Electronic Supplementary Information

Reversible Redox Inter-Conversion of Biologically Active Derivatives of NAD^+/NADH Bound to a Gold Electrode: ToF-SIMS Evidence

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1. Experimental Methods

1.1 Materials

NAD^+, NMN was obtained from Shanghai Boylechem Co.ltd. UHQ II system (Elga) was used to purify water to a resistivity of 18 MΩ·cm for preparation of all solutions. Phosphate buffer solution (PBS) was prepared using Na_2HPO_4 and NaH_2PO_4. All electrodes for electrochemical experiment were purchased from Shanghai Chenhua Co., Ltd., China. All chemical reagents for synthesis were of analytical grade and used without further purification unless otherwise noted. Triethylamine and morpholine were dried over potassium hydroxide, distilled, and then stored over potassium hydroxide pellets. ^1H NMR and ^{13}C NMR were acquired in D_2O, DMSO-d_6 or $CDCl_3$ on BRUKER AVANCE 500 spectrometer using TMS as an internal standard. HR-MS were obtained on HP 5989 mass spectrometer.

1.2 Synthesis of Benzyl Sulfide-modified NAD^+ Derivative PhSNAD

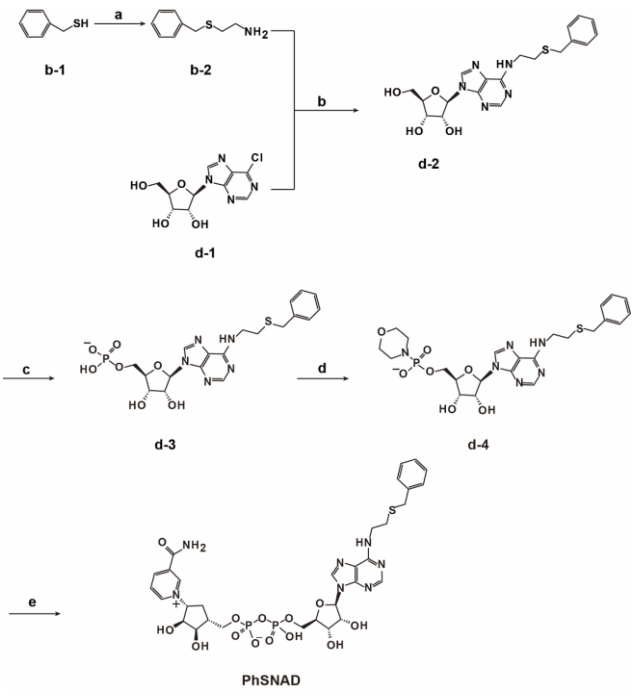


Fig. S1 The synthesis of benzyl sulfide-modified NAD^+ derivative. Reagents and conditions: a) 2-chloroethylamine hydrochloride, EtONa, EtOH, 85 %. b) b-2, EtOH, 83 ℃, 93.1 %; c) TEP, $POCl_3$, H_2O, 0 ℃, 45 %; d) PPh_3, dipyridyl disulfide, morpholine, room temperature, 78 %; e) $MnCl_2$/formamide, $MgSO_4$, β-NMN^+, room temperature, 55 %.

The Synthesis of PhSNAD. 2-(benzylthio)ethanamine (b-2). To an ice-cooled flask of ethanol (100 ml) fitted with a condenser was added sodium (3.16 g, 138 mmol, 1.1 eq.) in small pieces. After disappearing of sodium pieces, benzyl mercaptan b-1 (14.6 ml, 125 mmol) was added via a syringe, followed a solution of 2-chloroethylamine hydrochloride (10.0 g, 125 mmol) in ethanol (50 ml) was injected. The mixture was heated at reflux for 24 h then cooled and poured into saturated ammonium chloride solution (200 ml) and extracted with dichloromethane (3×200 ml). The combined organic phases were dried with magnesium sulfate, filtered and evaporated under reduced pressure to give an oil which was purified by flash chromatography on silica eluting with dichloromethane to 10 % methanol: dichloromethane (gradient) to give the title compound b-2 as a yellow oil (17.7 g, 85 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.23-7.37 (m, J = 29.3, 6.8 Hz, 5H), 6.66 (s, 2H), 3.78 (s, 2H), 2.87-2.92 (t, J = 7.3 Hz, 2H), 2.74 – 2.57 (t, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 138.87, 129.40, 128.88, 127.37, 37.08, 35.12, 29.69. MS (ESI): m/z calcd for ($M+H^+$) $C_9H_{13}NS$ 168.0847; found 168.0853.

(2R, 3R, 4S, 5R)-2-(6-((2-(benzylthio) ethyl) amino)-9H-purin-9-yl)-5-(hydroxylmethyl) tetrahydrofuran-3, 4-diol (d-2). To a solution containing b-2 (7 g, 41.9 mmol, 5 eq), 6-chloropurine riboside (2.4 g, 8.4 mmol) in ethanol (50 ml). The mixture was heated at reflux for 14h then cooled to room temperature and crystallized in refrigerator (4 ℃), filtering, washing with ethanol, drying give pury compound d-2 as a white soild (3.26 g, 93.1 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.38 (s, 1H), 8.26 (s, 1H), 8.02 (s, 1H), 7.41 – 7.14 (m, 5H), 5.92 (d, J = 5.6 Hz, 1H), 5.33 (d, J = 111.5 Hz, 2H), 4.63 (s, 1H), 4.37 (s, 1H), 4.17 (s, 1H), 3.99 (s, 1H), 3.79 (s, 2H), 3.69 (d, J = 8.8 Hz, 2H), 3.58 (s, 1H), 3.44 (dd, J = 13.5, 6.7 Hz, 1H), 2.65 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 154.88, 152.78, 148.85, 140.36, 139.06, 129.36, 129.29, 128.77, 127.21, 120.28, 88.49, 86.40, 74.02, 71.15, 62.15, 56.54, 35.22, 30.40. MS (ESI): m/z calcd for ($M+H^+$) $C_{19}H_{23}N_5O_4S$ 418.1549; found 418.1559.

((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio) ethyl) amino)-9H-purin-9-yl)-3, 4-dihydroxytetrahydrofuran-2-yl) methyl dihydrogen phosphate (d-3). The compound d-2 (3 g, 6.6 mmol) was dissolved in TEP (45 mL) by heating. It was then cooled to 0 ℃,

and then H_2O (0.01 mL) was added followed by $POCl_3$ (2.1 mL, 23 mmol). The mixture was stirred at 0 ℃ until starting material was completely consumed. Excess $POCl_3$ was then removed under vacuum, H_2O (10 mL) and pyridine (5 mL) were added at 0 ℃, and the mixture was stirred for 1 h. TEP was removed by partition between water and ice-cold ethyl acetate (5 mL). The aqueous layer was concentrated under vacuum, and the resulting white residue was redissolved in 0.1% aqueous LiOH (15 mL). Crude was precipitated in acetone (200 mL) and then purified by reverse-phase chromatography, eluted with a gradient of 0-30% MeCN in 0.05 M TEAB buffer. The appropriate fractions were collected and evaporated under reduced pressure. The residue obtained was dissolved in H_2O (10 mL) and treated with charcoal for 1 h to remove any residual inorganic phosphate. Elution with EtOH-H_2O-NH_3 (400 mL, 25:24:1) provided the crude compound which was further treated with Dowex-H^+ to give the desired compound d-3 as its free acid (1.48 g, 45 %): ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.38 (s, 1H), 8.29 (s, 1H), 8.11 (s, 1H), 7.48 – 7.11 (m, 5H), 5.96 (d, J = 5.4 Hz, 1H), 4.61 (t, J = 5.0 Hz, 1H), 4.20 (s, 1H), 4.16 – 4.04 (m, 2H), 3.99 (dd, J = 11.8, 5.9 Hz, 1H), 3.80 (s, 2H), 3.69 (s, 2H), 2.66 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 154.35, 152.63, 139.96, 139.05, 130.77, 129.37, 128.93, 128.80, 127.24, 119.77, 87.64, 83.59, 83.50, 73.87, 70.89, 65.88, 65.84, 35.20, 30.32. ^{31}P NMR (202 MHz, DMSO-d_6): δ = -1.19 (s). MS (ESI): m/z calcd for ($M+H^+$) $C_{19}H_{24}N_5O_7PS$ 498.1212; found 498.1138.

((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio) ethyl) amino)-9H-purin-9-yl)-3, 4-dihydr-oxytetrahydrofuran-2-yl) methyl hydrogen morpholinophosphonate (d-4). The compound d-3 (300 mg, 0.6 mmol) was dissolved in dry DMSO (1 mL) and coevaporated with dry DMF (2 mL). The resulting yellow residue was dissolved in DMSO (1 mL), and dipyridyl disulfide (397 mg, 1.8 mmol), morpholine (0.43 mL, 4.8 mmol), and triphenylphosphine (409 mg, 1.56 mmol) were added in sequence. The reaction mixture was stirred at room temperature for 3 h. Precipitation of the product occurred by dropwise addition of a solution of NaI in acetone (20 mL), and the resulting precipitate was filtered and washed with acetone to yield the desired compound as a yellow solid (264.8 mg, 78 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.50 (s, 1H), 8.26 (s, 1H), 7.96 (s, 3H), 7.53 – 6.95 (m, 5H), 5.94 (d, J = 5.4 Hz, 1H), 4.64 (s, 1H), 4.29 –

4.17 (m, 1H), 4.10 – 3.96 (m, 2H), 3.87 (s, 2H), 3.79 (s, 1H), 3.60 (s, 2H), 2.89 (s, 8H), 2.80 (s, 2H), 2.73 (s, 8H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 162.79, 152.87, 139.95, 139.08, 131.85, 130.77, 129.36, 128.90, 128.77, 128.18, 127.20, 87.47, 74.69, 71.36, 67.31, 66.19, 64.47, 63.28, 57.27, 45.87, 45.16, 36.25, 31.23. ^{31}P NMR (202 MHz, DMSO-d_6): δ = 4.58. MS (ESI): m/z calcd for ($M+H^+$) $C_{23}H_{31}N_6O_7PS$ 567.1791; found 567.1711.

Benzyl Sulfide-Modified NAD^+ Derivative (PhSNAD). To a solution of the compound d-4 (150 mg, 26 mmol) in $MnCl_2$/formamide (2 mL, 0.2 M) was added β-NMN^+ (97 mg, 29 mmol) and $MgSO_4$ (63.6 mg, 140 mmol) under an argon atmosphere. The resulting suspension was stirred at room temperature for 48 h after which HPLC analysis showed completion of the reaction. Precipitation of the product occurred by addition of MeCN (3 mL). It was filtered, dissolved in deionized water (2 mL), and treated with Chelex-Na^+ to remove any residual Mn^{2+} and then purified on a reverse-phase system, eluted with 0-30% MeCN against 0.05 M TEAB buffer. The appropriate fractions were combined and evaporated under reduced pressure, and the excess TEAB was destroyed by coevaporation with methanol to give the title compound PhSNAD as a white foam (118 mg, 55 %). ^{1}H NMR (400 MHz, D_2O): δ = 9.28 (s, 1H), 9.13 (s, 1H), 8.78 (s, 1H), 8.45 (s, 1H), 8.13 (s, 2H), 7.14 – 6.70 (m, 5H), 6.03 (s, 2H), 4.48 (s, 1H), 4.43 (d, J = 4.0 Hz, 2H), 4.35 (s, 1H), 4.29 (d, J = 13.1 Hz, 2H), 4.13 (s, 2H), 4.00 – 3.76 (m, 1H), 3.60 (s, 2H), 3.52 (s, 1H), 2.79 (s, 2H), 1.93 (s, 1H). ^{13}C NMR (101 MHz, D_2O): δ = 165.39, 149.17, 148.48, 146.59, 145.92, 145.01, 142.50, 141.53, 139.77, 138.53, 133.76, 128.59, 128.45, 128.07, 126.63, 118.51, 117.44, 99.87, 87.61, 86.98, 84.18, 77.51, 74.54, 70.63, 70.33, 65.23, 64.88, 41.17, 35.76, 29.54. ^{31}P NMR (202 MHz, D_2O): δ = -11.06, -11.19. MS (ESI): m/z calcd for ($M+H^+$) $C_{30}H_{37}N_7O_{14}P_2S$ 814.1673; found 814.1653.

1.3 The Electrochemical Behavior of NAD^+ Standard in Solution

In order to prove the electrochemistry characteristics of synthesic PhSNAD, the CVs of NAD^+ standard was measured and compared with the CVs of compound PhSNAD.

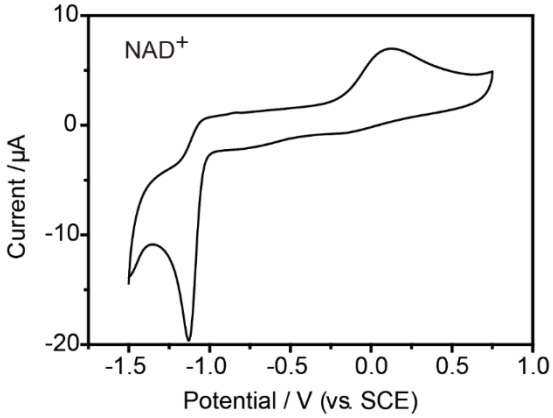


Fig. S2 CVs of GC electrode in PBS (0.1 M, pH 7.0) containing 2 mM NAD^+. Scan rates: 100 mV/s.

1.4 The Evaluation of PhSNAD Coenzyme Activities

To confirm that the synthetic PhSNAD was active in a biological system, UV-vis spectroscopy was used to investigate the catalytic reaction of ADH for ethanol in the presence of PhSNAD. To 10 mL phosphate buffer saline PBS (50 mM, pH 7.4, 20 % (v/v) ethanol), 5 μL PhSNAD aqueous solutions (10 mM) and 5 μL alcohol dehydrogenase (ADH) solutions (1 mg/mL) were added in sequence. After mixed evenly, the mixture was immediately measured continuously by UV-vis spectroscopy.

1.5 The Preparation of PhSNAD Modified Electrode

For the preparation of PhSNAD modified electrode, a 2 mm diameter gold electrode was first polished with different sizes of alumina suspension, then successively rinsed with ultrapure water, ethanol. Following, the polished gold electrode was immersed for 10 min in a hot "piranha" solution. After copious rinsing with ultrapure water, the gold electrode was further electrochemically cleaned by potential cycling in the potential range from -0.30 to 1.50 V vs SCE in 0.5 M H_2SO_4 until the typical cyclic voltammogram of clean gold electrode was obtained. After being rinsed with ultrapure water and ethanol and dried with N_2, the gold electrode was immersed in 2 mM PhSNAD aqueous solution over 24 hours. Finally, the formed PhSNAD modified electrode was successively rinsed with copious amounts of

ultrapure water and ethanol to remove excess adsorbate and then dried with N_2 to remove residual solvent.

2. Surface Characterization of PhSNAD Modified Electrode

2.1 X-ray Photoelectron Spectroscopy (XPS) Analysis

X-ray photoelectron spectroscopy (XPS) was utilized to characterize the modification of compound PhSNAD on gold electrodes. All XPS measurements were performed in an ultrahigh vacuum chamber of an M-probe surface spectrometer. In order to prevent excess contamination by carbon, oxygen and nitrogen species, the gold surface has been carefully precleaned with fresh hot Piranha solution prior to incubation in deaerated solutions containing compounds PhSNAD. The data were processed by specific XPS software.

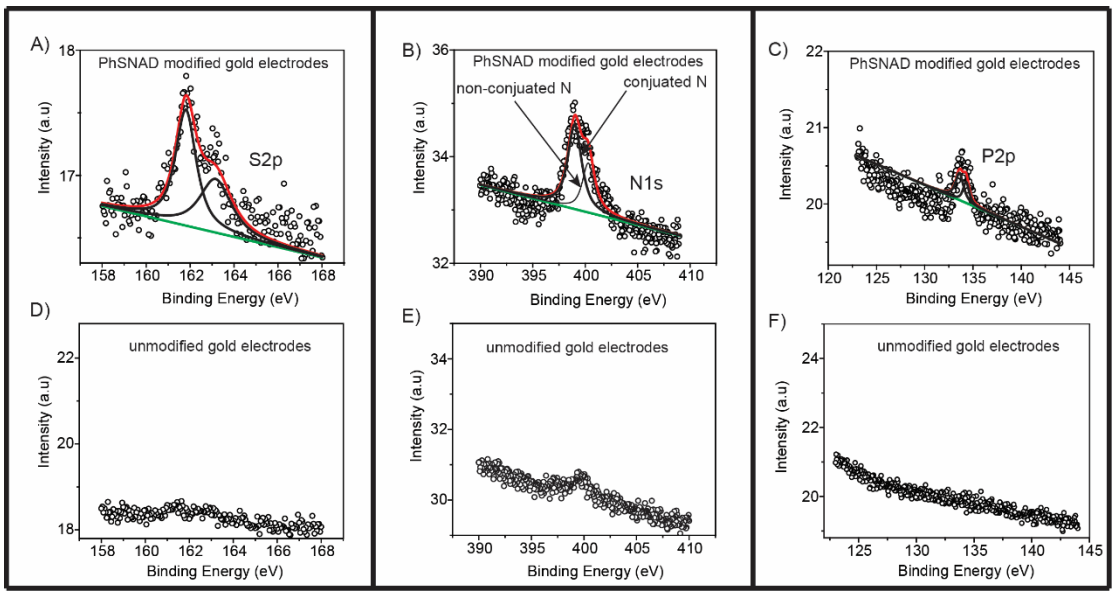


Fig. S3 High-resolution XPS spectra of S_{2p}, N_{1s} and P_{2p} for PhSNAD modified gold electrodes (A-C) and unmodified gold electrodes (D-F), respectively. Open circles represent experimental raw data, red solid lines are for the total fits, black lines are for the component-fitted peaks, and green lines are for the baselines.

2.2 Time-of-Flight Secondary-Ion-Mass-Spectrometry (ToF-SIMS) Analysis

ToF-SIMS spectra were acquired both in positive detection mode from identical

analysis areas. The mass scale was internally calibrated using a number of well- defined and easily assignable secondary ions ($C_4H_2^+$, $C_5H_5^+$, $C_6H_5^+$, $C_7H_7^+$) keeping the error in calibration for the selected secondary ions below 10 ppm. All sample measurements of ToF-SIMS were performed on a ToF-SIMS IV instrument (ION-TOF GmbH, Münster, Germany) of the reflectron-type, equipped with a 25 keV bismuth liquid metal ion gun (LMIG) as primary ion source mounted at 45° with respect to the sample surface. Bi_3++ was selected as primary ion by appropriate mass filter settings. To improve the focus of the primary ion beam (and hence the lateral resolution) the pulse width of the Bi_3++ (25 keV) ion pulse was reduced to 11 ns and the lens target was adjusted to obtain a sharp image on a structured sample (e.g., silver cross) in the secondary electron mode. The primary ion current was directly determined at 100 µs cycle time (i.e., a repetition rate of 10 kHz) using a Faraday cup located on a grounded sample holder. Operation conditions with these settings comprised a target current of 0.19 pA for the selected primary ion. The large area scans (3×3 mm) were performed in the so-called stitching mode where the total analysis area is divided into several small analysis areas, which are stitched together by a routine implemented in the SurfaceLab 6.4 (ION-TOF GmbH, Münster, Germany) software. The total primary ion dose density was 5×10^{10} ions/cm^2 for large area scans and 5.1×10^{11} ions/cm^2 for a standard measurement area (500×500 µm) ensuring static conditions. The vacuum in the analysis chamber was in the range of 10^{-9} mbar during all measurements.

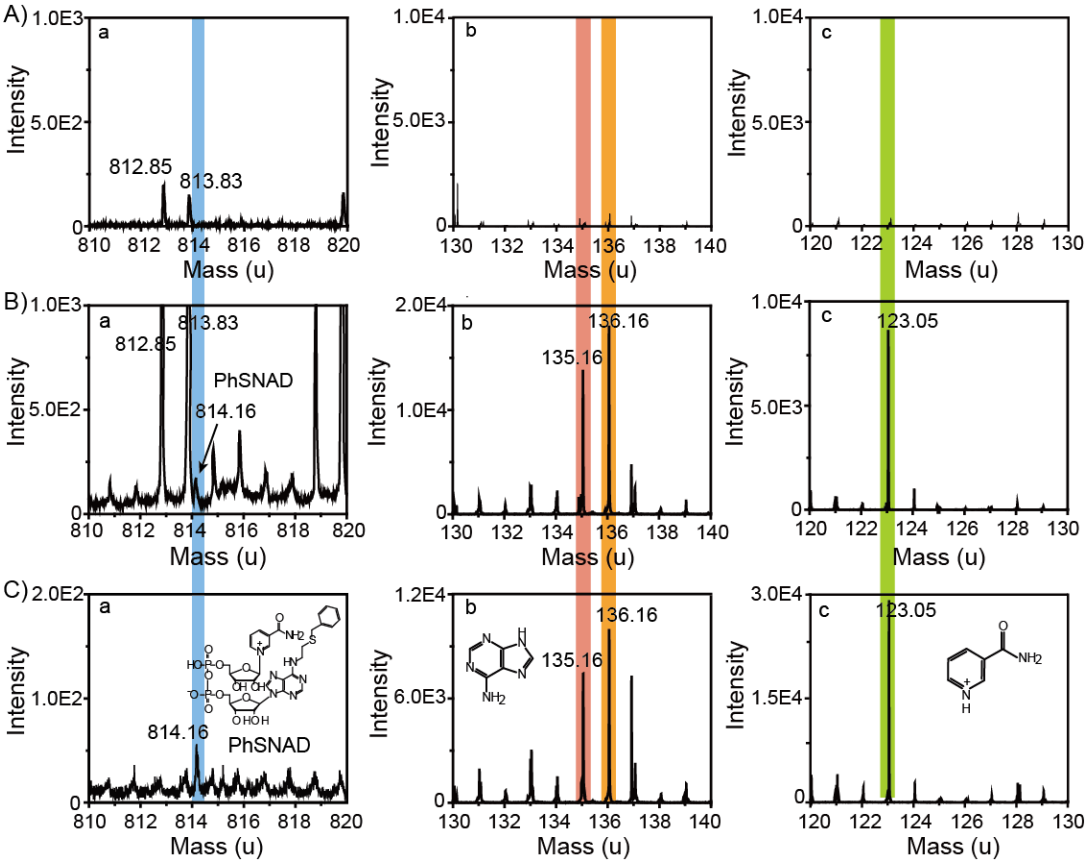


Fig. S4 ToF-SIMS spectra of bare gold electrodes (A), PhSNAD modified electrodes (B) and PhSNAD coated silicon wafer (C) obtained with Bi_3++ primary ion beams, respectively. (a, b, c) represents the molecular ion peak of PhSNAD (a), the characteristic fragment ion peaks of the nicotinamide (b) and the adenine (c), respectively.

3. Electrochemical Measurements of PhSNAD Modified Electrode

The cyclic voltammetric measurements were performed using a CHI660E electrochemical workstation (Shanghai Chenhua, China). All electrochemical experiments were performed with a conventional three-electrode system, using the glassy electrodes or modified gold electrodes as the working electrode, a platinum wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode.

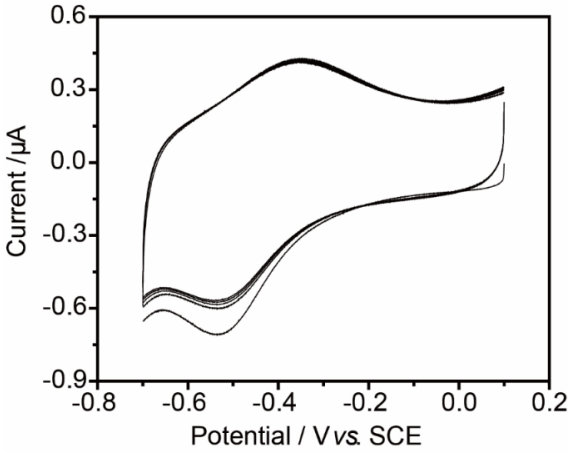


Fig. S5 CVs of PhSNAD modified gold in PBS (0.1 M, pH 7.0) for 20 sweeps. Scan rates: 100 mV s^{-1}.

4. ToF-SIMS Spectra of NAD^+ Dimer

In order to prove the reduced product of PhSNAD modified electrode surface was NADH, the ToF-SIMS spectra of NAD^+ dimer was recorded on the silicon wafer.

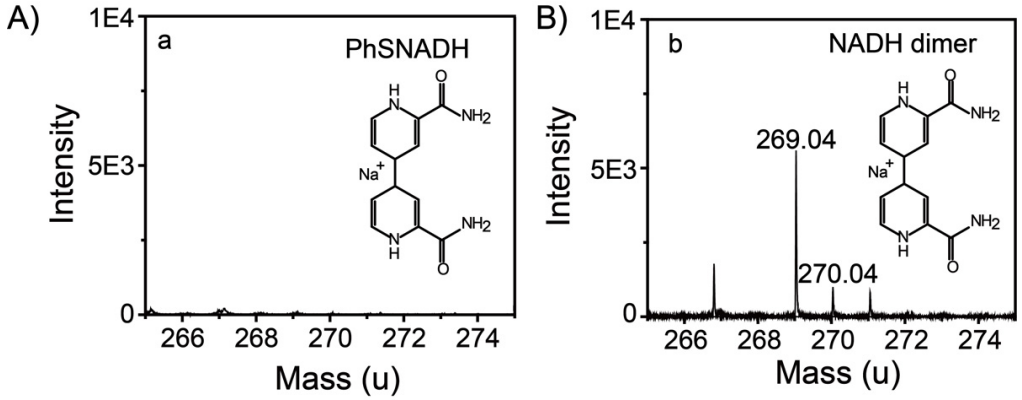


Fig. S6 ToF-SIMS spectra of generated NADH on the surface of gold electrode (A) and NAD^+ dimer coated silicon wafer (B) obtained with Bi3++ primary ion beams.

5. The Coenzyme Activity of Generated NADH on the Surface of Electrode

In order to investigate the coenzyme activity of generated NADH on the surface of electrode, an enzymatic experiment was performed by immersing the reduced PhSNAD modified electrode in the mixture solution of acetaldehyde and ADH. After 30 minutes, the electrode was removed and successively rinsed with copious amounts of ultrapure

water and ethanol to remove excess adsorbate and then dried with N_2 to remove residual solvent. Finally, the reacted electrode was analyzed using ToF-SIMS.

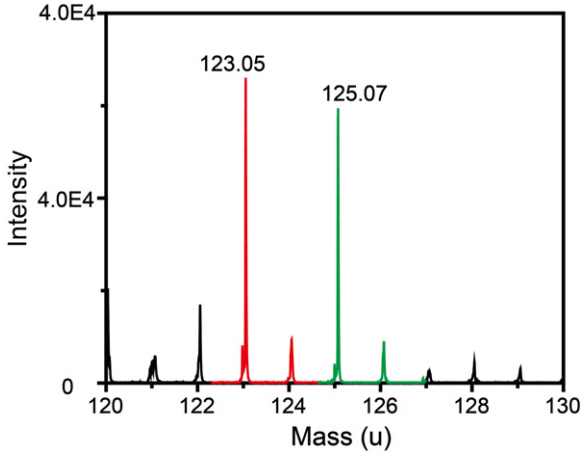


Fig. S7 ToF-SIMS monitored the coenzyme activities of electro-reduced NADH on electrode surface.

6. The ^{1}H NMR, ^{13}C NMR, ^{31}P NMR and MS Characterization of Target Compounds and Intermediates

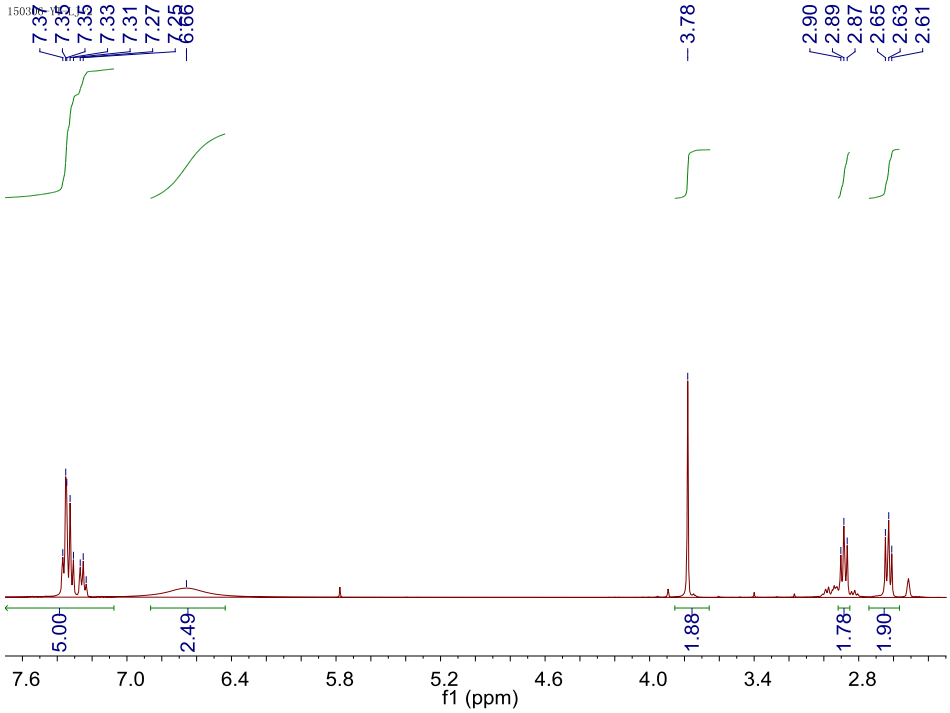


Fig. S8 ^{1}H NMR spectrum of 2-(benzylthio)ethanamine (b-2).

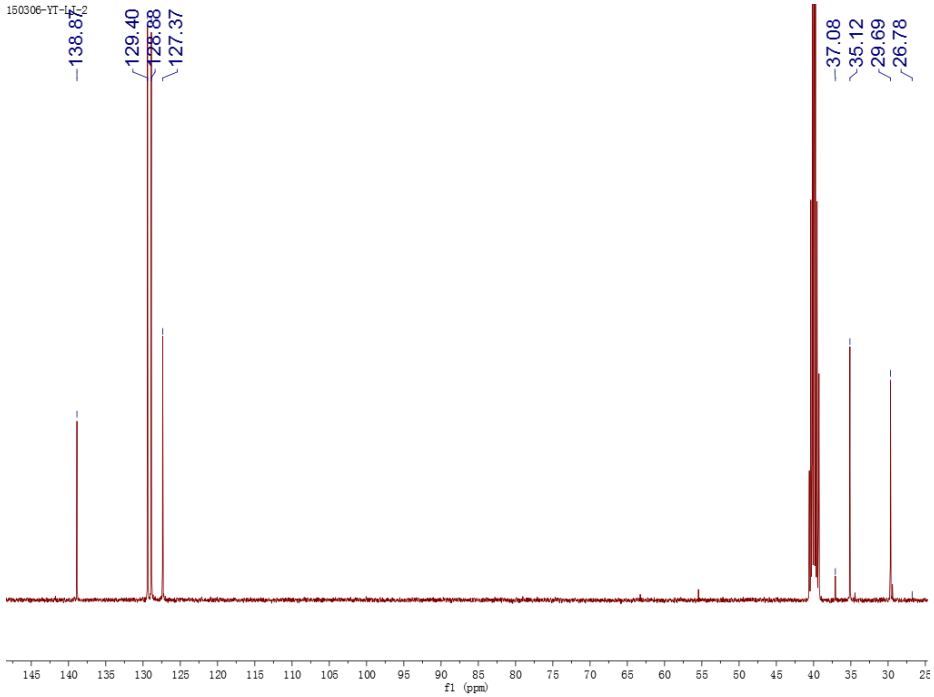
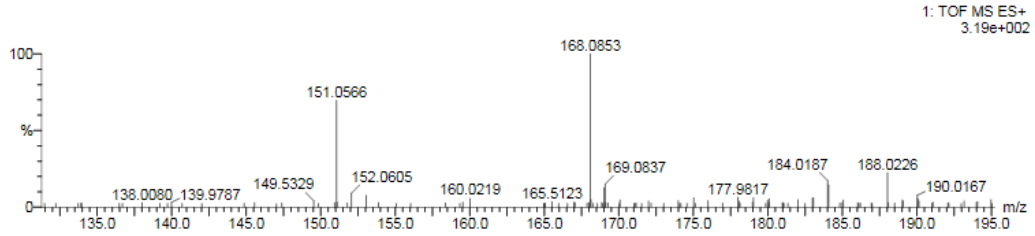


Fig.S9 ^{13}C NMR spectrum of 2-(benzylthio)ethanamine (b-2).



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
168.0853	168.0847	0.6	3.6	3.5	49.2	0.0	C9 H14 N S

Fig. S10 Mass spectrum of 2-(benzylthio)ethanamine (b-2).

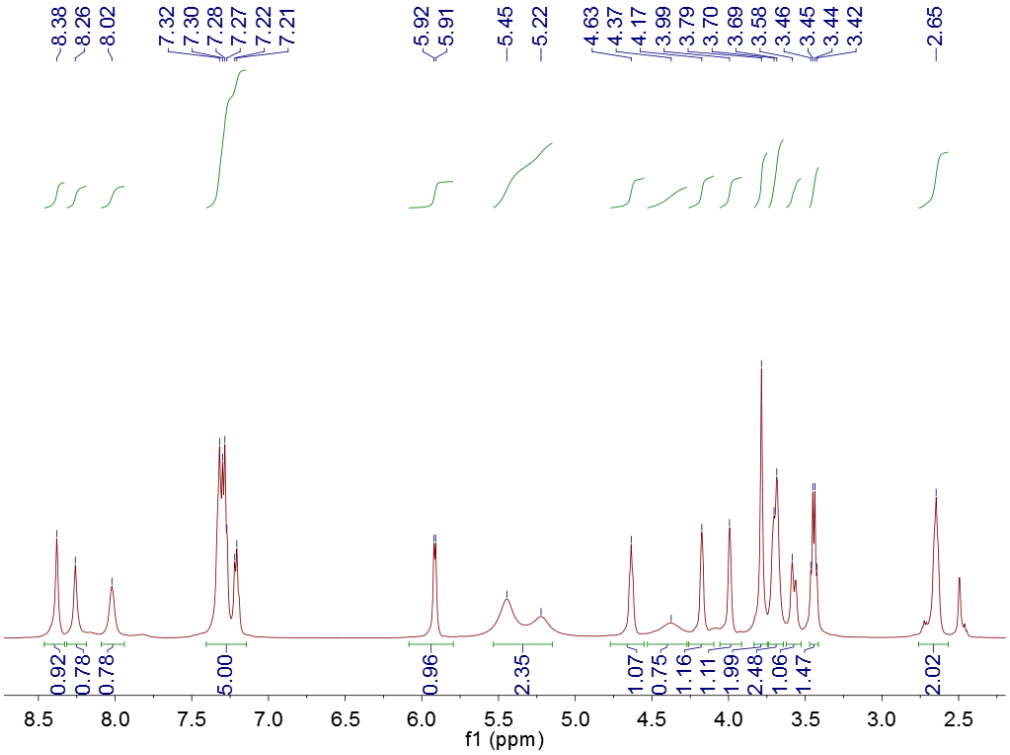


Fig. S11 [1]H NMR spectrum of (2R, 3R, 4S, 5R)-2-(6-((2-(benzylthio) ethyl) amino)-9H-purin-9-yl)-5-(hydroxymethyl) tetrahydrofuran-3, 4-diol (d-2).

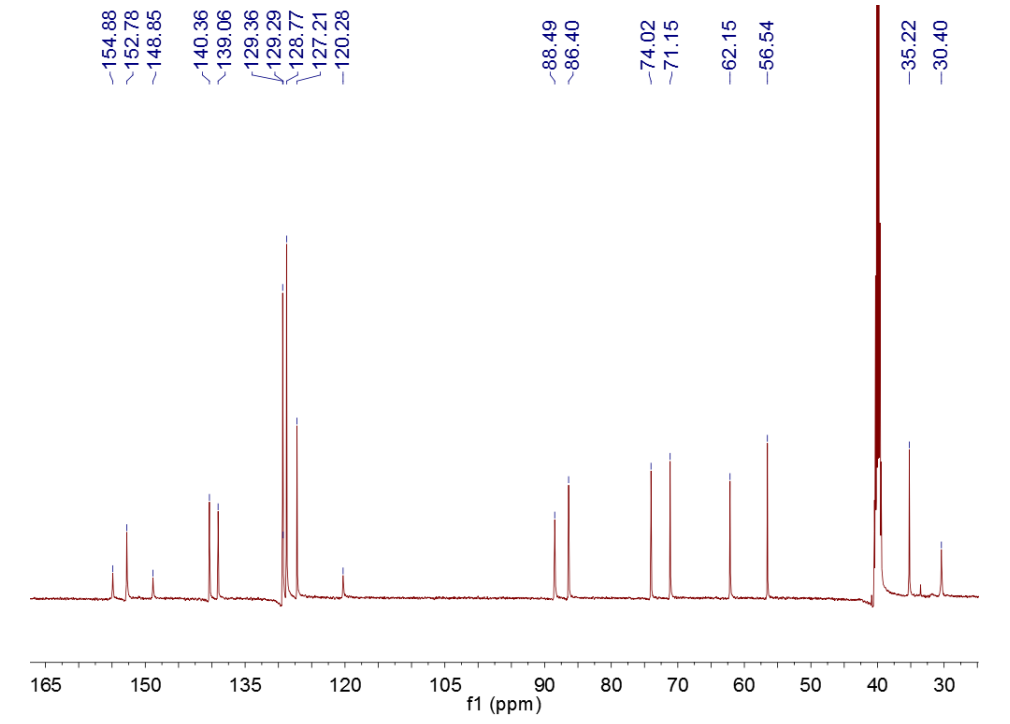
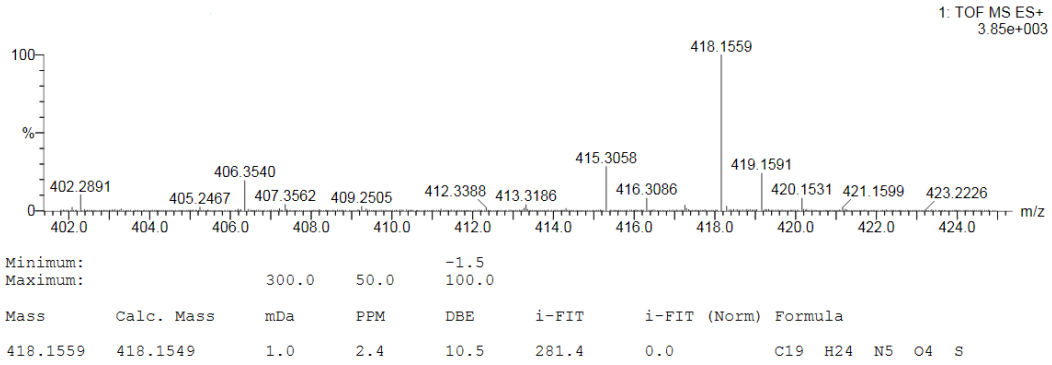


Fig. S12 [13]C NMR spectrum of (2R, 3R, 4S, 5R)-2-(6-((2-(benzylthio) ethyl) amino)-9H-purin-9-yl)-5-(hydroxymethyl) tetrahydrofuran-3, 4-diol (d-2).



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
418.1559	418.1549	1.0	2.4	10.5	281.4	0.0	C19 H24 N5 O4 S

Fig. S13 Mass spectrum of (2R, 3R, 4S, 5R)-2-(6-((2-(benzylthio) ethyl) amino)-9H-purin-9-yl)-5-(hydroxymethyl) tetrahydrofuran-3, 4-diol (d-2).

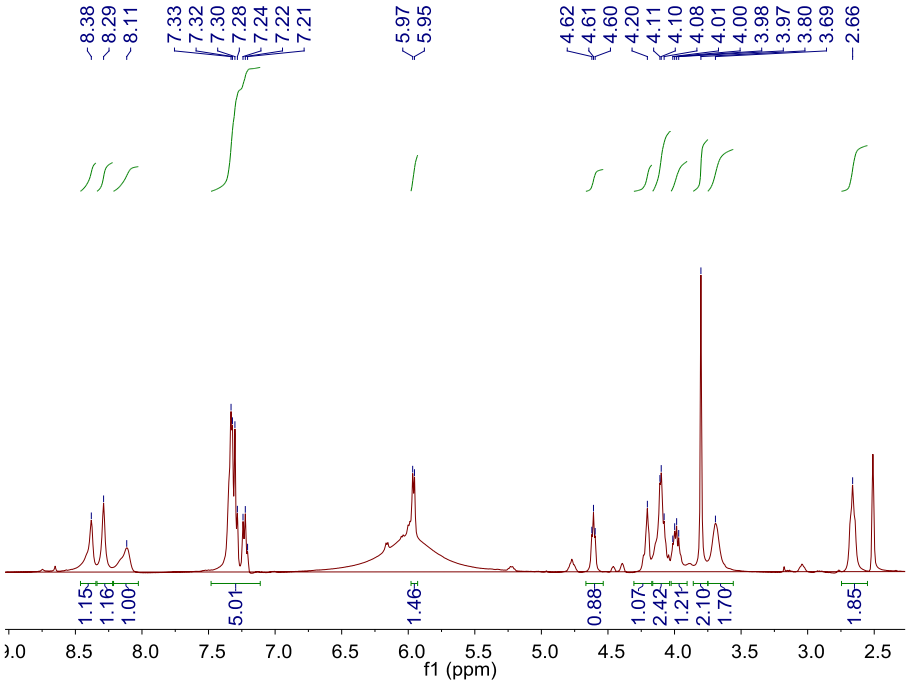


Fig. S14 ^{1}H NMR spectrum of ((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio) ethyl) amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl) methyldihydrogenphosphate (d-3).

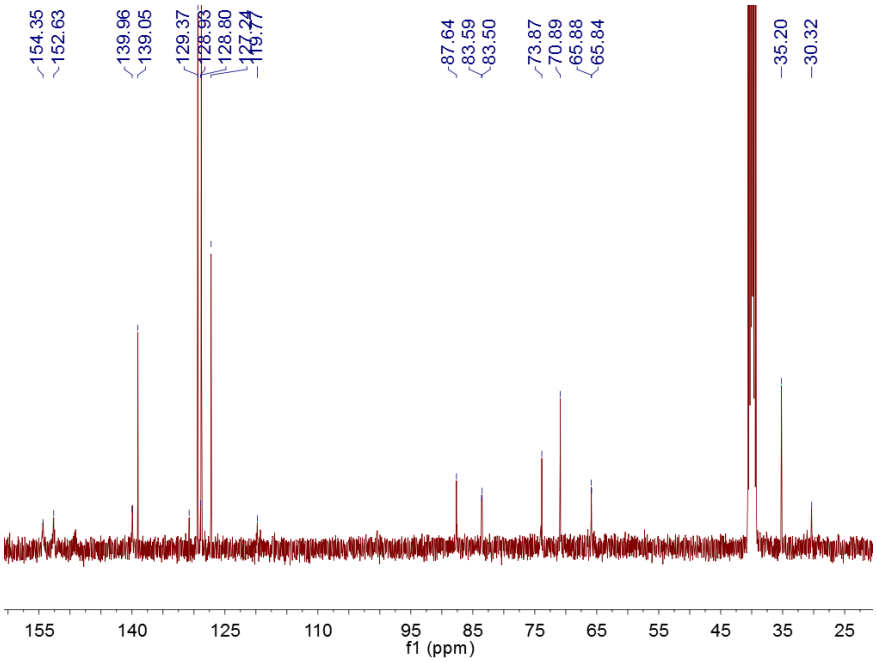


Fig. S15 ^{13}C NMR spectrum of ((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio)ethyl)ami no)-9H-purin-9-yl)-3, 4-dihydroxytetrahydrofuran-2-yl)methyldihydrogenphosphate (d-3).

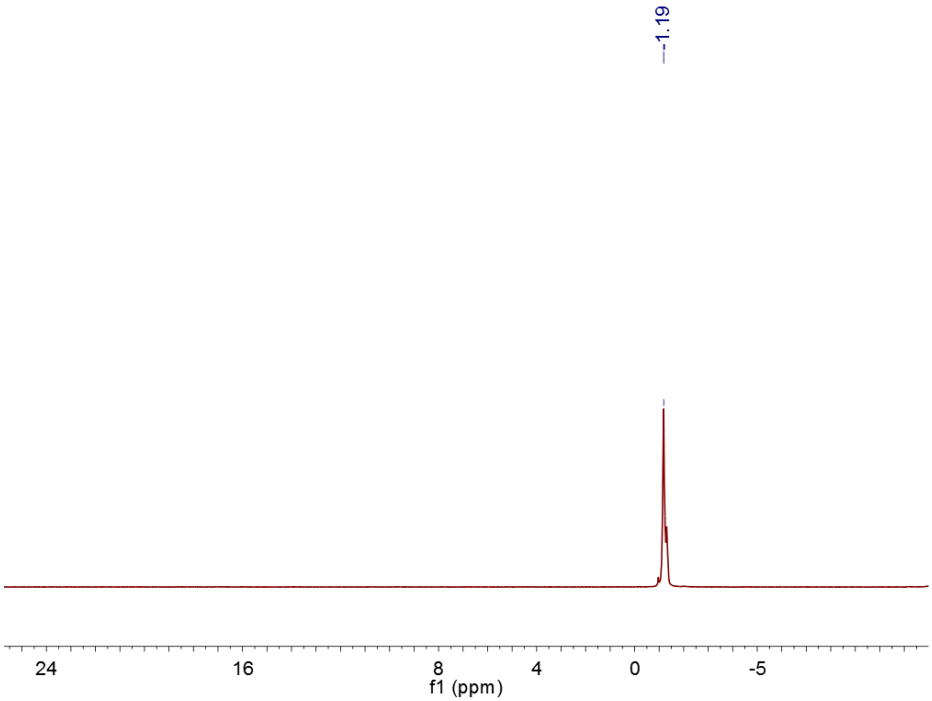
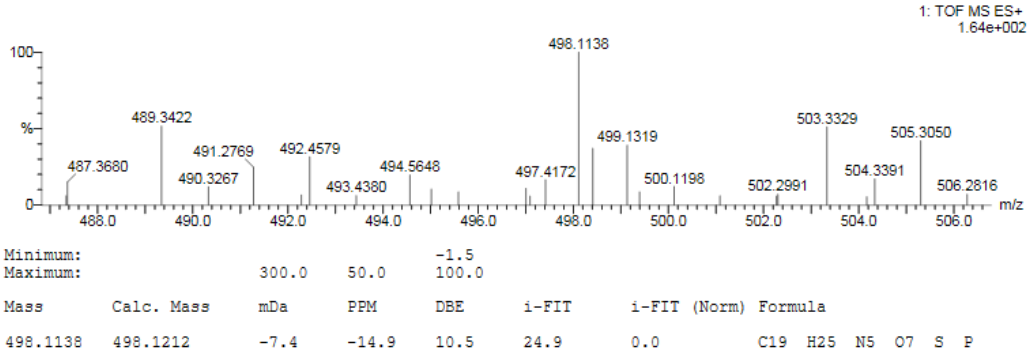


Fig. S16 ^{31}P NMR spectrum of ((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio) ethyl)ami no)-9H-purin-9-yl)-3, 4-dihydroxytetrahydrofuran-2-yl) methyldihydrogenphosphate (d-3).



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
498.1138	498.1212	-7.4	-14.9	10.5	24.9	0.0	C19 H25 N5 O7 S P

Fig. S17 Mass spectrum of ((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio) ethyl) amino)-9H-purin-9-yl)-3, 4-dihydroxytetrahydrofuran-2-yl) methyl dihydrogen phosphate (d-3).

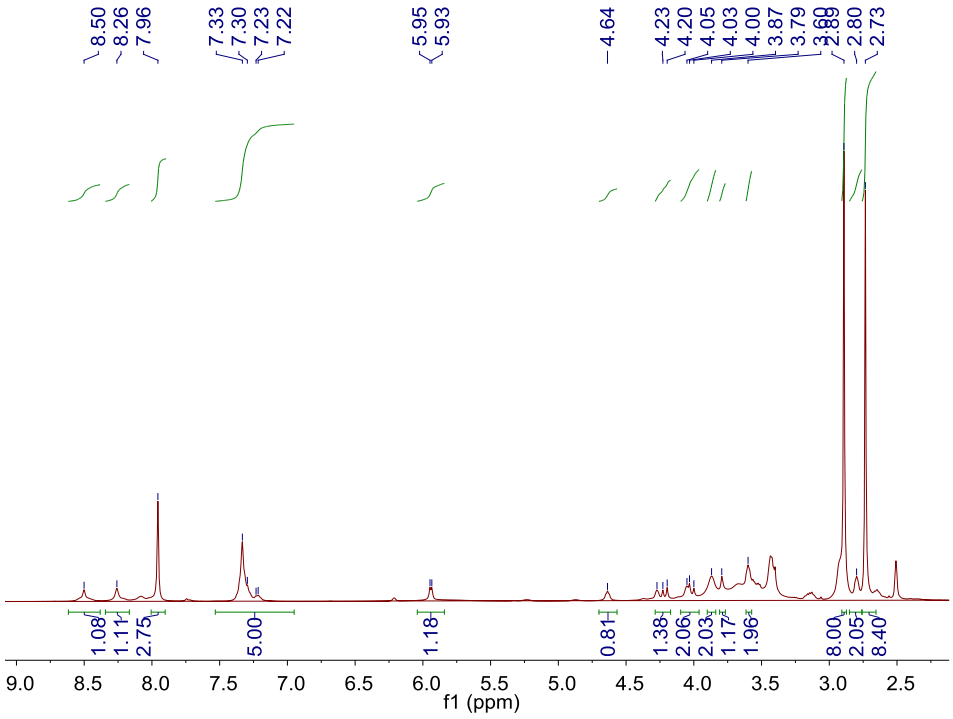


Fig. S18 ^{1}H NMR spectrum of ((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio)ethyl)amino)-9H-purin-9-yl)-3, 4-dihydroxytetrahydrofuran-2-yl)methyl hydrogen morpholinopho sphornate (d-4).

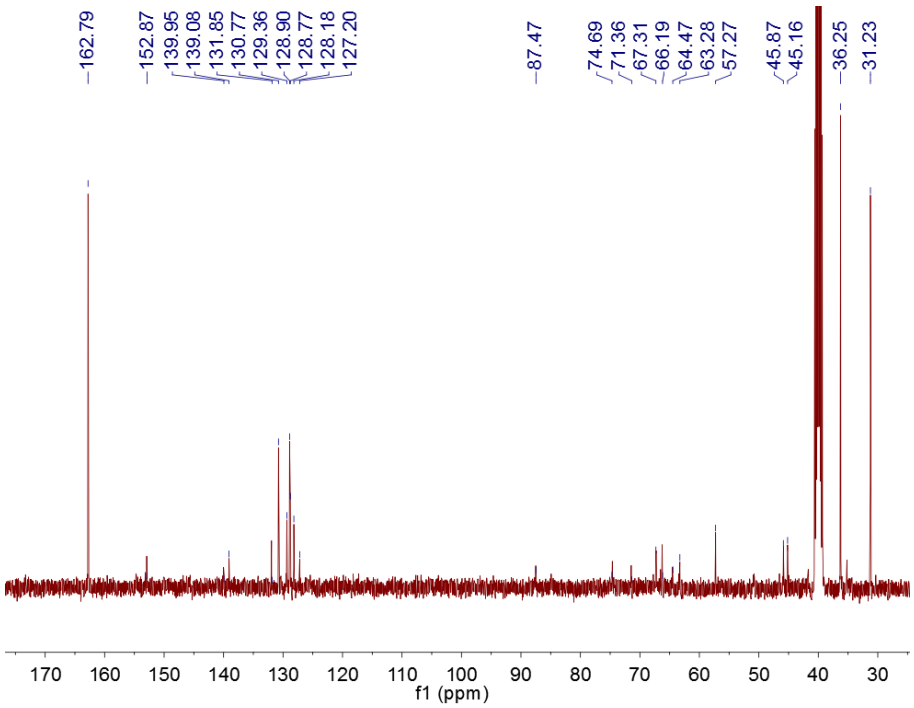


Fig. S19 ^{13}C NMR spectrum of ((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio)ethyl)amin o)-9H-purin-9-yl)-3, 4-dihydroxytetrahydrofuran-2-yl)methyl hydrogen morpholinoph osphornate (d-4).

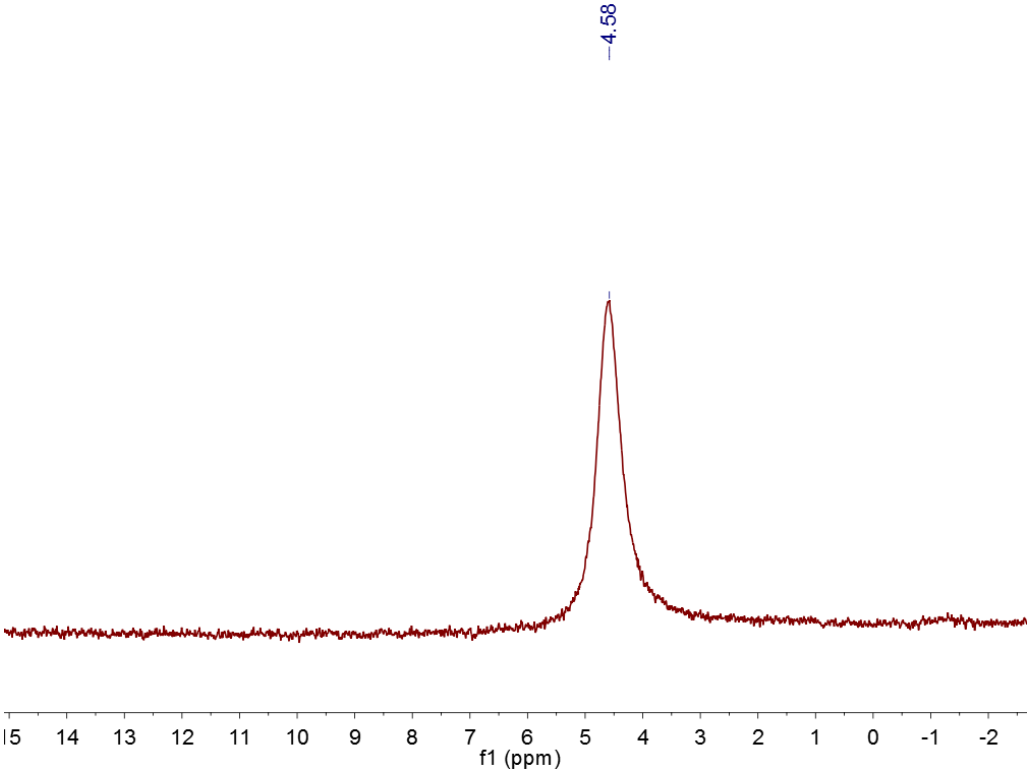
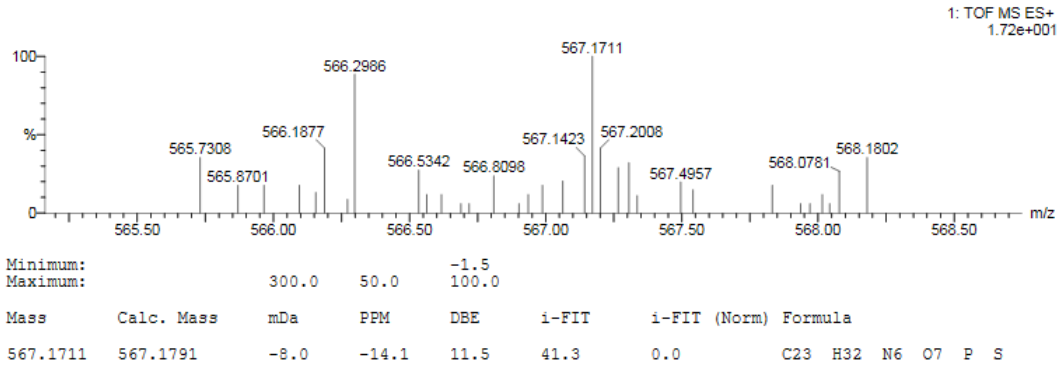


Fig. S20 ^{31}P NMR spectrum of ((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio)ethyl)amin o)-9H-purin-9-yl)-3, 4-dihydroxytetrahydrofuran-2-yl)methyl hydrogen morpholinoph osphornate (d-4).



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
567.1711	567.1791	-8.0	-14.1	11.5	41.3	0.0	C23 H32 N6 O7 P S

Fig. S21 Mass spectrum of ((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio)ethyl)amino)-9 H-purin-9-yl)-3, 4-dihydroxytetrahydrofuran-2-yl)methyl hydrogen morpholinophosp hornate (d-4).

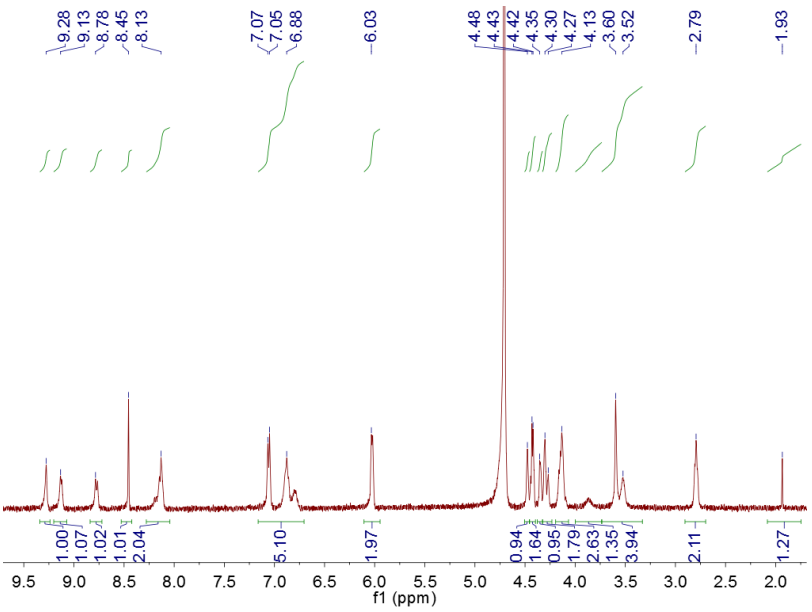


Fig. S22 ^{1}H NMR spectrum of 1-((2R,3R,4S,5R)-5-(((((((2R,3S,4R,5R)-5-(6-((2-(benzylthio)-ethyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)oxidophosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-3,4-dihydroxytetrahydrofuran-2-yl)-3-carbamoylpyridin-1-ium (PhSNAD).

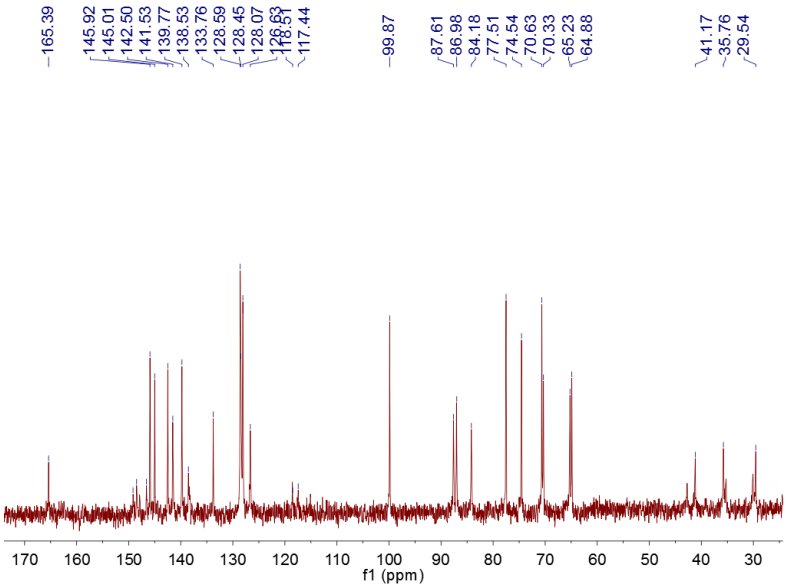


Fig. S23 ^{13}C NMR spectrum of 1-((2R, 3R, 4S, 5R)-5-(((((((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio)-ethyl) amino)-9H-purin-9-yl)-3, 4-dihydroxytetrahydrofuran-2-yl)methoxy)oxidophosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-3, 4-dihydroxytetrahydrofuran-2-yl)-3-carbamoylpyridin-1-ium (PhSNAD).

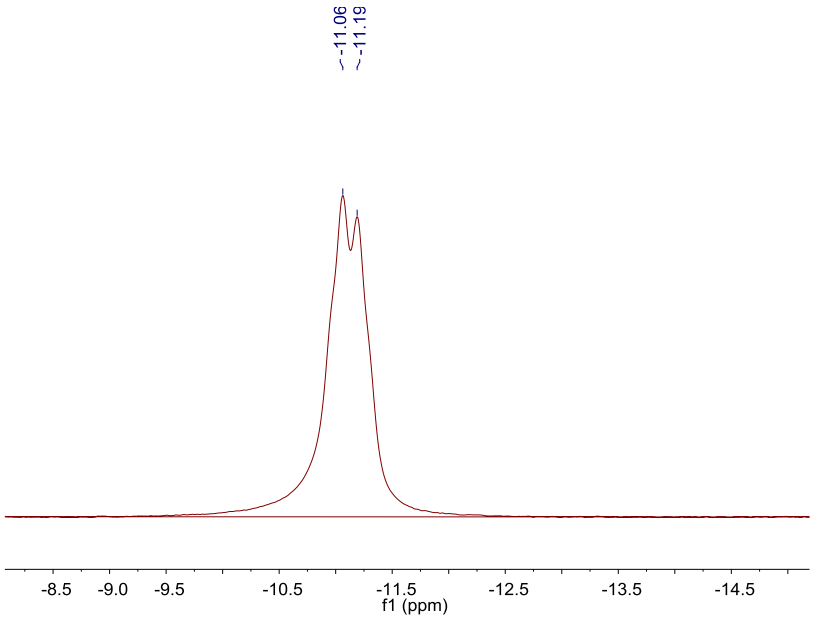


Fig. S24 ^{31}P NMR spectrum of 1-((2R,3R,4S,5R)-5-(((((((2R,3S,4R,5R)-5-(6-((2-(benzylthio)-ethyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)oxidophosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-3,4-dihydroxytetrahydrofuran-2-yl)-3-carbamoylpyridin-1-ium (PhSNAD).

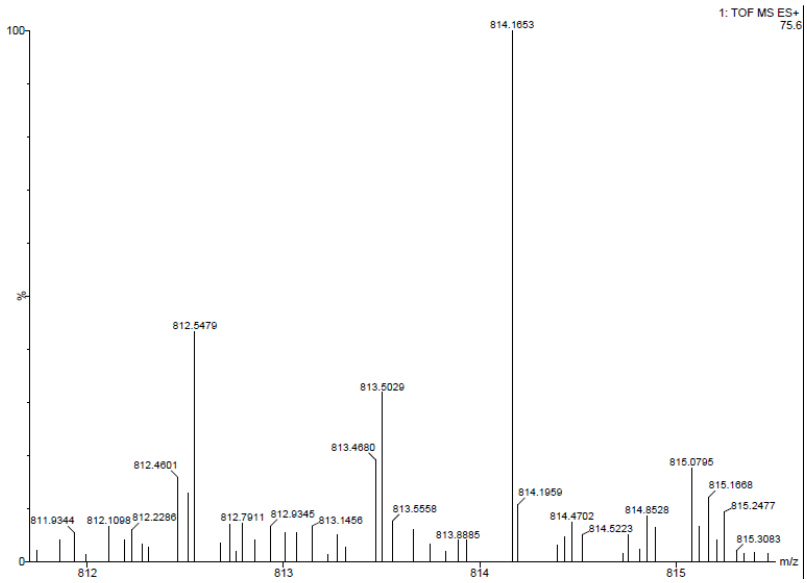


Fig. S25 Mass spectrum of 1-((2R, 3R, 4S, 5R)-5-(((((((2R, 3S, 4R, 5R)-5-(6-((2-(benzylthio)-ethyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methoxy)oxidophosphoryl)oxy)(hydroxy)phosphoryl)oxy)methyl)-3,4-dihydroxytetrahydrofuran-2-yl)-3-carbamoylpyridin-1-ium (PhSNAD).