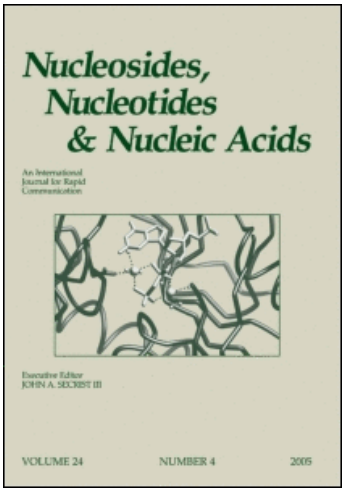


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and In Vitro Activity Evaluation of 2',3'-*C*-Dimethyl Carbocyclic Nucleoside Analogues as Potential Anti-HCV Agents

Ok Hyun Ko[a]; Joon Hee Hong[a]
[a] BK21-Project Team, College of Pharmacy, Chosun University, Kwangju, Republic of Korea

To cite this Article Ko, Ok Hyun and Hong, Joon Hee(2009) 'Synthesis and In Vitro Activity Evaluation of 2',3'-*C*-Dimethyl Carbocyclic Nucleoside Analogues as Potential Anti-HCV Agents', Nucleosides, Nucleotides and Nucleic Acids, 28: 8, 761 — 771
To link to this Article: DOI: 10.1080/15257770903155642
URL: http://dx.doi.org/10.1080/15257770903155642

Nucleosides, Nucleotides and Nucleic Acids, 28:761–771, 2009

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770903155642

Taylor & Francis
Taylor & Francis Group

SYNTHESIS AND IN VITRO ACTIVITY EVALUATION OF 2′,3′-*C*-DIMETHYL CARBOCYCLIC NUCLEOSIDE ANALOGUES AS POTENTIAL ANTI-HCV AGENTS

Ok Hyun Ko and Joon Hee Hong

BK21-Project Team, College of Pharmacy, Chosun University, Kwangju, Republic of Korea

□ *The first synthetic route of novel 2′(β),3′(β)-C-dimethyl carbodine analogues is described. The key intermediate cyclopentenyl alcohol* ***11****(β) prepared from Weinreb amide* ***4*** *via ring-closing metathesis (RCM) and vicinal dihydroxylation. Coupling of* ***12*** *with nucleosidic bases via the Pd(0) catalyzed reaction followed by stereoselective dihydoxylation and deprotection afforded the target carbocyclic nucleoside analogues. The synthesized compounds were evaluated as inhibitors of the hepatitis C virus (HCV) in Huh-7 cell line in vitro. However, the nucleosides failed to inhibit HCV RNA replication in the cell-based replicon assay ($EC_{50} > \mu M$).*

Keywords Weinreb amide; carbodine; anti-HCV agents; vicinal dihydroxylation

INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded, enveloped, positive sense RNA virus in the *flaviviridae* family.[1] The infection of HCV accounts for major proportions of hepatitis cases worldwide and is also strongly associated with the development of cirrhosis and hepatocellular carcinoma. Furthermore, there are no vaccines for the treatment of HCV. The current standard therapy for chronic HCV infection is interferon-α in combination with ribavirin; however, this is inadequate because of the low response rates as well as its side effects.[2]

The molecular virology of HCV has led to the identification of a number of antiviral molecular targets, including the NS5B RNA-dependent RNA polymerase. Inhibition of this enzyme results in the inhibition of the replication of HCV, making this enzyme crucial target for the development

Received 1 April 2009; accepted 17 June 2009.
This study was supported by research grant of Chosun University, 2008.
Address correspondence to Joon Hee Hong, College of Pharmacy, Chosun University, Kwangju 501-759, Republic of Korea. E-mail: hongjh@chosun.ac.kr

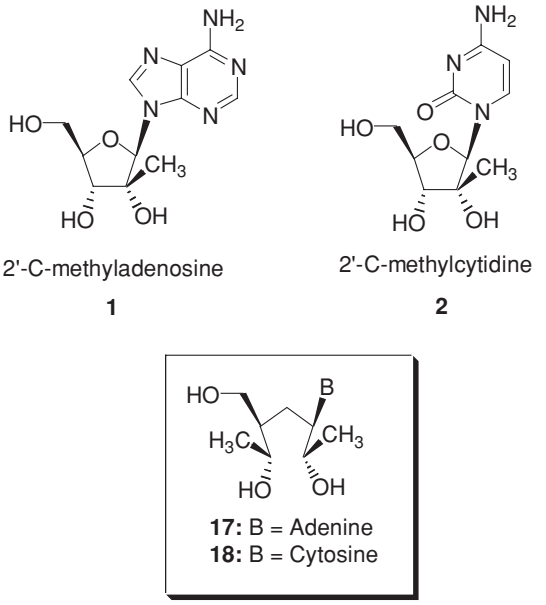
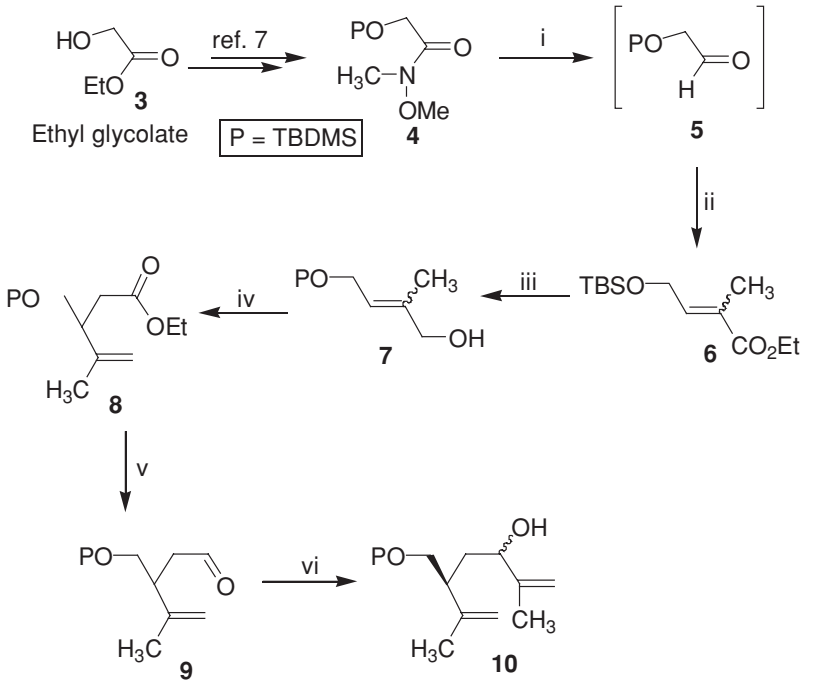


FIGURE 1 Structure of potent anti-HCV agents.

of new anti-HCV agent. Since nucleoside analogues have been used as a drug of choice in treating viral infection including, a number of nucleoside analogues have been synthesized and evaluated for anti-HCV agent.[3] These nucleosides are incorporated into proviral RNA after being converted to their corresponding triphosphates and act as chain terminators. Modification in the vicinity of the 2′-hydroxy of the ribose in natural ribonucleosides can produce effective RNA chain terminator.[4] For example, replacement of the 2′-hydrogen of natural ribonucleosides with a methyl group yields compounds with excellent chain-terminating properties. Among them, 2′-*C*-methyladenosine[5] **1** and 2′-*C*-methylcytidine[6] **2** were discovered as potent anti-HCV agents and are in clinical trials (Figure 1).

In order to explorer the effect of substitution in the sugar moiety of the aforementioned antiviral agents, we decided to synthesize the nucleoside analogues **17** and **18** containing a novel 2′,3′-dimethylcarbasugar. It was of interest to determine how the two methyl groups at 2′,3′-position influence the anti-HCV activity.

As depicted in Scheme 1, we used the Weinreb amide **4** as our starting material which was derived from ethyl glycolate **1**.[7] Conversion of the amide to the aldehyd **5** was achieved using diisobutylaluminum hydride (DIBAL-H). Olefination of **5** with triethyl 2-phosphonopropionate under Horner-Wadsworth-Emmons (HWE) reaction conditions[8] provided α,β-unsaturated ethyl ester **6** as cis/trans isomeric mixtures. It is unnecessary to separate the isomers, because they will be merged into one isomer in the



SCHEME 1 Synthesis of diene intermediate **10**. Reagents: i) DIBAL-H, THF; ii) triethyl 2-phosphonopropionate, NaH, THF, 0°C, 1 hour; iii) DIBAL-H, CH_2Cl_2, 0°C; iv) triethylorthoacetate, propionic acid, 140°C; v) DIBAL-H, toluene, −78°C; vi) isopropenylMgBr, THF.

stage of Claisen rearrangement reaction. Ester **6** was reduced to allylic alcohol **7** by using diisobutylaluminum hydride (DIBAL-H), which underwent [3,3]-sigmatropic rearrangement[9] under Johnson-Claisen rearrangement conditions to give γ,δ–unsaturated ester **8**. Direct reduction of the ester **8** to the aldehyde **9** was possible by slow addition of DIBAL-H in the toluene solvent system at –78°C. The aldehyde **9** was subjected to carbonyl addition by isopropenyl Grignard reagent to yield divinyl **10** as inseparable diastereomeric mixtures. Without separation, divinyl **10** was subjected to standard ring-closing metathesis conditions using 2nd generation Grubbs catalyst[10] to provide cyclopentenol **11**α and **11**β, respectively. As shown in Figure 2, the relative stereochemistry was readily determined on the basis of the NOE results between the proximal hydrogens. On irradiation of C_4-H, relatively weak nuclear overhauser enhancement (NOE) was observed at C_1-H of **11**(α) (0.09%), compared to that of **11**(β) (0.32%).

For the synthesis β-configuration of nucleoside analogues, cyclopentenol **11**β was converted to **12** using ethyl chloroformate, which was coupled with adenine or cytosine anions generated by NaH/DMSO with use of Pd(0) catalyst adduct to provide carbocyclic nucleoside analogues **13** and **14**. In order to synthesize the 2′,3′-dihydroxy carbocyclic nucleoside analogues **17**

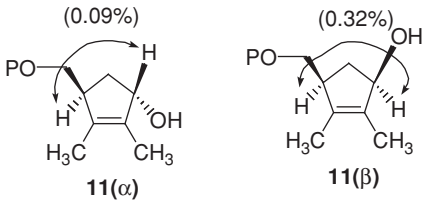
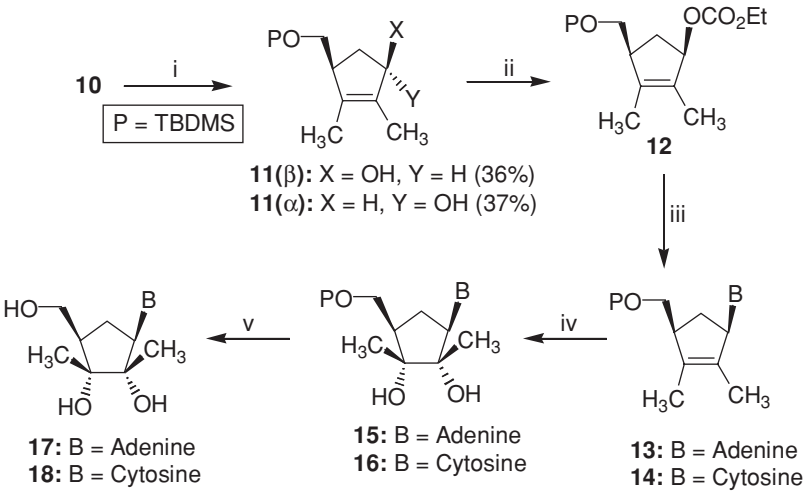


FIGURE 2 NOE data of compound **11**(α) and **11**(β).

and **18**, the double bonds of **13** and **14** were subjected to a catalytic amount of OsO_4 in the presence of stoichiometric amount of NMO to give the diol derivatives **15** or **16** as major products.[11] It is noteworthy that an unexpected higher stereoselectivity was observed in this study than what was reported in previously.[12] These stereochemical outcomes suggest that the bulky groups such as silylated hydroxymethyl group and nucleosidic base of **13** and **14** reinforce the steric hindrance of the β-faces. Removals of silyl protection group of **15** and **16** were preformed by the treatment of tetrabutylammonium fluoride (TBAF) to give target carbodine analogues **17** and **18** (Scheme 2).

The synthesized nucleoside analogues mentioned above were assayed for their ability to inhibit HCV RNA replication in a subgenomic replicon cell line (Huh-7 cell line).[13] However, the nucleosides failed to inhibit HCV RNA replication in the cell-based replicon assay ($EC_{50} > \mu M$).



SCHEME 2 Synthesis of 2′, 3′-*C*-dimethyl carbodine analogues. Reagents: i) 2nd-generation Grubbs catalyst, benzene; ii) ethylchloroformate, DMAP, pyridine; iii) adenine and cytosine, $Pd_2(dba)_3$. $CHCL_3$, P(O-*i*-Pr)$_3$, NaH, THF/DMSO, reflux, overnight; iv) OsO_4, NMO, acetone/water; v) TBAF, THF/CH_3CN, room temperature.

In summary, an efficient synthetic method of 2′,3′-*C*-dimethylated carbodine analogues from Weinreb amide was developed. We can conclude that the dimethyl groups at 2′,3′-position are attributed to the inability of the nucleoside kinase to catalyze the initial phosphorylation of the nucleosides to their monophosphates.

EXPERIMENTAL

Melting points were determined on a Mel-temp II laboratory device and are uncorrected. NMR spectra were recorded on a JEOL 300 Fourier transform spectrometer (JEOL, Tokyo, Japan); chemical shifts are reported in parts per million (δ) and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (doublet of doublets). UV spectra were obtained on a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). The elemental analyses were performed using a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA). TLC was performed on Uniplates (silica gel) purchased from Analtech Co. (7558, Newark, DE, USA). All reactions were carried out under an atmosphere of nitrogen unless specified. Dry dichloromethane, benzene, and pyridine were obtained by distillation from CaH_2. Dry THF was obtained by distillation from Na and benzophenone immediately prior to use.

(t-Butyldimethylsilanyloxy) Acetaldehyde (5)

To a solution of Weinreb amide **4** (3.0 g, 12.85 mmol) in dry THF (60 mL) was slowly added DIBALH (15.42 mL, 1.0 M solution in Hexane) at 0°C. After 2 hours, methanol (15 mL) was added, and the reaction mixture was slowly warmed to room temperature. The mixture was stirred at room temperature for 2 hours, and the resulting solid was filtered through a Celite pad. The filtrate was concentrated under vacuum and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:20) to give crude aldehyde **5** (1.77 g, 79%) as colorless oil. Without further purification, compound **5** was subject to next reaction.

(*E*) and (*Z*)-4-(t-Butyldimethylsilanyloxy)-2-methyl-but-2-enoic Acid Ethyl Ester (6)

To a suspension of sodium hydride (400 mg, 9.98 mmol, 60% in dispersion of oil) in distilled THF (50 mL) was added dropwise triethyl 2-phosphonopropionate (2.32 g, 9.98 mmol) at 0°C and the mixture was stirred at room temperature for 1 hour. The aldehyde **5** (1.74 g, 9.98 mmol) was added to this mixture and the mixture was for 2 hours. The solution was neutralized with AcOH (2.0 mL) and poured into H_2O (100 mL) and extracted with EtOAc (150 × 2). The combined organic layer was washed

with brine and dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **6** (1.8 g, 70%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.20 (dd, J = 4.2, 1.8 Hz, 1H), 4.49 (m, 2H), 4.14 (q, J = 7.0 Hz, 2H), 1.95 (s, 3H), 1.25 (t, J = 7.0 Hz, 3H), 0.83 (m, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 168.4, 137.9, 127.3, 67.3, 60.2. 25.5, 18.4, 17.2, 12.9, -5.5.

(*E*)and(*Z*)-3-(t-Butyldimethylsilyloxymethyl)-2-methyl-but-2-en-1-ol(7)

To a solution of **6** (2.7 g, 10.5 mmol) in CH_2Cl_2 (70 mL), DIBALH (23.1 mL, 1.0 M solution in hexane) was added slowly at −20°C, and stirred for 1 hour at the same temperature. To the mixture, methanol (23 mL) was added. The mixture was stirred at room temperature for 1 hour, and the resulting solid was filtered through a Celite pad. The filtrate was concentrated under vacuum and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give alcohol **7** (2.04 g, 90%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.62 (dd, J = 4.0, 1.6 Hz, 1H), 4.41–4.30 (m, 4H), 1.72 (s, 3H), 0.83 (m, 9H), 0.01 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 139.4, 122.2, 71.3, 65.3, 25.5, 18.4, 13.8, -5.6.

(±)-3-(t-Butyldimethylsilyloxymethyl)-2-methyl-pent-4-enoic Acid Ethyl Ester (8)

A solution of allylic alcohol **7** (3.4 g, 15.8 mmol) in triethyl orthoacetate (60 mL) and 0.05 mL of propionic acid was heated at 140°C overnight with stirring under condition for distillative removal of ethanol. The excess of triethyl orthoacetate was distilled off and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:20) to give **8** (3.62 g, 80%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.63–5.55 (m, 2H), 4.09 (q, J = 7.0 Hz, 2H), 3.53 (d, J = 9.6 Hz, 1H), 3.42 (d, J = 9.6 Hz, 1H), 2.60 (dd, J = 14.0, 5.2 Hz, 1H), 2.36 (dd, J = 14.0, 8.6 Hz, 1H), 2.27 (m, 1H), 1.79 (s, 3H), 1.23 (t, J = 7.0 Hz, 3H), 0.83 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 172.4, 139.4, 118.2, 66.4, 61.8, 45.9, 40.3, 25.7, 18.6, 17.4, 13.6, -5.6.

(±)-3-(t-Butyldimethylsilyloxymethyl)-2-methyl-pent-4-enal (9)

To a solution of **8** (3.0 g, 10.5 mmol) in toluene (50 mL), DIBALH (7.7 mL, 1.5 M solution in toluene) was added slowly at −78°C, and stirred for 10 minutes at the same temperature. To the mixture, methanol (8 mL) was added. The mixture was stirred at room temperature for 1 hour, and the resulting solid was filtered through a Celite pad. The filtrate was concentrated under vacuum and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **9** (2.54 g, 63%) as a

colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.71 (s, 1H), 5.60–5.53 (m, 2H), 3.62 (dd, J = 9.8, 5.0 Hz, 1H), 3.45 (d, J = 9.6, 5.6 Hz, 1H), 2.70 (m, 1H), 2.61 (dd, J = 13.6, 5.4 Hz, 1H), 2.39 (dd, J = 13.6, 5.6 Hz, 1H), 1.76 (s, 3H), 0.82 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 202.1, 140.7, 119.7, 69.5, 61.8, 46.1, 41.6, 25.7, 18.7, 17.9, -5.5.

(*rel*)-(3*R* and *3S*,5*S*)-5-(t-Butyldimethylsilanyloxymethyl)-2,6-dimethyl-hepta-1,6-dien-3-ol(10)

To a solution of **9** (1.33 g, 5.5 mmol) in dry THF (25 mL) was slowly added isopropenylmagnesium bromide (13.2 mL, 0.5 M solution in THF) at −40°C. After 5 hours, saturated NH_4Cl solution (7 mL) was added, and the reaction mixture was slowly warmed to room temperature. The mixture was extracted with EtOAc/water twice. The combined organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **10** (1.24 g, 79%) as a diastereomeric mixture: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.13 (s, 1H), 5.04 (s, 1H), 4.93 (s, 1H), 4.81 (s, 1H), 3.99–3.88 (m, 1H), 3.45–3.30 (m, 2H), 2.21–2.09 (m, 1H), 1.69 (s, 3H), 1.52 (s, 3H), 1.50–1.41 (m, 2H), 0.81 (s, 9H), 0.01 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 147.5, 142.3, 139.0, 117.7, 116.6, 110.4, 77.3, 72.5, 66.1, 51.76, 38.5, 25.7, 20.32, 18.6, 17.3, -5.6.

(*rel*)-(1*R*,4*S*)-4-(t-Butyldimethylsilyloxymethyl)-2,3-dimethyl-cyclopent-2-enol(11β);and(*rel*)-(1*S*,4*S*)-4-(t-Butyldimethylsilyloxymethyl)-2,3-dimethyl-cyclopent-2-enol(11α)

To a solution of **10** (3.51 g, 12.36 mmol) in dry benzene (15 mL) was added second generation Grubbs catalyst (183 mg 0.216 mmol). The reaction mixture was refluxed overnight, and cooled to room temperature. The mixture was concentrated in vacuum, and residue was purified by silica gel column chromatography (EtOAc/hexane, 1:12) to give cyclopentenol **11**β (1.14 g, 36%) and **11**α (1.17 g, 37%) as colorless oils, respectively. Cyclopentenol **11**β: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.31 (dd, J = 10.2, 7.8 Hz, 1H), 3.90 (d, J = 8.8 Hz, 1H), 3.79 (d, J = 9.0 Hz, 1H), 2.90 (m, 1H), 2.61 (d, J = 10.6 Hz, 1H), 2.20 (dd, J = 13.8, 7.4 Hz, 1H), 1.65 (s, 3H), 1.22 (s, 3H), 0.81 (s, 9H), 0.01 (s, 6H): ^{13}C NMR ($CDCl_3$) δ 138.8, 135.6, 78.2, 67.3, 49.2, 38.8, 25.5, 18.5, 11.6, 10.8, -5.5; Anal. Calcd. for $C_{14}H_{28}O_2Si$: C, 65.57; H, 11.00. Found: C, 65.65; H, 10.91.

Cyclopentenol **11**α: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.52 (s, 1H), 3.94 (d, J = 9.2 Hz, 1H), 3.75 (d, J = 9.1 Hz, 1H), 2.87 (m, 1H), 2.60 (dd, J = 13.8, 7.8 Hz, 1H), 1.74 (s, 3H), 1.65 (dd, J = 13.8, 6.0 Hz, 1H), 1.33 (s, 3H), 0.82 (s, 9H), 0.01 (s, 6H): ^{13}C NMR ($CDCl_3$) δ 137.9, 135.0, 77.8, 67.8, 48.9, 39.6, 25.6, 18.6, 11.1, 10.4, -5.6; Anal. Calcd. for $C_{14}H_{28}O_2Si$: C, 65.57; H, 11.00. Found: C, 65.48; H, 11.07.

(*rel*)-(1*R*,4*S*)-1-Ethoxy carbonyloxy-4-(t-butyldimethylsilyloxymethyl)-2,3-dimethyl-cyclopent-2-ene(12)

To a solution of **11**β (3.19 g, 12.45 mmol) in anhydrous pyridine (25 mL) was added ethyl chloroformate (2.68 g, 24.7 mmol) and DMAP (122 mg, 1.0 mmol). The reaction mixture was stirred overnight at 50°C. The reaction mixture was quenched with saturated $NaHCO_3$ solution (6 mL), stirred for 10 minutes and concentrated in reduced pressure. The residue was extracted with EtOAc/H_2O two times, and combined organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:20) to give **12** (3.06 g, 75%) as colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.03 (dd, J = 6.2, 4.2 Hz, 1H), 4.15 (q, J = 7.4 Hz, 2H), 3.45 (m, 2H), 2.34 (m, 1H), 2.20 (dd, J = 13.8, 8.2 Hz, 1H), 1.91 (s, 3H), 1.70 (dd, J = 13.8, 3.6 Hz, 1H), 1.52 (s, 3H), 1.27 (t. J = 7.4 Hz, 3H), 0.82 (s, 9H), 0.01 (s, 6H): ^{13}C NMR ($CDCl_3$) δ 155.5, 138.0, 134.5, 85.8, 66.9, 64.8, 51.3, 40.3, 25.4, 18.3, 12.3, 10.8, -5.5; Anal. Calcd. for $C_{17}H_{32}O_4Si$: C, 62.15; H, 9.82. Found: C, 62.23; H, 9.92.

(*rel*)-(1′*R*,4′*S*)-9-[4-(t-Butyldimethylsilyloxymethyl)-2,3-dimethyl-cyclopent-2-en-1-yl]adenine(13)

In order to generate nucleosidic base anion, adenine (194 mg, 1.13 mmol) was added to a hexane washed NaH (30.2 mg, 1.26 mmol) in anhydrous DMSO (7.0 mL). The reaction mixture was stirred for 40 minutes at 50–55°C and cooled to room temperature. Simultaneously, P(O-*i*-Pr)$_3$ (93 mg, 0.45 mmol) was added to a solution of $Pd_2(dba)_3 \cdot CHCl_3$ (58 mg, 5.65 μmol) in anhydrous THF (7.0 mL), which was stirred for 30 minutes. To the adenine solution of DMSO was sequentially added catalyst solution of THF and **12** (345 mg, 1.05 mmol) dissolved in anhydrous THF (8.0 mL). The reaction mixture was stirred overnight at refluxing temperature and quenched with water (4.0 mL). The reaction solvent was removed in vacuum. The residue was purified by silica gel column chromatography (MeOH/Hexane/EtOAc, 0.1:5:1) to give **13** (137.3 mg, 35%) as a white solid; m.p. 180–182°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (s, 1H), 8.11 (s, 1H), 4.86 (dd, J = 8.0, 4.6 Hz, 1H), 3.69 (d, J = 8.8 Hz, 1H), 3.49 (d, J = 8.8 Hz, 1H), 2.49 (m, 1H), 2.36 (dd, J = 13.6, 6.8 Hz, 1H), 2.09 (dd, J = 13.6, 8.6 Hz, 1H), 1.71 (s, 3H), 1.62 (s, 3H), 0.81 (s, 9H), 0.01 (s, 6H): ^{13}C NMR ($CDCl_3$) δ 155.6, 152.3, 148.6, 141.2, 138.1, 132.2, 118.2, 67.7, 66.1, 50.5, 46.8, 25.3, 23.6, 18.4, 13.4, 11.3, -5.6; Anal. Calcd. for $C_{19}H_{31}N_5OSi$: C, 61.09; H, 8.36; N, 18.75. Found: C, 60.89; H, 8.41; N, 18.62.

(*rel*)-(1′*R*,4′*S*)-1-[4-(t-Butyldimethylsilyloxymethyl)-2,3-dimethyl-cyclopent-2-en-1-yl]cytosine(14)

Cytosine nucleoside **14** analogue was synthesized from **12** by the similar procedure as described for **13**: yield 36%; m.p. 181–183°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37 (d, J = 7.2 Hz, 1H), 5.80 (d, J = 4.2 Hz, 1H), 5.42 (d, J = 7.2 Hz, 1H), 3.77 (d, J = 8.4, Hz, 1H), 3.51 (d, J = 8.4 Hz, 1H), 2.95 (m, 1H), 2.71 (dd, J = 13.6, 7.6 Hz, 1H), 2.15 (dd, J = 13.6, 2.4 Hz, 1H), 1.75 (s, 3H), 1.32 (s, 3H), 0.83 (s, 9H), 0.02 (s, 6H): ^{13}C NMR ($CDCl_3$) δ 165.5, 155.6, 143.1, 140.4, 133.1, 94.1, 67.5, 58.3, 41.6, 25.5, 18.5, 11.2, 10.1, -5.6; Anal. Calcd. for $C_{18}H_{31}N_3O_2Si$: C, 61.85; H, 8.94; N, 12.02. Found: C, 61.92; H, 8.89; N, 11.98.

(*rel*)-(1′*R*,2′*S*,3′*R*,4′*R*)-9-[4-(t-Butyldimethylsilyloxymethyl)-2,3-dimethyl-2,3-dihydroxy-cyclopentan-1-yl]adenine(15)

To a stirred solution of **13** (376 mg, 1.008 mmol) in cosolvent (4.0 mL, acetone:water/5:1) was added NMO (236 mg, 2.016 mmol), and OsO_4 (0.06 mL, 4% in water). The mixture was stirred overnight at 50°C, and quenched with saturated Na_2SO_3 solution (4 mL). Resulting solid was removed by filtration through a pad of Celite, and filtrate was concentrated in reduced pressure. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:7) to give **15** (291 mg, 71%) as a white solid: m.p. 194–196°C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.31 (s, 1H), 8.19 (s, 1), 7.19 (br s, 2H, D_2O exchangeable), 5.17 (s, 1H, D_2O exchangeable), 5.09 (s, 1H, D_2O exchangeable), 5.08 (dd, J = 5.2, 2.4 Hz, 1H), 3.46 (dd, J = 12.6, 7.2 Hz, 1H), 3.30 (dd, J = 12.6, 8.4 Hz, 1H), 2.44 (dd, J = 10.6, 4.2 Hz, 1H), 2.31 (dd, J = 10.6, 8.6 Hz, 1H), 1.60 (m, 1H), 1.41 (s, 3H), 1.23 (s, 3H), 0.82 (s, 9H), 0.01 (s, 6H): ^{13}C NMR (DMSO-d_6) δ 155.7, 152.0, 149.7, 140.5, 119.4, 83.3, 81.3, 69.7, 56.7, 25.6, 18.4, 17.1, 16.2, 14.3, -5.7; Anal calc for $C_{19}H_{33}N_5O_3Si$: C, 55.99; H, 8.16; N, 17.18. Found: C, 56.13; H, 8.03; N, 17.23.

(*rel*)-(1′*R*,2′*S*,3′*R*,4′*R*)-1-[4-(t-Butyldimethylsilyloxymethyl) 2,3-dimethyl-2,3-dihydroxy-cyclopentan-1-yl] cytosine (16)

Cytosine nucleoside **16** analogue was synthesized from **14** by the similar procedure as described for **15**: yield 69%; m.p. 189–192°C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.65 (d, J = 7.2 Hz, 1H), 7.05 (br d, 2H, D_2O exchangeable), 5.59 (d, J = 7.2 Hz, 1H), 5.12 (s, J = 4.6 Hz, 1H, D_2O exchangeable), 5.02 (s, 1H, D_2O exchangeable), 4.97 (m, 1H), 3.71 (dd, J = 13.2, 6.6 Hz, 1H), 3.42 (dd, J = 13.2, 8.8 Hz, 1H), 2.19 (dd, J = 13.2, 4.8 Hz, 1H), 1.97 (dd, J = 13.2, 8.0 Hz, 1H), 1.63 (m, 1H), 1.47 (s, 3H), 0.82 (s, 9H), 0.01 (s, 6H): ^{13}C NMR (DMSO-d_6) δ 165.5, 156.6, 144.3, 94.2, 83.2, 81.3, 62.7,

57.9, 41.3, 25.5, 18.7, 17.3, 13.5, 12.5, -5.6; Anal. Calcd. for $C_{18}H_{33}N_3O_4Si$: C, 56.37; H, 8.67; N, 10.96. Found: C, 56.43; H, 8.71; N, 11.04.

(*rel*)-(1′*R*,2′*S*,3′*R*,4′*R*)-9-[4-(Hydroxymethyl)-2,3-dimethyl-2,3-dihydroxy-cyclopentan-1-yl]adenine(17)

To a solution of **15** (171 mg, 0.42 mmol) in cosolvent (5.0 mL, THF:CH_3CN/1:1) THF (5 mL) was TBAF (0.63 mL, 1.0 M solution in THF) at 0°C. The mixture was stirred overnight at room temperature, and concentrated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:4) to give **17** (76 mg, 70%) as a white solid: m.p. 208–210°C; UV (H_2O) λ_{max} 259.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.32 (s, 1H), 8.12 (s, 1H), 7.20 (br s, 2H, D_2O exchangeable), 5.09 (s, 1H, D_2O exchangeable), 5.01 (s, 1H, D_2O exchangeable), 4.92 (m, 1H), 4.81 (t, J = 4.6 Hz, 1H, D_2O exchangeable), 3.51–3.39 (m, 2H), 2.20 (dd, J = 13.2, 4.4 Hz, 1H), 2.04 (dd, J = 13.2, 8.6 Hz, 1H), 1.69 (m, 1H), 1.40 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 155.3, 152.2, 147.6, 137.3, 118.6, 82.1, 80.4, 68.2, 56.2, 40.2, 16.0, 13.9, 12.1; Anal calc for $C_{13}H_{19}N_5O_3$+1.0 H_2O: C, 50.15; H, 6.79; N, 22.49. Found: C, 50.03; H, 6.81; N, 22.39.

(*rel*)-(1′*R*,2′*S*,3′*R*,4′*R*)-1-[4-(t-Hydroxymethyl)-2,3-dimethyl-cyclopent-2-en-1-yl]cytosine(18)

Cytosine nucleoside **18** analogue was synthesized from **16** by the similar condition as described for **17** as a white solid as a white solid: yield 73%; m.p. 197–199°C; UV (H_2O) λ_{max} 271.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.68 (d, J = 7.3 Hz, 1H), 7.11 (br d, 2H, D_2O exchangeable), 5.60 (d, J = 7.2 Hz, 1H), 5.09 (s, 1H, D_2O exchangeable), 5.00 (s, 1H, D_2O exchangeable), 4.89 (m, 1H), 4.80 (t, J = 4.2 Hz, 1H), 3.54 (dd, J = 13.2, 4.8 Hz, 1H), 3.30 (dd, J = 13.2, 8.0 Hz, 1H), 2.19–2.08 (m, 2H), 1.70 (m, 1H), 1.37 (s, 3H), 1.30 (s, 3H): ^{13}C NMR (DMSO-d_6) δ 165.8, 155.5, 145.1, 95.5, 81.1, 80.3, 68.2, 56.2, 41.3, 15.6, 14.3, 13.2,; Anal. Calcd. for $C_{12}H_{19}N_3O_4$+1.0 H_2O: C, 50.16; H, 7.36; N, 14.62. Found: C, 50.25; H, 7.27; N, 14.54.

REFERENCES

1. Tan, S.L.; Pause, A.; Shi, Y.; Sonenberg, N. Hepatitis C therapeutics: current status and emerging strategies. *Nat. Rev. Drug Discovery* **2002**, 1, 867–881.
2. Heathcote, E.; Shiffman, M.; Cooksley, W.; Dusheiko, G.M.; Lee, S.S.; Balart, L.; Reindollar, R.; Reddy, R.K.; Wright, T.L.; Lin, A.; Hoffman, J.; De Pamphilis, J. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. *N. Engl. J. Med.* **2000**, 343, 1673–1680.
3. a) Bera, S.; Malik, L.; Bhat, B.; Carroll, S.S.; MacCoss, M.; Olsen, D.B.; Tomassini, J.E.; Eldrup, A.B. Synthesis and evaluation of optically pure dioxolanes as inhibitors of hepatitis C virus RNA replication. *Bioorg. Med. Chem. Lett.* **2003**, 13, 4455–4458; b) Eldrup, A.B.; Prhavc, M.; Brooks, J.; Bhat, B.; Prakash, T.P.; Song, Q.; Bera, S.; Bhat, N.; Dande, P.; Cook, P.D.; Bennett, C.F.; Carroll, S.S.; Ball, R.G.; Bosserman, M.; Burlein, C.; Colwell, L.F.; Fay, J.F.; Flores, O.A.; Getty, K.; LaFemina,

R.L.; Leone, J.; MacCoss, M.; McMasters, D.R.; Tomassini, J.E.; Von Langen, D.; Wolanski, B.; Olsen, D.B. 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; c) Clark, J.L.; Hollecker, L.; Mason, J.C.; Stuyver, L.J.; Tharnish, P.M.; Lostia, S.; McBrayer, T.R.; Schinazi, R.F.; Watanabe, K.A.; Otto, M.J.; Furman, P.A.; Stec, W.J.; Patterson, S.E.; Pankiewicz, K.W. Design, synthesis, and antiviral activity of 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine, a potent inhibitor of hepatitis C virus replication. *J. Med. Chem.* **2005**, 48, 5504–5508. d) Smith, D.B.; Kalayanov, G.; Sund, C.; Winqvist, A.; Pinho, P.; Maltseva, T.; Morisson, V.; Leveque, V.; Rajyaguru, S.; Le Pogam, S.; Najera, I.; Benkestock, K.; Zhou, X.X.; Maag, H.; Cammack, N.; Martin, J.A.; Swallow, S.; Johansson, N.G.; Klumpp, K.; Smith, M. The design, synthesis, and antiviral activity of 4′-azidocytidine analogues against hepatitis C virus replication: the discovery of 4′-azidoarabinocytidine. *J. Med. Chem.* **2009**, 52, 219–223.

4. El Kouni, M.H. Trends in the design of nucleoside analogues as anti-HIV drugs. *Curr. Pharm. Des.* **2002**, 8, 581–593.
5. Carrol, S.S.; Tomassini, J.E.; Bosserman, M.; Getty, K.; Stahlhut, M.W.; Eldrup, A.B.; Bhat, B.; Hall, D.; Simcoe, A.L.; LaFemina, R.; Rutkowski, C.A.; Wolanski, B.; Yang, Z.; Migliaccio, G.; De Francesco, R.; Kuo, L.C.; MacCoss, M.; Olsen, D.B. Inhibition of hepatitis C virus RNA replication by 2′-modified nucleoside analogs. *J. Biol. Chem.* **2003**, 278, 11979–11984.
6. Eldrup, A.B.; Allerson, C.R.; Bennett, C.F.; Bera, S.; Bhat, B.; Bhat, N.; Bosserman, M.R.; Brooks, J.; Burlein, C.; Carrol, S.S.; Cook, P.D.; Getty, K.L.; MacCoss, M.; McMasters, D.R.; Olsen, D.B.; Prakash, T.P.; Prhavc, M.; Song, Q.L.; Tomassini, J.E.; Xia, J. Structure-activity relationship of purine ribonucleosides for inhibition of hepatitis C virus RNA-dependent RNA polymerase. *J. Med. Chem.* **2004**, 47, 2283–2295.
7. Liu, L.J.; Yoo, J.C.; Hong, J.H. Efficient synthesis of 4′-cyclopropylated carbovir analogues with use of ring-closing metathesis from glycolate. *Nucleosides Nucleotides Nucleic Acids* **2008**, 27, 1186–1196.
8. Hyatt, J.A. Convenient monoketalization of 1,4-cyclohexanedione. Synthesis of new quinone methides. *J. Org. Chem.* **1983**, 48, 129–131.
9. a) Johnson, W.S.; Werthmann, L.; Bartlett, W.R.; Brocksom, T.J.; Li, T.T.; Faulkner, D.J.; Petersen, M.R. Simple stereoselective version of the Claisen rearrangement leading to trans-trisubstituted olefinic bonds. Synthesis of squalene. *J. Am. Chem. Soc.* **1970**, 92, 741–743; b) Castro, A.M.M. Claisen rearrangement over the past nine decades. *Chem. Rev.* **2004**, 104, 2939–3002; c) Ziegler, F.E. The thermal, aliphatic Claisen rearrangement. *Chem. Rev.* **1988**, 88, 1423–1452; d) Lutz, R. Catalysis of the Cope and Claisen rearrangements. *Chem. Rev.* **1984**, 84, 205–297.
10. a) Furstner, A. Olefin metathesis and beyond. *Ange. Chem. Int. Ed. Engl.* **2000**, 39, 3012–3043; b) Nicolaou, K.C.; Bulger, P.G.; Sarlah, D. Metathesis reactions in total synthesis. *Ange. Chem. Int. Ed. Engl.* **2005**, 44, 4490–4527; c) Hansen, E.C.; Lee, D. Search for solutions to the reactivity and selectivity problems in enyne metathesis. *Acc. Chem. Res.* **2006**, 39, 509–519.
11. Hong, J.H.; Shim, M.J.; Ro, B.O.; Ko, O.H. An efficient synthesis of novel carbocyclic nucleosides with use of ring-closing metathesis from D-lactose. *J. Org. Chem.* **2002**, 67, 6837–6840.
12. Burlina, F.; Favre, A.; Fourrey, J.-L.; Thomas, M. An expeditious route to carbocyclic nucleosides: (−)-aristeromycin and (−)-carbodine. *Bioorg. Med. Chem. Lett.* **1997**, 7, 247–250.
13. Stuyver, L.J.; McBrayer, T.R.; Tharnish, P.M.; Hassan, A.E.A.; Chu, C.K.; Pankiewicz, K.W.; Watanabe, K.A.; Schinazi, R.F.; Otto, M.J. Dynamics of subgenomic hepatitis C virus replicon RNA levels in Huh-7 cells after exposure to nucleoside antimetabolite. *J. Virol.* **2003**, 77, 10689–10694.