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"Molecular Chameleons". Design and Synthesis of a Second Series of Flexible Nucleosides

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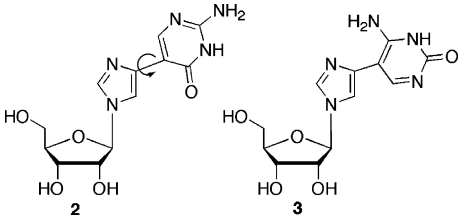

The second series of flexible shape-modified nucleosides is introduced. The "fleximers" feature the purine ring systems split into their individual imidazole and pyrimidine components. This structural modification serves to introduce flexibility to the nucleoside while still retaining the elements essential for recognition. As a consequence, these structurally innovative nucleosides can more readily adapt to their environment and should find use as bioprobes for investigating enzyme−coenzyme binding sites as well as nucleic acid and protein interactions. Their design and synthesis is described.

Introduction

One focus for our research has involved the design and synthesis of novel shape-modified nucleosides to explore fundamental aspects of nucleic acid structure, function, and stability, as well as to investigate enzyme binding site parameters. With the increasing number of crystal structures for various enzyme−substrate complexes, it has become apparent that many binding sites are more flexible than previously thought and can therefore adjust to fit a wide range of substrates.[1−3] This phenomenon causes inaccuracies when employing structure-based drug design of a potential enzyme−substrate complex from a crystallographic basis; although the fit may appear to be good, in reality the "best guess" drug often proves to be a poor inhibitor.

Significant to this observation, recent reports have shown that (i) flexible inhibitors can overcome drug resistance mutations in viral HIV[4−7] and (ii) the binding site of *S*-adenosylhomocysteine hydrolase (SAHase), an enzyme critical in the replication mechanism of viruses, exhibits a significant difference between the "open" and "closed" conformations.[8] As a possible means to explore this phenomenon, we have designed a series of structurally innovative nucleosides that possess a heteroaromatic purine ring split into its two components (for example, an imidazole and pyrimidine ring), thereby conferring additional degrees of conformational freedom and torsional flexibility to the ligand. As a result, these "molecular chameleons" can readily adapt to the environment of the flexible binding site in order to maximize complementary structural interactions, without losing the integrity of the basic scaffold required for the enzyme's mechanism of action.

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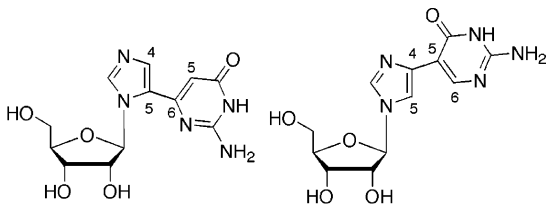

FIGURE 1. C-5- and C-4-substituted guanosine fleximers.

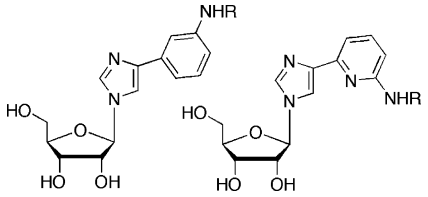

FIGURE 2. Weisz's C-4-substituted analogues.

The lead provided by the unusual inhibitory activity exhibited by our guanosine C-5 substituted imidazole fleximer **1** (Figure 1) against *S*-adenosylhomocysteine hydrolase (SAHase), an enzyme that recognizes adenosine analogues, prompted us to expand our initial efforts with these structurally unique analogues and this biologically significant enzyme. In contrast to our previously reported[9−11] C-5-substituted imidazole fleximers, the C-4 series is connected at the C-4 of the imidazole and the C-5 of the pyrimidine (as in **2**, Figure 1), rather than the C-5 of the imidazole and the C-6 of the pyrimidine (as in **1**).

To our knowledge, the only other example in the literature of a split nucleoside such as these was recently introduced by Weisz et al.;[12,13] however, as shown in Figure 2, the connectivity of the C-4 of the imidazole is substituted with either a pyridine or a benzene ring attached at the C-3 of the benzene ring (or the C-2 of the pyridine ring), rather than C-5 or C-6 of the pyrimidine ring, thereby differing from the standard purine motif. As a result, our analogues are more faithful to a classic purine design, and as such, they will provide a unique perspective on enzyme/ligand interactions. Their synthesis is described herein.

Results and Discussion

The aforementioned unusual activity exhibited[11] by our C-5-substituted imidazole guanosine fleximer (**1**) against SAHase, as well as the interesting conformation it adopted in our modeling studies when bound into the SAHase active site, prompted us to investigate the connectivity for the fleximer bond. Our modeling studies,[10,11,14] as well as other computational studies[15] involving our fleximers, had suggested that the C-4-substituted imidazole fleximers would adopt quite a different conformation than the C-5 analogues, so we were anxious to explore this experimentally. We also wanted to explore the arrangement of the substituents, since the "twisted" guanosine fleximer closely resembles the isoguanosine (isoG) ring system (Figure 3). As a result, it occurred to us that if we "prearranged" the substituents in the conformation they appeared to prefer, it might result in a more favorable binding energy, since theoretically the pyrimidine would not have to expend any energy to attain the desired conformation.

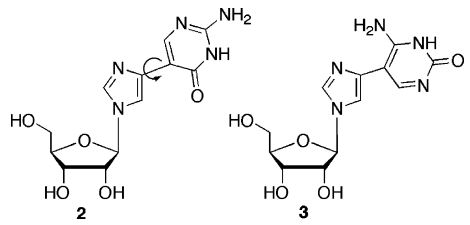

FIGURE 3. Twisted G and isoG fleximers.

Our initial calculations in SAHase had shown that the binding energy for the C-5 guanosine fleximer was −24.88 kcal/mol, but these had been carried out on a lower level of theory, using a class I force field (CVFF), so in an effort to increase our accuracy, we moved to a class II force field[16,17] (CFF) and repeated the calculations. The new results all showed lower binding energies than for the previous force field with the new energy for the C-5 guanosine being used as the reference in this study. The corresponding C-4 guanosine fleximer was 5.36 kcal/mol lower in energy that the C-5 guansosine; however, as we predicted, both the C-5- and C-4-substituted isoG's exhibited much more favorable binding energies. Relative to the C-5 guanosine fleximer, the C-5 and C-4 isoG's were more favorable by −20.59 and −21.97 kcal/mol, respectively.

Analogous to isoguanosine,[18] the isoG fleximers each have two tautomeric forms, so it was important for us to determine which would be preferred, since it would affect the hydrogen bonding and recognition.[19,20] Our calculations showed that when bound in the active site of SAHase, the C-5-substituted isoG fleximer preferred to exist as the N-3-H tautomer (shown in Figure 4), with a difference in energy between the two tautomers of 18.09 kcal/mol, while the C-4 isoG fleximer preferred the N-1-H tautomeric form (Figure 5) with a relative binding energy of −23.08 kcal/mol lower than the N-3-H tautomer.

Figure 6 depicts the C-5- and C-4-substituted imidazole G's, as well as the C-5- and C-4-substituted imidazole isoG's aligned in the binding site of SAHase by the residues they interact with. As mentioned above, the C-5 and C-4 isoG fleximers exhibit the most favorable binding energy values, but it is clear that they are both capable

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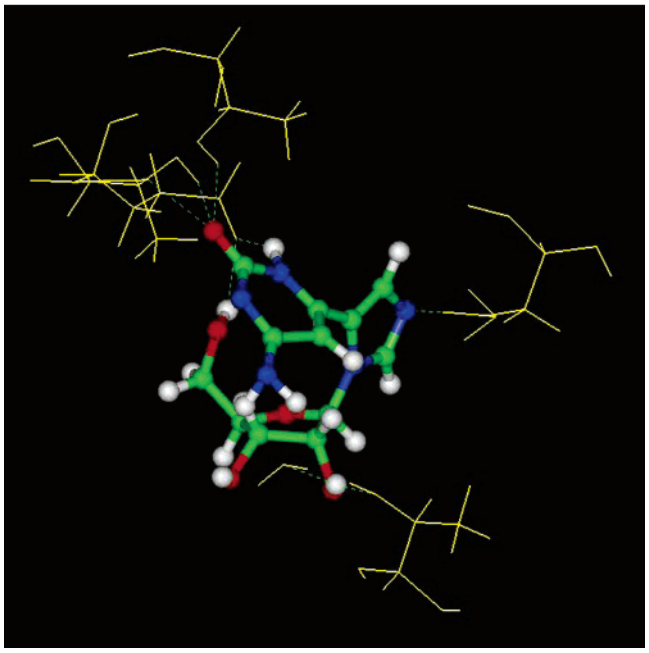


FIGURE 4. N3(H) tautomer for the C-5 IsoG fleximer in SAHase.

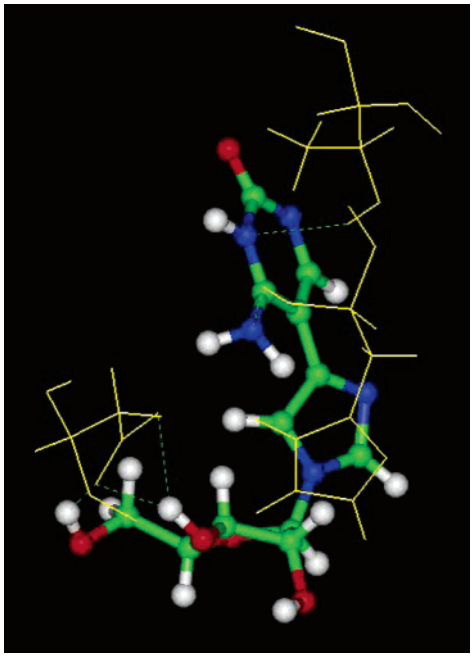


FIGURE 5. N1(H) tautomer for the C-4 IsoG fleximer in SAHase.

of adopting a significantly different conformation from each other, while still maintaining critical contacts necessary for recognition.

In light of this, we focused our initial efforts on the synthesis of the C-4-substituted guanosine (**2**) and isoG (**3**) analogues. While **2** and **3** could not be realized directly due to complications with the exocyclic amine group, they could be obtained by manipulation of either the diamino (**4**) or xanthosine (**5**) fleximers (Figure 7), which were of interest to us as well as they would allow a complete comparison of the effect of the amino and carbonyl groups.

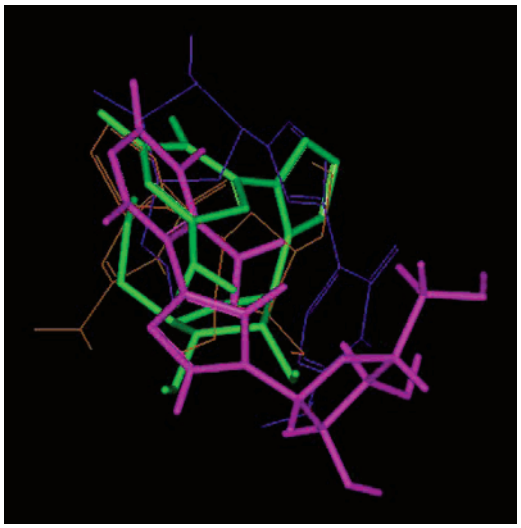


FIGURE 6. Relative energies of C-4- and C-5-substituted G and IsoG fleximers in SAHase: orange, C-5 guanosine fleximer, ΔG = reference; purple, C-4 guanosine fleximer, $\Delta G = -5.36$ kcal/mol; green, C-5 isoguanosine fleximer, $\Delta G = -20.59$ kcal/mol; pink: C-4 isoguanosine fleximer, $\Delta G = -21.97$ kcal/mol.

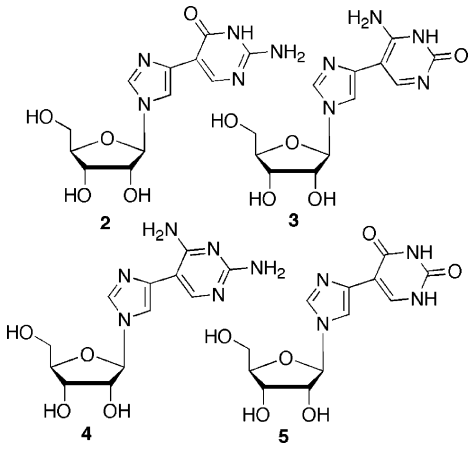
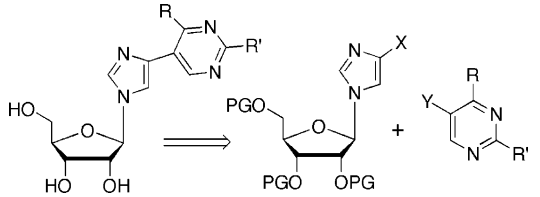


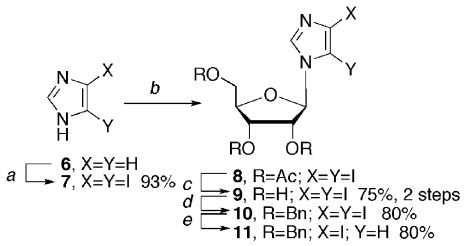
FIGURE 7. C-4-substituted fleximer targets.

SCHEME 1. Cross-Coupling Approaches: Stille, Negishi, Kumada, Grignard, and Suzuki



The initial approach to realize the C-4-substituted fleximer scaffold was envisioned from traditional organometallic coupling methods to attach the imidazole and pyrimidine rings (as depicted above in Scheme 1). The plethora of available catalysts and coupling methods, including Stille, Kumada, Negishi, Grignard, and Suzuki, made this effort more attractive and straightforward than the nontrivial and tedious linear synthesis that had been required to attain the C-5-substituted fleximers. While there were examples of both the imidazole and the pyrimidine components serving as either the electrophilic or nucleophilic coupling partner, we first chose to attempt

SCHEME 2[a]



[a] Reaction conditions: (a) KCl, ICl; (b) (i) β-D-ribofuranose 1,2,3,5-*O*-tetraacetate, BSA, (ii) TMSOTf; (c) NH_4OH, EtOH, 18 h; (d) (i) NaH, THF, 0 °C, 3 h; (ii) BnBr, TBAI; (e) EtMgBr, Et_2O, 3 h.

coupling with the imidazole moiety as the nucleophilic species, since we had ready access to the necessary starting materials and many of the examples using this approach had produced results in reasonably good yields.

Since many of the routes appeared to have a bias for the C-4 iodo-substituted imidazole rather than with the analogous 4-bromoimidazole for coupling procedures, we adapted our previously reported route,[9,10] which had featured a dibromoimidazole ribofuranosyl intermediate. This proved to be advantageous, since the workup and purification steps for the diiodoimidazole analogue were much less tedious than for the dibromoimidazole analogue.

Synthesis began with replacement of the C-4 and C-5 hydrogens with iodine using a facile literature procedure[21] to give **7** in 93% yield (Scheme 2). Next, coupling diiodoimidazole **7** with commercially available tetraacetate-protected ribofuranose using bis(trimethylsilyl)-acetamide (BSA) and trimethylsilyltriflate (TMSOTf) gave **8**.[9,10] It was then necessary to replace the acetate groups with a more robust protecting group, due to the various reaction conditions that would be encountered in subsequent steps that would not be tolerated by either an alcohol or ester functionality. Standard base-promoted deprotection afforded **9** in a 75% overall yield for the two steps. Reprotection of the three hydroxyls with the in situ[22] formation of benzyliodide, a method we have found to be superior to other traditionally used routes when attempting to benzylate all three hydroxyls, was then accomplished to give **10** (80% yield), followed by displacement of the C-5 iodide using Grignard conditions,[23] which yielded **11** also in an 80% yield.

All of the desired targets were available from the xanthosine fleximer. As a result, the Kumada and Negishi were the first cross-coupling methods tried as shown in Scheme 3 on the next page.[24,25] Unfortunately, all efforts to construct the C-4-substituted scaffold in this manner proved fruitless; altering the nature of the halouracil derivative, the choice of solvent, the reaction temperature, or the palladium catalyst failed to yield the desired intermediate, from which all four of the target fleximers could be realized. The failure of the Negishi conditions surprised us, since Negishi coupling had been reported[26] to work well with C-4 iodo-substituted imidazoles and was more tolerant of sensitive functional groups than traditional Grignard and organolithium approaches. We speculated that it could have been due to the π-electron-deficient nature of the dimethyluracil since we were successful in forming the nucleophilic imidazole moiety, both in situ and as an isolated intermediate. As a result, it appeared that the pyrimidine system was simply insufficiently activated to participate in the cross-coupling.

We turned next to the Stille[27−31] and Suzuki[32,33] coupling methods, as there were examples in the literature that had been obtained in reasonable yields. In some respects, Stille coupling has many advantages that made it an attractive choice: the conditions are tolerant of many functional groups, and the stannanes are readily prepared and purified and are known to be fairly stable.[27,28,33] Unfortunately, all efforts with Stille or Suzuki conditions failed; however, use of *n*-BuLi and EtMgBr provided the dehalogenated imidazole nucleoside **14** in a 90% yield (Scheme 4 on the next page).

At this point, we reversed our strategy to employ the pyrimidine as the nucleophilic component in an effort to obtain the critical xanthosine intermediate. Once again using traditional coupling approaches, we attempted to couple **11** to a variety of organometallic pyrimidine species, including the pyrimidinylstannane **15**,[34,35] as well as the corresponding Kumada and Negishi metalated pyrimidines (**16** and **17**, respectively, Scheme 5 on the next page).[24,25] Pyrimidinylstannane **15** was isolated in reasonable yield (66%) but failed to undergo coupling with the usual palladium reagents. A procedure[34] employing copper(I)thiophene carboxylate and 1-methyl-2-pyrrolidinone (NMP) was also tried, unfortunately, to the same end so this route was abandoned as well.

We speculated that the inherent electron-deficient nature of the pyrimidine ring system was the cause for the significant lack of reactivity noted to date. If this was indeed true, it was clear that to be successful, the electronics of the pyrimidine ring system had to be enhanced; therefore, electron-donating substituents such as ethers or thioethers might prove to be a possible solution. Suzuki coupling with 5-bromo-2,4-bis(methylthio)pyrimidine was tried,[37] but NMR and MS analysis of the product proved it to be a mixture of dimerized pyrimidines, which were the result of self-cross coupling at C-5/C-5′ and C-5/C-6′, a fate apparently not uncommon for boronic acid pyrimidines.[38]

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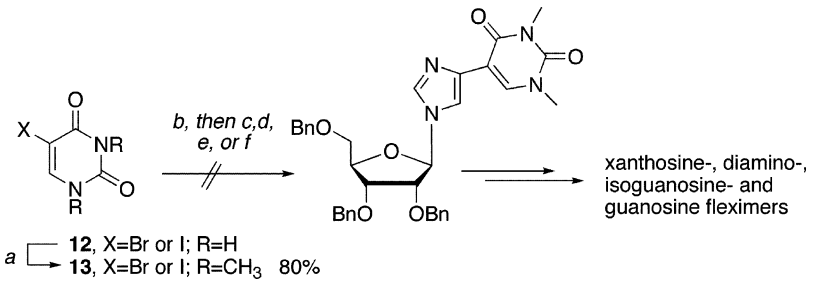
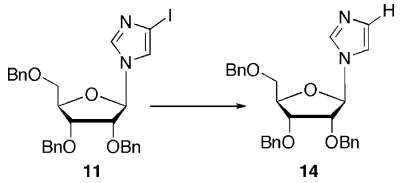
SCHEME 3[a]



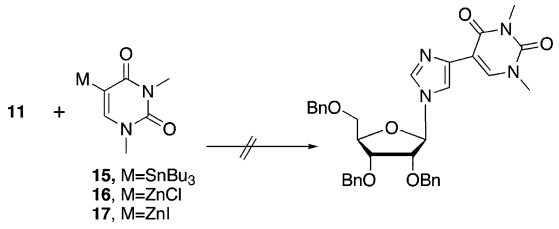
[a] Reaction conditions: (a) Me_2SO_4, NaOH, H_2O; (b) (i) **11** with EtMgBr or $ZnCl_2$ in Et_2O, then (ii) **13** with (c) $PdCl_2$(dppf); (e) $Pd(PPh_3)_4$; or (f) $Pd_2(dba)_3$.

SCHEME 4[a]



[a] Reaction conditions: *n*-BuLi, EtMgBr.

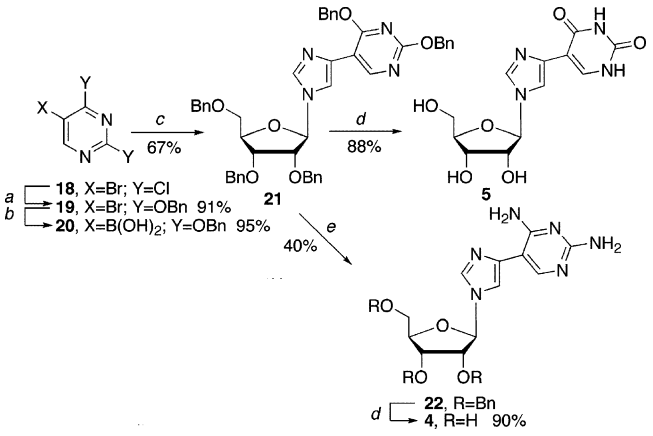
SCHEME 5[a]



[a] Reaction conditions: for **15**: (i) **13**, $(Bu_3Sn)_2$, 66%; then (ii) **11**, $Pd(PPh_3)_4$, CuI, DMF, or DME, 80 °C, NR; or (ii) **11**, $(PPh_3)_2PdCl_2$, CH_3CN, reflux, NR; or (ii) **11**, copper(I)thiophenecarboxylate, NMP, 0 °C to rt; for **16** or **17**: (i) **13**, EtMgBr, Et_2O, $ZnCl_2$, then (ii) **11**, (dppf)$PdCl_2$, DMF or (ii) **11**, $Pd(PPh_3)_4$, CuI, DMF, NR.

Attention then turned to the analogous dibenzyloxyether pyrimidine system. Beginning with 5-bromo-2,4-dichloropyrimidine (**18**) that is commercially available, but is also an intermediate in the synthesis[36] of 5-bromo-2,4-bis(thiomethyl)pyrimidine, conversion to the dibenzyloxy analogue **19** (Scheme 6) was accomplished in excellent yield (91%) using standard benzylation conditions, followed by conversion to the boronic acid **20**, which was also obtained in excellent yield (95%).[39] Coupling was then successfully carried out with tetrakis (triphenylphosphine)palladium (0) to give **21** (67%).[40] Removal of all five of the benzyl groups was accomplished in 88% yield to give the xanthosine fleximer **5** directly.[41] Alternatively, treatment of **21** with ammonia in butanol in a steel Parr bomb afforded **22** in moderate yield (40%), which, followed by deprotection,[10] gave the desired diamino fleximer **4** (90%).[42]

SCHEME 6[a]



[a] Reaction conditions: (a) NaH, BnOH; (b) (i) $B(O^iPr)_3$, THF/toluene (1:4), (ii) *n*-BuLi, THF, −78 °C, (iii) aq HCl; (c) $Pd(PPh_3)_4$, 1,2-dimethoxyethane (DME), $NaHCO_3$, reflux; (d) 10% NH_3, BuOH, heat.

With the first two C-4-substituted fleximers finally in hand, the remaining task was to manipulate the diamino fleximer to give us the guanosine and isoguanosine fleximers we had initially set our sights on. The C-4-substituted isoguanosine fleximer **3** was realized following selective conversion of the C-2 amino group of the protected diamino fleximer **22** to the desired carbonyl. As shown in Scheme 7, formation of the diazonium using standard conditions, followed by hydrolysis, and subsequent deblocking of the benzyl protecting groups gave **3** in a 41% yield (two steps).[10,41,42] Finally, the diamino fleximer **4** was converted directly to **2** using aqueous sodium bisulfite and heat in 88% yield.[37]

In summary, a series of traditional cross-coupling methods using a variety of catalysts and conditions were explored to realize the synthesis of the second series of fleximers. It is clear from the efforts outlined herein that the imidazole and pyrimidine ring systems are recalcitrant at best and that work remains to fully understand the ideal conditions to manipulate these heterocycles more efficiently. We are, however, encouraged by the reasonably good results finally obtained with the Suzuki system, and we will continue to optimize this promising route.

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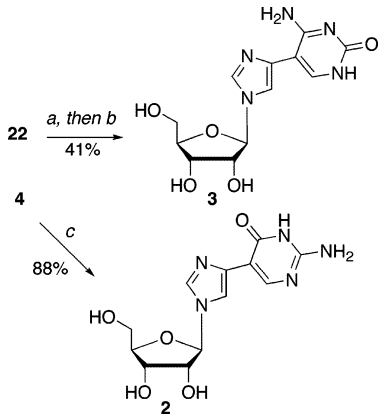
SCHEME 7[a]



[a] Reaction conditions: (a) $NaNO_2$, AcOH, H_2O/THF (1:1); (b) Pd/C, HCO_2NH_4, EtOH, reflux; (c) $NaHSO_3$, H_2O.

With the first four C-4 substituted fleximers in hand, we will be able to continue our investigations into exploring the confines of biologically significant enzymes, and by comparing the results already obtained with the C-5 substituted fleximers to see if altering the position of the fleximer bond has a significant effect on recognition with SAHase and the other enzymes we are studying. In the meantime, efforts are also underway to realize the C-4-substituted adenosine and inosine fleximers from a different route, since those are of interest to us as well. Results of those efforts and the results of the broad screen antiviral, anticancer, and antiparasitic testing presently underway will be reported in the future.

Experimental Section

Modeling. All potential inhibitors were docked into the SAHase crystal structure (PDB 1A7A) and calculations performed on an SGI Octane2 using Accelrys InsightII/Discover with a CFF force field, which is derived from ab initio calculations on the Hartree−Fock level of theory using the 6-31G* basis set. The inhibitors were optimized using steepest descent calculations. Then, using Monte Carlo techniques, up to 20 spatial conformers were generated for each inhibitor. The 10 lowest energy conformations were then minimized using simulated annealing techniques. The final structures were analyzed for the best ligand structure with the lowest energy value. The overlapped images depicted in Figure 6 are the best final structures docked into the SAHase binding site. The nonessential hydrogens have been removed for clarity.

General Procedures. Melting points are uncorrected. ^1H and ^{13}C spectra were operated at 300 and 75 MHz, respectively, all referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q(quartet), m (multiplet), and b (broad). Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm Whatman Diamond silica gel 60-F_{254} precoated plates. Column chromatography was performed on Whatman silica, 200−400 mesh, 60 and elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^1H and ^{13}C NMR) homogeneous materials.

4,5-Diiodoimidazole (7).[21] An aqueous solution (500 mL) of imidazole (30.0 g, 0.441 mol) was added dropwise to a solution of $KICl_2$ (2.0 M, 550 mL, 1.1 mol, prepared from dissolving ICl (79.9 g, 1.03 mol) in an aqueous solution of KCl (131.5 g, 1.77 mol in 550 mL of H_2O) at rt. The mixture was stirred for an additional 10 h, followed by slow addition of 2 M NaOH solution until the suspension was completely dissolved. The clear solution was then acidified to pH 9.50 by dropwise addition of concentrated HCl. The resulting product was filtered to afford an off-white powder, which, following recrystallization in EtOH, gave **7** as a white crystalline solid (131.2 g, 93%): mp 188−191 °C; ^1H NMR (DMSO-d_6) δ 7.76 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 141.1, 143.2.

2,3-Diacetoxy-5-acetoxymethyl-1-(4,5-diiodoimidazol-3-yl)-β-D-ribofuranose (8).[9,10] *N*,*O*-Bis(trimethylsilyl)acetamide (BSA) (73.9 mL, 0.30 mol) was added to a stirred solution of **7** (50.0 g, 156 mmol) and 1,2,3,5-tetra-*O*-acetate-β-D-ribofuranose (50.0 g, 157 mmol) in anhydrous acetonitrile (500 mL). The mixture was stirred for an additional 4 h at rt under Ar. The solution was then cooled to 0 °C, at which point trimethylsilyl trifluromethylsulfonate (TMSOTf) (31 mL, 172 mmol) was added dropwise. The mixture was heated at 60 °C under Ar for 18 h. The solvent was removed under reduced pressure, and the resulting residue was cooled to 0 °C, followed by portionwise addition of aqueous $NaHCO_3$ until production of gas ceased. The mixture was extracted with CH_2Cl_2 (3 × 400 mL); the organic extracts were combined, washed with brine (2 × 300 mL), and dried over $MgSO_4$; and the solvent was removed under reduced pressure to give **8** as a light brown syrup (67.6 g), which was used without further purification.

(4,5-Diiodoimidazol-3-yl)-1-β-D-ribofuranose (9). A mixture of crude **8** (67.6 g, 0.117 mol) and concentrated ammonium hydroxide (28%, 500 mL) in EtOH (300 mL) was stirred at rt for 18 h. The resulting precipitate was filtered and washed with cold H_2O to afford **9** as a white crystalline solid (52.8 g, 75% for two steps): ^1H NMR (DMSO-d_6) δ 3.49 (dd, 3.9 Hz, 12.0 Hz, 1 H), 3.57 (dd, 3.9 Hz, 12.0 Hz, 1 H), 3.88 (q, 3.6 Hz, 1 H), 4.01 (t, 3.6 Hz, 1 H), 4.24 (t, 5.0 Hz, 1 H), 5.19 (t, 5.1 Hz, 1 H), 5.33 (d, 4.8 Hz, 1 H), 5.46 (d, 5.1 Hz, 1 H), 5.60 (d, 6.3 Hz, 1 H), 8.15 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 61.1, 70.2, 75.0, 85.6, 92.4, 97.3, 130.5, 140.8.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-(4,5-diiodoimidazol-3-yl)-1-β-D-ribofuranose (10). To a stirred solution of **9** (21.0 g, 45.9 mmol) in anhydrous THF (300 mL) at 0 °C was added NaH (95% dry, 4.06 g, 161 mmol) in small portions over a period of 10 min. The resulting mixture was stirred at rt for 3 h, at which point tetrabutylammonium iodide (3.4 g, 9.20 mmol) was added, followed by dropwise addition of benzyl bromide (19.1 mL, 161 mmol).[22] The new mixture was stirred at rt for 18 h. The solvent was then removed under reduced pressure, and saturated aqueous NH_4Cl solution (200 mL) was added to the residue. The mixture was extracted with CH_2Cl_2 (3 × 250 mL); the organic extracts were combined, washed with brine (2 × 300 mL), and dried over $MgSO_4$; and the solvent was removed under reduced pressure to give a yellow syrup. Column chromatography eluting with hexane/EtOAc (3:1) gave **10** as a white crystalline solid (26.5 g, 80% yield): mp 86−88 °C; ^1H NMR ($CDCl_3$) δ 3.53 (dd, 2.5 Hz, 10.8 Hz, 1 H), 3.75 (dd, 2.4 Hz, 10.8 Hz, 1 H), 4.08−4.18 (m, 2 H), 4.31−4.34 (m, 1 H), 4.44−4.70 (m, 6 H), 5.82 (d, 3.6 Hz, 1 H), 7.21−7.37 (m, 15 H), 7.99 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 66.3, 72.6, 72.9, 73.5, 75.7, 79.8, 80.9, 81.9, 92.0, 127.8, 127.9, 128.2, 128.5, 128.6, 136.9, 137.2, 140.3. Anal. Calcd for $C_{29}H_{28}I_2N_2O_4$: C, 48.22; H, 3.91; N, 3.88; I, 35.14. Found: C, 48.48; H, 3.91; N, 3.86; I, 35.41.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-(4-iodoimidazol-3-yl)-1-β-D-ribofuranose (11). To a solution of **10** (34.7 g, 48.0 mmol) in anhydrous ether (500 mL) at rt was added EtMgBr (3.0 M, 19 mL, 57 mmol) in a dropwise manner.[23] The mixture was stirred at rt for 3 h and then quenched with anhydrous EtOH (50 mL). The solvent was removed under reduced pressure, saturated aqueous NH_4Cl solution (300 mL) added, and the mixture extracted with CH_2Cl_2 (3 × 200 mL). The organic layers were combined, washed with brine (2 × 400 mL), and dried over $MgSO_4$, and the solvent was removed under reduced pressure. Column chromatography of the residue, eluting with hexane/EtOAc (3:1), gave **11** as a colorless syrup (23 g, 80%): ^1H NMR ($CDCl_3$) δ 3.51 (dd, 2.4 Hz, 10.8 Hz, 1 H), 3.67 (dd, 3.0 Hz, 10.8 Hz, 1 H), 4.04−4.16 (m, 3 H),

4.30−4.66 (m, 6 H), 5.69 (d, 6.0 Hz, 1 H), 7.03 (d, 1.2 Hz, 1 H), 7.11 (dd, 2.0 Hz, 7.8 Hz, 2 H), 7.21−7.42 (m, 13 H), 7.46 (d, 1.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 60.3, 69.7, 72.3, 72.6, 73.7, 76.5, 81.6, 82.6, 88.8, 121.9, 127.8, 127.9, 128.0, 128.2, 128.5, 128.6, 136.6, 137.1, 137.2, 137.5. Anal. Calcd for $C_{29}H_{29}IN_2O_4$: C, 58.40; H, 4.90; N, 4.70; I, 21.28. Found: C, 58.29; H, 4.93; N, 4.61; I, 21.06.

5-Iodo-1,3-dimethyluracil (13). Dimethyl sulfate (13.0 mL, 137 mmol) was added dropwise to a stirring slurry of 5-iodoracil **12** (14.9 g, 62.6 mmol) and NaOH (7.4 g, 156 mmol) in H_2O (100 mL) at 0 °C.[34] The mixture was heated under reflux for 2 h, cooled to rt, and extracted with CH_2Cl_2 (3 × 200 mL). The organic layers were combined, washed with brine (300 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a white solid, which, following recrystallization in hot EtOH, gave **13** as a white crystalline solid (10.6 g, 63%): mp 224 °C (Aldrich, 225 °C); ^{1}H NMR ($CDCl_3$) δ 3.40 (s, 3 H), 3.41 (s, 3 H), 7.62 (s, 1 H); ^{13}C NMR ($CDCl_3$) 29.5, 37.3, 67.1, 147.2, 151.2, 160.2.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-(imidazol-3-yl)-1-β-D-ribofuranose (14). EtMgBr (3.0 M in ethyl ether, 0.62 mL, 1.84 mmol) was added dropwise to a solution of **11** (1.0 g, 1.67 mmol) in anhydrous CH_2Cl_2 (15 mL) at rt under Ar. The resulting solution was stirred at rt for 2 h, followed by dropwise addition of trimethyltin chloride (1.0 M in CH_2Cl_2, 2.0 mL, 2.0 mmol) at rt.[35] The mixture was allowed to stir at rt for 18 h and then quenched with water. The organic layer was separated and the aqueous layer washed with CH_2Cl_2 (2 × 20 mL). The organic extracts were combined and washed sequentially with saturated KF solution (30 mL) to form insoluble organotin fluoride and then brine (50 mL). The organic layer was dried over $MgSO_4$ and the solvent removed to afford **14** as a colorless syrup (714 mg, 91%): ^{1}H NMR ($CDCl_3$) δ 3.48 (dd, 2.4 Hz, 10.8 Hz, 1 H), 3.62 (dd, 3.0 Hz, 10.8 Hz, 1 H), 4.03 (dd, 2.8 Hz, 4.8 Hz, 1 H), 4.10 (br t, 5.4 Hz, 1 H), 4.29 (dd, 2.8 Hz, 5.4 Hz, 1 H), 4.36−4.61 (m, 6 H), 5.74 (d, 6.4 Hz, 1 H), 6.98 (d, 6.8 Hz, 2 H), 7.10−7.32 (m, 15 H), 7.59 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 69.5, 72.0, 72.3, 73.3, 76.4, 81.4, 82.1, 88.5, 116.1, 127.3, 127.4,-127.5, 127.6, 127.7, 128.1, 129.3, 135.7, 136.5, 137.0, 137.1.

5-Tri-*n*-butylstannyl-1,3-dimethyluracil (15). A mixture of 5-iodo-1,3-dimethyluracil (**13**) (2.66 g, 10.0 mmol), bis-(tributyltin) (10.1 mL, 20.0 mmol), and $PdCl_2(PPh_3)_4$ (280 mg, 0.40 mmol) in anhydrous toluene (300 mL) was heated under Ar at 90 °C for 4 h.[35] The mixture was filtered through a pad of alumina, the solvent removed under reduced pressure, and the residue purified by column chromatography (silica gel pretreated with 5% triethylamine in hexanes), eluting with 2% triethylamine in hexanes, to afford 5-tri-*n*-butylstannyl-1,3-dimethyluracil (**15**) as a colorless oil (2.7 g, 62%): ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 7.2 Hz, 9 H), 1.03 (t, 8.4 Hz, 6 H), 1.31 (q, 7.6 Hz, 6 H), 1.50 (q, 8.4 Hz, 6 H), 3.32 (s, 3 H), 3.36 (s, 3 H), 6.87 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 9.9, 13.8, 27.4, 27.7, 29.1, 36.8, 111.0, 146.7, 152.4, 166.5.

2,4-Dibenzyloxy-5-bromopyrimidine (19).[39] A stirred solution of benzyl alcohol (13.4 mL, 129 mmol) in anhydrous toluene (140 mL) was treated with NaH (60% in mineral oil, 4.84 g, 121 mmol) under Ar. The mixture was warmed to 50 °C to facilitate the formation of the sodium salt and stirred until all signs of gas evolution had subsided.[39] The suspension was cooled and 5-bromo-2,4-dichloropyrimidine[36] (9.1 g, 40 mmol) added dropwise, while maintaining the temperature below 25 °C. After being stirred for 18 h at rt, the reaction mixture was filtered to remove precipitated NaCl and thoroughly washed with toluene. The filtrate was then evaporated under reduced pressure to give an oil which solidified upon cooling. The crude solid was then recrystallized in EtOH to afford **19** as a white crystalline solid (16 g, 91%): mp 88−90 °C (lit.[39] mp 89−91 °C); ^{1}H NMR ($CDCl_3$) δ 5.40 (s, 2 H), 5.48 (s, 2 H), 7.32−8.48 (m, 10 H), 8.35 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 69.1, 69.7, 98.4, 127.7, 128.0, 128.1, 128.2, 128.5, 128.6, 135.5, 136.1, 159.4, 159.5, 163.5, 166.2.

5-(Dihydroxyboryl)-2,4-bis(benzyloxy)pyrimidine (20).[39] *N*-Butyllithium (1.3 M in hexane, 2.5 mL, 3.2 mmol) was dropwise added over a 1 h period to a solution of **19** (1.0 g, 2.7 mmol) and $B(O^iPr)_3$ (1.0 mL, 5.3 mmol) in a mixture of anhydrous THF and toluene (1:4 volume ratio, 50 mL total) at −78 °C under Ar.[39] The mixture was stirred for an additional 18 h at rt, followed by addition of dilute HCl (1 M). The reaction mixture was evaporated under reduced pressure. The residue was then treated with H_2O (50 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic extracts were combined, washed with brine (50 mL), and dried over $MgSO_4$, and the solvent was removed under reduced pressure to give **20** as a white powder (8.8 g, 95%). Spectral data are in agreement with the literature:[39] ^{1}H NMR ($CDCl_3$) δ 5.46 (s, 4 H), 7.25−7.48 (m, 10 H), 8.72 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 118.5, 160.1, 161.4, 165.9.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-[4-(2,4-dibenzyloxy-5-pyrimidinyl)imidazol-1-yl]-β-D-ribofuranose (21). A mixture of **11** (1.7 g, 2.9 mmol) and $Pd(PPh_3)_4$ (185 mg, 0.16 mmol) in 40 mL of 1,2-dimethoxyethane (DME) was stirred at rt under Ar for 10 min. To this mixture was added 5-(dihydroxyboryl)-2,4-bis(benzyloxy)pyrimidine (**20**) (3.2 mmol in 20 mL of DME).[40] Saturated aqueous $NaHCO_3$ (40 mL) was added and the mixture refluxed under Ar for 4 h. The solution was cooled to rt and the DME layer separated and set aside. The aqueous layer was then extracted with EtOAc (3 × 50 mL), and the organic extracts were combined with the DME layer, washed with brine (100 mL), and dried over $MgSO_4$. The solvent was removed to give a pale brown syrup. Column chromatography eluting with 2% EtOH in CH_2Cl_2 gave **21** as a colorless syrup (1.54 g, 70%): ^{1}H NMR ($CDCl_3$) δ 3.45 (dd, 3.0 Hz, 10.6 Hz, 1 H), 3.56 (dd, 3.3 Hz, 10.6 Hz, 1 H), 4.01−4.48 (m, 1 H), 4.11 (br t, 5.5 Hz, 1 H), 4.31−4.62 (m, 7 H), 5.42−5.52 (m, 4 H), 5.78 (d, 6.0 Hz, 1 H), 7.15−7.54 (m, 26 H), 7.73 (s, 1 H), 9.15 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 68.3, 68.9, 69.4, 72.1, 72.5, 73.1, 76.5, 81.5, 82.1, 88.7, 109.5, 115.6, 127.3, 127.6, 127.8, 127.9, 128.3, 134.0, 135.6, 136.1, 136.5, 136.7, 137.2, 137.3, 155.9, 162.7, 166.0. Anal. Calcd for $C_{47}H_{44}N_4O_6\cdot 1H_2O$: C, 72.48; H, 5.95; N, 7.19. Found: C, 72.82; H, 5.75; N, 7.03.

1-[4-(Uracil-5-yl)imidazol-1-yl]-1-β-D-ribofuranose-2,3,5-triol (5). A mixture of **21** (200 mg, 0.26 mmol), palladium (10% Pd/C, 200 mg), and ammonium formate (300 mg) in EtOH (50 mL) was heated under reflux for 2 h.[10] The solvent was then removed under reduced pressure and the residue purified by column chromatography eluting with EtOAc/acetone/EtOH/H_2O (7:1:1:0.5) to give **5** as a white crystalline solid (71 mg, 88%): mp 239−242 °C; ^{1}H NMR (DMSO-d_6) δ 3.49 (dd, 4.5 Hz, 12.3 Hz, 1 H), 3.55 (dd, 5.0 Hz, 12.3 Hz, 1 H), 3.86 (br d, 3.3 Hz, 1 H), 4.0 (dd, 4.0 Hz, 6.0 Hz, 1 H), 4.11 (br t, 5.1 Hz, 1 H), 5.00 (br t, 4.8 Hz, 1 H), 5.14 (br d, 3.9 Hz, 1 H), 5.36 (br d, 5.7 Hz, 1 H), 5.52 (d, 6.0 Hz, 1 H), 7.67 (s, 1 H), 7.81 (s, 1 H), 7.86 (s, 1 H). 11.0 (s, 1 H), 112.2 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 61.3, 70.3, 75.1, 85.2, 89.3, 107.3, 114.5, 133.5, 135.7, 136.3, 150.5, 162.2. Anal. Calcd for $C_{12}H_{14}N_4O_6\cdot 0.6H_2O$: C, 44.88; H, 4.80; N, 17.38. Found: C, 45.14; H, 4.60; N, 17.03.

1-[4-(2,4-Diaminopyrimidin-5-yl)imidazol-1-yl]-1-β-D-ribofuranose-2,3,5-triol (4). In a Parr bomb, anhydrous ammonia gas was bubbled in an anhydrous methanolic solution of **21** (1.0 g, 1.3 mmol in 50 mL of MeOH) at −78 °C for 5 min. The bomb was sealed and heated at 170 °C for 96 h. After the mixture was cooled to 0 °C, the solvent was removed under reduced pressure. The residue was purified by column chromatography eluting with 8% EtOH in CH_2Cl_2 to give **22** (398 mg, 52%) as a colorless syrup, which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 3.54 (dd, 2.4 Hz, 10.4 Hz, 1 H), 3.70 (dd, 2.8 Hz, 10.4 Hz, 1 H), 4.09 (dd, 2.5 Hz, 4.5 Hz, 1 H), 4.16 (br t, 4.8 Hz, 1 H), 4.33−4.36 (m, 1 H), 4.39 (d, 12.0, 1 H), 4.47−4.68 (m, 6 H), 5.06 (br s, 2 H), 5.66 (br s, 2 H), 5.74 (d, 6.4 Hz, 1 H), 7.01 (s, 1 H), 7.10−7.35 (m, 15 H), 7.60 (s, 1 H), 7.78 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 70.2, 72.7, 73.0, 74.0, 76.9, 81.7, 82.9, 89.2, 102.6, 110.4, 127.3, 127.8, 128.0, 128.1, 128.2,

128.3, 128.4, 128.5, 128.7, 128.8, 129.0, 135.3, 136.9, 137.4, 137.5, 138.9, 152.3, 161.2, 161.4.

In a similar fashion as was used to obtain **5**, the desired triol **4** was obtained in 88% yield (156 mg) as a white powder following deprotection of **22** with Pd/C and ammonium formate: mp 268−272 °C. ^{1}H NMR (DMSO-d_6) δ 3.72 (dd, 3.6 Hz, 12.0 Hz, 1 H), 3.80 (dd, 3.2 Hz, 12.0 Hz, 1 H), 4.05 (q, 3.2 Hz, 1 H), 4.20, (dd, 3.6 Hz, 4.2 Hz, 1 H), 4.27 (br t, 4.2 Hz, 1 H), 5.64 (d, 5.6 Hz, 1 H), 7.56 (s, 1 H), 7.91 (s, 1 H), 7.96 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 61.4, 70.4, 75.1, 85.4, 89.4, 100.6, 111.0, 135.5, 137.5, 150.4, 160.3, 160.5. Anal. Calcd for $C_{12}H_{16}N_6O_4 \cdot 1H_2O$: C, 45.42; H, 5.40; N, 26.48. Found: C, 45.71; H, 5.22; N, 26.26.

1-[4-(4-Aminopyrimidin-5-yl-2-one)imidazol-1-yl]-1-β-D-ribofuranose-2,3,5-triol (3). To a solution of **22** (850 mg, 1.46 mmol) in a 1:1 mixture of THF and H_2O (30 mL) was added sodium nitrite (486 mg, 7.03 mmol), followed by glacial acetic acid (0.62 mL, 10.69 mmol). The mixture was heated to 60 °C and stirred for 2 h, at which point the TLC showed no traces of starting material. The solution was cooled to rt, neutralized with concentrated NH_4OH (1 mL), and evaporated under reduced pressure. The crude product was used directly in the next step.

In a similar fashion as was used for the deprotection of **21** to get **5**, the benzyl groups were deblocked with Pd/C and ammonium formate and subsequently purified by column chromatography eluting with EtOAc/acetone/EtOH/H_2O (4:1:1:0.5) to give **3** as a white powder (42 mg, 41% for two steps): ^{1}H NMR (D_2O) δ 3.61 (dd, 3.6 Hz, 12.0 Hz, 1 H), 3.71 (dd, 3.2 Hz, 12.0 Hz, 1 H), 4.06 (q, 3.2 Hz, 1 H), 4.19, (dd, 3.6 Hz, 4.2 Hz, 1 H), 4.33 (dd, 1 H), 5.68 (d, 3.9 Hz, 1 H), 7.39 (s, 1 H), 7.56 (s, 1 H), 7.89 (s, 1 H), 8.23 (s, 2H); ^{13}C NMR (D_2O) δ 61.7, 70.3, 75.0, 85.1, 89.5, 101.1, 115.1, 134.2, 137.7, 158.5, 165.1, 171.1.

1-[4-(2-Aminopyrimidin-5-yl-4-one)imidazol-1-yl]-1-β-D-ribofuranose-2,3,5-triol (2). A solution of **4** (14 mg, 0.05 mmol) and saturated aqueous $NaHSO_3$ (5 mL) was heated at 60 °C for 10 h.[37] The solution was evaporated under reduced pressure to afford the crude product, which was purified by preparative TLC eluting with EtOAc/acetone/EtOH/H_2O (4:1:1:1) to obtain **2** as a white powder (12.4 mg, 88%): ^{1}H NMR (D_2O) δ 3.65 (dd, 3.6 Hz, 11.2 Hz, 1 H), 3.76 (dd, 3.3 Hz, 12.1 Hz, 1 H), 4.05−4.08 (m, 1 H), 4.13, (dd, 3.6 Hz, 4.2 Hz, 1 H), 4.31 (dd, 1 H), 5.99 (d, 1 H), 6.61 (s, 1 H), 7.46 (s, 1 H), 8.05 (s, 1 H); ^{13}C NMR (D_2O) δ 60.6, 69.3, 75.5, 85.4, 90.7, 103.1, 121.1, 132.9, 136.6, 153.9, 163.5, 168.4.

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