European Journal of Medicinal Chemistry 57 (2012) 126–133

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antiviral evaluation of *bis*(POM) prodrugs of (*E*)-[4′-phosphono-but-2′-en-1′-yl]purine nucleosides

Ugo Pradère [a], Vincent Roy [a], Aurélien Montagu [a], Ozkan Sari [a], Manabu Hamada [a], Jan Balzarini [b], Robert Snoeck [b], Graciela Andrei [b], Luigi A. Agrofoglio [a,*]

[a] Institut de Chimie Organique et Analytique, UMR 7311 CNRS, Université d'Orléans, 45067 Orléans, France
[b] Rega Institute for Medical Research, KU Leuven, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 27 April 2012
Received in revised form
28 August 2012
Accepted 29 August 2012
Available online 7 September 2012

Keywords:
Acyclic nucleoside phosphonate
Prodrugs
Mitsunobu reaction
Antiviral evaluation

ABSTRACT

Seventeen *hitherto unknown bis*(POM) prodrugs of novel (*E*)-[4′-phosphono-but-2′-en-1′-yl]purine nucleosides were prepared in a straight approach and at good yields. Those compounds were synthesized by the reaction of purine nucleobases directly with the phosphonate synthon **3** bearing POM biolabile groups under Mitsunobu conditions. All obtained compounds were evaluated for their antiviral activities against a large number of DNA and RNA viruses including herpes simplex viruses 1 and 2, varicella zoster virus, Feline herpes virus, human cytomegalovirus, HIV-1 and HIV-2. Among these molecules, some of them exhibit anti-VZV and anti-HIV activity at submicromolar concentrations. This class of compound will be of further interest for lead optimization as anti-infectious agents.

1. Introduction

Acyclonucleoside phosphonates (ANPs) [1] represent a key class of antiviral drugs which has attracted considerable attention through the large number of modified nucleobase and/or acyclic chain moieties. Among ANPs, 9-[2-(phosphonomethoxy)ethyl] adenine (PMEA) [2] or (*R*)-[2-(phosphonomethoxy)propyl]adenine (PMPA) [3] exhibited strong antiviral activities against of HIV and/or HBV infections (Fig. 1). However, their antiviral efficiency is mainly hampered by their low cell penetration (<5%) but also by their ability to be converted by human/viral kinases to their triphosphate active forms at a high concentration at the right location [4]. To circumvent the poor bioavailability of ANP which are negatively charged at physiological pH, several groups [5–8] have successfully developed biolabile promoieties in which the charged phosphonic acid group is transformed into a neutral phosphonate diester(s) with decreased polarity. Based on that, PMEA and PMPA have been commercialized as their prodrug *bis*(-POM)PMEA (Adefovir, HEPSERA™) and *bis*(POC)PMPA (Tenofovir, VIREAD™), respectively.

Part of our program to discover antiviral compounds, we have recently reported a new class of acyclic nucleoside phosphonates bearing the (*E*)-4′-phosphono-but-2′-en-1′-yl side chain moiety, which can mimic the conformation of the C1–O4–C4–C5 atoms from the 2-deoxyribose in dTMP [9,10]. Having in hand an acyclic phosphonate side chain, it was rational to connect this synthon to purine base analogues which exhibit the greatest activities as antiviral agents. In this work, we aim to identify a novel series of purine ANP analogues in their prodrug form, combining our biolabile phosphonate side-chain and purine analogues selected in the literature as the lead nucleobases which possesses antiviral properties (e.g., guanine [11] and its tricyclic analogues [12,13], N^6-substituted-purines [14,15]). Herein we report the biological evaluation and the efficient one step synthesis *via* Mitsunobu reaction of (*E*)-*bis*-(POM)-[4-phosphono-but-2′-en-1′-yl]purine analogues.

2. Chemistry

We have previously reported the use of cross metathesis reaction between *bis*(POM)-allyl phosphonate **2** and crotylated

Abbreviations: VZV, varicella zoster virus; VV, vaccinia virus; HSV, herpes simplex virus; VSV, vesicular stomatitis virus; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; CC_{50}, compound concentration affording 50% inhibition of cell growth; EC_{50}, compound concentration affording 50% inhibition of the viral cytopathicity; MCC, minimum cytotoxic concentration required to afford a microscopically detectable alteration of cell morphology; MDCK, Madin–Darby canine kidney.

* Corresponding author. Tel.: +33 2 3849 4582.
E-mail address: luigi.agrofoglio@univ-orleans.fr (L.A. Agrofoglio).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.08.042

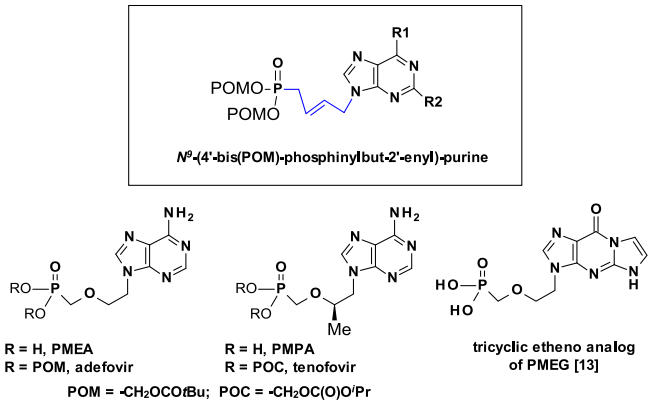


Fig. 1. Some nucleoside phosphonates and target compounds.

5-substituted uracil as an one-step efficient tool to obtain in good yield a large library of (*E*)-4-phosphono-but-2′-en-1′-yl pyrimidine nucleosides, which some of them exhibit submicromolar activities against VZV [9,16]. However, it is well-known that nitrogen containing heterocycles can decrease the catalytic metals through a poisoning mechanism. Usually, metathesis reaction involving purine nucleosides requires exocyclic amine protection to avoid catalyst poisoning and often gives very low yields. Thus, to circumvent this problem, we aimed to determine the most suitable conditions for an efficient synthesis of (*E*)-4-phosphono-but-2′-en-1′-yl purines in their prodrug form. We thought that selected purines could be directly introduced through a *N*-alkylation with the corresponding activated (*E*)-4-phosphono-but-2′-en-1′-yl counterpart either under SN2 or under Mitsunobu conditions.

Thus, starting with *bis*(POM)-allyl phosphonate (**2**), the (*E*)-*bis*(POM)-4-bromo-but-2-en-1-yl phosphonate (**3′**) was obtained in 73% yields by cross metathesis of *bis*-(POM)-allyl phosphonate (**2**) with (*E*)-1,4-dibromobut-2-ene. Unfortunately, the *N*-alkylation reaction of adenine in the presence of Cs_2CO_3 [17] and synthon **3′** in anhydrous DMF failed, because of major formation of *bis*(POM)-but-1,3-dienyl phosphonate **4** resulting from undesired bromine elimination (Scheme 1).

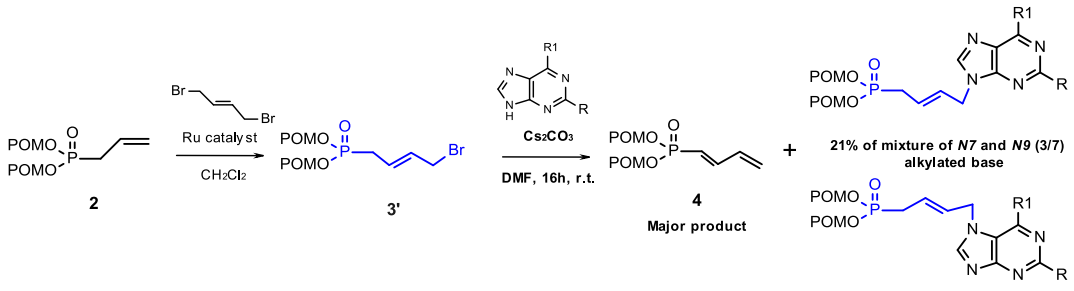
We then turned our attention to the introduction of the nucleobase under Mitsunobu conditions [18] (Scheme 2). Firstly, the *hitherto* unknown (*E*)-*bis*(POM)-4-hydroxy-but-2-en-1-yl phosphonate **3** bearing hydroxyl was prepared by cross metathesis reaction with *bis*(POM) allylphosphonate (**2**) and 2-buten-1,4-diol in 74% yields.

Starting from dimethylallylphosphonate, compound **2** was obtained according our previous procedure, using chloromethylpivalate in the presence of sodium iodine during 48 h in 84% yield. (*E*)-4-Hydroxy-*bis*(POM)-but-2-enylphosphonate **3**, the key compound of our strategy, was prepared through cross metathesis reaction between *cis*-but-2-en-1,4-diol and **2** in the presence of 5 mol% of Ru catalyst during 16 h in CH_2Cl_2 at reflux. The resulted mixture of two isomers (*E* and *Z*) was separable by column chromatography to afford the desired isomer *trans* **3** in 74% yield. A previous attempted cross metathesis reaction with crotylalcohol afforded also (*E*/*Z*) mixture in a disappointing 32% yield, which can be mainly ascribed to the formation of the unreactive crotyl phosphonate.

The synthesis of purine ANP analogues **9**–**13** was performed as shown in Scheme 3). The key intermediates **7** and **8** were obtained in 71% and 50% yield, respectively, through Mitsunobu reaction between appropriate 6-chloropurines (**5** or **6**) and synthon **3** in the presence of PPh_3 and DIAD in dioxane at room temperature. The Mitsunobu reaction condition gave a mixture of *N*-7 and *N*-9 alkylated bases, which were isolated by delicate column chromatography. Both isomers were determined by NMR spectroscopic data analysis including HMBC correlation between protons H2 and H2′, and carbons C4 and C5. *Bis*(POM)-ANP chloropurine derivatives **7** and **8** were first converted to hypoxanthine **9** and guanine **10** derivatives in 86% and 85% yields, respectively, using diluted HCOOH at 40 °C for 20 h. Compounds **7** and **8** underwent also nucleophilic substitution on the C6-position with cyclopropylamine to afford **11** and **12** in good yield. From compound **8**, a tricyclic derivative **13** was obtained in 48% yield by treatment with 2-chloroacetaldehyde (50 wt. % in H_2O) in a dioxane/water mixture at 70 °C for 6 h [13]. During our investigations, we did not observe the degradation of the (POM) biolabile group proving the efficiency of our strategy in terms of number of steps and overall yield. However, in order to facilitate the isolation of the *N*-9 isomers, substitutions at C6-position were alternatively proceeded before the Mitsunobu reaction. The desired C6-substituted-purine bases **14b**–**e** and **15c**–**e** were obtained from **5** or **6** by nucleophilic substitution with substituted primary amines and Et_3N in *n*BuOH with yields ranging from 80 to 92%. Compounds **14a**–**e** and **15a**,**c**,**d**,**e**,**f** can be readily converted in *bis*(POM) ANP derivatives **16a**–**e** and **17a**,**c**,**d**,**e**,**f** by coupling reaction with synthon **3** as described previously, in 38–52% overall yield. All the structures were confirmed by ^1H, ^{13}C, ^{31}P NMR and HRMS analysis.

The title *bis*(POM) (*E*)-4-phosphono-but-2′-en-1′-yl acyclic nucleosides **7**–**13** and **16**, **17** were subjected to an *in vitro* antiviral screening using a wide spectrum of DNA viruses, in HEL cell cultures, for vaccinia virus (VV), herpes simplex virus-1(KOS) (HSV-1), herpes simplex virus-2(G) (HSV-2), varicella-zoster virus (VZV TK^+ and TK^-) as summarized in Table 1.

Among the synthesized compounds some of them show potent inhibitory activities against several DNA viruses, in particular compounds **10**, **12** and **17f**, exhibiting submicromolar activities. Even if the active compounds against DNA virus replication do not cause a microscopically detectable alteration of cell morphology (MCC) at concentration up to 100 μM, they were found to be rather cytostatic. Therefore, it is currently unclear whether the activity observed for these compounds is due to a specific antiviral effect or to an antiproliferative activity. It is interesting to notice that the



Scheme 1. Purine base alkylation through the use of halogenated synthon **3′**.