Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Structure–Activity Relationships of Neplanocin A Analogues as *S*-Adenosylhomocysteine Hydrolase Inhibitors and Their Antiviral and Antitumor Activities

Girish Chandra,[†,‡,∥] Yang Won Moon,[§,∥] Yoonji Lee,[§,∥] Ji Yong Jang,[§] Jayoung Song,[†] Akshata Nayak,[†] Kawon Oh,[†,§] Varughese A. Mulamoottil,[§] Pramod K. Sahu,[†] Gyudong Kim,[†] Tong-Shin Chang,[§] Minsoo Noh,[†] Sang Kook Lee,[†] Sun Choi,*[,§] and Lak Shin Jeong*[,†]

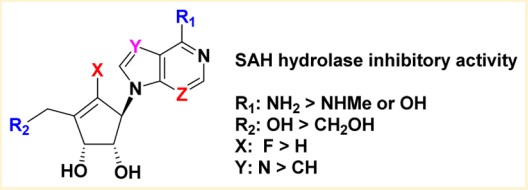
[†]Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Korea

[‡]Department of Chemistry, School of Chemical and Physical Sciences, Central University of Bihar, Gaya, Bihar, 823001, India

[§]College of Pharmacy, Graduate School of Pharmaceutical Sciences, Ewha Womans University, Seoul 120-750, Korea

Supporting Information

ABSTRACT: On the basis of the potent inhibitory activity of neplanocin A (**1**) against *S*-adenosylhomocysteine (AdoHcy) hydrolase, we analyzed the comprehensive structure–activity relationships by modifying the adenine and carbasugar moiety of **1** to find the pharmacophore in the active site of the enzyme. The introduction of 7-deazaadenine instead of adenine eliminated the inhibitory activity against the AdoHcy hydrolase, while 3-deazaadenine maintained the inhibitory activity of the enzyme, indicating that N-7 is essential for its role as a hydrogen bonding acceptor. The substitution of hydrogen at the 6′-position with fluorine increased the inhibitory activity of the enzyme. The one-carbon homologation at the 5′-position generally decreased the inhibitory activity of the enzyme, indicating that steric repulsion exists. A molecular docking study also supported these experimental data. In this study, 6′-fluoroneplanocin A (**2**) was the most potent inhibitor of AdoHcy hydrolase (IC_{50} = 0.24 μM). It showed a potent anti-VSV activity (EC_{50} = 0.43 μM) and potent anticancer activity in all the human tumor cell lines tested.



INTRODUCTION

S-Adenosyl-L-homocysteine (AdoHcy) hydrolase catalyzes the interconversion of AdoHcy to adenosine and L-homocysteine (Hcy).[1,2] The inhibition of AdoHcy hydrolase accumulates AdoHcy in the cell, which triggers the negative feedback inhibition to suppress the *S*-adenosyl-L-methionine (SAM) dependent transmethylation.[1,2] Because SAM-dependent transmethylation plays an essential role in forming the capped methylated structure at the 5′-terminus of viral mRNA, AdoHcy hydrolase has been an attractive target for the development of a broad-spectrum of antiviral agents.[3−5]

Neplanocin A (**1**)[6] is a representative carbocyclic nucleoside and is known to be one of the most potent inhibitors against AdoHcy hydrolase, and it exhibits potent and wide-ranging antiviral activities (Figure 1).[7]

However, despite the potent antiviral activities against several RNA and DNA viruses, its high cytotoxicity has hindered it from being further developed as a clinical agent.[5,7] To search for new carbocyclic nucleosides with a therapeutic index better than **1**, many modifications have been made on the carbasugar ring and on the purine base. Among these, fluoroneplanocin A (**2**) was discovered as a highly potent inhibitor of AdoHcy hydrolase, exhibiting both the type I mechanism based reversible cofactor (NAD) depletion and the type II mechanism based irreversible inhibition.[8]

Recently, 7-deazaneplanocin A (**3**) was reported to show potent antiviral activity against the hepatitis C virus (HCV) and cowpox along with enzymatic stability, indicating that the 7-deazaadenine moiety may serve as a good template for the design of antiviral agents.[9,10] Additionally, 3-deazaneplanocin A (**4**) is a powerful inhibitor of AdoHcy hydrolase with potent and selective in vitro and in vivo antiviral activities.[11] The one-carbon homologated analogues of **1** also exhibited a potent inhibitory activity against AdoHcy hydrolase with potent antiviral activities.[12]

Thus, it is of great interest to determine the systematic structure–activity relationships of AdoHcy hydrolase inhibitors by modifying the 5′ or 6′ position of the carbasugar moiety and/or the N^6, 3, or 7 position of the purine moiety of the carbocyclic nucleosides, **1**–**4**. Herein, we report the stereoselective synthesis of the neplanocin A analogue **5**, using stereoselective epoxidation, the regio- and stereoselective fluorination, and their AdoHcy hydrolase inhibitory activity along with their antiviral and anticancer activities.[13]

Received: April 8, 2015

DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

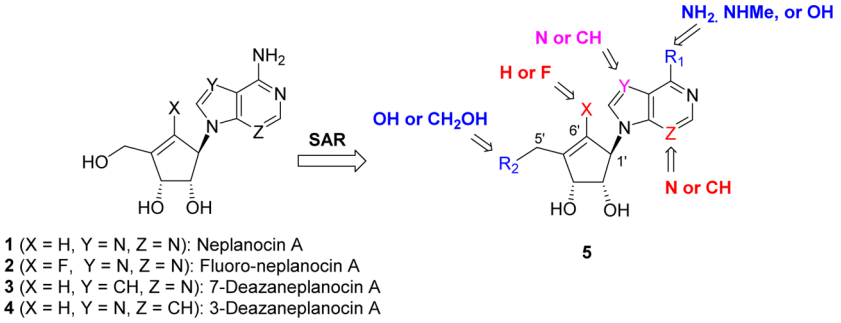
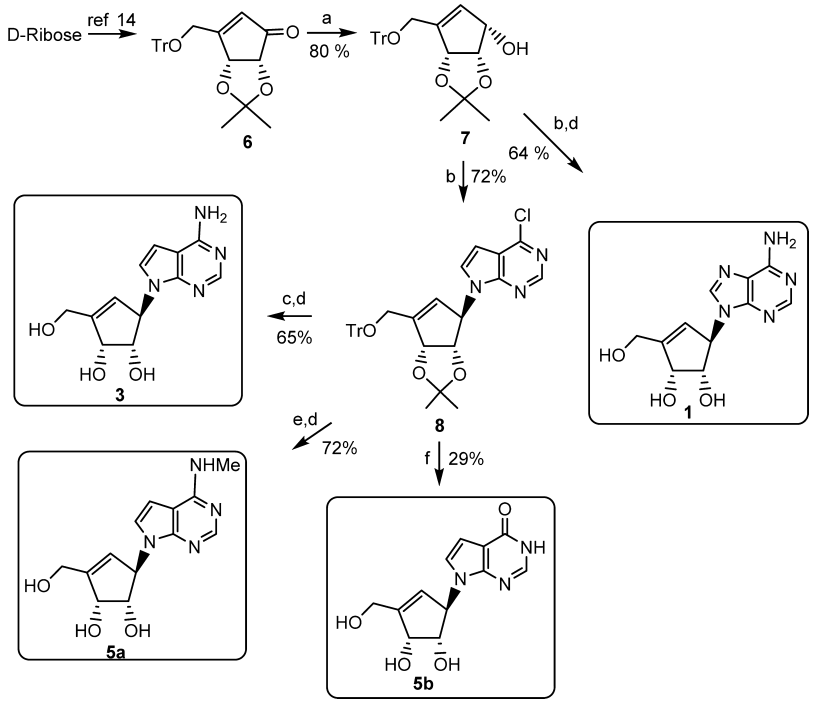


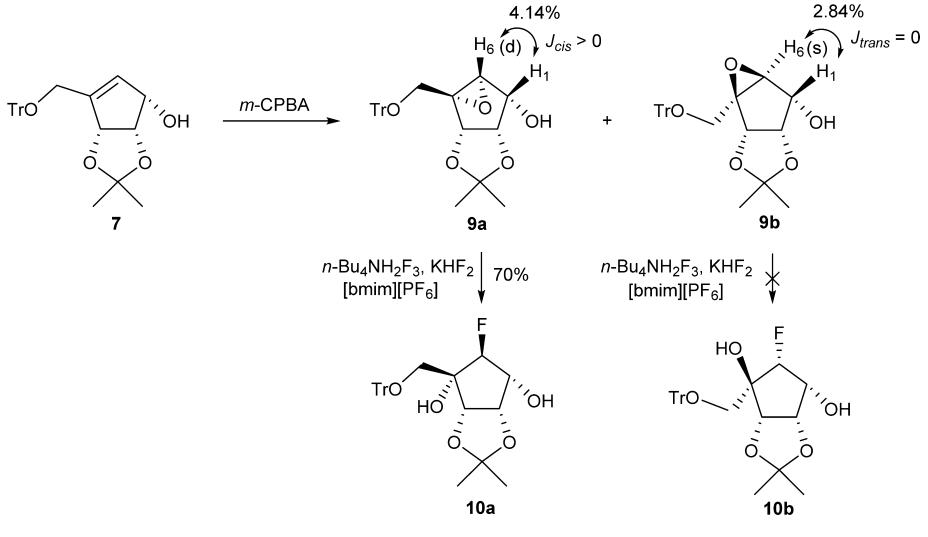
Figure 1. Rationale for the design of the target nucleoside **5**.

Scheme 1[a]



[a]Reagent and conditions: (a) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, 0 °C to rt, 1 h; (b) adenine (for **1**) or 7-deaza-6-chloropurine (for **3**), Ph_3P, DIAD, THF, 0 °C to rt, 15 h; (c) NH_3, MeOH, 100 °C, 24 h for **1**; NH_3, MeOH, 120 °C, 40 h for **3**; (d) 2 N HCl, THF, MeOH, 55 °C, 15 h followed by $NaHCO_3$ solution; (e) $MeNH_2$ (40% in H_2O), MeOH, 80 °C, 15 h; (f) 1,3-dioxane, 1 N HCl, 100 °C, 50 h.

Scheme 2



DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

RESULTS AND DISCUSSION

Chemistry. First, we synthesized the 7-deazaneplanocin A analogues, **5a** and **5b**, and the known analogues, **1** and **3**, starting from the key intermediate **6**, which was efficiently synthesized from D-ribose according to our previously published procedure (Scheme 1).[14]

The key intermediate **6** was reduced with $NaBH_4$ in the presence of $CeCl_3 \cdot 7H_2O$ to give the glycosyl donor **7** as a single diastereomer. The condensation of **7** with adenine under the standard Mitsunobu conditions was followed by the removal of the protecting groups under acidic conditions to yield **1**.[6] The glycosyl donor **7** was also condensed with 3-deaza-6-chloropurine to produce the protected nucleoside **8**. The treatment of **8** with methanolic ammonia was followed by acidic hydrolysis to yield **3**.[10] Compound **8** was then converted to the 7-deaza-N^6-methylneplanocin A (**5a**) by treating it with a 40% methylamine solution that was followed by acidic hydrolysis. Compound **8** was also converted to the 7-deazahypoxanthine derivative **5b** via heating with 1 N HCl in 1,4-dioxane.

Second, we synthesized compound **2** and 6′-fluoro-7-deazaneplanocin A derivatives **5c−e** by employing stereoselective epoxidation followed by regio- and stereoselective nucleophilic fluorination as the key steps, as shown in Scheme 2.

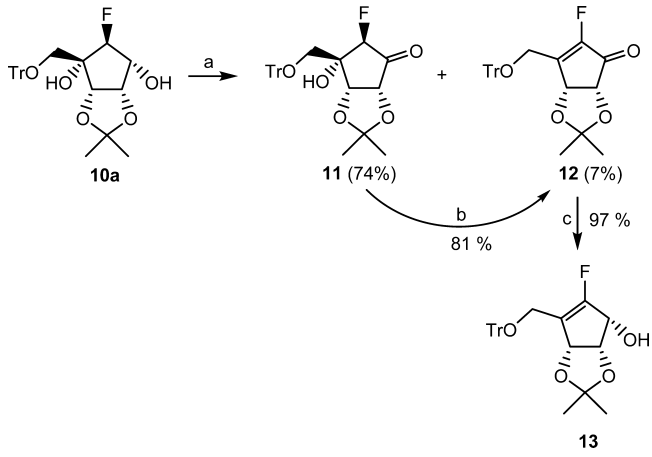
For the regio- and stereoselective nucleophilic fluorination, α-epoxide **9a** was favored over β-epoxide **9b** because of the steric factor. The epoxidation of the α-allylic alcohol **7** was expected to produce α-epoxide **9a** as a major product, based on the directing effect[15] of the α-hydroxyl group; however, the epoxidation with *m*-CPBA at room temperature yielded the desired α-epoxide **9a** with a modest degree of selectivity (**9a**/**9b** = 3/1, 96% total yield was based on the recovered starting material). This result indicated that the steric factor created by the bulky trityl group competed with the electronic factor of the α-hydroxyl group. There have been many attempts to improve the selectivity of the epoxidation. Because of the acid-labile trityl protecting group, the epoxidation with *m*-CPBA was conducted in the presence of $NaHCO_3$ to neutralize the *m*-chlorobenzoic acid, which is a byproduct of the reaction. This condition resulted in an improved total yield (80%) but a poor selectivity (**9a**/**9b** = 1.67/1). The configurations of two diastereomeric epoxides **9a** and **9b** were confirmed via the careful analysis of the ^{1}H NMR data and 1D NOE experiments. Two isomers showed the typical diagnostic coupling constants of the boat conformation of the bicyclo[3.1.0]hexane system, which has previously been elucidated using X-ray crystallography and ^{1}H NMR experiments.[16] The $J_{H1',H6}$ value in the α-epoxide **9a** should be greater than zero because of the cis relationship, while the corresponding coupling constant in the β-epoxide **9b** must be zero because of the $H_1-C-C-H_6$ dihedral angle that has a trans configuration close to 90°. Thus, the H-6 in **9a** should split into a doublet, whereas the H-6 in **9b** should appear as a singlet. Indeed, the ^{1}H NMR analysis of **9a** and **9b** indicated that the H-6 of **9a** was split into a doublet at δ 3.43 and the H-6 of **9b** was a singlet at δ 3.64. In the 1D NOE experiments, irradiation of the H-6 in **9a** enhanced the H-1 proton peak by 4.14%, while the same irradiation in **9b** produced only a 2.84% enhancement. This experiment confirmed that the H-1 and H-6 of **9a** are in a cis configuration, while those of **9b** are in a trans configuration.

After the synthesis of the epoxides, **9a** and **9b**, we attempted the nucleophilic opening of the epoxide with fluoride by employing the same conditions used in the synthesis of **2**.[13] However, unlike the successful openings of the one-carbon homologated α- and β-epoxides with fluoride (n-$Bu_4NH_2F_3$, KHF_2), the same fluorination reactions of **9a** and **9b** did not give the desired fluorinated compounds, **10a** and **10b**, respectively. The bulky trityl protecting group may have prevented the approach of the fluoride anion to the epoxide.

To circumvent this problem, we increased the nucleophilicity of the fluoride by changing the polarity of the solvent. To increase the polarity of the solvent, we used a room temperature ionic liquid (RTIL)[17] as the polar solvent in the fluorination reaction. The treatment of the α-epoxide **9a** with n-$Bu_4NH_2F_3$ and KHF_2 in the presence of 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]) at 140 °C in a glass seal tube produced the desired fluorinated derivative **10a** but in only a 19% yield. The low yield was attributed to the decomposition of the ionic liquid at high temperature; thus, we changed the ionic liquid [bmim][BF_4] to 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF_6]), which produced the desired fluoro derivative **10a** in a 70% yield. However, the β-epoxide **10b** was totally inert under various reaction conditions, such as the use of different ionic liquids, the increase of the reaction temperature, and microwave irradiation. The lack of reactivity may be attributed to the steric hindrance caused by the bulky trityl and the isopropylidene groups that make it difficult for the incoming fluoride nucleophile to approach from the concave side.

The fluorodiol **10a** was converted to another glycosyl donor **13**, as illustrated in Scheme 3. The oxidation of **10a** with

Scheme 3[a]



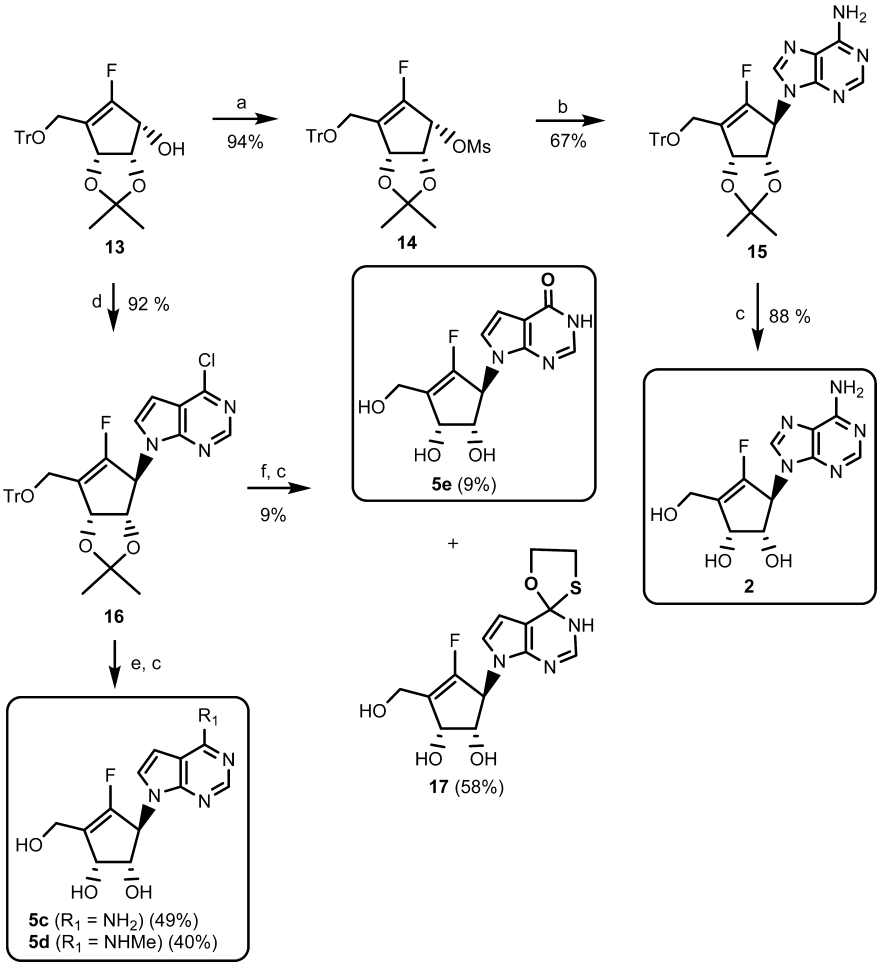
[a]Reagents and conditions: (a) DMSO, EDCI, TFAA, pyridine, benzene, 0 °C to rt, 15 h; (b) MsCl, Et_3N, MC, 0 °C, 2 min; (c) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, 0 °C to rt, 1 h.

DMSO in the presence of EDCI produced β-hydroxyketone **11** as the major product (74%) and α,β-unsaturated ketone **12** as the minor product (7%). Compound **11** was smoothly converted to **12** by treating it with MsCl and triethylamine.[13] The Luche[18] reduction of **12** yielded the glycosyl donor **13**, which can readily condense with the purine bases.

For the synthesis of compound **2**, the glycosyl donor **13** was first condensed with 6-chloropurine or adenine using the Mitsunobu conditions; however, this failed to produce the desired condensed product in a satisfactory yield (Scheme 4).

DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 4[a]



[a]Reagent and conditions: (a) MsCl, Et_3N, MC, 0 °C, 40 min; (b) adenine, Cs_2CO_3, DMSO, 80 °C, 15 h; (c) 2 N HCl, THF, MeOH, 55 °C, 15 h; (d) 7-deaza-6-chloropurine, Ph_3P, DIAD, THF, 0 °C to rt, 15 h; (e) NH_3, MeOH, 120 °C, 40 h for **5c** and $MeNH_2$ (40% in H_2O), MeOH, 80 °C, 15 h for **5d**; (f) $HSCH_2CH_2OH$, NaOMe, MeOH, 80 °C, 15 h.

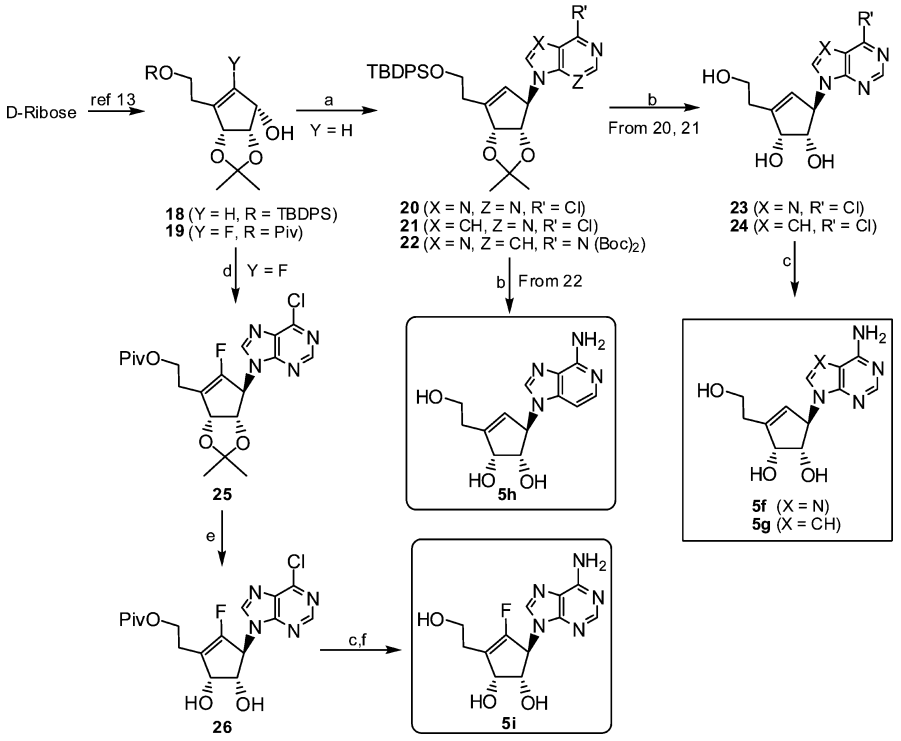
Thus, the direct S_N2 condensation using the mesylate **14** was employed. The condensation of **14** with the adenine anion, prepared by treating adenine with Cs_2CO_3 in DMSO yielded the desired nucleoside **15** in good yield. The removal of the protecting groups of **15** was achieved with 2 N HCl in methanol and THF at 55 °C to give the final compound **2**.[8] Then, 7-deaza-6-chloropurine was successfully condensed with the glycosyl donor **13** under the standard Mitsunobu conditions to yield the condensed nucleoside **16** in an excellent yield. The treatment of **16** with methanolic ammonia or 40% aqueous methylamine in MeOH, followed by the acidic hydrolysis yielded the 7-deazafluoroneplanocin A (**5c**) or the 7-deaza-N^6-methyfluoroneplanocin A (**5d**), respectively. Compound **16** was converted to the desired 7-deazahypoxanthine derivative **5e**, in addition to the formation of the undesired oxathiolanyl derivative **17**, by treating with 2-mercaptoethanol and sodium methoxide at 80 °C, followed by the removal of the protecting groups under acidic conditions. The structure of **17** was confirmed using ^{1}H NMR, which exhibited four extra protons at δ 3.51 (t, J = 6.4 Hz, 2 H) and δ 3.85 (t, J = 6.4 Hz, 2 H).

The homoneplanocin A analogues, **5f−i** were synthesized from D-ribose as shown in Scheme 5. D-Ribose was converted to the glycosyl donors, **18** and **19**, according to our previously reported procedure.[13] The condensation of **18**[13] with 6-chloropurine or 7-deaza-6-chloropurine using the Mitsunobu conditions yielded the condensed derivative, **20** or **21**, respectively. These compounds were then treated with trifluoroacetic acid (TFA), and the resulting 6-chloro derivatives **23** and **24** with ammonia in *t*-BuOH to yield homoneplanocin A (**5f**)[13] and 7-deazahomoneplanocin A (**5g**), respectively. The glycosyl donor **18** was also condensed with *N*,*N*-$(BOC)_2$-3-deazaadenine to produce the protected nucleoside **22**, which was converted to the final 3-deazahomoneplanocin A (**5h**), using a similar procedure as in the preparation of **5f** and **5g**. The condensation of **19** with 6-chloropurine produced the 6-chloro derivative **25**, whose protecting groups were removed to give **26**. Finally, the treatment of **26** with ammonia in *t*-BuOH yielded fluorohomoneplanocin A (**5i**). However, the desired 3-deazafluorohomoneplanocin A was not obtained because the condensation of **19** with 3-deaza-6-chloropurine,[11] 6-azido-3-deazapurine,[19] or *N*,*N*-$(BOC)_2$-3-deazaadenine[19] resulted in almost exclusive formation of the N^7-derivatives. The N^7-regioisomers were confirmed by comparing their UV or NOE data with those of the reported N^7-isomers.[11,19]

AdoHcy Hydrolase Inhibitory Activity. All the final compounds **1−5** were assayed for inhibitory activity against

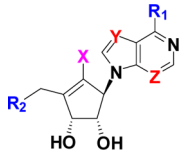
DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 5[a]



[a]Reagent and conditions: (a) 6-chloropurine or 7-deaza-6-chloropurine or 3-deaza-*N*-BOC-adenine, Ph_3P, DIAD, THF, 0 °C to rt, 15 h; (b) TFA, THF, rt, 18 h; (c) NH_3, *t*-BuOH, 120 °C, 24 h; (d) 6-chloropurine, Ph_3P, DIAD, THF, 0 °C to rt, 15 h; (e) 2 N HCl, THF, MeOH, 55 °C, 15 h; (f) NaOMe, MeOH, 45 °C, 4 h.

Table 1. AdoHcy Hydrolase Inhibitory Activity of the Neplanocin A Analogues 1−5



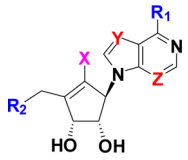
compd	X, Y, Z, R_1, R_2	IC_{50} (μM)
1 (neplanocin A)	X = H, Y = N, Z = N, R_1 = NH_2, R_2 = OH	0.47
2 (fluoroneplanocin A)	X = F, Y = N, Z = N, R_1 = NH_2, R_2 = OH	0.24
3 (7-deazaneplanocin A)	X = H, Y = CH, Z = N, R_1 = NH_2, R_2 = OH	>100
4 (3-deazaneplanocin A)	X = H, Y = N, Z = CH, R_1 = NH_2, R_2 = OH	0.44
5a (7-deaza series)	X = H, Y = CH, Z = N, R_1 = NHMe, R_2 = OH	>100
5b (7-deaza series)	X = H, Y = CH, Z = N, R_1 = OH, R_2 = OH	>100
5c (7-deaza series)	X = F, Y = CH, Z = N, R_1 = NH_2, R_2 = OH	>100
5d (7-deaza series)	X = F, Y = CH, Z = N, R_1 = NHMe, R_2 = OH	>100
5e (7-deaza series)	X = F, Y = CH, Z = N, R_1 = OH, R_2 = OH	>100
5f (homoneplanocin A)	X = H, Y = N, Z = N, R_1 = NH_2, R_2 = CH_2OH	0.48
5g (7-deazahomoneplanocin A)	X = H, Y = CH, Z = N, R_1 = NH_2, R_2 = CH_2OH	>100
5h (3-deazahomoneplanocin A)	X = H, Y = N, Z = CH, R_1 = NH_2, R_2 = CH_2OH	3.76
5i (fluorohomoneplanocin A)	X = F, Y = N, Z = N, R_1 = NH_2, R_2 = CH_2OH	0.91

AdoHcy hydrolase using the recombinant AdoHcy hydrolase protein, which was obtained from *E. coli* BL21 that harbored the plasmid pET14b containing the DNA-encoding human placental AdoHcy hydrolase (Table 1).[8] All the final compounds were preincubated with AdoHcy hydrolase at various concentrations for 10 min at 37 °C, and the hydrolytic activity of the enzyme was determined using a 5,5′-dithiobis-2-nitrobenzoate (DTNB) coupled assay as described by Lozada-Ramirez et al.[20]

The effect of the purine base modification (Y, Z) was first analyzed to determine its inhibitory activity against AdoHcy hydrolase. The 7-deazaadenine derivatives did not exhibit inhibitory activity, whereas the 3-deazaadenine and adenine derivatives exhibited potent inhibitory activity. In the 7-deazaadenine series, substitution of the N^6-amino group (R_1) with the N^6-methylamino or the 6-hydroxyl group did not improve the inhibitory activity. Additionally, the effect of the fluorine atom (X) on the sugar moiety was examined. The introduction of fluorine (X = F) at the 6′-position generally

DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 2. Anticancer Activity of the Neplanocin A Analogues 1−5



compd	IC_{50} (μM)					
	A549[a]	HCT116[b]	MDA-MB-231[c]	PC3[d]	SK-hep-1[e]	SNU-638[f]
1 (X = H, Y = N, Z = N, R_1 = NH_2, R_2 = OH)	1.22	0.9	0.4	3.7	4.5	0.7
2 (X = F, Y = N, Z = N, R_1 = NH_2, R_2 = OH)	1.20	2.8	1.4	1.4	0.8	2.2
3 (X = H, Y = CH, Z = N, R_1 = NH_2, R_2 = OH)	1.84	10.44	0.79	1.62	1.17	1.2
4 (X = H, Y = N, Z = CH, R_1 = NH_2, R_2 = OH)	1.40	0.26	0.30	9.39	0.72	0.91
5a (X = H, Y = CH, Z = N, R_1 = NHMe, R_2 = OH)	>100	>100	49.54	>100	99.18	>100
5b (X = H, Y = CH, Z = N, R_1 = OH, R_2 = OH)	>100	>100	>100	>100	>100	>100
5c (X = F, Y = CH,Z = N, R_1 = NH_2, R_2 = OH)	16.84	0.94	0.94	1.3	1.39	0.96
5d (X = F, Y = CH, Z = N, R_1 = NHMe, R_2 = OH)	>100	>100	>100	>100	>100	>100
5e (X = F, Y = CH, Z = N, R_1 = OH, R_2 = OH)	>100	>100	>100	>100	>100	>100
5f (X = H, Y = N, Z = N, R_1 = NH_2, R_2 = CH_2OH)	8.95	1.72	2.64	>100	6.12	2.31
5g (X = H, Y = CH, Z = N, R_1 = NH_2, R_2 = CH_2OH)	34.01	32.03	26.26	>100	42.09	30.19
5h (X = H, Y = N, Z = CH, R_1 = NH_2, R_2 = CH_2OH)	76.48	17.94	8.01	>100	28.89	28.16
5i (X = F, Y = N, Z = N, R_1 = NH_2, R_2 = CH_2OH)	11.70	2.90	6.95	>100	8.07	3.73
Ara-C	1.20	2.42	1.37	50.51	2.12	3.98
etoposide	0.4	1.9	7.7	ND[g]	2.8	0.3

[a]A549: human lung cancer cell line. [b]HCT-116: human colon cancer cell line. [c]MDA-MB-231: human breast cancer cell line. [d]PC3: human prostate cancer cell line. [e]SK-hep-1: human liver cancer cell line. [f]SNU-638: human stomach cancer cell line. [g]ND: not determined.

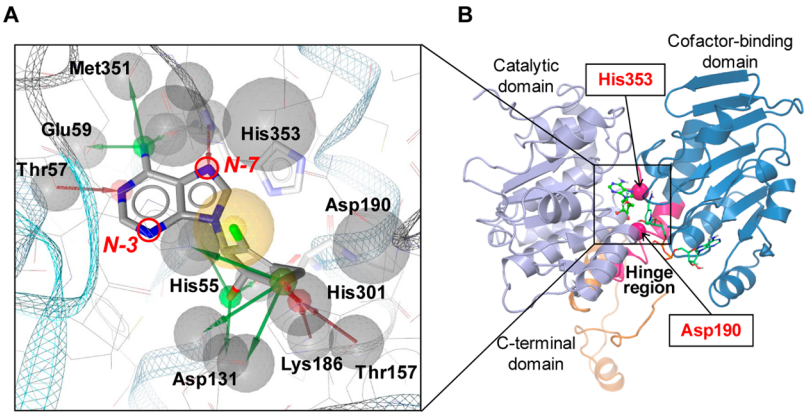


Figure 2. Structure of the AdoHcy hydrolase complexed with fluoroneplanocin A (**2**). (A) Key pharmacophores at the active site (red, hydrogen bond acceptor; blue, hydrogen bond donor; yellow, hydrophobe; gray sphere, exclusion volume). The cocrystallized ligand is displayed using sticks with the carbon color of gray, and its N-3 and N-7 positions are marked with red circles. The His353 and Asp190 residues are represented using sticks with their carbons in white. (B) Monomer structure of the enzyme. The secondary structures of the catalytic, cofactor binding, and C-terminal domains are colored in light blue, sky blue, and orange, respectively, while the hinge region is in pink. The locations of the His353 and Asp190 residues in the hinge region are depicted as the spheres. The bound ligand and cofactor are displayed as green balls-and-sticks and green blue sticks, respectively.

increased the inhibitory activity against the AdoHcy hydrolase. For example, the 6′-fluoro derivatives **2** and **5i** were more potent than the corresponding 6′-H analogues, **1** and **5f**, respectively. Finally, the effect of the homologation (R_2) was studied. Compound **2** (IC_{50} = 0.36 μM) exhibited a more potent inhibitory activity than the corresponding 5′-homo derivative **5i** (IC_{50} = 0.91 μM), indicating that homologation slightly decreased the inhibitory activity. Among the compounds tested, compound **2** exhibited the most potent inhibitory activity against the AdoHcy hydrolase.

Antiviral Activity. All of the synthesized compounds **1**−**5** were tested for antiviral activity against herpes simplex virus (HSV, Vero cell) 1 and 2, human immunodeficiency virus (HIV, MT-4 cells) 1, picornavirus (PC, CB-1 cell), and vesicular stomatitis virus (VSV, HeLa cells).[21] Half of the synthesized compounds exhibited toxicity-dependent antiviral activities. Among the compounds tested, **5c** showed potent anti-PC activity (EC_{50} = 2.34 μM) without cytotoxicity up to 100 μM, although it was inactive against AdoHcy hydrolase. However, compound **2** showed the most potent inhibitory activity against the AdoHcy hydrolase and exhibited potent anti-VSV activity (EC_{50} = 0.43 μM) without cytotoxicity up to 40 μM. Because most of the synthesized compounds exhibited high cytotoxicity in various cell lines, they were tested for anticancer activity in various cancer cell lines.

DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

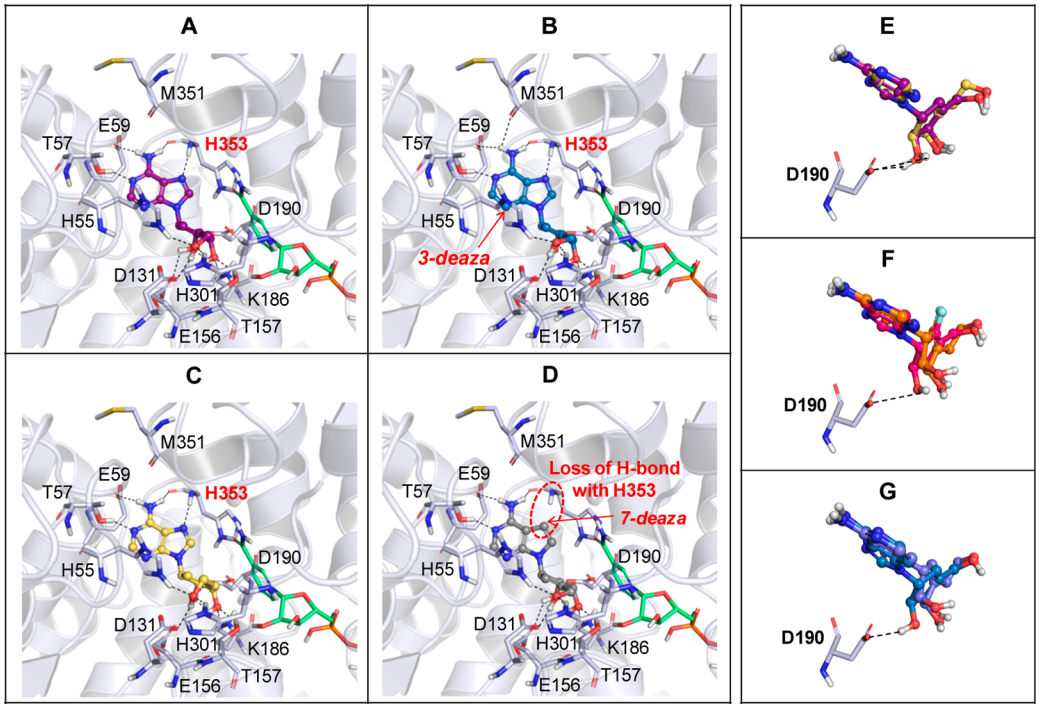


Figure 3. Binding mode comparison of the 3- or 7-deaza and the homologated neplanocin A analogues. The docked poses of compounds **1A**, **4B**, **4C**, **5f**, and **3D** are represented as balls-and-sticks, and their carbon atoms are colored in purple, sky blue, yellow, and gray, respectively. The secondary structure of the AdoHcy hydrolase is colored light blue, and the interacting residues are depicted as sticks. For a comparison of the carbocyclic ring conformation, the binding modes of (E) **1** and **5f**, (F) **2** and **5i**, and (G) **4** and **5h** are aligned. The colors of the carbon atoms of **2**, **5i**, and **5h** are in pink, orange, and slate, respectively. The H-bonds are displayed as black dashed lines, and the nonpolar hydrogens are not displayed for clarity.

Anticancer Activity. All of the synthesized neplanocin A analogues **1**−**5** were also assayed for anticancer activity against several human cell lines, such as human lung cancer cell line (A549), human colon cancer cell line (HCT-116), human breast cancer cell line (MDA-MB-231), human prostate cancer cell line (PC3), human liver cancer cell line (SK-hep-1), and human stomach cancer cell line (SNU-638).[22]

As shown in Table 2, all of the adenine, 3-deaza- and 7-deazaadenine derivatives except for the N^6-methyladenine derivatives, **5a** and **5d**, and hypoxanthine derivatives, **5b** and **5e**, exhibited potent anticancer activity in all of the human cancer cell lines tested. In the base-modified series (Y, Z), the anticancer activity is in the following order: **4** (Y = N, Z = CH) > **1** (Y = N, Z = N) > **3** (Y = CH, Z = N). Introduction of the fluorine (X = F) at the sugar moiety generally exhibited an improved anticancer activity over the corresponding hydrogen derivatives (X = H). For example, the 6′-F derivatives **2**, **5c**, and **5i** exhibited an improved anticancer activity over the corresponding 6′-H derivatives **1**, **3**, and **5f**, respectively. The one-carbon homologation at the 5′-position generally decreased the anticancer activity substantially. For example, the 5′-CH_2OH analogues **5f**, **5g**, **5h**, and **5i** were less potent than the corresponding 5′-OH analogues **1**, **3**, **4**, and **2**, respectively. Among the tested compounds, compound **4** exhibited the most potent anticancer activity against the human colon cancer (HCT116), breast cancer (MDA-MB-231), and liver cancer (SK-Hep-1) cell lines; however, compound **2** exhibited the most potent anticancer activity against the human lung cancer (A549) and prostate cancer (PC3) cell lines. Compound **1** was the most potent in the stomach cancer cell (SNU-638) line.

Molecular Modeling Study. To investigate the interactions between the AdoHcy hydrolase and the synthesized analogues, we utilized the X-ray crystal structure of AdoHcy hydrolase complexed with the most potent compound **2** (PDB code 3NJ4).[8c] The pharmacophore analysis of the complex structure clearly supports the activity differences between the 3-deaza and the 7-deaza compounds. As shown in Figure 2, the N-7 position of the ligand functions as a hydrogen bond acceptor, whereas the N-3 position does not play a role in the inhibitory activity.

For a more detailed comparison of the binding modes, flexible docking studies were performed using Glide (Schrödinger, LLC, NY, USA).

Compound **1** and its 3-deaza derivative **4**, which both showed inhibitory activities with the IC_{50} values of 0.47 μM and 0.44 μM, respectively, both bound well at the active site of the AdoHcy hydrolase with their adenine or 3-deaza adenine parts while maintaining the H-bonding interactions with Thr57, Glu59, and His353 (Figure 3A and Figure 3B). However, the 7-deaza derivative **3**, whose inhibitory activity was drastically diminished, could not maintain the H-bonding with His353 (Figure 3D). All of the 7-deaza compounds lost the H-bonding with His353 (Figure S1) and exhibited much lower docking scores compared with the neplanocin A and 3-deaza neplanocin A analogues (Figure S2). The interaction energies between the ligands and His353, especially the H-bonding and Coulombic scores, are important for their binding. Therefore, the N-7 of the adenine ring, which H-bonds with His353, functions as a critical pharmacophore for the enzyme inhibition.

For the homologated compounds, the structure−activity relationships are more complicated. The 5′-homo derivative **5f**, which has a similar inhibitory activity as compared with **1**, binds easily at the active site, retaining the H-bonding network of the adenine portion (Figure 3C). Additionally, its carbocyclic ring

DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

showed very similar conformation as **1** (Figure 3E). The region where the 5′-CH_2OH group binds is tolerable for the one-carbon homologation. However, the homologation of the fluorinated or 3-deaza analogues decreased the activities lightly. Interestingly, the docking results showed that these compounds, **5i** and **5h**, could not maintain the carbocyclic ring conformation, resulting in the loss of H-bonding with the Asp190 (Figure 3F, Figure 3G).

Thus, we confirmed that the His353 and Asp190 residues, which are located at the hinge region (Figure 2B), play critical roles in the inhibitory activities: (i) N-7 of the adenine ring H-bonds with the His353, and this interaction is essential for the activity; (ii) the one-carbon homologation at the 5′-position is tolerable at the binding site, but it reduces the activities for the fluorinated or 3-deaza analogues. This is because the H-bonding with Asp190 is critical for the inhibitory activity, and it requires a suitable carbocyclic ring conformation. These results agree with our previous work that identified these hinge region residues as hot spots for the open-to-closed domain motion,[23] which suggests their importance for enzymatic function.

CONCLUSIONS

The neplanocin A analogues **1**−**5** were synthesized from D-ribose, tested for their inhibitory activity against AdoHcy hydrolase, and tested for antiviral and anticancer activities. The introduction of fluorine at the 6′-position during the synthesis of the key fluorocarbasugars **13** and **19** was achieved by stereoselective epoxidation, stereo- and regioselective nucleophilic fluorination, and the simultaneous oxidation−elimination reaction. All of the glycosyl donors **7**, **13**, **18**, and **19** were condensed with 6-chloropurine or adenine using the Mitsunobu conditions or via the direct S_N2 reaction to give the neplanocin A analogues **1**−**5**. All of the carbocyclic nucleosides exhibited very potent inhibitory activity against AdoHcy hydrolase except for the 7-deazaneplanocin A analogues. Among these compounds, compound **2** exhibited the most inhibitory activity (IC_{50} = 0.24 μM) against the AdoHcy hydrolase. The pharmacophore analysis of the complex that was docked into the structure of the AdoHcy hydrolase clearly showed that the N-7 position of the ligand functions as a key hydrogen bond acceptor, whereas the N-3 position does not play a role in the inhibitory activity. Most of the compounds exhibited toxicity-dependent antiviral activity except for **2** (EC_{50} = 0.43 μM against VSV) and **5c** (EC_{50} = 2.34 μM against PC). All of the N^6-adenine derivatives exhibited potent anticancer activity in all of the human cancer cell lines tested, among which the 6′-F-adenine derivative **2** (X = F, Y = N, Z = N) and the 6′-H-3-deazaadenine derivative **4** (X = H, Y = N, Z = CH) exhibited the most potent anticancer activity. This comprehensive structure−activity relationship analysis study and the identification of the pharmacophore at the active site of the AdoHcy hydrolase will assist in the therapeutic development of carbocyclic nucleosides as antiviral and anticancer compounds.

EXPERIMENTAL SECTION

General Methods. ^{1}H NMR spectra ($CDCl_3$, CD_3OD, or DMSO-d_6) were recorded on Varian Unity Invoa 400 MHz instrument. The ^{1}H NMR data are reported as peak multiplicities: s for singlet, d for doublet, dd for doublet of doublets, t for triplet, q for quartet, br s for broad singlet, and m for multiplet. Coupling constants are reported in hertz. ^{13}C NMR spectra ($CDCl_3$, CD_3OD, or DMSO-d_6) were recorded on Varian Unity Inova 100 MHz instrument. ^{19}F NMR spectra ($CDCl_3$, CD_3OD) were recorded on Varian Unity Inova 376 MHz instrument. The chemical shifts were reported as parts per million (δ) relative to the solvent peak. Optical rotations were determined on Jasco III in appropriate solvent. UV spectra were recorded on a U-3000 instrument made by Hitachi in methanol or water. Infrared spectra were recorded on FT-IR (FTS-135) instrument made by Bio-Rad. Melting points were measured on a B-540 made by Buchi. Elemental analyses (C, H, and N) were used to determine purity of all synthesized compounds, and the results were within ±0.4% of the calculated values, confirming ≥95% purity. Reactions were checked with TLC (Merck precoated $60F_{254}$ plates). Spots were detected by viewing under a UV light, colorizing with charring after dipping in anisaldehyde solution with acetic acid, sulfuric acid, and methanol. Column chromatography was performed on silica gel 60 (230−400 mesh, Merck). Reagents were purchased from Aldrich Chemical Co. Solvents were obtained from local suppliers. All the anhydrous solvents were distilled over CaH_2, P_2O_5, or sodium/benzophenone prior to the reaction.

(1*S*,4*R*,5*S*)-4,5-(*O*-Isopropylidenedioxy)-3-(trityloxymethyl)-cyclopent-2-ene-1-ol (7). To a stirred solution of **6** (4.0 g, 9.38 mmol) in MeOH (80 mL, HPLC grade) were added $CeCl_3{\cdot}7H_2O$ (3.49 g, 9.38 mmol) and $NaBH_4$ (0.36 g, 9.38 mmol) at 0 °C, and the mixture was stirred at rt for 1 h, quenched with water (50 mL), and evaporated to a half volume. The mixture was extracted with EtOAC (50 mL × 3), and the combined organic layers were washed with brine (50 mL), dried over anhydrous $MgSO_4$, and evaporated. The crude residue was purified by flash column chromatography (hexanes/ethyl acetate = 4:1) to give **7** (3.20 g, 80%) as a white solid, whose spectroscopic data were identical with those of authentic sample.[14]

(1*S*,4*R*,5*S*)-1-(6-Chloro-7-deaza-purine-9-yl)-4,5-(*O*-isopropylidenedioxy)-3-(trityloxymethyl)-2-cyclopentene (8). To a stirred solution of PPh_3 (1.60 g, 6.13 mmol) in anhydrous THF (25 mL) was added DIAD (1.2 mL, 6.13 mmol) dropwise at 0 °C, and the mixture was stirred at the same temperature for 10 min. To this solution was added **7** (1.05 g, 2.45 mmol) in anhydrous THF (30 mL) at 0 °C, and the resulting solution was stirred at rt for 30 min. To this solution was added 6-chloro-7-deazapurine (0.38 g, 2.45 mmol), and the mixture was further stirred at rt for 15 h. The solvent was evaporated and the residue was purified by flash silica gel column chromatography (hexanes/ethyl acetate = 4:1) to afford **8** (0.88 g, with DIAD byproduct) as white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 3H), 1.45 (s, 3H), 3.83−3.87 (m, 1H), 4.01−4.05 (m, 1H), 4.61 (d, J = 5.6 Hz, 1H), 5.24 (d, J = 5.6 Hz, 1H), 5.89 (s, 1H), 6.02 (br s, 1H), 6.61 (d, J = 3.2 Hz, 1H), 7.08 (d, J = 3.6 Hz, 1H), 7.23−7.32 (m, 10H), 7.45−7.47 (m, 5H), 8.69 (s, 1H). HRMS (ESI) calculated for $C_{34}H_{31}ClN_3O_3$: 564.1981. Found $(M + H)^+$: 564.2054. Anal. Calcd for $C_{34}H_{31}ClN_3O_3$: C, 72.40; H, 5.36; N, 7.45. Found: C, 72.41; H, 5.43; N, 7.05.

(1*S*,2*R*,5*S*)-5-(6-Aminopurine-9-yl)-3-hydroxymethyl-cyclopent-3(4)-ene-1,2-diol (1). Compound **7** (1.00 g, 2.45 mmol) was condensed with adenine (0.798 g, 2.45 mmol), using the same procedure used in the preparation of **8** to give the trityl derivative (0.88 g, with DIAD byproduct) as white foam. To a solution of the trityl derivative (0.88 g) in MeOH and THF (10 mL, 1:1 v/v) was added 2 N HCl (0.2 mL) at 0 °C, and the mixture was heated at 55 °C for 15 h. After cooling to rt, solvents were evaporated and the residue was neutralized with saturated $NaHCO_3$ solution (5 mL). The mixture was evaporated and coevaporated with toluene and the crude residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH = 4:1) to give **1** (1.0 g, 64%) as a white solid, whose spectroscopic data were identical to those of authentic sample.[6]

(1*S*,2*R*,5*S*)-5-(7-Deaza-6-aminopurine-9-yl)-3-hydroxymethylcyclopent-3(4)-ene-1,2-diol (3). A solution of **8** (0.30 g, 0.98 mmol) in methanolic ammonia (10 mL) was heated at 120 °C for 40 h in a steel bomb. After cooling to rt, the mixture was evaporated, and the residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH = 9:1) to give the 6-amino derivative (0.17 g) as white foam. To a solution of the 6-amino derivative (0.17 g) in MeOH and THF solution (10 mL, 1:1 v/v) was added 2 N HCl (0.2 mL) at 0 °C, and the mixture was heated at 55 °C

DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

for 15 h. After cooling to rt, solvents were evaporated and the residue was neutralized with saturated $NaHCO_3$ solution (5 mL). The mixture was evaporated and coevaporated with toluene and the crude residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH = 4:1) to give **3** (0.17 g, 65%) as a white solid, whose spectroscopic data were identical to those of authentic sample.[10]

(1*S*,2*R*,5*S*)-5-(6-*N*-Methylamine-7-deazapurine-9-yl)-3-hydroxymethylcyclopent-3(4)-ene-1,2-diol (5a). To a solution of **8** (0.28 g, 0.92 mmol) in MeOH (20 mL) was added aqueous $MeNH_2$ (20 mL, 40% solution in H_2O), and the mixture was heated at 80 °C for 15 h in a steel bomb. Removal of the protecting groups using the same procedure used in the preparation of **3** and purification by silica gel column chromatography (CH_2Cl_2/MeOH = 4:1) afforded **5a** (0.1 g, 72%) as a white solid: mp 168−169 °C; $[\alpha]_D^{25}$ −90.8° (*c* 0.12, CH_3OH); UV (MeOH) λ_{max} 275.5 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.06 (s, 3H), 4.19 (pseudo t, *J* = 5.6 Hz, 1H), 4.29−4.30 (m, 2H), 4.59 (d, *J* = 6.8 Hz, 1H), 5.61 (m, 1H), 5.82−5.83 (m, 1H), 6.56 (d, *J* = 3.6 Hz, 1H), 7.03 (d, *J* = 3.6 Hz, 1H), 8.15 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 28.9, 61.1, 66.6, 75.1, 80.2, 100.9, 105.9, 123.4, 127.6, 150.8, 151.3, 153.0, 159.4. HRMS (ESI) calculated for $C_{13}H_{16}N_4O_3$: 276.1225. Found (2M + Na)$^+$: 575.2340. Anal. Calcd for $C_{13}H_{16}N_4O_3$: C, 56.51; H, 5.84; N, 20.28. Found: C, 56.87; H, 5.54; N, 20.01.

(1*S*,2*R*,5*S*)-5-(7-Deazahypoxanthine-9-yl)-3-hydroxymethylcyclopent-3(4)-ene-1,2-diol (5b). To a solution of **8** (0.30 g, 0.98 mmol) in 1,4-dioxane (15 mL) was added 1 N HCl (10 mL), and the mixture was heated at 100 °C for 50 h. After evaporating solvents, the residue was neutralized with saturated $NaHCO_3$ solution (20 mL) and evaporated. The residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH = 6:1) to give **5b** (0.4 g, 29%) as a white solid: mp 242−243 °C; $[\alpha]_D^{25}$ −34.0° (*c* 0.10, CH_3OH); UV(MeOH) λ_{max} 261.0 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 4.21 (t, *J* = 5.7 Hz, 1H), 4.31−4.32 (m, 2H), 4.60−4.61 (m, 1H), 5.67 (br s, 1H), 5.83 (d, *J* = 1.5 Hz, 1H), 6.70 (d, *J* = 3.5 Hz, 1H), 7.08 (d, *J* = 3.6 Hz, 1H), 7.96 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD + D_2O) δ 60.9, 66.7, 74.9, 80.2, 104.4, 110.2, 123.9, 127.7, 144.7, 150.1, 150.9, 162.4. HRMS (ESI) calculated for $C_{12}H_{13}N_3O_4$: 263.0906. Found (2M + Na)$^+$: 549.1703. Anal. Calcd for $C_{12}H_{13}N_3O_4$: C, 54.75; H, 4.98; N, 15.96. Found: C, 54.56; H, 5.04; N, 15.58.

(1*R*,2*S*,3*R*,4*R*,5*R*)-4,5-(*O*-Isopropylidenedioxy)-2(3)oxirane-3-(trityloxymethyl)cyclopent-1-ol (9a) and (1*R*,2*R*,3*R*,4*R*,5*S*)-4,5-(*O*-Isopropylidenedioxy)-2(3)oxirane-3-(trityloxymethyl)-cyclopent-1-ol (9b). To a suspension of **7** (4.03 g, 9.40 mmol) and $NaHCO_3$ (1.60 g) in anhydrous CH_2Cl_2 (60 mL) was added *m*-CPBA (6.45 g, 37.4 mmol) in anhydrous CH_2Cl_2 (60 mL) at 0 °C, and the reaction mixture was stirred at rt for 72 h and quenched with saturated $NaHCO_3$ solution (100 mL). The aqueous layer was extracted with methylene chloride, and the organic layer was washed with brine (50 mL), dried over anhydrous $MgSO_4$, and evaporated. The residue was purified by flash silica gel column chromatography (hexanes/ethyl acetate = 3:1) to give the α-epoxy derivative **9a** (2.2 g, 50%) as a white foam and the β-epoxy derivative **9b** (0.82 g, 17%) as a white foam with recovered starting material **7** (1.0 g).

Compound ***9a.*** $[\alpha]_D^{25}$ + 30.7° (*c* 8.25, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (s, 3H), 1.58 (s, 3H), 2.77 (d, *J* = 11.2 Hz, AB pattern, 1H), 3.33 (d, *J* = 11.2 Hz, AB pattern, 1H), 3.43−3.44 (m, 1H), 3.52 (d, part of AB pattern, *J* = 11.2 Hz, 1H), 4.09−4.16 (m, 1H), 4.52 (dt, J_1 = 6.8, J_2 = 0.8 Hz, 1H), 4.72 (d, *J* = 6.8 Hz, 1H), 7.22−7.32 (m, 10H), 7.41−7.44 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.2, 26.6, 61.4, 66.1, 66.6, 67.6, 70.0, 79.1, 87.3, 114.7, 127.4, 128.1, 128.8, 143.9. HRMS (ESI) calculated for $C_{28}H_{28}NaO_5$: 467.1829. Found (M + Na)$^+$: 467.1826. Anal. Calcd for $C_{28}H_{28}O_5$: C, 75.65; H, 6.35. Found: C, 75.54; H, 6.45.

Compound ***9b.*** $[\alpha]_D^{25}$ +8.0° (*c* 9.35, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 3H), 1.42 (s, 3H), 2.81 (d, *J* = 5.2 Hz, 1H), 3.20 (d, *J* = 10.8 Hz, 1H), 3.64 (s, 1H), 3.73 (d, *J* = 10.1 Hz, 1H), 4.10 (t, *J* = 5.6 Hz, 1H), 4.54 (t, *J* = 5.6 Hz, 1H), 4.82 (dd, J_1 = 5.6 Hz, J_2 = 0.8 Hz, 1H), 7.21−7.31 (m, 10H), 7.45−7.47 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.9, 26.4, 60.6, 64.1, 68.1, 68.6, 79.8, 80.3, 113.4, 127.2, 128.1, 128.3, 128.7, 129.0, 143.9. HRMS (ESI) calculated for $C_{28}H_{28}O_5Na$: 467.193. Found 467.1823 (M + Na)$^+$. Anal. Calcd for $C_{28}H_{28}O_5$: C, 75.65; H, 6.35. Found: C, 75.89; H, 6.67.

(1*S*,2*S*,3*R*,4*R*,5*S*)-2-Fluoro-4,5-(*O*-isopropylidenedioxy)-1-(trityloxymethyl)cyclopent-1,3-diol (10). To a solution of **9a** (1.4 g, 3.15 mmol) in dry DMF (2 mL) were added 1-butyl-3-methylimidazolium hexafluorophosphate (2 mL), KHF_2 (0.62 g, 7.88 mmol), and tetrabutylammonium dihydrogen trifluoride (2.38 g, 7.88 mmol), and the mixture was heated in a glass sealed tube at 140 °C for 72 h. After cooling to rt, the reaction mixture was diluted with water (50 mL) and extracted with EtOAc (50 mL × 3). The combined organic layers were washed with brine (25 mL), dried over anhydrous $MgSO_4$, and evaporated. The crude residue was purified by flash silica gel column chromatography (hexanes/ethyl acetate = 2:1) to give **10** (1.13 g, 70%) as a white foam: $[\alpha]_D^{25}$ −9.5° (*c* 0.70, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35 (s, 3H), 1.52 (s, 3H), 2.59 (d, *J* = 9.6 Hz, 1H), 2.94 (s, 1H), 3.24 (dd, *J* = 2.8, 9.6 Hz, 1H), 3.40 (dd, *J* = 0.8, 9.6 Hz, 1H), 4.22 (dd, *J* = 2.8, 6.0 Hz, 1H), 4.32−4.41 (m, 1H), 4.53 (dt, *J* = 2.0, 6.0 Hz, 1H), 4.70 (dd, *J* = 7.2, 7.6 Hz, 1H), 7.23−7.33 (m, 9 H), 7.40−7.43 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.6, 26.2, 63.9 (d, J_{C-F} = 6.0 Hz), 71.8 (d, J_{C-F} = 22.2 Hz), 76.0, 76.2, 79.6 (d, J_{C-F} = 18.4 Hz), 88.0, 103.8 (d, J_{C-F} = 193.1 Hz), 112.3, 127.5, 128.8, 128.9, 143.5; ^{19}F NMR (376 MHz) δ −219. HRMS (ESI) calculated for $C_{28}H_{29}FO_5$: 487.2003. Found (M + Na)$^+$: 487.1895. Anal. Calcd for $C_{28}H_{29}FO_5$: C, 72.40; H, 6.29. Found: C, 72.76; H, 6.01.

(2*R*,3*S*,4*S*,5*R*)-2-Fluoro-3-hydroxy-4,5-(*O*-isopropylidene-dioxy) -3-(trityloxymethyl)cyclopentanone (11) and (4*R*,5*R*)-2-Fluoro-4,5-(*O*-isopropylidenedioxy)-3-(trityloxymethyl)-cyclopent-2-ene-1-one (12). To a stirred solution of **10** (0.56 g, 1.21 mmol) in dry DMSO (10 mL) and benzene (12 mL) were added EDCI (0.70g, 3.63 mmol), pyridine (0.15 mL, 1.81 mmol), and TFAA (0.097 mL, 1.81 mmol), and the reaction mixture was stirred at rt for 15 h, quenched with water (15 mL), and extracted with EtOAc (30 mL). The organic layer was washed with brine (10 mL), dried over anhydrous $MgSO_4$, and evaporated. The crude residue was purified by flash silica gel column chromatography (hexanes/ethyl acetate = 5:1 to 3:1) to give **11** (0.411 g, 74%) and **12** (0.04 g, 7%) as colorless liquids.

Compound ***11.*** $[\alpha]_D^{25}$ −66.8° (*c* 0.25, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (s, 3H), 1.48 (s, 3H), 2.79 (s, 1H), 2.99 (dd, *J* = 2.0, 8.8 Hz, 1H), 3.77 (d, *J* = 8.8 Hz, 1H), 4.03 (dd, *J* = 4.4, 6.4 Hz, 1H), 4.38−4.40 (m, 1H), 5.32 (dd, *J* = 0.8, 49.6 Hz, 1 H), 7.22−7.26 (m, 4H), 7.29−7.36 (m, 11H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.0 (d, J_{C-F} = 3 Hz), 26.2 (d, J_{C-F} = 2.9), 62.4 (d, J_{C-F} = 6.6), 63.1(d, J_{C-F} = 4.4), 78.8−78.9 (m), 89.4, 94.6 (dd, J_{C-F} = 20.6, 26.4 Hz), 113.5 (d, J_{C-F} = 25.6), 127.3, 127.4, 128.0, 128.1, 128.6, 142.3, 146.9, 200.2 (d, J_{C-F} = 13.1); ^{19}F NMR (376 MHz) δ −223.66 . HRMS (ESI) calculated for $C_{28}H_{27}FO_5Na$: 485.1841. Found (M + Na)$^+$: 485.1734. Anal. Calcd for $C_{28}H_{27}FO_5$: C, 72.71; H, 5.88. Found: C, 72.78; H, 5.90.

Compound ***12.*** $[\alpha]_D^{25}$ +6.6° (*c* 0.30, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 3H), 1.41 (s, 3H), 4.13 (dd, *J* = 16.0, 31.6 Hz, 1H), 4.23 (dd, *J* = 2.0, 15.2 Hz, 1H), 4.41 (*J* = 2.4, 6.0 Hz, 1H) 5.21 (pseudo t, *J* = 5.6 Hz, 1H), 7.23−7.33 (m, 10 H), 7.47−7.50 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.4, 27.9, 58.3 (d, J_{C-F} = 1.5 Hz), 74.0 (d, J_{C-F} = 6.6), 74.8 (d, J_{C-F} = 6.6), 88.0, 115.6, 127.6, 128.1, 128.3, 128.8, 143.5, 146.4, 153.0 (d, J_{C-F} = 289.1 Hz), 192.4 (d, J_{C-F} = 16.9); ^{19}F NMR (376 MHz) δ −136.76. HRMS (ESI) calculated for $C_{28}H_{25}FO_4Na$: 467.1734. Found (M + Na)$^+$: 467.1626. Anal. Calcd for $C_{28}H_{25}FO_4$: C, 75.66; H, 5.67. Found: C, 75.98; H, 5.32.

(4*R*,5*R*)-2-Fluoro-4,5-(*O*-isopropylidenedioxy)-3-(trityloxymethyl)cyclopent-2-ene-1-one (12). To a stirred solution of **11** (0.4 g, 0.866 mmol) in methylene chloride (20 mL) was added Et_3N (0.49 mL, 3.463 mmol), and the mixture was stirred at rt for 10 min. To this solution was added a solution of MsCl (0.15 mL, 0.952 mmol) in anhydrous methylene chloride (10 mL) at −5 °C, and the mixture was stirred at the same temperature for 5 min, quenched with water (10 mL), and extracted with methylene chloride (25 mL). The organic layer was washed with brine (15 mL), dried over anhydrous $MgSO_4$, and evaporated. The crude residue was purified by flash silica gel column chromatography (hexanes/ethyl acetate = 3:1)

DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

to give **12** (0.38 g, 81%) as a colorless liquid, whose spectroscopic data were identical with those of authentic sample.[8]

(1*R*,4*R*,5*S*)-2-Fluoro-4,5-(*O*-isopropylidenedioxy)-3-(trityloxymethyl)cyclopent-2-ene-1-ol (13). To a solution of **12** (1.23 g, 2.76 mmol) in MeOH (25 mL) were added $CeCl_3 \cdot 7H_2O$ (1.02 g, 274 mmol) and $NaBH_4$ (0.1 g, 263 mmol) at 0 °C, and the mixture was stirred at rt for 1 h. The reaction mixture was quenched with water (30 mL) and evaporated to half volume. The residue was diluted with water (20 mL) and extracted with ethyl acetate (50 mL × 2). The combined organic layers were washed with brine (30 mL), dried over anhydrous $MgSO_4$, and evaporated. The crude residue was purified by flash silica gel column chromatography (hexanes/ethyl acetate = 3:1) to give **13** (1.195 g, 97%) as a colorless thick oil: $[\alpha]_D^{25}$ +19.4° (*c* 5.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 3H), 1.42 (s, 3H), 2.78 (d, *J* = 9.6 Hz, 1H), 3.74−3.78 (m, 1H), 3.91 (d, *J* = 12.0 Hz, 1H), 4.38−4.42 (m, 1H), 4.68 (superimposed ddd, *J* = 3.6, 9.6 Hz, 1H), 5.12 (pseudo t, *J* = 6.0 HZ, 1H), 7.21−7.31 (m, 9H), 7.45−7.48 (m, 6H); ^{13}C (100 MHz, $CDCl_3$) δ 26.8, 27.9, 56.4, 69.3 (d, J_{C-F} = 20.9 Hz), 74.0 (d, J_{C-F} = 7.7 Hz), 78.9 (d, J_{C-F} = 9.3 Hz), 87.3, 112.5, 115.9 (d, J_{C-F} = 4.6 Hz), 127.3, 128.0, 128.8, 143.9, 157.9 (d, J_{C-F} = 287.9 Hz); ^{19}F NMR (376 MHz) δ −128.34. HRMS (ESI) calculated for $C_{28}H_{27}FO_4Na$: 469.1890. Found $(M + Na)^+$: 469.1783. Anal. Calcd for $C_{28}H_{27}FO_4$: C, 75.32; H, 6.09. Found: C, 75.87; H, 6.49.

(1*S*,4*R*,5*S*)-1-(6-Aminopurine-9-yl)-2-fluoro-4,5-(*O*-isopropylidenedioxy)-3-(trityloxymethyl)-2-cyclopentene (15). To a stirred solution of **13** (0.1 g, 0.22 mmol) in anhydrous methylene chloride (10 mL) were added Et_3N (0.09 mL, 0.67 mmol) and MsCl (0.09 mL, 0.34 mmol) at 0 °C, and the reaction mixture was stirred at rt for 40 min, quenched with water (10 mL), and extracted with methlyene chloride (20 mL). The organic layer was washed with saturated $NaHCO_3$ solution (10 mL), dried over anhydrous $MgSO_4$, and evaporated. The crude residue was purified by flash silica gel column chromatography (hexanes/ethyl acetate = 4:1) to give **14** (0.11 g, 94%) as colorless oil, which was used for the next step immediately.

A suspension of adenine (0.06 g, 0.45 mmol) and cesium carbonate (0.22 g, 0.67 mmol) in anhydrous DMSO (3 mL) was heated at 80 °C for 30 min under N_2 atmosphere. To this solution was added a solution of **14** (0.11 g, 0.21 mmol) in DMSO (3 mL) dropwise at the same temperature, and the mixture was heated at 80 °C for 15 h. The reaction mixture was cooled to room temperature, quenched with water (5 mL), and extracted with methylene chloride (15 mL). The organic layer was washed with brine (20 mL), dried over anhydrous $MgSO_4$, and evaporated. The crude residue was purified by flash silica gel column chromatography (hexanes/ethyl acetate = 1:1) to give **15** (80 mg, 67%) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 3H), 1.48 (s, 3H), 3.86 (d, *J* = 12.6 Hz, 1H), 4.02 (d, *J* = 12.6, 1H), 4.73 (t, *J* = 10.4, 1H), 5.52 (s, 2H), 7.24−7.50 (m, 9H), 7.45−7.50 (m, 6H), 7.82 (s, 1H), 8.32 (s, 1H). Anal. Calcd for $C_{33}H_{30}FN_5O_3$: C, 70.32; H, 5.36; F, 3.37; N, 12.43. Found: C, 69.99; H, 5.76; N, 12.13.

(1*S*,2*R*,5*S*)-5-(6-Aminopurin-9-yl)-4-fluro-3-hydroxymethylcyclopent-3-ene-1,2-diol (2). To a stirred solution of **15** (0.08 g, 0.14 mmol) in MeOH and THF (10 mL, 1:1 v/v) was added 2 N HCl (0.1 mL in 0.5 mL H_2O), and the mixture was heated at 55 °C for 15 h. The mixture was evaporated and neutralized with methanolic ammonia. The mixture was evaporated and the crude residue was purified by flash silica gelcolumn chromatography (CH_2Cl_2/MeOH = 5.5:1) to give **2** (0.04 g, 88%) as a white solid, whose spectroscopic data were identical with those of authentic sample.[8]

(1*S*,4*R*,5*S*)-1-(6-Chloro-7-deazapurine-9-yl)-2-fluoro-4,5-(*O*-isopropylidenedioxy)-3-(trityloxymethyl)-2-cyclopentene (16). Compound **13** (0.32 g, 0.72 mmol) was condensed with 6-chloro-7-deazapurine (0.275 g, 1.79 mmol) according to the same procedure used in the preparation of **8** and purified by flash silica gel column chromatography (hexanes/ethyl acetate = 4:1) to give **16** (0.28 g, with DIAD byproduct) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.37 (s, 3H), 1.50 (s, 3H), 3.82−3.85 (m, 1H), 4.02 (d, *J* = 12.2 Hz, 1H), 4.67 (t, *J* = 5.0 Hz, 1H), 5.55 (pseudo t, *J* = 4.9 Hz, 1H), 5.75 (bs, 1H), 6.66 (d, *J* = 3.6 Hz, 1H), 7.12 (d, *J* = 3.6 Hz, 1H), 7.22−7.32 (m, 10H), 7.48−7.51 (m, 5H), 8.67 (s, 1H); ^{19}F NMR (376 MHz) δ −126.53. HRMS (ESI) calculated for $C_{34}H_{30}ClFN_3O_3$: 582.1883. Found $(M + H)^+$: 582.1956. Anal. Calcd for $C_{34}H_{29}ClFN_3O_3$: C, 70.16; H, 5.02; N, 7.22. Found: C, 70.56; H, 5.42; N, 7.02.

(1*S*,2*R*,5*S*)-5-(7-Deaza-6-aminopurine-9-yl)-4-fluoro-3-hydroxymethylcyclopent-3(4)-ene-1,2-diol (5c). Compound **16** (0.28 g, 0.8 mmol) was converted to **5c** (0.1 g, 49% for three steps) as a white solid, according to the same procedure used in the preparation of **3**: mp 215−216 °C; $[\alpha]_D^{25}$ −213.3° (*c* 0.13, CH_3OH); UV (MeOH) λ_{max} 271 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 4.14−4.18 (m, 1H), 4.36 (dt, *J* = 1,6, 5.6 Hz, 1H), 4.40 (d, *J* = 12.4 Hz, 1H), 4.75−4.80 (m, 1H), 5.68 (m, 1H), 6.66 (d, *J* = 3.6 Hz, 1H), 7.13 (d, *J* = 3.6 1H), 8.08 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) 54.2, 62.6 (d, J_{C-F} = 17.8 Hz), 70.9 (d, J_{C-F} = 34 Hz), 75.9 (d, J_{C-F} = 5.5 Hz), 101.2, 104.6, 121.2, 124.0, 150.9, 152.1, 155.9 (d, *J* = 285.6 Hz), 158.7; ^{19}F NMR (376 MHz) −130.59. HRMS(ESI) calculated for $C_{12}H_{14}FN_4O_3$: 281.0972. Found $(M + H)^+$: 281.1045. Anal. Calcd for $C_{12}H_{14}FN_4O_3$: C, 51.43; H, 4.68; N, 19.99. Found: C, 51.03; H, 4.98; N, 19.90.

(1*S*,2*R*,5*S*)-5-(7-Deaza-6-*N*-methylaminopurine-9-yl)-4-fluoro-3-hydroxymethylcyclopent-3(4)-ene-1,2-diol (5d). Compound **16** (0.30 g, 0.52 mmol) was converted to **5d** (0.106 g, 40% for 3 steps) as a white solid, according to the same procedure used in the preparation of **5a**: mp 114−116 °C; $[\alpha]_D^{25}$ −159.8° (*c* 0.10, CH_3OH); UV (MeOH) λ_{max} 274 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 3.06 (s, 3H), 4.16 (dt, *J* = 2.4, 12.8 Hz, 1H), 4.34−4.37 (m, 1H), 4.41 (d, *J* = 12.8 Hz, 1H), 4.76 (pseudo t, *J* = 6.0 Hz, 1H), 5.66−5.68 (m, 1H), 6.61−6.62 (d, *J* = 3.6 Hz, 1H), 7.09 (d, *J* = 4.0 Hz, 1H), 8.14 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 28.9, 55.1, 63.6 (d, J_{C-F} = 18 Hz), 71.7 (d, J_{C-F} = 8.7 Hz), 77.0 (d, J_{C-F} = 5.6 Hz), 101.5, 105.9, 122.7 (d, J_{C-F} = 2.8 Hz), 123.6, 151.3, 153.3, 156.8 (d, J_{C-F} = 285.6 Hz), 159.4; ^{19}F NMR (376 MHz) δ −132.0. HRMS (ESI) calculated for $C_{13}H_{16}FN_4O_3$: 295.1133. Found $(M + H)^+$: 295.1206. Anal. Calcd for $C_{13}H_{16}FN_4O_3$: C, 53.06; H, 5.14; N, 19.04. Found: C, 53.46; H, 4.84; N, 19.34.

(1*S*,2*R*,5*S*)-5-(7-Deazahypoxanthine-9-yl)-4-fluoro-3-hydroxymethylcyclopent-3(4)-ene-1,2-diol (5e) and (1*S*,2*R*,5*S*)-4-Fluoro-3-(hydroxylmethyl)-5-(spiro[[1,3]oxathiolane-2,4′-pyrrolo[2,3-*d*]pyrimidine]-7′(3′*H*)-yl)cyclopent-3-ene-1,2-diol (17). To a stirred solution of **16** (0.30 g, 0.52 mmol) in MeOH (10 mL) were added 2-mercaptoethanol (0.14 mL, 2.06 mmol) and sodium methoxide (0.11g, 2.06 mmol), and the reaction mixture was heated at 80 °C for 15 h. After cooling to rt, the mixture was neutralized with acetic acid and evaporated. The residue was purified by flash silica gel column chromatography (hexanes/ethyl acetate = 6:1) to give the trityl derivative as a white foam, whose protecting groups were removed using the same procedure used in the preparation of **3** to give **5e** (0.03 g, 9%) as a white solid and **17** (0.9 g, 58%) as a white foam.

*Compound **5e**.* Mp 210−212 °C; $[\alpha]_D^{25}$ −123.0° (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} 259.5 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 4.14−4.18 (m, 1H), 4.35 (pseudo t, *J* = 5.1 Hz, 1H), 4.39 (d, *J* = 13.0 Hz, 1H), 4.76 (pseudo t, *J* = 5.4 Hz, 1H), 5.74 (bs, 1H), 6.71 (d, *J* = 3.5 Hz, 1H), 7.11 (d, *J* = 3.5 Hz, 1H), 7.90 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 55.1, 63.9 (d, J_{C-F} = 18 Hz), 71.8 (d, J_{C-F} = 8.9 Hz), 77.2 (d, J_{C-F} = 5.3 Hz), 104.8, 110.5, 123.0 (d, J_{C-F} = 2.4 Hz), 123.6, 114.9, 151.0, 156.4 (d, J_{C-F} = 285.2 Hz), 162.2; ^{19}F NMR (376 MHz) δ −132.39. HRMS (ESI) calculated for $C_{12}H_{13}FN_3O_4$: 282.0814. Found $(M + H)^+$: 282.0887. Anal. Calcd for $C_{12}H_{13}FN_3O_4$: C, 51.25; H, 4.30; N, 14.94. Found: C, 5.65; H, 4.70; N, 14.65.

*Compound **17**.* ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.51 (t, *J* = 6.4 Hz, 2H), 3.85 (t, *J* = 6.4 Hz, 2H), 4.18 (td, J_1 = 12.8 Hz, J_2 = 2.4 Hz, 1H), 4.4−4.41 (m. 1H), 4.42−4.43 (m, 1H), 4.79 (pseudo t, *J* = 6.0 Hz, 1H), 5.8 (bs, 1H), 6.62 (d, *J* = 3.6 Hz, 1H), 7.38 (d, *J* = 3.2 Hz, 1H), 8.56 (s, 1H); ^{19}F NMR (376 MHz) −132.34.

Synthesis of 5′-Homoneplanocin A Analogues. 1-[(1*R*,2*S*,3*R*)-2,3-(*O*-Isopropylidenedioxy)-4-[2-(*tert*-butyldiphenylsilyloxy)ethyl]cyclopent-4(5)en]-(6-chloropurine-9-yl) (20) and Its *N*^{7}-Isomer.[13] To the suspension of **18** (697 mg, 1.59 mmol), PPh_3 (1.04 mg, 3.97 mmol), and 6-chloropurine (0.54 mg, 3.49 mmol) in anhydrous THF (75 mL) was added a solution of DIAD (0.78 mL, 3.977 mmol) in dry THF (30 mL)

DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

dropwise at 0 °C, and the reaction mixture was stirred at the same temperature for 15 min and then at room temperature for 12 h. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 1:1) to give **20** (1.0 g, with DIAD byproduct) as a white foam and its N^7-isomer (0.08 g, 9%) as a thick liquid.

*Compound **20**.* ^{1}H NMR (400 MHz, $CDCl_3$, with DIAD byproduct impurity) δ 1.03 (s, 3H), 1.08 (s, 3H), 1.27 (s, 9H), 2.54−2.64 (m, 2H), 3.92−3.97 (m, 2H), 4.64 (d, J = 5.2 Hz, 1H), 5.30 (d, J = 6.0 Hz, 1H), 5.59 (d, J = 8.8 Hz, 1H), 7.35−7.45 (m, 6H), 7.64−7.70 (m, 4H), 7.97 (s, 1H), 8.75 (s, 1H).

N^7-Isomer. $[\alpha]^{25}_D$ 36.25° (c 4.33, CH_2Cl_2); UV (MeOH) λ_{max} 265.0 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (s, 9H), 1.33 (s, 3H), 1.43 (s, 3H), 2.51−2.61 (m, 2H), 3.92−4.03 (s, 2H), 4.56 (d, J = 5.2 Hz, 1H), 5.14 (d, J = 5.2 Hz, 1H), 5.71 (s, 1H), 5.99 (s, 1H), 7.37−7.46 (m, 6H), 7.66−7.69 (m, 4H), 8.13 (s, 1H), 8.90 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.40, 26.30, 27.11, 27.62, 29.91, 31.97, 61.65, 67.22, 76.67, 84.68, 85.19, 112.90, 121.67, 128.05, 130.12, 133.55, 135.75, 143.85, 146.60, 152.62, 152.91. HRMS (ESI) calculated for $C_{31}H_{35}ClN_4O_3Na$: 597.2167. Found $(M + Na)^+$: 597.2045.

(1*S*,2*R*,5*S*) 5-(6-Chloropurine-9-yl)-3-hydroxyethylcyclopent-3-en-1,2-diol (23).[13] Compound **20** (1.0 g with DIAD byproduct) was dissolved in trifluoroacetic acid (5 mL) and water (5 mL) at 0 °C, and the mixture was stirred at room temperature for 4 h. The mixture was evaporated and coevaporated with MeOH. The crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) to give **23** (0.31 g, 66% from **18**) as a white solid: mp 152−153 °C; $[\alpha]^{25}_D$ −0.34° (c 5.8, MeOH); UV (MeOH) λ_{max} 269.0 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 2.52−2.55 (m, 2H), 3.80−3.84 (m, 2H), 4.39 (superimposed dd, J = 5.2 Hz, 1H), 4.62 (d, J = 5.6 Hz, 1H), 5.61−5.63 (m, 1H), 5.81−5.83 (m, 1H), 8.56 (s, 1H), 8.72 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 33.61, 60.81, 67.43, 76.32, 78.46, 126.36, 132.77, 147.04, 149.88, 151.29, 152.90, 153.45. HRMS (ESI) calculated for $C_{12}H_{13}ClN_4O_3Na$: 319.0673. Found $(M + Na)^+$: 319.0562.

(1*S*,2*R*,5*S*)-5-(6-Aminopurine-9-yl)-3-hydroxyethylcyclopent-3-en-1,2-diol (5f).[13] A solution of **23** (0.309 g, 0.538 mmol) in saturated ammonia in *t*-BuOH (10 mL) was stirred in a steel bomb at 120 °C for 24 h. The volatiles were evaporated, and the residue was purified by silica gel (neutralized with Et_3N) column chromatography (CH_2Cl_2/MeOH = 20:1) to give **5f** (0.1 g, 67%) as a white solid: mp 182−183 °C (lit.[12] 181−182 °C,[12] 202−203 °C [11]); $[\alpha]^{25}_D$ −9.692° (c 5.8, MeOH); UV (MeOH) λ_{max} 267.0 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 2.51−2.54 (m, 2H), 3.82 (dt, J_1 = 6.4, J_2 = 1.6 Hz, 2H), 4.32 (dd, J_1 = 5.6, J_2 = 4.8 Hz, 1H), 4.60 (d, J = 5.6 Hz, 1H), 5.51−5.52 (m, 1H), 5.77 (d, J = 1.6 Hz, 1H), 8.21 (s, 1H), 8.26 (s, 1H). HRMS (ESI) calculated for $C_{12}H_{15}N_5O_3$: 277.1171. Found $(M)^+$: 277.1131.

7-Deazahomoneplanocin A (**5g**) and 3-deazahomoneplanocin A (**5h**) were prepared according to the similar procedure used in the synthesis of homoneplanocin A (**5f**).

(1*S*,2*R*,5*S*)-5-(7-Deaza-6-aminopurine-9-yl)-3-hydroxyethylcyclopent-3-en-1,2-diol (5g). Yield: 60%; mp 114−116 °C; $[\alpha]^{25}_D$ +71.5° (c 0.2, MeOH); UV (MeOH) λ_{max} 272 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 2.49−2.52 (m, 2H), 3.77−3.83 (m, 2H), 4.15 (t, J = 5.2 Hz, 1H) 4.54 (d, J = 5.5 Hz, 1H), 5.59−5.60 (m, 1H), 5.69 (m, 1H), 6.58 (d, J = 3.6 Hz, 1H), 7.07 (s, 1H), 8.07 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 34.4, 61.7, 67.0, 77.3, 79.8, 101.1, 105.5, 124.1, 128.9, 149.2, 151.6, 152.6, 160.0. HRMS (ESI) calculated for $C_{13}H_{17}N_4O_3$: 277.1301. Found $(M + H)^+$: 277.1311. Anal. Calcd for $C_{13}H_{16}N_4O_3$: C, 56.51; H, 5.84; N, 20.28. Found: C, 56.76; H, 5.44; N, 19.99.

(1*S*,2*R*,5*S*)-5-(3-Deaza-6-aminopurine-9-yl)-3-hydroxyethylcyclopent-3-en-1,2-diol (5h). Yield: 50%; mp 200 °C (dec); $[\alpha]^{25}_D$ +22.9° (c 0.2, MeOH); UV (MeOH) λ_{max} 268 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 2.55 (t, J = 6.3 Hz, 1H), 3.81−3.84 (m, 2H), 4.19 (t, J = 5.5 Hz, 1H), 4.28 (s, 1H), 5.37−5.38 (m, 1H), 5.84−5.85 (m, 1H), 6.97 (d, J = 6.0 Hz, 1H), 7.07 (d, J = 6.0 Hz, 1H), 8.12 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 34.5, 61.5, 69.0, 76.7, 79.5, 100.3, 127.5, 129.1, 140.8, 141.1, 143.1, 150.3, 154.0. HRMS (ESI) calculated for $C_{13}H_{17}N_4O_3$: 277.1301. Found $(M + H)^+$: 277.1320. Anal. Calcd for $C_{13}H_{16}N_4O_3$: C, 56.51; H, 5.84; N, 20.28. Found: C, 56.55; H, 5.98; N, 20.48.

2,2-Dimethylpropionic Acid 2-[(2*R*,3*S*,4*S*)-4-(6-Chloropurine-9-yl)-5-fluoro-2,3-dihydroxycyclopent-1(5)-en]ethyl Ester (26).[13] To a suspension of **19** (230 mg, 0.76 mmol), PPh_3 (0.5 g, 1.90 mmol), and 6-chloropurine (294 mg, 1.90 mmol) in anhydrous THF (30 mL) was added a solution of DIAD (0.4 mL, 1.90 mmol) in dry THF (10 mL) at 0 °C, and the reaction mixture was stirred at the same temperature for 15 min and then at room temperature for 20 h. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 2:1) to give **25** (0.3 g mixed with DIAD byproduct) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$ mixed with DIAD byproduct) δ 1.14 (s, 9H), 1.37 (s, 3H), 1.51 (s, 3H), 2.55−2.73 (m, 2H), 4.23−4.29 (m, 1H), 4.35−4.41 (m, 1H), 4.80 (pseudo t, J = 5.6 Hz, 1h), 5.45 (dt, J = 2.0, 6.4 Hz, 1H), 5.56 (s, 1H), 8.09 (s, 1H), 8.75 (s, 1H); ^{19}F NMR (376 MHz, $CDCl_3$) δ −135.18.

To a solution of **25** (0.3 g mixed with DIAD byproduct) in anhydrous THF (10 mL) was added 2 N HCl (5 mL) at 0 °C, and the reaction mixture was stirred at 45 °C for 18 h. After completion of reaction (by TLC), the mixture was neutralized with 1 N NaOH solution and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1) to give **26** (150 mg, 50% from **19**) as a colorless solid: mp 153−154 °C; $[\alpha]_D^{20}$ −72.28° (c 5.7, CH_2Cl_2); UV (MeOH) λ_{max} 261.0 nm; ^{1}H NMR (400 MHz, MeOH-d_4) δ 1.16 (s, 9H), 2.53−2.60 (m, 1H), 2.63−2.71 (m, 1H), 4.25−4.35 (m, 2H), 4.64−4.70 (m, 1H), 4.80 (pseudo t, J = 4.4 Hz, 1H), 5.66−5.69 (m, 1H), 8.59 (s, 1H), 8.71 (s, 1H); ^{13}C (100 MHz, $CDCl_3$) δ 24.91, 27.53, 39.78, 62.79, 64.53 (d, J_{C-F} = 19.1 Hz), 73.0 (d, J_{C-F} = 9.2 Hz), 74.82, 126.76 (d, J_{C-F} = 122.38 Hz), 147.58, 151.66, 153.15, 155.84, 160.2, 180.10; ^{19}F NMR (376 MHz, $CDCl_3$) δ −314.85. HRMS (ESI) calculated for $C_{17}H_{21}ClFN_4O_4$: 399.116. Found $(M + H)^+$: 399.1232.

(1*S*,2*R*,5*S*)-5-(6-Aminopurine-9-yl)-4-fluoro-3-hydroxyethylcyclopent-3(4)-en-1,2-diol (5i).[13] A solution of **26** (150 mg, 0.37 mmol) in saturated ammonia in *t*-BuOH (10 mL) was stirred in a steel bomb at 120 °C for 1 d. The volatiles were evaporated, and the residue was purified by silica gel (neutralized with Et_3N) column chromatography (CH_2Cl_2/MeOH = 10:1) to give adenine derivative (135 mg, 95%) as a white solid. HRMS (ESI) calculated for $C_{17}H_{23}FN_5O_4$: 380.1657. Found $(M + H)^+$: 380.1730.

To a solution of the adenine derivative (135 mg) in anhydrous MeOH (3 mL) was added NaOMe (150 mg), and the reaction mixture was heated at 45 °C for 3 h. After completion of reaction (by TLC), the mixture was neutralized with glacial acetic acid and evaporated. The crude residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 4:1) to give **5i** (60 mg, 55%) as a colorless solid: mp 186−187 °C; $[\alpha]_D^{25}$ −124.9° (c 5.5, MeOH); UV (MeOH) λ_{max} 258.0 nm; ^{1}H NMR (400 MHz, MeOH-d_4) δ 2.48−2.54 (m, 2H), 3.76−3.81 (m, 2H), 4.51 (td, J = 1.6, 5.6 Hz, 1H), 4.69 (pseudo t, J = 4.4 Hz, 1H), 5.53−5.56 (m, 1H), 8.17 (s, 1H), 8.18 (s, 1H); ^{13}C (100 MHz, MeOH-d_4) δ 28.66, 60.39, 63.6 (d, J = 18.3 Hz), 72.5 (d, J = 9.6 Hz), 75.59 (d, J = 18.3 Hz), 120.58, 121.24 (d, J = 3.6 Hz), 141.78, 150.92, 153.58, 153.92, 157.0 (d, J = 108.4 Hz); ^{19}F NMR (376 MHz, MeOH-d_4) δ −135.0. HRMS (ESI) calculated for $C_{12}H_{14}FNO_3$: 295.1071. Found $(M + H)^+$: 296.1151. Anal. Calcd for $C_{12}H_{13}FNO_3$: C, 48.81; H, 4.78; N, 23.72. Found: C, 48.79; H, 4.80; N, 23.67.

AdoHcy Hydrolase Assay.[20] The reaction mixture (250 μL) containing 50 mM sodium phosphate (pH 8.0) and AdoHcy hydrolase (2 μM as monomer; 0.5 μM as tetramer) was preincubated with various concentrations of compounds for 10 min at 37 °C. The reaction was initiated by adding 100 μM AdoHcy and was allowed to run for 20 min. The reaction mixture was further incubated with 200 μM DTNB, and the maximum absorbance of the product 5-thio-2-nitrobenzoic acid (TNB) was measured at 412 nm using a spectrophotometer. The molar extinction coefficient for TNB (ε_{412}

DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

= 13 700 M^{-1} cm^{-1}) was used to measure TNB formation in all quantifications.

Antiviral Assay. Antiviral assay was briefly described based on previously published protocols.[21] First, 100 $CCID_{50}$ (50% cell culture inhibitory dose) per well of 96-well plates was prepared by infecting Vero cells with 100 μL of the virus. Each well was then diluted with DME/2% FBS. After the adsorption at 37 °C for 1 h, each well was concentrated in vacuo and treated with the test compound diluted with 100 μL of DME/2% FBS in duplicate for each concentration and further incubated for 3 d. Antiviral activity was measured by MTT assay, and the concentration of compound responsible for 50% reduction of viral replication was calculated and expressed as the EC_{50}. Cytotoxic assay was performed as a control experiment, using mock instead of virus for infection. Cell viability was measured by MTT assay, and CC_{50} (50% cytotoxic concentration) was calculated as the concentration of compound responsible for 50% reduction of cell viability.

Sulforhodamine B (SRB) Assay.[22] Cells (5×10^4 cells/mL) were treated with various concentrations of compounds in 96-well culture plates for 72 h, incubated, fixed with 10% trichloroacetic acid, dried, and stained with 0.4% SRB in 1% acetic acid. After washing the unbound dye out, the stained cells were dried and resuspended in 10 mM Tris (pH 10.0). The absorbance at 515 nm was measured, and cell proliferation was calculated using the following equation: cell proliferation (%) = [(average $absorbance_{compound}$ − average $absorbance_{day\ zero}$)/(average $absorbance_{control}$ − average $absorbance_{day\ zero}$)] × 100. IC_{50} values were calculated by nonlinear regression analysis using TableCurve 2D, versopm 5.01 (Systat Sofrware Inc., Richmond, CA, USA).

Molecular Modeling. *Protein Preparation and Active Site Analysis.* The X-ray crystal structure of AdoHcy hydrolase (PDB code 3NJ4)[8c] was prepared using the Protein Preparation Wizard in Maestro, version 9.2 (Schrödinger, LLC, NY, USA). During the preparation process, bond orders were assigned, hydrogen atoms were added, and protonation states of the residues at pH 7.4 were generated by Epik, version 2.6. According to Yamada et al.,[24] the His54 residue in rat appears to be protonated in its NE2 position and the protonation state of this residue is less influenced by the pH of solution. Therefore, the His55 residue was prepared to be protonated for the docking studies. All the hydrogen atoms were energy minimized with the optimized potential for liquid simulation (OPLS) 2005 force field until the average root-mean-square deviation for hydrogen atoms reached 0.30 Å. To evaluate the active site features, structure-based pharmacophore analysis was performed using LigandScout, version 3.1 (Inte:Ligand GmbH, Vienna, Austria).

Ligand Preparation. Three-dimensional structures of the ligand molecules were prepared by LigPrep, version 2.5, and their protonation states and tautomeric structures in aqueous solution at pH 7.4 were generated by Epik, version 2.6, in Maestro. The resulting structures were energy minimized in implicit solvent with OPLS2005 force field.

Flexible Docking. The ligands were docked to AdoHcy hydrolase using Glide, version 6.1, in Maestro with the following steps: (i) The grid for the active site was generated using the centroid of the cocrystallized ligand, fluoroneplanocin A (**2**). Since the structure of the cocrystallized ligand was very similar to that of tested compounds, we used the grid box size automatically calculated based on the cocrystallized ligand. (ii) For the initial docking stage, Glide SP (standard precision) docking was performed retaining a maximum number of 30 poses per ligand. (iii) Top 5 ranked poses per ligand in the previous step were selected and re-docked using Glide XP (extra precision).

All the molecular graphic figures were generated by PyMOL software (http://www.pymol.org) except the pharmacophore figure by LigandScout. All computational studies were undertaken on an Intel Xeonocta-Core 2.5 GHz workstation with Linux CentOS, release 5.8.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures of molecular modeling, showing binding modes and docking scores, and an xlsx file containing molecular formula strings and IC_{50} values. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00553.

■ AUTHOR INFORMATION

Corresponding Authors

*S.C.: phone, 82-2-3277-4503; fax, 82-2-3277-2851; e-mail, sunchoi@ewha.ac.kr.

*L.S.J.: phone, 82-2-880-7850; fax, 82-2-888-9122; e-mail, lakjeong@snu.ac.kr.

Author Contributions

‖G.C., Y.W.M., and Y.L. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the grants from Midcareer Research Program (Grant 370C-20130120) (L.S.J.) and National Leading Research Lab (NLRL) program (Grant 2011-0028885) (S.C.) through the Ministry of Science, ICT & Future Planning (MSIP) and National Research Foundation (NRF) of Korea.

■ ABBREVIATIONS USED

AdoHcy, *S*-adenosyl-L-homocysteine; SAM, *S*-adenosyl-L-methionine; HCV, hepatitis C virus; NOE, nuclear Overhauser effect; RTIL, room temperature ionic liquid; [bmim][BF_4], 1-butyl-3-methylimidazolium tetrafluoroborate; [bmim][PF_6], 1-butyl-3-methylimidazolium hexafluorophosphate; DTNB, 5,5′-dithiobis-2-nitrobenzoate; HSV, herpes simplex virus; HIV, human immunodeficiency virus; PC, picornavirus; VSV, vesicular stomatitis virus; DIAD, diisopropyl azodicarboxylate; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; TFAA, trifluoroacetic anhydride; SRB, sulforhodamine B; TNB, 5-thio-2-nitrobenzoic acid; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; FBS, fetal bovine serum; OPLS, optimized potential for liquid simulation

■ REFERENCES

(1) Turner, M. A.; Yang, X.; Yin, D.; Kuczera, K.; Borchardt, R. T.; Howell, P. L. Structure and function of S-adenosylhomocysteine hydrolase. *Cell Biohem. Biophys.* **2000**, *33*, 101−125.

(2) Cantoni, G. L. The centrality of S-adenosylhomocysteinase in the regulation of the biological utilization of S-adenosylmethionine. In *Biological Methylation and Drug Design*; Borchardt, R. T., Creveling, C. R., Ueland, P. M., Eds.; Humana Press: Clifton, NJ, 1986; pp 227−238.

(3) Snoeck, R.; Andrei, G.; Neyts, J.; Schols, D.; Cools, M.; Balzarini, J.; De Clercq, E. Inhibitory activity of S-adenosylhomocysteine hydrolase inhibitors against human cytomegalovirus replication. *Antiviral Res.* **1993**, *21*, 197−216.

(4) Liu, S.; Wolfe, M. S.; Borchardt, R. T. Rational approaches to the design of antiviral agents based on *S*-adenosyl-L-homocysteine hydrolase as a molecular target. *Antiviral Res.* **1992**, *19*, 247−265.

(5) Wolfe, M. S.; Borchardt, R. T. *S*-Adenosyl-L-homocysteine hydrolase as a target for antiviral chemotherapy. *J. Med. Chem.* **1991**, *34*, 1521−1530.

(6) Yaginuma, S.; Muto, N.; Tsujino, M.; Sudate, Y.; Hayashi, M.; Otani, M. Studies on neplanocin A, new antitumor antibiotic. I.

DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX

Producing organism, isolation and characterization. *J. Antibiot. (Tokyo)* **1981**, *34*, 359−366.
(7) Keller, B. T.; Borchardt, R. T. Metabolism and mechanism of action of neplanocin A—A potent inhibitor of *S*-adenosylhomocysteine hydrolase. In *Biological Methylation and Drug Design*; Borchardt, R. T., Creveling, C. R., Ueland, P. M., Eds.; Humana Press: Clifton, NJ, 1986; pp 385−396.
(8) (a) Jeong, L. S.; Moon, H. R.; Park, J. G.; Shin, D. H.; Choi, W. J.; Lee, K. M.; Kim, H. O.; Chun, M. W.; Kim, H. D.; Kim, J. H. Synthesis and biological evaluation of halo-neplanocin A as novel mechanism-based inhibitors of *S*-adenosylhomocysteine hydrolase. *Nucleosides, Nucleotides Nucleic Acids* **2003**, *22*, 589−592. (b) Jeong, L. S.; Yoo, S. J.; Lee, K. M.; Koo, M. J.; Choi, W. J.; Kim, H. O.; Moon, H. R.; Lee, M. Y.; Park, J. G.; Lee, S. K.; Chun, M. W. Design, synthesis, and biological evaluation of fluoroneplanocin A as the novel mechanism-based inhibitor of S-adenosylhomocysteine hydrolase. *J. Med. Chem.* **2003**, *46*, 201−203. (c) Lee, K. M.; Choi, W. J.; Lee, Y.; Lee, H. J.; Zhao, L. X.; Lee, H. W.; Park, J. G.; Kim, H. O.; Hwang, K. Y.; Heo, Y. X-ray crystal structure and binding mode analysis of human *S*-adenosylhomocysteine hydrolase complexed with novel mechanism-based inhibitors, haloneplanocin A analogues. *J. Med. Chem.* **2011**, *54*, 930−938.
(9) Eldrup, A. B.; Prhavc, M.; Brooks, J.; Bhat, B.; Prakash, T. P.; Song, Q.; Bera, S.; Bhat, N.; Dande, P.; Cook, P. D. Structure-activity relationship of heterobase-modified 2′-C-methyl ribonucleosides as inhibitors of hepatitis C virus RNA replication. *J. Med. Chem.* **2004**, *47*, 5284−5297.
(10) (a) Arumugham, B.; Kim, H.; Prichard, M. N.; Kern, E. R.; Chu, C. K. Synthesis and antiviral activity of 7-deazaneplanocin A against orthopoxviruses (vaccinia and cowpox virus). *Bioorg. Med. Chem. Lett.* **2006**, *16*, 285−287. (b) Kim, H.; Sharon, A.; Bal, C.; Wang, J.; Allu, M.; Huang, Z.; Murray, M. G.; Bassit, L.; Schinazi, R. F.; Korba, B. Synthesis and anti-hepatitis B virus and anti-hepatitis C virus activities of 7-deazaneplanocin A analogues in vitro. *J. Med. Chem.* **2008**, *52*, 206−213.
(11) Tseng, C. K. H.; Marquez, V. E.; Fuller, R. W.; Goldstein, B. M.; Haines, D. R.; McPherson, H.; Parsons, J. L.; Shannon, W. M.; Arnett, G. Synthesis of 3-deazaneplanocin A, a powerful inhibitor of *S*-adenosylhomocysteine hydrolase with potent and selective in vitro and in vivo antiviral activities. *J. Med. Chem.* **1989**, *32*, 1442−1446.
(12) (a) Shuto, S.; Obara, T.; Saito, Y.; Andrei, G.; Snoeck, R.; De Clercq, E.; Matsuda, A. New neplanocin analogues. 6. Synthesis and potent antiviral activity of 6′-homoneplanocin A. *J. Med. Chem.* **1996**, *39*, 2392−2399. (b) Yang, M.; Schneller, S. W.; Korba, B. 5′-Homoneplanocin A inhibits hepatitis B and hepatitis C. *J. Med. Chem.* **2005**, *48*, 5043−5046.
(13) Chandra, G.; Majik, M. S.; Lee, J. Y.; Jeong, L. S. Stereoselective synthesis of fluoro-homoneplanocin A as a potential antiviral agent. *Org. Lett.* **2012**, *14*, 2134−2137.
(14) Choi, W. J.; Moon, H. R.; Kim, H. O.; Yoo, B. N.; Lee, J. A.; Shin, D. H.; Jeong, L. S. Preparative and stereoselective synthesis of the versatile intermediate for carbocyclic nucleosides: effects of the bulky protecting groups to enforce facial selectivity. *J. Org. Chem.* **2004**, *69*, 2634−2636.
(15) (a) Hoveyda, A. H.; Evans, D. A.; Fu, G. C. Substrate-directable chemical reactions. *Chem. Rev.* **1993**, *93*, 1307−1370. (b) Adam, W.; Wirth, T. Hydroxyl group directivity in the epoxidation of chiral allylic alcohols: Control of diastereoselectivity through allylic strain and hydrogen bonding. *Acc. Chem. Res.* **1999**, *32*, 703−710.
(16) (a) Moon, H. R.; Kim, H. O.; Chun, M. W.; Jeong, L. S.; Marquez, V. E. Synthesis of cyclopropyl-fused carbocyclic nucleosides via the regioselective opening of cyclic sulfites. *J. Org. Chem.* **1999**, *64*, 4733−4741. (b) Marquez, V. E. In *Modified Nucleosides: in Biochemistry, Biotechnology and Medicine*; Herdewijn, P., Ed.; Wiley-VCH Verlag: Weinheim, Germany, 2008; pp 307−341.
(17) Jain, N.; Kumar, A.; Chauhan, S.; Chauhan, S. Chemical and biochemical transformations in ionic liquids. *Tetrahedron* **2005**, *61*, 1015−1060.
(18) Luche, J. L. Lanthanides in organic chemistry. 1. Selective 1,2 reductions of conjugated ketones. *J. Am. Chem. Soc.* **1978**, *100*, 2226−2227.
(19) Chu, C. K.; Cho, J.-H.; Kim, H.-J. Cyclopentenyl nucleoside compounds intermediates for their synthesis and methods of treating viral infections. U.S. Patent 0270431A1, Oct 29, 2009.
(20) Lozada-Ramírez, J. D.; Martínez-Martínez, I.; Sánchez-Ferrer, A.; García-Carmona, F. A colorimetric assay for *S*-adenosylhomocysteine hydrolase. *J. Biochem. Biophys. Methods* **2006**, *67*, 131−140.
(21) (a) Snoeck, R.; Sakuma, T.; De Clerq, E.; Rosenberg, I.; Holy, A. (*S*)-1-(3-Hydroxy-2-phosphonylmethoxypropyl)cytosine, a potent and selective inhibitor of human cytomegalovirus replication. *Antimicrob. Agents Chemother.* **1988**, *32*, 1839−1844. (b) Lee, H.-Y.; Lee, K.; Hah, J. H.; Moon, D. K.; Lee, C.-K. Design, synthesis and antiviral activity of 5-hydroxymethyl-3-phosphonyl-4,5-dihydrofuran analogs of nucleotides. *Bull. Korean Chem. Soc.* **2010**, *31*, 2139−2140.
(22) Lee, S. K.; Heo, Y. H.; Steelee, V. E.; Pezzuto, J. M. Induction of apoptosis by 1,4-phenylenebis(methylene)selenocyanate in cultured human colon cancer cells. *Anticancer Res.* **2002**, *22*, 97−102.
(23) Lee, Y.; Jeong, L. S.; Choi, S.; Hyeon, C. Link between allosteric signal transduction and functional dynamics in a multisubunit enzyme: *S*-adenosylhomocysteine hydrolase. *J. Am. Chem. Soc.* **2011**, *133*, 19807−19815.
(24) Yamada, T.; Takata, Y.; Komoto, J.; Gomi, T.; Ogawa, H.; Fujioka, M.; Takusagawa, F. Catalytic mechanism of *S*-adenosylhomocysteine hydrolase: roles of His 54, Asp130, Glu155, Lys185, and Asp189. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 2417−2435.

DOI: 10.1021/acs.jmedchem.5b00553
J. Med. Chem. XXXX, XXX, XXX−XXX