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The phosphoramidate ProTide approach greatly enhances the activity of β-2′-C-methylguanosine against hepatitis C virus

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ABSTRACT

β-2′-C-Methyl purines (**1**, **2**) are known inhibitors of hepatitis C virus (HCV). We herein report the synthesis, biological and enzymatic evaluation of their 5′-phosphoramidate ProTides. Described herein are seven L-alanine phosphoramidate derivatives with variations to the amino acid ester. The 1-naphthyl phosphoramidate of β-2′-methylguanosine containing the benzyl ester (**20**) was the most active at 0.12 μM, an 84-fold of increase in activity compared to the parent nucleoside (**2**) with no increase of cytotoxicity. The carboxypeptidase mediated hydrolysis of several ProTides showed a predictive correlation with their activity versus HCV in replicon.

The hepatitis C virus (HCV) was identified in 1989 as a member of the family of the Flaviviridae.[1] An estimated 180 million people are chronically infected with HCV and thus at increased risk of developing life threatening liver disease (including cirrhosis and hepatocellular carcinoma). HCV infections are the major reason for liver transplantation in industrialized countries. The current therapy for HCV (pegylated interferon and ribavirin) has limited efficacy and major side-effects.[2] Several examples of modified nucleosides have already been reported with potential anti-HCV activity.[3,4] Modified nucleosides need to be phosphorylated to their corresponding 5′-triphosphates by the host cell kinases. In many cases, however, nucleoside analogues are poor substrates for the kinases and the pharmacologically active triphosphate species cannot be considered as possible drug candidates due to their high instability and poor cellular permeation.[5] In many cases, the limiting step in this process is represented by the conversion to the corresponding 5′-monophosphate. Our group has developed in the past the aryloxy-phosphoramidate ProTide approach which allows the delivery of the monophosphorylated nucleoside analogue into the cell, bypassing the need of the first phosphorylation step.[5] We have previously reported the successful application of the ProTide approach to different nucleoside analogues[6,7,10,11] 2′-Methylpurines (adenosine and guanosine) have been shown to be potent anti-HCV agents:[3] β-2′-Methyladenosine (**1**) (Fig. 1) showed EC_{50} = 0.3 μM against HCV in replicon assay, and its corresponding 5′-triphosphate inhibited HCV RNA polymerase at 1.9 μM. β-2′-Methylguanosine (**2**), instead, showed IC_{50} = 0.13 μM (inhibition of RdRp), EC_{50} = 3.5 μM against HCV, but most importantly the detected level of its corresponding 5′-triphosphate was rather poor.[3] In the case of β-2′-methylguanosine (**2**), the low level of its intracellular 5′-triphosphate may be an indication that this nucleoside is a poor substrate for nucleoside kinases, limiting its antiviral efficacy.

We decided to apply the aryloxy-phosphoramidate approach to these two nucleoside analogues in order to explore the possibility of further increasing their activity against HCV. In the first instance, β-2′-methyladenosine (**1**) and β-2′-methylguanosine (**2**) were synthesized and evaluated for their effect on HCV (sub genomic) replicon replication.

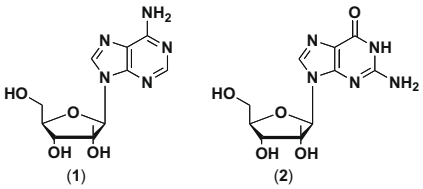

Figure 1. β-2′-Methyladenosine (**1**) and β-2′-methylguanosine (**2**).

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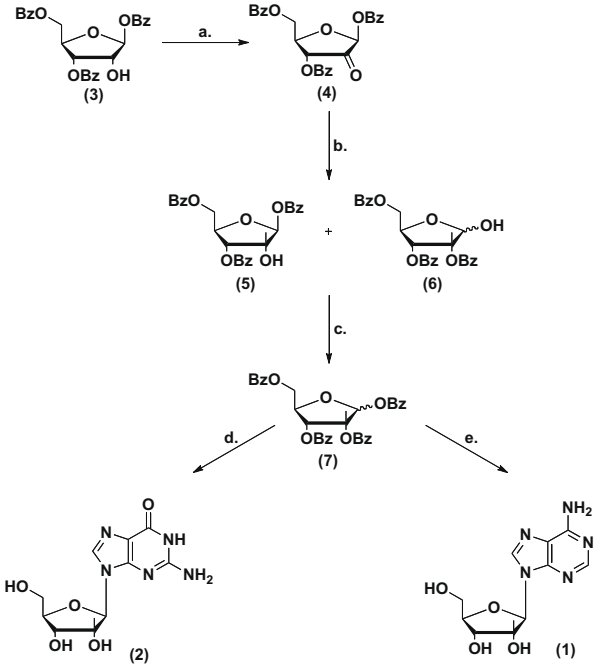

Figure 2. Synthesis of β-2′-methyladenosine (**1**) and β-2′-methylguanosine (**2**). Reagents: (a) Dess–Martin reagent, CH_2Cl_2 dry; (b) CH_3TiCl_3, Et_2O dry; (c) BzCl, DMAP, Et_3N; (d) (1) N2-acethylguanine, HMDS, TMS-triflate, *p*-xylene; (2) CH_3OH/NH_3; (e) (1) N6-pivaloyladenine, DBU, TMS-triflate, CH_3CN; (2) CH_3OH/NH_3.

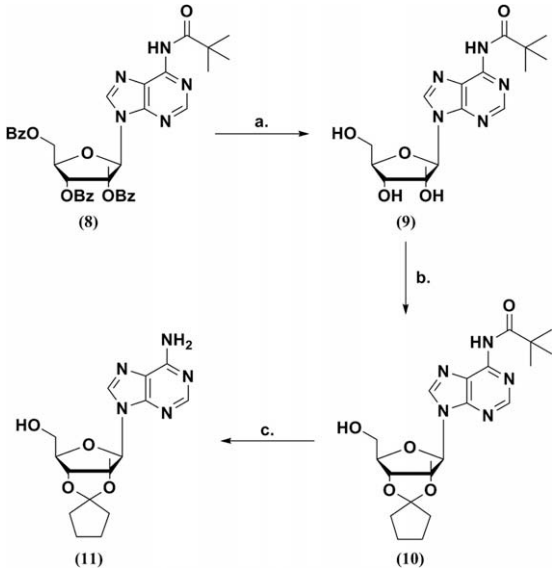

Figure 3. Synthesis of 2′,3′-protected-β-2′-methyladenosine (**11**). Reagents: (a) 1 M NaOH, ethanol, pyridine; (b) 1,1-dimethoxypentane, *p*-TSA; (c) NH_3, CH_3OH.

The synthesis of β-2′-methyladenosine (**1**) and β-2′-methylguanosine (**2**) was planned following the reported procedure (Fig. 2).[3]

The oxidation in the 2-position of the tri-benzoylated ribose (**3**) was performed in the presence of the synthesized Dess–Martin reagent.[7] The stereoselective addition of the methyl group in the β-2-position was performed by the addition of **4** to a solution of methyl titanium trichloride, synthesized in situ from an anhydrous solution of titanium tetrachloride and methylmagnesium bromide in diethyl ether, to give a mixture of **5** and **6** (Fig. 2).[8] The next benzoylation reaction was performed under standard conditions to give **7**.[3] In the case of β-2′-methyladenosine (**1**), in order to avoid a problem of selectivity in N^7- and N^9-positions, it was necessary to protect the NH_2 with the pivaloyl group. The following coupling reaction was performed in the presence of DBU and trimethylsilyl trifluoromethanesulfonate (Fig. 2). Heteronuclear multiple bond correlation (HMBC) showed the correlation between C4 and H1′, confirming the presence of the N^9-regioisomer. Nuclear Overhauser Enhancement Spectroscopy (NOESY), showed no correlation between the proton of H1′ and the three protons of the methyl group in the 2′-position confirming the presence of the β-nucleoside; instead, the correlation between H2′ and the three protons of the methyl group in the 2′-position confirmed the presence of the methyl group in the β-position. The final step was the removal of the pivaloyl group using NH_3/CH_3OH at room temperature in a sealed tube (Fig. 2). The fully benzoylated β-2′-methyl sugar (**7**) was also the substrate for the synthesis of 2′-methylguanosine (**2**). In order to avoid N^9- and N^7-regioisomers, the coupling reaction was performed in two steps: synthesis of the totally silylated acetylguanine and coupling reaction in the presence of the appropriate 2-methylribosugar (**7**) and trimethylsilyl trifluoromethanesulfonate (TMS-triflate) using *para*-xylene as solvent (Fig. 2).[9] The N^9-regioisomer was isolated without any traces of N^7-isomer. Also in this case the final step required the use of NH_3/CH_3OH to give the desired product (**2**) in quantitative yield (Fig. 2). In our previous work,[10,11] the phosphoramidate synthesis was greatly improved with the presence of a protecting group in the 2′- and 3′-position of the nucleoside. Consequently, the N^6-pivaloyl-2′,3′,5′-tribenzoate-β-2′methyladenosine (**8**) was selectively deprotected in the 2′-, 3′-, and 5′-position whilst keeping the pivaloyl group in the N^6-position, followed by introduction of the cyclopentylidene group in the 2′- and 3′-positions in the presence of 1,1-dimethoxypentane and *p*-TSA. The final deprotection of the 6-amino group was performed in the presence of NH_3/CH_3OH at room temperature in a sealed tube (Fig. 3).

The introduction of the cyclopentylidene group was attempted also in the case of β-2′-methylguanosine: this attempt was unsuccessful and consequently the use of isopropylidene as protecting group was considered. The synthesis was performed in the presence of a catalytic amount of perchloric acid in a solution of dry acetone (Fig. 4).

The synthesis of the phosphoramidate was performed following the Uchiyama procedure,[12] in the presence of *t*-butyl magnesium chloride (Figs. 5 and 6).[13]

The sugar deprotection in the case of the cyclopentylidene group (synthesis of β-2′-methyladenosine phosphoramidates, **15**–**17**) required an acidic hydrolysis using 80% formic acid at room temperature (Fig. 5).[14]

The hydrolysis of isopropylidene group (synthesis of β-2′-methyladenosine phosphoramidates, **20**–**23**), instead, was performed using acetic acid at 90 °C (Fig. 6).[15] The yields of the coupling reaction for the synthesis of β-2′-methyladenosine phosphoramidates (**15**–**17**) were 40–50%, whilst the deprotection reaction yields were 50–60%. Instead, in the case of the synthesis of β-2′-methylguanosine phosphoramidates (**20**–**23**) the yields for the coupling reaction ranged from 20–40%, while the yields for the deprotection in

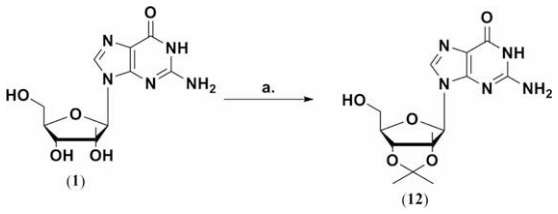

Figure 4. Synthesis of 2′,3′-protected-β-2′-methylguanosine (**12**). Reagents: $HClO_4$, acetone.