Bioorganic & Medicinal Chemistry 18 (2010) 5975–5980

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Concise synthesis of 5,6-dihydrovaltrate leading to enhanced Rev-export inhibitory congener

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ARTICLE INFO

Article history:
Received 3 June 2010
Revised 21 June 2010
Accepted 22 June 2010
Available online 1 July 2010

Keywords:
Rev-export inhibitor
Anti-HIV
5,6-Dihydrovaltrate
Iridoid
Genipin

ABSTRACT

The concise synthesis of 5,6-dihydrovaltrate (**2**), the bioisostere of valtrate (**1**) showing anti-HIV activity by inhibition for nuclear export of Rev, has been achieved from the commercially available iridoid genipin (**3**). Analysis of steric influence of the substituents linked to the three hydroxyl groups was conducted by the synthesized three analogs (**2a**–**2c**). Consequently, attenuation of steric hindrance around the epoxy portion was revealed to enhance inhibitory potency for Rev-export. In addition to this finding, 1-acetoxy analog **2b** was disclosed as the promising Rev-export inhibitor superior to **1**.

1. Introduction

Human immunodeficiency virus-type 1 (HIV-1) is the etiological agent of acquired immuno-deficiency syndrome (AIDS). The unique nature of the replicative cycle of HIV-1 provides many potential targets for chemotherapeutic intervention. One of these, the HIV-1 regulatory protein Rev catalyzes nuclear export of viral mRNA to produce the HIV-1 structural proteins.[1] Since this biological step is essential for viral replication, inhibition of nuclear export of Rev is an attractive and potential strategy for HIV-1 drug discovery efforts.[2] Under this circumstance, we have been engaged in search for Rev-export inhibitors originating in medicinal plants.[3–6] Previously, we disclosed the iridoid type monoterpene valtrate (**1**) as the inhibitor for nuclear export of Rev from the crude drug Valerianae Radix.[3] Furthermore, we have recently presented the synthetic bioisostere, 5,6-dihydrovaltrate (**2**), to conquest not only scarce supply from the natural resource but also extreme difficulty in chemical derivation of **1**; removal of the isovaleryl group at C-1 readily gave rise to decomposition via dial accompanied with elimination of the acetoxy function.[7] However, the synthetic route to the bioisostere **2** seems redundant to explore anti-HIV leads in spite of high optical outcome (95% ee). In this context, we have devoted ourselves to develop a simplified synthetic protocol for **2**. Herein, we describe the concise synthesis of 5,6-dihydrovaltrate (**2**) from the commercially available iridoid genipin (**3**). In addition, we wish to introduce the synthesized analog **2b** with superior Rev-export inhibitory activity to valtrate (**1**) as well as the bioisostere **2** by using the present synthetic protocol (Fig. 1).

2. Results and discussion

The synthetic strategy from genipin (**3**) to 5,6-dihydrovaltrate (**2**) is outlined in Figure 2. In the previous synthesis of **2**, 8α,10-epoxy moiety was introduced by stereospecific epoxidation with

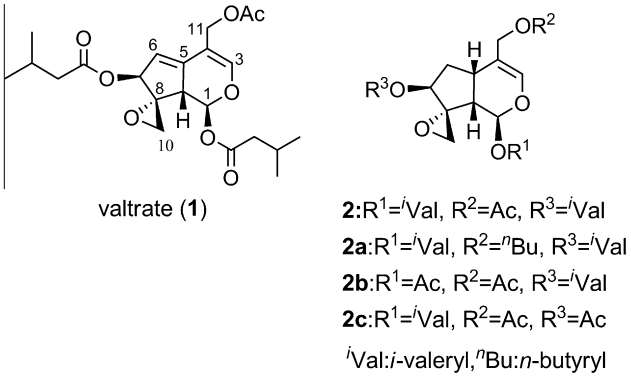

Figure 1. Chemical structures of valtrate (**1**) and bioisosteric analogs (**2, 2a**–**2c**).

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doi:10.1016/j.bmc.2010.06.079

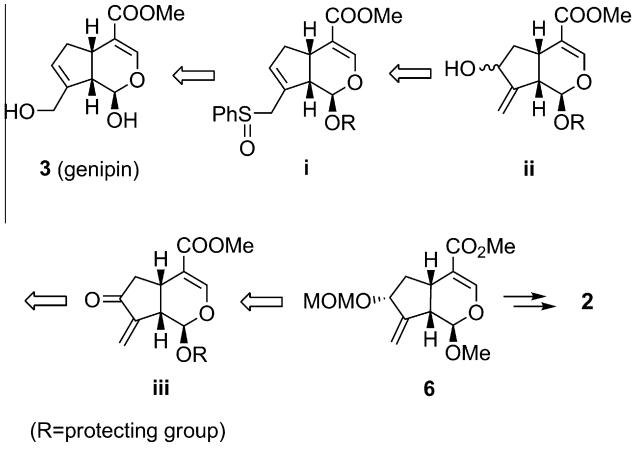


Figure 2. Synthetic strategy of 5,6-dihydrovaltrate (**2**) from genipin (**3**).

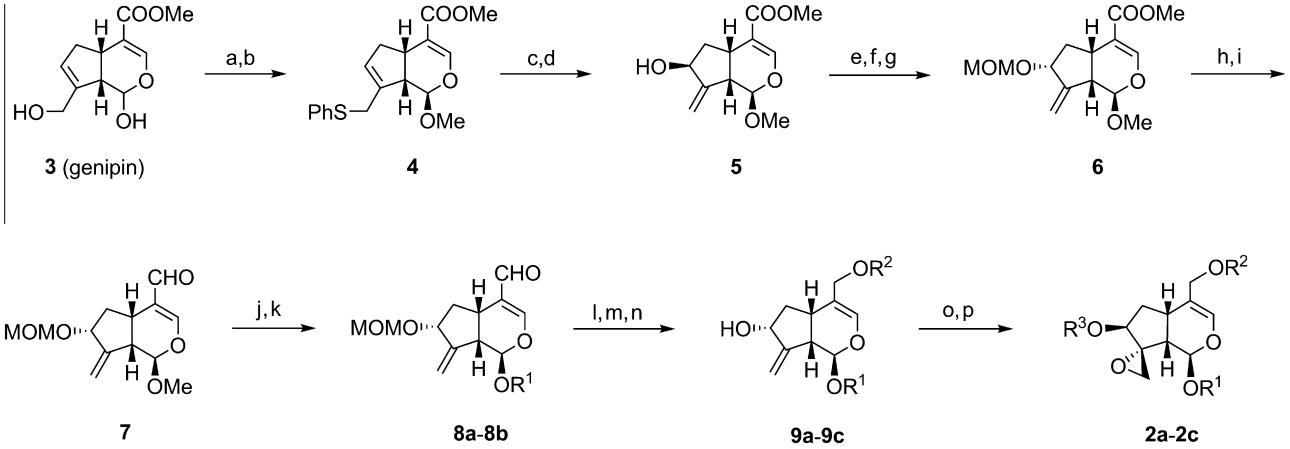
the aid of the adjacent 7α-hydroxyl function. Since the 7α-hydroxy-protected methyl ester (**6**) was utilized for the synthesis of **2**, we designated the ester **6** as the target compound. Therefore, construction of 7α-hydroxy-8,(10)-ene in **6** from 10-hydroxy-7-ene structure in **3** was regarded as a main issue. The planned 7α-hydroxy-8,(10)-ene structure in **6** would be provided by stereoselective 1,2-reduction of 8,(10)-en-7-one from the convex face. The conjugated enone **iii** was envisioned to be afforded from a mixture of allyl alcohols **ii**, which would be obtained by Mislow–Evans rearrangement[8] of phenyl sulfoxide **i**. The sulfoxide was planned to be converted from **3** by way of phenyl sulfide.

The synthesis of **6** from genipin (**3**) was executed as shown in Scheme 1. Treatment of **3** with Amberlyst-15 in MeOH provided methyl acetal, which was submitted to phenylsulfidation using diphenyldisulfide and tri-*n*-butylphosphine to give phenylsulfide **4**. After oxidation of **4** with Oxone in acetone–H_2O, the resulting sulfoxide was subjected to Mislow–Evans rearrangement with trimethyl phosphite in MeOH to afford allyl alcohol **5** as a sole product unexpectedly. However, the spectroscopic data of the methoxymethyl (MOM) ether prepared from **5** is in no accordance with those of **6**. Moreover, the NOESY spectrum of **5** displayed the cross peak between 6α-H and 7-H. Thus, the alcohol was elucidated to bear 7β-hydroxy function.

Dess–Martin oxidation of **5** followed by stereoselective reduction using $NaBH_4$ and $CeCl_3$ predominantly provided 7α-alcohol, of which the hydroxyl group was protected as MOM ether to give the envisioned methyl ester **6**. The chemical transformation from **6** to 5,6-dihydrovaltrate (**2**) was previously presented, therefore the synthetic protocol from genipin (**3**) to **2** was established. In the previous procedure, synthesis of **6** was performed in 7.0% overall yield for 18 steps from the starting material. It should be noted that the present protocol furnished **6** in 47.6% overall yield through seven chemical transformations.

Subsequently, we examined steric influence of substituents, linked to the three hydroxyl groups, on the Rev-export inhibitory potency of **2**. Thus, the three analogs (**2a**–**2c**) were designed and synthesized as depicted in Scheme 1. Successive diisobutylaluminum hydride (DIBAL) reduction and oxidation by tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine N-oxide (NMO)[9] of **6** afforded conjugated aldehyde **7**. After acidic hydrolysis of the methyl acetal with 10% HCl–THF, the resulting hemiacetal was coupled with isovaleric acid or AcOH in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and *N*,*N*′-carbonyldiimidazole (CDI) to yield isovaleryl and acetyl acetal (**8a** and **8b**), respectively. Sequential removal of the MOM group with BCl_3 and 1,2-reduction of the α,β-unsaturated formyl portion with $NaBH_4$ and $CeCl_3$ provided diols. Acylation of the primary hydroxyl group in the resulting diols with *n*-butyryl or acetyl chloride in the presence of *i*-Pr_2NEt provided diesters **9a**–**9c**. Stereoselective epoxidation associated with the adjacent hydroxyl group using *tert*-butyl hydroperoxide (TBHP) and vanadium oxyacetylacetonate [$VO(acac)_2$][10] afforded epoxyalcohols, of which the α-epoxy configurations were unequivocally confirmed by the NOE correlations between 7-H and 10-Ha, 9-H and 10-Hb in the NOESY spectra. Finally, introduction of the isovaleryloxy or acetoxy function to C-7 by Mitsunobu inversion[11] using diethyl azodicarboxylate (DEAD) and triphenylphosphine furnished the desired three analogs **2a**–**2c**.

Next, the synthesized three analogs (**2a**–**2c**) were assessed for inhibitory activity for nuclear export of Rev by an indirect fluorescent antibody technique. After transfection of plasmid[12] coding Rev tagged with human influenza haemagglutinin (HA) into HeLa cells, localization of Rev protein was examined with a fluorescence microscope using HA as a marker. Table 1 shows inhibitory potency (IC_{50}) of the synthesized analogs along with that of valtrate (**1**) and the bioisostere **2**. All the synthesized analogs more potently inhibited Rev-export than the scaffold **2** and the 7-acetoxy analog **2c** exhibited comparable biological activity to **1**. Notably, 1-acetoxy analog **2b** inhibited Rev-export in a concentration-dependent manner and showed more potent efficacy than



Scheme 1. Synthesis of analogs (**2a**–**2c**) of 5,6-dihydrovaltrate. Reagents and conditions: (a) Amberlyst-15, MeOH, rt, 95%; (b) PhSSPh, *n*-Bu_3P, toluene, rt, 82%; (c) Oxone, acetone–H_2O, rt; (d) $(MeO)_3P$, MeOH, reflux, 97%, two steps; (e) Dess–Martin periodinane, CH_2Cl_2, rt; (f) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, −78 °C; (g) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 63%, three steps; (h) DIBAL, CH_2Cl_2, −78 °C; (i) TPAP, NMO, CH_2Cl_2, rt, 90%, two steps; (j) 10% HCl–THF, rt, 50%, recovery of **7** 31%; (k) isovaleric acid or AcOH, Im_2CO, DBU, CH_2Cl_2, 0 °C, 85% for **8a**, quant for **8b**; (l) BCl_3, CH_2Cl_2, 0 °C; (m) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, −78 °C; (n) *n*-butyryl chloride or AcCl, *i*-Pr_2NEt, CH_2Cl_2, 0 °C, 48% for **9a**, 50% for **9b**, three steps; (o) TBHP, $VO(acac)_2$, C_6H_6, rt; (p) isovaleric acid or AcOH, DEAD, PPh_3, C_6H_6, rt, 70% for **2a**, 71% for **2b**, 65% for **2c**, two steps.

Table 1
Inhibitory activity of valtrate analogs for nuclear export of Rev

Compd	R^1	R^2	R^3	IC_{50} (μM)
1	*i*-Val	Ac	*i*-Val	2.5
2	*i*-Val	Ac	*i*-Val	4.4
2a	*i*-Val	*n*-Bu	*i*-Val	3.3
2b	Ac	Ac	*i*-Val	1.0
2c	*i*-Val	Ac	Ac	2.6

i-Val: *i*-valeryl, *n*-Bu: *n*-butyryl.

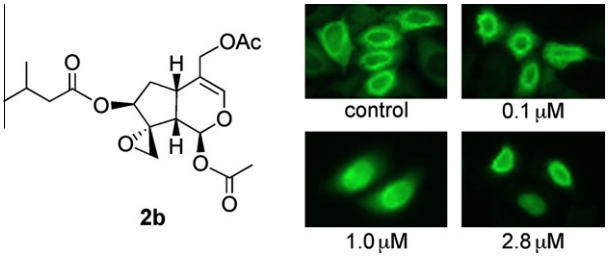


Figure 3. Inhibition for nuclear export of Rev by **2b**. In treatment with 0.1 μM of the analog **2b**, Rev protein is distributed in both nucleus and cytoplasm, while presence of 2.8 μM (MIC) of **2b** completely interrupts nuclear export of Rev.

valtrate (**1**) from the medicinal plant Valerianae Radix. In addition, the analog **2b** completely interrupted Rev-export at a concentration of 2.8 μM (MIC) as shown in Figure 3. In the previous paper, valtrate (**1**) was presumed to be linked to the Cys-529 residue in CRM1, the receptor of Rev, therefore the epoxy portion was deduced as the crucial function to exert the bioactivity of **1**.[3] These findings rationally support the present biological outcome that attenuation of steric hindrance around the epoxy portion of 5,6-dihydrovaltrate (**2**) results in enhancement of Rev-export inhibitory activity.

3. Conclusion

In summary, we developed the concise synthesis of 5,6-dihydrovaltrate (**2**), the bioisostere of valtrate (**1**) showing anti-HIV activity by inhibition for nuclear export of Rev, from the commercially available iridoid genipin (**3**). By ingenious use of this protocol, we analyzed steric influence of the substituents linked to the three hydroxyl groups by the synthesized three analogs (**2a**–**2c**). Consequently, attenuation of steric hindrance around the epoxy portion of **1** and **2** was revealed to enhance inhibitory potency for Rev-export. In addition to this finding, 1-acetoxy analog **2b** was disclosed as a promising Rev-export inhibitor superior to **1**. Our synthetic protocol enables to assemble analogs of valtrate (**1**), thereby exploration of more potent analogs is currently under investigation with reference to the present structure–activity relationship.

4. Experimental

4.1. General procedures

^{1}H NMR spectra were recorded on a JEOL JNM LA-500 (500 MHz) and NOESY spectra were recorded on a Varian Unity Inova 600 (600 MHz) spectrometer. All ^{1}H NMR data were referenced to the residual solvent signal (δ_H 7.26 ppm) as an internal standard. Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. 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. HPLC was performed on a JASCO PU2080 pump equipped with a JASCO UV2070 UV detector. Silica gel (Fuji Silysia Chemical, BW-200) and pre-coated thin-layer chromatography (TLC) plates (Merck, Kiesel gel 60F_{254}) were used for column chromatography and TLC, respectively. Spots on TLC plates were detected by spraying $Ce(SO_4)_2/H_2SO_4$ [$Ce(SO_4)_2{\cdot}nH_2O$ 10 g in 6.3% aqueous H_2SO_4 1.0 L] or acidic *para*-anisaldehyde solution (*para*-anisaldehyde 25 mL, *c*-H_2SO_4 25 mL, AcOH 5 mL, EtOH 425 mL) with subsequent heating. Distribution of HA-Rev was observed on an Olympus BX50 fluorescence microscope. HeLa cells were cultivated at 37 °C under 5% CO_2 atmosphere in SANYO MCO-17AIC CO_2 incubator.

4.2. Conversion from genipin (3) to phenylsulfide 4

A solution of **3** (1.19 g, 5.38 mmol) in MeOH (10.0 mL) was treated with Amberlyst-15 (3.51 g) at rt overnight. After filtration, removal of the solvent from the filtrate under reduced pressure gave a residue. The residue was purified by column chromatography (SiO_2 30 g, *n*-hexane/EtOAc = 2:1) to afford methyl acetal (1.21 g, 95% from **6**). To a solution of the methyl acetal (1.06 g, 4.41 mmol) in toluene (11.0 mL) was added PhSSPh (847 mg, 5.52 mmol) and tributylphosphine (1.32 mL, 5.31 mmol), then the reaction mixture was stirred at rt for 1 h. Removal of the solvent from the reaction mixture under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 30 g, *n*-hexane/EtOAc = 7:1) to afford **4** (1.20 g, 82%).

Compound **4**: colorless oil. $[\alpha]_D^{24}$ −12.0 (*c* 1.1, $CHCl_3$). IR ν_{max} (KBr) cm^{-1}: 1691, 1639. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.51 (1H, s, 3-H), 7.17–7.33 (5H, m, Ph), 5.68 (1H, br s, 7-H), 4.51 (1H, d, *J* = 8.2 Hz, 1-H), 3.82 (1H, d, *J* = 14.2 Hz, 10-Ha), 3.71 (3H, s, CO_2Me), 3.60 (1H, d, *J* = 14.2 Hz, 10-Hb), 3.56 (3H, s, OMe), 3.12 (1H, ddd, *J* = 8.2, 8.2, 7.3 Hz, 5-H), 2.75 (2H, m, 6-Ha, 9-H), 2.02 (1H, br dd, *J* = 16.0, 8.2 Hz, 6-Hb). FAB-MS (*m/z*): 333 $(M+H)^+$. HR FAB-MS (*m/z*): calcd for $C_{18}H_{20}O_4S$+H: 333.1161, found: 333.1150.

4.3. Conversion from 4 to allyl alcohol 5

To a solution of the **4** (1.11 g, 3.35 mmol) in acetone–H_2O [2:1(v/v), 10.0 mL] was added Oxone (2.23 g, 3.69 mmol) at −20 °C, then the reaction mixture was stirred at −20 °C for 1 h. After the reaction mixture was treated with aqueous saturated Na_2SO_3 for 10 min, the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave crude sulfoxide. A solution of the crude sulfoxide and $(MeO)_3P$ (0.57 mL, 5.04 mmol) in MeOH (33.3 mL) was heated under reflux for 5 h. The reaction mixture was cooled to rt and poured into aqueous saturated NaCl, then the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated 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 30 g, *n*-hexane/EtOAc = 2:1) to afford **5** (780 mg, 97% from **4**).

Compound **5**: colorless oil. $[\alpha]_D^{25}$ −34.6 (*c* 1.0, MeOH). IR ν_{max} (KBr) cm^{-1}: 3415, 1694, 1637. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.37 (1H, s, 3-H), 5.32 (1H, br s, 10-Ha), 5.26 (1H, br s, 10-Hb), 4.41 (1H, d, *J* = 6.6 Hz, 1-H), 4.36 (1H, dd, *J* = 6.4, 5.7 Hz, 7-H), 3.62 (3H, s, CO_2Me), 3.42 (3H, s, OMe), 3.11 (1H, ddd, *J* = 7.9, 7.5, 7.3 Hz, 5-H), 2.72 (1H, dd, *J* = 7.5, 6.6 Hz, 9-H), 2.06 (1H, ddd, *J* = 13.7, 7.3, 6.4 Hz, 6-Ha), 1.65 (1H, ddd, *J* = 13.7, 7.9, 5.7 Hz, 6-Hb). FAB-MS (*m/z*): 241 $(M+H)^+$. HR FAB-MS (*m/z*): calcd for $C_{12}H_{16}O_5$+H: 241.1076, found: 241.1060.

4.4. Conversion from 5 to 7α-MOM ether 6

A solution of **5** (750 mg, 3.13 mmol) in CH_2Cl_2 (15.5 mL) was treated with Dess–Martin periodinane (1.60 g, 3.75 mmol) at rt for 1 h.

After dilution of the reaction mixture with EtOAc, the whole was treated with aqueous saturated $NaHCO_3$ and aqueous saturated $Na_2S_2O_3$ for 30 min. The mixture was poured into aqueous saturated NaCl, then the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave crude ketone. A solution of the crude ketone in MeOH (15.0 mL) was treated with $NaBH_4$ (142 mg, 3.75 mmol) in the presence of $CeCl_3{\cdot}7H_2O$ (1.35 g, 3.75 mmol) at −78 °C for 30 min. The reaction mixture was poured into aqueous saturated NaCl, then the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave crude diol. To a solution of the diol in CH_2Cl_2 (31.2 mL) was successively added *i*-Pr_2NEt (2.78 mL, 15.7 mmol) and MOMCl (1.18 mL, 15.7 mmol) at 0 °C, then the reaction mixture was stirred at rt overnight. The reaction mixture was poured into aqueous saturated NaCl, then the whole was extracted with EtOAc. The EtOAc extract was successively washed with 5% HCl, aqueous saturated $NaHCO_3$, and aqueous saturated 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 20 g, *n*-hexane/EtOAc = 4:1) to afford **6** (560 mg, 63% from **5**).

Compound **6**: colorless oil. $[\alpha]_D^{24}$ 29.8 (*c* 0.95, MeOH). IR ν_{max} (KBr) cm^{-1}: 1692, 1637. ^1H NMR (500 MHz, $CDCl_3$), δ: 7.51 (1H, s, 3-H), 5.44 (1H, br s, 10-Ha), 5.37 (1H, br s, 10-Hb), 4.72 (2H, s, CH_3OCH_2O), 4.55 (1H, d, *J* = 7.3 Hz, 1-H), 4.41 (1H, m, 7-H), 3.72 (3H, s, CO_2CH_3), 3.57 (3H, s, CH_3O), 3.38 (3H, s, CH_3OCH_2O), 2.83 (1H, ddd, *J* = 7.3, 6.7, 6.7 Hz, 5-H), 2.69 (1H, ddd, *J* = 11.0, 6.7, 6.7 Hz, 6-Ha), 2.57 (1H, dd, *J* = 7.3, 7.3 Hz, 9-H), 1.36 (1H, *J* = 11.0, 6.7, 6.7 Hz, 6-Hb). FAB-MS (*m/z*): 285 $(M+H)^+$. HR FAB-MS (*m/z*): calcd for $C_{14}H_{20}O_6$+H: 285.1338, found: 285.1347.

4.5. Conversion from 6 to conjugated aldehyde 7

A solution of **6** (502 mg, 1.77 mmol) in CH_2Cl_2 (16.7 mL) was treated with DIBAL (1.77 mL, 1.5 M in toluene, 2.66 mmol) at −78 °C for 15 min. The reaction was quenched with MeOH, then the whole was stirred for 15 min. After the reaction mixture was treated with aqueous 1 N NaOH for 10 min, the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure gave crude allyl alcohol. A solution of the crude alcohol in CH_2Cl_2 (16.7 mL) was treated with TPAP (46.7 mg, 0.13 mmol) and NMO (2.15 g, 17.7 mmol) at rt for 48 h. After dilution of the reaction mixture with Et_2O, the whole was absorbed on SiO_2 column (5 g). The column was eluted with Et_2O, then the resulting eluate was concentrated under reduced pressure to give a residue, which was purified by column chromatography (SiO_2 15 g, C_6H_6/EtOAc = 5:1) to furnish aldehyde **7** (404 mg, 90% from **6**).

Compound **7**: colorless oil. $[\alpha]_D^{21}$ 28.6 (*c* 1.2, MeOH). IR ν_{max} (KBr) cm^{-1}: 1676, 1634. ^1H NMR (500 MHz, $CDCl_3$) δ: 9.30 (1H, s, 11-H), 7.25 (1H, s, 3-H), 5.44 (1H, br s, 10-Ha), 5.40 (1H, br s, 10-Hb), 4.75 (1H, d, *J* = 7.3 Hz, 1-H), 4.70 (2H, s, CH_3OCH_2O), 4.44 (1H, dd, *J* = 6.7, 6.7 Hz, 7-H), 3.61 (3H, s, CH_3O), 3.37 (3H, s, CH_3OCH_2O), 2.88 (1H, ddd, *J* = 7.3, 6.7, 6.7 Hz, 5-H), 2.67 (1H, ddd, *J* = 11.0, 6.7, 6.7 Hz, 6-Ha), 2.63 (1H, dd, *J* = 7.3, 7.3 Hz, 9-H), 1.44 (1H, ddd, *J* = 11.0, 6.7, 6.7 Hz, 6-Hb). FAB-MS (*m/z*): 255 $(M+H)^+$. HR FAB-MS (*m/z*): calcd for $C_{13}H_{18}O_5$+H: 255.1232, found: 255.1250.

4.6. Conversion from 7 to acyloxyacetals 8a and 8b

To a solution of **7** (394 mg, 1.55 mmol) in THF (15.8 mL) was added aqueous 10% HCl (7.8 mL) at 0 °C, then the reaction mixture was stirred at rt for 1 h. The reaction mixture was poured into aqueous saturated NaCl, then the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated 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 20 g, C_6H_6/EtOAc = 5:1) to provide a hemiacetal (160 mg, 50%, 31% recovery of **7**).

A solution of carbonyldiimidazole (1.25 g, 7.7 mmol) in CH_2Cl_2 (7.5 mL) was treated with isovaleric acid (0.9 mL, 8.2 mmol) and DBU (12.5 μL, 0.08 mmol) at 0 °C for 10 min. The mixture was added to a solution of the hemiacetal (100 mg, 0.42 mmol) in CH_2Cl_2 (0.75 mL), then the whole was stirred at rt overnight. After the reaction mixture was poured into aqueous saturated NaCl, the whole was extracted with EtOAc. The EtOAc extract was successively washed with 5% HCl, aqueous saturated $NaHCO_3$, and aqueous saturated 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 7 g, C_6H_6/EtOAc = 5:1) to furnish **8a** (114.5 mg, 85%).

A solution of carbonyldiimidazole (625 mg, 3.9 mmol) in CH_2Cl_2 (3.8 mL) was treated with acetic acid (0.25 mL, 4.3 mmol) and DBU (6.3 μL, 0.043 mmol) at 0 °C for 10 min. The mixture was added to a solution of the hemiacetal (50 mg, 0.21 mmol) in CH_2Cl_2 (0.38 mL) and the whole was stirred at rt overnight. Work-up in the same manner as preparation for **8a** gave a residue, which was purified by column chromatography (SiO_2 5 g, C_6H_6/EtOAc = 4:1) to furnish **8b** (58.5 mg, quant).

Compound **8a**: colorless oil. $[\alpha]_D^{21}$ +24.6 (*c* 1.1, MeOH). IR ν_{max} (KBr) cm^{-1}: 1759(sh), 1736, 1678, 1633. ^1H NMR (500 MHz, $CDCl_3$) δ: 9.31 (1H, s, 11-H), 7.19 (1H, s, 3-H), 6.13 (1H, d, *J* = 7.3 Hz, 1-H), 5.40 (1H, dd, *J* = 1.8, 1.8 Hz, 10-Ha), 5.36 (1H, dd, *J* = 1.8, 1.8 Hz, 10-Hb), 4.65 (1H, d, *J* = 10.4 Hz, CH_3OCH_2O), 4.63 (1H, d, *J* = 10.4 Hz, CH_3OCH_2O), 4.45 (1H, dddd, *J* = 6.7, 6.7, 1.8, 1.8 Hz, 7-H), 3.35 (3H, s, CH_3OCH_2O), 2.96 (1H, ddd, *J* = 7.3, 7.3, 7.3 Hz, 5-H), 2.76 (1H, dd, *J* = 7.3, 7.3 Hz, 9-H), 2.60 (1H, ddd, *J* = 13.4, 7.3, 6.7 Hz, 6-Ha), 2.28–2.31 (2H, m, $CO_2CH_2CH(CH_3)_2$), 2.14 (1H, m, $CO_2CH_2CH(CH_3)_2$), 1.69 (1H, *J* = 13.4, 7.3, 6.7 Hz, 6-Hb), 0.98 (3H, d, *J* = 6.7 Hz, $CO_2CH_2CH(CH_3)_2$), 0.97 (3H, d, *J* = 6.7 Hz, $CO_2CH_2CH(CH_3)_2$). FAB-MS (*m/z*): 325 $(M+H)^+$. HR FAB-MS (*m/z*): calcd for $C_{17}H_{24}O_6$+H: 325.1651, found: 325.1678.

Compound **8b:** colorless oil. $[\alpha]_D^{24}$ 28.9 (*c* 1.1, MeOH). IR ν_{max} (KBr) cm^{-1}: 1760(sh), 1738, 1674, 1631. ^1H NMR (500 MHz, $CDCl_3$) δ: 9.32 (1H, s, CHO), 7.19 (1H, s, 3-H), 6.10 (1H, d, *J* = 7.3 Hz, 1-H), 5.40 (1H, s, 10-Ha), 5.37 (1H, s, 10-Hb), 4.65 (2H, s, OCH_2OCH_3), 4.45 (1H, dd, *J* = 7.3, 6.7 Hz, 7-H), 3.35 (3H, s, OCH_2OCH_3), 2.97 (1H, ddd, *J* = 7.3, 7.3, 6.7, 5-H), 2.76 (1H, dd, *J* = 7.3, 6.7 Hz, 9-H). 2.61 (1H, ddd, *J* = 13.4, 7.3, 6.7 Hz, 6-Ha), 2.17 (3H, s, 1-OAc), 1.68 (1H, ddd, *J* = 13.4, 7.3, 7.3 Hz, 6-Hb). FAB-MS (*m/z*): 305 $(M+Na)^+$. HR FAB-MS (*m/z*): calcd for $C_{14}H_{18}O_6$+Na: 305.1001, found: 305.0998.

4.7. Conversion from 8a and 8b to diesters 9a–9c

A solution of **8a** (103.5 mg, 0.32 mmol) in CH_2Cl_2 (7.5 mL) was treated with BCl_3 (0.64 mL, 1.0 M in toluene, 0.64 mmol) at 0 °C for 10 min. The reaction mixture was poured into aqueous saturated NaCl, then the whole was extracted with EtOAc. The EtOAc extract was successively washed with aqueous saturated $NaHCO_3$ and aqueous saturated 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 5 g, C_6H_6/EtOAc = 2:1) to provide secondary alcohol (60.1 mg). A solution of the alcohol (58.0 mg, 0.21 mmol) in MeOH (4.0 mL) was treated with $NaBH_4$ (9.5 mg, 0.25 mmol) in the presence of $CeCl_3{\cdot}7H_2O$ (91.5 mg, 0.25 mmol) at −78 °C for 10 min. The reaction mixture was poured into aqueous saturated NaCl, then the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous

saturated NaCl and dried over $MgSO_4$. Removal of the solvent from the EtOAc extract under reduced pressure afforded crude diol (59.5 mg). A solution of the crude diol (21.0 mg) in CH_2Cl_2 (1.5 mL) was treated with *n*-butyryl chloride (31.0 μL, 0.30 mmol) in the presence of *i*-Pr_2NEt (65.0 μL, 0.38 mmol) at 0 °C for 10 min. The reaction mixture was poured into aqueous saturated NaCl, then the whole was extracted with EtOAc. The EtOAc extract was successively washed with 5% HCl, aqueous saturated $NaHCO_3$, and aqueous saturated 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 3 g, *n*-hexane/EtOAc = 2:1) to furnish **9a** (20.8 mg, 54% from **8a**).

A solution of the crude diol (26.0 mg) in CH_2Cl_2 (1.8 mL) was treated with AcCl (26.5 μL, 0.38 mmol) in the presence of *i*-Pr_2NEt (82.8 μL, 0.47 mmol) at 0 °C for 10 min. Work-up in the same manner as preparation for **9a** gave a residue, which was purified by column chromatography (SiO_2 3 g, *n*-hexane/EtOAc = 2:1) to furnish **9c** (21.1 mg, 48% from **8a**).

Removal of the MOM group of **8b** (48.0 mg, 0.17 mmol) was carried out in the same manner as preparation for **9a** gave a residue, which was purified by column chromatography (SiO_2 3 g, *n*-hexane/EtOAc = 3:2) to afford secondary alcohol (25.6 mg). $NaBH_4$ reduction of the alcohol (24.4 mg, 0.10 mmol) was conducted in the same manner as preparation for **9a** provided crude diol. A solution of the crude diol in CH_2Cl_2 (4.0 mL) was treated with AcCl (70.2 μL, 0.90 mmol) in the presence of *i*-Pr_2NEt (212 μL, 1.2 mmol) at 0 °C for 10 min. Work-up in the same manner as preparation for **9a** gave a residue, which was purified by column chromatography (SiO_2 3 g, *n*-hexane/EtOAc = 3:2) to furnish **9b** (22.8 mg, 50% from **8b**).

Compound **9a:** colorless oil. $[\alpha]_D^{24}$ 15.2 (*c* 0.92, MeOH). IR ν_{max} (KBr) cm^{-1}: 3314, 1758(sh), 1735, 1668. ^1H NMR (500 MHz, $CDCl_3$) δ: 6.42 (1H, s, 3-H), 6.04 (1H, d, *J* = 6.4 Hz, 1-H), 5.43 (1H, dd, *J* = 1.8, 1.8 Hz, 10-Ha), 5.32 (1H, dd, *J* = 1.8, 1.8 Hz, 10-Hb), 4.62 (1H, d, *J* = 12.1 Hz, 11-Ha), 4.50 (1H, dd, *J* = 6.1, 6.0 Hz, 7-H), 4.47 (1H, d, *J* = 12.1 Hz, 11-Hb), 2.79 (1H, dd, *J* = 6.4, 6.2 Hz, 9-H), 2.70 (1H, ddd, *J* = 6.2, 6.2, 6.2 Hz, 5-H), 2.34 (1H, ddd, *J* = 12.5, 6.2, 6.1 Hz, 6-Ha), 2.27–2.30 (2H, m, 1-OCOCH_2CH$(CH_3)_2$, 2.28 (2H, t, *J* = 7.6 Hz, 11-OCOC$H_2$$CH_2CH_3$), 2.13 (1H, m, 1-$OCOCH_2CH(CH_3)_2$), 1.67 (1H, ddd, *J* = 12.5, 6.2, 6.0 Hz, 6-Hb), 1.64 (2H, tq, *J* = 7.6, 7.3 Hz, 11-$OCOCH_2CH_2CH_3$), 0.98 (3H, d, *J* = 6.6 Hz, 1-$OCOCH_2$-$CH(CH_3)_2$), 0.97 (3H, d, *J* = 6.6 Hz, 1-$OCOCH_2CH(CH_3)_2$), 0.93 (3H, t, *J* = 7.3 Hz 11-$OCOCH_2CH_2CH_3$). FAB-MS (*m/z*): 353 $(M+H)^+$. HR FAB-MS (*m/z*): calcd for $C_{19}H_{28}O_6$+H: 353.1964, found: 353.1940.

Compound **9b:** colorless oil. $[\alpha]_D^{24}$ 19.2 (*c* 1.0, MeOH). IR ν_{max} (KBr) cm^{-1}: 3327, 1758(sh), 1731, 1672. ^1H NMR (500 MHz, $CDCl_3$) δ: 6.43 (1H, s, 3-H), 6.00 (1H, d, *J* = 6.4 Hz, 1-H), 5.42 (1H, dd, *J* = 2.2, 2.0 Hz, 10-Ha), 5.32 (1H, dd, *J* = 2.2, 2.0 Hz, 10-Hb), 4.62 (1H, d, *J* = 12.3 Hz, 11-Ha), 4.50 (1H, br dd, *J* = 6.8, 6.8 Hz, 7-H), 4.46 (1H, d, *J* = 12.3 Hz, 11-Hb), 2.79 (1H, dd, *J* = 7.3, 6.4 Hz, 9-H), 2.69 (1H, ddd, *J* = 7.7, 7.3, 6.6 Hz, 5-H) 2.34 (1H, ddd, *J* = 13.4, 6.8, 6.6 Hz, 6-Ha), 2.15 (3H, s, 1-OAc), 2.06 (3H, s, 11-OAc), 1.70 (1H, ddd, *J* = 13.4, 7.7, 6.8 Hz, 6-Hb). FAB-MS (*m/z*): 283 $(M+H)^+$. HR FAB-MS (*m/z*): calcd for $C_{14}H_{18}O_6$+H: 283.1182, found: 283.1172.

Compound **9c:** colorless oil. $[\alpha]_D^{24}$ 9.9 (*c* 0.90, MeOH). IR ν_{max} (KBr) cm^{-1}: 3311, 1759(sh), 1739, 1668. ^1H NMR (500 MHz, $CDCl_3$) δ: 6.42 (1H, s, 3-H), 6.03 (1H, d, *J* = 6.2 Hz, 1-H), 5.42 (1H, dd, *J* = 1.8, 1.8 Hz, 10-Ha), 5.32 (1H, dd, *J* = 1.8, 1.8 Hz, 10-Hb), 4.52 (1H, d, *J* = 12.1 Hz, 11-Ha), 4.50 (1H, br dd, *J* = 6.2, 6.1 Hz, 7-H), 4.46 (1H, d, *J* = 12.1 Hz, 11-Hb), 2.79 (1H, dd, *J* = 6.2, 6.2 Hz, 9-H), 2.70 (1H, ddd, *J* = 6.2, 6.2, 6.2 Hz, 5-H). 2.34 (1H, ddd, *J* = 13.1, 6.2, 6.2 Hz, 6-Ha), 2.27–2.30 (2H, m, 1-OCOCH_2CH$(CH_3)_2$), 2.13 (1H, m, 1-$OCOCH_2CH(CH_3)_2$), 2.06 (3H, s, 11-OCOCH_3), 1.71 (1H, ddd, *J* = 13.1, 6.2, 6.1 Hz, 6-Hb), 0.98 (3H, d, *J* = 6.7 Hz, 1-$OCOCH_2$-$CH(CH_3)_2$), 0.97 (3H, d, *J* = 6.7 Hz, 1-$OCOCH_2CH(CH_3)_2$). FAB-MS (*m/z*): 325 $(M+H)^+$. HR FAB-MS (*m/z*): calcd for $C_{17}H_{24}O_6$+H: 325.1651, found: 325.1622.

4.8. Preparation of bioisosteric analogs (2a–2c)

A solution of **9a** (17.6 mg, 0.05 mmol) in C_6H_6 (3.3 mL) was treated with *tert*-butyl hydroperoxide (45.3 μL, 3.3 M in toluene, 0.15 mmol) in the presence of $VO(acac)_2$ (1.3 mg, 0.005 mmol) for 20 min. The reaction mixture was poured into aqueous saturated $NaHCO_3$, then the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaCl and dried over Na_2SO_4. Removal of the solvent from the EtOAc extract under reduced pressure gave crude epoxide. To a solution of the crude epoxide in C_6H_6 (3.3 mL) was added PPh_3 (61.3 mg, 0.23 mmol), isovaleric acid (15.3 μL, 0.14 mmol), and diethyl azodicarboxylate (106 μL, 40% in toluene, 0.25 mmol) at 0 °C, then the reaction mixture was stirred at rt for 15 min. Removal of the solvent from the whole mixture under reduced pressure gave a residue, which was purified by column chromatography (SiO_2 2 g, *n*-hexane/EtOAc = 3:1) to furnish **2a** (15.8 mg, 70% from **9a**).

Epoxidation of **9b** (14.0 mg, 0.05 mmol) followed by Mitsunobu inversion were carried out in the same manner as preparation for **2a** gave a residue, which was purified by column chromatography (SiO_2 2 g, *n*-hexane/EtOAc = 7:3) to furnish **2b** (13.5 mg, 71% from **9b**). Epoxidation of **9c** (16.1 mg, 0.05 mmol) was carried out in the same manner as preparation for **2a** gave crude epoxide. To a solution of the crude epoxide in C_6H_6 (3.3 mL) was added PPh_3 (61.3 mg, 0.23 mmol), acetic acid (8.3 μL, 0.13 mmol), and diethyl azodicarboxylate (106 μL, 40% in toluene, 0.25 mmol) at 0 °C, then the reaction mixture was stirred at rt for 10 min. Work-up in the same manner as preparation for **2a** gave a residue, which was purified by column chromatography (SiO_2 2 g, *n*-hexane/EtOAc = 2:1) to furnish **2c** (12.4 mg, 65% from **9c**).

Compound **2a**: colorless oil. $[\alpha]_D^{24}$ −47.3 (*c* 0.48, MeOH). IR ν_{max} (KBr) cm^{-1}: 1760(sh), 1740, 1670. ^1H NMR (500 MHz, $CDCl_3$) δ: 6.51 (1H, s, 3-H), 5.83 (1H, d, *J* = 5.5 Hz, 1-H), 4.95 (1H, dd, *J* = 6.2, 5.4 Hz, 7-H), 4.67 (1H, d, *J* = 12.2 Hz, 11-Ha), 4.47 (1H, d, *J* = 12.2 Hz, 11-Hb), 3.05 (1H, d, *J* = 5.5 Hz, 10-Ha), 2.96 (1H, ddd, *J* = 8.6, 7.3, 6.1 Hz, 5-H), 2.82 (1H, d, *J* = 5.5 Hz, 10-Hb), 2.71 (1H, dd, *J* = 8.6, 5.5 Hz, 9-H), 2.31 (2H, t, *J* = 7.6 Hz, 11-OCOCH_2-CH_2CH_3), 2.25 (1H, ddd, *J* = 12.5, 6.2, 6.1 Hz, 6-Ha), 2.17–2.20 (4H, m, 1-OCOCH_2CH$(CH_3)_2$, 7-OCOCH_2CH$(CH_3)_2$), 2.00–2.10 (2H, m, 1-$OCOCH_2CH(CH_3)_2$, 7-$OCOCH_2CH(CH_3)_2$), 2.01 (1H, ddd, *J* = 12.5, 7.3, 5.4 Hz, 6-Hb), 1.66 (2H, sext-like, *J* = *ca.* 7.5 Hz, 11-$OCOCH_2CH_2CH_3$), 0.94–0.98 (15H, m, 1-$OCOCH_2CH(CH_3)_2$, 7-$OCOCH_2CH(CH_3)_2$, 11-$OCOCH_2CH_2CH_3$). FAB-MS (*m/z*): 475 $(M+Na)^+$. HR FAB-MS (*m/z*): calcd for $C_{24}H_{36}O_8$+Na: 475.2308, found: 475.2321.

Compound **2b:** colorless oil. $[\alpha]_D^{24}$ −56.9 (*c* 0.50, MeOH). IR ν_{max} (KBr) cm^{-1}: 1759(sh), 1738, 1671. ^1H NMR (500 MHz, $CDCl_3$) δ: 6.52 (1H, s, 3-H), 5.82 (1H, d, *J* = 4.8 Hz, 1-H), 4.97 (1H, dd, *J* = 6.2, 5.4 Hz, 7-H), 4.65 (1H, d, *J* = 12.2 Hz, 11-Ha), 4.47 (1H, d, *J* = 12.2 Hz, 11-Hb), 3.06 (1H, d, *J* = 4.9 Hz, 10-Ha), 2.98 (1H, ddd, *J* = 8.5, 6.1, 6.1 Hz, 5-H), 2.82 (1H, d, *J* = 4.9 Hz, 10-Hb), 2.72 (1H, dd, *J* = 8.5, 4.8 Hz, 9-H), 2.28 (1H, ddd, *J* = 13.4, 6.2, 6.1 Hz, 6-Ha), 2.18 (2H, d, *J* = 5.5 Hz, 7-OCOCH_2CH$(CH_3)_2$), 2.08 (6H, s, 1-OAc, 11-OAc), 2.06 (1H, m, 7-$OCOCH_2CH(CH_3)_2$), 1.99 (1H, ddd, *J* = 13.4, 6.1, 5.4 Hz, 6-Hb), 0.95 (6H, d, *J* = 5.5 Hz, 7-$OCOCH_2CH(CH_3)_2$). FAB-MS (*m/z*): 405 $(M+Na)^+$. HR FAB-MS (*m/z*): calcd for $C_{19}H_{26}O_8$+Na: .405.1525, found: 405.1511.

Compound **2c:** colorless oil. $[\alpha]_D^{24}$ −50.0 (*c* 0.49, MeOH). IR ν_{max} (KBr) cm^{-1}: 1760(sh), 1741, 1671. ^1H NMR (500 MHz, $CDCl_3$) δ: 6.52 (1H, s, 3-H), 5.84 (1H, d, *J* = 5.4 Hz, 1-H), 4.96 (1H, dd, *J* = 6.1, 5.5 Hz, 7-H), 4.64 (1H, d, *J* = 12.2 Hz, 11-Ha), 4.47 (1H, d, *J* = 12.2 Hz, 11-Hb), 3.07 (1H, d, *J* = 4.9 Hz, 10-Ha), 2.96 (1H, ddd, *J* = 8.5, 6.1, 6.1 Hz, 5-H), 2.82 (1H, d, *J* = 4.9 Hz, 10-Hb), 2.72 (1H,

dd, J = 8.5, 5.4 Hz, 9-H), 2.29 (1H, ddd, J = 13.5, 6.1, 6.1 Hz, 6-Ha), 2.18–2.20 (2H, m, 1-OCOCH_2CH(CH_3)$_2$), 2.05, 2.08 (3H each, both s, 7-OAc, 11-OAc), 2.00–2.10 (1H, m, 1-OCOCH_2CH(CH_3)$_2$), 1.99 (1H, ddd, J = 13.5, 6.1, 5.5 Hz, 6-Hb), 0.96 (6H, d, J = 6.7 Hz, 1-OCOCH_2CH(CH_3)$_2$). FAB-MS (m/z): 405 $(M+Na)^+$. HR FAB-MS (m/z): calcd for $C_{19}H_{26}O_8$+Na: 405.1525, found: 405.1526.

4.9. Evaluation for inhibitory activity for nuclear export of Rev protein

HeLa cells (1.0×10^5 cells) were maintained on coverslips in 24-well microplate with 1 mL of Dulbecco's MEM medium supplemented with 10% FBS at 37 °C in 5% CO_2 for 24 h. Transfection of pCG-HA-Rev (plasmid encoding HA-tagged Rev protein) and pCRRE/ΔRev (plasmid encoding Gag protein) plasmids into HeLa cells were performed using PolyFect® transfection reagent kit (QIAGEN) for 16 h according to the manufacturer's instructions. After the cells were washed, each solution of tested sample at an appropriate concentration in the medium containing 1% DMSO was inoculated and the whole was incubated at 37 °C for further 12 h. Cells were rinsed with cold D-PBS (−) twice and fixed with 4% formaldehyde/D-PBS (−) for 20 min. Then the cells were defatted with MeOH under shaking for 10 min and washed with cold D-PBS (−) thrice. After treatment with 10% FBS in Dulbecco's MEM medium for 30 min, the samples were incubated with anti-HA antibody (Roche) for 45 min followed by incubation with FITC-labeled anti-mouse IgG antibody (Vector) for 45 min. Localization of the HA-tagged Rev protein in the cells was examined under a fluorescence microscope, then image analysis was conducted by Scion image software (Scion) to determine Rev-export inhibitory activity.

Acknowledgments

We wish to thank Professor Tominori Kimura in College of Pharmaceutical Sciences, Ritsumeikan University for providing the plasmid of HA-Rev. This research was financially supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science. The authors are grateful to the Shorai Foundation for Science and Technology for financial support.

References and notes

1. Werstuck, G.; Zapp, M. L.; Green, M. R. *Chem. Biol.* **1996**, *3*, 129.
2. Kjems, J.; Askjaer, P. *Adv. Pharmacol.* **2000**, *48*, 251.
3. Murakami, N.; Ye, Y.; Kawanishi, M.; Aoki, S.; Kudo, N.; Yoshida, M.; Nakayama, E. E.; Shioda, T.; Kobayashi, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2807.
4. Tamura, S.; Shiomi, A.; Kaneko, M.; Ye, Y.; Yoshida, M.; Yoshikawa, M.; Kimura, T.; Kobayashi, M.; Murakami, N. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2555.
5. Tamura, S.; Kaneko, M.; Shiomi, A.; Yang, G. M.; Yamaura, T.; Murakami, N. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1837.
6. Tamura, S.; Fujitani, T.; Kaneko, M.; Kaneko, M.; Murakami, N. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3717.
7. Tamura, S.; Shimizu, N.; Fujiwara, K.; Kaneko, M.; Kimura, T.; Murakami, N. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2159.
8. Evans, D. A.; Andrews, G. C. *Acc. Chem. Res.* **1974**, *7*, 147.
9. Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625.
10. Sharpless, K. B.; Michaelson, R. C. *J. Am. Chem. Soc.* **1973**, *95*, 6136.
11. Mitsunobu, O. *Synthesis* **1981**, 1.
12. Kimura, T.; Hashimoto, I.; Yamamoto, T.; Nishikawa, M.; Fujisawa, J.-I. *Genes Cells* **2000**, *5*, 289.