Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1243–1254

BIOORGANIC & MEDICINAL CHEMISTRY

Total Synthesis and Stereochemistry of Cytoblastin

Ofir A. Moreno † and Yoshito Kishi *

Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA

Received 26 May 1998; accepted 5 June 1998

Abstract—The first total synthesis and stereochemical assignment of cytoblastin were reported. Key steps included the palladium-mediated coupling of *N*-SEM-7-bromoindolactam V ((−)-**11**) with allylstannane *c*-**13**, and osmium tetroxide-mediated dihydroxylation of **14**, both of which were stereoselective. The stereochemistry of cytoblastin was determined as **1A** via spectroscopic analysis of the pentacyclic derivative **21** of cytoblastin. A connection was then made between the stereochemistry so elucidated and the Kishi/Rando hypothesis for the structural correlation between (*S*)-1,2-diacylglycerol and tumor promoters for the process of protein kinase C activation.

Introduction

Protein kinase C (PKC) is a serine/threonine-specific kinase, central to regulatory processes in all cells.[1] PKC actually refers to a family of kinases which are generally activated by the simultaneous presence of (*S*)-1,2-diacylglycerol ((*S*)-DAG), Ca^{2+}, and an acidic phospholipid such as phosphatidylserine, although some isoforms have been reported to be calcium-independent. Under quiescent conditions, PKC resides in the cytoplasm, where it is inactive. PKC is transiently activated by the endogenous (*S*)-DAG, which is produced via the receptor-mediated activation of a phospholipase C, which cleaves phosphatidylinositol to generate (*S*)-DAG and phosphoinositol. Phosphorylation of (*S*)-DAG by a specific kinase, or hydrolysis at the 2-position then removes (*S*)-DAG from circulation, allowing for temporal regulation of PKC. PKC possesses a regulatory domain and a protein kinase domain. (*S*)-DAG binds to the regulatory domain of PKC, resulting in the translocation of the enzyme to the plasma membrane, where the enzyme becomes catalytically active. The regulatory domain contains a pseudosubstrate sequence which is thought to bind to the active site of the kinase domain, thus inhibiting catalytic function. An important function of the binding of (*S*)-DAG to the regulatory domain is to relieve this inhibitory constraint, resulting in the activation of PKC.

The regulatory domain of PKC is of exceptional interest because not only does it specifically recognize (*S*)-DAG's, but it binds to and is activated by an extremely diverse group of non-DAG's. These molecules include the phorbol esters, the ingenols, the aplysiatoxins, the teleocidins, and the bryostatins. The central issue for understanding the mechanism of PKC activation by (*S*)-DAG and these tumor promoters is to relate the structures of these dissimilar molecules to their identical function; namely, the occupation of the same binding site and the activation of PKC. A structural model in which the pharmacophores of the various PKC activators were identified had previously been presented by Kishi and Rando[2] (Fig. 1). This model was experimentally developed through structure–activity studies on debromoaplysiatoxin and its strategically-chosen analogues in reference to (*S*)-DAG and its analogues. In this model, the interaction of PKC with the hydrophilic atoms correlated in Fig. 1 represents the *sine qua non* for the activation of PKC. The circled hydrophobic portions are not necessary, but they significantly increase activity.

Of particular interest to the current studies is the teleocidin class of tumor promoters. The naturally occurring (−)-indolactam V (IL-V; **2**) contains the common

Key words: Natural products; cytoblastin; protein kinase C; tumor promoters.

*Corresponding author. Tel: (617) 495-4679; Fax: (617) 495-5150; E-mail: kishi@chemistry.harvard.edu

†Present address: Amgen, 1840 DeHavilland Drive, Thousand Oaks, California 91320-1789, USA

0968-0896/98/$—see front matter
PII: S0968-0896(98)00113-8

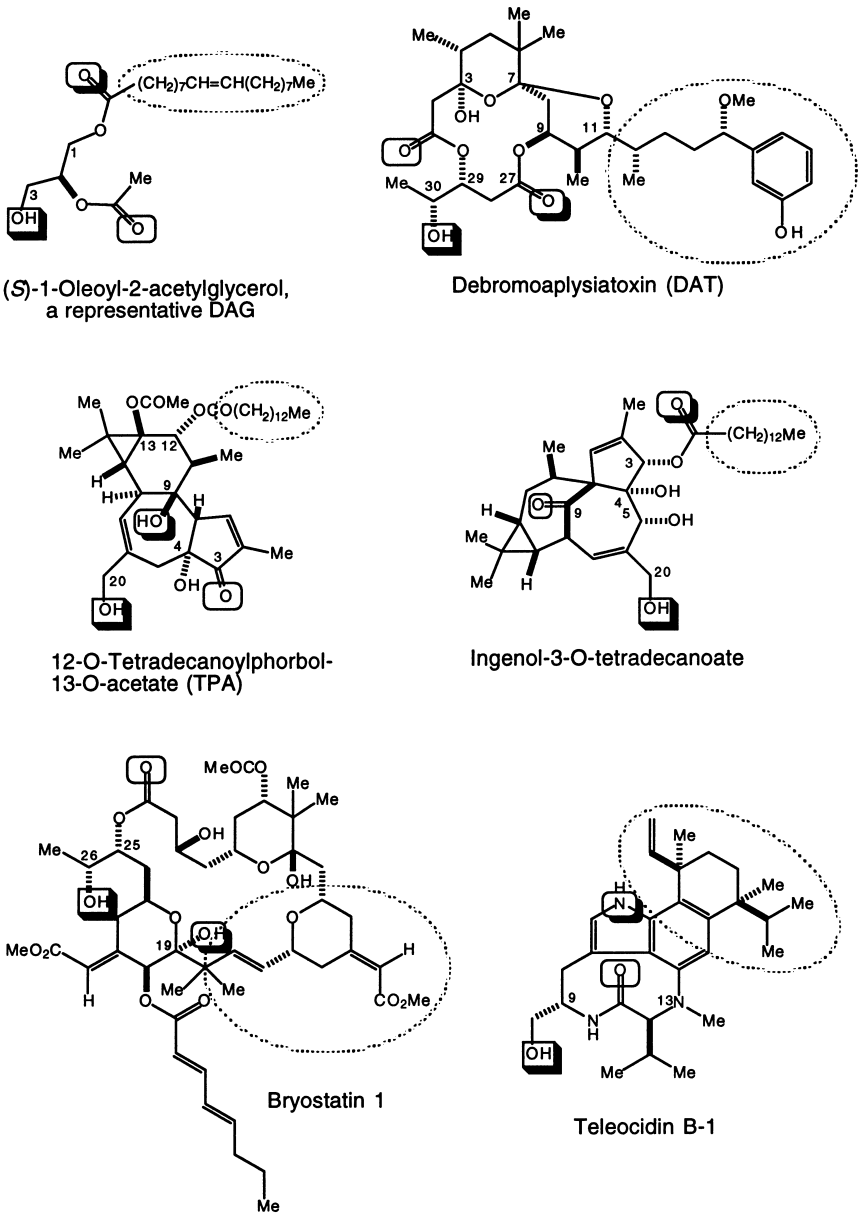


Figure 1. The pharmacophores of various tumor promoters.

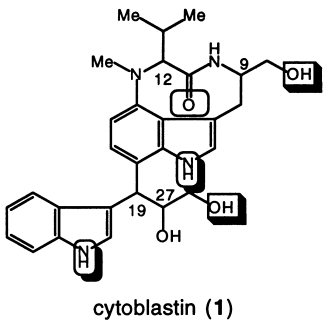
structural element of this class of tumor promoters. This natural product contains the minimum structural requirements for PKC activation.[3] It is widely recognized that the lactam ring of IL-V exists as an equilibrium of two solution conformations with the major conformer named TWIST and the minor conformer SOFA. Although the exact ratio of the two conformers varies with solvent and temperature, they typically exist as roughly a 3:1 ratio in $CDCl_3$ at room temperature.[4] It is this conformational issue which makes IL-V and the teleocidin class of tumor promoters a topic of controversy with respect to their structural correlation with (*S*)-DAG, and several different models concerning the IL-V pharmacophore have evolved.[5]

Cytoblastin[6,7] (**1**) is a natural product isolated from *Streptoverticillium eurocidium*, found in soil samples collected in Yokohama City, Kanagawa Prefecture, Japan. It was found to promote the proliferation of T cells, while showing low cytotoxicity and no antimicrobial activity. Although the gross structure of cytoblastin was reported, the stereochemistry remained

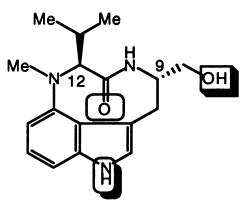
unknown. Interestingly, the upper half of cytoblastin apparently corresponds to (−)-IL-V (**2**), and we felt that a structural correlation between cytoblastin and (*S*)-DAG might give further insights into the pharmacophore of the PKC-based tumor promoters. Intriguingly, when the structural correlation proposed by Kishi and Rando is adopted and an imaginary acyl group is placed on the C.27 alcohol, the lower half of cytoblastin also corresponds to the pharmacophore of (*S*)-DAG. Thus, cytoblastin could be viewed as a pseudo-dimeric form of (*S*)-DAG (Fig. 2). Clearly, the validity of this hypothesis hinges on the stereochemistry of cytoblastin, and we report the first total synthesis and stereochemical assignment of cytoblastin.[8]

Total synthesis

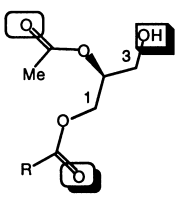
A priori, the upper half of cytoblastin was assumed to correspond structurally to (−)-IL-V (**2**). It is known that the nine-membered lactam ring present in the teleocidin family of natural products exists as a mixture of two conformers (vide ante), whereas inversion of one of the stereocenters on this ring results in a single conformer at room temperature. Cytoblastin (**1**) was reported to exist as a mixture of two conformers,[6] suggesting that at least the *relative* stereochemistry of the upper half of **1** corresponds to that of (−)-IL-V (**2**). Thus, (−)-IL-V (**2**) was chosen as a starting point for the synthesis. Two different approaches were explored.



cytoblastin (**1**)



(-)-indolactam V (**2**)



an (*S*)-diacylglycerol

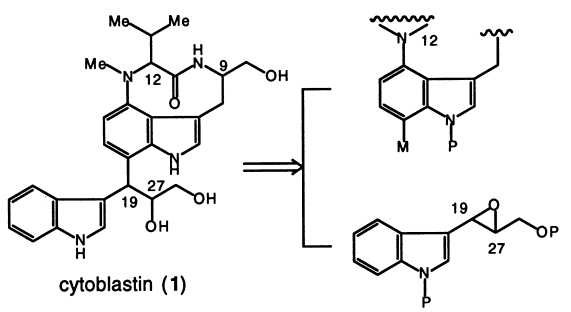
Figure 2. The structure of cytoblastin (**1**), (−)-indolactam V (**2**), and an (*S*)-diacylglycerol, and the proposed structural correlation among them.

First approach

The key bond formation in the first approach was envisioned to take place via the addition of a suitably protected organometallic derivative of the upper half to an epoxide (Scheme 1). The obvious advantage of this approach is prescience of the consequent relative stereochemistry.

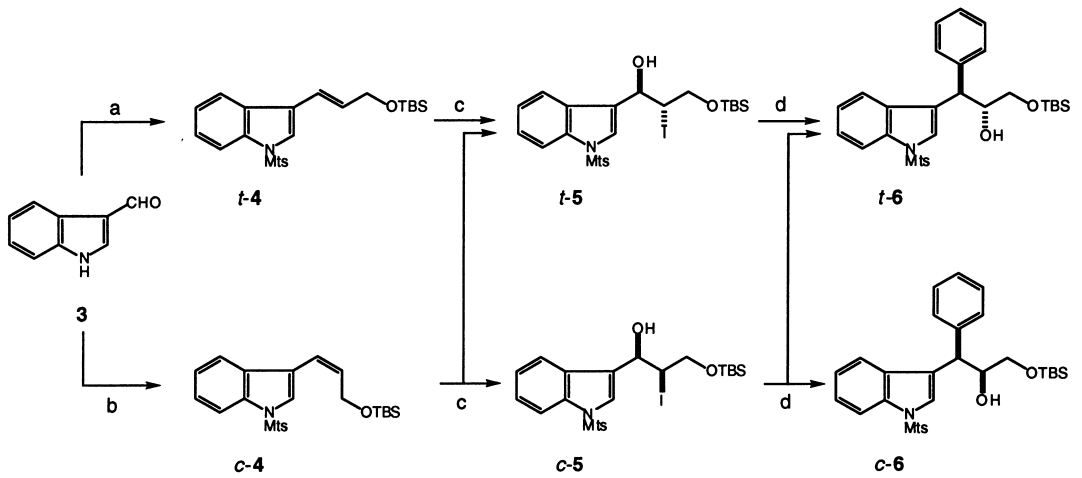
The feasibility of this approach was first examined by using a model system (Scheme 2). Synthesis of the epoxide was planned via the corresponding allylic alcohol, with the consideration that such an epoxide could be prepared in an optically active form via its asymmetric epoxidation. As the allylic alcohol and the epoxide might prove to be unstable due to conjugation to the indole ring, an electron-withdrawing protecting group was deemed appropriate for the indole nitrogen, and the mesitylsulfonyl (Mts) was chosen.

The allylic alcohols were synthesized as outlined in Scheme 2. Under phase-transfer conditions,[10] 3-formylindole (**3**) was protected as its mesitylsulfonamide and converted selectively to *cis*- and *trans*-α,β-unsaturated esters, DIBAL reduction of which furnished the *cis*- and *trans*-allylic alcohols. Although stable in solution, these allylic alcohols decomposed upon concentration, where the major decomposition product appeared to be the dimeric ether. Protection as the *t*-butyldimethylsilyl (TBS) ether resolved this problem. Attempts to epoxidize the allylic alcohols resulted in either



cytoblastin (**1**)

Scheme 1. The first retrosynthetic analysis of cytoblastin (**1**).



Scheme 2. Reagents and conditions. (a) 1. Mts-Cl, NaOH, Cetyltrimethylammonium chloride, CH_2Cl_2, 0 °C; 2. $Ph_3P=CHCO_2Et$, CH_2Cl_2, rt; 3. DIBAL, THF, −78 °C; 4. TBS-Cl, imidazole, DMF, rt. (b) 1. same as (a) 1; 2. $(CF_3CH_2O)_2P(O)CH_2CO_2Me$,[9] KHMDS, 18-Crown-6, THF, −78 °C; 3. same as (a) 3; 4. same as (a) 4. (c) I_2, Ag_2O, aq THF. (d) KHMDS, THF, −78 °C, followed by addition of PhMgBr, −78 °C→rt.

decomposition (MCPBA) or apparently clean conversion but followed by decomposition to the diol on isolation (dimethyldioxirane). In spite of the electron-withdrawing sulfonamide, it appeared that the epoxide was too unstable to isolate. However, it was possible to generate the intermediate iodohydrin via treatment of the allylic alcohols with iodine and silver oxide in wet THF. Interestingly, the TBS-*trans*-allylic alcohol *t*-**4** led exclusively to the iodohydrin *t*-**5**, which was assumed to be *trans*, but the *cis*-olefin *c*-**4** led to a chromatographically separable 5/3 mixture of *cis* and *trans*-iodohydrins *t*- and *c*-**5**.

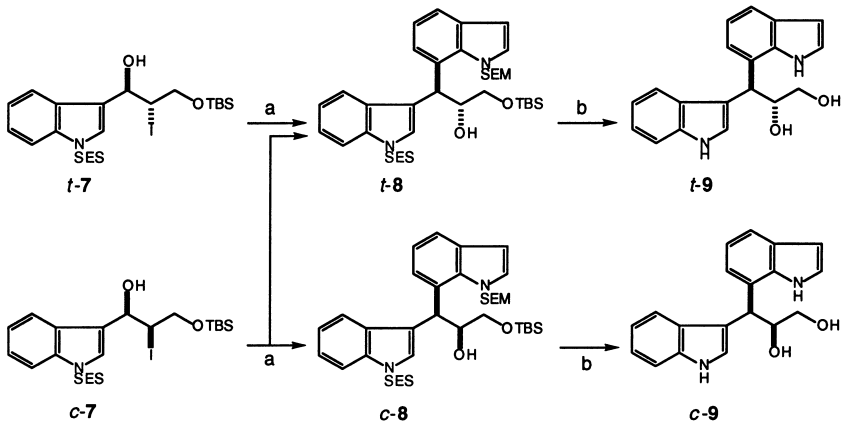
Treatment of the iodohydrin with KHMDS in THF under anhydrous conditions at −78 °C resulted in clean conversion to a less polar compound by silica gel thin-layer chromatography (TLC), which was presumed to be the epoxide. Treatment of the putative epoxide generated in situ with phenylmagnesium bromide resulted in successful coupling in 75% yield. Again, the *trans*-iodohydrin *t*-**5** led to a single adduct *t*-**6**, inferred to be *trans*, whereas the *cis*-iodohydrin *c*-**5** led to a chromatographically separable equimolar mixture of *cis*- and *trans*-products *t*- and *c*-**6**.

The feasibility of this approach was then tested by using an *N*-protected 7-metalloindole species (Scheme 3). At this point, it was desirable to select a more realistic protecting-group scheme for synthetic purposes. Since the mesitylsulfonamide provided adequate protection for the lower indole but did not allow for facile deprotection, it was replaced with the 2-(trimethylsilyl)-ethylsulfonamide (SES).[11] For the upper indole, the protecting group had to withstand the organometallic environment. It was our hope that the 2-(trimethylsilyl)-ethoxymethyl (SEM)[12] group would not only meet these requirements, but would pose the added potential benefit of stabilizing the organometallic center via coordination of its oxygen. Obviously, this silicon-based protecting group strategy should offer the opportunity of single-step global deprotection.

The Grignard reagent derived from *N*-SEM-7-bromoindole could be prepared either by lithium–halogen exchange followed by transmetallation with magnesium bromide, or by direct insertion of activated magnesium metal. Unfortunately, it failed to add to the in situ derived epoxide. Then the organozinc species, generated via lithium–halogen exchange followed by transmetallation with zinc chloride, was examined. This method was successful, but its reproducibility proved capricious, depending on the source of zinc chloride.[13]

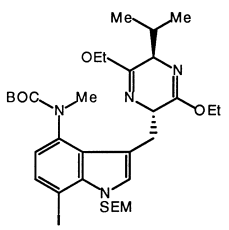
The scenario of batch-dependence of organometallic reagents has been a familiar one to this laboratory.[14] An additive was sought that would bestow reproducibility upon the arylzinc–epoxide coupling reaction. Ultimately, cerium chloride provided such a result, affording reproducible coupling. As before, the *trans*-iodohydrin *t*-**7** led to a single product *t*-**8** in 60–75% yield, whereas the *cis*-iodohydrin *c*-**7** led to a separable 1/1 mixture of *cis*- and *trans*-adducts *t*- and *c*-**8** in comparable yields.

Not only had the protecting-group strategy proved suitable for the organometallic coupling conditions, it



Scheme 3. Reagents and conditions. (a) KHMDS, THF, −78 °C, followed by addition of Ce reagent (prepared from *N*-SEM-7-bromoindole by treatment with *n*-Buli, THF, −78 °C, $ZnCl_2$, −78 °C→rt, then $CeCl_3$, rt), −78 °C→rt. (b) TBAF, EDA, DMF, 70 °C.

also proved suitable for facile single-step global deprotection. It had been hoped that the vicinal coupling constants for the *cis*- and *trans*-adducts would be indicative of the relative stereochemistries introduced during the coupling, providing a method to assign the *relative* stereochemistry between C.19 and C.27 of cytoblastin. Unfortunately, this turned out not to be the case. Although the ^{1}H NMR spectra of *t*-**9** and *c*-**9** were different from each other, the vicinal spin-coupling constants for the C.19 and C.27 protons were found to be 7.2 Hz for both isomers.



10

With the coupling and protecting-group strategies having been validated by the second model system, the next step was to extend this approach to the real system. For this purpose, coupling of the in situ generated epoxide with the iodide **10** was extensively studied. Unfortunately, although almost any metal could be directly inserted,[15] none proved fruitful towards the coupling.

Second approach

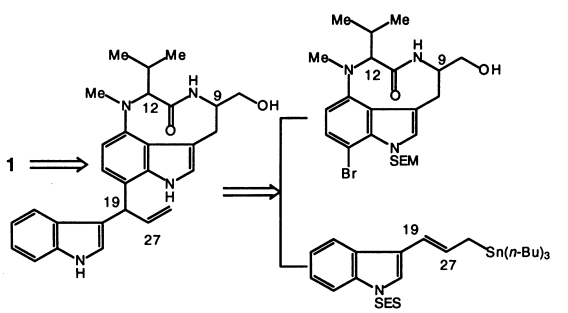
Because of the difficulty encountered in the first approach, we developed the revised strategy, involving the Heck coupling[16] of *N*-SEM-7-bromoindolactam V[17] with an allylstannane, followed by dihydroxylation (Scheme 4).

Among several synthetic routes studied, the synthesis of *N*-SEM-7-bromo-IL-V ((−)-**11**) was most effectively achieved as shown in Scheme 5.[18] Although bromination of (−)-IL-V (**2**) itself was recorded in the literature, its efficiency was rather poor.[19] However, with the specific protecting groups indicated, bromination was accomplished in 90% yield. Although not necessary, it was most convenient for purposes of post-coupling purification to deprotect the alcohol at this stage.

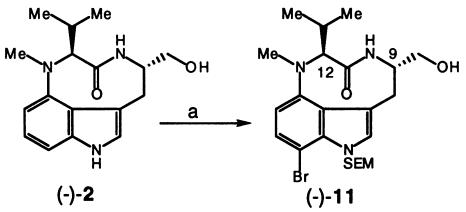
A concise synthesis of both *trans*- and *cis*-allylstannanes *t*- and *c*-**13** was also developed (Scheme 6).

The Heck coupling of (−)-**11** with *c*-**13** was initially effected under the standard conditions, yielding a 3:1 ratio of the desired and undesired regioisomers in excellent yields. Since *trans*- and *cis*-allylstannanes *t*-**13** and *c*-**13** were shown to behave equivalently towards (−)-**11**, the more readily available *cis*-allylstannane *c*-**13** was used for optimization studies.

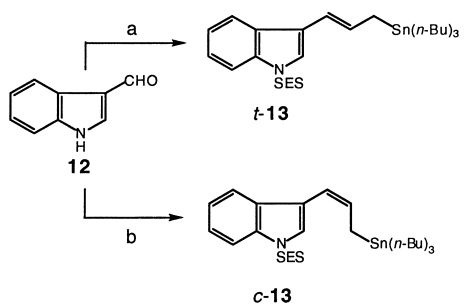
After several successful runs, this palladium-catalyzed coupling mysteriously ceased to function, leaving only



Scheme 4. The second retrosynthetic analysis of cytoblastin (**1**).



Scheme 5. Reagents and conditions. (a) 1. TBS-Cl, imidazole, DMF, rt; 2. SEM-Cl, NaH, THF, −15 °C; 3. NBS, DMF, −15 °C; 4. TBAF, THF, rt.



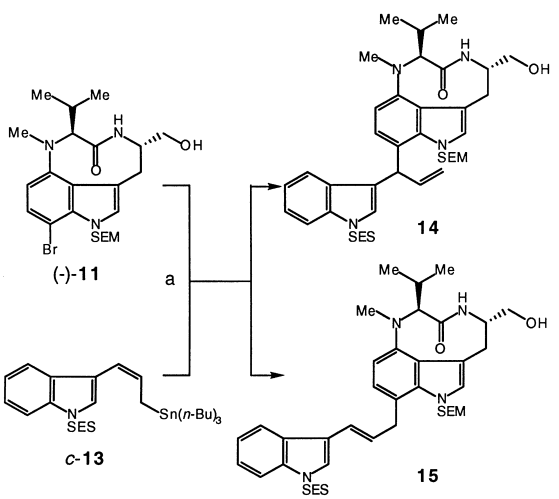
Scheme 6. Reagents and conditions. (a) 1. SES-Cl, NaOH, cetyltrimethylammonium chloride, CH_2Cl_2, 0 °C; 2. $CH_2=CHMgBr$, THF, −78 °C; 3. Ac_2O, Py, rt; 4. *n*-Bu_3Sn-Cu(*n*-Bu) $(CN)Li_2$, THF, −78 °C. (b) 1. same as (a) 1; 2. *n*-$Bu_3SnCH_2CH=PPh_3$, THF, −78 °C→rt.

unreacted starting material. Repeated efforts to restore function, including doping with various transition metal salts, proved fruitless. When a stoichiometric quantity of metal complex was employed, again no coupling was observed. However, some of the halide had been consumed and promoted to a less polar spot on TLC (silica gel). Chromatographic isolation (silica gel) and spectral characterization (^{1}H NMR, MS) revealed this compound to be the aryl-palladium species. Treatment of this compound with *c*-**13** and TBAI in refluxing benzene resulted in complete conversion to the coupled products in a 1:1 ratio of the two regioisomers. This experiment was repeated, without isolation of the aryl-palladium intermediate, producing a 3:1 ratio of the two regioisomers.

This study presented a unique opportunity to break the catalytic cycle down into discrete steps, thereby allowing multi-variable analysis of the reaction pathway. Extensive efforts were made to determine the effects of solvents, ligands, and allylstannanes, demonstrating that (1) polar solvents (DMF, CH_3CN) resulted in virtually exclusive formation of **15**, (2) weakly coordinating ligands [Ph_3As, $(2\text{-furyl})_3P$, $(o\text{-Tol})_3P$] also resulted in virtually exclusive formation of **15**, (3) stronger coordinating and bidentate ligands (PBu_3, DPPE, DPPP) inhibited the reaction after oxidative insertion, and (4) introduction of an oxygen functionality at the α-position of allylstannanes resulted in exclusive formation of the undesired regioisomer.[20]

The coupling of (−)-**11** with *c*-**13** transpired most effectively utilizing a stoichiometric quantity of tetrakis-(triphenylphosphine)palladium(0) in refluxing benzene in the presence of excess tetrabutylammonium iodide (Scheme 7) at approximately 0.1 M concentration. Under these conditions, the product distribution favored the desired regioisomer by at least 10:1. The coupling product **14** was isolated in 80% yield, along with less than 10% of the regioisomer **15**. Intriguingly, ^{1}H NMR analysis indicated that the desired regioisomer **14** consisted primarily of one diastereomer.

The next step in the synthesis was dihydroxylation of the terminal olefin of **14**. Catalytic osmylation (OsO_4/NMMO) was first attempted, but it was quickly realized that the substrate was too sensitive to the oxidizing environment required to regenerate the osmium tetroxide. The method employed by Sakai and co-workers (OsO_4, THF, pyridine, followed by aq $NaHSO_3$ workup)[21] was tried, as it reportedly affected a relatively hindered olefin preferentially over the indole ring. This method indeed resulted in the apparently desired product in 30–40% yield, along with about 20% recovered starting material. However, a serious difficulty was encountered with this method. The reproducibility in cleavage of the osmate ester in the workup proved capricious; addition of aq $NaHSO_3$ to the reaction sporadically generated the product but more typically resulted in complete decomposition. Observations made during repeated trials suggested that water might have



Scheme 7. Reagents and conditions. (a) $Pd(PPh_3)_4$, TBAI, PhH, 80 °C.

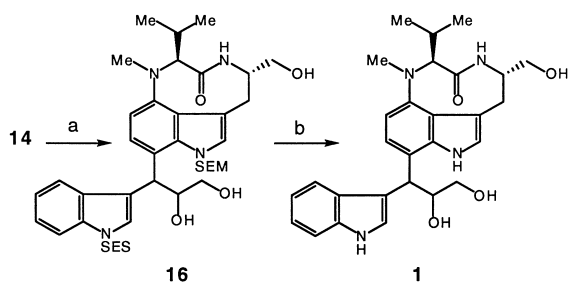
been the problem. Thus, 1,3-propanedithiol was used to cleave the osmate ester under anhydrous conditions, resulting in reproducible results.

With this workup procedure developed, the efficacy of the dihydroxylation remained to be addressed. It was envisioned that this might be achieved by enhancing the reactivity of OsO_4, which could be counteracted by cooling, allowing for fine-tuning. Thus, *N*,*N*′-tetramethyl-ethylenediamine (TMEDA)[22] was used to effect osmylation at −90 °C, and 1,3-propanedithiol was used under anhydrous conditions to cleave the osmate ester. This method consistently produced the dihydroxylated products in nearly quantitative yield. Surprisingly, a single product accounted for 70% of the mixture, with the other three comprising the remaining 30%.

These results show that both the palladium(0)-mediated coupling and the osmylation proceeded in a stereoselective manner. The bias observed in the osmylation could plausibly be attributed to complexation of osmium to the aminoindole ring prior to osmaoxetane formation, resulting in facial-selective attack on the olefin. This rationale is loosely analogous to the ready complexation of osmium with pyrrole as exploited by Harman.[23] The stereoselectivity exhibited by the Heck coupling, on the other hand, is perplexing. The steric bias must have arisen from the chirality in the lactam ring, and it is most likely that the transmetallation[24] occurred with some facial selectivity with respect to the planar allylstannane. However, any connectivity between these two suppositions remains unclear at this time.

Global deprotection was then studied. Under the standard TBAF conditions, removal of the SES protecting group was facile, but cleavage of the SEM protecting group was problematic. It was serendipitously found that, when a DMF solution of **16** containing excess TBAF was evaporated in vacuo and stored under high vacuum, the deprotection smoothly took place, to yield **1** in excellent yields. This result may suggest that the rate-determining step involves an equilibrium between the hemiaminal and the free indole NH, where removal of the formaldehyde accelerates the reaction and drives the reaction to completion. This rationale is supported by the report that addition of ethylenediamine to TBAF substantially improves the yields of SEM deprotections.[25]

Fortuitously, the global deprotection of the major product **16** furnished a single product which matched cytoblastin (**1**)[26] in every analysis performed (TLC, ^{1}H NMR (Fig. 3), $[\alpha]_D$, CD). Deprotection of the other three osmylation products led to three compounds which were clearly distinct from the natural product.



Scheme 8. Reagents and conditions. (a) OsO_4, TMEDA, CH_2Cl_2 −90 °C. (b) TBAF, neat at 0.1 mm Hg.

Stereochemical Elucidation

The fact that the synthesis reported led to the natural product demonstrated that the stereochemistry of the upper half of cytoblastin indeed corresponds to that of (−)-IL-V (**2**). However, the stereochemistry of the lower half remained to be elucidated. To this end, efforts to crystallize cytoblastin or its derivatives had thus far proved fruitless. Therefore, an alternative approach was explored.

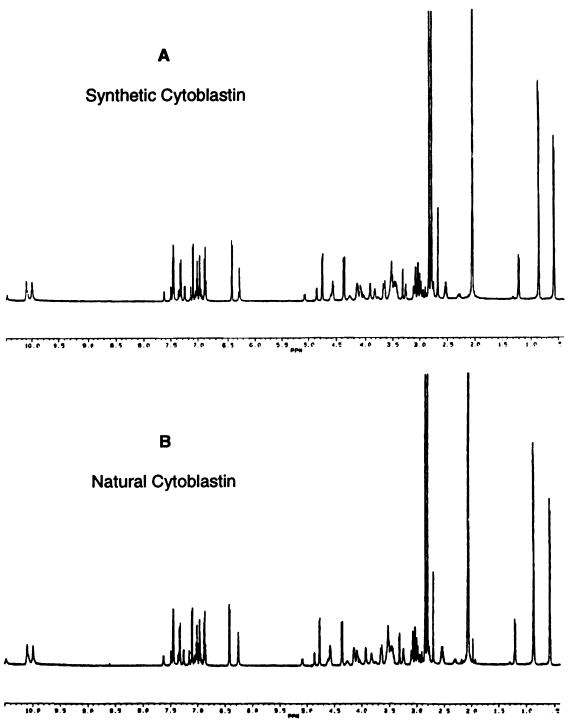
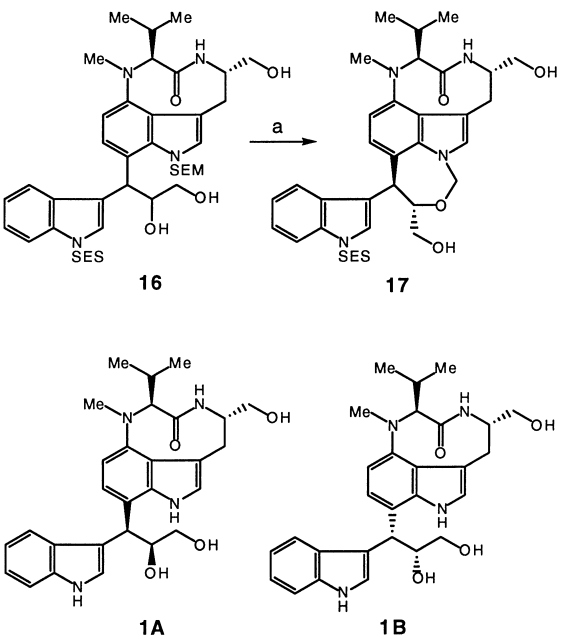


Figure 3. ^{1}H NMR spectra (5000 MHz) of the synthetic (Panel A) and natural (Panel B) cycloblastin (**1**) in acetone-d_6.

Determination of the *relative* configuration at the C.19 and C.27 positions was sought via spectroscopic studies on a cyclic derivative involving the upper indole nitrogen and the ethylene glycol moiety. To this end, it was found that treatment of **16** with lithium tetrafluoroborate ($LiBF_4$) and camphorsulfonic acid (CSA) in THF resulted in transketalization of the SEM group with the secondary alcohol, to give the seven-membered aminal **17**. ^1H NMR analysis of the diacetate of **17** clearly showed the vicinal spin-coupling constant of the C.19 and C.27 protons to be 10.0 Hz. Coupled with the lack of NOE between them, this experiment established the C.19 and C.27 protons in the seven-membered ring to be *trans*, which transposed to a *syn* relationship in the natural product. Thus, the complete structure of cytoblastin should be represented by either **1A** or **1B**.

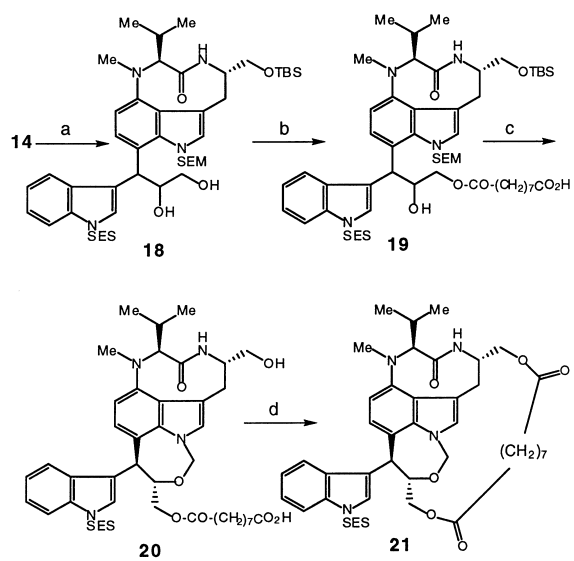
In order to differentiate **1A** from **1B**, a method of correlating the stereocenters in the lower half with those of the upper half was explored, which exploited the observation that the SOFA conformation orients the lactam hydroxymethyl on one side of the plane defined by the upper indole ring, whereas the TWIST conformation orients it on the other side. Therefore, by tethering the two primary hydroxyl groups of **17**, one might expect to deduce the relative spatial relationship between the upper and lower halves, which could then be extrapolated to the absolute configuration of the lower portion.



Scheme 9. Reagents and conditions. (a) $LiBF_4$ (MeCN), CSA, THF, rt.

Analysis of molecular models suggested that roughly a nine-carbon aliphatic chain should provide such a tether. Indeed, the olefin **14** was successfully converted into the macrolactone **21** (Scheme 9). Upon treatment with: 1. K_2CO_3/MeOH; 2. CSA/THF/H_2O; and 3. TBAF/THF, **21** gave cytoblastin (**1**), demonstrating that its complete structure was preserved in **21**.

2-D NOESY of **21** revealed that the lactam ring was locked into its minor (SOFA) conformation; a strong NOE was observed between the amide N-H and C.12-H but no NOE was detected between C.12-H and C.8-H. Importantly, distinct NOE's were detected between the C.19 proton and the protons of the aliphatic chain but no NOE was detected between the C.27 proton and the protons of the aliphatic chain (Fig. 4). Since the C.19 and C.27 relative stereochemistry was shown to be *trans* (vide supra), this NOE experiment allowed to establish on which side of the aminal ring the chain passes. With the lactam ring having provided the absolute spatial relationship between the chain and the upper half, and the NOESY having provided the orientation of the chain relative to the aminal ring, the absolute stereochemistry was deduced to be **1A**. This conclusion was further supported by the additional data provided by the 2D NOESY NMR experiment. The structural information thus provided is in agreement with that provided by the MacroModel molecular modelling program[27] (Fig. 5).



Scheme 10. Reagents and conditions. (a) 1. TBS-Cl, imidazole, DMF, rt. 2. OsO_4, TMEDA, CH_2Cl_2, −90 °C. (b) Azelaic acid, EDAC, DMAP, DMAP·HCl, CH_2Cl_2 rt. (c) $LiBF_4$ (MeCN), CSA, THF, rt. (d) EDAC, DMAP, DMAP·HCl, CH_2Cl_2, rt.

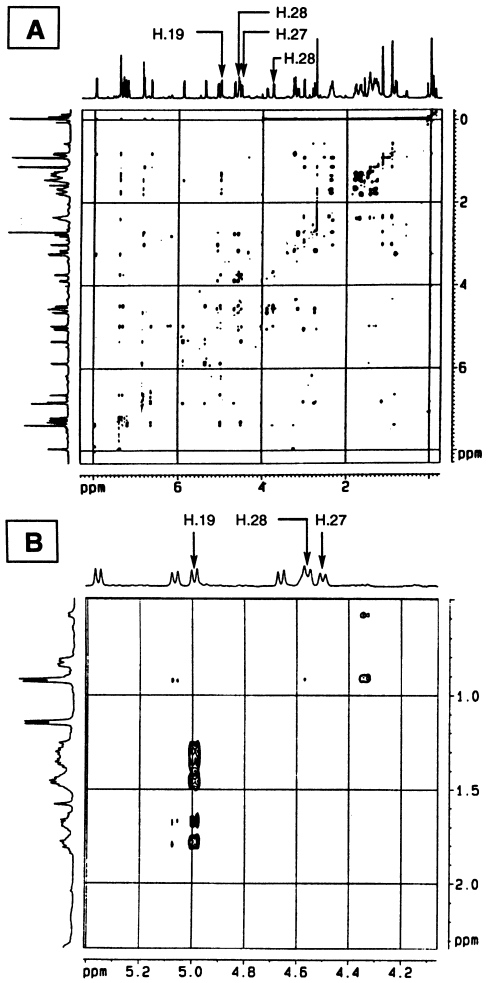


Figure 4. The NOSEY spectrum ($CDCl_3$) of **21**. The panel A shows the region of (−0.2–8.2 ppm)×(−0.2–8.2 ppm), whereas the panel B shows the region of (4.1–5.4 ppm)×(0.5–2.3 ppm). The chemical shift for the C.19–H, C.27–H, C.28–H, and C.28–H are 4.99 ppm (d), 4.49 (ddd), 4.56 (dd), and 3.73 (dd), respectively.

Conclusions

The two stereochemical hypotheses proposed at the outset have been confirmed. The stereochemistry about the lactam ring has been shown to be degenerate with that of the naturally occurring (−)-IL-V (**2**). Also, the chirality at the C.27 in cytoblastin (**1**) was shown to be equivalent to the C.9 stereochemistry of (−)-IL-V as well as the C.2 stereochemistry of (*S*)-DAG. Although it is generally agreed that (−)-IL-V (**2**) possesses the minimum structural requirements for PKC activation, it is interesting to note that cytoblastin had originally been reported to exhibit a complete lack of activity towards PKC.[6] However, assays of both natural and synthetic

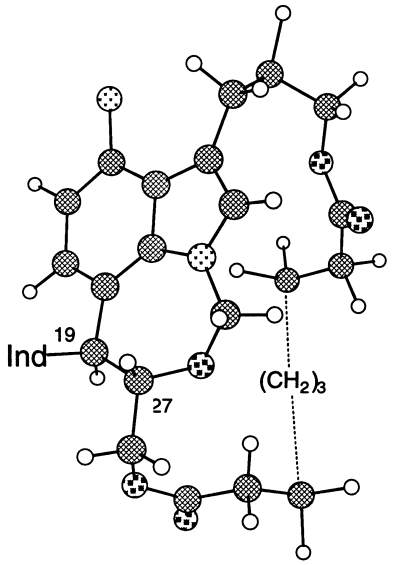


Figure 5. A partial stereoview of the lactam **21**. ⊗, ⊙, ⊙, ○, represent C, N, O, and H, respectively.

cytoblastin have shown an activity towards PKC that is roughly equivalent to (−)-IL-V.[28] Finally, the results reported immediately suggest new types of PKC activators, including C.27 acyl cytoblastin as well as dimeric and pseudo-dimeric (*S*)-DAG's and tumor promoters.

Experimental

General information

NMR spectra were recorded on a Bruker AM-500 (500 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane. The residual solvent peaks were used as internal references. Fast atom bombardment (FAB) mass spectra were obtained on either a JOEL JMS-AX505H or a JOEL JMS-SX102, using 3-nitrobenzyl alcohol or glycerol as the matrix. Sodium iodide was added when indicated. Chemical ionization (CI) mass spectra were recorded on a Kratos MS-50L double focusing instrument using ammonia as the reagent gas. Optical rotations were measured on a Perkin–Elmer 241 Polarimeter at room temperature using sodium D line.

Analytical TLC was performed with E. Merck precoated TLC plates, silica gel 60F-254, layer thickness 0.25 mm. Flash chromatography was performed with E. Merck Kieselgel 60 (230–400 mesh) silica gel.

Reagents and solvents were used as supplied, with the following exceptions: benzene, distilled from sodium

benzophenone ketyl; chlorotrimethylsilane, distilled from calcium hydride; ether, distilled from sodium benzophenone ketyl; methylene chloride, distilled from phosphorous pentoxide; pyridine, stored over anhydrous potassium hydroxide; tetrahydrofuran, distilled from sodium benzophenone ketyl; toluene, distilled from sodium.

All reactions were performed under argon. For moisture-sensitive reactions, reaction vessels were oven- or flame-dried and allowed to cool under vacuum after purging with argon.

Synthesis of (−)-11. To a stirring 0.2 M solution of (−)-IL-V ((−)-**2**)[18] in DMF was added 1.0 equiv TBS-Cl, 2.0 equiv imidazole, and 0.1 equiv TBAI. After 2 h, the reaction was diluted with EtOAc and washed with water, 5% $KHSO_4$, sat'd $NaHCO_3$, and brine. The organic phase was dried over $MgSO_4$, filtered through silica, and concentrated in vacuo to yield a slightly yellow crystalline solid, which was carried on without further purification.

To a stirring 0.1 M solution of the crude product in THF at −15 °C was added 3.0 equiv NaH. After 30 min, 1.2 equiv SEM-Cl was added, and stirring was continued for another 1 h at −15 °C. The reaction was poured into satd NH_4Cl and extracted with EtOAc. The organic phase was washed with water (2×) and brine, dried over $MgSO_4$, filtered through silica gel, and concentrated in vacuo to yield a yellow oil, which was carried on without further purification.

To a stirring 0.1 M solution of the crude product in DMF at −15 °C was added dropwise 1.5 equiv of a 1.0 M solution of NBS in DMF. After an additional 10 min of stirring, the reaction was poured into water and extracted into EtOAc. The organic phase was then washed with water, 5% $KHSO_4$, satd $NaHCO_3$, and brine, dried over $MgSO_4$, filtered through silica gel, and concentrated in vacuo to yield a viscous green oil, which was carried on without further purification.

To a stirring 0.2 M solution of the crude product in THF was added 1.5 equiv of a 1.0 M solution of TBAF in THF. After 30 min, the reaction was diluted with EtOAc, washed with water (2×) and brine, dried over $MgSO_4$, filtered and concentrated. Flash chromatography (silica gel; EtOAc) afforded (−)-**11** as a yellow foam in 80% yield over four steps. $[\alpha]_D$ −150° (*c* 0.51, EtOH). HRMS: calcd for $C_{23}H_{36}N_3O_3BrSiNa$ = 532.1607; found = 532.1603. ^1H NMR ($CDCl_3$): 7.26 (2H, m, (under $CDCl_3$ peak), 6.89 (1H , s), 6.43 (1H, d, J = 8.4 Hz), 5.88 (1H, d, J = 10.7 Hz), 5.72 (1H, d, J = 10.6 Hz), 4.25 (2H, m, d, J = 9.9 Hz), 3.72 (1H, m), 3.55 (3H, m, t, J = 8.2 Hz), 3.13 (1H, bd, J = 17.6 Hz), 3.0 (1H, dd, J = 3.6 and J = 17.6 Hz), 2.88 (3H, s), 2.76 (1H, t. J = 6.0 Hz), 2.57 (1H, m), 0.91 (5H, m, d, J = 6.4 Hz), 0.61 (3H, d, J = 6.7 Hz), 0.05 (9H, m).

Synthesis of *cis*-13. To a stirring 0.2 M solution of 3-formylindole, 2.5 equiv powdered NaOH, and 0.01 equiv cetyltrimethylammonium chloride in CH_2Cl_2 at 0 °C was added a 1.7 M solution of SES-Cl in CH_2Cl_2 dropwise. After 2 h, the reaction was acidified with 1 N HCl, and the phases separated. The organic phase was washed with sat'd $NaHCO_3$ and water, dried over $MgSO_4$, filtered and concentrated. Flash chromatography (silica gel; CH_2Cl_2) afforded a beige crystalline solid in 90% yield.

To a stirring 0.5 M solution of LDA in THF at 0 °C was added 1.0 equiv of $(n\text{-Bu})_3SnH$ dropwise. After 30 min, the yellow solution was cooled to −78 °C and added dropwise via cannula to 1.05 equiv of vinyl triphenylphosphorane 0.3 M in ether at −78 °C. After 15 min, to the resulting blood-red suspension was titrated the protected formylindole, as a 1 M solution in THF, until the red color was dissipitated. The reaction was allowed to warm to rt, poured into satd NH_4Cl and extracted twice with EtOAc. The organic phase was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. Flash chromatography (silica gel; 2% ether/hexanes) afforded (−)-**13** as a mobile colorless oil in 50–60% yield. HRMS: calcd. for $C_{28}H_{49}NO_2SSiSnNa$ = 634.2177; found = 634.2187. ^1H NMR ($CDCl_3$): 7.88 (1H, d, J = 7.9 Hz), 7.65 (1H, d, J = 7.2 Hz), 7.43 (1H,s), 7.33 (2H, m), 6.18 (1H, d, J = 10.7 Hz), 6.07 (1H, m), 3.17 (2H, m), 2.12 (2H, d, J = 9.6 Hz), 1.5–0.7 (20H, m), −0.05 (9H, m).

Heck coupling of (−)-11 with *c*-13. To a stirring 0.1 M solution of (−)-**11** in benzene under argon was added 1.0 equiv of tetrakis(triphenylphosphine)palladium(0), and the suspension was heated to reflux, whereupon the reaction homogenized. After 1 h, the reaction was allowed to cool to rt and 1.5 equiv of (*c*)-**13** was added, along with 5.0 equiv of TBAI, and refluxing was resumed overnight. The homogenous reaction was cooled to rt, resulting in a suspension, which was diluted with EtOAc and filtered through celite. The filtrate was then concentrated in vacuo, and flash chromatographed (silica gel; 75% EtOAc) affording **14** as a yellow amorphous solid in 80% yield. HRMS: calcd for $C_{39}H_{58}N_4O_5SSi_2Na$ = 773.3564; found = 773.3591. ^1H NMR ($CDCl_3$) (major conformer only): 7.86 (1H, d, J = 8.3 Hz), 7.34 (1H, d, J = 7.8 Hz) 7.30 (2H, m), 7.15 (1H,m), 6.97 (1H, s), 6.89 (1H, d, J = 8.1 Hz), 6.77 (1H, s), 6.51 (1H, d, J = 8.2 Hz), 6.37 (1H, m), 5.82 (1H, d, J = 4.9 Hz), 5.27 (2H, m), 5.16 (1H, d, J = 11.4 Hz), 4.86 (1H, d, J = 17.2 Hz), 4.35 (1H, m), 4.31 (1H, d, J = 10.1 Hz), 3.72 (1H, m), 3.53 (3H, m), 3.17 (3H, m), 3.02 (1H, m), 2.86 (3H, s), 2.57

(1H, m), 0.93 (5H, d, $J=6.3$ Hz), 0.78 (2H, m), 0.59 (3H, d, $J=6.7$ Hz), −0.04 (18H, m).

Osmylation of 14. To a stirring 0.1 M solution of **14** in CH_2Cl_2 was added 3 equiv of TMEDA, and the solution was cooled to −90 °C. OsO_4 (1.0 equiv, 0.75 M in CH_2Cl_2) was added dropwise over 30 min. After 2.5 h, 10 equiv of 1,3-propanedithiol was added, and the resulting dark-green mixture was allowed to warm to rt. The reaction was then diluted with methanol, 50% by volume, and allowed to stir overnight. The CH_2Cl_2 was removed in vacuo, and the mixture was filtered through celite, rinsing with methanol, and concentrated in vacuo, to afford a viscous dark green oil. Flash chromatography (silica gel; 2%CH_3OH/$CHCl_3$) afforded a colorless amorphous solid in 70% yield. $[\alpha]_D$ −140° (*c* 0.42, EtOH). HRMS: calcd for $C_{45}H_{74}N_4O_7SSi_3Na$ = 921.4484; found = 921.4474. ^{1}H NMR ($CDCl_3$) (major conformer only): 7.83 (1H, d, $J=8.3$ Hz), 7.69 (1H, d, $J=7.9$ Hz), 7.27 (2H, m), 7.19 (2H, m), 6.77 (1H, s), 6.57 (1H, d, $J=8.3$ Hz), 6.16 (1H, s), 5.63 (1H, d, $J=11.5$ Hz), 5.40 (1H, d, $J=11.3$ Hz), 5.38 (1H, d, $J=8.9$ Hz), 4.39 (1H, m), 4.22 (2H, m, d, $J=10.0$ Hz), 3.85 (1H, m), 3.72 (1H, m), 3.6 (3H, m), 3.46 (1H, dd, $J=9.8$ and 9.2 Hz), 3.1 (4H, m), 2.88 (3H, s), 2.55 (1H, m), 2.33 (1H, d, $J=2.7$ Hz), 2.22 (1H, m), 0.89 (16H, m), 0.50 (3H, d, $J=6.7$ Hz), −0.02 (24H, m).

Synthetic cytoblastin (1). To a solution of the triol in DMF (arbitrary concentration) was added solid TBAF (6 equiv), and the resulting red solution was concentrated in vacuo and placed on a vacuum line (≤0.1 mm Hg) overnight. The residue was partitioned between 5% $KHSO_4$ and EtOAc, and the phases were separated. The organic phase was washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated in vacuo. Flash chromatography (silica gel; 10% CH_3OH/$CHCl_3$) afforded **1** as a colorless amorphous solid in 90% yield. $[\alpha]_D$ −110° (*c* 0.30, MeOH). HRMS: calcd for $C_{28}H_{34}N_4O_4Na$ = 513.2478; found = 513.2473. ^{1}H NMR (acetone-d_6) (major conformer only): 10.14 (1H, br), 10.02 (1H, br), 7.45 (2H, m), 7.32 (1H, d, $J=8.1$ Hz), 7.10 (1H, d, $J=7.9$ Hz), 7.00 (1H, t, $J=8.1$ Hz), 6.96 (1H, s), 6.86 (1H, t, $J=8.1$ Hz), 6.41 (1H, d, 7.9 Hz), 6.28 (1H, s), 4.77 (1H, d, $J=5.3$ Hz), 4.56 (1H, m), 4.35 (1H, d, $J=10.2$ Hz), 4.12 (1H, m), 3.63 (1H, m), 3.50 (3H, m), 3.08 (1H, br d, $J=17.6$ Hz), 3.0 (1H, dd, $J=3.6$ and 17.2 Hz), 2.85 (3H, s), 2.52 (1H, m), 0.85 (3H, d, $J=6.4$ Hz), 0.55 (3H, d, $J=6.8$ Hz).

Preparation of 21 from 14. To a stirring 0.2 M solution of **14** in DMF was added 2 equiv imidazole, 1.2 equiv TBS-Cl, and 0.1 equiv TBAI. After 2 h, the reaction was diluted with EtOAc, washed with H_2O (2×) and brine, dried over $MgSO_4$, filtered and concentrated. Flash chromatography (silica gel; 20% EtOAc/hexanes) afforded TBS silyl ether as a yellow foam in 90% yield.

Osmylation of TBS silyl ether conducted under the same condition as the one given for osmylation of **14**, to give **18**.

To a stirring 0.01 M solution of **18** in CH_2Cl_2 was added 5 equiv azelaic acid, 2 equiv DMAP, 1.5 equiv DMAP·HCl, and 1.2 equiv EDAC. After 2 h, the reaction was washed with H_2O, dried over $MgSO_4$, filtered and concentrated in vacuo. Flash chromatography (silica gel; 50% EtOAc/hexanes→EtOAC) afforded a colorless foam in 85% yield. $[\alpha]_D$ −110° (*c* 0.36, EtOH). ^{1}H NMR ($CDCl_3$): 7.85 (1H, d, $J=8.3$ Hz), 7.56 (1H, d, $J=7.9$ Hz), 7.43 (1H, s), 7.26 (2H, m), 7.14 (1H, d, $J=8.0$ Hz), 6.78 (1H, s), 6.53 (1H, d, $J=8.3$ Hz), 6.23 (1H, d, $J=1.6$ Hz), 5.89 (1H, d, $J=11.8$ Hz), 5.39 (1H, d, $J=9.5$ Hz), 5.32 (1H, d, $J=11.8$ Hz), 4.55 (1H, m), 4.47 (1H, dd, $J=11.7$ and 1.8 Hz), 4.23 (3H, m, d, $J=9.9$ Hz), 3.63 (3H, m), 3.47 (1H, t, $J=9.8$ Hz), 3.19 (2H, t, $J=8.9$ Hz), 3.1 (1H, bd, $J=14.5$ Hz), 2.86 (4H, m, s), 2.56 (1H, m), 2.32 (4H, m), 1.61 (4H, m), 1.30 (6H, m), 0.92 (16H, m), 0.47 (3H, d, $J=6.8$ Hz), 0.0 (24H, m).

To a stirring 0.03 M solution of this product in THF was added 5 equiv of $LiBF_4$, 1.0 M in CH_3CN, followed by 5 equiv of CSA, and the reaction was allowed to stir overnight. The reaction was diluted with EtOAc, washed with H_2O (2×) and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Flash chromatography (silica gel; 9/0.2/0.2 CH_2Cl_2/CH_3OH/HOAc) afforded **20** as a colorless foam in 84% yield. $[\alpha]_D$ −110° (*c* 0.41, EtOH). HRMS: calcd for $C_{43}H_{60}N_4O_9SSiNa$ = 859.3748; found = 859.3766. ^{1}H NMR ($CDCl_3$) (major conformer only): 7.95 (1H, d, $J=8.3$ Hz), 7.47 (1H, d, $J=8.0$ Hz), 7.38 (1H, t, $J=8.0$ Hz), 7.30 (1H, m), 7.18 (2H, m), 6.79 (1H, s), 6.49 (1H, d, $J=8.1$ Hz), 6.30 (1H, d, $J=8.3$ Hz), 5.91 (1H, d, $J=10.7$ Hz), 5.33 (1H, d, $J=10.6$ Hz), 4.88 (1H, d, $J=9.3$ Hz), 4.65 (1H, br), 4.34 (1H, m), 4.28 (1H, d, $J=10.1$ Hz), 4.22 (1H, m), 4.05 (1H, br m), 3.76 (1H, br), 3.59 (1H, br), 3.22 (2H, m), 3.10 (2H, br m), 2.83 (3H, s), 2.56 (1H, br), 2.25 (4H, br m), 1.60 (4H, br), 1.31 (6H, br), 0.87 (5H, m), 0.58 (3H, d, $J=6.5$ Hz), −0.03 (9H, m).

To a stirring 1 mM solution of **20** in DMF was added 6 equiv DMAP, 4 equiv DMAP·HCl, and 4 equiv EDAC. After 5 days, the reaction was concentrated in vacuo, the residue was dissolved in EtOAc and washed with H_2O, 5% $KHSO_4$, satd $NaHCO_3$, H_2O, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. Flash chromatography (silica gel; CH_2Cl_2→20% EtOAC/CH_2Cl_2), followed by size exclusion chromatography, afforded **21** as a colorless, amorphous solid in 15%

yield. $[\alpha]_D$ −41° (*c* 0.39, $CHCl_3$). HRMS: calcd for $C_{43}H_{58}N_4O_8SSiNa$ = 841.3642; found = 841.3632. ^1H NMR ($CDCl_3$): 7.96 (1H, d, *J* = 8.2 Hz), 7.39 (2H, s), 7.38 (1H, t, *J* = 8.5 Hz), 7.32 (1H, d, *J* = 7.9 Hz), 7.20 (1H, t, *J* = 8.1 Hz), 6.85 (1H, s), 6.82 (1H, d, *J* = 7.9 Hz), 6.64 (1H, d, *J* = 7.9 Hz), 5.88 (1H, d, *J* = 10.3 Hz), 5.36 (1H, d, *J* = 10.3 Hz), 5.07 (1H, d, *J* = 10.6 Hz), 4.99 (1H, d, *J* = 10.0 Hz), 4.66 (1H, dd, *J* = 1.4 and 11.6 Hz), 4.56 (1H, m), 4.56 (1H, dd, *J* = 2.2 and 12.2 Hz), 4.49 (1H, ddd, *J* = 2.2 and 10.3 Hz), 3.88 (1H, dd, *J* = 4.4 and 11.4 Hz), 3.73 (1H, dd, *J* = 2.4 and 11.7 Hz), 3.25 (2H, m), 3.16 (1H, dd, *J* = 4.7 and 14.7 Hz), 3.00 (1H, d, *J* = 10.9 Hz), 2.76 (1H, dd, *J* = 1.9 and 14.5 Hz), 2.71 (3H, s), 2.36 (2H, m), 1.85–1.20 (12H, m), 1.14 (3H, d, *J* = 6.5 Hz), 0.92 (3H, d, *J* = 6.5 Hz), 0.83 (2H, m), −0.02 (9H, s).

Acknowledgement

Financial support from the National Institutes of Health (CA-22215) is gratefully acknowledged.

References and Notes

1. For recent reviews on PKC, see for example: Protein Kinase C: Current Concepts and Future Perspectives; Ellis Horwood, 1992.

2. Nakamura, H.; Kishi, Y.; Pajares, M. A.; Rando, R. R. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9672. For reviews on this work, see: Rando, R. R.; Kishi, Y. *Biochemistry* **1992**, *31*, 2211; Kishi, Y.; Rando, R. R. *Acc. Chem. Res.* **1998**, *31*, 163.

3. Fujiki, H.; Suganuma, M.; Tahira, T.; Yoshioka, A.; Nakayasu, M.; Endo, Y.; Shudo, K.; Takayama, S.; Moore, R. E.; Sugimura, T. *Cellular Interactions by Environmental Tumor Promoters*; Japan Sci. Soc.: Utrecht, 1984; pp 37–45.

4. Endo, Y.; Hasegawa, M.; Itai, A.; Shudo, K.; Tori, M.; Asakawa, Y.; Sakai, S. *Tetrahedron Lett.* **1985**, *26*, 1069.

5. These models are quoted in reviews cited under reference 2.

6. Kumagai, H.; Iijima, M.; Dobashi, K.; Naganawa, H.; Sawa, T.; Hamada, M.; Ishizuka, M.; Takeuchi, T. *J. Antibiotics* **1991**, *44*, 1029.

7. The numbering system adopted in this paper corresponds to that of cytoblastin.[6]

8. (a) Preliminary results were communicated in *J. Am. Chem. Soc.* **1996**, *118*, 8180. (b) This paper is largely based on the Dissertation of Moreno, O. A., Harvard University, July, 1996.

9. Still, W. C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405.

10. Fujii, N.; Futaki, S.; Yasamura, K.; Yajima, H. *Chem. Pharm. Bull.* **1984**, *32*, 2660.

11. Weinreb, S. M.; Demko, D. M.; Lessen, T. A. *Tetrahedron Lett.* **1986**, *27*, 2099.

12. Lipshutz, B. H.; Pegram, J. J. *Tetrahedron Lett.* **1980**, *21*, 3343.

13. When the addition failed, the epoxide was quantitatively rearranged to the homobenzylic ketone.

14. Jin, H. ; Uenishi, J.-I.; Christ, W. J.; Kishi, Y. *J. Am. Chem. Soc.* **1986**, *108*, 5644. Takai, K.; Tagashira, M.; Kuroda, T.; Oshima, K.; Utimoto, K.; Nozaki, H. *J. Am. Chem. Soc.* **1986**, *108*, 6048.

15. The organometallic derivatives prepared and tested for the coupling included: $MgCl_2$, $MgCl_2$/CuX, Zn/Cu, Zn/Cu/CuCN·2LiCl, $ZnCl_2$/$CeCl_3$, CuCN, and $MnCl_2$.

16. For a review on Heck reactions, see for example: Hegedus, L. S. *Transition Metals in the Synthesis of Complex Organic Molecules*; University Science Books: Mill Valley, 1994; pp 103–113.

17. *N*-SEM-7-bromo-IL-V was chosen because we had previously encountered difficulty isolating the corresponding *N*-SEM-7-iodo-IL-V.

18. (–)-IL-V was prepared from L-tryptophan methyl ester, according to the reported method: Kogan, T. P.; Somers, T. C.; Venuti, M. C. *Tetrahedron* **1990**, *46*, 6623.

19. Irie, K.; Hagiwara, N.; Koshimizu, K. *Tetrahedron* **1987**, *43*, 5251.

20. The electronic and steric effects of allylstannanes were tested; the coupling with α-acetoxy, α-methoxy, and α-*t*-butyldimethylsilyloxyallylstannanes resulted in exclusive formation of **15**, whereas tri-*sec*-butylstannane resulted in a 5/1 mixture of **14** and **15**.

21. Takayama, H.; Yuhshin, T.; Kitajima, M.; Aimi N.; Sakai, S. *J. Org. Chem.* **1994**, *59*, 4381.

22. (a) For diamine acceleration of osmylations: Corey, E. J.; DaSilva, J. P.; Virgil, S.; Yuen, P. W.; Connell, R. D. *J. Am. Chem. Soc.* **1989**, *111*, 9243. (b) For examples for the use of TMEDA or related diamines for low-temperature osmylation, see: Wang, Y.; Babirad, S. A.; Kishi, Y. *J. Org. Chem.* **1992**, *57*, 468.

23. Hodges, L. M.; Gonzalez, J.; Koontz, J. I.; Myers, W. H.; Harman, W. D. *J. Org. Chem.* **1993**, *58*, 4788 and references cited therein.

24. The extensive mechanistic studies conducted suggest that the observed regioselectivity of the coupling towards the desired adduct arises during the initial transmetallation step, whereas net leakage through a π-allylpalladium intermediate goes unilaterally towards the undesired adduct (ref [1b]).

25. Muchowski, J. M.; Solas, D. R. *J. Org. Chem.* **1984**, *49*, 203.

26. We are indebted to Drs. Kumagai and Naganawa at Microbial Chemistry Research Foundation for a sample of natural cytoblastin.

27. MacroModel version 45 provided courtesy of Professor Still, Columbia University Minimizations were performed on a Silicon Graphics Indigo workstation.

28. Assays performed by Professor Rando at Harvard Medical School and by Dr Gusovsky at Eisai Research Institute.