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Synthesis, conformational study and antiviral activity of L-like neplanocin derivatives

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ABSTRACT

The L-like enantiomer of 9-(trans-2′, trans-3′-dihydroxycyclopent-4′-enyl)-3-deazaadenine (DHCDA) (**1**), its 3-deaza-3-bromo derivative (**3**), and the conformational restricted methanocarba (MC) nucleoside analogues (**2** and **4**) were synthesized. X-ray crystal structures showed the L isomer MC analogue **4** adopts a similar North-like locked conformation as conventional D-MC nucleosides, while the DHCDA analogue **3** preferred south-like conformer. Compounds **1** and **4** showed potent antiviral activity against norovirus, while compound **2** and **3** were less potent or inactive. The conformational behavior of "sugar" puckering (north/south) and nucleobase orientation (syn /anti) may contribute to the antiviral activity differences. For compound **3**, antiviral activity was also found against Ebola virus.

Introduction

Nucleoside analogues play a vital role in chemotherapy of viral infectious diseases.[1] D-like carbocyclic[2] nucleosides, such as abacavir[3] and entecavir[4] (Fig. 1) with the similar configuration as naturally occurring nucleosides, effectively interact with target enzymes and, thus, possess interesting biological activities.[3,4] Despite acting as conventional polymerase inhibitors, the antiviral properties of adenine-derived carbocyclic nucleosides can act by the inhibition of S-adenosylhomocysteine (AdoHcy) hydrolase, a cellular enzyme involved in controlling viral transcription by regulating the mRNA cap structures.[2,5] However, the development of such carbocyclic nucleosides as therapeutics is limited by cytotoxicity that commonly arises from its C-5′ phosphate metabolites arising from various cellular enzymes.[5] In order to seek ways to circumvent this metabolic pathway, and, hence decrease unwanted toxicity, the uncommon L-like carbocyclic enantiomeric nucleosides arose.[6]

Recently, we reported the D-like (**5**) and L-like (**6**) enantiomers of 3-deaza- and 3-deaza-3-bomo-1′, 6′-isoneplanocin (Fig. 2), which have been found to possess potent and broad spectrum antiviral activities. Interestingly, the L-isomers (**6**) were equally or more potent than the D-isomers against human cytomegalovirus, hepatitis B virus (HBV), norovirus, measles and Ebola virus.[7,8]

To further investigate L-like carbocyclic nucleosides, based on compound **6**, we sought the analogues of DHCDA (that is **1** and **3**).[9] DHCDA is a neplanocin derivative whose broad spectrum antiviral activities were attributed to the inhibition of AdoHcy hydrolase, and reduced cytotoxicity due to lacking the 5′ hydroxyl group necessary for the 5′-phosphorylation and circumventing antiviral activity through polymerase inhibition.

It has been well recognized that the conformational behavior of ribonucleosides and modified nucleosides, including those in the category carbocyclic, owe their biological properties to sugar puckering and the orientation of the heterocyclic base. Among these properties are their metabolic pathways, enzyme binding affinity, and therapeutic effects and toxicity.[10] The sugar puckering of ribofuranosyl nucleosides is known to exist in dynamic equilibrium between north (3′-endo) and south (2′-endo) conformations. In most the cases, binding affinity to a pharmacological target favors one conformer over the other. Furthermore, nucleosides adopt either an anti or a syn nucleobase orientation. It has been reported[11] that nucleoside analogues with a syn orientation

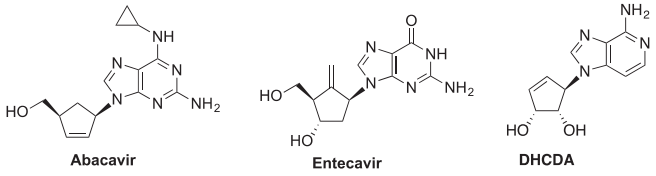

Fig. 1. Examples for carbocyclic nucleosides.

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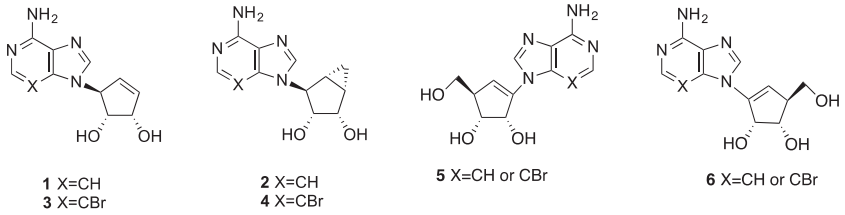


Fig. 2. D and L-like 1′, 6′-isoneplanocin derivatives and designed targer compounds.

exhibited much lower binding affinities to adenosine based receptors. Incorporating these parameters in our study, the 3-bromo substitution in **2** is envisioned to increase the stereo hindrance significantly and force the nucleobase to adopt an anti conformation (Fig. 3). In that direction, methanocarba (MC) nucleoside analogues (**2** and **4**, Fig. 2) were also included as target compounds in this study. The bicyclic[3.1.0]hexane ring in **2** and **4** was expected to constrain the pseudo sugar component and result in locked north-like conformers,[12] which are expected to show the different antiviral profile comparing with conformers **1** and **3**. Further Structure-Activity Relationship (SAR) study can be expected to provide valuable information for exploring the mechanism of carbocyclic nucleosides as antiviral agents.

We envisioned a versatile retrosynthetic plan (Scheme 1) to the proposed targets. The plan involved a coupling reaction between two main components: a cyclopentenol pseudo-sugar potion and a modified purine base. In the latter regard, synthesis of the 3-deazapurine base **7** was accomplished by adapting known procedures using 2-chloro-3,4-diaminopyridine (**11**) as the starting material (Scheme 2).[13,14] Construction of the carbocyclic coupling

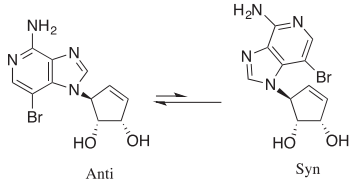
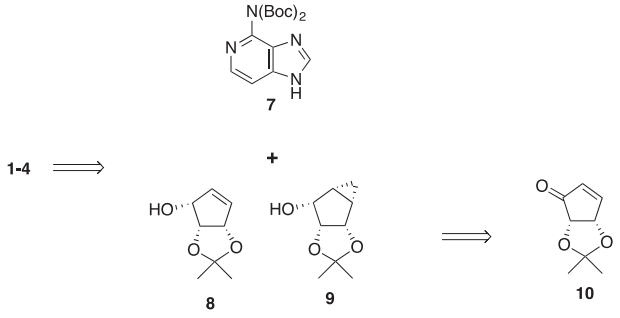
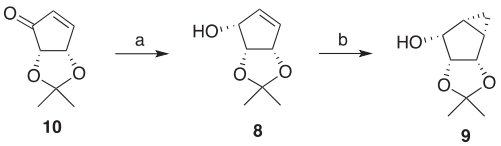


Fig. 3. Syn and Anti conformers of compound **3**.



Scheme 1. Retrosynthesis design for trager compounds.



Scheme 3. Synthesis of cyclopentenol derivatives. Reaction and conditions: a. $LiAlH_4$, $CeCl_3$, THF, 95%; b. Diethylzinc, CH_2I_2, DCM, 70%.

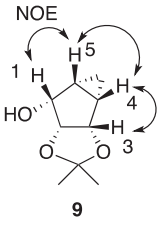


Fig. 4. Stereochemistry of bicycle[3.1.0]hexanol **9**.

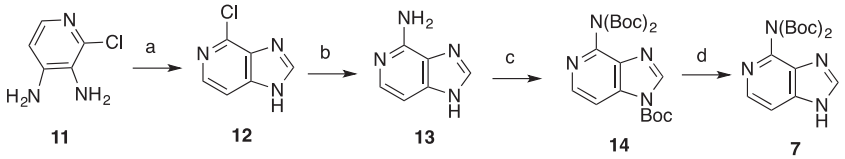
precursors **8** and **9** was achieved from L-cyclopentenone **10**[15] via a Luche condition reduction (for **8**), and a Simmons Smith reaction (for **9**) (Scheme 3).[16,17] The stereo chemistry of compound **9**[18] was confirmed by ROSEY (Fig. 4[18]) and X-ray crystallography of compound **4**.

Mitsunobu coupling reaction was preformed between **7** and L-form cyclopentenol **8** (Scheme 4) or **9** (Scheme 5) to provide coupled products **15** and **16**, which, upon deprotection, gave **1**[19] and **2**.[20] 3-Bromo functionalized targets **3**[21] and **4**[22] were accessible from **1** and **2** via suitable bromination conditions.

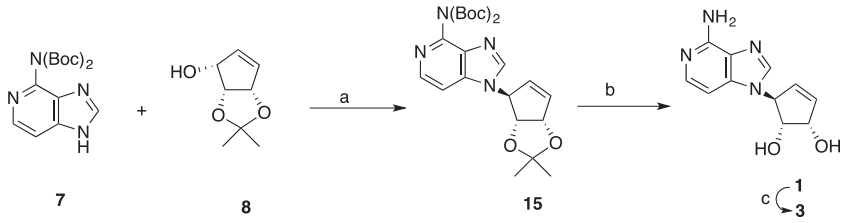
In addition to NMR data, X-ray crystallography structures[23] of **3** and **4** further confirmed the regiospecific bromination at the C-3 position of **1** and **2**.

Crystal structures also showed **3** adopting a south (3′-exo) conformation when posed with similar orientation with D-nucleosides, while more structural rigid bicyclo[3.1.0] hexane locked **4** into an north (2′-exo) conformation in the solid state (Fig. 5). As expected, the bromo group at the C-3 position forced both **3** and **4** to adopt the less congested anti conformation.

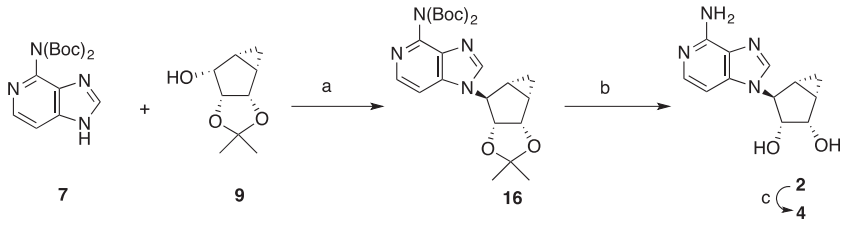
Compounds **1**–**4** were assayed for their antiviral potential.[24,25] Compounds **1** and **4** displayed activity against norovirus. Com-



Scheme 2. [13,14]Synthesis of 3-deazapurine base. Reaction and conditions: a. $CH(OMe)_3$, 85%; b. i)NH_2NH_2 ii) Raney Ni, H_2O, 70%; c. $(Boc)_2O$, THF, 90%; d. TBAF, THF, 88%.



Scheme 4. Synthesis of compound **3**. Reaction and conditions: a. DIAD, PPh_3, THF, 77%; b. 1 M HCl/MeOH, 87%; c. NBS, DCM/DMF, 74%.



Scheme 5. Synthesis of compound **4**. Reaction and conditions: a. DIAD, PPh_3, THF, 56%; b. 1 M HCl/MeOH, 81%; c. NBS, DCM/DMF, 71%.

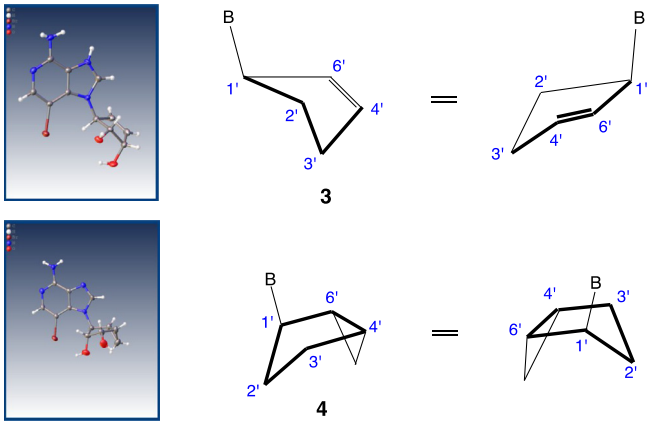


Fig. 5. North and South conformation for compound **3** and **4**.

pound **1** (EC_{50} = 4.2 μM) was more potent than its bromo analogue **3** (EC_{50} = 33 μM). On the other hand, the antiviral profile against norovirus for MC nucleosides **2** and **4** were reversed. In contrast to **1**, compound **2** (EC_{50} > 100 μM) lost anti-norovirus activity, the bromo derivative **4** (EC_{50} = 3.0 μM) showed stronger viral inhibition than **3**. As evidenced in the crystal structures, **3** and **4** are locked into the anti-conformation in solid state, while **1** and **2** are more flexible. The most active norovirus **4** represents the anti-north L-like nucleosides and suggests more extensive study is in order. In comparison with the D-like nucleosides, compound DHCDA (**1**) showed antiviral activity against vaccinia virus (EC_{50} = 0.7 μg/mL)[26] and vesicular stomatitis virus (EC_{50} = 0.2 μg/mL)[26], but no anti-norovirus activity was reported to our knowledge.

Additionally, **3** (EC_{50} = 8.3 μg/mL) showed stronger activity against Ebola virus than **1** (EC_{50} = 29.5 μg/mL), while MC analogues **2** and **4** were completely inactive. Compound **3** displayed toxicity related mild active towards the flavivirus family in vero cell line.[27] Compounds **1**,**2** and **4** showed no apparent toxicity towards the testing cell lines.

No effects were found for **1**–**4** towards AdoHcy hydrolase (IC_{50} > 10^5 mM for all compounds) which most likely correlated with the activity of D-carbocyclic nucleosides.[2]

Acknowledgments

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18. Structural assignments for **9** were accomplished by ^{1}H COSY and the stereochemistry was assigned by a ROSEY, which shows strong NOE correlations between H-1 and H-5, H-3 and H-4, H4 and H-5 protons (Fig. 4).
19. Compound **1**, white solid; ^{1}H NMR (600 MHz, MeOD): δ 8.07 (s, 1H), 7.68 (d, J = 6.1 Hz, 1H), 6.90 (d, J = 6.1 Hz, 1H), 6.32 (dt, J = 6.2, 2.52 Hz, 1H), 6.20 (dd, J = 6.2, 1.7 Hz, 1H), 5.41 (m, 1H), 4.66 (dd, J = 2.7, 1.3 Hz, 1H), 4.65 (dd, J = 2.6, 1.3 Hz, 1H); ^{13}C NMR (150.9 MHz, MeOD): δ153.5, 142.3, 141.0, 140.2, 137.9, 133.7, 128.4, 99.5, 78.9, 74.5, 68.3; HRMS Calcd for $C_{11}H_{13}N_4O_2$ $[M+H]^+$ 233.1039, found 233.1035. Anal. Calcd for $C_{11}H_{12}N_4O_2$. 0.3H_2O: C, 55.60; H, 5.34; N, 23.58. found: C, 55.83; H, 5.32; N, 23.34..
20. Compound **2**, white solid; ^{1}H NMR (600 MHz, MeOD): δ 8.24 (s, 1H), 7.73 (d, J = 6.0 Hz, 1H), 6.97 (d, J = 6.0 Hz, 1H), 4.68 (d, J = 1.2 Hz, 1H), 4.60 (t, J = 6.0 Hz, 1H), 3.84 (d, J = 6.6 Hz, 1H), 2.02 (m, 1H), 1.77 (m, 1H), 1.84 (dd, J = 9.0, 4.2 Hz, 1H), 0.83 (m, 1H); ^{13}C NMR (150.9 MHz, MeOD): δ 153.5, 141.5, 141.1, 140.0, 128.1, 98.8, 77.9, 72.9, 65.5, 24.7, 19.6, 8.43; HRMS Calcd for $C_{12}H_{15}N_4O_2$ $[M+H]^+$ 247.1195, found 247.1188.
21. Compound **3**, white solid; ^{1}H NMR (600 MHz, MeOD): δ 8.11 (s, 1H), 7.78 (s, 1H), 6.36 (m, 1H), 6.29 (m, 1H), 6.13 (m, 1H), 4.71 (m, 1H), 4,27 (m, 1H); ^{13}C

NMR (150.9 MHz, MeOD):δ 153.2, 143.6, 142.1, 138.4, 137.2, 133.6, 129.7, 90.9, 79.8, 74.8, 67.2; HRMS Calcd for $C_{11}H_{12}N_4O_2Br$ $[M+H]^+$ 311.0144, found 311.0132. Anal. Calcd for $C_{11}H_{11}N_4O_2Br$: C, 42.46; H, 3.56; N, 18.01. found: C, 42.63; H, 3.63; N, 17.74.

22. Compound **4**, white solid; ^1H NMR (600 MHz, MeOD): δ 8.32 (s, 1H), 7.77 (s, 1H), 4.64 (d, *J* = 1.2 Hz, 1H), 4.58 (t, *J* = 5.4 Hz, 1H), 3.94 (d, *J* = 6.0 Hz, 1H), 2.0 (m, 1H), 1.76 (m, 1H), 1.40 (dd, *J* = 9.0, 4.2 Hz, 1H), 0.79 (m, 1H); ^{13}C NMR (150.9 MHz, MeOD): δ 151.7, 141.9, 140.6, 135.0, 128.3, 89.3, 75.9, 71.4, 63.6, 22.9, 19.2, 6.69; HRMS Calcd for $C_{12}H_{14}N_4O_2Br$ $[M+H]^+$ 325.0300, found 325.0313.
23. Crystallographic data (excluding structure factors) for 3 and 4 has been deposited with the Cambridge Crystallographic Data Centre with the following deposition number CCDC 1544960 and CCDC 1544961 respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.
24. For the antiviral and hydrolase assay details: Chen Q, Liu C, Komazin G, Bowlin SW, Schneller SW. *Bioorg Med Chem*. 2014;22:6961.
25. No significant activities were found for **1**–**4** towards Adenovirus, Chikungunya virus, Dengue virus, Poliovirus, Punta Toro virus, Rift Valley fever virus, Tacaribe virus, Usutu virus, Venezuelan equine encephalitis virus, West Nile virus, Yellow fever virus, Japanese encepahalitis virus, Zika virus and measles virus.
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27. Compound **3** towards Dengue virus (Vero IC_{50}=7.2 μg/mL, CC_{50}=28 μg/mL); Rift Valley fever virus (Vero IC_{50}=31 μg/mL, CC_{50}=39 μg/mL); Venezuelan equine encephalitis virus (Vero IC_{50}=27 μg/mL, CC_{50}=27 μg/mL); West Nile virus (Vero IC_{50}=32 μg/mL, CC_{50}=32 μg/mL); Yellow fever virus (Vero IC_{50}=32 μg/mL, CC_{50}=32 μg/mL); Japanese encepahalitis virus (Vero IC_{50}=32 μg/mL, CC_{50}=32 μg/mL).