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An Improved Preparation Process for Gemcitabine

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Abstract:

An improved, cost-effective, and convenient process, using cinnamoyl as hydroxyl protective group and tosyl as the leaving group for gemcitabine (1) is described. The overall yield obtained from this newly developed process is around 10%, including two stereospecific crystallizations, and the quality of the product complies with the requirements of USP30.

Introduction

Gemcitabine, **1** (Figure 1), is a nucleoside analogue of cytidine with wide-ranging activity in a variety of solid tumors such as nonsmall cell lung cancer (NSCLC), head and neck cancer, genitourinary tract cancer.[1,2] Gemzar, marketed by Eli Lilly for intravenous use, contains gemcitabine, mannitol, and sodium acetate as a sterile lyophilized powder.

The synthesis of gemcitabine was originally accomplished by Hertel and co-workers, by using *tert*-butyldimethyl chlorosilane as the protecting agent for the hydroxyl groups.[3,4] However, this method was not suitable for large-scale production, since the major product so obtained was undesired **1a** (**1**:**1a** = 1:4). Several other syntheses of **1** have been reported with relatively low yield.[5−7]

Chou and co-workers selected benzoyl as the protecting group for the hydroxyls at C-3 and C-5 positions,[8] and the ratio of **1** and **1a** was increased to 1:1 (Scheme 1). Therefore, benzoyl is believed to be a qualified protective group in the process of preparing gemcitabine. Nevertheless, Chou's process suffered from industrial inconveniences: (a) the impurities contained in the oil form of intermediate **5a/b** were not easy to get rid of, and the remaining impurities not only decreased the yield of subsequent coupling reaction but also consumed the expensive trimethylsilyl trifluoromethanesulfonate (TMSOTf); (b) the mixture of **6a/b** (1:1) was used in following step, and the undesired isomer, **6a**, resulted in unnecessary material cost; (c) overall yield was at 5% from compound **2** over eight steps, and the purity of the final product (98%) did not comply with the requirement of USP30 (≥99.8%).

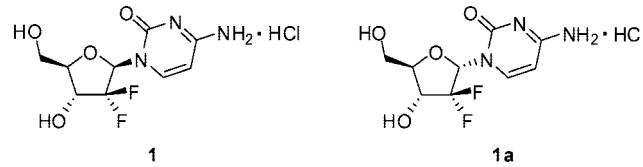

Figure 1. **Structures of gemcitabine and undesired isomer.**

Herein, we report a new process for producing gemcitabine by using cinnamoyl as hydroxyl protective group and tosyl as a leaving group. The improved process has some industrial advantages such as economical material cost, convenient operation, and relatively high yield, etc.

Results and Discussion

Compound **8**, 2-deoxy-2,2-difluoropentofuranos-1-ulose,[3,9] was acylated by cinnamoyl chloride in the presence of pyridine to give both isomers at C-3 position, **9a** and **9b**, in ethyl acetate (Scheme 2). Workup of this reaction furnished the solution of **9a/b** in toluene, which was cooled to ambient temperature (5−10 °C) with stirring, and the desired **9a** slowly crystallized out as powder, in excellent purity (97.1%), ee (99.3%), and yield (43%).

Compound **9a** was reduced to get the hydroxyl intermediate of 2-deoxy-2,2-difluoro-D-ribofuranose-3,5-dicinnamonate **10** by lithium tri-*tert*-butoxyaluminohydride (LTBA), which was prepared *in situ* by using *tert*-butanol and $LiAlH_4$ in tetrahydrofuran (THF). Other reduction reagents, such as lithium aluminohydride, lithium triacetoxyaluminohydride, and Vitride solution also worked well with almost similar yields. The hydroxyl intermediate **10**, in a mixture of α and β isomers (1:1), was used directly to the next step of tosylation without further purification.

The crude product from the reduction step was tosylated with *p*-toluene sulfonyl chloride (TsCl) in ethyl acetate to give **11a/b**. The base used in this reaction had obvious effect on the ratio of **11a** and **11b** (Figure 2), but the ratio did not affect the result of the following coupling reaction.

The tosyl of **11a/b** was replaced with mesyl to check which one was better for large-scale preparation. It was found that the tosylate easily crystallized in ethyl acetate/petroleum ether with reasonable yield (63% based on **9a**); nevertheless, the mesylate was in oil form. Otherwise, the corresponding tosylate of **5a/b** was in solid form. This implied that **11a/b** was in solid form due to the effect of tosyl.

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(1) Van Moorsel, C. J.; Peters, G. J.; Pinedo, H. M. *Oncologist* **1997**, *2*, 127.

(2) Vogelzang, N. J. *Semin. Oncol.* **2002**, *29* (1 Suppl. 3), 40.

(3) Hertel, L. W. U.S. Patent 4,808,614A1, 1989.

(4) Hertel, L. W.; Kroin, J. S.; Misner, J. W.; Tustin, J. M. *J. Org. Chem.* **1988**, *53*, 2406.

(5) Hertel, L. W.; Jones, C. D.; Kroin, J. S.; Mabry, T. E. U.S. Patent 5,480,992A1, 1996.

(6) Lee, J.; Park, G. S.; Lee, M.; Bang, H. J.; Lee, J. C.; Kim, C. K.; Choi, C. J.; Kim, H. K.; Lee, H. C.; Chang, Y. K.; Lee, G. S. WO Patent 2006/071090A1, 2005.

(7) Shen, J. S.; Li, Y. F.; Kaspi, J. WO Patent 2007/027564A2, 2006.

(8) Chou, T. S.; Heath, P. C.; Patterson, L. E.; Poteet, L. M.; Lakin, R. E.; Hunt, A. H. *Synthesis* **1992**, *6*, 565.

(9) Berton, C. P.; Laurens, A.; Henry, A. L. *J. Am. Chem. Soc.* **1950**, *72*, 2404.

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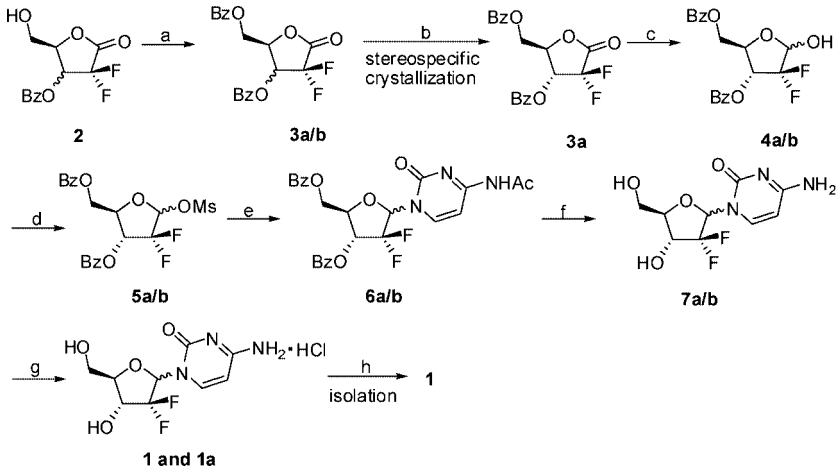
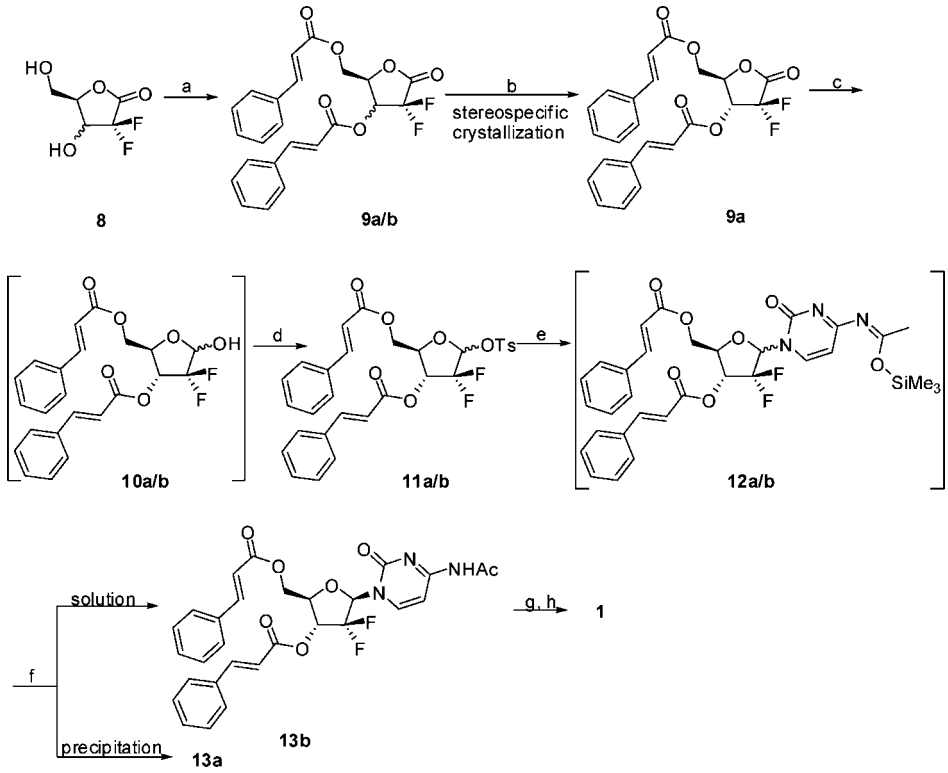
Scheme 1. **Reported route for gemcitabine**[a]



[a] Reagents and conditions: (a) benzoyl chloride, pyridine, *N*,*N*-dimethyl-4-amino-pyridine, 65 °C; (b) dichloromethane/heptane, 5−35 °C, 26%, over two steps; (c) LTBA, ethyl ether, THF, −10 °C; (d) MsCl, Et_3N, dichloromethane, 0 °C; (e) *N*-acetyl cytosine, HMDS, TMSOTf, DCE, reflux; (f) NH_3, methanol, rt; (g) concd. hydrochloric acid, *i*-PrOH, 70 °C, 49% over four steps; (h) acetone/H_2O, 37%.

Scheme 2. **Improved route for gemcitabine**[a]



[a] Reagents and conditions: (a) cinnamoyl chloride, pyridine, ethyl acetate, 60 °C; (b) toluene, 43% over two steps; (c) LTBA, THF, −10 °C; (d) TsCl, Et_3N, toluene, −10 °C, 62% over two steps; (e) *N*-acetyl cytosine, HMDS, TMSOTf, DCE, reflux; (f) 5% sodium dicarbonate; (g) NH_3, methanol, rt; (h) 1 N hydrochloric acid, acetone, rt, 80% over two steps.

It was interesting that a mixture of **13a** and **13b** (1:1) was obtained, regardless of the ratio of **11a** and **11b**. The phenomenon indicates that the coupling reaction proceeds via S_N1-like pathway, which is consistent with the hypothesis of Chou's group.[5] Excessive TMSOTf (1.5 equiv to **5a/b**) was needed in the reported procedure,[8] as the impurities contained in **5a/b** consumed this expensive reagent. In the present procedure, pure **11a/b** was used to reduce the consumption of TMSOTf to 1 equiv and improve the yield of this coupling step.

The unstable **12a/b** was treated by 5% sodium carbonate solution to give **13a/b** in situ. Most of the **13a** precipitated from the mixture, thanks to its poor solubility in 1,2-dichloroethane (DCE). Thereby, the undesired isomer **13a** was simply removed from the reaction mixture by filtration. This advantage reduces the cost of materials in the following steps and makes the process more efficient.

Crude **13b** was treated with NH_3/methanol at room temperature to give crude gemcitabine base form in high yield. The

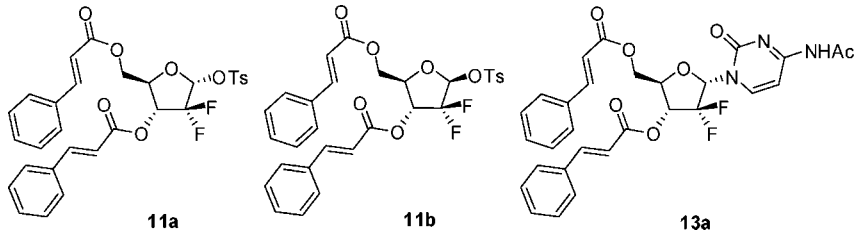


Figure 2. **Intermediates and byproduct of the improved procedure.**

main byproduct of the deprotection were methyl cinnamate and cinnamide, which can be removed by extraction with dichloromethane during the workup step. The crude gemcitabine base form **7b**, containing a trace amount of α-isomer **7a**, was acidified with hydrochloric acid and crystallized in acetone/H_2O to give **1** of 99.9% purity and any individual impurity of not more than 0.1%. The improved process showed a yield of 23% over the five steps from compound **9a** to gemcitabine. Comparatively, for the Chou's process, the corresponding yield was 18% from mesylate **3a**.

Conclusion

An improved process for gemcitabine, including two stereoselective crystallizations, has been provided with the total yield of 10% over six steps. Because of the affect of cinnamoyl and tosyl, the resulting new intermediates have some industrially favorable physical properties. For example, **9a** and **13a** can be simply separated from their isomer mixtures, and the intermediate **11a/b** can be purified conveniently. The advantages ensure that the efficient, cost-effective, and industrially convenient process will be employed for commercial production of gemcitabine.

Experimental Section

Materials and Instruments. All commercially available materials and solvents were used as received without any further purification. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 at room temperature on a Bruker AMX-400/600 at 400 MHz using TMS as an internal standard. ^{13}C NMR spectra were obtained from a Gemini-300 spectrometer in $CDCl_3$ or DMSO-d_6 at room temperature. The chemical-shift scale is based on internal TMS. The mass spectrum was recorded on a Finnigan MAT-95/711spectrometer. Melting points were measured on a Buchi-510 melting point apparatus, which are uncorrected. TLC analyses were performed on Merck silica gel 60 F_{254} plate.

2-Deoxy-2,2-difluoro-D-erythropentofuranos-1-ulose-3,5-dicinnamonate (9a). The solution of 2 kg (12 mol) of cinnamyl chloride in 2 L of ethyl acetate was added to the mixture of 1 kg (6 mol) of **8** and 1.4 L (18 mol) of pyridine in 8 L of ethyl acetate. The reaction mixture was heated to 30 °C for 3 h, cooled to 5 °C, filtrated to obtain the wet cake, which was washed by 3 L of cooled toluene. The combined filtration was concentrated under reduced pressure at 40 °C to obtain 5 L of residue, which was slowly cooled to ambient temperature (5−10 °C), stirred overnight, and filtrated to obtain the off-white powder; the solid was washed by toluene, and oven-dried to obtain 1.1 kg of off-white solid **9a**; yield 43.0%, purity 97.1%, ee = 99.3% (HPLC).

9a: mp 130−131 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.59 (m, 2H), 4.87 (m, 1H), 5.64 (m, 1H), 6.45 (d, 1H, J = 16.0 Hz), 6.49 (d, 1H, J = 16.0 Hz), 7.42 (m, 6H), 7.54 (m, 4H), 7.74 (d, 1H, J = 16.0 Hz), 7.82 (d, 1H, J = 16.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 61.62, 68.85, 78.23, 111.52, 114.83, 116.17, 128.31, 128.48, 128.94, 129.06, 130.79, 131.28, 133.52, 133.87, 146.79, 162.41, 164.78, 165.90. EI-MS (*m*/*z*) 428 (M^+).

2-Deoxy-2,2-difluoro-D-ribofuranose-3,5-dicinnamonate (10a/b). The solution of 5.84 kg (78 mol) of *tert*-butanol in 2 L of THF was carefully added to the mixture of 8 L of absolute THF and 1 kg (26 mol) of $LiAlH_4$ at 10 °C. The reaction mixture was stirred for 2 h and then concentrated to obtain 6.68 kg of off-white solid LTBA.

The suspension of 1 kg (2.3 mol) of **3a** in 8 L of THF was cooled to 10 °C, and the previously prepared reduction reagent was added in portions. After the suspension became clear, the reaction mixture was stirred for another 2 h, and 1 L of H_2O was carefully added to quench the reaction. The resulted mixture was washed by 1 N hydrochloric acid and 5% sodium bicarbonate, dried over Na_2SO_4, filtrated to obtain the filtration, which was used directly in next step.

2-Deoxy-2,2-difluoro-D-ribofuranos-3,5-dicinnamonyl-1-(4-methylbenzene) Sulfonate (11a/b). To the resulted filtration of previous step, 480 mL (3.4 mol) of triethylamine was added at 0 °C. The solution of 440 g (2.3 mol) of *p*-toluene sulfonyl chloride in 500 mL of toluene was added to the resulted mixture in 2 h, and stirred for 5 h more. The reaction mixture was washed by 1 N hydrochloric acid and 5% sodium bicarbonate. The organic phase was concentrated to obtain the residue as a yellow oil. The residue was dissolved in 4 L of ethyl acetate at 50 °C under reduced pressure, cooled to ambient temperature (5−10 °C), and 3 L of petroleum ether was slowly added. The resulted mixture was stirred for 3 h, and filtrated to obtain 850 g of **11a/b**, yield 62.3% of two steps, purity 97.31% of two isomers on HPLC.

11b: mp 120−121 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 4.12 (dd, 1H), 4.28 (dd, 1H), 4.39−4.44 (m, 1H), 5.68 (m, 1H), 6.01 (d, 1H, J 6.0 Hz), 6.34 (d, 1H, J = 16.4 Hz), 6.46 (d, 1H, J = 16.4 Hz), 7.27 (d, 1H, J = 8.8 Hz), 7.39 (m, 8H), 7.52 (m, 5H), 7.67 (d, 1H, J = 16.4 Hz), 7.76 (d, 1H, J = 16.4 Hz), 7.80 (d, 1H, J = 8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.63, 62.90, 69.31, 79.23, 98.55, 115.38, 116.92, 127.95, 128.21, 128.36, 128.90, 128.98, 129.90, 130.55, 131.02, 133.65, 133.74, 134.06, 145.74, 147.78, 164.98, 165.91. ESI-MS (*m*/*z*) 607 (M + Na).

11a: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.42 (s, 3H), 4.09 (dd, 1H), 4.28 (dd, 1H), 4.35−4.37 (m, 1H), 5.31 (dd, 1H), 6.03 (d, 1H, J = 7.2 Hz), 6.41 (d, 1H, J = 16.4 Hz), 6.49 (d, 1H, J = 16.4 Hz), 7.34 (d, 1H, J = 8.8 Hz), 7.39 (m, 6H), 7.52 (m, 4H), 7.70 (d, 1H, J = 16.4 Hz), 7.78 (d, 1H, J = 16.4 Hz), 7.85 (d, 1H, J = 8.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ

21.63, 62.05, 69.31, 79.30, 99.72, 115.54, 116.76, 127.83, 128.21, 128.36, 128.90, 128.98, 129.84, 130.55, 131.02, 133.65, 133.74, 134.06, 145.58, 147.78, 164.98, 165.91. ESI-MS (*m*/*z*) 607 (M + Na).

***N*-1-Acetyl-2-deoxy-2,2-difluorocytidine-3,5-dicinnamonate (13b).** The mixture of 500 g (3.3 mol) of acetyl cytosine, 1.36 L (6.5 mol) of hexamethyldisilazane (HMDS), 8.7 g (66 mmol) of ammonium sulfate, and 5 L of 1,2-dichloroethane was heated to reflux for 4 h, concentrated to obtain a white solid, 774 g. Another 8 L of DCE, 770 g (1.3 mol) of **11a/b** and 470 mL of TMSOTf was added, and the resulted mixture was heated to reflux for 12 h; the reaction mixture was washed by 3 L of 5% sodium bicarbonate, filtrated to obtain 320 g of **13a** as off-white solid, purity at 93.4%; no **13b** was found by HPLC. The filtration was washed by saturated brine, dried over Na_2SO_4, and concentrated under reduced pressure to obtain 340 g of **13b** as a buff solid, yield 46.5%, ee = 90%.

13b: mp 205−207 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.29 (s, 3H), 4.51 (m, 1H), 4.63 (m, 1H), 5.49 (m, 1H), 6.45 (d, 1H, J = 6.6 Hz), 6.51 (d, 1H, J = 6.6 Hz), 6.57 (m, 1H), 7.40 (m, 6H), 7.54 (m, 4H), 7.74−7.86 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.89, 62.16, 71.24, 78.55, 84.00, 97.42, 115.40, 116.51, 128.26, 128.39, 128.94, 128.99, 130.72, 131.05, 133.68, 133.90, 144.67, 146.51, 147.85, 154.69, 163.39, 164.89, 166.16, 170.86. ESI-MS (*m*/*z*) 588 (M + Na).

2′-Deoxy-2′,2′-difluorocytidine, Monohydrochloride (Gemcitabine, 1). The mixture of 250 g (440 mmol) of **13b** and 2.5 L of 11% ammonia in methanol was stirred overnight and concentrated to obtain an oily residue. The residue was dissolved in 2 L of 1 N hydrochloric acid and washed by dichloromethane two times. The aqueous layer was concentrated to 300 mL, 1.0 L of acetone was added; the mixture stirred for 12 h and was filtrated to obtain 106 g of pure gemcitabine **1** (99.8%, initial impurity no more than 0.1%), yield 80%.

1: mp 271−273 °C dec. $[\alpha]^{20}_D$ = (+) 49.3 (c 1.0, H_2O). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.60 (dd, 1H, J = 12.8, 4.0 Hz), 3.76 (d, 1H, J = 12.0 Hz), 3.89 (dd, 1H, J = 5.6, 1.8 Hz), 4.17 (m, 1H), 6.05 (m, 1H,), 6.25 (d, 1H, J = 8.0 Hz), 8.15 (d, 1H, J = 8.0 Hz), 8.91 (s, 1H), 10.07 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 58.69, 68.12, 81.59, 83.80, 94.76, 143.38, 146.98, 159.58. ESI-MS (*m*/*z*) 298 (M − H).

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