Synthesis and Anti-Hepatitis B Virus and Anti-Hepatitis C Virus Activities of 7-Deazaneplanocin A Analogues in Vitro

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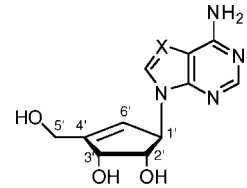
A series of 7-deazaneplanocin A (7-DNPA, **2**) analogues were synthesized and evaluated for in vitro antiviral activity against HBV and HCV. The syntheses of target carbocyclic nucleosides were accomplished via a convergent procedure. 7-Substitutions were introduced by using 7-substituted-7-deaza heterocyclic base precursors (F, Cl, Br, and I) or via substitution reactions after the synthesis of the carbocyclic nucleosides. Among the synthesized compounds, **2**, **13**−**15**, **24**, and **27** exhibited significant anti-HCV activity (EC_{50} ranged from 1.8 to 20.1 μM) and compounds **2**, **15**, **22**, and **24** demonstrated moderate to potent anti-HBV activity (EC_{50} = 0.3−3.3 μM). In addition, compound **24** also showed activity against lamivudine- and adefovir-associated HBV mutants.

Introduction

Neplanocin A (NPA,[a] Figure 1, **1**), a fermentation-derived carbocyclic nucleoside, exhibits a broad-spectrum antiviral activity and may be associated with the inhibition of *S*-adenosylhomocysteine hydrolase (AdoHcyase), a key enzyme regulating biologically important transmethylation processes.[1] However, the therapeutic utility of NPA has been limited because of its cytotoxicity, which may be attributed mainly to the phosphorylation of NPA at the 5′-position[2] as well as the lack of selective inhibition of AdoHcyase.[1] The corresponding NPA-triphosphate may interfere with the RNA synthesis of the host-cell and be further metabolized to cytotoxic *S*-neplanocylmethionine.[2] On the basis of these observations, numerous efforts have been made to modify the structure of NPA to produce the compounds with better therapeutic efficacy.[3] Recently, our group has also been involved in discovering biologically interesting carbocyclic nucleosides.[4]

Hepatitis B and hepatitis C virus (HBV and HCV, respectively) belong to *hepadnaviridae*[5] and *flaviviridae*[6] families, respectively. The infection of the two viruses accounts for major proportions of hepatitis cases worldwide and is also strongly associated with the development of cirrhosis and hepatocellular carcinoma. Although important advances have been made in the prevention of HBV infection, the current therapies remain still unsatisfactory.[7] Furthermore, there are no vaccines as well as no effective therapeutic agents that are available for the treatment of HCV. The current standard therapy for chronic HCV infection is interferon α (or pegylated-interferon α) in combination with ribavirin, which is inadequate because of the low response rates as well as its side effects.[8] Although a number of inhibitors of HCV replication have recently been reported, their therapeutic effectiveness and safety profile remain to be demonstrated in clinical trials.[9] As a part of our ongoing antiviral drug discovery program, a number of carbocyclic nucleoside analogues have been synthesized and evaluated for their potential antiviral activity against HBV and HCV.[10]



1 X= N NPA
2 X= CH 7-DNPA

Figure 1. Structures of neplanocin A (NPA) and 7-deazaneplanocin A (7-DNPA).

Recently, we described the synthesis and antiviral activity of 7-deazaneplanocin A (7-DNPA, Figure 1, **2**) against orthopoxviruses (vaccinia and cowpox virus).[4e] In addition, the further screening of the compound **2** revealed significant anti-HBV and anti-HCV activity with low cytotoxicity. In view of these interesting biological results, it was of interest to explore the structure−activity relationships of 7-substituted-7-DNPA analogues as potential antiviral agents. Herein, we report the synthesis and anti-HBV and anti-HCV activities of 7-deazaneplanocin A analogues. The most potent compound against wild type HBV was further evaluated against drug-resistant mutant strains of HBV.

Results and Discussion

Chemistry. The modification of carbocyclic nucleosides 4-amino-7-(2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (7-DNPA, Figure 1, **2**) was investigated by synthesizing the corresponding 7-substituted-7-DNPA analogues, as shown in Schemes 1−3. For the

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[a] Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; NPA, neplanocin A; 7-DNPA, 7-deazaneplanocin A; 2′-*C*-Me-C, 2′-*C*-Me-cytosine; 2′-F-*C*-Me-C, 2′-F-*C*-Me-cytosine; 2′-*C*-Me-A, 2′-*C*-methyladenosine; RdRp, RNA dependent RNA polymerase; AdoHcyase, *S*-adenosylhomocysteine hydrolase; DIAD, diisopropyl azodicarboxylate; TPP, triphenylphosphine; PMB, *p*-methoxybenzyl; DDQ, 2,3-dichloro-5,6-dicyano-1,6-benzoquinone; HMPA, hexamethylphosphoramide; TBAF, tetrabutylammonium fluoride; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; RI, replication intermediates.

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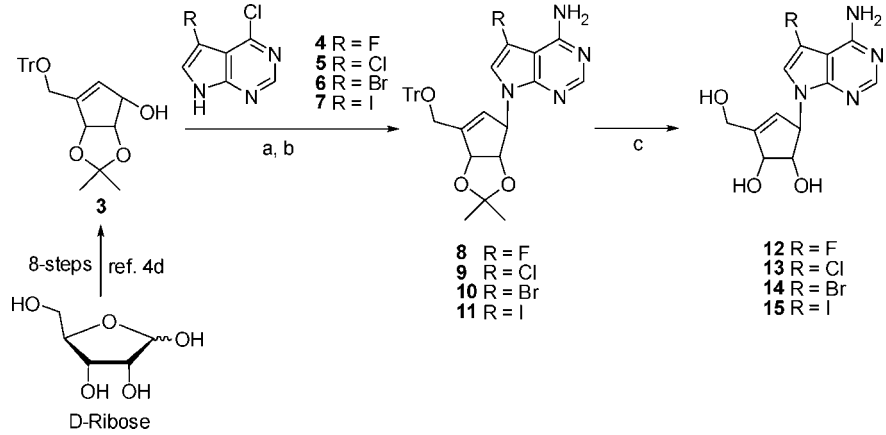
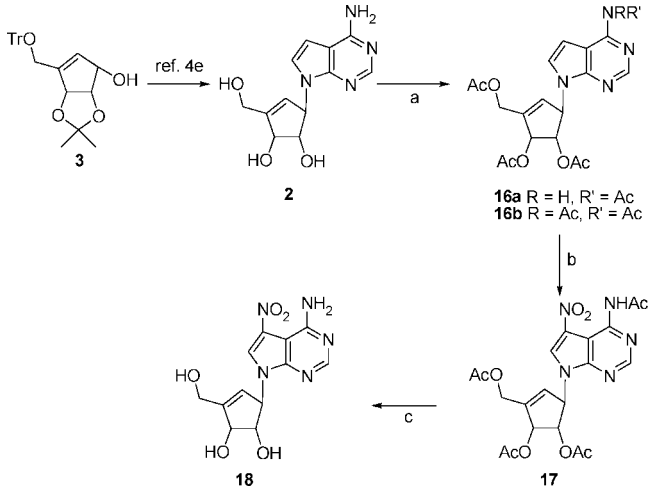
Scheme 1[a]



[a] Reagent and conditions: (a) PPh_3, DIAD, THF, room temp; (b) NH_3, MeOH, 80 °C; (c) HCl, MeOH, THF, 50 °C.

Scheme 2[a]



[a] Reagent and conditions: (a) Ac_2O, pyridine, 60 °C; (b) HNO_3, H_2SO_4, CH_2Cl_2; (c) NH_3, MeOH, 80 °C.

synthesis of 7-halogenated-7-DNPA analogues (Scheme 1), the key intermediate 2,3-isopropylidenedioxy-4-trityloxymethyl-4-cyclopenten-1-ol **3** was utilized as the starting material. The cyclopentenol intermediate **3** was synthesized from D-ribose in eight steps with 52% overall yield, as previously reported from our group.[4d] The Mitsunobu coupling reaction of the cyclopentenol intermediate **3** with 7-halogenated-7-deaza-6-chloropurines **4**, **5**, **6**, and **7** provided corresponding coupling products as protected carbocyclic nucleosides, which were aminated at elevated temperature to give the amino derivatives **8**, **9**, **10**, and **11** in 75−95% yield. The deprotection of the trityl and acetonide groups of **8**−**11** was accomplished using hydrochloric acid in a mixture of methanol and THF at 50 °C to yield the desired carbocyclic nucleosides **12**, **13**, **14**, and **15**.

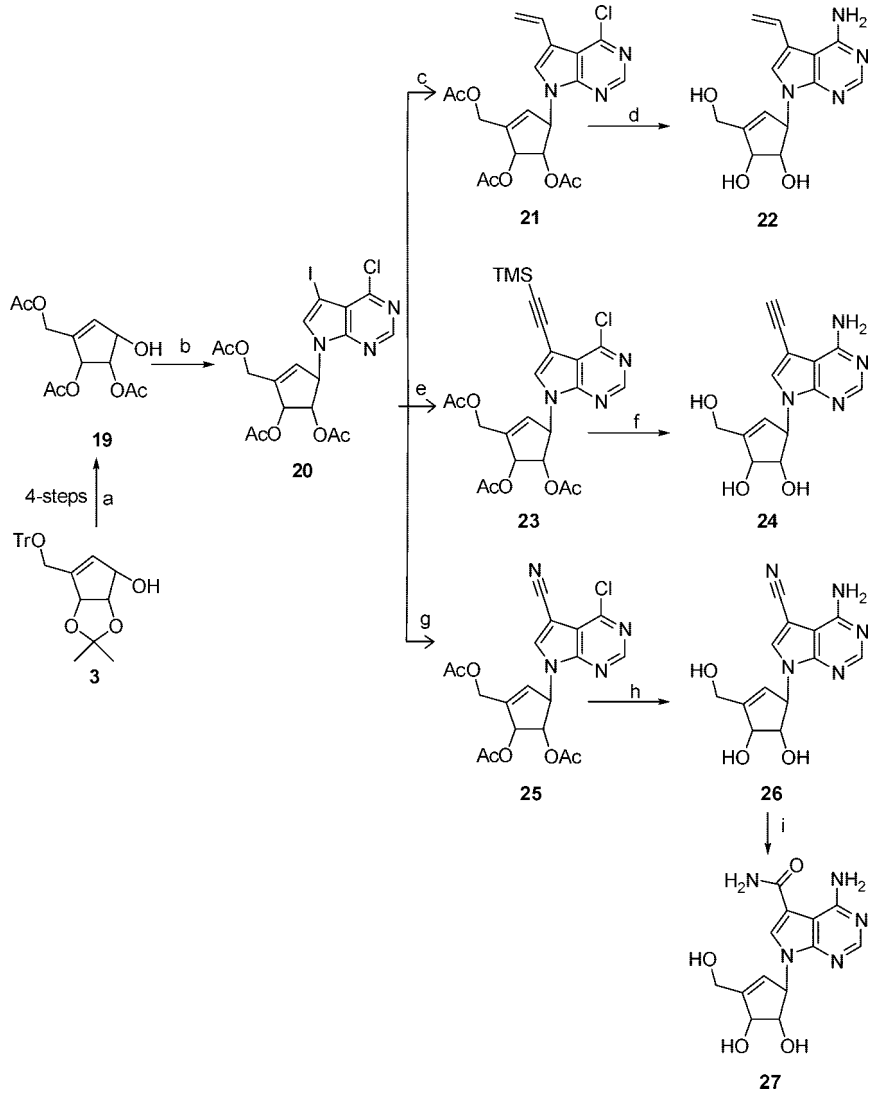
The 7-nitro analogues of 7-DNPA **18** was synthesized as shown in Scheme 2. The acetylation of 7-DNPA **2** with acetic anhydride in pyridine at 60 °C gave a mixture of **16a** and **16b**, which were treated with fuming nitric acid and sulfuric acid in methylene chloride to give the 7-nitro derivative **17** in good yield. The removal of acetyl groups of **17** was accomplished with saturated methanolic ammonia to give 7-nitro-7-DNPA **18** in 66% yield.

The syntheses of vinyl, acetylene, and cyano substituted derivatives of 7-DNPA, **22**, **24**, and **26**, are illustrated in Scheme 3. During our first attempt, we used our strategy based on Scheme 1 to produce the vinyl and acetylene derivatives from the precursor of iodo compound **11** by a palladium catalyzed reaction followed by ammonolysis. However, the acidic hydrolysis of the coupled products by HCl in MeOH and THF gave a complex mixture that could not be separated. To avoid the deprotection step in acidic conditions, a new key intermediate **19** (acetyl protected) was prepared from the cyclopentenol intermediate **3** by alkylation with *p*-methoxybenzyl bromide and deprotection of the trityl and acetonide groups followed by acetylation and deprotection of *p*-methoxybenzyl (PMB) group using 2,3-dichloro-5,6-dicyano-1,6-benzoquinone (DDQ). Subsequently, the acetyl protected cyclopentenol intermediate **19** was treated with 7-iodo-7-deaza-6-chloropurine (**7**) under the Mitsunobu conditions and provided the coupling product **20** in 54% yield. The iodo group of **20** was converted to the vinyl group by the treatment of tetravinyltin in hexamethylphosporamide (HMPA) at 80 °C for 16 h to obtain **21** in 26% yield, which was deprotected under basic conditions to give 7-vinyl-7-DNPA **22**. Compound **20** was reacted with trimethylsilylacetylene under palladium catalyzed conditions to give the TMS protected acetylene substituted derivative **23** in 57% yield, which was in turn converted to 7-acetylene-7-DNPA **24** by ammonolysis.

Additionally, the iodo group in **20** was used to the obtain the cyano moiety by a palladium catalyzed reaction with tributyltin cyanide to give **25** in moderate yield. The ammonolysis reaction of **25** gave the desired product 7-cyano-7-DNPA **26** in 73% yield from **20**. The 7-cyano group of **26** was further converted to carboxamide analogues by the treatment with hydrogen peroxide in ammonium hydroxide solution to yield a 7-carboxamide-7-DNPA **27** in quantitative yield.

Anti-HCV Activity. The anti-HCV activity and cytotoxicity of synthesized nucleosides were evaluated in the HCV RNA replicon assay system in Huh7 ET cells, as previous described,[11] and the results are summarized in Table 1. Compounds were initially assessed at a single concentration of 20 μM. Any potentially active or cytotoxic compounds in the primary assay were further evaluated in the dose−response assay. From these studies we found that 6 out of 10 NPA analogues (**2**, **13**−**15**, **24**, and **27**) exhibited significant anti-HCV activity with EC_{50} ranging from 1.8 to 20.1 μM. 7-DNPA **2** displayed anti-HCV activity with an EC_{50} value of 2.5 μM without any cytotoxicity at a concentration up to 100 μM (Table 1). This result was confirmed independently in a separate anti-HCV evaluation, in which the anti-HCV activity of 7-DNPA **2** is at least comparable to, if not better than, the positive controls 2′-*C*-Me-cytosine (2′-*C*-Me-C)[9f] and 2′-F-*C*-Me-cytosine (2′-F-*C*-Me-C)[9g,h] (Table 2).

Scheme 3[a]

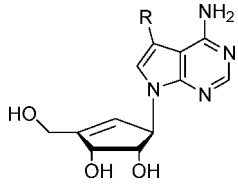


[a] Reagents and conditions: (a) (i) NaH, *p*-methoxybenzyl chloride, DMF; (ii) HCl/MeOH/THF; (iii) Ac_2O, pyridine; (iv) DDQ, CH_2Cl_2, H_2O; (b) PPh_3, DIAD, THF, room temp, **7**; (c) $Bu_3Sn(CHCH_2)$, $Pd(PPh_3)_4$, Et_3N, CuI, DMF; (d) NH_3, MeOH, 80 °C; (e) TMSC≡CH, $Pd(PPh_3)_4$, Et_3N, CuI; (f) NH_3, MeOH, 80 °C; (g) Bu_3SnCN, $(PPh_3)_4Pd$, $ClCH_2CH_2Cl$, reflux; (h) NH_3, MeOH, 80 °C; (i) H_2O_2, NH_4OH.

As the modifications at the 7-position of the 7-deaza analogues of 2′-*C*-methyladenosine (2′-*C*-Me-A) exhibited enhanced anti-HCV activity,[9d] it was of interest to explore the electrostatic and steric requirements at the 7-position in the 7-DNPA analogues. Electronegative substituents, such as 7-fluoro-**12**, 7-chloro-**13**, 7-bromo-**14**, and 7-iodo-**15** analogues, were also synthesized. In comparison to the parent compound **2**, the fluoro analogue **12** was devoid of anti-HCV activity. The anti-HCV potency tends to be increased with decreasing electronegativity as well as with increasing size of halogens at the 7-position (**13**, EC_{50} = 14.7 μM; **14**, EC_{50} = 16.7 μM; **15**, EC_{50} = 2.1 μM), while a concomitant increase in cytotoxicity was also observed (**13**, IC_{50} = 50.1 μM; **14**, IC_{50} = 41.7 μM; **15**, IC_{50} = 15.0 μM). Compounds with other substituents on the 7-position, such as carboxamide **27**, cyano **26**, vinyl **22**, and acetylene **24** derivatives, were also investigated, in which the 7-carboxamide derivative **27** appeared to be the most active compound against HCV (EC_{50} = 1.8 μM); however, it was also the most cytotoxic (IC_{50} = 11.8 μM). The anti-HCV activity decreased significantly when electron-withdrawing substituents (CN or NO_2) were present at the 7-position, such as the cyano-**26** and nitro-**18** derivatives. From these results, it appears that the electron-withdrawing groups have deleterious effects on the anti-HCV activity in the 7-DNPA, which is clearly contradictory to the observations of the 2′-*C*-methyl-7-substituted-7-deaza adenosine analogues, in which electronegative substituents on the 7-position appeared to be favored.[9d] These results suggest that the two classes of compounds may have different mode of actions, although the structures of heterocyclic moiety are same. However, investigating the mode of action of 7-DNPAs is beyond the scope of the current structure−activity relationships studies and would be the future subject of study.

Anti-HBV Activity. The synthesized carbocyclic nucleosides were also screened for their antiviral activity against wild type HBV in vitro, and the results are summarized in Table 3. The most potent compound was further evaluated against HBV drug-resistant mutants. As shown in Table 3, compounds **2**, **15**, **22**, and **24** exhibited significant anti-HBV activity against wild-type HBV with EC_{50} values of 0.60, 0.43, 3.3, and 0.32 μM, respectively, in an extracellular virion HBV DNA assay. 7-DNPA **2** showed moderate cytotoxicity (IC_{50} = 22 μM), whereas the compounds **15**, **22**, and **24** did not show any significant cytotoxicity up to 300 μM. The most potent compound **24** (7-ethynyl derivative) was also evaluated against

Table 1. Anti-HCV Activity of 7-DNPA Analogues Based on an HCV RNA Replicon Assay and Cytotoxicity



compd	R	anti-HCV activity, EC_{50} (μM)	cytotoxicity, IC_{50} (μM)	SI_{50}
2	H	2.5	>100	>40
12[a]	F	9.5%	112.7%	
13	Cl	14.7	50.1	3.4
14	Br	16.7	41.7	2.5
15	I	2.1	15.0	7.1
18[a]	NO_2	31.5	101.5%	
22[a]	vinyl	49.2%	99.7%	
24	ethynyl	20.1	48.9	2.4
26[a]	CN	0.5%	103.3%	
27	$CONH_2$	1.8	11.8	6.6
2′-*C*-Me-A		0.15	>10.0	>66.7

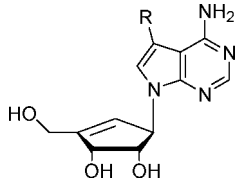
[a] Compounds assessed at a single-concentration of 20 (μM) with percentage donating inhibition level compared to control cells.

Table 2. Additional Studies of Anti-HCV Activity and Cytoxicity of 7-DNPA **2** in the HCV RNA Replicon Huh7 Assay in Comparison to the Known Agents (2′-F-*C*-Me-C and 2′-*C*-Me-C)

compd	anti-HCV activity, EC_{50} (μM)[a]	cytotoxicity, EC_{90} (μM)[a]	CC_{50} (μM)[b]
2	0.9	8.2	>50
2′-F-*C*-Me-C	1.7	5.3	>50
2′-*C*-Me-C	3.5	11	>50

[a] Effective concentrations required for reducing HCV level by 50% and 90% in 5 days. [b] Cytotoxicity concentration required for reducing the rRNA levels by 50% in 5 days.

Table 3. In Vitro Antiviral Activity Against Wild Type HBV and Cytotoxicity of 7-DNPA Analogues

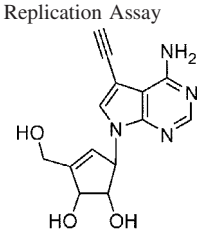


		anti-HBV activity		
compd	R	virion, EC_{50} (μM)	HBV RI, EC_{50} (μM)	cytotoxicity, IC_{50} (μM)
2	H	0.60	ND[a]	22
12	F	>10	ND[a]	>300
13	Cl	>10	ND[a]	>300
14	Br	>10	ND[a]	>300
15	I	0.43	1.6	>300
18	NO_2	>10	ND[a]	>300
22	vinyl	3.3	10	>300
24	acetylene	0.32	1.8	>300
26	CN	>10	ND[a]	>300
27	$CONH_2$	>10	ND[a]	>300
3TC		0.048	0.15	>2000

[a] ND, not determined.

drug resistant HBV mutants (Table 4), in which compound **24** did not lose activity against all the lamivudine (3TC) and adefovir associated HBV mutants, with an EC_{50} value comparable to that of adefovir (ADV). It was also found that compound **24** confers a slight resistance (2-fold) to the ADV mutant (rtN236T). Again, the mode of action of the compound has not been studied, but it will be the future subject of investigation.

Table 4. In Vitro Antiviral Activity against HBV Mutants Based on the Intracellular HBV DNA Replication Assay



24

		EC_{50} (μM)	
strain	anti-HBV activity	3TC	adefovir
WT	2.5	0.2	1.3
rtL180M	2.0	10.0	1.6
rtLM/rtMV[a]	2.8	>100	1.2
rtM204I	3.0	>100	1.8
rtM204V	2.1	>100	1.5
rtN236T	5.6	0.3	7.7

[a] rtLM/rtMV = rtL180M/rtM204V double mutant.

In summary, to study the structure−activity relationships, a series of novel 7-deazaneplanocin A analogues have been synthesized and their anti-HCV and anti-HBV activity were determined in vitro. Several synthesized compounds exhibited significant anti-HCV activity, among which the most potent analogue was the 7-unsubstituted compound **2** without any cytotoxicity. The anti-HBV activities were also determined, from which the ethynyl derivative **24** was found to be the most significant against the wild-type, maintaining the potency against drug-resistant HBV mutants. The present findings warrant future investigation of the mechanism of action as well as additional biological evaluation of these analogues.

Experimental Section

Chemistry. Melting points were determined on a Mel-temp II apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian Inova 500 spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR with tetramethylsilane as the internal standard. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or bs (broad singlet). UV spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. Column chromatography was performed using either silica gel-60 (220−440 mesh) for flash column chromatography or silica gel G (TLC grade, >440 mesh) for vacuum column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

(−)-(1*R*, 2*S*, 3*R*)-4-Amino-5-fluoro-7-[2,3-(isopropylidenedioxy)-4-(trityloxymethyl)-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (8). To a mixture of compound **3** (318 mg, 0.74 mmol), compound **4** (140 mg, 0.82 mmol), and Ph_3P (388 mg, 1.48 mmol) in THF (30 mL) was added DIAD (299 mg, 1.48 mmol) at 0 °C. After being stirred for 30 min at room temperature, the mixture was evaporated under reduced pressure and purified by column chromatography on silica gel. The appropriate fraction was collected and evaporated. The residue was treated with saturated methanolic ammonia and heated to 80 °C in a steal bomb for 16 h. After cooling, the mixture was purified via flash column chromatography on silica gel (eluting up to 50% ethyl acetate in hexane) to give **8** (350 mg, 84%) as a white solid: mp 100−102 °C; $[\alpha]_D^{27}$ −25.33 (*c* 0.13, $CHCl_3$); UV ($CHCl_3$) λ_{max} 283.0 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.4−7.5 (m, 6H), 7.2−7.35 (m, 9H), 6.48 (d, J = 2.0 Hz, 1H), 5.99 (s, 1H), 5.88 (s, 1H), 5.37 (s, 2H), 5.18 (d, J = 6.0 Hz, 1H), 4.54 (d, J = 6.0 Hz, 1H), 4.01 (dt, J = 15 and

2 Hz, 1H), 3.83 (d, J = 15.5 Hz, 1H), 1.42 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.51, 155.49, 153.20, 149.12, 145.84, 145.81, 144.14, 143.78, 141.71, 128.54, 128.09, 127.94, 127.20, 123.07, 112.48, 104.25, 103.99, 93.81, 93.66, 87.26, 84.99, 83.94, 77.24, 63.97, 61.48, 27.52, 26.10. HRMS ([M + H]$^+$) calcd, 563.2453; found, 563.2458.

(−)-(1*R*,2*S*,3*R*)-4-Amino-5-chloro-7-[2,3-(isopropylidenedioxy)-4-(trityloxymethyl)-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (9). To a mixture of **3** (193 mg, 0.45 mmol), **5** (110 mg, 0.59 mmol), and Ph_3P (177 mg, 0.68 mmol) in THF (20 mL) was added DIAD (0.13 mL, 0.68 mmol) at 0 °C. After being stirred for 30 min at room temperature, the mixture was evaporated under reduced pressure and purified by silica gel column chromatography. The isolated product was treated with saturated methanolic ammonia and heated to 80 °C in a steal bomb for 16 h. After cooling, the mixture was purified by flash column chromatography on silica gel (eluting up to 50% ethyl acetate in hexane) to give **9** (205 mg, 79%) as a white solid: mp 94−95 °C; $[\alpha]_D^{28}$ −28.55 (*c* 0.20, $CHCl_3$); UV ($CHCl_3$) λ_{max} 284.0 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.4−7.5 (m, 6H), 7.2−7.35 (m, 9H), 6.68 (s, 1H), 5.99 (s, 1H), 5.85 (s, 1H), 5.57 (br s, 2H), 5.17 (d, J = 5.5 Hz, 1H), 4.54 (d, J = 5.5 Hz, 1H), 4.03 (m, 1H), 3.84 (d, J = 16.0 Hz, 1H), 1.43 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.62, 153.20, 149.51, 149.02, 143.81, 128.58, 127.98, 127.25, 122.80, 118.67, 112.56, 103.10, 101.34, 87.35, 84.99, 83.98, 64.39, 61.54, 27.55, 26.13. HRMS ([M + H]$^+$) calcd, 579.2157; found, 579.2163.

(−)-(1*R*,2*S*,3*R*)-4-Amino-5-bromo-7-[2,3-(isopropylidenedioxy)-4-(trityloxymethyl)-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (10). To a mixture of **3** (144 mg, 0.34 mmol), **6** (102 mg, 0.44 mmol), and Ph_3P (134 mg, 0.51 mmol) in THF (20 mL) was added DIAD (0.10 mL, 0.51 mmol) at 0 °C. After being stirred for 30 min at room temperature, the mixture was evaporated and purified by column chromatography on silica gel. The appropriate fraction was collected and evaporated. The residue was treated with saturated methanolic ammonia and heated to 80 °C in a steal bomb for 16 h. After cooling, the mixture was purified by flash column chromatography on silica gel (eluting up to 50% ethyl acetate in hexane) to give **10** (197 mg, 93%) as a white solid: mp 112−114 °C; $[\alpha]_D^{26}$ −39.47 (*c* 0.15, MeOH); UV (MeOH) λ_{max} 284.0 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.4−7.5 (m, 6H), 7.2−7.35 (m, 9H), 6.75 (s, 1H), 5.99 (s, 1H), 5.85 (s, 1H), 5.61 (br s, 2H), 5.17 (d, J = 5.5 Hz, 1H), 4.54 (d, J = 6.0 Hz, 1H), 4.03 (d, J = 15.0 Hz, 1H), 3.85 (d, J = 15.5 Hz, 1H), 1.43 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.75, 153.01, 149.57, 149.51, 143.81, 128.58, 127.98, 127.26, 122.75, 121.14, 112.56, 103.10, 87.36, 86.71, 84.99, 83.97, 64.51, 61.55, 27.55, 26.13. HRMS ([M + H]$^+$) calcd, 623.1652; found, 623.1657.

(−)-(1*R*,2*S*,3*R*)-4-Amino-5-iodo-7-[2,3-(isopropylidenedioxy)-4-(trityloxymethyl)-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (11). To a mixture of **3** (380 mg, 0.89 mmol), **7** (310 mg, 1.11 mmol), and Ph_3P (467 mg, 1.78 mmol) in THF (40 mL) was added DIAD (360 mg, 1.78 mmol) at 0 °C. After being stirred for 30 min at room temperature, the mixture was evaporated and purified by column chromatography on silica gel to give a white solid (580 mg). A portion of solid (550 mg) was treated with saturated methanolic ammonia and heated to 80 °C for 16 h. After cooling, the mixture was purified by flash column chromatography on silica gel (eluting up to 50% ethyl acetate in hexane) to give **11** (545 mg, 94%) as a white solid: mp 114−116 °C; $[\alpha]_D^{27}$ −34.68 (*c* 0.34, $CHCl_3$); UV ($CHCl_3$) λ_{max} 287.0 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.4−7.5 (m, 6H), 7.2−7.35 (m, 9H), 6.84 (s, 1H), 6.00 (s, 1H), 5.85 (s, 1H), 5.71 (br s, 2H), 5.18 (d, J = 5.5 Hz, 1H), 4.54 (d, J = 6.0 Hz, 1H), 4.03 (d, J = 15.0 Hz, 1H), 3.85 (d, J = 16.0 Hz, 1H), 1.44 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.93, 152.63, 150.16, 149.56, 143.82, 128.59, 127.98, 128.00, 127.26, 126.41, 122.77, 112.55, 104.59, 87.31, 85.03, 83.98, 64.64, 61.58, 49.36, 27.56, 26.14. HRMS ([M + H]$^+$) calcd, 671.1514; found, 671.1519.

(−)-(1*R*,2*S*,3*R*)-4-Amino-5-fluoro-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (12). A mixture of **8** (300 mg) and HCl (0.2 mL) in MeOH (10 mL), THF (10 mL), and water (1 mL) was heated to 50 °C for 16 h. It was neutralized by $NaHCO_3$ and purified by flash column chromatography on silica gel (eluting up to 10% methanol in dichloromethane) to give **12** (140 mg, 94%) as a white solid: mp 224−225 °C; $[\alpha]_D^{26}$ −102.29 (*c* 0.14, MeOH); UV (H_2O) λ_{max} 282 nm (pH 2), 282 nm (pH 7), 283 nm (pH 11); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.01 (d, J = 2.5 Hz, 1H), 6.94 (br s, 2H), 5.59 (br s, 2H), 5.02 (d, J = 6.5 Hz, 1H), 4.92 (d, J = 6.5 Hz, 1H), 4.90 (t, J = 5.5 Hz, 1H), 4.42 (t, J = 6.0 Hz, 1H), 4.12 (m, 2H), 4.08 (m,, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.22, 156.20, 152.96, 150.52, 146.15, 146.12, 143.86, 141.44, 124.47, 104.73, 104.47, 92.57, 92.42, 77.34, 72.70, 63.71, 58.99. Anal. Calcd for ($C_{12}H_{13}FN_4O_3 \cdot 0.2H_2O$).

(−)-(1*R*,2*S*,3*R*)-4-Amino-5-chloro-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (13). A mixture of compound **9** (190 mg) and HCl (0.2 mL) in MeOH (10 mL), THF (10 mL), and water (1 mL) was heated to 50 °C for 16 h. It was neutralized by $NaHCO_3$ and purified by flash column chromatography on silica gel (eluting up to 10% methanol in dichloromethane) to give **13** (87 mg, 90%) as a white solid: mp 236−237 °C; $[\alpha]_D^{27}$ −140.51 (*c* 0.14, MeOH); UV (H_2O) λ_{max} 285 nm (pH 2), 285 nm (pH 7), 283 nm (pH 11); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.21 (s, 1H), 6.90 (br s, 2H), 5.56 (d, J = 1.2 Hz, 1H), 5.52 (br s, 1H), 5.01 (d, J = 7.2 Hz, 1H), 4.89 (d, J = 6.0 Hz, 1H), 4.85 (t, J = 5.6 Hz, 1H), 4.39 (t, J = 5.6 Hz, 1H), 4.1−4.15 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.16, 152.87, 150.74, 149.33, 124.29, 119.56, 102.04, 100.19, 77.39, 72.74, 64.32, 59.00. Anal. Calcd for ($C_{12}H_{13}ClN_4O_3$).

(−)-(1*R*,2*S*,3*R*)-4-Amino-5-bromo-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (14). A mixture of **10** (100 mg) and HCl (0.1 mL) in MeOH (5 mL), THF (5 mL), and water (0.5 mL) was heated to 50 °C for 16 h. It was neutralized by $NaHCO_3$ and purified by flash column chromatography on silica gel (eluting up to 10% methanol in dichloromethane) to give **14** (49 mg, 90%) as a white solid: mp 242−243 °C; $[\alpha]_D^{26}$ −142.06 (*c* 0.21, MeOH); UV (H_2O) λ_{max} 285 nm (pH 2), 283 nm (pH 7), 284 nm (pH 11); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.26 (s, 1H), 6.70 (br s, 2H), 5.56 (br s, 1H), 5.53 (br s, 1H), 5.02 (d, J = 6.4 Hz, 1H), 4.90 (d, J = 6.4 Hz, 1H), 4.86 (t, J = 5.6 Hz, 1H), 4.39 (t, J = 6.4 Hz, 1H), 4.0−4.15 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.34, 152.68, 150.79, 149.81, 124.28, 122.10, 101.38, 86.02, 77.42, 72.75, 64.47, 59.00. Anal. Calcd for ($C_{12}H_{13}BrN_4O_3 \cdot 0.8H_2O$).

(−)-(1*R*,2*S*,3*R*)-4-Amino-5-iodo-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (15). A mixture of **11** (130 mg) and HCl (0.2 mL) in MeOH (10 mL), THF (10 mL), and water (1 mL) was heated to 50 °C for 16 h. It was neutralized by $NaHCO_3$ and purified by flash column chromatography on silica gel (eluting up to 10% methanol in dichloromethane) and recrystallized with ethanol to give **15** (65 mg, 87%) as a white solid: mp 230−231 °C; $[\alpha]_D^{26}$ −98.93 (*c* 0.12, MeOH); UV (H_2O) λ_{max} 290 nm (pH 2), 286 nm (pH 7), 285 nm (pH 11); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.28 (s, 1H), 6.60 (br s, 2H), 5.56 (d, J = 1.2 Hz, 1H), 5.51 (br s, 1H), 5.00 (d, J = 6.8 Hz, 1H), 4.89 (d, J = 6.0 Hz, 1H), 4.85 (t, J = 5.6 Hz, 1H), 4.39 (t, J = 5.6 Hz, 1H), 4.0−4.15 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.59, 152.19, 150.74, 150.42, 127.34, 124.37, 103.60, 77.48, 72.75, 64.56, 59.00, 50.91. Anal. Calcd for ($C_{12}H_{13}IN_4O_3 \cdot 0.15C_2H_5OH$).

(−)-(1*R*,2*S*,3*R*)-4-Acetylamino-5-nitro-7-[2,3-diacetoxy-4-acetoxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (17). A mixture of **2** (183 mg, 0.70 mmol) and acetic anhydride (1 mL) in pyridine (8 mL) was heated to 60 °C for 2 days. The mixture was evaporated and purified by column chromatography on silica gel to give mono- and diacetyl derivatives **16a** and **16b** (260 mg) as a white solid. To a mixture of **16a** and **16b** (260 mg) in methylene chloride (30 mL) were added H_2SO_4 (0.5 mL) and HNO_3 (fuming, 0.5 mL) (prepared from the addition of H_2SO_4 into HNO_3) at 0 °C. The mixture was stirred at 0 °C for 15 min and neutralized by $NaHCO_3$ (excess) and MeOH (5 mL). It was filtered through Celite and washed with ethyl acetate. The combined filtrate was

purified by flash column chromatography on a silica gel to give **17** (225 mg, 68%) as a light-yellow solid: mp 66−67 °C; $[\alpha]_D^{27}$ −141.94 (*c* 0.19, $CHCl_3$); UV ($CHCl_3$) λ_{max} 347, 279 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.69 (br s, 1H), 8.74 (s, 1H), 8.17 (s, 1H), 6.16 (m, 1H), 6.06 (m, 1H), 5.97 (m, 1H), 5.44 (t, J = 6.0 Hz, 1H), 4.78 (m, 2H), 2.53 (s, 3H), 2.15 (s, 3H), 2.14 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.29, 170.11, 169.67, 169.56, 153.95, 151.16, 151.12, 142.86, 128.91, 128.73, 128.05, 98.34, 75.84, 73.04, 62.91, 60.25, 26.21, 20.72, 20.61, 20.38. Anal. Calcd for ($C_{20}H_{21}N_5O_9$).

(−)-(1*R*,2*S*,3*R*)-4-Amino-5-nitro-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (18). A mixture of **17** (215 mg) in saturated methanolic ammonia was heated to 80 °C for 16 h in a steal bomb. After cooling, the mixture was purified by flash column chromatography on silica gel (eluting up to 10% methanol in dichloromethane) to give **18** (92 mg, 66%) as a yellow solid: mp 245−246 °C; $[\alpha]_D^{23}$ −195.08 (*c* 0.28, DMSO); UV (H_2O) λ_{max} 339, 261 nm (pH 2), 371, 276 nm (pH 7), 371, 275 nm (pH 11); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (s, 1H), 8.23 (s, 1H), 7.80 (br s, 1H), 7.20 (br s, 1H), 5.64 (d, J = 2.0 Hz, 1H), 5.59 (m, 1H), 5.13 (d, J = 6.8 Hz, 1H), 4.98 (d, J = 6.0 Hz, 1H), 4.92 (t, J = 5.6 Hz, 1H), 4.41 (t, J = 6.0 Hz, 1H), 4.22 (q, J = 6.4 Hz, 1H), 4.10 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.98, 154.49, 152.02, 150.65, 128.98, 128.33, 123.08, 95.44, 77.41, 72.76, 65.69, 59.04. Anal. Calcd for ($C_{12}H_{13}N_5O_5 \cdot 0.4H_2O$).

(+)-(1*S*,2*S*,3*R*)-2,3-Diacetoxy-4-acetoxymethyl-4-cyclopenten-1-ol (19). To a solution of compound **3** (6.00 g, 14.0 mmol) in DMF (60 mL) was added a 60% dispersion of sodium hydride (1.12 g, 28 mmol) at 0 °C. After 10 min, the mixture was treated with 4-methoxybenzyl chloride (2.1 mL, 15.4 mmol) and stirred at room temperature for 3 h. It was poured into water (300 mL) and extracted with ethyl ether (100 mL × 2). The combined ether layer was dried ($MgSO_4$) and evaporated. The resulting solid was dissolved in MeOH (150 mL), THF (150 mL), and water (15 mL) and treated with concentrated HCl (0.5 mL). The mixture was heated to 55−60 °C for 4 h and neutralized by $NaHCO_3$. After evaporation, the mixture was purified by vacuum column chromatography on a silica gel to give the triol compound (2.61 g, 70%) as a white solid. Part of the triol (101 mg, 0.38 mmol) and acetic anhydride (0.25 mL) in pyridine (2 mL) and methylene chloride (2 mL) was stirred at room temperature overnight. The mixture was evaporated and purified by flash column chromatography on a silica gel. The appropriate fraction was collected and evaporated and dissolved in CH_2Cl_2/H_2O (10 mL/0.5 mL). It was treated with DDQ (260 mg, 1.14 mmol) and stirred at room temperature for 3 h. The mixture was mixed with water (20 mL) and extracted with methylene chloride. The combined organic layer was dried over Na_2SO_4 and purified by flash column chromatography on a silica gel to give a reddish sticky liquid. It was dissolved in chloroform and the insoluble solid was filtered off and the filtrate was evaporated to give **19** (80 mg, 78%) as a white solid: mp 47 °C; $[\alpha]_D^{24}$ +24.95 (*c* 0.45, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14 (s, 1H), 5.68 (d, J = 6 Hz, 1H), 5.29 (t, J = 5.6 Hz, 1H), 4.69 (br s, 3H), 2.13 (s, 3H), 2.10 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.45, 170.06, 139.84, 133.36, 73.14, 72.43, 72.00, 60.15, 20.74, 20.71, 20.63. Anal. Calcd for ($C_{12}H_{16}O_7$).

(−)-(1*R*,2*S*,3*R*)-4-Chloro-5-iodo-7-[2,3-diacetoxy-4-acetoxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (20). A mixture of **19** (470 mg, 1.72 mmol), **7** (290 mg, 1.03 mmol), and Ph_3P (1.35 g, 5.16 mmol) was taken in THF (30 mL), and DIAD (1.04 g, 5.16 mmol) was added at 0 °C to the reaction mixture. The reaction mixture was stirred for 1 h at room temperature and then concentrated under reduced pressure. The crude was further purified by flash column chromatography on silica gel (eluting up to 30% ethyl acetate in hexane) to yield **20** (500 mg, 54%) as a light-yellow solid: mp 49 °C; $[\alpha]_D^{26}$ −126.83 (*c* 0.25, $CHCl_3$); UV ($CHCl_3$) λ_{max} 308, 269, 240 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 7.35 (s, 1H), 6.11 (m, 1H), 6.03 (m, 1H), 5.95 (dd, J = 5.6, 0.8 Hz, 1H), 5.42 (t, J = 5.6 Hz, 1H), 4.75 (m, 2H), 2.14 (s, 3H), 2.13 (s, 3H), 2.00 (s, 3H). Anal. Calcd for ($C_{18}H_{17}ClIN_3O_6$).

(−)-(1*R*,2*S*,3*R*)-4-Chloro-5-ethenyl-7-[2,3-diacetoxy-4-acetoxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (21). A mixture of **20** (550 mg. 1.03 mmol), tri-*n*-butylvinyltin (0.9 mL, 3.0 mmol), CuI (39 mg, 0.206 mmol), triethylamine (0.29 mL, 2.0 mol), and $(PPh_3)_4Pd$ (119 mg, 0.10 mmol) in DMF (10 mL) was stirred at 60 °C for 8 h. The mixture was poured into water (10 mL) and extracted with ethyl acetate (100 mL). The organic layer was dried over Na_2SO_4, evaporated under reduced pressure, and purified by flash column chromatography on a silica gel (eluting up to 30% ethyl acetate in hexane) to yield **21** (310 mg, 69%) as a light-yellow sticky liquid: $[\alpha]_D^{27}$ −142.24 (*c* 0.21, $CHCl_3$); UV ($CHCl_3$) λ_{max} 243 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 7.35 (s, 1H), 7.22 (ddd, J = 17.6, 12, 0.8 Hz, 1H), 6.15 (m, 1H), 6.05 (m, 1H), 5.97 (dd, J = 6, 1.2 Hz, 1H), 5.45 (t, J = 5.6 Hz, 1H), 5.29 (dd, J = 10.8, 1.2 Hz, 1H), 4.76 (m, 2H), 2.14 (s, 3H), 2.13 (s, 3H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.35, 170.26, 169.74, 152.54, 151.79, 150.78, 140.97, 130.72, 126.91, 121.71, 115.83, 115.29, 114.75, 75.85, 73.00, 61.60, 60.45, 20.75, 20.65, 20.46. HR-MS calcd for $(M + H)^+$ 434.1113, found 434.1110.

(−)-(1*R*,2*S*,3*R*)-4-Amino-5-ethenyl-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (22). A mixture of **21** (290 mg), methanol (5 mL), dioxane (3 mL), and liquid ammonia (∼15 mL) was heated to 80 °C for 16 h in a steel bomb. After cooling, the mixture was purified by flash column chromatography on silica gel (eluting up to 10% methanol in dichloromethane) to give **22** (106 mg, 55%) as a light-yellow solid: mp 168−170 °C; $[\alpha]_D^{26}$ −187.84 (*c* 0.11, MeOH); UV (H_2O) λ_{max} 294 nm (pH 2), 287 nm (pH 7), 287 nm (pH 11); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.32 (s, 1H), 7.09 (dd, J = 17.6, 10.8 Hz, 1H), 6.65 (br s, 2H), 5.5−5.6 (m, 3H), 5.0−5.1 (m, 2H), 4.85−4.95 (m, 2H), 4.40 (t, J = 5.6 Hz, 1H), 4.05−4.15 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.99, 151.77, 151.02, 150.32, 129.63, 124.78, 119.07, 113.93, 112.68, 100.86, 77.62, 72.76, 63.97, 59.02. Anal. Calcd for ($C_{14}H_{16}N_4O_3 \cdot 0.15H_2O$).

(−)-(1*R*,2*S*,3*R*)-4-Chloro-5-trimethylsilanylethynyl-7-[2,3-diacetoxy-4-acetoxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (23). A mixture of **20** (350 mg. 0.66 mmol), trimethylsilylacetylene (0.186 mL, 1.32 mmol), CuI (13 mg, 0.066 mmol), triethylamine (0.184 mL, 1.32 mol), and $(PPh_3)_4Pd$ (38 mg, 0.033 mmol) in DMF (7 mL) was stirred at 60 °C for 3 h. The mixture was poured into water and extracted with ethyl acetate. The organic layer was evaporated and purified by flash column chromatography on a silica gel (eluting up to 20% ethyl acetate in hexane) to yield **23** (190 mg, 57%) as a light-yellow solid: mp 56−57 °C; $[\alpha]_D^{25}$ −156.83 (*c* 0.22, $CHCl_3$); UV ($CHCl_3$) λ_{max} 310, 235 nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.42 (s, 1H), 6.10 (br s, 1H), 6.02 (br s, 1H), 5.94 (d, J = 6 Hz, 1H), 5.40 (t, J = 6 Hz, 1H), 4.74 (s, 2H), 2.133 (s, 3H), 2.128 (s, 3H), 1.99 (s, 3H), 0.27 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.61, 170.46, 169.88, 153.85, 151.98, 151.09, 141.68, 130.46, 130.31, 117.46, 99.17, 98.93, 96.03, 76.14, 73.27, 62.25, 60.66, 21.02, 20.92, 20.70, 0.00. Anal. Calcd for ($C_{23}H_{26}ClN_3O_6Si$).

(−)-(1*R*,2*S*,3*R*)-4-Amino-5-ethynyl-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (24). A mixture of **23** (180 mg), methanol (3 mL), dioxane (3 mL), and liquid ammonia (∼10 mL) was heated to 80 °C for 16 h in a steel bomb. After cooling, the mixture was purified by flash column chromatography on silica gel (eluting up to 10% methanol in dichloromethane) to give **24** (75 mg, 73%) as a light-yellow solid: mp 126−128 °C; $[\alpha]_D^{26}$ −194.42 (*c* 0.10, MeOH); UV (H_2O) λ_{max} 287 nm (pH 2), 281 nm (pH 7), 279 nm (pH 11); ^{1}H NMR (400 MHz, CD_3OD) δ 8.12 (s, 1H), 7.37 (s, 1H), 5.80 (m, 1H), 5.63 (m, 1H), 4.58 (d, J = 5.6 Hz, 1H), 4.29 (m, 2H), 4.19 (t, J = 5.2 Hz, 1H), 3.71 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.92, 153.08, 150.83, 149.84, 127.71, 124.17, 102.84, 93.52, 83.20, 78.08, 77.41, 72.77, 64.58, 58.99. Anal. Calcd for ($C_{14}H_{14}N_4O_3 \cdot 0.3H_2O$).

(−)-(1*R*,2*S*,3*R*)-4-Amino-5-cyano-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (26). A mixture of Bu_3SnCN (499 mg, 1.58 mmol) and $Pd(PPh_3)_4$ (91 mg, 0.079 mmol) in dichloroethane (15 mL) was heated to reflux for

30 min. To this mixture **20** (425 mg, 0.79 mmol) in dichloromethane (10 mL) was added and refluxed overnight. The mixture was evaporated and purified by flash column chromatography on a silica gel to give a crude oily compound **25**. A mixture of crude oily compound was saturated with methanolic ammonia and was heated to 80 °C for 16 h in a steal bomb. After cooling, the mixture was purified by flash column chromatography on a silica gel (eluting up to 10% methanol in dichloromethane) to give **26** (100 mg, 44%) as a white solid: mp 178−180 °C; $[\alpha]_D^{27}$ −161.12 (*c* 0.13, MeOH); UV (H_2O) λ_{max} 277 nm (pH 2), 278 nm (pH 7), 278 nm (pH 11); ^{1}H NMR (500 MHz, CD_3OD) δ 8.26 (s, 1H), 7.95 (s, 1H), 5.86 (m, 1H), 5.73 (m, 1H), 4.64 (d, J = 5.5 Hz, 1H), 4.3−4.4 (m, 2H), 4.26 (t, J = 5.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 157.45, 153.84, 151.45, 150.71, 132.96, 123.62, 116.01, 101.84, 82.41, 77.43, 72.78, 65.32, 59.03. Anal. Calcd for ($C_{13}H_{13}N_5O_3 \cdot 0.6H_2O$).

(−)-(1*R*,2*S*,3*R*)-4-Amino-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (27). A mixture of **26** (50 mg) and 30% H_2O_2 (0.4 mL) in NH_4OH (4 mL) was stirred at room temperature for 1 h. The mixture was evaporated and the residue was dispersed in MeOH/EtOH (2 mL/ 4 mL) and filtered to give **27** (47 mg, 92%) as a light-yellow solid: mp 260−261 °C; $[\alpha]_D^{27}$ −134.41 (*c* 0.20, H_2O); UV (H_2O) λ_{max} 280 nm (pH 2), 282 nm (pH 7), 283 nm (pH 11); ^{1}H NMR (400 MHz, CD_3OD) δ 8.10 (s, 1H), 7.87 (s, 1H), 5.86 (m, 1H), 5.67 (m, 1H), 4.63 (d, J = 5.6 Hz, 1H), 4.26 − 4.40 (m, 2H), 4.19 (t, J = 5.2 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.58, 158.21, 152.10, 150.64, 150.17, 125.44, 124.31, 110.43, 101.51, 77.88, 72.91, 64.71, 58.79. Anal. Calcd for ($C_{13}H_{15}N_5O_4 \cdot 0.6H_2O$).

Virology. Anti-HCV Assay Using 2′-C-Me-A as Positive Control. The subgenomic HCV replicon cell line, ET, was obtained from Dr. Ralf Bartenschlager and ReBLikon GmbH. The cell line harbors a dicistronic self-replicating HCV RNA replicon with a firefly luciferase gene and three cell culture-adaptive mutations. Measurement of the activity of the luciferase reporter is directly proportional to the replication of the RNA replicon in the experimental conditions used. Antiviral and cytotoxicity assays were performed as described previously[11] with minor modifications. For the marginally active compounds, we were unable to calculate the exact EC_{50} values. This is the reason that we expressed them as "percentage of level of inhibition to control cells at 20 μM".[12] Briefly, the ET cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 2 mM L-glutamine, 1× nonessential amino acids, 10% fetal bovine serum (FBS), and 0.25mg/mL G418 (Invitrogen, Carlsbad, CA). The cells were seeded in 96-well tissue culture plates dedicated for analysis of antiviral activity or cell numbers (cytotoxicity) at a density of 5000/well in 100 μL of DMEM without G418 overnight. Serial dilutions of compounds were prepared in DMEM and added to the appropriate wells. Following 3 days of incubation, the cells were processed to assess the replicon-derived luciferase activity with the Steady-Glo luciferase assay system (Promega, Madison, WI) according to manufacturer's instruction. Cytotoxic effect of the compound on the viability of the replicon cells was determined using a tetrazolium-based CytoTox-1 cell proliferation assay (Promega). Each data point represents an average of four replicates in cell culture to derive applicable EC_{50} (concentration of compound inhibiting luciferase activity by 50%), IC_{50} (concentration of compound decreasing cell viability by 50%), and SI_{50} (selective index, EC_{50}/IC_{50}) values.

A Further Anti-HCV Assay Using 2′-*C*-Me-C and 2′-F-*C*-Me-C as Positive Controls. Subgenomic HCV replicon RNA-containing Huh-7 cells (clone B, kindly provided by Dr. C. M. Rice) were seeded in a 96-well plate at 3000 cells/well, and the compounds were added in duplicate at two different dilutions immediately after seeding. Following 5 days of incubation (37 °C, 5% CO_2), total cellular RNA was isolated by using RNeasy 96-kit, Qiagen. Replicon RNA and an internal control (TaqMan rRNA contro reagents, Applied Biosystems) were amplified in a single step multiplex real time RT-PCR assay.[13] The antiviral effectiveness of the compounds was calculated by subtracting the threshold RT-PCR cycle of the test compound from the threshold RT-PCR cycle of the no-drug control (ΔCtHCV). A ΔCt of 3.3 equals a 1 log reduction (equal to 90% less starting material) in replicon RNA levels. The cytotoxicity of the compounds was also calculated by using the ΔCt rRNA values. Two compounds (2′-*C*-Me-C and 2′-F-*C*-Me-C) were used as positive controls. To determine EC_{50}, EC_{90}, and CC_{50} values, ΔCt values were first converted into fraction of starting material by using CalcuSyn computer software (Biosoft, Ferguson, MO).

HBV Antiviral Assays. HBV antiviral assays were conducted as previous described.[14] Briefly, confluent cultures of 2.2.15 cells were maintained on 96-well flat-bottomed tissue culture plates (confluence in this culture system is required for active, high levels of HBV replication equivalent to that observed in chronically infected individuals). Cultures were treated with nine consecutive daily doses of the test compounds. HBV DNA levels were assessed by quantitative blot hybridization 24 h after the last treatment. Cytotoxicity was assessed by uptake of neutral red dye 24 h following the last treatment.

Activity Against Drug-Resistant HBV Mutants. Activity against lamivudine-resistant and adefovir-resistant HBV mutants was performed in a 5-day assay using a transient transfection method as previously described.[15] Antiviral activity was determined by quantitative Southern blot hybridization of intracellular HBV DNA replication intermediates (HBV RI) following 4 days of drug treatment.

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Supporting Information Available: Elemental analysis data for the final compounds and few key intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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