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Synthesis of a Complete Janus-type Guanosine–Cytosine Base and Its 2′-Deoxyribonucleoside

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Novel Janus-type guanosine–cytosine pyrimido[4,5-*d*]pyrimidine and its 2′-deoxyribonucleoside have been synthesized in a viable route employing the Vorbruggen method.

Nucleoside analogs have played an important role in the treatment of viral disease for many years.[1,2] The mechanisms of these compounds to inhibit viruses are mainly through incorporating and altering natural DNA/RNA macromolecules, inhibiting various enzymes which participate in the synthesis of nucleic acid and terminating the synthesis of the genome.[3] Apart from these, Crotty and his colleagues[4] have demonstrated that ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) has an unique antiviral mechanism: its antiviral activity can be fully attributed to lethal mutagenesis[5] due to dual base pairing which causes transition or transversion mutations (A to G and C to U). However, the efficiency of incorporating into a viral genome is relatively low for ribavirin,[6] due to its pseudo base structure being significant different from nature nucleoside. Inspired by this consideration, we designed a novel Janus guanine–cytosine (J-GC) nucleoside. The base moiety of this nucleoside has both a Watson–Crick H-bond donor–donor–acceptor (DDA) pattern of guanine and a Watson–Crick H-bond acceptor–acceptor–donor (AAD) pattern of cytosine, which will base-pair with either guanosine or cytosine when it adopts either syn or anti conformation upon the rotation of the glycosyl bond. Our laboratory has synthesized the Janus guanine–cytosine ribonucleoside by a linear method, and preliminary in vivo testing shows that it has anti HBV potential.[7] On this basis, we would like to expand this J-GC systems into deoxyribonucleoside to see if it can also be incorporated into viral DNA genome and to investigate their base-pair properties in synthetic DNA fragments (Figure 1). For this purpose, compound **1** is needed. Surprisingly, the synthesis of the free Janus GC pyrimido-[4,5-*d*]pyrimidine base moiety and its corresponding 2′-deoxyribonucleoside have not been reported before. Therefore, we will describe synthesis of them.

There are two major parts for the synthesis of J-GC 2′-deoxyribonucleoside. First, we successfully synthesized the free guanosine–cytosine base (4,7-diaminopyrimido[4,5-*d*]pyrimidine-2,5(1*H*,6*H*)-dione (**2**)), then we adopted direct glycosylation to obtain the target compound, 4,7-diamino-1-(2-deoxy-β-D-*erythro*-pentofuranoyl)pyrimido[4,5-*d*]pyrimidine-2,5(1*H*,6*H*)-dione (**1**) using the Vorbruggen method by employing the silylated Janus-type base moieties as sugar acceptor and 1-α-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-furanose as sugar donor in the presence of Friedel–Crafts catalysts.[8,9]

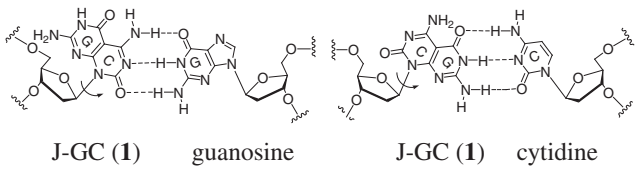
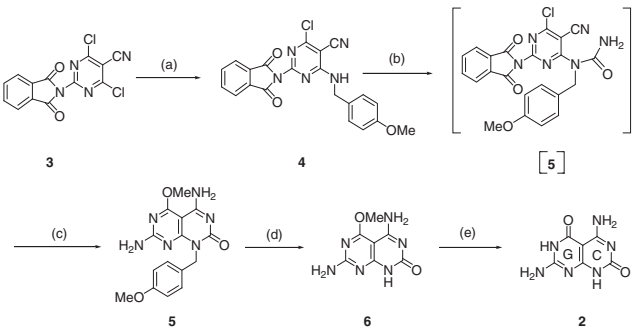


Figure 1. The designed J-GC deoxyribonucleoside is supposed to pair with either guanosine or cytidine through the specific pattern of hydrogen-bonding patterns.

To prepare the free Janus-type guanosine–cytosine nucleobase **2**, we adopted a procedure starting from commercially available 2-amino-4,6-dihydroxypyrimidine (Scheme 1). The synthesis of 2-*N*-phthaloyl-5-cyano-4,6-dichloropyrimidine (**3**) has been reported by our group.[7] As compound **3** was difficult to purify by flash chromatography, we attempted to purify it by recrystallizing from organic solvent. After a series of experiments, ethyl acetate was finally found to be the best choice.

In order to construct the second pyrimidine ring, a suitable protected amine needs to be introduced into the first pyrimidine counterpart. At the beginning, we tried to use benzylamine, but actually the removal of the benzyl group proved to be problematic and unsuccessful, such as treatment with Lewis acids or hydrogenation. To overcome this problem, *p*-methoxybenzylamine (PMB) was selected as a solution due to its removability under mild conditions. When compound **3** was treated with PMB (2 M) in THF at 50 °C for about 1 h, compound **4** was obtained with a yield of 76% after flash column chromatography. Initially, we chose *N*-(chlorocarbonyl) isocyanate (CCI) as a reagent to obtain the the urea intermediate [**5**] for the subsequent cyclization which offers compound **5** as the fully constructed pyrimido[4,5-*d*]pyrimidine. However, the total yield was not ideal (20%) and the CCI was neither stable nor



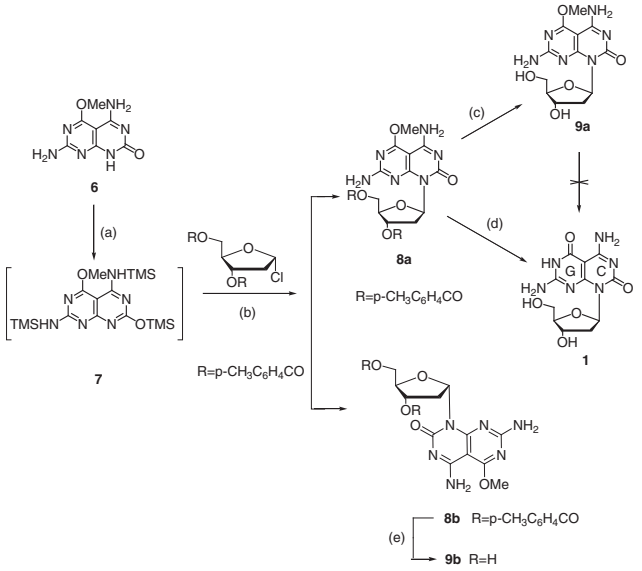
Scheme 1. Synthesis of Janus-type guanosine–cytosine base: (a) *p*-methoxybenzylamine (PMB), THF, 50 °C, 1 h, 76%; (b) *i*-Pr_2EtN, trichloroacetyl isocyanate, dichloromethane (DCM), rt, 1 h; (c) 0.5 M NaOMe, 75 °C, 2 h, 65%; (d) CH_3CN–H_2O (3:1, v/v), CAN, rt, 16 h, 73%; (e) CH_3CN, NaI, TMSCl, rt, 16 h, 94%.

economical. For this reason, we tried to find a better route to obtain **5**. After several failed attempts, we finally found trichloroacetyl isocyanate to be the best choice. In this case, compound **4** was treated with trichloroacetyl isocyanate and *i*-Pr_2EtN as base catalyst in dry DCM for about 1 h at rt to give the urea intermediate [**5**], which was used directly in the cyclization reaction (refluxed in 0.5 M NaOMe for about 2 h) to lead to the formation of the key compound pyrimido[4,5-*d*]-pyrimidine **5**. During the cyclization the phthaloyl protecting group of the 2-amino group was removed and the remaining chloride at 4-position of the first pyrimidine ring was replaced by a methoxy group simultaneously. Compared to CCI, preparation of **5** with trichloroacetyl isocyanate was more economical and produced a higher yield (total yield of 65% from **4**).

Next, the PMB group was removed under mild conditions by treating compound **5** with $AlCl_3$ in anisole[10,11] or ceric ammonium nitrate (CAN) in 3:1 CH_3CN and H_2O.[12] However, the $AlCl_3$ was difficult to remove completely when compound **6** was purified, and it may affect the glycosylation in the later stages because $AlCl_3$ is a strong Lewis acid. Therefore, we treated **5** with CAN (4 equiv) in 3:1 CH_3CN and H_2O, and the product precipitated from the reaction solution. Pure compound **6** was obtained by washing with MeOH thoroughly (73%). The removal of the methoxy group was as follows. Compound **6** was treated with NaI and trimethylchlorosilane (TMSCl) in acetonitrile, the mixture was stirred overnight at rt, and the white precipitate was collected and washed with methanol to get compound **2** (94%).

Synthesis of the J-GC 2′-deoxyribonucleoside (**1**) was achieved by the route shown in Scheme 2. Compound **6** was reacted with hexamethyldisilazane (HMDS) to provide the intermediate **7** which was used directly in the later glycosylation reaction without any purification due to its sensitivity to moisture. Compared with the pyrimidine system, the silylation step was rather slow due to the additional exocyclic amino and oxy groups. Therefore, we attempted to find a catalyst to overcome this problem. After several experiments (ammonium sulfate,[13,14] trimethylchlorosilane,[8] and pyridine + TMSCl[15]), HMDS and TMSCl was finally found to be a suitable combination for this reaction. As mentioned before, attention should be paid not to introduce moisture when evaporating HMDS due to the sensitivity of compound **7** to moisture.

The silylated base **7** was used as sugar acceptor and 1-α-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-furanose was used as sugar donor.[16] In the case of riboside, because of the steric hindrance of the 2′-acyloxy group, only β-anomer nucleoside was formed. But in the case of deoxyribonucleoside, without the neighboring group participation, usually, 1:1 mixtures of α:β anomers will be formed. It has been reported that CuI can facilitate the β-configuration selectivity with an electronic push–pull processs in $CHCl_3$ for the normal purine and pyrimidine system.[17] However, this method did not give satisfactory results in this pyrimido-[4,5-*d*]pyrimidine system. We tried the reaction of silylated base **7** treating with 1-α-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-furanose and with CuI as catalyst, however, the yield was low. Therefore, we attempted to obtain **8a** by reaction of **7** with 1-α-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-furanose by employing either $SnCl_4$ or TMSOTf as catalyst. Finally, we found trimethylsilyl trifluoromethanesulfonate (TMSOTf) was superior to $SnCl_4$ as catalyst for the synthesis of deoxyribonucleoside **8a**.



Scheme 2. Synthesis of J-GC 2′-deoxyribonucleoside: (a) HMDS, TMSCl, 140 °C; (b) TMSOTf, CH_3CN–1,2-dichloroethane (1:1, v/v), rt, 1 h; (c) 0.5 M NaOMe, 70 °C, 92%; (d) 2 M NaOH, 1,4-dioxane, 70 °C, 6 h, 90%; (e) 0.5 M NaOMe, 70 °C, 90%.

Silylated **7** was treated with 1-α-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-furanose and TMSOTf in anhydrous CH_3CN and 1,2-dichloroethane. Subsequently, the reaction was stirred at room temperature for about 1 h, saturated sodium bicarbonate was added to quench the reaction at 0 °C, then extracted with DCM and washed with saturated saline, H_2O, dried with anhydrous Na_2SO_4, and the compound **8a** (β, 37%) and **8b** (α, 11%) was obtained by flash chromatography. Compound **8a** and **8b** was treated with 0.5 M NaOMe at 70 °C until the solution become clear, the reaction was neutralized with acetic acid, then the precipitate was collected and washed with MeOH to get compound **9a** (92%) and **9b** (90%), the α and β configuration was clearly established by their NMR spectra. 1′-H of **9a** produced a strong NOE at 2′-H_a and 2′-H_b while 1′-H of **9b** showed an NOE only at 2′-H_a, simultaneously, 1′-H of **9b** show an NOE at 3′-H. It is worthwhile to mention that initially we tried the same method as in the case of compound **6** to replace the methoxy group of compound **9a** by treating it with NaI and TMSCl in CH_3CN. Unfortunately, this reaction did not proceed, instead of compound **1** we finally obtained compound **2**. Obviously, the glycosyl bond was unstable under such conditions. Consequently, we found a way to deprotect the Tol group and to replace the methoxyl group as follows. **8a** was treated with 2 M NaOH and 1,4-dioxane at 70 °C for about 6 h, then neutralized with 2 M HCl, the white precipitate was collected and washed with MeOH to give pure compound **1** (90%).

In conclusion, we have developed a viable route to obtain the complete Janus-type guanosine–cytosine base and its 2′-deoxyribonucleoside. Our next step is to investigate their biological activity, base-pairing properties, and enzymatic reactions in the context of DNA. These experiments are under investigation currently and will be published in the near future.

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References

1 T. Cihlar, A. S. Ray, *Antiviral Res.* **2010**, *85*, 39.
2 E. D. Clercq, *Med. Res. Rev.* **2009**, *29*, 571.
3 C. M. Galmarini, J. R. Mackey, C. Dumontet, *Lancet Oncol.* **2002**, *3*, 415.
4 S. Crotty, C. Cameron, R. Andino, *J. Mol. Med.* **2002**, *80*, 86.
5 J. D. Graci, C. E. Cameron, *Future Virol.* **2008**, *3*, 553.
6 J. D. Graci, C. E. Cameron, *Rev. Med. Virol.* **2006**, *16*, 37.
7 H.-Z. Yang, M.-Y. Pan, D.-W. Jiang, Y. He, *Org. Biomol. Chem.* **2011**, *9*, 1516.
8 U. Niedballa, H. Vorbrueggen, *J. Org. Chem.* **1974**, *39*, 3654.
9 H. Vorbrüggen, K. Krolikiewicz, B. Bennua, *Chem. Ber.* **1981**, *114*, 1234.
10 T. Tsuji, T. Kataoka, M. Yoshioka, Y. Sendo, Y. Nishitani, S. Hirai, T. Maeda, W. Nagata, *Tetrahedron Lett.* **1979**, *20*, 2793.
11 T. Akiyama, M. Kumegawa, Y. Takesue, H. Nishimoto, S. Ozaki, *Chem. Lett.* **1990**, 339.
12 A. L. LaFrate, J. A. Katzenellenbogen, *J. Org. Chem.* **2007**, *72*, 8573.
13 M. Okabe, R.-C. Sun, S. Y. K. Tam, L. J. Todaro, D. L. Coffen, *J. Org. Chem.* **1988**, *53*, 4780.
14 J.-Q. Wang, Q.-H. Zheng, X. Fei, X. Liu, T. A. Gardner, C. Kao, S. P. Raikwar, B. E. Glick-Wilson, M. L. Sullivan, B. H. Mock, G. D. Hutchins, *Synth. Commun.* **2004**, *34*, 917.
15 U. Niedballa, H. Vorbrueggen, *J. Org. Chem.* **1974**, *39*, 3672.
16 V. Rolland, M. Kotera, J. Lhomme, *Synth. Commun.* **1997**, *27*, 3505.
17 J. N. Freskos, *Nucleosides, Nucleotides Nucleic Acids* **1989**, *8*, 549.

