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Is Simpler Better? Synthetic Nicotinamide Cofactor Analogues for Redox Chemistry

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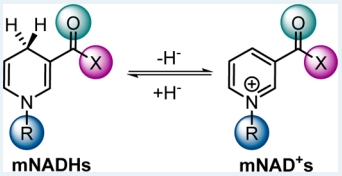
ABSTRACT: The topic of synthetic nicotinamide cofactor analogues is resurfacing as new approaches are being explored, especially in the areas of organic chemistry and biocatalysis. By changing the adenine dinucleotide moiety for a simpler alkyl or aryl group and taking advantage of their ability for hydride transfer, these cofactor biomimetics are used in redox reactions in catalytic or stoichiometric amounts. Alteration of the amide functional group on the pyridine ring, thus varying their electronic properties, and the presence of divalent metal ions also enable rate acceleration in enzyme-catalyzed and chemical reactions. Herein, an overview of the synthesis, mechanism, and applications of nicotinamide cofactor NAD(P)H analogues in redox chemistry, particularly 1,4-dihydronicotinamide derivatives and their oxidized counterpart, is presented. These compounds have been extensively studied as models of NAD(P)H for enzymatic reactions with oxidoreductases as well as nonenzymatic reactions, and the focus of this review is placed mainly on the scope and limitations of these synthetic analogues in biocatalysis.



KEYWORDS: *nicotinamide cofactors, 1,4-dihydronicotinamides, mNADH, biomimetic, biocatalysis, oxidoreductases, metal-free catalysis*

1. INTRODUCTION

The rapidly expanding field of redox chemistry in biocatalysis has led to major changes with respect to the availability of oxidoreductase enzymes (EC 1). Nowadays, the use of these enzymes, such as an alcohol dehydrogenase (ADH, EC 1.1.1.1) is becoming quite trivial,[1,2] thanks to a wide collection of commercially available enzymes and large libraries of recombinant plasmids,[3] including robust expression systems such as *Escherichia coli* and *Pichia pastoris*. Nevertheless, with recent efforts toward improving existing enzymes for use in chemical synthesis through protein engineering[4,5] and redesign,[6,7] the focus has scarcely been placed toward optimizing the cofactors these enzymes use.[8,9] Oxidoreductases require the cofactor β-nicotinamide adenine dinucleotide, existing as a phosphorylated NADP$^+$ and nonphosphorylated form NAD$^+$ as well as in its reduced form NAD(P)H (Scheme 1), a ubiquitous central redox cofactor in living cells involved in many cellular processes, such as electron transport and oxidative phosphorylation.

A distinction between NADH and NADPH is drawn in biochemical reactions in living cells, NADPH being used in anabolic reactions whereas NAD$^+$ is used in catabolic reactions. The nicotinamide cofactor can act as an electron donor NAD(P)H or acceptor NAD(P)$^+$ by releasing or accepting a hydride from or onto the C-4 position of its nicotinamide moiety (Scheme 1). In oxidoreductase-catalyzed reactions, NAD(P)H must be added to the reaction in either stoichiometric amount, with the subsequent inhibitory problems and economic issues, or in catalytic amount in combination with an in situ regeneration system. Thus, one of the challenges when employing NAD(P)-dependent oxidoreductases remains the regeneration of that particular cofactor or the use of a model in an efficient and economical way in terms of availability, cost, and the removal of byproducts, although several efficient recycling methods already exist using enzymatic, photo- and electrochemical, chemical, and biological approaches.[10−12] The development of synthetic nicotinamide cofactor analogues (mNADHs) has led to a major breakthrough not only in the field of biocatalysis but also in organic chemistry and for medicinal applications, as well. With the current cost of nicotinamide cofactors (Table 1),[11] and in the interest of bioorthogonality, the renewal of interest in these mimics as cofactors in oxidoreductase-catalyzed reactions is showing great promise.[13−15] This review will discuss the synthesis and mechanism of nicotinamide cofactor analogues as

Scheme 1. Structure of the Nicotinamide Cofactor in Its Oxidized NAD(P)$^+$ and Reduced NAD(P)H Form

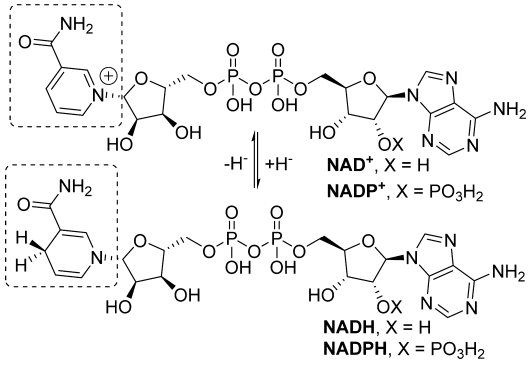


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Table 1. Current Cost of Nicotinamide Cofactors

nicotinamide cofactor	price (euros/mol)[a]
NAD^+	1 410
NADH	2 625
$NADP^+$	18 500
NADPH	70 835

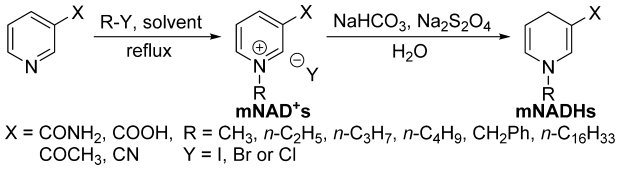
[a]As seen with Alfa Aesar, a Johnson Matthey Company.

well as applications and recent achievements in biocatalysis; organic chemistry; and, more succinctly, in medicine.

2. STATE-OF-THE-ART OF SYNTHETIC NICOTINAMIDE COFACTORS

2.1. 1,4-Dihydronicotinamides Derivatives As Cofactor Models. In 1936, Warburg, Karrer, and co-workers first demonstrated that the pyridine ring of the nicotinamide moiety in NAD(P)H, previously called diphosphopyridine nucleotide DPNH, was reduced during enzymatic reactions.[16−19] An initial series of purely synthetic 1,4-dihydronicotinamide derivatives, mNADHs, was then prepared through reduction of N-substituted pyridinium salts with sodium dithionite under alkaline conditions (Scheme 2) and used to chemically reduce methylene blue to its colorless leuco base.[20] These compounds were referred to as NAD(P)H models to simulate oxidoreductase-catalyzed reactions.

Scheme 2. Preparation of Synthetic $mNAD^+$s and mNADHs



However, it was only in 1955 that Westheimer and co-workers investigated more in-depth the reduced analogue 1-benzyl-1,4-dihydronicotinamide (BNAH) through isotope labeling in the reduction reaction of malachite green to its leuco base and compared BNAH with the natural nicotinamide cofactor to elucidate its mode of action during hydrogen transfer.[21] This study was one of the first mechanistic examples of the direct hydride transfer occurring from the C-4 of the reduced nicotinamide ring and showed that these derivatives could be used as models of NAD(P)H. Further work by Westheimer confirmed the 1,4-dihydronicotinamide structure assigned to 1-methyl-1,4-dihydronicotinamide (MNAH) by NMR spectroscopy.[22] Thereafter, from the 1950s throughout the 1970s 1,4-dihydronicotinamide models were used extensively to elucidate the mechanism of hydrogen transfer by the nicotinamide cofactor and its role in biological systems.[23,24]

More conserved nicotinamide cofactor analogues, semisynthetic $mNAD^+$s, were first prepared in the 1950s and 1960s by the group of Kaplan, this time used as the sole cofactor in oxidoreductase-catalyzed reactions to determine the mode of action of the cofactor and its steric and electronic properties within the enzymatic environment (vide infra).[9,25−35] Other types of 1,4-dihydropyridine derivatives, such as the Hantzsch ester 2,6-dimethyl-3,5-dicarboethoxy-1,4-dihydropyridine, are also used extensively as synthetic NAD(P)H analogues and for hydride transfer in catalytic metal-free reduction reactions.[23,36−38] The discussion on Hantzsch esters is beyond the scope of this review; however, it is relevant to mention that Hantzsch ester and its 1,4-dihydropyridine derivatives were discovered as effective calcium channel blockers and have wide applications in biomedical applications (see Section 5).[39] Throughout the 1980s and 90s, Ohno and co-workers have published extensively on the use of NAD(P)H analogues in chemical synthesis for the reduction of a wide variety of compounds. A review relating the studies of these nicotinamide derivatives as NAD(P)H models covers most of this research,[40] and more detailed reactions are discussed in the Applications to Organic Chemistry section.

Finally, stereoselective syntheses of nicotinamide β-riboside and nucleoside analogues were also described as substitutes for the natural nicotinamide cofactor (Figure 1).[41−45] Sugar-

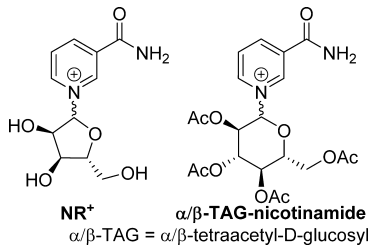
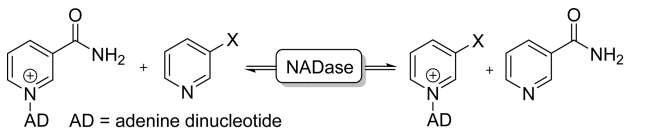


Figure 1. Structure of mNADHs with a sugar residue.

containing mNADHs offer an intermediate alternative between the more conserved semisynthetic analogues and the truncated synthetic mimics and played a role in determining cofactor moiety requirements in oxidoreductases. Overall, these synthetic and semisynthetic $mNAD^+$s and mNADHs have been used as models to simulate enzyme-catalyzed reactions, for hydride transfer in metal-free catalyzed reactions, and as direct replacement of NAD(P)H, with applications for therapeutics.

2.2. Preparation of 1,4-Dihydronicotinamides Derivatives. Kaplan and co-workers produced a series of semisynthetic mNADHs, which were obtained from the natural nicotinamide cofactor with pig brain NADase to substitute the 3-carbamoyl moiety on the pyridine ring for different substituents (Scheme 3).[9,25−31] The results of these semisynthetic $mNAD^+$s in enzymatic reactions are discussed further below.

Scheme 3. Preparation of semi-synthetic $mNAD^+$s from $NAD(P)^+$



Synthetic $mNAD^+$s and mNADHs are quite straightforward to prepare. Using an adapted procedure from Karrer in the 1930s (Scheme 2), commercially available pyridine derivatives are alkylated with an alkyl halide, such as benzyl bromide, under reflux in a solvent to obtain the desired halide pyridinium salts. Simple filtration and washing with diethyl ether lead to the pure products in generally high yields.[15] The pyridinium ring is then reduced into the corresponding 1,4-dihydropyridine with sodium dithionite under aqueous basic conditions and inert atmosphere, obtaining the mNADHs products in moderate to

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high yields. For mechanistic studies, the monodeuterated 1,4-dihydropyridine has been obtained by reduction of pyridinium ring in deuterium oxide.[21] Since their first introduction, a wide variety of synthetic mNADHs have been designed and synthesized (Figure 2): N-substituted-1,4-dihydronicotinamides where the R group is alkyl,[46] benzyl or 2,6-dichlorobenzyl,[47,48] alkoxymethyl, 2-chloro- or 2-hydroxyethyl, or a sugar residue;[23] and 1-benzyl-3-substituted-1,4-dihydropyridines.

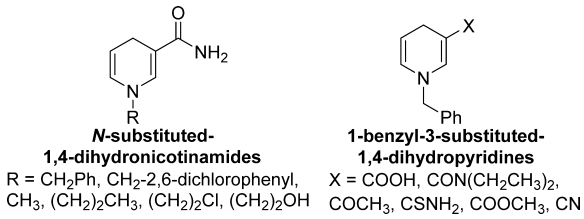
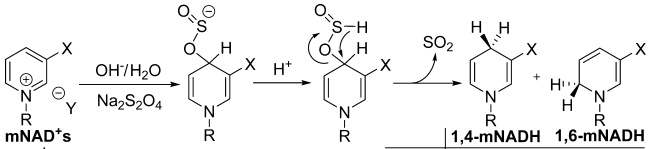


Figure 2. Series of popular synthetic mNADHs.

Reduction of pyridinium salts bearing electron-withdrawing groups (EWGs), such as $-CONH_2$, $-COOR$, $-CN$, with sodium dithionite has been shown to afford almost exclusively the corresponding 1,4-dihydropyridines. This high regioselectivity can be explained by the greater thermodynamic stability of the 1,4-dihydropyridine products with respect to their isomeric 1,2- and 1,6-dihydropyridine derivatives.[49] The reaction of $mNAD^+$s with sodium dithionite occurs with the attack of a dithionite oxyanion at carbon 4 of the pyridinium moiety and proceeds through a sulfinate anion intermediate (Scheme 4), which is stable in alkaline solution; however, under neutral or acidic conditions, the salt is converted to an unstable acid that decomposes.[22,50,51]

Scheme 4. Sodium Dithionite Reduction Mechanism of $mNAD^+$s[52,53]



	1,4-mNADH	1,6-mNADH
BNAH	95	5
MNAH	82	18
1b	94	6
2b	92	8

An intramolecular hydrogen transfer occurs, releasing sulfur dioxide, to generate the 1,4-dihydropyridine products with high regioselectivity, especially for 1-benzyl-substituted derivatives BNAH and **2a**, with 95% and 94% of the 1,4-dihydro compound, respectively (Scheme 4). Nevertheless, a reduction affording a regioselectivity lower than 99.9% does not allow for an efficient recycling system because of the evident loss of the 1,4-dihydro product and accumulation of the 1,6-dihydro derivative over time. Moreover, sodium dithionite is a very potent reductant and therefore can also reduce C=C bonds, leading to racemic and side-products in ER-catalyzed reactions. The regioselective reduction of NAD^+ and $mNAD^+$ derivatives can also be achieved with $[Cp^*Rh(bpy)H]^+$ to obtain exclusively the 1,4-dihydronicotinamide.[54−57] The group of Steckhan was the first to report chemically driven metal-catalyzed regeneration of NAD(P)H using formate as an electron source to generate $[Cp^*Rh(bpy)H]^+$ in situ.[57]

In terms of chemical and physical properties, the reaction of mNADHs and their pyridinium salt derivatives with alkali[58,59] and acid[60−64] has been extensively documented.[11,65] The stability of the natural and substituted pyridinium ions and their 1,4-dihydro reduction products in aqueous buffers was also largely reported.[66] The presence of more electron-donating groups on the nitrogen leads to more acid-labile compounds, whereas EWGs result in base-labile products. Overall, the series of synthetic mNADHs were described as being hygroscopic and photosensitive, thus requiring storage at −20 °C. Conformation preferences and rotational barriers about both the ring-amide bond and the C−N bond in $mNAD^+$s and mNADHs were investigated by NMR spectroscopy and molecular mechanics calculations to show that the cis conformation is strongly preferred, as with NAD(P)H.[67,68] Synthetic mNADHs can therefore be rather simply prepared in good yield with high regioselectivity and purity, whereas semisynthetic $mNAD^+$s require the natural nicotinamide cofactor as starting material, thus increasing the overall cost.

3. NICOTINAMIDE COFACTOR ANALOGUES IN BIOCATALYSIS

3.1. Applications to NAD(P)H-Dependent Enzymes: NAD(P)H As a Direct Prosthetic Group. NAD(P)H can add a hydride directly to the substrate itself or accept/donate reducing equivalents from/to another cofactor such as flavin as part of an electron transport chain. Each electron acceptor or donor displays a specific redox potential, E^0, that determines the ability of the compound to donate or accept electrons (Table 2).

With these redox potentials in mind, Jones's group implemented a system using NAD(P)H mimics with the sugar moiety β-TAG (Figure 2) to recycle catalytic amounts of NAD^+ for preparative scale HLADH-catalyzed oxidation of alcohols, achieving up to 25 turnover number for NAD^+.[72] This study was further developed using purely synthetic mNADHs in a NAD(P)H regeneration system (Scheme 5).[69,73,74]

In the HLADH-catalyzed reaction, NAD^+ was added in its oxidized form; thus, no reduction of the ketone was possible until the addition of an mNADH. Additional control experiments ensured that the mNADHs did not directly reduce the ketone substrate. This recycling system allowed up to 14 recycles of NAD^+. Jones also noted that the values of the second-order rate constants of the reaction reflected the magnitudes of the respective redox potential difference between the acceptor (NAD^+) and the donor (mNADHs).[73]

In another study, Kaplan and co-workers used semisynthetic $NAD(P)^+$ analogues as the sole cofactor, with the carbamide moiety substituted to an isobutyryl, a thioamide ($SNAD^+$), and other groups (Scheme 3).[9,26−28,30,31] Acceleration of the rate in the HLADH-catalyzed oxidation of ethanol was observed with the butyryl- and thioamide-substituted mimics, performing the reaction 8- and 3.5-fold faster, respectively, than with NAD^+. Semisynthetic $mNAD^+$s with lower redox potentials were therefore found to outperform NAD^+ by a few fold. These semisynthetic $mNAD^+$s were used with dehydrogenases, such as HLADH by Kaplan and others,[9,26,27,75−79] and glyceraldehyde 3-phosphate dehydrogenase by Kirtley,[80−83] and 5-methyl nicotinamide analogues were used with HLADH by Samama.[84] The effect of these mimics on the enantioselectivity of ADHs was also evaluated.[85] Structural modifications of NAD^+ in HLADH-catalyzed reactions were investigated more in depth by Luisi[75] and Kazlauskas.[8] The latter produced 13 analogues of

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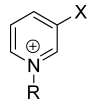
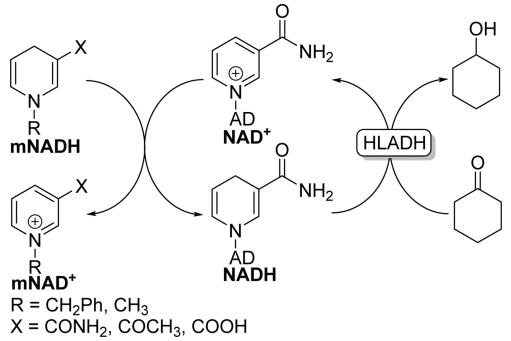
Table 2. Biochemical Redox Potentials, E^0, of Common Reagents, Cofactors,[11] and mNAD$^+$s[8,9,69,70]



X	R	reductive half-reaction	E^0 (mV)[a]
		O_2/H_2O	816
		methylene blue$^{(ox)}$/methylene blue$^{(red)}$	11
		O_2/H_2O_2	295
CN	AD	3-cyanopyridine adenine dinucleotide	−200
		$FMN/FMNH_2$	−211
		$FAD/FADH_2$	−219
$COCH_3$	β-TAG	β-TAG-3-acetylpyridine	−222
$COCH(CH_3)_2$	AD	3-isobutyrylpyridine adenine dinucleotide	−248
COC_5H_6	AD	3-benzoylnicotinamide adenine dinucleotide	−250
$COCH_3$	AD	3-acetylpyridine adenine dinucleotide (APAD$^+$)	−258
CHO	AD	3-formylpyridine adenine dinucleotide	−262
$CONH_2$	β-TAG	β-TAG-nicotinamide	−267
$CSNH_2$	AD	thionicotinamide adenine dinucleotide (SNAD$^+$)	−285
CONHOH	AD	*N*-hydroxynicotinamide adenine dinucleotide	−320
$CONH_2$	AD	NAD$^+$/NADH	−320
$CONH_2$	ADP	NADP$^+$/NADPH	−320
$CONHNH_2$	AD	nicotinohydrazide adenine dinucleotide	−344
C(NOH)H	AD	3-aldoximepyridine adenine dinucleotide	−347
I	AD	3-iodopyridine adenine dinucleotide	−354
$CONH_2$	CH_2Ph	BNA$^+$/BNAH	−361
$CONH_2$	n-C_3H_7	PNA$^+$/PNAH	−387
COOH	AD	nicotinic acid adenine dinucleotide	−400
$CONH_2$	CH_3	MNA$^+$/MNAH	−403
		CO_2/formate	−420
		$2H^+/H_2$	−421
$CON(CH_3)_2$	n-C_3H_7	1-propyl-3-dimethylnicotinamide	−434
COOH	n-C_3H_7	1-propylnicotinic acid	−442
		CL4[71]	−880

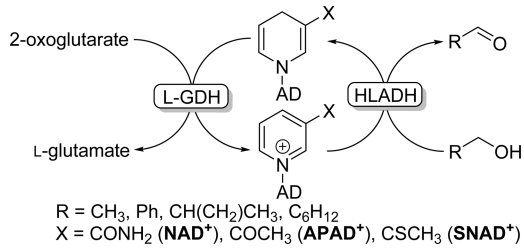
[a]Redox potentials measured under standard conditions according to the references. AD = adenine dinucleotide; ADP = adenine dinucleotide phosphate; TAG = 2,3,4,6-tetraacetyl-D-glucosyl.

Scheme 5. NADH Regeneration with mNADHs for HLADH-Catalyzed Reductions



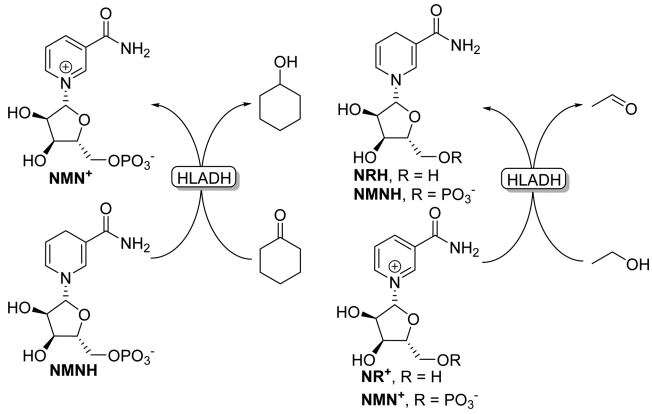
NAD$^+$ using the same procedure as Kaplan and found up to 9-fold rate acceleration when the natural NAD$^+$ was replaced by a stronger oxidant, such as SNAD$^+$ and APAD$^+$, in the HLADH-catalyzed oxidation of alcohols to aldehydes, with using L-glutamate dehydrogenase (L-GDH) for cofactor recycling (Scheme 6).

Scheme 6. NAD$^+$ and Analogues SNAD$^+$ and APAD$^+$ in HLADH-Catalyzed Oxidations



Employing yet simpler nicotinamide cofactor analogues, the group of Sicsic investigated HLADH-catalyzed reactions with nicotinamide mononucleotide (NMN$^+$) and nicotinamide mononucleoside (NR$^+$).[86,87] NMN$^+$ was obtained by enzymatic cleavage of NAD$^+$ with a nucleotide pyrophosphatase and reduced to obtain NMNH, whereas NR$^+$ was synthesized. The reduction of cyclohexanone as well as the oxidation of ethanol catalyzed by HLADH using NMNH or NMN$^+$ was analyzed (Scheme 7).

Scheme 7. HLADH-Catalyzed Reduction of Cyclohexanone with NMNH and Oxidation of Ethanol with NMN$^+$ or NR$^+$



The rate of reaction with NMNH and NMN$^+$ as cofactors was improved by the addition of adenosine in cyclohexanone reduction by 14-fold and in ethanol oxidation by 5-fold. NR$^+$ was not active in ethanol oxidation; however, addition of adenosine monophosphate (AMP) promoted the reaction. Therefore the presence of a phosphate group, whether in NMNH or in AMP, played an essential role for the enzymatic reaction to occur.[86] Similarly, addition of adenosine improved the v_{max} without changing the K_M of NMN$^+$; thus, the position of NMN$^+$ in the enzyme active site was not influenced by the presence of adenosine.[87] Recently, NMN$^+$ was also used for bioelectrocatalysis in enzymatic biofuel cells with an engineered ADH from *Pyrococcus furiosus* (ADHD).[88] The ADHD double mutant K249G/H255R was found to have a lower affinity for NMN$^+$ than for NAD$^+$; nevertheless, the biofuel cells using NMN$^+$ as cofactor performed as well as with NAD$^+$.

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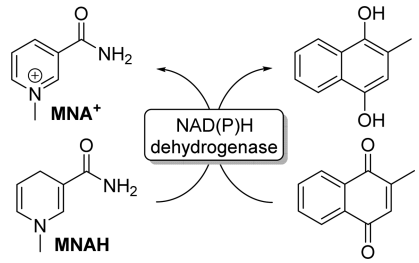
An even simpler nicotinamide cofactor analogue, purely synthetic mNADHs such as MNAH, were used for the enzyme DT diaphorase (EC 1.6.5.2), a NAD(P)H dehydrogenase (quinone). This enzyme accepts equally NADH or NADPH as a cofactor for reduction reactions without distinction. In this study, mNADH both with the adenine dinucleotide (AD) moiety and without, or with the riboside or ribotide moiety, was analyzed.[89] The NAD(P)H dehydrogenase (quinone) was used to reduce menadione with MNAH as cofactor and cytochrome *c* as terminal electron acceptor (Scheme 8).[89,90]

Scheme 8. Simplified Scheme of the Reduction of Menadione with MNAH



Knox and co-workers were therefore the first to demonstrate that simple synthetic mNADHs are taken up as efficient cofactors with an oxidoreductase. MNAH was also found to be as effective as NAD(P)H for an *E. coli* nitroreductase as with NAD(P)H dehydrogenase (quinone), whereas the NMNH and NRH analogues slightly underperformed.[90,91]

The scope of using purely synthetic mNAD$^+$s with enzymes has been extended by the group of Lowe with triazine-based NAD(P)H analogues (Figure 3).[71,92−98] Up to 4% activity was

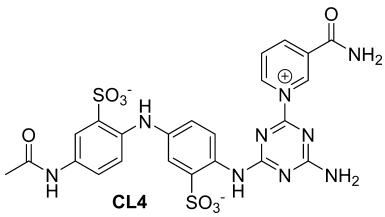


Figure 3. Structure of the NAD$^+$ mimic CL4 by Lowe and co-workers.

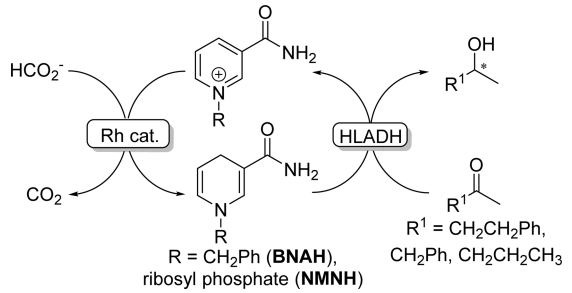
reported with HLADH, using the synthetic CL4 mimic, as compared with the native cofactor.[71,97,98] However, a low v_{max} and high K_M value (1 order of magnitude) for the oxidation of butanol resulted in poor performance of CL4 as a cofactor.

Lo et al. showed that BNAH and NMNH, generated in situ with [Cp*Rh (bpy)H]$^+$, can be used by HLADH to catalyze the reduction of achiral ketones to chiral alcohols (Scheme 9).[99] Both of these mimics displayed very similar results when compared with reactions with NAD$^+$; however, it should be noted that the enzyme preparation employed in this study possibly contained natural nicotinamide cofactor.

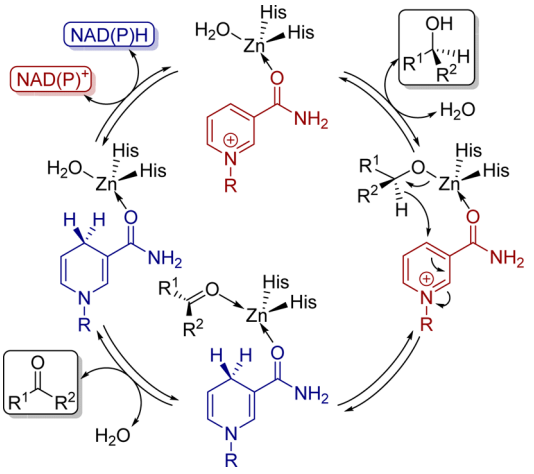
Perhaps the reason for the poor acceptance of simple synthetic mNADHs by ADHs resides in the catalytic mechanism of the enzymes. The nicotinamide cofactor is used as a direct source or acceptor of a hydride with the substrate (Scheme 10).

For the reduction of a carbonyl group by an ADH, the hydride is delivered stereospecifically to one enantiotopic face of the prochiral ketone from NAD(P)H, which is consequently

Scheme 9. HLADH-Catalyzed Oxidation of Alcohols with mNAD$^+$s



Scheme 10. Simplified Catalytic Mechanism of a Zinc-Dependent ADH[100]



aromatized to NAD(P)$^+$. An enzyme is usually specific for either NADH or NADPH, showing higher affinity for one over the other. Binding of the nicotinamide cofactor and the presence of a phosphate group seems to be essential for the enzymatic reaction to proceed. In addition, it was suggested that NAD(P)H is positioned in the ADH in such a way that the ribose backbone of the cofactor has many hydrogen bonding interactions within the active site, therefore explaining the poor activity observed with truncated synthetic mimics.[101] Overall, the semisynthetic mNAD$^+$s are well accepted by ADHs, whereas synthetic mNADHs failed to act as cofactors. In the case of NAD(P)H dehydrogenase (quinone), which does not distinguish between NADH and NADPH, MNAH performed as well as the natural nicotinamide cofactors.

3.2. Applications to NAD(P)H-Dependent Enzymes: NAD(P)H As an Electron Donor. Monooxygenases (1.14.*x*.*x*) require a flavin cofactor and NAD(P)H to specifically incorporate an oxygen atom from molecular oxygen into nonactivated carbon−hydrogen bonds. As replacement for NAD(P)H, BNAH was used to promote a monooxygenase-catalyzed hydroxylation reaction.[13] In this study, Lutz et al. reported that BNAH could supply the electrons necessary for phenol oxidation by 2-hydroxybiphenyl 3-monooxygenase, HbpA (EC 1.14.13.44, Scheme 11). A reduction in the specific hydroxylation activity of the enzyme from ∼4 to 0.4 U/mg was reported. This lower activity may be mainly due to highly increased uncoupling due to the lack of stabilization of the 4α-hydroxyperoxoflavin observed.[102] In another study, Clark and co-workers used the cytochrome P450 BM3 (EC 1.14.14.1) as wild-type and a mutant W1064S/R966D with two synthetic

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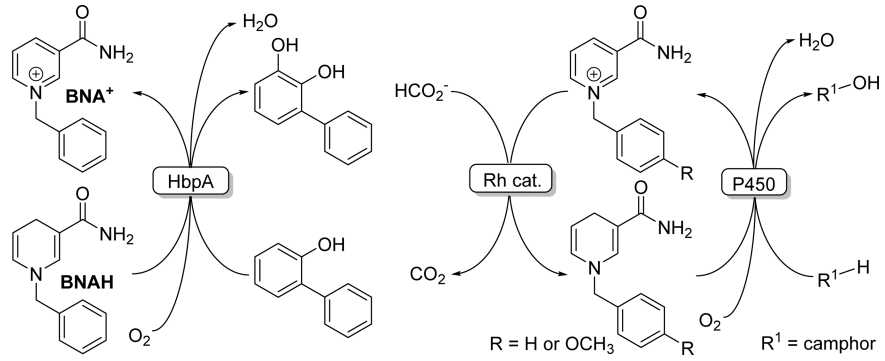
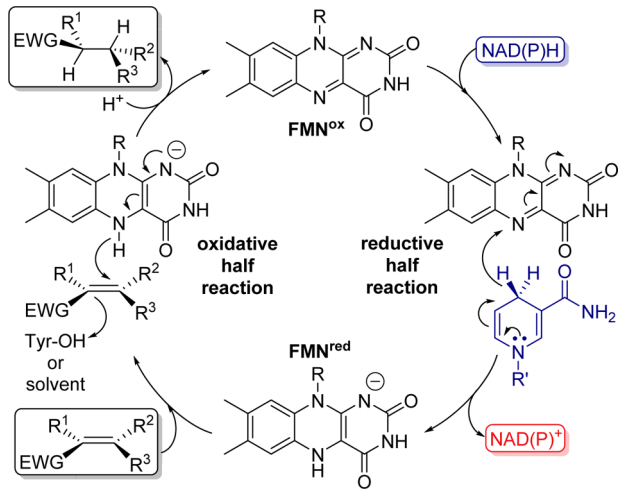
Scheme 11. HbpA-Catalyzed Oxidation with BNAH[13] and in Situ Regeneration System of mNADHs with P450s



mNADHs as cofactors, a rhodium catalyst precursor, and sodium formate as the hydride source for oxidation reactions to determine whether the activity of P450s toward biomimetic cofactors could be improved by protein engineering (Scheme 11).[14] The results showed that the mutant P450 BM3 accepted the two NADH mimics with rates comparable to that of NADH.

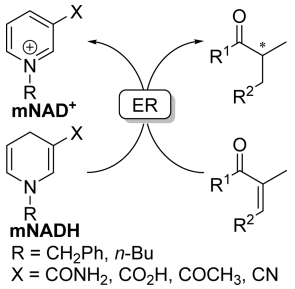
Other flavoproteins, enoate reductases (ERs, EC 1.3.1.31), selectively reduce C=C bonds and tend to exhibit significant cofactor promiscuity. Unlike ADHs, which can show a pronounced preference for either NADH or NADPH, ERs seem to be less specific, and its catalytic mechanism includes flavin mononucleotide (FMN), which plays the role of mediator between the nicotinamide cofactor and the substrate (Scheme 12).

Scheme 12. Proposed Catalytic Mechanism of ER-Catalyzed Reduction of Activated C=C Double Bonds[100]



Recently, we presented a study on a series of mNADHs as replacement for NAD(P)H with ERs (Scheme 13). BNAH and other derivatives could, in fact, replace the natural cofactors with a range of ERs without impairing the final yield or stereospecificity of the reaction. Control experiments were carried out to ensure that in the absence of either cofactor or enzyme, no conversion was detected. In this study, the use of inexpensive synthetic mNADHs represents a true alternative to the established flavin regeneration systems to promote ER-catalyzed reduction reactions and apply them to preparative scale.

Scheme 13. Asymmetric Bioreduction of Conjugated C=C Double Bonds Using Synthetic Nicotinamide Cofactor Mimics (mNADHs)



Another very interesting opportunity with mNADHs lies in the bioorthogonality of these reaction schemes. The low activity of most enzyme classes tested so far with mNADHs with ADHs and monooxygenases enables the use of poorly purified and, hence, inexpensive ER preparations without hampering the selectivity of the desired reactions. Another promising approach for a bioorthogonal redox system was recently developed by Zhao and co-workers through modification of the AMP moiety in NAD^+ to obtain the nicotinamide flucytosine dinucleotide ($NFCD^+$). Various enzymes, such as malate dehydrogenase, D-lactate dehydrogenase, and malic enzyme, were mutated to accept $NFCD^+$ over NAD^+ with excellent activity.[103,104] Moreover, coupling of the mutants of malic enzyme and D-lactate dehydrogenase resulted in the successful conversion of L-malate to D-lactate with only catalytic amounts of $NFCD^+$.[103]

In conclusion, enzymes that do use the nicotinamide cofactor as a direct source of electron seem to accept synthetic mNADHs, such as is the case with the monooxygenases and ERs. Further screenings of other heme- and flavin-dependent enzymes would perhaps establish the full scope and limitation of these NADH mimics in biocatalysis.

4. APPLICATIONS TO ORGANIC CHEMISTRY

In several studies, the synthetic mNADHs were used as models to determine the oxidation reaction, substituent effect,[105] kinetics, and mechanism process with FMN,[106−109] flavin analogues,[110] riboflavin,[111] lumiflavin,[112] flavopapain,[113] quinones,[114] reactions with oxygen, and the requirement for divalent metal ions.[115] Following the study on the enzymatic hydrogen transfer between NAD(P)H and substrate,[116−118] the cofactor analogues were studied to simulate an enzyme-catalyzed reaction and catalyze the hydride exchange between its nicotinamide moiety and a substrate without enzyme.[21] In

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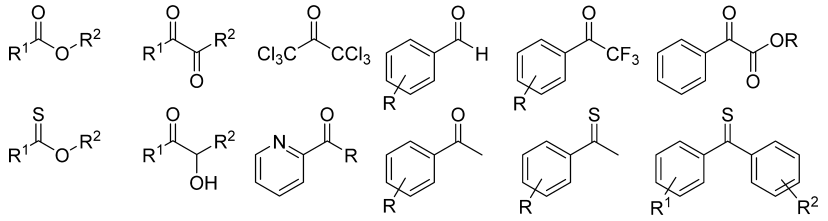
Scheme 14. Selected Examples of Substrates Reduced Using mNADH as a Hydride Donor/Acceptor



addition, the rate of reaction when using mNADHs was found to be enhanced by the presence of divalent metal ions (Ni^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}).[119−128] The role played by a divalent metal ion such as magnesium may be the activation of the substrate and the 1,4-dihydronicotinamide, as well as the stabilization of the transition state.[129,130]

mNADHs, especially MNAH, PNAH, and BNAH, have been used in the reduction of a wide range of compounds (Scheme 14), such as α-keto esters,[121,131,132] α-diketones,[133] α-hydroxy ketones,[133] trifluoroacetophenone,[134,135] benzaldehydes and acetophenone,[136] 2-acylpyridines,[137] and cinnamoylpyridines (with Mg^{2+} or Zn^{2+}).[138] The reduction of different imines to amines has also been carried out, with α-imino esters,[139] α-imino acids,[140] α,β-unsaturated iminium salts,[141] and reductive aminations.[142] Thiol esters,[120] thio ketones,[143] the aromatic ring in 1,3,5-trinitrobenzene,[144] and thiobenzophenone[145] can also be successfully reduced by mNADHs. Pyridoxal phosphate and analogues,[146] keto acids, quinones, dyes, benzyl, derivatives of maleic and fumaric acids, bromotrichloromethane,[147] chloro-substituted acetone,[47,148] 2,5-dihydroxybenzoquinone,[114] olefins, dimethyl maleate, and fumarate were also reduced with NAD(P)H mimics.[149] A review on mNADHs for the stereoselective reduction of benzoyl formates to their corresponding mandelates depicts well how to achieve directional and orientational control through divalent metal ions using an axis of symmetry and chiral dihydronicotinamide derivatives.[129,150]

Oxidoreductases can catalyze redox reactions with high stereoselectivity. For example, an ADH can transfer either the *pro*-R or *pro*-S hydrogen to and from the nicotinamide cofactor to afford the product with high enantiopurity. To simulate this stereoselectivity, several chiral 1,4-dihydronicotinamide derivatives were designed to achieve asymmetric reductions (Figure 4).[129,130,151] In these redox reactions, a bivalent metal ion is required, therefore reproducing the enzymatic environment of a zinc-dependent ADH to some extent.

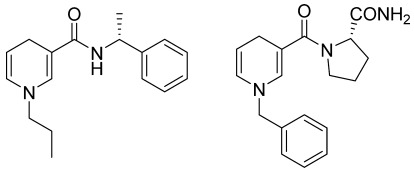
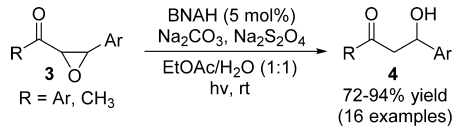


Figure 4. Examples of chiral synthetic mNADHs.

Among other familiar examples of mNADH-catalyzed reactions in redox chemistry is the catalytic hydrogenation of α,β-epoxy ketones **3** with BNAH, which can be regenerated from BNA^+ through chemical reduction with $Na_2S_2O_4$ (Scheme 15).[152,153] The corresponding β-hydroxyketone products **4** were isolated in high yields. More recently, the oxidized form of BNAH was used in catalytic amounts for the hydrogenation of

Scheme 15. Catalytic Hydrogenation of α,β-Epoxyketones with BNAH



α,β-epoxy ketones and 1,2-diketones and regenerated with a mixture of HCOOH/Et_3N.[153] Another example includes the iron-catalyzed hydrogenation for in situ regeneration of a NAD(P)H biomimetic for the reduction of α-keto-/α-iminoesters.[154] Biomimetic in situ regeneration of $NAD(P)^+$ and NAD(P)H models, Hantzsch esters, and dihydrophenanthridine was also reported.[155]

Evidently, many redox reactions can be performed with synthetic mNADHs. This metal-free synthetic route is advantageous for its simplicity, low cost, and low toxicity with respect to other procedures. Moreover, optically pure products can be obtained when combining a chiral mNADH with a divalent metal ion. Clearly, new redox reactions will continue to be discovered with the use of mNADHs.

5. BIOMEDICAL APPLICATIONS

In the topic of therapeutics, a series of 1,4-dihydropyridine redox systems was described as a general and flexible method for site-specific and sustained delivery of anticancer drugs to the brain.[156] Artemisinins were transformed by leucomethylene blue generated from BNAH in situ in aqueous buffer at physiological pH. The authors concluded that artemisinins may act as antimalarial drugs by disturbing the redox balance within the malaria parasite, and BNAH helped to elucidate the mechanism of malarial drugs.[157−159] Nicotinamide mononucleoside and nucleotide analogues were synthesized and tested as microbial and human pyridine nucleotide adenyltransferase inhibitors for potential chemotherapeutics.[160] Recently, Knox and co-workers described the use of synthetic mNADHs in a pro-drug activation system endogenous in human tumor cells.[161] Using the antitumor pro-drug named CB 1954 [5-(aziridin-1-yl)2,4-dinitrobenzamide], a series of synthetic mNADH mimics, particularly the 1-carbamoylmethyl-1,4-dihydronicotinamide, was shown to be a cosubstrate for the enzyme NAD(P)H quinone oxidoreductase 2 (NQO2) with the ability to enter cells and potentiate the cytotoxicity of CB 1954. Other studies involving 1,4-dihydropyridines describe the applications of these compounds in more detail.[39]

6. CONCLUSIONS

Nicotinamide cofactor analogues were shown to be essential as NAD(P)H models to elucidate structural and mechanistic aspects of enzymatic reactions and useful as a hydride donor or acceptor in redox enzymatic and chemical reactions. Although many NAD(P)H regeneration systems are already well

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established, simpler and less expensive synthetic mimics are a viable alternative and can offer many advantages. Synthetic mNADHs are now used as replacements of natural NAD(P)H cofactors in various enzymatic reactions. Similarly, mNADHs are also increasingly used as a hydride acceptor and donor for a wide range of metal-free redox reactions in organic chemistry. Moreover, recent research has shown mNADHs are useful in therapeutics and cover a large array of biomedical applications.

The in situ regeneration of catalytic amounts of mNADHs is already being addressed for nonenzymatic reactions, and a viable, simple, and economical regeneration system will need to be applied in enzymatically catalyzed reactions to fully exploit their catalytic potential. Can we design simple synthetic nicotinamide cofactor analogues to be faster than naturally designed?

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

ADH, alcohol dehydrogenase; BNAH, 1-benzyl-1,4-dihydronicotinamide; BNA^+, oxidized form of BNAH; ER, enoate reductase; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; mNADHs, nicotinamide cofactor analogues; MNAH, 1-methyl-1,4-dihydronicotinamide; NMN^+, nicotinamide mononucleotide; NR^+, nicotinamide mononucleoside; PNAH, 1-propyl-1,4-dihydronicotinamide

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