Synthesis of Gemcitabine Analogues as Potential Anti-tumor Agents

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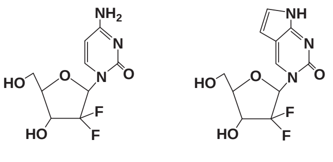
Gemcitabine analogue was designed to keep the anti-tumor activities but not to be the substrate of deoxycytidine deaminase. By coupling the 2-deoxy-2,2-difluoro sugar derivative with a bicyclic base, the gemcitabine analogue was synthesized, and its configuration was confirmed by 2D NMR studies.

The deoxycytidine analogue gemcitabine (2′-deoxy-2′,2′-difluorocytidine) is an anti-tumor drug marketed in many countries.[1] Deoxycytidine kinase and deoxycytidine deaminase are two key enzymes in the activation and inactivation of deoxycytidine derivatives, consequently, in their antiviral and anti-cancer activities. Once inside the cell, gemcitabine was phosphorylated by deoxycytidine kinase to the monophosphate, which was further phosphorylated to the active form triphosphate which could be incorporated into DNA and inhibit DNA synthesis causing DNA chain termination and DNA stand breaks.[2] Gemcitabine has shown activity against a wide spectrum of human solid tumors including non-small cell lung, pancreatic, colon, breast, bladder, overian, head and neck, cervical, and hepato cellular cancers.[3]

Unfortunately, gemcitabine is also a good substrate of deoxycytidine deaminase and it can be deaminated to an inactive metabolite. Therefore, the tumor tissues with high level of this deoxycytidine deaminase enzyme, such as colorectal cancer and small cell lung cancer were not the targets for gemcitabine therapy.[4]

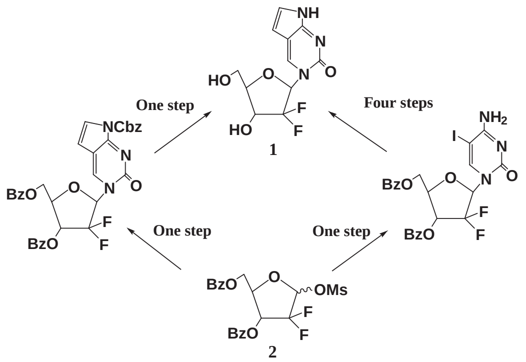
Based on this consideration, it is necessary to design and synthesize the gemcitabine analogues, to keep the anti-tumor activity but not to be the substrates of deoxycytidine deaminase. Compound **1** was designed as our synthetic target to meet this requirement (Figure 1).

Compound **1** can be synthesized through two different synthetic strategies (Scheme 1). From the coupling reaction of 3,5-di-*O*-benzoyl-2-deoxy-2,2-difluoro-*O*-(methylsulfonyl)-D-*erythro*-pentofuranose (**2**)[5] with 5-iodocytosine, we could get the 5-iodocytidine derivative. By using the procedure,[6] the 5-iodocytidine derivative can be transformed to compound **1** in four steps. However, the presence of the electron-withdrawing difluoro substituents at *C*-2 of compound **2** should make dissociation of mesylate ion from the sugar more difficult, and the yield of coupling reaction would be very lower.[7] Therefore, coupling the compound **2** with the bicyclic base **4** directly may be a better strategy for the synthesis of compound **1**.



Gemcitabine **1**

Figure 1.



Scheme 1. Synthetic strategy of compound **1**.

The synthesis of 2*H*-pyrrolo[2,3-*d*]pyrimidin-2-one base **3** was synthesized by using the procedure (Figure 2).[8]

In Scheme 2, the bicyclic base **3** first was silylated with BSA in the mixture of acetonitrile and 1,2-dichloroethane (2:1) at 70 °C for 5 h, and then the mesylate derivative **2** was added, followed by the addition of 3 equiv. of TMSOTf. This reaction mixture was stirred at 80 °C for 15 h. TLC indicated the starting material using up. Triethylamine was added to quench the reaction. After work up, the coupling products were isolated as a mixture of β and α anomers **4** and **5**, which were inseparable by chromatographic method. After deprotecting the benzoyl and Cbz groups with methanolic ammonia, compound **1** was isolated in 17% yield as the β anomer, and compound **6** was isolated in 7% yield as the α anomer from flash column chromatography. Due to the 2,2-difluoro groups of the sugar, the β anomer **4** is less hindered than the α anomer **5** and was formed as a major product.

The proton NMR spectral data of compounds **1** and **6** were assigned by COSY spectral studies, and the anomeric configurations of compound **1** and **6** were confirmed by NOESY studies. For compound **1**, the signal for H-4 at 4.34 ppm (dt, 1H, $J = 8.2$, 7.2 Hz) and the signal for H-1 at 6.45 ppm (t, 1H, $J = 7.2$ Hz) had the NOE in NOESY spectrum; for compound **6**, the signal for H-4 at 4.34 (dt, 1H, $J = 8.5$, 7.8 Hz) and the signal for H-1 at 6.55 ppm (t, 1H, $J = 6.9$ Hz) did not have the NOE in its NOESY spectrum, which indicated compound **1** has β configuration and compound **6** has α configuration.[9] Their anti-tumor studies are under progress.

In conclusion, in order to find a compound keeping the anti-

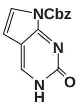


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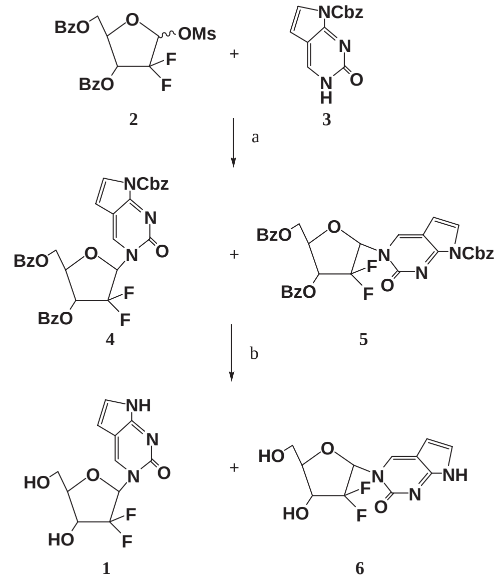
Figure 2.



Scheme 2. Regents and conditions: (a) i: **3**, BSA, CH_3CN, 70 °C, 5 h; ii: **2**, TMSOTf, 15 h, 80 °C; (b) $MeOH/NH_3$.

tumor activity of gemcitabine but not being substrate of deoxycytidine deaminase, a gemcitabine analogue was designed. By coupling the 2-deoxy-2,2-difluoro sugar derivative with bicycle base, the target compound **1** was synthesized, and its configuration was confirmed by 2D NMR studies.

References and Notes

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9 Spectral data for the selected compounds: **1** ^{1}H NMR (CD_3OD, 300 MHz): δ 8.77 (s, 1H), 7.12 (d, 1H, $J = 4.0$ Hz), 6.45 (t, 1H, $J = 6.9$ Hz), 6.34 (d, 1H, $J = 4.0$ Hz), 4.34 (dt, 1H, $J = 8.3$, 7.2 Hz), 4.04 (m, 2H), 3.85 (dd, 1H, $J = 12.5$, 2.5 Hz); MS: 288 $[M + 1]^+$. **6** ^{1}H NMR (CD_3OD, 300 MHz): δ 8.47 (s, 1H), 7.12 (d, 1H, $J = 4.2$ Hz), 6.55 (t, 1H, $J = 6.9$ Hz), 6.38 (d, 1H, $J = 4.2$ Hz), 4.41 (m, 1H), 4.04 (m, 2H), 3.68 (m, 1H); MS: 288 $[M + 1]^+$.

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