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A Facile Synthetic Approach to 7-Deazaguanine Nucleosides via a Boc Protection Strategy

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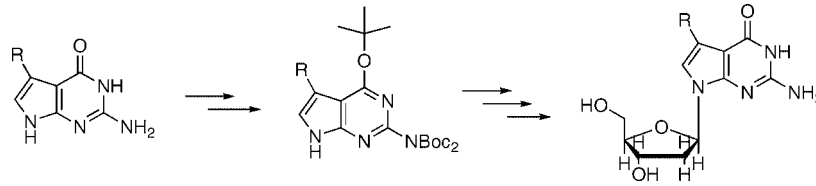
ABSTRACT



An efficient route to the preparation of 5-substituted 2-amino-7-((2*R*,4*R*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one compounds has been developed by the condensation of ω-substituted aldehydes with 2,6-diaminopyrimidin-4(3*H*)-one, followed by Boc protection to afford the corresponding N^2,N^2,N^7-tris-Boc-O^4-*t*-Bu-5-substituted 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one, which is amenable to direct condensation with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose. This route affords an efficient synthesis to 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one, 2-amino-5-alkyl-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one, and guanine nucleosides.

The 5-position of 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one (aka, 7-deazaguanine) is well-suited to introduce functionalized appendages into the major groove of DNA for the purposes of structural and stability studies,[1] DNA fluorescence labeling,[2] DNA sequencing,[3] and the production of DNA-based nanoarrays.[4] There are also a number of naturally occurring 5-substituted 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one nucleosides, including Queuosine (**I**), and related glycosylated analogues,[5] and Archaeosine (**II**)[6] found in tRNA (Figure 1). Accordingly, there have been numerous studies on routes to the synthesis of 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one modified nucleosides.[7]

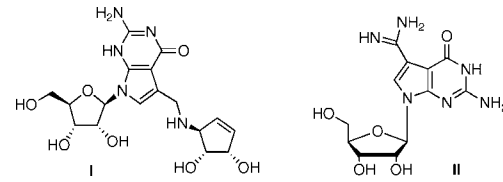


Figure 1. Queuosine (**I**) and Archaeosine (**II**).

There are several barriers to the synthesis of 5-substituted 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one nucleosides.

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Unlike the N1 of pyrimidines and the N9 of adenine, the N9-position of guanine, which is incorporated into a number of antiviral and anticancer nucleoside compounds,[8] and the 5-position of 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one derivatives are not sufficiently activated for direct glycosylation.[9] The classic solution to the preparation of 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one nuclosides is to convert the 5-functionalized-2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one to the 4-chloro compound, which is suitable for reaction with an activated sugar (e.g., 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose).[10] The 4-chloro nucleoside is then converted back to the keto derivative by hydrolysis.[7] In addition to the extra synthetic steps, the 4-chloro derivatives have very poor solubility characteristics,[11] which confounds their functionalization.[7b,8b] If the synthesis of 5-substituted 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one starts with 4-chloro-2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one, it must be first converted to the 5-iodo compound prior to the introduction of modifications to the 5-position by metal-mediated Sonogashira,[7e,12] Stille,[13] or related cross-coupling reactions.

In an effort to prepare a series of 5-substituted 2-amino-7-((2*R*,4*R*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)-furan-2-yl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one modified DNAs (Figure 2) to (1) extend studies on how cationic and

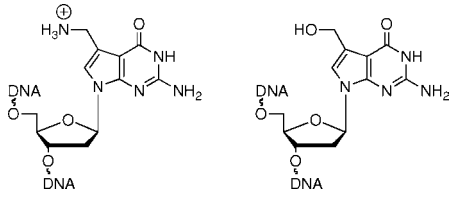

Figure 2. 5-Aminomethyl- (left) and 5-hydroxymethyl- (right) 2-amino-7-((2*R*,4*R*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-ones in DNA.

polar groups located near the floor of the major groove affect the thermodynamic stability, reactivity, and structure of DNA[14] and (2) generate stable interstrand cross-links,[15] we found that the existing synthetic schemes were not suitable.

We report herein the synthesis of 5-aminomethyl- and 5-hydroxymethyl-2-amino-7-((2*R*,4*R*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-ones as examples of a convenient, efficient, and general route to 5-substituted 2-amino-7-((2*R*,4*R*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-ones that involves mild reaction conditions. It is also demonstrated that the approach is amenable to the preparation of guanine nucleosides.

The synthesis started with 5-substituted 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one compounds (**1** and **2**) that were prepared by condensation of the ω-substituted aldehydes[16] with 2,6-diaminopyrimidin-4(3*H*)-one.[17] As mentioned above, normally, the 5-substituted 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one would be transformed to the 4-halo derivative to activate the 7-position for reaction with a protected 1-chloro-2-deoxy-α-D-*erythro*-pentofuranose.[10] Attempts to convert **1** and **2** to the 4-chloro compounds were unsuccessful.

It was envisioned that the tetra-Boc derivatives of **1** and **2** could be prepared and then selectively deprotected to reveal the pyrrole NH-7 for coupling with a reactive Cl sugar (Figure 3).

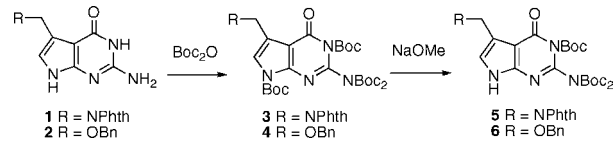

Figure 3. Design of 5-substituted 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one as new coupling precusors with improved solubility.

Compounds **1** and **2** have very limited solubility, so they were treated as a suspension in MeCN with excess Boc_2O. After several days at rt, all of the solid starting material had gone into solution. Instead of the anticipated tetra-Boc derivative, it was found that **1** and **2** afforded the tris-Boc-protected O^4-*t*-Bu ether compounds **7** and **8**, respectively (Scheme 1). Fortuitously, formation of the *O*-*t*-Bu ethers negates the need to convert the 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one nucleus into the 4-chloro derivative prior to sugar coupling.

To elucidate the origin of the O^4-*t*-Bu ethers **7** and **8**, the reactions were monitored by TLC and LC/MS analysis. Time

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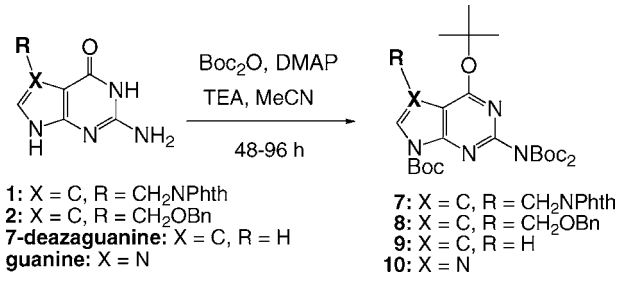
Scheme 1. Preparation of *O*-*t*-Bu Ethers



course studies clearly showed the buildup of the tetra-Boc derivatives (presumably **3** and **4**) and subsequent loss of CO_2 with ether formation to give **7** and **8**, respectively (Figure 4).

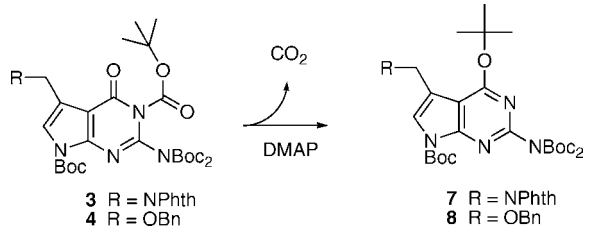
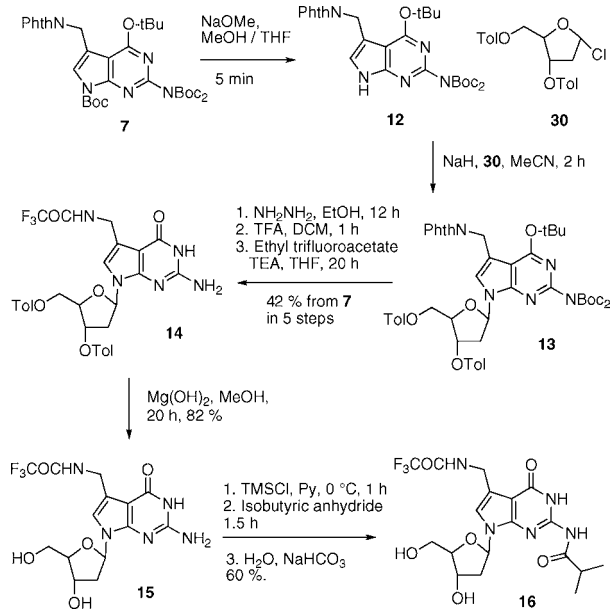


Figure 4. Products **7** and **8** formed from the reactions of **1** and **2** with t-Boc_2O, respectively, that arise via intermediates **3** and **4**.

In terms of the scope of the reaction, the tetra-Boc intermediate and the butyl ether product were also observed in the reaction of unsubstituted 2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one, indicating that the functionality attached to the 5-position does not play a role in the conversion of the carbamate to the ether (**9**). The reaction with guanine was explored despite previous reports on the difficulty of protecting guanine with Boc_2O.[10] After 96 h, the N^2,N^2,N^7-tris-Boc-O^4-*t*-Bu ether derivative of guanine was isolated and characterized. Earlier time points in the reaction were analyzed by LC/MS and clearly showed that formation of ether product (**10**) proceeds through the initial formation of the tetra-Boc derivative.

The conversion of **7** to the protected 2′-deoxynucleoside is shown in Scheme 2. To reveal the pyrrole NH-7, the N^7-Boc was selectively deprotected by sodium methoxide to give pyrrolo[2,3-*d*]pyrimidinone **12**, which is an efficient precursor, with good solubility, for the sugar coupling reaction. Compound **12** was condensed with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (**30**) to give the desired β-anomer of the protected 2′-deoxynucleoside **13**. The phthalimide and Boc protecting groups were sequentially removed with hydrazine and TFA, and the primary amine was then protected as the trifluoroacetamide **14**. From compound **7**, compound **14** was prepared in five steps in 42% yield, and only one column purification was required.

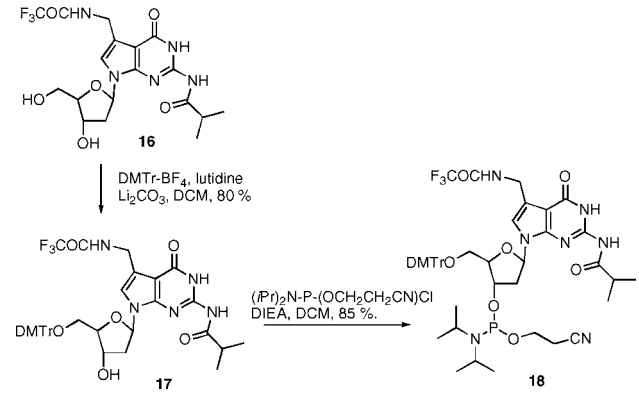
Scheme 2. Synthesis of **16**



The toluoyl groups were selectively removed with $Mg(OMe)_2$ to give trifluoroacetamide **15**.[18] The N^2-amino group in **15** was selectively protected to give isobutyric amide **16**. The overall yield from **1** to **16** is 6.2%.

Compound **16** was converted into the *O*3′-(2-cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-(4,4′-dimethoxytrityl) derivative **18** (Scheme 3) by standard procedures to

Scheme 3. Syntheses of Phosphamidite **18**



provide the required intermediate for solid phase DNA synthesis.[19]

Similarly, the conversion of **8** to the deoxynucleoside **27** involved its coupling to the chlorosugar after selective removal of the N^7-Boc group with NaOMe (Scheme 4).

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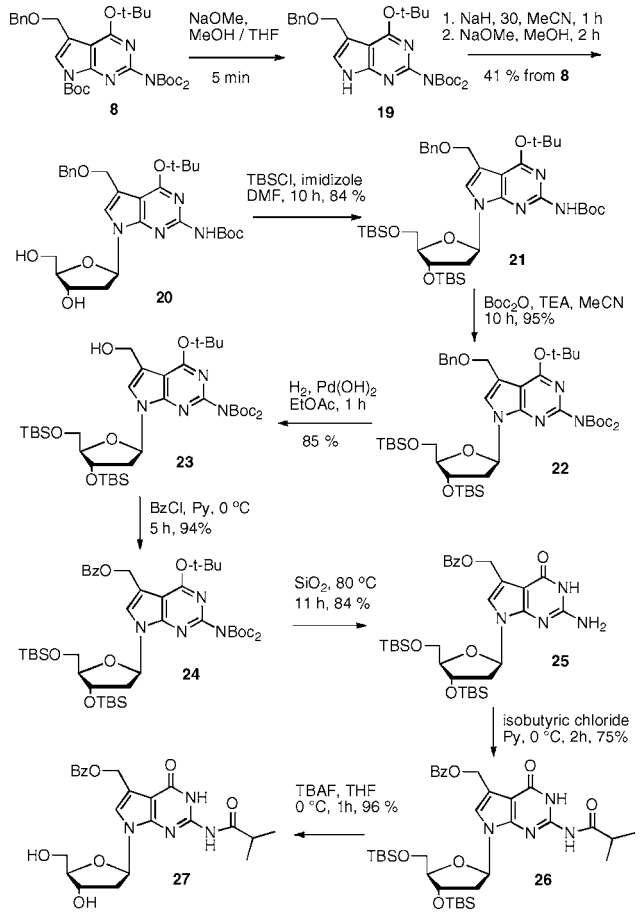
Scheme 4. Syntheses of Compound **27**



The *O*3′- and *O*5′-toluoyl protection was converted to TBDMS protection by sequential treatment with NaOMe and TBSMSCl. Reaction with Boc_2O restored the bis-N^2-Boc protection, and the O-Bn group was then reductively removed by $Pd(OH)_2$-catalyzed hydrogenation. The free primary alcohol **23** was reprotected as the benzoate **24**, and the N^2-Boc and O^4-*t*-butyl groups were removed by heating at 80 °C in vacuo on silica.[20] The N^2-position was reprotected with *i*-PrCOCl and the silyl protection removed with TBAF. The overall yield of **27** from **2** is 6.4% via 14 steps. The primary alcohol **23** can, if desired, be further reduced to give 5-methyl-2-amino-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one, a useful isosteric analogue of 7-methylguanine. The Bz group of ester **24** can also be removed in acidic conditions used for removal of *N*-Boc groups. The synthesis of compound **27** illustrated that Boc protection strategy provides an efficient route to the preparation of those 5-substituted 2-amino-7-((2*R*,4*R*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one with senstive functionalities since Boc and O^4-*t*-butyl groups can be efficiently removed under mild condition.

In conclusion, we have synthesized 5-aminomethyl-2-amino-7-((2*R*,4*R*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one (**16**) and 5-hydroxymethyl-2-amino-7-((2*R*,4*R*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one (**27**) from **1** and **2**, respectively, in 6% yield via key O^4-*t*-Bu ether intermediates **7** and **8**. These syntheses illustrate an efficient and general route to the preparation of 5-substituted 2-amino-7-((2*R*,4*R*,5*R*)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3*H*-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-ones. Time course studies showed that the tetra-Boc derivatives were converted to the O^4-*t*-Bu ethers via intramolecular transformation.

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Supporting Information Available: Experimental procedure and spectral data for synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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