site. These procedures allowed to identify few compounds with high predicted affinity for the Hel new site. Subsequently, anti-Hel activity of the identified compounds was shown through *in vitro* enzymatic assays. More specifically, we showed unequivocally that the MM3 compound (previously known for more than 20 years for an unrelated therapeutic application) is an efficient inhibitor of flavivirus Hel. We further analyzed the effect of MM3 on Flavivirus replication in cell culture. MM3 was shown to inhibit the replication of several flaviviruses, including YFV, DENV, JEV and TBEV. Results from virus yield assays, after which viral RNA was assessed by means of quantitative RT-PCR, revealed that EC50 values for inhibition of flavivirus replication are for a number of viruses in the nM range, in particular highly potent activity was observed against the YFV (EP 09174368).

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Stereoselective Synthesis of Different Types of Nucleotide Prodrugs

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Since the discovery of cancer and antiviral diseases many efforts have been done in order to infer with their propagation. One of them uses pronucleotides as potential lipophilic nucleotide precursors to deliver phosphorylated metabolites to cells. In cases in which the phosphorus atom has four different substituents the pronucleotides are P-chiral, e.g. in *cyclo*Sal-derivatives, phosphoramidates or in the HepDirect compounds (Meier, 2006; Cahard et al., 2004; Erion et al., 2004). Since very recently, these pronucleotides could not be prepared in the form of single diastereomers due to lacking control of the stereochemistry at the phosphorus atom during synthesis. Their separation using liquid chromatography was almost impossible. However, it has been proven that the configuration at the phosphorus atom has an influence on the biological activity. For this reason the stereoselective synthesis of such compounds is of extreme relevance. Here, we present new diastereoselective syntheses of pronucleotides by using a convergent strategy and a linear strategy. Using chiral auxiliaries it was possible to prepare successfully optically active compounds with asymmetric phosphorus atoms. On these ways *cyclo*Sal-nucleotides, arylphosphoramidates and HepDirect-pronucleotides with different substitution patterns were synthesised with very high diastereomeric excesses (higher 95% *d.e.*) (Arbelo Roman et al., 2010; Rios Morales et al., 2010). In addition to this, biological activities of several compounds against HIV-1 and HIV-2 infected CEM/0 and HIV-2 infected CEM/TK$^-$ cells will be presented and discussed.

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A Comparison of the ability of wild-type and S282T mutant HCV NS5B to incorporate 2′-α-F-2′-β-*C*-methylguanosine-5′-monophosphate and 2′-α-OH-2′-β-*C*-methylguanosine-5′-monophosphate

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Several nucleoside/tide analogs targeting HCV RNA-dependent RNA polymerase (RdRp) in clinical development possess either a 2′-α-OH-2′-β-*C*-methyl or 2′-α-F-2′-β-*C*-methyl substitution in the sugar moiety. The S282T mutation confers resistance to certain nucleoside/tide analogs containing the 2′-α-*C*-methyl substitution including the guanosine monophosphate prodrugs, IDX-184 and INX-189. Both compounds are metabolized to a common triphosphate, 2′-α-OH-2′-β-*C*-methylGTP. PSI-352938 and PSI-353661 are prodrugs of 2′-α-F-2′-β-*C*-methylGMP analogs that are metabolized to 2′-α-F-2′-β-*C*-methylGTP. PSI-352938 and PSI-353661 demonstrate potent anti-HCV replicon activity against both the wild-type and the S282T mutant replicons. To better understand the difference in activity *in vitro* conveyed by the 2′-α-fluorine to the activity of PSI-352938 and PSI-353661 against the S282T mutant, biochemical assays monitoring incorporation of 2′-α-F-2′-β-*C*-methylGMP or 2′-α-OH-2′-β-*C*-methylGMP into a nascent RNA chain were performed under single turnover conditions where the enzyme concentration is in excess over the RNA substrate. This primer-dependent assay resembles the elongation step during RNA replication. The wild-type RdRp incorporated 2′-α-F-2′-β-*C*-methylGMP and 2′-α-OH-2′-β-*C*-methylGMP at similar efficiencies. Substrate specificity was calculated as a ratio of incorporation efficiency for the nucleotide analog to that for the natural GMP. The substrate specificity for 2′-α-F-2′-β-*C*-methylGTP was similar for wild type and S282T RdRp with less than a 2-fold difference. The ability of the S282T mutant enzyme to incorporate 2′-α-OH-2′-β-*C*-methylGMP was significantly different from that with the wild-type. The kinetic parameters for incorporation efficiency of 2′-α-OH-2′-β-*C*-methylGMP were not determined because the 2′-α-OH-2′-β-*C*-methylGTP was highly discriminated against by the S282T RdRp. Therefore, our *in vitro* data suggest that the mechanism of S282T resistance to 2′-β-*C*-methylguanosine nucleotide analogs involves discrimination against the 2′-α-OH-2′-β-*C*-methylGTP, but not the 2′-α-F-2′-β-*C*-methylGTP by the mutant enzyme during RNA elongation.

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