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

The 5-position of 2-amino-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-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,<sup>1</sup> DNA fluorescence labeling,<sup>2</sup> DNA sequencing,<sup>3</sup> and the production of DNA-based nanoarrays.<sup>4</sup> There are also a number of naturally occurring 5-substituted 2-amino-3H-pyrrolo[2,3d]pyrimidin-4(7H)-one nucleosides, including Queuosine (I), and related glycosylated analogues,<sup>5</sup> and Archaeosine (II)<sup>6</sup>



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

found in tRNA (Figure 1). Accordingly, there have been numerous studies on routes to the synthesis of 2-amino-3Hpyrrolo[2,3-d]pyrimidin-4(7H)-one modified nucleosides.<sup>7</sup>

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

<sup>(1)</sup> Rosemeyer, H.; Ramzaeva, N.; Becker, E. M.; Feiling, E.; Seela, F. Bioconjugate Chem. 2002, 13, 1274-1285.

<sup>(2)</sup> Ju, J.; Kim, D. H.; Bi, L.; Meng, Q.; Bai, X.; Li, Z.; Li, X.; Marma, M. S.; Shi, S.; Wu, J.; Edwards, J. R.; Romu, A.; Turro, N. J. *Proc. Natl.* Acad. Sci. U.S.A. 2006, 103, 19635-19640.

<sup>(3)</sup> Kawate, T.; Allerson, C. R.; Wolfe, J. L. Org. Lett. 2005, 7, 3865-3868.

<sup>(4) (</sup>a) Seeman, N. C. J. Theor. Biol. 1982, 99, 237-247. (b) Feldkamp, U.; Niemeyer, C. M. Angew. Chem., Int. Ed. 2006, 45, 1856-1876. (c) Seeman, N. C. Nature 2003, 421, 427-431.

<sup>(5)</sup> Okada, N.; Noguchi, S.; Nishimura, S.; Ohgi, T.; Goto, T.; Crain, P. F.; McCloskey, J. A. Nucleic Acids Res. 1977, 7, 2289-2296. (b) Okada, N.; Yasuda, T.; Nishimura, S. Nucleic Acids Res. 1977, 4, 4063-4075.

<sup>(6)</sup> Gregson, M.; Crain, P. F.; Edmonds, C. G.; Gupta, R.; Hashizume, T.; Phillipson, D. W.; McCloskey, J. A. J. Biol. Chem. 1993, 268, 10076-10086.

<sup>(7) (</sup>a) Seela, F.; Winkeler, H. D. J. Org. Chem. 1983, 48, 3119-3122. (b) Ramasamy, K.; Joshi, R. V.; Robins, R. K.; Revankar, G. R. J. Chem. Soc., Perkin Trans. 1 1989, 2375–2384. (c) Ramasamy, K.; Robins, R. K.; Revankar, G. R. J. Chem. Soc., Chem. Commun. 1989, 560-562. (d) Seela, F.; Peng, X. J. Org. Chem. 2006, 71, 81-90. (e) Meng, Q.; Kim, D. H.; Bai, X.; Bi, L.; Turro, N. J.; Ju, J. J. Org. Chem. 2006, 71, 3248-3252. (f) Vorbrüggen, H.; Ruh-Polenz, C. Org. React. 2000, 55, 1-630.

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,<sup>8</sup> and the 5-position of 2-amino-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one derivatives are not sufficiently activated for direct glycosylation.<sup>9</sup> The classic solution to the preparation of 2-amino-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one nuclosides is to convert the 5-functionalized-2-amino-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-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- $\alpha$ -D-erythro-pentofuranose).<sup>10</sup> The 4-chloro nucleoside is then converted back to the keto derivative by hydrolysis.<sup>7</sup> In addition to the extra synthetic steps, the 4-chloro derivatives have very poor solubility characteristics,<sup>11</sup> which confounds their functionalization.<sup>7b,8b</sup> If the synthesis of 5-substituted 2-amino-3H-pyrrolo[2,3d]pyrimidin-4(7H)-one starts with 4-chloro-2-amino-3Hpyrrolo[2,3-d]pyrimidin-4(7H)-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,<sup>7e,12</sup> Stille,<sup>13</sup> or related cross-coupling reactions.

In an effort to prepare a series of 5-substituted 2-amino-7-((2R,4R,5R)-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-((2R,4R,5R)-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<sup>14</sup> and (2) generate stable interstrand cross-links,<sup>15</sup> we found that the existing synthetic schemes were not suitable. We report herein the synthesis of 5-aminomethyl- and 5-hydroxymethyl-2-amino-7-((2R,4R,5R)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-ones as examples of a convenient, efficient, and general route to 5-substituted 2-amino-7-((2R,4R,5R)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-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  $\omega$ -substituted aldehydes<sup>16</sup> with 2,6-diaminopyrimidin-4(3*H*)-one.<sup>17</sup> 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- $\alpha$ -D-*erythro*-pentofuranose.<sup>10</sup> 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

<sup>(8) (</sup>a) Sims, K. A.; Woodland, A. M. *Pharmacotherapy* **2006**, *12*, 1745–1757. (b) Field, A. K.; Tuomari, A. V.; McGeever-Rubin, B.; Terry, B. J.; Mazina, K. E.; Haffey, M. L.; Hagen, M. E.; Clark, J. M.; Braitman, A.; Slusarchyk, W. A. *Antiviral Res.* **1990**, *13*, 41–52. (c) Brigden, D.; Fiddian, P.; Rosling, A. E.; Ravenscroft, T. *Antiviral Res.* **1981**, *1*, 203–212. (d) Vere Hodge, R. A.; Sutton, D.; Boyd, M. R.; Harnden, M. R.; Jarvest, R. L. *Antimicrob. Agents Chemother.* **1989**, *33*, 1765–7173. (e) Lee, J.; Chuang, T.-H.; Redecke, V.; She, L.; Pitha, P. M.; Carson, D. A.; Raz, E.; Cottam, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6646–6651.

<sup>(9) (</sup>a) Tolman, R. L.; Tolman, G. L.; Robins, R. K.; Townsend, L. B. *J. Heterocycl. Chem.* **1970**, *7*, 799–806. (b) Seela, F.; Westermann, B.; Bindig, U. J. Chem. Soc., Perkin Trans. 1 **1988**, 697–702.

<sup>(10)</sup> Ramasamy, K.; Imamura, N.; Robins, R. K.; Revankar, G. R. *Tetrahedron Lett.* **1987**, *43*, 5107–5100.

<sup>(11)</sup> Dey, S.; Garner, P. J. Org. Chem. 2000, 65, 7697-7699.

<sup>(12) (</sup>a) Jager, S.; Rasched, G.; Kornreich-Leshem, H.; Engeser, M.; Thum, O.; Famulok, M. J. Am. Chem. Soc. **2005**, 127, 15071–15082. (b) Seela, F.; Shaikh, K. I. Tetrahedron **2005**, 61, 2675–2681.

<sup>(13)</sup> Angelov, T.; Guainazzi, A.; Scharer, O. D. Org. Lett. 2009, 11, 661–664.

<sup>(14) (</sup>a) Manning, G. S. Q. Rev. Biophys. 1978, 2, 179–246. (b) Record,
M. T.; Anderson, C. F.; Lohman, T. M. Q. Rev. Biophys. 1978, 2, 103–179. (c) Honig, B.; Nicholls, A. Science 1995, 268, 1144–1149. (d) Gold,
B. Biopolymers 2002, 65, 173–179. (e) Gold, B.; Marky, L. M.; Stone,
M. P.; Williams, L. D. Chem. Res. Toxicol. 2006, 19, 1402–1414.

<sup>(15) (</sup>a) Wilds, C. J.; Noronha, A. M.; Robidoux, S.; Miller, P. S. *Nucleosides Nucleotides Nucleic Acids* **2005**, *4*, 965–969. (b) Räschle, M.; Knipsheer, P.; Enoiu, M.; Angelov, T.; Sun, J.; Griffith, J. D.; Ellenberger, T. E.; Schärer, O. D.; Walter, J. C. *Cell* **2008**, *134*, 969–980.

<sup>(16)</sup> De Luca, L.; Giacomelli, G.; Porcheddu, A. Org. Lett. 2001, 3, 3041–3043.

<sup>(17) (</sup>a) Klepper, F.; Polborn, K.; Carell, T. *Helv. Chim. Acta* **2005**, *88*, 2610–2616. (b) Barnett, C. J.; Grubb, L. M. *Tetrahedron Lett.* **2000**, *41*, 9741–9745.





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<sub>2</sub>O, 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<sub>2</sub>O.<sup>10</sup> 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-3,5di-*O-p*-toluoyl- $\alpha$ -*D-erythro*-pentofuranose (**30**) to give the desired  $\beta$ -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)<sub>2</sub> to give trifluoroacetamide **15**.<sup>18</sup> 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 O3'-(2-cyanoethoxy)(diisopropylamino)phosphino]-5'-O-(4,4'-dimethoxytrityl) derivative **18** (Scheme 3) by standard procedures to





provide the required intermediate for solid phase DNA synthesis.<sup>19</sup>

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).

<sup>(18)</sup> Xu, Y.-C.; Lebeau, E.; Walker, C. Tetrahedron Lett. 1994, 35, 6207–6210.

<sup>(19) (</sup>a) BeaucageS. L. In *Protocols for Oligonucleotides and Analogs*; Agrawal, S., Ed.; Humana Press: Totowa, NJ, 1993; pp 33–62. (b) Bleasdale, B.; Ellwood, S. B.; Golding, B. T. *J. Chem. Soc., Perkin Trans. 1* **1990**, 803–805.

Scheme 4. Syntheses of Compound 27



The O3'- and O5'-toluoyl protection was converted to TBDMS protection by sequential treatment with NaOMe and TBSMSCl. Reaction with Boc<sub>2</sub>O restored the bis- $N^2$ -Boc protection, and the O-Bn group was then reductively removed by Pd(OH)<sub>2</sub>-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.<sup>20</sup> 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-2amino-7-((2R,4R,5R)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**16**) and 5-hydroxymethyl-2-amino-7-((2R,4R,5R)-tetrahydro-4hydroxy-5-(hydroxymethyl)furan-2-yl)-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-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-((2R,4R,5R)-tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-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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<sup>(20)</sup> Apelqvist, T.; Wensbo, D. Tetrahedron Lett. 1996, 37, 1471-1472.