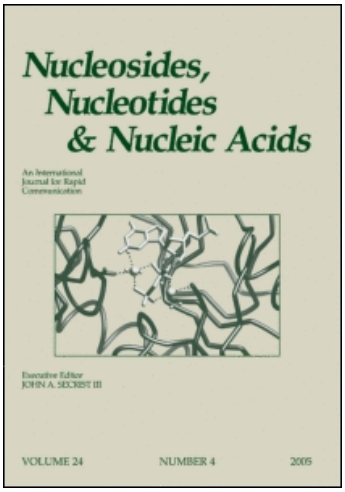

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TRUNCATED FLUOROCYCLOPENTENYL PYRIMIDINES AS *S*-ADENOSYLHOMOCYSTEINE HYDROLASE INHIBITORS

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□ *On the basis of inhibitory activity of truncated cyclopentenyl cytosine against* S-*adenosylhomocysteine hydrolase (SAH), its fluorocyclopentenyl pyrimidine derivatives were efficiently synthesized from* D*-ribose via electrophilic fluorination as a key step. The final nucleosides were evaluated for SAH inhibitory activity, among which the uracil derivative* ***9*** *showed significant inhibitory activity (IC_{50} = 8.53 μM). They were also evaluated for cytotoxic effects in several human cancer cell lines such as fibro sarcoma, stomach cancer, leukemia, and colon cancer, but they did not show any cytotoxic effects up to 100 μM, indicating that 4′-hydroxymethyl groups are essential for the anticancer activity.*

Keywords Truncated nucleosides; S-adenosylhomocysteine hydrolase; electrophilic fluorination

INTRODUCTION

The carbocyclic nucleoside,[1–4] whose furanose oxygen of the sugar moiety is replaced by a methylene possesses inherent advantages such as a stabilized glycosyl bond, resistance to phosphorylases, and increased lipophilicity, but their syntheses have always been challenging because of lengthy steps, low overall yields, and harsh reaction conditions. Recently, thanks to several elegant syntheses of the carbocyclic moiety, several carbocyclic nucleosides showing potent biological activities have been reported in the literature.[5]

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In honor of and in celebration of Morris J. Robins' 70th birthday.

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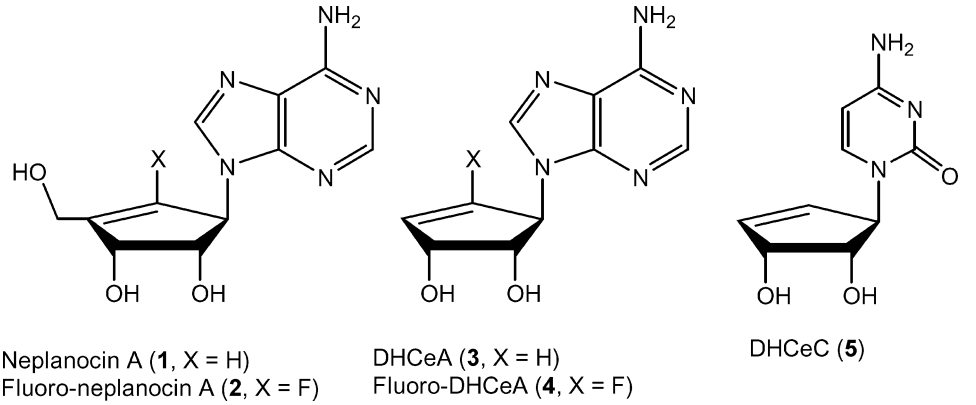


FIGURE 1 Cyclopentenyl nucleosides showing potent inhibitory activity against SAH.

S-adenosylhomocysteine hydrolase (SAH) catalyzes the hydrolysis of *S*-adenosylhomocysteine into adenosine and L-homocysteine.[6] Inhibition of SAH leads to accumulation of *S*-adenosylhomocysteine in the cell, resulting in the inhibition of *S*-adenosylmethionine (SAM) mediated transmethylations.[6]

Because transmethylation is essential for replication of most animal viruses, SAH has been a promising target for the development of broad-spectrum antiviral agents.[7,8]

A naturally occurring carbocyclic nucleoside, neplanocin A (**1**)[9] is one of the most potent inhibitors of SAH.[10] Unfortunately, compound **1** exhibited significant toxicity due to phosphorylation of the 5′-hydroxyl group by cellular kinases (Figure 1).[10] Recently, we have reported its fluoro analogue **2** as a new type of mechanism-based irreversible inhibitor of SAH.[11] Compound **2** showed two times more potent SAH inhibitory activity than compound **1**, but it was also cytotoxic.[11] In order to overcome the cytotoxicity caused by the 5′-hydroxyl groups in compounds **1** and **2**, their truncated derivatives **3**[12] and **4**[13] were also synthesized. Both compounds were also potent inhibitors of SAH although they were less potent than their corresponding 4′-hydroxymethyl derivatives.[12,13] However, neither served as a substrate for adenosine kinase nor adenosine deaminase, thus did not show the toxicity associated with neplanocin A.[12] Interestingly, truncated cytosine analogue **5** was also reported to show significant inhibitory activity against SAH despite being a pyrimidine analogue, indicating that pyrimidines might serve as substrates for SAH in addition to adenine.[14]

The base-modified analogues of neplanocin A were also reported to show potent biological activity (Figure 2). For example, the cytosine derivative **6**[15] showed potent antitumor activity by reducing cytidine-5′-triphosphate (CTP) pools, resulting from the inhibition of CTP synthetase and its 5-fluorocytosine analogue **7**[16] exhibited potent antiviral activity

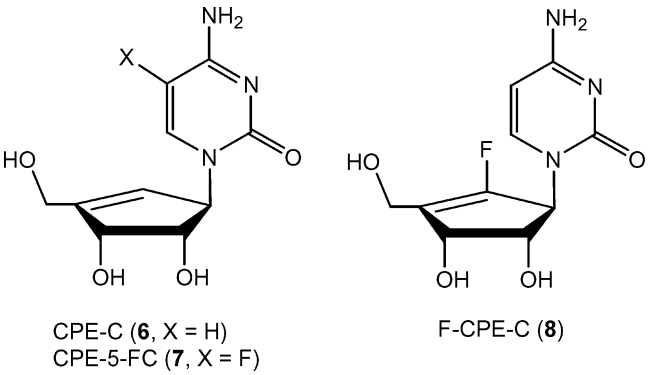


FIGURE 2 Cyclopentenyl nucleosides showing antiviral and antitumor activities.

against West Nile virus. On the other hand, another cytosine analogue **8**[17] with fluorocyclopentenyl ring showed potent antitumor activity against most human cancer cell lines.

Thus, on the basis of potent SAH inhibitory activity of the truncated cyclopentenyl pyrimidine **5**, it was of interest to synthesize its truncated fluorocyclopentenyl pyrimidines **9** and **10** and to compare their biological activity (Figure 3). It was also of interest to compare the antitumor activity of truncated **9** and **10** with that of the cytosine derivative **8** showing potent antitumor activity to determine if 4′-hydroxymethyl group is essential for antitumor activity. Herein, we report the synthesis and biological activity of the truncated fluorocyclopentenyl pyrimidines, **9** and **10**.

RESULTS AND DISCUSSION

Synthesis started from D-ribose, as shown in Scheme 1. D-Ribose was efficiently converted to the key intermediate, D-cyclopentenone **10** according to our previously reported procedure,[18] which was then converted to the glycosyl donor **15**.

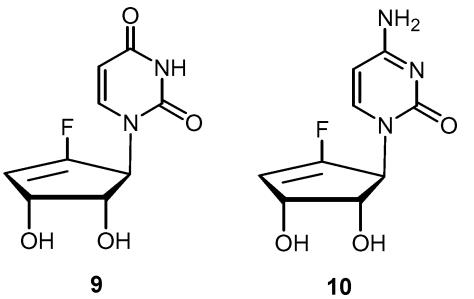
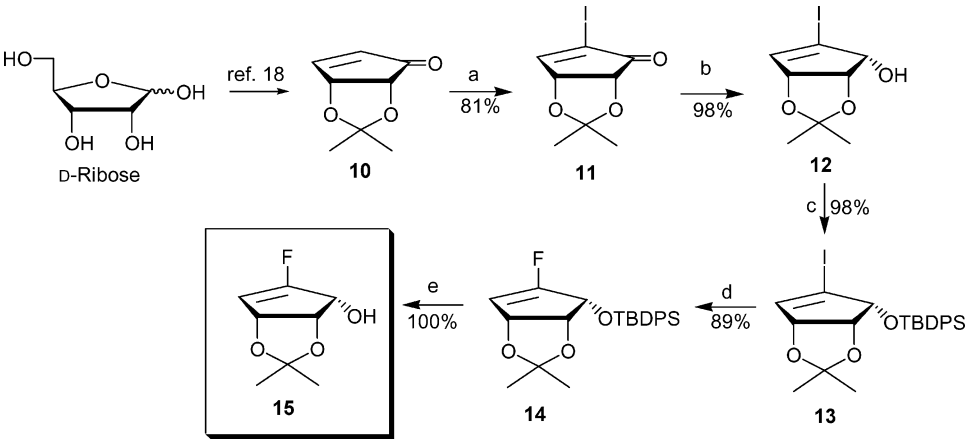


FIGURE 3 Structures of the target nucleosides.

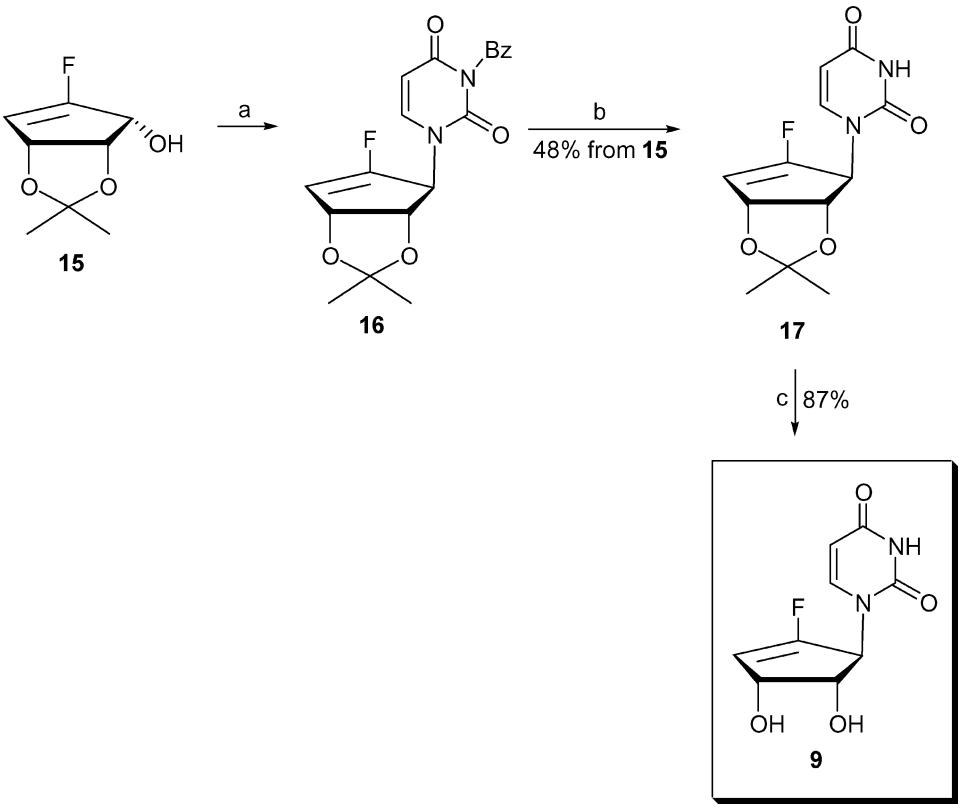


SCHEME 1 Reagents and conditions: a) I_2, pyridine, CCl_4, rt, 1.5 h; b) $NaBH_4$, $CeCl_3$–H_2O, MeOH, 0°, 40 min; c) TBDPSCl, imidazole, DMF, rt, overnight; d) NFSI, n-BuLi, THF, −78°, 10 min; e) TBAF, THF, rt, 1 h.

Our initial strategy to the introduction of the fluorine was to use direct electrophilic fluorination on cyclopentenone **10**, but this method failed to give the desired fluorinated compound under various conditions. Thus, we decided to utilize halogen-metal exchange reaction followed by electrophilic fluorination. Treatment of **10** with iodine in pyridine gave iodocyclopentenone **11**. Reduction of **11** with $NaBH_4$ in the presence of cerium (III) chloride heptahydrate at 0°C produced the iodocyclopentenol **12**. Protection of the hydroxyl group in **12** with TBDPSCl in DMF afforded TBDPS ether **13**. Electrophilic fluorination reaction[11] was achieved by adding *n*-BuLi to a solution of **13** and *N*-fluorobenzenesulfonimide (NFSI) in THF at −78°C to give an inseparable mixture of vinyl fluoride **14** and its hydrogen derivative in 4/1 ratio in 89% yield. Treatment of **14** with tetra-*n*-butylammonium fluoride afforded the glycosyl donor **15**[13] in quantitative yield.

Synthesis of the uracil derivative **9** from the glycosyl donor **15** was achieved using a Mitsunobu reaction[19] as the key step, as illustrated in Scheme 2. Condensation of **15** with N^3-benzoyluracil under the standard Mitsunobu condition[19] gave N^3-Bz-uracil derivative **16**, which was treated with methanolic ammonia to afford the uracil derivative **17** (48% from **15**). Treatment of **17** with 50% aqueous trifluoroacetic acid (TFA) gave the final uracil derivative **9**.

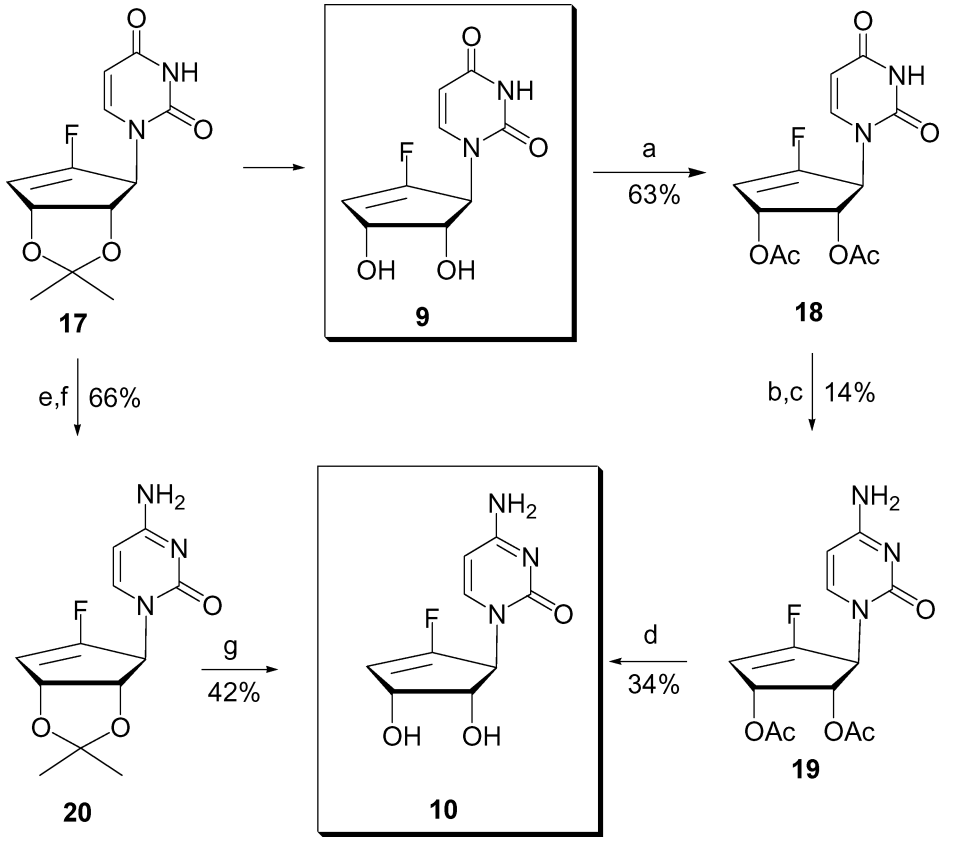
For the synthesis of the cytosine derivative **10**, two synthetic routes were employed, as shown in Scheme 3. The first route began with the uracil derivative **9**. Compound **9** was treated with acetic anhydride in pyridine to give triacetate **18** (63%). Treatment of **18** with 1,2,4-triazole, phosphorus oxychloride, and triethyl amine (TEA) gave the N^4-triazole derivative, which without purification, was reacted with aqueous ammonia in 1,4-dioxane



SCHEME 2 Reagents and conditions: a) N^3-benzoyl-uracil, PPh_3, DEAD, THF, rt, 2.5 h; b) NH_3 in MeOH, rt, overnight; c) 50% aq. TFA, rt, 3 h.

to afford the cytosine analogue **19** in only 14% yield. Deprotection of **19** with methanolic ammonia gave the final nucleoside **10** (34%). Because of poor overall yield of the first route, the second route starting from 2,3-acetonide **17** was explored. Treatment of **17** with 1,2,4-triazole in the presence of 4-chlorophenyldichlorophosphate in pyridine gave the triazole derivative, which was then immediately treated with aqueous ammonia in 1,4-dioxane to give the cytosine derivative **20** in 66% yield. Removal of the 2,3-isopropylidene group in **20** using 50% aqueous TFA yielded the final cytosine nucleoside **10** (42%). The second route turned out to be better than the first route in view of number of steps and overall yields (27.7 vs. 2.6).

The final nucleosides **9** and **10** were assayed for SAH inhibitory activity. Enzyme activity was measured using pure recombinant enzyme obtained from human placenta.[11] The residual activity of the enzyme was determined in the synthetic direction toward *S*-adenosylhomocysteine using adenosine and L-homocysteine.[11] Both compounds were preincubated with the enzyme at 100 μM for 5 minutes at 37°C. Surprisingly, the uracil



SCHEME 3 Reagents and conditions: a) Ac_2O, pyridine, rt, overnight; b) $POCl_3$, 1,2,4-triazole, TEA, CH_3CN, rt, overnight; c) 1,4-dioxane: 28% NH_4OH = 10 : 1, rt, overnight; d) NH_3 in MeOH, rt, overnight; e) 4-chlorophenyldichlorophosphate, 1,2,4-triazole, pyridine, rt, overnight; f) 1,4-dioxane: 28% NH_4OH = 1 : 2, rt, 3 h; g) 50% aq. TFA, rt, 2.5 h.

derivative **9** exhibited potent inhibitory activity (IC_{50} = 8.53 μM) against SAH, while the cytosine derivative **10** was inactive.

The growth inhibition of the final nucleosides **9** and **10** against a variety of human tumor cells such as lung cancer, fibrosarcoma, stomach cancer, leukemia, and colon cancer was also evaluated using the Sulforhodamine B (SRB) method. Unlike the potent anticancer activity of compound **8**, none of the synthesized compounds showed anticancer activity, indicating that 4′-hydroxyl methyl group is essential for the anticancer activity.

In conclusion, we have accomplished the synthesis of novel fluorocyclopentenyl pyrimidines from D-ribose, using electrophilic fluorination and Mitsunobu condensation as key steps. Among compounds tested, the uracil derivative showed potent SAH inhibitory activity, indicating that pyrimidine base can serve as a good template like a purine base. All synthesized compounds showed no cytotoxic activity in several human cancer cell lines,

probably due to the absence of the 4′-hydroxylmethyl group, which may be essential for phosphorylation by cellular kinases.

EXPERIMENTAL

General Methods

Melting points were measured and are uncorrected. UV spectra were measured in methylene chloride or methanol. ^{1}H NMR spectra ($CDCl_3$, CD_3OD) were recorded on Varian Unity Inova 400 MHz NMR spectrometers. Chemical shifts were reported in parts per million (δ) units relative to the solvent peak. The ^{1}H NMR data were reported as peak multiplicities: s for singlet; d for doublet; dd for doublet of doublets; ddd for doublet of doublet of doublets; t for triplet; pseudo t for pseudo triplet; br s for broad singlet; and m for multiplet. Coupling constants were reported in hertz. ^{13}C NMR spectra ($CDCl_3$, CD_3OD) were recorded on Varian Unity Inova 100 MHz. The chemical shifts were reported as ppm (δ) relative to the solvent peak. FAB mass spectra were recorded on Jeol HX 110 spectrometer (Korea). Optical rotations were determined on Jasco III (Korea) in methanol. Elementary analyses were measured on EA1110. Reactions were checked with TLC (Merck precoated 60F254 plates, Korea). Spots were detected by viewing under a UV light, colorizing with charring after dipping in anisaldehyde solution with acetic acid and sulfuric acid and methanol. Column chromatography were performed on silica gel 60 (230–400 mesh, Merck). Reagents were purchased from Aldrich Chemical Company, Korea. Solvents were obtained from local suppliers. All the anhydrous solvents were distilled over CaH_2, P_2O_5, or Na/benzophenone prior to the reaction.

5-Iodo-2,2-dimethyl-3a,6a-dihydro-cyclopenta[1,3]dioxol-4-one (11)

To a stirred solution of **10** (9.81 g, 63.64 mmol) and iodine (56.53 g, 222.7 mmol) in carbon tetrachloride (200 mL) was added pyridine (5.15 mL, 63.64 mmol) under nitrogen atmosphere at 0°C. The reaction mixture was stirred at room temperature for 1.5 hours. The resulting mixture was diluted with methylene chloride and water. The mixture was washed with water, saturated solution of sodium thiosulfate and brine. The organic layer was dried and filtered through a pad of celite. The filtrates were concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 7 : 1) to afford **11** (14.48 g, 81%) as a white solid: m.p. 80–82°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 2.8 Hz, 1 H), 5.21 (dd, J = 2.8, 5.6 Hz, 1 H), 4.51 (d, J = 5.6 Hz, 1 H), 1.39 (s, 3 H), 1.36 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.7, 165.0, 115.9,

105.8, 79.8, 73.8, 27.4, 26.5; MS (FAB) m/z 281 $[M + H]^+$; $[\alpha]^{23}_D$ +14.5 (c 0.53, MeOH).

5-Iodo-2,2-dimethyl-4,6a-dihydro-3aH-cyclopenta[1,3]dioxol-4-ol (12)

To a stirred solution of **11** (14.0 g, 50.0 mmol) and cerium (III) chloride heptahydrate (13.56 g, 55.0 mmol) in methanol (150 mL) was added sodium borohydride (2.08 g, 55.0 mmol) at 0°C. The reaction mixture was stirred at 0°C for 40 minutes. After adding water, the mixture was evaporated, which was extracted with ethyl acetate and dried, filtered through a pad of celite. The filtrates were concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 6 : 1) to give **12** (13.7 g, 98%) as a white solid: m.p. 55–59°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.30–6.29 (m, 1 H), 4.91 (dd, J = 2.0, 5.6 Hz, 1 H), 4.67 (t, J = 5.6 Hz, 1 H), 4.41–4.40 (m, 1 H), 2.80 (br s, 1 H), 1.40 (s, 3 H), 1.38 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.8, 112.9, 107.6, 83.8, 78.6, 76.4, 27.7, 26.9; MS (FAB) m/z 283 $(M + H^+)$; $[\alpha]^{24}_D$ +1.05 (c 2.76, MeOH).

tert-Butyl-(5-iodo-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta [1,3]dioxol-4-yloxy)-diphenyl-silane (13)

To a stirred solution of **12** (13.68 g, 48.50 mmol) and imidazole (10.01 g, 145.5 mmol) in anhydrous *N,N*-dimethylformamide (100 mL) was added *tert*-butyl diphenylchlorosilane (14.6 mL, 58.20 mmol) at room temperature under nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight. After adding water, the mixture was evaporated, which was extracted with ethyl acetate and dried, filtered through a pad of celite. The filtrates were concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 30 : 1) to give **13** (24.74 g, 98%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84–7.80 (m, 4 H), 7.44–7.26 (m, 6 H), 6.23–6.22 (m, 1 H), 4.61 (dd, J = 1.6, 5.2 Hz, 1 H), 3.91 (t, J = 6.0 Hz, 1 H), 1.40 (s, 3 H), 1.21 (s, 3 H), 1.16 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.7, 136.7, 136.2, 135.9, 134.0, 133.7, 129.9, 127.6, 127.4, 111.9, 107.1, 83.7, 79.5, 27.7, 27.2, 27.1, 26.9, 19.8; MS (FAB) m/z 519 $[M-H]^+$, 543 $[M + Na]^+$; $[\alpha]^{24}_D$ −23.16 (c 1.87, MeOH).

tert-Butyl-(5-fluoro-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta [1,3]dioxol-4-yloxy)-diphenyl-silane (14)

To a stirred solution of **13** (23.05 g, 44.29 mmol) and *N*-fluorobenzenesulfonimide (21.60 g, 66.43 mmol) in tetrahydrofuran (400 mL) was slowly added a 1.6 M solution of *n*-butyllithium in hexanes (83 mL,

132.9 mmol) at −78°C under nitrogen gas. The reaction mixture was stirred at -78°C for 10 minutes. After saturated solution of ammonium chloride was added at 0°C, the mixture was extracted with ethyl acetate and dried, filtered through a pad of celite. The filtrates were concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 30 : 1) to give **14** (16.24 g, 89%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82–7.79 (m, 2 H), 7.76–7.72 (m, 2 H), 7.46–7.26 (m, 6 H), 5.27 (br s, 1 H), 4.81–4.75 (m, 1 H), 4.40–4.37 (m, 1 H), 4.28–4.24 (m, 1 H), 1.53 (s, 3 H), 1.35 (s, 3 H), 1.11 (br s, 9 H); MS (FAB) m/z 411 $[M-H]^+$, 435 $[M + Na]^+$; $[\alpha]^{24}_D$−59.93 (c 6.76, MeOH).

5-Fluoro-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta[1,3] dioxol-4-ol (15)

To a stirred solution of **14** (30.18 g, 71.76 mmol) in tetrahydrofuran (400 mL) was added dropwise a 1.0 M solution of tetra-*n*-butylammoniumfluoride in tetrahydrofuran (107.64 mL, 107.64 mmol) at 0°C. The reaction mixture was stirred at room temperature for 1 hour. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 4 : 1) to give **15** (12.74 g, 100%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27–5.26 (m, 1 H), 4.98–4.92 (m, 1 H), 4.72–4.68 (m, 1 H), 4.40 (br s, 1 H), 2.77 (br s, 1 H), 1.45 (s, 3 H), 1.36 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$, mixed with 5-hydrogenated compound) δ 165.2, 162.3, 136.5, 132.1, 112.5, 105.2, 83.7, 77.9, 76.9, 75.0, 69.1, 27.8, 27.7, 26.7, 26.4; MS (FAB) m/z 173 $[M-H]^+$, 197 $[M + Na]^+$; $[\alpha]^{25}_D$ +0.833 (c 1.20, MeOH); Anal. Calcd for $C_8H_{11}FO_3$: C, 55.17; H, 6.37. Found C, 55.36; H, 6.53.

3-Benzoyl-1-(5-fluoro-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta [1,3]dioxol-4-yl)-1*H*-pyrimidine-2,4-dione (16)

To a stirred solution of **15** (3.45 g, 19.84 mmol), N^3-benzoyl-uracil (8.58 g, 39.68 mmol), triphenylphosphine (13.00 g, 49.60 mmol) in tetrahydrofuran (35 mL) was added dropwise diethyl azodicarboxylate (8.05 mL, 49.60 mmol) at 0°C. The reaction mixture was stirred at room temperature for 2.5 hours. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 1.5 : 1) to give **16** as a colorless oil: UV (MeOH) λ_{max} 255.0 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.98–7.95 (m, 2 H), 7.74–7.70 (m, 1 H), 7.61 (d, J = 8.0 Hz, 1 H), 7.57–7.53 (m, 2 H), 5.86 (d, J = 8.4 Hz, 1 H), 5.67 (br s, 1 H), 5.33 (br s, 1 H), 5.28–5.25 (m, 1 H), 4.83–4.80 (m, 1 H), 1.44 (s, 3 H), 1.31 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 164.2, 159.2, 156.4, 151.2, 144.8, 136.6, 132.9, 131.6, 130.6, 113.1, 112.9, 103.3, 82.5, 80.5, 67.6, 49.7, 48.5,

27.7, 25.7; MS (FAB) m/z 373 $[M + H]^+$, 395 $[M + Na]^+$; $[\alpha]^{24}_D$ −137.26 (c 4.92, MeOH).

1-(5-Fluoro-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta[1,3]dioxol-4-yl)-1*H*-pyrimidine-2,4-dione (17)

To **16** (5.9 g, impure) in a 25 mL of round bottom flask was added methanolic ammonia (5 mL) rapidly. The reaction mixture was stirred at room temperature overnight. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (methylene chloride/methanol = 70: 1 to hexanes/ethyl acetate = 1 : 1) to give **17** (2.08 g, 48% for 2 steps) as a white solid: m.p. 105–110°C; UV (MeOH) λ_{max} 262.5 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 (dd, J = 0.4, 8.0 Hz, 1 H), 5.70 (d, J = 8.0 Hz, 1 H), 5.31–5.28 (m, 1 H), 4.75–4.72 (m, 1 H), 1.45 (s, 3 H), 1.32 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 159.7, 156.9, 152.5, 144.3, 113.1, 112.4, 103.4, 82.8, 80.7, 66.8, 27.7, 25.8; MS (FAB) m/z 269 $[M + H]^+$, 291 $[M + Na]^+$; $[\alpha]^{25}_D$ −199.59 (c 2.42, MeOH).

1-(2-Fluoro-4,5-dihydroxy-cyclopent-2-enyl)-1H-pyrimidine-2,4-dione (9)

A solution of **17** (107 mg, 0.399 mmol) in 50% aqueous trifluoroacetic acid solution (4 mL) was stirred at room temperature for 3 hours. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (methylene chloride/methanol = 10 : 1) to give **9** (79.5 mg, 87%) as a white crystal: m.p. 138–140°C; UV (MeOH) λ_{max} 264.5 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (dd, J = 0.8, 8.0 Hz, 1 H), 5.73 (d, J = 8.0 Hz, 1 H), 5.55–5.53 (m, 1 H), 5.43–5.39 (m, 1 H), 4.85–4.59 (m, 1 H), 4.27 (dt, J = 1.2, 6.0 Hz, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.2, 162.3, 159.4, 152.9, 144.3, 110.3, 103.4, 75.7, 70.5, 65.1; MS (FAB) m/z 251 $[M + Na]^+$; $[\alpha]^{25}_D$ −74.31 (c 5.59, MeOH); Anal. Calcd for $C_9H_9FN_2O_4$: C, 47.37; H, 3.98. Found C, 47.46; H, 4.03.

Acetic Acid 5-acetoxy-4-(2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-3-fluoro-cyclopent-2-enyl ester (18)

A solution of **9** (628 mg, 2.75 mmol) in pyridine (10 mL) was added dropwise acetic anhydride (1.02 mL, 8.25 mmol) at 0°C. The reaction mixture was stirred at room temperature overnight under nitrogen atmosphere. After removal of the solvent, the mixture was extracted with ethyl acetate and dried, filtered through a pad of celite. The filtrates were concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 1 : 2) to give **18** (540 mg, 63%) as a white solid: m.p. 150–152°C; UV (MeOH) λ_{max} 260.5 nm; ^{1}H NMR (400 MHz, CD_3OD)

δ 7.49 (dd, J = 0.8, 8.0 Hz, 1 H), 5.81–5.77 (m, 1 H), 5.69 (d, J = 8.0 Hz, 1 H), 5.59–5.56 (m, 2 H), 5.51–5.48 (m, 1 H), 2.04 (s, 3 H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 172.1, 171.7, 166.0, 162.9, 160.0, 152.7, 144.4, 107.1, 103.7, 74.0, 72.0, 63.6, 20.7, 20.4; MS (FAB) m/z 313 $[M + H]^+$, 335 $[M + Na]^+$; $[\alpha]^{24}_D$ −203.41 (c 0.82, MeOH).

Acetic Acid 5-acetoxy-4-(4-amino-2-oxo-2*H*-pyrimidin-1-yl)-3-fluoro-cyclopent-2-enyl ester (19)

A solution of 1,2,4-triazole (0.27 g, 3.85 mmol) and phosphorus oxychloride (0.32 mL, 3.50 mmol) in acetonitrile (3 mL) was treated with triethylamine (0.44 mL, 3.15 mmol) and **18** (109 mg, 0.35 mmol) in acetonitrile (2 mL). The reaction mixture was stirred at room temperature overnight, followed by addition of triethylamine (0.54 mL, 3.85 mmol) and water (0.54 mL), and then the reaction mixture was stirred at room temperature for 10 minutes. After the addition of methylene chloride, the mixture was washed with saturated solution of sodium bicarbonate and brine. The organic layer was dried over anhydrous magnesium sulfate, filtered through a pad of celite. The filtrates were concentrated in vacuo, and the residue was used in the next step without further purification.

To a stirred solution of triazole derivative in 1,4-dioxane (10 mL) was added 28% ammonium hydroxide (1 mL) at 0°C, and the reaction mixture was stirred at room temperature overnight. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (methylene chloride/methanol = 10 : 1) to give **19** (23.5 mg, 14% for 2 steps) as a yellowish solid: m.p. 180°C (decomp.); UV (MeOH) λ_{max} 272.5 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 (d, J = 8.0 Hz, 1 H), 5.91 (d, J = 7.2 Hz, 1 H), 5.84–5.80 (m, 1 H), 5.61–5.55 (m, 3 H), 2.07 (s, 3 H), 2.04 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 172.2, 171.6, 167.9, 163.7, 160.9, 158.6, 148.1, 145.3, 106.2, 97.0, 74.1, 72.2, 20.7, 20.5; MS (FAB) m/z 312 $[M + H]^+$, 334 $[M + Na]^+$; $[\alpha]^{25}_D$ −154.31 (c 0.58, MeOH).

4-Amino-1-(2-fluoro-4,5-dihydroxy-cyclopent-2-enyl)-1*H*-pyrimidin-2-one (10)

A solution of **19** (20 mg, 0.06 mmol) in saturated methanolic ammonia (5 mL) was stirred at room temperature overnight. The solvent was removed in vacuo, and the residue was purified by reverse phase silica gel column chromatography using water as the eluent to give **10** (5.0 mg, 34%) as a white crystal: m.p. 200°C (decomp.); UV (MeOH) λ_{max} 274.0 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (d, J = 7.6 Hz, 1 H), 5.95 (d, J = 7.6 Hz, 1 H), 5.51–5.50 (m, 1 H), 5.39 (m, 1 H), 4.64–4.61 (m, 1 H), 4.32 (t, J = 5.2 Hz, 1 H); ^{13}C NMR (100 MHz, CD_3OD) δ 167.5, 163.0, 160.1, 158.7, 145.2, 109.7, 96.8, 75.8, 70.5, 66.4; MS (FAB) m/z 250 $[M + Na]^+$; $[\alpha]^{25}_D$ −116.79 (c 1.96,

MeOH); Anal. Calcd for $C_9H_{10}FN_3O_3$: C, 47.58; H, 4.44. Found C, 47.46; H, 4.53.

4-Amino-1-(5-fluoro-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta [1,3]dioxol-4-yl)-1*H*-pyrimidin-2-one (20)

To a stirred solution of **17** (380 mg, 1.415 mmol) in pyridine (9 mL) was added dropwise 4-chlorophenyldichlorophosphate (0.92 mL, 5.66 mmol) at room temperature, following by addition of 1,2,4-triazole (0.39 g, 5.66 mmol), the reaction mixture was stirred at room temperature overnight. After removal of the solvent, the mixture was extracted with saturated solution of sodium bicarbonate and ethyl acetate, dried, and filtered through a pad of celite. The filtrates were concentrated in vacuo, and the residue was used without further purification for the next step. To a stirred solution of triazole derivative in 1,4-dioxane (4 mL) was added 28% ammonium hydroxide (2 mL) at 0°C, and the reaction mixture was stirred at the same temperature for 3 hours. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (methylene chloride/methanol = 50: 1 to 10: 1) to give **20** (250 mg, 66% for 2 steps) as a yellowish solid: m.p. 240°C (decomp.); UV (MeOH) λ_{max} 272.5 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 (d, J = 7.2 Hz, 1 H), 5.90 (d, J = 7.2 Hz, 1 H), 5.61 (s, 1 H), 5.31–5.27 (m, 2 H), 4.71–4.68 (m, 1 H), 1.45 (s, 3 H), 1.31 (s, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 168.0, 160.4, 158.5, 157.6, 145.1, 112.8, 111.8, 96.7, 83.1, 80.8, 68.3, 27.2, 25.8; MS (FAB) m/z 268 $[M + H]^+$, 290 $[M + Na]^+$; $[\alpha]^{25}_D$ −46.90 (c 2.42, MeOH).

4-Amino-1-(2-fluoro-4,5-dihydroxy-cyclopent-2-enyl)-1*H*-pyrimidin-2-one (10)

A solution of **17** (250 mg, 0.935 mmol) in 50% aqueous trifluoroacetic acid solution (4 mL) was stirred at room temperature for 2.5 hours. The solvent was removed in vacuo, to the mixture was added toluene (3 mL) then evaporated. Several times this work was replayed. And the residue was purified by silica gel column chromatography (methylene chloride/methanol = 10: 1 to 5: 1) to give **10** (110 mg, 42%) as a white crystal: All the analytical data was identical to **10** obtained from the previous aminolysis reaction.

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