Total Synthesis and Stereochemistry of Cytoblastin

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Cytoblastin (**1A**), a low molecular weight immunomodulator produced by *Streptoverticillium eurocidium*, was recently reported.[1] Structurally, the upper half of cytoblastin apparently corresponds to (−)-indolactam V (**2**), a natural product containing the minimum pharmacophore for protein kinase C (PKC) activation found in the teleocidin class of tumor promoters.[2] We felt that a structural correlation between cytoblastin and (*S*)-1,2-diacylglycerol (DAG), an endogenous PKC activator, may give further insights on the pharmacophore of the PKC-based tumor promoters.[3] Intriguingly, when the structural correlation proposed from these laboratories[4] is adopted and an imaginary acyl group is placed on the C.27 alcohol,[5] the lower half of cytoblastin also corresponds to the pharmacophore of DAG. Thus, cytoblastin can be viewed as a pseudodimeric form of DAG (Figure 1). Clearly, the validity of this hypothesis hinges on the stereochemistry of cytoblastin. In this communication, we report the first total synthesis and stereochemical elucidation of cytoblastin.

A priori, the upper half of cytoblastin was assumed to correspond structurally to indolactam V. It is known that the nine-membered lactam ring present in the teleocidin family of natural products exists as a mixture of two conformers, dubbed twist (major) and sofa (minor), whereas inversion of one of the stereocenters on this ring results in a conformationally locked system.[6] Cytoblastin was reported to exist as a mixture of two conformers, suggesting that at least the relative stereochemistry of its upper half corresponds to that of indolactam V. Thus, (−)-indolactam V (**2**) was chosen as a starting point for the synthesis.[7] The most expedient approach from **2** to cytoblastin was then envisioned to take place via palladium-mediated coupling of 7-bromoindolactam V (**3**) with allylstannane **4** (Scheme 1).[8] The resultant terminal olefin would then be subjected to dihydroxylation and deprotection to furnish the natural product.

Synthesis of 7-bromoindolactam V (**3**) was achieved via a four-step sequence in 80% overall yield. Thus, protection of the hydroxyl group of **2** as its *tert*-butyldimethylsilyl (TBS) ether, and of the indole nitrogen with 2-(trimethylsilyl)-ethoxymethyl (SEM) chloride,[9] furnished a suitably protected substrate for nuclear bromination, which was efficiently effected with NBS in DMF at low temperature.[10] Although not necessary, it was most convenient for purposes of postcoupling purification to deprotect the alcohol at this juncture (TBAF/THF). The allylstannane **4** was prepared from 3-formylindole, via protection with the (trimethylsilyl)ethylsulfonyl (SES)[11,12] group, followed by treatment with an *in situ* derived 2-(tributylstannyl)ethyl Wittig reagent[13] in 50% overall yield.[14]

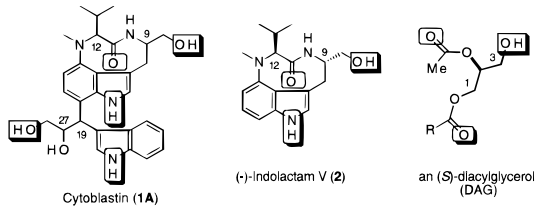
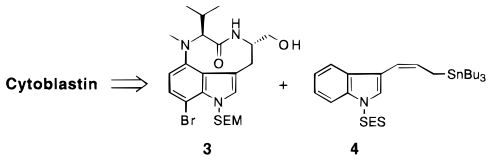


Figure 1. Proposed PKC activation model for cytoblastin (**1A**), (−)-indolactam V (**2**), and an (*S*)-diacylglycerol (DAG). Identical symbols indicate equivalent atoms.

Scheme 1. Retrosynthesis of Cytoblastin



The coupling of **3** with **4** transpired most effectively utilizing a stoichiometric quantity of tetrakis(triphenylphosphine)-palladium(0) in refluxing benzene in the presence of excess tetrabutylammonium iodide (Scheme 2). Thus, **5** was isolated in good yield as an inseparable mixture of diastereomers, along with less than 10% of the regioisomer **6**. Interestingly, polar solvents (DMF, CH_3CN) and/or more weakly coordinating ligands [Ph_3As, $(2\text{-furyl})_3P$, $(o\text{-Tol})_3P$] resulted in virtually exclusive formation of **6** with roughly equivalent yields, whereas stronger coordinating and bidentate ligands (PBu_3, DPPE, DPPP) completely inhibited the reaction.[15] Surprisingly, osmylation of **5** resulted in the formation of triol **7** as the major product (70%), with the remainder (∼25%) comprised of the other three triols. These results demonstrated that both the coupling and the osmylation proceeded in a non-stereorandom fashion, although the sources of the observed stereoselectivities are not obvious at this time.

Although removal of the SES protecting group was facile, cleavage of the SEM protecting group was problematic under standard conditions.[9,16] This difficulty was overcome by performing the deprotection in neat tetrabutylammonium fluoride under vacuum, where the rate of deprotection was observed

(1) Kumagai, H.; Iijima, M.; Dobashi, K.; Naganawa, H.; Sawa, T.; Hamada, M.; Ishizuka, M.; Takeuchi, T. *J. Antibiot.* **1991**, *44*, 1029−1032.

(2) 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 Scientific Societies Press: Utrecht, 1984; pp 37−45.

(3) For some recent activities in this area, see: Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917−924. Kozikowski, A. P.; Ma, D.; Du, L.; Lewin, N. E.; Blumberg, P. M. *J. Am. Chem. Soc.* **1995**, *117*, 6666−6672. Irie, K.; Ishii, T.; Ohigashi, H.; Wender, P. A.; Miller, B. L.; Takeda, N. *J. Org. Chem.* **1996**, *61*, 2164−2173. Endo, Y.; Ohno, M.; Hirano, M.; Itai, A.; Shudo, K. *J. Am. Chem. Soc.* **1996**, *118*, 1841−1855. Endo, Y.; Yamaguchi, M.; Hirano, M.; Shudo, K. *Chem. Pharm. Bull.* **1996**, *44*, 1138−1149. Sugita, K.; Sawada, D.; Sodeoka, M.; Sasai, H.; Shibasaki, M. *Chem. Pharm. Bull.* **1996**, *44*, 463−465.

(4) Nakamura, H.; Kishi, Y.; Pajares, M. A.; Rando, R. R. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9672−9676. For a review on this work, see: Rando, R. R.; Kishi, Y. *Biochemistry* **1992**, *31*, 2211−2218.

(5) The cytoblastin numbering[1] is adopted in this paper.

(6) Endo, Y.; Hasegawa, M.; Itai, A.; Shudo, K.; Tori, M.; Asakawa, Y.; Sakai, S. *Tetrahedron Lett.* **1985**, *26*, 1069−1072.

(7) Although **2** can be purchased at considerable expense, it is readily prepared via published protocols, e.g.: Kogan, T. P.; Somers, T. C.; Venuti, M. C. *Tetrahedron* **1990**, *46*, 6623−6632.

(8) Kosugi, M.; Sasazawa, K.; Shimizu, Y.; Migita, T. *Chem. Lett.* **1977**, 301−302.

(9) Lipshutz, B. H.; Pegram, J. J. *Tetrahedron Lett.* **1980**, *21*, 3343−3346.

(10) Mitchell, R. H.; Lai, Y. H.; Williams, R. V. *J. Org. Chem.* **1979**, *44*, 4733−4735.

(11) Weinreb, S. M.; Demko, D. M.; Lessen, T. A. *Tetrahedron Lett.* **1986**, *27*, 2099−2102.

(12) Fujii, N.; Futaki, S.; Yasamura, K.; Yajima, H. *Chem. Pharm. Bull.* **1984**, *32*, 2660−2665.

(13) Hannon, S. T.; Traylor, T. G. *J. Chem. Soc., Chem. Commun.* **1975**, 630−631.

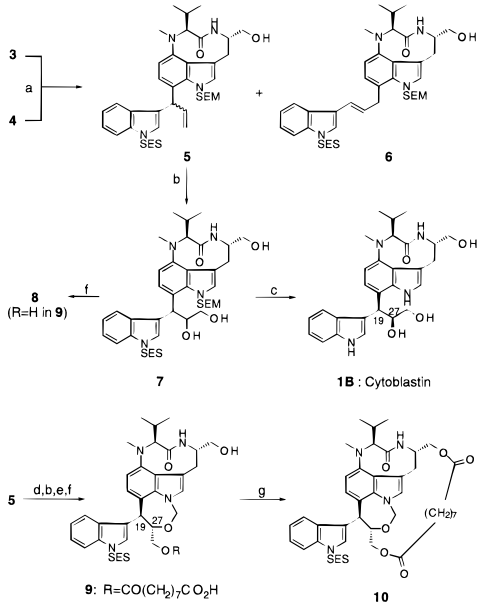
(14) This method produced the *cis*-allylstannane with, at most, only a trace (<5%) of the *trans* compound (^{1}H NMR). The analogous *trans*-allylstannane was prepared via conjugate addition of Lipshutz's mixed higher order stannylcuprate (Lipshutz, B. H.; Ellsworth, E. L.; Dimock, S. H.; Reuter, D. C. *Tetrahedron Lett.* **1989**, *30*, 2065−2068) to the allylic acetate derived from the same protected formylindole; this longer sequence was forsaken, as the two allylstannanes produced degenerate results in the subsequent coupling.

(15) Multivariable and mechanistic analyses of the reaction pathway will be given in detail: Moreno, O. A. Ph.D. Dissertation, Harvard University, 1996.

(16) Muchowski, J. M.; Solas, D. R. *J. Org. Chem.* **1984**, *49*, 203−205.

S0002-7863(96)01841-0 CCC: $12.00

Scheme 2[a]

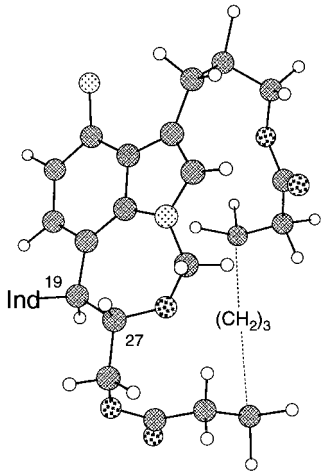


[a] Reagents, reaction conditions, and yields: (a) $Pd(PPh_3)_4$, TBAI, PhH, Δ (80%). (b) OsO_4, TMEDA, CH_2Cl_2, −90 °C (70%). (c) TBAF, 0.1 mmHg (90%). (d) TBS−Cl, imidazole, DMF, rt (90%). (e) Azelaic acid, EDAC, DMAP, DMAP·HCl, CH_2Cl_2 (80%). (f) $LiBF_4$, CH_3CN, CSA, THF (85%). (g) EDAC, DMAP, DMAP·HCl, CH_2Cl_2 (15%).

to increase with the degree of vacuum. 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. Fortuitously, deprotection of the major osmylation product led to a compound (**1B**) which matched the naturally occurring cytoblastin[17] in all analyses performed (TLC, HRMS, ^{1}H NMR, α_D, CD).

The fact that **3** led to the natural product demonstrated that the stereochemistry of the upper half of cytoblastin indeed corresponds to that of (−)-indolactam 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. It was found that treatment of **7** with a combination of lithium tetrafluoroborate and camphorsulfonic acid (CSA) resulted in transketalization of the SEM group with the secondary hydroxyl group to yield **8**. The vicinal coupling constant (J = 10.0 Hz) observed for the C.19 and C.27 protons of the resultant seven-membered cyclic aminal, coupled with the lack of NOE between them, demonstrated a *trans* relationship between these protons. This relationship was further supported by an NOE between the C.19 proton and only one of the aminal methylene protons, and another NOE between the C.27 proton and only the other aminal methylene proton.

In order to elucidate the absolute stereochemistry of the lower half, 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, while the twist conformation orients it on the other side.[5] To this end, the hydroxyl group of **5** was protected as its TBS ether prior to osmylation, and the primary hydroxyl introduced during the osmylation was then selectively esterified with azelaic acid. Transketalization with the $LiBF_4$/CSA reagent system was accompanied by TBS removal to produce the hydroxy acid **9**, which was then macrolactonized[18] to provide **10**. Upon treatment with (1) K_2CO_3/MeOH, (2) CSA/THF/H_2O, and (3) TBAF/THF, **10** was reverted to **1B**, demonstrating preservation of the structural unit of cytoblastin. 2D NOESY of **10** revealed that the lactam ring was locked into its sofa conformation.[19] Since it had been established that C.19 and C.27 were *trans* (*vide supra*), NOEs between the C.19 proton and the aliphatic chain (none were observed with C.27) indicate on which side of the aminal ring the chain passes. Thus, with the lactam ring conformation 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 could be deduced to be that indicated in **10**. This conclusion was further endorsed by the additional data provided via the 2D NOESY and COSY NMR experiments.[20] It is worth mentioning that this structural information agrees with that provided by the MacroModel molecular modeling program (Figure 2).[21] Thus, as hypothesized, the C.27 stereochemistry of cytoblastin was determined to be as shown in **1B**, consistent with that of C.9 of indolactam V (**2**) and, of course, cytoblastin.



, , , represent C, N, O, and H, respectively

Figure 2. Partial stereoview of the lactam **10**.

Finally, although it is generally agreed that (−)-indolactam V (**2**) possesses the minimum structural requirements for PKC activation,[2] it is interesting to note that cytoblastin was originally reported to exhibit a complete lack of activity toward PKC. However, assays of both natural and synthetic cytoblastin have shown an activity toward PKC that is roughly equivalent to that of (−)-indolactam V.[22]

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Supporting Information Available: Experimental details and spectroscopic data for all compounds reported, as well as ^{1}H NMR spectra of synthetic and natural cytoblastin and 2D COSY and NOESY spectra of **10** (9 pages). See any current masthead page for ordering and Internet access instructions.

JA9618412

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

(18) Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394−2395.

(19) For ^{1}H NMR and diagnostic NOEs of the two indolactam V conformations, see ref 5.

(20) For the details, see the 2D COSY and NOESY spectra included in the supporting information.

(21) MacroModel version 4.5 provided courtesy of Prof. Still, Columbia University. Minimizations were performed on a Silicon Graphics Indigo workstation.

(22) Assays performed by Prof. Rando at Harvard Medical School and by Dr. Gusovsky at Eisai Research Institute. Details of these assays are published elsewhere.