Electronic Supplementary Information

Discovery of an NAD$^+$ Analogue with Enhanced Specificity for PARP1

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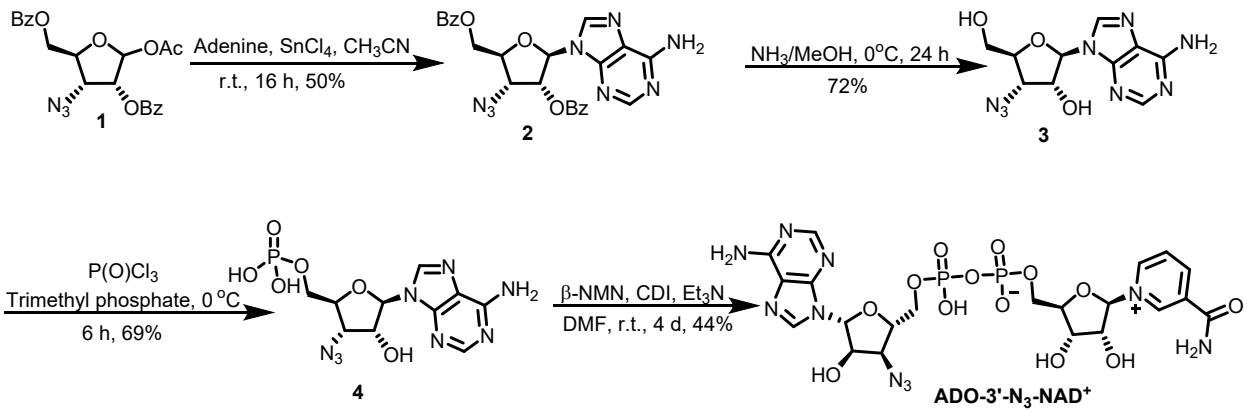


Scheme S1. Chemical synthesis of ADO-3′-N_3-NAD^+.

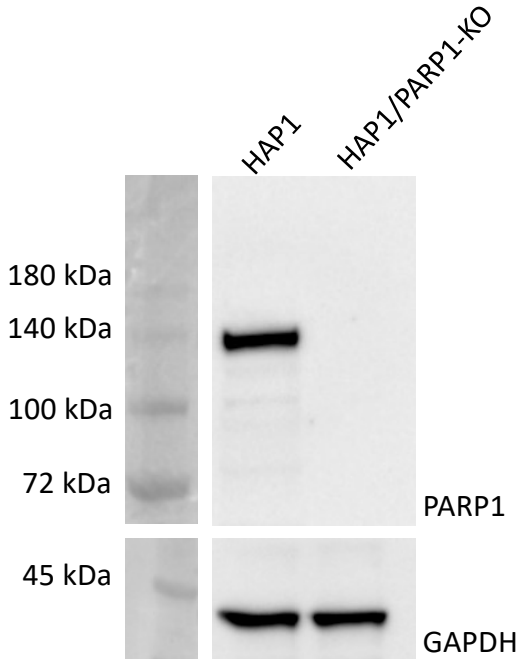
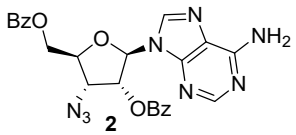


Figure S1. Immunoblot analysis of human PARP1 expression in HAP1 and HAP1/PARP1-KO cell lines. An anti-human PARP1 monoclonal antibody (top panel) and an anti-human GAPDH monoclonal antibody (bottom panel) were used for detection.

Chemical synthesis and characterization of ADO-3′-N_3-NAD^+.

General materials and methods for compound synthesis. ^{1}H NMR spectra were recorded on an Oxford AM-400 spectrometer for solution in CD_3OD or D_2O. Coupling constants *J* are shown in Hz. ^{13}C NMR spectra were recorded on an Oxford AM-400 spectrophotometer (100 MHz) with complete proton decoupling spectrophotometer (CD_3OD: 49.0 ppm). Flash column chromatography was performed using 230-400 mesh silica gel (Sigma-Aldrich, St. Louis, MO). For thin-layer chromatography (TLC), silica gel plates (Sigma-Aldrich: GF254) were used. HPLC was performed on a Waters 2487 series with a C18 Kinetex column (5 μm, 100 Å, 150×10.0 mm, Phenomenex Inc, Torrance, CA). All other reagents were purchased from readily available commercial sources and used without further purification.

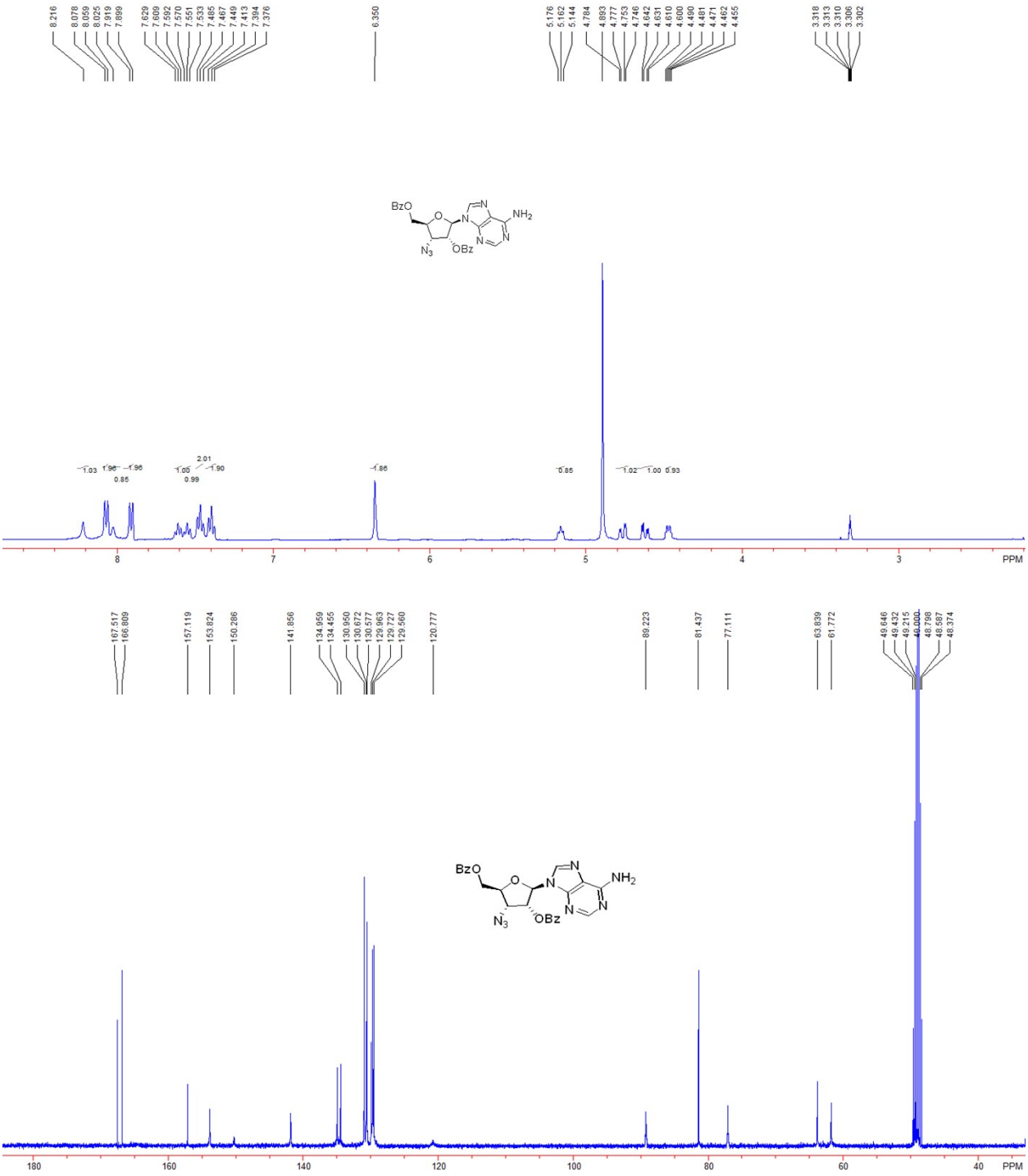


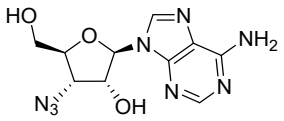
2

*General procedure for the synthesis of compound **2***: Adenine (324 mg, 2.4 mmol, 1.2 eq) was suspended in acetonitrile (40 mL) with compound **1** ((2S,3R,4R)-5-acetoxy-3-azido-4-(benzoyloxy)tetrahydrofuran-2-yl)methyl benzoate[1] (851 mg, 2.0 mmol, 1.0 eq). To this suspension was added stannic chloride fuming (304 μL, 2.6 mmol, 1.3 eq) at room temperature. After 16 hours, the reaction was concentrated to a small volume (about 10 mL), and $NaHCO_3$ (10 g) and water (12 mL) were added. The resulting white solid (tin salts) was extracted with hot chloroform (5×20 mL). The combined extracts were filtered on a cellite bed. The organic phase was washed with 5% $NaHCO_3$ solution and water, dried over sodium sulfate. Then the solvent was removed under reduced pressure and the residue was purified by a flash column chromatography to afford the desired products **2** (500 mg, yield: 50%) as a colorless solid.

((2S,3R,4R,5R)-5-(6-amino-9H-purin-9-yl)-3-azido-4-(benzoyloxy)tetrahydrofuran-2-yl)methyl benzoate (**2**). ^{1}H NMR (400 MHz, CD_3OD): δ 4.46-4.49 (m, 1H, CH), 4.62 (dd, 1H, *J* = 12.4, 4.0 Hz, CH_2), 4.77 (dd, 1H, *J* = 12.4, 2.8 Hz, CH_2), 5.14-5.18 (m, 1H, CH), 6.35 (s, 2H, 2CH), 7.39 (t, 2H, *J* = 7.2 Hz, ArH), 7.47 (t, 2H, *J* = 7.2 Hz, ArH), 7.55 (t, 1H, *J* = 7.2 Hz, ArH),

7.61 (t, 1H, J = 7.2 Hz, ArH), 7.91 (d, 2H, J = 8.0 Hz, ArH), 8.03 (s, 1H, ArH), 8.07 (d, 2H, J = 7.6 Hz, ArH), 8.22 (s, 1H, ArH); ^{13}C NMR (100 MHz, CD_3OD): δ 61.8, 63.8, 77.1, 81.4, 89.2, 120.8, 129.6, 129.7, 130.0, 130.6, 130.7, 130.9, 134.5, 135.0, 141.9, 150.3, 153.8, 157.1, 166.8, 167.5. MS (ESI) *m/z*: 501.3 $(M+H)^+$.

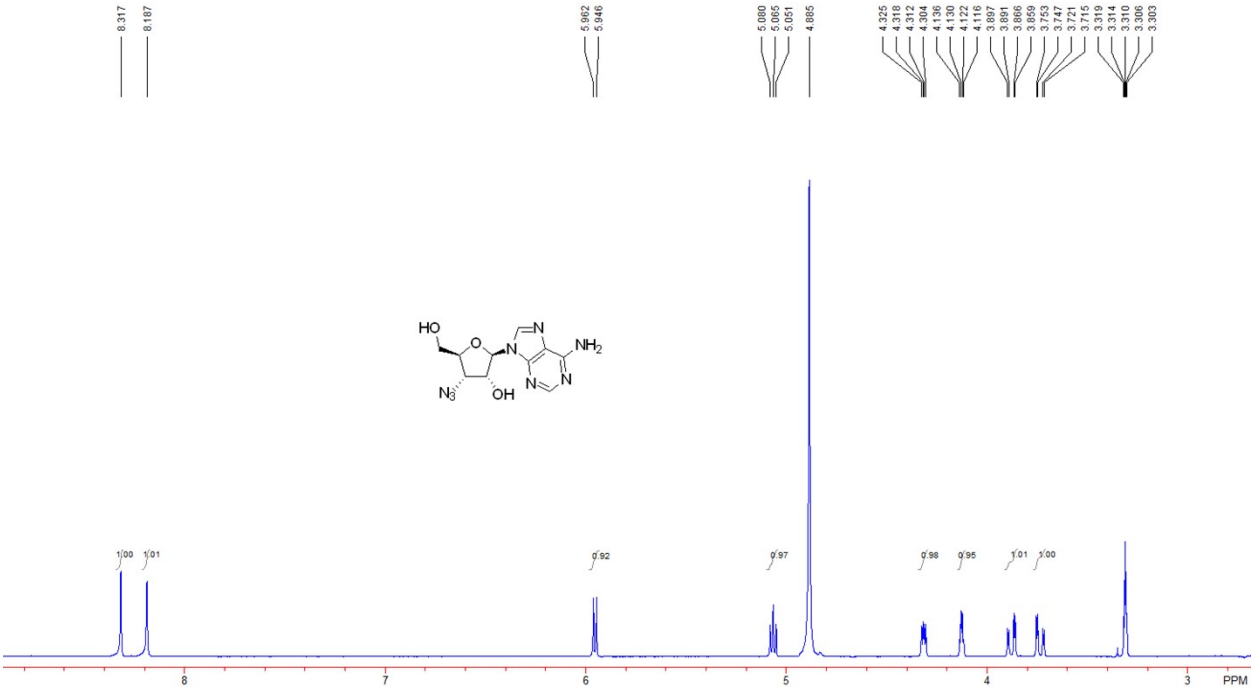


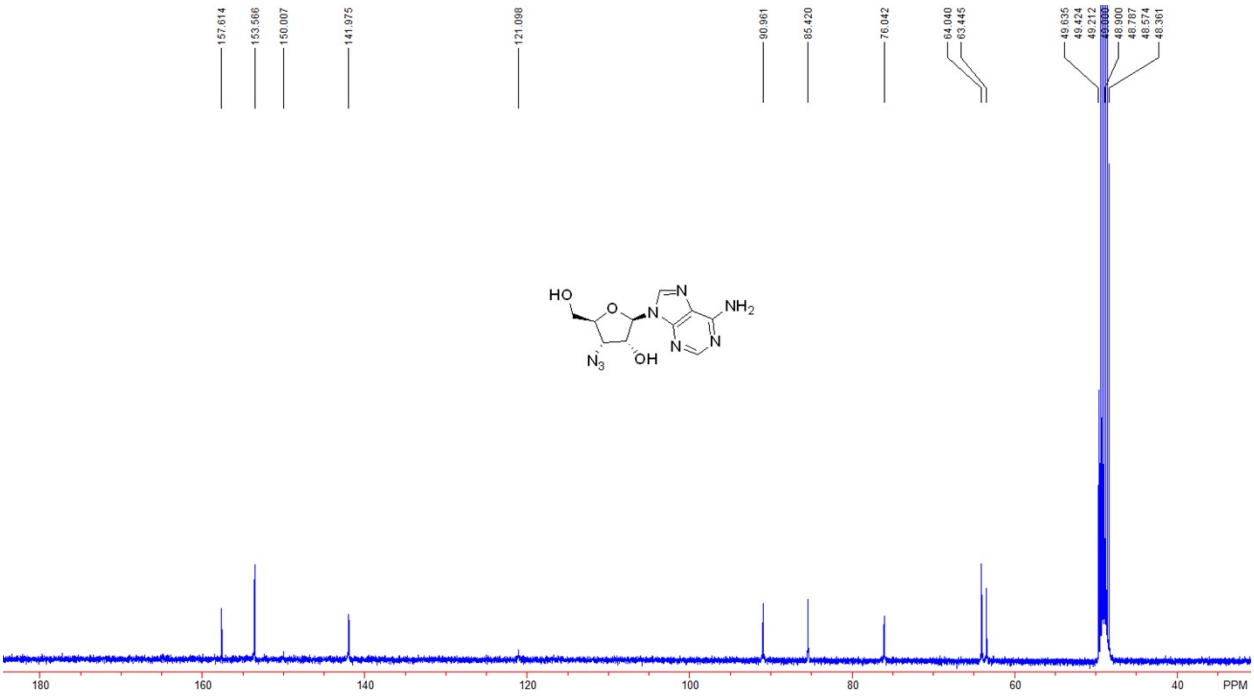


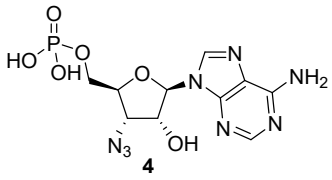
3

*General procedure for the synthesis of compound **3***: Compound **2** (250 mg, 0.5 mmol) was dissolved in ammonia (10 mL, 7 N in MeOH) and the reaction was stirred at 0°C for 24 hours. The reaction was then concentrated *in vacuo* and the crude product was purified via HPLC using the C18 Kinetex column (5 µm, 100 Å, 150×10.0 mm) (mobile phase A: 0.1% formic acid (aq), mobile B: 0.1% formic acid in acetonitrile; flow rate = 2.0 mL min^{-1}; 0-2 min: 0-4% B, 2-4 min: 4-10% B; 4-8 min: 10-20% B; 8-9 min: 20% B; 9-12 min: 20-50% B; 12-14 min: 50-0% B). Fractions containing the desired product were concentrated and lyophilized to yield the desired products **3** (105 mg, yield: 72%) as a colorless solid.

(2R,3R,4S,5S)-2-(6-amino-9H-purin-9-yl)-4-azido-5-(hydroxymethyl)tetrahydrofuran-3-ol (3). ^{1}H NMR (400 MHz, CD_3OD): δ 3.73 (dd, 1H, J = 12.8, 2.4 Hz, CH_2), 3.88 (dd, 1H, J = 12.8, 2.4 Hz, CH_2), 4.13 (dd, 1H, J = 5.6, 2.4 Hz, CH), 4.31 (dd, 1H, J = 5.6, 3.2 Hz, CH), 5.06 (t, 1H, J = 5.6 Hz, CH), 5.95 (d, 1H, J = 6.4 Hz, CH), 8.19 (s, 1H, ArH), 8.32 (s, 1H, ArH); ^{13}C NMR (100 MHz, CD_3OD): δ 63.4, 64.0, 76.0, 85.4, 91.0, 121.1, 142.0, 150.0, 153.6, 157.6; MS (ESI) *m/z*: 293.2 $(M+H)^+$.



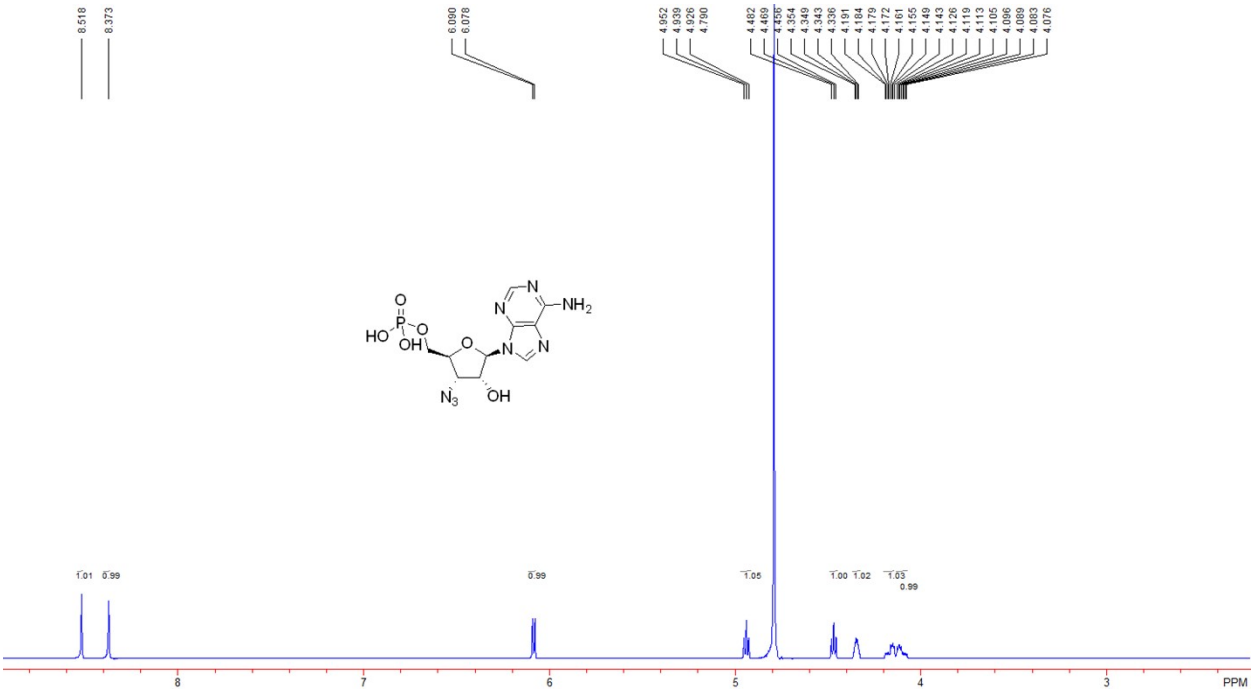


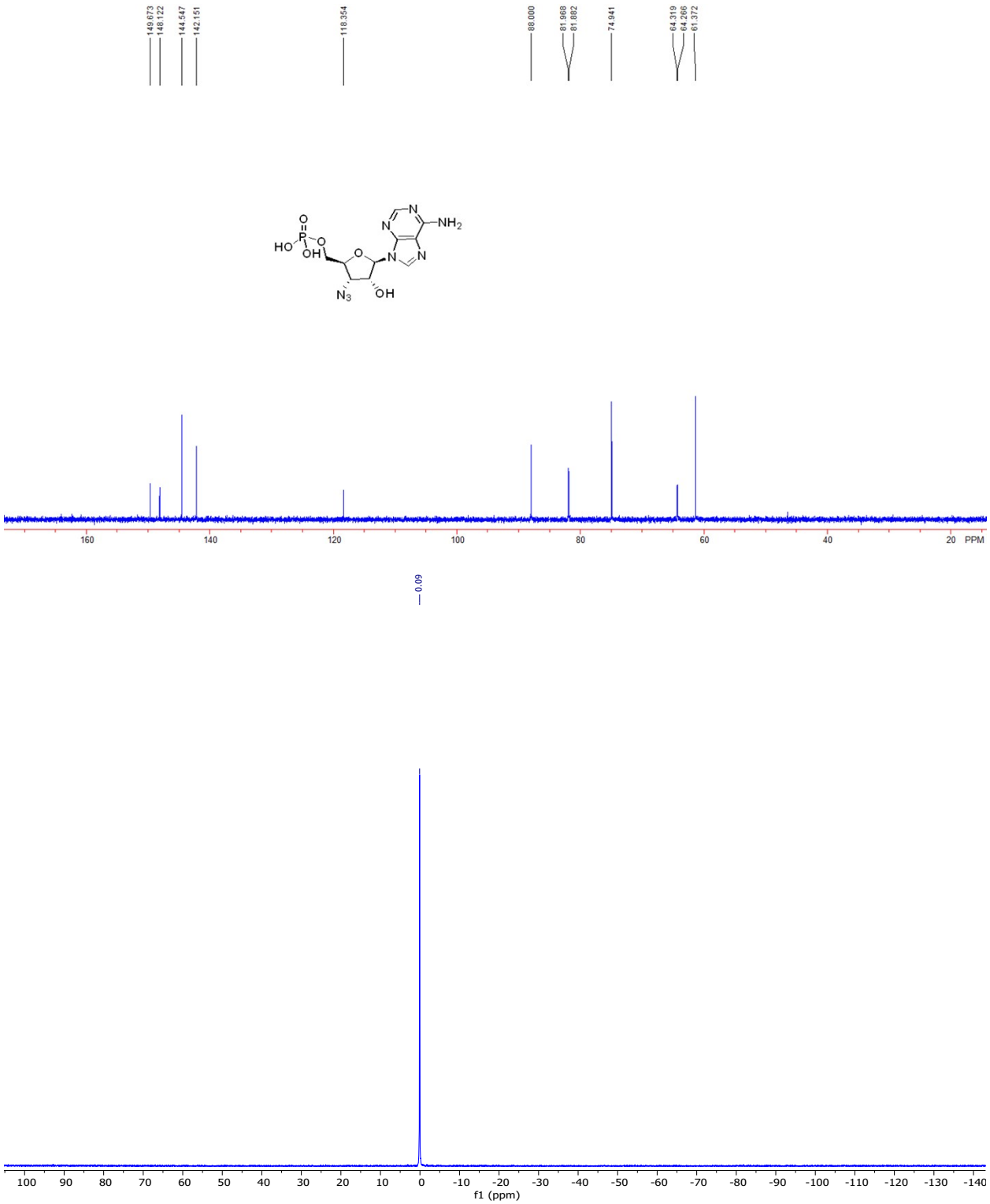


4

*General procedure for the synthesis of compound **4***: To a stirred solution of compound **3** (58 mg, 0.2 mmol) in trimethylphosphate (2 mL, purchased from Sigma-Aldrich with a quality level of 200 and purity assay of 97% (catalog number: 132195-250G) and used without further purification) was added $P(O)Cl_3$ (56 µL, 0.6 mmol, 3 eq, purchased from Sigma-Aldrich with a quality level of 200 and purity assay of 99% (catalog number: 201170-5G) and used without further purification) at 0˚C and the resulting mixture was stirred at 0˚C for 6 hours. A few drops of water were then added to quench the reaction. The reaction was then concentrated *in vacuo* and the crude product was purified via HPLC using the C18 Kinetex column (5 µm, 100 Å, 150×10.0 mm) (mobile phase A: 0.1% formic acid (aq), mobile B: 0.1% formic acid in acetonitrile; flow rate = 2.0 mL min^{-1}; 0-2 min: 0-4% B, 2-4 min: 4-10% B; 4-8 min: 10-20% B; 8-9 min: 20% B; 9-12 min: 20-50% B; 12-14 min: 50-0% B). Fractions containing the desired product were concentrated and lyophilized to yield the desired products **4** (47 mg, yield: 69%) as a colorless solid.

((2S,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3-azido-4-hydroxytetrahydrofuran-2-yl)methyl dihydrogen phosphate (4). ^{1}H NMR (400 MHz, D_2O): δ 4.10 (ddd, 1H, *J* = 12.0, 5.2, 2.8 Hz, CH_2), 4.17 (ddd, 1H, *J* = 12.0, 4.8, 2.4 Hz, CH_2), 4.33-4.36 (m, 1H, CH), 4.47 (t, 1H, *J* = 5.2 Hz, CH), 4.94 (t, 1H, *J* = 5.2 Hz, CH), 6.08 (d, 1H, *J* = 5.2 Hz, CH), 8.37 (s, 1H, ArH), 8.52 (s, 1H, ArH); ^{13}C NMR (100 MHz, D_2O): δ 61.4, 64.3 (d, *J* = 5.3 Hz), 74.9, 81.9 (d, *J* = 8.6 Hz), 88.0, 118.4, 142.2, 144.5, 148.1, 149.7; ^{31}P NMR (162 MHz, D_2O): δ 0.09; MS (ESI) *m/z*: 373.2 $(M+H)^+$.

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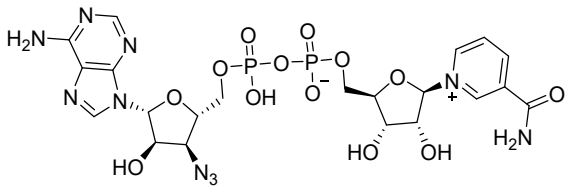
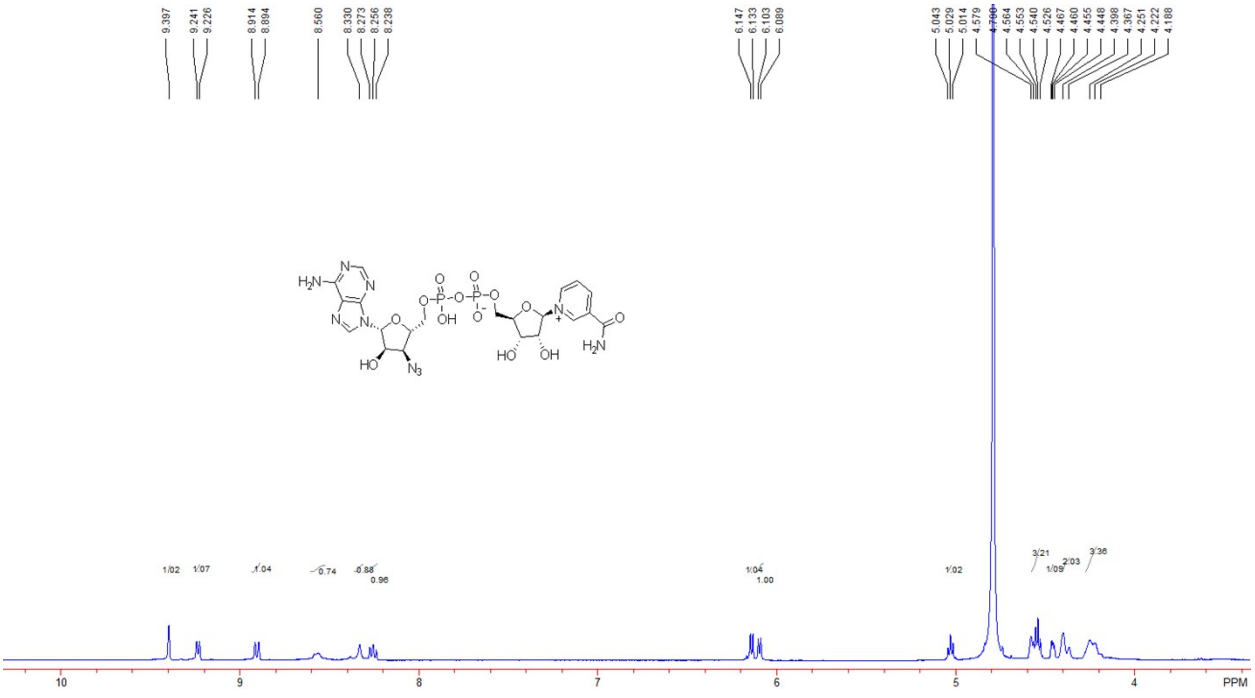


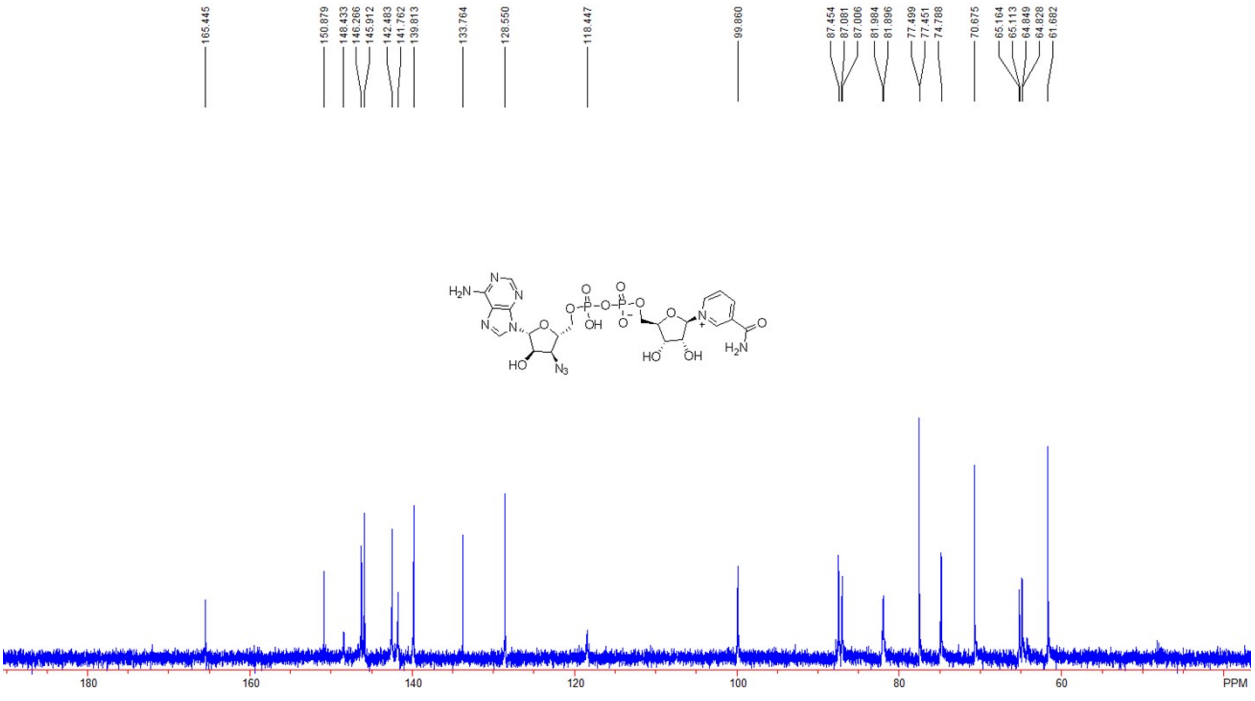
ADO-3'-N_3-NAD^+

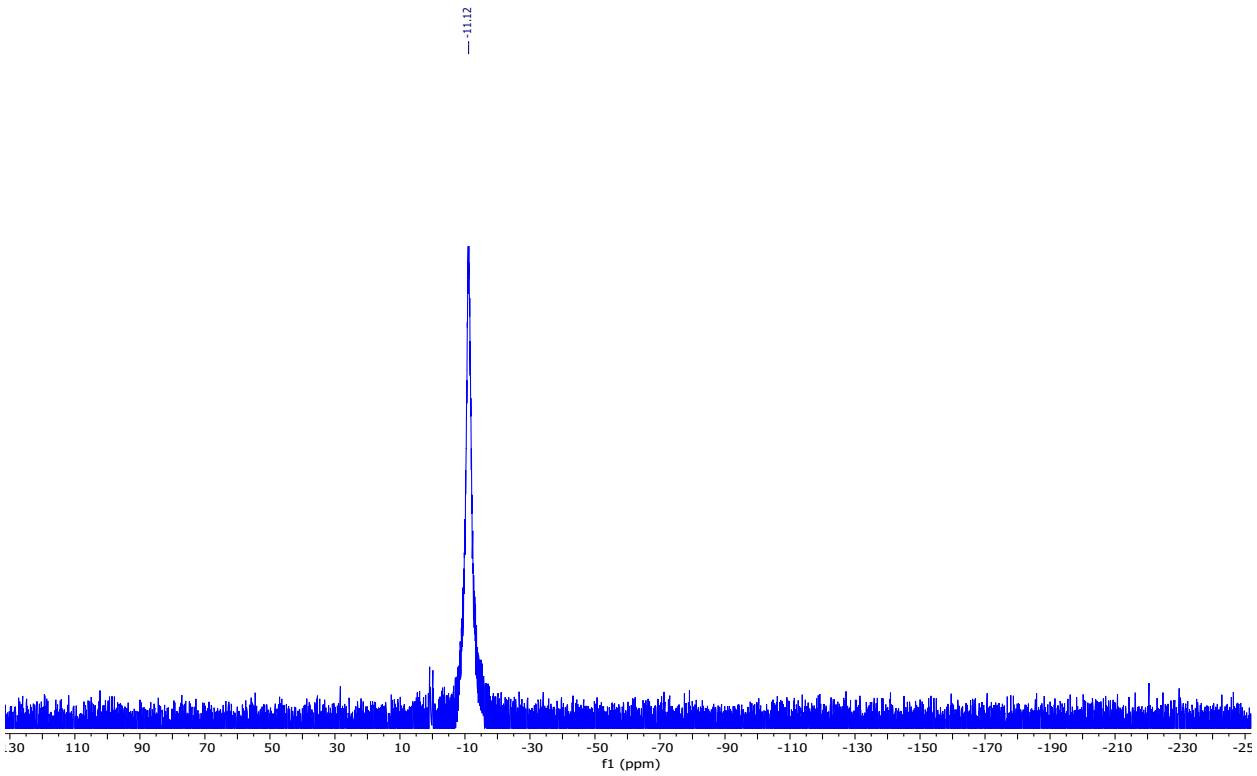
*General procedure for the synthesis of **ADO-3′-N_3-NAD^+***: To a stirred solution of **4** (37 mg, 0.1 mmol) in dried DMF (2 mL, purchased from Acros Organics with a purity assay of 99.8% (catalog number: 348435000) and used without further purification) were added 1,1-carbonyldiimidazole (CDI) (63 mg, 0.50 mmol, 5 eq) and triethylamine (23 μL, 0.16 mmol. 1.6 eq). The reaction mixture was stirred at room temperature for 10 hours, and then quenched with 0.1 mL dried methanol. The solvent was removed under vacuum and the residue was co-evaporated 3 times each with 1 mL of dried DMF. The activated 5′-AMP analogue was dissolved in dried DMF (1 mL) and NMN (50 mg, 0.15 mmol, 1.5 eq) was added. After stirring at room temperature for 4 days, H_2O was added to quench the reaction at 0˚C. The resulting mixture was continued stirring at room temperature for 24 hours. The reaction was then concentrated *in vacuo* and the crude product was purified via HPLC using the C18 Kinetex column (5 μm, 100 Å, 150×10.0 mm) (mobile phase A: 0.1% formic acid (aq), mobile B: 0.1% formic acid in acetonitrile; flow rate = 2.0 mL min^{-1}; 0-2 min: 0-4% B, 2-4 min: 4-10% B, 4-6 min: 10-20% B, 6-12 min: 20-50% B, 12-17 min: 50-100% B, 17-20 min: 100-0% B) with detection of UV absorbance at 260 nm. Fractions containing the desired product were concentrated and lyophilized to yield the **ADO-3′-N_3-NAD^+** (30 mg, yield: 44%) as a colorless solid.

1-((2R,3R,4S,5R)-5-(((((((((2S,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3-azido-4-hydroxytetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)oxidophosphoryl)oxy)methyl)-3,4-dihydroxytetrahydrofuran-2-yl)-3-carbamoylpyridin-1-ium (ADO-3′-N_3-NAD^+). ^{1}H NMR (400 MHz, D_2O): δ 4.19-4.25 (m 3H, CH_2+CH_2), 4.37-4.40 (m, 2H, CH_2+CH), 4.46 (dd, 1H, *J* = 4.8, 2.8 Hz, CH), 4.53-4.58 (m, 3H, 3CH), 5.03 (t, 1H, *J* = 5.6 Hz, CH), 6.10 (d, 1H, *J* = 5.6 Hz, CH), 6.14 (d, 1H, *J* = 5.6 Hz, CH), 8.24-8.27 (m, 1H, ArH), 8.33 (s, 1H, ArH), 8.56 (br, 1H, ArH), 8.90 (d, 1H, *J* = 8.0 Hz, ArH), 9.23 (d, 1H, *J* = 6.0 Hz, ArH), 9.40 (s, 1H, ArH); ^{13}C NMR (100 MHz, D_2O): δ 61.7, 64.8 (m), 65.1 (m), 70.7, 74.8, 77.5, 81.9 (m), 87.0 (m), 87.5, 99.9, 118.4, 128.6, 133.8, 139.8, 141.8, 142.5, 145.9, 146.3, 148.4, 150.9, 165.4; ^{31}P NMR (162 MHz, D_2O):

δ -11.12 (br); HRMS (ESI) for $C_{21}H_{26}N_{10}NaO_{13}P_2^+$ $(M+Na)^+$: Calcd.: 711.1054 Da; Obs: 711.1044 Da.



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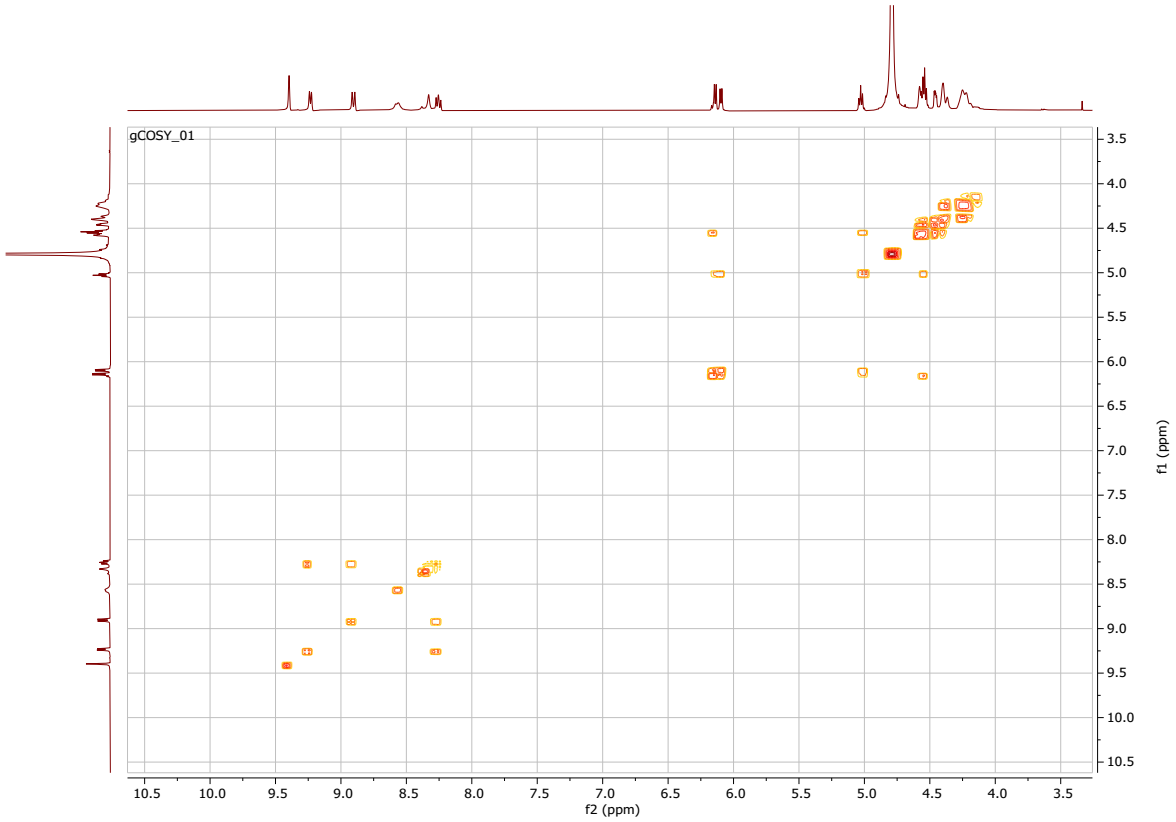
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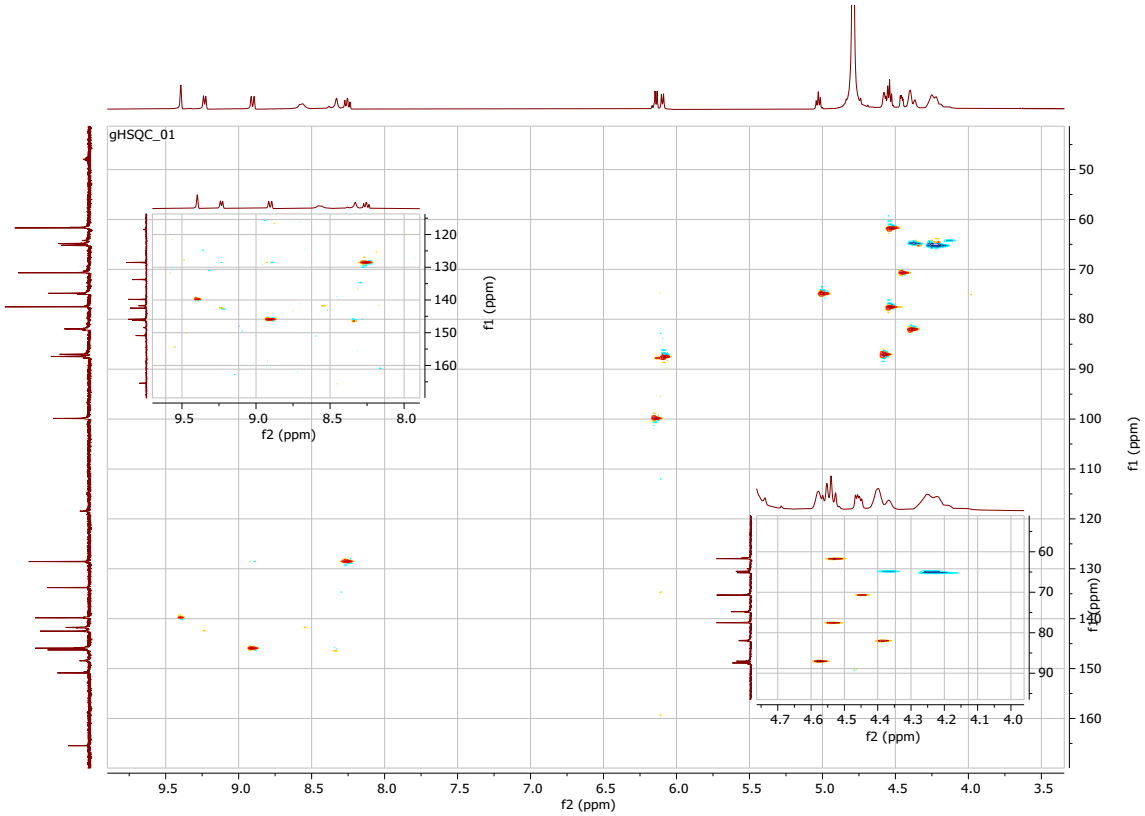
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Reference

1. Zhang, X.N.; Cheng, Q.; Chen, J.; Lam, A. T.; Lu, Y.; Dai, Z.; Pei, H.; Evdokimov, N. M.; Louie, S. G.; Zhang, Y., A ribose-functionalized NAD^+ with unexpected high activity and selectivity for protein poly-ADP-ribosylation. *Nature communications* **2019,** *10* (1), 1-13.