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Participation of the Conjugated Diene Part for Potent Cytotoxicity of Callystatin A, a Spongean Polyketide

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Abstract—5-*epi*, 10-*epi*, 8-Deethyl, and 10-demethyl analogues of callystatin A, a potent cytotoxic spongean polyketide, were synthesized to elucidate structure-requirement for cytotoxic potency. Inversion of the asymmetric center at C-10 in callystatin A minimally affected the activity, while lack of the 10-methyl group in callystatin A decreased cytotoxicity. In addition, the C-5 epimer and the 8-deethyl analogue of callystatin A showed weaker cytotoxicity.

Introduction

During the course of our search for new biologically active principles from marine organisms, we found an extremely potent cytotoxic polyketide, callystatin A (**1**, $IC_{50} = 0.022$ nM against KB cells), from the marine sponge *Callyspongia truncata*.[1] We determined the absolute stereostructure of **1** by the physicochemical properties and comparative analysis of the CD spectra with those of the synthesized model compounds.[2] In addition, we achieved the first total synthesis of **1** and confirmed the absolute stereostructure of **1**.[3] The planar structure of callystatin A (**1**), having an α,β-unsaturated-δ-lactone, two conjugated dienes, and a β-hydroxyketone moiety, was very alike those of several anti-tumor antibiotics (e.g., leptomycin,[4] kazusamycin,[5] anguinomycin,[6] leptofuranin[7]) previously isolated from *Streptomyces* sp. As for these anti-tumor antibiotics, only planar structures have been elucidated. Among them, leptomycin B (**2**) was recently shown to inhibit nuclear export signal (NES)-dependent transport of proteins from the nucleus to cytoplasm through prevention of direct binding between NES and the chromosome maintenance region 1 (CRM1) protein.[8] Hence, we undertook to clarify the absolute stereostructure of leptomycin B (**2**) and succeeded in establishing it by the first total synthesis.[9] As a result, it was found that callystatin A (**1**) possessed the same stereostructure as leptomycin B (**2**). Additionally, we revealed that callystatin A (**1**) also exhibited the same biological properties as **2**.[10]

Although some other related anti-tumor antibiotics designated as the leptomycin family were found from *Streptomyces* sp. as mentioned above, their structure–activity relationships have not so far been investigated because of ambiguous stereostructures. Thus, we explored the structure–activity relationship of callystatin A (**1**) in order to analyze the structure-requirement for the potent cytotoxicity of **1** and the inhibitory activity of NES-dependent export of nuclear protein. This paper deals with the participation of two conjugated diene parts from C-5 to C-15 in the cytotoxic activity of **1** (Chart 1).

Chemistry

First of all, our interest was focused on the contribution of the two asymmetric centers (C-5 and C-10) to the potent cytotoxicity of **1**, since three conjugated chromophores (an α,β-unsaturated-δ-lactone and two conjugated dienes) of **1** were shown to be characteristically oriented to indicate an intense CD spectrum.[2] Thus, 5-*epi*- (**20**) and 10-*epi*-callystatin A (**12**) were synthesized and evaluated for their cytotoxicity. According to the synthetic protocol of **1**, 10-*epi*-callystatin A (**12**) was synthesized from the condensation of segment C_1–C_6 (**3a**), segment C_7–C_{12} (**4b**), and segment C_{13}–C_{22} (**10**) as shown in Scheme 1. The segment C_7–C_{12} (**4b**) was prepared as shown in Scheme 2. Namely, the known alcohol **13b** was converted by *p*-methoxybenzyl (PMB) ether protection and subsequent *n*-Bu_4NF treatment to a primary alcohol **14b**. Swern oxidation[11] of **14b** followed by Still–Wittig reaction[12] gave a conjugated *Z*-ester **15b**

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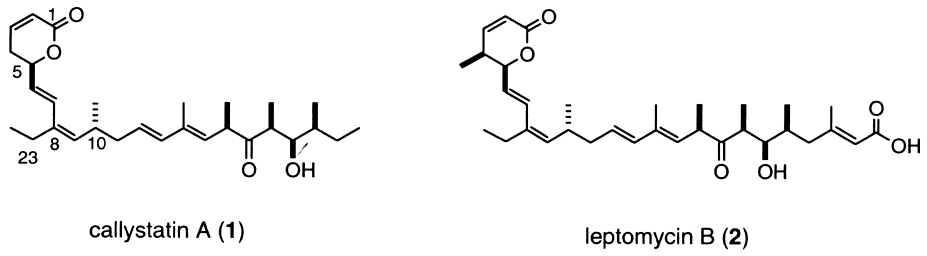


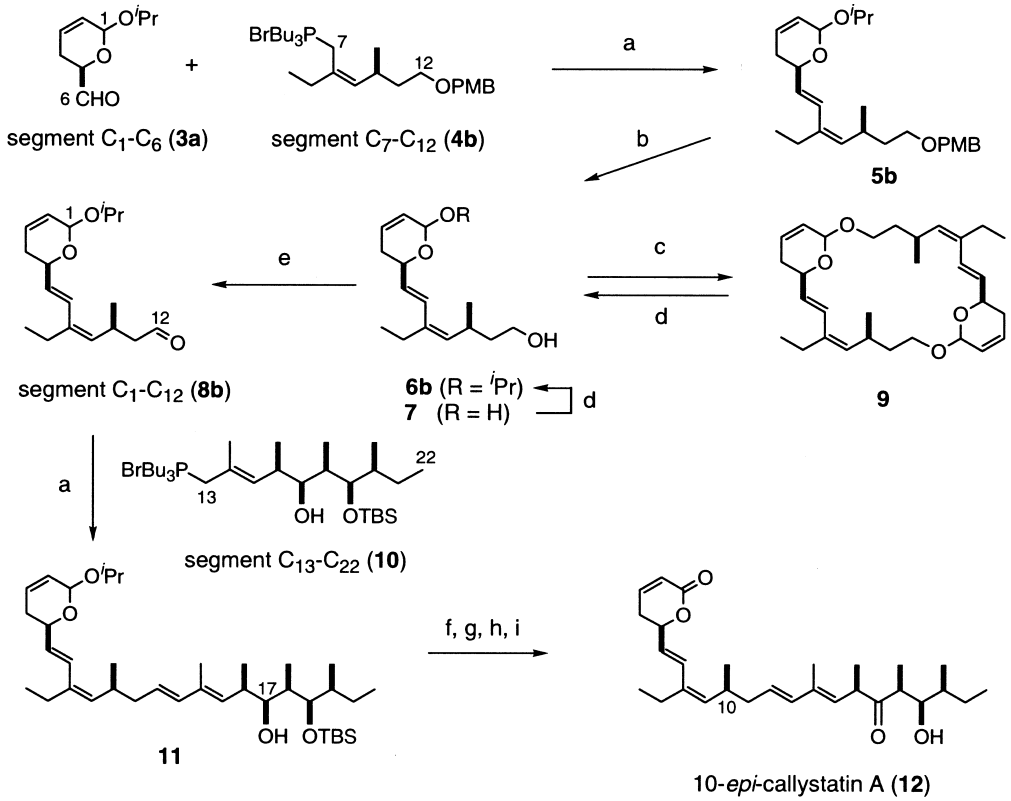
Chart 1.

predominantly (Z:E = 16:1). The olefinic geometry was established by the NOE enhancements observed between H-9 and H-23. The ester **15b** was successively submitted to diisobutylaluminum hydride (DIBAL-H) reduction and bromination using CBr_4 and PPh_3 in the presence of 2,6-lutidine[13] to provide an allylic bromide. Finally, treatment of this bromide with Bu_3P furnished the desired tributylphosphorus ylide **4b**.

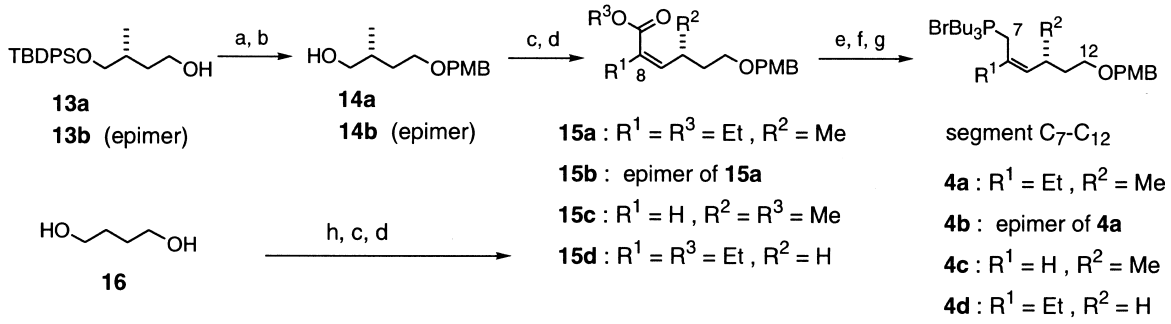
The two segments (**3a** and **4b**) were coupled in the presence of $LiCH_2S(O)CH_3$ to afford a 6-*E* conjugated diene (**5b**) with complete stereoselectivity.[14] After deprotection of the PMB group in **5b** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ),[15] the resulting alcohol **6b** was subjected to Swern oxidation to give a segment C_1–C_{12} (**8b**). A second Wittig reaction using allylic tributylphosphorus ylide (**10**) also constructed a 12,14-conjugated diene portion selectively to give **11** in moderate yield. Tetrapropylammonium perruthenate (TPAP) oxidation[16] of **11** followed by hydrolytic cleavage of the isopropyl acetal moiety gave a keto-lactol, which was further oxidized by Ag_2CO_3–Celite[17] to construct an α,β-unsaturated δ-lactone moiety. Finally, removal of the *t*-butyldimethylsilyl (TBS) group by HF:pyridine (5:1) treatment[18] furnished 10-*epi*-callystatin A (**12**).

In the course of synthesis of **12**, the removal of the PMB group of **5b** partly involved hydrolysis of the acetal portion to afford a diol (**7**) together with **6b**. In addition, the dimeric macrocyclic acetal (**9**) was also formed during SiO_2 column separation of **6b**, secondarily. Both **7** and **9** were, therefore, converted to the acetal **6b** by treatment with pyridinium *p*-toluenesulfonate (PPTS) in isopropanol. However, the reaction on a large scale reduced the yield of the desired alcohol **6b** in spite of the subsequent acidic conversion.

This finding led us to modify a synthetic route of 5-*epi*-callystatin A (**20**), in which a lactone moiety was constructed before the condensation with segment C_{13}–C_{22}



Scheme 1. Reagents and conditions: (a) $LiCH_2S(O)CH_3$, toluene, −78 °C, rt, **5b**: 72% from **4b**, **11**: 71% from **6b**; (b) DDQ, CH_2Cl_2: 0.5% aq $NaHCO_3$ (9:1), 82%; (c) SiO_2 column chromatography; (d) PPTS, ^iPrOH, 72%; (e) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (f) TPAP, NMO, CH_2Cl_2; (g) Dowex HCR-W2, acetone:H_2O (5:1), 40 °C; (h) Ag_2CO_3–Celite, PhH, 50 °C, three steps 65%; (i) HF:Py (5:1), THF, 90%.



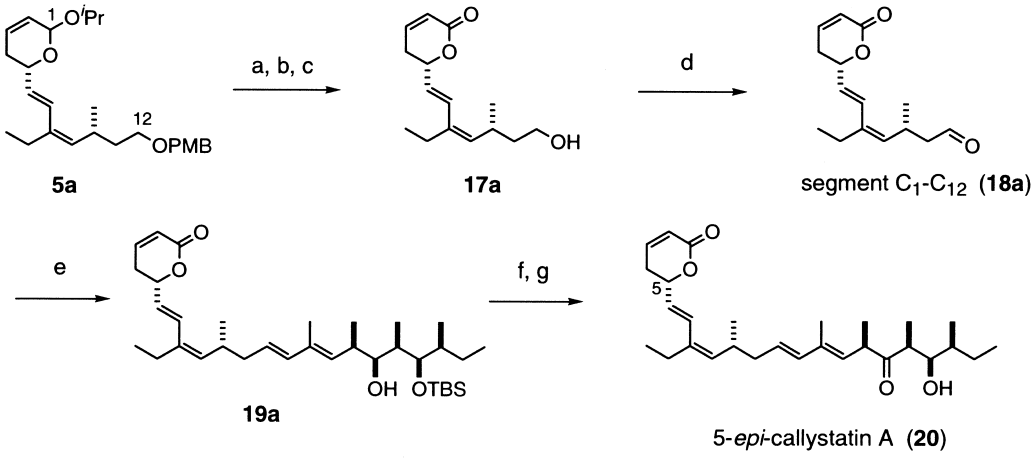
Scheme 2. Reagents and conditions: (a) PMBBr, NaH, THF, 96%; (b) $^{n}Bu_4NF$, THF, 97%; (c) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (d) $R^3OCOCH(R^1)PO(OCH_2CF_3)_2$, $KN(SiMe_3)_2$, 18-crown-6, THF, −78–0 °C, **15a**: two steps 92% (*Z*:*E* = 12:1), **15c**: two steps quant. (*Z*:*E* = 7.7:1), **15d**: two steps 95% (*Z*:*E* = 5:1); (e) DIBAL-H, CH_2Cl_2, −78 °C; (f) CBr_4, PPh_3, 2,6-lutidine, CH_3CN; (g) Bu_3P, CH_3CN, three steps **4a**: 92%, **4c**: 85%, **4d**: 98%; (h) KH, PMBCl, THF, 77%.

(**10**) as depicted in Scheme 3. Deprotection of the isopropyl acetal group in **5a** with acidic ion-exchange resin (Dowex HCR-W2, H^+ form) and subsequent oxidation using Ag_2CO_3–Celite gave a lactone, whose PMB group was readily cleaved by treatment with DDQ to furnish the desired alcohol **17a** in 82%, three steps. Then, **17a** was converted to segment C_1–C_{12} (**18a**) by Dess–Martin oxidation.[19] The lactone aldehyde (**18a**) was coupled with segment C_{13}–C_{22} (**10**) under the reaction conditions established by us[3] to give a lactone-alcohol (**19a**) having the same carbon framework as callystatin A (**1**). Oxidation of **19a** using Dess–Martin periodinane provided a ketone without concomitant isomerization on the asymmetric centers. Finally, the TBS group was removed by use of HF:pyridine (5:1) to give 5-*epi*-callystatin A (**20**). In the final deprotection of the TBS group giving callystatin A (**1**) and/or the two analogues (**12**, **20**), a minor stereoisomer was produced in any case. However, the chemical structure of the stereoisomer was not established due to its almost superimposable NMR spectra. Fortunately, the stereoisomer obtained in the deprotection of 19-*O*-TBS-callystatin A was found to be identical with 5-*epi*-callystatin A (**20**) by CD and HPLC analyses. Since the 10-*epi*-analogue (**12**) was not detected in this reaction, the isomerization would mainly proceed via a C-5 carbocation intermediate formed by cleavage of the lactone ring. Taking into account an exploration of the structure–activity relationship around the β-hydroxyketone moiety in **1** by synthetic means, it should be noted that the overall yield from **5a** to **20** was improved from 34 to 46%.

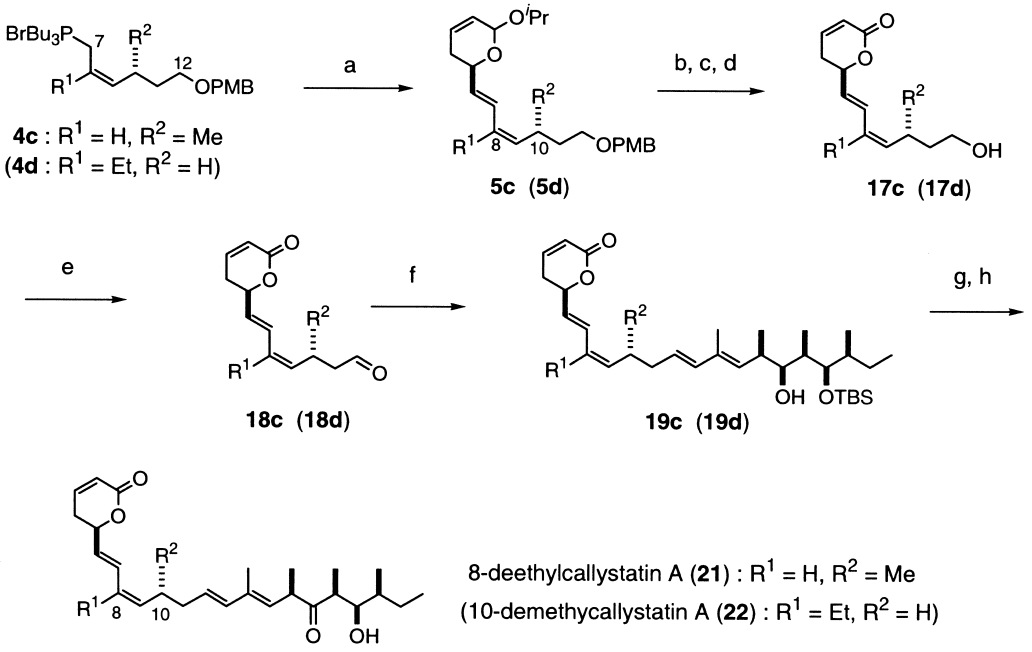
Next, we synthesized 8-deethyl (**21**) and 10-demethyl analogues (**22**) and assessed their cytotoxicity as the first step toward a search for more simplified lead compounds. The same protocol utilizing the corresponding segment C_7–C_{12} (**4c** and **4d**) as in the synthesis of 5-*epi*-callystatin A (**20**), as illustrated in Scheme 4, furnished 8-deethyl- (**21**) and 10-demethylcallystatin A (**22**). The two segments **4c** and **4d** were prepared from **13a** and 1,4-butanediol (**16**), respectively, as shown in Scheme 2.

Biological properties and discussion

The cytotoxic activity against KB cells of 5-*epi*-callystatin A (**20**) was significantly reduced to 7.6 nM, while 10-*epi*-callystatin A (**12**) showed almost the same activity (IC_{50} = 0.076 nM) as that of callystatin A (**1**). Furthermore, 8-deethyl (**21**: IC_{50} = 7.9 nM) and 10-demethyl analogues (**22**: IC_{50} = 1.4 nM) approximately exhibited 360-



Scheme 3. Reagents and conditions: (a) Dowex HCR-W2, acetone:H_2O (5:1), 40 °C; (b) Ag_2CO_3–Celite, PhH, 50 °C, two steps 91%; (c) DDQ, CH_2Cl_2:^{t}BuOH:pH 6.9 phosphate buffer (90:1:9), 91%; (d) Dess–Martin periodinane, CH_2Cl_2; (e) **10**, $LiCH_2S(O)CH_3$, toluene, −20 °C, two steps 62%; (f) Dess–Martin periodinane, CH_2Cl_2; (g) HF:Py (5:1), THF, two steps 90%.



Scheme 4. Reagents and conditions: (a) **3a**, $LiCH_2S(O)CH_3$, toluene, −20 °C; **5c**: 75%; **5d**: 81%; (b) Dowex; HCR-W2, acetone:H_2O (5:1), 40 °C; (c) Ag_2CO_3–Celite, PhH, 50 °C; (d) DDQ, CH_2Cl_2:^{t}BuOH:pH 6.9 phosphate buffer (90:1:9); **17c**: three steps 81%, **17d**: three steps 76%; (e) Dess–Martin periodinane, CH_2Cl_2; (f) **10**, $LiCH_2S(O)CH_3$, toluene, −20 °C, **19c**: two steps 66%, **19d**: two steps 65%; (g) Dess–Martin periodinane, CH_2Cl_2; (h) HF:Py (5:1), THF; **21**: two steps 85%, **22**: two steps 80%.

and 60-fold weaker cytotoxicity as compared with that of callystatin A (**1**), respectively.

On the other hand, the CD spectra of callystatin A (**1**) and the three analogues (**12**, **20** and **22**) are depicted in Figure 1. As for **1** and the two epimers (**12**, **20**), split Cotton effects at 230 and 250 nm due to the interaction between π–π* transition of the two conjugated diene chromophores were distinctly observed and their sign reflected the configuration at C-10 as reported previously.[2,20] It is, therefore, presumed that these three compounds have predominant conformations, in which two conjugated diene moieties divided by C-10 are optimally arranged. In contrast, the CD profile of 10-demethyl analogue (**22**) suggests that the absence of a 10-methyl group permits the single bond between C-10 and C-11 to rotate flexibly.

Based on these findings, the following structure–activity relationship of **1** is assumed: (1) 5*R*-configuration and an 8-alkyl functional group are important structural factors for the potent cytotoxicity of **1**; (2) the cytotoxicity of **1** is minimally affected by the configuration at C-10; (3) lack of a 10-methyl group reduced the cytotoxic activity of **1**; (4) the potent cytotoxicity of **1** is not affected by the configuration of the asymmetric C-10 carbon but by the spatial arrangement between the α,β-unsaturated-δ-lactone and 6,8-conjugated diene moieties.

Mitogen-activated protein kinase kinase (MAPKK) is a threonine/tyrosine specific kinase belonging to the MAP kinase cascade that begins with the stimulation of the proto-oncoprotein Ras in response to a wide variety of extracellular stimuli such as expression of some oncogenes. It was found that MAPKKs which contain an

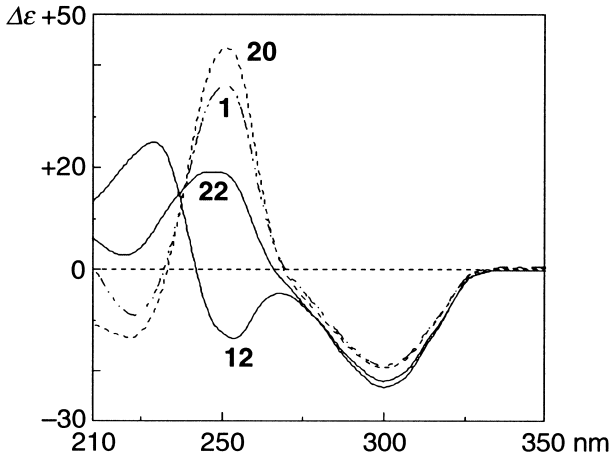


Figure 1. CD spectra of callystatin A (**1**) and 10-*epi* (**12**), 5-*epi* (**20**), and 10-demethyl (**22**) analogues in MeOH.

NES sequence and are transported in cytoplasm by CRM1 protein thus fulfill their inherent function.[21,22] Taking the above-described findings into consideration comprehensively, callystatin A (**1**) is presumed to prevent MAPKKs from binding toward CRM1 to restrict their location in the nucleus, which may relate to the remarkably potent cytotoxicity of **1**. In fact, the inhibitors of MAPKKs suppressed the growth of several human epithelial tumor lines both in vitro and in vivo.[23,24] Therefore, preventing the function of this kinase would be considered to lead to potential anti-cancer chemotherapy.

In summary, we have analyzed the participation of the two asymmetric centers at C-5 and C-10 in callystatin A (**1**), and the two alkyl residues at C-8 and C-10, in the efficacy of potent cytotoxicity using synthetic analogues,

indicating that 5-*R* configuration and 8-ethyl residue contribute in particular. Investigation of the participation of the β-hydroxyketone part on structure requirement for the potent cytotoxicity of callystatin A (**1**) aiming at a search for promising leads is currently in progress.

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 (500 MHz), a JEOL JNM-AL300, and JNM-EX270 NMR spectrometers for ^{1}H NMR[25] and ^{13}C NMR spectra (^{1}H NMR: $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard unless otherwise specified. ^{13}C NMR: $CDCl_3$ solution with $CHCl_3$ (δ_c 77.0) 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.

Condensation of 3a and 4b giving 5b. A solution of *n*-BuLi (1.54 M in *n*-hexane, 0.79 mL, 1.22 mmol) was added to a solution of DMSO (0.35 mL) in dry toluene (6.1 mL) at rt, then the whole was stirred for 45 min. A solution of **4b** (264 mg, 0.49 mmol) and **3a** (207 mg, 1.22 mmol) in dry toluene (5.0 mL) was added to the solution of dimesylcarbanion at −78 °C, then the whole was stirred warming from −60 °C to 0 °C overnight. 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 7 g, *n*-hexane:Et_2O = 15:1) to furnish **5b** (144 mg, 72%). Compound **5b**: colorless oil; IR ν_{max} (KBr) cm^{-1}: 2968, 1612, 1514, 1367, 1248. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.97 (3H, d, J = 6.6 Hz, 10-CH_3), 1.05 (3H, t, J = 7.4 Hz, 8-CH_2CH_3), 1.17, 1.24 (both 3H, d, J = 6.2 Hz, 1-OCH(CH_3)$_2$), 1.43, 1.70 (both 1H, m, H-11), 2.02 (2H, m, H-4), 2.19 (2H, q, J = 7.4 Hz, 8-CH_2CH_3), 2.80 (1H, m, H-10), 3.35 (2H, m, H-12), 3.80 (3H, s, CH_2PhOCH_3), 4.01 (1H, m, 1-OCH(CH_3)$_2$), 4.36, 4.39 (both 1H, d, J = 11.3 Hz, CH_2PhOCH_3), 4.52 (1H, m, H-5), 5.11 (1H, br s, H-1), 5.72–5.76 (2H, m, H-2, H-6), 5.98 (1H, m, H-3), 6.61 (1H, d, J = 15.6 Hz, H-7), 6.86, 7.23 (both 2H, d, J = 8.4 Hz, CH_2PhOCH_3). EI–MS *m*/*z*: 414 (M^+, 6.3), 121 (100). EI–HRMS *m*/*z*: calcd for $C_{26}H_{38}O_4$: 414.2770; found: 414.2767.

Removal of *p*-methoxybenzyl group in 5b. A solution of **5b** (9.9 mg, 0.024 mmol) in CH_2Cl_2:0.5% aqueous $NaHCO_3$ (9:1, 0.8 mL) was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (8.2 mg, 0.036 mmol) at rt for 40 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 3 g, *n*-hexane:EtOAc:Et_2NH = 7:1:0.08) to furnish a mixture of **6b** (5.8 mg, 82%), **7** (0.2 mg, 4%), and **9** (0.3 mg, 6%). Because of lability of the diol **7**, acidic transformation to **6b** as described below was carried out without characterization. The same conversion on a large scale (**5b**, 416 mg, 1.00 mmol) was carried out to give **6b** (71.0 mg, 24%), **7** (20.1 mg, 8%), and **9** (49.1 mg, 21%). Compound **6b**: colorless oil; IR ν_{max} (KBr) cm^{-1}: 3621, 2969, 1653, 1458, 1181, 1100, 1032, 999. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.99 (3H, d, J = 6.6 Hz, 10-CH_3), 1.05 (3H, t, J = 7.4 Hz, 8-CH_2CH_3), 1.17, 1.24 (both 3H, d, J = 6.2 Hz, 1-OCH(CH_3)$_2$), 1.45, 1.66 (both 1H, m, H-11), 2.07 (2H, m, H-4), 2.20 (2H, q, J = 7.4 Hz, 8-CH_2CH_3), 2.79 (1H, m, H-10), 3.58 (2H, t, J = 6.4 Hz, H-12), 4.00 (1H, m, 1-OCH(CH_3)$_2$), 4.50 (1H, m, H-5), 5.15 (1H, br s, H-1), 5.74 (2H, m, H-2, H-6), 6.00 (1H, dd, J = 9.7, 5.2 Hz, H-3), 6.62 (1H, d, J = 15.9 Hz, H-7). FAB–MS *m*/*z*: 317 $(M+Na)^+$. FAB–HRMS *m*/*z*: calcd for $C_{18}H_{30}O_3Na$: 317.2092; found: 317.2104. Compound **9**: colorless oil; IR ν_{max} (KBr) cm^{-1} : 2969, 1663, 1458, 1181, 1110, 1042. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.00 (6H, d, J = 6.8 Hz, 10-CH_3), 1.06 (6H, t, J = 7.4 Hz, 8-CH_2CH_3), 1.45, 1.65 (both 2H, m, H-11), 2.05 (4H, m, H-4), 2.20 (4H, q, J = 7.4 Hz, 8-CH_2CH_3), 2.76 (2H, m, H-10), 3.56 (4H, t, J = 6.6 Hz, H-12), 4.60 (2H, m, H-5), 5.05 (2H, br s, H-1), 5.74 (4H, m, H-2, H-6), 6.04 (2H, dd, J = 9.7, 5.2 Hz, H-3), 6.68 (2H, d, J = 15.9 Hz, H-7). FAB–MS *m*/*z*: 475 $(M+Li)^+$. FAB–HRMS *m*/*z*: calcd for $C_{30}H_{44}LiO_4$: 475.3400; found: 475.2671.

Acidic treatment of 7 or 9 giving 6b. A solution of **9** (7.0 mg, 0.015 mmol) in dry ^{i}PrOH (4.0 mL) was treated with pyridinium *p*-toluenesulfonate (PPTS) (5.0 mg, 0.020 mmol) at rt for 2 h. The reaction mixture was poured into saturated aqueous $NaHCO_3$, 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 in the presence of a slight amount of pyridine under reduced pressure gave **6b** (6.3 mg, 72%). Similarly, compound **7** (4.3 mg, 0.017 mmol) was converted to **6b** (2.6 mg, 52%).

Swern oxidation of 6b followed by Wittig reaction with 10 giving 11. DMSO (0.023 mL) was added to a solution of $(COCl)_2$ (0.020 mL, 0.165 mmol) in dry CH_2Cl_2 (2.7 mL) at −78 °C, then the whole was stirred for 20 min. After adding a solution of **6b** (16.2 mg, 0.055 mmol) in dry CH_2Cl_2 (2.0 mL) to the reaction mixture at −78 °C, the whole was stirred for 30 min. Then, the reaction mixture was treated with Et_3N (0.061 mL,

0.44 mmol) at −78 °C for 2 h. After the reaction mixture was diluted with dry Et_2O, a filtrate given through Na_2SO_4 column was concentrated under reduced pressure to afford an aldehyde **8b**. A solution of *n*-BuLi (1.54 M in *n*-hexane, 0.28 mL, 0.43 mmol) was added to a solution of DMSO (0.19 mL) in dry toluene (6.4 mL) at rt, then the whole was stirred for 60 min. A solution of **8b** and **10** (103 mg, 0.17 mmol) in dry toluene (3.0 mL) was added to the solution of dimesylcarbanion 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 compound **5b** gave a product, which was purified by column chromatography (SiO_2 1 g, *n*-hexane:EtOAc = 30:1) to furnish **11** (24.0 mg, 71%). Compound **11**: colorless oil; IR ν_{max} (KBr) cm^{-1}: 3534, 2934, 1462, 1379, 1260, 1098, 1018. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.08 (6H, s, 19-OSi($C\underline{H}_3$)$_2$C(CH_3), 0.80–1.06 (34H, m, 1-OCH($C\underline{H}_3$)$_2$, 8-$CH_2C\underline{H}_3$, 10-CH_3, 16-CH_3, 18-CH_3, 19-OSi(CH_3)$_2$C($C\underline{H}_3$)$_3$, 20-CH_3, H-21, H-22), 1.20 (2H, m, H-20, 21), 1.70 (3H, s, 14-CH_3), 1.72 (1H, m, H-18), 2.04–2.21 (6H, m, H-4, 8-$C\underline{H}_2CH_3$, H-11), 2.57 (1H, m, H-10), 2.67 (1H, m, H-16), 3.35 (1H, d, *J* = 9.2 Hz, H-19), 3.63 (1H, t, *J* = 3.7 Hz, H-17), 4.02 (1H, m, 1-OC$\underline{H}$(CH_3)$_2$), 4.49 (1H, m, H-5), 5.05 (1H, d, *J* = 9.8 Hz, H-15), 5.12 (1H, br s, H-1), 5.16 (1H, d, *J* = 10.1 Hz, H-9), 5.47 (1H, dt, *J* = 15.3, 7.9 Hz, H-12), 5.75 (2H, m, H-2, H-6), 5.98 (2H, m, H-3, H-13), 6.56 (1H, d, *J* = 15.9 Hz, H-7). FAB–MS *m*/*z*: 639 $(M+Na)^+$. FAB–HRMS *m*/*z*: calcd for $C_{38}H_{68}O_4SiNa$: 639.4784; found: 639.4751.

Conversion from 11 to 10-*epi*-callystatin A (12). *N*-Methyl morpholine *N*-oxide (NMO) (13.1 mg, 0.112 mmol) was added to a solution of **11** (17.2 mg, 0.028 mmol) in CH_2Cl_2 (2.8 mL), then the whole was stirred at rt for 10 min. The reaction mixture was treated with tetrapropylammonium perruthenate (TPAP) (11.8 mg, 0.034 mmol) at rt for 36 h. After dilution with Et_2O, a filtrate given through SiO_2 column was concentrated under reduced pressure to give a product, which was purified by column chromatography (SiO_2 1 g, *n*-hexane:EtOAc = 10:1) to furnish a ketone (17.1 mg). A solution of the ketone (4.2 mg, 0.0068 mmol) in acetone:H_2O (5:1) (1.2 mL) was treated with Dowex HCR-W2 (H^+ form) (30 mg) at 40 °C for 5 h. After removing the residue by filtration, the filtrate was poured into saturated aqueous $NaHCO_3$ and 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 lactol. A solution of the lactol in dry benzene (1.7 mL) was treated with Ag_2CO_3 (94 mg, 0.34 mmol) and Celite (47 mg) at 45 °C for 6 h in the dark. After removing the residue by filtration with Na_2SO_4, the filtrate was concentrated under reduced pressure to give a product, which was purified by column chromatography (SiO_2 1 g, *n*-hexane:EtOAc = 5:1) to furnish a lactone (2.5 mg, 65%). To a solution of the lactone (1.9 mg, 0.0033 mmol) in dry THF (2.0 mL) was added HF:pyridine (5:1) (0.7 mL) at 0 °C, then the whole was stirred at rt for 80 h. The reaction mixture was neutralized with $NaHCO_3$ at 0 °C, then the residue was removed by filtration. Removal of solvent from the filtrate 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 = 2:1; detection; UV (λ = 250 nm); flow rate; 3.0 mL/min) to furnish 10-*epi*-callystatin A (**12**, 1.3 mg, 90%).[26] 10-*epi*-Callystatin A (**12**): colorless oil; $[\alpha]_D$ −288.9° (*c* = 0.05, MeOH, 26 °C). UV λ_{max} (MeOH) nm (ε) : 242 (30900), 300 (1300). CD (MeOH) nm (Δε) : 334 (−0.3), 300 (−22.0), 268 (−4.8), 254 (−13.6), 242 (0), 229 (+25.0), 210 (+13.1). IR ν_{max} (KBr) cm^{-1}: 3563, 2965, 2928, 1730, 1713, 1456, 1377, 1262, 1084, 1051, 1020. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.84 (3H, t, *J* = 7.4 Hz, H-22), 0.89 (3H, d, *J* = 6.6 Hz, 20-CH_3), 0.97 (3H, d, *J* = 6.4 Hz, 10-CH_3), 1.04 (3H, t, *J* = 7.4 Hz, 8-$CH_2C\underline{H}_3$), 1.08 (1H, m, H-21), 1.11 (3H, d, *J* = 7.1 Hz, 18-CH_3), 1.14 (3H, d, *J* = 6.7 Hz, 16-CH_3), 1.32 (1H, m, H-21), 1.40 (1H, m, H-20), 1.80 (3H, s, 14-CH_3), 2.08 (2H, dd, *J* = 7.4, 6.7 Hz, H-11), 2.18 (2H, q, *J* = 7.4 Hz, 8-$C\underline{H}_2CH_3$), 2.46 (2H, m, H-4), 2.67 (1H, m, H-10), 2.84 (1H, dq, *J* = 4.3, 7.1 Hz, H-18), 3.56 (1H, m, H-19), 3.64 (1H, dq, *J* = 10.0, 6.7 Hz, H-16), 5.00 (1H, ddd, *J* = 7.6, 7.6, 6.4 Hz, H-5), 5.13 (1H, d, *J* = 10.0 Hz, H-15), 5.25 (1H, d, *J* = 9.7 Hz, H-9), 5.58 (1H, dt, *J* = 15.8, 7.4 Hz, H-12), 5.76 (1H, dd, *J* = 6.4, 16.0 Hz, H-6), 6.01 (1H, d, *J* = 15.8 Hz, H-13), 6.07 (1H, d, *J* = 10.2 Hz, H-2), 6.64 (1H, d, *J* = 16.0 Hz, H-7), 6.90 (1H, dt, *J* = 10.2, 4.8 Hz, H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 10.9 (q, C-22), 11.0 (q, 18-CH_3), 13.1 (q, 14-CH_3), 13.4 (q, 8-$CH_2C\underline{H}_3$), 14.3 (q, 20-CH_3), 16.3 (q, 16-CH_3), 20.9 (q, 10-$C\underline{H}_3$), 25.7 (t, C-21), 26.4 (t, 8-$C\underline{H}_2CH_3$), 30.0 (t, C-4), 32.2 (d, C-10), 36.7 (d, C-20), 40.8 (t, C-11), 45.7 (d, C-16, C-18), 74.5 (d, C-19), 78.6 (d, C-5), 121.7 (d, C-2), 124.8 (d, C-6), 127.9 (d, C-12), 128.3 (d, C-15), 129.8 (d, C-7), 135.2 (d, C-13), 135.3 (s, C-8), 136.2 (s, C-14), 137.1 (d, C-9), 144.6 (d, C-3), 164.2 (s, C-1), 216.4 (s, C-17). FAB–MS *m*/*z*: 457 $(M+H)^+$. FAB–HRMS *m*/*z*: calcd for $C_{29}H_{45}O_4$: 457.3318; found: 457.3332.

Conversion from 13b to 14b. To a solution of **13b** (21.0 g, 61.4 mmol) in dry THF (150 mL) was added NaH (3.0 g, 123 mmol) at 0 °C, then the whole mixture was stirred at rt for 15 min. The reaction mixture was treated with *p*-methoxybenzyl bromide (PMBBr) (15.0 mL, 74.6 mmol) at rt in the dark for 24 h. The reaction mixture was poured into saturated aqueous NaCl, then the whole was extracted with Et_2O. The Et_2O extract was 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_2 750 g, *n*-hexane:EtOAc = 30:1) to afford a PMB ether (27.3 g, 96%). Tetrabutylammonium fluoride (1.0 M in THF, 118 mL, 118 mmol) was added to a solution of PMB ether (27.2 g, 58.9 mmol) in dry THF (240 mL), then the whole mixture was stirred at rt for 17 h. The reaction mixture was worked up in the same manner as preparation for PMB ether of **13b** to give a product, which was purified by column chromatography (SiO_2 350 g, *n*-hexane:EtOAc = 5:2) to furnish **14b** (12.8 g, 97%). Compound **14b**: colorless oil; $[\alpha]_D$ −7.9° (*c* = 2.34, $CHCl_3$, 25 °C). IR ν_{max} (KBr) cm^{-1}: 3487, 2928, 1514, 1248. ^1H NMR (270 MHz, $CDCl_3$) δ: 0.91 (3H, d, *J* = 6.9 Hz, 10-CH_3), 1.65 (2H, m, H-11), 1.78 (1H, m, H-10), 3.37–3.61 (4H, m, H-9, H-12), 3.80 (3H,

s, OCH_3), 4.45 (2H, s, $C\underline{H}_2PhOCH_3$), 6.88, 7.25 (both 2H, d, $J=8.6$ Hz, $CH_2\underline{Ph}OCH_3$). FAB–MS m/z: 225 $(M+H)^+$. FAB–HRMS m/z: calcd for $C_{13}H_{21}O_3$: 225.1491; found: 225.1538.

Conversion from 14b to 15b. DMSO (0.89 mL) was added to a solution of $(COCl)_2$ (0.55 mL, 6.29 mmol) in dry CH_2Cl_2 (24 mL) at −78 °C, then the whole was stirred for 20 min. After adding a solution of **14b** (470 mg, 2.10 mmol) in dry CH_2Cl_2 (2.0 mL) to the reaction mixture at −78 °C, the whole was stirred for 30 min. Then, the reaction mixture was treated with Et_3N (2.3 mL, 16.8 mmol) at −78 °C for 1 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. After 18-Crown-6 ether/CH_3CN complex (5.55 g, 21.0 mmol) was added to a solution of $EtOCOCHEtPO(OCH_2CF_3)_2$ (1.89 g, 5.25 mmol) in dry THF (71 mL) at rt, the reaction mixture was treated with potassium bis(trimethylsilyl)amide (0.5 M in toluene, 8.40 mL, 4.20 mmol) at −78 °C for 5 min. Then, a solution of aldehyde in dry THF (23 mL) was added and 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 Na_2SO_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:Et_2O = 10:1) to furnish **15b** (620 mg, 92%, *E*:*Z* = 12:1). Compound **15b**: colorless oil, $[\alpha]_D$ +2.0° (*c* = 0.75, $CHCl_3$, 25 °C). IR ν_{max} (KBr) cm^{-1}: 2967, 1713, 1512, 1248. ^1H NMR (270 MHz, $CDCl_3$) δ: 0.99 (3H, d, $J=6.3$ Hz, 10-CH_3), 1.00 (3H, t, $J=7.5$ Hz, 8-$CH_2C\underline{H}_3$), 1.28 (3H, t, $J=7.3$ Hz, $OCH_2C\underline{H}_3$), 1.61 (2H, m, H-11), 2.25 (2H, q, $J=7.3$ Hz, 8-$C\underline{H}_2CH_3$), 3.10 (1H, m, H-10), 3.41 (2H, t, $J=7.1$ Hz, H-12), 3.80 (3H, s, OCH_3), 4.18 (2H, q, $J=7.1$ Hz, $OC\underline{H}_2CH_3$), 4.39 (2H, s, $C\underline{H}_2PhOCH_3$), 5.55 (1H, d, $J=10.2$ Hz, H-9), 6.86, 7.24 (both 2H, d, $J=8.6$ Hz, $CH_2\underline{Ph}OCH_3$). FAB–MS m/z: 321 $(M+H)^+$. FAB–HRMS m/z: calcd for $C_{19}H_{29}O_4$: 321.2066; found: 321.1996.

Conversion from 15b to 4b. A solution of **15b** (590 mg, 1.85 mmol) in dry CH_2Cl_2 (9 mL) was treated with diisobutylaluminum hydride (DIBAL-H) (1.5 M in toluene, 2.7 mL, 4.06 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 stop of precipitate-formation, 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 10 g, *n*-hexane:EtOAc = 5:1) to furnish an allyl alcohol (472 mg, 92%). Triphenylphosphine (622 mg, 2.37 mmol) was added to a solution of the allyl alcohol (264 mg, 0.95 mmol) in dry CH_3CN (14 mL) at rt, then 2,6-lutidine (0.03 mL, 0.28 mmol) and CBr_4 (962 mg, 2.85 mmol) were added at 0 °C. The whole was further stirred at rt 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 NaCl, then dried over Na_2SO_4. Removal of solvent from this 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 bromide. A solution of the bromide in dry CH_3CN (4.0 mL) was treated with tri-*n*-butylphosphine (0.71 mL, 2.85 mmol) at 0 °C for 2 h. The reaction mixture was evaporated under reduced pressure to afford a product, which was purified by column chromatography (SiO_2 10 g, $CHCl_3$:MeOH = 50:1) to furnish **4b** (515 mg, quant.). Because of lability of this tributylphosphonium bromide, next transformation was carried out without further purification and characterization.

Preparation of 15c. After 18-Crown-6 ether/CH_3CN complex (15.0 g, 56.7 mmol) was added to a solution of $MeOCOCH_2PO(OCH_2CF_3)_2$ (3.43 mL, 16.2 mmol) in dry THF (35 mL) at rt, the reaction mixture was treated with potassium bis(trimethylsilyl)amide (0.5 M in toluene, 30.8 mL, 16.0 mmol) at −78 °C for 10 min. Then, a solution of the aldehyde (1.8 g, 8.1 mmol), which was prepared from **14a** for synthesis of **15a**, in dry THF (35 mL) was added and the whole was stirred warming from −78 °C to 0 °C overnight. Work up in the same manner as preparation for **15a** gave a product, which was purified by column chromatography (SiO_2 90 g, *n*-hexane:EtOAc = 3:1) to furnish **15c** (2.25 g, quant., *Z*:*E* = 7.7:1). Compound **15c**: colorless oil; $[\alpha]_D$ +12.6° (*c* = 3.83, $CHCl_3$, 25 °C). IR ν_{max} (KBr) cm^{-1} : 2953, 2861, 1723, 1514, 1248. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.03 (3H, d, $J=6.8$ Hz, 10-CH_3), 1.66 (2H, m, H-11), 3.43 (2H, m, H-12), 3.61 (1H, m, H-10), 3.70 (3H, s, 7-OCH_3), 3.80 (3H, s, $CH_2PhOC\underline{H}_3$), 4.39 (2H, s, $C\underline{H}_2PhOCH_3$), 5.71 (1H, dd, $J=11.4$, 0.8 Hz, H-8), 6.00 (1H, dd, $J=11.4$, 10.3 Hz, H-9), 6.86, 7.24 (both 2H, d, $J=8.6$ Hz, $CH_2\underline{Ph}OCH_3$). FAB–MS m/z: 301 $(M+Na)^+$. FAB–HRMS m/z: calcd for $C_{16}H_{22}O_4Na$: 301.1416; found: 301.1409.

Conversion from 15c to 4c. The same procedure as preparation for **4a** was conducted using **15c** (1530 mg, 5.50 mmol) giving a product, which was purified by column chromatography (SiO_2 20 g, *n*-hexane:EtOAc = 2:1) to furnish an allyl alcohol (233 mg, 85%). The allyl alcohol (150 mg, 0.60 mmol) was treated in the same manner as preparation for **15a** to give a product, which was purified by column chromatography (SiO_2 6 g, benzene:Et_2O = 100:1) to furnish a bromide. The same treatment of the bromide as preparation for **4a** afforded a product, which was purified by washing with *n*-hexane to furnish **4c** (309 mg, quant.). Because of lability of this tributylphosphonium bromide, next transformation was carried out without further purification and characterization.

Conversion from 1,4-butanediol (16) to 15d. A solution of 1,4-butanediol (**16**) (3.0 g, 33.3 mmol) in dry THF (300 mL) was treated with KH (1.47 g, 36.7 mmol) at 0 °C for 10 min. To the reaction mixture was added *p*-methoxybenzyl chrolide (PMBCl) (4.54 mL, 33.3 mmol), then the whole was stirred at rt for 1 h. Work up in the

same manner as preparation for **14a** except for using EtOAc in extraction gave a product, which was purified by column chromatography (SiO_2 100 g, *n*-hexane:EtOAc = 1:1) to furnish mono-PMB ether (5.42 g, 77%). After the same treatment for oxidation of mono-PMB ether (932 mg, 4.44 mmol) as preparation for **15a**, the reaction mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with saturated aqueous NaCl:5.0% aqueous HCl (1:1), then dried over $MgSO_4$. Removal of solvent from the CH_2Cl_2 extract under reduced pressure gave an aldehyde. The same procedure as preparation for **15a** was conducted using EtO-$COCHEtPO(OCH_2CF_3)_2$ (3.43 g, 9.5 mmol) to give a product, which was purified by column chromatography (SiO_2 25 g, *n*-hexane:EtOAc = 5:1) to furnish **15d** (1.29 g, 95%, *Z*:*E* = 5:1). Compound **15d**: colorless oil; IR ν_{max} (KBr) cm^{-1}: 2969, 2849, 1709, 1613, 1462, 1375, 1300, 1248, 1094. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.00 (3H, t, *J* = 7.6 Hz, 8-$CH_2C\underline{H}_3$), 1.29 (3H, t, *J* = 7.0 Hz, $OCH_2C\underline{H}_3$), 1.73 (2H, m, H-11), 2.30 (2H, m, H-10), 2.48 (2H, q, *J* = 7.6 Hz, 8-$C\underline{H}_2CH_3$), 3.46 (2H, dd, *J* = 6.7, 6.4 Hz, H-12), 3.80 (3H, s, $CH_2PhOC\underline{H}_3$), 4.19 (2H, q, *J* = 7.0 Hz, $OC\underline{H}_2CH_3$), 4.43 (2H, s, $C\underline{H}_2PhOCH_3$), 5.83 (1H, t, *J* = 7.6 Hz, H-9), 6.87, 7.25 (both 2H, d, *J* = 8.5 Hz, $CH_2\underline{Ph}OCH_3$). FAB–MS *m*/*z*: 329 $(M+Na)^+$. FAB–HRMS *m*/*z*: calcd for $C_{18}H_{26}O_4Na$: 329.1728; found: 329.1723.

Conversion from 15d to 4d. The same procedure as preparation for **4a** was conducted using **15d** (375 mg, 1.23 mmol) giving a product, which was purified by column chromatography (SiO_2 7 g, *n*-hexane:EtOAc = 5:2) to furnish an allyl alcohol (318 mg, 98%). The allyl alcohol (243 mg, 0.92 mmol) was treated in the same manner as preparation for **4a** to give a product, which was purified by column chromatography (SiO_2 13 g, benzene:Et_2O = 100:1) to furnish a bromide. The same treatment of the bromide as preparation for **4a** afforded a product, which was purified by washing with *n*-hexane to furnish **4d** (300 mg, quant.). Because of lability of this tributylphosphonium bromide, next transformation was carried out without further purification and characterization.

Conversion from 5a to 17a. A solution of **5a** (153 mg, 0.37 mmol) in acetone:H_2O (5:1) (45 mL) was treated with Dowex HCR-W2 (H^+ form) (1.97 g) at 40 °C for 5 h. Work up in the same manner as preparation for **12** gave a lactol. A solution of the lactol in dry benzene (60 mL) was treated with Ag_2CO_3 (5.08 g, 18.4 mmol) and Celite (2.53 g) at 45 °C for 6 h in the dark. Work up in the same manner as preparation for **12** gave a product, which was purified by column chromatography (SiO_2 9 g, *n*-hexane:EtOAc = 3:1) to furnish a lactone (124 mg, 91%). A solution of the lactone (104 mg, 0.28 mmol) in CH_2Cl_2:*t*-BuOH:pH 6.9 phospate buffer (90:1:9) (13.5 mL) was treated with DDQ (127 mg, 0.56 mmol) at rt for 30 min. Work up in the same manner as preparation for **6b** except for using EtOAc in extraction gave a product, which was purified by column chromatography (SiO_2 10 g, *n*-hexane:EtOAc = 1:1) to furnish **17a** (64 mg, 91%). Compound **17a**: colorless oil; $[\alpha]_D$ −55.3° (*c* = 1.94, $CHCl_3$, 20 °C). IR ν_{max} (KBr) cm^{-1}: 3414, 2963, 2928, 2874, 1725, 1456, 1381, 1246, 1051, 961. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.00 (3H, d, *J* = 6.7 Hz, 10-CH_3), 1.05 (3H, t, *J* = 7.3 Hz, 8-$CH_2C\underline{H}_3$), 1.48 (1H, m, H-11), 1.63 (1H, m, H-11), 2.19 (2H, q, *J* = 7.3 Hz, 8-$C\underline{H}_2CH_3$), 2.50 (2H, m, H-4), 2.80 (1H, m, H-10), 3.58 (2H, m, H-12), 5.01 (1H, ddd, *J* = 7.3, 7.3, 6.7 Hz, H-5), 5.23 (1H, d, *J* = 9.8 Hz, H-9), 5.78 (1H, dd, *J* = 6.7, 15.9 Hz, H-6), 6.06 (1H, d, *J* = 9.8 Hz, H-2), 6.70 (1H, d, *J* = 15.9 Hz, H-7), 6.92 (1H, dd, *J* = 9.8, 4.3 Hz, H-3). FAB–MS *m*/*z*: 251 $(M+H)^+$. FAB–HRMS *m*/*z*: calcd for $C_{15}H_{23}O_3$: 251.1648; found: 251.1651.

Dess–Martin oxidation of 17a followed by Wittig reaction with 10 giving 19a. A solution of **17a** (10 mg, 0.04 mmol) in dry CH_2Cl_2 (2.2 mL) was treated with Dess–Martin periodinane (54 mg, 0.12 mmol) at rt for 30 min. The reaction mixture was poured into saturated aqueous $NaHCO_3$:saturated aqueous $Na_2S_2O_3$ (1:1), then the whole was extracted with Et_2O. The Et_2O extract was washed with saturated aqueous NaCl:saturated aqueous $Na_2S_2O_3$ (1:1), then dried over $MgSO_4$. Removal of solvent from the Et_2O extract under reduced pressure gave **18a**. A solution of *n*-BuLi (1.53 M in *n*-hexane, 0.06 mL, 0.097 mmol) was added to a solution of DMSO (0.07 mL) in dry toluene (1.3 mL) at rt, then the whole was stirred for 45 min. A solution of **18a** and **10** (60.8 mg, 0.098 mmol) in dry toluene (3.0 mL) was added to the solution of dimesylcarbanion at −78 °C, then the whole was stirred warming from −60 °C to 0 °C overnight. Work up in the same manner as preparation for **5b** gave a product, which was purified by column chromatography (SiO_2 1 g, *n*-hexane:EtOAc = 3:1) to furnish **19a** (14.2 mg, 62%). Compound **19a**: colorless oil; ^1H NMR (500 MHz, $CDCl_3$) δ: 0.07, 0.08 (both 3H, s, 19-$OSi(C\underline{H}_3)_2$ $C(CH_3)_3$), 0.83 (3H, d, *J* = 7.4 Hz, 20-CH_3), 0.84 (3H, d, *J* = 7.3 Hz, 18-CH_3), 0.87 (3H, t, *J* = 7.3 Hz, H-22), 0.91 (9H, s, 19-$OSi(CH_3)_2C(C\underline{H}_3)_3$), 0.98 (3H, d, *J* = 7.3 Hz, 16-CH_3), 1.08 (1H, m, H-21), 1.03 (3H, d, *J* = 6.7 Hz, 10-CH_3), 1.05 (3H, t, *J* = 7.4 Hz, 8-$CH_2C\underline{H}_3$), 1.58 (3H, m, H-20, 21), 1.72 (3H, s, 14-CH_3), 1.76 (1H, m, H-18), 2.07 (2H, m, H-11), 2.18 (2H, q, *J* = 7.3 Hz, 8-$C\underline{H}_2CH_3$), 2.48 (2H, m, H-4), 2.58 (1H, m, H-16), 2.67 (1H, m, H-10), 3.36 (1H, d, *J* = 9.2 Hz, H-19), 3.64 (1H, dd, *J* = 7.9, 3.7 Hz, H-17), 5.00 (1H, ddd, *J* = 7.4, 7.4, 6.7 Hz, H-5), 5.07 (1H, d, *J* = 9.8 Hz, H-15), 5.27 (1H, d, *J* = 9.8 Hz, H-9), 5.49 (1H, dt, *J* = 15.3, 7.3 Hz, H-12), 5.76 (1H, dd, *J* = 6.6, 15.9 Hz, H-6), 5.99 (1H, d, *J* = 15.3 Hz, H-13), 6.08 (1H, d, *J* = 9.8 Hz, H-2), 6.65 (1H, d, *J* = 15.9 Hz, H-7), 6.90 (1H, dt, *J* = 9.8, 4.9 Hz, H-3). FAB–MS *m*/*z*: 573 $(M+H)^+$. FAB–HRMS *m*/*z*: calcd for $C_{35}H_{61}O_4Si$: 573.4340; found: 573.4337.

Conversion from 19a to 5-*epi*-callystatin A (20). A solution of **19a** (9.2 mg, 0.016 mmol) in dry CH_2Cl_2 (1.8 mL) was treated with Dess–Martin periodinane (24 mg, 0.57 mmol) at rt for 1.5 h. The reaction mixture was worked up in the same manner as preparation for **18a** to give a ketone. To a solution of the ketone in dry THF (5.0 mL) was added HF:pyridine (5:1) (1.67 mL) at 0 °C, then the whole was stirred at rt for 80 h. Work up in the same manner as preparation for **12** gave a product, which was purified by HPLC (column: COSMOSIL 5SL (10 mm i.d.×250 mm), mobile phase: *n*-hexane:EtOAc = 2:1,

detection: UV ($\lambda = 250$ nm), flow rate: 3.0 mL/min) to furnish 5-*epi*-callystatin A (**20**, 6.1 mg, 90%).[26] 5-*epi*-Callystatin A (**20**): colorless oil; $[\alpha]_D$ −71.4° ($c = 0.27$, MeOH, 25 °C). UV λ_{max} (MeOH) nm (ε): 244 (22500), 296 (2200). CD (MeOH) nm (Δε): 330 (0), 300 (−24.6), 270 (0), 252 (+57.5), 233 (0), 222 (−17.4), 210 (−13.1). IR ν_{max} (KBr) cm^{-1}: 3475, 2925, 2857, 1725, 1459, 1381, 1247, 1018, 965. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.85 (3H, t, $J = 7.4$ Hz, H-22), 0.89 (3H, d, $J = 6.6$ Hz, 20-CH_3), 0.96 (3H, d, $J = 6.6$ Hz, 10-CH_3), 1.04 (3H, t, $J = 7.4$ Hz, 8-$CH_2C\underline{H}_3$), 1.07 (1H, m, H-21), 1.11 (3H, d, $J = 7.1$ Hz, 18-CH_3), 1.14 (3H, d, $J = 6.6$ Hz, 16-CH_3), 1.36 (1H, m, H-21), 1.40 (1H, m, H-20), 1.81 (3H, s, 14-CH_3), 2.08 (2H, t, $J = 7.9$ Hz, H-11), 2.18 (2H, q, $J = 7.4$ Hz, 8-$C\underline{H}_2CH_3$), 2.47 (2H, m, H-4), 2.67 (1H, m, H-10), 2.86 (1H, dq, $J = 4.2$, 7.1 Hz, H-18), 3.57 (1H, dd, $J = 6.9$, 4.2 Hz, H-19), 3.65 (1H, dq, $J = 10.0$, 6.6 Hz, H-16), 5.00 (1H, ddd, $J = 7.7$, 7.4, 6.6 Hz, H-5), 5.13 (1H, d, $J = 10.0$ Hz, H-15), 5.25 (1H, d, $J = 9.8$ Hz, H-9), 5.59 (1H, dt, $J = 15.9$, 7.9 Hz, H-12), 5.76 (1H, dd, $J = 6.6$, 15.9 Hz, H-6), 6.01 (1H, d, $J = 15.9$ Hz, H-13), 6.07 (1H, dd, $J = 9.8$, 1.9 Hz, H-2), 6.65 (1H, d, $J = 15.9$ Hz, H-7), 6.90 (1H, dt, $J = 9.8$, 4.2 Hz, H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 10.9 (q, C-22), 11.1 (q, 18-CH_3), 13.1 (q, 14-CH_3), 13.4 (q, 8-$CH_2\underline{C}H_3$), 14.2 (q, 20-CH_3), 16.2 (q, 16-CH_3), 20.7 (q, 10-$\underline{C}H_3$), 25.8 (t, C-21), 26.4 (t, 8-$\underline{C}H_2CH_3$), 30.1 (t, C-4), 32.1 (d, C-10), 36.7 (d, C-20), 40.7 (t, C-11), 45.7 (d, C-16, C-18), 74.5 (d, C-19), 78.6 (d, C-5), 121.7 (d, C-2), 124.8 (d, C-6), 127.7 (d, C-12), 128.3 (d, C-15), 129.7 (d, C-7), 135.3 (d, C-13), 135.4 (s, C-8), 136.2 (s, C-14), 137.2 (d, C-9), 144.6 (d, C-3), 164.0 (s, C-1), 216.4 (s, C-17). FAB–MS *m/z*: 457 $(M+H)^+$. FAB–HRMS *m/z*: calcd for $C_{29}H_{45}O_4$: 457.3318; found: 457.3330.

Preparation of 5c and 5d. A solution of *n*-BuLi (1.54 M in *n*-hexane, 7.7 mL, 12.0 mmol) was added to a solution of DMSO (0.42 mL) in dry toluene (7.1 mL) at rt, then the whole was stirred for 45 min. A solution of **4c** (309 mg, 0.60 mmol) and **3a** (254 mg, 1.49 mmol) in dry toluene (6.9 mL) was added to the solution of dimesylcarbanion at −78 °C, then the whole was stirred warming from −60 °C to 0 °C overnight. Work up in the same manner as preparation for **5b** gave a product, which was purified by column chromatography (SiO_2 50 g, *n*-hexane:EtOAc = 8:1) to furnish **5c** (172 mg, 75%). The same procedure as preparation for **5c** was conducted using **4d** (213 mg, 0.40 mmol) and **3a** (171 mg, 1.00 mmol) giving a product, which was purified by column chromatography (SiO_2 7 g, *n*-hexane:EtOAc = 10:1) to furnish **5d** (130 mg, 81%). Compound **5c**: colorless oil; IR ν_{max} (KBr) cm^{-1}: 2926, 2899, 2864, 1512, 1248, 1101, 1032, 993. ^1H NMR (300 MHz, $CDCl_3$) δ: 0.92 (3H, d, $J = 6.7$ Hz, 10-CH_3), 1.09, 1.14 (both 3H, d, $J = 6.1$ Hz, 1-OCH($C\underline{H}_3$)$_2$), 1.40 (1H, m, H-11), 1.60 (1H, m, H-11), 1.96 (2H, m, H-4), 2.77 (1H, m, H-10), 3.30 (2H, m, H-12), 3.73 (3H, s, $CH_2PhOC\underline{H}_3$), 3.89–3.97 (1H, m, 1-OC$\underline{H}$(CH_3)$_2$), 4.28, 4.34 (both 1H, d, $J = 11.6$ Hz, $C\underline{H}_2PhOCH_3$), 4.41 (1H, m, H-5), 5.04 (1H, s, H-1), 5.13 (1H, t, $J = 10.4$ Hz, H-9), 5.66 (2H, m, H-2, 6), 5.90 (2H, m, H-3, 8), 6.50 (1H, dd, $J = 15.3$, 10.7 Hz, H-7), 6.79, 7.17 (both 2H, d, $J = 8.5$ Hz, $CH_2\underline{Ph}OCH_3$). FAB–MS *m/z*: 409 $(M+Na)^+$. FAB–HRMS *m/z*: calcd for $C_{24}H_{34}O_4Na$: 409.2555; found: 409.2303. Compound **5d**: colorless oil; IR ν_{max} (KBr) cm^{-1}: 2969, 2930, 2878, 1400, 1248, 1100, 1032, 1001. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.05 (3H, t, $J = 7.3$ Hz, 8-$CH_2C\underline{H}_3$), 1.17, 1.24 (both 3H, d, $J = 6.1$ Hz, 1-OCH($C\underline{H}_3$)$_2$), 1.63 (2H, m, H-11), 2.02–2.32 (6H, m, H-4, H-10, 8-$C\underline{H}_2CH_3$), 3.44 (2H, t, $J = 6.1$ Hz, H-12), 3.80 (3H, s, $CH_2PhOC\underline{H}_3$), 4.01 (1H, m, 1-OC$\underline{H}$(CH_3)$_2$), 4.42 (2H, s, $C\underline{H}_2PhOCH_3$), 4.51 (1H, m, H-5), 5.12 (1H, s, H-1), 5.36 (1H, t, $J = 7.3$ Hz, H-9), 5.75 (2H, m, H-2, 6), 6.00 (1H, m, H-3), 6.60 (1H, d, $J = 15.9$ Hz, H-7), 6.87, 7.25 (both 2H, d, $J = 8.5$ Hz, $CH_2\underline{Ph}OCH_3$). FAB–MS *m/z*: 423 $(M+Na)^+$. FAB–HRMS *m/z*: calcd for $C_{25}H_{36}O_4Na$: 423.2512; found: 423.2523.

Preparation of 17c and 17d. A solution of **5c** (154 mg, 0.40 mmol) in acetone:H_2O (5:1) (47 mL) was treated with Dowex HCR-W2 (H^+ form) (2.0 g) at 40 °C for 5 h. Work up in the same manner as preparation for **12** gave a lactol. A solution of the lactol in dry benzene (61 mL) was treated with Ag_2CO_3 (2.2 g, 8.0 mmol) and Celite (1.1 g) at 45 °C for 6 h in the dark. Work up in the same manner as preparation for **12** gave a product, which was purified by column chromatography (SiO_2 5 g, *n*-hexane:EtOAc = 2:1) to furnish a lactone (130 mg). A solution of the lactone in CH_2Cl_2:*t*-BuOH: pH 6.9 phospate buffer (90:1:9) (17.5 mL) was treated with DDQ (173 mg, 0.76 mmol) at rt for 30 min. Work up in the same manner as preparation for **17a** gave a product, which was purified by column chromatography (SiO_2 4 g, *n*-hexane:EtOAc = 1:1) to furnish **17c** (68.4 mg, 81%). The same procedure as preparation for **17c** was conducted using **5d** (100 mg, 0.25 mmol) giving a product, which was purified by column chromatography (SiO_2 5 g, *n*-hexane:EtOAc = 3:2) to furnish **17d** (44.8 mg, 76%). Compound **17c**: colorless oil; $[\alpha]_D$ +77.5° ($c = 0.70$, $CHCl_3$, 25 °C). IR ν_{max} (KBr) cm^{-1}: 3402, 2961, 2930, 1715, 1383, 1250, 1046. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.01 (3H, d, $J = 6.6$ Hz, 10-CH_3), 1.47 (1H, m, H-11), 1.65 (1H, m, H-11), 2.09 (1H, s, 12-OH), 2.47 (2H, m, H-4), 2.84 (1H, m, H-10), 3.57 (1H, m, H-12), 3.61 (1H, m, H-12), 4.99 (1H, ddd, $J = 10.7$, 6.6, 5.1 Hz, H-5), 5.33 (1H, t, $J = 10.4$ Hz, H-9), 5.73 (1H, dd, $J = 6.6$, 15.2 Hz, H-6), 5.97 (1H, dd, $J = 11.2$, 10.4 Hz, H-8), 6.04 (1H, d, $J = 9.9$, Hz, H-2), 6.67 (1H, dd, $J = 15.2$, 11.2 Hz, H-7), 6.91 (1H, ddd, $J = 9.9$, 5.1, 3.0 Hz, H-3). FAB–MS *m/z*: 223 $(M+H)^+$. FAB-HRMS *m/z*: calcd for $C_{13}H_{19}O_3$: 223.1334; found: 223.1320. Compound **17d**: colorless oil; $[\alpha]_D$ +27.0° ($c = 1.74$, $CHCl_3$, 20 °C). IR ν_{max} (KBr) cm^{-1}: 3437, 2967, 2934, 2874, 1719, 1383, 1248, 1055, 1020, 966. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.06 (3H, t, $J = 7.3$ Hz, 8-$CH_2C\underline{H}_3$), 1.66 (2H, m, H-11), 2.20 (2H, q, $J = 7.3$ Hz, 8-$C\underline{H}_2C\underline{H}_3$), 2.27 (2H, m, H-10), 2.49 (2H, m, H-4), 3.65 (2H, t, $J = 6.7$ Hz, H-12), 5.02 (1H, ddd, $J = 7.3$, 7.3, 7.3 Hz, H-5), 5.47 (1H, t, $J = 7.3$ Hz, H-9), 5.78 (1H, dd, $J = 7.3$, 15.9 Hz, H-6), 6.07 (1H, d, $J = 9.8$, Hz, H-2), 6.70 (1H, d, $J = 15.9$ Hz, H-7), 6.91 (1H, dt, $J = 9.8$, 4.3 Hz, H-3). FAB–MS *m/z*: 259 $(M+Na)^+$. FAB–HRMS *m/z*: calcd for $C_{14}H_{20}O_3Na$: 259.1310; found: 259.1329.

Preparation of 19c and 19d. A solution of **17c** (3.6 mg, 0.016 mmol) in dry CH_2Cl_2 (0.43 mL) was treated with Dess–Martin periodinane (20.2 mg, 0.048 mmol) at rt

for 30 min. Work up in the same manner as preparation for **18a** gave **18c**. A solution of *n*-BuLi (1.54 M in *n*-hexane, 0.065 mL, 0.043 mmol) was added to a solution of DMSO (0.18 mL) in dry toluene (0.5 mL) at rt, then the whole was stirred for 45 min. A solution of **18c** (3.6 mg, 0.016 mmol) and **10** (16 mg, 0.026 mmol) in dry toluene (0.5 mL) was added to the solution of dimesylcarbanion at −78 °C, then the whole was stirred warming from −60 °C to 0 °C overnight. Work up in the same manner as preparation for **19a** gave a product, which was purified by column chromatography (SiO_2 2 g, *n*-hexane:EtOAc = 4:1) to furnish **19c** (5.9 mg, 66%). The same procedure as preparation for **19c** was conducted using **17d** (4.0 mg, 0.017 mmol) giving a product, which was purified by column chromatography (SiO_2 4 g, *n*-hexane:EtOAc = 3:1) to furnish **19d** (6.2 mg, 65%). Compound **19c**: colorless oil; $[\alpha]_D$ +59.9° (*c* = 0.25, $CHCl_3$, 20 °C). IR ν_{max} (KBr) cm^{-1}: 3501, 2963, 2928, 1725, 1460, 1383, 1256, 1053, 837. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.07, 0.08 (both 3H, s, 19-OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.83 (3H, t, *J* = 7.3 Hz, H-22), 0.85 (3H, d, *J* = 7.3 Hz, 20-CH_3), 0.87 (3H, d, *J* = 7.3 Hz, 18-CH_3), 0.97 (9H, s, 19-OSi(CH_3)$_2$C($C\underline{H}_3$)$_3$), 0.99 (3H, d, *J* = 6.7 Hz, 16-CH_3), 1.03 (3H, d, *J* = 6.1 Hz, 10-CH_3), 0.95–1.15 (3H, m, H-20, H-21), 1.54 (1H, m, H-18), 1.72 (3H, s, 14-CH_3), 2.09 (2H, m, H-11), 2.45 (2H, m, H-4), 2.58 (1H, m, H-16), 2.69 (1H, m, H-10), 3.36 (1H, br d, *J* = 9.2 Hz, H-19), 3.63 (1H, m, H-17), 4.97 (1H, ddd, *J* = 9.1, 6.7, 6.4 Hz, H-5), 5.07 (1H, d, *J* = 10.4 Hz, H-15), 5.37 (1H, dd, *J* = 11.0, 10.4 Hz, H-9), 5.49 (1H, dt, *J* = 15.9, 7.3 Hz, H-12), 5.72 (1H, dd, *J* = 15.3, 6.7 Hz, H-6), 5.94 (1H, t, *J* = 11.0 Hz, H-8), 5.99 (1H, d, *J* = 15.9 Hz, H-13), 6.01 (1H, d, *J* = 9.8 Hz, H-2), 6.63 (1H, dd, *J* = 15.3, 11.0 Hz, H-7), 6.89 (1H, ddd, *J* = 9.8, 4.9, 3.7 Hz, H-3). FAB–MS *m*/*z*: 567 $(M+Na)^+$. FAB–HRMS *m*/*z*: calcd for $C_{33}H_{56}O_4SiNa$: 567.3846; found: 567.3836. Compound **19d**: colorless oil; $[\alpha]_D$ +14.9° (*c* = 0.84, $CHCl_3$, 21 °C). IR ν_{max} (KBr) cm^{-1}: 3511, 2959, 2930, 2851, 1726, 1462, 1383, 1252, 1055, 1020, 964. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.07, 0.08 (both 3H, s, 19-OSi($C\underline{H}_3$)$_2$C(CH_3)$_3$), 0.82 (3H, d, *J* = 6.7 Hz, 20-CH_3), 0.83 (3H, d, *J* = 6.7 Hz, 18-CH_3), 0.87 (3H, t, *J* = 6.7 Hz, H-22), 0.91 (9H, s, 19-OSi (CH_3)$_2$C($C\underline{H}_3$)$_3$), 1.03 (3H, d, *J* = 6.7 Hz, 16-CH_3), 1.05 (3H, t, *J* = 7.3 Hz, 8-$CH_2C\underline{H}_3$), 1.09 (1H, m, H-21), 1.54 (2H, m, H-20, H-21), 1.73 (3H, s, 14-CH_3), 1.75 (1H, m, H-18), 2.15–2.31 (6H, m, 8-$C\underline{H}_2CH_3$, H-10, H-11), 2.47 (2H, m, H-4), 2.57 (1H, m, H-16), 3.37 (1H, br d, *J* = 9.2 Hz, H-19), 3.64 (1H, dd, *J* = 3.7, 4.3 Hz, H-17), 5.00 (1H, ddd, *J* = 7.3, 7.3, 6.7 Hz, H-5), 5.09 (1H, d, *J* = 9.8 Hz, H-15), 5.47 (1H, t, *J* = 7.3 Hz, H-9), 5.55 (1H, dt, *J* = 15.9, 6.7 Hz, H-12), 5.77 (1H, dd, *J* = 15.9, 6.7 Hz, H-6), 6.04 (1H, d, *J* = 15.9 Hz, H-13), 6.06 (1H, d, *J* = 9.8 Hz, H-2), 6.66 (1H, d, *J* = 15.9 Hz, H-7), 6.89 (1H, dt, *J* = 9.8, 4.9 Hz, H-3). FAB–MS *m*/*z*: 559 $(M+H)^+$. FAB–HRMS *m*/*z*: calcd for $C_{34}H_{59}O_4Si$: 559.4183; found: 559.4185.

Preparation of 8-deethylcallystatin A (21) and 10-demethylcallystatin A (22). A solution of **19c** (3.1 mg, 0.006 mmol) in dry CH_2Cl_2 (1.0 mL) was treated with Dess–Martin periodinane (7.3 mg, 0.17 mmol) at rt for 1.5 h. The reaction mixture was worked up in the same manner as preparation for **20** to give a ketone. To a solution of the ketone in dry THF (0.78 mL) was added HF:pyridine (5:1) (0.49 mL) at 0 °C, then the whole was stirred at rt for 80 h. Work up in the same manner as preparation for **12** gave a product, which was purified by HPLC (column: COSMOSIL 5SL (10 mm i.d. ×250 mm), mobile phase: *n*-hexane:EtOAc = 2:1, detection: UV (λ = 250 nm), flow rate: 3.0 mL/min) to furnish 8-deethylcallystatin A (**21**, 2.2 mg, 85%).[26] The same procedure as preparation for **21** was conducted using **19d** (6.0 mg, 0.011 mmol) giving a product, which was purified by HPLC (*n*-hexane:EtOAc = 2:1) to furnish 10-demethylcallystatin A (**22**, 3.2 mg, 80%).[26] 8-Deethylcallystatin A (**21**): colorless oil; $[\alpha]_D$ −85.7° (*c* = 0.22, MeOH, 27 °C). UV λ_{max} (MeOH) nm (ε): 231 (26700), 289 (3070). CD (MeOH) nm (Δε): 331 (0), 300 (−16.1), 268 (0), 247 (+31.7), 228 (0), 220 (−7.1), 210 (−3.7). IR ν_{max} (KBr) cm^{-1}: 3503, 2957, 2928, 2855, 1732, 1715, 1458, 1381, 1258, 1032. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.85 (3H, t, *J* = 6.3 Hz, H-22), 0.88 (3H, d, *J* = 6.7 Hz, 20-CH_3), 0.99 (3H, d, *J* = 6.7 Hz, 10-CH_3), 1.10 (1H, m, H-21), 1.11 (3H, d, *J* = 7.3 Hz, 18-CH_3), 1.14 (3H, d, *J* = 6.7 Hz, 16-CH_3), 1.16–1.46 (2H, m, H-20, 21), 1.83 (3H, s, 14-CH_3), 2.10 (2H, t, *J* = 6.7 Hz, H-11), 2.47 (2H, m, H-4), 2.70 (1H, m, H-10), 2.85 (1H, dq, *J* = 7.3, 4.5 Hz, H-18), 3.57 (1H, dd, *J* = 6.7, 4.5 Hz, H-19), 3.65 (1H, dq, *J* = 10.2, 6.7 Hz, H-16), 4.97 (1H, ddd, *J* = 10.5, 6.4, 4.4 Hz, H-5), 5.13 (1H, d, *J* = 10.2 Hz, H-15), 5.35 (1H, dd, *J* = 11.1, 10.2 Hz, H-9), 5.59 (1H, dt, *J* = 15.2, 6.7 Hz, H-12), 5.72 (1H, dd, *J* = 15.2, 6.4 Hz, H-6), 5.95 (1H, t, *J* = 11.1 Hz, H-8), 6.03 (1H, d, *J* = 15.2 Hz, H-13), 6.06 (1H, dt, *J* = 9.6, 1.7 Hz, H-2), 6.62 (1H, dd, *J* = 15.2, 11.1 Hz, H-7), 6.89 (1H, ddd, *J* = 9.6, 4.4, 3.7 Hz, H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 10.9 (q, C-22), 11.2 (q, 18-CH_3), 13.1 (q, 14-CH_3), 14.2 (q, 20-CH_3), 16.1 (q, 16-CH_3), 20.6 (q, 10-CH_3), 25.8 (t, C-21), 29.9 (t, C-4), 32.8 (d, C-10), 36.7 (d, C-20), 40.5 (t, C-11), 45.7 (d, C-16), 45.8 (d, C-18), 74.5 (d, C-19), 77.8 (d, C-5), 121.7 (d, C-2), 125.9 (d, C-6), 127.5 (d, C-12), 128.5 (d, C-15), 128.7 (d, C-9), 128.9 (d, C-7), 135.5 (d, C-13), 136.2 (s, C-14), 140.3 (d, C-8), 144.6 (d, C-3), 163.9 (s, C-1), 216.4 (s, C-17). FAB–MS *m*/*z*: 429 $(M+H)^+$. FAB–HRMS *m*/*z*: calcd for $C_{27}H_{41}O_4$: 429.3005; found: 429.2991. 10-Demethylcallystatin A (**22**): colorless oil; $[\alpha]_D$ −186.2° (*c* = 0.37, MeOH, 21 °C). UV λ_{max} (MeOH) nm (ε): 243 (36900), 291 (3600). CD (MeOH) nm (Δε): 335 (−0.2), 300 (−23.2), 266 (0), 246 (+19.2), 220 (+2.8), 210 (+6.1). IR ν_{max} (KBr) cm^{-1}: 3497, 2965, 2932, 2876, 1707, 1456, 1383, 1244, 1055, 1022, 965. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.85 (3H, t, *J* = 7.4 Hz, H-22), 0.90 (3H, d, *J* = 6.7 Hz, 20-CH_3), 1.05 (3H, t, *J* = 7.4 Hz, 8-$CH_2C\underline{H}_3$), 1.07 (1H, m, H-21), 1.12 (3H, d, *J* = 7.1 Hz, 18-CH_3), 1.15 (3H, d, *J* = 6.7 Hz, 16-CH_3), 1.31–1.44 (2H, m, H-20, H-21), 1.83 (3H, s, 14-CH_3), 2.20 (2H, t, *J* = 6.7 Hz, H-11), 2.26 (4H, m, 8-$C\underline{H}_2CH_3$, H-10), 2.47 (2H, m, H-4), 2.86 (1H, dq, *J* = 4.3, 7.1 Hz, H-18), 3.57 (1H, m, H-19), 3.65 (1H, dq, *J* = 10.2, 6.7 Hz, H-16), 5.00 (1H, ddd, *J* = 7.4, 7.4, 6.7 Hz, H-5), 5.16 (1H, d, *J* = 10.2 Hz, H-15), 5.45 (1H, t, *J* = 7.6 Hz, H-9), 5.66 (1H, dt, *J* = 15.7, 6.7 Hz, H-12), 5.77 (1H, dd, *J* = 6.7, 16.0 Hz, H-6), 6.05 (1H, d, *J* = 15.7 Hz, H-13), 6.07 (1H, d, *J* = 9.8 Hz, H-2), 6.65 (1H, d, *J* = 16.0 Hz, H-7), 6.90 (1H, dt, *J* = 9.8, 4.3 Hz, H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 10.9 (q, C-22),

11.1 (q, 18-CH_3), 13.0 (q, 14-CH_3), 13.3 (q, 8-$CH_2\underline{C}H_3$), 14.3 (q, 20-CH_3), 16.3 (q, 16-CH_3), 25.8 (t, C-21), 26.4 (t, 8-$\underline{C}H_2CH_3$), 27.5 (t, C-11), 30.1 (t, C-4), 32.9 (t, C-10), $\overline{36}$.7 (d, C-20), 45.7 (d, C-18), 45.8 (d, C-16), 74.5 (d, C-19), 78.7 (d, C-5), 121.8 (d, C-2), 125.0 (d, C-6), 128.4 (d, C-15), 128.9 (d, C-12), 129.5 (d, C-7), 130.4 (s, C-8), 134.5 (d, C-13), 136.1 (s, C-14), 136.9 (d, C-9), 144.5 (d, C-3), 164.0 (s, C-1), 216.4 (s, C-17). FAB–MS *m/z*: 443 $(M+H)^+$. FAB–HRMS *m/z*: calcd for $C_{28}H_{43}O_4$: 443.3161; found: 443.3154.

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 a 5% CO_2 atmosphere at 37 °C, 25 μL of MTT solution (2 mg/mL in PBS) was added to each well and incubated for a 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.

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25. Compound **3a** consists of two diastereomers on C-1 in a ratio of 8:1. Since all compounds with 1-acetal and/or 8-ene functions except for the four analogues (**12**, **20**, **21** and **22**) contained stereoisomers, 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**).
26. The yields were determined by taking into account that minor 8-*E* isomers given by Still–Wittig condensation were removed with final HPLC separation.