Organic & Biomolecular Chemistry

PAPER

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Cite this: *Org. Biomol. Chem.*, 2014, **12**, 9439

Conformationally locked bicyclo[4.3.0]nonane carbanucleosides: synthesis and bio-evaluation†

Tony K. M. Shing,*[a] Anthony W. H. Wong,[a] Huiyan Li,[b] Z. F. Liu[b] and Paul K. S. Chan[c]

Received 18th August 2014,
Accepted 25th September 2014

DOI: 10.1039/c4ob01763c

www.rsc.org/obc

D-Ribose was converted into 3 novel carbobicyclic 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 enzymatic 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 structure–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]

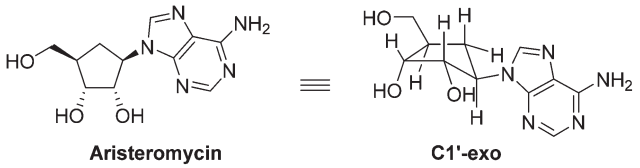


Fig. 1 Naturally occurring carbocyclic nucleoside aristeromycin.

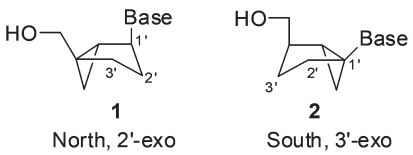


Fig. 2 Conformationally locked nucleosides with a bicyclo[3.1.0]hexane system.

Aristeromycin is a representative and its conformation is illustrated in Fig. 1.[6] It has been reported to be a potent inhibitor of *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase, which plays an important regulatory role in the *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]

There are not many accounts on the syntheses of bicyclic carbanucleosides. In 1993, Rodriguez *et al.* pioneered the construction of dideoxyribonucleoside analogues with a bicyclo [3.1.0]hexane backbone (Fig. 2) which constrains the conformation of the analogue back to the C2′-*exo* (North).[11] From

[a]*Department of Chemistry and Center of Novel Functional Molecules, The Chinese University of Hong Kong, Shatin, Hong Kong, China*
[b]*Department of Chemistry and Centre for Scientific Modeling and Computation, The Chinese University of Hong Kong, Shatin, Hong Kong, China*
[c]*Department of Microbiology, The Chinese University of Hong Kong, China*

†Electronic supplementary information (ESI) available: Experimental procedures, characterisation data, copies of NMR spectra for all compounds, detailed DFT modelling and CIF files. CCDC 727043 and 1000117. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4ob01763c

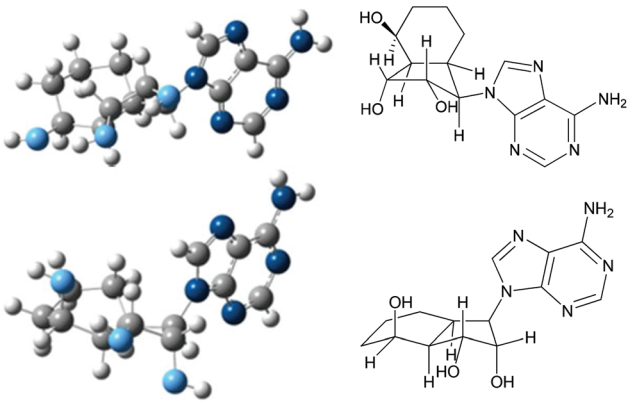


Fig. 3 Conformation of bicyclic carbanucleosides calculated using the DFT model: top: C1′-*exo* conformation; bottom: C3′-*endo* conformation.

then on, different kinds of conformationally restricted carbocyclic nucleosides that lock the conformation as either North or South were reported.[12]

Coxsackie B viruses[13] 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 potential drugs to treat the viruses.[14]

However, to date, only carbobicyclic nucleosides with a bicyclo[3.1.0]hexane and a bicyclo[3.3.0]octane system have been described. There is still no publication on the syntheses of carbanucleosides 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 calculations, carbanucleosides 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 with the North conformer of conventional nucleosides (Fig. 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 hydrindenones were not an easy task *via* an intramolecular cycloaddition.[15] To date, only a few publications have reported the intramolecular Diels–Alder (IMDA) reaction on sugars and most of these papers documented the syntheses of *trans*-decaline systems.[16] Only one report described the synthesis of a bicyclo[4.3.0]-nonenone, involving a lengthy synthetic route.[17] Jarosz *et al.* reported the syntheses of chiral bicyclo[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.[18] In this paper, we report a shorter route with milder conditions for the construction of chiral hydrindenone intermediates.

Results and discussion

Theoretical calculation

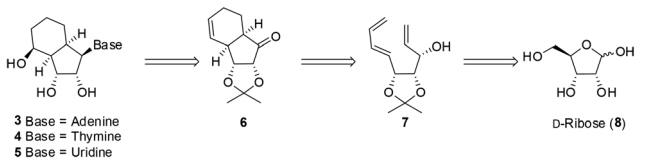
Gaussian 09 program package[19] was used to calculate the structures of the carbanucleosides with the density functional theory (DFT) quantum chemical method. Equilibrium geometries of all molecules were fully optimized at the B3LYP/6-31G (d) level of theory.[20] Stationary points were characterized 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) calculations.[21]

Synthesis

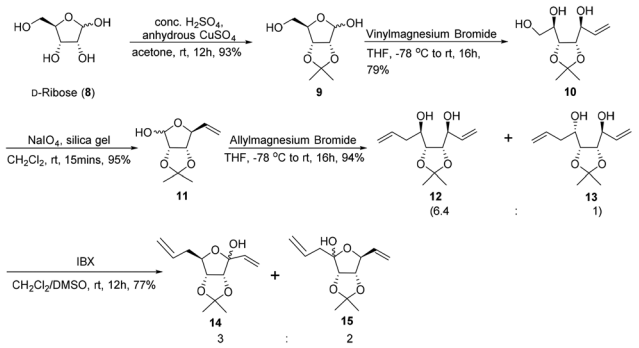
Retrosynthesis of the target carbanucleosides (**3–5**) shows that it could be synthesized from cycloadduct **6**, which would be prepared from D-(−)-ribose (**8**) *via* triene **7**, using an IMDA reaction as the key step (Scheme 1).

Starting from D-(−)-ribose (**8**), the 2,3-diol in **8** was isopropylidenated to afford acetonide **9**,[22] which was then subjected to vinylation to give alkene **10** in good yield.[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 the 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.

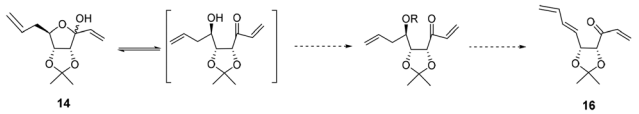
Since the ring opening–elimination approach failed, we changed our synthetic avenue to a protection and deprotection approach (Scheme 4). The allylic hydroxyl group in the diol **12** was protected as silyl ether **17** in good yield. The homoallylic hydroxyl group was then attempted to be eliminated. 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



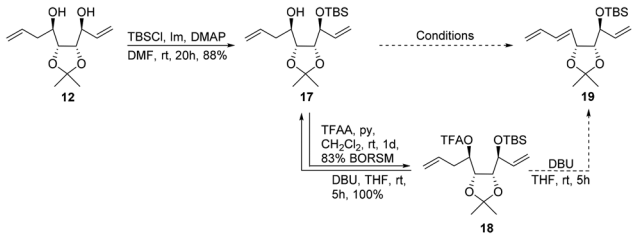
Scheme 1 Retrosynthesis of the target nucleosides.



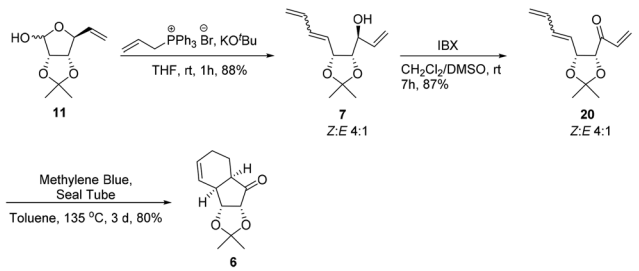
Scheme 2 Synthesis of lactol **14** and **15**.



Scheme 3 Equilibrium between the ring and chain form of **14**.



Scheme 4 Attempted synthesis of triene **19**.



Scheme 5 Synthesis of **6**.

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 the Wittig reaction[24] with allyltriphenylphosphonium bromide[25] to introduce the diene functionality (Scheme 5). After screening with different bases, potassium *tert*-butoxide (KO^{t}Bu) in THF gave the best yield of triene **7** of 88% with a *Z* to *E* ratio of 4 : 1. The geometric isomers were not separable by column chromatography. In order to enhance the selectivity, Horner–Wadsworth–

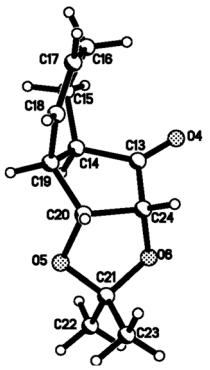


Fig. 4 X-ray structure of **6**.

Emmons (HWE) olefination[26] was tried but no reaction was observed with different kinds of bases such as dimsyl sodium and *n*-butyllithium. The mixture of trienes **7** was subjected to oxidation with IBX as an oxidant and furnished trienone **20** in 87% yield. Trienone **20** was highly unstable and polymerized quickly after standing in vacuum. Trienone **20** underwent the IMDA reaction in toluene in the presence of 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 (Fig. 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^{-1}), which was 50 kcal mol^{-1} higher than the published value.[27] Therefore, the stereoselectivity of the cycloadduct could be explained by the proposed transition state models as shown in Fig. 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-membered 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-membered ring and in **TS-6**, the highly twisted conformation might inhibit the formation of the *trans*-fused cycloadduct **22**.

Since there was no *trans*-fused cycloadduct **22** isolated in the previous step, epimerization was tried but failed to occur after many attempts and under a wide variety of conditions (Scheme 6).[28]

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.[29]

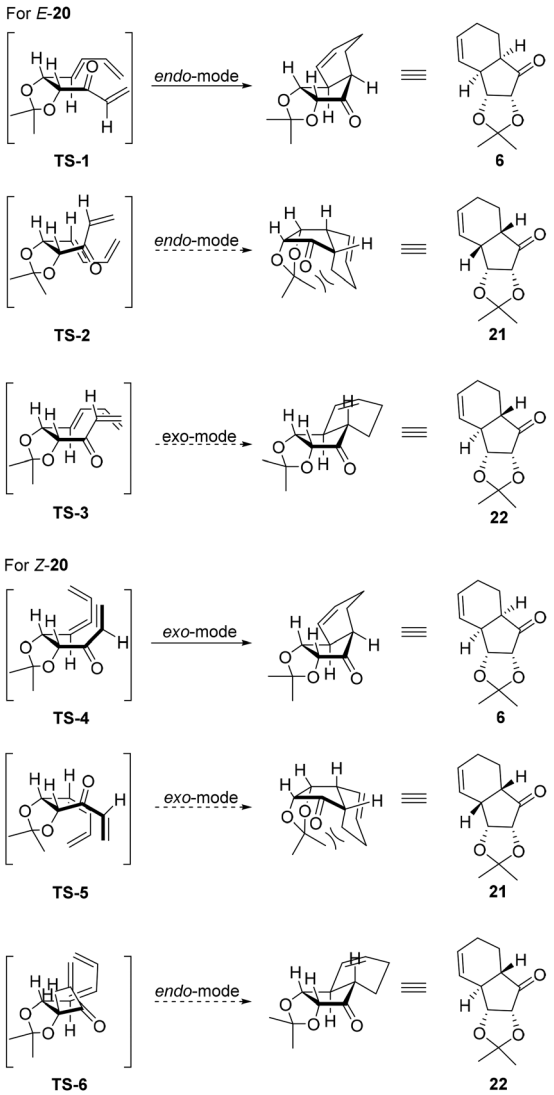
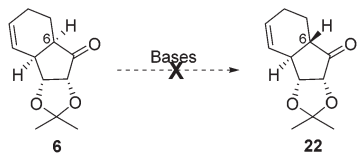
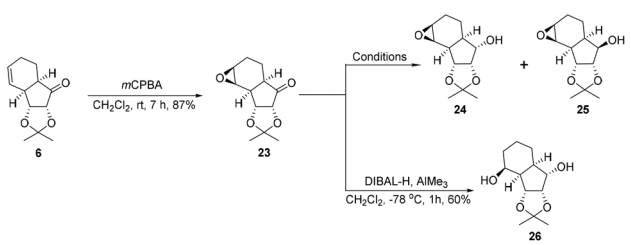


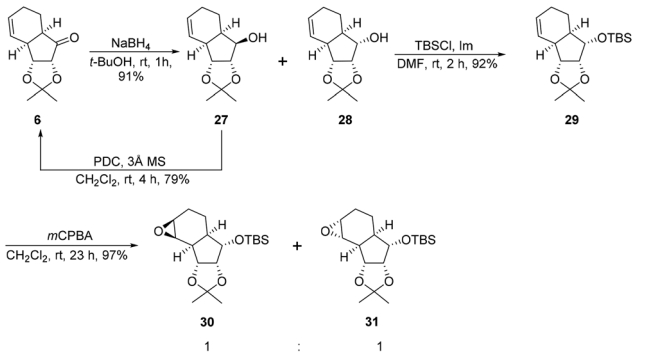
Fig. 5 Proposed transition state models of the cyclization of trienone **20**.



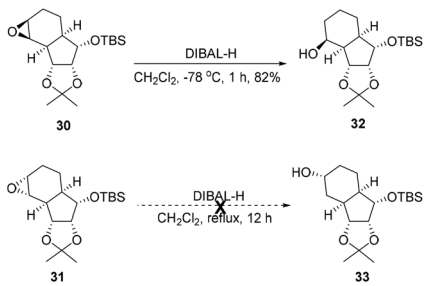
Scheme 6 Attempted epimerization of **6** to **22**.



Scheme 7 Synthesis of diol **26**.



Scheme 8 Synthesis of epoxides **30** and **31**.



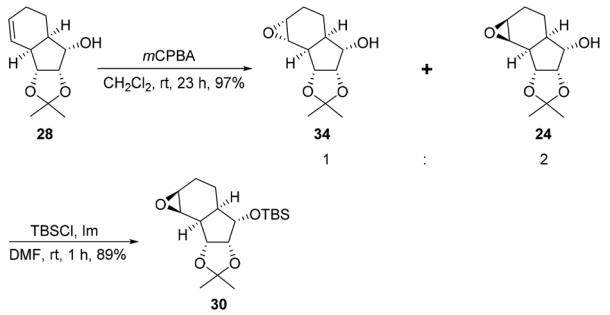
Scheme 9 Reduction of epoxides **32** and **33**.

Reduction of epoxide was attempted with $LiBH_4$, $NaBH_4$, super hydride or $LiAlH_4$ under various conditions, but unfortunately only alcohols **24** and **25** were isolated. Interestingly, reduction with DIBAL-H in the presence of $AlMe_3$ provided the desired diol **26** in moderate yield.

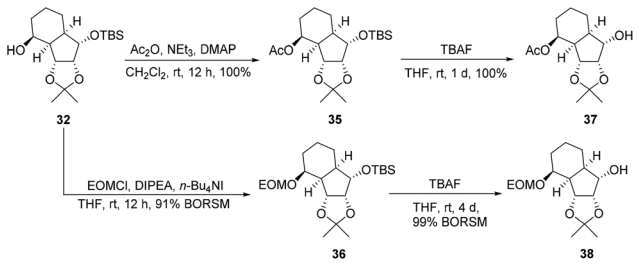
As the reduction was not as easy as expected and the yield was not satisfactory, an alternative approach was performed (Scheme 8). Hydrindenone **6** was firstly reduced with $NaBH_4$ to the corresponding alcohols **27** and **28** in a 1 : 4 ratio. Alcohol **27** could be oxidized back to **6** by PDC 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 a 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 CH_2Cl_2 (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 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 TBS ether to furnish silyl ether **30** again.

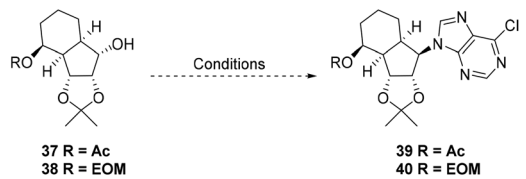
After the reduction of the epoxide, the newly formed hydroxyl group in **32** was protected as acetate **35** and as ethoxy methyl (EOM) ether **36**, respectively, in excellent yields (Scheme 11). Removal of the silyl protecting group in both



Scheme 10 Epoxidation of **28**.



Scheme 11 Synthesis of **37** and **38**.

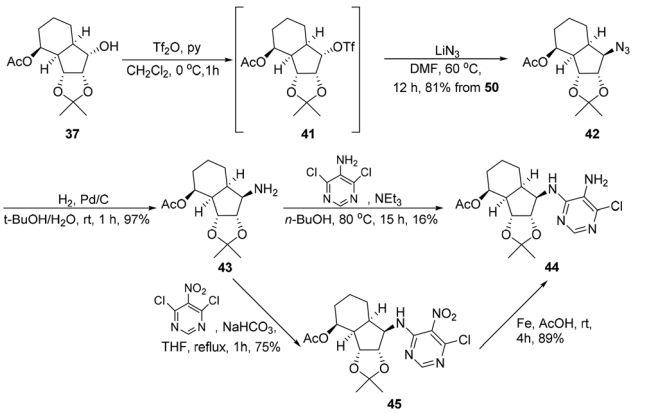


Scheme 12 Attempted to synthesize pyrimidine **39** and **40**.

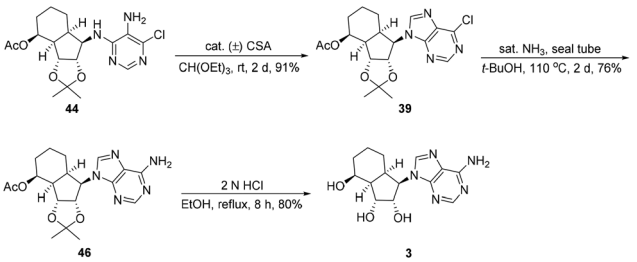
substrates proceeded smoothly to furnish alcohols **37** and **38** in excellent yields, which were the precursors for coupling with the nucleobases.

With alcohol **37** and **38** in hand, direct coupling of nucleobases with the free alcohols was examined (Scheme 12). Firstly, the Mitsunobu reaction[30] 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.

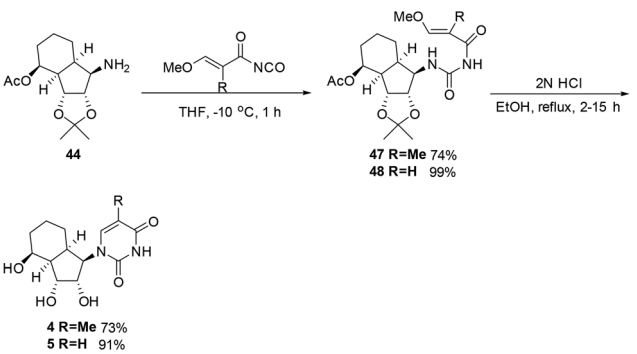
As the convergent route failed to give any desired nucleoside, the linear approach was adopted to synthesize the target molecules. Triflate **41** was successfully converted into azide **42** in good overall yield from alcohol **37** (Scheme 13). Azide **42** was reduced to the corresponding amine **43** by catalytic hydrogenation in excellent yield. Reaction of amine **43** with 5-amino-4,6-dichloropyrimidine furnished pyrimidine **44** in miserable yield and could be explained by the low electrophilicity of 5-amino-4,6-dichloropyrimidine. In order to improve



Scheme 13 Synthesis of pyrimidine **44**.



Scheme 14 Synthesis of the target adenosine analogue **3**.



Scheme 15 Syntheses of thymidine analogue **4** and uridine analogue **5**.

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 using iron powder as a reductant in AcOH (Scheme 13).[31] Using a standard procedure, pyrimidine **44** was transformed into the target carbanucleoside **3** (Scheme 14).

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-methoxymethacryloyl isocyanate[32] and 3-methoxy-2-propenoyl isocyanate[33] 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. Thymi-

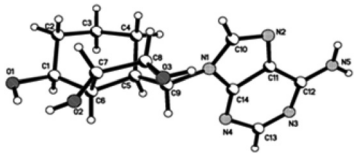


Fig. 6 X-ray structure of adenosine **3**.

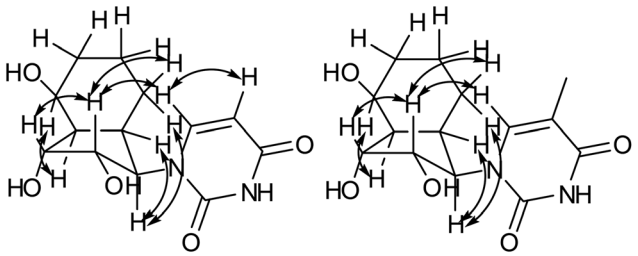


Fig. 7 NOE results of nucleosides **4** and **5**.

dine 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, their conformations 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 **3** is shown in Fig. 6. The NOE results of both nucleosides **4** and **5** also agreed with the C1′-*exo* conformation (Fig. 7).

Biological studies

Antiviral activity against enterovirus, Coxsackie B3 virus, was assessed using the 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. The percentage of reduction in the number of plaques in wells with compounds was calculated. Compounds suspected to have antiviral effect were further examined for cytotoxicity using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

As a result, 3 nucleosides **3**, **4** and **5** showed screening results suggestive of having weak *in vitro* antiviral activity against Coxsackie B3 virus. Adenosine **3** (concentration = 1 μg mL^{-1}) showed 4.9% of inhibition and thymidine **4** (concentration = 1 μg mL^{-1}) showed 5.5% of inhibition and higher concentration (concentration = 100 μg mL^{-1}) led to highly toxic uridine **5** (concentration = 1 μg mL^{-1}) which gave the best result, showing more than 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 key steps. The cycloadduct **6**, a chiral functionalized hydrindenone (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 worth modifying the compounds and reassess for a wider range of viruses. Research in this direction is in progress.

Acknowledgements

This work was supported by a Hong Kong RGC General Research Fund (Ref. CUHK 4030/09P).

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