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A Heterocyclic Molecule with Significant Activity Against Dengue Virus

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The etiological agents of dengue fever (DF) and dengue hemorrhagic fever (DHF) are four dengue viruses, which are antigenically similar but immunologically distinct serotypes of the family called Flavivirus. Estimates make the number of cases of dengue fever as high as 100 million annually, which makes this arthropod-borne viral infection a serious global health problem. Dengue virus genome is a positive-stranded, 5′-capped RNA of about 11 kD. After fusion and entry, translation of genomic RNA occurs in infected cells. Viral polyprotein processing is apparently catalyzed by both viral and cellular enzymes. For example, the NS3 viral protease is essential for viral replication. The NS5 conserved protein has a methyltransferase motif in the N-terminal domain and an RNA-dependent RNA polymerase in the C-terminal domain. The transferase and polymerase are potential targets in antiviral strategies. However, there are no specific approved drugs or vaccines for the treatment or prevention of DF and DHF. This presentation describes the concise synthesis of a uracil-based multifunctional compound, which has strong activity against dengue virus. Interestingly, this heterocyclic compound also exhibits low activity against a few other RNA viruses, but is highly active against yellow fever virus, a related flavivirus. It is likely that the mechanism of action of the antiviral activity of our compound is through its inhibition of the enzyme, inosine monophosphate dehydrogenase (IMPDH). Molecular modeling studies reveal that the compound can have specific hydrogen bonding interactions with a number of amino acids in the active site of IMPDH, a stacking interaction with the bound natural substrate, IMP, and the ability to interfere with the binding of NAD^+ with IMPDH, prior to the hydration step. Details of the chemistry, enzymology and antiviral activity will be discussed.

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Synthesis and Properties of *Cyclo*Sal-phosphatetriesters of Fluorescent Bicyclic Nucleoside Analogues (BCNAS)

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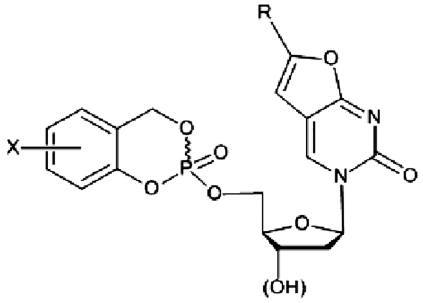
*Cyclo*Sal-pronucleotides are well known for the efficient delivery of nucleoside monophosphates of antivirally active nucleoside analogues inside cells. The *cyclo*Sal-concept is based on the use of substituted salicyl alcohols as lipophilic masking units for the negative charges of the phosphate group and therefore allowing the pronucleotide to diffuse through the cell membrane. Intracellularly, the masking unit is then cleaved by a purely chemically driven hydrolysis mechanism and the nucleoside monophosphate is released. Latest developments include "lock-in"-modifications and enzymatically activated *cyclo*Sal-pronucleotides.

For further development of the concept it is important to acquire information about the cell uptake and the intracellular fate of these pronucleotides. However, for such studies, the compounds should be labelled or should bear a probe. A convenient way for these investigations is the usage of fluorescent probes that show close structural similarity to the antivirally active nucleoside analogues. A class of compounds that may be suitable to act as probes are the bicyclic nucleoside analogues (BCNAs). Originally developed as antiviral agents by the group of *C. McGuigan*, these compounds also exhibit strong intrinsic fluorescence (McGuigan et al., 2007).

Here, we report on the synthesis and properties of the first *cyclo*Sal-phosphatetriesters of these bicyclic nucleoside analogues.



Reference

McGuigan, C., Pathirana, R.N., Migliore, M., Adak, R., Luoni, G., Jones, A.T., Díez-Torrubia, A., Camarasa, M.-J., Vélazquez, S., Henson, G., Verbeken, E., Sienaert, R., Naesens, L., Snoeck, R., Andrei, G., Balzarini, J., 2007. J. Antimicrob. Chemother. 60, 1316–1330.

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Anti-H5N1 Influenza Virus Activity of Carbocyclic Cytosine Nucleosides

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The carbocyclic analog of cytosine (carbodine) was earlier reported as a racemic mixture and has been shown to possess inhibitory activity against human influenza type A virus. Previously, at this meeting in 2008, we have reported enantiomeric D-(−)-carbodine as a potent antiviral agent against various strains of H5N1 influenza virus. This interesting biological result prompts us to synthesize various analogs of carbodine to study the structure-activity relationships against various strains of avian influenza virus (H5N1). Among the synthesized analogs, carbodine and the 5-fluoro-derivative showed potent activity against influenza A/Duck/MN/1525/81 (H5N1), influenza A/Hongkong/213/03 (H5N1) as well as other strains (H1N1, H3N2 and influenza B virus). The 5-fluoro derivative was active in the range of 0.3–2.0 μg/ml against multiple viruses in cytopathic effect inhibition assays. Its potent activity was confirmed by virus yield reduction. No cytotoxicity was evident at 100 μg/ml. Substitution by other groups like Cl, Br, I, vinyl and ethynyl results to the complete loss of activity.

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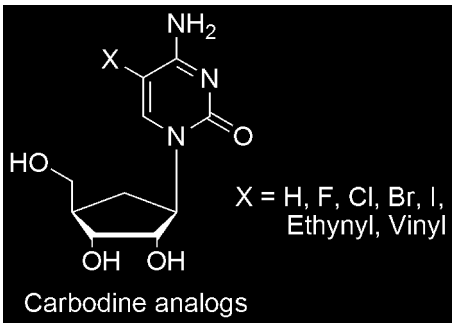

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Synthesis of CADA Analog Prodrugs Designed as Novel Down-modulators of the CD4 Receptor

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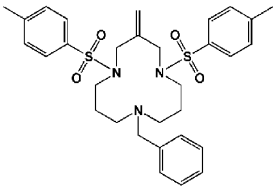
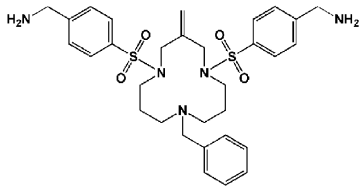
Cyclotriazadisulfonamide (CADA) inhibits HIV replication by specifically down-modulating expression of the of the CD4 receptor protein on host cells. Many analogs of CADA have been synthesized in order to enhance potency, reduce toxicity, and improve physical properties, especially solubility and cell permeability (Bell et al., 2006). These analogs have also been used to develop a three-dimensional, quantitative structure–activity relationship (3D-QSAR) computer model. Current studies are aimed at developing a pro-drug approach involving novel CADA analog ES02. This compound is expected to have a CD4 down-modulation potency that is similar to that of CADA, according to our 3D-QSAR model. ES02 is the parent compound for prodrugs bearing dipeptide chains that are covalently bonded to the two amino groups of the aminomethylbenzenesulfonyl side arms. Cleavage of these chains by dipeptidyl-peptidase IV (Garcia-Aparicio et al., 2006) is expected to convert the prodrugs into ES02. The synthesis of ES02 involves a new macrocyclization method using palladium as a catalyst. This technique avoids large solvent volumes, long reaction times, and polymer side products associated with the conventional, Richman–Atkins macrocyclization method. The anti-HIV and CD4 down-modulation activities of the novel CADA compounds will be presented.





Reference

Bell, et al., 2006. J. Med. Chem. 49, 1291–1312.
Garcia-Aparicio, et al., 2006. J. Med. Chem. 49, 5339–5351.

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Bioreversible Protection of Nucleosidediphosphates—Synthesis and Properties

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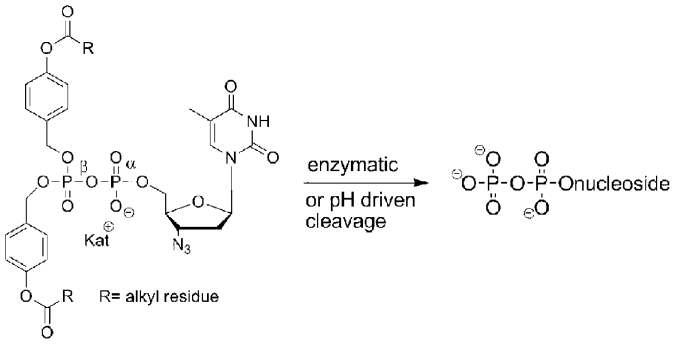
Nucleoside analogs are widely applied in antiviral and antitumor therapy. A severe limitation of these compounds arises from the need of biotransformation to the eventually active nucleoside triphosphates (NTP) by stepwise addition of phosphate groups by kinases which often proceeds insufficient. Prodrugs (e.g. the *cyclo*Sal- or phosphoamidate-approach) can possibly enhance the antiviral or antitumor activity of nucleotide analogs, enabling the nucleotide analogs to penetrate cellular membranes and protecting them from degradation by unspecific plasma phosphatases. They bypass the limited bioactivation of nucleoside kinases, hence rising the intracellular level of nucleoside monophosphates. However the subject of nucleosidediphosphate prodrugs has been addressed very rarely and unsuccessful.

This is remarkable, considering that, e.g. 3′-azido-3′-deoxythymidine (AZT) is only very slowly diphosphorylated by thymidylate kinase resulting in the loss of antiviral activity and unwanted side effects. For these reasons we turned our interest on the bioreversible protection of nucleoside diphosphates. This way of protection might also be very desirable for other nucleotide analogs.

We will present the synthesis and biological properties (pH- and cell extract stability, cytotoxicity, antiviral activity) of newly designed bis-(4-acyloxybenzyl)nucleoside-diphosphates which should act as nucleoside diphosphate prodrugs. Once insight the cell, the pyrophosphate protecting groups should be cleaved by enzymes resulting in a spontaneous release of the nucleotide.



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Multivalent Synthetic Lectin Polymers Against HIV

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While Phase III trials of microbicides proceed there still remain critical gaps in the therapeutic pipeline. Specifically there is a lack of affordable, and broadly efficacious entry inhibitors that target the HIV ENV complex, and are safe for repeated topical delivery. Cyanovirin-N, one of the most potent anti-HIV agents known, inac-