Supporting Information

Bi-substate Inhibitors of Severe Acute Respiratory Syndrome Coronavirus-2 Nsp14 Methyltransferase

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Abbreviations

MTase, methyltransferase; RNMT, mRNA cap guanine-*N*7 methyltransferase; CMTR1, mRNA cap 2'-*O*-Me methyltransferase; cAMP, cyclic AMP; DTT, dithiothreitol; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; ACE2, angiotensin converting enzyme 2; TMPRSS2, transmembrane serine protease 2; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; NEAA, non-essential amino acids; DAPI, 4′,6-diamidino-2-phenylindole; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; TSA, thermal shift assay; NADPH, nicotinamide adenine dinucleotide phosphate; UDPGA, uridine 5'-diphosphoglucuronic acid; PAMPA, parallel artificial membrane permeability assay.

Table S1. Shifts in melting temperature of selected inhibitors.

Compd	MTase IC_{50} (μM)	Shift in melting temperature at 200 μM (°C)
3	0.061	8.38 ± 0.14
6	0.048	8.81 ± 0.01
10	0.093	7.96 ± 0.24
sinefungin	0.26	1.84 ± 0.01

Table S2. Screening of compound **3** against a panel of 10 human MTases.

Human MTase	% Inhibition at 10 μM	Reference compound
G9a	11 ± 1	UNC0646
DOT1L	10 ± 5	SGC0946
MLL1 Complex	1 ± 1	SAH
PRMT1	5 ± 3	MS023
PRMT3	-5 ± 3	SGC707
SUV39H1	11 ± 1	Chaetocin
METTL3/METTL14	10 ± 6	SAH
DNMT1	5 ± 1	SAH
DNMT3A/DNMT3L	4 ± 1	SAH
EZH1	2 ± 7	GSK343

Assays were performed by BPS Bioscience.

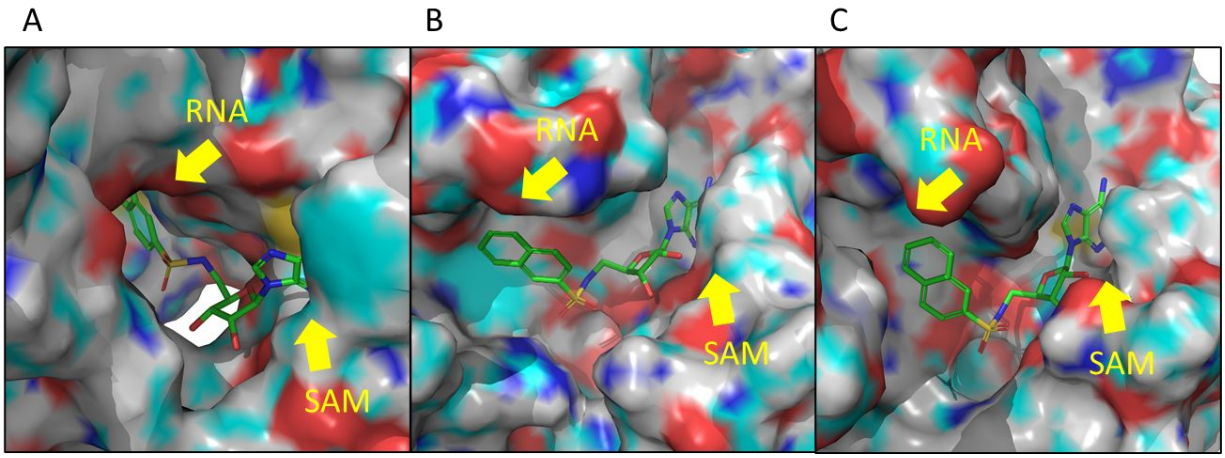


Figure S1. The overall geometry of the MTase active sites. (**A**) SARS-CoV-2 Nsp14 MTase (PDB 7EGQ) with compounds **3**. (**B**) Human RNMT (PDB 3EPP) with compound **3**. (**C**) Human CMTR1 (PDB 4N49) with compound **3**. RNA and SAM approximately indicate the RNA- and SAM-binding pockets, respectively.

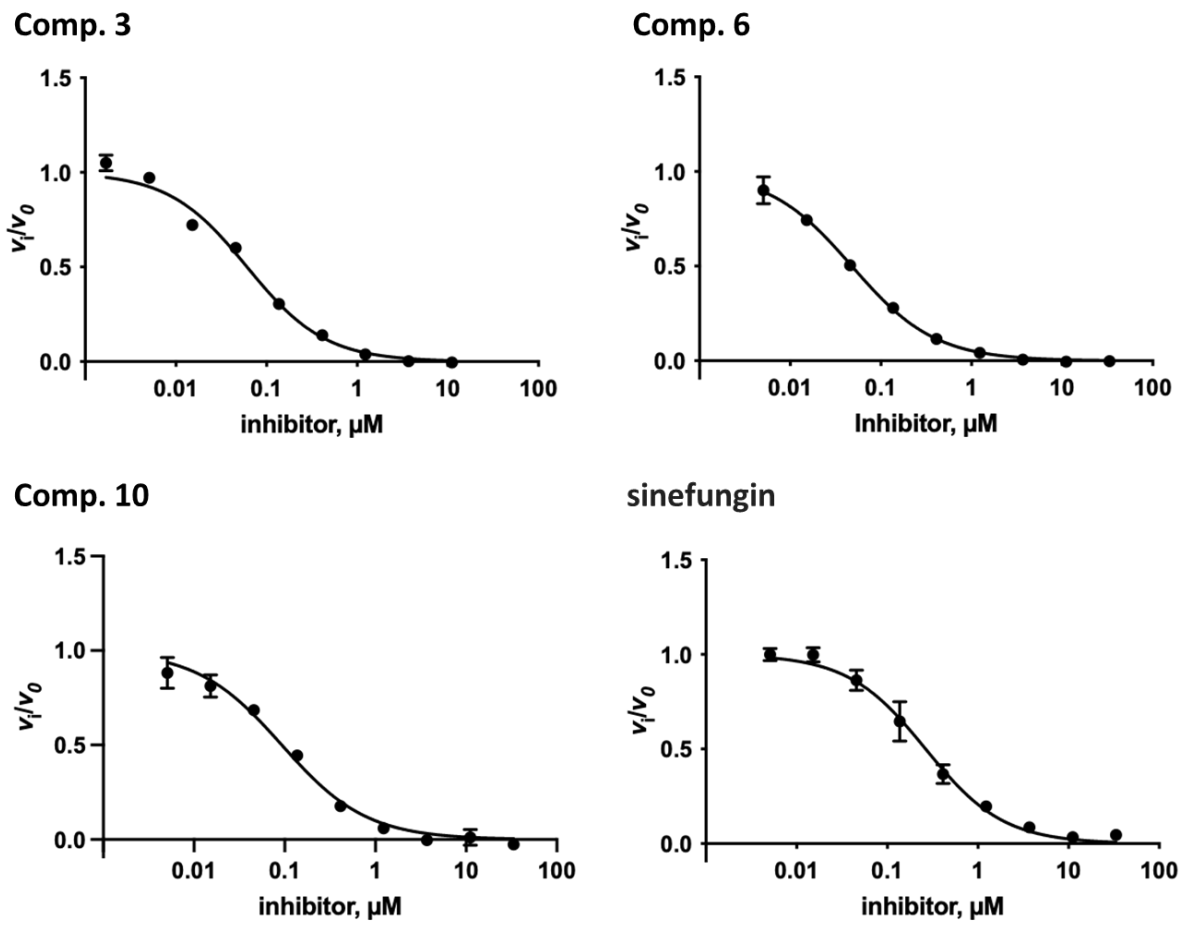


Figure S2. Dose-response curves of representative compounds tested in the biochemical methyltransferase assay using LC-MS/MS.

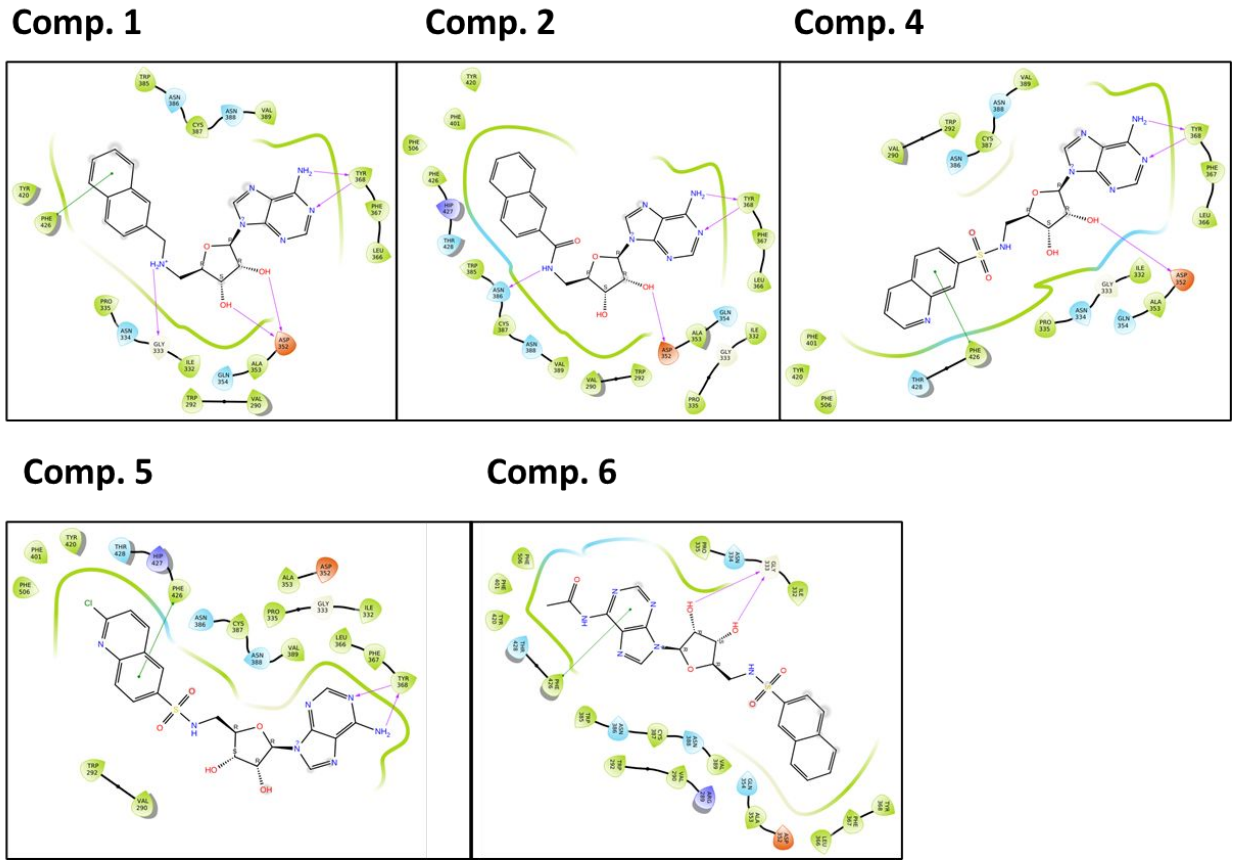


Figure S3. Protein-ligand interaction diagrams of selected nucleosides in the active site of SARS-CoV-2 Nsp14 MTase (PDB 7EGQ).

EXPERIMENTAL METHODS

SARS-CoV-2 Nsp14 MTase Assay

Protein expression and purification. The plasmid pCDFDuet-1-nsp14S2 was transformed into *E. coli* BL21(DE3) STAR for expression. All media was supplemented with 50 μg/mL streptomycin for plasmid maintenance. Overnight cultures were used to inoculate 800 mL LB, 1% final concentration in a 3L Fernbach flask. The culture was grown at 37 °C 220 RPM to an OD600 0.6. The flask was moved to 4 °C for 10 minutes and then induced with IPTG to a final concentration of 0.4 mM. The culture was incubated overnight at 18°C and 220 RPM. Cultures were centrifuged and the cell pellet was frozen at –80 °C until protein isolation.

The cell pellet was sonicated 4 times 2 min using a power setting of 8 and 30% duty cycle on a Branson 250 Sonifier (Brookfield, CT, USA) in 40 mL of lysis buffer (50 mM Tris pH 8.0, 200 mM NaCl, 20 mM imidazole). Cell debris was removed by centrifugation at 45000 g for 15 min at 4 °C. The protein was purified using a HisTrap HP 5 mL column (Cytiva) on a Bio-Rad NGS system (Hercules, CA, USA). Protein was eluted using a linear gradient from buffer A (50 mM Tris pH 8.0, 200 mM NaCl, 5 mM imidazole) to buffer B (50 mM Tris pH 8.0, 200 mM NaCl, 500 mM imidazole). The purified protein was concentrated using an Amicon Ultra 15 3,000 MWCO filter and desalted using a PD-10 column into storage buffer (10 mM HEPES pH 7.5, 150 mM NaCl). Glycerol was added to 50 % and the protein was stored at –20 °C.

MTase assay. The assay was carried out generally following the procedures reported previously.[1] Assays were set up in 1.5 mL centrifuge tubes at a final volume of 50 μL and consisted of 40 mM Tris pH 8.0, 0.5 mM DTT, 2 mM $MgCl_2$, 100 nM cAMP (mass spec internal standard), 3 μM GpppA, and 10 nM Nsp14. A negative control reaction was included that lacked GpppA. Reactions were preincubated with DMSO or inhibitors (0.5 μL) for 15 min at room temperature and initiated by adding SAM to 0.4 μM. Reactions were incubated for 15 min at 30 °C and quenched with 3 volumes (150 μL) acetonitrile.

LC-MS/MS method. The acetonitrile quenched samples were analyzed using a LC-MS/MS system consisting of an AB Sciex QTrap 5500 mass spectrometer and an Agilent 1260 Infinity HPLC (Santa Clara, CA, USA). Chromatographic separation of the analytes was achieved on a Thermo Aquasil C18 column (150 × 2.1 mm, 3 µm) using two eluents: (A) water with 0.1% formic acid, and (B) acetonitrile with 0.1% formic acid. The mobile phase was delivered at a flow rate of 0.3 mL/min with a gradient of A and B as follows: 0–2.3 min, 5–30% B (v/v); 2.3–2.8 min, 30% B (v/v); 2.8–3.0 min, 30–5% B (v/v); 3.0–6.0 min, 5% B (v/v). MS/MS detection of SAH and SAM was conducted using positive electrospray ionization with mass transitions of m/z 385.2→136.0 and m/z 399.2→250.1, respectively. Cyclic AMP (cAMP, m/z 330.0→136.0) was used as an internal standard. Quantitation of SAH and SAM were performed using matrix-matched 7-points standard curves. SAH concentrations were normalized to the cAMP signal and data was fit using GraphPad Prism (GraphPad Software; San Diego, CA, USA) to determine IC_{50} values.

Cells and Viruses

A549/ACE2/TMPRSS2 cells[2] (provided by M. Saeed, Boston University; Boston, MA, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM) high glucose supplemented with 10% fetal bovine serum (FBS), 100 IU streptomycin/penicillin per mL, 10 mM HEPES, 1X Non-Essential Amino Acids (NEAA), 1X Glutamax, 1mM sodium pyruvate, 5 µg/mL plasmocin, 0.5 µg/mL puromycin and 0.5 µg/mL blasticidin). SARS-CoV-2 isolate USA-WA1/2020 (NR-52281) was obtained through BEI Resources **(**Manassas, VA, USA) and propagated in Vero-E6 cells.

SARS-CoV-2 Immunofluorescence Antiviral Assay

$1.5x10^4$ A549/ACE2/TMPRSS2 cells per well were plated in a 96 well plate. Next day, the medium was replaced with 50 µL of SARS-CoV-2 infection medium (DMEM high glucose supplemented with 5% FBS, 100 IU streptomycin/penicillin per mL, 10 mM HEPES, 1X NEAA, 1X Glutamax and 1mM sodium pyruvate). Cells were treated with compound in 50 µL infection medium, transferred to the BSL-3 facility and inoculated with 50 µL infection medium containing SARS-CoV-2 at an MOI of 0.01. Forty-eight hours post infection, the cells were fixed with 4% PFA in 1X PBS for 30 min and processed for immunofluorescence using an anti- SARS-CoV-2 nucleoprotein antibody (Sino Biologicals 40588-T62; Wayne, PA, USA) as described.[3] Images

were acquired using a BioTek Cytation 1™ imaging reader (Winooski, VT, USA) and the total number of DAPI-stained cells and infected cells for each well were quantified in Gen5 software. Percentage of infected cells was determined by dividing the number of infected cells by the total number of cells per well. The percentage of infected cells and total number of cells for each treatment was plotted into GraphPad prism (GraphPad Software; San Diego, CA, USA) to perform a non-linear regression analysis, generate infectious dose-response curves and to calculate EC_{50} and CC_{50} values.

Cell Viability Assay

Viability assays for the non-infected A549/ACE2/TMPRSS2 cells were performed using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS)-based tetrazolium reduction CellTiter 96 Aqueous Non-Radioactive cell proliferation assay (Promega G5430; Madison, WI) as described.[3-4] Assays were conducted using parallel plates to those used in the immunofluorescence assay. Experimental details (cell line, number of cells seeded and incubation time) corresponded to those used in the immunofluorescence assay above. The data obtained were used to calculate the CC_{50} value using GraphPad Prism software.

Thermal Shift Assay (TSA)

Assays were run in duplicate in an ABI 96 well 0.2 mL PCR plate in 25 µL final volume. Master mixes containing 5 µM Nsp14 in buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1 mM $MgCl_2$) with 5.6 X Sypro Orange (Sigma Aldrich) were added to the plates containing 0.5 µL DMSO or ligand (200 µM final concentration). The final DMSO concentration was 3.1% including both ligand and Sypro Orange. Reactions were mixed with a multi-channel pipette. The plate was sealed using Pure Amp P1001-Q sealing film (MTC Bio) and centrifuged at 3000 g for 3 min to remove any air bubbles. TSA assays were run on a QuantStudio 3 (Applied Biosystems) in standard run mode and melt curve experiment type. The plate was held at 25 °C for 1 min and then ramped to 95 °C at 0.015 °C/s. The instrument was set to detect ROX dye. Data was analyzed using Thermofisher Protein Thermal Shift Software using the Boltzman method to generate T_ms.

Human MTase Screening

Assay conditions. The human methyltransferase inhibition assays were performed at BPS Bioscience (San Diego, CA, USA). All of the enzymatic reactions were conducted in duplicate at room temperature for 60-960 min in a 50 µL mixture containing proper methyltransferase assay buffer, *S*-adenosylmethionine (SAM), enzyme, and the test compound. These 50 µL reactions were carried out in wells of a substrate pre-coated plate. First, enzyme and inhibitor were added to assay wells and preincubated for 30 min at room temperature, then SAM was added to start the reaction. Final DMSO concentration was 1% in all reactions.

After enzymatic reactions, the reaction mixtures were discarded and each of the wells was washed three times with TBST buffer, and slowly shaken with Blocking Buffer for 10 min. Wells were emptied, and 100 µL of diluted primary antibody was added. The plate was then slowly shaken for 60 minutes at room temperature. As before, the plate was emptied and washed three times with TBST, and shaken with Blocking Buffer for 10 minutes at room temperature. After discarding the Blocking Buffer, 100 µL of diluted secondary antibody was added. The plate was then slowly shaken for 30 min at room temperature. As before, the plate was emptied and washed three times with TBST, and shaken with Blocking Buffer for 10 minutes at room temperature. Blocking Buffer was discarded and a mixture of the HRP chemiluminescent substrates was freshly prepared. 100 µL of this mixture was added to each empty well. Immediately, the luminescence of the samples was measured in a BioTek Synergy™ 2 (Winooski, VT, USA) microplate reader using Gen5 software.

Data analysis. Enzyme activity assays were performed in duplicates at each concentration. The luminescence data/alpha counts were analyzed and compared. In the absence of the compound, the intensity (C_e) in each data set was defined as 100% activity. In the absence of enzyme, the intensity (C_0) in each data set was defined as 0% activity. The percent activity in the presence of each compound was calculated according to the following equation: % activity = $(C-C_0)/(C_e-C_0)$, where C= the luminescence/alpha counts in the presence of the compound.

In vitro Metabolic Stabilities and Permeability

Plasma stability assay. The plasma stability assay was performed in triplicate by incubating each selected compound (1 µmol/L final concentration) in normal mouse (CD-1) or human plasma

(Innovative Research; Novi, MI, USA) diluted to 80% with 0.1 mol/L potassium phosphate buffer (pH 7.4) at 37 °C. At 0, 1, 3, 6, and 24 h, a 50 μL aliquot of the plasma mixture was taken and quenched with 150 μL of acetonitrile containing 0.1% formic acid. The samples were then vortexed and centrifuged at 15,000 rpm (Eppendorf centrifuge 5424R; Enfield, CT, USA) for 5 min. The supernatants were collected and analyzed by LC-MS/MS to determine the *in vitro* plasma half-life ($t_{1/2}$).

Liver S9 stability assay. The *in vitro* liver S9 stability assay was conducted in duplicate in commercially available mouse and human liver S9 fractions (Sekisui XenoTech; Kansas City, KS, USA), which were supplemented with either nicotinamide adenine dinucleotide phosphate (NADPH) or uridine 5'-diphosphoglucuronic acid (UDPGA) as a cofactor. Briefly, a compound (1 μmol/L final concentration) was spiked into the reaction mixture containing liver microsomal protein (1 mg/mL final concentration), $MgCl_2$ (1 mmol/L final concentration), and/or alamethicin (50 μg/mg protein, only in incubation with cofactor UDPGA) in 0.1 mol/L potassium phosphate buffer (pH 7.4). For phase I metabolism, the reaction was initiated by addition of 1 mmol/L NADPH, followed by incubation at 37 °C. A negative control was performed in parallel without NADPH to reveal any chemical instability or non-NADPH dependent enzymatic degradation for each compound. A reaction with positive control verapamil was also performed as an in-house quality control to confirm the proper functionality of the incubation systems. For phase II metabolic conjugation, the mixture was first pre-incubated on ice for 10 min to allow the pore-formation peptide alamethicin to penetrate the membrane of microsomes and to expose UGT enzymes. The reaction was then initiated by the addition of 5 mmol/L UDPGA, followed by incubation at 37 °C. A negative control was performed in parallel without UDPGA to reveal any chemical instability or non-UDPGA dependent enzymatic degradation for each compound. A reaction with positive control umbelliferone was also performed as an in-house quality control to confirm the proper functionality of the incubation systems. At various time points (0, 5, 15, 30, 45 or 60 min), a 50 μL aliquot of reaction was taken and quenched with 150 μL of acetonitrile containing 0.1% formic acid. The samples were then vortexed and centrifuged at 15,000 rpm for 5 min at 4 °C. The supernatants were collected and analyzed by LC-MS/MS to determine the *in vitro* metabolic half-life ($t_{1/2}$).

Parallel artificial membrane permeability assay (PAMPA).[5] The membrane permeability of selected compounds was evaluated using the Corning® BioCoat™ Pre-coated PAMPA Plate System (Corning 353015; Glendale, AZ, USA). The pre-coated plate assembly, which was stored at –20°C, was taken to thaw for 30 min at room temperature. The permeability assay was carried out in accordance with the manufacturer's protocol. Briefly, the 96-well filter plate, pre-coated with lipids, was used as the permeation acceptor and a matching 96 well receiver plate was used as the permeation donor. Compound solutions were prepared by diluting the 10 mmol/L DMSO stock solutions with 10% methanol in DPBS to a final concentration of 10 μmol/L. The compound solutions were added to the wells (300 μL/well) of the receiver plate and DPBS with 10% methanol was added to the wells (200 μL/well) of the pre-coated filter plate. The filter plate was then coupled with the receiver plate and the plate assembly was incubated at 25 °C without agitation for 5 h. At the end of the incubation, the plate was separated and the final concentrations of compounds in both donor wells and acceptor wells were analyzed using LC-MS/MS. Permeability of a compound was calculated using the Eq. (1):

$$Pe = \{-ln\,[1-C_A(t)/C_{eq}]\}/[A\times(1/V_D+1/V_A)\times t] \quad (1)$$

where A = filter area (0.3 cm^2), V_D = donor well volume (0.3 mL), V_A = acceptor well volume (0.2 mL), t = incubation time (seconds), $C_A(t)$ = compound concentration in acceptor well at time t, $C_D(t)$ = compound concentration in donor well at time t, and C_{eq} was calculated using the Eq. (2):

$$C_{eq} = [C_D(t)\times V_D+C_A(t)\times V_A]/(V_D+V_A) \quad (2)$$

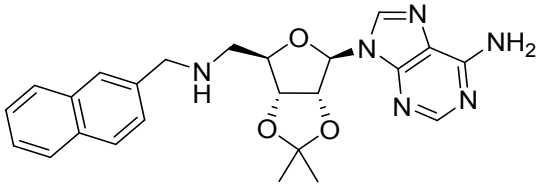
A cutoff criterion of *Pe* value at 1.5×10^{-6} cm/s was used to classify the compounds into high and low permeability according to the literature report of this PAMPA plate system.[5]

Molecular Modeling

The docking study was carried out using the Schrodinger modeling package (release 2019-3). The cryo-EM structure of SARS-CoV-2 Nsp14 (PDB 7EGQ)[6] was taken from the Protein Data Bank. The missing residues were added to the Nsp14 structure using Prime and the energy minimization was performed using OPLS3e forcefield. Nsp14 MTase inhibitors were generated by LigPrep and docked into the Glide grid encompassing the SAM and RNA substrate binding pockets of the SARS2-CoV-2 Nsp14 MTase active site. Docking was carried out in the XP mode with no constraints. Structural visualization and representation were performed using PyMOL.

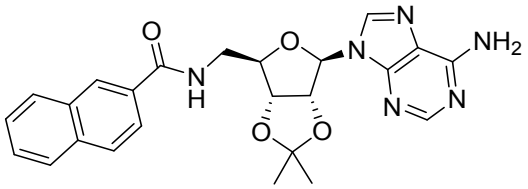
Chemical Syntheses

General Procedures. All commercial reagents were used as provided unless otherwise indicated. An anhydrous solvent dispensing system (J. C. Meyer) using 2 packed columns of neutral alumina was used for drying THF, Et_2O, and CH_2Cl_2, whereas 2 packed columns of molecular sieves were used to dry DMF. Solvents were dispensed under argon. Flash chromatography was performed with RediSep R_f silica gel columns on a Teledyne ISCO CombiFlash® R_f system using the solvents as indicated. Nuclear magnetic resonance spectra were recorded on a Varian 600 MHz or Bruker 400 MHz spectrometer with Me_4Si or signals from residual solvent as the internal standard for ^1H or ^{13}C. Chemical shifts are reported in ppm, and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br s (broad singlet), and dd (double doublet). Values given for coupling constants are first order. High resolution mass spectra were recorded on an Agilent TOF II TOF/MS instrument equipped with either an ESI or APCI interface at the Center for Drug Design, University of Minnesota (Minneapolis, MN, USA). Analysis of sample purity was performed on an Agilent 1200 Infinity series HPLC system with a Phenomenex Gemini C18 column (5 μ, 4.6×250 mm). HPLC conditions were as follows: solvent A = water with 0.1% acetuc acid, solvent B = MeCN or MeOH with 0.1% acetic acid, and flow rate = 2.0 mL/min. Nucleosides were eluted with a gradient of from 10% to 100% MeCN/water or from 10 to 100% MeOH/water in 8 min. Nucleoside prodrugs were eluted with a gradient of from 40% to 100% MeCN/water or from 40 to 100% MeOH/water in 8 min. Purity was determined by the absorbance at 280 nm. All tested compounds have a purity of ≥ 95%. No unexpected or unusually high safety hazards were encountered.

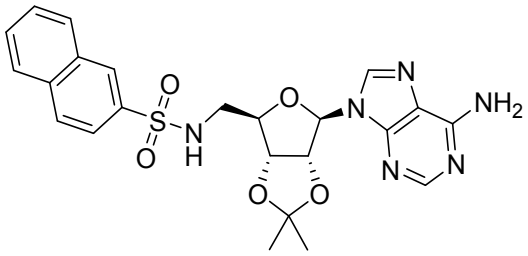


9-((3aR,4R,6R,6aR)-2,2-Dimethyl-6-(((naphthalen-2-ylmethyl)amino)methyl)tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purin-6-amine (15).[7] To a solution of compound **14**[8] (170 mg, 0.55 mmol, 1.0 eq) in methanol (20 mL) at room temperature under argon atmosphere was added 2-naphthaldehyde (130 mg, 0.83 mmol, 1.5 eq). After 3 h,

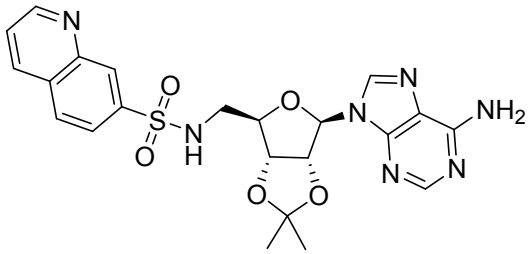
$NaBH_4$ (84 mg, 2.22 mmol, 4.0 eq) was added, and the reaction mixture was stirred for 2 h at room temperature. Water (20 mL) was added, the aqueous layer was extracted with ethyl acetate and the combined organic extracts were washed with brine, dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by column chromatography (10% MeOH in CH_2Cl_2) to give compound **15** as a white solid (210 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.88 (s, 1H), 7.84 – 7.76 (m, 3H), 7.71 (s, 1H), 7.48 – 7.39 (m, 4H), 5.99 (d, *J* = 3.3 Hz, 1H), 5.82 (s, 2H), 5.48 (dd, *J* = 6.4, 3.3 Hz, 1H), 5.07 (dd, *J* = 6.5, 3.2 Hz, 1H), 4.45 – 4.40 (m, 1H), 3.96 (s, 2H), 2.98 (dd, *J* = 12.8, 4.2 Hz, 1H), 2.91 (dd, *J* = 12.6, 6.0 Hz, 1H), 1.61 (s, 3H), 1.38 (s, 3H). HRMS (ESI$^+$): m/z calcd for $C_{24}H_{27}N_6O_3$ $[M+H]^+$ 447.2139, found 447.2128.



***N*-(((3aR,4R,6R,6aR)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-naphthamide (16).**[9] To a solution of compound **14**[8] (95 mg, 0.31 mmol, 1.0 eq) in CH_2Cl_2 (20 mL) at room temperature under argon atmosphere was added triethylamine (0.13 mL, 0.93 mmol, 3.0 eq). The reaction mixture was cooled to 0 ℃ and 2-naphthoyl chloride (88 mg, 0.47 mmol, 1.5 eq) was added. The reaction mixture was stirred for 18 h at room temperature. Water (10 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by column chromatography (5% MeOH in CH_2Cl_2) to give compound **16** as a white solid (135 mg, 93%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.28 (dd, *J* = 8.4, 2.6 Hz, 1H), 7.96 – 7.86 (m, 5H), 7.82 (s, 1H), 7.58 – 7.52 (m, 2H), 5.92 (s, 2H), 5.88 (d, *J* = 4.4 Hz, 1H), 5.40 (dd, *J* = 6.3, 4.4 Hz, 1H), 5.05 (dd, *J* = 6.4, 2.7 Hz, 1H), 4.60 (d, *J* = 3.1 Hz, 1H), 4.38 (ddd, *J* = 14.5, 8.3, 3.4 Hz, 1H), 3.60 (dt, *J* = 14.5, 2.7 Hz, 1H), 1.64 (s, 3H), 1.37 (s, 3H). HRMS (ESI$^+$): m/z calcd for $C_{24}H_{25}N_6O_4$ $[M+H]^+$ 461.1932, found 461.1928.

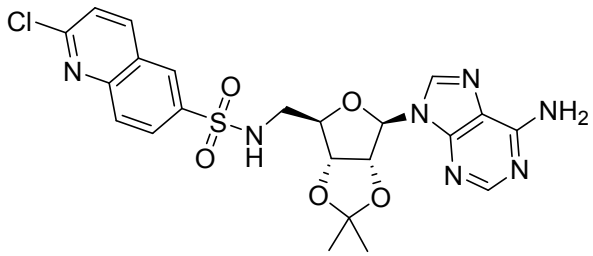


***N*-(((3aR,4R,6R,6aR)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1, 3]dioxol-4-yl)methyl)naphthalene-2-sulfonamide (17).** To a solution of compound **14**[8] (94 mg, 0.31 mmol, 1.0 eq) in CH_2Cl_2 (20 mL) at room temperature under argon atmosphere was added triethylamine (0.13 mL, 0.92 mmol, 3.0 eq). The reaction mixture was cooled to 0 ℃ and 2-naphthalenesulfonyl chloride (110 mg, 0.46 mmol, 1.5 eq) was added. The reaction mixture was stirred for 18 h at room temperature. Water (10 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by column chromatography (5% MeOH in CH_2Cl_2) to give compound **17** as a white solid (130 mg, 93%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.31 (d, *J* = 10.0 Hz, 1H), 8.66 (s, 1H), 8.42 (s, 1H), 8.01 – 7.85 (m, 3H), 7.80 – 7.77 (m, 2H), 7.67 – 7.56 (m, 2H), 5.74 (d, *J* = 5.1 Hz, 1H), 5.64 (s, 2H), 5.28 (t, *J* = 5.7 Hz, 1H), 4.95 (dd, *J* = 6.2, 1.7 Hz, 1H), 4.50 (d, *J* = 1.7 Hz, 1H), 3.56 – 3.48 (m, 1H), 3.18 (dd, *J* = 13.0, 2.5 Hz, 1H), 1.59 (s, 3H), 1.25 (s, 3H). HRMS (ESI$^+$): m/z calcd for $C_{23}H_{25}N_6O_5S$ $[M+H]^+$ 497.1607found 497.1601.

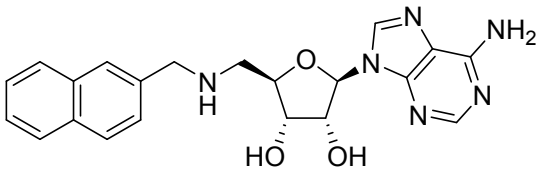


***N*-(((3aR,4R,6R,6aR)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)quinoline-7-sulfonamide (18).** To a solution of compound **14** (84 mg, 0.27 mmol, 1.0 eq.) in CH_2Cl_2 (20 mL) at room temperature under argon atmosphere was added triethylamine (0.12 mL, 0.82 mmol, 3.0 eq.). The mixture was cooled to 0 ℃ and quinoline-7-sulfonyl chloride (93 mg, 0.41 mmol, 1.5 eq.) was added. The reaction mixture was stirred for 18 h at room temperature. Water (10 mL) was added, and the aqueous layer was extracted with CH_2Cl_2

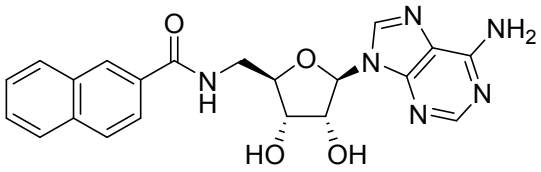
and the combined organic extracts were washed with brine, dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by column chromatography (5% MeOH in CH_2Cl_2) to give compound **18** as a white solid (41 mg, 33%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.53 (d, *J* = 10.0 Hz, 1H), 9.03 (dd, *J* = 4.1, 1.7 Hz, 1H), 8.64 (s, 1H), 8.63 (s, 1H), 8.19 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.92 (s, 2H), 7.80 (s, 1H), 7.52 (dd, *J* = 8.3, 4.2 Hz, 1H), 5.96 (s, 2H), 5.76 (d, *J* = 5.0 Hz, 1H), 5.29 (d, *J* = 6.2 Hz, 1H), 5.00 (dd, *J* = 6.1, 1.8 Hz, 1H), 4.51 (d, *J* = 2.1 Hz, 1H), 3.58 (ddd, *J* = 12.7, 10.1, 2.2 Hz, 1H), 3.20 (dd, *J* = 12.9, 2.5 Hz, 1H), 1.59 (s, 3H), 1.28 (s, 3H). HRMS (ESI$^+$): m/z calcd for $C_{22}H_{24}N_7O_5S$ $[M+H]^+$ 498.1554, found 498.1552.



***N*-(((3aR,4R,6R,6aR)-6-(6-Amino-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-chloroquinoline-6-sulfonamide (19).** To a solution of compound **14** (107 mg, 0.35 mmol, 1.0 eq.) in CH_2Cl_2 (20 mL) at room temperature under argon atmosphere was added triethylamine (0.15 mL, 1.05 mmol, 3.0 eq.). The mixture was cooled to 0 ℃ and 2-chloroquinoline-6-sulfonyl chloride (109 mg, 0.42 mmol, 1.2 eq.) was added. The reaction mixture was stirred for 18 h at room temperature. Water (10 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by column chromatography (5% MeOH in CH_2Cl_2) to give compound **19** as a white solid (105 mg, 54%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.51 (d, *J* = 9.9 Hz, 1H), 8.62 (s, 1H), 8.40 (d, *J* = 2.0 Hz, 1H), 8.19 (d, *J* = 8.6 Hz, 1H), 8.09 (d, *J* = 8.9 Hz, 1H), 8.04 (dd, *J* = 8.9, 2.0 Hz, 1H), 7.80 (s, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 5.77 (d, *J* = 5.0 Hz, 1H), 5.73 (s, 2H), 5.27 (t, *J* = 5.6 Hz, 1H), 5.00 (dd, *J* = 6.4, 1.9 Hz, 1H), 4.51 (d, *J* = 2.2 Hz, 1H), 3.54 (ddd, *J* = 12.6, 9.9, 2.2 Hz, 1H), 3.19 (dd, *J* = 13.1, 2.5 Hz, 1H), 1.60 (s, 3H), 1.30 (s, 3H). HRMS (ESI$^+$): m/z calcd for $C_{22}H_{23}ClN_7O_5S$ $[M+H]^+$ 532.1164, found 532.1161.

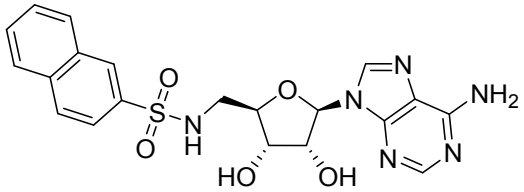


(2R,3R,4S,5R)-2-(6-Amino-9*H*-purin-9-yl)-5-(((naphthalen-2-ylmethyl)amino)methyl)tetrahydrofuran-3,4-diol (1). To compound **15** (104 mg, 0.22 mmol) was added a solution of TFA/H_2O (5 mL, 4:1) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, allowed to warm to room temperature and stirred for a further 3 h. The reaction mixture was diluted with methanol (10 mL) and the solvents were removed under vacuum. The crude product was purified by column chromatography (20% MeOH in CH_2Cl_2) to give compound **1** as a white solid (90 mg, 67%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (s, 1H), 7.96 (s, 1H), 7.84 – 7.77 (m, 4H), 7.49– 7.45 (m, 3H), 5.97 (d, *J* = 5.6 Hz, 1H), 4.84 – 4.82 (m, 1H), 4.36 (t, *J* = 4.7 Hz, 1H), 4.30 (dd, *J* = 7.4, 3.6 Hz, 1H), 4.12 (d, *J* = 15.2 Hz, 2H), 3.19 (dd, *J* = 12.8, 7.4 Hz, 1H), 3.13 (dd, *J* = 12.8, 3.6 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 157.4, 153.8, 150.4, 142.1, 135.5, 134.8, 134.4, 129.4, 128.9, 128.8, 128.7, 127.6, 127.4, 127.2, 121.2, 91.2, 84.2, 74.5, 73.3, 54.0, 51.2. HRMS (ESI$^+$): m/z calcd for $C_{21}H_{23}N_6O_3$ $[M+H]^+$ 407.1826, found 407.1816.

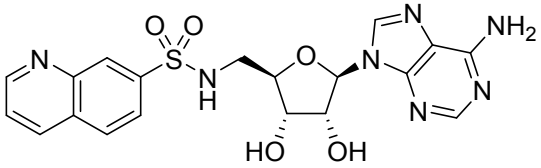


***N*-(((2R,3S,4R,5R)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-2-naphthamide (2).** To compound **16** (135 mg, 0.29 mmol) was added a solution of TFA/H_2O (6.25 mL, 4:1) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, allowed to warm to room temperature and stirred for a further 3 h. The reaction mixture was diluted with methanol (10 mL) and the solvents were removed under vacuum. The crude product was purified by column chromatography (15% MeOH in CH_2Cl_2) to give compound **2** as a white solid (95 mg, 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (t, *J* = 5.8 Hz, 1H), 8.46 (d, *J* = 1.6 Hz, 1H), 8.38 (s, 1H), 8.07 (s, 1H), 8.02 – 7.93 (m, 4H), 7.63 – 7.57 (m, 2H), 7.35 (s, 2H), 5.89 (d, *J* = 6.2 Hz, 1H), 5.47 (s, 1H), 5.30 (s, 1H), 4.78 (t, *J* = 5.7 Hz, 1H), 4.23 (dd, *J* = 5.1, 3.2 Hz, 1H), 4.15– 4.13 (m, 1H), 3.68 (t, *J* = 5.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8, 156.0, 152.3, 149.3, 140.4, 134.1,

132.1, 131.8, 128.8, 127.9, 127.6 (×2), 127.5, 126.7, 124.2, 119.4, 87.7, 83.3, 72.6, 71.3, 41.8. HRMS (ESI$^+$): m/z calcd for $C_{21}H_{21}N_6O_4$ $[M+H]^+$ 421.1619, found 421.1608.

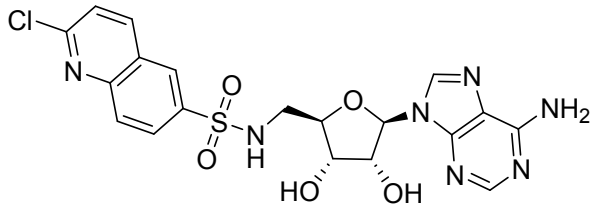


***N*-(((2R,3S,4R,5R)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)naphthalene-2-sulfonamide (3).** To compound **17** (130 mg, 0.26 mmol) was added a solution of TFA/H_2O (6.25 mL, 4:1) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, allowed to warm to room temperature and stirred for a further 3 h. The reaction mixture was diluted with methanol (10 mL) and the solvents were removed under vacuum. The crude product was purified by column chromatography (15% MeOH in CH_2Cl_2) to give compound **3** as a white solid (90 mg, 75%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.41 (d, *J* = 1.9 Hz, 1H), 8.29 (s, 1H), 8.18 (s, 1H), 7.98 – 7.95 (m, 2H), 7.91 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.79 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.65 – 7.56 (m, 2H), 5.86 (d, *J* = 6.7 Hz, 1H), 4.86 (d, *J* = 5.6 Hz, 1H), 4.25 (dd, *J* = 5.4, 2.5 Hz, 1H), 4.21 (q, *J* = 3.2 Hz, 1H), 3.36 (dd, *J* = 13.5, 3.6 Hz, 1H), 3.20 (dd, *J* = 13.5, 3.3 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 157.7, 153.6, 149.9, 142.4, 138.4, 136.1, 133.5, 130.6, 130.2, 129.8, 129.1, 129.0, 128.7, 123.2, 121.2, 91.4, 85.8, 74.3, 73.0, 46.0. HRMS (ESI$^+$): m/z calcd for $C_{20}H_{21}N_6O_5S$ $[M+H]^+$ 457.1289, found 457.1289.

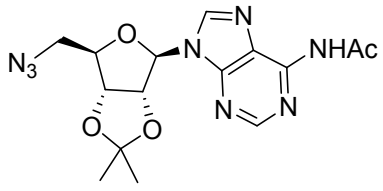


***N*-(((2R,3S,4R,5R)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)quinoline-7-sulfonamide (4).** To compound **18** (41 mg, 0.08 mmol) was added a solution of TFA/H_2O (2.5 mL, 4:1) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, allowed to warm to room temperature and stirred for a further 3 h. The reaction mixture was diluted with methanol (5 mL) and the solvents were removed under vacuum. The crude product was purified by column chromatography (20% MeOH in CH_2Cl_2) to give compound **4** as a white solid (16 mg, 44%). ^{1}H NMR (600 MHz, CD_3OD) δ 8.98 (dd, *J* = 4.3, 1.6 Hz, 1H), 8.53 (t, *J* = 1.2 Hz,

1H), 8.43 (dd, *J* = 8.3, 1.6 Hz, 1H), 8.28 (s, 1H), 8.17 (s, 1H), 8.09 (d, *J* = 8.6 Hz, 1H), 7.95 (dd, *J* = 8.6, 1.8 Hz, 1H), 7.67 (dd, *J* = 8.4, 4.3 Hz, 1H), 5.83 (d, *J* = 6.6 Hz, 1H), 4.83 (t, *J* = 6.2 Hz, 1H), 4.22 (dd, *J* = 5.5, 2.7 Hz, 1H), 4.19 (q, *J* = 3.4 Hz, 1H), 3.41 (dd, *J* = 13.5, 3.8 Hz, 1H), 3.26 (dd, *J* = 13.5, 3.4 Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 157.8, 153.7, 153.3, 147.9, 143.0, 142.3,142.0, 138.2, 137.7, 131.6, 131.1, 128.9, 124.8, 124.5, 91.4, 85.7, 74.3, 72.9, 46.2. HRMS (ESI$^+$): m/z calcd for $C_{19}H_{20}N_7O_5S$ $[M+H]^+$ 458.1241, found 458.1241.

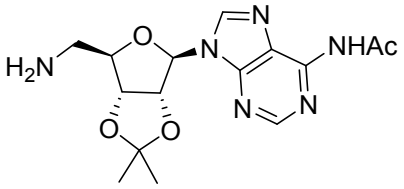


***N*-(((2R,3S,4R,5R)-5-(6-Amino-9*H*-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-2-chloroquinoline-6-sulfonamide (5).** To compound **19** (105 mg, 0.20 mmol) was added a solution of TFA/H_2O (5 mL, 4:1) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, allowed to warm to room temperature and stirred for a further 3 h. The reaction mixture was diluted with methanol (10 mL) and the solvents were removed under vacuum. The crude product was purified by column chromatography (10% MeOH in CH_2Cl_2) to give compound **5** as a white solid (45 mg, 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (t, *J* = 5.6 Hz, 1H), 8.66 (d, *J* = 8.6 Hz, 1H), 8.59 (d, *J* = 1.8 Hz, 1H), 8.28 (s, 1H), 8.14 – 8.08 (m, 3H), 7.74 (d, *J* = 8.6 Hz, 1H), 7.40 (s, 2H), 5.80 (d, *J* = 6.4 Hz, 1H), 5.47 (s, 1H), 5.27 (s, 1H), 4.66 (t, *J* = 6.4 Hz, 1H), 4.06 – 4.04 (m, 1H), 4.00 – 3.97 (m, 1H), 3.19 – 3.13 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.1, 152.4, 152.1, 148.8, 148.2, 141.2, 140.4, 138.6, 129.5, 127.8, 127.2, 126.0, 123.9, 119.5, 88.1, 83.5, 72.4, 71.1, 44.9. HRMS (ESI$^+$): m/z calcd for $C_{19}H_{19}ClN_7O_5S$ $[M+H]^+$ 492.0851, found 492.0833.

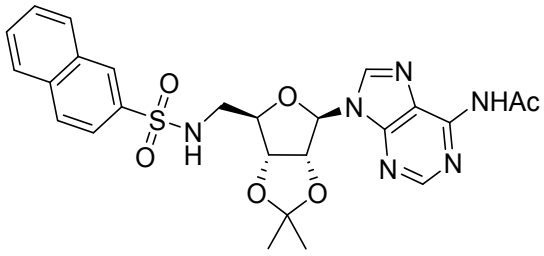


***N*-(9-((3aR,4R,6R,6aR)-6-(Azidomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purin-6-yl)acetamide (20).** To a solution of compound **13**[8] (240 mg, 0.72 mmol, 1.0 eq.) in dry pyridine (10 mL) at room temperature was added acetic anhydride (0.68 mL, 7.22 mmol,

10.0 eq.). The resultant solution was stirred at room temperature for 24 h. The solvents were removed under vacuum. The residue was co-evaporated with methanol. The crude product was purified by column chromatography (95% ethyl acetate in hexane) to give compound **20** as a white solid (180 mg, 67%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.70 (s, 1H), 8.17 (s, 1H), 6.16 (d, *J* = 2.4 Hz, 1H), 5.44 (dd, *J* = 6.4, 2.4 Hz, 1H), 5.05 (dd, *J* = 6.4, 3.5 Hz, 1H), 4.40 (td, *J* = 5.3, 3.5 Hz, 1H), 3.61 – 3.55 (m, 2H), 2.63 (s, 3H), 1.62 (s, 3H), 1.39 (s, 3H). HRMS (ESI$^+$): m/z calcd for $C_{15}H_{19}N_8O_4$ $[M+H]^+$ 375.1524, found 375.1519.

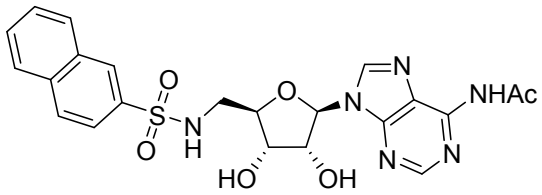


***N*-(9-((3aR,4R,6R,6aR)-6-(Aminomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purin-6-yl)acetamide (21).** To a solution of compound **20** (180 mg, 0.48 mmol) in ethanol (5 mL) was added 10 % Pd/C (36 mg, 20% w/w) and the mixture stirred under an atmosphere of hydrogen for 18 h. The mixture was filtered through Celite, washed with methanol and the solvent removed under vacuum. The crude product was purified by column chromatography (35% MeOH in CH_2Cl_2) to give compound **21** as a white solid (110 mg, 69%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.62 (s, 1H), 8.50 (s, 1H), 6.27 (d, *J* = 2.8 Hz, 1H), 5.50 (dd, *J* = 6.4, 2.7 Hz, 1H), 5.09 (dd, *J* = 6.4, 3.4 Hz, 1H), 4.34 (ddd, *J* = 7.4, 5.0, 3.5 Hz, 1H), 3.19 – 3.00 (m, 2H), 2.36 (s, 3H), 1.61 (s, 3H), 1.39 (s, 3H). HRMS (ESI$^+$): m/z calcd for $C_{15}H_{21}N_6O_4$ $[M+H]^+$ 349.1619, found 349.1619.

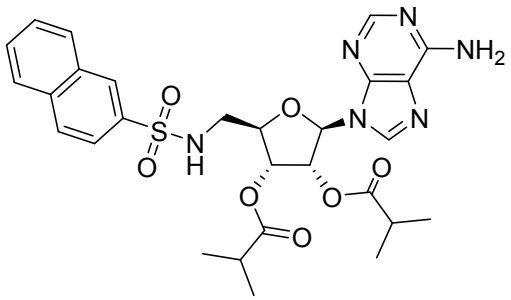


***N*-(9-((3aR,4R,6R,6aR)-2,2-Dimethyl-6-((naphthalene-2-sulfonamido)methyl)tetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purin-6-yl)acetamide (22).** To compound **21** (110 mg, 0.32 mmol, 1.0 eq.) in CH_2Cl_2 (25 mL) under argon atmosphere at room temperature was added triethylamine (0.13 mL, 0.95 mmol, 3.0 eq.). The mixture was cooled

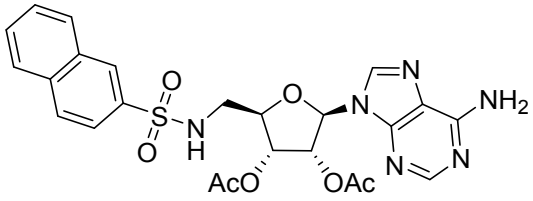
to 0 ℃ and 2-naphthalenesulfonyl chloride (107 mg, 0.47 mmol, 1.5 eq.) was added. The reaction mixture was stirred for 24 h at room temperature. Water (20 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by column chromatography (5% MeOH in CH_2Cl_2) to give compound **22** as a white solid (74 mg, 44%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.34 (s, 1H), 8.96 (s, 1H), 8.41 (d, *J* = 1.8 Hz, 1H), 8.10 (s, 1H), 7.97 – 7.83 (m, 3H), 7.77 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.65 – 7.51 (m, 2H), 5.83 (d, *J* = 4.7 Hz, 1H), 5.26 (dd, *J* = 6.2, 4.8 Hz, 1H), 4.95 (dd, *J* = 6.3, 2.1 Hz, 1H), 4.47 (d, *J* = 2.4 Hz, 1H), 3.51 (dd, *J* = 12.8, 2.8 Hz, 1H), 3.19 (dd, *J* = 12.8, 2.2 Hz, 1H), 2.66 (s, 3H), 1.57 (s, 4H), 1.24 (s, 3H). HRMS (ESI$^+$): m/z calcd for $C_{25}H_{27}N_6O_6S$ $[M+H]^+$ 539.1707, found 539.1711.



***N*-(9-((2R,3R,4S,5R)-3,4-Dihydroxy-5-((naphthalene-2-sulfonamido)methyl)tetrahydrofuran-2-yl)-9*H*-purin-6-yl)acetamide (6).** To compound **22** (70 mg, 0.13 mmol) was added a solution of TFA/H_2O (3.75 mL, 4:1) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, allowed to warm to room temperature and stirred for a further 3 h. The reaction mixture was diluted with methanol (10 mL) and the solvents were removed under vacuum. The crude product was purified by column chromatography (10% MeOH in CH_2Cl_2) to give compound **6** as a white solid (24 mg, 38%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.65 (s, 1H), 8.43 (s, 1H), 8.42 (d, *J* = 2.1 Hz, 1H), 8.03 – 7.97 (m, 2H), 7.94 (d, *J* = 8.1 Hz, 1H), 7.82 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.67 – 7.59 (m, 2H), 5.94 (d, *J* = 6.3 Hz, 1H), 4.86 (*t*, J = 5.8 Hz, 1H), 4.29 (dd, *J* = 5.4, 3.1 Hz, 1H), 4.18 (q, *J* = 4.1 Hz, 1H), 3.36 (dd, J = 13.7, 4.1 Hz, 1H), 3.26 (dd, J = 13.7, 3.7 Hz, 1H), 2.39 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 171.9, 153.7, 152.8, 149.9, 145.1, 142.3, 138.6, 136.2, 133.6, 130.6, 130.2, 129.9, 129.1, 129.0, 128.7, 123.2, 91.4, 85.6, 74.2, 73.0, 46.1, 24.8. HRMS (ESI$^+$): m/z calcd for $C_{22}H_{23}N_6O_6S$ $[M+H]^+$ 499.1394, found 499.1386.

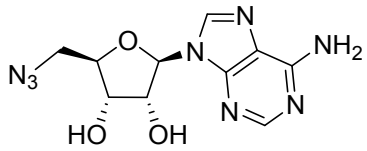


(2R,3R,4R,5R)-2-(6-Amino-9*H*-purin-9-yl)-5-((naphthalene-2-sulfonamido)methyl)tetrahydrofuran-3,4-diyl bis(2-methylpropanoate) (7). To a solution of compound **3** (120 mg, 0.26 mmol, 1.0 eq.) in dry pyridine (10 mL) at room temperature was added isobutyric anhydride (0.44 mL, 2.63 mmol, 10.0 eq.). The resultant solution was stirred at room temperature for 2 h. The mixture was diluted with MeOH (20 mL) and the solvents were removed under vacuum. The crude product was purified by column chromatography (95-100% ethyl acetate in hexane) to give compound **7** as a white solid (43 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (t, J = 6.2 Hz, 1H), 8.46 (d, J = 1.8 Hz, 1H), 8.33 (s, 1H), 8.17 – 8.08 (m, 3H), 8.03 (d, J = 8.0 Hz, 1H), 7.81 (dd, J = 8.7, 1.9 Hz, 1H), 7.73 – 7.65 (m, 2H), 7.44 (s, 2H), 6.14 (d, J = 6.3 Hz, 1H), 5.93 (t, J = 6.0 Hz, 1H), 5.49 (dd, J = 5.7, 3.5 Hz, 1H), 4.23 (q, J = 4.4 Hz, 1H), 3.26 (s, 2H), 2.57 (s, J = 7.0 Hz, 1H), 2.44 (s, J = 7.0 Hz, 1H), 1.11 (t, J = 6.8 Hz, 6H), 0.98 (dd, J = 11.4, 7.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.9, 174.6, 156.3, 152.5, 148.6, 140.1, 137.1, 134.2, 131.7, 129.5, 129.2, 128.8, 127.8, 127.6, 127.5, 122.0, 119.4, 86.0, 81.2, 71.6, 71.1, 44.2, 33.0, 32.9, 18.6, 18.5, 18.4 (× 2). HRMS (ESI$^+$): m/z calcd for $C_{28}H_{33}N_6O_7S$ $[M+H]^+$ 597.2126, found 597.2126.

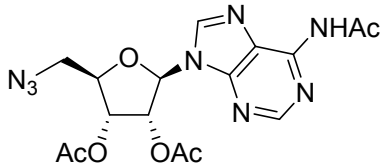


(2R,3R,4R,5R)-2-(6-Amino-9*H*-purin-9-yl)-5-((naphthalene-2-sulfonamido)methyl)tetrahydrofuran-3,4-diyl diacetate (8). To a solution of compound **3** (46 mg, 0.10 mmol, 1.0 eq.) in dry pyridine (4 mL) at room temperature was added acetic anhydride (0.1 mL, 0.60 mmol, 6.0 eq.). The resultant solution was stirred at room temperature for 2 h. The mixture was diluted with methanol (10 mL) and the solvents were removed under vacuum. The

crude product was purified by column chromatography (95-100% ethyl acetate in hexane) to give compound **8** as a white solid (28 mg, 52%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.46 (d, J = 1.9 Hz, 1H), 8.34 (s, 1H), 8.17 – 8.08 (m, 3H), 8.03 (d, J = 8.0 Hz, 1H), 7.80 (dd, J = 8.7, 1.9 Hz, 1H), 7.74 – 7.64 (m, 2H), 7.45 (s, 2H), 6.16 (d, J = 6.5 Hz, 1H), 5.96 (t, J = 6.2 Hz, 1H), 5.46 (dd, J = 5.8, 3.4 Hz, 1H), 4.24 (q, J = 4.7 Hz, 1H), 3.27 – 3.16 (m, 2H), 2.06 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.4, 169.1, 156.3, 152.5, 148.7, 140.2, 137.1, 134.2, 131.0, 129.5, 129.2, 128.8, 127.8, 127.6, 127.5, 122.1, 119.4, 85.7, 81.0, 71.4, 71.2, 44.2, 20.3, 20.1. HRMS (ESI$^+$): m/z calcd for $C_{24}H_{25}N_6O_7S$ $[M+H]^+$ 541.1500, found 541.1498.

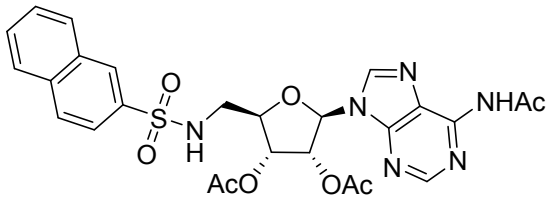


(2R,3R,4S,5R)-2-(6-Amino-9*H*-purin-9-yl)-5-(aminomethyl)tetrahydrofuran-3,4-diol (23).[10] To compound **13**[8] (260 mg, 0.78 mmol) was added a solution of TFA/H_2O (10 mL, 4:1) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, allowed to warm to room temperature and stirred for a further 4 h. The reaction mixture was diluted with methanol (20 mL) and the solvents were removed under vacuum. The crude product was purified by column chromatography (20% MeOH in CH_2Cl_2) to give compound **23** as a yellow oil (190 mg, 86%). ^{1}H NMR (600 MHz, CD_3OD) δ 8.29 (s, 1H), 8.22 (s, 1H), 6.03 (d, J = 4.7 Hz, 1H), 4.79 (t, J = 5.0 Hz, 1H), 4.38 (t, J = 5.1 Hz, 1H), 4.19 (d, J = 4.5 Hz, 1H), 3.73 – 3.61 (m, 2H). HRMS (ESI$^+$): m/z calcd for $C_{10}H_{13}N_8O_3$ $[M+H]^+$ 293.1105, found 293.1105.

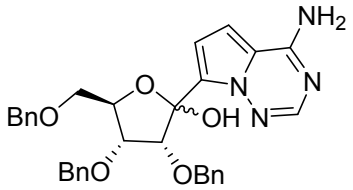


(2R,3R,4R,5R)-2-(6-Acetamido-9*H*-purin-9-yl)-5-(azidomethyl)tetrahydrofuran-3,4-diyl diacetate (24). To a solution of compound **23** (120 mg, 0.41 mmol, 1.0 eq.) in dry pyridine (6 mL) at 50 ℃ was added acetic anhydride (0.58 mL, 6.15 mmol, 15.0 eq.). The resultant solution was stirred for 4 h. The mixture was diluted with methanol (10 mL) and the solvents were removed under vacuum. The crude product was purified by column chromatography (70% ethyl acetate in hexane) to give compound **24** as a white solid (95 mg, 56%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.51

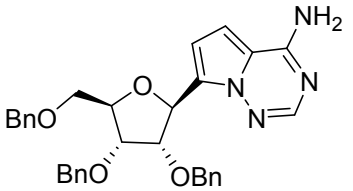
(s, 1H), 8.70 (s, 1H), 8.36 (s, 1H), 6.24 (d, *J* = 5.6 Hz, 1H), 5.95 (t, *J* = 5.7 Hz, 1H), 5.60 (dd, *J* = 5.7, 4.3 Hz, 1H), 4.35 (q, *J* = 4.1 Hz, 1H), 3.73 (dd, *J* = 13.3, 3.6 Hz, 1H), 3.70 (dd, *J* = 13.3, 3.5 Hz, 1H), 2.61 (s, 3H), 2.13 (s, 3H), 2.05 (s, 3H). HRMS (ESI$^+$): m/z calcd for $C_{16}H_{19}N_8O_6$ $[M+H]^+$ 419.1422, found 419.1424.



(2R,3R,4R,5R)-2-(6-Acetamido-9*H*-purin-9-yl)-5-((naphthalene-2-sulfonamido)methyl)tetrahydrofuran-3,4-diyl diacetate (9). To a solution of compound **24** (84 mg, 0.20 mmol) in dioxane (5 mL) was added 10 % Pd/C (17 mg, 20% w/w) and the mixture stirred under an atmosphere of hydrogen for 18 h. The mixture was filtered through Celite, washed with methanol and the solvent removed under vacuum. The crude product **25** was used for next step without further purification. To compound **25** (96 mg, 0.24 mmol, 1.0 eq) in CH_2Cl_2 (15 mL) at room temperature under argon atmosphere was added triethylamine (0.1 mL, 0.73 mmol, 3.0 eq). The mixture was cooled to 0 ℃ and 2-naphthalenesulfonyl chloride (83 mg, 0.37 mmol, 1.5 eq) was added. The reaction mixture was stirred for 5 h at room temperature. Water (10 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by column chromatography (95-100% ethyl acetate in hexane) to give compound **9** as a white solid (58 mg, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.94 (s, 1H), 8.61 (dd, *J* = 9.7, 2.0 Hz, 1H), 8.47 (d, *J* = 2.0 Hz, 1H), 8.08 (s, 1H), 8.01 – 7.82 (m, 4H), 7.66 – 7.56 (m, 2H), 5.98 (d, *J* = 6.8 Hz, 1H), 5.95 (t, *J* = 6.2 Hz, 1H), 5.43 (dd, *J* = 5.6, 3.0 Hz, 1H), 4.36 (q, *J* = 2.8 Hz, 1H), 3.50 (ddd, *J* = 13.4, 9.7, 2.5 Hz, 1H), 3.32 (dt, *J* = 13.4, 2.4 Hz, 1H), 2.68 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 169.5, 169.2, 152.7, 150.3, 150.2, 142.6, 136.7, 134.9, 132.3, 129.9, 129.4, 128.9, 128.1, 127.7, 123.1, 122.4, 88.5, 82.7, 72.4, 71.8, 60.5, 44.2, 26.0, 20.6, 20.4. HRMS (ESI$^+$): m/z calcd for $C_{26}H_{27}N_6O_8S$ $[M+H]^+$ 583.1606, found 583.1607.

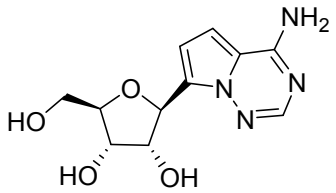


(3R,4R,5R)-2-(4-Aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-ol (28).[11-12] To a solution of 7-iodopyrrolo[2,1-*f*][1,2,4]triazin-4-amine (**27**, 1.45 g, 5.58 mmol, 1.0 eq.) in THF (30 mL) under argon atmosphere was added TMSCl (1.41 mL, 11.15 mmol, 2.0 eq.) and the resulting mixture was stirred for 10 min at room temperature. The mixture was cooled to 0 ℃, and PhMgCl (2 M in THF, 5.57 mL, 11.15 mmol, 2.0 eq.) was added dropwise. The reaction mixture was stirred for 20 min and ^{i}PrMgCl·LiCl (1.3 M in THF, 4.28 mL, 5.58 mmol, 1.0 eq.) was added. After 15 min, the reaction mixture was cooled to approximately –20 °C and a solution of 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone (**26**, 2.33 g, 5.58 mmol, 1.0 eq.) in THF (10 mL) was added dropwise. After 2 h, the reaction mixture was quenched with methanol (5 mL), followed by acetic acid (5 mL) and water (5 mL). The mixture was concentrated under vacuum, then partitioned between EtOAc (100 mL) and 1 M HCl (50 mL). The organic layer was washed with $NaHCO_3$ (50 mL), brine, dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by column chromatography (70% ethyl acetate in hexane) to give compound **28** as a pale yellow solid (1.3 g, 42%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.01 (s, 2H), 7.98 (s, 1H), 7.38 – 7.24 (m, 10H), 7.18 – 7.12 (m, 2H), 7.03 – 6.97 (m, 2H), 6.94 (d, *J* = 4.8 Hz, 1H), 5.38 (d, J = 6.1 Hz, 1H), 4.98 (d, *J* = 5.2 Hz, 1H), 4.68 – 4.56 (m, 2H), 4.48 – 4.45 (m, 4H), 4.06 – 3.98 (m, 2H), 3.93 (dd, *J* = 6.1, 4.4 Hz, 1H), 3.70 (dd, *J* = 10.1, 3.5 Hz, 1H), 3.48 (dd, J = 10.1, 6.5 Hz, 1H). HRMS (ESI$^-$): m/z calcd for $C_{32}H_{31}N_4O_5$ $[M-H]^-$ 551.2300, found 551.2300.

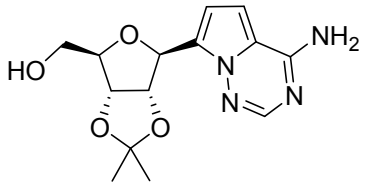


7-((2S,3S,4R,5R)-3,4-Bis(benzyloxy)-5-((benzyloxy)methyl)tetrahydrofuran-2-yl)pyrrolo[2,1-*f*][1,2,4]triazin-4-amine (29).[11-12] To a solution of compound **28** (1.1 g, 1.99 mmol, 1.0 eq.) in CH_2Cl_2 (30 mL) at 0 ℃ under an argon atmosphere was added triethylsilane (1.6 mL,

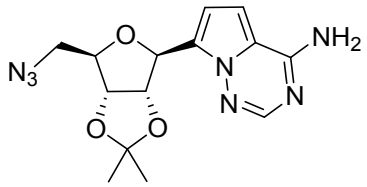
9.95 mmol, 5.0 eq.), followed by slow addition of $BF_3{\cdot}OEt_2$ (0.056 mL, 5.97 mmol, 3eq.). The resulting solution was stirred for 1 h at 0 ℃. Saturated aqueous $NaHCO_3$ solution (30 mL) was added and the aqueous mixture was extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over $MgSO_4$ and the solvent was removed under vacuum. The crude product was purified by column chromatography (70% ethyl acetate in hexane) to give compound **29** as a yellowish oil (0.91 g, 87%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.34 – 7.27 (m, 15H), 6.68 (d, *J* = 4.5 Hz, 1H), 6.50 (d, *J* = 4.5 Hz, 1H), 5.72 (s, 2H), 5.70 (d, *J* = 4.2 Hz, 1H), 4.78 – 4.68 (m, 2H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.58 – 4.52 (m, 2H), 4.45 (d, *J* = 12.0 Hz, 1H), 4.43 – 4.39 (m, 1H), 4.29 (t, *J* = 4.6 Hz, 1H), 4.16 – 4.12 (m, 1H), 3.79 (dd, *J* = 10.7, 3.5 Hz, 1H), 3.67 (dd, *J* = 10.7, 4.1 Hz, 1H). HRMS (ESI$^+$): m/z calcd for $C_{32}H_{33}N_4O_4$ $[M+H]^+$ 537.2496, found 537.2495.



(2S,3R,4S,5R)-2-(4-Aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (30).[11-12] To a solution of compound **29** (0.91 g, 1.69 mmol, 1.0 eq.) in CH_2Cl_2 (30 mL) under an atmosphere of argon at –78 ℃, was added BBr_3 (1.0 M in CH_2Cl_2, 8.5 mL, 8.48 mmol, 5.0 eq.) The mixture was stirred for 2 h at –78 °C. Methanol (5 mL) was added and the mixture was warmed to room temperature. The resulting mixture was concentrated under vacuum, and the residue co-evaporated with methanol. The crude product was purified by column chromatography (20% MeOH in CH_2Cl_2) to give compound **30** as a yellowish solid (0.41 g, 91%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.82 (s, 1H), 7.70 (s, 2H), 6.85 (d, *J* = 4.4 Hz, 1H), 6.68 (d, *J* = 4.5 Hz, 1H), 5.10 (d, *J* = 6.5 Hz, 1H), 4.96 (s, 1H), 4.86 (s, 1H), 4.76 (s, 1H), 4.23 (t, *J* = 5.5 Hz, 1H), 3.94 (t, *J* = 4.9 Hz, 1H), 3.78 (q, *J* = 4.5 Hz, 1H), 3.54 (dd, *J* = 11.7, 4.0 Hz, 1H), 3.45 (dd, *J* = 11.7, 4.4 Hz, 1H). HRMS (ESI$^+$): m/z calcd for $C_{11}H_{15}N_4O_4$ $[M+H]^+$ 267.1088, found 267.1088.

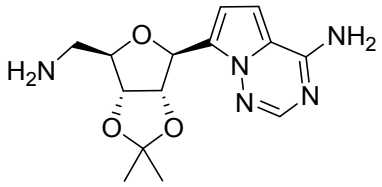


((3aR,4R,6S,6aS)-6-(4-Aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methanol (31). To a solution of compound **30** (0.56 g, 2.10 mmol, 1.0 eq.) in acetone (20 mL) was added 2,2-dimethoxypropane (1.29 mL, 10.5 mmol, 5.0 eq.), followed by addition of sulfuric acid (18 M, 0.15 mL, 2.73 mmol, 1.3 eq.). The reaction mixture was stirred for 30 min, and was warmed to 45 °C. After 6 h, a saturated aqueous $NaHCO_3$ solution (20 mL) was added and the aqueous mixture was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over $MgSO_4$ and the solvent was removed under vacuum. The crude product was purified by column chromatography (95-100% ethyl acetate in hexane) to give compound **31** as a colorless oil (0.91 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 6.69 (d, *J* = 4.5 Hz, 1H), 6.56 (d, *J* = 4.5 Hz, 1H), 5.86 (s, 2H), 5.41 – 5.13 (m, 2H), 5.03 (dd, *J* = 5.7, 2.4 Hz, 1H), 4.56 (s, 1H), 4.39 (q, *J* = 2.2 Hz, 1H), 3.92 (dd, *J* = 12.2, 2.2 Hz, 1H), 3.77 (dd, *J* = 12.3, 2.0 Hz, 1H), 1.63 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 147.4, 127.4, 116.0, 114.2, 112.8, 100.3, 84.6, 82.6, 82.3, 81.2, 63.5, 27.9, 25.6. HRMS (ESI$^+$): m/z calcd for $C_{14}H_{19}N_4O_4$ $[M+H]^+$ 307.1401, found 307.1402.

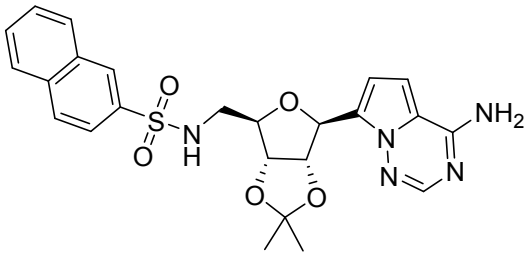


7-((3aS,4S,6R,6aR)-6-(Azidomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)pyrrolo[2,1-*f*][1,2,4]triazin-4-amine (32). To a solution of compound **31** (0.26 g, 0.85 mmol, 1.0 eq.) in 1,4-dioxane (10 mL) under an atmosphere of argon was added diphenylphosphoryl azide (0.37 mL, 1.69 mmol, 2.0 eq.) and DBU (0.38 mL, 2.54 mmol, 3.0 eq.), and the reaction mixture was stirred for 18 h at room temperature. Sodium azide (0.28 g, 4.24 mmol, 5.0 eq.) and 15-crown-5 (1.6 µmL, 0.008 mmol, 0.01 eq.) were added, and the reaction mixture heated at 110°C for 4 h. The solid was removed by filtration and the solvent was removed under vacuum. The crude product was purified by column chromatography (70% ethyl acetate in hexane) to give compound **32** as a white solid (0.23 g, 82%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 (s, 1H), 6.68 (d, *J* = 4.5 Hz,

1H), 6.62 (d, *J* = 4.5 Hz, 1H), 6.38 (s, 2H), 5.46 (d, *J* = 4.4 Hz, 1H), 5.11 (dd, *J* = 6.7, 4.4 Hz, 1H), 4.79 (dd, *J* = 6.7, 4.5 Hz, 1H), 4.23 (q, *J* = 4.7 Hz, 1H), 3.57 (dd, *J* = 13.0, 4.4 Hz, 1H), 3.44 (dd, *J* = 13.0, 5.2 Hz, 1H), 1.59 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.7, 147.6, 127.6, 115.6, 115.0, 110.5, 100.3, 83.9, 83.2, 82.4, 78.6, 52.4, 27.6, 25.7. HRMS (ESI$^-$): m/z calcd for $C_{14}H_{16}N_7O_3$ $[M-H]^-$ 330.1320, found 330.1330.

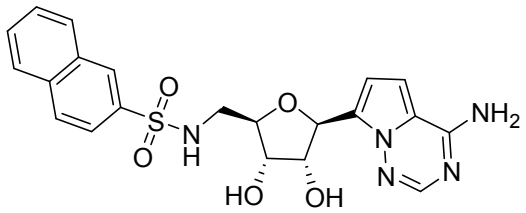


7-((3aS,4S,6R,6aR)-6-(Aminomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)pyrrolo[2,1-*f*][1,2,4]triazin-4-amine (33). To a solution of compound **32** (0.23 g, 0.69 mmol) in ethanol (20 mL) was added 10 % Pd/C (0.046 g, 20% w/w) and the mixture stirred under an atmosphere of hydrogen for 18 h. The mixture was filtered through Celite, washed with methanol and the solvent removed under vacuum. The crude product was purified by column chromatography (20% MeOH in CH_2Cl_2) to give compound **33** as a white solid (0.21 g, 95%). ^{1}H NMR (600 MHz, CD_3OD) δ 7.81 (s, 1H), 6.85 (d, *J* = 4.5 Hz, 1H), 6.72 (d, *J* = 4.5 Hz, 1H), 5.32 (d, *J* = 4.6 Hz, 1H), 5.14 (dd, *J* = 6.7, 4.7 Hz, 1H), 4.84 (s, 2H), 4.76 (dd, *J* = 6.7, 4.2 Hz, 1H), 4.06 (dt, *J* = 6.4, 4.3 Hz, 1H), 2.87 (dd, *J* = 13.4, 4.4 Hz, 1H), 2.80 (dd, *J* = 13.4, 6.6 Hz, 1H), 1.56 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 157.2, 148.2, 129.2, 116.7, 115.6, 111.7, 102.5, 86.6, 84.9, 84.0, 79.6, 44.7, 27.8, 25.8. HRMS (ESI$^+$): m/z calcd for $C_{14}H_{20}N_5O_3$ $[M+H]^+$ 306.1561, found 306.1561.



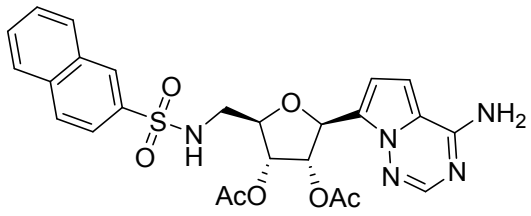
***N*-(((3aR,4R,6S,6aS)-6-(4-Aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)naphthalene-2-sulfonamide (34).** To compound **33** (0.21 g, 0.69 mmol, 1.0 eq.) in CH_2Cl_2 (30 mL) under argon atmosphere at room

temperature was added triethylamine (0.29 mL, 2.06 mmol, 3.0 eq.). The mixture was cooled to 0 ℃ and 2-naphthalenesulfonyl chloride (0.23 g, 1.03 mmol, 1.5 eq.) was added. The reaction mixture was stirred for 24 h at room temperature. Water (20 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 and the combined organic extracts were washed with brine, dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by column chromatography (5% MeOH in CH_2Cl_2) to give compound **34** as a white solid (0.27 g, 61%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.42 (d, *J* = 1.9 Hz, 1H), 8.32 (s, 1H), 7.93 – 7.89 (m, 2H), 7.85 (d, *J* = 8.2 Hz, 1H), 7.83 (d, *J* = 9.2 Hz, 1H), 7.78 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.61 – 7.56 (m, 2H), 6.66 (d, *J* = 4.4 Hz, 1H), 6.59 (d, *J* = 4.4 Hz, 1H), 6.19 (s, 2H), 5.26 (t, *J* = 6.4 Hz, 1H), 5.02 (d, *J* = 6.4 Hz, 1H), 4.84 (dd, *J* = 6.4, 2.5 Hz, 1H), 4.36 (d, *J* = 2.6 Hz, 1H), 3.48 – 3.41 (m, 1H), 3.17 (dd, *J* = 12.5, 2.9 Hz, 1H), 1.56 (s, 3H), 1.22 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.0, 147.9, 136.8, 134.9, 132.3, 129.7, 129.3, 128.8, 128.4, 128.0, 127.6, 126.5, 122.3, 116.2, 114.6, 113.2, 100.4, 82.9, 81.7, 81.6, 81.3, 45.3, 27.8, 25.4. HRMS (ESI$^+$): m/z calcd for $C_{24}H_{26}N_5O_5S$ $[M+H]^+$ 496.1649, found 496.1647.



***N*-(((2R,3S,4R,5S)-5-(4-Aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)naphthalene-2-sulfonamide (10).** To compound **34** (100 mg, 0.20 mmol) was added a solution of TFA/H_2O (5 mL, 4:1) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, allowed to warm to room temperature and stirred for a further 4 h. The reaction mixture was diluted with methanol (20 mL) and the solvents were removed under vacuum. The crude product was purified by column chromatography (5% MeOH in CH_2Cl_2) to give compound **10** as a white solid (54 mg, 59%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.42 (d, *J* = 2.0 Hz, 1H), 7.99 (t, *J* = 8.4 Hz, 2H), 7.94 (d, *J* = 6.8 Hz, 2H), 7.81 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.72 – 7.57 (m, 2H), 6.84 (d, *J* = 4.5 Hz, 1H), 6.67 (d, *J* = 4.4 Hz, 1H), 5.08 (d, *J* = 7.6 Hz, 1H), 4.70 (dd, *J* = 7.6, 5.7 Hz, 1H), 4.13 (dd, *J* = 5.7, 3.2 Hz, 1H), 4.08 (q, *J* = 3.6 Hz, 1H), 3.25 (dd, *J* = 13.0, 3.8 Hz, 1H), 3.15 (dd, *J* = 13.0, 3.6 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD) δ 157.3, 148.2, 138.3, 136.2, 133.6, 130.6, 130.2, 129.8, 129.1, 129.0, 128.9, 128.7, 123.3, 117.0, 113.3, 102.8, 84.6,

79.3, 74.2, 73.8, 46.4. HRMS (ESI$^+$): m/z calcd for $C_{21}H_{22}N_5O_5S$ $[M+H]^+$ 456.1336, found 456.1336.



(2S,3S,4R,5R)-2-(4-Aminopyrrolo[2,1-*f*][1,2,4]triazin-7-yl)-5-((naphthalene-2-sulfonamido)methyl)tetrahydrofuran-3,4-diyl diacetate (11). To a solution of compound **10** (51 mg, 0.11 mmol, 1.0 eq.) in dry pyridine (6 mL) at room temperature was added acetic anhydride (0.12 mL, 0.67 mmol, 6.0 eq.). The resultant solution was stirred for 2 h. The mixture was diluted with methanol (10 mL) and the solvents were removed under vacuum. The crude product was purified by column chromatography (65% ethyl acetate in hexane) to give compound **11** as a white solid (21 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 1.4 Hz, 1H), 8.21 (s, 1H), 7.98 – 7.86 (m, 3H), 7.84 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.68 – 7.55 (m, 3H), 6.70 (d, *J* = 4.5 Hz, 1H), 6.63 (d, *J* = 4.5 Hz, 1H), 5.93 (dd, *J* = 8.1, 5.9 Hz, 1H), 5.39 (dd, *J* = 5.9, 3.2 Hz,, 3H), 5.22 (d, *J* = 8.0 Hz, 1H), 4.25 (q, *J* = 3.0 Hz, 1H), 3.43 (dd, *J* = 12.5, 3.2 Hz, 1H), 3.24 (dd, *J* = 12.5, 3.2 Hz, 1H), 2.06 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 169.5, 155.4, 147.5, 136.6, 135.0, 132.3, 129.8, 129.4, 128.8, 128.7, 128.0, 127.6, 125.5, 122.5, 116.2, 113.5, 100.8, 81.7, 76.4, 73.3, 71.4, 44.6, 20.8, 20.6. HRMS (ESI$^+$): m/z calcd for $C_{25}H_{26}N_5O_7S$ $[M+H]^+$ 540.1547, found 540.1548.

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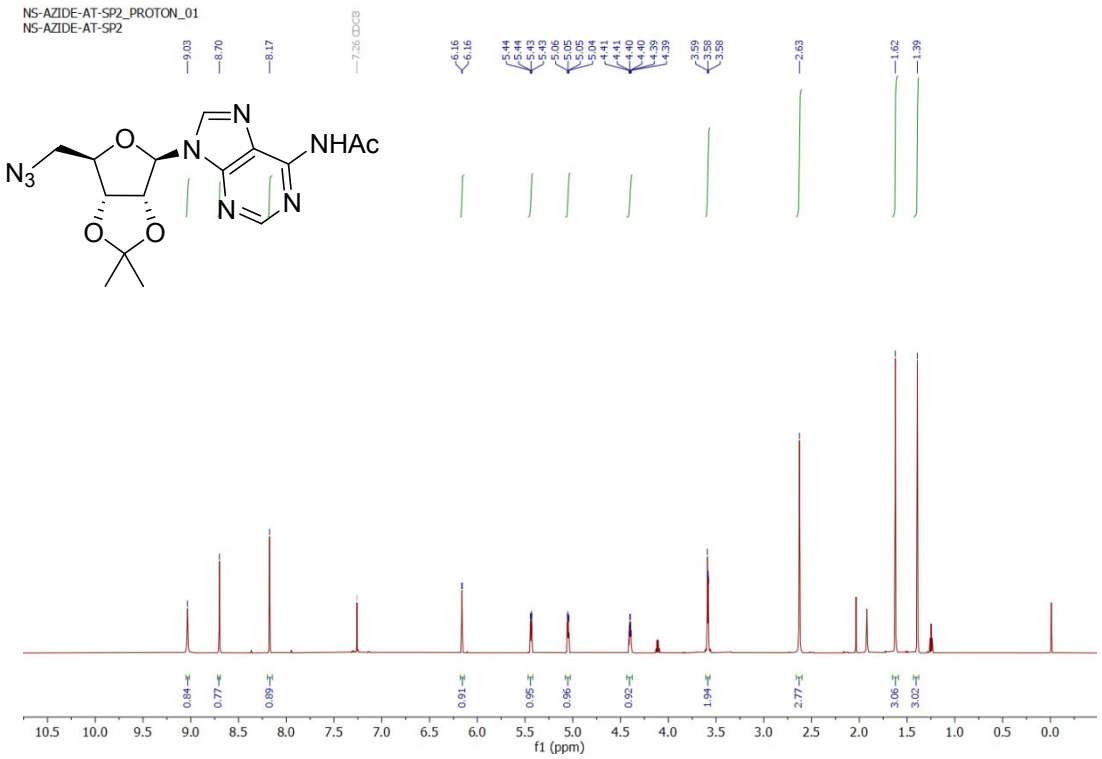
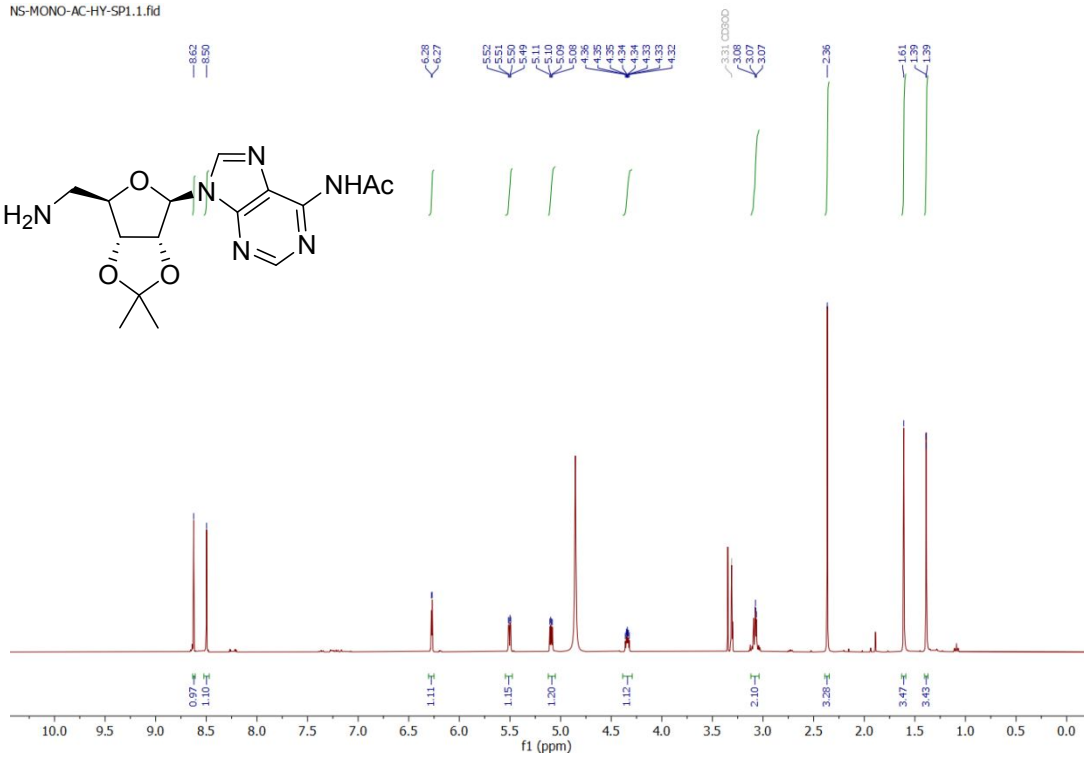
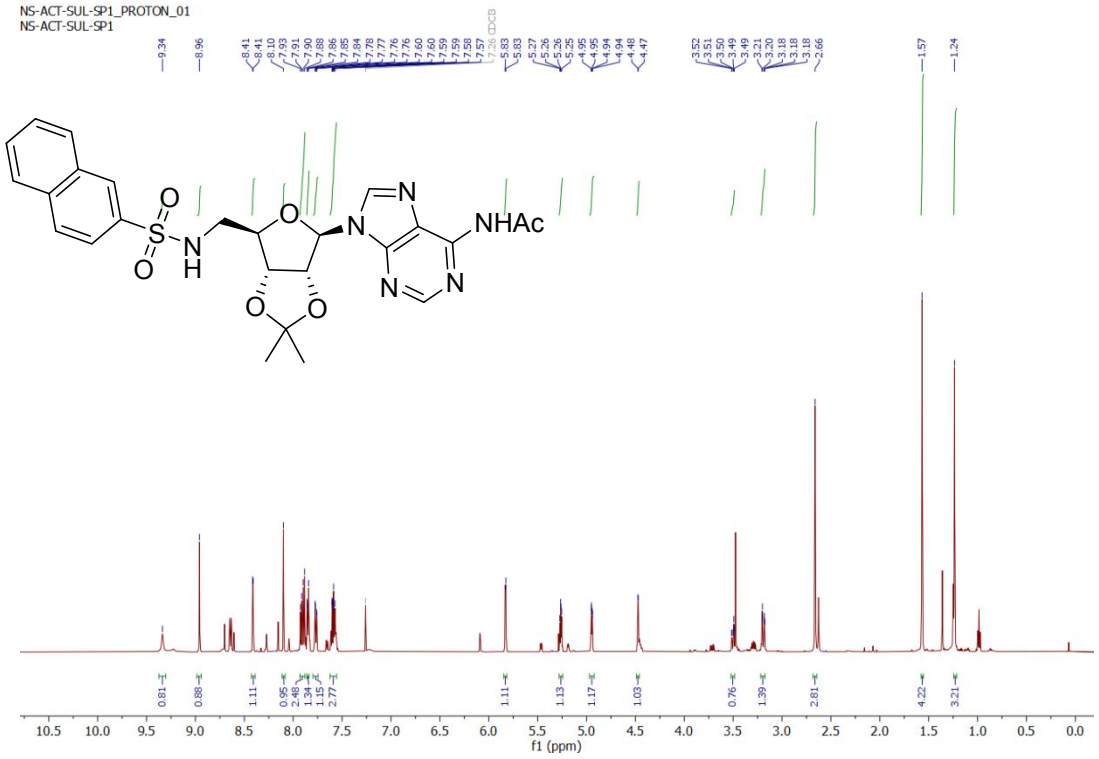


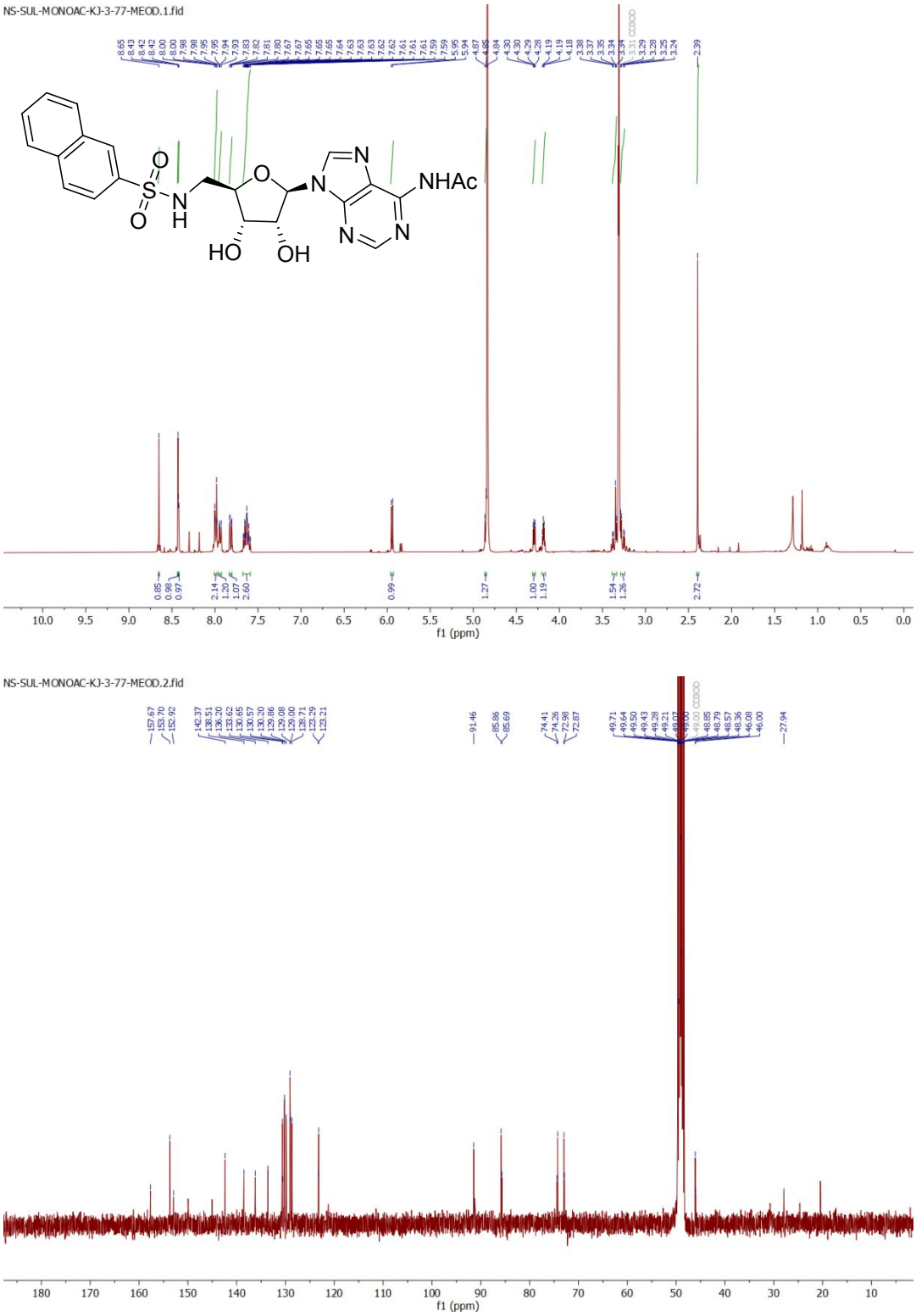
^{1}H NMR spectrum of compound **20**



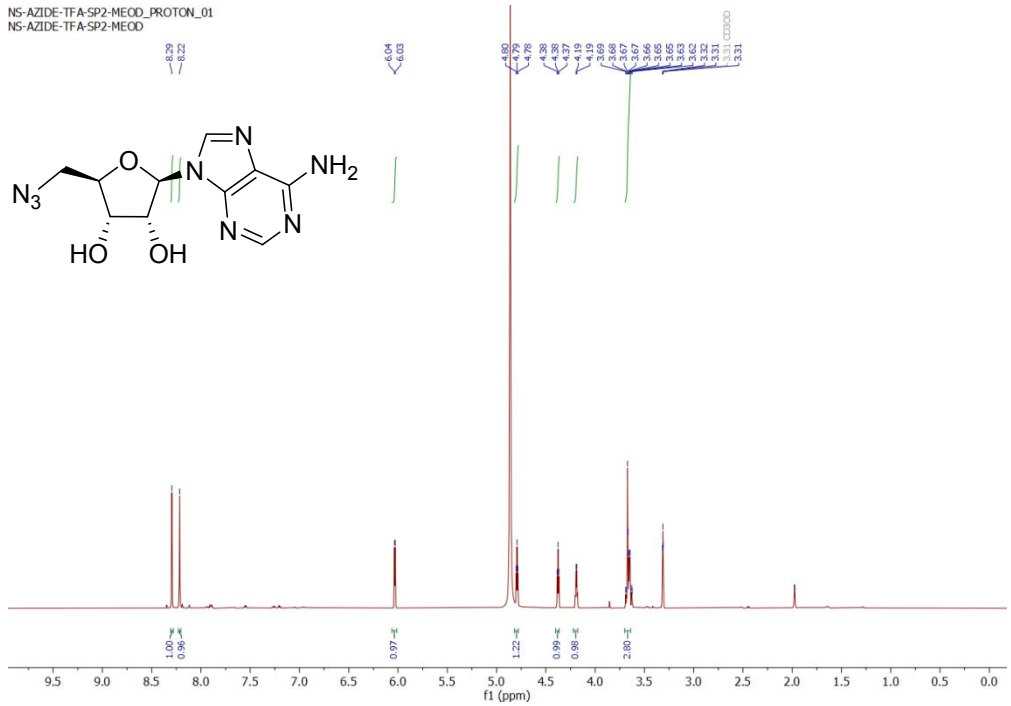
^{1}H NMR spectrum of compound **21**



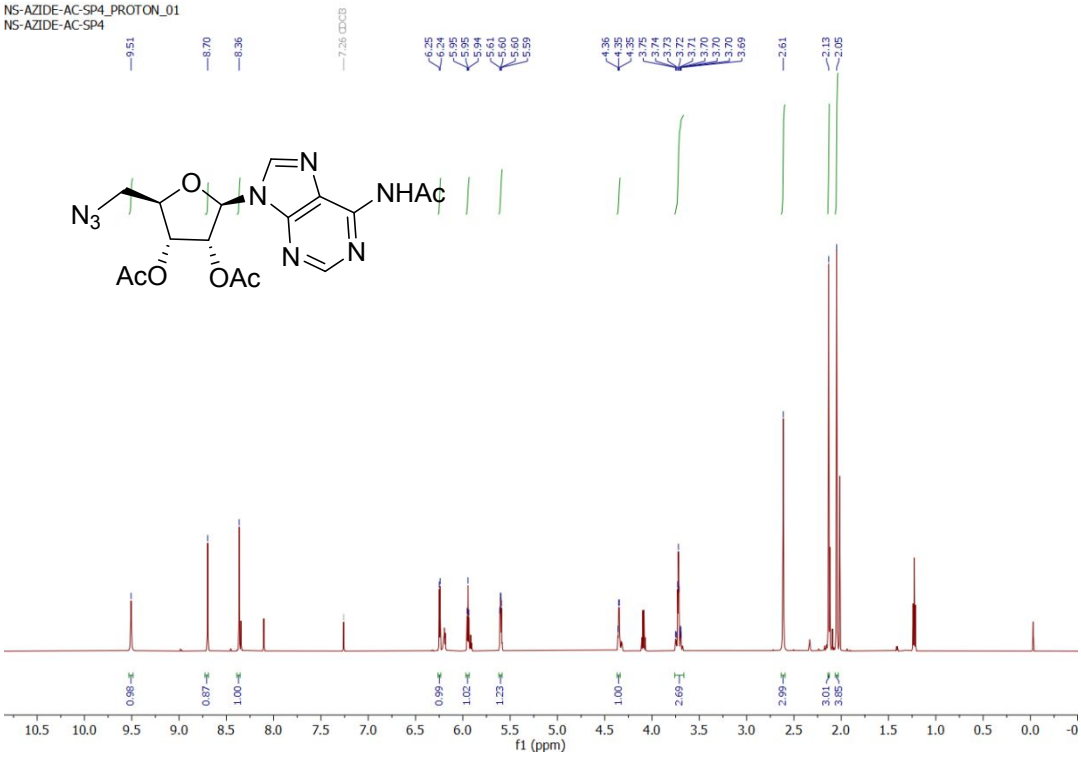
^{1}H NMR spectrum of compound **22**



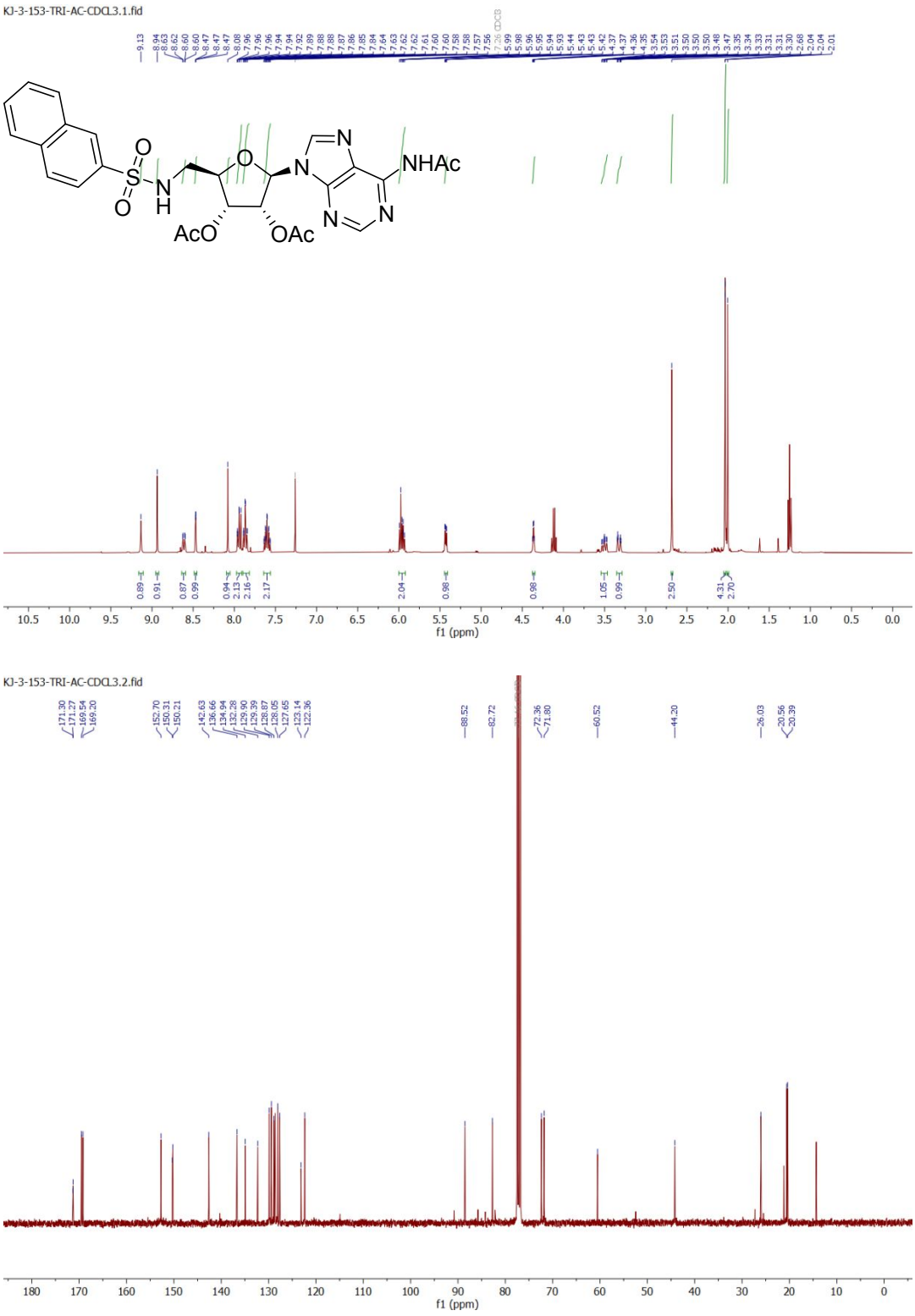
^{1}H and ^{13}C NMR spectra of compound **6**



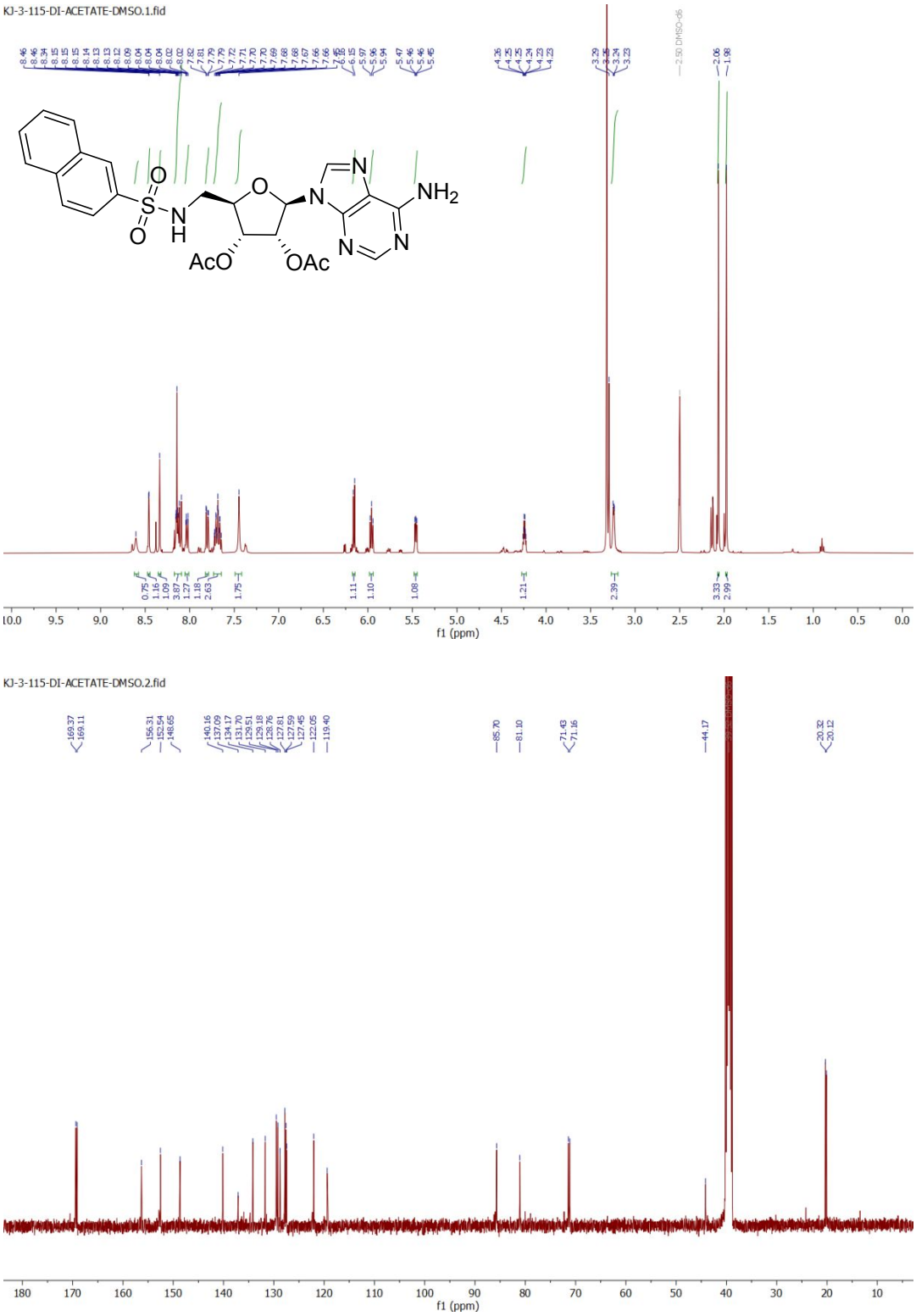
^{1}H NMR spectrum of compound **23**



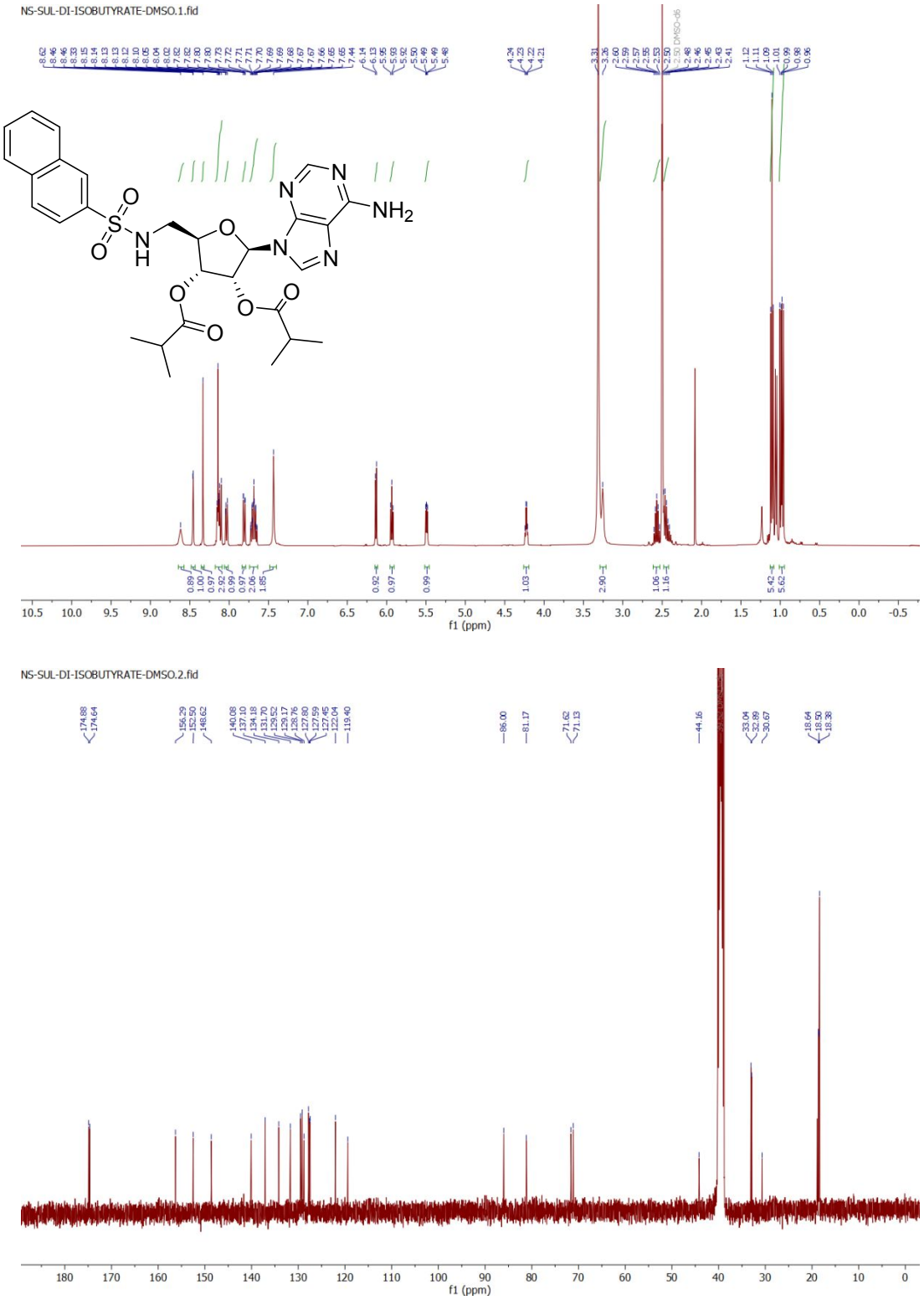
^{1}H NMR spectrum of compound **24**



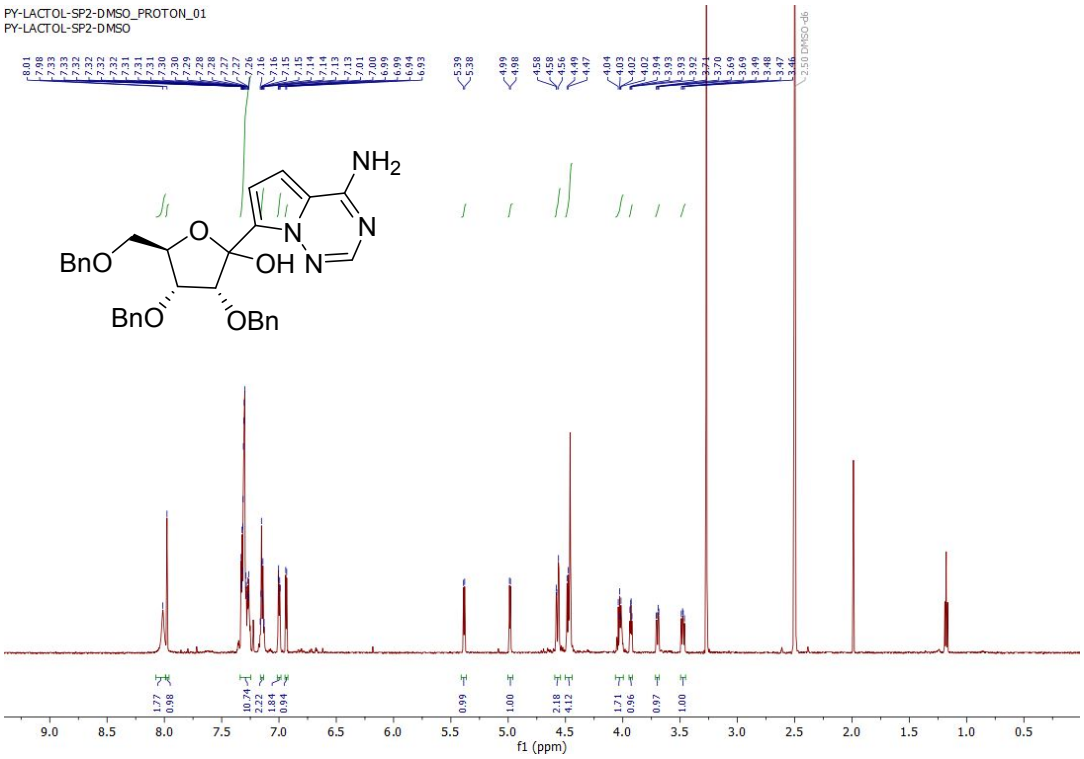
^{1}H and ^{13}C NMR spectra of compound **9**



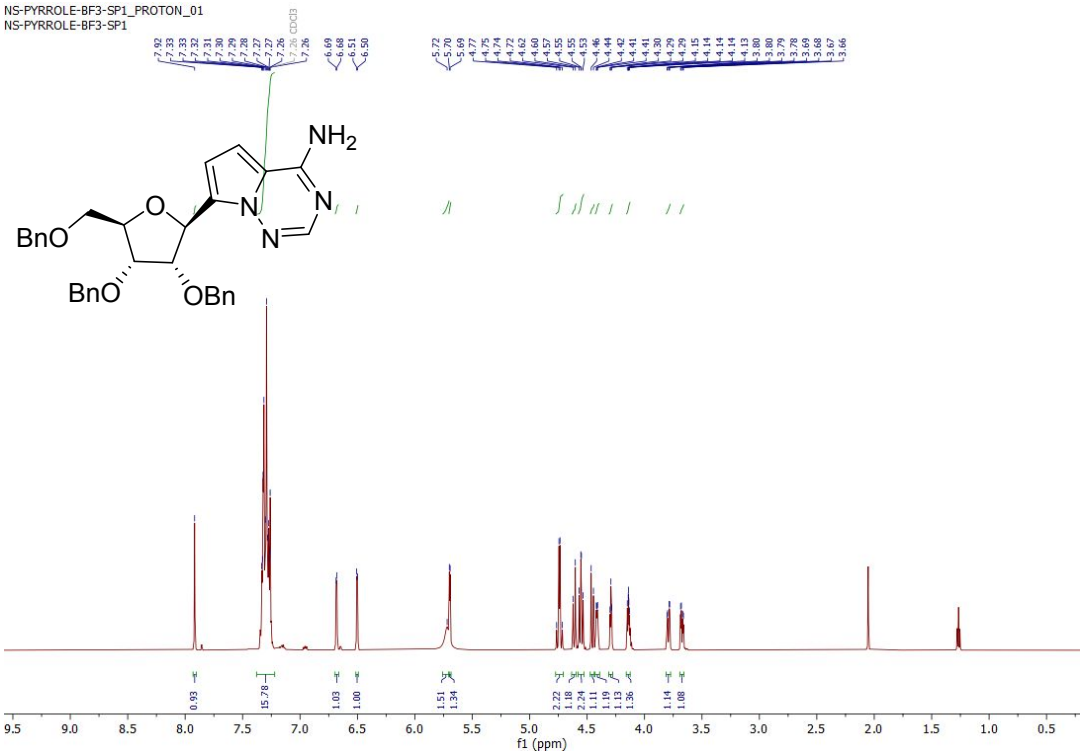
^{1}H and ^{13}C NMR spectra of compound **8**



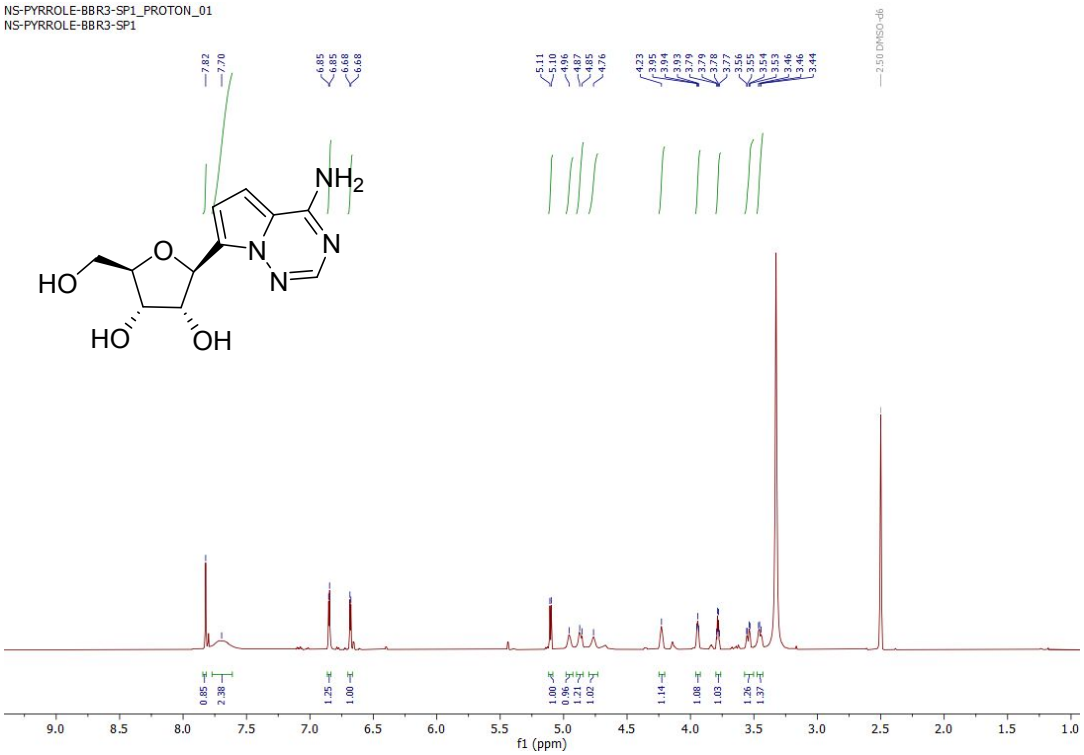
^{1}H and ^{13}C NMR spectra of compound **7**



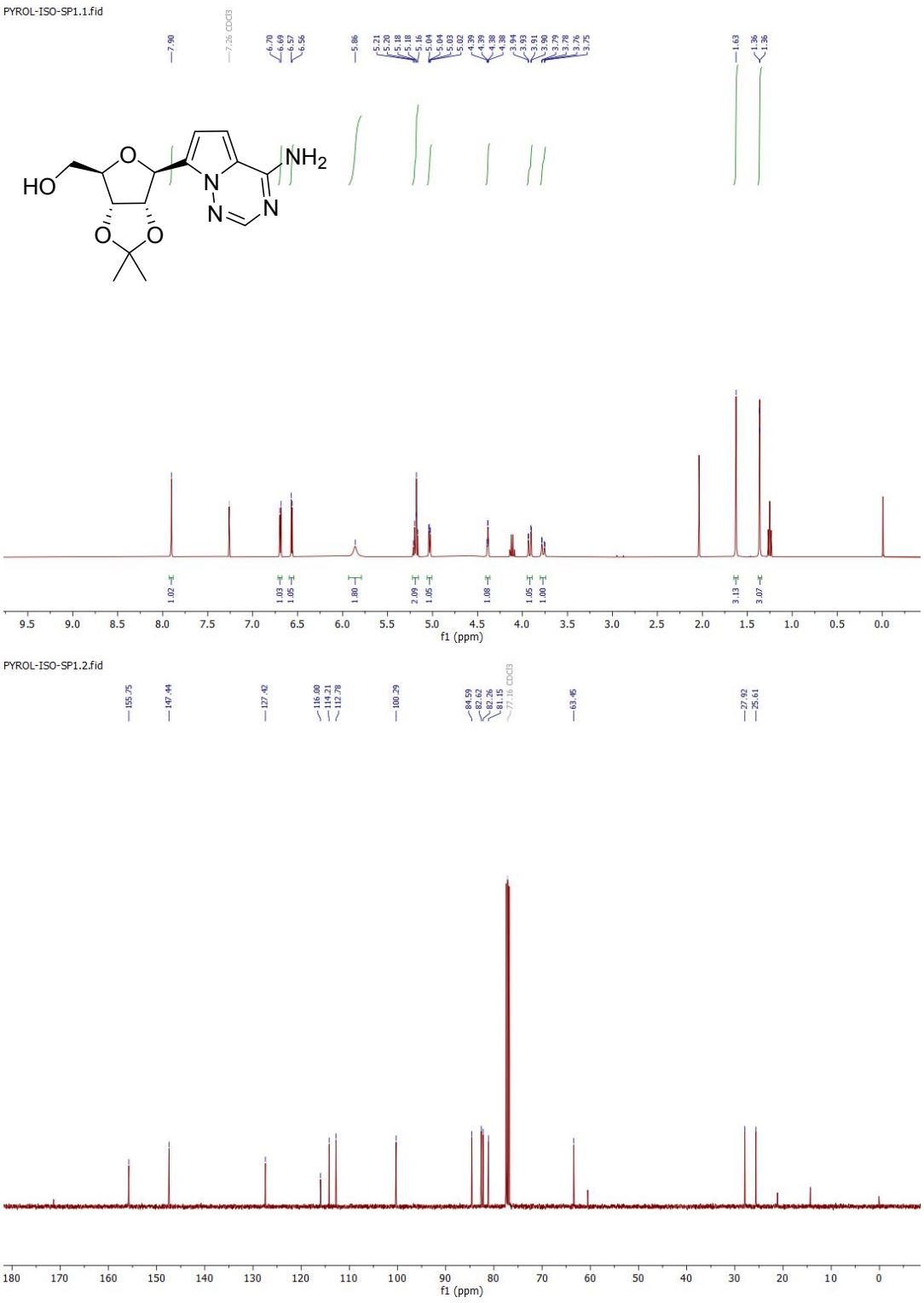
^{1}H NMR spectrum of compound **28**



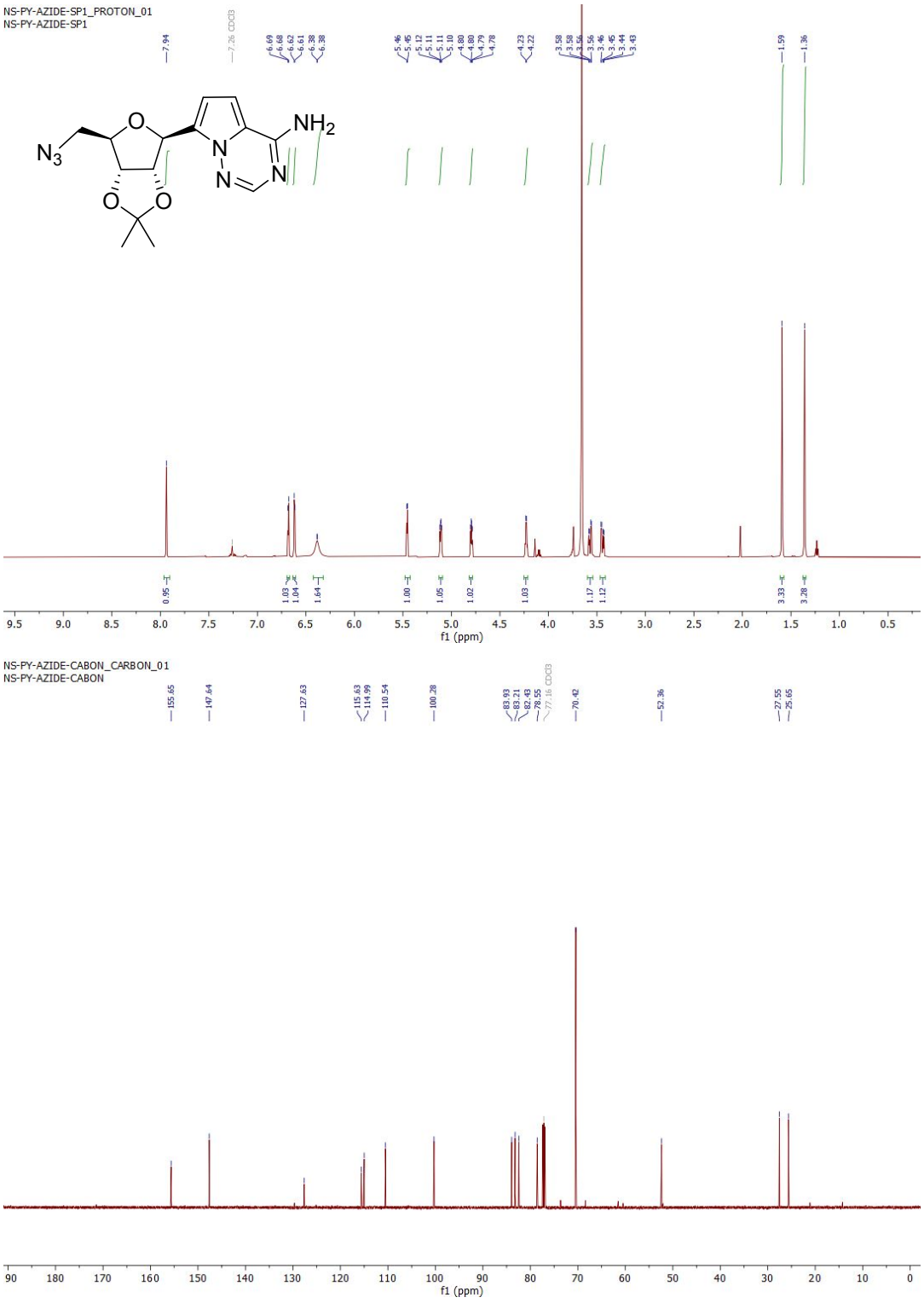
^{1}H NMR spectrum of compound **29**



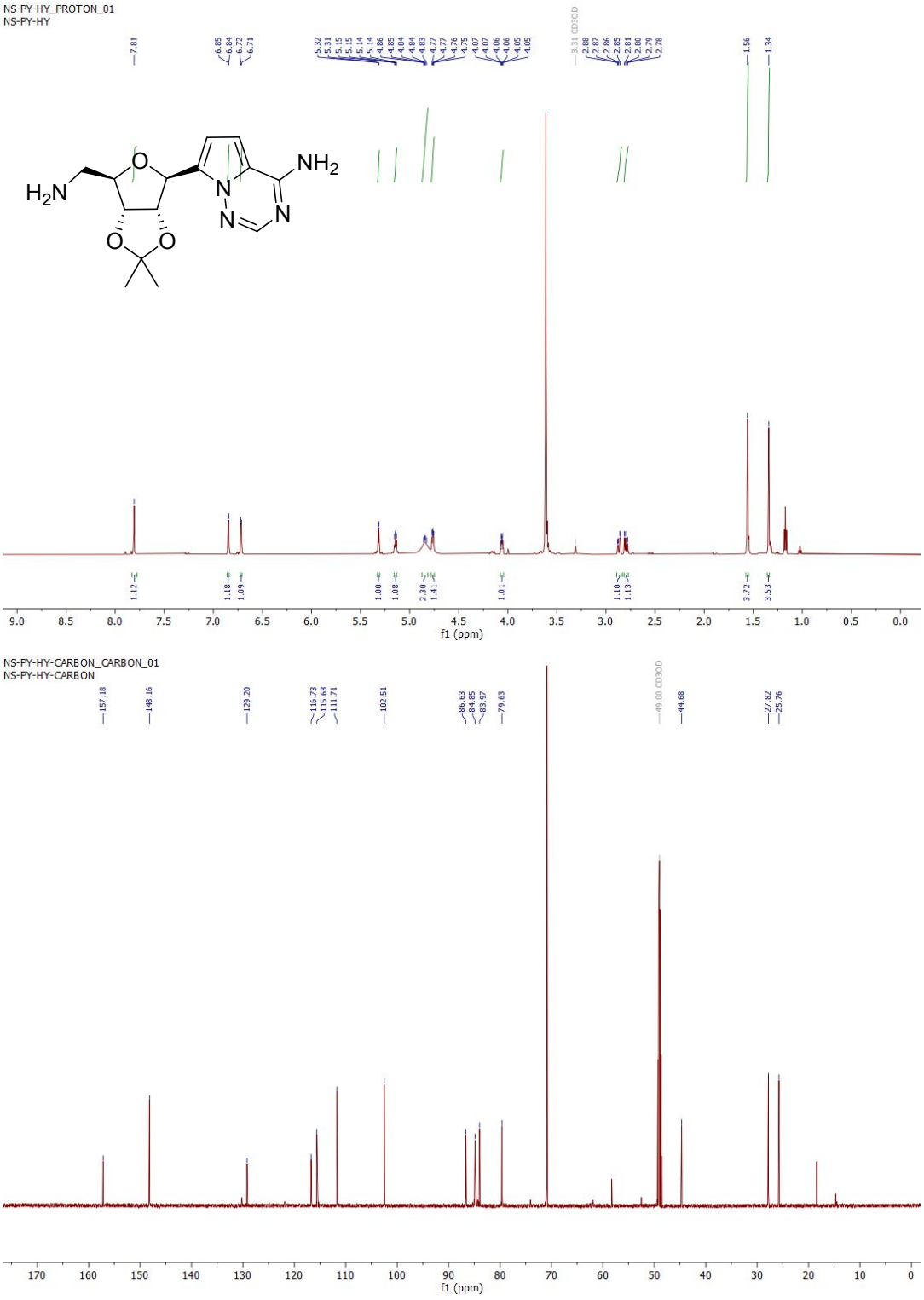
^{1}H NMR spectrum of compound **30**



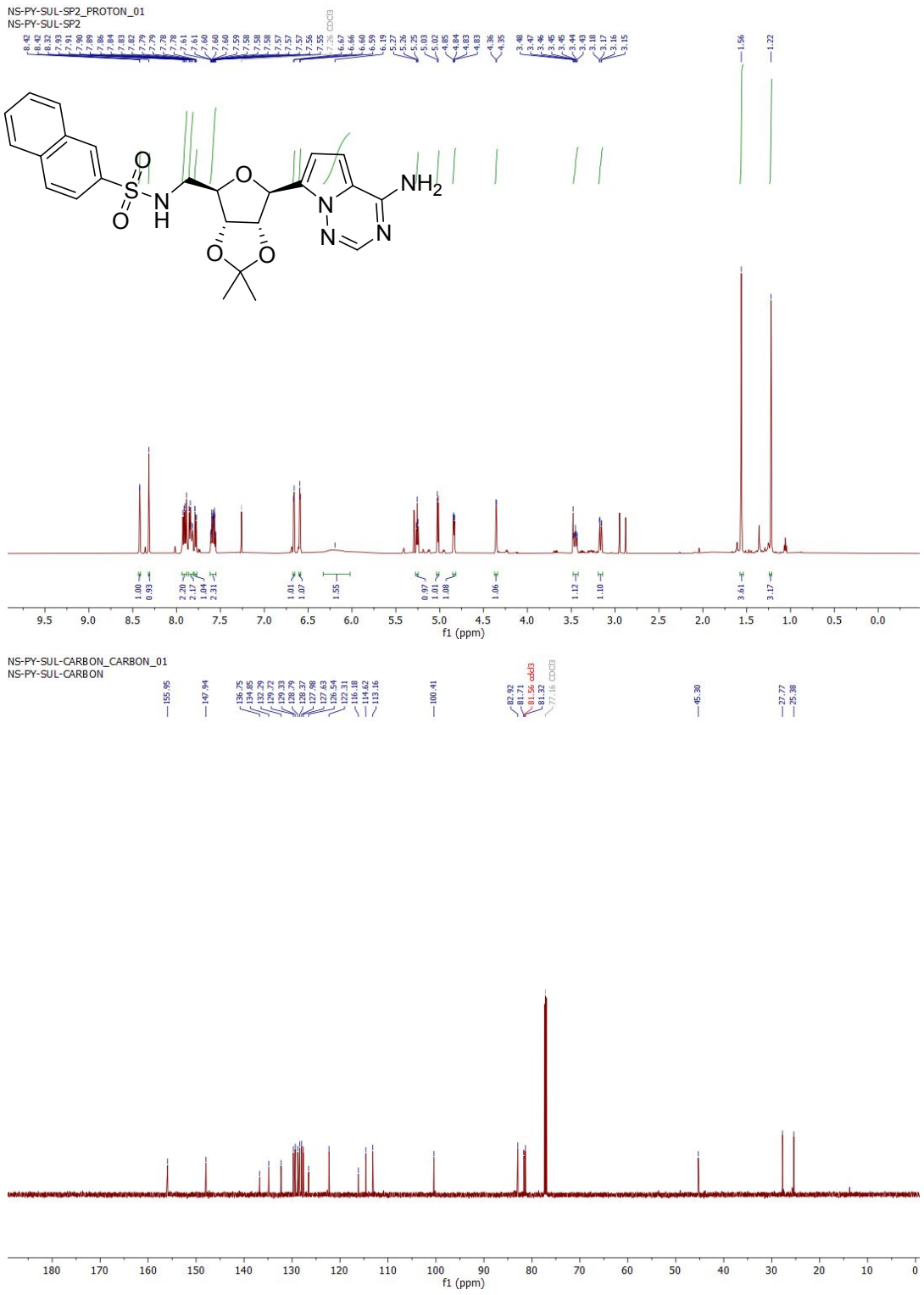
^{1}H and ^{13}C NMR spectra of compound **31**



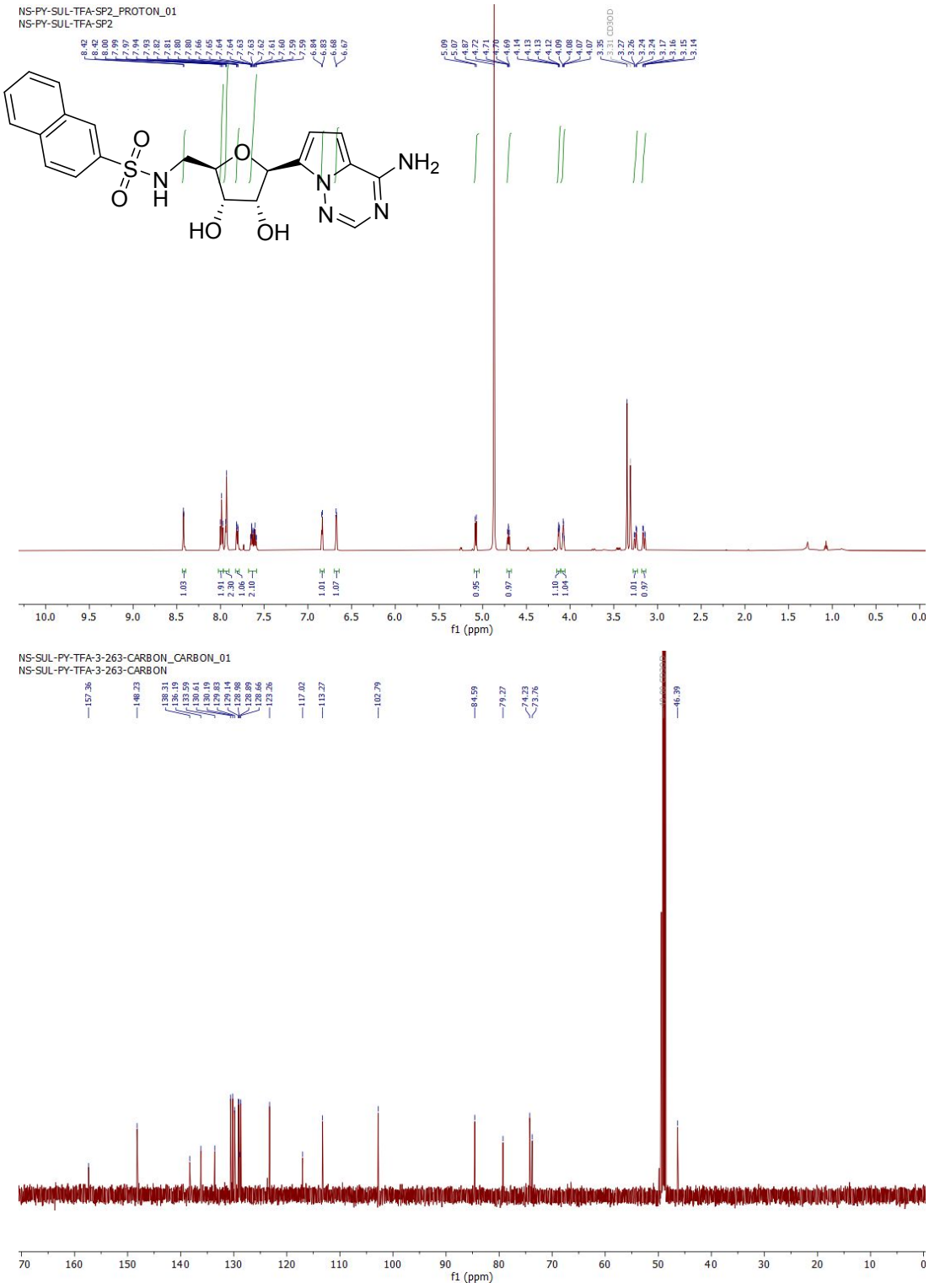
^{1}H and ^{13}C NMR spectra of compound **32**



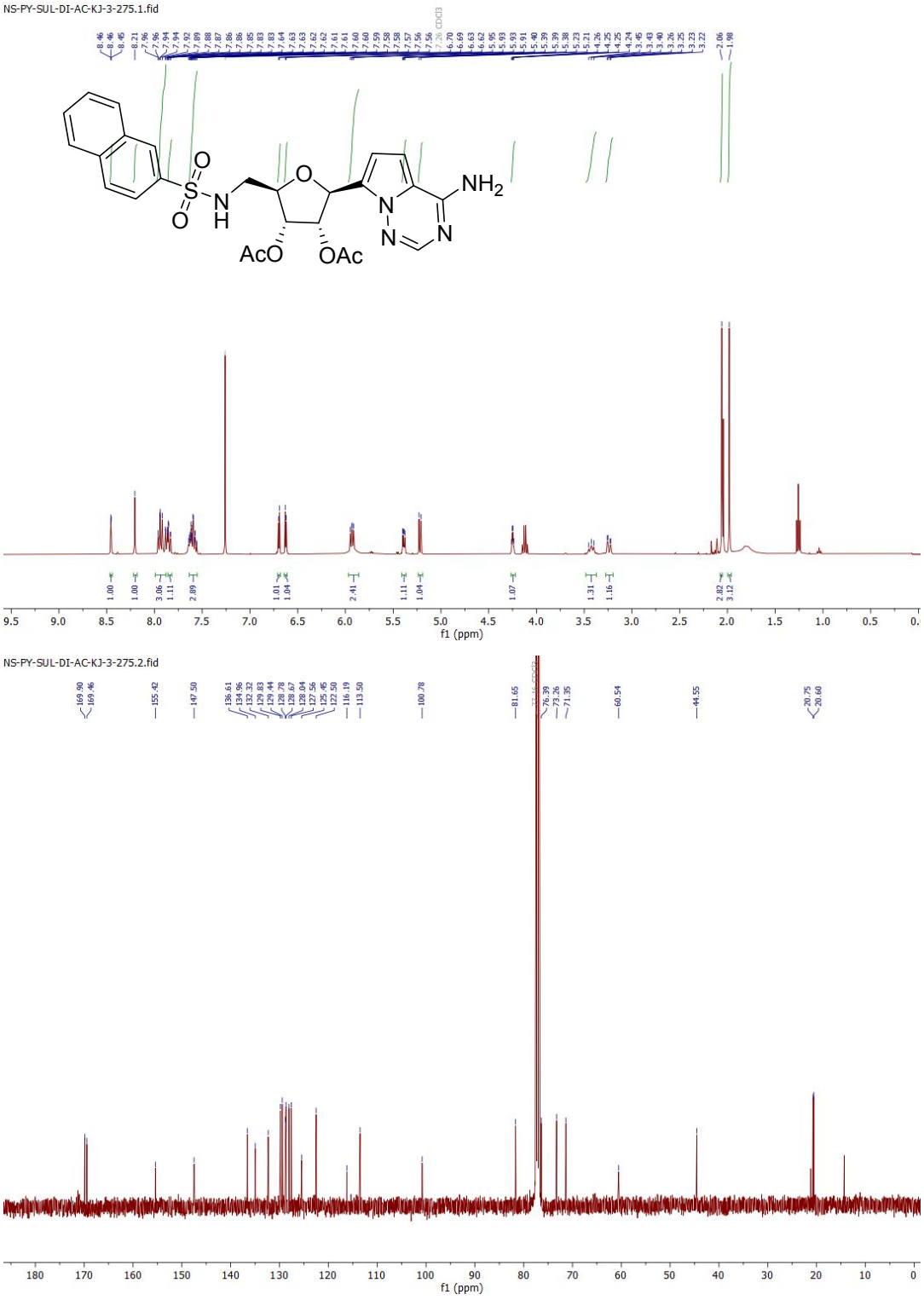
^{1}H and ^{13}C NMR spectra of compound **33**



^{1}H and ^{13}C NMR spectra of compound **34**



^{1}H and ^{13}C NMR spectra of compound **10**



^{1}H and ^{13}C NMR spectra of compound **11**