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Synthesis of phosphodiester-type nicotinamide adenine dinucleotide analogs

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ABSTRACT

Fourteen phosphodiester-type β-nicotinamide adenine dinucleotide (NAD^+) analogs were prepared starting from nicotinamide. The phosphodiester linkage was effectively assembled in 69–93% yields via condensation reaction between 2′,3′-di-*O*-acetyl nicotinamide mononucleotide and alcohols in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride. The analog β-nicotinamide ribose-5-(2-phenylethyl) phosphate showed beneficial effects on cell growth of model microorganisms.

1. Introduction

Nicotinamide adenine dinucleotide (NAD^+, Fig. 1) and its reduced form, NADH, serve as cofactors of many oxidoreductive enzymes.[1] They are also functional molecules in many aspects of life. It has been established that NAD^+ plays critical roles in calcium homeostasis,[2] cell proliferation,[3] aging,[4] apoptosis,[5] covalent protein modification,[6] gene expression, and regulation of numerous NAD^+-dependent non-oxidoreductive enzymes.[7] Furthermore, recent studies showed that NAD^+ could act as an immune modulator or induce T cell death.[8] NAD^+ uptake by mammalian cells has also been firmly recognized.[9] These findings have greatly enriched our understanding on the essential functions of pyridine nucleotides.[10] When NAD^+ functions as a coenzyme of dehydrogenase, the adenosine pocket and the NAR binding site are specially isolated.[11] This appeals to engineering of the adenosine binding pocket of dehydrogenase[12] to match elaborated NAD^+ analogs in an approach as found in kinases research.[13] In such scenario, NAD^+ analogs are of great potential to be developed for elucidation of complicated problems at the interface of chemistry and biology.

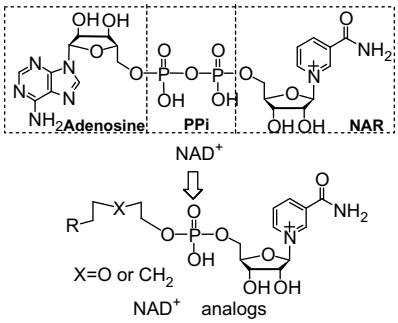

Figure 1. Structural function of NAD^+ and its phosphodiester-type analogs.

Although modifications on adenosine, ribose, PPi, and nicotinamide moiety of the NAD^+ structure have been achieved, those with substantial changes of the NAD^+ skeleton are limited.[14] In particular, preparation of diversified phosphodiester-type NAD^+ analogs (Fig. 1) remains challenging in terms of efficient coupling strategy and product purification. The simplest phosphodiester-type NAD^+ analog, β-nicotinamide ribose-5′-methyl phosphate, was prepared using the *N*,*N*′-dicyclohexylcarbodiimide (DCC)/4-dimethylaminopyridine (DMAP) system.[15] The reduced form of the analog acted as a biomimic cofactor binding to horse liver alcohol dehydrogenase and facilitated reduction of prochiral ketones in moderate yields.[16] Although the results were stimulating, no other phosphodiester-type NAD^+ analogs have been documented so far. Because a methyl group could barely provoke tight interactions with protein, more complex structures are intriguing to mimic the adenosine moiety. Here, we designed novel phosphodiester-type NAD^+ analogs (Fig. 1), of which the adenine moiety, the ribose moiety, and the PPi linkage was replaced with other organic constituents, ether or alkane chains[17] and monophosphate functionality, respectively.

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We now report our efforts on preparation of these NAD^+ analogs. We also present preliminary results of microbial cell growth and activity assay of dehydrogenases in the presence of a typical asymmetrical phosphodiester NAD^+ analog.

2. Results and discussion

2.1. Synthetic plan

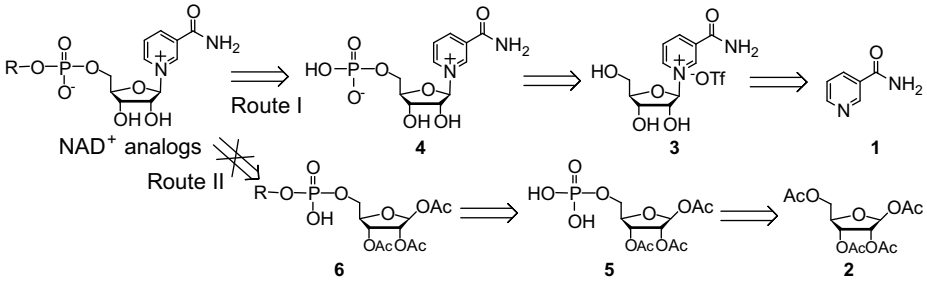
Construction of phosphatediesters including positive charged constituents remains challenge, although many strategies have been developed to assemble phosphodiester via either P(III)[18] or P(V)[19] reagents in nucleic acid chemistry.[20] P(III) strategy required more steps such as oxidation, protection, and deprotection. This resulted in tedious purification work and breakdown of the anomeric C–N bond in NAD^+ analog assembly. Thus, we focused on P(V) chemistry. Based on the retrosynthetic analysis, we planned our synthesis work by one of the two routes (Scheme 1). Intermolecular condensation reaction between nicotinamide mononucleotide (NMN, **4**) and alcohols was designed as the key step in Route I. However, it was difficult to find an activation system to realize the coupling reaction, because NMN is liable to form an inner salt and has notorious solubility in most organic solvents. Moreover, breakdown of **4** via scission of the C–N bond was substantial in many experiments. According to Route II, we successfully prepared **6** in dichloromethane. However, attempts were in vain to assemble the C–N bond via glycosylation reaction with nicotinamide or its *N*-protected derivatives in the presence of Lewis acids. Therefore, these NAD^+ analogs were prepared in a way similar to Route I with NMN derivatives (vide infra).

2.2. Synthesis

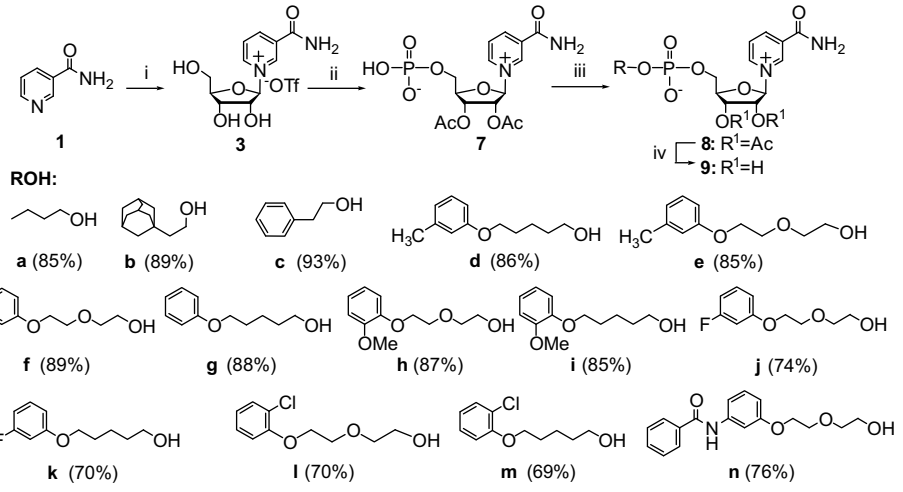
In the literatures, **4** has been prepared by selective hydrolysis[21] of NAD^+ or chemical synthesis.[22] To facilitate in-house preparation of various NAD^+ analogs, we prepared **4** starting from commercially available nicotinamide according to a modified procedure and obtained a total isolated yield of 63%.[22c,23] Briefly, nicotinamide was treated with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and TMSCl, then reacted with 1,2,3,5-tetra-*O*-acetylribofuranose (**2**) in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) to form 2′,3′,5′-tri-*O*-acetyl nicotinamide riboside via a glycosylation mechanism. The intermediate was deacetylated by methanolic ammonia to give nicotinamide ribose (NAR, **3**), which was phosphorylated with $OPCl_3$ to afford **4**. With NMN in hand, our attention turned to assemble the phosphodiester linkage en route to the NAD^+ analogs.

The DCC/DMAP strategy was first applied to couple **4** with 2-phenylethanol (**c**), a conceptive adenosine analog, in various solvents.[15] It was found that long reaction time (>3 d) and large excess DCC were required to achieve good conversion, yet low yield and complicated byproducts were observed based on ^{31}P NMR analysis. In the reaction, significant amount of **4** dimerized to form the symmetric pyrophosphate product. Reactions involved the hydroxyl groups at 2′- and 3′-positions of **4** also occurred. Consequently, it was difficult to recover β-nicotinamide ribose-5′-(2-phenylethyl) phosphate **9c**.

We then prepared 2′,3′-di-*O*-acetyl nicotinamide mononucleotide (Ac_2NMN, **7**) in around 62% overall yield started from nicotinamide (Scheme 2).[24] It was found that Ac_2NMN had reasonable solubility and high reactivity in solvents such as DMF,



Scheme 1. Retrosynthetic analysis for phosphodiester-type NAD^+ analogs.



Scheme 2. Synthesis of phosphodiester-type NAD^+ analogs. Reagents and conditions: (i) (a) TMSCl, HMDS, 120 °C, 8 h; (b) **2**, 1,2-dichloroethane, TMSOTf, 45 °C, 2 h; (c) NH_3/CH_3OH, −5 °C, 20–48 h; (ii) (a) $POCl_3$ (4 equiv), $PO(OMe)_3$, −5 to 0 °C, 12 h, 63% for four steps; (b) Ac_2O/pyridine (1:1), 0–5 °C, 24 h; (iii) (a) ROH (**a**–**n**), coupling reagent, DMF/pyridine (1:1), 25 °C, 6 h; (iv) 1 M NH_3/CH_3OH, −5 °C, 4 h, 96–98%. Data in the parentheses indicated the coupling yield.

DMSO, and pyridine, which were routinely used in coupling reactions. Moreover, blocking the free hydroxyl groups on the ribose ring also eliminated side reactions and improved the stability of the precursor.

The alcohols (**a**–**n**) used in NAD^+ analogs preparation were either commercially available (**a**–**c**) or obtained via Williamson ether synthesis (**d**–**n**) using the corresponding phenol and the halide (Scheme 2).[25]

To attain these analogs, we evaluated commercially available condensation agents found in the preparations of DNA or RNA fragments (Fig. 2). Table 1 summarizes the results of the coupling reaction between Ac_2NMN (**7**) and 2-phenylethanol (**c**) with various condensation reagents using an anhydrous DMF/pyridine solvent system at room temperature. Conventional carbodiimides derivatives including DCC, *N*,*N*′-diisopropylcarbodiimide (DIC) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) showed unsatisfactory results (Table 1, entries 1–3). Although about 100% conversion for **7** was achieved with these mediators, the product **8c** was less than 10% based on ^{31}P NMR analysis. Difficulties also occurred to recover **8c** in the presence of excess byproducts and the coupling reagent derivatives. Acyl chlorides, commonly used for assembling of *H*-phosphonate linkage in nucleic acid chemistry, such as pivaloyl chloride (Piv-Cl) and 1-adamantanecarbonyl chloride (AC-Cl) were estimated.[20] Both Piv-Cl and AC-Cl facilitated the coupling reaction, yet the reaction was rather slow. It took over 10 d for Piv-Cl or AC-Cl to reach ^{31}P NMR yields of 51% and 34%, respectively (Table 1, entries 4 and 5). Moreover, large excess 2-phenylethanol and the acyl chlorides were required. Chlorophosphates were also tested, including diphenyl phosphorochloridate (DPP-Cl) and bis(2-oxo-3-oxazolidinyl) phosphinic chloride (BOP-Cl), which are known to prompt asymmetrical pyrophosphate synthesis. Near quantitative conversion of **7** was achieved within 24 h in the presence of DPP-Cl or BOP-Cl (Table 1, entries 6 and 7).

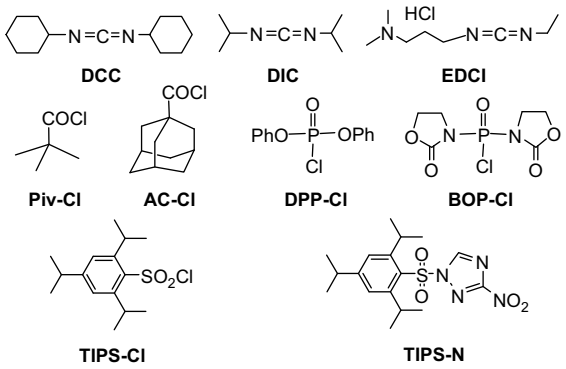


Figure 2. Structures of condensation agents used in this work.

Table 1
Results and conditions with different condensation reagents[a]

Entry	Reagents	Loading (equiv)	Time (h)	NMR yield (%)
1	DCC	3	36	10
2	DIC	3	76	10
3	EDC	3	78	8
4	Piv-Cl	10	240	51
5	AC-Cl	10	240	34
6	DPP-Cl	3	24	39
7	BOP-Cl	3	20	33
8	TIPS-Cl	3	6	93
9	TIPS-N	3	20	24

[a] Conditions: Ac_2NMN and 3 equiv of 2-phenylethanol were employed in the presence of a condensation agent in DMF/pyridine (1:1) at 25 °C.

When Ac_2NMN and 2-phenylethanol was treated with 2,4,6-triisopropylbenzenesulfonyl chloride (TIPS-Cl), ^{31}P NMR analysis indicated that the singlet at 3.7 ppm of the starting material disappeared within 6 h and signals at 0.3 and −11.2 ppm, corresponding to the product and the pyrophosphate byproduct, respectively, evolved. A typical yield was about 93% (Table 1, entry 8) that was comparable to nucleotide or glycosyl phosphate diester synthesis using P(III) agents.[18] Seth et al. improved the yield of phosphodiester in nucleotide synthesis using 1*H*-tetrazole and TIPS-Cl.[26] However, dimerization of Ac_2NMN increased noticeably if 1*H*-tetrazole was applied.

Another agent with a similar structure to that of TIPS-Cl, 1-(2,4,6-triisopropylbenzenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (TIPS-N) was estimated, however, the NMR yield was only 24% after 20 h (Table 1, entry 9).

As demonstrated herein, TIPS-Cl was the favored agent to prepare **8c**. When it was applied for the coupling reaction with other alcohols, NAD^+ analogs **8a**–**8n** were obtained in isolated yields ranged from 69% to 93% (Scheme 2). It was interesting to note that analogs **8j**–**8n**, resulted from alcohols with an electron withdrawing or bulky group, were obtained in slightly lower yields. We reasoned that nucleophilic attack on the phosphorus atom of phosphate–sulfonate anhydride intermediate was less effective due to stronger repulsion forces between these alcohol substrates and the TIPS group.

2.3. Preliminary bioactivity assays

Preliminary tests were performed using the analog **9c** for its effects on cell growth of model microorganisms. Compared to the control samples, *Escherichia coli* DH5α and *Saccharomyces cerevisiae* ATCC 26108 cells grew faster in the presence of 100 μM **9c** (Fig. 3). The maximal growth increments for *E. coli* and *S. cerevisiae* cells reached 16% and 52%, respectively. It is known that NAD^+ or NAR can have beneficial effects on the culture of mammalian cells, because these compounds can change the metabolism of pyridine nucleotide cofactors.[9] The analog **9c** promoted microbial cell growth might also suggest a similar mechanism.

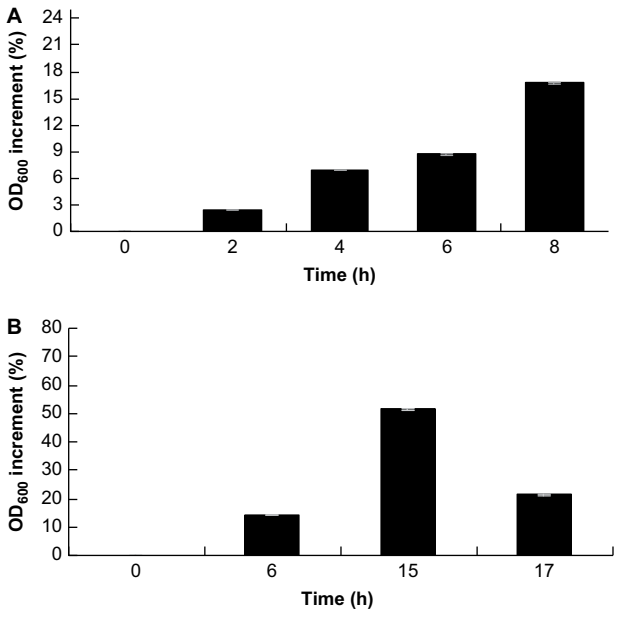


Figure 3. Growth improvements of (A) *E. coli* DH5α and (B) *S. cerevisiae* ATCC 26108 in the presence of 100 μM NAD^+ analog **9c**. Cell growth was indicated using OD_{600} data and normalized with the control cultures.

Enzymatic activity of alcohol dehydrogenase (ADH) from *S. cerevisiae* was also tested in the presence of analog **9c**. Compared to the control sample, there was no discernible reaction rate change even the concentration of **9c** was fivefold more than that of NAD^+ (Fig. 4). Similar results were also observed in the case of lactate dehydrogenase (LDH) from *Lactobacillus leichmanii* (data not shown). These results indicated that the synthetic analogs were unlikely recognized by wild-type NAD^+-dependent oxidoreductases. Extensive screening work is ongoing to find mutated enzymes from designed libraries.[12]

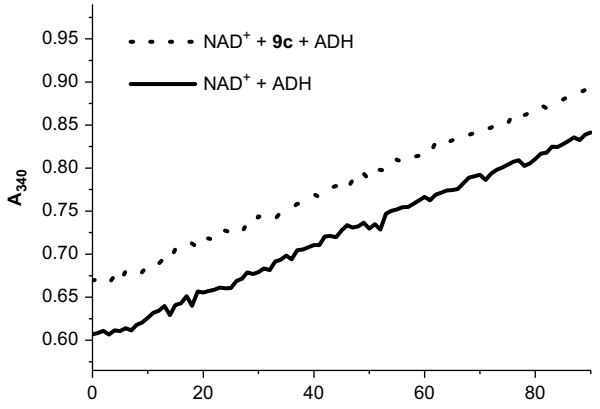


Figure 4. Time course of ADH activity in the presence of **9c**.

3. Conclusions

We have developed an effective and general strategy to prepare phosphodiester-type NAD^+ analogs starting from nicotinamide through coupling of Ac_2NMN with alcohols in the presence of TIPS-Cl. Preliminary study indicated that one representative analog **9c** promoted microbial cell growth. We are now exploring the biological function with these compounds in a wide variety of chemical biology areas, and detailed results will be reported in due course.

4. Experimental

4.1. General

All reagents were analytical grade and obtained from commercial suppliers (ABCR, ACROS or Sigma). NMR spectra were measured with a Bruker DRX-400 spectrometer (400.3 MHz for ^1H, 100.6 MHz for ^{13}C, 160.1 MHz for ^{31}P, and 376.4 for ^{19}F) at 298 K. HRMS was obtained on a Q-TOF-MS and operated with an electrospray source in positive ion mode. Optical density at 600 nm (OD_{600}) was recorded on JASCO V-530 UV–vis spectrophotometer. F_{254} thin-layer and silica gel (400 mesh) were purchased from Yantai Jiangyou Silica Co., Ltd., China. Octyl-functionalized silica gel was purchased from Sigma. Ion exchange resin (100–200 mesh) was purchased from the Chemical Plant of Nankai University, Tianjin, China. Bio-Gel P2 resin (45 μm) was obtained from Bio-Rad Laboratories, Inc. *E. coli* DH5α and *S. cerevisiae* ATCC 26108 were purchased from Beijing Ding Guo Biotech. Co., Ltd and Invitrogen Co., Ltd, respectively. ADH from *S. cerevisiae* (CAS No. 9031-72-5) and LDH from *L. leichmanii* (CAS No. 9028-36-8) were purchased from Sigma. All organic reactions were carried out under a nitrogen atmosphere.

4.2. Procedure for preparation of intermediate compounds

Adenosine analogs **d**–**n** in Scheme 2 were synthesized via Williamson ether synthesis using corresponding phenol and the halide according to known procedure (yield 80–92%).[25]

Ac_2NMN **7** was prepared starting from nicotinamide in five steps using a modified procedure of the literatures.[22c,23]

4.3. General procedure for preparation of NAD^+ analogs (9a–9n)

All NAD^+ analogs were synthesized following the procedure described for **9c**. All purifications were performed at 25 °C except size exclusion chromatography (4 °C).

*4.3.1. β-Nicotinamide ribose-5-(2-phenylethyl) phosphate (**9c**).* Ac_2NMN **7** (263 mg, 0.63 mmol) and 2-phenylethanol **c** (200 μL, 1.67 mmol) were dissolved in pyridine/DMF (1:1, 25 mL). TIPS-Cl (604 mg, 2 mmol) was added and the suspension was stirred at room temperature for 6 h. After removing the solvents, the residue was taken by H_2O (15 mL), extracted with DCM (3×15 mL). The supernatant was purified by column chromatography on octyl-functionalized silica gel eluted with CH_3OH/H_2O (30:1) to give the crude intermediate **8c** (306 mg, 0.58 mmol). This was treated with 1 M NH_3/MeOH (0.2 mL) at −5 °C for 4 h. The solvent was evaporated and the residue was purified by column chromatography on anion resin (201×2, HCO_3^- form, H_2O). The corresponding fraction was pooled and lyophilized to give syrupy solid **9c** (251 mg, 91%). ^1H NMR (400 MHz, D_2O) δ (ppm) 3.65 (m, 3H), 3.94 (m, 3H), 4.11 (t, *J*=2.2 Hz, 1H), 4.35 (s, 1H), 6.01 (d, *J*=5.4 Hz, 1H), 6.68 (m, 3H), 7.15 (m, 2H), 8.06 (t, *J*=7.6 Hz, 1H), 8.80 (d, *J*=8.0 Hz, 1H), 9.06 (d, *J*=6.0 Hz, 1H), 9.22 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 62.3, 65.3, 66.7, 69.2, 73.4, 80.1, 89.6, 89.7, 102.2, 128.9, 130.8, 131.0, 131.6, 136.2, 141.2, 141.9, 144.7, 148.4, 167.0. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.2. HRMS (ESI) calcd for $C_{19}H_{23}N_2O_8P$ $[M+H]^+$ 439.1270; found: 439.1284.

*4.3.2. β-Nicotinamide ribose-5-(1-butyl) phosphate (**9a**).* Compound **9a** (syrupy solid, 203 mg, 82%) was obtained from **7** (263 mg, 0.63 mmol) as described for the synthesis of **9c**, followed by size exclusion chromatography (polyacrylamide gel, 25 mM NH_4HCO_3). ^1H NMR (400 MHz, D_2O) δ (ppm) 0.73 (t, *J*=7.2 Hz, 2H), 1.19 (m, 2H), 1.44 (m, 2H), 3.73 (q, 2H), 4.01 (d, *J*=5.3 Hz, 1H), 4.18 (d, *J*=10.3 Hz, 1H), 4.3 (s, 1H), 4.40 (t, *J*=5.1 Hz, 1H), 4.49 (s, 1H), 6.08 (d, *J*=4.3 Hz, 1H), 8.15 (t, *J*=6.9 Hz, 1H), 8.86 (d, *J*=7.9 Hz, 1H), 9.14 (d, *J*=6.0 Hz, 1H), 9.34 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 15.4, 20.8, 34.4, 66.8, 68.7, 73.5, 80.2, 89.7, 102.3, 130.9, 136.4, 142.2, 144.9, 148.4, 168.2. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.5. HRMS (ESI) calcd for $C_{15}H_{23}N_2O_8P$ $[M+H]^+$ 391.1270; found: 391.1277.

*4.3.3. β-Nicotinamide ribose-5-[2-(1-adamantane)ethyl] phosphate (**9b**).* Compound **9b** (syrupy solid, 271 mg, 87%) was obtained from **7** (263 mg, 0.63 mmol) as described for the synthesis of **9c**, followed by size exclusion chromatography (polyacrylamide gel, 25 mM NH_4HCO_3). ^1H NMR (400 MHz, D_2O) δ (ppm) 1.20 (t, *J*=7.4 Hz, 2H), 1.28 (d, *J*=2.0 Hz, 6H), 1.39–1.51 (m, 6H), 1.69 (s, 3H), 3.74 (q, 2H), 4.01 (ddd, *J*=1.9, 4.9, 12.0 Hz, 1H), 4.17 (ddd, *J*=2.2, 4.0, 12.1 Hz, 1H), 4.28 (s, 1H), 4.43 (s, 1H), 4.48 (s, 1H), 6.12 (d, *J*=5.0 Hz, 1H), 8.21 (t, *J*=7.2 Hz, 1H), 8.92 (d, *J*=7.9 Hz, 1H), 9.19 (d, *J*=5.8 Hz, 1H), 9.37 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 30.8, 33.7, 39.1, 44.5, 46.5, 65.0, 66.8, 73.3, 80.2, 89.7, 102.5, 131.1, 136.5, 142.3, 145.2, 148.7, 167.8. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.6. HRMS (ESI) calcd for $C_{23}H_{33}N_2O_8P$ $[M+H]^+$ 497.2053; found: 497.2061.

*4.3.4. β-Nicotinamide ribose-5-[5-(m-tolyloxy)pentyl] phosphate (**9d**).* Compound **9d** (syrupy solid, 266 mg, 83%) was obtained from **7** (263 mg, 0.63 mmol) as described for the synthesis of **9c**. ^1H NMR (400 MHz, D_2O) δ (ppm) 1.34 (m, 2H), 1.56 (m, 4H), 2.10 (s, 3H), 3.77 (m, 4H), 4.00 (ddd, *J*=2.5, 4.8, 10.4 Hz, 1H), 4.14 (ddd, *J*=2.0, 4.0, 12.0 Hz, 1H), 4.27 (m, 1H), 4.32 (t, *J*=4.8 Hz, 1H), 4.44 (s, 1H), 5.99 (d, *J*=5.1 Hz, 1H), 6.53 (t, *J*=7.2 Hz, 2H), 6.65 (d, *J*=7.5 Hz, 1H), 7.01 (t,

J=7.8 Hz, 1H), 8.07 (t, J=6.4 Hz, 1H), 8.73 (d, J=8.1 Hz, 1H), 9.07 (d, J=6.2 Hz, 1H), 9.20 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 22.9, 24.3, 30.4, 31.9, 66.8, 68.7, 70.5, 73.1, 80.1, 89.6, 102.3, 114.0, 117.7, 124.5, 130.9, 132.1, 136.2, 142.2, 142.7, 144.7, 148.3, 160.5, 167.7. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.6. HRMS (ESI) calcd for $C_{23}H_{31}N_2O_9P$ $[M+H]^+$ 511.1845; found: 511.1839.

*4.3.5. β-Nicotinamide ribose-5-{2-[2-(m-tolyloxy)ethoxy]ethyl} phosphate (**9e**).* Compound **9e** (syrupy solid, 267 mg, 83%) was obtained from **7** (263 mg, 0.63 mmol) as described for the synthesis of **9c**. ^{1}H NMR (400 MHz, D_2O) δ (ppm) 2.04 (s, 3H), 3.59 (t, J=4.2 Hz, 2H), 3.67 (t, J=7.2 Hz, 2H), 3.88 (m, 4H), 3.97 (ddd, J=1.9, 4.8, 12.1 Hz, 1H), 4.15 (ddd, J=1.8, 3.4, 12.1 Hz, 1H), 4.21 (m, 1H), 4.25 (t, J=5.1 Hz, 1H), 4.34 (s, 1H), 5.86 (d, J=5.2 Hz, 1H), 6.43 (m, 2H), 6.57 (d, J=7.5 Hz, 1H), 6.93 (t, J=8.3 Hz, 1H), 7.99 (t, J=7.5 Hz, 1H), 8.63 (d, J=8.1 Hz, 1H), 9.00 (d, J=6.2 Hz, 1H), 9.08 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 66.8, 67.6, 69.4, 71.6, 72.7, 73.0, 80.1, 89.5, 102.2, 117.5, 124.6, 129.2, 130.9, 132.0, 136.0, 141.9, 142.6, 144.6, 148.1, 160.1, 162.8, 167.6. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.3. HRMS (ESI) calcd for $C_{22}H_{29}N_2O_{10}P$ $[M+H]^+$ 513.1638; found: 513.1632.

*4.3.6. β-Nicotinamide ribose-5-{2-[2-(2-phenoxy)ethoxy]ethyl} phosphate (**9f**).* Compound **9f** (syrupy solid, 242 mg, 86%) was obtained from **7** (263 mg, 0.63 mmol) as described for the synthesis of **9c**. ^{1}H NMR (400 MHz, D_2O) δ (ppm) 3.64 (t, J=4.3 Hz, 2H), 3.74 (t, J=4.2 Hz, 2H), 3.91 (m, 2H), 3.99 (m, 3H), 4.15 (dd, J=2.5, 5.5 Hz, 2H), 4.25 (d, J=5.3 Hz, 1H), 4.29 (d, J=2.6 Hz, 1H), 4.38 (s, 1H), 5.93 (d, J=5.4 Hz, 1H), 6.73 (d, J=8.2 Hz, 2H), 6.82 (t, J=7.4 Hz, 1H), 7.13 (t, J=7.7 Hz, 1H), 8.05 (t, J=6.7 Hz, 1H), 8.69 (d, J=8.1 Hz, 1H), 9.06 (d, J=6.2 Hz, 1H), 9.15 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 66.9, 67.6, 69.6, 71.7, 72.7, 73.2, 80.2, 89.6, 102.3, 117.0, 124.0, 129.4, 131.0, 132.3, 136.2, 142.1, 144.8, 148.3, 160.2, 167.9. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.3. HRMS (ESI) calcd for $C_{21}H_{27}N_2O_{10}P$ $[M+H]^+$ 449.1482; found: 449.1493.

*4.3.7. β-Nicotinamide ribose-5-(5-phenoxypentyl) phosphate (**9g**).* Compound **9g** (syrupy solid, 265 mg, 85%) was obtained from **7** (263 mg, 0.63 mmol) as described for the synthesis of **9c**. ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.30 (m, 2H), 1.48 (m, 2H), 1.54 (m, 2H), 3.71 (q, 2H), 3.75 (t, J=6.4 Hz, 2H), 3.95 (ddd, J=2.2, 4.9, 12.0 Hz, 1H), 4.10 (ddd, J=2.4, 4.2, 12.0 Hz, 1H), 4.22 (m, 1H), 4.28 (t, J=5.0 Hz, 1H), 4.39 (s, 1H), 5.96 (d, J=5.2 Hz, 1H), 6.68 (d, J=8.1 Hz, 2H), 6.77 (t, J=7.4 Hz, 1H), 7.09 (t, J=8.4 Hz, 2H), 8.02 (t, J=7.8 Hz, 1H), 8.69 (d, J=8.1 Hz, 1H), 9.02 (d, J=6.3 Hz, 1H), 9.16 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 24.2, 30.4, 31.8, 31.9, 66.8, 68.7, 70.5, 73.0, 80.1, 89.4, 102.3, 117.0, 123.7, 130.9, 132.2, 136.2, 142.1, 144.7, 148.3, 160.4, 162.7, 167.8. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.5. HRMS (ESI) calcd for $C_{22}H_{29}N_2O_9P$ $[M+H]^+$ 497.1689; found: 497.1680.

*4.3.8. β-Nicotinamide ribose-5-{2-[2-(2-methoxyphenoxy)ethoxy]ethyl} phosphate (**9h**).* Compound **9h** (syrupy solid, 297 mg, 86%) was obtained from **7** (263 mg, 0.63 mmol) as similar procedure described for the synthesis of **9c** (anion resin, aqueous 5% HCO_2NH_4), followed by column chromatography on octyl-functionalized silica gel with CH_3OH/H_2O (30:1) as eluent. ^{1}H NMR (400 MHz, D_2O) δ (ppm) 3.61 (s, 3H), 3.64 (t, J=2.7 Hz, 2H), 3.75 (t, J=4.2 Hz, 2H), 3.87–4.08 (m, 5H), 4.20 (ddd, J=2.1, 4.5, 12.2 Hz, 1H), 4.24 (dd, J=2.3, 4.7 Hz, 1H), 4.26 (t, J=5.1 Hz, 1H), 4.38 (s, 1H), 5.88 (d, J=5.3 Hz, 1H), 6.77–6.91 (m, 4H), 8.03 (t, J=7.7 Hz, 1H), 8.68 (d, J=8.0 Hz, 1H), 9.02 (d, J=6.2 Hz, 1H), 9.14 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 57.8, 66.9, 67.7, 70.2, 71.6, 72.7, 73.2, 80.1, 89.5, 102.2, 114.4, 115.5, 123.8, 124.2, 130.9, 136.1, 141.8, 144.7, 148.1, 149.3, 150.4, 162.8. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.3. HRMS (ESI) calcd for $C_{22}H_{29}N_2O_{11}P$ $[M+H]^+$ 551.1407; found: 551.1402.

*4.3.9. β-Nicotinamide ribose-5-[5-(2-methoxyphenoxy)pentyl] phosphate (**9i**).* Compound **9i** (syrupy solid, 277 mg, 82%) was obtained from **7** (263 mg, 0.63 mmol) as similar procedure described for the synthesis of **9c** (anion resin, aqueous 5% HCO_2NH_4), followed by column chromatography on octyl-functionalized silica gel with CH_3OH/H_2O (30:1) as eluent. ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.37 (q, 2H), 1.54 (m, 2H), 1.63 (m, 2H), 3.66 (s, 3H), 3.76 (q, 2H), 3.84 (t, J=6.2 Hz, 2H), 4.01 (dd, J=4.5, 12.2 Hz, 1H), 4.16 (dd, J=4.1, 12.0 Hz, 1H), 4.27 (m, 1H), 4.34 (t, J=5.0 Hz, 1H), 4.45 (s, 1H), 5.99 (d, J=5.2 Hz, 1H), 6.81 (m, 4H), 8.08 (t, J=7.3 Hz, 1H), 8.72 (d, J=8.0 Hz, 1H), 9.06 (d, J=6.2 Hz, 1H), 9.22 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 24.2, 30.3, 31.9, 57.9, 66.8, 68.7, 71.2, 73.1, 80.1, 89.6, 114.5, 115.7, 123.9, 124.0, 130.6, 130.9, 136.2, 142.2, 148.3, 149.7, 150.7, 162.7, 167.8. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.6. HRMS (ESI) calcd for $C_{23}H_{31}N_2O_{10}P$ $[M+H]^+$ 527.1795; found: 527.1802.

*4.3.10. β-Nicotinamide ribose-5-{2-[2-(3-fluorophenoxy)ethoxy]ethyl} phosphate (**9j**).* Compound **9j** (syrupy solid, 234 mg, 72%) was obtained from **7** (263 mg, 0.63 mmol) as similar procedure described for the synthesis of **9c** (anion resin, aqueous 5% HCO_2H). ^{1}H NMR (400 MHz, D_2O) δ (ppm) 3.65 (s, 2H), 3.75 (t, J=3.8 Hz, 2H), 3.91 (m, 2H), 3.99 (m, 3H), 4.17 (dd, J=3.9, 11.9 Hz, 1H), 4.26 (m, 1H), 4.33 (m, 1H), 4.40 (m, 1H), 5.97 (d, J=5.2 Hz, 1H), 6.56 (m, 3H), 7.12 (m, 1H), 8.10 (t, J=8.1 Hz, 1H), 8.73 (d, J=8.7 Hz, 1H), 9.08 (d, J=6.2 Hz, 1H), 9.21 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 66.9, 67.6, 69.9, 71.5, 72.7, 72.8, 73.2, 80.2, 89.5, 89.6, 102.3, 104.4, 104.7, 110.3, 110.6, 112.9, 131.0, 133.1, 133.2, 136.2, 142.1, 144.8, 148.3, 161.6, 167.8. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.3. ^{19}F NMR (376 MHz, D_2O) δ (ppm) −111.5. HRMS (ESI) calcd for $C_{21}H_{26}FN_2O_{10}P$ $[M+H]^+$ 517.1406; found: 517.1397.

*4.3.11. β-Nicotinamide ribose-5-[5-(3-fluorophenoxy)pentyl] phosphate (**9k**).* Compound **9k** (syrupy solid, 217 mg, 67%) was obtained from **7** (263 mg, 0.63 mmol) as similar procedure described for the synthesis of **9c** (anion resin, aqueous 5% HCO_2H). ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.37 (q, 2H), 1.57 (m, 2H), 1.64 (m, 2H), 3.78 (q, 2H), 3.87 (t, J=6.2 Hz, 2H), 4.05 (dd, J=4.5, 11.6 Hz, 1H), 4.20 (dd, J=4.3, 12.0 Hz, 1H), 4.31 (m, 1H), 4.38 (t, J=5.0 Hz, 1H), 4.48 (s, 1H), 6.05 (d, J=5.3 Hz, 1H), 6.60 (m, 3H), 7.15 (m, 1H), 8.14 (t, J=7.2 Hz, 1H), 8.78 (d, J=8.0 Hz, 1H), 9.12 (d, J=6.1 Hz, 1H), 9.28 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 66.8, 68.8, 71.0, 73.2, 80.2, 89.6, 89.7, 102.4, 104.5, 104.7, 110.1, 110.3, 113.1, 131.0, 133.1, 133.2, 136.3, 142.2, 144.8, 148.4, 162.4, 167.5. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.6. ^{19}F NMR (376 MHz, D_2O) δ (ppm) −111.7. HRMS (ESI) calcd for $C_{22}H_{28}FN_2O_9P$ $[M+H]^+$ 515.1595; found: 515.1592.

*4.3.12. β-Nicotinamide ribose-5-{2-[2-(2-chlorophenoxy)ethoxy]ethyl} phosphate (**9l**).* Compound **9l** (syrupy solid, 231 mg, 69%) was obtained from **7** (263 mg, 0.63 mmol) as similar procedure described for the synthesis of **9c** (anion resin, aqueous 5% HCO_2H). ^{1}H NMR (400 MHz, D_2O) δ (ppm) 3.61 (t, J=4.1 Hz, 2H), 3.70 (t, J=4.2 Hz, 2H), 3.86 (m, 2H), 3.99 (m, 3H), 4.13 (ddd, J=2.4, 3.9, 11.9 Hz, 2H), 4.18 (m, 1H), 4.23 (t, J=5.1 Hz, 1H), 4.32 (t, J=2.4 Hz, 1H), 5.87 (d, J=5.2 Hz, 1H), 6.70 (t, J=7.6 Hz, 2H), 6.79 (d, J=8.3 Hz, 1H), 7.03 (m, 2H), 8.00 (t, J=6.4 Hz, 1H), 8.63 (d, J=8.1 Hz, 1H), 8.99 (d, J=6.3 Hz, 1H), 9.10 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 66.8, 67.7, 67.8, 71.0, 71.5, 72.8, 73.0, 80.1, 89.3, 89.4, 102.2, 116.6, 124.0, 124.6, 130.8, 130.9, 132.6, 136.0, 142.0, 144.7, 148.2, 155.7, 162.7, 167.6. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.2. HRMS (ESI) calcd for $C_{21}H_{26}ClN_2O_{10}P$ $[M+H]^+$ 533.1092; found: 533.1092.

*4.3.13. β-Nicotinamide ribose-5-[5-(2-chlorophenoxy)pentyl] phosphate (**9m**).* Compound **9m** (syrupy solid, 224 mg, 68%) was obtained from **7** (263 mg, 0.63 mmol) as similar procedure described for the synthesis of **9c** (anion resin, aqueous 5% HCO_2H). ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.42 (q, 2H), 1.56 (m, 2H), 1.66 (m, 2H), 3.80 (q, 2H), 3.93 (t, J=5.6 Hz, 2H), 4.00 (ddd, J=2.3, 4.6, 12.1 Hz, 1H), 4.16 (ddd, J=2.0, 4.1, 12.0 Hz 1H), 4.29 (m, 1H), 4.36 (m, 1H), 4.46 (m,

1H), 6.03 (d, *J*=5.1 Hz, 1H), 6.81 (t, *J*=7.7 Hz, 1H), 6.92 (d, *J*=8.3 Hz, 1H), 7.13 (t, *J*=7.7 Hz, 1H), 7.2 (d, *J*=9.9 Hz, 1H), 8.11 (t, *J*=7.7 Hz, 1H), 8.73 (d, *J*=8.1 Hz, 1H), 9.11 (d, *J*=6.2 Hz, 1H), 9.27 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 24.3, 30.3, 31.9, 66.8, 68.8, 71.8, 73.1, 80.2, 89.5, 102.4, 116.9, 124.5, 130.8, 131.0, 132.6, 136.2, 142.3, 144.8, 148.3, 156.0, 167.7. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.6. HRMS (ESI) calcd For $C_{22}H_{28}ClN_2O_9P$ $[M+H]^+$ 533.1119; found: 533.1111.

*4.3.14. β-Nicotinamide ribose-5-{2-[2-(N-benzoyl-3-aminophenoxy)-ethoxy]ethyl} phosphate (**9n**).* Compound **9n** (syrupy solid, 287 mg, 74%) was obtained from **7** (263 mg, 0.63 mmol) as similar procedure described for the synthesis of **9c** (octyl-functionalized silica gel, CH_3OH/H_2O, 30:1), followed by size exclusion chromatography (polyacrylamide gel, 25 mM NH_4HCO_3). ^{1}H NMR (400 MHz, D_2O) δ (ppm) 3.69 (m, 2H), 3.79 (m, 2H), 4.02 (m, 4H), 4.08 (m, 1H), 4.17 (d, *J*=11.9 Hz 1H), 4.30 (m, 2H), 4.39 (s, 1H), 5.89 (d, *J*=5.2 Hz, 1H), 6.64 (d, *J*=8.3 Hz, 1H), 6.94 (d, *J*=8.0 Hz, 2H), 6.99 (s, 1H), 7.18 (t, *J*=8.1 Hz, 2H), 7.43 (t, *J*=7.5 Hz, 2H), 7.53 (d, *J*=7.4 Hz, 1H), 7.72 (d, *J*=8.0 Hz, 2H), 8.06 (t, *J*=7.2 Hz, 1H), 8.70 (d, *J*=8.0 Hz, 1H), 9.06 (d, *J*=6.1 Hz, 1H), 9.13 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ (ppm) 66.9, 67.7, 69.7, 71.6, 72.8, 73.2, 80.1, 89.6, 102.3, 110.4, 113.7, 117.0, 129.8, 130.9, 131.3, 132.6, 134.9, 136.0, 136.2, 140.9, 142.1, 144.6, 148.2, 160.7, 171.5. ^{31}P NMR (160 MHz, D_2O) δ (ppm) 0.2. HRMS (ESI) calcd for $C_{28}H_{32}N_3O_{11}P$ $[M+H]^+$ 618.1853; found: 618.1857.

4.4. Preliminary tests of the effects of 9c on microbial cell growth

The detailed procedure for cell culture in the presence of NAD^+ analog **9c** was as follows: 55 mL sterilized Luria–Bertani medium (peptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, pH 7.2) supplemented with 100 μM analog **9c** was inoculated with 8-h-old preculture of *E. coli* DH5α cells (500 μL), and cultured at 37 °C, 200 rpm. The control sample was run in the absence of analog **9c**. Aliquots were withdrawn every 2 h and OD_{600} was recorded on a UV–vis spectrophotometer.

For yeast *S. cerevisiae* ATCC 26108, 50 mL sterilized YEPD medium (glucose 20 g/L, yeast extract 10 g/L, peptone 20 g/L, pH 6.2) supplemented with 100 μM analog **9c** was inoculated with 18-h-old preculture (250 μL), and cultured at 30 °C, 200 rpm. The control sample was run in the absence of analog **9c**. Aliquots were withdrawn every 5 h and OD_{600} was recorded on a UV–vis spectrophotometer.

4.5. Activity assay of ADH in the presence of analog 9c

To a solution of ethanol (10 mM), NAD^+ (5 mM), and analog **9c** (25 mM) in Tris–HCl buffer (50 mM, pH 8.8) was added ADH (0.002 U). The mixture was vortexed and transferred into a colorimetric cuvette. Time course measurement was recorded for 90 s at 340 nm on a UV–vis spectrophotometer at 25 °C. Activity of LDH was similarly tested.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.08.007.

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