Organic LETTERS

Letter

Cite This: Org. Lett. XXXX, XXX, XXX–XXX

pubs.acs.org/OrgLett

Nicotinamide-Containing Di- and Trinucleotides as Chemical Tools for Studies of NAD-Capped RNAs

Agnieszka Mlynarska-Cieslak,[†,∥] Anais Depaix,[†,∥] Ewa Grudzien-Nogalska,[‡] Pawel J. Sikorski,[§] Marcin Warminski,[†] Megerditch Kiledjian,[‡] Jacek Jemielity,[§] and Joanna Kowalska*[,†]

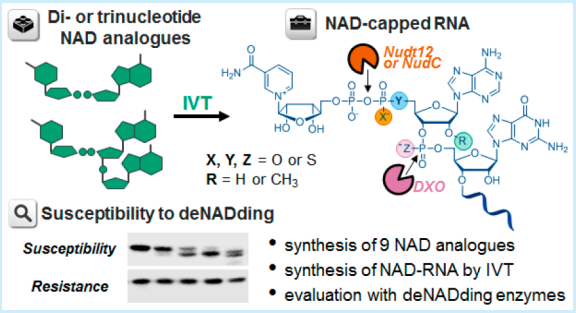
[†]Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Pasteura 5, 02-093 Warsaw, Poland

[‡]Department of Cell Biology and Neuroscience, Rutgers University, Piscataway, New Jersey 08854, United States

[§]Centre of New Technologies, University of Warsaw, Banacha 2c, 02-097 Warsaw, Poland

Supporting Information

ABSTRACT: We report the chemical synthesis of a set of nicotinamide adenine dinucleotide (NAD) cap analogues containing chemical modifications that reduce their susceptibility to NAD-RNA-degrading enzymes. These analogues can be incorporated into transcripts in a similar way as NAD. Biochemical characterization of RNAs carrying these caps with DXO, NudC, and Nudt12 enzymes led to the identification of compounds that can be instrumental in unraveling so far unaddressed biological aspects of NAD-RNAs.



Nicotinamide adenine dinucleotide (NAD), discovered over 100 years ago as a cofactor in cellular redox reactions, has since been thoroughly studied and demonstrated to be a highly important biomolecule with multiple biological functions. Besides participation in redox biotransformations, NAD serves as a substrate for protein post-translational modifications (poly(ADP-ribosylation, deacetylation), for signal transduction (as a precursor of cyclic ADP ribose),[1] and, as was shown recently, as a 5′-end-modifying molecule in some RNAs.[2] NAD-capped RNAs (Figure 1A) were first found in bacteria[2] and thus are termed "bacterial caps" because of some structural analogy to the 7-methylguanosine (m^7G) cap present at the 5′-end of eukaryotic mRNAs.[3] More recently, NAD-capped RNAs have also been discovered in other organisms ranging from bacteria to eukaryotes,[4,5] including mammals. The latest studies indicated that up to 10% of human mitochondrial transcripts are NAD-capped.[6] The biological roles of this new, fascinating RNA modification remain elusive and likely differ among organisms. Nonetheless, it has been established that in both bacteria and eukaryotes, the 5′-terminal NAD moiety influences RNA stability. Accordingly, several deNADding enzymes that participate in RNA degradation have been identified.[7]

In *Escherichia coli*, the presence of the 5′-NAD moiety arguably increases the stability of RNA by decreasing its susceptibility to the action of RppH,[8] a bacterial pyrophosphatase that participates in dephosphorylation of RNA 5′-triphosphate to 5′-monophosphate, thereby triggering 5′-decay. However, NAD-capped RNAs are targeted by NudC, a bacterial enzyme capable of specific 5′-deNADding of RNA in vitro and in vivo via pyrophosphate bond cleavage (Figure 1A).[8,9]

In contrast, in eukaryotes the presence of NAD at the 5′-end appears to destabilize RNA and direct it for decay. The decay is initiated by the removal of the whole 5′-terminal NAD moiety by DXO nuclease, which cleaves the first phosphodiester bond in RNA (Figure 1A).[4] Nudt12 pyrophosphatase, an eukaryotic homologue of NudC, also cleaves NAD-RNA in vitro and contributes to NAD-RNA decay in human cells.[10] Despite the increasing number of novel NAD-related analytical and chemical biology tools,[11] the biological significance of NAD-capped-RNAs in different organisms remains unclear. One of the most important questions to be addressed is whether this modification has specific biological functions in cells (e.g., to act as a signaling response to stress conditions) or is merely a result of aberrant transcription initiation that is rapidly eliminated by RNA quality control mechanisms.

We envisaged that investigations of the structure and function of NAD-terminated RNAs and their specific binding proteins can be expedited by the use of rationally designed molecular tools enabling the synthesis of NAD-RNAs that are less susceptible to enzymatic cleavage than natural NAD-RNAs. Such tools would provide access to NAD-RNAs with increased cellular stability suitable for structural and functional studies, pull-down experiments, and many others.

Here we report the synthesis of a set of novel NAD analogues that can be incorporated into RNA by in vitro

Received: October 23, 2018

DOI: 10.1021/acs.orglett.8b03386
Org. Lett. XXXX, XXX, XXX–XXX

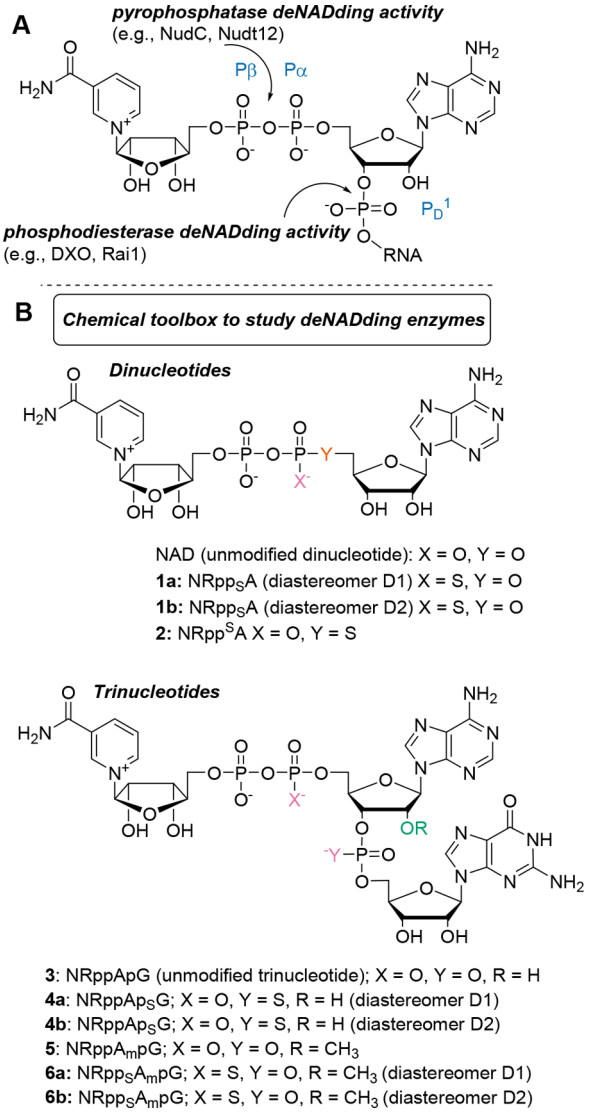


Figure 1. (A) Structure of NAD-linked RNA and activity of deNADding enzymes identified to date. (B) Structure of NAD analogues synthesized in this study. Abbreviations: NR, nicotinamide riboside; A, adenosine; G, guanosine.

transcription (IVT). These analogues were designed to be either partially or completely resistant to hydrolytic enzymes that target NAD-RNAs. We further demonstrate efficient incorporation of these NAD analogues into RNA by polymerase T7. Finally, we examine the susceptibility of the modified NAD-linked RNAs to several deNADding enzymes (NudC, Nudt12, and DXO) and identify analogues that are most suitable for future biological applications.

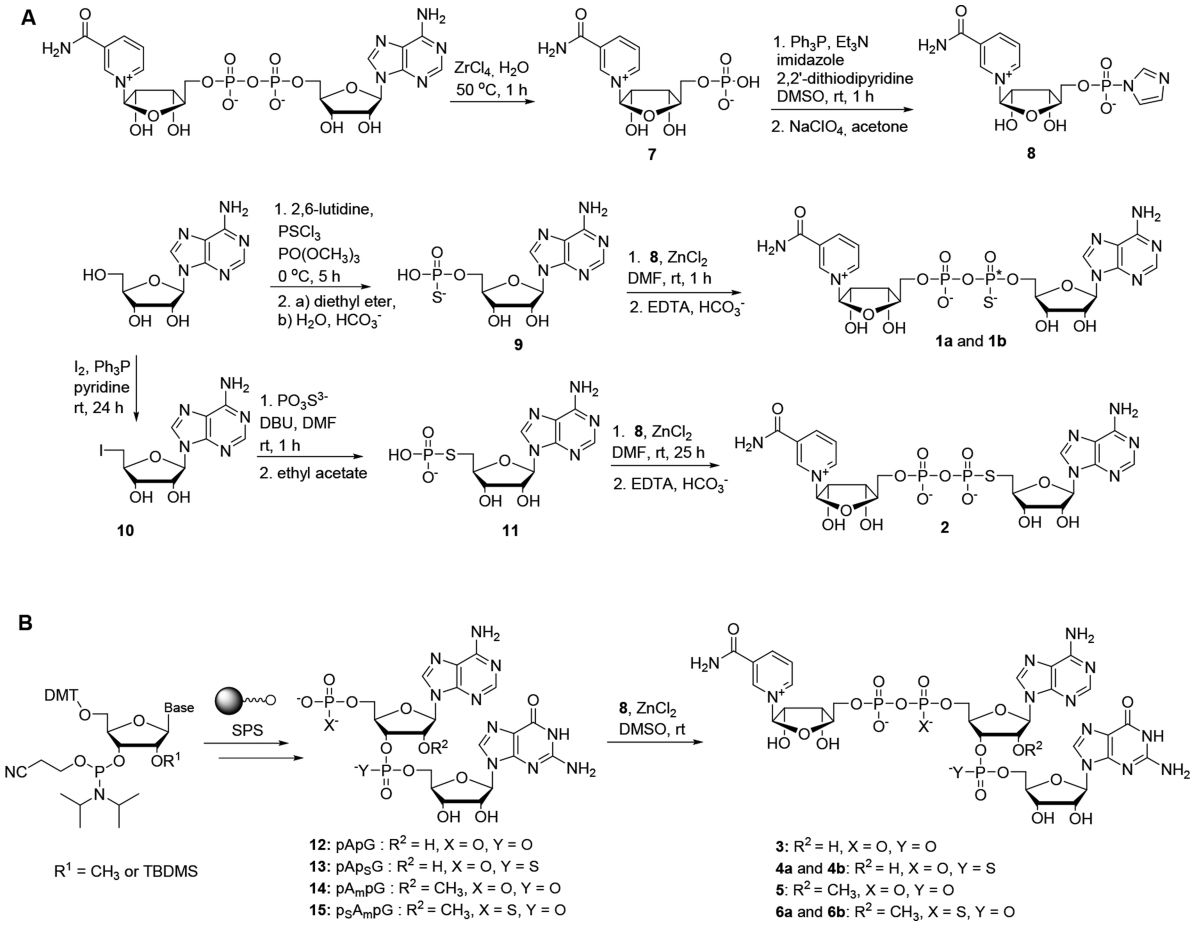
To achieve resistance to NUDIX-family pyrophosphatases such as NudC and Nudt12, we designed dinucleotide NAD analogues carrying an O-to-S substitution at the nonbridging position of the pyrophosphate moiety or at the 5′-bridging position of the phosphoester (Figure 1B). This type of modification has been previously shown to be a viable strategy for stabilizing m^7G-capped RNAs against decapping enzymes without disrupting their translational activity.[12] To this end, three dinucleotide NAD analogues were synthesized: two stereoisomers of NAD carrying the α-phosphorothioate (α-PS) moiety (compounds **1a** and **1b**) and one NAD analogue carrying 5′-phosphorothiolate (PSL) (compound **2**). To confer resistance to phosphodiesterases such as DXO, stabilizing modifications were introduced within the first phosphodiester bond (PD1) by utilizing trinucleotide NAD analogues (compounds **3**–**6**; Figure 1B). In addition to replacing the first phosphodiester bond with the PS moiety (two diastereomers of compound **4**), we also explored 2′-O-methylation of the first nucleoside in the RNA body (compound **5**), as it has been recently reported to decrease the susceptibility to DXO for m^7GpppN and GpppN-capped RNAs.[13] Finally, expecting to achieve resistance to both types of deNADding enzymes, we combined the O-to-S substitution at the α-phosphate (α-PS) with 2′-*O*-methylation (compounds **6a** and **6b**).

A key intermediate in the synthesis of all of the NAD analogues was imidazole-activated nicotinamide mononucleotide (**8**), obtained from nicotinamide riboside (NR) 5′-monophosphate (**7**). The latter can be either synthesized de novo by a multistep procedure,[14] prepared enzymatically,[15] or obtained by hydrolysis of commercially available NAD in the presence of aqueous $ZrCl_4$.[16] We chose the last of these methods, as it appeared the most time- and cost-efficient for gram-scale synthesis. The resulting mixture of AMP and nicotinamide mononucleotide was separated by ion-exchange chromatography (IEC) using DEAE Sephadex A25 to give compound **7** as a triethylammonium salt in 30% yield (Scheme 1A). Reaction of **7** with imidazole in the presence of triphenylphosphine and 2,2′-dithiodipyridine in DMSO followed by precipitation with $NaClO_4$ in acetone gave the sodium salt of *P*-imidazolide **8** in 56% yield. Adenosine 5′-phosphorothioate (**9**) was synthesized by thiophosphorylation of adenosine with $PSCl_3$ in the presence of 2,6-lutidine followed by hydrolysis[17] and purification by IEC. Finally, to yield α-substituted NAD dinucleotide **1**, compounds **8** and **9** were reacted in the presence of a 3-fold excess of $ZnCl_2$ in DMF as the solvent. RP-HPLC analysis of the reaction mixture revealed the formation of two P-diastereomers of **1** (**1a** and **1b**) in a ratio of ∼1:1.4 (Figure 2A) and nicotinamide nucleotide dimer $(NRMP)_2$ as a side product. The diastereomers were labeled D1 (**1a**) and D2 (**1b**) according to their elution order from the RP HPLC column. The diastereometric product was isolated by IEC, after which the diastereomers were separated by semireparative RP-HPLC. Compound **2** was synthesized in an analogous coupling reaction of **8** and adenosine 5′-phosphorothiolate (**11**), which was obtained by S-alkylation of triethylammonium phosphorothioate by 5′-deoxy-5′-iodoadenosine.[18]

Dinucleotide precursors required for the synthesis of trinucleotide NAD analogues were obtained by phosphoramidite solid-phase synthesis (Figure S1) using iodine and DDTT as oxidating and sulfurizing reagents, respectively. The coupling reactions with **8** that led to trinucleotides were carried out under conditions similar to those described above (Scheme 1B). However, these reactions proceeded notably slower, requiring 2–3 days for maximal conversion. The yields of all final compounds (**1**–**6**) were moderate to low (8–33%), mainly because of the two-step purification required before biochemical studies. The spectroscopic and high-resolution mass spectrometry data for all of the analogues were consistent with the presence of the oxidized form (NAD^+) with trace amounts of NADH (<5%). ^{31}P NMR analysis confirmed the presence of phosphorothioate and phosphorothiolate moieties, as noted by the shifts of the resonance signals by approximately +55 and +20 ppm, respectively, compared with the unmodified

DOI: 10.1021/acs.orglett.8b03386
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of (A) Dinucleotide and (B) Trinucleotide NAD Analogues



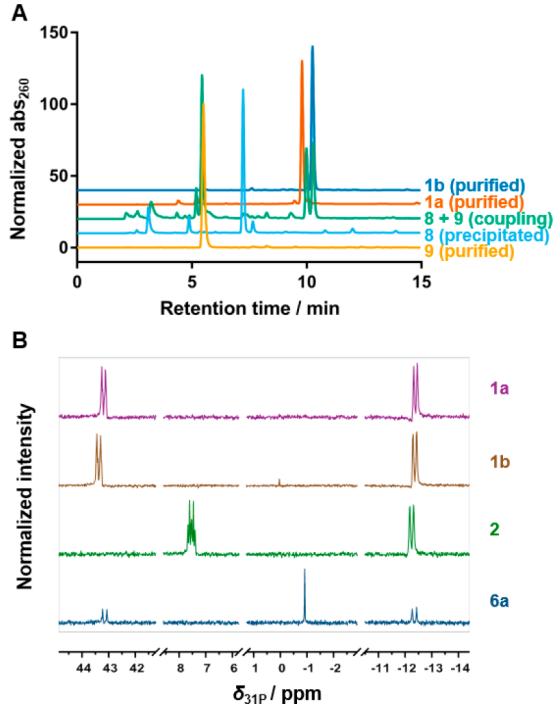


Figure 2. (A) Sample HPLC profiles from the synthesis of NAD analogues **1a** and **1b**. (B) Comparison of ^{31}P NMR spectra for selected NAD analogues.

phosphate moieties (Figure 2B) and by the increase in the $^{2}J_{P-P}$ coupling constant from 19–20 to 27–28 Hz. The absolute configurations of isomers D1 and D2 were not assigned, but ^{31}P and ^{1}H NMR data confirmed that the separation was successful (Table S1).

Next, we tested whether the newly synthesized NAD analogues can be incorporated into RNA 5′ ends. Transcription reactions were performed by T7 RNA polymerase from a DNA template containing the T7 Aϕ2.5 promoter sequence, defining A as the first transcribed nucleotide and G as the second (Figure 3A).[19] Transcripts were trimmed at the 3′ end by DNAzyme 10–23 to decrease the 3′-end heterogeneity, followed by PAGE under conditions that

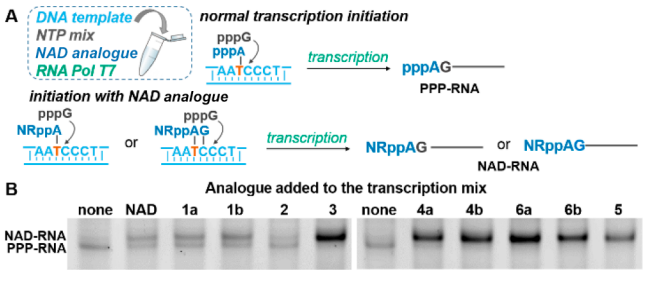


Figure 3. (A) Schematic representation of transcription initiation leading to either uncapped or NAD-capped RNA. (B) RNAs (25 nt) produced by IVT in the presence of different NAD analogues analyzed by PAGE (SybrGold staining was used for the visualization).

DOI: 10.1021/acs.orglett.8b03386
Org. Lett. XXXX, XXX, XXX−XXX

enabled separation of NAD-RNAs from uncapped (ATP-initiated) RNAs (Figure 3B). Analogues **1a** and **1b** were incorporated into RNA with efficiencies comparable to that for unmodified NAD with good 5′-end homogeneity.

The 5′-triphosphorylated fraction of these RNAs constituted less than 40% of the total RNA. In the case of analogue **2**, a higher portion of uncapped RNA was observed, indicating that the analogue was less efficiently recognized by T7 polymerase. In contrast, the unmodified trinucleotide **3** was a very efficient transcription initiator because uncapped RNA was at a negligible level. Notably, the efficiency of trinucleotide NAD analogue incorporation was superior to that of dinucleotide NAD analogue incorporation[19] and of the previously reported method for chemical addition of nicotinamide riboside 5′-monophosphate to 5′-phosphorylated RNA.[20] Therefore, the use of trinucleotide **3** may be the preferred method for NAD-RNA synthesis when RNA with high 5′-end homogeneity is required for biochemical experiments. Trinucleotides **4a**, **4b**, **5**, **6a**, and **6b** were also efficiently incorporated into RNA, indicating that the introduced modifications did not affect the interaction with T7 polymerase.

Next, we assessed the susceptibility of RNAs capped with various NAD analogues to previously described deNADding enzymes, including two NUDIX-family pyrophosphatases (NudC from *E. coli* and murine Nudt12)[8−10] and a phosphodiesterase (murine DXO).[4] Recombinant proteins were incubated with IVT ^{32}P-labeled RNA, and the resulting products were resolved using PAGE to assess the remaining amount of NAD-capped RNA (Figures 4−6). RNA capped with unmodified NAD was efficiently deNADded by DXO to RNA 5′-monophosphate and further degraded by its 5′ → 3′ exonuclease activity, which was particularly well manifested at the highest enzyme concentration (50 nM) (Figure 4A).

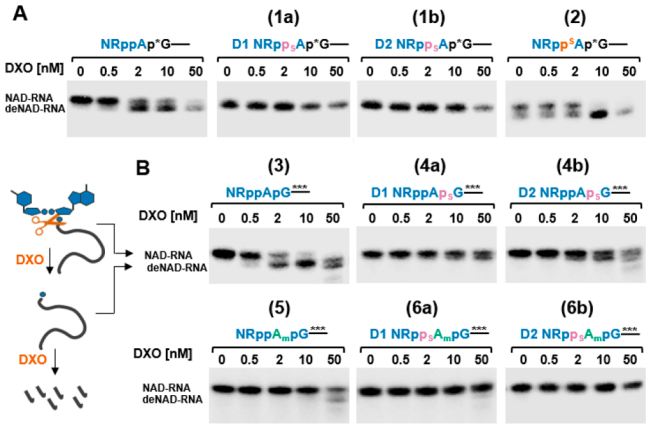


Figure 4. Susceptibility of RNAs capped with NAD analogues to DXO. A schematic of NAD-capped RNA hydrolysis by DXO is shown at the left. Reaction products of in vitro deNADding assays with mouse DXO protein and the indicated ^{32}P-labeled RNAs capped with (A) NAD dinucleotide analogues or (B) NAD trinucleotide analogues were resolved on 20% urea polyacrylamide gels.

As expected, RNAs capped with analogues **1a** and **1b** were also efficiently hydrolyzed, although a higher concentration of the enzyme was required. Interestingly, the transient/initial deNADding product was not observed, suggesting a reduced efficiency of deNADding with a subsequent rapid decay of the deNADded product by the DXO 5′ → 3′ exonuclease activity compared with RNA capped with unmodified NAD. RNA carrying analogue **2** was also susceptible to the action of DXO, and despite noticeable 5′-end inhomogeneity at time point 0, it behaved similarly to NAD-capped RNA. As expected, RNA carrying trinucleotide **3** at the 5′ end was equally readily degraded by DXO as RNA capped with unmodified NAD (Figure 4B). RNA capped with trinucleotides **4a** and **4b** containing a phosphorothioate moiety at the site of DXO-mediated cleavage showed notably diminished susceptibility to DXO. However, partial degradation was clearly visible at higher enzyme concentration (50 nM), especially for stereoisomer D2. Similarly, 2′-O-methylated NAD-capped RNA obtained with analogue **5** was notably less susceptible but still partially degraded by DXO at the highest concentration. The combination of 2′-*O*-Me and α-PS modifications turned out to be additive, as RNAs capped with analogues **6a** and **6b** were less susceptible to DXO than the corresponding RNAs carrying NADs with only single modification (**4a**, **4b**, or **5**). As a result, transcripts capped with **6a** and **6b** were the most resistant to DXO activity, with almost negligible degradation even at the highest DXO concentration, and thus were assigned as "DXO-resistant" (Table 1).

Table 1. Enzymatic Susceptibilities of Different NAD-Capped RNAs

		susceptibility to deNADding[b] by		
entry	compound at the RNA 5′ end[a]	DXO	NudC	NUDt12
1	NAD	cleaved	cleaved	cleaved
2	**1a**	partially resistant	resistant	resistant
3	**1b**	partially resistant	resistant	resistant
4	**2**	cleaved	resistant	resistant
5	**3**	cleaved	cleaved	cleaved
6	**4a**	partially resistant	n.d.[c]	n.d.[c]
7	**4b**	partially resistant	n.d.[c]	n.d.[c]
8	**5**	partially resistant	cleaved	cleaved
9	**6a**	resistant	resistant	resistant
10	**6b**	resistant	resistant	resistant

[a]Incorporated by IVT with polymerase T7; [b]"cleaved" indicates that the RNA susceptibility was similar to that of unmodified NAD-capped RNA; "partially resistant" indicates that the susceptibility was reduced but cleavage was observed at higher enzyme concentrations; "resistant" indicates that cleavage was not observed at the concentrations tested. [c]The analogue was not investigated in this assay.

Next, we assessed the susceptibility of modified RNAs to pyrophosphate bond cleavage mediated by NudC and Nudt12. RNAs uniformly labeled with ^{32}P and carrying different NAD analogues were exposed to increasing concentrations of NudC (Figure 5) or Nudt12 (Figure 6).

For NudC, RNA capped with unmodified NAD, regardless of the synthesis method (using NAD dinucleotide or trinucleotide **3**), was completely deNADded even at the lowest enzyme concentration tested (50 nM). Similar susceptibility to degradation was observed for analogue **5**, which was in agreement with our expectation that modifications outside the pyrophosphate moiety would not influence the susceptibility to pyrophosphatase activity. All of the analogues carrying α-PS or PSL modifications within the pyrophosphate were resistant to NudC at concentrations up to 250 nM (Table 1).

DOI: 10.1021/acs.orglett.8b03386
Org. Lett. XXXX, XXX, XXX−XXX

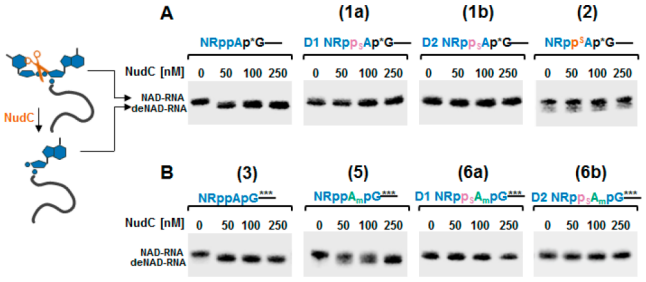


Figure 5. Susceptibility of RNAs capped with (A) NAD dinucleotide analogues or (B) NAD trinucleotide analogues to NudC. A schematic of NAD-capped RNA hydrolysis by NudC is shown at the left. Reaction products of NAD-capped RNA were resolved as in Figure 4.

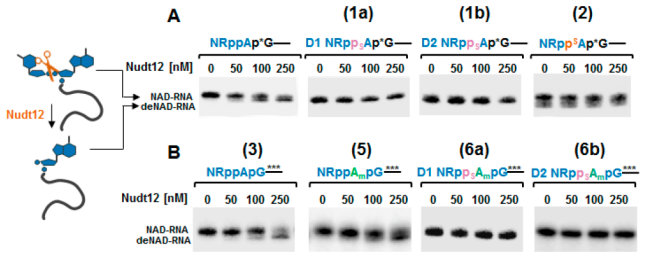


Figure 6. Susceptibility of RNAs capped with (A) NAD dinucleotide analogues or (B) NAD trinucleotide analogues to Nudt12. A schematic of NAD-capped RNA hydrolysis by NudC is shown at the left. Reaction products of NAD-capped RNA were resolved as in Figure 4.

Mouse Nudt12 showed a similar substrate preference as NudC, i.e., RNAs capped with NAD, analogue **3**, and **5** were susceptible to cleavage, whereas all of the other tested analogues were resistant (Table 1).

In conclusion, we synthesized and biochemically characterized eight novel NAD analogues that enabled the generation of NAD-capped RNAs with reduced susceptibility to enzymatic cleavage. These analogues can be incorporated into RNA using a simple IVT reaction to produce modified NAD-capped RNAs. The use of the trinucleotide NRppApG (**3**) or its analogues (**4**–**6**) in IVT improved the efficiency of RNA capping compared with initiation with NAD, which makes the synthesized analogues particularly useful for obtaining NAD-capped RNAs with superior 5′-end homogeneity. Studies of the susceptibility of RNAs with different NAD caps to deNADding enzymes revealed compounds with distinct characteristics. Among the eight new chemically modified NAD analogues, we identified a compound that conferred resistance to the pyrophosphatase activity of NudC/Nudt12 on RNA but kept it susceptible to the phosphodiesterase activity of DXO (compound **2**), a compound that was susceptible to pyrophosphatases but conferred reduced susceptibility to DXO (compound **5**), and compounds that conferred reduced (to various degrees) susceptibility to both types of enzymes (compounds **1a**, **1b**, **6a**, and **6b**). Thus, the chemical toolbox of our NAD analogues should be particularly useful in biochemical studies of the contributions of different deNADding enzymes to overall NAD-RNAs decay given that in human cells different RNA sequences are targeted by DXO and Nudt12.[10] Other potential applications of the synthesized analogues include studies of biological functions of NAD-RNAs and identification of putative effector proteins that recognize the 5′-terminal NAD moiety in RNA as a molecular hallmark.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b03386.

Experimental procedures and spectroscopic data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jkowalska@fuw.edu.pl

ORCID

Jacek Jemielity: 0000-0001-7633-788X

Joanna Kowalska: 0000-0002-9174-7999

Author Contributions

‖A.-M.C. and A.D. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank M. Baranowski (University of Warsaw) for recording NMR spectra and B. E. Nickels (Rutgers University) for providing recombinant NudC. This work was supported by funding from the National Science Centre, Poland (2015/18/E/ST5/00555 to J.K.), the Foundation for Polish Science (TEAM/2016-2/13 to J.J.), and the National Institutes of Health (Grant GM126488 to M.K.).

■ REFERENCES

(1) Berger, F.; Ramírez-Hernández, M. H.; Ziegler, M. The new life of a centenarian: signalling functions of NAD(P). *Trends Biochem. Sci.* **2004**, *29*, 111−118.

(2) Chen, Y. G.; Kowtoniuk, W. E.; Agarwal, I.; Shen, Y.; Liu, D. R. LC/MS analysis of cellular RNA reveals NAD-linked RNA. *Nat. Chem. Biol.* **2009**, *5*, 879−881.

(3) (a) Luciano, D. J.; Belasco, J. G. NAD in RNA: unconventional headgear. *Trends Biochem. Sci.* **2015**, *40* (5), 245−247. (b) Jaschke, A.; Hofer, K.; Nubel, G.; Frindert, J. Cap-like structures in bacterial RNA and epitranscriptomic modification. *Curr. Opin. Microbiol.* **2016**, *30*, 44−49. (c) Cahova, H.; Winz, M. L.; Hofer, K.; Nubel, G.; Jaschke, A. NAD captureSeq indicates NAD as a bacterial cap for a subset of regulatory RNAs. *Nature* **2015**, *519*, 374−377.

(4) Jiao, X.; Doamekpor, S. K.; Bird, J. G.; Nickels, B. E.; Tong, L.; Hart, R. P.; Kiledjian, M. 5′ End Nicotinamide Adenine Dinucleotide Cap in Human Cells Promotes RNA Decay through DXO-Mediated deNADding. *Cell* **2017**, *168* (6), 1015−1027.

(5) (a) Walters, R. W.; Matheny, T.; Mizoue, L. S.; Rao, B. S.; Muhlrad, D.; Parker, R. Identification of NAD+ capped mRNAs in Saccharomyces cerevisiae. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (3), 480−485. (b) Frindert, J.; Zhang, Y. Q.; Nubel, G.; Kahloon, M.; Kolmar, L.; Hotz-Wagenblatt, A.; Burhenne, J.; Haefeli, W. E.; Jaschke, A. Identification, Biosynthesis, and Decapping of NAD-Capped RNAs in B. subtilis. *Cell Rep.* **2018**, *24*, 1890−1901.

(6) Bird, J. G.; Basu, U.; Kuster, D.; Ramachandran, A.; Grudzien-Nogalska, E.; Kiledjian, M.; Temiakov, D.; Patel, S. S.; Ebright, R. H.; Nickels, B. E. Mitochondrial RNA capping: highly efficient 5′-RNA capping with NAD^+ and NADH by yeast and human mitochondrial RNA polymerase. 2018, bioRχiv 381160. bioRχiv, The Preprint Server for Biology. DOI: 10.1101/381160.

(7) Kiledjian, M. Eukaryotic RNA 5′-End NAD+ Capping and DeNADding. *Trends Cell Biol.* **2018**, *28* (6), 454−464.

DOI: 10.1021/acs.orglett.8b03386
Org. Lett. XXXX, XXX, XXX−XXX

(8) Hofer, K.; Li, S. S.; Abele, F.; Frindert, J.; Schlotthauer, J.; Grawenhoff, J.; Du, J. M.; Patel, D. J.; Jaschke, A. Structure and function of the bacterial decapping enzyme NudC. *Nat. Chem. Biol.* **2016**, *12*, 730−734.
(9) Zhang, D. L.; Liu, Y. X.; Wang, Q.; Guan, Z. Y.; Wang, J.; Liu, J.; Zou, T. T.; Yin, P. Structural basis of prokaryotic NAD-RNA decapping by NudC. *Cell Res.* **2016**, *26*, 1062−1066.
(10) Grudzien-Nogalska, E.; Jiao, Y.; Wu, X.; Cui, H.; Hart, R. P.; Tong, L.; Kiledjian, M. 2018, Biorxiv, https://doi.org/10.1101/474478.
(11) (a) Winz, M. L.; Cahova, H.; Nubel, G.; Frindert, J.; Hofer, K.; Jaschke, A. Capture and sequencing of NAD-capped RNA sequences with NAD captureSeq. *Nat. Protoc.* **2017**, *12*, 122−149. (b) Halle, F.; Fin, A.; Rovira, A. R.; Tor, Y. Emissive Synthetic Cofactors: Enzymatic Interconversions of tzA Analogues of ATP, NAD+, NADH, NADP+, and NADPH. *Angew. Chem., Int. Ed.* **2018**, *57*, 1087−1090. (c) Vvedenskaya, I. O.; Bird, J. G.; Zhang, Y. C.; Zhang, Y.; Jiao, X. F.; Barvik, I.; Krasny, L.; Kiledjian, M.; Taylor, D. M.; Ebright, R. H.; Nickels, B. E. CapZyme-Seq Comprehensively Defines Promoter-Sequence Determinants for RNA 5′ Capping with NAD+. *Mol. Cell* **2018**, *70*, 553−564. (d) Grudzien-Nogalska, E.; Bird, J. G.; Nickels, B.; Kiledjian, M. NAD-capQ" detection and quantitation of NAD caps. *RNA* **2018**, *24*, 1418−1425.
(12) (a) Grudzien-Nogalska, E.; Jemielity, J.; Kowalska, J.; Darzynkiewicz, E.; Rhoads, R. E. Phosphorothioate cap analogs stabilize mRNA and increase translational efficiency in mammalian cells. *RNA* **2007**, *13*, 1745−1755. (b) Kowalska, J.; Lewdorowicz, M.; Zuberek, J.; Grudzien-Nogalska, E.; Bojarska, E.; Stepinski, J.; Rhoads, R. E.; Darzynkiewicz, E.; Davis, R. E.; Jemielity, J. Synthesis and characterization of mRNA cap analogs containing phosphorothioate substitutions that bind tightly to eIF4E and are resistant to the decapping pyrophosphatase. *RNA* **2008**, *14*, 1119−1131. (c) Wojtczak, B. A.; Sikorski, P. J.; Fac-Dabrowska, K.; Nowicka, A.; Warminski, M.; Kubacka, D.; Nowak, E.; Nowotny, M.; Kowalska, J.; Jemielity, J. 5′-Phosphorothiolate Dinucleotide Cap Analogues: Reagents for Messenger RNA Modification and Potent Small-Molecular Inhibitors of Decapping Enzymes. *J. Am. Chem. Soc.* **2018**, *140* (18), 5987−5999.
(13) Picard-Jean, F.; Brand, C.; Tremblay-Letourneau, M.; Allaire, A.; Beaudoin, M. C.; Boudreault, S.; Duval, C.; Rainville-Sirois, J.; Robert, F.; Pelletier, J.; Geiss, B. J.; Bisaillon, M. *PLoS One* **2018**, *13* (3), e0193804.
(14) Tanimori, S.; Ohta, T.; Kirihata, M. An efficient chemical synthesis of nicotinamide riboside (NAR) and analogues. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1135−1137.
(15) Preiss, J.; Handler, P. Enzymatic synthesis of nicotinamide mononucleotide. *J. Biol. Chem.* **1957**, *225*, 759−770.
(16) Liu, R. H.; Visscher, J. A Novel Preparation of Nicotinamide Mononucleotide. *Nucleosides Nucleotides* **1994**, *13*, 1215−1216.
(17) Moran, J. R.; Whitesides, G. M. A practical enzymic synthesis of (S_P)-adenosine 5′-*O*-(1-thiotriphosphate) ((S_P)-ATP-α-S). *J. Org. Chem.* **1984**, *49*, 704−706.
(18) Patel, B. K.; Eckstein, F. 5′-Deoxy-5′-thioribonucleoside-5′-triphosphates. *Tetrahedron Lett.* **1997**, *38*, 1021−1024.
(19) Huang, F. Efficient incorporation of CoA, NAD and FAD into RNA by in vitro transcription. *Nucleic Acids Res.* **2003**, *31* (3), e8.
(20) Hofer, K.; Abele, F.; Schlotthauer, J.; Jaschke, A. Synthesis of 5′-NAD-Capped RNA. *Bioconjugate Chem.* **2016**, *27*, 874−877.

DOI: 10.1021/acs.orglett.8b03386
Org. Lett. XXXX, XXX, XXX−XXX