Strongylophorine-26, an Inhibitor of Cancer Cell Invasion: SAR Revealed by Synthesis of Analogues

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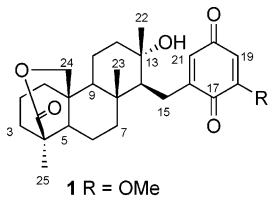
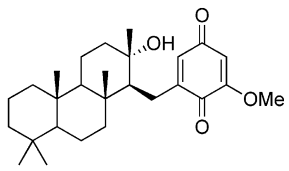
The absolute configuration of strongylophorine-26 (**1**) was determined to be 4*S*, 5*R*, 8*R*, 9*S*, 10*S*, 13*S*, 14*S* by single-crystal X-ray diffraction analysis of the derivative **7** prepared from the co-occurring metabolite strongylophorine-8 (**4**) and chemical interconversion to the bislactone **8**. Synthetic analogues (+)- and (−)-**3** have been prepared in order to explore the structure−activity relationship for the anti-invasion pharmacophore of stronglylophorine-26. These studies revealed the unanticipated importance of the A ring lactone moiety for the anti-invasion activity of **1**.

The ability of cells to invade adjacent tissues is a biological phenomenon that is crucial for angiogenesis and metastasis. Under the control of angiogenic factors produced by tumor cells, vascular endothelial cells invade solid tumor masses to establish new blood vessels that supply nutrients and oxygen to tumor cells. These newly formed blood vessels can also provide a conduit for metastatic spread, a process that requires the tumor cells themselves to become invasive. Thus, inhibitors of cell invasion may be useful in cancer therapy by preventing both neoangiogenesis and metastasis.[1] In the course of our ongoing search for anti-invasive natural products from marine organisms, strongylophorine-26 (**1**) was found in the lipophilic extract of the marine sponge *Petrosia* (*Strongylophora*) *corticata* as an inhibitor of cell invasion by MDA-MB-231 breast carcinoma cells.[2] Investigation of the biological mechanism revealed that **1** induces nonpolarized lamellipodial extensions, resulting in inhibition of cell motility, and that the anti-invasive activity of **1** depends on the small GTPase Rho.[3] Other inhibitors of cell migration such as fumagillin, TNP-470, thronbospondin-1, endostatin, EMAP II, and dihydromotuporamine C[4,5] all cause an induction of actin stress fibers. By contrast, **1** decreases actin stress fibers and increases focal adhesions while causing a dense meshwork of actin filaments to form at the cell periphery, indicating a distinct mechanism of action.

Our interest in identifying the structural features responsible for this unique cellular response and the molecular target of the compound required the availability of larger amounts of **1** than were available from the natural sponge source, so we turned to synthesis to provide the material. At the outset, our hypothesis was that the quinone substructure was an essential part of the anti-invasive pharmacophore, that the diterpenoid skeleton and the tertiary alcohol at C-13 were also likely important, but that the A ring lactone was probably not critical. On the basis of this hypothesis, the strongylophorine-26 analogue **3** was chosen as the synthetic target. This analogue had the advantage of being much easier to synthesize than **1** because installation of the A ring lactone was viewed as a significant challenge. As part of our synthetic efforts, we planned to investigate the relative bioactivities of the two enantiomers of **3** to determine if the configuration of the terpenoid fragment was important. In order to know which enantiomer of **3** corresponded to the natural product, it was necessary to first determine the absolute configuration of strongylophorine-26 (**1**). Herein, we describe the determination of the absolute configuration of **1**, the synthesis of its analogues (+)- and (−)-**3**, and some unanticipated SAR for its anti-invasive pharmacophore.



1 R = OMe
2 R = H



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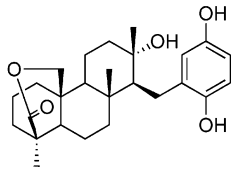

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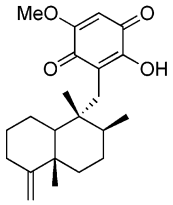

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Results and Discussion

The absolute configuration of **1** was determined via chemical degradation and interconversion experiments using strongylophorine-8 (**4**), which was isolated along with **1** from *P. corticata*.[2] Treatment of **4** with *tert*-butyldimethylsilyl chloride followed by benzyl bromide yielded **7** as needle-like crystals (Scheme 1). Single-crystal X-ray diffraction analysis of **7** revealed that its relative configuration was 4*S**, 5*R**, 8*R**, 9*S**, 10*S**, 13*S**, and 14*S** (Supporting Information). The absolute configuration of **7** was determined on the basis of the refined Flack parameter value, 0.12(9).[6] Ideally this value should be 0 for the correct configuration or 1 for its enantiomer. Values between 0 and 1 may indicate racemic twinning; however, in this case modeling the structure as a racemic twin did not improve the refinement (i.e., R1 values) at all. The slightly elevated Flack value is likely due to weak anomalous dispersion factors for the heaviest atom, Si. Therefore, the absolute configuration of **4** is 4*S*, 5*R*, 8*R*, 9*S*, 10*S*, 13*S*, and 14*S*. Since strongylophorine-8 (**4**) and strongylophorine-26 (**1**) come from the same extract and they have identical diterpenoid fragments, it was assumed that they had the same absolute configuration. In order to confirm this assumption, strongylophorine-8 (**4**) was oxidatively degraded by treatment with $RuCl_3$ and $NaIO_4$, yielding the optically active bislactone **8** ($[\alpha]_D^{23}$ −46 (*c* 0.13 CH_2Cl_2)). When strongylophorine-26 (**1**) was chemically degraded using the same $RuCl_3$ and $NaIO_4$ treatment, the reaction product was identical by ^1H NMR comparison. The small amount of bislactone **8** obtained from **1**

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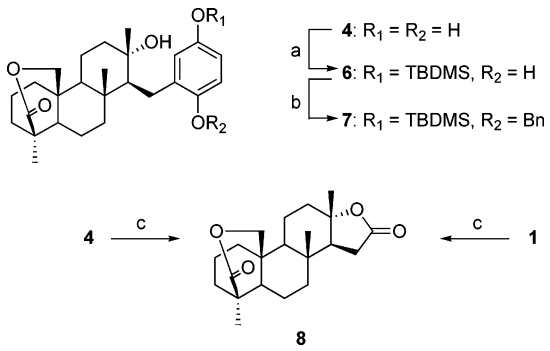
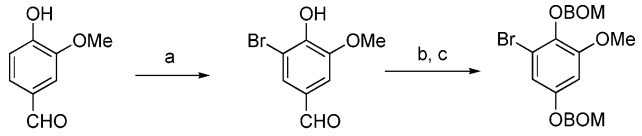
Scheme 1[a]



[a] Reagents and conditions: (a) TBDMSCl, *i*-Pr_2NEt, pyridine, THF, rt, 59%; (b) BnBr, NaH, THF, 0 °C to rt, 81%; (c) $RuCl_3$, $NaIO_4$, CCl_4, CH_3CN, H_2O, rt.

Scheme 2[a]



[a] Reagents and conditions: (a) Br_2, MeOH, rt; (b) H_2O_2, NaOH, H_2O, rt; (c) BOMCl, DMAP, *i*-Pr_2NEt, CH_2Cl_2, rt, 27% for 2 steps.

precluded an accurate determination of the magnitude of its specific rotation; however, it was possible to ascertain that it had the same sign of optical rotation as the bislactone **8** obtained from strongylophorine-8 (**4**). Therefore, strongylophorine-26 (**1**) has been assigned the absolute configuration 4*S*, 5*R*, 8*R*, 9*S*, 10*S*, 13*S*, and 14*S*.

A biomimetic approach was adopted for the synthesis of the strongylophorine analogue **3** missing the A ring lactone. The tricyclic diterpenoid fragment of **3** was constructed from geranylgeranylacetate (**12**) via polyene cyclization, while the quinone moiety was synthesized from vanillin (**9**) (Schemes 2 and 3). Bromination of vanillin (**9**) was carried out as described in the literature,[7] yielding 5-bromovanillin (**10**). Baeyer−Villiger oxidation of **10** under alkaline conditions generated the bromohydroquinone, whose phenolic groups were subsequently protected with BOM, affording the bromobenzene **11**.

The racemic diol **13** was synthesized following a literature precedent[8] involving cyclization of geranylgeranylacetate (**12**) with mercury ditrifluoroacetate and *N*,*N*-dimethylaniline, followed by treatment with KBr, and reduction in sequence (Scheme 3). The primary hydroxyl group of **13** was protected as a TBDMS ether, while the tertiary hydroxyl group was protected with BOM. Deprotection of the primary hydroxyl group of **15** with tetrabutylammonium fluoride followed by PCC oxidation yielded the aldehyde **17**, the precursor for the subsequent coupling reaction.

Treatment of **11** with *tert*-butyllithium generated the corresponding phenyllithium reagent, which was coupled with the aldehyde **17** to furnish a mixture of the alcohols **18a** and **18b** (C-15 epimers) in the ratio 1:1. After acylation of the secondary hydroxyl groups of **18a** and **18b** with trifluoroacetic anhydride, the resulting ester mixture was hydrogenated to afford the hydroquinone, which spontaneously oxidized to the quinone **3** in air. In order to obtain the individual enantiomers of **3**, chiral HPLC separation of one of the epimers of **18** was performed on Chiralpak IA (DAICEL), resulting in two distinct peaks corresponding to (+)- and (−)-**18a**. Acylation of (+)- and (−)-**18a** followed by hydrogenation in a similar manner to racemic **18** afforded (+)- and (−)-**3**, respectively.

The ^1H NMR spectrum of quinone **3** showed two sets of minor signals in the regions of δ 2.35−2.75, 3.70−3.72, and 5.23−5.94, which mirrored the ^1H NMR data of strongylophorine-26 (**1**). Analysis of COSY, HMQC, and HMBC correlations for **3** indicated the existence of a tautomeric equilibrium between the open form **3** and the two cyclic forms **3a**/**b** (Scheme 4).[9] The open tautomer, the major cyclic form, and the minor cyclic form were present in the ratio 6.1:2.2:1, respectively. Since the same equilibrium was earlier observed for strongylophorine-26 (**1**), it appears that the absence of the A ring γ-lactone of **1** does not affect the reactivity of the quinone moiety.

In the CD spectrum, (+)-**3** showed a positive Cotton effect around 280 nm, which can be attributed to the configuration of the chiral centers around the quinone moiety (Supporting Information). Since strongylophorine-26 (**1**) also exhibited a positive Cotton effect around 280 nm in the CD spectrum (Supporting Information), (+)-**3** must have the same absolute configuration as **1**, while (−)-**3** must be the antipode.

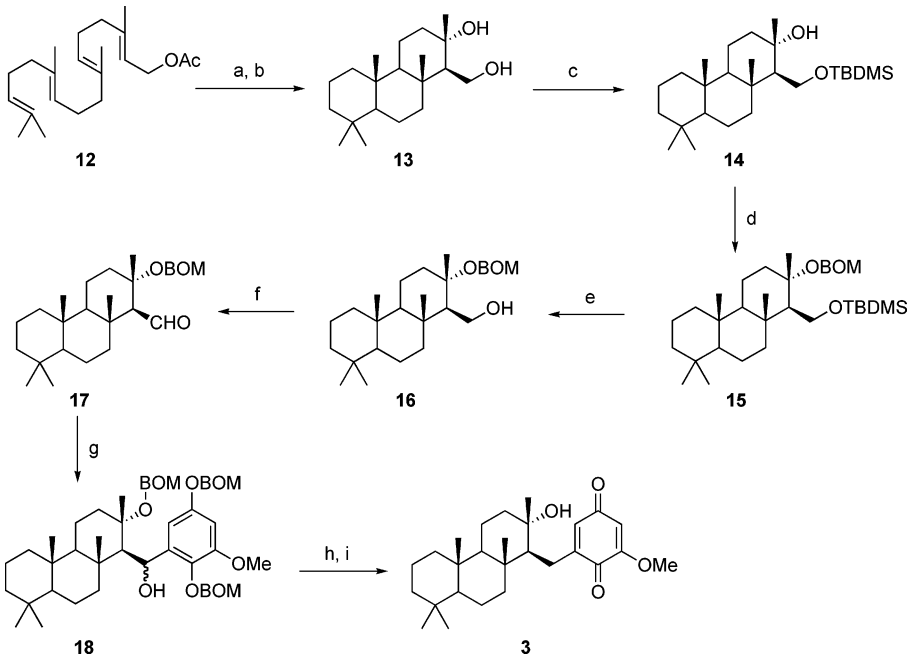
In the initial study of the structure and bioactivity of strongylophorine (**1**), it was found that strongylophorine-8 (**4**) and the corresponding quinone **2** were active in the cell-based anti-invasion assay, but with IC_{50}'s roughly an order of magnitude greater than **1**, while ilimaquinone (**5**) was completely inactive.[2] It was assumed that the similar activities of **2** and **4** resulted from the hydroquinone **4** being oxidized to the quinone **2** during the assay. On the basis of relative potencies of **1** and **2**, it was apparent that the methoxy substituent on the quinone in **1** enhanced the anti-invasive activity. The lack of activity in ilimaquinone (**5**) seemed to suggest that the structure of the terpenoid fragment in **1** and **2** was also important. When the anti-invasive activity of synthetic (+)- and (−)-**3** was examined in the cell-based assay using MDA-MB-231 cells, it was found that neither enantiomer showed anti-invasion activity at concentrations below their IC_{50}'s for cytotoxicity ($\approx$0.3 μg/mL for both enantiomers). This result suggests that removal of the A ring lactone from **1** has diminished its selectivity for binding to the anti-invasion molecular target, and as a consequence, the synthetic analogues **3** display only cytotoxicity to cancer cells. Therefore, in strongylophorine-26 (**1**) the A ring lactone and the methoxy-substituted quinone moieties are both indispensable components of its anti-invasion pharmacophore, in contrast to our initial hypothesis.

Experimental Section

General Experimental Procedures. Synthetic reagents were obtained from commercial sources and were used without further purification. THF was distilled from sodium/benzophenone under Ar gas before use. CH_2Cl_2 was distilled from CaH_2 under N_2 gas. Chemical reactions were monitored on Si gel 60 F-254 precoated aluminum plates (Merk). Normal-phase chromatography was carried out on 120−230 mesh Si gel. Measurement of melting points was performed on a Fisher-Johns melting point apparatus. Optical rotations were measured with a JASCO P-1010 polarimeter. UV absorptions were recorded on a Waters 2487 dual λ absorbance detector. CD spectra ware obtained using a JASCO J-700 spectropolarimeter. Proton and carbon NMR spectra were obtained using Bruker AM-400, AMX-500, or AV-600 spectrometers. ESIMS and EIMS spectra were obtained using an ESI micromass LCT spectrometer and a Kratos MS-50 mass spectrometer, respectively.

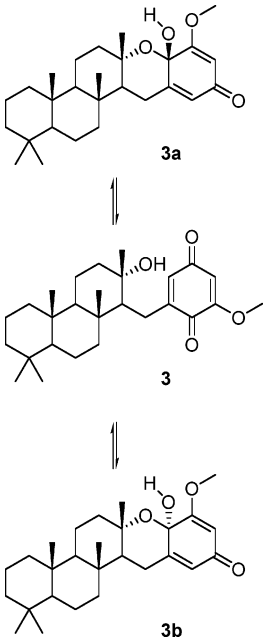
Compound 6. Strongylophorine-8 (**4**) (22.9 mg, 53.8 μmol) was dissolved in a mixed solvent system of THF (0.5 mL), *N*,*N*-diisopropylethylamine (1.5 mL), and pyridine (2.5 mL). To this solution, *t*-$BuMe_2SiCl$ (157.8 mg, 1.047 mmol) was added. After the reaction solution was stirred at room temperature for 12 h, additional *t*-$BuMe_2$-SiCl (70.0 mg, 0.464 mmol) was added and the reaction solution was stirred for an additional 7.5 h. The reaction was quenched with saturated aqueous $NaHCO_3$, and the resulting solution was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$, passed through a cotton filter to remove $MgSO_4$, and concentrated in vacuo. The residue was purified on a Si gel column, eluted in a stepwise gradient manner with EtOAc/hexanes (0:100 to 40:60) to yield **6** (17.1 mg, 59% yield) as a colorless solid: ^1H NMR (DMSO-d_6) δ 9.16 (s, 17-OH), 6.69 (d, J = 2.8 Hz, H21), 6.53 (d, J = 8.6 Hz, H18), 6.44 (dd, J = 2.8, 8.6 Hz, H19), 5.36 (s, 13-OH), 4.57 (d, J = 12, H24), 3.97 (d, J = 12 Hz, H24), 2.60 (d, J = 15 Hz, H15), 2.48 (m, H15),

Scheme 3[a]



[a] Reagents and conditions: (a) $Hg(OTf)_2$, *N*,*N*-dimethylaniline, $MeNO_2$, then KBr.; (b) NaOH, $NaBH_4$, EtOH/CH_2Cl_2/H_2O (5:5:2); (c) TBDMSCl, DMAP, Et_3N, CH_2Cl_2, rt, 62%; (d) BOMCl, DMAP, *i*-Pr_2NEt, CH_2Cl_2, rt, 95%; (e) TBAF, THF, 60−70 °C, 96%; (f) PCC, AcONa, CH_2Cl_2; (g) **11**, *t*-BuLi, THF, −78 °C, 65% (**18a**:**18b** = 1:1); (h) H_2, $Pd(OH)_2$, EtOH/AcOEt (1:4), rt; (i) [O], 50% for 2 steps from **18a**.

Scheme 4. Possible Tautomeric Equibilium of **3**



2.08 (d, *J* = 12, H1), 1.81 (d, *J* = 13 Hz, H12), 1.73 (d, *J* = 14 Hz, H7), 1.69 (d, *J* = 13 Hz, H11), 1.63 (m, H2), 1.61 (m, H3), 1.49 (m, H6), 1.47 (m, H14), 1.43 (m, H12), 1.43 (m, H2), 1.41 (m, H3), 1.19 (m, H5), 1.16 (m, H6), 1.13 (s, H22), 1.03 (m, H1), 1.01 (m, H9), 1.00 (s, H25), 0.99 (s, H23), 0.92 (s, H4′, H5′, H6′), 0.62 (dd, *J* = 10, 10 Hz, H7), 0.12 (s, H2′), 0.12 (s, H1′); HRESIMS $[M + Na]^+$ *m*/*z* 565.3329 ($C_{32}H_{50}O_5NaSi$, calcd 565.3325).

Compound 7. To compound **6** (17.1 mg, 31.5 μmol) in THF (2.0 mL) on an ice bath were added NaH (60% dispersion in mineral oil, 101.4 mg) and benzyl bromide (0.5 mL, 4.2 mmol). The reaction solution was stirred at room temperature for 16 h. The reaction was quenched with saturated aqueous $NaHCO_3$ on an ice bath, and then the solution was extracted with EtOAc. The organic layer was washed with saturated aqueous NH_4Cl and brine, successively, and then dried over $MgSO_4$. After the solution was passed through a cotton filter to remove $MgSO_4$, the filtrate was concentrated in vacuo. The residue was subjected to Si gel column chromatography, eluted in a stepwise gradient manner with EtOAc/hexanes (0:100 to 40:60) to afford **7** (16.2 mg, 81% yield) as colorless needles: mp 195−196 °C; HRESIMS $[M + Na]^+$ *m*/*z* 655.3781 ($C_{39}H_{56}O_5NaSi$, calcd 655.3795).

Compound 8 from Strongylophorine-8 (4). To strongylophorine-8 (**4**) (17.5 mg, 41.4 mmol) in a mixed solvent system of CCl_4 (0.5 mL), CH_3CN (0.5 mL), and H_2O (0.5 mL) were added catalytic amounts of $NaIO_4$ (167.1 mg, 0.781 mmol) and $RuCl_3$. The reaction solution was stirred at 40 °C for 25 h and then diluted with H_2O (10 mL). The resulting solution was extracted with Et_2O, and the organic layer was concentrated in vacuo. The residue was fractionated with a Sepak (Waters Sepak 12 cm^3 Si gel 2 g) eluted using a stepwise gradient of EtOAc/hexanes (40:60 to 100:0). The fraction containing **8** was subjected to normal-phase HPLC (Alltech Econosil Si 5 μm) eluted with EtOAc/hexanes (44:56) to afford **8** (0.2 mg, 1% yield) as a colorless solid: $[\alpha]_D^{23}$ −46 (*c* 0.13, CH_2Cl_2); ^1H NMR (CD_2Cl_2) δ 4.74 (dd, *J* = 2.0, 12 Hz, H-19), 4.03 (d, *J* = 12 Hz, H-19), 2.43 (dd, *J* = 15, 16 Hz, H-15), 2.23 (dd, *J* = 7.0, 16 Hz, H-15), 2.12 (m, H-1), 2.10 (m, H-12), 2.03 (m, H-11), 1.97 (dd, *J* = 7.0, 15, H-14), 1.82 (m, H-3), 1.78 (m, H-6), 1.72 (m, H-2), 1.68 (m, H-12), 1.65 (m, H-2), 1.57 (m, H-7), 1.52 (m, H-3), 1.36 (m, H-6), 1.33 (m, H-5), 1.33 (s, H-17), 1.24 (m, H-9), 1.24 (m, H-11), 1.18 (m, H-7), 1.16 (m, H-1), 1.16 (s, H-20), 1.05 (s, H-18); HREIMS *m*/*z* 346.21465 ($C_{21}H_{30}O_4$, calcd 246.21441).

Compound 8 from Strongylophorine-26 (1). Chemical degradation of strongylophorine-26 (**1**) (3.5 mg, 7.7 μmol) in a similar manner to strongylophorine-8 described above yielded **8** (0.1 mg, 4% yield).

Compound 11. To the compound **10** (1.5244 g, 0.6980 mmol) in H_2O (25 mL) were added KOH (587 mg, 10.5 mmol) in H_2O (3.8 mL) and aqueous H_2O_2 (50% (w/w), 1.7 mL). The reaction solution was stirred at 90 °C for 5.3 h. After being cooled to room temperature, the reaction solution was acidified with aqueous HCl and then extracted with Et_2O. The organic layer was concentrated under reduced pressure and then dried in vacuo. To the residue were added 4-(dimethyamino)-pyridine (5.1 mg, 0.041 mmol), CH_2Cl_2 (16.0 mL), and *N*,*N*-diisopropylethylamine (10.0 mL). After sonication for a few minutes, benzyl chloromethyl ether (techn. ca. 60%, 5.4 mL) was added to the solution. The reaction solution was stirred at room temperature for 4.5 h. After quenching with saturated aqueous $NaHCO_3$, the solution was extracted with Et_2O. The organic layer was washed with brine and then concentrated under reduced pressure. The residue was subjected to Si

gel chromatography, eluted in a stepwise gradient manner with EtOAc/hexanes (0:100 to 13:87) to furnish **11** (805 mg, 27% yield for 2 steps) as a colorless oil: ^{1}H NMR (acetone-d_6) δ 7.33−7.27 (10H, H4′/H5′/H6′/H7′/H8′/H4″/H5″/H6″/H7″/H8″), 6.92 (d, J = 2.7 Hz, 1H, H6), 6.77 (d, J = 2.7 Hz, 1H, H4), 5.33 (s, 2H, H1″), 5.21 (s, 2H, H1′), 4.94 (s, 2H, H2′), 4.74 (s, 2H, H2″), 3.83 (s, 3H, H7); HREIMS m/z 460.07127 ($C_{23}H_{23}O_5{}^{81}Br$, calcd 460.07084), m/z 458.07291 ($C_{23}H_{23}O_5{}^{79}Br$, calcd 458.07289).

Compound 14. To compound **13** (48.0 mg, 0.156 mmol) in CH_2Cl_2 (4.0 mL) were added 4-(dimethylamino)pyridine (8.3 mg, 0.068 mmol), triethylamine (4.0 mL), and *t*-BuMe_2SiCl (473 mg, 3.14 mmol). The reaction solution was stirred at room temperature for 2 h, and then additional *t*-BuMe_2SiCl (146 mg, 0.967 mmol) was added to the solution. After 1 h, the reaction was quenched with saturated aqueous $NaHCO_3$ and brine. The resulting mixture was extracted with Et_2O, and the organic layer was concentrated under reduced pressure. The residue was subjected to Si gel column chromatography eluted in a stepwise gradient manner with Et_2O/hexanes (0:100 to 10:90) to furnish **14** (40.5 mg, 62% yield) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 4.00 (dd, J = 10, 10 Hz, 1H, H15), 3.89 (dd, J = 3.9, 10 Hz, 1H, H15), 1.85 (ddd, J = 3.4, 3.4, 13 Hz, 1H, H12), 1.77 (ddd, J = 3.5, 3.5, 12 Hz, 1H, H1), 1.70 (m, 1H, H7), 1.61 (m, 1H, H14), 1.60 (m, 1H, H6), 1.60 (m, 1H, H11), 1.54 (m, 1H, H2), 1.52 (m, 1H, H12), 1.41 (m, 1H, H6), 1.37 (m, 1H, H3), 1.35 (m, 1H, H2), 1.30 (s, 3H, H16), 1.22 (m, 1H, H11), 1.17 (m, 1H, H1), 1.15 (m, 1H, H3), 0.95 (m, 1H, H9), 0.91 (s, 9H, H4′/H5′/H6′), 0.86 (s, 3H, H20), 0.84 (m, 1H, H5), 0.83 (m, 1H, H7), 0.82 (s, 3H, H17), 0.81 (s, 3H, H18), 0.81 (s, 3H, H19), 0.11 (s, 3H, H1′), 0.10 (s, 3H, H2′); HRESIMS [M + Na]$^+$ m/z 445.3471 ($C_{26}H_{50}O_2NaSi$, calcd 445.3478).

Compound 15. To compound **14** (41 mg, 0.096 mmol) in CH_2Cl_2 (10.0 mL) were added 4-(dimethylamino)pyridine (1.9 mg, 0.016 mmol), *N*,*N*-diisopropylethylamine (2.0 mL), and benzyl chloromethyl ether (techn. ca. 60%, 0.8 mL). The solution was stirred at 50−70 °C for 21 h. After cooling to room temperature, the reaction was quenched with saturated aqueous $NaCO_3$ and brine. The resulting solution was extracted with Et_2O, and then the organic layer was concentrated under reduced pressure. The residue was purified by Si gel column chromatography eluted in a stepwise gradient manner with Et_2O/hexanes (0:100 to 3:97) to afford **15** (49 mg, 95% yield) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 7.32 (m, 5H, H4″/H5″/H7″/H8″), 4.93 (d, J = 7.7 Hz, 1H, H1″), 4.78 (d, J = 7.6 Hz, 1H, H1″), 4.66 (d, J = 12 Hz, 1H, H2″), 4.55 (d, J = 12 Hz, 1H, H2″), 3.88 (dd, J = 1.7, 11 Hz, 1H, H15), 3.71 (dd, J = 5.7, 11, 1H, H15), 1.90 (m, 2H, H2), 1.85 (m, 1H, H12), 1.83 (m, 1H, H7), 1.66 (m, 1H, H12), 1.65 (m, 1H, H1), 1.50 (m, 2H, H6), 1.44 (d, J = 4.9 Hz, 1H, H14), 1.37 (m, 2H, H11), 1.35 (m, 1H, H3), 1.17 (s, 3H, H16), 1.13 (m, 1H, H7), 1.11 (m, 1H, H3), 0.93 (s, 3H, H17), 0.87 (m, 1H, H9), 0.87 (s, 9H, H4′/H5′/H6′), 0.84 (s, 3H, H20), 0.83 (m, 1H, H5), 0.79 (m, 1H, H1), 0.79 (s, 3H, H18), 0.79 (s, 3H, H19), 0.03 (s, 3H, H1′), 0.02 (s, 3H, H2′); HREIMS m/z 542.41501 ($C_{34}H_{58}O_3Si$, calcd 542.41552).

Compound 16. To compound **15** (46 mg, 0.084 mmol) in THF (4.0 mL) was added 1 M tetrabutylammonium fluoride/THF (1.0 mL). The solution was stirred at 60−70 °C for 21 h. After cooling to room temperature, the solution was diluted with H_2O, and then the THF was removed under reduced pressure. To the resulting aqueous solution, brine was added, and then the solution was extracted with Et_2O. The organic layer was concentrated under reduced pressure. The residue was purified by Si gel column chromatography eluted in a stepwise gradient manner with EtOAc/hexanes (0:100 to 20:80) to afford **16** (35 mg, 96% yield) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 7.35−7.30 (m, 5H, H4′/H5′/H6′/H7′/H8′), 4.86 (s, 2H, H2′), 4.60 (s, 2H, H1′), 3.87 (dd, J = 8.5, 11 Hz, 1H, H15), 3.73 (dd, J = 2.6, 11 Hz, 1H, H15), 2.02 (ddd, J = 3.1, 3.1, 12 Hz, 1H, H12), 1.88 (ddd, J = 3.0, 3.0, 13 Hz, 1H, H7), 1.65 (m, 1H, H12), 1.63 (m, 1H, H2), 1.63 (m, 1H, H11), 1.61 (m, 1H, H1), 1.58 (m, 1H, H14), 1.55 (m, 1H, H6), 1.37 (s, 3H, H16), 1.35 (m, 1H, H2), 1.32 (m, 1H, H3), 1.22 (m, 1H, H7), 1.19 (m, 1H, H11), 1.11 (m, 1H, H3), 0.90 (d, J = 12 Hz, 1H, H9), 0.84 (s, 3H, H20), 0.83 (s, 3H, H5), 0.80 (m, 1H, H1), 0.79 (s, 3H, H17), 0.78 (s, 3H, H19), 0.77 (s, 3H, H18); HREIMS m/z 428.32859 ($C_{28}H_{44}O_3$, calcd 428.32905).

Compound 18a,b. To compound **16** (32 mg, 0.074 mmol) in CH_2Cl_2 (5.0 mL) on an ice bath were added CH_3COONa (52 mg, 0.63 mmol) and PCC (130 mg, 0.60 mmol). The solution was stirred on the ice bath for 4.5 h. The reaction solution was filtered through a Florisil column and eluted with Et_2O. The filtrate was concentrated under reduced pressure. The residue (102 mg) was used for the next reaction without purification.

To the aromatic compound **11** in THF (3.0 mL) at −78 °C was added dropwise 1.7 M *t*-BuLi/pentane (0.3 mL). The solution was stirred at −78 °C for 30 min. To the resulting solution was added dropwise the residue containing **17** (102 mg) in THF (2.0 mL), and the solution was stirred at −78 °C for 3 h. The reaction was quenched with saturated aqueous NH_4Cl, and the solution was stirred at room temperature overnight. After addition of brine to the quenched reaction solution, the resulting solution was extracted with Et_2O, and the organic layer was concentrated under reduced pressure. The residue was purified by Si gel column chromatography eluted in a stepwise manner with EtOAc/hexanes (0:100 to 20:80) to furnish **18a** (19 mg, 32% yield in 2 steps) and **18b** (20 mg, 33% yield for 2 steps). Compound **18a**: colorless oil; ^{1}H NMR (C_6D_6) δ 7.37 (d, J = 2.7 Hz, 1H, H21), 7.36 (d, J = 7.1 Hz, 2H, H4′, H8′), 7.29 (d, J = 7.2 Hz, 2H, H4″, H8″), 7.24 (d, J = 7.1 Hz, 2H, H4‴, H8‴), 7.18−7.04 (9H, H5′, H6′, H7′, H5″, H6″, H7″), 6.59 (d, J = 2.7, 1H, H19), 5.96 (dd, J = 3.5, 5.7 Hz, 1H, H15), 5.28 (d, J = 5.6 Hz, 1H, H1″), 5.22 (d, J = 5.6 Hz, 1H, H1″), 5.11 (d, J = 7.0 Hz, 1H, H1‴), 5.09 (d, J = 7.0 Hz, 1H, H1‴), 4.84 (d, J = 12 Hz, 1H, H2′), 4.76 (d, J = 12 Hz, 1H, H2′), 4.72 (d, J = 7.6 Hz, 1H, H1′), 4.60 (s, 2H, H2″), 4.58 (s, 2H, H2‴), 4.28 (d, J = 7.6 Hz, 1H, H1′), 3.21 (s, 3H, H27), 3.06 (d, J = 5.7 Hz, 1H, 15-OH), 2.74 (d, J = 12 Hz, 1H, H7), 2.39 (d, J = 3.5 Hz, 1H, H14), 2.01 (d, J = 13 Hz, 1H, H12), 1.73 (ddd, J = 2.8, 13, 13 Hz, 1H, H12), 1.62 (s, 3H, H22), 1.52 (m, 1H, H1), 1.49 (m, 1H, H11), 1.41 (m, 1H, H6), 1.41 (s, 3H, H23), 1.33 (m, 1H, H6), 1.30 (m, 1H, H3), 1.29 (m, 1H, H7), 1.21 (m, 1H, H11), 1.07 (ddd, J = 3.1, 13, 13 Hz, 1H, H3), 0.86 (m, 1H, H9), 0.80 (s, 3H, H24), 0.77 (s, 3H, H25), 0.71 (s, 3H, H26), 0.67 (dd, J = 2.5, 11 Hz, 1H, H5), 0.63 (ddd, J = 2.9, 13, 13 Hz, 1H, H1); HRESIMS [M + Na]$^+$ m/z 829.4656 ($C_{51}H_{66}O_8Na$, calcd 829.4655). Compound **18b**: colorless oil; ^{1}H NMR (C_6D_6) δ 7.59 (d, J = 2.7 Hz, 1H, H21), 7.47 (d, J = 7.1 Hz, 2H, H4″, H8″), 7.38 (d, J = 7.1 Hz, 2H, H4′, H8′), 7.23−7.03 (9H, H5′, H6′, H7′, H5″, H6″, H7″), 7.19 (2H, H4‴, H8‴), 6.64 (d, J = 2.7 Hz, 1H, H19), 5.80 (dd, J = 6.3, 10 Hz, 1H, H15), 5.59 (d, J = 10 Hz, 1H, H1″), 5.46 (d, J = 10 Hz, 1H, H1″), 5.16 (d, J = 12 Hz, 1H, H2″), 5.14 (d, J = 7.1 Hz, 1H, H1‴), 5.10 (d, J = 7.1 Hz, 1H, H1‴), 5.08 (d, J = 12 Hz, 1H, H2″), 4.82 (d, J = 7.6 Hz, 1H, H1′), 4.73 (d, J = 10 Hz, 1H, 15-OH), 4.73 (d, J = 7.6 Hz, 1H, H1′), 4.67 (d, J = 12 Hz, 1H, H2′), 4.60 (d, J = 12 Hz, 1H, H2′), 4.55 (s, 2H, H2‴), 2.45 (d, J = 6.3 Hz, 1H, H14), 2.05 (m, 1H, H7), 1.94 (ddd, J = 2.7, 3.3, 12 Hz, 1H, H12), 1.72 (s, 3H, H22), 1.65 (ddd, J = 2.7, 12, 13 Hz, 1H, H12), 1.51 (m, 1H, H11), 1.45 (dd, J = 13, 13 Hz, 1H, H1), 1.44 (m, 1H, H2), 1.30 (m, 1H, H11), 1.24 (m, 1H, H3), 1.20 (m, 1H, H6), 1.14 (s, 3H, H23), 1.13 (m, 1H, H6), 1.09 (m, 1H, H2), 0.99 (ddd, J = 4.1, 14, 14 Hz, H3), 0.73 (m, 1H, H7), 0.72 (m, 1H, H8), 0.71 (s, 3H, H26), 0.70 (s, 3H, H24), 0.56 (m, 1H, H1), 0.56 (s, 3H, H25), 0.45 (m, 1H, H5); HRESIMS [M + Na]$^+$ m/z 829.4659 ($C_{51}H_{66}O_8Na$, calcd 829.4655).

Compound 3. To the alcohol **18a** (4 mg, 5 μmol) in pyridine (1.0 mL) on ice was added trifluoroacetic anhydride (100 μL, 719 μmol). After the reaction solution was stirred on ice for 50 min, the reaction was quenched with saturated aqueous $NaHCO_3$. To the quenched solution was added brine, and then the resulting solution was extracted with Et_2O/toluene (1:1). The organic layer was concentrated under reduced pressure, and the resulting residue was used for the next step without further purification. The residue was dissolved in EtOAc/EtOH (1:4) (2.0 mL). To this solution was added $Pd(OH)_2$/C (21 mg). Hydrogenation was undertaken using a hydrogen gas pressure of 450 psi. The reaction solution was stirred at room temperature for 16 h. The reaction solution was filtered though a Celite 545 column to remove the $Pd(OH)_2$/C powder. The column was washed with EtOAc (10 mL), which was combined with the filtrate. The combined organic solution was concentrated under reduced pressure. The residue was purified by Si gel column chromatography eluted in a stepwise gradient manner with EtOAc/hexanes (0:100 to 40:60) to afford **3** (0.6 mg, 50% yield over 2 steps) as a yellow oil: UV (MeOH) λ_{max} (log ϵ) 244 nm (3.86), 278 nm (3.81); ^{1}H NMR (acetone-d_6) δ 6.55 (ddd, J = 1.4, 1.4, 2.5 Hz, 1H, H21), 5.87 (d, J = 2.5 Hz, 1H, H19), 3.81 (s, 3H, H27), 3.19 (s, 1H, 13-OH), 2.57 (dd, J = 1.4, 5.0 Hz, 1H, H15), 2.56 (dd, J = 1.4, 6.6 Hz, 1H, H15), 1.80 (ddd, J = 1.3, 1.3, 6.3 Hz, 1H, H12), 1.69 (m, 1H, H1), 1.67 (m, 1H, H2), 1.67 (m, 1H, H7), 1.67 (m, 1H, H14), 1.59 (m, 1H, H6), 1.50 (m, 1H, H2), 1.47 (m, 1H, H12), 1.38 (m, 1H,

H6), 1.35 (m, 1H, H3), 1.30 (m, 2H, H11), 1.18 (s, 3H, H22), 1.16 (m, 1H, H3), 1.08 (m, 1H, H7), 0.95 (m, 1H, H9), 0.92 (s, 3H, H23), 0.85 (m, 1H, H5), 0.85 (s, 3H, H24), 0.83 (m, 1H, H1), 0.83 (s, 3H, H26), 0.82 (3H, H25); HREIMS *m*/*z* 428.29235 ($C_{27}H_{40}O_4$, calcd 428.29266).

Chiral Separation of the Compound 18a. A 14 mg portion of the fraction containing **18a** was subjected to normal-phase chiral HPLC (DAICEL Chiralpak IA) eluted with EtOAc/hexanes/triethylamine (13:87:0.03), furnishing (−)-**18a** (3.7 mg) and (+)-**18a** (3.4 mg) at retention times of 29.4 and 37.0 min, respectively. (−)-**18a**: $[\alpha]_D^{24}$ −10 (*c* 1.0, CH_2Cl_2). (+)-**18a**: $[\alpha]_D^{24}$ +10 (*c* 1.0, CH_2Cl_2).

Compound (+)-3. Treatment of (−)-**18a** (2.2 mg, 2.7 μmol) in a similar manner to racemic **18a** afforded (+)-**3** (0.4 mg, 35% yield): $[\alpha]_D^{23}$ +16 (*c* 0.27, EtOAc).

Compound (−)-3. Treatment of (+)-**18a** (2.0 mg, 2.5 μmol) in a similar manner to racemic **18a** afforded (−)-**3** (0.8 mg, 70% yield): $[\alpha]_D^{23}$ −16 (*c* 0.33, EtOAc).

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Supporting Information Available: ^{13}C NMR data for synthetic compounds; experimental details and data for single-crystal X-ray diffraction analysis of **7**; CD spectra for **1** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Partial ^{1}H and ^{13}C chemical shift values were assigned as follows: C-15 [δ 2.35, dd, J = 2.5, 13 Hz and 2.72, ddd, J = 1.8, 13, 14 Hz (major); 2.35, d, J = 15 Hz and 2.75, m (minor)], C-19 [δ 5.25, d, J = 1.6 Hz; 99.7 (major); 5.23, d, J = 1.9 Hz; 98.6 (minor)], C-21 [δ 5.76, dd, J = 1.8, 1.6 Hz; 122.5 (major); 5.94, brd; 125.6 (minor)], C-27 [δ 3.70, s (major); 3.72, s (minor)].

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