

4-22-2015


# Synthesis of $\beta$ -triphosphotriester pronucleotides

Yousef A. Beni  
*Tennessee State University*

Chandravanu Dash  
*Meharry Medical College*

Keykavous Parang  
*Chapman University, parang@chapman.edu*

Follow this and additional works at: [http://digitalcommons.chapman.edu/pharmacy\\_articles](http://digitalcommons.chapman.edu/pharmacy_articles)

 Part of the [Macromolecular Substances Commons](#), [Medical Biochemistry Commons](#), [Nucleic Acids, Nucleotides, and Nucleosides Commons](#), and the [Polycyclic Compounds Commons](#)

---

## Recommended Citation

Beni, Y., Dash, C., Parang, K., Synthesis of  $\beta$ -triphosphotriester pronucleotides, *Tetrahedron Letters* (2015)  
DOI: 10.1016/j.tetlet.2015.03.036

This Article is brought to you for free and open access by the School of Pharmacy at Chapman University Digital Commons. It has been accepted for inclusion in Pharmacy Faculty Articles and Research by an authorized administrator of Chapman University Digital Commons. For more information, please contact [laughtin@chapman.edu](mailto:laughtin@chapman.edu).

---

# Synthesis of $\beta$ -triphosphotriester pronucleotides

## Comments

NOTICE: this is the author's version of a work that was accepted for publication in *Tetrahedron Letters*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version will subsequently be published in *Tetrahedron Letters* in 2015. DOI: [10.1016/j.tetlet.2015.03.036](https://doi.org/10.1016/j.tetlet.2015.03.036)

The Creative Commons license below applies only to this version of the article.

## Creative Commons License



This work is licensed under a [Creative Commons Attribution-Noncommercial-No Derivative Works 4.0 License](https://creativecommons.org/licenses/by-nc-nd/4.0/).

## Copyright

Elsevier

## Accepted Manuscript

Synthesis of  $\beta$ -triphosphotriester pronucleotides

Yousef Beni, Chandravanu Dash, Keykavous Parang

PII: S0040-4039(15)00482-7  
DOI: <http://dx.doi.org/10.1016/j.tetlet.2015.03.036>  
Reference: TETL 46039

To appear in: *Tetrahedron Letters*

Received Date: 27 January 2015  
Revised Date: 8 March 2015  
Accepted Date: 10 March 2015



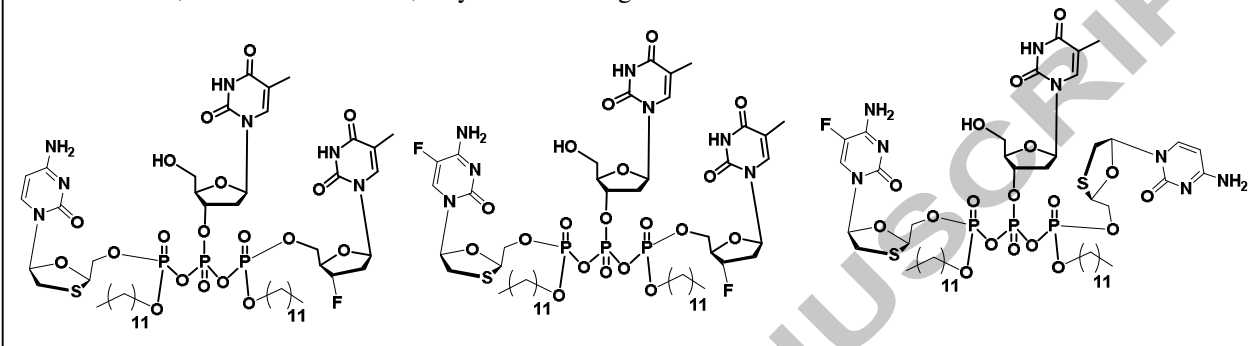
Please cite this article as: Beni, Y., Dash, C., Parang, K., Synthesis of  $\beta$ -triphosphotriester pronucleotides, *Tetrahedron Letters* (2015), doi: <http://dx.doi.org/10.1016/j.tetlet.2015.03.036>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

## Graphical Abstract

Synthesis of  $\beta$ -triphosphotriester pronucleotides

Leave this area blank for abstract info.

Yousef Beni<sup>1,\*</sup>, Chandravanu Dash<sup>2</sup>, Keykavous Parang<sup>3,4,\*</sup>



Tetrahedron Letters  
journal homepage: www.elsevier.com

## Synthesis of $\beta$ -triphosphotriester pronucleotides

Yousef Beni<sup>1,\*</sup>, Chandravanu Dash<sup>2</sup>, Keykavous Parang<sup>3,4,\*</sup>

<sup>1</sup>Department of Chemistry, Tennessee State University, Nashville, TN, USA 37209

<sup>2</sup>Center for AIDS Health Disparities Research, Department of Biochemistry and Cancer Biology, Meharry Medical College, Nashville, TN, USA 37208

<sup>3</sup>Chapman University School of Pharmacy, Irvine, CA, USA 92618

<sup>4</sup>Chao Family Comprehensive Cancer Center, School of Medicine, University of California, Irvine, Shambrom Hall, 101 The City Drive, Orange, CA, USA 92868

### ARTICLE INFO

#### Article history:

Received

Received in revised form

Accepted

Available online

#### Keywords:

Fatty chains

Nucleosides

Nucleotides

Pronucleotides

Triphosphotriesters

### ABSTRACT

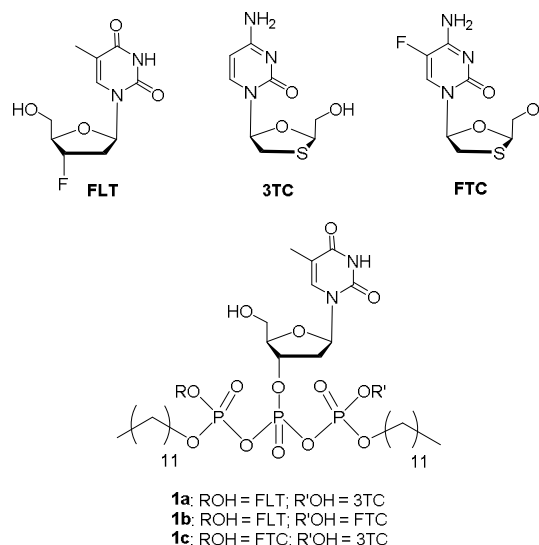
Dinucleoside phosphorochloridite were synthesized from phosphorus trichloride and three nucleoside analogues, 3'-fluoro-2',3'-dideoxythymidine (FLT), 2',3'-dideoxy-5-fluoro-3'-thiacytidine (FTC), and 2',3'-dideoxy-3'-thiacytidine (3TC), in a multistep synthesis. Polymer-bound *N*-Boc *p*-acetoxybenzyl 5'-*O*-2'-deoxythymidine was reacted with dinucleoside phosphorochloridite in the presence of 2,6-lutidine, followed by the reaction with dodecyl alcohol and 5-(ethylthio)-1*H*-tetrazole, oxidation with *tert*-butyl hydroperoxide, and acidic cleavage, respectively, to afford the  $\beta$ -triphosphotriester derivatives containing three different nucleosides.

2009 Elsevier Ltd. All rights reserved.

During HIV-1 replication, the viral RNA genome is reverse transcribed into a double stranded DNA by the virally encoded multifunctional enzyme reverse transcriptase (RT).<sup>1</sup> HIV-1 RT remains a major target for continued development of antagonists to inhibit virus replication and stem the devastating consequences of AIDS.

Two classes of drugs belonging either to the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) or to the non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been used in the clinic as part of the antiretroviral therapy against HIV/AIDS.<sup>2</sup> NRTIs compete with the natural deoxynucleoside triphosphates (dNTPs) during DNA synthesis and act as chain terminators.<sup>3</sup> In contrast, NNRTIs are non-competitive inhibitors that bind at an allosteric nonsubstrate binding site, which is distinct from the substrate binding site of HIV-1 RT.<sup>4</sup> While the unique pharmacology of these inhibitors has rendered their use in highly active antiretroviral therapy (HAART) therapy, HIV-1 has the ability to develop drug resistance mutations for both NRTI and NNRTIs.<sup>5</sup> Thus, design of novel lead compounds that can inhibit wild-type and drug resistant HIV-1 RTs is a subject of major interest in anti-HIV research.

The structural similarity of modified nucleotides to natural ribo- and deoxyribonucleoside triphosphates makes them useful reagents as substrates or inhibitors for DNA or RNA polymerases.<sup>6,7</sup> A number of approaches have focused on modifications and/or substitutions on the base,<sup>8,9</sup> carbohydrate<sup>10-15</sup> and linear triphosphate moieties<sup>16-21</sup> to design modified nucleotides for diverse applications in nucleic acid and antiviral research.



#### \*Corresponding authors.

(1) 3500 John A Merritt Blvd, Department of Chemistry, Tennessee State University, Nashville, TN, USA 37209, USA; Tel.: +1-615-963-5333; Fax: +1-615-963-5326; E-mail address: [ybeni@tnstate.edu](mailto:ybeni@tnstate.edu). (3) Chapman University School of Pharmacy, Irvine, CA, USA 92618; Tel.: +1-714-516-5489; Fax: +1-714-516-5481; E-mail address: [parang@chapman.edu](mailto:parang@chapman.edu).

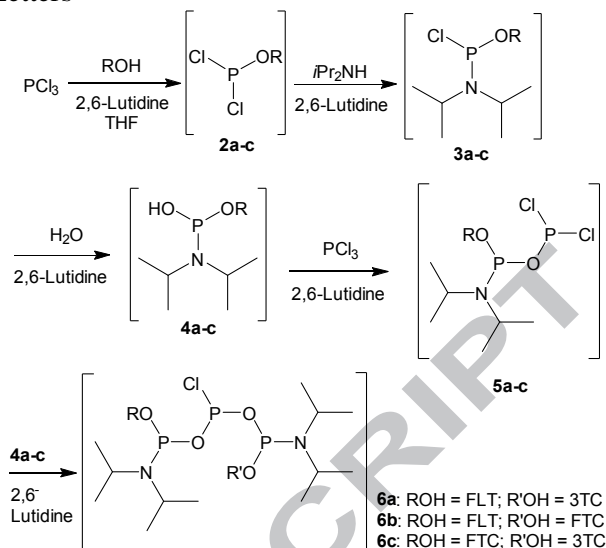
Fig. 1. Chemical structures of  $\beta$ -triphosphotriester pronucleotides (1a-c).

Negatively-charged nucleotides have limited cell-permeability. Masking the phosphate residues with a lipophilic chain could generate pronucleotides with improved cellular permeability. Prodrugs are chemically modified analogues of the active metabolite that can improve pharmacokinetics and pharmacodynamics (PK/PD) properties of the active drug. However, intracellular chemical transformation needs to be occurred in the presence of different enzymes to convert prodrugs to their corresponding pharmacologically potent compounds in *in vivo* systems. Prodrug approach offers several advantageous, such as enhancing water solubility, improved chemical stability, decreased toxicity, and insufficient brain penetration.<sup>22</sup> Herein we hypothesized that lipophilic pronucleotides can act as prodrugs of nucleotide analogs.

We have previously reported the synthesis of nucleoside 5'- $\alpha,\beta$ -methylene- $\beta$ -triphosphates and 5'- $O$ - $\beta,\gamma$ -methylenetriphosphates and their potency towards the enzymatic function of wild-type HIV-1 RT.<sup>23,24</sup> In continuation of our efforts to design a diverse array of modified nucleoside triphosphates as RT inhibitors, we report here the synthesis of  $\beta$ -triphosphotriester pronucleotides (**1a-c**) of NRTIs, including 3'-fluoro-3'-deoxythymidine (Alovudine, FLT), 2',3'-dideoxy-3'-thiacytidine (Lamivudine, 3TC), and 2',3'-dideoxy-5-fluoro-3'-thiacytidine (Emtricitabine, FTC) (Fig. 1). To the best of our knowledge, this is the first report of the synthesis of  $\beta$ -triphosphotriester pronucleotides containing two RT inhibitors.

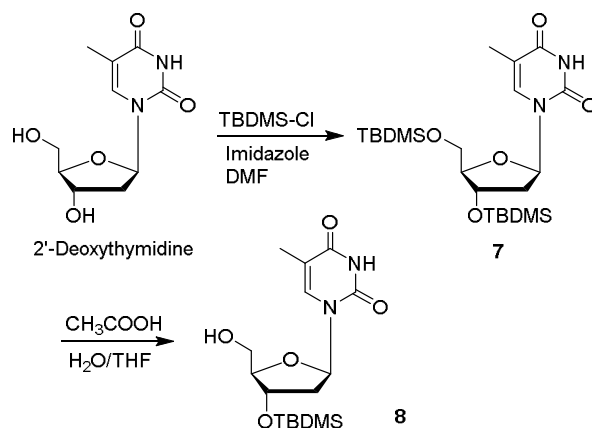
Scheme 1 illustrates the synthesis of nucleoside  $\beta$ -triphosphitylating reagents containing NRTIs (**6a-c**). Phosphorus trichloride ( $\text{PCl}_3$ , 2 mmol) was reacted with the nucleosides e.g. FLT, 3TC, or FTC (2 mmol) in the presence of 2,6-lutidine (2 mmol) to yield intermediate 5'- $O$ -nucleoside phosphorus dichloride (**2a-c**). In situ reaction of **2a-c** with *N,N*-diisopropylamine (2 mmol) in the presence of 2,6-lutidine (2 mmol) afforded intermediate 5'- $O$ -nucleoside *N,N*-diisopropylphosphoramidochloridite (**3a-c**). Addition of water (2 mmol) and 2,6-lutidine (2 mmol) gave 5'- $O$ -nucleoside *N,N*-diisopropyl hydroxyphosphoramidate (**4a-c**) that were reacted with phosphorus trichloride (2 mmol) in situ in the presence of 2,6-lutidine (2 mmol) to afford intermediate compounds **5a-c**. The intermediates were used immediately for the next reaction under extremely dry conditions and nitrogen.

The reaction of equimolar amounts of **4a-c** and **5a-c** produced 5'- $O$ -5'- $O$ -dinucleoside phosphorochloridite (ROH = FLT, R'-OH = 3TC (**6a**); ROH = FLT, R'-OH = FTC (**6b**); and ROH = FTC, R'-OH = 3TC (**6c**). The chemical structures of **6a-c** were confirmed by high-resolution time-of-flight electrospray mass spectrometry of hydroxyl form of the compounds as shown in the Supporting Information.



**Scheme 1.** Preparation of nucleoside  $\beta$ -triphosphitylating reagents containing NRTIs **6a-c**.

To accomplish the synthesis of dendritic  $\beta$ -triphosphotriester pronucleotides, a differentially protected 3'- $O$ -TBDMS-2'-deoxythymidine (**8**) was synthesized according to the previously reported procedures<sup>25-27</sup> (Scheme 2). In this regard, the 5'- and 3'-hydroxyl groups of 2'-deoxythymidine were protected by *tert*-butyldimethylsilyl (TBDMS) by the reaction of the unprotected nucleoside with *tert*-butyldimethylsilyl chloride in the presence of imidazole in DMF.<sup>25,26</sup> The selective removal of 5'- $O$ -TBDMS group in the presence of  $\text{AcOH}/\text{H}_2\text{O}/\text{THF}$ <sup>27</sup> afforded 3'- $O$ -TBDMS-2'-deoxythymidine (**8**).



**Scheme 2.** Preparation of 3'- $O$ -TBDMS-2'-deoxythymidine **8**.

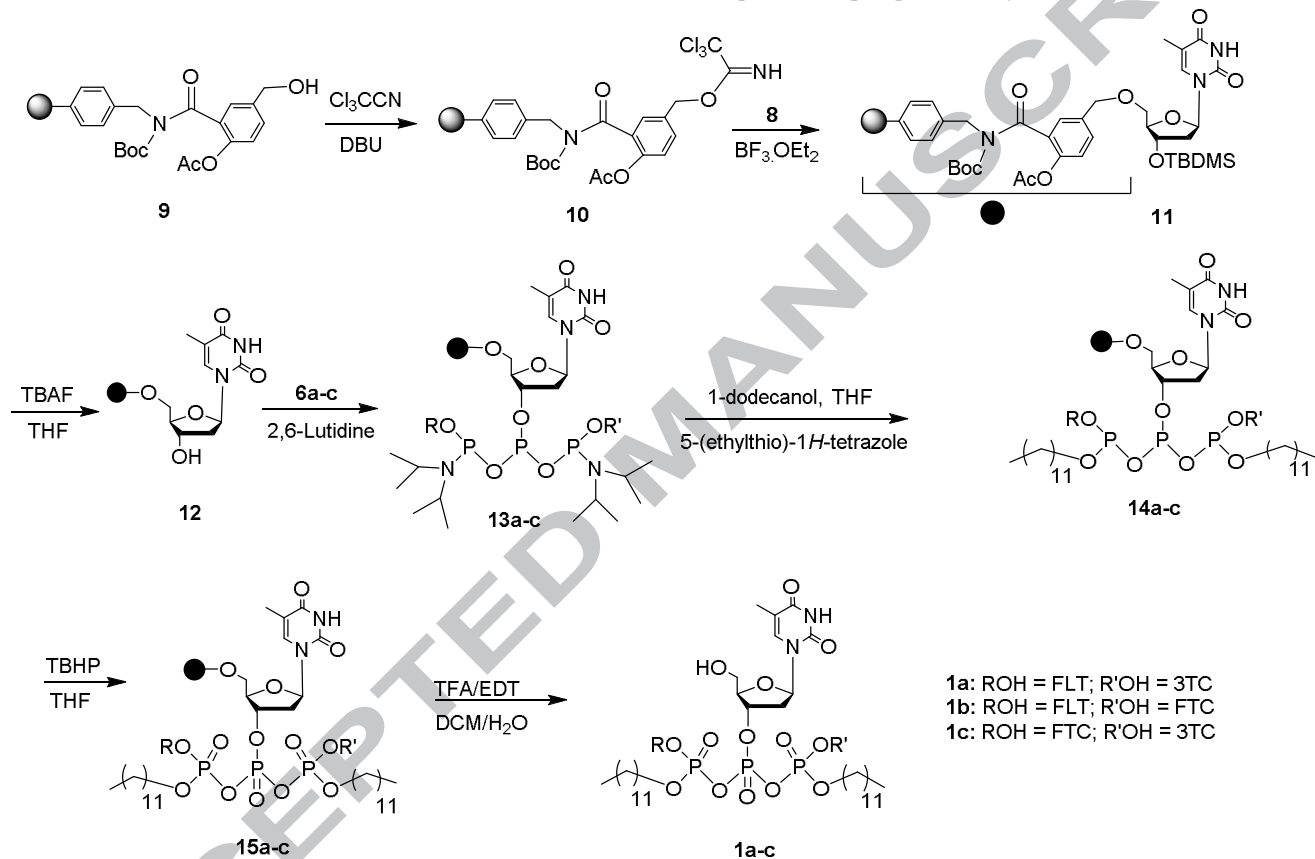
Our research on the solid-phase synthesis of organophosphorus and organosulfur compounds revealed that the polymer-bound *N*-Boc *p*-acetoxybenzyl alcohol (**9**) is a versatile solid-phase linker system for the phosphorylation of organic compounds<sup>27</sup>. In this regard the polymer-bound *N*-Boc *p*-acetoxybenzyl alcohol (**9**) was prepared according to our previously reported procedure<sup>26</sup> and was used as a loading system in this study. Then the polymer-bound *N*-Boc *p*-acetoxybenzyl trichloroacetimidate (**10**) was prepared from the reaction of (**9**) with trichloroacetonitrile in the presence of DBU according to reported procedure.<sup>28</sup>

Scheme 3 illustrates the synthesis of dendritic nucleoside  $\beta$ -triphosphate analogs (**1a-c**). The 2'-deoxy TBDMS protected 2'-deoxythymidine (**8**, 3 mmol) was attached to **10** (1.5 mmol) through 5'-hydroxyl group in the presence of  $\text{BF}_3 \cdot \text{OEt}_2$  as acidic catalyst<sup>29</sup> to afford **11**. The deprotection of 3'- $O$ -TBDMS group in **11** with tetrabutylammonium fluoride (TBAF) in THF afforded (**12**, ~1.50 mmol), which was divided to three portions

(~0.50 mmol each). Each portion of **12** underwent  $\beta$ -triphosphitylation of 3'-hydroxyl group with  $\beta$ -triphosphitylating reagents (**6a-c**, ~2 mmol, 4 equiv. of 2'-deoxy functions) under extremely dry conditions and nitrogen to afford (**13a-c**). In this regard, the prepared reaction mixture containing **6a-c** in THF (~2 mmol) was added to a swelled solution of polymer-bound *N*-Boc *p*-acetoxybenzyl 5'-*O*-2'-deoxythymidine **12** (~0.50 mmol) and 2,6-lutidine (2 mmol) in anhydrous THF. The mixture was shaken for 28 h with increasing of temperature from -20 °C to room temperature. The resin was collected by filtration, washed with THF and MeOH, respectively, and was dried overnight under vacuum to give **13a-c**.

1-Dodecanol was used to mask the negatively-charged cell-impermeable phosphate residues and to improve the lipophilicity of the pronucleotides. Thus, 1-dodecanol (4.0 mmol) and 5-

(ethylthio)-1*H*-tetrazole (4.0 mmol) were added to **13a-c** in anhydrous THF. The mixtures were shaken for 24 h at room temperature, then the resins were collected by filtration, washed with DCM and MeOH, respectively, and dried under vacuum to give **14a-c**. *tert*-Butyl hydroperoxide in decane was used for the oxidation of **14a-c** to **15a-c**. Finally, the cleavage of polymer-bound compounds was carried out under acidic conditions (DCM/TFA/H<sub>2</sub>O/1,2-ethanedithiol). The linker-trapped resin **10** was separated from the final products by filtration. After filtration, the solvents were removed using lyophilization and the crude products were purified (>98%) using HPLC system to afford pure **1a-c** products in 51-53% overall yield calculated from **10**. The chemical structures of the final products (**1a-c**) were determined by nuclear magnetic resonance spectra (<sup>1</sup>H NMR, <sup>13</sup>C NMR, and <sup>31</sup>P NMR), SELDI-TOF mass spectrometer, and quantitative phosphorus analysis.



**Scheme 3.** Preparation of pronucleotide derivatives of 3'-fluoro-3'-deoxythymidine, 2',3'-dideoxy-5-fluoro-3'-thiacytidine, and 2',3'-dideoxy-3'-thiacytidine **1a-c**.

In conclusion, a polymer-bound *N*-Boc *p*-acetoxybenzyl 5'-*O*-2'-deoxythymidine was reacted with three dinucleoside phosphorochloridites containing FLT, FTC, and 3TC in the presence of 2,6-lutidine in a solid phase reaction. Subsequent conjugation with dodecyl alcohol to mask the negatively charged

phosphate in the presence of 5-(ethylthio)-1*H*-tetrazole, oxidation with *tert*-butyl hydroperoxide, and acidic cleavage, respectively, afforded the  $\beta$ -triphosphotriester nucleotide derivatives **1a-c** containing three different nucleosides and a dodecyl chain. The compounds will be further evaluated for anti-HIV activities.

## References and notes

- Telesnitsky, A.; Goff, S. P., In Coffin, J. M.; Hughes, S. H.; Varmus, H. E. (eds.), *Retroviruses* **1997**, Cold Spring Harbor Laboratory Press, Plainview, New York, 121–160.
- De Clercq, E. *Biochimica et Biophysica Acta* **2002**, 1587, 258–275.
- Cihlara, T.; Ray, A. S. *Antiviral Res.* **2010**, 85, 39–58.
- Prajapati, D. G.; Ramajayam, R.; Yadav, M. R.; Giridhar, R. *Bioorg. Med. Chem.* **2009**, 17, 5744–5762.
- Menéndez-Arias, L. *TRENDS in Pharmacol Sci.* **2002**, 23, 381–388.
- Spelta, V.; Mekhalfia, A.; Rejman, D.; Thompson, M.; Blackburn, G. M.; North, R. A. *J. Pharmacol.* **2003**, 140, 1027–1034.
- Wang, G.; Boyle, N.; Chen, F.; Rajappan, V.; Fagan, P.; Brooks, J. L.; Hurd, T.; Leeds, J. M.; Rajwanshi, V. K.; Jin, Y.; Prhac, M.; Bruice, T. W.; Cook, P. D. *J. Med. Chem.* **2004**, 47, 6902–6913.
- Kawate, T.; Allerson, C. R.; Wolfe, J. L. *Org. Lett.* **2005**, 7, 3865–3868.
- Hoffmann, C.; Genieser, H. G.; Veron, M.; Jastorff B. *Bioorg. Med. Chem. Lett.* **1996**, 6, 2571–2271.
- Wu, W.; Meyers, C. L. F.; Borch, R. F. *Org. Lett.* **2004**, 6, 2257–2260.
- Camplo, M.; Faury, P.; Charvet, A. S.; Graciet, J. C.; Chermann, J. C.; Kraus, J. L. *Eur. J. Med. Chem.* **1994**, 29, 357–362.
- Chong, Y.; Gumina, G.; Mathew, J. S.; Schinazi, R. F.; Chu, C. K. *J. Med. Chem.* **2003**, 46, 3245–3256.

13. Von Janta-Lipinski, M.; Costisella, B.; Ochs, H.; Hubscher, U.; Hafkemeyer, P.; Matthes, E. *J. Med. Chem.* **1998**, *41*, 2040–2046.
14. Anastasi, C.; Quelever, G.; Burlet, S.; Garino, C.; Souard, F.; Kraus, J. L. *Curr. Med. Chem.* **2003**, *10*, 1825–1843.
15. Van Aerschot, A.; Herdewijn, P.; Balzarini, J.; Pauwels, R.; De Clercq, E. *J. Med. Chem.* **1989**, *32*, 1743–1749.
16. Okruszek, A.; Olesiak, M.; Balzarini, J. *J. Med. Chem.* **1994**, *37*, 3850–3854.
17. Barai, V. N.; Zinchenko, A. I.; Kvach, S. V.; Titovich, O. I.; Rubinova, E. B.; Kalinichenko, E. N.; Mikhailopulo, I. A. *Helv. Chim. Acta* **2003**, *86*, 2827–2832.
18. Ludwig, J.; Eckstein, F. *Nucleosides & Nucleotides* **1991**, *10*, 663–665.
19. Ludwig, J.; Eckstein, F. *J. Org. Chem.* **1991**, *56*, 5860–5865.
20. Ludwig, J.; Eckstein, F. *J. Org. Chem.* **1991**, *56*, 1777–1783.
21. Ma, Q. F.; Bathurst, I. C.; Barr, P. J.; Kenyon, G. L. *J. Med. Chem.* **1992**, *35*, 1938–1941.
22. Raurio, J.; Kumpulainen, H.; Heimbach, T.; Oliyai, R.; Oh, D.; Jarvinen, T.; S, J. *Nature Rev.* **2008**, *7*, 255–270.
23. Ahmadibeni, Y.; Dash, C.; Le Grice, S.; Parang, K. *Tet. Lett.* **2010**, *51*, 3010–3013.
24. Ahmadibeni, Y.; Dash, C.; Hanley, M. J.; Le Grice, S.; Agarwal, H. K.; Parang, K. *Org. Biomol. Chem.* **2010**, *8*, 1271–1274.
25. Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1976**, *94*, 17, 6190–6191.
26. Sekine, M.; Masuda, N.; Hata, T. *Tetrahedron* **1985**, *41*, 5445–5453.
27. Kawai, A.; Hara, O.; Hamada, Y.; Shioiri, T. *Tet. Lett.* **1988**, *29*, 6331–6334.
28. Ahmadibeni, Y.; Parang, K. *J. Org. Chem.* **2005**, *70*, 1100–1103.
29. Hanessian, S.; Xie, F. *Tet. Lett.* **1998**, *39*, 733–736.

### Supplementary Material

Supplementary data associated with this article including experimental procedures and characterization of compounds can be found in the online version.