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Preparation of the 2′-Deoxynucleosides of 2,6-Diaminopurine and Isoguanine by Direct Glycosylation

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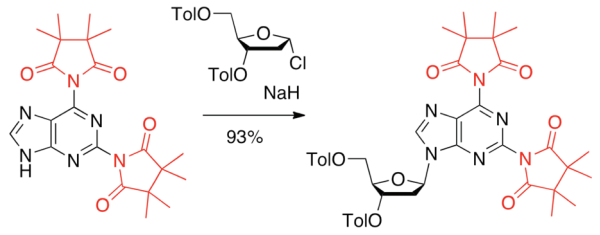
Received December 14, 2009



The purine nucleoside 2,6-diaminopurine-2′-deoxyriboside is prepared by the direct glycosylation of the 2,6-bis(tetramethylsuccinimide) derivative of the parent purine heterocycle **4** with 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride **5** using the sodium salt method. 2′-Deoxyisoguanosine is prepared from 2,6-diaminopurine by a five-step procedure. The purine heterocycle isoguanine is prepared by selective diazotization of 2,6-diaminopurine and then converted to the N9-trityl derivative to increase solubility. After silylation of the O^2-carbonyl with TMSCl, the N^6-amino group is protected as the tetramethylsuccinimide (M_4SI). The O^2-carbonyl is protected as the DPC derivative, and the trityl group is removed. The resulting product is glycosylated in good yield to generate fully protected 2′-deoxyisoguanosine.

Introduction

Among their many roles, nucleoside analogues can be used to modulate stability for DNA, RNA, or DNA/RNA duplexes. 2,6-Diaminopurine-2′-deoxyriboside or the corresponding ribonucleoside is often used in this role because of the presence of a minor groove amino group that can provide a third hydrogen bond for dA−dT base pairs, or corresponding RNA A−U base pairs, and enhance overall helix stability.[1] 2′-Deoxyisoguanosine has also been of interest for its hydrogen-bonding capability but perhaps more so for the observation that it pairs with isoC and provides a fifth base pair to expand the genetic code in artificial genetic systems.[2,3]

An early description of the preparation of 2,6-diaminopurine riboside was a multistep pathway beginning with the N1-oxide of adenosine.[4] A much simpler synthesis of the 2′-deoxynucleoside and corresponding phosphoramidite was described by Gaffney and Jones;[5] it involved sulfonation of the O^6 carbonyl of dG followed by treatment with trimethylamine and then ammonia to generate the 2,6-diaminopurine-2′-deoxynucleoside. That procedure has been improved by preparation of the O^6-pentafluorophenyl derivative, which can be converted in high yield to 2,6-diaminopurine-2′-deoxyriboside by treatment with ammonia. They also report that this synthon can be incorporated into DNA and then converted to 2,6-diaminopurine.[6] A recent report also describes the preparation of the ribonucleoside directly from guanosine in high yield under high pressure conditions using HMDS and triflic acid.[7] One drawback to all of these procedures is that they use a nucleoside starting material, making them less cost-effective and less flexible if, for example, a nucleoside bearing a modified sugar were desired.

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Published on Web 02/10/2010 DOI: 10.1021/jo902616s

The alternative approach involves glycosylation of 2,6-diaminopurine or related derivative using for example, 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride[8] (**5**) to generate a precursor to the diaminonucleoside. To achieve highly diastereomeric yields of 2′-deoxynucleosides the sodium salt method[9] is often used, requiring that the purine be activated by a base (commonly NaH). This procedure is untenable when free amines or even amides are present, but this problem can be eliminated by using 2,6-dichloropurine (available from xanthine) for glycosylation.[8,10,11] Treatment of chloropurines with NaH generates the isomeric N9/N7 salt, which upon reaction with the chlorinated carbohydrate typically results in a mixture of N9 and N7 purine nucleosides. The major product is the N9-β-isomer; the minor product is the N9-α-isomer.[8] The sodium salt method requires polar solvents, which can accelerate the anomerization of the chloro-sugar and generate some of the diastereomeric α-nucleoside. Reaction of the product 2,6-dichloropurine nucleoside with ammonia displaces the chlorine at the C6 position, but not the one at C2.[11] The latter site requires a harsher hydrazine treatment followed by Raney nickel reduction. Alternatively, NaN_3 can be used to displace the chlorines at both positions; the product diazide can then be reduced to the desired 2,6-diaminopurine nucleoside.[10] One drawback with this overall approach is the larger number of steps needed, first to eliminate the amines and then to regenerate them in order to obtain the desired 2,6-diaminopurine-2′-deoxyriboside.

Syntheses of 2′-deoxyisoguanosine[12] and isoguanosine suffer from similar issues. Reported syntheses generally employ a nucleoside starting material. For example, after preparing the N1-oxide of 2′-deoxyadenosine a photolytic rearrangement generates the desired 2′-deoxyisoguanosine[13,14] in a reported 42% yield.[15] A second reported synthesis requires four steps from guanosine to generate 2-iodoadenosine, which could then be photolyzed to isoguanosine in a reported 55% yield.[16] The other common approach to obtain the isoguanine nucleosides is to selectively deaminate 2,6-diaminopurine riboside or the 2′-deoxy derivative with nitrous acid.[14,17,18] These deaminations still require a costly nucleoside starting material and are limited to ribose or 2′-deoxyribose sugars.

Reported procedures for the glycosylation of isoguanine are limited. To the best of our knowledge, there has been no report of direct glycosylation of isoguanine to prepare 2′-deoxyisoguanosine. Bookser and Raffaele describe[19] a Vorbrüggen glycosylation using microwave acceleration to generate isoguanosine in 38% yield and in 79% purity; the noted purity results from product isolation by precipitation. The absence of other reports for isoguanine glycosylations likely stems from the extremely poor solubility of isoguanine, estimated to be 2.2 mg/L of water,[20] and virtual insolubility in organic solvents.

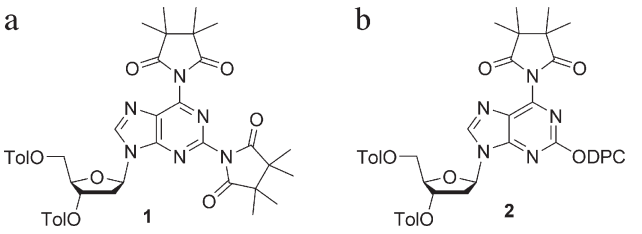
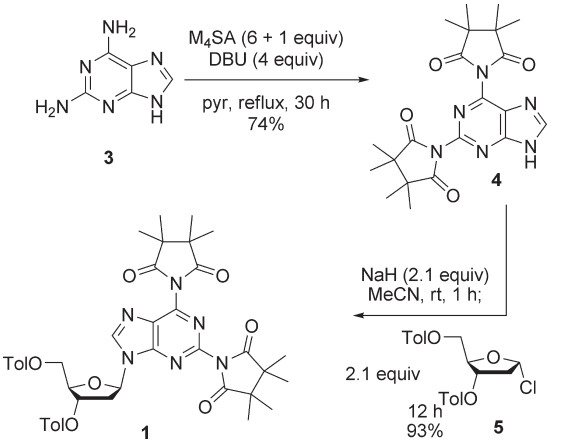


FIGURE 1. Protected derivatives of (a) 2,6-diaminopurine-2′-deoxyriboside and (b) 2′-deoxyisoguanosine resulting from glycosylation of the corresponding purines.

SCHEME 1



Here we describe simple and straightforward procedures involving glycosylation of protected aminopurine derivatives to generate the fully protected nucleosides of 2,6-diaminopurine (**1**) and isoguanine (**2**) (Figure 1). The use of the tetramethylsuccinimide (M_4SI) protecting group[21] affords purine derivatives that display excellent solubility and are excellent coupling partners in the sodium salt procedure. These derivatives can be further elaborated to DMT-protected nucleoside phosphoramidites for DNA synthesis, or can be fully deprotected to generate the parent nucleosides 2,6-diaminopurine-2′-deoxyriboside and 2′-deoxyisoguanosine.

Results and Discussion

Glycosylation reactions to form purine 2′-deoxynucleosides commonly use the sodium salt method[9] whereby the purine is treated with base (e.g., NaH) and the purine salt thus formed performs an S_N2 displacement of chloride from 2-deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-*erythro*-pentofuranosyl chloride (**5**, Scheme 1); the reaction usually generates a mixture of N9 and N7 nucleosides in the β-configuration,

(8) Wright, G. E.; Hildebrand, C.; Freese, S.; Dudycz, L. W.; Kazimierczuk, Z. *J. Org. Chem.* **1987**, *52*, 4617–4618.
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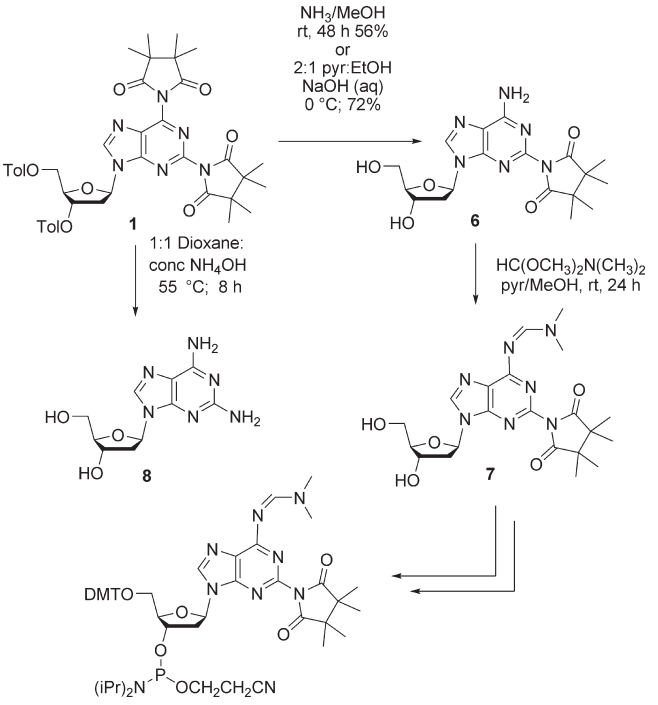
(21) Arico, J.; Calhoun, A. K.; Salandria, K.; McLaughlin, L. W. *Org. Lett.* **2010**, *12*, 120–122.

SCHEME 2



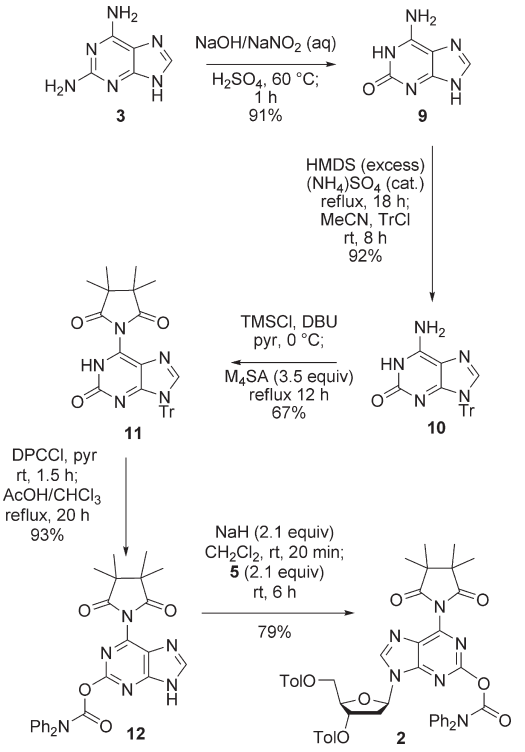
with α-anomers sometimes formed due to anomerization of the sugar. Purines containing free amines or amides generally have poor solubility and give low product yield with the sodium salt method.

We wished to avoid the use of the costly 2,6-dichloropurine glycosylation partner and examine direct glycosylation of the readily available 2,6-diaminopurine. To accomplish this task, we needed to protect the exocyclic amines in such a manner that sodium hydride treatment generated only the N7/N9 purine salt. We chose to use tetramethylsuccinic anhydride (M_4SA)[21] to form the bis(tetramethylsuccinimide) of 2,6-diaminopurine. After refluxing in the presence of DBU for 22 h we obtained the fully protected purine heterocycle **4** (Scheme 1) as a mixture with approximately 10% of the corresponding amide. After partial purification, this mixture was fully converted to **4** in an overall yield of 74% by refluxing in anhydrous pyridine an additional 8 h.

The glycosylation of **4** occurred under standard conditions using sodium hydride to activate the fully protected heterocycle and 2.1 equivalents of the glycosylating sugar **5**. Under these conditions and at ambient temperature we obtained the β-N9 product **1** in 93% yield (Scheme 1). None of the N7 glycosylation product and neither of the possible α-anomers were observed.

The toluoyl groups could be removed from **1** using ammonia/methanol (Scheme 2), but during this reaction partial deprotection of the N^6-M_4SI was observed (→**6**). However, it was possible to convert **1** to **6** employing aq NaOH in 2:1 pyridine/ethanol, and after purification protect the exocyclic amine as the dimethylformamidine derivative (**7**). The material obtained in this manner can be converted to a DMT-protected nucleoside phosphoramidite using standard protocols. The coupling product **1** could also be fully deprotected to yield the 2′-deoxynucleoside **8**.

SCHEME 3

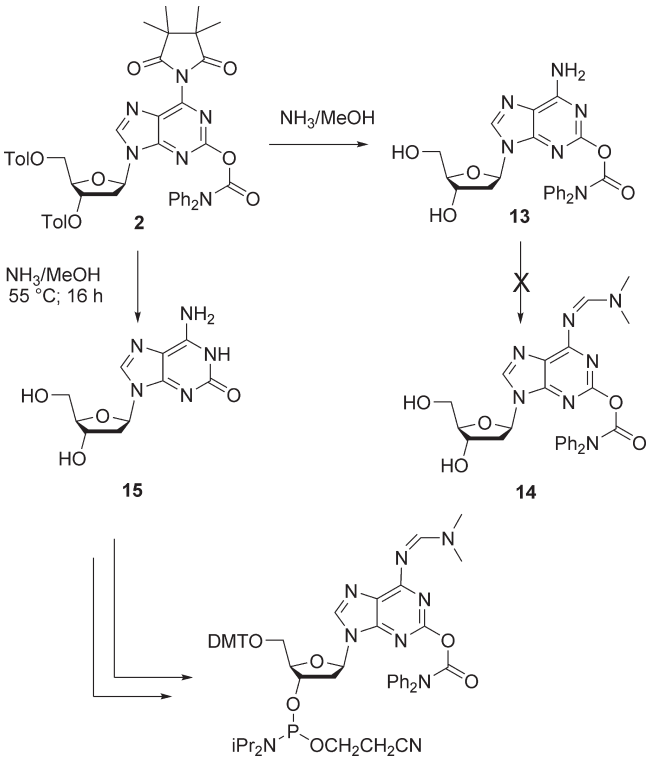


Glycosylation of isoguanine has proven difficult with a single report noted above using high temperatures facilitated by microwave treatment.[19] 2,6-Diaminopurine (Scheme 3) is a readily available starting material for 2′-deoxyisoguanosine, and similar to reports[14,17,18] for the corresponding nucleoside, we could selectively deaminate it by dripping a solution of 2,6-diaminopurine in aqueous $NaOH/NaNO_2$ into an acidic solution and then heating at 60 °C for 1 h. Precipitation gave isoguanine (**9**) in excellent yield. Owing to its poor solubility, isoguanine was treated with excess HMDS under reflux and then treated with trityl chloride to generate **10**. The N9-trityl derivative of isoguanine exhibited much improved solubility but the exocyclic amine could not be protected with M_4SA; the low reactivity of the amino group of isoguanosine has also been noted by Seela.[22] Transient protection of isoguanosine by treatment with TMSCl has been reported to enhance the reactivity of N^6,[22] presumably as the result of silylation of the O^2-carbonyl. We adopted a similar course of action and treated **10** with TMSCl and DBU followed by M_4SA and obtained **11** in 67% yield. Removal of the trityl group from **11** with acetic acid followed by glycosylation of the resulting M_4SI-protected nucleobase generated at least four different products, suggesting the presence of reactive nucleophiles in addition to N9. This observation is also consistent with reactions described by Seela[22] who noted that alkylation could take place at N1, N3, and N9, suggesting extensive delocalization of the isoguanyl anion.

As an alternative, the DPC group (one of the preferred O^2-protecting groups for monomers of isoguanine used in DNA synthesis protocols[13]) was introduced at O^2 (→**12**) and

(22) Seela, F.; Wei, C.; Kazimierczuk, Z. *Helv. Chim. Acta* **1995**, *78*, 1843–1854.

SCHEME 4



the trityl group removed. We then glycosylated the fully protected heterocycle (**12**) using **5**. Glycosylation of **12** proceeded smoothly to generate a quantitative yield of coupling products with 70−79% of the desired β-N9 product, 5−15% α-N9, and 15% α/β-N7 in an approximately 1:1 ratio. The wide variation in yield of the α-N9 product prompted us to optimize the reaction conditions to both minimize this product and render the reaction more reproducible.

The same amount of α-N9 (12−15%) was produced with either acetonitrile or dichloromethane as the solvent after allowing 1 h for formation of the purine sodium salt with NaH and followed with an overnight (16 h) reaction time after addition of **5**. Stopping the reaction at 1, 5, or 16 h after addition of the sugar indicated that the highest yield of coupling products (quantitative) was obtained after 5 h; the same amount of α-N9 (12−15%) was obtained in each case. To our surprise, the least amount of α-N9 (4−5%) was produced when the reaction time of **12** with NaH was minimized (20 min) and all other conditions were kept identical, while allowing 1 h or more for reaction with NaH gave the most α-N9 product. Either acetonitrile or dichloromethane could be used as the reaction solvent with identical yields and identical ratios of products obtained. Therefore, the reaction time after addition of the sugar was not important except in terms of the overall yield. This result is surprising given that the sugar **5** is reported to anomerize at a faster rate in polar solvents[23] and thus an increasing yield of α-nucleosides should result with increasing reaction times. This rationale does not adequately explain our observed trend in coupling product ratios; it may be that another factor such as aggregation of the sodium salt of **12** during long reaction times with NaH leads to the observed effect. No similar effects were observed either with coupling of **4** or with other M_4SI-protected purine substrates.[21]

The final step of the synthesis required the selective cleavage of the toluoyl esters (Scheme 4), but this proved infeasible under a number of conditions (NH_3/MeOH, NaOH at 4 °C or K_2CO_3 at ambient temperature) as the M_4SI group was the most labile protecting group. The DPC group was stable enough that **13** could be isolated in ∼50% yield. However, Seela has reported[24] that **13** cannot be converted to the amidine derivative (**14**). Therefore, the simplest course of action was to fully deprotect **2** using ammonia in methanol at 60° overnight to obtain **15.** The conversion of 2′-deoxyisoguanosine (**15**) to the fully protected DMT-nucleoside phosphoramidite (Scheme 4) has been described.[15,24]

Conclusions

We describe simple and straightforward procedures for the glycosylation of protected derivatives of 2,6-diaminopurine and isoguanine. Both procedures rely upon the use of the bidentate protecting group M_4SI. The bidentate nature of the M_4SI group eliminates any side reactions that result from proton abstraction at the exocyclic amines (or corresponding amides); it also provides enhanced solubility for purine heterocycles particularly for isoguanine. The M_4SI protecting group exhibits exceptional directing ability such that high ratios of the N9 vs N7 glycosylation products result. These valuable nucleosides can be further elaborated to generate nucleoside phosphoramidites for solid-phase DNA syntheses using standard protocols.

Experimental Section

General Procedures. All reactions were carried out in oven-dried glassware under an inert atmosphere with dry solvents and anhydrous conditions, unless otherwise noted. Dry tetrahydrofuran (THF), diethyl ether (Et_2O), *N*,*N*-dimethylformamide (DMF), pyridine (pyr), acetonitrile (MeCN), and dichloromethane (DCM) were obtained by passing commercially available predried, oxygen-free formulations through activated alumina columns. Dry methanol (MeOH) was obtained by distillation from $Mg(OMe)_2$. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Silicycle TLG-R10011B-323 60 Å plates using UV light as visualizing agent and ceric ammonium molybdate (CAM) stain and heat as developing agents. Dynamic Adsorbents silica gel (particle size 32−63 μm) was used for flash column chromatography. Difficult separations were carried out using "chromatospec silica gel" (E. Merck Chromatospec silica gel (60 Å pore size, particle size 15−40 μm). NMR spectra were recorded on 400, 500, or 600 instruments and calibrated using residual undeuterated solvent ($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.16 ppm, acetone-d_6: δ_H = 2.05 ppm, δ_C = 29.84, DMSO-d_6: δ_H = 2.50 ppm, δ_C = 39.52 ppm, methanol-d_4: δ_H = 3.34 ppm, δ_C = 49.00 ppm) as an internal reference. The following abbreviations are used to designate

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(24) Seela, F.; Wei, C. *Helv. Chim. Acta* **1997**, *80*, 73–85.

the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were recorded using ESI (electrospray ionization) or DART (direct analysis in real time).

***N*,*N*′-(9*H*-Purine-2,6-diyl)bis(2,2,3,3-tetramethylsuccinimide) (4).** To a flask containing 2,6-diaminopurine **3** (257 mg, 1.71 mmol) and 2,2,3,3-tetramethylsuccinic anhydride (1.604 g, 10.27 mmol) were added 13 mL of pyridine and 1.02 mL of DBU (6.84 mmol). The mixture was heated to reflux with stirring for 20 h. The starting material gradually dissolved as the reaction proceeded. TLC (9:1 DCM/MeOH containing 0.7 M NH_3) indicated that some starting material remained. Another 267 mg (1.71 mmol) of anhydride was added, and heating continued for another 10 h. TLC showed that no starting material remained. Volatiles were removed in vacuo, and the residue coevaporated with toluene (3 × 3 mL). The resulting brown oil was purified by flash chromatography eluting with 95:5 DCM/MeOH) to obtain 738 mg of white solid. This material contained the amides (~10% by ^{1}H NMR) that coeluted with the desired product. The solid was thoroughly dried in vacuo and treated with $SOCl_2$ (3 mL) at reflux for 2 h with a drying tube attached or dissolved in anhydrous pyridine (10 mL) and heated to reflux for 8 h. Volatiles were removed in vacuo, and the residue was coevaporated with EtOAc or toluene, respectively (3 × 3 mL). The residue was purified by flash chromatography eluting with 95:5 DCM/MeOH to obtain 546 mg (74%) of **4** as a white amorphous powder. **4**: R_f = 0.7 (silica gel, 9:1 DCM/MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 14.25 (brs, 1H), 8.84 (brs, 1H), 1.29 (s, 12H), 1.24 (s, 12H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 180.5, 180.1, 146.4, 47.7, 47.2, 21.1, 20.9; HRMS (ESI-TOF) calcd for $C_{21}H_{26}N_6O_4Na^+$ [M + Na]$^+$ 449.1940, found 449.1920.

9-[2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentofuranosyl]-2,6-di-(3,3,4,4-tetramethyl-2,5-dioxopyrrolidin-1-yl)purine (1). To a flask containing **4** (200 mg, 0.467 mmol) and NaH (45 mg, 0.984 mmol, 50% dispersion in oil) was added 20 mL of MeCN. The mixture was allowed to stir for 1 h, and then 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranosyl chloride **5** (382 mg, 0.984 mmol) was added. Stirring was continued overnight. The reaction mixture was filtered through Celite and the filter cake washed with acetone (25 mL). The filtrate was evaporated in vacuo, and the resulting yellow foam was purified by flash column chromatography eluting with 98.25:1.75 DCM/MeOH to obtain 338 mg (93%) of **1** as a white foam. **1**: R_f = 0.55 (silica gel, 97:3 DCM/MeOH); ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.79 (s, 1H), 7.97 (d, J = 8.2 Hz, 2H), 7.87 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 7.26 (d, J = 8.1 Hz, 2H), 6.80 (s, 1H), 5.99 (s, 1H), 4.79 (dd, J = 10.5 Hz, J = 5.0 Hz, 1H), 4.74 − 4.59 (m, 2H), 3.55 − 3.42 (m, 1H), 3.01 (ddd, J = 14.5, 6.6, 3.4 Hz, 1H), 2.42 (s, 3H), 2.38 (s, 3H), 1.35 (s, 12H), 1.31 (s, 12H); ^{13}C NMR (acetone-d_6, 101 MHz) δ 180.9, 180.6, 166.5, 166.2, 155.2, 148.7, 148.3, 147.2, 145.1, 144.7, 131.7, 130.5, 130.4, 130.1, 130.0, 128.1, 127.9, 86.6, 83.7, 76.0, 65.0, 49.0, 48.4, 37.1, 21.6, 21.5, 21.5, 21.5, 21.4; HRMS (ESI-TOF) calcd for $C_{42}H_{46}N_6O_9Na^+$ [M + Na]$^+$ 801.3224, found 801.3207.

6-Amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2-(3,3,4,4-tetramethyl-2,5-dioxopyrrolidin-1-yl)purine (6). *Note: anhydrous conditions not needed.* To a flask containing **1** (123 mg, 0.158 mmol) dissolved in 2:1 pyr/EtOH (1.4 mL) at 0 °C was added 1 M NaOH (0.7 mL) dropwise. The mixture was allowed to stir at 0 °C for 2 h. The reaction was quenched by adding NH_4Cl (45 mg, 0.19 mmol). Volatiles were removed in vacuo, and the residue was redissolved in minimal MeOH. Silica gel (700 mg) was added, and volatiles were removed in vacuo. The thoroughly dried powder was loaded onto a flash column and purified by elution with 9:1 DCM/MeOH then 6:1→5:1 DCM/EtOH to obtain 45 mg (72%) of **6** as a white foam. **6**: R_f = 0.13 (silica gel, 9:1 DCM/MeOH); ^{1}H NMR (acetone-d_6, 500 MHz) δ 8.27 (s, 1H), 7.01 (brs, 2H), 6.41 (dd, J = 8.8, 5.7 Hz, 1H), 5.00 (brs, 1H), 4.59 (m, 1H), 4.42 (brs, 1H), 4.05 (dd, J = 4.2, 2.6 Hz, 1H), 3.74 (dd, J = 12.3, 2.4 Hz, 1H), 3.68−3.56 (m, 1H), 2.83 (ddd, J = 13.2, 8.9, 5.4 Hz, 2H), 2.35 (s, 1H), 1.26 (s, 12H); ^{13}C NMR (acetone-d_6, 126 MHz) δ 181.1, 158.4, 150.8, 148.6, 142.4, 90.2, 87.4, 73.2, 63.7, 48.1, 41.3, 21.6, 21.4; HRMS (ESI-TOF) calcd for $C_{18}H_{24}N_6O_5Na^+$ [M + Na]$^+$ 427.1706, found 427.1709.

9-[2-Deoxy-β-D-*erythro*-pentofuranosyl]-6-[[1-(dimethylamino)-ethylidene]amino]-2-(3,3,4,4-tetramethyl-2,5-dioxopyrrolidin-1-yl)-purine (7). To a flask containing **6** (36 mg, 0.089 mmol) was added MeOH (0.5 mL) followed by *N*,*N*-dimethylformamide dimethyl acetal (18 μL, 0.133 mmol). The mixture was allowed to stir at rt for 24 h, at which time TLC indicated that all starting material had been consumed. Volatiles were removed in vacuo, and the residue was purified by flash chromatography eluting with 9:1 DCM/MeOH to obtain 35 mg (85%) of **7** as a white foam. **7**: R_f = 0.29 (silica gel, 9:1 DCM/MeOH); ^{1}H NMR (acetone-d_6, 500 MHz) δ 8.98 (s, 1H), 8.39 (s, 1H), 6.46 (dd, J = 8.8, 5.7 Hz, 1H), 4.92 (brs, 1H), 4.65 − 4.56 (m, 1H), 4.46 (brs, 1H), 4.07 (dd, J = 4.5, 2.7 Hz, 1H), 3.76 (dd, J = 12.3, 2.6 Hz, 1H), 3.65 (d, J = 11.9 Hz, 1H), 3.26 (s, 3H), 3.21 (d, J = 0.6 Hz, 3H), 2.86 (ddd, J = 13.2, 8.8, 5.4 Hz, 1H), 2.37 (ddd, J = 13.1, 5.8, 1.9 Hz, 1H), 1.28 (d, J = 1.1 Hz, 12H); ^{13}C NMR (acetone-d_6, 126 MHz) δ 181.3, 162.2, 159.9, 152.8, 148.1, 143.6, 127.3, 90.1, 87.2, 73.2, 63.7, 48.1, 41.3, 41.3, 35.1, 21.6, 21.4; HRMS (ESI-TOF) calcd for $C_{21}H_{29}N_7O_5Na^+$ [M + Na]$^+$ 482.2128, found, 482.2122.

Isoguanine (6-Amino-1*H*-purin-2(9*H*)-one) (9). 2,6-Diaminopurine (2.0 g, 13.3 mmol) was dissolved in 1.67 M NaOH (20 mL), and $NaNO_2$ (1.19 g, 17.3 mmol) was added. The resulting solution was transferred to an addition funnel and added dropwise over 1 h, with stirring, to a flask containing 78% H_2SO_4 (8 mL) immersed in a water bath at 20 °C. The temperature was carefully regulated at 20 °C during this time. After the solution of 2,6-diaminopurine had been added, the temperature was raised to 60 °C and allowed to stir for 1 h. The mixture was allowed to cool to room temperature and filtered to remove the precipitate that had formed. The precipitate was washed with 10 mL H_2O, air-dried, then redissolved in 0.9 M NaOH (40 mL). To this solution was added $NaHSO_3$ (122 mg). The solution was heated to 90 °C with stirring and slowly neutralized to pH 7−8 by adding 50% H_2SO_4 dropwise. A precipitate formed, and after the neutralized mixture was allowed to cool to room temperature it was filtered. The precipitate was washed with 50 mL of H_2O and dried overnight in a vacuum oven at 50 °C to yield **9** as an off-white amorphous solid (1.837 g, 91%). The product was virtually insoluble in organic solvents and H_2O but readily soluble in alkali. The physical data were in agreement with that reported.[25] **9**: mp > 360 °C; UV λ_{max} = 283 (0.1 M NaOH) (8.0); IR (KBr pellet) 3051, 2785, 1828, 1698, 1666, 1524, 1450, 1398, 1236, 1179, 1119, 1028, 940, 852, 775 cm^{-1}.

6-Amino-9-trityl-1*H*-purin-2(9*H*)-one (10). To a flask containing isoguanine **9** (530 mg, 3.50 mmol) and $(NH_4)_2SO_4$ (35 mg, 0.26 mmol) was added HMDS (53 mL). The mixture was heated to reflux with stirring for 18 h. The excess HMDS was removed in vacuo and the flask purged with nitrogen. Trityl chloride (988 mg, 3.54 mmol) and MeCN (53 mL) were added, and the mixture was allowed to stir for 8 h at rt under inert atmosphere. Volatiles were removed in vacuo, and the residue was purified by flash chromatography eluting with 9:1 DCM/MeOH to obtain 1.268 g (92%) of **10** as a white amorphous powder. **10**: R_f = 0.38 (silica gel, 9:1 DCM/MeOH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.21 (brs, 1H), 7.39 − 7.23 (m, 12H), 7.18−7.11 (m, 6H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 141.4,

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129.4, 127.8, 127.3, 74.2; HRMS (ESI-TOF) calcd for $C_{24}H_{19}N_5OH^+$ $[M + H]^+$ 394.1668, found 394.1662.

***N*-(2-Oxo-9-trityl-2,9-dihydro-1*H*-purin-6-yl)-2,2,3,3-tetramethylsuccinimide (11).** To a flask containing **10** (593 mg, 1.51 mmol) and DBU (0.9 mL, 6.03 mmol) in 16 mL of anhydrous pyridine at 0 °C was added TMSCl (476 μL, 3.77 mmol) dropwise by syringe. The mixture was allowed to stir for 30 min. The ice bath was removed and the mixture allowed to stir for another 30 min. 2,2,3,3-Tetramethylsuccinic anhydride (823 mg, 5.27 mmol) was added and the mixture heated to reflux for 12 h. Volatiles were removed in vacuo, and the residue was coevaporated with toluene (3 × 5 mL). The residue was redissolved in DCM (15 mL) and washed with satd $NaHCO_3$ (2 × 5 mL) and brine (1 × 5 mL). The organic layer was dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography eluting with 98.25:1.75 DCM/MeOH to obtain 700 mg of a white foam. The product contained 23% of the unreacted anhydride M_4SA by weight as determined by ^1H NMR; the yield of **11** was thus 539 mg (67%). Some of the anhydride could be removed by repeated chromatography, but **11** could be used in subsequent reactions without further purification. An analytical sample of **11** was obtained by recrystallization from acetone containing a small amount of MeOH. **11**: $R_f = 0.44$ (silica gel, 97:3 DCM/MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 10.27 (brs, 1H), 7.93 (s, 1H), 7.36−7.25 (m, 9H), 7.24−7.08 (m, 6H), 1.28 (s, 12H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 180.1, 159.9, 157.1, 145.8, 145.26, 140.6, 129.9, 128.2, 128.1, 126.0, 76.4, 48.3, 21.4; HRMS (ESI-TOF) calcd for $C_{32}H_{29}N_5O_3Na^+$ $[M + Na]^+$ 554.2168, found 554.2147.

6-(3,3,4,4-Tetramethyl-2,5-dioxopyrrolidin-1-yl)-9*H*-purin-2-yl Diphenylcarbamate (12). To a flask containing **11** (781 mg, 77% **11** by weight, 1.13 mmol) and diphenylcarbamoyl chloride (289 mg, 1.25 mmol) were added pyridine (8 mL) and DIPEA (0.4 mL, 2.26 mmol). The mixture was allowed to stir at room temperature. Full conversion to a UV-active spot with $R_f = 0.32$ in 99:1 DCM/MeOH was observed by TLC after 1.5 h. Water (5 mL) was added and stirring continued for 10 min. Volatiles were removed in vacuo, and the residue was coevaporated with toluene (3 × 3 mL). $CHCl_3$ (not anhydrous, 20 mL) and AcOH (4 mL) were added, and the mixture was heated to reflux with stirring until TLC indicated consumption of starting material (20 h). Volatiles were removed in vacuo. The residue was redissolved in DCM (25 mL) and washed with satd $NaHCO_3$ (3 × 8 mL) and brine (10 mL). The organic layer was dried with Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography eluting with 97:3/95:5 DCM/MeOH to obtain 508 mg (93%) of **12** as a white foam. **12**: $R_f = 0.42$ (silica gel, 95:5 DCM/MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 12.13 (s, 1H), 7.97 (s, 1H), 7.34 (m, 8H), 7.30 − 7.15 (m, 2H), 1.32 (s, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.1, 155.8, 155.4, 152.0, 146.0, 145.8, 141.5 (br), 129.4, 128.0, 127.5 (br), 126.3 (br), 48.4, 21.4; HRMS (ESI-TOF) calcd for $C_{26}H_{24}N_6O_4Na^+$ $[M + Na]^+$ 507.1757, found 507.1745.

9-[2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-β-D-*erythro*-pentofuranosyl]-6-(3,3,4,4-tetramethyl-2,5-dioxopyrrolidin-1-yl)-9*H*-purin-2-yl Diphenylcarbamate (2). To a flask containing **12** (201 mg, 0.414 mmol) and NaH (41 mg, 0.871 mmol, 50% dispersion in oil) was added 20 mL of DCM. The mixture was allowed to stir for 20 min, and then **5** (338 mg, 0.871 mmol) was added. Stirring was continued for 5 h, after which time the reaction mixture was filtered through Celite and the filter cake washed with acetone (25 mL). The filtrate was evaporated in vacuo, and the resulting yellow foam was purified by flash chromatography (20 × 5 cm column, chromatospec silica gel) eluting with 98.25:1.75 DCM/MeOH to obtain 273 mg (79%) of **2** as a white foam. Also obtained were 17 mg (5%) of α-N9 product and 55 mg (16%) of α/β-N7 products. **2**: $R_f = 0.38$ (silica gel, 97:3 DCM/MeOH); ^1H NMR (acetone-d_6, 400 MHz) δ 8.66 (s, 1H), 8.00 (d, $J = 8.3$ Hz, 2H), 7.87 (d, $J = 8.3$ Hz, 2H), 7.53 (d, $J = 7.5$ Hz, 4H), 7.43−7.32 (m, 6H), 7.32−7.19 (m, 4H), 6.75 (t, $J = 6.8$ Hz, 1H), 6.06 (dt, $J = 6.4$, 3.2 Hz, 1H), 4.84 (dd, $J = 10.5$, 4.5 Hz, 1H), 4.76−4.61 (m, 2H), 3.47 (dt, $J = 14.0$, 6.8 Hz, 1H), 3.01 (ddd, $J = 14.4$, 6.5, 3.4 Hz, 1H), 2.43 (s, 3H), 2.37 (s, 3H), 1.34 (s, 12H); ^{13}C NMR (acetone-d_6, 101 MHz) δ 180.5, 166.58, 166.4, 156.6, 155.6, 151.9, 147.6, 147.1, 145.1, 144.7, 143.1, 130.6, 130.4, 130.1, 130.0, 128.0, 128.0, 127.8 (br), 86.4, 83.6, 76.0, 65.0, 49.0, 37.1, 21.6, 21.6, 21.5, 21.5; HRMS (ESI-TOF) calcd for $C_{47}H_{44}N_6O_9Na^+$ $[M + Na]^+$ 859.3067, found 859.3039.

9-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-6-amino-9*H*-purin-2-yl Diphenylcarbamate (13). Obtained in approximately 50% yield by deprotection of **2** in NH_3/MeOH at rt for 15 h. Purification of the residue by flash chromatography eluting with 92.5:7.5 DCM/MeOH gave **13** as a white foam. Physical data were in agreement with those reported.[23]

2′-Deoxyisoguanosine (15)[14,26]**.** To a flask containing **2** (212 mg, 0.253 mmol) was added 7 M NH_3/MeOH (8 mL). The mixture was allowed to stir at 60 °C for 15 h, and volatiles were removed in vacuo. The residue was washed with DCM (2 × 5 mL) and EtOAc (2 × 5 mL) and dried in vacuo to yield 58 mg of impure product. This off-white solid was recrystallized from MeOH to yield 49 mg (73%) of **15** as an off-white powder.

Acknowledgment. We thank Dr. Nick Greco for critical discussions. This work was supported by an award from the NSF to L.W.M. (MCB 0451488).

Supporting Information Available: ^1H NMR and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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