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Chemistry and biology of wortmannin

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Recent synthetic and biological studies of the viridin class of steroidal furans have revealed multiple opportunities for fundamental discoveries as well as advanced drug design. Wortmannin is a potent enzyme inhibitor that binds to the ATP site of important regulatory kinases such as PI-3 kinase and Polo-like kinase. The natural product shares a unique mechanism-based biological activation pathway with other viridins. Furthermore, while there have been several encouraging approaches toward the total synthesis of these compounds, there is still ample room for improvements in synthetic strategies and tactics, and the development of structurally simplified analogs that exert more specific biological effects and are devoid of toxicity issues that have thwarted the clinical development of the parent compounds.

1 Introduction and mechanism of action of wortmannin

Wortmannin (**1**) was isolated in 1957 by Brian and co-workers from the broth of *Penicillium wortmanni* Klocker.[1] Preliminary assays demonstrated that the natural product extract had significant antifungal properties, but at the time no further work on the biological activity or the structure elucidation was pursued. In the late 1960s, MacMillan and co-workers completed the structure assignment[2] and established the relative configuration at all but one stereocenter by ^{1}H NMR spectroscopy and degradation experiments.[3] Independently and concurrently, Petcher and co-workers at Sandoz laboratories established the absolute configuration of wortmannin by X-ray crystallography.[4]

Wortmannin is a member of the structurally closely-related class of steroidal furanoids which include viridin (**5**), viridiol (**6**), demethoxyviridin (**7**), demethoxyviridiol (**8**), and wortmannolone (**9**) (Fig. 1).[5] Viridiol (**6**) is identical to the more recently isolated TAEMC-161,[6] which was found to inhibit 5'-hydroxyaverantin dehydrogenase, an enzyme involved in aflatoxin biosynthesis. Halenaquinol (**2**), halenaquinone (**3**), and xestoquinone (**4**) are also included in the coverage of wortmannin chemistry and biology, since they share many of the structural and biological properties of wortmannin and its congeners. Both **2** and **3** demonstrated some antibacterial activity, as well as cardiotonic properties in early assays.[7] (+)-Xestoquinone (**4**) was isolated from *Xestospongia sapra* in 1960[8,9] and has been shown to be an inhibitor of both the oncogenic protein tyrosine kinase pp60$^{v\text{-}src}$ and the human epidermal growth factor kinase. Quinone **4** also possesses cardiotonic activity and inhibits Ca^{2+} ATPase of skeletal muscle myosin.[10,11]

Halenaquinone and halenaquinol were isolated in 1983 from *Xestospongia exigua*;[7] the former compound was found to induce apoptosis in PC-12 cells, possibly due to inhibition of phosphatidylinositol 3-kinase (PI-3K).[12] Not surprisingly, as a consequence of the presence of quinones in the polycyclic scaffold,[13] halenaquinone and xestoquinone analogs were recently shown to inhibit the dual specificity phosphatase Cdc25.[14]

In 1998, noelaquinone (**10**) was obtained from an Indonesian *Xestospongia* sp.; this compound is clearly closely related to the halenaquinones, but no specific biological activities have been reported.[15]

The structural origin of wortmannin's biological activity, and quite likely of other members of the viridin class of steroidal furans,[16] arises at least in part from the electrophilic character of the strained tricyclic furan moiety. The naphtho[1,8-*bc*]furan

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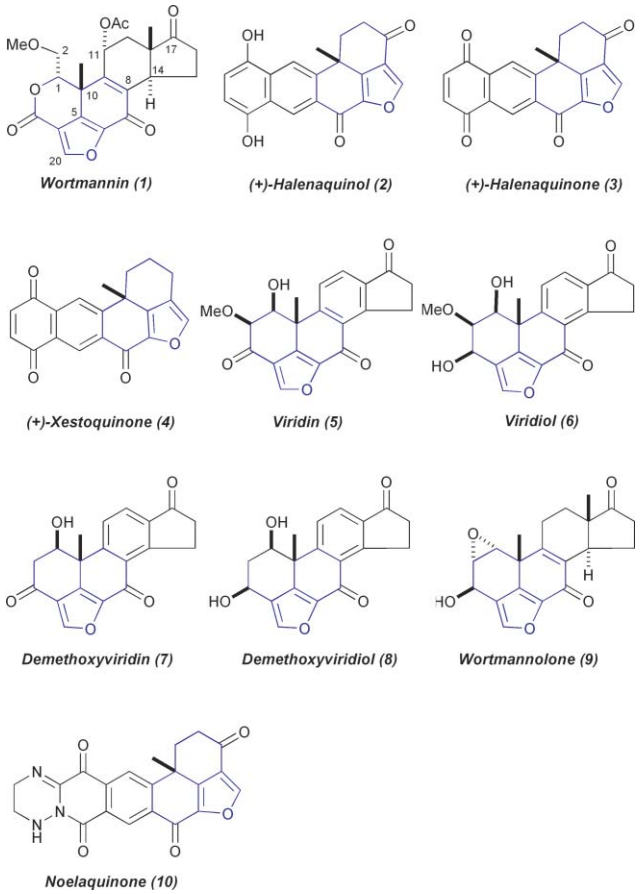


Fig. 1 Structures of furanosteroids as well as related natural products that contain the characteristic naphtho[1,8-*bc*]furan scaffold.

subunit is generally flanked by electron-withdrawing carbonyl groups that increase the electrophilicity of the α-carbon of the furan ring, and the strained nature of the tricycle further enhances furan reactivity (Fig. 2). The condensed nature of the contiguous sp^2-hybridized carbons on the tricyclic ring system leads to a strain energy of approximately 12.1 kcal mol^{-1}, based on a comparison of the relative differences in the energy of hydrogenation of **11** and **13**.

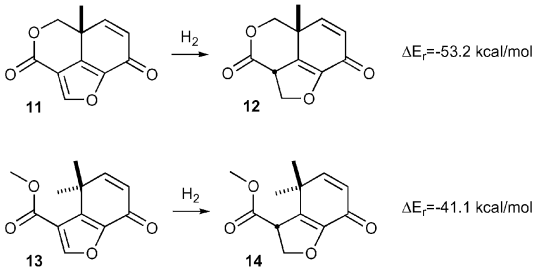


Fig. 2 Approximate reaction enthalpies determined at the B3LYP/6-31G* level in Spartan 04.

Wortmannin interacts with many biological targets, but binds *in vitro* most strongly to PI-3 kinase. The PI-3K enzyme is part of a signalling cascade that is essential for cell growth and differentiation, and wortmannin is therefore a potent antiproliferative agent (Fig. 3). PI-3 kinase is present in cellular complexes with almost all ligand activated growth factor receptor and oncogene protein tyrosine kinases. Its activity has been found to increase in response to platelet-derived growth factor (PDGF), insulin, insulin-like growth factor 1 (IGF-1), colony stimulating factor 1 (CSF-1), nerve growth factor (NGF), hepatocyte growth factor (HGF), stem cell growth factor (Steel), and epidermal growth factor (EGF).[17] Similarly, PI-3K activity is found to be elevated

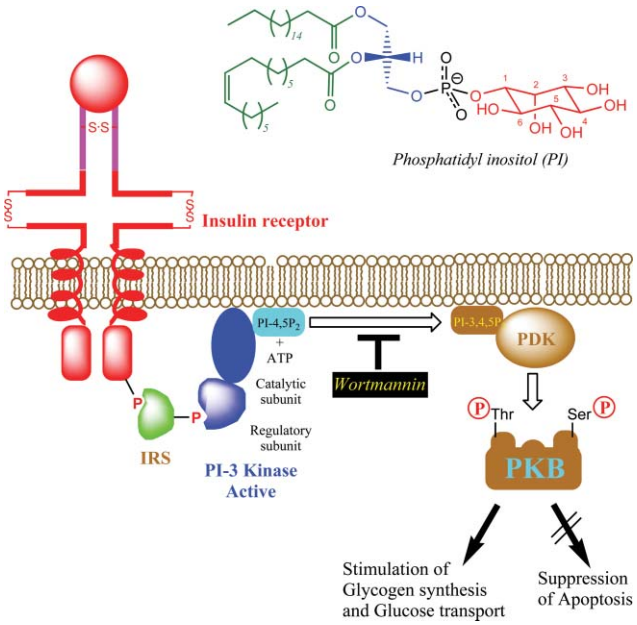


Fig. 3 Regulation of protein kinase B (PKB, Akt) through inhibition of the phosphorylation of the 3-position of the inositol ring of phosphoinositides (PI), and structure of phosphatidylinositol (PI, PtdIns).

in cells transformed by *v-src*, *v-ros*, *v-yes*, and *v-abl* as well as the polyomavirus middle T antigen/*c-src* complex,[18,19] and it is also regulated by and associated with members of the non-receptor family of tyrosine-kinases (*i.e.*, *lck*, *fyn*, *lyn*, and *c-yes*) in response to cellular activators that stimulate these enzymes. Unfortunately, wortmannin is not selective toward transformed cells, exhibiting a level of toxicity that renders it unsuitable as an anticancer drug.[20] Despite the toxicity issues, the nanomolar inhibition of PI-3 kinase by wortmannin has inspired numerous synthetic and biological studies.[5,21]

In a very recent and extremely exciting discovery, wortmannin was also found to be a potent inhibitor of mammalian Polo-like kinase (PLK1).[22] This enzyme is involved in cell cycle progression of rapidly proliferating, nontransformed cells as well as tumor cells. In cancer, overexpression of PLK1 contributes to the malignant state by aberrant cell cycle regulation at the G2/M phase. Observation of the wortmannin–PLK1 interaction was enabled by a tetramethylrhodamine–wortmannin conjugate, and it was shown that wortmannin inhibits PLK1 in an *in vitro* kinase assay with an IC_{50} of 24 nM. These results clearly indicate that, at the concentrations of wortmannin that are commonly used to inhibit PI-3 kinases, PLK1 is also significantly inhibited.

Even prior to the PLK1 findings, wortmannin's target selectivity has been called into question as it was shown to inhibit other serine/threonine kinases of the PI-3-kinase family, such as mTOR and DNA-dependent protein kinase, with IC_{50} values of 2 and 4 μM, respectively, in intact cells (the IC_{50} is considerably lower for the isolated enzymes, 250 nM for mTOR and 16 nM for DNA-PK).[23,24] Functionally, the mammalian target of rapamycin (mTOR) is involved in the control of protein synthesis through activation of a number of targets including p70 S6 kinase. Recently, a direct link between mTOR and the PI-3 kinase–AKT signalling pathway in transformed cells has also been established.[25] Wortmannin is therefore expected to affect other kinases with homologies within the PI-3 kinase ATP recognition region. Furthermore, the natural product has been reported to be an inhibitor of myosin light chain kinase (MLCK) with an IC_{50} of 0.17 μM and a membrane-bound form of PI-4 kinase at high nanomolar concentrations.[26,27]

Proteolysis and mutagenesis studies of PI-3 kinase revealed that Lys-802 was the target for a covalent attachment of wortmannin.[28] This lysine residue resides in the ATP binding site of the p110 catalytic subunit and thus has a crucial role in the

phosphotransfer reaction. Specifically, the irreversible inhibition of PI-3 kinase involves the formation of a vinylogous carbamate by an attack of the lysine side chain amino group onto the furan ring (Fig. 4). This mechanism was supported through structure activity relationship studies.[29] Wymann *et al.* also proposed a model for the noncovalent interactions of wortmannin with PI-3 kinase supported by the X-ray crystallographic structures of PI-3K inhibitors bound in the ATP binding pocket (Fig. 5).[30] It is important to note that wortmannin is a bimodal inhibitor of PI-3Ks. The inherent affinity of the natural product to the ATP-pocket would in itself be sufficient to inhibit the kinase domain, but the process then proceeds toward covalent modification of the lysine residue and formation of the irreversible adduct. Accordingly, the noncovalent binding interaction is sufficient to inhibit the enzyme, and Lys modification only makes it irreversible.

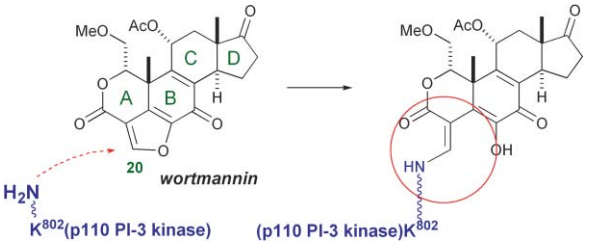


Fig. 4 Wortmannin is a mechanism-based inhibitor of PI-3 kinase.

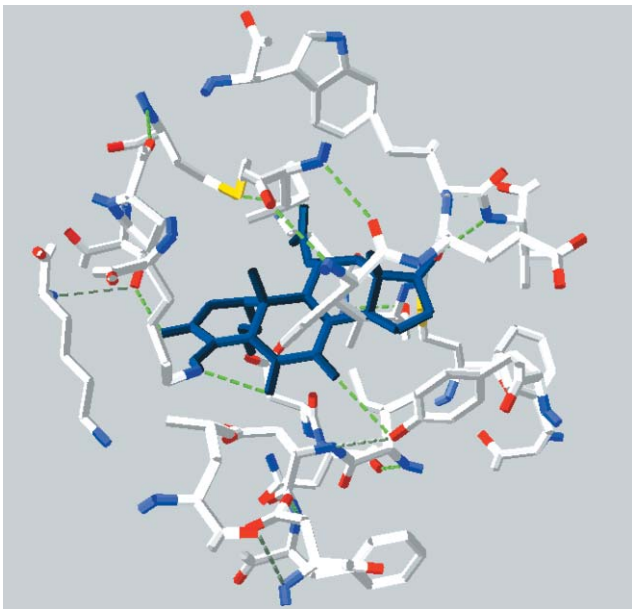


Fig. 5 The ATP-binding site of PI-3 kinase binds tightly to wortmannin through covalent attachment as well as a series of hydrogen bonds.

Aside from selectivity and toxicity issues, another obstacle to the use of wortmannin and other viridins as clinical candidates is their instability. Both wortmannin and demethoxyviridin, when stored as aqueous solutions at either 37 or 0 °C at neutral pH (Tris–HCl buffer, pH 7.4), are subject to decomposition by hydrolytic opening of the furan ring. This chemical instability is much more pronounced in demethoxyviridin than wortmannin, mirroring their relative potency. Furthermore, lactone hydrolysis in wortmannin followed by elimination of methoxyacetaldehyde leads to aromatization of the B-ring and loss of PI-3K inhibition (Fig. 6).[2,3]

Both viridin and wortmannin were originally characterized as antifungal agents. Viridin was also found to exhibit antibiotic activity against certain plant pathogens; however, co-metabolites viridiol and demethoxyviridiol exhibited phytotoxic activity,

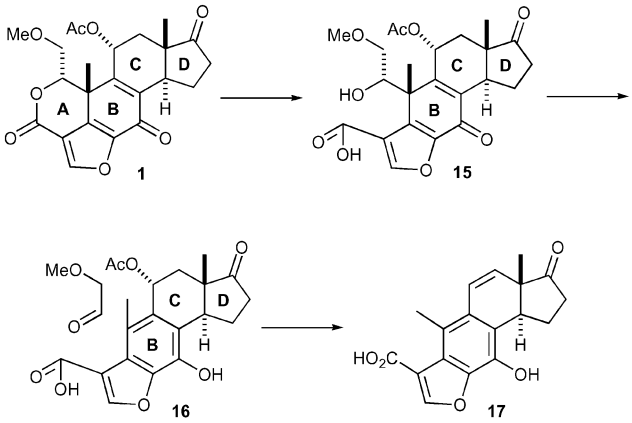


Fig. 6 Fragmentation of the A-ring of wortmannin leads to an inactive phenolic product.

rendering the parent fungal species unsuitable for biocontrol. Wortmannin and its 11-desacetoxy analog were subsequently identified as potent anti-inflammatory agents.[31] After early but limited studies of wortmannin at Sandoz laboratories,[32] considerably more extensive investigations were pursued at Eli Lilly & Co.[29,33] The first systematic SAR studies of wortmannin established that slight structural modifications distant from the furan ring had little or no effect on its *in vitro* efficacy (Fig. 7). Conversely, modifications that modulated the electrophilicity of the furan ring had a profound impact on both the toxicity and the efficacy of **1**. Interestingly, only one of the synthetic analogues showed a higher level of activity against PI-3 kinase than the parent wortmannin. A significant demonstration of the biological importance of the electrophilic site in wortmannin was obtained by addition of diazomethane. The resulting C(20)-methylated product, presumably formed through a cyclic intermediate, was deprived of all activity (Fig. 7). Expansion of the furan ring to a pyran can be accomplished with trimethylsulfoxonium ylide. The pyran, which remains a reactive Michael acceptor, retains significant activity against PI-3 kinase.[33] In view of these structure–activity relationships it becomes apparent that efficacy and electrophilicity of wortmannin derivatives are closely correlated. The cardio-, hepato-, and nephrotoxicity of all active compounds and their rapidly formed metabolites presumably remained too high to be medically useful.

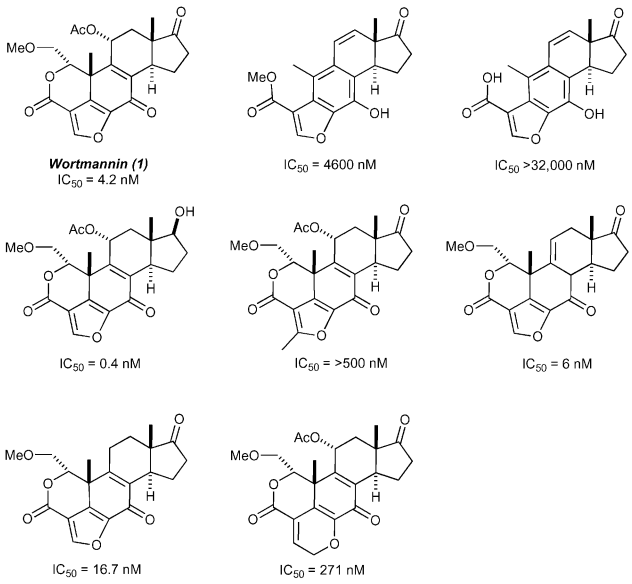
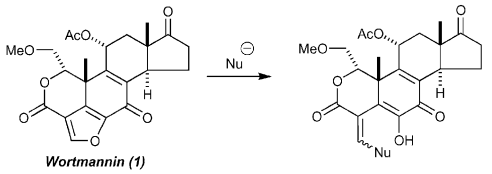
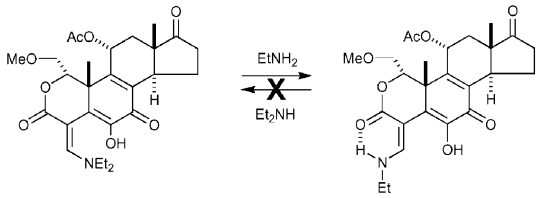


Fig. 7 Structural analogs and IC_{50} values for in vitro PI-3 kinase inhibition.

The strained furan heterocycle can be opened with a variety of nucleophiles.[33a,c] The ring opening with amines and thiols is particularly rapid and results in orange colored diosphenols (Scheme 1). Addition of secondary amines yields compounds with similar activity as wortmannin. After primary amine treatment, however, little to no biological activity is observed. Researchers at Lilly correlated this discrepancy with the conformation of the newly formed enamine.[33c] The vinylogous carbamate derived from primary amines prefers a hydrogen bond stabilized (*Z*)-conformation. This orientation was thought to sterically hinder the attack of nucleophilic residues on PI-3 kinase. The secondary amine adducts, which do not benefit from hydrogen bonding interactions, give rise to (*E*)-conformations that remain sterically accessible to nucleophilic substitutions. The difference in thermodynamic stability between the two derivatives is illustrated by the ability of secondary amine-derived analogues to exchange with primary amines, whereas the inverse exchange does not occur (Scheme 2).[33c] In agreement with this hypothesis, thiol adducts also prove to be active compounds, residing in the (*E*)-thioether conformation. The recent preparation of a 95-membered combinatorial library of synthetic viridins took advantage of the ease of furan ring opening, and several biologically promising derivatives were identified in subsequent screens (Fig. 8).[34] Most significantly, some of these derivatives demonstrated lower unselective toxicity compared to the wortmannin lead structure.[35]



Scheme 1 The furan ring of wortmannin is susceptible to nucleophilic addition.



Scheme 2 The reactivity of amine adducts is conformation and *N*-substituent dependent.

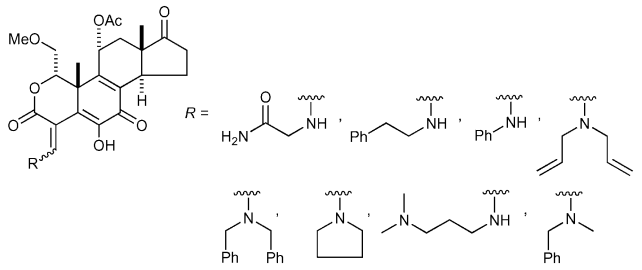


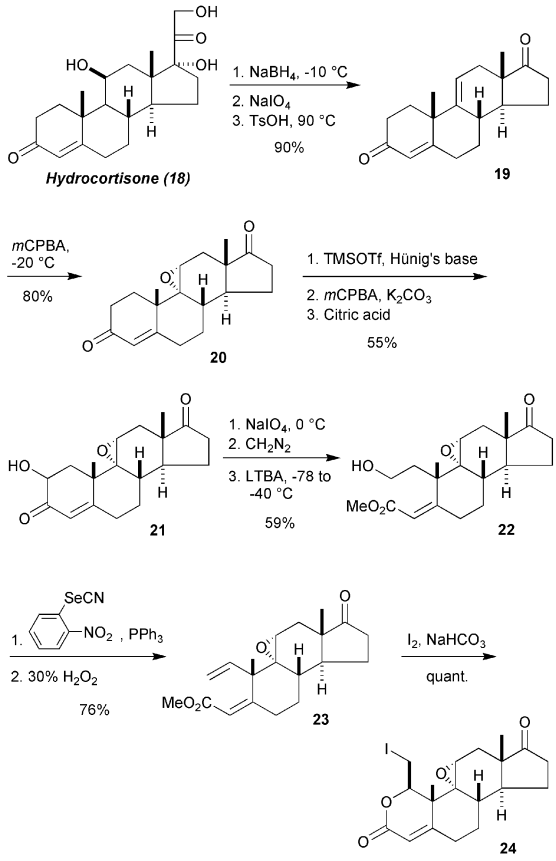
Fig. 8 A focused library of synthetic wortmannin derivatives furnished several nanomolar enzyme inhibitors with high selectivity for PI-3 kinase and high cytotoxic activity against cancer cell lines.[34]

2 Synthetic approaches toward wortmannin

In spite of the great advances in steroid synthesis in the second part of the 20th century,[36] the structural complexity of the pentacyclic wortmannin renders it a challenging target even for modern total synthesis. The timing of the introduction of the highly substituted and reactive furan moiety presents the greatest strategic conundrum. Only two total syntheses, both by Shibasaki and coworkers,[37,38] and one partial synthesis by Broka and Ruhland[39] have been reported to date. In the mid 1990s, Shibasaki and co-workers published the first stereoselective, albeit low-yielding, synthesis of **1**, starting from commercially available hydrocortisone. More recently, this group succeeded in a total synthesis of the racemic product. In addition to wortmannin, related natural products have also served as target molecules for the development of innovative synthetic strategies. A synthesis of the core of viridin (**5**) was published by Souza and Rodrigo,[40] and a total synthesis of this target was recently accomplished by Anderson, Alexanian and Sorensen.[41] Several protocols for the preparation of xestoquinone and halenaquinone have appeared (*vide infra*).[10,42] In addition to the accomplishments of Shibasaki[37,38] and Broka and coworkers,[39] Rodrigo's partial synthesis of viridin[40] and the total syntheses of **2** and **5** will be discussed in more detail.[42a]

2.1 Shibasaki's 1st synthesis of wortmannin

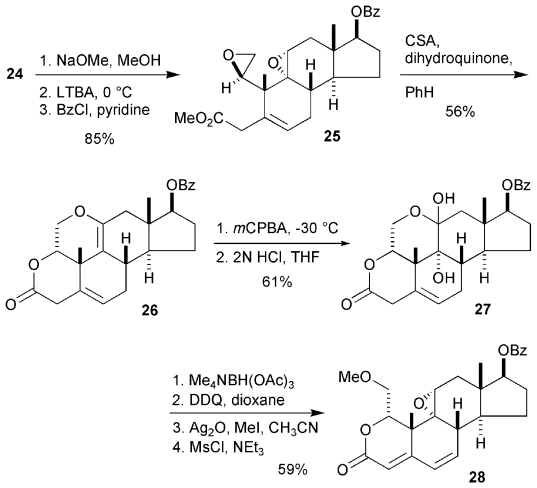
Shibasaki's initial synthesis of wortmannin started with commercially available hydrocortisone.[37] The difficulty in installing the furan ring became apparent in the second part of the *ca.* 35 step synthesis. First, the hydrocortisone side chain was reduced to the triol and cleaved chemoselectively with sodium periodate (Scheme 3). The remaining secondary hydroxyl group was eliminated, providing the trisubstituted C-ring alkene **19**. According to a series of known transformations, the C-ring double bond was epoxidized stereoselectively with *m*CPBA to give **20**. Introduction of the lactone A-ring was initiated by treatment of the α,β-unsaturated ketone with Hünig's base and TMSOTf to give the TMS enol ether, which was subjected to a Rubottom oxidation with *m*CPBA. The epoxide was opened during citric acid work-up to provide hydroxy ketone **21**. Carbon–carbon bond cleavage with sodium periodate opened the six-membered ring, providing an aldehyde and a carboxylic



Scheme 3 Conversion of the A-cyclohexane ring of hydrocortisone to a lactone.

acid. The acid was protected as the methyl ester and the aldehyde was reduced to give alcohol **22**. Grieco-elimination gave terminal alkene **23**, and treatment with I_2 led to the iodo-lactonization product **24**. Unfortunately, the stereochemistry of the side chain of the lactone ring was opposite to what was desired. A variety of reaction conditions were explored to obtain the proper configuration, but none were successful.

The lactone configuration was corrected through a multi-step sequence. Methanolysis under basic conditions provided the epoxy ester (Scheme 4). The D-ring ketone was then selectively reduced and protected as the benzoate **25**. Exposure of epoxide **25** to camphorsulfonic acid and dihydroquinone regenerated the lactone, with inversion of the stereochemistry (in relation to **24**) and also opened the C-ring epoxide, leading to pyran **26**. The newly formed double bond was epoxidized with *m*CPBA and then treated with HCl to provide diol **27**. Reduction of the diol, followed by oxidation and treatment with methyl iodide provided the primary methyl ether. The C-ring epoxide was then reformed by treatment with MsCl and triethyl amine, providing the desired lactone **28**.

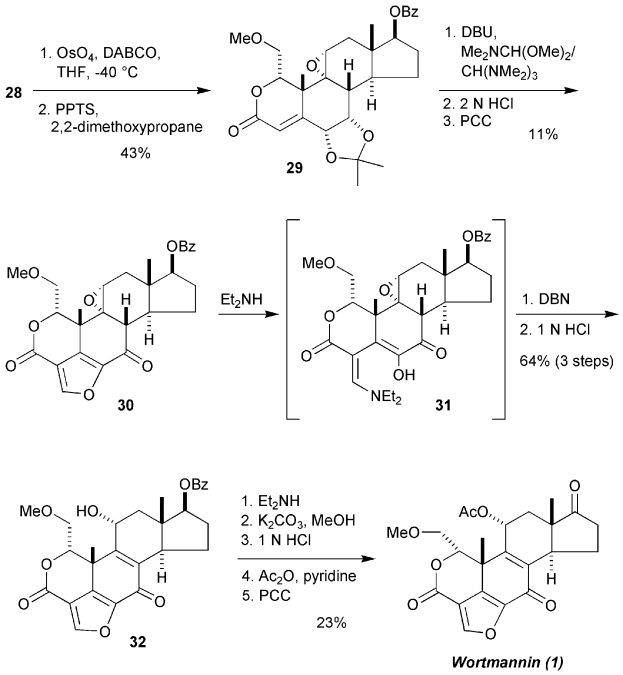


Scheme 4 Correction of the configuration of the A-ring lactone.

At this stage of the synthesis, the furan ring of wortmannin had yet to be introduced. Dihydroxylation of the γ,δ-double bond with OsO_4 provided a diol which was protected as the acetonide (Scheme 5). Enone **29** was treated with DBU and tris(dimethylamino)methane in the presence of dimethylformamide dimethyl acetal at 100 °C for 1 h to give the aminomethylene lactone. Further treatment with 2 N HCl and oxidation with PCC provided furan **30** in low yield. Unfortunately, the reactivity of the furan ring was the source of significant problems that prevented an expedient completion of the synthesis. Eventually, a somewhat circuitous solution was identified. Furan **30** was treated with diethylamine to open the furan ring, in effect protecting it as the enamine **31**. Treatment with DBN eliminated the epoxide to the allylic alcohol, and HCl was used to reestablish the furan ring, providing **32** in good yield. Another furan opening with diethyl amine was followed by deprotection of the benzoate. Renewed HCl promoted formation of the furan and selective acetylation of the alcohol at C(11) was followed by PCC oxidation of the remaining D-ring hydroxyl group to the ketone, providing enantiomerically pure wortmannin in *ca.* 35 steps and 0.02% overall yield.

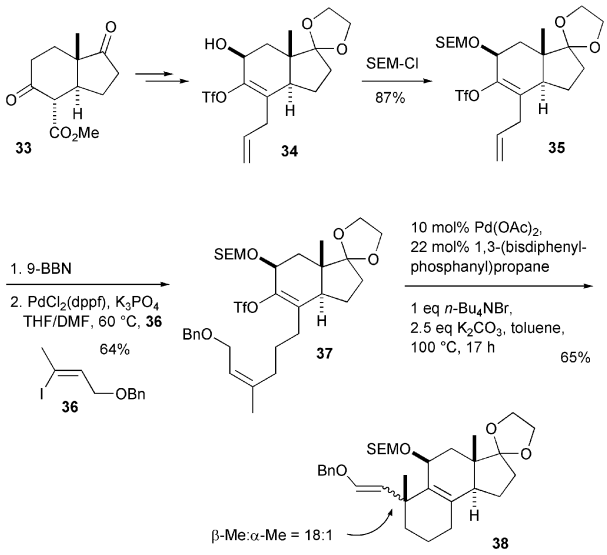
2.2 Shibasaki's 2nd synthesis of wortmannin

The goal of the second synthesis of the natural product in the Shibasaki lab was to prepare wortmannin from non-steroidal starting materials and to showcase intramolecular Heck coupling methodology. In this synthesis,[37] the key furan ring was also installed late in the sequence, in almost the same manner



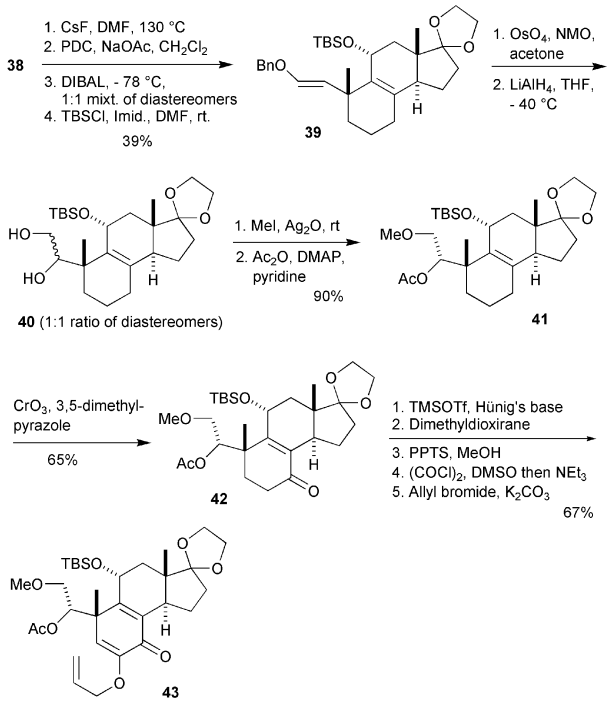
Scheme 5 Completion of the 1st synthesis of wortmannin.

as in the previous approach. The known intermediate *rac*-**34**,[43] derived from indane **33** in 8 steps and 27% overall yield, was protected as a SEM-ether in preparation for a Suzuki coupling (Scheme 6). Pd-catalyzed coupling of the borane derived from **35** and the easily obtainable iodide **36** set the stage for a Heck reaction. Subjecting the triflate **37** to typical Heck conditions resulted in the formation of the six-membered ring in good yield, with the desired stereochemistry at the newly formed quaternary center as the major product. Unfortunately, this approach did not tolerate the opposite α-configuration of the SEM-ether in the C-ring. Thus, it became necessary to invert the stereochemistry at that position.



Scheme 6 Intramolecular diastereoselective Heck coupling for B-ring synthesis.

Inversion of the SEM-ether was accomplished *via* deprotection of **38**, oxidation to the ketone, DIBAL reduction and re-protection as a TBS-ether to give **39** (Scheme 7). Unfortunately, the DIBAL reduction was not selective, giving a 1 : 1 mixture of diastereomers. The undesired diastereomer was recycled to improve the yield of the desired product. Dihydroxylation of the enol ether, followed by reduction of the resulting aldehyde with

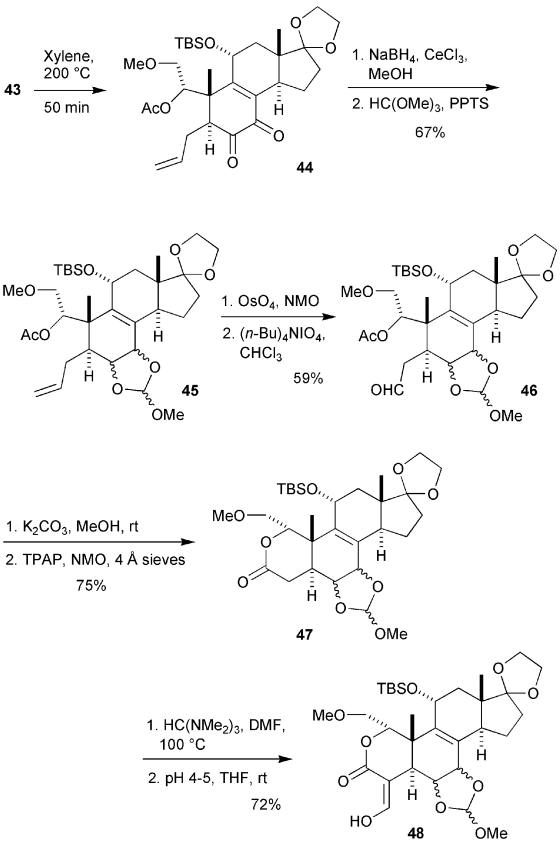


Scheme 7 Inversion of alcohol configuration and oxidation of the B-ring.

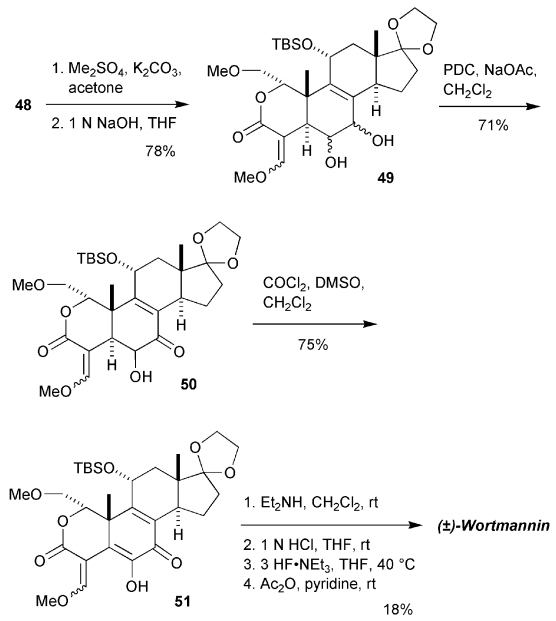
$LiAlH_4$ gave diol **40**. The undesired diastereomer could again be recycled. Selective protection of the primary alcohol as a methyl ether and acetylation of the secondary alcohol allowed an allylic oxidation with CrO_3 to introduce the carbonyl group in the B-ring. Formation of the TMS enol ether with Hünig's base, followed by oxidation with dimethyldioxirane, hydrolysis, and Swern oxidation generated the diosphenol, which was condensed with allyl bromide to provide **43**, in preparation for a Claisen rearrangement.

[3,3] Sigmatropic rearrangement of allyl ether **43** in xylene provided diketone **44** as a single isomer (Scheme 8). Reduction of both carbonyl groups with $NaBH_4$ followed by protection with trimethylorthoformate yielded the ortho ester **45** as a mixture of several diastereomers. Johnson–Lemieux oxidation of the vinyl group led to aldehyde **46**. The acetate was saponified and oxidative cyclization with TPAP provided δ-lactone **47**. Treatment with tris(dimethylamino)methane followed by hydrolysis provided the vinylogous acid **48**, in preparation for installment of the fused furan ring.

O-Methylation and basic deprotection of the ortho ester provided diol **49** (Scheme 9). Selective PDC oxidation of the allylic alcohol followed by Swern oxidation gave diosphenol **51**. Methyl ether exchange with diethyl amine, a reaction reminiscent of the way wortmannin interacts with PI-3 kinase, was followed by acid treatment and ring closure to the furan. Finally, deprotection of the TBS ether, followed by acetylation led to (±)-wortmannin. Starting from intermediate **34**, the racemic natural product was thus produced in *ca.* 35 chemical steps and 0.04% overall yield.



Scheme 8 Formation of the A-ring valerolactone.

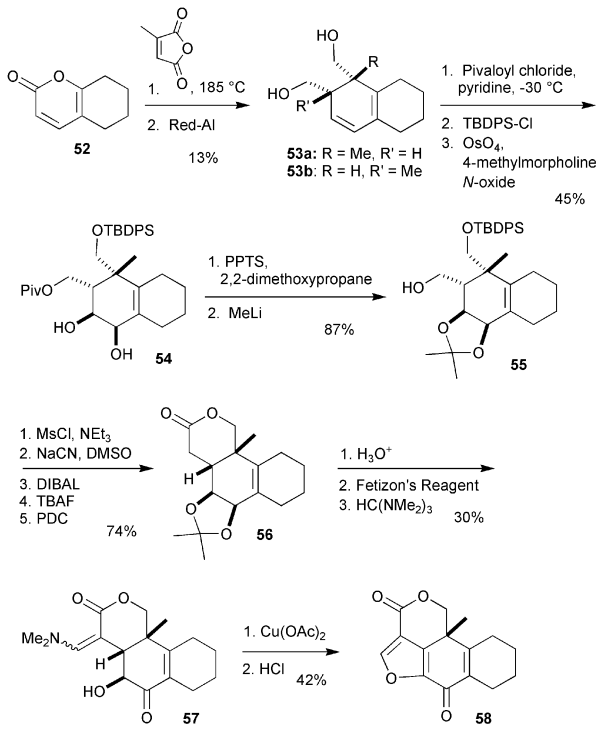


Scheme 9 Completion of the total synthesis of racemic wortmannin.

2.3 Partial synthesis of wortmannin

A partial synthesis of the wortmannin core structure featuring a Diels–Alder reaction as a key step has been published by Broka and Ruhland.[39] The known tetrahydrocoumarin **52** was subjected to a cycloaddition reaction with citraconic anhydride to give a mixture of regioisomers, **53a** and **53b**, in low yield (Scheme 10). Only **53a** was carried on toward the intermediate **56**. In spite of the low yield and lack of regioselectivity of the intermolecular Diels–Alder reaction, an intramolecular version of this sequence might be more successful and could well be useful for a second generation approach toward the natural product.

Mono-protection of the diol **53a** as the pivaloate and silylation of the remaining hydroxyl group allowed for the selective dihydroxylation of the disubstituted double bond to give **54**. The diol was protected as an acetonide and the pivaloate was cleaved under nucleophilic conditions. Homologation of the side chain proceeded *via* formation of the mesylate and substitution with sodium cyanide. Reduction of the cyano moiety with DIBAL resulted in the formation of a hemiacetal after deprotection with TBAF. Oxidation with PDC then led to lactone **56**.

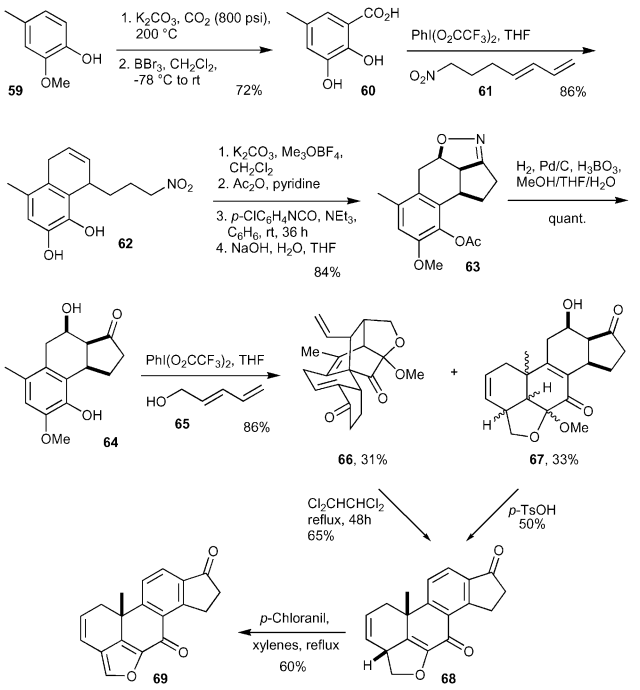


Scheme 10 Diels–Alder reaction with an α-pyrone for B-ring formation in an approach toward the tetracyclic core structure of wortmannin.

For the installment of the furan moiety, the acetonide was deprotected and selectively oxidized with Fetizon's reagent or, more efficiently, with Dess–Martin reagent to provide the enone. This intermediate reacted with tris(dimethylamino)methane to give the enamide **57**. In the presence of $Cu(OAc)_2$, the hydroxy enone was converted to the diosphenol, and treatment with HCl in dioxane under the conditions originally developed by Haefliger and Hauser provided the desired furan product **58**.[44] Overall, this synthesis represents a concise approach to the core structure of wortmannin. Compound **58** failed to block fMet–Leu–Phe-stimulated generation of superoxide by neutrophils, under conditions where wortmannin demonstrated an IC_{50} of 0.1 μM, but unfortunately PI-3 K inhibition was not tested.

2.5 Viridin and halenaquinone

2.5.1 Viridin. Rodrigo and co-workers assembled the pentacyclic ring system of viridin (**5**), in an elegant approach with nine steps, from 4-methylguaicol by means of successive cycloadditions with *in situ*-generated ortho-benzoquinone intermediates (Scheme 11).[45,46] Diol **60** was obtained by carboxylation and demethylation of **59**. Oxidation with a hypervalent iodine reagent, followed by condensation with 1-nitrohepta-4,6-diene (**61**) allowed for an intramolecular Diels–Alder reaction, whereby the primary cycloadduct underwent further decarboxylation and rearomatization to provide diol **62**. The hydroxyl groups were selectively protected as methyl ether and acetate ester. The isoxazoline **63** was formed by intramolecular nitrile oxide 1,3-dipolar cycloaddition upon treatment with *p*-chlorophenyl isocyanate, followed by a basic workup and hydrolytic hydrogenolysis, giving aldol **64**. A second hypervalent iodine oxidation–Diels–Alder sequence with penta-2,4-dienol (**65**) gave a mixture of **66** and **67**; while the former was formed as a single compound, the latter was isolated as a mixture of two diastereomers. Treatment of these diastereomers with *p*-TsOH converged both into **68**, and a Cope rearrangement of **66** also gave **68**. A final oxidation step completed the formation of the furan moiety and provided the viridin core **69**. Although this route would need to be expanded to include some of the functionality of the natural product, it coincides with a late-



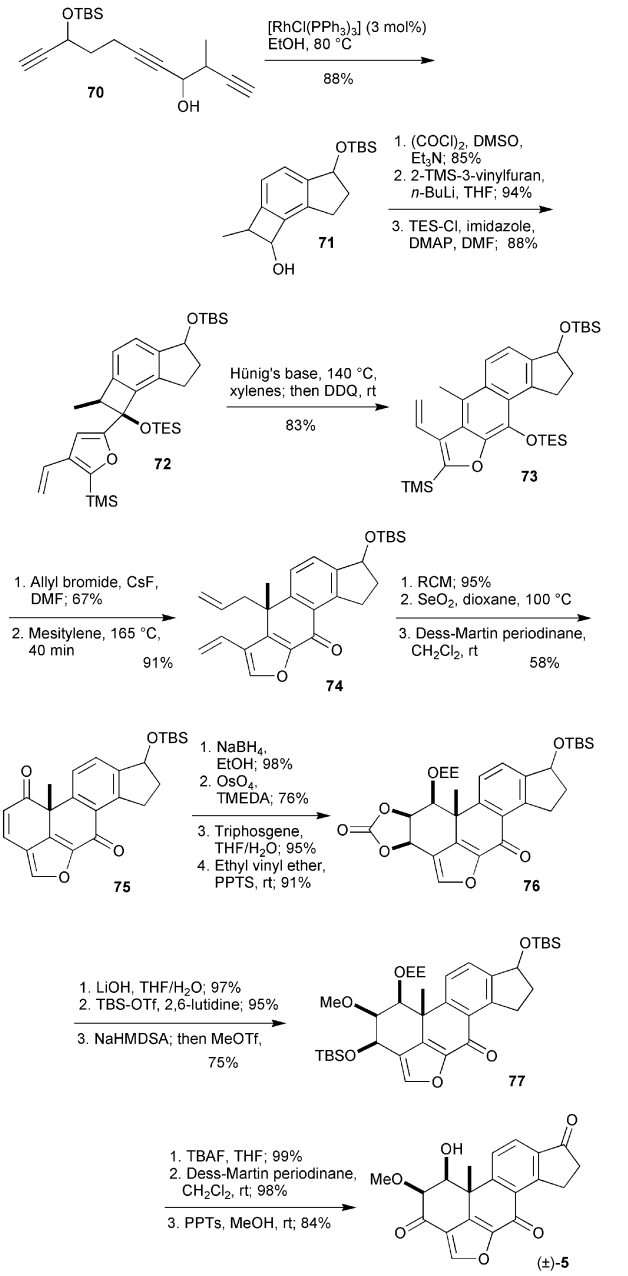
Scheme 11 Assembly of the pentacyclic core structure of viridin based on *ortho*-quinone Diels–Alder processes.

stage intermediate in the Sorenson approach (*vide infra*), and the chemistry clearly provides an elegant entry into the pentacyclic system.

Quite recently, the Sorensen group completed the first total synthesis of racemic viridin based on a rhodium-catalyzed cyclotrimerization (Scheme 12).[41] Triyne **70** was converted into the tricycle **71** as a mixture of four diastereomers with Wilkinson's catalyst, in analogy to the work of Vollhardt *et al.* on alkyne cyclotrimerization in steroid synthesis.[47] Swern oxidation of **71**, nucleophilic addition of vinyl furan, and TES-protection of the tertiary alcohol led to the *anti*-product **72**, which underwent a tandem conrotatory electrocyclic ring-opening 6π-disrotatory electrocyclization to tetracycle **73** in 83% yield. Phenol *O*-desilylation under concurrent *C*-desilylation and *O*-allylation conditions led, after *para*-Claisen rearrangement,[48] to dienone **74**.

A ruthenium-catalyzed ring-closing metathesis (RCM)[49] reaction produced the pentacycle **75** after allylic hydroxylation to the α-alcohol and Dess–Martin oxidation to the enone. Reduction to the β-alcohol was followed by hydroxy-directed dihydroxylation[50] to give the all-*syn* triol which was selectively protected as the cyclic carbonate. The remaining hydroxyl group was masked as the ethoxyethyl (EE) ether to give **76**. Saponification of the carbonate, silylation of the more accessible secondary alcohol, and methylation provided the fully protected intermediate **77**. Finally, fluoride cleavage of the silyl ethers, double oxidation with Dess–Martin periodinane, and hydrolysis of the ethoxyethyl ether led to racemic viridin in 27 steps and 5% overall yield from commercially available starting materials.

2.5.2 Halenaquinone. The closely related halenaquinone (**3**) and xestoquinone (**4**) have been synthesized by a number of groups.[10,42b,46,51,52,53,54,55] One of the shortest and most efficient routes originated from the Rodrigo labs (Scheme 13).[42a] This approach was based on a Diels–Alder–Cope sequence to quickly form the polycyclic scaffold of the natural product. Condensation of the known diene **78** and *in situ* prepared *ortho*-benzoquinone monoketal derived from hypervalent iodine oxidation of methylguaiacol **79** gave an inseparable mixture of **80** and **81**. Upon heating at reflux in trimethylbenzene for 48 h, **81** was converted to **80** *via* a Cope rearrangement. In the

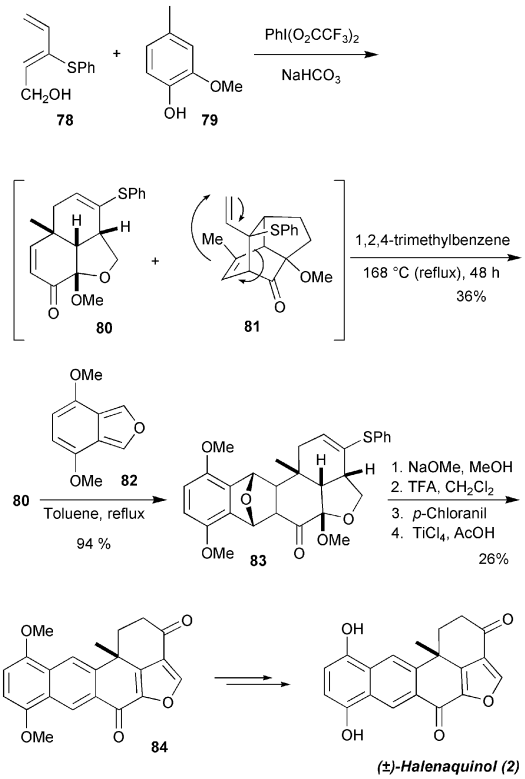


Scheme 12 A rhodium-catalyzed cyclotrimerization and a thermal electrocyclic rearrangement as key steps in the preparation of (±)-viridin.

presence of benzofuran **82**, **80** underwent a cycloaddition to the bridged Diels–Alder adduct **83**. The polycyclic compound could be aromatized by treatment with sodium methoxide in methanol at reflux. Elimination of the angular methoxy group with trifluoroacetic acid, followed by oxidation with *p*-chloranil adjusted the oxidation state of the furan ring. Hydrolysis of the marcaptan with titanium tetrachloride led to the known dimethylhydroquinone **84**,[42c] which can be converted to halenaquinone *via* oxidation, or to halenaquinol *via* subsequent reduction.

3 Conclusions

The viridin class of natural products and its quinone congeners serve as powerful reminders of the potential of natural products to bridge chemistry and biology and serve as inspiration for both fundamental and applied scientists in biomedical research.[56] Wortmannin, in particular, has a rich history of significant biological discoveries that are relevant for medicinal chemistry and drug development, and its mechanism of action is a great



Scheme 13 Two Diels–Alder reactions provide a rapid access to the pentacyclic framework of halenaquinone.

example of the ingenuity of biosynthetic evolution and natural enzymatic pathway targeting processes. Moreover, new potential biomedical applications of wortmannin and its analogs continue to be identified.[57] It is clear from a review of the synthetic endeavors in this field that chemical innovation continues to be challenged by the structural and mechanistic subtleties present in polycyclic natural products, and that our synthetic tool chest has yet to deliver the level of efficiency and practicality that would allow a *de novo* practical synthesis[58] of any viridin scaffold that could possibly match the biosynthesis[59] of these compounds.

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References

1 P. W. Brian, P. J. Curtis, H. G. Hemming and G. L. F. Norris, *Trans. Brit. Mycol. Soc.*, 1957, **40**, 365–368.
2 J. MacMillan, A. E. Vanstone and S. K. Yeboah, *J. Chem. Soc., Chem. Commun.*, 1968, 613–614.
3 (*a*) J. MacMillan, T. J. Simpson and S. K. Yeboah, *J. Chem. Soc., Chem. Commun.*, 1972, 1063; (*b*) J. MacMillan, A. E. Vanstone and S. K. Yeboah, *J. Chem. Soc., Perkin Trans. 1*, 1972, 2898–2903; (*c*) J. MacMillan, T. J. Simpson, A. E. Vanstone and S. K. Yeboah, *J. Chem. Soc., Perkin Trans. 1*, 1972, 2892–2898.
4 T. J. Petcher, H.-P. Weber and Z. Kis, *J. Chem. Soc., Chem. Commun.*, 1972, 1061–1062.
5 For a review of steroidal furans, see: J. R. Hanson, *Nat. Prod. Rep.*, 1995, **12**, 381–384.
6 (*a*) P. Wipf and A. D. Kerekes, *J. Nat. Prod.*, 2003, **66**, 716–718; (*b*) E. Sakuno, K. Yabe, T. Hamasaki and H. Nakajima, *J. Nat. Prod.*, 2000, **63**, 1677–1678.
7 D. M. Roll and P. J. Scheuer, *J. Am. Chem. Soc.*, 1983, **105**, 6177–6188.
8 H. Nakamura, J. Kobayashi, M. Kobayashi, Y. Ohizumi and Y. Hirata, *Chem. Lett.*, 1985, 713–716.
9 See also: Y. C. Shen, C. V. S. Prakash and J.-H. Guh, *Tetrahedron Lett.*, 2004, **45**, 2463–2466.

10 S. P. Maddaford, N. G. Andersen, W. A. Cristofoli and B. A. Keay, *J. Am. Chem. Soc.*, 1996, **118**, 10766–10773.
11 M. Nakamura, T. Kakuda, Y. Oba, M. Ojika and H. Nakamura, *Bioorg. Med. Chem.*, 2003, **11**, 3077–3082.
12 H. Fujiwara, K. Matsunaga, M. Saito, S. Hagiya, K. I. Furukawa, H. Nakamura and Y. Ohizumi, *Eur. J. Pharmacol.*, 2001, **413**, 37–45.
13 M. A. Lyon, A. P. Ducruet, P. Wipf and J. S. Lazo, *Nat. Rev. Drug Discovery*, 2002, **1**, 961–976.
14 S. Cao, C. Foster, M. Brisson, J. S. Lazo and D. G. I. Kingston, *Bioorg. Med. Chem.*, 2005, **13**, 999–1003.
15 Y. Zhu, W. Y. Yoshida, M. Kelly-Borges and P. J. Scheuer, *Heterocycles*, 1998, **49**, 355–360.
16 K. A. Alvi, J. Rodriguez, M. C. Diaz, R. Moretti, R. S. Wilhelm, R. H. Lee, D. L. Slate and P. Crews, *J. Org. Chem.*, 1993, **58**, 4871–4880.
17 R. Kapeller and L. C. Cantley, *BioEssays*, 1994, **16**, 565–576.
18 L. Rameh and L. C. Cantley, *J. Biol. Chem.*, 1999, **274**, 8347–8350.
19 H. Yano, S. Nakanishi, K. Kimura, N. Hanai, Y. Saitoh, Y. Fukui, Y. Nonomura and Y. Matsuda, *J. Biol. Chem.*, 1993, **34**, 25846–25856.
20 For some preliminary metabolism studies, see: J. L. Holleran, J. Fourcade, M. J. Egorin, J. L. Eiseman, R. A. Parise, S. M. Musser, K. D. White, J. M. Covey, G. L. Forrest and S.-S. Pan, *Drug Metab. Dispos.*, 2004, **32**, 490–496.
21 A. Giaccia, B. G. Siim and R. S. Johnson, *Nat. Rev. Drug Discovery*, 2003, **2**, 803–811.
22 Y. Liu, K. R. Shreder, W. Gai, S. Corral, D. K. Ferris and J. S. Rosenblum, *Chem. Biol.*, 2005, **12**, 99–107.
23 G. J. Brunn, J. Williams, C. Sabers, G. Wiederrecht, J. C. Lawrence and R. T. Abraham, *EMBO J.*, 1996, **15**, 5256–5267.
24 S. Boulton, S. Kyle, L. Yalcintepe and B. W. Durkacz, *Carcinogenesis*, 1996, **17**, 2285–2290.
25 A. Sekulic, C. C. Hudson, J. L. Homme, P. Yin, D. M. Otterness, L. M. Karnitz and R. T. Abraham, *Cancer Res.*, 2000, **60**, 3504–3513.
26 S. Nakanishi, S. Kakita, I. Takahashi, K. Kawahara, E. Tsukuda, T. Sano, K. Yamada, M. Yoshida, H. Kase, Y. Matsuda, Y. Hashimoto and Y. Nonomura, *J. Biol. Chem.*, 1992, **267**, 2157–2163.
27 S. Corvera and M. P. Czech, *Trends Cell. Biol.*, 1998, **8**, 442–446.
28 M. P. Wymann, G. Bulgarelli-Leva, M. J. Zvelebil, L. Pirola, B. VanHaese-broeck, M. D. Waterfield and G. Panayotou, *Mol. Cell. Biol.*, 1996, **16**, 1722–1733.
29 B. H. Norman, J. Paschal and C. J. Vlahos, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 1183–1186.
30 E. H. Walker, M. E. Pacold, O. Perisic, L. Stephens, P. T. Hawkins, M. P. Wymann and R. L. Williams, *Mol. Cell*, 2000, **6**, 909–919.
31 D. Wiesinger, H. U. Gubler, W. Haefliger and D. Hauser, *Experientia*, 1974, **30**, 135–136.
32 (*a*) W. Haefliger and D. Hauser, *Helv. Chim. Acta*, 1973, **56**, 2901–2904; (*b*) W. Haefliger, Z. Kis and D. Hauser, *Helv. Chim. Acta*, 1975, **58**, 1620–1628; (*c*) W. Haefliger and D. Hauser, *Helv. Chim. Acta*, 1975, **58**, 1629–1633.
33 (*a*) J. A. Dodge, H. U. Byrant, J. Kim, W. F. Matter, B. H. Norman, U. Srinivasan, C. J. Vlahos and M. Sato, *Bioorg. Med. Chem. Lett.*, 1995, **5**, 1713–1718; (*b*) B. H. Norman, C. Shih, J. E. Toth, J. E. Ray, J. A. Dodge, D. W. Johnson, P. G. Rutherford, R. M. Schultz, J. F. Worzalla and C. J. Vlahos, *J. Med. Chem.*, 1996, **39**, 1106–1111; (*c*) L. C. Creemer, H. A. Kirst, C. J. Vlahos and R. M. Schultz, *J. Med. Chem.*, 1996, **39**, 5021–5024.
34 P. Wipf, D. J. Minion, R. J. Halter, M. I. Berggren, C. B. Ho, G. G. Chiang, L. Kirkpatrick, R. Abraham and G. Powis, *Org. Biomol. Chem.*, 2004, **2**, 1911–1920.
35 N. T. Ihle, R. Williams, S. Chow, W. Chew, M. I. Berggren, G. Paine-Murrieta, D. J. Minion, R. J. Halter, P. Wipf, R. Abraham, L. Kirkpatrick and G. Powis, *Mol. Cancer Ther.*, 2004, **3**, 763–772.
36 R. Maltais, M. R. Tremblay, L. C. Ciobanu and D. Poirier, *J. Comb. Chem.*, 2004, **6**, 443–456.
37 S. Sato, M. Nakada and M. Shibasaki, *Tetrahedron Lett.*, 1996, **73**, 6141–6144.
38 T. Mizutani, S. Honzawa, S.-y. Tosaki and M. Shibasaki, *Angew. Chem., Int. Ed.*, 2002, **41**, 4680–4681.
39 C. A. Broka and B. Ruhland, *J. Org. Chem.*, 1992, **57**, 4888–4894.
40 F. E. S. Souza and R. Rodrigo, *Chem. Commun.*, 1999, 1947–1948.
41 E. A. Anderson, E. J. Alexanian and E. J. Sorensen, *Angew. Chem., Int. Ed.*, 2004, **43**, 1998–2001.
42 (*a*) H. S. Sutherland, F. E. S. Souza and R. G. A. Rodrigo, *J. Org. Chem.*, 2001, **66**, 3639–3641; (*b*) A. Kojima, T. Takemoto, M. Sodeoka and M. Shibasaki, *Synthesis*, 1998, 581–589; (*c*) N. Harada, T. Sugioka, Y. Ando, H. Uda and T. Kuriki, *J. Am. Chem. Soc.*, 1988, **110**, 8483–8487.
43 A. S. Caselli, D. J. Collins and G. M. Stone, *Aust. J. Chem.*, 1982, **35**, 799–808.
44 W. Haefliger, Z. Kis and D. Hauser, *Helv. Chim. Acta*, 1975, **58**, 1620–1628.
45 F. E. S. Souza and R. Rodrigo, *Chem. Commun.*, 1999, 1947–1948.
46 R. Carlini, K. Higgs, C. Older, S. Randhawa and R. Rodrigo, *J. Org. Chem.*, 1997, **62**, 2330–2331.
47 S. H. Lecker, N. H. Nguyen and K. P. C. Vollhardt, *J. Am. Chem. Soc.*, 1986, **108**, 856–858.
48 P. Wipf, in *Claisen Rearrangements, vol. 5*, ed. B. M. Trost, I. Fleming, and L. A. Paquette, Oxford, 1991, pp. 827–874.
49 T. M. Trnka and R. H. Grubbs, *Acc. Chem. Res.*, 2001, **34**, 18–29.
50 T. J. Donohoe, K. Blades, P. R. Moore, M. J. Waring, J. J. G. Winter, M. Helliwell, N. J. Newcombe and G. Stemp, *J. Org. Chem.*, 2002, **67**, 7946–7956.
51 R. Carlini, K. Higgs, C. Older, S. Randhawa and R. Rodrigo, *J. Org. Chem.*, 1997, **62**, 2330–2331.
52 A. Kojima, T. Takemoto, M. Sodeoka and M. Shibasaki, *Synthesis*, 1998, 581.
53 N. Toyooka, M. Nagaoka, E. Sasaki, H. Qin, H. Kakuda and H. Nemoto, *Tetrahedron*, 2002, **58**, 6097–6102.
54 H. S. Sutherland, K. C. Higgs, N. J. Taylor and R. Rodrigo, *Tetrahedron*, 2001, **57**, 309–317.
55 A. Kojima, T. Takemoto, M. Sodeoka and M. Shibasaki, *J. Org. Chem.*, 1996, **61**, 4876–4877.
56 (*a*) D. G. I. Kingston and D. J. Newman, *Curr. Opin. DrugDiscovery Dev.*, 2005, **8**, 207–227; (*b*) P. Wipf, J. T. Reeves and B. W. Day, *Curr. Pharm. Des.*, 2004, **10**, 1417–1437; (*c*) K. C. Nicolaou, T. Montagnon and S. A. Snyder, *Chem. Commun.*, 2003, 551–564.
57 For example, in stent implantation: R.-H. Zhou, T.-S. Lee, T.-C. Tsou, F. Rannou, Y.-S. Li, C. Shu and J. Y. J. Shyy, *Arterioscler., Thromb., Vasc. Biol.*, 2003, **23**, 2015–2020; or in targeting HIF-1: ref. 21.
58 I. Paterson, O. Delgado, G. J. Florence, I. Lyothier, M. O'Brien, J. P. Scott and N. Sereinig, *J. Org. Chem.*, 2005, **70**, 150–160.
59 (*a*) T. J. Simpson, M. W. Lunnon and J. MacMillan, *J. Chem. Soc., Perkin Trans. 1*, 1979, 931–934; (*b*) J. R. Hanson, M. A. O'Leary and H. J. Wadsworth, *J. Chem. Soc., Perkin Trans. 1*, 1983, 867–870; (*c*) W. S. Golder and T. R. Watson, *J. Chem. Soc., Perkin Trans. 1*, 1980, 422–425; (*d*) M. M. Blight, J. J. W. Coppen and J. F. Grove, *J. Chem. Soc., Chem. Commun.*, 1968, 1117–1118.