Electronic Supplementary Information

Facile chemoenzymatic synthesis of a novel stable mimic of NAD^+

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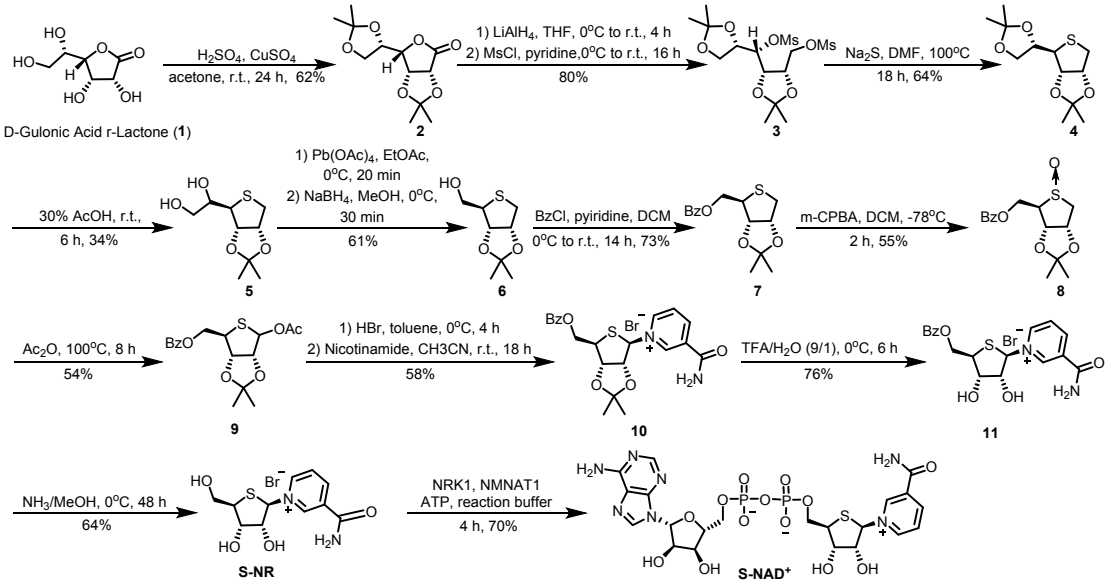
Experimental Procedures.

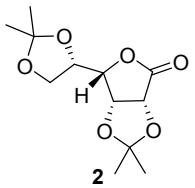
Chemoenzymatic synthesis of S-NAD$^+$.



Scheme S1. Chemoenzymatic synthesis of S-NAD$^+$.

Materials and Methods.

^{1}H NMR spectra were recorded on an Oxford AM-400 spectrometer for solution in $CDCl_3$, 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 a complete proton decoupling spectrophotometer ($CDCl_3$: 77.0 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, from Phenomenex Inc, Torrance, CA). All other reagents were purchased from readily available commercial sources and used without further purification.

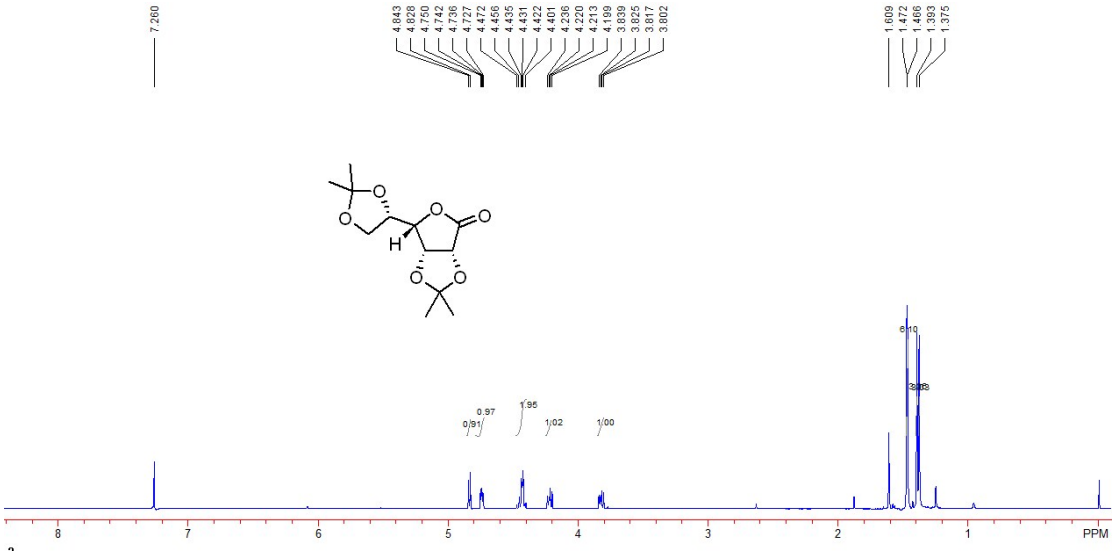


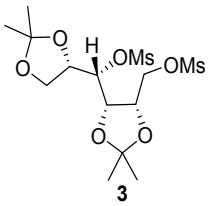
2

General procedure for the synthesis of compound ***2***. To a stirred solution of compound **1** (8.9 g, 50 mmol) in dry acetone (200 mL) was added $CuSO_4$ (16.0 g, 100

mmol, 2 eq) followed by the addition of concentrated H_2SO_4 (266 μL, 0.1 eq) at 0°C. Then, the reaction mixture was allowed to warm to room temperature and stirred at the same temperature for 24 hours. The pH of the solution was adjusted to 7 with $NaHCO_3$, and the resulting slurry was filtered and evaporated to give a residue. The residue was dissolved in ethyl ether (200 mL) and washed with water (3×50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated to give the compound **2** (8.0 g, 62%) as a colorless oil.

(3a*R*,6*S*,6a*R*)-6-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-4(3a*H*)-one (2).[1] NMR (400 MHz, $CDCl_3$): δ 1.38 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.466 (s, 3H, CH_3), 1.472 (s, 3H, CH_3), 3.82 (dd, 1H, J = 8.8, 5.6 Hz, CH_2), 4.22 (dd, 1H, J = 8.8, 6.0 Hz, CH_2), 4.40-4.47 (m, 2H, 2CH), 4.74 (dd, 1H, J = 6.0, 3.2 Hz, CH), 4.83 (d, 1H, J = 6.0 Hz, CH).



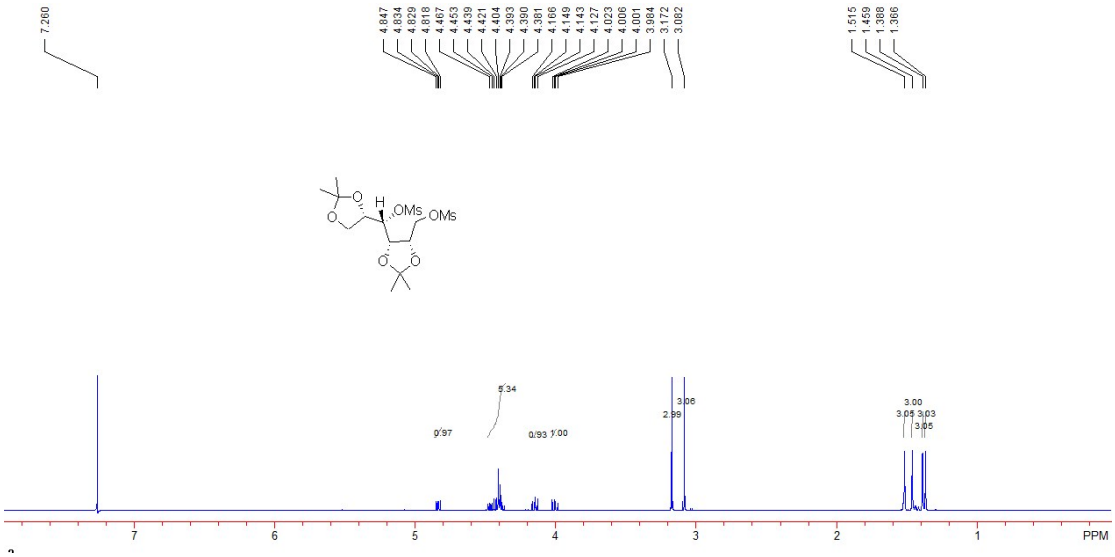


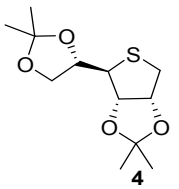
3

*General procedure for the synthesis of compound **3***. To a stirred solution of compound **2** (4.5 g, 17.4 mmol) in anhydrous THF (50 mL) was added $LiAlH_4$ (9.9 mL, 34.8 mmol, 2 eq, a 3.5 M solution in THF) in several portions at 0°C. Then, the

reaction mixture was allowed to warm to room temperature and stirred at the same temperature for 8 hours. The reaction mixture was then quenched with ice, dried over anhydrous $MgSO_4$, filtered through a celite pad and evaporated to give a residue. The residue was dissolved in pyridine (20 mL) and the mixture was cooled to 0°C. MsCl (6.8 mL, 52.2 mmol, 3 eq) was added dropwise and the resulting mixture was then allowed to warm to room temperature. After stirring for 16 hours, the reaction was quenched with MeOH (10 mL) and the mixture was concentrated under reduced pressure to give a residue. The residue was dissolved in EtOAc (50 mL), and the organic phase was washed successively with saturated aqueous $CuSO_4$ (3×50 mL), brine (50 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and purified by a flash column chromatography on silica gel to afford the compound **3** (5.8 g, 80%) as a colorless oil.

(*S*)-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)((4*S*,5*S*)-2,2-dimethyl-5-(((methylsulfonyl)oxy)methyl)-1,3-dioxolan-4-yl)methyl methanesulfonate (3). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 3.08 (s, 3H, CH_3), 3.17 (s, 3H, CH_3), 4.00 (dd, 1H, *J* = 8.8, 6.8 Hz, CH_2), 4.15 (dd, 1H, *J* = 8.8, 6.8 Hz, CH_2), 4.36-4.49 (m, 5H, CH), 4.83 (dd, 1H, *J* = 6.8, 4.8 Hz, CH).

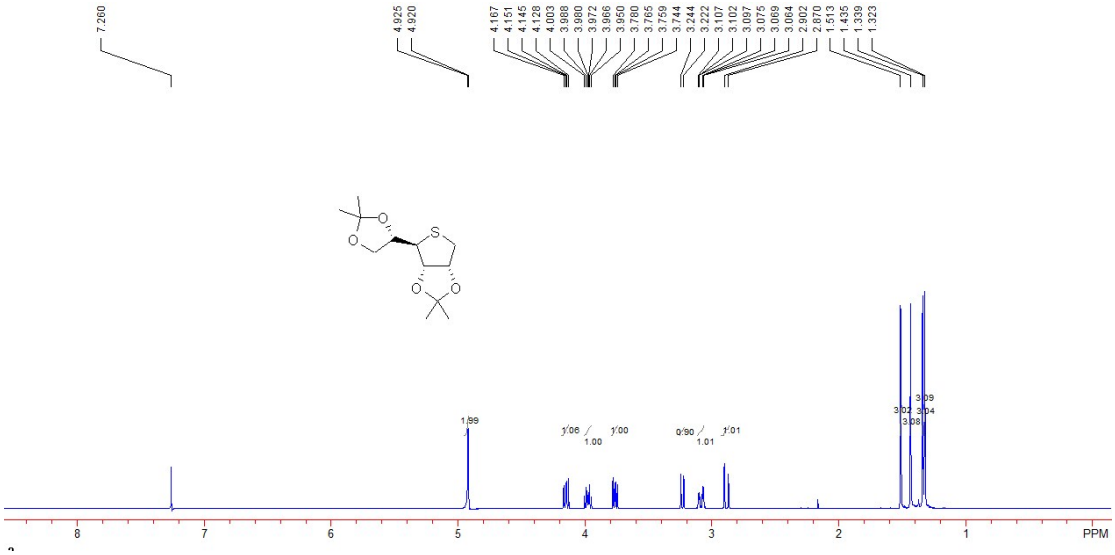


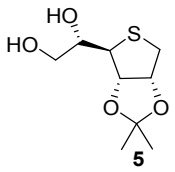


4

*General procedure for the synthesis of compound **4***. To a stirred solution of compound **3** (5.8 g, 13.9 mmol) in DMF (100 mL) was added sodium sulfide nonahydrate (4.0 g, 16.7 mmol, 1.2 eq) and the reaction mixture was heated to 100°C for 18 h. After being cooled to room temperature, the mixture was diluted with ethyl ether (300 mL) and washed with water (5×50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated and purified by a flash column chromatography on silica gel to afford the compound **4** (2.3 g, 64%) as a colorless oil.

(3a*S*,4*R*,6a*R*)-4-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxole (4).[1] ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.32 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 2.89 (d, 1H, J = 12.8 Hz, CH_2), 3.09 (dt, 1H, J = 12.8, 2.0 Hz, CH_2), 3.23 (d, 1H, J = 8.8 Hz, CH), 3.76 (dd, 1H, J = 8.4, 6.0 Hz, CH_2), 3.98 (dt, 1H, J = 8.8, 6.4 Hz, CH), 4.15 (dd, 1H, J = 8.8, 6.4 Hz, CH_2), 4.92 (d, 2H, J = 2.0 Hz, 2CH).

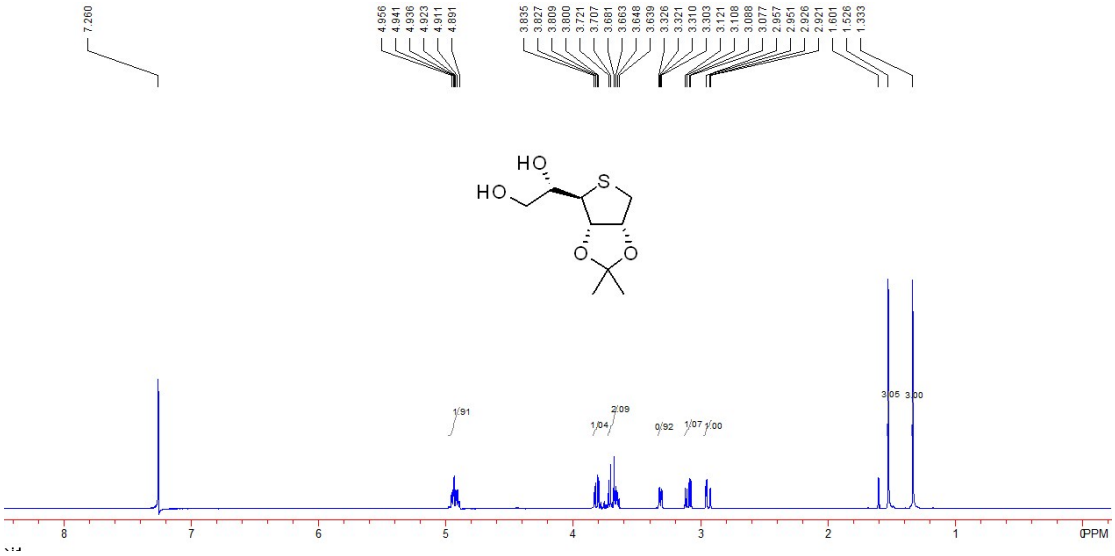


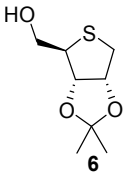


5

General procedure for the synthesis of compound ***5***. Compound **4** (2.1 g, 8.0 mmol) was dissolved in 30% aqueous AcOH (60 mL) and the resulting mixture was stirred at room temperature for 6 h. The reaction was then concentrated under reduced pressure to give a residue. The residue was purified by a flash column chromatography on silica gel to afford the compound **5** (599 mg, 34%) as a colorless oil.

(*S*)-1-((3a*S*,4*R*,6a*R*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)ethane-1,2-diol (5).[1] ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 2.94 (dd, 1H, *J* = 12.4, 2.0 Hz, CH_2), 3.09 (dt, 1H, *J* = 12.4, 4.8 Hz, CH_2), 3.32 (dd, 1H, *J* = 6.8, 2.4 Hz, CH), 3.64-3.72 (m, 2H, CH_2+CH), 3.82 (dd, 1H, *J* = 10.4, 3.6 Hz, CH_2), 4.89-4.96 (m, 2H, 2CH).



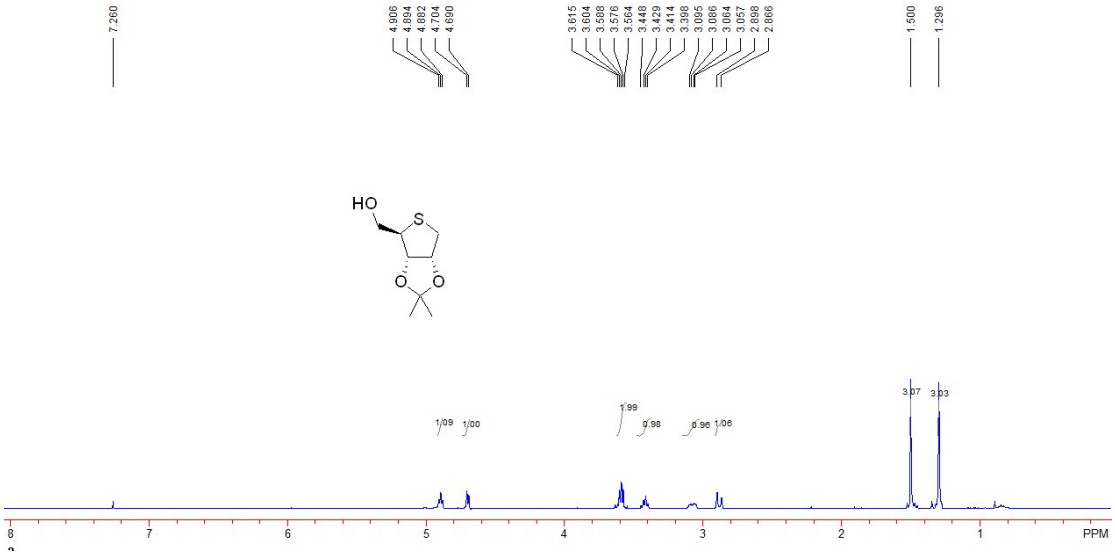


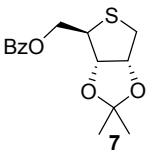
6

General procedure for the synthesis of compound ***6***. To a stirred solution of compound **5** (1.1 g, 5.0 mmol) in ethyl acetate (25 mL) was added $Pb(OAc)_4$ (2.4 g,

5.5 mmol, 1.1 eq) at 0°C and the reaction mixture was stirred at same temperature for 20 min. The reaction mixture was filtered, the filtrate was diluted with EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ solution (3×20 mL), water (20 mL) and brine (15 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated to give a residue. The residue was dissolved in MeOH (20 mL) and $NaBH_4$ (246 mg, 6.5 mmol, 1.3 eq) was added carefully in several portions. The resulting mixture was stirred for 30 min at the same temperature and neutralized with glacial AcOH (1 mL). The mixture was diluted with ethyl ether (200 mL) and washed with water (3×20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated and purified by a flash column chromatography on silica gel to afford the compound **6** (580 mg, 61%) as a colorless oil.

((3a*S*,4*R*,6a*R*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)methanol (6).[1] ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 2.88 (d, 1H, J = 12.8 Hz, CH_2), 3.07 (dd, 1H, J = 12.8, 3.6 Hz, CH_2), 3.40-3.45 (m, 1H, CH), 3.50-3.62 (m, 2H, CH_2), 4.70 (d, 1H, J = 5.6 Hz, CH_2), 4.89 (t, 1H, J = 4.8 Hz, CH).



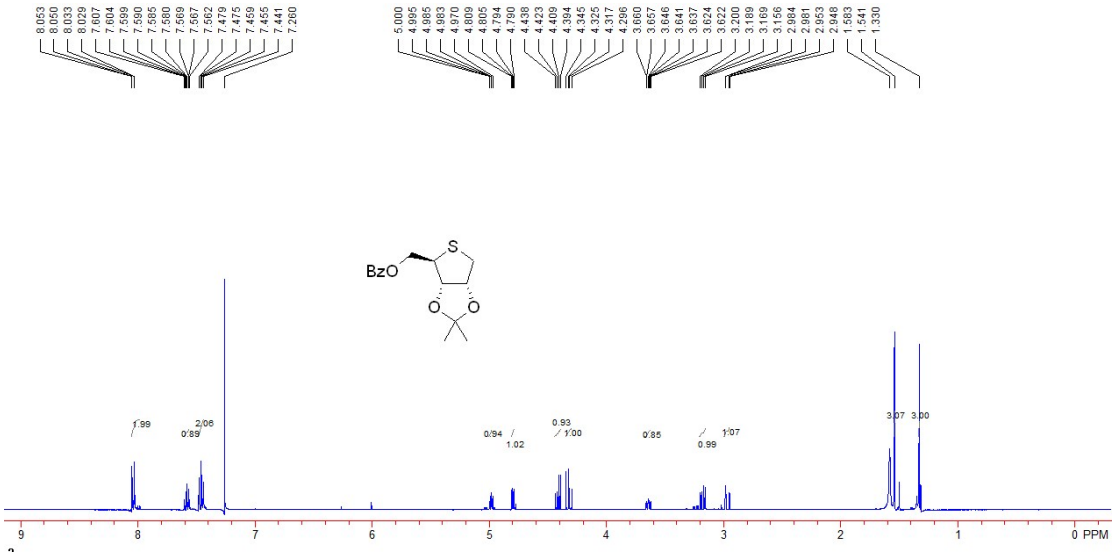


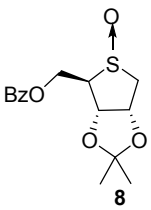
7

General procedure for the synthesis of compound 7. To a stirred solution of compound **6** (570 mg, 3.0 mmol) in a mixture of anhydrous DCM (15 mL) and

pyridine (15 mL) was added BzCl (523 μL, 4.5 mmol, 1.5 eq) at 0°C. Then, the reaction mixture was allowed to warm to room temperature. After stirring for 14 hours, the reaction was quenched with MeOH (2 mL) and the mixture was concentrated under reduced pressure to give a residue. The residue was dissolved in EtOAc (80 mL), and the organic phase was washed successively with saturated aqueous $CuSO_4$ (3×20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and purified by a flash column chromatography on silica gel to afford the compound **7** (645 mg, 73%) as a colorless oil.

((3a*S*,4*R*,6a*R*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)methyl benzoate (7).[1] ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 2.97 (dd, 1H, *J* = 12.8, 1.6 Hz, CH_2), 3.18 (dd, 1H, *J* = 12.8, 4.8 Hz, CH_2), 3.62-3.66 (m, 1H, CH), 4.32 (dd, 1H, *J* = 11.6, 8.0 Hz, CH_2), 4.42 (dd, 1H, *J* = 11.6, 6.0 Hz, CH_2), 4.80 (dd, 1H, *J* = 6.0, 1.6 Hz, CH), 4.97-5.00 (m, 1H, CH), 7.44-7.48 (m, 2H, ArH), 7.56-7.61 (m, 1H, ArH), 8.03-8.05 (m, 2H, ArH).



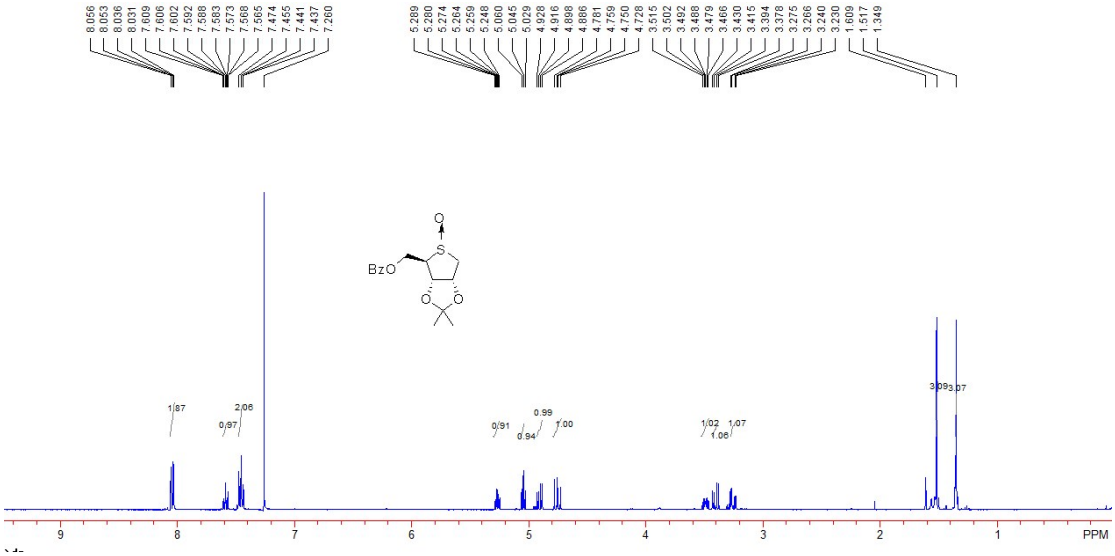


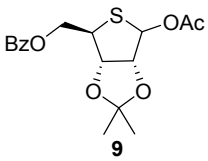
8

*General procedure for the synthesis of compound **8***. To a stirred solution of compound **7** (589 mg, 2.0 mmol) in anhydrous DCM (15 mL) was added *m*-CPBA

(380 mg, 2.2 mmol, 1.1 eq) in several portions at -78°C and the resulting mixture was stirred at the same temperature for 2 h. Then, the reaction was quenched with saturated aqueous $NaHCO_3$ (15 mL) and extracted with DCM (3×20 mL). The combined organic layers were washed with brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and purified by a flash column chromatography on silica gel to afford the compound **8** (341 mg, 55%) as a colorless oil.

Compound 8.[1] ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 3.25 (dd, 1H, *J* = 14.4, 4.0 Hz, CH_2), 3.40 (dd, 1H, *J* = 14.4, 6.0 Hz, CH_2), 3.49 (dt, 1H, *J* = 8.8, 5.2 Hz, CH), 4.75 (dd, 1H, *J* = 12.0, 8.8 Hz, CH_2), 4.91 (dd, 1H, *J* = 12.0, 4.8 Hz, CH_2), 5.05 (t, 1H, *J* = 6.0 Hz, CH), 5.27 (dt, 1H, *J* = 4.0, 6.0 Hz, CH), 7.44-7.47 (m, 2H, ArH), 7.56-7.61 (m, 1H, ArH), 8.03-8.06 (m, 2H, ArH).



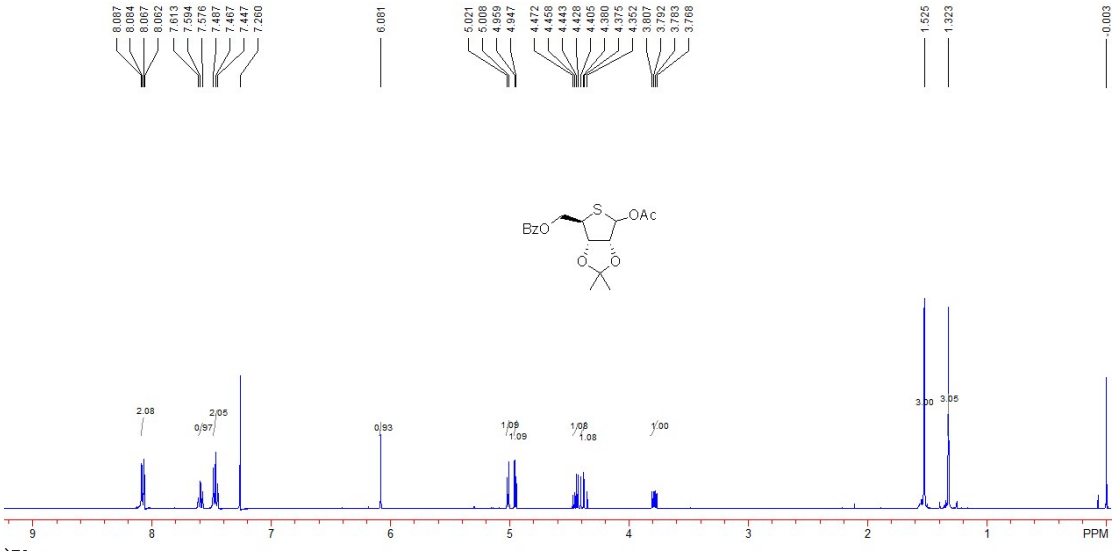


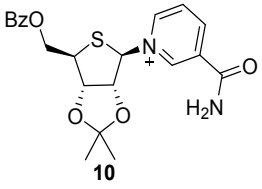
9

*General procedure for the synthesis of compound **9***. Compound **8** (310 mg, 1.0 mmol) was dissolved in AcOH (10 mL) and the mixture was heated to 100°C for 8 h. Then, the reaction was concentrated to give a residue. The residue was dissolved in EtOAc (80 mL), and the organic phase was washed successively with saturated aqueous $NaHCO_3$ (3×10 mL), brine (20 mL), dried over anhydrous Na_2SO_4, filtered, concentrated and purified by a flash column chromatography on silica gel to afford

the compound **9** (190 mg, 54%) as a colorless oil.

((3a*S*,4*R*,6a*R*)-6-acetoxy-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)methyl benzoate (9).[1] ^{1}H NMR (400 MHz, $CDCl_3$) of one isomer: δ 1.32 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 3.79 (dd, 1H, *J* = 9.6, 6.0 Hz, CH), 4.38 (dd, 1H, *J* = 12.0, 9.6 Hz, CH_2), 4.45 (dd, 1H, *J* = 12.0, 6.0 Hz, CH_2), 4.95 (d, 1H, *J* = 4.8 Hz, CH_2), 5.01 (d, 1H, *J* = 4.8 Hz, CH), 6.08 (s, 1H, CH), 7.47 (t, 2H, *J* = 8.0 Hz, ArH), 7.57-7.61 (m, 1H, ArH), 8.06-8.09 (m, 2H, ArH).



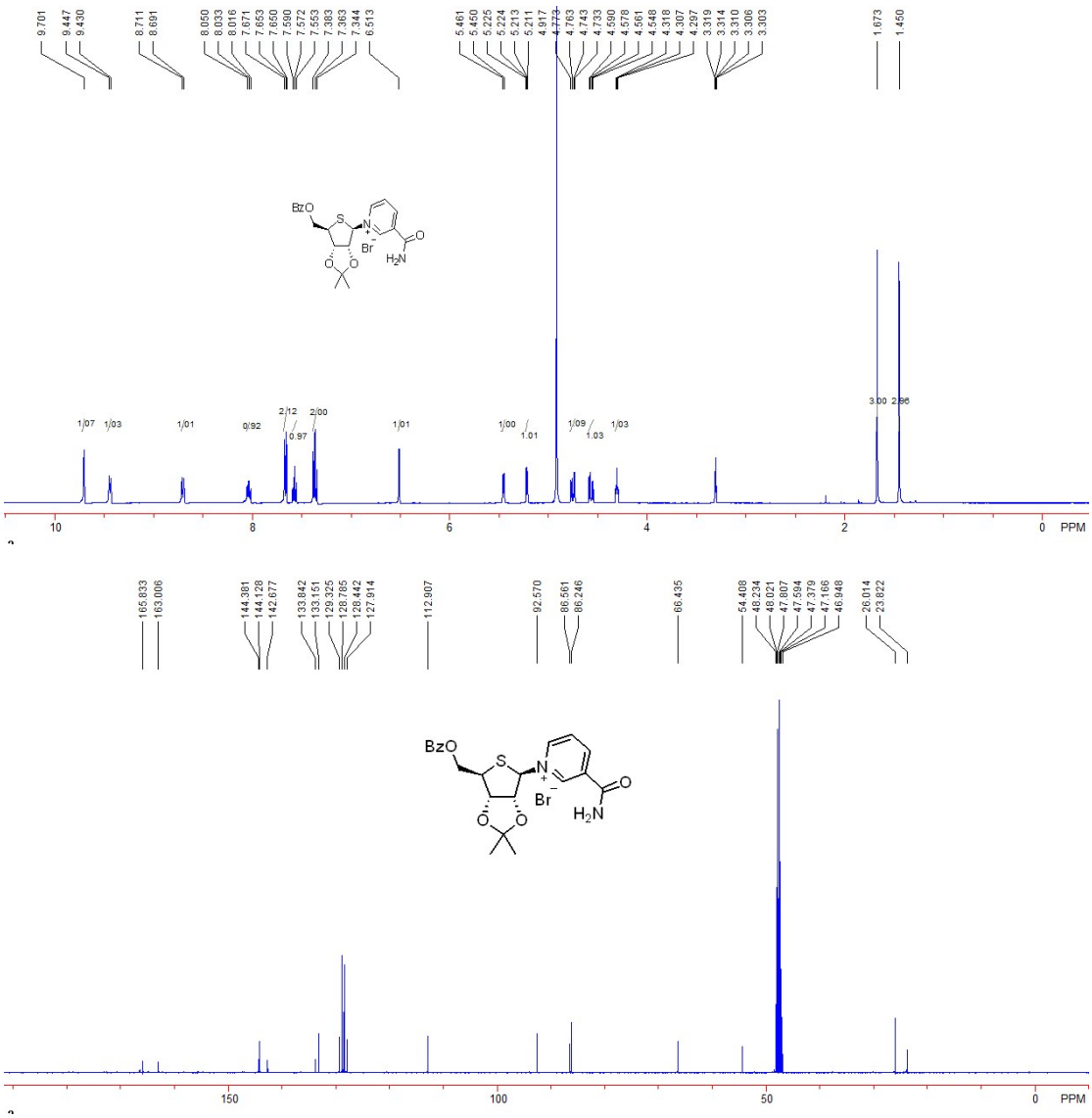


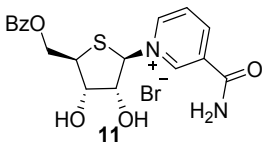
10

*General procedure for the synthesis of compound **3***. Compound **9**[1] (106 mg, 0.3 mmol) was dissolved in toluene (10 mL) and cooled to 0°C. HBr (33% (wt) in acetic acid) (110 mg, 0.45 mmol, 1.5 eq) was added dropwise and the reaction was stirred at 0°C for 5 hours. After the starting material was consumed, the reaction was concentrated under reduced pressure to give a residue. The residue was azeotroped with toluene (3×20 mL) to remove remaining acetic acid and dried *in vacuo*. The crude product and nicotinamide (44 mg, 0.36 mmol, 1.2 eq) was dissolved in CH_3CN (10 mL). The reaction was stirred under Ar gas at room temperature for 24 hours. The reaction was concentrated in vacuo (the temperature was kept below 35°C) and

purified by a flash column chromatography on silica gel to afford the desired compound **10** (86 mg, 58% yield) as a colorless solid.

1-((3a*R*,4*R*,6*R*,6a*S*)-6-((benzoyloxy)methyl)-2,2-dimethyltetrahydrothieno[3,4-d][1,3]dioxol-4-yl)-3-carbamoylpyridin-1-ium bromide (10). ^{1}H NMR (400 MHz, CD_3OD): δ 1.45 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 4.31 (t, 1H, *J* = 4.4 Hz, CH), 4.57 (dd, 1H, *J* = 12.0, 4.4 Hz, CH_2), 4.75 (dd, 1H, *J* = 12.0, 4.4 Hz, CH_2), 5.22 (dd, 1H, *J* = 4.8, 0.8 Hz, CH), 5.46 (d, 1H, *J* = 4.8 Hz, CH), 6.51 (s, 1H, CH), 7.36 (t, 2H, *J* = 8.0 Hz, ArH), 7.55-7.59 (m, 1H, ArH), 7.65-7.67 (m, 2H, ArH), 8.03 (t, 1H, *J* = 6.8 Hz, ArH), 8.70 (d, 1H, *J* = 8.0 Hz, ArH), 9.44 (d, 1H, *J* = 6.8 Hz, ArH), 9.70 (s, 1H, ArH); ^{13}C NMR (100 MHz, CD_3OD): δ 23.8, 26.0, 54.4, 66.4, 86.2, 86.6, 92.6, 112.9, 127.9, 128.4, 128.8, 129.3, 133.2, 133.8, 142.7, 144.1, 144.4, 163.0, 165.8.

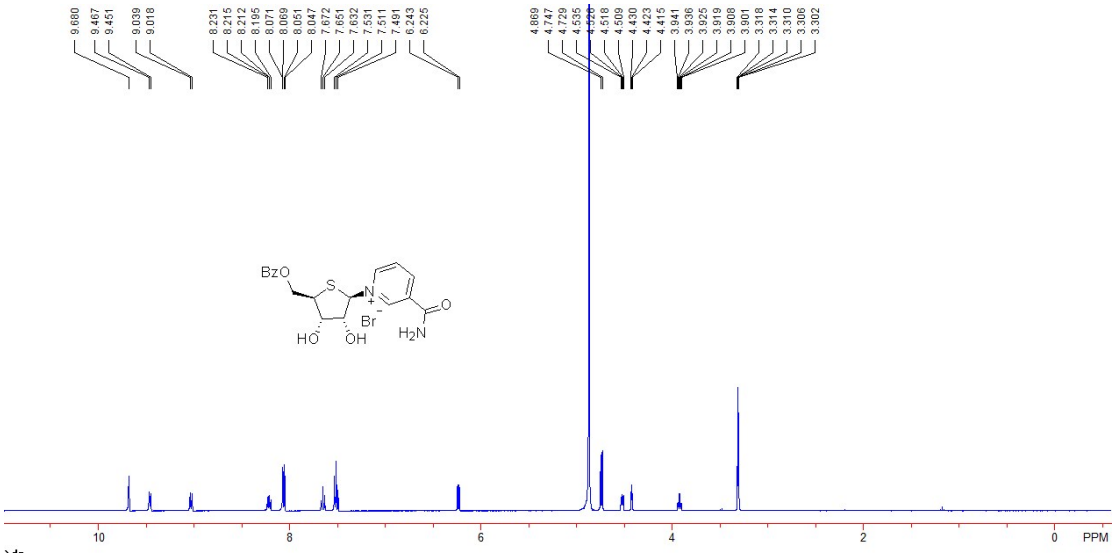


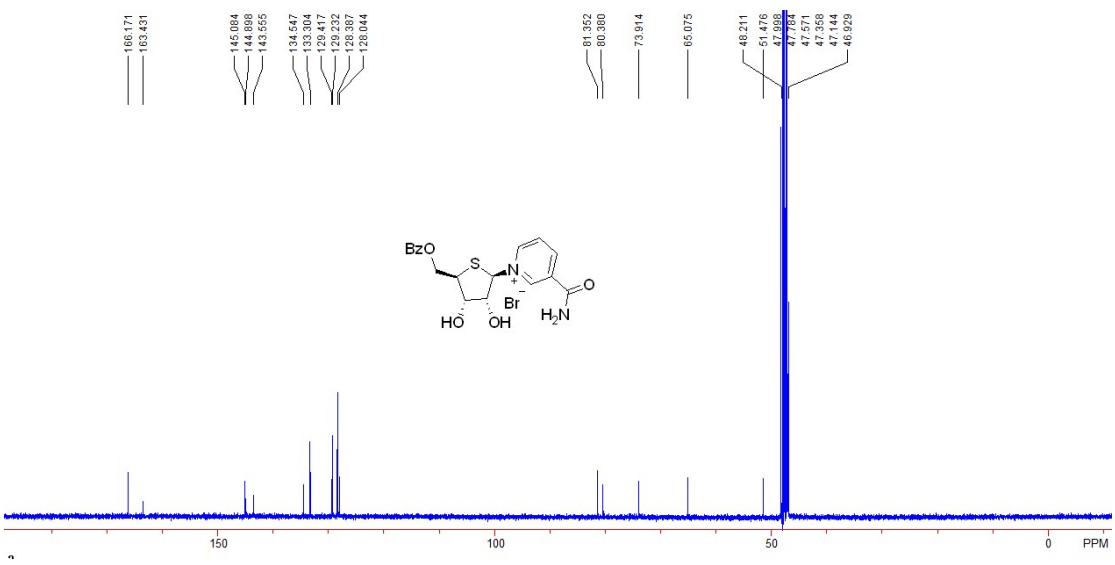


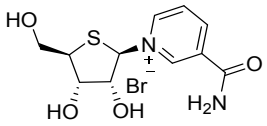
11

*General procedure for the synthesis of compound **11***. Compound **10** (50 mg, 0.1 mmol) was dissolved in a mixture of TFA/H2O (9/1, 15 mL) at 0°C and the resulting mixture was stirred at the same temperature until the reaction complete (monitoring by TLC). The reaction was concentrated under reduced pressure and the crude product was dissolved in MeOH (0.3 mL). Addition of ethyl ether (20 mL) resulted in ppt of the desired product. The procedure was repeated four times to yield the desired product **11** (35 mg, 76%) as a colorless solid**.**

1-((2*R*,3*R*,4*S*,5*R*)-5-((benzoyloxy)methyl)-3,4-dihydroxytetrahydrothiophen-2-yl)-3-carbamoylpyridin-1-ium bromide (11). ^{1}H NMR (400 MHz, CD_3OD): δ 3.92 (td, 1H, *J* = 6.8, 2.8 Hz, CH), 4.43 (t, 1H, *J* = 3.2 Hz, CH), 4.52 (dd, 1H, *J* = 6.8, 3.2 Hz, CH), 4.74 (d, 2H, *J* = 6.8 Hz, CH_2), 6.23 (d, 1H, *J* = 6.8 Hz, CH), 7.51 (t, 2H, *J* = 8.0 Hz, ArH), 7.63-7.67 (m, 1H, ArH), 8.05-8.07 (m, 2H, ArH), 8.21 (dd, 1H, *J* = 8.0, 6.4 Hz, ArH), 9.03 (d, 1H, *J* = 8.0 Hz, ArH), 9.46 (d, 1H, *J* = 6.4 Hz, ArH), 9.68 (s, 1H, ArH); ^{13}C NMR (100 MHz, CD_3OD): δ 51.5, 65.1, 73.9, 80.4, 81.4, 128.0, 128.4, 129.2, 129.4, 133.3, 134.5, 143.6, 144.9, 145.1, 163.4, 166.2.



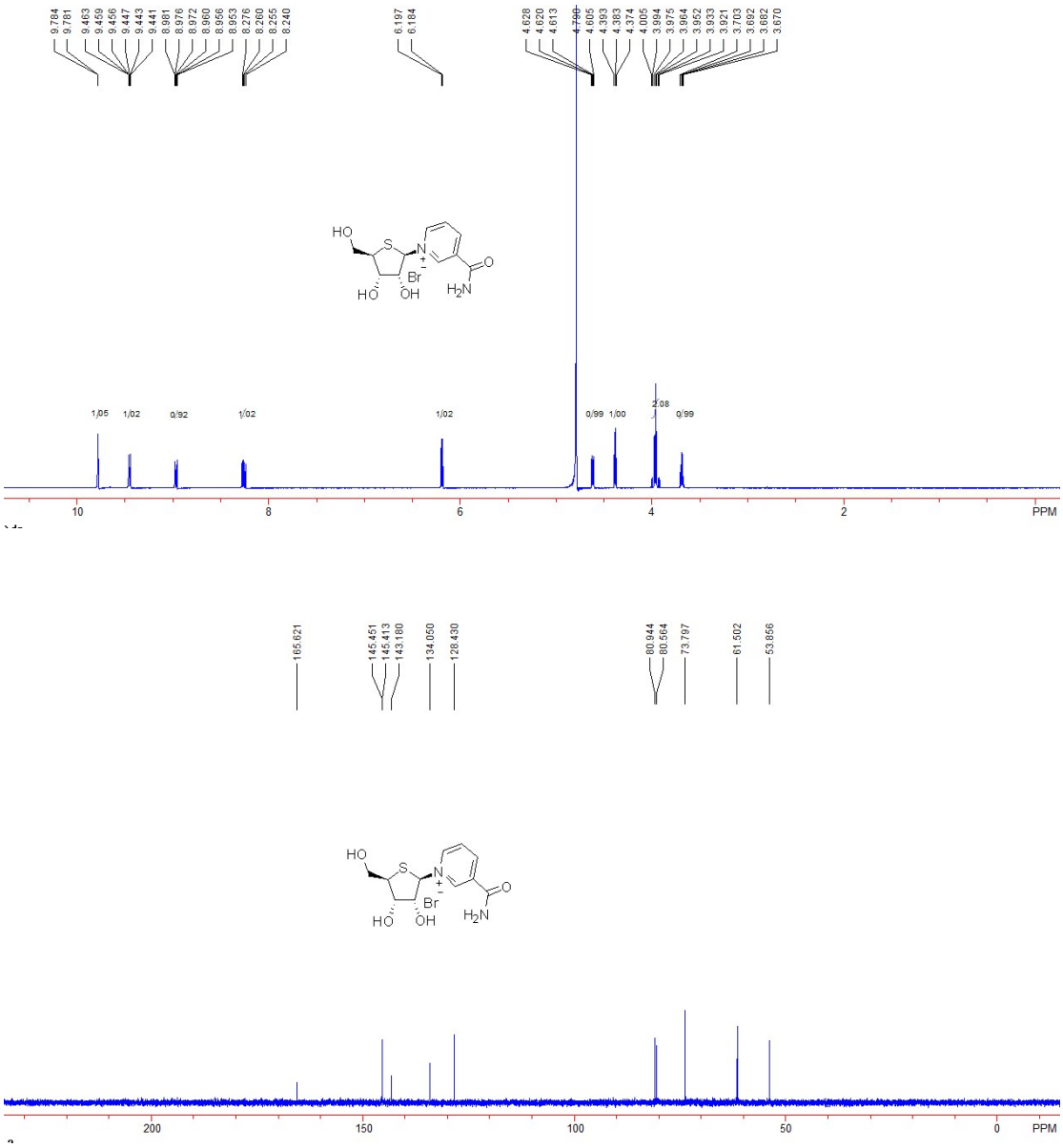




S-NR

General procedure for the synthesis of ***S-NR***. Compound **11** (23 mg, 0.05 mmol) was dissolved in ammonia (5 mL, 7 N in MeOH) and the reaction was stirred at 0°C for 48 hours. The reaction was concentrated under reduced pressure and the crude product was dissolved in MeOH (0.2 mL). Addition of ethyl ether (20 mL) resulted in ppt of the desired product. The procedure was repeated five times to yield the desired product **S-NR** (11 mg, 64%) as a colorless solid**.**

3-carbamoyl-1-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrothiophen-2-yl)pyridin-1-ium (S-NR). ^{1}H NMR (400 MHz, D_2O): δ 3.67-3.70 (m, 1H, CH), 3.92-4.01 (m, 2H, CH_2), 4.38 (t, 1H, J = 3.6 Hz, CH), 4.62 (dd, 1H, J = 5.6, 3.6 Hz, CH), 6.19 (d, 1H, J = 5.6 Hz, CH), 8.26 (dd, 1H, J = 8.0, 6.4 Hz, ArH), 8.97 (dt, 1H, J = 8.0, 1.2 Hz, ArH), 9.45 (dt, 1H, J = 6.4, 1.2 Hz, ArH), 9.78 (t, 1H, J = 1.2 Hz, ArH); ^{13}C NMR (100 MHz, D_2O): δ 53.9, 61.5, 73.8, 80.6, 80.9, 128.4, 134.1, 143.2, 145.4, 145.5, 165.6; HRMS (ESI) for $C_{11}H_{15}N_2O_4S^+$ $(M)^+$ Calcd.: 271.0763 Da; Obs: 271.0753 Da.



Enzymatic Conversion of S-NR to S-NAD$^+$.

Human nicotinamide riboside kinase 1 (NRK1) and nicotinamide mononucleotide adenylyltransferase 1 (NMNAT1) were expressed and purified based on previous publications.[2,3]

Table S1. List of primer sequences used for molecular cloning of human NRK1 and NMNAT1. Restriction enzyme sites for NcoI and XhoI are underlined and in italics.

Name	Sequence
NRK1-Fw	5′-*CCATGG*ATGAAAACATTTATCATTGGAATCAGTGG-3′
NRK1-Rv	5′-*CTCGAG*TGCTGTCACTTGCAAACACTTTTG-3′
NMNAT1-Fw	5′- *CCATGG*ATGCACCACCACCACCACCACGAAAATTCCGAG AAGACTGAAGTG-3′
NMNAT1-Rv	5′-*CTCGAG*CTACTACTATGTCTTAGCTTCTGCAGTGTTTC-3′

Protein sequences of human NRK1 and NMNAT1:

NRK1

MKRFVIGIGGVTNGGKTTLAKSLQKHLPNCSVISQDDFFKPESEIDIDENGFLQ
YDVLEALNMEKMMSAVSCWMENPGSSAGPAALESAQGVPILIIEGFLLFNYK
PLDTIWNRSYFLTVPYEECKRRRSTRVYEPPDPPGYFDGHVWPMYLKHRQE
MSSITWDIVYLDGTRSEEDLFSQVYEDVKQELEKQNGLHHHHHH

NMNAT1

MENSEKTEVVLLACGSFNPITNMHLRLFELAKDYMNGTGRYTVVKGIISPVG
DAYKKKGLIPAYHRVIMAELATKNSKWVEVDTWESLQKEWKETLKVLRHH
QEKLEASDCDHQQNSPTLERPGRKRKWTETQDSSQKKSLEPKTKAVPKVKLL
CGADLLESFAVPNLWKSEDITQIVANYGLICVTRAGNDAQKFIYESDVLWKH
RSNIHVVNEWIANDISSTKIRRALRRGQSIRYLVPDLVQEYIEKHNLYSSESED
RNAGVILAPLQRNTAEAKTHHHHHH

Molecular Cloning and Protein Expression and Purification. The open reading frames (ORFs) of human NRK1 (528 bp) and NMNAT1 (840 bp) with N-terminal His_6-tags were amplified through polymerase chain reaction (PCR) using primers of NRK1-Fw/Rv and NMNAT1-Fw/Rv, respectively (Table S1), which contained NcoI and XhoI restriction enzyme sites at 5'- and 3'-end, respectively. The amplified DNA fragments were digested by NcoI and XhoI restriction enzymes and then ligated into pET-28a (+) using T4 DNA ligase (New England Biolabs, Ipswich, MA). All

generated expression vectors were confirmed by DNA sequencing provided by Genewiz LLC (South Plainfield, NJ).

BL21 (DE3) cells were transformed with the generated constructs for bacterial protein expression in LB Broth supplemented with kanamycin (50 μg mL^{-1}). The overnight bacterial culture (5 mL) was diluted into 1 liter of LB Broth with kanamycin (50 μg mL^{-1}) for growth at 37°C in an incubator shaker at a speed of 250 rpm (Series 25, New Brunswick Scientific, NJ). When OD_{600nm} reached 0.6-0.8, protein expression was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for overnight at 18°C. Cells were then harvested by centrifugation at 4,550 g (Beckman J6B Centrifuge, JS-4.2 rotor), resuspended in equilibrium buffer (20 mM Tris-HCl, pH 8.0, 200 mM NaCl, 20 mM imidazole), and lysed using a French Press (GlenMills, NJ) at 25,000 psi for three cycles. Cell debris was removed by centrifugation at 14,000 g for 1 hour (Beckman Coulter centrifuge, JA-17 rotor) and supernatants were filtered through 0.45 μm membranes. The filtrate was loaded on a gravity flow column packed with 1 mL Ni-NTA agarose resin (Thermo Fisher Scientific, Waltham, MA), followed by washing with 15 column volumes of wash buffer (20 mM Tris-HCl, pH 8.0, 200 mM NaCl, 30 mM imidazole). Proteins were then eluted in 15 column volumes of elution buffer (20 mM Tris-HCl, pH 8.0, 200 mM NaCl, 400 mM imidazole), dialyzed in storage buffer (20 mM Tris-HCl, pH 8.0, 300 mM NaCl, 1 mM DTT, 10% glycerol) at 4℃ for overnight and another 6 hours in fresh storage buffer, and concentrated using Amicon centrifugal concentrators (EMD Millipore, Temecula, CA) with 10 kDa cut-off. Purified proteins were examined by SDS-PAGE and a NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific,

Waltham, MA), and aliquoted and flash-frozen in liquid nitrogen for storage at -80°C. Calculated molecular extinction coefficient values are 1.537 for NRK1-6×His and 1.592 for NMNAT1-6×His.

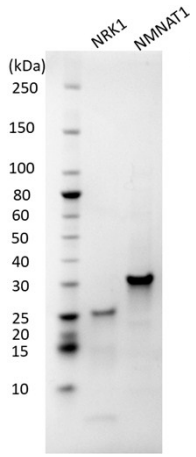
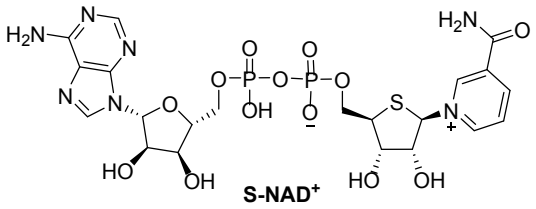
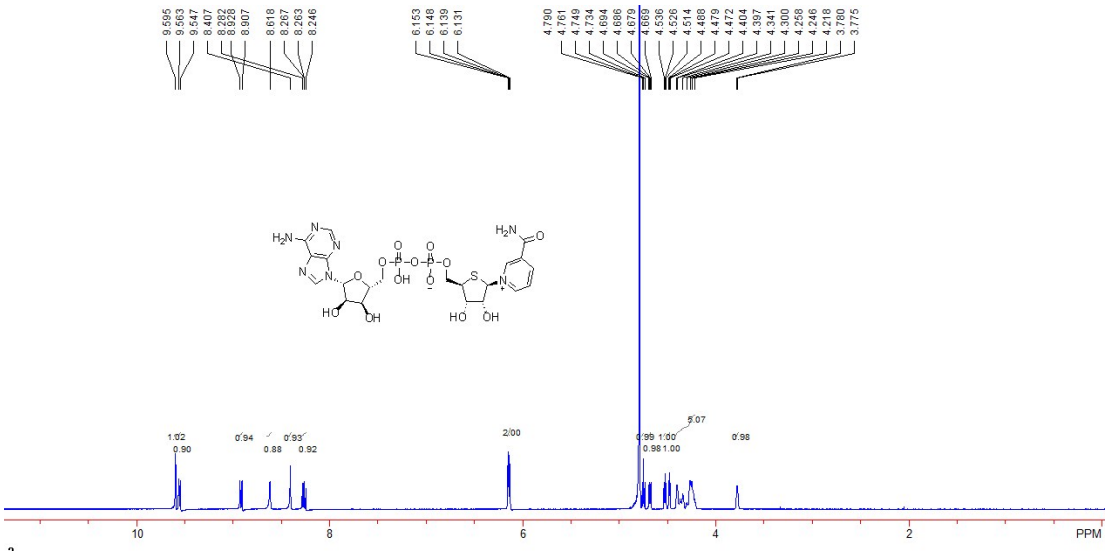


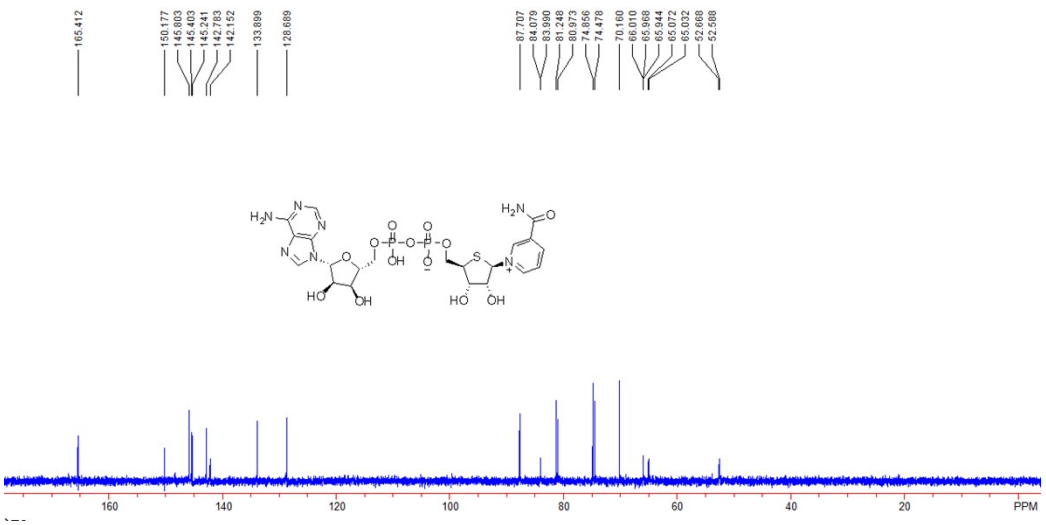
Figure S1. SDS-PAGE gel of the purified human NRK1 and NMNAT1.

Purified S-NR (0.5 mg in the form of bromide salt) was incubated with 6 mM ATP, 5 uM of purified NRK1 and NMNAT1 at the final concentration of 2 mM at room temperature for overnight in the reaction buffer (50 mM Tris, 100 mM NaCl, 12 mM $MgCl_2$, 1 mM DTT, and pH 7.5). After centrifugation at 4,000 g for 5 minutes, supernatants were analyzed by HPLC and the fraction containing S-NAD^+ (0.68 mg, 70%) were pooled and confirmed by both NMR and mass spectrometry. All analysis and purifications on HPLC were performed with a semipreparative C18 Kinetex column (5 μm, 100 Å, 150×10.0 mm, from Phenomenex Inc, Torrance, CA) using the following method, mobile phase A: 0.1% formic acid (aq); mobile phase B: 0.1% formic acid in methanol; flow rate: 2.0 ml/min; 0-2 min: 0-4% B, 2-4 min: 4-10% B; 4-6 min: 10-20% B; 6-9 min: 20% B; 9-12 min: 20-50% B; and 12-14 min: 50-0% B.



S-NAD$^+$. ^{1}H NMR (400 MHz, D_2O): δ 3.76-3.79 (m, 1H, CH), 4.22-4.40 (m, 5H, 2CH_2+CH), 4.48 (t, 1H, *J* = 3.2 Hz, CH), 4.51-4.54 (m, 1H, CH), 4.68 (dd, 1H, *J* = 6.4, 3.6 Hz, CH), 4.75 (t, 1H, *J* = 5.2 Hz, ArH), 6.13-6.15 (m, 2H, 2CH), 8.26 (dd, 1H, *J* = 8.0, 6.4 Hz, ArH), 8.41 (s, 1H, ArH), 8.62 (s, 1H, ArH), 8.92 (d, 1H, *J* = 8.0 Hz, ArH), 9.55 (d, 1H, *J* = 6.4 Hz, ArH), 9.60 (s, 1H, ArH); ^{13}C NMR (100 MHz, D_2O): δ 52.6 (d, *J* = 8.0 Hz), 65.0-65.1 (m), 65.9-66.1 (m), 70.2, 74.5, 74.9, 81.0, 81.2, 84.0 (d, *J* = 8.9 Hz), 87.7, 128.7, 133.9, 142.2, 142.8, 145.2, 145.4, 145.8, 150.2, 165.4; HRMS (ESI) for $C_{21}H_{27}N_7O_{13}P_2SNa^+$ $(M+Na)^+$: Calcd.: 702.0766 Da; Obs: 702.0760 Da.

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80.973
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74.478
70.160
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20
PPM

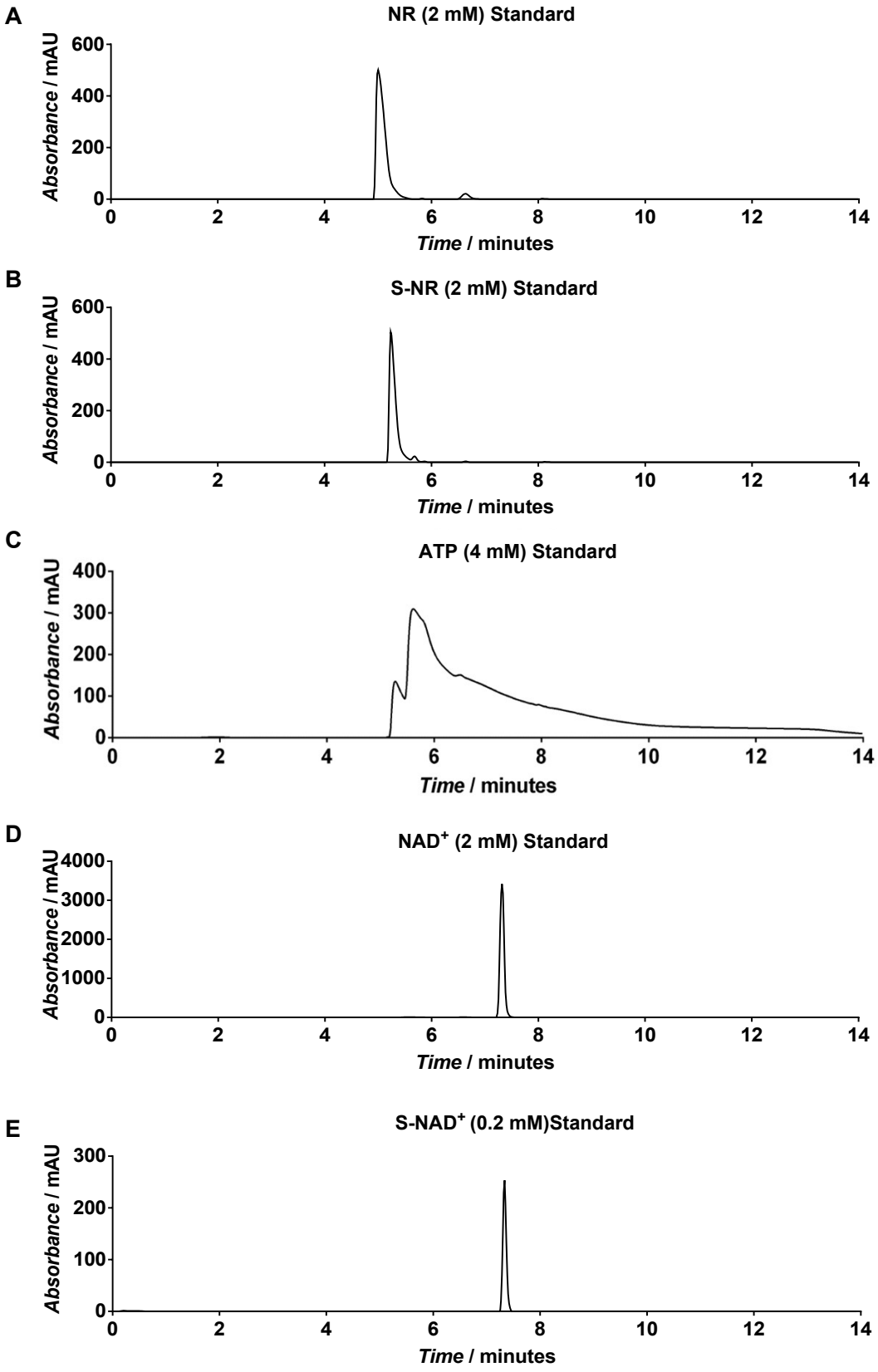

Figure S2. HPLC analysis of standard compounds as measured by UV absorbance at 260 nm. (A) NR, (B) S-NR, (C) ATP, (D) NAD^+, and (E) S-NAD^+.

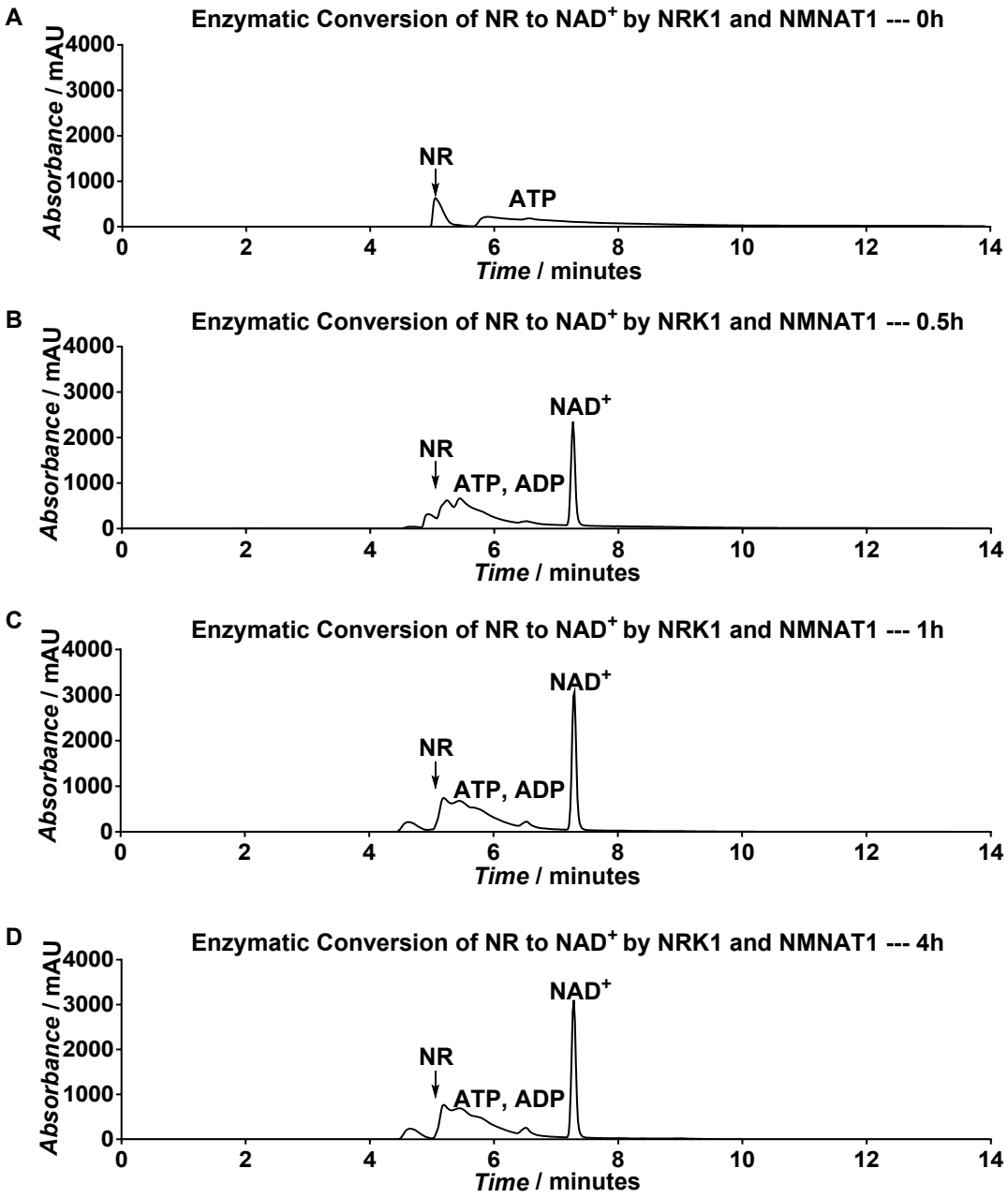

Figure S3. HPLC analysis of enzymatic synthesis of NAD^+ by the purified NRK1 and NMNAT1 as measured by UV absorbance at 260 nm. 2 mM NR was incubated with 6 mM ATP and 5 μM NRK1 and 5 μM NMNAT1 at RT for 0h (A), 0.5h (B), 1h (C), and 4h (D), followed by HPLC analysis.

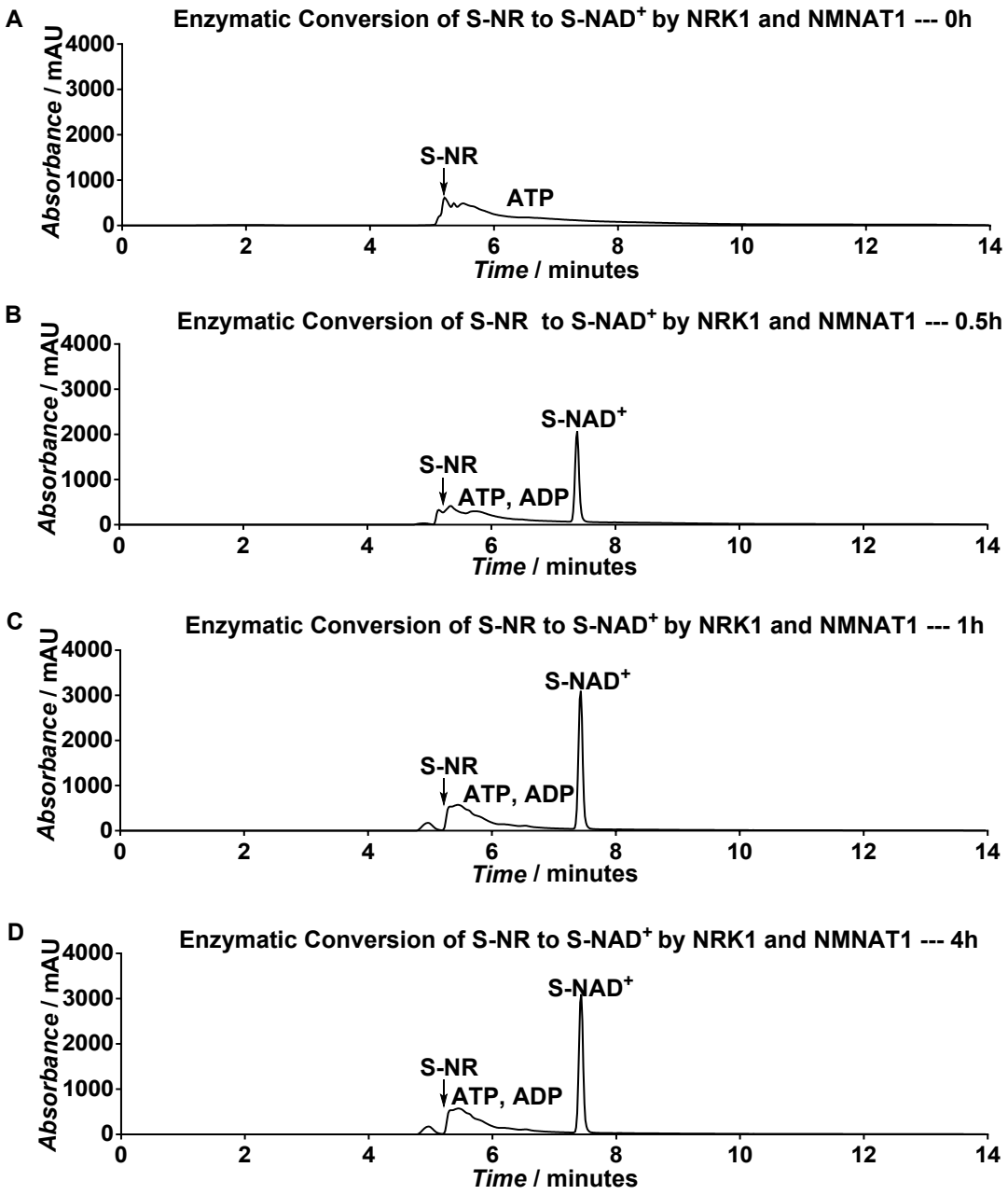

Figure S4. HPLC analysis of enzymatic synthesis of S-NAD$^+$ by the purified NRK1 and NMNAT1 as measured by UV absorbance at 260 nm. 2 mM S-NR was incubated with 6 mM ATP and 5 μM NRK1 and 5 μM NMNAT1 at RT for 0h (A), 0.5h (B), 1h (C), and 4h (D), followed by HPLC analysis.

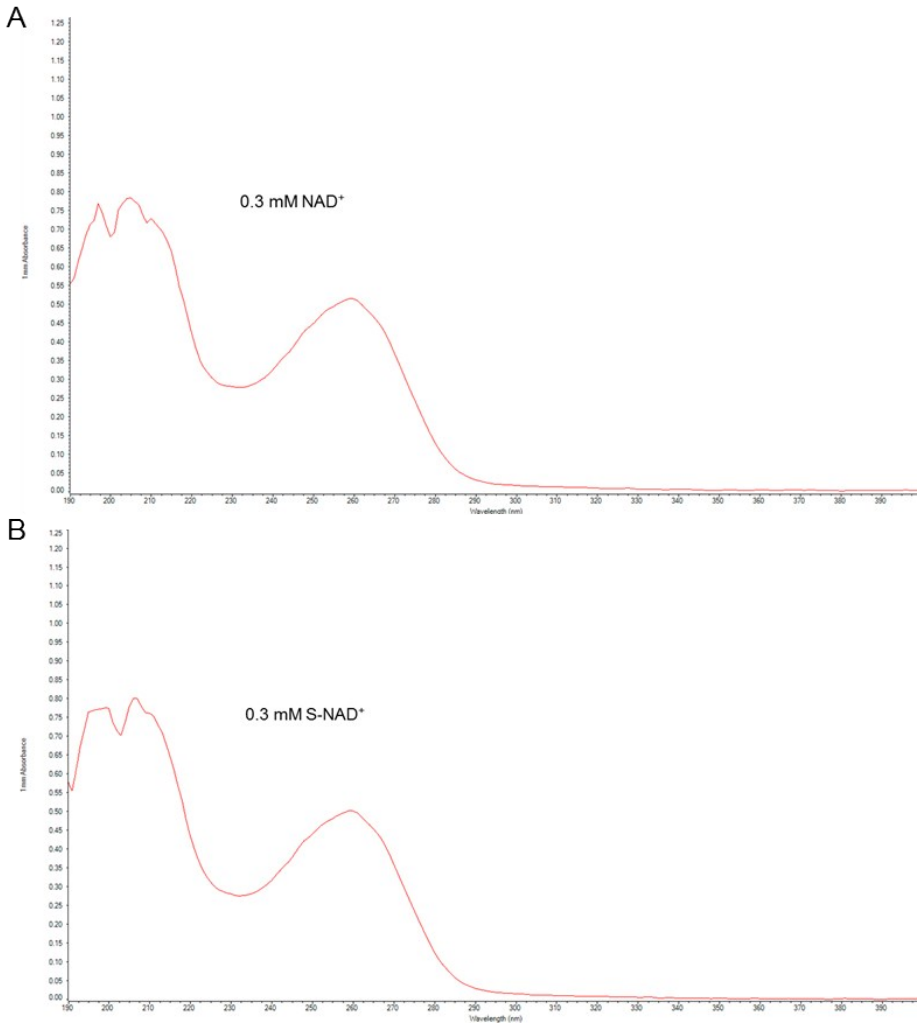

Figure S5. UV absorption spectra of NAD^+ and S-NAD^+. 0.3 mM NAD^+ (A) or S-NAD^+ (B) was used to measure the UV absorption spectra (190-400 nm) by a NanoDrop 2000C spectrophotometer.

Expression and purification of recombinant human CD38.

Synthetic DNA encoding extracellular domain of human CD38 (R45-I300) was purchased from Integrated DNA Technologies, Inc. (Coralville, Iowa), in which four asparagine residues were mutated to eliminate *N*-glycosylation. Below is the amino acid sequence of human CD38 used for expression and purification: RWRQQWSGPGTTKRFPETVLARCVKYTEIHPEMRHVDCQSVWDAFKGAFIS KHPCDITEEDYQPLMKLGTQTVPCNKILLWSRIKDLAHQFTQVQRDMFTLED

TLLGYLADDLTWCGEFATSKINYQSCPDWRKDCSNNPVSVFWKTVSRRFAE AACDVVHVMLDGSRSKIFDKDSTFGSVEVHNLQPEKVQTLEAWVIHGGRED SRDLCQDPTIKELESIISKRNIQFSCKNIYRPDKFLQCVKNPEDSSCTSEI.[4] An N-terminal His_6-tag followed by a TEV protease cleave site was placed in front of the gene encoding human CD38. The designed insert was amplified by PCR using primers (1) 5′-AGTCTTGCACTTGTCACGAATTCGCATCATCAC-3′ and (2) 5′-ATGTCTGGCCAGCTAGCACTTATCAG-3′, followed by enzymatic digestion using NheI and EcoRI (New England Biolabs, Ipswich, MA), and ligation into pFuse mammalian expression vector using T4 DNA ligase (New England Biolabs, Ipswich, MA). The expression vector expressing human CD38 was confirmed by DNA sequencing provided by Genewiz LLC (South Plainfield, NJ).

Recombinant CD38 was expressed in Expi293 expression system (Thermo Scientific, MA) through transient transfection by following manufacturer's instruction. Culture media of Expi293 cells containing secreted recombinant human CD38 were collected 4 days post transfection. Expressed human CD38 was purified by Ni-NTA affinity chromatography by following the same procedures as described above for purification of human NRK1 and NMNAT1. The N terminal poly-histidine tag was removed by incubating purified CD38 with TEV protease (Thermo Scientific, MA) at 30°C for 4 hours, followed by passing through a Ni-NTA column to collect His_6-tag-free CD38. Purified CD38 was then dialyzed against storage buffer (25 mM HEPES, 250 mM NaCl, pH 7.5) overnight at 4°C and another 6 hours in fresh storage buffer, and concentrated using Amicon centrifugal concentrators (EMD Millipore, Temecula,

CA) with 10 kDa cut-off. Purified human CD38 was examined by SDS-PAGE and a NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA), and aliquoted and flash-frozen in liquid nitrogen for storage at -80^{o}C. The calculated molecular extinction coefficient is 1.756 for human CD38. For crystallization, His_6-tag-free CD38 was further purified by gel filtration chromatography using Superdex 75 Increase 10/300 GL (GE Healthcare Life Sciences, Pittsburgh, PA) in 15 mM HEPES pH 7.0 and 50 mM NaCl. Fractions with CD38 were pooled and concentrated using Amicon centrifugal concentrators with 10 kDa cut-off.

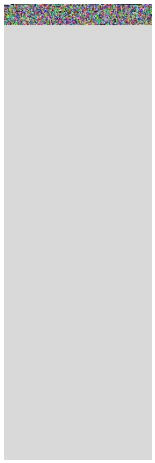

Figure S6. SDA-PAGE gel of the purified extracellular domain of human CD38.

CD38 kinetic studies with nicotinamide guanine dinucleotide (NGD^+) as the substrate.

NGD^+ (Sigma-Aldrich, MO) was used as a substrate to determine ADP-ribosyl cyclase activity of purified CD38, because cyclic GDP-ribose (cGDPR) product is characterized by distinctive UV absorbance at 295 nm and fluorescence emission at 410 nm (excitation at 300 nm).[5] To characterize kinetic parameters, reactions were initiated by additions of recombinant CD38 (8.3 nM final) into assay wells with various concentrations of NGD^+ in 20 mM MES, pH 6.5, followed by monitoring the reactions through fluorescence at 410 nm (excitation at 300 nm) to determine initial reaction rates. Inhibition activities of S-NAD^+ were determined by carried out enzymatic formation of cyclic GDP-ribose in the presence of S-NAD^+. Reactions were initiated by additions of recombinant CD38 (8.3 nM final) into assay wells with 50 uM of NGD and various concentrations of S-NAD^+ in 20 mM MES, pH 6.5. All reactions were carried out in black 96-well plates and the resulting fluorescence was measured using a BioTek Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek, VT) in the kinetic mode. The K_m of NGD^+ for CD38 was calculated by fitting data with Michaelis–Menten function implemented in GraphPad Prism (La Jolla, CA). The inhibition constant (K_i) of NGD^+ was determined by fitting the initial reaction rates and inhibitor concentrations to the expression for competitive inhibition: $(V'_0/V_0) = (K_m + [S])/(K_m + [S] + (K_m[I]/K_i))$, where V'_0 is the initial reaction rate in the presence of inhibitor, V_0 is the initial reaction rate in the absence of inhibitor, [S] is the substrate concentration, and [I] is the inhibitor concentration.

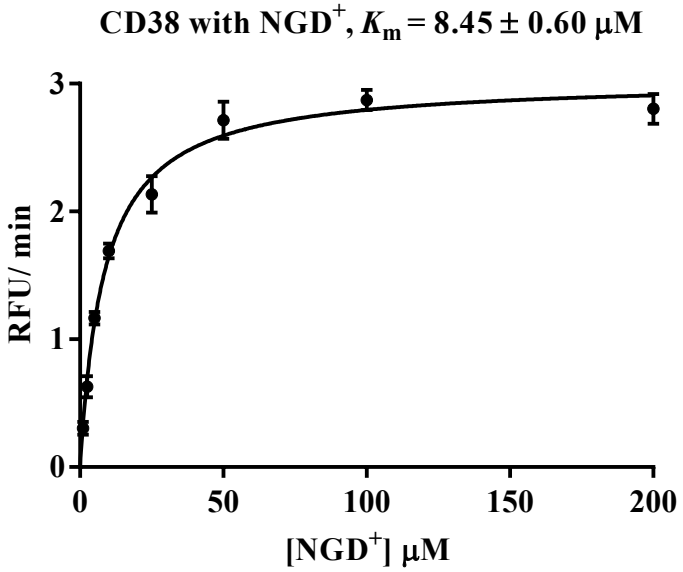

Figure S7. Enzymatic parameter of the ADP-ribosyl cyclase activity of the purified human CD38 with NGD^+ as a substrate.

Substrate activity of S-NAD^+ for human CD38.

1 mM S-NAD^+ and NAD^+ in 50 mM MES, pH 6.5 were incubated with 13.4 nM recombinant human CD38 for overnight at room temperature, followed HPLC analysis of the reaction mixtures. HPLC analysis was performed with a semipreparative C18 Kinetex column (5 μm, 100 Å, 150×10.0 mm, from Phenomenex Inc, Torrance, CA) using following method: mobile phase A: 0.1% formic acid (aq); mobile phase B: 0.1% formic acid in methanol; flow rate: 2.0 ml/min; 0-2 min: 0-4% B, 2-4 min: 4-10% B; 4-6 min: 10-20% B; 6-9 min: 20% B; 9-12 min: 20-50% B; and 12-14 min: 50-0% B.

Substrate activity of S-NAD^+ for human sirtuins 2 (SIRT2).

Recombinant human SIRT2 and a fluorogenic peptide substrate Ac-RGK(Ac)-AMC were purchased from R&D Systems (Minneapolis, MN). Bovine trypsin was purchased from Sigma-Aldrich (St. Louis, MO). To determine the substrate activities

of NAD^+ and S-NAD^+, 0.25 mM NAD^+ or S-NAD^+ was incubated with 250 μM Ac-RGK(Ac)-AMC peptide and 45.4 nM SIRT2 in buffer containing 25 mM Tris, 150 mM NaCl, 1 mM DTT, pH 8.0 for overnight at room temperature, followed by additions of 50 nM bovine trypsin to the reaction mixtures and measurements of fluorescence signals (excitation at 380 nm and emission at 460 nm) using a BioTek Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek, VT) in a kinetic mode. To analyze inhibition activity of S-NAD^+, 0.5 mM NAD^+ or S-NAD^+ or a mixture of 0.5 mM NAD^+ and 2 mM S-NAD^+ was incubated with 250 μM Ac-RGK(Ac)-AMC peptide and 45.4 nM SIRT2 in buffer containing 25 mM Tris, 150 mM NaCl, 1 mM DTT, pH 8.0 for 30 minutes at room temperature, followed by incubation with 50 nM bovine trypsin for 15 minutes. The fluorescence signals were then measured using the BioTek Synergy H1 Hybrid Multi-Mode Microplate Reader.

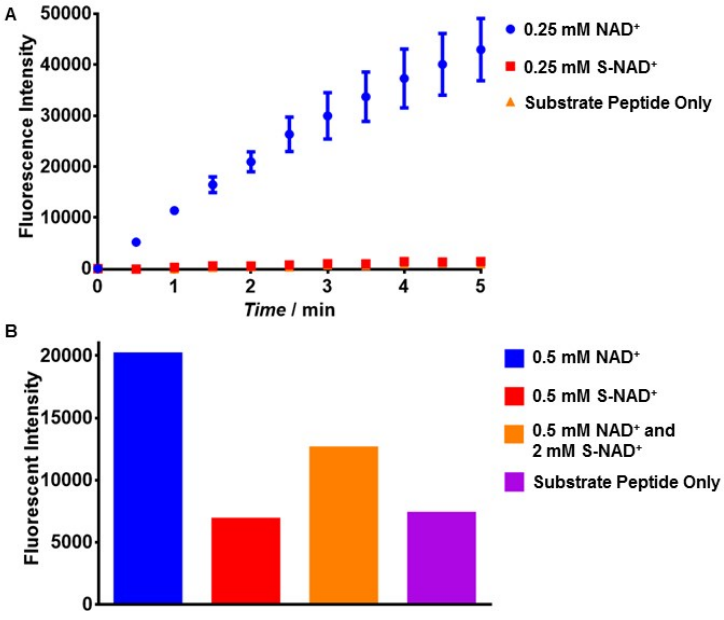

Figure S8. Substrate activities of NAD^+ and S-NAD^+ with human SIRT2. The deacetylation activity for recombinant human SIRT2 was measured using a trypsin-

coupled fluorescence-based assay at 460 nm on the basis of the released 7-amino-4-methylcoumarin (AMC) from a deacetylate peptide substrate cleaved by trypsin. (A) 0.25 mM NAD^+ or S-NAD^+ was incubated with 250 μM Ac-RGK(Ac)-AMC peptide and 45.4 nM SIRT2 in reaction buffer (25 mM Tris, 150 mM NaCl, 1 mM DTT, pH 8.0) for overnight at room temperature, followed by additions of 50 nM bovine trypsin and measurements of fluorescence signals for 5 minutes. (B) 0.5 mM NAD^+ or S-NAD^+ or a mixture of 0.5 mM NAD^+ and 2 mM S-NAD^+ was incubated with 250 μM Ac-RGK(Ac)-AMC peptide and 45.4 nM SIRT2 in reaction buffer (25 mM Tris, 150 mM NaCl, 1 mM DTT, pH 8.0) for 30 minutes at room temperature. Following 15-minute incubation with 50 nM bovine trypsin, fluorescence intensities were determined.

Substrate activities of S-NAD^+ with redox enzymes.

Bovine glutamate dehydrogenase (GDH) and *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenase (G6PDH) were purchased from Sigma-Aldrich (St. Louis, MO) and used for determining the substrate activities of NAD^+ and S-NAD^+. To examine substrate activity with bovine GDH, 0.1 mM NAD^+ or S-NAD^+ was incubated with 1 mM L-glutamate and 0.1 unit/ml GDH in buffer containing 2 mM DTT, 50 mM Tris, pH 7.5 for 15 minutes at room temperature. To determine substrate activity with G6PDH, 0.5 mM NAD^+ or S-NAD^+ was incubated with 5 mM D-glucose 6-phosphate and 0.01 unit/ml G6PDH in buffer containing 5 mM $MgCl_2$, 50 mM Tris, pH 7.5 for 15 minutes at room temperature. Formation of NADH and S-NADH were monitored by UV absorbance at 340 nm under kinetic mode using a

NanoDrop 2000C spectrophotometer (Thermo Scientific, MA).

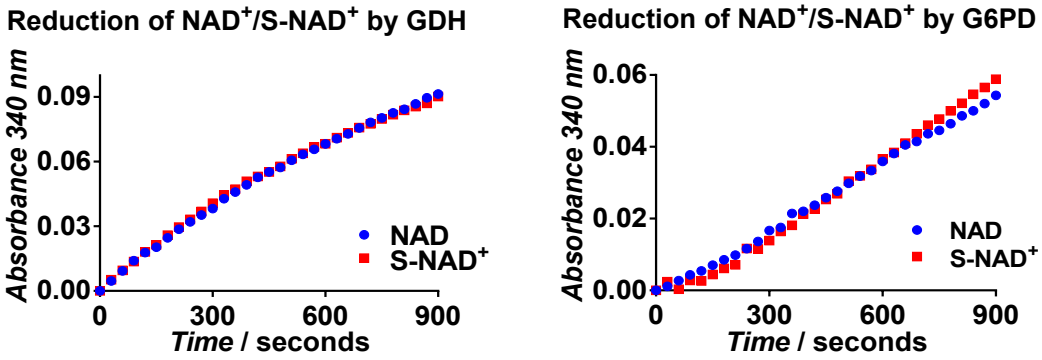

Figure S9. Substrate activities of NAD^+ and S-NAD^+ with bovine GDH and *L. mesenteroides* G6PD enzymes. The reactions were monitored by UV absorbance at 340 nm.

HPLC analysis of reduction of NAD^+ and S-NAD^+ by redox enzymes.

To examine the bovine GDH-catalyzed reactions, 0.5 mM NAD^+ or S-NAD^+ was incubated with 2 mM L-glutamate and 20 unit/ml GDH in buffer containing 2 mM DTT, 50 mM Tris, pH 7.5 for 30 minutes at room temperature, followed by HPLC analysis with detection of UV absorbance at 340 nm. To evaluate the *L. mesenteroides* G6PDH-catalyzed reactions, 0.5 mM NAD^+ or S-NAD^+ was incubated with 5 mM D-glucose 6-phosphate and 10 unit/ml G6PDH in buffer containing 5 mM $MgCl_2$, 50 mM Tris, pH 7.5 for 30 minutes at room temperature, followed by HPLC analysis with detection of UV absorbance at 340 nm. The HPLC method used for analysis of reactions by GDH and G6PDH was the same as described above.

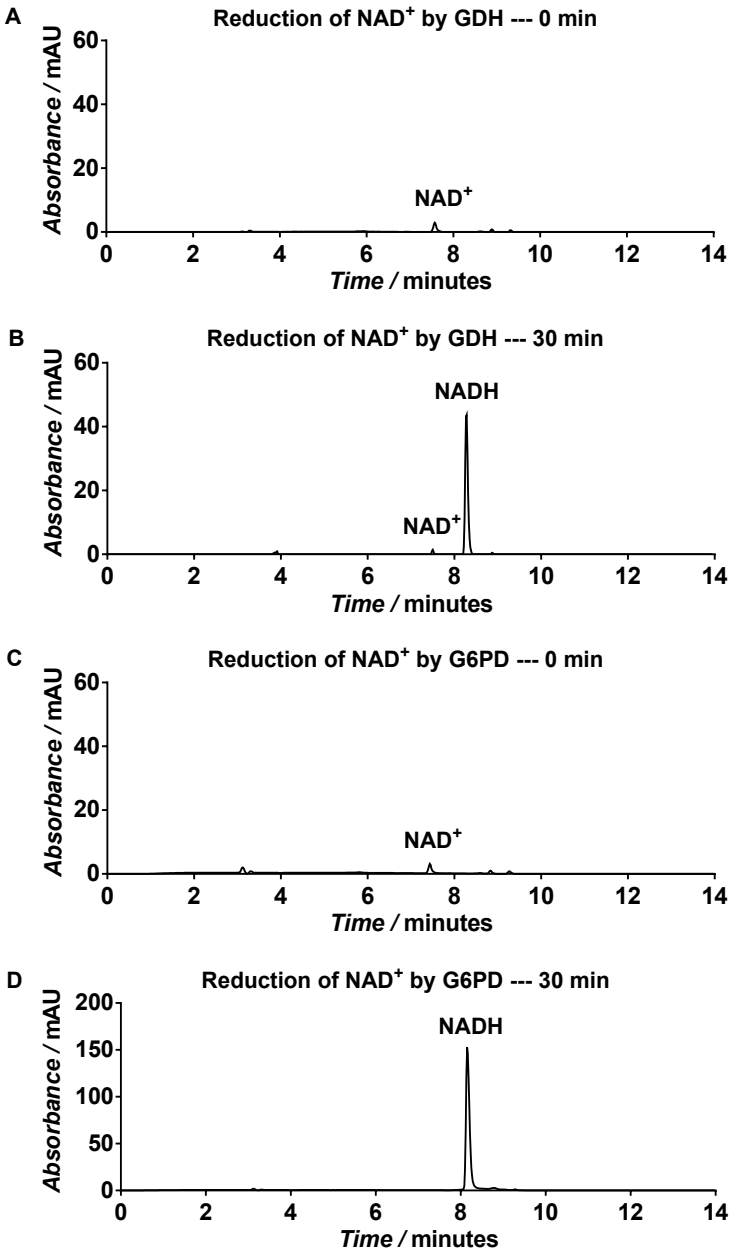

Figure S10. HPLC analysis of reduction of NAD^+ by bovine GDH and *L. mesenteroides* G6PDH as measured by UV absorbance at 340 nm. (A) and (B) 0.5 mM NAD^+ was incubated with 2 mM L-glutamate and 20 unit/ml GDH in buffer containing 2 mM DTT, 50 mM Tris, pH 7.5 for 0 (A) or 30 minutes (B) at room temperature, followed by HPLC analysis. (C) and (D) 0.5 mM NAD^+ was incubated with 5 mM D-glucose 6-phosphate and 10 unit/ml G6PDH in buffer containing 5 mM $MgCl_2$, 50 mM Tris, pH 7.5 for 0 (C) or 30 minutes (D) at room temperature,

followed by HPLC analysis.

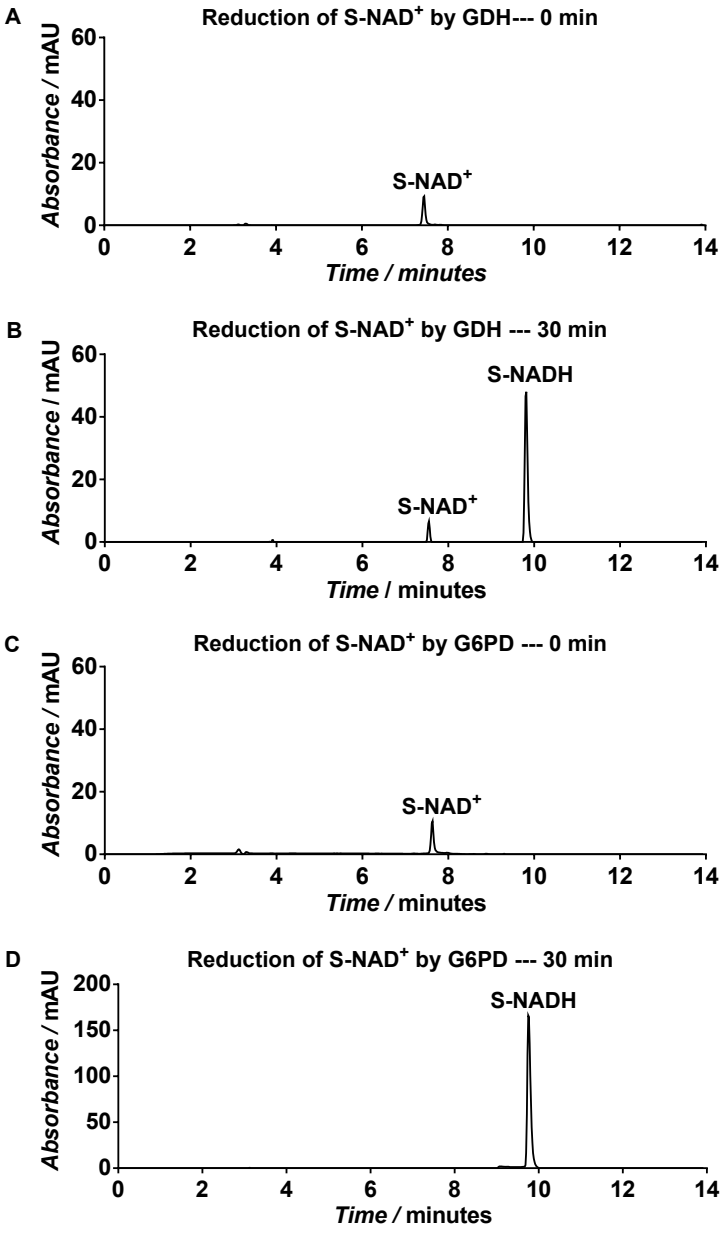

Figure S11. HPLC analysis of reduction of S-NAD$^+$ by bovine GDH and *L. mesenteroides* G6PDH as measured by UV absorbance at 340 nm. (A) and (B) 0.5 mM S-NAD$^+$ was incubated with 2 mM L-glutamate and 20 unit/ml GDH in buffer containing 2 mM DTT, 50 mM Tris, pH 7.5 for 0 (A) or 30 minutes (B) at room temperature, followed by HPLC analysis. (C) and (D) 0.5 mM S-NAD$^+$ was incubated

with 5 mM D-glucose 6-phosphate and 10 unit/ml G6PDH in buffer containing 5 mM $MgCl_2$, 50 mM Tris, pH 7.5 for 0 (C) or 30 minutes (D) at room temperature, followed by HPLC analysis.

X-ray crystallographic study of S-NAD$^+$ with human CD38.

Crystallization was performed using a vapor diffusion method in a hanging-drop manner with a 1:1 ratio of 1 μL of reservoir solution and 1 μL of protein-ligand solution. The initial crystals grew under the previously published conditions.[6,7] To prepare the Protein-Ligand complex, 18.5 μl of 0.1 mM protein in 15 mM HEPES, pH 7.0, 50 mM NaCl was mixed with 1.5 μl of 69 mM ligand in the same buffer. This produced a mixture of the protein: ligand close to 1:50 molar ratio at their final concentration. The complex was incubated on ice for 30 minutes before crystallization. Single crystals appeared after 5 days and grew to their maximum size within 10-40 days. The best crystals grew in 100 mM HEPES, pH 7.0, 14-18% PEG 4000 at 22°C. Crystals were dipped into a 10% glycerol cryosolution and were flash-frozen in liquid nitrogen before they were mounted for data collection.

X-Ray diffraction data collection and structure determination.

Data was collected at Synchrotron Radiation Light Source (SSRL) using beamline 12-2 equipped with a Dectris-Pilatus 6M detector. The collected data were indexed and integrated with XDS and scaled using Scala, a part of the CCP4 suite.[8-10] Initial phase information was obtained by molecular replacement using PHASER with the previously solved structure of human cyclic-ADP-ribosyl synthetase/NAD$^+$ glycohydrolase (PDB ID code 1ZVM) as the search model.[11] Waters were added

using ArpWarp during the initial round of the refinement.[12-14] The Ligand was built using Ligand Builder in Coot and restrain generation and optimization by elbow part of Phenix Crystallography suit and also ProDrg part of CCP4 suit.[9,14-16] The structure was improved by iterative rounds of model building and refinement using the programs Coot and Refmac5.[13,14] The crystals belong to space group P1 2_1 1 and it contains two molecules per asymmetric unit. Crystallographic details and statistics are listed in Table S2.

Table S2. Crystallographic statistics for the S-NAD$^+$ with human CD38.

Data collection[a]	
Wavelength (Å)	0.97946
Space group	P 1 2_1 1
Unit cell dimensions [*a, b, c* (Å)]	*a*= 57.74 b=51.11 c=100.67
Resolution range (Å)	29.31-2.40
Highest resolution shell (Å)	2.53-2.40
No. of observed reflections	202072
No. of unique reflections	23158
Multiplicity	8.7 (8.7)
Completeness (%)	99.9 (99.9)
$<I/\sigma I>$	6.0 (1.3)
R_{merge} (%)	36.8 (146.8)
R_{pim} (%)	13.3 (52.5)
$CC_{1/2}$ (%)	96.1 (73.6)
Wilson B-factor	48.5
Refinement	
R_{work} (%)	21.57
R_{free} (%)	26.17
No. atoms	
Macromolecules	3908
Ligand	88
Water	87
B-factor (Å^2)	
Macromolecule	A: 48.1 B: 54.3
Solvent	C: 42.3
R.m.s. deviations	
Bond lengths (Å)	0.01
Bond angles (deg)	1.42
Ramachandran statistics (%)	
Favored	97.15
Outliers	0.20
Molprobity score	1.03
PDB ID	6EDR

[a] Values in parentheses are for the highest-resolution shell.

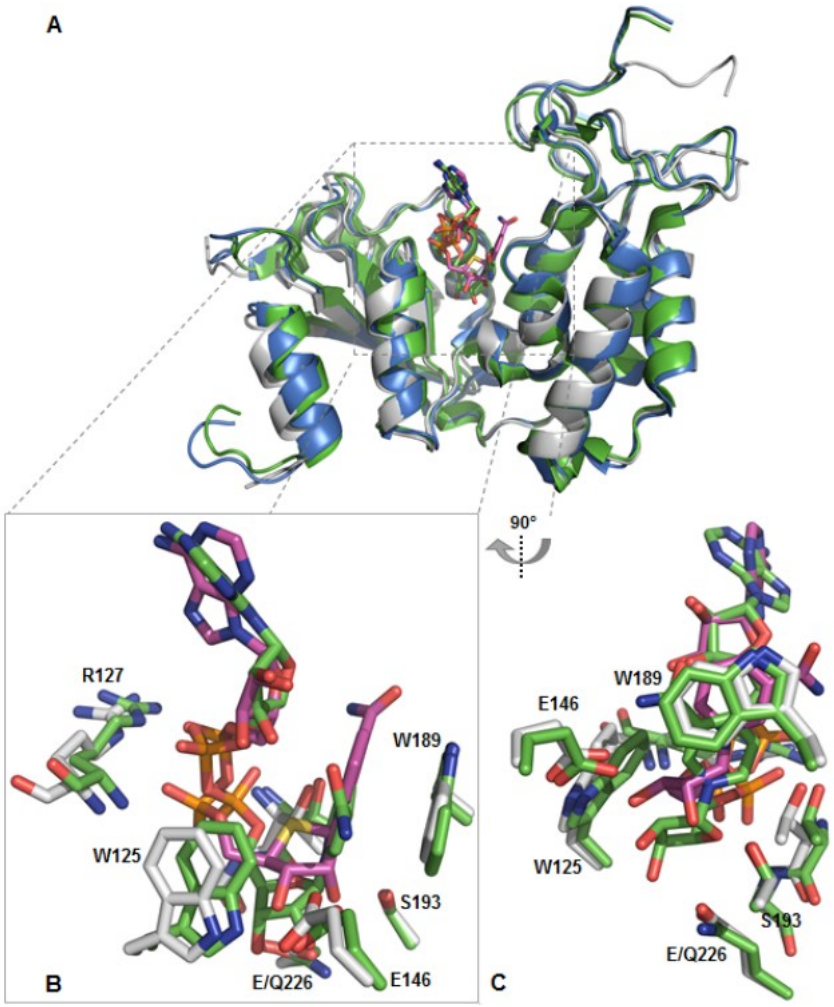

Figure S12. Structural comparison of S-NAD$^+$-bound CD38 with NAD$^+$-bound CD38 and apo-CD38. (A) Overlaid x-ray structures of S-NAD$^+$-bound catalytically active CD38 (grey), NAD$^+$-bound catalytically inactive CD38 (green) (E226Q) (PDB ID: 2I65), and apo-CD38 (blue) (PDB ID: 1YH3). (B) and (C) NAD$^+$ (green) and S-NAD$^+$ (magenta) at the binding sites of catalytically inactive CD38 (E226Q) (green) and active CD38 (grey) with indicated key interacting residues.

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