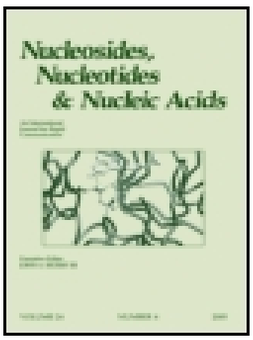

Nucleosides, Nucleotides and Nucleic Acids

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Regioselective Synthesis of Pyrazolo[3,4-*D*]Pyrimidine Based Carbocyclic Nucleosides as Possible Antiviral Agent

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ABSTRACT

Carbocyclic nucleosides are considered as nucleoside mimetic having high therapeutic potentials, however diverse exploration is still limited due to their synthetic difficulties. The major challenges are associated with the preparation of new base and carbocyclic sugar key intermediates. The modified base may provide conformational advantage to achieve better nucleoside mimetics and may also help in increasing the drug-like properties. In this manuscript, we report the use of acetamidine hydrochloride to synthesize 6-methyl-4-amino-pyrazolo[3,4-*d*]pyrimidine base and regioselective synthesis of six new carbocyclic nucleosides (**6a-f**) for antiviral evaluation. Theoretical investigations were carried out on the basis of thermodynamic and kinetic stability using MM based energy optimizations and QM based transition state search for the significant regioselectivity, which was further experimentally analyzed by NOE and UV spectroscopy.

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Mitsunobu reaction; neplanocin; conformation; nucleoside mimetic; antiviral agent

Abbreviations: 4-APP; 4-amino-pyrazolo[3,4-*d*]pyrimidine; 6-methyl-4-APP; 6-methyl-4-amino-pyrazolo[3,4-*d*]pyrimidine; HCV; Hepatitis C Virus

1. Introduction

Carbocyclic nucleosides are nucleoside analogs in which -O- of the furanoside is replaced by its bioisostere -CH_2- to generate a carbocyclic moiety which has an increased metabolic stability due to an improvement in resistance against the enzymatic hydrolysis[1] and a ring-puckering which may be useful to exhibit new biological properties.[2] Hence, these molecules may serve as better nucleoside mimic for competitive inhibiton of viral polymerase.[3] It is important to note that the naturally occuring carbocyclic nucleosides aristeromycin[4] and neplanocin A[5] exhibits significant antivral activities[6] but were found to be toxic.[7,8] In addition, abacavir

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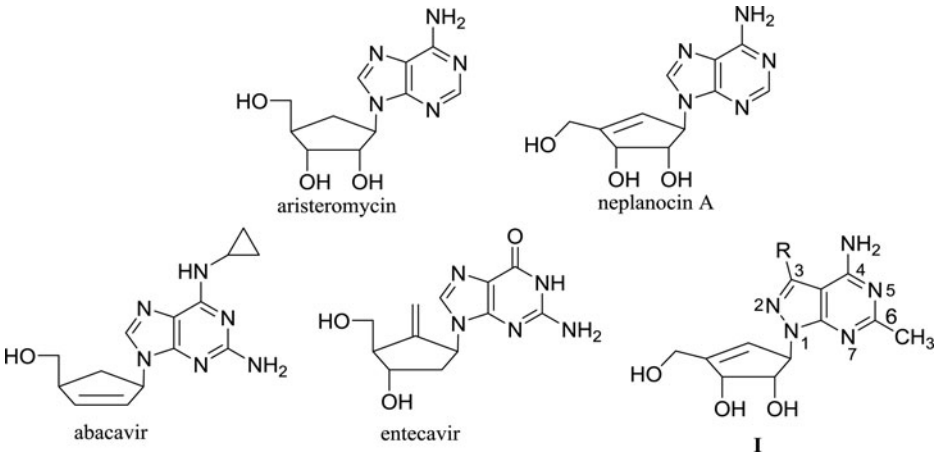


Figure 1. The chemical structure of aristeromycin, neplanocin A, abacavir (approved anti-HIV drug), entecavir (approved anti-HBV drug) and target molecules based on 6-methyl-4-amino-1*H*-pyrazolo[3,4-*d*] pyrimidine based carbocyclic nucleosides (**I**) for synthesis and biological evaluation.

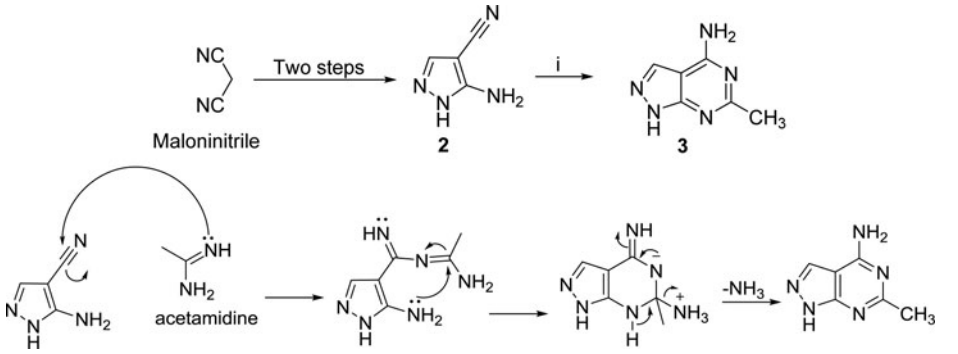
(approved anti-HIV drug), entecavir (approved anti-HBV drug) belongs to this class of nucleoside which provides us a vision to explore novel carbocyclic nucleosides as possible antivirals.

Recently, we have synthesized and explored less toxic neplanocin based analogs with 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (4-APP) base having some antiviral activity.[9] Therefore, in continuation to our previous work, we chose to synthesize a new class of more hydrophobic carbocyclic analogs based on 6-methyl-4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (6-methyl-4-APP, **I**, Figure 1) as possible antiviral agents. Synthesis of this base was earlier reported[10] starting from 3-amino-4-cyano pyrazole with relatively harsh conditions and low yield (~30%). Here, we present a new high yield synthesis of 6-methyl-4-APP followed by regioselective synthesis of new carbocyclic nucleosides **I**.

2. Results and discussion

2.1. *Chemistry*

The carbocyclic sugar key intermediate (**1**) as a single isomer was prepared starting from the commercially available D-ribose in eight consecutive steps.[11,12] The target base **3** was synthesized starting from malononitrile followed by cyclization of **2** with acetamidine hydrochloride (Scheme 1). According to our knowledge this is the first report to use acetamidine hydrochloride as an imidine precursor to construct the pyrimidine ring. In brief, acetamidine hydrochloride converts into acetamidine upon dissolving in ethanolic solution of sodium ethoxide. The imidine group of acetamidine act as a nucleophile to attack the carbon atom of the cyano group followed by cyclization with the release of ammonia giving **3** in 82% yield (Scheme 1).

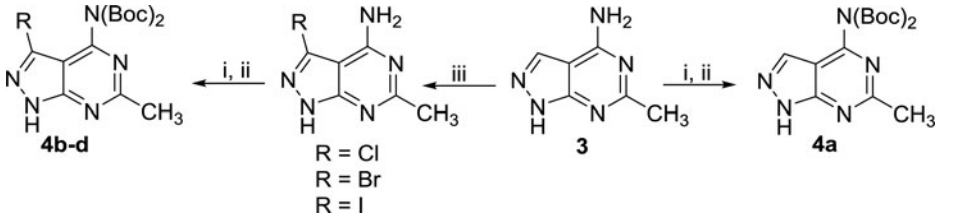


Scheme 1. **Reagents** and **Condition**: (i) Acetamidine hydrochloride, NaOEt, EtOH, 110°C, 7 h.

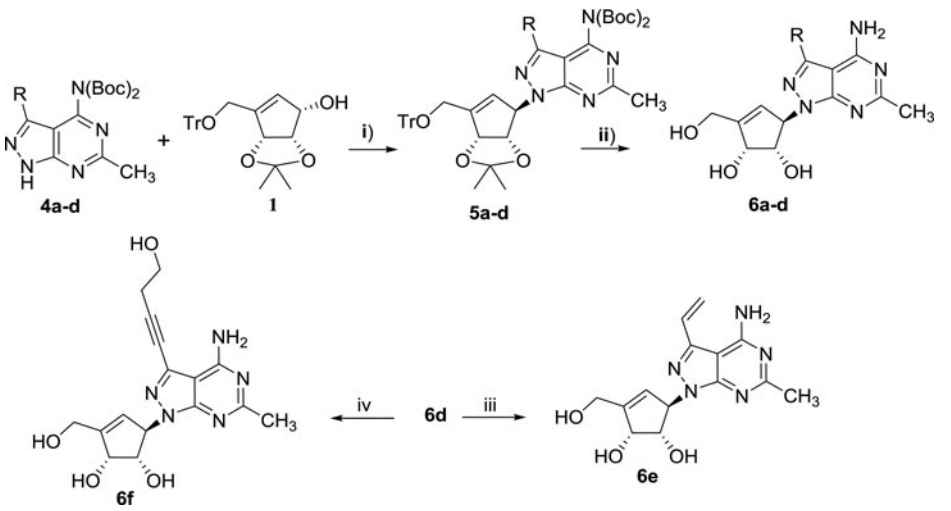
Reaction of **3** with di-*tert*-butyl dicarbonate [$(Boc)_2O$] in presence of DMAP as catalyst in THF followed by treatment with aq. $NaHCO_3$ in methanol[13] yielded the di-Boc protected base **4a** (Scheme 2). The di-Boc derivatives of 3-halo analogs (**4b**-**d**) were synthesized by treating **3** with respective *N*-halosuccinimide in DMF followed by reaction with di-*tert*-butyl dicarbonate [$(Boc)_2O$] in presence of DMAP and with aq. $NaHCO_3$ in methanol.

Mitsunobu coupling of **4a-d** with **1** yielded regio-selective products **5a-d** (Scheme 3). The UV spectra of **5a**-**d** were close to their respective Boc protected aglycons **(4a-d)**, which indicates only N1 glycosylation.[9,14] To further support the selectivity, we performed NOE experiment. According to Seela et al. the irradiation of H3 of the N2 glycosylated product should exhibit NOEs with sugar ring protons.[15] In our case, the irradiation of H3 (on base moiety) of **5a** showed nocorrelation with any sugar ring protons (in Supporting information).

To further support our assignment, we performed the conformational search analysis for N1 and N2 isomers that could be theoretically formed during the coupling of **4a** with **1**. We found that the distances between H3 and sugar ring protons are in the NOE range for N2 isomer while they are out of NOE range in case of N1. The rate determining step for the coupling of **4a** with **1** was studied by means of computational calculations and reaction mechanism (Figure 2) before assigning and understanding the selectivity for N1 isomers (5a-d) (discussed in **2.2**). These experimental data provides strong evidences for the formation of N1 isomer.



Scheme 2. Reagents and conditions: (**i**) $(Boc)_2O$, DMAP, THF, rt, 6 h (**ii**) aq. $NaHCO_3$, MeOH, rt, 3 h (**iii**) NCS/NBS/NIS, DMF, rt, 4 h.



Scheme 3. Synthesis of 6-methy-4-APP based carbocyclic nucleoside analogs **6a-e**.

Reagents and conditions: (**i**) Ph_3P, DIAD, THF, 0–5°C, 2 h; (ii) Methanolic HCl, rt, 5–10 h; (**iii**) Tributyl(vinyl) stannane, $Pd(PPh_3)_4$, CuI, Triethyl amine, 110°C, 5 h; (**iv**) 3-butyn-1-ol, $Pd(PPh_3)_4$, CuI, Triethyl amine, 110°C, 10 h.

The deprotection of 5a-d was carried out by treating them in methanolic HCl to yield the desired carbocyclic nucleosides (**6a-d**). Stille and Sonogashira coupling reactions were utilized for the synthesis of **6e** and **6f** from **6d**, respectively (Scheme 3). In brief, aryl halide **6d** was treated with tributylvinyltin and homopropargyl alcohol to introduce the vinyl and the homopropargyl alcohol groups, respectively.

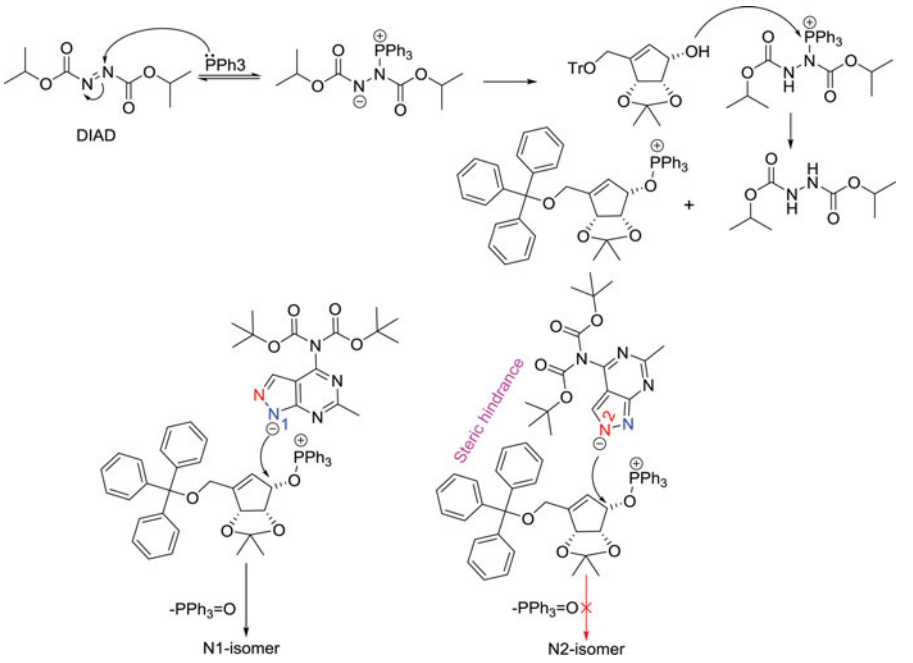


Figure 2. The plausible mechanism for Mitsunobu coupling of **4a** with **1**.

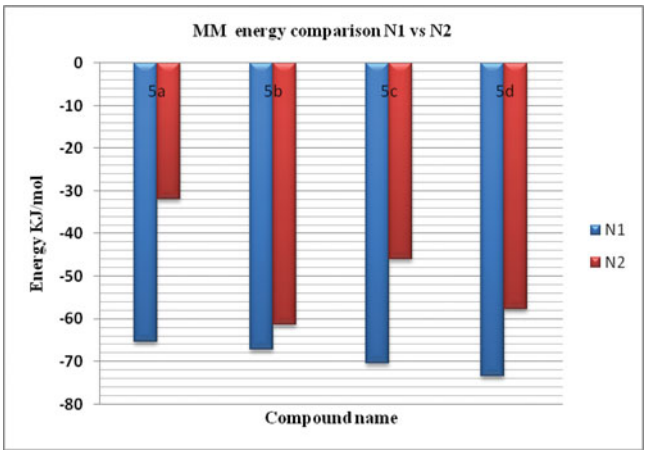


Figure 3. MM energy analyses showing the N1-regioisomer to have lower energy than N2-isomer.

2.2. *Computational studies*

To investigate the regioselectivity of Mitsunobu coupling, energetic investigation was performed. The molecular mechanics (MM) energy optimization indicated that the potential energy of the N1 isomer is lower than the corresponding N2 as shown in Figure 3. This suggests that the selective formation of N1 isomer is due to its higher stability with respect to N2.

The quantum mechanics (QM) calculation for the activation energy for the formation of **5a** (N1) and **5a** (N2) were carried out. The result shows that the transition state for N1 is 0.0016 Ha (4.27 kJ/mol) lower in energy than that of N2 (schematic representation in Figure 4). In addition, the relative energy of N1 isomer is less than that of N2 isomer. Thus, QM calculation also supports the formation of N1 product. In contrast, when Yang et al. performed the Mitsunobu coupling of the same sugar

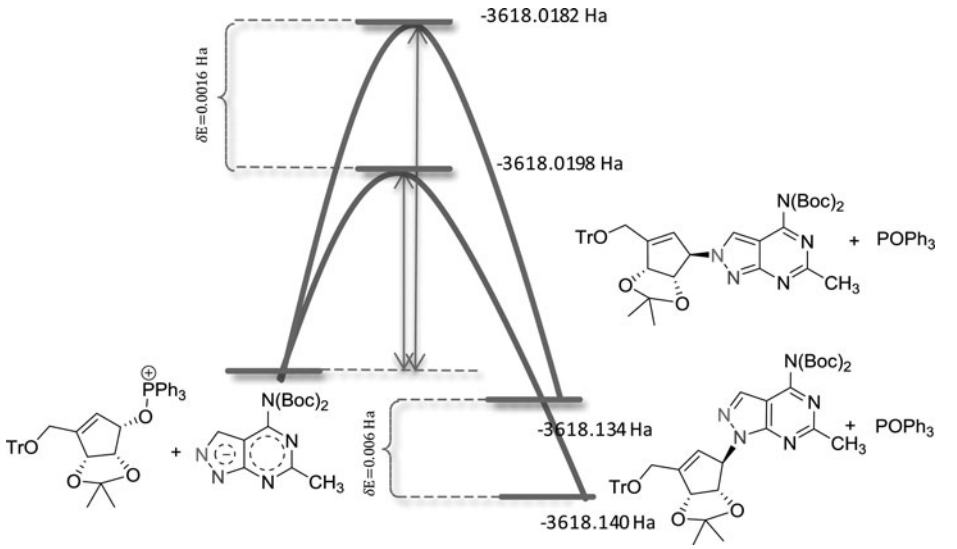


Figure 4. Schematic representation of the energy barrier for the formation of N1 and N2 isomer.

with a 4-chloro substituted base, obtained both the N1 and N2 isomers in a ratio of 42:53.[16] It implies that the steric crowding at 4-postion (bulky Boc groups on nitrogen) reduces nucleophilicity of N2 base and raises the activation energy for the formation of N2 isomer. Furthermore, the steric hinderence between the bulky OTr and $N(Boc)_2$ groups destabilizes the N2 isomer (Figure 2). Therefore, the absolute regioselectivity can be accounted to the fact that the N1 is not only thermodynamically stable but also kinetically favored.

3. Experimental

3.1. *General methods*

All reactions. were carried out in oven-dried glassware under nitrogen atmosphere. The chemicals and solvents were purchased from Spectrochem, Acros, Rankem or Sigma-Aldrich. Melting points were recorded on Veego melting point apparatus. Analytical thin layer chromatography (TLC) was performed on precoated plates (silica gel 60 F-254) purchased from Merck Inc. Purification by gravity column chromatography was carried out on silica gel (100–200 mesh). Eleco UV/Vis spectrophotometer was used for recording the UV spectra. $^{1}H/^{13}C$ NMR were obtained from a Varian (400 MHz) spectrometer or Bruker spectrometer using $CDCl_3$ or DMSO-d6, as solvents. Peaks are recorded with the following abbreviations: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (hertz).

3.2. *General procedure for the synthesis of 6a-d*

To a mixture of appropriate **4a-d** (1.57 mmol), sugar key intermediate **1** (1.50 mmol) and Ph_3P (3.75 mmol) in THF was added DIAD (3.75 mmol) dropwise at 0°C. The reaction mixture was thereafter brought up to rt and stirring was continued. Completion of the reaction was analyzed by TLC, solvent was evaporated under reduced pressure and crude was purified by column chromatography on silica gel by eluting up to 30% ethyl acetate in hexane to get coupled products (**5a-d**). The trityl, acetonide and Boc groups of **5a-d** were deprotected by stirring at rt in methanolic HCl. After completion (monitored by TLC), the reaction mixture was concentrated under reduced pressure and solid was dissolved in methanol; neutralized with $NaHCO_3$ and the crude was purified by silica gel column chromatography using dichloromethane/methanol (5–10%) to yield pure carbocyclic nucleosides **6a-d**. To a solution of **6d** (1.5 mmol) in dry DMF, tributylvinyltin (3.1 mmol), $Pd(PPh_3)_4$ (0.07 mmol) were added and stirred at 110°C for 5 h to obtaine **6e**. Synthesis of **6f** was carried out using the same procedure wherein the homopropargyl alcohol was added instead of tributylvinyltin, CuI (0.3 mmol), triethylamine (7.5 mmol).

Di-Boc protected 1-((3aS,4R,6aR)-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)-6-methyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-amine (5a) Yield: 70%; mp: 68–70°C; MS-ESI (*m*/*z*): 760.2 $[M^{+}+1]$; UV (MeOH): λ_{max} 214 nm, 270 nm; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (s, 1H,

3-CH), 7.21–7.46 (m, 15H, Tr-H), 6.04–6.10 (m, 2H, 1′, 6′-CH), 5.36–5.37 (d, J^3= 5.9 Hz, 1H, 2′-CH), 4.87–4.89 (d, J^3= 5.7 Hz, 1H, 3′-CH), 3.95–3.99 (d, J^2= 14.9 Hz, 1H, 5′-CH), 3.77–3.81 (d, J^2= 15.4 Hz, 1H, 5′-CH), 2.71 (s, 3H, 6-CH_3), 1.55 (s, 18H, Boc-6CH_3), 1.45 (s, 3H, CH_3), 1.34 (s, 3H, CH_3).

Di-Boc protected 3-chloro-1-((3aS,4R,6aR)-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)-6-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (5b) Yield: 80%; mp: 70–74°C; MS-ESI (*m*/*z*): [M^+-100] 694.2, [M^++2–100] 696.2; UV (MeOH) λ_{max}: 215.0, 271.0 nm; ^{1}H NMR (400 MHz, $CDCl_3$): 7.21–7.46 (m, 15H, Tr-H), 6.02–6.07 (m, 2H, 1′, 6′-CH), 5.32–5.34 (d, *J*= 5.3 Hz, 1H, 2′-CH), 4.85–4.86 (d, J^3= 5.7 Hz, 1H, 3′-CH), 3.94–3.98 (d, J^2= 15.3 Hz, 1H, 5′-CH), 3.77–3.80 (d, J^2= 15.3 Hz, 1H, 5′-CH), 2.81 (s, 3H, 6-CH_3), 1.46 (s, 18H, Boc-6CH_3), 1.45 (s, 3H, CH_3), 1.33 (s, 3H, CH_3).

Di-Boc protected 3-bromo-1-((3aS,4R,6aR)-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)-6-methyl-1*H*-pyrazolo [3,4-*d*]pyrimidin-4-amine (5c) Yield: 77%; mp: 71–72°C; MS (*m*/*z*): [M^+] 837.8, [M^++2] 839.8; UV (MeOH) λ_{max}: 216.9, 269.4nm; ^{1}H NMR (400 MHz, $CDCl_3$): 7.21–7.46 (m, 15H, Tr-H) 6.02–6.07 (m, 2H, 1′, 6′-CH), 5.33–5.35 (d, J^3= 5.7 Hz, 1H, 2′-CH), 4.87–4.88 (d, J^3= 5.7 Hz, 1H, 3′-CH), 3.95–3.98 (d, J^2= 15.3 Hz, 1H, 5′-CH), 3.78–3.82 (d, J^2= 15.3 Hz, 1H, 5′-CH), 2.82 (s, 3H, 6-CH_3), 1.45 (s, 18H, Boc-6CH_3), 1.43 (s, 3H, CH_3), 1.33 (s, 3H, CH_3).

Di-Boc protected 3-iodo-1-((3aS,4R,6aR)-2,2-dimethyl-6-(trityloxymethyl)-4,6a-dihydro-3aH-cyclopenta[d][1,3]dioxol-4-yl)-6-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (5d) Yield: 73%; mp: 74–75 °C; MS-ESI (*m*/*z*): [M^+] 885.7; UV (MeOH) λ_{max}: 210.2, 269.3 nm; ^{1}H NMR (400 MHz, $CDCl_3$): 7.21–7.47 (m, 15H, Tr-H), 6.02–6.06 (m, 2H, 1′, 6′-CH), 5.34–5.36 (d, J^3= 5.3 Hz, 1H, 2′-CH), 4.88–4.90 (d, J^3= 5.5 Hz, 1H, 3′-CH), 3.94–3.98 (d, J^2= 15.4 Hz, 1H, 5′-CH), 3.78–3.82 (d, J^2= 14.9 Hz, 1H, 5′-CH), 2.82 (s, 3H, 6-CH_3), 1.44 (s, 18H, Boc-6CH_3), 1.39 (s, 3H, CH_3), 1.33 (s, 3H, CH_3).

(1S,2R,5R)-5-(4-amino-6-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3-(hydroxymethyl) cyclopent-3-ene-1,2-diol (6a) Yield: 80%, mp > 250°C; HR-MS (m/z): [M^++1] 278.0670; $[\alpha]_D^{19}$ −26.39 (c 0.082 MeOH); UV (MeOH) λ_{max} 215, 270.2 nm; ^{1}H NMR (400 MHz, DMSO-d6) δ 10.06 (bs, 1H, NH), 8.64 (bs, 1H, NH), 8.49 (s, 1H, 3-CH), 5.65–5.66 (m, 1H, 1′-CH), 5.53–5.54 (m, 1H, 6′-CH), 4.40–4.42 (m, 1H, 2′-CH), 4.29–4.31 (m, 1H, 3′-CH), 4.10–4.13 (m, 2H, 5′-CH), 3.16–3.65 (bs, 3H, 3-OH), 2.57 (s, 3H, 6-CH_3); ^{1}H NMR (400 MHz, DMSO-d6, D_2O exchange) δ 8.32 (s, 1H, 3-CH), 5.62–5.63 (m, 1H, 1′-CH), 5.54–5.55 (m, 1H, 6′-CH), 4.39–4.40 (m, 1H, 2′-CH), 4.26–4.29 (m, 1H, 6′-CH), 4.08–4.10 (m, 2H, 5′-CH), 2.51 (s, 3H, 6-CH_3).

(1S,2R,5R)-5-(4-amino-3-chloro-6-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (6b) Yield: 78%; mp: >250°C; MS-ESI (m/z): [M^+] 311.6, [M^++2] 313.6; UV (MeOH) λ_{max} 215.0, 278.0nm; $[\alpha]_D^{20}$ −70.22 (c 0.08 MeOH); ^{1}H NMR (400 MHz, DMSO-d6) δ 7.8–8.2 (bs, 1H, NH), 6.8–7.2 (bs, 1H, NH), 5.59–5.60 (m, 1H, 1′-CH), 5.50–5.51 (m, 1H, 6′-CH), 4.90–5.04 (m, 3H, 3-OH), 4.33–4.36 (m, 1H, 2′-CH), 4.19–4.24 (m, 1H, 3′-CH),

4.05–4.06 (m, 2H, 5′-CH), 2.37 (s, 3H, 6-CH_3); ^{1}H NMR (400 MHz, DMSO-d6, D_2O exchange) 5.58–5.63 (m, 2H, 1′, 6′-CH), 4.41–4.42 (m, 1H, 2′-CH), 4.24–4.27 (m, 1H, 3′-CH), 4.10–4.13 (m, 2H, 5′-CH), 2.42 (s, 3H, 6-CH_3).

(1S,2R,5R)−5-(4-amino-3-bromo-6-methyl-1H-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (6c) Yield: 75%; mp: >250°C; MS-ESI (*m*/*z*): [M^+] 355.5, [M^++2] 357.5; $[\alpha]_D^{26}$ −42.23 (c 0.092 MeOH); UV (MeOH) λ_{max} 215.7nm, 279.4nm; ^{1}H NMR (400 MHz, DMSO-d6) δ 7.7–8.2 (bs, 1H, NH), 6.6–7.2 (bs, 1H, NH), 5.62–5.63 (m, 1H, 1′-CH), 5.52–5.53 (m, 1H, 6′-CH), 4.93–5.06 (m, 3H, 3-OH), 4.33–4.36 (m, 1H, 2′-CH), 4.23–4.26 (m, 1H, 3′-CH), 4.07–4.08 (m, 2H, 5′-CH), 2.40 (s, 3H, 6-CH_3); ^{1}H NMR (400 MHz, DMSO-d6, D_2O exchange) 5.63–5.64 (m, 1H, 1′-CH), 5.58–5.59 (m, 1H, 6′-CH), 4.40–4.42 (m, 1H, 2′-CH), 4.25–4.28 (m, 1H, 3′-CH), 4.12–4.13 (m, 2H, 5′-CH), 2.43 (s, 3H, 6-CH_3).

(1S,2R,5R)-5-(4-amino-3-Iodo-6-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (6d) Yield: 71%; mp: >250°C; HR-MS-ESI (*m*/*z*): [M^+] 403.9974; $[\alpha]_D^{24}$ −99.07 (c 0.18 MeOH); UV (MeOH) λ_{max} 215.7nm, 282.9nm; ^{1}H NMR (400 MHz, DMSO-d6) δ 7.2–7.8 (bs, 1H, NH), 6.2–6.8 (bs, 1H, NH), 5.60–5.61 (m, 1H, 1′-CH), 5.51–5.52 (m, 1H, 6′-CH_3), 4.94–5.06 (m, 3H, 3-OH), 4.36–4.38 (m, 1H, 2′-CH), 4.25–4.30 (m, 1H, 3′-CH_3), 4.08–4.10 (m, 2H, 5′-CH), 2.40 (s, 3H, 6-CH_3); ^{1}H NMR (400 MHz, DMSO-d6, D_2O exchange) 5.59–5.63 (m, 2H, 1′, 6′-CH), 4.41–4.43 (m, 1H, 2′-CH), 4.28–4.30 (m, 1H, 3′-CH), 4.09–4.13 (m, 2H, 5′-CH), 2.43 (s, 3H, 6-CH_3); ^{13}C NMR (100 MHz, DMSO-d6) δ 165.52, 157.74, 155.39, 149.89, 124.80, 101.88, 89.89, 76.75, 72.39, 66.23, 58.93, 26.03.

(1S,2R,5R)-5-(4-amino-6-methyl-3-vinyl-1H-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (6e) Yield: 41%; mp: >250°C; HR-MS-ESI (m/z): [M^++1] 304.0576; $[\alpha]_D^{24}$ −82.07 (c 0.12 MeOH); UV (MeOH) λ_{max} 214.7nm, 284.9nm; ^{1}H NMR (400 MHz, DMSO-d6) δ 7.20–7.27 (dd, 1H, CH), 5.93–5.98 (t, 1H, CH), 5.68–5.69 (m, 1H, 1′-CH), 5.543–5.546 (d, 1H, 6′-CH), 5.34–5.37 (d, 1H, CH), 4.37–4.40 (q, 2H, 2′ & 3′-CH), 4.10 (m, 2H, 5′-CH), 2.39 (s, 3H, 2-CH_3); ^{1}H NMR (400 MHz, DMSO-d6, D_2O exchange) δ 7.16–7.23 (dd, 1H, CH), 5.95–5.99 (d, 1H, CH), 5.69–5.71 (m, 1H, 1′-CH), 5.58–5.59 (m, 1H, 6′-CH), 5.42–5.45 (d, 1H, CH), 4.36–4.43 (m, 2H, 2′ & 3′-CH), 4.12 (m, 2H, 5′-CH), 2.41 (s, 3H, 6-CH_3); ^{13}C NMR (100 MHz, DMSO-d6) δ 164.82, 158.20, 156.16, 149.43, 141.18, 128.17, 125.23, 117.59, 96.52, 76.59, 72.49, 65.60, 58.99, 25.97.

(1S,2R,5R)-5-(4-amino-3-(4-hydroxybut-1-ynyl)-6-methyl-1H-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (6f) Yield: 30%; mp: >250°C; MS-ESI (*m*/*z*): [M^++1] 346.18; $[\alpha]_D^{20}$ −79.06 (c 0.11 MeOH); UV (MeOH) λ_{max} 210.5 nm, 279.3 nm; ^{1}H NMR (400 MHz, DMSO-d6) δ 6.56–6.58 (bs, 1H, OH), 5.45–5.49 (d, 1H, *J*= 15.6 Hz, 1′-CH), 5.30–5.32 (d, 1H, *J*= 6.4 Hz, 6′-CH), 5.06–5.08 (bs, 1H, OH), 4.71–4.75 (bs, 1H), 4.61–4.66 (dd, 1H, *J*= 15.6 Hz, *J*= 5.6 Hz, 2′-CH), 4.02–4.13 (m, 3H, 5′ & 3′-CH), 3.61–3.63 (t, 2H, CH_2), 2.64–2.66 (t, 2H, CH_2), 2.41–2.43 (d, 3H, CH_3) ^{1}H NMR (400 MHz, DMSO-d6, D_2O exchange) 5.50–5.54 (d, 1H, *J*= 15.6 Hz, 1′-CH), 5.39–5.40 (d, 1H, *J*= 6.4 Hz,

6′-CH), 4.70–4.73 (dd, 1H, *J*= 15.6 Hz, *J*= 5.6 Hz, 2′-CH), 4.02–4.20 (m, 3H, 5′ & 3′-CH), 3.63–3.66 (t, 2H, CH_2), 2.64–2.67 (t, 2H, CH_2), 2.42–2.44 (d, 3H, CH_3).

3.3. *Computer aided computational studies*

To study the absolute selectivity obtained in Mitsunobu reaction from coupling of **4a-d** with **1**, the structures of N1 and N2 isomers that could be formed were build using Maestro interface of Schrödinger. Preliminary energy optimizations were carried out using classical mechanics. The structures were minimized using Macromodel[17] with Optimized Potentials for Liquid Simulations (OPLS) 2005 force field. The minimized structures were subjected to conformational search with OPLS 2005 force field using Polak-Ribiere Conjugate Gradient method with a maximum of 5000 iterations and convergence threshold of 0.05. Further, the reaction kinetics study for the rate determing step was performed using Transition State Search protocol of Jaguar Module[18] Schrodinger. The linear synchronous transit (LST) method was used to elucidate the energetic pathways from reactant(s) to formation of product(s) through transition state. The phosphonium ion intermediate of the sugar and the deprotonated base were used as the reactants and the corresponding N1 and N2 isomers with triphenyl phosphine oxide as products. The calculations were performed using spin restricted DFT with B3LYP (Becke-3-Lee-Yang-Parr) density functional and the 6–31-G** basis set.

4. Conclusions

The 6-methyl-4-APP based carbocyclic nucelosides (**6a**-**f**) were successfully prepared for antiviral evaluation. The base (**3**) was prepared using acetamidine hydrochloride as an imidine precursor for the preparation of various 4-$N(Boc)_2$ bases (**4a-d**). The Boc group at 4-position resulted in exclusive regioselective glycosylation during Mitsunobu reaction to yield only N1 products (**5a**-**d**). Regioselectivity was investigated both theoretically by studying thermodynamic and kinetic stability and experimentally by analyzing NOE and UV spectra. The final compounds **6a**-**f** were evaluated against HCV in subgenome HCV RNA replicon cells containing the luciferase gene. However none of the compounds was found to be significantly active.

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