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NUCLEOSIDES AND NUCLEOTIDES. 128. (2'*S*)-2'-DEOXY-2'-*C*-METHYL-5-IODOURIDINE (SMIU) AS A NOVEL POTENT ANTI-HERPES VIRUS AGENT[1]

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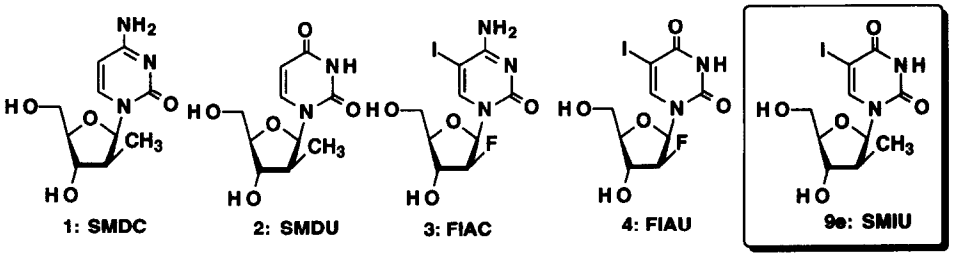
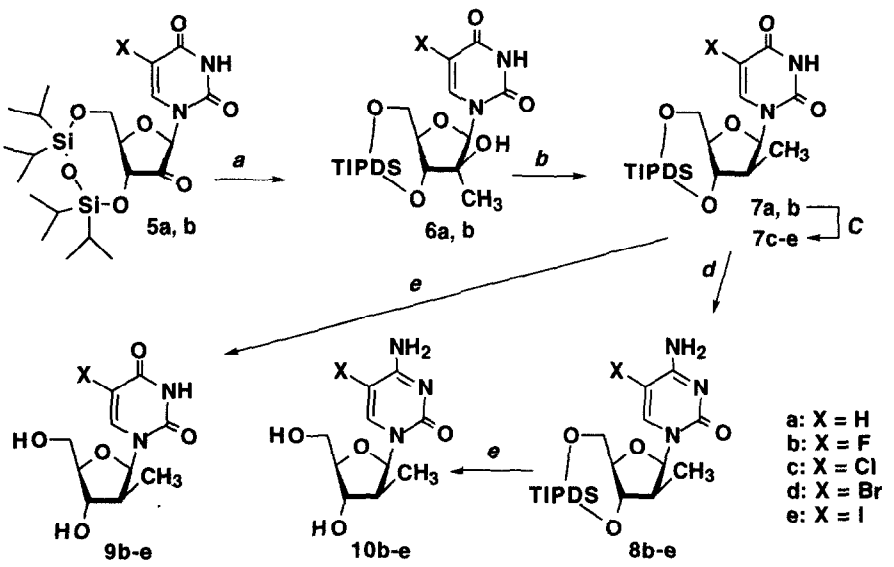
Abstract: (2'*S*)-2'-deoxy-2'-*C*-methyl-5-halogenopyrimidine nucleosides were designed and synthesized as anti-herpes virus agents. Of these compounds, (2'*S*)-2'-deoxy-2'-*C*-methyl-5-iodouridine (SMIU, **9e**) showed the greatest anti-HSV-1 activity *in vitro* while showing no cytotoxicity. SMIU is a candidate drug for clinical treatment of herpes virus infection disease.

In the last decade, several efficient antiviral agents have been developed for the clinical treatment of infection diseases caused by herpes viruses.[2] However, these are not always sufficient in herpes virus chemotherapy. For instance, herpes viruses often develop resistance to the drugs and the antiviral spectra of drugs are not broad enough.[2] Therefore, much attention has been focused on developing more efficient anti-herpes virus agents.

In recent years, we have engaged in the synthesis of 2'-modified nucleoside analogs as potential antitumor or antiviral agents.[3] Throughout these studies, we have disclosed that (2'*S*)-2'-deoxy-2'-*C*-methylcytidine (SMDC, **1**) had potent antineoplastic activities against tumor cells *in vitro*.[3a] On the other hand, it has been recognized that simultaneous introduction of a substituent at both 5-and 2'-β-position of pyrimidine nucleosides often have brought notable anti-herpes virus activity (*e.g.* 5-bromovinylarabinosyluridine, BVAU;[4] (2'*S*)-2'-deoxy-2'-fluoro-5-iodocytidine, FIAC, **3**;[5] (2'*S*)-2'-deoxy-2'-fluoro-5-iodouridine, FIAU, **4**[6]). Thus, we have planned synthesizing 5-halogeno derivatives of SMDC as well as the corresponding uracil congener SMDU (**2**), and evaluating their anti-herpes virus activities.

3',5'-*O*-Protected 2'-ketouridine derivative **5a**, prepared readily from uridine,[3h] was treated with methyl magnesium bromide in diethyl ether to give (2'*S*)-2'-*C*-methyluridine derivative **6a** exclusively in 81% yield.[7]





SCHEME 1

a) MeMgBr, Et_2O, -18°C; *b*) (i) MeO_2CCOCl, DMAP, CH_2Cl_2, rt, (ii) nBu_3SnH, AIBN, toluene, reflux; *c*) NXS (X = Cl, Br, I), AcOH, 80°C; *d*) (i) TPSCl, DMAP, Et_3N, MeCN, rt, then 28%NH_4OH; *e*) nBu_4NF, THF, rt.

Compound **6a** was treated by the stereoselective radical deoxygenation reaction of the 2'-tertiary hydroxyl of 2'-branched-chain sugar nucleosides that has been recently developed by us.[2a,d,e,h] This was treatment of **6a** with methoxalyl chloride and DMAP in CH_2Cl_2 to give 2'-*O*-methoxalylate, which was immediately reacted under reflux in toluene with tributyltin hydride and AIBN. This afforded the desired (2'*S*)-2'-deoxy-2'-*C*-methyl nucleoside **7a** in 73 % yield.[8] The corresponding 2'-diastereoisomer was not isolated from this reaction. Halogenation at the 5-position of the uracil moiety of **7a** was done with *N*-halogenosuccinimides as electrophilic reagents. Compound **7a** was heated with NCS in acetic acid at 80°C for 6 h to give 5-chloro derivative **7c** in 82% yield. In similar way the 5-bromo and 5-iodo derivatives (**7d** and **7e**) were obtained in 64% and 68% yields, respectively. The corresponding 5-fluoro derivative **7b** was synthesized starting from commercially available 5-fluorouridine in 16% over-all yield, by the same route for synthesizing **7a** from uridine as outlined in Scheme 1. Conversions of uracil nucleosides **7** to the corresponding cytosine congeners **8** were done by a known procedure: treatment of **7b-e** with triisopropylbenzenesulfonyl chloride (TPSCl) in the presence of DMAP and Et_3N in CH_3CN followed by ammonolysis afforded cytidine derivative **8b-e** in good yields (**8b**, 76%; **8c**, 83%; **8d**, 85%; **8e**, 56%, respectively). The silyl protecting group of **7** and **8** was removed by a standard method to furnish the target (2'*S*)-2'-deoxy-2-*C*-methyl-5-halogenopyrimidine nucleosides **9b-e** and **10b-e** in excellent yields.[9]

All the free nucleosides newly synthesized, together with some known potent antiviral agents, were evaluated for anti-HSV-1 activity *in vitro*,[10] and the results are summarized in Table 1. Of these compounds, 5-iodo uracil derivative **9e** (SMIU) had an EC_{50} value of 0.14 μg/mL while not being toxic to the host cells at

concentration up to 100 μg/mL, which was comparable with the potency of acyclovir, the most useful anti-herpes virus drug in clinical use. Although FIAU (**4**), having a fluoro atom instead of the methyl group at the 2'-β-position of SIAU, also showed a significant EC_{50} value (0.06 μg/mL), it was cytotoxic against host cells as reported previously.[6] 5-Iodocytosine derivative **10e** showed only a moderate activity,[11] in spite of its considerable structural similarity to FIAC (**3**), a well-known potent anti-herpes virus agent, which had a notable ED_{50} value of 0.10 μg/mL in this evaluation system. It has been recognized that FIAC was deaminated easily by cytidine deaminase to yield cytotoxic FIAU *in vivo*[12] as well as *in vitro*,[13] though it had a excellent chemotherapeutic index *in vitro*. Compound **10b** also had a significant ED_{50} value (0.22 μg/mL), but was quite toxic to the host cells. All other compounds tested, including 5-iodo-2'-deoxyuridine (IDU) which is a prototype for designing FIAU or SMIU, had only insignificant effects in this evaluation system.[14] These results indicated the 5-iodouracil base would be essential for the efficient anti-HSV-1 activity in the (2'*S*)-2'-deoxy-2'-*C*-methyl pyrimidine nucleoside derivatives.

Table 1. Inhibitory Effects on Replication of HSV-1 (KOS strain) in NC-37 cells[a]

compound	EC_{50}[b] (μg/mL)	CC_{50}[c] (μg/mL)
9b	>100	>100
9c	>100	>100
9d	11	>100
9e	0.14	>100
10b	0.22	0.22
10c	>100	>100
10d	>100	>100
10e	4.8	>100
FIAC (**3**)	0.10	63
FIAU (**4**)	0.06	6.7
IDU	> 80	80
Acyclovir	0.17	>100

[a]Antiviral assay was done by previously reported method (*Ref.* 10). [b]Concentration required to reduce virus-induced cytopathogenicity by 50%. [c]Concentration required to inhibit cell-growth by 50%.

In conclusion, SMIU showed a significant anti-HSV-1 effect *in vitro*, especially in terms of selective toxicity. SMIU should be further pursued for its therapeutic potential as an antiviral agent.

References and Notes

1. Part 127: Ono, A.; Haginoya, N.; Kiyokawa, M.; Minakawa, N.; Matsuda, A. *BioMed. Chem. Lett.* preceding paper.
2. *Antiviral Drug Development; A Multidisciplinary Approach*; De Clercq, E.; Walker, R. T. Ed; Plenum Press **1988.**
3. a) Matsuda, A.; Takenuki, K.; Itoh, H.; Sasaki, T.; Ueda, T. *Chem. Pharm. Bull.* **1987**, *35*, 3967-3970. b) Matsuda, A.; Itoh, H.; Takenuki, K.; Sasaki, T.; Ueda, T. *Chem. Pharm. Bull.* **1988**, *36*, 945-953. c) Ueda, T.; Matsuda, A.; Yoshimura, Y.; Takenuki, K. *Nucleosides Nucleotides.* **1989**, *8*, 743-752. d) Takenuki, K.; Itoh, H.; Matsuda, A.; Ueda, T. *Chem. Pharm. Bull.* **1990**, *38*, 2497-2952. e) Matsuda, A.; Takenuki, K.; Sasaki, T.; Ueda, T. *J. Med. Chem.* **1991**, *34*, 234-239. f) Matsuda, A.; Yasuoka, J.; Sasaki, T.; Ueda, T. *J. Med. Chem.* **1991**, *34*, 999-1002. g) Matsuda, A.; Nakajima. A.; Azuma, A.; Tanaka, M.; Sasaki, T. *J. Med. Chem.* **1991**, *34*, 2917-2919. h) Yoshimura, Y.; Iino, T.; Matsuda, A. *Tetrahedron Lett.* **1991**, *32*, 6003-6006.
4. Machida, H.; Sakata, S. In *Nucleosiides and Nucleotides as Antitumor and Antiviral Agents;* Chu, C. K.; Baker, D. C., Ed.; Plenum Press: 1993; pp. 245-264.

5. Lopez, C.; Watanabe, K. A.; Fox, J. J. *Antimicrob. Agents Chemother.* **1980**, *17*, 303-806.
6. Watanabe, K. A.; Su, T. L.; Klein, R. S.; Shun, C. K.; Matsuda, A.; Chuh, M. W.; Lopez, D.; Fox, J. J. *J. Med. Chem.* **1983**, *28*, 152-156.
7. Although synthesis of compound **6a** had been reported by addition reaction of Me_3Al to keto-nucleoside **5a** (Hayakawa, H.; Tanaka, H.; Itoh, H.; Nakajima, M.; Miyasaka, T.; Yamaguchi, K.; Iitaka, Y. *Chem. Pharm. Bull.* **1987**, *35*, 2605-2608.), we have used the Grignard reagent, instead of the aluminum reagent, because of its ease of handling in large scale experiments.
8. In ^1H NMR spectrum of **7a**, $J_{1',2'}$ value was 7.3 Hz. This demonstrated the 2'-β-configuration of **7a**. Compound **7a** was also derivatized to SMDC (**1**) which was identical with an authentic sample (*Ref.* 3a).
9. All the free nucleosides synthesized were analyzed for C, H, and N, and were within ±0.3% of theoretical value. ^1H NMR (DMSO-d_6) data were as follows. **9b**: δ 11.84 (1 H, br s, NH), 8.48 (dd, H, H-6, $J_{6,F}$ = 7.7, $J_{6,NH}$ = 2.2 Hz), 6.05 (1 H, dd, H-1', $J_{1',F}$ = 1.6, $J_{1',2'}$ = 7.7 Hz), 5.40-5.36 (2 H, m, 3'- and 5'-OH), 3.80-3.59 (4 H, m, H-3', 4', 5'), 2.48-2.38 (1 H, m, H-2'), 0.85 (3 H, d, 2'-CH_3, J = 7.1 Hz). **9c**: δ 11.86 (1 H, br s, NH), 8.60 (1 H, s, H-6), 6.08 (1 H, d, H-1', $J_{1',2'}$ = 7.7 Hz), 5.41-5.39 (2 H, m, 3'- and 5'-OH), 3.82-3.61 (4 H, m, H-3', 4', 5'), 2.45-2.39 (1 H, m, H-2'), 0.86 (3 H, d, 2'-CH_3, J = 7.1 Hz). **9d**: δ 11.86 (1 H, br s, NH), 8.70 (1 H, s, H-6), 6.08 (1 H, d, H-1', $J_{1',2'}$ = 7.2 Hz), 5.44-5.22 (2 H, m, 3'- and 5'-OH), 3.79-3.63 (4 H, m, H-3', 4', 5'), 2.47-2.38 (1 H, m, H-2'), 0.87 (3 H, d, 2'-CH_3, J = 7.1 Hz). **9e**: δ 11.86 (1 H, br s, NH), 8.67 (1 H, s, H-6), 6.05 (1 H, d, H-1', $J_{1',2'}$ = 7.3 Hz), 5.40-5.36 (2 H, m, 3'- and 5'-OH), 3.80-3.61 (4 H, m, H-3', 4', 5'), 2.48-2.35 (1 H, m, H-2'), 0.83 (3 H, d, 2'-CH_3, J = 7.0 Hz). **10b**: δ 8.29 (1 H, d, H-6, $J_{6,F}$ = 7.7 Hz), 7 69 (1 H, br s, NH), 7.48 (1 H, br s, NH), 6.06 (1 H, d, H-1', $J_{1',2'}$ = 7.7 Hz), 5.31 (1 H, d, 3'-OH, $J_{3,OH}$ = 6.1 Hz), 5.26 (1 H, t, 5'-OH, J = 5.1 Hz), 3.78-3.57 (4 H, m, H-3', 4', 5'), 2.42-2.33 (1 H, m, H-2'), 0.78 (3 H, d, 2'-CH_3, J = 7.2 Hz). **10c**: δ 8.41(1 H, s ,H-6), 7.79 (1 H, br s, NH), 7.18 (1 H, br s, NH), 6.07 (1 H, d, H-1', $J_{1',2'}$ = 7.7 Hz), 5.33-5.28 (2 H, m, 3'- and 5'-OH), 3.78-3.59 (4 H, m, H-3', 4', 5'), 2.49-2.34 (1 H, m, H-2'), 0.77 (3 H, d, 2'-CH_3, J = 7.1Hz). **10d**: δ 8.48 (1 H, s, H-6), 7.80 (1 H, br s, NH), 6.97 (1 H, br s, NH), 6.07 (1 H, d, H-1', $J_{1',2'}$ = 7.7 Hz), 5.33-5.31 (2 H, m, 3'- and 5'-OH), 3.78-3.59 (4 H, m, H-3', 4', 5'), 2.43-2.31 (1 H, m, H-2'), 0.77 (3 H, d, 2'-CH_3, J = 7.1 Hz). **10e**: δ 8.50 (1 H, s, H-6), 7.77 (1 H, br s, NH), 6.58 (1 H, br s, NH), 6.07 (1 H, d, H-1', $J_{1',2'}$ = 7.7 Hz), 5.32-5.27 (2 H, m, 3'- and 5'-OH), 3.73-3.37 (4 H, m, H-3', 4', 5'), 2.41-2.32 (1 H, m, H-2'), 0.75 (3 H, d, 2'-CH_3, J = 6.6 Hz).
10. Takeuchi, H.; Baba, M.; Shigeta, S. *J. Virol. Methods* **1991**, *33*, 61-67.
11. Since we found SMDC was not deaminated by cytidine deaminase from rat liver, **10e** would not be a substrate of the enzyme.
12. Chou, T-C.; Feinberg, A.; Grant, A. J.; Vidal, P.; Reichman, U.; Watanabe, K. A.; Fox, J. J.; Philips, F. S. *Cancer Res.* **1981**, *41*, 3336-3342.
13. a) Chen, Y-C.; Tan, R-S.; Ruth, J. L.; Dutschman, D. *Biochemical Pharmacol.* **1983**, *32*, 726-729. b) Kreis, W.; Damin, L.; Colacino, J.; Lopez, C. *ibid.* **1983**, *31*, 767-773.
14. IDU did not show any anti-HSV-1 activity up to 80 μg/mL in this evaluation system using a B-lymphoblastoid cell line, NC-37, as host cells (*Ref.* 10).

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