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Synthesis and evaluation against hepatitis C virus of 7-deaza analogues of 2′-*C*-methyl-6-*O*-methyl guanosine nucleoside and L-Alanine ester phosphoramidates

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ABSTRACT

7-Deazapurines are known to possess broad antiviral activity, however the 2′-*C*-methylguanosine analogue displays poor cell permeation and limited phosphorylation, thus is not an efficient inhibitor of hepatitis C virus (HCV) replication. We previously reported the 6-*O*-methyl entity as a prodrug moiety to increase liphophilicity of guanine nucleosides and the ProTide approach applied to 2′-*C*-methyl-6-*O*-methylguanosine has lead to potent HCV inhibitors now in clinical trials. In this Letter, we report the synthesis and biological evaluation of 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine and ProTide derivatives. In contrast to prior studies, removal of the N-7 of the nucleobase entirely negates anti-HCV activity compared to the 2′-*C*-methyl-6-*O*-methylguanosine analogues. To understand better this significant loss of activity, enzymatic assays and molecular modeling were carried out and suggested 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine and related ProTides do not act as efficient prodrugs of the free nucleotide, in marked contrast to the case of the parent guanine analogue.

Identified in 1989,[1] Hepatitis C is an infectious disease affecting approximately 180 million people worldwide.[2] The standard treatment of care currently administered to patients consists of the general antiviral agent ribavirin combined with pegylated interferon and one of the recently discovered non-nucleoside inhibitors telaprevir or boceprevir. This treatment is not only long but has limited efficacy[3] and causes numerous side effects,[4] hence the need of more efficient therapy. Several modified purine nucleosides, such as β-2′-*C*-methyl purines and nucleobase modified purines, have exhibited significant anti-HCV activity.[5] After phosphorylation to their triphosphates β-2′-*C*-methyl modified nucleosides are direct inhibitors of HCV replication,[6] and they are known as Direct Acting Antivirals (DAAs). This includes several 7-deaza purines, particularly β-2′-*C*-methyl-7-deaza adenosine (**1**) (Fig. 1) which is reported to be a potent inhibitor of HCV replication (EC_{50} = 0.25 μM, CC_{50} >100 μM),[5,6] whereas its guanosine analogue is inactive (EC_{50} >100 μM, CC_{50} >100 μM). The loss of inhibitory activity is likely to be due to its poor cell uptake and inefficient intracellular metabolism to its 5′-triphosphate form.[6,7] This membrane permeation issue was previously addressed and modifications at the 6-position of nucleobase lead us to develop β-2′-*C*-methyl-6-*O*-methyl guanosine (**2**) (Fig. 1), being however fivefold less potent in HCV replicon than its β-2′-*C*-methyl guanosine analogue.[8] Nevertheless the ProTide technology applied to this nucleoside not only boosted the lipophilicity but also enabled by-pass of the limiting first phosphorylation step, delivering efficiently inside the host cell the corresponding 5′-monophosphate form.[9] The L-Alanine neopentyl phosphoramidate of β-2′-*C*-methyl-6-*O*-methyl guanosine (**3**) (Fig. 1) ,[8] also known as INX-08189 or BMS-986094, is one of the examples of a ProTide developed for HCV treatment.

It has previously been reported that both 2′-*C*-methyl guanosine 7-deaza and 7-aza guanosine triphosphates exhibited sub-micromolar inhibitory potencies against the polymerase (respectively IC_{50} = 0.12 μM and IC_{50} = 0.13 μM).[6] Hence, the polymerase does not discriminate between 7-aza and 7-deaza derivatives. We thus

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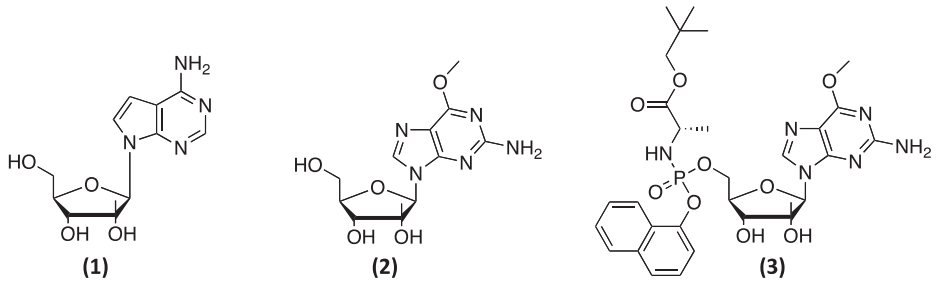

Figure 1. Structures of potent anti-HCV β-2′-*C*-methyl nucleosides (**1**–**2**) and ProTide (**3**).

decided to synthesise 7-deaza analogues of 2′-*C*-methyl-6-*O*-methyl guanosine and related ProTides in order to increase inhibitory potency against HCV.

β-2′-*C*-Methyl-6-*O*-methyl-7-deaza guanosine nucleoside (**8**) was synthesized in two stages: first the formation of the nucleobase (**4**) (Fig. 2), followed by coupling with the commercial 3,5-bis-*O*-(2,4-dichlorophenylmethyl)-2′-*C*-methyl-1-*O*-methyl-α-D-ribofuranose (**5**) (Fig. 2).

7-Deaza-6-chloroguanine (**4**) was formed in moderate yield over two steps as described in the literature.[10,11] Displacement of the 6-chlorine of **4** by sodium methoxide was undertaken under reflux for 5 days but lead mainly to degradation and generation of 6-methoxy-7-deaza guanine in very poor yield (3%). It was then decided to perform this step directly at the nucleoside level. Condensation with the ribofuranose moiety (**5**) was performed, as described in the literature,[6] by in situ conversion to the 1-bromo analogue followed by subsequent reaction with 7-deaza-6-chloroguanine potassium salt (formed in situ with potassium hydroxide powder) to afford only the β-anomer of **6** (Fig. 2). Treatment of **6** with boron trichloride in dichloromethane resulted in the deprotection of dichlorophenylmethyl group to yield 2′-*C*-methyl-6-chloro-7-deazaguanosine (**7**). The latter was then converted to the 6-*O*-methyl analogue by substitution of the chlorine with sodium methoxide in methanol under reflux,[8] affording 2′-*C*-methyl-6-*O*-methoxy-7-deaza guanosine (**8**) after column chromatography, in 50–60% yield. Without prior protection of the nucleoside **8**, three 5′-ProTides (**9**–**11**) bearing the L-Alanine as amino acid moiety were synthesized following our standard procedure[12] using *tert*-butyl magnesium chloride as a base[13] (Fig. 2). Purification by column chromatography and preparative TLC were required to obtain pure compounds. NMR and analytical HPLC confirmed the structure with purity of at least 95% for all phosphoramidates. They were each obtained in 12–13% yield as a mixture of two diastereoisomers, which were only separated in the case of the L-alanine-*O*-cyclohexyl ester ProTide (**9a** and **9b**).

The three phosphoramidates (**9**–**11**) and their parent nucleoside (**8**) were evaluated in a HCV replicon assay (EC_{50}) and for cytotoxicity in Huh-7 cells (CC_{50}) (Table 1). Table 1 compares the biological results of synthesized compounds (**9**–**11**) and their 2′-*C*-methyl-6-*O*-methyl guanosine analogues (**2**, **3**, **12**–**13**).

In vitro results (Table 1) suggest that 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine (**8**) is not an inhibitor of HCV replication whereas its corresponding ProTides (**9**–**11**) boost the antiviral activity from 3- to 10-fold. So, to some extent the ProTide approach applied to 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine is effective and the results confirm the delivery of the 5′-triphosphate inside the cells, whereas the N-7 modification of the parent nucleoside (**8**) does not show the improvement of potency desired and either lacks of cell permeation or is a poor substrate for nucleoside kinases responsible for phosphorylation. The separated diastereoisomers **9a** and **9b** exhibit similar potency in replicon assays showing little if any influence of phosphorus stereochemistry on bioactiva-

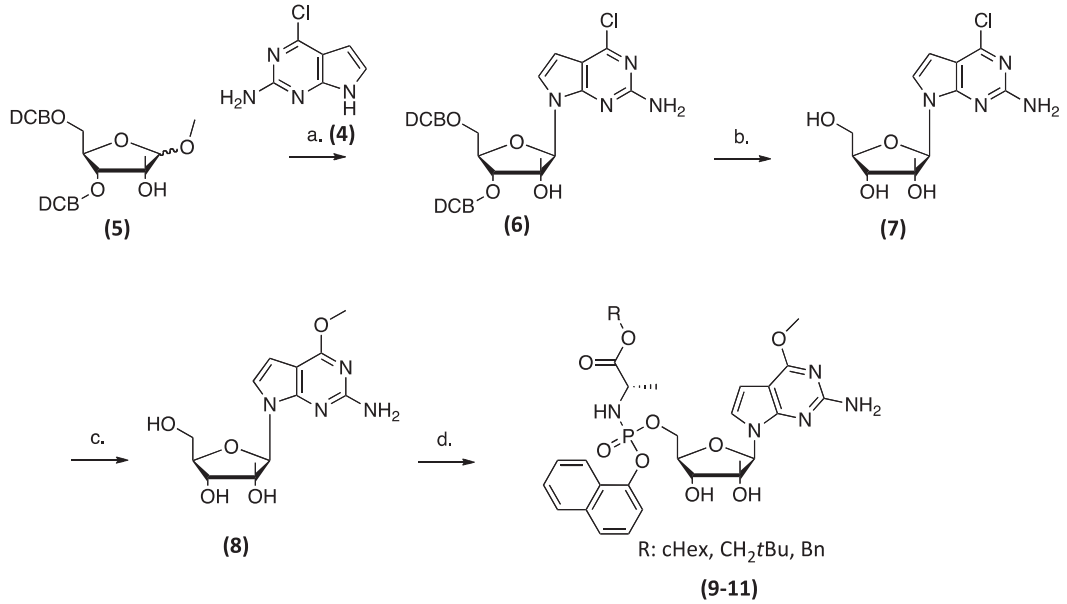

Figure 2. Synthesis of 2′-*C*-methyl-6-*O*-methyl-7-deaza guanosine (**8**) and ProTides (**9**–**11**). Reagents and conditions: (a) anhyd CH_2Cl_2, HBr (33% in acetic acid, 6.7 equiv), 0 °C to rt, 2 h; anhyd ACN, KOH (3.0 equiv), TDA-1 (0.2 equiv), rt, 1 h; (b) anhyd CH_2Cl_2, BCl_3 (10 equiv), −78 °C, 2 h then −20 °C, 2 h 30 min; (c) anhyd MeOH, NaOMe (3.0 equiv), reflux, overnight; (d) anhyd THF, *t*BuMgCl (1.2 equiv), naphthyl L-Alanine ester phosphochloridate (1.2 equiv), rt, overnight.