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Efficient synthesis of triazole moiety-containing nucleotide analogs and their inhibitory effects on a malic enzyme

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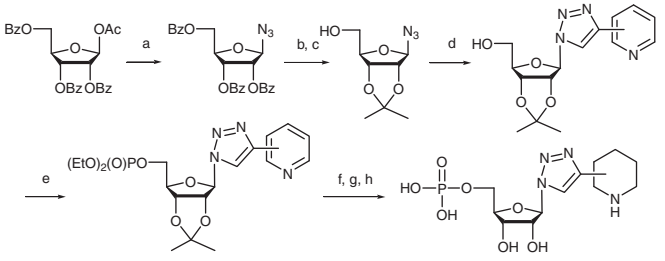
ABSTRACT

Eleven triazole moiety-containing nucleotide analogs were synthesized starting form tetra-*O*-acetylribose in 55–63% total yields. The synthesis involved two key steps, the lipase-mediated selective deacylation of 1-azido-2,3,5-tri-*O*-acetyl-β-D-ribofuranoside and the Huisgen 1,3-dipolar cycloaddition between terminal alkynes and the 1-azido ribofuranoside derivative. These analogs showed inhibitory effects against a recombinant *Escherichia coli* NAD-dependent malic enzyme.

New nucleoside and nucleotide analogs have high potentials in medicinal chemistry to develop therapeutic agents targeting diseases caused by viruses such as human immunodeficiency virus, hepatitis C virus and hepatitis B virus. Some of those analogs are also valuable in terms of treatment of cancers.[1–5] In most cases, the inhibition of viral replication and cell proliferation is resulted from the conversion of nucleoside analogs into their corresponding nucleotides and nucleoside triphosphates in vivo.[1,2,6,7] Subsequently, those analogs establish their biological function through inhibition of enzymes involved in the de novo biosynthesis of nucleotides.[8–10] Yet, such intracellular conversions were usually the rate-limiting step and were strongly depending on molecular interactions between those nucleoside analogs and kinases from the host cell. Although it would be much more problematic for preparation of nucleotide than nucleoside, nucleotide analogs should be pursued to test their biological usefulness.

Structural diversification of nucleoside and nucleotide has been extensively pursued over the years.[2,11] Among those analogs, compounds bearing five-membered heterocyclic nucleobases deserve more attention.[12,13] Recently, much effort has been put into preparation of nucleosides contained a triazole moiety, because those compounds had various biological activities.[14–18] However, the corresponding nucleotides were less scrutinized.[10,19] Nucleotide analogs contained 1,2,3-triazole moiety are intriguing to assemble. In the literature, some representative nucleotide analogs of this type were prepared (Scheme 1).[19] In this method, the Huisgen 1,3-dipolar cycloaddition was exerted in the forth step, followed by phosphorylation and deprotection. The terminal alkynes should be free of reactive groups such as amino group or hydroxyl group, because they might interfere with the phosphorylation reaction using either electrophilic P(III) or P(V) reagents.[20] This method was laborious in terms of structural diversification, because each nucleotide analog had to go through a three-step sequence of cycloaddition, phosphorylation and deprotection.



Scheme 1. Synthesis of pyridine and piperidine containing 1,2,3-triazolide adducts.[19] Reagents and conditions: (a) $TMSN_3$, $AlCl_3$, CH_3CN, 95%; (b) MeONa, MeOH, 90%; (c) acetone, H_2SO_4, 91%; (d) ethynylpyridine, $CuSO_4$ (0.1 equiv), sodium ascorbate (1.0 equiv), H_2O, 100 °C, 12–50%; (e) $(EtO)_2PCl$, Et_3N, tBuOOH, 90%; (f) Pd/C, MeOH/HCO_2H (9/1, v/v), H_2, quant.; (g) TMSBr, CH_2Cl_2, rt, quant.; (h) TFA/H_2O (1/1, v/v), 95–99%.

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Malic enzyme (ME) catalyzes the oxidative decarboxylation of malate to pyruvate and carbon dioxide with the simultaneous reduction of $NAD(P)^+$ to NAD(P)H. In both tumors and highly proliferating cells, the mitochondrial $NAD(P)^+$-dependent ME is overexpressed, suggesting that the activity of this isoform enzyme are involved in energy metabolism for cancer cells.[21,22] Selective inhibition of this isoform enzyme is of potential approach to develop an alternative and specific strategy for cancer therapy.[23] The NAD-dependent ME (NAD-ME) from *Escherichia coli* (NCBI No. NP_415996.1) shares a 38.4% sequence identity with that of the human mitochondrial $NAD(P)^+$-dependent ME (GenBank No. AAA36197.1).

In this Letter, we report an efficient synthesis of nucleotide analogs containing 1,2,3-triazole moiety. The synthesis involved two key steps, the lipase-mediated selective deacylation of 1-azido-2,3,5-tri-*O*-acetyl-β-D-ribofuranoside and the Huisgen 1,3-dipolar cycloaddition between 1-azido-5-*O*-phosphate-2,3-di-*O*-acetyl-β-D-ribofuranoside and terminal alkynes. These analogs showed inhibitory effects against a recombinant NAD-ME from *E. coli*.[24]

The synthetic strategy of the targeted products was depicted in Scheme 2. It is known that copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction is robust and compatible with various reactive groups including amino group, hydroxyl group and carboxylic group.[25–30] However, to the best of our knowledge, no example was documented that used azido phosphates as the reactant. Obviously, our method should be more efficient in terms of the construction of the library of nucleotide analogs because the formation of the 1,2,3-triazole moiety were done in the late stage of the synthetic sequence.

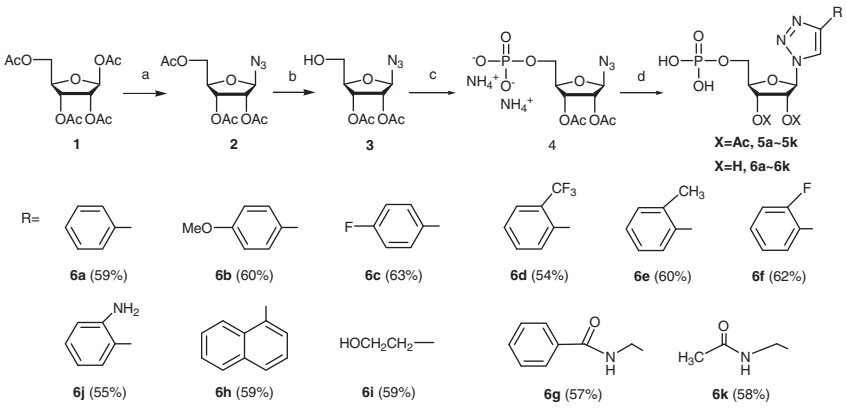
Treatment of the commercially available tetra-*O*-acetylribose (**1**) with $TMSN_3$ in the presence of $SnCl_4$ gave the azide **2** in high yield.[31] The reaction was completely stereoselective, and only the β-anomer was isolated. With **2** in hand, we set to remove the acetyl group at the 5-position in order to run the phosphorylation reaction. A conventional multi-step approach can be envisioned that involves complete deacetylation of **2** followed by selective protection of the hydroxyl groups at the 2- and 3-positions. It can be tedious and end up with low yields. In the literature, it was known that lipase from *Candida rugosa* could remove the primary *O*-acetyl group from tetra-*O*-acetylribose (**1**)[32] and methyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranoside.[33] Therefore, we tried hydrolysis of **2** using lipase. Fortunately, the lipase from Sigma (Cat No. L1754) was indeed effective in selective deacetylation of **2** to afford the 5-position deprotected product **3**.

Phosphorylation of **3** with phosphoryl chloride in THF provided the intermediate **4**, which was used directly in the next step without purification. The survey of the 1,3-dipolar cycloaddition conditions was examined using the model reaction between **4** and phenylacetylene. In the presence of sodium ascorbate (60 mol %) and copper(II) sulfate (10 mol %) in water/methanol (1:4, v/v), the reaction was incomplete at room temperature regardless of overloading phenylacetylene and the catalyst. It was very difficult to separate **4** and **5a** because of their similar behavior over a number of separation matrixes. However, when the reaction was held under reflux condition for 24 h, **4** was disappeared completely according to ^1H NMR analysis. Thus, intermediates **5b**–**5k** were prepared under almost identical conditions.

To purify the compound **5a**, we loaded the cycloaddition reaction mixture on silica gel and eluted with 6 M aqueous ammonia contained 60% isopropanol.[34] Upon removal of solvents, the product was identified as **6a** with excellent purity, and the yield of **6a** from **3** was 91%. Apparently, the acetyl groups of **5a** were cleaved due to the presence of the basic eluent. This serendipitous finding also enabled the synthesis of other nucleotide analogs **6b–6k** from **3** in 83−96% yields without the isolation of the precursor **4** and their corresponding diacetyl intermediates. Thus, the current process was highly efficient. It should be noted that the reaction between **4** and 3-butyn-1-ol leading to **6i** went well at room temperature because both reactants were soluble in the water/ethanol mixture. Lastly, it should be pointed out that only 1,4-regioisomers of the Huisgen 1,3-dipolar cycloaddition product were isolated, because the catalysts were the ascorbate/copper(II) system. The regioisomers were further confirmed by HMBC experiments showing a correlation between the C-5′ triazole carbon atom and H-1 ribose hydrogen atom.

Nucleotide analogs **6a–6k** represented a reasonable structural diversity. The R groups in **6a–6h** contained various substituted phenyl groups, and these substituents were of electron-donating or electron-withdrawing nature. The R groups in **6d–6j** had different *ortho*-substitutions. Compounds **6i–6k** should be more flexible because the R groups at 4′-position of the 1,2,3-triazole ring were linear structures. Compounds **6j**, and **6i–6k** had a hydroxyl group or an amino group, should be useful to establish additional molecular interactions when they are exposed to a biological system.

The fact that nucleotide analogs **6a–6k** were all obtained in good overall yields starting from readily available starting material tetra-*O*-acetylribose **1** suggested that our strategy was versatile and powerful. Specifically, the Huisgen 1,3-dipolar cycloaddition between the 1-azido-5-*O*-phosphate-2,3-di-*O*-acetyl-β-D-ribofuranoside and terminal alkynes was efficient because yields were over 83% for all terminal alkynes used herein. It is likely that this strategy will be generally applicable for preparation of a wide variety of base modified nucleotide analogs containing the 1,2,3-triazole ring structure.



Scheme 2. Preparation of nucleotide analogs. Reagents and conditions: (a) $TMSN_3$, $SnCl_4$, CH_2Cl_2, rt, quant.; (b) lipase, DMF, 0.1 M phosphate buffer (pH 7.0), 37 °C, 200 rpm, 65%; (c) $POCl_3$, Et_3N, THF, 0 °C to rt; (d) sodium ascorbate (60−70 mol %) and copper sulfate (10 mol %), RC≡CH, MeOH/H_2O, 83–96%.

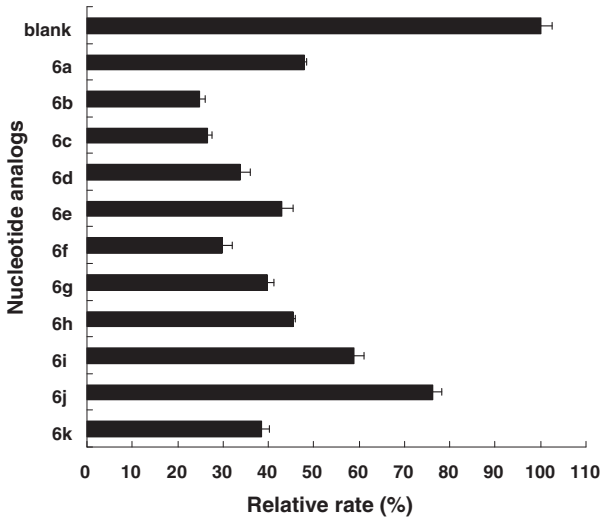


Figure 1. Data of **6a**–**6k** inhibition of the recombinant *E. coli* NAD-ME. Concentration of NAD^+ was 0.4 mM. The concentrations of **6a**–**6k** was 0.2 mM, and **6a** was substituted by water at blank. Blank was 100%. Each data point was performed in triplicates.

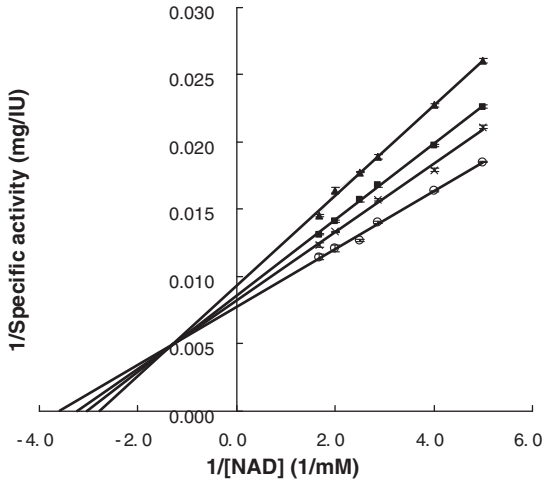


Figure 2. Dixon plot the data of **6b** inhibition of the recombinant *E. coli* NAD-ME at varying concentrations of NAD^+. The concentrations of **6b**, from bottom to top, were 0, 5, 10, 30 μM, respectively. Each data point was performed in triplicates.

To test the biological activity of nucleotide analogs **6a**–**6k**, we evaluated their inhibitory effects against the recombinant *E. coli* NAD-ME.[24] Most of the compounds showed some inhibition on this enzyme. Among them, **6b** showed the strongest inhibition (Fig. 1). Detailed inhibition kinetics indicated that **6b** acted with respect to NAD as a linear mixed inhibitor with a K_i value of 0.057 mM (Fig. 2). Early data showed that ATP had a competitive-inhibition to human mitochondrial $NAD(P)^+$-dependent ME, and the K_i value was 0.081 mM with respect to NAD.[35] Thus, these triazole moiety-containing nucleotide analogs were close to ATP in terms of inhibition on ME. Because the ME from *E. coli* shares high sequence identity with that of the human enzyme, it will be interesting to test these nucleotide analogs against the human mitochondrial $NAD(P)^+$-dependent ME in the future.

In summary, we developed an efficient, four-step synthetic strategy for preparation of nucleotide analogs contained the 1,2,3-triazole moiety starting form tetra-*O*-acetylribose in 55–63% total yields. Similar strategy should be applicable for medicinal chemists to quickly assemble a large number of biologically active nucleotide derivatives. We are currently synthesizing more complex molecules using those analogs as the building blocks.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.107.

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