Synthesis of 4'/5'-Spirocyclopropanated Uridine and D-Xylouridine Derivatives and their Activity against the Human Respiratory Syncytial Virus

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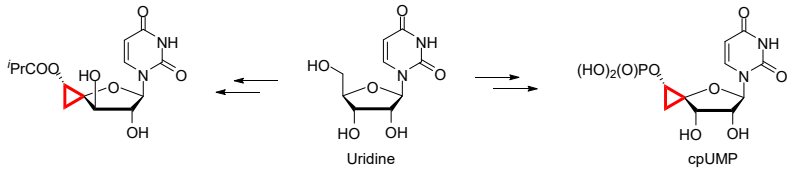
Supporting Information Placeholder



ABSTRACT: The Simmons-Smith-Furukawa reaction was used to generate 4'/5'-spirocyclopropanated uridine analogs from electron-rich exocyclic enol esters. During synthesis the native hydroxylation pattern of the nucleoside is preserved and offers the possibility for a late stage 5'-phosphorylation at the spirocyclopropanol moiety. All synthesized spirocyclopropanated uridine derivatives, including the corresponding 5'-monophosphate (cpUMP), were evaluated with respect to their antiviral activity in an HRSV assay showing modest, but promising activity.

The synthesis of nucleoside derivatives for medical therapy has been of major interest for several decades;[1,2] nucleosides have become a striking and powerful group of therapeutic agents, especially in case of viral diseases and anti-cancer therapy. While antiviral agents against certain viruses such as HIV-1, Hepatitis C virus, CMV or Influenza virus are available, treatment options for human respiratory syncytial virus (HRSV) are still limited.[3]

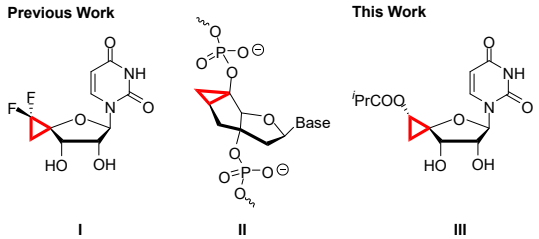
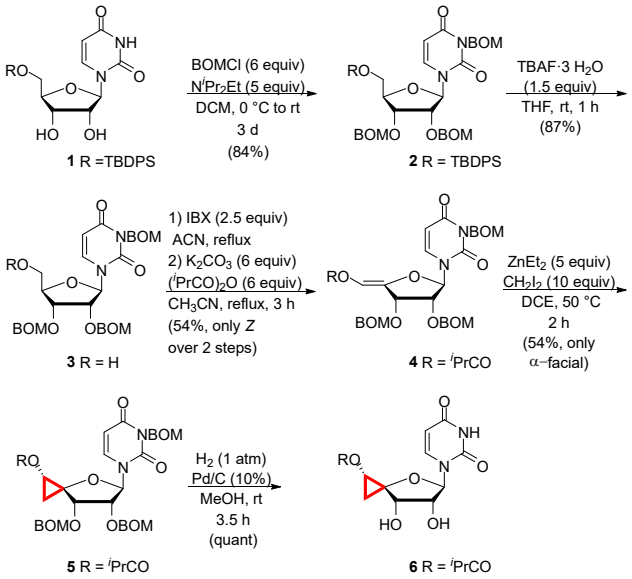


Figure 1. Conformational restricted cyclopropanated nucleoside derivatives.

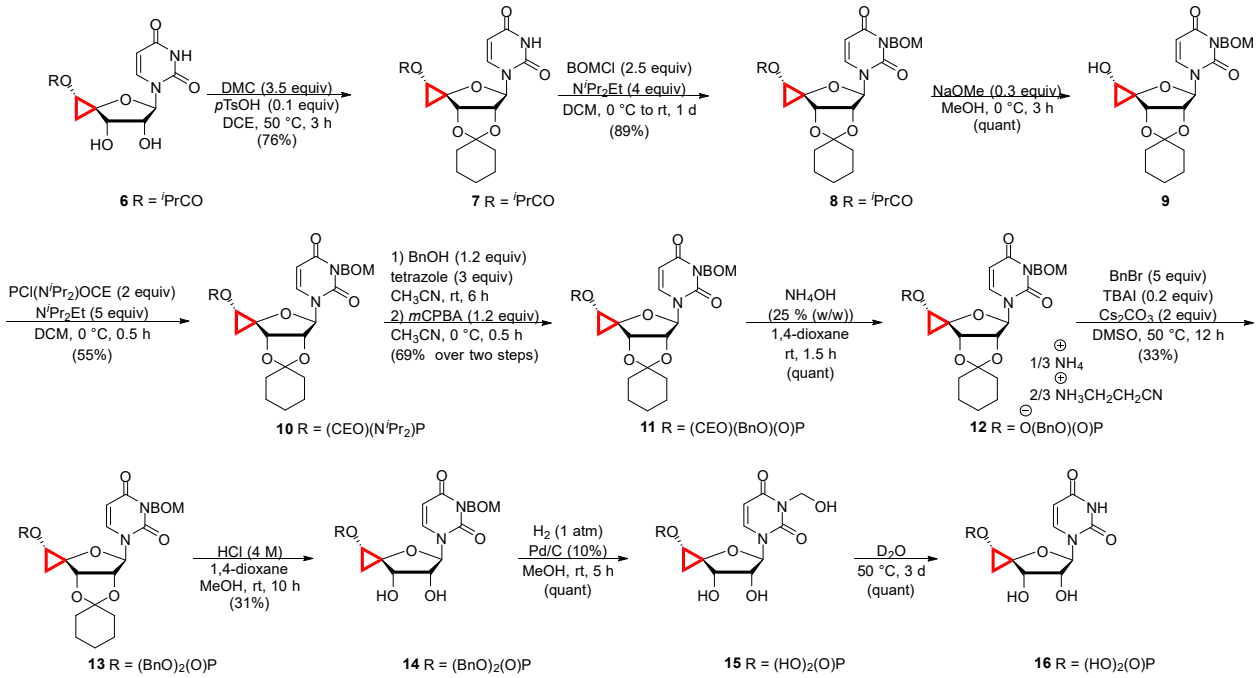
HRSV is a globally distributed negative strand RNA virus of the family *Pneumoviridae* that causes a severe disease burden among infants, the elderly and in immunocompromised patients.[4–6] Apart from a prophylactic monoclonal antibody that is

Scheme 1. Synthesis of Spirocyclopropanated Uridine Derivative 6.

given to children at high risk of severe infection, only Ribavirin, a guanosine analog with broad antiviral activity, is approved for



Scheme 2. Synthesis of Spirocyclopropanated Uridine Monophosphate (cpUMP) 16.



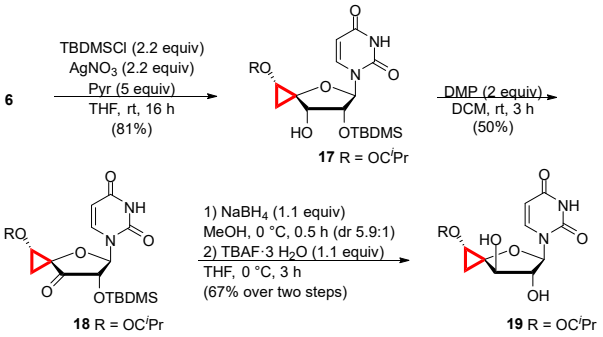
treatment of RSV-infected patients.[7] Nevertheless, side effects of established therapeutics and the evolution of viral drug resistance have revealed a lack of appropriate new drug classes. During the last few decades the cyclopropyl motif has shown astonishing biological effects in drug development and was finally introduced as a spirocyclopropanation motif of the D-ribose scaffold by Mulard et al.[8–11] Since then, spirocyclopropanations have been realized at carbons 2', 3' and 4' by the addition of difluoromethylene (**I**) or methylene units, generated from metal reagents or diazomethane (Figure 1).[8,12–14] Although 4'/5'-methylene spirocyclopropanated uridine derivatives have been synthesized by the groups of Boyer and Robins, one aspect of the native substitution pattern of the nucleoside, namely the 5'-hydroxyl group at the spirocyclopropane, has been neglected.[8,15] In fact, conservation of the 5'-hydroxy motif for polycyclic (**II**) or spirocyclopropanated systems (**III**) proves challenging, as literature-known spirocyclopropanation protocols for nucleosides require electronically neutral, exocyclic, primary alkenes. Starting materials containing electron-rich alkenes are associated with low yields under reported reaction conditions.[16] Recently, our lab successfully introduced the Simmons-Smith-Furukawa method for the spirocyclopropanation of electron-rich enol ester systems of various monosaccharides for the purpose of reducing conformational freedom along the C5/C6-bond or achieving 5-*C*-methylation.[17–19] Further, we have shown that the stability of a free spirocyclopropanol motif depends on the carbohydrate scaffold. As isobutyric esters are widely used in prodrug strategies, releasing the final drug *in vivo* after enzyme-catalyzed ester cleavage, this protecting group is suitable for preserving the spirocyclopropanol motif.[20] Nevertheless, the activation of nucleosides in biological systems is realized by enzyme-promoted phosphorylation giving 5'-phosphorylated nucleotides. Therefore, we envisioned a late stage phosphorylation protocol to generate 4'/5'-spirocyclopropanated uridine monophosphate (cpUMP) from a cyclopropanated precursor.

In this work, we present a synthetic approach to 4'/5'-spirocyclopropanated uridine derivatives bearing a 5'-oxy substitution pattern. The secondary alcohol at the spirocyclopropane motif was masked by a carboxylic ester or a monophosphate. Finally, we investigated the potential application of compounds **6**, **16**, **19**, **20** and **21** as antiviral agents against infection with HRSV.

As starting material we used the O5-silylated nucleoside **1**, which was prepared from uridine according to the reported procedure by van Otterlo et al.[21] Treatment with benzyloxymethyl chloride (BOMCl) afforded the fully protected nucleoside **2** (Scheme 1). BOM was chosen to enable simultaneous deprotection of the nucleobase and the 2,3-*syn*-diol motif by hydrogenolysis with palladium on charcoal in a single step. Use of this hydrogenolytically cleavable ether proved to be superior to other common protecting strategies lacking orthogonality towards carboxylic esters or the spirocyclopropane. Removal of the O5-silyl group by TBAF afforded compound **3** in 87% yield. After oxidation of the primary alcohol by IBX, the sensitive aldehyde was directly subjected to further reaction without purification. Enolisation by potassium carbonate and subsequent esterification led to compound **4** (54%). Although high diastereoselectivity was reported for the corresponding literature-known enol acetates, only the (*Z*)-configured diastereomer was isolated with the isobutyrates.[22,23] Cyclopropanation of the exocyclic enol ester was carried out by application of the Simmons-Smith-Furukawa protocol, providing compound **5** exclusively as the *α*-facial cyclopropanated diastereomer in 54% yield.[24,25] The striking diastereoselectivity is assumed to be controlled by the ether-protected O2 and O3, directing the zinc carbenoid during cyclopropanation. Following global deprotection by hydrogenolysis, using palladium on charcoal, secured the spirocyclopropanated uridine derivative **6** (Figure 2). The configuration of the isobutyric ester at the spirocyclopropane motif and the previous *α*-facial diastereoselective cyclopropanation were confirmed by NOE and single-crystal X-ray analysis (Figure 2 and Supporting Information). Determined by X-ray analysis, the D-ribose scaffold showed a $_3$E pucker, with carbon 3' lying 0.57 Å out of the ring plane (5'-O-1'-2'). The χ-value (-140.40(17)°) of the nucleoside derivative corresponds to the common *anti*-conformation. In comparison to free uridine (1.507(3) Å), no significant effect of the spirocyclopropane on the 4'-5' bond length was observed (1.502(3) Å).

The isobutyric ester could not be replaced by a phosphoric ester without reprotection of both N5 of the nucleobase and the *syn*-diol motif at the furanose. Therefore, we used an acid-catalyzed spiroketal formation with 1,1-dimethoxycyclohexane (DMC) leading to compound **7** in 76% yield. The following reprotection of N5 with BOMCl proceeded easily, giving compound **8** (89%). The isobutyric ester was finally cleaved by alkaline reaction conditions using sodium methoxide in methanol at 0 °C; ester cleavage at room temperature promoted decomposition of the secondary alcohol at the spirocyclopropane and hampered formation of product **9**. Addition of phosphorus (V) species such as phosphoryl chlorides afforded only traces of the desired phosphorylated uridine derivative. However, phosphorus (III) species neatly converted the secondary alcohol to the phosphoramidite **10**, after which the diisopropylamino group was substituted by benzylic alcohol in a tetrazole-mediated reaction. The emerging phosphite was subsequently oxidized to the diastereomeric mixture of the corresponding phosphate **11** by the addition of *m*CPBA at 0 °C (69%). An aqueous solution of ammonium hydroxide was used for selective cleavage of the cyanoethyl protecting group in quantitative yield.[25] The resulting phosphate ester was obtained as a mixed salt with ammonium and ammonium propionitrile cations. Column purification using triethylamine as an additive in the eluant led to the respective triethylammonium salt but still remained difficult (**12a**, see the Supporting Information). The following steps were performed without further purification as this proved to be needless. Benzylation of compound **12** was applied for reasons of purification, as partially unprotected phosphates prevented proper aqueous work-up after ketal cleavage. Treatment with hydrogen chloride in dioxane delivered the deprotected 1,2-*syn* diol motif of the spirocyclopropanated uridine monophosphate. In contrast to compound **6**, only the partially deprotected nucleotide **15** was obtained after palladium-mediated hydrogenolysis of **14**, leaving a hemiaminal at N5. Dissolving the nucleotide in D_2O at 50 °C furnished complete hydrolysis of the hemiaminal under formation of the desired cpUMP nucleotide **16** (Scheme 2).[27] The process of temperature-promoted hydrolysis, giving rise to an increasing signal set of cpUMP, was monitored by ^{1}H NMR spectroscopy (see Supporting Information).

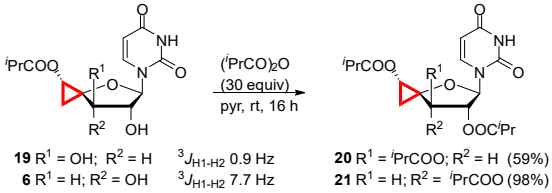
Scheme 3. Synthesis of Spirocyclopropanated D-Xylouridine Scaffold 19.



D-Xylose scaffolds are valuable synthetic precursors for the preparation of further 3'-substituted derivatives.[28] Structural elucidation experiments by NMR revealed D-xylose nucleosides to adopt strictly a 3'-*endo* conformation.[29] In order to investigate the conformational impact of 4'/5'-spirocyclopropanations on the ring flexibility of uridine, we submitted **6**, showing a $_3$E-conformation, to a four-step 3'-epimerisation. Thereby, **17** was prepared by a modified procedure of an analogous synthesis by Kreutz.[30] Epimerisation of carbon 3' was performed by prior oxidation of the hydroxy group using Dess–Martin periodinane (50%).[31] The 3-oxonucleoside **18** was diastereoselectively reduced by $NaBH_4$ delivering the desired D-xylose nucleoside as the major diastereomer.[32,33] Successive desilylation by application of TBAF at lower temperature afforded **19** in 67% yield over two steps (Scheme 3).

Interestingly, NMR investigations showed that 4'/5'-spirocyclopropanation effectively guided the uridine structure from the native 3'-*endo* conformation to a $_3$E pucker, as observed in X-ray analysis of compound **6**.[34] Nevertheless, this effect does not survive the 3'-epimerization. Changing the molecular configuration at 3' causes a retransformation to a 3'-*endo* pucker, as indicated by the strongly reduced coupling constant between H1 and H2 (Scheme 4).[35] Converting **6** to the respective 5'-phosphate **16** led only to a minor effect on the ring conformation ($^3J_{H1-H2}$ = 6.9 Hz). For a more detailed and comparative view on the torsion angles of compound **6** and other conformational restricted ribose and 2'-deoxyribose nucleosides in DNA, see the Supporting Information.[36,37] Compared to the 2'-deoxyribose backbone of DNA, the *δ*-value of compound **6** (137.6 °) fits the region of the DNA B-form given by the scatterplot of Dickerson et al.[38]

Scheme 4. Preparation of Esterificated D-Xylo Nucleoside 20 and D-Ribo Nucleoside 21.



According to the established prodrug strategy we sought to increase membrane solubility and permeability of our nucleoside

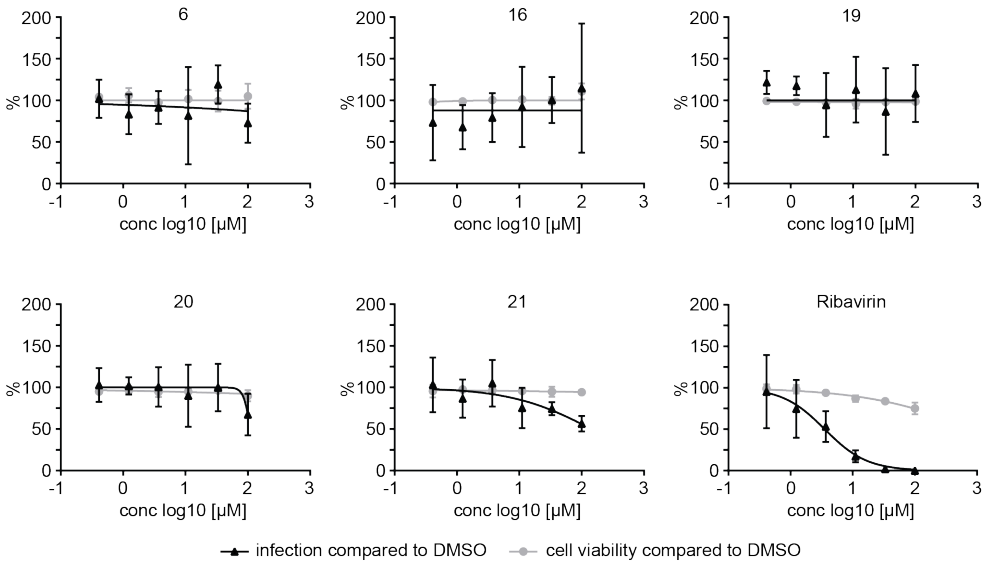


Figure 3. Antiviral activity and cell toxicity of compounds **6**, **16**, **19**, **20** and **21** compared to the antiviral guanosine analog ribavirin. Mean and standard deviations of three to four independent experiments are shown.

derivatives by decreasing the hydrophilicity. For this purpose, both spirocyclopropanated compounds **19** and **6** were esterificated twice by application of isobutyric anhydride in pyridine. Compounds **20** and **21** were obtained in 59% and 98% yield, respectively.

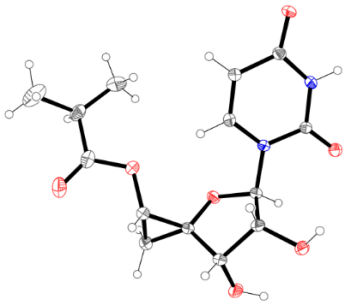


Figure 2. X-ray crystal structure of compound **6** (50% ellipsoid probability).

To determine the antiviral activity of the spirocyclopropanated uridine nucleosides, their inhibitory effect was exemplarily tested against the negative strand RNA virus HRSV. Therefore, HEp2 cells were infected with HRSV[39] in the presence of various compound concentrations. Ribavirin, the only nucleoside analogue licensed as RSV targeting antiviral therapeutic, was used as assay control. In the tested dose range, compounds **6**, **16** and **19** did not show any antiviral effect (Figure 3). In contrast, compounds **20** and **21** inhibited viral infection when used at concentrations of 100 µM or 33 µM, respectively. Although the antiviral effect was modest, it occurred independently of any overt cytotoxic effect, suggesting that the observed effect was attributable to direct inhibition of HRSV infection rather than to indirect effects caused by compromised cell viability. Moreover, nucleoside **21** was more effective than compound **20**, providing the first hints for optimizing the anti-HRSV activity of these spirocyclopropanated uridine nucleosides.

Antiviral activity of nucleoside analogs depends on several factors. The compounds have to permeate the cell membrane[40] and be phosphorylated by the host cell machinery[41,42] in order to be incorporated into the nascent viral RNA, thus causing chain termination or mutations by base mispairing in genome replication.[43] In our case, extracellular phosphorylation of compound **6** did not render compound **16** antiviral. However, compounds **20** and **21**, which are more lipophilic than their derivatives, compound **19** and **6**, possess an antiviral activity at high doses. Further experiments are needed to conclude whether this is because of improved cell membrane penetration of these molecules. Moreover, it will be interesting to see whether spirocyclopropanated uridine nucleosides have broad spectrum antiviral activity or are HRSV specific inhibitors.

In conclusion, we have demonstrated an access to 4'/5'-spirocyclopropanated uridine derivatives while preserving the native hydroxylation pattern of the nucleoside. Further, we have generated the first spirocyclopropanated nucleotide of uridine applying the Simmons-Smith-Furukawa protocol as the key step. NMR analysis of a spirocyclopropanated D-xylose scaffold showed a minor impact of the spirocyclopropane on the ring conformation in comparison to an axially oriented 3'-hydroxy group. For two uridine derivatives, we observed moderate antiviral activity in an HRSV inhibition assay. For a transfer of our developed methodology to cytosine, guanine and adenine nucleosides we expect a proper protecting strategy for the nucleobase to be necessary.

ASSOCIATED CONTENT

Supporting Information Detailed experimental procedures, analytical data for all new compounds, and crystal data (CIF) for **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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