

Synthesis of 1-(2-Deoxy-2-C-fluoromethyl-β-D-arabinofuranosyl)cytosine As a Potential Antineoplastic Agent

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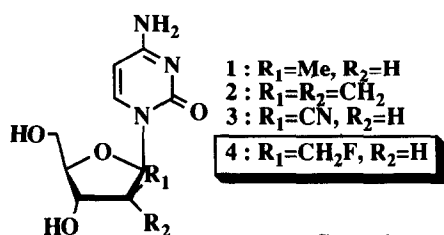
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Abstract: 2'-β-Spiroepoxy-uridine was obtained from the reaction between 2'-ketouridine and dimethylsulfoxonium methylid. The oxirane ring was cleaved by KFHF and the resulting tertiary hydroxyl group was removed by radical deoxygenation using a *t*-methyl oxalyl-tributyltin hydride system to give 2-deoxy-2-C-fluoromethyl-1-β-D-arabinofuranosyl-uracil derivative 7. Finally, the uracil moiety was converted to a cytosine counterpart, followed by deprotection to yield the title compound.

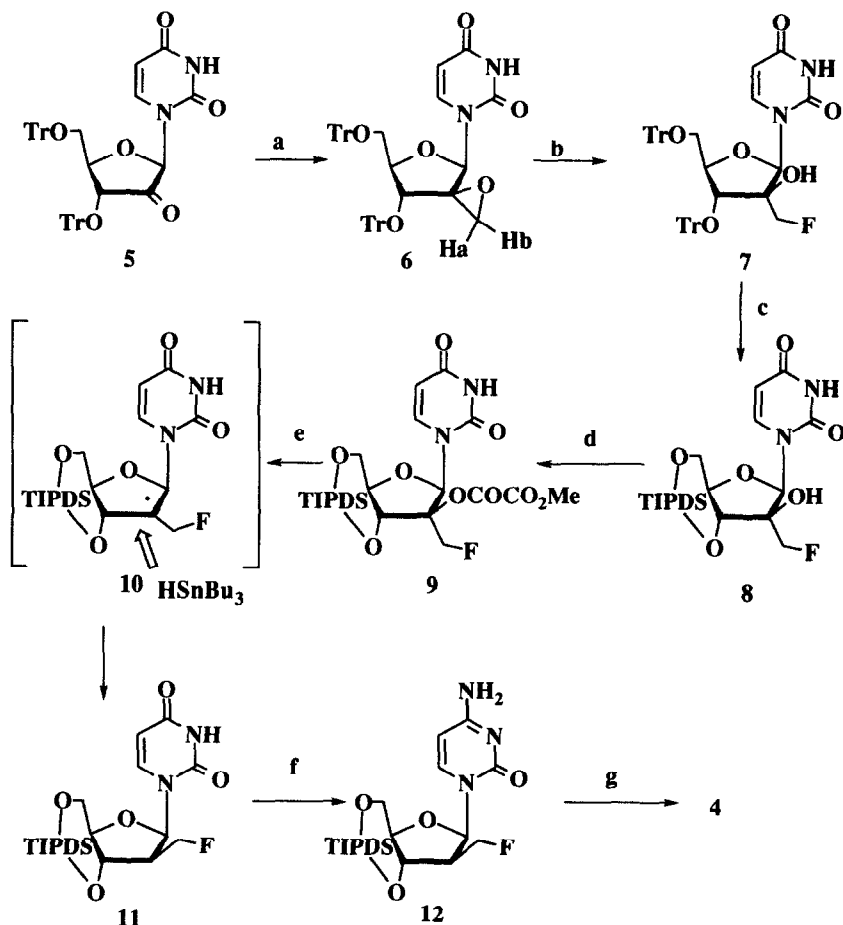
New drugs effective for solid tumors and leukemia are essential for further development of cancer chemotherapy. The synthesis of modified nucleosides is one promising field for the development of new chemotherapy drugs. While synthesizing antineoplastic nucleosides, we found that some 2'-substituted cytidine analogues showed potent antitumor activity; e.g., 1-(2-deoxy-2-C-methyl-β-D-arabinofuranosyl)cytosine (SMDC) **1** showed potent cytotoxicity toward several leukemic cell lines,¹ and



1-(2-deoxy-2-methylene-erythro-pentofuranosyl)-cytosine (DMDC) **2** showed broad cytotoxicity toward a variety of tumor cell lines.² Although SMDC revealed potent antileukemic activity *in vitro*, its *in vivo* activity was negligible.³ In contrast, 1-(2-C-cyano-2-deoxy-β-D-arabinofuranosyl)cytosine (CNDAC) **3** had strong anti-neoplastic activity against both leukemia and solid

tumors both *in vivo* and *in vitro*.⁴ This suggests that introduction of an electron-withdrawing substituent at the 2'-position of 2'-deoxycytidine would be useful in the development of antineoplastic nucleosides. Hence, we designed a 2'- β -fluoromethyl derivative to improve the activity of SMDC. The present report describes the synthesis of the target compound **4** using radical deoxygenation of 2'-*tert*-alcohol and its cytotoxicity to tumor cell lines.

Scheme 1.^a



^aa: $\text{Me}_3\text{S}^+(\text{O})\text{I}^-$, NaH, DMSO, THF. 65%. b: KFHF, 2-methoxyethanol, reflux. 42%. c: 1) 80% AcOH, 2) TIPDSCl₂, pyridine. 59%. d: methyl oxalyl chloride, DMAP, CH₂Cl₂. e: Bu_3SnH , AIBN, toluene. 86%. f: POCl₃, triazole, Et₃N, CH₃CN, then NH_4OH . 59%. g: TBAF, THF. 61%.

3',5'-Di-O-trityl-2'-ketouridine⁵ **5** was treated with dimethylsulfoxonium methylide to yield the spiro-epoxy derivative **6** in a 65 % yield. Compound **6** was diastereomerically pure, according to its ¹H-NMR spectrum, and the stereochemistry of the 2'-position was determined by n.O.e. experiment : 7.1% of an n.O.e was observed between H-1' and oxirane proton Ha while a weak n.O.e. (3.6%) appeared between

H-3' and the other oxirane proton H_b (Scheme 1). This result revealed that the stereochemistry of the 2'-epoxy-ring was like that of the β -epoxide. Therefore, the nucleophilic attack of the sulfur ylide is considered to direct from the α -face selectively, due to steric bulkiness of the 1'-heterocyclic moiety. This result is consistent with a previous report.⁶ The epoxy-ring of **6** was cleaved by treatment with KFHF to give fluoromethyl derivative **7** in a 42% yield.⁷ The choice of fluoride salts and solvents was important in achieving effective cleavage of the epoxide ring. In the case of potassium fluoride, the reaction gave a complex mixture, instead of KFHF. Similarly, 2-methoxyethanol was the best choice of solvents, while the use of DMF as a solvent decreased the yield of **7**.

We attempted to remove a *tert*-hydroxyl group at the 2'-position of **7**. However, radical deoxygenation of the corresponding methyl oxalyl ester of the *tert*-alcohol did not give the desired 2'-deoxygenated compounds. Therefore, the trityl group of **7** was deprotected once by acid treatment and the resulting free nucleoside was protected by 1,1,3,3-tetraisopropylidisiloxane-1,3-diyl (TIPDS) group to yield **8**. Compound **8** was acylated by methyl oxalyl chloride in the presence of a stoichiometric amount of DMAP, and the resulting 2'-methyl oxalate was further treated, without purification, with tributyltin hydride^{1,8} in the presence of AIBN as a radical initiator to give the desired 2'-deoxy-2'-fluoromethyl derivative **11** in an 86% yield,⁹ along with a small amount of inseparable by-product. The radical deoxygenation of **8** appeared to occur selectively from the α -side, judging from the ¹H-NMR spectrum, and showed a coupling constant of 7.3 Hz between H-1' and H-2' in **11**. This value is similar to those of SMDC and CNDAC.^{1,4} The stereochemistry of **11** was further confirmed by n.O.e experiments after removal of the protecting group. It is noteworthy that the fluorine atom remains intact through the radical deoxygenation. This was confirmed by the ¹H-NMR spectrum which showed a large coupling constant (47.9 Hz) between the fluorine and geminal proton. Amination at the 4-position of the uracil ring of **11** using the triazole method,¹⁰ followed by deprotection of the TIPDS group yielded 1-(2-deoxy-2-C-fluoromethyl- β -D-arabinofuranosyl)cytosine **4**.¹¹

As a preliminary result, compound **4** showed potent cytotoxicity toward the human T-cell line, CCRF-HSB-2 (IC₅₀ = 0.030 μ g/ml). The compound revealed moderate activities toward several solid tumor cell lines: KB cell, IC₅₀ = 4.47 μ g/ml; Colo320DM (colon adenocarcinoma), IC₅₀ = 1.6 μ g/ml; and A-375 (melanoma), IC₅₀ = 1.6 μ g/ml. On the other hand, it showed only weak cytotoxicity towards PC-8 (lung adeno-carcinoma, IC₅₀ = 26 μ g/ml) and KATO-III (stomach adenocarcinoma, IC₅₀ = 11 μ g/ml).¹² The antitumor activities of **4** varied with the cell lines, but the compound showed potent activity against the leukemic cell line.

In summary, we synthesized 1-(2-deoxy-2-C-fluoromethyl- β -D-arabinofuranosyl)cytosine and found that this compound had potent cytotoxicity. Further investigation, including comparison with SMDC, is now in progress.

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References and Notes

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7. Typical procedure: To a solution of 3.09 g of **6** (4.17 mmol) in 80 ml of 2-methoxyethanol was added 3.26 g of KFHF (41.7 mmol) and the mixture was kept in gentle reflux for 1 day in an argon atmosphere. The solvent was removed under reduced pressure and the residue was partitioned between AcOEt and water. The organic phase was dried (Na₂SO₄), and the filtrate was concentrated and the residue was purified over a silica gel column, giving 1.33 g of **7** (42%).
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9. Typical procedure: To a solution of 1.02 g of **8** (1.96 mmol) and 720 mg of DMAP (5.88 mmol) in 35 ml of CH₂Cl₂ was added 540 ml of methy oxalyl chloride (5.88 mmol) at 0°C in an argon atmosphere. The mixture was stirred at room temperature overnight. The reaction was quenched by water, the whole was extracted by CHCl₃, and the separated organic phase was dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was dissolved in 45 ml of anhydrous toluene. To the solution, 1.60 ml of tributyltin hydride (5.88 mmol) and 85 mg of AIBN were added and the mixture was kept reflux for 2 h. in an argon atmosphere. After the solvent was removed under reduced pressure, the residue was purified over a silica gel column, giving 850 mg of **11** (86%).
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11. **5**: Mp >180°C (dec) ¹H-NMR (D₂O) δ 7.85 (1 H, d, *J* = 7.8 Hz), 6.37 (1 H, d, *J* = 7.3 Hz), 6.04 (1 H, d, *J* = 7.8 Hz), 4.63-4.64 (2 H, m, *J*_{H,F} = 46.9 Hz), 4.26 (1 H, t, *J* = 8.3 Hz), 4.01 (1 H, dd, *J* = 2.4, 12.5 Hz), 3.98-3.94 (1 H, m), 3.87 (1 H, dd, *J* = 4.4, 12.5 Hz), 3.00-2.86 (1 H, m). *Anal.* Calcd for C₁₀H₁₄N₃FO₄: C, 46.33; H, 5.44; N, 16.21. Found: C, 46.13; H, 5.49; N, 15.93. n.O.e. data for **4**: 15.8% between H-1' and H-2', 4.5% between H-2' and H-4', 2.2% between one of the -CH₂F and H-6, 3.4% between one of the -CH₂F and H-3'.
12. The inhibitory effects of compound **4** on the growth of various human tumor cell lines, except KB cell, was determined by Professor T. Sasaki, Cancer Research Institute, Kanazawa University. The authors gratefully acknowledge this contribution to the present study.

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