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Synthesis and evaluation of novel phosphoramidate prodrugs of 2′-methyl cytidine as inhibitors of hepatitis c virus NS5B polymerase

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ABSTRACT

A variety of new prodrugs of 2′-methyl cytidine based on acyloxy ethylamino phosphoramidates have been synthesized and tested in vitro and in vivo for their biological activity. Compared with the parent drug a 10- to 20-fold increase in formation of nucleotide triphosphate in rat and human hepatocytes could be achieved.

Hepatitis C virus (HCV) infection is a serious worldwide health problem affecting about 2% of the world population (WHO data).[1] While the early stages of the disease are typically asymptomatic, the majority of HCV infections progress to chronic infection, which is associated with an increased risk of cirrhosis, hepatocellular carcinoma, and liver failure. At present, the standard of care available to patients with chronic HCV infection is a combination of ribavirin and interferon-based therapies, which leads to a sustained virologic response in only about half of the patients treated.[2]

Efforts to discover more effective drugs to treat HCV-infected patients have focused on several possible targets, including the NS5B RNA-dependent RNA polymerase.[3] 2′-Methyl cytidine[4] has been shown to be an inhibitor of hepatitis virus C NS5B in cell culture.[5] The 5′-triphosphate of this nucleoside is a potent active site inhibitor of hepatitis virus C polymerase.[6] To enhance the bioavailability of 2′-methyl cytidine its 3′-valine ester has been prepared (NM283 = Valopicitabine **1**, Fig. 1)[7] and a 1.2 $\log_{10}$ viral reduction in HCV RNA was observed in patients upon dosing 800 mg qd.[8] Its development has been stopped in phase II due to an unfavorable risk/benefit profile observed in clinical testing.[3c]

For a variety of nucleosides with modified core structure (e.g., d4T or ara-C) a poor turnover to the nucleoside triphosphate (NTP) has been observed.[9,10] In some cases this is attributed to the inability of the nucleoside kinase to catalyze the initial phosphorylation of the nucleoside to its monophosphate (NMP). To circumvent this problem, a suitable prodrug strategy would be one aimed at the delivery of the nucleoside monophosphate inside the target cell.[11]

To this end, different approaches have been described in the literature. For example, Metabasis Therapeutics has reported cyclic cytochrome P450-3A-cleavable phosphate esters as liver targeted-prodrugs (so-called HepDirect prodrugs).[12] Furthermore, in the antiviral field, McGuigan and co-workers have described aryloxy phosphoramidates (incorporating an amino acid ester) as suitable pro-moieties, specifically to improve delivery of AZT and d4T (ProTide approach).[13]

In the search for HCV NS5B polymerase inhibitors we applied an analogous phosphoramidate approach to 2′-methyl cytidine.[14] As a variation on the theme we also evaluated replacing the amino acid moiety with 2-aminoethanol. Although Imbach et al. reported this

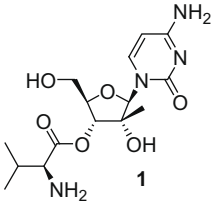

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Figure 1. Valopicitabine, NM283.

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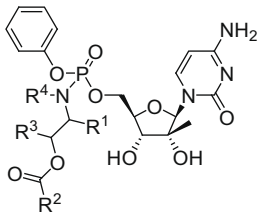


Figure 2. Acyloxyethylamino phosphoramidate prodrugs.

approach failed in their study on AZT derivatives,[15] we were pleased to find that this strategy proved successful for our substrate. Herein we report the design, synthesis and biological evaluation of this novel 2′-Me-C-based series (Fig. 2).

The synthesis of the compounds was first performed under classical conditions by reaction of the unprotected nucleoside with a phosphoramide chloride. This approach suffered from two major liabilities. The first is a physical issue related to the limited solubility of 2′-Me-C in organic solvents. The second derives from the poor yield in the reaction of the amino alcohol fragments with phenyl phosphorodichloridate. While the latter reacted smoothly with amino acid derivatives, only poor results were observed with amino alcohol derivatives. To circumvent these issues, a different route was developed. We found that the compounds could be conveniently prepared by first masking the secondary and tertiary alcohols on the nucleoside **2** as a 2′,3′-acetonide (Scheme 1).

The resulting 5′-unprotected nucleoside **3** was then reacted with diphenyl phosphite to give the common intermediate **4**. This material was not isolated but subjected directly to an Atherton-Todd reaction with an *O*-acyl aminoethanol in the presence of tetrachloromethane.[19] Careful deprotection of the phosphoramidates **5** afforded the desired prodrugs **6–19** in moderate overall yield (25% from nucleoside **2**). Separation of the two diastereoisomers was achieved for each derivative by reversed phase HPLC.

All compounds were routinely tested in the replicon assay.[16] For representative compounds the formation of nucleoside triphosphate was assessed in screening mode in human and rat hepatocytes.[17] Those compounds showing promising results were then profiled further.

In this report, we focus on the structure–activity relationship around the amino alcohol moiety, with the phenoxy functionality on the phosphoramidate remaining unvaried. In particular, initial studies were directed towards the preparation of a series of esters of unsubstituted ethanolamine ($R^1 = R^3 = H$).

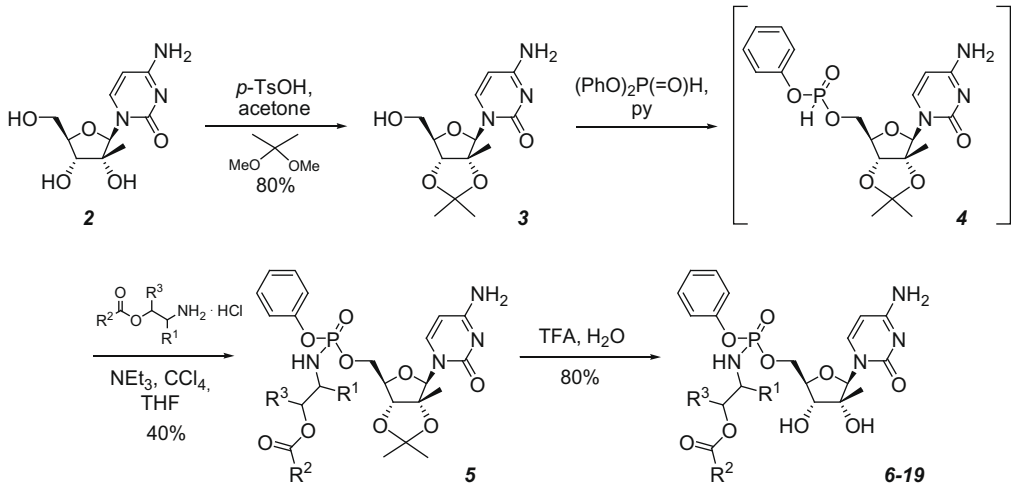
Benzoyl and acetyl esters were prepared (examples **6–7**). These compounds exhibited encouraging improvements in cell-based activity, when compared with NM283. A significant step forward in terms of activity could then be made with the introduction of branched chain alkanoic esters **8–12** (Table 1). We found that not only small esters like those based on *iso*-butanoic acid were tolerated, but also longer chain esters like 2-ethylbutanoates and 2-propylpentanoates gave rise to very active compounds in the cell-based assay.

Substitution of the ethanolamine (**13–19**) was at best tolerated (**16b**) but still compatible with sub-μM levels of inhibition in the replicon. However, simple *N*-methylation of the ethanolamine nitrogen (R^4 = Me, **20**) or exchanging the ethanolamine moiety with 3-hydroxy-prolinol (example **21**) completely abrogated activity.

Due to the chirality of the phosphorus all compounds were obtained as a mixture of diastereomers. These could be separated by HPLC or SFC. In most cases the second eluting diastereomer (e.g., **6b**, **9b**, **10b** and **16b**) turned out to be more active in the replicon assay than the first eluting product although in the end both diastereomers should yield the same nucleotide. Thus, a plausible hypothesis for pro-moiety activation would be via a stereoselective enzymatic first step (as for the McGuigan prodrug), whereby this stereochemical preference is then reflected in the replicon activity. The data reported in Table 1 refer to the second eluted diastereoisomer if not otherwise denoted.

For representative compounds the actual formation of nucleoside triphosphate from the prodrug was studied in rat and human hepatocytes, (Table 2). We were very pleased to observe that the replicon data could be associated with levels of NTP formation in comparison with the reference compound NM283. Compounds which had been found to be inactive in the replicon assay also did not give any appreciable level of NTP formation in hepatocytes (e.g., **20b**). It should be noted that the difference in activity between the first and the second eluting diastereomers observed in the replicon assay could not be fully explained by the hepatocyte assay data. However, the interspecies variability observed in the hepatocytes would be in agreement with an enzymatic activation. Taking into consideration the replicon and hepatocyte data we identified **10,** 11, 12 and **16** as the most promising compounds.

Though **11** and **16** showed very satisfactory NTP formation in human hepatocytes, we considered the release of one equivalent of pivalic acid or tryptanol per molecule as a potential liability from a toxicity point of view,[18] which led us to prefer compounds **10** and **12**.



Scheme 1. Preparation of new amino alcohol based arylphosphoramidate diester prodrugs.[20]