SUPPORTING INFORMATION

Two-Step Synthesis of Novel, Bioactive Derivatives of the Ubiquitous Cofactor Nicotinamide Adenine Dinucleotide (NAD)

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(i) .....Chromatography conditions

(ii) .....Preparation of adenosine and AMP derivatives **4a**, **4b**, **5a** & **5b**

(iii) .....Stability testing of 8-Bromo NAD **2**

(iv) .....NMR spectra of NAD derivatives **3a-g**

(v) .....Additional figures:

Figure S1: Representative chromatogram for the one-pot synthesis of **3a**

Figure S2: Sequence alignment of four human sirtuins

Figure S3: Superimposition of SIRT2–**3b** and SIRT3–ADPR

Figure S4: Superimposition of SIRT2–**3b** and Sir2-Af2–NAD

Figure S5: Molecular interactions SIRT2–**3b**

Figure S6: Molecular interactions SIRT3–NAD

(vi) .....Docking scores

(i) CHROMATOGRAPHY CONDITIONS

Preparative chromatography was performed on a Biologic LP chromatography system equipped with a peristaltic pump and a 254nm UV Optics Module under the following conditions:

Purification method 1 – Ion-pair chromatography was performed using Lichroprep RP-18 as the stationary phase. The resin was equilibrated with 0.05 M TEAB (triethylammonium bicarbonate), and the sample solution, in a small volume of TEAB, was applied with a Pasteur pipette. Purification of the sample was carried out with a gradient of 0–10 % acetonitrile (or methanol) against 0.05 M TEAB, over a total volume of 480 mL (flow rate: 5 mL/min). Product-containing fractions were combined and reduced to dryness. The solid residue was co-evaporated repeatedly with methanol to remove residual TEAB.

Purification method 2 – Ion-pair chromatography was performed using Lichroprep RP-18 as the stationary phase. The resin was equilibrated with 0.05 M TEAB (triethylammonium bicarbonate), and the sample solution, in a small volume of TEAB, was applied with a Pasteur pipette. Purification of the sample was carried out with a gradient of 0–25 % acetonitrile (or methanol) against 0.05 M TEAB, over a total volume of 480 mL (flow rate: 5 mL/min). Product-containing fractions were combined and reduced to dryness. The solid residue was co-evaporated repeatedly with methanol to remove residual TEAB.

Purification method 3 – Anion-exchange chromatography was performed using a MacroPrep 25Q resin as the stationary phase. The resin was washed rigorously with MilliQ H_2O and loaded with a dilute solution of the sample ($\Omega < 200$ μSi). Purification of the sample was carried out with a gradient of 0–100 % TEAB (1M, pH 7.3) against H_2O, over a total volume of 480 mL (flow rate: 5 mL/min). Product-containing fractions were combined and reduced to dryness. The solid residue was co-evaporated repeatedly with methanol to remove residual TEAB.

Analytical chromatography (HPLC) was carried out on an Agilent 1200 machine equipped with a Supelcosil LC-18T column (25cm ×4.6mm, particle size 5μm), a diode array detector (detection wavelengths: 254 and 280 nm) and a column oven (temperature: 25 ºC). Samples (injection volume: 20 μL) were analysed using a mobile phase composed of phosphate buffer (0.05M, pH 8) and methanol according to the following gradient steps (flow rate: 1.5 mL/min):

0 to 2 min	10% methanol, 90% phosphate buffer
2 to 17 min	10-50% methanol
17 to 19 min	50% methanol
19 to 21 min	50-10% methanol
21 to 25 min	10% methanol.

(ii) PREPARATION OF ADENOSINE AND AMP DERIVATIVES **4** & **5**

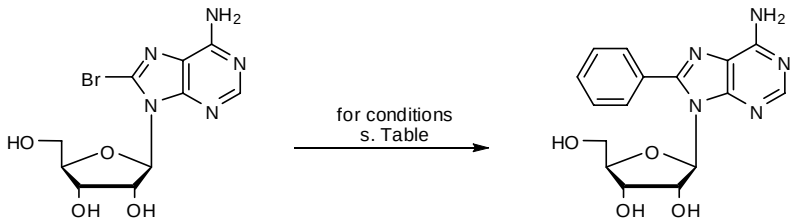
8-Bromoadenosine (4a). Saturated aqueous bromine (75 mL) was added to a solution of adenosine (2.00 g, 7.48 mmol) in sodium acetate buffer (0.5M, 45 ml, pH 4.0). The reaction was stirred at room temperature for 28 hours, when TLC indicated completion. The reaction was decolourised with sodium thiosulfate solution (5 M) and the pH was adjusted to 7 with aqueous NaOH (5M). The precipitate was collected by filtration and dried in the oven to give the title compound as a solid in 80% yield (2.09 g, 6.04 mmol). HPLC: 12.67 mins (98 %). δ_H (300 MHz, DMSO-d_6) 8.13 (s, 1H, H-2), 7.66 (br s, 2H, NH_2), 5.84 (d, 1H, J = 5.4, H-1'), 5.42 (br s, 3H, OH-5', OH-2', OH-3'), 5.09 (m, 1H, H-2'), 4.19 (m, 1H, H-3'), 3.98 (m, 1H, H-4'), 3.68 (d, 1H, J = 12.2, H-5'), 3.52 (d, 1 H, J = 9.1, H-5'); δ_C (100 MHz, DMSO-d_6) 155.7 (C-6), 153.0 (C-2), 150.5 (C-4), 127.9 (C-8), 120.3 (C-5), 91.0 (C-1'), 87.4 (C-4'), 71.8 (C-2'), 71.5 (C-3'), 62.8 (C-5'). *m/z* (ESI) 346.0145, [M+H], $C_{10}H_{13}{}^{79}BrN_5O_4$ requires 346.0145.

8-Bromo AMP (5a). The title compound was prepared from **4a** (200.2 mg, 0.58 mmol), proton sponge (727.7 mg, 3.40 mmol) and $POCl_3$ (205 µl, 2.26 mmol) in triethylphosphate (12 ml). The reaction was quenched with TEAB buffer (0.2M, 120 mL). The aqueous solution was washed with ethyl acetate (80 mL) and purified by ion-pair chromatography. The triethylammonium salt of the title compound was obtained in 61% yield (150.1 mg, 0.35 mmol, 1.4 equiv. of TEA as determined by NMR). HPLC: 4.92 mins (97 %). δ_H (300 MHz, D_2O) 7.89 (s, 1H, H-2), 5.90 (d, 1H, J = 5.9, H-1'), 5.09 (m, 1H, H-2'), 4.47 (m, 1H, H-3'), 4.07 (m, 3H, H-4',H_2-5'), 3.03 (m, 11H, CH_2 TEA), 1.12 (m, 16H, CH_3 TEA); δ_C (100 MHz, D_2O) 154.6 (C-6), 153.7 (C-2), 151.0 (C-4), 129.0 (C-8), 119.7 (C-5), 90.5 (C-1'), 84.6 (C-4'), 71.9 (C-2'), 70.7 (C-3'), 65.4 (C-5'), 47.5 (CH_2 TEA), 9.1 (CH_3 TEA); δ_P (121.5 MHz, D_2O) 7.4. *m/z* (ESI) 423.9663 [monoanion], $C_{10}H_{12}{}^{79}BrN_5O_7P$ requires 423.9663.

8-Phenyladenosine (4b). The title compound was prepared from **4a** (1 equiv.), phenylboronic acid (1.2 equiv.), Na_2PdCl_4 (see Table 1), TPPTS or TXPTS (see Table 1), and K_2CO_3 (1.5 equiv.). A two-necked flask was charged with the reagents and purged with nitrogen. Degassed water (6 or 3 mL) was added *via* a syringe, and the reaction was stirred at the appropriate temperature under N_2 for the given time (see Table 1). Upon completion of the reaction, the aqueous reaction was cooled down to room temperature, the pH was adjusted to pH 7 with 1 % aq. HCl, and the solvent was removed *in vacuo*. The residue was dissolved in MeOH and purified by column chromatography (SiO_2, CH_2Cl_2:MeOH, 8:1). The material was re-crystallised from MeOH to give 8-phenyladenosine as colourless crystals. HPLC: 18.20 mins (99%). δ_H (300 MHz, DMSO-d_6) 3.64 (m, 2H, H-5'), 3.94 (1H, m, H-4'), 4.17 (1H, m, H-3'), 5.19 (2H, m, H-2', OH-3'), 5.50 (1H, d, J = 8.4 Hz, OH-2'), 5.76 (1H, d, J = 9.6 Hz, H-1'), 5.84 (1H, dd, J = 4.4 and 4.8 Hz, OH-5'), 7.57 (5H, m, Ph), 7.76 (2H, m, Ph), 8.16 (1H, s, H-2); δ_C (100 MHz, DMSO-d_6) 63.0 (C-5'), 71.8 (C-3'), 71.9 (C-2'), 87.4 (C-4'), 89.8 (C-1'), 119.8 (C-5), 129.4 (C-4_{Ph}), 130.1 (C-$2_{Ph,}$ 6_{Ph}), 130.3 (C-3_{Ph}, 5_{Ph}), 130.8 (C-1_{Ph}), 150.5 (C-8), 151.6 (C-4), 152.7 (C-2), 156.9 (C-6). *m/z* (ESI) 344.1356 $[M+H]^+$, $C_{16}H_{18}N_5O_4$ requires 344.1353.

8-Phenyl AMP (5b). The title compound was prepared from **5a** (67 mg, 0.12 mmol), Na_2PdCl_4, TPPTS, and phenyl boronic acid in water at 80 °C. The reaction product was purified by ion-pair chromatography to give the triethylammonium salt of the title compound in 66% yield (43.0 mg, 0.08 mmol, 1.3 equiv. of TEA as determined by NMR). HPLC: 11.22 mins (96 %). δ_H (400 MHz, D_2O) 8.08 (s, 1H, H-2), 7.43 (m, 5H, Ph), 5.77 (d, 1H, J = 5.6, H-1'), 5.07 (m, 1H, H-2'), 4.32 (m, 1H, H-3'), 4.11 (m, 3H, H-4', H_2-5'), 2.92 (q, 8H, J = 7.4, CH_2 TEA), 1.18 (t, 18H, J = 7.4, CH_3 TEA); δ_C (100 MHz, D_2O) 155.0 (C-6), 153.8 (C-2), 152.3 (C-4), 150.6 (C-8), 131.8 (C-1_{Ph}), 130.0 (C-3_{Ph},5_{Ph}), 129.7 (C-2_{Ph},6_{Ph}), 128.1 (C-4_{Ph}), 119.0 (C-5), 89.8 (C-1'), 84.0 (C-4'), 71.1 (C-2'), 70.5 (C-3'), 65.5 (C-5'), 47.5 (TEA CH_2), 9.07 (TEA CH_3); δ_P (121.5 MHz, D_2O) 10.23. *m/z* (ESI) 422.0869 [monoanion], $C_{16}H_{17}N_5O_7P$ requires 422.0871.

Table 1. Optimisation of cross-coupling conditions.



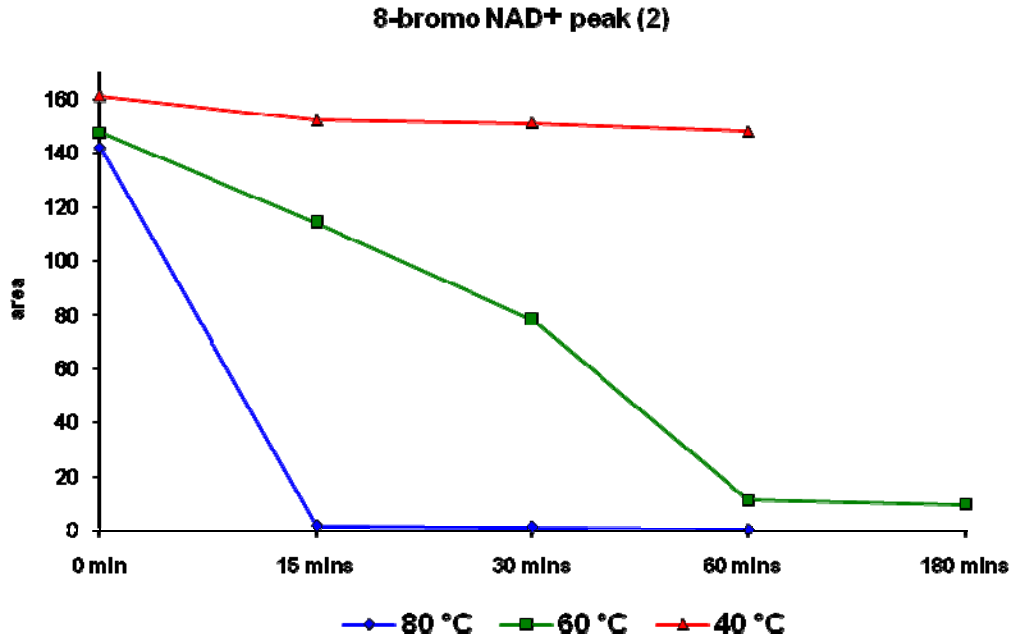
8-bromoadenosine 8-phenyladenosine

Ligand (2.5 equiv. to Pd)	**Na_2PdCl_4** (mol%)	**Temp.** (°C)	**Time** (h)	**8-Br adenosine** (mg)	**8-Ph adenosine** (mg)	**Yield** (%)[a]
TPPTS	2.5	80	1	100	71	72
TPPTS	10	40	4	50	32	64
TXPTS	2.5	80	0.5	100	76	76
TXPTS	10	40	0.5	50	20	41

[a]Isolated yield

(iii) STABILITY TESTING OF 8-BROMO NAD (**2**)

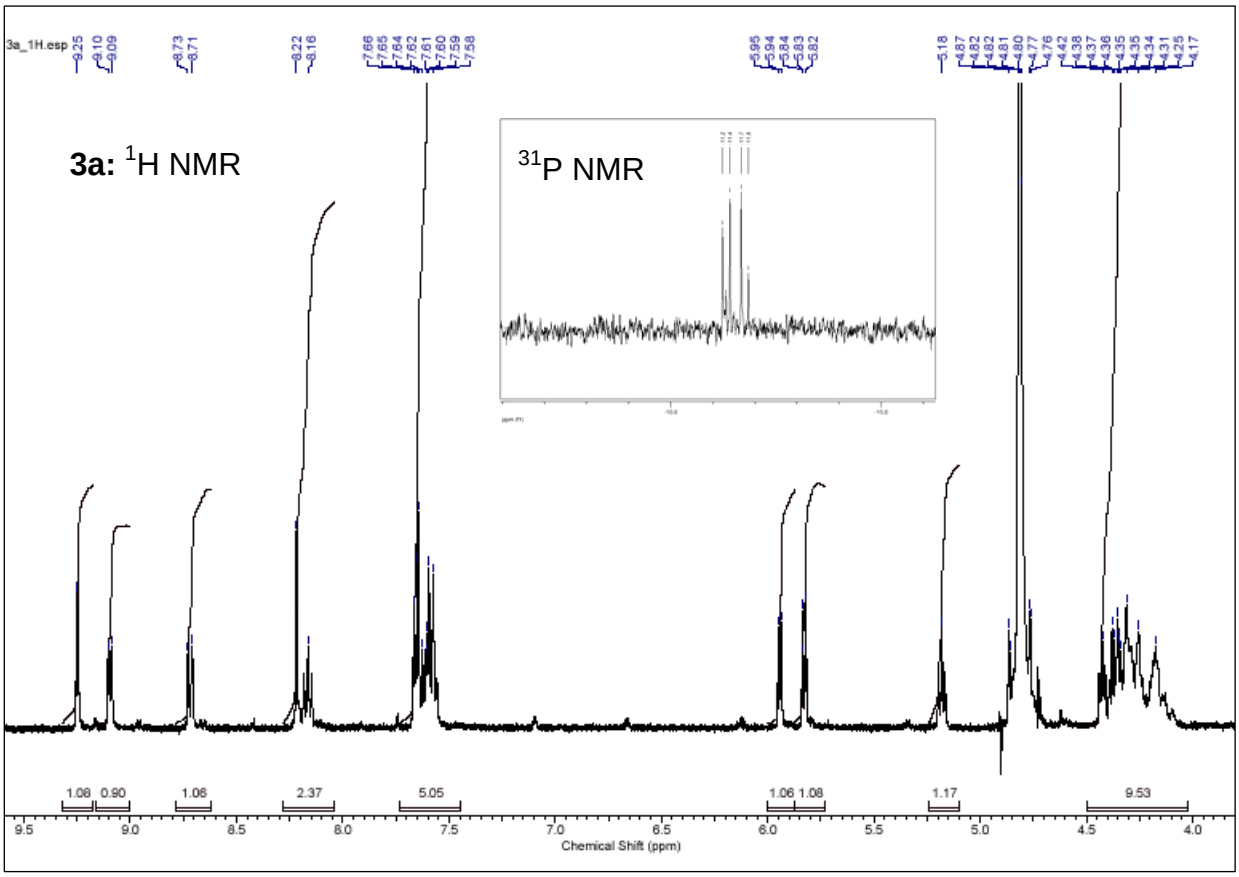
K_2CO_3 (1.2 mg) was added to individual aliquots (1 mL) of a stock solution of 2 (18 mg) in MilliQ water (9 mL). Individual samples were heated at 80°C, 60°C, and 40°C respectively. The relative concentration of **2** in each sample was monitored by HPLC (see below for conditions). Samples were taken prior to heating (0 min), and at 15, 30 and 60 mins (the 60 °C reaction was also measured after 3h). Almost complete degradation was observed after 15 mins at 80°C, and very advanced degradation after 1h at 60°C, while the concentration of **2** remained nearly unchanged after 1h at 40°C (Figure).

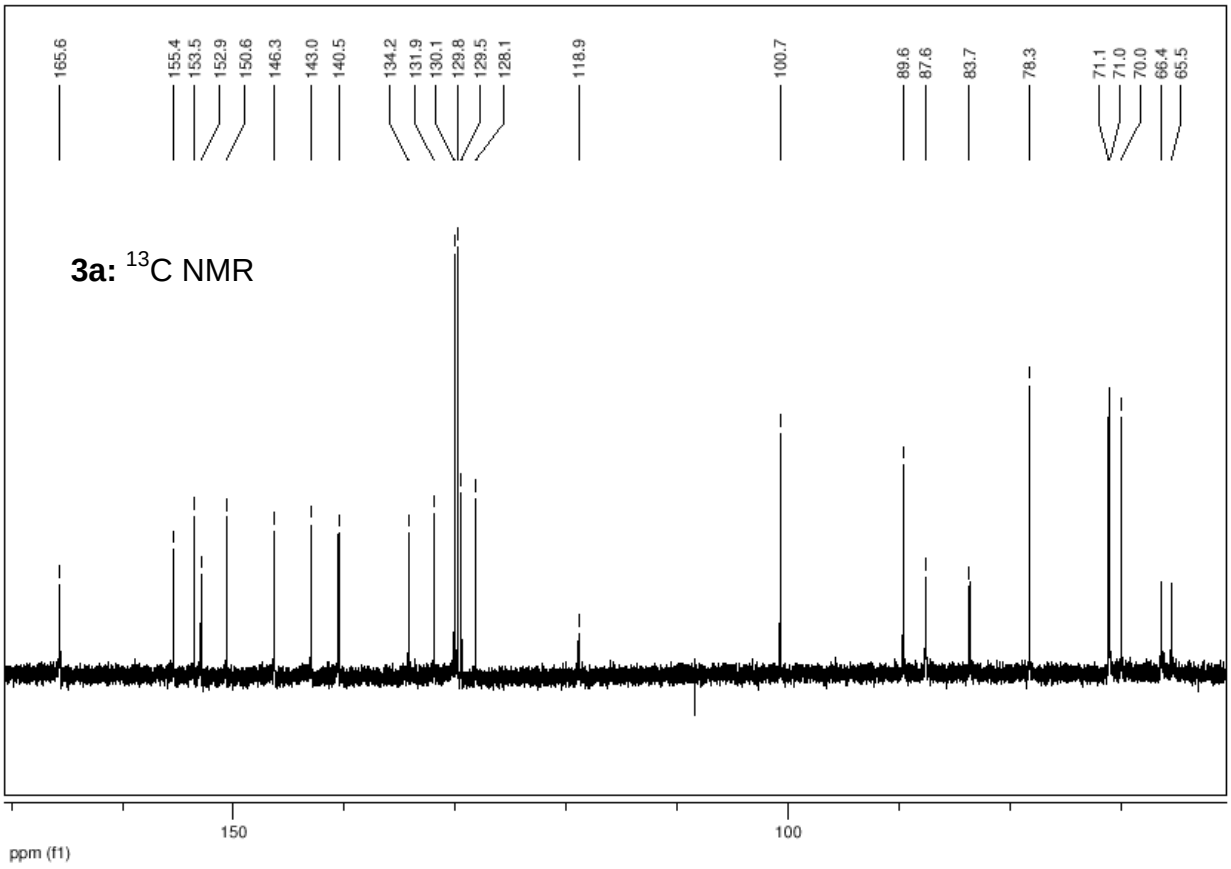


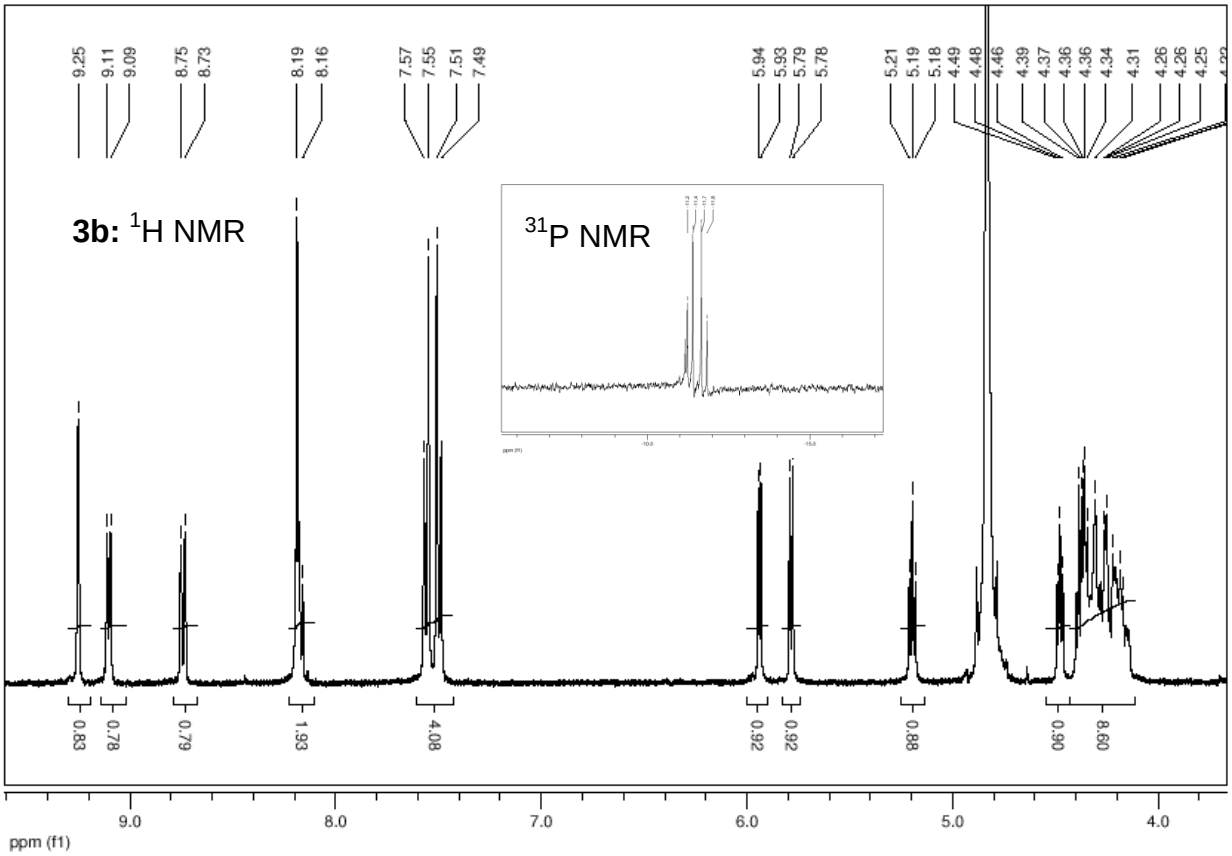
HPLC conditions

Analytical chromatography was carried out on an Agilent 1200 machine equipped with a Supelcosil LC-18T column (25cm × 4.6mm, particle size 5 µm); gradient: MeOH against 0.1 M TEA phosphate (pH 8.0); detection (diode array detector): 254/280 nm. For the preparation of samples, the reaction mixture (100 µL) was diluted with MilliQ water (900 µL) and filtered prior to injection (injection volume: 5 µL). HPLC analyses were carried out under the following conditions – flow rate: 1.5 mL/min; solvent A: MeOH; solvent B: 0.1 M TEA phosphate (pH 8.0); column temperature: 25 °C; gradient: 0-2 min – 90 % B, 2-8 min – 90 % B, 8-10 min – 80 % B, 10-12 min – 80 % B, 12-13 min – 90 % B.

(iv) NMR SPECTRA OF NAD DERIVATIVES



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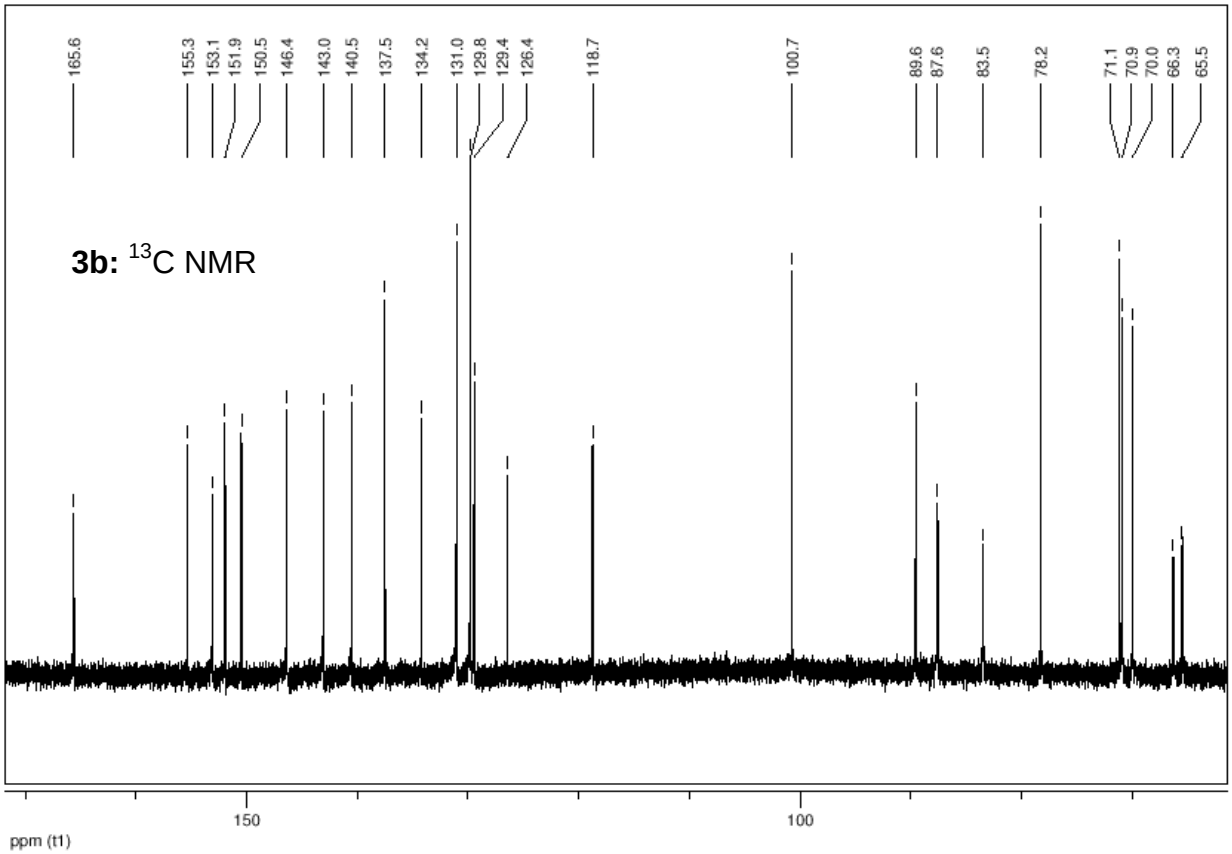
9.11
9.09
8.75
8.73
8.19
8.16
7.57
7.55
7.51
7.49
5.94
5.93
5.79
5.78
5.21
5.19
5.18
4.49
4.48
4.46
4.39
4.37
4.36
4.36
4.34
4.31
4.26
4.26
4.25
3b: 1H NMR
31P NMR
0.83
0.78
0.79
1.93
4.08
0.92
0.92
0.88
0.90
8.60
9.0
8.0
7.0
6.0
5.0
4.0
ppm (f1)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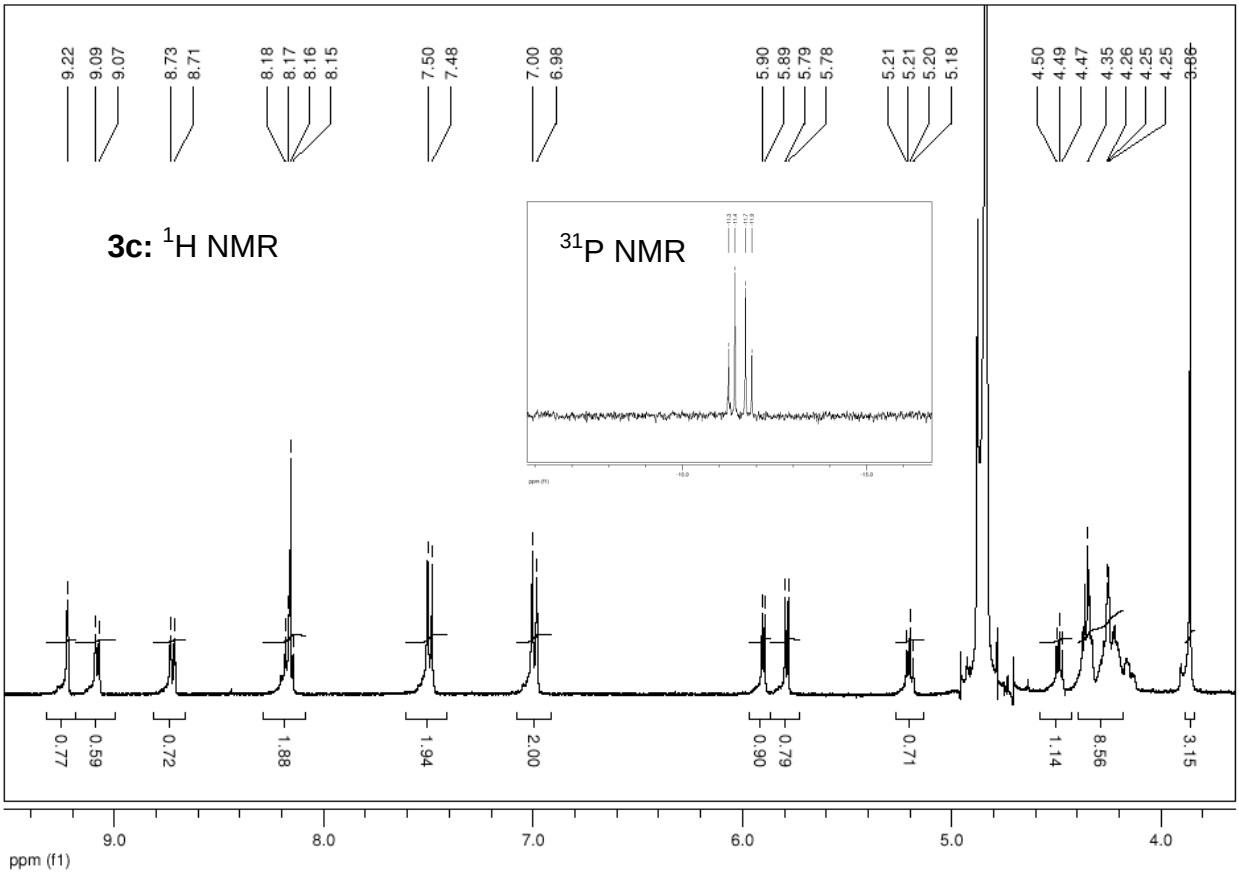
155.3
153.1
151.9
150.5
146.4
143.0
140.5
137.5
134.2
131.0
129.8
129.4
126.4
118.7
100.7
89.6
87.6
83.5
78.2
71.1
70.9
70.0
66.3
65.5
3b: 13C NMR
150
100
ppm (t1)


9.22
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9.07
8.73
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8.18
8.17
8.16
8.15
7.50
7.48
7.00
6.98
5.90
5.89
5.79
5.78
5.21
5.21
5.20
5.18
4.50
4.49
4.47
4.35
4.26
4.25
4.25
3.86
3c: 1H NMR
31P NMR
0.77
0.59
0.72
1.88
1.94
2.00
0.90
0.79
0.71
1.14
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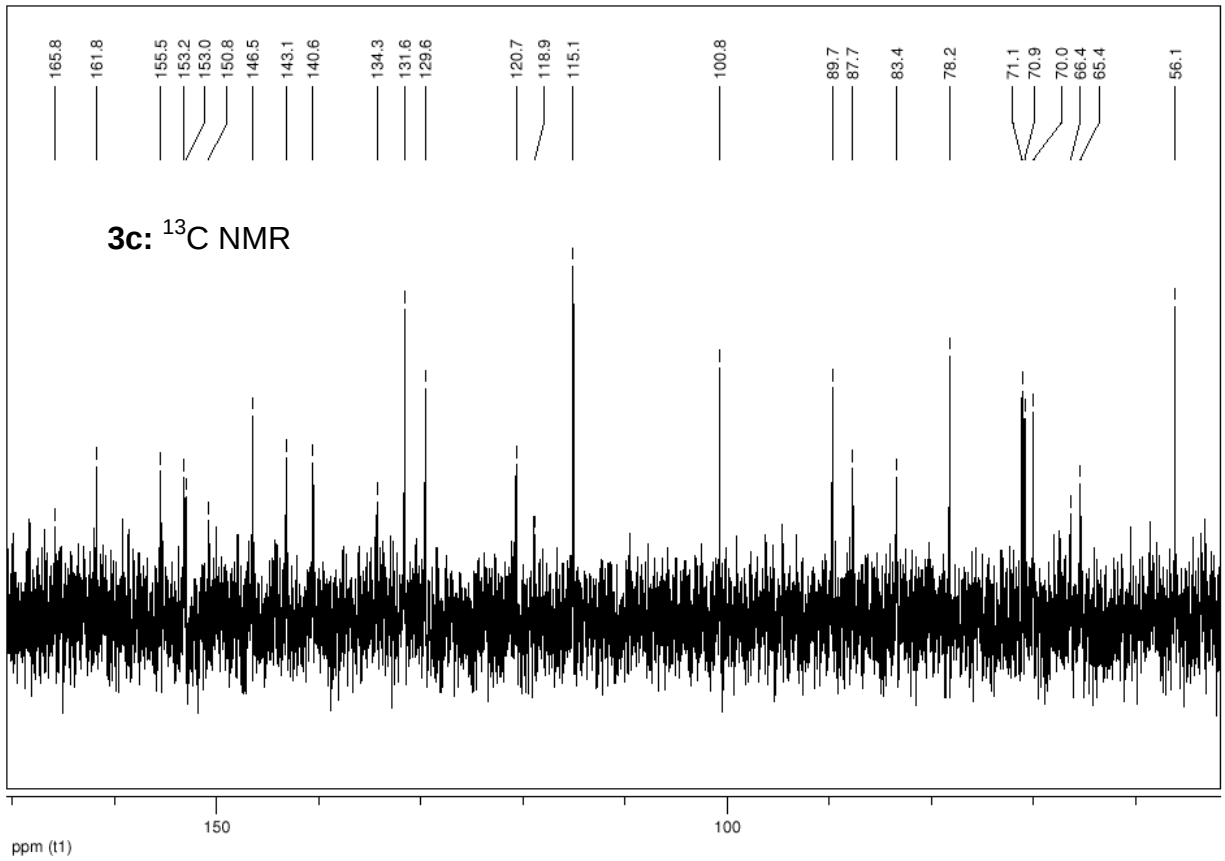
9.0
8.0
7.0
6.0
5.0
4.0
ppm (f1)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161.8
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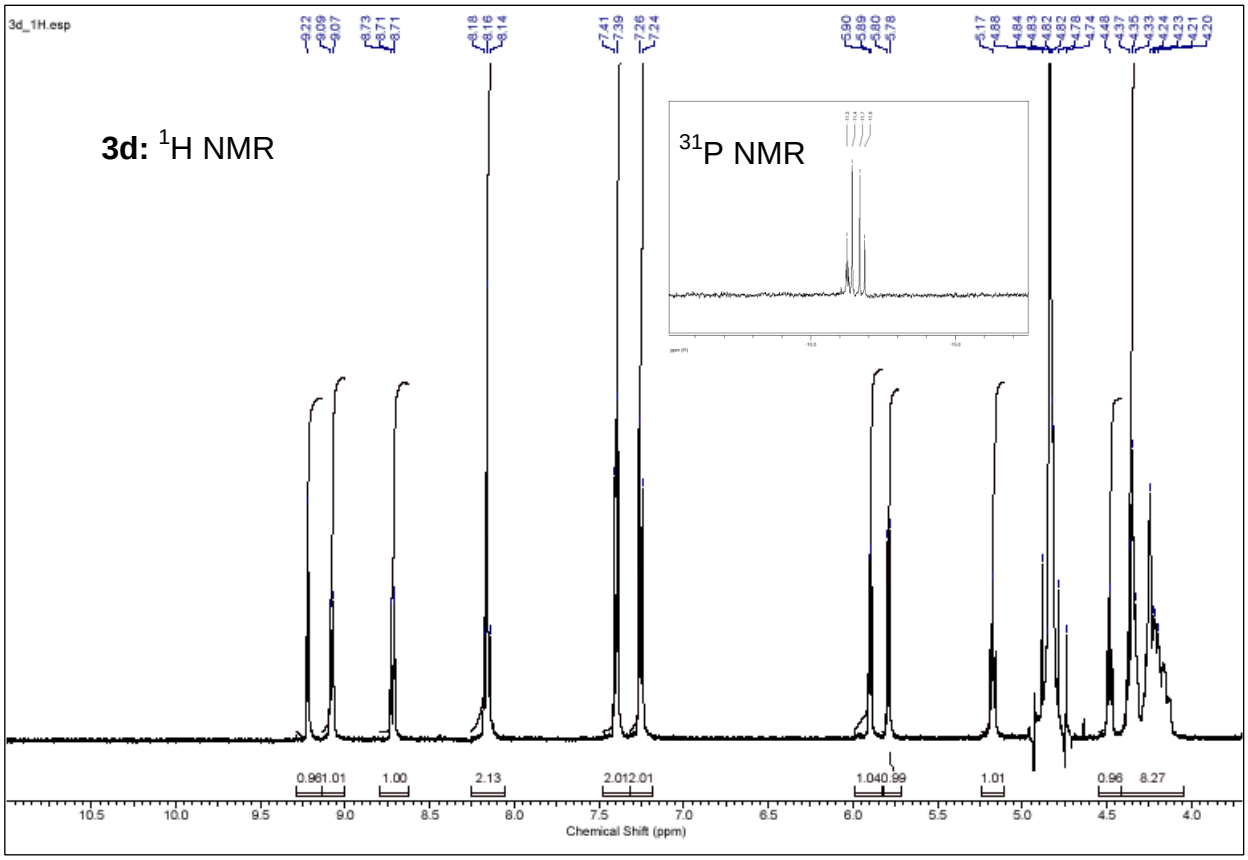
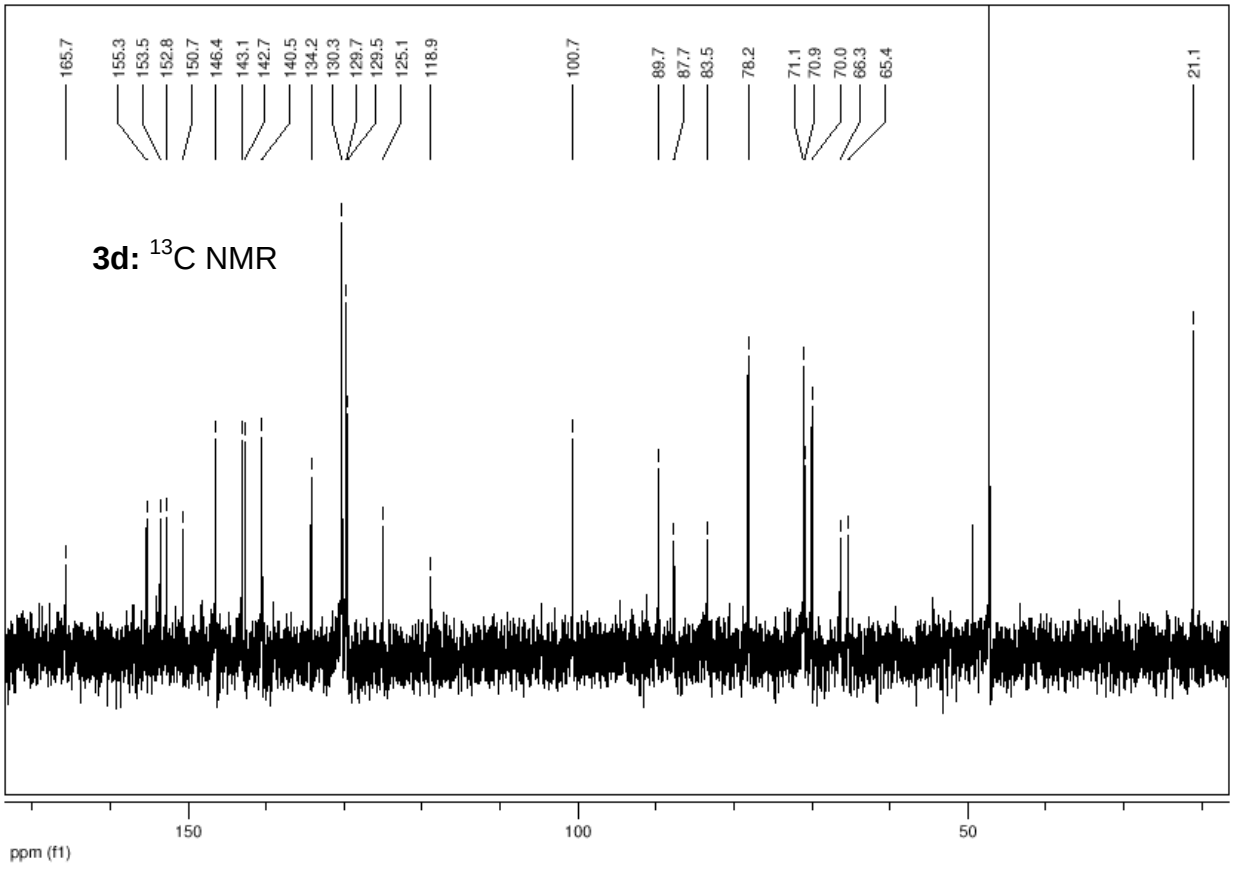
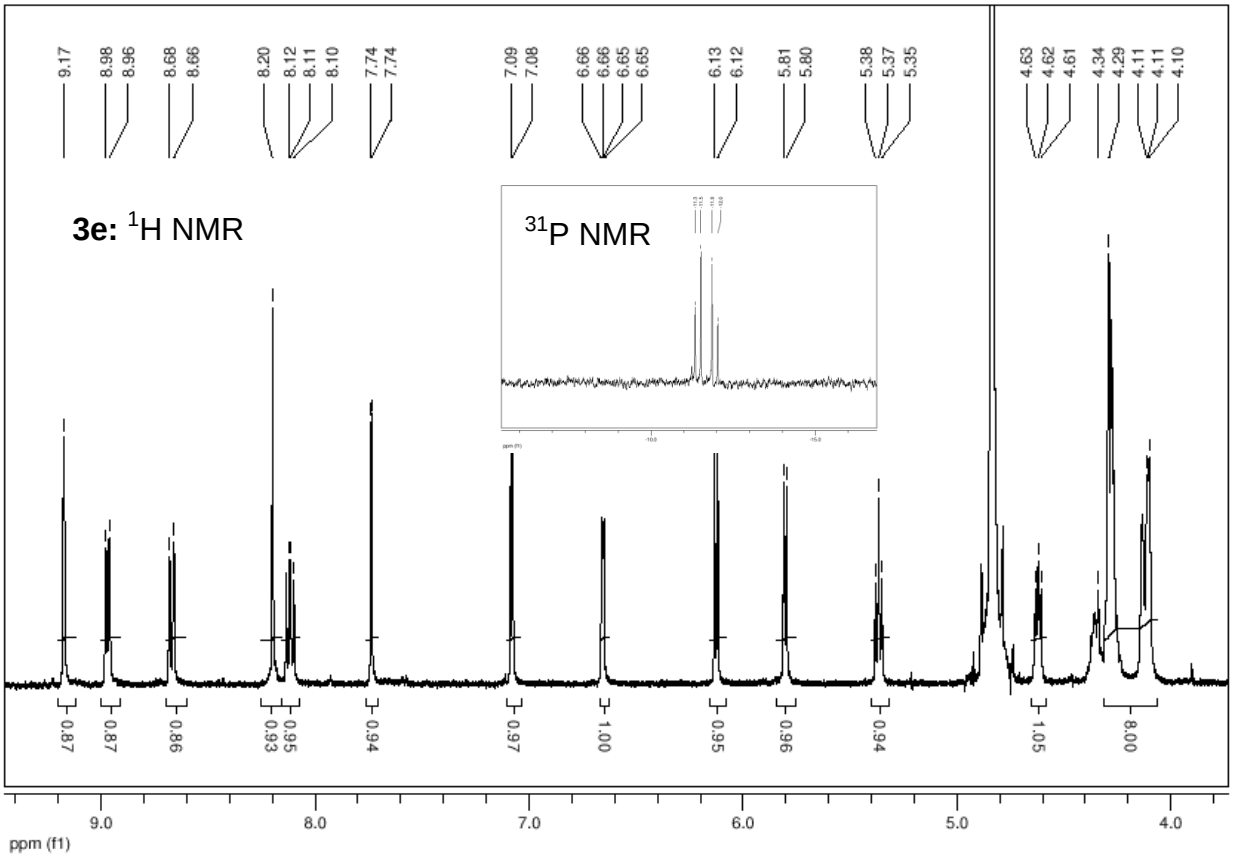
153.2
153.0
150.8
146.5
143.1
140.6
134.3
131.6
129.6
120.7
118.9
115.1
100.8
89.7
87.7
83.4
78.2
71.1
70.9
70.0
66.4
65.4
56.1
3c: 13C NMR
150
100
ppm (t1)


3d_1H.esp
3d: 1H NMR
31P NMR
Chemical Shift (ppm)


3d: 13C NMR
ppm (f1)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8.20
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8.11
8.10
7.74
7.74
7.09
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6.66
6.66
6.65
6.65
6.13
6.12
5.81
5.80
5.38
5.37
5.35
4.63
4.62
4.61
4.34
4.29
4.11
4.11
4.10
3e: 1H NMR
31P NMR
0.87
0.87
0.86
0.93
0.95
0.94
0.97
1.00
0.95
0.96
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9.0
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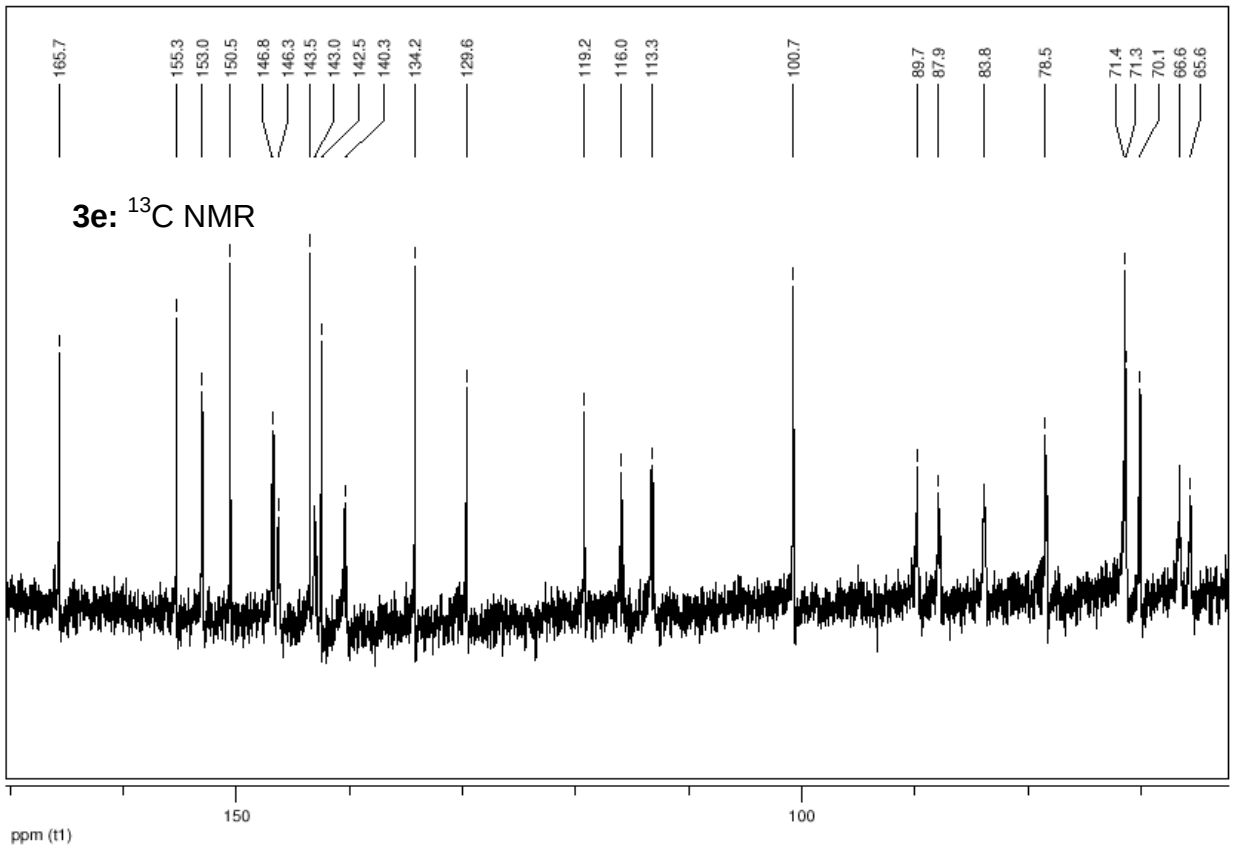
6.0
5.0
4.0
ppm (f1)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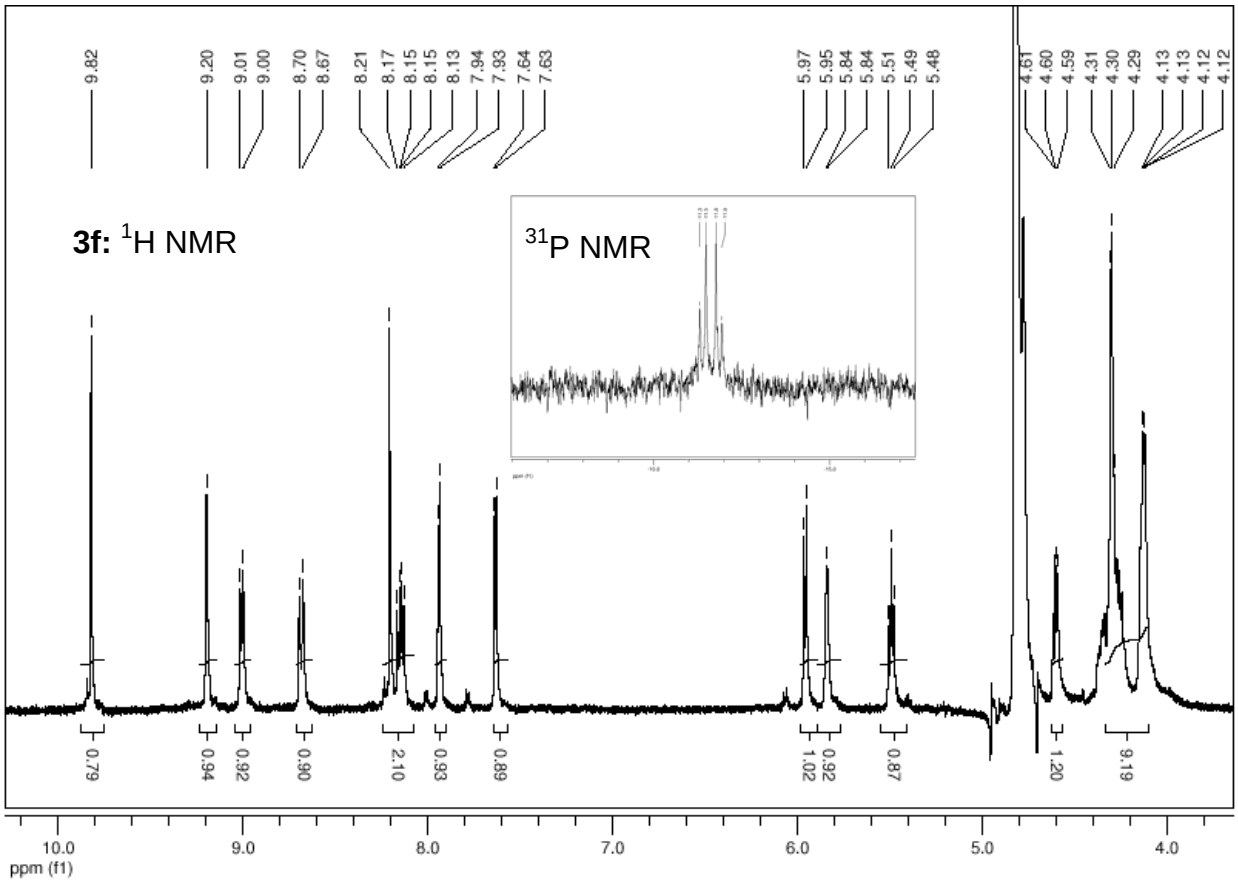
155.3
153.0
150.5
146.8
146.3
143.5
143.0
142.5
140.3
134.2
129.6
119.2
116.0
113.3
100.7
89.7
87.9
83.8
78.5
71.4
71.3
70.1
66.6
65.6
3e: 13C NMR
150
100
ppm (t1)


3f: ^{1}H NMR
^{31}P NMR
9.82
9.20
9.01
9.00
8.70
8.67
8.21
8.17
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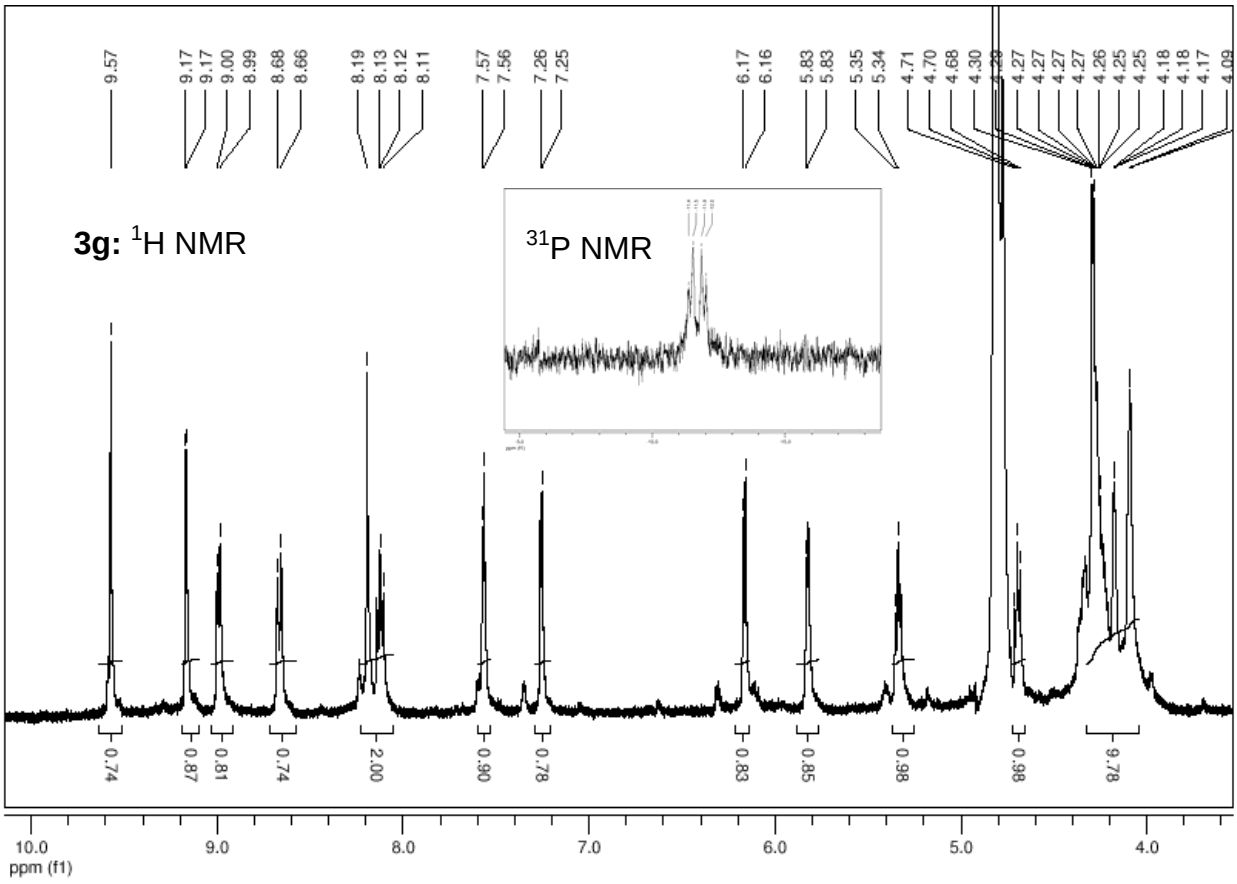
8.13
7.94
7.93
7.64
7.63
5.97
5.95
5.84
5.84
5.51
5.49
5.48
4.61
4.60
4.59
4.31
4.30
4.29
4.13
4.13
4.12
4.12
0.79
0.94
0.92
0.90
2.10
0.93
0.89
1.02
0.92
0.87
1.20
9.19
10.0
9.0
8.0
7.0
6.0
5.0
4.0
ppm (f1)


3g: ^{1}H NMR
^{31}P NMR
9.57
9.17
9.17
9.00
8.99
8.68
8.66
8.19
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8.12
8.11
7.57
7.56
7.26
7.25
6.17
6.16
5.83
5.83
5.35
5.34
4.71
4.70
4.68
4.30
4.27
4.27
4.27
4.27
4.26
4.25
4.25
4.18
4.18
4.17
4.09
0.74
0.87
0.81
0.74
2.00
0.90
0.78
0.83
0.85
0.98
0.98
9.78
10.0
9.0
8.0
7.0
6.0
5.0
4.0
ppm (f1)

(v) ADDITIONAL FIGURES

FIGURE S1: Representative chromatogram for the one-pot synthesis of 8-phenyl NAD **3a** from NAD (blue: bromination step; red: cross-coupling step; UV detection: 280 nm; other HPLC conditions as described above)

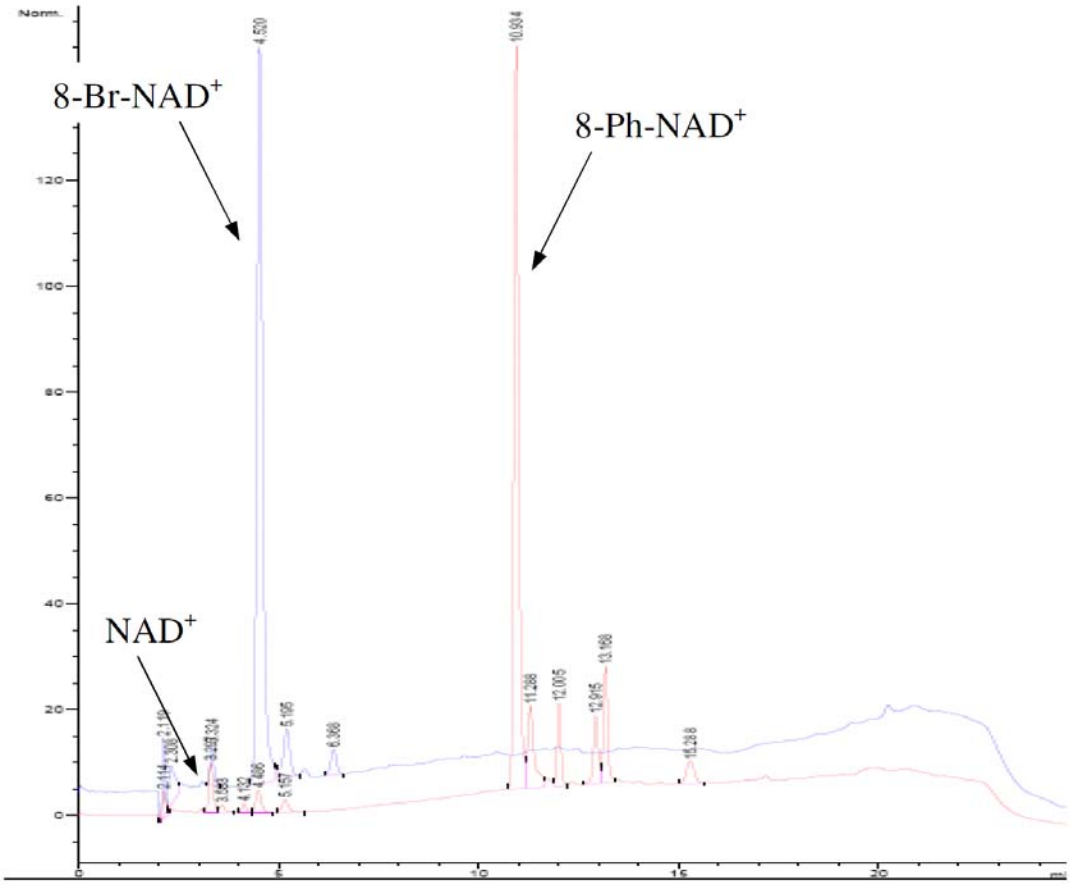


FIGURE S2: Sequence alignment of four human sirtuins (SIRT1, 2, 3, and 5). The secondary structural elements for SIRT2 are shown below the sequence alignment; Alpha helices are indicated with red lines, beta sheets are indicated with green arrows and loop parts are indicated with black lines. Residues that participate in NAD binding are colored blue, residues that participate in acetyl-lysine peptide binding are colored purple, and residues involved in zinc binding are colored in orange. Solid lines above the sequence alignment indicate which regions of the proteins compose the Rossmann-fold domain (magenta), cofactor binding loop (black), small domain (blue) and connecting domain (green).

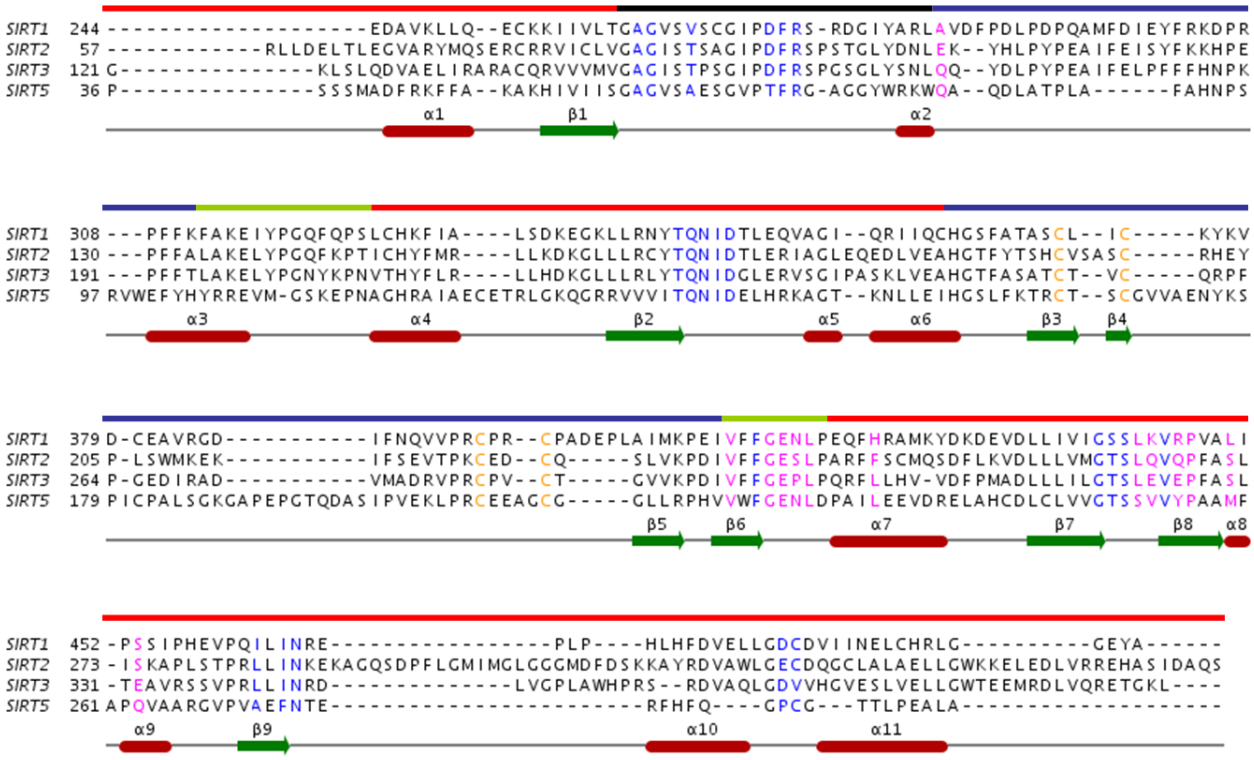


FIGURE S3: Superimposition of the SIRT3 crystal structure, in complex with ADPR (*green ribbon*, PDB code 3GLT, ADPR omitted for clarity), and human SIRT2 (*purple ribbon*, PDB code 1J8F) with docked inhibitor **3b** (*orange balls and sticks*). The steric clash of the 8-(4-chlorophenyl) substituent in **3b** with the flexible loop in the SIRT3-ADPR complex is indicated with a circle.

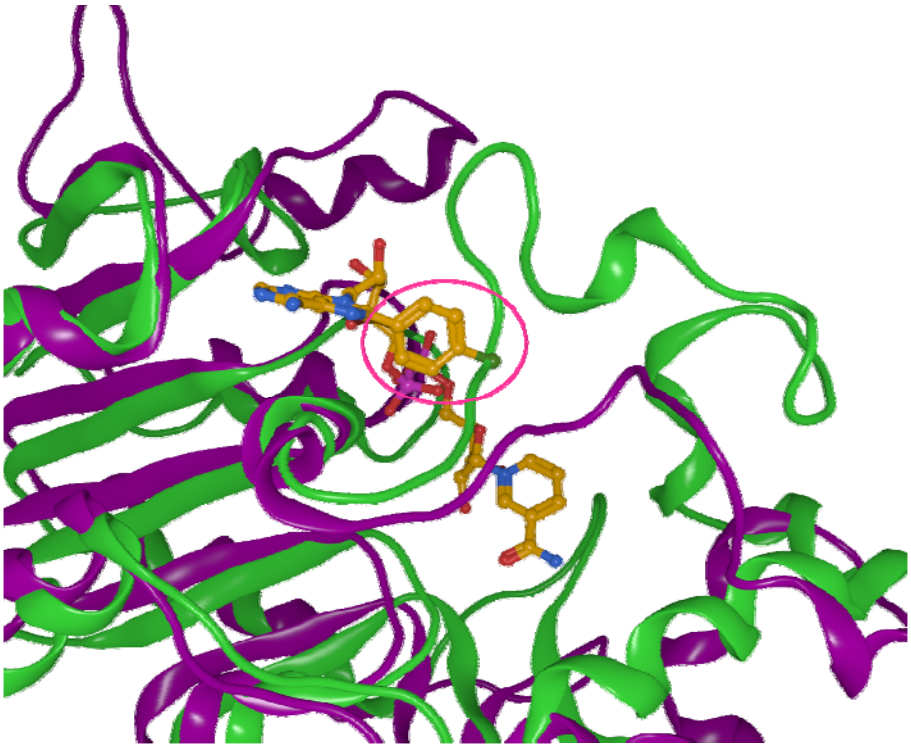


FIGURE S4: Superimposition of 8-(4-chlorophenyl) NAD **3b** (*green balls and sticks*), docked into the cofactor binding site of human SIRT2 (PDB code 1J8F), and NAD (*light pink balls and sticks*), in complex with the archaeal enzyme Sir2-Af2, (PDB code 1YC2). **3b** binds at the cofactor binding in a very similar orientation to NAD (SIRT2 backbone atoms are represented by the blue ribbon, Sir2-Af2 backbone atoms by the orange ribbon).

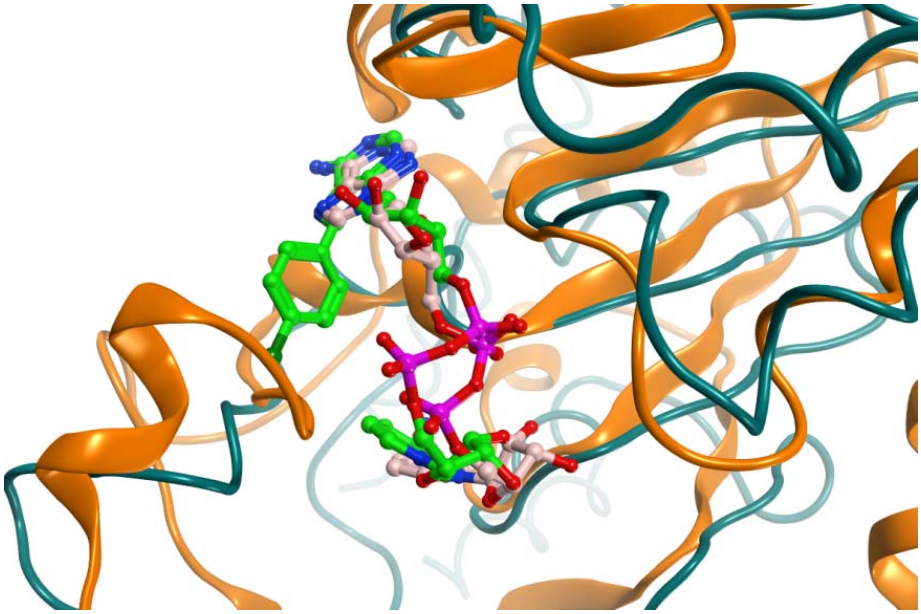


FIGURE S5: Interaction of inhibitor **3b** (*orange balls and sticks*) with human SIRT2 (PDB code 1J8F) based on molecular docking. Only residues near the 8-chlorophenyl group are displayed. Whereas the residues D95-S98 are conserved among all sirtuins, P99-T101 represent a variable region. NB that for human SIRT2, no structure in complex with NAD is currently available.

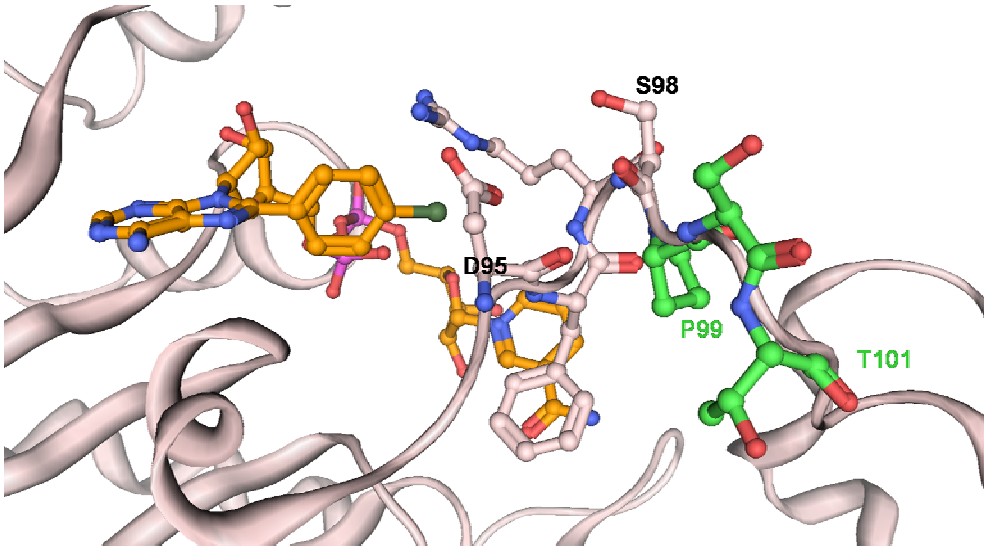
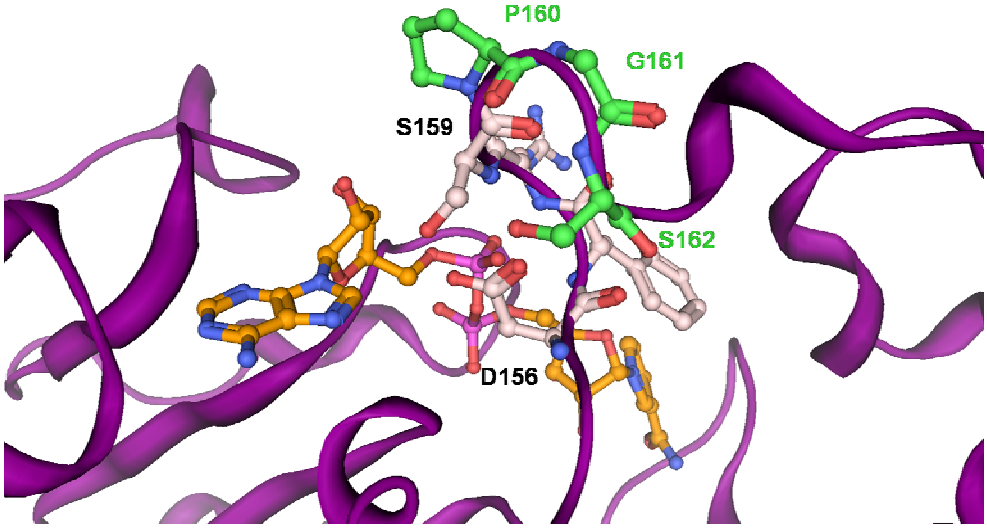


FIGURE S6: Crystal structure of SIRT3 with bound NAD (*orange balls and sticks*). The structure shows an entirely different conformation of the flexible NAD-binding loop compared to apo-SIRT2 (see above), with residues P160-S162 (corresponding to P99-T101 in SIRT2) now located near the 8-position of NAD.



(vi) DOCKING SCORES

Calculated docking scores for inhibitors tested against SIRT2. Goldscore and Chemscore are calculated using the GOLD program. DrugScore and DrugScores_CSD[1] are calculated for the top-ranked docking pose derived from the Goldscore ranking.

Cmpd	Goldscore	Chemscore	Drugscore	Drugscore_CSD	IC_{50} SIRT2
2	69.87	29.27	-557068	-297236	28 ± 7
3a	50.63	33.27	-603895	-310674	123 ± 4
3b	72.72	33.23	-635100	-325602	35 ± 8
3c	62.43	33.97	-636472	-319692	43 ± 5
3d	65.00	32.84	-628687	-326215	52 ± 12
3e	57.38	29.61	-586272	-296412	35%
4a	46.25	22.81	-359957	-166816	inactive
4b	49.99	25.35	-458107	-199471	29 ± 3
5a	27.05	18.16	-300715	-115393	16%
5b	28.06	20.03	-370269	-151119	inactive

[1]DrugScore(CSD)-knowledge-based scoring function derived from small molecule crystal data with superior recognition rate of near-native ligand poses and better affinity prediction. Velec, H. F., Gohlke, H., Klebe, G. *J. Med. Chem.* **2005**, *48*, 6296-303.