JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

ARTICLE

pubs.acs.org/JACS

Total Synthesis and Biological Evaluation of Pederin, Psymberin, and Highly Potent Analogs

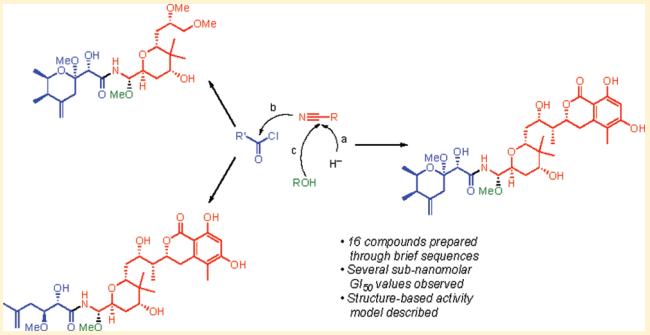
Shuangyi Wan,[†,‡] Fanghui Wu,[†,‡] Jason C. Rech,[†,§] Michael E. Green,[†,∥] Raghavan Balachandran,[⊥] W. Seth Horne,[*,†] Billy W. Day,[*,†,⊥] and Paul E. Floreancig[*,†]

[†] Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, United States

[⊥] Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, United States

Supporting Information

ABSTRACT: The potent cytotoxins pederin and psymberin have been prepared through concise synthetic routes (10 and 14 steps in the longest linear sequences, respectively) that proceed via a late-stage multicomponent approach to construct the *N*-acyl aminal linkages. This route allowed for the facile preparation of a number of analogs that were designed to explore the importance of the alkoxy group in the *N*-acyl aminal and functional groups in the two major subunits on biological activity. These analogs, including a pederin/psymberin chimera, were analyzed for their growth inhibitory effects, revealing several new potent cytotoxins and leading to postulates regarding the molecular conformational and hydrogen bonding patterns that are required for biological activity. Second generation analogs have been prepared based on the results of the initial assays and a structure-based model for the binding of these compounds to the ribosome. The growth inhibitory properties of these compounds are reported. These studies show the profound role that organic chemistry in general and specifically late-stage multicomponent reactions can play in the development of unique and potent effectors for biological responses.



INTRODUCTION

Pederin[1] (**1**) and psymberin,[2] also known as irciniastatin A,[3] (**2**) are potent cytotoxins that contain a densely functionalized tetrahydropyran subunit and an *N*-acyl aminal linkage. These structural features are also found in the mycalamide/theopederin/onnamide class of molecules, represented in Figure 1 by mycalamide A (**3**) and onnamide A (**4**).[4] While the mycalamides, theopederins, onnamides and psymberin were isolated from marine sponges, pederin was originally isolated from the *Paederus* beetle. The Piel group has explained this curious observation by demonstrating that these compounds are actually synthesized by symbiotic bacteria.[5]

Pederin was shown[6] to be a mitotic poison in 1966, but further studies on the biological activity of this family of compounds were not reported until 1989,[7] when the mycalamides were identified as potent cytotoxins that showed efficacy in vivo against leukemia and solid tumor models. This study also demonstrated that these compounds are protein synthesis inhibitors. Ogawara and co-workers showed[8] that the mycalamides change *ras*-transformed cells to normal morphology and correlated this activity to the inhibition of the synthesis of p21, a cyclin-dependent kinase inhibitor that regulates cell growth. The Kocienski group reported[9] that pederin and the mycalamides induce necrosis in squamous carcinoma cells but not in fibroblasts. Further mechanistic work[10] led to the conclusion that these compounds induce apoptosis in a number of cell lines by activating the c-Jun kinase (JNK) and the p31 mitogen-activated protein kinase.[11] Usui and co-workers showed[12] that psymberin induces a similar JNK activation, that the activation is a response to the accumulation of reactive oxygen species in the mitochondria, and that apoptosis is at least partially induced by caspase-8. Ribosome binding was demonstrated[13] by the displacement of radiolabeled 13-deoxytedanolide (independently shown to be a ribosome binding molecule) from the 60S subunit by pederin, and by the crystal structure of mycalamide A in the ribosomal binding site for the CCA end of tRNAs that occupy the E-site.[14]

The interesting biological activity and unique structures of these compounds, coupled with their scarcity from natural sources, has resulted in a number of total, formal, and partial syntheses of pederin,[15] the mycalamides,[16] and psymberin.[17] Our interest in this molecular class arose from our efforts[18] in oxidative approaches to cyclic acyl aminal formation. While this work culminated in the total synthesis of theopederin D,[16k] we were cognizant that the approach we developed to prepare the amido trioxadecalin unit in theopederin D would be ineffective for constructing the *N*-acyl aminal groups in pederin and

Received: August 4, 2011
Published: September 08, 2011

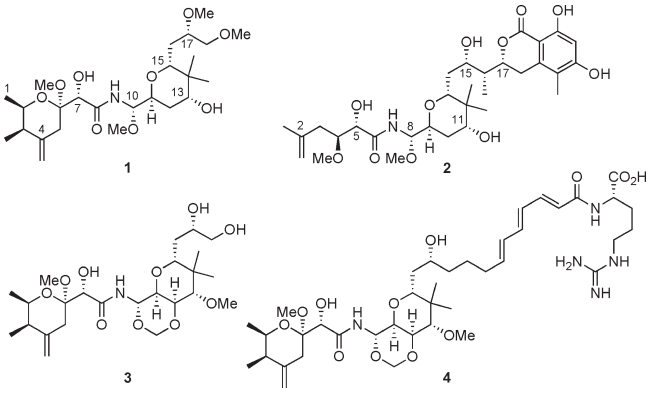
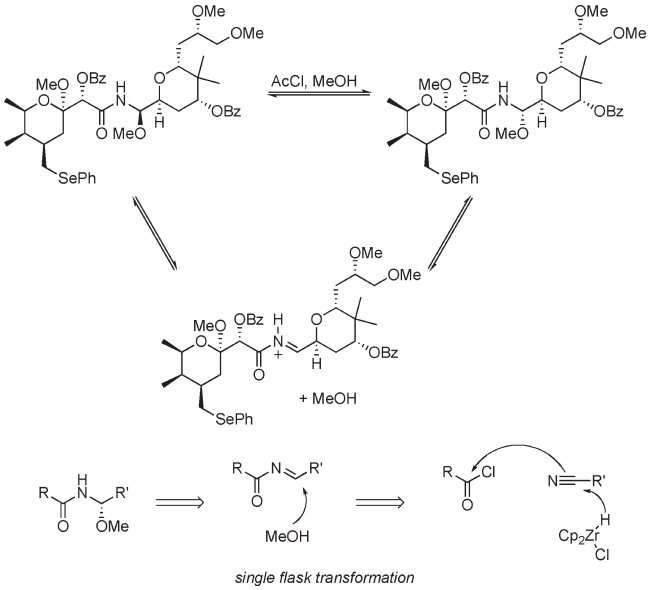


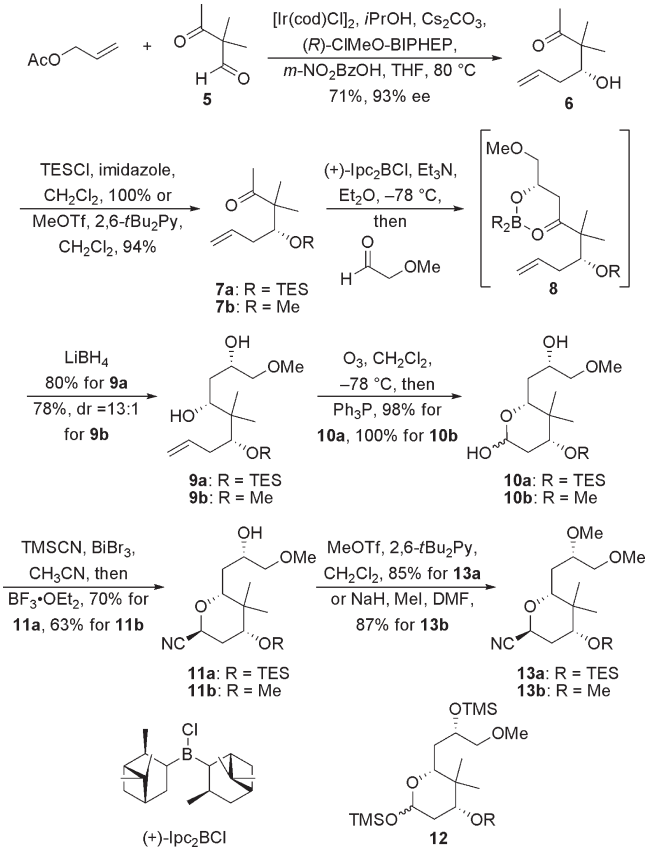
Figure 1. Cytotoxic *N*-acyl aminal-containing natural products.

Scheme 1. Matsuda's Epimerization Studies and a Multicomponent Approach to *N*-Acyl Aminal Construction



psymberin. Inspired by Matsuda's studies[15c] on acidic MeOH-mediated *N*-acyl aminal stereochemical inversion in advanced pederin intermediates, we envisioned an approach to these compounds that would proceed through a late stage addition of MeOH to an acylimine that would arise from the union of the two major subunits of the natural products. The acylimine would arise through a sequence of nitrile hydrozirconation[19] and acylation (Scheme 1).[20] We have applied[21] the multicomponent sequence of nitrile hydrozirconation, acylation, and nucleophilic addition to the synthesis of several amide structural classes. In this manuscript we provide a full account of our application of the multicomponent acyl aminal construction to the total syntheses of pederin, psymberin, and analogs. The abilities of these compounds to inhibit cell growth have been determined, and many of the analogs are extremely potent cytotoxins. The results of these assays were used to generate postulates regarding the roles that several structural features in these molecules play in causing the biological response. These postulates guide the design of second generation analogs that were synthesized and evaluated for activity.

Scheme 2. Synthesis of the Right Fragments for Pederin and Analogs



RESULTS AND DISCUSSION

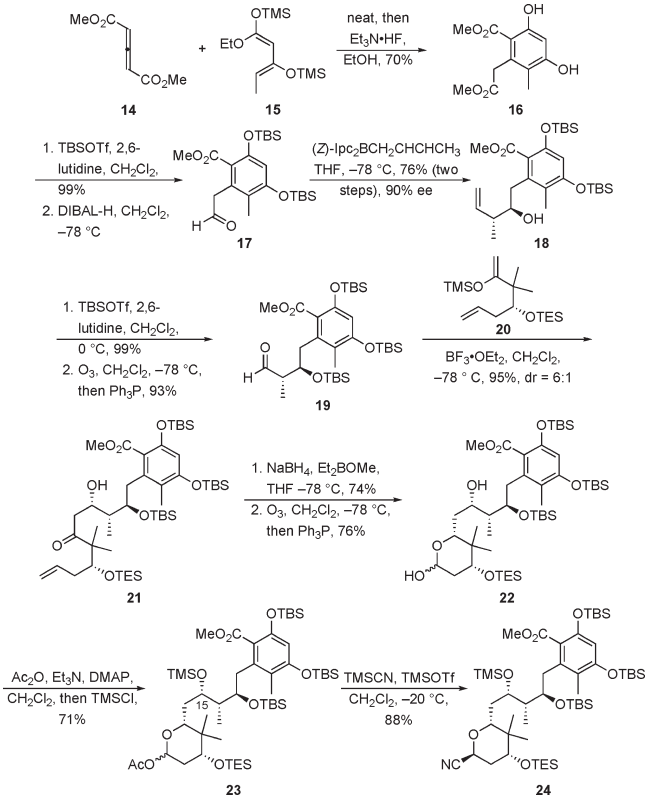
Subunit Syntheses. The synthesis of the right fragment of pederin and its analogs is shown in Scheme 2.[22] The sequence began with the asymmetric allylation of keto aldehyde **5**,[23] prepared on multigram scale from the condensation of acetyl chloride with the morpholine enamine of isobutyraldehyde. Several methods were shown to be successful for this transformation. The Leighton allylation[24] provided superb enantioselectivity and yield, though accessing sufficient quantities of the pseudoephedrine-based reagent for large scale studies proved to be difficult. The abundance of tartaric acid derivatives rendered the Roush allylation[25] a more practical alternative for large scale reactions, though the enantioselectivity was lower and reagent preparation required significant effort. The Krische allylation[26] ultimately provided an ideal solution with respect to reagent availability and reaction enantioselectivity. Exposing **5** to allyl acetate, under reductive conditions (*i*PrOH, $[Ir(cod)Cl]_2$, (*R*)-Cl,MeO-BIPHEP, Cs_2CO_3, *m*-NO_2BzOH, THF) provided alcohol **6** in 71% yield and 93% ee. Thus a key early stage intermediate that is required for the syntheses of all molecules in this study can be accessed from inexpensive precursors on multigram scale by using asymmetric catalysis. Allylation of the primary alcohol corresponding to **4** was possible under these conditions in the absence of *i*PrOH, though the overall efficiency from the aldehyde proved to be superior. Conversion of the alcohol to silyl ether **7a**, the required intermediate for the total synthesis, and to methyl ether **7b**, an intermediate for analog

dx.doi.org/10.1021/ja207331m |*J. Am. Chem. Soc.* 2011, 133, 16668–16679

synthesis, proceeded readily under standard conditions. The resulting ketones are well-suited for chain extension through aldol reactions. Numerous studies have shown that boron enolates of β-alkoxy methyl ketones react with aldehydes to provide good levels of 1,5-asymmetric induction,[27] as required for pederin synthesis. Unfortunately the diastereomeric ratios that we observed for reactions between the dibutylboron or the dicyclohexylboron enolates of **7a** and **7b** never exceeded 3:1. We therefore explored the pinene-derived boron enolates that have been reported by the Paterson group.[28] These readily prepared compounds substantially enhanced the stereoselectivity of the process to provide product diastereomeric ratios in excess of 15:1. Purification of the product from the pinene-derived by-product proved to be difficult, however, so we exploited the stability of chelated structure **8**, the initial product of the aldol reaction, to effect a stereoselective reduction with $LiBH_4$.[29] These one flask aldol/reduction reactions provided readily purified diols **9a** and **9b** in 80% yield as a single stereoisomer and 78% yield as a 13:1 mixture of stereoisomers, respectively. Although we observed low levels of stereoinduction from the β-substituent and enol diiopinocamphenylborinates from methyl ketones have been shown to react with moderate levels of control, the reenforcing effects led to very satisfying levels of stereocontrol. Ozonolytic alkene cleavage provided lactols **10a** and **10b** in excellent yield. Cyano group incorporation proceeded without converting the anomeric hydroxyl group to a leaving group or protecting the C17 by ionizing the lactol with $BiBr_3$[30] in the presence of TMSCN. Yields of nitriles **11a** and **11b** were low, however, because of the competitive formation of bis-silyl ether **12**. No reaction was observed when **12** was isolated and resubjected to the reaction conditions, indicating that the efficiency of the process was compromised by forming the bis-silyl ether or that anhydrous HBr, formed through the reaction of $BiBr_3$ with the hydroxy groups,[31] is the relevant catalyst in the early stages of the reaction. We reasoned, however, that a stronger Lewis acid would be able to induce ionization of **12**. Therefore, we added $BF_3 \cdot OEt_2$ to the reaction mixture after the initial process had occurred. This led to suitable yields of nitriles **11a** and **11b**. Directly subjecting **10a** or **10b** to $BF_3 \cdot OEt_2$ and TMSCN led to decomposition, indicating that C13 silyl ether formation through the $BiBr_3$-mediated pathway is a prerequisite for efficient cyanation. The stereochemical outcomes of thse reactions are consistent with Woerpel's studies[32] of cyclic oxocarbenium ion cyanation in acetonitrile. Methylation of the secondary alcohols completed the syntheses of multicomponent reaction substrates **13a** and **13b**. Competitive silyl transfer plagued the methylation of **13a** under standard Williamson etherification conditions (NaH, DMF, then MeI), causing us to employ MeOTf and di-*tert*-butylpyridine in CH_2Cl_2 for this transformation. The Williamson conditions were appropriate for the more robust substrate **13b**.

The synthesis of the right fragment for psymberin is shown in Scheme 3.[33] An efficient synthesis of this unit requires a rapid construction of the pentasubstituted arene. Rather than modify commercially available arenes, we chose to employ a cycloaddition-based approach to this unit based on work from the Langer group.[34] Thus, allene **14**[35] and diene **15**,[36] each available in one step from commercially available materials, were mixed in the absence of solvent to form a cycloadduct that, upon exposure to $Et_3N \cdot HF$, directly yielded **16** in 70% yield. This procedure was very attractive at this point in the synthesis because it could readily be run on large (30 g) scale. The hydroxyl groups were

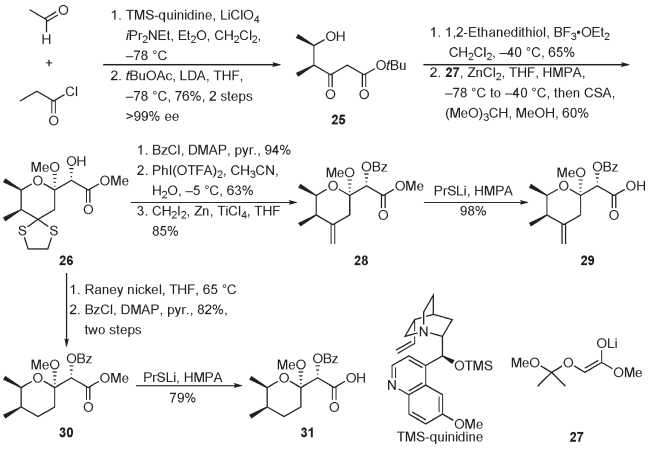
Scheme 3. Synthesis of the Right Fragment of Psymberin



protected as silyl ethers under standard conditions and the sterically less hindered aliphatic ester group reacted with one equiv of DIBAL-H to provide aldehyde **17**. A Brown crotylation[37] to yield **18** in suitable yield and enantioselectivity followed by protection of the resulting hydroxyl group as a TBS ether and ozonlytic cleavage of the alkene produced aldehyde **19**. The boron enolate of **7a** did not react with **19** smoothly, presumably as a result of enhanced steric hindrance in comparison to the reaction with methoxy acetaldehyde. Therefore we pursued an approach in which the chirality of the aldehyde controls the approach of an enolsilane nucleophile. Applying the Felkin-Anh model[38] to **19** leads to the prediction that nucleophilic addition should provide the desired 1,2-*syn*-stereochemical relationship. Evans' polar extended Felkin model,[39] however, predicts that the silyloxy group at the β-position of the aldehyde should guide nucleophiles to produce the undesired 1,3-*anti*-stereoisomer. Adding enolsilane **20**, prepared in quantitative yield from **7a** under standard conditions, to **19** in the presence of $BF_3 \cdot OEt_2$ produced aldol product **21** in excellent yield as an inseparable 6:1 mixture of diastereomers that favored the *syn*, *syn*-stereoisomer. This result was consistent with observations from the Evans group[40] that Felkin-Anh selectivity overrides β-alkoxy induction when sterically hindered enolsilanes are used as nucleophiles. The resulting hydroxy ketone was reduced with $NaBH_4$ and Et_2BOMe[41] to provide the expected diol, which could be isolated as a single stereoisomer with high *syn*-control being confirmed by forming the acetonide and analyzing its ^{13}C NMR spectrum.[42] As in Scheme 2, ozonolysis was employed to form lactol **22**. The $BiBr_3$-mediated lactol cyanation conditions from Scheme 2, however, proved to be ineffective in this system and led to substantial decomposition. The strategy of acylating both alcohols

dx.doi.org/10.1021/ja207331m |J. Am. Chem. Soc. 2011, 133, 16668–16679

Scheme 4. Synthesis of Pederic Acid and Des-methylene Pederic Acid

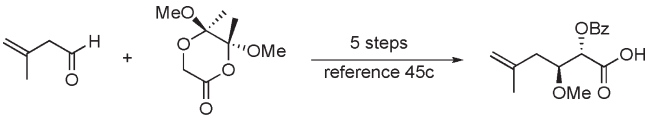


and selectively ionizing to yield the tetrahydropyranyl cation[33] worked well, but the subsequent multicomponent reaction was not compatible with the presence of the acetoxy group at C15. The observation that the anomeric hydroxyl group undergoes acylation much faster than the C15 hydoxyl group led us to conduct a selective acylation followed by trimethylsilyl ether formation at C15 to form **23**. Cyanation of **23** with TMSOTf provided **24**, the key fragment for the multicomponent reaction, in 88% yield.

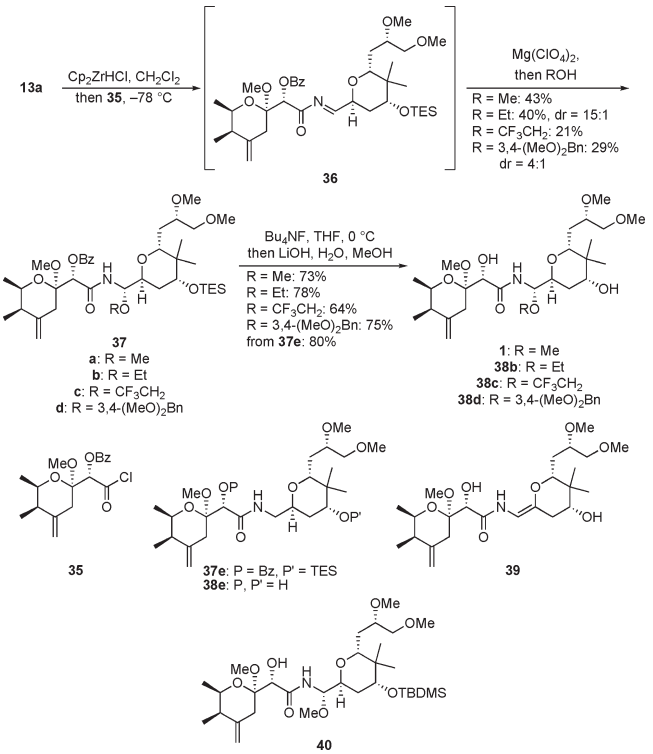
Numerous approaches to the acyl fragment of pederin, in addition to those that were developed for the total syntheses,[15,16] have been reported.[43] Asymmetric induction in these sequences invariably arose from the use of stoichiometric chiral reagents or auxiliaries, or from chiral pool starting materials. Our objective for the synthesis of this subunit was to develop a route that relies upon asymmetric catalysis to set the absolute stereochemistry.[44] We based our approach on the excellent route from the Nakata group,[43c] in which asymmetric induction arose from the use of an Evans aldol reaction. β-Lactones are effective surrogates for aldol products that can be prepared enantio- and diastereoselectively through the condensation of aldehydes with acid chlorides in the presence of cinchona alkaloid derivatives.[45] Thus acetaldehyde and propionyl chloride were combined in the presence of Et_3N, trimethylsilyl quinidine, and $LiClO_4$ to yield a volatile β-lactone that was exposed to the lithium enolate of *tert*-butyl acetate to provide keto ester **25** in 76% yield as a single stereoisomer to the limits of gas chromatographic detection. In accord with Nakata's route, **25** was converted to ester **26** through a sequence of thioacetal formation, stereoselective Claisen condensation with enolate **27**[43a] in the presence of $ZnCl_2$, and acidic methanol treatment. Benzoylation, thioacetal cleavage, and methylenation provided benzoylated pederic ester **28**. Thiolate-mediated cleavage produced acid **29**. The presence of the exocyclic alkene in the pederic acid unit causes instability toward a number of common reagents. Therefore, we prepared an analog that lacks the alkene as a potentially more versatile subunit. Desulfurization of **26** with Raney nickel followed by benzoylation provided ester **30**, which was treated with LiSPr to yield des-methylene pederic acid derivative **31** (Scheme 4).

The acyl fragment of psymberin (psymberic acid) has also been prepared through several approaches.[17,46] In consideration of our desire to minimize late stage deprotection efforts we employed

Scheme 5. Synthesis of Psymberic Acid



Scheme 6. Synthesis of Pederin and Acyl Aminal Analogs

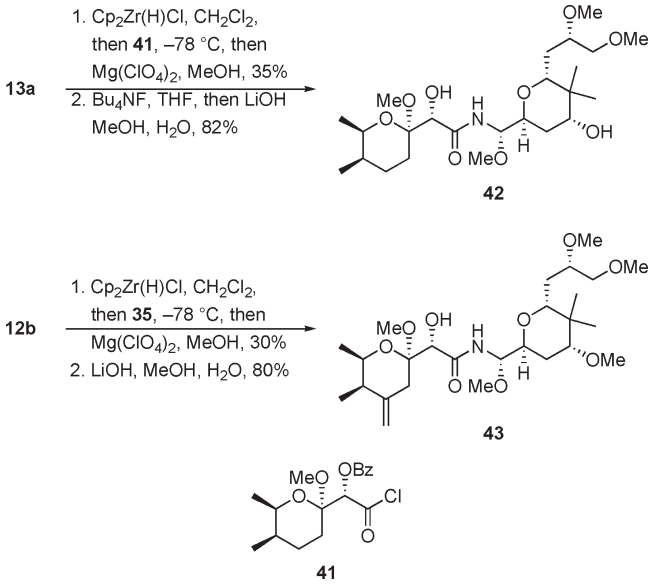


Pietruszka's exceptional route[46c] (Scheme 5) that is based on a stereoselective aldol reaction between β,γ-unsaturated aldehyde **32** and glycolic acid acetal **33**.[47] This route provided abundant quantities of acid **34** in which the hydroxyl group at C5 was protected as a benzoate ester, in accord with our projected final deprotection strategy.

Fragment coupling and total synthesis. The fragment coupling in the pederin series is shown in Scheme 6. Hydrozirconation of nitrile **13a** with $Cp_2Zr(H)Cl$ followed by the addition of acid chloride **35**, freshly prepared from **29** prior to the reaction and used without purification, resulted in the transient formation of acylimine **36**. The addition was conducted at −78 °C due to the propensity of **36** to undergo tautomerization to form a stable enamide product. Competitive tautomerization was not observed in simpler systems, even when a tetrahydropyranyl nitrile was used as the substrate.[21a] Adding structurally simpler acid chlorides to the hydrozirconation product of **13a** also promoted the formation enamide products, indicating that tautomerization can be attributed to structural elements in the nitrile component. $Mg(ClO_4)_2$ was added to lock the acylimine conformation by chelation between the nitrogen of the imine and the oxygen of the tetrahydropyran, thereby promoting nucleophilic attack through the desired, less hindered trajectory. Alcohol addition led to the

dx.doi.org/10.1021/ja207331m |J. Am. Chem. Soc. 2011, 133, 16668–16679

Scheme 7. Variations in the Left and Right Fragments of Pederin



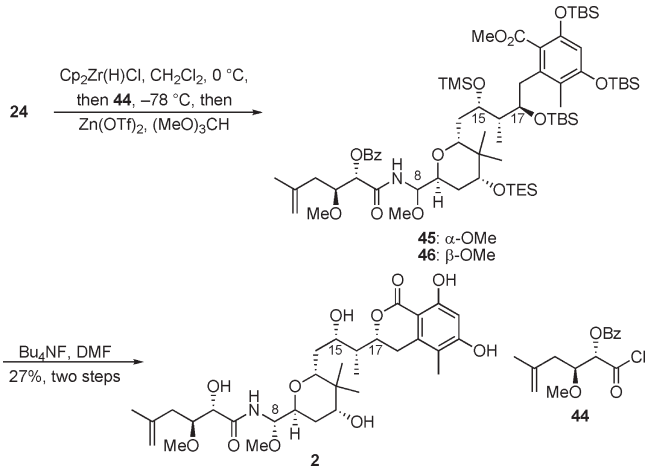
desired acyl aminals **37a**–**d**. Ethanol and trifluoroethanol were selected as nucleophiles to determine whether the ease of acyl aminal ionization, a proposed mechanism of action for these species,[16a] would cause a difference in biological activity for structurally similar species. Dimethoxybenzyl alcohol was selected because it provides potential access to an acyl hemiaminal analog that should be a biosynthetic precursor to pederin. Water could be used directly as a nucleophile in the multocomponent reaction but the resulting *N*-acyl hemiaminal was not stable toward the final deprotection conditions. An additional benefit of the dimethyoxybenzyl analog is that the final product will provide information on the tolerance of the binding site for larger groups that could provide useful handles for probe development. The yields for these reactions were moderate but the stereocontrol was good to excellent and products were readily isolated in sufficient quantities to complete the syntheses and conduct biological evaluations. Reduction of acylimine **36** by residual $Cp_2Zr(H)Cl$ to form amide **37e** was a side reaction in each of these reactions, allowing us to accrue sufficient quantities of the amide for subsequent studies. The syntheses of pederin and analogs were completed by a one flask protocol that was inspired by Rawal's synthesis[15h] whereby silyl group cleavage was effected by Bu_4NF in THF followed by the addition of aqueous LiOH and MeOH to cleave the benzoate ester. Pederin (**1**) and analogs **38b**–**e** were prepared through this route. The efficiency of the process was high for all substrates except **37c**, in which the basic conditions promoted the elimination of the trifluoroethoxy group to form enamide analog **39** in 29% yield as a side product. The geometry of the enamide group was confirmed by NOESY experiments.[22] We isolated silyl ether **40**, which was resistant toward cleavage, when the sequence was conducted with a TBDMS ether at C13 rather than a TES ether. The longest linear sequence for these syntheses was 10 steps, and the overall sequence required 18 steps. Pederin was prepared in 10.4% overall yield from keto aldehyde **5**.

Through related sequences (Scheme 7) **13a** was coupled with acid chloride **41**, prepared from **31** immediately prior to coupling, to form des-methylene pederin analog **42**. Nitrile **13b** was converted to methyl ether analog **43**. The syntheses of these analogs demonstrates late stage that the multicomponent assembly of the acyl aminal group can be used to introduce structural variations in all sections of the natural product, thereby greatly facilitating structure–activity relationship studies.

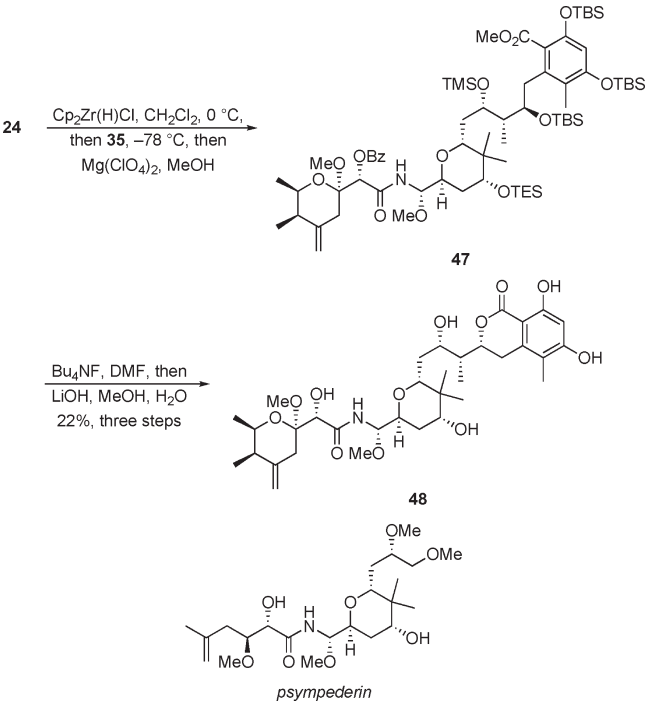
The multicomponent approach to the acyl aminal of psymberin proved to be much more challenging than the corresponding reaction in the pederin series. Difficulties in controlling the stereochemical outcome of the reaction could be predicted based on De Brabander's approach to a pederin/psymberin hybrid strucure[48] in which the approach of $NaBH_4$ to acylated imidate intermediates that are structurally related to the acylimines in our procedure proceeded with opposite trajectories for the pederin and psymberin subunits. This study provided further evidence[49] that remote interactions can exert a strong influence over the reactivity at the acyl aminal site. Exposing nitrile **24** to $Cp_2Zr(H)Cl$, acyating with acid chloride **44**, and adding MeOH provided diastereomeric acyl aminals **45** and **46**, in a 1:3 ratio in which the undesired stereoisomer was the major product. Adding one equivalent of $Mg(ClO_4)_2$ improved the ratio to 1:2. Increasing the equivalents of $Mg(ClO_4)_2$ incrementally increased the stereocontrol, with 2 equivalents providing a 1:1 ratio and 10 equivalents leading to a 3:1 ratio, albeit at the expense of the overall efficiency of the reaction. We hypothesized that replacing MeOH with a less reactive or bulkier surrogate would improve the stereoselectivity. Conducting the addition with 2 eq $Mg(ClO_4)_2$ and $(MeO)_3CH$ as the source of the methoxy group provided a 3:1 mixture of **45** and **46**, as determined by crude ^1H NMR, though the yield for the transformation was low (~20%). Changing the Lewis acid to $Zn(OTf)_2$ resulted in lower stereocontrol (~1.5:1) but higher overall efficiency. For simplicity the crude mixture was carried to the next step without purification. Exposing the acyl aminal mixture to Bu_4NF in DMF led to the cleavage of all silyl groups, formation of the dihydroisocoumarin, and removal of the benzoate to yield psymberin in 27% yield along with 8-*epi*-psymberin in 12% yield for the two steps. The benzoate cleavage most likely resulted from the generation of Bu_4NOH during the cleavage of the silyl ethers. The identity of the protecting group at C15 proved to be critical for the success of this transformation. Cleavage at of the silyl ether at C17 was not observed when the C15 oxygen was protected as an acetate or a bulkier silyl ether. We postulate that the cleavage of the labile C15 TMS ether allows for intramolecular silyl transfer form C17. Deprotection of the resulting C15 silyl ether then leads to the final product. Thus psymberin is available from commercially available materials through a route that is only 14 steps in its longest linear sequence (28 steps total) and proceeds in an overall yield of 4.4% (Scheme 8). As with the pederin synthesis the modest efficiency of the multicomponent reaction did not impede further studies since tens of milligrams of the natural product can be readily accessed.

The availability of the left and right fragments for pederin and psymberin led us to undertake the synthesis of chimeric structures. Psympederin, a pederin/psymberin chimera that contains the left fragment of psymberin and the right fragment of pederin, was prepared and shown to be far less cytotoxic than either pederin or psymberin.[48] This led the authors to conclude that pederin and psymberin do not share a common biological target. While this conclusion is perfectly reasonable, we posited an alternative explanation in which the dihydroisocoumarin group in psymberin contributes additional binding affinity to compensate

Scheme 8. Completion of the Psymberin Synthesis



Scheme 9. Synthesis of the Pederin–Psymberin Chimera Pedestatin



for a reduced binding contribution from the less-organized acyl fragment. Therefore we prepared a new analog that contains the left fragment of pederin and the right fragment of psymberin (Scheme 9). The multicomponent reaction employed nitrile **24** and acid chloride **35**. MeOH was a suitable nucleophile in this reaction since stereocontrol did not prove to be a problem when 2 equivalents of $Mg(ClO_4)_2$ were employed. Crude product **47** was subjected to Bu_4NF in DMF to cleave the silyl ethers and form the dihydroisocoumarin unit. The benzoate ester was only partially cleaved under these conditions, in contrast to our observations with the less hindered ester in psymberin. The crude mixture was treated with LiOH in MeOH and H_2O to complete the synthesis of chimera **48** that we have named pedestatin (pederin + irciniastatin).[50] The statin suffix was selected as an homage to the remarkably influential contributions from the Pettit group to the isolation and development of antineoplastic agents.

Table 1. GI_{50} Values of the Natural Products and Analogs against HCT116 Cells

entry	compound	description	GI_{50} (nM)
1	**1**	pederin	0.6 ± 0.1
2	**38b**	10-ethoxy pederin	0.34 ± 0.1
3	**38c**	10-trifluoroethoxy pederin	0.55 ± 0.02
4	**38d**	10-dimethoxybenzyloxy pederin	0.32 ± 0.09
5	**38e**	10-desmethoxy pederin	7.7 ± 0.2
6	**39**	pederin enamide	27 ± 0.9
7	**42**	C4-desmethylene pederin	6.5 ± 0.8
8	**43**	13-*O*-methyl pederin	0.084 ± 0.01
9	**40**	13-OTBS pederin	3.1 ± 0.1
10	**2**	psymberin	0.052 ± 0.02
11	**48**	pedestatin	0.004 ± 0.003[a]

[a] Result of averaging two independent experiments.

Cytotoxicity Studies. The potency of these compounds was measured in cell viability studies using HCT-116 colon cancer cells by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl-2H-tetrazolium) dye reduction assay with PMS (phenozine methosulfate) as an electron acceptor. The assays were conducted over a three day incubation period at 37 °C and were run in triplicate or quintuplicate. Growth inhibition (GI) was calculated as defined by the National Cancer Institute ($GI = 100(T - T_0)/(C - T_0)$, where T_0 = cell density at time zero, T = cell density after 72 h with psymberin, and C = cell density at 72 h with vehicle). The results are shown in Table 1.

These studies showed that all of the compounds in this series are potent antiproliferative agents. The identity of the alkoxy group in the acyl aminal subunit appears to exert little influence over the biological activity, but the presence of the alkoxy group enhances potency by at least 1 order of magnitude (entries 1–4 vs entry 5). The reasonable potency of desmethoxy analog **38e** suggests that acyl aminal ionization is not a requirement for biological activity. The most potent pederin analog contains a methyl ether at the C13 position rather than a hydroxyl group, suggesting that reducing hydrophilicty at that site is beneficial to activity. This has also been observed in psymberin analogs that lack a substituent at that position.[51] Dramatic increases to the size and hydrophobicity at the C13 position proved to be modestly detrimental to activity, as seen in the TBS ether analog **40** in entry 9. Remarkably the enamide analog also showed reasonable activity (entry 6) despite the structural changes that result from the elimination reaction. The approximately 1 order of magnitude potency reduction of the des-methylene analog (entry 7), though consistent with results from the Nakata group,[52] was somewhat surprising based on the minimal structural perturbation that this change effects on the hydrogen bond donors and acceptors in the molecule. Psymberin is more potent than pederin (entries 1 and 10). While the GI_{50} value that we observed was somewhat lower than the value in the literature for this cell line (0.16 nM)[53] the values were within a factor of 3 and were consistent over several assays. The primary data are shown in Figure 2. Chimera **48** is even more potent than psymberin (entry 11),

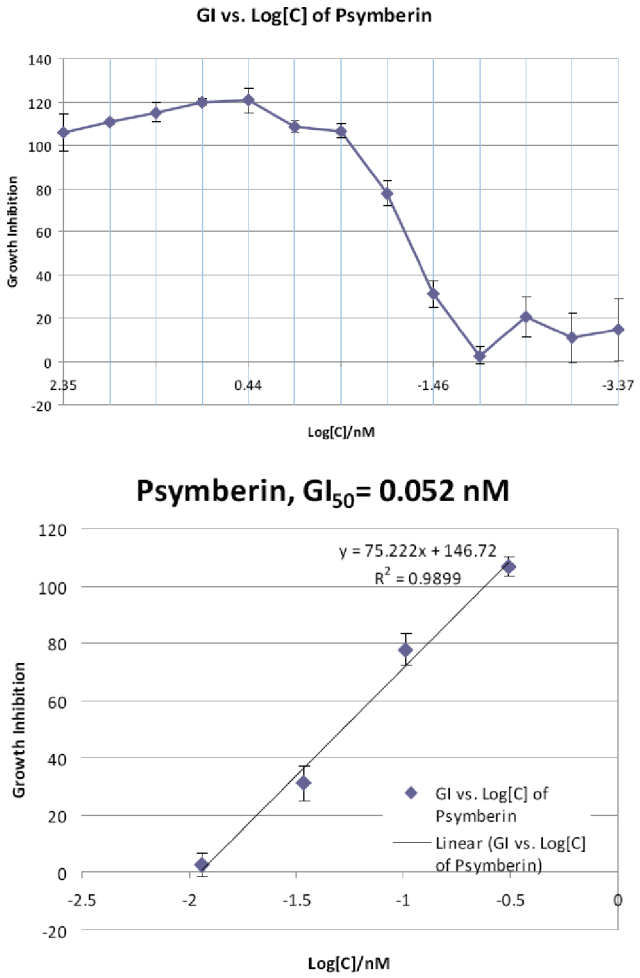


Figure 2. Growth inhibition data for psymberin against HCT-116 cells. (Top) Growth inhibition vs log[psymberin], where [psymberin] is reported as nM. (Bottom) Growth inhibition in the responsive range of concentrations.

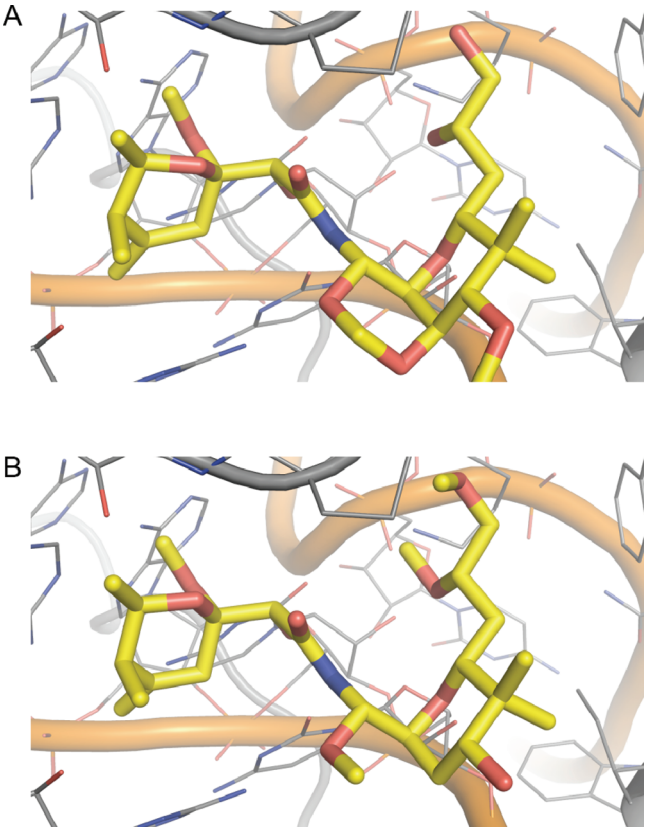


Figure 3. Binding models of mycalamide A and pederin bound to the ribosome. (A) Revised structure of mycalamide A based on a reinterpretation of the electron density from the crystal structure. (B) Modeled structure of pederin derived from mutating the structure of mycalamide A.

showing a 4 pM GI_{50} value. In addition to the remarkable potency, this result is quite significant in consideration of De Brabander's results[47] showing that a chimera containing the left fragment of psymberin and the right fragment of pederin is not a potent cytotoxin. Our results suggest that pederin and psymberin share a common binding site on the ribosome and that the left fragment of pederin and the right fragment of psymberin are the essential components for the biological activity of these natural products. Compounds **1**, **38b**−**e**, **39**, **42**, and **43** were tested against a p53 knockout variant of the HCT116 cell line[54] and showed essentially identical activity. These results indicate that the apoptotic pathway is not dependent on p53.

Structural Basis for Biological Activity. The published crystal structure of the complex between mycalamide A (**3**) and the large ribosomal subunit[14] provides an opportunity to identify structural bases for the differences in biological activity among the molecules reported here. Such analysis should prove to be valuable in the design of second-generation analogs with superior activity or greater accessibility. Given the fact that the structures of mycalamide A and pederin are closely related, we reasoned that the two natural products share similar binding modes. The coordinates published for mycalamide A in complex with the ribosome[14] differ from the natural product by epimerization at C10. Since the prior study reported that material of natural origin was used in crystallization,[14] we reinterpreted the published electron density using coordinates for the correct diastereomer (see Supporting Information). With the refined structure of the mycalamide A/ribosome complex in hand (Figure 3A), pederin was docked into the binding site by superposition of the backbone atoms shared between the two molecules (Figure 3B). Analysis of the resulting model provides several insights into the structural basis for the biological activity of a variety of natural *N*-acyl aminal-containing cytotoxins and their synthetic analogs.

The potency of 10-desmethoxy pederin (**38e**) was somewhat surprising in consideration of reports that related compounds that lack oxygenation[55] or are epimeric at C10[9] show significantly diminished activity. An examination of the bioactive conformation of pederin in the bound structure suggests multiple roles for oxygenation at C10 (Figure 4). Foremost, the C10 alkoxy group promotes a conformation about the C10−C11 bond that matches the ribosome-bound structure by forcing the less bulky C10-hydrogen to occupy the position over the tetrahydropyran ring (Figure 4A). Evidence for this conformational preorganization in solution is provided by the large coupling constant (~8 Hz) between the C10 and C11 hydrogens in the ^{1}H NMR spectrum of pederin. Epimerization at C10 forces the amide group into an unproductive binding orientation to relieve the energetic penalty of placing the methoxy group over the tetrahydropyran ring. The 10-deoxy compound lacks a conformational bias around the C10−C11 bond. This loss of conformational preorganization is a

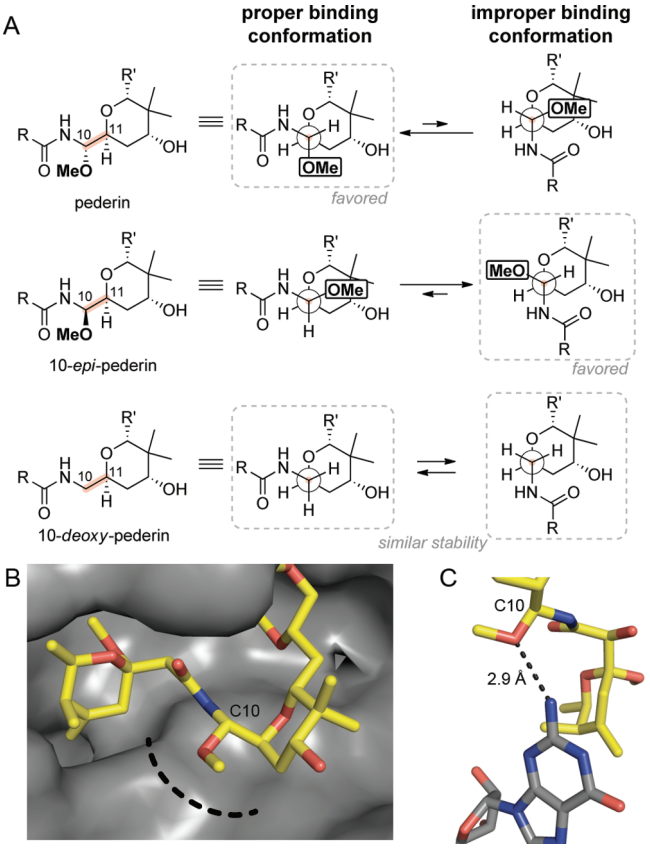


Figure 4. C10 methoxy group in pederin plays multiple roles in ribosome binding. (A) C10 substituent acts as a conformational control element for rotation about the C10−C11 bond; (B) pederin binding pocket has space to accommodate larger substituents at C10; (C) C10 oxygen forms a hydrogen-bond to a nearby ribosome nucleobase.

likely contributor to the weaker activity observed for 10-desmethoxy pederin compared to the natural product.

Two other features around C10 in the model for pederin bound to the ribosome are noteworthy. The binding region for the C10 methoxy group appears capable of accommodating larger substituents (Figure 4B), in accord with our observation that larger alkoxy groups do not reduce activity. The structure also shows a hydrogen bond between the oxygen at C10 and the amino group of a nearby guanine residue (Figure 4C). Thus, the role of the alkoxy group may transcend simple conformational restriction by providing direct contacts that enhance binding affinity. Collectively, the results of our structural analysis provide a picture of the role of the C10 substituent that is consistent with available biological data for pederin and its analogs. Closer examinations of the known natural products that lack oxygen at the C10 position reveal the presence of other structural perturbations that are the likely source of their diminished biological activity.

The origin of the strong influence of the pederic acid component on pederin bioactivity, and the exocyclic alkene in particular, can be proposed based on the bound structure. In the model of the pederin/ribosome complex, the exocyclic alkene of the pederic acid ring intercalates between adjacent adenine and guanine residues in the binding pocket (Figure 5). We propose that this interaction acts as an anchor that organizes other functional groups of the acyl fragment into a suitable binding orientation. Deletion of the exocyclic methylene (as in **42**) or

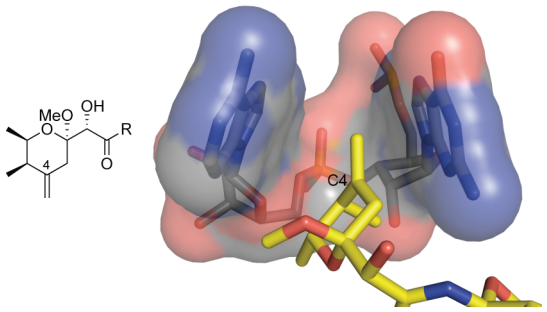


Figure 5. Exocyclic alkene at C4 in the left-hand fragment of pederin intercalates between two sequential bases in the ribosome.

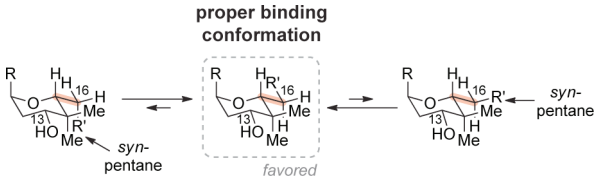


Figure 6. Geminal methyl groups in the right-hand tetrahydropyran ring in pederin restrict rotation about C15−C16 and thereby the orientation of the branched side chain (R′).

removal of ring conformational restraints (as in psymberic acid) attenuates the binding contribution of the acyl fragment and reduces activity. The pocket between the nucleobases appears sufficiently large to accommodate the products of exocyclic alkene reduction, which have been shown[56] to retain cytotoxic activity.

The lack of hydrogen-bonding contacts involving the C13 hydroxyl group suggests that it serves no role in ribosome binding. This is consistent with results from the Schering group[51] showing that deoxygenation at the corresponding position of psymberin actually increased activity. Replacement of the C13 hydroxyl group in pederin with a methyl ether led to the highly cytotoxic analog **43**. Taken together, these observations suggest that increasing the hydrophobicity at C13 could be a general strategy to enhance potency in this family of natural products.

We propose that the geminal methyl groups at C14 set the orientation of the branched side chain projecting off the right fragment of pederin (Figure 6). Conformational constraint of the C15−C16 bond arises from the minimization of the *syn*-pentane interactions that would be present in conformations that are not relevant for binding. The existence of this conformational constraint in solution is supported by observed ^{1}H NMR coupling constants of 10.4 and 1.8 Hz for the C15 hydrogen that indicate a rigid conformation with one *anti* and one *gauche* relationship to the hydrogens on C16. Thus, the tetrahydropyran ring appears to serve as a scaffold to align the left and right arms into the proper binding orientation.

Although we have no information regarding the bound conformation of psymberin, we note the presence of a sizable cavity adjacent to the end of the right fragment of pederin (Figure 7) that could accommodate the dihydroisocoumarin group of psymberin and the long chain of the onnamides.[4b,e] Further crystallographic studies are required to determine the precise structural basis for the potency enhancement that the dihydroisocoumarin group effects.

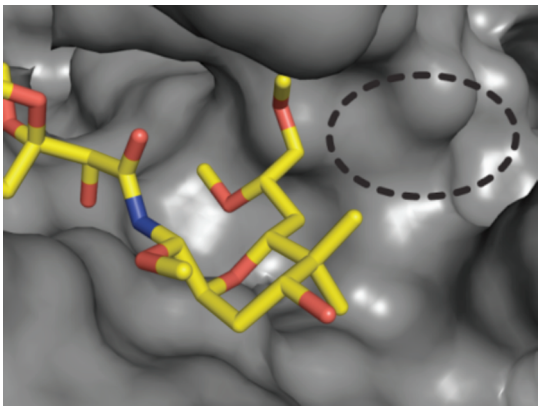
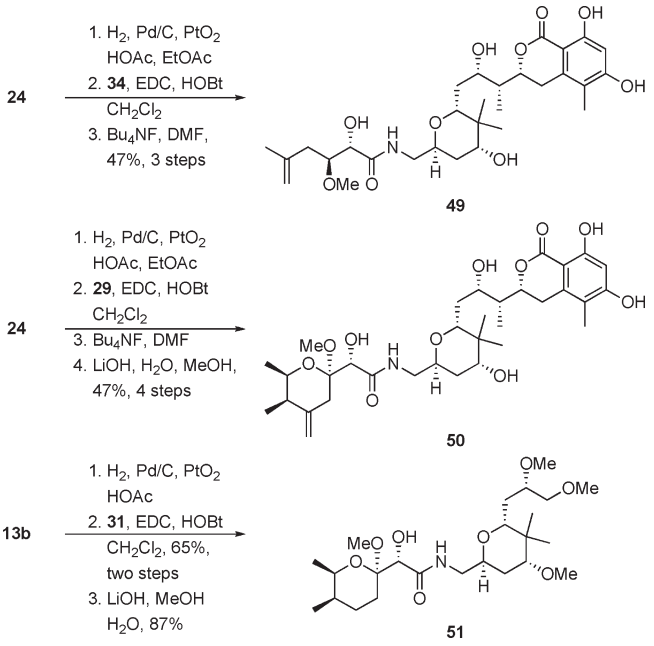


Figure 7. Unoccupied pocket adjacent to the right-hand fragment of pederin suggests a possible basis for the enhanced efficacy of psymberin and pedestatin.

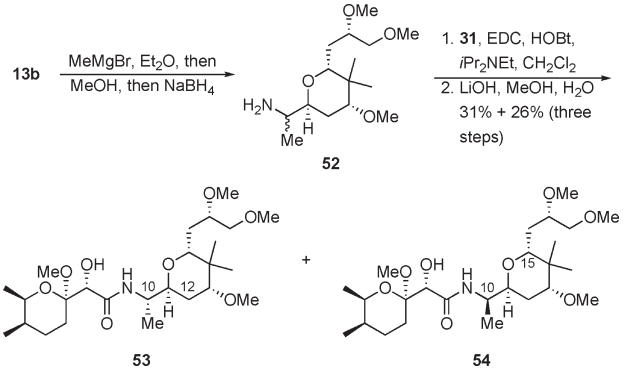
Scheme 10. Synthesis of Desmethoxy Analogs



Second Generation Analogs. The results from the initial round of biological assays and the structural details that were gleaned from the modeling studies provide a basis for the design and synthesis of a second wave of analogs. These compounds were developed to test hypotheses regarding ribosome binding and/or to facilitate synthesis.

The respectable activity of C10-desmethoxy analog **38e** led us to consider other analogs that lack oxygenation at the C10 site (C8 site of psymberin). Since **38e** was formed from side-products of the multicomponent reaction we changed the route so that the nitrile was reduced to a primary amine through catalytic hydrogenation followed by conventional amide coupling (Scheme 10). The reduction of nitrile **24** proceeded with H_2 (1 atm) in the presence of a mixture of Pd/C and PtO_2 in HOAc and EtOAc[57] to provide the corresponding amine. Coupling the crude amine to psymberic acid derivative **34** with EDC and HOBt led to the amide, which was treated with Bu_4NF in DMF to yield 8-desmethoxy psymberin, **49**. The yield was 47% for the three step sequence in which only one quick filtration through silica gel was required. A similar sequence was executed with pederic acid derivative **30**. An additional step was required to complete the cleavage of the sterically hindered benzoate group, similar to the synthesis of chimera **48**. Through this route 10-desmethoxy pedestatin **50** was prepared, again in 47% overall yield and with minimal purification. We also constructed a pederin analog that lacks the exocyclic alkene at C4 and the C10 methoxy group to facilitate synthesis and handling, and contains the C13 methyl ether to compensate for the potency loss that these changes would accrue. Reduction of nitrile **12b** followed by coupling of the resulting amine with desmethylene pederic acid derivative **31** in the presence of EDC and HOBt provided the desired amide in 65% yield. Cleavage of the benzoate group provided the desired analog in 87% yield.

Scheme 11. Synthesis of Alkyl Branched Amide Analogs



The final set of analogs was designed to test our hypotheses regarding the importance of conformation constraint and a hydrogen bonding interaction between the C10 methoxy group and the ribosome. This was achieved by replacing the methoxy group with a methyl group (Scheme 11). Exposing nitrile **13b** to MeMgBr followed by quenching with MeOH and reducing the resulting imine with $NaBH_4$[58] provided amine **52** as an inseparable 1.5:1 mixture of diastereomers (as determined by NMR). Acylation of the mixture with acid **31** followed by benzoate cleavage with LiOH provided methyl analogs **53** and **54** in 31 and 26% yields, respectively, for the three-step sequence. The stereochemical assignments for these structures were based on the observation of a cross peak between the C10 methyl group and the equatorial hydrogen of C12 in the NOESY spectrum of **53** and between the C10 methyl group and the C15 hydrogen in the NOESY spectrum of **54**.

Biological Evaluation of Second Generation Analogs. The second generation analogs were evaluated for their ability to inhibit HCT116 cell growth through the same protocol that was used to evaluate the initial set of compounds. The results are shown in Table 2.

The results of these studies revealed some highly valuable information. The potency of the psymberin and pedestatin cores is sufficient to provide extremely strong cytotoxicity even in the absence of the *N*-acyl aminal subunit. These results could be useful if large scale preparation of these agents is desired since catalytic nitrile hydrogenation is more economical and experimentally facile than the nitrile hydrozirconation sequence. The significantly improved activity of pederic acid-containing analog **50** in comparison to psymberic acid-containing analog **49** again

Table 2. GI_{50} Values for Second Generation Analogs against HCT116 Cells

entry	compound	description	GI_{50} (nM)
1	**49**	8-desmethoxy psymberin	0.83 ± 0.1
2	**50**	10-desmethoxy pedestatin	0.068 ± 0.02
3	**51**	4-desmethylene-10-desmethoxy 13-*O*-methyl pederin	79 ± 8
4	**53**	4-desmethylene-10-(*S*)-methyl 13-*O*-methyl pederin	42 ± 5
5	**54**	4-desmethylene-10-(*R*)-methyl 13-*O*-methyl pederin	>9000

highlights the importance of structural organizization in this part of the molecule. Additional evidence for this point is the loss of activity for **51**. Removing two structural elements that promote ribosome binding amplified the detrimental effects, even in the presence of the activity-enhancing C13 methyl ether. C10-Methyl ether analogs **53** and **54** show the essential role that the conformational organization that was illustrated in Figure 4 plays in biological activity. The 10-(*S*) isomer **53** shows slightly enhanced activity in comparison to the desmethoxy analog **51** while the 10-(*R*) isomer **54** is completely inactive because of the energetic penalty that results from placing the methyl group over the tetrahydropyran ring in the biologically active conformation. The diminished activity of **53** in comparison to C4-desmethylene pederin (**42**) also shows that either the hydrogen bond to the guanine residue in the ribosome (Figure 4) adds to the activity, that a steric clash between the alkyl group and this residue diminishes activity, or both. Regardless of the exact origin of the differential activity, these results suggest that the indirect biosynthetic sequence of oxidizing a glycine subunit to an *N*-acyl aminal as opposed to using an α-alkyl amino acid subunit provides the producing organism with a superior defensive agent. The question of whether the enhanced activity resulted from evolutionary pressure or through chance must remain open to speculation.

CONCLUSIONS

The hydrozirconation-initiated multicomponent assembly of nitriles, acid chlorides, and alcohols into acyl aminals serves as a versatile reaction that allows for the synthesis of pederin, psymberin, and several analogs. The linear sequences for for pederin (10 steps) and psymberin (14 steps) are the shortest reported synthetic routes to these well-studied natural products. The brevity of these sequences can in part be attributed to the facility by which the nitrile intermediates can be prepared. Employing the multicomponent reaction as the penultimate step in diverted total synthesis[59] sequences provides unprecedented access to analogs where the effects of variations in the acyl-, nitrile-, and alcohol-derived fragments on biological activity could be studied. These analogs include the pederin/psymberin chimeric structures that we have named the pedestatins.

Biological assays showed several important structure−activity trends including: (1) the pederic acid subunit confers greater activity on compounds than the psymberic acid subunit, (2) the identity of the alkoxy group in the *N*-acyl aminal linkage can be altered significantly, but activity is diminished when the alkoxy group is replaced by a hydrogen atom, (3) enhancing the hydrophobicity at the C13 site of pederin (C11 site of psymberin) increases activity, (4) the dihydroisocoumarin group of psymberin yields stronger activity than the structurally simpler righthand fragment of pederin, and (5) combining the most potent fragments of pederin and psymberin produces exceedingly powerful antineoplastic agents. Initial studies show that the chimeric structure pedestatin is among the most potent cytotoxins that have been recorded, including the natural product spongistatin-1[60] and the synthetic agent meayamycin B.[61]

The biological results were analyzed in conjunction with a model for the complex between pederin and the ribosome that was derived from a crystal structure between the structurally related natural product mycalamide A and the ribosome. This analysis identified the structural basis for several of our observations and showed that the main function of the central tetrahydropyran ring is to act as a scaffold to project the left and right fragments of the molecules into the proper binding orientations. Second generation analogs were designed from our initial conclusions and showed that compounds in which the C10/C8 alkoxy group is deleted are still quite potent when they contain the appropriate left and right fragments. Additionally replacing the C10 alkoxy group of pederin with a methyl group causes a reduction in activity as a result of losing a hydrogen-bonding interaction, accruing a steric interaction, or both. This study highlights the uniquely powerful role that chemical synthesis in general and specifically late stage multicomponent reactions can play in understanding the structural basis for and improving upon the biological activity of natural products and other medicinal agents.

ASSOCIATED CONTENT

Supporting Information. Experimental procedures, compound characterization data, and spectra for all new compounds, experimental procedures for cytotoxicity studies, and protocols for molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

florean@pitt.edu, bday@pitt.edu, horne@pitt.edu.

Present Addresses

[§]Johnson & Johnson, Pharmaceutical Research and Development, 3210 Merryfield Row, San Diego, CA 92121

[∥]Pfizer Global Research and Development, Groton, CT 06340

Author Contributions

[‡]These authors contributed equally to this work.

ACKNOWLEDGMENT

This work was supported by generous funding from the National Science Foundation (CHE-0848299, P.E.F.) and the University of Pittsburgh (W.S.H.) We thank Professor Kazunori Koide and Mr. Sami Osman (University of Pittsburgh) for assistance in the cytotoxicity assays and the National Institutes of Health (S10RR023404) for the 700 MHz NMR at the University of Pittsburgh.

REFERENCES

(1) (a) Pavan, M.; Bo, G. *Physiol. Comp. Oecol.* **1953**, *3*, 307. (b) Cardani, C.; Ghiringhelli, D.; Mondelli, R.; Quilico, A. *Tetrahedron Lett.* **1965**, *6*, 2537. (c) Furusaki, A.; Watanabé, T.; Matsumoto, T.; Yanagiya, M. *Tetrahedron Lett.* **1968**, *9*, 6301.

(2) Cichewicz, R. H.; Valeriote, F. A.; Crews, P. *Org. Lett.* **2004**, *6*, 1951.

(3) Petit, G. R.; Xu, J.; Chapuis, J.; Petit, R. K.; Tackett, L. P.; Doubek, D. L.; Hooper, J. N. A.; Schmidt, J. M. *J. Med. Chem.* **2004**, *47*, 1149.

(4) (a) Perry, N. B.; Blunt, J. W.; Munro, M. H. G.; Pannell, L. K. *J. Am. Chem. Soc.* **1988**, *110*, 4850. (b) Sakemi, S.; Ichiba, T.; Kohmoto, S.; Saucy, G.; Higa, T. *J. Am. Chem. Soc.* **1988**, *110*, 4851. (c) Perry, N. B.; Blunt, J. W.; Munro, M. H. G.; Thompson, A. M. *J. Org. Chem.* **1990**, *55*, 223. (d) Fusetani, N.; Sugawara, T.; Matsunaga, S. *J. Org. Chem.* **1992**, *57*, 3828. (e) Kobayashi, J.; Itagaki, F.; Shigemori, H.; Sasaki, T. *J. Nat. Prod.* **1993**, *56*, 976. (f) Tsukamoto, S.; Matsunaga, S.; Fusetani, N.; Toh-e, A. *Tetrahedron* **1999**, *55*, 13697.

(5) (a) Piel, J.; Butzke, D.; Fusetani, N.; Hui, D.; Platzer, M.; Wen, G.; Matsunaga, S. *J. Nat. Prod.* **2005**, *68*, 472. (b) Piel, J.; Hui, D.; Wen, G.; Butzke, D.; Platzer, M.; Fusetani, N.; Matsunaga, S. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 16222. (c) Piel, J.; Wen, G.; Platzer, M.; Hui, D. *Chembiochem* **2004**, *5*, 93. (d) Fisch, K. M.; Gurgui, C.; Heycke, N.; van der Sar, S. A.; Anderson, S. A.; Webb, V. L.; Taudien, S.; Platzer, M.; Rubio, B. K.; Robinson, S. J.; Crews, P.; Piel, J. *Nat. Chem. Biol.* **2009**, *5*, 494.

(6) Soldati, M.; Fioretti, A.; Ghione, M. *Experimentia* **1966**, *22*, 176.

(7) Burres, N. S.; Clement, J. J. *Cancer Res.* **1989**, *49*, 2935.

(8) Ogawara, H.; Higashi, K.; Uchino, K.; Perry, N. B. *Chem. Pharm. Bull.* **1991**, *39*, 2152.

(9) Richter, A.; Kocienski, P.; Raubo, P.; Davies, D. E. *Anti-Cancer Drug Des.* **1997**, *12*, 217.

(10) Hood, K. A.; West, L. M.; Northcote, P. T.; Berridge, M. V.; Miller, J. H. *Apoptosis* **2001**, *6*, 207.

(11) Lee, K.-H.; Nishimura, S.; Matsunaga, S.; Fusetani, N.; Horinouchi, S.; Yoshida, M. *Cancer Sci.* **2005**, *96*, 357.

(12) Chinen, T.; Nagumo, Y.; Watanabe, T.; Imaizumi, T.; Shibuya, M.; Kataoka, T.; Kanoh, N.; Iwabuchi, Y.; Usui, T. *Toxicol. Lett.* **2010**, *199*, 341.

(13) Nishimura, S.; Matsunaga, S.; Yoshida, M.; Hirota, H.; Yokoyama, S.; Fusetani, N. *Bioorg. Med. Chem.* **2005**, *13*, 449.

(14) Gürel, G.; Blaha, G.; Steitz, T. A.; Moore, P. B. *Antimicrob. Agents Chemother.* **2009**, *53*, 5010.

(15) (a) Meinwald, J. *Pure Appl. Chem.* **1977**, *49*, 1275. (b) Nakata, T.; Nagao, S.; Oishi, T. *Tetrahedron Lett.* **1985**, *26*, 6465. (c) Matsuda, F.; Tomiyoshi, N.; Yanagiya, M.; Matsumoto, T. *Tetrahedron* **1988**, *44*, 7063. (d) Jarowicki, K.; Kocienski, P.; Marczak, S.; Willson, T. *Tetrahedron Lett.* **1990**, *31*, 3433. (e) Hoffmann, R. W.; Schlapbach, A. *Tetrahedron* **1992**, *48*, 1959. (f) Takemura, T.; Nishii, Y.; Takahashi, S.; Kobayashi, J.; Nakata, T. *Tetrahedron* **2002**, *58*, 6359. (g) Rolle, T.; Hoffmann, R. W. *Helv. Chim. Acta* **2004**, *87*, 1214. (h) Jewett, J. C.; Rawal, V. H. *Angew. Chem., Int. Ed.* **2007**, *46*, 6502.

(16) (a) Hong, C. Y.; Kishi, Y. *J. Org. Chem.* **1990**, *55*, 4242. (b) Hong, C. Y.; Kishi, Y. *J. Am. Chem. Soc.* **1991**, *113*, 9693. (c) Hoffmann, R. W.; Schlapbach, A. *Tetrahedron Lett.* **1993**, *34*, 7903. (d) Nakata, T.; Matsukura, H.; Jian, D.; Nagashima, H. *Tetrahedron Lett.* **1994**, *35*, 8229. (e) Marron, T. G.; Roush, W. R. *Tetrahedron Lett.* **1995**, *36*, 1581. (f) Roush, W. A.; Pfeifer, L. A. *Org. Lett.* **2000**, *2*, 859. (g) Kocienski, P.; Narquizian, R.; Raubo, P.; Smith, C.; Farrugia, L. J.; Muir, K.; Boyle, F. T. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2357. (h) Trost, B. M.; Yang, H.; Probst, G. D. *J. Am. Chem. Soc.* **2004**, *126*, 48. (i) Sohn, J.-H.; Waizumi, N.; Zhong, H. M.; Rawal, V. H. *J. Am. Chem. Soc.* **2005**, *127*, 7290. (j) Kagawa, N.; Ihara, M.; Toyota, M. *J. Org. Chem.* **2006**, *71*, 6796. (k) Green, M. E.; Rech, J. C.; Floreancig, P. E. *Angew. Chem., Int. Ed.* **2008**, *47*, 7317. (l) Nishii, Y.; Higa, T.; Takahashi, S.; Nakata, T. *Tetrahedron Lett.* **2009**, *50*, 3597. (m) Jewett, J. C.; Rawal, V. H. *Angew. Chem., Int. Ed.* **2010**, *49*, 8682.

(17) (a) Jiang, X.; Garcia-Fortanet, J.; De Brabander, J. K. *J. Am. Chem. Soc.* **2005**, *127*, 11254. (b) Shangguan, N.; Kiren, S.; Williams, L. J. *Org. Lett.* **2007**, *9*, 1093. (c) Huang, X.; Shao, N.; Palani, A.; Aslanian, R.; Buevich, A. *Org. Lett.* **2007**, *9*, 2597. (d) Lachance, H.; Marion, O.; Hall, D. G. *Tetrahedron Lett.* **2008**, *49*, 6061. (e) Smith, A. B., III; Jurica, J. A.; Walsh, S. P. *Org. Lett.* **2008**, *10*, 5625. (f) Crimmins, M. T.; Stevens, J. M.; Schaaf, G. M. *Org. Lett.* **2009**, *11*, 3990.

(18) (a) Aubele, D. L.; Floreancig, P. E. *Org. Lett.* **2002**, *4*, 3443. (b) Rech, J. C.; Floreancig, P. E. *Org. Lett.* **2003**, *5*, 1495. (c) Aubele, D. L.; Rech, J. C.; Floreancig, P. E. *Adv. Synth. Catal.* **2004**, *346*, 359.

(19) (a) Erker, G.; Frömberg, W.; Atwood, J. L.; Hunter, W. E. *Angew. Chem., Int. Ed.* **1984**, *23*, 68. (b) Frömberg, W.; Erker, G. *J. Organomet. Chem.* **1985**, *280*, 343. (c) Anbhaikar, N. B.; Herold, M.; Liotta, D. C. *Heterocycles* **2004**, *62*, 217.

(20) Maraval, A.; Igau, A.; Donnadieu, B.; Majoral, J. P. *Eur. J. Org. Chem.* **2003**, 385.

(21) (a) Wan, S.; Green, M. E.; Park, J.-H.; Floreancig, P. E. *Org. Lett.* **2007**, *9*, 5385. (b) Xiao, Q.; Floreancig, P. E. *Org. Lett.* **2008**, *10*, 1139. (c) DeBenedetto, M. V.; Green, M. E.; Wan, S.; Park, J.-H.; Floreancig, P. E. *Org. Lett.* **2009**, *11*, 835. (d) Lu, C.; Xiao, Q.; Floreancig, P. E. *Org. Lett.* **2010**, *12*, 5112.

(22) Portions of this material were previously reported. Please see: Wu, F.; Green, M. E.; Floreancig, P. E. *Angew. Chem., Int. Ed.* **2011**, *50*, 1131.

(23) Inukai, T.; Yoshizawa, R. *J. Org. Chem.* **1967**, *32*, 404.

(24) Kinnaird, J. W. A.; Ng, P. Y.; Kubota, K.; Wang, X.; Leighton, J. A. *J. Am. Chem. Soc.* **2002**, *124*, 7920.

(25) Roush, W. R.; Palkowitz, A. D.; Ando, K. *J. Am. Chem. Soc.* **1990**, *112*, 6348.

(26) Kim, I. S.; Ngai, M.-Y.; Krische, M. J. *J. Am. Chem. Soc.* **2008**, *130*, 14891.

(27) (a) Paterson, I.; Gibson, K. R.; Oballa, R. M. *Tetrahedron Lett.* **1996**, *37*, 8585. (b) Evans, D. A.; Coleman, P. J.; Côté, B. *J. Org. Chem.* **1997**, *62*, 788. (c) Evans, D. A.; Côté, B.; Coleman, P. J.; Connell, B. T. *J. Am. Chem. Soc.* **2003**, *125*, 10893. (d) Paton, R. S.; Goodman, J. M. *J. Org. Chem.* **2008**, *73*, 1253. (e) Dias, L. C.; Pinheiro, S. M.; de Oliveira, V. M.; Ferreira, M. A. B.; Tormena, C. F.; Aguilar, A. M.; Zukerman-Schpector, J.; Tiekink, E. R. T. *Tetrahedron* **2009**, *65*, 8714 and references therein.

(28) Paterson, I.; Goodman, J. M.; Lister, M. A.; Schumann, R. C.; McClure, C. K.; Norcross, R. D. *Tetrahedron* **1990**, *46*, 4663.

(29) Paterson, I.; Perkins, M. V. *Tetrahedron Lett.* **1992**, *33*, 801.

(30) (a) Komatsu, N.; Uda, M.; Suzuki, H.; Takahashi, T.; Domae, T.; Wada, M. *Tetrahedron Lett.* **1997**, *38*, 7215. (b) Jung, H. H.; Seiders, J. R., II; Floreancig, P. E. *Angew. Chem., Int. Ed.* **2007**, *46*, 8464.

(31) Evans, P. A.; Cui, J.; Gharpure, S. J.; Hinkle, R. J. *J. Am. Chem. Soc.* **2003**, *125*, 11456.

(32) Shenoy, S. R.; Smith, D. M.; Woerpel, K. A. *J. Am. Chem. Soc.* **2006**, *128*, 8671.

(33) A portion of this work has been reported previously. Please see: Rech, J. C.; Floreancig, P. E. *Org. Lett.* **2005**, *7*, 5175.

(34) (a) Langer, P.; Kracke, B. *Tetrahedron Lett.* **2000**, *41*, 4545. (b) Hussain, I.; Yawer, M. A.; Appel, B.; Sher, M.; Mahal, A.; Villinger, A.; Fischer, C.; Langer, P. *Tetrahedron* **2008**, *64*, 8003.

(35) Node, M.; Fujiwara, T.; Ichihashi, S.; Nishide, K. *Tetrahedron Lett.* **1998**, *39*, 6331.

(36) Barker, D.; Brimble, M.; Do, P.; Turner, P. *Tetrahedron* **2003**, *59*, 2441.

(37) Brown, H. C.; Bhat, K. S. *J. Am. Chem. Soc.* **1986**, *108*, 5919.

(38) (a) Cherest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.* **1968**, *9*, 2199. (b) Anh, N. T.; Eisenstein, O. *Nouv. J. Chem.* **1977**, *1*, 61. (c) Lodge, E. P.; Heathcock, C. H. *J. Am. Chem. Soc.* **1987**, *109*, 3353.

(39) Evans, D. A.; Duffy, J. L.; Dart, M. J. *Tetrahedron Lett.* **1994**, *35*, 8537.

(40) (a) Evans, D. A.; Allison, B. D.; Yang, M. G.; Masse, C. E. *J. Am. Chem. Soc.* **2001**, *123*, 10840. (b) Evans, D. A.; Dart, M. J.; Duffy, J. L.; Yang, M. G. *J. Am. Chem. Soc.* **1996**, *118*, 4322.

(41) Chen, K.-M.; Hardtmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. *Tetrahedron Lett.* **1987**, *28*, 155.

(42) (a) Rychnovsky, S. D.; Skalitzky, D. J. *Tetrahedron Lett.* **1990**, *31*, 945. (b) Evans, D. A.; Rieger, D. L.; Gage, J. R. *Tetrahedron Lett.* **1990**, *31*, 7099.
(43) (a) Roush, W. R.; Marron, T. G.; Pfeifer, L. A. *J. Org. Chem.* **1997**, *62*, 474. (b) Trotter, N. S.; Takahashi, S.; Nakata, T. *Org. Lett.* **1999**, *1*, 957. (c) Breitfelder, S.; Schuemacher, A. C.; Rolle, T.; Kikuchi, M.; Hoffmann, R. W. *Helv. Chim. Acta* **2004**, *87*, 1202.
(44) A portion of this work has been reported in ref 16k.
(45) Zhu, C.; Shen, X.; Nelson, S. G. *J. Am. Chem. Soc.* **2004**, *126*, 5352.
(46) (a) Kiren, S.; Williams, L. J. *Org. Lett.* **2005**, *7*, 2905. (b) Green, M. E.; Rech, J. C.; Floreancig, P. E. *Org. Lett.* **2005**, *7*, 4117. (c) Pietruszka, J.; Simon, R. C. *Eur. J. Org. Chem.* **2009**, 3628.
(47) Ley, S. V.; Dixon, D. J.; Guy, R. T.; Palomero, M. A.; Polara, A.; Rodriguez, F.; Sheppard, T. D. *Org. Biomol. Chem.* **2004**, *2*, 3618.
(48) Jiang, X.; Williams, N.; De Brabander, J. K. *Org. Lett.* **2007**, *9*, 227.
(49) For other examples please see refs 16a, 16k and 17f.
(50) The structure of pedestatin was reported in a patent by the Schering-Plough group though it has yet to appear in the peer-reviewed literature. See: Huang, X.; Shao, N.; Seidel-Dugan, C.; Palani, A.; Aslanian, R. G.; Huryk, R. *PCT Int. Appl.*, WO 2009158381 A1 20091230, 2009. We thank an anonymous referee for bringing this to our attention.
(51) Huang, X.; Shao, N.; Huryk, R.; Palani, A.; Aslanian, R.; Seidel-Dugan, C. *Org. Lett.* **2009**, *11*, 867.
(52) Fukui, H.; Tsuchiya, Y.; Fujita, K.; Nakagawa, T.; Koshino, H.; Nakata, T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2081.
(53) Robinson, S. J.; Tenney, K.; Yee, D. F.; Martinez, L.; Media, J. E.; Valeriote, F. A.; van Soest, R. W. M.; Crews, P. *J. Nat. Prod.* **2007**, *70*, 1002.
(54) We thank Dr. Bert Vogelstein from Johns Hopkins University for supplying this cell line.
(55) (a) Matsunaga, S.; Fusetani, N.; Nakao, Y. *Tetrahedron* **1992**, *48*, 8369. (b) Simpson, J. S.; Garson, M. J.; Blunt, J. W.; Munro, M. H. G.; Hooper, J. N. A. *J. Nat. Prod.* **2000**, *63*, 704.
(56) Thomson, A. M.; Blunt, J. W.; Munro, M. H. G.; Perry, N. B. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1233.
(57) Bergeron, R. J.; Ludin, C.; Müller, R.; Smith, R. E.; Phanstiel, O., IV *J. Org. Chem.* **1997**, *62*, 3285.
(58) Krepski, L. R.; Jensen, K. M.; Heilmann, S. M.; Rasmussen, J. K. *Synthesis* **1986**, 301.
(59) (a) Gaul, C.; Njardarson, J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 6042. (b) Szpilman, A. M.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2010**, *49*, 9592.
(60) Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Herald, C. L.; Boyd, M. R.; Schmidt, J. M.; Hooper, J. N. A. *J. Org. Chem.* **1993**, *58*, 1302.
(61) Osman, S.; Albert, B. J.; Wang, Y.; Li, M.; Czaicki, N. L.; Koide, K. *Chem.—Eur. J.* **2011**, *17*, 895.