Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 57–67

BIOORGANIC & MEDICINAL CHEMISTRY

Participation of the β-Hydroxyketone Part for Potent Cytotoxicity of Callystatin A, a Spongean Polyketide

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Received 22 June 2000; accepted 4 August 2000

Abstract—The participation of the β-hydroxyketone part of callystatin A in the potent cytotoxicity was analyzed through the analogue-syntheses and the assessment of their biological potencies. The ketonic carbonyl, the 19-hydroxyl, and the three asymmetric methyl groups located in the β-hydroxyketone part of callystatin A were revealed to contribute to the cytotoxic potency, respectively. Moreover, the α,β-unsaturated δ-lactone portion was shown to serve as a conclusive functional group for the cytotoxic activity.

Introduction

In the course of our search for new biologically active principles from marine organisms, we found the potent cytotoxic polyketide, callystatin A (**1**; $IC_{50} = 0.022$ nM against KB cells), from the marine sponge *Callyspongia truncata* and characterized its absolute stereostructure.[1,2] Subsequently, our first total synthesis of **1** confirmed the presented stereochemistry of **1**.[3] On the other hand, several antitumor antibiotics[4–7] designated as the leptomycin family have been isolated from several strains of *Streptomyces* sp., only the planar structures of which, similar to that of callystatin A (**1**), had been elucidated. Since leptomycin B was recently shown to inhibit nuclear export signal (NES)-dependent nucleus–cytoplasm transport of proteins,[8] we studied the stereochemistry of leptomycin B having 4-methyl-α,β-unsaturated δ-lactone and, by our first total synthesis, clarified that leptomycin B had the same absolute stereostructure as callystatin A (**1**).[9] Additionally, we revealed that these two congeners showed the same biological behavior.[10]

In view of the limited examination of the structure–activity relationships of the leptomycin family polyketides, we devoted ourselves to analyze the structure-requirement for the potent cytotoxicity of **1**, also aiming at the exploration of new promising analogues. In the preceding paper,[11] we examined the contribution of two conjugated diene parts to the potent cytotoxicity of **1**, which led to the outcome that the 5*R*-configuration and 8-ethyl residue were significantly responsible for the cytotoxic activity. Here, we present the participation of the β-hydroxyketone part containing four asymmetric centers in the biological potency of **1**.

Chemistry

At first, we executed the syntheses of analogue-I (**8**) and analogue-II (**12**), as illustrated in Scheme 1, and carried out an evaluation of their biological activity. These two analogues (**8** and **12**) respectively correspond to the partial structure from the C-11 position of **1** and that from C-5, which can ascertain the contribution of the β-hydroxyketone portion to the potent cytotoxicity of **1** in particular.

Lithium salt of dimsylcarbanion promoted Wittig condensation of acetaldehyde and an allylic tributylphosphonium bromide **7**,[12] which was the right-half segment in the total synthesis of callystatin A (**1**), to afford a conjugated diene with high selectivity. Dess–Martin oxidation[13] of the diene alcohol followed by deprotection of the *t*-butyldimethylsilyl (TBS) group with HF:pyridine[14] (5:1) furnished the desired analogue-I (**8**).

Taking into consideration the exploration of a more promising functional group than α,β-unsaturated δ-lactone in the case of the synthesis of analogue-II (**12**), we adopted a synthetic strategy in which the construction of the 6*E*-double bond was carried out in the final stage. Thus, segment C_7–C_{12} (**9**) was prepared from ethyl *R*-(*Z*)-2-ethyl-4-methyl-6-*O*-*para*-methoxybenzylhex-2-

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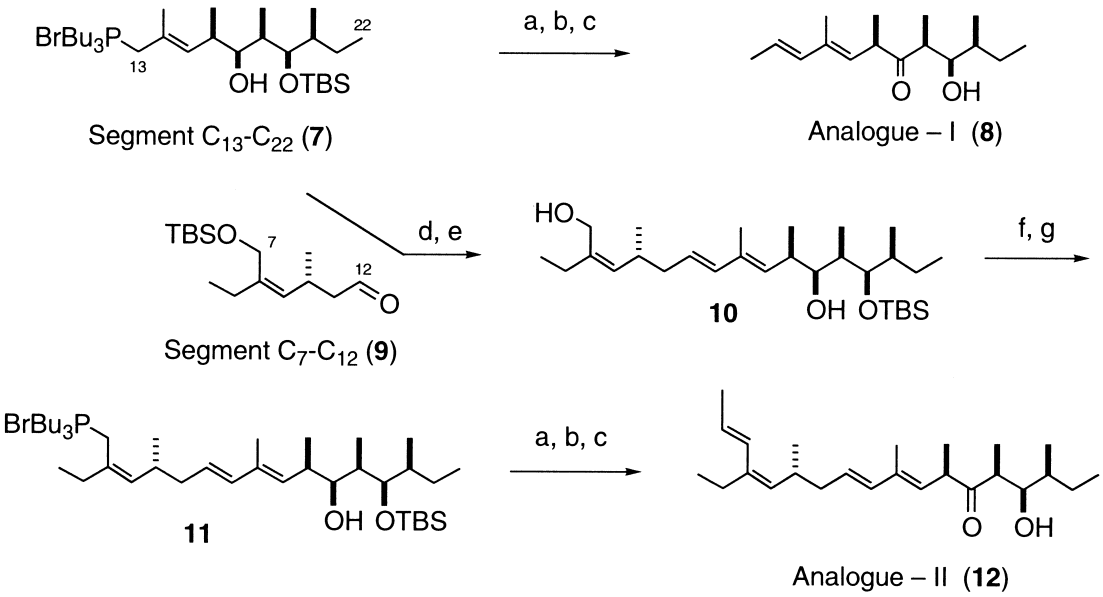
PII: S0968-0896(00)00220-0



Scheme 1. (a) CH_3CHO, $LiCH_2S(O)CH_3$, toluene; (b) Dess–Martin periodinane, CH_2Cl_2; (c) HF:Py (5:1), THF, **8**: 3 steps 58%, **12**: 3 steps 63%; (d) **9**, $LiCH_2S(O)CH_3$, toluene; (e) HF:Py (1:1), THF, 87% from **7**; (f) CBr_4, Ph_3P, 2,6-lutidine, CH_3CN; (g) Bu_3P, CH_3CN, 2 steps 94%.

enoate[15] by the following transformation: i) DIBAL reduction, ii) protection of the newly formed hydroxyl group as a TBS ether, iii) removal of the *p*-methoxybenzyl group by DDQ treatment,[16] and iv) Dess–Martin oxidation. The resulting segment C_7–C_{12} (**9**) was linked to **7** by the previous Wittig reaction to afford a di-*O*-TBS ether, which was submitted to selective deprotection with HF:pyridine (1:1) to give a diol **10** in 87% yield from **7**. Subsequent bromination by use of CBr_4 and Ph_3P in the presence of 2,6-lutidine[17] provided an allylic bromide, which was converted with tributylphosphorus to **11** in 94% yield for two steps. The same transformation from **7** to **8** was conducted for **11** to furnish analogue-II (**12**) in 63% yield for three steps. The cytotoxic potency of both analogues (**8** and **12**) was surprisingly lower relative to that of callystatin A (**1**) as shown in Table 1, even though the analogue-II (**12**) lacked only the α,β-unsaturated δ-lactone moiety.

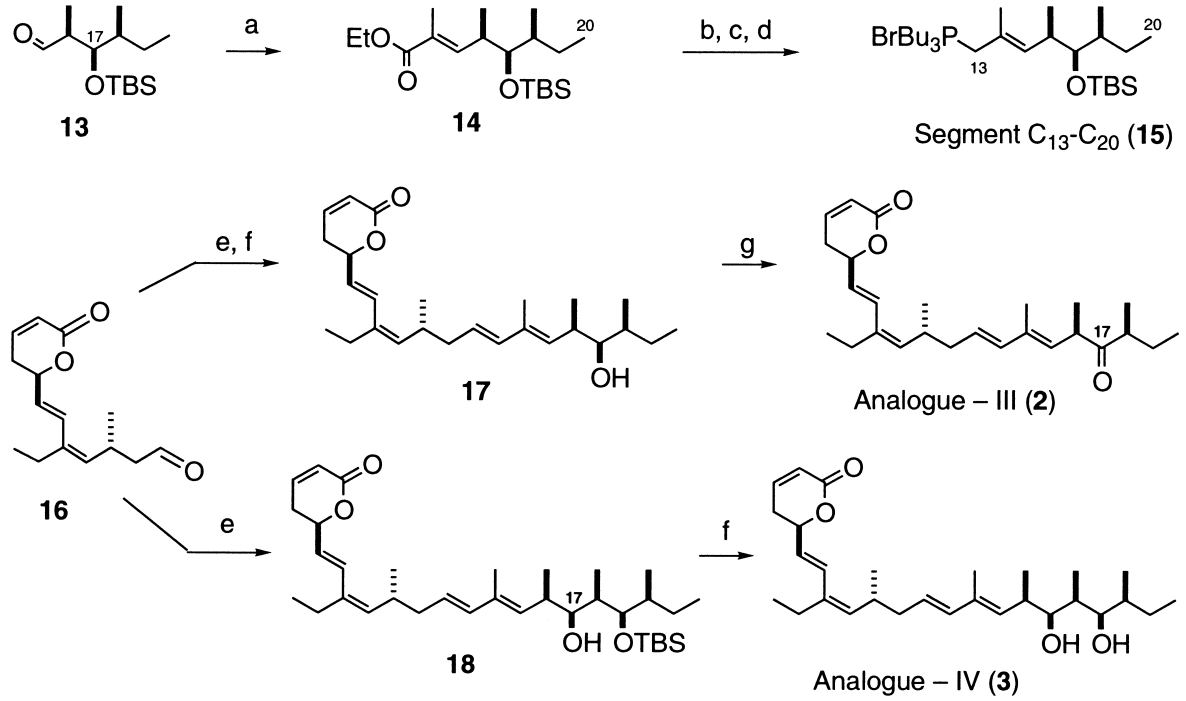
This finding directed us to elucidate the contribution of the β-hydroxyketone portion (the 17-ketonic carbonyl residue, the 19-hydroxyl, and the three methyl groups on C-16, C-18, and C-20 of callystatin A (**1**)) by comparison of the synthetic analogues possessing the α,β-unsaturated δ-lactone moiety. Hence, we designed the following three analogues (analogue-III (**2**), analogue-IV (**3**), and analogue-V (**4**)).

Table 1. Cytotoxic activity of callystatin A and its analogues against KB cells

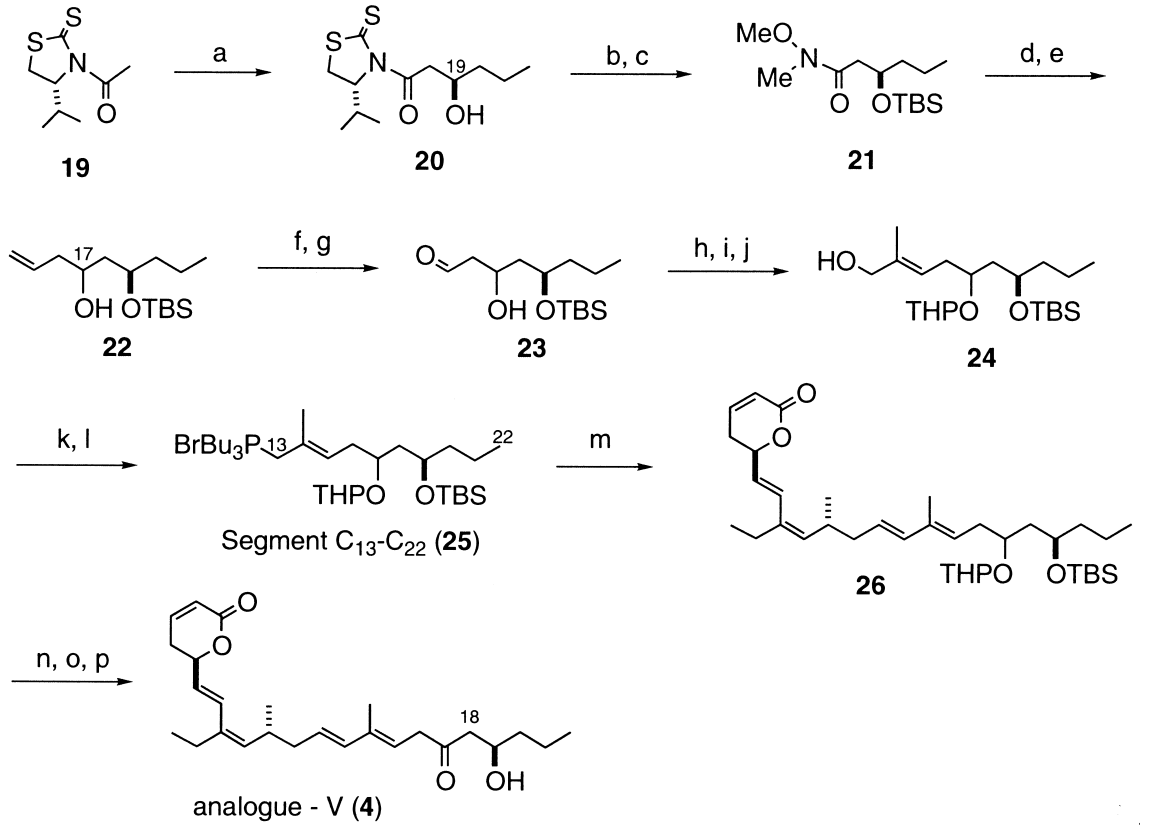
Compound	IC_{50} (M)
1	2.2×10^{-11}
2	1.3×10^{-8}
3	1.3×10^{-8}
4	7.5×10^{-8}
5	3.7×10^{-7}
6	3.5×10^{-8}
8	1.1×10^{-4}
12	1.7×10^{-5}
17	1.9×10^{-8}

Analogue-III (**2**) lacking the 19-hydroxyl and the terminal ethyl moieties was synthesized as depicted in Scheme 2. An optically active aldehyde **13**, which was prepared from *S*-amyl alcohol by five steps,[3] was conjugated with ethyl 2-(trimethylphosphoranylidene)propionate to give an α,β-unsaturated ester **14** in 97% yield. DIBAL reduction of this ester afforded an allyl alcohol, which was subjected to bromination and subsequent Bu_3P treatment to provide segment C_{13}–C_{20} (**15**) in 92% yield for three steps. After coupling of segment C_1–C_{12} (**16**) and **15** under the same conditions as mentioned above, the resulting *O*-silylated lactone was submitted to deprotection of the TBS group to afford an alcohol **17**. Finally, Dess–Martin oxidation of **17** furnished the desired analogue-III (**2**) in 97% yield. On the other hand, coupling of segment C_1–C_{12} (**16**) and segment C_{12}–C_{22} (**7**) followed by TBS-deprotection using HF pyridine provided analogue-IV (**3**), in which the ketonic carbonyl of **1** was exchanged with a β-hydroxyl group.

The synthesis of tridemethyl analogue-V (**4**) was achieved by use of asymmetric aldol condensation as a key reaction. Thus, optically active 1,3-thiazolidine-2-thione[18] was utilized as a chiral auxiliary to build up the 19*R*-asymmetric center as shown in Scheme 3. Butanal was conjugated with (*R*)-3-acetyl-4-isopropyl-1,3-thiazolidine-2-thione (**19**) in the presence of $Sn(OTf)_2$ to afford predominantly a secondary alcohol **20** with the desired stereochemistry quantitatively (19*R*:19*S* = 97:3). The chiral auxiliary was converted by the MeONHMe•HCl and Et_3N treatment to a Weinreb amide,[18] and then a hydroxyl group of which was protected as a TBS ether to afford **21** in 95% yield. The amide **21** was treated by allyl magnesium bromide and subsequent DIBAL reduction provided a vinylogous alcohol **22** as a mixture of diastereomers (17*R*:17*S* = 1:1) in 95% yield.



Scheme 2. (a) $EtOCOC(CH_3)=PPh_3$, toluene, 97%; (b) DIBAL-H, CH_2Cl_2; (c) CBr_4, Ph_3P, 2,6-lutidine, CH_3CN; (d) Bu_3P, CH_3CN, 3 steps 92%; (e) **15** or **7**, $LiCH_2S(O)CH_3$, toluene, **18**: 62%; (f) HF:Py (5:1), THF, **17**: 2 steps 58%, **3**: 98%; (g) Dess–Martin periodinane, CH_2Cl_2, 97%.



Scheme 3. (a) $Sn(OTf)_2$, 1-ethylpiperidine, ^{n}PrCHO, CH_2Cl_2, quant.; (b) MeONHMe-HCl, Et_3N, CH_2Cl_2; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2, 2 steps 95%; (d) allylmagnesium bromide, THF; (e) DIBAL-H, CH_2Cl_2, 2 steps 95%; (f) MC OsO_4, NMO, acetone–CH_3CN–H_2O; (g) $NaIO_4$, Et_2O–H_2O, 2 steps 95%; (h) $EtOCOC(CH_3)=PPh_3$, toluene, 94%; (i) DHP, PPTS, CH_2Cl_2; (j) DIBAL-H, CH_2Cl_2, 2 steps 85%; (k) CBr_4, Ph_3P, 2,6-lutidine, CH_3CN; (l) Bu_3P, CH_3CN, 2 steps 83%; (m) **16**, $LiCH_2S(O)CH_3$, toluene, 73%; (n) PPTS, ^{t}BuOH, 30 °C; (o) PCC, MS4A, CH_2Cl_2; (p) HF:Py (5:1), THF, 3 steps 94%.

Oxidative cleavage of the double bond of **22** with microencapsulated osmium tetroxide (MC OsO_4)[19] followed by $NaIO_4$ treatment afforded a hydroxyaldehyde **23** in 95% yield. Elongation of three carbons by Wittig reaction and subsequent protection of the hydroxyl group of **23** using dihydropyran (DHP) in the presence of pyridinium-*p*-toluenesulfonate (PPTS) provided an *O*-tetrahydropyanyl ether, which was subjected to DIBAL reduction to give an allyl alcohol **24** in 85% yield. The alcohol **24** was then converted to segment C_{13}–C_{22} (**25**) via bromination and tributylphosphine treatment in 83% yield. Coupling of the two segments (**16** and **25**) afforded a lactone **26** with a carbon-framework of tridemethyl analogue. After selective deprotection of the THP group with PPTS in *t*-BuOH, the resulting alcohol was oxidized with Dess–Martin periodinane to afford a ketone in unsatisfactory yield. In contrast, PCC oxidation coexistent with molecular sieve 4 Å[20] quantitatively gave the ketone, which was subsequently converted to analogue-V (**4**) by HF–pyridine treatment in 94% yield for three steps.

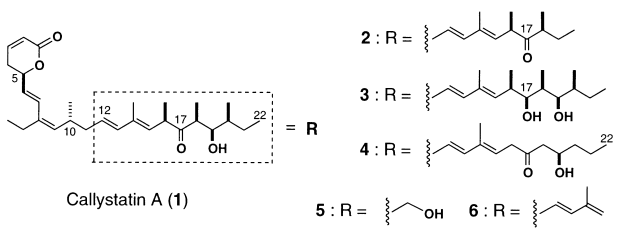


Chart 1. Chemical structures of callystatin A and its analogues.

Biological Results and Discussion

Table 1 summarizes the cytotoxic activity against KB cells of analogues-I–V (**8**, **12**, **2**, **3**, **4**) as well as the three congeners **5**,[15] **6**,[21] and **17** lacking the β-hydroxyketone portion. Judging from the cytotoxic potency of analogues-I (**8**) and II (**12**), the α,β-unsaturated δ-lactone moiety was proved to be the pharmacophore of callystatin A (**1**). Recently, the 2,3-dihydroderivative of leptomycin B with saturated δ-lactone was reported to show 6200-fold weaker cytotoxicity against HeLa cells.[22] Additionally, the Michael adduct to α,β-unsaturated δ-lactone of leptomycin B with CH_3NO_2 was shown to reduce markedly the inhibitory activity of leptomycin B on NES-dependent nucleus–cytoplasm transport of proteins.[8] These two observations would support our presumption. However, it is surprising that commercially available 5,6-dihydro-2*H*-pyran-2-one exhibited only similar efficacy ($IC_{50} = 4.5 \times 10^{-5}$ M) as compared with **8** and **12**. Furthermore, the two analogues (**5**, **6**) lacking the β-hydroxyketone portion showed moderate cytotoxicity. This evidence suggested that the β-hydroxyketone portion is requisite for the rigid binding of **1** to the receptor molecule leading to remarkable cytotoxic potency.

From the comparison of the cytotoxic activities of analogues-III (**2**), IV (**3**), and V (**4**) with that of **1**, each of the 17-ketonic carbonyl, the 19-hydroxyl, and the three methyl groups settled around the β-hydroxyketone moiety have similar contributions, while the participation of the three methyl residues may be slightly greater. Moreover, the comprehensive comparison of cytotoxic activity of the analogues shown in Chart 1 implies that each functional group in the β-hydroxyketone portion is involved in the rigid binding of **1** with the receptor molecule.

The ^1H NMR spectrum of **1** showed the proton signal due to the 17-hydroxyl group remained regardless of D_2O treatment.[23] In addition, the NOE enhancements among the protons located around the β-hydroxyketone function were observed in the NOESY spectrum as illustrated in Figure 1. These spectral features of **1** would disclose the predominant conformation mediated by intramolecular hydrogen bonding. The β-hydroxyketone moiety of **1** might function to form a stable complex with the receptor molecule through strong lipophilic interaction rather than hydrogen bonding with the amino acid residues of the receptor molecule. Hence, the combination of the α,β-unsaturated δ-lactone part and the β-hydroxyketone moiety including four asymmetric centers is assumed to result in the potent cytotoxic activity of callystatin A (**1**).

In conclusion, we have analyzed the contribution of the β-hydroxyketone part of callystatin A (**1**) to the potent cytotoxicity through the analogue-syntheses and the assessment of their biological potency. Based on the above-described findings, it was deduced that the α,β-unsaturated δ-lactone portion of **1** is bound to the domain in the receptor molecule and the rigid conformation of the β-hydroxyketone part plays an important role in forming a more stable complex between **1** and the receptor protein. Combining previous observations,[11] the participation of each functional group is outlined as shown in Figure 2.

Experimental

The following instruments were used to obtain physical data: a JASCO DIP-370 digital polarimeter for specific rotations; a Hitachi 330 spectrophotometer for UV spectra; a JASCO FT/IR-5300 infrared spectrometer for IR spectra; a JEOL JMS SX-102 mass spectrometer for FAB–MS and EI–MS; a JASCO J-720W circular dichroism spectrometer for CD spectra; a JEOL JNM LA-500

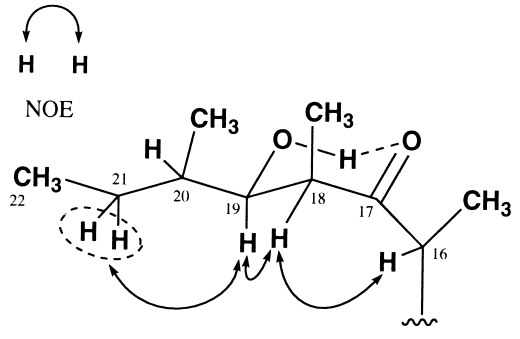


Figure 1. NOEs observed in NOESY spectrum of callystatin A.

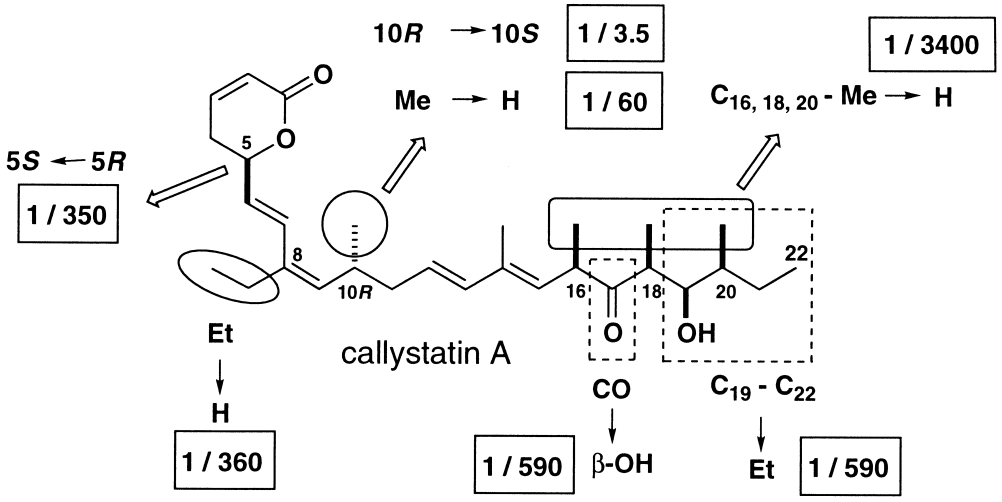


Figure 2. Structure requirement for potent cytotoxicity of callystatin A.

(500 MHz) and a JEOL JNM-AL300 (300 MHz) spectrometers for ^{1}H NMR spectra[24] [^{1}H NMR: $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard unless otherwise specified]. HPLC was performed using a Hitachi L-6000 pump equipped with Hitachi L-4000H UV detector. Silica gel (Merck 60–230 mesh) and pre-coated thin layer chromatography (TLC) plates (Merck, Kiesel gel, $60F_{254}$) were used for column chromatography and TLC. Spots on TLC plates were detected by spraying vanillin/H_2SO_4 (vanillin 5 g, *c*-H_2SO_4 95 mL) or acidic *p*-anisaldehyde solution (*p*-anisaldehyde 25 mL, *c*-H_2SO_4 25 mL, AcOH 5 mL, EtOH 425 mL) with subsequent heating.

Preparation of analogue-I (8). A solution of *n*-BuLi (1.53 M in *n*-hexane, 0.74 mL, 1.15 mmol) was added to a solution of DMSO (0.32 mL) in dry toluene (3.0 mL) at room temperature, then the whole was stirred for 45 min. A solution of **7** (141 mg, 0.23 mmol) in dry toluene (1.5 mL) was added to the solution of dimsylcarbanion at −78 °C, and stirred for 1 h. After adding CH_3CHO (0.10 mL, 2.26 mmol), the whole was stirred warming from −78 °C to 0 °C overnight. The reaction mixture was poured into saturated aqueous NH_4Cl, then the whole was extracted with Et_2O. The Et_2O extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the Et_2O extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 10 g, *n*-hexane:EtOAc = 10:1) to furnish a diene alcohol (51.3 mg, 61%). A solution of the alcohol (42.0 mg, 0.11 mmol) in dry CH_2Cl_2 (3.0 mL) was treated with Dess–Martin periodinane (154 mg, 0.34 mmol) at room temperature for 30 min. The reaction mixture was poured into a mixture of saturated aqueous $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$ (1:1), then the whole was extracted with Et_2O. The Et_2O extract was successively washed with saturated aqueous $Na_2S_2O_3$ and saturated aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the Et_2O extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 5 g, *n*-hexane:EtOAc = 30:1) to furnish a ketone (41.8 mg, quant.). To a solution of the ketone (14.1 mg, 0.039 mmol) in dry THF (6 mL) was added HF:pyridine (5:1, 2.5 mL) at 0 °C, then the whole was stirred at room temperature for 70 h. The reaction mixture was neutralized with $NaHCO_3$ at 0 °C, then poured into saturated aqueous NaCl. The whole was extracted with Et_2O, then the Et_2O extract was successively washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the Et_2O extract under reduced pressure gave a product, which was purified by HPLC [column; COSMOSIL 5SL (10 mm i.d. ×250 mm), mobile phase; *n*-hexane:EtOAc = 10:1, detection; UV (λ = 240 nm), flow rate; 5.0 mL/min] to furnish analogue-I (**8**, 9.3 mg, 96%). **8**: colorless oil, $[\alpha]_D$ −321° (*c* = 0.88, MeOH, 26 °C). UV λ_{max} (MeOH) nm (ε): 237 (18100), 296 (2400). CD (MeOH) nm (Δε): 334 (0), 300 (−21.4), 263 (0), 243 (+20.9), 210 (0). IR ν_{max} (KBr) cm^{-1}: 3491, 2965, 2928, 1707, 1456, 984, 963. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.85 (3H, t, *J* = 7.2 Hz, H-22), 0.90 (3H, d, *J* = 6.5 Hz, 20-CH_3), 1.06 (1H, m, H-21), 1.11 (3H, d, *J* = 7.2 Hz, 18-CH_3), 1.14 (3H, d, *J* = 6.9 Hz, 16-CH_3), 1.31–1.43 (2H, m, H-20, 21), 1.77 (3H, d, *J* = 6.7 Hz, H-11), 1.83 (3H, d, *J* = 1.2 Hz, 14-CH_3), 2.66 (1H, br, 19-OH), 2.86 (1H, dq, *J* = 4.2, 7.2 Hz, H-18), 3.57 (1H, dd, *J* = 7.0, 4.2 Hz, H-19), 3.64 (1H, dq, *J* = 10.2, 6.9 Hz, H-16), 5.12 (1H, d, *J* = 10.2 Hz, H-15), 5.69 (1H, dq, *J* = 15.7, 6.7 Hz, H-12), 6.03 (1H, dq, *J* = 15.7, 1.2 Hz, H-13). FAB-MS *m*/*z*: 253 $(M+H)^+$, FAB-HRMS *m*/*z*: calcd for $C_{16}H_{29}O_2$: 253.2167; found: 253.2179.

Preparation of 9. A solution of ethyl *R*-(*Z*)-2-ethyl-4-methyl-6-*O*-*para*-methoxybenzylhex-2-enoate (494 mg, 1.55 mmol) in dry CH_2Cl_2 (7.5 mL) was treated with diisobutylaluminum hydride (DIBAL-H) (1.5 M in toluene, 2.26 mL, 3.40 mmol) at −78 °C for 15 min. After the reaction mixture was diluted with Et_2O, saturated aqueous NaCl and 4.0 N aqueous NaOH were added. The reaction mixture was stirred vigorously until formation of precipitate was stopped, then the residue was removed by filtration. After drying the filtrate over Na_2SO_4, removal of solvent from the filtrate under reduced pressure gave a product, which was purified by column chromatography (SiO_2 12 g, *n*-hexane:

EtOAc = 5:1) to furnish an allyl alcohol (398 mg, 92%). A solution of the allyl alcohol (366 mg, 1.32 mmol) in dry CH_2Cl_2 (14.3 mL) was treated with *t*-butyldimethylsilyl chloride (301 mg, 2.00 mmol) and imidazole (166 mg, 2.43 mmol) at room temperature for 30 min. The reaction mixture was poured into saturated aqueous NH_4Cl, then the whole was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a TBS-ether. A solution of the TBS-ether in CH_2Cl_2:H_2O (20:1, 28 mL) was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (486 mg, 2.15 mmol) at room temperature for 30 min. The reaction mixture was poured into saturated aqueous $NaHCO_3$, then the whole was extracted with Et_2O. The Et_2O extract was washed with saturated aqueous $NaHCO_3$, then dried over $MgSO_4$. Removal of solvent from the Et_2O extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 12 g, *n*-hexane:EtOAc = 20:1) to furnish an alcohol (351 mg, 98%). A solution of the alcohol in dry CH_2Cl_2 (7.0 mL) was treated with Dess–Martin periodinane (1.1 g, 2.58 mmol) at room temperature for 30 min. Work-up in the same manner as preparation for **8** gave a segment C_7–C_{12} (**9**) (347 mg, quant.). Because of lability of this aldehyde, characterization was carried out using the alcohol prior to Dess–Martin oxidation. (*R*)-*Z*-1-*O*-*t*-Butyldimethylsilyl-2-ethyl-4-methyl-2-hexene-1,6-diol. Colorless oil, $[\alpha]_D$ −73° (*c* = 0.52, $CHCl_3$, 27 °C). IR ν_{max} (KBr) cm^{-1}: 3476, 2961, 2932, 1462, 1256, 1065. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.09, 0.10 (both 3H, s, 7-OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.91 (9H, s, 7-OSi (CH_3)$_2$ C($C\underline{H}_3$)$_3$), 0.97 (3H, d, *J* = 6.7 Hz, 10-CH_3), 1.02 (3H, t, *J* = 7.3 Hz, 8-$CH_2C\underline{H}_3$), 1.29 (1H, m, H-11), 1.70 (1H, m, H-11), 2.13 (2H, m, 8-$C\underline{H}_2CH_3$), 2.63 (1H, t, *J* = 6.7 Hz, 12-OH), 2.74 (1H, m, H-10), 3.56 (2H, m, H-12), 3.95 (1H, brd, *J* = 11.6 Hz, H-7), 4.32 (1H, d, *J* = 11.6 Hz, H-7), 5.01 (1H, d, *J* = 10.4 Hz, H-9). FAB-MS *m*/*z*: 273 $(M+H)^+$. FAB-HRMS *m*/*z*: calcd for $C_{15}H_{33}O_2Si$: 273.2250; found: 273.2240.

Conversion of 7 to 10. A solution of *n*-BuLi (1.53 M in *n*-hexane, 0.86 mL, 1.32 mmol) was added to a solution of DMSO (0.56 mL) in dry toluene (10 mL) at room temperature, then the whole was stirred for 60 min. A solution of **7** (410 mg, 0.66 mmol) and **9** (347 mg, 1.28 mmol) in dry toluene (10 mL) was added to the solution of dimsylcarbanion at −78 °C, then the whole was stirred warming from −78 °C to 0 °C overnight. Work-up in the same manner as preparation of **8** gave a product, which was purified by column chromatography (SiO_2 15 g, *n*-hexane:Et_2O = 20:1) to furnish a diene (349 mg, 89%). To a solution of the diene (44.8 mg, 0.075 mmol) in dry THF (1.0 mL) was added HF:pyridine (1:1) (0.3 mL) at −78 °C, then the whole was stirred for 70 h. Work-up in the same manner as preparation for **8** except for using EtOAc in extraction gave a product, which was purified by column chromatography (SiO_2 5 g, *n*-hexane:EtOAc = 5:1) to furnish **10** (31.6 mg, 87%). **10**: colorless oil, $[\alpha]_D$ +10.7° (*c* = 4.77, $CHCl_3$, 27 °C). IR ν_{max} (KBr) cm^{-1}: 3403, 2959, 2930, 2874, 1458, 1254, 1053, 1016. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.06, 0.08 (both 3H, s, 19-OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.82 (3H, d, *J* = 7.7 Hz, 20-CH_3), 0.83 (3H, d, *J* = 7.1 Hz, 18-CH_3), 0.86 (3H, t, *J* = 7.3 Hz, H-22), 0.90 (9H, s, 19-OSi(CH_3)$_2$C($C\underline{H}_3$)$_3$), 0.97 (3H, d, *J* = 6.6 Hz, 16-CH_3), 1.02 (6H, m, 10-CH_3, 8-$CH_2C\underline{H}_3$), 1.09 (1H, m, H-21), 1.52–1.56 (2H, m, H-20, 21), 1.72 (3H, d, *J* = 1.1 Hz, 14-CH_3), 1.98–2.08 (2H, m, H-11), 2.13 (4H, m, 8-$C\underline{H}_2CH_3$, H-18), 2.49–2.59 (2H, m, H-10, 16), 3.36 (1H, brd, *J* = 8.9 Hz, H-19), 3.63 (1H, dd, *J* = 4.7, 4.1 Hz, H-17), 4.06, 4.10 (both 1H, d, *J* = 11.5 Hz, H-7), 5.06 (1H, d, *J* = 9.8 Hz, H-15), 5.07 (1H, d, *J* = 9.6 Hz, H-9), 5.49 (1H, ddd, *J* = 15.5, 7.4, 7.3 Hz, H-12), 5.98 (1H, brd, *J* = 15.5 Hz, H-13). FAB-MS *m*/*z*: 467 $(M+H)^+$. FAB-HRMS *m*/*z*: calcd for $C_{28}H_{55}O_3Si$: 467.3920; found: 467.3935.

Conversion of 10 to 11. To a solution of **10** (6.7 mg, 0.014 mmol) in dry CH_3CN (0.8 mL) was added triphenylphosphine (10.1 mg, 0.038 mmol) at room temperature. To the reaction mixture were added 2,6-lutidine (0.5 μL, 0.0042 mmol) and CBr_4 (15.4 mg, 0.046 mmol) at 0 °C, then the whole was stirred at room temperature for 15 min. The reaction mixture was poured into saturated aqueous NaCl, then extracted with *n*-hexane:Et_2O (1:1). The organic layer was washed with saturated aqueous $NaHCO_3$, then dried over $MgSO_4$. Removal of solvent from the extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 2 g, benzene:Et_2O = 100:1) to furnish a bromide. A solution of bromide in dry CH_3CN (0.13 mL) was treated with tri-*n*-butylphosphine (9.9 μL, 0.040 mmol) at 0 °C for 12 h. The reaction mixture was evaporated in vacuo to afford a product, which was purified by column chromatography (SiO_2 2 g, $CHCl_3$:MeOH = 100:1) to furnish **11** (9.5 mg, 94%). Because of lability of this tributylphosphonium bromide, next transformation was carried out without further purification and characterization.

Preparation of analogue-II (12). A solution of *n*-BuLi (1.53 M in *n*-hexane, 0.025 mL, 0.038 mmol) was added to a solution of DMSO (0.0043 mL) in dry toluene (0.26 mL) at room temperature, then the whole was stirred for 45 min. A solution of CH_3CHO (2.2 μL, 0.050 mmol) and **11** (7.4 mg, 0.012 mmol) in dry toluene (0.7 mL) was added to the solution of dimsylcarbanion at −78 °C, then the whole was stirred warming from −78 °C to 0 °C overnight. Work-up in the same manner as preparation for **8** gave a product, which was purified by column chromatography (SiO_2 3 g, *n*-hexane:EtOAc = 20:1) to furnish an alcohol (4.3 mg, 74%). A solution of the alcohol in dry CH_2Cl_2 (1.0 mL) was treated with Dess–Martin periodinane (8.1 mg, 0.018 mmol) at room temperature for 90 min. Work-up in the same manner as preparation of **8** gave a ketone. To a solution of the ketone in dry THF (4.5 mL) was added HF:pyridine (5:1) (1.0 mL) at 0 °C, then the whole was stirred at room temperature for 36 h. Work-up in the same manner as preparation of **8** gave a product, which was purified by HPLC [column; YMC ODS-A (4.6 mm i.d. ×250 mm), mobile phase; MeOH:H_2O = 85:15, detection; UV (λ = 220 nm), flow rate; 1.0 mL/min] to furnish analogue-II (**12**, 2.8 mg, 85%).[25] **12**: colorless

oil, $[\alpha]_D$ −145° (c = 0.14, MeOH, 26 °C). IR ν_{max} (KBr) cm^{-1}: 3472, 2926, 1705, 1495, 1462, 1219. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.85 (3H, t, J = 7.4 Hz, H-22), 0.90 (3H, d, J = 6.6 Hz, 20-CH_3), 0.96 (3H, d, J = 6.8 Hz, 10-CH_3), 1.03 (3H, t, J = 7.4 Hz, 8-$CH_2C\underline{H}_3$), 1.08 (1H, m, H-21), 1.11 (3H, d, J = 7.3 Hz, 18-$C\underline{H}_3$), 1.14 (3H, d, J = 6.6 Hz, 16-CH_3), 1.32–1.42 (2H, m, H-20, 21), 1.79 (3H, dd, J = 6.6, 1.5 Hz, H-5), 1.81 (3H, s, 14-CH_3), 2.07 (2H, m, H-11), 2.17 (2H, m, 8-$C\underline{H}_2CH_3$), 2.66 (1H, m, H-10), 2.85 (1H, m, H-18), 3.57 (1H, m, H-19), 3.65 (1H, m, H-16), 5.01 (1H, d, J = 9.0 Hz, H-15), 5.11 (1H, d, J = 10.0 Hz, H-9), 5.62 (1H, ddd, J = 15.3, 7.5, 7.3 Hz, H-12), 5.71 (1H, dq, J = 15.6, 6.6 Hz, H-6), 6.01 (1H, d, J = 15.3 Hz, H-13), 6.31 (1H, brd, J = 15.6 Hz, H-7). FAB-MS m/z: 397 $(M+Na)^+$. FAB-HRMS m/z: calcd for $C_{25}H_{42}O_2Na$: 397.3082; found: 397.3108.

Conversion of 13 to 14. A solution of **13** (263 mg, 1.02 mmol) in dry toluene (5 mL) was added to a solution of ethyl-2-(trimethylphosphoranylidene)propionate (924 mg, 2.55 mmol) in dry toluene (5 mL) at room temperature, then the whole was stirred for 36 h. After diluting the reaction mixture with a mixture of *n*-hexane and EtOAc (30:1), the whole was filtered. Removal of solvent from the filtrate under reduced pressure gave a product, which was purified by column chromatography (SiO_2 15 g, *n*-hexane:EtOAc = 40:1) to furnish **14** (338 mg, 97%). **14**: colorless oil, $[\alpha]_D$ +23° (c = 1.41, $CHCl_3$, 26 °C). IR ν_{max} (KBr) cm^{-1}: 2959, 1709, 1651, 1464, 1387, 1256, 1103. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.05, 0.06 (both 3H, s, 17-OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.82 (3H, d, J = 6.7 Hz, 18-CH_3), 0.87 (3H, t, J = 7.4 Hz, H-20), 0.92 (9H, s, 17-OSi(CH_3)$_2$C($C\underline{H}_3$)$_3$), 0.99 (3H, d, J = 6.9 Hz, 16-CH_3), 1.15 (1H, m, H-19), 1.29 (3H, t, J = 6.9 Hz, 13-OCH$_2C\underline{H}_3$), 1.37–1.46 (2H, m, H-18, 19), 1.84 (3H, s, 14-CH_3), 2.66 (1H, m, H-16), 3.43 (1H, dd, J = 6.9, 2.7 Hz, H-17), 4.20 (2H, m, 13-OC$\underline{H}_2$CH$_3$), 6.61 (1H, d, J = 10.4 Hz, H-15). FAB-MS m/z: 343 $(M+H)^+$. FAB-HRMS m/z: calcd for $C_{19}H_{39}O_3Si$: 343.2669; found: 343.2664.

Conversion of 14 to segmemt C_{13}–C_{20} (15). A solution of **14** (320 mg, 0.94 mmol) in dry CH_2Cl_2 (9.4 mL) was treated with diisobutylaluminum hydride (1.5 M in toluene, 1.87 mL, 2.81 mmol) at −78 °C for 10 min. Work-up in the same manner as preparation for **11** gave a product, which was purified by column chromatography (SiO_2 10 g, *n*-hexane:EtOAc = 5:1) to furnish an allyl alcohol (256 mg, 92%). To a solution of the allyl alcohol (31.1 mg, 0.10 mmol) in dry CH_3CN (4.2 mL) were added triphenylphosphine (81.2 mg, 0.31 mmol) at room temperature, then 2,6-lutidine (3.8 μL, 0.031 mmol) and CBr_4 (139 mg, 0.41 mmol) were added at 0 °C. The whole was stirred at room temperature for 15 min. Work-up in the same manner as preparation of **11** except for using Et_2O in extraction gave a product, which was purified by column chromatography (SiO_2 10 g, *n*-hexane:Et_2O = 20:1) to furnish a bromide. A solution of the bromide in dry CH_3CN (1.0 mL) was treated with tri-*n*-butylphosphine (96.2 μL, 0.38 mmol) at 0 °C for 13 h. The reaction mixture was evaporated in vacuo to afford a product, which was purified by column chromatography (SiO_2 5 g, $CHCl_3$:MeOH = 40:1) to furnish **15** (58.5 mg, quant.). Because of lability of this tributylphosphonium bromide, next transformation was carried out without further purification and characterization.

Condensation of 15 and 16 giving 17. A solution of *n*-BuLi (1.54 M in *n*-hexane, 0.16 mL, 0.25 mmol) was added to a solution of DMSO (0.071 mL) in dry toluene (0.58 mL) at room temperature, then the whole was stirred for 45 min. A solution of **15** (58.5 mg, 0.10 mmol) and **16** (7.7 mg, 0.031 mmol) in dry toluene (1.5 mL) was added to the solution of dimsylcarbanion at −78 °C, then the whole was stirred warming from −78 °C to 0 °C overnight. Work-up in the same manner as preparation for **8** gave a product, which was purified by column chromatography (SiO_2 5 g, *n*-hexane:EtOAc = 4:1) to furnish a TBS ether (10.0 mg, 62%). To a solution of the TBS ether (4.9 mg, 0.0095 mmol) in dry THF (2.8 mL) was added HF:pyridine (5:1, 0.36 mL) at 0 °C, then the whole was stirred at room temperature for 48 h. Work-up in the same manner as preparation of **8** gave a product, which was successively purified by column chromatography (SiO_2 2 g, *n*-hexane:EtOAc = 5:2) and HPLC [column: COSMOSIL 5SL (10 mm i.d. ×250 mm), mobile phase: *n*-hexane:EtOAc = 5:1, detection: UV (λ = 250 nm), flow rate; 6.0 mL/min] to furnish an alcohol **17** (3.3 mg, 94%)[25] **17**: colorless oil, $[\alpha]_D$ +67° (c = 0.31, $CHCl_3$, 27 °C). IR ν_{max} (KBr) cm^{-1}: 3451, 2961, 2919, 1728, 1653, 1562, 1456, 1381, 1246. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.81 (3H, d, J = 6.5 Hz, 18-CH_3), 0.90 (3H, t, J = 7.4 Hz, H-20), 0.98 (3H, d, J = 6.5 Hz, 16-CH_3), 1.03 (3H, d, J = 6.5 Hz, 10-CH_3), 1.05 (3H, t, J = 7.4 Hz, 8-$CH_2C\underline{H}_3$), 1.15 (1H, m, H-19), 1.36–1.47 (2H, m, H-18, 19), 1.72 (3H, s, 14-CH_3), 2.08 (2H, dd, J = 6.5, 6.8 Hz, H-11), 2.19 (2H, m, 8-$C\underline{H}_2CH_3$), 2.47 (2H, m, H-4), 2.58–2.70 (2H, m, H-10, 16), 3.30 (1H, brd, J = 5.0 Hz, H-17), 4.99 (1H, ddd, J = 7.2, 7.1, 6.8 Hz, H-5), 5.14 (1H, d, J = 9.9 Hz, H-15), 5.26 (1H, d, J = 9.7 Hz, H-9), 5.49 (1H, ddd, J = 15.4, 7.5, 6.8 Hz, H-12), 5.76 (1H, dd, J = 15.9, 6.8 Hz, H-6), 6.02 (1H, d, J = 15.4 Hz, H-13), 6.06 (1H, dt, J = 9.9, 1.8 Hz, H-2), 6.65 (1H, d, J = 15.9 Hz, H-7), 6.90 (1H, ddd, J = 9.9, 4.4, 4.2 Hz, H-3). FAB-MS m/z: 401 $(M+H)^+$. FAB-HRMS m/z: calcd for $C_{26}H_{41}O_3$: 401.3055; found: 401.3087.

Preparation of analogue-III (2). A solution of **17** (2.4 mg, 0.006 mmol) was treated with Dess–Martin periodinane (8.1 mg, 0.018 mmol) in dry CH_2Cl_2 (2.4 mL) at room temperature for 30 min. Work-up in the same manner as preparation for **8** gave a product, which was purified by HPLC [column: COSMOSIL 5SL (10 mm i.d. ×250 mm), mobile phase: *n*-hexane:EtOAc = 3:1, detection: UV (λ = 250 nm), flow rate: 4.0 mL/min] to furnish analogue-III (**2**, 2.1 mg, 97%). **2**: colorless oil, $[\alpha]_D$ −74° (c = 0.17, $CHCl_3$, 26 °C). UV λ_{max} (MeOH) nm (ε): 243 (28600), 297 (2300). CD (MeOH) nm ($\Delta\varepsilon$): 326 (0), 299 (−14.2), 269 (0), 252 (+28.2), 233 (0), 223 (−9.6), 210 (−4.8). IR ν_{max} (KBr) cm^{-1}: 2920, 1730, 1704, 1653, 1460, 1381, 1242, 965. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.79 (3H, t, J = 7.3 Hz, H-20), 0.97 (3H, d, J = 6.7 Hz, 10-CH_3), 1.04 (3H, d, J = 6.7 Hz, 18-CH_3), 1.05 (3H, t, J = 7.2 Hz, 8-$CH_2C\underline{H}_3$), 1.13 (3H, d, J = 6.7 Hz, 16-CH_3), 1.30, 1.64 (both 1H, m, H-19), 1.79 (3H, d, J = 1.2 Hz, 14-CH_3), 2.08 (2H, t, J = 7.0 Hz,

H-11), 2.19 (2H, m, 8-C$\underline{H}_2$CH$_3$), 2.47 (2H, m, H-4), 2.56 (1H, sext.-like, J = ca. 7.0 Hz, H-18), 2.66 (1H, m, H-10), 3.62 (1H, dq, J = 10.0, 6.7 Hz, H-16), 4.98 (1H, ddd, J = 7.3, 7.2, 7.0 Hz, H-5), 5.20 (1H, d, J = 10.0 Hz, H-15), 5.25 (1H, d, J = 10.0 Hz, H-9), 5.55 (1H, ddd, J = 15.7, 8.0, 7.2 Hz, H-12), 5.76 (1H, dd, J = 16.0, 7.0 Hz, H-6), 6.02 (1H, brd, J = 15.7 Hz, H-13), 6.06 (1H, dt, J = 10.0, 1.7 Hz, H-2), 6.63 (1H, d, J = 16.0 Hz, H-7), 6.90 (1H, ddd, J = 10.0, 4.2, 4.0 Hz, H-3). FAB-MS m/z: 399 $(M+H)^+$. FAB-HRMS m/z: calcd for $C_{26}H_{39}O_3$: 399.2899; found: 399.2923.

Condensation of 7 and 16 giving 18. A solution of *n*-BuLi (1.54 M in *n*-hexane, 0.029 mL, 0.045 mmol) was added to a solution of DMSO (0.024 mL) in dry toluene (0.35 mL) at room temperature, then the whole was stirred for 45 min. A solution of **7** (21.2 mg, 0.034 mmol) and **16** (4.2 mg, 0.017 mmol) in dry toluene (0.9 mL) was added to the solution of dimsylcarbanion at −78 °C, then the whole was stirred warming from −78 °C to 0 °C overnight. Work-up in the same manner as preparation for **8** gave a product, which was purified by column chromatography (SiO_2 3 g, *n*-hexane:EtOAc = 4:1) to furnish **18** (6.0 mg, 62%). **18**: colorless oil, $[\alpha]_D$ +50.5° (c = 1.63, $CHCl_3$, 26 °C). IR ν_{max} (KBr) cm^{-1}: 3531, 2932, 1738, 1462, 1383, 1244, 1055, 963. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.06, 0.08 (both 3H, s, 19-OSi(C$\underline{H}_3$)$_2$C(CH$_3$)$_3$), 0.82 (3H, d, J = 6.6 Hz, 20-CH$_3$), 0.84 (3H, d, J = 7.1 Hz, 18-CH$_3$), 0.87 (3H, t, J = 7.5 Hz, H-22), 0.91 (9H, s, 19-OSi(CH$_3$)$_2$C(C$\underline{H}_3$)$_3$), 0.97 (3H, d, J = 6.6 Hz, 16-CH$_3$), 1.03 (3H, d, J = 6.5 Hz, 10-CH$_3$), 1.04 (3H, t, J = 7.5 Hz, 8-CH$_2$C$\underline{H}_3$), 1.09 (1H, m, H-21), 1.52–1.55 (2H, m, H-20, 21), 1.72 (3H, s, 14-CH$_3$), 2.07 (2H, q, J = 6.7 Hz, 8-C$\underline{H}_2$CH$_3$), 2.16–2.21 (3H, m, H-11, 18), 2.47 (2H, m, H-4), 2.58 (1H, m, H-16), 2.67 (1H, m, H-10), 3.36 (1H, brd, J = 9.1 Hz, H-19), 3.63 (1H, dd, J = 3.8, 3.9 Hz, H-17), 4.99 (1H, ddd, J = 7.5, 7.4, 6.7 Hz, H-5), 5.07 (1H, d, J = 9.9 Hz, H-15), 5.26 (1H, d, J = 9.9 Hz, H-9), 5.49 (1H, dt, J = 15.5, 7.2 Hz, H-12), 5.76 (1H, dd, J = 15.7, 6.7 Hz, H-6), 5.99 (1H, d, J = 15.5 Hz, H-13), 6.06 (1H, d, J = 9.7 Hz, H-2), 6.65 (1H, d, J = 15.7 Hz, H-7), 6.90 (1H, dt, J = 9.7, 4.0 Hz, H-3). FAB-MS m/z: 573 $(M+H)^+$. FAB-HRMS m/z: calcd for $C_{35}H_{61}O_4Si$: 573.4339; found: 573.4290.

Preparation of analogue-IV (3). To a solution of **18** (4.3 mg, 0.0075 mmol) in dry THF (2.2 mL) was added HF:pyridine (5:1, 0.29 mL) at 0 °C, then the whole was stirred at room temperature for 48 h. Work-up in the same manner as preparation for **8** gave a product, which was purified by HPLC [column: COSMOSIL 5SL (10 mm i.d. ×250 mm), mobile phase: *n*-hexane:EtOAc = 1:1, detection; UV (λ = 250 nm), flow rate: 4.0 mL/min] to furnish analogue-IV (**3**, 3.1 mg, 98%).[25] **3**: colorless oil, $[\alpha]_D$ +51.5° (c = 0.15, $CHCl_3$, 26 °C). UV λ_{max} (MeOH) nm (ε): 227 (29600). CD (MeOH) nm (Δε): 350 (+0.2), 267 (+1.5), 248 (+11.3), 235 (0), 226 (−8.8), 210 (−0.2). IR ν_{max} (KBr) cm^{-1}: 3468, 2962, 1723, 1456, 1381, 1244. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.84 (3H, d, J = 7.0 Hz, 20-CH$_3$), 0.85 (3H, t, J = 7.5 Hz, H-22), 0.95 (3H, d, J = 6.7 Hz, 18-CH$_3$), 0.97 (3H, d, J = 6.7 Hz, 16-CH$_3$), 1.00 (1H, m, H-21), 1.04 (3H, t, J = 7.5 Hz, 8-CH$_2$C$\underline{H}_3$), 1.05 (3H, d, J = 7.2 Hz, 10-CH$_3$), 1.33–1.38 (2H, m, H-20, 21), 1.74 (3H, s, 14-CH$_3$), 1.81 (1H, m, H-18), 2.08 (2H, dd, J = 7.7, 7.0 Hz, H-11), 2.21 (2H, m, 8-C$\underline{H}_2$CH$_3$), 2.48 (2H, m, H-4), 2.65 (2H, m, H-10, 16), 3.44 (1H, dd, J = 8.5, 2.6 Hz, H-19), 3.49 (1H, d-like, J = 9.8 Hz, H-17), 4.99 (1H, ddd, J = 7.1, 7.0, 6.7 Hz, H-5), 5.06 (1H, d, J = 10.1 Hz, H-15), 5.26 (1H, d, J = 9.5 Hz, H-9), 5.50 (1H, dt, J = 15.7, 7.2 Hz, H-12), 5.76 (1H, dd, J = 15.7, 6.7 Hz, H-6), 5.99 (1H, d, J = 15.7 Hz, H-13), 6.06 (1H, dt, J = 9.8, 1.8 Hz, H-2), 6.65 (1H, d, J = 15.7 Hz, H-7), 6.90 (1H, dt, J = 9.8, 4.1 Hz, H-3). FAB-MS m/z: 459 $(M+H)^+$. FAB-HRMS m/z: calcd for $C_{29}H_{47}O_4$: 459.3474; found: 459.3411.

Condensation of 19 and butanal giving 20. To a solution of $Sn(OTf)_2$ (2.67 g, 6.4 mmol) in dry CH_2Cl_2 (10.6 mL) was successively added *N*-ethylpiperidine (0.93 mL, 6.8 mmol) and a solution of (*R*)-3-acetyl-4-isopropyl-1,3-thiazolidine-2-thione (**19**, 767 mg, 3.78 mmol) in dry CH_2Cl_2 (6.5 mL) at −40 °C, then the whole was stirred for 4 h. After cooling the reaction mixture to −78 °C, butanal (0.67 mL, 7.6 mmol) was added to the reaction mixture and the whole was stirred for 10 min. The reaction mixture was poured into H_2O, then the whole was extracted with Et_2O. The Et_2O extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the Et_2O extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 20 g, *n*-hexane:EtOAc = 6:1) to furnish **20** (1.04 g, quant.). **20**: colorless oil, $[\alpha]_D$ −405° (c = 1.21, $CHCl_3$, 22 °C). IR ν_{max} (KBr) cm^{-1}: 3470, 2963, 1690, 1366, 1304, 1260, 1165. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.94 (3H, t, J = 7.1 Hz, H-22), 0.99, 1.07 (both 3H, d, J = 6.9 Hz, -CH(C$\underline{H}_3$)$_2$), 1.35–1.62 (4H, m, H-20, 21), 2.37 (1H, m, C$\underline{H}$(CH$_3$)$_2$), 2.86 (1H, brs, 19-OH), 3.04 (1H, dd, J = 11.5, 1.0 Hz, SC$\underline{H}_2$CHN), 3.13 (1H, dd, J = 17.6, 9.2 Hz, H-18), 3.53 (1H, dd, J = 11.5, 7.9 Hz, SC$\underline{H}_2$CHN), 3.63 (1H, dd, J = 17.6, 2.3 Hz, H-18), 4.14 (1H, m, H-19), 5.17 (1H, ddd, J = 7.9, 6.4, 1.0 Hz, SCH$_2$C$\underline{H}$N). FAB-MS m/z: 276 $(M+H)^+$. FAB-HRMS m/z: calcd for $C_{12}H_{22}O_2NS_2$: 276.1092; found: 276.1099.

Conversion of 20 to 21. To a solution of *N*,*O*-dimethylhydroxylamine hydrochloride (7.08 g, 72.7 mmol) in dry CH_2Cl_2 (7.2 mL) was added Et_3N (9.6 mL) at 0 °C, then the whole was stirred at room temperature for 1 h. The reaction mixture was added to **20** (953 mg, 3.46 mmol) at room temperature and further stirred for 21 h. After cooled to 0 °C, the reaction mixture was poured into 5% aqueous HCl and extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 18 g, *n*-hexane:EtOAc = 2:3) to furnish a Weinreb amide (576 mg, 95%). To a solution of the amide in dry CH_2Cl_2 (16 mL) were added *tert*-butyldimethylsilyl trifluoromethanesulfonate (1.5 mL, 6.6 mmol) and 2,6-lutidine (0.95 mL, 8.2 mmol) at −20 °C, then the whole was stirred warming from −20 °C to room temperature over 20 min. The reaction mixture was poured into 0.5 N aqueous $NaHSO_4$, then the whole was extracted with EtOAc. The EtOAc extract was successively washed with saturated aqueous $NaHCO_3$ and saturated

aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 20 g, *n*-hexane:EtOAc = 5:1) to furnish **21** (950 mg, quant.). **21**: colorless oil, $[\alpha]_D$ −19.4° (c = 1.54, $CHCl_3$, 22 °C). IR ν_{max} (KBr) cm^{-1}: 2959, 2936, 1671, 1464, 1385, 1254. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.03, 0.06 (both 3H, s, 19-OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.86 (9H, s, 19-OSi(CH_3)$_2$C ($C\underline{H}_3$)$_3$), 0.91 (3H, t, J = 7.2 Hz, H-22), 1.36 (2H, m, H-21), 1.49 (2H, m, H-20), 2.39 (1H, dd, J = 14.7, 5.3 Hz, H-18), 2.71 (1H, dd, J = 14.7, 5.0 Hz, H-18), 3.17 (3H, s, 17-NCH_3), 3.69 (3H, s, 17-$NOCH_3$), 4.23 (1H, m, H-19). FAB-MS m/z: 290 $(M+H)^+$. FAB-HRMS m/z: calcd for $C_{14}H_{32}O_3NSi$: 290.2152; found: 290.2132.

Grignard reaction followed by DIBAL reduction giving 22. To a solution of **21** (339 mg, 1.17 mmol) in dry THF (3.8 mL) was added allylmagnesium bromide (3.69M in THF, 1.59 mL, 5.85 mmol) at 0 °C, then the whole was stirred for 30 min. After adding 0.5 N aqueous $NaHSO_4$ to the reaction mixture at 0 °C, the whole was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 10 g, *n*-hexane:EtOAc = 25:1) to furnish a ketone (317 mg, quant.). A solution of the ketone (107 mg, 0.39 mmol) in dry CH_2Cl_2 (1.9 mL) was treated with diisobutylaluminum hydride (1.0 M in toluene, 0.39 mL, 0.39 mmol) at −78 °C for 10 min. then the whole was stirred for 30 min. After adding 0.1 N aqueous Rochelle salt (5.0 mL) to the reaction mixture at 0 °C, the whole was stirred at 0 °C for 30 min. The reaction mixture was extracted with EtOAc and the EtOAc extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 10 g, *n*-hexane:EtOAc = 20:1) to furnish **22** (100 mg, 95%). **22**: colorless oil, IR ν_{max} (KBr) cm^{-1}: 3476, 2934, 2861, 1464, 1256, 1067. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.04, 0.09 (both 3H, s, 19-OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.89 (9H, s, 19-OSi(CH_3)$_2$C ($C\underline{H}_3$)$_3$), 0.90 (3H, t, J = 7.3 Hz, H-22), 1.30 (2H, m, H-21), 1.46–1.66 (4H, m, H-18, 20), 2.15–2.28 (2H, m, H-16), 3.81, 3.92 (both 1/2H, m, H-17 or 19), 4.00 (total 1 H, m, H-17 or 19), 5.10 (2H, m, H-14), 5.84 (1H, m, H-15). FAB-MS m/z: 273 $(M+H)^+$. FAB-HRMS m/z: calcd for $C_{15}H_{33}O_2Si$: 273.2250; found: 273.2249.

Conversion of 22 to 23. A solution of **22** (52.3 mg, 0.19 mmol) in H_2O:acetone:CH_3CN (1:1:1, 1.9 mL) was treated with microencapsulated osmium tetroxide (24.6 mg, 0.0095 mmol) in the presence of *N*-methylmorpholine *N*-oxide (1.9 mg, 0.95 mmol) at room temperature for 7 days. After filtration, removal of solvent from the filtrate under reduced pressure gave a triol. A solution of triol in Et_2O:H_2O (1:1, 21 mL) was treated with $NaIO_4$ (203 mg, 0.95 mmol) at room temperature for 1.5 h. The reaction mixture was poured into H_2O, then the whole was extracted with Et_2O. The Et_2O extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the Et_2O extract under reduced pressure gave an aldehyde **23** (50.2 mg, 95%). Because of lability of this aldehyde, next transformation was carried out without further purification and characterization.

Conversion of 23 to 24. A solution of **23** (47.5 mg, 0.17 mmol) in dry toluene (3.0 mL) was treated with ethyl 2-(trimethylphosphoranylidene)propionate (161 mg, 0.45 mmol) at room temperature, then the whole was stirred for 22 h. After diluting the reaction mixture with a mixture of *n*-hexane and EtOAc (30:1), the whole was filtered. Removal of solvent from the filtrate under reduced pressure gave a product, which was purified by column chromatography (SiO_2 8 g, *n*-hexane:EtOAc = 6:1) to furnish an ester. A solution of the ester in dry CH_2Cl_2 (2.0 mL) was treated with dihydropyran (29 μL, 0.36 mmol) in the presence of PPTS (43 mg, 0.17 mmol) at room temperature for 3 h. The reaction mixture was poured into saturated aqueous $NaHCO_3$, then the whole was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 5 g, *n*-hexane:EtOAc = 15:1) to furnish a tetrahydropyranylated alcohol (72 mg, 94%). To a solution of the tetrahydropyranylated alcohol in dry CH_2Cl_2 (3.0 mL) was added diisobutylaluminum hydride (1.0 M in toluene, 0.49 mL, 0.49 mmol) at −78 °C, then the whole was stirred for 20 min. After adding 1 N aqueous NaOH (5 mL) to the reaction mixture at 0 °C, the whole was stirred at 0 °C for 30 min. The reaction mixture was extracted with EtOAc and the resulting organic layer was washed with saturated aqueous NaCl, then dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a product, which was purified by column chromatography (SiO_2 5 g, *n*-hexane:EtOAc = 5:1) to furnish **24** (55 mg, 85%). **24**: colorless oil, IR ν_{max} (KBr) cm^{-1}: 3470, 2938, 2857, 1076, 1024. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.04, 0.05 (both 3H, s, 19-OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.87–0.90 (12H, m, 19-OSi(CH_3)$_2$C($C\underline{H}_3$)$_3$, H-22), 1.67, 1.68 (both 3/2H, s, 14-CH_3), 1.30–1.82 (12H, m, H-18, 20, 21, 17-OCH($C\underline{H}_2$)$_3$$CH_2$O-), 2.22–2.45 (2H, m, H-16), 3.45–3.54 (1H, m, H-19), 3.62–3.97 (3H, m, H-17, 17-OCH(CH_2)$_3$-$C\underline{H}_2$O-), 4.00 (2H, s, H-13), 4.59, 4.63, 4.70 (total 1H, m, 17-OC$\underline{H}$(CH_2)$_3$$CH_2$O-), 5.43, 5.50, 5.51 (total 1H, m, H-15). FAB-MS m/z: 401 $(M+H)^+$. FAB-HRMS m/z: calcd for $C_{22}H_{45}O_4Si$: 401.3087; found: 401.3095.

Conversion of 24 to 26. Triphenylphosphine (33.0 mg, 0.13 mmol) was added to a solution of **24** (16.9 mg, 0.042 mmol) in dry CH_3CN (1.7 mL) at room temperature. To the reaction mixture were added 2,6-lutidine (1.4 μL, 0.013 mmol) and CBr_4 (56.7 mg, 0.17 mmol) at 0 °C, then the whole was stirred at room temperature for 10 min. Work-up in the same manner as preparation for **11** gave a product, which was purified by column chromatography (SiO_2 2 g, *n*-hexane:EtOAc = 25:1) to furnish a bromide. A solution of the bromide in dry CH_3CN (1.1 mL) was treated with tri-*n*-butylphosphine (0.026 mL, 0.105 mmol) at room temperature for 2 h in

the dark. The reaction mixture was evaporated in vacuo to afford a product, which was purified by column chromatography (SiO_2 5 g, $CHCl_3$:MeOH = 40:1–10:1) to furnish **25** (25.0 mg, 83%). A solution of *n*-BuLi (1.54 M in *n*-hexane, 0.042 mL, 0.064 mmol) was added to a solution of DMSO (0.023 mL) in dry toluene (1.0 mL) at −78 °C, then the whole was stirred for 45 min. A solution of **25** (23.0 mg, 0.032 mmol) and **16** (5.0 mg, 0.020 mmol) in dry toluene (1.0 mL) was added to the solution of dimsylcarbanion at −78 °C, then the whole was stirred warming from −78 °C to 0 °C overnight. Work-up in the same manner as preparation for **8** except for using EtOAc in extraction gave a product, which was purified by column chromatography (SiO_2 2 g, *n*-hexane:EtOAc = 4:1) to furnish **26** (9.0 mg, 73%). **26**: colorless oil, IR ν_{max} (KBr) cm^{-1}: 2957, 1734, 1262, 1026. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.04–0.10 (6H, m, 19-OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.87–0.89 (12H, m, 19-OSi(CH_3)$_2$ C($C\underline{H}_3$)$_3$, H-22), 0.96 (3H, d, J = 6.4 Hz, 10-CH_3), 1.05 (3H, t, J = 7.4 Hz, 8-$CH_2C\underline{H}_3$), 1.40–1.63 (12H, m, H-18, 20, 21, 17-OCH($C\underline{H}_2$)$_3$$CH_2$O-), 1.70 (3H, s, 14-$CH_3$), 2.07 (2H, m, H-11), 2.18 (2H, m, 8-$C\underline{H}_2CH_3$), 2.35 (2H, m, H-16), 2.47 (2H, m, H-4), 2.66 (1H, s, H-10), 3.49 (1H, m, H-17), 3.74–3.88 (3H, m, H-19, 17-OCH(CH_2)$_3$-$C\underline{H}_2$O-), 4.67 (1H, m, 17-OC$\underline{H}$(CH_2)$_3$$CH_2$O-), 4.99 (1H, dt, J = 6.9, 7.3 Hz, H-5), 5.26 (1H, d, J = 9.9 Hz, H-9), 5.32–5.52 (2H, m, H-12, 15), 5.76 (1H, dd, J = 15.8, 6.9 Hz, H-6), 6.05 (1H, d, J = 16.0 Hz, H-13), 6.06 (1H, d, J = 9.7 Hz, H-2), 6.64 (1H, d, J = 15.8 Hz, H-7), 6.90 (1H, dt, J = 9.7, 4.3 Hz, H-3). FAB-MS m/z: 637 $(M+Na)^+$. FAB-HRMS m/z: calcd for $C_{37}H_{62}O_5SiNa$: 637.4264; found: 637.4258.

Preparation of analogue-V (4). A solution of **26** (6.2 mg, 0.010 mmol) in dry *t*-BuOH (2.0 mL) was treated with PPTS (10.0 mg, 0.040 mmol) at 30 °C for 18 h. Work-up in the same manner as tetrahydropyranylation of **23** to **24** gave a product, which was purified by column chromatography (SiO_2 3 g, *n*-hexane:EtOAc = 5:1–2:1) to furnish an alcohol (2.2 mg, quant. based on transformed starting material) along with the recovered **26** (3.8 mg, 61%). A solution of the alcohol (2.2 mg, 0.0042 mmol) in CH_2Cl_2 (2.0 mL) was treated with PCC (5.0 mg, 0.023 mmol) in the presence of MS 4 Å (15 mg) at room temperature for 10 min. After filtration through Florisil column using Et_2O as eluent, removal of solvent from the filtrate under reduced pressure gave a ketone. To a solution of the ketone in dry THF (0.76 mL) was added HF:pyridine (5:1, 0.42 mL) at 0 °C, then the whole was stirred at room temperature for 3 h. Work-up in the same manner as preparation of **8** gave a product, which was purified by HPLC [column: COSMOSIL 5SL-II (10 mm i.d. ×250 mm), mobile phase: *n*-hexane: EtOAc = 2:1, detection: UV (λ = 250 nm), flow rate: 5.0 mL/min] to furnish analogue-V (**4**, 1.4 mg, 94%).[25] **4**: colorless oil, $[\alpha]_D$ +60° (c = 0.02, $CHCl_3$, 25 °C). UV λ_{max} (MeOH) nm (ε): 242 (28500), 297 (2300). CD (MeOH) nm (Δε): 338 (0), 286 (−1.0), 274 (0), 247 (+10.3), 234 (0), 225 (−5.7), 212 (0), 210 (+0.2). IR ν_{max} (KBr) cm^{-1}: 3426, 2926, 1729, 1458, 1381, 1262, 1096, 1024. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.92 (3H, t, J = 7.3 Hz, H-22), 0.98 (3H, d, J = 6.5 Hz, 10-CH_3), 1.05 (3H, t, J = 7.2 Hz, 8-$CH_2C\underline{H}_3$), 1.20–1.57 (4H, m, H-20, 21), 1.71 (3H, s, 14-CH_3), 2.09 (2H, q, J = 7.2 Hz, 8-$C\underline{H}_2CH_3$), 2.19 (2H, m, H-11), 2.46 (2H, m, H-4), 2.53 (1H, dd, J = 17.6, 9.3 Hz, H-18), 2.63 (1H, dd, J = 17.6, 2.8 Hz, H-18), 2.65 (1H, m, H-10), 2.95 (1H, d, J = 2.8 Hz, 19-OH), 3.27 (2H, d, J = 7.6 Hz, H-16), 4.05 (1H, m, H-19), 4.98 (1H, ddd, J = 6.6, 6.9, 7.2 Hz, H-5), 5.24 (1H, d, J = 9.3 Hz, H-9), 5.50 (1H, t, J = 7.6 Hz, H-15), 5.56 (1H, dt, J = 15.5, 7.2 Hz, H-12), 5.75 (1H, dd, J = 16.5, 7.2 Hz, H-6), 6.05 (1H, ddd, J = 9.6, 2.1, 1.7 Hz, H-2), 6.08 (1H, d, J = 15.5 Hz, H-13), 6.62 (1H, d, J = 16.5 Hz, H-7), 6.89 (1H, dt, J = 9.6, 4.1 Hz, H-3). FAB-MS m/z: 437 $(M+Na)^+$. FAB-HRMS m/z: calcd for $C_{26}H_{38}O_4Na$: 437.2668; found: 437.2681.

Bioassay

Human epidermoid carcinoma KB cells were cultured in RPMI 1640 medium with 0.58 mg/mL of glutamine, 50 μg/mL of kanamycin sulfate, supplemented with 10% fetal bovine serum. Cytotoxic activity was measured by means of MTT colorimetric assay performed in 96-well plates. Equal numbers of cells (2×10^4) were inoculated into each well with 100 μL of the culture medium, then a 100 μL solution of each tested compound was added to each well. After 72 h incubation under 5% of CO_2 atmosphere at 37 °C, 25 μL of MTT solution (2 mg/mL in PBS) was added to each well and incubated for further 3 h. The medium was removed by aspiration, then the resulting formazan was dissolved with 200 μL of DMSO. The percentage of cell growth inhibition was calculated from the absorbance at 540 nm and IC_{50} value was determined by linear interpolation from the inhibition curve.

Acknowledgements

The authors are grateful to the Naito Foundation, the Houansha Foundation, and the Ministry of Education, Science, Sports, and Culture of Japan for financial support.

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21. Compounds **5** and **6** were synthesized by use of 5-*R* segment C_1–C_6[3] according to the procedure of preparation for 5-*epi*-congener.[2] Full preparative details and characteristics in the syntheses of **5** and **6** will be presented in our forthcoming full paper.
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23. D_2O treatment was conducted in d_6-DMSO and the integration for signals ascribable to hydroxyl protons in **1** and **4** was unchanged after 24 h.
24. Since all compounds except for analogues II–V (**2**, **3**, **4**, **12** and **17**) contained a small amount of stereoisomer at C-8, the NMR data are assigned with respect to each major isomer in significant proportion. Numbering used for assignment of NMR data is in accordance with that of callystatin A (**1**).
25. The yields were determined by taking into account that minor 8-*E* isomers given by Still–Wittig condensation were removed with final HPLC separation.