Synthesis and *in Vitro* Evaluation of New Wortmannin Esters: Potent Inhibitors of Phosphatidylinositol 3-Kinase

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New C-11 esters of the fermentation product wortmannin have been synthesized, with some of them further derivatized at C-17. The new esters show greater inhibition of isolated phosphatidylinositol 3-kinase and increased cell cytotoxicity in a rapidly proliferating leukemia cell line, when compared to wortmannin. Reduction of the C-17 ketone caused a slight increase in activity, while acylation of this new alcohol caused severe loss of activity. With their increased activity, the new C-11 esters may be good candidates to explore the *in vivo* antitumor effects of phosphatidylinositol 3-kinase inhibitors.

Introduction

In order to improve current cancer therapy, biological activities beyond simple cell cytotoxicity are being investigated. One current area of interest is the regulation of cell proliferation and the signaling pathways within cells that control it.[1] If drugs can be developed to interfere with signal transduction, then cancer cells may be made nonproliferating, and new cancer therapies may emerge. Phosphatidylinositol 3-kinase (PI 3-kinase) is an enzyme that functions within signal transduction pathways; therefore, inhibitors of this enzyme may be useful for new cancer and tumor therapies.[2] It has recently been shown that the fermentation product wortmannin (**1**), produced by *Penicillium wortmanni*, is a potent inhibitor of PI 3-kinase.[3−5] Wortmannin is a potent cell cytotoxic agent and causes toxic effects in rats including weight loss, internal hemorrhaging, and death.[6] More potent inhibitors of PI 3-kinase with less general toxicity and/or lower dose requirements may be useful in investigating the inhibition of PI 3-kinase for *in vivo* antitumor activity.[7] We have therefore expanded the chemistry of wortmannin and synthesized a group of new esters derived from 11-*O*-deacylated wortmannin (**4**). We have also synthesized derivatives of the 11-*O*-propionyl ester to explore substitution at C-17 (Table 1).[8] In comparison to wortmannin, the most active derivatives show much greater inhibition of isolated PI 3-kinase and a general increase in cell cytotoxicity in a rapidly proliferating leukemia cell line.

Chemistry

Following the literature procedure,[8] **1** was treated with diethylamine in dichloromethane giving 20-(*N*,*N*-diethylimino)-20-desoxowortmannin (**2**), which was deacylated with potassium carbonate in methanol to give 11-*O*-desacetyl-20-(*N*,*N*-diethylimino)-20-desoxowortmannin (**3**). The furan ring was reclosed under acidic conditions in dioxane to give 11-*O*-desacetylwortmannin (**4**) in a 61% yield from wortmannin.[8] **4** was produced more easily by treatment of wortmannin with excess diethylamine in methanol, followed by evaporation of the solvent and treatment with dilute acid in dioxane, and reacylated by reaction with the appropriate acid chloride or anhydride to give the title compounds. **4** was also oxidized with pyridinium dichromate in dichloromethane to give 11-*O*-desacetyl-11-dehydrowortmannin (**5**) in 79% yield (Scheme 1).[8]

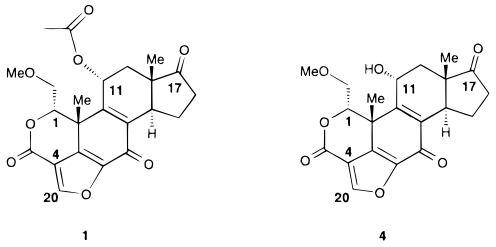
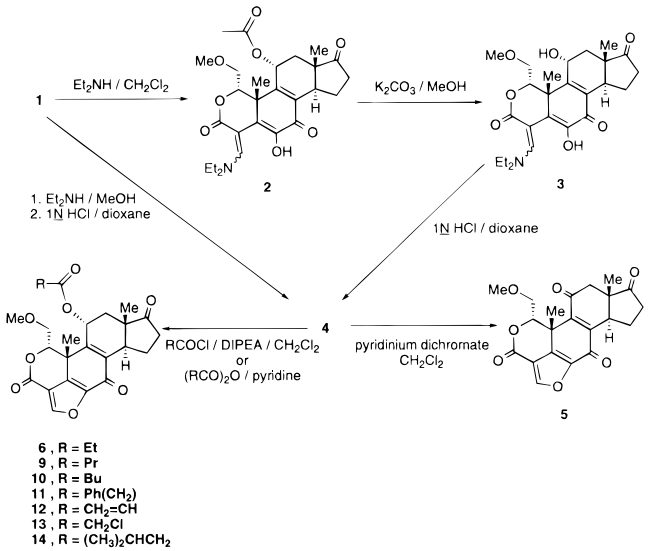


Figure 1. Wortmannin (**1**) and 11-*O*-desacetylwortmannin (**4**).

Scheme 1. Deacetylation of Wortmannin (**1**) and Reacylation and Oxidation of 11-*O*-Desacetylwortmannin (**4**)



The C-17 ketone of 11-*O*-desacetyl-11-*O*-propionylwortmannin (**6**) was reduced by borane in THF to give *β*-11-*O*-desacetyl-11-*O*-propionyl-17-dihydrowortmannin (**7**).[8] The C-17 hydroxyl group in **7** was then acetylated with acetic anhydride in pyridine to give *β*-11-*O*-desacetyl-11-*O*-propionyl-17-dihydro-17-*O*-acetylwortmannin (**8**) (Scheme 2).

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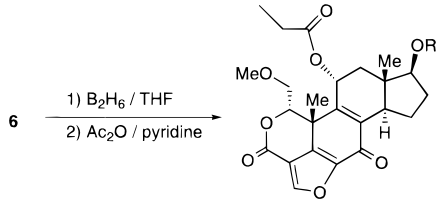
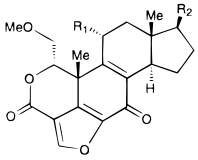
Scheme 2. Reduction and Acylation of C-17 in 11-*O*-Desacetyl-11-*O*-propionylwortmannin (**6**)



7 ; R = H
8 ; R = Ac

Table 1. *In Vitro* Inhibition of Isolated PI 3-Kinase and Cell Cytotoxicity in a Rapidly Proliferating Leukemia Cell Line of a Group of 11-*O*-Desacetylwortmannin Esters, Substituted at C-11 and with Further Reduction and Acylation at C-17



compd	R_1	R_2	IC_{50}[a] (ng/mL)	CT_{50}[b] (μg/mL)
1	CH_3COO	=O	1.8	0.9
4	OH	=O	10	2.0
5	=O	=O	1.6 (1.1)	2.8
6	CH_3CH_2COO	=O	0.6 (1.00)	0.2
7	CH_3CH_2COO	OH	0.38	0.2
8	CH_3CH_2COO	OAc	56.5	3.1
9	$CH_3(CH_2)_2COO$	=O	0.4 (0.76)	0.3
10	$CH_3(CH_2)_3COO$	=O	1.2 (0.85)	0.8
11	$PhCH_2COO$	=O	1.53 (1.59)	1.0
12	CH_2=$CHCOO$	=O	0.95 (0.6)	0.4
13	$ClCH_2COO$	=O	1.3 (1.7)	0.7
14	$(CH_3)_2CHCH_2COO$	=O	1.16	0.6

[a] IC_{50} calculated against pure PI 3-kinase isolate from bovine brain. Numbers in parentheses represent repeat tests with different lots of material. [b] CT_{50} calculated against CCRF-CEM cells after 72 h exposure.

Biological Activity and Discussion

PI 3-kinase is a heterodimeric enzyme that binds to a number of proteins containing intrinsic or associated tyrosine kinase activities, including the receptors for platelet-derived growth factor (PDGF)[9] and CSF-1,[10] the insulin receptor substrate-1 (IRS-1),[11] the products of oncogenes *v-src* and *v-yes*,[12] and the polyomavirus middle T antigen/pp60^{c-src} complex.[13] PI 3-kinase transfers the terminal phosphate of ATP to the D-3 position of phosphatidylinositol (PI), PI-4-P, or PI-4,5-P_2 to yield the products PI-3-P, PI-3,4-P_2, or PI-3,4,5-P_3, respectively. These novel products are not directly involved in the traditional pathway for generating the second-messenger inositol-1,4,5-trisphosphate or for generating substrates for phospholipase C.[14]

The PI 3-kinase inhibitory activity of C-11-substituted ester derivatives increased with increasing lipophilic character. 11-*O*-desacetylwortmannin (**4**), which is more polar than wortmannin, showed a reduction in activity, suggesting a lipophilic pocket in this portion of the enzyme active site. The reduction of the C-17 ketone in **6** to the 17-dihydro derivative **7** gave a slight increase in activity, while further acetylation of the C-17 hydroxyl group to **8** showed a dramatic loss in activity. This reduction in activity suggests that the active site cannot accommodate lipophilicity or steric bulk at C-17.

Selected compounds (**4, 6**−**8**) were tested for their ability to inhibit PI 4-kinase as previously described.[5] In the presence of 10 μg/mL of compound, no inhibition of PI 4-kinase was detected. This concentration is 200−1000-fold higher than the IC_{50} values of these compounds against PI 3-kinase. These findings are consistent with observations that wortmannin has no effect on PI 4-kinase activity *in vitro*.[5] In addition, selected compounds (**1**, **6**, **7**, **9**, **10**) were tested for their ability to inhibit the PI 3-kinase homolog mTOR. The rank order of potency of these compounds essentially mirrors that obtained with PI 3-kinase as the target, with the exception that, like wortmannin, these analogs are 100-fold less potent when tested against the mTOR kinase.[15] The analogs were not tested for their ability to inhibit myosin light chain kinase (MLCK) because at the concentrations that inhibit PI 3-kinase, wortmannin has no effect on MLCK. The IC_{50} of wortmannin versus MLCK has been reported to be 170 nM or 73 ng/mL, which is 40 times higher than the IC_{50} of wortmannin against purified PI 3-kinase (see Table 1).[5,16]

These new derivatives show both superior inhibitory activity of PI 3-kinase and increased cell cytotoxicity in a rapidly proliferating leukemia cell line, when compared to wortmannin. This improved *in vitro* activity makes these new ester derivatives good candidates to explore the *in vivo* antitumor effects of PI 3-kinase inhibitors.

Experimental Section

PI 3-Kinase Inhibitory Assay. The inhibition of PI 3-kinase was determined using purified bovine brain PI 3-kinase and PI as substrate as previously described.[5,17] Wortmannin or analogs were preincubated with the kinase for 10 min prior to initiation of the reaction by addition of [γ-^{32}P]ATP. After 10 min, the reaction was quenched, and lipid products were extracted as described previously.[17] Radioactive lipid products were analyzed by thin-layer chromatography and autoradiography.[18] The PI-3-P was scraped from the plate and quantitated using liquid scintillation spectroscopy. The level of inhibition for wortmannin and its analogs was determined as the percentage of ^{32}P counts/min compared to controls.

Cytotoxicity Determination Assay. CCRF-CEM cells, a human leukemia cell line, was obtained from St. Jude Children's Research Hospital, Memphis, TN. The cells were maintained as suspension cultures in RPMI-1640 medium supplemented with 10% dialyzed fetal calf serum and 25 mM HEPES buffer (Whittaker Bioproducts, Walkersville, MD). Dose-response curves were generated to determine the concentration required for 50% inhibition of growth (CT_{50}). Test compounds were dissolved initially in DMSO at a concentration of 4 mg/mL and further diluted with solvent to the desired concentration. Cells in complete medium were added to 24-well Cluster plates at a final concentration of 4.8×10^4/well in a total volume of 2.0 mL. Test compounds at various concentrations were added to duplicate wells so that the final volume of DMSO was 0.5%. The plates were incubated for 72 h at 37 °C in a 5% CO_2-in-air atmosphere. At the end of the incubation, cell numbers were determined on a ZBI Coulter counter. Control wells usually contained $4-6 \times 10^5$ cells at the end of the incubation.

Chemical Synthesis. ^{1}H-NMR spectra were measured in $CDCl_3$ solution on a Varian Gemini-300 spectrometer and are scaled from internal TMS. IR spectra were obtained on a Nicolet 510P optical bench spectrometer. Field desorption mass spectra (FD-MS) were obtained on a VG ZAB-3F mass spectrometer. Elemental analyses were performed on a Control Equipment Corporation 440 elemental analyzer for carbon and hydrogen.

The preparation of wortmannin was carried out at the Lilly fermentation facilities in Indianapolis, IN. All synthetic reagents were obtained from Aldrich Chemical Co., Milwaukee, WI.

11-*O*-Desacetylwortmannin (4). A suspension of wortmannin (12.13 g, 28.3 mmol) in MeOH (700 mL) was treated with diethylamine (70 mL, 67.7 mmol). The mixture was stirred at room temperature for 22 h and then evaporated. The crude **3** was dissolved in dioxane (900 mL), 1 N HCl (240 mL) was added, and the mixture was stirred at room temperature for 19 h. The mixture was concentrated to a small volume and diluted with water. The aqueous mixture was extracted with CH_2Cl_2. The CH_2Cl_2 was dried with Na_2SO_4 and evaporated. The crude product was purified by chromatography on silica gel eluting with 25% hexane in ethyl acetate to give **4** (7.35 g, 67%, based on wortmannin) as a yellow glass: MS (M^+) 386; ^{1}H-NMR ($CDCl_3$) δ 0.97 (s, 3H), 1.55 (dd, J = 12.57, 2.38 Hz, 2H), 1.84 (s, 3H), 2.0−2.18 (m, 1H), 2.24 (dt, J = 19.48, 8.98 Hz, 1H), 2.44 (dd, J = 12.3, 6.6 Hz, 1H), 2.60 (dd, J = 19.66, 8.37 Hz, 1H), 2.79−2.84 (m, 1H), 2.88 (t, J = 9.34 Hz, 1H), 3.19 (s, 3H), 3.51 (dd, J = 9.47, 3.91 Hz, 2H), 4.8−4.9 (m, 1H), 5.61 (dd, J = 9.15, 3.9 Hz, 1H), 8.25 (s, 1H). Anal. ($C_{21}H_{22}O_7$) C,H.

11-*O*-Desacetyl-11-dehydrowortmannin (5). A solution of **4** (401.1 mg, 1.04 mmol) in dry CH_2Cl_2 (25 mL) was treated with pyridinium dichromate (1.96 g, 5.2 mmol), and the mixture was stirred at room temperature for 2.5 h. The mixture was then filtered through Celite, and the Celite was washed with fresh CH_2Cl_2. The CH_2Cl_2 was combined and evaporated, and the crude product was purified by chromatography on silica gel eluting with 50% hexane in ethyl acetate to give **5** (313.9 mg, 79%) as an off-white solid: MS (M^+) 384; IR ($CHCl_3$) 1469, 1546, 1680, 1748 cm $^{-1}$; ^{1}H-NMR ($CDCl_3$) δ 1.00 (s, 3H), 1.62 (s, 1H), 1.78 (s, 3H), 2.12−2.48 (m, 3H), 2.65 (dd, J = 19.72, 9.21 Hz, 1H), 2.90 (d, J = 16.73 Hz, 1H), 2.99 (s, 3H), 3.09 (dd, J = 12.21, 5.92 Hz, 1H), 3.20 (d, J = 5.07 Hz, 1H), 3.28−3.40 (m, 1H), 5.68 (t, J = 4.46 Hz, 1H), 8.24 (s, 1H). Anal. ($C_{21}H_{20}O_7$) C,H.

11-*O*-Desacetyl-11-*O*-propionylwortmannin (6). A solution of **4** (483.6 mg, 1.25 mmol) in pyridine (25 mL) was treated with propionic anhydride (680 mL, 5.3 mmol), and the mixture was stirred at room temperature for 26 h. The mixture was then evaporated, and the residue was purified by chromatography on silica gel eluting with 50% hexane in ethyl acetate to give **6** (512.8 mg, 93%) as an off-white solid: MS (M^+) 442; IR ($CHCl_3$) 1378, 1467, 1541, 1681, 1746 cm $^{-1}$; ^{1}H-NMR ($CDCl_3$) δ 0.96 (s, 3H), 1.19 (t, J = 7.45 Hz, 3H), 1.58 (dd, J = 12.94, 8.66 Hz, 2H), 1.71 (s, 3H), 2.05 (dt, J = 3.05, 12.45 Hz, 1H), 2.16−2.46 (m, 3H), 2.52−2.68 (m, 2H), 2.84−2.92 (m, 1H), 3.02 (dd, J = 11.11, 6.89 Hz, 1H), 3.18 (s, 3H), 3.46 (dd, J = 1.77, 11.11 Hz, 1H), 4.74 (dd, J =1.77, 6.84 Hz, 1H), 6.18 (dt, J = 2.5, 9.95 Hz, 1H), 8.26 (s, 1H). Anal. ($C_{24}H_{26}O_8$) C,H.

***β*-11-*O*-Desacetyl-11-*O*-propionyl-17-dihydrowortmannin (7).** An ice cold solution of **6** (345 mg, 0.78 mmol), in dry THF (12 mL) under N_2, was treated with 1 M borane in THF (750 mL, 0.75 mmol), and the mixture was stirred at ∼0 °C for 2.5 h. The reaction was quenched by addition of water (1 mL) at 0 °C. After warming to room temperature, the mixture was diluted with water and extracted with ethyl acetate. The ethyl acetate was washed with brine, dried with Na_2SO_4, and then evaporated, giving crude product which was purified by chromatography on silica gel eluting with 50% hexane in ethyl acetate to give **7** (309.9 mg, 89%) as a light yellow solid: MS (ionspray; M + H)$^+$ 445; ^{1}H-NMR ($CDCl_3$) δ 0.89 (s, 3H), 1.18 (t, J = 7.76 Hz, 3H), 1.35−1.65 (m, 4H), 1.72 (s, 3H), 2.12−2.68 (m, 4H), 2.70−2.84 (m, 1H), 2.96 (dd, J = 10.93, 7.15 Hz, 1H), 3.18 (s, 3H), 3.48 (dd, J = 2.02, 11.11 Hz, 1H), 3.88 (t, J = 8.67 Hz, 1H), 4.79 (dd, J = 2.02, 7.57 Hz, 1H), 6.10−6.24 (m, 1H), 8.21 (s, 1H). Anal. ($C_{24}H_{28}O_8$) C,H.

***β*-11-*O*-Desacetyl-11-*O*-propionyl-17-dihydro-17-acetylwortmannin (8).** A solution of **7** (104.9 mg, 0.24 mmol) in pyridine (5 mL) was treated with acetic anhydride (95 mL, 1.0 mmol), and the mixture was stirred at room temperature for 27 h. The mixture was then evaporated, and the residue was purified by chromatography on silica gel eluting with 40% ethyl acetate in hexane to give **8** (89.9 mg, 77%) as a white solid: MS (M + H)$^+$ 487, 412; ^{1}H-NMR ($CDCl_3$) δ 0.92 (s, 3H), 1.19 (t, J = 7.51 Hz, 3H), 1.42−1.86 (m, 6H), 2.09 (s, 3H), 2.24−2.58 (m, 4H), 2.59−2.68 (m, 1H), 2.75−2.92 (m, 1H), 2.98 (dd, J = 11.05, 7.26 Hz, 1H), 3.20 (s, 3H), 3.48 (dd, J = 10.99, 1.89 Hz, 1H), 4.75 (dd, J = 7.21, 1.9 Hz, 1H), 4.84 (dd, J = 9.65, 7.51 Hz, 1H), 6.08−6.20 (m, 1H), 8.24 (s, 1H). Anal. ($C_{26}H_{30}O_9$) C,H.

11-*O*-Desacetyl-11-*O*-butyrylwortmannin (9). This reaction was run as with **6**, starting with **4** (457.5 mg, 1.19 mmol) and butyric anhydride to give **9** (486.6 mg, 90%) as a white solid: MS (M^+) 456; ^{1}H-NMR ($CDCl_3$) δ 0.82−1.10 (s, broad, 6H), 1.51−1.81 (m, 6H), 1.94−2.15 (m, 1H), 2.16−2.42 (m, 3H), 2.50−2.68 (m, 2H), 2.89 (ddd, J = 12.64, 6.05, 2.81 Hz, 1H), 3.02 (dd, J = 11.18, 6.9 Hz, 1H), 3.18 (s, 3H), 3.48 (dd, J = 11.05, 2.02 Hz, 1H), 4.77 (dd, J = 6.89, 1.95 Hz, 1H), 6.12−6.28 (m, 1H), 8.26 (s, 1H). Anal. ($C_{25}H_{28}O_8$) C,H.

11-*O*-Desacetyl-11-*O*-valerylwortmannin (10). This reaction was run as with **6**, starting with **4** (454.1 mg, 1.18 mmol) and valeric anhydride to give **10** (517.7 mg, 93%) as a white solid: MS (M + H)$^+$ 471; ^{1}H-NMR ($CDCl_3$) δ 0.92−0.95 (m, 6H), 1.28−1.42 (m, 2H), 1.48−1.68 (m, 3H), 1.72 (s, 3H), 1.94−2.12 (m, 1H), 2.14−2.41 (m, 3H), 2.50−2.64 (m, 2H), 2.89 (ddd, J = 12.64, 5.98, 2.75 Hz, 1H), 3.00 (dd, J = 11.11, 6.96 Hz, 1H), 3.10−3.24 (m, 4H), 3.46 (dd, J = 11.11, 2.02 Hz, 1H), 4.77 (dd, J = 7.02, 2.02 Hz, 1H), 6.11−6.24 (m, 1H), 8.22 (s, 1H). Anal. ($C_{26}H_{30}O_8$) C,H.

11-*O*-Desacetyl-11-*O*-(phenylacetyl)wortmannin (11). A solution of **4** (100.2 mg, 0.26 mmol) in dry CH_2Cl_2 (8 mL) was treated with diisopropylethylamine (150 mL, 0.86 mmol) followed by phenylacetyl chloride (120 μL, 0.91 mmol). The mixture was stirred at room temperature for 23 h, then diluted with CH_2Cl_2, washed sequentially with saturated $NaHCO_3$ and brine, and dried with Na_2SO_4. The solution was evaporated giving crude product which was purified by chromatography on silica gel eluting with 50% hexane in ethyl acetate to give **11** (81.4 mg, 62%) as a light yellow glass: MS (M + H)$^+$ 505; ^{1}H-NMR ($CDCl_3$) δ 0.92 (s, 3H), 1.54 (dd, J = 12.82, 8.67 Hz, 2H), 1.70 (s, 3H), 1.92−2.30 (m, 2H), 2.44−2.64 (m, 2H), 2.85 (ddd, J = 12.58, 5.98, 2.69 Hz, 1H), 2.95 (dd, J = 11.29, 6.53 Hz, 1H), 3.14 (s, 3H), 3.36 (dd, J = 11.3, 2.02 Hz, 1H), 3.69 (s, 2H), 4.72 (dd, J = 6.53, 2.01 Hz, 1H), 6.11−6.22 (m, 1H), 7.19−7.40 (m, 5H + solvent), 8.24 (s, 1H). Anal. ($C_{29}H_{28}O_8$) C,H.

11-*O*-Desacetyl-11-*O*-acryloylwortmannin (12). This reaction was run as with **11**, starting with **4** (476.7 mg, 1.23 mmol) and acryloyl chloride to give **12** (470 mg, 87%) as a light yellow solid: MS (M + H)$^+$ 441; ^{1}H-NMR ($CDCl_3$) δ 1.01 (s, 3H), 1.68−1.72 (m, 4H), 1.98−2.15 (m, 1H), 2.25 (dt, J = 19.6, 8.97 Hz, 1H), 2.51−2.68 (m, 2H), 2.91 (ddd, J = 12.45, 5.98. 2.81 Hz, 1H), 3.08 (dd, J = 11.17, 6.59 Hz, 1H), 3.10−3.24 (m, 4H), 3.44 (dd, J = 11.23, 2.26 Hz, 1H), 4.79 (dd, J = 6.53, 2.25 Hz, 1H), 5.96 (dd, J = 10.44, 1.4 Hz, 1H), 6.12 (dd, J = 17.1, 10.44 Hz, 1H), 6.18−6.30 (m, 1H), 6.50 (dd, J = 17.03, 1.35 Hz, 1H), 8.24 (s, 1H). Anal. ($C_{24}H_{24}O_8$) C,H.

11-*O*-Desacetyl-11-*O*-(1-chloroacetyl)wortmannin (13). This reaction was run as with **11**, starting with **4** (442.8 mg, 1.15 mmol) and chloroacetyl chloride to give **13** (200.1 mg, 38%) as a light beige solid: MS (M^+) 462; ^{1}H-NMR ($CDCl_3$) δ 0.98 (s, 3H), 1.69 (dd, J = 13.13, 8.36 Hz, 1H), 1.76 (s, 3H), 1.98−2.18 (m, 1H), 2.26 (dt, J = 19.72, 9.04 Hz, 1H), 2.52−2.72 (m, 2H), 2.92 (ddd, J = 12.58, 6.04, 2.44 Hz, 1H), 3.02 (dd, J = 11.42, 6.54 Hz, 1H), 3.19 (s, broad, 4H), 3.44 (dd, J = 11.36, 1.77 Hz, 1H), 4.13 (dd, J = 22.46, 14.77 Hz, 2H), 4.75 (dd, J = 6.53, 1.71 Hz, 1H), 6.23 (dt, J = 2.57, 8.06 Hz, 1H), 8.24 (s, 1H). Anal. ($C_{23}H_{23}O_8Cl$) C,H.

11-*O*-Desacetyl-11-*O*-isovalerylwortmannin (14). This reaction was run as with **11**, starting with **4** (479.9 mg, 1.24 mmol) and isovaleryl chloride to give **14** (350.3 mg, 60%) as a light orange solid: MS (M + H)$^+$ 471; ^{1}H-NMR ($CDCl_3$) δ 0.91−1.06 (s, broad, 9H), 1.56 (dd, J = 12.64, 8.91 Hz, 1H), 2.72 (s, 3H), 1.92−2.36 (m, 5H), 2.52−2.68 (m, 2H), 2.82−2.94 (m, 1H), 3.01 (dd, J = 11.05, 6.96 Hz, 1H), 3.11−3.28 (s, broad, 4H), 3.46 (d, J = 11.11 Hz, 1H), 4.78 (d, J = 5.37 Hz, 1H), 6.12−6.24 (m, 1H), 8.26 (s, 1H). Anal. ($C_{26}H_{30}O_8$) C,H.

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