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ORIGINAL PAPER

Oenothein B, dimeric hydrolysable tannin inhibiting HCV invasion from *Oenothera erythrosepala*

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Abstract
The envelope proteins of the hepatitis C virus (HCV), E1 and E2, have been revealed to be essential for invasion of HCV. Thus, we were engaged in the search for the inhibitors against HCV invasion through the assay system using the model virus expressing recombinant HCV envelopes, E1 and E2. Now, we disclosed dimeric hydrolysable tannin oenothein B (**1**) from MeOH extract of *Oenothera erythrosepala* as an active principle for inhibition of HCV invasion and its potency was almost the same as that of monomeric hydrolysable tannin, tellimagrandin I (**2**). Furthermore, by use of stereoselectively prepared 1-β- and 1-α-*O*-methyl tellimagrandin Is (**4** and **5**), the introduction of methyl moiety into 1-hydroxy group of **2** was clarified to result in slightly reduction of activity and β-isomer was revealed to exhibit a little stronger activity than α-one.

Keywords Hepatitis C virus · Envelope protein E1 and E2 · *Oenothera erythrosepala* · Oenothein B · Invasion inhibitor

Introduction

Hepatitis C virus (HCV) infection, the important causative agent of posttransfusion, becomes a major health problem and 71 million people are estimated to have chronic hepatitis C infection worldwide [1]. Additionally, it may eventually bring about hepatitis, liver cirrhosis, hepatic failure and hepatocellular carcinoma [2]. In 1990's, the treatment of interferon-β (INF-β) or its PEG-modified form in combination with ribavirin has been the only recommended therapy for chronic HCV infection which included an unsatisfactory cure rate, effective in about 50% of patients at best [3]. Recently, NS3 inhibitors and NS5B inhibitors exhibiting noticeable anti-HCV effects were developed and currently treated in many case for HCV patients. However, it isn't the case that these new drugs can be applied to all patients and they cost comparatively high. Thus, the exploration for promising anti-HCV candidates is continuously demanded all over the world. The most difficulty for exploration of anti-HCV seeds through the cell-based bioassay is that no efficient culture system of HCV in vitro has developed for a long period although replicon system of HCV was established [4]. In this circumstance, we were interested in invasion process of HCV with the aid of envelop protein E1 and E2. In this context, we deal with inhibitors against HCV invasion utilizing model virus expressing E1 and E2 proteins.

Result and discussion

Recently, Matsuura et al. clarified that deficiency of either E1- or E2-envelope protein of HCV resulted in significant reduction of invasion efficacy into hepatocytes and established a model virus (E1E2 virus) encoding two envelope glycoproteins E1 and E2 responsible for virus invasion [5]. By use of this E1E2 virus, we were engaged in search for unprecedented HCV invasion inhibitors from medicinal plants and found hydrolysable tannin tellimagrandin I (**2**)

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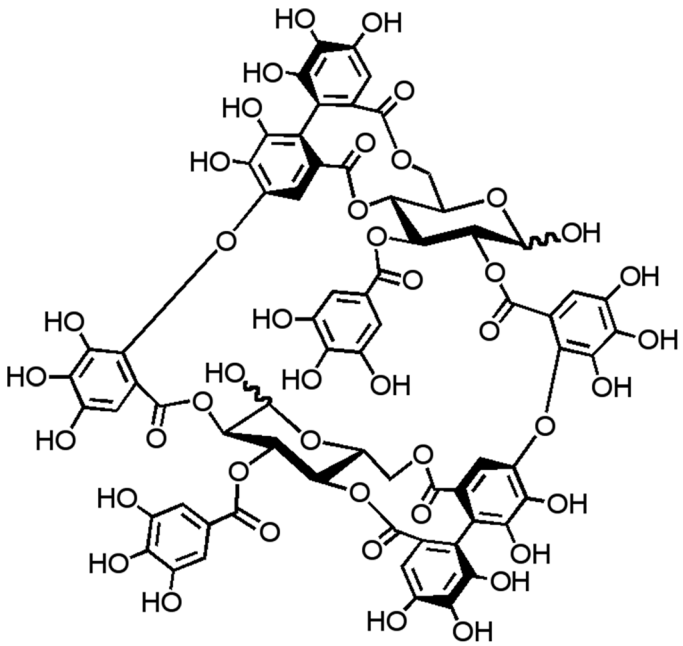
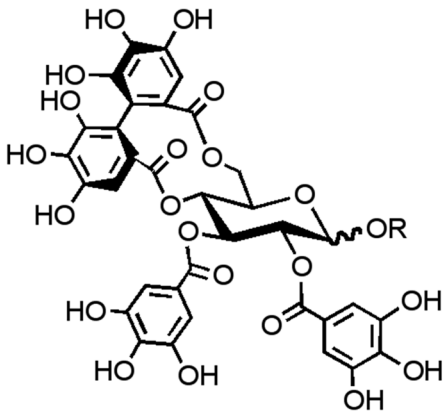
as an active constituent from *Rosa rugosa* by established assay method in previous report [6]. In the course of further exploration for active principles by the same assay procedure, methanolic extract of *Oenothera erythrosepala* [7] was clarified to show promising potency. The bioassay-guided separation of this extract through DIAION HP-20, silica gel, and ODS column chromatography gave active fraction, which was subjected to final purification by ODS HPLC to disclose an active compound.

Isolated active principle was presumed to be a hydrolysable tannin because of its similar ^{1}H- and ^{13}C-NMR spectra to those of tellimagrandin I (**2**) [6]. HH COSY indicated the existence of sugar moiety and hydroxy groups on sugar residues were suggested to be esterified by the chemical shift of ^{1}H NMR. Further detailed analysis of the 1D and 2D NMR spectra exhibited galloyl and valoneoyl groups. HR-FAB MS revealed the molecular formula to be $C_{68}H_{48}O_{44}$ and the absorption bands due to hydroxy (3144 cm^{-1}), ester carbonyl groups (1732, 1718 cm^{-1}) and aromatic ring (1604 cm^{-1}) were observed in IR spectrum. On the basis of these spectroscopic data together with UV spectra and optical rotation, this active constituent was suggested to be oenothein B (**1**) [8, 9] (Fig. 1). Furthermore, since a negative split Cotton around 250 nm was observed in the CD spectrum, configurations of both veloneoyl moieties in **1** were presumed to be *S* [9] (Fig. 2). In order to achieve complete identification, CD spectrum of trimethyl octa-*O*-methylvaloneoate (**3**) derived from natural product **1** was measured. After per-methylation of phenolic hydroxy groups of **1** by trimethylsilyldiazomethane ($TMSCHN_2$) in the presence of diisopropylethylamine, resulting permethyl ether was subjected to methanolysis by NaOMe in MeOH to provide desired **3**. Prepared **3** exhibited a negative split Cotton [$\Delta\varepsilon = -7.5\times10^{4}$ (247 nm), 1.4×10^{5} (220 nm)] due to *S* configurations of the two valoneoyl functions. The CD data was in good accordance with the reported data [10]. Consequently, active principle was securely identified as oenothein B (**1**) (Fig. 2).

Oenothein B (**1**) inhibited dose-dependently invasion of HCV model virus (E1E2 virus) with IC_{50} of 2.1 μM (98.4% at 10 μM, 92.3% at 3 μM, and 30.8% at 1 μM) whereas tellimagrandin I (**2**) showed activity with IC_{50} of 1.7 μM. These active hydrolysable tannins were disclosed to possess free 1-hydroxy moiety leading to inseparable stereo mixtures. Next, we prepared β- and α-1-*O*-methyltellimagrandin Is (**4**, **5**) separately and compared their inhibitory activity against HCV invasion. Some researchers already reported synthesis of hydrolysable tannins bearing hexahydroxydiphenoyl (HHDP) moieties, and in many of these reports, HHDP groups were prepared by intramolecularly oxidative coupling between benzoyl groups at hydroxy portions of glucose [11, 12]. Although configurations of resulting HHDPs were confirmed by CD spectra to be *S*, they were not constructed through the artificially stereo-controlled methods. Most recently, Yamada's group investigated synthesis of



oenothein B (**1**)



tellimagrandin I (**2**, R=H)
1-α-*O*-methyltellimagrandin I (**4**, R=α-Me)
1-β-*O*-methyltellimagrandin I (**5**, R=β-Me)

Fig. 1 Hydrolysable tannins inhibiting HCV invasion

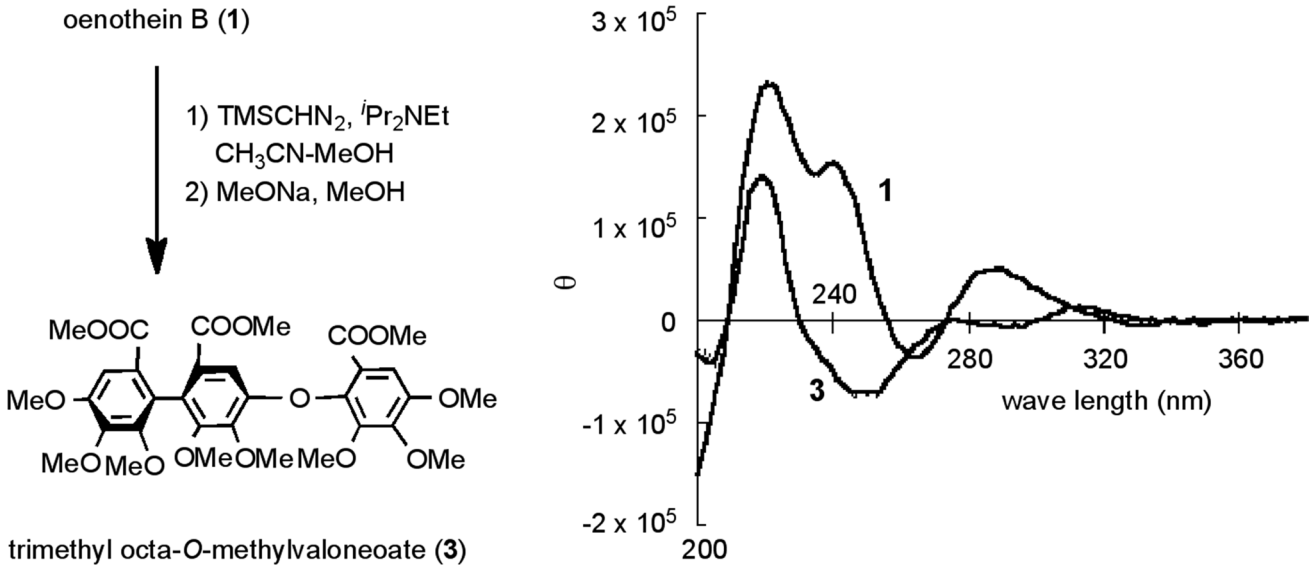
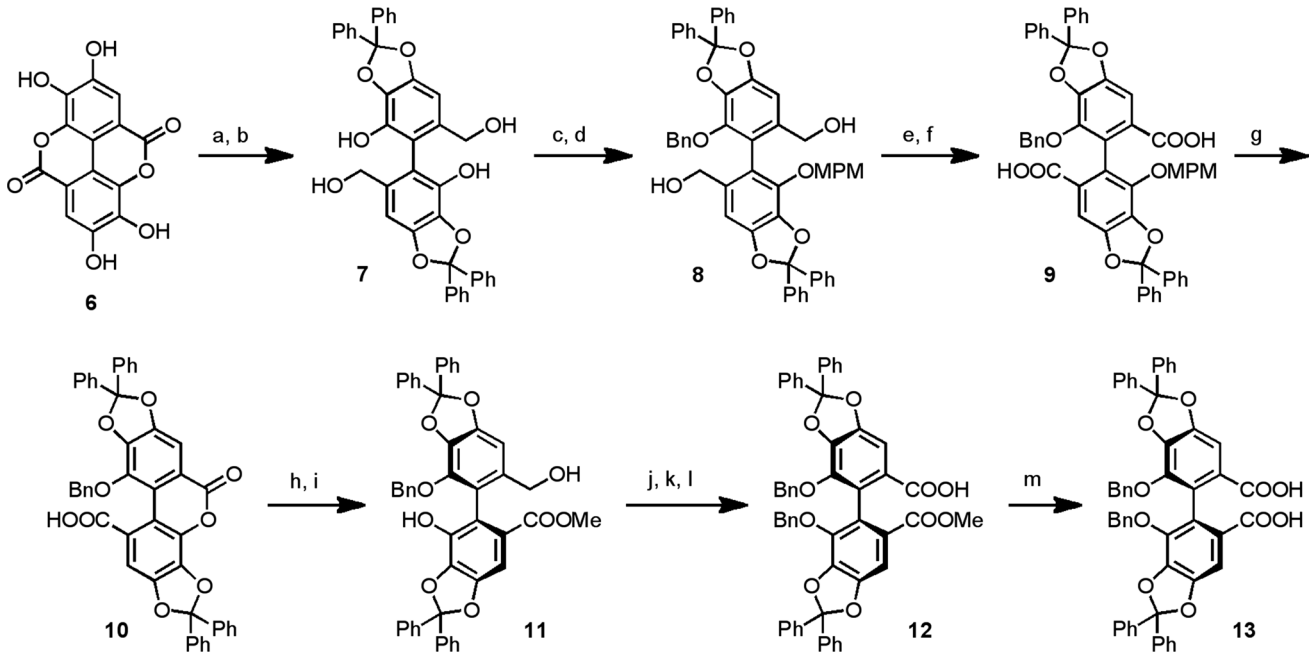


Fig. 2 Preparation for **3** from **1** and CD spectra of them

many kinds of hydrolysable tannins comprehensively and they reported stereo-selective synthesis of HHDP [13–18]. However, their strategy was based on the usage of chiral auxiliary groups referring to the sugar structure [19]. Thus, at first we performed directly stereoselective synthesis of HHDP moiety without chiral auxiliary groups, consulting Bringmann's method [20] as depicted in Scheme 1. Two catechol portions of ellagic acid **6** were protected by diphenyl acetal, then lactone rings were reductively opened by $LiAlH_4$ to furnish tetraol **7** in 95% yield. One phenolic hydroxy



Scheme 1 Asymmetric synthesis of HHDP. Reagents and conditions: **a** Ph_2CCl_2, 180 °C; **b** $LiAlH_4$, THF, 95% 2 steps; **c** BnBr, K_2CO_3, nBu_4NI, acetone; **d** MPMCl, K_2CO_3, nBu_4NI, acetone, 60% 2 steps; **e** Dess–Martin periodinane, CH_2Cl_2; **f** $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, H_2O, *t*BuOH, 89% 2 steps; **g** DDQ, $(CH_2Cl)_2$, H_2O, reflux, 55%; **h** $TMSCHN_2$, MeOH, CH_3CN, 81%; **i** (*R*)-2-methyl-CBS-oxazaborolidine, BH_3·THF, THF, 89%, > 99% ee; **j** BnBr, K_2CO_3, nBu_4NI, acetone; **k** TPAP, NMO, CH_2Cl_2; **l** $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, H_2O, *t*BuOH, 88% 3 steps; **m** KOH, MeOH, 65%

group of **7** was protected as benzyl (Bn) ether by treatment with benzyl bromide (BnBr) in the presence of potassium carbonate with tetra-*n*-butylammonium iodide (TBAI) and the other was protected by *p*-methoxybenzyl (PMB) moiety through the similar reaction condition to benzylation. Both of primary hydroxy groups of resulting diol **8** were oxidized by Dess–Martin periodinane and following $NaClO_2$ to give dicarboxylic acid **9** in 89% yield. Removal of PMB group of **9** by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) derived consequent lactonization to form **10**. After methylation of **10**, asymmetric reduction by BH_3·THF with the aid of (*R*)-2-methyl-CBS-oxazaborolidine [21] in 89% yield with more than 99% ee to furnish (*S*)-**11**. Resultant phenolic hydroxy group of **11** was benzylated and primary hydroxy moiety was oxidized to furnish carboxylic acid **12** in 88% yield for 3 steps. Finally, methyl ester group of **12** was hydrolyzed by KOH to provide desired protected HHDP **13** in 65% yield. (Scheme 1).

Next, prepared HHDP unit **13** was condensed with glucose part as shown in Scheme 2. Benzylidene acetal was introduced into 4- and 6-hydroxy groups of 1-*O*-methylglucose **14** by treatment with benzaldehyde in the presence of zinc chloride in 65% yield. After esterification of 2- and 3-hydroxy groups of resultant **15** with protected gallic acid **16** by use of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride salt (EDCI·HCl) and 4-dimethylaminopyridine (DMAP), benzylidene acetal portion was removed by iodine in MeOH to afford **17** in 97% yield for 2 steps. Protected HHDP **13** was condensed with **17** by use of 2-methyl-6-nitrobenzoic anhydride (MNBA) [22] to afford 1-β- and α-*O*-methyltellimagrandin relatives **18a** and **b**, separately. Finally, hydrogenation of **18a** and **b** in the presence of palladium black as a catalyst gave desired 1-β- and α-*O*-methyltellimagrandin Is (**4** and **5**) in 95% and 75% yield, respectively.

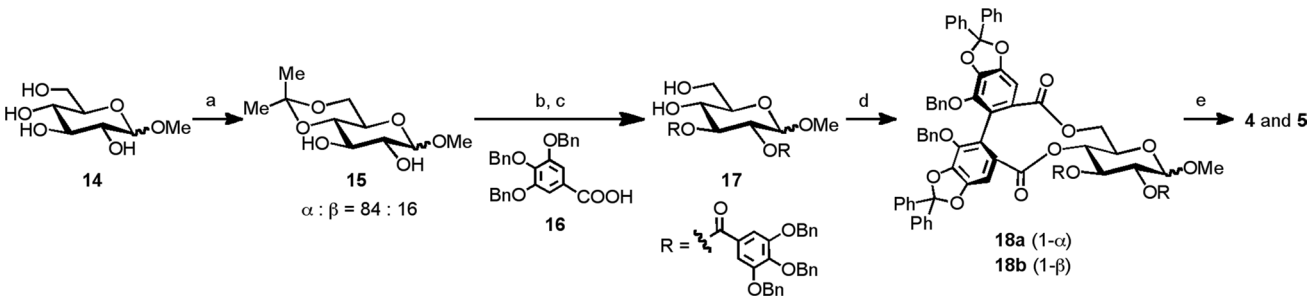
Inhibitory activities of 1-β- and α-*O*-methyltellimagrandin Is (**4** and **5**) against HCV invasion were evaluated to be IC_{50} of 4.1 μM and 7.7 μM, respectively. Therefore, introduction of methyl group into 1-hydroxy moiety of tellimagrandin I (**2**) was revealed to reduce their potency and 1-β-*O*-methyltellimagrandin I (**4**) was disclosed to show a little stronger activity than α-epimer (**5**) (Table 1).

In conclusion, we disclosed oenothein B (**1**) as the HCV invasion inhibitor using HCV model virus expressing E1 and E2 envelope proteins through bioassay guided separation from the MeOH extract of *O. erythrosepala*. Fortunately, **1** and tellimagrandin I (**2**) were reported to show little cytotoxicity at the concentration that they inhibited HCV invasion. [23, 24] Ellagitannins were also reported to block the HCV NS3/4A protease activity, [25] thus, **1** and **2** may effectively annihilate HCV at the both points, invasion and replication, which is thought to be one of the promising HCV seeds. In addition, the anomeric hydroxy group in **2** was revealed to play a little role in its potent inhibition against HCV invasion. Further investigation is now in progress because we should clarify the proof of concept in the near future that these active compounds really inhibit the HCV invasion.

Materials and methods

General procedure

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM LA-500 (^{1}H: 500 MHz, ^{13}C: 125 MHz) and a Varian inova 600 (600 MHz) spectrometer. All ^{1}H-NMR data were referenced to tetramethylsilane (δ_H 0.00 ppm) as an internal standard. IR spectra were recorded on a JASCO FT/IR-5300 infrared spectrometer. FAB-MS and HR FAB-MS data were acquired on a JEOL JMS SX-102 mass spectrometer. Circular dichroism (CD) spectra were measured in 1 mm length cell with a JASCO J-720W Spectropolarimeter. Optical rotation was determined in 0.5 dm length cell with a JASCO DIP-370 digital polarimeter. HPLC was performed on a JASCO PU2080 equipped with a JASCO UV2070 UV detector. Silica gel (Fuji Silysia Chemical, BW-200) and precoated thin-layer chromatography (TLC) plates (Merck, Kiesel gel 60F_{254}) were used for column chromatography and



Scheme 2 Synthesis of 1-*O*-methyltellimagrandin Is. Reagents and conditions: **a** $Me_2C(OMe)_2$, *p*-TsOH, DMF, 65%; **b 16**, EDCI·HCl, DMAP, CH_2Cl_2; **c** I_2, MeOH, 97% 2 steps; **d 13**, MNBA, Et_3N, DMAP, CH_2Cl_2, 61% for **18a**, 12% for **18b**; **e** H_2, Pd-black, 95% for **4** from **18a**, 75% for **5** from **18b**

TLC, respectively. Spots on TLC plates were detected by staining with phosphomolybdic acid in EtOH. The reagents and solvents for organic synthesis were purchased and used without any purification. The biological assay were performed in the same manner as previous our report (*Bioorg. Med. Chem. Lett.* **2010**, *20*, 1598–1600).

Isolation of oenothein B (1)

The dried leaves (500 g) of *Oenothera erythrosepala* were soaked with methanol under room temperature for 6 h and then extracted twice under reflux for 4 h. The maceration extract and the decoction were combined and concentrated *in vacuo* to afford 77 g methanol extract. The extract was charged on DIAION HP-20 (Mitsubishi Chemical) column as water solution, which was washed with water then eluted by methanol. After concentration of methanolic elution under the reduced pressure, the resultant 6.7 g of the extract was applied on silica gel (SiO_2) column chromatography (*n*-hexane:EtOAc = 1:1 → $CHCl_3$:MeOH:H_2O = 10:3:1 [lower layer] → 4:4:1 → MeOH). Three fractions were gained, OE-1 (0.86 g), OE-2 (0.81 g) and OE-3 (5.3 g). Bioassay-guided results indicated that OE-3 was the active fraction and it was subjected to ODS gel column chromatography (25% MeOH → 40% MeOH → 60% MeOH → MeOH) to give four fractions, OE3–1 (2.5 g), OE3–2 (1.4 g), OE3–3 (0.35 g) and OE3–4 (0.28 g). Fraction OE3–2 was further separated by reversed phase HPLC [column: Cosmosil 5C_{18}-AR-II (10 mm i.d.×250 mm, Nacalai), mobile phase: CH_3OH:H_2O = 12.5:87.5 with 0.05% trifluoroacetic acid (TFA), detection: UV (λ = 254 nm), flow rate: 4 mL/min] to afford oenothein B (**1**, 10.3 mg). The structure was confirmed by its ^{1}H-NMR data which are consistent with those reported in literature [8, 9].

Preparation of tetraol 7

A solution of ellagic acid (**6**, 2.6 g, 8.6 mmol) was treated with β,β-dichlorodiphenylmethane (13 mL, 68.9 mmol) at 180 °C for 3 h. After cooling down to room temperature, the reaction mixture was diluted with *n*-hexane, then filtrated. The resulting residue was washed with *n*-hexane giving crude acetal product (5.5 g). Next, to a solution of this crude acetal (5.5 g) in THF (86 mL), $LiAlH_4$ (2.0 M in THF, 8.6 mL, 17.2 mmol) was added dropwise at 0 °C for 10 min, then the reaction mixture stirred at room temperature for 5 h. The reaction was quenched with ice-cold aq. satd. NH_4Cl, then the whole was extracted with EtOAc. The EtOAc extract was washed with aq satd NaCl and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 200 g, *n*-hexane:EtOAc = 3:1) to afford tetraol **7** (5.2 g, 95%).

Tetraol **7**; a colorless amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 4.20 (4H, s), 6.77 (2H, s), 7.38–7.64 (20H, m). IR (KBr): 3063, 1630 cm^{-1}; FAB-MS *m/z*: 639 $(M+H)^+$. HRFAB-MS *m/z*: 639.1944 (Calcd for $C_{40}H_{31}O_8^+$: 639.1941).

Preparation of diol 8

A solution of tetraol **7** (5.2 g, 8.1 mmol) in acetone (81 mL) was treated with benzyl bromide (1.1 mL, 8.9 mmol) and tetra-*n*-butylammonium iodide (TBAI, 299 mg, 0.81 mmol) in the presence of K_2CO_3 (980 mg, 8.1 mmol) at room temperature for 5 h. The reaction mixture was poured into aq. satd. NaCl, then the whole was extracted with EtOAc. The EtOAc extract was washed with aq. satd. NaCl and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude benzyl ether (7.1 g). Next, a solution of crude benzyl ether (7.1 g) in acetone (81 mL) was treated with *p*-methoxybenzyl chloride (1.2 mL, 8.9 mmol) and TBAI (299 mg, 0.81 mmol) in the presence of K_2CO_3 (980 mg, 8.1 mmol) at room temperature for 5 h. The reaction mixture was poured into aq. satd. NaCl, then the whole was extracted with EtOAc. The EtOAc extract was washed with aq. satd. NaCl and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 250 g, *n*-hexane:EtOAc = 3:1) to afford diol **8** (4.1 g, 60%).

Diol **8**; a colorless amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 3.65 (3H, s), 4.78 (1H, d, J = 11.3 Hz), 4.89 (1H, d, J = 11.7 Hz), 5.04 (1H, d, J = 11.7 Hz), 5.14 (1H, d, J = 11.3 Hz), 6.47 (2H, d, J = 8.7 Hz), 6.74 (2H, d, J = 8.7 Hz), 6.81–7.21 (7H, m), 7.40–7.64 (20H). IR (KBr): 3304, 3063, 1613 cm^{-1}. FAB-MS *m/z*: 849 $(M+H)^+$. HRFAB-MS *m/z*: 849.2989 (Calcd for $C_{55}H_{45}O_9^+$: 849.2985).

Preparation of dicarboxylic acid 9

A solution of diol **8** (4.1 g, 4.8 mmol) in CH_2Cl_2 (48 mL) was treated with Dess–Martin periodinane (6.1 g, 14.4 mmol) at room temperature for 3 h. To the reaction mixture, aq. satd. $Na_2S_2O_3$ and aq. satd. $NaHCO_3$ were added at 0 °C, then the whole was stirred at room temperature for 30 min. The whole was extracted with EtOAc and the EtOAc extract was washed with aq. satd. NaCl then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude aldehyde (4.5 g). Next, a solution of this crude aldehyde (4.5 g) in *t*-BuOH (9.6 mL) was mixed with 2-methyl-2-butene (5.1 mL, 48 mmol), then to the solution, the aqueous solution (9.6 mL) of NaH_2PO_4 (5.8 g, 48 mmol) and $NaClO_2$ (4.3 g, 48 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 5 h. The

reaction mixture was poured into aq. satd. NaCl, then the whole was extracted with CH_2Cl_2. Removal of the solvent from the CH_2Cl_2 extract under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 150 g, *n*-hexane:EtOAc = 1:1) to afford dicarboxylic acid **9** (3.8 g, 89%).

Dicarboxylic **9**; a colorless amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 3.55 (3H, s), 4.88 (2H, s), 4.94 (2H, d, J = 7.7 Hz), 6.41 (2H, d, J = 8.7 Hz), 6.74 (2H, d, J = 8.7 Hz), 6.81–7.00 (7H, m), 7.32–7.60 (20H, m). IR (KBr): 3296, 3063, 2924, 1718 cm^{-1}. FAB-MS *m/z*: 899 $(M + Na)^+$. HRFAB-MS *m/z*: 899.2574 (Calcd for $C_{55}H_{40}O_{11}Na^+$: 899.2571).

Preparation of lactone 10

A solution of dicarboxylic acid **9** (3.8 g, 4.3 mmol) in $(CH_2Cl)_2$ (43 mL) was treated with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 1.8 g, 8.1 mmol) in the presence of distilled water (2.7 mL) under reflux for 9 h. After cooling to room temperature, aq. satd. NaCl was added to the reaction mixture, then the whole was extracted with EtOAc. The EtOAc extract was washed with aq. satd. NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 100 g, $CHCl_3$:MeOH:H_2O = 50:3:1 [lower layer]) to afford lactone **10** (1.8 g, 55%).

Lactone **10**; a colorless amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 5.07 (2H, s), 6.66 (2H, brs), 7.29–7.63 (25H, m). IR (KBr): 3065, 2930, 1690 cm^{-1}. FAB-MS *m/z*: 761 $(M + Na)^+$. HRFAB-MS *m/z*: 761.1893 (Calcd for $C_{47}H_{30}O_9Na^+$: 761.1890).

Preparation of optical pure biphenyl 11

A solution of lactone **10** (1.8 g, 2.4 mmol) in CH_3CN:MeOH = 9:1 (24 mL) was treated with trimethylsilyldiazomethane ($TMSCHN_2$, 2.0 M in Et_2O, 1.2 mL, 2.4 mmol) at room temperature for 10 min. Removal of the solvent from the reaction mixture under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 60 g, *n*-hexane:EtOAc = 6:1) to afford methyl ester (1.5 g, 81%). Next, to a solution of methyl ester (1.5 g, 1.9 mmol) in THF (19 mL), (*R*)-2-methyl-CBS-oxazaborolidine (2.0 M in toluene, 0.05 mL, 0.1 mmol) was added. Then the solution was treated with BH_3·THF (1.0 M in THF, 3.8 mL, 3.8 mmol) at − 40 °C for 10 h. The reaction mixture was poured into aq. satd. NH_4Cl, then the whole was extracted with EtOAc. The EtOAc extract was washed with aq. satd. NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 50 g, *n*-hexane:EtOAc = 2:1) to afford optical pure biphenyl **11** (1.3 g, 89%). The optical purity of **11** was determined by HPLC with chiral column (Chiralcel OD 4.6 mm *i.d.* × 250 mm, mobile phase; *n*-hexane:*i*PrOH = 10:1, flow rate; 1.0 mL/min, detection; UV λ = 254 nm) to be > 99% *ee*.

Optical pure biphenyl **11**; a colorless amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 3.51 (3H, s), 4.23 (2H, s), 5.04 (1H, d, J = 11.7 Hz), 5.12 (1H, d, J = 11.7 Hz), 6.88–7.15 (7H, m), 7.26–7.64 (20H, m). IR (KBr): 3735, 2930, 1717 cm^{-1}. FAB-MS *m/z*: 757 $(M + H)^+$. HRFAB-MS *m/z*: 757.2363 (Calcd for $C_{48}H_{37}O_9^+$: 757.2359). $[\beta]_D^{24}$ + 3.8° (*c* 1.21, MeOH). CD (MeOH) λ_{max} ($\Delta\varepsilon$) 203 (+ 47.4), 229 (− 31.5) nm.

Preparation of carbomethoxy carboxylic acid 12

A solution of biphenyl **11** (1.6 g, 2.1 mmol) in acetone (21 mL) was treated with benzyl bromide (0.37 mL, 3.0 mmol) and TBAI (77 mg, 0.21 mmol) in the presence of K_2CO_3 (442 mg, 3.2 mmol) at room temperature for 2 h. The reaction mixture was poured into aq. satd. NaCl, then the whole was extracted with EtOAc. The EtOAc extract was washed with aq. satd. NaCl and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude bisbenzyl ether (2.7 g). A solution of crude bisbenzyl ether (2.7 g) in CH_2Cl_2 (21 mL) was treated with tetra-*n*-propylammonium perruthenate (TPAP, 74 mg, 0.21 mmol) and *N*-methylmorpholine *N*-oxide (NMO, 1.2 g, 10 mmol) at room temperature for 2 h. The reaction mixture was filtered through short pad of SiO_2, then removal of solvent from the filtrate under reduced pressure gave a crude aldehyde (2.8 g). Next, a solution of this crude aldehyde (2.8 g) in *t*-BuOH (11 mL) was mixed with 2-methyl-2-butene (3.0 mL, 28 mmol), then to the solution, the aqueous solution (11 mL) of NaH_2PO_4 (3.4 g, 28 mmol) and $NaClO_2$ (2.5 g, 28 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 6 h. The reaction mixture was poured into aq. satd. NaCl, and the whole was extracted with CH_2Cl_2, then dried over $MgSO_4$. The Removal of the solvent from the CH_2Cl_2 extract under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 100 g, *n*-hexane:EtOAc = 2:1) to afford carbomethoxy carboxylic acid **12** (1.6 g, 88%).

Carbomethoxy carboxylic acid **12**; a colorless amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 3.51 (3H, s), 4.99 (4H, brs), 6.83–7.09 (12H, m), 7.39–7.60 (20H, m). IR (KBr): 2920, 1724 cm^{-1}. FAB-MS *m/z*: 883 $(M + Na)^+$. HRFAB-MS *m/z*: 883.2625 (Calcd for $C_{55}H_{40}O_{10}Na^+$: 883.2621). $[\beta]_D^{24}$ − 38.0° (*c* 0.11, MeOH).

Preparation of protected HHDP 13

Carbomethoxy carboxylic acid **12** (1.6 g, 1.8 mmol) was treated with methanolic KOH (3 M, 9.0 mL, 27 mmol) at 40 °C for 3 h. After cooling to room temperature, aq. satd. $KHSO_4$ was added to the reaction mixture, then the whole was extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried over $MgSO_4$. Removal of the solvent from the CH_2Cl_2 extract under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 50 g, $CHCl_3$:MeOH: H_2O = 30:3:1 [lower layer]) to afford lactone **13** (990 mg, 65%).

Protected HHDP **13**; a colorless amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 5.08 (4H, s), 6.91–7.09 (12H, m), 7.45–7.66 (20H, m). IR (KBr): 3063, 3032, 1698, 1611 cm^{-1}. FAB-MS *m/z*: 869 $(M+Na)^+$. HRFAB-MS *m/z*: 869.2469 (Calcd for $C_{54}H_{38}O_{10}Na^+$: 869.2465). $[\beta]_D^{24}$ −36.6° (*c* 0.50, MeOH).

Preparation of 4,6-*O*-acetonide-1-*O*-methyl glucose 15

A solution of 1-*O*-methyl glucose **14** (96 mg, 0.49 mmol) in DMF (1.0 mL) was treated with 2,2-dimethoxypropane (0.48 mL, 3.9 mmol) and *p*-toluenesulfonic acid (4.2 mg, 0.025 mmol) at room temperature for 1 h. To the reaction mixture, aq. satd. $NaHCO_3$ was added, then the whole was extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried over $MgSO_4$. Removal of the solvent from the CH_2Cl_2 extract under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 50 g, $CHCl_3$:MeOH: H_2O = 50:3:1 [lower layer]) to afford **15** (75 mg, 65%). The structure of **15** was identified by comparison of spectroscopic data with reported one [26].

Preparation of 2,3-*O*-bis-tribenylgalloyl-1-*O*-methyl glucose 17

A solution of **15** (75 mg, 0.32 mmol) in CH_2Cl_2 (3.2 mL) was treated with tri-*O*-benzylgallic acid (**16**, 352 mg, 0.8 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride salt (EDCI·HCl, 368 mg, 1.9 mmol) in the presence of 4-dimethylaminopyridine (DMAP, 19 mg, 0.16 mmol) at room temperature overnight. To the reaction mixture, aq. satd. NaCl was added, then the whole was extracted with EtOAc. The EtOAc extract was washed with 5% HCl, aq. satd. $NaHCO_3$ and aq. satd. NaCl sequentially, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a crude bis-tribenylgalloyl product (488 mg). A solution of the crude product (488 mg) in MeOH (6.4 mL) was treated with I_2 (81.2 mg, 0.32 mmol) under reflux for 1 h. To the reaction mixture, aq. satd. $Na_2S_2O_3$ was added, then the whole was extracted with EtOAc. The EtOAc extract was washed with aq. satd. NaCl, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 15 g, *n*-hexane:EtOAc = 2:3) to afford **17** (335 mg, 97%). 2,3-*O*-Bis-tribenylgalloyl-1-*O*-methyl glucose **17**; a colorless amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 3.38 (2.52H, s, β-isomer), 3.45 (0.48H, s, α-isomer), 3.78–3.98(4H, m), 4.55 (0.16H, d, *J* = 7.9 Hz, α-isomer), 4.86–5.00 (12H, m), 5.06–5.08 (1.68H, m, β-isomer), 5.20 (0.16H, dd, *J* = 9.2, 9.2 Hz, α-isomer), 5.32 (0.16H, dd, *J* = 7.9, 9.2 Hz, α-isomer), 5.58 (0.84H, dd, *J* = 9.2, 9.2 Hz, β-isomer), 7.13–7.35 (34H, m). IR (KBr): 2922, 1734 cm^{-1}; FAB-MS *m/z*: 1041 $(M+H)^+$. HRFAB-MS *m/z*: 1041.3987 (Calcd for $C_{63}H_{61}O_{14}^+$: 1041.3983). $[\beta]_D^{24}$ +40.8° (*c* 0.72, MeOH).

Preparation of 2,3-*O*-bis-tribenylgalloyl-4,6-*O*-protected HHDP-1-*O*-methyl glucose 18

A solution of **17** (335 mg, 0.31 mmol) and protected HHDP (263 mg, 0.31 mmol) in CH_2Cl_2 (3.1 mL) was treated with 2-methyl-6-nitrobenzoic anhydride (MNBA, 640 mg, 1.9 mmol) in the presence of triethylamine (0.43 mL, 3.1 mmol) and DMAP (113 mg, 0.93 mmol) at room temperature for 3 h. To the reaction mixture, aq. satd. NaCl was added, then the whole was extracted with EtOAc. The EtOAc extract was washed with 5% HCl, aq. satd. $NaHCO_3$ and aq. satd. NaCl sequentially, then dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 15 g, benzene:*n*-hexane:EtOAc = 10:10:1) to afford **18a** (β-isomer, 349 mg, 61%) and **18b** (α-isomer, 69 mg, 12%).

18a; a colorless amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 3.42 (3H, s), 3.94 (1H, d, *J* = 12.8 Hz), 4.43 (1H, dd, *J* = 7.9, 9.7 Hz), 4.67–5.09 (17H, m), 5.23 (1H, d, *J* = 3.7 Hz), 5.28–5.34 (2H, m), 5.91 (1H, dd, *J* = 9.8, 10.4 Hz), 6.69 (1H, s), 6.73 (1H, s), 6.82–7.59 (64H, m). IR (KBr): 3090, 3034, 1747 cm^{-1}. FAB-MS *m/z*: 1849 $(M+H)^+$. HRFAB-MS *m/z*: 1849.6080 (Calcd for $C_{117}H_{93}O_{22}^+$: 1849.6114). $[\beta]_D^{24}$ +12.5° (*c* 0.78, MeOH).

18b; a colorless amorphous powder, ^{1}H-NMR ($CDCl_3$) δ: 3.53 (3H, s), 4.03 (1H, d, *J* = 12.8 Hz), 4.35 (1H, dd, *J* = 6.1, 10.4 Hz), 4.61 (1H, d, *J* = 7.3 Hz), 4.91–5.12 (16H, m), 5.31 (1H, dd, *J* = 9.8, 10.4 Hz), 5.38 (1H, dd, *J* = 6.1, 12.8 Hz), 5.44 (1H, dd, *J* = 7.3, 9.8 Hz), 5.59 (1H, dd, *J* = 9.8, 9.8 Hz), 6.74 (1H, s), 6.81 (1H, s), 6.82–7.59 (64H, m). IR (KBr): 3063, 2932, 1732 cm^{-1}. FAB-MS *m/z*: 1849 $(M+H)^+$. HRFAB-MS *m/z*: 1849.6110 (Calcd for $C_{117}H_{93}O_{22}^+$: 1849.6114). $[\beta]_D^{24}$ +11.6° (*c* 0.88, MeOH).

Table 1 Inhibitory activity for invasion of model HCV

Compounds	IC_{50} (μM)
oenothein B (**1**)	2.1
1-β-*O*-methyltellimagrandin I (**4**)	4.1
1-α-*O*-methyltellimagrandin I (**5**)	7.7
Tellimagrandin I (**2**)*	1.7

*Positive control

Preparation of 1α-*O*-methyl tellimagrandin I 4

A solution of **18a** (349 mg, 0.19 mmol) in THF (19 mL) was treated with Pd-black (35 mg) under H_2 atmosphere at room temperature overnight. The reaction mixture was filtered and removal of the solvent from the filtrate under reduced pressure gave a residue, which was purified by column chromatography (octadecylsilica gel [ODS] 5 g, 20% CH_3CN) to afford **4** (144 mg, 95%).

1α-*O*-Methyl tellimagrandin I **4**; a colorless amorphous powder, ^{1}H-NMR (acetone-d_6) δ: 3.43 (3H, s), 3.84 (1H, d, J=12.8 Hz), 4.43 (1H, dd, J=6.7, 9.8 Hz), 5.10–5.13 (2H, m), 5.15 (1H, d, J=3.7 Hz), 5.31 (1H, dd, J=6.7, 12.8 Hz), 5.80 (1H, dd, J=9.8, 9.8 Hz), 6.43 (1H, s), 6.64 (1H, s), 6.99 (2H, s), 7.05 (2H, s). IR (KBr): 3314, 2965, 1701, 1606 cm^{-1}. FAB-MS *m/z*: 823 $(M+Na)^+$. HRFAB-MS *m/z*: 823.1055 (Calcd for $C_{35}H_{28}O_{22}Na^+$: 823.1046). $[\beta]_D^{24}$ +47.1° (*c* 0.40, MeOH);

Preparation of 1β-*O*-methyl tellimagrandin I 5

A solution of **18b** (69 mg, 0.037 mmol) in THF (3.7 mL) was treated with Pd-black (6.9 mg) under H_2 atmosphere at room temperature overnight. The reaction mixture was filtered and removal of the solvent from the filtrate under reduced pressure gave a residue, which was purified by column chromatography (ODS 1 g, 20% CH_3CN) to afford **5** (22 mg, 75%).

1β-*O*-Methyl tellimagrandin I **5**; a colorless amorphous powder, ^{1}H-NMR (acetone-d_6) δ: 3.48 (3H, s), 3.90 (1H, d, J=12.8 Hz), 4.29 (1H, dd, J=6.1, 9.8 Hz), 4.82 (1H, d, J=7.9), 5.11 (1H, dd, J=9.8, 9.8 Hz), 5.28 (1H, dd, J=7.9, 9.8 Hz), 5.34 (1H, dd, J=6.1, 12.8 Hz), 5.63 (1H, dd, J=9.8, 9.8 Hz), 6.42 (1H, s), 6.64 (1H, s), 6.92 (2H, s), 7.04 (2H, s). IR (KBr): 3291, 2926, 1717, 1615 cm^{-1}. FAB-MS *m/z*: 799 $(M-H)^-$. HRFAB-MS *m/z*: 799.0990 (Calcd for $C_{35}H_{27}O_{22}^-$: 799.0992). $[\beta]_D^{24}$ +32.0° (*c* 0.20, MeOH).

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Compliance with ethical standards

Conflicts of interest The authors declare no conflicts of interest.

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