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Synthesis, Anti-Hepatitis B Virus and Anti-Hepatitis C Virus Activities of 7-Deazaneplanocin A Analogues *In Vitro*

Hyo-Joong Kim^a, Ashoke Sharon^a, Chandralata Bai^a, Jianing Wang^a, Madhan Allu^a, Zhuhui Huang^b, Michael G. Murray^b, Leda Bassit^c, Raymond F. Schinazi^c, Brent Korba^d, and Chung K. Chu^{a,*}

^aThe University of Georgia College of Pharmacy, Athens, GA 30602

^bSouthern Research Institute, Frederick, MD 21701

^cEmory University School of Medicine/VA Medical Center, Atlanta, GA 30033

^dGeorgetown University Medical Center, Rockville, MD 20850.

Abstract

A series of 7-deazaneplanocin A (7-DNPA, **2**) analogues were synthesized and evaluated for *in vitro* antiviral activity against HBV and HCV. The synthesis 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 (EC₅₀ ranged from 1.8 to 20.1 μM), and compounds **2**, **15**, **22** and **24** demonstrated moderate to potent anti-HBV activity (EC₅₀ 0.3 to 3.3 μM). In addition, the compound **24** also showed the activity against lamivudine- and adefovir- associated HBV mutants.

Introduction

Neplanocin A (NPA, Figure 1, **1**), a fermentation-derived carbocyclic nucleoside exhibits a broad-spectrum antiviral activity and may 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 due to its cytotoxicity, which may be attributed mainly to the phosphorylation of NPA at the 5'-position² 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 to discover 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, while the current therapies remain still unsatisfactory.⁷ Furthermore, there is no vaccines as well as no effective therapeutic agents are available for

Corresponding Author: Dr. C. K. Chu, Distinguished Research Professor, Emeritus, College of Pharmacy, The University of Georgia, Athens, GA 30602, USA., Tel.: (706) 542-5379, Fax: (706) 542-5381, E-Mail: DCHU@RX.UGA.EDU .

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 due to 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 as well as safety profile remains 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

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, anti-HBV and anti-HCV activity 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)-7H-pyrrolo[2,3-*d*]pyrimidine (7-DNPA, Figure 1, **2**), was investigated by synthesizing the corresponding 7-substituted-7-DNPA analogues, as shown in Scheme 1, Scheme 2 and Scheme 3. For the 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 8 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 acetone groups of **8–11** was accomplished using hydrochloric acid in a mixture of methanol and THF at 50 °C to yield a 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** were accomplished with saturated methanolic ammonia to give 7-nitro-7-DNPA **18** in 66 % yield.

The synthesis of vinyl, acetylene and cyano substituted derivatives of 7-DNPA, **22**, **24**, and **26**, were illustrated in Scheme 3. During our first attempt, we used our strategy based on the 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, which could not be separated. To avoid the deprotection step in acidic conditions, a new key intermediate **19** (acetyl protected) were prepared from the cyclopentenol intermediate **3** by alkylation with *p*-methoxy benzyl bromide, deprotection of the trityl and acetone groups followed by acetylation and deprotection of *p*-methoxybenzyl (PMB) group using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). Subsequently, the acetyl protected cyclopentenol intermediate **19** was treated with 7-iodo-7-deaza-6-chloropurine (**7**) under the Mitsunobu conditions 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 hexamethylphosphoramide (HMPA) at 80

°C for 16 h to obtain **21** in 26 % yield, which was deprotected under the basic condition to give 7-vinyl-7-DNPA **22**. The 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** used to 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 analogs 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 previously described,¹¹ and the results are summarized in Table 1. Compounds were initially assessed at a single-concentration of 20 μ M. Any potentially active or cytotoxic compounds in the primary assay were further evaluated in the dose-response assay. From these studies we found that six out of ten NPA analogues (**2**, **13–15**, **24** and **27**) exhibited significant anti-HCV activity with EC₅₀ ranged from 1.8 to 20.1 μ M. 7-DNPA **2** displayed anti-HCV activity with an EC₅₀ value of 2.5 μ M without any cytotoxicity at a concentration up to 100 μ M (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)^{9h} or 2'-F-C-Me-cytosine (2'-F-C-Me-C)^{9i, 9j} (Table 2). 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,^{9f} it was of interest to explore the electrostatic as well as 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 increasing size of halogens at the 7-position (**13**, EC₅₀ 14.7 μ M; **14**, EC₅₀ 16.7 μ M; **15**, EC₅₀ 2.1 μ M), while a concomitant increase in cytotoxicity was also observed (**13**, IC₅₀ 50.1 μ M; **14**, IC₅₀ 41.7 μ M; **15**, IC₅₀ 15.0 μ M). 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 (EC₅₀ 1.8 μ M), however, it was also the most cytotoxic (IC₅₀ 11.8 μ M). The anti-HCV activity decreased significantly when electron-withdrawing substituents (CN or NO₂) 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.^{9f} 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-DNPA 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-resistance mutants. As shown in Table 3, the compounds **2**, **15**, **22** and **24** exhibited significant anti-HBV activity against wild-type HBV with EC₅₀ values of 0.60, 0.43, 3.3 and 0.32 μ M, respectively in an extracellular virion HBV DNA assay. 7-DNPA **2** showed moderate cytotoxicity (IC₅₀ 22 μ M), whereas the compounds

15, **22** and **24** didn't show any significant cytotoxicity up to 300 μ M. The most potent compound **24** (7-ethynyl derivative) was also evaluated against drug resistant HBV mutants (Table 4), in which the compound **24** did not lose activity against all the lamivudine (3TC) and adefovir associated HBV-mutants, with an EC₅₀ value comparable to that of adefovir (ADV). It was also found that compound **24** confers a slight resistance (two 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.

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** were found most significant against wild-type as well as it maintained 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 ¹H NMR and 125 MHz for ¹³C 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.

(-)-(1R, 2S, 3R)-4-Amino-5-fluoro-7-[2,3-(isopropylidenedioxy)-4-(trityloxymethyl)-4-cyclopenten-1-yl]-7H-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 Ph₃P (388 mg, 1.48 mmol) in THF (30 mL), DIAD (299 mg, 1.48 mmol) was added at 0 °C. After stirring 30 min at rt, the mixture was evaporated under reduced pressure and purified by column chromatography on a silica gel. The appropriate fraction was collected and evaporated. The residue was treated with saturated methanolic ammonia and heated to 80 °C in a sealed 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; [α]_D²⁷ -25.33 (c 0.13, CHCl₃); UV (CHCl₃) $\lambda_{\max}$ 283.0 nm; ¹H NMR (500 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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.

(-)-(1R, 2S, 3R)-4-Amino-5-chloro-7-[2,3-(isopropylidenedioxy)-4-(trityloxymethyl)-4-cyclopenten-1-yl]-7H-pyrrolo[2,3-d]pyrimidine (9**)**

To a mixture of **3** (193 mg, 0.45 mmol), **5** (110 mg, 0.59 mmol) and Ph₃P (177 mg, 0.68 mmol) in THF (20 mL), DIAD (0.13 mL, 0.68 mmol) was added at 0 °C. After stirring 30 min at rt,

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 steel 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, CHCl₃); UV (CHCl₃) $\lambda_{\max}$ 284.0 nm; ¹H NMR (500 MHz, CDCl₃) 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); ¹³C NMR (125 MHz, CDCl₃) 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 Ph₃P (134 mg, 0.51 mmol) in THF (20 mL), DIAD (0.10 mL, 0.51 mmol) was added at 0 °C. After stirring 30 min at rt, the mixture was evaporated and purified by column chromatography on a silica gel. The appropriate fraction was collected and evaporated. The residue was treated with saturated methanolic ammonia and heated to 80 °C in a steel 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) $\lambda_{\max}$ 284.0 nm; ¹H NMR (500 MHz, CDCl₃) 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); ¹³C NMR (125 MHz, CDCl₃) 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 Ph₃P (467 mg, 1.78 mmol) in THF (40 mL) DIAD (360 mg, 1.78 mmol) was added at 0 °C. After stirring 30 min at rt, the mixture was evaporated and purified by column chromatography on a 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, CHCl₃); UV (CHCl₃) $\lambda_{\max}$ 287.0 nm; ¹H NMR (500 MHz, CDCl₃) 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); ¹³C NMR (125 MHz, CDCl₃) 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 NaHCO₃ 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 (H₂O) $\lambda_{\max}$ 282 nm (pH 2), 282 nm (pH 7), 283 nm (pH 11); ¹H 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 ($\text{C}_{12}\text{H}_{13}\text{FN}_4\text{O}_3 \cdot 0.2\text{H}_2\text{O}$).

(-)-(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); ^1H 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 ($\text{C}_{12}\text{H}_{13}\text{ClN}_4\text{O}_3$).

(-)-(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); ^1H 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 ($\text{C}_{12}\text{H}_{13}\text{BrN}_4\text{O}_3 \cdot 0.8\text{H}_2\text{O}$).

(-)-(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); ^1H 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 ($\text{C}_{12}\text{H}_{13}\text{IN}_4\text{O}_3 \cdot 0.15\text{C}_2\text{H}_5\text{OH}$).

(-)-(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 a silica gel to give mono- and di-acetyl derivatives **16a**, **16b** (260 mg) as a white solid. To a mixture of **16a**, **16b** (260 mg) in methylene chloride (30 mL), H_2SO_4 (0.5 mL) and HNO_3 (Fuming, 0.5 mL) (prepared from the addition of H_2SO_4 into HNO_3) was added 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, CHCl₃); UV (CHCl₃) λ_{max} 347, 279 nm; ¹H NMR (500 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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 (C₂₀H₂₁N₅O₉).

(-)-(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 steel 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 (H₂O) λ_{max} 339, 261 nm (pH 2), 371, 276 nm (pH 7), 371, 275 nm (pH 11); ¹H 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); ¹³C NMR (100 MHz, CDCl₃) 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 (C₁₂H₁₃N₅O₅·0.4H₂O).

-(+)-(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), 60 % dispersion of sodium hydride (1.12 g, 28 mmol) was added at 0 °C. After 10 min, the mixture was treated with 4-methoxy benzylchloride (2.1 mL, 15.4 mmol) and stirred at rt for 3h. It was poured into water (300 mL) and extracted with ethyl ether (100 mL X 2). The combined ether layer was dried (MgSO₄) and evaporated. The resulting solid was dissolved in MeOH (150 mL), THF (150 mL) and water (15 mL) and treated with conc. HCl (0.5 mL). The mixture was heated to 55–60 °C for 4h and neutralized by NaHCO₃. After evaporation, the mixture was purified by vacuum column chromatography on a silica gel to give 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 rt 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 CH₂Cl₂/H₂O (10 mL/0.5 mL). It was treated with DDQ (260 mg, 1.14 mmol) and stirred at rt for 3 h. The mixture was mixed with water (20 mL) and extracted with methylene chloride. The combined organic layer was dried over Na₂SO₄ 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, CHCl₃); ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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 (C₁₂H₁₆O₇).

(-)-(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)

The mixture of **19** (470 mg, 1.72 mmol), **7** (290 mg, 1.03 mmol) and Ph₃P (1.35 g, 5.16 mmol) was taken in THF (30 mL) DIAD (1.04 g, 5.16 mmol) was added at 0 °C into the reaction mixture. The reaction mixture was stirred for 1 h at rt, and then concentrated under reduced pressure. The crude were 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, CHCl₃); UV (CHCl₃) $\lambda_{\max}$ 308, 269, 240 nm; ¹H NMR (400 MHz, CDCl₃) 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 (C₁₈H₁₇ClIN₃O₆).

(-)-(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), triethyl amine (0.29 mL, 2.0 mol) and (PPh₃)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 Na₂SO₄, 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, CHCl₃); UV (CHCl₃) $\lambda_{\max}$ 243 nm; ¹H NMR (400 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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-hydroxy methyl-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. 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 (H₂O) $\lambda_{\max}$ 294 nm (pH 2), 287 nm (pH 7), 287 nm (pH 11); ¹H 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); ¹³C 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 (C₁₄H₁₆N₄O₃·0.15H₂O).

(-)-(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), trimethylsilyl acetylene (0.186 mL, 1.32 mmol), CuI (13 mg, 0.066 mmol), triethyl amine (0.184 mL, 1.32 mol) and (PPh₃)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, CHCl₃); UV (CHCl₃) $\lambda_{\max}$ 310, 235 nm; ¹H NMR (500 MHz, CDCl₃) 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); ¹³C NMR (100 MHz, CDCl₃) 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 (C₂₃H₂₆ClN₃O₆Si).

(-)-(1R, 2S, 3R)-4-Amino-5-ethynyl-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7H-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. 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 (H₂O) λ_{max} 287 nm (pH 2), 281 nm (pH 7), 279 nm (pH 11); ¹H NMR (400 MHz, CD₃OD) 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); ¹³C 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 (C₁₄H₁₄N₄O₃·0.3H₂O).

(-)-(1R, 2S, 3R)-4-Amino-5-cyano-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7H-pyrrolo[2,3-d]pyrimidine (26)

A mixture of Bu₃SnCN (499 mg, 1.58 mmol) and Pd(PPh₃)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 steel 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 (H₂O) λ_{max} 277 nm (pH 2), 278 nm (pH 7), 278 nm (pH 11); ¹H NMR (500 MHz, CD₃OD) 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); ¹³C 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 (C₁₃H₁₃N₅O₃·0.6H₂O).

(-)-(1R, 2S, 3R)-4-Amino-7-[2,3-dihydroxy-4-hydroxymethyl-4-cyclopenten-1-yl]-7H-pyrrolo[2,3-d]pyrimidine-5-carboxamide (27)

A mixture of **26** (50 mg) and 30% H₂O₂ (0.4 mL) in NH₄OH (4 mL) was stirred at rt 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, H₂O); UV (H₂O) λ_{max} 280 nm (pH 2), 282 nm (pH 7), 283 nm (pH 11); ¹H NMR (400 MHz, CD₃OD) 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); ¹³C 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 (C₁₃H₁₅N₅O₄·0.6H₂O).

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 previously¹¹ with minor modifications. For the marginally active compounds, we were unable to calculate the exact EC₅₀ values. This is the reason why we expressed as “percentage of level of inhibition to control cells at 20 μ M.”¹² Briefly, the ET cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 2 mM L-glutamine, 1 $\times$ 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 5,000/well in 100 μ l DMEM without G418 overnight. Serial dilutions of compounds were prepared in DMEM and added to the appropriate wells. Following three 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 EC₅₀ (concentration of compound inhibiting luciferase activity by 50%), IC₅₀ (concentration of compound decreasing cell viability by 50%) and SI₅₀ (Selective Index: EC₅₀/IC₅₀) 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. CM Rice) were seeded in a 96-well plate at 3,000 cells/well, and the compounds were added in duplicate at two different dilutions immediately after seeding. Following five days incubation (37 °C, 5% CO₂), 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 (Δ CtHCV). A Δ Ct 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 Δ Ct rRNA values. Two compounds (2'-C-Me-C and 2'-F-C-Me-C) were used as positive controls. To determine EC₅₀, EC₉₀ and CC₅₀ values, Δ Ct 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 hr. after the last treatment. Cytotoxicity was assessed by uptake of neutral red dye 24 hr. 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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, triphenyl phosphine
 PMB, *p*-methoxybenzyl
 DDQ, 2,3-dichloro-5,6-dicyano-1,6-benzoquinone
 HMPA, tetravinyltin in hexamethylphosphoramide
 TBAF, tetrabutylammonium fluoride
 DMEM, Dulbecco's modified Eagle's medium
 FBS, fetal bovine serum
 RI, replication intermediates.

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1 $\text{X} = \text{N}$ **NPA**
2 $\text{X} = \text{CH}$ **7-DNPA**

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

Scheme 1.

Reagent and conditions: a) PPh_3 , DIAD, THF, rt; b) NH_3 , MeOH, 80 °C; c) HCl, MeOH, THF, 50 °C.

Scheme 2.

Reagent and conditions: a) Ac₂O, pyridine, 60 °C; b) HNO₃, H₂SO₄, CH₂Cl₂; c) NH₃, MeOH, 80 °C.

Scheme 3.

Reagents and conditions a) i. NaH, *p*-methoxybenzyl chloride, DMF ii. HCl/MeOH/THF iii. Ac₂O, pyridine iv. DDQ, CH₂Cl₂, H₂O; b) PPh₃, DIAD, THF, rt, 7; c) Bu₃Sn(CHCH₂), Pd (PPh₃)₄, Et₃N, CuI, DMF; d) NH₃, MeOH, 80 °C; e) TMS-C≡CH, Pd(PPh₃)₄, Et₃N, CuI; f) NH₃, MeOH, 80 °C; g) Bu₃SnCN, (PPh₃)₄Pd, ClCH₂CH₂Cl, reflux h) NH₃, MeOH, 80 °C; i) H₂O₂, NH₄OH.

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 (μ M) with percentage donating inhibition level compared to control cells

^b Cyclopentenyl-7-deazaguanosine analogue (refer to Scheme 3)

Table 2

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

Compd	Anti-HCV activity		Cytotoxicity
	EC ₅₀ (μ M) ^a	EC ₉₀ (μ M) ^a	CC ₅₀ (μ M) ^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

^aEffective concentrations required for reducing HCV level by 50% and 90% in 5 days.

^bCytotoxicity 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

Compd	R	Anti-HCV activity		Cytotoxicity IC ₅₀ (μM)
		EC ₅₀ (μM) virion	EC ₅₀ (μM) HBV RI	
2	H	0.60	ND ^a	22
12	F	>10	ND	>300
13	Cl	>10	ND	>300
14	Br	>10	ND	>300
15	I	0.43	1.6	>300
18	NO ₂	>10	ND	>300
22	Vinyl	3.3	10	>300
24	Acetylene	0.32	1.8	>300
26	CN	>10	ND	>300
27	CONH ₂	>10	ND	>300
3TC	-	0.048	0.15	>2000

^aND, not determined

Table 4*In vitro* Antiviral Activity Against HBV Mutants Based on the Intracellular HBV DNA Replication Assay

Compd	Strain	Anti-HCV activity EC ₅₀ (μ M)	3TC EC ₅₀ (μ M)	Adefovir EC ₅₀ (μ M)
24	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.