Tetrahedron Letters 55 (2014) 1573–1576

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Concise and efficient synthesis of 3′-*O*-triphosphates of 2′-deoxyadenosine and 2′-deoxycytidine

Anilkumar R. Kore *, Bo Yang, Balasubramanian Srinivasan

Life Technologies Corporation, Bioorganic Chemistry Division, 2130 Woodward Street, Austin, TX 78744-1832, USA

ARTICLE INFO

Article history:
Received 24 December 2013
Revised 16 January 2014
Accepted 18 January 2014
Available online 25 January 2014

Keywords:
Nucleoside triphosphate
Phosphorylation
Concise synthesis
One-pot methodology
Deprotection

ABSTRACT

We describe concise and efficient synthesis of 2′-deoxyadenosine-3′-*O*-triphosphate (2′-d-3′-ATP) and 2′-deoxycytidine-3′-*O*-triphosphate (2′-d-3′-CTP) which are well known for their various biological applications. One-pot synthetic methodology was used to convert N^6-Benzoyl-5′-*O*-levulinoyl-2′-deoxyadenosine into N^6-Benzoyl-5′-*O*-levulinoyl-2′-deoxyadenosine-3′-*O*-triphosphate in 72% yield. One-step concurrent deprotection of N^6-Benzoyl and 5′-*O*-levulinoyl groups using concentrated aqueous ammonia resulted in 2′-d-3′-ATP in 75% yield. The same synthetic strategy was successfully employed to convert N^4-Benzoyl-5′-*O*-levulinoyl-2′-deoxycytidine into 2′-d-3′-CTP in 66% yield.

Nucleoside triphosphate (NTP) binding studies to a phosphorylating enzyme can provide valuable information about the enzyme's active site nature and mechanism of action. Cytosolic deoxycytidine kinase (dCK), cytosolic thymidine kinase (TK1), mitochondrial thymidine kinase (TK2), and mitochondrial deoxyguanosine kinase (dGK) are the four deoxyribonucleoside kinases possessed by mammalian cells.[1] These are the key enzymes of the salvage pathway and are involved in phosphorylation (metabolic activation) of antitumor and/or antiviral nucleoside analogues.[2] dCK and dGK display overlapping substrate specificities for dA and dG phosphorylations while dC was phosphorylated by both dCK and TK2. TK1 is highly specific for dT and dU phosphorylations. Although substrate specificities of all these enzymes are extensively studied,[2,3] less attention was paid toward phosphate donor specificity. It has been widely and implicitly assumed that ATP is the universal phosphate donor for protein and nucleoside kinases essentially because of relative high abundance of ATP in the cellular systems.[4] In reality, this assumption is not always true and there are few well studied exceptions as well which were recently reviewed.[5] One of the most notable examples is preference of UTP over ATP as phosphate donor for dCK.[6] These observations, in turn, led to the search of other classes of potential phosphate donors, not necessarily confined to 5′-NTPs. 2′-Deoxynucleoside-3′-*O*-triphosphate (2′-d-3′-NTPs) are one class of such promising phosphate donors recently investigated. Sahyoun et al.[7] isolated a naturally occurring specific inhibitor of adenylyl cyclase (represent one of the major families of effector enzymes for G protein-coupled receptors) from amphibian and mammalian cells, identified as 2′-deoxyadenosine-3′-*O*-triphosphate (2′-d-3′-ATP). This observation was followed by numerous reports describing the inhibition of enzymes by nucleotides bearing 3′-*O*-triphosphate and 3′-*O*-polyphosphate groups. 2′-d-3′-ATP- and other related analogues are recently shown to be potent inhibitors of adenylyl cyclases by reducing cellular levels of 3′,5′-cAMP-dependant protein kinases.[8] Identification of new agents regulating 3′,5′-cAMP signaling pathway is of much current interest to treat various diseases.[9] 2′-d-3′-ATP and related analogues are recently demonstrated as promising phosphate donors to replace ATP for all four human and the *Drosophila Melanogaster* deoxyribonucleoside kinases.[10] These findings emphasize the ever increasing need to obtain these important 2′-d-3′-NTPs in high yields and purities by simple methods.

Upon literature search, it turned out to be the only available chemical method for the synthesis of 2′-d-3′-NTPs that was reported by Josse and Moffatt[11] and this low-yielding procedure essentially involved homologation of respective nucleoside-3′-*O*-monophosphates by the use of phosphoromorpholidate.[12] Modifications to the Josse and Moffatt method involving 3′-*O*-diphosphates and morpholinylphosphonate[13] were of limited success for thymidine and guanosine series and cannot be extended to the amino group bearing adenine and cytidine series. Nucleoside-3′-*O*-monophosphorylation of N-unprotected nucleosides via magnesium alkoxide formation by Uchiyama et al. involves treatment of nucleosides with equimolar amounts of *tert*-butylmagnesium chloride and tribromoethylphos-

* Corresponding author. Tel.: +1 512 721 3589; fax: +1 512 651 0201.
E-mail address: anil.kore@lifetech.com (A.R. Kore).

http://dx.doi.org/10.1016/j.tetlet.2014.01.077
0040-4039/

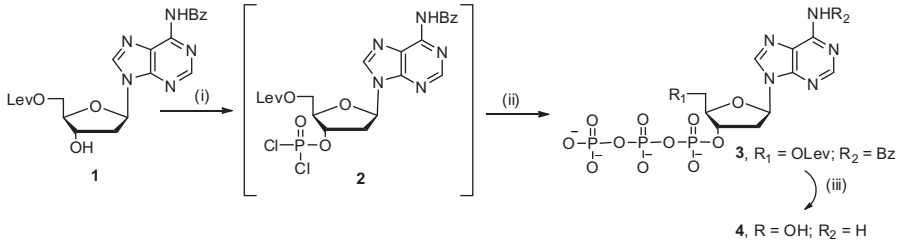
phoromorpholinochloridate and zinc mediated pyrophosphate coupling steps.[14] This method is less attractive as it involves multiple steps, pyrophoric Grignard reagents, moisture sensitive reagents, transition metals, protection-deprotection steps, and low over all yields. Using this alkoxide activation method, 2′-d-3′-ATP was synthesized in reported 22% yield.[8a] Hence, it is very important to develop a concise and high-yielding synthetic route to easily access these biologically very important 2′-d-3′-NTPs from readily available cheap starting materials.

Our laboratory recently reported an improved protection free, gram-scale, one-pot methodology for the chemical synthesis of 2′-deoxynucleoside-5′-*O*-triphosphates.[15] The chemistry involves the formation of nucleoside dichlorophosphoridate using $POCl_3$ as the reagent at the monophosphorylation step followed by reaction with tributylammonium pyrophosphate and hydrolysis of the resulting cyclic intermediate leading to dNTPs. In the one-pot procedure for all dNTPs, trimethyl phosphate is identified as the suitable solvent for the monophosphorylation and acetonitrile as the suitable solvent and tributylamine as the base for the step involving tributylammonium pyrophosphate. Our recent finding[16] successfully demonstrated the utility of this one-pot methodology by synthesizing various natural and non-natural nucleoside 5′-*O*-triphosphates in high yields and purity. We hypothesized that a highly concise synthesis of 2′-d-3′-NTPs can be easily obtained by using this one-pot protocol[15,16] when 5′-OH (of deoxyribose), N^6- (in case of adenosine), and N^4- (in case of cytidine) suitably protected commercially available cheap starting nucleosides are used. This protection is necessary as 3′-OH is less reactive than the 5′-OH of the 2′-deoxyribose moiety. The preferred protection groups for the 5′-OH of deoxyribose and the amino group on the nucleobase are the one that can be concurrently removed with ease under conditions that are compatible with triphosphates stability. Dimethoxytrityl (DMT) and levulinoyl protection groups are our preferred choices for the 5′-O-protection and the benzoyl group is the choice for the nucleobase amino group protection. In this Letter, we report a concise and efficient synthesis of 2′-deoxyadenosine-3′-*O*-triphosphate (2′-d-3′-ATP, Scheme 1) and 2′-deoxycytidine-3′-*O*-triphosphate (2′-d-3′-CTP, Scheme 2) using our recently published one-pot methodology.

During our initial experiments, we began the synthesis of 2′-deoxyadenosine-3′-*O*-triphosphate **4** with the monophosphorylation of commercially available N^6-Benzoyl-5′-*O*-dimethoxytrityl-2′-deoxyadenosine using $POCl_3$ in the presence of tributylamine as the base and trimethylphosphate as solvent. Immediate formation of orange color was noticed indicating falling apart of the 5′-*O*-DMT group during the monophosphorylation step and the reaction was sluggish. Hence acid labile 5′-*O*-DMT protection was replaced with 5′-*O*-Lev protection for monophosphorylation step. Commercially readily available N^6-Benzoyl-5'-*O*-levulinoyl-2′-deoxyadenosine **1** was monophosphorylated using $POCl_3$ at room temperature to form N^6-Benzoyl-5′-*O*-levulinoyl-2′-deoxyadenosine-3′-*O*-dichlorophosphoridate **2** intermediate in 98% yield (by HPLC).[17] Trimethylphosphate was used as the solvent and tributylamine was used as the base for the monophosphorylation step. In a one-pot fashion, without isolating the intermediate **2**, to the same flask, a prechilled cocktail containing tributylammonium pyrophosphate, tributylamine, and acetonitrile was added to the reaction mixture. The reaction mixture was quenched by slow addition of ice-cold water followed by extraction with dichloromethane. The collected aqueous solution was adjusted to pH 6.5 and loaded on a DEAE Sepharose column. The desired product was eluted using a linear gradient of 0–1 M triethylammonium biocarbonate (TEAB) and the fraction containing the product was pooled, concentrated. and coevaporated with water. The triethylammonium salt thus obtained was subjected to ion-exchange with sodium perchlorate in acetone for twice to afford the sodium salt of N^6-Benzoyl-5′-*O*-levulinoyl-2′-deoxyadenosine-3′-*O*-triphosphate **3** in 72% yield.[17] Treatment of compound **3** with concentrated aqueous ammonia for one hour resulted in concurrent deprotection of N^6-Benzoyl and 5′-*O*-levulinoyl groups in one-step. After purification and ion-exchange procedures, 2′-deoxyadenosine-3′-*O*-triphosphate (2′-d-3′-ATP, **4**) was obtained as sodium salt in 75% yield (Scheme 1).[18] Deprotection step does not require the use of purified protected 2′-d-3′-ATP (**3**). The aqueous portion containing the protected 2′-d-3′-ATP (**3**) after reaction workup can be concentrated under reduced pressure. The crude product **3** thus obtained was subjected to deprotection by the treatment with concentrated aqueous ammonia to afford 2′-d-3′-ATP, **4** in very comparable yield. Hence the purification of protected 2′-d-3′-ATP **3** is not required and we end up doing the purification of compound **3** just to obtain characterization data.

Using the same synthetic strategy, N^4-Benzoyl-5′-*O*-levulinoyl-2′-deoxycytidine **5** was first converted into N^4-Benzoyl-5′-*O*-levulinoyl-2′-deoxycytidine-3′-*O*-dichlorophosphoridate **6** intermediate in 98% yield (by HPLC) by $POCl_3$ monophosphorylation (Scheme 2).[18] In a one-pot fashion, without isolating the intermediate **6**, to the same flask, a prechilled cocktail containing tributylammonium pyrophosphate, tributylamine, and acetonitrile was added leading to the formation of N^4-Benzoyl-5′-*O*-levulinoyl-2′-deoxycytidine-3′-*O*-triphosphate **7**. After reaction work up and purification, compound **7** was isolated in 69% yield.[19] One-step concurrent deprotection of N^4-Benzoyl and 5′-*O*-levulinoyl groups using concentrated aqueous ammonia resulted in 2′-d-3′-CTP in 66% yield.[20]

In summary, concise and efficient synthesis of 2′-deoxynucleoside-3′-*O*-triphosphate (2′-d-3′-NTPs) reported in this letter is very attractive as it (i) utilizes high yielding one-pot procedure for the key triphosphate formation step, (ii) concurrent one-step deprotection of N-Benzoyl and 5′-*O*-levulinoyl groups of NTPs, (iii) requires only one final column purification after deprotection, and (iv) works equally well on both pyrimidine and purine nucleosides. This method can be efficiently extended to obtain other nucleoside-3′-*O*-triphosphates in high yields and purities for substrate evaluation against enzymes and other applications.



Scheme 1. Synthesis of 2′-deoxyadenosine-3′-*O*-triphosphate. Reagents and conditions: (i) Bu_3N, $(CH_3O)_3PO$, $POCl_3$, rt, 0.5 h, 98% (by HPLC); (ii) $(NHBu_3)_2H_2P_2O_7$, Bu_3N, CH_3CN, rt, 0.5 h, 72%; (iii) Concn NH_4OH, rt, 1 h, 75%.