Bioorganic & Medicinal Chemistry 26 (2018) 2365–2371

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Chemo-enzymatic synthesis of the exocyclic olefin isomer of thymidine monophosphate

Dibyendu Mondal, Eric M. Koehn [a], Jiajun Yao, David F. Wiemer *, Amnon Kohen *

Department of Chemistry, University of Iowa, Iowa City, IA 52242-1294, United States

ARTICLE INFO

Article history:
Received 3 February 2018
Revised 15 March 2018
Accepted 21 March 2018
Available online 22 March 2018

Keywords:
Thymidylate
Metabolism
Nucleotide analogue
Synthesis

ABSTRACT

Exocyclic olefin variants of thymidylate (dTMP) recently have been proposed as reaction intermediates for the thymidyl biosynthesis enzymes found in many pathogenic organisms, yet synthetic reports on these materials are lacking. Here we report two strategies to prepare the exocyclic olefin isomer of dTMP, which is a putative reaction intermediate in pathogenic thymidylate biosynthesis and a novel nucleotide analog. Our most effective strategy involves preserving the existing glyosidic bond of thymidine and manipulating the base to generate the exocyclic methylene moiety. We also report a successful enzymatic deoxyribosylation of a non-aromatic nucleobase isomer of thymine, which provides an additional strategy to access nucleotide analogs with disrupted ring conjugation or with reduced heterocyclic bases. The strategies reported here are straightforward and extendable towards the synthesis of various pyrimidine nucleotide analogs, which could lead to compounds of value in studies of enzyme reaction mechanisms or serve as templates for rational drug design.

1. Introduction

Nucleotides are essential molecules commonly referred to as the building blocks of life because they make up DNA and RNA as well as cell machinery for expression, regulation, and generation of gene products. Thus, structural analogs of nucleotides have seen a variety of uses including, but not limited to, cancer therapeutics, mechanistic bioreagents, and *in vivo* probes for metabolic studies.[1–3] Unnatural and noncanonical nucleotides have been produced through modification of the phosphate,[4–7] the sugar,[8–11] and the nucleobase[3,12–15] moieties resulting in a range of biologically relevant compounds with a variety of activities and functions.[9] The focus of this report involves nucleobase modifications that result in a new structural variant of the thymidyl moiety. In the past nucleobase analogs that mimic thymidyl moieties have produced antiviral agents (e.g., trifluorothymidine[16,17]), antitumor agents (e.g., 5-F-uridine[18,19]), and biological fluorophores (e.g., 5-aminoallyluridine[20]). These past examples showcase the importance of modifications of this pyrimidine nucleotide in particular, and the targeting of thymidyl biological processes in general.

Thymine is unique among the DNA nucleosides. Deoxyguanosine, deoxyadenosine, and deoxycytosine can be produced directly from their respective ribosides through the action of ribonucleotide reductases, but thymidyl moieties must be produced *de novo* by methylation of uridyl moieties.[21] Several enzymes catalyze this reductive methylation, including thymidylate synthase (TSase),[22] flavin-dependent thymidylate synthase (FDTS),[23] and methylenetetrahydrofolate-tRNA-(uracil-5-)-methyltransferase (TrmFO),[24] to provide the thymidine that is essential for DNA production and cell proliferation.[22] As a result, thymidyl biosynthetic enzymes are often the target of chemotherapeutic agents[19] and they have shown potential as antibacterial[25] and antiviral[26] drug targets as well. However, while the well-studied "classical" thymidylate synthase (TSase) has been the focus of cancer drug development (e.g., 5-F-dU together with leucovorin), less is known regarding the inhibition of alternative thymidyl biosynthesis enzymes, which limits the development of antibacterial and antiviral compounds that target these enzymes.[23] For example, the enzyme FDTS, which is present in several important human pathogens, functions by a remarkably different chemical mechanism from classical TSase. At this time there are no known potent inhibitors that selectively target FDTS.[27] Compounds that mimic the unique intermediates for this alternative thymidylate biosynthesis may advance our ability to exploit this attractive target.

All of the known biosynthetic pathways that lead to thymidylic acid (**1**) by reductive methylation, whether catalyzed by FDTS

* Corresponding authors.
E-mail address: david-wiemer@uiowa.edu (D.F. Wiemer).
[a] Current address: Department of Chemistry and California Institute for Quantitative Biosciences, University of California Berkeley, Berkeley, CA 94720-3220, United States.

https://doi.org/10.1016/j.bmc.2018.03.032
0968-0896/

(from **2**) or by TrmFO or TSase (from **3**), have in common an exocyclic methylene group at the C5-C7 position of the pyrimidine ring (Fig. 1). The existence of exocyclic methylene intermediates has been supported by nucleophilic trapping experiments which disrupt the enzymatic turnover and allow isolation of derivatives of the reaction intermediates.[25,28,29] Many of these putative intermediate species can only be hypothesized based on these chemical trapping experiments along with the known reactivity of an α,β-unsaturated system, and therefore direct access to this analogue of dTMP would greatly facilitate mechanistic study of thymidyl biosynthesis.[29] Exocyclic olefin analogs of thymidine have appeared in studies by Greenberg and his group (e.g. **4**), but all the currently known compounds are also substituted at the C-6 position. Nevertheless, these doubly-modified thymidine analogs have been used to demonstrate the formation of interstrand cross-linking and biocongugates in RNA and DNA via a mechanism involving exocyclic methylene-modified thymidines.[12,13,30,31] Thus there has been substantial interest in designing synthetic routes that can generate modified nucleotides,[9,32] and one modified only by transposition of the olefin to an exocyclic position (i.e. compound **2**) would be especially valuable in studies of enzyme mechanism(s). Unfortunately, due to the various functional groups present in the starting materials, formation of an exocyclic methylene moiety is challenging and traditional reactions proceeding by installation and removal of orthogonal blocking groups ultimately impact synthetic feasibility.[9]

To the best of our knowledge, only transient olefin isomers of thymidine or thymidylate have been proposed,[23] occurring either as intermediates in enzyme reactions or along the pathway to DNA labeling via photolysis. Furthermore, all of these examples have a heteroatom substituent at C-6 of the pyrimidine ring and therefore have a different C-6 oxidation state than the dihydro species proposed as an intermediate during alternative thymidylate biosynthesis (i.e. compound **2**).[23,33] In 1973 Klötzer[34] reported the synthesis of an exocyclic methylene nucleobase isomer of thymine, but synthesis of the exocyclic methylene isomer of thymidine or thymidylate has not been reported. Herein we present two different synthetic routes to achieve the unique C-5 isomer of dTMP (**1**) bearing an exocyclic olefin. This isomer has been proposed[25,29,35,36] as an intermediate during the FDTS catalyzed reaction and therefore represents an important structural motif in pathogenic dTMP biosynthesis. We also report on the chemical and pH stability of this compound, along with derivatization at the C-7 methylene terminus that results in novel nucleophilic addition products and may serve as a possible gateway to novel compounds with antibacterial or antiviral activities.[23] Finally, the reactivity reported herein suggests that compound **2** may have potential use in reaction with nucleophilic residues of thymidyl binding proteins or in DNA.

2. Results and discussion

Two complementary synthetic approaches have been developed to prepare the target compound **2**. Although the approaches are orthogonal in strategy, both afford the exocyclic C-5 olefin isomer of thymidine (**10**) and then follow the same phosphorylation pathway via an enzymatic kinase. Exploration of these two orthogonal synthetic strategies paves the way for future synthetic modifications that can easily afford novel isotopic labeling patterns in compound **2**.

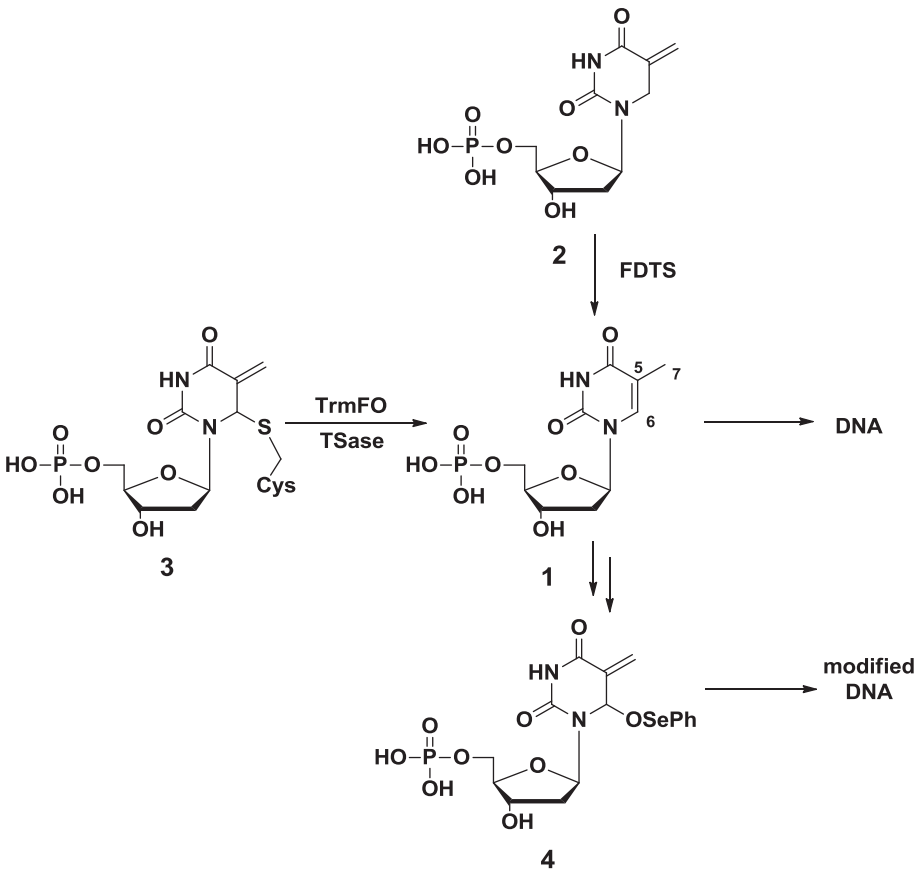

Fig. 1. Thymidylic acid and some olefin analogs of biological significance.