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5,6-Diaminocytidine, a Versatile Synthon for Pyrimidine-Based Bicyclic Nucleosides†

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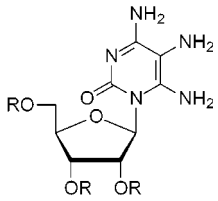
ABSTRACT



This communication describes a convenient, facile, and high-yield synthesis of 3-(β-D-ribofuranosyl)isoguanine and its 8-methyl derivative, as well as nucleoside analogues of pteridines, from a common precursor, 5,6-diaminocytidine. 5,6-Diamino-2′,3′,5′-tri-*O*-benzoylcytidine was synthesized from 4,6-diamino-2-oxopyrimidine in three steps.

Incorporation of 9-(β-D-ribofuranosyl)isoguanine into oligonucleotides and its ability to form parallel duplex,[1] tetraplex[1b−e,2] and quintet[3] structures have been well recognized. It is also known that 9-(β-D-ribofuranosyl)isoguanine by itself and in combination with berberin is active against various tumor cell lines.[4] It would be interesting to know the effect of position of glycosylation of isoguanine on its structural properties and antitumor activity. As a part of an ongoing project leading to the synthesis of anti-HIV polynucleotides, we became interested in 3-(β-D-ribofuranosyl)isoguanine (**6a**) and its analogues. A synthesis of **6a** has been reported by Schmidt and Townsend.[5] However, the effect of **6a** on the structure of oligonucleotides and its biological relevance are lacking, perhaps because of the synthetic difficulties in obtaining substantial quantities of the compound. The present work discusses a convenient, facile, and high-yield synthesis of **6a** and its 8-substituted analogues from a pyrimidine nucleoside, 5,6-diaminocytidine (**4**).

Also described is a convenient route to the synthesis of 4-amino-2-oxo-3-(β-D-ribofuranosyl)-2,3-dihydropteridine and its 6,7-dimethyl derivative from **4**. Synthesis of analogues of pteridine nucleosides starting from pteridines are well-known in the literature.[6] Recently, the fluorescent properties of pteridine nucleosides have stimulated interest in their use as fluorophores in nucleic acid interaction studies.[7] Glyco-

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(1) (a) Seela, F.; He, Y.; Wei, C. *Tetrahedron* **1999**, *55*, 9481−9500. (b) Seela, F.; Wei, C.; Melenewski, A. Leonard, P. *Nucleic Acids Symp. Ser.* **1997**, *37*, 149−150. (c) Yang, X.-L.; Sugiyama, H.; Ikeda, S.; Saito, I.; Wang, A. H.-J. *Biophys. J.* **1998**, *75*(3), 1163−1171. (d) Seela, F.; Wei, C.; Melenewski, A.; Feiling, E. *Nucleosides Nucleotides* **1998**, *17*, 2045−2052. (e) Sugiyama, H.; Ikeda, S.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 9994−9995. (f) Roberts, C.; Bandaru, R.; Switzer, C. *J. Am. Chem. Soc.* **1997**, *119*, 4640−4649. (g) Seela, F.; Wei, C. *Helv. Chim. Acta* **1997**, *80*, 73−85.

(2) (a) Seela, F.; Wei, C.; Melenewski, A. *Origins Life Evol. Biosphere* **1997**, *27*, 597−608. (b) Davis, J. T.; Tirumala, S.; Jenssen, J. R.; Radler, E.; Fabris, D. *J. Org. Chem.* **1995**, *60*, 4167−76.

(3) Chaput, J. C.; Switzer, C. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 10614−10619.

(4) (a) Liebich, H. M.; Lehmann, R.; Di Stefano, C.; Haring, H. U.; Kim, J. H.; Kim, K. R. *J. Chromatogr., A* **1998**, *795*, 388−393. (b) Kim, J. H.; Lee, S. J.; Han, Y. B.; Moon, J. J.; Kim, J. B. *Arch. Pharmacal Res.* **1994**, *17*, 115−18.

(5) Schmidt, C. L.; Townsend, L. B. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1257−1260.

(6) (a) Pfleiderer, F. *Chemistry of Nucleosides and Nucleotides*; Townsend, L. B., Ed.; Plenum: New York, 1988; Vol. 3 (rev), pp 214−255 and references cited therein. (b) Harzer, von K.; Pfleiderer, W. *Helv. Chim. Acta* **1973**, *56*, 1225−1234.

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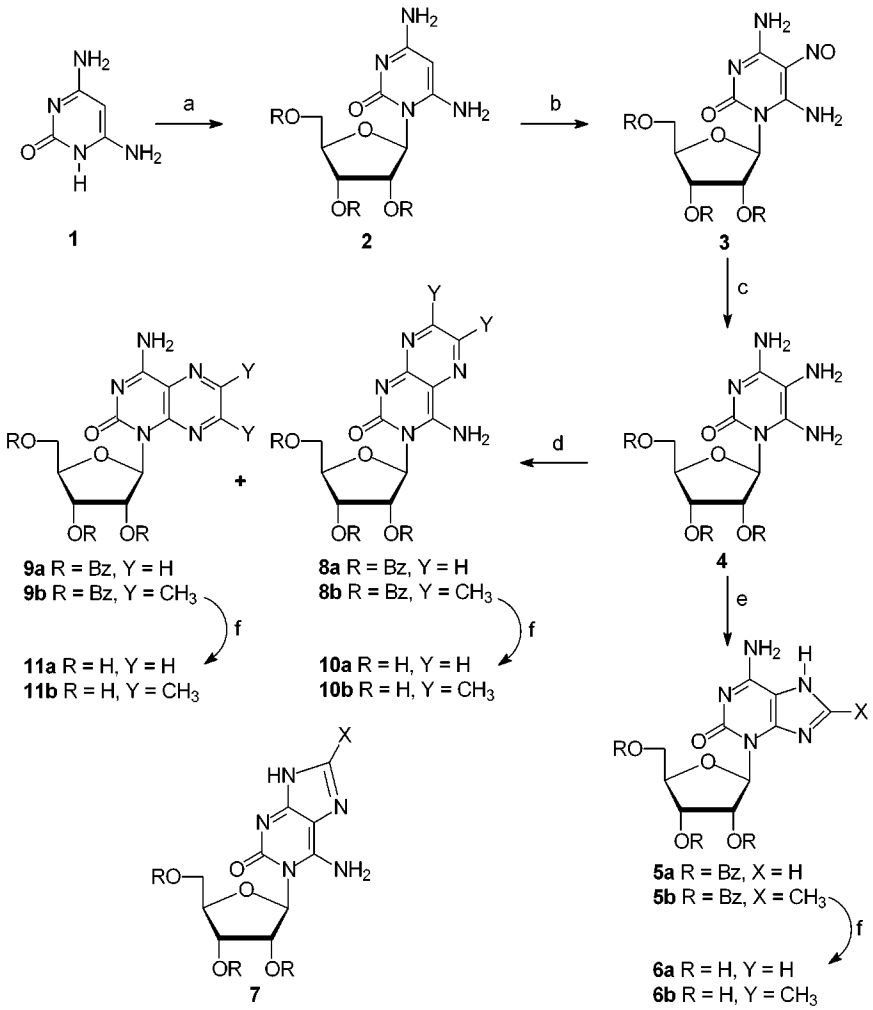
Scheme 1[a]



[a] (a) i. HMDS, TMS−Cl (cat.) reflux, 6 h; ii. 1-*O*-acetyl-2.3.5-(tri-*O*-benzoyl)-*β*-D-ribofuranose, Sn(IV)Cl, rt, 24 h (yield 88%). (b) $NaNO_2$ (2 equiv), HOAc, H_2O (10%), < 10 °C, 4 h (yield > 95%). (c) $Na_2S_2O_4$ (2 equiv), DMF, H_2O (10%), 50 °C, 4 h (yield > 95%). (d) Y-CO-CO-Y, DMF (yield 80−90%, overall). (e) X-CO-N$(CH_3)_2$, $POCl_3$ (1.2 equiv) (yield 80−90%). (f) NaOMe−MeOH or NaOH−H_2O/MeOH.

sylation of silylated 4-amino-2-oxo-1,2-dihydropteridine gave predominantly the N1-glycosylated pteridine nucleoside; it is difficult to synthesize the corresponding N3 nucleoside analogue with the existing methodology.[6b] The present approach depicts a convenient way of synthesizing N3-glycosylated 4-amino-2-oxopteridines.

5,6-Diamino-2′,3′,5′-tri-*O*-benzoylcytidine (**4**) was synthesized from 4,6-diamino-2-oxopyrimidine (**1**) as shown in Scheme 1. Trimethylsilylated **1** was allowed to react with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose in 1,2-dichloroethane under Vorbrüggen coupling conditions[8] to yield 6-amino-2′,3′,5′-tri-*O*-benzoylcytidine (**2**) in 90% yield. The synthesis of 6-aminocytidine[9] from 5-bromocytidine and 6-amino-2′-*O*-methylcytidine[10] from **1** and the corresponding 2-*O*-methyl sugar have been reported. Nitrosation of **2** in acetic acid below 10 °C for 4 h gave the purple 5-nitroso derivative **3** in quantitative yield.[11,12] Quantitative reduction of nitroso to amine was achieved by the treatment of **3** with sodium hydrosulfite and water in DMF at 50 °C for 4 h to yield **4** as a yellowish green solid.

(7) (a) Hawkins, M. PCT Int. Appl. WO 9826093 A2. 18 Jun 1998. (b) Hawkins, M. E.; Pfleiderer, W.; Balis, F. M.; Porter, D.; Knutson, J. R. *Anal. Biochem.* **1997**, *244*, 86−95.

(8) Niedballa, U.; Vorbruggen, H. *J. Org. Chem.* **1974**, *39*, 3654−3660.

(9) Goldman, D.; Kalman, T. I. *Nucleosides Nucleotides* **1983**, *2*, 175−187.

(10) Pudlo, J. S.; Wadwani, S.; Milligan, J. F.; Matteucci, M. D. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1025−1028.

(11) Rogers, G. T.; Ulbricht, T. L. V. *J. Chem. Soc., C* **1971**, 2364−2366.

(12) (a) The reaction proceeded more slowly when there was methanol in the reaction mixture. It is recommended that the reaction be performed in an acetic acid−water solvent system at a temperature less than 10 °C to obtain the nitroso derivative in quantitative yield. The analytically pure product gave unusually complex proton and carbon NMR spectra, with many signals split into two peaks. This may arise from the presence of tautomers such as those described by Pfleiderer[12b] involving the imino-oxime at the 6- and 5-positions, respectively. (b) Pfleiderer, W.; Kempter, F. E. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 259−260.

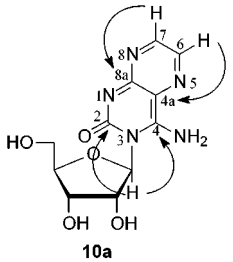
Treatment of **4** with 1 equiv[13] of $POCl_3$ in DMF (Vilsmeier−Haack reagent) at ambient temperature gave predominantly 6-amino-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-purine-2-one (**5a**).[14] One might expect a mixture of **5a** and its isomer **7** from the reaction, but the major product isolated was **5a** in 85% yield. However, a small amount of a slightly faster moving component was observed on TLC.[15] This could be the isomer **7**, but the very low yield made isolation and characterization impractical. Debenzoylation of **5a** in methanolic sodium methoxide gave the free nucleoside **6a**. The structure of compound **6a** has been established by HMQC and gradient HMBC (GHMBC) NMR experiments and by the identity of its UV spectra with those reported in the literature.[5] In the reported procedure, the authors took advantage of the steric directing effect of iodide present at position 8 of 8-iodoisoguanine to selectively glycosylate at N3 of the base. This was followed by catalytic hydrogenolysis of the iodide to obtain the target compound.[5]

Reaction of **4** with 1 equiv of $POCl_3$ and DMAc gave the corresponding 8-methyl derivative **5b**. Synthesis of 8-substituted 3-(β-D-ribofuranosyl)isoguanine, while possible, would be challenging using the earlier method.[5] The present methodology thus provides an excellent route to 3-(β-D-ribofuranosyl)isoguanine and its 8-methyl derivative in high yield (and fewer steps than in the previous procedure) by application of Vilsmeier−Haack reagents. The position of the sugar was defined by its position on the starting pyrimidine nucleoside.

Reaction of **4** with 1 equiv of glyoxal in DMF at ambient temperature for 1 h gave a 3:2 mixture of 4-amino-2-oxo-1-(2,3,5-tri-*O*-benzoyl-β-D-ribufuranosyl)-1,2-dihydropteridine[6b] (**9a**) and 4-amino-2-oxo-3-(2,3,5-tri-*O*-benzoyl-β-D-ribufuranosyl)-2,3-dihydropteridine (**8a**), respectively. The slower eluting compound on a silica gel column was debenzoylated and characterized by HMQC and GHMBC NMR experiments as **11a.** Its UV spectrum matched that of the reported compound.[6b] The faster eluting compound was debenzoylated,[16] and its structure was established as **10a** by HMQC and GHMBC NMR experiments (vide infra). Reaction of **4** with 1 equiv of butane-2,3-dione in DMF at ambient temperature gave the 3-(β-D-ribofuranosyl)pteridine derivative **8b** as the major product (80% yield, faster eluting fraction on silica gel column) and 1-(β-D-ribofuranosyl)-pteridine[17] derivative **9b** as the minor product (less than 10%). The predominant formation of **8b** rather than **9b** is probably due to steric constraints in the transition state between the methyl group and the sugar in the case of **9b**. Preliminary studies on the fluorescent properties of the N3-glycosylated pteridines revealed that the fluorescence intensity of these analogues is greater than that of the corresponding N1-glycosylated derivatives.[7]

In the ^1H NMR spectra in $CDCl_3$, the H1′ of **8a** and **8b** appeared as broad singlets at δ 6.98 and 6.94, and one of the amino protons appeared as a sharp peak at δ 9.70 and 9.46, respectively. The appearance of the sharp N*H* peak in $CDCl_3$ at around 9.5 ppm probably results from intramolecular H-bonding between the N*H* (of **8a**/**8b**) and the carbonyl of the 5′-*O*-benzoate. On the other hand, the amino protons were not detected in $CDCl_3$ for **9a**, a more typical finding when specific hydrogen bonding is not present. These observations are consistent with the assignment of **8a** and **8b** as 3-ribosylated pteridines, but additional proof was sought using HMQC and GHMBC NMR spectroscopy.

Debenzoylation of **8a** afforded nucleoside **10a**. An HMQC experiment in DMSO-d_6 readily established all the expected 1-bond correlations, of which those of interest were H1′ to C1′ (δ 6.44 to 88.9) and the two aromatic protons (δ 8.42 to 138.6 and 8.56 to 147.6). GHMBC NMR experiment in DMSO-d_6 unequivocally established the structure of **10a** by means of the following connectivities: (1) two three-bond correlations involving H1′(δ 6.44) to C2(δ 156.7) and C4(δ 150.3); (2) a one-bond correlation between H6(δ 8.42) and C6(δ 138.6); a two-bond correlation between H6 and C7(δ 147.6) and a three-bond correlation between H6 and C4a(δ 125.8); and (3) a one-bond correlation between H7(δ 8.56) and C7(δ 147.), a two-bond correlation between H7 and C6(δ 138.6), and a three-bond correlation between H7 and C8a (δ 147.1). The critical three-bond couplings are illustrated by arrows in the structure of **10a** (Figure 1).



10a

Figure 1. Important connectivities observed in the GHMBC NMR spectra.

There was no cross-peak seen between H6 and/or H7 with C4 (which gave a cross-peak with H1′). Also, no cross-peak was seen between H7 and C4a or between H6 and C8a. At the same time, the strong cross-peak between H6 and C4a and between H7 and C8a along with the cross-peak of H1′ with C2 and C4 clearly support the structure **10a**.

In conclusion, 5,6-diaminocytidine can be used as a general synthon for the synthesis of a variety of 8-substituted 3-(β-D-ribofuranosyl)isoguanines and N3-glycosylated 4-amino-2-oxopteridines. The incorporation of 3-(β-D-ribofuranosyl)-

(13) Addition of excess $POCl_3$ led to the formation of multiple products. It is recommended that only enough $POCl_3$ be added to the reaction mixture to force the reactant **4** to react completely to avoid a very difficult chromatographic separation.

(14) Anderson, G. L.; Rizkalla, B. H.; Broom, A. D. *J. Org. Chem.* **1974**, *39*, 937−939.

(15) The reaction was performed at 0 and 50 °C, but no noticeable change was observed.

(16) The debenzoylation of **8a** in methanolic sodium methoxide was very slow and the 5′-mono-*O*-benzoate of **8a** was isolated as a major product. Prolonged treatment of **8a** with sodium methoxide in methanol led to partial deglycosylation. The debenzoylation of **9a** was complete within 3 h. Details of the successful debenzoylation procedure are provided in the Supporting Information.

(17) Angelika, R.; Pfleiderer, W. *Collect. Czech. Chem. Commun.* **1996**, *61*, S230−S233.

isoguanine, which has H-bond acceptor/donor switches at N1 and N7, into oligonucleotides will be interesting for comparison with the corresponding 9-(β-D-ribofuranosyl)-isoguanine.[1e,18] Further studies on these aspects and the evaluation of the anticancer properties of all the isoguanine and pteridine derivatives are underway. The fluorescent properties of the pteridine derivatives will also be evaluated in detail.

(18) (a) Robinson, H.; Gao, Y.-G.; Bauer, C.; Roberts, C.; Switzer, C.; Wang, A. H.-J. *Biochemistry* **1998**, *37*, 10897−10905. (b) Liaw, Y− C.; Chern, J.-W.; Lin, G.-S.; Wang, A. H.-J. *FEBS Lett.* **1992**, *297*, 4−8.

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Supporting Information Available: Experimental detail and characterization data for all compounds are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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