Pegylated Wortmannin and 17-Hydroxywortmannin Conjugates as Phosphoinositide 3-Kinase Inhibitors Active in Human Tumor Xenograft Models

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Phosphoinositide 3-kinase (PI3K) is an important target for cancer chemotherapy due to the deregulation of its signaling pathway in a wide spectrum of human tumors. Wortmannin and its analogues are potent PI3K inhibitors whose therapeutic use has been impeded by inherent defects such as instability and toxicity. Pegylation of wortmannin and 17-hydroxywortmannin gives rise to conjugates with improved properties, including a higher therapeutic index. Pegylated 17-hydroxywortmannin (**8**, PWT-458) has been selected for further development.

The phosphoinositide 3-kinase (PI3K)/Akt signaling pathway is involved in cell cycle progression and apoptosis and is therefore of great interest as a target for cancer chemotherapy.[1] PI3K is a heterodimer comprised of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit and is responsible for the production of phosphatidylinositol 3,4,5-triphosphate (PI-$(3,4,5)P_3$), which in turn activates the downstream target Akt. Deregulation of the PI3K/Akt pathway has been cited in a wide spectrum of human cancers. Tumors that are deficient in the tumor suppressor gene PTEN, a negative regulator of PI3K signaling (through dephosphorylation of $PI(3,4,5)P_3$), are prevalent in cancer, and estimated to occur in 50% of all human cancers. In addition, activation mutations and constitutive overexpression of PI3K has been observed in several major solid tumors such as colon, breast, lung, ovarian, and pancreatic cancer. Thus, inhibition of PI3K is a promising avenue for cancer treatment.

The natural product wortmannin (**1**, Figure 1) is a potent inhibitor of PI3K (IC_{50} = 4.2 nM) that binds irreversibly to a lysine in the ATP binding pocket of PI3K via opening of the electrophilic furan ring at its C-20 position.[2,3] This was confirmed by X-ray crystallography of wortmannin irreversibly bound to Lys833 of PI3K-γ.[2] Wortmannin has played a major part in elucidating the role of PI3K in signal transduction pathways. In addition, it has demonstrated cytotoxic activity against human tumor cell lines in vitro and in vivo in xenograft models in mice.[4,5] However, its toxicity (exemplified by a low therapeutic index),[5] insolubility, and aqueous instability (through hydrolytic ring opening of its reactive furan ring) have hampered its development into a viable anticancer agent.

Structure−activity relationship (SAR) studies with wortmannin derivatives have shown that the furan ring is crucial for potent biological activity, consistent with its mechanism of PI3K inhibition.[5,6] Thus, some analogues with modifications at positions 17 or 11 (e.g. **2** and **3**, respectively) remote from the furan ring can retain PI3K activity. In contrast, modification of the furan ring by methylation at position 20 (**4**) or ring expansion to a pyran ring (**5**) led to a loss or reduction of activity, respectively. These SAR studies have failed to produce analogues with improved properties over wortmannin. Opening of the wortmannin furan ring with nucleophiles such as secondary amines leads to analogues that retain significant PI3K inhibition (**6**, IC_{50} = 80 nM).[5] Recently, furan ring-opened analogues have been reported to have reduced toxicity relative to wortmannin (i.e., **7**).[7,8]

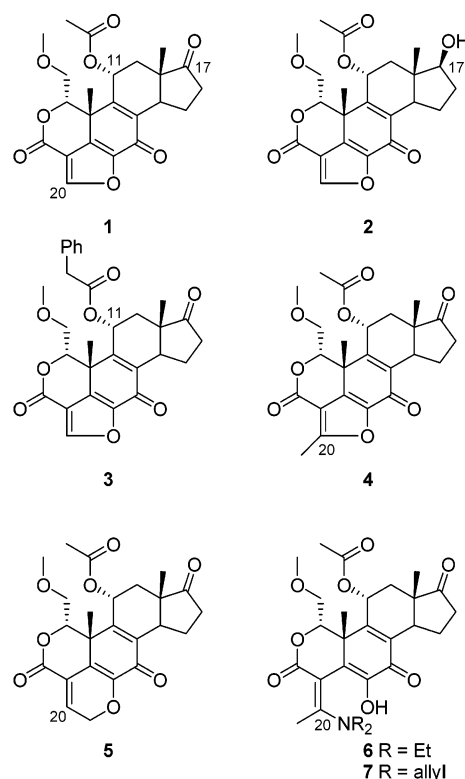

Figure 1. Wortmannin (**1**) and synthetic analogues.

The utility of poly(ethylene glycol) (PEG) for the covalent modification of proteins is well-established and has resulted in several clinically used therapeutic agents.[9,10] PEG is soluble in both water and organic solvents and is available in a variety of molecular weights of low polydispersity. It is relatively nontoxic and is cleared by a combination of renal and hepatic pathways

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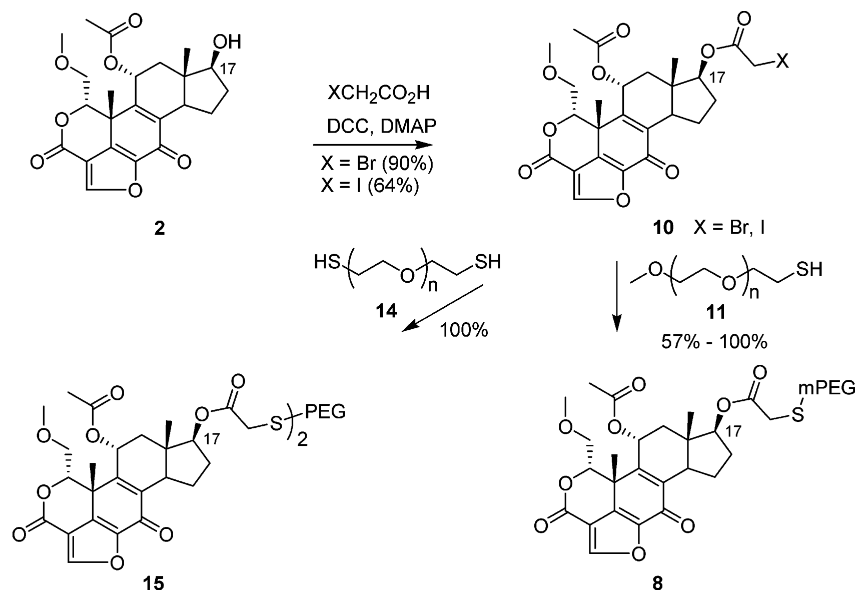
Scheme 1. Synthesis of Pegylated 17-Hydroxywortmannin Analogues **8** and **15**

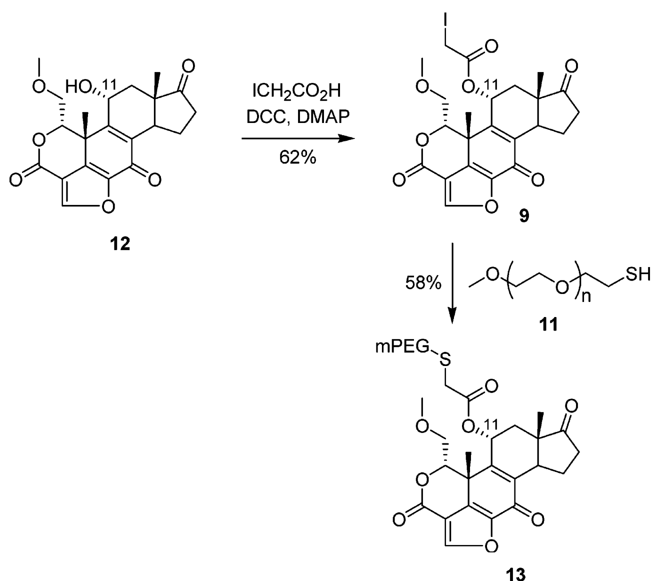


and is thus used for iv and oral pharmaceutical applications. Although conjugation of PEG to small molecule therapeutics, especially anticancer agents, has been investigated, only one compound, pegylated camptothecin, has advanced to the clinic.[9] Pegylation of organic molecules has been reported to confer beneficial properties such as enhanced aqueous solubility, improved plasma half-life, increased stability, improved biological distribution, and reduced toxicity.[9,10] We report here the first successful application of pegylation technology to wortmannin and its analogue, 17-hydroxywortmannin (**2**), to produce conjugates that overcome the defects of the parent compounds. One compound, pegylated 17-hydroxywortmannin (**8**, PWT-458), has been selected for further development.[11]

Synthesis

The reactivity of the furan ring of wortmannin with nucleophiles required a mild method of derivatization that would lead to a conjugate capable of releasing the active component in vivo. A novel method of pegylation was used in which **1** or **2** was chemically modified, by coupling to an activated acetyl group, to form a derivative (**9** or **10**, respectively) that could form a covalent bond with PEG thiols under mild conditions (Schemes 1 and 2). Thus, derivatization of **2** at the 17-hydroxy group by coupling with α-iodoacetic acid, in the presence of DCC and DMAP in acetonitrile, gave the 17-α-iodoacetate derivative **10** (X = I) in 64% yield after HPLC. Similarly, the α-bromoacetate derivative (**10**, X = Br) was prepared in 90% yield. Reaction of **10** (X = I) with mPEGSH (**11**, MW ∼ 5000) in the presence aqueous sodium bicarbonate in acetonitrile selectively produced the desired PEG-thioacetate **8** in 57% yield after HPLC. Alternatively, **8** could be prepared from **10** (X = Br) and **11** by treatment with triethylamine in acetonitrile in quantitative yield. Under these mild conditions, no evidence of reaction at the furan ring though ring opening by **11** was seen in the ^{1}H NMR spectra.[5] Similarly, derivatization at the 11-hydroxy group of 11-desacetylwortmannin (**12**)[6] with iodoacetic acid gave **9** in 62% yield after HPLC. Treatment of **9** with **11** in the presence of aqueous sodium bicarbonate in acetonitrile gave the 11-pegylated-wortmannin derivative **13** in 58% yield after HPLC. Doubling the loading of the active component in the conjugate was accomplished by reaction of difunctionalized PEG$(SH)_2$ (**14**, MW ∼ 6000) with 2 mol equiv of **10** (X = Br) to give **15**

Scheme 2. Synthesis of Pegylated Wortmannin Analogue **13**



in quantitative yield. Analytical HPLC analysis of **10** showed it to be 91% pure with ∼7% of a side peak with the same UV spectra as the main peak. However, the ^{1}H NMR spectra of **15** showed no impurities. These data are consistent with a minor amount of a PEG component in **15** containing only 1 equiv of **2**. As such, this minor component would have a similar activity to **15**. PEG thiols **11** and **14** were commercially available in small quantities in a limited selection of molecular weights. Alternatively, they could be prepared from the more readily available PEG alcohols by a literature route.[12]

Results and Discussion

Application of pegylation technology to wortmannin (**1**) and 17-hydroxywortmannin (**2**) gave the pegylated wortmannin conjugates **8**, **13**, and **15** as white amorphous powders that are completely miscible with water. These compounds thus have the advantage of administration as solutions in saline without the need for complex formulations. In contrast, the parent compounds, **1** and **2**, have aqueous solubilities of <1 mg/mL. These compounds were evaluated in in vitro and in vivo

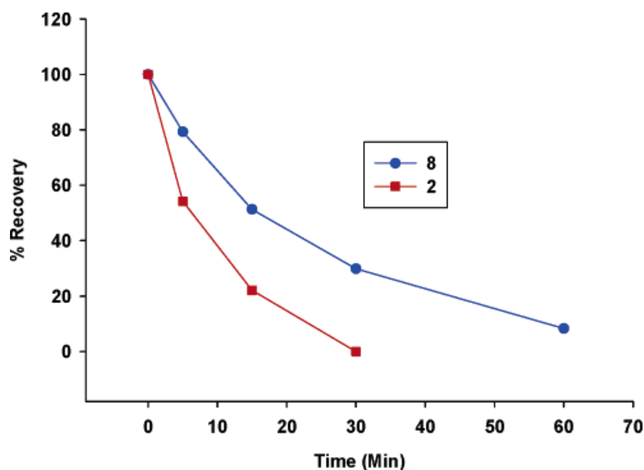


Figure 2. In vitro stability of **2** and **8** in nude mouse plasma. A concentration of 100 μg/mL of **2** and **8** (wortmannin equivalents) was incubated in female nude mouse plasma at room temperature.

biological assays.[11] As expected, compounds **8**, **13**, and **15** show no in vitro inhibitory activity versus PI3K or in vitro growth inhibition of H157 or A549 tumor cells at 30 μg/mL. Similarly, no inhibition of downstream targets in the PI3K/Akt pathway, including Akt phosphorylation, was seen at 6.6 μg/mL in the cellular IGF model. The lack of in vitro enzyme activity correlates with the incompatibility of the large polymeric PEG group with the enzyme-binding pocket.[2] Indeed, addition of even small substituents (e.g. acetyl) at the 17-position has been reported to lead to a decrease of activity.[6] This is consistent with the PI3K/wortmannin X-ray crystal structure that shows a lack of space at this position in the enzyme. The lack of cellular activity is consistent with the inability of PEG to penetrate cell membranes.

In vivo, **8** shows potent antitumor activity and a profound decrease in toxicity relative to **2**.[11] Reduction of toxicity of the parent compound by pegylation has been reported for other anticancer agents (e.g. paclitaxel).[13] This effect may be associated with a reduction of the peak plasma concentration through gradual release of the active component from the PEG. In addition, PEG alters the clearance and distribution of organic molecules, including tumor accumulation, in a way that is dependent on the MW of the PEG.[14,15] This may serve to decrease the effective dose and/or to target the active component more efficiently to the tumor.[15] The anticancer effects of these conjugates (**8**, **13**, and **15**) in vivo were studied using PTEN-negative U87MG glioma cells in a xenograft model in athymic mice. The loss of the PTEN tumor suppressor in these cells results in deregulation of the PI3K/Akt pathway. Thus, in the U87MG glioma xenograft model, **8** showed potent antitumor activity (50% inhibition of tumor growth on day 7) with a minimally efficacious dose (MED) of 0.5 mg/kg based on equivalents of **2** (daily × 5 dosing, iv).[11] Tumor growth inhibition increased in a dose-dependent manner, up to a maximally tolerated dose (MTD) of 15 mg/kg. In contrast, **2** had the same MED of 0.5 mg/kg (daily × 5, ip) but was not tolerated at doses >1.5 mg/kg. Thus, the therapeutic index of **8** is greatly enhanced by at least 10-fold over the therapeutic index for **2**.

The plasma stability of **8** was greatly improved over those of **1** and **2**. After incubation of 100 μg/mL of **2** and **8** (wortmannin equivalents) at room temperature in female nude mouse plasma, **8** remained detectable after 60 min, while **2** disappeared within 30 min (Figure 2). After a 20 mg/kg dose of **8** (formulated in saline) was administered iv to female nude

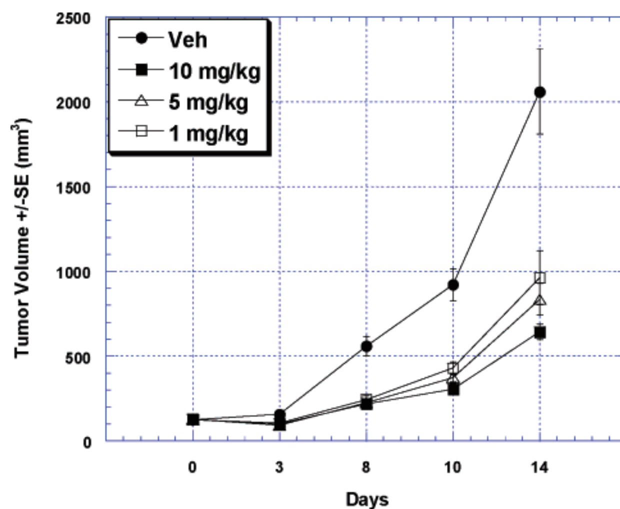


Figure 3. Compound **15** inhibits in vivo growth of U87MG glioma. U87MG glioma subcutaneous xenografts were staged on day 0 and dosed iv on days 0−4 at 1, 5, and 10 mg/kg (wortmannin equivalents). Data of a single experiment are shown (n = 10 for all treatment groups).

mice, compound **8** could be detected up to 30 min postinjection. After 15 min, 18.8 μg/mL (wortmannin equivalents) of **8** was detected. After 30 min the concentration of **8** in plasma fell to 2.6 μg/mL (wortmannin equivalents). The levels of released **2** were below the limits of detection (0.5 μg/mL) in this experiment. In contrast to the results observed with **8**, plasma levels of **1** have been reported to be undetectable after iv administration to mice.[7]

Incorporation of two molecules of **2** onto a single PEG led to compound **15**, with double the loading of drug, thus enabling a lower dose to be administered as compared to compound **8**. As desired, **15** showed in vivo efficacy comparable to that of **8**. Compound **15** showed dose-dependent inhibition in the U87MG glioma xenograft model at 1, 5, and 10 mg/kg (daily × 5, iv) based on the equivalents of compound **2** (Figure 3). Thus, as for **8**, **15** showed a greatly improved therapeutic index relative to **2**. Compound **15** was also active using a less frequent dosing regimen and by oral administration. It was active in the non-small-cell lung cancer (NSCLC) A549 xenograft model by iv administration at 1 and 5 mg/kg (2× per week) (Figure 4) as well as by oral administration at 5 mg/kg (2× weekly) (Figure 5). The wortmannin analogue pegylated at the 11-position (**13**) also demonstrated in vivo activity when dosed in the human U87MG glioma xenograft model at 0.7 mg/kg (daily × 5, iv) based on the number of wortmannin equivalents (Figure 6). However, further studies with **13**, beyond this initial proof of efficacy, were not conducted due to the known, 6-fold weaker PI3K inhibition of 11-desacetylwortmannin (**12**) relative to **1** (IC_{50} = 26 and 4.2 nM, respectively).[6]

In summary, a novel, mild pegylation methodology has enabled the pegylation of wortmannin and 17-hydroxywortmannin for the first time. The resulting conjugates overcome many of the deficits of the parent compounds. They are water-soluble, with improved plasma stability and most importantly reduced toxicity, as exemplified by the greatly improved therapeutic index of **8** and **15**. Pegylated wortmannins target the PI3K/Akt pathway and are potent anticancer agents of potential utility in the clinic.

Experimental Section

Materials and Instruments. Wortmannin was obtained from fermentation broths of the fungal culture ZIMV298 of the Wyeth microbial collection. 1,3-Dicyclohexylcarbodiimide (DCC) and

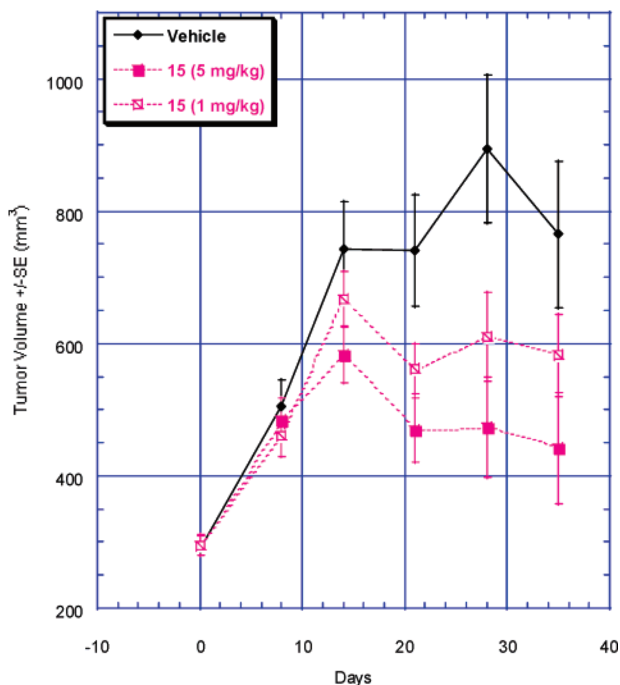


Figure 4. Compound **15** inhibits in vivo growth of A549. Human NSCLC A549 subcutaneous xenografts were staged on day 0 and dosed iv at 1 and 5 mg/kg twice weekly for 2 weeks (wortmannin equivalents). Data of a single experiment are shown ($n = 10$ for all treatment groups).

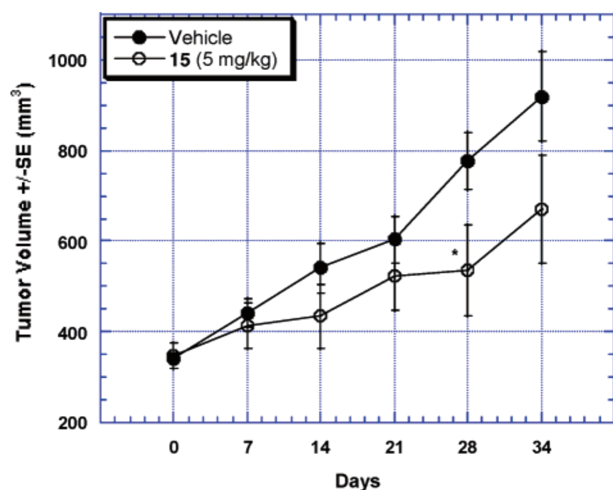


Figure 5. Oral dosing of compound **15** inhibits in vivo growth of A549. Human NSCLC A549 subcutaneous xenografts were staged on day 0 and dosed po at 10 mg/kg twice weekly for 2 weeks (wortmannin equivalents). Data of a single experiment are shown ($n = 10$).

4-(dimethylamino)pyridine were purchased from Aldrich Chemical Co. (Milwaukee, WI). Poly(ethylene glycol)s were purchased from Shearwater Polymers, Inc. (Huntsville, Al) or were synthesized in-house according to literature methods.[12] All solvents were HPLC grade and all other chemicals were analytical reagents or equivalent. The preparative HPLC consisted of two Dynamax solvent delivery systems (Model SD-1) and one Dynamax absorbance detector (Model UV-1) from Rainin Instrument Inc. (Woburn, MA). Preparative HPLC was done on a Prep Nova-pak HR C18 column (300 × 19 mm from Waters) using a gradient method that held 80% A and 20% B for the first 5 min then changed to 30% A and 70% B in 30 min. Buffer A is 90% water and 10% acetonitrile. Buffer B is 10% water and 90% acetonitrile. The flow rate is 20 mL/min and UV detection was at 254 nm. An automatic Speed-Vac concentrator (Savant, Model AS 160) was from Savant Instruments, Inc. (Holbrook, NY) and a Buchi rotary evaporation system (RE 260 and R 124) was from Buchi (Flawil, Switzerland).

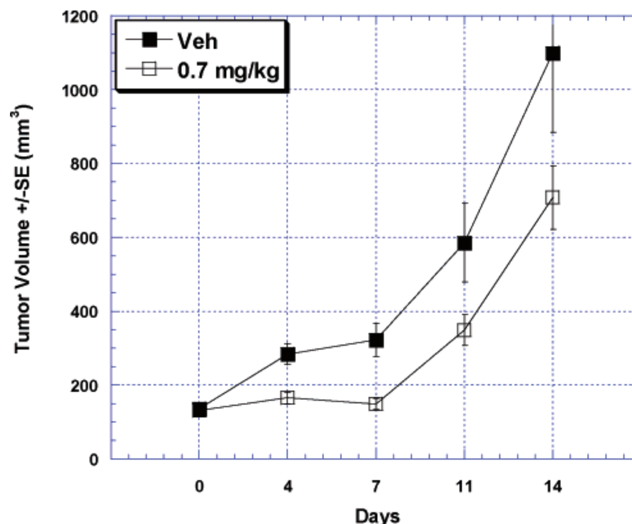


Figure 6. Compound **13** inhibits in vivo growth of U87MG glioma. U87MG glioma subcutaneous xenografts were staged on day 0 and dosed iv on days 0−4 at 0.7 mg/kg (wortmannin equivalents). Data of a single experiment are shown ($n = 10$).

^{1}H NMR spectra were recorded on a 400 MHz NMR spectrophotometer using $CDCl_3$ as solvent. Electrospray (ES) mass spectra were recorded on a Micromass Platform spectrometer. High-resolution mass spectra were recorded on a Finnigen MAT-90 spectrometer.

Biology. In vitro and in vivo assays were carried out as described in ref 11.

Preparation of 17-Dihydro-17-(1-iodoacetyl)wortmannin (10, X = I). To a solution of wortmannin (**1**) (60 mg, 0.14 mmol) in THF (12 mL) at 0 °C under nitrogen was added 1 M borane in THF (134 μL, 0.14 mmol). After 3.5 h at 0 °C, water (1 mL) was added. The reaction mixture was allowed to warm to room temperature. Additional water was added and the mixture was extracted with ethyl acetate. Concentration in vacuo gave **2** (60 mg, 90% pure by HPLC) as a solid. 17-Hydroxywortmannin (**2**) (~0.126 mmol) was dissolved in dichloromethane (15 mL) and treated with iodoacetic acid (24 mg, 0.13 mmol), DCC (27 mg, 0.13 mmol), and DMAP (0.1 mg as catalyst). The reaction mixture was kept at room temperature for 1 h. After work up, **10** (X = I) was obtained as a yellow solid (75 mg, 89%). Purification by preparative HPLC gave **10** (X = I) as a white solid (54 mg, 64%): MS m/z 599 $(M + H)^+$, 616 $(M + NH_4)^+$; HRMS (ESI) calculated for $C_{25}H_{28}O_9I$ 599.0772 $(M + H)^+$, found 599.0758; ^{1}H NMR ($CDCl_3$) δ 0.94 (s, 3H), 1.54 (dd, $J = 12.16, 10.06$, 1H), 1.69 (m, 1H), 1.69 (m, 3H), 1.78 (m, 1H), 2.15 (s, 3H), 2.31 (m, 1H), 2.56 (dd, $J = 12.16, 7.36$, 1H), 2.63 (ddd, $J = 2.7$, 1H), 2.85 (ddd, $J = 20.12, 9.91, 2.7$, 1H), 2.99 (dd, $J = 11.11, 7.21$, 1H), 3.19 (s, 3H), 3.46 (dd, $J = 11.11, 1.8$, 1H), 3.69 (d, $J = 10.6$, 1H), 3.72 (d, $J = 10.6$, 1H), 4.76 (dd, $J = 7.21, 1.8$, 1H), 4.87 (dd, $J = 7.51, 9.46$, 1H), 6.10 (ddd, $J = 10.06, 7.36, 3.0$, 1H), 8.23 (s, 1H); ^{13}C NMR ($CDCl_3$) δ −5.62, 12.79, 21.14, 24.65, 26.58, 27.04, 40.11, 40.72, 44.07, 44.99, 59.44, 72.90, 88.88, 114.25, 141.11, 142.72, 144.93, 148.68, 149.84, 157.66, 168.94, 169.54, 172.77.

Preparation of Pegylated 17-Hydroxywortmannin (8). To **10** (X = I) (40 mg, 0.067 mmol) in acetonitrile (15 mL) and 0.1 M aqueous sodium bicarbonate (10 mL) under nitrogen was added mPEGSH (345 mg, 0.069 mmol, MW ~ 5000) over 1 h. After an additional hour at room temperature, the reaction mixture was extracted with dichloromethane to give 320 mg of crude product. A total of 209 mg (57%) of pure **8** was obtained from 260 mg (71%) of crude product after preparative HPLC: ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 3H), 1.53 (dd, 1H), 1.68 (m, 1H), 1.75 (s, 3H), 1.77 (m, 1H), 2.14 (s, 3H), 2.32 (m, 1H), 2.53 (dd, 1H), 2.63 (s, 1H), 2.85 (overlap, 1H), 2.85 (t, $J = 6.56$, 2H), 2.99 (dd, $J = 11.03, 7.3$, 1H), 3.2 (s, 3H), 3.31 (s, 2H), 3.38 (s, 3H), 3.47 (dd, $J = 11.03, 1.79$, 1H), 3.55 (s, 2H), 3.64 (m), 3.7 (s, 2H), 4.76 (dd, $J = 7.3$,

1.79, 1H), 4.86 (dd, 1H), 6.15 (s, 1H), 8.24 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.85, 21.11, 24.68, 26.48, 27.39, 34.01, 40.19, 40.68, 44.05, 44.81, 59.03, 59.42, 70.3, 70.35, 70.57, 70.88, 71.93, 72.88, 80.69, 88.86, 114.21, 141.19, 142.68, 144.92, 148.63, 149.88, 157.63, 169.58, 170.52, 172.75.

Preparation of 17-Dihydro-17-(1-bromoacetyl)wortmannin (10, X = Br). To a solution of **2** (43.0 g, 100 mmol) in acetonitrile at 0 °C were added α-bromoacetic acid (19.46 g, 140 mmol) and DMAP (610 mg, 5 mmol). The reaction mixture was then treated with a solution of DCC (29.87 g, 145 mmol) in acetonitrile (200 mL) dropwise over 30 min. After the addition was complete, the reaction mixture was stirred at 0 °C for 2.5 h. The white solid that formed was removed by filtration and washed with acetonitrile (2 × 100 mL). The combined acetonitrile washes were then added to H_2O (4000 mL) over 15 min. After stirring for another 15 min, the resulting solid was collected and washed with H_2O (2 × 250 mL) and IPA (2 × 200 mL) and then dried in vacuo. Compound **10** (X = Br) was obtained as an off-white powder (49.5 g, 90%): HRMS (ESI) calcd for $C_{25}H_{28}O_9Br$ 551.0911 $(M + H)^+$, found 551.0894; ^{1}H NMR ($CDCl_3$) δ 0.95 (s, 3H), 1.6−1.52 (m, 1 H), 1.82−1.68 (m, 5H), 2.16 (s, 3H), 2.34−2.30 (m, 1H), 2.59−2.52 (dd, *J* = 7.2, 12.3 Hz, 1H), 2.69−2.62 (m, 1H), 2.87−2.81 (m, 1H), 3.04−2.98 (dd, *J* = 7.5, 11.4 Hz, 1H), 3.21 (s, 3H), 3.5−3.46 (dd, *J* = 1.2, 11.4 Hz, 1H), 3.88 (d, *J* = 1.2 Hz, 2H). 4.8−4.77 (dd, *J* = 1.2, 7.2 Hz, 1H), 4.93−4.87 (dd, *J* = 7.2, 9.3 Hz, 1H), 6.14−6.08 (m, 1H), 8.29 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 173.04, 169.98, 167.63, 157.96, 150.36, 148.98, 145.20, 143.02, 141.36, 114.51, 89.16, 81.99, 73.20, 70.58, 59.72, 45.19, 44.27, 41.02, 40.38, 27.61, 26.85, 26.24, 25.01, 21.47, 13.11 ppm.

Preparation of Pegylated 17-Hydroxywortmannin (8) from 10 (X = Br). To a solution of mPEGSH (105 g, MW ∼ 5000) in acetonitrile (225 mL) under nitrogen was added *N,N*-diisopropylethylamine (4.5 mL). Compound **10** (X = Br) (10.0 g, 18.15 mmol) was then added in portions over 15 min. The reaction mixture was stirred at room temperature for 2 h, whereupon 2-propanol (2250 mL) was added over 45 min. The resulting mixture was then cooled to 10−15 °C and maintained at that temperature for 1 h. The resulting solid was collected on a Buchner funnel and washed with 2 × 250 mL of 2-propanol. The resulting wet cake was then redissolved in acetonitile (200 mL) and precipitated by addition of 2-propanol/ethyl acetate (2000 mL/75 mL). The resulting solid was collected and washed with 2-propanol and dried in vacuo. Compound **8** (110 g, quantitative yield) was obtained as an off-white powder.

Preparation of 11-Desacetyl-11-(1-iodoacetyl)wortmannin (9, X = I). To a solution of 11-desacetylwortmannin[6] (12) (42 mg, 0.11 mmol) in dichloromethane (8 mL) were added iodoacetic acid (24 mg, 0.13 mmol), DCC (27 mg, 0.13 mmol), and DMAP (0.1 mg). The reaction mixture was kept at room temperature for 2 h. After work up, about 80 mg (quantitative yield) of crude product was obtained as a yellow solid. Pure compound **9** (X = I) was isolated by preparative HPLC as a yellowish solid (41 mg, 62%): MS *m/z* 555 $(M + H)^+$, 572 $(M + NH_4)^+$; HRMS (ESI) calcd for $C_{23}H_{27}O_8NI$ 572.0775 $(M + H)^+$, found 572.0783. ^{1}H NMR ($CDCl_3$) δ 0.97 (s, 3H), 1.66 (dd, *J* = 12.84, 8.80 Hz, 1H), 1.75 (s, 3H), 2.06 (ddd, *J* = 22.25, 12.72, 8.93 Hz, 1H), 2.27 (dt, *J* = 19.68, 8.93 Hz, 1H), 2.65 (dd, *J* = 12.84, 7.58 Hz, 1H), 2.61−3.18 (m, 2H), 2.92 (ddd, *J* = 12.72, 5.99, 2.57 Hz, 1H), 3.01 proR (ddd, *J* = 11.25, 6.72 Hz, 1H), 3.23 (s, 3H), 3.46 proS (dd, *J* = 11.25, 1.59 Hz, 1H), 3.65 (d, *J* = 9.9 Hz, 1H), 3.89 (d, *J* = 9.9 Hz, 1H), 4.83 (dd, *J* = 6.72, 1.59 Hz, 1H), 6.15 (ddd, *J* = 8.8, 7.58, 2.57 Hz, 1H), 8.26 (s, 1H); ^{13}C NMR δ −6.68, 14.65, 22.93, 26.48, 35.05, 35.74, 40.87, 44.11, 49.04, 59.69, 71.84, 73.31, 88.54, 114.28, 140.92, 142.74, 144.81, 148.77, 150.09, 157.52, 167.8, 172.48, 215.89.

Preparation of Pegylated Wortmannin (13). To **9** (X = I) (30 mg, 0.054 mmol) in acetonitrile (15 mL) and aqueous 0.1 M sodium bicarbonate (10 mL) under nitrogen was added mPEGSH (300 mg, 0.060 mmol, MW ∼ 5000) over 1 h. After stirring for 1 h at room temperature, the reaction mixture was extracted with dichloromethane to give 274 mg of crude product. Preparative HPLC gave 172 mg of pure **13** (172 mg, 58%): ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 3H), 1.64 (dd, *J* = 12.88, 8.87, 1H), 1.74 (s, 3H), 2.06 (ddd, *J* = 22.25, 12.72, 9.03, 1H), 2.27 (dd, *J* = 19.58, 9.37, 1H), 2.6 (dd, *J* = 19.58, 8.53, 1H), 2.63 (dd, *J* = 12.88, 7.53, 1H), 2.84 (t, *J* = 6.36, 2H), 2.91 (ddd, *J* = 12.72, 5.86, 2.68, 1H), 3.01 proR (dd, *J* = 11.38, 6.36, 1H), 3.16 (s, 3H), 3.19 (m, 1H), 3.38 (s, 3H), 3.46 proS (dd, *J* = 11.38, 6.36, 1H), 3.55 (s, 2H), 3.65 (m), 3.7 (s, 2H), 3.34 (d, *J* = 9.87, 2H), 4.91 (dd, *J* = 6.36, 1.84, 1H), 6.15 (ddd, *J* = 8.87, 7.53, 2.68, 1H), 8.27 (s, 1H); ^{13}C NMR δ 14.6, 22.93, 26.51, 31.99, 33.64, 35.72, 35.76, 40.82, 44.08, 49.1, 59.02, 59.47, 70.36, 70.55, 70.87, 71.18, 71.92, 73.05, 88.35, 114.35, 140.52, 142.97, 144.74, 149.08, 150.07, 157.68, 169.02, 172.52, 215.97.

Preparation of Pegylated 17-Hydroxywortmannin (15). To a solution of **14** (35 g, MW 6000) and *N,N*-diisopropylethylamine (2.7 mL) in acetonitrile (90 mL) under nitrogen at 0 °C was added **10** (X = Br) (6.06 g, 11 mmol) portionwise over 30 min. After the addition, the ice bath was removed and the mixture was allowed to warm to room temperature. After 3−4 h, 2-propanol (1200 mL) was added over 30 min. After an additional 1.5 h, the resulting solid was collected on a Buchner funnel and washed with 2 × 150 mL of 2-propanol. The wet cake was then dissolved in acetonitrile (80 mL) containing 0.5% $^iPr_2NEt$ at 0−5 °C and precipitated by addition of 2-propanol (1000 mL). The resulting solid was collected and washed with 2-propanol and dried in vacuo to give **15** as light yellow powder (38.2 g, 100%): ^{1}H NMR ($CDCl_3$) δ 0.92 (s, 3H), 1.57−1.49 (dd, *J* = 12.3, 10.2 Hz, 1H), 1.73 (s, 3H), 1.82−1.65 (m, 2H), 2.14 (s, 3H), 2.38−2.30 (m, 1H), 2.56−2.50 (dd, *J* = 12.6, 7.2 Hz, 1H), 2.67−2.60 (m, 1H), 2.88−2.82 (m, 3H), 3.01−2.95 (dd, *J* = 10.8, 7.2 Hz, 1H), 3 0.19 (s, 3H), 3.31 (s, 2H), 3.42−3.39 (t, *J* = 4.8 Hz, 2H), 3.49−3.44 (dd, *J* = 11.1, 1.8 Hz, 1H), 3.64 (backbone of PEG, ∼321H), 3.89−3.86 (t, *J* = 4.8 Hz, 2H)), 4.77−4.74 (dd, *J* = 10.2, 1.2 Hz, 1H), 4.89−4.86 (dd, *J* = 7.2, 9.6 Hz, 1H), 6.13−6.06 (dt, *J* = 9.6, 2.4 Hz, 1H), 8.25 (s, 1H).

Supporting Information Available: Mass spectra and HPLC purity data. This material is available free of charge via the Internet at http://pubs.acs.org.

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