 (45.8)
CC _{1/2} (%)	99.8 (57.7)
Completeness (%)	99.7 (97.8)
$\Delta I/\sigma(I)$	19.1 (1.8)
Multiplicity	11.1 (10.0)
Wilson <i>B</i> factor	35.8
Refinement	
Resolution range (Å)	29.90 - 1.90 (1.95 - 1.90)
Completeness (%)	99.7 (98.3)
No. of reflections:	
Working set	110,798 (8,329)
Free R test set ^b	6,205 (528)
<i>R</i> _{work} / <i>R</i> _{free} , (%)	16.4/18.8 (26.4/27.9)
Protein chains/atoms	4/6,390
Ligand/Solvent atoms	63/768
Mean temperature factor (Å ²)	35.1
Coordinate Deviations	
R.m.s.d. bonds (Å)	0.005
R.m.s.d. angles (°)	1.325
Ramachandran plot	
Favored (%)	97.0
Allowed (%)	3.0
Outside allowed (%)	0.0

^a Values in parentheses are for the outer shell.

^b Free R value test set size is 5.0%

Supporting Table 2. Qvina docking nsp14 7RV2 **4p** qvinia w rigid

Mode	Affinity (kcal/mol)	Dist. from best mode (rmsd l.b.)	Dist from best mode (rmsd u.b.)
1	-11.4	0	0
2	-11	1.933	2.625
3	-10.6	3.414	5.027
4	-10.4	6.483	8.601
5	-10.2	2.539	3.763
6	-10.2	1.868	2.77
7	-10.2	8.216	10.427
8	-10	8.374	10.368
9	-10	9.666	12.712

Supporting Table 3. Qvina docking nsp14 7RV2 **4p** qvinia 2 rigid

Mode	Affinity (kcal/mol)	Dist. from best mode (rmsd l.b.)	Dist from best mode (rmsd u.b.)
1	-10.9	0	0
2	-10.8	1.868	2.442
3	-10.5	1.667	2.853
4	-10.5	5.981	8.203
5	-10.5	3.077	4.782
6	-10.2	3.173	4.826
7	-10.1	6.27	7.984
8	-10.1	7.997	10.256
9	-10	2.047	2.687

Supporting Table 4. Qvina docking nsp14 7RV2 **4h** qvinia w rigid

Mode	Affinity (kcal/mol)	Dist. from best mode (rmsd l.b.)	Dist from best mode (rmsd u.b.)
1	-9.6	0.000	0.000
2	-9.6	1.208	2.019
3	-9.3	7.377	10.052
4	-9.2	1.937	2.822
5	-9.1	8.082	10.870
6	-9.1	4.495	7.576
7	-8.9	2.717	4.860
8	-8.8	6.865	9.649
9	-8.8	6.059	8.603

Supporting Table 5. Qvina docking nsp14 7RV2 **4h** qvinia 2rigid

Mode	Affinity (kcal/mol)	Dist. from best mode (rmsd l.b.)	Dist from best mode (rmsd u.b.)
1	-9.6	0.000	0.000
2	-9.4	4.843	8.248
3	-9.2	6.836	8.416
4	-9.2	1.339	1.992
5	-9.0	2.555	3.562
6	-9.0	1.209	1.378
7	-9.0	-2.939	4.706
8	-8.9	7.523	9.661
9	-8.9	3.487	4.706

Supporting Table 6. Qvina2 docking nsp16 **4p** w rigid

Mode	Affinity (kcal/mol)	Dist. from best mode (rmsd l.b.)	Dist from best mode (rmsd u.b.)
1	-10.9	0	0
2	-10.8	1.868	2.442
3	-10.5	1.667	2.853
4	-10.5	5.981	8.203
5	-10.5	3.077	4.782
6	-10.2	3.173	4.826
7	-10.1	6.27	7.984
8	-10.1	7.997	10.256
9	-10	2.047	2.687

Supporting Table 7. Qvina2 docking nsp16 **4p** rigid

Mode	Affinity (kcal/mol)	Dist. from best mode (rmsd l.b.)	Dist from best mode (rmsd u.b.)
1	1	-8.8	0
2	2	-8.3	2.03
3	3	-8	5.345
4	4	-8	4.971
5	5	-7.7	6.142
6	6	-7.7	10.631
7	7	-7.6	5.413
8	8	-7.6	1.361
9	9	-7.5	2.362

Supporting Table 8. Qvina2 docking nsp16 **4h** w rigid

Mode	Affinity (kcal/mol)	Dist. from best mode (rmsd l.b.)	Dist from best mode (rmsd u.b.)
1	-9.3	0	0
2	-9	1.82	2.462
3	-9	1.395	1.997
4	-8.9	1.485	2.026
5	-8.6	4.888	9.808
6	-8.6	4.925	9.732
7	-8.6	9.467	13.42
8	-8.4	3.107	4.115
9	-8.4	2.691	3.771

Supporting Table 9. Qvina2 docking nsp16 **4h** rigid

Mode	Affinity (kcal/mol)	Dist. from best mode (rmsd l.b.)	Dist from best mode (rmsd u.b.)
1	-9.6	0	0
2	-9.2	1.227	1.73
3	-8.9	1.629	2.45
4	-8.8	4.492	9.581
5	-8.7	5.099	9.46
6	-8.5	7.757	11.58
7	-8.4	2.514	3.786
8	-8.3	2.246	3.437
9	-8.3	3.381	4.474

3. Supporting Movie Legends 1-8

Supporting Movie 1. Nsp14-Qvinia-W and 4p poses. Nsp14 is depicted as a pale blue solvent-exposed surface. Carbons of compound **4p** are depicted as pink sticks and teal sticks for SAH. In all cases, other atoms are shown as sticks: red, oxygen; blue, nitrogen; yellow, sulfur; and deep red, bromide.

Supporting Movie 2. Nsp14-Qvinia-2 and 4p poses. Nsp14 is depicted as a pale blue solvent-exposed surface. Carbons of compound **4p** are shown as orange sticks and teal sticks for SAH. In all cases, other atoms are shown as sticks: red, oxygen; blue, nitrogen; yellow, sulfur; and deep red, bromide.

Supporting Movie 3. Nsp14-Qvinia-W and 4h poses. Nsp14 as a pale blue solvent-exposed surface. Carbons of compound **4h** are depicted as cyan sticks and teal sticks for SAH. In all cases, other atoms are shown as sticks: red, oxygen; blue, nitrogen; yellow, sulfur; and green, chlorine.

Supporting Movie 4. Nsp14-Qvinia-2 and 4h poses. Nsp14 is depicted as a pale blue solvent-exposed surface. Carbons of compound **4h** are depicted as magenta sticks, and teal sticks for SAH. In all cases, other atoms are shown as sticks: red, oxygen; blue, nitrogen; yellow, sulfur; and green, chlorine.

Supporting Movie 5. Nsp16-Qvinia-W and 4p poses. Nsp16 is depicted as solvent-exposed surface (gray). The carbons in the compound **4p** are depicted as orange sticks, and teal for SAH. In all cases, other atoms are represented as sticks and colored as: red, oxygen; blue, nitrogen; yellow, sulfur; and deep red, bromide.

Supporting Movie 6. Nsp16-Qvinia-2 and 4p poses. Nsp16 is depicted as solvent-exposed surface (gray). The carbons in the compound **4p** are depicted as pale blue sticks, and teal for SAH. In all cases, other atoms are represented as sticks and colored as: red, oxygen; blue, nitrogen; yellow, sulfur; and deep red, bromide.

Supporting Movie 7. Nsp16-Qvinia-W and 4h poses. Nsp16 is depicted as solvent-exposed surface (gray). The carbons in the compound **4h** are depicted as yellow sticks, and teal for SAH. In all cases, other atoms are represented as sticks and colored as: red, oxygen; blue, nitrogen; yellow, sulfur; and green, chlorine.

Supporting Movie 8. Nsp16-Qvinia-2 and 4h poses. Nsp16 is depicted as solvent-exposed surface (gray). The carbons in the compound **4h** are depicted as magenta sticks, and teal for SAH. In all cases, other atoms are represented as sticks and colored as: red, oxygen; blue, nitrogen; yellow, sulfur; and green, chlorine.

4. Experimental Procedures – Biological and Biochemical Assays

MHV Infection and Inhibitor Screening. Inhibition of MHV replication was conducted as previously described.¹ Briefly, L2 cells (rat lung fibroblasts, RRID:CVCL_0383, a kind gift from Dr. Pablo Penaloza-MacMaster) were transduced to stably express mCherry fluorescent protein were cultured in Dulbecco's Modified Eagle's Medium 10 (DMEM10) supplemented with 1% glutamine, 1% penicillin-streptomycin (P/S), and 10% heat-inactivated fetal bovine serum (FBS). Cells were infected at multiplicity of infection (MOI) = 0.1 with 50 μ L of MHV-p38-GFP (a kind gift from Dr. Pablo Penaloza) for 2 hr. Cells were then washed and media replaced with DMEM10 containing 6.25-200 μ M compound. The 384-well plates were placed in a Molecular Devices ImageXpress High Content Imaging microscope. Images were collected at 10 $\times$ on bright, green, and red channels every 4 h for 24 h postinfection (p.i.). Maximum peak of infection was detected at 16 h p.i. and the analysis was performed at this time point. The GFP intensity was analyzed using Molecular Devices software counting the number of green foci and normalized to 100% infection assigned to DMSO controls. Plots were generated, and curves were fitted using GraphPad Prism. Experiments were performed in duplicates by plate and at least repeated in 2 plates ($n=4$).

SARS-CoV-2 Infection. Calu-3 cells (human epithelial lung adenocarcinoma, RRID:CVCL_0609) were cultured in Modified Eagle's Medium 10 (MEM10) supplemented with 1% glutamine, 1% P/S, 1% non-essential amino acids, and 10% FBS, and maintained in 75 cm² flasks until 90% confluence at 37 °C, 5% CO₂. Cells were harvested by trypsinization (0.25% trypsin + EDTA), counted and seeded as needed for SARS-CoV-2 drug screening and cytotoxicity assays.

Calu-3 cells were seeded at a density of 30,000 cells/ well in a 96 well plate, after 24 h, the cells were infected with SARS-CoV-2 at MOI= 5 and incubated at 37°C and 5% CO₂ for 24 h. Infected cells and controls (non-infected) were treated with the compounds 2 h postinfection and fixed after 24 h incubation using 4% paraformaldehyde for 30 min. Nuclei were stained with Hoechst 33342. Infected cells were detected by immunofluorescence using anti-N primary (Sino Biological 40143-T62) and 594/647 secondary (Jackson 711-585-152). Positive cells were counted using Molecular Devices software, with infection normalized to DMSO. Plots were generated and curves fitted using GraphPad Prism. Experiments were performed in quadruplicates per plate ($n= 4$).

Cytotoxicity Determination. Cytotoxicity of L2 cells was determined by crystal violet staining (L2). Cells were washed with 50 μ L of phosphate buffered saline (PBS) and stained with crystal violet (1% in 70% ethanol) for 15 min, then washed 3 times with 100 mL of water. Stained cells were dissolved in 50 μ L of 10% acetic acid. The absorbance of the solution was measured in a transparent plate at 590 nm in a Tecan Infinite M100 plate reader.

Cytotoxicity of Calu-3 cells was determined by the MTT assay. Cells were plated in a 384-well plate and incubated for 24h after treatment with inhibitors. The MTT assay was performed using the Promega CellTiter 96® Non-Radioactive Cell Proliferation Assay (catalog number G4000), according to the manufacturer's instructions, and the absorbance was measured at 570 nm.

Methyltransferase activity. The methyltransferase activity of SARS-CoV-2 nsp14 complex were assessed as previously described.² Briefly, the assays were performed at 1.5 nM nsp14, 250 nM ³H-SAM, and 50 nM Biotin-RNA substrate (5'-GpppACCCCCCCCC-Biotin 3') in 20 mM Tris-

HCl, pH 7.5, 250 μ M MgCl₂, 5 mM dithiothreitol and 0.01% Triton X-100. The reaction time was 20 min at 23 °C. The signal detection and data analysis were performed as described before.

The methyltransferase activity of SARS-CoV-2 nsp16 was assessed as previously described.³ Briefly, the assays were performed at 250 nM nsp16-nsp10 complex, 4 μ M ³H-SAM, and 2 μ M of Biotin-RNA (5'-N^{7-me}GpppACCCCC-biotin) in 50 mM Tris HCl, pH 7.5, 1.5 mM MgCl₂, 0.01% Triton X-100, 0.01% bovine serum albumin and 5 mM dithiothreitol. The reaction time was 30 min at 23 °C. The signal detection and data analysis were performed as described before

The methyl transfer assay activity across a panel of mammalian methyltransferase was done as described⁴

5. Experimental Procedures – X-ray Structure determination

Expression, Purification, Crystallization of apo-nsp16-nsp10 complex. Protein expression and purification of nsp16-nsp10 complex for crystallization was performed as previously described.⁵

⁶ To form nsp16-nsp10 complex free of SAM or SAH, the pure proteins were mixed at a 1:1 molar ratio at about 2 mg/ml in loading buffer, incubated for 2 hours with ribavirin (2 mM in DMSO), and then the buffer was exchanged three times [10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM MgCl₂, 1 mM TCEP, and 5% glycerol]. The complex was concentrated to 5.3 mg/ml and set up for crystallization immediately in 150 mM NaCl, 10 mM Tris pH 7.5, 5% glycerol. The protein crystallized in the NeXtal ComPAS Suite at 0.4 M Potassium/Sodium tartrate (condition F10).

Crystallography. The data set was collected from the single crystal at beam line 21ID-D of the Life Sciences-Collaborative Access Team (LS-CAT) at the Advanced Photon Source, Argonne National Laboratory. The data were processed using HKL-3000.⁷ The structure was determined by Molecular Replacement method in PHASER.⁸ using crystals structure of nsp16-nsp10 (PDB code 6w4h) as a search model. The crystal belongs to the trigonal space group P3₂21 with four protein chains in the asymmetric unit, which form two nsp16-nsp10 heterodimers. The initial model went through several rounds of refinement in REFMAC.⁹ Manual model corrections were done using Coot.¹⁰ The water molecules were generated automatically using ARP/wARP.¹¹ followed by additional rounds of refinement in REFMAC. Sodium ions and formate molecules were fit into electron density maps manually and the structure was further refined in REFMAC. The Translation–Libration–Screw (TLS) group were generated by the TLS Motion Determination (TLSMD) server.¹² (<https://doi.org/10.1107/S0021889805038987>) and used in the final refinement cycles. The quality of the model during refinement and the final validation of the

structure was done using MolProbity.¹³ (<http://molprobity.biochem.duke.edu/>). Data quality, structure refinement, and the final model statistics are shown in Suppl Table 1. The nsp16-nsp10 apo- structure was deposited to the RCSB PDB (<https://www.rcsb.org/>) with the assigned PDB code 7ult.

6. Experimental Procedures – Molecular modeling and docking

High Throughput Screening of Drug Candidates. The high throughput screening of drug candidates was performed with the FRED docking program from OpenEye toolkit, version 2025.1.1.¹⁴ The screening was carried out on Intel(R) Xeon(R) Gold 6148 CPU using 10 cores. We prepared both nsp14 protein monomer (PDB code 7r2z) and nsp16/nsp10 complex (PDB code 7jz0) as the receptor for the docking experiment. The SAM binding pocket was treated as the active site for the docking experiment. The SMILES strings of 70 SAH analogs were generated into 3D structures and compressed into a multiconformer database using OpenEye OMEGA tool.¹⁵ The small molecules were docked into the active site using exhaustive search algorithm. The generated poses were scored with Chemgauss4 score function, which represents the attributions from molecular shape, hydrogen bonds and solvent effect. The top pose was retained for each SAH analog. The overall score of each molecule was then used to rank against other molecules in the dataset.

Molecular Docking and AlphaFold 3 modeling. Binary files for AlphaFold3 (<https://github.com/google-deepmind/alphafold3>), QuickVina-2 and QuickVina-W (<https://qvina.github.io/>) were downloaded to a DELL Precision 5820 workstation, which runs Ubuntu 22.4.05 LTS (Jammy) and has the following specifications: processor - Intel Xeon W-2245 (8 physical, 16 hyperthreaded cores, 3.9GHz), RAM - 64G, graphics - NVIDIA RTX A4000 GPU.

Our first step was to get predicted models of the protein-ligand complexes using AF3. We used the full sequences for nsp10, nsp14 and nsp16 as inputs for protein and the SMILES strings for the compounds (**4h**:

(O[C@@H]1[C@@H](CSCC2=C(Cl)C=C(Cl)C=C2)O[C@@H](N3C(N=CN=C4N)=C4N=C3)[C@@H]1O and **4p**:

O[C@@H]1[C@@H](CSCC2=CC=C(C=CC=C3)C3=C2Br)O[C@@H](N4C(N=CN=C5N)=C5N=C4)[C@@H]1O). The results of AF3 predicted five models were visualized using PyMol (www, version). For the further analysis for each compound we used the model with the highest confidence score.

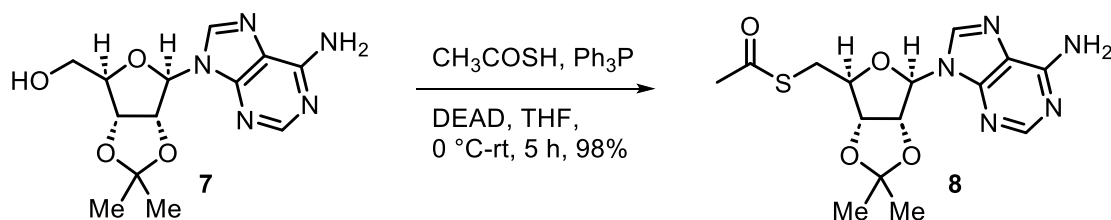
The results of the first step were used to choose parameters for our docking studies. We used a rigid receptor model and flexible ligand to run QVina-2 and QVina-W. The crystal structures with PDB codes 7r2v and 7jz0 were used as the nsp14 and nsp16-nsp10 rigid receptors, respectively, and AlphaFold3 generated models of the 4h and 4p as the ligands. The center of the box was defined based on position of C5' atom of the SAH in crystal structures of nsp14/SAH (7r2v) and nsp16-nsp10/SAH (7jz0) complexes, and the size of the box was set to 40x40x40 (Å). The results of QVina-2 and QVina-W docking runs were visualized in PyMol. Only top ranked solutions, based on software calculated affinity, were used for further analysis and structural comparison. Docking score data are found in Supporting Tables 2-9.

7. Experimental Procedures – Chemical synthesis

Unless stated otherwise, reactions were performed under a nitrogen atmosphere using oven- or flame-dried glassware and stir bars. Ambient temperature refers to 22–24 °C. Pre-heated oil baths were used to maintain temperatures above ambient. Lower temperatures were maintained using acetone/CO₂ (s) (–78 °C), and water/ice (0 °C) baths. Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. Thin-layer chromatography (TLC) was performed using EMD Millipore silica gel 60 Å plates with UV fluorescence quenching (254 nm), KMnO₄, or Seebach's stain. Flash column chromatography was performed on Sili Cycle Sili Flash P60 (40–63 µm particle size) using ACS or HPLC grade solvents purchased from Fisher Scientific.

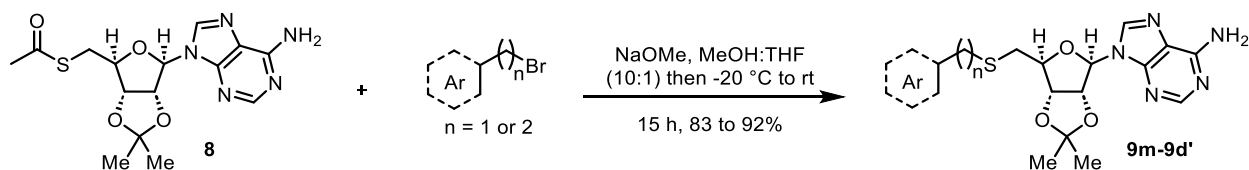
Nuclear magnetic resonance (NMR) spectra were recorded on Bruker 400 and 500 MHz instruments and calibrated using deuterated solvent (CDCl₃: ¹H NMR: 7.26 ppm, ¹³C NMR: 77.16 ppm, DMSO-d₆: ¹H NMR: 2.50 ppm, ¹³C NMR: 39.52 ppm, CD₃OD: ¹H NMR: 3.31 ppm, ¹³C NMR: 49.00 ppm). NMR data are reported as: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), and integration). High-resolution mass spectral analysis was measured on Agilent Technologies 6224 TOF LC/MS (electrospray ionization).

S-(((3aS,4S,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl) methyl) ethanethioate (8):



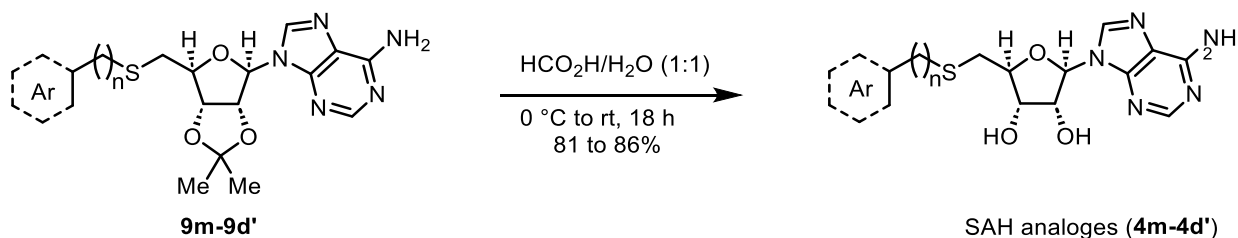
To an ice-cold solution of TPP (9.38 g, 35.8 mmol) in THF (32 mL) was added diethyl azodicarboxylate (16.3 mL, 40% in toluene, 35.8 mmol) and the resulting reaction mixture was stirred for 5 min. A solution of compound **7** (5.0 g, 16.2 mmol) in THF (10 mL) was then introduced to the above mixture, and the resulting yellow suspension was stirred for another 10 min. Then, a solution of thioacetic acid (2.06 mL, 28.8 mmol) in THF (5.0 mL) was added, and the reaction mixture was stirred for another 2 h at the same temperature. During this time, the yellow suspension cleared, affording an orange solution. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography (3- 5% DCM/MeOH) to afford **8** (5.8 g, 98% yield) as a viscous oil. ¹H NMR (400 MHz, CDCl₃) δ 8.33 (s, 1H), 7.89 (s, 1H), 6.26 (bs, 2H), 6.06 (d, J = 2.1 Hz, 1H), 5.50 (dd, J = 6.4, 2.1 Hz, 1H), 4.96 (dd, J = 6.3, 3.1 Hz, 1H), 4.32 (d, J = 3.1 Hz, 1H), 3.25 (d, J = 7.2 Hz, 1H), 3.16 (dd, J = 13.8, 6.6 Hz, 1H), 2.32 (s, 3H), 1.57 (s, 3H), 1.36 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 194.5, 155.6, 153.2, 149.2, 140.0, 120.3, 114.5, 90.9, 86.1, 84.2, 83.7, 31.2, 30.5, 27.0, 25.3. The spectral data are consistent with those reported in the literature.¹⁶

General procedure A:



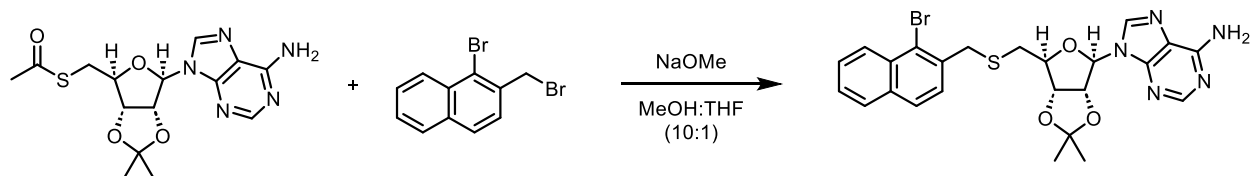
To a mixture of thioester compound **8** (1.0 equiv.) and aryl halides (1.4 equiv.) in MeOH/THF (2:1, 3 mL) at $-20\text{ }^{\circ}\text{C}$, was added NaOMe (2.5 equiv.), and the resulting pale-yellow reaction mixture was stirred at room temperature for 18 h. Silica gel was then added to the mixture to form a slurry, which was dried under vacuum and subsequently loaded onto a silica gel column. Elution with 4–5% MeOH/DCM afforded the desired SAH analog precursors (**9m** to **9d'**).

General procedure B:



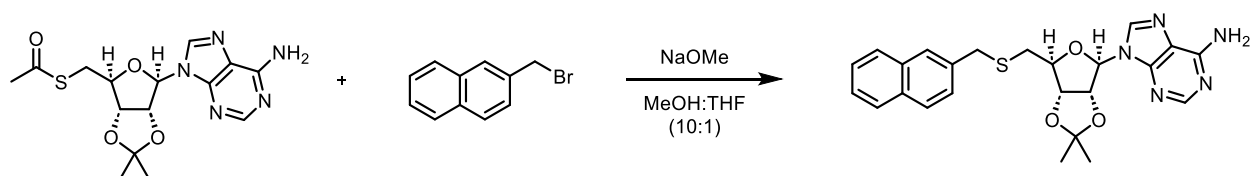
To the SAH analog precursors (**9m** to **9d'**, 1.0 equiv.), a mixture of formic acid and water (1:1, 10 mL/mmol) was added at $0\text{ }^{\circ}\text{C}$. The resulting mixture was stirred at either room temperature or $50\text{ }^{\circ}\text{C}$ for 18 to 24 hours. Upon completion of the reaction, the solvent was co-evaporated with ethanol, and the resulting crude product was redissolved in a 10% MeOH/DCM solution. A slurry was prepared and directly loaded onto a silica gel column. Elution with 5–10% MeOH/DCM afforded the desired SAH analogs (**4m** to **4d'**).

9-((3aR,4R,6S,6aS)-6-((((1-bromonaphthalen-2-yl)methyl)thio)methyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purin-6-amine (9p).



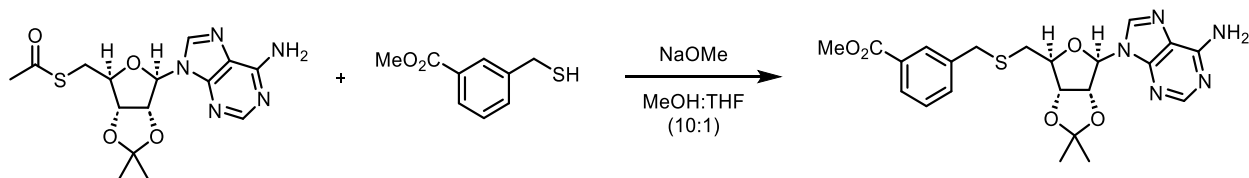
The product was prepared from thioester **8** (100 mg, 0.27 mmol) and 1-bromo-2-(bromomethyl)naphthalene (97 mg, 0.33 mmol) according to **General Procedure A**. After purification of the crude product by column chromatography (2 to 5% MeOH/DCM), the desired compound **9p** was isolated as a white solid (127.5 mg, 86% yield). ^1H NMR (400 MHz, CDCl_3) δ 8.30 – 8.25 (m, 2H), 7.90 (s, 1H), 7.80 – 7.76 (m, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.58 (m, 1H), 7.50 (m, 1H), 7.41 (d, J = 8.4 Hz, 1H), 6.05 (d, J = 2.3 Hz, 1H), 5.70 (bs, 2H), 5.46 (dd, J = 6.4, 2.3 Hz, 1H), 5.00 (dd, J = 6.4, 3.2 Hz, 1H), 4.43 (td, J = 6.8, 3.2 Hz, 1H), 4.10 (ABq, J = 13.5, 24.0 Hz, 2H), 2.86 (dd, J = 13.8, 7.2 Hz, 1H), 2.77 (dd, J = 13.7, 6.3 Hz, 1H), 1.58 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 155.5, 153.1, 149.2, 140.0, 135.5, 133.6, 132.4, 128.0, 127.8, 127.7, 127.6, 127.5, 126.5, 124.2, 120.3, 114.4, 90.8, 86.7, 84.0, 83.8, 37.5, 33.6, 27.0, 25.3. HRMS (ESI): calcd for $\text{C}_{24}\text{H}_{24}\text{BrN}_5\text{O}_3\text{SNa}$ $[\text{M}+\text{Na}]^+$: 564.0680, found: 566.0634.

9-((3aR,4R,6S,6aS)-2,2-dimethyl-6-(((naphthalen-2-yl)methyl)thio)methyl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purin-6-amine (9v).



The product was prepared using thioester **8** (100 mg, 0.27 mmol) and 2-(bromomethyl) naphthalene (73 mg, 0.33 mmol) according to **General Procedure A**. After purification of the crude product by column chromatography (2 to 5% MeOH/DCM), the desired compound **9v** was isolated as a white solid (102.7 mg, 81% yield). **¹H NMR (500 MHz, CDCl₃)** δ 8.28 (s, 1H), 7.92 (s, 1H), 7.83 – 7.72 (m, 3H), 7.62 (s, 1H), 7.44 (ddd, *J* = 15.7, 7.3, 2.1 Hz, 3H), 6.24 (s, 2H), 6.07 (d, *J* = 2.3 Hz, 1H), 5.45 (dd, *J* = 6.5, 2.2 Hz, 1H), 4.98 (dd, *J* = 6.5, 3.3 Hz, 1H), 4.38 (td, *J* = 6.7, 3.3 Hz, 1H), 3.87 (s, 2H), 2.79 (dd, *J* = 13.7, 7.2 Hz, 1H), 2.69 (dd, *J* = 13.7, 6.2 Hz, 1H), 1.59 (s, 3H), 1.37 (s, 3H). **¹³C NMR (126 MHz, CDCl₃)** δ 155.8, 153.1, 149.2, 139.8, 135.1, 133.1, 132.5, 128.5, 127.6, 127.6, 127.4, 126.9, 126.2, 125.8, 120.2, 114.5, 90.7, 86.5, 84.0, 83.7, 36.8, 33.2, 27.1, 25.3. The spectral data are consistent with those reported in the literature.^{S2}

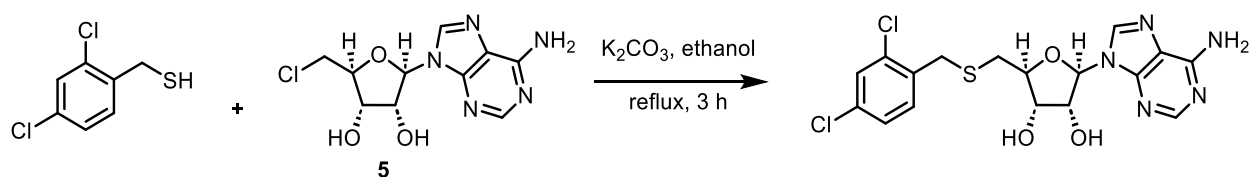
methyl 3-((((3a*S*,4*S*,6*R*,6a*R*)-6-(6-amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3] dioxol-4-yl) methyl)thio)methyl) benzoate (9x**).**



The product was prepared using thioester **8** (200 mg, 0.54 mmol) and methyl 2-(bromomethyl) benzoate (150.6 mg, 0.66 mmol) according to **General Procedure A**. After purification of the crude product by column chromatography (2 to 5% MeOH/DCM) the desired compound **9x** was isolated as a white solid (214.0 mg, 83% yield). **¹H NMR (400 MHz, CDCl₃)** δ 8.29 (s, 1H), 7.96 – 7.85 (m, 3H), 7.42 – 7.40 (m, 1H), 7.33 (t, *J* = 7.7 Hz, 1H), 6.05 (d, *J* = 2.2 Hz, 1H), 5.77 (s, 2H), 5.46 (dd, *J* = 6.4, 2.2 Hz, 1H), 5.01 (dd, *J* = 6.4, 3.4 Hz, 1H), 4.35 (td, *J* = 6.7, 3.4 Hz, 1H), 3.91 (s, 3H), 3.74 (s, 2H), 2.78 (dd, *J* = 13.7, 7.0 Hz, 1H), 2.67 (dd, *J* = 13.7, 6.5 Hz, 1H), 1.60 (s,

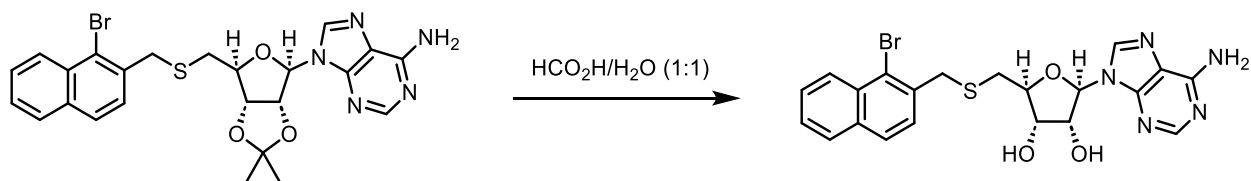
3H), 1.38 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 166.9, 155.6, 153.2, 149.3, 140.1, 138.4, 133.4, 130.6, 130.0, 128.7, 128.5, 120.5, 114.7, 90.8, 86.8, 84.1, 83.9, 52.3, 36.3, 33.5, 27.2, 25.4. HRMS (ESI): calcd for C₂₂H₂₆N₅O₅S [M+H]⁺: 472.1655, found: 472.1657.

(2R,3R,4S,5S)-2-(6-amino-9H-purin-9-yl)-5-(((2,4-dichlorobenzyl)thio)methyl) tetrahydrofuran-3,4-diol (4h).



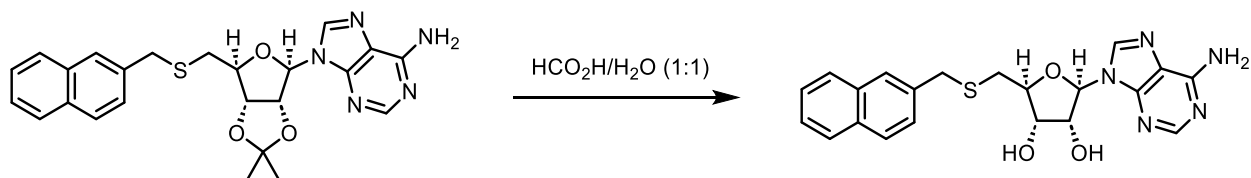
To a mixture of chloro adenosine **5** (100 mg, 0.35 mmol, 1.0 equiv.) and 2,4-Dichlorobenzyl mercaptan (81 mg, 0.42 mmol, 1.2 equiv.) in EtOH (3.0 mL), was added K₂CO₃ (97 mg, 0.70 mol, 2.0 equiv.) at room temperature. The reaction mixture was then heated to reflux for 3 h. After completion of the reaction, it was allowed to cool to room temperature and subsequently washed with isopropanol (3 x 3 mL), affording the desired product **4h** (120 mg, 78%) as a white solid. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.34 (s, 1H), 8.14 (s, 1H), 7.58 (s, 1H), 7.37 – 7.30 (m, 2H), 5.88 (d, *J* = 5.4 Hz, 1H), 4.74 (t, *J* = 5.0 Hz, 1H), 4.17 (t, *J* = 4.6 Hz, 1H), 4.04 – 4.00 (m, 1H), 3.85 – 3.756 (m, 2H), 2.91– 2.75 (m, 2H); ¹³C NMR (101 MHz, DMSO-*d*₆): δ 156.0, 152.6, 149.3, 139.9, 135.3, 133.9, 132.3, 132.2, 128.9, 127.16, 119.19, 87.7, 83.9, 72.6, 72.5, 33.6, 32.8. HRMS (ESI): calcd for C₁₇H₁₈Cl₂N₅O₃S [M+H]⁺: 442.0507, found: 442.0500.

(2R,3R,4S,5S)-2-(6-amino-9H-purin-9-yl)-5-(((1-bromonaphthalen-2-yl)methyl)thio)methyl) tetrahydrofuran-3,4-diol (4p).



Following **General Procedure B**, compound **9p** (100 mg, 0.18 mmol) was treated with formic acid/water (1:1, 2.0 mL/mmol) and stirred the reaction mixture at room temperature for 18 h. After purification of the crude product by column chromatography (3 to 8% MeOH/DCM), the desired compound **4p** was isolated as a white solid (78.7 mg, 85% yield). **¹H NMR (400 MHz, DMSO-*d*₆):** δ 8.36 (s, 1H), 8.19 (s, 1H), 8.13 (s, 1H), 7.97 (d, *J* = 6.9 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.70 – 7.68 (m, 1H), 7.61 – 7.57 (m, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.29 (s, 2H), 5.90 (d, *J* = 5.5 Hz, 1H), 5.50 (d, *J* = 5.8 Hz, 1H), 5.32 (d, *J* = 5.0 Hz, 1H), 4.78 (q, *J* = 5.5 Hz, 1H), 4.19 (q, *J* = 4.7 Hz, 1H), 4.11 (s, 2H), 4.08 (m, 1H), 2.96 (dd, *J* = 13.9, 5.7 Hz, 1H), 2.85 (dd, *J* = 13.9, 6.9 Hz, 1H). **¹³C NMR (126 MHz, DMSO-*d*₆):** δ 156.5, 153.1, 149.8, 140.4, 136.9, 133.6, 132.1, 128.8, 128.5, 128.2, 127.1, 127.0, 123.6, 119.7, 88.1, 84.3, 73.1, 72.9, 37.6, 34.3. **HRMS (ESI):** calcd for C₂₁H₂₀BrN₅O₃SSNa [M+Na]⁺: 524.0367, found: 527.0352

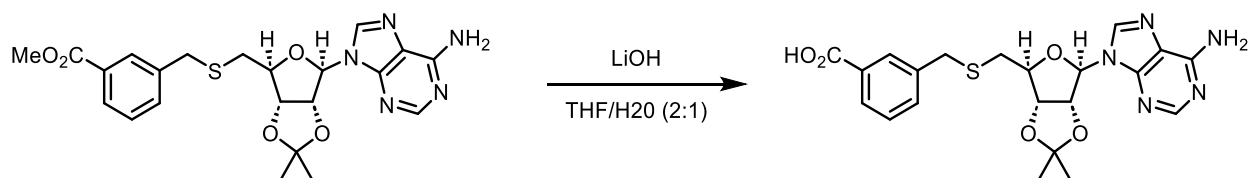
(2R,3R,4S,5S)-2-(6-amino-9H-purin-9-yl)-5-(((naphthalen-2-ylmethyl)thio)methyl)tetrahydrofuran-3,4-diol (4v).



Following the **General Procedure B**, compound **9v** (100 mg, 0.21 mmol) was treated with formic acid/water (1:1, 2.0 mL/mmol) and the reaction mixture was stirred at room temperature for 18 h. Upon completion of the reaction, the solvent was co-evaporated with ethanol, and the resulting crude product was redissolved in a 10% MeOH/DCM solution. A slurry was prepared,

which was directly loaded onto a silica gel column chromatography. A slurry was prepared and directly loaded onto a silica gel column. Elution with 5–10% MeOH/DCM afforded the desired compound **4v** as an off-white solid (76.0 mg, 83%). **¹H NMR (400 MHz, DMSO):** δ 8.35 (s, 1H), 8.11 (d, J = 1.0 Hz, 1H), 7.86 (dd, J = 12.0, 6.9 Hz, 2H), 7.82 – 7.77 (m, 1H), 7.69 (d, J = 1.8 Hz, 1H), 7.50 – 7.43 (m, 3H), 7.30 (s, 2H), 5.90 (dd, J = 5.4, 1.2 Hz, 1H), 5.50 (dd, J = 5.9, 1.6 Hz, 1H), 5.30 (dd, J = 5.3, 1.5 Hz, 1H), 4.74 (q, J = 5.5 Hz, 1H), 4.19 (q, J = 4.9 Hz, 1H), 4.04 (q, J = 5.8 Hz, 1H), 3.95 – 3.88 (m, 2H), 2.86 (dd, J = 13.9, 5.7 Hz, 1H), 2.70 (dd, J = 13.9, 6.9 Hz, 1H); **¹³C NMR (400 MHz, CDCl₃):** δ 156.0, 152.6, 149.4, 139.9, 135.9, 132.7, 131.9, 128.0, 127.5, 127.4, 127.3, 127.0, 126.2, 125.7, 119.2, 87.6, 83.7, 72.6, 72.5, 35.8, 33.2. The spectral data are consistent with those reported in the literature.²

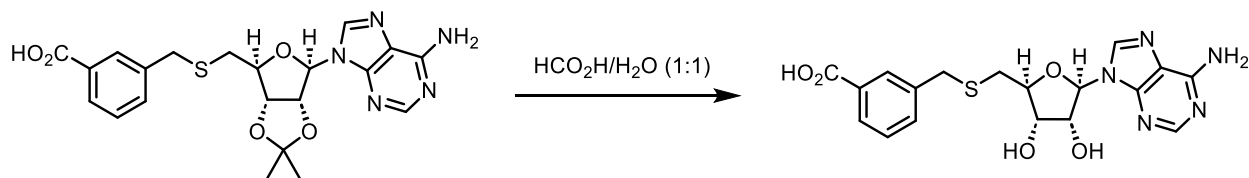
3-((((3aS,4S,6R,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d] [1,3] dioxol-4-yl) methyl)thio)methyl) benzoic acid (4x'**).**



To a solution of ester **9x** (150 mg, 0.32 mmol, 1.0 equiv.) in THF:H₂O (5 mL, 2:1) at room temperature, LiOH (23 mg, 0.96 mmol, 3.0 equiv.) was added, and stirred the reaction mixture for 24 h at the same temperature. The reaction mixture was then acidified with 1N HCl (5 mL) and the aqueous phase was extracted with DCM (3 x 20 mL), the combined organic phases were washed with brine (4.0 mL) and dried over Na₂SO₄, and concentrated in vacuo. The resulting crude product was purified by silica gel column chromatography (5 to 10% MeOH/DCM) to provide the acid **4x'** (141.0 mg, 97 % yield) as a white solid. **¹H NMR (400 MHz, CD₃OD)** δ 8.24 (s, 1H),

8.15 (s, 1H), 7.94 (s, 1H), 7.93 (s, 1H), 7.85 (d, $J = 7.6$ Hz, 1H), 7.37 (m, 1H), 7.3 (t, $J = 7.6$ Hz, 1H), 6.14 (d, $J = 2.4$ Hz, 1H), 5.46 (dd, $J = 6.4, 2.4$ Hz, 1H), 4.97 (dd, $J = 6.4, 3.2$ Hz, 1H), 4.29 (td, $J = 6.8, 3.2$ Hz, 1H), 3.76 (s, 2H), 2.72 (qd, $J = 13.8, 6.8$ Hz, 2H), 1.56 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 170.2, 157.3, 153.9, 150.1, 141.8, 140.2, 134.1, 133.0, 131.1, 129.4, 129.3, 120.5, 115.5, 91.5, 88.1, 85.1, 36.8, 34.5, 27.3, 25.5. The spectral data are consistent with those reported in the literature.²

3-((((2S,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)thio)methyl benzoic acid (4x).



Following **General Procedure B**, compound **4x'** (110 mg, 0.20 mmol) was treated with formic acid/water (1:1, 2.0 mL/mmol) and the reaction mixture was stirred at room temperature for 18 h. Upon completion of the reaction, the crude product was subjected to washing with hot MeOH (3 x 2 mL), followed by a rinse with acetonitrile (2 x 3 mL). The resulting material was then dried under vacuum, yielding the desired compound **4x** as a white solid (86.3 mg, 86% yield). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 12.94 (s, 1H), 8.34 (s, 1H), 8.14 (s, 1H), 7.90 (s, 1H), 7.80 (d, $J = 7.7$ Hz, 1H), 7.49–7.45 (m, 1H), 7.39 (t, $J = 7.6$ Hz, 1H), 7.30 (s, 2H), 5.89 (d, $J = 5.5$ Hz, 1H), 5.49 (d, $J = 5.9$ Hz, 1H), 5.30 (d, $J = 5.2$ Hz, 1H), 4.74 (q, $J = 5.4$ Hz, 1H), 4.16 (q, $J = 4.6$ Hz, 1H), 4.02 (m, 1H), 3.83 (s, 2H), 2.85 (dd, $J = 13.8, 5.9$ Hz, 1H), 2.71 (dd, $J = 13.8, 6.9$ Hz, 1H); ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$): δ 167.6, 163.5, 156.2, 152.7, 149.8, 140.5, 139.6, 133.7, 131.4,

130.1, 129.0, 128.2, 119.6, 88.0, 84.0, 73.0, 73.0, 35.6, 33.8. The spectral data are consistent with those reported in the literature.¹⁷

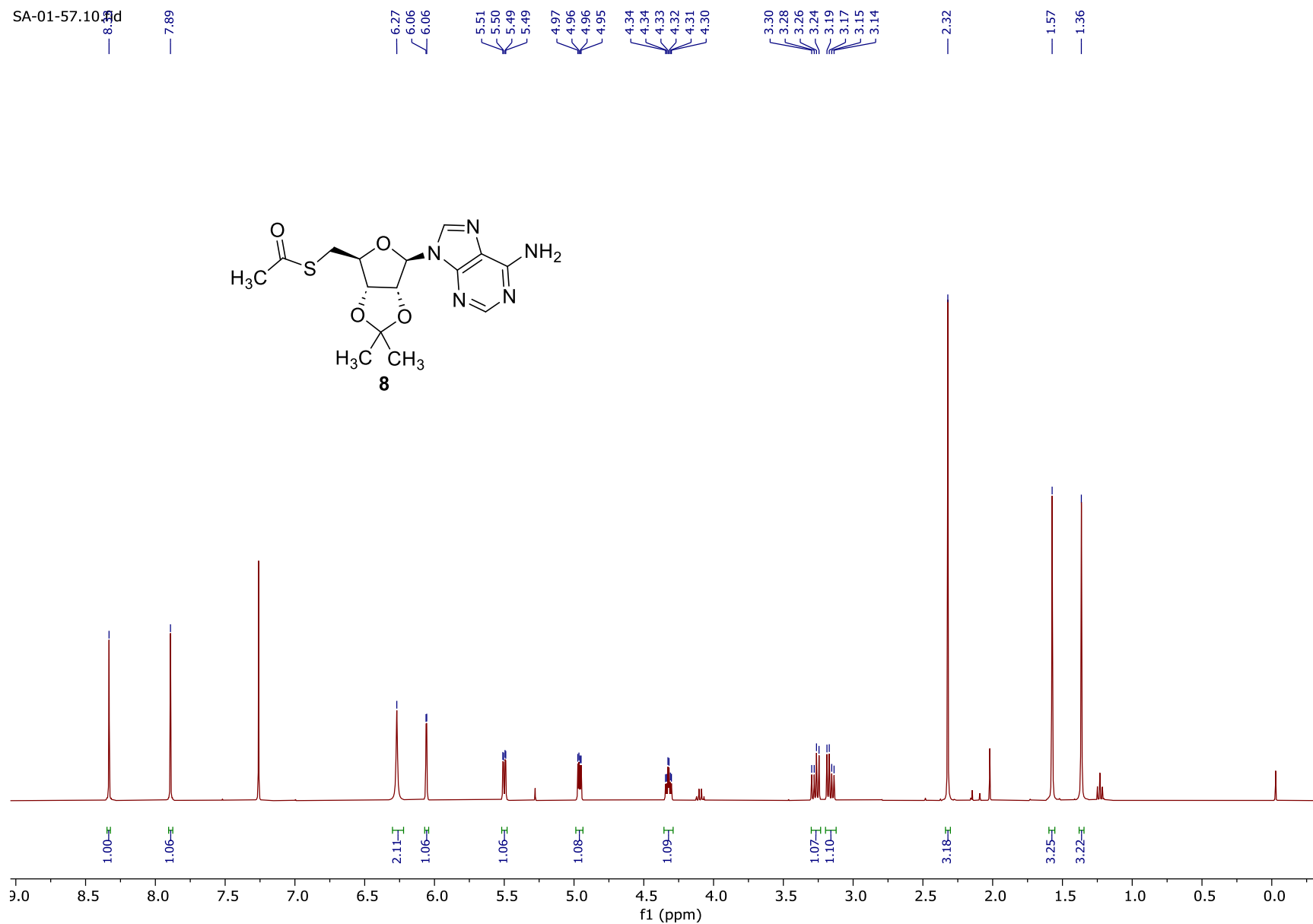
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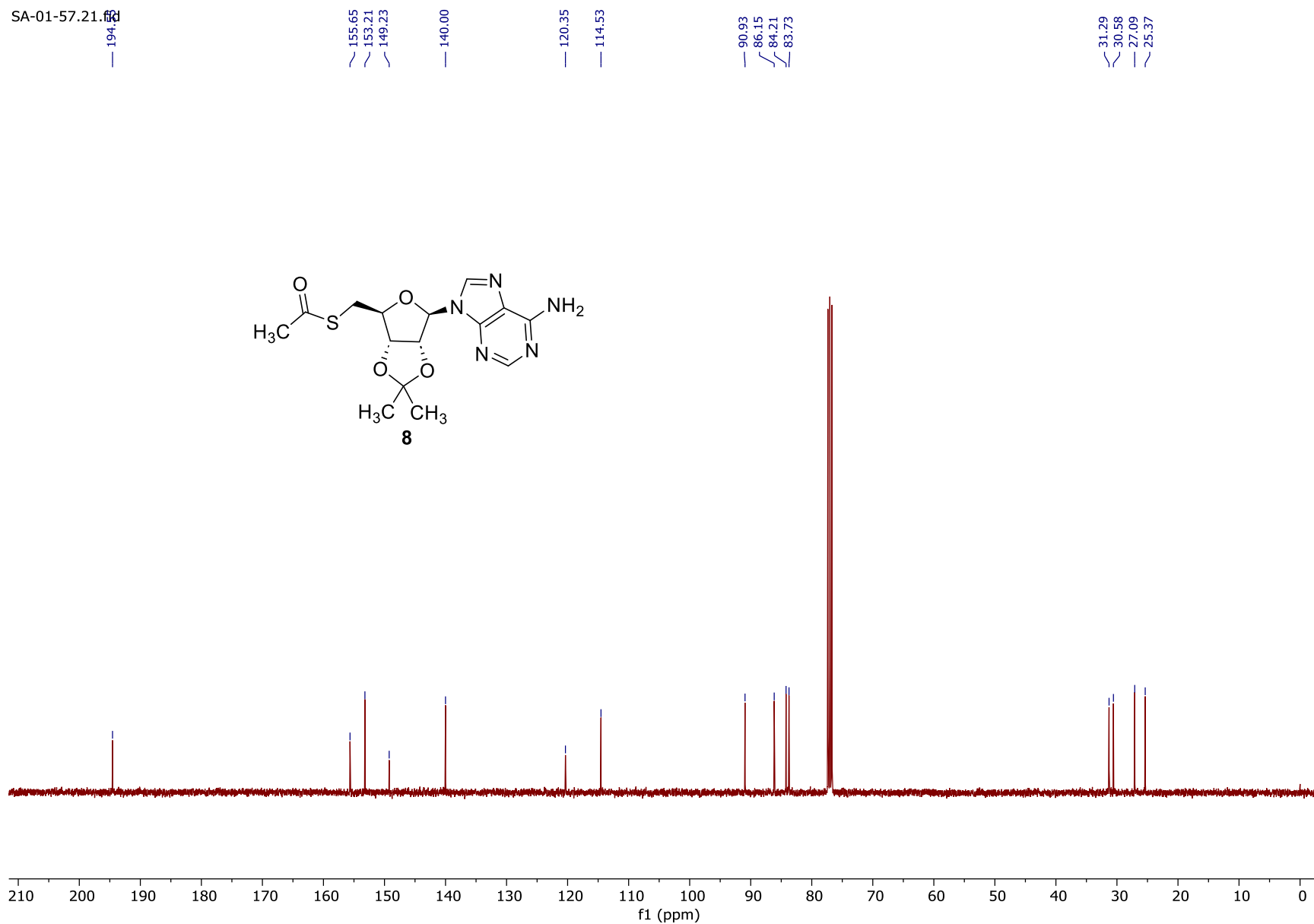
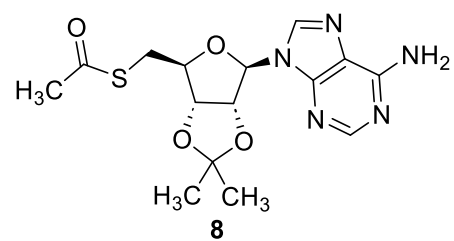
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9. Chemical Validation NMR spectra

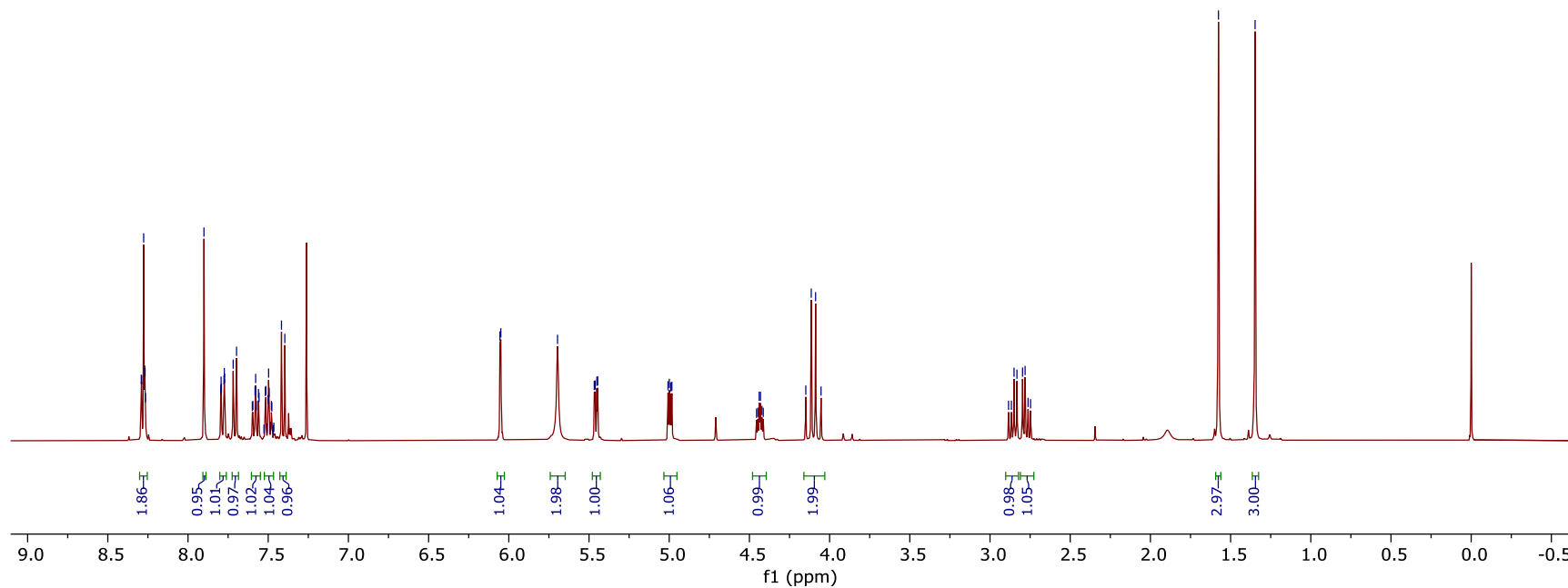
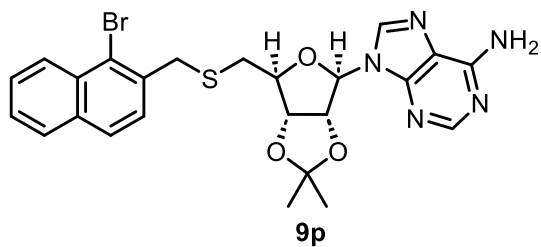
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SA-01-57.21.61



SA-01111072



SA-01-111f.20.fid

155.53
153.13
149.22

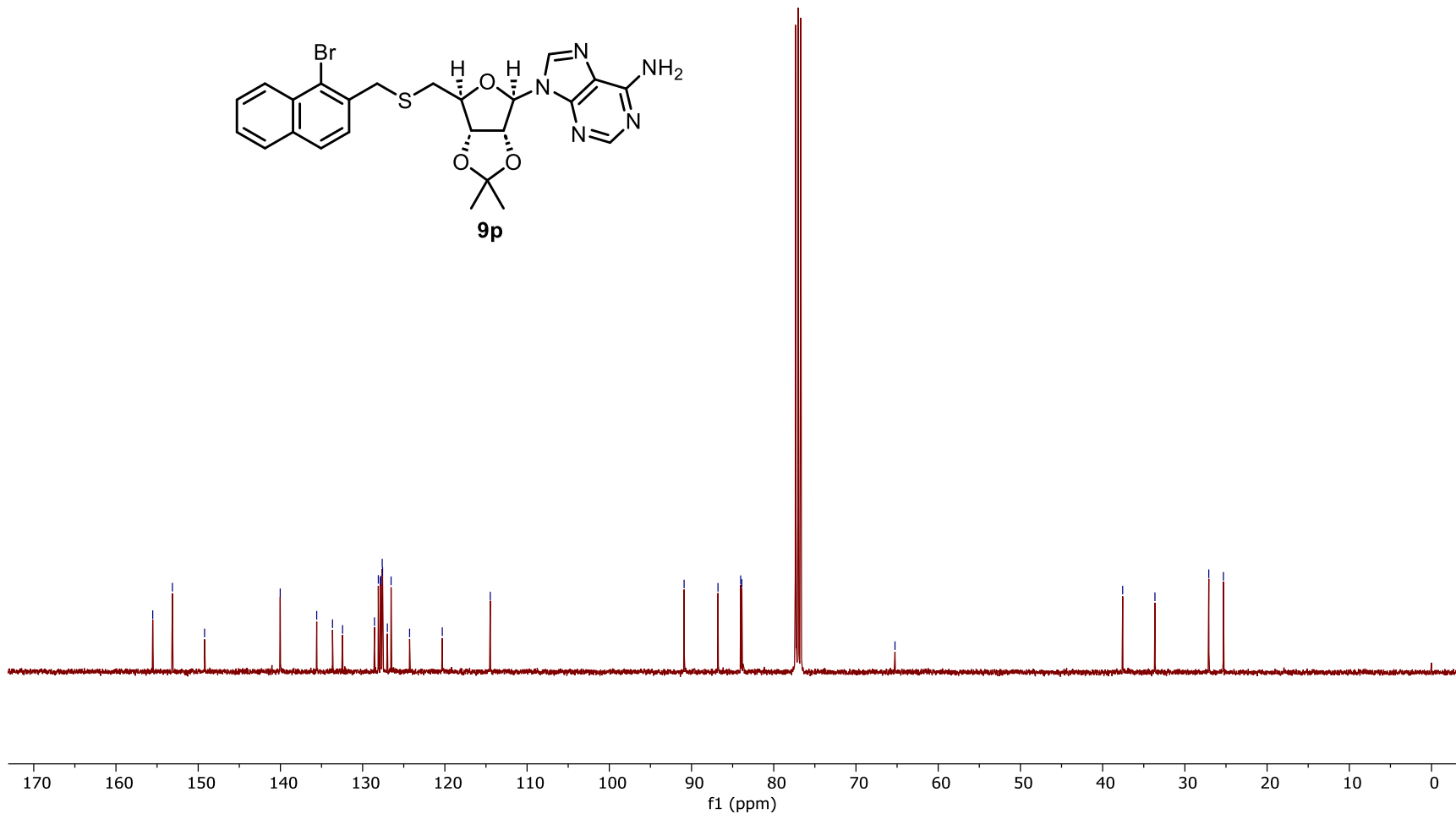
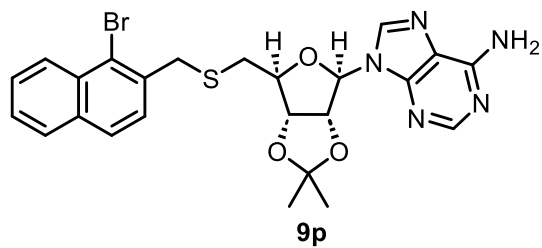
140.02
135.59
133.66
132.44
128.56
128.09
127.85
127.78
127.62
127.57
126.99
126.54
124.29
120.31
114.48

90.90
86.78
84.02
83.88

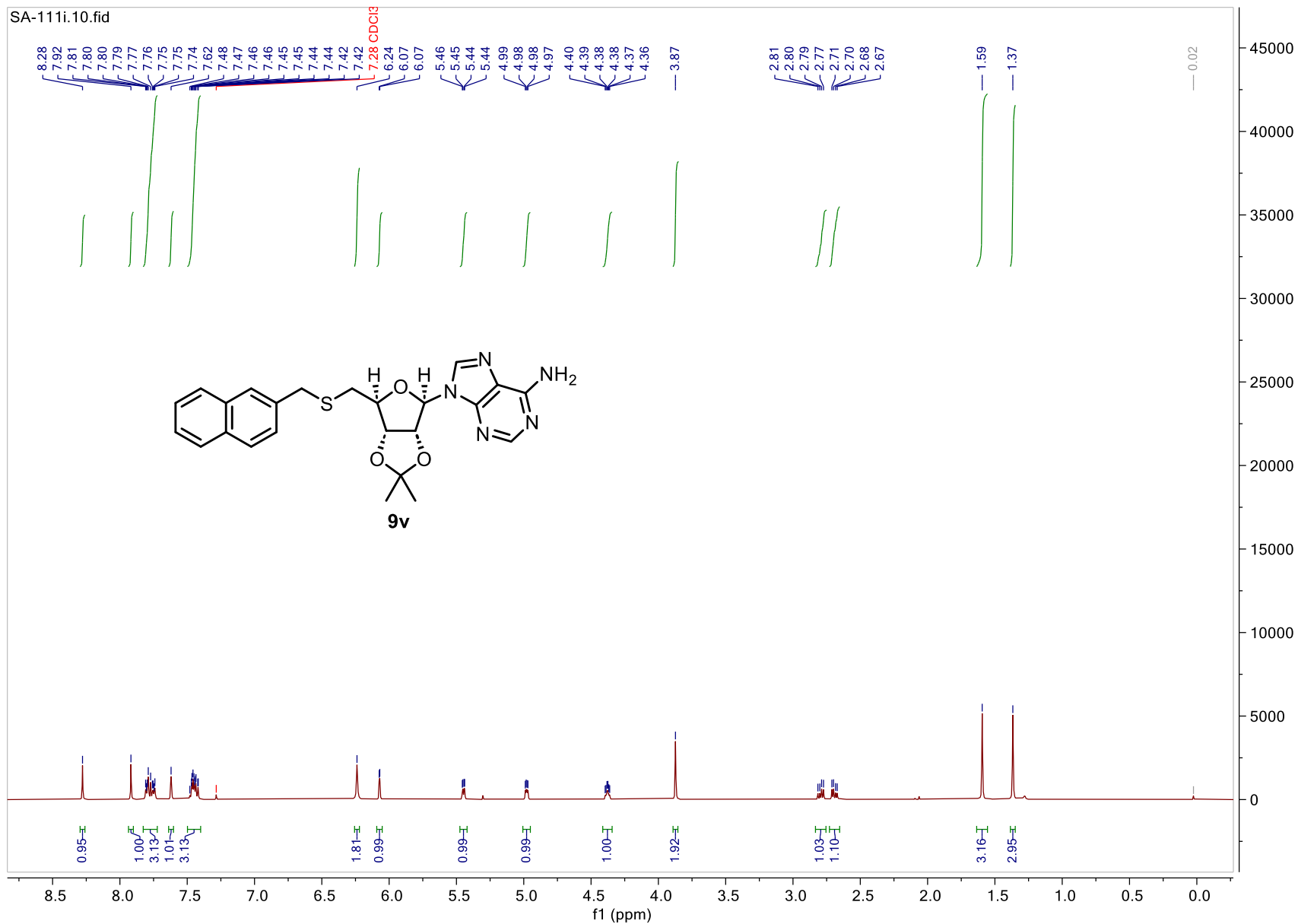
65.25

37.56
33.64

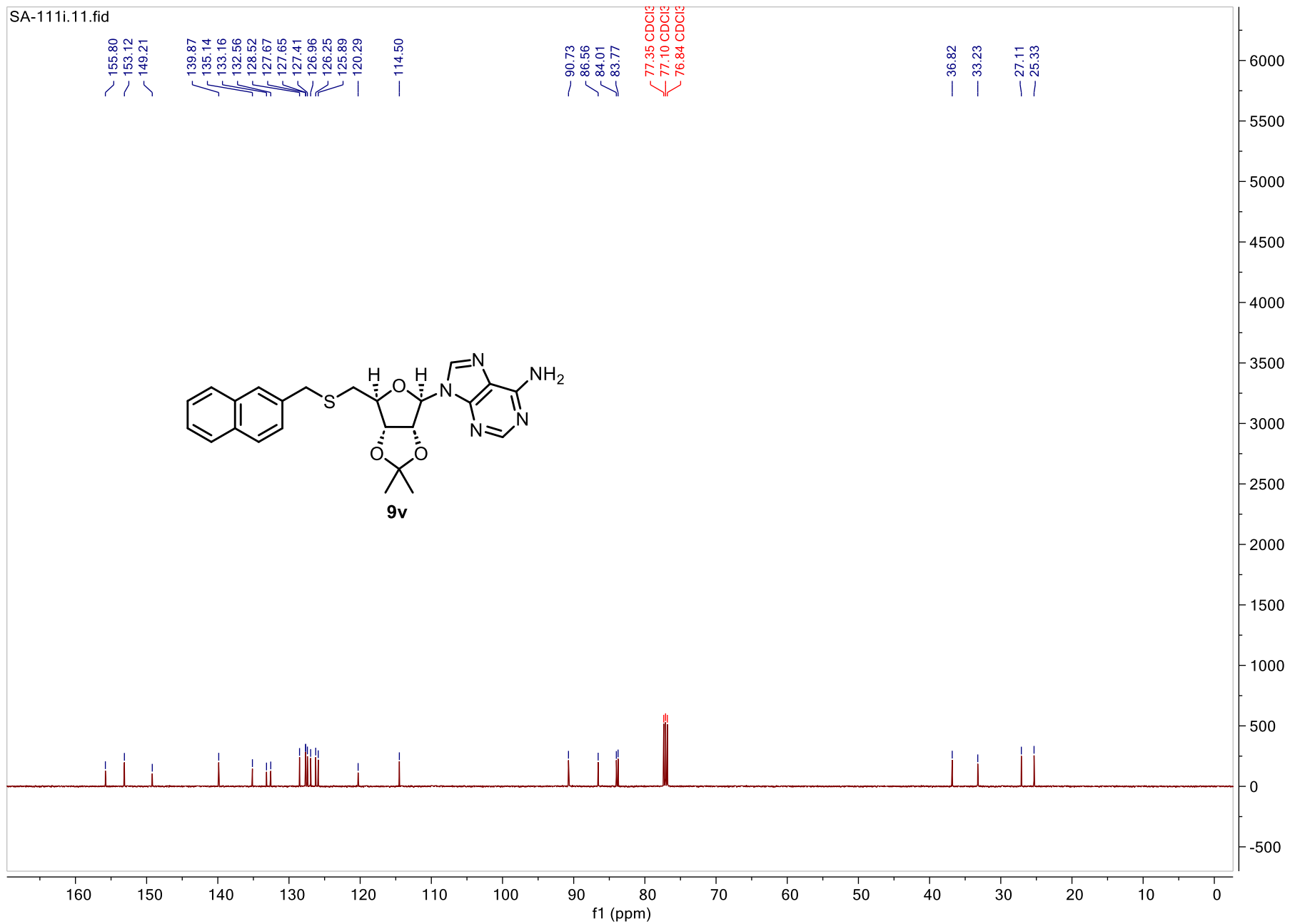
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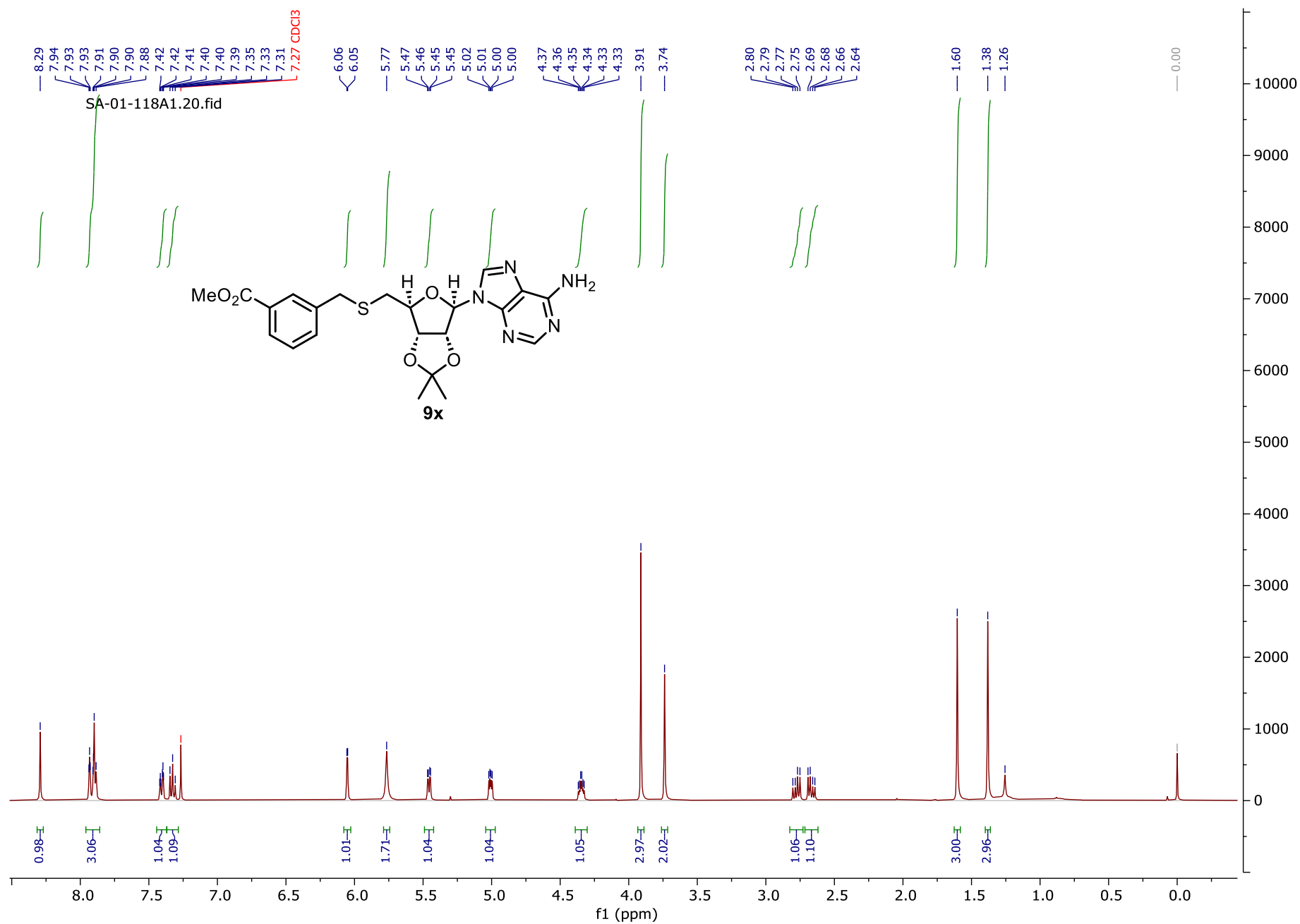


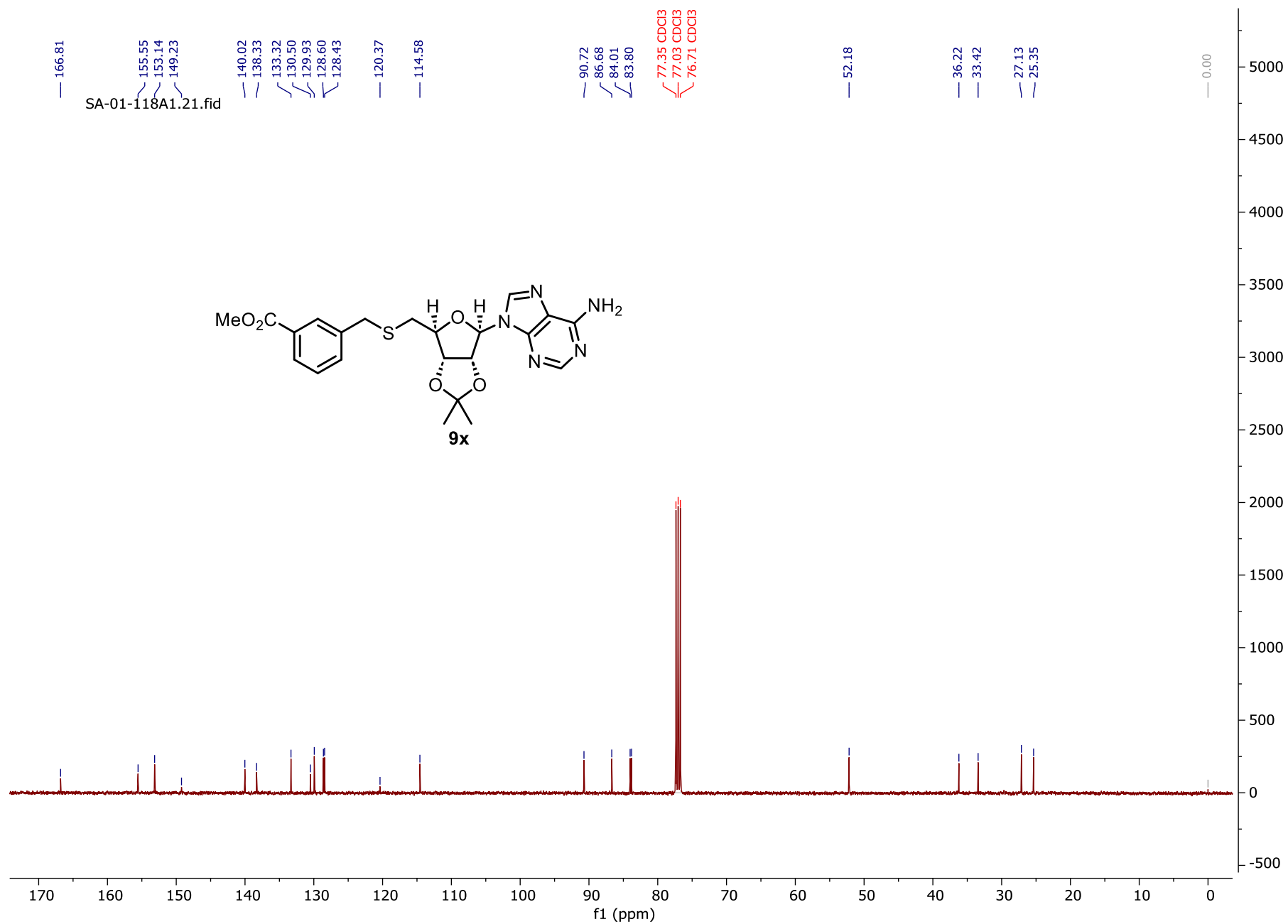
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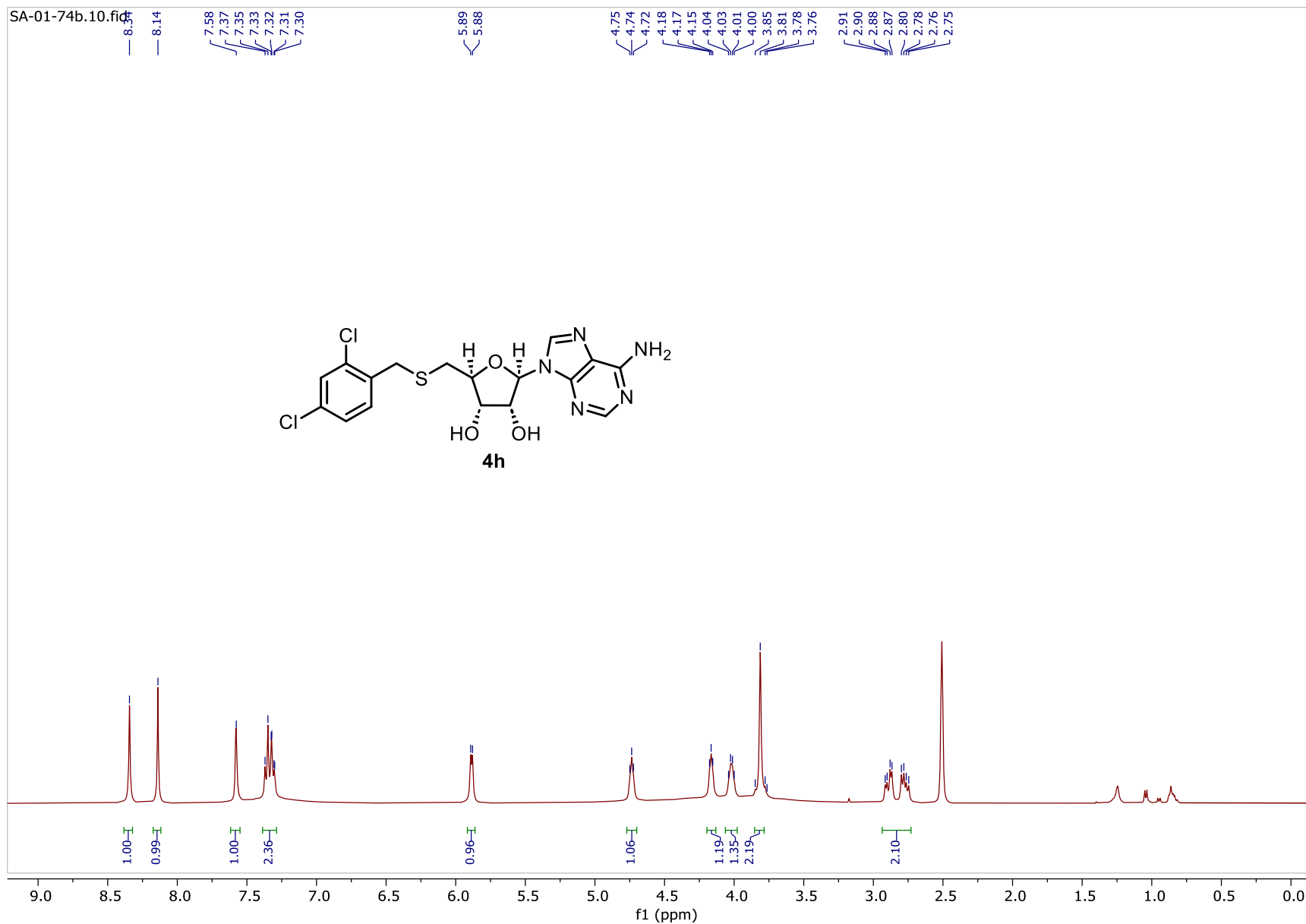
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SA-01-74b.11.fid

156.07
152.62
149.37

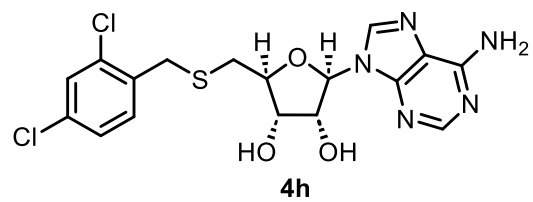
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135.32
133.95
132.32
132.21
128.97
127.16

119.19

87.73
83.91

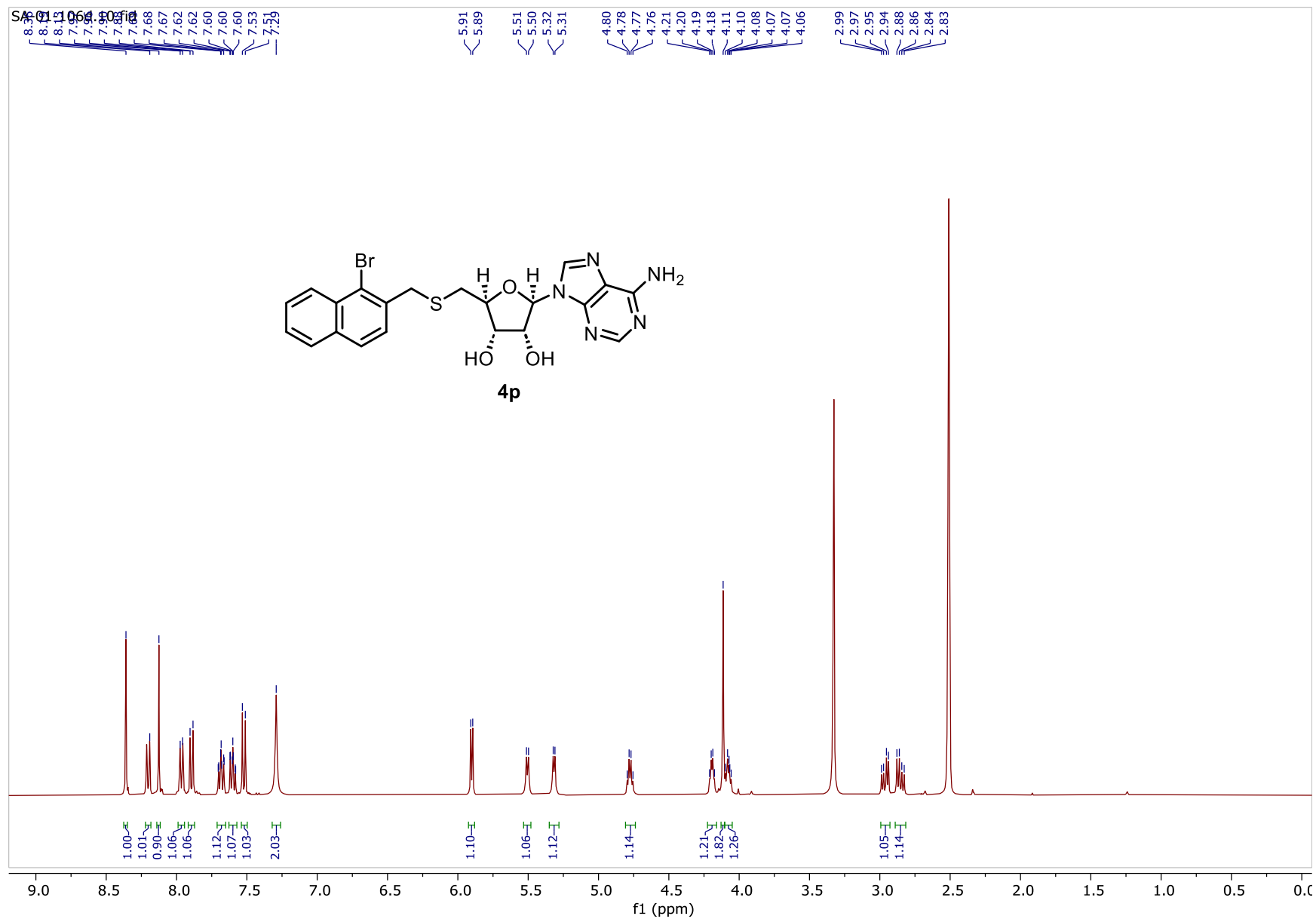
72.68
72.55

33.63
32.83



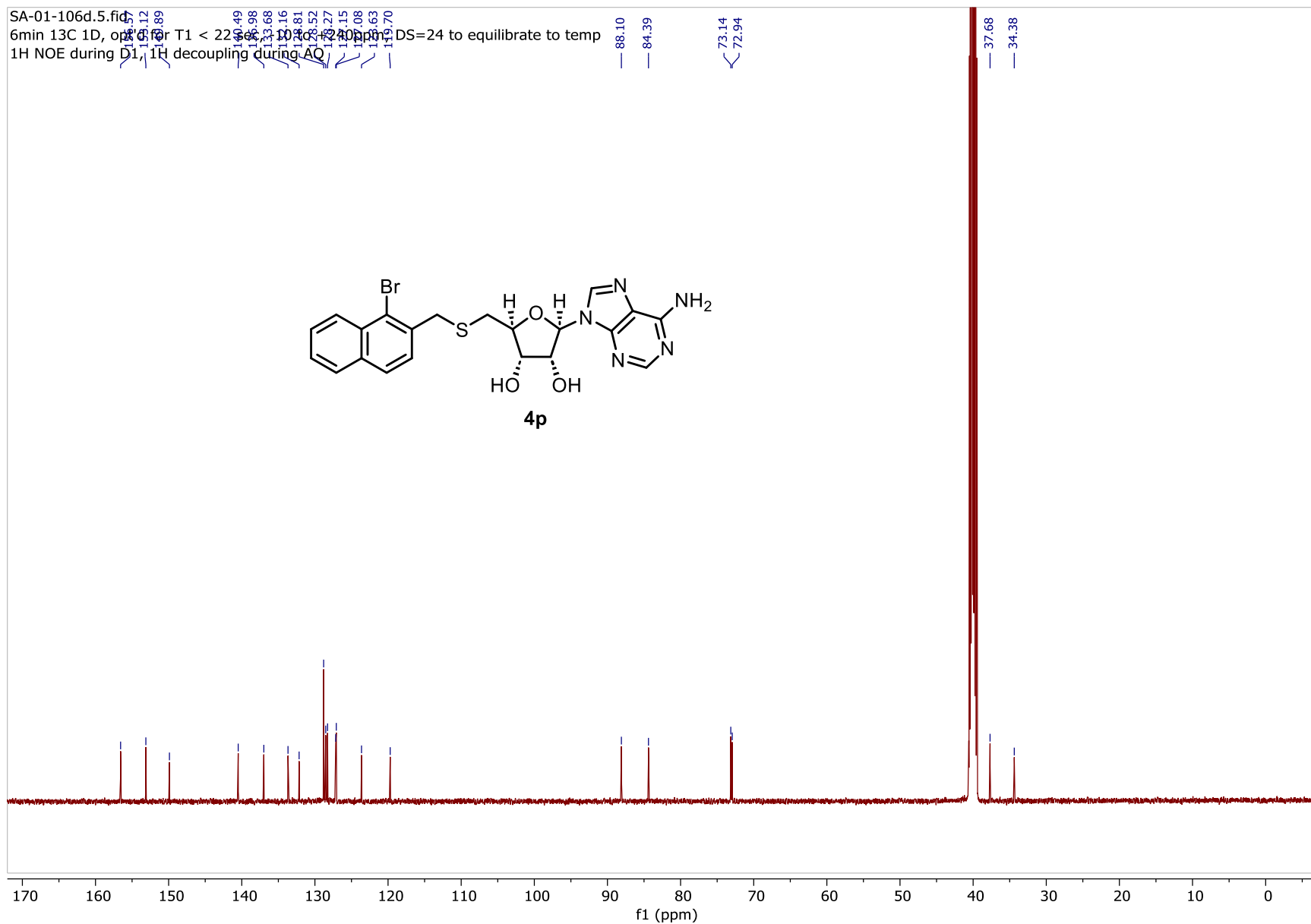
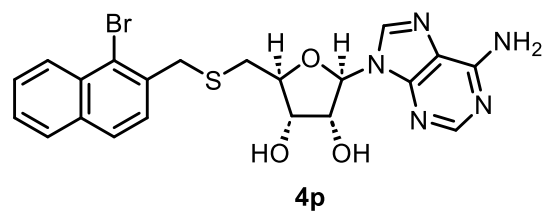
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f1 (ppm)

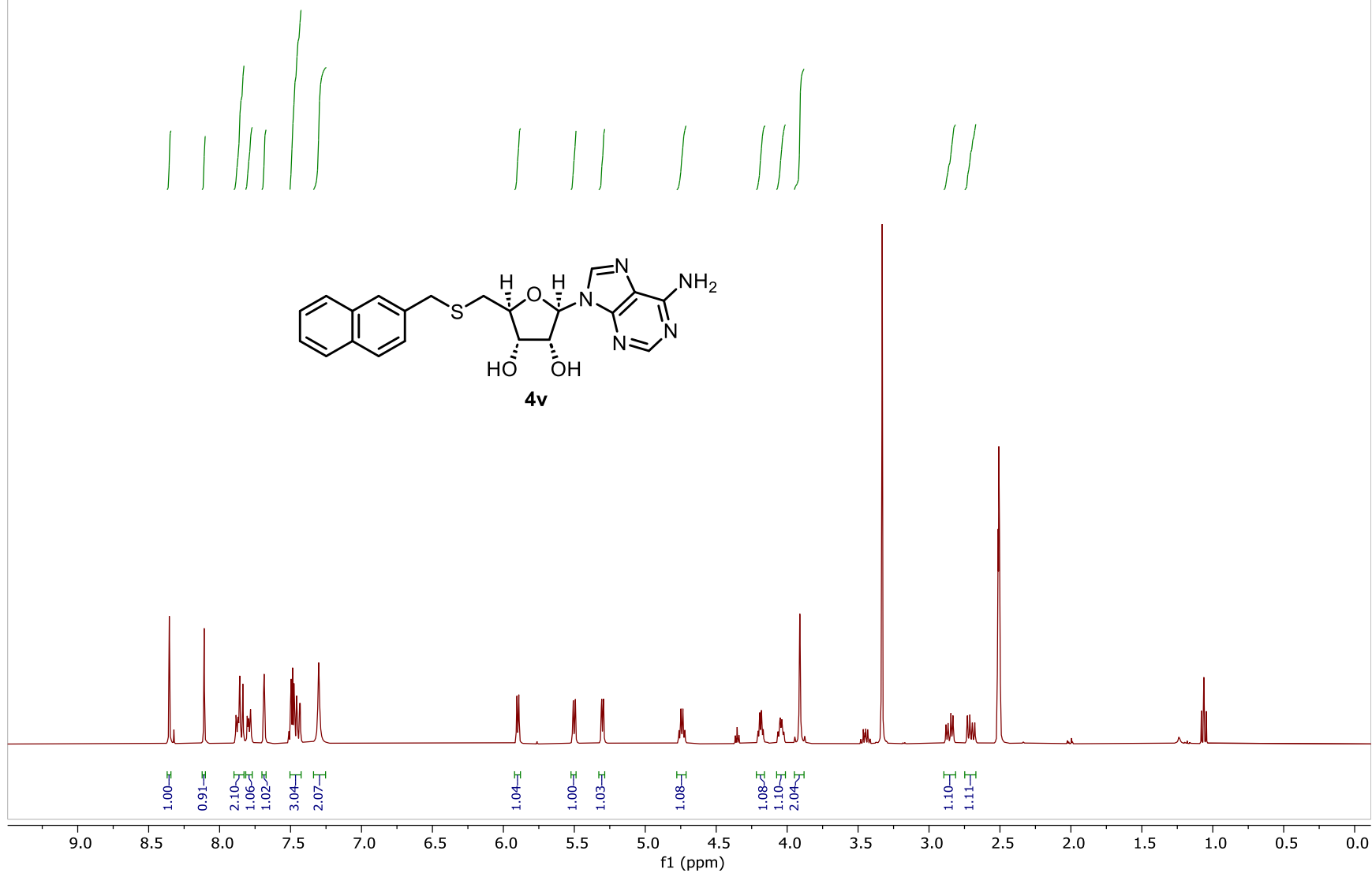


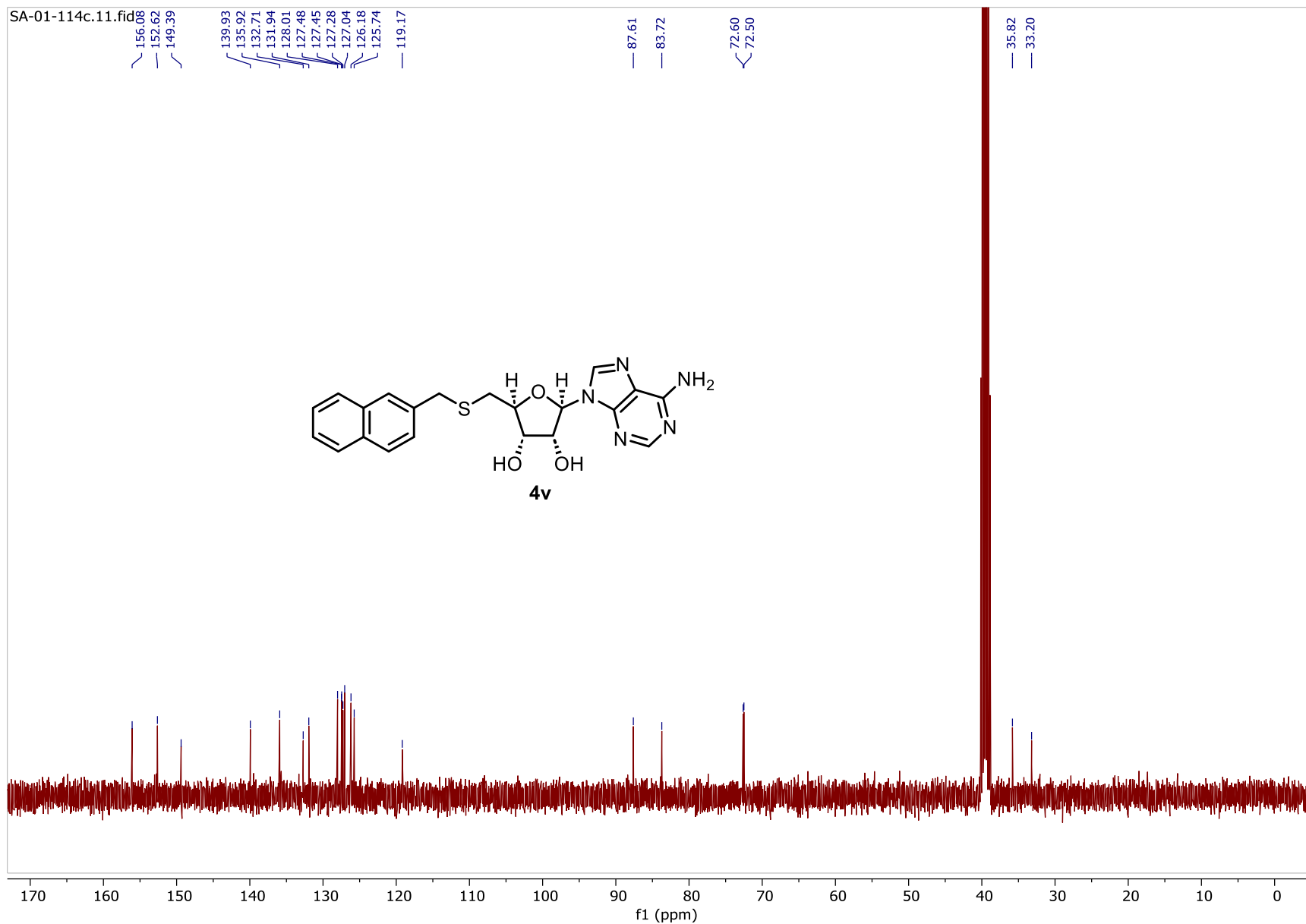
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6min 13C 1D, op 6 for T1 < 22 sec, 10% to 100% DS=24 to equilibrate to temp
1H NOE during D1, 1H decoupling during AQ



SA-01-114c.10.fid





SA-01-118A.21.fid

170.84

157.37

153.95

150.19

141.86

140.21

134.16

133.00

131.12

129.45

129.34

120.59

115.52

91.59

88.15

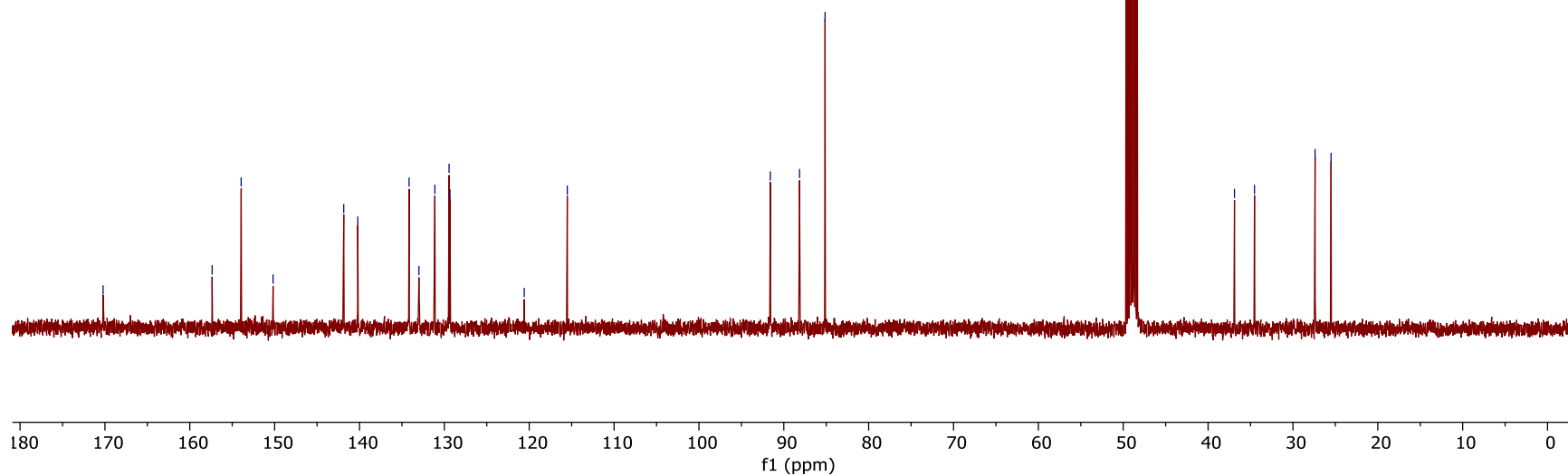
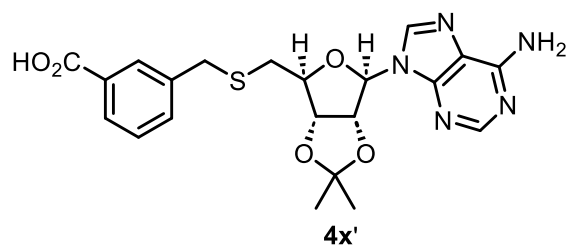
85.14

36.89

34.53

27.39

25.51



SA-01-118.30.fid

