JOC Article

Conformationally Constrained Purine Mimics. Incorporation of Adenine and Guanine into Spirocyclic Nucleosides

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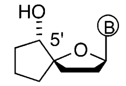
Directed syntheses of spirocyclic nucleosides featuring adenine and guanine as the nucleobases have been successfully developed. The key starting materials are the enantiomerically pure spiro lactones **4**, **15**, and **28**, which have proven amenable to conversion to anomeric mixtures of chloro sugars. The latter can enter into glycosidation by S_N2 displacement with the sodium salts of 6-chloropurine or 2-amino-6-chloropurine. In the saturated series, the chromatographic separation of **18** from **19** was possible. Where **33** and **34** are concerned, their relatively rapid rate of epimerization precluded this. Mimics **35**–**37** resisted isolation as pure anomers. The configurational assignments are based on the thermal interconversions and supporting MM3 steric energy calculations. Added corroboration was gained from a crystallographic analysis of **8**. Although removal of the TBS protecting groups in all late-stage guanine intermediates proved to be problematical, the pair of hydroxyl groups in **37** could be introduced by proper recourse to the oxidation of **36** with ruthenium tetraoxide.

Supplantation of the C5′ hydroxymethyl group resident in natural nucleosides by a spirocyclopentanol ring has given rise to a newly emerging class of analogues having attractive structural advantages.[1,2] These include the option of incorporating the otherwise conformationally flexible $-CH_2OH$ substituent specifically into an α or β arrangement as in **1** and **2**. Due to the inherent atomic arrangement, some conformational biasing operates, but not to the level of structural rigidification. In addition, the spirocyclic architecture holds promise of being less subject to free radical degradation, of contributing to the enhancement of duplex stability, and of offering greater resistance to degradation by cellular nucleases. Molecular modeling studies provide further indication that backbone and glycosyl torsional angles are minimally perturbed by the spirocyclic framework, thereby allowing this compound class to be considered as useful isosteres of natural nucleosides.[1f,g]



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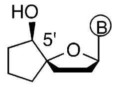
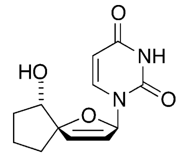


2

Several of the spirocyclic nucleosides synthesized to date in our laboratory have displayed significant antiviral activity.[3] One of the more exciting leads uncovered to the present time is the inhibitory capacity exhibited by **3** against human coronavirus. High selectivity and low cytotoxicity are additional characteristics of this substance. As part of the expansion of our synthetic program, a number of complementary dideoxy and didehydrodideoxy spironucleosides were targeted for preparation. In this context, notable use was made of the capacity for introducing unsaturation in the furanose sector via phenylsulfenylation. The application of Vorbrüggen technology[4] involving the $SnCl_4$-induced ionization of anomeric acetates in the presence of silylated derivatives of uracil and thymine proved to be notably serviceable.[1d] Although these objectives were met without serious drawback, related attempts to involve persilylated cytosine, benzoyladenine, and guanine proved uniformly unsuccessful because of the extensive decomposition incurred during the sulfoxide pyrolytic extrusion step. An alternative synthetic approach to the purine spironucleosides was therefore mandated, and the routes reported herein have proven feasible.



3

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10.1021/jo049413z CCC: \$27.50

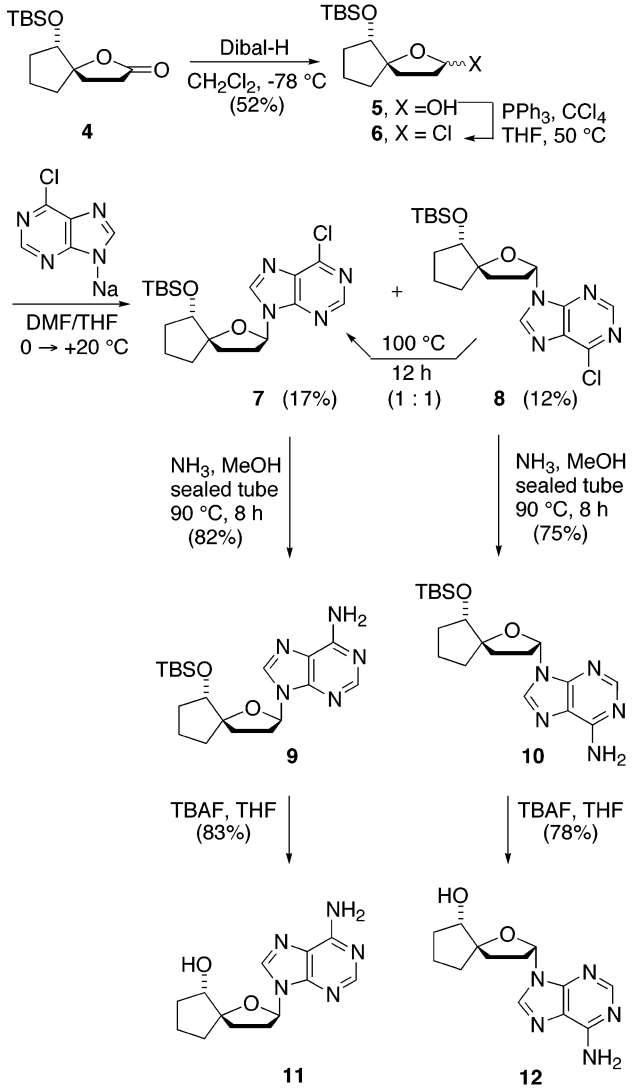
Results and Discussion

In light of the significant anti-HIV activity of the purine nucleoside β-D-2′,3′-dideoxyadenosine (D-ddA)[5] and its recognized susceptibility to degradation by the action of adenosine deaminase[5] and purine nucleoside phosphorylase,[6] the realization of possible resistance of inactive metabolite formation in this manner was initially pursued. The strategy used for the synthesis of spirocyclic congeners **11** and **24** involved S_N2-type displacement of halide ion from chlorides such as **6** with the sodium salt of 6-chloropurine.[7−10] This reactive 1-chloro spirofuranose was prepared nonstereoselectively by Dibal-H reduction of the enantiopure lactone **4**[11] followed by exposure to triphenylphosphine and carbon tetrachloride in THF at 50 °C.[12] The generation of an epimeric mixture was not a major concern in that Hildebrand and Wright have demonstrated that epimerization occurs readily in such systems.[8]

Glycosylation with **6** proceeded with the formation of **7** and **8** in nearly comparable amounts (Scheme 1). Chromatographic separation was possible and provided us with pure samples of two products. This pair of compounds was shown to be related as anomers by heating **8** to 100 °C for 12 h. Under these conditions, conversion into a 1:1 mixture with **7** materialized. This thermal lability was not observed in **7**, in keeping with the fact that anomerism in this series is rare. When detailed 1D and 2D NMR studies failed to establish the structural features of these purines beyond a reasonable doubt, recourse was made to MM3 calculations for assessing the relative thermodynamic stabilities of the four possible isomers including the N^7 and N^9 options. Monte Carlo simulations (MacroModel version 5.0) of 1500 different conformers in each instance showed **7** to be the global minimum by a rather small margin relative to **8** (0.8 kcal/mol). The N^7 isomers **13** and **14** exhibited significantly more elevated steric energies, presumably due to the greater proximity of the chlorine atoms to the furanose sector (Figure 1). Ultimately, it proved possible to grow crystals of **8** suitable for X-ray analysis, and thereby to confirm the earlier experimental and computational findings.

With the configurational issues resolved, substitutive amination was accomplished by heating **7** and **8** at 90 °C with methanolic ammonia in a sealed vessel for 8 h.[13−15] These nucleophilic displacements delivered **9** and **10** in isolated yields of 82% and 75%, respectively. Only when the temperature was increased to 100 °C was rearrangement observed. Unmasking of the hydroxyl substituents to give **11** and **12** was accomplished by routine desilylation with tetra-*n*-butylammonium fluoride. At this point, a further check of our configurational assignments was undertaken by comparing the C5 and C8 shifts to those of adenosine. Kjellberg and Johansson have described a method for identifying quite clear trends for both signals.[16] Thus, C5 in the N^7 isomer appears approximately 5 ppm upfield from that in N^9 isomer. The reverse is true for C8, which in N^7 derivatives appears 8−10 ppm downfield from that in N^9 systems. As seen in Table 1, the data for **11** and **12** compare so closely to the relevant adenosine shifts as to discount alternative options.

SCHEME 1



When recourse was next made to the diastereomeric levorotatory lactone **15**,[11] somewhat more efficient conversion to chloro sugar **17** and subsequently to the adenosines **18** and **19** was noted (Scheme 2). When careful chromatography on silica gel led to the separation of these anomers, the observation of their ready equili-

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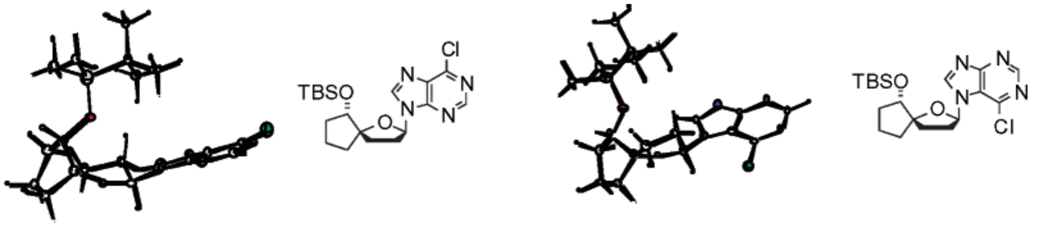
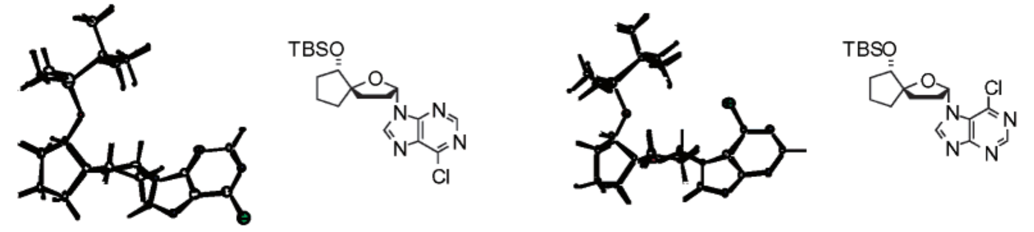


7
Steric energy = 51.6 kcal/mol

13
Steric energy = 62.0 kcal/mol



8
Steric energy = 52.4 kcal/mol

14
Steric energy = 62.2 kcal/mol

FIGURE 1. Global energy minima and associated steric energies for **7**, **8**, **13**, and **14** (1500 simulations per conformational search).

TABLE 1. Comparison of ^{13}C NMR Shifts for Adenosine, 11, and 12 ($CDCl_3$ Solution)

carbon	adenosine[a]	**11**	**12**
C8	140.2	138.9	139.9
C5	119.6	120.7	121.2

[a] Values recorded in DMSO-d_6 in this case.

bration on standing at room temperature or in the presence of acid was made possible. We assume that this interconversion proceeds by transient formation of the oxonium ion[17] and that the β/α distribution of 1.7:1 represents the thermodynamic equilibrium ratio. This interconversion is assumed to be an indication that both isomers share a common linkage to a specific nitrogen. The structural assignment to **18** and **19** follows in part from the results of MM3 calculations that show **18** to possess the lowest steric energy among those purines that constitute the **18/19** and **26/27** subsets (Figure 2).

Treatment of either pure **18** or pure **19** with methanolic ammonia at 100 °C resulted identically in the generation of 1.7:1 anomeric mixtures of both the methoxy (**20/22**) and amino derivatives (**21/23**). The same distribution resulted when the mixtures of **18** and **19** were employed directly. The production of **20** and **22** was favored at shorter reaction times. When heating was prolonged, a greater proportion of **21** and **23** was observed, but losses arising from decomposition became clearly apparent. Deprotection of the OH groups gave rise to **24** and **25** whose C8 (141.1, 138.9) and C5 shifts (121.8, 121.8) share close similarity with adenosine as expected (Table 1).

With a variable route to the saturated spirocyclic nucleosides in hand, the focus of our attention turned to the preparation of their unsaturated (sd4N) and RNA (sN) congeners. To this end, the previously described α,β-unsaturated lactone **28**[11] was transformed into the desired glycosyl chloride **30** in the predescribed manner (Scheme 3). In this instance, glycosidation with the sodium salt of 2-amino-6-chloropurine furnished **31** and **32** as a 2.3:1 mixture of anomers in 50% overall yield. Careful chromatography on silica gel led to isolation of enriched samples of both nucleosides. When samples of **31** and **32** purified in this manner were allowed to stand at room temperature for 12 h, reconversion to the original 2.3:1 mixture took place in both cases. As before, the structural assignments are based on the results of MM3 calculations (Figure 3).

Complete NOE studies were performed on most of the spironucleosides amenable to purification. In no instance was reliable data recorded. Ultraviolet spectra also proved inconclusive as found elsewhere.[16]

Synthetic use of the **31/32** isomer pair was undertaken in two directions. The first involved conversion to the diols **33** and **34**. This transformation was most expediently achieved by treatment with ruthenium tetraoxide in a two-phase solvent system constituted of ethyl acetate, acetonitrile, and water as developed by Shing and co-workers.[18] Reaction was complete in only 5 min, presumably because approach from the unhindered α face could operate. The separation of **33** from **34** was realized by MPLC on silica gel.

The second application targeted the possible concurrent preparation of unsaturated and RNA guanine relatives. For this purpose, the **31/32** mixture was treated with 2-mercaptoethanol and sodium methoxide in aqueous methanol according to precedent.[19,20] However, these conditions did not result in direct guanine formation (Scheme 4). Instead, sulfides **35** (ratio 2.3:1) were isolated

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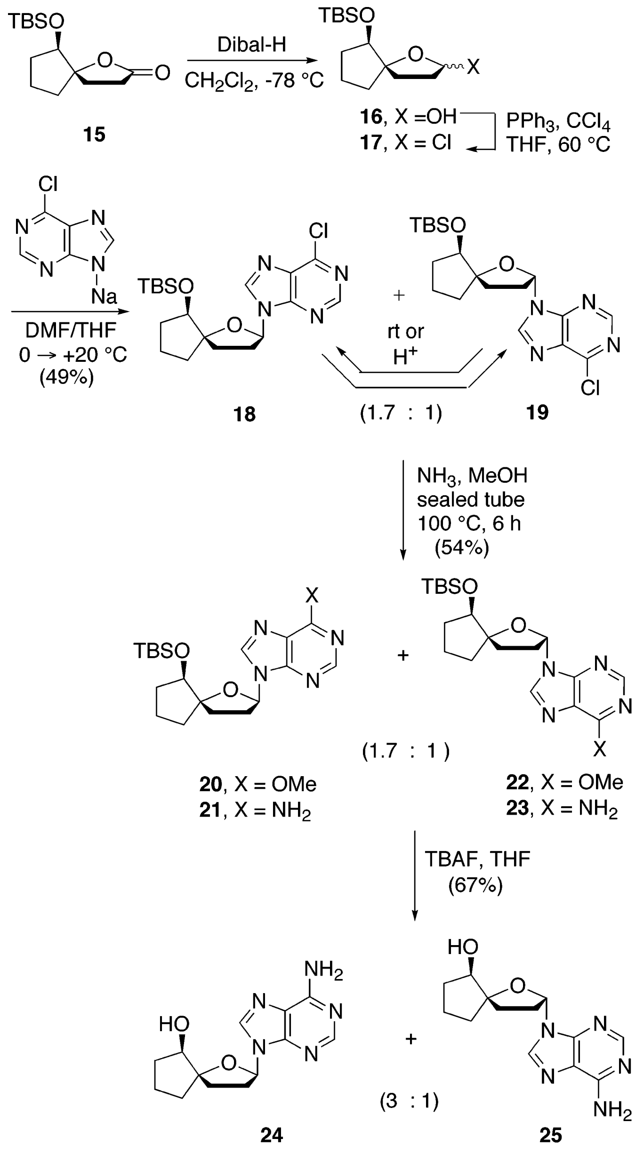
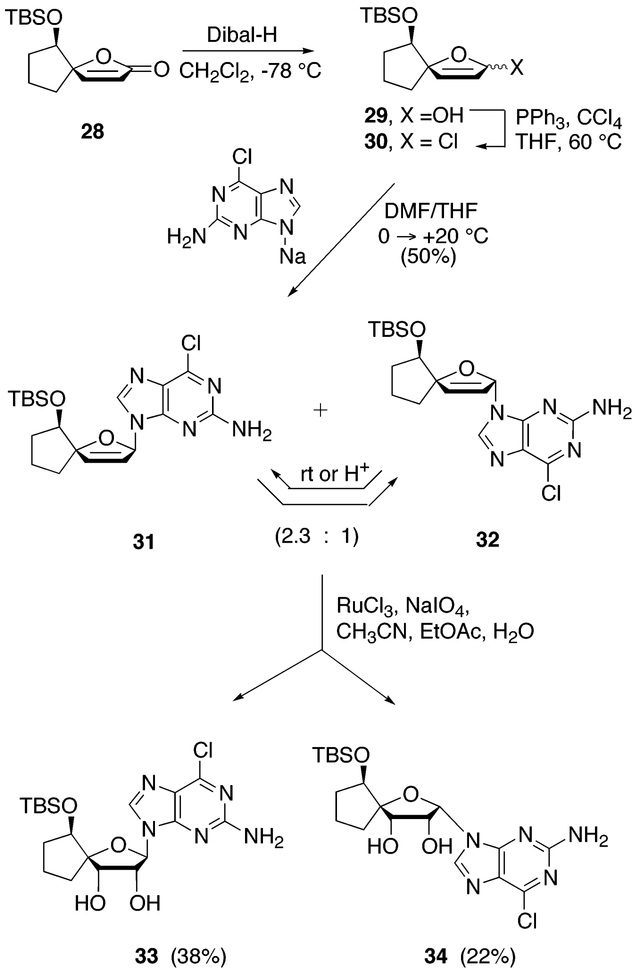
SCHEME 2



in 90% yield. When prolonged reaction times and/or introduction of added water and sodium methoxide were found to be ineffective in leading to **36**, a solvent change from methanol to the higher boiling dioxane was investigated. Satisfyingly, this modification caused the targeted conversion to be complete within 30 min. Subsequent acidification with acetic acid led to the precipitation of **36**, which proved amenable to dihydroxylation with osmium tetraoxide. The use of hydrogen sulfide to reduce the osmate ester was necessary because of the need to avoid an aqueous workup due to elevated water solubility and difficulty of isolation from water. After chromatography, the diol was obtained as a 3:1:1 mixture of diastereomers, with the major constituent having the configuration defined by **37** as determined by NOE methods.

As a pair, **33** and **36** proved notably sensitive to conditions required for the deprotection of their silyl ether functionality. The use of TBAF, HF−pyridine, KF/18-crown 6, CsF, and related reagents resulted either in no reaction or total decomposition. In no instance was heating tolerated.

SCHEME 3

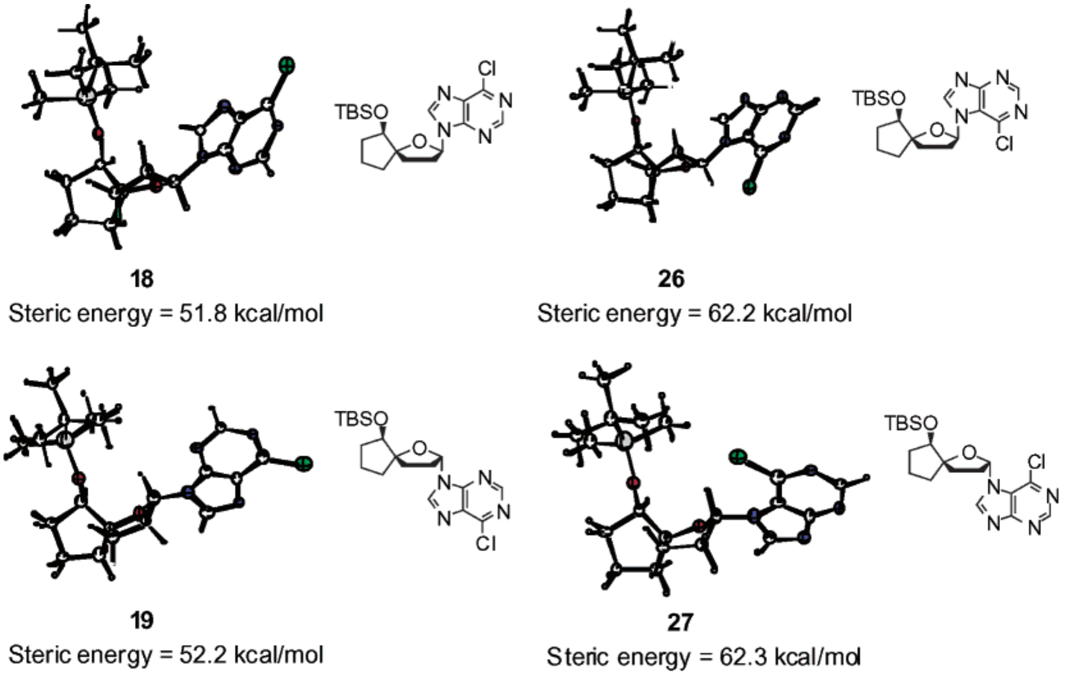


Conclusion

A viable approach to the synthesis of spirocyclic nucleosides carrying adenine and guanine as the nucleobases has been developed. The methodology takes advantage of the availability of saturated (e.g., **6** and **17**) and unsaturated (e.g., **30**) chlorides and their susceptibility to S_N2-type displacement with the sodium salts of 6-chloropurine and 2-amino-6-chloropurine. Although the glycosylations are not stereocontrolled, it has proven possible to effect chromatographic separation of the α and *β* anomers at this step (e.g., **7** from **8**) or one immediately following (e.g., **33** from **34**). The absolute configurational assignments originate with our knowledge of the stereochemistry inherent to enantiopure spiro lactones **4**, **15**, and **28**,[11] evolve further on the basis of ^{13}C NMR shifts of C5 and C8 and steric energies derived from molecular mechanics (MM3) calculations, and are supported by an X-ray crystallographic study. The sensitivity of **33** and **36** to fluoride ion has precluded ultimate arrival at two guanine unprotected spironucleosides.

Experimental Section

Formation of 6 and Coupling with the Sodium Salt of 6-Chloropurine. A solution of **4**[11] (1.00 g, 3.70 mmol) in dry CH_2Cl_2 (200 mL) was treated dropwise at −78 °C with Dibal-H (1 M in hexanes, 7.4 mL). The reaction was allowed to proceed for 1 h and was followed by the addition of a



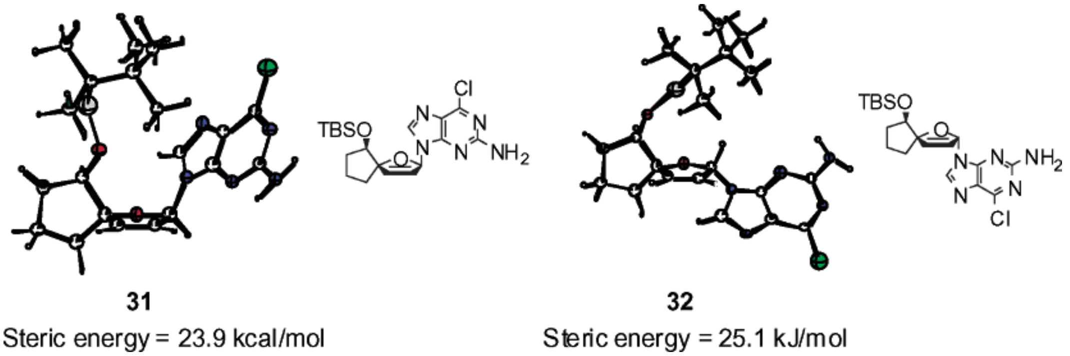
18
Steric energy = 51.8 kcal/mol

26
Steric energy = 62.2 kcal/mol

19
Steric energy = 52.2 kcal/mol

27
Steric energy = 62.3 kcal/mol

FIGURE 2. Global energy minima and associated steric energies for **18**, **19**, **26**, and **27** (1500 simulations per conformational search).

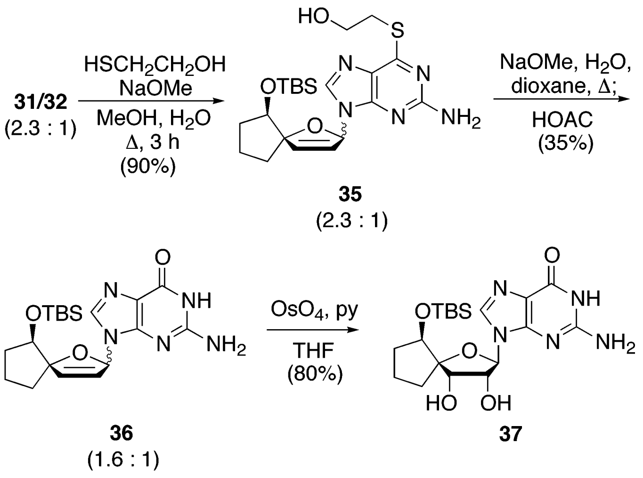


31
Steric energy = 23.9 kcal/mol

32
Steric energy = 25.1 kJ/mol

FIGURE 3. Global energy minima and associated steric energies for **31** and **32** (1500 simulations per conformational search).

SCHEME 4



saturated solution of Rochelle's salt (100 mL). After warming to rt, the bilayer was stirred until both phases were clear (~3 h). The separated aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL), dried, and concentrated. After chromatography on silica gel (eluent 10% ether in hexanes), **5** was isolated as a colorless oil (0.52 g, 52%) alongside the overreduced diol (0.23 g, 23%).

Lactol **5** (0.30 g, 1.1 mmol) was dissolved in dry THF (10 mL), treated with triphenylphosphine (0.5 g, 1.9 mmol) and carbon tetrachloride (1.5 mL), and heated at 50 °C for 3 h during which time a white precipitate formed. The suspension was allowed to cool, and the supernatant solution was cannulated into a solution of the sodium salt of 6-chloropurine. The latter was prepared by reaction of 6-chloropurine (0.30 g, 1.8 mmol) in DMF (8 mL) with sodium hydride (0.18 g, 7.6 mmol) at 0 °C. The reaction mixture was stirred at rt for 3 h, diluted with water (5 mL), and extracted with ether (3 × 20 mL) prior to drying and solvent evaporation. The brown tar was taken up in benzene and purified by column chromatography. Elution with hexane/ethyl acetate 9:1 afforded 218 mg of a 4:3 mixture of anomers. The latter was subjected to MPLC on silica gel (elution with 10% ethyl acetate in hexanes) to give 78 mg (17%) of pure **7** and 52 mg (12%) of pure **8** in addition to mixed fractions.

For **7**: colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.87 (s, 1 H), 8.67 (s, 1 H), 6.39−6.36 (m, 1 H), 3.95 (t, J = 8.2 Hz, 1 H), 2.61−2.57 (m, 1 H), 2.42−2.37 (m, 1 H), 2.15−2.11 (m, 1 H), 1.95−1.59 (series of m, 7 H), 0.87 (s, 9 H), 0.10 (s, 3 H), 0.06 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.4, 150.8, 150.5, 144.6, 132.2, 93.5, 85.6, 78.1, 34.1, 33.9, 31.1, 30.2, 26.0 (3C), 18.4, 18.1, −4.5 (2C); ES HRMS *m/z* (M + Na)$^+$ calcd 431.1646, obsd 431.1645; [α]$^{20}_{D}$ +6.9 (*c* 1.5, $CHCl_3$).

For **8**: white solid; mp 90.1−90.8 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (s, 1 H), 8.27 (s, 1 H), 6.41 (dd, *J* = 7.1, 2.7 Hz, 1 H), 3.82 (t, *J* = 8.0 Hz, 1 H), 2.83−2.72 (m, 1 H), 2.45−2.37 (m, 1 H), 2.37−2.20 (m, 1 H), 2.07−1.51 (series of m, 7 H), 0.93 (s, 9 H), 0.10 (s, 3 H), 0.09 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.7, 150.9, 150.8, 143.8, 132.4, 94.0, 86.9, 80.0, 33.7, 31.4 (2C), 25.9 (3C), 18.7, 18.0, −4.3, −4.8; ES HRMS *m/z* (M + Na)$^+$ calcd 431.1646, obsd 431.1642; [α]$^{20}_{D}$ +5.2 (*c* 0.83, $CHCl_3$). For X-ray crystallographic analysis, see the Supporting Information.

Amination of 7. A solution of **7** (41.5 mg, 0.101 mmol) in methanol saturated with ammonia (2.8 mL) was transferred to a 5 mL screw-top pressure tube, which was sealed and heated at 90 °C for 12 h. The solvent was removed under reduced pressure, and the residue was purified by chromatography on silica gel. Elution with 50% hexanes in ethyl acetate to pure ethyl acetate gave **9** (34.0 mg, 82%) as a white solid: mp 186.7−187.5 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (s, 1 H), 8.32 (s, 1 H), 6.34 (dd, *J* = 5.9, 4.7 Hz, 1 H), 5.86 (br s, 2 H), 3.95 (t, *J* = 8.2 Hz, 1 H), 2.63−2.51 (m, 1 H), 2.40−2.30 (m, 1 H), 2.18−2.03 (m, 1 H), 1.94−1.78 (series of m, 5 H), 1.64−1.59 (m, 2 H), 0.91 (s, 9 H), 0.12 (s, 3 H), 0.10 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.3, 152.5, 149.1, 139.8, 120.0, 92.9, 85.0, 78.1, 34.1, 33.9, 31.2, 30.3, 26.0 (3C), 18.4, 18.1, −4.3, −4.5; ES HRMS *m/z* (M + Na)$^+$ calcd 390.2325, obsd 390.2332; [α]$^{18}_{D}$ +14.8 (*c* 0.50, $CHCl_3$).

Amination of 8. A sample of **8** (56.4 mg, 0.138 mmol) was dissolved in methanol saturated with ammonia (3.8 mmol), heated in a sealed vessel at 90 °C for 8 h, and freed of solvent under reduced pressure. The residue was chromatographed on silica gel (elution with ethyl acetate) to provide 40.3 mg (75%) of **10** as a white solid: mp 217.8−218.5 °C dec; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (s, 1 H), 8.02 (s, 1 H), 6.39 (dd, *J* = 6.9, 2.7 Hz, 1 H), 6.04 (s, 2 H), 3.85 (dd, *J* = 8.6, 7.1 Hz, 1 H), 2.81−2.73 (m, 1 H), 2.47−2.42 (m, 1 H), 2.87−2.70 (m, 1 H), 2.07−2.01 (m, 2 H), 1.91−1.83 (m, 3 H), 1.81−1.74 (m, 1 H), 1.63−1.55 (m, 1 H), 0.97 (s, 9 H), 0.19 (s, 3 H), 0.14 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.9, 153.2, 149.7, 139.4, 120.8, 93.9, 86.9, 80.2, 34.3, 33.5, 32.0, 31.9, 26.4 (3C), 19.1, 18.5, −3.9, −4.3; ES HRMS *m/z* (M + Na)$^+$ calcd 390.2325, obsd 390.2325; [α]$^{18}_{D}$ +9.3 (*c* 0.94, $CHCl_3$).

Desilylation of 9. Protected adenosine **9** (34.0 mg, 0.083 mmol) was dissolved in THF (4.2 mL), treated with tetra-*n*-butylammonium fluoride (1 M solution in THF, 0.29 mL), and allowed to stir for 3 h. The volatiles were removed under reduced pressure, and the residue was purified by chromatography on silica gel (elution with 50% ethyl acetate in acetone to 100% acetone) to yield **11** (18.9 mg, 83%) as a white solid: mp 184.0−186.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (s, 1 H), 7.90 (s, 1 H), 6.19 (dd, *J* = 7.3, 5.6 Hz, 1 H), 5.75 (br s, 2 H), 3.95 (t, *J* = 7.2 Hz, 1 H), 2.75−2.69 (m, 1 H), 2.42−2.31 (m, 2 H), 2.13−2.02 (m, 3 H), 2.00−1.55 (m, 4 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.8, 153.4, 149.9, 138.9, 120.7, 93.9, 86.1, 78.1, 35.5, 33.4, 33.1, 32.2, 19.8; ES HRMS *m/z* (M + Na)$^+$ calcd 298.1274, obsd 298.1281; [α]$^{20}_{D}$ +22.8 (*c* 0.18, $CHCl_3$).

Desilylation of 10. Protected adenosine **10** (9.6 mg, 0.02 mmol) was dissolved in THF (1 mL), treated with tetra-*n*-butylammonium fluoride (1 M solution in THF, 0.07 mL), and allowed to stir for 3 h. The volatiles were removed under reduced pressure, and the residue was purified by chromatography on silica gel (elution with 50% ethyl acetate in acetone to pure acetone) to yield **12** (5.3 mg, 78%) as a white solid: mp 200.5−202.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1 H), 7.97 (s, 1 H), 6.35 (dd, *J* = 4.1, 2.0 Hz, 1 H), 5.71 (br s, 2 H), 8.78 (t, *J* = 5.8 Hz, 1 H), 2.67−2.58 (m, 2 H), 2.17−2.12 (m, 2 H), 2.09−1.54 (series of m, 6 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.0, 153.1, 149.6, 139.9, 121.2, 93.2, 86.9, 78.4, 35.7, 33.1 (2C), 32.1, 19.3; ES HRMS *m/z* (M + Na)$^+$ calcd 298.1274, obsd 298.1266; [α]$^{20}_{D}$ +7.0 (*c* 0.27, $CHCl_3$).

Formation of 17 and Coupling with the Sodium Salt of 6-Chloropurine. A cold (−78 °C) solution of **15**[11] (0.50 g, 1.8 mmol) in CH_2Cl_2 (20 mL) was treated with Dibal-H (2.2 mL of 1 M in hexanes, 2.2 mmol), stirred at this temperature for 30 min, and quenched with saturated Rochelle's salt solution (50 mL). The separated aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL), and the combined organic phases were dried and evaporated. The resulting lactol **16** was dissolved in dry THF (20 mL), treated with triphenylphosphine (0.97 g, 3.7 mmol) and carbon tetrachloride (2 mL), and heated at 60 °C for 3 h. The reaction mixture was cooled to rt, at which point the supernatant was transferred via cannula to a prepared solution of 6-chloropurine (0.56 g, 3.7 mmol) and sodium hydride (0.15 g, 3.7 mmol) in dry DMF (20 mL) at 0 °C. After 5 h of stirring, the solvent was removed under reduced pressure and the residue was taken up in CH_2Cl_2 (200 mL). This solution was washed with water (40 mL), dried, and evaporated. Chromatography of the residue on silica gel (elution with 5:1 hexane/ether) furnished 0.23 g of **18** and 0.13 g of **19** (49% total).

18: white solid; mp 108.0−110 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (s, 1 H), 8.41 (s, 1 H), 6.40−6.38 (m, 1 H), 4.18 (t, *J* = 6.0 Hz, 1 H), 2.65−2.63 (m, 1 H), 2.56−2.51 (m, 2 H), 2.03−1.72 (series of m, 6 H), 1.59−1.54 (m, 1 H), 0.92 (s, 9 H), 0.10 (s, 3 H), 0.08 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.2, 151.5, 151.4, 143.9, 132.7, 96.5, 85.8, 78.0, 35.2, 32.9, 32.8, 28.8, 26.2 (3C), 19.6, 18.4, −3.9, −4.3; ES HRMS *m/z* (M + Na)$^+$ calcd 431.1640, obsd 431.1664; [α]$^{18}_{D}$ −32.1 (*c* 1.0, $CHCl_3$).

19: colorless glass; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (s, 1 H), 8.31 (s, 1 H), 6.38−6.36 (m, 1 H), 4.05 (t, *J* = 6.2 Hz, 1 H), 2.71−2.67 (m, 1 H), 2.59−2.51 (m, 2 H), 2.03−1.87 (m, 4 H), 1.78−1.71 (m, 2 H), 1.59−1.54 (m, 1 H), 0.95 (s, 9 H), 0.14 (s, 3 H), 0.13 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.2, 151.4, 151.3, 143.8, 132.8, 96.8, 86.9, 78.4, 35.4, 33.6, 32.2, 29.0, 26.2 (3C), 19.5, 18.4, −4.1, −4.5; ES HRMS *m/z* (M + Na)$^+$ calcd 431.1640, obsd 431.1659; [α]$^{18}_{D}$ −21.5 (*c* 1.0, $CHCl_3$).

Amination of 18/19. The chloropurines **18** and **19** (0.37 g of a 1.7:1 mixture, 0.90 mmol) were dissolved in methanol that had been saturated with NH_3 (40 mL) and stirred in a sealed heavy wall glass tube at 100 °C for 6 h. After removal of the solvent, the residue was subjected to chromatography on silica gel (elution with 3:2 hexane/ether). There were isolated 1.7:1 anomeric mixtures of both the methoxy compounds **20/22** (0.02 g, 5%) and the amino derivatives **21/23** (0.17 g, 48%).

20/22: colorless syrup; ^{1}H NMR (500 MHz, C_6D_6) δ 8.63 (s, 0.6 H), 8.62 (s, 0.4 H), 7.97 (s, 0.6 H), 7.87 (s, 0.4 H), 6.12−6.09 (m, 0.4 H), 5.96−5.94 (m, 0.6 H), 4.08 (t, *J* = 5.2 Hz, 0.6 H), 3.87 (s, 1.2 H), 3.85 (s, 1.8 H), 3.79 (t, *J* = 5.8 Hz, 0.4 H), 2.36−2.17 (m, 4 H), 1.92−1.35 (series of m, 6 H), 0.91 (s, 3.6 H), 0.90 (s, 5.4 H), 0.01 (s, 1.8 H), 0.00 (s, 1.2 H), −0.01 (s, 1.2 H), −0.04 (s, 1.8 H); ^{13}C NMR (125 MHz, C_6D_6) δ 161.8, 161.7, 152.2 (2C), 152.1 (2C), 141.0, 140.5, 123.1 (2C), 95.6 (2C), 85.9, 85.4, 78.1, 78.0, 53.8, 53.7, 35.2, 35.1, 32.9 (2C), 32.1, 31.8, 28.9, 28.7, 26.1 (3C), 26.0 (3C), 19.9, 19.5, 18.2 (2C), −4.3, −4.5, −4.7, −4.8; ES HRMS *m/z* (M + Na)$^+$ calcd 427.2976, obsd 427.2154; [α]$^{18}_{D}$ −22.9 (*c* 1.0, $CHCl_3$).

21/23: white solid; mp 205.2−207.0 °C dec; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.38 (br s, 1 H), 8.10 (s, 0.63 H), 8.02 (s, 0.37 H), 6.34−6.31 (m, 1 H), 6.03 (br s, 2 H), 4.17 (t, *J* = 5.8 Hz, 0.63 H), 4.03 (t, *J* = 6.0 Hz, 0.37 H), 2.66−2.57 (m, 1 H), 2.54−2.30 (m, 2 H), 2.04−1.71 (series of m, 6 H), 1.58−1.52 (m, 1 H), 0.94 (s, 3.3 H), 0.92 (s, 5.7 H), 0.13 (s, 1.1 H), 0.12 (s, 1.1 H), 0.09 (s, 1.9 H), 0.08 (s, 1.9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.7 (2C), 152.9 (2C), 149.8 (2C), 139.2, 139.0, 120.5 (2C), 96.4, 96.0, 86.2, 85.1, 78.2, 78.1, 35.5, 35.3, 33.7, 32.9, 32.8, 32.4, 29.1, 29.0, 26.3 (3C), 26.2 (3C), 19.7, 19.6, 18.4 (2C), −3.9, −4.1, −4.3, −4.5; ES HRMS *m/z* (M + Na)$^+$ calcd 390.2320, obsd 390.2328; [α]$^{18}_{D}$ −31.4 (*c* 1.50, $CHCl_3$).

Desilylation of 21/23. A 0.04 g (0.10 mmol) sample of the **21/23** mixture generated above was dissolved in dry THF (3 mL) and treated with TBAF (0.2 mL of 1 M in THF, 0.2 mmol). The reaction mixture was stirred for 3 h, quenched with water (10 mL), and extracted with ethyl acetate (3 × 10 mL). The combined organic phases were dried and evaporated. The residue was triturated with ether (3 × 5 mL) to give **24/25** as a 3:1 mixture (0.02 g, 67%): white solid; mp 159.5−161.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (s, 0.25 H), 8.37 (s, 0.75 H), 8.01 (s, 0.25 H), 7.88 (s, 0.75 H), 6.58 (br s, 0.75 H), 6.36−6.34 (m, 0.25 H), 6.14 (dd, $J = 6.4$, 8.2 Hz, 0.75 H), 5.73 (br s, 1.5 H), 5.64 (br s, 0.5 H), 4.26 (t, $J = 8.8$ Hz, 0.75 H), 4.11 (t, $J = 5.7$ Hz, 0.25 H), 3.10−3.02 (m, 0.75 H), 2.79−2.74 (m, 0.75 H), 2.70−2.41 (series of m, 1.75 H), 2.20−1.98 (series of m, 3 H), 1.90−1.57 (m, 4 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.3, 155.7, 153.3, 152.8, 148.9, 148.5, 141.1, 138.9, 121.8 (2C), 96.6, 95.1, 87.9, 85.7, 78.4, 78.0, 36.6, 35.3, 33.3, 32.5, 32.1, 31.8, 28.8, 28.7, 19.7, 18.2; EI HRMS *m/z* (M^+) calcd 275.1377, obsd 275.1378; $[\alpha]^{18}_{D}$ −25.1 (*c* 0.3, C_2H_5OH).

Formation of 30 and Coupling with the Sodium Salt of 2-Amino-6-chloropurine. Lactone **28**[11] (0.50 g, 1.7 mmol) was dissolved in CH_2Cl_2 (20 mL), cooled to −78 °C, and treated with Dibal-H (2.1 mL, 1 M in hexanes, 2.1 mmol). After 30 min, the reaction mixture was quenched with saturated Rochelle's salt solution (50 mL), the separated aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL), and the combined organic layers were dried and evaporated. The resulting lactol **31** was dissolved in dry THF (20 mL), treated with triphenylphosphine (0.97 g, 3.7 mmol) and carbon tetrachloride (2 mL), and heated at 60 °C for 3 h. The reaction mixture was cooled to rt, at which point the supernatant was transferred via cannula to a prepared solution of 2-amino-6-chloropurine (0.63 g, 3.7 mmol) and sodium hydride (0.15 g, 3.7 mol) in dry DMF (20 mL) at 0 °C. After 5 h of stirring, the solvent was taken up in CH_2Cl_2 (200 mL) prior to a water wash (40 mL), drying, and solvent evaporation. Chromatography of the residue on silica gel (elution with 3:1 hexane/ether) furnished an inseparable mixture of **31/32** (ratio 2.3:1) (0.37 g, 50%) as a colorless glass: ^{1}H NMR (500 MHz, C_6D_6) δ 7.72 (s, 0.32 H), 7.48 (s, 0.68 H), 6.74−6.73 (m, 0.68 H), 6.70−6.68 (m, 0.32 H), 6.10 (dd, $J = 1.7$, 5.9 Hz, 0.32 H), 6.06 (dd, $J = 1.7$, 5.9 Hz, 0.68 H), 5.20−5.18 (m, 1 H), 4.64 (br s, 0.64 H), 4.58 (br s, 1.36 H), 3.87−3.58 (m, 0.68 H), 3.81−3.79 (m, 0.32 H), 1.87−1.74 (m, 2 H), 1.64−1.35 (m, 4 H), 0.90 (s, 6.12 H), 0.88 (s, 2.88 H), −0.03 (s, 2.04 H), −0.04 (s, 2.04 H), −0.06 (s, 0.96 H), −0.12 (s, 0.96 H); ^{13}C NMR (125 MHz, C_6D_6) δ 159.8 (2C), 154.2, 154.1, 152.2, 152.1, 139.7, 139.6, 138.5, 138.4, 126.5, 126.4, 123.7 (2C), 101.6, 87.6 (2C), 81.3, 81.0, 35.4, 35.2, 34.0, 33.7, 26.0 (6C), 21.4, 21.2, 18.2, 18.1, −4.4, −4.6, −4.8 (2C); ES HRMS *m/z* $(M + Na)^+$ calcd 444.1593, obsd 444, 1588; $[\alpha]^{18}_{D}$ −75.9 (*c* 0.75, $CHCl_3$).

Dihydroxylation of 31/32. Following the dissolution of the **31/32** mixture in a combination of acetonitrile (4 mL), ethyl acetate (4 mL), and water (1.2 mL), the reaction mixture was cooled to 0 °C and treated sequentially with sodium periodate (0.15 g, 0.70 mmol) and 5 min later $RuCl_3 \cdot xH_2O$ (10 mg, 0.047 mmol). After an additional 5 min of stirring, a saturated solution of sodium thiosulfate (25 mL) was introduced and the products were extracted into ethyl acetate (3 × 15 mL). The combined organic layers were dried and evaporated to leave a residue that was chromatographed on silica gel (elution with 1:2 hexane/ether) to give pure samples of **33** (82 mg, 38%) and **34** (48 mg, 22%).

33: white solid; mp 190.5−192 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (s, 1 H), 5.84 (d, $J = 5.8$ Hz, 1 H), 5.19 (br s, 2 H), 4.73 (t, $J = 5.3$ Hz, 1 H), 4.55 (d, $J = 4.7$ Hz, 1 H), 4.09 (t, $J = 5.7$ Hz, 1 H), 2.41−2.35 (m, 1 H), 2.06−2.02 (m, 1 H), 1.89−1.76 (m, 3 H), 1.63−1.58 (m, 1 H), 0.86 (s, 9 H), 0.08 (s, 3 H) (neither OH is observed); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 153.1, 152.5, 140.9, 125.9, 98.2, 90.2, 79.6, 76.6, 72.1, 33.1, 30.8, 26.1 (3C), 19.6, 18.2, −3.9, −4.5; ES HRMS *m/z* $(M + Na)^+$ calcd 478.1653, obsd 478.1594; $[\alpha]^{18}_{D}$ −22.9 (*c* 0.35, $CHCl_3$).

34: white solid; mp 195−197.2 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (s, 1 H), 5.95 (d, $J = 6.2$ Hz, 1 H), 5.16 (br s, 2 H), 4.89 (dd, $J = 4.5$, 6.2 Hz, 1 H), 4.85 (br s, 1 H), 4.47 (d, $J = 4.5$ Hz, 1 H), 4.44 (t, $J = 5.6$ Hz, 1 H), 2.07−2.03 (m, 2 H), 1.99−1.77 (m, 4 H), 0.98 (s, 9 H), 0.23 (s, 3 H), 0.19 (s, 3 H) (1 OH not observed) ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.9, 153.2, 152.5, 140.9, 125.9, 98.3, 90.7, 79.6, 76.6, 72.1, 33.1, 30.8, 26.1 (3C), 19.6, 18.2, −3.9, −4.5; ES HRMS *m/z* $(M + Na)^+$ calcd 478.1653, obsd 478.1593; $[\alpha]^{18}_{D}$ −17 (*c* 0.2, $CHCl_3$).

2-[2-Amino-9-[6-(*tert*-butyldimethylsilanyloxy)-1-oxaspiro[4.4]non-3-en-2-yl]-9*H*-purin-6-ylsulfanyl]ethanol (35). Sodium methoxide (0.10 g, 2.0 mmol) and 2-mercaptoethanol (0.16 mL) were added to a solution of **31/32** (0.20 g, 0.48 mmol) in methanol (4 mL). After the addition of two drops of water, the mixture was heated under reflux for 1.5 h. Another portion of sodium methoxide (0.08 g, 1.5 mmol) was added, and heating was continued for another 1 h. All volatile materials were removed in vacuo. The residue was purified by column chromatography on silica gel (1:3 hexanes/ether) to give **35** (0.20 g, 90%) as a colorless oil: anomeric ratio 2.3:1; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 0.7 H), 7.72 (s, 0.3 H), 6.95−6.94 (m, 0.7 H), 6.93−6.92 (m, 0.3 H), 6.55 (dd, $J = 1.8$, 5.9 Hz, 0.7 H), 6.52 (dd, $J = 1.7$, 5.9 Hz, 0.3 H), 5.99−5.96 (m, 1 H), 5.09 (br s, 2 H), 4.06−4.01 (m, 2.3 H), 3.91−3.89 (m, 0.7 H), 3.53−3.51 (m, 2 H), 2.14−1.59 (series of m, 6 H), 0.94 (s, 6.3 H), 0.93 (s, 2.7 H), 0.09 (br s, 6 H), (OH not observed); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.6, 159.1, 151.2, 151.1, 139.5, 139.3, 138.9, 138.8, 126.4, 126.3, 123.8, 123.7, 116.8, 116.4, 102.3, 102.2, 87.6, 85.5, 81.5, 81.2, 63.7 (2C), 35.6, 35.4, 34.2, 34.0, 32.9, 32.8, 26.2 (3C), 26.1 (3C), 21.6, 21.5, 18.4, 18.3, −4.0, −4.2, −4.3, −4.4; ES HRMS *m/z* $(M + Na)^+$ calcd 486.1966, obsd 486.1971; $[\alpha]^{18}_{D}$ −48.3 (*c* 1.4, $CHCl_3$).

2-Amino-9-[6-(*tert*-butyldimethylsilanyloxy)-1-oxaspiro[4.4]non-3-en-2-yl]-1,9-dihydropurin-6-one (36). To a solution of **35** (0.20 g, 0.48 mmol) in dioxane (4 mL) were added sodium methoxide (0.10 g, 2.0 mmol) and two drops of water. The mixture was heated under reflux for 1.5 h when another portion of sodium methoxide (0.08 g, 1.5 mmol) was added and heating was continued for a second hour. All volatile materials were removed in vacuo. The residue was dissolved in water (2 mL) and acidified with acetic acid. The resulting solid was collected by vacuum filtration and washed with water to give **36** (0.06 g, 35%) as a white solid: mp 175.0−180.2 °C; ^{1}H NMR (500 MHz, C_5D_5N) δ 7.92 (s, 1 H), 7.11−7.09 (m, 1 H), 6.49 (dd, $J = 1.8$, 6.0 Hz, 0.38 H), 6.46 (dd, $J = 1.7$, 5.9 Hz, 0.62 H), 6.03−6.00 (m, 1 H), 4.04−4.02 (m, 1 H), 3.08−2.85 (m, 2 H), 2.11−1.55 (series of m, 4 H), 0.90 (s, 5.6 H), 0.89 (s, 3.4 H), 0.07 (br s, 4.8 H), 0.01 (s, 1.2 H); ^{13}C NMR (125 MHz, C_5D_5N) δ 160.3, 160.2, 156.9, 156.8, 153.6, 153.5, 150.7 (2C), 139.5, 139.3, 136.4, 136.1, 120.1, 120.0, 102.8, 102.7, 89.0, 88.9, 82.6, 82.5, 36.7, 36.6, 35.3, 34.9, 27.3 (3C), 27.2 (3C), 22.7, 22.5, 19.5, 19.4, −3.1, −3.3, −3.4, −3.5; ES HRMS *m/z* $(M + Na)^+$ calcd 426.1932, obsd 426.1914; $[\alpha]^{18}_{D}$ −79.1 (*c* 0.1, $CHCl_3$).

2-Amino-9-[6-(*tert*-butyldimethylsilanyloxy)-3,4-dihydroxy-1-oxaspiro[4.4]non-2-yl]-1,9-dihydropurin-6-one (37). A solution of **36** (7.5 mg, 0.02 mmol) was dissolved in a 4:1 mixture of THF/pyridine (1 mL), and osmium tetraoxide (0.005 g, 0.02 mmol) was added. The resulting brown solution was stirred for 2 h, at which point no starting material was observed by TLC. The reaction mixture was quenched by bubbling H_2S gas through for 10 min, filtered through a plug of Celite, and evaporated. The residue was purified by column chromatography on silica gel (elution with 20% methanol in benzene) to give **37** (0.007 g, 80%) as a white solid: mp 91−112 °C; diastereomer ratio of 3:1:1; ^{1}H NMR (500 MHz, C_5D_5N)* δ 6.72 (d, $J = 6.6$ Hz, 0.33 H), 6.68−6.66 (m, 0.67 H), 5.60−5.55 (m, 0.33 H), 5.28−5.26 (m, 0.33 H), 5.12 (t, $J = 5.9$ Hz, 0.34 H), 4.94 (d, $J = 5.0$ Hz, 0.33 H), 4.85 (d, $J = 5.4$ Hz, 0.33 H), 4.63−4.59 (m, 0.34 H), 4.41 (t, $J = 5.7$ Hz, 0.33 H), 4.17−4.15 (m, 0.33 H), 2.79−2.75 (m, 1 H), 2.11−1.61 (series of multiplets, 5 H), 0.98 (s, 3 H), 0.87 (s, 6 H), 0.34 (s, 1 H), 0.22 (s, 1 H), 0.11 (s, 1 H), 0.09 (s, 2 H), 0.08 (s, 1 H); ^{1}H NMR (500 MHz, CD_3OD) δ 8.04 (s, 0.2 H), 7.85 (s, 0.2 H), 7.82 (s, 0.6 H), 6.12 (d, $J = 6.6$ Hz, 0.2 H), 5.91 (d, $J = 7.4$ Hz, 0.2 H), 5.83 (d, $J = 7.2$ Hz, 0.6 H), 4.71 (dd, $J = 7.2$, 4.9 Hz, 0.6 H), 4.40 (d, $J = 5.0$ Hz, 0.6 H), 4.36 (d, $J = 5.4$ Hz, 0.2 H), 4.23 (d, $J = 4.2$ Hz, 0.2 H), 4.16 (t, $J = 5.8$ Hz, 0.6 H), 4.04 (t, $J = 5.8$ Hz, 0.2 H), 2.45−2.28 (m, 1 H), 2.06−1.99 (m, 1 H), 1.80−1.10 (series of multiplets, 4 H), 0.97 (s, 1.8 Hz), 0.94 (s, 7.2 H), 0.19 (s, 0.6 H), 0.16 (s, 0.6 H), 0.15 (s, 0.6 H), 0.14 (s, 0.6 H), 0.12 (s, 1.8 H), 0.11 (s, 1 H); ^{13}C NMR (125 MHz, CD_3OD) major isomer δ 164.7, 154.6, 152.3, 136.3, 131.4, 117.0, 96.5, 86.9, 79.0, 75.1, 71.3, 32.7, 30.0, 29.7, 25.4, 18.7, 17.8, −5.1, −5.6; ES HRMS m/z (M + Na)$^+$ calcd 460.1987, obsd 460.2000; $[\alpha]^{18}_{D}$ −1.1 (c 0.5, CH_3OH).

(*Note: a 3:1:1 mixture was observed by ^{1}H NMR in CD_3OD. However, the residual peaks from methanol and water obscured most of the proton signals for the substrates. In pyridine-d_5, the major product was less soluble giving a 1:1:1 mixture.)

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Supporting Information Available: Copies of the high-field ^{1}H and ^{13}C NMR spectra of all compounds and details of the X-ray crystallographic analysis of **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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