SUPPORTING INFORMATION

for

Transition state of ADP-ribosylation of acetyllysine catalyzed by *Archeaglobus fulgidus* Sir2 determined by kinetic isotope effects and computational approaches

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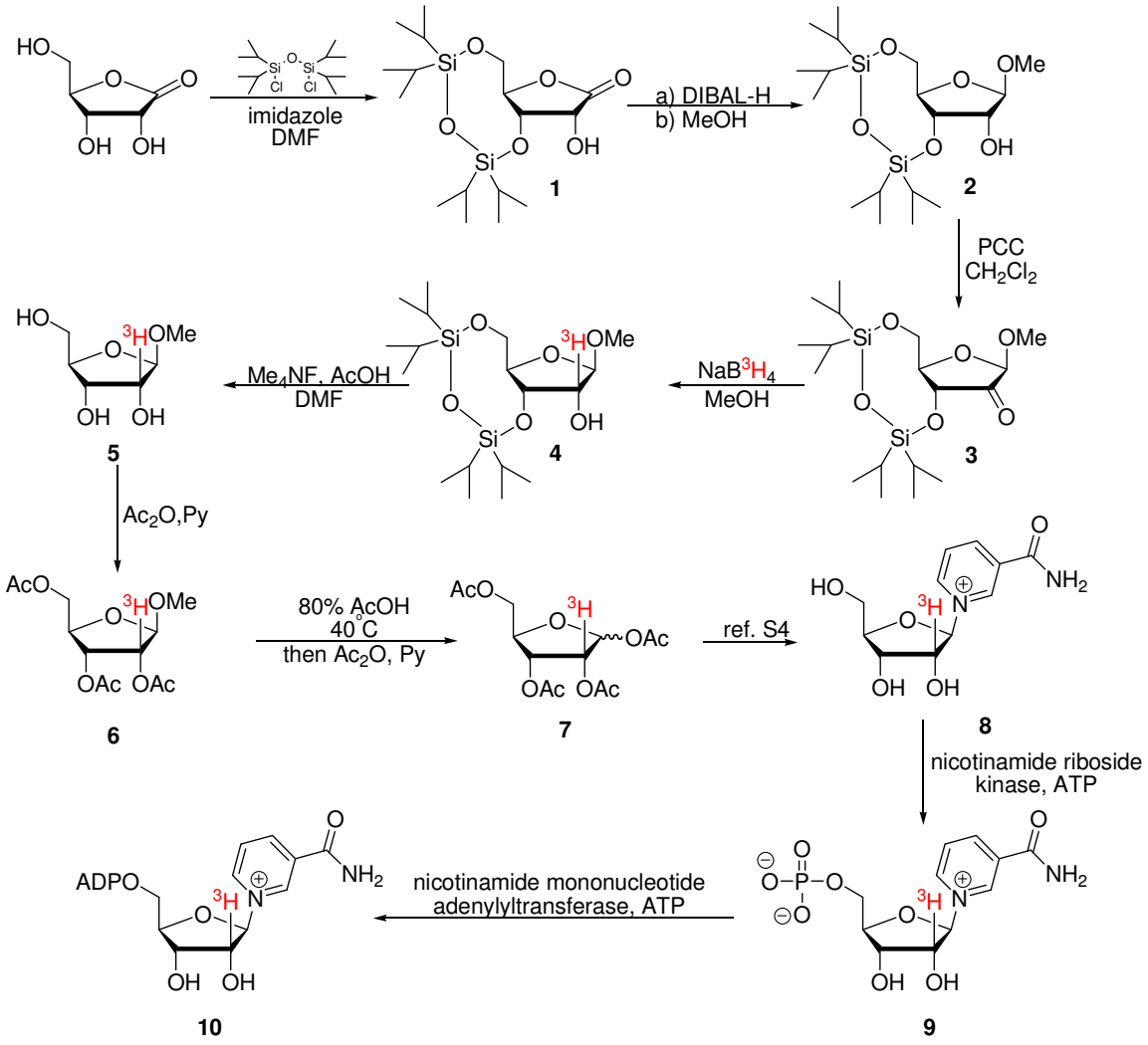
Enzymes and Reagents for NAD^+ Synthesis. Hexokinase, glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphoriboisomerase, myokinase and pyruvate kinase were purchased from Sigma. The enzymes *E.Coli* 5-phosphoribosylpyrophosphate synthetase,[S1] human CD38 (cell developmental protein 38, a transmembrane glycoprotein with NAD glycohydrolase activity),[S2] and yeast NAD^+ synthetase[S3] (received by gift from Professor Charlie Brenner) were expressed from vectors by published methods. Nicotinate phosphoribosyltransferase and nicotinic acid mononucleotide adenylyltransferase were cloned from yeast genomic DNA and integrated into pet28 vectors for expression. Purification of enzymes was achieved by Ni columns and purified enzyme was frozen as aliquots in 1 mM DTT and 20% glycerol. [2-^{3}H]-glucose, [5-^{3}H]-glucose, [6-^{3}H]-glucose, [6-^{14}C]-glucose, [2,8-^{3}H]-ATP, [8-^{14}C]-ATP were purchased from American Radiolabeled Chemicals, Inc. ^{15}N-ammonium chloride (99 %), ^{18}O-water (97% ^{18}O) were purchased from Cambridge Isotope Laboratories. ATP, α-ketoglutarate, glucose, β-nicotinamide adenine dinucleotide sodium salt (NAD^+), phosphoenolpyruvic acid (PEP), potassium chloride, potassium phosphate, magnesium chloride, ammonium chloride, DL-dithiothreitol (DTT), porcine liver esterase were purchased from Sigma. Phosphordiesterase I was purchased from Worthington biochemical corporation.

Synthesis of [2'$_N$-^{3}H]-NAD$^+$.

[2'$_N$-^{3}H]-NAD$^+$ was synthesized according to **Scheme S1**. A non-radiolabeled synthesis was done in parallel to obtain NMR data for each single intermediate along the synthetic pathway. The procedures described below are for non-radiolabeled synthesis.



Scheme S1

Ribonolactone (500 mg, 3.38 mmol) and imidazole (1.12g, 16.4 mmol) were dissolved in 35 mL of dry DMF, to this solution was added 1,3-dichloro-1,1,3,3-tetraisopropylsiloxane (1.25 g, 4 mmol) dropwise at 0°C. Then the reaction was allowed to warm to room temperature. When the reaction was complete, the reaction mixture was

partitioned between water and ethyl acetate, the aqueous layer was extracted with ethyl acetate. Combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure and the residue was purified by column chromatography (hexanes: ethyl acetate 5:1) to afford **1** (800 mg, 2.05 mmol, 61%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.04 (stack, 28 H), 2.89 (d, *J*= 2.6 Hz, 1H), 3.96 (dd, *J*= 6.3, 12.6 Hz, 1H), 4.13 (dd, *J*= 3.7, 12.5 Hz, 1H), 4.22 (dd, *J*= 2.6, 5.9 Hz, 1H), 4.40 (m, 1H), 4.49 (t, *J*= 6.2 Hz, 1H).

Compound **1** (125 mg, 0.32 mmol) was dissolved in 2 mL of toluene and cooled to -78°C. To this solution was added DIBAL-H (1 M in THF, 2.3 mmol) at -78°C. The reaction mixture was held at -78°C for another hour, then quenched with methanol at -20°C. The mixture was then allowed to warm to room temperature and washed with 0.1 M HCl. The aqueous layer was extracted with ether, combined organic layer was washed with saturated $NaHCO_3$, water, brine and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure, crude product was purified by column chromatograph (hexanes: ethyl acetate 3:1) to afford **2** (123 mg, 0.303 mmol, 95%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.06 (stack, 28 H), 2.11 (d, *J*= 10.2 Hz, 1H), 3.12 (d, *J*= 4.3 Hz, 1H), 3.76 (s, 3H), 3.80 (dd, *J*= 2.1, 11.8 Hz, 1H), 3.85 (t, *J*= 9.6 Hz, 1H), 4.04 (dd, *J* = 1.4, 9.7 Hz, 1H), 4.09 (d, *J*= 11.7 Hz, 1H), 4.51 (d, *J*= 1.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 12.48, 12.52, 13.3, 17.1, 17.18, 17.24, 17.4, 52.4, 62.3, 70.4, 72.9, 73.0, 73.6; NOE identified between H_1 and H_4 in NOESY; HRMS (ESI) calcd for $C_{18}H_{38}O_6Si_2$ 406.2207, found 406.2215.

PCC (330 mg, 1.53 mmol) was suspended in 5 mL of anhydrous CH_2Cl_2, to this solution was added a solution of **2** (400 mg, 1 mmol) in 2 mL of anhydrous CH_2Cl_2, the

reaction was kept at room temperature overnight. The reaction mixture was then filtered through a short pad of silica gel, the filtrate was concentrated and purified by column chromatography (hexanes: ethyl acetate 5:1) to give **3** (280 mg, 0.69 mmol, 69%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.04 (stack, 28 H), 3.75 (s, 3H), 3.89 (d, *J*= 11.9 Hz, 1H), 4.66 (stack, 2H), 4.77 (m, 1H), 4.86 (d, *J*= 2.5 Hz, 1H).

Compound **3** (100 mg, 0.25 mmol) was dissolved in 5 mL of methanol, to this solution was added sodium borohydride (14 mg, 0.37 mmol) in one portion. The reaction was kept at room temperature for 4 hours before solvent was removed in vacuo. The residue was redissolved in ethyl acetate and washed with water. Organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. ^{1}H NMR of **4** is identical to the ^{1}H NMR of **1**. Crude product **4** (92 mg, 0.23 mmol, 92%) was used for the next step without further purification.

Compound **4** (150 mg, 0.37 mmol) was dissolved in 6 mL of dry DMF, to this solution was added acetic acid (110 mg, 1.83 mmol) and tetramethylammonium fluoride (170 mg, 1.83 mmol). After 2 hours' at room temperature, TLC indicated that the reaction was complete, solvent was evaporated in vacuo, the residue was redissolved in ethyl acetate and washed with water. Organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Crude product **5** was used for the next step without further purification. ^{1}H NMR (500 MHz, *d*$_6$-DMSO) δ (ppm): 3.29 (s, 3H), 3.32 (m, 1H), 3.47 (stack, 2H), 3.53 (d, *J*= 3.4 Hz, 1H), 3.64 (stack, 2H), 3.68 (t, *J*= 3.8 Hz, 1H), 4.43 (d, *J*= 2.2 Hz, 1H).

To a solution of **5** (60 mg, 0.37 mmol) in 2 mL of anhydrous pyridine was added acetic anhydride (300 mg, 2.9 mmol) at 0°C. The reaction was then allowed to warm to room

temperature and stir for another hour. The solvent was removed under reduced pressure and crude product **6** was used for the next step without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.98 (s, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 3.38 (s, 3H), 3.73 (dd, *J*= 3.7, 12.5 Hz, 1H), 3.93 (d, *J*= 12.5 Hz, 1H), 4.66 (d, *J*= 2.4 Hz, 1H), 4.97 (s, 1H), 5.10 (s, 1H), 5.31 (s, 1H).

Compound **6** (50 mg, 0.17 mmol) was dissolved in 1 mL of 80% acetic acid, the reaction mixture was kept at 40°C for about 2 hours before solvent was removed in vacuo. The residue was redissolved in dry pyridine and concentrated under reduced pressure. Once again, the residue was dissolved in anhydrous pyridine (1 mL) and cooled to 0°C before acetic anhydride (35 mg, 0.34 mmol) was added. The reaction was then allowed to warm to room temperature and was kept at room temperature for another hour. Solvent was evaporated in vacuo to afford a crude product **7** which has the identical ^{1}HNMR spectrum as the authentic **7** purchased from Aldrich. Crude product was further purified by column chromatography (hexanes: ethyl acetate 8:1) to afford **7** (38 mg, 0.12 mmol, 71%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.01 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 2.15 (s, 3H), 4.14 (dd, *J*= 4.7, 12.5 Hz, 1H), 4.25 (d, *J*= 2.6 Hz, 1H), 4.32 (dd, *J*= 2.5, 12.5 Hz, 1H), 5.32 (stack, 2H), 6.21 (s, 1H).

Compound **8** was synthesized according to the reported procedure.[S4]

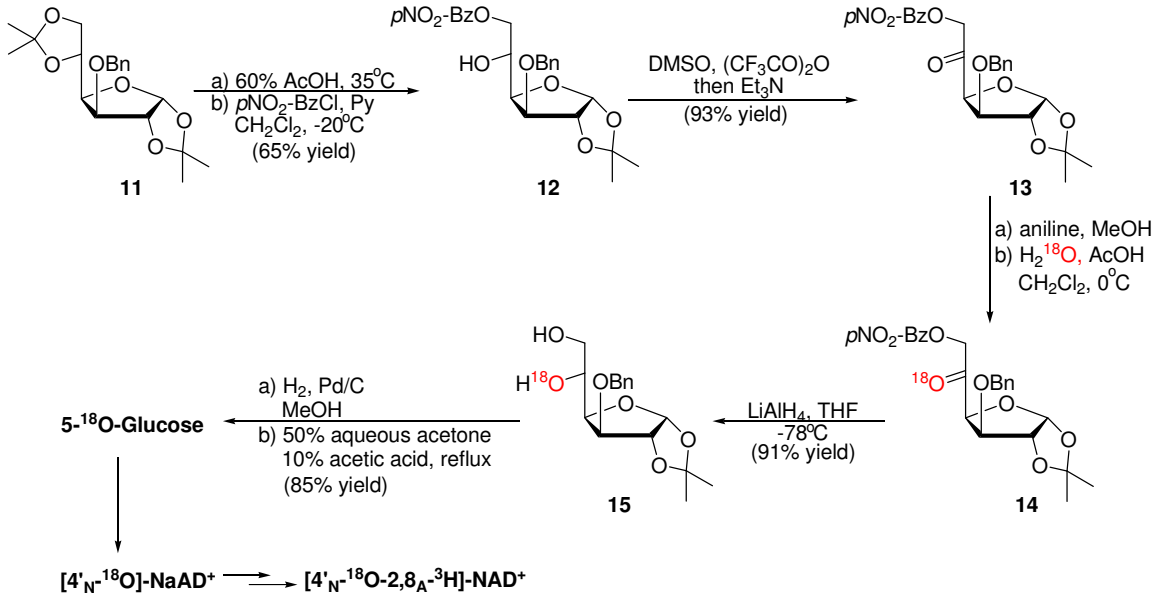
A single reaction (100 μL) containing 100 μM of **8**, 200 μM of ATP, 400 μM of $MgCl_2$, 14.6 μM of human nicotinamide riboside kinase-1, and 100 mM phosphate buffer (pH~7.5) was incubated at 37°C for 2 hours. The reaction was terminated by addition of 8 μL of 10% TFA and purified by HPLC on a Macherey-Nagel Nucleosil 250/4.6 C18

column (solvent was 20 mM ammonium acetate, compound **9** was eluted at a flow rate of 1 mL/min, t_R = 5 min, 85%).

A single reaction (100 μL) containing 150 μM of **9**, 400 μM of ATP, 4 mM of $MgCl_2$, 15 μM of yeast nicotinamidemononucleotide adenylyltransferase, 1 unit of pyrophosphatase, and 100 mM phosphate buffer (pH~7.5) was incubated at 37°C for 1.5 hours. The reaction was terminated by addition of 8 μL of 10% TFA and purified by HPLC on a Macherey-Nagel Nucleosil 250/4.6 C18 column (solvent was 20 mM ammonium acetate, compound **10** was eluted at a flow rate of 1 mL/min, t_R = 18 min, 90%). **10** has the same retention time as the authentic NAD^+ standard, and upon treatment with phosphordiesterase I, **10** can be converted to nicotinamide mononucleotide and adenosine monophosphate.

[$2'_N$-^{3}H]-NAD^+ was synthesized according to the procedures described above. The reduction of compound **3** with sodium borotritide allows the incorporation of ^{3}H-label at 2'-position.

Synthesis of [4'$_N$-^{18}O-2,8$_A$-^{3}H]-NAD$^+$.



Scheme S2

[4'$_N$-^{18}O-2,8$_A$-^{3}H]-NAD$^+$ was synthesized according to **Scheme S2**. Compound **11** was synthesized according to the method described before.[S5] Selective hydrolysis of compound **11** (35 mg, 0.1 mmol) in 2 mL of 60% aqueous acetic acid at 35°C for 5 hours removed the 5,6-isopropylidene group. The reaction mixture was then concentrated to a syrup. This syrup was redissolved in 2 mL of CH_2Cl_2 and 50 μL of anhydrous pyridine. The solution was cooled to -20°C before *p*-nitrobenzoyl chloride (19.4 mg, 0.105 mmol) was added slowly. The reaction was maintained at -20°C until completion. Water was added, the aqueous phase was extracted with CH_2Cl_2, the combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure. Crude product was purified by column chromatography (hexanes: ethyl acetate 3:1) to afford **12** (30 mg, 0.065 mmol, 65%) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.31 (s, 3H), 1.46 (s, 3H), 2.39 (s, broad, 1H), 4.12 (s, 1H), 4.20

(m, 1H), 4.29 (m, 1H), 4.44 (m, 1H), 4.54 (d, *J* = 10 Hz, 1H), 4.64 (stack, 2H), 4.74 (d, *J* = 10 Hz, 1H), 5.94 (s, 1H), 7.34 (stack, 5H), 8.18 (d, *J* = 5 Hz, 2H), 8.24 (d, *J* = 5 Hz, 2H).

To a solution of DMSO (17.7 mg, 0.23 mmol) in 1 mL of CH_2Cl_2 was added trifluoroacetic anhydride (27.3 mg, 0.13 mmol) at -78°C. After 20 minutes, a solution of compound **12** (30 mg, 0.065 mmol) in 1 mL of CH_2Cl_2 was added dropwise to the reaction mixture. The reaction was kept at -78°C for one hour, then triethylamine (26.3 mg, 0.26 mmol) was introduced and the reaction was allowed to warm to room temperature. Water was added, the aqueous phase was extracted with CH_2Cl_2, the combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure. Crude product was purified by column chromatography (hexanes: ethyl acetate 6:1) to afford **13** (28 mg, 0.061 mmol, 93%) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.32 (s, 3H), 1.47 (s, 3H), 4.31 (d, *J* = 3.6 Hz, 1H), 4.58 (stack, 3H), 4.79 (d, *J* = 3.6 Hz, 1H), 5.18 (d, *J* = 18 Hz, 1H), 5.37 (d, *J* = 18 Hz, 1H), 6.08 (d, *J* = 3.5 Hz, 1H), 7.30 (stack, 5H), 8.24 (d, *J* = 8.9 Hz, 2H), 8.29 (d, *J* = 8.9 Hz, 2H).

For ^{18}O incorporation, ketone **13** (5 mg, 0.011 mmol) and aniline (1.3 mg, 0.014 mmol) were dissolved in 0.8 mL of methanol, the reaction was allowed to stir at room temperature for 2 hours, then concentrated under reduced pressure. The resulting viscous oil was taken up in 0.6 mL of anhydrous CH_2Cl_2, to this solution were added ^{18}O–water (Cambridge Isotope Laboratories, Inc, 97%, 20 μL) and acetic acid (1 μL) at 0°C. The reaction was monitored by TLC, upon completion the reaction mixture was concentrated in *vacuo*. The exchange was carried out twice, the ^{1}H NMR spectrum of crude product **14**

matches that of ketone **13**. The crude **14** was used for the next step without further purification.

A suspension of $LiAlH_4$ (1.2 mg, 0.033 mmol) in 1 mL of anhydrous THF was cooled to -78°C, to this mixture was added a solution of **14** (~ 5 mg, 0.011 mmol) in 1 mL of THF. Upon completion, excess $LiAlH_4$ was decomposed by dropwise addition of water at -78°C, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure. Crude product was purified by column chromatography (hexanes: ethyl acetate 3:1) to afford **15** (3.1 mg, 0.01 mmol, 91%) as a colorless oil. The ^{18}O enrichment (95%) was determined from the relative intensities of the ions at *m/z* 333 and 335 (M + Na) in the mass spectrum of **15**. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.31 (s, 3H), 1.47 (s, 3H), 4.15 (m, 1H), 4.24 (stack, 2H), 4.57 (m, 1H), 4.80 (stack, 4H), 5.98 (s, 1H), 8.20 (d, J = 5.2 Hz, 2H), 8.26 (d, J = 5.2 Hz, 2H).

Palladium (10%) on activated charcoal (1 mg, 10 mol%) was placed in a 10 mL round-bottom flask under argon and carefully wetted with 600 μL of methanol. A solution of **15** (3 mg, 0.01 mmol) in 0.5 mL of methanol was added, after the system was purge with hydrogen three times, it was allowed to stir at room temperature until H_2 uptake was complete. The catalyst was filtered over a Celite pad and rinsed with methanol. The filtrate was concentrated under reduce pressure. The residue was redissolved in 1 mL of 50% aqueous acetone with 10% acetic acid and was refluxed overnight. The crude product was recrystallized in warm ethanol. The ^1H NMR spectrum of 5-^{18}O-glucose matches that of the authentic glucose.

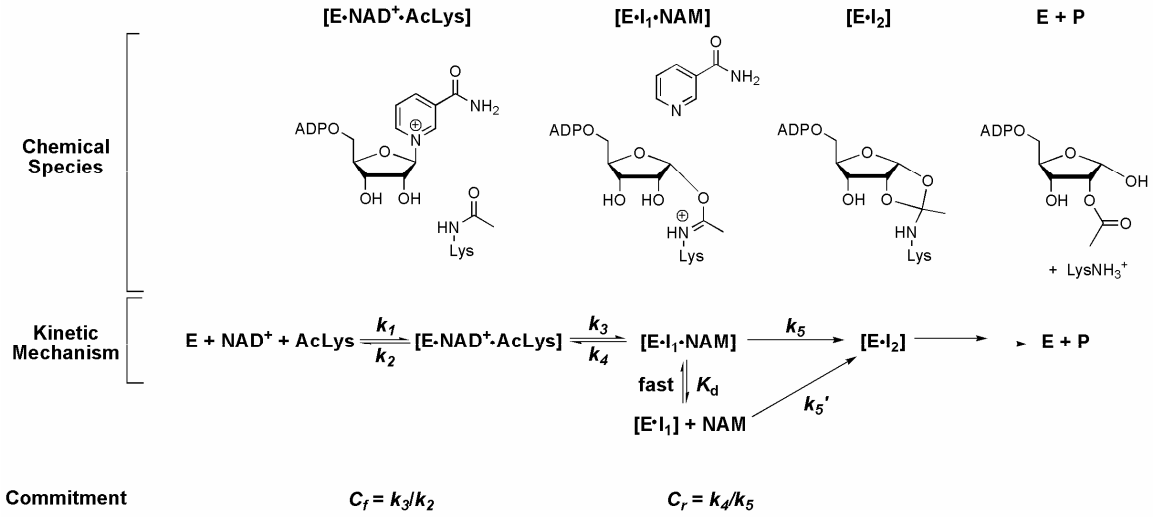
5-^{18}O-glucose was converted to [4'$_N$-^{18}O]-NaAD$^+$ enzymatically as described previously.[S6] [4'$_N$-^{18}O]-NaAD$^+$ was then decomposed to [4'$_N$-^{18}O]-NaMN using phosphordiesterase I. A single reaction (100 μL) containing 200 μM of [4'$_N$-^{18}O]-NaAD$^+$, 0.2 units of phosphordiesterase in 100 mM phosphate buffer (pH~7.5) was incubated at 37°C for 30 minutes. The reaction was terminated by addition of 8 μL of 10% TFA and purified by HPLC on a Vydac reverse phase C18 column (solvent was 20 mM ammonium acetate, NaMN was eluted at a flow rate of 1 mL/min, t_R = 6 min).

The synthesis of [4'$_N$-^{18}O-2,8$_A$-^{3}H]-NAD$^+$ was done in two steps to allow the incorporation of ^{3}H-label from [2,8-^{3}H]-ATP. In the first step, [4'$_N$-^{18}O]-NaMN was coupled with [2,8-^{3}H]-ATP enzymatically as described previously[S6]. The [4'$_N$-^{18}O-2,8$_A$-^{3}H]-NaAD$^+$ was purified and converted to [4'$_N$-^{18}O-2,8$_A$-^{3}H]-NAD$^+$ as described previously.[S6]

Determination of Forward Committment

Briefly, a 180 μL reaction mixture containing 10 μM Af2Sir2, 400 μM NAD^+ (1.5×10^6 cpm [8_A-^{14}C]-NAD^+) in 100 mM phosphate buffer (pH=7.5) and 500 μM of p53A peptide (HLKSKKGQSTSRHK(KAc)LMFK) was incubated at 37 °C for 10 s (0.03 of a catalytic turnover) and then diluted with 270 μL buffered solution containing 16 mM unlabeled NAD^+. Reaction was allowed to continue. Following dilution with unlabeled NAD^+, aliquots (50 μL) from the reaction mixture was removed periodically over the next 300 s and stopped by addition of 8 μL of 10% TFA. Control reactions were performed with the chase solution added before the enzyme, and allowed to proceed for the same length of time. To quantitate enzymatic activity during formation of the Michaelis complex, an 20 μL reaction containing 10 μM of Af2Sir2 and 400 μM of NAD^+ including 1.5×10^5 cpm [8_A-^{14}C]-NAD^+ in 100 mM phosphate buffer (pH = 7.5) was incubated at 37 °C for 10 s, and then stopped by addition of 8 μL of 10% TFA. Samples were then neutralized to pH 7.5 by addition of 3 M of NaOH and 1 M phosphate buffer (pH = 9), to the neutralized sample was added 1 unit of esterase and the sample was allowed to incubate at 37 °C for two hours. The reactions were then stopped by addition of 8 μL of 10% TFA. The product ADP-ribose and remaining NAD^+ were separated on Waters C18-reverse phase column. Fractions of eluent (2 mL) were collected, the radioactivity was determined by scintillation counting.

Derivation of Commitment Equation 4



Scheme S3

In the absence of a forward commitment, where $k_2 >> k_3$ and $C_f = k_3/k_2$, then the equation for KIE that corrects for reverse commitment on the nicotinamide cleavage step is

$$KIE_{obs} = (KIE_{int} + C_r * KIE_{eq})/(1 + C_r)$$ (Equation S1)

which is equation 3 provided in the text, where KIE_{obs} is the observed isotope effect, corrected for depletion, KIE_{int} is the intrinsic isotope effect, KIE_{eq} is the equilibrium isotope effect of $E{\cdot}I_1$ (the imidate complex) with the free enzyme and reactants and C_r is the reverse commitment defined by the rate of the intermediate proceeding forward, before $E{\cdot}I_1{\cdot}$NAM can react backward. However, in a dynamic reaction coordinate, where the $E{\cdot}I_1{\cdot}$NAM complex is in rapid equilibrium with an $E{\cdot}I_1$ complex, there is additional complexity. The reassociation of NAM does not affect the observed isotope effect unless it leads to actual reversal. We treat the equilibration of NAM to the $E{\cdot}I_1$ complex as being governed by a dissociation constant K_d. We have shown by viscosity studies on page S23 that the product release and association of nicotinamide are fast relative to the

base-exchange chemical steps, supporting this assumption. As such the observed commitment is sensitive to the fraction of $E{\cdot}I_1$ that resides in the $E{\cdot}I_1{\cdot}NAM$ complex. i.e.

$C_{r\ (obs)} = k_4[E{\cdot}I_1{\cdot}NAM]/(k_5[E{\cdot}I_1{\cdot}NAM]+k_5'[E{\cdot}I_1])$ (Equation S2)

We have found for sirtuin enzymes that binding occupancy to the NAM site does not generally affect the rate forward i.e. $k_5' \approx k_5$.[S7, S8] We provide additional support for this in demonstrating that isonicotinamide interferes with base exchange but does not affect the deacetylation rate on page S25-S27. Therefore we argue this is a reasonable assumption This allows us to simplify Equation S2 to:

$C_{r\ (obs)} = k_4[E{\cdot}I_1{\cdot}NAM]/k_5([E{\cdot}I_1{\cdot}NAM]+[E{\cdot}I_1])$ (Equation S3)

$[E{\cdot}I_1{\cdot}NAM]/([E{\cdot}I_1{\cdot}NAM]+[E{\cdot}I_1]) = [NAM]/([NAM]+K_d)$

Therefore, $C_{r\ (obs)} = (k_4/k_5)([NAM]/([NAM]+K_d)$ (Equation S4)

Substituting into Equation S1 and simplifying we derive a relation for the KIE_{obs} that takes into account a dependency on NAM and its binding constant to the imidate complex:

$KIE_{obs.} = (KIE_{int.}+(KIE_{eq}*C_r*[NAM])/(K_d+[NAM]))/(1.+ (C_r*[NAM]/(K_d+[NAM])))$

(Equation S5)

This is equation 4 in the text, used to curve fit the KIE dependence with respect to NAM in Figure 1 in the text.

Expression and purification of human nicotinamide riboside kinase-1.

The gene encoding nicotinamide riboside kinase-1 (Nrk1) from human was cloned from human cDNA using PCR. The coding sequence was initially cloned into a pSTblue vector and subsequently recloned into Pet28a to obtain protein expressed with an N-terminal poly-histidine tag. The insert was verified by nucleotide sequencing and checked against published sequence. PetNrk1 vector was transfected into Codon-plus® RIPL cells (Stratagene) and protein synthesis was induced by addition of 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at OD_{600} = 0.7. Cells were grown for 4 hours at 37°C, pelleted and lysed by freeze-thaw cycles. The protein was purified by Ni-affinity chromatography, aliquoted in 20% glycerol and 2 mM DTT and stored at -80°C.

Table S1: Mulliken charges acetyllysine ribosyl nicotinamide (2.65 Å distances LG, NU)

Atom	Reactant state Mulliken Atomic Charge	Transition state Mulliken Atomic Charge	Δ Mulliken Atomic Charge
C_N1'	0.321778	0.378176	0.056398
C_N2'	0.130053	0.11578	-0.01427
C_N3'	0.112205	0.123465	0.01126
C_N4'	0.174501	0.104084	-0.07042
C_N5'	0.036563	0.043055	0.006492
O_N4'	-0.52495	-0.35631	0.168646
H_N1'	0.175982	0.193482	0.0175
H_N2'	0.113839	0.199141	0.085302
H_N3'	0.1544	0.168872	0.01447
H_N4'	0.1544	0.171806	0.017406
H_N5'	0.131057	0.1394	0.008343
H_N5'	0.13304	0.128016	-0.00502
2'-hydroxy O	-0.54088	-0.53389	0.006993
3'-hydroxy O	-0.54678	-0.5794	-0.03262
5'-hydroxy O	-0.55552	-0.53396	0.021555
2'-hydroxy H	0.339536	0.327936	-0.0116
3'-hydroxy H	0.338192	0.354639	0.016447
5'-hydroxy H	0.335958	0.33757	0.001612
Ribose group	0.483374	0.781871	0.298496
N_N1	-0.484315	-0.523213	-0.0389
C-2	0.189836	0.090692	-0.09914
C-3	-0.036374	0.008211	0.044585
C-4	-0.032822	-0.044399	-0.01158
C-5	-0.141773	-0.119746	0.022027
C-6	0.191557	0.156716	-0.03484
H-2	0.215060	0.114556	-0.1005
H-4	0.155510	0.159743	0.004233
H-5	0.166172	0.124899	-0.04127
H-6	0.218193	0.102271	-0.11592
carbonyl C	0.600200	0.556012	-0.04419
carbonyl O	-0.479354	-0.482584	-0.00323
Amide N	-0.621499	-0.620947	0.000552
Amide H	0.314688	0.296617	-0.01807
Amide H'	0.286162	0.270266	-0.0159
NAM group	0.541241	0.089094	-0.45214
Nu-O (carbonyl)	-0.517657	-0.598974	-0.08132
Nu-C (carbonyl)	0.576935	0.597145	0.02021
N	-0.528389	-0.495663	0.032726
Amide H	0.254195	0.294008	0.039813
Methyl C	-0.381733	-0.398059	-0.01633
Methyl H	0.091345	0.163401	0.072056
Methyl H'	0.143878	0.138176	-0.0057
Methyl H''	0.143875	0.160937	0.017062

N-methyl C	-0.169220	-0.179640	-0.01042
N-methyl H	0.112380	0.148202	0.035822
N-methyl H'	0.112380	0.151513	0.039133
N-methyl H''	0.162011	0.147846	-0.01417
N-methyl acetamide group	0	0.128892	0.128882

Table S2: Mulliken charges trifluoro-acetyllysine ribosyl nicotinamide (2.65 Å distances LG, NU)

	Reactant state	"Dissociated Structure"	
Atom	**Mulliken Atomic Charge**	**Mulliken Atomic Charge**	**Δ Mulliken Atomic Charge**
C_N1'	0.321778	0.373174	0.051396
C_N2'	0.130053	0.116276	-0.01378
C_N3'	0.112205	0.119982	0.007777
C_N4'	0.174501	0.102921	-0.07158
C_N5'	0.036563	0.043496	0.006933
O_N4'	-0.52495	-0.354695	0.170255
H_N1'	0.175982	0.199311	0.023329
H_N2'	0.113839	0.199086	0.085247
H_N3'	0.1544	0.173905	0.019505
H_N4'	0.1544	0.171973	0.017573
H_N5'	0.131057	0.139937	0.00888
H_N5'	0.13304	0.130443	-0.0026
2'-hydroxy O	-0.54088	-0.536609	0.004271
3'-hydroxy O	-0.54678	-0.567846	-0.02107
5'-hydroxy O	-0.55552	-0.533878	0.021642
2'-hydroxy H	0.339536	0.328620	-0.01092
3'-hydroxy H	0.338192	0.342912	0.00472
5'-hydroxy H	0.335958	0.338308	0.00235
Ribose group	0.483374	0.78732	0.303942
N_N1	-0.484315	-0.524026	-0.03971
C-2	0.189836	0.091608	-0.09823
C-3	-0.036374	0.008084	0.044458
C-4	-0.032822	-0.043925	-0.0111
C-5	-0.141773	-0.119768	0.022005
C-6	0.191557	0.157437	-0.03412
H-2	0.215060	0.114862	-0.1002
H-4	0.155510	0.160019	0.004509
H-5	0.166172	0.125266	-0.04091
H-6	0.218193	0.102036	-0.11616
carbonyl C	0.600200	0.555099	-0.0451
carbonyl O	-0.479354	-0.481885	-0.00253
Amide N	-0.621499	-0.620882	0.000617
Amide H	0.314688	0.296836	-0.01785
Amide H'	0.286162	0.271063	-0.0151
NAM group	0.541241	0.09182	-0.44942
Nu-O (carbonyl)	-0.499185	-0.578826	-0.07964
Nu-C (carbonyl)	0.492976	0.543663	0.050687
N	-0.533963	-0.505391	0.028572
Amide H	0.279780	0.314264	0.034484
CF3 C	0.803976	0.795506	-0.00847
CF3 F	-0.287723	-0.243745	0.043978
CF3 F'	-0.252137	-0.254832	-0.0027

CF3 F''	-0.252155	-0.244954	0.007201
N-methyl C	-0.174075	-0.183776	-0.0097
N-methyl H	0.128122	0.159882	0.03176
N-methyl H'	0.128120	0.159323	0.031203
N-methyl H''	0.166265	0.159744	-0.00652
N-methyl acetamide group	0	0.120858	0.120857

Table S3: Mulliken charges acetyllysine ribosyl nicotinamide (3.55 Å distances LG, 3.48 Å distances NU)

	Reactant state	Transition state	
Atom	**Mulliken Atomic Charge**	**Mulliken Atomic Charge**	**Δ Mulliken Atomic Charge**
C_N1'	0.321778	0.371814	0.050036
C_N2'	0.130053	0.084183	-0.04587
C_N3'	0.112205	0.130048	0.017843
C_N4'	0.174501	0.081612	-0.09289
C_N5'	0.036563	0.056470	0.019907
O_N4'	-0.52495	-0.329585	0.195365
H_N1'	0.175982	0.220804	0.044822
H_N2'	0.113839	0.227337	0.113498
H_N3'	0.1544	0.176550	0.02215
H_N4'	0.1544	0.186863	0.032463
H_N5'	0.131057	0.143928	0.012871
H_N5'	0.13304	0.138427	0.005387
2'-hydroxy O	-0.54088	-0.523527	0.017353
3'-hydroxy O	-0.54678	-0.582949	-0.03617
5'-hydroxy O	-0.55552	-0.533544	0.021976
2'-hydroxy H	0.339536	0.335894	-0.00364
3'-hydroxy H	0.338192	0.359724	0.021532
5'-hydroxy H	0.335958	0.341482	0.005524
Ribose group	0.483374	0.88553	0.402157
N_N1	-0.484315	-0.490613	-0.0063
C-2	0.189836	0.075344	-0.11449
C-3	-0.036374	0.019090	0.055464
C-4	-0.032822	-0.052358	-0.01954
C-5	-0.141773	-0.105256	0.036517
C-6	0.191557	0.102522	-0.08904
H-2	0.215060	0.103696	-0.11136
H-4	0.155510	0.154248	-0.00126
H-5	0.166172	0.118764	-0.04741
H-6	0.218193	0.088238	-0.12996
carbonyl C	0.600200	0.550699	-0.0495
carbonyl O	-0.479354	-0.486182	-0.00683
Amide N	-0.621499	-0.620640	0.000859
Amide H	0.314688	0.293523	-0.02117
Amide H'	0.286162	0.269616	-0.01655
NAM group	0.541241	0.02069	-0.52055
Nu-O (carbonyl)	-0.517657	-0.595081	-0.07742
Nu-C (carbonyl)	0.576935	0.592026	0.015091
N	-0.528389	-0.499082	0.029307
Amide H	0.254195	0.288251	0.034056
Methyl C	-0.381733	-0.399344	-0.01761
Methyl H	0.091345	0.153369	0.062024
Methyl H'	0.143878	0.148557	0.004679
Methyl H''	0.143875	0.153262	0.009387

N-methyl C	-0.169220	-0.172537	-0.00332
N-methyl H	0.112380	0.139234	0.026854
N-methyl H'	0.112380	0.145567	0.033187
N-methyl H''	0.162011	0.139560	-0.02245
N-methyl acetamide group	0	0.09378	0.093782

Table S4: Experimental and intrinsic KIEs for AfSir2 catalyzed deacetylation

label of interest	KIE type	experimental KIE[a,b]	intrinsic KIE[c]	intrinsic KIE[d]
1_N-^{15}N	primary	1.024(2)	1.024(2)[e]	1.024(2)[e]
$1'_N$-^{14}C	primary	1.014(4)	1.014(4)	1.011(4)
$1'_N$-^{3}H	α-secondary	1.289(1)	1.300(1)	1.296(1)
$2'_N$-^{3}H	β-secondary	1.095(5)	1.099(5)	1.095(5)
$4'_N$-^{3}H	γ-secondary	0.997(2)	0.997(2)	0.993(2)
$5'_N$-^{3}H	δ-secondary	1.019(5)	1.020(5)	1.016(5)
$4'_N$-^{18}O	α-secondary	0.985(5)	0.984(5)	0.981(5)
$2,8_A$-^{3}H	remote	1.001(4)	1.001(4)	1.001(4)

[a]KIE determined from at least three experiments and from correction for isotopic depletion. [b]The number in the parenthesis represents the error in the last digit. [c]KIE determined from experimental KIE and corrected for commitment factors assuming C_r = 0.038 and KIE_{eq} = 1. [d]KIE determined from experimental KIE and corrected for commitment factors assuming C_r = 0.038 and KIE_{eq} = 1.1. [e] KIE determined from experimental KIE and corrected for commitment factors assuming C_r = 0.038 and KIE_{eq} = 1.027.

The effect of forward and reverse commitment factors govern observed KIEs and are predicted by the Northrop's equation (1):

$$KIE_{obs} = (KIE_{int} + C_f + C_r*KIE_{eq})/(1 + C_f + C_r) \quad (1)$$

Where KIE_{obs} is the observed KIE, KIE_{int} is the intrinsic KIE, C_f and C_r are the forward and reverse commitment factors, respectively, and KIE_{eq} is the equilibrium KIE. Because the forward commitment factor is negligible, equation (1) is reduced to equation (2):

$$KIE_{obs} = (KIE_{int} + C_r*KIE_{eq})/(1 + C_r) \quad (2)$$

In the case of AfSir2 catalyzed reactions, the reverse commitment was determined to be 0.038, the observed KIEs are corrected for the reverse commitment according to equation (3):

$$KIE_{int} = KIE_{obs}(1 + C_r) - C_r*KIE_{eq} \quad (3)$$

The intrinsic KIEs assuming KIE_{eq} = 1 or 1.1 are listed in Table S4.

Viscosity Study to Establish the Fast Equilibration of Nicotinamide During Steady State Base Exchange

Reactions containing 400 μM of NAD^+, 250 μM of p53A with 5 mM of nicotinamide including 50,000 cpm of [carbonyl-^{14}C]-nicotinamide in 100 mM phosphate buffer with 0, 5, 10, 17.5, 25 and 35% w/v sucrose were initiated by addition of Af2Sir2 enzyme to a concentration of 4.1 μM. Reactions were incubated for one and half hours at 37 °C and quenched by addition of 10% TFA to pH 2. After centrifugation to remove precipitates, reactions were injected on HPLC (0.1% TFA eluent) to separate nicotinamide and NAD^+. Fractions containing nicotinamide and NAD^+ were collected and radioactivity was determined by scintillation counting. Rates were determined as cpm/s incorporated into NAD^+, and then converted to a turnover rate (1/s) by adjustment for specific radioactivity of nicotinamide and enzyme concentration. The ratio of $k_{B.E.}$ measured at 0% sucrose to the $k_{B.E.}$ observed at each concentration of sucrose was plotted against the relative viscosities of the corresponding sucrose solution. The relative viscosities of each sucrose percentage were taken from those measured by Murray et al.[S9]

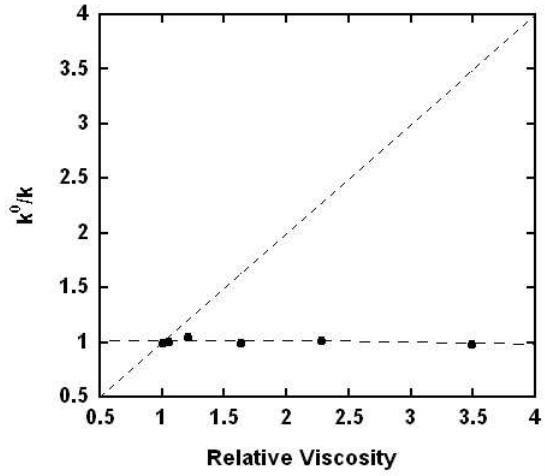


Figure S1: Viscosity Effects on AfSir2 catalyzed base-exchange

We believe there is a fast equilibrium between [E·I_1·NAM] and [E·I_1] + NAM, to test this hypothesis, solvent viscosity studies were carried out. A slope equals to unity in a plot of the inverse of the relative rate constant (k^0/k) versus the relative viscosity of the solution reflects product release as rate limiting step, whereas a slope of zero reflects a non-diffusion mediated step. Indeed, using sucrose as a viscogen, we found that the $k_{B.E.}$ displays no viscosity dependence (Figure S1).

Demonstration of Selective Isonicotinamide Inhibition of Base Exchange and with no inhibition of Deacetylation

It has been known that AfSir2 catalyzes both base-exchange and deacetylation from a common intermediate, $[E{\cdot}I_1{\cdot}NAM]$ or $[E{\cdot}I_1]$ + NAM, even at saturating nicotinamide concentration.[S7] Ratio of base-exhange to deacetylation can be altered by increasing isonicotinamide- a nicotinamide antagonist-concentration. We have found that 20 mM isonicotinamide concentration led to a 18% reduction of base-exchange versus control, but only a 0% reduction in the level of deacetylation (Figures S2 and S3).

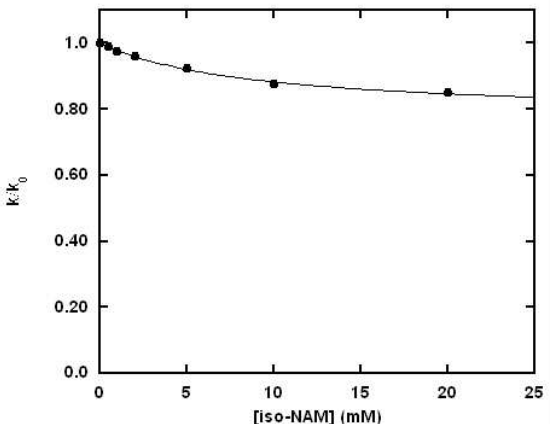


Figure S2: Iso-NAM Effect on AfSir2 Catalyzed Base-Exchange

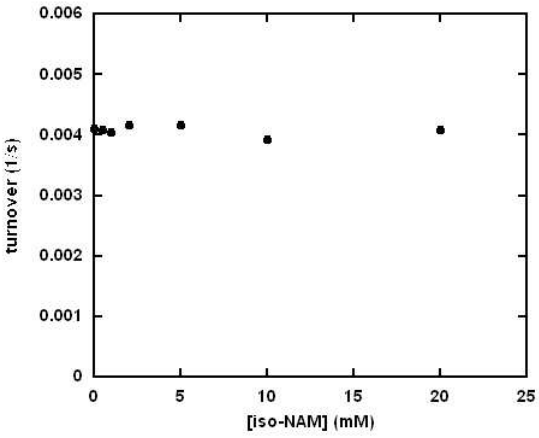


Figure S3: Iso-NAM Effect on AfSir2 Catalyzed Deacetylation

Experimental

Inhibition of Base Exchange by Isonicotinamide Isonicotinamide was added to reaction mixtures in concentrations of 0, 0.5, 1.0, 2.5, 5, 10 and to 20 mM to solutions containing 400 μM of NAD^+, 250 μM of p53A with 5 mM of nicotinamide including 50,000 cpm of [carbonyl-^{14}C]-nicotinamide in 100 mM phosphate buffer. Reactions were initiated by addition of Af2Sir2 enzyme to a concentration of 4.1 μM. Reactions were incubated for one and half hours at 37 °C and quenched by addition of 10% TFA to pH 2. After centrifugation to remove precipitates, reactions were injected on HPLC (0.1% TFA eluent) to separate nicotinamide and NAD^+. Fractions containing nicotinamide and NAD^+ were collected and radioactivity was determined by scintillation counting. Rates were determined as cpm/s incorporated into NAD^+, and then converted to a turnover rate (1/s) by adjustment for specific radioactivity of nicotinamide and enzyme concentration. The ratio of $k_{B.E.}$ measured at each isonicotinamide concentration was divided by the $k_{B.E.}$ observed at 0 isonicotinamide concentration. The points were plotted against the isonicotinamide concentration and fit to the curve for competitive inhibition with a K_m for nicotinamide of 1.13 mM, as described in the text.

Evaluation of Effect of Isonicotinamide on Deacetylation

Similar to above, isonicotinamide was added to reaction mixtures in concentrations of 0, 0.5, 1.0, 2.5, 5, 10 and to 20 mM to solutions containing 400 μM of NAD^+, 250 μM of p53A containing 25,000 cpm of [2,8$_A$-^{3}H]-NAD^+ in 100 mM phosphate buffer. Reactions were initiated by addition of Af2Sir2 enzyme to a concentration of 4.1 μM. Reactions were incubated for one and half hours at 37 °C and quenched by addition of 10% TFA to pH 2. After centrifugation to remove precipitates, reactions were loaded

onto 1 mL DEAE columns preequilibrated with acetic acid and neutralized by washing with 5 mM ammonium acetate until pH = 7. Columns were eluted sequentially with 7 × 2 mL volumes of 20 mM ammonium acetate pH 7, and then eluted with 3 × 2 mL of 250 mM ammonium acetate pH 7. The last three volumes contain [2,8_A-^{3}H]-AADPR, and are counted by scintillation counting. Rates were determined as cpm/s incorporated into NAD^+, and then converted to a turnover rate (1/s) by adjustment for specific radioactivity of nicotinamide and enzyme concentration and plotted.

Table S5: Intrinsic KIEs, calculated KIEs and calculated EIEs for AfSir2 catalyzed deacetylation

label of interest	intrinsic KIE[a]	Calculated KIEs (NR model)[b]	Calculated KIEs (NMN model)[c]	Calculated EIEs[d]
1_N-^{15}N	1.024(2)	1.025	1.025	
$1'_N$-^{14}C	1.014(4)	1.013	1.010	1.006
$1'_N$-^{3}H	1.300(1)	1.424	1.410	1.069
$2'_N$-^{3}H	1.099(5)	1.0998	1.098	0.969
$4'_N$-^{3}H	0.997(2)	1.003	1.003	1.030
$5'_N$-^{3}H	1.020(5)	1.035	1.038	1.03
$4'_N$-^{18}O	0.984(5)	0.993	0.995	1.007

[a]KIE determined from experimental KIE and corrected for commitment factors assuming $C_r = 0.038$ and $KIE_{eq} = 1$. [b]KIEs calculated using nicotinamide riboside (NR) as the cutoff model, the distance between leaving group and C1', incoming nucleophile and C1' are both 2.65 Å. [c]KIEs calculated using nicotinamide mononucleotide (NMN) as the cutoff model, the distance between leaving group and C1', incoming nucleophile and C1' are both 2.65 Å. . [d]EIEs calculated using nicotinamide riboside (NR) as the cutoff model, the distance between leaving group and C1', incoming nucleophile and C1' are both 2.65 Å.

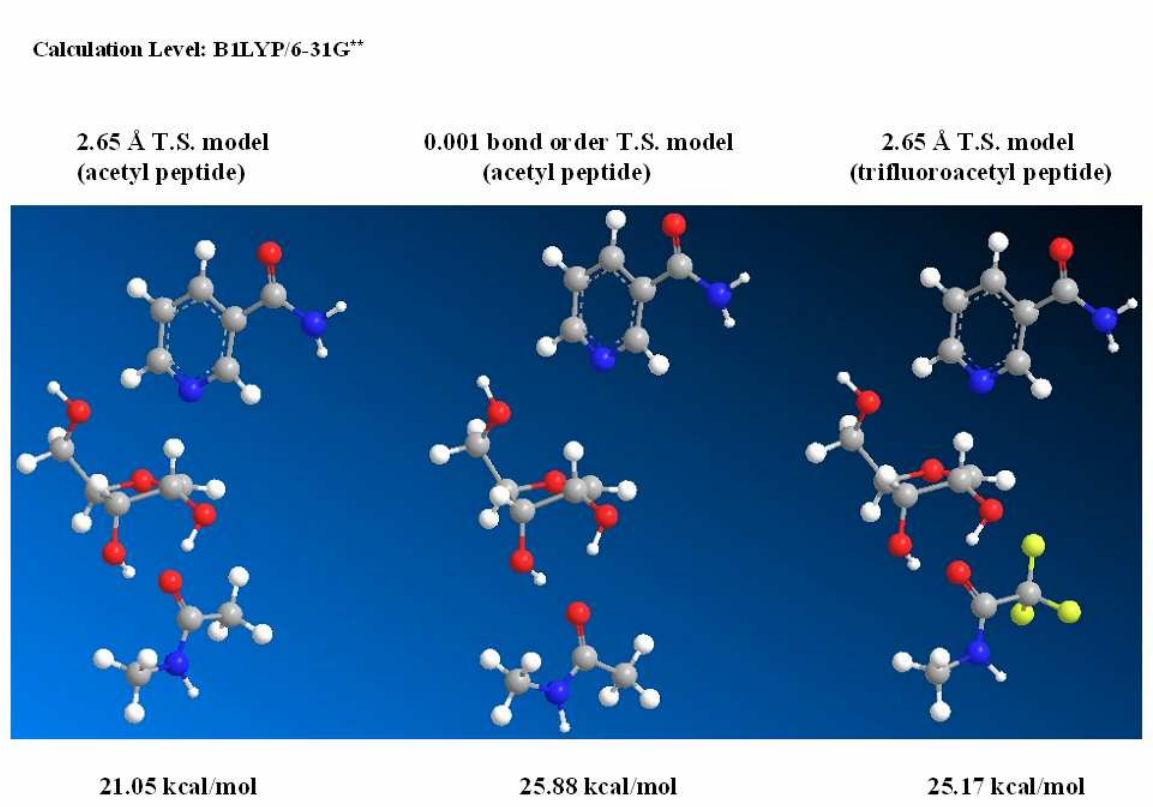


Figure S4: Energies and structures of optimal, fully dissociated, and trifluoroacetyl putative transition state structures.

Thermochemical calculations of energies of complexes as described in the experimental in text.

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