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Tetrahedron 62 (2006) 4262–4271
Tetrahedron

A structure–activity relationship study of brusatol, an antitumor quassinoid

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Received 8 November 2005; accepted 10 January 2006

Available online 3 March 2006

Abstract—Analogues of brusatol (**2**) were prepared and examined for their cytotoxic activity by using P-388 murine leukemia cells. The following structure–activity relationships were noted: (i) an enone carbonyl oxygen or an enolic oxygen at C-2 is essential, but an oxygen at C-3 not essential for the activity; (ii) the C-11 β-hydroxyl group is important for the activity; and (iii) the length of the ester alkoxy side chain at C-21 seems to have a slight effect on the activity.

1. Introduction

Bruceantin (**1**, NSC 165563),[1] isolated from *Brucea antidysenterica* (Simaroubaceae), is a quassinoid having a promising antitumor activity (Fig. 1).[2] Its mode of action is attributed to inhibition of protein synthesis through interference at the peptidyl transferase site.[3,4] Phase I and II clinical trials of **1** were conducted in the late 1970s and early 1980s in the United States.[5–9] Brusatol (**2**),[10] a closely related congener of **1**, having also a potent antitumor activity,[11] and a different ester side chain at C-15, has been obtained from *B. sumatrana*[10] and *B. javanica*.[12] In spite of their promising antitumor activity, no systematic structure–activity relationship (SAR) studies have been reported: such information should be inevitable for designing their analogues with more improved medicinal profiles.[13]

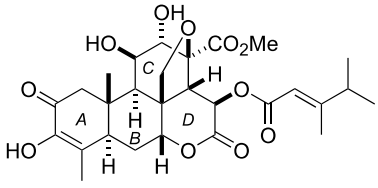
In the present report, we describe preparation of analogues of **2** with a modified ring A or ring C structure, or different C-21 alkoxy chain lengths and some observations on the chemical reactivity of the ring C moiety, and on the effects of chemical structures on the cytotoxic activity.

2. Results and discussion

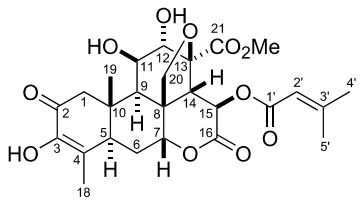
Brusatol (**2**) was obtained from the ground seeds of *B. javanica* as it was[14] or by partial hydrolysis of bruceoside-A,[15] the 2-*O*-glucoside of **2**, also obtained more abundantly from this plant source. Throughout the present transformation reactions, the senecioyl side chain at C-15 of brusatol (**2**) was retained, as it is reported to be essential for the compound to express potent antitumor activity.[1,13a,16]

2.1. Modification of ring A

Brusatol (**2**) possesses a diosphenol (3-hydroxy-3-en-2-one) group in the ring A. An earlier SAR study by Lee et al.[16]



bruceantin (**1**)



brusatol (**2**)

Figure 1.

Keywords: Bruceantin; Brusatol; Quassinoid; SAR; Cytotoxicity.
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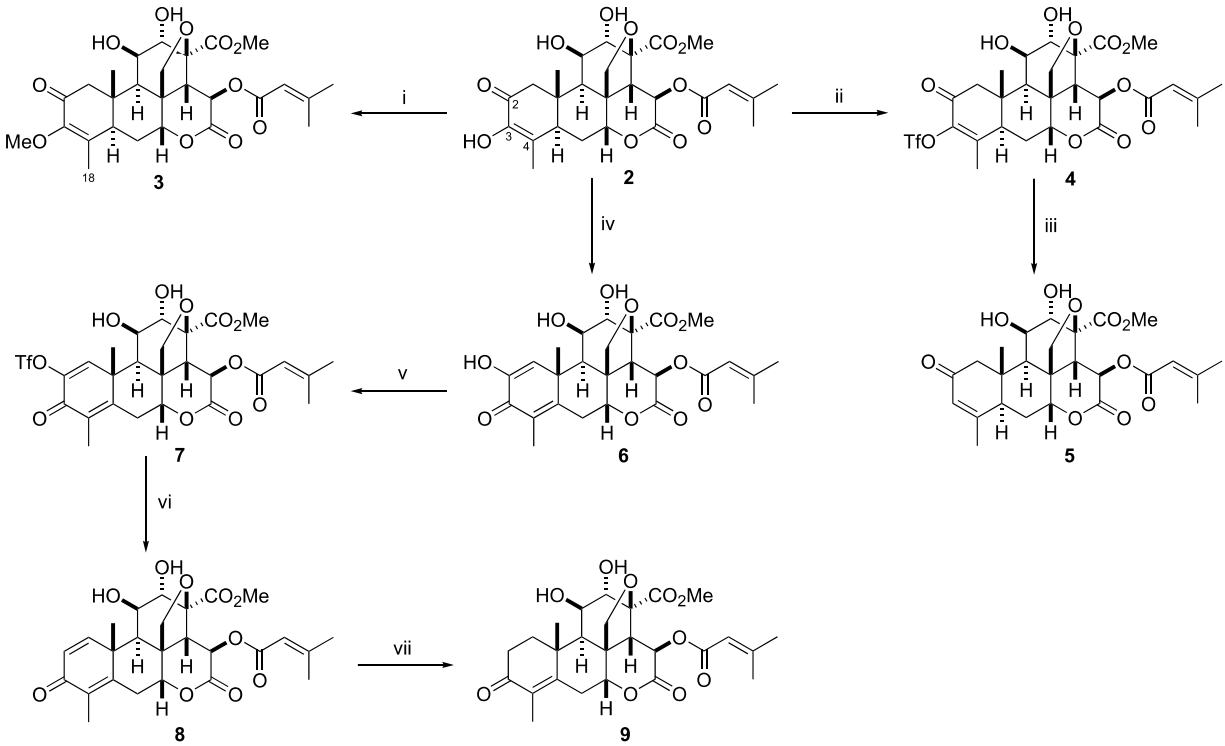
doi:10.1016/j.tet.2006.01.083

revealed that saturation of the 3,4-double bond resulted in loss of the antitumor activity, which indicates that the presence of a π bond system in this region is essential. Therefore, in the present study, we first prepared several analogues in which the tautomerizable diosphenol structure in **2** was modified. Analogue **3** in which this conjugate system was fixed and delineated was prepared by introduction of a methyl group onto the enolic oxygen (Scheme 1). Confirmation of the structure of **3** was made by observation of a NOESY correlation between the methoxyl protons and H_3-18. Enone **5** was prepared by treatment of brusatol (**2**) with *N*-phenyl-bis(trifluoromethanesulfonimide) to afford 3-*O*-trifrate **4**, and subsequent palladium-mediated reduction of **4**.[17] Dehydrobrusatol (**6**), having a ketone group at C-3, obtainable also as a minor quassinoid from this plant source, was prepared by DDQ oxidation of **2**.[18] A series of C-3 carbonyl analogues were prepared as shown in Scheme 1: **8** was prepared by converting the enol group in **6** into hydrogen via 2-*O*-trifrate **7**, by the same procedure used for the conversion of **2** into **5**. Partial hydrogenation of **8** by using Wilkinson's rhodium catalyst provided enone **9**.

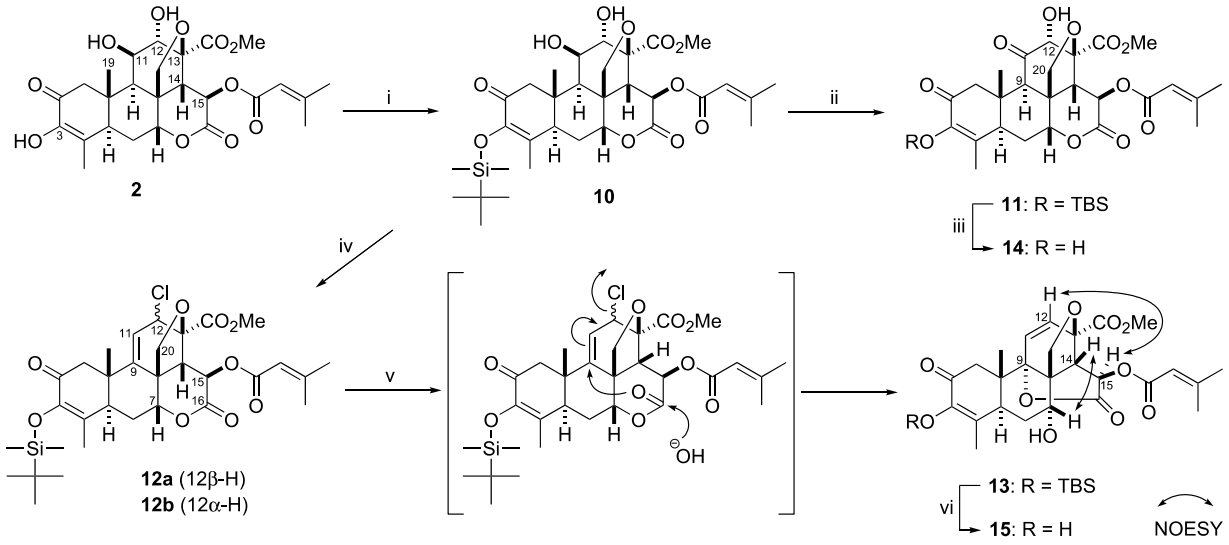
2.2. Modification of ring C

The ring C of brusatol (**2**) is characterized by the C-11 β- and C-12 α-hydroxyl groups. As regards the modification involving those hydroxyl groups, several acylated analogues have been prepared, which are reported to have a lower antitumor activity.[16] In the present work, prior to performing transformations of the ring C structure, the enolic hydroxyl group at C-3 was selectively protected as a *tert*-butyldimethylsilyl (TBS) ether, **10** (Scheme 2).[19] Oxidation of **10** with Jones reagent afforded a ketone, **11**, in 77% yield. The location of the ketone group in **11** was determined by the HMBC cross-peaks observed between the carbonyl signal (δ_C 201.5) and H-9 and H-12. The H-9 signal observed as a singlet signal in the ^{1}H NMR spectrum also supported this structure. The selective oxidation of the C-11 hydroxyl group may be due to its steric environment. Of the two hydroxyl groups on the ring C, both axially oriented, the C-11 hydroxyl group is sterically more congested than the C-12 hydroxyl group. The C-11 hydroxyl group is influenced by three 1,3-diaxial interactions involving the carbon atoms, C-19 and C-20, and the oxygen atom at C-13, whereas the C-12 hydroxyl group suffers only one 1,3-diaxial interaction with the C-15 atom. Steric congestion is known to accelerate oxidation of hydroxyl groups by chromium(VI) oxidation.[20]

Chlorination of those hydroxyl groups with thionyl chloride caused simultaneous elimination of the C-11 hydroxyl group to afford two epimeric allyl chlorides **12a** and **12b** in 54 and 25% yields, respectively (Scheme 2). The presence of an olefinic bond between C-9 and C-11 was confirmed by the HMBC experiments. The α- and β-orientations of the chlorine atoms of **12a** and **12b** at C-12 were determined by observation of NOESY correlations between H-12 and H_a-20 for **12a** and between H-12 and H-15 for **12b**, respectively. Steric congestion around the C-11 hydroxyl group mentioned above may facilitate the elimination of the axial hydroxyl group at C-11. Several attempts at the



Scheme 1. Reagents and conditions: (i) *p*-TsOMe, K_2CO_3, acetone, 50 °C, 7 days, 37%; (ii) Tf_2NPh, Et_3N, DMAP, dioxane, room temperature, 30 min, 75%; (iii) $Pd(OAc)_2$, 1,1′-bis(diphenylphosphino)ferrocene, HCO_2H, Et_3N, DMF, 45 °C, 2 h, 68%; (iv) DDQ, dioxane, room temperature, 1 h, 55%; (v) Tf_2NPh, Et_3N, DMAP, dioxane, room temperature, 1 h, 90%; (vi) $Pd(OAc)_2$, 1,1′-bis(diphenylphosphino)ferrocene, HCO_2H, Et_3N, DMF, 50 °C, 1 h, 87%; (vii) H_2, $(PPh_3)_3RhCl$, benzene, 45 °C, 30 h, 62%.



Scheme 2. Reagents and conditions: (i) *tert*-butyl(chloro)dimethylsilane, imidazole, DMF, room temperature, 24 h, 91%; (ii) Jones reagent, acetone, room temperature, 1 h, 77%; (iii) *n*-Bu_4NF, THF, room temperature, 15 min, 46%; (iv) $SOCl_2$, pyridine, $CHCl_3$, 60 °C, 10 h, 54% of **12a** and 25% of **12b**; (v) K_2CO_3, acetone–H_2O (10/1), room temperature, 6 h, 54%; (vi) *n*-Bu_4NF, THF, room temperature, 20 min, 46%.

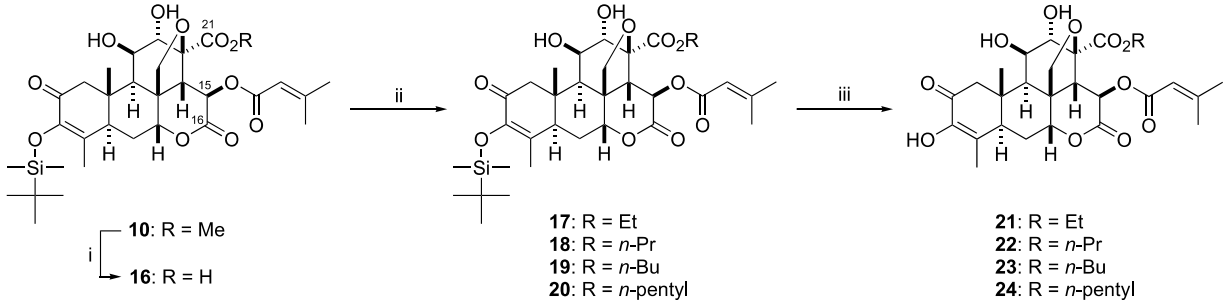
nucleophilic displacement at C-12 of **12a**,**b** were unsuccessful. Treatment of **12a**,**b** with potassium carbonate in aqueous acetone at room temperature, however, unexpectedly produced lactone **13** in 54% yield. Its structure was established by the spectroscopic methods. The molecular formula, $C_{32}H_{44}O_{10}Si$, determined by the HRESIMS, suggested that a hydroxyl group substituted the chlorine atom in the reaction. Its HMBC spectra showed the presence of an olefinic bond between C-11 (δ_H 6.10, d, J=9.8 Hz; δ_C 128.1) and C-12 (δ_H 6.38, dd, J=9.8, 1.2 Hz; δ_C 130.5) in **13**. The chemical shift value of C-9 (δ 85.4) and a DEPT experiment indicated that C-9 was an oxygen-bearing sp^3 quaternary carbon. The chemical shift of H-7 (δ 4.21) was in higher field than that in **10** (δ 4.78) or in **12a**,**b** (δ 5.00, 4.92), suggesting deacylation at the C-7 oxygen. NOESY correlations between H-7 and H-14 and between H-12 and H-15 indicated that the configuration at C-7 and C-15 did not change through the reactions. From these observations, structure **13** given in Scheme 2 was assigned for this product. The possible steps from **12** to **13** are shown in Scheme 2. The hydroxide anion first attacked the C-16 carbonyl carbon, and the resultant oxide anion subsequently attacked the proximal C-9 carbon in an intramolecular S_N' displacement fashion to give **13**. Compound **13** is unique in that it has an unusual 9,16-lactone linkage. Desilylation of **11** and **13** produced analogues **14** and **15**, respectively.

2.3. Modifications at C-21 ester side chain

Brusatol (**2**) possess a methyl ester group at C-21 along with two other ester functionalities at the C-15 side chain and C-16. Of those three ester groups, the C-15 side chain ester is the most susceptible to alkaline hydrolysis.[21] Therefore, to modify the C-21 ester side chain, first, we had to establish a method of selective cleavage of the C-21 ester group. This problem was successfully solved by an S_N2 displacement reaction on the ester–methyl group.[22] When compound **10** was treated with lithium iodide in pyridine at 100 °C for 24 h, acid **16** was obtained in 72% yield (Scheme 3), which was then converted by O-alkylation of the carboxylic acid into brusatol analogues having different alkyl chains. By treatment with potassium carbonate and iodoethane, 1-iodopropane, 1-iodobutane, and 1-iodopentane at 50 °C, it afforded corresponding esters **17**, **18**, **19**, and **20**, respectively, in yields of 88–92%, which were then desilylated to afford ethyl (**21**), propyl (**22**), butyl (**23**), and pentyl (**24**) ester analogues, respectively.

2.4. Cytotoxicity of brusatol analogues on P-388 leukemia cells

Brusatol (**2**) and its analogues thus prepared were evaluated for their cytotoxicity by using P-388 murine leukemia cells.



Scheme 3. Reagents and conditions: (i) LiI, pyridine, 100 °C, 24 h, 72%; (ii) RI, K_2CO_3, acetone, 50 °C, 6–24 h, 88–92%; (iii) *n*-Bu_4NF, THF, room temperature, 15 min, 61–79%.

Table 1. Cytotoxicity of brusatol (**2**) and its analogues **3**, **5**, **6**, **8**, **9**, **14**, **15**, and **21**–**24** on P-388 leukemia cells

Compound	IC_{50} (μg/mL)
Brusatol (**2**)	0.0061
3	0.23
5	0.057
6	0.072
8	1.5
9	5.0
14	10
15	18
21	0.014
22	0.050
23	0.063
24	0.071

The results are summarized in Table 1. As regards modifications of ring A (**3**, **5**, **6**, **8**, and **9**), replacement of the enol group with a methoxyl group (**3**) gave a marked decrease in activity, but replacement with a hydrogen atom (**5**) gave less decrease. In dehydrobrusatol (**6**), the decrease was less, whereas in **8**, with the enol group oxygen of **6** replaced by a hydrogen atom, and in **9**, with the 1,2-double bond of **8** saturated, the decrease in activity was more significant. Thus, the presence of an enone carbonyl oxygen or an enolic oxygen at C-2 appears to be essential for the compounds to express the activity, but the oxygen at C-3 not essential.

As regards the ring C-modified analogues, 11-ketone analogue **14** showed only a very weak activity, indicating that disposition of a β-hydroxyl group at C-11 is important, and the 9,16-lactone analogue **15** also only a marginal activity.

The table shows that the lengths of the ester alkyl side chains at C-21 apparently had a weak effect on the activity: the activity generally decreases as the alkyl chain length increases from methyl (**2**) to pentyl (**24**).

In the present study, we prepared several analogues of brusatol (**2**) and evaluated their cytotoxic activity. Thus, we established methods of preparation of new analogues of **2**, having modified ring A conjugate systems, modified ring C structures, or different C-21 alkoxy chain lengths, and by detailed assay of their effect on leukemia cells, obtained information on the relation between the chemical structure and the cytotoxic activity of the compounds of this series. Such knowledge should be useful for designing and synthesizing novel analogues of brusatol (**2**) and bruceantin (**1**).

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP-360 digital polarimeter, IR spectra on a Perkin-Elmer 1710 spectrophotometer, mass spectra on a Micromass LCT spectrometer, and NMR spectra on a Bruker DRX-500 spectrometer at 300 K. ^{1}H chemical shifts in $CDCl_3$ or methanol-d_4 were referenced to the residual $CHCl_3$ (7.26 ppm) or CD_2HOD (3.31 ppm); ^{13}C chemical shifts were referenced to the solvent ($CDCl_3$, 77.03 ppm; methanol-d_4, 49.0 ppm). Preparative HPLC was performed on a Shimadzu LC-6AD system equipped with a SPD-10A UV detector (at 254 nm) and a reverse-phase column, Inertsil PREP-ODS (10 μm, 20×250 mm), by using a mixed solvent system of MeOH–H_2O or MeCN–H_2O, at a flow rate of 10 mL/min.

3.2. Material

Brusatol (**2**) was obtained by chromatographic separation of a methanol extract of the seeds of *Brucea javanica* (L.) Merr. as described before[14] or by acid hydrolysis of bruceoside-A from the same plant source, according to the reported procedure.[15]

3.3. Synthesis of brusatol analogues

3.3.1. 3-*O*-Methylbrusatol (3). To a solution of brusatol (**2**) (30 mg, 0.058 mmol) in acetone (1 mL) were added potassium carbonate (16 mg, 0.12 mmol) and methyl *p*-toluenesulfonate (88 μL, 0.58 mmol). After stirring at 50 °C under an argon atmosphere for 7 days, the reaction mixture was diluted with chloroform (10 mL) and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative ODS-HPLC using MeOH–H_2O (33/67) as an eluent to afford **3** (11.4 mg, 37%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +35.2 (*c* 0.66, $CHCl_3$); IR (film) ν_{max} 3473, 1736, 1671 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.28 (1H, br s, H-15), 5.62 (1H, s, H-2′), 4.79 (1H, s, H-7), 4.73 (1H, d, J=7.9 Hz, H_a-20), 4.26 (1H, m, H-11), 4.19 (1H, s, H-12), 3.79 (1H, dd, J=7.9, 1.3 Hz, H_b-20), 3.79 (3H, s, CH_3O-21), 3.64 (3H, s, CH_3O-3), 3.13 (1H, br s, H-14), 2.96 (1H, d, J=13.0 Hz, H-5), 2.93 (1H, d, J=15.9 Hz, H_β-1), 2.38 (1H, dt, J=14.6, 2.8 Hz, H_α-6), 2.36 (1H, d, J=15.9 Hz, H_α-1), 2.19 (3H, d, J=0.8 Hz, H-5′), 2.07 (1H, br s, H-9), 1.93 (3H, d, J=1.0 Hz, H-4′), 1.87 (3H, d, J=1.4 Hz, H-18), 1.77 (1H, ddd, J=14.6, 13.0, 2.5 Hz, H_β-6), 1.39 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.4 (C-2), 172.0 (C-21), 167.0 (C-16), 164.5 (C-1′), 161.1 (C-3′), 149.4 (C-3), 143.4 (C-4), 114.0 (C-2′), 82.4 (C-7), 81.4 (C-13), 75.9 (C-12), 74.1 (C-20), 70.9 (C-11), 65.7 (C-15), 59.9 (CH_3O-3), 53.1 (CH_3O-21), 51.8 (C-14), 51.2 (C-1), 45.4 (C-8), 42.7 (C-5), 42.0 (C-9), 40.5 (C-10), 29.1 (C-6), 27.7 (C-4′), 20.6 (C-5′), 15.4 (C-19), 14.0 (C-18); HRESIMS *m*/*z* 535.2147 $[M+H]^+$ (calcd for $C_{27}H_{35}O_{11}$, 535.2179).

3.3.2. Compound 4. A solution of brusatol (**2**) (100 mg, 0.192 mmol) and *N*-phenyl-bis(trifluoromethanesulfonimide) (206 mg, 0.577 mmol) in 1,4-dioxane (1 mL) was treated with triethylamine (81 μL, 0.58 mmol) and 4-(dimethylamino)pyridine (23 mg, 0.19 mmol), and the resulting reaction mixture was stirred at room temperature under an argon atmosphere for 30 min. The mixture was diluted with chloroform (30 mL), washed sequentially with 1 M HCl (5 mL), water (5 mL), and brine (5 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative ODS-HPLC using MeOH–H_2O (54/46) as an eluent to afford **4** (94.2 mg, 75%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +31.8 (*c* 0.60, $CHCl_3$); IR (film) ν_{max} 3526, 1733, 1697 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.26 (1H, br s, H-15), 5.63

(1H, s, H-2′), 4.82 (1H, s, H-7), 4.72 (1H, d, J=8.0 Hz, H_a-20), 4.27 (1H, s, H-11), 4.20 (1H, s, H-12), 3.81 (1H, d, J=8.0 Hz, H_b-20), 3.79 (3H, s, OCH_3), 3.16 (1H, br s, H-14), 3.11 (1H, d, J=12.9 Hz, H-5), 3.08 (1H, d, J=16.4 Hz, H_β-1), 2.47 (1H, d, J=16.4 Hz, H_α-1), 2.43 (1H, dt, J=14.6, 2.8 Hz, H_α-6), 2.19 (3H, d, J=0.9 Hz, H-5′), 2.10 (1H, br s, H-9), 2.00 (3H, s, H-18), 1.94 (3H, d, J=1.1 Hz, H-4′), 1.88 (1H, ddd, J=14.6, 12.9, 2.4 Hz, H_β-6), 1.46 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 187.2 (C-2), 171.8 (C-21), 166.7 (C-16), 164.4 (C-1′), 161.5 (C-3′), 149.4 (C-3), 141.9 (C-4), 118.5 (CF_3SO_2), 113.9 (C-2′), 81.7 (C-7), 81.2 (C-13), 75.8 (C-12), 73.8 (C-20), 70.8 (C-11), 65.7 (C-15), 53.1 (OCH_3), 51.7 (C-14), 49.9 (C-1), 45.3 (C-8), 43.7 (C-5), 41.6 (C-9), 40.4 (C-10), 28.8 (C-6), 27.7 (C-4′), 20.7 (C-5′), 15.6 (C-18), 15.4 (C-19); HRESIMS m/z 653.1509 $[M+H]^+$ (calcd for $C_{27}H_{32}O_{13}F_3S$, 653.1516).

3.3.3. Compound 5. Triethylamine (64 μL, 0.46 mmol) and formic acid (17 μL, 0.45 mmol) were added to a solution of compound **4** (30 mg, 0.046 mmol), 1,1′-bis(diphenylphosphino)ferrocene (38 mg, 0.069 mmol), and palladium(II) acetate (10.3 mg, 0.046 mmol) in *N*,*N*-dimethylformamide (0.5 mL), and the resulting mixture was stirred at 45 °C under an argon atmosphere for 2 h. The volatiles were removed in vacuo, and the residue was dissolved in chloroform (30 mL). The solution was washed sequentially with 1 M HCl (5 mL), saturated aqueous $NaHCO_3$ (5 mL), and brine (5 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative ODS-HPLC using MeOH–H_2O (37/63) as an eluent to afford **5** (15.8 mg, 68%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +35.6 (*c* 0.67, $CHCl_3$); IR (film) ν_{max} 3466, 1738, 1650 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.27 (1H, br s, H-15), 5.97 (1H, s, H-3), 5.63 (1H, s, H-2′), 4.79 (1H, s, H-7), 4.74 (1H, d, J=7.9 Hz, H_a-20), 4.28 (1H, m, H-11), 4.20 (1H, s, H-12), 3.80 (1H, dd, J=7.9, 1.4 Hz, H_b-20), 3.79 (3H, s, OCH_3), 3.13 (1H, br s, H-14), 2.85 (1H, d, J=15.7 Hz, H_β-1), 2.85 (1H, d, J=13.0 Hz, H-5), 2.41 (1H, dt, J=14.6, 2.8 Hz, H_α-6), 2.29 (1H, d, J=15.7 Hz, H_α-1), 2.19 (3H, d, J=1.0 Hz, H-5′), 2.10 (1H, br s, H-9), 1.93 (3H, d, J=1.1 Hz, H-4′), 1.91 (3H, s, H-18), 1.78 (1H, ddd, J=14.6, 13.0, 2.6 Hz, H_β-6), 1.38 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.1 (C-2), 172.0 (C-21), 167.0 (C-16), 164.5 (C-1′), 161.1 (C-3′), 160.1 (C-4), 127.4 (C-3), 114.0 (C-2′), 82.4 (C-7), 81.3 (C-13), 75.8 (C-12), 74.1 (C-20), 70.9 (C-11), 65.8 (C-15), 53.1 (OCH_3), 51.8 (C-14), 50.6 (C-1), 45.5 (C-8), 43.2 (C-5), 42.1 (C-9), 40.9 (C-10), 28.6 (C-6), 27.7 (C-4′), 22.2 (C-18), 20.6 (C-5′), 15.5 (C-19); HRESIMS m/z 527.1880 $[M+Na]^+$ (calcd for $C_{26}H_{32}O_{10}Na$, 527.1893).

3.3.4. Dehydrobrusatol (6). To a solution of brusatol (**2**) (25 mg, 0.048 mmol) in 1,4-dioxane (1 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (11 mg, 0.048 mmol), and the resulting mixture was stirred at room temperature for 1 h. After dilution with chloroform (30 mL), the mixture was washed successively with saturated aqueous $NaHCO_3$ (5 mL) and brine (5 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative ODS-HPLC using MeOH–H_2O (36/64) as an eluent to afford dehydrobrusatol (**6**) (13.6 mg, 55%) as a colorless amorphous solid, whose ^{1}H and ^{13}C NMR spectra were identical with those in the literature.[18]

3.3.5. Dehydrobrusatol 2-*O*-triflate (7). A solution of compound **6** (90 mg, 0.17 mmol) and *N*-phenylbis(trifluoromethanesulfonimide) (186 mg, 0.52 mmol) in 1,4-dioxane (1 mL) was treated with triethylamine (73 μL, 0.52 mmol) and 4-(dimethylamino)pyridine (21 mg, 0.17 mmol), and the resulting reaction mixture was stirred at room temperature under an argon atmosphere for 1 h. The mixture was diluted with chloroform (30 mL), washed successively with 1 M HCl (5 mL), water (5 mL), and brine (5 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative ODS-HPLC using MeOH–H_2O (49/51) as an eluent to afford **7** (102 mg, 90%) as a colorless amorphous solid: $[\alpha]_D^{21}$ +51.2 (*c* 0.25, $CHCl_3$); IR (film) ν_{max} 3510, 1736, 1658 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (1H, s, H-1), 5.98 (1H, br s, H-15), 5.61 (1H, s, H-2′), 4.88 (1H, s, H-7), 4.84 (1H, d, J=8.0 Hz, H_a-20), 4.37 (1H, br s, H-11), 4.25 (1H, s, H-12), 3.93 (1H, dd, J=8.0, 1.1 Hz, H_b-20), 3.79 (3H, s, OCH_3), 3.34 (1H, dd, J=15.0, 3.1 Hz, H_α-6), 3.22 (1H, br s, H-14), 2.60 (1H, d, J=15.0 Hz, H_β-6), 2.16 (3H, d, J=0.8 Hz, H-5′), 2.09 (1H, br s, H-9), 2.02 (3H, s, H-18), 1.92 (3H, d, J=0.8 Hz, H-4′), 1.77 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.4 (C-3), 171.4 (C-21), 166.0 (C-16), 164.5 (C-1′), 161.3 (C-3′), 153.9 (C-5), 143.9 (C-2), 140.6 (C-1), 133.2 (C-4), 118.7 (CF_3SO_2), 113.9 (C-2′), 82.8 (C-7), 81.5 (C-13), 75.8 (C-12), 73.3 (C-20), 73.0 (C-11), 65.7 (C-15), 53.2 (OCH_3), 51.2 (C14), 45.9 (C-8), 44.9 (C-10), 40.5 (C-9), 32.4 (C-6), 27.7 (C-4′), 22.9 (C-19), 20.6 (C-5′), 11.1 (C-18); HRESIMS m/z 651.1343 $[M+H]^+$ (calcd for $C_{27}H_{30}O_{13}F_3S$, 651.1359).

3.3.6. Compound 8. Triethylamine (100 μL, 0.72 mmol) and formic acid (27 μL, 0.72 mmol) were added to a solution of compound **7** (47 mg, 0.072 mmol), 1,1′-bis(diphenylphosphino)ferrocene (60 mg, 0.11 mmol), and palladium(II) acetate (16 mg, 0.071 mmol) in *N*,*N*-dimethylformamide (0.5 mL), and the resulting mixture was stirred at 50 °C under an argon atmosphere for 1 h. The volatiles were removed in vacuo, and the residue was dissolved in chloroform (30 mL). The solution was washed successively with 1 M HCl (5 mL), saturated aqueous $NaHCO_3$ (5 mL), and brine (5 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative ODS-HPLC using MeOH–H_2O (44/56) as an eluent to afford **8** (31.6 mg, 87%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +87.2 (*c* 0.87, $CHCl_3$); IR (film) ν_{max} 3478, 1742, 1659 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (1H, d, J=10.1 Hz, H-1), 6.35 (1H, d, J=10.1 Hz, H-2), 6.01 (1H, br s, H-15), 5.60 (1H, s, H-2′), 4.85 (1H, d, J=8.0 Hz, H_a-20), 4.84 (1H, s, H-7), 4.42 (1H, d, J=4.2 Hz, H-11), 4.23 (1H, s, H-12), 3.90 (1H, d, J=8.0 Hz, H_b-20), 3.78 (3H, s, OCH_3), 3.32 (1H, dd, J=14.8, 3.0 Hz, H_α-6), 3.15 (1H, br s, H-14), 2.56 (1H, d, J=14.8 Hz, H_β-6), 2.15 (3H, d, J=0.7 Hz, H-5′), 2.02 (1H, br s, H-9), 1.96 (3H, s, H-18), 1.91 (3H, s, H-4′), 1.65 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.6 (C-3), 171.7 (C-21), 166.3 (C-16), 164.5 (C-1′), 161.0 (C-3′), 152.8 (C-5), 152.4 (C-1), 133.4 (C-4), 127.5 (C-2), 114.0 (C-2′), 83.3 (C-7), 81.6 (C-13), 75.9 (C-12), 73.5 (C-20), 72.9 (C-11), 65.8 (C-15), 53.1 (OCH_3), 51.4 (C-14), 45.9 (C-8), 43.3

(C-10), 40.2 (C-9), 32.2 (C-6), 27.7 (C-4′), 22.6 (C-19), 20.6 (C-5′), 10.8 (C-18); HRESIMS *m/z* 503.1868 $[M+H]^+$ (calcd for $C_{26}H_{31}O_{10}$, 503.1917).

3.3.7. Compound 9. Compound **8** (32 mg, 0.064 mmol) and tris(triphenylphosphine)rhodium(I) chloride (59 mg, 0.064 mmol) were dissolved in benzene (5 mL), and the mixture was stirred at 45 °C under a hydrogen atmosphere for 30 h. The solvent was removed in vacuo, and the residue was separated by preparative ODS-HPLC using MeOH–H_2-O (38/62) as an eluent to afford **9** (19.8 mg, 62%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +120.8 (*c* 0.59, $CHCl_3$); IR (film) ν_{max} 3486, 1742, 1659 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.16 (1H, br s, H-15), 5.62 (1H, s, H-2′), 4.80 (1H, d, J=7.9 Hz, H_a-20), 4.78 (1H, s, H-7), 4.38 (1H, m, H-11), 4.23 (1H, s, H-12), 3.88 (1H, dd, J=7.9, 1.5 Hz, H_b-20), 3.79 (3H, s, OCH_3), 3.23 (1H, dd, J=15.5, 3.5 Hz, H_α-6), 3.11 (1H, br d, J=10.9 Hz, H-14), 2.53 (1H, ddd, J=16.7, 14.2, 5.1 Hz, H_β-2), 2.48 (1H, ddd, J=16.7, 5.4, 3.4 Hz, H_α-2), 2.41 (1H, d, J=15.5 Hz, H_β-6), 2.34 (1H, m, H_α-1), 2.18 (3H, d, J=1.1 Hz, H-5′), 1.99 (1H, br s, H-9), 1.97 (1H, dd, J=14.4, 5.6 Hz, H_β-1), 1.92 (3H, d, J=1.1 Hz, H-4′), 1.83 (3H, d, J=0.8 Hz, H-18), 1.68 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 197.2 (C-3), 171.9 (C-21), 166.5 (C-16), 164.5 (C-1′), 161.0 (C-3′), 154.3 (C-5), 132.6 (C-4), 114.1 (C-2′), 82.3 (C-7), 81.6 (C-13), 75.9 (C-12), 73.6 (C-20), 71.3 (C-11), 65.9 (C-15), 53.1 (OCH_3), 51.9 (C-14), 45.7 (C-8), 42.4 (C-9), 39.9 (C-10), 34.9 (C-1), 32.8 (C-2), 32.0 (C-6), 27.7 (C-4′), 21.1 (C-19), 20.6 (C-5′), 11.3 (C-18); HRESIMS *m/z* 505.2074 $[M+H]^+$ (calcd for $C_{26}H_{33}O_{10}$, 505.2074).

3.3.8. Compound 10. To a solution of **2** (100 mg, 0.192 mmol) in *N*,*N*-dimethylformamide (0.5 mL) were added *tert*-butyl(chloro)dimethylsilane (87 mg, 0.58 mmol) and imidazole (90 mg, 1.3 mmol), and the mixture was stirred at room temperature under an argon atmosphere for 24 h. The mixture was diluted with water (0.5 mL), and the precipitate was collected by filtration. This was purified by preparative ODS-HPLC using MeOH–H_2O (76/24) as an eluent to afford **10** (111 mg, 91%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +18.5 (*c* 0.44, $CHCl_3$); IR (film) ν_{max} 3475, 1743, 1676 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.25 (1H, br s, H-15), 5.63 (1H, s, H-2′), 4.78 (1H, s, H-7), 4.72 (1H, d, J=7.9 Hz, H_a-20), 4.25 (1H, br s, H-11), 4.19 (1H, s, H-12), 3.79 (1H, d, J=7.9 Hz, H_b-20), 3.78 (3H, s, OCH_3), 3.13 (1H, br s, H-14), 2.93 (1H, br d, J=14 Hz, H-5), 2.89 (1H, d, J=16.0 Hz, H_β-1), 2.38 (1H, dt, J=14.7, 2.7 Hz, H_α-6), 2.33 (1H, d, J=16.0 Hz, H_α-1), 2.19 (3H, d, J=0.8 Hz, H-5′), 2.07 (1H, br s, H-9), 1.92 (3H, d, J=0.8 Hz, H-4′), 1.84 (3H, d, J=1.7 Hz, H-18), 1.75 (1H, ddd, J=14.7, 13.8, 2.5 Hz, H_β-6), 1.39 (3H, s, H-19), 0.95 (9H, s, Me_3CSi), 0.17 and 0.13 (3H each, s, Me_2Si); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.0 (C-2), 172.0 (C-21), 167.1 (C-16), 164.5 (C-1′), 160.9 (C-3′), 145.0 (C-3), 135.7 (C-4), 114.1 (C-2′), 82.5 (C-7), 81.4 (C-13), 75.8 (C-12), 74.1 (C-20), 70.9 (C-11), 65.8 (C-15), 53.0 (OCH_3), 51.7 (C-14), 50.6 (C-1), 45.4 (C-8), 42.7 (C-5), 42.0 (C-9), 40.4 (C-10), 29.4 (C-6), 27.7 (C-4′), 26.0 (Me_3CSi, 3C), 20.6 (C-5′), 18.8 (Me_3CSi), 15.5 (C-19), 14.4 (C-18), −3.8, −3.9 (Me_2Si); HRESIMS *m/z* 635.2880 $[M+H]^+$ (calcd for $C_{32}H_{47}O_{11}Si$, 635.2888).

3.3.9. Compound 11. To an ice-cooled solution of **10** (50 mg, 0.079 mmol) in acetone (0.5 mL) was added Jones reagent [30 μL, prepared by dissolving chromium(VI) oxide (267 mg) in sulfuric acid–H_2O (23/77, 1 mL)], and the mixture was stirred at room temperature for 1 h. The mixture was diluted with chloroform (30 mL), which was washed sequentially with saturated aqueous $NaHCO_3$ (5 mL) and brine (5 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by silica gel column chromatography using chloroform–methanol (20/1) as an eluent to afford **11** (38.3 mg, 77%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +52.0 (*c* 0.78, $CHCl_3$); IR (film) ν_{max} 3446, 1734, 1672 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.36 (1H, br s, H-15), 5.68 (1H, s, H-2′), 4.89 (1H, s, H-7), 4.30 (1H, s, H-12), 4.29 (1H, d, J=8.2 Hz, H_a-20), 3.80 (1H, d, J=8.2 Hz, H_b-20), 3.78 (3H, s, OCH_3), 3.47 (1H, d, J=16.5 Hz, H_β-1), 3.38 (1H, br s, H-14), 3.02 (1H, s, H-9), 2.97 (1H, d, J=12.9 Hz, H-5), 2.41 (1H, dt, J=14.8, 3.0 Hz, H_α-6), 2.21 (3H, d, J=0.7 Hz, H-5′), 2.18 (1H, d, J=16.5 Hz, H_α-1), 1.95 (3H, d, J=0.9 Hz, H-4′), 1.85 (3H, d, J=1.7 Hz, H-18), 1.64 (1H, ddd, J=14.8, 12.9, 2.3 Hz, H_β-6), 1.21 (3H, s, H-19), 0.95 (9H, s, Me_3CSi), 0.18 and 0.14 (3H each, s, Me_2Si); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.5 (C-11), 191.8 (C-2), 169.7 (C-21), 166.7 (C-16), 164.7 (C-1′), 161.4 (C-3′), 145.4 (C-3), 134.8 (C-4), 114.0 (C-2′), 81.3 (C-13), 80.7 (C-7), 77.8 (C-12), 73.6 (C-20), 65.8 (C-15), 53.1 (OCH_3), 51.3 (C-14), 51.2 (C-1), 50.6 (C-9), 46.7 (C-8), 40.7 (C-5), 38.9 (C-10), 28.6 (C-6), 27.7 (C-4′), 26.0 (Me_3CSi, 3C), 20.7 (C-5), 18.9 (Me_3CSi), 14.3 (C-18), 13.3 (C-19), −3.7, −3.9 (Me_2Si); HRESIMS *m/z* 633.2723 $[M+H]^+$ (calcd for $C_{32}H_{45}O_{11}Si$, 633.2731).

3.3.10. Treatment of 10 with thionyl chloride. Thionyl chloride (12 μL, 0.16 mmol) and pyridine (26 μL, 0.32 mmol) were added to a solution of **10** (20 mg, 0.032 mmol) in chloroform (0.5 mL) at 0 °C, and the resultant mixture was stirred at 60 °C under an argon atmosphere for 10 h. After dilution with chloroform (30 mL), the mixture was washed with saturated aqueous $NaHCO_3$ (5 mL) and brine (5 mL), dried over Na_2SO_4, filtered and evaporated in vacuo. The residue was shown to be a mixture of **12a** and **12b** in a ratio of ca. 2:1. The mixture was subjected to preparative ODS-HPLC using MeOH–H_2-O (70/30) as an eluent to afford **12a** (10.8 mg, 54%) and **12b** (5.0 mg, 25%). Compound **12a**: colorless amorphous solid, $[\alpha]_D^{24}$ −113.5 (*c* 0.18, $CHCl_3$); IR (film) ν_{max} 1752, 1677 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.91 (1H, d, J=4.4 Hz, H-11), 5.75 (1H, s, H-2′), 5.24 (1H, br s, H-15), 5.00 (1H, s, H-7), 4.71 (1H, d, J=4.4 Hz, H-12), 4.01 (1H, d, J=7.2 Hz, H_a-20), 3.86 (1H, d, J=7.2 Hz, H_b-20), 3.81 (3H, s, OCH_3), 3.66 (1H, br s, H-14), 3.07 (1H, br d, J=13.0 Hz, H-5), 2.91 (1H, d, J=16.1 Hz, H_β-1), 2.49 (1H, dt, J=14.3, 2.9 Hz, H_α-6), 2.47 (1H, d, J=16.1 Hz, H_α-1), 2.18 (3H, s, H-5′), 1.93 (3H, s, H-4′), 1.90 (3H, d, J=1.1 Hz, H-18), 1.77 (1H, dd, J=14.3, 13.0 Hz, H_β-6), 1.16 (3H, s, H-19), 0.95 (9H, s, Me_3CSi), 0.16 (6H, s, Me_2Si); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.1 (C-2), 167.1 (C-21), 166.6 (C-16), 165.3 (C-1′), 159.6 (C-3′), 145.3 (C-3), 144.1 (C-9), 136.0 (C-4), 122.3 (C-11), 115.0 (C-2′), 83.2 (C-13), 79.6 (C-7), 78.4 (C-20), 67.1 (C-15), 53.3 (C-12), 53.0 (OCH_3), 49.6 (C-1), 47.2 (C-8), 45.2 (C-14), 40.8 (C-10), 38.2 (C-5), 28.7 (C-6), 27.6 (C-4′), 26.0 (Me_3CSi, 3C), 23.0

(C-19), 20.6 (C-5′), 18.8 (Me_3CSi), 14.6 (C-18), −3.8×2 (Me_2Si); HRESIMS *m/z* 635.2415 $[M+H]^+$ (calcd for $C_{32}H_{44}O_9ClSi$, 635.2443). Compound **12b**: colorless amorphous solid, $[\alpha]_D^{24}$ +27.8 (*c* 0.19, $CHCl_3$); IR (film) ν_{max} 1742, 1675 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 5.73 (1H, d, *J*=2.3 Hz, H-11), 5.70 (1H, br s, H-15), 5.67 (1H, s, H-2′), 5.30 (1H, s, H-12), 4.92 (1H, s, H-7), 4.38 (1H, d, *J*=7.3 Hz, H_a-20), 4.12 (1H, dd, *J*=7.3, 0.9 Hz, H_b-20), 3.75 (3H, s, OCH_3), 3.11 (1H, d, *J*=12.6 Hz, H-14), 2.96 (1H, d, *J*=13.0 Hz, H-5), 2.91 (1H, d, *J*=16.1 Hz, H_β-1), 2.48 (1H, dt, *J*=14.7, 3.0 Hz, H_α-6), 2.43 (1H, d, *J*=16.1 Hz, H_α-1), 2.22 (3H, d, *J*=1.0 Hz, H-5′), 1.96 (3H, d, *J*=1.0 Hz, H-4′), 1.88 (3H, d, *J*=1.8 Hz, H-18), 1.79 (1H, ddd, *J*=14.7, 13.0, 2.2 Hz, H_β-6), 1.21 (3H, s, H-19), 0.95 (9H, s, Me_3CSi), 0.18 and 0.15 (3H, each, s, Me_2Si); HRESIMS *m/z* 635.2444 $[M+H]^+$ (calcd for $C_{32}H_{44}O_9ClSi$, 635.2443).

3.3.11. Treatment of 12a and 12b with potassium carbonate. To a solution of a mixture of **12a** and **12b** (a product of Section 3.3.10 without HPLC, a ca. 2:1 mixture, 20 mg, 0.031 mmol) in acetone–H_2O (10/1, 1 mL) was added potassium carbonate (85 mg, 0.62 mmol), and the mixture was stirred at room temperature for 6 h. The mixture was diluted with chloroform (20 mL), washed sequentially with 1 M HCl (5 mL) and brine (5 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative ODS-HPLC using MeCN–H_2O (65/35) as an eluent to afford **13** (10.4 mg, 54%) as a colorless amorphous solid: $[\alpha]_D^{24}$ −106.0 (*c* 0.20, $CHCl_3$); IR (film) ν_{max} 3444, 1753, 1731, 1680 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.38 (1H, dd, *J*=9.8, 1.2 Hz, H-12), 6.10 (1H, d, *J*=9.8 Hz, H-11), 5.77 (1H, s, H-2′), 5.41 (1H, d, *J*=1.9 Hz, H-15), 4.21 (1H, br s, H-7), 4.18 (1H, d, *J*=9.8 Hz, H_a-20), 3.83 (3H, s, OCH_3), 3.78 (1H, d, *J*=9.8 Hz, H_b-20), 3.74 (1H, br d, *J*=13.1 Hz, H-5), 3.19 (1H, d, *J*=16.2 Hz, H_α-1), 2.72 (1H, dd, *J*=1.9, 1.2 Hz, H-14), 2.56 (1H, br s, HO-7), 2.52 (1H, d, *J*=16.2 Hz, H_β-1), 2.18 (1H, d-like, *J*=14.3 Hz, H_α-6), 2.18 (3H, d, *J*=0.6 Hz, H-5′), 1.92 (3H, d, *J*=0.6 Hz, H-4′), 1.85 (3H, d, *J*=1.7 Hz, H-18), 1.64 (1H, ddd, *J*=14.3, 13.1, 2.6 Hz, H_β-6), 1.07 (3H, s, H-19), 0.94 (9H, s, Me_3CSi), 0.15 (6H, s, Me_2Si); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.4 (C-2), 169.1 (C-21), 167.2 (C-16), 164.4 (C-1′), 159.9 (C-3′), 144.6 (C-3), 136.0 (C-4), 130.5 (C-12), 128.1 (C-11), 114.7 (C-2′), 85.4 (C-9), 82.2 (C-13), 72.8 (C-7), 71.1 (C-20), 63.1 (C-15), 54.5 (C-14), 53.2 (OCH_3), 48.2 (C-8), 45.0 (C-1), 44.4 (C-10), 34.2 (C-5), 30.5 (C-6), 27.6 (C-4′), 26.0 (Me_3CSi, 3C), 20.6 (C-5′), 18.9 (Me_3CSi), 17.3 (C-19), 14.5 (C-18), −3.8×2 (Me_2Si); HRESIMS *m/z* 617.2754 $[M+H]^+$ (calcd for $C_{32}H_{45}O_{10}Si$, 617.2782).

3.3.12. Compound 14. A tetrabutylammonium fluoride solution (1.0 M in tetrahydrofuran, 120 μL, 0.120 mmol) was added to a solution of **11** (15 mg, 0.024 mmol) in tetrahydrofuran (0.5 mL), and the mixture was stirred at room temperature under an argon atmosphere for 15 min. The mixture was diluted with chloroform (30 mL), washed sequentially with water (5 mL) and brine (5 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative ODS-HPLC using MeCN–H_2O (35/65) as an eluent to afford **14** (5.6 mg, 46%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +52.8 (*c* 0.04, $CHCl_3$); IR (film) ν_{max} 3431, 1735, 1644 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.38 (1H, br s, H-15), 5.68 (1H, s, H-2′), 4.89 (1H, s, H-7), 4.31 (1H, d, *J*=8.4 Hz, H_a-20), 4.30 (1H, s, H-12), 3.79 (3H, s, OCH_3), 3.78 (1H, d, *J*=8.4 Hz, H_b-20), 3.58 (1H, d, *J*=16.8 Hz, H_β-1), 3.40 (1H, br s, H-14), 3.05 (1H, s, H-9), 2.98 (1H, d, *J*=13.1 Hz, H-5), 2.41 (1H, dt, *J*=14.8, 3.0 Hz, H_α-6), 2.24 (1H, d, *J*=16.8 Hz, H_α-1), 2.21 (3H, d, *J*=1.1 Hz, H-5′), 1.95 (3H, d, *J*=1.1 Hz, H-4′), 1.85 (3H, d, *J*=1.9 Hz, H-18), 1.65 (1H, ddd, *J*=14.8, 13.1, 2.4 Hz, H_β-6), 1.21 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.2 (C-11), 191.9 (C-2), 169.9 (C-21), 166.6 (C-16), 164.7 (C-1′), 161.6 (C-3′), 144.4 (C-3), 126.7 (C-4), 113.9 (C-2′), 81.2 (C-13), 80.6 (C-7), 77.8 (C-12), 73.7 (C-20), 65.7 (C-15), 53.2 (OCH_3), 51.4 (C-14), 50.5 (C-9), 49.3 (C-1), 46.8 (C-8), 39.9 (C-5), 39.7 (C-10), 28.3 (C-6), 27.7 (C-4′), 20.7 (C-5′), 13.2×2 (C-18, C-19); HRESIMS *m/z* 519.1853 $[M+H]^+$ (calcd for $C_{26}H_{31}O_{11}$, 519.1866).

3.3.13. Compound 15. To a solution of **13** (15 mg, 0.024 mmol) in tetrahydrofuran (0.5 mL) was added a tetrabutylammonium fluoride solution (1.0 M in tetrahydrofuran, 120 μL, 0.120 mmol), and the mixture was stirred at room temperature under an argon atmosphere for 20 min. The mixture was diluted with chloroform (30 mL), washed sequentially with water (5 mL) and brine (5 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative ODS-HPLC using MeCN–H_2O (35/65) as an eluent to afford **15** (5.6 mg, 46%) as a colorless amorphous solid: $[\alpha]_D^{24}$ −113.4 (*c* 0.11, $CHCl_3$); IR (film) ν_{max} 3429, 1745, 1643 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.39 (1H, d, *J*=9.8 Hz, H-12), 6.08 (1H, d, *J*=9.8 Hz, H-11), 5.78 (1H, s, H-2′), 5.43 (1H, d, *J*=1.5 Hz, H-15), 4.20 (1H, s, H-7), 4.16 (1H, d, *J*=9.8 Hz, H_a-20), 3.82 (3H, s, OCH_3), 3.78 (1H, d, *J*=9.8 Hz, H_b-20), 3.73 (1H, d, *J*=12.8 Hz, H-5), 3.20 (1H, d, *J*=16.5 Hz, H_α-1), 2.74 (1H, s, H-14), 2.59 (1H, d, *J*=16.5 Hz, H_β-1), 2.22 (1H, br d, *J*=14.5 Hz, H_α-6), 2.18 (3H, s, H-5′), 1.93 (3H, s, H-4′), 1.84 (3H, d, *J*=1.1 Hz, H-18), 1.59 (1H, dd, *J*=14.5, 12.8 Hz, H_β-6), 1.06 (3H, s, H-19); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.4 (C-2), 169.0 (C-21), 167.9 (C-16), 164.5 (C-1′), 159.8 (C-3′), 143.5 (C-3), 130.7 (C-12), 128.5 (C-4), 127.8 (C-11), 114.8 (C-2′), 85.6 (C-9), 82.1 (C-13), 72.5 (C-7), 71.0 (C-20), 63.1 (C-15), 54.4 (C-14), 53.1 (OCH_3), 48.2 (C-8), 45.1 (C-10), 43.3 (C-1), 33.5 (C-5), 30.0 (C-6), 27.6 (C-4′), 20.6 (C-5′), 17.3 (C-19), 13.4 (C-18); HRESIMS *m/z* 503.1882 $[M+H]^+$ (calcd for $C_{26}H_{31}O_{10}$, 503.1917).

3.3.14. Compound 16. A solution of **10** (60 mg, 0.095 mmol) and lithium iodide (63 mg, 0.47 mmol) in pyridine (1 mL) was stirred at 100 °C under an argon atmosphere for 24 h. The solvent was removed in vacuo, and the residue was dissolved in chloroform (30 mL). The solution was washed sequentially with 1 M HCl (5 mL) and brine (5 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative ODS-HPLC using MeCN–H_2O–AcOH (51/48/1) as an eluent to afford acid **16** (42.4 mg, 72%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +15.3 (*c* 0.85, $CHCl_3$); IR (film) ν_{max} 3467, 1728, 1672 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.20 (1H, br s, H-15), 5.69 (1H, s, H-2′), 4.91 (1H, s, H-7), 4.70 (1H, br s, H_a-20), 4.17 (1H, s, H-11), 4.12 (1H, s, H-12), 3.74 (1H, br s, H_b-20), 3.24 (1H, br s, H-14),

2.99 (1H, d, J=12.2 Hz, H-5), 2.82 (1H, d, J=15.9 Hz, H_β-1), 2.51 (1H, d, J=15.9 Hz, H_α-1), 2.31 (1H, dd, J=14.6, 2.4 Hz, H_α-6), 2.19 (1H, s, H-9), 2.15 (3H, s, H-5′), 1.92 (3H, s, H-4′), 1.89 (3H, s, H-18), 1.88 (1H, m, H_β-6), 1.39 (3H, s, H-19), 0.97 (9H, s, Me_3CSi), 0.17 and 0.12 (3H each, s, Me_2Si); ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.1 (C-2), 174.6 (C-21), 170.0 (C-16), 166.5 (C-1′), 160.0 (C-3′), 146.0 (C-3), 139.1 (C-4), 116.1 (C-2′), 84.9 (C-7), 82.6 (C-13), 77.1 (C-12), 74.5 (C-20), 72.4 (C-11), 68.1 (C-15), 51.4×2 (C-1, C-14), 46.6 (C-8), 44.0 (C-5), 42.6 (C-9), 41.7 (C-10), 30.3 (C-6), 27.5 (C-4′), 26.5 (Me_3CSi, 3C), 20.5 (C-5′), 19.7 (Me_3CSi), 15.9 (C-19), 14.8 (C-18), −3.4, −3.6 (Me_2Si); HRESIMS m/z 621.2755 $[M+H]^+$ (calcd for $C_{31}H_{45}O_{11}Si$, 621.2731).

3.3.15. Compound 17. To a solution of **16** (20 mg, 0.032 mmol) in acetone (0.5 mL) were added potassium carbonate (22 mg, 0.16 mmol) and iodoethane (26 μL, 0.33 mmol), and the mixture was stirred at 50 °C under an argon atmosphere for 6 h. The mixture was diluted with chloroform (20 mL) and the insoluble matter was removed by filtration. The filtrate was concentrated in vacuo, and the crude product was purified by silica gel column chromatography using chloroform–methanol (20/1) as an eluent to afford **17** (18.9 mg, 90%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +11.3 (c 0.23, $CHCl_3$); IR (film) ν_{max} 3473, 1739, 1676 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.29 (1H, br s, H-15), 5.63 (1H, s, H-2′), 4.78 (1H, s, H-7), 4.72 (1H, d, J=7.9 Hz, H_a-20), 4.28–4.20 (3H, m, H-11 and H-1″), 4.18 (1H, s, H-12), 3.79 (1H, d, J=7.9 Hz, H_b-20), 3.10 (1H, br s, H-14), 2.93 (1H, d, J=12.5 Hz, H-5), 2.90 (1H, d, J=15.9 Hz, H_β-1), 2.38 (1H, dt, J=14.7, 2.7 Hz, H_α-6), 2.33 (1H, d, J=15.9 Hz, H_α-1), 2.19 (3H, s, H-5′), 2.07 (1H, br s, H-9), 1.92 (3H, s, H-4′), 1.84 (3H, d, J=1.6 Hz, H-18), 1.75 (1H, ddd, J=14.7, 12.5, 2.5 Hz, H_β-6), 1.39 (3H, s, H-19), 1.32 (3H, t, J=7.2 Hz, H-2″), 0.95 (9H, s, Me_3CSi), 0.17 and 0.14 (3H each, s, Me_2Si); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.9 (C-2), 171.7 (C-21), 167.1 (C-16), 164.5 (C-1′), 161.0 (C-3′), 145.1 (C-3), 135.6 (C-4), 114.1 (C-2′), 82.5 (C-7), 81.3 (C-13), 75.9 (C-12), 74.1 (C-20), 70.9 (C-11), 65.8 (C-15), 62.5 (C-1″), 51.7 (C-14), 50.6 (C-1), 45.4 (C-8), 42.7 (C-5), 42.0 (C-9), 40.5 (C-10), 29.4 (C-6), 27.7 (C-4′), 26.0 (Me_3CSi, 3C), 20.6 (C-5′), 18.8 (Me_3CSi), 15.5 (C-19), 14.4 (C-18), 14.0 (C-2″), −3.8, −3.9 (Me_2Si); HRESIMS m/z 649.3077 $[M+H]^+$ (calcd for $C_{33}H_{49}O_{11}Si$, 649.3044).

3.3.16. Compound 18. As described for **17**, the reaction of compound **16** (10 mg, 0.016 mmol), potassium carbonate (11 mg, 0.080 mmol), and 1-iodopropane (16 μL, 0.16 mmol) in acetone (0.5 mL) at 50 °C for 16 h afforded **18** (9.7 mg, 91%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +10.8 (c 0.19, $CHCl_3$); IR (film) ν_{max} 3460, 1738, 1674 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.30 (1H, br s, H-15), 5.62 (1H, s, H-2′), 4.79 (1H, s, H-7), 4.72 (1H, d, J=7.9 Hz, H_a-20), 4.25 (1H, m, H-11), 4.18 (1H, s, H-12), 4.12 (2H, m, H-1″), 3.79 (1H, d, J=7.9 Hz, H_b-20), 3.09 (1H, br s, H-14), 2.93 (1H, d, J=12.6 Hz, H-5), 2.90 (1H, d, J=15.9 Hz, H_β-1), 2.38 (1H, dt, J=14.7, 2.8 Hz, H_α-6), 2.33 (1H, d, J=15.9 Hz, H_α-1), 2.18 (3H, d, J=0.9 Hz, H-5′), 2.07 (1H, br s, H-9), 1.92 (3H, d, J=1.1 Hz, H-4′), 1.84 (3H, d, J=1.7 Hz, H-18), 1.75 (1H, ddd, J=14.7, 12.6, 2.6 Hz, H_β-6), 1.75–1.67 (2H, m, H-2″), 1.39 (3H, s, H-19), 0.96 (3H, t, J=7.5 Hz, H-3″), 0.95 (9H, s, Me_3CSi), 0.18 and 0.14 (3H each, s, Me_2Si); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.9 (C-2), 171.8 (C-21), 167.1 (C-16), 164.5 (C-1′), 160.9 (C-3′), 145.1 (C-3), 135.6 (C-4), 114.1 (C-2′), 82.5 (C-7), 81.4 (C-13), 76.0 (C-12), 74.1 (C-20), 70.9 (C-11), 68.0 (C-1″), 65.8 (C-15), 52.0 (C-14), 50.6 (C-1), 45.4 (C-8), 42.7 (C-5), 42.0 (C-9), 40.5 (C-10), 29.4 (C-6), 27.7 (C-4′), 26.0 (Me_3CSi, 3C), 21.8 (C-2″), 20.6 (C-5′), 18.8 (Me_3CSi), 15.5 (C-19), 14.4 (C-18), 10.3 (C-3″), −3.8, −3.9 (Me_2Si); HRESIMS m/z 663.3204 $[M+H]^+$ (calcd for $C_{34}H_{51}O_{11}Si$, 663.3201).

3.3.17. Compound 19. As described for **17**, the reaction of compound **16** (10 mg, 0.016 mmol), potassium carbonate (11 mg, 0.080 mmol), and 1-iodobutane (18 μL, 0.16 mmol) in acetone (0.5 mL) at 50 °C for 16 h afforded **19** (10 mg, 92%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +2.0 (c 0.20, $CHCl_3$); IR (film) ν_{max} 3483, 1730, 1672 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.30 (1H, br s, H-15), 5.63 (1H, s, H-2′), 4.79 (1H, s, H-7), 4.72 (1H, d, J=7.9 Hz, H_a-20), 4.25 (1H, m, H-11), 4.17 (1H, s, H-12), 4.16 (2H, m, H-1″), 3.79 (1H, d, J=7.9 Hz, H_b-20), 3.08 (1H, br s, H-14), 2.93 (1H, d, J=12.5 Hz, H-5), 2.90 (1H, d, J=15.9 Hz, H_β-1), 2.38 (1H, dt, J=14.7, 2.8 Hz, H_α-6), 2.32 (1H, d, J=15.9 Hz, H_α-1), 2.19 (3H, d, J=1.0 Hz, H-5′), 2.07 (1H, br s, H-9), 1.92 (3H, d, J=1.0 Hz, H-4′), 1.84 (3H, d, J=1.7 Hz, H-18), 1.75 (1H, ddd, J=14.7, 12.5, 2.6 Hz, H_β-6), 1.66 (2H, m, H-2″), 1.39 (3H, s, H-19), 1.38 (2H, m, H-3″), 0.95 (3H, t, J=7.5 Hz, H-4″), 0.95 (9H, s, Me_3CSi), 0.18 and 0.14 (3H each, s, Me_2Si); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.9 (C-2), 171.8 (C-21), 167.1 (C-16), 164.4 (C-1′), 160.9 (C-3′), 145.1 (C-3), 135.6 (C-4), 114.2 (C-2′), 82.5 (C-7), 81.4 (C-13), 76.0 (C-12), 74.1 (C-20), 70.9 (C-11), 66.4 (C-1″), 65.8 (C-15), 51.8 (C-14), 50.6 (C-1), 45.4 (C-8), 42.7 (C-5), 42.0 (C-9), 40.5 (C-10), 30.4 (C-2″), 29.4 (C-6), 27.7 (C-4′), 26.0 (Me_3CSi, 3C), 20.6 (C-5′), 19.1 (C-3″), 18.8 (Me_3CSi), 15.5 (C-19), 14.4 (C-18), 13.7 (C-4″), −3.8, −3.9 (Me_2Si); HRESIMS m/z 677.3362 $[M+H]^+$ (calcd for $C_{35}H_{53}O_{11}Si$, 677.3357).

3.3.18. Compound 20. As described for **17**, the reaction of compound **16** (10 mg, 0.016 mmol), potassium carbonate (11 mg, 0.080 mmol), and 1-iodopentane (21 μL, 0.16 mmol) in acetone (0.5 mL) at 50 °C for 24 h afforded **20** (9.8 mg, 88%) as a colorless amorphous solid: $[\alpha]_D^{24}$ −11.8 (c 0.26, $CHCl_3$); IR (film) ν_{max} 3417, 1739, 1672 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.29 (1H, br s, H-15), 5.62 (1H, s, H-2′), 4.79 (1H, s, H-7), 4.72 (1H, d, J=7.9 Hz, H_a-20), 4.25 (1H, m, H-11), 4.18 (1H, s, H-12), 4.15 (2H, m, H-1″), 3.78 (1H, d, J=7.9 Hz, H_b-20), 3.09 (1H, br s, H-14), 2.93 (1H, d, J=12.3 Hz, H-5), 2.90 (1H, d, J=15.8 Hz, H_β-1), 2.38 (1H, d, J=14.5 Hz, H_α-6), 2.32 (1H, d, J=15.8 Hz, H_α-1), 2.18 (3H, s, H-5′), 2.06 (1H, br s, H-9), 1.91 (3H, s, H-4′), 1.84 (3H, d, J=1.2 Hz, H-18), 1.75 (1H, ddd, J=14.5, 12.3, 2.6 Hz, H_β-6), 1.68 (2H, m, H-2″), 1.39 (3H, s, H-19), 1.37–1.28 (4H, m, H-3″ and H-4″), 0.95 (9H, s, Me_3CSi), 0.92 (3H, t, J=7.0 Hz, H-5″), 0.17 and 0.13 (3H each, s, Me_2Si); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.0 (C-2), 171.7 (C-21), 167.1 (C-16), 164.5 (C-1′), 160.9 (C-3′), 145.1 (C-3), 135.7 (C-4), 114.2 (C-2′), 82.5 (C-7), 81.4 (C-13), 75.9 (C-12), 74.0 (C-20), 70.9 (C-11), 66.7 (C-1″), 65.8 (C-15), 51.8 (C-14), 50.6 (C-1), 45.4 (C-8), 42.7 (C-5), 42.0 (C-9), 40.5 (C-10), 29.4 (C-6), 28.0 (C-2″), 27.9 (C-3″),

27.7 (C-4′), 26.0 (Me_3CSi, 3C), 22.3 (C-4″), 20.6 (C-5′), 18.8 (Me_3CSi), 15.5 (C-19), 14.4 (C-18), 13.9 (C-5″), −3.8, −3.9 (Me_2Si); HRESIMS *m/z* 691.3505 $[M+H]^+$ (calcd for $C_{36}H_{55}O_{11}Si$, 691.3514).

3.3.19. Compound 21. To a solution of **17** (10 mg, 0.015 mmol) in tetrahydrofuran (0.5 mL) was added a tetrabutylammonium fluoride solution (1.0 M solution in tetrahydrofuran, 80 μL, 0.080 mmol), and the mixture was stirred at room temperature under an argon atmosphere for 15 min. Water (5 mL) was added to the mixture, and the whole was extracted with chloroform (3×10 mL). The combined organic layers were washed sequentially with water (5 mL) and brine (5 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo, and the crude product was purified by preparative ODS-HPLC using MeCN–H_2O (30/70) as an eluent to afford **21** (6.3 mg, 76%) as a colorless amorphous solid: $[\alpha]_D^{24}$ +2.4 (*c* 0.13, $CHCl_3$); IR (film) ν_{max} 3452, 1731, 1644 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.31 (1H, br s, H-15), 5.63 (1H, s, H-2′), 4.79 (1H, s, H-7), 4.72 (1H, d, *J*=8.0 Hz, H_a-20), 4.27–4.23 (3H, m, H-11 and H-1″), 4.19 (1H, s, H-12), 3.80 (1H, dd, *J*=8.0, 1.2 Hz, H_b-20), 3.11 (1H, br s, H-14), 2.98 (1H, d, *J*=16.2 Hz, H_β-1), 2.96 (1H, d, *J*=12.6 Hz, H-5), 2.39 (1H, d, *J*=16.2 Hz, H_α-1), 2.39 (1H, d, *J*=15.1 Hz, H_α-6), 2.19 (3H, d, *J*=0.8 Hz, H-5′), 2.12 (1H, br s, H-9), 1.92 (3H, d, *J*=0.8 Hz, H-4′), 1.85 (3H, d, *J*=1.8 Hz, H-18), 1.76 (1H, ddd, *J*=15.1, 12.6, 2.6 Hz, H_β-6), 1.40 (3H, s, H-19), 1.32 (3H, t, *J*=7.2 Hz, H-2″); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.9 (C-2), 171.7 (C-21), 167.0 (C-16), 164.5 (C-1′), 161.1 (C-3′), 144.1 (C-3), 127.6 (C-4), 114.1 (C-2′), 82.3 (C-7), 81.3 (C-13), 75.9 (C-12), 74.1 (C-20), 70.9 (C-11), 65.7 (C-15), 62.6 (C-1″), 51.8 (C-14), 48.7 (C-1), 45.5 (C-8), 42.0 (C-9), 41.9 (C-5), 41.2 (C-10), 29.1 (C-6), 27.7 (C-4′), 20.6 (C-5′), 15.5 (C-19), 14.0 (C-2″), 13.3 (C-18); HRESIMS *m/z* 535.2159 $[M+H]^+$ (calcd for $C_{27}H_{35}O_{11}$, 535.2179).

3.3.20. Compound 22. As described for **21**, desilylation of **18** (10 mg, 0.015 mmol) afforded **22** (6.5 mg, 79%) as a colorless amorphous solid: $[\alpha]_D^{24}$ −1.3 (*c* 0.15, $CHCl_3$); IR (film) ν_{max} 3442, 1731, 1644 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.32 (1H, br s, H-15), 5.62 (1H, s, H-2′), 4.79 (1H, s, H-7), 4.72 (1H, d, *J*=7.9 Hz, H_a-20), 4.25 (1H, s, H-11), 4.19 (1H, s, H-12), 4.13 (2H, m, H-1″), 3.80 (1H, d, *J*=7.9 Hz, H_b-20), 3.10 (1H, br s, H-14), 2.99 (1H, d, *J*=16.2 Hz, H_β-1), 2.96 (1H, d, *J*=12.8 Hz, H-5), 2.39 (1H, d, *J*=16.2 Hz, H_α-1), 2.39 (1H, d-like, *J*=15.2 Hz, H_α-6), 2.19 (3H, s, H-5′), 2.11 (1H, br s, H-9), 1.92 (3H, s, H-4′), 1.85 (3H, d, *J*=1.4 Hz, H-18), 1.76 (1H, ddd, *J*=15.2, 12.8, 2.4 Hz, H_β-6), 1.71 (2H, m, H-2″), 1.40 (3H, s, H-19), 0.96 (3H, t, *J*=7.5 Hz, H-3″); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.0 (C-2), 171.7 (C-21), 167.1 (C-16), 164.4 (C-1′), 161.0 (C-3′), 144.1 (C-3), 127.6 (C-4), 114.1 (C-2′), 82.3 (C-7), 81.4 (C-13), 75.9 (C-12), 74.1 (C-20), 70.9 (C-11), 68.0 (C-1″), 65.8 (C-15), 51.8 (C-14), 48.7 (C-1), 45.5 (C-8), 42.0 (C-9), 41.9 (C-5), 41.2 (C-10), 29.1 (C-6), 27.7 (C-4′), 21.8 (C-2″), 20.6 (C-5′), 15.5 (C-19), 13.3 (C-18), 10.3 (C-3″); HRESIMS *m/z* 549.2338 $[M+H]^+$ (calcd for $C_{28}H_{37}O_{11}$, 549.2336).

3.3.21. Compound 23. As described for **21**, desilylation of **19** (10 mg, 0.015 mmol) afforded **23** (6.1 mg, 73%) as a colorless amorphous solid: $[\alpha]_D^{24}$ 0 (*c* 0.04, $CHCl_3$); IR (film) ν_{max} 3443, 1732, 1645 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.33 (1H, br s, H-15), 5.63 (1H, s, H-2′), 4.79 (1H, s, H-7), 4.72 (1H, d, *J*=8.0 Hz, H_a-20), 4.25 (1H, br s, H-11), 4.19 (1H, s, H-12), 4.17 (2H, m, H-1″), 3.80 (1H, d, *J*=8.0 Hz, H_b-20), 3.09 (1H, br s, H-14), 2.99 (1H, d, *J*=16.2 Hz, H_β-1), 2.96 (1H, d, *J*=12.3 Hz, H-5), 2.39 (1H, d, *J*=16.2 Hz, H_α-1), 2.39 (1H, d, *J*=14.9 Hz, H_α-6), 2.20 (3H, s, H-5′), 2.12 (1H, br s, H-9), 1.93 (3H, s, H-4′), 1.85 (3H, d, *J*=1.8 Hz, H-18), 1.76 (1H, ddd, *J*=14.9, 12.3, 2.6 Hz, H_β-6), 1.67 (2H, m, H-2″), 1.41 (3H, s, H-19), 1.38 (2H, m, H-3″), 0.96 (3H, t, *J*=7.4 Hz, H-4″); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.9 (C-2), 171.8 (C-21), 167.1 (C-16), 164.5 (C-1′), 161.1 (C-3′), 144.1 (C-3), 127.5 (C-4), 114.1 (C-2′), 82.3 (C-7), 81.4 (C-13), 76.0 (C-12), 74.1 (C-20), 70.9 (C-11), 66.4 (C-1″), 65.7 (C-15), 51.9 (C-14), 48.7 (C-1), 45.5 (C-8), 42.0 (C-9), 41.9 (C-5), 41.2 (C-10), 30.4 (C-2″), 29.1 (C-6), 27.7 (C-4′), 20.6 (C-5′), 19.1 (C-3″), 15.5 (C-19), 13.7 (C-4″), 13.3 (C-18); HRESIMS *m/z* 563.2471 $[M+H]^+$ (calcd for $C_{29}H_{39}O_{11}$, 563.2492).

3.3.22. Compound 24. As described for **21**, desilylation of **20** (25 mg, 0.036 mmol) afforded **24** (12.7 mg, 61%) as a colorless amorphous solid: $[\alpha]_D^{24}$ −2.8 (*c* 0.25, $CHCl_3$); IR (film) ν_{max} 3452, 1731, 1644 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.31 (1H, br s, H-15), 5.62 (1H, s, H-2′), 4.79 (1H, s, H-7), 4.72 (1H, d, *J*=8.0 Hz, H_a-20), 4.25 (1H, br s, H-11), 4.19 (1H, s, H-12), 4.15 (2H, m, H-1″), 3.80 (1H, d, *J*=8.0 Hz, H_b-20), 3.09 (1H, br s, H-14), 2.98 (1H, d, *J*=16.1 Hz, H_β-1), 2.96 (1H, d, *J*=12.8 Hz, H-5), 2.39 (1H, d, *J*=16.1 Hz, H_α-1), 2.39 (1H, d, *J*=15.2 Hz, H_α-6), 2.19 (3H, s, H-5′), 2.12 (1H, br s, H-9), 1.92 (3H, s, H-4′), 1.85 (3H, d, *J*=1.5 Hz, H-18), 1.76 (1H, ddd, *J*=15.2, 12.8, 2.4 Hz, H_β-6), 1.68 (2H, m, H-2″), 1.40 (3H, s, H-19), 1.38–1.28 (4H, m, H-3″ and H-4″), 0.92 (3H, t, *J*=7.0 Hz, H-5″); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.0 (C-2), 171.7 (C-21), 167.1 (C-16), 164.4 (C-1′), 161.0 (C-3′), 144.1 (C-3), 127.6 (C-4), 114.1 (C-2′), 82.3 (C-7), 81.4 (C-13), 75.9 (C-12), 74.1 (C-20), 70.9 (C-11), 66.7 (C-1″), 65.7 (C-15), 51.9 (C-14), 48.7 (C-1), 45.5 (C-8), 42.1 (C-9), 41.9 (C-5), 41.2 (C-10), 29.1 (C-6), 28.0 (C-2″), 27.9 (C-3″), 27.7 (C-4′), 22.3 (C-4″), 20.6 (C-5′), 15.5 (C-19), 13.9 (C-5″), 13.3 (C-18); HRESIMS *m/z* 577.2626 $[M+H]^+$ (calcd for $C_{30}H_{41}O_{11}$, 577.2649).

3.4. Cytotoxicity assays

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay was performed on a 96-well plate. Murine P-388 leukemia cells (3×10^3 cells) in 100 μL of RPMI-1640 medium (Nissui Pharmaceutical Company, Ltd, Tokyo, Japan) supplemented with 5% fetal calf serum (Mitsubishi Chemical Industry Co., Ltd, Tokyo, Japan) and kanamycin (100 μg/mL) were inoculated into each well and incubated at 37 °C in a humidified atmosphere of 7% CO_2. Test samples of various concentrations (10 μL) were added to the cultures 24 h after incubation. The medium was incubated for 48 h at 37 °C and then 20 μL of the MTT solution (5 mg/mL) was added to each well. After a further incubation for 4 h, 100 μL of 10% sodium dodecyl sulfate–0.01 M HCl solution was added to each well, and the formazan crystals that were formed in each well were dissolved by stirring with a pipette. Optical density was

recorded on a microplate reader (Tosoh MPR-A4i) at 550 nm. In the assay for cytotoxicity, each data point represents the average of three replicate measurements.

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