Bioorganic & Medicinal Chemistry Letters 24 (2014) 2720–2723

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Phospho-carboxylic anhydride of a homologated nucleoside leads to primer degradation in the presence of a polymerase

Dhuldeo Kachare, Xiao-Ping Song, Piet Herdewijn *

Medicinal Chemistry, Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 20 February 2014
Revised 9 April 2014
Accepted 10 April 2014
Available online 19 April 2014

Keywords:
Phospho-carboxyl anhydride
Nucleosides
Polymerases
Phosphorolysis
Homologated AZT

ABSTRACT

Starting from thymidine, through a series of key synthetic transformations (e.g., Wittig reaction, hydroboration, Mitsunobu reaction and TEMPO oxidation) a nucleoside homologue bearing a phospho-carboxylic anhydride group at 6′ position was synthesized. The potential of polymerases to catalyze amide bond formation was investigated by using a modified primer with an amino group at 3′ position and the synthesized phosphoanhydro compound as substrate. Unfortunately, we did not observe the desired product either by gel electrophoresis or mass spectrometry. In contrast, the instability of the phosphoanhydro compound could lead to pyrophosphate formation and thus, to pyrophosphorolysis of the primer rather than to primer extension.

In coeval life, proteins and nucleic acids are intricately dependent upon each other for a host of functions. Protein enzymes (polymerases) are necessary for the synthesis of DNA and RNA, while nucleic acids (ribosomes) are necessary for the synthesis of proteins. According to the RNA world hypothesis, early life used nucleic acids for both information storage and chemical catalysis before the emergence of protein enzymes. However, it still remains unclear how nucleic acids were able to assemble and replicate before the advent of protein enzymes. This means that in contemporary life, proteins can catalyze phosphodiester bond formation (nucleic acid synthesis) and nucleic acids can catalyze amide bond formation (protein synthesis). Our motivation in this context comes from the recently proposed 'Molecular Midwife' hypothesis[1] and has the aim to investigate if polymerases can also catalyze amide bond formation. Furthermore, investigations on alternative information systems in the field of synthetic biology ask for a careful mapping of the substrate specificity of natural enzymes, such as polymerases.[2]

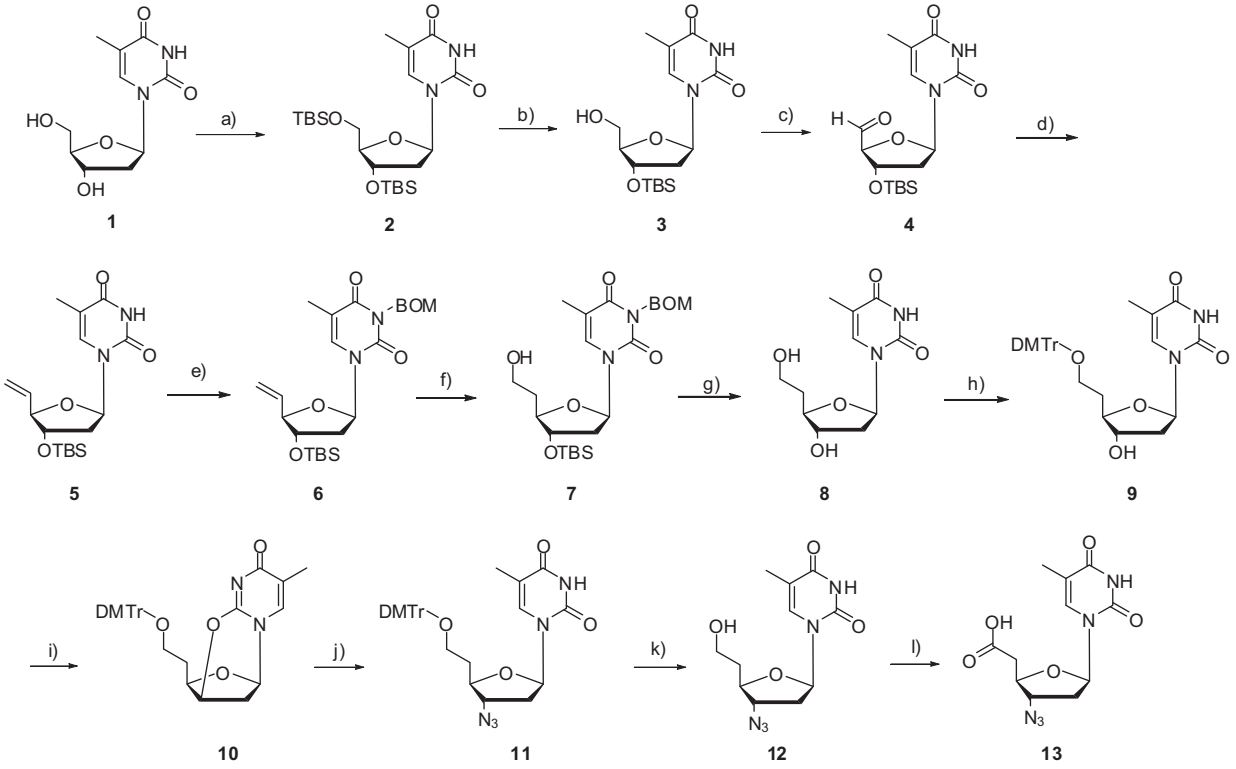
In a first effort to test the potential of polymerases to catalyze an amide bond, we considered the synthesis of a modified nucleoside with a carboxylate group at 6′ position and an amine group at 3′ position. The carboxylate group needs to be activated (for example as mixed anhydride with phosphate) before the compound can be tested as substrate for polymerases. The selection of a 3′-amino group is based on (a) the possibility to get chain elongation reaction and (b) the introduction of a positive charge in the oligonucleotide (after incorporation reaction) to facilitate detection by gel chromatography and mass spectrometry. Due to the expected difficulties in obtaining a carboxylate activated, unprotected amino acid, we decided to test the reaction with a 3′-azido congener. The azido group could then be reduced to an amino group after incorporation, if that would be necessary for analytic purposes.

The synthetic scheme started from commercially available thymidine **1**, in which compounds **2** and **3** are synthesized by using literature procedures reported by Tronchet et. al.[3]

Compound **3** was oxidized to **4** using Dess–Martin periodinane (DMP) reagent. Wittig reaction was carried out on **4**, using potassium *tert*-butoxide and methyl triphenylphosphonium bromide in THF to afford the vinyl compound **5**. The vinyl compound **5** was reacted with benzylchloromethyl ether (BOM-Cl) to protect the NH group in thymine ring to furnish compound **6**. BOM protected compound **6** was reacted with 9-borabicyclo[3.3.1]nonane (9-BBN) and subsequent treatment with hydrogen peroxide in alkaline medium to give **7**.[4] The removal of BOM group by treatment with Pd/C 10% wt. in MeOH in presence of cyclohexene under reflux conditions, also lead to removal of the TBS group at the 3′ position affording **8**. Homologated thymidine **8** was protected at the 6′-O-position with 4,4′-dimethoxytrityl chloride in pyridine yielding compound **9**. The subsequent synthesis of compound **11** involved an inversion of the 3′-carbon atoms from S-configuration to R-configuration which was accomplished in two steps. Cyclisation of compound **9** under Mitsunobu condition gave the O^2, 3′-anhydro derivative **10**, which is a useful intermediate for

* Corresponding author. Tel.: +32 16 337387; fax: +32 16 337340.
E-mail address: Piet.Hedewijn@rega.kuleuven.be (P. Herdewijn).

http://dx.doi.org/10.1016/j.bmcl.2014.04.042
0960-894X/



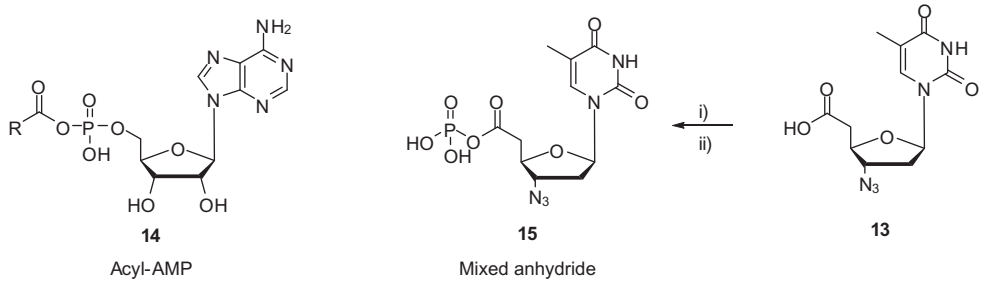
Scheme 1. Reagents and conditions: (a) TBDMSCl, Py, added at rt for 1 h, then stirred at 60–70 °C, 22 h, 98.8%; (b) TFA/H_2O (10:1, v/v), DCM, rt, 45 min then ice bath for 30 min, 80%; (c) Dess–Martin periodinane, MeCN, 80 °C; (d) methyltriphenylphosphonium bromide, potassium *tert*-butoxide, THF, added at 0 °C and stirred at rt, overnight, 53%; (e) BOM-Cl, DBU, MeCN, 0 °C to rt, 2 h, 80.6%; (f) 9-BBN, THF, added at 0 °C and stirred at rt, overnight; then added NaOH and H_2O_2 at 0 °C and stirred at rt, 6 h, 57.2%; (g) Pd/C 10% wt., cyclohexene, MeOH, reflux, 57%; (h) DMTr-Cl, pyridine, rt, 70.7%; (i) PPh_3, DIAD, MeCN, 0 °C, 12 h, 87.7%; (j) NaN_3, DMF, 140 °C, 12 h, 85.3%; (k) 3% trichloroacetic acid, DCM, rt, 66.7%; (l) TEMPO, bis(acetoxy)iodobenzene, MeCN/H_2O (1:1, v/v), rt, overnight, 57.7%.

substitutions at the 3′-position. Opening of the aza-eno–ether in **10** with sodium azide in DMF gave protected AZT derivative **11**.[5] Compound **11** was deprotected with 3% trichloroacetic acid to furnish homologated alcohol **12**, which was subsequently oxidized at 6′ position to afford the corresponding carboxylic acid **13** (Scheme 1).

Acyl monophosphates (**14**) are known as intermediates in biosynthesis, but unfortunately due to the low stability their synthesis and purification are not much explored. As far as we know, there is no literature dealing with the synthesis of mixed anhydrides (**15**) in which the nucleoside moiety contains a carboxylic acid functionality (Scheme 2).

Our first attempt to obtain anhydride **15** from compound **13** by using bis(trimethylsilyl)-tributylstannyl phosphate was unsuccessful.[6] Further efforts for its synthesis by using diethyl chlorophosphate, diphenyl chlorophosphonate, and bis(tetraethyl ammonium) ethyl phosphate were also unsuccessful.[7,8] Therefore we focused on a two-step reaction. The acid **13** was first reacted with ethylchloroformate and tri-*n*-butylamine to furnish the corresponding anhydride, then the obtained intermediate was reacted with tri-*n*-butylammonium phosphate (0.5 M in DMF) affording the desired mixed anhydride **15** (Scheme 2).[9,10] The tri-*n*-butylammonium phosphate (0.5 M in DMF) reagent was synthesized by using a reported procedure by El-Tayeb et al.[11] Compound **15**, however, was unstable and was isolated by partitioning the reaction mixture between ethyl acetate and cold water. The product was collected in the aqueous layer and isolated by freeze drying. Further purification of compound **15** was carried out by HPLC with C18 column using a gradient elution buffer of triethylammonium acetate (TEAA) and acetonitrile.

In the context of probing template-directed amide bond formation by polymerases, compound **15** bearing a phospho-carboxylic acid anhydride function at 6′-position of sugar moiety was used as substrate, a modified primer (P1) (see Table 1) was installed with 3′-NH_2-2′,3′-dideoxycytidine at 3′ terminal and template T1



Scheme 2. Chemical structure of acyl phosphate (**14**) and mixed anhydride (**15**); (i) ethylchloroformate, *n*-Bu_3N, 1,4-dioxane, 0 °C–rt; (ii) 0.5 M tri-*n*-butylammonium phosphate, DMF.