Design and Divergent Synthesis of Aza Nucleosides from a Chiral Imino Sugar

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*Supporting Information

ABSTRACT: Several novel nucleoside analogues as potential inhibitors of glycosidases and purine nucleoside phosphorylase (PNP) have been synthesized via selective coupling of an appropriate nucleobase at different positions of an orthogonally protected imino sugar as a common precursor. This synthetic strategy offers a straightforward protocol for the assembly of imino sugar containing nucleosides, establishing a new repertoire of molecules as potential therapeutics.

INTRODUCTION

The synthesis and development of glycosidase inhibitors have been the focus of attention, due to their vital role played by carbohydrates in a variety of biological processes. The glycosidases are responsible for catalyzing the cleavage of the glycosidic bonds in oligosaccharides and glycoconjugates. Thus, these enzymes are involved in a wide range of biochemical process such as intestinal digestion, post-translational processing of glycoproteins, and the lysosomal catabolism of glycoconjugates. Consequently, compounds that are able to inhibit or modulate the activity of glycosidases have proven to be promising anticancer, antidiabetes, and antiviral agents.

One of the most potent glycosidase inhibitors belongs to the class of imino sugars (or aza sugars), with a demonstrated ability to interact with glycosidases and glycotransferases. Radicamine A (2) and B (3) are representative examples together with naturally occurring 3,4-dihydroxy-2,5-bis(hydroxymethyl)pyrrolidine (DMDP, 4) and its corresponding analogues 5 and 6 exhibiting potent glycosidase inhibition.

Additionally, the therapeutic potential of aza sugars has been further exploited by Schramm and co-workers in the design of purine nucleoside phosphorylase (PNP, E.C. 2.4.2.1) inhibitors. PNP is a ubiquitous nucleoside processing enzyme essential for DNA and RNA synthesis and energy metabolism. It is involved in the reversible phosphorolysis of purine nucleosides to their respective bases and ribose(deoxyribose)-α-1-phosphate. Inhibition of PNP leads to an accumulation of 2′-deoxyguanosine triphosphate (dGTP) in T-cells that are not able to metabolize dGTP because of their low nucleotidase activity, causing high concentrations of this compound and ultimately leading to cellular apoptosis. Therefore, PNP is a key target for the control of T-cell proliferative disorders and consequently, for the treatment of leukemia and autoimmune related diseases.

The seminal work by Schramm in understanding the transition state for the natural PNP-catalyzed hydrolysis of inosine has resulted in the rational design and synthesis of a series of PNP inhibitors. A variety of imino sugar containing nucleoside analogues have been identified to mimic the oxocarbenium ion character at the transition state. Some of imino sugars are the polyhydroxypyrrolidines, which are known to interact with glycosidases and glycotransferases.
them have shown to exhibit high PNP inhibitory activity. In fact, two nucleoside analogues are currently in human clinical trials for the treatment of T- and B-cell cancers and other autoimmune disorders. These are first-generation Immucillin-H (7, Figure 2) and second-generation DADMe-immucillin-H (8) analogues. Use of an imino sugar and 9-deazapurine base was a central feature in the design of these two nucleoside analogues.

The overall biological importance of polyhydroxypyrrolidines has sparked significant efforts in their synthesis. As a result, further studies on the synthesis and biological evaluation of polyhydroxypyrrolidines and related compounds are of broad interest. Additionally, one of the key objectives of modern drug discovery is to increase the diversity and number of small molecules available for biological screening. Diversity-oriented synthesis (DOS) is already playing a major role in medicinal chemistry. Although DOS is usually directed toward oriented synthesis (DOS) is already playing a major role in the goal, one approach involves generation of inhibitors of different target enzymes. To achieve this goal, one approach involves generation of inhibitors of different enzymes from a common synthetic precursor.

Given the central role of the polyhydroxypyrrolidine moiety in the design of glycosidase, PNP type inhibitors and our ongoing efforts for discovering biologically active nucleosides, we embarked on the synthesis of novel structures. The focus of this study is to use a common and versatile polyhydroxypyrrolidine precursor and introduce a suitable nitrogenated base at different positions of the imino sugar to generate potential enzyme inhibitors. Herein, we describe a practical synthetic strategy leading to a fast and efficient preparation of structurally diverse nucleoside analogues from a single imino sugar precursor.

■ RESULTS AND DISCUSSION

An efficient synthesis of imino sugar 5 has been described by Kumar and Ramesh as a strong α-fucosidase inhibitor from commercially available tri-O-benzyl-D-glucal 9 (Scheme 1). The synthesis of 5 was accomplished in a few steps from the protected imino sugar 10. We noted that chiral imino sugar 10 is perfectly set up with three orthogonal protecting groups that could be cleaved or manipulated selectively to expose a single functionality at a time. Also, we were inspired by the fact that five-membered imino sugars have shown glycosidase inhibitory activity. Therefore, it was of interest to expand the study to construct nucleosides containing imino sugars. Herein, we describe the utility of 10 as an ideal starting material for building novel nucleoside analogues (Figure 3).

We envisioned synthesizing two types of nucleoside analogues starting from imino sugar 10. The first set of C-linked nucleosides was built via attachment of a natural adenine base to the C2 position of 10, resulting in compounds 15, 20, and 25. In the second set of N-linked nucleosides, we utilized the unnatural 9-deazapurine base and connected it to the ring nitrogen to furnish compounds 28 and 31.

Synthesis of C-Linked Nucleosides. The synthesis of imino sugar containing nucleoside 15 was accomplished in five steps, starting with 10. First, selective hydrolysis of the acetyl group with Na2CO3 in MeOH furnished 11 in quantitative yield. The deprotection of N-Ts was not observed under the mild conditions used for the hydrolysis of an acetyl group. Second, the primary hydroxyl group in 11 was tosylated under standard conditions to furnish 12 in excellent yield. In the third step, glycosylation of protected 12 with adenine base afforded protected nucleoside 13 in good yield (ca. 81%) after silica gel chromatography. It is noteworthy that N9-substituted product was isolated as the major product upon glycosylation. The deprotection of 13 into 15 was achieved in a two-step process. First, detosylation with Mg/MeOH and then debenzylation with an acid offered unprotected nucleoside 15 in good yield (Scheme 1). The structure of nucleoside 15 was established by 1H and 13C NMR data.

Wong et al. reported that the introduction of a methyl group into the N1 position of the imino sugars resulted in improved biological properties.16a,18 Therefore, we also became interested in developing a method to synthesize the N-methylated analogue of 15. As shown in Scheme 2, the reaction of compound 10 with Mg in MeOH removed the tosyl group with concomitant cleavage of the acetyl protecting group to afford 16 in high yield (ca. 89%). Interestingly, when we tried the N-detosylation of 10 using Mg turnings activated with iodine, we observed the formation of 17 in moderate yield (46%). We postulate that in situ iodine mediated oxidation of MeOH to formaldehyde may have contributed to the formation of bicyclic structure 17.19

![Figure 2. Structures of the two PNP inhibitors currently in clinical trials.](image)

![Figure 3. Three classes of orthogonal protecting groups of imino sugar 10.](image)
However, the synthesis of 17 was unequivocally established by treatment of 16 with aqueous formaldehyde in the presence of acid, giving 94% of the desired product. Next, selective hydrogenolysis of the hemiaminal afforded N-methylated aza sugar 18 without cleaving the benzylic groups. Subsequent tosylation of 18 under conditions identical with those described for 11 furnished a mixture of products. The $^1$H NMR spectra of the mixture failed to show the presence of the tosyl signals. Gratifyingly, mesylation of the mixture not only deblocked the acetal but also triggered an inversion at the C3 position. Moreover, the NOESY spectrum of the final compound 20 confirmed the configuration suitable for an elimination reaction. To the best of our knowledge, this is the first example of a locked bicyclic homoaza nucleoside synthesized in a straightforward manner.

The expeditious synthesis of nucleoside 15 and the C3-hydroxyl group configuration suitable for an elimination followed by ring closure encouraged us to assemble locked nucleic acid structures that are key building blocks for antisense oligonucleotides as therapeutic agents.20 Additionally, examples of natural and synthetic bicycle imino sugars with glycosidase inhibitory activity prompted us to undertake the synthesis of a locked aza nucleoside.96,21 Therefore, installation of a leaving group on the C3 hydroxyl group may allow ring closure onto the hydroxymethyl group, furnishing the novel bicyclic locked nucleoside analogue 25 (Scheme 3).

The synthesis of nucleoside 25 was accomplished in five steps starting with 13. First, acid treatment of 13 successfully cleaved the three benzyl groups simultaneously to furnish the hydroxymethyl group, furnishing the novel bicyclic locked nucleoside analogue 24 in excellent yield. Removal of the tosyl group from 24 following the same protocol as before yielded the final compound 25. To the best of our knowledge, this is the first example of a locked bicyclic homoaza nucleoside synthesized in a straightforward manner.

The structures of 24 and 25 were confirmed by extensive analysis of 1D and 2D NMR spectroscopy. After cyclization, HMBC spectra of compound 24 clearly showed a three-bond correlation between C3 and CH$_3$O hydrogen atoms of the new five-membered ring together with other expected cross-peaks. The structure was further confirmed on the basis of the NOESY spectrum of the final compound 25, which showed a correlation between H3 and H4 hydrogen atoms, supporting the change in the previous trans relative configuration between them. These data supported the inversion at the C3 position (Figure 4).

The synthesis of N-Linked Nucleosides. The first and second generations of PNP inhibitors containing an imino sugar moiety have already demonstrated their potential in the design of drug molecules, where Immucillin-H was granted orphan drug status in the United States for the treatment of various leukemias. Subsequently, Schramm and co-workers described a new class of inhibitors (8, Figure 2) for PNP in which the 9-deazapurine moiety is directly attached to the N atom of the aza sugar via a methylene bridge.22 Currently, DADMe-Immucillin-H is under phase II clinical development for the treatment of gout. The promising PNP inhibitory activity of N-linked nucleosides prompted us to undertake the synthesis of the twoaza sugar nucleoside analogues 28 and 31 (Schemes 4 and 5, respectively).

Orthogonally protected aza sugar 10 also serves as an attractive starting material for the synthesis of proposed PNP inhibitors, increasing the molecular diversity that can be created from a single imino sugar structure. The aza sugar 10 was conveniently transformed into 16 (Scheme 2) with the unprotected imino group ready for coupling with the base. Schramm et al. have utilized the Mannich reaction for the assembly of immucillin analogues starting with imino sugars.23 Surprisingly, the reaction of 16, 9-deazapurine, and form-
aldehyde under Mannich conditions led to the formation of the bicyclic 17 (Scheme 2) as a major product instead of the desired nucleoside. We believe that the iminium intermediate formed after the reaction of 16 with formaldehyde underwent concomitant intramolecular attack of the free hydroxyl group to furnish 17. Therefore, an alternative approach was conceived, starting with the previously reported 9-deazapurine 26\(^\text{8a}\) prefunctionalized with an aldehyde group that will be easier to link with imino sugar 16 using reductive amination conditions. As expected, the coupling of 16 with aldehyde 26 in the presence of NaBH\(_4\)CN furnished the desired nucleoside 27 in 84% isolated yield after chromatography (Scheme 4).

Next, the treatment of 27 with acid cleanly removed all five protecting groups in 2.5 h under reflux to furnish the N-linked nucleoside 28 as a hydrochloride salt. The structure of 28 was established by extensive NMR and MS data. \(^1\)H NMR spectrum of compound 28 shows only half of the expected signals, confirming the C\(_3\) axis of symmetry that was further supported by NOESY experiments. It is striking to note that the synthesis of a complex nucleoside such as 28 was accomplished in three simple steps starting with 10 in high yield.

In recent years, use of the fluorine atom has proven to be an indispensable tool for drug discovery efforts.\(^{24}\) For this reason, Schramm et al. have described the synthesis and biological evaluation of an inhibitor of human PNP where one of the hydroxy groups of the pyrrolidine moiety has been replaced by a fluorine atom.\(^{25}\) In a similar way, we wished to synthesize the fluoro nucleoside 31 as a structural analogue of nucleoside 28, expanding the repertoire ofaza nucleosides accessible for drug discovery efforts. The prior success of the synthesis of 28 prompted us to install the fluoro substituent first and then carry out the coupling with the formyl-functionalized 26. Therefore, treatment of 11 with DAST at a low temperature followed by N-detsylation of the crude product furnished 29 in moderate yield (ca. 55%) over two steps (Scheme 5).

Fluoro aza sugar 29 was then reductively alkylated with aldehyde 26 in the presence of NaBH\(_4\)CN in a MeOH/THF mixture to afford the protected product 30 in excellent yield. Next, the acid-mediated deprotection of 30 gave the fluoro nucleoside 31 in 84% yield, isolated as the hydrochloride salt.

**CONCLUSIONS**

In summary, we have synthesized a series of five nucleoside analogues using divergent and high-yielding protocols. We started from the common chiral imino sugar 10 by selective removal of the protecting groups followed by glycosylation with appropriate bases. The homoaza nucleoside 15 and its bicyclic analogue 25 were assembled by starting from 10, where the O-acetyl group was deblocked selectively. A serendipitous reaction led to the formation of the bicyclic structure 17, which was further transformed into the N-methyl derivative 20. On the other hand, concomitant deprotection of N-tosylate and O-acetyl groups from 10 offered a short synthesis of potential PNP inhibitors 28 and its fluorine analogue 31 via reductive amination. This study illustrates the successful utility and applications of the easily accessibleaza sugar 10 for the preparation of novel N- and C-linked nucleoside analogues with potential for interesting biological activity. Clearly, aza sugar 10 is a desirable scaffold containing orthogonal protecting groups that will enable the discovery of divergent reaction pathways and synthesis of structurally diverse compounds needed for modern day drug discovery and synthetic organic chemistry applications. Full exploitation of this unique class of nucleosides as potential therapeutics and their biological screening is currently in progress.

**EXPERIMENTAL SECTION**

General Considerations. All reagents were the highest commercial quality and were used without further purification. All nonaqueous reactions were carried out under anhydrous conditions in dry, freshly distilled solvents. Reactions were monitored by TLC with commercial quality and were used without further purification.

**Procedures and Experimental Data.** \((2S,3R,4R,5R)-3,4-Bis(benzoyloxy)-5-(benzoyl oxy)methyl)-2-(hydroxy)methyl-N-(p-toluenesulfonyl)oxy-N-(p-toluenesulfonyl)pyrrolidine (11).\) A suspension of 10 (400 mg, 0.635 mmol) and Na\(_2\)CO\(_3\) (508 mg, 4.8 mmol) in MeOH (8 mL) was stirred at reflux for 1.5 h. After completion of the reaction, the mixture was filtered over Celite and washed with MeOH and CH\(_2\)Cl\(_2\). Solvents were evaporated to give 11\(^1\) as a colorless viscous liquid (372 mg, 99%) without purification. \((2S,3R,4R,5S)-3,4-Bis(benzoyloxy)-5-(benzoyl oxy)methyl)-2-[(4-methylphenyl)sulfonyl]oxy-N-(p-toluenesulfonyl)pyrrolidine (12).\) A solution of 11 (373 mg, 0.635 mmol) and p-TsCl (607 mg, 3.2 mmol) in dry pyridine (6.5 mL) was stirred overnight at 50 °C. Pyridine was evaporated under vacuum, and the resulting residue was subjected to flash chromatography (20% EtOAc/hexane) to yield 12 (424 mg, 90%) as a colorless viscous liquid. \(R\_f\) (50% EtOAc/hexanes): 0.69 (KBr): \(\nu\) 3.063, 3034, 2924, 2871, 1736, 1598, 1496 cm\(^{-1}\); \(\alpha\_D\)\(^\text{20}\) = -14° (c 0.5, CH\(_2\)Cl\(_2\)). \(^1\)H NMR (CDCl\(_3\), 300.13 MHz): \(\delta\) 2.34, (s, 3H), 2.38 (s, 3H), 3.58 (d, 1H, \(J\_HH = 10.0 Hz\)), 3.80 (dd, 1H, \(J\_HH = 10.0 Hz, J\_HH = 3.1 Hz\)), 3.98 (m, 2H), 4.23 (m, 5H), 4.63 (m, 6H), 7.03 (m, 2H), 7.24 (m, 16H), 7.70 (d, 2H, \(J\_HH = 8.4 Hz\)), 7.77 (s, 3H).
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(2H, J_H, 6.9 Hz). 13C NMR (CDCl3, 75.5 MHz): δ 21.3, 21.4, 56.9, 58.2, 65.8, 67.2, 72.5, 73.0, 73.3, 80.0, 80.2, 126.8–129.4 (23 C), 132.8, 137.2, 137.8, 138.0, 138.2, 143.4, 144.6. MS (ESI+, m/z) 742 [(M + H)+ 100%], 764 [(M + Na)+ 60%]. HRMS (ESI+): calc for C9H14N2O5S [M + Na]+ 764.2322, found 764.2299.

(25,3R,4R,5S)-2-(9-Aminopyrimidin-4-yl)-3-benzoxazolyl-5-(benzoxazolylmethyl)-N-(p-toluenesulfonyl)sulfonamide (13). A mixture of compound 12 (300 mg, 0.426 mmol), adenine (86 mg, 0.639 mmol), K2CO3 (118 mg, 0.852 mmol), and 18-crown-6 (168 mg, 0.639 mmol) in DMF (4.3 mL) was heated to 90 °C overnight. DMF was evaporated under vacuum, and flash column chromatography (2% MeOH/CH2Cl2) of the residue gave pure 13 (23 mg, 81%) as a white foam. Rf (10% MeOH/90% CH2Cl2) = 0.48. [α]D210° = +20° (c 0.5, CHCl3). 1H NMR (CDCl3, 300.13 MHz): 2.32 (s, 3H), 3.57 (d, 1H, J_H, 9.0 Hz), 3.97–4.17 (m, 4H), 4.25–4.70 (m, 7H), 4.96 (dd, 1H, J_H, 3.2 Hz, J_H, 13.8 Hz), 6.26 (br s, 2H), 6.88 (m, 2H, J_H, 9.0–7.32 (m, 16H), 7.93 (d, 1H, J_H, 9.7 Hz), 7.97 (s, 1H), 8.39 (s, 1H). 13C NMR (CDCl3, 75.5 MHz): δ 21.5, 44.3, 57.2, 59.0, 65.3, 72.6, 73.2, 80.1, 80.5, 119.3, 126.8–129.4 (20C), 136.9, 137.3, 137.5, 137.8, 142.1, 143.4, 150.5, 152.7, 155.6. MS (ESI+, m/z): 705 [(M + H)+ 100%]; 727 [(M + Na)+ 50%]. HRMS (ESI): calc for C38H34N4O5S [M + H]+ 705.2885, found 705.2809.

(25,3R,4R,5S)-2-(9-Aminopyrimidin-4-yl)-3-benzoxazolyl-5-(benzoxazolylmethyl)-N-methylpyrrolidine (14). Mg (turnings) was added (68 mg, 2.8 mmol) to a solution of compound 13 (200 mg, 0.28 mmol) in dry MeOH (2.8 mL), and the mixture was refluxed for 2 h. Water was evaporated, and purification by flash chromatography (10% MeOH/90% CH2Cl2) afforded 14 (141 mg, 90%) as a white foam. Rf (10% MeOH/CH2Cl2) = 0.32. [α]D210° = −19° (c 0.5, CHCl3). 1H NMR (CDCl3, 300.13 MHz): 0.62–2.33 (br s, 1H), 3.53 (dd, 1H, J_H, 9.0 Hz, J_H, 9.0 Hz), 3.61 (dd, 1H, J_H, 4.6 Hz, J_H, 9.2 Hz), 3.69 (q, 1H, J_H, 6.4 Hz), 3.94 (m, 1H), 4.00 (m, 1H), 4.04 (m, 1H), 4.18 (dd, 1H, J_H, 8.0 Hz, J_H, 14.0 Hz), 4.21–4.59 (m, 9H), 5.86 (br s, 2H), 7.22–7.39 (m, 15H), 7.81 (s, 1H), 8.33 (s, 1H). 13C NMR (CDCl3, 75.5 MHz): δ 45.1, 58.0, 59.0, 68.8, 72.3, 73.4, 82.1, 84.2, 104.0, 125.7–128.5 (15 C), 138.0, 137.9, 138.1, 141.8, 150.1, 152.7, 156.4. HRMS (ESI): calc for C27H27N5O2S [M + H]+ 515.2175, found 515.2502; 1H NMR (CDCl3, 300.13 MHz): 0.63 (dd, 1H, J_H, 4.4 Hz, J_H, 12.0 Hz), 0.70 (dd, 1H, J_H, 4.4 Hz, J_H, 12.0 Hz), 0.73 (q, 1H, J_H, 4.4 Hz), 4.25 (dd, 1H, J_H, 1.6 Hz, J_H, 3.2 Hz), 4.31 (m, 1H), 4.36 (dd, 1H, J_H, 1.6 Hz, J_H, 4.0 Hz), 4.62 (dd, 1H, J_H, 7.6 Hz, J_H, 15.2 Hz), 4.70 (dd, 1H, J_H, 6.5 Hz, J_H, 15.2 Hz), 8.18 (s, 2H). 13C NMR (CDCl3, 100.6 MHz): δ 40.7, 50.7, 57.5, 60.3, 63.9, 74.3, 74.6, 118.4, 142.3, 150.0, 152.3, 155.3. MS (ESI+, m/z): 281 [(M + H)+ 100%]; 303 [(M + Na)+ 80%]. HRMS (ESI): calc for C19H19N3O3S [M + H]+ 281.1387, found 281.1387.

(25,3R,4R,5S)-3,4-Dihydroxy-5-(hydroxymethyl)-N-methylpyrrolidine (15). A mixture of 14 (100 mg, 0.18 mmol) and concentrated aqueous HCl (38%; 1.8 mL) was refluxed for 2 h. Water was evaporated, and aqueous NH3 (32%; 1 mL) was added to the residue. After the aqueous phase was evaporated, the resulting solid was purified by column chromatography (CH2Cl2/MeOH/NH4OH, 88/10/2) to afford 15 (41 mg, 82%) as a white solid. Rf (CH2Cl2/MeOH/NH4OH, 6:3:1) = 0.32. [α]D210° = −21° (c 0.5, MeOH). 1H NMR (CDCl3, 400.13 MHz): δ 3.30 (dd, 1H, J_H, 4.4 Hz, J_H, 12.0 Hz), 0.70 (dd, 1H, J_H, 4.4 Hz, J_H, 12.0 Hz), 0.73 (q, 1H, J_H, 4.4 Hz), 4.25 (dd, 1H, J_H, 1.6 Hz, J_H, 3.2 Hz), 4.31 (m, 1H), 4.36 (dd, 1H, J_H, 1.6 Hz, J_H, 4.0 Hz), 4.62 (dd, 1H, J_H, 7.6 Hz, J_H, 15.2 Hz), 4.70 (dd, 1H, J_H, 6.5 Hz, J_H, 15.2 Hz), 8.18 (s, 2H). 13C NMR (CDCl3, 100.6 MHz): δ 40.7, 50.7, 57.5, 60.3, 63.9, 74.3, 74.6, 118.4, 142.3, 150.0, 152.3, 155.3. MS (ESI+, m/z): 281 [(M + H)+ 100%]; 303 [(M + Na)+ 80%]. HRMS (ESI): calc for C19H19N3O3S [M + H]+ 281.1387, found 281.1387.

(25,3R,4R,5S)-3,4-Bis(benzoxazolyl)-5-(benzoxazolylmethyl)-N-(p-toluenesulfonyl)sulfonamide (21). Compound 13 (220 mg, 0.31 mmol) was refluxed in concentrated aqueous HCl (38%; 2.0 mL) for 2 h. Water was evaporated, and aqueous NH3 (32%; 1 mL) was added to the residue. After the aqueous phase was evaporated, the resulting solid was purified by column chromatography (10% MeOH/90% CH2Cl2) to give 21 (89 mg, 66%) as a white solid. Rf (10% MeOH/CH2Cl2) = 0.39. 1H NMR (DMSO-d6, 300.13 MHz):
R2, chromatography (1% MeOH/99% CH2Cl2) to give 119.5, 127.0 (2C), 130.0 (2C), 138.7, 141.2, 143.7, 149.3, 152.5, 156.1. 75.5 MHz): δ (1H, 4.30 (br s, 1H), 4.45 (dd, 1H, JHH 2.9 Hz, JHH 11.4 Hz), 5.16 (br s, 1H), 5.68 (br s, 1H), 7.23 (br s, 2H), 7.36 (d, 2H, JHH 8.1 Hz), 7.90 (d, 2H, JHH 8.1 Hz), 8.01 (s, 1H), 8.22 (s, 1H). 13C NMR (DMSO-d6, 75.5 MHz): δ 20.3, 24.3, 56.7, 56.9, 63.2, 78.3, 73.1, 118.5, 127.2 (2C), 129.4 (2C), 137.5, 141.0, 142.1, 143.9, 149.2, 152.4, 155.9. MS (ESI, m/z): 435 [M + H]+ 100%, 457 [M + Na]+ 10%. HRMS (ESI): calcd for C26H27N2O5S [M + H]+ 435.4435, found 435.4435.

(4aS,6R,7R)-6-(4-Hydroxy-3-methylpyrrolidine)-2,2-dimethyl-5-tosylhexahydro-1,3,5,7-tetrahydroimidazo[1,2,3,4]benzodiazepine (27). A mixture of compound 26 (56 mg, 0.19 mmol) was dissolved in 4/1 mixture of MeOH and THF (4 mL). Three drops of acetic acid were added, and the solution was stirred at 40 °C overnight. Solvents were evaporated in vacuo, and the residue was purified by flash chromatography (3% MeOH/97% CH2Cl2) to afford compound 27 (113 mg, 84%). 1H NMR (CDCl3, 300.13 MHz): δ 3.25 (m, 1H), 3.46 (tt, 1H, JHH 3.7 Hz, 3JHH 10.1 Hz), 3.79 (m, 2H), 8.66 (d, 1H, JHH 3.7 Hz, 3JHH 10.1 Hz), 4.13 (m, 1H), 4.57 (m, 2H, CH2O+C H2N). MS (ESI, m/z): 671 [M + H]+ 100%, 683 [M + Na]+ 20%. HRMS (ESI): calcd for C19H21F3N6O6S 607.1251, found 607.1242.

(25S,3R,4R,5S)-3,4-Bis(benzoyl)-5-(benzoyl)methyl-2-(hydroxymethyl)-N-(7-benzoyl)-6-methoxy-9-deazapurin-9-ylpyrrolidine (28). To a solution of 21 (89 mg, 0.205 mmol) in anhydrous THF (4.0 mL) and DMF (0.4 mL) were added 2-methoxypyridine (57 µL, 0.60 mmol) and p-TsOH (0.41 mmol, 72 mg) under a nitrogen atmosphere. The solution was stirred at room temperature. After 30 min, additional 2-methoxypyridine (97 µL, 1.02 mmol) was added and the solution was allowed to react for an additional 30 min. Solvents were evaporated under vacuum, and the residue was purified by flash chromatography (2% MeOH/CH2Cl2) to afford pure 22 (95 mg, 98%). 1H NMR (DMSO-d6, 600.13 MHz): δ 3.38 (s, 1H, H-5), 3.52 (t, 1H, JHH 8.2 Hz), 3.76 (d, 1H, CH2O JHH 8.4 Hz), 3.98 (m, 2H, CH2O+ CH2N), 4.03 (s, 1H, H-3), 4.10 (dd, 1H, CH2O JHH 12.6 Hz, 2JHH 8.4 Hz) 4.28 (s, 1H, H-2), 4.29 (d, 1H, H-4), 7.98 (s, 1H, 1H, JHH 13.3 Hz, 4JHH 8.4 Hz) 10.5 Hz), 5.66 (d, 1H, JHH 8.1 Hz), 7.23 (m, 2H, JHH 8.1 Hz), 7.74 (m, 2H, JHH 8.1 Hz), 8.01 (s, 1H), 8.22 (s, 1H). 13C NMR (DMSO-d6, 150.92 MHz): δ 41.8, 53.4, 57.3, 60.7, 62.6, 66.6, 69.9, 72.4, 72.8, 73.3, 82.3, 83.7, 115.3, 116.0, 127.2–128.3 (22C), 131.5, 136.8, 138.4, 138.6, 146.9, 150.0, 156.1. MS (ESI, m/z): 715 [M + H]+ 100%, 737 [M + Na]+ 10%. HRMS (ESI): calcd for C23H26F3N6O7S2 [M + H]+ 715.3490, found 715.3526.

(25S,3R,4R,5S)-3,4-DiHydroxy-2,5-bis(hydroxymethyl)-N-(9-deaza-hypoxanthin-9-yl)pyrrolidine (29). Compound 27 (112 mg, 0.16 mmol) was heated under reflux in concentrated HCl (38%, 2 mL) for 2.5 h. After it was cooled, the solution was concentrated in vacuo and aq. NH4OH (32%, 1 mL) was added to the residue. Water was evaporated, and the resulting solid was purified by column chromatography (CH2Cl2/MeOH/ NH4OH, 88/10/2) to afford 26 (36 mg, 74%), which was then converted with 10% aqueous HCl to 28. 1H NMR (CDCl3, 300.13 MHz): δ 3.80 (br s, 4H, CH2O), 4.58 (s, 2H, CH2E 8.1 Hz), 4.90 (d, 2H, H-4, JHH 3.4 Hz, 3JHH 15.3 Hz), 5.10 (d, 1H, H-5, JHH 13.4 Hz), 5.28 (d, 1H, CH2N JHH 13.5 Hz), 8.22 (s, 1H, H-8), 8.54 (s, 1H, H-2). 13C NMR (DMSO-d6, 150.92 MHz): δ 49.1 (CH3), 55.3 (CH2O), 69.0 (C-2 + C-5), 71.7 (s, 1H), 72.1–73.8 (3C), 117.7 (C-4B), 131.4 (C-8B), 143.1 (C-2B), 144.1 (C-5B), 155.1 (C-6B). MS (APCI, m/z): 311 [M + H]+ 20%. HRMS (ESI): calcd for C16H16N2O4 [M + H]+ 263.1243, found 263.1244.

(25S,3R,4R,5S)-3,4-Bis(benzoyl)-5-(benzoyl)methyl-2-(fluoromethyl)-N-(7-benzoyl)-6-methoxy-9-deazapurin-9-ylpyrrolidine (30). Sodium cyanoanhydride (8 mg, 0.13 mmol) was added to a stirred solution of 29 (31 mg, 0.07 mmol)


