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Short communication

Cyclic phosphoramidates as prodrugs of 2′-*C*-methylcytidine

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ABSTRACT

The currently approved treatment for hepatitis C virus infections is a combination of Ribavirin and pegylated Interferon. It leads to a sustained virologic response in approximately only half of the patients treated. For this reason there is an urgent need of new therapeutic agents. 2′-*C*-Methylcytidine is the first nucleoside inhibitor of the HCV NS5B polymerase that was efficacious in reducing the viral load in patients infected with HCV. The application of a monophosphate prodrug approach based on unprecedented cyclic phosphoramidates is reported. Our SAR studies led to compounds that are efficiently converted to the active triphosphate in human hepatocytes.

1. Introduction

Hepatitis C virus is a 9.5 kb positive-strand RNA virus that undergoes rapid mutation, making treatment difficult. Chronic hepatitis C viral infection can lead to cirrhosis and hepatocellular carcinoma. Estimates of the total number of infected individuals are currently 170–200 million worldwide [1]. While the incidence of new infections is declining, mortality is expected to increase in the middle of the next decade [2a]. The currently approved treatment available for patients with chronic hepatitis C is a combination of Ribavirin and pegylated Interferon, leading to a sustained virologic response (SVR) in approximately half of the patients treated. Side effects such as fatigue, flu-like symptoms, depression, and hemolytic anemia can not only be dose limiting but also lead to a premature discontinuation of therapy prior to achievement of sustained viral response [2a,b].

HCV inhibition can be achieved by blocking essential virally-encoded non-structural enzymes (NS2-3 and NS3-4A proteases, the NS3 helicase as well as the NS5B RNA-dependent RNA polymerase) [2c,d]. The NS3-4A protease and NS5B RNA enzymes have been the focus of intense drug discovery efforts over the years [2a]. These have culminated in the identification of antiviral compounds with which significant reductions in HCV viral load have been demonstrated in the clinic [2a,b].

The RNA-dependent RNA polymerase (RdRp) is at the heart of the viral replication complex. Nucleoside as well as non-nucleoside inhibitors of this enzyme have been described. The former offer the advantage of pan-genotype inhibition due to the high conservation of the RdRp active site across all HCV genotypes. A nucleoside needs to be converted efficiently into the corresponding nucleotide triphosphate anabolite via cellular kinases. This newly synthesized nucleotide must be a substrate for the HCV RdRp, and needs to be incorporated into the nascent nucleic acid chain causing chain termination.

The efficiency of NTP formation of a given nucleoside often rests on the rate-limiting first phosphorylation which converts the nucleoside into its corresponding monophosphate anabolite by cellular kinases (Fig. 1; from **4** to **5**). Several approaches have been

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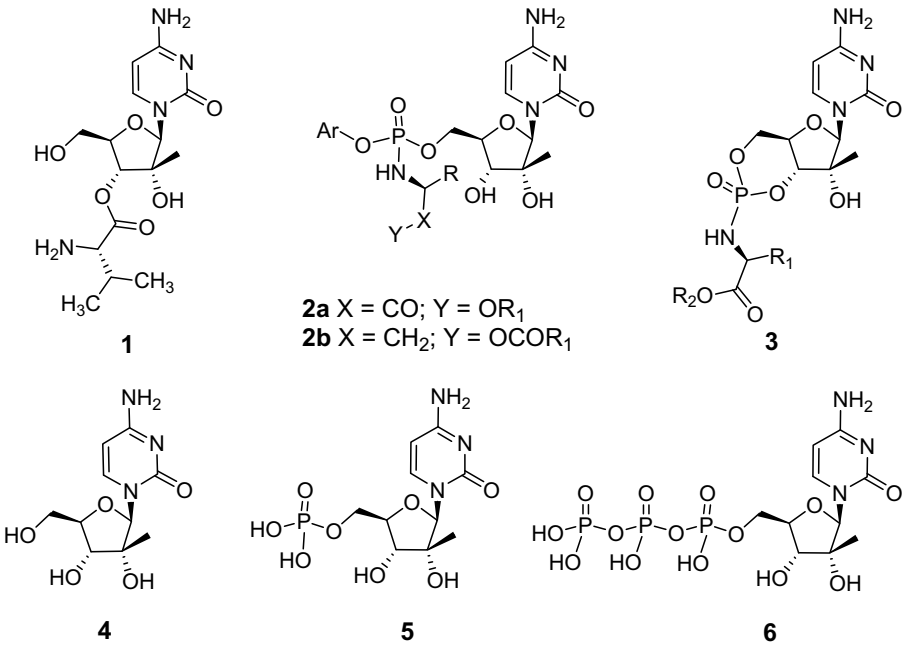


Fig. 1. 2′-*C*-Methylcytidine (**4**), its prodrugs (**1**, **2a**, **2b**, **3**) and anabolites (**5** and **6**).

described in the literature which address this problem by delivering a masked nucleoside monophosphate (SATE approach [3a], cycloSal approach [3b], HepDirect approach [3c] as well as the use of phosphoramidates [3d]).

We have recently been involved in a prodrug based nucleoside inhibitor program focusing on 2′-*C*-methylcytidine **4**, a potent and selective inhibitor of HCV replication in cell culture. We used Valopicitabine **1** [4], the 3′-*O*-L-valine ester derivative of 2′-*C*-methylcytidine, as our reference compound. Although **1** has been evaluated in Phase IIb clinical studies, further development has been put on hold due to an unfavourable risk/benefit profile [2c]. Initially we based our approach on Protide type structures **2a** [5a,5b], and on acyloxy ethylamino phosphoramidates **2b** [5c], as we were aware that initial, first phosphorylation to **5** was the rate-limiting step in cellular processing to form active triphosphate **6** [6a,b]. This approach in literature has been addressed as 'kinase-bypass-effect' [3c,7a–c].

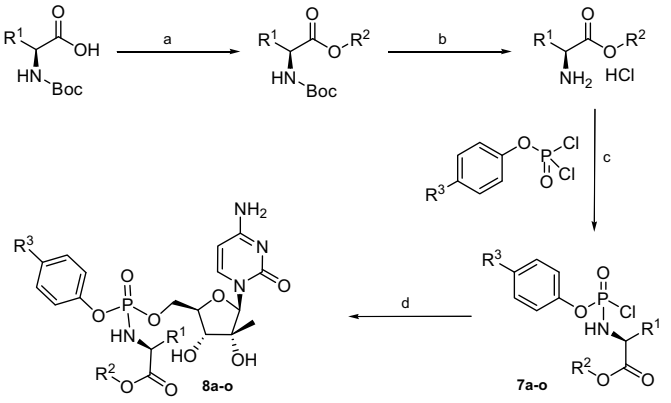
Over the course of our program, we could eliminate the need for a phenolic leaving group from our prodrugs, designing the cyclic phosphoramidates of type **3** (Fig. 1) [8]. This generated an additional level of diversity in our SAR studies and this approach might have two additional advantages: the cyclisation reduces the degree of rotational freedom as compared to generally described Protide prodrugs **2a**, possibly allowing for improved entry into the cell, and less phenol related cytotoxic side effects [9a,b] might occur.

In this communication we describe the synthesis and structure–activity relationship studies around cyclic phosphoramidates **3** and show that they efficiently generate high levels of 2′-methylcytidineTP **6** in hepatocytes.

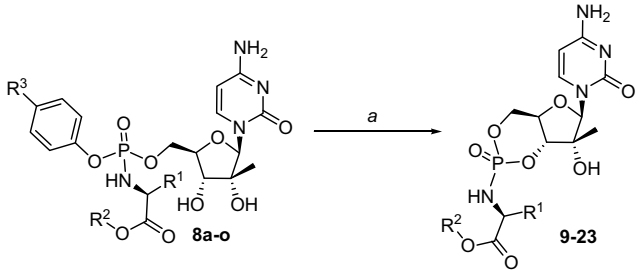
2. Results and discussion

2.1. Chemistry

The synthesis of the precursors **8a**–**o** is described in Scheme 1 [5b]. The ester coupling between amino acids and alcohols as well as derivatisation to form phosphoramidate chlorides **7a**–**o** were modified literature procedures giving generally good yields [10]. Selective *t*BuMgCl-mediated reaction between phosphoramidate chlorides **7a**–**o** and 2′-*C*-methylcytidine [11] to form intermediate nucleoside derived phosphoramidates **8a**–**o** proceeded in discrete to moderate yields as a mixture of diastereomers that were separated or taken on as a mixture to the cyclisation step.



Scheme 1. Reagents and conditions: (a) R_2OH, TEA, DMAP, DCM 80–97%; (b) 4 N HCl dioxane, 95–100%; (c) Et_3N, DCM, −78 °C to rt, 72–91%; (d) *t*BuMgCl, **4**, THF, −78 °C to rt, 5–36%.



Scheme 2. Reagents and conditions: (a) KO*t*Bu, DMSO, 41–78%.