and reduced the accumulation of viral DNA by less than 10-fold at 10 μM. Combinations of CMX001 and GCV were synergistic, when concentrations of CMX001 as low as 3 picomolar were added to GCV. No significant changes in cytotoxicity were observed for any of the concentrations tested confirming that the combination was not toxic. The exceptional potency of CMX001 observed in these assays was confirmed in a quantitative real-time RT-PCR-based array that determined levels of all viral transcripts. Reductions in the levels of viral transcripts were consistent with the reductions in genome copy number and reflected the marked inhibition of viral replication in vitro relative to GCV. These results clearly indicated that combinations using suboptimal concentrations of CMX001 with GCV are synergistic in vitro. In vivo studies should be performed to further explore this combination, especially in drug resistant HCMV.

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In Vivo Efficacy of Twice Daily Oral Treatment with N-MCT Against Herpes Simplex Virus Type 2 in Balb/C Mice

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N-MCT has been previously reported to have excellent activity both in vitro and in vivo against human herpesviruses (HSV). Mice were lethally infected intranasally with herpes simplex virus (HSV), type 2, strain MS and treatments were initiated 24, 48 or 72 h post-viral infection. Compound was suspended in 0.4% carboxymethylcellulose to yield desired dosages in a 0.2 ml volume. N-MCT was administered orally twice daily at 100, 50 or 25 mg/kg and continued for 7 days. Acyclovir (ACV) was given similarly at 100 mg/kg as a positive control. No toxicity was observed in uninfected mice treated with N-MCT. All dosages of N-MCT were highly effective ($p < 0.001$) in reducing mortality when treatments were initiated 24, 48 or 72 h post-viral infection. Pathogenesis studies were performed to determine the effect on viral replication in target organs of lung, liver, spleen, kidney and sections of brain. Treatment with N-MCT significantly reduced viral replication of HSV-2 better than ACV in all organs, particularly within the CNS. In order to determine the lowest effective dose, additional studies using lower doses were performed with N-MCT from 25 to 0.01 mg/kg given twice daily beginning 24 h post-viral inoculation. All doses of N-MCT greater than 0.03 mg/kg significantly reduced mortality ($p < 0.001$). The lowest dose evaluated was 0.01 mg/kg which also reduced mortality to 40% ($p = 0.001$) and increased the mean day to death from 7.5 to 10 days ($p = 0.001$). N-MCT is a potent antiviral for herpesvirus and has potential for the treatment of serious HSV type 1 and 2 infections in humans.

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Enhanced Efficacy Using Combinations of CMX001 with Acyclovir Against Herpes Simplex Virus Infections *In Vitro* and In Mice

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Previous studies have shown that either CMX001 (HDP-cidofovir) or acyclovir (ACV) is effective in vitro against herpes simplex virus (HSV) isolates and in preventing mortality of mice infected intranasally with HSV-1or 2. Evaluation of efficacy using suboptimal doses of these two agents in combination has not been previously reported. In cell culture, CMX001 was evaluated against a panel of both wild-type and ACV-resistant isolates of HSV-1 and HSV-2 and found to be highly effective with EC_{50} values ranging from 0.008 to 0.03 μM. These same virus isolates were also inhibited by concentrations of ACV ranging from 2.0 to >100 μM. Using various concentrations of CMX001 and ACV in combination in tissue culture, we demonstrated synergistic efficacy without an increase in toxicity in cell culture experiments. To determine if this combination would result in enhanced efficacy in an animal model, CMX001 was given once daily at 1.25, 0.42 or 0.125 mg/kg with or without ACV to mice infected intranasally with HSV-2. ACV was given twice daily at 30, 10 or 3 mg/kg. Treatments were initiated 72 h post-viral infection by oral gavage for 7 days. As expected from previous work where 5 mg/kg was an optimal dose of CMX001 in this model, CMX001 as a single therapy at 1.25, 0.42 or 0.125 mg/kg did not significantly improve survival or increase the mean day to death (MDD). ACV alone improved survival at 30 mg/kg ($p = 0.06$) and significantly increased the mean day to death at 30 or 10 mg/kg ($p < 0.01$), but not at 3 mg/kg. Suboptimal doses of CMX001 and ACV given together significantly enhanced protection from mortality or increased the MDD when compared with either drug alone in 8 of 9 combination groups. No additive toxicity was detected. Our results indicate that low dose combinations of these two agents act synergistically in vitro and in vivo and should be considered for use in herpesvirus infections in humans.

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First Diastereoselective Synthesis of Pronucleotides

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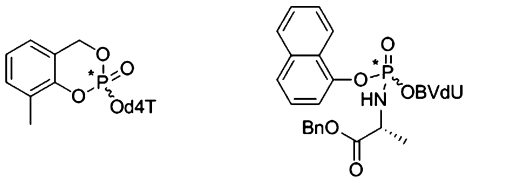
Pronucleotides represent a promising alternative to improve the biological activity of nucleoside analogs in antiviral and cancer chemotherapy. Two of the most successful prodrug-systems are the *cyclo*Sal-pronucleotides (Meier, 2002) as well as the nucleoside arylphosphoramidates (Congiatu et al., 2006). As the first approach is based on a selective chemical hydrolysis, the second technology requires an enzyme-mediated activation. Due to their synthesis pathways, so far derivatives belonging to these two classes of pronucleotides were obtained as 1:1 mixtures of diastereomers

with respect to the configuration at the phosphorus center. The mixtures of diastereomers can only be separated in limited cases and in addition to this, the diastereomers have different antiviral activity, toxicity and hydrolysis stabilities (see below) (Meier, 2002; Congiatu et al., 2006). For this reason developing strategies to synthesize isomerically pure pronucleotides is very mandatory. Here, we present new diastereoselective syntheses of pronucleotides by using a convergent strategy and a linear strategy. Both strategies are based on the use of chiral auxiliaries. We could synthesize for the first time 3-methyl-*cyclo*Sal-nucleotides as well as several nucleoside arylphosphoramidates with very high diastereoselectivities. Beside the antiviral activity, biophysical properties will be presented and discussed in dependence on the different chirality at the phosphate.



References

Meier, C., 2002. Mini Rev. Med. Chem. 2, 219–234.
Congiatu, C., et al., 2006. J. Med. Chem. 49, 452–455.

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L-β-1-(5-Bromovinyl-2-hydroxymethyl-1,3-dioxolanyl) Uracil (L-Bhdu) Prevents Varicella-Zoster Virus Replication in Fibroblasts, Skin Organ Culture, and Scid-Hu Mice with Human Skin Xenografts

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The αα-herpesvirus varicella-zoster virus (VZV) causes chickenpox and shingles. Current treatments are acyclovir, valaciclovir (VACV), famciclovir and brivudin (Europe). Vaccines are also approved that lower the incidence of primary and recurrent infections. Additional antiviral compounds with increased potency and specificity are needed to treat VZV and for strains resistant to the existing drugs. L-BHDU (MW 319.1) had anti-VZV activity in pilot studies. We evaluated L-BHDU in 3 models of VZV replication: primary human foreskin fibroblasts (HFFs), skin organ culture (SOC) and in SCID-Hu mice with skin xenografts. In HFFs, 100 μM L-BHDU was noncytotoxic over 3 days, and the antiviral effects of 2 μM treatment were reversible. Virus replication was measured by bioluminescence imaging of the VZV-BAC-Luc strain. The EC_{50} in HFFs was ~0.03 μM and in SOC was <0.1 μM. In mouse studies, L-BHDU in DMSO was administered by oral gavage once daily for 7 days, or 3 mg/mL VACV was added to drinking water, starting 2 dpi. Groups (n = 5) were given 8, 15, or 150 mg/kg/day L-BHDU, and all doses significantly reduced VZV growth compared to VACV or DMSO. VACV was not effective and the group lost >20% of body weight, suggesting mice were averse to the drug in water. L-BHDU and DMSO caused moderate weight loss, which did not correspond to dose, and mortality was 1/8 in the DMSO group and 1/5 in the 8 mg/kg/day group. Mouse organs were harvested 2 h after the last dose. C_{max} values in the human skin xenografts were 0.7 ± 0.1 and 11.3 ± 1.1 for the 8 and 150 mg/kg/day doses, respectively. Comparison of concentration ratios of tissue to plasma indicated saturation of uptake at the higher dose. To determine the phase of VZV replication blocked by L-BHDU, virus proteins were evaluated by immunoblot and all were reduced in treated HFFs. The effects of drug on VZV genome copy number will be measured by quantitative PCR. L-BHDU was effective and well tolerated in mice, therefore it has potential as a novel anti-VZV agent.

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Synthesis and Antiviral Evaluation of Alkoxyalkyl Esters of (*R*)-[2-(Phosphonomethoxy)propyl]-Nucleosides

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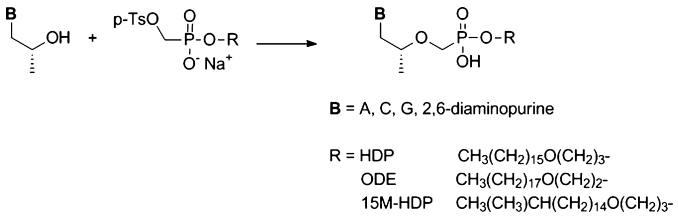
PMPA, (9-(*R*)-[2-(phosphonomethoxy)propyl]adenine, tenofovir), administered orally as its disoproxil fumarate prodrug, is a potent anti-HIV therapy used most often in combination with other antiretroviral drugs. We previously reported the synthesis of a lipophilic tenofovir prodrug, hexadecyloxypropyl 9-(*R*)-[2-(phosphonomethoxy)propyl]adenine (HDP-(*R*)-PMPA, CMX157), that was more potent than tenofovir versus in vitro replication of HIV, and was orally bioavailable in rats. Encouraged by these results, we have synthesized several more alkoxyalkyl esters in the PMP-series. We first prepared hexadecyloxypropoyl (HDP), octadecyloxyethyl (ODE) and 15-methyl-hexadecyloxypropyl (15M-HDP) esters of p-toluenesulfonyloxymethylphosphonate. These synthons reacted with hydroxypropyl derivatives of adenine, cytosine, 6-*O*-benzylguanine and 2,6-diaminopurine to afford alkoxyalkyl monoesters of (*R*)-PMPA, (*R*)-PMPC, (*R*)-PMPG and (*R*)-PMPDAP, respectively. The antiviral activity of the new compounds was evaluated in PBMCs. The octadecyloxyethyl ester of (*R*)-PMPA proved slightly more potent (EC_{50} = 1 nM) than the HDP ester. However, because the cytotoxicity of ODE-PMPA also increased, the selectivity index (CC_{50}/EC_{50}) was less than observed with the HDP ester. The most active compounds were ODE-(*R*)-PMPDAP and ODE-(*R*)-PMPG with EC_{50} values of 0.3–2 nM. The HDP and 15M-HDP-esters of (*R*)-PMPG were also highly active. ODE-(*R*)-PMPC was not active against HIV.



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