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The chemistry of the vitamin B3 metabolome

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Abstract

The functional cofactors derived from vitamin B3 are nicotinamide adenine dinucleotide (NAD^+), its phosphorylated form, nicotinamide adenine dinucleotide phosphate ($NADP^+$) and their reduced forms (NAD(P)H). These cofactors, together referred as the NAD(P)(H) pool, are intimately implicated in all essential bioenergetics, anabolic and catabolic pathways in all forms of life. This pool also contributes to post-translational protein modifications and second messenger generation. Since NAD^+ seats at the cross-road between cell metabolism and cell signaling, manipulation of NAD^+ bioavailability through vitamin B3 supplementation has become a valuable nutritional and therapeutic avenue. Yet, much remains unexplored regarding vitamin B3 metabolism. The present review highlights the chemical diversity of the vitamin B3-derived anabo-lites and catabolites of NAD^+ and offers a chemical perspective on the approaches adopted to identify, modulate and measure the contribution of various precursors to the NAD(P)(H) pool.

Introduction

Niacin and niacinamide, also known as nicotinic acid (NA) and nicotinamide (Nam), are the better known forms of vitamin B3 [1,2]. Along with tryptophan (trp), they are biosynthetic precursors to nicotinamide adenine dinucleotide (NAD^+), nicotinamide adenine dinucleotide phosphate ($NADP^+$) and their respective reduced forms (NAD(P)H), altogether referred as the NAD(P)(H) pool. The vitamin B3 metabolome includes the biosynthetic precursors of NAD^+ (anabolites; Table 1a), the cofactors derived from NAD^+ (i.e. the NAD(P)(H) pool; Table 1a) and the derivatives generated through catabolic processes (catabolites; Table 1b) [3–8]. Altogether, the NAD^+-derived cofactors are central to cellular homeostasis and growth through their roles in intermediary metabolism, mitochondrial respiration, the Krebs' cycle, ATP production, reactive oxygen species generation and inhibition, and additional roles in post-translational protein modifications, protein regulation and second messengers' generation [9–16]. Sub-optimal intracellular levels of these cofactors yield to cellular dysfunction, while acute vitamin B3 deficiency leads to pellagra [17,18], a debilitating and deadly disease still endemic in some regions of the world where malnutrition is common

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place. In more affluent countries, clinical vitamin B3 deficiency is due to poor food choices, adverse drug reactions, alcoholism and infectious or autoimmune diseases [19–22]. There are several additional excellent publications covering in detail the biological and physiological roles of the NAD(P)(H) pool and that of its biosynthetic precursors [23–30]. The present review covers the breadth of the vitamin B3 metabolome and presents an overview of the tools used to modulate the NAD(P)(H) pool and therefore the vitamin B3 metabolome in biological systems with a focus on mammalian systems. First, the known vitamin B3 metabolites (anabolites and catabolites) and the biosynthetic pathways to NAD(P)(H) will be summarized. A brief foray in the chemical and chemoenzymatic routes to NAD^+ precursors will then follow along with an overview of isotopically labeled metabolic NAD^+ intermediates, which have been used to report on the vitamin B3 metabolomic profiles..

The chemistry of the NAD(P)(H) pool

The anabolites and catabolites of the vitamin B3 metabolome

Once generated from vitamin B3 derivatives via independent biosynthetic pathways, NAD^+ can be converted to its reduced form NADH via redox processes or to its phosphorylated form $NADP^+$, which, in turn, can enter redox processes to generate its reduced form, NADPH. Alternatively, NADH can be phosphorylated to NADPH. This constitutes the anabolic pathways to the NAD(P)(H) pool. Upon a range of biochemical and chemically driven processes, the components of the NAD(P)(H) pool are converted to nicotinamide or to catabolites which are either eliminated through excretion or recycled. The following describes these components in greater detail.

Vitamin B3 anabolites—Niacin (NA) and niacinamide (Nam) fall under the vitamin B3 denomination [1]. Intracellularly, NAD^+ is generated from dietary vitamin B3 or trp (Figure 1) with the contribution made by the latter, known as the kynurenine pathway, varying greatly between species and organs [31–36]. Via the kynurenine pathway, biosynthetic precursors to NAD^+ include kynurenine, 3-hydroxykinurenine, 3-hydroxyanthranilate and quinolinate, leading to nicotinic acid mononucleotide (NAMN) [37]. NAMN is also an NAD^+ anabolite through the Preiss-Handler pathway, which uses NA [38], while nicotinamide mononucleotide (NMN) is generated in the salvage pathway, which uses Nam. Additional NAD^+ anabolites include nicotinamide riboside (NR), nicotinic acid (NAR) and nicotinic acid adenine dinucleotide (NAAD) [8,39,40].

Vitamin B3 functional catabolites—NAD^+ and $NADP^+$ are substrates of enzymes capable of cleaving the glycosidic linkage between the northern ribose of the dinucleotide and nicotinamide, and replacing the latter with water, nucleophilic nucleobases or side chains of peptidic residues (e.g. hydroxyl or carboxylate) [41–49]. Unless chemical hydrolysis occurs, this cleavage is a finely orchestrated nucleophilic enzymatic process, leading to an exquisitely specific derivative. These derivatives are unique with regard to the biology they regulate [50]. NAADP is generated by an as-yet undiscovered biosynthetic pathway either from NAAD or from $NADP^+$, regulating intracellular Ca^{2+} signaling processes [51]. The cyclic form of adenosine diphosphoribose, cADPR, produced by a cyclase [5], specifically mobilizes Ca^{2+} from ryanodine receptors [52], while its linear form, ADPR (adenosine diphosphoribose), generated from NAD^+ by glycohydrolases, promotes Ca^{2+} cellular uptake [53]. Unlike ADPR itself, acylated forms of ADPR are products of NAD^+- dependent post-translation modification catalyzed by sirtuins [10,26,54] hydrolyzed to ADPR by esterases [55]. Finally, the polymeric forms of ADPR, product of PARP enzymes, either covalently bound to proteins or free in solution, act as major complex recruiting agents in DNA repair [56–58].

Vitamin B3 catabolites—Many catabolic pathways are responsible for the loss of vitamin B3-derived cofactors and of their anabolites. Upon high NA intake, excess NA is converted to nicotinuric acid (NUA, Table 1b) in a phase 2 metabolic process when conjugated to glycine [59]. Excess Nam is readily oxidized to *N*-oxide-Nam (Table 1b) by cytochrome P450 [60,61]. Yet, under standard dietary conditions, the bigger contributor to vitamin B3 catabolism in human physiology is the methylation of Nam, leading to *N*-methyl-Nam (*N*-Me-Nam; Table 1b). The formation of *N*-methyl-Nam requires S-adenosylmethionine. Therefore, in conjunction with homocysteine, *N*-methyl-Nam is a reporter of both the 1-carbon pathway efficacy and the vitamin B3 dietary status [62,63].

Trigonelline is *N*-methyl nicotinic acid, found abundantly in fenugreek and thought to be generated during coffee bean processing [64,65]. Trigonelline is also a catabolite found in tissues but less often measured [66] and for which the physiological properties remain unexplored. Oxidation of circulating *N*-methyl-Nam by aldehyde oxidase yields *N*-methyl-4-pyridone3-carboxamide (*N*-Me-4PY) and *N*-methyl-2-pyridone-5-carboxamide (*N*-Me-2PY) [6,67–69]. Much confusion exists in the literature as to the nomenclature of these two entities. The relative production of these catabolites is species-specific as well as driven by age and health status [70]. *N*-Me-2PY has been described as a uremic toxin because of the correlation between its abundance in blood and kidney disease states [71]. Critically, these two pyridones are produced systemically [72,73]. There, *N*-Me-2PY is thought to be an inhibitor of PARP function at physiologically relevant concentrations [67,74,75]. Another catabolite of vitamin B3 is *N*-ribosyl-3-carboxamide 4-pyridone (4PYR, Table 1b). This ribosylated pyridone is also found abundantly in circulation in uremic patients. Importantly, it is easily converted to its nucleotide forms (4PYR-MP, 4PYR-DP, 4PYR-TP, Table 1b) or adenylated to generate pyridone adenine dinucleotide species [NAD(P)O, Table 1b] [76–82]. Both the phosphorylated forms of 4PYR and its dinucleotide forms are endogenously generated. The synthesis of NADPO has been shown to occur as a side-reaction on $NAD(P)^+$ catalyzed by flavin-dependent oxidases, such as ferrodoxin reductase [83–85]. *In vitro,*, the nucleotide forms show substantial ability to inhibit ATP-dependent kinases, while the dinucleotides are inhibitors of $NAD(P)^+$-dependent metabolic redox enzymes at physiologically relevant concentrations [76,86]. A similar class of $NAD(P)^+$ catabolites capable of inhibiting key metabolic enzymes are hydroxylated NAD(P)H (NAD(P)HX, Table 1b). The generation of these catabolites, which occurs chemically, is sufficiently critical to warrant a repair mechanism in all forms of life and the regeneration of NAD(P)H as accumulation of these catabolites causes central metabolomic perturbations [87,88]. Finally, other even less explored NAD(P)H catabolites are the 1,2-NAD(P)H and the 1,6-NAD(P)H [89]. These isomers can be mistaken for the α-anomeric forms of NAD(P)H [90–92] and are excellent inhibitors of isolated NAD(P)H-dependent redox enzymes. Renalase has been shown to re-oxidize these NAD(P)H isomers *in vitro*. It can then be viewed as a NAD(P)H repair enzyme directly affecting intracellular metabolism [93].

Overall, except for *N*-Me-Nam and *N*-Me-2PY, the catabolites of the NAD(P)(H) metabolome are rarely accounted for in metabolomic studies [94–96]. Furthermore, these compounds react readily under standard analytical conditions used for cell and tissues metabolomic measurements unless special care is applied and therefore go undetected. As such, an in-depth account of the detection protocols of the vitamin B3 metabolome is warranted but is beyond the scope of this review. Furthermore, mammalian cells have in place at least two known repair mechanisms to control dinucleotidic catabolite levels, renalase and NAD(P)HX dehydratase/ epimerase [87,88,93]. Dysregulation of such repair processes and accumulation of these catabolites surely impacts cellular homeostasis. Yet, the function, regulation and impact of the multiple vitamin B3 repair mechanisms have been vastly under-explored.

Biochemical pathways known to sustain the NAD(P)(H) pool

Notably, tryptophan, NA and Nam employ three convergent pathways which require molar equivalents of 5-phospho-1-pyrophosphoriboside (Scheme 1; PRPP) to convert quinolinic acid and NA to nicotinic acid adenine dinucleotide (NAAD) or Nam to NAD^+ (Scheme 1) [34].

While cofactors derived from riboflavin (vitamin B2) [97] and pyridoxine (vitamin B6) [98] are required by enzymes of the kynurenic pathway to generate quinolinic acid from tryptophan, $NADP^+$ and thiamine (vitamin B1) diphosphate are cofactors required for the synthesis of PRPP from glucose 6-phosphate [36]. This highlights the dependency of the NAD(P)(H) biosynthetic pathways on the bioavailability of three other metabolic cofactors, all derived from water soluble B-vitamins.

Along with NA, Nam and trp, NAR and NR are also precursors of NAD^+ (Scheme 1). Noticeably, QA, NA and Nam require phosphoribosylation as means of biosynthetic activation to NAMN [99] and NMN [100–102], while NR and NAR require phosphorylation by a specific kinase (Scheme 1) [8,39,103,104]. NAMN and NMN are biosynthetic intermediates to NAAD and NAD^+, following an adenylyl transfer (Scheme 1) [105–109]. NAAD, acting as a pre-NAD^+ storage pool [40], is converted to NAD^+ by a ligase (NADS) (Scheme 1) [35,110]. $NADP^+$ is generated from NAD^+ by NAD^+ kinase for which NADH is a weak substrate yielding NADPH [111]. It must be noted that while NR, NAR and NMN are PRPP-independent precursors to the NAD^+, they are only molar equivalent precursors to NAD^+. It is the generation of Nam through NAD^+ consuming enzymes and its recycling to NAD^+ which enables sustained NAD^+ levels [112]. To sustain increased NAD^+ levels through NR supplementation, NRK, NMNAT and NamPRT (nicotinamide phosphori- bosyl transferase; Scheme 1) must be functional, with turn-over in excess of that of Nam methylation by NNMT and cellular export mechanisms.

It is only recently that NR and NMN, both found in milk [113,114], have gained recognition as nutraceutical precursors of NAD^+. NR supplementation in cell-based assays was evidenced to boost the NAD(P)(H) pool with a specific effect on the mitochondrial pool and function. Supplementation with NA and Nam, while critical in acute vitamin B3 deficiency, does not demonstrate the same physiological outcomes compared with that of supplementation with NR or NMN [7,15,34,94], indicative of additional controlling factors, such as intracellular biodistribution, expression of key biosynthetic enzymes and/or bioavailability of PRPP. To explore the parameters controlling functionalization and conversion of these NAD^+ precursors and their biological endpoints in cell-based assays as well as in animals, an extensive synthetic program has been implemented over the past 50 years.

Accessing biosynthetic precursors of NAD^+

NA and Nam can be readily obtained from bacterial broth, foodstuffs or generated from petroleum sources [1]. They are now widely available commercially along with some more clinically focused versions and formulations [115]. The ribosylated forms of NA or Nam have required the development of more substantial synthetic routes.

Enzymatic syntheses—NR may be prepared enzymatically from NAD^+ and NMN by using snake venom phosphodiesterase and subsequent transformation of NMN to NR with prostatic monoesterase [116] or with 5′-nucleotidase [117]. Alternatively, NR can be generated from α-D-ribose-1-phosphate and Nam using purine nucleoside phosphor-ylase and sucrose phosphorylase [118]. There are only few reports in the literature describing the efficient chemical generation of NMN from NR [119–121]. In general, this process is often low yielding and associated with difficulties in removing phosphate contaminants. As such, enzymatic conversions with isolated NRK or whole cell production have been explored, but they too remain challenging. Accessing NAR has been even less explored. Yet, the generation of NAMN from NMN using a new cross-linked deamidase aggregate biocatalyst has been reported [122]. This offers new opportunities for a facile access to NAR via enzymatic routes using phosphatases such as 5′-nucleotidase [8].

Chemical syntheses of nicotinoyl ribosides and derivatives—Two main synthetic strategies have been developed to access NR salt forms (NR^+X^-) (Figure 2). One proceeds via a reaction between Nam or derivative **A** and a peracylated (halo)-D-ribofuranoside **B** resulting in acylated intermediate **C** that is subsequently converted into the desired $NR^+X^–$ salt. This approach was also applied for the synthesis of NAR (NAR zwitterion). The other proceeds via the condensation of *N*-(2,4-dinitrophenyl)-3-carbamoylpyridinium salt **D** with derivatives of D-ribofuranosylamine **E** [120]. To date, the first approach has proved the most efficient in terms of overall yields and chemo-selectivity. We will summarize advances made with this first approach.

Two anomeric α- and β-forms of NR (α-C and β-C; Figure 2) can be generated by glycosylation reactions with the stereochemical outcome of the synthesis being dependent on the nature and stereochemical position of the leaving group X, nature of the substituents at amide nitrogen atom in Nam and conditions of glycosylation, such as solvent and temperature. Because only the β-form of NR or NAR is of biochemical relevance, the most valuable synthetic methods offer β-stereoselectivity.

The first chemical syntheses of NR salts (NR^+X^-) was described by Todd and coworkers [123,124] and entailed the glycosylation of nicotinamide (Nam) **1a** with either 1-bromo-2,3,5-tri-*O*-acetyl-D-ribofuranose to yield the bromide salt, 1-chloro-2,3,5-tri-*O*-acetyl-D-ribofuranose to yield the triacetylated chloride salt or 1-chloro-2,3,5-tri-*O*-benzoyl-D-ribofuranose to yield the tribenzoylated chloride salt. The halosugars were obtained from 1,2,3,5-tetra-*O*-acetyl-D-β-ribofuranose (2a) or 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-β-ribofuranose (2b) [125]. Such chemistry resulted in the generation of both pyridinium riboside anomers, with the best results in terms of β-/α-anomer stereoselectivity obtained when chlorosugars were used as precursors. Removal of the protecting groups in anhydrous methanol saturated with dry ammonia at 0°C yielded $NR^+Cl^—$ as a 4: 1 mixture of β- and α-anomers [124]. Low temperature was required to minimize Nam release (Scheme 2).

While several routes and optimization studies have been conducted [126] since the first synthetic route development, the most versatile uses tetra-acylated ribosides and TMSOTf as a catalyst [127,128]. Sauve and coworkers improved on the method and reported a very efficient one-pot procedure for the synthesis of β-NR from ethyl nicotinate [129,130]. Both routes generate the triflate salt forms of NR. The triflate salts, deemed unsuitable for pharmacological use, must be exchanged for pharmaceutically acceptable anions. Anion exchange either by liquid/liquid extraction [131] or by treatment with ion exchange resin such as using Amberlite IRA400-Cl have been successfully applied to generated $NR^+Cl^—$ [132]. Oxidation of the reduced form of NR 7 on charcoal in the presence of protic salts, such as ammonium salts NH_4X^-, is an alternative method to anion exchange resulting in different salt forms of $NR^+X^—$. Acylated NR-triflate and acylated NAR prepared via mechanochemical methods, reduced to the acylated 1,4-dihydronicotinamide and dihydronicoti- noyl riboside, can be readily extracted in pure form in organic solvents (Scheme 3) [133]. The reduced forms of NR **6a-b** and NAR are stable to Bronsted bases and, therefore, the acyl groups can be removed at room temperature at increased rates [134,135]. 1,4-Dihydronicotinamide riboside derivatives may be also oxidized with hexachloroacetone or cobalt(II) acetate in the presence of hydrogen peroxide. The later process requires removal of cobalt cations with QuadraSil AP resin [136].

Chemical reduction in *N*-substituted pyridinium salts results in three possible isomeric products: 1,2-, 1,4- and 1,6-dihydropyridines (DHP) as illustrated in Figure 3 for corresponding dihydro-1-P-D-ribofuranosyl-3- pyridinecarboxamides.

Reduction in pyridinium salts to dihydropyridines has been extensively reviewed in the literature [137–140]. Sodium borohydride ($NaBH_4$) and sodium dithionite ($Na_2S_2O_4$) are the most commonly used reducing agents to reduce NAD(P), NMN and NR. However, these reagents are not equivalent. $Na_2S_2O_4$ regioselectively reduces NAD^+ to 1,4-dihydronicotinamide adenine dinucleotide (NADH), and NR to the 1,4–3-carboxamide dihydropyridinyl riboside, while reduction in NAD^+ and NR^+ with $NaBH_4$ or milder hydride-based reducing agents results in a mixture of the 1,2-, 1,4- and 1,6-isomers.

Synthesis of pyridones—While the hydroxylated forms of 1,4–3-carboxamide dihydropyridinyl riboside derivatives (e.g. NAD(P)HX) are readily generated from the ribosylated species, the pyridone-derived catabolites (N-Me-2/4-PY, 4-PYR, NAD(P) O, 4-PYR-M/D/TP; Table 1b) require chemical syntheses. 4-Pyridone-3-carboxamide (4PY) and 2-pyridone-5- carboxamide (2PY) are generated from 4-chloro-3-carboxypyridine and 2-hydroxy-5-cyanopyridine, respectively [79]. These can then be used to prepare the nucleosides [79,141]. Critically, the nucleotide- and dinucleotide-derived pyridones are only prepared on analytical scale, generated as enzymatic side-reaction products [142].

Synthesis of isotope-labeled NAD^+ precursors (isotopomers and isotopologues)

Decaying and stable isotope-labeled derivatives [143–146] have been used to study metabolic pathways and bio-distribution processes. Combining separation to detection and quantification allows for complex product distributions to be measured. To differentiate between biosynthetic components and pathways of the NAD(P)(H) pool, anabolites incorporating different profiles of stable isotopes can be used if their incorporation into NAD^+ leads to versions of NAD^+ which can be differentiated by mass (MS) or fragmentation patterns (MS^2). Here, enter two critical definitions: that of isotopomers which are molecules which vary in the position of labeled atom, such as 2′–^{2}H-NR versus 1′–^{2}H-NR and isotopologues which are molecules which differ by containing different isotopes, such as ^{2}H-NR versus ^{13}C-NR. These isotopically labeled compounds will possess different exact molecular mass and/or fragments' exact mass.

Presently, the rationale applied to selecting appropriate isotopologues is driven by the question being asked and the levels of the isotopically labeled derivatives to be detected. Bioavailability and biodistribution studies in animals are often addressed using decaying isotopomers. Combined to liquid chromatography, this highly sensitive method differentiates between the bio-transformed radio-isotopically labeled products, e.g. [147]. Furthermore, the uniformly labeled NAD^+ is commercially available and amenable to chemoenzymatic transformations. For instance, radio-isotopically labeled NAD^+ can be converted to its $NADP^+$ parent by NAD^+ kinase [148]. It is also reduced enzymatically to NADPH [148]. These can be used as starting materials in some of the enzymatic processes described below.

Non-decaying isotopomers provide the necessary versatility to interrogate fluxes if the building blocks and products can be traced with enough statistical confidence at levels above natural isotopic abundance. For instance, to establish in mammals whether NR was directly converted to NMN, or hydrolyzed to Nam prior to it being incorporated into NMN, the use of a doubly labeled isotopomer of NR was used [94]. This isotopomer incorporated one heavier isotope on the nicotinamide ring and one heavier isotope on the furanose. The glyco-sidic breakage led to mono-labeled NAD^+, while the direct incorporation led to doubly labeled NAD^+ being detected. In cell work study, this type of multi-site labeling informs on the regulation of the biosynthetic pathways and of the turn-over of NAD^+ by consuming and biosynthetic enzymes [34,40,107].

Isotopically labeled forms of Nam, NA and trp are available commercially and can thus be selected at will. Similarly, ribosylated derivatives, for which isotope labels may be incorporated into the sugar residue (^{2}H, ^{3}H, ^{18}O, ^{13}C and ^{14}C isotopes) and the nicotinamide or nicotinic core (^{18}O, ^{13}C, ^{14}C and ^{15}N isotopes), or both (Figure 4), require dedicated chemoenzymatic or chemical syntheses.

Decaying isotopically labeled NAD^{+} anabolites, such as synthetic tritiated nucleotides and ^{32}P-containing ATP, have been combined to chemoenzymatic preparations to allow for the efficient generation of radiolabeled NAD(P)(H) pools [149–155]. The enzymatic methods allow preparation of not only NR derivatives labeled in the Nam core but in the ribosyl moiety as well when labeled phosphoribosyl pyrophosphate (PRPP) is used. An example of this methodology is found in work of *Kašarov and Moat* describing preparation of *[carbonyl-^{14}C]NR* from corresponding ^{14}C-labeled NAD^{+} catalyzed by enzymes from *Proteus vulgaris* OX-19 [156]. Saunders et al. [117] describe the preparation of *[carbonyl-^{14}C]NR* and [4-^{3}H]NR by the treatment of corresponding radiolabeled NMN with 5′-nucleotidase. Chemical synthesis of tritium-labeled NR and subsequent enzymatic synthesis of tritium-labeled NMN as well as corresponding [2′–^{3}H]-NAD^{+} are described in work of Cen and Sauve [157], which also describes the synthesis of NAD^{+} containing ^{18}O-label in the NR portion of the molecule and originating from [5-^{18}O]glucose. Bull et al. [158] describes the chemical synthesis of deuterium-labeled [1′–^{2}H]NR+Br$^{—}$ and [1′–^{2}H]NMN. Once purified, this was used to prepare [1′–^{2}H]NAD^{+} enzymatically, which was then enzymatically converted to [*carbonyl-^{14}C,1′*–^{2}H]NAD^{+} with [carbonyl-^{14}C]Nam. In a series of papers by Schramm et al. dealing with the enzymatic synthesis of [^{3}H,^{14}C] NAD^{+} isotopomers, the authors used [2-^{3}H]-, [5-^{3}H]-, [6-^{3}H]-, [2-^{14}C]- and [6-^{14}C]glucose and nicotinic acid to generate corresponding [1′–^{3}H]-, [2′–^{3}H]-, [4′–^{3}H]-, [5′–^{3}H]-, [1′–^{14}C]-, [5′–^{14}C]NAD^{+}; they also describe preparation of ^{15}N-labeled NAD^{+} isotopologues, such as [1′–^{14}C,1-^{15}N]NAD^{+} and [5′–^{14}C,1-^{15}N]NAD^{+} (primed numbers indicate atomic locations in the ribosyl residue of NR part of NAD^{+}), using ^{15}N-labeled NA as a source of the label [159–162]. The enzymatic synthesis of [^{14}C]NR was achieved from unlabeled NAD^{+} and [carbonyl-^{14}C] Nam in the presence of ADP-ribosylcyclase to give ^{14}C-NAD^{+}, followed by treatment with phosphodiesterase I and alkaline phosphatase [163].

While extremely sensitive, detection of radiation-emitting entities requires special laboratory set-up and therefore limits its use by the wider research community. Detection of non-decaying isotopic modifications are less sensitive but rely on more generally adopted protocols [145]. Yet, accurate measurements of the vitamin B3 metabolome in biological systems have been limited by the chemical availability of chemical standards and tailor-made vitamin B3 metabolites. However synthetic efforts have been undertaken towards achieving higher availability of labeled and non-labeled standards for an increased coverage, characterization and quantification of the metabolome. The use of isotopically labeled vitamin B3 metabolites combined to powerful targeted metabolomic analytical methods has proved particularly suited to improving our knowledge of vitamin B3 both at cellular and organismal levels, allowing rapid translational discoveries [34,164]. This has been enabled by dramatic advances in the field of mass spectroscopy, metabolomics and large data set management along with an increased access to molecules purposefully incorporating isotopes that enable their detection and quantification as well as inform of their modifications.

According to the approach described by Tran et al. [163], [^{13}C,^{18}O]NR can be generated from [U-^{13}C] glucose and NA enzymatically converted to [^{13}C]NAAD containing fully ^{13}C-labeled ribosyl residue in NAR part of the NAAD molecule. This synthesis requires usage of 10 enzymes, along with ATP, phospho(enol)pyru-vate, NADP^{+} and α-ketoglutarate. In the second — again enzymatic — step, purified ^{13}C-labeled NAAD was transformed to corresponding [^{13}C]NAD^{+} by NAD^{+} synthetase. Then, purified [^{13}C]NAD^{+} was incubated with [^{18}O]Nam (prepared by chemical reaction of 3-cyanopyridine with ^{18}O-water) in the presence of ADP-ribosylcyclase to give [^{13}C,^{18}O]NAD^{+} that was subsequently degraded by using phosphodiesterase I and alkaline phosphatase to quantitatively afford [^{13}C,^{18}O]NR. Furthermore, the enzymatic synthesis of ^{18}O-labeled NAD^{+} from non-labeled NAD^{+} can be achieved using glycohydrolase/cyclase CD38 and ^{18}O-nicotinamide (20-fold excess) [165]. Mills et al. [105] used double-labeled NMN prepared via a procedure based on the work of Lee et al., while Ratajczak et al. mention the synthesis of ^{18}O-labeled NR from [^{18}O]Nam and subsequent synthesis of ^{18}O-labeled NNM by phosphorylation with NRK1 [94,107]. Chemical sequences described above were applied to generate [^{18}O]NR from ^{18}O-labeled Nam and [2′–^{2}H,*carbonyl*-^{13}C]NR generated from the 2′–^{2}H-1,2,3,5-tetra-O-acetyl-β-D-ribofuranose and [*carbonyl*-^{13}C]Nam to establish biodistribution and function [104]. Finally, [2’–^{2}H,*carbonyl*-^{13}C]NAR was synthesized and compared with [2′–^{2}H, ^{18}O]NR in metabolic fluxes and organelle transport experiments [34,40].

Conclusion

Overall, many chemical syntheses and chemoenzymatic syntheses have been developed to identify and trace the metabolites and precursors of NAD(P)(H) and quantify the metabolic distribution following supplementation. Current limitations associated with establishing a true representation of the vitamin B3 metabolome are associated with the breadth of molecules which this metabolome includes, the synthetic challenges associated with their individual preparation, the cost of the isotopically labeled reagents and the scale on which syntheses are carried out. However, these limitations appear to slowly fade as more efficient syntheses become available and enable cell-based kinetic studies and animal pharmacokinetics investigations. This review aimed to update our view of the vitamin B3 metabolome and the current chemical efforts undertaken in the field of NAD^{+} biology to better understand its role in cellular biology and physiology.

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Abbreviations

4PYR	*N*-ribosyl-3-carboxamide 4-pyridone
ADPR	adenosine diphosphoribose

ADPRP	adenosine diphosphoribose phosphate
N-Me-2PY	*N*-methyl-2-pyridone-5-carboxamide
***N*-Me-4PY**	*N*-methyl-4-pyridone3-carboxamide
NA	nicotinic acid
NAAD	nicotinic acid adenine dinucleotide
NAADP	nicotinic acid adenine dinucleotide phosphate
NAD	adenine dinucleotide
NADH	1,4-dihydronicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NADS	nicotinamide Adenine dinucleotide synthase
Nam	nicotinamide
NAMN	nicotinic acid mononucleotide
NAPRT	Nicotinic acid phosphoribosyl transferase
NAR	nicotinic acid
NMN	nicotinamide mononucleotide
NMNAT	nicotinamide mononucleotide adenylyl transferase
NR	nicotinamide riboside
NRK	nicotinamide riboside kinase
PRPP	5-phospho-1-pyrophosphoriboside

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Perspectives

1. Since NAD^+ seats at the cross-road of metabolism and cellular signaling, there is an urgent need to acquire a greater evidence-based understanding of vitamin B3 metabolism and of its role in health, diseases and ageing.

2. Increased access to fit-for-purpose chemical entities and biosynthetic intermediates has greatly enabled the recent discoveries in the NAD^+ field and facilitated translational research in ageing, metabolic diseases and nutrition.

3. As further analytical refinements are achieved, and analytical standards become more widely available, the cellular functions of endogenously generated vitamin B3 catabolites will come under greater scrutiny. Furthermore, as the functional co-dependence between the NAD(P)(H) pool and cofactors derived from vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B5 (pantothenate), vitamin B6 (pyridoxine) and vitamin B9 (folate) becomes more apparent, vitamin B-targeted metabolomics will offer new functional perspectives on the B-vitaminome.

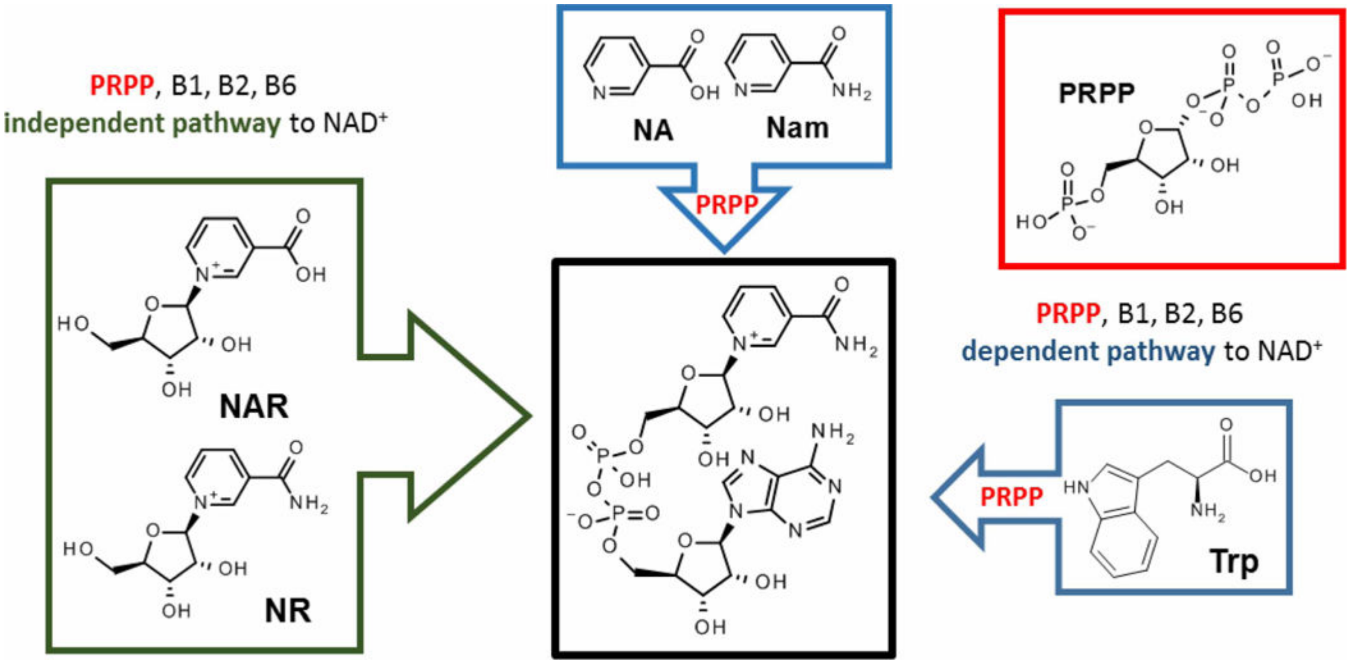


Figure 1. Precursors to NAD$^+$.

Blue box: PRPP-dependent NAD$^+$ biosynthetic pathways; Green box: PRPP and vitamin B1, B2 and B6-independent pathways; PRPP, 5-phospho-1-pyrophosphoriboside; vitamin B1, thiamine; vitamin B2, riboflavin; vitamin B6, pyridoxine; NA, niacin/nicotinic acid; Nam, niacinamide/nicotinamide; NR, nicotinamide riboside; NAR, nicotinic acid riboside.

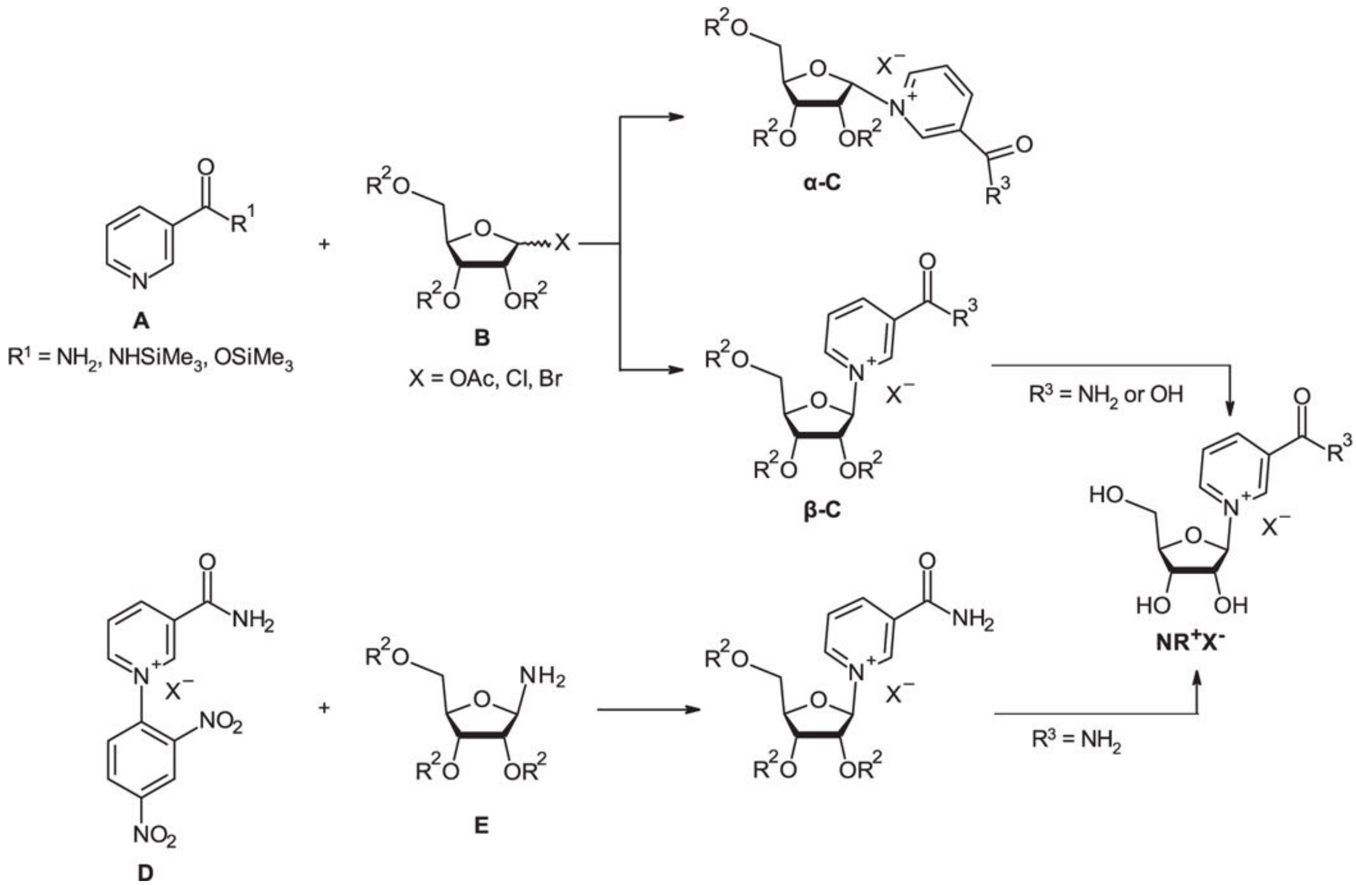


Figure 2.
Synthetic routes to nicotinamide riboside (NR^+X^-).

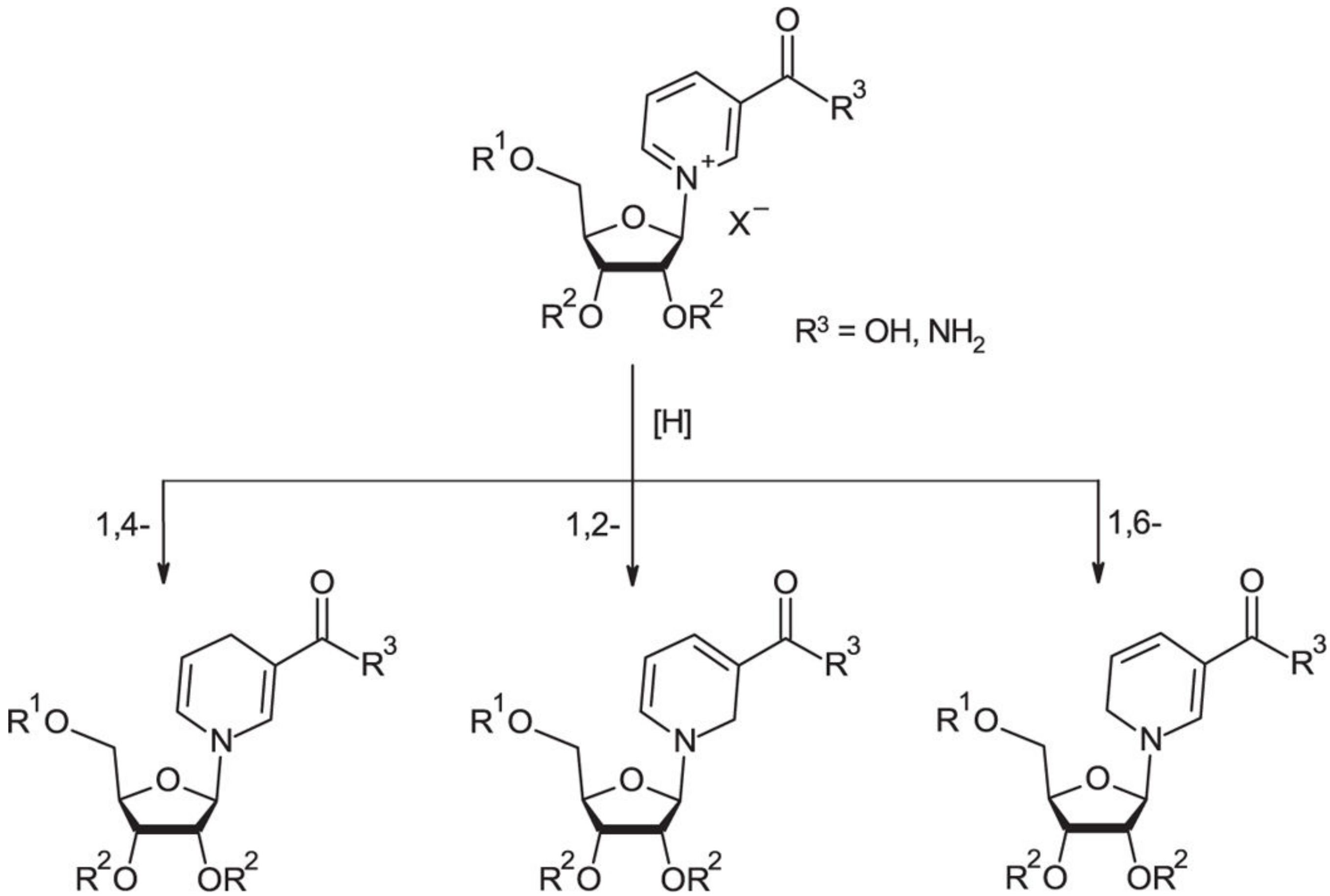


Figure 3.
Reduction in derivatives of $NR^{+}X^{—}$ into corresponding NRH derivatives.

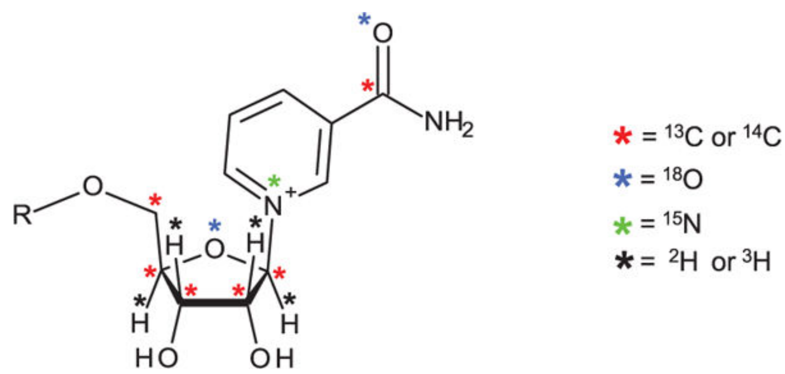
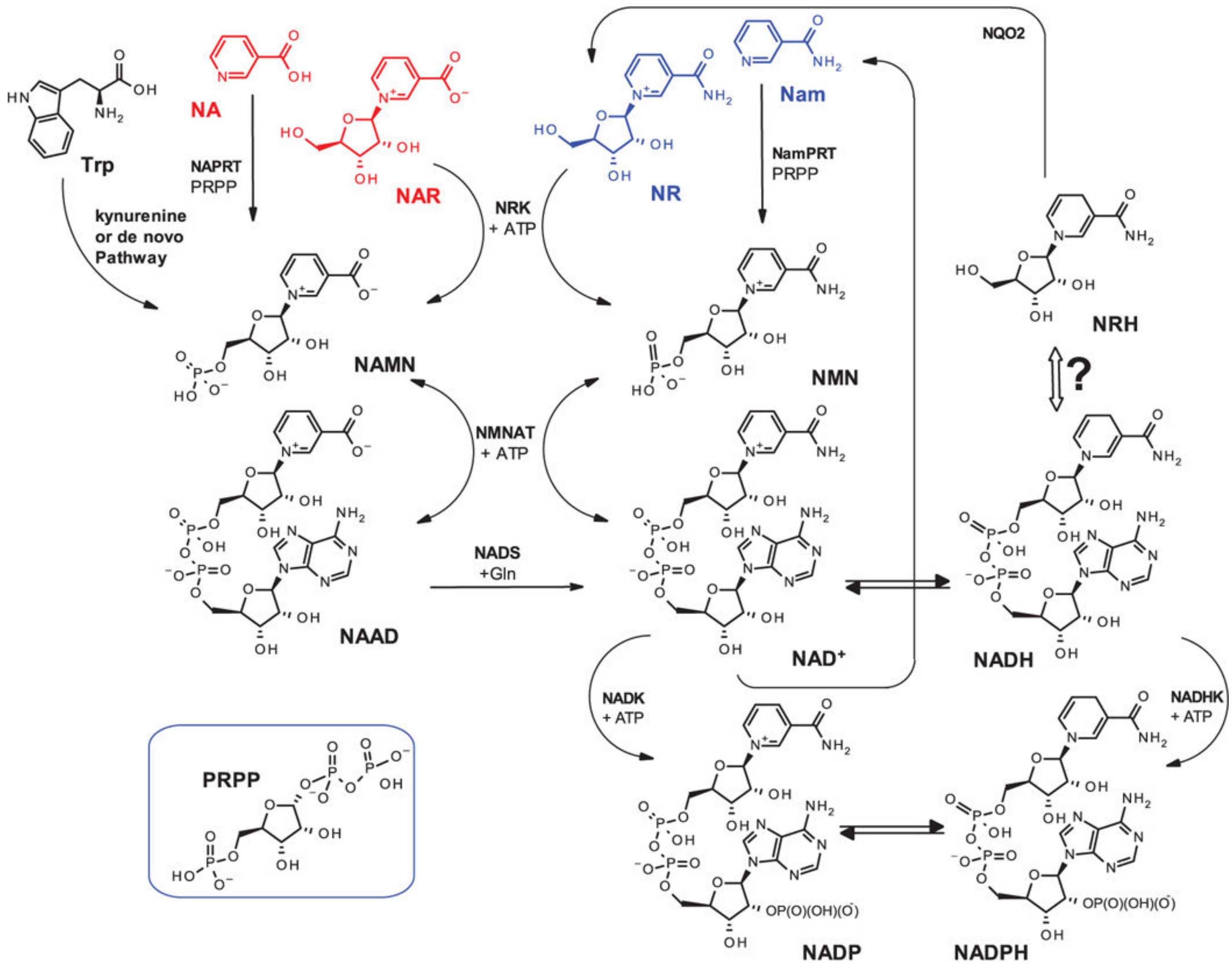
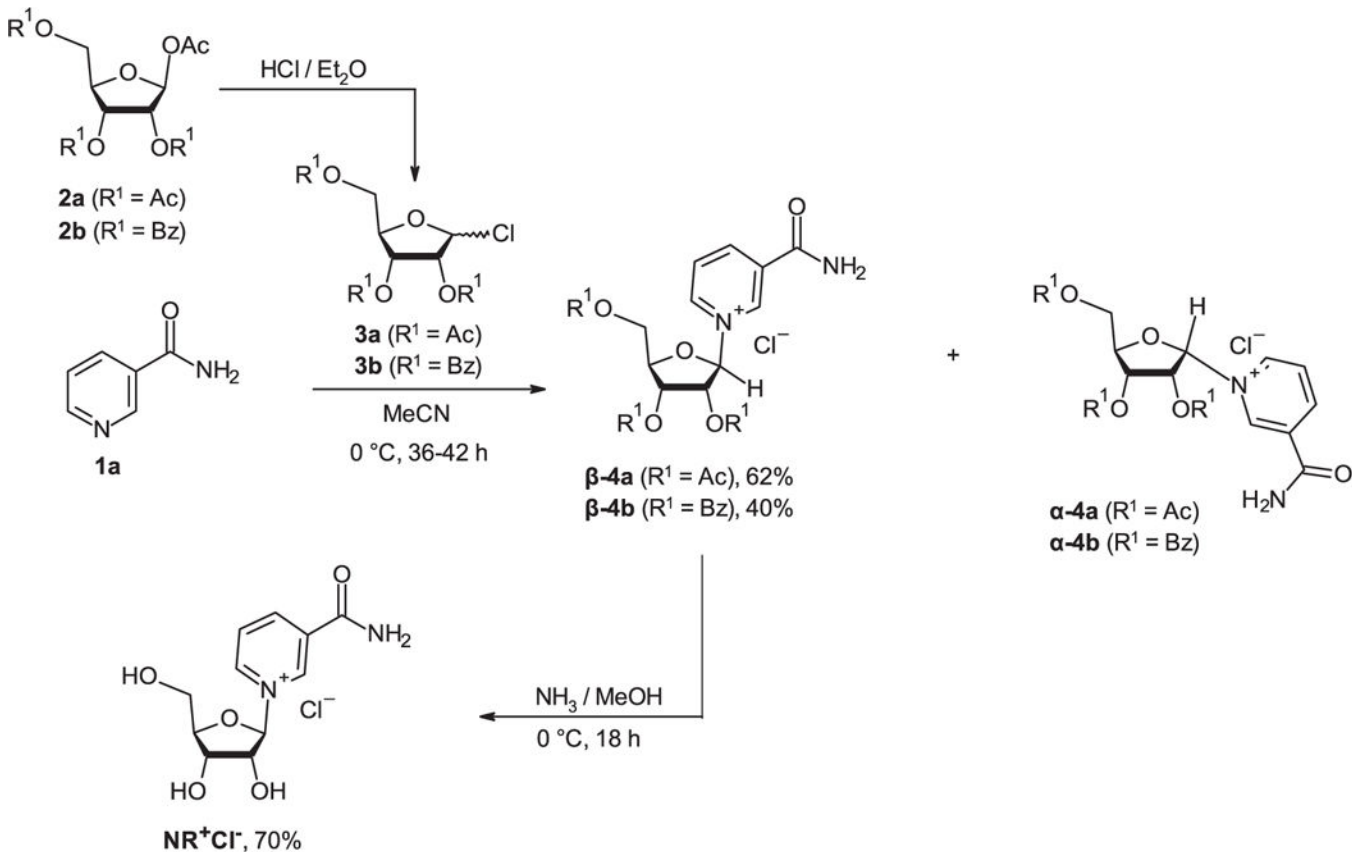


Figure 4.
General representation of isotope-labeled NR derivative; illustrative labeled sites are shown by colored asterisks.

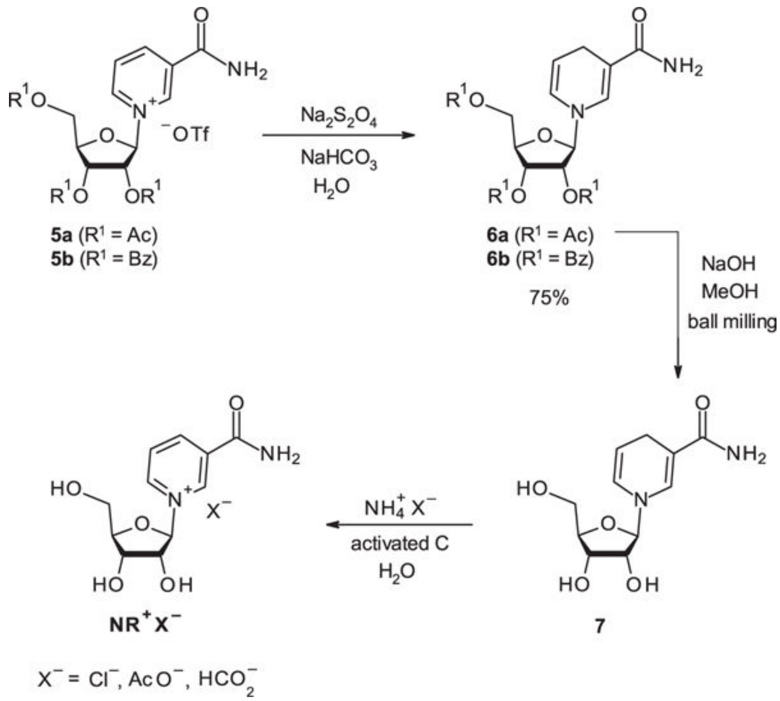


Scheme 1. Detailed biosynthetic pathways to the NAD(P)(H) pool components.

trp, tryptophan; Gln, glutamine; PRPP, 5-phosphoriboside pyrophosphate; NAPRT, nicotinic acid phosphoribosyl transferase; NamPRT, nicotinamide phosphoribosyl transferase; NMNAT, nicotinamide mononucleotide adenylyl transferase; NRK, nicotinamide riboside kinase; NADS, nicotinamide adenine dinucleotide synthase; NADK, nicotinamide adenine dinucleotide kinase; NADHK, NADH kinase; NQO2: *N*-ribosyldihydronicotinamide : quinone reductase 2.



Scheme 2.
Synthetic sequence to 1-β-D-ribofuranoside nicotinamide chloride.



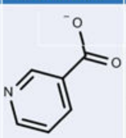
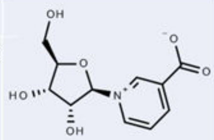
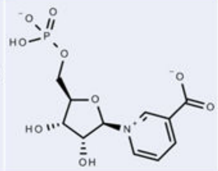
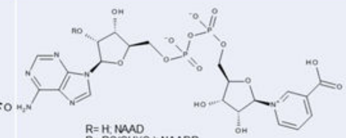
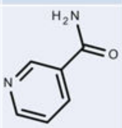
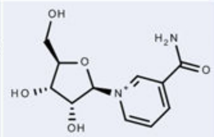
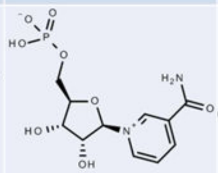
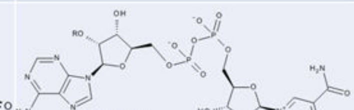
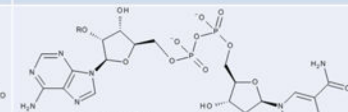
Scheme 3.

Synthesis of the reduced form of NR as a synthetic intermediate to NR.

Table 1a

Chemical structures and abbreviations of the anabolites constituting the vitamin B3 metabolome

Anabolites of the vitamin B3 metabolome, precursor to the NAD(P)(H) pool

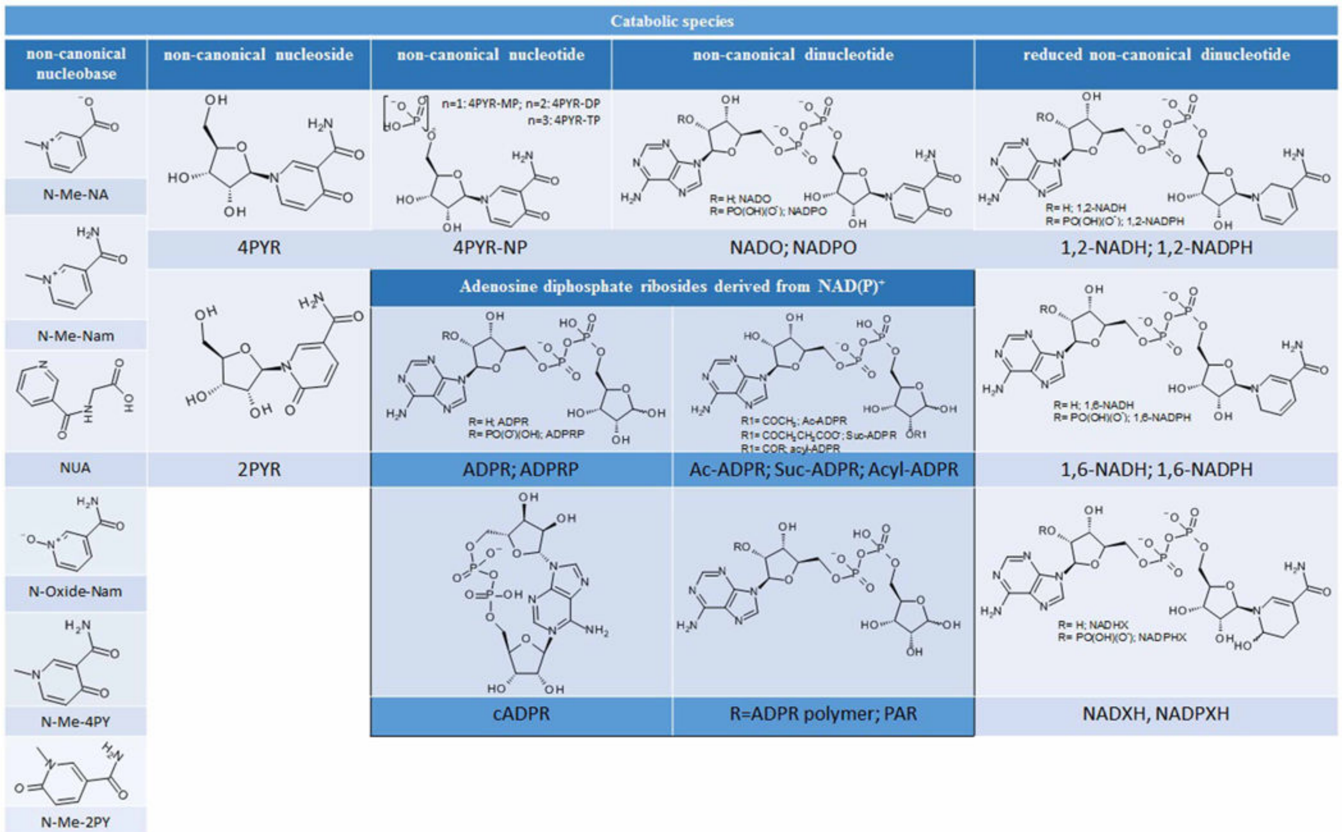
	Anabolic species				
VitaminB3	Nucleobase	Nucleoside	Nucleotide	Dinucleotide	Reduced dinucleotide
Niacin containing derivatives					
	NA	NAR	NAMN	NAAD, NAADP	
Nicotinamide containing derivatives					
	Nam	NR	NMN	NAD^+, NADP	NADH, NADPH

Abbreviations: NA, niacin/nicotinic acid; Nam, niacinamide/nicotinamide; NR, nicotinamide riboside; NAR, nicotinic acid riboside; NAMN, nicotinic acid mononucleotide; NMN, nicotinamide mononucleotide; NAAD, nicotinic acid adenine dinucleotide; NAADP*, nicotinic acid adenine dinucleotide phosphate; NAD^+, nicotinamide adenine dinucleotide; $NADP^+$, nicotinamide adenine dinucleotide phosphate; NADH, nicotinamide adenine dinucleotide reduced form; NADPH, nicotinamide adenine dinucleotide phosphate reduced form. *Generated via a yet unknown mechanism.

Table 1b

Chemical structures and abbreviations of the catabolites constituting the vitamin B3 metabolome

Catabolites of the vitamin B3 metabolome



Abbreviations: *N*-Me-Nam, *N*-methyl nicotinamide or trigonellinamide; *N*-methyl-NA, *N*-methyl nicotinic acid or trigonelline; *N*-Oxide-Nam, *N*-oxide nicotinamide; *N*-Me-4PY, *N*-methyl-4-pyridone-3-carboxamide; *N*-Me-2PY, *N*-methyl-2-pyridone-4-carboxamide; 4PYR, 1-β-D-ribofuranosyl 4-pyridone-3-carboxamide; 4PYR-MP ($n = 1$), 1 -β-D-ribofuranosyl 4-pyridone-3-carboxamide monophosphate; 4PYR-DP ($n = 2$), 1 -β-D-ribofuranosyl 4-pyridone-3-carboxamide diphosphate; 4PYR-TP ($n = 3$), 1 -β-D-ribofuranosyl 4-pyridone-3-carboxamide triphosphate; NADO, 4-pyridone-3-carboxamide adenine dinucleotide; NADPO, 4-pyridone-3-carboxamide adenine dinucleotide phosphate; 1,2-NADH, 1,2-dihydronicotinamide adenine dinucleotide; 1,2-NADPH, 1,2-dihydronicotinamide adenine dinucleotide phosphate; 1,6-NADH, 1, 6-dihydronicotinamide adenine dinucleotide; 1,6-NADPH, 1,6-dihydronicotinamide adenine dinucleotide phosphate; NADHX, adenosine 5 ′-(trihydrogen diphosphate), $P' \longrightarrow 5'$ -ester with 1,4,5,6-tetrahydro-6-hydroxy-1 -β-D-ribofuranosyl-3-pyridinecarboxamide also known as 6-hydroxylated nicotinamide adenine dinucleotide reduced form. NADPHX, adenosine 5 ′-(trihydrogen diphosphate), $P' \longrightarrow 5'$-ester with 1,4,5,6-tetrahydro-6-hydroxy-1-β-D-ribofuranosyl-3-pyridinecarboxamide phosphate also known as 6-hydroxylated nicotinamide adenine dinucleotide phosphate reduced form. cADPR, cyclic adenosine diphosphoriboside; ADPR, adenosine diphosphoribose; ADPRP, adenosine diphosphoribose phosphate; PAR, poly adenosine diphosphoriboside; Ac-ADPR, acetyl adenosine diphosphoribose; Suc-ADPR, succinyl adenosine diphosphoribose; acyl-ADPR, acyl adenosine diphosphoribose.