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Synthesis of fluorinated cyclopentenyladenine as potent inhibitor of *S*-adenosylhomocysteine hydrolase☆

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Abstract—Fluoro-DHCeA (**4**) was efficiently synthesized from D-cyclopentenone derivative **5** using electrophilic fluorination as a key step. Fluoro-DHCeA (**4**) was found to be as potent as DHCeA (**3**), but exhibited irreversible inhibition of enzyme unlike DHCeA (**3**) showing reversible inhibition. From this study, 4′-hydroxymethyl groups of neplanocin A and fluoro-neplanocin A played an important role in binding to the active site of the enzyme.

1. Introduction

S-Adenosylhomocysteine hydrolase (SAH) is the enzyme catalyzing the interconversion of *S*-adenosylhomocysteine into adenosine and L-homocysteine.[2] SAH in conjunction with cellular *S*-adenosyl-L-methionine (SAM) dependent transmethylase plays its essential role in forming the 5′-terminal methylated N^7-guanosine *m*RNA cap of most animal infecting viruses, which is necessary for viral replication.[2] Thus, SAH has been promising target for the development of broad-spectrum antiviral agents.[3,4]

Neplanocin A (**1**)[5] is recognized to be one of the most potent inhibitors of SAH.[6] This compound inhibits SAH by depleting cofactor NAD^+ and its inhibition is reversed by the addition of excess NAD^+.[7] On the basis of the potent inhibitory activity of neplanocin A, we have recently reported the synthesis of fluoro-neplanocin A (**2**) and its novel mechanism of action.[8] It was found that fluoro-neplanocin A (**2**) was about twofold more potent than neplanocin A (**1**) against SAH and demonstrated novel irreversible inhibition of SAH, unlike neplanocin A (**1**), showing reversible inhibition. On the other hand, 9-(*trans*-2′-,*trans*-3′-dihydroxycyclopent-4′-enyl)adenine (DHCeA, **3**) is the another representative of carbocyclic nucleoside showing potent inhibitory activity against SAH.[9,10] DHCeA is also reported to show type I mechanism of action inhibiting SAH by depleting cofactor NAD^+ like neplanocin A.[4] Its type I mechanism of action was recently confirmed by the X-ray structure of the co-crystals of DHCeA and SAH.[11]

On the basis of the potent inhibitory activity of fluoro-neplanocin A and DHCeA against SAH, it was of interest to design the fluoro analogue **4** of DHCeA and to compare the inhibitory activity and mechanism of action with those of DHCeA (**3**). It was also interesting to find out the importance of the 4′-hydroxymethyl substituent by comparing the inhibitory activities of fluoro-neplanocin A (**3**) and fluoro-DHCeA (**4**). Herein, we wish to report the synthesis of fluoro-DHCeA (**4**) via electrophilic fluorination reaction as a key step, its mechanism of action, and the importance of the 4′-hydroxymethyl substituent in inhibiting SAH (Fig. 1).

2. Results and discussion

2.1. Chemistry

Synthesis of the target nucleoside **4** started from D-cyclopentenone derivative **5**, which was readily available from

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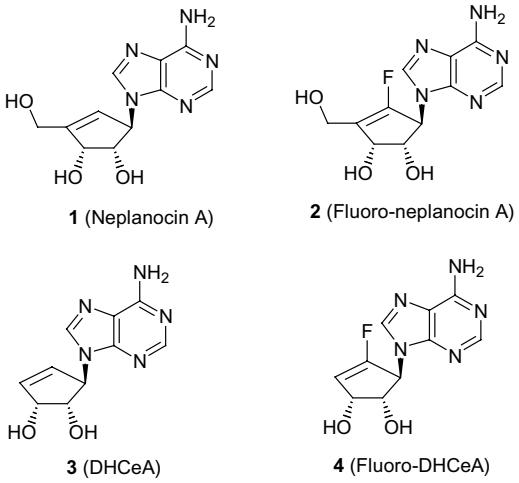


Figure 1. Rationale for the design of the desired nucleoside **4**.

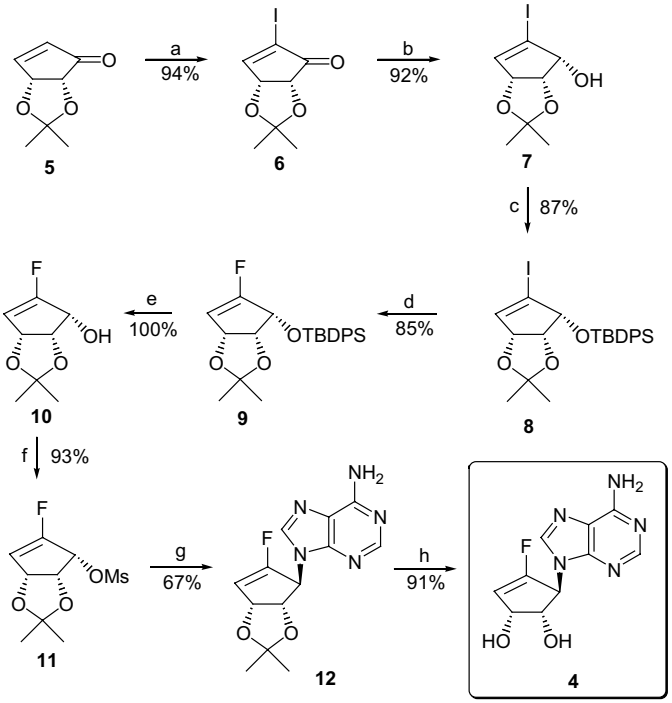
D-erythrose or D-ribose by the efficient procedure[12,13] developed by our laboratory (Scheme 1). Compound **5** was iodinated using iodine and pyridine to give iodocyclopentenone **6**. Reduction of **6** with $NaBH_4$ at 0 °C followed by treating the resultant **7** with TBDPSCl in DMF yielded silyl ether **8**. Electrophilic fluorination reaction was achieved by adding *n*-BuLi to a mixture of **8** and *N*-fluorobenzenesulfonimide (NFSI) in THF at −78 °C to afford an inseparable mixture of vinyl fluoride **9** and its hydrogen substituted derivative in 7/1 ratio.[14] Treatment of **9** with tetra-*n*-butylammonium fluoride gave **10**, which was mesylated to obtain mesylate **11** as the glycosyl donor. Condensation of **11** with adenine anion in DMF at 80 °C produced the protected nucleoside **12**. At this stage, fluoro derivative **12** could be separated from its hydrogen substituted derivative by silica gel column chromatography. Deprotection of **12** using aqueous trifluoroacetic acid afforded the final nucleoside, fluoro-DHCeA **4**.[15]

2.2. Biological evaluation

Inhibitory activity of SAH by DHCeA (**3**) and its fluoro analogue **4** was measured using pure recombinant enzyme obtained from human placenta (Table 1).[8] Both compounds were preincubated with the enzyme at various concentrations ranging from 0.5 to 12 μM for 5 min at 37 °C.

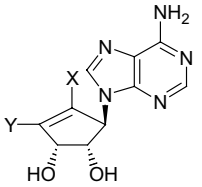
The residual activity of the enzyme was determined in the synthetic direction toward *S*-adenosylhomocysteine using adenosine and L-homocysteine. Incubation of enzyme with fluoro-DHCeA (**4**) resulted in concentration- and time-dependent inhibition of the enzyme like fluoro-neplanocin A (**2**). Fluoro-DHCeA (**4**) (IC_{50} = 8.9 μM) was almost equipotent as DHCeA (**3**) (IC_{50} = 8.7 μM), but exhibited about 20-fold less potent than fluoro-neplanocin A (**2**) (IC_{50} = 0.47 μM), indicating that 4′-hydroxymethyl group played a major role in binding to the active site of the enzyme (Table 1). The similar trend was observed in case of neplanocin A (**1**) and DHCeA (**3**).

Fluoro-DHCeA (**4**) was subjected to several experiments such as dialysis, ^{19}F NMR, and incubation with excess



Scheme 1. Reagents and conditions: (a) I_2, pyridine, CH_2Cl_2, rt, 1 h; (b) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, 0 °C, 1 h; (c) TBDPSCl, Imidazole, DMF, rt, 5 h; (d) NFSI, *n*-BuLi, THF, −78 °C, 1 h; (e) TBAF, THF, rt, 1 h; (f) MsCl, NEt_3, CH_2Cl_2, 0 °C, 20 min; (g) adenine, K_2CO_3, 18-crown-6-ether, DMF, 80 °C, 12 h; (h) 10% CF_3CO_2H, THF, rt, overnight.

Table 1. SAH inhibitory activity of the target nucleosides



Compound	IC_{50} (μM)[a]
1 (X = H, Y = CH_2OH)	0.89
2 (X = F, Y = CH_2OH)	0.47
3 (X = H, Y = H)	8.7
4 (X = F, Y = H)	8.9

[a] Determined using pure recombinant enzyme obtained from human placenta.

cofactor NAD^+ to investigate whether its mechanism of action is irreversible as fluoro-neplanocin A (**2**). As expected, fluoro-DHCeA (**4**) exhibited the same irreversible inhibition of SAH as fluoro-neplanocin A (**2**), while DHCeA (**3**) exhibited the same reversible inhibition as neplanocin A (**1**).

3. Summary

We have synthesized fluoro-DHCeA (**4**) using electrophilic fluorination reaction as a key step. Fluoro-DHCeA (**4**) was almost as potent as DHCeA (**3**), but exhibited irreversible inhibition of enzyme unlike DHCeA (**3**) showing reversible inhibition. From this study, we have discovered the important role of the 4′-hydroxymethyl group in binding to the active site of the enzyme. This result will contribute greatly to the design of the potent nucleoside analogues inhibiting SAH.

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14. To a stirred solution of **8** (1.50 g, 2.88 mmol) and *N*-fluorobenzenesulfonimide (1.09 g, 3.46 mmol) in dry tetrahydrofuran (30 mL) was added slowly *n*-butyl lithium (1.6 M solution in hexanes, 5.40 mL, 8.64 mmol) at −78 °C under nitrogen atmosphere and the reaction mixture was stirred at the same temperature for 1 h. After usual work-up, the residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 30:1) to give an inseparable mixture (1.16 g) of **9** and its hydrogen substituted derivative in 7/1 ratio.
15. $[\alpha]_D^{25}$ = −172.39 (*c* 0.77, MeOH); UV (MeOH) λ_{max} 260.0 nm; ^{19}F NMR (376 MHz, MeOH-d_4) δ −121.59; ^1H NMR (400 MHz, MeOH-d_4) δ 8.19 (s, 1H, 8-H), 8.18 (s, 1H), 5.63 (m, 1H), 5.60 (m, 1H), 4.75 (m, 1H), 4.68 (td, J = 1.2, 6.0 Hz, 1H); ^{13}C NMR (100 MHz, MeOH-d_4) δ 163.67, 163.33, 162.12, 159.26, 157.42, 153.82, 151.15, 142.07, 120.65, 110.11 (d, J = 4.9 Hz), 76.56 (d, J = 4.2 Hz), 70.71 (d, J = 10.4 Hz), 62.93 (d, J = 18.2 Hz). Anal. Calcd for $C_{10}H_{10}FN_5O_2$: C, 47.81; H, 4.01; N, 27.88. Found: C, 47.88; H, 4.11; N, 27.54.