Natural Products Synthesis

Total Synthesis and Configurational Assignment of (−)-Dictyostatin, a Microtubule-Stabilizing Macrolide of Marine Sponge Origin**

Ian Paterson, Robert Britton, Oscar Delgado, Arndt Meyer, and Karine G. Poullennec*

Dictyostatin (**1**, Scheme 1) is a potent cytotoxic macrolide first isolated by Pettit et al.[1] from a marine sponge of the genus *Spongia sp.*, collected in the Republic of Maldives, and, subsequently, isolated by Wright and co-workers[2] from a *lithistida* sponge of the family *Corallistidae,* harvested at great depths off the Jamaican coast. The planar structure of this unsaturated 22-membered macrolactone, featuring 11 stereogenic centers, an endocyclic 2*Z*,4*E* dienoate and a pendant *Z*-diene moiety at C21, was deduced by the Pettit group, primarily on the basis of 2D NMR spectroscopic data.[1] Recently, we proposed a full stereochemical assignment for dictyostatin, as indicated in **1**, based on the use of extensive high-field NMR experiments, including the Murata *J*-based configuration analysis, in combination with molecular modeling.[3] Although it was not possible to ascertain the absolute configuration, our assignment was based on dictyostatin being biogenetically related to discodermolide.[4]

Notably, dictyostatin displays powerful growth-inhibitory activity against a number of murine and human cancer cells at low nanomolar concentrations and, importantly, retains

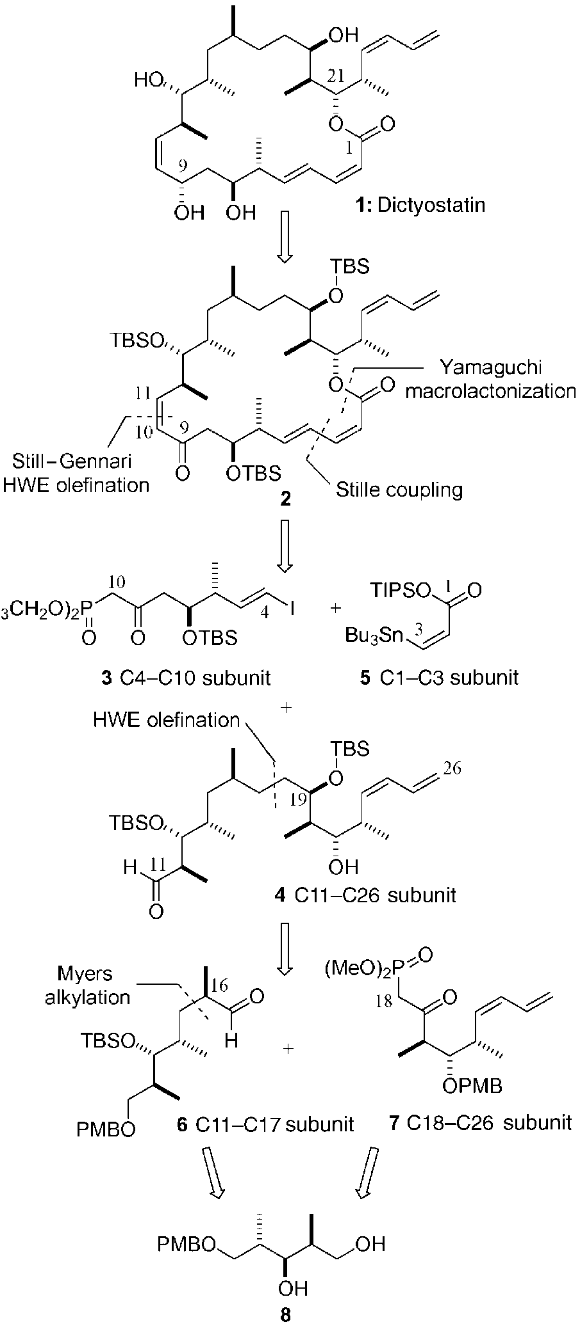
---

[*] Prof. Dr. I. Paterson, Dr. R. Britton, Dr. O. Delgado, A. Meyer, Dr. K. G. Poullennec
University Chemical Laboratory
Lensfield Road, Cambridge, CB2 1EW (UK)
Fax: (+44) 1223-336-362
E-mail: ip100@cam.ac.uk

[**] This research was supported by the EPSRC, the NSERC-Canada (R.B.), the Gobierno de Canarias, the Cambridge European Trust (O.D.), the EC (Network HPRN-CT-2000-18 and IE Marie Curie Fellowship to K.G.P.), Novartis Pharma AG, and the Merck Research Council. We thank Dr. Amy Wright (Harbor Branch Oceanographic Institution) for helpful discussions and for providing an authentic sample of dictyostatin, and Kate Ashton (Cambridge) for molecular modeling assistance.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

DOI: 10.1002/ange.200460589

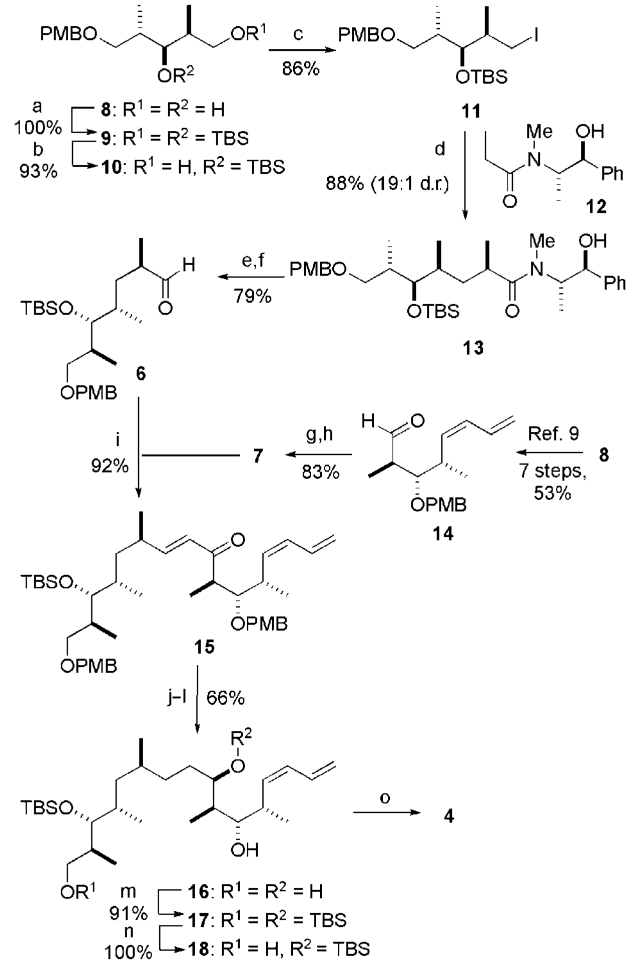


Scheme 1. Retrosynthetic analysis for dictyostatin (**1**) leading to key building blocks **3**, **6**, and **7**.

activity against taxol-resistant cancer cells that express active P-glycoprotein. Preliminary studies into the mechanism of action demonstrated that dictyostatin, in a similar manner to taxol (paclitaxel), arrests cells in the G2/M phase by potently inducing tubulin polymerization and suppressing normal microtubule dynamics, leading to apoptosis.[2] Hence, dictyostatin currently represents a promising antimitotic natural product lead for development in cancer chemotherapy, joining an elite group of microtubule-stabilizing polyketides that include laulimalide,[5] peloruside A,[6] and discodermolide.[7] Unfortunately, evaluation of its antitumor properties has been precluded so far by the very low natural abundance. Herein, we disclose the first total synthesis of dictyostatin (**1**) and establish unequivocally the relative and absolute configuration of this rare polyketide metabolite. Furthermore, the modular synthetic approach employed is flexible, highly convergent, and stereocontrolled, and thus offers the potential to provide useful quantities of dictyostatin as well as a range of structural derivatives to initiate structure–activity relationship studies.

As outlined retrosynthetically in Scheme 1, our approach relies on a late-stage reduction of the enone **2**, controlled by the macrocyclic conformation adopted by the 22-membered ring; molecular modeling studies[8] indicated that hydride addition should occur selectively to install the requisite C9 hydroxyl-bearing stereocenter. The 10*Z* alkene moiety in **2** was planned to arise from a Still–Gennari-type olefination between the β-keto phosphonate **3** and aldehyde **4**, in conjunction with a Stille cross-coupling with vinyl stannane **5** to deliver the 2*Z*,4*E* dienoate. In principle, either of these two reactions could be employed to close the macrocycle as an alternative to a more conventional Yamaguchi macrolactonization, thus offering considerable flexibility in the synthesis. In turn, the C11–C26 subunit **4** should be accessible by a Horner–Wadsworth–Emmons (HWE) coupling between aldehyde **6** and phosphonate **7**, which contains the terminal *Z* diene. As these latter segments share a common stereochemical triad, it was anticipated that both **6** and **7** could be prepared from common intermediate **8**, which is available in multigram quantities through efficient aldol methodology reported previously by our group.[9]

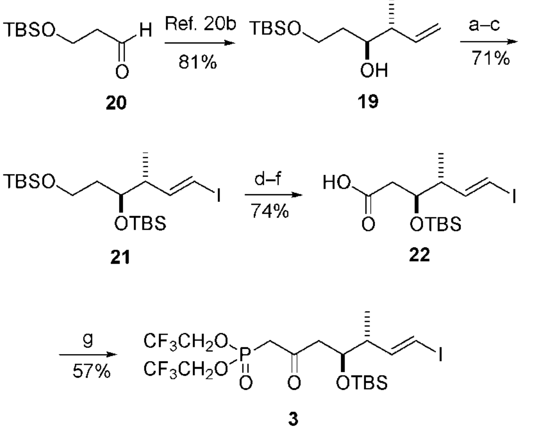
Synthesis of the C11–C26 subunit **4**, as shown in Scheme 2, commenced with conversion of 1,3-diol **8** into bis-TBS ether **9** and liberation of the primary hydroxy group under mildly acidic conditions. The resulting alcohol **10** was readily converted into iodide **11** through a modified Mitsunobu protocol (PPh_3, I_2, imid).[10] Subsequent alkylation of the lithium enolate of the Myers propionamide **12**[11] effected a three-carbon homologation, delivering **13** with excellent diastereoselectivity and yield (19:1 d.r., 88%).[12] Reductive removal of the pseudoephedrine auxiliary ($LiNH_2BH_3$)[11] afforded a primary alcohol, which was, in turn, oxidized with Dess–Martin periodinane to provide aldehyde **6**. The HWE coupling partner **7** was synthesized by addition of $(MeO)_2P(O)CH_2Li$ to aldehyde **14**, which was prepared from 1,3-diol **8** as described previously.[9,13] The resulting epimeric mixture of alcohols was converted into β-ketophosphonate **7** (Dess–Martin periodinane, 83% over two steps). Following a HWE coupling between aldehyde **6** and phosphonate **7**, employing $Ba(OH)_2$ in wet THF, enone **15** was isolated in 92% yield.[14] With the entire C11–C26 backbone assembled, we next focused on the stereocontrolled reduction of the C19 ketone directed by the proximal hydroxy-bearing stereocenter through metal chelation. To this end, conjugate reduction of enone **15** with the Stryker reagent $\{[Ph_3PCuH]_6\}$[15] and oxidative removal of both PMB groups with DDQ provided the intermediate β-hydroxyketone, which underwent 1,3-*syn*-selective reduction when treated with a cold (−30°C) ethereal solution of $Zn(BH_4)_2$,[16] generating triol **16** (>20:1 d.r.).[17] Finally, the synthesis of subunit **4** was completed by a three-step sequence involving



Scheme 2. Synthesis of C11–C26 subunit **4**. a) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0°C→RT, 15 min; b) *p*-TSA (30 mol%), 20:1 THF/H_2O, 24 h; c) PPh_3, I_2, imid, PhMe, 0°C, 15 min; d) 1) **12**, LiCl, LDA, THF, −78°C→RT; 2) **11**, THF, 0°C→RT, 18 h; e) 1) LDA, BH_3NH_3, THF, 0°C; 2) **13**, THF, 0°C→RT, 18 h; f) DMP, $NaHCO_3$, CH_2Cl_2, 0°C, 2 h; g) 1) *n*BuLi, $(MeO)_2P(O)Me$, THF, −78°C, 30 min; 2) **14**, THF, −78°C ; h) DMP, CH_2Cl_2, room temperature, 10 min; i) 1) **7**, $Ba(OH)_2\cdot 8H_2O$, THF, 1 h; 2) **6**, 40:1 THF/H_2O, 20 h; j) $[Ph_3PCuH]_6$, PhH, H_2O, room temperature, 2 h; k) DDQ, pH 7 buffer, CH_2Cl_2, 0°C, 6 h; l) $Zn(BH_4)_2$, Et_2O, −30°C, 2 h; m) TBSOTf, 2,6-lutidine, −78→0°C, 30 min; n) TBAF, AcOH, THF, room temperature, 24 h; o) TEMPO, $PhI(OAc)_2$, CH_2Cl_2, room temperature, 18 h. TBS = *tert*-butyldimethylsilyl, Tf = trifluoromethanesulfonyl, *p*-TSA = *p*-toluenesulfonic acid, imid = imidazole, LDA = lithium diisopropylamide, DMP = Dess–Martin periodinane, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, TBAF = tetrabutylammonium fluoride, TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical.

selective TBS protection of the C11 and C19 hydroxy groups over that at C21 (TBSOTf), cleavage (TBAF, AcOH) of the primary TBS ether in **17**, and oxidation of resulting alcohol **18** (TEMPO/$PhI(OAc)_2$).[18]

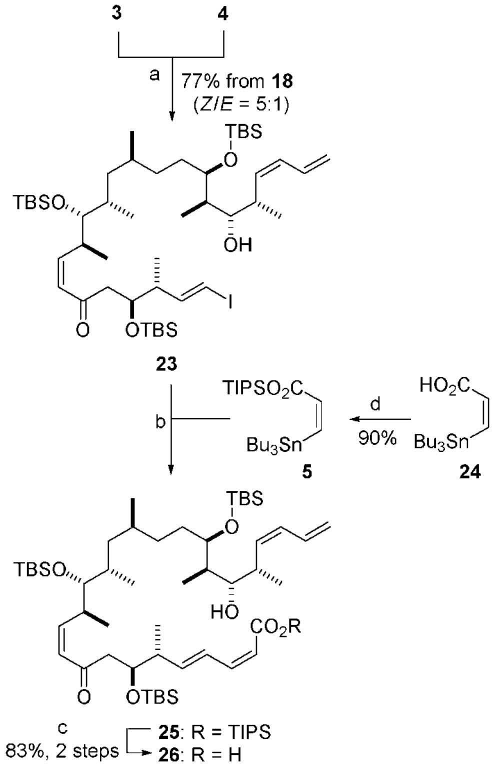
As our primary objective was an efficient and convergent synthesis of dictyostatin, it was crucial to install the 10*Z* alkene function by employing advanced coupling partners. Although a number of protocols exist to effect such coupling, most require strongly basic conditions. Our concerns regarding the epimerizable center at C12 in aldehyde **4** led us to investigate the mild Still–Gennari modification of the HWE olefination.[19] The requisite F_3CCH_2O-substituted phosphonate **3** was prepared from alcohol **19**, which was obtained with 95% *ee* and 20:1 d.r. through a Brown asymmetric crotylation of aldehyde **20** (Scheme 3).[20] Silyl ether formation (TBSOTf), ozonolysis, and Takai methylenation[21] of the intermediate aldehyde provided *E* vinyl iodide **21** in 71% yield. After selective cleavage of the primary silyl ether, oxidation of the resulting alcohol afforded acid **22**, which was transformed into its acid chloride by using the Ghosez reagent ($Me_2C{=}C(Cl)NMe_2$).[22] Next, the acid chloride was added to a solution of $(F_3CCH_2O)_2P(O)CH_2Li$ in THF,[23] generated at −100°C, to afford phosphonate **3** containing the required functionality for the pivotal fragment assembly.



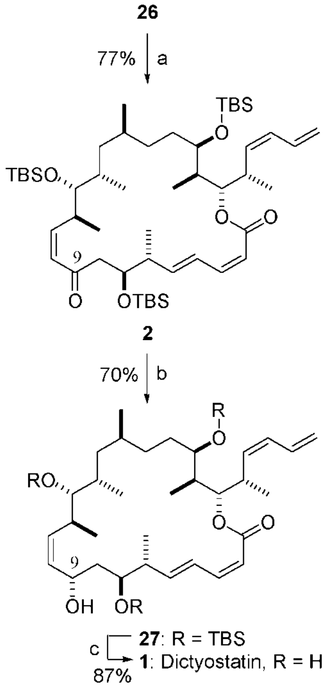
Scheme 3. Synthesis of C4–C10 subunit **3**. a) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78°C, 30 min; b) 1) O_3, CH_2Cl_2, −78°C; 2) Ph_3P, −78°C→RT, 2 h; c) $CrCl_2$, CHI_3, THF, dioxane, 0°C, 18 h; d) TBAF, AcOH, THF, room temperature, 14 h; e) DMP, py, CH_2Cl_2, room temperature, 3 h; f) $NaClO_2$, NaH_2PO_4, 10:1 *t*BuOH/H_2O, 0°C, 2 h; g) 1) $Me_2C{=}C(Cl)NMe_2$, CH_2Cl_2, room temperature, 15 min; 2) $(F_3CCH_2O)_2P(O)CH_2Li$, THF, −100°C, 1 h.

To our delight, when a mixture of aldehyde **4** and phosphonate **3** was subjected to an excess of K_2CO_3 in the presence of [18]crown-6 in toluene (Scheme 4),[19] the HWE coupling proceeded smoothly to produce *Z* enone **23** efficiently, with good levels of selectivity (*Z*/*E* = 5:1, 77%). Notably, this constitutes one of the first examples of a *Z*-selective *intermolecular* Still–Gennari olefination employing such an elaborate β-ketophosphonate. Construction of the dictyostatin carbon backbone was completed through a copper-mediated, Liebeskind-type Stille coupling[24] (CuTC in NMP) of vinyl iodide **23** and *Z* alkenyl stannane **5**, which is readily accessible from known acid **24**.[25] Finally, TIPS ester **25** was converted into acid **26** (KF, THF/MeOH) in 83% overall yield.

With **26** in hand, attention was now directed towards the completion of the synthesis, as shown in Scheme 5. Seco acid **26** was subjected to a standard Yamaguchi macrolactonization protocol (2,4,6-trichlorobenzoyl chloride, NEt_3, DMAP, PhMe, 60°C, 2 h) to furnish the 22-membered macrocycle **2** cleanly in 77% yield.[26] Next, we investigated the reduction of the C9 ketone, in a bid to exploit macrocyclic stereocontrol.[8]



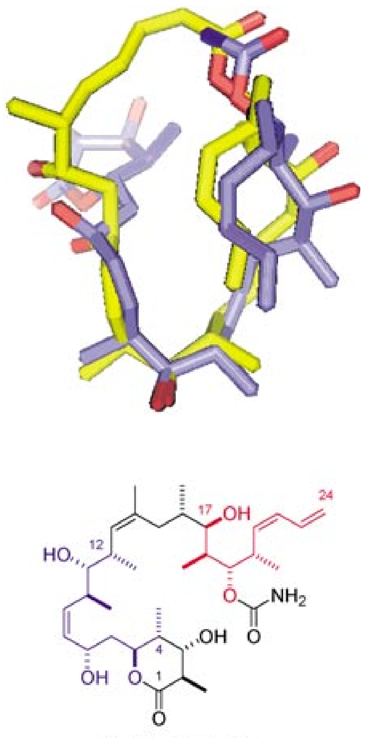
Scheme 4. Synthesis of seco acid **26**. a) K_2CO_3 (10 equiv), [18]crown-6 (25 equiv), toluene, room temperature, 48 h; b) CuTC, NMP, room temperature, 1 h; c) KF, THF, MeOH, room temperature, 2 h; d) TIPSCl, Et_3N, CH_2Cl_2, room temperature, 45 min. CuTC = copper(I) thiophenecarboxylate, NMP = 1-methyl-2-pyrrolidinone, TIPS = triisopropylsilyl.



Scheme 5. Completion of the synthesis of dictyostatin (**1**). a) 2,4,6-trichlorobenzoyl chloride, NEt_3, DMAP, PhMe, 60 °C, 2 h; b) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, EtOH, −30 °C; c) HCl (3 N), MeOH, room temperature, 6 h. DMAP = 4-dimethylaminopyridine.

Gratifyingly, Luche reduction of enone **2** ($NaBH_4$, $CeCl_3$, EtOH)[27] provided the expected alcohol **27** (70%). Finally, global deprotection with HCl (3 N) in methanol gave dictyostatin (**1**) in 87% yield. The spectroscopic data of our synthetic material were in complete agreement with those of an authentic sample obtained from the *Corallistidae* sponge source (^1H (700 MHz), ^{13}C NMR (125 MHz), MS), and the specific rotation of $[\alpha]_D = -32.7$ ($c = 0.22$, MeOH) agreed with that measured in our laboratory for the authentic sample,[28] thus allowing confident assignment of the relative and absolute configuration of dictyostatin and validating our earlier proposal.[3a]

Significantly, this configurational assignment for dictyostatin is fully consistent with a common biogenesis for the structurally related, but open-chain, polyketide discodermolide (**28**, Figure 1), presently in clinical development as a new-generation microtubule-stabilizing anticancer agent.[29] There is an exact configurational match of the C19–C26 and C6–C14 regions of dictyostatin with those at C17–C24 (shown in red) and C4–C12 (shown in blue) of discodermolide, respectively. As shown by the overlay in Figure 1, the preferred conformation for dictyostatin in solution[3a] resembles closely that determined for discodermolide in the solid state, suggesting that they may interact in a similar fashion with the taxol-binding site on β-tubulin.[30] As a conformationally constrained macrocyclic analogue of discodermolide, dictyostatin represents a highly attractive template for rational drug design.



28: Discodermolide

Figure 1. Overlay of preferred solution conformation[3a] of dictyostatin (in yellow, C1/C2 s-*trans* rotamer) in water with conformation of discodermolide (**28**) obtained from the X-ray crystal structure (in blue).

In conclusion, this expedient total synthesis of (−)-dictyostatin proceeds in 27 steps and 3.8% overall yield (longest linear sequence from the Roche ester[9]) and establishes unequivocally the relative and absolute configuration. Key transformations include an unprecedented Still–Gennari-type HWE coupling with a complex β-ketophosphonate, a Liebeskind-type Stille cross-coupling to install the 2*Z*,4*E* dienoate, a facile Yamaguchi macrolactonization to construct the macrocycle, and subsequent reduction of the C9 ketone under macrocyclic stereocontrol. Importantly, this modular, convergent synthesis should be amenable to producing useful quantities and designed analogues of this novel microtubule-stabilizing agent, enabling extensive exploration of its anticancer properties.

Received: May 7, 2004

Keywords: antitumor agents · conformation analysis · macrocycles · natural products · total synthesis

---

[1] G. R. Pettit, Z. A. Cichacz, F. Gao, M. R. Boyd, J. M. Schmidt, *J. Chem. Soc. Chem. Commun.* **1994**, 1111.

[2] R. A. Isbrucker, J. Cummins, S. A. Pomponi, R. E. Longley, A. E. Wright, *Biochem. Pharmacol.* **2003**, *66*, 75.

[3] a) I. Paterson, R. Britton, O. Delgado, A. E. Wright, *Chem. Commun.* **2004**, 632; b) G. R. Pettit, Z. A. Cichacz, WO 5430053, **1995** [*Chem. Abstr.* **1995**,733500; this patent contains a partial stereochemical assignment for dictyostatin that differs significantly from our findings.

[4] The structural similarities between discodermolide and dictyostatin and the scope for preparing hybrid structures have been highlighted before: Y. Shin, N. Choy, R. Balachandran, C. Madiraju, B. W. Day, D. P. Curran, *Org. Lett.* **2002**, *4*, 4443. For an alternative total synthesis of dictyostatin, which independently confirms our earlier stereochemical assignment,[3a] see the following Communication in this issue: Y. Shin, J.-H. Fournier, Y. Fukui, A. M. Brückner, D. P. Curran, *Angew. Chem.* **2004**, *116*, 4734; *Angew. Chem. Int. Ed.* **2004**, *43*, 4634. We thank Professor Curran for the exchange of information.

[5] S. L. Mooberry, G. Tien, A. H. Hernandez, A. Plubrukarn, B. S. Davidson, *Cancer Res.* **1999**, *59*, 653.

[6] K. A. Hood, L. M. West, B. Rouwe, P. T. Northcote, M. V. Berridge, S. J. Wakefield, J. H. Miller, *Cancer Res.* **2002**, *62*, 3356.

[7] a) E. ter Haar, R. J. Kowalski, E. Hamel, C. M. Lin, R. E. Longley, S. P. Gunasekera, H. S. Rosenkranz, B. W. Day, *Biochemistry* **1996**, *35*, 243; b) for a review, see: I. Paterson, G. J. Florence, *Eur. J. Org. Chem.* **2003**, *12*, 2193.

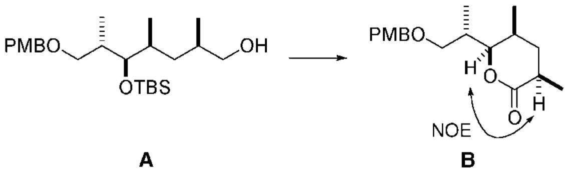
[8] Macromodel (Version 8.0) was used in a 1700-step Monte Carlo search with **2** using the MM2* force field. The accessible low-energy conformations found all had the lower (*Si*) face of the ketone blocked by the bulky TBS ether attached to C7, such that hydride attack was expected from the upper (*Re*) face.

[9] 1,3-Diol **8** was prepared on a multigram scale in five steps and 52% yield from methyl (*S*)-3-hydroxy-2-methylpropionate (Roche ester): I. Paterson, O. Delgado, G. J. Florence, I. Lyothier, J. P. Scott, N. Sereinig, *Org. Lett.* **2003**, *5*, 35.

[10] P. J. Garegg, B. Samuelson, *J. Chem. Soc. Perkin Trans. 1* **1980**, 2866.

[11] A. G. Myers, B. H. Yang, H. Chen, L. McKinstry, D. J. Kopecky, J. L. Gleason, *J. Am. Chem. Soc.* **1997**, *119*, 6496.

[12] The 1,3-*syn* stereochemistry of amide **13** was confirmed by NOE enhancements on lactone **B**, obtained by a two-step sequence (HCl (2 N), EtOH; TEMPO, PhI(OAc)$_2$) from alcohol **A**.



[13] a) I. Paterson, G. J. Florence, K. Gerlach, J. P. Scott, *Angew. Chem.* **2000**, *112*, 385; *Angew. Chem. Int. Ed.* **2000**, *39*, 377; b) I. Paterson, G. J. Florence, K. Gerlach, J. P. Scott, N. Sereinig, *J. Am. Chem. Soc.* **2001**, *123*, 9535.

[14] I. Paterson, K.-S. Yeung, J. B. Smaill, *Synlett* **1993**, 774.

[15] W. S. Mahoney, D. M. Brestensky, J. M. Stryker, *J. Am. Chem. Soc.* **1988**, *110*, 291.

[16] T. Oishi, T. Nakata, *Acc. Chem. Res.* **1984**, *17*, 338.

[17] The relative stereochemistry of **16** was confirmed unequivocally by NMR spectroscopy experiments performed on the corresponding acetonide.

[18] A. De Mico, R. Margarita, L. Parlanti, A. Vescovi, G. Piancatelli, *J. Org. Chem.* **1997**, *62*, 6974.

[19] W. C. Still, C. Gennari, *Tetrahedron Lett.* **1983**, *24*, 4405.

[20] a) H. C. Brown, K. S. Bhat, R. S. Randad, *J. Org. Chem.* **1989**, *54*, 1570; b) I. Paterson, K. Ashton, R. Britton, H. Knust, *Org. Lett.* **2003**, *5*, 1963.

[21] K. Takai, K. Nitta, K. Utimoto, *J. Am. Chem. Soc.* **1986**, *108*, 7408.

[22] A. Devos, J. Remion, A. M. Frisque-Hesbain, A. Colens, L. Ghosez, *J. Chem. Soc. Chem. Commun.* **1979**, 1180.

[23] C. Patois, P. Savignac, E. About-Jadet, N. Collignon, *Synth. Commun.* **1991**, *21*, 2391.

[24] G. D. Allred, L. S. Liebeskind, *J. Am. Chem. Soc.* **1996**, *118*, 2748.

[25] D. J. Crichter, G. Pattenden, *Tetrahedron Lett.* **1996**, *37*, 9107.

[26] J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.

[27] J. L. Luche, *J. Am. Chem. Soc.* **1978**, *100*, 2226.

[28] In comparison, the specific rotation measured for a natural sample of dictyostatin-1 was $[\alpha]_D = -27.4$ ($c = 0.16$, MeOH), whereas that reported by Pettit et al.[1] was -20 ($c = 0.12$, MeOH). For copies of ^{1}H and ^{13}C NMR spectra, see the Supporting Information.

[29] For the recent large-scale synthesis of discodermolide, see: S. J. Mickel, D. Niederer, R. Daeffler, A. Osmani, E. Kuesters, E. Schmid, K. Schaer, R. Gamboni, W. Chen, E. Loeser, F. R. Kinder, K. Konigsberger, K. Prasad, T. M. Ramsey, O. Repic, R.-M. Wang, G. J. Florence, I. Lyothier, I. Paterson, *Org. Process Res. Dev.* **2004**, *8*, 122.

[30] Note added in proof: Preliminary biological evaluation suggests that dictyostatin is also a ligand for the taxoid binding site on β.tutublin and binds more strongly than taxol. We thank Dr. Fernando Díaz and Ruben M. Buey (Centro de Investigaciones Biológicas, CSIC, Madrid) for carrying out this study with synthetic dictyostatin.