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Expedient synthesis and biological evaluation of alkenyl acyclic nucleoside phosphonate prodrugs

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ABSTRACT

The importance of phosphonoamidate prodrugs (ProTides) of acyclic nucleoside phosphonate (ANPs) is highlighted by the approval of Tenofovir Alafenamide Fumarate for the treatment of HIV and HBV infections. In the present paper we are reporting an expedient, one-pot, two-steps synthesis of allyl phosphonoamidates and diamidates that offers a time saving strategy when compared to literature methods. The use of these substrates in the cross metathesis reactions with alkenyl functionalised thymine and uracil nucleobases is reported. ANPs prodrugs synthesized via this methodology were evaluated for their antiviral activities against DNA and RNA viruses. It is anticipated that the use of 5,6,7,8-tetrahydro-1-napthyl as aryloxy moiety is capable to confer antiviral activity among a series of otherwise inactive uracil ProTides.

1. Introduction

The ProTide approach, pioneered by Chris Mcguigan's group,[1,2] is a powerful technology aimed to optimize intracellular drug delivery and circumvent metabolic bottlenecks in the activation of nucleoside-based antiviral and anticancer drugs. In the last years this technology has displayed a great deal of success in the antiviral field with two compounds in the market: the phosphoramidate Sofosbuvir [3,4] (Sovaldi®) approved in 2013 against HCV infections and the phosphonoamidate tenofovir alafenamide fumarate[5] (TAF, Vemlidy®) approved in 2015 for the treatment of HIV[6,7] and later in 2016 for HBV infections[8,9] (Fig. 1).

Several other ProTides have entered in clinical trials while many others are in preclinical evaluation either as antiviral or anticancer drugs.[2,10,11] Given the tremendous importance of phosphor(n)oamidate prodrugs in the antiviral arena and beyond, after the approval of Sofosbuvir and TAF, the application of the ProTide technology has grown dramatically and it has started to show very promising results in other therapeutic areas as well.[12–14] While there are several efficient procedures to synthesize phosphoroamidate nucleosides, the phosphonoamidate cognate class especially of acyclic nucleoside phosphonates (ANPs) lacks of such plethora of synthetic methodologies.[15]

ANPs play a key role in the treatment of viral infections, and this class of compounds can be regarded as one of the most significant group of drugs in the antiviral field.[16,17] Discovered almost 30 years ago, a great wealth of research has been dedicated to the development of efficient synthetic methodologies that resulted in a great variety of ANPs.[18–22] These new structures offer a potential for the discovery of more effective drugs against a variety of infectious diseases including antiparasitic,[23–29] antimicrobial,[30–33] and antitubercolous[34,35] medicines. Among these synthetic strategies, quite recently, Agrofoglio's group has elaborated a novel, efficient and straightforward synthesis of C5-alkenyl substituted ANPs via olefin cross-metathesis.[36–42] Although structure-activity relationship (SAR) studies on acyclic nucleosides have not clarified their pharmacophore model, the introduction of a rigid structural element such as the double bond has proved to be extremely important for their antiviral activity.[43,44] Precisely, the *trans*-alkene skeleton is able to mimic the three-dimensional geometry of the ribose ring maintaining also an electronic contribution similar to the one provided by the oxygen.[45] There are considerable evidences that the *trans*-alkenyl acyclic nucleotide motif has a strong affinity with recombinant human thymidylate kinase (hTMPK) active site, responsible for the nucleotide phosphorylation and consequently correlated to its antiviral activity.[41]Interestingly, Agrofoglio's group employed the olefin cross-metathesis methodology also for the direct synthesis of a vast array of unsaturated ANPs analogues including *bis*-POM, *bis*-POC, and alkoxyesters prodrugs.[36,38–41,46,47] Although adopting a different procedure, our group extended the range of prodrugs of (*E*)-but-2-enyl-pyrimidine, by synthesising their ProTide and bisamidate derivatives.[48] In this study we showed that the ProTide technology was able to broaden the spectrum of antiviral activity when compared to other

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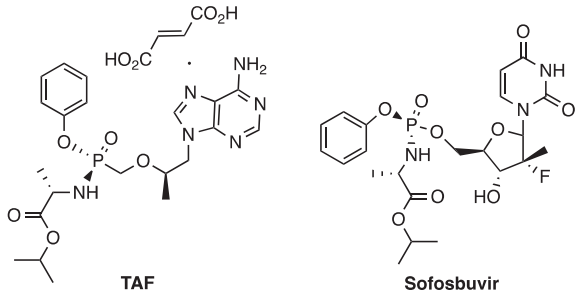


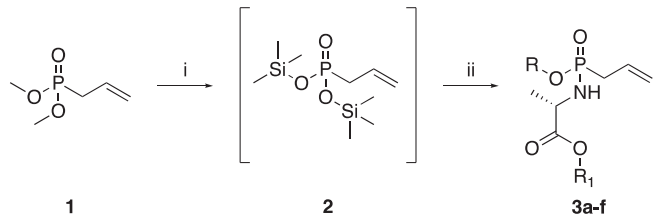
Figure 1. Structures of Sofosbuvir and TAF.

phosphate prodrug approaches. However, we discovered that this methodology suffers from the limitation that only linear olefin must be employed, as with trisubstituted alkenyl derivatives we observed only formation of traces of the desired ProTides. This finding prompted us to investigate the possibility of using the cross-metathesis for the direct synthesis of unsaturated branched ANP phosphonoamidates. At the time we started this investigation, no application of such procedure for the synthesis of ProTides was yet reported. However, during the preparation of this manuscript, a paper reporting the use of the cross metathesis for the synthesis of ProTide derivatives of linear (*E*)-but-2-enyl nucleoside scaffold, was published.[49] The prodrugs described in this work belong to the same family of compounds previously reported by us,[48] and indeed their antiviral profile was in agreement with our published results. In the present article, we would like to report an effective and improved methodology for the synthesis of allyl phosphonoamidate and their further application in olefin cross-metathesis for the synthesis of ANP ProTides. We also anticipate that our two-steps, one-pot methodology can also be applied to the synthesis of symmetrical allyl phosphonodiamidates. Compared with the recently published procedure,[49] our synthetic strategy presents some advantages which we believe, merit consideration.

2. Results and discussion

2.1. Chemistry

Our research began with the synthesis of the aryloxy allylphosphonoamidate synthon **3a**, for which the only literature procedure available is a long and tedious multistep sequence.[50,51] Based on our experience in the application of Holy's one-pot procedure for the direct synthesis of phosphonodiamidates,[52] we envisaged that this protocol could be used to get access to the desired synthon starting from the commercially available dimethyl allylphosphonate **1** (Scheme 1). This methodology was already adapted in our laboratory for the synthesis of adefovir and tenofovir phosphonoamidate prodrugs[53] and more recently for the preparation of (*E*)-but-2-enyl pyrimidine ProTides.[48] Briefly, commercial dimethyl allylphosphonate **1** was converted into the corresponding silyl ester **2**, by reaction with an excess of bromotrimethylsilane (5.0 equivalents). Due to the hydrolytically instability of this ester, **2** was not isolated but immediately dissolved in a mixture



Scheme 1. Synthesis of *O*-Aryl-(L-alanine-ester)-allylphosphonate. *Reagents and conditions*: *i.* TMSBr (5.0 equiv), 2,6-Lutidine (4.0 equiv), CH_3CN, rt, 16 h; *ii.* Amino acid ester hydrochloride (1.0 equiv), aryl-alcohol (6.0 equiv), Et_3N (15.0 equiv), aldrithiol-2 (6.0 equiv), PPh_3 (6.0 equiv), pyridine, 50 °C, 16 h.

Table 1
Substitution pattern and isolated yields of allyl phosphonoamidates **3a–f**.

Entry	Cpds	Aryl	Amino acid	Ester	Yield[a]
1	**3a**	1-Naph	L-Ala	*i*-Pr	79%
2	**3b**	1-Naph	L-Ala	Bz	78%
3	**3c**	Ph	L-Ala	*i*-Pr	65%
4	**3d**	Ph	L-Ala	Bz	42%
5	**3e**	TH-1-Naph	L-Ala	*i*-Pr	55%
6	**3f**	TH-1-Naph	L-Ala	Bz	55%

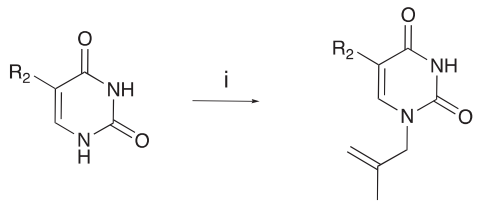
[a] Yield are determined for isolated, purified compounds; see experimental part for details.

of pyridine/Et_3N and treated with the L-alanine isopropyl ester hydrochloride (1.0 equivalents), an excess of 1-naphthol (6.0 equivalents), and a premade solution of PPh_3 (6.0 equivalents) and aldrithiol-2 (6.0 equivalents) in pyridine. After 16 h, the crude mixture did not show the presence of either the desired product or phosphonodiamidate compound (which, based on our experience, is almost invariably formed). We attributed this lack of reactivity to the decomposition of the disilyl ester **2** caused by the release of hydrobromic acid, generated by the hydrolysis of the excess of TMSBr used. Pleasingly, when we attempted the reaction in the presence of 2,6-lutidine (4.0 equivalents) as acid scavenger, the formation of the desired product **3a** was observed (^{31}P NMR and LC-MS analysis of the crude mixture). **3a** was isolated by flash chromatography in excellent yield (79%) (Table 1, Entry 1). Quite surprisingly, no evidence of side reactions[48] (bromination of the double bond and formation of the phosphonodiamidate) have been observed.

With the above methodology, we prepared six different allyl phosphonate analogues **3a-f** in which a variety of aryloxy groups were introduced in combination with two different amino acid esters (L-alanine isopropyl or benzyl esters). From Table 1 it can be appreciated that our method worked well with aryl alcohols with different steric requirements. In particular, we were able to prepare the allyl phosphonoamidates bearing the 5,6,7,8-tetrahydro-1-napthol **3e** and **3f** (Entries 5 and 6, Table 1), which have shown to impart remarkable antiviral activities in compounds of previous series.[48,53]

This procedure is short and efficient, representing an improvement of the literature method, which accounts for a 29% overall yield in four steps.[49]

With these allyl phosphonoamidates in hand we began the synthesis of (*E*)-methylbut-2-enyl pyrimidine **6** and **7**, selected as the other partner for the cross-metathesis reaction. These nucleosides and their *bis*-POM prodrugs were originally prepared by Agrofoglio and colleagues,[38] which found the latest to have moderate activities against feline herpes virus (FHV) and feline corona virus (FCoV). Considering that ProTides of alkenyl pyrimidine with "linear" (*E*)-but-2-enyl double bond have shown improved antiviral activities and a broad antiviral spectrum when compared to the corresponding *bis*-POM derivatives, we were now interested in investigating whether ProTide of branched alkenyl pyrimidine might have the same effect. We therefore synthesised a thymine and uracil derivative **6** and **7** as reported in Scheme 2.



Scheme 2. Synthesis of N^1-2′methylallylpyrimidine. *Reagents and conditions*: *i.* 3-Bromo-2-methylpropene (2.0 equiv), BSA (2.5 equivalents), NaI (1.1 equiv), TMSCl (1 equiv), CH_3CN, reflux temperature, 16 h.