Synthesis and electrochemical behavior of triazole-containing nicotinamide adenine dinucleotide analogs

Wujun Liu, Shuhua Hou, and Zongbao Kent Zhao

Abstract: The coupling of 2′,3′-di-*O*-acetyl nicotinamide mononucleotide with 3-butyn-1-ol in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride quantitatively afforded a terminal alkyne-containing intermediate. Furthermore, copper(I)-mediated Huisgen [3 + 2] cycloaddition with a series of azido compounds in a two-phase solvent system gave eight triazole-containing nicotinamide adenine dinucleotide analogs with yields over 88%. The cyclic voltammetric behaviors of these novel analogs were investigated with a glassy carbon electrode, and structural features of these analogs on their electrochemical properties were briefly discussed.

Key words: nicotinamide adenine dinucleotide, analog, click chemistry, cyclic voltammetry.

Résumé : Le couplage du mononucléotide du 2′,3′-di-O-acétylnicotinamide avec le but-3-yn-1-ol, en présence de chlorure de 2,4,6-triisopropylbenzènesulfonyle conduit à la formation pratiquement quantitative d'un intermédiaire comportant un alcyne terminal. Une cycloaddition ultérieure [3 + 2] de Huisgen, catalysée par le cuivre(I), avec une série de composés azide, dans un système liquide à deux phases, conduit à la formation, avec des rendements globaux supérieurs à 88 %, d'analogues dinucléotides d'adénine nicotinamide contenant un triazole en position 8. On a étudié les comportements en voltampérométrie cyclique de ces nouveaux analogues à l'aide d'une électrode de carbone vitreux et on discute brièvement des caractéristiques structurales de ces analogues sur leurs propriétés électrochimiques.

Mots-clés : dinucléotide de l'adénine nicotinamide, analogue, chimie par clic, voltampérométrie cyclique.

[Traduit par la Rédaction]

Introduction

Nicotinamide adenine dinucleotide (NAD^+) and its reduced form, NADH, are involved in numerous biological systems, especially as cofactors for enzymes mediating oxidoreductive reactions.[1] In those oxidoreductive reactions, NAD(H) transfers hydrogen and electron shuttles, along with chemical potential change. The chemical potential of NAD^+ or its derivatives play critical roles in calcium homeostasis,[2] cross-membrane transportation,[3] oxidative stress resistance,[4,5] and reactive oxygen species clearance.[6] Although electrochemical studies have been done on a few model compounds,[7] and on applying a novel method[8] or materials[9] for modification of the surface of the electrode to improve the sensitivity of electrode response to NAD(H), there are few reports on testing the electrocatalytic reduction of synthetic NAD^+ analogs.

NAD^+ is a coupled product of the nicotinamide mononucleotide (NMN) part and the adenosine monophosphate (AMP) moiety (Fig. 1). The NMN part bestows its oxidoreductive property, whereas the AMP moiety is largely involved in binding in the biological environment. Early study showed that a simple phosphodiester-type NAD^+ analog could be recognized by horse liver alcohol dehydrogenase, and that the system could be applied for reduction of prochiral ketones in moderate yields.[10,11] Thus, it is essential to have the NMN part in NAD^+ analogs so that their prospects as a redox chemistry mediator can be further explored. In these reactions, a synthetic cofactor was regenerated with valuable rhodium complexes, but low-cost electrochemical or photo driven regeneration of the NAD^+ cofactor led to a complex reaction.[12,13] One strategy to overcome these deficiencies is to modify the structure of the cofactor to improve electrode reaction activity.

We were recently interested in the preparation of NAD^+ analogs in which one phosphorus atom is removed from the original skeleton to meliorate electrode reaction activity of the natural coenzyme. In this work, we wish to report our efforts on the construction of 1,2,3-triazole-containing NAD^+ analogs (Fig. 1) and their electrochemical properties. We developed an efficient synthetic strategy for synthesis of these analogs based on $CuSO_4$/sodium ascorbate-mediated Huisgen's 1,3-dipolar cycloaddition, or click reaction.[14]

Received 10 July 2009. Accepted 14 September 2009. Published on the NRC Research Press Web site at canjchem.nrc.ca on 16 December 2009.

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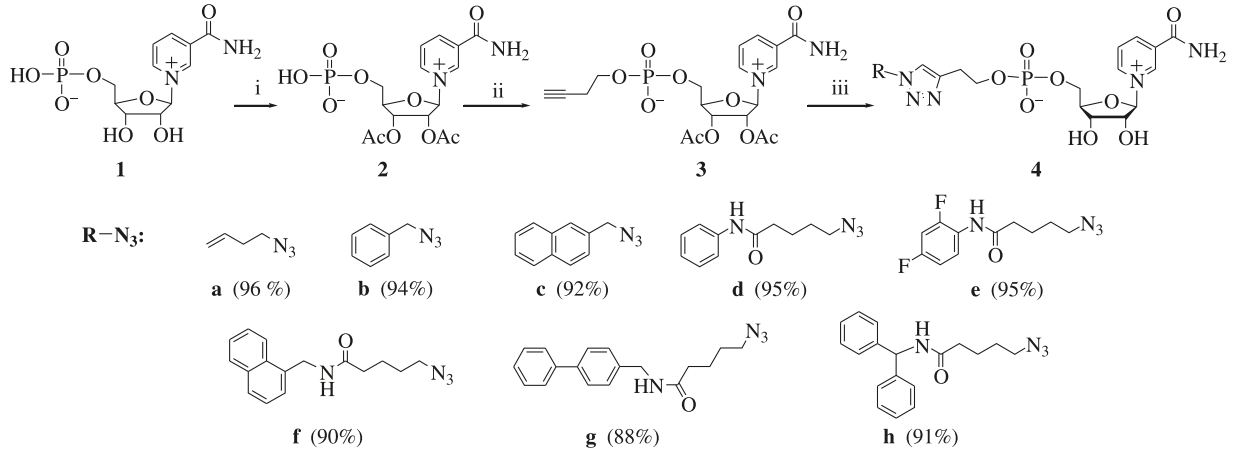
Scheme 1. Synthesis of triazole-containing NAD+ analogs. Reagents and conditions: (i) Ac_2O/Py (1:1), 0–5 °C, 24 h, 98%; (ii) 3-butyn-1-ol (3 equiv.), TIPS-Cl (3 equiv.), DMF/Py (1:1), RT, 3 h, 99%; (iii) (*a*) R–N_3, $CuSO_4$/sodium ascorbate, H_2O/DCM (1:1), RT, 0.5–6 h, 90%–98%, (*b*) NH_4HCO_3 (1 mol/L), in H_2O/MeOH (1:10), 98%. Data in the parentheses indicate the total isolated yield of each compound on Ac_2NMN.



Compared to NAD+, these analogs showed relatively low reductive peak potentials (E_p) and a distinct improvement of the electrode reaction rate constant (K_s).

Results and discussion

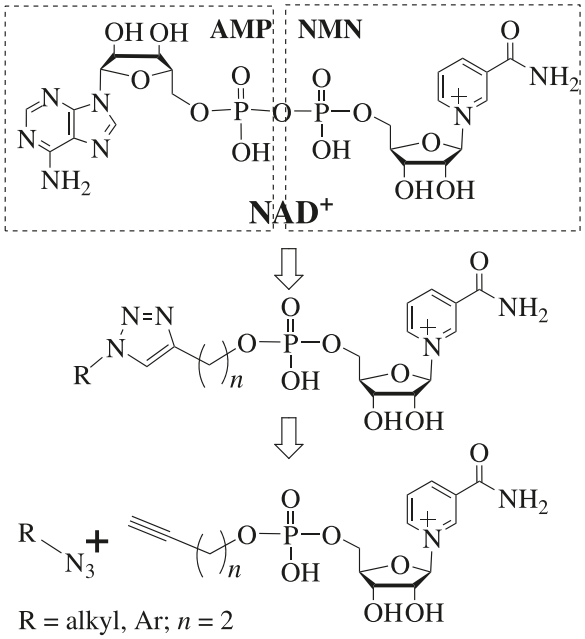
Synthesis of triazole-containing NAD+ analogs

Our in-house preparation of NMN was realized via selective hydrolysis of NAD+ using $ZrCl_4$ as catalyst.[15] To synthesize the triazole-containing NAD+ analogs, we originally tried to couple NMN with the corresponding alkynyl alcohol followed by click reaction with azides (Fig. 1). Because NMN is liable to form an inactive inner salt and has notoriously low solubility in most organic solvents (i.e., DMF (dimethylformamide), pyridine, MeCN), it was difficult to find an appropriate solvent system to realize the coupling reaction. Moreover, C–N bond breakage of the NMN molecule was substantial in many experiments. We then treated NMN with a mixture of Ac_2O and pyridine at 0–5 °C, leading to the acetylated product of NMN, 2′,3′-di-*O*-acetyl nicotinamide mononucleotide (Ac_2NMN). It turned out that Ac_2NMN had much better solubility and higher activity. Thus, the coupling of Ac_2NMN with excess 3-butyn-1-ol in DMF/Py (1:1, *v*/*v*) in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TIPS-Cl) at room temperature for 3 h afforded the key intermediate of 2′,3′-di-*O*-acetyl β-nicotinamide ribose-5′-3-butynyl phosphate, **3**, in a 99% yield based on ^{31}P NMR analysis (Scheme 1).

To convert compound **3** into triazole-containing NAD+ analogs, we prepared eight azides (Scheme 1). These compounds were selected to mimic the adenine moiety of the NAD+ structure, mainly depending on their accessibility and aromatic ring structures. Azides **a**–**c** were obtained from the corresponding halides via a nucleophilic substitution using sodium azide, whereas azides **d**–**h** were made via acylation of the corresponding amines with 5-chloropentanoyl chloride followed by a nucleophilic substitution reaction using sodium azide.[16]

With intermediate **3** and azide compounds in hand, we

Fig. 1. Structures of NAD+ and its triazole-containing analogs.



carried out cycloaddition experiments in the presence of copper sulfate and sodium ascorbate according to the strategy described by Sharpless and co-workers.[17] Upon testing a couple of solvent systems, i.e., water mixed with DMSO, DMF, DCM, THF, MeCN, or *t*-BuOH, we found that there was no significant difference whether the organic solvent was soluble or insoluble in water. This was likely because compound **3** and the coupled products had excellent solubility in water. To facilitate an easier recycling of azides, our synthesis was carried out in a two-phased system made of DCM and H_2O. Using this strategy, we were successful in coupling azides **a**–**h** with **3** at room temperature in over 90% yields within 6 h. For most reactions, 5 mol% Cu(I) catalyst was enough to convert **3** within 2 h. The bulky azides **f** and **h** required 4 h for better yields. Because azide **g** had a poor solubility, up to 6 h was necessary to achieve

excellent conversion. We also tried the combination $CuSO_4$/Cu to mediate the coupling reaction. However, the products seemed liable to coordinate with copper, leading to a tedious purification process and low isolated yields.

It was interesting to note that those triazole-containing intermediates could be easily deacetylated by 1 mol/L NH_4HCO_3 solution in H_2O/MeOH (1:10, *v*/*v*). Thus, crude compound **4** was achieved during the reverse phase silica gel chromatography purification.

Because NAD^+ analogs are amphiphilic molecules containing both positive and negative charges, purification usually presents a major barrier to achieve an appreciable quantity of target compounds in the lab. Thus, successful procedures varied from case to case for the isolation of analogs **4a**–**4h**. Chromatography with various supporting materials, including activated charcoal, silica gel, octyl-functionalized silica gel, ion exchange resins, and Bio-Gel P-2 resin has been applied in different combinations to fulfill valid purification. Compound **4a** was purified with octyl-functionalized silica gel and 201 × 2 HCO_2^- form of the anion resin followed by Bio-Gel P-2 resin. For compounds **4b**, **4d**, and **4e**, chromatography on octyl-functionalized silica gel and anion resin, at different elution conditions, gave the best results. For compounds **4c** and **4f**–**4h**, chromatography on octyl-functionalized silica gel and Bio-Gel P-2 resin gave our target molecules.

Electrochemical behavior of triazole-containing NAD^+ analogs

With novel analogs in hand, we investigated their electrochemical performance on a glassy carbon (GC) electrode in aqueous solution. Oxido-reductive behaviors of these triazole-containing NAD^+ analogs were estimated by cyclic voltammetry. The redox curves were also a two electron mechanism according to the Angulo et. al method.[18] Upon a reversal scan of the NAD^+ analogs, both the reduction peak and reoxidation peak were observed, albeit the latter was much weaker than the former. The corresponding reoxidation peaks were stronger than that of NAD^+ under the same conditions. Thus, the overall processes are not reversible.

The reductive potential (E_p) of the triazole-containing analog (–1.141 V) was much lower than that of natural NAD^+ (–1.269 V; Table 1). Upon four cyclic reversal scans, marginal decreases in the reductive peak currents of NAD^+ analogs were observed, and the decrease range of **4h** was much smaller than that of NAD^+ under the same conditions (Fig. 2). This indicated that triazole-containing analogs had relatively weak absorption on GC than natural NAD^+.

The cathodic peak currents (i_p) of triazole-containing analogs increased curvilinearly with the square root of the scan rate (v), an upper warp curve in the range of 20–200 mV s^{-1}. All the upper data resulted in the reduction of triazole-containing NAD^+, which was an absorption-controlled irreversible process under our conditions.

According to Laviron theory,[19] absorption-controlled irreversible process potential (E_p) is determined based on the following equation:

$$[1] \qquad E_p = E^\circ - (RT/\alpha nF)\ln(\alpha nF/RTK_s) - (RT/\alpha nF)\ln v$$

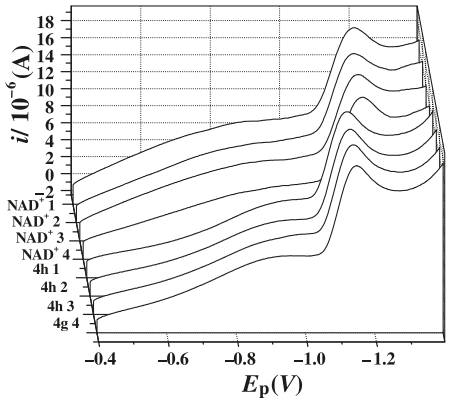
where E°, ln v, R, T, F, n, K_s, and α, represent the standard potential, logarithm of the scan rate (v), gas constant, absolute temperature, Faraday constant, the transfer electron number (n = 2 for NAD^+ or its derivatives), electrode reaction rate constant, and electron transfer coefficient, respectively. The value of E° of all compounds (vs the standard calomel electrode (SCE)) was obtained from the intercept of the plot of E_p vs. v (data not shown). The electrode reaction rate constant (K_s) and the electron transfer coefficient (α) were calculated from the slope and intercept of E_p vs. ln v according to the above equation (Fig. 3*a*). Accordingly, all triazole-containing NAD^+ analogs and related compounds were tested and results were listed in Table 1.

Table 1. Electrochemical dynamic constants of triazole-containing NAD^+ analogs (**4a**–**4h**) and related compounds.

Compound	E_p (V)	K_s (s^{-1})	α
4a	–1.157	0.24	0.39
4b	–1.145	0.23	0.38
4c	–1.148	0.23	0.36
4d	–1.154	0.23	0.35
4e	–1.159	0.20	0.34
4f	–1.142	0.23	0.36
4g	–1.145	0.23	0.36
4h	–1.141	0.24	0.40
NAD^+	–1.269	0.11	0.22
NMN	–1.183	0.12	0.28
NAR	–1.211	0.12	0.21

Note: All data were the average value of three replicates.

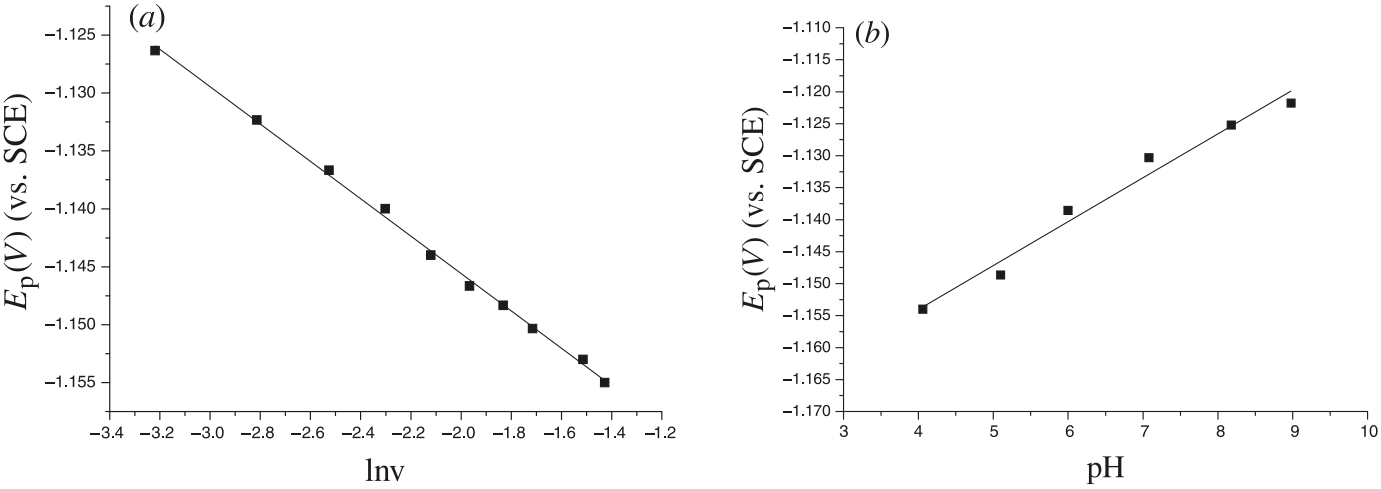
Fig. 2. Overlay of the continuous reductive curves of NAD^+ and **4h** under the same conditions.



It was obvious that analogs with bulky aromatic rings and **4b** have relatively low reductive peak potentials. Compound **4a** and **4h** have the largest K_s and α. Compound **4e** shows the lowest K_s and α in tris(hydroxymethyl)aminomethane (Tris)/NaCl buffer, which may be due to the presence of aromatic fluorine atoms in the molecule. To investigate the electrochemical property in more detail, we carried out additional experiments on **4h**.

To make a comparison, we also obtained the correlative constants of NAD^+, NMN, and nicotinamide ribose (NAR) under identical conditions. It was clear that NAD^+ and NAR had similar reduction peak potentials (E_p) and NMN had a more positive E_p value. However, all NAD^+ analogs had even more positive E_p values. Thus, substitution of

Fig. 3. (*a*) Linear plot of E_p vs. ln v of NAD$^+$ analog **4h** (r = 0.9986). (*b*) Linear plot of E_p vs. pH of the NAD$^+$ analog **4h**.



AMP moiety of the NAD$^+$ skeleton with a triazole-containing structure could increase the E_p of the corresponding product.

K_s and α of **4h** were more than twofold larger than that of other compounds, especially to natural NAD$^+$. That indicated that the reduction of NAD$^+$ analog **4h** on a GC electrode was faster than natural NAD$^+$ and related compounds. Thus, modification of pyrophosphate made NAD$^+$ reduce at a relatively lower potential and the triazole ring in the analog molecule made it reduce faster than natural NAD$^+$. The triazole ring increased the charge transfer efficiency, which is similar to Zhou et al.'s[20] results. Thus, we concluded that triazole-containing NAD$^+$ analogs were more liable to be reduced than other compounds on a GC electrode.

To investigate the reoxide stability of **4h** at different pHs, the peak reductive potential of the NAD$^+$ analog was measured in 1 mol/L phosphate buffer containing 10^{-3} mol/L **4h** at different pHs (pH = 4–9). It was found that the E_p increased linearly with pH and that reduction of **4h** would be much easier at a higher pH (Fig. 3*b*). It should be noted that **4h** was stable at these selected pHs and could be recovered by reverse-phase column chromatography in a 96% yield. However, no such correlations were observed for NAD$^+$, NMN, or NAR at identical conditions (data not shown).

Based on the pK_a of nicotinamide and triazole, the amino group of nicotinamide and nitrogen atom of the triazole ring was protonated below pH 6. Thus, the NAD$^+$ analogs existed as an inner salt in solution at a wide range of pHs. The triazole ring had an interaction with H$^+$ and promoted conductance of the charge at a relatively high concentration of H$^+$, so a higher E_p was needed to reduce analogs. When the pH was higher than 7, interaction between the triazole ring and proton on the NAD$^+$ analog gradually disappeared. The repulsion of the triazole ring with the hydroxide anion made analog **4h** reduce at a relatively lower potential.

Conclusion

An efficient strategy to prepare novel triazole-containing NAD$^+$ analogs was developed. These compounds had a triazole ring and phosphatediester linkage as mimics of the adenosine heterocyclic ring and pyrophosphate, respectively. These analogs were more liable to reduce than NAD$^+$ on a GC electrode in Tris/NaCl buffer. Triazole analogs showed a stable redoxide property at different pHs. The reductive peak potential of **4h** increased linearly with the solution pH. The triazole-containing analogs could be broadly applied in cofactor regeneration or biosensor. We are now exploring other special biological functions with these compounds in a wide variety of chemical and biological areas, and results will be reported in due course.

Experimental section

General

All reagents were analytical grade, obtained from commercial suppliers (ABCR, ACROS, or Sigma-Aldrich), and used without further purification. NMR spectra were measured with a Bruker DRX-400 spectrometer (400.3 MHz for ^{1}H NMR, 100.6 MHz for ^{13}C NMR, 160.1 MHz for ^{31}P NMR, and 376 MHz for ^{19}F NMR) at 298 K. High resolution mass spectroscopy was obtained on a LC/Q-TOF-MS and operated with an electrospray source in positive ion mode. F254 thin-layer and silica gel (400 mesh) were purchased from Yantai Jiangyou Silica Co., Ltd., China. Octyl-functionalized silica gel was purchased from Sigma-Aldrich. Ion exchange resin (100 ~ 200 mesh) was purchased from the Chemical Plant of Nankai University, Tianjin, China. Bio-Gel P-2 resin (45 μm) was obtained from Bio-Rad Laboratories, Inc. All reactions were carried out under a nitrogen atmosphere. UV–vis spectra were obtained at room temperature on a JASCO V-530 UV–vis spectrophotometer. The electrochemical measurements were performed with a CHI 600C electrochemical analyzer (CH Instruments, USA) connected to a personal computer. All experiments were performed using a conventional three-electrode system with a glassy carbon (GC) disc (CHI 104, 3 mm diameter) working electrode, a saturated calomel reference electrode (CHI 150), and a platinum wire as the counter electrode (CHI 115).

Preparation of Ac_2NMN (2)

To a mixture of pyridine (7.5 mL) and Ac_2O (7.5 mL) was added NMN (275 mg, 0.82 mmol) in H_2O (160 μL)

at –5 °C. The suspension was kept at 0 °C until all solids dissolved. The solvents were removed and the residue was dissolved in a mixture of H_2O (2 mL) and pyridine (2 mL). The yellow solution was stirred at room temperature for 2 h and the solvents were evaporated at reduced pressure. The residue was lyophilized to give Ac_2NMN as an amorphous solid, which was used directly in the next reaction without additional purification.[21]

Preparation of alkynyl ester (3)

Ac_2NMN (344 mg, 0.82 mmol) and 3-butyn-1-ol (3 equiv. to Ac_2NMN) were dissolved in Py/DMF (1:1, *v*/*v*) under a nitrogen atmosphere. TIPS-Cl (3 equiv. to Ac_2NMN) was added and the mixture was stirred at room temperature for 3 h in a 99% yield based on ^{31}P NMR. The solvent was removed, and the residue was added to 10 mL of H_2O and extracted with DCM (15 mL × 3). The aqueous solution was concentrated, purified via ion exchange column chromatography on anion resin (201 × 2, HCO_2^- form) eluted with H_2O. Fractions were concentrated and lyophilized to give crude **3**, and it was used in the next reaction without further purification.

General method for the preparation of azide compounds (a–h)

Azides (**a**–**c**) in Scheme 1 were synthesized via a nucleophilic substitution using sodium azide, whereas azides **d**–**h** were made via acylation of the corresponding amines with 5-chloropentanoyl chloride followed by a nucleophilic substitution reaction using sodium azide.[16]

General method for the preparation of NAD$^+$ analogs (4a–4h)

A solution of compound **3** in H_2O and azide in DCM (or other solvent) was mixed. To the mixture was added an appropriate amount of $CuSO_4$/sodium ascorbate (5 mol%) in one portion. After the reaction was stirred at room temperature for 0.5–6 h, the organic layer was removed, and the aqueous phase was purified by octyl-functionalized silica gel eluted with water and 1 mol/L NH_4HCO_3 solution in CH_3OH/H_2O (1:10, *v*/*v*) to give the crude product. The product was further purified via anion resin (201 × 2, HCO_2^- form) or size-exclusion chromatography Bio-Gel® P-2 Gel polyacrylamide gel. The corresponding fractions were pooled and freeze dried to give the triazole-containing analogs.

Compound 4a

Compound **4a** was purified by anion resin (HCO_2^- form) column chromatography eluted with H_2O and the fraction was concentrated, followed by size-exclusion chromatography Bio-Gel® P-2 Gel polyacrylamide gel, eluted with a 25 mmol/L NH_4HCO_3 water solution, to give **4a** as a colorless syrupy solid in a 96% yield. ^{1}H NMR (400 MHz, D_2O, ppm) δ: 2.46 (q, 2H), 2.85 (t, *J* = 6.16 Hz, 2H), 3.83 (ddd, *J* = 11.96, 5.00, 2.2 Hz, 1H), 3.94 (q, 2H), 3.96 (ddd, *J* = 11.96, 4.56, 2.4 Hz, 1H), 4.22 (dd, *J* = 4.96, 2.72 Hz, 1H), 4.29 (t, *J* = 6.68 Hz, 2H), 4.34 (t, *J* = 5.2 Hz, 1H), 4.41 (t, *J* = 2.44 Hz, 1H), 4.82 (dd, *J* = 8.84, 1.48 Hz, 1H), 4.87 (d, *J* = 1.52 Hz, 1H), 5.62 (ddd, *J* = 23.96, 17.28, 10.44 Hz, 1H), 6.06 (d, *J* = 5.44 Hz, 1H), 7.72 (s, 1H), 8.12 (t, *J* = 6.48 Hz, 1H), 8.84 (d, *J* = 8.12 Hz, 1H), 9.09 (d, *J* = 6.28 Hz, 1H), 9.28 (s, 1H). ^{13}C NMR (100 MHz, D_2O, ppm) δ: 168.05, 162.82, 148.47, 147.04, 144.86, 142.22, 136.41, 136.28, 130.95, 126.58, 120.34, 102.28, 89.59, 89.51, 80.13, 73.30, 67.21, 66.76, 52.03, 36.21, 28.83, 28.75, 22.56. ^{31}P NMR (160 MHz, D_2O, pp,) δ: 0.067. HR-MS (ESI) calcd. for $C_{19}H_{26}N_5O_8P$ [M + H]$^+$: 484.1597; found: 484.1588.

Compound 4b

Compound **4b** was purified by anion resin (HCO_2^- form) column chromatography eluted with water to give **4b** as a colorless solid syrup in a 94% yield. ^{1}H NMR (400 MHz, D_2O, ppm) δ: 2.84 (t, *J* = 5.92 Hz, 2H), 3.52 (ddd, *J* = 1.84, 4.64, 11.88 Hz, 1H), 3.70 (ddd, *J* = 2.24, 4.40, 12 Hz, 1H), 3.91 (dd, *J* = 5.92, 11.84 Hz, 2H), 4.10 (dd, *J* = 2.48, 4.76 Hz, 1H), 4.15 (t, *J* = 2.16 Hz, 1H), 4.25 (t, *J* = 5.16 Hz, 1H), 5.35 (s, 2H), 5.93 (d, *J* = 5.48 Hz, 1H), 7.2 (m, 5H), 7.76 (s, 1H), 7.98 (dd, *J* = 6.48, 7.76 Hz, 1H), 8.73 (d, *J* = 8.12 Hz, 1H), 8.94 (d, *J* = 7.88 Hz, 1H), 9.16 (s, 1H). ^{13}C NMR (100 MHz, D_2O, ppm) δ: 168.0, 148.42, 147.53, 144.70, 142.22, 142.22, 137.50, 136.43, 131.64, 131.26, 130.95, 130.72, 126.67, 102.29, 89.52, 80.15, 73.33, 67.20, 66.71, 56.33, 28.82. ^{31}P NMR (160 MHz, D_2O, ppm) δ: 0.01. HR-MS (ESI) calcd. for $C_{22}H_{27}N_5O_8P$ [M + H]$^+$: 520.1597; found: 520.1617.

Compound 4c

Compound **4c** was purified by size-exclusion chromatography Bio-Gel® P-2 Gel polyacrylamide gel, eluted with 25 mmol/L NH_4HCO_3 water solution, to give **4c** as a colorless solid syrup in a 92% yield. ^{1}H NMR (400 MHz, D_2O, ppm) δ: 2.91 (t, 2H), 3.61 (t, 2H), 3.94 (dd, *J* = 10.64, 5.16 Hz, 1H), 3.99 (dd, *J* = 2.76 Hz, 1H), 4.02 (t, 2H), 4.07 (t, 1H), 5.51 (d, *J* = 2.76 Hz, 1H), 5.56 (d, *J* = 4.36 Hz, 1H), 7.31 (d, *J* = 8.6 Hz, 1H), 7.35 (dd, *J* = 6.16, 3.0 Hz, 2H), 7.47 (t, *J* = 7.52 Hz, 1H), 7.62 (m, 4H), 7.90 (s, 1H), 8.39 (d, *J* = 6.16 Hz, 1H), 8.44 (d, *J* = 8.04 Hz, 1H), 8.61 (s, 1H). ^{13}C NMR (100 MHz, D_2O, ppm) δ: 167.32, 147.92, 147.49, 143.43, 141.31, 135.46, 134.98, 134.84, 131.22, 130.33, 130.14, 130.02, 129.94, 129.43, 128.38, 126.41, 101.91, 89.46, 79.93, 73.32, 67.04, 66.83, 56.36, 28.83. ^{31}P NMR (160 MHz, D_2O, ppm) δ: 0.14. HR-MS (ESI) calcd. for $C_{26}H_{28}N_5O_8P$ [M + H]$^+$: 570.1754; found: 570.1735.

Compound 4d

Compound **4d** was purified by anion resin (HCO_2^- form) column chromatography eluted with water to give **4d** as a colorless solid syrup in a 95% yield. ^{1}H NMR (400 MHz, D_2O, ppm) δ: 1.35 (m, 2H), 1.68 (m, 2H), 2.15 (t, 2H), 2.77 (t, 2H), 3.70 (ddd, *J* = 2.08, 5.08, 11.96 Hz, 1H), 3.78–3.86 (m, 3H), 4.13–4.18 (m, 3H), 4.26 (t, 1H), 5.87 (d, *J* = 5.24 Hz, 1H), 6.92 (m, 1H), 7.10 (d, 5H), 7.64 (s, 1H), 7.94 (t, *J* = 6.6 Hz, 1H), 8.67 (d, *J* = 8.12 Hz, 1H), 8.89 (d, *J* = 6.24 Hz, 1H), 9.08 (s, 1H). ^{13}C NMR (100 MHz, D_2O, ppm) δ: 177.13, 173.46, 168.11, 167.85, 162.90, 148.26, 147.17, 144.61, 142.04, 139.20, 136.22, 131.55, 130.84, 127.82, 126.42, 124.07, 102.17, 89.34, 80.01, 73.10, 67.21, 66.68, 52.33, 37.96, 31.24, 28.70, 24.51. ^{31}P NMR (160 MHz, D_2O, ppm) δ: 0.01. HR-MS (ESI) calcd. for $C_{26}H_{33}N_6O_9P$ [M + H]$^+$: 605.2125; found: 605.2103.

Compound 4e

Compound **4e** was purified by anion resin (HCO_2^- form) column chromatography, eluted with 5% HCO_2NH_4 solution, followed by octyl-functionalized silica gel eluted with water, to give **4e** as a colorless solid syrup in a 95% yield. ^{1}H NMR (400 MHz, D_2O, ppm) δ: 1.48 (m, 2H), 1.80 (m, 2H), 2.31 (t, 2H), 2.86 (t, 2H), 3.80 (dd, *J* = 3.72, 10.6 Hz, 1H), 3.91–3.96 (m, 3H), 4.13–4.18 (m, 3H), 4.20 (s, 1H), 4.26–4.32 (m, 3H), 4.38 (s, 1H), 6.02 (d, *J* = 5.24 Hz, 1H), 6.84–6.91 (m, 2H), 7.28 (dd, *J* = 2.72, 8.76 Hz, 1H), 7.31 (s, 1H), 8.09 (t, *J* = 6.72 Hz, 1H), 8.81 (d, *J* = 8.12 Hz, 1H), 9.05 (d, *J* = 6.28 Hz, 1H), 9.24 (s, 1H). ^{13}C NMR (100 MHz, D_2O, ppm) δ: 178.13, 168.01, 148.45, 144.82, 142.19, 136.40, 130.94, 130.05, 126.52, 114.00, 113.78, 107.08, 106.83, 106.58, 102.30, 89.56, 80.13, 73.30, 67.26, 66.81, 52.38, 37.43, 31.26, 28.78, 24.60. ^{31}P NMR (160 MHz, D_2O, ppm) δ: 0.08. ^{19}F NMR (376 MHz, D_2O, ppm) δ: –111.8, –118.9. HR-MS (ESI) calcd. for $C_{26}H_{31}F_2N_6O_9P$ $[M + H]^+$: 641.1936; found: 641.1960.

Compound 4f

Compound **4f** was purified by size-exclusion chromatography Bio-Gel® P-2 Gel polyacrylamide gel, eluted with 25 mmol/L NH_4HCO_3 water solution, to give **4f** as a colorless solid syrup in a 90% yield. ^{1}H NMR (400 MHz, D_2O) δ: 1.36 (m, 2H), 1.54 (m, 2H), 2.10 (t, 2H), 2.82 (t, 2H), 3.80 (ddd, *J* = 11.96, 5.16, 1.92 Hz, 1 H), 3.94 (m, 3H), 4.05 (t, 2H), 4.17 (dd, *J* = 4.52, 2.64 Hz, 1H), 4.22 (t, 1H), 4.34 (s, 1H), 4.52 (s, 2H), 5.88 (d, *J* = 5.32 Hz, 1H), 7.32–7.17 (m, 4H), 7.44 (s, 1H), 7.64 (d, *J* = 2.44 Hz, 1H), 7.66 (dd, *J* = 3.6 Hz, 1H), 7.87 (t, *J* = 7.64 Hz, 1H), 8.58 (d, *J* = 8.08 Hz, 1H), 8.87 (d, *J* = 6.24 Hz, 1H), 9.04 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ: 178.03, 167.54, 162.80, 148.02, 146.99, 144.49, 141.87, 135.85, 135.46, 132.88, 131.16, 130.64, 129.01, 128.66, 128.40, 128.12, 126.13, 125.48, 102.21, 89.37, 80.05, 73.11, 67.21, 66.74, 52.18, 43.43, 37.40, 31.21, 28.81, 24.82. ^{31}P NMR (160 MHz, D_2O, ppm) δ: 0.11. HR-MS (ESI) calcd. for $C_{31}H_{37}O_9N_6P$ $[M + H]^+$: 669.2438; found: 669.2444.

Compound 4g

Compound **4g** was purified by size-exclusion chromatography Bio-Gel® P-2 Gel polyacrylamide gel, eluted with 25 mmol/L NH_4HCO_3 water solution, to give **4g** as a colorless solid syrup in an 88% yield. ^{1}H NMR (400 MHz, D_2O, ppm) δ: 1.46 (m, 2H), 1.70 (m, 2H), 2.18 (t, *J* = 6.96 Hz, 2H), 2.80 (t, *J* = 6.62 Hz, 2H), 3.77 (dd, *J* = 11.36, 4.04 Hz, 1H), 3.88–3.93 (m, 3H), 4.17–4.22 (m, 5H), 4.27 (t, *J* = 5.16 Hz, 1H), 4.32 (s, 1H), 5.90 (d, *J* = 5.48 Hz, 1H), 7.16 (d, *J* = 7.96 Hz, 2H), 7.21 (d, *J* = 7.12 Hz, 1H), 7.27 (t, *J* = 7.24 Hz, 2H), 7.41 (m, 5H), 7.64 (s, 1H), 8.01 (t, *J* = 6.88 Hz, 1H), 8.72 (d, *J* = 8.08, 1H), 8.98 (d, *J* = 6.2 Hz, 1H), 9.16 (s, 1H). ^{13}C NMR (100 MHz, D_2O, ppm) δ: 177.21, 167.63, 148.39, 144.79, 142.09, 141.24, 140.09, 136.32, 131.16, 130.97, 130.33, 129.61, 129.02, 128.81, 102.36, 89.63, 80.17, 73.23, 67.15, 66.76, 52.15, 44.87, 37.43, 31.51, 28.98, 24.88. ^{31}P NMR (160 MHz, D_2O, ppm) δ: 0.01. HR-MS (ESI) calcd. for $C_{33}H_{39}N_6O_9P$ $[M + H]^+$: 695.2594; found: 695.2569.

Compound 4h

Compound **4h** was purified by size-exclusion chromatography Bio-Gel® P-2 Gel polyacrylamide gel, eluted with 25 mmol/L NH_4HCO_3 water solution, to give **4h** as a colorless solid syrup in a 91% yield. ^{1}H NMR (400 MHz, D_2O, ppm) δ: 1.45 (m, 2H), 1.62 (m, 2H), 2.17 (t, *J* = 7 Hz, 2H), 2.86 (t, *J* = 6 Hz, 2H), 3.75 (dd, *J* = 11.4, 4.15 Hz, 1H), 3.92–4.11 (m, 3H), 4.14 (t, *J* = 6.8 Hz, 2H), 4.18 (t, *J* = 2.4 Hz, 1H), 4.24 (t, *J* = 5.4 Hz, 1H), 4.35 (s, 1H), 5.91 (d, *J* = 5.2 Hz, 1H), 7.28–7.39 (m, 5H), 7.5 (s, 3H), 7.68 (d, *J* = 7.8 Hz, 2H), 7.75 (t, *J* = 8.2 Hz, 2H), 7.91 (t, *J* = 6.8 Hz, 2H), 8.63 (d, *J* = 8.08 Hz, 1H), 8.92 (d, *J* = 6.16 Hz, 1H), 9.08 (s, 1H). ^{13}C NMR (100 MHz, D_2O, ppm) δ: 176.29, 167.64, 148.39, 146.84, 144.82, 143.86, 142.1, 136.33, 130.95, 129.60, 126.01, 102.35, 89.66, 80.16, 73.22, 67.16, 66.76, 59.23, 52.04, 37.19, 31.47, 28.94, 24.83. ^{31}P NMR (160 MHz, D_2O, ppm) δ: 0.15. HR-MS (ESI) calcd. for $C_{33}H_{39}N_6O_9P$ $[M + H]^+$: 695.2594; found: 695.2615.

Electrochemical experiment

All data were recorded on a CHI 600C electrochemical analyzer, and further processed using the software Origin 8.0 when necessary.[22] The working concentration of analytes was 2×10^{-3} mol/L. Solutions of either 0.01 mol/L Tris/NaCl (0.005 mol/L) or phosphate (1 mol/L) buffer were used as supporting electrolytes. The pH was adjusted with NaOH or H_3PO_4. Stock solutions of all compounds were stored at 4 °C to avoid decomposition. Solutions were sonicated and purged with purified nitrogen and the temperature was kept at 25 ± 0.2 °C.

To use this electrode it was necessary to activate its surface. For this purpose, several methods were tried, namely, polishing with alumina powder, sonication, activation with sulfochromic mixture, etc. The treatment selected for the electrode, made before each measurement, was the following: washing with distilled water, putting the electrode into a sulfochromic mixture for 30 s, washing with distilled water, polishing with 0.3 μm alumina powder and 0.05 μm alumina powder, sonication for 2 min in distilled water, washing with distilled water, and drying with soft paper. Under such conditions measurements were highly reproducible.

To maintain the activity of NAD^+, all buffer pHs must be in the range of 5–7. Tris/NaCl (pH 7) was a better choice than the same pH phosphate for a sensitive GC electrode response. In NaCl (0.01 mol/L) / Tris (0.005 mol/L) buffer at pH 7, peak currents was invariable. The potential range used for NAD^+ analog detection was –0.4 to –1.4 V, which was the most suitable (small peak current changes). The scan rate was 0.1 V/s.

Cyclic voltammogram for determination of dynamic constants was performed with a glassy carbon electrode vs. SCE in supporting electrolyte (0.01 mol/L NaCl / 0.005 mol/L Tris, pH 7). The response to 2×10^{-3} mol/L NAD^+ analogs and corresponding compounds were measured in 10 mL of supporting electrolyte at 298 ± 0.2 °C under a nitrogen atmosphere.

The cyclic voltammogram for pH dependence of **4h** was performed in 1 mol/L phosphate buffer at different pHs (pH 4–9) containing 2×10^{-3} mol/L **4h**.

Acknowledgement

We are grateful for the support of the National Natural Science Foundation of China (No. 20472084).

References

(1) Haigis, M. C.; Mostoslavsky, R.; Haigis, K. M.; Fahie, K.; Christodoulou, D. C.; Murphy, A. J.; Valenzuela, D. M.; Yancopoulos, G. D.; Karow, M.; Blander, G.; Wolberger, C.; Prolla, T. A.; Weindruch, R.; Alt, F. W.; Guarente, L. *Cell* **2006**, *126* (5), 941. doi:10.1016/j.cell.2006.06.057. PMID:16959573.

(2) Liu, Q.; Kriksunov, I. A.; Graeff, R.; Lee, H. C.; Hao, Q. *J. Biol. Chem.* **2007**, *282* (8), 5853. doi:10.1074/jbc.M609093200. PMID:17182614.

(3) Alano, C. C.; Ying, W. H.; Swanson, R. A. *J. Biol. Chem.* **2004**, *279* (18), 18895. doi:10.1074/jbc.M313329200. PMID:14960594.

(4) Olek, R. A.; Ziolkowski, W.; Kaczor, J. J.; Greci, L.; Popinigis, J.; Antosiewicz, J. *J. Biochem. Mol. Biol.* **2004**, *37* (4), 416. PMID:15469728.

(5) Waypa, G. B.; Schumacker, P. T. *J. Appl. Physiol.* **2005**, *98* (1), 404. doi:10.1152/japplphysiol.00722.2004. PMID:15591310.

(6) (a) Barker, C. D.; Reda, T.; Hirst, J. *Biochemistry (Mosc.)* **2007**, *46* (11), 3454. doi:10.1021/bi061988y.; (b) Zhang, Z.; Blake, D. R.; Stevens, C. R.; Kanczler, J. M.; Winyard, P. G.; Symons, M. C.; Benboubetra, M.; Harrison, R. *Free Radic. Res.* **1998**, *28* (2), 151. doi:10.3109/10715769809065801. PMID:9645392.

(7) Damian, A.; Omanovic, S. *J. Mol. Catal. Chem.* **2006**, *253* (1–2), 222. doi:10.1016/j.molcata.2006.03.020.

(8) Xu, X. H.; Chen, J. H.; Li, W.; Nie, Z.; Yao, S. Z. *Electrochem. Commun.* **2008**, *10* (10), 1459. doi:10.1016/j.elecom.2008.07.033.

(9) Salimi, A.; Izadi, M.; Hallaj, R.; Soltanian, S.; Hadadzadeh, H. *J. Solid State Electrochem.* **2009**, *13* (3), 485. doi:10.1007/s10008-008-0583-6.

(10) Lo, H. C.; Leiva, C.; Buriez, O.; Kerr, J. B.; Olmstead, M. M.; Fish, R. H. *Inorg. Chem.* **2001**, *40* (26), 6705. doi:10.1021/ic010562z. PMID:11735482.

(11) Lo, H. C.; Fish, R. H. *Angew. Chem. Int. Ed.* **2002**, *41* (3), 478. doi:10.1002/1521-3773(20020201)41:3<478::AID-ANIE478>3.0.CO;2-K.

(12) Song, H. K.; Lee, S. H.; Won, K.; Park, J. H.; Kim, J. K.; Lee, H.; Moon, S. J.; Kim, D. K.; Park, C. B. *Angew. Chem. Int. Ed.* **2008**, *47* (9), 1749. doi:10.1002/anie.200703632.

(13) Lee, S. H.; Nam, D. H.; Kim, J. H.; Baeg, J. O.; Park, C. B. *ChemBioChem* **2009**, *10* (10), 1621. doi:10.1002/cbic.200900156. PMID:19551795.

(14) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40* (11), 2004. doi:10.1002/1521-3773(20010601)40:11<2004::AID-ANIE2004>3.0.CO;2-5.

(15) Liu, R. H.; Visscher, J. *Nucleosides Nucleotides* **1994**, *13* (5), 1215. doi:10.1080/15257779408011891. PMID:11539878.

(16) (a) Theocharis, A. B.; Alexandrou, N. E.; Terzis, A. *J. Heterocycl. Chem.* **1990**, *27* (6), 1741. doi:10.1002/jhet.5570270643.; (b) van der Peet, P.; Gannon, C. T.; Walker, I.; Dinev, Z.; Angelin, M.; Tam, S.; Ralton, J. E.; McConville, M. J.; Williams, S. J. *ChemBioChem* **2006**, *7* (9), 1384. doi:10.1002/cbic.200600159. PMID:16841351.; (c) Gunter, M. J.; Farquhar, S. M.; Mullen, K. M. *N. J. Chem.* **2004**, *28* (12), 1443. doi:10.1039/b411583j.

(17) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41* (14), 2596. doi:10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4.

(18) Angulo, M.; Ruiz Montoya, M.; Marin Galvin, R.; Rodriguez Mellado, J. *Electroanalysis* **1997**, *9* (4), 345. doi:10.1002/elan.1140090417.

(19) Laviron, E. *J. Electroanal. Chem.* **1974**, *52* (3), 355. doi:10.1016/S0022-0728(74)80448-1.

(20) Zhou, Z.; Li, S. W.; Zhang, Y. L.; Liu, M. L.; Li, W. *J. Am. Chem. Soc.* **2005**, *127* (31), 10824. doi:10.1021/ja052280u. PMID:16076176.

(21) Graham, S. M.; Macaya, D. J.; Sengupta, R. N.; Turner, K. B. *Org. Lett.* **2004**, *6* (2), 233. doi:10.1021/ol036152r. PMID:14723536.

(22) OriginLab Corporation. *Origin 8.0;* MicroCal, Inc.: Northampton, MA, USA, 2007.