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Is the Anomeric Effect an Important Factor in the Rate of Adenosine Deaminase Catalyzed Hydrolysis of Purine Nucleosides? A Direct Comparison of Nucleoside Analogues Constructed on Ribose and Carbocyclic Templates with Equivalent Heterocyclic Bases Selected to Promote Hydration

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Abstract—The aglycone of (North)-methanocarbadeoxyadenosine [(N)-MCdA, (**5**)], a relatively weak substrate for adenosine deaminase (ADA)—relative rate of deamination ca. 100 times lower than adenosine—was modified with substitutions at positions 6 (6-fluoro, compound **6**) and 8 (8-aza, compound **7**) with the intent to improve the level of hydration and hence hydrolysis by ADA. In these substrates the fused cyclopropane moiety constrains the cyclopentane ring to mimic the conformation of a furanose sugar in the North hemisphere of the pseudorotational cycle, which matches the conformation of the ribose ring of adenosine in complex with ADA. The order of susceptibility to ADA hydrolysis was adenosine > > (N)-MCdA (**5**)≈(N)-6F-MCdP (**6**) > (N)-8-aza-MCdA (**7**). Despite the known fact that 8-azaadenosine is hydrolyzed twice as fast as adenosine, the corresponding carbocyclic analogue **7** was hydrolyzed at approximately half the rate of the parent **5**. These results argue in favor of the critical role of the O(4′) oxygen atom and its associated anomeric effect in assisting hydrolysis by ADA.

Introduction

Adenosine deaminase (ADA, EC 3.5.4.4) is a critical enzyme of the purine metabolic pathway that catalyzes the irreversible hydrolytic deamination of adenosine (Ado, **1**) and 2′-deoxyadenosine (dAdo, **2**)—as well as other exogenous substrates—to their hypoxanthine derivatives **3** and **4**, and ammonia (Scheme 1).[1,2] The importance of ADA in maintaining purine nucleotide pools is evident in several hereditary conditions associated with either overproduction or deficiency of the enzyme. Excess of ADA function has been associated with hemolytic anemia,[3] while ADA deficiency results in impaired B- and T-cell-based immunity.[4] The complete lack of T lymphoblasts characterized by ADA deficiency provided the rationale for the use of ADA inhibitors in the treatment of T-lymphoblastic leukemias.[5,6] However, the limited success of this approach justifies the continued search for more effective and less toxic inhibitors of ADA. An alternative way to achieve a similar goal is to employ effective adenosine analogues that are resistant to ADA deamination. Along these lines, our laboratory has recently reported the synthesis of two classes of conformationally locked North and South carbocyclic 2′-deoxyadenosine analogues [(N)-MCdA (**5**) and (*S*)-MCdA (**8**)] that are, respectively, poorly deaminated or almost impervious to the action of ADA.[7] Remarkably, these compounds can be recognized by various adenosine receptors as bona fide adenosine analogues, sometimes even displaying higher binding affinity than ribosides themselves.[8,9] Relative to their resistance to deamination, our initial observations suggest that replacing the ribose or deoxyribose with a carbocyclic moiety significantly lessens the rate of deamination, thus pointing to the important role of the O(4′) oxygen in the mechanism of enzymatic deamination by ADA.[7] The role of this oxygen appears to be strictly mechanistic since ADA crystal structures complexed with a transition-state inhibitor (2′-deoxycoformycin) or a reaction coordinate analogue [(6*S*)-hydroxyl-1,6-dihydropurine

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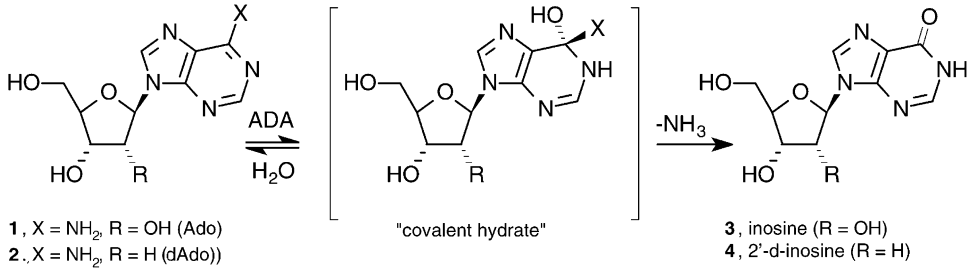
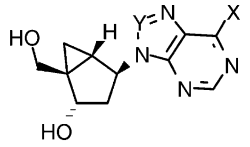
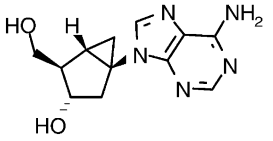
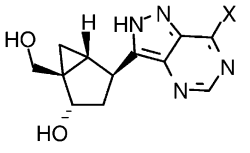


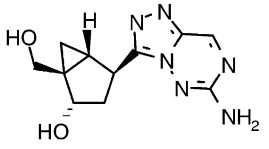
Scheme 1.

riboside] reveal absolutely no contacts between O(4′) and any amino acid residue at the active site.[10]









The confirmed mechanism for ADA-catalyzed deamination is via formation of an unstable hydrated intermediate engendered by the addition of water across the purine N1–C6 double bond (Scheme 1).[10,11] Hence, the efficiency of a substrate in facilitating formation of this critical covalent hydrate should be directly proportional to its capacity to undergo hydrolysis. Heteroaromatic hydration is controlled by multiple factors.[12–14] Indeed, the extent of hydration is dependent on the presence and position of heteroatoms on the ring, their protonation state, and the position and the nature of substituents attached to the ring. Hydration of purines is not a favorable event due to loss of resonance energy and hence hydrated forms are almost undetectable in solution.[12–14] However, the presence of substituents or additional heteroatoms appears to facilitate the process.[12–14] For example, the calculated difference in resonance energy loss between 8-aza-9-methylpurine and 9-methylpurine is 6.6 Kcal/mol less for the former, suggesting that the extent of hydration increases by the addition of the heteroatom.[12,13] With regard to ADA, the additional heteroatom resulted in a more than 2-fold increase in the rate of deamination (rel. V_{max}) of 8-azaadenosine relative to adenosine,[15] and in a 400-fold increase in the inhibitory potency of 8-azapurine riboside ($K_i = 4.0\times10^{-8}$ M) versus purine riboside ($K_i = 1.6\times10^{-5}$ M),[16] reflecting the important role of the 8-aza modification in facilitating formation of a covalent hydrate at the active site. In addition, studies of substituted 9-methylpurine analogues suggested that electron withdrawing groups at C6 (i.e., fluoro or trifluoromethyl) can also produce large enhancements in hydration.[14]

Since the propensity to form a covalent hydrate must depend on the interplay of stereoelectronic forces between the purine ring and the ribofuranosyl moieties, we surmised that the carbocyclic analogues were poorer substrates for ADA because these interacting forces were nullified by the absence of the O(4′) oxygen. The question we seek to answer in the present study is whether it is possible to manipulate one component of the system (i.e., the purine ring) to compensate for the absence of the other [i.e., the O(4′) oxygen]. If we accept as a premise that the O(4′) oxygen is critical for the formation of the covalent hydrate, our goal is to compensate for its absence by using modified purine bases linked to an appropriate carbocyclic moiety to overcome the absence of the electron withdrawing effect of the sugar. In this report we provide evidence that these two structural elements act in concert and that effective covalent hydration of the heteroaromatic ring is strongly dependent on the presence on the O(4′) oxygen, regardless of the electronic nature of the modified heteroaromatic ring.

In this work, we chose to modify the best of the carbocyclic ADA substrates, (N)-MCdA (**5**),[7] with substitutions at positions 6 (6-fluoro, **6**) and 8 (8-aza, **7**) on the purine ring with the intent to improve the level of hydration and hence hydrolysis by ADA. MCdA (**5**) belongs to a class of nucleosides where the fused cyclopropane moiety constrains the cyclopentane ring to mimic the conformation of a furanose sugar in the North hemisphere of the pseudorotational cycle.[7] The selection of the North analogue was based on the 100-fold difference in the rate of deamination favoring the constrained North analogue over its antipodal South (**8**).[7] This conformational preference for a North, C3′-*endo* pucker is also in agreement with the crystal structure of ADA–inhibitor complexes[10] and enzymatic studies on conformationally biased substrates.[17]

Chemistry

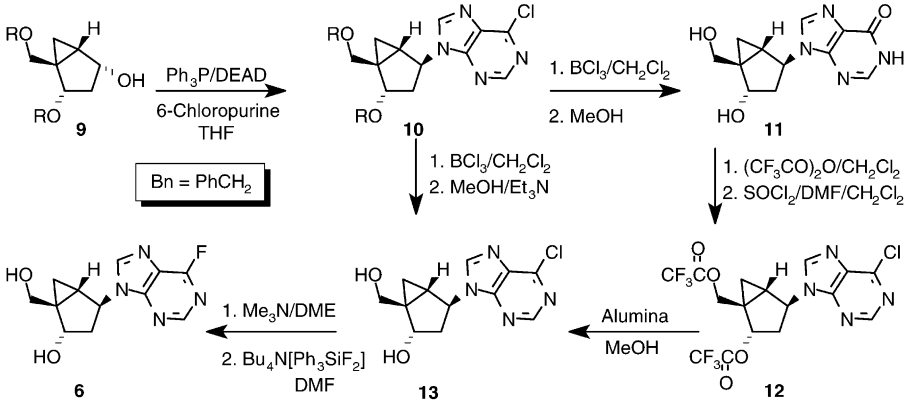
The synthesis of the 6-fluorinated analogue **6** was based on the recently published work of Harris et al.[18] for the efficient conversion of 6-chloropurine nucleosides to 6-fluoropurine derivatives using tetrabutylammoium triphenyldifluorosilicate (TBAT). Our requisite

carbocyclic 6-chloropurine **13** (Scheme 2) was synthesized from the known protected intermediate **10**[19] prepared earlier in our laboratory by Mitsunobu coupling of **9** with 6-chloropurine. Removal of the *O*-benzyl protection in **10** with boron trichloride without neutralization with triethylamine during the quenching step produced exclusively the hypoxanthine analogue **11**, which was then transformed into the desired 6-chloropurine **13** by direct halogenation with thionyl chloride using the method of Robins and Basom.[20] More directly, **13** was obtained as the exclusive product if triethylamine was included during the quenching step. The conversion of **13** to **6** proceeded according to the method of Harris et al.[18] in excellent yield.

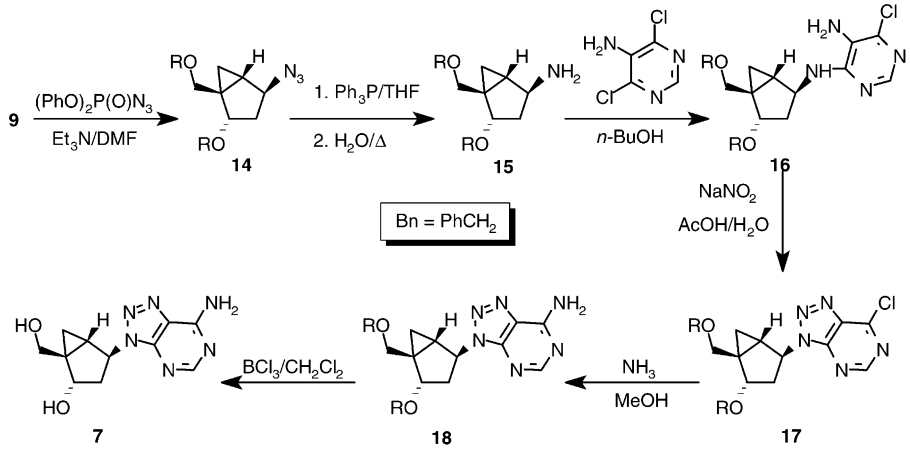
For the synthesis of the 8-aza analogue **7**, a linear approach was chosen to avoid obtaining mixtures of products from the coupling of **9** with 8-azapurine. Hence, the first step involved formation of azide **14** (Scheme 3) with diphenylphosphoryl azide which proceeded with the expected inversion of configuration. Reduction of the azide via formation of the iminophosphorane as reported by Carrié et al.[21] proceeded very smoothly to give the corresponding carbocyclic amine **15**. From this intermediate, the stepwise construction of the 8-azapurine ring continued along a well established route using nitrous acid for the ring closing step from **16**.[22] Ammnolysis of **17** and removal of the *O*-benzyl protection afforded the target carbocyclic 8-azaadenosine analogue **7**.

Biological Results and Discussion

As reported earlier, the ADA-mediated relative rate of hydrolysis for the parent conformationally locked (N)-MCdA (**5**) was 100 times slower than that for the natural substrate adenosine (Table 1).[7] The two new, carbocyclic nucleoside analogues **6** and **7** showed rates that were either essentially unchanged compared to **5**, or 200 times slower than adenosine (Ado, **1**) (Table 1). The order of susceptibility to ADA hydrolysis was Ado (**1**) > > (N)-MCdA (**5**)≈(N)-6F-MCdP (**6**) > (N)-8-aza-MCdA (**7**). Despite the literature report showing that 8-azaadenosine was hydrolyzed twice as fast as adenosine,[15] the corresponding carbocyclic analogue **7** was in fact hydrolyzed at approximately half the rate of the parent carbocyclic adenosine analogue **5**. These results argue in favor of the important role of the oxygen atom in assisting hydrolysis as we previously suggested for the parent carbocyclic nucleoside, (N)-MCdA (**5**).[7] We propose that formation of the requisite covalent hydrate intermediate depends, among other factors, on the critical role of the anomeric effect, which allows for the efficient transmission of electronic effects between the sugar and the base. Chattopadhyaya et al.[23] have clearly shown



Scheme 2.



Scheme 3.

Table 1. Relative rate of ADA hydrolysis

Substrate	Kinetic data[a] (37 °C)		UV analytical data λ_{max} (nm)[d]	
	K (min^{-1})[b]	Relative rate[c]	Substrate	Product
Ado (**1**)	—	100	260	250
(N)MCdA (**5**)	0.30	0.99	261	251
(N)-6F-MCdP (**6**)	0.27	0.90	250	250
(N)-8-aza-MCdA (**7**)	0.13	0.42	279	256

[a]Data was fitted to 1st order exponential decay using ($A = Be^{-kt}$), where B is initial substrate concentration, A is the concentration at time (t), and k is the rate constant.
[b]Rate constant normalized to 1 Unit of ADA.
[c]Relative rates calculated using normalized rate constants.
[d]UV maxima obtained on-the-fly or with spectrophotometer.

that the purine base of adenosine and its sugar moiety communicate electronically via the anomeric effect. By measuring the change of ΔG° for the North/South equilibrium, they showed that as the pH of the solution decreases, the protonated purine favors the North conformer in which the orbital overlap between one of the O(4′)oxygen's lone pair and the σ* antibonding orbital of the C–N glycosyl bond is maximized (anomeric effect, Fig. 1). Since protonation weakens the glycosyl bond via the anomeric effect (increased electron density in the antibonding orbital), this means that the resulting increase in negative charge on the purine ring would help stabilize the protonated base at the active site of ADA. Such effect would be absent in the case of the carbocyclic nucleoside since any interplay of stereoelectronic forces between the purine ring and the ribofuranosyl moiety is completely absent.

For ADA to catalyze the effective hydration of a heteroaromatic base the formation of a stable protonated species is essential. This is achieved at the active site of ADA by Glu217, which is perfectly aligned to donate a proton to N1, thereby making the C6 carbon more susceptible to nucleophilic attack and hydration.[10]

It is clear that structural changes to the purine base that would otherwise expedite hydrolysis in purine nucleosides fail to improve hydrolysis rates when the same purine bases are presented to the enzyme as carbocyclic nucleosides, even when these have the locked North platform (**6** and **7**) preferred by ADA.[7,10] Additional evidence of the importance of the anomeric effect in the generation of the critical covalent hydrate intermediate will probably require syntheses of compounds with heterocyclic bases that deviate from conventional purines, but which per se facilitate even more the formation of a covalent hydrate. These compounds, such as **19**, **20** and **21**, bearing identical heterocyclic bases to the natural product formycin[24] and its deaminoformycin analogue,[25] and the recently developed imidotriazine riboside,[25] will ultimately help determine the role of the anomeric effect in hydration. It is important to highlight that the anomeric effect could still be an important factor in the efficient deamination of the C-nucleoside formycin,[24] since the transmission of electronic effects in C-nucleosides is mediated by an equivalent π→σ* anomeric effect.[26] The syntheses of the corresponding bicylo[3.1.0] hexane nucleosides bearing these new modified bases (**19**–**21**), which will be devoid of anomeric effect, will

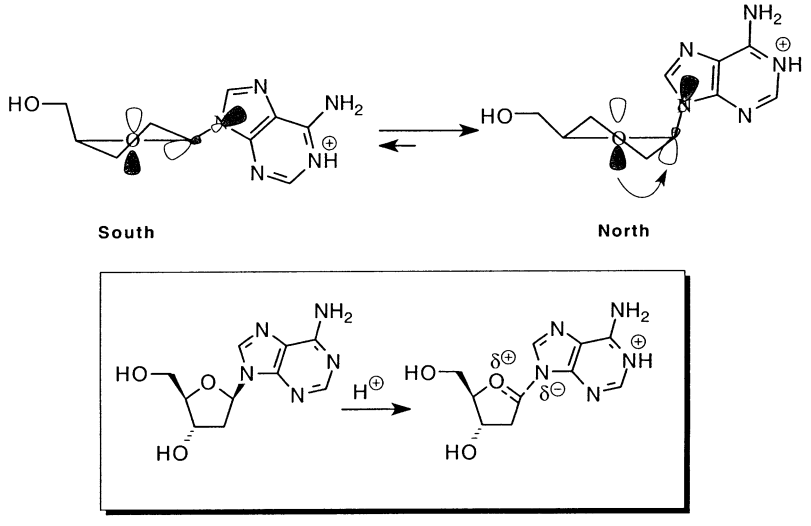


Figure 1. O4′–C1′–N9 anomeric effect. The lone pair orbitals are sp^2 [higher energy $^1n^2_{sp}$(p-type), lower energy $^2n^2_{sp}$(s-type)]. The $n_{O4'} \rightarrow \sigma^*$ overlap stabilizes the North conformation following protonation of the base (box).[23] Orbital interaction in the North conformation is possible due to a near anti-periplanar orientation.

ultimately show whether effective hydration of the base is possible at the active site of ADA in the absence of this driving force. The synthesis of these compounds is currently under investigation.

Experimental

All chemical reagents were commercially available. Reported melting points were determined on a Fisher-Johnson melting point apparatus and are uncorrected. Column chromatography was performed on silica gel 60, 230–240 mesh (E. Merck), and analytical TLC was performed on Analtech Uniplates silica gel GF. Routine IR and 1H, ^{13}C and ^{19}F NMR spectra were recordered using standard methods. Specific rotation were measured in a Perkin-Elmer model 241 polarimeter. Low resolution, positive-ion fast atom-bombardment mass spectra (FABMS) were obtained on a VG 7070-EHF mass spectrometer operated under control of a MSS MASPEC-II[32] data system, while accurate mass FABMS determinations were conducted on a JEOL SX102 instrument. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, USA.

Adenosine deaminase (ADA) reaction

In a 1.5 mL Eppendorf vial, 14.2 μL of an aqueous solution of the nucleoside (**5**–**7**, final concentration 50 μM) and 965.8 μL of phosphate buffer (pH 7.4, 50 mM) was incubated at 37 °C. To this solution, 20 μL diluted ADA was added to reach a final concentration of enzyme in the reaction vessel of ~0.3 U/mL. Aliquots (50 μL) were removed at predetermined time intervals and mixed with the stopping solution, which contained 450 μL of water and 2 μL of 2′-deoxycoformycin at a concentration of 0.5 mg/mL. The ADA-mediated hydrolysis was monitored by HPLC at 255 nm. Chromatography and on-the-fly UV scans were obtained using a Thermo Separations Products HPLC system with a Perkin-Elmer LC235 photodiode array detector. ADA reaction mixtures were separated on a LC-18S 4.6×250 mm column with a linear gradient of 0–15% acetonitrile over 20 min, with a 5 min re-equilibration time. Due to peak interference, an isocratic system of 92:8 (water/acetonitrile) was used for compound **6**. The flow rate was 1 mL/min and a 50 μL injection from each aliquot was applied to follow the disappearance of substrate and formation of product.

(1*R*,2*S*,4*S*,5*S*)-4-(6-Chloropurin-9-yl)-1-(hydroxymethyl) bicyclo[3.1.0]hexan-2-ol (13). A stirred solution of **10**[19] (0.29 g, 0.67 mmol) in anhyd CH_2Cl_2 (5 mL) was cooled to −78 °C under a blanket of argon and treated with a solution of BCl_3 (1 M/CH_2Cl_2, 7 mL). The reaction mixture was stirred for 5 h at −78 °C, allowed to warm up to −20 °C, and further stirred for 1 h. After cooling back to −78 °C, the reaction was quenched by the slow addition of a 1:1 mixture of MeOH/Et_3N (12 mL) and stirred for 15 min. *Note: when the reaction was quenched with just methanol, the inosine analogue **11** was obtained as the only product of the reaction (vide infra).* After evaporation of the volatiles, the off-white residue was pre-adsorbed on silica gel, packed on the top of a silica gel column and chromatographed with EtOAc/10% MeOH to give **13** (0.21 g, 99%) as an off-white solid: mp 179–180 °C. ^1H NMR (CD_3OD) δ 9.09 (s, 1H, H-8), 8.78 (s, 1H, H-2), 5.26 (d, J = 6.6 Hz, 1H, H-4′), 4.97 (t, J = 8.5 Hz, 1H, H-2′), 4.35 (AB d, J = 11.7 Hz, 1H, C*H*HOH), 3.41 (AB d, J = 11.7 Hz, 1H, CH*H*OH), 2.19 (dd, J = 14.8, 8.0 Hz, 1H, H-3′$_a$), 1.90 (ddd, J = 15.0, 9.0, 6.6 Hz, 1H, H-3′$_b$), 1.79 (dd, J = 8.3, 3.4 Hz, 1H, H-5′), 1.15 (dd, J = 5.8, 3.9 Hz, 1H, H-6$_{endo}$), 0.88 (dd, J = 8.0, 6.1 Hz, 1H, H-6$_{exo}$); ^{13}C NMR (CD_3OD) δ 152.8, 151.1, 146.7, 132.5, 71.6, 63.6, 57.3, 38.8, 37.4, 26.7, 10.7; DEPT (CD_3OD) δ 152.6 (CH), 146.5 (CH), 71.3 (CH), 63.3 (CH_2), 57.0 (CH), 38.5 (CH_2), 26.4 (CH), 10.3 (CH_2); FABMS m/z (relative intensity) 281 (MH^+, 100), 155 (b + 2H, 53). Anal. calcd for $C_{12}H_{13}ClN_4O_2 \cdot 0.75H_2O$: C, 48.98; H, 4.96; N, 19.04. Found: C, 49.26; H, 4.89; N, 18.76.

(1*S*,2*S*,4*S*,5*R*)-9-[4-Hydroxy-5-(hydroxymethyl)bicyclo [3.1.0]hex-2-yl]hydropurin-6-one (11). This compound was obtained using the same protocol as for compound **13**, except that quenching of the reaction was performed without Et_3N; mp 132–134 °C; $[\alpha]_D^{25}$ −6.5° (*c* 0.32, MeOH). ^1H NMR (DMSO) δ 12.3 (br s, 1H, NH, D_2O exchanged), 8.43 (s, 1H, H-2), 8.07 (s, 1H, H-8), 5.04 (app t, 1H, OH, D_2O exchanged), 4.90 (d, J = 6.3 Hz, 1H, H-2′), 4.77 (br d, 1H, OH, D_2O exchanged), 4.66 (t, J = 8.3 Hz, 1H, H-4′), 4.09 (AB d, J = 11.5 Hz, 1H, C*H*HOH), 3.20 (AB d, J = 11.7 Hz, 1H, CH*H*OH), 1.88 (dd, J = 14.4, 8.0 Hz, 1H, H-3′$_a$), 1.73 (ddd, J = 15.1, 8.8, 6.6 Hz, 1H, H-3′$_b$), 1.57 (dd, J = 8.3, 3.4 Hz, 1H, H-1′), 0.94 (dd, J = 5.1, 4.0 Hz, 1H, H-6′$_{endo}$), 0.69 (dd, J = 8.0, 5.9 Hz, 1H, H-6$_{exo}$); ^{13}C NMR (DMSO-d_6) δ 156.5, 147.6, 145.1, 137.9, 123.8, 69.1, 61.4, 54.2, 37.8, 35.8, 24.9, 9.3. FAB MS m/z (relative intensity) 263 (MH^+, 100), 137 (b + 2H, 67). Anal. calcd for $C_{12}H_{14}N_4O_3 \cdot 0.75H_2O$: C, 50.34; H, 5.87; N, 19.57. Found: C, 50.19; H, 5.59; N, 19.40.

Synthesis of 13 from 11

A magnetically stirred suspension of **11** (0.074 g, 0.28 mmol) in anhydrous CH_2Cl_2 (5 mL) was cooled to 0 °C (ice bath) under an argon atmosphere. Trifluoroacetic anhydride (0.8 mL) was added and the mixture was stirred at 0 °C for 1 h. The reaction vessel was removed from the cold bath and the mixture was further stirred at room temperature for 1 h until starting material was consumed. All volatiles were removed under high vacuum and the glassy residue was taken up in anhydrous CH_2Cl_2 (5 mL) and maintained under argon. A solution of $SOCl_2$ (0.5 mL, 6.9 mmol) in anhydrous DMF (0.5 mL, 6.4 mmol) was added via syringe, and the mixture was heated to 55 °C for 4 h with monitoring by TLC (1:1 hexane/EtOAc) until completion. After cooling to rt, the solution was poured into a vigorously stirred, ice-cold solution of $NaHCO_3$ and stirring continued for 15 min until the phases separated. The aqueous layer was extracted with CH_2Cl_2 (2×5 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The crude solid was dissolved in cold MeOH (2 mL) and this solution was passed

through a short column of neutral alumina packed in MeOH. After further elution with MeOH, the solution was evaporated and the residue was purified by column chromatography with the identical system as described above to provided an off-white solid having the same mp and all spectral characteristics of **13**.

(1*R*,2*S*,4*S*,5*S*)-4-(6-Fluoropurin-9-yl)-1-(hydroxymethyl)-bicyclo[3.1.0]hexan-2-ol (6). A stirred solution of **13** (0.085 g, 0.30 mmol) in dimethoxyethane (3 mL) and anhyd DMF (1 mL) was maintained at rt under argon and treated via cannula with condensed Me_3N. The solution was stirred at room temperature and monitored by TLC (10% EtOH in EtOAc) until completion (ca. 1.75 h) after which time the trimethylammonium salt precipitated. All volatiles were removed under high vacuum to give the intermediate salt as a white solid. A solution of tetrabutylammonium triphenyldifluorosilicate (TBAT, 0.32 mg, 0.60 mmol) dissolved in anhydrous DMF (5 mL) was added to the above salt and the reaction was stirred at room temperature for 3 h. The volatiles were removed under reduced pressure and traces of DMF were removed after several cycles of evaporation with toluene. The residue was purified by flash column chromatography on silica gel using 100% EtOAc, followed by elution with a mixture of EtOAc/10% EtOH to afford **6** (0.065 g, 82%) as a white solid: mp 120–121 °C; $[\alpha]_D^{25}+17.1°$ (*c* 0.32, MeOH). ^{1}H NMR (CD_3OD) δ 9.05 (s, 1H, H-8), 8.68 (d, $J_{2,F}=0.7$ Hz, 1H, H-2), 5.28 (d, $J=6.8$ Hz, 1H, H-4′), 4.97 (t, $J=8.3$ Hz, 1H, H-2′), 4.35 (AB d, $J=11.7$ Hz, 1H, C*H*HOH), 3.41 (AB d, $J=11.7$ Hz, 1H, CH*H*OH), 2.18 (dd, $J=14.8$, 8.0 Hz, 1H, H-3′$_a$), 1.91 (ddd, $J=15.3$, 9.0, 6.6 Hz, 1H, H-3′$_b$), 1.79 (dd, $J=8.3$, 3.4 Hz, 1H, H-5′), 1.15 (dd, $J=5.8$, 3.9 Hz, 1H, H-6′$_{endo}$), 0.89 (dd, $J=8.0$, 6.3 Hz, 1H, H-6′$_{exo}$); ^{13}C NMR (CD_3OD) δ 160.6 (d, $J=257.5$ Hz), 156.5 (d, $J=11.5$ Hz), 152.6 (d, $J=14.1$ Hz), 146.3 (d, $J=3.0$ Hz), 120.9 (d, $J=28.7$ Hz), 71.5, 63.6, 57.4, 38.9, 37.3, 26.7, 10.6; DEPT (CD_3OD) δ 152.4 (d, $J=14.1$ Hz, CH), 146.1 (d, $J=2.9$ Hz, CH), 71.3 (CH), 63.3 (CH_2), 57.1 (CH), 38.6 (CH_2), 26.4 (CH), 10.3 (CH_2); ^{19}F NMR (CD_3OD) δ 0.20. FABMS *m*/*z* (relative intensity) 265 (MH^+, 100), 139 (b+2H, 58); HRMS (FAB, MH^+) calcd for $C_{12}H_{14}FN_4O_2$: 265.1101; found 265.1097.

(1*R*,2*S*,4*S*,5*S*)-[4-Azido-2-(phenylmethoxy)bicyclo[3.1.0]-hexyl] (phenylmethoxy)methane (14). A stirred solution of **9**[7] (0.50 g, 1.61 mmol) in anhydrous DMF (8 mL) was maintained at rt under argon and treated dropwise with diphenylphosphoryl azide (DPPA, 0.85 mL, 4.02 mmol). Immediately after, Et_3N (0.65 mL, 4.35 mmol) was added via syringe and the resulting mixture was heated to 60 °C for 2.5 days. After allowing it to reach rt, the reaction mixture was extracted with Et_2O (4×10 mL), washed with water (2×5 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a yellow oil. Purification by flash column chromatography on silica gel with 1:4 EtOAc/hexanes as eluant afforded **14** (0.42 g,74%) as a pale-yellow oil: IR (neat) 2926, 2095 (N_3), 1099 cm^{-1}. ^{1}H NMR (CDCl3) δ 7.32–7.51 (m, 10H, Ph), 4.57–4.71 (m, 5H, H-2′, 2×PhC*H*$_2$O), 4.00 (d, $J=6.1$ Hz, 1H, H-4′), 3.94 (AB d, $J=10.7$ Hz, 1H, PhCH$_2$O C*H*H), 3.44 (AB d, $J=10.7$ Hz, 1H, PhCH$_2$OCH*H*), 2.17 (dd, $J=14.4$, 7.5 Hz, 1H, H-3′$_a$), 1.56–1.67 (m, 2H, H-3′$_b$, H 5′) 0.96 (dd, $J=5.6$, 4.1 Hz, 1H, H-6′$_{endo}$), 0.78 (dd, $J=8.3$, 5.8 Hz, 1H, H-6′$_{exo}$); ^{13}C NMR ($CDCl_3$) δ 138.7, 138.6, 128.5, 128.4, 127.8, 127.7, 127.6, 79.6, 72.7, 72.5, 71.4, 62.3, 35.2, 32.4, 26.7, 10.1. FAB MS *m*/*z* (relative intensity) 350 (MH^+, 10), 91 (100); HRMS (FAB, MH^+) calcd for $C_{21}H_{24}N_3O_2$ 350.1869; found 350.1874.

(1*S*,2*S*,4*S*,5*R*)-4-(Phenylmethoxy)-5-[(phenylmethoxy)-methyl)bicyclo [3.1.0]hex-2-ylamine (15). A solution of azide **14** (0.26 g, 0.74 mmol) in anhydrous THF (7 mL) maintained under argon was treated with triphenylphosphine (0.49 g, 1.85 mmol) and stirred overnight at room temperature. Water (0.1 mL, 5.5 mmol) was added and the solution was heated to reflux for 4 h. After cooling to room temperature, the solvent was removed under reduced pressure and the crude material was dissolved in Et_2O, dried ($MgSO_4$) and concentrated. The oily residue was chromatographed on silica gel using first EtOAc, followed by 15% MeOH in $CHCl_3$ to afford amine **15** (0.19 g, 80%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.39 (br s, 10H, Ph), 4.54–4.69 (m, 5H, H-4′, 2×PhC*H*$_2$O), 4.00 (AB d, $J=10.2$ Hz, 1H, PhCH$_2$O C*H*H), 3.42 (d, $J=5.8$ Hz, 1H, H-2′), 3.28 (AB d, $J=10.2$ Hz, 1H, PhCH$_2$OCH*H*), 1.66 (br s, 1H, NH_2), 1.65–1.93 (m, 2H, H-3′$_a$), 1.55 (ddd, $J=14.4$, 8.7, 6.3 Hz, 1H, H-3′$_b$), 1.29–1.34 (m, 1H, H-1′), 0.91 (apparent t, $J=5.1$ Hz, 1H, H-6′$_{endo}$), 0.63 (dd, $J=8.0$, 5.8 Hz, 1H, H-6′$_{exo}$); ^{13}C NMR ($CDCl_3$) δ 139.0, 138.7, 128.3 (×2), 127.7, 127.6, 127.5 (×2), 79.3, 72.8, 72.1 (×2), 52.0, 38.4, 32.0, 30.8, 10.7. FAB MS *m*/*z* (relative intensity) 324 (MH^+, 79), 91 (100); HRMS (FAB, MH^+) calcd for $C_{21}H_{26}NO_2$ 324.1964; found 324.1952.

(1*S*,2*S*,4*S*,5*R*)-(5-Amino-6-chloropyrimidin-4-yl){4-(phenylmethoxy)-5 [(phenylmethoxy)methyl]bicyclo[3.1.0]hex-2-yl}amine (16). A mixture of freshly prepared **15** (0.11 g, 0.35 mmol), 5-amino-4,6-dichloropyrimidine (0.08 g, 0.5 mmol), triethylamine (2 mL) and *n*-butanol (3 mL) was refluxed under a blanket of argon for 48 h. After removal of all volatiles, the residue was preadsorbed on silica gel, packed on the top the column and chromatographed with a 1:1 mixture of EtOAc/hexanes to afford the desired product **16** (0.13 g, 81%). ^{1}H NMR ($CDCl_3$) δ 8.10 (s, 1H, H-2), 7.32–7.45 (m, 10H, Ph), 5.22 (d, $J=7.0$ Hz, 1H, H-2′), 4.48–4.65 (m, 5H, H-4′, 2PhC*H*$_2$O), 4.30 (AB d, $J=10.0$ Hz, 1H, PhCH$_2$OC*H*H), 3.21 (AB d, $J=10.0$ Hz, 1H, Ph CH$_2$OCH*H*), 2.19 (dd, $J=13.9$, 7.3 Hz, 1H, H-3′$_a$), 1.65 (ddd, $J=14.4$, 8.7, 5.8 Hz, 1H, H-3′$_b$), 1.45 (dd, $J=8.7$, 4.3 Hz, 1H, H-1′), 1.15 (dd, $J=5.8$, 4.4 Hz, 1H, H-6′$_{endo}$), 0.81 (dd, $J=8.0$, 6.1 Hz, 1H, H-6′$_{exo}$); ^{13}C NMR ($CDCl_3$) δ 153.5, 149.1, 142.0, 138.7, 138.3, 128.6, 128.5, 128.0 (×2), 127.7, 122.0, 78.6, 73.3, 72.2, 72.1, 52.2, 35.2, 32.7, 27.3, 11.0; DEPT ($CDCl_3$) δ 148.2 (CH), 127.7 (CH), 127.5 (CH), 127.1 (CH), 127.0 (CH), 126.8 (CH), 77.6 (CH), 72.3 (CH_2), 71.2 (CH_2), 71.1 (CH_2), 51.2 (CH), 34.1 (CH_2), 26.8 (CH), 9.9 (CH_2). FABMS *m*/*z* (relative intensity) 453 (^{37}Cl MH^+, 33), 451 (MH^+, 100), 91 (97). HRMS (FAB, MH^+) calcd for $C_{25}H_{28}ClN_4O_2$ 451.1901, found 451.1894.

(1*R*,2*S*,4*S*,5*S*)-[4-(7-chloro(1,2,3-triazolo[5,4-*d*]pyrimidin-3-yl))-2-(phenylmethoxy)bicyclo[3.1.0]hexyl](phenylmethoxy)methane (17). Pyrimidine **16** (0.128 g, 0.28 mmol) was dissolved in a mixture of water (1 mL) and acetic acid (2 mL). The solution was cooled to 0 °C, and then a solution of sodium nitrite (0.028 g, 0.4 mmol) in water (0.5 mL) was added dropwise via syringe. The ice bath was removed, and the milky suspension was stirred at room temperature for 3 h. After removal of the volatiles, the crude mixture was pre-adsorbed on silica gel, packed on the top of a silica gel column and chromatographed with 1:1 EtOAc/hexanes as eluant to yield azapurine **17** (0.075 g, 57%). ^{1}H NMR ($CDCl_3$) δ 8.95 (s, 1H, H-2), 7.31–7.44 (m, 10H, Ph), 5.56 (d, J = 7.3 Hz, 1H, H-4′), 5.07 (t, J = 8.0 Hz, 1H, H-2′), 4.51–4.77 (m, 4H, PhCH_2O), 3.88 and 3.75 (AB q, J = 10.2 Hz, 2H, Ph CH_2OCH_2), 2.69 (dd, J = 14.9, 7.8 Hz, 1H, H-3′$_a$), 2.08–2.21 (m, apparent pentuplet, 1H, H-3′$_b$), 1.77 (dd, J = 8.3, 3.9, 1H, H-5′), 1.26 (dd, J = 5.8, 4.1 Hz, 1H, H-6′$_{endo}$), 1.08 (dd, J = 8.3, 6.1 Hz, 1H, H-6′$_{exo}$); ^{13}C NMR ($CDCl_3$) δ 155.2, 154.1, 149.3, 138.6, 138.4, 134.3, 128.4, 128.3, 127.9, 127.7, 127.5 (×2), 81.1, 72.8, 72.7, 71.2, 59.8, 35.7, 33.8, 27.6, 10.3; DEPT ($CDCl_3$) δ 155.3 (CH), 128.5 (CH), 128.4 (CH), 127.9 (CH), 127.8 (CH), 127.6 (×2CH), 81.1 (CH), 72.8 (CH_2), 72.7 (CH_2), 71.2 (CH_2), 59.7 (CH), 35.7 (CH_2), 27.5 (CH), 10.2 (CH_2). FABMS m/z (relative intensity) 462 (MH^+, 36); 91 (100). HRMS (FAB, MH^+) calcd for $C_{25}H_{25}ClN_5O_2$ 462.1697, found 462.1697.

(1*R*,2*S*,4*S*,5*S*)-1-(Phenylmethoxy)methyl-2-(Phenylmethoxy)bicyclo[3.1.0]hex-4-yl)-1,2,3-triazolino[4,5-d]pyrimidine-7-ylamine (18). Chloroazapurine **17** (0.075 g, 0.16 mmol) was dissolved in a minimum amount of MeOH and added to a saturated NH_3/MeOH solution (10 mL). The resulting solution was heated in a sealed pressure Pyrex tube at 80 °C for 22 h. After cooling, the solution was evaporated and co-evapored twice with MeOH (10 mL). The crude mixture was pre-adsorbed on silica gel, packed on top of a silica gel column and chromatographed with EtOAc as eluant to give **18** (0.064 g, 90%) as an off-white solid: mp 141 °C; ^{1}H NMR ($CDCl_3$) δ 8.50 (s, 1H, H-2), 7.28–7.44 (m, 10H, Ph), 6.96 (br s, 2H, NH_2), 5.43 (d, J = 7.3 Hz, 1H, H-4′), 5.10 (t, J = 8.0 Hz, 1H, H-2′), 4.52–4.78 (m, 4H, 2×PhCH_2O), 3.90 and 3.75 (AB q, J = 10.5 Hz, 2H, $PhCH_2OCH_2$), 2.68 (dd, J = 14.4, 7.5 Hz, 1H, H-3′$_a$), 2.03–2.16 (m, apparent pentuplet, 1H, H-3′$_b$), 1.76 (dd, J = 8.3, 3.9, 1H, H-5′), 1.22 (dd, J = 5.6, 4.3 Hz, 1H, H-6′$_{endo}$), 1.03 (dd, J = 8.3, 6.3 Hz, 1H, H-6′$_{exo}$); ^{13}C NMR ($CDCl_3$) δ 156.4, 156.0, 148.8, 138.8, 138.6, 128.4 (×2), 127.8, 127.7, 127.6, 127.5, 124.7, 81.3, 72.7, 72.6, 71.6, 58.5, 35.7, 33.6, 27.9, 10.2; DEPT ($CDCl_3$) δ 156.5 (CH), 128.5 (CH), 128.4 (CH), 127.9 (CH), 127.7 (CH), 127.6 (×2CH), 81.3 (CH), 72.7 (CH_2), 72.6 (CH_2), 71.6 (CH_2), 58.5 (CH), 35.7 (CH_2), 27.8 (CH), 10.2 (CH_2). FABMS m/z (relative intensity) 443 (MH^+, 100); 91 (97); HRMS (FAB, MH^+) calcd for $C_{25}H_{27}N_6O_2$ 443.2195, found 443.2184.

(1*R*,2*S*,4*S*,5*S*)-4-(7-Amino(1,2,3-triazolo[4,5-d]pyrimidin-3-yl))-1-(hydroxymethyl)bicyclo[3.1.0]hexan-2-ol (7). Compound **18** (0.060 g, 0.13 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL), cooled to −78 °C and maintained under argon with stirring. To this solution, BCl_3 (1 M/CH_2Cl_2, 1.5 mL) was added and the reaction mixture was stirred for 5 h. After allowing the temperature to reach −20 °C, stirring was continued for an additional 1 h. MeOH (5 mL) was added and the solution was evaporated to dryness. The resulting crude product was neutralized with saturated methanolic ammonia and reduced to dryness again. The residue was purified by reverse phase chromatography (Baker octadecyl C-18) using 15% MeOH in water to give **7** (0.025 g, 75%) as an off-white solid: mp 178 °C; $[\alpha]_D^{25}$ −80° (c 0.15, DMSO). ^{1}H NMR (DMSO-d_6) δ 8.41 (br s, 1H, N*H*H, D_2O exchanged), 8.32 (s, 1H, H-2), 8.10 (br s, 1H, NH*H*, D_2O exchanged), 5.21 (d, J = 7.0 Hz, 1H, H-4′), 4.88 (t, J = 8.3 Hz, 1H, H-2′), 4.73 (d, J = 5.8, 1H, OH, D_2O exchanged), 4.44 (dd, J = 6.3, 4.8, 1H, OH, D_2O exchanged), 3.81 (dd, J = 11.4, 6.1 Hz, 1H, C*H*HOH) 3.46 (dd, J = 11.4, 4.3 Hz, 1H, CH*H*OH), 2.34 (dd, J = 14.8, 7.8 Hz, 1H, H-3′$_a$), 1.82–1.94 (m, 1H, apparent pentuplet, 1H, H-3′$_b$), 1.57 (dd, J = 8.3, 3.4, 1H, H-5′), 0.92 (dd, apparent t, J = 4.8 Hz, 1H, H-6′$_{endo}$), 0.73 (dd, J = 8.0, 5.1, 1H, H-6′$_{exo}$); ^{13}C NMR (DMSO-d_6) δ 156.2, 156.1, 148.0, 124.0, 71.3, 61.2, 58.0, 36.6, 35.9, 25.5, 8.3. HRMS (FAB, MH^+) calcd for $C_{11}H_{15}N_6O_2$ 263.1256, found 263.1253.

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