

# Synthesis of $N^7$ -Alkyl-9-deaza-2'-deoxyguanosines Containing Polar $N^7$ Chains. Examples of Chemically Stable Analogues of $N^7$ -Hydroxyethyl and $N^7$ -Oxoethyl Adducts of 2'-Deoxyguanosine

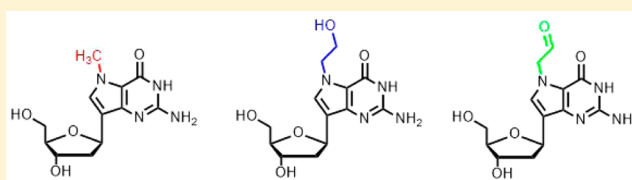
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## Supporting Information

**ABSTRACT:** Development of chemically stable analogues of unstable DNA lesions enables accurate study of polymerase bypass. We report the design and synthesis of  $N^7$ -hydroxyethyl-9-deaza-2'-deoxyguanosine and  $N^7$ -oxoethyl-9-deaza-2'-deoxyguanosine as the analogues of  $N^7$ -hydroxyethyl-2'-deoxyguanosine and  $N^7$ -oxoethyl-2'-deoxyguanosine, respectively. We also developed the synthesis of these two nucleosides whose  $N^7$  side chains are protected by TBS for the convenience of conversion to phosphoramidites.

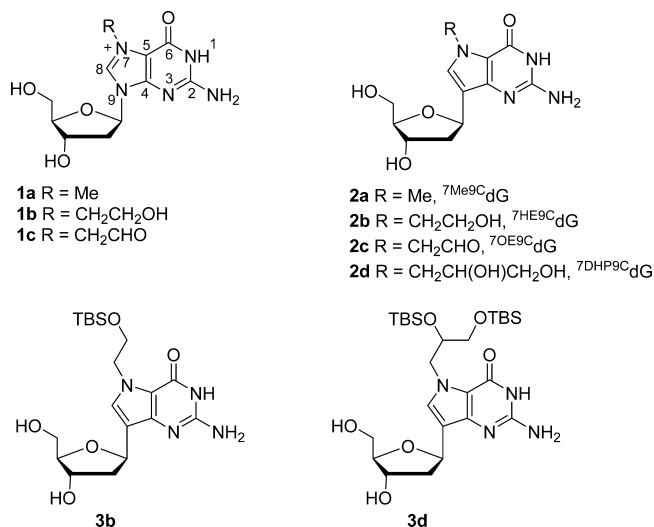


## INTRODUCTION

DNA alkylation is one of the most common forms of DNA damage.<sup>1</sup> Many alkylating agents that are found in consumer products or released from industrial plants may present a threat to public health. At the molecular level, these agents or their metabolites are known to react with DNA nucleobases to form alkyl adducts.<sup>2–7</sup> The biological effects of DNA alkyl adducts, however, are difficult to evaluate at the cellular level due to the uncontrolled distribution of the adducts within the whole genome and the involvement of multiple DNA polymerases and repair enzymes.<sup>6,8,9</sup> Furthermore,  $N^7$ -alkylguanines, the major reaction products produced by most alkylating agents, are converted to highly mutagenic abasic sites and weakly mutagenic  $N^7$ -alkyl-FAPY-guanines through spontaneous or enzymatic hydrolysis.<sup>10–15</sup> The conversion rate is dependent on the conditions of the study and the repair activities of the cell line. Therefore, comprehensive understanding of the biological picture of  $N^7$ -alkylguanines at the molecular level, especially how fast and accurately polymerases bypass the lesions, is essential for connecting the chemistry of DNA damage to its biological consequences.

Synthesis of oligonucleotides that contain a single  $N^7$ -alkylguanine has become a vital tool to achieve the above goal. However, the instability of  $N^7$ -alkylguanines is not compatible with the phosphoramidite chemistry used by solid-phase oligonucleotide synthesis. Two studies have been reported in which a short oligonucleotide containing a single guanine reacted with alkylating agents to form the desired  $N^7$ -adducts.<sup>16,17</sup> However, depurination may occur if the  $N^7$ -alkylguanine-containing oligonucleotide needs to be ligated to a long template and annealed to a primer for a standard polymerase kinetic assay where the incorporation accuracy and rate can be determined. Furthermore, the requirement for a single G site limited the possibility of investigating different local sequences. In the past,

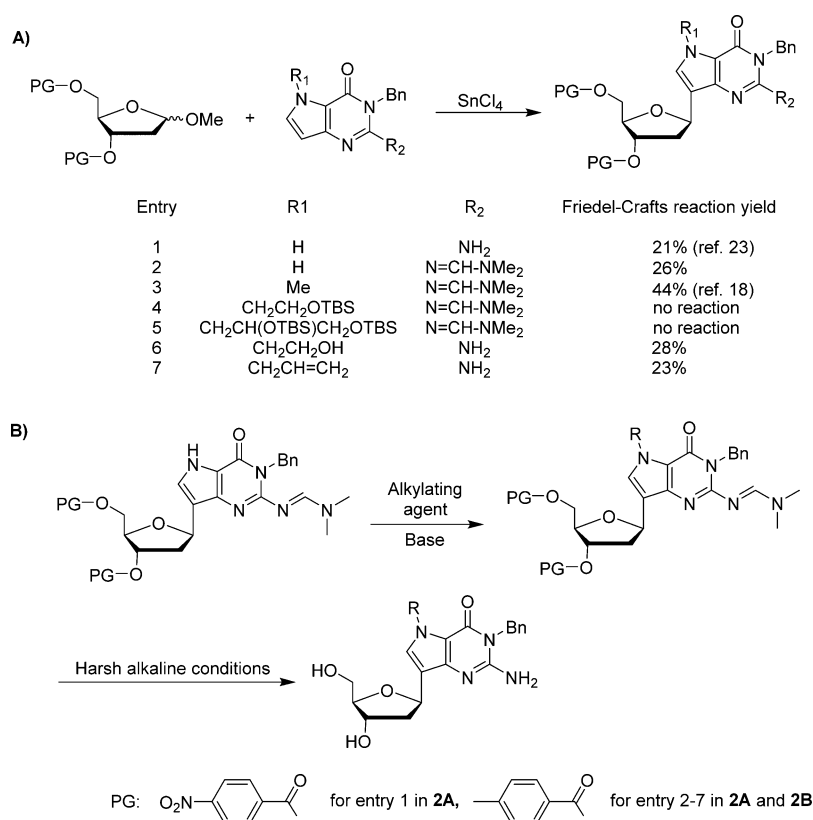
## Scheme 1. Compounds of Interest



we have used  $N^7$ -methyl-9-deazaguanine (**2a**) as the model of  $N^7$ -methylguanine (**1a**) to study its polymerase replication (Scheme 1).<sup>18</sup> We now would like to expand the DNA lesions of interest to the adducts formed between guanine and epoxides, particularly those generated *in vivo* from various olefins. Specifically we are interested in  $N^7$ -hydroxyethyl-2'-deoxyguanine (**1b**) and  $N^7$ -oxoethyl-2'-deoxyguanosine (**1c**) (Scheme 1). The former is formed via a reaction with ethylene oxide.<sup>19</sup> The latter can be generated from multiple sources in the chemical industry, among which the most widely used are vinyl chloride, vinyl bromide, urethane, and acrylonitrile.<sup>20–22</sup> Herein we report

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Scheme 2. (A) Formation of 9-Deazaguanine Nucleosides through Friedel–Crafts Reactions.<sup>a</sup>(B) Alternative Method To Introduce *N*<sup>7</sup>-Alkyl Groups after C-Glycosidic Bond Formation

<sup>a</sup>PG in entry 1 is a *p*-nitrobenzoyl group. PG in entries 2–7 is a *p*-toluoyl group. Reaction details are described in Results and Discussion.

the synthesis of *N*<sup>7</sup>-hydroxyethyl-9-deaza-2'-deoxyguanosine (<sup>7</sup>HE<sup>9</sup>CdG, **2b**) and *N*<sup>7</sup>-oxoethyl-9-deaza-2'-deoxyguanosine (<sup>7</sup>OE<sup>9</sup>CdG, **2c**) as two *N*<sup>7</sup>-alkyl-dG analogues that are resistant to glycosidic bond cleavage (Scheme 1). We also report the side-chain-protected nucleosides (**3b** and **3d**) that can be readily converted to phosphoramidites for oligonucleotide synthesis (Scheme 1). Notably, <sup>7</sup>OE<sup>9</sup>CdG contains an aldehyde group and is not compatible with solid-phase oligonucleotide synthesis. Therefore, its vicinal dihydroxyl precursor (**3d**) was the focus of the synthesis.

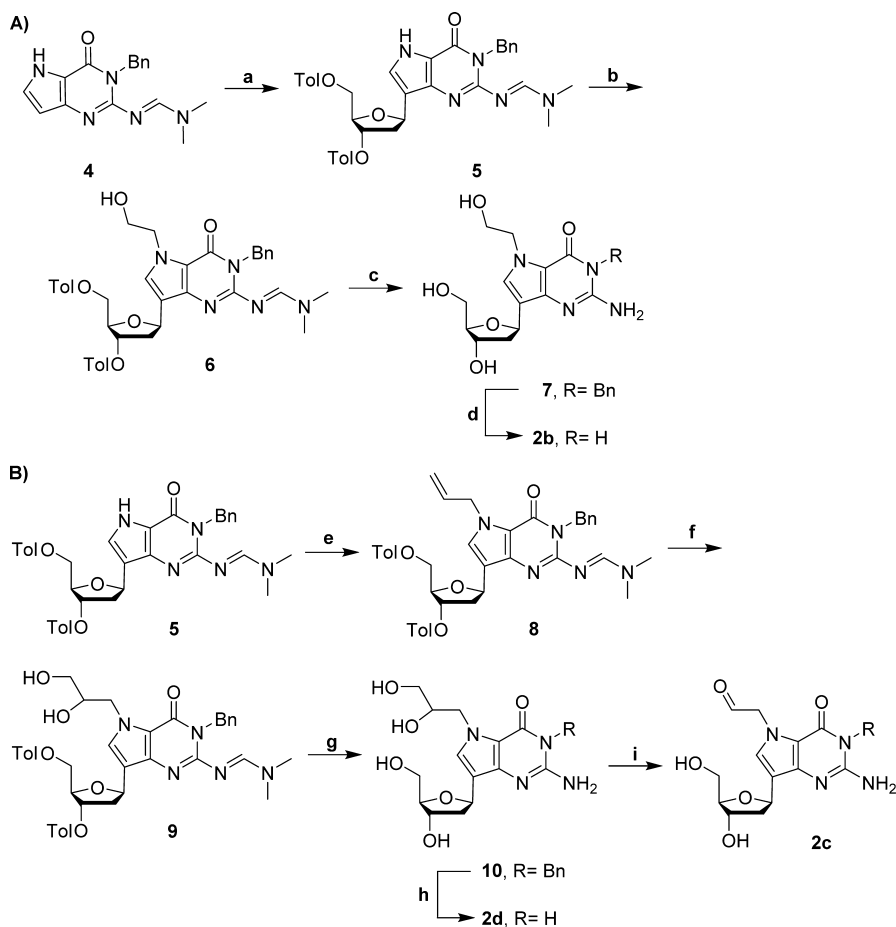
TBS is an evident choice of protecting group for the side chains of **3b** and **3d**, as it is commonly used to protect the 2'-OH of ribonucleosides in RNA synthesis. However, our preliminary studies showed that TBS is not compatible with the Friedel–Crafts reaction that is responsible for the formation of C-glycosidic bonds of 9-deaza-dG (Scheme 2A, entries 4 and 5).<sup>18,23</sup> We examined several possible solutions to this problem. The immediate alternative solution was to directly introduce alkyl group to the *N*<sup>7</sup> position of fully protected 9-deaza-dG (Scheme 2B). Using this strategy, **2b**, **2d**, and **2c** were prepared successfully.

Unfortunately, we experienced difficulties when preparing **3b** and **3d**. It is well documented that the *N,N*-dimethylformamide (dmf) group is a mild protecting group for the exocyclic amine of nucleobases.<sup>24,25</sup> However, during the course of our study of <sup>7</sup>Me<sup>9</sup>CdG (**2a**), we found that when *N*<sup>1</sup> of 9-deazaguanine was protected with a benzyl (Bn) group, strong basic conditions and high temperature were required for complete removal of *N*<sup>2</sup>-dmf.<sup>18</sup> Under the same conditions, TBS was also cleaved from the side chains of **3b** and **3d**. Therefore, we attempted alkylation using dmf-free 9-deaza-dG.<sup>23,26</sup> The reaction conditions used

to introduce the 7-hydroxyethyl group to 9-deaza-dG caused serious epimerization, while in the other case, the 7-allyl group was added to 9-deaza-dG nonselectively at the *N*<sup>2</sup> and *N*<sup>7</sup> positions at the same time. As the last resort, we revisited the possibility of using alkylated 9-deazaguanines that do not contain TBS or dmf groups for C-glycosidic bond formation. Here, we are pleased to report the successful Friedel–Crafts reactions of *N*<sup>7</sup>-hydroxyethyl-9-deazaguanine and *N*<sup>7</sup>-allyl-9-deazaguanine (Scheme 2A, entries 6 and 7). To our surprise, the unprotected hydroxyl group of *N*<sup>7</sup>-hydroxyethyl-9-deazaguanine did not significantly affect the yield of glycosylation. The free hydroxyl group was then protected as a TBS ether. Compound **3b** was obtained by selectively removing the O3' and O5' toluoyl and the *N*<sup>1</sup>-benzyl groups. The product in entry 7 contained an allyl group, which was later dihydroxylated to generate a vicinal diol (Scheme 2A).<sup>27–29</sup> The diol was protected by a pair of TBS groups, yielding compound **3d** after removal of the toluoyl and benzyl groups.

## RESULTS AND DISCUSSION

The *N*<sup>2</sup>-dmf-protected 9-deazaguanine **4**, previously obtained from 2-amino-4-hydroxy-6-methylpyrimidine in five steps,<sup>23</sup> underwent Lewis-acid-mediated Friedel–Crafts reaction with a 1-*O*-methyl-3,5-*O*-ditoluoylated deoxyribose derivative to yield C-nucleoside **5** (Scheme 3A). This coupling mainly afforded the  $\beta$ -anomer in 26% yield. This yield is comparable to the reaction of *N*<sup>2</sup>- and *N*<sup>7</sup>-naked 9-deazaguanine (Scheme 2A, entry 1)<sup>23</sup> but significantly lower than what we observed for *N*<sup>2</sup>-dmf-*N*<sup>7</sup>-methyl-9-deazaguanine (Scheme 2A, entry 3).<sup>18</sup> The hydroxyethyl group was introduced to the deprotonated *N*<sup>7</sup>

Scheme 3<sup>a</sup>

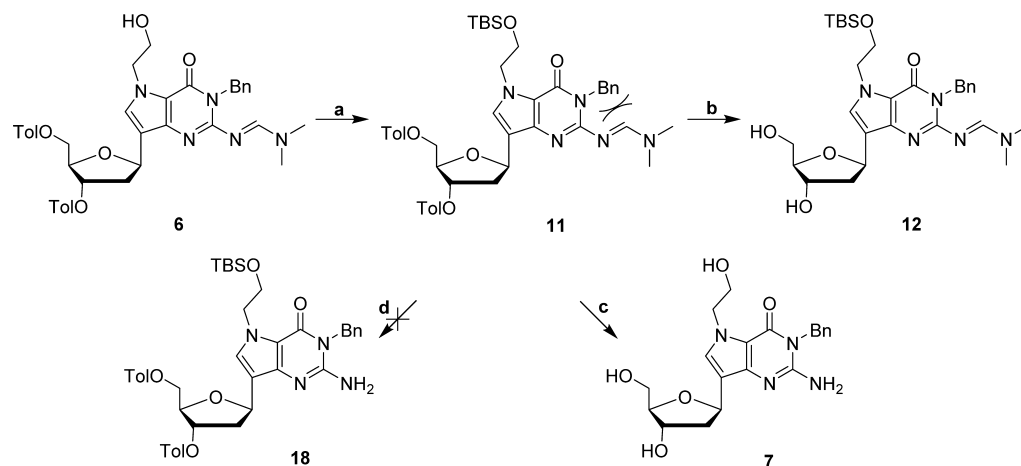
<sup>a</sup>(a) 1-( $\alpha,\beta$ )-*O*-methyl-3,5-di-(*O*-*p*-toluoyl)-2-deoxy-D-ribose, SnCl<sub>4</sub>, 1:1 (v/v) dry CH<sub>3</sub>CN–CH<sub>2</sub>Cl<sub>2</sub>, 65 °C, 16 h, 26%; (b) ethylene carbonate, DBU, dry DMF, 90 °C, 4 h, 49%; (c) 1 M NaOH, 2:1 (v/v) CH<sub>3</sub>OH–H<sub>2</sub>O, 70 °C, 16 h, 72%; (d) ammonium formate, Pd/C, CH<sub>3</sub>OH, 75 °C, 16 h, 48%; (e) allyl bromide, NaH, dry THF, rt, 16 h, 48%; (f) osmium tetroxide, TBHP, TBAF, 4:1 (v/v) acetone–H<sub>2</sub>O, rt, 76%; (g) 1 M NaOH, 6:1 (v/v) CH<sub>3</sub>OH–H<sub>2</sub>O, 70 °C, 16 h, 54%; (h) ammonium formate, Pd/C, CH<sub>3</sub>OH, 75 °C, 16 h, 74%; (i) KIO<sub>4</sub>, 1:1 (v/v) CH<sub>3</sub>OH–H<sub>2</sub>O, rt, 30 min, 85%.

position using ethylene carbonate as the alkylating agent, and then the toluoyl and dmf groups were removed via strong alkaline hydrolysis to afford 7. Pd/C-catalyzed hydrogenolysis by ammonium formate was employed to deprotect the N<sup>1</sup>-Bn group under mild heating, which was previously demonstrated by us to be an efficient way to remove the N<sup>1</sup>-Bn group.<sup>18</sup> This reaction led to the formation of 7<sup>HE9C</sup>dG (2b). The synthesis of 7<sup>OE9C</sup>dG followed the same strategy. Alkylation was performed on 5 to generate N<sup>7</sup>-allyl nucleoside 8. Then the olefin underwent dihydroxylation to form a vicinal diol (9). After deprotection of dmf, toluoyl, and benzyl groups successively, 7<sup>DHP9C</sup>dG (2d) was obtained. The vicinal diol was cleaved by potassium periodate to generate 7<sup>OE9C</sup>dG (2c) (Scheme 3B). Nucleoside 7<sup>OE9C</sup>dG was not very stable at room temperature. It was generated and purified immediately before characterization.

The stabilities of the glycosidic bonds of 7<sup>HE9C</sup>dG and 7<sup>DHP9C</sup>dG were examined after incubation under the following three conditions for 8 h: (i) HCl (pH 2.5), rt, (ii) NaOH (pH 11.7), rt, and (iii) phosphate buffer (pH 7.2), 70 °C. The NMR results confirmed that the glycosidic bond was stable under these conditions, and no sign of epimerization was detected. This result demonstrated the feasibility of using these structures as stable analogues of N<sup>7</sup>-alkyl-dG. 7<sup>OE9C</sup>dG was excluded from the study due to the chemical instability of the aldehyde group.

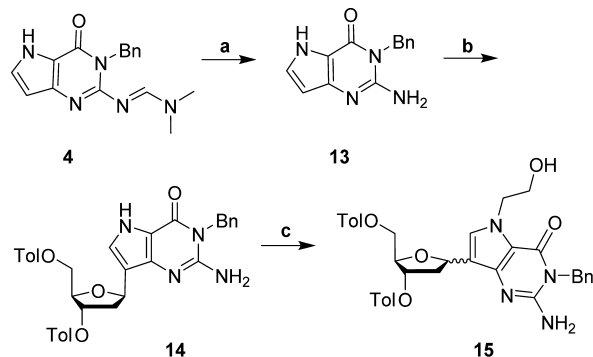
Having completed the synthesis of 7<sup>HE9C</sup>dG and 7<sup>OE9C</sup>dG, we sought to add TBS groups to the 7-alkyl chains of 6 and 9 to generate side-chain-protected nucleosides. However, efforts to hydrolyze the toluoyl and dmf groups simultaneously while keeping the OTBS ether intact were futile (Scheme 4). Different bases (concentrated ammonia, diluted or 1 M KOH/NaOH), temperatures (0–75 °C), and solvent ratios (water–ethanol–THF mixture) were tested. We found that in general, mild conditions only enabled the removal of the toluoyl groups, while harsher conditions caused deprotection of toluoyl, dmf, and TBS at the same time. This was consistent with our past observation that hydrolysis of N<sup>2</sup>-dmf in the presence of N<sup>1</sup>-Bn was difficult.<sup>18</sup> Anticipating that removal of the N<sup>1</sup>-Bn group first should facilitate the removal of the dmf group, we treated 11 with Pd/C–ammonium formate in refluxing methanol overnight. Unfortunately, the same reaction that worked efficiently in the syntheses of 2b and 2d did not show any effect on 11. Change of the conditions to refluxing DMF resulted in partial removal of the N<sup>2</sup>-dmf group (48 h, less than 10% conversion). The N<sup>1</sup>-Bn group, however, remained intact. Therefore, it appears that N<sup>1</sup>-Bn and N<sup>2</sup>-dmf of 11 have remarkable steric effect on each other, which blocks facile deprotection of either group.

To circumvent this problem, we decided to remove the dmf group prior to alkylation, on condition that the unprotected N<sup>2</sup>-amino group would not compete with the N<sup>7</sup> alkylation under

Scheme 4<sup>a</sup>

<sup>a</sup>(a) TBSCl, imidazole, 4-DMAP, DMF, rt, 16 h, 75%; (b) 5:1 (v/v) CH<sub>3</sub>OH–H<sub>2</sub>O, 0.05 M NaOH, rt, 1 h, 75%; (c) 1 M NaOH, 2:1 (v/v) CH<sub>3</sub>OH–H<sub>2</sub>O, 70 °C, 16 h, 72%; (d) different bases (ammonia, KOH, or NaOH), temperatures (0–75 °C), reaction times (1–48 h), and solvent ratios (water–ethanol–THF mixture).

basic conditions. The dmf-free 9-deaza-dG (**14**) was treated with DBU, followed by the addition of ethylene carbonate. Surprisingly, the reaction did not proceed under the previously adopted hydroxyethylation temperature (90 °C). This indicated that removal of *N*<sup>2</sup>-dmf group rendered the *N*<sup>7</sup>-H more difficult to dissociate. At an elevated temperature (110 °C), the desired hydroxyethylated product was obtained (Scheme 5), however, as

Scheme 5<sup>a</sup>

<sup>a</sup>(a) 1 M NaOH, 1:4 (v/v) H<sub>2</sub>O–CH<sub>3</sub>OH, reflux, 20 h, 75%; (b) 1-( $\alpha,\beta$ )-*O*-methyl-3,5-di-(*O*-*p*-toluoyl)-2-deoxy-D-ribose, SnCl<sub>4</sub>, 1:1 (v/v) dry CH<sub>3</sub>CN–CH<sub>2</sub>Cl<sub>2</sub>, 65 °C, 16 h, 26%; (c) ethylene carbonate, DBU, dry DMF, 110 °C, 4 h, 46%,  $\alpha:\beta = 1:3$ .

a mixture of inseparable  $\alpha/\beta$  isomers (1:3, <sup>1</sup>H NMR integration). Therefore, an alternative solution to the synthesis of **3b** was then developed.

It is interesting to compare the epimerization of **14** with other C-nucleosides reported by us and others.<sup>18,23,26,31–33</sup> Hamm et al. first reported epimerization of unalkylated 9-deaza-dG resulting from treatment in concentrated ammonia at 55 °C for 15 h. The same observation was not found at room temperature.<sup>26</sup> In contrast, we previously observed that *N*<sup>7</sup>-methyl-9-deaza-dG (**2a**) was not prone to epimerization under strong alkaline conditions at high temperature.<sup>18</sup> Therefore, we concluded that deprotonating *N*<sup>7</sup>-H through either general base catalysis or specific base catalysis is required for the epimerization of 9-deazaguanine nucleosides. Here we further provide evidence to show that 9-deaza-dG epimerizes at 110 °C

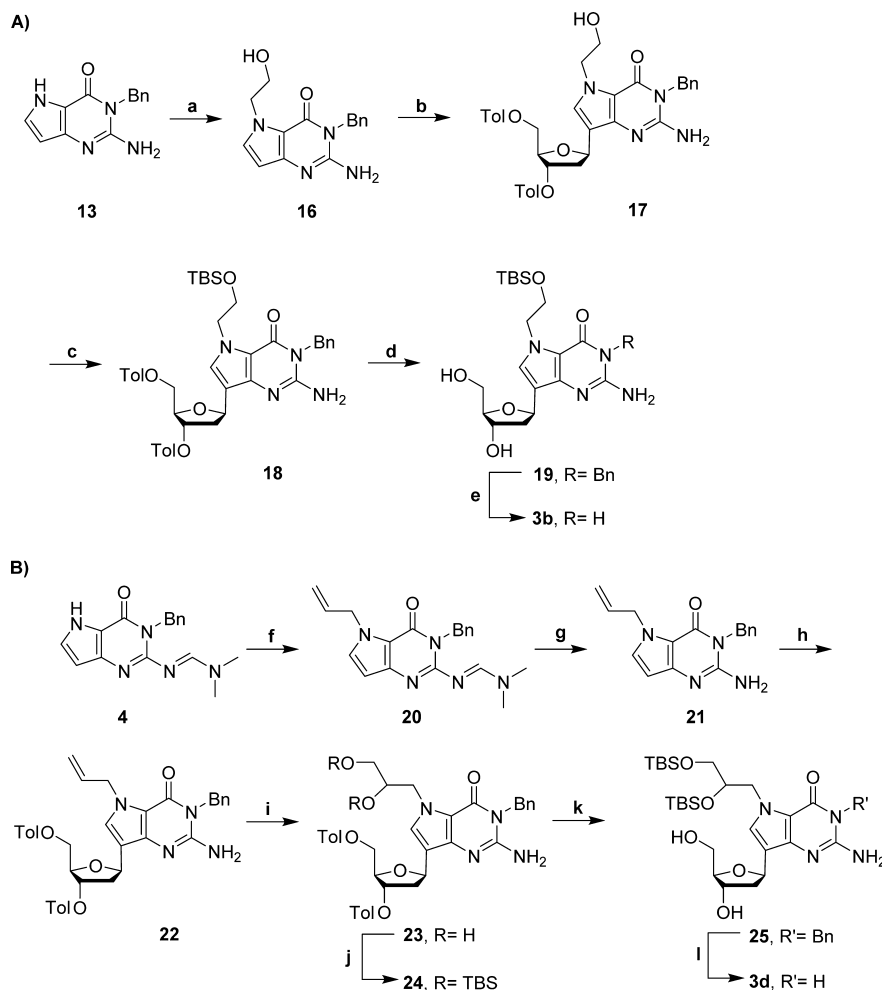
in the presence of only 1 mol equiv of DBU, while the same treatment at 90 °C did not result in detectable epimerization. Furthermore, the less electron-donating effect exerted by *N*<sup>2</sup>-dmf can attenuate epimerization. This electronic effect is consistent with the literature record of the C-glycoside of 6-aminopyridone.<sup>34</sup> In short, the availability of *N*<sup>7</sup>-H, alkaline pH, high temperature, and electron-donating groups on the nucleobase have in combination created a favorable condition for the epimerization of 9-deaza-dG.

Nonreacting hydroxyl groups are often protected in a Lewis acid-promoted glycosylation reaction. However, when the glycosyl donor is an *O*-glycoside, attack of the alkoxy group to the oxocarbenium intermediate is reversible. As such, formation of a more stable C-glycosidic bond will be favored over an *O*-glycosidic bond. On the basis of this analysis, we treated the unprotected *N*<sup>7</sup>-hydroxyethyl-9-deazaguanine (**16**) with the sugar donor successfully with no significantly lower yield (Scheme 6A). The hydroxyl group was then protected as a TBS ether, followed by removal of the two toluoyl groups at room temperature (**19**). The *N*<sup>1</sup>-Bn group of **19** was deprotected smoothly using Pd/C-catalyzed hydrogenolysis to afford **3b**.

The synthesis of **3d** was carried out using a similar strategy (Scheme 6B). As we observed that allylation of **13** produced a mixture of *N*<sup>2</sup>- and *N*<sup>7</sup>-allyl products, the *N*<sup>2</sup>-dmf-protected 9-deazaguanine (**4**) was used as the starting material instead. After the allyl group was specifically introduced to *N*<sup>7</sup>, the *N*<sup>2</sup>-dmf group was removed under strong alkaline conditions (**21**). The *N*<sup>7</sup>-allyl group survived the next Friedel–Crafts reaction. Then the olefin was dihydroxylated to form a vicinal diol **23**, followed by TBS protection of both hydroxyl groups (**24**). The two toluoyl groups and the *N*<sup>1</sup>-Bn were successively removed under the same conditions as described for the synthesis of **3b**.

## CONCLUSION

In summary, we have successfully synthesized two polar-chain-containing *N*<sup>7</sup>-alkyl-9-deaza-dGs and expanded the series of chemically stable analogues of *N*<sup>7</sup>-alkyl-dGs. The C-glycosidic bonds of the two new compounds were proved to be stable under strong acidic and basic conditions and at high temperatures. In addition, we overcame the difficulties in stereoselectivity and regioselectivity when the exocyclic amine of 9-deazaguanine was

Scheme 6<sup>a</sup>

<sup>a</sup>(a) Ethylene carbonate, DBU, dry DMF, 90 °C, 4 h, 47%; (b) 1-( $\alpha,\beta$ )-*O*-methyl-3,5-di-(*O*-*p*-toluoyl)-2-deoxy-D-ribose, SnCl<sub>4</sub>, 1:1 (v/v) dry CH<sub>3</sub>CN–CH<sub>2</sub>Cl<sub>2</sub>, reflux, 16 h, 28%; (c) TBSCl, imidazole, 4-DMAP, DMF, rt, 16 h, 93%; (d) 0.05 M NaOH, 10:1 (v/v) CH<sub>3</sub>OH–H<sub>2</sub>O, rt, 1 h, 74%; (e) ammonium formate, Pd/C, CH<sub>3</sub>OH, 75 °C, 16 h, 43%; (f) allyl bromide, NaH, dry THF, rt, 4 h, 64%; (g) 1 M NaOH, 1:4 (v/v) H<sub>2</sub>O–CH<sub>3</sub>OH, 70 °C, 16 h, 70%; (h) 1-( $\alpha,\beta$ )-*O*-methyl-3,5-di-(*O*-*p*-toluoyl)-2-deoxy-D-ribose, SnCl<sub>4</sub>, 1:1 (v/v) dry CH<sub>3</sub>CN–CH<sub>2</sub>Cl<sub>2</sub>, 65 °C, 16 h, 23%; (i) osmium tetroxide, TBHP, TBAF, 4:1 (v/v) acetone–H<sub>2</sub>O, rt, 16 h, 40%; (j) TBSCl, imidazole, 4-DMAP, DMF, rt, 16 h, 76%; (k) 0.1 M NaOH, 10:1 (v/v) CH<sub>3</sub>OH–H<sub>2</sub>O, rt, 1 h, 68%; (l) ammonium formate, Pd/C, CH<sub>3</sub>OH, 75 °C, 16 h, 56%.

not protected and developed an efficient synthesis of the two nucleosides whose N<sup>7</sup> polar side chains were protected by TBS groups. These side-chain-protected nucleosides can be readily converted to phosphoramidites for solid-phase oligonucleotide synthesis, which will play essential roles in elucidating the polymerase actions on N<sup>7</sup>-hydroxyethyl and N<sup>7</sup>-oxoethyl adducts of 2'-deoxyguanosine.

## EXPERIMENTAL SECTION

**General Information.** <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a NMR spectrometer operating at 500 or 600 MHz for <sup>1</sup>H and 125 or 150 MHz for <sup>13</sup>C using the solvent as an internal reference. The coupling constants (*J*) for <sup>1</sup>H NMR are recorded in hertz. High resolution mass spectra (HRMS) of compounds 9, 11, 12, 18, and 24 were obtained using a MALDI-TOF spectrometer and all others with an ICR (ESI) spectrometer. Melting points were recorded on a microscopic instrument.

THF was distilled freshly from LiAlH<sub>4</sub>. Acetonitrile, dichloromethane, and DMF were distilled freshly from CaH<sub>2</sub>. 1-Benzyl-9-deazaguanine (4) was prepared following Rana's protocol, and its characterization was described previously by Gibson et al.<sup>18,23</sup> 1-( $\alpha,\beta$ )-*O*-Methyl-3,5-di-(*O*-*p*-toluoyl)-2-deoxy-D-ribose was prepared according to a reported procedure.<sup>30</sup>

**Stability Test.** <sup>7</sup>HE<sup>9C</sup>dG and <sup>7</sup>DHP<sup>9C</sup>dG were incubated under the following three conditions for 8 h: (i) HCl (pH 2.5), rt; (ii) NaOH (pH 11.7), rt; (iii) phosphate buffer (pH 7.2), 70 °C. After that, the reaction mixtures were neutralized, concentrated, and passed through a silica gel column using a relatively polar eluent (methanol/ethyl acetate 1:2, compared to 1:5 for 2b and 2d) to elute possible cleaved products. The NMR spectra were compared with the spectra of 2b and 2d to determine any possible bond cleavage and epimerization.

(2*R*,3*S*,5*R*)-5-(3-Benzyl-2-(((dimethylamino)methylene)amino)-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-Methylbenzoate (5). To a suspension of *N'*-(3-benzyl-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-2-yl)-*N,N*-dimethylformimidamide 4 (3.52 g, 12.0 mmol) and 1-( $\alpha,\beta$ )-*O*-methyl-3,5-di-(*O*-*p*-toluoyl)-2-deoxy-D-ribose (6.6 g, 17.2 mmol) in a mixture of methylene chloride (20.0 mL) and acetonitrile (20.0 mL) was added a solution of SnCl<sub>4</sub> (23.2 mL, 23.2 mmol, 1 M in CH<sub>2</sub>Cl<sub>2</sub>). The reaction mixture was heated at 65 °C for 16 h. The reaction mixture was diluted with methylene chloride and washed successively with sat. NaHCO<sub>3</sub> and brine. The organic layer was separated and dried over MgSO<sub>4</sub>. The solution was concentrated and purified by column chromatography (SiO<sub>2</sub>, 0.06–0.20 mm, eluting with hexane/ethyl acetate 2:1 to 1:2) to give compound 5 as a light yellow solid (1.96 g, 26%). mp 92–93 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm

2.36 (s, 3H), 2.42 (s, 3H), 2.49–2.53 (m, 1H), 2.91–2.95 (m, 1H), 3.05 (s, 3H), 3.12 (s, 3H), 4.50 (s, 1H), 4.58 (dd,  $J = 10.5, 5.0$  Hz, 1H), 4.66 (dd,  $J = 11.0, 4.5$  Hz, 1H), 5.49–5.56 (m, 3H), 5.74 (s, 1H), 7.15–7.36 (m, 10H), 7.90 (d,  $J = 7.0$  Hz, 2H), 8.01 (s, 2H), 8.58 (s, 1H), 10.36 (s, 1H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 21.6, 21.7, 35.0, 37.9, 40.8, 45.6, 60.4, 64.8, 73.8, 81.8, 114.4, 116.2, 126.8, 127.0, 127.2, 127.23, 128.0, 128.1, 129.1, 129.14, 129.7, 129.8, 138.9, 143.6, 144.0, 153.7, 156.2, 156.8, 166.2, 166.5. ESI-MS ( $M + H$ )<sup>+</sup> for  $\text{C}_{37}\text{H}_{37}\text{N}_5\text{O}_6$ : expected 648.2822, found 648.2852.

(2*R*,3*S*,5*R*)-5-(3-Benzyl-2-(((dimethylamino)methylene)amino)-5-(2-hydroxyethyl)-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-Methylbenzoate (**6**). To a solution of **5** (0.52 g, 0.8 mmol) in dry DMF (5.0 mL) were added DBU (280.0  $\mu\text{L}$ , 1.8 mmol) and ethylene carbonate (0.21 g, 2.4 mmol). The reaction was heated under 90 °C for 4 h. After removal of DMF, the reaction mixture was diluted with ethyl acetate and washed successively with water and brine. The solution was concentrated and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with hexane/ethyl acetate 1:4 to 1:8) to give compound **6** as a white solid (0.27 g, 49%). mp 75–76 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 2.40 (s, 3H), 2.44 (s, 3H), 2.52–2.57 (m, 1H), 2.85–2.89 (m, 1H), 3.05 (s, 3H), 3.15 (s, 3H), 3.90 (s, 2H), 4.46 (s, 2H), 4.51 (s, 1H), 4.59 (dd,  $J = 11.0, 4.0$  Hz, 1H), 4.70 (dd,  $J = 11.3, 4.5$  Hz, 1H), 5.53 (s, 3H), 5.76 (s, 1H), 7.09 (s, 1H), 7.19–7.21 (m, 3H), 7.26–7.29 (m, 4H), 7.35 (d,  $J = 5.0$  Hz, 2H), 7.94 (d,  $J = 7.5$  Hz, 2H), 8.01 (d,  $J = 7.5$  Hz, 2H), 8.60 (s, 1H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 21.5, 21.6, 34.9, 37.8, 40.7, 45.2, 50.8, 63.0, 64.5, 73.5, 81.7, 113.4, 115.2, 126.6, 127.0, 127.04, 127.6, 128.0, 129.0, 129.4, 129.6, 130.7, 138.6, 143.6, 143.8, 153.7, 156.3, 156.6, 166.0, 166.3. ESI-MS ( $M + H$ )<sup>+</sup> for  $\text{C}_{39}\text{H}_{41}\text{N}_5\text{O}_7$ : expected 692.3084, found 692.3122.

2-Amino-3-benzyl-7-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-5-(2-hydroxyethyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**7**). A suspension of **6** (0.15 g, 0.22 mmol) in 1 M sodium hydroxide in a mixture of methanol (2.0 mL) and water (1.0 mL) was heated under 70 °C for 16 h and allowed to cool to room temperature. The reaction mixture was concentrated and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with hexane/ethyl acetate 1:5 to 1:10) to give **7** as a white solid (0.063 g, 72%). mp 99–100 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  ppm 2.08 (dd,  $J = 13.0, 5.0$  Hz, 1H), 2.46–2.52 (m, 1H), 3.69 (d,  $J = 12.0$  Hz, 1H), 3.80–3.85 (m, 3H), 4.02 (s, 1H), 4.36–4.41 (m, 3H), 4.45 (d,  $J = 5.0$  Hz, 1H), 5.28–5.32 (m, 3H), 7.22 (d,  $J = 7.5$  Hz, 2H), 7.25 (d,  $J = 7.5$  Hz, 2H), 7.31 (t,  $J = 8.0$  Hz, 2H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  ppm 42.1, 43.7, 50.5, 61.7, 63.4, 74.4, 74.7, 87.9, 112.4, 112.8, 126.17, 127.2, 128.4, 131.7, 135.7, 142.6, 151.0, 154.7. ESI-MS ( $M + H$ )<sup>+</sup> for  $\text{C}_{20}\text{H}_{24}\text{N}_4\text{O}_5$ : expected 401.1825, found 401.1839.

2-Amino-7-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-5-(2-hydroxyethyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**2b**). To a mixture of **7** (0.063 g, 0.16 mmol) and 10% palladium on carbon (0.03 g) in methanol (2.0 mL) was added ammonium formate (0.10 g, 1.5 mmol) under Ar. The reaction mixture was heated under 75 °C for 16 h and then filtered through Celite. The filtrate was concentrated and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with methanol/ethyl acetate 1:5 to 1:5) to give **2b** as a white solid (0.024 g, 48%). mp 124–125 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  ppm 2.04 (dd,  $J = 11.0, 4.5$  Hz, 1H), 2.43 (ddd,  $J = 11.0, 9.5, 4.5$  Hz, 1H), 3.70 (dd,  $J = 10.0, 2.0$  Hz, 1H), 3.79–3.83 (m, 3H), 4.00 (s, 1H), 4.34–4.37 (m, 2H), 4.44 (d,  $J = 4.5$  Hz, 1H), 5.26 (dd,  $J = 9.5, 4.5$  Hz, 1H), 7.20 (s, 1H).  $^{13}\text{C}$  NMR was not available due to the poor solubility. ESI-MS ( $M + H$ )<sup>+</sup> for  $\text{C}_{13}\text{H}_{18}\text{N}_4\text{O}_5$ : expected 311.1355, found 311.1377.

(2*R*,3*S*,5*R*)-5-(5-Allyl-3-benzyl-2-(((dimethylamino)methylene)amino)-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-Methylbenzoate (**8**). To a suspension of **5** (1.61 g, 2.4 mmol) in dry THF (25.0 mL) was added sodium hydride (0.1 g, 2.3 mmol, 60% in mineral oil). The mixture was stirred for 15 min before allyl bromide (150  $\mu\text{L}$ , 3.5 mmol) was added. The mixture was stirred at room temperature for 16 h. After removal of solvent, the reaction was diluted with methylene chloride and washed successively with water and brine. The organic layer

was separated, dried over  $\text{MgSO}_4$ , concentrated, and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with hexane/ethyl acetate 5:1 to 3:1) to give compound **8** as a white solid (0.82 g, 48%). mp 94–95 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 2.41 (s, 3H), 2.45 (s, 3H), 2.53–2.57 (m, 1H), 2.89–2.92 (m, 1H), 3.04 (s, 3H), 3.12 (s, 3H), 4.52–4.55 (m, 1H), 4.60 (dd,  $J = 11.5, 4.0$  Hz, 1H), 4.71 (dd,  $J = 11.5, 5.0$  Hz, 1H), 5.01–5.09 (m, 2H), 5.13–5.23 (m, 2H), 5.55 (s, 3H), 5.77 (t,  $J = 5.0$  Hz, 1H), 6.00–6.05 (m, 1H), 7.06 (s, 1H), 7.19–7.23 (m, 3H), 7.26–7.29 (m, 4H), 7.38–7.39 (m, 2H), 7.95 (d,  $J = 7.5$  Hz, 2H), 8.01 (d,  $J = 7.5$  Hz, 2H), 8.59 (s, 1H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 21.8, 21.83, 35.2, 38.4, 41.0, 45.3, 50.6, 64.8, 68.6, 73.8, 82.1, 114.0, 117.6, 126.8, 127.3, 127.9, 128.3, 129.0, 129.2, 129.23, 129.26, 129.3, 129.85, 129.9, 130.1, 134.5, 143.8, 144.0, 154.2, 166.5. ESI-MS ( $M + H$ )<sup>+</sup> for  $\text{C}_{40}\text{H}_{41}\text{N}_5\text{O}_6$ : expected 688.3135, found 688.3202.

(2*R*,3*S*,5*R*)-5-(3-Benzyl-5-(2,3-dihydroxypropyl)-2-(((dimethylamino)methylene)amino)-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-Methylbenzoate (**9**). To the suspension of **8** (0.82 g, 1.2 mmol) in a mixture of acetone (4.0 mL) and water (1.0 mL) were added TBHP (220  $\mu\text{L}$ , 1.1–1.3 mmol, 5.0–6.0 M in decane), TBAF (0.028 g, 0.11 mmol), and  $\text{OsO}_4$  (trace). The reaction was stirred at room temperature for 16 h. The reaction mixture was diluted with methylene chloride and washed successively with water and brine. The organic layer was separated, dried over  $\text{MgSO}_4$ , concentrated, and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with hexane/ethyl acetate 1:1 to 1:3) to give compound **9** as a light yellow solid (0.66 g, 76%). mp 80–81 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 2.40 (s, 3H), 2.44 (s, 3H), 2.52–2.60 (m, 1H), 2.84–2.93 (m, 1H), 3.06 (s, 3H), 3.15 (s, 3H), 3.51 (s, 2H), 3.88–3.94 (m, 2H), 4.30–4.34 (m, 1H), 4.51–4.60 (m, 3H), 4.69–4.72 (m, 1H), 5.54 (s, 3H), 7.11 (s, 1H), 7.19–7.35 (m, 9H), 7.93 (d,  $J = 8.0$  Hz, 2H), 8.01 (s, 2H), 8.58 (s, 1H).  $^{13}\text{C}$  NMR for both diastereomers (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 21.0, 21.6, 35.0, 37.8, 40.8, 45.4, 50.0, 50.1, 60.4, 62.9, 63.0, 64.6, 71.8, 73.5, 73.6, 81.8, 113.8, 126.8, 127.08, 127.1, 127.7, 128.1, 129.0, 129.09, 129.1, 129.2, 129.68, 129.72, 131.7, 138.7, 143.7, 143.9, 153.8, 156.8, 166.4. MALDI-MS ( $M + H$ )<sup>+</sup> for  $\text{C}_{40}\text{H}_{43}\text{N}_5\text{O}_8$ : expected 722.3190, found 722.3193.

2-Amino-3-benzyl-5-(2,3-dihydroxypropyl)-7-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**10**). A suspension of **9** (0.66 g, 0.91 mmol) in 1 M sodium hydroxide in a mixture of methanol (3 mL) and water (0.5 mL) was heated under 70 °C for 16 h and allowed to cool to room temperature. The reaction mixture was concentrated and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with methanol/ethyl acetate 1:20 to 1:10) to give **10** as a white solid (0.21 g, 54%). mp 93–94 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  ppm 2.07 (dd,  $J = 13.0, 5.5$  Hz, 1H), 2.51 (ddd,  $J = 13.0, 11.5, 5.5$  Hz, 1H), 3.48 (ddd,  $J = 11.5, 5.5, 3.0$  Hz, 1H), 3.54 (ddd,  $J = 11.5, 5.0, 2.0$  Hz, 1H), 3.69 (dd,  $J = 9.5, 2.5$  Hz, 1H), 3.82 (dd,  $J = 9.5, 2.5$  Hz, 1H), 3.93–3.97 (m, 1H), 4.00–4.02 (m, 1H), 4.26 (dt,  $J = 14.0, 5.0$  Hz, 1H), 4.45–4.46 (d,  $J = 5.0$  Hz, 1H), 4.51–4.56 (m, 1H), 5.29–5.35 (m, 3H), 7.23–7.29 (m, 4H), 7.32–7.35 (m, 2H).  $^{13}\text{C}$  NMR for both diastereomers (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  ppm 44.18, 44.2, 45.9, 52.6, 62.2, 65.4, 65.5, 73.9, 76.5, 76.8, 90.1, 114.9, 115.1, 128.3, 129.3, 130.5, 134.3, 137.9, 145.3, 153.1, 157.2. ESI-MS ( $M + H$ )<sup>+</sup> for  $\text{C}_{21}\text{H}_{26}\text{N}_4\text{O}_6$ : expected 431.1931, found 431.1966.

2-Amino-5-(2,3-dihydroxypropyl)-7-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**2d**). To a mixture of **10** (0.21 g, 0.49 mmol) and 10% palladium on carbon (0.1 g) in methanol (5 mL) was added ammonium formate (0.29 g, 4.5 mmol) under Ar. The reaction mixture was heated under 75 °C for 16 h and then filtered through Celite. The filtrate was concentrated and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with methanol/ethyl acetate 1:20 to 1:5) to give **2d** as a white solid (0.12 g, 74%). mp 135–136 °C.  $^1\text{H}$  NMR (500 MHz,  $d_6$ -DMSO)  $\delta$  ppm 1.90 (dd,  $J = 13.8, 4.8$  Hz, 1H), 2.11–2.17 (m, 1H), 3.27 (t,  $J = 5.0$  Hz, 2H), 3.45 (s, 2H), 3.71 (s, 2H), 4.03–4.09 (m, 1H), 4.18 (s, 1H), 4.32–4.35 (m, 1H), 4.66 (s, 1H), 4.91 (s, 2H), 5.07 (dd,  $J = 11.2, 6.0$  Hz, 1H), 5.20 (br, 1 H), 5.72 (s, 2H), 7.15 (s, 1H), 10.53 (s, 1H).  $^{13}\text{C}$  NMR for both of the diastereomers (150 MHz,  $d_6$ -DMSO)

$\delta$  ppm 42.7, 51.6, 59.9, 63.9, 64.4, 72.3, 73.5, 74.0, 88.4, 113.3, 114.4, 131.3, 132.3, 151.6, 156.3. ESI-MS ( $M + H$ )<sup>+</sup> for C<sub>14</sub>H<sub>20</sub>N<sub>4</sub>O<sub>6</sub>: expected 341.1461, found 341.1486.

2-(2-Amino-7-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)-4-oxo-3,4-dihydro-5*H*-pyrrolo[3,2-*d*]pyrimidin-5-yl)acetaldehyde (**2c**). To compound **2d** (0.050 g, 0.15 mmol) in a mixture of methanol (1 mL) and water (1 mL) was added potassium periodate (0.035 g, 0.15 mmol). The reaction mixture was stirred under room temperature for 30 min, concentrated, and purified by column chromatography (SiO<sub>2</sub>, 0.06–0.20 mm, eluting with methanol/ethyl acetate 1:20 to 1:5) to give **2c** as a white solid (0.039 g, 85%). mp 117–118 °C. <sup>1</sup>H NMR (600 MHz, *d*<sub>6</sub>-DMSO)  $\delta$  ppm 1.91–1.99 (m, 1H), 2.10–2.13 (m, 1H), 3.44 (s, 2H), 3.72 (s, 1H), 4.20–4.22 (m, 1H), 4.93–4.95 (m, 1H), 5.07 (s, 2H), 5.84–6.01 (m, 2H), 7.16 (s, 1H), 9.60 (s, 1H), 10.66–10.72 (s, 1H). <sup>13</sup>C NMR was not available due to the poor solubility. ESI-MS ( $M + H$ )<sup>+</sup> for C<sub>13</sub>H<sub>16</sub>N<sub>4</sub>O<sub>5</sub>: expected 309.1199, found 309.1220.

(2*R*,3*S*,5*R*)-5-(3-Benzyl-5-(2-((*tert*-butyldimethylsilyloxy)ethyl)-2-(((dimethylamino)methylene)amino)-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-(((4-methylbenzoyloxy)methyl)-tetrahydrofuran-3-yl 4-Methylbenzoate (**11**). To a solution of **6** (0.45 g, 0.65 mmol) in DMF were added imidazole (0.13 g, 1.9 mmol), 4-DMAP (0.002 g, 0.018 mmol), and TBSCl (0.29 g, 1.9 mmol). The reaction was stirred under room temperature for 16 h. After removal of the solvent, the mixture was diluted with methylene chloride and washed with water and brine. The solution was concentrated and purified by column chromatography (SiO<sub>2</sub>, 0.06–0.20 mm, eluting with hexane/ethyl acetate 5:1 to 3:1) to give compound **11** as a light yellow solid (0.43 g, 82%). mp 50–51 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm –0.12 (s, 6H), 0.83 (s, 9H), 2.40 (s, 3H), 2.44 (s, 3H), 2.49–2.52 (m, 1H), 2.87–2.89 (m, 1H), 3.03 (s, 3H), 3.10 (s, 3H), 3.93 (s, 2H), 4.44 (s, 2H), 4.52 (s, 1H), 4.60 (dd, *J* = 11.3, 4.5 Hz, 1H), 4.66 (dd, *J* = 11.5, 5.0 Hz, 1H), 5.56 (s, 3H), 5.78 (s, 1H), 7.12 (s, 1H), 7.19–7.21 (m, 3H), 7.25–7.29 (m, 4H), 7.35–7.37 (m, 2H), 7.96 (d, *J* = 7.5 Hz, 2H), 8.01 (d, *J* = 6.5 Hz, 2H), 8.59 (s, 1H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  ppm –5.4, 18.4, 21.9, 21.94, 26.1, 31.8, 35.2, 41.0, 45.3, 51.3, 63.5, 65.0, 73.6, 82.1, 113.0, 114.7, 126.8, 127.46, 127.5, 127.9, 128.3, 129.3, 129.4, 129.96, 130.0, 139.3, 143.1, 143.9, 144.1, 154.2, 156.0, 156.8, 166.4, 166.6. MALDI-MS ( $M + H$ )<sup>+</sup> for C<sub>45</sub>H<sub>55</sub>N<sub>5</sub>O<sub>7</sub>Si: expected 806.3943, found 806.3944.

*N'*-(3-Benzyl-5-(2-((*tert*-butyldimethylsilyloxy)ethyl)-7-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-2-yl)-*N,N*-dimethylformimidamide (**12**). To compound **11** (0.43 g, 0.53 mmol) in a mixture of methanol (5 mL) and water (1 mL) was added sodium hydroxide (0.05 M). The reaction mixture was stirred under room temperature for 1 h. The reaction mixture was concentrated and purified by column chromatography (SiO<sub>2</sub>, 0.06–0.20 mm, eluting with methanol/ethyl acetate 1:50 to 1:20) to give **12** as a light yellow solid (0.20 g, 72%). mp 65–66 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm –0.11 (s, 3H), –0.09 (s, 3H), 0.83 (s, 9H), 2.04 (dd, *J* = 11.0, 5.0 Hz, 1H), 2.60–2.66 (m, 1H), 3.03 (s, 3H), 3.14 (s, 3H), 3.72–3.75 (m, 1H), 3.86–3.90 (m, 3H), 4.12 (s, 1H), 4.32–4.35 (m, 1H), 4.56–4.63 (m, 2H), 5.32 (dd, *J* = 11.0, 5.0 Hz, 1H), 5.49 (s, 2H), 7.06 (s, 1H), 7.19–7.33 (m, 5H), 8.28 (s, 1H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  ppm –5.4, 18.4, 26.1, 35.1, 41.0, 43.8, 45.6, 51.1, 63.5, 64.1, 75.7, 88.5, 113.5, 115.1, 127.0, 127.9, 128.4, 131.4, 138.7, 154.8, 155.8, 157.1. MALDI-MS ( $M + H$ )<sup>+</sup> for C<sub>29</sub>H<sub>43</sub>N<sub>5</sub>O<sub>5</sub>Si: expected 570.3112, found 570.3108.

2-Amino-3-benzyl-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**13**). A suspension of **6** (3 g, 10.2 mmol) in 1 M sodium hydroxide in a mixture of methanol (12 mL) and water (6 mL) was heated under 70 °C for 20 h and allowed to cool to room temperature. The reaction mixture was concentrated and purified by column chromatography (SiO<sub>2</sub>, 0.06–0.20 mm, eluting with ethyl acetate) to give **13** as a white solid (1.86 g, 75%). The characterization of this compound was previously described by Gibson et al.<sup>23</sup>

(2*R*,3*S*,5*R*)-5-(2-Amino-3-benzyl-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-(((4-methylbenzoyloxy)methyl)-tetrahydrofuran-3-yl 4-Methylbenzoate (**14**). To compound **13** (0.31 g, 1.3 mmol) and 1-( $\alpha,\beta$ )-*O*-methyl-3,5-di(*O-p*-toluoyl)-2-deoxy-D-ribose

(0.7 g, 1.9 mmol) in a mixture of methylene chloride (3.0 mL) and acetonitrile (3.0 mL) was added a solution of SnCl<sub>4</sub> (2.5 mL, 2.5 mmol, 1 M in CH<sub>2</sub>Cl<sub>2</sub>). The reaction mixture was heated at 65 °C for 16 h. The reaction mixture was diluted with methylene chloride and washed successively with sat. NaHCO<sub>3</sub> and brine. The organic layer was separated and dried over MgSO<sub>4</sub>. The solution was concentrated and purified by column chromatography (SiO<sub>2</sub>, 0.06–0.20 mm, eluting with hexane/ethyl acetate 1:1 to 1:2) to give compound **14** as a light yellow solid (0.20 g, 26%). mp 84–85 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 2.37 (s, 3H), 2.41 (s, 3H), 2.60–2.63 (m, 2H), 4.53 (s, 1H), 4.64 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.78 (dd, *J* = 11.5, 4.5 Hz, 1H), 5.34–5.42 (m, 2H), 5.47–5.50 (m, 1H), 5.65–5.66 (m, 1H), 7.18–7.33 (m, 10H), 7.91 (d, *J* = 7.5 Hz, 2H), 7.98 (d, *J* = 8.0 Hz, 2H), 10.54 (s, 1H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 21.9, 22.0, 30.0, 39.3, 45.1, 65.1, 73.6, 82.9, 113.0, 113.7, 127.0, 127.2, 127.3, 128.5, 129.3, 129.39, 129.4, 129.5, 130.2, 134.8, 143.7, 144.1, 144.2, 151.3, 166.5, 166.8. ESI-MS ( $M + H$ )<sup>+</sup> for C<sub>34</sub>H<sub>32</sub>N<sub>4</sub>O<sub>6</sub>: expected 593.2401, found 593.2422.

(2*R*,3*S*,5*R*,5*S*)-5-(2-Amino-3-benzyl-5-(2-hydroxyethyl)-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-(((4-methylbenzoyloxy)methyl)tetrahydrofuran-3-yl 4-Methylbenzoate (**15**). To compound **14** (0.100 g, 0.17 mmol) in dry DMF (2 mL) were added DBU (28  $\mu$ L, 0.18 mmol) and ethylene carbonate (0.04 g, 0.45 mmol). The reaction was heated under 110 °C for 4 h. After removal of the solvent, the reaction mixture was diluted with ethyl acetate and washed with water and brine. The residue was concentrated and purified by column chromatography (SiO<sub>2</sub>, 0.06–0.20 mm, eluting with hexane/ethyl acetate 1:5 to 1:8) to give compound **15** as a white solid (0.050 g, 46%). The ratio of the epimers ( $\alpha:\beta$  = 1:3) was determined by <sup>1</sup>H NMR. <sup>1</sup>H NMR for both epimers (600 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 2.38 (s, 4.78H), 2.41 (s, 3H), 2.50 (dd, *J* = 11.2, 5.6 Hz, 1.35H), 2.62–2.65 (m, 1.38H), 3.35 (s, 0.66H), 3.67 (s, 2H), 3.89 (t, *J* = 5.6 Hz, 2.88H), 4.42 (t, *J* = 5.6 Hz, 2H), 4.46–4.47 (m, 1.59H), 4.51–4.53 (m, 0.53H), 4.55 (dd, *J* = 11.2, 5.6 Hz, 1H), 4.64–4.65 (m, 0.39H), 4.75 (dd, *J* = 11.2, 5.6 Hz, 1H), 5.11 (s, 0.61H), 5.20–5.29 (m, 3.21H), 5.42 (dd, *J* = 9.6, 4.8 Hz, 1H), 5.48 (t, *J* = 6.6 Hz, 0.38H), 5.52–5.58 (m, 0.34H), 5.64 (d, *J* = 5.4 Hz, 1H), 7.08 (s, 1H), 7.16–7.36 (m, 13.72H), 7.78 (d, *J* = 7.2 Hz, 0.62H), 7.93 (d, *J* = 7.8 Hz, 2.47H), 7.97 (d, *J* = 8.2 Hz, 2H). <sup>13</sup>C NMR for both epimers (150 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 21.80, 21.82, 42.2, 44.6, 51.3, 62.2, 64.7, 73.2, 83.2, 110.6, 110.9, 112.4, 126.9, 127.1, 127.3, 128.6, 129.2, 129.3, 129.8, 129.9, 130.1, 130.2, 131.2, 133.4, 144.0, 144.1, 144.2, 151.36, 151.40, 152.5, 166.5, 166.6, 166.7.

2-Amino-3-benzyl-5-(2-hydroxyethyl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**16**). To compound **13** (1.3 g, 4.4 mmol) in dry DMF (5 mL) were added DBU (280  $\mu$ L, 1.83 mmol) and ethylene carbonate (1.0 g, 11.7 mmol). The reaction was heated under 90 °C for 4 h. After removal of the solvent, the reaction mixture was diluted with ethyl acetate and washed with water and brine. The residue was chromatographed by ethyl acetate to give compound **16** as a white solid (0.6 g, 47%). mp 168–169 °C. <sup>1</sup>H NMR (500 MHz, *d*<sub>6</sub>-DMSO)  $\delta$  ppm 3.65 (dd, *J* = 9.5, 5.0 Hz, 2H), 4.30 (t, *J* = 4.5 Hz, 2H), 5.23 (s, 2H), 5.90 (s, 1H), 6.25 (s, 1H), 7.21–7.26 (m, 4H), 7.32 (t, *J* = 6.0 Hz, 2H). <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD)  $\delta$  ppm 45.1, 51.9, 63.2, 100.3, 112.7, 127.5, 128.5, 129.8, 134.6, 137.3, 146.9, 152.8, 156.2. ESI-MS ( $M + H$ )<sup>+</sup> for C<sub>15</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub>: expected 285.1352, found 285.1337.

(2*R*,3*S*,5*S*)-5-(2-Amino-3-benzyl-5-(2-hydroxyethyl)-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-(((4-methylbenzoyloxy)methyl)tetrahydrofuran-3-yl 4-Methylbenzoate (**17**). To compound **16** (0.6 g, 2.2 mmol) and 1-( $\alpha,\beta$ )-*O*-methyl-3,5-di(*O-p*-toluoyl)-2-deoxy-D-ribose (2.4 g, 3.3 mmol) in a mixture of methylene chloride (5.0 mL) and acetonitrile (5.0 mL) was added a solution of SnCl<sub>4</sub> (4.5 mL, 4.5 mmol, 1 M in CH<sub>2</sub>Cl<sub>2</sub>). The reaction mixture was heated at 65 °C for 16 h. The reaction mixture was diluted with methylene chloride and washed successively with sat. NaHCO<sub>3</sub> and brine. The organic layer was separated and dried over MgSO<sub>4</sub>. The solution was concentrated and purified by column chromatography (SiO<sub>2</sub>, 0.06–0.20 mm, eluting with hexane/ethyl acetate 1:1 to 1:2) to give compound **17** as a white solid (0.39 g, 28%). mp 89–90 °C. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  ppm 2.40 (s, 3H), 2.43 (s, 3H), 2.50 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.67 (ddd, *J* = 10.5, 10.5, 4.5 Hz, 1H), 3.60 (s, 1H), 3.90 (s, 2H), 4.42 (s, 3H), 4.55 (dd, *J* = 10.5, 4.5 Hz, 1H), 4.73 (dd, *J* = 10.5, 4.5 Hz, 1H), 5.02 (s, 2H),

5.19–5.26 (m, 2H), 5.45 (dd,  $J = 10.5, 5.0$  Hz, 1H), 5.65 (d,  $J = 5.0$  Hz, 1H), 7.09 (s, 1H), 7.20–7.32 (m, 9H), 7.95 (d,  $J = 8.0$  Hz, 2H), 7.99 (d,  $J = 8.0$  Hz, 2H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 21.87, 21.9, 38.6, 44.8, 51.3, 63.0, 65.0, 73.5, 82.6, 112.8, 113.1, 126.7, 127.3, 127.4, 128.2, 129.38, 129.4, 129.9, 130.0, 131.0, 135.3, 144.1, 144.3, 150.9, 155.2, 166.4, 166.7. ESI-MS ( $\text{M} + \text{H}^+$ ) for  $\text{C}_{36}\text{H}_{36}\text{N}_4\text{O}_7$ : expected 637.2663, found 637.2697.

(2*R*,3*S*,5*S*)-5-(2-Amino-3-benzyl-5-(2-((*tert*-butyldimethylsilyloxy)ethyl)-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-(((4-methylbenzoyloxy)methyl)tetrahydrofuran-3-yl) 4-Methylbenzoate (**18**). To compound **17** (0.39 g, 0.6 mmol) in DMF were added imidazole (0.13 g, 1.9 mmol), 4-DMAP (0.002 g, 0.018 mmol), and TBSCl (0.3 g, 2.0 mmol). The reaction was stirred under room temperature for 16 h. After removal of the solvent, the mixture was diluted by methylene chloride and washed with water and brine. The solution was concentrated and purified by column ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with hexane/ethyl acetate 5:1 to 3:1) to give compound **18** as a gummy solid (0.42 g, 93%). mp 55–56 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm –0.11 (d,  $J = 5.0$  Hz, 6H), 0.82 (s, 9H), 2.39 (s, 3H), 2.41 (s, 3H), 2.44 (dd,  $J = 11.5, 4.5$  Hz, 1H), 2.62 (ddd,  $J = 11.5, 11.5, 4.5$  Hz, 1H), 3.90 (t,  $J = 3.5$  Hz, 2H), 4.31–4.37 (m, 2H), 4.44 (dt,  $J = 11.5, 3.5$  Hz, 1H), 4.51 (dd,  $J = 9.5, 3.5$  Hz, 1H), 4.67 (dd,  $J = 9.5, 4.0$  Hz, 1H), 5.14–5.28 (m, 3H), 5.45 (dd,  $J = 9.5, 4.5$  Hz, 1H), 5.65 (d,  $J = 4.5$  Hz, 1H), 7.08 (s, 1H), 7.19–7.30 (m, 9H), 7.94 (d,  $J = 7.0$  Hz, 2H), 7.97 (d,  $J = 6.5$  Hz, 2H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm –5.6, 18.1, 21.6, 21.65, 25.8, 37.8, 44.3, 51.1, 53.5, 63.1, 64.7, 72.6, 82.3, 111.7, 112.2, 126.1, 127.1, 127.3, 127.8, 129.0, 129.1, 129.7, 129.74, 130.9, 135.3, 143.7, 144.0, 151.4, 154.7, 166.0, 166.2. MALDI-MS ( $\text{M} + \text{H}^+$ ) for  $\text{C}_{42}\text{H}_{50}\text{N}_4\text{O}_7\text{Si}$ : expected 751.3528, found 751.3505.

2-Amino-3-benzyl-5-(2-((*tert*-butyldimethylsilyloxy)ethyl)-7-((2*S*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**19**). To compound **18** (0.42 g, 0.56 mmol) in a mixture of methanol (10.0 mL) and water (1.0 mL) was added sodium hydroxide (0.05 M). The reaction mixture was stirred under room temperature for 1 h. The reaction mixture was concentrated and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with methanol/ethyl acetate 1:50 to 1:20) to give **19** as a white solid (0.21 g, 74%). mp 57–58 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  ppm –0.09 (d,  $J = 10.0$  Hz, 6H), 0.82 (s, 9H), 2.02 (dd,  $J = 11.0, 5.0$  Hz, 1H), 2.51–2.56 (m, 1H), 3.68 (d,  $J = 12.0$  Hz, 1H), 3.87–3.90 (m, 2H), 4.08 (s, 1H), 4.31 (dt,  $J = 14.0, 5.0$  Hz, 1H), 4.53–4.58 (m, 2H), 5.13 (d,  $J = 16.0$  Hz, 1H), 5.26 (dd,  $J = 11.5, 5.5$  Hz, 1H), 5.40 (d,  $J = 16.0$  Hz, 1H), 7.06 (s, 1H), 7.22–7.35 (m, 5H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  ppm –4.5, 19.7, 27.2, 44.4, 45.7, 52.6, 64.9, 65.5, 76.7, 89.9, 114.3, 114.7, 128.2, 129.2, 130.4, 134.0, 137.7, 144.9, 152.9, 156.6. ESI-MS ( $\text{M} + \text{H}^+$ ) for  $\text{C}_{26}\text{H}_{38}\text{N}_4\text{O}_5\text{Si}$ : expected 515.2690, found 515.2706.

2-Amino-5-(2-((*tert*-butyldimethylsilyloxy)ethyl)-7-((2*S*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**3b**). To compound **19** (0.21 g, 0.41 mmol) and 10% palladium on carbon (0.11 g) in methanol (5.0 mL) was added ammonium formate (0.28 g, 4.5 mmol) under Ar. The reaction mixture was heated under 75 °C for 16 h and then filtered through Celite. The filtrate was concentrate and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with methanol/ethyl acetate 1:20 to 1:5) to give **3b** as a white gummy solid (0.075 g, 43%). mp 49–50 °C.  $^1\text{H}$  NMR (600 MHz,  $d_6$ -DMSO)  $\delta$  ppm –0.13 (s, 6H), 0.79 (s, 9H), 1.88 (dd,  $J = 12.2, 5.4$  Hz, 1H), 2.06–2.11 (m, 1H), 3.42 (s, 2H), 3.72 (s, 1H), 3.81 (t,  $J = 5.4$  Hz, 2H), 4.17 (s, 1H), 4.25 (t,  $J = 4.8$  Hz, 2H), 4.90 (s, 1H), 5.07 (dd,  $J = 12.0, 5.4$  Hz, 1H), 5.71 (s, 1H), 7.12 (s, 1H), 10.78 (s, 1H).  $^{13}\text{C}$  NMR (150 MHz,  $d_6$ -DMSO)  $\delta$  ppm –4.7, 18.8, 26.7, 42.8, 51.1, 63.9, 73.4, 74.0, 88.4, 112.8, 114.5, 130.9, 145.8, 151.6, 155.6. ESI-MS ( $\text{M} + \text{H}^+$ ) for  $\text{C}_{19}\text{H}_{32}\text{N}_4\text{O}_5\text{Si}$ : expected 425.2221, found 425.2260.

*N'*-(*S*-Allyl-3-benzyl-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-2-yl)-*N,N*-dimethylformimidamide (**20**). To a suspension of compound **6** (2.0 g, 6.8 mmol) in dry THF (25.0 mL) was added sodium hydride (0.28 g, 6.7 mmol, 60% in mineral oil). The mixture was stirred for 15 min before allyl bromide (330.0  $\mu\text{L}$ , 8.0 mmol) was added. The mixture was stirred at room temperature for 16 h. After removal of

solvent, the reaction was diluted with methylene chloride and washed successively with water and brine. The organic layer was separated, dried over  $\text{MgSO}_4$ , concentrated, and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with hexane/ethyl acetate 5:1 to 3:1) to give compound **20** as a light yellow solid (1.4 g, 64%). mp 93–94 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 3.06 (s, 3H), 3.15 (s, 3H), 5.06–5.09 (m, 3H), 5.19 (d,  $J = 10.5$  Hz, 1H), 5.52 (s, 2H), 6.05 (ddt,  $J = 16.5, 10.5, 5.0$  Hz, 1H), 6.31 (s, 1H), 7.03 (s, 1H), 7.19–7.21 (m, 1H), 7.27 (t,  $J = 7.5$  Hz, 2H), 7.37 (d,  $J = 7.5$  Hz, 2H), 8.61 (s, 1H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 35.0, 40.8, 45.1, 50.4, 101.3, 114.3, 116.9, 126.7, 127.9, 128.1, 130.4, 134.8, 139.0, 144.4, 154.3, 155.8, 156.2. ESI-MS ( $\text{M} + \text{H}^+$ ) for  $\text{C}_{19}\text{H}_{21}\text{N}_5\text{O}$ : expected 336.1824, found 336.1832.

5-Allyl-2-amino-3-benzyl-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**21**). To compound **20** (1.0 g, 3 mmol) in a mixture of methanol (20.0 mL) and water (5.0 mL) was added sodium hydroxide (1.0 M). The reaction mixture was heated under 70 °C for 16 h. The reaction mixture was concentrated and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with ethyl acetate) to give **21** as a white solid (0.58 g, 70%). mp 121–122 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  ppm 4.99–5.05 (m, 3H), 5.16 (dd,  $J = 10.5, 1.5$  Hz, 1H), 5.33 (s, 2H), 6.04–6.12 (m, 2H), 7.21–7.30 (m, 4H), 7.35 (t,  $J = 7.5$  Hz, 2H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 44.5, 50.5, 101.3, 112.3, 117.0, 126.5, 127.9, 129.1, 131.1, 134.6, 135.7, 144.9, 151.1, 154.8. ESI-MS ( $\text{M} + \text{H}^+$ ) for  $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}$ : expected 281.1403, found 281.1387.

(2*R*,3*S*,5*R*)-5-(*S*-Allyl-2-amino-3-benzyl-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-(((4-methylbenzoyloxy)methyl)tetrahydrofuran-3-yl) 4-Methylbenzoate (**22**). To compound **21** (0.58 g, 2.1 mmol) and 1-( $\alpha,\beta$ )-*O*-methyl-3,5-di(*O*-*p*-toluoyl)-2-deoxy-*D*-ribose (2.3 g, 3.1 mmol) in a mixture of methylene chloride (5.0 mL) and acetonitrile (5.0 mL) was added a solution of  $\text{SnCl}_4$  (4.2 mL, 4.2 mmol, 1 M in  $\text{CH}_2\text{Cl}_2$ ). The reaction mixture was heated at 65 °C for 16 h. The reaction mixture was diluted with methylene chloride and washed successively with sat.  $\text{NaHCO}_3$  and brine. The organic layer was separated, dried over  $\text{MgSO}_4$ , concentrated, and purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with hexane/ethyl acetate 2:1 to 1:2) to give compound **22** as a light yellow solid (0.30 g, 23%). mp 59–60 °C.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 2.40 (s, 3H), 2.41 (s, 3H), 2.56–2.59 (m, 2H), 4.47 (dt,  $J = 4.2, 1.8$  Hz, 1H), 4.57 (dd,  $J = 12.0, 4.2$  Hz, 1H), 4.76 (dd,  $J = 12.0, 4.2$  Hz, 1H), 4.94–4.96 (m, 2H), 5.09 (d,  $J = 10.4$  Hz, 1H), 5.17 (d,  $J = 10.4$  Hz, 1H), 5.29 (s, 2H), 5.48 (t,  $J = 8.4$  Hz, 1H), 5.64–5.66 (m, 1H), 5.95–6.02 (m, 1H), 7.04 (s, 1H), 7.23–7.29 (m, 7H), 7.31–7.34 (m, 2H), 7.94 (d,  $J = 8.4$  Hz, 2H), 7.97 (d,  $J = 8.4$  Hz, 2H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 21.75, 21.8, 38.6, 44.5, 50.6, 64.8, 73.2, 77.4, 82.5, 112.8, 113.1, 117.6, 126.4, 127.2, 127.3, 128.0, 129.0, 129.2, 129.23, 129.8, 134.2, 135.4, 143.8, 144.1, 151.3, 154.7, 166.2, 166.4. ESI-MS ( $\text{M} + \text{H}^+$ ) for  $\text{C}_{37}\text{H}_{36}\text{N}_4\text{O}_6$ : expected 633.2714, found 633.2745.

(2*R*,3*S*,5*R*)-5-(2-Amino-3-benzyl-5-(2,3-dihydroxypropyl)-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-(((4-methylbenzoyloxy)methyl)tetrahydrofuran-3-yl) 4-Methylbenzoate (**23**). To the suspension of compound **22** (0.30 g, 0.48 mmol) in a mixture of acetone (4.0 mL) and water (1.0 mL) were added TBHP (100  $\mu\text{L}$ , 0.5–0.6 mmol, 5.0–6.0 M in decane), TBAF (0.012 g, 0.048 mmol), and  $\text{OsO}_4$  (trace). The reaction was stirred at room temperature for 16 h. The reaction mixture was diluted with methylene chloride and washed successively with water and brine. The organic layer was separated, dried over  $\text{MgSO}_4$ , and concentrated. The solution was purified by column chromatography ( $\text{SiO}_2$ , 0.06–0.20 mm, eluting with hexane/ethyl acetate 1:1 to 1:3) to give compound **23** as a light yellow solid (0.13 g, 40%). mp 61–62 °C.  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 2.36 (s, 3H), 2.39 (s, 3H), 2.50–2.58 (m, 2H), 3.46 (dd,  $J = 12.0, 5.5$  Hz, 1H), 3.52 (ddd,  $J = 11.5, 4.5, 1.5$  Hz, 1H), 3.88–3.93 (m, 1H), 4.18 (dt,  $J = 14.0, 7.0$  Hz, 1H), 4.40 (dt,  $J = 5.0, 2.0$  Hz, 1H), 4.44 (dd,  $J = 14.0, 3.5$  Hz, 1H), 4.54 (ddd,  $J = 12.0, 5.0, 2.0$  Hz, 1H), 4.67 (dt,  $J = 12.0, 5.4$  Hz, 1H), 5.26 (s, 2H), 5.43 (dd,  $J = 10.0, 6.0$  Hz, 1H), 5.59–5.60 (m, 1H), 7.19–7.28 (m, 10H), 7.89 (d,  $J = 8.5$  Hz, 2H), 7.94 (d,  $J = 8.0$  Hz, 2H).  $^{13}\text{C}$  NMR for both diastereomers (125 MHz,  $\text{CDCl}_3$ )  $\delta$  ppm 22.5, 22.56, 46.0, 52.79, 52.8, 65.40, 65.44, 66.7, 73.8, 73.85, 75.5, 79.5, 84.6, 114.1, 114.3, 128.3, 129.0, 129.1, 129.40, 130.6, 131.1, 131.5, 131.53,



133.7, 137.7, 146.2, 146.3, 153.4, 156.9, 168.4, 168.6. ESI-MS ( $M + H$ )<sup>+</sup> for  $C_{37}H_{38}N_4O_8$ : expected 667.2769, found 667.2786.

(2*R*, 3*S*, 5*R*)-5-(2-Amino-3-benzyl-5-(2,3-bis((*tert*-butyldimethylsilyloxy)propyl)-4-oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-2-((4-methylbenzoyloxy)methyl)tetrahydrofuran-3-yl 4-Methylbenzoate (**24**). To compound **23** (0.13 g, 0.19 mmol) in DMF were added imidazole (0.08 g, 1.1 mmol), 4-DMAP (0.002 g, 0.018 mmol), and TBSCl (0.17 g, 1.1 mmol). The reaction was stirred under room temperature for 16 h. After removal of the solvent, the mixture was diluted by methylene chloride and washed with water and brine. The solution was concentrated and purified by column chromatography ( $SiO_2$ , 0.06–0.20 mm, eluting with hexane/ethyl acetate 5:1 to 3:1) to give compound **24** as a white gummy solid (0.13 g, 76%). mp 44–45 °C. <sup>1</sup>H NMR (600 MHz,  $CDCl_3$ )  $\delta$  ppm –0.31 (s, 3H), –0.07 (d,  $J = 10.2$  Hz, 3H), 0.07 (s, 6H), 0.78 (d,  $J = 2.4$  Hz, 9H), 0.91 (s, 9H), 2.39 (s, 3H), 2.40 (s, 4H), 2.42–2.44 (m, 1H), 3.52–3.55 (m, 1H), 3.61 (dd,  $J = 10.2$ , 3.6 Hz, 1H), 3.99–4.14 (m, 2H), 4.50–4.52 (m, 1H), 4.60–4.64 (m, 2H), 4.77 (dd,  $J = 11.4$ , 4.8 Hz, 1H), 5.39–5.43 (m, 3H), 5.61–5.63 (m, 1H), 7.11 (s, 1H), 7.18–7.20 (m, 2H), 7.24–7.27 (m, 4H), 7.32–7.35 (m, 5H), 7.91 (d,  $J = 7.8$  Hz, 2H), 8.02 (d,  $J = 7.8$  Hz, 2H). <sup>13</sup>C NMR for both diastereomers (150 MHz,  $CDCl_3$ )  $\delta$  ppm –5.1, –5.0, –4.5, 18.2, 18.6, 21.9, 22.0, 26.1, 26.2, 29.9, 38.5, 44.6, 52.3, 52.6, 65.2, 65.8, 72.9, 73.0, 73.6, 82.6, 112.2, 112.3, 126.7, 126.8, 127.4, 127.5, 128.3, 129.4, 129.5, 130.0, 130.1, 131.4, 131.6, 135.5, 143.9, 144.2, 150.9, 151.2, 166.4, 166.6. MALDI-MS ( $M + H$ )<sup>+</sup> for  $C_{49}H_{66}N_4O_8Si_2$ : expected 895.4498, found 895.4525.

2-Amino-5-(2,3-bis((*tert*-butyldimethylsilyloxy)propyl)-7-((2*R*,4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3,5-dihydro-4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (**3d**). To compound **24** (0.13 g, 0.14 mmol) in a mixture of methanol (5 mL) and water (0.5 mL) was added sodium hydroxide (0.1 M). The reaction mixture was stirred under room temperature for 1 h. The reaction mixture was concentrated and purified by column chromatography ( $SiO_2$ , 0.06–0.20 mm, eluting with methanol/ethyl acetate 1:50 to 1:20) to give **25** (0.065 g, 68%). To compound **25** (0.065 g, 0.10 mmol) and 10% palladium on carbon (0.030 g) in methanol (3.0 mL) was added ammonium formate (0.063 g, 1.0 mmol) under Ar. The reaction mixture was heated under 75 °C for 16 h and then filtered through Celite. The filtrate was concentrated and purified by column chromatography ( $SiO_2$ , 0.06–0.20 mm, eluting with methanol/ethyl acetate 1:20 to 1:5) to give **3d** as a white gummy solid (0.031 g, 56%). mp 53–54 °C. <sup>1</sup>H NMR (600 MHz,  $d_6$ -DMSO)  $\delta$  ppm –0.28 (d,  $J = 6.0$  Hz, 3H), –0.08 (s, 3H), 0.05 (s, 6H), 0.79 (s, 9H), 0.89 (s, 9H), 1.88–1.91 (m, 1H), 2.02–2.11 (m, 1H), 3.46–3.50 (m, 2H), 3.70 (d,  $J = 15.6$  Hz, 1H), 3.97–4.03 (m, 2H), 4.18 (d,  $J = 12.0$  Hz, 1H), 4.34 (d,  $J = 10.2$  Hz, 1H), 4.90 (s, 1H), 5.06–5.10 (m, 1H), 5.69 (d,  $J = 20.0$  Hz, 1H), 7.02 (s, 1H), 10.50 (d,  $J = 15.2$  Hz, 1H). <sup>13</sup>C NMR (150 MHz,  $d_6$ -DMSO)  $\delta$  ppm –4.5, –3.9, 18.6, 19.0, 26.7, 49.6, 52.1, 52.2, 63.9, 64.0, 66.3, 73.6, 73.9, 74.2, 88.3, 113.2, 114.8, 131.2, 145.8, 151.4, 155.3. ESI-MS ( $M + H$ )<sup>+</sup> for  $C_{26}H_{48}N_4O_8Si_2$ : expected 569.3191, found 569.3234.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02110.

<sup>1</sup>H and <sup>13</sup>C NMR spectra (PDF)

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### Notes

The authors declare no competing financial interest.

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