Supplementary Methods

General Materials and Methods

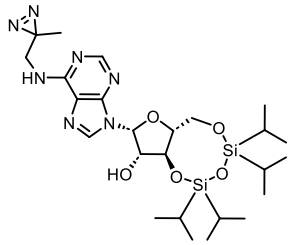
All solvents were anhydrous ($H_2O \leq 10$ ppm) and purchased from Energy Chemical (AnNaiJi), commercially available reagents were used as received without further purification unless otherwise stated, and all reactions were monitored by TLC on silica gel G-25 UV254 plates (0.25 mm). Spots were visualized under UV light. Flash column chromatography was performed using silica gel (200-300 mesh, 100 Å). The ratio of silica gel to crude product ranged from 80:1 to 50:1 (w/w). Analytical HPLC was performed on a Fuli Instruments LC5090 system equipped with a Welch C18 column (4.6×250 mm, 5 μm). Preparative HPLC was conducted on a Shimadzu LC-20 system using a Nan Micr C18 column (21.2×250 mm, 5 μm).

^{1}H NMR (300 or 600 MHz), ^{13}C NMR (151 MHz), and ^{31}P NMR (243 MHz) spectra were recorded on Bruker AVANCE 300 and Bruker AVANCE NEO 600 NMR spectrometers. All spectra were acquired in deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD), or deuterated water (D_2O). Chemical shifts (d_H, d_C, and d_P) are reported in parts per million (ppm) relative to residual solvent signals, and coupling constants (*J*) are reported in hertz (Hz). Splitting patterns in ^{1}H NMR spectra are designated as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet).

cDNAs of human RPLP1, FCF1, PRIM2, and EIF2S1 were purchased from Jiutian Gene. Dithiothreitol (DTT), L-ascorbic acid sodium salt, olaparib, β-NAD^+, and activated calf thymus DNA were purchased from Aladdin (Shanghai). Streptavidin-HRP conjugate was purchased from Proteintech (Wuhan). The anti-RPLP1 antibody (A6725) and anti-His antibody (AE003) were purchased from ABclonal Technology. The anti-GST antibody (K000478P) was purchased from Solarbio. HeLa cells were obtained from Quicell (Shanghai) and tested negative for mycoplasma. All other reagents were obtained from commercially available sources and used without further purificati.

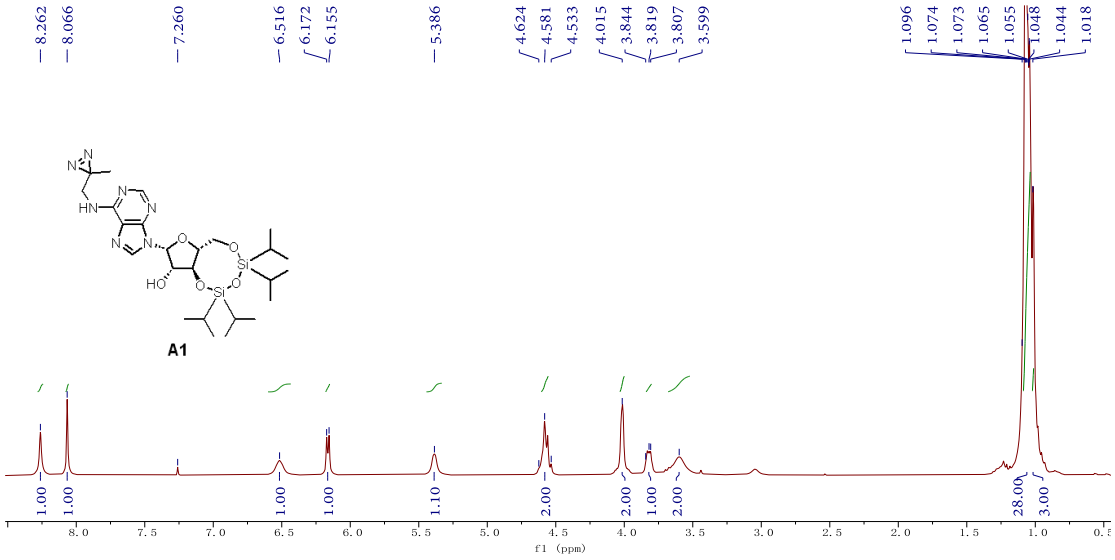
Chemical Synthesis and Characterization of PCCT-NAD^+

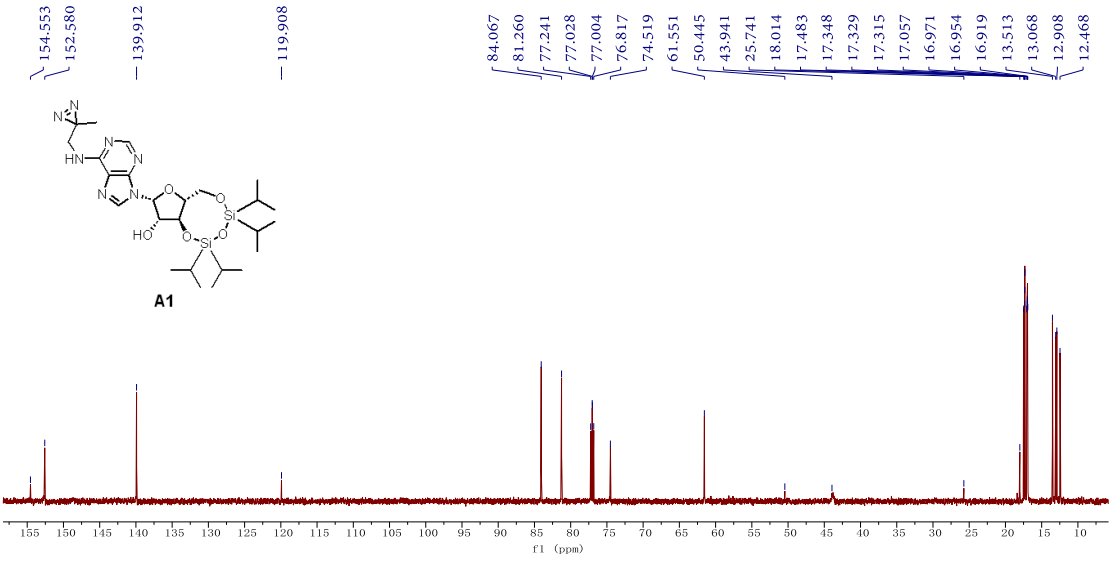
SM-1 is a known compound and prepared according to a procedure in the literature.[1] **SM-2** is a known compound and prepared according to a procedure in the literature.[2]

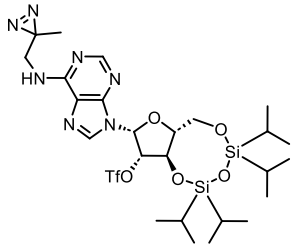


A1

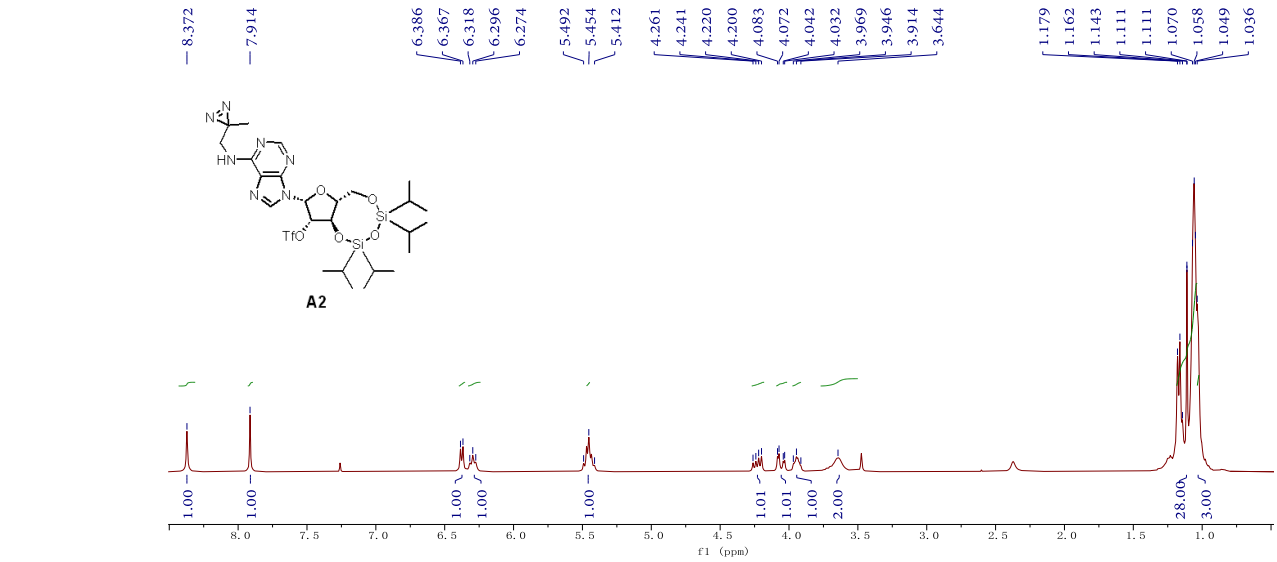
*General procedure for the synthesis of compound **A1***: To a stirred solution of **SM-1** (5.7 g, 10.77 mmol) in EtOH (20 mL) was added DIPEA (3.75 mL, 21.54 mmol), followed by addition of **SM-2** (1.38 g, 16.16 mmol). The reaction mixture was heated at 55 °C and stirred overnight (12 h). After completion, the solvent was removed under reduced pressure. The residue was diluted with EtOAc (100 mL), and the organic phase was washed with H_2O (3×50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to give a residue. The residue was purified by flash column chromatography on silica gel to afford compound **A1** (5.17 g, 83%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (s, 1H, ArH), 8.07 (s, 1H, ArH), 6.52 (s, 1H, NH), 6.16 (d, 1H, J = 5.1 Hz, CH), 5.39 (s, 1H, OH), 4.62-4.53 (m, 2H, CH_2), 4.02 (s, 2H, CH_2), 3.84-3.81 (m, 1H, CH), 3.60 (br, 2H, CH_2), 1.09-1.04 (m, 28H, 4CH+8CH_3), 1.02 (s, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.6 152.6, 139.9, 119.9, 84.1, 81.3, 74.5, 61.6, 50.4, 43.9, 25.7,18.0, 17.5, 17.35, 17.33, 17.32, 17.1, 16.97, 16.95, 16.92, 13.5, 13.1, 12.9, 12.5. HRMS (ESI$^+$) *m/z*: calcd for $C_{25}H_{44}N_7O_5Si_2$ $[M+H]^+$578.2937, found 578.2945.

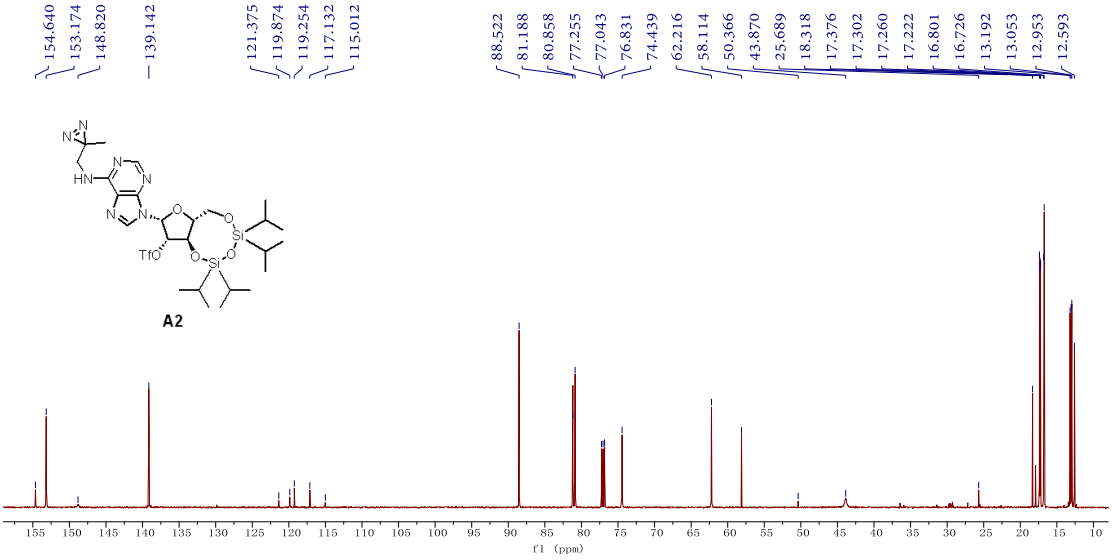


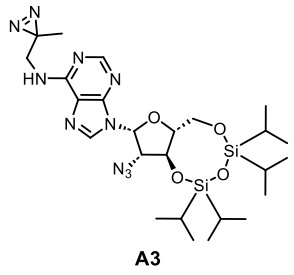




*General procedure for the synthesis of compound **A2***: To a stirred solution of compound **A1** (5.17 g, 8.95 mmol) and 4-(dimethylamino)pyridine (3.28 g, 26.84 mmol) in anhydrous DCM (30 mL) under a nitrogen atmosphere was cooled in an ice bath for 10 min. Trifluoromethane-sulfonic anhydride (3.03 g, 10.74 mmol, 1.81 mL) was then added dropwise at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. After completion (monitored by TLC), the solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel to afford compound **A2** (5.09 g, 7.31 mmol, 82%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.37 (s, 1H, ArH), 7.91 (s, 1H, ArH), 6.38 (d, *J* = 5.7 Hz, 1H, CH), 6.30 (t, *J* = 6.6 Hz, 1H, CH), 5.49-5.41 (m, 1H, CH), 4.26-4.20 (dd, 1H, *J* =12.3,6Hz,CH), 4.08-4.03 (dd, 1H, *J* =12.3,3.3Hz,CH)3.97-3.91 (m, 1H, CH), 3.64 (br, 2H, CH_2), 1.18-1.05 (m, 28H, 4CH+8CH_3), 1.04 (s, 3H, CH_3, CH_3).^{13}C NMR (151 MHz, $CDCl_3$): δ 154.6, 153.2, 148.8, 139.1, 119.9, 118.2 (q, CF_3, *J* = 320 Hz), 88.5, 81.2, 80.9, 74.4, 62.2, 58.1,50.4, 43.9,25.7, 18.3, 17.37, 17.30, 17.26, 17.2, 16.8, 16.7, 13.2, 13.1, 13.0, 12.6. HRMS (ESI$^+$) *m/z*: calcd for $C_{25}H_{44}N_7O_5Si_2$ $[M+H]^+$ 710.2430, found 710.2441.

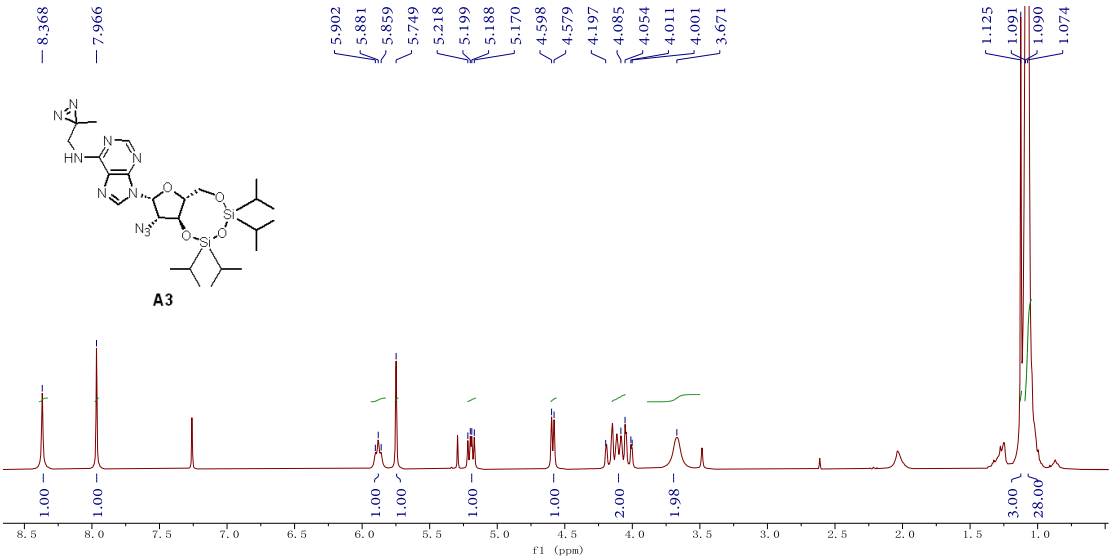


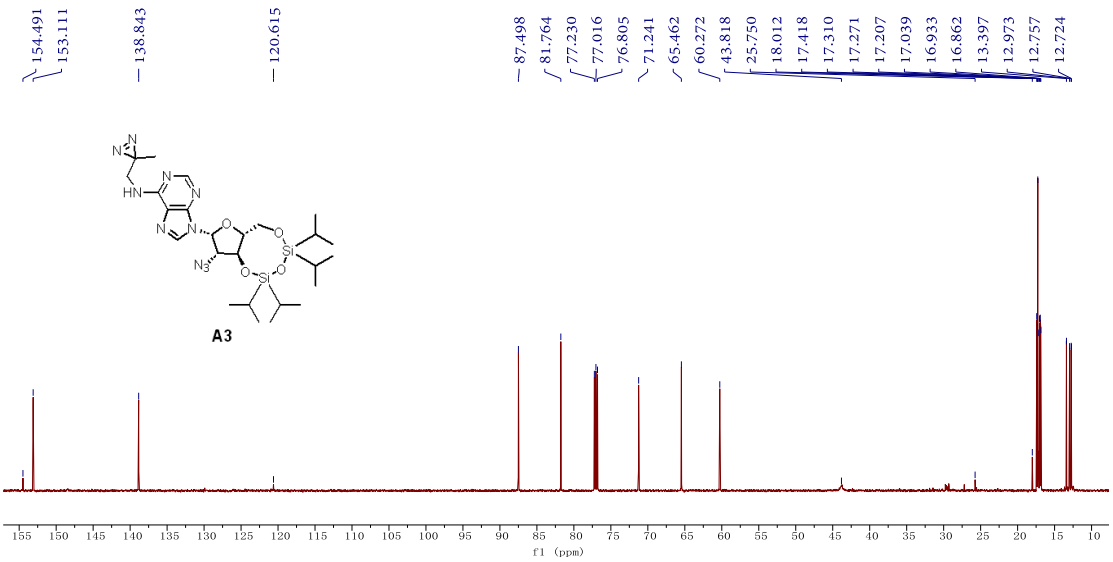


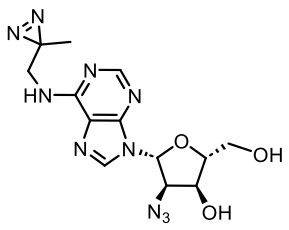


*General procedure for the synthesis of compound **A3***: To a stirred solution of compound **A2** (5.09 g, 7.31 mmol) in anhydrous DMF (20 mL) under a nitrogen atmosphere was added sodium azide (1.19 g, 18.29 mmol). The reaction mixture was heated at 55 °C (oil bath) and stirred overnight. After completion (monitored by TLC), the reaction mixture was diluted with EtOAc (150 mL), and the organic phase was washed with saturated brine (3×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to afford compound **A3** (2.54 g, 4.31 mmol, 59%) as a pale yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.37 (s, 1H, ArH), 7.97 (s, 1H, ArH),

5.88 (t, 1H, J = 6.5 Hz, CH), 5.75 (s, 1H, CH), 5.19 (dd, 1H, J = 8.9, 5.6 Hz, CH), 4.59 (d, 1H, J = 5.7 Hz, CH), 4.20-4.00 (m, 2H, CH_2), 3.67 (br, 2H, CH_2), 1.13 (s, 3H, CH_3), 1.09-1.07 (m, 28H,4CH+8CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 154.5, 153.1, 138.8, 120.6, 87.5, 81.8, 71.2, 65.5, 60.3, 43.8, 25.8, 18.0, 17.4, 17.31, 17.27, 17.2, 17.0, 16.93, 16.86, 13.4, 13.0, 12.8, 12.7. HRMS (ESI$^+$) *m/z*: calcd for$C_{25}H_{44}N_7O_5Si_2$ $[M+H]^+$603.3002, found 603.3017.

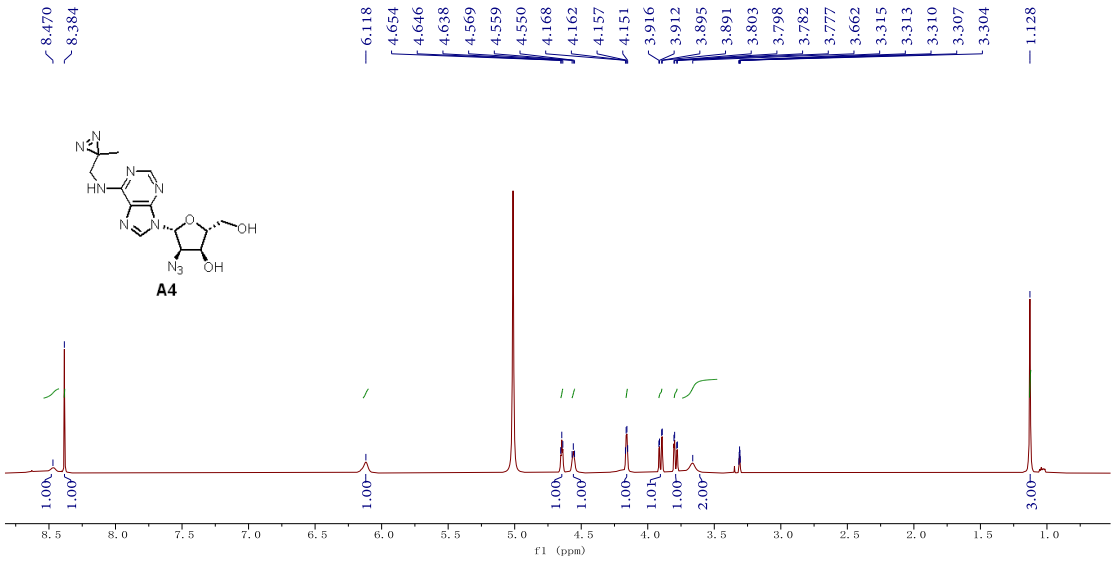


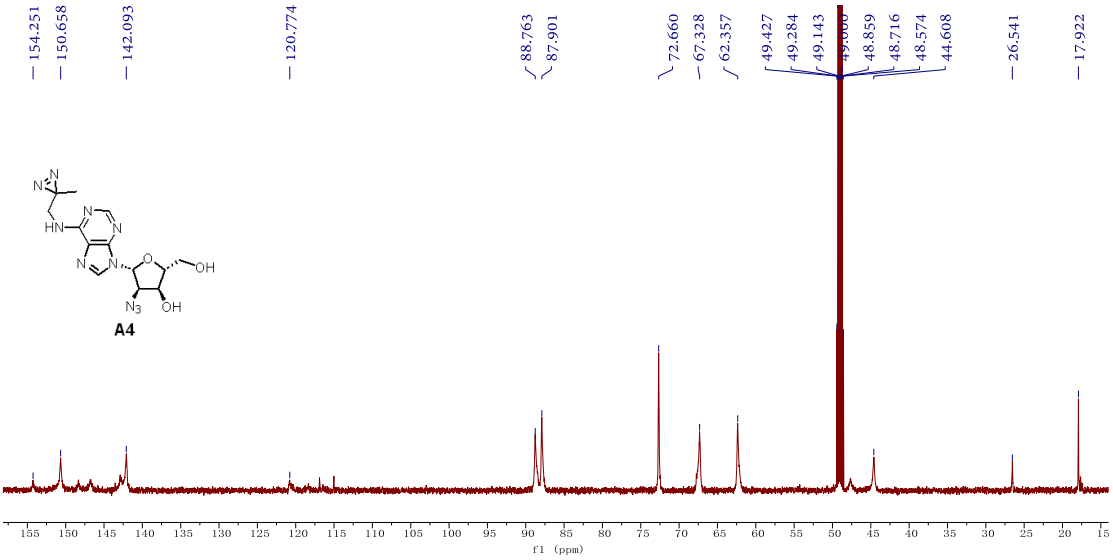


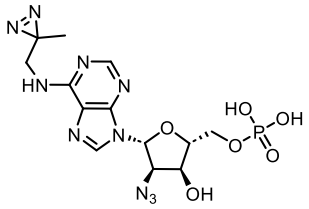


A4

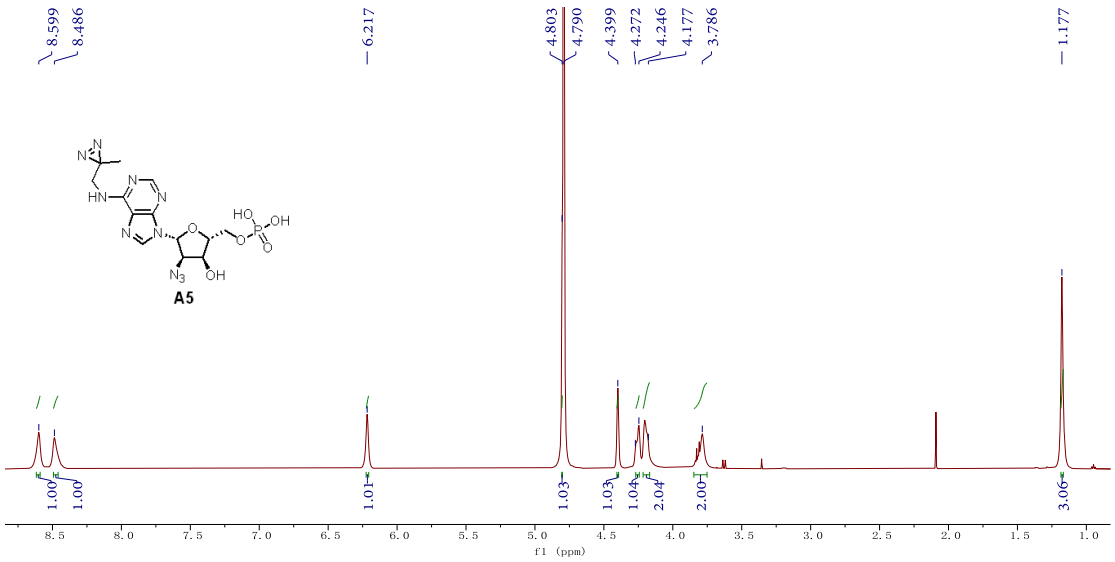
*General procedure for the synthesis of compound **A4***: To a stirred solution of compound **A3** (2.54 g, 4.21 mmol) in anhydrous THF (35 mL) under a nitrogen atmosphere was cooled in an ice bath. Tetrabutylammonium fluoride (1.0 M in THF, 8.43 mL, 8.43 mmol) and acetic acid (506.04 mg, 8.43 mmol, 482.40 μL) were added sequentially at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. After completion (monitored by TLC), the solvent was removed under reduced pressure. The residue was diluted with EtOAc (150 mL), and the organic phase was washed with saturated aq $NaHCO_3$ (3×50 mL) and saturated brine (3×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel to afford compound **A4** (900 mg, 2.50 mmol, 58%) as a white foam. ^{1}H NMR (600 MHz, CD_3OD): δ 8.47 (s, 1H, ArH), 8.38 (s, 1H, ArH), 6.12 (s, 1H, CH), 4.65 (t, 1H, J = 4.8 Hz, CH), 4.56 (t, 1H, J = 5.7 Hz, CH), 4.17-4.15 (m, 1H, CH), 3.90 (dd, 1H, J = 12.6, 2.4 Hz, CH), 3.79 (dd, 1H, J = 12.6, 3.0 Hz, CH), 3.66 (br, 2H, CH_2), 1.13 (s, 3H, CH_3). ^{13}C NMR (151 MHz, CD_3OD): δ 154.3, 150.7, 142.1,120.8, 88.8, 87.9, 72.7, 67.3, 62.4, 44.6, 26.5, 17.9. HRMS (ESI^+) *m/z*: calcd for $C_{25}H_{44}N_7O_5Si_2$ $[M+H]^+$ 361.1480, found 361.1488.







*General procedure for the synthesis of compound **A5***: To a stirred solution of compound **A4** (300 mg, 832.55 μmol) in trimethyl phosphate (5 mL) under a nitrogen atmosphere at 0 °C was added phosphoryl chloride ($POCl_3$, 382.95 mg, 2.50 mmol, 232.09 μL) dropwise. The reaction mixture was maintained at 0 °C and stirred for 6 h until completion (monitored by HPLC). The reaction was quenched by addition of H_2O (500 μL) at 0 °C, then concentrated in vacuo. The crude product was purified by preparative HPLC (Shimadzu LC-20, Nan Micr C18, 21.2×250 mm, 5 μm) using mobile phase A (0.1% TFA in H_2O) and mobile phase B (0.1% TFA in MeOH) with the following gradient: 0-5 min, 15% B, 5-15 min, 20% B, 15-25 min, 25% B, 25-30 min, 15% B. Concentration of the appropriate fractions afforded compound **A5** (144 mg, 327.04 μmol, 39%) as a white solid. ^{1}H NMR (600 MHz, D_2O): δ 8.60 (s, 1H, ArH), 8.49 (s, 1H, ArH), 6.22 (s, 1H, CH), 4.80 (m, 1H, CH), 4.40 (s, 1H, CH), 4.27-4.18 (m, 3H, CH+CH_2 overlap), 3.79 (br, 2H, CH_2), 1.18 (s, 3H, CH_3). ^{13}C NMR (151 MHz, D_2O): δ 149.4, 145.2, 142.0, 118.8, 87.0, 84.2 (d, J = 8.6 Hz), 70.8, 66.1, 64.4 (d, J = 4.9 Hz), 44.4, 25.3, 16.7. ^{31}P NMR (243 MHz, D_2O): δ -0.11. HRMS (ESI^+) *m/z*: calcd for $C_{25}H_{44}N_7O_5Si_2$ $[M+H]^+$ 441.1143, found 441.1158.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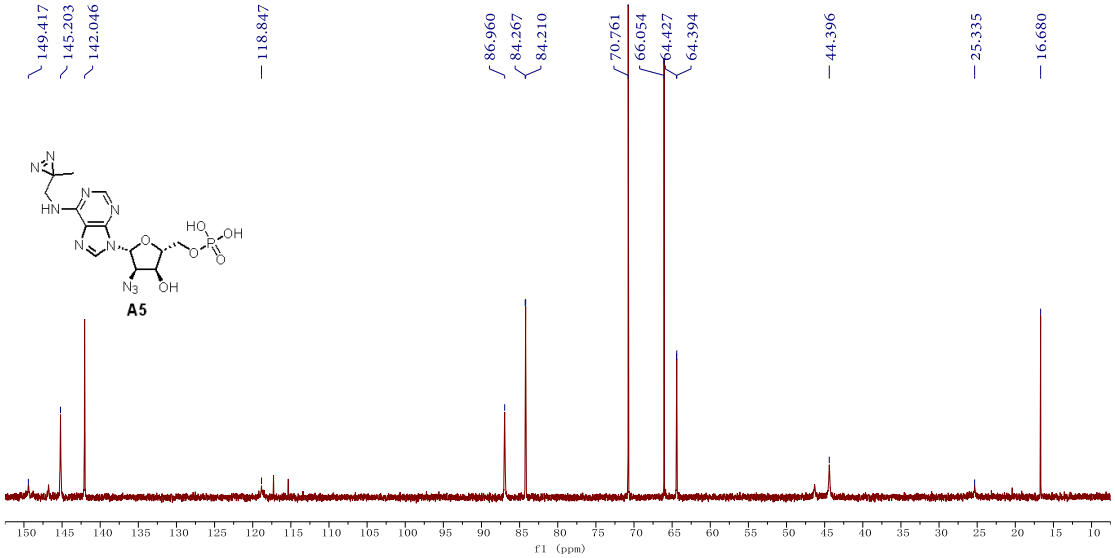
1.03
1.04
2.04
2.00
3.06
f1 (ppm)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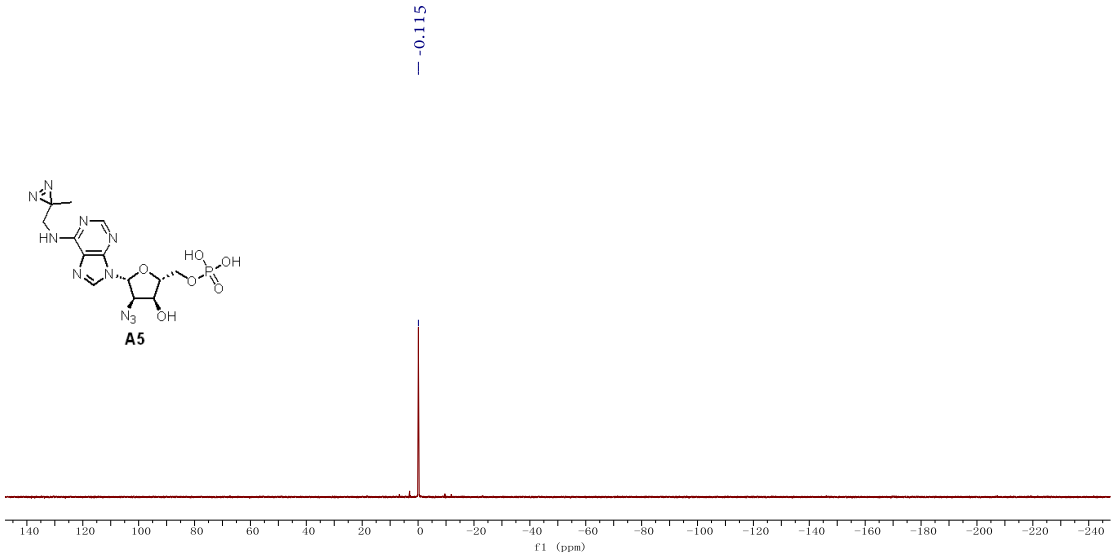
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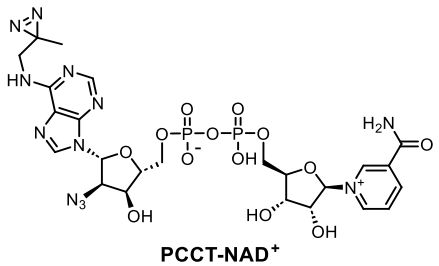
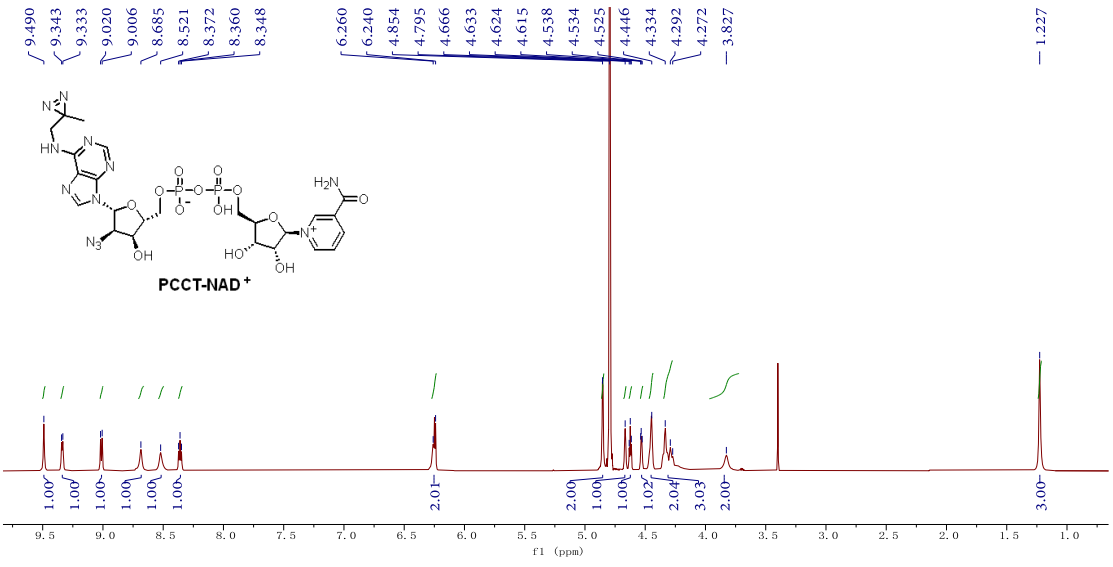
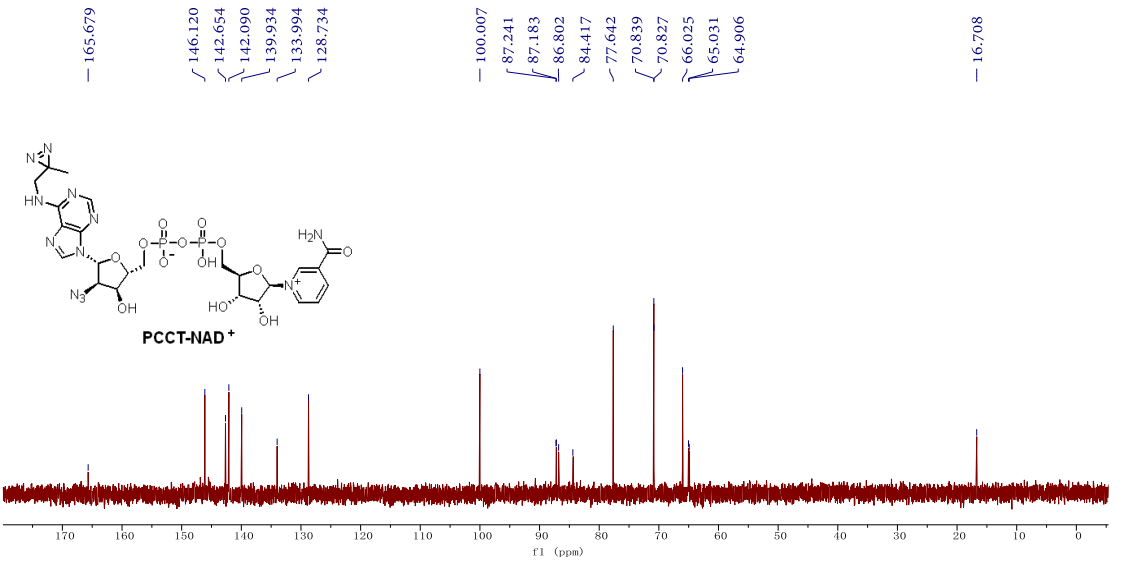
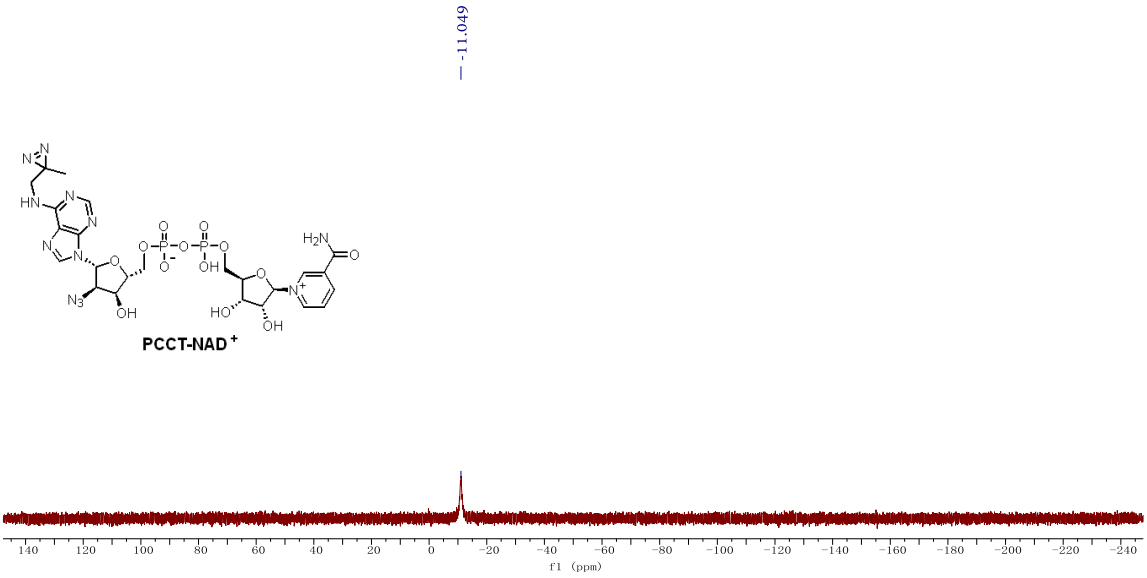


PCCT-NAD$^+$

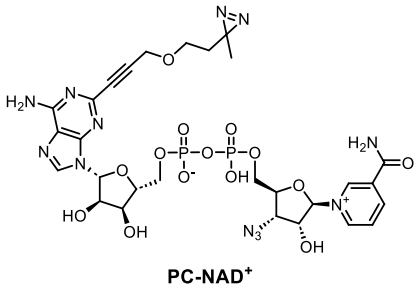
General procedure for the synthesis of compound ***PCCT-NAD***$^+$: To a stirred solution of β-nicotinamide mononucleotide (NMN) (274.08 mg, 817.59 μmol) in anhydrous DMF under a nitrogen atmosphere was added 1,1′-carbonyldiimidazole (CDI) (265.15 mg, 1.64 mmol). The reaction mixture was stirred at room temperature for 24 h until completion (monitored by HPLC), then quenched with a few drops of MeOH. The solvent was removed under reduced pressure to afford the activated intermediate as a residue. The residue was dissolved in anhydrous DMF, and compound **A5** (144.00 mg, 327.04 μmol) was added. The reaction mixture was stirred at room temperature under nitrogen for 4 days until completion (monitored by HPLC). The reaction mixture was then purified by HPLC (FuLi Instruments LC5090, Welch C18, 4.6 × 250 mm, 5 μm) using mobile phase A (0.1% TFA in H_2O) and mobile phase B (0.1% TFA in MeOH) with the following gradient: 0-5 min, 5% B, 5-15 min, 15% B, 15-25 min, 20% B, 25-30 min, 5% B. Fractions containing the desired product were combined, concentrated, and lyophilized to afford PCCT-NAD$^+$ (61 mg, 80.52 μmol, 25%) as a pale yellow powder. ^{1}H NMR (600 MHz, D_2O): δ 9.49 (s, 1H, ArH), 9.34 (d, 1H, J = 6.0 Hz, ArH), 9.01 (d, 1H, J = 8.4 Hz, ArH), 8.69 (s, 1H, ArH), 8.52 (s, 1H, ArH), 8.36 (t, 1H, J = 7.2 Hz, ArH), 6.26-6.24 (m, 2H, 2CH), 4.85 (m, 2H, 2CH), 4.67 (s, 1H, CH), 4.62 (t, 1H, J = 5.4 Hz, CH), 4.54-4.53 (m, 1H, CH), 4.45 (m, 2H, CH_2), 4.33-4.27 (m, 3H, CH+CH_2), 3.83 (br, 2H, CH_2), 1.23 (s, 3H, CH_3).^{13}C NMR (151 MHz, D_2O): δ 165.7, 146.1, 142.7, 142.1, 139.9, 134.0, 128.7, 100.0, 87.2, 87.2, 86.8, 84.4, 77.6, 70.8, 70.8, 66.0, 65.0, 64.9, 16.7. ^{31}P NMR (243 MHz, D_2O): δ -11.05. HRMS (ESI$^+$) *m/z*: calcd for$C_{25}H_{44}N_7O_5Si_2$ $[M+H]^+$ 757.1603, found 757.1614.





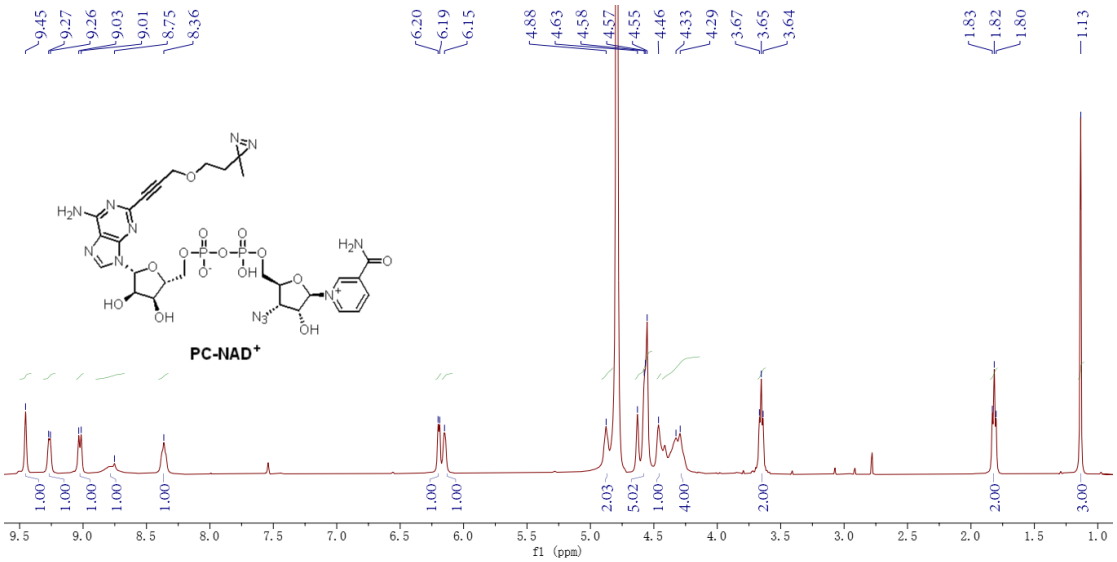


Chemical Synthesis and Characterization of PC-NAD$^+$



PC-NAD$^+$ is a known compound and prepared according to a procedure in the literature[3] ^{1}H NMR (400 MHz, D_2O) δ 9.45 (s, 1H,ArH), 9.26 (d, J = 5.8 Hz, 1H, ArH), 9.02 (d, J = 7.7 Hz, 1H, ArH), 8.75 (s, 1H, ArH), 8.36 (s, 1H, ArH), 6.19 (d, J = 5.2 Hz, 1H,CH), 6.15 (s, 1H,CH), 4.88 (s, 2H,2CH), 4.63-4.55 (m,5H,3CH+CH_2), 4.46 (s, 1H,CH), 4.41-4.29 (m, 4H,2CH_2), 3.65 (t, J = 5.9 Hz, 2H,CH_2), 1.82 (t, J = 6.0 Hz, 2H,CH_2), 1.13 (d, J = 2.0

Hz, 3H,CH_3).



No unexpected or unusually high safety hazards were encountered.

References

(1) Kittaka, A., Yamada, N., Tanaka, H., Nakamura, K. T., Miyasaka, T. "Radical-Mediated Cyclization of A 6-Chloro-9-(2-Deoxy--d erythro-pent-1-enofuranosyl)-8-(2, 2-dibromovinyl) purine". *Nucleosides, Nucleotides & Nucleic Acids,**15*** (1996), 1447.

(2) Walter, M., Lindhorst, T. K. "A modular system for the preparation of diazirine-labeled mannose derivatives using thiourea bridging". *Synthesis,**2006*** (2006), 952.

(3) 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*, 10 (2019), 4196.