Synthesis of 3'(β)-C-methyl Carbodine Analogues as Potential Anti-HCV Agents

Hua Li, Young Chan Baik, and Joon Hee Hong

BK21-Project Team, College of Pharmacy, Chosun University, Kwangju 501-759, Korea. E-mail: hongjh@chosun.ac.kr
Hawon Pharmaceuticals Co., Pyeongtaeksi, Gyeoggi 451-860, Korea

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The synthetic route of novel 3'-C-methyl carbodine analogue is described. The construction of tertiary alcohol at 3'-position of carbodine analogues was successfully made via sequential [3,3]-sigmatropic rearrangement, ring-closing metathesis (RCM) and stereoselective dihydroxylation reactions starting from ethyl glycolate.

Key Words: Carbodine, Anti-HCV agents, Ring-closing metathesis, Vicinal dihydroxylation

Introduction

Hepatitis C virus (HCV) infection is a leading cause of chronic hepatitis, liver cirrhosis and hepatoma carcinoma.1 However, there is no effective chemotherapy for the treatment of HCV-infected people except immunotherapy using ribavirin in combination with interferon-α, which leads to a sustained virological response in only about half of the patients treated.2 Recent advances in the molecular virology of HCV have 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 of new anti-HCV agent.3 Since nucleoside analogues have been used as a drug of choice in curing viral infection, a number of nucleoside analogues have been synthesized and evaluated for anti-HCV agent.4 These nucleosides are incorporated into proviral RNA like a substrate 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.5 For example, 2'-methylribonucleosides yield compounds with excellent chain-terminating properties. Among them, 2'-C-methyladenosine6 1 and 2'-C-methylcytidine7 2 were discovered as potent anti-HCV agents and are in clinical trials.

Natural as well as synthetic carbocyclic nucleosides8 are well known for their interesting biological activities, including antitumor and antiviral activities against a wide variety of RNA and DNA viruses. Carbocyclic nucleosides are chemically more stable and are subject to the action of the enzymes that cleave the glycosyl linkage in conventional nucleosides.

On the basis of these findings that the methyl group of 2'-position could impose favorable steric as well as electronic effect on the interaction with HCV polymerase, we have determined to synthesize novel classes of nucleosides comprising 3'(β)-C-methylated carbodine analogues, which transpose the methyl group from 2'- to 3'-position.

To this end, we describe a very convenient and general synthetic procedure for carbocyclic nucleosides using repetitive three-step sequences ([3,3]-sigmatropic rearrangement8, RCM9,10 and vicinal dihydroxylation11). As shown in Scheme 1, we used the Weinreb amide 4 as starting material, which could be readily synthesized via silyl protection of the alcohol could be readily synthesized via silyl protection of the alcohol of commercially available starting material 3 followed by hydrolysis and amidation using DCC and DMAP coupling reagent as described in previous report.12 Conversion of the amide 4 to aldehyde derivative 5 turned out to be successful under the usual carbonyl reduction condition (DIBALH, THF, 0 °C). Subjection of 5 to Horner-Wadsworth-Emmons (HWE) reaction condition13 provided α,β-unsaturated ester 6 as cis/trans isomeric mixtures. It is unnecessary to separate the isomers, because they will be merged into one isomer in next reaction. Ester 6 was reduced to allylic alcohol 7 by using disobutylaluminum hydride, which underwent regular [3,3]-sigmatropic rearrangement using triethyl orthoacetate to give γ,δ-unsaturated ester 8. Direct conversion of the ester 8 to the aldehyde 9 was possible by slow addition of DIBALH in the toluene solvent system at -78 °C. The aldehyde 9 was subjected to carbonyl addition by CH₂=CHMgBr to yield divinyl 10 as inseparable diastereomeric mixtures.

Scheme 1. Synthesis of diene intermediate 10. Reagents: i) DIBALH, THF; ii) triethyl 2-phosphonopropionate, NaH, THF, 0°C, 1 h, iii) DIBAL-H, CH₂Cl₂, 0 °C, iv) triethylthioacetate, propionic acid, 140 °C; v) DIBAL-H, toluene, -78 °C; vi) vinylMgBr, THF.
OH

analytic amount of OsO₄ and NMO to give the dihydroxylated
to provide nucleoside analogues
chloroformate, which was coupled with cytosine or adenine
the protected nucleosides
previously. These stereochemical outcomes suggest that the
activity was observed in this study than what was reported in
ment. It is noteworthy that an unexpected higher stereoselec-
tivity was readily determined by NOE experi-
relatively weak NOE was observed at C 1-H of
unambiguously assigned on the basis of the NOE correlations
between the proximal hydrogens. On irradiation of C4-H,
unambiguously assigned on the basis of the NOE correlations
respectively. As shown in Figure 2, the stereochemistry was
fully determined by NOE experi-

Figure 1. Structure of potent anti-HCV agents.

Figure 2. NOE results of compound 11(β) and 11(α).

Figure 3. NOE result of compound 16.

Without separation, divinyl 10 was subjected to standard RCMin a standard
RCMcondition using 2nd generation Grubbs catalyst [(1m)-
Cl₂PCy₃RuCHPh] to provide cyclopentenol 11a and 11β, respectively. As shown in Figure 2, the stereochemistry was
unambiguously assigned on the basis of the NOE correlations
between the proximal hydrogens. On irradiation of C1-H, relatively weak NOE was observed at C1-H of 11(α) (0.1%
NOE), but not at C1-H of 11(β) (0.4% NOE).

Cyclopentenol 11β was transformed to 12 using ethyl chloroformate, which was coupled with cytosine or adenine
anions generated by NaH/DMSO with use of catalyst [tris-
(dibenzylidene-acetone)-dipalladium(0)-chloroform] adduct
to provide nucleoside analogues 13 and 14. In order to syn-
thesize the 2',3'-dihydroxy nucleoside analogues 17 and 18,
the protected nucleosides 13 and 14 were subjected to a cata-
ylytic amount of OsO₄ and NMO to give the dihydroxylated
15 and 16 as the only reaction products. As shown in Figure 3, the stereochemistry was readily determined by NOE experi-
ment. It is noteworthy that an unexpected higher stereoselec-
tivity was observed in this study than what was reported in
previously. These stereochemical outcomes suggest that the
bulky groups such as silylated hydroxymethyl group and nucelosidic base of 13 and 14 reinforce the steric hindrance of
the β-faces.

Scheme 2. Synthesis of 3'-methyl carbocyclic nucleosides. Rea-
gents: i) 2nd Grubbs catalyst, CH₂Cl₂; ii) ethylchloroformate, DMAP,
pyridine, iii) nucleobases, Pd₂(dba)₃, P(O-i-Pr), NaH, THF/ DMSO,
reflux, overnight, iv) OsO₄, NMO; v) TBAF, THF, rt.

Removals of silyl protection group of 15 and 16 were pre-
formed by the treatment of tetrabutylammonium fluoride
(TBAF) to give target nucleosides 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 Huh7 cell line (LucNeo2). These
cells contain an HCV subgenomic replicon RNA encoding a
luciferase reporter gene as a marker. The antiviral potency of
the analogues against the HCV replicon is expressed as EC₅₀,
which was quantified by a luciferase assay after a two-day
incubation period with the corresponding compound. In addi-
tion, the associated cytoxicity was evaluated in a tetrazolium
(XTT)-based assay according to the manufacturer’s protocol. However, the synthesized nucleosides neither showed any
significant antiviral activity nor toxicity up to 100 µM.
In summary, an efficient synthetic method of 3'(β)-C-
methyloxycarbocyclic nucleosides from ethyl glycolate was
developed. We can conclude that the methyl group at 3'-
position is responsible for the inability of the nucleoside
kinase to catalyze the initial phosphorylation of the nucleosides
to their monophosphates.

Experimental Section

Melting points were determined on a Mel-temp II labor-
atory device and are uncorrected. NMR spectra were recorded
on a JEOL 300 Fourier transform; chemical shifts are reported
in parts per million (δ) and signals are quoted as s (singlet), d
doublet), t (triplet), q (quartet), m (multiplet) and dd (doublet
do
doublets). UV spectra were obtained on a Beckman DU-7
spectrophotometer. The elemental analyses were performed
using an Elemental Analyzer System (EA1112). TLC was
performed on Uniplates (silica gel) purchased from Analtech
Co. All reactions were carried out under an atmosphere of
nitrogen unless specified. Dry dichloromethane, benzene and
pyridine were obtained by distillation from CaH₂. Dry THF
was obtained by distillation from Na and benzophenone
immediately prior to use.

(tert-Butyldimethylsilyl)acetaldehyde (5). To a solu-
tion 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 h, methanol (15 mL) was added, and the reaction mixture was slowly warmed to rt. The mixture was stirred at room temperature for 2 h, 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-(tert-Butyldimethylsilyl oxy)-2-methyl-2-enolic 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 drop wise triethyl 2-phosphono propionate (2.38 g, 9.98 mmol) at 0 °C and the mixture was stirred for 2 h. THF (50 mL) was added and the mixture was stirred at room temperature for 1 h. The aldehyde 5 (1.74 g, 9.98 mmol) was added to this mixture and the mixture was stirred for 2 h. The solution was neutralized with AcOH (2.0 mL) and poured into H2O (100 mL) and extracted with EtOAc (150 × 2). The combined organic layer was washed with brine and dried over anhydrous MgSO4, 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: 1H NMR (CDCl3, 300 MHz) δ 6.20 (dd, J = 4.2, 1.8 Hz, 1H), 4.49 (m, 2H), 4.14 (q, δ J = 7.0 Hz, 2H), 1.95 (s, 3H), 1.25 (t, δ J = 7.0 Hz, 3H), 0.83 (m, 9H), 0.01 (s, 6H); 13C NMR (CDCl3) δ 137.9, 127.3, 67.3, 60.2, 25.5, 18.4, 17.2, 12.9, -5.5. (E) and (Z)-3-(tert-Butyldimethylsiloxy)-2-methyl-but-2-en-1-ol (7). A solution of allylic alcohol (±)-3-(tert-Butyldimethylsiloxy)-2-methyl-but-2-en-1-ol (7) in dry CH2Cl2 (70 mL) Dibal-H (23.1 mL, 1.0 M solution in hexane) was added slowly at -20 °C, and stirred for 1 h at the same temperature. To the mixture, methanol (23 mL) was added. The mixture was stirred at room temperature for 1 h, 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 (2.7 g, 10.5 mmol) in triethyl orthoacetate (50 mL) was distilled off and the residue was purified by distillative removal of ethanol. The excess ethyl orthoacetate was distilled off and the residue was concentrated in reduced pressure. 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: 1H NMR (CDCl3, 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); 13C NMR (CDCl3) δ 202.1, 201.0, 140.7, 119.7, 69.5, 61.8, 46.1, 41.6, 25.7, 18.7, 17.9, -5.5. (E)-(1R,4S)-4-(tert-Butyldimethylsilyloxy)-3-methyl-cyclopent-2-en-1-ol (11R); and (E)-(1S,4S)-4-(tert-Butyldimethylsilyloxy)-3-methyl-cyclopent-2-en-1-ol (11S). A solution of 10 (2.78 g, 10.3 mmol) in dry CH2Cl2 (20 mL) was added 2nd generation Grubbs catalyst (152 mg, 0.18 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, 1:10) to give cyclopentenol 11 (1.58 g, 76%) as a diastereomeric mixture: 1H NMR (CDCl3, 300 MHz) δ 5.71-5.63 (m, 2H), 5.30-5.18 (m, 4H), 4.11 (m, 1H), 3.56-3.40 (m, 2H), 2.37 (m, 1H), 2.22-1.78 (m, 1H), 1.66 (s, 3H), 1.58-1.49 (m, 1H), 0.82 (s, 9H), 0.01 (s, 6H); 13C NMR (CDCl3) δ 147.1, 139.2, 139.1, 115.7, 115.6, 111.4, 73.8, 73.7, 66.5, 43.0, 27.3, 27.2, 25.8, 18.3, 17.3, -5.5. (E)-(1R,4S)-4-(tert-Butyldimethylsilyloxy)-3-methyl-cyclopent-2-en-1-ol (11R); and (E)-(1S,4S)-4-(tert-Butyldimethylsilyloxy)-3-methyl-cyclopent-2-en-1-ol (11S). A solution of 10 (2.78 g, 10.3 mmol) in dry CH2Cl2 (20 mL) was added 2nd generation Grubbs catalyst (152 mg, 0.18 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, 1:10) to give cyclopentenol 11 (1.07 g, 43%) and 11a (1.09 g, 44%) as colorless oils, respectively. Cyclopentenol 11R: 1H NMR (CDCl3, 300 MHz) δ 5.67 (dd, J = 5.4, 2.4 Hz, 1H), 4.52 (d, J = 4.8 Hz, 1H), 3.45 (dd, J = 13.8, 8.4 Hz, 2H), 2.88 (m, 1H), 1.98 (dd, J = 13.4, 6.8 Hz, 1H), 1.77 (dd, J = 13.4, 8.2 Hz, 1H), 1.40 (s, 3H), 0.81 (s, 9H), 0.01 (s, 6H); 13C NMR (CDCl3) δ 145.7, 131.4, 71.7, 67.1, 47.4, 38.7, 26.5, 18.4, 14.5, -5.7. Cyclopentenol 11a: 1H NMR (CDCl3, 300 MHz) δ 5.60 (d, J = 5.2 Hz, 1H), 4.48 (m, 1H), 3.47 (d, J = 13.6 Hz, 1H), 3.33 (d, J = 13.6 Hz, 1H), 2.82 (m, 1H), 1.92 (dd, J = 13.6, 8.4 Hz, 1H), 1.71 (dd, J = 13.6, 7.2 Hz, 1H), 1.49 (s, 3H), 0.82 (s, 9H), 0.01 (s, 6H); 13C NMR (CDCl3) δ 143.6, 130.2, 76.3, 66.3, 47.2, 38.8, 25.5, 18.4, 14.2, -5.6. (E)-(1R,4S)-1-Ethoxy carboxyloxy-4-(tert-butyldimethylsilyloxy)-3-methyl-cyclopent-2-ene (12). To a solution of 11R (2.51 g, 10.38 mmol) in anhydrous pyridine (20 mL) was added ethyl chloroformate (2.25 g, 20.7 mmol) and DMAP (122 mg, 1.0 mmol). The reaction mixture was stirred overnight at 50 °C. The reaction mixture was quenched with saturated NaHCO3 solution (5 mL), stirred for 10 minute and concentrated in reduced pressure. The residue was extracted with EtOAc/H2O two times, and combined organic layer was dried over MgSO4, filtered, and concentrated. The residue was...
purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give 12 (2.55 g, 78%) as colorless syrup: 1H NMR (CDCl3, 300 MHz) δ 6.92 (dd, J = 5.4 Hz, 1H), 5.72 (dd, J = 4.8, 1.4 Hz, 1H), 4.14 (q, J = 7.2 Hz, 2H), 3.40 (d, J = 13.2 Hz, 2H), 2.98 (m, 1H), 2.11-1.90 (m, 2H), 1.71 (s, 3H), 1.26 (t, J = 7.2 Hz, 3H), 0.82 (s, 9H), 0.01 (s, 6H); 13C NMR (CDCl3) δ 155.3, 138.5, 133.2, 85.7, 66.3, 63.5, 46.5, 35.3, 25.8, 18.3, 14.3, 12.8, -5.5.

(ref)-(1'R,4'S)-9-[4-(tert-Butyldimethylsilyloxy)methyl]-3-methyl-cyclopent-2-en-1-yl) cytosine (13). Cytosine nucleoside analogue 13 was synthesized from 14 by the similar procedure as described for 14: yield 39%; 1H NMR (CDCl3, 300 MHz) δ 8.30 (s, 1H), 5.12 (d, J = 4.6 Hz, 1H, D2O exchangeable), 5.04 (m, 1H), 4.78 (t, J = 9.8, 4.4 Hz, 1H), 3.48 (dd, J = 10.8, 8.8 Hz, 1H), 1.61 (s, 3H); 13C NMR (CDCl3) δ 155.7, 152.0, 150.7, 147.4, 90.9, 78.4, 65.1, 63.3, 47.4, 34.5, 25.6, 18.4, 14.0, -5.5; Anal. Calcd for C17H29N3O2Si: C, 60.86; H, 8.13; N, 19.48. Found: C, 60.32; H, 8.76; N, 11.28.

References

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