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D-Ribose has been converted into 3 novel carbocyclic nucleosides bearing a bicyclo[4.3.0]nonane framework in 16-19 steps with 5-12% overall yields involving a Wittig olefination and an intramolecular Diels-Alder reaction as the key steps. The present synthesis also provides an efficient entry for chiral hydrindenones. The conformation studies of these carbanucleosides and their bio-evaluation as potential antiviral agents are reported.

Introduction

In recent years, carbocyclic nucleosides or carbanucleosides have warranted tremendous attention from both synthetic and medicinal chemists.1-2 The replacement of the oxygen atom in the furanose ring by a methylene unit provides both enzymic and chemical stability, attributable to the loss of a true glycosidic linkage.3 However, there is a significant change in the conformation due to the loss of the gauche and anomeric effects which exist in conventional nucleosides. These effects maintain the sugar moiety of the nucleoside in either a C3' -endo (North) or a C2' -endo (South) conformation.4 In the absence of these effects, the conformation of carbocyclic nucleosides is mainly governed by the steric bulk of the nucleobase, which prefers to occupy the equatorial position and adopts an atypical C1' -exo conformation. The conformation of nucleosides is intriguing since any structural−activity relationship (SAR) study of antiretroviral nucleosides is complicated by the complexity of the anabolic process of activation. It involves three enzymatic steps to transform the nucleoside into its 5'-triphosphate (NTP), plus the final interaction of the NTP with the target enzyme, the reverse transcriptase (RT). Therefore, the preferences of conformation exhibited by the nucleoside, or its nucleotides, must be identified at each intervening enzymatic step. The only step which is invariant and common to all nucleoside RT inhibitors, is the final interaction of NTP with RT. The route to NTP anabolite, however, involves different kinases, all of which are highly dependent on the nature of the heterocyclic base.5

Aristeromycin is a representative and its conformation is illustrated in Figure 1.6 It has been reported to be potent inhibitors of S-adenosyl-L-homocysteine (AdoHcy) hydrolase, which plays an important regulatory role in S-adenosyl-L-methionine (AdoMet)-dependent methylation process.7 The methylation process is important as most plant and animal viruses require a methylated cap structure at the 5'-terminus of their mRNA for viral replication. However, their cytotoxicity precludes clinical use.8-10

![Figure 1. Naturally occurring carbocyclic nucleoside Aristeromycin.](image)

There are not many accounts on the syntheses of bicyclic carbanucleosides. In 1993, Rodriguez et al. pioneered the construction of dideoxynucleoside analogues with a bicyclo[3.1.0]hexane backbone (Figure 2) which constrains the conformation of the analogue back to the C2'-exo (North).11 From then on, different kinds of conformationally restricted carbocyclic nucleosides that lock the conformation as either North or South were reported.12

Coxsackie B viruses13 are single strand positive-sense RNA viruses and contain six serotypes of pathogenic enteroviruses that trigger illness ranging from gastrointestinal distress to full-
fledged pericarditis and myocarditis. They resist a wide variety of chemical treatment and to the best of our knowledge, there is still no well-accepted treatment for the Coxsackie B viruses and it is interesting to see whether our compounds could be a potential drug to treat the viruses.\textsuperscript{14}

However, up to date, only carbobicyclic nucleosides with a bicyclo[3.1.0]hexane and a bicyclo[3.3.0]octane system were described. There is still no publication on the syntheses of carbocoumarines with a bicyclo[4.3.0]nonane system. Therefore, it is fascinating to see if they exhibit any antiviral activity. Furthermore, according to our theoretical calculation, carbocoumarines with a cyclohexane ring cis-fused to a cyclopentane ring should exist in either a C1'-exo or a C3'-endo conformation, which is in close agreement to the North conformer of conventional nucleosides (Figure 3) and may show RNA antiviral activity.

To synthesize this class of carbobicyclic nucleosides for biological studies, we first have to synthesize the bicyclo[4.3.0]nonane core. Functionalized hydrindenones (bicyclo[4.3.0]nonenones) are versatile intermediates in complex natural product synthesis, e.g., the CD ring in steroids. It is noteworthy that syntheses of chiral functionalized hydrindenone intermediates in complex natural product synthesis, e.g., the CD ring in steroids. It is noteworthy that syntheses of chiral functionalized hydrindenones were not an easy task via an intramolecular cycloaddition.\textsuperscript{15} To date, only a few publications reported the intramolecular Diels–Alder (IMDA) reaction on sugars and most of these papers documented the syntheses of trans-decaline systems.\textsuperscript{16} Only one report described the synthesis of a bicyclo[4.3.0]nonene, involving a lengthy synthetic route.\textsuperscript{17}

Jarosz \textit{et al.} reported the syntheses of chiral bicycle[4.3.0]non-2-enes from a sugar allyltin. However, the choice for protecting groups was limited as Lewis acid was employed in the preparation of the IMDA precursors.\textsuperscript{18} In this paper, we reported a shorter route with milder conditions for the construction of chiral hydrindenone intermediates.

\section*{Results and Discussion}

\subsection*{Theoretical Calculation}

Gaussian 09 program package\textsuperscript{19} was used to calculate the structures of the carbocoumarines with density functional theory (DFT) quantum chemical method. Equilibrium geometries of all molecules were fully optimized at the B3LYP/6-31G(d) level of theory.\textsuperscript{20} Stationary points were characterized as either minima (no imaginary frequency) or transition structures (only one imaginary frequency) by calculation of the vibrational frequencies using analytical gradient procedures at the same level. Minimum energy pathways connecting the reactants and products were confirmed by intrinsic reaction coordinate (IRC) calculation.\textsuperscript{21}

\subsection*{Synthesis}

Retrosynthesis of the target carbocoumarines (3-5) shows that it could be synthesized from cycloadduct 6, which would be prepared from d-(−)-ribose (8) via triene 7, using an IMDA reaction as the key step (Scheme 1).

Starting from d-(−)-ribose (8), the 2,3-diol in 8 was isopropylidenated to afford acetonide 9,\textsuperscript{22} which was then subjected to vinylation to give alkene 10 in good yield.\textsuperscript{23} Glycol cleavage oxidation of the vicinal diol in 10 gave lactol 11, which with allylmagnesium bromide furnished dienes 12 and 13 in a ratio of 6:1, respectively and in excellent overall yields. Aqueous indium allylation only led to decomposition of starting material. Oxidation of 12 with IBX provided a mixture of lactols 14 and 15 in a 3:2 ratio, which could not be separated by column chromatography (Scheme 2). We hoped to utilize the equilibrated open-chain form of 14 and to eliminate the homoallylic alcohol to form IMDA precursor 17 (Scheme 3). Different elimination conditions were examined, but no positive results were observed after exhaustive experimentation.
Since the ring opening-elimination approach failed, we changed our synthetic avenue to a protection and deprotection approach (Scheme 4). The allylic hydroxyl group in the diol 12 was protected as silyl ether 17 in good yield. The homoallylic hydroxyl group was then attempted to be eliminated. However, after trying different methods, silyl ether 17 only reacted with trifluoroacetic anhydride (TFAA) to give trifluoroacetate 18 which was then subjected to elimination in the presence of DBU. However, no triene 19 was detected, beyond recovery of alcohol 17 quantitatively. The use of other bases did not yield any desired 19.

We therefore revised our synthetic strategy and discovered that lactol 11 was a good candidate for Wittig Reaction\textsuperscript{4} with allyltriphenylphosphonium bromide\textsuperscript{25} to introduce the diene functionality (Scheme 5). After screening with different bases, potassium tert-butoxide (KO‘Bu) in THF gave the best yield of triene 7 in 88% with Z to E ratio in 4:1. The geometric isomers were not separable by column chromatography. In order to enhance the selectivity, Horner-Wadsworth-Emmons Olefination (HWE)\textsuperscript{26} was tried but no reaction was observed with different kind of bases such as dimsyl sodium and t-butylithium. The mixture of trienes 7 was subjected to oxidation with IBX as oxidant and furnished trienone 20 in 87% yield. Trienone 20 was highly unstable and polymerized quickly after standing in vacuum. Trienone 20 underwent IMDA reaction in toluene in the presence methylene blue in a sealed tube to give hydrindenone 6 in good yield. The structure and absolute configuration of 6 was confirmed by X-ray crystallography (Figure 4).

Initially we believed that cis to trans isomerization of the diene in 20 should precede the cycloaddition. However, theoretical calculations disagreed with this idea since the relative energy of the isomerization is surprisingly high (99 kcal/mol), which was 50 kcal/mol higher than the published value.\textsuperscript{27} Therefore, the stereoselectivity of the cycloadduct could be explained by the proposed transition state models as shown in Figure 5. For E-20, two possible transition states, TS-1 and TS-2, in the endo mode of cycloaddition, would lead to the formation of two thermodynamically favorable cis-fused cycloadducts 6 and 21. Theoretical calculations also agreed with these results. However, the steric repulsion between the isopropylidene and the newly formed 6-memberd ring in the transition state might inhibit the formation of 21. TS-3 might give 22 but unfortunately 22 was unable to detect. For Z-20, TS-5 was not favorable due to the steric repulsion between the isopropylidene and the newly formed 6-memberd ring and in TS-6, the highly twisted conformation might inhibit the formation of the trans-fused cycloadduct 22.
epoxide was attempted with LiBH₄, NaBH₄, super hydride or LiAlH₄ under various conditions, but unfortunately only alcohols 24 and 25 were isolated. Interestingly, reduction with DIBAL-H in the presence of AlMe₃ provided the desired diol 26 in moderate yield.

As the reduction was not as easy as expected and the yield was not satisfactory, an alternative approach was performed (Scheme 8). Hydrindenone 6 was firstly reduced with NaBH₄ to the corresponding alcohols 27 and 28 in an 1:4 ratio. Alcohol 27 could be oxidized back to 6 by PDC in good yield. Protection of 28 as silyl ether 29 proceeded smoothly and this was followed by epoxidation to give epoxides 30 and 31 in an 1:1 ratio. When both epoxides were subjected to reduction, only the β-epoxide 30 was susceptible to reduction, and alcohol 32 was obtained in good yield. The α-epoxide 31 was reluctant to be opened with DIBAL-H even under refluxing CH₂Cl₂ (Scheme 9).

The difficulty of ring-opening in α-epoxide 33 might be attributable to the steric hindrance of the adjacent cyclopentane ring so that the hydride was unable to approach the reacting

Since epimerization was not successful, syntheses of bicyclic carbanucleosides were carried on with the cis-fused bicyclic carbocycle 6. The hydrindenone 6 was epoxidized to give epoxide 23 in good yield (Scheme 7). The β-epoxide was the only isolated product and no α-epoxide was detected. The extremely high diastereoselectivity might be explained by the ketone directing effect proposed by Armstrong. Reduction of
site. To minimize the formation of α-epoxide, we chose to epoxidize alkene-alcohol 28, and the ratio of epoxide 34 to 24 was slightly increased to 1:2 (Scheme 10). The hydroxyl group in 24 was protected as a TBS ether to furnish silyl ether 30 again.

\[ \text{Scheme 10. Epoxidation of 28.} \]

After reduction of the epoxide, the newly formed hydroxyl group in 32 was protected as acetate 35 and as ethoxy methyl (EOM) ether 36 respectively in excellent yields (Scheme 11). Removal of the silyl protecting group in both substrates proceeded smoothly to furnish alcohols 37 and 38 in excellent yields, which were the precursors for coupling with the nucleobases.

\[ \text{Scheme 11. Synthesis of 37 and 38.} \]

With alcohol 37 and 38 in hand, direct coupling of nucleobases with the free alcohols was examined (Scheme 12). Firstly, Mitsunobu reaction 39 was used but no desired product was isolated using either PPh\(_3\) or PBU\(_3\) with DIAD in refluxing toluene. Alcohols 37 and 38 were then activated by conversion into sulfonate esters (R = Ms, Tf) which were displaced with purine bases. Unfortunately, both sulfonate esters failed to form purine 39 and 40 respectively.

\[ \text{Scheme 12. Attempted to synthesize pyrimidine 39 and 40.} \]

As the convergent route failed to give any desired nucleoside, the linear approach was adopted to synthesize the target molecules. Triflate 41 was successfully converted into azide 42 in good overall yield from alcohol 37 (Scheme 13). Azide 42 was reduced to the corresponding amine 43 by catalytic hydrogenation in excellent yield. Reaction of amine 43 with 5-amino-4,6-dichloropyrimidine furnished pyrimidine 44 in miserable yield could be explained by the low electrophilicity of 5-amino-4,6-dichloropyrimidine. In order to improve the reactivity, a stronger electrophile, 5-nitro-4,6-dichloropyrimidine was used instead. Pyrimidine 45 was then obtained in good yield, and reduction of the nitro group to the amino group was achieved by using iron powder as an reductant in AcOH (Scheme 13). Using standard procedure, pyrimidine 44 was transformed into the target carbanucleoside 3 (Scheme 14).

\[ \text{Scheme 13. Synthesis of pyrimidine 44.} \]

\[ \text{Scheme 14. Synthesis of the target adenosine analogue 3.} \]

The syntheses of thymidine analogue 4 and uridine analogue 5 were similar and the results are shown in Scheme 15. Amine 44 was reacted with 3-methoxymethylacryloyl isocyanate\(^{12}\) and 3-methoxy-2-propenoyl isocyanate\(^{31}\) to produce urea 47 and 48 in 74% and 99% respectively. Both ureas 47 and 48 were then subjected to acid hydrolysis to remove all the protecting groups as well as to facilitate the cyclization. Thymidine carbanucleoside 4 was obtained in moderate yield (73%) and uridine carbanucleoside 5 was obtained in excellent yield (91%).
Determination of conformation

With all the carbanucleosides in hand, the conformations of them were determined by both X-ray crystallography and NOE experiments. Disappointing results were obtained as all the carbanucleosides displayed the undesired C1'-exo conformation. The crystal structure of adenine was shown in Figure 6. The NOE results of both nucleosides 4 and 5 also agreed with the C1'-exo conformation (Figure 7).

Biological Studies

Antiviral activity against enterovirus, Coxsackie B3 virus, was assessed by plaque reduction assay. Briefly, African green monkey cells (Vero) were used to cultivate virus-compound mixtures at various dilutions. After 72 hours of incubation at 37°C, infected cells were fixed and the number of plaques was counted. Percentage of reduction in number of plaques in wells with compounds was calculated. Compounds suspected to have antiviral effect were further examined for cytotoxicity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

As a result, 3 compounds nucleosides 3, 4 and 5 showed screening results suggestive of having weak in-vitro antiviral activity against Coxsackie B3 virus. Adenosine 3 (concentration = 1µg/mL) showed 4.9% of inhibition and thymidine 4 (concentration = 1µg/mL) showed 5.5% of inhibition and higher concentration (concentration = 100µg/mL) led to high toxicity. Uridine 5 (concentration = 1µg/mL) gave the best result, which showed more 12.3% of inhibition and further structural modification is recommended.

Conclusion

To conclude, bicyclo[4.3.0]nonane carbanucleosides 3, 4, 5 were synthesized from D-(−)-ribose in 16 steps or 19 steps with 5%, 7% and 12% overall yield respectively, involving a Wittig alkenation and an IMDA reaction as the keys steps. The cycloaduct 6, a chiral functionalized hydrindene (bicyclo[4.3.0]nonenone) should open an avenue as a versatile intermediate in complex natural product synthesis. X-ray crystallography and NOE studies of these carbanucleosides show that they display the C1'-exo conformation. These compounds demonstrated weak in vitro antiviral activity against Coxsackie B3 virus. It is worthwhile to modify the compounds and reassessed for a wider range of viruses. Research in this direction is in progress.

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Notes and references


28 Several bases were tried such as NaH, NaOMe, LDA, KOtBu and DBU. We also tried to deprotonate the α-H with NaH and quenched by D2O. The proton signal disappeared which proved that deprotonation did exist.


