"Fleximers". Design and Synthesis of a New Class of Novel Shape-Modified Nucleosides[1]

Katherine L. Seley,* Liang Zhang, Asmerom Hagos, and Stephen Quirk†

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332-0400

katherine.seley@chemistry.gatech.edu

Received January 22, 2002

A new class of shape-modified nucleosides is introduced. These novel "fleximers" feature the purine ring systems of adenosine, inosine, and guanosine split into their individual imidazole and pyrimidine components (as in **1**−**3**). This structural modification serves to introduce flexibility into the nucleoside while still retaining the elements essential for recognition. As a consequence, these novel fleximers should find use as bioprobes for investigating enzyme−coenzyme binding sites as well as nucleic acid and protein interactions. Their design and synthesis are described.

Introduction

Understanding the structural interactions between enzymes and their substrates or cofactors is vital to the design of more effective medicinal agents. Purines are one of the most ubiquitous heterocyclic ring systems found in nature; they are components in numerous biologically significant molecules and thus present an excellent scaffold upon which to construct bioprobes. Pioneering work in this area began with Leonard's *lin*-, *dist*-, and *prox*-benzo-separated adenosine analogues shown in Figure 1.[2−4]

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 availability 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.[5−12] This phenomenon causes inaccuracies when employing the structure-based drug design method for a potential enzyme−substrate complex using crystallographic data; although the fit may appear to be good, in reality, the "best guess" drug often proves to be a poor inhibitor.[13,14]

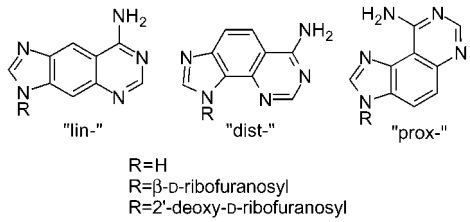


Figure 1. Leonard's benzo-separated analogues.

As a first step toward a potential solution to this dilemma, we set out to design a flexible substrate to pair with a flexible binding site. Since the sugar moieties of nucleosides inherently possess the ability to adapt their conformations, we instead chose to focus our attention on the heterobases. Separation of the components of the purine ring while maintaining some modicum of connectivity would allow the purine ring to become "flexible", thereby allowing it to adapt to spatial confines of the binding site more readily. In addition, it was essential that the nucleoside still possess the molecular elements needed for recognition; in essence, the nucleobase moiety could be "split" but still retain the maximum number of its key structural features. Then, by allowing both the enzyme and the substrate to adjust to each other, the ideal fit would more likely be achieved and maintained, thereby providing a unique perspective on enzyme−substrate binding conformations. This information could then be used to design more effective inhibitors.

These novel fleximers feature a purine ring split into the individual components; the imidazole and pyrimidine rings of adenosine, inosine, and guanosine (as in **1**−**3**, respectively, Figure 2) are connected by a single carbon−carbon bond, thereby creating a separation of 1.50 Å

* To whom correspondence should be addressed. Phone: 404-894-4013. Fax: 404-894-2295.

† Present Address: Kimberly-Clark Corporation, 1400 Holcomb Bridge Rd., Roswell, GA 30076-2199.

(1) A preliminary report of this work was recently published: Seley, K. L.; Zhang, L.; Hagos, A. *Org. Lett.* **2001**, *3*, 3209.

(2) Leonard, N. J.; Morrice, A. G.; Sprecker, M. A. *J. Org. Chem.* **1975**, *40*, 356.

(3) Leonard, N. J. *Acct. Chem. Res.* **1982**, *15*, 128.

(4) Leonard, N. J.; Hiremath, S. P. *Tetrahedron* **1986**, *42*, 1917.

(5) Hu, Y.; Yang, X.; Yin, D. H.; Mahadevan, J.; Kuczera, K.; Schowen, R. L.; Borchardt, R. T. *Biochem.* **2001**, *40*, 15143.

(6) Hu, Y.; Komoto, J.; Huang, Y.; Gomi, T.; Ogawa, H.; Takata, Y.; Fujioka, M.; Takusagawa, F. *Biochem.* **1999**, *38*, 8323.

(7) Yin, D.; Yang, X.; Hu, Y.; Kuczera, K.; Schowen, R. L.; Borchardt, R. T.; Squier, T. C. *Biochem.* **2000**, *39*, 9811.

(8) Bugg, C. E.; Carson, W. M.; Montgomery, J. A. *Sci. Am.* **1993**, *269*, 92.

(9) Ealick, S. E.; Babu, Y. S.; Bugg, C. E.; Erion, M. D.; Guida, W. C.; Montgomery, J. A.; Secrist, J. A., III. *Proc. Natl. Acad. Sci., USA* **1991**, *88*, 11540.

(10) Ealick, S. E.; Rule, S. A.; Carter, D. C.; Greenhough, T. J.; Babu, Y. S.; Cook, W. J.; Habash, J.; Helliwell, J. R.; Stoeckler, J. D.; Parks, R. E., Jr.; Chen, S.-f.; Bugg, C. E. *J. Biol. Chem.* **1990**, *265*, 1812.

(11) Erion, M. D.; Stoeckler, J. D.; Guida, W. C.; Walter, R. L.; Ealick, S. E. *Biochemistry* **1997**, *36*, 11735.

(12) Erion, M. D.; Takabayashi, K.; Smith, H. B.; Kessi, J.; Wagner, S.; Hönger, S.; Shames, S. L.; Ealick, S. E. *Biochemistry* **1997**, *36*, 11725.

(13) Montgomery, J. A. *Med. Res. Rev.* **1993**, *13*, 209.

(14) Montgomery, J. A.; Niwas, S.; Rose, J. D.; Secrist, J. A., III; Babu, Y. S.; Bugg, C. E.; Erion, M. D.; Guida, W. C.; Ealick, S. E. *J. Med. Chem.* **1993**, *36*, 55.

10.1021/jo0255476 CCC: $22.00
Published on Web 04/18/2002

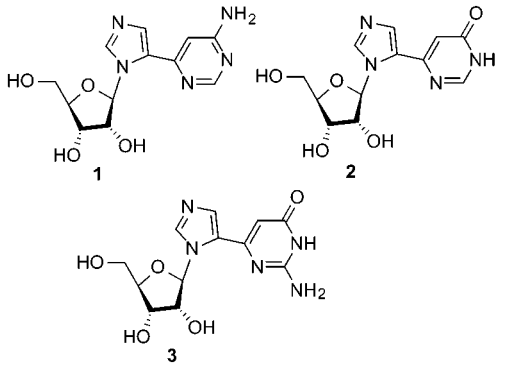


Figure 2. Fleximer targets.

Table 1. Various Properties of the Hartree−Fock-Optimized Fleximer Structures[a]

fleximer	1	2	3
total energy (AU)	−1035.5 ± 22	−1110.5 ± 37	−1055.4 ± 28
dipole (Debye)	4.9	11.7	9.7
dihedral angle (deg) C2′−C1′−N1−C2	223.2 ± 3.4	225.6 ± 2.1	228.7 ± 3.3
dihedral angle (deg) N1−C5−C6′−N1′	−12.9 ± 4.1	−19.1 ± 6.6	−26.6 ± 5.4
volume (Å^3)	305.9	317.1	302.9
bond (Å) rms dev	0.009	0.004	0.007
angles (deg) rms dev	0.012	0.013	0.017

[a] A 6-311G* basis set was utilized for the optimizations. Values are the average (±SD) of the 16 optimized conformations.

between the rings. Analogous to Leonard's *dist*-benzo analogues, these fleximers are connected at the C-5 of the imidazole and the C-6 of the pyrimidine. To our knowledge, there is only one other example of a split nucleoside in the literature;[15] however, it differs significantly in its structure. There is also a very recent report in the literature[16] of a biphenyl nucleoside; however, we believe our novel fleximers more closely resemble the naturally occurring purine nucleosides, and that as such, they will provide a unique perspective on enzyme/substrate interactions.

Results and Discussion

We initially carried out a series of *ab initio* quantum mechanical structure optimization calculations. In the case of all three fleximers, sixteen initial structures converged to a similar final conformation. Three-dimensional least squares analysis was employed to fully analyze the structural deviations of the sixteen optimized structures for each fleximer. Some structural and energetic properties of the optimized fleximer nucleosides are shown in Table 1. Bond lengths and angles are similar among the optimized structures as is evidenced by the low root-mean-squared deviations. In all cases, there was little effect of the initial pyrimidine/imidazole rotation or of the initial *syn*/*anti* conformation on the final optimized structure.

All three of the fleximers proved to be quite similar in their optimized structures, and Figure 3 shows the final conformation for the adenosine fleximer **1**. The structures were optimized using RHF SCF gradient optimization at the 6-311G* level of theory from an initial model. The

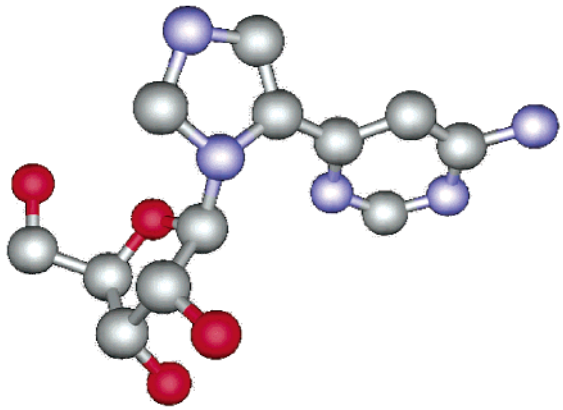


Figure 3. Optimized structure of the adenosine fleximer.

fleximer nucleosides all adopt a high *anti* conformation with a corresponding dihedral angle in the range of 225°, and the ribose, in all instances, maintained a C2′ *endo* conformation. There are no significant structural or energetic differences between the final optimized fleximer structures; in all 48 final structures, the pyrimidine is rotated out of the plane defined by the imidazole ring. This rotation dips the N1 portion of the pyrimidine ring below the imidazole plane. The inosine fleximer shows an average of −26.6° for the rotation, which is greater than either the guanosine (−19.1°) or the adenosine (−12.9°) fleximers. The observation that, at the 6-311G* level of theory, all three purine fleximers adopt a nonplanar base conformation in the gas phase is encouraging.

Next, to gauge the level of conformational flexibility in the context of an enzyme active site, the adenosine fleximer was chosen for further modeling. Automated molecular docking reveals that the molecule occupies the *S*-adenosylhomocysteine hydrolase active site in an orientation that is similar, but not identical, to the position of the known nucleoside inhibitor in the crystal structure published by Turner et al.[17] The RMS deviation between the two molecules is 1.1 Å. Most of this difference is accounted for by a 0.4 Å translation of the adenosine fleximer toward His 353 and a 12° rotation of the entire molecule toward Asp 190. Most notably is the observation that the docked adenosine fleximer pyrimidine ring is rotated to be nearly perpendicular to the imidazole ring (87°). Figure 4 shows the conformation the molecule preferred when docked in the enzyme active site. Although the gas-phase-optimized structure reveals a low-energy conformation with that angle at −12.9°, the fleximer can sample a high degree of conformational space and adopt alternate conformations in order to maximize intermolecular contacts. The docking search resulted in two main clusters; the primary group (−60 kcal/mol of intermolecular energy) accounted for 80% of the solution orientations. The second group (−40 kcal/mol of intermolecular energy) showed an orientation closer to the inhibitor in the crystal structure. Full details of the molecular docking and binding characteristics of the fleximers will be given elsewhere.

Next, construction of the fleximers was undertaken. The initial approach was envisioned from a Suzuki or Stille coupling to attach the imidazole and pyrimidine

(15) Van Calenbergh, S.; De Bruyn, A.; Schrami, J.; Blaton, N.; Peeters, O.; De Keukeleire, D.; Busson, R.; Herdewijn, P. *Nucleosides Nucleotides* **1997**, *16*, 291.

(16) Singh, I.; Hecker, W.; Prasad, A. K.; Parmar, V. S.; Seitz, O. *Chem. Commun.* **2002**, 500.

(17) Turner, M. A.; Yuan, C.-S.; Borchardt, R. T.; Hershfield, M. S.; Smith, D. G.; Howell, P. L. *Nat. Struct. Biol.* **1998**, *5*, 369.

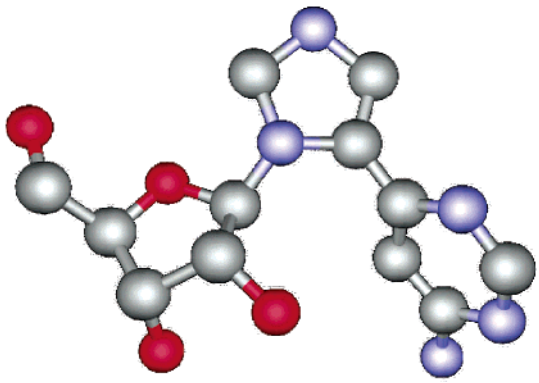
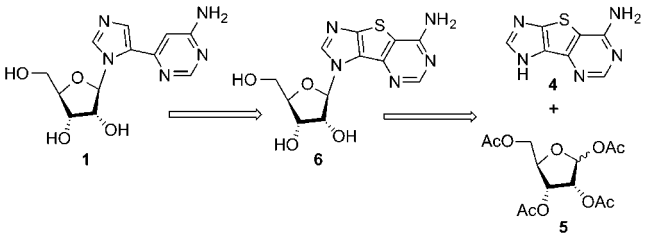


Figure 4. Conformation of the adenosine fleximer adopted in the *S*-adenosylhomocysteine hydrolase active site determined by automated molecular docking. Shown is the best-fit conformation, corresponding to −60 kcal/mol in the active site.

Scheme 1

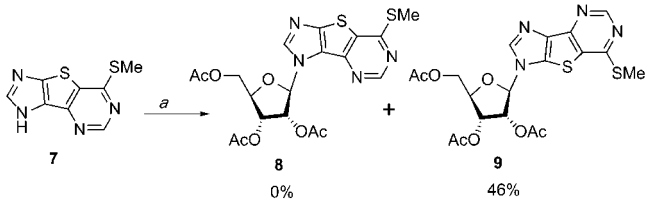


rings, an approach we chose for the synthesis of the *prox*-analogues (manuscript in preparation). Unfortunately, the requisite pyrimidine rings substituted in the necessary position for coupling were unavailable commercially or tedious to obtain synthetically. We then turned our attention toward construction of a tricyclic ring system that could be preferentially cleaved at only one of the connecting bonds.

Although the targets resembled Leonard's *dist*-benzo analogues in their attached ring motif, the benzene spacer ring was, of course, unsuitable for our purposes since it did not possess a single carbon−carbon bond connection. It quickly became apparent, however, that insertion of a thiophene spacer ring offered an exceptional alternative. The carbon−carbon single-bond connection of the thiophene spacer ring was as we desired, and removal of the sulfur would then result in the partial cleavage of the nucleobase while keeping the two components connected. Admittedly, there was some concern that the extended aromatic system of the tricyclic system would be resistant to cleavage since loss of aromaticity would occur, but we felt confident that treatment with Raney nickel would be successful.

As depicted retrosynthetically in Scheme 1, we initially intended to follow Leonard's approach by coupling the preconstructed heterobase (**4**) to the commercially available tetraacetate-protected sugar (**5**), which, following deprotection, would provide us with the desired tricyclic nucleoside intermediate **6**. Subsequent removal of the sulfur would yield the fleximer nucleoside. As we have previously reported,[18] the synthesis of **4** and the corresponding tricyclic guanine heterobase was successful, but unfortunately, coupling proved to be problematic. Initial attempts at coupling **4** failed due to problems with

Scheme 2



solubility, so we reverted to coupling the thiomethyl intermediate **7** (as shown in Scheme 2), something Leonard[19] and others[20−23] have found necessary in the synthesis of other *lin*-benzoadenosine analogues.

Use of a coupling method[24] that employs bis(trimethylsilyl)acetamide (BSA) and trimethylsilyl triflate successfully coupled **7** to tetraacetate **5**. We expected two products, **8** and **9**, would be formed, resulting from N-1 and N-3 coupling, since both nitrogens of the heterobase are available for glycolization. This is a common occurrence in nucleoside synthesis, but to our surprise, only one product was formed. Upon structural characterization, the product was shown by two-dimensional NMR to be **9**, the N-1 coupled product, rather than the desired N-3 product (**8**), so our focus then turned to coupling at an earlier step in the construction of the base.

Several of the heterobase intermediates were investigated, but dibromoimidazole proved to be advantageous for two reasons: one, 4,5-dibromoimidazole is symmetrical, and therefore coupling at either nitrogen would result in only one product, thereby foregoing the need for tedious structure proof, and two, it would circumvent the difficulties we had previously encountered during deprotection of the imidazole nitrogen during the synthesis of the bases. Coupling was successful, and the synthesis of the nucleoside resumed with a series of reactions analogous to that used previously for the construction of the bases.[18]

As shown in Scheme 3, coupling of 4,5-dibromoimidazole (**10**) again using BSA and trimethylsilyl triflate produced **11**.[24] Removal of the acetate groups was then accomplished quantitatively with methanolic ammonia overnight to afford the triol **12**.

Reprotection of the hydroxyl groups was then undertaken. The correct choice of protecting group was critical, since it would be required to withstand both acidic and basic conditions, as well as attack from Grignard reagents. We initially chose to protect the hydroxyls as methyl ethers, since methoxy groups are robust to the various conditions we were facing but not sterically hindering for adjacent hydroxyls. Using standard methylation procedures, triol **12** was then easily converted to **13a**. Formylation of the C-5 position was then carried out using Grignard conditions to provide **14a**. As we have previously noted, this reaction gives primarily the desired C-5 aldehyde, along with a C-4 monobrominated side product, which is readily separated from the aldehyde. Despite numerous attempts at optimization of this step,

(18) Seley, K. L.; Januszczyk, P.; Hagos, A.; Zhang, L.; Dransfield, D. T. *J. Med. Chem.* **2000**, *43*, 4877.

(19) Leonard, N. J.; Laursen, R. A. *Biochem.* **1965**, *4*, 354.

(20) Zhang, J.; Nair, V. *Nucleosides Nucleotides* **1997**, *16*, 1091.

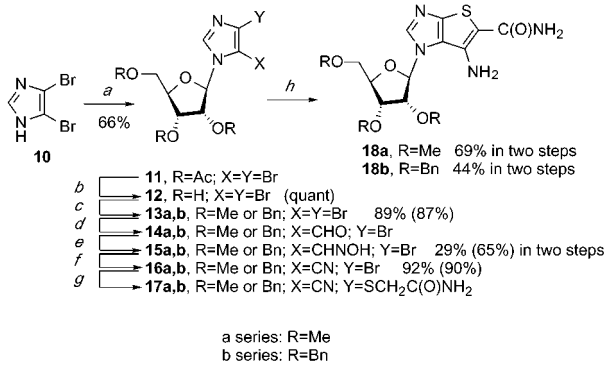
(21) Zhang, J.; Neamati, N.; Pommier, Y.; Nair, V. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1887.

(22) Rajappan, V. P.; Schneller, S. W. *Tetrahedron* **2001**, *57*, 9049.

(23) Rajappan, V. P.; Schneller, S. W. *Nucleosides, Nucleotides and Nucleic Acids* **2001**, *20*, 1117.

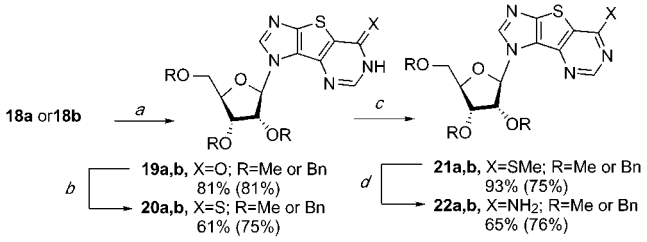
(24) Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607.

Scheme 3



[a] (i) 1,2,3,5-Tetra-*O*-acetyl-*β*-D-ribofuranose, BSA, acetonitrile; (ii) TMSOTf. [b] NH_3, MeOH. [c] For series **a**, NaH and then CH_3I; for series **b**, Bu_4NI, NaH, BnBr. [d] EtMgBr, anhydrous DMF. [e] Hydroxylamine hydrochloride. [f] Ac_2O. [g] $NH_2C(O)CH_2SH$, K_2CO_3. [h] NaOEt, EtOH.

Scheme 4



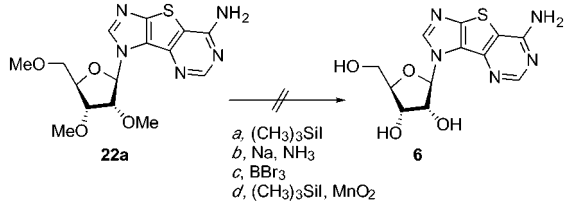
[a] $CH(OEt)_3/Ac_2O$ (1:1). [b] P_2S_5, pyridine. [c] K_2CO_3, MeI. [d] NH_3, BuOH.

we have been unable to limit the formation of this side product. Next, **14a** was converted to the oxime **15a** and subsequently dehydrated to yield nitrile **16a**. Introduction of the thiol side chain using thioglycolamide gave **17a**, which was then immediately subjected to ring closure to provide the bicyclic imidazole-thiophene intermediate **18a**. We have found this step to be quite facile; ring closure occurs to a large extent even before treatment with base most likely due to the extended aromaticity that results.

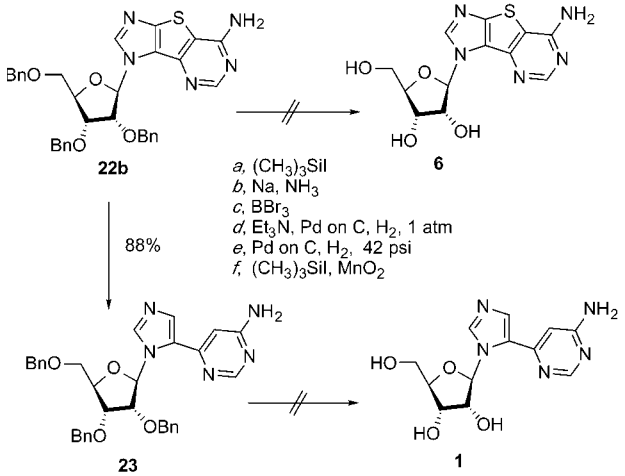
Ring closure of the pyrimidine ring to form the inosine intermediate **19a** was accomplished with a 1:1 mixture of acetic anhydride and triethyl orthoformate (Scheme 4).[25] Conversion of the carbonyl of **19a** was carried out using standard conditions with phosphorus pentasulfide to give the thiocarbonyl **20a**, which was then immediately methylated to afford **21a**. Standard ammonolysis then provided **22a**. At this point we attempted to deblock the hydroxyls; however, the methyl protecting groups proved to be completely recalcitrant to any attempts at removal (Scheme 5). Several standard methods using a variety of conditions were tried but all failed to produce **6**. Realizing that the benzyl group, although more sterically hindering than the methyl group, would be stable to all of the reaction conditions in the pathway and removal is typically is more facile than with a methyl group, we were forced to return to the beginning of the synthetic pathway, this time using the benzyl protecting group.

Using standard benzylation conditions employing sodium hydride and benzyl bromide proved to be difficult

Scheme 5



Scheme 6



at best. Incomplete benzylation of the three hydroxyls in various combinations occurred, thereby resulting in an inseparable mixture of products; therefore, we adjusted our procedure to utilize an *in situ* formation of benzyl iodide with benzyl bromide and tetrabutylammonium iodide.[26] This modification proved to be successful, and **13b** was obtained in good yield (87%). In addition, purification was accomplished with ease, since there were few side products formed.

Proceeding onward with **13b**, we obtained tricyclic intermediate **22b** by way of a series of reactions identical to that used before (Scheme 4). Grignard addition of the formyl group gave **14b**, which was then converted to the oxime **15b**. Dehydration of **15b** with acetic anhydride then provided **16b**. Next, addition of the thioglycolamide group, followed by base-catalyzed ring closure of the thiophene ring, was accomplished to give **18b**. Closure of the pyrimidine ring was carried out as before to afford **19b**. Treatment with phosphorus pentasulfide, followed by methylation, provided **21b**. Ammonolysis then gave **22b**.

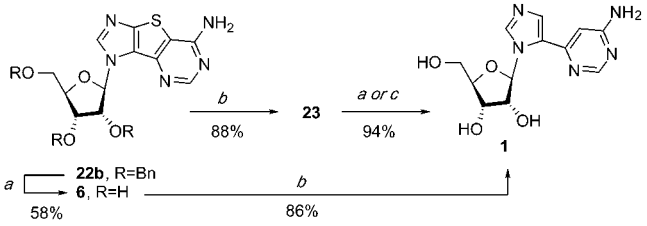
Once **22b** was in hand, removal of the benzyl groups was undertaken. Due to the presence of the sulfur, we suspected that standard conditions employing palladium might be unsuccessful (which proved to be true); however, several alternative methods were tried. Once again, we met with numerous failures as summarized in Scheme 6. Taking a different approach, we then tried removal of the sulfur to form the protected split nucleoside **23** in the hopes this would overcome the poisoning of the palladium deprotection methods, but this failed as well. The split nucleoside **23** was readily formed, but all procedures employed failed to remove the benzyl groups.

Finally, as outlined in Scheme 7, a procedure was found that utilized an excess of boron trifluoride etherate

(25) Rousseau, R. J.; Robins, R. K. *J. Heterocycl. Chem.* **1965**, *2*, 196.

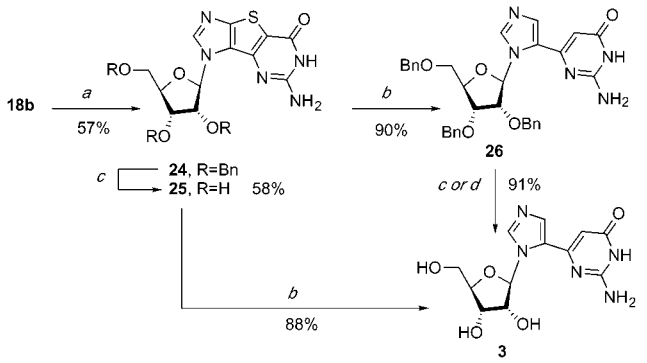
(26) Czernecki, S.; Georgoulis, C.; Provelenghiou, C. *Tetrahedron Lett.* **1976**, *17*, 3535.

Scheme 7



[a] BF_3·OEt_2, EtSH, CH_2Cl_2. [b] Raney Ni, MeOH, reflux. [c] Pd on C, ammonium formate, MeOH.

Scheme 8

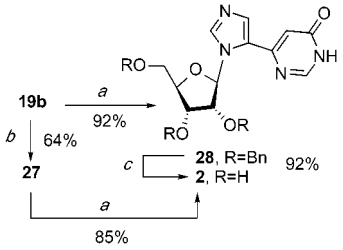


[a] (i) NaOH, MeOH; (ii) CS_2, heat; (iii) H_2O_2; (iv) NH_3. [b] Raney Ni, MeOH, reflux. [c] BF_3·OEt_2, EtSH, CH_2Cl_2. [d] Pd on C, ammonium formate, MeOH.

and ethanethiol to deblock the hydroxyls.[27,28] This proved to be successful, and tricyclic adenosine analogue **6** was obtained, albeit in very low yield. Next, removal of the sulfur in the spacer ring of **6** was accomplished by treatment with Raney nickel and refluxing in methanol for 8 h to provide fleximer **1**. Since we were curious to see if we could improve the yield, we reversed the steps and carried out the desulfurization to give **23** before removal of the benzyl groups. Again employing the boron trifluoride etherate/ethanethiol method, we were successful in deprotecting **23**; however, the yields were again quite low, and the purification was difficult even employing HPLC methods.

Once again searching for a new method, we uncovered a recent paper[29] employing palladium on charcoal and ammonium formate. To our delight, this method proved to be outstanding: the yields were high, and purification was accomplished with ease; **1** was obtained from **23** in 94% yield. To obtain the guanosine fleximer, intermediate **18b** was subjected to an alternative procedure for closure of the pyrimidine ring (Scheme 8). Our previously reported[18] method employed chloroformamidine hydrochloride, but this could not be used for these analogues since the acidic conditions inherent in this procedure resulted in nearly quantitative cleavage of the glycosidic bond of the nucleoside; therefore, we turned our attention to an alternative procedure.[30] Sequential treatment of **18b** with sodium hydroxide and methanol and then heating with

Scheme 9



[a] Raney Ni, MeOH, reflux. [b] BF_3·OEt_2, EtSH, CH_2Cl_2. [c] Pd on C, ammonium formate, MeOH.

carbon disulfide, followed by addition of hydrogen peroxide and finally ammonia, yielded **24**. Removal of the protecting groups using the boron trifluoride etherate method gave the desired guanosine tricyclic intermediate **25**, which was then subjected to treatment with Raney nickel to give fleximer **3**.

Once again repeating the reversal of the steps, cleavage of the sulfur in the spacer ring gave the protected guanosine fleximer **26**, but only deprotection with palladium and ammonium formate consistently afforded **3**. Finally, the inosine analogue **2** was obtained from **19b** in a manner analogous to that shown in Scheme 9. Treatment of **19b** with the boron trifluoride etherate method gave the deprotected tricyclic nucleoside **27**, which could then be converted to **2**; however, the best yield once again came from treatment of **19b** with Raney nickel and then deprotection with the palladium method to provide the inosine fleximer **2**.

We have now shown that in all three cases, (i) the tricyclic nucleosides were completely resistant to the palladium method of deprotection (most probably due to poisoning of the catalyst by the sulfur), as well as many other commonly used standard methods, (ii) the use of the boron trifluoride etherate deprotection method worked consistently well for the tricyclics, (iii) the use of the boron trifluoride etherate method did not produce consistent or acceptable yields for the fleximers, and purification was difficult at best, and (iv) the protected fleximers reacted quickly and cleanly and gave excellent yields when using the palladium method of deprotection.

In summary, we have successfully synthesized the first examples of a new class of nucleosides that can be used as dimensional bioprobes of a wide variety of biological systems. Broad-screen testing to determine medicinal properties, as well as other biological and biophysical investigations, for both the tricyclics and the fleximers has just begun, and reports of these and other studies will be forthcoming.

Experimental Section

General. Melting points are uncorrected. Combustion analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. ^1H and ^{13}C NMR spectra were recorded 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 silica gel 60-F_{254} precoated plates. Column chromatography was performed on silica (200−400 mesh, 60 Å) and elution with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^1H and ^{13}C NMR) homogeneous materials.

2,3-Diacetoxy-5-acetoxymethyl-1-(7-thiomethylimidazo-[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-3-yl)-*β*-D-ribofuranose (8)

(27) Fuji, K.; Ichikawa, K.; Node, M.; Fujita, E. *J. Org. Chem.* **1979**, *44*, 1661.
(28) Node, M.; Nishide, K.; Fuji, K.; Fujita, E. *J. Org. Chem.* **1980**, *45*, 4275.
(29) Koshkin, A. A.; Fensholdt, J.; Pfundheller, H. M.; Lomholt, C. *J. Org. Chem.* **2001**, *66*, 8506.
(30) Bennett, S. M.; Nguyen-Ba, N.; Ogilvie, K. K. *J. Med. Chem.* **1990**, *33*, 2162.

and 2,3-Diacetoxy-5-acetoxymethyl-1-(7-thiomethylimidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-1-yl)-*β*-D-ribofuranose (9). To a stirred mixture of **7**[18] (178 mg, 0.80 mmol) and 1,2,3,5-tetra-*O*-acetate-*β*-D-ribofuranose (0.28 g, 0.88 mmol) in anhydrous acetonitrile (20 mL) was added *N*,*O*-bis(trimethylsilyl)-acetamide (BSA) (1.20 mL, 4.8 mmol). The mixture was stirred at room temperature under Ar for 6 h. The mixture was cooled to 0 °C, at which point trimethylsilyltriflate (TMS−OTf) (174 *μ*L, 0.9 mmol) was added. The mixture was further stirred at 60 °C for 18 h. The solvent was removed under reduced pressure, and the residue was cooled to 0 °C and quenched with cold, saturated $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (30 mL × 3), and the organic extracts were combined and washed with brine. The solvent was removed under reduced pressure to give a brown syrup. Column chromatography eluting with hexane/ethyl acetate (2:1) gave a single compound as a colorless syrup (180 mg, 46% yield), which, following structural characterization with two-dimensional NMR, proved to be **9**: ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3 H, H−Ac), 2.09 (s, 3 H, H−Ac), 2.19 (s, 3 H, H−Ac), 2.78 (s, 1 H, H−SMe), 3.68 (dd, 3.6 Hz, 11.0 Hz, 1 H, H-5), 4.23 (dd, 3.6 Hz, 11.0 Hz, 1 H, H-5), 4.46 (dd, 3.6 Hz, 8.4 Hz, 1 H), 4.52 (d, 8.4 Hz, 1 H), 4.59−4.64 (m, 1 H), 5.86 (dd, 3.9 Hz, 4.8 Hz, 1 H, H-2), 8.18 (s, 1 H), 8.94 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 11.9, 20.0, 20.2, 20.4, 62.9, 70.1, 73.0, 80.2, 88.0, 128.9, 143.5, 144.3, 150.7, 152.97, 153.03, 163.9, 168.8, 169.2, 170.1. Anal. Calcd for $C_{19}H_{20}N_4O_7S_2$·0.4EtOAc: C, 47.80; H, 4.41; N, 11.15; S, 12.76. Found: C, 47.98; H, 4.53; N, 10.97; S, 12.76.

2,3-Diacetoxy-5-acetoxymethyl-1-(4,5-dibromoimidazol-3-yl)-*β*-D-ribofuranose (11). To a stirred solution of 1,2,3,5-tetra-*O*-acetate-*β*-D-ribofuranose (25.4 g, 79.7 mmol) and 4,5-dibromoimidazole **10**[18] (18.0 g, 79.7 mmol) in anhydrous acetonitrile (500 mL) was added BSA (1.5 mL, 6.0 mmol). The reaction mixture was stirred for 5 h at room temperature and cooled to 0 °C. TMS−OTf (0.30 mL, 1.6 mmol) was added. The mixture was further stirred at 60 °C for 8 h, at which point it was chilled to 0 °C, and saturated aqueous $NaHCO_3$ solution (15 mL) was added slowly. The mixture was extracted with CH_2Cl_2 (3 × 30 mL). The extracts were combined and washed with brine (50 mL). The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure to give a brown syrup. Flash column chromatography eluting with petroleum ether/EtOAc (1:1) gave **11** as a pale yellow syrup (23.0 g, 47.5 mmol; 66%), which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 9 H), 4.25−4.30 (m, 2 H), 4.33−4.38 (m, 1 H), 5.31 (dt, 2.7 Hz, 5.2 Hz, 1 H), 5.46 (dt, 2.2 Hz, 5.2 Hz, 1 H), 5.77 (dd, 2.6 Hz, 4.6 Hz, 1 H), 7.76 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 20.2, 20.3, 20.5, 62.3, 69.6, 73.5, 80.0, 88.0, 102.4, 118.2, 135.2, 168.8, 169.3, 170.0.

(4,5-Dibromoimidazol-3-yl)-1-*β*-D-ribofuranose (12). A solution of triacetate **11** (13.7 g, 28.3 mmol) in saturated methanolic ammonia (300 mL) in a steel bomb was heated at 100 °C for 3 h. The reaction was cooled and the solvent removed under reduced pressure to give a light brown foam. Column chromatography eluting with $CHCl_3$/MeOH (9:1) gave **12** as a colorless syrup (10.1 g, quantitative): ^{1}H NMR (CD_3OD) δ 3.72 (dd, 3.2 Hz, 12.2 Hz, 1 H), 3.84 (dd, 3.0 Hz, 12.2 Hz, 1 H), 4.03−4.07 (m, 1 H), 4.22 (t, 5.1 Hz, 1 H), 4.32 (t, 4.8 Hz, 1 H), 5.69 (d, 4.5 Hz, 1 H), 8.24 (s, 1 H); ^{13}C NMR (CD_3OD) δ 61.9, 71.0, 76.8, 86.6, 92.4, 104.3, 117.5, 138.1. Anal. Calcd for $C_8H_{10}Br_2N_2O_4$·0.25MeOH: C, 27.05; H, 3.02; N, 7.64; Br, 43.68. Found: C, 26.99; H, 2.83; N, 7.62; Br, 43.57.

2,3-Dimethoxy-5-methoxymethyl-1-(4,5-dibromoimidazol-3-yl)-*β*-D-ribofuranose 13a. Sodium hydride (95%, 2.12 g, 88.5 mmol) was added to a stirred solution of **12** (10.1 g, 28.2 mmol) in anhydrous THF (200 mL) at 0 °C under Ar. Then, the mixture was stirred at room temperature for 1 h. Methyl iodide (12.57 g, 88.5 mmol) was added dropwise. The mixture was stirred at room temperature for 18 h and poured over ice to quench the reaction. The volatiles were removed under reduced pressure, and H_2O was added (200 mL). The resulting mixture was extracted with CH_2Cl_2 (3 × 200 mL). The organic extracts were combined, washed with brine (300 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a pale brown syrup. Flash column chromatography eluting with hexane/ethyl acetate (2:1) gave **13a** as a colorless syrup (10.4 g, 89%), which was used directly in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 3.41 (s, 3 H), 3.44 (s, 3 H), 3.53 (s, 3 H), 3.55 (dd, 2.4 Hz, 11.1 Hz, 1 H), 3.73 (dd, 2.7 Hz, 11.1 Hz, 1 H), 3.95−4.01 (m, 2 H), 4.19−4.23 (m, 1 H), 5.75 (d, 3.0 Hz, 1 H), 8.07 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 58.3, 58.8, 59.2, 71.1, 77.4, 81.3, 83.3, 89.1, 101.4, 117.7, 136.4.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-(4,5-dibromoimidazol-3-yl)-*β*-D-ribofuranose 13b. Sodium hydride (95%, 2.9 g, 0.115 mol) was added to a stirred solution of **12** (13.7 g, 38.3 mmol) in anhydrous THF (100 mL) at 0 °C under Ar. Then, the mixture was stirred at room temperature for 3 h. Tetrabutylammonium iodide (0.5 g, 1.35 mmol) and benzyl bromide (19.7 g, 0.115 mol) was added. The mixture was stirred at room temperature for 3 h, followed by quenching with ethanol (20 mL). The solvent was removed under reduced pressure; H_2O (200 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 100 mL). The organic extracts were combined, washed with brine (200 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a pale brown syrup. Column chromatography eluting with hexane/ethyl acetate (3:1) gave **13b** as a colorless syrup (20.9 g, 87% yield): ^{1}H NMR ($CDCl_3$) δ 3.54 (dd, 2.4 Hz, 10.8 Hz, 1 H), 3.75 (dd, 2.7 Hz, 10.8 Hz, 1 H), 4.10−4.16 (m, 2 H), 4.31−4.34 (m, 1 H), 4.43−4.66 (m, 6 H), 5.83 (d, 3.3 Hz, 1 H), 7.22−7.38 (m, 15 H), 7.88 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 68.3, 72.5, 72.6, 73.5, 75.5, 80.5, 81.9, 89.5, 101.8, 117.6, 127.8, 127.9, 128.0, 128.2, 128.5, 128.6, 136.2, 136.7, 137.1. Anal. Calcd for $C_{29}H_{28}Br_2N_2O_4$: C, 55.43; H, 4.49; N, 4.46; Br, 25.43. Found: C, 55.33; H, 4.60; N, 4.29; Br, 25.15.

2,3-Dimethoxy-5-methoxymethyl-1-[(5-bromo-4-carbaldehydeoxime)imidazol-3-yl]-*β*-D-ribofuranose 15a. Ethylmagnesium bromide (3.0 M in Et_2O, 2.4 mL, 7.2 mmol) was added to a stirred solution of **13a** (2.8 g, 7.0 mmol) in anhydrous Et_2O (50 mL) under Ar at room temperature. The mixture was then stirred for 5 h. Anhydrous DMF (3.0 mL) was added, and the mixture was further stirred for 18 h. The solvent was removed under reduced pressure; saturated NH_4Cl aqueous solution (100 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 60 mL). The organic fractions were combined, washed with brine (2 × 100 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a pale brown syrup. Column chromatography eluting with hexane:ethyl acetate (1:1) gave **14a** as a tan crystalline solid (1.4 g, 58% yield), which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 3.39 (s, 3 H), 3.45 (s, 3 H), 3.72 (s, 3 H), 3.61 (dd, 2.0 Hz, 11.4 Hz, 1 H), 3.82 (br d, 3.9 Hz, 1 H), 3.93 (dd, 1.8 Hz, 11.4 Hz, 1 H), 3.98 (dd, 4.2 Hz, 9.0 Hz, 1 H), 4.23 (dt, 2.0 Hz, 9.0 Hz, 1 H), 6.38 (br s, 1 H), 8.60 (s, 1 H), 9.75 (d, 1.2 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 58.4, 59.1, 69.8, 75.7, 80.7, 82.4, 89.2, 125.7, 141.3, 169.0, 179.3. A solution of **14a** (0.50 g, 1.4 mmol) in ethanol (20 mL) was added to a mixture of hydroxylamine hydrochloride (0.69 g, 10.0 mmol) and $NaHCO_3$ (0.84 g, 10.0 mmol) in H_2O (5 mL). The mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure; H_2O (30 mL) was added and the mixture filtered. The solid collected was washed further with H_2O to give **15a** as a hygroscopic off-white crystalline solid (0.27 g, 50% yield), mp 159−161 °C: ^{1}H NMR (DMSO-d_6) δ 3.32 (s, 3 H), 3.32 (s, 3 H), 3.36 (s, 3 H), 3.49 (dd, 3.6 Hz, 11.0 Hz, 1 H), 3.59 (dd, 3.6 Hz, 11.0 Hz, 1 H), 3.94 (t, 4.8 Hz, 1 H), 4.09 (dd, 3.6 Hz, 8.4 Hz, 1 H), 4.21 (t, 4.8 Hz, 1 H), 6.28 (d, 4.5 Hz, 1 H), 7.99 (s, 1 H), 8.18 (s, 1 H), 11.64 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 57.7, 58.2, 58.8, 71.5, 77.3, 80.9, 81.8, 87.8, 119.3, 121.6, 130.5, 138.1. Anal. Calcd for $C_{12}H_{18}BrN_3O_5$·0.125H_2O: C, 39.32; H, 5.02; N, 11.46. Found: C, 39.36; H, 5.05; N, 11.19.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-[(5-bromo-4-carbaldehydeoxime)imidazol-3-yl]-*β*-D-ribofuranose 15b. Ethylmagnesium bromide (12.8 mL, 3.0 M in Et_2O, 38.4 mmol) was added to a stirred solution of **13b** (24.0 g, 38.2 mmol) in anhydrous diethyl ether (200 mL) under Ar at room temperature. The mixture was then stirred for 5 h. Anhydrous DMF (20.0 mL) was added, and the mixture was further stirred for

18 h. The solvent was removed under reduced pressure. Saturated NH_4Cl solution (100 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 200 mL). The organic extracts were combined, washed with brine (2 × 200 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a pale brown syrup. Column chromatography eluting with hexane/ethyl acetate (3:1) gave a colorless syrup whose ^1H NMR showed that it was a mixture of **14b** and the starting material **13b**, which proved to be inseparable, so the mixture was used directly without further purification. A solution of the mixture containing **13b** and **14b** (2.0 g) in ethanol (200 mL) was added to a mixture of hydroxylamine hydrochloride (6.9 g, 0.10 mol) and $NaHCO_3$ (8.4 g, 0.10 mol) in H_2O (30 mL). The mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure; the residue was treated with H_2O (200 mL) and the mixture extracted with CH_2Cl_2 (3 × 200 mL). The organic extracts were combined, washed with brine (300 mL), and dried over $MgSO_4$. The solvent was removed under reduced pressure to give a pale brown syrup, which was purified by column chromatography eluting with hexane/ethyl acetate (3:1) to afford **15b** as a white crystalline solid (14.6 g, 65% yield in two steps), mp 178−180 °C: ^1H NMR ($CDCl_3$) δ 3.59 (dd, 2.4 Hz, 11.1 Hz, 1 H), 3.86 (dd, 2.4 Hz, 11.1 Hz, 1 H), 4.03 (dd, 2.0 Hz, 4.3 Hz, 1 H), 4.22 (dd, 2.0 Hz, 4.5 Hz, 1 H), 4.34 (dt, 2.0 Hz, 7.5 Hz, 1 H), 4.38−4.79 (m, 6 H), 6.41 (d, 2.1 Hz, 1 H), 7.22−7.39 (m, 15 H), 7.56 (s, 1 H), 8.12 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 67.5, 68.3, 72.7, 72.9, 73.4, 75.0, 80.8, 81.0, 89.6, 90.3, 89.6, 90.3, 120.7, 121.7, 127.7, 127.8, 128.0, 128.5, 128.6, 137.3, 137.8, 140.3. Anal. Calcd for $C_{30}H_{30}BrN_3O_5$: C, 60.80; H, 5.10; N, 7.09; Br, 13.49. Found: C, 60.76; H, 5.12; N, 7.12; Br, 13.74.

2,3-Dimethoxy-5-methoxymethyl-1-[(5-bromo-4-carbonitrile)imidazol-3-yl]-*β*-D-ribofuranose 16a. A mixture of oxime **15a** (2.10 g, 5.76 mmol) in acetic anhydride (200 mL) was heated under reflux for 3 h. The volatiles were removed under reduced pressure to give a brown syrup. Column chromatography eluting with CH_2Cl_2/MeOH (25:1) gave **16a** as a light brown syrup (1.84 g, 92%): ^1H NMR ($CDCl_3$) δ 3.37 (s, 3 H), 3.38 (s, 3 H), 3.47 (s, 3 H), 3.51 (dd, 2.7 Hz, 10.8 Hz, 1 H), 3.68 (dd, 2.7 Hz, 10.8 Hz, 1 H), 3.92 (br t, 2.1 Hz, 1 H), 3.99 (br t, 4.2 Hz, 1 H), 4.19−4.23 (m, 1 H), 5.74 (d, 3.9 Hz, 1 H), 8.00 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 57.9, 58.4, 59.0, 71.7, 77.0, 80.6, 81.5, 119.0, 119.9, 120.9, 130.2. Anal. Calcd for $C_{12}H_{16}BrN_3O_4$: C, 41.63; H, 4.67; N, 12.14; Br, 23.08. Found: C, 41.84; H, 4.76; N, 12.30; Br, 23.31.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-[(5-bromo-4-carbonitrile)imidazol-3-yl]-*β*-D-ribofuranose (16b). Using a procedure identical to that used for **16a**, we obtained **16b** as a colorless syrup (16.0 g, 90%) from **15b** (18.2 g, 30.6 mmol): ^1H NMR ($CDCl_3$) δ 3.50 (dd, 2.7 Hz, 10.8 Hz, 1 H), 3.75 (dd, 3.3 Hz, 10.8 Hz, 1 H), 4.07−4.14 (m, 2H), 4.36 (dd, 3.0 Hz, 7.5 Hz, 1 H), 4.43−4.66 (m, 6H), 5.82 (d, 4.2 Hz, 1 H), 7.20−7.38 (m, 15 H), 7.80 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 67.9, 68.3, 71.6, 71.7, 71.8, 71.9, 72.7, 74.9, 80.1, 82.2, 88.9, 90.3, 103.1, 109.3, 126.7, 126.8, 127.3, 127.5, 127.8, 136.1, 136.7, 138.3, 147.3, 165.7. Anal. Calcd for $C_{30}H_{28}Br_2N_2O_4$: C, 62.72; H, 4.91; N, 7.32; Br, 13.91. Found: C, 62.82; H, 4.91; N, 7.17; Br, 13.66.

2,3-Dimethoxy-5-methoxymethyl-1-[(5-carboxamide)-thieno[2,3-*d*]imidazol-3-yl]-*β*-D-ribofuranose (18a). A mixture of **16a** (4.10 g, 11.40 mmol), freshly prepared thioglycolamide (4.80 g, 52.10 mmol), and potassium carbonate (6.0 g, 43.2 mmol) in anhydrous DMF (300 mL) was heated at 55 °C for 18 h. The mixture was cooled, and the solid was filtered off. Then, the solvent was removed under reduced pressure to give a dark brown syrup. Column chromatography eluting with CH_2Cl_2/MeOH (15:1) gave a white solid **17a** (3.2 g, 75%), which was used directly in the next step: ^1H NMR (DMSO-d_6) δ 3.29 (s, 3 H), 3.32 (s, 3 H), 3.36 (s, 3 H), 3.50 (dd, 4.5 Hz, 11.1 Hz, 1 H), 3.55 (dd, 4.2 Hz, 11.1 Hz, 1 H), 3.77 (s, 2 H), 3.95 (dd, 4.2 Hz, 8.7 Hz, 1 H), 4.18 (dd, 4.2 Hz, 8.1 Hz, 1 H), 4.27 (br t, 8.1 Hz, 1 H), 5.77 (d, 2.7 Hz, 1 H), 7.14 (br s, 1H), 7.54 (br s, 1 H), 8.28 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 36.2, 58.0, 58.3, 59.2, 72.1, 78.1, 82.2, 84.2, 88.7, 101.3, 111.5, 141.0, 148.5, 169.7. A mixture of **17a** (3.2 g, 8.9 mmol) and sodium ethoxide (2 mL, 21 wt % in ethanol) in anhydrous ethanol (400 mL) was heated under reflux for 3.0 h. The solvent was removed under reduced pressure to give a brown solid. Purification via column chromatography eluting with CH_2Cl_2/MeOH (15:1) afforded **18a** as a white crystalline solid following recrystallization from MeOH (2.94 g, 92%), mp 57 °C: ^1H NMR (DMSO-d_6) δ 3.29 (s, 3 H), 3.32 (s, 3 H), 3.38 (s, 3 H), 3.55 (dd, 3.3 Hz, 9.0 Hz, 1 H), 3.61 (dd, 3.0 Hz, 9.0 Hz, 1 H), 4.00−4.05 (m, 2 H), 4.20 (br d, 1 H), 5.94 (d, 3.0 Hz, 1 H), 6.68 (br s, 1H), 6.88 (br s, 1 H), 8.23 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 58.6, 59.1, 59.3, 72.1, 77.5, 83.0, 84.1, 88.8, 98.9, 128.3, 139.8, 143.4, 146.5, 168.7. Anal. Calcd for $C_{14}H_{20}N_4O_5S$·0.5MeOH: C, 46.76; H, 5.95; N, 15.09; S, 8.61. Found: C, 46.43; H, 5.67; N, 15.11; S, 8.80.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-[(5-carboxamide)-thieno[2,3-*d*]imidazol-3-yl]-*β*-D-ribofuranose (18b). In a procedure analogous to that for **18a**, use of **16b** (16.0 g, 27.3 mmol), thioglycolamide (3.7 g, 41.0 mmol) and potassium carbonate (3.7 g, 27 mmol) gave **17b**, which was shown by TLC to be a mixture of **17b** and **18b** (7.8 g). The mixture was then treated with sodium ethoxide (21 wt % in ethanol, 1.0 g, 3.1 mmol) in anhydrous ethanol (100 mL) and refluxed for 4 h. The solvent was removed in vacuo to provide **18b** as a colorless syrup (5.0 g, 88%): ^1H NMR (DMSO-d_6) δ 3.59 (dd, 3.0 Hz, 11.1 Hz, 1H), 3.74 (dd, 3.9 Hz, 11.1 Hz, 1H), 4.24−4.34 (m, 3H), 4.45−4.66 (m, 6H), 6.09 (d, 2.7 Hz, 1 H), 6.52 (br s, 1H), 6.84 (br s, 1 H), 7.15−7.35 (m, 15H), 8.22 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 36.2, 58.0, 58.2, 58.3, 59.2, 72.1, 78.1, 82.2, 88.7, 101.3, 111.5, 141.0, 148.5, 169.7. Anal. Calcd for $C_{32}H_{32}N_4O_5S$: C, 65.74; H, 5.52; N, 9.58; S, 5.48. Found: C, 65.65; H, 5.62; N, 9.47; S, 5.43.

2,3-Dimethoxy-5-methoxymethyl-1-(imidazo[4′,5′:4,5]-thieno[3,2-*d*]pyrimidin-3-yl-7-one)-*β*-D-ribofuranose (19a). A mixture of **18a** (2.5 g, 6.9 mmol) and diethoxymethyl acetate (3.45 mL, 20.7 mmol) was refluxed for 3 h. The excess diethoxymethyl acetate was then evaporated and the residue purified by chromatography eluting with CH_2Cl_2/MeOH (8:1) to afford **19a** as a white solid (2.07, 81%), mp 82 °C: ^1H NMR (DMSO-d_6) δ 3.31 (s, 3 H), 3.34 (s, 3 H), 3.38 (s, 3 H), 3.55 (dd, 4.8 Hz, 10.8 Hz, 1H), 3.66 (dd, 4.8 Hz, 10.8 Hz, 1H), 4.04 (br t, 4.2 Hz, 1 H), 4.18 (br t, 3.9 Hz, 1 H), 4.69 (br t, 5.5 Hz, 1 H), 6.25 (d, 3.0 Hz, H-1), 8.30 (s, 1 H), 8.53 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 57.9, 58.4, 59.3, 72.6, 78.5, 81.8, 82.3, 88.0, 121.8, 128.6, 141.3, 143.5, 147.9, 149.3, 158.4. Anal. Calcd for $C_{15}H_{18}N_4O_5S$: C, 49.17; H, 4.95; N, 15.29; S, 8.75. Found: C, 49.00; H, 4.95; N, 15.26; S, 8.74.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-(imidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-3-yl-7-one)-*β*-D-ribofuranose (19b). A mixture of **18b** (2.0 g, 3.4 mmol), triethyl orthoformate (20 mL), and acetic anhydride (20 mL) was refluxed for 3 h. The excess solvent was evaporated and the residue purified by column chromatography eluting with ethyl acetate/hexane (3:1) to afford **19b** as a hygroscopic white foam (1.7 g, 81%): ^1H NMR ($CDCl_3$) δ 3.63 (dd, 4.1 Hz, 10.5 Hz, 1 H), 3.84 (dd, 5.0 Hz, 10.5 Hz, 1 H), 4.22 (t, 4.8 Hz, 1 H), 4.46 (q, 4.4 Hz, 1 H), 4.52−4.74 (m, 6 H), 4.94 (t, 5.5 Hz, 1 H), 6.28 (d, 5.1 Hz, 1 H), 7.02−7.12 (m, 5 H), 7.24−7.34 (m, 10 H), 7.86 (s, 1 H), 8.16 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 61.2, 61.6, 61.9, 68.5, 71.9, 73.2, 75.1, 79.1, 81.8, 88.7, 121.4, 127.5, 127.6, 127.8, 128.2, 136.9, 137.2, 137.4, 143.2, 144.4, 144.6, 151.2, 160.2. Anal. Calcd for $C_{33}H_{30}N_4O_5S$·0.33H_2O: C, 65.98; H, 5.14; N, 9.33, S, 5.33. Found: C, 65.97; H, 5.05; N, 9.11, S, 5.22.

2,3-Dimethoxy-5-methoxymethyl-1-(7-thiomethylimidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-3-yl)-*β*-D-ribofuranose (21a). A solution of P_2S_5 (2.3 g), dry pyridine (250 mL), and **19a** (2.1 g, 5.77 mmol) was refluxed for 24 h, cooled, and evaporated to dryness under vacuum, and the residue was purified by column chromatography eluting with CH_2Cl_2/MeOH (10:1) to afford **20a** as a grayish solid (1.33 g, 61%), which was used without purification. To a stirring solution of **20a** (1.1 g, 2.9 mmol) and potassium carbonate (486 mg, 3.5 mmol) in MeOH (100 mL) was added methyl iodide (446 mg, 3.19 mmol). After 15 min, the solvent was removed by rotary evaporation and the residue purified via column chromatography eluting with CH_2Cl_2/MeOH (15:1) to give **21a** as a colorless syrup (1.09 g, 93%), which was used directly without

further purification: ^{1}H NMR (DMSO-d_6) δ 2.71 (s, 3H), 3.30 (s, 3 H), 3.35 (s, 3 H), 3.38 (s, 3 H), 3.58 (dd, 5.4 Hz, 10.8 Hz, 1 H), 3.70 (dd, 5.0 Hz, 10.8 Hz, 1 H), 4.08 (br t, 4.5 Hz, 1 H), 4.22 (dd, 4.8 Hz, 8.7 Hz, 1 H), 4.80 (br t, 5.7 Hz, 1 H), 6.31 (d, 2.7 Hz, 1H), 8.62 (s, 1 H), 9.04 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 55.0, 58.0, 58.3, 59.2, 72.6, 78.4, 81.9, 82.2, 88.4, 116.7, 127.6, 146.3, 147.5, 150.4, 154.9, 164.6.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-(7-thiomethylimidazo-[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-3-yl)-*β*-D-ribofuranose (21b). A mixture of **19b** (2.8 g, 6.2 mmol) and P_2S_5 (2.8 g, 6.3 mmol) in anhydrous pyridine (200 mL) was heated under reflux for 24 h. The solvent was removed under reduced pressure to give a dark brown syrup. Column chromatography eluting with ethyl acetate/hexane (2:1) gave **20b** as a light brown foam (2.2 g, 75%), which was used without further purification. Methyl iodide (142 mg, 1.0 mmol) was added to a mixture of **20b** (292 mg, 0.5 mmol) and K_2CO_3 (138 mg, 1.0 mmol) in anhydrous MeOH (20 mL) and stirred for 10 min. The solvent was removed under reduced pressure, and the residue was purified by column chromatography eluting with ethyl acetate/hexane (2:1) to provide **21b** (1.69 g, 75%) as a light brown syrup: ^{1}H NMR (DMSO-d_6) δ 2.78 (s, 3 H), 3.68 (dd, 4.3 Hz, 10.5 Hz, 1 H), 3.92 (dd, 5.1 Hz, 10.5 Hz, 1 H), 4.25 (t, 4.8 Hz, 1 H), 4.48 (q, 4.5 Hz, 1 H), 4.55−4.87 (m, 6 H), 6.26 (d, 5.1 Hz, 1 H), 7.01−7.06 (m, 5 H), 7.26−7.37 (m, 10 H), 8.17 (s, 1 H), 8.68 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 12.6, 69.0, 72.3, 73.7, 75.5, 79.2, 82.3, 89.3, 126.7, 127.8, 127.9, 128.0, 128.2, 128.7, 137.4, 137.7, 137.9, 144.2, 145.1, 151.2, 153.1, 163.9. Anal. Calcd for $C_{14}H_{20}N_4O_5S$: C, 65.36; H, 5.16; N, 8.97; S, 10.26. Found: C, 65.49; H, 5.24; N, 8.77; S, 10.05.

2,3-Dimethyloxy-5-methyloxymethyl-1-[(7-amino)imidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-3-yl]-*β*-D-ribofuranose (22a). A solution of **21a** (500 mg, 1.26 mmol) in saturated butanolic ammonia (20 mL) was sealed in a steel bomb and heated at 160 °C for 90 h. The solvent was removed by rotary evaporation, and the residue was purified via column chromatography eluting with CH_2Cl_2/MeOH (8:1) to give **22a** as a white solid (426 mg, 92%), mp 84 °C: ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.27 (s, 3H), 3.28 (s, 3H), 3.29 (s, 3H), 3.47 (dd,, 1 H, H−C5), 3.72 (dd, 1 H, H−C5), 4.05 (t, 1H), 4.19 (t, 1H), 4.84 (t, 1H), 6.27 (d, 1H), 7.48 (s, 2H), 8.40 (s, 1H), 8.47 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 57.9, 58.3, 59.2, 72.6, 78.5, 81.8, 88.1, 100.1, 113.4, 128.0, 145.2, 145.7, 148.7, 154.9, 159.3.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-[(7-amino)imidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-3-yl]-*β*-D-ribofuranose (22b). A solution of **21b** (1.0 g, 1.6 mmol) in saturated butanolic ammonia (50 mL) was sealed in a steel bomb and heated at 160 °C for 90 h. The solvent was removed by rotary evaporation, and the residue was purified via column chromatography eluting with CH_2Cl_2/MeOH (8:1) to give **22** as a hygroscopic white foam (720 mg, 76%): ^{1}H NMR ($CDCl_3$) δ 3.64 (m, 1 H), 3.96 (m, 1 H), 4.25 (m, 1H), 4.62−4.89 (m, 2 H), 4.44−4.58 (m, 6H), 6.29 (d, 1 H), 7.06−7.09 (m, 5 H), 7.25−7.32 (m, 10 H), 8.14 (s, 1 H), 8.34, (s, 1H); ^{13}C NMR ($CDCl_3$) δ 68.9, 72.3, 73.6, 79.5, 82.2, 89.4, 114.3, 137.6, 137.7, 137.9, 146.2, 149.5, 154.3, 158.3. Anal. Calcd for $C_{33}H_{31}N_5O_4S\cdot 0.33H_2O$: C, 66.09; H, 5.32; N, 11.68; S, 5.35; Found: C, 66.04; H, 5.32; N, 11.56; S, 5.27.

1-[(7-Aminoimidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-3-yl)]-2,3,5-*β*-D-ribofuranose Triol (6). A mixture of **22b** (84 mg, 0.14 mmol), $BF_3\cdot OEt_2$ (250 μL, 1.2 mmol), and EtSH (1.2 mL, 16.0 mmol) in anhydrous CH_2Cl_2 (3.0 mL) was stirred at room temperature for 24 h. The solvent and excess reagents were removed under reduced pressure, and the residue was dissolved in H_2O (10 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The aqueous layer was evaporated to dryness to afford **6** as a hygroscopic white powder, following recrystallization in H_2O (26 mg, 58%): ^{1}H NMR (DMSO-d_6) δ 3.56 (dd, 3.3 Hz, 10.5 Hz, 1 H), 3.71 (dt, 3.3 Hz, 10.5 Hz, 1H), 3.99 (br s, 1H), 4.15 (br s, 1 H), 4.64 (q, 2.7 Hz, 1H), 5.23 (br s, 1H), 5.45 (br d, 6.9 Hz, 1H), 5.78 (dd, 3.3 Hz, 7.5 Hz, 1H), 5.98 (d, 7.2 Hz, 1H), 7.51 (s, 2H), 8.34 (s, 1H), 8.49 (s, 1H); ^{13}C NMR (D_2O) δ 60.9, 69.7, 75.8, 87.1, 90.7, 113.0, 123.0, 127.6, 129.9, 130.4, 146.8, 147.3; HRMS (FAB) calcd for (M + 1) $C_{12}H_{14}N_5O_4S$ 324.0766, found 324.0764. Anal. Calcd for $C_{12}H_{13}N_5O_4S\cdot 0.4H_2O$: C, 43.61; H, 4.21; N, 21.17; S, 9.70. Found: C, 43.93; H, 4.15; N, 20.77; S, 9.51.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-[5-(4-aminopyrimidin-6-yl)imidazol-1-yl]-1-*β*-D-ribofuranose (23). A mixture of **22b** (0.25 g, 0.42 mmol) and freshly prepared Raney Ni (1.0 g) in MeOH/H_2O (50:50 mL) was heated under reflux for 18 h. The mixture was filtered and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography eluting with CH_2Cl_2/MeOH (10:1) to afford **23** as a white hygroscopic foam (0.21 g, 88%): ^{1}H NMR (DMSO-d_6) δ 3.61 (dd, 3.0 Hz, 10.2 Hz, 1 H), 3.76 (dd, 4.5 Hz, 10.2 Hz, 1 H), 4.23 (br s, 2 H), 4.31−4.35 (m, 1 H), 4.49−4.68 (m, 6 H), 6.66 (s, 1 H), 6.87 (d, 3.3 Hz, 1 H), 6.95 (br s, 2 H), 7.19−7.33 (m, 15 H), 7.43 (s, 1 H), 8.12 (s, 1 H), 8.36 (s, 1 H); ^{13}C NMR (CD_3OD) δ 68.4, 72.2, 72.5, 73.2, 75.5, 81.7, 101.4, 127.8, 128.1, 128.2, 128.3, 128.6, 137.8, 137.9, 138.0, 158.0, 164.3. Anal. Calcd for $C_{33}H_{33}N_5O_4$: C, 70.32; H, 5.90; N, 12.42. Found: C, 70.27; H, 5.91; N, 12.29.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-[(5-aminoimidazo-[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-3-yl-7-one)]-*β*-D-ribofuranose (24). In a steel bomb, a mixture of **18b** (1.35 g, 0.344 mmol) and NaOH (0.68 g, 17 mmol) in anhydrous MeOH (50 mL) was stirred for 30 min. at room temperature until the mixture became homogeneous. Carbon disulfide (1.05 mL, 17.5 mmol) was added, and the bomb was sealed and heated in an oil bath at 145 °C for 18 h. The solvent was removed under reduced pressure and MeOH (80 mL) added to the residue. The mixture was cooled to 0 °C; hydrogen peroxide (30%, 7.5 mL) was added dropwise and the mixture stirred at 0 °C for 2 h. The mixture was then transferred to a steel bomb and anhydrous ammonia bubbled through for 10 min. The bomb was sealed and heated in an oil bath at 120 °C for 18 h. The solvent was removed in vacuo, and the residue was purified by column chromatography eluting with hexane/ethyl acetate (1:3). The fractions containing product were combined and concentrated, and a second column was run eluting with CH_2Cl_2/EtOH (95:5) to give **24** as a hygroscopic white foam (0.8 g, 57%): ^{1}H NMR ($CDCl_3$) δ 3.64 (dd, 3.0 Hz, 9.9 Hz, 1 H), 3.84 (dd, 4.5 Hz, 9.9 Hz, 1 H), 4.25 (t, 5.0 Hz, 1 H), 4.44 (dd, 4.0, Hz, 8.7 Hz, 1 H), 4.52−4.79 (m, 6 H), 5.26 (br s, 2 H), 6.30 (d, 4.2 Hz, 1 H), 7.14−7.37 (m, 15 H), 8.12 (s, 1 H), 11.69 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 68.0, 71.5, 71.7, 72.7, 74.7, 79.2, 81.0, 88.3, 110.2, 126.3, 127.0, 127.3, 127.6, 127.8, 136.7, 143.3, 145.5, 150.6, 152.8, 160.0. Anal. Calcd for $C_{33}H_{31}N_5O_5S\cdot 1.0H_2O$: C, 63.14; H, 5.14; N, 11.16; S, 5.11. Found: C, 63.11; H, 5.05; N, 11.13; S, 5.09.

1-[(5-Aminoimidazo[4′,5′:4,5]thieno[3,2-*d*]pyrimidin-3-yl-7-one)]-*β*-D-ribofuranose 2,3,5-Triol (25). In a procedure analogous to that used to obtain **6**, deprotection of **24** gave **25** as a hygroscopic white crystalline solid (57 mg, 58%), mp 233−235 °C: ^{1}H NMR (D_2O) δ 3.74 (dd, 2.4 Hz, 10.5 Hz, 1H), 3.82 (dd, 1.8 Hz, 10.5 Hz, 1H), 4.08−4.16 (m, 3H), 5.82 (d, 4.5 Hz, 1 H), 8.46 (s, 1H); ^{13}C NMR (CD_3OD) δ 62.2, 70.7, 77.0, 88.6, 92.5, 113.6, 129.4, 133.1, 138.8, 149.0, 154.3, 158.4; HRMS (ESI) calcd for $C_{12}H_{15}N_5O_5S$ 339.0637, found 339.0637. Anal. Calcd for $C_{12}H_{13}N_5O_5S\cdot 1.0H_2O$: C, 40.33; H, 4.23; N, 19.60; S, 8.97. Found: C, 40.52; H, 3.95; N, 19.32; S, 9.27.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-[5-(2-aminopyrimidin-6-yl-4-one)imidazol-1-yl]-1-*β*-D-ribofuranose (26). A mixture of **24** (0.20 g, 0.33 mmol) and Raney Ni in MeOH (50 mL) was heated under reflux for 18 h. The mixture was filtered, and the filtrate was evaporated under reduced pressure. Chromatography eluting with CH_2Cl_2/MeOH (10:1) gave the protected fleximer **26** as a sticky white foam (0.17 g, 90%): ^{1}H NMR ($CDCl_3$) δ 3.60 (dd, 3.0 Hz, 9.0 Hz, 1 H), 3.83 (dd, 4.5 Hz, 9.0 Hz, 1 H), 4.10 (br s, 1 H), 4.20−4.23 (m, 1 H), 4.34−4.38 (m, 1 H), 4.45−4.69 (m, 6 H), 5.68 (br s, 2 H), 6.05 (d, 4.2 Hz, 1 H), 6.88 (s, 1 H), 7.20−7.38 (m, 15 H), 7.50 (s, 1 H), 8.23 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 68.3, 72.7, 73.5, 76.0, 81.1, 81.8, 89.3, 98.4, 127.5, 127.9, 128.6, 132.9, 137.3, 137.4, 155.1, 165.4. Anal. Calcd for $C_{33}H_{33}N_5O_5\cdot 0.25$MeOH: C, 67.95; H, 5.83; N, 11.91. Found: C, 67.68; H, 5.78; N, 11.95.

1-[5-(Pyrimidin-6-yl-4-one)imidazol-1-yl]-1-*β*-D-ribofuranose 2,3,5-Triol (27). In a procedure analogous to that used to obtain **6**, deprotection of **19b** gave **27** as hygroscopic white

needles (142 mg, 64%), mp 242−244 °C: ^{1}H NMR (DMSO-d_6) δ 3.56 (dd, 3.3 Hz, 10.5 Hz, 1 H), 3.71 (dt, 3.3 Hz, 10.5 Hz, 1 H), 3.99 (br s, 1 H), 4.15 (br s, 1 H), 4.64 (q, 2.7 Hz, 1 H), 5.23 (br s, 1 H), 5.45 (br d, 6.9 Hz, 1 H), 5.78 (dd, 3.3 Hz, 7.5 Hz, 1 H), 5.98 (d, 7.2 Hz, 1 H), 7.51 (s, 2 H), 8.34 (s, 1 H), 8.49 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 61.5, 70.2, 74.1, 86.0, 89.0, 107.8, 120.9, 126.3, 128.2, 129.5, 142.7, 157.7; HRMS (ESI) calcd for (M + H) $C_{12}H_{13}N_4O_5S$ 325.0607, found 325.0611. Anal. Calcd for $C_{12}H_{12}N_4O_5S\cdot 1.0H_2O$: C, 42.10; H, 4.12; N, 16.37; S, 9.36. Found: C, 42.25; H, 4.01; N, 16.03; S, 9.10.

2,3-Dibenzyloxy-5-benzyloxymethyl-1-[5-(pyrimidin-6-yl-4-one)imidazol-1-yl]-1-β-D-ribofuranose (28). In a procedure analogous to that used to obtain **26**, cleavage of **19b** gave **28** as a hygroscopic foam (180 mg, 92%): ^{1}H NMR ($CDCl_3$) δ 3.59 (dd, 2.2 Hz, 10.8 Hz, 1 H), 3.80 (dd, 2.3 Hz, 10.8 Hz, 1 H), 4.18−4.25 (m, 2 H), 4.36−4.39 (m, 1 H), 4.45−4.67 (m, 6 H), 6.71 (s, 1 H), 6.73 (d, 3.0 Hz, 1 H), 7.18−7.36 (m, 15 H), 7.54 (s, 1 H), 8.00 (s, 1 H), 8.22 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 68.3, 72.4, 72.7, 73.4, 75.7, 81.2, 81.5, 88.9, 110.2, 127.6, 127.7, 127.8, 127.9, 128.4, 133.0, 137.2, 139.5, 148.6, 155.1, 163.7. Anal. Calcd for $C_{33}H_{32}N_4O_5\cdot 1.25H_2O$: C, 67.50; H, 5.88; N, 9.54. Found: C, 67.41; H, 5.57; N, 9.80.

1-[5-(4-Aminopyrimidin-6-yl)imidazol-1-yl]-1-β-D-ribofuranose 2,3,5-Triol (1). A mixture of **23** (0.20 g, 0.62 mmol), Pd/C (10% Pd on C, 0.20 g), and ammonium formate (0.20 g, 3.2 mmol) in MeOH (50 mL) was heated under reflux for 18 h. The mixture was filtered over Celite and the filtrate evaporated under reduced pressure. The residue was purified via column chromatography eluting with EtOAc/EtOH/acetone/H_2O (4:1:1:0.5) to give hygroscopic white needles (98 mg, 94%), mp 186 °C: ^{1}H NMR (D_2O) δ 3.65 (dd, 3.3 Hz, 12.9 Hz, 1 H), 3.82 (dd, 2.4 Hz, 12.9 Hz, 1 H), 4.00−4.13 (m, 2 H), 4.28 (dd, 1.8 Hz, 4.5 Hz, 1 H), 6.29 (d, 2.1 Hz, 1 H), 7.12 (s, 1 H), 8.00 (s, 1 H), 8.46 (s, 1 H), 9.19 (s 1 H); ^{13}C NMR (D_2O) δ 59.7, 68.1, 75.9, 84.6, 92.5, 106.8, 123.9, 127.5, 136.4, 150.0, 157.8, 161.8; HRMS (EI) calcd for $C_{12}H_{15}N_5O_4$ 293.1124, found 293.1127. Anal. Calcd for $C_{12}H_{15}N_5O_4\cdot 0.5H_2O$: C, 47.68; H, 5.33; N, 23.17. Found: C, 47.87; H, 5.37; N, 23.14.

1-[5-(2-Aminopyrimidin-6-yl-4-one)imidazol-1-yl]-1-β-D-ribofuranose 2,3,5-Triol (2): hygroscopic white needles (53 mg, 92%), mp > 212 °C dec; ^{1}H NMR (D_2O) δ 3.71 (dd, 3.3 Hz, 13.2 Hz, 1 H), 3.88 (br d, 13.2 Hz, 1 H), 4.04−4.16 (m, 2 H), 4.27−4.30 (m, 1 H), 6.42 (s, 1 H), 6.80 (s, 1 H), 7.90 (s, 1 H), 8.28 (s, 1 H), 9.17 (s, 1 H); ^{13}C NMR (D_2O) δ 59.6, 68.0, 75.8, 84.2, 92.5, 113.8, 122.1, 129.6, 135.5, 150.5, 152.1, 163.9. Anal. Calcd for $C_{12}H_{14}N_4O_5\cdot 1.0\ H_2O$: C, 46.15; H, 5.16; N, 17.94. Found: C, 46.33; H, 5.09; N, 17.85.

1-[5-(2-Aminopyrimidin-6-yl-4-one)imidazol-1-yl]-1-β-D-ribofuranose 2,3,5-Triol (3): hygroscopic white needles (0.155 g, 91%), mp > 204 °C dec; ^{1}H NMR (D_2O) δ 3.70 (dd, 3.3 Hz, 10.2 Hz, 1H), 3.87 (dd, 2.4 Hz, 10.2 Hz, 1H), 4.07−4.16 (m, 2H), 4.29 (dd, 2.4 Hz, 4.5 Hz, 1H), 6.11 (s, 1 H), 6.38 (d, 5.7 Hz, 1 H), 7.84 (d, 1.2 Hz, 1H), 9.14 (dd, 0.6 Hz, 1.5 Hz, 1 H); ^{13}C NMR (D_2O) δ 60.1, 69.0, 75.9, 85.4, 91.6, 105.0, 122.5, 130.0, 135.8, 145.3, 154.0, 163.4; HRMS (ESI) calcd for $C_{12}H_{15}N_5O_5$ 309.1073, found 309.1068. Anal. Calcd for $C_{12}H_{15}N_5O_5\cdot 0.75H_2O$: C, 44.65; H, 5.15; N, 21.71. Found: C, 44.53; H, 5.09; N, 21.57.

Ab Initio Quantum Mechanical Calculations. The starting geometries for the three fleximer nucleosides (**1**−**3**) were based on standard bond lengths and angles for purine nucleosides.[31] Each fleximer structure was initially optimized as a series of syn and anti conformations via unconstrained gradient geometry optimization using a 3-21G basis set. A total of eight syn and eight anti initial structures were generated that differed from one another by a 45° rotation of the pyrimidine moiety about the imidazole ring. This family of multiple conformations was used to assay the energetics of pyrimidine ring conformational freedom with respect to the rest of the fleximer nucleoside structure. The sixteen starting structures therefore served to minimize the uncertainty in the energetically favored values of the two dihedral angles. Specifically, the dihedral angle defined as C2′−C1′−N1−C2 (the glycosidic bond that defines the syn-anti conformation of the fleximer pyrimidine base about the ribose) and the dihedral defined by N1−C5−C6′−N1′ (the fleximer bond that defines the pyrimidine ring rotation about the imidazole ring) were initially altered and ultimately analyzed. Thus, the set of structures was also used to assess any effects that the starting geometry had on the final Hartree−Fock-optimized conformation. All furanose conformations were initially set to C2′ endo.

The sixteen 3-21G-optimized structures were subjected to self-consistent field (SCF) geometry optimizations using restricted Hartree−Fock (RHF) SCF gradient optimization at the 6-311G* level of theory. All internal degrees of freedom were optimized without constraints or parameterization. For the simulation, the gradient convergence was set to <0.01 Hartree/Bohr for the change in molecular energy as a function of the displacement forces. Electric dipole moments and molecular energies were calculated by single-point SCF optimization using an RHF 6-311G* basis set. All calculations were performed using SPARTAN software.

Automated Molecular-Docking Methods. The crystal structure of *S*-adenosylhomocysteine hydrolase complexed with the inhibitor (1′*R*, 2′*S*, 3′*R*)-9-(2′,3′-dihydroxycyclopentan-1′-yl)adenine (PDB code 1A7A) was the source of protein atomic coordinates. Prior to docking computations, the coordinate file was edited to remove all water molecules and the inhibitor. Docking was carried out using the program suite LigandFit from MSI, Inc. A search grid of 35 × 35 × 35 Å was constructed in the enzyme active site. Grid points were separated by 0.2 Å. All rotatable dihedrals of the adenosine fleximer were allowed to move freely, and the molecule was docked via a Monte Carlo configuration search. The docking run was allowed to save up to 1000 possible solutions, which were then automatically clustered into groups. A group was defined by a root-mean-squared (rms) deviation of less than or equal to 0.3 Å. The clusters were then scored on the basis of the calculated intermolecular energy of the solution.

Acknowledgment. Project support provided in part by the Georgia Tech/Emory Biomedical Research program and the Georgia Institute of Technology is greatly appreciated. The authors thank Professors Piet Herdewijn (Rega Institute, Leuven), Stewart W. Schneller (Auburn University), Donald Doyle (Georgia Tech), as well as Dr. Bal Bhat (ISIS Pharmaceuticals) and Drs. Kyo Watanabe, Krystoff Pankiewicz, and Steve Patterson (Pharmasset Inc.) for many helpful discussions. The efforts of Ms. Elizabeth Gooding, Ms. Marion Götz, and Ms. Julia Sponaugle in making starting materials are appreciated.

Supporting Information Available: Two-dimensional NMR spectra and structural analysis for compound **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0255476

(31) Saenger, W. *Principles of Nucleic Acids*; Springer-Verlag: New York, 1984.